

MULTI-CHAIN CHIMERIC POLYPEPTIDES AND USE THEREOF IN THE TREATMENT OF LIVER DISEASES

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims priority to U.S. Provisional Application Serial No. 63/232,140, filed on August 11, 2021, and U.S. Provisional Application Serial No. 63/330,757, filed on April 13, 2022, each of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

10 This application contains a Sequence Listing that has been submitted electronically as an XML file named 47039-0025WO1_SL_ST26.XML. The XML file, created on August 9, 2022, is 83,507 bytes in size. The material in the XML file is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

15 The present disclosure relates to the field of biotechnology, and more specifically, to antigen-binding molecules and the treatment of liver diseases.

BACKGROUND

20 Tissue factor (TF), a 263 amino acid integral membrane glycoprotein with a molecular weight of ~46 kDa and the trigger protein of the extrinsic blood coagulation pathway, is the primary initiator of coagulation *in vivo*. Tissue factor, normally not in contact with circulating blood, initiates the coagulation cascade upon exposure to the circulating coagulation serine protease factors. Vascular damage exposes sub-endothelial
25 cells expressing tissue factor, resulting in the formation of a calcium-dependent, high-affinity complex with pre-existing plasma factor VIIa (FVIIa). Binding of the serine protease FVIIa to tissue factor promotes rapid cleavage of FX to FXa and FIX to FIXa. The proteolytic activity of the resulting FXa and an active membrane surface then inefficiently converts a small amount of prothrombin to thrombin. The thrombin
30 generated by FXa initiates platelet activation and activates minute amounts of the pro-

cofactors factor V (FV) and factor VIII (FVIII) to become active cofactors, factor Va (FVa) and factor VIIIa (FVIIIa). FIXa complexes with FVIIIa on the platelet surface forming the intrinsic tenase complex, which results in rapid generation of FXa. FXa complexes with FVa to form the pro-thrombinase complex on the activated platelet surface which results in rapid cleavage of prothrombin to thrombin.

In addition to the tissue factor-FVIIa complex, a recent study showed that the tissue factor-FVIIa-FXa complex can activate FVIII, which would provide additional levels of FVIIIa during the initiation phase. The extrinsic pathway is paramount in initiating coagulation via the activation of limited amounts of thrombin, whereas the intrinsic pathway maintains coagulation by dramatic amplification of the initial signal.

Much of the tissue factor expressed on a cell surface is “encrypted,” which must be “decrypted” for full participation in coagulation. The mechanism of “decryption” of cell-surface tissue factor is still unclear at this time, however, exposure of anionic phospholipids plays a major role in this process. Healthy cells actively sequester anionic phospholipids such as phosphatidyl serine (PS) to the inner leaflet of the plasma membrane. Following cellular damage, activation, or increased levels of cytosolic Ca^{2+} , this bilayer asymmetry is lost, resulting in increased PS exposure on the outer leaflet, which increases the specific activity of cell-surface tissue factor-FVIIa complexes. PS exposure is known to decrease the apparent K_m for activation of FIX and FX by tissue factor-FVIIa complexes, but additional mechanisms could include conformational rearrangement of tissue factor or tissue factor-FVIIa and subsequent exposure of substrate binding sites.

SUMMARY

Provided herein are methods of treating a liver disease or a metabolic syndrome in a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric

polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII. In some embodiments of any of the methods

5 described herein, the liver disease is selected from the group of: fatty liver disease, hepatic steatosis, acute hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy,

10 hepatitis A, hepatitis B, hepatitis C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage

15 disease, and Wilson's disease. In some embodiments of any of the methods described herein, the metabolic syndrome is selected from the group of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial hypertension, stroke, and cataracts.

20 Also provided herein are methods of reducing one or more of the rate of: progression from non-alcoholic fatty liver disease (NAFL) to non-alcoholic steatohepatitis (NASH), progression from NASH to cirrhosis, and progression from cirrhosis to hepatocellular carcinoma, that include administering to a subject identified or diagnosed as having NAFL, NASH, or cirrhosis, a therapeutically effective amount of a

25 multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric

30 polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a

ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII. In some embodiments of any of the methods described herein, the method results in a decreasing in the rate of progression from NAFL to NASH. In some embodiments of any of the methods described herein, the method
5 results in decreasing the rate of progression of NASH to cirrhosis. In some embodiments of any of the methods described herein, the method results in decreasing the rate of progression from cirrhosis to hepatocellular carcinoma.

Also provided herein are methods of reducing inflammation in a liver of a subject that include administering to the subject a therapeutically effective amount of a multi-
10 chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate
15 through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

Also provided herein are methods of decreasing gluconeogenesis in a liver of a
20 subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-
25 binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

Also provided herein are methods of decreasing lipogenesis in a liver of a subject
30 that include administering to the subject a therapeutically effective amount of a multi-

chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

Also provided herein are methods of decreasing hepatocytic senescence in a liver of a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

Also provided herein are methods of rebalancing metabolic function in a liver of a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

Also provided herein are methods of modulating expression of one or more genes in Tables 1-4 in a liver of a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII. In some embodiments of any of the methods described herein, the administering results in a decrease in the expression of one or more genes in the liver of the subject selected from the group consisting of: ACSS1, RETN, SLC2A4, PDK4, PNPLA3, GADD45B, PPARGC1A, CAV1, ENDOD1, REG3G, IGHG3, IGHG2B, SCGB3A1, GLYCAM1, IGHG2C, IGKC, LTF, MS4A1, JCHAIN, CD19, IGHM, IFI27L2A, ACKR3, LSP1, PMEPA1, CORO1A, GPX3, MYH8, NPPA, TCAP, FLNC, SLC36A2, MYH6, ACTC1, ACTA2, and TPM2, as compared to the level of expression of the one or more genes in the subject prior to the administering. In some embodiments of any of the methods described herein, the administering results in an increase in the expression of one or more genes in the liver of the subject selected from the group consisting of: SLC34A2, and CISH, as compared to the level of expression of the one or more genes in the subject prior to the administering. In some embodiments of any of the methods described herein, the administering results in a decrease in the expression of one or more genes in the liver of the subject selected from the group consisting of: CSF3R, IFI27L2A, GM17066, GNL3, FABP1, GM14303, AURKA, RPL14-PS1, QTRT2, G6PC, C8B, DYNLL1, LCN2, LRG1, CEBPD, COL4A3, ST3GAL5, RSAD2, 9330162G02RIK, PINX1, SRA1, SPATA2L, PNRC1, MUP20, IL6RA, APOA1, IL1B, WDR54, CTCFLOS, GM16973, 4632427E13RIK, IGHG2B, TGFB1I1, SELENBP2, SEMA6B, NEXN, ZFP653, NOB1, PCK1, FAM25C, MAPK15, GM16551, ESM1, RPL37RT, FAM133B, PDE8B, TUT1, S100A11, PDILT, PPARD, IER2, GM15401, MX2, WNK4, G0S2, BC005561, AA986860, JDP2,

GM26982, NOP58, ACTB, GM14586, RPP38, GM13436, NT5DC2, IMPDH1, CYTIP, AI846148, CHKA, GM37963, NR0B2, CYP4A32, ALKBH2, FAU-PS2, PPP1R15A, KLF2, SLC25A22, GM13341, IGHM, SATB1, SNRPF, DNASE1L2, CD3EAP, GM2788, DANCR, ZFP612, NOP56, JUND, ID1, HSPB1, KLHDC8A, KLF10, 5 ANGPT2, THBS1, GM44891, GM9752, ABLIM3, PTGES, GM28438, 2410002F23RIK, FOSL2, CRIP3, JUN, ALAS1, GM2000, RHOC, LMCD1, GM2061, GM42595, GM11478, IKZF2, PNLDC1, COMTD1, SNORA31, COL20A1, AKAP12, C1QTNF12, 1810032O08RIK, 2310033P09RIK, GM47528, SERPINE2, NPFF, SERPINA3K, RFXANK, IGKV5-39, NAB2, MAFF, CEP85, CSAD, LTB4R1, 10 1810012K08RIK, BCL7C, NRBP2, NLE1, ALKBH1, ARID5A, CFAP43, GM45767, CD8A, PPRC1, GM26870, TMC7, BCL6B, GM16348, GM26981, SLC16A3, TNFRSF12A, CYP2J9, NR4A2, MMP9, MIR17HG, TMEM191C, PCDH11X, HILPDA, RAPGEF4, GM17300, SLC25A47, KCNJ2, NYAP1, LAX1, RPS19-PS3, HES1, RGS16, DUSP1, GM43323, ASB4, MUC6, GM15502, UNG, FOXQ1, GM17936, 15 UBE2C, SLC16A6, MIR7052, NLRP12, GM14286, FGF21, KLF5, GM37969, PF4, GM21738, HOTAIRM1, GM6493, LOR, MFSD2B, MATK, SYNE4, GM44694, TRBC1, GM37274, PLN, CXCR4, PHF24, SNORD104, SERPINA7, RGS4, TCIM, EGFR, GM37760, FBXL22, TEDC2, ENHO, GM26917, GM43775, 4833411C07RIK, GM45053, INHBB, OPN3, SNHG15, B230206H07RIK, KCNE3, GM43305, 20 C530043K16RIK, KLF4, LEPR, JCHAIN, TSKU, LGALS4, PCP4L1, GM44829, DUSP8, GM44620, IGFBP1, JUNB, GM32017, GM2814, GM37144, MYADM120S, GM37666, HDC, SLFN4, A530041M06RIK, GM43359, GM2602, GM10277, FAM222A, FOXA3, AOC2, SERPINA1E, CTXN1, RAPGEF4OS2, SOCS2, PPAN, PRKAG2OS1, GADD45B, HOXA5, GRHL1, EIF4EBP3, OSGIN1, GM28513, 25 MAP3K6, SLC34A2, B630019A10RIK, IGKC, PLIN4, ANGPTL4, DUSP5, EGR1, GM42507, GM14257, APOLD1, IER3, ZBTB16, GM37033, IGLC1, GADD45G, IGLC3, GM45244, RGS1, CXCL1, RNF225, GM44005, ANKRD37, NR4A1, GM8893, GM26762, CDKN1A, 5330406M23RIK, IGLV1, IGKV3-2, FOS, GM43637, IGKV3-10, S100A9, GM15622, S100A8, MT1, RETNLG, MT2, IGKV19-93, GM45774, and 30 SERPINA4-PS1, as compared to the level of expression of the one or more genes in the subject prior to the administering. In some embodiments of any of the methods described

herein, the administering results in an increase in the expression of one or more genes in the liver of the subject selected from the group consisting of: DBP, IGKV4-55, PER3, MUP-PS10, GPAM, TMPRSS4, MUP-PS14, AC166078.1, MUP-PS12, GM2065, A530020G20RIK, ACSS2OS, DCLK3, KLF12, GM44669, MFSD9, B4GALNT3,

5 GM3776, TMEM167-PS1, KRT23, LMBRD2, GM22935, SULT2A-PS1, SNAI3, GM15908, MIR6392, ACSS2, NR1D1, BC049987, CCDC85C, CES2C, ACPP, MUP2, PTK6, UGT1A5, 1810008I18RIK, IL22RA1, ACSS3, ADNP, RDH16, SNTB1, 4933411K16RIK, NTRK2, EXTL1, PSTPIP2, RASSF6, AQP4, UGT1A9, PROM1, ZFP608, FAM13A, NFE2, TEF, TNFAIP8L3, SCD1, MMD2, SYNE3, ACLY,

10 C330021F23RIK, STON2, LRFN4, HHIPL1, WNT9B, NR1D2, 1810049J17RIK, PDPR, NA, GM45884, SLC2A5, FAM83F, ZFP526, SGK2, GM43080, DEAF1, ME1, BMF, WDFY2, ADCY9, CLSTN3, ACOT11, LYST, LRTM1, OAT, VPS13C, E330011O21RIK, P2RY4, GM11437, RWDD2A, SVIL, ECHDC1, TRIM14, SLC10A5, TRHDE, MASP1, 2900097C17RIK, NDST1, RDH9, 1110002L01RIK, ABTB2, RGR,

15 ACACB, SACM1L, DYRK2, ROBO1, GM44744, EIF4EBP2, KLHL24, CYP2A5, TIAM2, RAB43, GM13855, 9130409I23RIK, STON1, USP9X, UGT3A1, 9030616G12RIK, DOCK8, KLB, ACE, VLDLR, PCDHGC3, ABCA6, 4932422M17RIK, GM45838, FARP2, GM47205, SP4, UGT1A6B, KLHL28, D130043K22RIK, ASIC5, PM20D2, A1CF, SORBS1, SLC10A2, GM10642, UTP14B,

20 GM38394, AFP, INSIG1, HNF1AOS2, METTL4, LSS, MTMR9, HMGCR, GDAP10, ADRA1A, ZFP773, CRKL, CHRNE, STARD13, CRY2, FADS2, COG5, FV1, RCAN2, ABCB1A, PPARA, ATP7A, MVD, 2610037D02RIK, TNFRSF14, SUCNR1, ECI3, ABCC4, LNCBATE1, MINDY2, BTBD7, 4933404O12RIK, ABCD1, FMN1, FNIP2, ABHD15, NKX2-6, C77080, GM43611, SGTB, ACSL3, NR5A2, FAM198A, KCTD7,

25 ACACA, ZFP955B, SULT2A3, FZD4, FASN, CYP3A59, ZFP354B, TNFSF10, SESN3, MN1, RNF152, DHCR24, SPHK2, SYTL5, GM6652, BAHCC1, GAREM1, MFSD4A, HGF, GM3571, NOS1AP, DIXDC1, KANK1, REPS2, ASAH2, SEMA3B, RNF103, ZC3H12C, CDS2, DCUN1D4, 2900026A02RIK, CYR1, EEPD1, P2RY2, CYP2C39, SEC22C, EHHADH, ABCA3, HIPK2, RBM20, GRAMD4, FCHSD2, MOB3A,

30 HMGN3, KLHDC7A, VCP-RS, TERT, CYP3A41B, ARL13B, ZC3H12D, TLCD2, SNHG11, SORL1, GPR157, DNAJA4, TMEM253, TACO1, SPATA5L1, RHBG,

COL15A1, PCDH12, IRS1, ASCC3, KIF16B, and MR1, as compared to the level of expression of the one or more genes in the subject prior to the administering.

In some embodiments of any of the methods described herein, the subject has been previously identified or diagnosed as having a liver disease or a metabolic syndrome. In some embodiments of any of the methods described herein, the subject has been previously identified or diagnosed as having a liver disease. In some embodiments of any of the methods described herein, the liver disease is selected from the group of: fatty liver disease, hepatic steatosis, acute hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease. In some embodiments of any of the methods described herein, the subject has been previously identified or diagnosed as having a metabolic syndrome. In some embodiments of any of the methods described herein, the metabolic syndrome is selected from the group consisting of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial hypertension, stroke, and cataracts.

In some embodiments of any of the methods described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of any of the methods described herein,

the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

5 In some embodiments of any of the methods described herein, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of any of the methods described herein, the second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

10 In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

15 In some embodiments of any of the methods described herein, the first chimeric polypeptide further comprises one or more additional target-binding domain(s). In some embodiments of any of the methods described herein, the second chimeric polypeptide further comprises one or more additional target-binding domain(s).

In some embodiments of any of the methods described herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some embodiments of any of the methods described herein, the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1. In some embodiments of any of the methods described herein, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15R α) and a soluble IL-15.

25 In some embodiments of any of the methods described herein, the first target-binding domain comprises a soluble TGF- β RII. In some embodiments of any of the methods described herein, the first target-binding domain comprises a first sequence that is at least 80% identical to SEQ ID NO: 2 and a second sequence that is at least 80% identical to SEQ ID NO: 2, wherein the first and second sequence are separated by a linker. In some embodiments of any of the methods described herein, the first target-binding domain comprises a first sequence that is at least 90% identical to SEQ ID NO: 2

and a second sequence that is at least 90% identical to SEQ ID NO: 2. In some embodiments of any of the methods described herein, the first target-binding domain comprises a first sequence of SEQ ID NO: 2 and a second sequence of SEQ ID NO: 2. In some embodiments of any of the methods described herein, the linker comprises a
5 sequence of SEQ ID NO: 3. In some embodiments of any of the methods described herein, the first target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4. In some embodiments of any of the methods described herein, the first target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4. In some embodiments of any of the methods described herein, the first target-binding
10 domain comprises a sequence of SEQ ID NO: 4. In some embodiments of any of the methods described herein, the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 6. In some embodiments of any of the methods described herein, the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 6. In some embodiments of any of the methods described
15 herein, the first chimeric polypeptide comprises a sequence of SEQ ID NO: 6. In some embodiments of any of the methods described herein, the first chimeric polypeptide comprises a sequence of SEQ ID NO: 7.

In some embodiments of any of the methods described herein, the second target-binding domain comprises a soluble TGF- β RII. In some embodiments of any of the
20 methods described herein, the second target-binding domain comprises a first sequence that is at least 80% identical to SEQ ID NO: 2 and a second sequence that is at least 80% identical to SEQ ID NO: 2, wherein the first and second sequence are separated by a linker. In some embodiments of any of the methods described herein, the second target-binding domain comprises a first sequence that is at least 90% identical to SEQ ID NO: 2
25 and a second sequence that is at least 90% identical to SEQ ID NO: 2. In some embodiments of any of the methods described herein, the second target-binding domain comprises a first sequence of SEQ ID NO: 2 and a second sequence of SEQ ID NO: 2. In some embodiments of any of the methods described herein, the linker comprises a sequence of SEQ ID NO: 3. In some embodiments of any of the methods described
30 herein, the second target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4. In some embodiments of any of the methods described

herein, the second target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4. In some embodiments of any of the methods described herein, the second target-binding domain comprises a sequence of SEQ ID NO: 4. In some embodiments of any of the methods described herein, the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 5. In some embodiments of any of the methods described herein, the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 6. In some embodiments of any of the methods described herein, the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 5. In some embodiments of any of the methods described herein, the second chimeric polypeptide comprises a sequence of SEQ ID NO: 5. In some embodiments of any of the methods described herein, the first chimeric polypeptide comprises a sequence of SEQ ID NO: 6. In some embodiments of any of the methods described herein, the second chimeric polypeptide comprises a sequence of SEQ ID NO: 8.

As used herein, the term “chimeric” refers to a polypeptide that includes amino acid sequences (e.g., domains) originally derived from two different sources (e.g., two different naturally-occurring proteins, e.g., from the same or different species). For example, a chimeric polypeptide can include domains from at least two different naturally occurring human proteins. In some examples, a chimeric polypeptide can include a domain that is a synthetic sequence (e.g., an scFv) and a domain that is derived from a naturally-occurring protein (e.g., a naturally-occurring human protein). In some embodiments, a chimeric polypeptide can include at least two different domains that are synthetic sequences (e.g., two different scFvs).

An “antigen-binding domain” is one or more protein domain(s) (e.g., formed from amino acids from a single polypeptide or formed from amino acids from two or more polypeptides (e.g., the same or different polypeptides) that is capable of specifically binding to one or more different antigen(s). In some examples, an antigen-binding domain can bind to an antigen or epitope with specificity and affinity similar to that of naturally-occurring antibodies. In some embodiments, the antigen-binding domain can be an antibody or a fragment thereof. In some embodiments, an antigen-binding domain can include an alternative scaffold. Non-limiting examples of antigen-binding domains

are described herein. Additional examples of antigen-binding domains are known in the art.

A “soluble tissue factor domain” refers to a polypeptide having at least 70% identity (e.g., at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 99% identity, or 100% identical) to a segment of a wildtype mammalian tissue factor protein (e.g., a wildtype human tissue factor protein) that lacks the transmembrane domain and the intracellular domain. Non-limiting examples of soluble tissue factor domains are described herein.

The term “soluble interleukin receptor” is used herein in the broadest sense to refer to a polypeptide that lacks a transmembrane domain (and optionally an intracellular domain) that is capable of binding one or more of its natural ligands (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a soluble interleukin receptor can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to an extracellular domain of wildtype interleukin receptor and retains its ability to specifically bind to one or more of its natural ligands, but lacks its transmembrane domain (and optionally, further lacks its intracellular domain). Non-limiting examples of soluble interleukin receptors are described herein.

The term “soluble cytokine receptor” is used herein in the broadest sense to refer to a polypeptide that lacks a transmembrane domain (and optionally an intracellular domain) that is capable of binding one or more of its natural ligands (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a soluble cytokine receptor can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to an extracellular domain of wildtype cytokine receptor and retains its ability to specifically bind to one or more of its natural ligands, but lacks its transmembrane domain (and optionally, further lacks its intracellular domain). Non-limiting examples of soluble cytokine receptors are described herein.

The term “antibody” is used herein in its broadest sense and includes certain types of immunoglobulin molecules that include one or more antigen-binding domains that specifically bind to an antigen or epitope. An antibody specifically includes, e.g., intact antibodies (e.g., intact immunoglobulins), antibody fragments, and multi-specific antibodies. One example of an antigen-binding domain is an antigen-binding domain formed by a VH -VL dimer. Additional examples of an antibody are described herein. Additional examples of an antibody are known in the art.

“Affinity” refers to the strength of the sum total of non-covalent interactions between an antigen-binding site and its binding partner (e.g., an antigen or epitope). Unless indicated otherwise, as used herein, “affinity” refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of an antigen-binding domain and an antigen or epitope. The affinity of a molecule X for its partner Y can be represented by the dissociation equilibrium constant (K_D). The kinetic components that contribute to the dissociation equilibrium constant are described in more detail below. Affinity can be measured by common methods known in the art, including those described herein. Affinity can be determined, for example, using surface plasmon resonance (SPR) technology (e.g., BIACORE®) or biolayer interferometry (e.g., FORTEBIO®). Additional methods for determining the affinity for an antigen-binding domain and its corresponding antigen or epitope are known in the art.

A “multi-chain polypeptide” as used herein refers to a polypeptide comprising two or more (e.g., three, four, five, six, seven, eight, nine, or ten) protein chains (e.g., at least a first chimeric polypeptide and a second polypeptide), where the two or more proteins chains associate through non-covalent bonds to form a quaternary structure.

The term “pair of affinity domains” is two different protein domain(s) that bind specifically to each other with a K_D of less than of less than 1×10^{-7} M (e.g., less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, or less than 1×10^{-11} M). In some examples, a pair of affinity domains can be a pair of naturally-occurring proteins. In some embodiments, a pair of affinity domains can be a pair of synthetic proteins. Non-limiting examples of pairs of affinity domains are described herein.

The term “epitope” means a portion of an antigen that specifically binds to an antigen-binding domain. Epitopes can, e.g., consist of surface-accessible amino acid

residues and/or sugar side chains and may have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter may be lost in the presence of denaturing solvents. An epitope may comprise
5 amino acid residues that are directly involved in the binding, and other amino acid residues, which are not directly involved in the binding. Methods for identifying an epitope to which an antigen-binding domain binds are known in the art.

An “immune effector cell” refers to a cell of the immune system of a mammal that is capable, directly or indirectly, of recognizing and/or causing cytostasis or cell
10 death of a pathogenic cell (e.g., a cancer cell) in the mammal. Non-limiting examples of immune effector cells include macrophages, T-lymphocytes (e.g., cytotoxic T-lymphocytes and T-helper cells), natural killer cells, neutrophils, monocytes, and eosinophils. Additional examples of immune effector cells are known in the art.

The term “treatment” means to ameliorate at least one symptom of a disorder. In
15 some examples, the disorder being treated is cancer and to ameliorate at least one symptom of cancer includes reducing aberrant proliferation, gene expression, signaling, translation, and/or secretion of factors. Generally, the methods of treatment include administering a therapeutically effective amount of composition that reduces at least one symptom of a disorder to a subject who is in need of, or who has been determined to be in
20 need of such treatment.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The
25 materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the
30 following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows exemplary diagrams for a multi-chain chimeric polypeptide: (i) a first chimeric polypeptide including a first target-binding domain (A), a soluble tissue factor domain, a first domain of an affinity pair of domains (soluble interleukin IL-15), and an additional target-binding domain (B); and (ii) second chimeric polypeptide including a second domain of an affinity pair of domains (IL-15 receptor alpha sushi domain), a second target-binding domain (C), and an additional antigen-binding domain (D). The top cartoon diagram depicts the association of the first and the second chimeric polypeptides through the pair of affinity domains. The bottom schematic diagrams show the order of the domains in the first and second chimeric polypeptides.

Figure 2 shows exemplary diagrams for a multi-chain chimeric polypeptide: (i) a first chimeric polypeptide including a first target-binding domain (A), a soluble tissue factor domain including five amino acid substitutions in order to remove binding of the soluble tissue factor domain to FVIIa, a first domain of an affinity pair of domains (soluble interleukin IL-15 including a D8N or D8A amino acid substitution), and an additional target-binding domain (B); and (ii) second chimeric polypeptide including a second domain of an affinity pair of domains (IL-15 receptor alpha sushi domain), a second target-binding domain (C), and an additional antigen-binding domain (D). The top cartoon diagram depicts the association of the first and the second chimeric polypeptides through the pair of affinity domains. The bottom schematic diagrams show the order of the domains in the first and second chimeric polypeptides. In other embodiments of any of the multi-chain chimeric polypeptides described herein the soluble tissue factor domain can comprise or consists of a soluble wildtype human tissue factor domain (comprising or consisting of a contiguous sequence within wildtype human tissue factor).

Figure 3 shows a schematic of the TGFRt15-TGFRs construct.

Figure 4 shows an additional schematic of the TGFRt15-TGFRs construct.

Figure 5 shows results of TGF β 1 inhibition by TGFRt15-TGFRs and TGFR-Fc.

Figure 6 shows results of 32D β cell proliferation assay with TGFRt15-TGFRs or recombinant IL-15

Figures 7A and 7B show results of detecting IL-15 and TGF β RII in TGFRt15-TGFRs with corresponding antibodies using ELISA.

Figure 8 is a line graph showing the chromatographic profile of TGFRt15-TGFRs protein containing cell culture supernatant following binding and elution on anti-TF
5 antibody resin.

Figure 9 shows the analytical SEC profile of TGFRt15-TGFRs.

Figure 10 shows TGFRt15-TGFRs before and after deglycosylation as analyzed by reduced SDS-PAGE.

Figures 11A and 11B show spleen weight and the percentages of immune cell
10 types in TGFRt15-TGFRs-treated and control-treated mice. Figure 85A shows spleen weight in mice treated with TGFRt15-TGFRs as compared to PBS control. Figure 85B shows the percentage of CD4⁺ T cells, CD8⁺ T cells, and NK cells in mice treated with TGFRt15-TGFRs as compared to PBS control.

Figure 12A and 12B show the spleen weight and immunostimulation over 92
15 hours in mice treated with TGFRt15-TGFRs. Figure 86A shows spleen weight of mice treated with TGFRt15-TGFRs at 16, 24, 48, 72, and 92 hours after treatment. Figure 86B shows the percentages of immune cells in mice treated with TGFRt15-TGFRs at 16, 24, 48, 72, and 92 hours after treatment.

Figure 13A and 13B show Ki67 and Granzyme B expression in mice treated with
20 TGFRt15-TGFRs over time.

Figure 14 shows enhancement of cytotoxicity of splenocytes by TGFRt15-TGFRs in C57BL/6 Mice.

Figure 15 shows changes in tumor size in response to PBS treatment, chemotherapy alone, TGFRt15-TGFRs alone, or chemotherapy and TGFRt15-TGFRs
25 combination, in a pancreatic cancer mouse model.

Figure 16 shows the cytotoxicity of NK cells isolated from mice treated with TGFRt15-TGFRs.

Figures 17A-17C show in vivo stimulation of Tregs, NK cells, and CD8⁺ T cells in ApoE^{-/-} mice fed with a Western diet and treated with TGFRt15-TGFRs.

Figures 18A-18C show immunostimulation in C57BL/6 mice following treatment
30 with TGFRt15-TGFRs.

Figures 19A and 19B show in vivo induction of proliferation of NK cells and CD8+ T cells in ApoE^{-/-} mice fed with a Western diet and treated with TGFRT15-TGFRs.

Figures 20A and 20B show enhancement of cytotoxicity of NK cells following treatment of NK cells with TGFRT15-TGFRs.

Figures 21A and 21B show enhancement of ADCC activity of NK cells following treatment of NK cells with TGFRT15-TGFRs.

Figures 22A-22H show antitumor activity of TGFRT15-TGFRs plus anti-TRP1 antibody (TA99) in combination with chemotherapy in a melanoma mouse model.

Figures 23A-23C show amelioration of the Western diet-induced hyperglycemia in ApoE^{-/-} mice by TGFRT15-TGFRs.

Figure 24 shows upregulation of CD44^{hi} memory T cells upon treatment with TGFRT15-TGFRs.

Figure 25 shows RNA-seq analysis of differentially expressed genes between the PBS (control group) or TGFRT15-TGFRs (TGFRT15-TGFRs group) in the liver of db/db mice.

Figure 26 shows RNA-seq analysis of differentially expressed genes between the PBS (control group) or TGFRT15-TGFRs (TGFRT15-TGFRs group) in aged mice liver.

Figure 27 shows a volcano plot of RNA-seq analysis of the livers of *db/db* mice.

Figure 28 shows a heatmap representing differentially expressed genes as measured by RNA-seq analysis of livers of *db/db* mice treated with TGFRT15-TGFRs (HCW9218) and PBS negative controls.

Figure 29 shows a heat map of differentially expressed senescence-related and inflammation-related genes measured by RNA-seq in livers of aged mice.

Figure 30 shows a schematic of a study design for investigating TGFRT15-TGFRs (HCW9218) treatment in a *db/db* mouse model.

Figure 31 shows relative mRNA expression of *IL1 β* , *IL1 α* , *PAI-1*, *IL6*, and *Tnfa* in liver as measured by quantitative PCR after treatment with TGFRT15-TGFRs (HCW9218) compared to control at day 10 or day 60.

Figure 32 shows relative mRNA expression of *IL1 β* , *IL1 α* , *PAI-1*, *IL6*, and *Cdkn1a* in liver after treatment with one or two TGFRt15-TGFRs (HCW9218) doses compared to control at day 120 as measured by quantitative PCR.

Figure 33 shows ELISA data of protein levels of IL-1 α , IL-6, IL-8, in liver tissue
5 after treatment with TGFRt15-TGFRs (HCW9218) compared to control 120 days after treatment.

Figure 34 shows ELISA data of protein levels of PAI-1, and Fibronectin in liver tissue after treatment with TGFRt15-TGFRs (HCW9218) compared to control 120 days after treatment.

Figure 35 shows shows immunofluorescent staining of liver tissue cells
10 expressing p21+ after treatment with two doses of TGFRt15-TGFRs (HCW9218) compared to PBS negative control.

Figure 36 shows heatmaps of differentially expressed genes as detected by RNA-seq data generated from the livers of aged mice receiving either TGFRt15-TGFRs
15 (HCW9218) treatment of PBS-only negative control.

Figure 37 shows flow cytometric analysis of Ki67 expression in CD4, CD8, Treg, and CD16+ NK cells in blood from Cynomolgus monkeys following treatment with TGFRt15-TGFRs (HCW9218).

Figure 38 shows flow cytometric analysis of absolute numbers of CD4, CD8,
20 Treg, and CD16+ NK cells in blood from Cynomolgus monkeys following treatment with TGFRt15-TGFRs (HCW9218).

Figure 39 shows TGFRt15-TGFRs treatment enhances immune cell populations in *db/db* mice.

Figure 40 shows the effect of TGFRt15-TGFRs treatment or TGFRt15*-TGFRs
25 treatment on cytotoxic activity of splenocytes in *db/db* mice after day 4 post-treatment.

Figure 41 shows the effect of TGFRt15-TGFRs and TGFRt15*-TGFRs treatment on interferon-gamma production of splenocytes in *db/db* mice after day 4 post-treatment and *in vitro* α CD3/CD28 stimulation assays.

Figure 42 shows the effect of TGFRt15-TGFRs on the glycolytic activity of
30 splenocytes in *db/db* mice after day 4 post-treatment.

Figure 43 shows the effect of TGF α 15-TGFRs on mitochondrial respiration of splenocytes in *db/db* mice after day 4 post-treatment.

Figure 44 shows the effect of TGF α 15-TGFRs on plasma TGF β 1 and TGF β 2 levels in *db/db* mice after day 4 post-treatment.

5 Figure 45 shows the effect of TGF α 15-TGFRs (HCW9218) from chemical induced liver damages.

DETAILED DESCRIPTION

Nonalcoholic fatty liver disease (NAFLD) is emerging as the leading chronic liver disease worldwide and is estimated to affect one billion individuals globally (Younossi et al., *Hepatology* 69(6):2672-2682, 2019). NAFLD represents a spectrum of liver diseases
10 ranging from non-alcoholic fatty liver (NAFL), in the case of isolated steatosis to non-alcoholic steatohepatitis (NASH), fibrotic NASH, advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC) (Meijnikman et al., *JHEP Rep.* 3(4):100301, 2021).

The metabolic mechanism leading to NAFLD reflects an imbalance of energy
15 metabolism in the liver. The inability of the liver to oxidize the excess energy, mostly in the form of carbohydrates and fat, to CO₂ or to export it as very-low-density lipoproteins. This leads to a net accumulation of energy in the liver as triglycerides, which explains the widespread presence of NAFLD in obese individuals and in individuals with lipodystrophy. Skeletal muscle insulin resistance, one of the earliest defects associated
20 with metabolic syndrome and prediabetes, can also promote development of NAFLD through increase hepatic de novo lipogenesis (DNL) and hypertriglyceridemia by diverting ingested glucose away from skeletal muscles glycogen synthesis and into the liver for DNL. Development of hepatic insulin resistance, where insulin activation of glycogen synthase is impaired (Loomba et al., *Cell* 184(10):2537-2564, 2021), redirects
25 glucose into lipogenic pathways and further promotes NAFLD.

Adipocyte dysfunction also promotes development of NAFLD. Severe NAFLD/NASH is a complication of congenital lipodystrophies, where the absence of adipose tissue forces the liver to store excess fatty acids, leading to severe insulin resistance. Other metabolic causes of hepatic steatosis include (1) defects in intrahepatic
30 lipolysis, (2) defects in triglyceride export, (3) increased glucokinase activity resulting in

hepatic DNL, and (4) reductions in hepatic mitochondrial/peroxisomal β -oxidation (Loomba et al., *Cell* 184(10):2537-2564, 2021).

Hepatocytes begin to amass fat when they synthesize new lipids through the DNL pathway, an adaptive response to counter the generation of toxic lipid metabolites and balance free fatty acid excess (Piccinin et al., *Nat. Rev. Gastroenterol. Hepatol.* 16(3):160-174, 2019). The accumulative toxic metabolites promote a hepatic inflammatory state that is further exacerbated by endotoxins derived from increased gut permeability and dysbiosis and release of IL-6 and TNF from inflamed adipose tissues (Loomba et al., *Cell* 184(10):2537-2564, 2021; Yki-Jarvinen et al., *Nat. Rev. Gastroenterol. Hepatol.* 2021). Thus, the hepatic inflammatory microenvironment plays a critical role in the development of NAFLD and progression toward HCC. In addition, cytokine mediated hepatocytes injury and death are followed by hepatic progenitor cell population growth, which, in an inflammatory environment, induces the fibrogenic response in hepatic stellate cells, thereby promoting progression toward liver fibrosis and NASH (Loomba et al., *Cell* 184(10):2537-2564, 2021). Recently, there is also evidence showing that hepatocyte cellular senescence is also a causal factor in NAFLD development.

NAFLD is associated with several markers of senescence in hepatocytes, such as increased senescence-associated damage foci, increased senescence-associated distention of satellites and larger nuclear areas (Ogrodnik et al., *Nat. Comm.* 8:15691, 2017). Hepatocytic senescence was also shown to impair hepatic mitochondrial β -oxidation, thereby hindering fatty acid elimination and promoting triglyceride accumulation (Ogrodnik et al., *Nat. Comm.* 8:15691, 2017). The paracrine effects of proinflammatory factors secreted from the senescence-associated secretory phenotype of senescent hepatocytes disrupt the normal metabolism of the normal hepatocytes (Loomba et al., *Cell* 184(10):2537-2564, 2021). Finally, a causal link between hepatocytic senescence and hepatic steatosis was unraveled using transgenic mice and a senolytic cocktail (Childs et al., *Nat. Rev. Drug Discov.* 16(10):718-735, 2017).

Herein, we describe that subcutaneous administration of TGF α 15-TGFRs can eliminate hepatocytic senescence, lower the inflammatory microenvironment of the liver, rebalance the metabolic functions of the liver, and reduce gluconeogenesis and

lipogenesis in Db/Db and naturally-aging mouse models. Thus, TGF β 15-TGFRs has the potential to treat a variety of liver diseases and metabolic syndrome.

Provided herein are methods of treating a liver disease or a metabolic syndrome in a subject diagnosed as having the liver disease or the metabolic syndrome; methods of
5 reducing one or more of the rate of progression from non-alcoholic fatty liver disease (NAFL) to non-alcoholic steatohepatitis (NASH), progression from NASH to cirrhosis, and progression from cirrhosis to hepatocellular carcinoma; methods of reducing inflammation in a liver of a subject identified as being in need thereof; methods of decreasing gluconeogenesis in a liver of a subject identified as being in need thereof;
10 methods of decreasing lipogenesis in a liver of a subject identified as being in need thereof; methods of decreasing hepatocytic senescence in a liver of a subject identified as being in need thereof; methods of rebalancing metabolic function in a liver of a subject identified as being in need thereof; and methods of modulating expression of one or more genes in Tables 1-4 in a liver of a subject identified as being in need thereof, that include
15 administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first
20 chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

In some examples of any of the multi-chain chimeric polypeptides described
25 herein the total length of first chimeric polypeptide and/or the second chimeric polypeptide can each independently be about 50 amino acids to about 3000 amino acids, about 50 amino acids to about 2500 amino acids, about 50 amino acids to about 2000 amino acids, about 50 amino acids to about 1500 amino acids, about 50 amino acids to about 1000 amino acids, about 50 amino acids to about 950 amino acids, about 50 amino
30 acids to about 900 amino acids, about 50 amino acids to about 850 amino acids, about 50 amino acids to about 800 amino acids, about 50 amino acids to about 750 amino acids,

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

28

[illegible]

[illegible]

[illegible]

800 amino acids to about 850 amino acids, about 850 amino acids to about 3000 amino acids, about 850 amino acids to about 2500 amino acids, about 850 amino acids to about 2000 amino acids, about 850 amino acids to about 1500 amino acids, about 850 amino acids to about 1000 amino acids, about 850 amino acids to about 950 amino acids, about 5 850 amino acids to about 900 amino acids, about 900 amino acids to about 3000 amino acids, about 900 amino acids to about 2500 amino acids, about 900 amino acids to about 2000 amino acids, about 900 amino acids to about 1500 amino acids, about 900 amino acids to about 1000 amino acids, about 900 amino acids to about 950 amino acids, about 950 amino acids to about 3000 amino acids, about 950 amino acids to about 2500 amino acids, about 950 amino acids to about 2000 amino acids, about 950 amino acids to about 1500 amino acids, about 950 amino acids to about 1000 amino acids, about 1000 amino acids to about 3000 amino acids, about 1000 amino acids to about 2500 amino acids, about 1000 amino acids to about 2000 amino acids, about 1000 amino acids to about 1500 amino acids, about 1500 amino acids to about 3000 amino acids, about 1500 amino acids to about 2500 amino acids, about 1500 amino acids to about 2000 amino acids, about 2000 amino acids to about 3000 amino acids, about 2000 amino acids to about 2500 amino acids, or about 2500 amino acids to about 3000 amino acids. Diagrams of exemplary multi-chain chimeric polypeptides provided herein are depicted in Figures 1 and 2.

20 In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the first target-binding domains described herein) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) directly abut each other in the first chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further comprises a linker sequence (e.g., 25 any of the exemplary linker sequences described herein or known in the art) between the first target-binding domain (e.g., any of the exemplary first target-binding domains described herein) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) in the first chimeric polypeptide.

30 In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor

domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) directly abut each other in the first chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) and the second target-binding domain (e.g., any of the exemplary second target-binding domains described herein) directly abut each other in the second chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) and the second target-binding domain (e.g., any of the exemplary second target-binding domains described herein) in the second chimeric polypeptide.

Non-limiting aspects of these chimeric polypeptides, nucleic acids, vectors, cells, and methods are described below, and can be used in any combination without limitation. Additional aspects of these chimeric polypeptides, nucleic acids, vectors, cells, and methods are known in the art.

Tissue Factor

Human tissue factor is a 263 amino-acid transmembrane protein containing three domains: (1) a 219-amino acid N-terminal extracellular domain (residues 1-219); (2) a 22-amino acid transmembrane domain (residues 220-242); and (3) a 21-amino acid

cytoplasmic C-terminal tail (residues 242-263) ((UniProtKB Identifier Number: P13726). The cytoplasmic tail contains two phosphorylation sites at Ser253 and Ser258, and one S-palmitoylation site at Cys245. Deletion or mutation of the cytoplasmic domain was not found to affect tissue factor coagulation activity. Tissue factor has one S-palmitoylation
5 site in the intracellular domain of the protein at Cys245. The Cys245 is located at the amino acid terminus of the intracellular domain and close to the membrane surface. The tissue factor transmembrane domain is composed of a single-spanning α -helix.

The extracellular domain of tissue factor, composed of two fibronectin type III domains, is connected to the transmembrane domain through a six-amino acid linker.

10 This linker provides conformational flexibility to decouple the tissue factor extracellular domain from its transmembrane and cytoplasmic domains. Each tissue factor fibronectin type III module is composed of two overlapping β sheets with the top sheet domain containing three antiparallel β -strands and the bottom sheet containing four β -strands. The β -strands are connected by β -loops between strand β A and β B, β C and β D, and β E
15 and β F, all of which are conserved in conformation in the two modules. There are three short α -helix segments connecting the β -strands. A unique feature of tissue factor is a 17-amino acid β -hairpin between strand β 10 and strand β 11, which is not a common element of the fibronectin superfamily. The N-terminal domain also contains a 12 amino acid loop between β 6F and β 7G that is not present in the C-terminal domain and is unique to
20 tissue factor. Such a fibronectin type III domain structure is a feature of the immunoglobulin-like family of protein folds and is conserved among a wide variety of extracellular proteins.

The zymogen FVII is rapidly converted to FVIIa by limited proteolysis once it binds to tissue to form the active tissue factor-FVIIa complex. The FVIIa, which
25 circulates as an enzyme at a concentration of approximately 0.1 nM (1% of plasma FVII), can also bind directly to tissue factor. The allosteric interaction between tissue factor and FVIIa on the tissue factor-FVIIa complex greatly increases the enzymatic activity of FVIIa: an approximate 20- to 100-fold increase in the rate of hydrolysis of small, chromogenic peptidyl substrates, and nearly a million-fold increase in the rate of
30 activation of the natural macromolecular substrates FIX and FX. In concert with allosteric activation of the active site of FVIIa upon binding to tissue factor, the

formation of tissue factor-FVIIa complex on phospholipid bilayer (i.e., upon exposure of phosphatidyl-L-serine on membrane surfaces) increases the rate of FIX or FX activation, in a Ca^{2+} -dependent manner, an additional 1,000-fold. The roughly million-fold overall increase in FX activation by tissue factor-FVIIa-phospholipid complex relative to free FVIIa is a critical regulatory point for the coagulation cascade.

FVII is a ~50 kDa, single-chain polypeptide consisting of 406 amino acid residues, with an N-terminal γ -carboxyglutamate-rich (GLA) domain, two epidermal growth factor-like domains (EGF1 and EGF2), and a C-terminal serine protease domain. FVII is activated to FVIIa by a specific proteolytic cleavage of the Ile¹⁵⁴-Arg¹⁵² bond in the short linker region between the EGF2 and the protease domain. This cleavage results in the light and heavy chains being held together by a single disulfide bond of Cys¹³⁵ and Cys²⁶². FVIIa binds phospholipid membrane in a Ca^{2+} -dependent manner through its N-terminal GLA-domain. Immediately C-terminal to the GLA domain is an aromatic stack and two EGF domains. The aromatic stack connects the GLA to EGF1 domain which binds a single Ca^{2+} ion. Occupancy of this Ca^{2+} -binding site increases FVIIa amidolytic activity and tissue factor association. The catalytic triad consist of His¹⁹³, Asp²⁴², and Ser³⁴⁴, and binding of a single Ca^{2+} ion within the FVIIa protease domain is critical for its catalytic activity. Proteolytic activation of FVII to FVIIa frees the newly formed amino terminus at Ile¹⁵³ to fold back and be inserted into the activation pocket forming a salt bridge with the carboxylate of Asp³⁴³ to generate the oxyanion hole. Formation of this salt bridge is critical for FVIIa activity. However, oxyanion hole formation does not occur in free FVIIa upon proteolytic activation. As a result, FVIIa circulates in a zymogen-like state that is poorly recognized by plasma protease inhibitors, allowing it to circulate with a half-life of approximately 90 minutes.

Tissue factor-mediated positioning of the FVIIa active site above the membrane surface is important for FVIIa towards cognate substrates. Free FVIIa adopts a stable, extended structure when bound to the membrane with its active site positioned ~80 Å above the membrane surface. Upon FVIIa binding to tissue factor, the FVIIa active site is repositioned ~6 Å closer to the membrane. This modulation may aid in a proper alignment of the FVIIa catalytic triad with the target substrate cleavage site. Using GLA-domainless FVIIa, it has been shown that the active site was still positioned a similar

distance above the membrane, demonstrating that tissue factor is able to fully support FVIIa active site positioning even in the absence of FVIIa-membrane interaction. Additional data showed that tissue factor supported full FVIIa proteolytic activity as long as the tissue factor extracellular domain was tethered in some way to the membrane surface. However, raising the active site of FVIIa greater than 80Å above the membrane surface greatly reduced the ability of the tissue factor-FVIIa complex to activate FX but did not diminish tissue factor-FVIIa amidolytic activity.

Alanine scanning mutagenesis has been used to assess the role of specific amino acid side chains in the tissue factor extracellular domain for interaction with FVIIa (Gibbs et al., *Biochemistry* 33(47): 14003-14010, 1994; Schullek et al., *J Biol Chem* 269(30): 19399-19403, 1994). Alanine substitution identified a limited number of residue positions at which alanine replacements cause 5- to 10-fold lower affinity for FVIIa binding. Most of these residue side chains were found to be well-exposed to solvent in the crystal structure, concordant with macromolecular ligand interaction. The FVIIa ligand-binding site is located over an extensive region at the boundary between the two modules. In the C-module, residues Arg¹³⁵ and Phe¹⁴⁰ located on the protruding B-C loop provide an independent contact with FVIIa. Leu¹³³ is located at the base of the fingerlike structure and packed into the cleft between the two modules. This provides continuity to a major cluster of important binding residues consisting of Lys²⁰, Thr⁶⁰, Asp⁵⁸, and Ile²². Thr⁶⁰ is only partially solvent-exposed and may play a local structural role rather than making a significant contact with ligand. The binding site extends onto the concave side of the intermodule angle involving Glu²⁴ and Gln¹¹⁰, and potentially the more distant residue Val²⁰⁷. The binding region extends from Asp⁵⁸ onto a convex surface area formed by Lys⁴⁸, Lys⁴⁶, Gln³⁷, Asp⁴⁴, and Trp⁴⁵. Trp⁴⁵ and Asp⁴⁴ do not interact independently with FVIIa, indicating that the mutational effect at the Trp⁴⁵ position may reflect a structural importance of this side chain for the local packing of the adjacent Asp⁴⁴ and Gln³⁷ side chain. The interactive area further includes two surface-exposed aromatic residues, Phe⁷⁶ and Tyr⁷⁸, which form part of the hydrophobic cluster in the N-module.

The known physiologic substrates of tissue factor-FVIIa are FVII, FIX, and FX and certain proteinase-activated receptors. Mutational analysis has identified a number of

residues that, when mutated, support full FVIIa amidolytic activity towards small peptidyl substrates but are deficient in their ability to support macromolecular substrate (i.e., FVII, FIX, and FX) activation (Ruf et al., *J Biol Chem* 267(31): 22206-22210, 1992; Ruf et al., *J Biol Chem* 267(9): 6375-6381, 1992; Huang et al., *J Biol Chem* 271(36): 21752-21757, 1996; Kirchhofer et al., *Biochemistry* 39(25): 7380-7387, 2000). The tissue factor loop region at residues 159-165, and residues in or adjacent to this flexible loop have been shown to be critical for the proteolytic activity of the tissue factor-FVIIa complex. This defines the proposed substrate-binding exosite region of tissue factor that is quite distant from the FVIIa active site. A substitution of the glycine residue by a marginally bulkier residue alanine, significantly impairs tissue factor-FVIIa proteolytic activity. This suggests that the flexibility afforded by glycine is critical for the loop of residues 159-165 for tissue factor macromolecular substrate recognition.

The residues Lys¹⁶⁵ and Lys¹⁶⁶ have also been demonstrated to be important for substrate recognition and binding. Mutation of either of these residues to alanine results in a significant decrease in the tissue factor co-factor function. Lys¹⁶⁵ and Lys¹⁶⁶ face away from each other, with Lys¹⁶⁵ pointing towards FVIIa in most tissue factor-FVIIa structures, and Lys¹⁶⁶ pointing into the substrate binding exosite region in the crystal structure. Putative salt bridge formation between Lys¹⁶⁵ of and Glu³⁵ of FVIIa would support the notion that tissue factor interaction with the GLA domain of FVIIa modulates substrate recognition. These results suggest that the C-terminal portion of the tissue factor ectodomain directly interacts with the GLA-domain, the possible adjacent EGF1 domains, of FIX and FX, and that the presence of the FVIIa GLA-domain may modulate these interactions either directly or indirectly.

25 Soluble Tissue Factor Domain

In some embodiments of any of the polypeptides, compositions, or methods described herein, the soluble tissue factor domain can be a wildtype tissue factor polypeptide lacking the signal sequence, the transmembrane domain, and the intracellular domain. In some examples, the soluble tissue factor domain can be a tissue factor mutant, wherein a wildtype tissue factor polypeptide lacking the signal sequence, the transmembrane domain, and the intracellular domain, and has been further modified at

selected amino acids. In some examples, the soluble tissue factor domain can be a soluble human tissue factor domain. In some examples, the soluble tissue factor domain can be a soluble mouse tissue factor domain. In some examples, the soluble tissue factor domain can be a soluble rat tissue factor domain. Non-limiting examples of soluble

5 human tissue factor domains, a mouse soluble tissue factor domain, a rat soluble tissue factor domain, and mutant soluble tissue factor domains are shown below.

Exemplary Soluble Human Tissue Factor Domain (SEQ ID NO: 1)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTD

10 TECDLTDEIVKDKVQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQ

PTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGK

KTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRE

Exemplary Nucleic Acid Encoding Soluble Human Tissue Factor Domain (SEQ ID NO: 9)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA

ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTAAACCAAGTTTACACC

GTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCAC

CGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACC

20 TACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTC

CGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGA

CCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGT

GAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTT

CTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGG

25 AAGTCCTCTTCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTT

TAATCGACGTGGATAAAGGCGAAAATACTGTTTCAGCGTGCAAGCTGTGATC

CCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGG

GCCAAGAAAAGGGCGAGTTCCGGGAG

30

Exemplary Mutant Soluble Human Tissue Factor Domain (SEQ ID NO: 10)

SGTTNTVAAYNLTWKSTNFATALEWEPKPVNQVYTVQISTKSGDWKSKCFYTT
 DTECALTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNL
 GQPTIQSFEQVGTKVNVTVEDERTLVARNNTALSLRDVFGKDLIYTLYYWKSSSS
 5 GKKTAKTNTNEFLIDVDKGENYCFVQAVIPSRTVNRKSTDSPVECMGQEKGEF
 RE

Exemplary Mutant Soluble Human Tissue Factor Domain (SEQ ID NO: 11)

SGTTNTVAAYNLTWKSTNFATALEWEPKPVNQVYTVQISTKSGDAKSKCFYTTD
 TECALTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLAENSPEFTPYLETNLG
 10 QPTIQSFEQVGTKVNVTVEDERTLVARNNTALSLRDVFGKDLIYTLYYWKSSSSG
 KKTAKTNTNEFLIDVDKGENYCFVQAVIPSRTVNRKSTDSPVECMGQEKGEFR
 E

Exemplary Soluble Mouse Tissue Factor Domain (SEQ ID NO: 12)

15 agipekafnltwistdfktilewqpkptnytytvqisdrsrnwknkcfstt
 dtecdltdeivkdvtwayeakvlsvprnrsvhgdgdlvihgeppftnap
 kflpyrdtnlgqpviqqfeqdgrklvkvkdsftlvrkngtftlrlqvfg
 dlgyiityrkgsstgkktntntnefsidveegvsycffvqamifsrktnq
 nspgsstvcetqwkswlge
 20

Exemplary Soluble Rat Tissue Factor Domain (SEQ ID NO: 13)

agtppgkafnltwistdfktilewqpkptnytytvqisdrsrnwkykctgt
 ttecdltdeivkdvnwtyearvlsvpwrnsthgketlfgthgeppftna
 rkflpyrdtkigqpviqkyeqggtklkvtvkdsftlvrkngtftlrlqvfg
 25 ndlgyiltyrkdsstgrktnthtneflidvekgvsycffaavifsrktn
 hkspesitkcteqwkswlge

In some embodiments, a soluble tissue factor domain can include a sequence that
 is at least 70% identical, at least 72% identical, at least 74% identical, at least 76%
 30 identical, at least 78% identical, at least 80% identical, at least 82% identical, at least
 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at
 least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical,
 at least 99% identical, or 100% identical to SEQ ID NO: 1, 10, 11, 12, or 13. In some

embodiments, a soluble tissue factor domain can include a sequence of SEQ ID NO: 1, 10, 11, 12, or 13, with one to twenty amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from its N-terminus and/or one to twenty amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from its C-terminus.

As can be appreciated in the art, one skilled in the art would understand that mutation of amino acids that are conserved between different mammalian species is more likely to decrease the activity and/or structural stability of the protein, while mutation of amino acids that are not conserved between different mammalian species is less likely to decrease the activity and/or structural stability of the protein.

In some examples of any of the multi-chain chimeric polypeptides described herein, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some examples of any of the multi-chain chimeric polypeptides described herein, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

In some examples, the soluble tissue factor domain can be a soluble human tissue factor domain. In some embodiments, the soluble tissue factor domain can be a soluble mouse tissue factor domain. In some embodiments, the soluble tissue factor domain can be a soluble rat tissue factor domain.

In some examples, the soluble tissue factor domain does not include one or more (e.g., two, three, four, five, six, or seven) of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that

corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some embodiments, the mutant soluble tissue factor possesses the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 11.

In some examples, the soluble tissue factor domain can be encoded by a nucleic acid including a sequence that is at least 70% identical, at least 72% identical, at least 74% identical, at least 76% identical, at least 78% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 9.

In some embodiments, the soluble tissue factor domain can have a total length of about 20 amino acids to about 220 amino acids, about 20 amino acids to about 215 amino acids, about 20 amino acids to about 210 amino acids, about 20 amino acids to about 205 amino acids, about 20 amino acids to about 200 amino acids, about 20 amino acids to about 195 amino acids, about 20 amino acids to about 190 amino acids, about 20 amino acids to about 185 amino acids, about 20 amino acids to about 180 amino acids, about 20 amino acids to about 175 amino acids, about 20 amino acids to about 170 amino acids, about 20 amino acids to about 165 amino acids, about 20 amino acids to about 160 amino acids, about 20 amino acids to about 155 amino acids, about 20 amino acids to about 150 amino acids, about 20 amino acids to about 145 amino acids, about 20 amino acids to about 140 amino acids, about 20 amino acids to about 135 amino acids, about 20 amino acids to about 130 amino acids, about 20 amino acids to about 125 amino acids, about 20 amino acids to about 120 amino acids, about 20 amino acids to about 115 amino acids, about 20 amino acids to about 110 amino acids, about 20 amino acids to about 105 amino acids, about 20 amino acids to about 100 amino acids, about 20 amino acids to about 95 amino acids, about 20 amino acids to about 90 amino acids, about 20 amino acids to about 85 amino acids, about 20 amino acids to about 80 amino acids, about 20 amino acids to about 75 amino acids, about 20 amino acids to about 70 amino acids, about 20 amino acids to about 60 amino acids, about 20 amino acids to about 50 amino acids, about 20 amino acids to about 40 amino acids, about 20 amino acids to about 30 amino acids, about 30 amino acids to about 220 amino acids, about 30 amino acids to about 215

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amino acids to about 155 amino acids, about 80 amino acids to about 150 amino acids,
about 80 amino acids to about 145 amino acids, about 80 amino acids to about 140 amino
acids, about 80 amino acids to about 135 amino acids, about 80 amino acids to about 130
amino acids, about 80 amino acids to about 125 amino acids, about 80 amino acids to
5 about 120 amino acids, about 80 amino acids to about 115 amino acids, about 80 amino
acids to about 110 amino acids, about 80 amino acids to about 105 amino acids, about 80
amino acids to about 100 amino acids, about 80 amino acids to about 95 amino acids,
about 80 amino acids to about 90 amino acids, about 90 amino acids to about 220 amino
acids, about 90 amino acids to about 215 amino acids, about 90 amino acids to about 210
10 amino acids, about 90 amino acids to about 205 amino acids, about 90 amino acids to
about 200 amino acids, about 90 amino acids to about 195 amino acids, about 90 amino
acids to about 190 amino acids, about 90 amino acids to about 185 amino acids, about 90
amino acids to about 180 amino acids, about 90 amino acids to about 175 amino acids,
about 90 amino acids to about 170 amino acids, about 90 amino acids to about 165 amino
15 acids, about 90 amino acids to about 160 amino acids, about 90 amino acids to about 155
amino acids, about 90 amino acids to about 150 amino acids, about 90 amino acids to
about 145 amino acids, about 90 amino acids to about 140 amino acids, about 90 amino
acids to about 135 amino acids, about 90 amino acids to about 130 amino acids, about 90
amino acids to about 125 amino acids, about 90 amino acids to about 120 amino acids,
20 about 90 amino acids to about 115 amino acids, about 90 amino acids to about 110 amino
acids, about 90 amino acids to about 105 amino acids, about 90 amino acids to about 100
amino acids, about 100 amino acids to about 220 amino acids, about 100 amino acids to
about 215 amino acids, about 100 amino acids to about 210 amino acids, about 100
amino acids to about 205 amino acids, about 100 amino acids to about 200 amino acids,
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amino acids, about 100 amino acids to about 185 amino acids, about 100 amino acids to
about 180 amino acids, about 100 amino acids to about 175 amino acids, about 100
amino acids to about 170 amino acids, about 100 amino acids to about 165 amino acids,
about 100 amino acids to about 160 amino acids, about 100 amino acids to about 155
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about 145 amino acids, about 100 amino acids to about 140 amino acids, about 100

amino acids to about 135 amino acids, about 100 amino acids to about 130 amino acids,
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amino acids, about 100 amino acids to about 115 amino acids, about 100 amino acids to
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about 220 amino acids, about 115 amino acids to about 215 amino acids, about 115
amino acids to about 210 amino acids, about 115 amino acids to about 205 amino acids,
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about 185 amino acids, about 115 amino acids to about 180 amino acids, about 115
amino acids to about 175 amino acids, about 115 amino acids to about 170 amino acids,
about 115 amino acids to about 165 amino acids, about 115 amino acids to about 160
amino acids, about 115 amino acids to about 155 amino acids, about 115 amino acids to
25 about 150 amino acids, about 115 amino acids to about 145 amino acids, about 115
amino acids to about 140 amino acids, about 115 amino acids to about 135 amino acids,
about 115 amino acids to about 130 amino acids, about 115 amino acids to about 125
amino acids, about 115 amino acids to about 120 amino acids, about 120 amino acids to
about 220 amino acids, about 120 amino acids to about 215 amino acids, about 120
30 amino acids to about 210 amino acids, about 120 amino acids to about 205 amino acids,
about 120 amino acids to about 200 amino acids, about 120 amino acids to about 195

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amino acids to about 175 amino acids, about 150 amino acids to about 170 amino acids,
about 150 amino acids to about 165 amino acids, about 150 amino acids to about 160
amino acids, about 150 amino acids to about 155 amino acids, about 155 amino acids to
about 220 amino acids, about 155 amino acids to about 215 amino acids, about 155
5 amino acids to about 210 amino acids, about 155 amino acids to about 205 amino acids,
about 155 amino acids to about 200 amino acids, about 155 amino acids to about 195
amino acids, about 155 amino acids to about 190 amino acids, about 155 amino acids to
about 185 amino acids, about 155 amino acids to about 180 amino acids, about 155
amino acids to about 175 amino acids, about 155 amino acids to about 170 amino acids,
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25 amino acids to about 215 amino acids, about 170 amino acids to about 210 amino acids,
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amino acids to about 180 amino acids, about 170 amino acids to about 175 amino acids,
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amino acids, about 175 amino acids to about 210 amino acids, about 175 amino acids to

about 205 amino acids, about 175 amino acids to about 200 amino acids, about 175 amino acids to about 195 amino acids, about 175 amino acids to about 190 amino acids, about 175 amino acids to about 185 amino acids, about 175 amino acids to about 180 amino acids, about 180 amino acids to about 220 amino acids, about 180 amino acids to about 215 amino acids, about 180 amino acids to about 210 amino acids, about 180 amino acids to about 205 amino acids, about 180 amino acids to about 200 amino acids, about 180 amino acids to about 195 amino acids, about 180 amino acids to about 190 amino acids, about 180 amino acids to about 185 amino acids, about 185 amino acids to about 220 amino acids, about 185 amino acids to about 215 amino acids, about 185 amino acids to about 210 amino acids, about 185 amino acids to about 205 amino acids, about 185 amino acids to about 200 amino acids, about 185 amino acids to about 195 amino acids, about 185 amino acids to about 190 amino acids, about 190 amino acids to about 220 amino acids, about 190 amino acids to about 215 amino acids, about 190 amino acids to about 210 amino acids, about 190 amino acids to about 205 amino acids, about 190 amino acids to about 200 amino acids, about 190 amino acids to about 195 amino acids, about 195 amino acids to about 220 amino acids, about 195 amino acids to about 215 amino acids, about 195 amino acids to about 210 amino acids, about 195 amino acids to about 205 amino acids, about 195 amino acids to about 200 amino acids, about 200 amino acids to about 220 amino acids, about 200 amino acids to about 215 amino acids, about 200 amino acids to about 210 amino acids, about 200 amino acids to about 205 amino acids, about 205 amino acids to about 220 amino acids, about 205 amino acids to about 215 amino acids, about 205 amino acids to about 210 amino acids, about 210 amino acids to about 220 amino acids, about 210 amino acids to about 215 amino acids, or about 215 amino acids to about 220 amino acids.

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Linker Sequences

In some embodiments, the linker sequence can be a flexible linker sequence. Non-limiting examples of linker sequences that can be used are described in Klein et al., *Protein Engineering, Design & Selection* 27(10):325–330, 2014; Priyanka et al., *Protein Sci.* 22(2):153–167, 2013. In some examples, the linker sequence is a synthetic linker sequence.

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In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide can include one, two, three, four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences, e.g., any of the exemplary linker sequences described herein or known in the art). In some
5 embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide can include one, two, three, four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences, e.g., any of the exemplary linker sequences described herein or known in the art).

In some embodiments, a linker sequence can have a total length of 1 amino acid
10 to about 100 amino acids, 1 amino acid to about 90 amino acids, 1 amino acid to about 80 amino acids, 1 amino acid to about 70 amino acids, 1 amino acid to about 60 amino acids, 1 amino acid to about 50 amino acids, 1 amino acid to about 45 amino acids, 1 amino acid to about 40 amino acids, 1 amino acid to about 35 amino acids, 1 amino acid to about 30 amino acids, 1 amino acid to about 25 amino acids, 1 amino acid to about 24
15 amino acids, 1 amino acid to about 22 amino acids, 1 amino acid to about 20 amino acids, 1 amino acid to about 18 amino acids, 1 amino acid to about 16 amino acids, 1 amino acid to about 14 amino acids, 1 amino acid to about 12 amino acids, 1 amino acid to about 10 amino acids, 1 amino acid to about 8 amino acids, 1 amino acid to about 6 amino acids, 1 amino acid to about 4 amino acids, about 2 amino acids to about 100
20 amino acids, about 2 amino acids to about 90 amino acids, about 2 amino acids to about 80 amino acids, about 2 amino acids to about 70 amino acids, about 2 amino acids to about 60 amino acids, about 2 amino acids to about 50 amino acids, about 2 amino acids to about 45 amino acids, about 2 amino acids to about 40 amino acids, about 2 amino acids to about 35 amino acids, about 2 amino acids to about 30 amino acids, about 2
25 amino acids to about 25 amino acids, about 2 amino acids to about 24 amino acids, about 2 amino acids to about 22 amino acids, about 2 amino acids to about 20 amino acids, about 2 amino acids to about 18 amino acids, about 2 amino acids to about 16 amino acids, about 2 amino acids to about 14 amino acids, about 2 amino acids to about 12 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 8
30 amino acids, about 2 amino acids to about 6 amino acids, about 2 amino acids to about 4 amino acids, about 4 amino acids to about 100 amino acids, about 4 amino acids to about

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amino acids to about 30 amino acids, about 30 amino acids to about 100 amino acids, about 30 amino acids to about 90 amino acids, about 30 amino acids to about 80 amino acids, about 30 amino acids to about 70 amino acids, about 30 amino acids to about 60 amino acids, about 30 amino acids to about 50 amino acids, about 30 amino acids to about 45 amino acids, about 30 amino acids to about 40 amino acids, about 30 amino acids to about 35 amino acids, about 35 amino acids to about 100 amino acids, about 35 amino acids to about 90 amino acids, about 35 amino acids to about 80 amino acids, about 35 amino acids to about 70 amino acids, about 35 amino acids to about 60 amino acids, about 35 amino acids to about 50 amino acids, about 35 amino acids to about 45 amino acids, about 35 amino acids to about 40 amino acids, about 40 amino acids to about 100 amino acids, about 40 amino acids to about 90 amino acids, about 40 amino acids to about 80 amino acids, about 40 amino acids to about 70 amino acids, about 40 amino acids to about 60 amino acids, about 40 amino acids to about 50 amino acids, about 40 amino acids to about 45 amino acids, about 45 amino acids to about 100 amino acids, about 45 amino acids to about 90 amino acids, about 45 amino acids to about 80 amino acids, about 45 amino acids to about 70 amino acids, about 45 amino acids to about 60 amino acids, about 45 amino acids to about 50 amino acids, about 50 amino acids to about 100 amino acids, about 50 amino acids to about 90 amino acids, about 50 amino acids to about 80 amino acids, about 50 amino acids to about 70 amino acids, about 50 amino acids to about 60 amino acids, about 60 amino acids to about 100 amino acids, about 60 amino acids to about 90 amino acids, about 60 amino acids to about 80 amino acids, about 60 amino acids to about 70 amino acids, about 70 amino acids to about 100 amino acids, about 70 amino acids to about 90 amino acids, about 70 amino acids to about 80 amino acids, about 80 amino acids to about 100 amino acids, about 80 amino acids to about 90 amino acids, or about 90 amino acids to about 100 amino acids.

In some embodiments, the linker is rich in glycine (Gly or G) residues. In some embodiments, the linker is rich in serine (Ser or S) residues. In some embodiments, the linker is rich in glycine and serine residues. In some embodiments, the linker has one or more glycine-serine residue pairs (GS), e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GS pairs. In some embodiments, the linker has one or more Gly-Gly-Gly-Ser (GGGS) sequences, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGGS sequences. In some

embodiments, the linker has one or more Gly-Gly-Gly-Gly-Ser (GGGGS) sequences, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGGGS sequences. In some embodiments, the linker has one or more Gly-Gly-Ser-Gly (GGSG) sequences, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGSG sequences.

- 5 In some embodiments, the linker sequence can comprise or consist of GGGGSGGGGSGGGGS (SEQ ID NO: 3). In some embodiments, the linker sequence can be encoded by a nucleic acid comprising or consisting of:
GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT (SEQ ID
NO: 14). In some embodiments, the linker sequence can comprise or consist of:
10 GGGSGGGS (SEQ ID NO: 15).

Target-Binding Domains

- In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and/or the
15 additional one or more target-binding domains can be an antigen-binding domain that binds specifically to a ligand of TGF- β RII (e.g., any of the exemplary antigen-binding domains described herein or known in the art) or a soluble interleukin or cytokine receptor that binds specifically to a ligand of TGF- β RII (e.g., any of the exemplary soluble interleukin receptors or soluble cytokine receptors described herein).

- 20 In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and/or the one or more additional target-binding domains can each independent have a total number of amino acids of about 5 amino acids to about 1000 amino acids, about 5 amino acids to about 950 amino acids, about 5 amino acids to about 900 amino acids, about 5 amino
25 acids to about 850 amino acids, about 5 amino acids to about 800 amino acids, about 5 amino acids to about 750 amino acids, about 5 amino acids to about 700 amino acids, about 5 amino acids to about 650 amino acids, about 5 amino acids to about 600 amino acids, about 5 amino acids to about 550 amino acids, about 5 amino acids to about 500 amino acids, about 5 amino acids to about 450 amino acids, about 5 amino acids to about
30 400 amino acids, about 5 amino acids to about 350 amino acids, about 5 amino acids to about 300 amino acids, about 5 amino acids to about 280 amino acids, about 5 amino

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amino acids to about 180 amino acids, about 70 amino acids to about 175 amino acids,
about 70 amino acids to about 170 amino acids, about 70 amino acids to about 165 amino
acids, about 70 amino acids to about 160 amino acids, about 70 amino acids to about 155
amino acids, about 70 amino acids to about 150 amino acids, about 70 amino acids to
5 about 145 amino acids, about 70 amino acids to about 140 amino acids, about 70 amino
acids to about 135 amino acids, about 70 amino acids to about 130 amino acids, about 70
amino acids to about 125 amino acids, about 70 amino acids to about 120 amino acids,
about 70 amino acids to about 115 amino acids, about 70 amino acids to about 110 amino
acids, about 70 amino acids to about 105 amino acids, about 70 amino acids to about 100
10 amino acids, about 70 amino acids to about 95 amino acids, about 70 amino acids to
about 90 amino acids, about 70 amino acids to about 85 amino acids, about 70 amino
acids to about 80 amino acids, about 70 amino acids to about 75 amino acids, about 75
amino acids to about 1000 amino acids, about 75 amino acids to about 950 amino acids,
about 75 amino acids to about 900 amino acids, about 75 amino acids to about 850 amino
15 acids, about 75 amino acids to about 800 amino acids, about 75 amino acids to about 750
amino acids, about 75 amino acids to about 700 amino acids, about 75 amino acids to
about 650 amino acids, about 75 amino acids to about 600 amino acids, about 75 amino
acids to about 550 amino acids, about 75 amino acids to about 500 amino acids, about 75
amino acids to about 450 amino acids, about 75 amino acids to about 400 amino acids,
20 about 75 amino acids to about 350 amino acids, about 75 amino acids to about 300 amino
acids, about 75 amino acids to about 280 amino acids, about 75 amino acids to about 260
amino acids, about 75 amino acids to about 240 amino acids, about 75 amino acids to
about 220 amino acids, about 75 amino acids to about 200 amino acids, about 75 amino
acids to about 195 amino acids, about 75 amino acids to about 190 amino acids, about 75
25 amino acids to about 185 amino acids, about 75 amino acids to about 180 amino acids,
about 75 amino acids to about 175 amino acids, about 75 amino acids to about 170 amino
acids, about 75 amino acids to about 165 amino acids, about 75 amino acids to about 160
amino acids, about 75 amino acids to about 155 amino acids, about 75 amino acids to
about 150 amino acids, about 75 amino acids to about 145 amino acids, about 75 amino
30 acids to about 140 amino acids, about 75 amino acids to about 135 amino acids, about 75
amino acids to about 130 amino acids, about 75 amino acids to about 125 amino acids,

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amino acids to about 160 amino acids, about 110 amino acids to about 155 amino acids,
about 110 amino acids to about 150 amino acids, about 110 amino acids to about 145
amino acids, about 110 amino acids to about 140 amino acids, about 110 amino acids to
about 135 amino acids, about 110 amino acids to about 130 amino acids, about 110
5 amino acids to about 125 amino acids, about 110 amino acids to about 120 amino acids,
about 110 amino acids to about 115 amino acids, about 115 amino acids to about 1000
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amino acids to about 220 amino acids, about 115 amino acids to about 200 amino acids,
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amino acids to about 850 amino acids, about 120 amino acids to about 800 amino acids,
30 about 120 amino acids to about 750 amino acids, about 120 amino acids to about 700
amino acids, about 120 amino acids to about 650 amino acids, about 120 amino acids to

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amino acids to about 700 amino acids, about 145 amino acids to about 650 amino acids,
about 145 amino acids to about 600 amino acids, about 145 amino acids to about 550
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amino acids to about 450 amino acids, about 185 amino acids to about 400 amino acids,
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amino acids to about 850 amino acids, about 650 amino acids to about 800 amino acids, about 650 amino acids to about 750 amino acids, about 650 amino acids to about 700 amino acids, about 700 amino acids to about 1000 amino acids, about 700 amino acids to about 950 amino acids, about 700 amino acids to about 900 amino acids, about 700 amino acids to about 850 amino acids, about 700 amino acids to about 800 amino acids, about 700 amino acids to about 750 amino acids, about 750 amino acids to about 1000 amino acids, about 750 amino acids to about 950 amino acids, about 750 amino acids to about 900 amino acids, about 750 amino acids to about 850 amino acids, about 750 amino acids to about 800 amino acids, about 800 amino acids to about 1000 amino acids, about 800 amino acids to about 950 amino acids, about 800 amino acids to about 900 amino acids, about 800 amino acids to about 850 amino acids, about 850 amino acids to about 1000 amino acids, about 850 amino acids to about 950 amino acids, about 850 amino acids to about 900 amino acids, about 900 amino acids to about 1000 amino acids, about 900 amino acids to about 950 amino acids, or about 950 amino acids to about 1000 amino acids.

Any of the target-binding domains described herein can bind to a ligand of TGF- β RII with a dissociation equilibrium constant (K_D) of less than 1×10^{-7} M, less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, less than 1×10^{-11} M, less than 1×10^{-12} M, or less than 1×10^{-13} M. In some embodiments, the antigen-binding protein construct provided herein can bind to an identifying antigen with a K_D of about 1×10^{-3} M to about 1×10^{-5} M, about 1×10^{-4} M to about 1×10^{-6} M, about 1×10^{-5} M to about 1×10^{-7} M, about 1×10^{-6} M to about 1×10^{-8} M, about 1×10^{-7} M to about 1×10^{-9} M, about 1×10^{-8} M to about 1×10^{-10} M, or about 1×10^{-9} M to about 1×10^{-11} M (inclusive).

Any of the target-binding domains described herein can bind to a ligand of TGF- β RII (e.g., TGF- β) with a K_D of between about 1 pM to about 30 nM (e.g., about 1 pM to about 25 nM, about 1 pM to about 20 nM, about 1 pM to about 15 nM, about 1 pM to about 10 nM, about 1 pM to about 5 nM, about 1 pM to about 2 nM, about 1 pM to about 1 nM, about 1 pM to about 950 pM, about 1 pM to about 900 pM, about 1 pM to about 850 pM, about 1 pM to about 800 pM, about 1 pM to about 750 pM, about 1 pM to about 700 pM, about 1 pM to about 650 pM, about 1 pM to about 600 pM, about 1 pM to about 550 pM, about 1 pM to about 500 pM, about 1 pM to about 450 pM, about 1 pM to about

400 pM, about 1 pM to about 350 pM, about 1 pM to about 300 pM, about 1 pM to about 250 pM, about 1 pM to about 200 pM, about 1 pM to about 150 pM, about 1 pM to about 100 pM, about 1 pM to about 90 pM, about 1 pM to about 80 pM, about 1 pM to about 70 pM, about 1 pM to about 60 pM, about 1 pM to about 50 pM, about 1 pM to about 40 pM, about 1 pM to about 30 pM, about 1 pM to about 20 pM, about 1 pM to about 10 pM, about 1 pM to about 5 pM, about 1 pM to about 4 pM, about 1 pM to about 3 pM, about 1 pM to about 2 pM, about 2 pM to about 30 nM, about 2 pM to about 25 nM, about 2 pM to about 20 nM, about 2 pM to about 15 nM, about 2 pM to about 10 nM, about 2 pM to about 5 nM, about 2 pM to about 2 nM, about 2 pM to about 1 nM, about 2 pM to about 950 pM, about 2 pM to about 900 pM, about 2 pM to about 850 pM, about 2 pM to about 800 pM, about 2 pM to about 750 pM, about 2 pM to about 700 pM, about 2 pM to about 650 pM, about 2 pM to about 600 pM, about 2 pM to about 550 pM, about 2 pM to about 500 pM, about 2 pM to about 450 pM, about 2 pM to about 400 pM, about 2 pM to about 350 pM, about 2 pM to about 300 pM, about 2 pM to about 250 pM, about 2 pM to about 200 pM, about 2 pM to about 150 pM, about 2 pM to about 100 pM, about 2 pM to about 90 pM, about 2 pM to about 80 pM, about 2 pM to about 70 pM, about 2 pM to about 60 pM, about 2 pM to about 50 pM, about 2 pM to about 40 pM, about 2 pM to about 30 pM, about 2 pM to about 20 pM, about 2 pM to about 10 pM, about 2 pM to about 5 pM, about 2 pM to about 4 pM, about 2 pM to about 3 pM, about 5 pM to about 30 nM, about 5 pM to about 25 nM, about 5 pM to about 20 nM, about 5 pM to about 15 nM, about 5 pM to about 10 nM, about 5 pM to about 5 nM, about 5 pM to about 2 nM, about 5 pM to about 1 nM, about 5 pM to about 950 pM, about 5 pM to about 900 pM, about 5 pM to about 850 pM, about 5 pM to about 800 pM, about 5 pM to about 750 pM, about 5 pM to about 700 pM, about 5 pM to about 650 pM, about 5 pM to about 600 pM, about 5 pM to about 550 pM, about 5 pM to about 500 pM, about 5 pM to about 450 pM, about 5 pM to about 400 pM, about 5 pM to about 350 pM, about 5 pM to about 300 pM, about 5 pM to about 250 pM, about 5 pM to about 200 pM, about 5 pM to about 150 pM, about 5 pM to about 100 pM, about 5 pM to about 90 pM, about 5 pM to about 80 pM, about 5 pM to about 70 pM, about 5 pM to about 60 pM, about 5 pM to about 50 pM, about 5 pM to about 40 pM, about 5 pM to about 30 pM, about 5 pM to about 20 pM, about 5 pM to about 10 pM, about 10 pM to about 30 nM, about 10 pM to about 25 nM,

about 10 pM to about 20 nM, about 10 pM to about 15 nM, about 10 pM to about 10 nM,
about 10 pM to about 5 nM, about 10 pM to about 2 nM, about 10 pM to about 1 nM,
about 10 pM to about 950 pM, about 10 pM to about 900 pM, about 10 pM to about 850
pM, about 10 pM to about 800 pM, about 10 pM to about 750 pM, about 10 pM to about
5 700 pM, about 10 pM to about 650 pM, about 10 pM to about 600 pM, about 10 pM to
about 550 pM, about 10 pM to about 500 pM, about 10 pM to about 450 pM, about 10
pM to about 400 pM, about 10 pM to about 350 pM, about 10 pM to about 300 pM, about
10 pM to about 250 pM, about 10 pM to about 200 pM, about 10 pM to about 150 pM,
about 10 pM to about 100 pM, about 10 pM to about 90 pM, about 10 pM to about 80
10 pM, about 10 pM to about 70 pM, about 10 pM to about 60 pM, about 10 pM to about 50
pM, about 10 pM to about 40 pM, about 10 pM to about 30 pM, about 10 pM to about 20
pM, about 15 pM to about 30 nM, about 15 pM to about 25 nM, about 15 pM to about 20
nM, about 15 pM to about 15 nM, about 15 pM to about 10 nM, about 15 pM to about 5
nM, about 15 pM to about 2 nM, about 15 pM to about 1 nM, about 15 pM to about 950
15 pM, about 15 pM to about 900 pM, about 15 pM to about 850 pM, about 15 pM to about
800 pM, about 15 pM to about 750 pM, about 15 pM to about 700 pM, about 15 pM to
about 650 pM, about 15 pM to about 600 pM, about 15 pM to about 550 pM, about 15
pM to about 500 pM, about 15 pM to about 450 pM, about 15 pM to about 400 pM, about
15 pM to about 350 pM, about 15 pM to about 300 pM, about 15 pM to about 250 pM,
20 about 15 pM to about 200 pM, about 15 pM to about 150 pM, about 15 pM to about 100
pM, about 15 pM to about 90 pM, about 15 pM to about 80 pM, about 15 pM to about 70
pM, about 15 pM to about 60 pM, about 15 pM to about 50 pM, about 15 pM to about 40
pM, about 15 pM to about 30 pM, about 15 pM to about 20 pM, about 20 pM to about 30
nM, about 20 pM to about 25 nM, about 20 pM to about 20 nM, about 20 pM to about 15
25 nM, about 20 pM to about 10 nM, about 20 pM to about 5 nM, about 20 pM to about 2
nM, about 20 pM to about 1 nM, about 20 pM to about 950 pM, about 20 pM to about
900 pM, about 20 pM to about 850 pM, about 20 pM to about 800 pM, about 20 pM to
about 750 pM, about 20 pM to about 700 pM, about 20 pM to about 650 pM, about 20
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30 20 pM to about 450 pM, about 20 pM to about 400 pM, about 20 pM to about 350 pM,
about 20 pM to about 300 pM, about 20 pM to about 250 pM, about 20 pM to about 20

pM, about 200 pM to about 150 pM, about 20 pM to about 100 pM, about 20 pM to about 90 pM, about 20 pM to about 80 pM, about 20 pM to about 70 pM, about 20 pM to about 60 pM, about 20 pM to about 50 pM, about 20 pM to about 40 pM, about 20 pM to about 30 pM, about 30 pM to about 30 nM, about 30 pM to about 25 nM, about 30 pM to about 5
5 30 nM, about 30 pM to about 15 nM, about 30 pM to about 10 nM, about 30 pM to about 5 nM, about 30 pM to about 2 nM, about 30 pM to about 1 nM, about 30 pM to about 950 pM, about 30 pM to about 900 pM, about 30 pM to about 850 pM, about 30 pM to about 800 pM, about 30 pM to about 750 pM, about 30 pM to about 700 pM, about 30 pM to about 650 pM, about 30 pM to about 600 pM, about 30 pM to about 550 pM, about 30
10 pM to about 500 pM, about 30 pM to about 450 pM, about 30 pM to about 400 pM, about 30 pM to about 350 pM, about 30 pM to about 300 pM, about 30 pM to about 250 pM, about 30 pM to about 200 pM, about 30 pM to about 150 pM, about 30 pM to about 100 pM, about 30 pM to about 90 pM, about 30 pM to about 80 pM, about 30 pM to about 70 pM, about 30 pM to about 60 pM, about 30 pM to about 50 pM, about 30 pM to about 40
15 pM, about 40 pM to about 30 nM, about 40 pM to about 25 nM, about 40 pM to about 30 nM, about 40 pM to about 15 nM, about 40 pM to about 10 nM, about 40 pM to about 5 nM, about 40 pM to about 2 nM, about 40 pM to about 1 nM, about 40 pM to about 950 pM, about 40 pM to about 900 pM, about 40 pM to about 850 pM, about 40 pM to about 800 pM, about 40 pM to about 750 pM, about 40 pM to about 700 pM, about 40 pM to about 650 pM, about 40 pM to about 600 pM, about 40 pM to about 550 pM, about 40
20 pM to about 500 pM, about 40 pM to about 450 pM, about 40 pM to about 400 pM, about 40 pM to about 350 pM, about 40 pM to about 300 pM, about 40 pM to about 250 pM, about 40 pM to about 200 pM, about 40 pM to about 150 pM, about 40 pM to about 100 pM, about 40 pM to about 90 pM, about 40 pM to about 80 pM, about 40 pM to about 70 pM, about 40 pM to about 60 pM, about 40 pM to about 50 pM, about 50 pM to about 30
25 nM, about 50 pM to about 25 nM, about 50 pM to about 30 nM, about 50 pM to about 15 nM, about 50 pM to about 10 nM, about 50 pM to about 5 nM, about 50 pM to about 2 nM, about 50 pM to about 1 nM, about 50 pM to about 950 pM, about 50 pM to about 900 pM, about 50 pM to about 850 pM, about 50 pM to about 800 pM, about 50 pM to about 750 pM, about 50 pM to about 700 pM, about 50 pM to about 650 pM, about 50
30 pM to about 600 pM, about 50 pM to about 550 pM, about 50 pM to about 500 pM, about

50 pM to about 450 pM, about 50 pM to about 400 pM, about 50 pM to about 350 pM, about 50 pM to about 300 pM, about 50 pM to about 250 pM, about 50 pM to about 200 pM, about 50 pM to about 150 pM, about 50 pM to about 100 pM, about 50 pM to about 90 pM, about 50 pM to about 80 pM, about 50 pM to about 70 pM, about 50 pM to about 60 pM, about 60 pM to about 30 nM, about 60 pM to about 25 nM, about 60 pM to about 30 nM, about 60 pM to about 15 nM, about 60 pM to about 10 nM, about 60 pM to about 5 nM, about 60 pM to about 2 nM, about 60 pM to about 1 nM, about 60 pM to about 950 pM, about 60 pM to about 900 pM, about 60 pM to about 850 pM, about 60 pM to about 800 pM, about 60 pM to about 750 pM, about 60 pM to about 700 pM, about 60 pM to about 650 pM, about 60 pM to about 600 pM, about 60 pM to about 550 pM, about 60 pM to about 500 pM, about 60 pM to about 450 pM, about 60 pM to about 400 pM, about 60 pM to about 350 pM, about 60 pM to about 300 pM, about 60 pM to about 250 pM, about 60 pM to about 200 pM, about 60 pM to about 150 pM, about 60 pM to about 100 pM, about 60 pM to about 90 pM, about 60 pM to about 80 pM, about 60 pM to about 70 pM, about 70 pM to about 30 nM, about 70 pM to about 25 nM, about 70 pM to about 30 nM, about 70 pM to about 15 nM, about 70 pM to about 10 nM, about 70 pM to about 5 nM, about 70 pM to about 2 nM, about 70 pM to about 1 nM, about 70 pM to about 950 pM, about 70 pM to about 900 pM, about 70 pM to about 850 pM, about 70 pM to about 800 pM, about 70 pM to about 750 pM, about 70 pM to about 700 pM, about 70 pM to about 650 pM, about 70 pM to about 600 pM, about 70 pM to about 550 pM, about 70 pM to about 500 pM, about 70 pM to about 450 pM, about 70 pM to about 400 pM, about 70 pM to about 350 pM, about 70 pM to about 300 pM, about 70 pM to about 250 pM, about 70 pM to about 200 pM, about 70 pM to about 150 pM, about 70 pM to about 100 pM, about 70 pM to about 90 pM, about 70 pM to about 80 pM, about 80 pM to about 30 nM, about 80 pM to about 25 nM, about 80 pM to about 30 nM, about 80 pM to about 15 nM, about 80 pM to about 10 nM, about 80 pM to about 5 nM, about 80 pM to about 2 nM, about 80 pM to about 1 nM, about 80 pM to about 950 pM, about 80 pM to about 900 pM, about 80 pM to about 850 pM, about 80 pM to about 800 pM, about 80 pM to about 750 pM, about 80 pM to about 700 pM, about 80 pM to about 650 pM, about 80 pM to about 600 pM, about 80 pM to about 550 pM, about 80 pM to about 500 pM, about 80 pM to about 450 pM, about 80 pM to about 400 pM, about 80 pM to about 350 pM,

about 80 pM to about 300 pM, about 80 pM to about 250 pM, about 80 pM to about 200 pM, about 80 pM to about 150 pM, about 80 pM to about 100 pM, about 80 pM to about 90 pM, about 90 pM to about 30 nM, about 90 pM to about 25 nM, about 90 pM to about 30 nM, about 90 pM to about 15 nM, about 90 pM to about 10 nM, about 90 pM to about 5 nM, about 90 pM to about 2 nM, about 90 pM to about 1 nM, about 90 pM to about 950 pM, about 90 pM to about 900 pM, about 90 pM to about 850 pM, about 90 pM to about 800 pM, about 90 pM to about 750 pM, about 90 pM to about 700 pM, about 90 pM to about 650 pM, about 90 pM to about 600 pM, about 90 pM to about 550 pM, about 90 pM to about 500 pM, about 90 pM to about 450 pM, about 90 pM to about 400 pM, about 90 pM to about 350 pM, about 90 pM to about 300 pM, about 90 pM to about 250 pM, about 90 pM to about 200 pM, about 90 pM to about 150 pM, about 90 pM to about 100 pM, about 100 pM to about 30 nM, about 100 pM to about 25 nM, about 100 pM to about 30 nM, about 100 pM to about 15 nM, about 100 pM to about 10 nM, about 100 pM to about 5 nM, about 100 pM to about 2 nM, about 100 pM to about 1 nM, about 100 pM to about 950 pM, about 100 pM to about 900 pM, about 100 pM to about 850 pM, about 100 pM to about 800 pM, about 100 pM to about 750 pM, about 100 pM to about 700 pM, about 100 pM to about 650 pM, about 100 pM to about 600 pM, about 100 pM to about 550 pM, about 100 pM to about 500 pM, about 100 pM to about 450 pM, about 100 pM to about 400 pM, about 100 pM to about 350 pM, about 100 pM to about 300 pM, about 100 pM to about 250 pM, about 100 pM to about 200 pM, about 100 pM to about 150 pM, about 150 pM to about 30 nM, about 150 pM to about 25 nM, about 150 pM to about 30 nM, about 150 pM to about 15 nM, about 150 pM to about 10 nM, about 150 pM to about 5 nM, about 150 pM to about 2 nM, about 150 pM to about 1 nM, about 150 pM to about 950 pM, about 150 pM to about 900 pM, about 150 pM to about 850 pM, about 150 pM to about 800 pM, about 150 pM to about 750 pM, about 150 pM to about 700 pM, about 150 pM to about 650 pM, about 150 pM to about 600 pM, about 150 pM to about 550 pM, about 150 pM to about 500 pM, about 150 pM to about 450 pM, about 150 pM to about 400 pM, about 150 pM to about 350 pM, about 150 pM to about 300 pM, about 150 pM to about 250 pM, about 150 pM to about 200 pM, about 200 pM to about 30 nM, about 200 pM to about 25 nM, about 200 pM to about 30 nM, about 200 pM to about 15 nM, about 200 pM to about 10 nM, about 200 pM to about 5

nM, about 200 pM to about 2 nM, about 200 pM to about 1 nM, about 200 pM to about 950 pM, about 200 pM to about 900 pM, about 200 pM to about 850 pM, about 200 pM to about 800 pM, about 200 pM to about 750 pM, about 200 pM to about 700 pM, about 200 pM to about 650 pM, about 200 pM to about 600 pM, about 200 pM to about 550 pM, about 200 pM to about 500 pM, about 200 pM to about 450 pM, about 200 pM to about 400 pM, about 200 pM to about 350 pM, about 200 pM to about 300 pM, about 200 pM to about 250 pM, about 300 pM to about 30 nM, about 300 pM to about 25 nM, about 300 pM to about 20 nM, about 300 pM to about 15 nM, about 300 pM to about 10 nM, about 300 pM to about 5 nM, about 300 pM to about 2 nM, about 300 pM to about 1 nM, about 300 pM to about 950 pM, about 300 pM to about 900 pM, about 300 pM to about 850 pM, about 300 pM to about 800 pM, about 300 pM to about 750 pM, about 300 pM to about 700 pM, about 300 pM to about 650 pM, about 300 pM to about 600 pM, about 300 pM to about 550 pM, about 300 pM to about 500 pM, about 300 pM to about 450 pM, about 300 pM to about 400 pM, about 300 pM to about 350 pM, about 400 pM to about 30 nM, about 400 pM to about 25 nM, about 400 pM to about 20 nM, about 400 pM to about 15 nM, about 400 pM to about 10 nM, about 400 pM to about 5 nM, about 400 pM to about 2 nM, about 400 pM to about 1 nM, about 400 pM to about 950 pM, about 400 pM to about 900 pM, about 400 pM to about 850 pM, about 400 pM to about 800 pM, about 400 pM to about 750 pM, about 400 pM to about 700 pM, about 400 pM to about 650 pM, about 400 pM to about 600 pM, about 400 pM to about 550 pM, about 400 pM to about 500 pM, about 500 pM to about 30 nM, about 500 pM to about 25 nM, about 500 pM to about 20 nM, about 500 pM to about 15 nM, about 500 pM to about 10 nM, about 500 pM to about 5 nM, about 500 pM to about 2 nM, about 500 pM to about 1 nM, about 500 pM to about 950 pM, about 500 pM to about 900 pM, about 500 pM to about 850 pM, about 500 pM to about 800 pM, about 500 pM to about 750 pM, about 500 pM to about 700 pM, about 500 pM to about 650 pM, about 500 pM to about 600 pM, about 500 pM to about 550 pM, about 600 pM to about 30 nM, about 600 pM to about 25 nM, about 600 pM to about 20 nM, about 600 pM to about 15 nM, about 600 pM to about 10 nM, about 600 pM to about 5 nM, about 600 pM to about 2 nM, about 600 pM to about 1 nM, about 600 pM to about 950 pM, about 600 pM to about 900 pM, about 600 pM to about 850 pM, about 600 pM to about 800 pM, about 600 pM

to about 750 pM, about 600 pM to about 700 pM, about 600 pM to about 650 pM, about 700 pM to about 30 nM, about 700 pM to about 25 nM, about 700 pM to about 30 nM, about 700 pM to about 15 nM, about 700 pM to about 10 nM, about 700 pM to about 5 nM, about 700 pM to about 2 nM, about 700 pM to about 1 nM, about 700 pM to about 950 pM, about 700 pM to about 900 pM, about 700 pM to about 850 pM, about 700 pM to about 800 pM, about 700 pM to about 750 pM, about 800 pM to about 30 nM, about 800 pM to about 25 nM, about 800 pM to about 30 nM, about 800 pM to about 15 nM, about 800 pM to about 10 nM, about 800 pM to about 5 nM, about 800 pM to about 2 nM, about 800 pM to about 1 nM, about 800 pM to about 950 pM, about 800 pM to about 900 pM, about 800 pM to about 850 pM, about 900 pM to about 30 nM, about 900 pM to about 25 nM, about 900 pM to about 30 nM, about 900 pM to about 15 nM, about 900 pM to about 10 nM, about 900 pM to about 5 nM, about 900 pM to about 2 nM, about 900 pM to about 1 nM, about 900 pM to about 950 pM, about 1 nM to about 30 nM, about 1 nM to about 25 nM, about 1 nM to about 20 nM, about 1 nM to about 15 nM, about 1 nM to about 10 nM, about 1 nM to about 5 nM, about 2 nM to about 30 nM, about 2 nM to about 25 nM, about 2 nM to about 20 nM, about 2 nM to about 15 nM, about 2 nM to about 10 nM, about 2 nM to about 5 nM, about 4 nM to about 30 nM, about 4 nM to about 25 nM, about 4 nM to about 20 nM, about 4 nM to about 15 nM, about 4 nM to about 10 nM, about 4 nM to about 5 nM, about 5 nM to about 30 nM, about 5 nM to about 25 nM, about 5 nM to about 20 nM, about 5 nM to about 15 nM, about 5 nM to about 10 nM, about 10 nM to about 30 nM, about 10 nM to about 25 nM, about 10 nM to about 20 nM, about 10 nM to about 15 nM, about 15 nM to about 30 nM, about 15 nM to about 25 nM, about 15 nM to about 20 nM, about 20 nM to about 30 nM, and about 20 nM to about 25 nM).

Any of the target-binding domains described herein can bind to a ligand of TGF β RII with a K_D of between about 1 nM to about 10 nM (e.g., about 1 nM to about 9 nM, about 1 nM to about 8 nM, about 1 nM to about 7 nM, about 1 nM to about 6 nM, about 1 nM to about 5 nM, about 1 nM to about 4 nM, about 1 nM to about 3 nM, about 1 nM to about 2 nM, about 2 nM to about 10 nM, about 2 nM to about 9 nM, about 2 nM to about 8 nM, about 2 nM to about 7 nM, about 2 nM to about 6 nM, about 2 nM to about 5 nM, about 2 nM to about 4 nM, about 2 nM to about 3 nM, about 3 nM to about

10 nM, about 3 nM to about 9 nM, about 3 nM to about 8 nM, about 3 nM to about 7 nM, about 3 nM to about 6 nM, about 3 nM to about 5 nM, about 3 nM to about 4 nM, about 4 nM to about 10 nM, about 4 nM to about 9 nM, about 4 nM to about 8 nM, about 4 nM to about 7 nM, about 4 nM to about 6 nM, about 4 nM to about 5 nM, about 5 nM to about 10 nM, about 5 nM to about 9 nM, about 5 nM to about 8 nM, about 5 nM to about 7 nM, about 5 nM to about 6 nM, about 6 nM to about 10 nM, about 6 nM to about 9 nM, about 6 nM to about 8 nM, about 6 nM to about 7 nM, about 7 nM to about 10 nM, about 7 nM to about 9 nM, about 7 nM to about 8 nM, about 8 nM to about 10 nM, about 8 nM to about 9 nM, and about 9 nM to about 10 nM).

10 A variety of different methods known in the art can be used to determine the K_D values of any of the antigen-binding protein constructs described herein (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a biomolecular binding kinetics assay, etc.).

15 ***Antigen-Binding Domains***

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain are each antigen-binding domains.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the antigen-binding domain includes or is a scFv or a single domain antibody (e.g., a VHH or a VNAR domain).

In some examples, an antigen-binding domain (e.g., any of the antigen-binding
5 domains described herein) can bind specifically to a ligand of TGF- β RII (see, e.g.,
antigen-binding domains that can bind specifically to TGF- β described in US
2021/0061897, US 2020/0399358, US 2020/0392221, US 2019/0315850, and US
2019/0177406, each of which is herein incorporated by reference).

The antigen-binding domains present in any of the multi-chain chimeric
10 polypeptides described herein are each independently selected from the group consisting
of: a VHH domain, a VNAR domain, and a scFv. In some embodiments, any of the
antigen-binding domains described herein is a BiTe, a (scFv)₂, a nanobody, a nanobody-
HSA, a DART, a TandAb, a scDiabody, a scDiabody-CH3, scFv-CH-CL-scFv, a
HSAbody, scDiabody-HAS, or a tandem-scFv. Additional examples of antigen-binding
15 domains that can be used in any of the multi-chain chimeric polypeptide are known in the
art.

A VHH domain is a single monomeric variable antibody domain that can be
found in camelids. A VNAR domain is a single monomeric variable antibody domain
that can be found in cartilaginous fish. Non-limiting aspects of VHH domains and VNAR
20 domains are described in, e.g., Cromie et al., *Curr. Top. Med. Chem.* 15:2543-2557, 2016;
De Genst et al., *Dev. Comp. Immunol.* 30:187-198, 2006; De Meyer et al., *Trends*
Biotechnol. 32:263-270, 2014; Kijanka et al., *Nanomedicine* 10:161-174, 2015; Kovaleva
et al., *Expert. Opin. Biol. Ther.* 14:1527-1539, 2014; Krah et al., *Immunopharmacol.*
Immunotoxicol. 38:21-28, 2016; Mujic-Delic et al., *Trends Pharmacol. Sci.* 35:247-255,
25 2014; Muyldermans, *J. Biotechnol.* 74:277-302, 2001; Muyldermans et al., *Trends*
Biochem. Sci. 26:230-235, 2001; Muyldermans, *Ann. Rev. Biochem.* 82:775-797, 2013;
Rahbarizadeh et al., *Immunol. Invest.* 40:299-338, 2011; Van Audenhove et al.,
EBioMedicine 8:40-48, 2016; Van Bockstaele et al., *Curr. Opin. Investig. Drugs* 10:1212-
1224, 2009; Vincke et al., *Methods Mol. Biol.* 911:15-26, 2012; and Wesolowski et al.,
30 *Med. Microbiol. Immunol.* 198:157-174, 2009.

In some embodiments, each of the antigen-binding domains in the multi-chain chimeric polypeptides described herein are both VHH domains, or at least one antigen-binding domain is a VHH domain. In some embodiments, each of the antigen-binding domains in the multi-chain chimeric polypeptides described herein are both VNAR domains, or at least one antigen-binding domain is a VNAR domain. In some embodiments, each of the antigen-binding domains in the multi-chain chimeric polypeptides described herein are both scFv domains, or at least one antigen-binding domain is a scFv domain.

In some embodiments, two or more of polypeptides present in the multi-chain chimeric polypeptide can assemble (e.g., non-covalently assemble) to form any of the antigen-binding domains described herein, e.g., an antigen-binding fragment of an antibody (e.g., any of the antigen-binding fragments of an antibody described herein), a VHH-scAb, a VHH-Fab, a Dual scFab, a F(ab')₂, a diabody, a crossMab, a DAF (two-in-one), a DAF (four-in-one), a DutaMab, a DT-IgG, a knobs-in-holes common light chain, a knobs-in-holes assembly, a charge pair, a Fab-arm exchange, a SEEDbody, a LUZ-Y, a Fcab, a κλ-body, an orthogonal Fab, a DVD-IgG, a IgG(H)-scFv, a scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zyboby, DVI-IgG, Diabody-CH3, a triple body, a miniantibody, a minibody, a TriBi minibody, scFv-CH3 KIH, Fab-scFv, a F(ab')₂-scFv₂, a scFv-KIH, a Fab-scFv-Fc, a tetravalent HCAb, a scDiabody-Fc, a Diabody-Fc, a tandem scFv-Fc, an Intrabody, a dock and lock, a lmmTAC, an IgG-IgG conjugate, a Cov-X-Body, and a scFv1-PEG-scFv₂. See, e.g., Spiess et al., *Mol. Immunol.* 67:95-106, 2015, incorporated in its entirety herewith, for a description of these elements. Non-limiting examples of an antigen-binding fragment of an antibody include an Fv fragment, a Fab fragment, a F(ab')₂ fragment, and a Fab' fragment. Additional examples of an antigen-binding fragment of an antibody is an antigen-binding fragment of an IgG (e.g., an antigen-binding fragment of IgG1, IgG2, IgG3, or IgG4) (e.g., an antigen-binding fragment of a human or humanized IgG, e.g., human or humanized IgG1, IgG2, IgG3, or IgG4); an antigen-binding fragment of an IgA (e.g., an antigen-binding fragment of IgA1 or IgA2) (e.g., an antigen-binding fragment of a human or humanized IgA, e.g., a human or humanized IgA1 or IgA2); an antigen-binding fragment of an IgD (e.g., an antigen-

binding fragment of a human or humanized IgD); an antigen-binding fragment of an IgE (e.g., an antigen-binding fragment of a human or humanized IgE); or an antigen-binding fragment of an IgM (e.g., an antigen-binding fragment of a human or humanized IgM).

5 An “Fv” fragment includes a non-covalently-linked dimer of one heavy chain variable domain and one light chain variable domain.

A “Fab” fragment includes, the constant domain of the light chain and the first constant domain (CH1) of the heavy chain, in addition to the heavy and light chain variable domains of the Fv fragment.

10 A “F(ab')₂” fragment includes two Fab fragments joined, near the hinge region, by disulfide bonds.

A “dual variable domain immunoglobulin” or “DVD-Ig” refers to multivalent and multispecific binding proteins as described, e.g., in DiGiammarino et al., *Methods Mol. Biol.* 899:145-156, 2012; Jakob et al., *MABs* 5:358-363, 2013; and U.S. Patent Nos. 7,612,181; 8,258,268; 8,586,714; 8,716,450; 8,722,855; 8,735,546; and 8,822,645, each
15 of which is incorporated by reference in its entirety.

DARTs are described in, e.g., Garber, *Nature Reviews Drug Discovery* 13:799-801, 2014.

In some embodiments of any of the antigen-binding domains described herein can bind to an antigen selected from the group consisting of: a protein, a carbohydrate, a
20 lipid, and a combination thereof.

Additional examples and aspects of antigen-binding domains are known in the art.

Soluble Receptor

In some embodiments of any of the multi-chain chimeric polypeptides described
25 herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin receptor, a soluble cytokine receptor or a ligand receptor. In some embodiments, the soluble receptor is a soluble TGF- β receptor II (TGF- β RII) (see, e.g., those described in Yung et al., *Am. J. Resp. Crit. Care Med.* 194(9):1140-1151, 2016) or a soluble TGF- β RIII (see, e.g., those described in Heng et al., *Placenta* 57:320,
30 2017).

Additional examples of soluble interleukin receptors and soluble cytokine receptors are known in the art.

Additional Target-Binding Domains

5 In some embodiments of any of the multi-chain chimeric polypeptides, the first chimeric polypeptide further includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domain(s) (e.g., any of the exemplary target-binding domains described herein or known in the art), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein). In some embodiments, the first chimeric polypeptide can further include a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the at least one of the one or more additional target-binding domain(s) (e.g., any of the exemplary target-binding domains described herein or known in the art), and/or a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the at least one of the one or more additional target-binding domain(s) (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein).

25 In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide. In some embodiments, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first

chimeric polypeptide. In some embodiments, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein). In some embodiments, the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art).

In some embodiments of any of the multi-chain chimeric polypeptides described herein, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains

described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the linker sequences described herein or known in the art) disposed between the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) disposed between the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the first domains described herein or any of the exemplary pairs of affinity domains described herein), directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains. In some embodiments, the first chimeric polypeptide further comprises a linker sequence

(e.g., any of the exemplary linker sequences described herein or known in the art) disposed (i) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described
5 herein or known in the art) positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein), and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional
10 target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains (e.g., any of
15 the exemplary target-binding domains described herein or known in the art) at the N-terminal end and/or the C-terminal end of the second chimeric polypeptide. In some embodiments, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the second domain of the pair of affinity domains (e.g., any of the exemplary
20 second domains of any of the exemplary pairs of affinity domains described herein) in the second chimeric polypeptide. In some embodiments, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or
25 known in the art) and the second domain of the pair of affinity domains (e.g., any of the second domains described herein of any of the exemplary pairs of affinity domains described herein) in the second chimeric polypeptide. In some embodiments, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the second target-binding domain (e.g., any of the target-binding domains described herein or known in the
30 art) in the second chimeric polypeptide. In some embodiments, the second chimeric

polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between at least one of the one or more additional target-binding domains (e.g., any of the exemplary target binding domains described herein or known in the art) and the second target-binding domain (e.g., any of the exemplary target binding domains described herein or known in the art) in the second chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, two or more (e.g., three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some embodiments, two or more (e.g., three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments, two or more (e.g., three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains include the same amino acid sequence. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each include the same amino acid sequence.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens. In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or more (e.g., two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the

second target-binding domain, and the one or more target-binding domains is an antigen-binding domain. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain (e.g., a scFv or a single-domain antibody).

5

Pairs of Affinity Domains

In some embodiments, a multi-chain chimeric polypeptide includes: 1) a first chimeric polypeptide that includes a first domain of a pair of affinity domains, and 2) a second chimeric polypeptide that includes a second domain of a pair of affinity domains
10 such that the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains. In some embodiments, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) and a soluble IL-15. A sushi domain, also known as a short consensus repeat or type 1 glycoprotein motif, is a common motif in
15 protein-protein interaction. Sushi domains have been identified on a number of protein-binding molecules, including complement components C1r, C1s, factor H, and C2m, as well as the nonimmunologic molecules factor XIII and β 2-glycoprotein. A typical Sushi domain has approximately 60 amino acid residues and contains four cysteines (Ranganathan, Pac. Symp Biocomput. 2000:155-67). The first cysteine can form a
20 disulfide bond with the third cysteine, and the second cysteine can form a disulfide bridge with the fourth cysteine. In some embodiments in which one member of the pair of affinity domains is a soluble IL-15, the soluble IL15 has a D8N or D8A amino acid substitution. In some embodiments in which one member of the pair of affinity domains is an alpha chain of human IL-15 receptor (IL15R α), the human IL15R α is a mature full-
25 length IL15R α . In some embodiments, the pair of affinity domains is barnase and barnstar. In some embodiments, the pair of affinity domains is a PKA and an AKAP. In some embodiments, the pair of affinity domains is an adapter/docking tag module based on mutated RNase I fragments (Rossi, *Proc Natl Acad Sci USA*. 103:6841-6846, 2006; Sharkey et al., *Cancer Res*. 68:5282-5290, 2008; Rossi et al., *Trends Pharmacol Sci*.
30 33:474-481, 2012) or SNARE modules based on interactions of the proteins syntaxin,

synaptotagmin, synaptobrevin, and SNAP25 (Deyev et al., *Nat Biotechnol.* 1486-1492, 2003).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide includes a first domain of a pair of affinity domains and a second chimeric polypeptide of the multi-chain chimeric polypeptide includes a second domain of a pair of affinity domains, wherein the first domain of the pair of affinity domains and the second domain of the pair of affinity domains bind to each other with a dissociation equilibrium constant (K_D) of less than 1×10^{-7} M, less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, less than 1×10^{-11} M, less than 1×10^{-12} M, or less than 1×10^{-13} M. In some embodiments, the first domain of the pair of affinity domains and the second domain of the pair of affinity domains bind to each other with a K_D of about 1×10^{-4} M to about 1×10^{-6} M, about 1×10^{-5} M to about 1×10^{-7} M, about 1×10^{-6} M to about 1×10^{-8} M, about 1×10^{-7} M to about 1×10^{-9} M, about 1×10^{-8} M to about 1×10^{-10} M, about 1×10^{-9} M to about 1×10^{-11} M, about 1×10^{-10} M to about 1×10^{-12} M, about 1×10^{-11} M to about 1×10^{-13} M, about 1×10^{-4} M to about 1×10^{-5} M, about 1×10^{-5} M to about 1×10^{-6} M, about 1×10^{-6} M to about 1×10^{-7} M, about 1×10^{-7} M to about 1×10^{-8} M, about 1×10^{-8} M to about 1×10^{-9} M, about 1×10^{-9} M to about 1×10^{-10} M, about 1×10^{-10} M to about 1×10^{-11} M, about 1×10^{-11} M to about 1×10^{-12} M, or about 1×10^{-12} M to about 1×10^{-13} M (inclusive). Any of a variety of different methods known in the art can be used to determine the K_D value of the binding of the first domain of the pair of affinity domains and the second domain of the pair of affinity domains (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a biomolecular binding kinetics assay, etc.).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide includes a first domain of a pair of affinity domains and a second chimeric polypeptide of the multi-chain chimeric polypeptide includes a second domain of a pair of affinity domains, wherein the first domain of the pair of affinity domains, the second domain of the pair of affinity domains, or both is about 10 to 100 amino acids in length. For example, a first domain of a pair of affinity domains, a second domain of a pair of affinity domains, or both can be about 10 to 100 amino acids in length, about 15 to 100 amino acids in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids

in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in length, about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length, about 10 to 65 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a first domain of a pair of affinity domains, a second domain of a pair of affinity domains, or both is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

In some embodiments, any of the first and/or second domains of a pair of affinity domains disclosed herein can include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at its N-terminus and/or C-terminus, so long as the function of the first and/or second domains of a pair of affinity domains remains intact. For example, a sushi domain from an alpha chain of human IL-15 receptor (IL15R α) can include one or more additional amino acids at the N-terminus and/or the C-terminus, while still retaining the ability to bind to a soluble IL-15. Additionally or alternatively, a soluble IL-15 can include one or more additional amino acids at the N-terminus and/or

the C-terminus, while still retaining the ability to bind to a sushi domain from an alpha chain of human IL-15 receptor (IL15R α).

A non-limiting example of a sushi domain from an alpha chain of IL-15 receptor alpha (IL15R α) can include a sequence that is at least 70% identical, at least 75%

5 identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH
WTPPSLKCIR (SEQ ID NO: 16). In some embodiments, a sushi domain from an alpha chain of IL15R α can be encoded by a nucleic acid including

10 ATTACATGCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAG
CTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAACAGCGGCTTCAAGAGGA
AGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGT
GGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 17).

In some embodiments, a soluble IL-15 can include a sequence that is at least 70%
15 identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESGD
ASIHDTVENLIILANNSLSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMFINT
S (SEQ ID NO: 18). In some embodiments, a soluble IL-15 can be encoded by a nucleic

20 acid including the sequence of

AACTGGGTGAACGTCATCAGCGATTAAAGAAGATCGAAGATTAAATTCAGTC
CATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTA
GGTGACCGCCATGAAATGTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAG
CGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATA
25 ACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGA
AGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTG
TCCAGATGTTTCATCAATACCTCC (SEQ ID NO: 19).

In some embodiments, a soluble IL-15 can include a D8N amino acid substitution.

In some embodiments, the soluble IL-15 with D8N mutant (IL15D8N) can include a

30 sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 92% identical, at least 94% identical,

at least 95% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to

NWVNVISNLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESGD
ASIHDTVENLIILANNSLSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMFINT

5 S (SEQ ID NO: 70). In some embodiments, the soluble IL-15 with D8N mutant (IL15D8N) can be encoded by a nucleic acid including the sequence of

AACTGGGTGAATGTAATAAGTAATTTGAAAAAATTGAAGATCTTATTC
AATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTACCCCCAGTTG
CAAAGTAACAGCAATGAAGTGCTTTCTCTTGAGTTACAAGTTATTTCACTTG
10 AGTCCGGAGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATCCTAGCAA
ACAACAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCAAAGAATGT
GAGGAAGTGGAGGAAAAAATATTAAAGAATTTTGCAGAGTTTTGTACATAT
TGTCCAAATGTTTCATCAACACTTCT (SEQ ID NO: 71).

15 **Signal Sequence**

In some embodiments, a multi-chain chimeric polypeptide includes a first chimeric polypeptide that includes a signal sequence at its N-terminal end. In some embodiments, a multi-chain chimeric polypeptide includes a second chimeric polypeptide that includes a signal sequence at its N-terminal end. In some embodiments, both the
20 first chimeric polypeptide of a multi-chain chimeric polypeptide and a second chimeric polypeptide of the multi-chain chimeric polypeptide include a signal sequence. As will be understood by those of ordinary skill in the art, a signal sequence is an amino acid sequence that is present at the N-terminus of a number of endogenously produced proteins that directs the protein to the secretory pathway (e.g., the protein is directed to
25 reside in certain intracellular organelles, to reside in the cell membrane, or to be secreted from the cell). Signal sequences are heterogeneous and differ greatly in their primary amino acid sequences. However, signal sequences are typically 16 to 30 amino acids in length and include a hydrophilic, usually positively charged N-terminal region, a central hydrophobic domain, and a C-terminal region that contains the cleavage site for signal
30 peptidase.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence

MKWVTFISLLFLFSSAYS (SEQ ID NO: 20). In some embodiments, a first chimeric

5 polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence encoded by the nucleic acid sequence

ATGAAATGGGTGACCTTTATTTCTTTACTGTTTCCTCTTTAGCAGCGCCTACTCC
(SEQ ID NO: 21),

10 ATGAAGTGGGTCACATTTATCTCTTTACTGTTTCCTCTTCTCCAGCGCCTACAGC
(SEQ ID NO: 22), or

ATGAAATGGGTGACCTTTATTTCTTTACTGTTTCCTCTTTAGCAGCGCCTACTCC
(SEQ ID NO: 23).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric
15 polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence

MKCLLYLAFLFLGVNC (SEQ ID NO: 24). In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino

20 acid sequence

MGQIVTMFEALPHIIDEVINIVLIITSIKAVYNFATCGILALVSFLFLAGRSCG
(SEQ ID NO: 25). In some embodiments, a first chimeric polypeptide of a multi-chain

chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence

25 MPNHQSGSPTGSSDLLSGKKQRPHLALRRKRRREMRKINRKVRRMNLAPIKEK
TAWQHLQALISEAEVLKTSQTPQNSLTLFLALLSVLGPPVTG (SEQ ID NO: 26).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence having an amino acid sequence

30 MDSKGSSQKGSRLLLLLVVSNLLLCQGVVS (SEQ ID NO: 27). Those of ordinary skill in the art will be aware of other appropriate signal sequences for use in a first

chimeric polypeptide and/or a second chimeric polypeptide of multi-chain chimeric polypeptides described herein.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence that is about 10 to 100 amino acids in length. For example, a signal sequence can be about 10 to 100 amino acids in length, about 15 to 100 amino acids in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in length, about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length, about 10 to 65 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a signal sequence is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

In some embodiments, any of the signal sequences disclosed herein can include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at its N-terminus and/or C-terminus, so long as the function of the signal sequence remains

intact. For example, a signal sequence having the amino acid sequence MKCLLYLAFLFLGVNC (SEQ ID NO: 28) can include one or more additional amino acids at the N-terminus or C-terminus, while still retaining the ability to direct a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both to the secretory pathway.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a signal sequence that directs the multi-chain chimeric polypeptide into the extracellular space. Such embodiments are useful in producing multi-chain chimeric polypeptides that are relatively easy to be isolated and/or purified.

Peptide Tags

In some embodiments, a multi-chain chimeric polypeptide includes a first chimeric polypeptide that includes a peptide tag (e.g., at the N-terminal end or the C-terminal end of the first chimeric polypeptide). In some embodiments, a multi-chain chimeric polypeptide includes a second chimeric polypeptide that includes a peptide tag (e.g., at the N-terminal end or the C-terminal end of the second chimeric polypeptide). In some embodiments, both the first chimeric polypeptide of a multi-chain chimeric polypeptide and a second chimeric polypeptide of the multi-chain chimeric polypeptide include a peptide tag. In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both include two or more peptide tags.

Exemplary peptide tags that can be included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both include, without limitation, AviTag (GLNDIFEAQKIEWHE; SEQ ID NO: 29), a calmodulin-tag (KRRWKKNFIAVSAANRFKKISSSGAL; SEQ ID NO: 30), a polyglutamate tag (EEEEEE; SEQ ID NO: 31), an E-tag (GAPVPYPDPLEPR; SEQ ID NO: 32), a FLAG-tag (DYKDDDDK; SEQ ID NO: 33), an HA-tag, a peptide from hemagglutinin (YPYDVDPDYA; SEQ ID NO: 34), a his-tag (HHHHH (SEQ ID NO: 35); HHHHHH (SEQ ID NO: 36); HHHHHHH (SEQ ID NO: 37); HHHHHHHH (SEQ ID NO: 38);

HHHHHHHHH (SEQ ID NO: 39); or HHHHHHHHHH (SEQ ID NO: 40)), a myc-tag (EQKLISEEDL; SEQ ID NO: 41), NE-tag (TKENPRSNQEESYDDNES; SEQ ID NO: 42), S-tag, (KETAAAKFERQHMDS; SEQ ID NO: 43), SBP-tag (MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP; SEQ ID NO: 44),
 5 Softag 1 (SLAELLNAGLGGS; SEQ ID NO: 45), Softag 3 (TQDPSRVG; SEQ ID NO: 46), Spot-tag (PDRVRAVSHWSS; SEQ ID NO: 47), Strep-tag (WSHPQFEK; SEQ ID NO: 48), TC tag (CCPGCC; SEQ ID NO: 49), Ty tag (EVHTNQDPLD; SEQ ID NO: 50), V5 tag (GKIPNPLLGLDST; SEQ ID NO: 51), VSV-tag (YTDIEMNRLGK; SEQ ID NO: 52), and Xpress tag (DLYDDDDK; SEQ ID NO: 53). In some embodiments,
 10 tissue factor protein is a peptide tag.

Peptide tags that can be included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both can be used in any of a variety of applications related to the multi-chain chimeric polypeptide. For example, a peptide tag can be used in the purification of
 15 a multi-chain chimeric polypeptide. As one non-limiting example, a first chimeric polypeptide of a multi-chain chimeric polypeptide (e.g., a recombinantly expressed first chimeric polypeptide), a second chimeric polypeptide of the multi-chain chimeric polypeptide (e.g., a recombinantly expressed second chimeric polypeptide), or both can include a myc tag; the multi-chain chimeric polypeptide that includes the myc-tagged
 20 first chimeric polypeptide, the myc-tagged second chimeric polypeptide, or both can be purified using an antibody that recognizes the myc tag(s). One non-limiting example of an antibody that recognizes a myc tag is 9E10, available from the non-commercial Developmental Studies Hybridoma Bank. As another non-limiting example, a first chimeric polypeptide of a multi-chain chimeric polypeptide (e.g., a recombinantly
 25 expressed first chimeric polypeptide), a second chimeric polypeptide of the multi-chain chimeric polypeptide (e.g., a recombinantly expressed second chimeric polypeptide), or both can include a histidine tag; the multi-chain chimeric polypeptide that includes the histidine-tagged first chimeric polypeptide, the histidine-tagged second chimeric polypeptide, or both can be purified using a nickel or cobalt chelate. Those of ordinary
 30 skill in the art will be aware of other suitable tags and agent that bind those tags for use in purifying multi-chain chimeric polypeptide. In some embodiments, a peptide tag is

removed from the first chimeric polypeptide and/or the second chimeric polypeptide of the multi-chain chimeric polypeptide after purification. In some embodiments, a peptide tag is not removed from the first chimeric polypeptide and/or the second chimeric polypeptide of the multi-chain chimeric polypeptide after purification.

5 Peptide tags that can be included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both can be used, for example, in immunoprecipitation of the multi-chain chimeric polypeptide, imaging of the multi-chain chimeric polypeptide (e.g., via Western blotting, ELISA, flow cytometry, and/or immunocytochemistry), and/or solubilization of
10 the multi-chain chimeric polypeptide.

 In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both includes a peptide tag that is about 10 to 100 amino acids in length. For example, a peptide tag can be about 10 to 100 amino acids in length, about 15 to 100 amino acids in
15 length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in
20 length, about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in
length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length, about 10 to 65
25 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in
length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about
30 30 to 40 amino acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in

length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a peptide tag is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

Peptide tags included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both can be of any suitable length. For example, peptide tags can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids in length. In embodiments in which a multi-chain chimeric polypeptide includes two or more peptide tags, the two or more peptide tags can be of the same or different lengths. In some embodiments, any of the peptide tags disclosed herein may include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at the N-terminus and/or C-terminus, so long as the function of the peptide tag remains intact. For example, a myc tag having the amino acid sequence EQKLISEEDL (SEQ ID NO: 54) can include one or more additional amino acids (e.g., at the N-terminus and/or the C-terminus of the peptide tag), while still retaining the ability to be bound by an antibody.

Exemplary Multi-Chain Chimeric Polypeptides

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF- β . In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain

chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

5 In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second
10 domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of
15 affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain each independently bind specifically to TGF- β . In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to
20 the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain is a soluble TGF- β receptor
25 (e.g., a soluble TGF β RII receptor, e.g., a soluble human TGF β RII). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGF β RII includes a first sequence of soluble human TGF β RII and a second sequence of soluble human TGF β RII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGF β RII includes a linker disposed between the first sequence of
30 soluble human TGF β RII and the second sequence of soluble human TGF β RII. In

some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 3).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFR β RII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:
 IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
 KPQEVCAVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGET
 FFMCS CSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 2).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFR β RII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:
 IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
 KPQEVCAVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGET
 FFMCS CSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 2).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFR β RII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:
 ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
 CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
 GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACCTGCAGCATCACCTCCATCT
 GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
 ACATCACCTGGAGACCGTGTGTACGACCCCAAGCTCCCTTATCACGACTTC
 ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAGC
 CCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGTAACGACAAC
 ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 55).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFR β RII receptor is encoded by a sequence that is at least

80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

5 ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCTTGGAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATT
10 CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO:
56).

In some embodiments of these multi-chain chimeric polypeptides, the soluble
15 TGF- β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
20 KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 4).

25 In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF- β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

30 ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAAGTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATACCCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
35 ATTCTGGAGGACGCTGCCTCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC

CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
 AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
 CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
 CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
 5 TCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTGGAAACCGTCTG
 CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
 CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTTCATGTGC
 TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
 TACCAGCAACCCCGAC (SEQ ID NO: 57).

10 In some embodiments, the first chimeric polypeptide can include a sequence that
 is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86%
 identical, at least 88% identical, at least 90% identical, at least 92% identical, at least
 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or
 15 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
 KPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGET
 FFMCSRSSDECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSIPPHVQKSVNNDM
 IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRK
 20 NDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSSSDECND
 NIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKS
 GDWKSCKFYTTDTECDLTDEIVKDVQTYLARVFSYPAGNVESTGSAGEPLYEN
 SPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLI
 YTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFVSQAVIPSRVTNRKSTDSPV
 25 ECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKC
 FLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECELEEKNIKEFL
 QSFVHIVQMFINTS (SEQ ID NO: 6).

30 In some embodiments, a first chimeric polypeptide is encoded by a sequence that
 is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86%
 identical, at least 88% identical, at least 90% identical, at least 92% identical, at least
 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or
 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
 ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTT
 35 AGCACCTGCGATAATCAGAAAGTCCTGCATGTCCAAGTGCAGCATCACCTCCA
 TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
 AGAACATCACCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
 CTTATTCTGGAGGACGCTGCCTCCCCAAATGCATCATGAAGGAGAAGAAG
 AAGCCCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGTAACG

ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
 CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
 CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
 AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
 5 CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
 AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTGGA
 AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTTCATCCTGGAAGAC
 GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
 TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
 10 CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
 TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
 GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
 GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCATCTCAC
 CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
 15 TACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
 AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
 CATCCAAAGCTTTGAGCAAGTTGGCACAAGGTGAATGTGACAGTGGAGGAC
 GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
 TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCTCCGGC
 20 AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
 GGCGAAAACACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
 ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
 AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
 TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
 25 ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
 ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
 TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
 TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
 TCCTTTGTGCACATTGTCCAGATGTTTCATCAATACCTCC (SEQ ID NO: 58).

30 In some embodiments, a first chimeric polypeptide can include a sequence that is
 at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86%
 identical, at least 88% identical, at least 90% identical, at least 92% identical, at least
 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or
 100% identical) to:

35 MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
 CDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED
 AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
 GGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
 TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKK
 40 PGETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEW
 EPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYP

AGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLV
RRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSV
QAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATL
YTESDVHPSCKVTAMKCFLELQVISLESGDASIHDTVENLIILANNSLSSNGNVT
5 ESGCKECEEELEEKNIKEFLQSFVHIVQMFINITS (SEQ ID NO: 7).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or
10 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCTCCTGTTCTCCAGCGCCTACTC
CATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACCTGCAGCATCACCTCC
15 ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
GAGAACATCACCTGGAGACCGTGTGTACGACCCCAAGCTCCCTTATCACG
ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
GAAGCCCGGAGAGACCTTCTTTATGTGTTCTGTTAGCAGCGACGAGTGTAAC
GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
20 GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCTCCCCACGT
GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
CCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTGGA
25 AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
30 GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCAC
CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
TACCCCGCCGGAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
35 CATCCAAAGCTTTGAGCAAGTTGGCACAAGGTGAATGTGACAGTGGAGGAC
GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGC
AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
40 ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA

TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
 ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
 ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
 TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
 5 TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
 TCCTTTGTGCACATTGTCCAGATGTTTCATCAATACCTCC (SEQ ID NO: 59).

In some embodiments, the second chimeric polypeptide can include a sequence
 that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least
 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at
 10 least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical,
 or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
 KPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPKGET
 FFMCSRSSDECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSIPPHVQKSVNNDM
 15 IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVVRK
 NDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPKGETFFMCSRSSDECND
 NIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLT
 ECVLNKATNVAHWTPSLKCIR (SEQ ID NO: 5).

In some embodiments, a second chimeric polypeptide is encoded by a sequence
 20 that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least
 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at
 least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical,
 or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
 25 ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
 AGCACCTGCGATAATCAGAAAGTCTGTCATGTCCAACGTCAGCATCACCTCCA
 TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
 AGAACATCACCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
 CTTCAATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
 30 AAGCCCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGTAACG
 ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
 CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCTCTCCACGTG
 CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
 AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTCCACCTGCGACAAC
 35 CAGAAGTCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
 AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTGGA
 AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTTCATCCTGGAAGAC
 GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC

TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
 CGAGGAATACAATACCAGCAACCCCGACATTACATGCCCCCTCCCATGAGC
 GTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGA
 GGTATATCTGTAAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCAC
 5 CGAGTGCCTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCT
 TTAAAGTGCATCCGG (SEQ ID NO: 60).

In some embodiments, a second chimeric polypeptide can include a sequence that
 is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86%
 identical, at least 88% identical, at least 90% identical, at least 92% identical, at least
 10 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or
 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
 CDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED
 AASPKCIMKEKKKPGETFFMCSSSDECNDNIIFSEYNTSNPDGGGGSGGGGSG
 15 GGGSSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
 TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKK
 PGETFFMCSSSDECNDNIIFSEYNTSNPDITCPPPMSVEHADIWVKSYSLSYRER
 YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPLK CIR (SEQ ID NO: 8).

In some embodiments, a second chimeric polypeptide is encoded by a sequence
 20 that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least
 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at
 least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical,
 or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCTCTCCAGCGCCTACTC
 25 CATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
 AACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
 CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAAGTGCAGCATCACCTCC
 ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
 GAGAACATCACCTGGAGACCGTGTGTACGACCCCAAGCTCCCTTATCACG
 30 ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
 GAAGCCCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGTAAC
 GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
 GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
 GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
 35 AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
 CCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
 AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTGGA
 AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTTCATCCTGGAAGAC

GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
 TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
 CGAGGAATACAATACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGC
 GTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGA
 5 GGTATATCTGTAAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCAC
 CGAGTGCCTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCT
 TTAAAGTGCATCCGG (SEQ ID NO: 61).

In some embodiments, the first chimeric polypeptide can include a sequence that
 10 is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 85%
 identical, at least 86% identical, at least 88% identical, at least 90% identical, at least
 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at
 least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
 15 KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGET
 FFMCS CSSDECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSIPPHVQKSVNNDM
 IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
 NDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECND
 NIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLT
 20 ECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 62).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that
 is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 85%
 identical, at least 86% identical, at least 88% identical, at least 90% identical, at least
 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at
 25 least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
 ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
 AGCACCTGCGATAATCAGAAAGTCCTGCATGTCCAAGTGCAGCATCACCTCCA
 TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
 30 AGAACATCACCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
 CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
 AAGCCCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGTAACG
 ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG

CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
 CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
 AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
 CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
 5 AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTGGA
 AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTTCATCCTGGAAGAC
 GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
 TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
 CGAGGAATACAATACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGC
 10 GTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGA
 GGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCAC
 CGAGTGCCTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCT
 TTAAAGTGCATCCGG (SEQ ID NO: 63).

In some embodiments, the first chimeric polypeptide can include a sequence that
 15 is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 85%
 identical, at least 86% identical, at least 88% identical, at least 90% identical, at least
 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at
 least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
 20 CDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED
 AASPKCIMKEKKKPGETFFMCSSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
 GGGSSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
 TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKK
 PGETFFMCSSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRER
 25 YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 64).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that
 is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 85%
 identical, at least 86% identical, at least 88% identical, at least 90% identical, at least
 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at
 30 least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCTCCTGTTCTCCAGCGCCTACTC
 CATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
 AACAAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
 CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACCTGCAGCATCACCTCC
 5 ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
 GAGAACATCACCTGGAGACCGTGTGTACGACCCCAAGCTCCCTTATCACG
 ACTTCATTCTGGAGGACGCTGCCTCCCCAAATGCATCATGAAGGAGAAGAA
 GAAGCCCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGTAAC
 GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
 10 GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
 GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
 AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
 CCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
 AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTGGA
 15 AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTTCATCCTGGAAGAC
 GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
 TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
 CGAGGAATACAATACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGC
 GTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGA
 20 GGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCAC
 CGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCT
 TTAAAGTGCATCCGG (SEQ ID NO: 65).

In some embodiments, a second chimeric polypeptide can include a sequence that
 is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 85%
 25 identical, at least 86% identical, at least 88% identical, at least 90% identical, at least
 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at
 least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
 KPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGET
 30 FFMCSRSSDECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSIPPHVQKSVNNDM
 IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVVRK

NDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECND
 NIIFSEYNTSNPDSGTTNTVAAYNL TWKSTNFKTILEWEPKPVNQVYTVQISTKS
 GDWKS KCFYTTDTECDLTDEIVKDV KQTYLARVFSYPAGNVESTGSAGEPLYEN
 SPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLI
 5 YTLYYWKSSSSGKK TAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPV
 ECMGQEKGEFRENWVNVISNLKKIEDLIQSMHIDATLYTESDVHPSCCKVTAMKC
 FLLELQVISLESGDASIHD TVENLIILANNSLSSNGNVTESGCKECELEEKNIKEFL
 QSFVHIVQMFINTS (SEQ ID NO: 66).

In some embodiments, a second chimeric polypeptide is encoded by a sequence
 10 that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least
 85% identical, at least 86% identical, at least 88% identical, at least 90% identical, at
 least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical,
 at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCACCGCACGTT CAGAAGTCGGTGAATAACGACATGATAGTCACTGACA
 15 ACAACGGTGCAGTCAAGTTTCCACA ACTGTGTAAATTTTGTGATGTGAGATTT
 TCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCA
 TCTGTGAGAAGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGA
 GAACATAACACTAGAGACAGTTTGCCATGACCCCAAGCTCCCCTACCATGAC
 TTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAAAAAAAA
 20 AGCCTGGTGAGACTTTCTTCATGTGTTCTGTAGCTCTGATGAGTGCAATGAC
 AACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACGGAGGTGGCG
 GATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCA
 GAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAA
 ATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACC
 25 AGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCA
 GGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTGGAA
 ACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTTCATCCTGGAAGACG
 CCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCT
 TTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGC
 30 GAGGAATACAATACCAGCAACCCCGACTCAGGCACTACAAATACTGTGGCAG
 CATATAATTTAACTTGGAAATCAACTAATTTCAAGACAATTTTGGAGTGGGA

ACCCAAACCCGTCATCAAGTCTACACTGTTCAAATAAGCACTAAGTCAGGA
 GATTGGAAAAGCAAATGCTTTTACACAACAGACACAGAGTGTGACCTCACCG
 ACGAGATTGTGAAGGATGTGAAGCAGACGTACTTGGCACGGGTCTTCTCCTA
 CCCGGCAGGGAATGTGGAGAGCACCGGTTCTGCTGGGGAGCCTCTGTATGAG
 5 AACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGGACAGCCAACAA
 TTCAGAGTTTTGAACAGGTGGGAACAAAAGTGAATGTGACCGTAGAAGATGA
 ACGGACTTTAGTCAGAAGGAACAACACTTTTCCTAAGCCTCCGGGATGTTTTTG
 GCAAGGACTTAATTTATACACTTTATTATTGGAAATCTTCAAGTTCAGGAAAG
 AAAACAGCCAAAACAAACACTAATGAGTTTTTGATTGATGTGGATAAAGGAG
 10 AAAACTACTGTTTCAGTGTTCAGCAGTGATTCCCTCCCGAACAGTTAACCGG
 AAGAGTACAGACAGCCCGGTAGAGTGTATGGGCCAGGAGAAAGGGGAATTC
 AGAGAAAACCTGGGTGAATGTAATAAGTAATTTGAAAAAAATTGAAGATCTTA
 TTCAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTACCCCC
 AGTTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTATTTCT
 15 ACTTGAGTCCGGAGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATC
 CTAGCAAACAACAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCA
 AAGAATGTGAGGAACTGGAGGAAAAAAATATTAAAGAATTTTTGCAGAGTTT
 TGTACATATTGTCCAAATGTTTCATCAACACTTCT (SEQ ID NO: 67).

In some embodiments, a second chimeric polypeptide can include a sequence that
 20 is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 85%
 identical, at least 86% identical, at least 88% identical, at least 90% identical, at least
 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at
 least 98% identical, at least 99% identical, or 100% identical) to:

MGVKVLFALICIAVAEAIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
 25 CDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED
 AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
 GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
 TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKK
 PGETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLWKSTNFKTILEW
 30 EPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYP
 AGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLV

RRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSV
QAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISNLKKIEDLIQSMHIDATL
YTESDVHPSCKVTAMKCFLELQVISLESGDASIHDTVENLIILANNSLSSNGNVT
ESGCKECEEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 68).

- 5 In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

10 ATGGGAGTGAAAGTTCTTTTTGCCCTTATTTGTATTGCTGTGGCCGAGGCC
ATCCCACCGCACGTTTCAGAAGTCGGTGAATAACGACATGATAGTCACTGACA
ACAACGGTGCAGTCAAGTTTCCACAACCTGTGTAAATTTGTGATGTGAGATTT
TCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCA
TCTGTGAGAAGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGA
15 GAACATAACACTAGAGACAGTTTGCCATGACCCCAAGCTCCCCTACCATGAC
TTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAAAAAAAA
AGCCTGGTGAGACTTTCTTCATGTGTTCTGTAGCTCTGATGAGTGCAATGAC
AACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACGGAGGTGGCG
GATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCA
20 GAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAA
ATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACC
AGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCA
GGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTGGAA
ACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACG
25 CCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCT
TTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGC
GAGGAATACAATACCAGCAACCCGACTCAGGCACTACAAATACTGTGGCAG
CATATAATTTAACTTGGAATCAACTAATTTCAAGACAATTTTGGAGTGGGA
ACCCAAACCCGTCAATCAAGTCTACACTGTTCAAATAAGCACTAAGTCAGGA
30 GATTGGAAAAGCAAATGCTTTTACACAACAGACACAGAGTGTGACCTACCG
ACGAGATTGTGAAGGATGTGAAGCAGACGTACTTGGCACGGGTCTTCTCCTA

CCCGGCAGGGAATGTGGAGAGCACCGGTTCTGCTGGGGAGCCTCTGTATGAG
AACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGGACAGCCAACAA
TTCAGAGTTTTGAACAGGTGGGAACAAAAGTGAATGTGACCGTAGAAGATGA
ACGGACTTTAGTCAGAAGGAACAACACTTTCCTAAGCCTCCGGGATGTTTTTG
5 GCAAGGACTTAATTTATACACTTTATTATTGGAAATCTTCAAGTTCAGGAAAG
AAAACAGCCAAAACAAACACTAATGAGTTTTTTGATTGATGTGGATAAAGGAG
AAAACACTACTGTTTCAGTGTTCAGCAGTGATTCCCTCCCGAACAGTTAACCGG
AAGAGTACAGACAGCCCGGTAGAGTGTATGGGCCAGGAGAAAGGGGAATTC
AGAGAAAACACTGGGTGAATGTAATAAGTAATTTGAAAAAAATTGAAGATCTTA
10 TTCAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTACCCCC
AGTTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTATTTC
ACTTGAGTCCGGAGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATC
CTAGCAAACAACAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCA
AAGAATGTGAGGAACTGGAGGAAAAAAATATTAAAGAATTTTTGCAGAGTTT
15 TGTACATATTGTCCAAATGTTTCATCAACACTTCT (SEQ ID NO: 69).

Compositions/Kits

Also provided herein are compositions (e.g., pharmaceutical compositions) that include at least one of any multi-chain chimeric polypeptides, any of the cells, or any of the nucleic acids described herein. In some embodiments, the compositions include at least one of any of the multi-chain chimeric polypeptides described herein. In some
20 embodiments, the compositions include any of the immune cells (e.g., any of the immune cells described herein, e.g., any of the immune cells produced using any of the methods described herein).

25 In some embodiments, the pharmaceutical compositions are formulated for different routes of administration (e.g., intravenous, subcutaneous). In some embodiments, the pharmaceutical compositions can include a pharmaceutically acceptable carrier (e.g., phosphate buffered saline).

Single or multiple administrations of pharmaceutical compositions can be given to
30 a subject in need thereof depending on for example: the dosage and frequency as required

and tolerated by the subject. The formulation should provide a sufficient quantity of active agent to effectively treat, prevent or ameliorate conditions, diseases or symptoms.

Also provided herein are kits that include any of the multi-chain chimeric polypeptides, compositions, nucleic acids, or cells (e.g., immune cells) described herein.

- 5 In some embodiments, the kits can include instructions for performing any of the methods described herein. In some embodiments, the kits can include at least one dose of any of the pharmaceutical compositions described herein.

Nucleic Acids/Vectors

- Also provided herein are nucleic acids that encode any of the multi-chain
10 chimeric polypeptides described herein. In some embodiments, a first nucleic acid can encode the first chimeric polypeptide and a second nucleic acid can encode the second chimeric polypeptide. In some embodiments, a single nucleic acid can encode both the first chimeric polypeptide and the second chimeric polypeptide.

- Also provided herein are vectors that include any of the nucleic acids encoding
15 any of the multi-chain chimeric polypeptides described herein. In some embodiments, a first vector can include a nucleic acid encoding the first chimeric polypeptide and a second vector can include a nucleic acid encoding the second chimeric polypeptide. In some embodiments, a single vector can include a first nucleic acid encoding the first chimeric polypeptide and a second nucleic acid encoding the second chimeric
20 polypeptide.

Any of the vectors described herein can be an expression vector. For example, an expression vector can include a promoter sequence operably linked to the sequence encoding the first chimeric polypeptide and the second chimeric polypeptide.

- Non-limiting examples of vectors include plasmids, transposons, cosmids, and
25 viral vectors (e.g., any adenoviral vectors (e.g., pSV or pCMV vectors), adeno-associated virus (AAV) vectors, lentivirus vectors, and retroviral vectors), and any Gateway® vectors. A vector can, e.g., include sufficient cis-acting elements for expression; other elements for expression can be supplied by the host mammalian cell or in an in vitro expression system. Skilled practitioners will be capable of selecting suitable vectors and

mammalian cells for making any of the multi-chain chimeric polypeptides described herein.

Cells

Also provided herein are cells (e.g., any of the exemplary cells described herein or
5 known in the art) comprising any of the nucleic acids described herein that encode any of
the multi-chain chimeric polypeptides described herein (e.g., encoding both the first and
second chimeric polypeptides). Also provided herein are cells (e.g., any of the exemplary
cells described herein or known in the art) comprising any of the nucleic acids described
herein that encode any of the first chimeric polypeptides described herein. Also provided
10 are cells (e.g., any of the exemplary cells described herein or known in the art)
comprising any of the nucleic acids described herein that encode any of the second
chimeric polypeptides described herein.

Also provided herein are cells (e.g., any of the exemplary cells described herein or
known in the art) that include any of the vectors described herein that encode any of the
15 multi-chain chimeric polypeptides described herein (e.g., encoding both the first and
second chimeric polypeptides). Also provided herein are cells (e.g., any of the exemplary
cells described herein or known in the art) that include any of the vectors described
herein that encode any of the first chimeric polypeptides described herein. Also provided
herein are cells (e.g., any of the exemplary cells described herein or known in the art) that
20 include any of the vectors described herein that encode any of the second chimeric
polypeptides described herein).

In some embodiments of any of the methods described herein, the cell can be a
eukaryotic cell. As used herein, the term “eukaryotic cell” refers to a cell having a
distinct, membrane-bound nucleus. Such cells may include, for example, mammalian
25 (e.g., rodent, non-human primate, or human), insect, fungal, or plant cells. In some
embodiments, the eukaryotic cell is a yeast cell, such as *Saccharomyces cerevisiae*. In
some embodiments, the eukaryotic cell is a higher eukaryote, such as mammalian, avian,
plant, or insect cells. Non-limiting examples of mammalian cells include Chinese
hamster ovary cells and human embryonic kidney cells (e.g., HEK293 cells).

Methods of introducing nucleic acids and expression vectors into a cell (e.g., a eukaryotic cell) are known in the art. Non-limiting examples of methods that can be used to introduce a nucleic acid into a cell include lipofection, transfection, electroporation, microinjection, calcium phosphate transfection, dendrimer-based transfection, cationic
5 polymer transfection, cell squeezing, sonoporation, optical transfection, impalefection, hydrodynamic delivery, magnetofection, viral transduction (e.g., adenoviral and lentiviral transduction), and nanoparticle transfection.

Methods of Producing Multi-Chain Chimeric Polypeptides

Also provided herein are methods of producing any of the multi-chain chimeric
10 polypeptides described herein that include culturing any of the cells described herein in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Also provided herein are method of producing any of the multi-chain chimeric
15 polypeptides described herein that include: culturing any of cells described herein in a first culture medium under conditions sufficient to result in the production of the first chimeric polypeptide; recovering the first chimeric polypeptide from the cell and/or the first culture medium; culturing any of the cells described herein in a second culture medium under conditions sufficient to result in the production of the second chimeric
20 polypeptide; recovering the second chimeric polypeptide from the cell and/or the second culture medium; and combining (e.g., mixing) the recovered first chimeric polypeptide and the recovered second chimeric polypeptide to form the multi-chain chimeric polypeptide (e.g., any of the multi-chain chimeric polypeptides described herein).

The recovery of the multi-chain chimeric polypeptide, the first chimeric
25 polypeptide, or the second chimeric polypeptide from a cell (e.g., a eukaryotic cell) can be performed using techniques well-known in the art (e.g., ammonium sulfate precipitation, polyethylene glycol precipitation, ion-exchange chromatography (anion or cation), chromatography based on hydrophobic interaction, metal-affinity chromatography, ligand-affinity chromatography, and size exclusion chromatography).

Methods of culturing cells are well known in the art. Cells can be maintained *in vitro* under conditions that favor proliferation, differentiation and growth. Briefly, cells can be cultured by contacting a cell (e.g., any cell) with a cell culture medium that includes the necessary growth factors and supplements to support cell viability and growth.

Also provided herein are multi-chain chimeric polypeptides (e.g., any of the multi-chain chimeric polypeptides described herein), first chimeric polypeptides (e.g., any of the first chimeric polypeptides), or second chimeric polypeptides (e.g., any of the second chimeric polypeptides described herein) produced by any of the methods described herein.

Senescent Cells

Senescence is a form of irreversible growth arrest accompanied by phenotypic changes, resistance to apoptosis and activation of damage-sensing signaling pathways. Cellular senescence was first described in cultured human fibroblast cells that lost their ability to proliferate, reaching permanent arrest after about 50 population doublings (referred to as the Hayflick limit). Senescence is considered a stress response that can be induced by a wide range of intrinsic and extrinsic insults, including oxidative and genotoxic stress, DNA damage, telomere attrition, oncogenic activation, mitochondrial dysfunction, or chemotherapeutic agents.

Senescent cells remain metabolically active and can influence the tissue hemostasis, disease and aging through their secretory phenotype. Senescence is considered as a physiologic process and is important in promoting wound healing, tissue homeostasis, regeneration, and fibrosis regulation. For instance, transient induction of senescent cells is observed during wound healing and contributes to wound resolution. Perhaps one of the most important roles of senescence is its role in tumor suppression. However, the accumulation of senescent cells also drives aging- and aging-related diseases and conditions. The senescent phenotype also can trigger chronic inflammatory responses and consequently augment chronic inflammatory conditions to promote tumor growth. The connection between senescence and aging was initially based on observations that senescent cells accumulate in aged tissue. The use of transgenic models

has enabled the detection of senescent cells systematically in many age-related pathologies. Strategies to selectively eliminate senescent cells has demonstrated that senescent cells can indeed play a causal role in aging and related pathologies.

Senescent cells display important and unique properties which include changes in morphology, chromatin organization, gene expression, and metabolism. There are several biochemical and functional properties associated with cellular senescence, such as (i) increased expression of p16 and p21, inhibitors of cyclin-dependent kinases, (ii) presence of senescence-associated β -galactosidase, a marker of lysosomal activity, (iii) appearance of senescence-associated heterochromatin foci and downregulation of lamin B1 levels, (iv) resistance to apoptosis caused by an increased expression of anti-apoptotic BCL-family protein, and (v) upregulation of CD26 (DPP4), CD36 (Scavenger receptor), forkhead box 4 (FOXO4), and secretory carrier membrane protein 4 (SCAMP4). Senescent cells also express an inflammatory signature, the so-called senescence-associated secretory phenotype (SASP). Through SASP, the senescent cells produce a wide range of inflammatory cytokines (IL-6, IL-8), growth factors (TGF- β), chemokines (CCL-2), and matrix metalloproteinases (MMP-3, MMP-9) that operate in a cell-autonomous manner to reinforce senescence (autocrine effects) and communicate with and modify the microenvironment (paracrine effects). SASP factors can contribute to tumor suppression by triggering senescence surveillance, an immune-mediated clearance of senescent cells. However, chronic inflammation is also a known driver of tumorigenesis, and accumulating evidence indicates that chronic SASP can also boost cancer and aging-related diseases.

The secretion profile of senescent cells is context dependent. For instance, the mitochondrial dysfunction-associated senescence (MiDAS), induced by different mitochondrial dysfunction in human fibroblasts, led to the appearance of a SASP that was deficient in IL-1-dependent inflammatory factors. A decrease in the NAD⁺/NADH ratio activated AMPK signaling which induced MiDAS through the activation of p53. As a result, p53 inhibited NF- κ B signaling which is a crucial inducer of pro-inflammatory SASP. In contrast, the cellular senescence caused by persistent DNA damage in human cells induced an inflammatory SASP, which was dependent on the activation of ataxia-telangiectasia mutated (ATM) kinase but not on that of p53. In particular, the expression

and secretion levels of IL-6 and IL-8 were increased. It was also demonstrated that cellular senescence caused by the ectopic expression p16INK4a and p21CIP1 induced the senescent phenotype in human fibroblasts without an inflammatory SASP indicating that the growth arrest itself did not stimulate SASP.

5 One of the most defining characteristics of senescence is stable growth arrest. This is achieved by two important pathways, the p16/Rb and the p53/p21, both of which are central in tumor suppression. DNA damage results in: (1) high deposition of γ H2Ax (histone coding gene) and 53BP1 (involved in DNA damage response) in chromatin: this leads to activation of a kinase cascade eventually resulting in p53 activation, and (2)
10 activation of p16INK4a and ARF (both encoded by CDKN2A) and P15INK4b (encoded by CDKN2B): p53 induces transcription of cyclin-dependent kinase inhibitor (p21) and along with both p16INK4a and p15INK4b block genes for cell cycle progression (CDK4 and CDK6). This eventually leads to hypophosphorylation of Retinoblastoma protein (Rb) and cell cycle arrest at the G1 phase.

15 Selectively killing senescent cells has been shown to significantly improve the health span of mice in the context of normal aging and ameliorates the consequences of age-related disease or cancer therapy (Ovadya, *J Clin Invest.* 128(4):1247-1254, 2018). In nature, the senescent cells are normally removed by the innate immune cells. Induction of senescence not only prevents the potential proliferation and transformation
20 of damaged/altered cells, but also favors tissue repair through the production of SASP factors that function as chemoattractants mainly for Natural Killer (NK) cells (such as IL-15 and CCL2) and macrophages (such as CFS-1 and CCL2). These innate immune cells mediate the immunosurveillance mechanism for eliminating stressed cells. Senescent cells usually up-regulate the NK-cell activating receptor NKG2D and DNAM-1 ligands,
25 which belong to a family of stress-inducible ligands: an important component of the frontline immune defense against infectious diseases and malignancies. Upon receptor activation, NK cells can then specifically induce the death of senescent cells through their cytolytic machinery. A role for NK cells in the immune surveillance of senescent cells has been pointed out in liver fibrosis (Sagiv, *Oncogene* 32(15): 1971-1977, 2013),
30 hepatocellular carcinoma (Iannello, *J Exp Med* 210(10): 2057-2069, 2013), multiple myeloma (Soriani, *Blood* 113(15): 3503-3511, 2009), and glioma cells stressed by

dysfunction of the mevalonate pathway (Ciaglia, *Int J Cancer* 142(1): 176-190, 2018). Endometrial cells undergo acute cellular senescence and do not differentiate into decidual cells. The differentiated decidual cells secrete IL-15 and thereby recruit uterine NK cells to target and eliminate the undifferentiated senescent cells thus helping to re-model and rejuvenate the endometrium (Brighton, *Elife* 6: e31274, 2017). With a similar mechanism, during liver fibrosis, p53-expressing senescent liver satellite cells skewed the polarization of resident Kupfer macrophages and freshly infiltrated macrophages toward the pro-inflammatory M1 phenotype, which display senolytic activity. F4/80+ macrophages have been shown to play a key role in the clearance of mouse uterine senescent cells to maintain postpartum uterine function.

Senescent cells recruit NK cells by mainly upregulating ligands to NKG2D (expressed on NK cells), chemokines, and other SASP factors. In vivo models of liver fibrosis have shown effective clearance of senescent cells by activated NK cells (Krizhanovsky, *Cell* 134(4): 657-667, 2008). Studies have described various models to study senescence including liver fibrosis (Krizhanovsky, *Cell* 134(4): 657-667, 2008), osteoarthritis (Xu, *J Gerontol A Biol Sci Med Sci* 72(6): 780-785, 2017), and Parkinson's disease (Chinta, *Cell Rep* 22(4): 930-940, 2018). Animal models for studying senescent cells are described in: Krizhanovsky, *Cell* 134(4): 657-667, 2008; Baker, *Nature* 479(7372): 232-236, 2011; Farr, *Nat Med* 23(9): 1072-1079, 2017; Bourgeois, *FEBS Lett* 592(12): 2083-2097, 2018; Xu, *Nat Med* 24(8): 1246-1256, 2018).

Methods of Treating a Liver Disease or a Metabolic Syndrome in a Subject

Also provided herein are methods of treating a liver disease or a metabolic syndrome in a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds

specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII (e.g., any of the exemplary multi-chain chimeric polypeptides described herein). In some embodiments, the subject is diagnosed or identified as having the liver disease or the metabolic syndrome.

5 In some embodiments, the multi-chain chimeric polypeptide is administered via intramuscular administration, subcutaneous administration, intravenous administration, intrahepatic administration, or intraperitoneal administration.

 In some embodiments, the liver disease is selected from the group of: fatty liver disease, hepatic steatosis, acute hepatic porphyria, Alagille syndrome, alcohol-related
10 liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer,
15 newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease. In some embodiments, the metabolic syndrome is selected from the group of: coronary heart disease, pulmonary disease, gall bladder disease,
20 dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial hypertension, stroke, and cataracts. Methods for accessing successful treatment of the liver diseases and metabolic syndromes are known in the art.

 In some embodiments, the subject has not been previously identified or diagnosed
25 as having type 2 diabetes mellitus. In some embodiments, the subject has not been previously identified or diagnosed as having adipose atrophy. In some embodiments, the subject has not been previously identified or diagnosed as having lipodystrophy. In some embodiments, the subject has not been previously identified or diagnosed as having liver cirrhosis. In some embodiments, the subject has not been previously identified or
30 diagnosed as having NAFLD. In some embodiments, the subject has not been previously identified or diagnosed as having non-alcoholic steatohepatitis.

Methods of Reducing One or More of the Rate of Progression from NAFL to NASH, Rate of Progression from NASH to Cirrhosis, and Rate of Progression from Cirrhosis to Hepatocellular Carcinoma

5 Also provided are methods of reducing one or more of the rate of: progression from non-alcoholic fatty liver disease (NAFL) to non-alcoholic steatohepatitis (NASH), progression from NASH to cirrhosis, and progression from cirrhosis to hepatocellular carcinoma, that include administering to a subject identified or diagnosed as having NAFL, NASH, or cirrhosis, a therapeutically effective amount of a multi-chain chimeric
10 polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding
15 of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII (e.g., any of the exemplary multi-chain chimeric polypeptides described herein).

 In some embodiments, the multi-chain chimeric polypeptide is administered via
20 intramuscular administration, subcutaneous administration, intravenous administration, intrahepatic administration, or intraperitoneal administration.

 In some embodiments, the method results in a decrease (e.g., about a 1% decrease to about a 100% decrease, about a 1% decrease to about a 95% decrease, about a 1% decrease to about a 90% decrease, about a 1% decrease to about a 85% decrease,
25 about a 1% decrease to about a 80% decrease, about a 1% decrease to about a 75% decrease, about a 1% decrease to about a 70% decrease, about a 1% decrease to about a 65% decrease, about a 1% decrease to about a 60% decrease, about a 1% decrease to about a 55% decrease, about a 1% decrease to about a 50% decrease, about a 1% decrease to about a 45% decrease, about a 1% decrease to about a 40% decrease, about a
30 1% decrease to about a 35% decrease, about a 1% decrease to about a 30% decrease, about a 1% decrease to about a 25% decrease, about a 1% decrease to about a 20% decrease, about a 1% decrease to about a 15% decrease, about a 1% decrease to about a

[illegible]

[illegible]

[illegible]

decrease to about a 100% decrease, about a 70% decrease to about a 95% decrease, about a 70% decrease to about a 90% decrease, about a 70% decrease to about a 85% decrease, about a 70% decrease to about a 80% decrease, about a 70% decrease to about a 75% decrease, about a 75% decrease to about a 100% decrease, about a 75% decrease to about a 95% decrease, about a 75% decrease to about a 90% decrease, about a 75% decrease to about a 85% decrease, about a 75% decrease to about a 80% decrease, about a 80% decrease to about a 100% decrease, about a 80% decrease to about a 95% decrease, about a 80% decrease to about a 90% decrease, about a 80% decrease to about a 85% decrease, about a 85% decrease to about a 100% decrease, about a 85% decrease to about a 95% decrease, about a 85% decrease to about a 90% decrease, about a 90% decrease to about a 100% decrease, about a 90% decrease to about a 95% decrease, about a 95% decrease to about a 100% decrease, or about a 95% to about a 99% decrease) in the rate of progression from NAFL to NASH, e.g., as compared to the rate of progression before treatment or the rate of progression in a similar subject identified as having NAFL and receiving no treatment or a different treatment.

In some embodiments, the method results in a decrease (e.g., about a 1% decrease to about a 100% decrease, or any of the subranges of this range described herein) in the rate of progression from NASH to cirrhosis, e.g., as compared to the rate of progression before treatment or the rate of progression in a similar subject identified as having NASH and receiving no treatment or a different treatment.

In some embodiments, the method results in a decrease (e.g., about a 1% decrease to about a 100% decrease, or any of the subranges of this range described herein) in the rate of progression from cirrhosis to hepatocellular carcinoma, e.g., as compared to the rate of progression before treatment or the rate of progression in a similar subject identified as having cirrhosis and receiving no treatment or a different treatment.

Methods of Reducing Inflammation in a Liver of a Subject

Also provided herein are methods of reducing inflammation in a liver of a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain;

(ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain
5 and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII (e.g., any of the exemplary multi-chain chimeric polypeptides described herein). In some embodiments, the subject is identified as being in need of a reduction in inflammation in their liver. Methods for
10 determining a level of inflammation in the liver of a subject are known in the art and include, e.g., detecting the level of expression of one or more inflammatory cytokines in the liver of the subject.

In some embodiments, the multi-chain chimeric polypeptide is administered via intramuscular administration, subcutaneous administration, intravenous administration,
15 intrahepatic administration, or intraperitoneal administration.

In some embodiments, the method results in a decrease (e.g., about a 1% decrease to about a 100% decrease, or any of the subranges of this range described herein) in the level of inflammation in the liver of a subject (e.g., any of the subjects described herein), e.g., as compared to the level of inflammation in the liver of the subject prior to the
20 administering.

In some embodiments, the subject has been previously identified or diagnosed as having a liver disease (e.g., any of the exemplary liver diseases described herein or known in the art) or a metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the subject has
25 been previously identified or diagnosed as having a liver disease (e.g., any of the exemplary liver diseases described herein or known in the art). In some embodiments, the liver disease is selected from the group of: fatty liver disease, hepatic steatosis, acute hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary
30 atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis

C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease. In some embodiments, the subject has been previously identified or diagnosed as having a metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the metabolic syndrome is selected from the group of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial hypertension, stroke, and cataracts.

In some embodiments, the subject has not been previously identified or diagnosed as having type 2 diabetes mellitus. In some embodiments, the subject has not been previously identified or diagnosed as having adipose atrophy. In some embodiments, the subject has not been previously identified or diagnosed as having lipodystrophy. In some embodiments, the subject has not been previously identified or diagnosed as having liver cirrhosis. In some embodiments, the subject has not been previously identified or diagnosed as having NAFLD. In some embodiments, the subject has not been previously identified or diagnosed as having non-alcoholic steatohepatitis.

Methods of Decreasing Gluconeogenesis in a Liver of a Subject

Also provided herein are methods of decreasing gluconeogenesis in a liver of a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain

binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII (e.g., any of the exemplary multi-chain chimeric polypeptides described herein). In some embodiments, the subject is identified as being in need of a decrease in gluconeogenesis in their liver. Methods of
5 detecting the level of gluconeogenesis in a liver of a subject are known in the art.

In some embodiments, the multi-chain chimeric polypeptide is administered via intramuscular administration, subcutaneous administration, intravenous administration, intrahepatic administration, or intraperitoneal administration.

In some embodiments, the method results in a decrease (e.g., about a 1% decrease
10 to about a 100% decrease, or any of the subranges of this range described herein) in the level of gluconeogenesis in the liver of a subject (e.g., any of the subjects described herein), e.g., as compared to the level of gluconeogenesis in the liver of the subject prior to the administering.

In some embodiments, the subject has been previously identified or diagnosed as
15 having a liver disease (e.g., any of the exemplary liver diseases described herein or known in the art) or a metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the subject has been previously identified or diagnosed as having a liver disease (e.g., any of the exemplary liver diseases described herein or known in the art). In some embodiments,
20 the liver disease is selected from the group of: fatty liver disease, hepatic steatosis, acute hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis
25 C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease. In
30 some embodiments, the subject has been previously identified or diagnosed as having a metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or

known in the art). In some embodiments, the metabolic syndrome is selected from the group of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial
5 hypertension, stroke, and cataracts.

In some embodiments, the subject has not been previously identified or diagnosed as having type 2 diabetes mellitus. In some embodiments, the subject has not been previously identified or diagnosed as having adipose atrophy. In some embodiments, the subject has not been previously identified or diagnosed as having lipodystrophy. In some
10 embodiments, the subject has not been previously identified or diagnosed as having liver cirrhosis. In some embodiments, the subject has not been previously identified or diagnosed as having NAFLD. In some embodiments, the subject has not been previously identified or diagnosed as having non-alcoholic steatohepatitis.

15 **Methods of Decreasing Lipogenesis in a Liver of a Subject**

Also provided herein are methods of decreasing lipogenesis in a liver of a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a
20 pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, wherein: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII
25 (e.g., any of the exemplary multi-chain chimeric polypeptides described herein). In some embodiments, the subject is identified as being in need of a decrease in lipogenesis in their liver. Methods for detecting the level of lipogenesis in a liver of a subject are known in the art.

In some embodiments, the multi-chain chimeric polypeptide is administered via intramuscular administration, subcutaneous administration, intravenous administration, intrahepatic administration, or intraperitoneal administration.

5 In some embodiments, the method results in a decrease (e.g., about a 1% decrease to about a 100% decrease, or any of the subranges of this range described herein) in the level of lipogenesis in the liver of a subject (e.g., any of the subjects described herein), e.g., as compared to the level of lipogenesis in the liver of the subject prior to the administering.

10 In some embodiments, the subject has been previously identified or diagnosed as having a liver disease (e.g., any of the exemplary liver diseases described herein or known in the art) or a metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the subject has been previously identified or diagnosed as having a liver disease (e.g., any of the exemplary liver diseases described herein or known in the art). In some embodiments, 15 the liver disease is selected from the group of: fatty liver disease, hepatic steatosis, acute hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis 20 C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease. In some embodiments, the subject has been previously identified or diagnosed as having a 25 metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the metabolic syndrome is selected from the group of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial 30 hypertension, stroke, and cataracts.

In some embodiments, the subject has not been previously identified or diagnosed as having type 2 diabetes mellitus. In some embodiments, the subject has not been previously identified or diagnosed as having adipose atrophy. In some embodiments, the subject has not been previously identified or diagnosed as having lipodystrophy. In some
5 embodiments, the subject has not been previously identified or diagnosed as having liver cirrhosis. In some embodiments, the subject has not been previously identified or diagnosed as having NAFLD. In some embodiments, the subject has not been previously identified or diagnosed as having non-alcoholic steatohepatitis.

10 **Methods of Decreasing Hepatocytic Senescence in a Liver of a Subject**

Also provided herein are methods of decreasing hepatocytic senescence in a liver of a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a
15 first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a
20 ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII (e.g., any of the exemplary multi-chain chimeric polypeptides described herein). In some embodiments, the subject is identified as being in need of decreased hepatocytic senescence in their liver. Methods for determining a level of hepatocytic senescence in a liver of a subject are known in the art.

25 In some embodiments, the multi-chain chimeric polypeptide is administered via intramuscular administration, subcutaneous administration, intravenous administration, intrahepatic administration, or intraperitoneal administration.

In some embodiments, the method results in a decrease (e.g., about a 1% decrease to about a 100% decrease, or any of the subranges of this range described herein) in the
30 level of hepatocytic senescence in the liver of a subject (e.g., any of the subjects

described herein), e.g., as compared to the level of hepatocytic senescence in the liver of the subject prior to the administering.

In some embodiments, the subject has been previously identified or diagnosed as having a liver disease (e.g., any of the exemplary liver diseases described herein or
5 known in the art) or a metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the subject has been previously identified or diagnosed as having a liver disease (e.g., any of the exemplary liver diseases described herein or known in the art). In some embodiments, the liver disease is selected from the group of: fatty liver disease, hepatic steatosis, acute
10 hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid
15 lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease. In some embodiments, the subject has been previously identified or diagnosed as having a
20 metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the metabolic syndrome is selected from the group of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial
25 hypertension, stroke, and cataracts.

In some embodiments, the subject has not been previously identified or diagnosed as having type 2 diabetes mellitus. In some embodiments, the subject has not been previously identified or diagnosed as having adipose atrophy. In some embodiments, the subject has not been previously identified or diagnosed as having lipodystrophy. In some
30 embodiments, the subject has not been previously identified or diagnosed as having liver cirrhosis. In some embodiments, the subject has not been previously identified or

diagnosed as having NAFLD. In some embodiments, the subject has not been previously identified or diagnosed as having non-alcoholic steatohepatitis.

Methods of Rebalancing Metabolic Function in a Liver of a Subject

5 Also provided herein are methods of rebalancing metabolic function in a liver of a subject that include administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide
10 comprising: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds
15 specifically to a ligand of TGF- β RII (e.g., any of the exemplary multi-chain chimeric polypeptides described herein). In some embodiments, the subject is identified as being in need of rebalancing of metabolic function in their liver. Methods for determining the rebalancing of metabolic function in a liver in the subject are known in the art. Non-limiting embodiments of rebalancing of metabolic function include normalizing blood
20 glucose levels (e.g., hemoglobin A1c levels or fasting glucose levels in a subject), reducing insulin resistance, and normalizing gene expression of Retn (Resistin).

 In some embodiments, the multi-chain chimeric polypeptide is administered via intramuscular administration, subcutaneous administration, intravenous administration, intrahepatic administration, or intraperitoneal administration.

25 In some embodiments, the subject has been previously identified or diagnosed as having a liver disease (e.g., any of the exemplary liver diseases described herein or known in the art) or a metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the subject has been previously identified or diagnosed as having a liver disease (e.g., any of the
30 exemplary liver diseases described herein or known in the art). In some embodiments, the liver disease is selected from the group of: fatty liver disease, hepatic steatosis, acute

hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease. In some embodiments, the subject has been previously identified or diagnosed as having a metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the metabolic syndrome is selected from the group of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial hypertension, stroke, and cataracts.

In some embodiments, the subject has not been previously identified or diagnosed as having type 2 diabetes mellitus. In some embodiments, the subject has not been previously identified or diagnosed as having adipose atrophy. In some embodiments, the subject has not been previously identified or diagnosed as having lipodystrophy. In some embodiments, the subject has not been previously identified or diagnosed as having liver cirrhosis. In some embodiments, the subject has not been previously identified or diagnosed as having NAFLD. In some embodiments, the subject has not been previously identified or diagnosed as having non-alcoholic steatohepatitis.

25

Methods of Modulating Expression of One or More Genes in Tables 1-4 in a Liver of a Subject

Also provided herein are methods of modulating expression of one or more (e.g., two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, fifteen or more, twenty or more, or thirty or more) genes in Tables 1-4 in a liver of a subject that include administering to the subject a

30

therapeutically effective amount of a multi-chain chimeric polypeptide comprising: (a) a first chimeric polypeptide comprising: (i) a first target-binding domain; (ii) soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide comprising: (i) a second domain of a pair of affinity domains; and
5 (ii) a second target-binding domain, where: the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and
the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII
10 (e.g., any of the exemplary multi-chain chimeric polypeptides described herein). In some embodiments, the subject is identified as being in need of modulation of expression of one or more genes listed in Tables 1-4 in their liver.

In some embodiments, the multi-chain chimeric polypeptide is administered via intramuscular administration, subcutaneous administration, intravenous administration,
15 intrahepatic administration, or intraperitoneal administration.

In some embodiments, the administering results in a decrease (e.g., about a 1% decrease to about a 100% decrease, or any of the subranges of this range described herein) in the expression of one or more (e.g., two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, fifteen
20 or more, or twenty or more) genes in the liver of the subject selected from the group of: ACSS1, RETN, SLC2A4, PDK4, PNPLA3, GADD45B, PPARGC1A, CAV1, ENDOD1, REG3G, IGHG3, IGHG2B, SCGB3A1, GLYCAM1, IGHG2C, IGKC, LTF, MS4A1, JCHAIN, CD19, IGHM, IFI27L2A, ACKR3, LSP1, PMEPA1, CORO1A, GPX3, MYH8, NPPA, TCAP, FLNC, SLC36A2, MYH6, ACTC1, ACTA2, and TPM2, as
25 compared to the level of expression of the one or more genes in the subject prior to the administering.

In some embodiments,, the administering results in an increase (e.g., about a 1% increase to about a 500% increase, about a 1% increase to about a 400% increase, about a 1% increase to about a 300% increase, about a 1% increase to about a 200% increase,
30 about a 1% increase to about a 150% increase, about a 1% increase to about a 100% increase, about a 1% increase to about a 80% increase, about a 1% increase to about a

60% increase, about a 1% increase to about a 40% increase, about a 1% increase to about
a 20% increase, about a 1% increase to about a 10% increase, about a 1% increase to
about a 5% increase, about a 5% increase to about a 500% increase, about a 5% increase
to about a 400% increase, about a 5% increase to about a 300% increase, about a 5%
5 increase to about a 200% increase, about a 5% increase to about a 150% increase, about a
5% increase to about a 100% increase, about a 5% increase to about a 80% increase,
about a 5% increase to about a 60% increase, about a 5% increase to about a 40%
increase, about a 5% increase to about a 20% increase, about a 5% increase to about a
10% increase, about a 10% increase to about a 500% increase, about a 10% increase to
10 about a 400% increase, about a 10% increase to about a 300% increase, about a 10%
increase to about a 200% increase, about a 10% increase to about a 150% increase, about
a 10% increase to about a 100% increase, about a 10% increase to about a 80% increase,
about a 10% increase to about a 60% increase, about a 10% increase to about a 40%
increase, about a 10% increase to about a 20% increase, about a 20% increase to about a
15 500% increase, about a 20% increase to about a 400% increase, about a 20% increase to
about a 300% increase, about a 20% increase to about a 200% increase, about a 20%
increase to about a 150% increase, about a 20% increase to about a 100% increase, about
a 20% increase to about a 80% increase, about a 20% increase to about a 60% increase,
about a 20% increase to about a 40% increase, about a 40% increase to about a 500%
20 increase, about a 40% increase to about a 400% increase, about a 40% increase to about a
300% increase, about a 40% increase to about a 200% increase, about a 40% increase to
about a 150% increase, about a 40% increase to about a 100% increase, about a 40%
increase to about a 80% increase, about a 40% increase to about a 60% increase, about a
60% increase to about a 500% increase, about a 60% increase to about a 400% increase,
25 about a 60% increase to about a 300% increase, about a 60% increase to about a 200%
increase, about a 60% increase to about a 150% increase, about a 60% increase to about a
100% increase, about a 60% increase to about a 80% increase, about a 80% increase to
about a 500% increase, about a 80% increase to about a 400% increase, about a 80%
increase to about a 300% increase, about a 80% increase to about a 200% increase, about
30 a 80% increase to about a 150% increase, about a 80% increase to about a 100% increase,
about a 100% increase to about a 500% increase, about a 100% increase to about a 400%

increase, about a 100% increase to about a 300% increase, about a 100% increase to about a 200% increase, about a 100% increase to about a 150% increase, about a 150% increase to about a 500% increase, about a 150% increase to about a 400% increase, about a 150% increase to about a 300% increase, about a 150% increase to about a 200% increase, about a 200% increase to about a 500% increase, about a 200% increase to about a 400% increase, about a 200% increase to about a 300% increase, about a 300% increase to about a 500% increase, about a 300% increase to about a 400% increase, or about a 400% increase to about a 500% increase, in the expression of one or more genes in the liver of the subject selected from the group consisting of: SLC34A2, and CISH, as compared to the level of expression of the one or more genes in the subject prior to the administering.

In some embodiments, the administering results in a decrease (e.g., about a 1% decrease to about a 100% decrease, or any of the subranges of this range described herein) in the expression of one or more (e.g., two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, fifteen or more, twenty or more, or thirty or more) genes in the liver of the subject selected from the group consisting of: CSF3R, IFI27L2A, GM17066, GNL3, FABP1, GM14303, AURKA, RPL14-PS1, QTRT2, G6PC, C8B, DYNLL1, LCN2, LRG1, CEBPD, COL4A3, ST3GAL5, RSAD2, 9330162G02RIK, PINX1, SRA1, SPATA2L, PNRC1, MUP20, IL6RA, APOA1, IL1B, WDR54, CTCFLOS, GM16973, 4632427E13RIK, IGHG2B, TGFB1I1, SELENBP2, SEMA6B, NEXN, ZFP653, NOB1, PCK1, FAM25C, MAPK15, GM16551, ESM1, RPL37RT, FAM133B, PDE8B, TUT1, S100A11, PDILT, PPARD, IER2, GM15401, MX2, WNK4, G0S2, BC005561, AA986860, JDP2, GM26982, NOP58, ACTB, GM14586, RPP38, GM13436, NT5DC2, IMPDH1, CYTIP, AI846148, CHKA, GM37963, NR0B2, CYP4A32, ALKBH2, FAU-PS2, PPP1R15A, KLF2, SLC25A22, GM13341, IGHM, SATB1, SNRPF, DNASE1L2, CD3EAP, GM2788, DANCER, ZFP612, NOP56, JUND, ID1, HSPB1, KLHDC8A, KLF10, ANGPT2, THBS1, GM44891, GM9752, ABLIM3, PTGES, GM28438, 2410002F23RIK, FOSL2, CRIP3, JUN, ALAS1, GM2000, RHOC, LMCD1, GM2061, GM42595, GM11478, IKZF2, PNLDC1, COMTD1, SNORA31, COL20A1, AKAP12, C1QTNF12, 1810032O08RIK, 2310033P09RIK, GM47528, SERPINE2, NPFF,

SERPINA3K, RFXANK, IGKV5-39, NAB2, MAFF, CEP85, CSAD, LTB4R1, 1810012K08RIK, BCL7C, NRBP2, NLE1, ALKBH1, ARID5A, CFAP43, GM45767, CD8A, PPRC1, GM26870, TMC7, BCL6B, GM16348, GM26981, SLC16A3, TNFRSF12A, CYP2J9, NR4A2, MMP9, MIR17HG, TMEM191C, PCDH11X, HILPDA, 5 RAPGEF4, GM17300, SLC25A47, KCNJ2, NYAP1, LAX1, RPS19-PS3, HES1, RGS16, DUSP1, GM43323, ASB4, MUC6, GM15502, UNG, FOXQ1, GM17936, UBE2C, SLC16A6, MIR7052, NLRP12, GM14286, FGF21, KLF5, GM37969, PF4, GM21738, HOTAIRM1, GM6493, LOR, MFSD2B, MATK, SYNE4, GM44694, TRBC1, GM37274, PLN, CXCR4, PHF24, SNORD104, SERPINA7, RGS4, TCIM, 10 EGFR, GM37760, FBXL22, TEDC2, ENHO, GM26917, GM43775, 4833411C07RIK, GM45053, INHBB, OPN3, SNHG15, B230206H07RIK, KCNE3, GM43305, C530043K16RIK, KLF4, LEPR, JCHAIN, TSKU, LGALS4, PCP4L1, GM44829, DUSP8, GM44620, IGFBP1, JUNB, GM32017, GM2814, GM37144, MYADML2OS, GM37666, HDC, SLFN4, A530041M06RIK, GM43359, GM2602, GM10277, 15 FAM222A, FOXA3, AOC2, SERPINA1E, CTXN1, RAPGEF4OS2, SOCS2, PPAN, PRKAG2OS1, GADD45B, HOXA5, GRHL1, EIF4EBP3, OSGIN1, GM28513, MAP3K6, SLC34A2, B630019A10RIK, IGKC, PLIN4, ANGPTL4, DUSP5, EGR1, GM42507, GM14257, APOLD1, IER3, ZBTB16, GM37033, IGLC1, GADD45G, IGLC3, GM45244, RGS1, CXCL1, RNF225, GM44005, ANKRD37, NR4A1, GM8893, 20 GM26762, CDKN1A, 5330406M23RIK, IGLV1, IGKV3-2, FOS, GM43637, IGKV3-10, S100A9, GM15622, S100A8, MT1, RETNLG, MT2, IGKV19-93, GM45774, and SERPINA4-PS1, as compared to the level of expression of the one or more genes in the subject prior to the administering.

In some embodiments, the administering results in an increase in the expression 25 of one or more genes in the liver of the subject selected from the group consisting of: DBP, IGKV4-55, PER3, MUP-PS10, GPAM, TMPRSS4, MUP-PS14, AC166078.1, MUP-PS12, GM2065, A530020G20RIK, ACSS2OS, DCLK3, KLF12, GM44669, MFSD9, B4GALNT3, GM3776, TMEM167-PS1, KRT23, LMBRD2, GM22935, SULT2A-PS1, SNAI3, GM15908, MIR6392, ACSS2, NR1D1, BC049987, CCDC85C, 30 CES2C, ACPP, MUP2, PTK6, UGT1A5, 1810008I18RIK, IL22RA1, ACSS3, ADNP, RDH16, SNTB1, 4933411K16RIK, NTRK2, EXTL1, PSTPIP2, RASSF6, AQP4,

UGT1A9, PROM1, ZFP608, FAM13A, NFE2, TEF, TNFAIP8L3, SCD1, MMD2,
 SYNE3, ACLY, C330021F23RIK, STON2, LRFN4, HHIPL1, WNT9B, NR1D2,
 1810049J17RIK, PDPR, NA, GM45884, SLC2A5, FAM83F, ZFP526, SGK2,
 GM43080, DEAF1, ME1, BMF, WDFY2, ADCY9, CLSTN3, ACOT11, LYST,
 5 LRTM1, OAT, VPS13C, E330011O21RIK, P2RY4, GM11437, RWDD2A, SVIL,
 ECHDC1, TRIM14, SLC10A5, TRHDE, MASP1, 2900097C17RIK, NDST1, RDH9,
 1110002L01RIK, ABTB2, RGR, ACACB, SACM1L, DYRK2, ROBO1, GM44744,
 EIF4EBP2, KLHL24, CYP2A5, TIAM2, RAB43, GM13855, 9130409I23RIK, STON1,
 USP9X, UGT3A1, 9030616G12RIK, DOCK8, KLB, ACE, VLDLR, PCDHGC3,
 10 ABCA6, 4932422M17RIK, GM45838, FARP2, GM47205, SP4, UGT1A6B, KLHL28,
 D130043K22RIK, ASIC5, PM20D2, A1CF, SORBS1, SLC10A2, GM10642, UTP14B,
 GM38394, AFP, INSIG1, HNF1AOS2, METTL4, LSS, MTMR9, HMGCR, GDAP10,
 ADRA1A, ZFP773, CRKL, CHRNE, STARD13, CRY2, FADS2, COG5, FV1, RCAN2,
 ABCB1A, PPARA, ATP7A, MVD, 2610037D02RIK, TNFRSF14, SUCNR1, ECI3,
 15 ABCC4, LNCBATE1, MINDY2, BTBD7, 4933404O12RIK, ABCD1, FMN1, FNIP2,
 ABHD15, NKX2-6, C77080, GM43611, SGTB, ACSL3, NR5A2, FAM198A, KCTD7,
 ACACA, ZFP955B, SULT2A3, FZD4, FASN, CYP3A59, ZFP354B, TNFSF10, SESN3,
 MN1, RNF152, DHCR24, SPHK2, SYTL5, GM6652, BAHCC1, GAREM1, MFSD4A,
 HGF, GM3571, NOS1AP, DIXDC1, KANK1, REPS2, ASAH2, SEMA3B, RNF103,
 20 ZC3H12C, CDS2, DCUN1D4, 2900026A02RIK, CYR1, EEPD1, P2RY2, CYP2C39,
 SEC22C, EHHADH, ABCA3, HIPK2, RBM20, GRAMD4, FCHSD2, MOB3A,
 HMGN3, KLHDC7A, VCP-RS, TERT, CYP3A41B, ARL13B, ZC3H12D, TLCD2,
 SNHG11, SORL1, GPR157, DNAJA4, TMEM253, TACO1, SPATA5L1, RHBG,
 COL15A1, PCDH12, IRS1, ASCC3, KIF16B, and MR1, as compared to the level of
 25 expression of the one or more genes in the subject prior to the administering.

In some embodiments, the subject has been previously identified or diagnosed as
 having a liver disease (e.g., any of the exemplary liver diseases described herein or
 known in the art) or a metabolic syndrome (e.g., any of the exemplary metabolic
 syndromes described herein or known in the art). In some embodiments, the subject has
 30 been previously identified or diagnosed as having a liver disease (e.g., any of the
 exemplary liver diseases described herein or known in the art). In some embodiments,

the liver disease is selected from the group of: fatty liver disease, hepatic steatosis, acute hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease. In some embodiments, the subject has been previously identified or diagnosed as having a metabolic syndrome (e.g., any of the exemplary metabolic syndromes described herein or known in the art). In some embodiments, the metabolic syndrome is selected from the group of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial hypertension, stroke, and cataracts.

In some embodiments, the subject has not been previously identified or diagnosed as having type 2 diabetes mellitus. In some embodiments, the subject has not been previously identified or diagnosed as having adipose atrophy. In some embodiments, the subject has not been previously identified or diagnosed as having lipodystrophy. In some embodiments, the subject has not been previously identified or diagnosed as having liver cirrhosis. In some embodiments, the subject has not been previously identified or diagnosed as having NAFLD. In some embodiments, the subject has not been previously identified or diagnosed as having non-alcoholic steatohepatitis.

Additional Therapeutic Agents

Some embodiments of any of the methods described herein can further include administering to a subject (e.g., any of the subjects described herein) a therapeutically effective amount of one or more additional therapeutic agents. The one or more additional therapeutic agents can be administered to the subject at substantially the same

time as the multi-chain chimeric polypeptide (e.g., any of the multi-chain chimeric polypeptides described herein). In some embodiments, one or more additional therapeutic agents can be administered to the subject prior to administration of the multi-chain chimeric polypeptide (e.g., any of the multi-chain chimeric polypeptides described herein). In some embodiments, one or more additional therapeutic agents can be administered to the subject after administration of the multi-chain chimeric polypeptide (e.g., any of the multi-chain chimeric polypeptides described herein) to the subject.

Non-limiting examples of additional therapeutic agents include: anti-inflammatory agents, anti-cancer drugs, activating receptor agonists, immune checkpoint inhibitors, agents for blocking HLA-specific inhibitory receptors, Glucogen Synthase Kinase (GSK) 3 inhibitors, antibodies, and ex-vivo activated immune cells.

Non-limiting examples of anticancer drugs include antimetabolic drugs (e.g., 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), capecitabine, cytarabine, floxuridine, fludarabine, gemcitabine, hydroxycarbamide, methotrexate, 6-thioguanine, cladribine, nelarabine, pentostatin, or pemetrexed), plant alkaloids (e.g., vinblastine, vincristine, vindesine, camptothecin, 9-methoxycamptothecin, coronaridine, taxol, nucleosides, diprenylated indole alkaloid, montamine, schischkiniin, protoberberine, berberine, sanguinarine, chelerythrine, chelidonine, liriodenine, clivorine, β -carboline, antofine, tylophorine, cryptolepine, neocryptolepine, corynoline, sampangine, carbazole, crinamine, montanine, ellipticine, paclitaxel, docetaxel, etoposide, teniposide, irinotecan, topotecan, or acridone alkaloids), proteasome inhibitors (e.g., lactacystin, disulfiram, epigallocatechin-3-gallate, marizomib (salinosporamide A), oprozomib (ONX-0912), delanzomib (CEP-18770), epoxomicin, MG132, beta-hydroxy beta-methylbutyrate, bortezomib, carfilzomib, or ixazomib), antitumor antibiotics (e.g., doxorubicin, daunorubicin, epirubicin, mitoxantrone, idarubicin, actinomycin, plicamycin, mitomycin, or bleomycin), histone deacetylase inhibitors (e.g., vorinostat, panobinostat, belinostat, givinostat, abexinostat, depsipeptide, entinostat, phenyl butyrate, valproic acid, trichostatin A, dacinostat, mocetinostat, pracinostat, nicotinamide, cambinol, tenovin 1, tenovin 6, sirtinol, ricolinostat, tefinostat, kevetrin, quisinostat, resminostat, tacedinaline, chidamide, or selisistat), tyrosine kinase inhibitors (e.g., axitinib, dasatinib, encorafenib, erlotinib, imatinib, nilotinib, pazopanib, and sunitinib), and chemotherapeutic agents

(e.g., all-trans retinoic acid, azacitidine, azathioprine, doxifluridine, epothilone, hydroxyurea, imatinib, teniposide, tioguanine, valrubicin, vemurafenib, and lenalidomide). Additional examples of chemotherapeutic agents include alkylating agents, e.g., mechlorethamine, cyclophosphamide, chlorambucil, melphalan, ifosfamide, 5 thiotepa, hexamethylmelamine, busulfan, altretamine, procarbazine, dacarbazine, temozolomide, carmustine, lumustine, streptozocin, carboplatin, cisplatin, and oxaliplatin.

Non-limiting examples of activating receptor agonists include any agonists for activating receptors which activate and enhance the cytotoxicity of NK cells, including 10 anti-CD16 antibodies (e.g., anti-CD16/CD30 bispecific monoclonal antibody (BiMAb)) and Fc-based fusion proteins. Non-limiting examples of checkpoint inhibitors include anti-PD-1 antibodies (e.g., MEDI0680), anti-PD-L1 antibodies (e.g., BCD-135, BGB-A333, CBT-502, CK-301, CS1001, FAZ053, KN035, MDX-1105, MSB2311, SHR-1316, anti-PD-L1/CTLA-4 bispecific antibody KN046, anti-PD-L1/TGFβRII fusion 15 protein M7824, anti-PD-L1/TIM-3 bispecific antibody LY3415244, atezolizumab, or avelumab), anti-TIM3 antibodies (e.g., TSR-022, Sym023, or MBG453) and anti-CTLA-4 antibodies (e.g., AGEN1884, MK-1308, or an anti-CTLA-4/OX40 bispecific antibody ATOR-1015). Non-limiting examples of agents for blocking HLA-specific inhibitory receptors include monalizumab (e.g., an anti-HLA-E NKG2A inhibitory receptor 20 monoclonal antibody). Non-limiting examples of GSK3 inhibitor include tideglusib or CHIR99021. Non-limiting examples of antibodies that can be used as additional therapeutic agents include anti-CD26 antibodies (e.g., YS110), anti-CD36 antibodies, and any other antibody or antibody construct that can bind to and activate an Fc receptor (e.g., CD16) on a NK cell. In some embodiments, an additional therapeutic agent can be 25 insulin or metformin.

Non-limiting examples of in-vitro activated immune cells include regulatory T cells, CAR-regulatory T cells, NK cells, CAR-NK cells, cytotoxic T cells, and CAR-cytotoxic T cells.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

5 **Example 1. Construction of exemplary multi-chain chimeric polypeptides and evaluation of properties thereof**

Two multi-chain chimeric polypeptides were generated and their properties were evaluated. Each of the two multi-chain chimeric polypeptides includes a first chimeric polypeptide that includes a soluble tissue factor domain covalently linked a first target-
10 binding domain and a first domain of an affinity pair of domains. The second chimeric polypeptide in each of the two multi-chain chimeric polypeptides includes a second domain of the affinity pair of domains, and a second target-binding domain.

Description of logic underlying construction of multi-chain chimeric polypeptides

15 Tissue Factor (TF) is a stable, transmembrane protein containing 236 amino acid residues. The truncated, recombinant 219-amino-acid extracellular domain of tissue factor is soluble and is known to be expressed at high levels in bacteria or mammalian cells. Without wishing to be bound to a particular theory, the applicants speculated that the 219-aa tissue factor could be used as a connector linker for creation of unique multi-
20 chain chimeric polypeptides.

First chimeric polypeptides including soluble tissue factor domain were produced at high levels by CHO cells grown in fermentation broth. These first chimeric polypeptides were purified by an anti-tissue factor monoclonal antibody (mAb) coupled on a solid matrix. Notably, tissue factor contains binding sites for FVIIa and FX. The
25 catalytic activity of the tissue factor-FVIIa complex for FX is approximately 1 million-fold lower when tissue factor is not anchored to a phospholipid bilayer. Thus, without wishing to be bound to a particular theory, applicants speculated that using the 219-aa extracellular domain of tissue factor without the transmembrane in construction of the first chimeric polypeptides may eliminate the pro-coagulation activity of tissue factor in
30 the first chimeric polypeptides. In an effort to further reduce or eliminate the pro-coagulation activity of the 219-aa tissue factor, select mutations in tissue factor can be

made, specifically at seven amino acid residues that are known to contribute to binding energy of the FVIIa binding site.

Characterization of binding interactions for described chimeric polypeptides

5 To determine if the first and second chimeric polypeptides bind to each other to form multi-chain chimeric polypeptides, *in vitro* binding assays were performed. To determine if the first chimeric polypeptide comprising soluble tissue factor domain are recognized and bound by anti-TF mAb, *in vitro* binding assays were performed. Notably, the data indicated that the mutated tissue factor proteins are still recognized and
10 selectively bound by the anti-TF mAb which is known to bind to the FX binding site on tissue factor. To determine if the first chimeric polypeptides comprising soluble tissue factor domain covalently linked to scFvs or cytokines (see Figure 1 and Figure 2) possess functional scFvs or cytokines, *in vitro* binding assays were performed. The data from the aforementioned assays were consistent with the purified first chimeric polypeptides
15 having the expected biological activities (e.g. scFvs selectively bind expected target antigens or cytokines selectively bind expected receptors or binding proteins).

 In addition, experiments performed using the two multi-chain chimeric polypeptides including a first and second chimeric polypeptide bound to each other demonstrate the expected target binding activity (e.g., the multi-chain chimeric
20 polypeptide binds specifically to the target specifically recognized by the first target-binding domain and the target specifically recognized by the second target-binding domain).

 Based on the aforementioned results, applicants concluded that the soluble tissue factor connector linker provided or enabled appropriate display of the polypeptides
25 encoding either scFvs, interleukins, cytokines, interleukin receptors, or cytokine receptors in three-dimensional space relative to soluble tissue factor domain and relative to one another such that each retained expected biological properties and activities.

 When both the first and second chimeric polypeptides were co-expressed, the heterodimeric complexes were secreted into the fermentation broths at high levels. The
30 complexes were captured and readily purified by anti-TF mAb conjugated to a solid matrix using affinity chromatography. The first and second target-binding domains of

these multi-chain chimeric polypeptides retained their expected biological activities as assayed by *in vitro* binding assays. Thus, the assembly of the multi-chain chimeric polypeptides provides the appropriate spatial display and folding of the domains for biological activities. Importantly, the spatial arrangement of the multi-chain chimeric polypeptides does not interfere with the FX binding site on tissue factor which enables the use of anti-TF mAb for affinity purification.

Characterization of stability for described chimeric polypeptides

Both purified multi-chain chimeric polypeptides are stable. These multi-chain chimeric polypeptides are structurally intact and fully biologically active when they are incubated in human serum at 37 °C for 72 hours.

Characterization of propensity of described chimeric polypeptides to aggregate

Both purified multi-chain chimeric polypeptides developed do not form aggregates when stored at 4 °C in PBS.

Characterization of viscosity of described chimeric polypeptides

There is no viscosity issue when the multi-chain chimeric polypeptides are formulated at a concentration as high as 50 mg/mL in PBS.

Additional applications of the multi-chain chimeric polypeptide platform

The data from these studies show that the platform technologies described herein can be utilized to create molecules that could be fused to target-binding domains derived from antibodies, in any of the formats as described herein including, without limitation, adhesion molecules, receptors, cytokines, ligands, and chemokines. With the appropriate target-binding domain, the resulting multi-chain chimeric polypeptides could promote conjugation of various immune effector cells and mediate destruction of target cells, including cancer cells, virally-infected cells, or senescent cells. Other domains in the multi-chain chimeric polypeptides stimulate, activate, and attract the immune system for enhancing cytotoxicity of effector cells for the targeted cells.

Example 2: TGF β Rt15-TGFRs fusion protein generation and characterization

A fusion protein complex was generated comprising of TGF β Receptor II/IL-15R α Su and TGF β Receptor II/TF/IL-15 fusion proteins (Figure 3 and Figure 4). The human TGF β Receptor II (Ile24-Asp159), tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGF β Receptor II sequences with a G4S(3) linker to generate a single chain version of TGF β Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGF β Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the two TGF β Receptor II/TF/IL-15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCTCCTGTTCTCCAGCGCCT
ACTCC

(Two Human TGF β Receptor II fragments)

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACCTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCTGGAGACCGTGTGTACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC

TGGAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

5 (*Human Tissue Factor 219*)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCATCTCACCGATGAGATCGTGAAAGATGTGAA
10 ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTTCGGCAAAGATTTAATCTACACACT
15 GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

 (*Human IL-15*)

20 AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
25 GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTTCATCAATACCTCC (SEQ ID NO: 59)

The amino acid sequence of TGF β Receptor II/TF/IL-15 fusion protein (including the leader sequence) is as follows:

30 (*Signal peptide*)

MKWVTFISLLFLFSSAYS

(Human TGF β Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
 TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKK
 PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSIPPHVQKSVN
 5 NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAV
 WRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGGETFFMCSCSSDE
 CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
 10 FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
 ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
 KSSSSGKKTAKTNTNEFLIDVDKGENYCFQAVIPSRTVNRKSTDSPVECMGQE
 KGEFRE

(Human IL-15)

15 NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCCKVTAMKCFLELQVIS
 LESGDASIHDVTENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
 MFINTS (SEQ ID NO: 7)

Constructs were also made by attaching two TGF β Receptor II directly to the IL-
 20 15R α Su chain which was synthesized by Genewiz. The nucleic acid and protein
 sequences of a construct comprising the TGF β Receptor II linked to the N-terminus of
 IL-15R α Su are shown below.

The nucleic acid sequence of the TGF β Receptor II/IL-15 R α Su construct
 (including signal peptide sequence) is as follows:

25 *(Signal peptide)*

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCTCCTGTTCTCCAGCGCCT
 ACTCC

(Two human TGF β Receptor II fragments)

ATCCCCCCCCATGTGCAAAGAGCGTGAACAACGATATGATCGTGACC
 30 GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
 GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAAGTGCAGCATCAC

CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
 GACGAGAACATCACCCCTGGAGACCGTGTGTACGACCCCAAGCTCCCTTATC
 ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
 GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGT
 5 AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
 GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCTCTCCCA
 CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
 GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
 CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
 10 CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
 TGGAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
 AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
 GACCTTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
 TTAGCGAGGAATAACAATACCAGCAACCCCGAC
 15 (*Human IL-15R α sushi domain*)
 ATTACATGCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
 AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
 AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCCTGCTGAATAAGGCTA
 CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO:
 20 61)

The amino acid sequence of the two TGF β Receptor II/IL-15R α Su construct
 (including signal peptide sequence) is as follows:

(*Signal peptide*)
 25 MKWVTFISLLFLFSSAYS
 (*Two human TGF β Receptor II extra-cellular domains*)
 IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
 TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKK
 PGETFFMCSSSDECNDNIIFSEEYNTSNPDGGGSGGGSGGGGSIPPHVQKSVN
 30 NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAV

WRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKAT

5 NVAHWTTPSLKCIR (SEQ ID NO: 8)

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The TGF β R/IL-15R α Su and TGF β R/TF/IL-15 constructs were cloned into a
10 modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation
15 and secretion of the soluble TGF β R/TF/IL-15:TGF β R/IL-15R α Su protein complex (referred to as TGF β Rt15-TGFRs), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Effect of TGF β Rt15-TGFRs on TGF β 1 activity in HEK-Blue TGF β cells

20 To evaluate the activity of TGF β Rt15-TGFRs, the effect of TGF β Rt15-TGFRs on the activity of TGF β 1 in HEK-Blue TGF β cells was analyzed. HEK-Blue TGF β cells (Invivogen) were washed twice with pre-warmed PBS and resuspended in the testing medium (DMEM, 10% heat-inactivated FCS, 1x glutamine, 1x anti-anti, and 2x glutamine) at 5×10^5 cells/mL. In a flat-bottom 96-well plate, 50 μ L cells were added to
25 each well (2.5×10^4 cells/well) and followed with 50 μ L 0.1nM TGF β 1 (R&D systems). TGF β Rt15-TGFRs or TGF β -Fc (R&D Systems) prepared at a 1:3 serial dilution was then added to the plate to reach a total volume of 200 μ L. After 24hrs of incubation at 37°C, 40 μ L of induced HEK-Blue TGF β cell supernatant was added to 160 μ L pre-warmed QUANTI-Blue (Invivogen) in a flat-bottom 96-well plate, and incubated at 37°C for 1-3
30 hrs. The OD values were then determined using a plate reader (Multiscan Sky) at 620-655 nM. The IC₅₀ of each protein sample was calculated with GraphPad Prism 7.04. The

IC₅₀ of TGF β Rt15-TGFRs and TGF β R-Fc were 216.9 pM and 460.6 pM respectively. These results showed that the TGF β Rt15 domain in TGF β Rt15-TGFRs was able to block the activity of TGF β 1 in HEK-Blue TGF β cells (Figure 5).

5 *The IL-15 in TGF β Rt15-TGFRs promotes IL-2R β and common γ chain containing 32D β cell proliferation*

To evaluate the activity of IL-15 in TGF β Rt15-TGFRs, the IL-15 activity of TGF β Rt15-TGFRs was compared to recombinant IL-15 using 32D β cells that express IL2R β and common γ chain, and evaluating their effects on promoting cell proliferation.

10 IL-15 dependent 32D β cells were washed 5 times with IMDM-10% FBS and seeded in the wells at 2×10^4 cells/well. Serially-diluted TGF β Rt15-TGFRs or IL-15 were added to the cells (Figure 6). Cells were incubated in a CO₂ incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 μ L of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO₂ incubator at 37°C. The absorbance at 450

15 nm was measured by analyzing the amount of formazan dye produced. As shown in Figure 5, TGF β Rt15-TGFRs and IL-15 promoted 32D β cell proliferation, with the EC₅₀ of TGF β Rt15-TGFRs and IL-15 being 1901 pM and 10.63 pM, respectively.

20 *Detection of IL-15 and TGF β Rt15 domains in TGF β Rt15-TGFRs with corresponding antibodies using ELISA*

A 96-well plate was coated with 100 μ L (8 μ g/mL) of anti-TF IgG1 in R5 (coating buffer) and incubated at room temperature (RT) for 2 hrs. The plates were washed 3 times and blocked with 100 μ L of 1% BSA in PBS. TGF β Rt15-TGFRs was added at a 1:3 serial dilution, and incubated at RT for 60 min. After 3 washes, 50 ng/mL

25 of biotinylated-anti-IL-15 antibody (BAM247, R&D Systems), or 200 ng/mL of biotinylated-anti-TGF β Rt15 antibody (BAF241, R&D Systems) was added to the wells and incubated at RT for 60 min. Next the plates were washed 3 times, and 0.25 μ g/mL of HRP-SA (Jackson ImmunoResearch) at 100 μ L per well was added and incubated for 30 min at RT, followed by 4 washes and incubation with 100 μ L of ABTS for 2 mins at RT.

30 Absorbance at 405 nm was read. As shown in Figure 7A and 7B, the IL-15 and TGF β Rt15 domains in TGF β Rt15-TGFRs were detected by the individual antibodies.

Purification elution chromatograph of TGFRt15-TGFRs from anti-TF antibody affinity column

TGFRt15-TGFRs harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample
5 loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 8, the anti-TF antibody affinity column bound to
10 TGFRt15-TGFRs which contains TF as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF
15 antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Analytical size exclusion chromatography (SEC) analysis of TGFRt15-TGFRs

A Superdex 200 Increase 10/300 GL gel filtration column (from GE Healthcare)
20 was connected to an AKTA Avant system (from GE Healthcare). The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.7 mL/min. A sample containing TGFRt15-TGFRs in PBS was injected into the Superdex 200 column using a capillary loop, and analyzed by SEC. The SEC chromatograph of the sample is shown in Figure 9. The SEC results showed four protein peaks for TGFRt15-TGFRs.

25

Reduced SDS-PAGE analysis of TGFRt15-TGFRs

To determine the purity and molecular weight of the TGFRt15-TGFRs protein, protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-
30 PAGE) method under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the TGF α 15-TGFRs protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs and the manufacturer's instructions. Figure 10 shows the reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. The results showed that the TGF α 15-TGFRs protein is glycosylated when expressed in CHO cells. After deglycosylation, the purified sample showed expected molecular weights (69 kDa and 39 kDa) in the reduced SDS gel. Lane M was loaded with 10 μ l of SeeBlue Plus2 Prestained Standard.

Immunostimulatory activity of TGF α 15-TGFRs in C57BL/6 mice

TGF α 15-TGFRs is a multi-chain polypeptide (a type A multi-chain polypeptide described herein) that includes a first polypeptide that is a soluble fusion of two TGF β RII domains, human tissue factor 219 fragment and human IL-15, and the second polypeptide that is a soluble fusion of two TGF β RII domains and sushi domain of human IL-15 receptor alpha chain.

Wild type C57BL/6 mice were treated subcutaneously with either control solution or with TGF α 15-TGFRs at a dosage of 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg. Four days after treatment, spleen weight and the percentages of various immune cell types present in the spleen were evaluated. As shown in Figure 11A, the spleen weight in mice treated with TGF α 15-TGFRs increased with increasing dosage of TGF α 15-TGFRs. Moreover, the spleen weight in mice treated with 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGF α 15-TGFRs were higher as compared to mice treated with the control solution, respectively. In addition, the percentages of CD4 $^{+}$ T cells, CD8 $^{+}$ T cells, NK cells, and CD19 $^{+}$ B cells present in the spleen of control-treated and TGF α 15-TGFRs-treated mice were evaluated. As shown in Figure 11B, in the spleens of mice treated with TGF α 15-TGFRs, the percentages of CD8 $^{+}$ T cells and NK cells both increased with increasing dosage of TGF α 15-TGFRs. Specifically, the percentages of CD8 $^{+}$ T cells were higher in mice treated with 0.3 mg/kg, 3 mg/kg, and 10 mg/kg of TGF α 15-TGFRs compared to control-treated mice, and the percentages of NK cells were higher in mice treated with 0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGF α 15-TGFRs compared to control-

treated mice. These results demonstrate that TGF α 15-TGFRs is able to stimulate immune cells in the spleen, in particular CD8 $^{+}$ T cells and NK cells.

The pharmacokinetics of TGF α 15-TGFRs molecules were evaluated in wild type C57BL/6 mice. The mice were treated subcutaneously with TGF α 15-TGFRs at a dosage of 3 mg/kg. The mouse blood was drained from tail vein at various time points and the serum was prepared. The TGF α 15-TGFRs concentrations in mouse serum was determined with ELISA (capture: anti-human tissue factor antibody; detection: biotinylated anti-human TGF β receptor antibody and followed by peroxidase conjugated streptavidin and ABTS substrate). The results showed that the half-life of TGF α 15-TGFRs was 12.66 hours in C57BL/6 mice.

The mouse splenocytes were prepared in order to evaluate the immunostimulatory activity of TGF α 15-TGFRs over time in mice. As shown in Figure 12A, the spleen weight in mice treated with TGF α 15-TGFRs increased 48 hours posttreatment and continued to increase over time. In addition, the percentages of CD4 $^{+}$ T cells, CD8 $^{+}$ T cells, NK cells, and CD19 $^{+}$ B cells present in the spleen of control-treated and TGF α 15-TGFRs-treated mice were evaluated. As shown in Figure 12B, in the spleens of mice treated with TGF α 15-TGFRs, the percentages of CD8 $^{+}$ T cells and NK cells both increased at 48 hours after treatment and were higher and higher overtime after the single dose treatment. These results further demonstrate that TGF α 15-TGFRs is able to stimulate immune cells in the spleen, in particular CD8 $^{+}$ T cells and NK cells.

Furthermore, the dynamic proliferation of immune cells based on Ki67 expression of splenocytes and cytotoxicity potential based on granzyme B expression were evaluated in splenocytes isolated from mice following a single dose (3 mg/kg) of TGF α 15-TGFRs. As shown in Figure 13A and 13B, in the spleens of mice treated with TGF α 15-TGFRs, the expression of Ki67 and granzyme B by NK cells increased at 24 hours after treatment and its expression of CD8 $^{+}$ T cells and NK cells both increased at 48 hours and later time points after the single dose treatment. These results demonstrate that TGF α 15-TGFRs not only increases the numbers of CD8 $^{+}$ T cells and NK cells but also enhance the cytotoxicity of these cells. The single dose treatment of TGF α 15-TGFRs led CD8 $^{+}$ T cells and NK cells to proliferate for at least 4 days.

The cytotoxicity of the splenocytes from TGF α 15-TGFRs-treated mice against tumor cells was also evaluated. Mouse Moloney leukemia cells (Yac-1) were labeled with CellTrace Violet and were used as tumor target cells. Splenocytes were prepared from TGF α 15-TGFRs (3 mg/kg)-treated mouse spleens at various time points post
5 treatment and were used as effector cells. The target cells were mixed with effector cells at an E:T ratio = 10:1 and incubated at 37°C for 20 hours. Target cell viability was assessed by analysis of propidium iodide positive, violet-labeled Yac-1 cells using flow cytometry. Percentage of Yac-1 tumor inhibition was calculated using the formula, (1-
[viable Yac-1 cell number in experimental sample]/[viable Yac-1 cell number in the
10 sample without splenocytes]) x 100. As shown in Figure 14, splenocytes from TGF α 15-TGFRs-treated mice had stronger cytotoxicity against Yac-1 cells than the control mouse splenocytes.

Tumor size analysis in response to chemotherapy and/or TGF α 15-TGFRs

15 Pancreatic cancer cells (SW1990, ATCC® CRL-2172) were subcutaneously (s.c.) injected into C57BL/6 scid mice (The Jackson Laboratory, 001913, 2x10⁶ cells/mouse, in 100 μ L HBSS) to establish the pancreatic cancer mouse model. Two weeks after tumor cell injection, chemotherapy was initiated in these mice intraperitoneally with a combination of Abraxane (Celgene, 68817-134, 5 mg/kg, i.p.) and Gemcitabine (Sigma
20 Aldrich, G6423, 40 mg/kg, i.p.), followed by immunotherapy with TGF α 15-TGFRs (3 mg/kg, s.c.) in 2 days. The procedure above was considered one treatment cycle and was repeated for another 3 cycles (1 cycle/week). Control groups were set up as the SW1990-injected mice that received PBS, chemotherapy (Gemcitabine and Abraxane), or
TGF α 15-TGFRs alone. Along with the treatment cycles, tumor size of each animal was
25 measured and recorded every other day, until the termination of the experiment 2 months after the SW1990 cells were injected. Measurement of the tumor volumes were analyzed by group and the results indicated that the animals receiving a combination of
chemotherapy and TGF α 15-TGFRs had significantly smaller tumors comparing to the PBS group, whereas neither chemotherapy nor TGF α 15-TGFRs therapy alone work as
30 sufficiently as the combination (Figure 15).

In vitro senescent B16F10 melanoma model

Next, *in vitro* killing of senescent B16F10 melanoma cells by activated mouse NK cells was evaluated. B16F10 senescence cells (B16F10-SNC) cells were labelled with CellTrace violet and incubated for 16 hrs with different E:T ratio of *in vitro* 2t2-
5 activated mouse NK cells (isolated from spleen of C57BL/6 mice injected with TGFRt15-TGFRs 10 mg/kg for 4 days). The cells were trypsinized, washed and resuspended in complete media containing propidium iodide (PI) solution. The cytotoxicity was assessed by flow cytometry (Figure 16).

10 Example 3: Stimulation of NK cells *in vivo* by TGFRt15-TGFRs

A set of experiments was performed to determine the effect of the TGFRt15-TGFRs construct on immune stimulation in ApoE^{-/-} mice fed with a Western diet. In these experiments, 6-week-old female B6.129P2-ApoE^{tm1Unc}/J mice (Jackson Laboratory) were fed with a Western diet containing 21% fat, 0.15% cholesterol, 34.1% sucrose,
15 19.5% casein, and 15% starch (TD88137, Envigo Laboratories). After 8-weeks of the Western diet, the mice were injected subcutaneously with TGFRt15-TGFRs at 3 mg/kg. Three days post treatment, mice were fasted for 16 hours and then blood samples were collected through retro-orbital venous plexus puncture. The blood was mixed with 10 µL 0.5 M EDTA, and 20 µL blood was taken for lymphocyte subsets analysis. The red
20 blood cells were lysed with ACK (0.15 M NH₄Cl, 1.0 mM KHCO₃, 0.1 mM Na₂EDTA, pH 7.4) and the lymphocytes were stained with anti-mouse CD8a and anti-mouse NK1.1 antibodies for 30 minutes at 4 °C in FACS staining buffer (1% BSA in PBS). The cells were washed once and analyzed with a BD FACS Celesta. For Treg staining, ACK treated blood lymphocytes were stained with anti-mouse CD4 and anti-mouse CD25
25 antibodies for 30 minutes at 4 °C in FACS staining buffer. The cells were washed once and resuspended in fixation/permeabilization working solution and incubated at room temperature for 60 minutes. The cells were washed once and resuspended in permeabilization buffer. The samples were centrifuged at 300-400 x g for 5 minutes at room temperature and the supernatant was then discarded. The cell pellet was
30 resuspended in residual volume and the volume adjusted to about 100 µL with 1 x permeabilization buffer. Anti-Foxp3 antibody was added to the cells, and the cells were

incubated for 30 minutes at room temperature. Permeabilization buffer (200 μ L) was added to the cells, and the cells were centrifuged at 300-400 $\times$ g for 5 minutes at room temperature. The cells were resuspended in flow cytometry staining buffer and analyzed on a flow cytometer. Figures 17A-17C show that treatment with TGFRT15-TGFRs increased the percentage of NK cells and CD8⁺ T cells in ApoE^{-/-} mice fed with Western diet.

Example 4: Induction of proliferation of immune cells *in vivo*

A set of experiments was performed to determine the effect of the TGFRT15-TGFRs construct on immune stimulation in C57BL/6 mice. In these experiments, C57BL/6 mice were subcutaneously treated with control solution (PBS) or TGFRT15-TGFRs at 0.1, 0.3, 1, 3, and 10 mg/kg. The treated mice were euthanized 4 days post-treatment. Spleen weight was measured and splenocyte suspensions were prepared. The splenocyte suspensions were stained with conjugated anti-CD4, anti-CD8, and anti-NK1.1 (NK) antibodies. The cells were additionally stained for proliferation marker Ki67. Figure 18A shows that spleen weight in mice treated with TGFRT15-TGFRs increased with increasing dosage of TGFRT15-TGFRs. Additionally, spleen weight in mice treated with 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRT15-TGFRs was higher as compared to mice treated with just the control solution. The percentages of CD8⁺ T cells and NK cells both increased with increasing dosage of TGFRT15-TGFRs (Figure 18B). Finally, TGFRT15-TGFRs significantly upregulated expression of cell proliferation marker Ki67 in both CD8⁺ T cells and NK cells at all doses of TGFRT15-TGFRs tested (Figure 18C). These results demonstrate that TGFRT15-TGFRs treatment induced proliferation of both CD8⁺ T cells and NK cells in C57BL/6 mice.

A set of experiments was performed to determine the effect of the TGFRT15-TGFRs construct on immune stimulation in ApoE^{-/-} mice fed with a Western diet. In these experiments, 6-week-old female B6.129P2-ApoE^{tm1Unc}/J mice (Jackson Laboratory) were fed with a Western diet containing 21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Envigo Laboratories). After 8-week of the Western diet, the mice were injected subcutaneously with TGFRT15-TGFRs at 3 mg/kg. Three days post-treatment, the mice were fasted for 16 hours and then blood samples

were collected through retro-orbital venous plexus puncture. The blood was mixed with 10 μ L 0.5 M EDTA and 20 μ L blood was taken for lymphocyte subsets analysis. The red blood cells were lysed with ACK (0.15 M NH_4Cl , 1.0 mM KHCO_3 , 0.1 mM Na_2EDTA , pH 7.4) and the lymphocytes were stained with anti-mouse CD8a and anti-mouse NK1.1 antibodies for 30 minutes at 4 °C in FACS staining buffer (1% BSA in PBS). The cells were washed once and resuspended in Fixation Buffer (BioLegend Cat# 420801) for 20 minutes at room temperature. The cells were centrifuged at 350 x g for 5 minutes, the fixed cells were resuspended in Intracellular Staining Permeabilization Wash Buffer (BioLegend Cat# 421002) and then centrifuged at 350 x g for 5 minutes. The cells were then stained with anti-Ki67 antibody for 20 minutes at RT. The cells were washed twice with Intracellular Staining Permeabilization Wash Buffer and centrifuged at 350 x g for 5 minutes. The cells were then resuspended in FACS staining buffer. Lymphocyte subsets were analyzed with a BD FACS Celesta. As described in Figure 19A and 19B, treatment of ApoE^{-/-} mice with TGFRT15-TGFRs induced proliferation (Ki67-positive staining) in NK and CD8⁺ T cells.

Example 5: NK-mediated cytotoxicity following treatment with multi-chain construct

A set of experiments was performed to determine if treatment of NK cells with TGFRT15-TGFRs enhanced cytotoxicity of NK cells. In these experiments, Human Daudi B lymphoma cells were labeled with CellTrace Violet (CTV) and used as tumor target cells. Mouse NK effector cells were isolated with NK1.1-positive selection using a magnetic cell sorting method (Miltenyi Biotec) of C57BL/6 female mouse spleens 4 days post TGFRT15-TGFRs subcutaneous treatment at 3 mg/kg. Human NK effector cells were isolated from peripheral blood mononuclear cells derived from human blood buffy coats with the RosetteSep/human NK cell reagent (Stemcell Technologies). The target cells (Human Daudi B lymphoma cells) were mixed with effector cells (either mouse NK effector cells or human NK effector cells) in the presence of 50 nM TGFRT15-TGFRs or in the absence of TGFRT15-TGFRs (control) and incubated at 37 °C for 44 hours for mouse NK cells and for 20 hours for human NK cells. Target cell (Daudi) viability was assessed by analysis of propidium iodide-positive, CTV-labeled cells using flow

cytometry. The percentage of Daudi inhibition was calculated using the formula (1-viable tumor cell number in experimental sample/viable tumor cell number in the sample without NK cells) x 100. Figure 20 shows that mouse (Figure 20A) and human (Figure 20B) NK cells had significantly stronger cytotoxicity against Daudi B cells following NK cell activation with TGFRt15-TGFRs than in the absence of TGFRt15-TGFRs activation.

A set of experiments was performed to determine antibody-dependent cellular cytotoxicity (ADCC) of mouse and human NK cells following treatment with TGFRt15-TGFRs. In these experiments, human Daudi B lymphoma cells were labeled with CellTrace Violet (CTV) and used as tumor target cells. Mouse NK effector cells were isolated with NK1.1-positive selection using a magnetic cell sorting method (Miltenyi Biotec) of C57BL/6 female mouse spleens 4 days post-TGFRt15-TGFRs subcutaneous treatment at 3 mg/kg. Human NK effector cells were isolated from peripheral blood mononuclear cells derived from human blood buffy coats with the RosetteSep/human NK cell reagent (Stemcell Technologies). The target cells (Daudi B cells) were mixed with effector cells (either mouse NK effector cells or human NK effector cells) in the presence of anti-CD20 antibody (10 nM Rituximab, Genentech) and in the presence of 50 nM TGFRt15-TGFRs, or in the absence of TGFRt15-TGFRs (control) and incubated at 37 °C for 44 hours for mouse NK cells and for 20 hours for human NK cells. The Daudi B cells express the CD20 targets for the anti-CD20 antibody. Target cell viability was assessed after incubation by analysis of propidium iodide-positive, CTV-labeled target cells using flow cytometry. The percentage of Daudi inhibition was calculated using the formula (1-viable tumor cell number in experimental sample/viable tumor cell number in the sample without NK cells) x 100. Figure 21 shows that mouse NK cells (Figure 21A) and human NK cells (Figure 21B) had stronger ADCC activity against Daudi B cells following NK cell activation with TGFRt15-TGFRs than in the absence of TGFRt15-TGFRs activation.

Example 6: Treatment of Cancer

A set of experiments was performed to assess antitumor activity of TGFRt15-TGFRs plus anti-TRP1 antibody (TA99) in combination with chemotherapy in a melanoma mouse model. In these experiments, C57BL/6 mice were subcutaneously injected with 0.5×10^6 B16F10 melanoma cells. The mice were treated with three doses

of chemotherapy docetaxel (10 mg/kg) (DTX) on day 1, day 4, and day 7, followed by treatment with single dose of combination immunotherapy TGFRt15-TGFRs (3 mg/kg) + anti-TRP1 antibody TA99 (200 µg) on day 9. Figure 22A shows a schematic of the treatment regimen. Tumor growth was monitored by caliper measurement, and tumor volume was calculated using the formula $V = (L \times W^2)/2$, where L is the largest tumor diameter and W is the perpendicular tumor diameter. Figure 22B shows that treatment with DTX + TGFRt15-TGFRs + TA99 significantly reduced tumor growth compared to saline control and DTX treatment groups (N=10, ****p <0.001, Multiple t test analyses).

To assess immune cell subsets in the B16F10 tumor model, peripheral blood analysis was performed. In these experiments, C57BL/6 mice were injected with B16F10 cells and treated with DTX, DTX + TGFRt15-TGFRs + TA99, or saline. Blood was drawn from the submandibular vein of B16F10 tumor-bearing mice on days 2, 5, and 8 post-immunotherapy for the DTX + TGFRt15-TGFRs + TA99 group and day 11 post-tumor injection for the DTX and saline groups. RBCs were lysed in ACK lysis buffer and the lymphocytes were washed and stained with anti-NK1.1, anti-CD8, and anti-CD4 antibodies. The cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figures 22C-22E show that DTX + TGFRt15-TGFRs + TA99 treatment induced an increase in the percentage of NK cells and CD8⁺ T cells in the tumors compared to the saline and DTX treatment groups.

On day 17, total RNA was extracted from tumors of mice treated with saline, DTX or DTX + TGFRt15-TGFRs + TA99 using Trizol. Total RNA (1 µg) was used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR was carried out with CFX96 Detection System (Bio-Rad) using FAM-labeled predesigned primers for senescence cell markers, (F) p21 (G) DPP4 and (H) IL6. The housekeeping gene 18S ribosomal RNA was used as an internal control to normalize the variability in expression levels. The expression of each target mRNA relative to 18S rRNA was calculated based on Ct as $2^{-\Delta(\Delta C_t)}$, in which $\Delta C_t = C_{t\text{target}} - C_{t18S}$. The data is presented as fold-change as compared to saline control. Figure 22F-22H show that DTX treatment induced an increase in senescent tumor cells that were subsequently reduced following treatment with TGFRt15-TGFRs + TA99 immunotherapy.

A set of experiments was performed to investigate amelioration of Western diet-induced hyperglycemia in ApoE^{-/-} mice by TGF α 15-TGFRs. In these experiments, 6-week-old female B6.129P2-ApoE^{tm1Unc}/J mice (Jackson Laboratory) were fed with a Western diet containing 21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Envigo Laboratories). After 8-weeks of the Western diet, the mice were injected subcutaneously with TGF α 15-TGFRs at 3 mg/kg. Three days post-treatment, the mice were fasted for 16 hours and then blood samples were collected through retro-orbital venous plexus puncture. Blood glucose was detected with a glucose meter (OneTouch UltraMini) and GenUltimated test strips using a drop of fresh blood. As shown in Figure 23A, TGF α 15-TGFRs treatment reduced hyperglycemia induced by the Western diet. The plasma insulin and resistin levels were analyzed with Mouse Rat Metabolic Array by Eve Technologies. HOMA-IR was calculated using the following formula: homeostatic model assessment-insulin resistance = Glucose (mg/dL) * Insulin (mU/mL)/405. As shown in Figure 23B, TGF α 15-TGFRs treatment reduced insulin resistance compared to the untreated group.

Example 7: Upregulation of CD44 memory T cells

A set of experiments was performed to assess upregulation of CD44 memory T cells upon treatment with TGF α 15-TGFRs. In these experiments, C57BL/6 mice were subcutaneously treated with TGF α 15-TGFRs. The treated mice were euthanized and the single splenocyte suspensions were prepared 4 days (TGF α 15-TGFRs) following the treatment. The prepared splenocytes were stained with fluorochrome-conjugated anti-CD4, anti-CD8 and anti-CD44 antibodies and the percentages of CD44^{high} T cells in CD4⁺ T cells or CD8⁺ T cells were analyzed by flow cytometry. The results show that TGF α 15-TGFRs upregulated expression of the memory marker CD44 on CD4⁺ and CD8⁺ T cells (Figure 24). These findings indicate that TGF α 15-TGFRs was able to induce mouse T cells to differentiate into memory T cells.

Example 8: Regulation of transcriptomes in the liver of db/db mice following treatment with TGFRt15-TGFRs

Five-week-old male BKS.Cg-Dock7m +/- Leprdb/J (db/db) mice were fed with standard chow diet and received drinking water ad libitum. At the age of six weeks, mice
5 were randomly assigned to control and treatment groups (n = 5/group). The treatment group received TGFRt15-TGFRs by subcutaneous injection at 3 mg/kg at 6 and 12 weeks of age, while control group received vehicle (PBS) only. At end of study (4-weeks post the 2nd dose), mice were euthanized and livers were collected. The half of liver was homogenized with the TRIzol reagent (Invitrogen) and total tissue RNA was purified
10 with RNeasy Mini Kit (Qiagen). Extracted RNA samples were quantified using Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and RNA integrity was checked using Agilent TapeStation 4200 (Agilent Technologies, Palo Alto, CA, USA).

RNA sequencing libraries were prepared using the NEBNext Ultra II RNA Library Prep Kit for Illumina following manufacturer's instructions (NEB, Ipswich, MA,
15 USA). Briefly, mRNAs were first enriched with Oligo(dT) beads. Enriched mRNAs were fragmented for 15 minutes at 94 °C. First strand and second strand cDNAs were subsequently synthesized. cDNA fragments were end repaired and adenylated at 3' ends, and universal adapters were ligated to cDNA fragments, followed by index addition and library enrichment by limited-cycle PCR. The sequencing libraries were validated on the
20 Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA), and quantified by using Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) as well as by quantitative PCR (KAPA Biosystems, Wilmington, MA, USA).

The sequencing libraries were clustered on 1 flowcell lane. After clustering, the flowcell was loaded on the Illumina HiSeq instrument (4000 or equivalent) according to
25 manufacturer's instructions. The samples were sequenced using a 2x150bp Paired End (PE) configuration. Image analysis and base calling were conducted by the HiSeq Control Software (HCS). Raw sequence data (.bcl files) generated from Illumina HiSeq was converted into fastq files and de-multiplexed using Illumina's bcl2fastq 2.17 software. One mismatch was allowed for index sequence identification.

30 Sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic v.0.36. The trimmed reads were

mapped to the Mus musculus GRCm38 reference genome available on ENSEMBL using the STAR aligner v.2.5.2b. The STAR aligner is a splice aligner that detects splice junctions and incorporates them to help align the entire read sequences. BAM files were generated as a result of this step.

5 Unique gene hit counts were calculated by using featureCounts from the Subread package v.1.5.2. The hit counts were summarized and reported using the gene_id feature in the annotation file. Only unique reads that fell within exon regions were counted. If a strand-specific library preparation was performed, the reads were strand-specifically counted.

10 After extraction of gene hit counts, the gene hit counts table was used for downstream differential expression analysis. Using DESeq2, a comparison of gene expression between the treatment-specific groups of samples was performed. The Wald test was used to generate p-values and log2 fold changes. Genes with an adjusted p-value < 0.05 and absolute log2 fold change > 1 were called as differentially expressed genes for
15 each comparison.

A gene ontology analysis was performed on the statistically significant set of genes by implementing the software GeneSCF v.1.1-p2. The mgi GO list was used to cluster the set of genes based on their biological processes and determine their statistical significance.

20 To estimate the expression levels of alternatively spliced transcripts, the splice variant hit counts were extracted from the RNA-seq reads mapped to the genome. Differentially spliced genes were identified for groups with more than one sample by testing for significant differences in read counts on exons (and junctions) of the genes using DEXSeq. For groups with only one sample, the exon hit count tables were
25 provided.

The significant genes downregulated or upregulated were divided into four groups according to the function. The heatmaps were constructed with GraphPad in accordance with gene functions. As shown in Figure 25 and Tables 1 and 2, the six genes involved in glucose regulation were downregulated; the three genes related to senescence regulation
30 were downregulated and one gene was upregulated; the nineteen genes involved in

inflammation were mostly downregulated excepting one gene; the nine genes related to vascular regulation were downregulated.

Among six genes regulating glucose, four of them (Pdk4, Pnpla3, Gadd45b, and Ppargc1a) were related to the gluconeogenesis. Downregulation of these four genes may
5 cause the reduction of gluconeogenesis and therefore reduce the circulating glucose. Downregulation of Retn was related to the reduction of insulin resistance. Downregulation of Slc2a4 may slow glucose transported to adipose tissue and striate muscle.

Downregulation of Cav1 and Endod1 along with upregulation of Slc34a2 promote
10 cell proliferation and reduce senescence. Downregulation of Acss1 may reduce glucose-independent acetate-mediated cell survival and tumor growth.

Downregulation of eighteen genes and upregulation of Cish are associated with downregulation of the cells and molecules involved in inflammatory responses. Downregulation of nine genes related to vascular regulation may reflect a different
15 vascular environment in the liver changed by TGF α 15-TGFRs treatment.

These findings indicate that TGF α 15-TGFRs treatment suppresses gene expression related to glucose regulation, senescence, inflammation and vascular regulation in the liver of db/db mice.

Table 1. Regulation of transcriptomes in the liver of db/db mice following treatment with TGFRt15-TGFRs

	Regulation	log2 Fold Change	Adj.Pval	Symbol	Control	TGFRt15-TGFRs
Glucose regulation	Down	-2.096289344	3.42E-02	Retn	5.61934746	3.974617084
	Down	-1.804543391	8.02E-03	Slc2a4	6.648145442	5.116575093
	Down	-1.346756214	9.80E-07	Pdk4	10.15283618	8.812808986
	Down	-1.319230698	1.82E-03	Pnpla3	6.820864253	5.58617444
	Down	-1.049951428	3.42E-02	Gadd45b	8.902818086	7.936747039
	Down	-1.037624414	2.49E-03	Ppargc1a	9.94205237	8.965423502
Senescence regulation	Up	1.471480309	7.29E-02	Slc34a2	5.768859731	7.064236232
	Down	-2.169290256	7.11E-02	Acsc1	5.443295282	3.721690488
	Down	-1.610400236	4.18E-02	Cav1	7.326547509	5.988279063
	Down	-1.229365392	1.47E-02	Endod1	7.446602038	6.192808898
Inflammation regulation	Up	1.45707262	7.62E-05	Cish	6.321861131	7.704905566
	Down	-3.06345987	3.82E-02	Reg3g	4.932412574	2.829725656
	Down	-2.886992871	8.02E-03	Ighg3	5.715159646	3.106258191
	Down	-2.826778789	3.00E-14	Ighg2b	7.437317137	4.709171503
	Down	-2.791929301	3.76E-02	Scgb3a1	5.023078231	3.024485524
	Down	-2.604831835	8.38E-04	Glycam1	6.095276793	3.867397849
	Down	-2.562008178	5.67E-05	Ighg2c	6.725829809	4.258477821
	Down	-2.469457569	1.41E-11	Igkc	8.457634718	6.060534075
	Down	-2.425601648	9.11E-02	Ltf	5.274677778	3.477435403
	Down	-2.399351243	4.85E-02	Ms4a1	5.017374783	3.304853422
	Down	-2.047374161	8.63E-05	Jchain	6.660474662	4.797517709
	Down	-2.039004438	7.47E-02	Cd19	5.303977874	3.593322805
	Down	-2.036901274	3.47E-07	Ighm	8.299180551	6.411154088
	Down	-1.909054054	1.65E-03	Ifi2712a	6.420560573	4.753540666
	Down	-1.706656106	4.71E-03	ACKR3	6.465216788	4.982147331
	Down	-1.467633284	3.97E-02	Lsp1	5.884533344	4.544074275
	Down	-1.11979923	4.71E-03	Pmepa1	6.90863891	5.841136195
	Down	-1.046058196	7.11E-02	Coro1a	7.076869734	6.124311808
	Down	-1.038663712	2.19E-02	GPX3	8.488888196	7.517625851
Vascular regulation	Down	-5.614042335	2.87E-02	Myh8	4.469324747	2.214290342
	Down	-5.507281406	1.65E-03	Nppa	5.06772092	2.19472816
	Down	-3.436742187	2.65E-03	Tcap	5.595187029	3.260187895
	Down	-2.764903666	9.49E-02	Flnc	4.862671393	3.129487218
	Down	-2.518127305	8.16E-03	Slc36a2	6.244224409	4.402606708
	Down	-2.317372389	3.00E-10	Myh6	6.928245867	4.797511367
	Down	-2.093059568	2.02E-02	Actc1	5.784720054	4.123883209
	Down	-1.550587332	2.25E-02	Acta2	7.25724117	5.925475021
	Down	-1.281068362	1.07E-03	Tpm2	8.878431842	7.68227915

Table 2. Regulation of transcriptomes in the liver of db/db mice following treatment with TGF α 15-TGFRs

Group	Regulation	Ensemble ID	Log2 Fold Change	Adj. Pval	Symbol	Control-6	Control-7	Control-8	Control-C-Mean	X9218-11	X9218-12	X9218-13	X-Mean	Function	References	Potential Effects
Glucose Regulation	Down	ENSMUSG000000027452	-2.1692902567	1.1E-02	Acs1	5.85530	5.08400	4.39015	5.43229	4.16813	2.99868	3.99824	3.72169	Essential for glucose-independent acetate-mediated cell survival. A key component of the glyoxylate shunt. Glucose-starved melanoma cells are highly dependent on acetate to sustain ATP levels, cell viability and proliferation. Conversely, depletion of ACS1 or ACS2 reduced melanoma tumor growth in mice.	Shkher, A.I., et al., JBC VOL 291, NO. 42, pp. 21869-21873, October 14, 2016	Reduce glucose-independent acetate related cell survival and proliferation
	Down	ENSMUSG000000012705	-2.096289344	3.42E-02	Retn	5.97458	5.30069	4.58275	5.61934	3.69884	4.31982	3.90517	3.97461	Resistin is related to the pathogenesis of inflammation.	J. Biol. Chem. (2019) 294(30) 11369-11381	Increase circulating glucose by reducing diffusion to fat and muscle
	Down	ENSMUSG000000018566	-1.804543391	8.02E-03	Sic2a4	7.11614	7.21600	5.61225	6.64814	5.35547	4.95190	5.04234	5.11657	Solute carrier family 2, member 4, also known as glucose transporter found primarily in adipose tissue and striated muscle (skeletal and cardiac). At the cell surface, GLUT4 permits the facilitated diffusion of circulating glucose.	Diabetes 53:899-910, 2004	Increase glucose to convert pyruvate reduce circulating glucose by consuming more.
	Down	ENSMUSG000000019577	-1.346756214	3.80E-07	Pdk4	9.97298	9.92306	10.56210	10.1528	8.39860	8.91028	8.12953	8.81280	Pyruvate dehydrogenase liponamide kinase isozyme 4, is located in the matrix of the mitochondria and inhibits the pyruvate dehydrogenase complex by phosphorylating one of its subunits, reducing the conversion of pyruvate. Starvation and diabetes increase pyruvate dehydrogenase kinase-4 (PDK4) expression.	Liver International. 2020; 40:1098-1110.	Reduce circulating glucose by reducing gluconeogenesis from glycerol by triacylglycerol hydrolis.
	Down	ENSMUSG000000041653	-1.319230698	1.82E-03	Pnpla3	6.91607	7.04124	6.50526	6.82086	5.39023	5.67000	5.69828	5.58617	Patatin-like phospholipase domain-containing protein 3 also known as adiponutrin (ADPN) is a triacylglycerol lipase that mediates triacylglycerol hydrolis in adipocytes and may also play a role in energy metabolism. In NASH biopsies, PNPLA3 significantly correlated with fibrosis stage and SMA levels independently of PNPLA3 genotype. In line, PNPLA3 expression was higher in SMA positive cells. Low glucose increased	Kim H. et al., GADD45 regulates hepatic gluconeogenesis via modulating the protein stability of FoxO1. Biomolecules 2021; 9:50.	Reduced liver gluconeogenesis and reduced blood glucose levels.
	Down	ENSMUSG000000015312	-1.049951428	3.42E-02	Gadd45b	8.81732	8.34108	9.55008	8.90281	7.81152	8.22622	7.77249	7.93674	Growth arrest and DNA-damage-inducible, beta is involved in the regulation of growth and apoptosis. Both whole-body knockout (KO) mice and adenovirus-mediated knockdown (KD) mice of GADD45 exhibited decreased hepatic gluconeogenic gene expression concomitant with reduced blood glucose levels under fasting and HFD conditions, but showed a more pronounced effect in GADD45 KD mice. Further, in primary hepatocytes, GADD45 KD reduced glucose output, whereas GADD45 overexpression increased it. Mechanistically, GADD45 did not affect Akt-mediated forkhead box protein O1 (FoxO1) phosphorylation and forskolin-induced cAMP response element-binding protein (CREB) phosphorylation. Rather it increased FoxO1 transcriptional activity via enhanced protein stability of FoxO1. Further GADD45 colocalized and physically interacted with FoxO1. Additionally, GADD45 deficiency potentiated	Besse-Patin A et al., PGCL1 regulates the IRS1:IRS2 ratio during fasting to influence hepatic metabolism downstream of insulin PNAS 2019; 116:4284-4290.	Reduce circulating glucose by reducing liver gluconeogenesis.
	Down	ENSMUSG000000029167	-1.037624414	2.49E-03	Ppargc1a	9.94111	9.42722	10.45719	9.94205	9.13982	8.84288	8.91356	8.96542	Peroxisome proliferation-activated receptor gamma coactivator 1-alpha (PGC-1a) is the master regulator of mitochondrial biogenesis and is also the primary regulator of gluconeogenesis in liver. The protein encoded by this gene is a transcriptional coactivator that regulates the genes involved in energy metabolism. This protein interacts with PPARgamma, which permits the interaction of this protein with multiple transcription factors. This protein can interact with, and regulate the activities of cAMP response element binding protein (CREB) and nuclear respiratory factors (NRFs). It provides a direct link between external physiological stimuli and the regulation of mitochondrial biogenesis, and is a major factor that regulates muscle fiber type determination. This protein may be also involved in controlling blood pressure, regulating cellular cholesterol homeostasis, and the development of obesity. PGC1A is key for insulin-mediated suppression of hepatic glucose production. While increased hepatic PGC1A levels are largely believed to negatively impact blood glucose control due to induction of enzymes that drive gluconeogenesis,		

Table 2 (cont'd). Regulation of transcriptomes in the liver of db/db mice following treatment with TGF α 15-TGFRs

[illegible]

Table 2 (cont'd). Regulation of transcriptomes in the liver of db/db mice following treatment with TGF α 15-TGFRs

[illegible]

Example 9 - RNA-seq analysis of differentially expressed genes between the PBS (Control group) or TGFRt15-TGFRs (TGFRt15-TGFRs group) in aged mice liver

C57BL/6, 76-week-old mice were purchased from the Jackson Laboratory. Mice were housed in a temperature and light controlled environment. Mice were divided into two groups and treated subcutaneously with either PBS (PBS control group) or TGFRt15-TGFRs at a dosage of 3 mg/kg (TGFRt15-TGFRs group). At day 60 post treatment, mice were euthanized, and livers were harvested. Harvested livers were stored in liquid nitrogen in 1.7 mL Eppendorf tubes. Samples were homogenized by using homogenizer in 1 mL of Trizol (Thermo Fischer). Homogenized tissues were transferred in fresh Eppendorf tubes. Total RNA was extracted using RNeasy Mini Kit (Qiagen #74106) according to the manufacturer's instructions.

Library preparations, sequencing reactions and bioinformatic analysis were conducted at GENEWIZ, LLC. (South Plainfield, NJ, USA) as follows: Library preparation with poly A selection and HiSeq sequencing extracted RNA samples were quantified using Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and RNA integrity was checked using Agilent TapeStation 4200 (Agilent Technologies, Palo Alto, CA, USA). RNA sequencing libraries were prepared using the NEBNext Ultra II RNA Library Prep Kit for Illumina following manufacturer's instructions (NEB, Ipswich, MA, USA). Briefly, mRNAs were first enriched with oligo(dT) beads. Enriched mRNAs were fragmented for 15 minutes at 94 °C. First strand and second strand cDNAs were subsequently synthesized and cDNA fragments were end repaired and adenylated at 3'ends. Universal adapters were ligated to cDNA fragments, followed by index addition and library enrichment by limited-cycle PCR. The sequencing libraries were validated on the Agilent TapeStation (Agilent Technologies, Palo Alto, CA, USA), and quantified by using Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) as well as by quantitative PCR (KAPA Biosystems, Wilmington, MA, USA). The sequencing libraries were clustered on 1 flowcell lane. After clustering, the flowcell was loaded on the Illumina HiSeq instrument (4000 or equivalent) according to manufacturer's instructions. The samples were sequenced using a 2x150bp Paired End (PE) configuration. Image analysis and base calling were conducted by the HiSeq Control Software (HCS). Raw sequence data (.bcl files) generated from Illumina HiSeq was converted into fastq files and de-multiplexed

using Illumina's bcl2fastq 2.17 software. One mismatch was allowed for index sequence identification. Sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic v.0.36. The trimmed reads were mapped to the *Mus musculus* GRCm38 reference genome available on ENSEMBL using the STAR aligner v.2.5.2b. The STAR aligner is a splice aligner that detects splice junctions and incorporates them to help align the entire read sequences. BAM files were generated as a result of this step. Unique gene hit counts were calculated by using feature counts from the Subread package v.1.5.2. The hit counts were summarized and reported using the gene_id feature in the annotation file. Only unique reads that fell within exon regions were counted. If a strand-specific library preparation was performed, the reads were strand-specifically counted. After extraction of gene hit counts, the gene hit counts table was used for downstream differential expression analysis. Using DESeq2, a comparison of gene expression between the treatment-specific groups of samples was performed. The Wald test was used to generate p-values and log2 fold changes. Genes with an adjusted p-value < 0.05 and absolute log2 fold change > 1 were called as differentially expressed genes for each comparison. A gene ontology analysis was performed on the statistically significant set of genes by implementing the software GeneSCF v.1.1-p2. The mgi GO list was used to cluster the set of genes based on their biological processes and determine their statistical significance. To estimate the expression levels of alternatively spliced transcripts, the splice variant hit counts were extracted from the RNA-seq reads mapped to the genome. Differentially spliced genes were identified for groups with more than one sample by testing for significant differences in read counts on exons (and junctions) of the genes using DEXSeq. For groups with only one sample, the exon hit count tables were provided.

The significant genes downregulated or upregulated were divided into four groups according to the function. The mean fold change was calculated by dividing the experimental group by the mean the control group. The heatmaps were constructed with GraphPad in accordance with gene functions. As showed in Figure 26 and Tables 3 and 4, most senescence and inflammation genes were downregulated in livers of the TGFRt15-TGFRs treated group compared to the PBS control group.

Table 3. RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRt15-TGFRs (TGFRt15-TGFRs group) in aged mice liver

ID	log2FoldChange	pvalue	padj	agedliver92181tox	agedliver92182tox	agedliver92183tox	agedliverpbs1	agedliverpbs2	agedliverpbs3	Gene.name
ENSMUSG00000000204	-2.005879896	3.88E-05	0.000867087	8.974563618	11.65468178	11.93770314	61.79909158	46.97322185	22.5130033	Sifr4
ENSMUSG000000000317	-1.462090887	1.76E-06	7.11E-05	60.82759785	43.70505667	55.0970914	92.69863737	140.9196656	205.5535084	Bcl6b
ENSMUSG000000000686	1.151464201	2.84E-06	0.000104278	355.9910235	571.0794072	469.2435618	171.5457542	161.796653	294.6266953	Ablc15
ENSMUSG000000001227	-1.099312542	1.49E-06	6.21E-05	100.7145473	67.98564371	92.74677053	171.5457542	203.550628	184.9981575	Sema6b
ENSMUSG0000000001403	-1.612087212	0.002110809	0.017174878	16.9519535	6.798564371	11.01941828	51.14407579	37.57857748	17.61887214	Ube2c
ENSMUSG000000001983	1.013926416	1.20E-05	0.000332241	308.1266842	343.8131125	299.3608633	128.9256911	122.1303768	219.2570756	Taco1
ENSMUSG0000000002233	-1.270303057	1.05E-10	1.46E-08	133.6212805	104.892136	141.4158679	330.3054895	288.1024274	298.5420002	Rhoc
ENSMUSG000000002250	-1.142106413	9.77E-06	0.000282853	303.1408155	156.3669805	415.9830401	627.58043	657.6251059	647.9829644	Ppard
ENSMUSG000000002289	-2.433615292	5.35E-35	1.82E-31	340.0362437	320.5037489	528.0137926	1865.693265	2130.496573	2428.467877	Angptl4
ENSMUSG000000002831	-2.428567233	6.40E-14	1.90E-11	67.807814	71.87053764	51.42395197	228.0173379	574.117156	226.1088592	Plin4
ENSMUSG000000003032	-1.814709673	6.30E-10	7.35E-08	33.903907	28.16548097	44.99595798	155.5632305	107.5164856	114.5226689	Klrf4
ENSMUSG000000003348	1.035062446	0.006673687	0.038727262	47.86433929	51.47484453	59.68851569	13.85152053	30.27163186	33.28009183	Mob3a
ENSMUSG000000003500	-1.169914769	9.39E-05	0.001721093	55.84172918	53.41729149	52.34223683	174.742259	99.16569058	90.05201318	Impch1
ENSMUSG000000003541	-2.579058324	2.05E-08	1.65E-06	9.971737353	11.65468178	11.01941828	105.4845653	45.92937248	44.04718036	Irb1
ENSMUSG000000003848	-1.114660438	1.53E-05	0.000400378	168.5223613	191.3310259	135.9061588	522.0957737	291.2339755	260.3677772	Nob1
ENSMUSG000000004100	-2.152000208	2.40E-05	0.000578522	177.4969249	181.6187911	165.2912742	1249.833352	729.6507128	351.3986167	Ppan
ENSMUSG000000004933	-1.661199536	0.00641801	0.037574902	11.96608482	8.741011335	3.673139427	42.62006316	14.61389124	19.57652461	Matk
ENSMUSG000000004951	-1.245761383	7.16E-05	0.001407433	177.4969249	194.2446963	114.7856071	237.6068521	636.7481184	278.9654756	Hspb1
ENSMUSG000000005148	-1.641216558	0.00407345	0.027163637	8.974563618	3.884893927	9.182848567	19.17902842	27.14008374	22.5130033	Klf5
ENSMUSG000000005547	1.316837452	9.30E-15	3.02E-12	9129.125547	13948.71164	12101.15784	5271.036311	4402.956662	4447.78639	Cyp2a5
ENSMUSG000000005580	1.4483211	2.75E-08	2.14E-06	248.2962601	371.00737	337.0105424	86.3056279	93.9464437	169.3369378	Acdy9
ENSMUSG000000006050	-1.042916307	2.32E-07	1.27E-05	992.1878666	1015.899762	869.6157953	2542.286767	2021.936238	1365.462591	Sra1
ENSMUSG000000006134	1.201348738	1.44E-09	1.52E-07	677.0809663	610.8995699	678.6125091	225.8863347	252.6115486	375.8692724	Crkl
ENSMUSG000000006517	1.182376585	1.39E-07	8.18E-06	2010.30225	1472.374798	1714.437827	1097.466626	579.3364028	613.7240464	Mwd
ENSMUSG000000006587	1.922052303	0.003206819	0.022932036	30.91238579	14.56835222	21.1205517	10.65501579	2.087989749	4.894131151	Sna13
ENSMUSG000000006711	1.246016282	4.07E-11	5.91E-09	435.7649223	410.8275327	410.4733309	210.9683126	141.9635149	177.1675477	D130043K22Rik
ENSMUSG000000006777	1.982368887	0.000106088	0.001901083	22.93499591	53.41729149	33.05825484	8.524012632	8.350794996	10.76708853	Krt23
ENSMUSG000000008153	1.444860417	1.72E-08	1.42E-06	278.2114721	339.9282186	185.4935411	96.96064369	120.0426781	78.30609842	C1stn3
ENSMUSG000000009013	-1.025154388	6.49E-07	3.04E-05	138.6071492	163.1655449	192.8398199	329.2399879	396.6627623	281.9019543	Dynl1l
ENSMUSG000000009633	-1.150697898	7.41E-07	3.38E-05	1177.662181	1721.008009	1060.619009	2971.683904	3862.242686	1956.673634	Gob2
ENSMUSG000000014547	1.47853344	5.88E-06	0.00018548	76.78237762	107.8058065	108.3576131	21.31003158	36.53472811	46.9836595	Wefy2
ENSMUSG000000014609	1.198939285	0.004891473	0.030982358	34.90108074	33.99282186	34.89482455	14.91702211	14.61389124	15.66121968	Chme
ENSMUSG000000015224	-1.47719251	7.85E-07	3.55E-05	57.83607665	125.2878291	79.80878253	318.5849721	255.7430968	158.5698493	Cyp2j9
ENSMUSG000000015312	-2.170653759	1.83E-07	1.04E-05	27.92086459	16.51079919	10.10113342	121.46718	59.4994435	63.62370497	Gadd45b
ENSMUSG000000016128	-1.194768611	1.81E-09	1.86E-07	486.6207828	535.1441384	631.7799814	199.2487953	218.1645193	304.4149576	Statd13
ENSMUSG000000016356	-1.303561468	6.94E-06	0.00021464	50.8558605	59.24463238	46.83252769	90.56763421	172.2351468	124.3109312	Col2a1
ENSMUSG000000017737	-1.495519922	0.005185903	0.032307064	19.94347471	7.769787853	5.50970914	38.35806684	32.35933061	22.5130033	Mmp9
ENSMUSG000000017868	1.520950393	2.42E-16	1.03E-13	1574.537328	1919.1376	1179.077756	543.4058053	586.6433485	498.2225512	Sgk2
ENSMUSG000000018486	1.561119026	0.006908525	0.039768921	26.92369085	21.3669166	27.5485457	3.196504737	15.65774062	6.851783612	Wnt5b
ENSMUSG000000019082	-1.21454588	1.93E-12	4.05E-10	2583.677148	3303.131061	2721.796315	7723.820946	7053.290223	5201.482588	Slc25a22
ENSMUSG000000019726	1.436923305	3.39E-10	4.31E-08	430.7790536	552.626161	627.1885571	179.0042653	147.1827618	268.193871	Lyst
ENSMUSG000000019737	-1.672592181	0.00414668	0.027410702	8.974563618	9.712234816	6.427993997	45.8165679	15.65774062	18.59769837	Syne4
ENSMUSG000000019883	1.383035463	1.73E-09	1.79E-07	790.7587721	685.683778	707.0793397	200.3142968	230.6907118	405.2340593	Enchc1
ENSMUSG000000020018	-1.224865075	5.55E-07	2.68E-05	76.78237762	100.0360186	117.5404617	268.5063979	250.5238499	170.3157641	Snrp1
ENSMUSG000000020027	-2.148540831	0.000201757	0.00309142	308.1266842	206.8706016	291.0962996	2138.461669	846.5618427	589.253906	Soes2
ENSMUSG000000020091	1.323362559	0.00594886	0.035409378	1482.797344	1694.784975	1814.530877	393.1700826	471.8199173	1129.56547	Eif4ebp2
ENSMUSG000000020122	-1.730853584	6.04E-21	5.49E-18	534.4851221	692.4823424	689.6319274	2670.146957	1807.947117	1885.219319	Egfr
ENSMUSG000000020335	1.099463556	0.002629472	0.020167667	41.88129688	88.38133683	80.80906739	24.50653632	41.75397498	32.3012656	Zfp354b
ENSMUSG000000020429	-1.914789163	0.005628577	0.034083881	1082.930677	219.4965068	977.0550875	2643.509417	2014.629293	3936.839098	Igfbp1
ENSMUSG000000020441	-1.319584205	1.17E-07	7.17E-06	96.72585232	114.6043708	87.23706139	303.66795	273.4885361	168.3581116	2310033P09Rik
ENSMUSG000000020532	1.125678465	6.99E-06	0.000215157	6805.710743	2936.008585	5607.047335	2810.793165	1739.053058	2484.260972	Acaca
ENSMUSG000000020641	-1.035196027	2.26E-05	0.00055792	307.1295105	181.6187911	139.5792982	408.0871047	425.8905448	452.2177184	Statd13
ENSMUSG000000020656	-2.17958622	1.43E-08	1.21E-06	16.9519535	7.769787853	23.87540627	71.38860579	76.20100434	73.41196727	Grlh1
ENSMUSG000000020681	1.270736177	0.003452779	0.024158824	30.91238579	35.93526882	52.34223683	14.91702211	21.92083686	12.72474099	Ace
ENSMUSG000000020692	-1.373008874	7.68E-06	0.000232298	53.84738171	65.07197327	76.21764311	249.3273695	152.4020087	104.7344066	Nle1
ENSMUSG000000020812	-1.318469089	0.000692163	0.007515219	32.90673326	43.70505667	47.75081255	180.0697668	73.06945621	57.75074759	1810032008Rik
ENSMUSG000000020889	1.857219295	5.65E-19	3.50E-16	1005.151125	1714.209445	1803.511459	443.2486569	371.6103773	433.620002	Nrlc1
ENSMUSG000000020917	1.579449336	7.98E-12	1.40E-09	34685.69121	17873.42573	22782.64729	9135.610538	6014.660096	10059.39717	Acly
ENSMUSG000000020948	1.248898073	0.003935164	0.026500655	67.807814	53.41729149	56.93366111	9.589514211	20.87698749	44.04718036	Klf128
ENSMUSG000000020961	1.570215029	7.42E-07	3.38E-05	116.669327	116.5468178	141.4158679	25.5720379	35.49087873	64.6025312	Ston2
ENSMUSG000000021250	-3.196150425	1.40E-18	7.95E-16	14.95760603	8.741011335	25.71197599	204.5763032	115.8672806	135.0780198	Fos
ENSMUSG000000021260	1.563177947	0.002093122	0.017081929	31.90955953	29.13670445	23.87540627	6.393009474	11.48234312	10.76708853	Hhip11
ENSMUSG000000021416	1.171425747	1.31E-08	1.13E-06	910.4196203	675.9715432	629.0251268	404.8906	324.6371555	254.4948199	Ecd3
ENSMUSG000000021453	-2.706074058	5.59E-23	6.28E-20	149.5760603	61.18707934	130.3964497	979.1959511	568.8979091	678.3265776	Gadd45g
ENSMUSG000000021611	1.027777308	1.21E-09	1.30E-07	416.8186214	423.453438	475.6715558	220.5588268	227.5591636	197.7228985	Tert
ENSMUSG000000021670	1.206582161	2.43E-07	1.30E-05	4843.272832	4623.994996	5239.733392	3051.596522	1273.496237	2047.704474	Hmgcr
ENSMUSG000000021684	-1.138260866	0.001182935	0.011115132	32.90673326	25.25181052	22.03883656	54.34058053	72.02560684	49.92013774	Pde8b
ENSMUSG000000021773	-1.294178405	0.002373717	0.01846284	17.94912724	12.62590526	22.03883656	44.75106632	55.32401685	29.36478691	Comt1
ENSMUSG000000021775	1.550962326	2.41E-10	3.16E-08	1168.687618	1405.360378	1119.38924	635.0389411	274.5323855	351.3986167	Nrlc2
ENSMUSG000000021804	1.339955479	0.00532128	0.032924815	26.92369085	35.93					

Table 3 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGF α 15-TGFRs (TGF α 15-TGFRs group) in aged mice liver

ENSMUSG00000022528	-1.557702695	1.66E-19	1.13E-16	205.4177895	185.503685	197.4312442	643.5629537	588.7310472	500.1802037	Hes1
ENSMUSG00000022651	-4.171544663	2.34E-09	2.36E-07	3.988694941	2.913670445	0	52.20957737	37.57857748	32.3012656	Retnlg
ENSMUSG00000022704	-1.016808687	0.000177103	0.002808323	94.73150485	108.7770299	100.0930494	291.9474326	188.9367368	134.0991935	Qrt2
ENSMUSG00000022853	1.04479536	2.29E-11	3.59E-09	9525.00352	10073.52995	7460.146176	4279.054341	3944.706786	4891.194673	Hehach
ENSMUSG00000022883	1.330834067	1.00E-09	1.09E-07	280.2058196	336.0433246	355.3762395	166.2182463	116.9111299	103.7555804	Robo1
ENSMUSG00000022887	1.363326654	1.68E-07	9.74E-06	1196.608482	1241.22361	1538.127135	350.5500195	374.7419254	819.2775547	Satp1
ENSMUSG00000022911	1.024722285	3.96E-05	0.000880701	133.6212805	188.4173554	193.7581048	105.4846563	73.06945621	75.36961973	Ar113b
ENSMUSG00000023034	-2.875895339	0.000200077	0.003084527	58.83325038	34.96404534	22.95712142	572.1743479	134.6556593	149.7604132	Nr4a1
ENSMUSG00000023044	-1.350199698	7.38E-12	1.31E-09	1875.683796	2461.080302	2315.914409	4109.63959	5560.585618	7290.297763	Csad
ENSMUSG00000023052	-1.334599319	0.004914148	0.031082668	13.96043229	11.65468178	17.44741228	36.22705368	52.19246872	20.55535084	Npff
ENSMUSG00000023067	-0.022867976	2.66E-57	3.63E-53	136.6128017	95.1799012	134.9878739	1114.514652	1009.402345	858.4306039	Cckn1a
ENSMUSG00000023072	1.22582592	2.02E-05	0.00050363	443.7423122	525.4319036	592.2937326	369.7290479	140.9196656	157.5910231	Slc10a2
ENSMUSG00000023341	-1.14606456	1.95E-06	7.71E-05	89.74563618	99.06479513	85.40049167	264.2443916	178.489243	164.4428067	Mc1r
ENSMUSG00000023571	-1.317554169	0.00212852	0.017761424	18.94630097	21.3669166	14.69255771	53.27507895	58.45556497	25.44848199	Cttnf12
ENSMUSG00000023800	1.312092825	2.45E-12	4.99E-10	459.697092	410.8275327	456.3875738	160.8907384	217.1206699	156.6121968	Tiam2
ENSMUSG00000023905	-1.475845141	9.44E-06	0.000275028	54.84455544	95.1799012	81.72735225	328.1744863	207.7260255	109.6285378	Tnfrsf12a
ENSMUSG00000023927	-1.218211099	0.003077602	0.022366209	27.92086459	26.223034	12.85598799	61.79909158	60.54326372	33.28009183	Satb1
ENSMUSG00000023968	-1.267291327	0.008148531	0.04468095	10.96891109	11.65468178	15.61084256	20.24453	38.62242686	33.28009183	Crip3
ENSMUSG00000024118	-1.757053931	5.31E-18	2.79E-15	442.7451385	592.4463238	352.621385	1463.99917	1896.674313	1329.246021	Tec2
ENSMUSG00000024130	1.042830636	1.30E-05	0.000354612	2989.526858	2745.468783	3530.805274	1006.889892	1228.610714	2261.088592	Abca3
ENSMUSG00000024136	-1.225506878	0.002002269	0.016518413	17.92086459	19.42446963	17.44741228	64.99559632	52.19246872	34.25891806	Dnase12
ENSMUSG00000024190	-1.579039564	2.17E-13	5.48E-11	281.2029934	502.12254	460.0607132	1247.702349	1464.520672	1004.275712	Dusp1
ENSMUSG00000024236	1.389584652	9.49E-13	2.12E-10	1344.190195	1041.151572	1299.373072	484.8032184	345.514143	575.5498234	Sufl
ENSMUSG00000024411	1.68012077	5.07E-05	0.001068968	63.81911906	48.56117408	35.81310941	14.91702211	13.57004187	17.61887214	Acp4
ENSMUSG00000024440	1.007637941	0.000827422	0.008613399	107.6947634	103.9209125	147.8438619	35.16155211	70.98175747	72.4331404	Pcsh12
ENSMUSG00000024665	1.18951247	3.11E-12	6.05E-10	9244.7977	8635.147975	9906.457034	3625.901873	3398.773563	5157.435407	Fads2
ENSMUSG00000024843	-1.175648724	2.45E-11	3.79E-09	1421.969747	1016.870985	1263.559963	3171.998201	2942.611387	2249.342677	Chka
ENSMUSG00000024887	1.067323547	1.55E-06	6.39E-05	398.8694941	436.0793433	565.6634717	191.7902842	179.5420924	296.5843478	Asah2
ENSMUSG00000024924	1.265415535	2.60E-12	5.20E-10	642.1798855	816.7989481	589.538878	269.5718995	262.006193	320.0716773	Vlelr
ENSMUSG00000024970	-1.174479585	0.000322102	0.004379373	31.90955953	39.82016275	25.71197599	90.56763421	63.67481184	65.58135743	Al84a148
ENSMUSG00000024978	2.527869097	4.51E-08	3.23E-06	10405.50793	5475.75989	8311.396238	1324.418463	791.2378259	2079.026913	Gpm
ENSMUSG00000025003	1.045766059	5.74E-07	2.75E-05	301.1464681	286.5109271	410.4733309	152.3667258	144.0512137	186.95581	Cyp2c39
ENSMUSG00000025006	1.229102652	2.44E-06	9.41E-05	288.1832095	294.2807149	416.9013249	107.6156595	110.6480337	207.5111608	Sorbs1
ENSMUSG00000025153	1.113433094	9.44E-07	4.18E-05	85604.37365	41744.15646	57005.28733	35454.56504	23677.63536	26074.95195	Fasn
ENSMUSG00000025161	-1.470478397	0.001962044	0.016285081	18.94630097	9.712234816	14.69255771	61.79909158	26.09623436	32.3012656	Slc16a3
ENSMUSG00000025240	1.333245056	1.72E-06	6.99E-05	553.4314231	682.7701076	696.0599214	183.2662716	163.8843518	418.9376266	Sacm1l
ENSMUSG00000025233	1.252220218	7.14E-07	3.28E-05	138.6071492	127.2302761	172.6375531	71.38860579	52.19246872	60.68722628	Sp4
ENSMUSG00000025402	-1.340941683	6.59E-15	2.25E-12	217.3838743	245.7195409	214.8786565	646.7594584	568.8979091	502.1378561	Nab2
ENSMUSG00000025429	1.693186246	2.17E-13	5.48E-11	624.2307583	540.0002558	651.9822483	188.5937795	118.9988287	253.5159936	Ptprtp2
ENSMUSG00000025450	-1.257708301	2.83E-05	0.000669372	30.91238579	62.15830282	55.0970914	145.9737163	103.3410881	105.7132329	Gm9752
ENSMUSG00000025997	1.284615532	0.009041141	0.049049118	26.93269085	9.712234816	12.85598799	39.42355842	58.4556497	22.5130323	Ikrf2
ENSMUSG00000026020	-1.156643623	9.59E-08	6.11E-06	250.2906076	294.2807149	269.9757479	828.9602284	520.8808379	466.9001118	Nop58
ENSMUSG00000026249	-1.326755053	0.000451208	0.005606296	36.89542821	50.50362104	26.63026084	45.8165659	122.1303768	117.4591476	Serpine2
ENSMUSG00000026358	-2.793399328	5.66E-13	1.33E-10	12.96325856	11.65468178	12.85598799	104.4191547	58.4556497	96.9037968	Rgs1
ENSMUSG00000026398	1.137826301	6.48E-12	1.16E-09	1403.023446	1667.590718	1539.04542	615.8599126	631.5288716	846.6846892	Nr5a2
ENSMUSG00000026471	1.000583106	1.46E-05	0.000384933	269.2369085	237.949753	315.8899907	181.1352684	104.3849374	126.2658537	Mr1
ENSMUSG00000026475	-1.56210025	0.004964709	0.031300593	735.9142167	388.4893927	994.5024998	1039.929541	2826.744106	2390.293654	Rgs16
ENSMUSG00000026525	-1.790734538	0.001947167	0.0161813	7.977389882	8.741011335	6.427993997	43.68556474	19.83313812	16.64004591	Opr3
ENSMUSG00000026822	1.02651459	5.14E-06	0.00016724	122.6523694	119.4604882	110.1941828	304.7334516	223.3837661	189.8922887	Len2
ENSMUSG00000026826	-1.483020728	0.008821676	0.047306802	15.95477976	10.6834583	11.93770314	69.25760263	25.05238499	13.70356722	Nr4a2
ENSMUSG00000026832	-1.174113684	0.001522033	0.013425591	30.91238579	34.96404534	25.71197599	101.22265	42.79782435	62.64487874	Cytip
ENSMUSG00000027360	-1.979964656	1.70E-12	3.69E-10	54.84455544	93.23745424	72.54450368	370.7945495	319.4179086	181.0828526	Hic
ENSMUSG00000027398	-1.057691022	0.001937845	0.016123489	78.77672509	37.87771578	32.13996998	98.02614527	82.46410059	128.2262362	Il1b
ENSMUSG00000027405	-1.241634744	7.40E-06	0.000226395	225.3612642	254.4605522	248.8551962	520.5933642	450.9429298	352.3774429	Nop56
ENSMUSG00000027496	-1.007831872	0.004782077	0.030501955	38.88977568	37.87771578	17.44741228	55.40608211	66.80635997	66.56018366	Aurka
ENSMUSG00000027513	-1.115800194	1.04E-10	1.46E-08	16805.36896	18645.5484	18901.97549	48173.45739	29794.5927	39823.54518	Pek1
ENSMUSG00000027605	1.86697993	9.67E-16	3.77E-13	27327.54622	18714.50527	21561.32844	8380.169919	3799.611723	6353.561061	Acsc2
ENSMUSG00000027762	1.17286147	2.98E-15	1.13E-12	1319.260852	1520.935972	1404.975831	637.1699442	555.3278672	690.0724923	Sunr1
ENSMUSG00000027907	-1.140239221	0.000303295	0.004203282	47.86433929	56.33096193	106.5210434	207.7728079	117.9549793	139.9721509	S100a11
ENSMUSG00000027947	-1.05537492	2.15E-06	8.45E-05	434.7677486	468.1297181	461.8972829	713.8860579	763.0538928	1358.610808	Il6ra
ENSMUSG00000028008	1.245889	0.000553467	0.00652119	54.84455544	81.58277246	55.0970914	20.24453	24.00853561	36.21657052	Asic5
ENSMUSG00000028339	1.011959422	2.14E-08	1.71E-06	706.961783	505.0362104	521.5857986	295.1439374	274.5323855	289.7325642	Col15a1
ENSMUSG00000028445	-1.767301879	0.003925136	0.026446185	70.79933521	71.87053764	91.82848967	91.63313579	280.7954817	425.7894102	Enho
ENSMUSG00000028630	1.331166045	1.46E-07	8.59E-06	373.9401507	358.3814647	406.8001915	124.6636847	101.2533893	226.1088592	Dyrk2
ENSMUSG00000028838	1.693729472	3.36E-09	3.22E-07	304.1379893	151.5108631	198.349529	74.58511053	49.0690296	78.30609842	Extl1
ENSMUSG00000028859	-1.001034699	0.004539011	0.029294129	74.78803015	57.30218542	29.38511541	152.3667258	96.03414245	74.3907935	Csf3r
ENSMUSG00000028862	-2.278242763	1.62E-07	9.45E-06	8.974563618	9.712234816	17.44741228	77.78161526	40.71021561	57.75074759	Map3k6
ENSMUSG00000028864	1.08474786	0.000792949	0.008337279	131.6269331	134.0228405	203.8592382	39.42355842	69.93790809	111.586022	Hgf
ENSMUSG00000028957	2.966674755	1.69E-27	3.84E-24	314.1097266	608.9283465	341.6019667	62.86459316	53.2363181	46.00483282	Pcr3
ENSMUSG00000028976	1.532387058	3.09E-07	1.58E-05	157.5534502	94.20867772	101.9296191	42.62006316	34.44702936	45.02600659	Slc2a5
ENSMUSG00000029086	1.631659452	1.01E-18	6.00E-16	713.9763945	617.6981343	593.2120				

Table 3 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRT15-TGFRs (TGFRT15-TGFRs group) in aged mice liver

ENSMUSG00000029373	-1.650047987	0.002725238	0.020577678	7.977389882	5.82734089	9.182848567	25.5720379	28.18393311	18.59769837	Pf4
ENSMUSG00000029380	-2.803403219	8.38E-26	1.43E-22	53.84738171	128.2014996	71.62621882	615.8599126	454.0744779	700.8395809	Cxcl1
ENSMUSG00000029580	-1.162469269	9.11E-12	1.55E-09	8321.414821	7155.974613	6927.540959	16369.30076	20515.81561	13266.0319	Actb
ENSMUSG00000029591	-1.593385799	3.01E-08	2.29E-06	43.87564435	60.21585586	35.81310941	177.9387637	131.5250212	112.5650165	Ung
ENSMUSG00000029656	-1.019716092	0.000134204	0.002299171	189.4630097	409.8563092	189.1666805	429.3971363	614.8272816	554.0156463	C8b
ENSMUSG00000030032	-1.061169015	0.007631272	0.042668167	23.93216965	14.56835222	25.71197599	43.68556474	55.32401685	35.23774429	Wdr54
ENSMUSG00000030055	1.305889925	4.54E-06	0.000151075	239.3216965	263.2015635	292.9328693	75.65061211	74.11330559	171.2945903	Rab43
ENSMUSG00000030691	1.035229834	1.01E-08	8.79E-07	365.9627609	443.8491311	415.0647552	189.6592811	180.5859418	227.0876854	Fchs2
ENSMUSG00000030782	-1.087204324	0.00053618	0.006369234	38.88977568	39.82016275	40.40453369	115.0741705	67.85020934	70.47548858	Tgfb11
ENSMUSG00000030814	-1.357189589	8.78E-10	9.81E-08	261.2595186	283.5972566	291.0962996	814.0432063	869.526529	459.069502	Bcl7c
ENSMUSG00000030827	-1.631143551	1.45E-05	0.000383464	26.92369085	20.39569311	33.05825484	120.4016784	80.37640184	48.94131151	Fgf21
ENSMUSG00000030934	1.434845811	0.000702548	0.007652274	13671.25191	24780.76713	9134.17947	5865.586192	5683.759844	6052.082582	Oat
ENSMUSG00000030968	-1.140744433	0.000133867	0.002296287	34.90108074	72.84176112	54.17880654	31.0566942	132.5688706	93.9673181	Polr1
ENSMUSG00000031010	1.281779593	3.26E-07	1.66E-05	953.2980909	1398.561814	1420.586673	414.4801142	361.1718836	775.2303744	Usp9x
ENSMUSG00000031271	-1.719181951	2.91E-23	3.97E-20	339.03907	470.0721651	364.5590881	1418.182602	1115.874981	1330.224847	Serpina7
ENSMUSG00000031378	1.154152634	6.32E-15	2.21E-12	1014.125689	912.9500727	885.2266019	429.3971363	392.4873648	441.4506298	Abcc1
ENSMUSG00000031465	-1.254345174	0.0029265	0.021654196	21.93782218	11.65468178	20.2026685	50.07857421	30.27163186	47.96248528	Angpt2
ENSMUSG00000031762	-4.701992063	1.08E-05	0.000305681	348.9090618	188.4173554	32.13996998	9477.636545	4672.2698	1607.23267	M2
ENSMUSG00000031765	-4.008275951	6.98E-08	4.64E-06	1129.797842	691.5111189	337.0105424	20312.7221	10174.39985	4243.211708	Mt1
ENSMUSG00000032009	1.096135097	4.98E-08	3.50E-06	816.6852892	873.12991	1044.089882	377.187559	338.2071973	562.8250824	Sesn3
ENSMUSG00000032064	1.076164714	3.75E-10	4.69E-08	659.131839	690.5398954	661.1650968	268.5063979	302.7163186	381.7422289	Dxadc1
ENSMUSG00000032083	-1.056710751	2.91E-09	2.86E-07	57416.2665	56826.28591	68671.17815	163165.5843	112907.9676	104422.1611	Apoa1
ENSMUSG00000032091	2.476316256	2.69E-06	0.000101175	102.7088947	50.5062104	32.13996998	20.24453	8.350794996	4.894131152	Ptprss4
ENSMUSG00000032285	1.014640895	0.008899206	0.047498424	51.85303424	70.89931416	52.34223683	26.63753947	15.65774062	44.04718036	Dnajl4
ENSMUSG00000032417	1.412452049	0.000231229	0.003394264	102.7088947	50.5062104	80.80906739	26.63753947	43.84167373	17.61887214	Rwdd2a
ENSMUSG00000032418	1.488424738	1.96E-09	2.00E-07	17569.20404	7712.485668	13799.06654	6053.11447	3739.068459	4136.519649	Me1
ENSMUSG00000032500	2.09734091	1.65E-20	1.25E-17	413.8271001	367.1224761	372.8236518	75.65061211	69.93790809	123.332105	Dclk3
ENSMUSG00000032561	1.808480512	0.000942567	0.009458265	1361.142149	660.4319675	1176.322901	453.9036726	141.9635149	317.1396986	Acpp
ENSMUSG00000032702	1.072973429	8.45E-08	5.48E-06	1328.235415	1308.23803	1494.049462	468.8206947	629.4411728	864.3035613	Kank1
ENSMUSG00000032724	1.34458697	3.25E-10	4.18E-08	647.1657542	540.0002558	680.4490788	234.4103474	176.4105443	323.9914822	Abtb2
ENSMUSG00000032735	-1.26252931	1.21E-08	1.04E-06	191.4573572	257.3742226	294.769439	436.8556474	741.1330559	606.8722628	Abilim3
ENSMUSG00000032786	-1.26794906	3.89E-08	2.81E-06	1778.957944	2029.857077	2206.638511	7150.581096	4035.521682	3301.580875	Alas1
ENSMUSG00000032849	1.170479352	7.00E-10	8.09E-08	403.8553628	338.9569951	399.4539127	168.3492495	143.0073643	195.7652461	Abcc4
ENSMUSG00000032860	1.046181292	6.20E-08	4.19E-06	339.03907	263.2015635	290.1780147	155.5632305	134.6556693	141.9298034	P2ry2
ENSMUSG00000032883	1.139189345	1.88E-11	3.09E-09	1754.0286	1475.288469	1528.944286	586.6632695	580.3802522	723.3525842	Acsl3
ENSMUSG00000033105	1.210153299	1.43E-10	1.97E-08	3593.814142	2741.763889	3240.627259	1574.811334	996.8761526	1567.100795	Lss
ENSMUSG00000033594	-1.045179866	5.30E-08	3.67E-06	379.9231931	342.841889	348.9482455	852.4012632	820.4656084	539.3332529	Scpt2l
ENSMUSG00000033624	1.545958738	3.43E-10	4.33E-08	290.177557	397.230404	428.8390281	105.4846563	98.1218412	178.1463739	Popa1
ENSMUSG00000033792	1.183185841	0.000561984	0.006589659	63.81911906	67.98564371	74.38107339	21.31003158	26.09623436	43.06835413	Atp7a
ENSMUSG00000033855	1.291796081	0.000477464	0.005865923	117.6665008	69.92809068	198.349529	37.29255526	42.79782435	77.32727219	Ston1
ENSMUSG00000033967	-2.822221219	4.36E-05	0.000952114	4.985868677	3.884893927	1.836569713	15.98252368	19.8313812	39.15304921	Rnf225
ENSMUSG00000034066	1.2566902	2.38E-07	1.29E-05	275.2195909	346.7267829	310.3802816	96.96064369	107.5164856	184.9981575	Farp2
ENSMUSG00000034110	1.131917427	3.20E-05	0.000736514	99.71737353	110.7194769	115.7038919	55.40608211	37.57857748	55.79309512	Kctd7
ENSMUSG00000034271	-1.155793079	0.004733656	0.030334993	36.89542821	29.13670445	40.40453369	135.3187005	37.57857748	64.6025132	Jcp2
ENSMUSG00000034755	-1.51005032	0.006510973	0.037993212	7.977389882	5.82734089	12.85598799	19.17902842	36.5347281	20.55535084	Pcchl1x
ENSMUSG00000034765	-2.457798355	3.78E-06	0.000131464	23.93216965	12.62590526	9.182848567	166.2182463	53.2363181	31.32243937	Dusp5
ENSMUSG00000034853	1.437062013	9.64E-14	2.63E-11	450.7225284	335.0721012	434.3487372	165.1527447	137.7881174	147.8027608	Acot11
ENSMUSG00000034926	1.093495822	1.31E-06	5.56E-05	17902.26007	19066.08817	17627.39611	6118.110066	6691.074491	12774.66113	Dhcr24
ENSMUSG00000035078	1.209517873	6.16E-05	0.001249206	322.0871165	384.6044987	346.193391	94.82964053	101.2533893	258.4101248	Mtmr9
ENSMUSG00000035112	-1.146398835	0.004042609	0.02701081	23.93216965	14.56835222	18.36569713	43.68556474	45.92937248	36.21657052	Wrnk4
ENSMUSG00000035164	1.06213892	8.60E-06	0.000256206	131.6269331	166.0792154	141.4158679	61.79909158	70.98175747	77.32727219	Zc3h12c
ENSMUSG00000035165	-1.798528938	1.18E-06	5.05E-05	32.90673326	22.33814008	28.46683056	144.9082147	55.32401685	91.03083941	Kone3
ENSMUSG00000035284	1.432291641	4.22E-09	3.95E-07	725.9424793	940.1443302	1017.459621	293.0129342	218.1645193	482.5613315	Vpsl3c
ENSMUSG00000035900	1.041020638	2.29E-08	1.81E-06	343.0277649	318.561302	367.3139427	147.0392179	160.7528037	191.8499411	Gramd4
ENSMUSG00000035933	1.189062904	7.63E-07	3.47E-05	188.465836	221.4389538	244.2637719	77.78161526	81.42025121	127.2474099	Cog5
ENSMUSG00000035948	1.741049509	1.78E-10	2.38E-08	816.6852892	893.5256031	857.6780562	193.9212874	154.4897074	418.9376266	Acsc3
ENSMUSG00000036062	-1.708320866	0.004715534	0.030261525	7.977389882	9.712234816	3.673139427	27.70304105	29.2778249	12.72474099	PfH24
ENSMUSG00000036120	-1.340248384	3.85E-09	3.64E-07	263.2538661	219.4965068	224.9797899	773.5541463	626.3096247	392.5093183	Rfxank
ENSMUSG00000036611	1.04665245	4.08E-07	2.03E-05	846.6005013	616.7269108	585.8657386	415.5456158	267.2254399	309.3090888	Eepd1
ENSMUSG00000037035	-1.785829601	2.14E-10	2.84E-08	59.83042412	30.10792793	50.50566712	141.71171	149.2704606	192.8287674	Inhbb
ENSMUSG00000037071	1.600161226	3.89E-11	5.71E-09	372268.8876	280319.3774	340820.5063	157237.1335	63623.66322	106805.6029	Scd1
ENSMUSG00000037095	-1.027049761	7.90E-10	8.97E-08	3492.102421	3177.843232	2991.772063	5413.813523	8132.630477	6142.134595	Lrg1
ENSMUSG00000037157	1.742314913	8.42E-06	0.000251859	51.85303424	60.21585586	58.77023083	11.72051737	14.61389124	24.47065576	Il22ra1
ENSMUSG00000037336	-1.65964734	0.000936801	0.009424872	14.95760603	7.769787853	8.26456371	23.44103474	42.79782435	31.32243937	Mfsd2b
ENSMUSG00000037443	-1.349128808	2.89E-12	5.72E-10	750.8718227	1078.058065	1089.08584	3091.020081	2288.117829	2056.51391	Cep85
ENSMUSG00000037447	-1.380120078	0.000135203	0.002310483	30.91238579	23.30936356	25.71197599	99.09164684	54.28016747	54.81426889	Arid5a
ENSMUSG00000037465	-1.250556349	2.44E-12	4.99E-10	342.0305912	272.9137983	297.5242936	592.4188779	817.3340602	760.5479809	Klf10
ENSMUSG00000037583	-1.178511493	2.71E-06	0.000101645	181.4856198	270.0001279	213.9603716	304.7334516	631.5288716	569.676866	Nrb2
ENSMUSG00000037709	1.621036788	2.49E-19	1.62E-16	850.5891962	1019.784656	857.6780562	239.7378553	289.1462767	357.271574	Fam13a
ENSMUSG00000037887	-1.887921138	0.000434734	0.005464027	15.95477976	8.741011335	11.01941828	80.97812	20.87698749	30.34361314	Dusp8
ENSMUSG00000038217	1.023783748	9.74E-10	1.07E-07							

Table 3 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGF α Rt15-TGFRs (TGF α Rt15-TGFRs group) in aged mice liver

ENSMUSG00000038418	-2.528561862	1.50E-17	7.58E-15	95.72867859	79.64032549	131.3147345	515.7027642	342.3825948	912.2660466	Egr1
ENSMUSG00000038473	1.076370271	0.000625033	0.007061263	117.6665008	63.12952631	89.99191596	45.8165679	35.49087873	46.98365905	Nosl1ap
ENSMUSG00000038530	-1.722746304	4.58E-05	0.000989869	17.94912724	16.51079919	13.77427285	64.99559632	30.27163186	63.62370497	Rgs4
ENSMUSG00000038583	-1.702074711	0.00088397	0.009002758	12.96325856	13.59712874	11.93770314	67.12659947	41.75397498	16.64004591	Pln
ENSMUSG00000038587	-1.31455808	3.12E-06	0.000113043	97.72302606	73.8129846	141.4158679	349.4845179	176.4105443	253.5159936	Akap12
ENSMUSG00000038751	1.774904606	6.46E-12	1.16E-09	167.5251875	125.2878291	171.7192682	46.88206947	44.8855231	44.04718036	Ptk6
ENSMUSG00000038768	1.297337324	1.64E-05	0.000422782	329.0673326	229.2087417	124.8867405	106.5501579	77.24485371	93.9673181	9130409123Rik
ENSMUSG00000038774	1.007283954	3.14E-06	0.00011311	450.7225284	497.2664226	488.5275438	194.986789	189.9805862	328.8856134	Ascc3
ENSMUSG00000038844	1.003328537	1.78E-06	7.13E-05	364.9655871	380.7196048	386.5979247	169.4147511	146.1389124	248.6218625	Kif16b
ENSMUSG00000038895	-1.113485639	6.66E-08	4.45E-06	100.7145473	103.9209125	94.58334024	230.1483411	226.5153143	190.8711149	Zfp653
ENSMUSG00000039103	-1.109845105	0.002277587	0.018174053	37.89260194	21.3669166	26.63026084	68.19210105	74.11330559	43.06835413	Nexn
ENSMUSG00000039304	1.09876453	1.10E-06	4.76E-05	197.4403996	176.7626737	181.8204016	105.4846563	73.06945621	81.2425771	Trnf10
ENSMUSG00000039533	1.597388363	4.59E-08	3.26E-06	367.9571083	378.7771578	293.8511541	167.2837479	54.2801677	122.3532788	Mmd2
ENSMUSG00000039601	1.185885146	6.77E-14	1.97E-11	707.933521	644.8923918	732.7913156	337.7640005	298.5409211	280.9231281	Rcan2
ENSMUSG00000039704	1.942291849	0.00175377	0.014994461	267.2425611	391.4030631	463.7338526	59.66808842	50.10476998	182.0616788	Lmbre2
ENSMUSG00000039741	1.087450189	5.12E-08	3.58E-06	264.2510399	270.0001279	292.0145844	105.4846563	146.1389124	137.0356722	Bahcc1
ENSMUSG00000039853	1.374596414	3.52E-09	3.36E-07	477.6462192	438.0217902	491.2823983	126.7946879	156.5774062	258.4101248	Trnf1a
ENSMUSG00000039981	1.024216498	5.34E-05	0.001113305	116.669327	128.2014996	147.8438619	53.27507895	77.24485371	62.64487874	Zc3h12a
ENSMUSG00000040093	1.487860391	2.33E-07	1.27E-05	234.358278	188.4173554	357.2128093	75.6061211	69.9379089	132.1415411	Bmf
ENSMUSG00000040128	-1.050165381	1.95E-11	3.16E-09	1378.094102	1310.180477	1381.100424	3368.050491	2671.210549	2388.336002	Pncr1
ENSMUSG00000040152	-1.254644006	0.001007039	0.00915514	35.89825447	19.42446963	35.81310941	91.63313579	40.71012561	85.15788203	Thbs1
ENSMUSG00000040435	-1.205190782	4.71E-06	0.000155637	119.6608482	131.11517	127.6415951	430.4626379	241.1292055	201.6382034	Ppp1r15a
ENSMUSG00000040584	1.185136822	2.65E-06	9.97E-05	302.1436418	375.8634874	406.8001915	118.2706753	124.2180756	233.939469	Acb1a
ENSMUSG00000040855	1.071792937	4.60E-07	2.25E-05	584.3438089	647.8060622	645.5542543	259.9823853	223.3873661	409.1493642	Reps2
ENSMUSG00000040891	-2.088765162	3.05E-41	2.08E-37	406.846884	431.2232258	360.8894897	1650.461946	1922.770548	1525.990093	Foxa3
ENSMUSG00000041134	1.050155799	0.006573499	0.03826156	41.88129688	57.30218542	84.48220682	18.11352684	31.31548123	39.15304921	Cyp11
ENSMUSG00000041372	2.023614953	1.33E-05	0.000359411	51.85303424	49.53239756	28.46683056	9.580514211	12.52619249	9.788262303	Bqalp1t3
ENSMUSG00000041695	-1.532466883	9.09E-05	0.001683702	33.903907	18.45324615	23.87540627	101.22265	78.28870309	41.11070167	Kcnj2
ENSMUSG00000041702	1.159708962	1.79E-05	0.000451095	125.6438906	178.7051206	179.0655471	59.66808842	58.45556497	97.88262303	Btbd7
ENSMUSG00000041920	-1.612549242	5.78E-11	8.29E-09	130.6297593	116.5468178	162.5364196	586.0258684	355.9526367	312.2455675	Sic16a6
ENSMUSG00000041930	-2.069670852	7.78E-10	8.92E-08	29.91521206	25.25181052	29.8511541	157.6942337	126.3057743	71.45431481	Fam22a
ENSMUSG00000041945	-2.034435588	0.000645414	0.007184911	35.89825447	23.30936356	20.20226685	3.196504737	5.129246872	10.76708853	Mfs9
ENSMUSG00000042010	1.335313237	1.72E-08	1.42E-06	9652.641758	5265.973717	7079.05796	4018.006454	2149.285862	2550.821156	Acab
ENSMUSG00000042115	1.249312452	6.34E-07	3.01E-05	57.83607665	47.5899506	51.42395197	117.2051737	124.2180756	131.1627149	Llchc8a
ENSMUSG00000042246	-1.458803387	0.000333225	0.004503661	16.9519535	19.42446963	23.87540627	77.78161526	33.40317998	54.81462889	Tmc7
ENSMUSG00000042333	1.17659167	0.000212792	0.003210009	83.76259377	76.72665505	64.27993997	30.89954579	27.14008374	41.11070167	Trnf1f14
ENSMUSG00000042354	-1.004338102	0.000195353	0.003017019	312.1153791	284.5684801	269.057463	922.7243674	424.8466954	389.5728396	Gm13
ENSMUSG00000042379	-1.128744357	0.001182685	0.011115132	63.81911906	39.82016275	89.99191596	140.6462084	79.33255246	203.5958559	Em1
ENSMUSG00000042444	1.165704626	4.84E-10	5.85E-08	592.3211988	520.5757862	674.0210848	255.720379	221.2960674	319.0973511	Mindy2
ENSMUSG00000042510	-1.154445757	0.002024484	0.016651324	28.91803832	29.13670445	26.63026084	94.82964053	45.92937248	47.96248528	A996860
ENSMUSG00000042607	-1.584091426	0.001074406	0.010435667	12.96325856	6.798564371	14.69255771	37.29255526	24.00835361	42.0895279	Asb4
ENSMUSG00000042622	-1.343729967	0.00016867	0.001912519	29.91521206	22.33814008	31.22168513	89.50213263	50.10476998	72.43314104	Meff
ENSMUSG00000042680	1.086909564	0.000262276	0.00374505	262.2566924	328.2735368	358.1310941	87.37112948	114.8234312	243.7277313	Grem1
ENSMUSG00000042743	1.143476031	0.005459928	0.03421057	39.88694941	35.93526882	50.5066712	15.98252368	14.6189124	26.42830822	Sgltb
ENSMUSG00000042745	-1.243661764	2.94E-08	2.25E-06	425.793185	215.6116129	317.7265604	726.6720769	889.3596671	654.834748	lcl1
ENSMUSG00000043165	-1.65740279	0.002445867	0.019147761	6.980216147	13.59712874	9.182848567	13.85152053	41.75397498	38.17422298	Lor
ENSMUSG00000043421	-1.515224307	3.07E-05	0.000711553	31.90955953	28.16548097	33.9765397	138.5152053	55.32401685	75.36961973	Hilpa
ENSMUSG00000043639	1.042566706	0.00209562	0.017092091	83.76259377	70.89931416	87.23706139	20.24453	46.97322185	49.92013774	Rbm20
ENSMUSG00000043681	-1.124534949	2.43E-09	2.42E-07	201.4290945	238.9209765	202.0226685	543.4058053	474.9514654	382.721056	Fam25c
ENSMUSG00000044042	1.154045613	3.06E-05	0.000711225	258.2679974	274.8562453	353.5396698	108.681161	85.59564871	203.5958559	Fmn1
ENSMUSG00000044186	1.148916053	0.009243418	0.048746497	35.89825447	33.99282186	30.30340027	17.04802526	10.43849473	17.61887214	Nkx2-6
ENSMUSG00000044339	-1.202694639	0.000240284	0.0035008	44.87281809	27.19425749	28.46683056	61.79909158	89.77104621	79.28492465	Alkbh2
ENSMUSG00000044349	1.020247724	0.000864482	0.008883909	70.5167087	218.5252834	182.7386865	148.1047195	83.50794996	50.8986397	Snhg11
ENSMUSG00000044359	1.419594491	2.01E-07	1.12E-05	221.3725692	303.9929497	263.5477539	69.25760263	76.20100434	148.781587	P2y2
ENSMUSG00000044676	-1.235532178	3.06E-06	0.000111153	173.5082299	206.8706016	146.0072922	609.4669032	360.1280342	270.1560396	Zfp612
ENSMUSG00000044749	1.260739468	0.003720434	0.025546608	2581.682801	3424.533996	3631.816608	1050.584557	874.7457758	2096.645785	Abca6
ENSMUSG00000044948	-1.380324256	9.28E-05	0.001711935	28.91803832	18.45324615	23.87540627	73.51960895	52.19246872	59.70840005	Cfap43
ENSMUSG00000045045	1.563883127	9.68E-08	6.14E-06	106.6975897	117.5180413	171.7192682	38.35805684	38.62242686	56.77192136	Lrn4
ENSMUSG00000045294	1.216661032	2.49E-17	1.21E-14	16820.32657	15644.46784	18334.47545	8335.418852	7066.860265	6456.337815	Insig1
ENSMUSG00000045348	-1.535176629	0.002140436	0.017319005	17.94912724	8.741011335	9.182848567	38.35805684	44.8855231	20.55535084	Nvap1
ENSMUSG00000045382	-1.705364762	8.30E-05	0.001575106	17.94912724	15.53957571	23.87540627	89.50213263	68.89405872	29.36478691	Cxcr4
ENSMUSG00000045411	-1.265704961	6.98E-06	0.000215157	217.3838743	180.6475676	227.7346445	798.0606826	403.9697079	303.4361314	2410002F23Rik
ENSMUSG00000045776	1.435732199	4.30E-15	1.59E-12	1029.083295	1166.439401	1214.890865	514.6372626	321.5056073	424.8105839	Lrtm1
ENSMUSG00000045875	1.205673082	0.000127777	0.002749319	91.73998365	131.11517	117.5404617	28.76854263	45.92937248	72.43314104	Aclra1a
ENSMUSG00000046541	1.523461987	5.84E-06	0.000185032	69.80216147	95.1799012	84.48220682	22.37553316	22.96468624	41.11070167	Zfp526
ENSMUSG00000046721	-1.016110295	0.006334733	0.037187581	17.94912724	26.223034	22.95712142	46.88206947	46.97322185	42.0895279	Rpl14-ps1
ENSMUSG00000046908	-1.354651164	0.005396039	0.033236578	15.95477976	8.741011335	10.10113342	36.22705368	28.18393311	24.70655756	Lrb41
ENSMUSG00000047496	1.093888518	1.32E-05	0.00035939	433.7705749	520.5757862	451.7961495	124.6636847	234.8661093	298.542002	Rnf152
ENSMUSG00000047649	-1.227032883	5.45E-06	0.000175137	72.79368268	67.01442023	64.27993997	120.9683126	155.5335568	111.5861902	Cd3eap
ENSMUSG00000047875	1.019701978	0.000156296	0.002567963	101.711721	104.892136	85.40049167				

Table 3 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRt15-TGFRs (TGFRt15-TGFRs group) in aged mice liver

ENSMUSG00000049580	-1.860685334	2.05E-30	5.59E-27	548.4455544	407.9138623	479.3446952	1707.999031	1943.647535	1562.206663	Tsku
ENSMUSG00000049791	1.116973741	1.01E-06	4.43E-05	636.1968431	678.8852137	759.4215765	215.231319	290.1901261	450.2600659	Fzc4
ENSMUSG00000049950	-1.164244145	0.007174451	0.040867998	18.94630097	37.87771578	21.1205517	94.82964053	44.8855231	35.23774429	Rpp38
ENSMUSG00000050390	1.148547066	4.80E-15	1.72E-12	1574.537328	1571.439593	1292.026793	692.5760263	634.6604197	674.4112726	C7080
ENSMUSG00000050503	-1.747508686	0.001612805	0.014098606	6.980216147	9.712234816	7.346278854	25.5720379	38.6224686	16.64004591	Fbxl22
ENSMUSG00000050663	1.366962359	0.003061462	0.022321797	20.94064844	51.47484453	44.07767312	15.98252368	13.57004187	15.66121968	Trhee
ENSMUSG00000050737	-1.262831751	0.008424555	0.045643887	26.92369085	13.59712874	44.99595798	119.3361768	55.32401685	31.32243937	Ptges
ENSMUSG00000050914	-2.867341906	0.000724516	0.007829023	13.96043229	23.30936356	14.68255771	273.8339058	58.45556497	46.98365905	Ankrd37
ENSMUSG00000051149	1.736018564	0.001879809	0.015833824	31.90955953	33.02159838	39.48624884	6.393009474	3.131548123	21.53417707	Adnp
ENSMUSG00000051339	1.051356464	2.17E-16	9.53E-14	1923.548135	1976.439785	1850.343986	917.3968595	914.4120521	942.6096597	2900026A02Rik
ENSMUSG00000051452	1.412708477	1.55E-10	2.11E-08	276.2171247	240.8634234	283.7500027	92.69863737	80.37640184	127.2474099	Gm11437
ENSMUSG00000051674	1.054513908	9.53E-09	8.39E-07	515.5388212	743.9571869	608.82286	295.1439374	270.356988	333.7797445	Dcn1e4
ENSMUSG00000051998	1.5364341	0.002646663	0.020230437	9.971737353	19.42446963	6.427993997	42.62006316	39.66627623	21.53417707	Lax1
ENSMUSG00000052085	1.272820714	3.30E-11	4.99E-09	423.7988375	546.7988202	446.2864404	197.1177921	161.796653	227.0876854	Dcfc8
ENSMUSG00000052595	1.233431784	0.003777269	0.025781088	1666.277312	2467.878867	2847.601341	821.5017174	661.8005034	1485.85218	Aclf
ENSMUSG00000052656	1.063492715	4.09E-12	7.63E-10	4427.451385	3448.814583	3382.043127	1950.933391	1650.325861	1785.379044	Rnf103
ENSMUSG00000052684	1.26752533	1.09E-07	6.78E-06	161.5421451	161.223098	181.8204016	547.6678116	268.2692892	399.3611019	Jun
ENSMUSG00000052713	1.631077965	2.29E-06	8.94E-05	74.78803015	84.4964429	102.8479039	23.44103474	18.78928874	42.0895279	Zfp608
ENSMUSG00000052837	1.91589408	1.98E-18	1.08E-15	205.4177895	302.0505028	276.4037419	1233.850828	697.2918322	1027.767542	Junb
ENSMUSG00000053560	-1.145431158	4.41E-10	5.44E-08	324.081464	268.0576809	242.4272022	725.6065733	569.9417585	550.1003414	Ier2
ENSMUSG00000053964	-1.860738134	2.78E-14	8.62E-12	110.7145473	119.4604882	153.3535711	348.4190163	621.0903778	388.5940134	Lgals4
ENSMUSG00000053977	-1.428502316	0.007562752	0.042337131	8.974563618	28.16548097	7.346278854	57.53708526	21.92083686	40.13187544	Col8a
ENSMUSG00000054008	1.359856014	0.005424595	0.033352211	3190.955953	3047.699285	3392.144261	753.3096163	842.386445	2156.354185	Nest1
ENSMUSG00000054150	1.583267643	1.23E-07	7.44E-06	98.72019979	105.8633595	78.97249768	33.03054895	29.22778249	32.3012656	Syne3
ENSMUSG00000054422	-1.006699851	5.17E-07	2.51E-05	21378.40771	26089.97639	27702.81756	57173.74923	60050.56682	33819.42508	Fabp1
ENSMUSG00000054453	1.090734081	0.000462815	0.005711685	93.73433112	87.41011335	126.7233102	69.25760263	36.53472811	39.15304021	Sytl5
ENSMUSG00000054659	1.244126007	0.002259781	0.018053098	79.77389882	73.8129846	100.0930494	15.98252368	25.05238499	65.58135743	Pm2ox2
ENSMUSG00000054932	1.219377704	9.42E-05	0.001742007	87.75128871	202.0144842	233.2443536	72.45410737	72.02560684	80.26375088	Afp
ENSMUSG00000055148	-1.210391107	1.04E-07	6.56E-06	210.4036581	153.4533101	137.7427285	476.2792058	390.3996661	293.6478691	Klf2
ENSMUSG00000055254	1.702350158	1.36E-13	3.56E-11	1260.427601	1100.396205	2139.603716	409.1526063	418.5835992	554.9944726	Ntrk2
ENSMUSG00000055491	-1.429157403	5.36E-08	3.69E-06	191.4573572	205.8993781	144.1707225	706.4275469	442.5921348	309.3090888	Pprc1
ENSMUSG00000055660	1.21539125	9.47E-05	0.001725945	86.75411497	97.12234816	112.0307525	29.83404421	36.53472811	60.68722628	Mett14
ENSMUSG00000055692	-1.507668302	6.91E-05	0.001367589	23.93216965	24.28058704	19.28398199	90.56763421	57.417156	44.04718036	Tmem191c
ENSMUSG00000055980	1.007478258	8.74E-05	0.001638705	336.0475488	377.8059344	381.0882155	114.0086689	159.7089543	270.1560396	Irs1
ENSMUSG00000056054	-4.00774487	1.39E-20	1.11E-17	6.980216147	6.798564371	4.591424283	100.1571484	98.1218412	95.92497057	S100a8
ENSMUSG00000056071	-3.639131514	1.15E-24	1.74E-21	15.95477976	8.741011335	12.85598799	140.6462084	203.350628	123.332105	S100a9
ENSMUSG00000056091	-1.031452251	1.62E-09	1.68E-07	1990.358776	1481.115809	2190.109383	3671.718441	3506.290049	4394.929774	St3gal5
ENSMUSG00000056148	1.3596028	0.000119043	0.002094688	75.78520388	1088.7770299	71.62621882	23.44103474	50.89896397	30.34361314	Nfe2
ENSMUSG00000056313	-1.726808914	2.85E-11	4.37E-09	435.7649223	244.7483174	415.0647552	1762.339612	990.6130564	874.0918236	Tcm
ENSMUSG00000057342	1.093040789	8.77E-15	2.92E-12	1269.402165	1384.964685	1236.011417	564.7158369	644.0550641	614.7028726	Sphk2
ENSMUSG00000057604	-1.272122676	0.008168023	0.044709526	10.96891109	10.6834583	11.93770314	25.5720379	28.18393311	27.40713445	Lmnc1
ENSMUSG00000057722	-1.840565518	1.78E-07	1.02E-05	45.86999182	35.93526882	110.1941828	323.91248	199.3752305	166.4045951	Lepr
ENSMUSG00000057969	1.066228289	0.000219235	0.003267437	95.7867859	74.78420809	99.17476452	49.01307263	42.79782435	37.19539675	Sema3b
ENSMUSG00000058207	-1.338541988	1.08E-13	2.88E-11	23432.58561	23413.28447	28330.92444	48372.70618	79528.79614	62218.1105	Serpina3k
ENSMUSG00000058503	-1.136818503	6.67E-07	3.10E-05	130.6297593	164.1367684	153.3535711	376.1220574	383.0927204	227.0876854	Fam133b
ENSMUSG00000058793	1.056167061	4.57E-10	5.57E-08	3494.096768	3071.008649	3164.409616	1397.938072	1320.469459	1959.610113	Cds2
ENSMUSG00000058794	1.61977926	2.23E-06	8.73E-05	144.5901916	102.9466891	144.1707225	71.38860579	26.09623436	30.34361314	Nfe2
ENSMUSG00000058921	1.372756574	4.51E-07	2.22E-05	911.4167941	1126.619239	1263.559963	261.0478868	329.8564023	683.2207087	Sic10a5
ENSMUSG00000059149	1.084964814	0.00015449	0.002547498	343.0277649	296.2231619	348.9482455	147.0392179	80.37640184	237.854774	Mfsd4a
ENSMUSG00000059824	4.510710868	5.51E-13	1.32E-10	248.2962601	730.3600582	248.8551962	24.50653632	16.70158999	12.72474099	Dbp
ENSMUSG00000060429	1.717674566	1.22E-12	2.68E-10	734.9170429	831.3673003	944.9151175	201.3797984	180.5859418	380.7634036	Sntb1
ENSMUSG00000061175	1.152852085	3.29E-14	9.98E-12	1100.879804	976.079599	989.9110755	495.4852342	405.0135573	478.6406266	Frip2
ENSMUSG00000061292	1.112980568	1.04E-05	0.000296719	1260.427601	1520.935972	1350.797024	523.1612753	399.7943104	986.6568401	Cyp3a59
ENSMUSG00000061436	1.042732241	2.40E-05	0.000578522	326.0758114	431.2232258	663.9199514	228.0173379	186.849038	275.0501707	Hipk2
ENSMUSG00000061536	1.045389319	1.00E-05	0.000287463	219.3782218	260.2878931	199.2678139	88.43663105	94.99029308	144.8662821	Sec22c
ENSMUSG00000061825	1.826944112	9.41E-14	2.63E-11	718.9622632	690.5398954	678.6125091	298.3404421	160.7528037	130.1838886	Ces2c
ENSMUSG00000062901	1.319464488	6.22E-09	5.70E-07	788.7644246	893.5256031	992.6659301	297.2749405	259.9184942	513.8837709	Klhl24
ENSMUSG00000063535	1.201850675	0.000151738	0.002508185	62.82194532	71.87053764	70.70793397	27.70304105	29.22778249	32.3012656	Zfp773
ENSMUSG00000063704	-1.127471271	2.47E-07	1.32E-05	294.1662519	311.7627376	224.061505	581.7638621	784.9747296	446.344761	Mapk15
ENSMUSG00000063929	-1.198836957	5.83E-06	0.000185032	50.8558605	105.8633595	82.6456371	208.8383095	167.0158999	174.231069	Cyp4a32
ENSMUSG00000065126	-1.713055356	1.38E-07	8.18E-06	29.91521206	48.56117408	40.40453369	154.497729	158.6651049	17.32727219	Snord104
ENSMUSG00000065147	-1.294541943	0.007255788	0.041125179	14.95760603	12.62590526	15.61084256	54.34058053	32.35933061	19.57652461	Snora31
ENSMUSG00000065952	1.07267976	1.62E-10	2.19E-08	199.4347471	157.338204	188.2483956	51.14407579	55.32401685	76.34844596	C30021F23Rik
ENSMUSG00000066456	1.032022635	0.005405213	0.033278057	76.78237762	64.10074979	150.5987165	70.32310421	34.44702936	38.17422298	Hmgn3
ENSMUSG00000066477	-1.128543552	2.29E-05	0.000561201	57.83607665	44.67628015	49.58738226	93.76413895	118.9988287	119.4168001	Gm16551
ENSMUSG00000066687	-2.591322343	5.98E-23	6.28E-20	139.6043229	122.3741587	161.6181348	472.0171995	943.6398345	1137.39608	Zbrb16
ENSMUSG00000066944	1.544707207	0.000412457	0.005261624	43.87564435	49.53239756	40.40453369	10.65501579	10.43849374	24.47065576	NA
ENSMUSG00000067149	-1.843573184	0.000282252	0.003980428	113.6778058	169.9641093	162.5364196	437.921149	878.9211733	284.838433	Jchain
ENSMUSG00000068463	2.382320454	0.000472976	0.005821273	4.985868677	3.884893927	5.50970914	46.88206947	15.65774062	12.72474099	B630019A10Rik
ENSMUSG00000068742	1.190690012	3.19E-12	6.14E-10	611.2674997	770.1802209	691.4684971	312.1919626	260.9623436	334.7585707	Cry2
ENSMUSG00000068877	-1.095647878	6.50E-07	3.04E-05	98.7						

Table 3 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGF α Rt15-TGFRs (TGF α Rt15-TGFRs group) in aged mice liver

ENSMUSG000000071076	-1.243260003	3.56E-08	2.62E-06	1324.24672	1340.288405	938.4871235	3733.517533	2948.874483	1847.045097	Jund
ENSMUSG000000071456	1.345823726	0.001263422	0.011712633	45.86999182	70.89931416	48.6690974	12.78601895	16.70158999	35.23774429	Nt5ec2
ENSMUSG000000071547	-1.168738292	0.00025901	0.003706323	44.87281809	25.25181052	47.75081255	90.56763421	104.3849374	70.47548858	Cebpd
ENSMUSG000000071637	-1.028910519	0.000250761	0.0036106	107.6947634	64.10074979	89.99191596	239.7378553	123.1742262	171.2945903	Tut1
ENSMUSG000000071645	-1.14015686	5.54E-07	2.68E-05	206.4149632	273.8850218	188.2483956	605.2048969	525.0562354	343.5680068	Klf12
ENSMUSG000000072294	2.084673623	3.53E-08	2.62E-06	97.72302606	148.5971927	196.5129593	24.50653632	18.78928874	60.68722628	Tmem253
ENSMUSG000000072571	1.01455077	0.003907572	0.026379983	56.83890291	47.5899506	55.0970914	20.24453	29.22778249	29.36478691	Ugr3a1
ENSMUSG000000072664	1.275253331	1.57E-06	6.41E-05	951.3037435	1401.475484	1131.326943	273.8339058	440.504436	724.3314104	Rpl37r
ENSMUSG000000072692	-1.135811828	0.000365333	0.004818223	34.90108074	29.13670445	28.46683056	73.51960895	70.98175747	58.72957382	Serpina1e
ENSMUSG000000072849	-2.106422019	7.14E-21	6.08E-18	100.7145473	96.15112468	102.8479039	570.0433447	342.3825948	378.8057511	Gm15401
ENSMUSG000000072999	-1.145851135	1.86E-07	1.05E-05	116.669327	176.7626737	168.0461288	294.0784358	417.5397498	310.287915	Prndc1
ENSMUSG000000073460	-1.28577089	0.005211876	0.032439227	18.94630097	39.82016275	10.10113342	51.14407579	79.33255246	69.83790809	Mup-ps12
ENSMUSG000000073835	2.295483419	0.000631649	0.007089542	332.0588539	185.530685	325.0728393	85.24012632	69.93790809	16.64004591	Gm22935
ENSMUSG000000074024	1.08003527	4.28E-05	0.000936631	43.87564435	61.18707934	53.26052169	118.2706753	117.9549793	98.86144926	Osgin1
ENSMUSG000000074063	-2.210318446	1.23E-07	7.44E-06	989.1963454	819.7126185	1493.131177	2843.823714	6567.900264	5870.020903	Gm10642
ENSMUSG000000074213	1.225081814	0.003513884	0.024460869	87.75128871	38.84893927	37.64967912	24.50653632	27.14008374	18.59769837	Tnfrsf813
ENSMUSG000000074345	1.610694413	1.86E-06	7.43E-05	95.1799012	56.93366111	26.63753947	24.00853561	29.36478691	70.47548858	Sult2a3
ENSMUSG000000074375	1.123374183	1.71E-16	7.76E-14	1739.070994	2059.965005	1850.343986	860.9252758	876.8334746	855.4941252	Spat51l
ENSMUSG000000074876	1.013446872	0.000205306	0.003120937	101.711721	119.4604882	117.5404617	71.38860579	41.75397498	54.81426889	Deaf1
ENSMUSG000000075470	1.495450545	0.008033121	0.044154642	346.0192861	387.5181692	422.4110341	77.78161526	80.37640184	251.5583142	Cyp3a41b
ENSMUSG000000075552	1.02486804	2.18E-07	1.20E-05	1632.373405	1558.813688	2640.068963	1023.947017	947.815232	894.6471745	Nrbp2
ENSMUSG000000075590	-1.36961476	1.11E-14	3.51E-12	556.4229443	571.0794072	507.8115258	1682.426993	1456.169877	1087.475942	Tbrc1
ENSMUSG000000076490	-1.690670704	0.002848332	0.021272015	9.971737353	4.856117408	8.26456371	35.1615521	20.87688749	18.59769837	Igkv3-10
ENSMUSG000000076569	-1.340585852	0.000314538	0.004311858	26.92369085	70.89931416	46.83252769	72.45410737	110.6480337	183.0405051	Igkv3-39
ENSMUSG000000076596	-3.365323343	1.13E-07	6.96E-06	3.988694941	6.798564371	9.182848567	152.3667258	28.18393311	26.42830822	Igkv3-10
ENSMUSG000000076609	-2.421594655	3.22E-06	0.000115075	484.6264354	675.0003197	704.3244851	4135.211628	4467.675323	1384.06029	Igkc
ENSMUSG000000076613	-1.082440472	0.000721125	0.007817157	31.90955983	61.18707934	74.38107339	100.1571484	155.5335568	99.84027549	Ighg2b
ENSMUSG000000076617	-1.218011643	6.44E-08	4.32E-06	1755.025774	1885.144778	1859.526835	5037.691465	5276.658588	2480.345667	Ighm
ENSMUSG000000076934	-3.037010136	1.44E-09	1.52E-07	7.977389882	2.913670445	10.10113342	77.78161526	36.53472811	58.72957382	Igvl1
ENSMUSG000000077148	1.93809708	8.47E-06	0.000252998	48.86151303	38.84893927	69.78964911	9.589514211	19.8313812	11.74591476	Gm22935
ENSMUSG000000078193	-1.269506646	0.004582659	0.029506003	13.96043229	9.712234816	21.1205517	42.62006316	35.49087873	30.34361314	Gm2000
ENSMUSG000000078234	1.031251449	6.47E-07	3.04E-05	557.420118	503.0937635	599.6400114	197.1177921	263.0500424	351.3986167	Hnf1a2
ENSMUSG000000078650	-1.017019231	3.99E-07	2.00E-05	5816.514398	3628.490927	4180.950953	6922.563759	10279.82864	10371.64274	G6pc
ENSMUSG000000078651	-2.099102297	6.20E-07	2.95E-05	8.974563618	10.6834583	16.52912742	55.40680211	57.4117156	43.06835413	Aoc2
ENSMUSG000000078672	-1.051061383	1.66E-05	0.000428362	108.6919371	197.1583668	152.4352862	337.7640005	374.7419254	237.854774	Mup2
ENSMUSG000000078688	1.787709931	3.76E-11	5.58E-09	119.6608482	143.7410753	173.558379	49.01307263	38.6224686	39.15304921	Mup2
ENSMUSG000000078817	-1.628755428	1.37E-11	2.31E-09	107.6947634	79.64032549	80.8090639	204.5763032	280.7954817	342.5891806	Nlrp12
ENSMUSG000000079017	-1.001509959	0.000541038	0.006404635	74.78803015	60.21585586	44.07767312	145.9737163	124.2180756	88.09436072	Ifi272a
ENSMUSG000000079036	-1.378588057	0.008915895	0.047568881	170.5167087	124.3166056	106.5210434	575.3708526	288.1024274	180.1040264	Alkbh1
ENSMUSG000000079065	-1.153356662	4.39E-06	0.00146637	123.6495432	164.1367684	125.8050254	371.8600511	353.864938	194.7864198	Bco05561
ENSMUSG000000079465	-1.030415111	0.006780187	0.03914539	18.94630097	27.19425749	22.03883656	46.8806947	56.36786622	36.21657052	Col4a3
ENSMUSG000000079470	1.222302248	2.09E-11	3.33E-09	498.5868677	463.2736007	474.7532709	201.3797984	169.1035897	244.7065576	Utp14b
ENSMUSG000000080059	-1.546208951	0.008392591	0.045561211	7.977389882	9.712234816	6.427993997	37.29255526	13.57004187	19.5765218	Rps19-ps3
ENSMUSG000000081344	-1.007584184	0.007335419	0.041406148	22.93499591	19.42446963	38.56796398	64.9959632	55.32401685	43.06835413	Gm14303
ENSMUSG000000082065	2.441656125	0.003600075	0.02496215	179.4912724	141.7986283	255.2831902	60.73359	39.66627623	5.872957382	Mup-ps14
ENSMUSG000000082173	2.591199516	2.10E-11	3.33E-09	150.573234	114.6043708	224.061505	41.55456158	31.3154821	8.809436072	Mup-ps10
ENSMUSG000000082586	1.935822229	3.52E-06	0.000123572	53.84738171	55.35973845	47.75081255	15.98252368	7.306945621	17.61887214	Sult2a-ps1
ENSMUSG000000082658	-1.204694651	0.004700851	0.030195718	67.807814	23.30936356	23.87540627	85.24012632	129.4373224	49.92013774	Fau-ps2
ENSMUSG000000083327	1.031096879	0.000937166	0.009424872	102.7088947	96.15112468	82.6456371	36.22705368	34.44702936	66.56018366	Vcp-ps
ENSMUSG000000083621	-1.163358375	0.003315291	0.023547198	19.94347471	19.42446963	20.20226685	54.34058053	48.01707123	31.32243937	Gm14586
ENSMUSG000000083716	-1.168157812	0.001666635	0.01443041	25.92651712	25.25181052	35.81310941	70.32310421	86.63949808	39.15304921	Gm13436
ENSMUSG000000083813	-1.588176086	8.35E-05	0.001580916	11.96608482	53.41729149	22.03883656	86.3056279	84.55179933	92.00966564	Gm15502
ENSMUSG000000083863	-1.215047234	0.000627696	0.007061263	24.92934338	19.42446963	27.5485457	64.9959632	54.28016747	47.96248528	Gm13341
ENSMUSG000000083992	-1.283026783	0.003457083	0.024176532	11.96608482	12.62590526	20.20226685	39.42355842	40.71012561	29.36478691	Gm11478
ENSMUSG000000084822	-1.954137616	1.52E-07	8.88E-06	16.9519535	25.25181052	13.77427285	66.0610979	91.85874496	58.72957382	Myadml2os
ENSMUSG000000084883	1.839966406	9.14E-09	8.09E-07	157.5534502	141.7986283	126.7233102	24.50653632	30.27163186	63.62370497	Cecce8c
ENSMUSG000000085001	-2.14213554	3.42E-08	2.56E-06	29.91521206	16.51079919	21.1205517	156.6287321	70.98175747	70.47548858	Rapgef4os2
ENSMUSG000000085156	-1.793249778	0.00014396	0.002408808	24.2934338	31.07915141	16.52912742	157.6942337	59.49941435	34.25891806	Snhg15
ENSMUSG000000085445	-1.469856228	8.55E-08	5.52E-06	45.86999182	46.61872712	72.54450368	126.7946879	143.0073643	187.9343662	Gm16348
ENSMUSG000000085834	-3.692490083	4.07E-39	1.85E-35	50.8558605	56.33096193	71.62621882	1120.907661	744.264604	450.2600659	Gm15622
ENSMUSG000000085995	-1.230019641	9.11E-06	0.000268289	189.4630097	167.0504388	353.5396698	444.3141584	772.4485371	450.2600659	Gm2788
ENSMUSG000000086140	1.216209638	0.003436989	0.024085413	34.90108074	60.21585586	44.99595798	11.72051737	22.96468624	25.44948199	Hnf1a2os2
ENSMUSG000000086446	-2.158985376	0.000113634	0.002012508	17.94912724	6.798564371	9.182848567	27.70304105	97.07799183	26.42830822	Prkg2os1
ENSMUSG000000086529	2.155075918	4.95E-05	0.001053778	27.92086459	38.84893927	44.07767312	13.85152053	8.350794996	2.936478691	Acss2os
ENSMUSG000000086786	1.909493382	0.004138057	0.027366965	31.90955953	14.56835222	33.05825484	2.131003158	3.131548123	15.66121968	Gm15908
ENSMUSG000000086844	-1.797314635	1.20E-05	0.000331862	10.96891109	17.48202267	18.36569713	73.51960895	42.79782435	46.98365905	B230206H07Rik
ENSMUSG000000087382	-1.074896574	1.10E-05	0.000308734	161.5421451	285.5397036	136.8244436	414.4801142	369.5226786	445.3659348	Ctcfios
ENSMUSG000000087445	-1.629934605	4.98E-05	0.001058506	21.93782218	12.62590526	15.61084256	61.79909158	54.28016747	39.15304921	Gm14286
ENSMUSG000000087595	-1.356715257	0.000782501	0.008272069	15.95477976	21.3669166	17.44741228	41.55456158	62.63096247	36.21657052	1810012K08Rik
ENSMUSG000000087613	1.3004									

Table 3 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRt15-TGFRs (TGFRt15-TGFRs group) in aged mice liver

ENSMUSG000000090145	1.252091278	1.05E-06	4.56E-05	514.5416474	669.1729788	606.0680054	154.497729	220.252218	375.8692724	Ugr1a6b
ENSMUSG000000090175	1.660039728	0.006419999	0.037574902	840.6174589	1098.453758	415.0647552	125.7291863	209.8137243	409.1493642	Ugr1a9
ENSMUSG000000090264	2.18334921	1.38E-07	8.18E-06	7.977389882	12.62590526	21.1205517	57.53708526	80.37640184	52.85661643	Eif4ebp3
ENSMUSG000000090369	1.713925914	3.56E-08	2.62E-06	82.76542003	126.2590526	106.5210434	27.70304105	27.14008374	41.11070167	4933411K16Rik
ENSMUSG000000090555	2.901973235	9.10E-10	1.01E-07	121.6551957	110.7194769	103.7661888	1396.87257	624.2219259	491.3707676	Gm3893
ENSMUSG000000090610	1.076721434	0.00270073	0.020438322	79.77389882	53.41729149	47.75081255	35.16155211	28.18393311	22.5130033	Gm3571
ENSMUSG000000090698	2.55687976	1.21E-08	1.04E-06	20.94064844	19.42446963	24.79369113	247.1963663	60.54326372	76.34844596	Apold1
ENSMUSG000000091021	1.527245218	0.003008341	0.022044462	10.96891109	16.51079919	18.36569713	82.04362158	27.14008374	23.49182953	Gm17300
ENSMUSG000000091509	1.002937779	0.000805178	0.008420412	69.80216147	64.10074979	68.87136425	143.8427132	183.7174899	79.28492465	Gm17066
ENSMUSG000000092075	5.716673555	4.92E-16	1.97E-13	2.991521206	2.913670445	0	83.10912316	163.8843518	57.75074759	Serpina4-ps1
ENSMUSG000000094410	1.220382244	0.001002234	0.009882478	53.84738171	132.0863935	116.6221768	47.94757105	25.05238499	56.77192136	Gm38394
ENSMUSG000000095280	1.650205335	4.03E-05	0.000893784	58.83325038	25.25181052	24.79369113	126.7946879	158.6651049	55.79309512	Gm21738
ENSMUSG000000095351	3.092804449	0.006028299	0.035789253	6.980216147	60.21585586	166.2095591	629.7114332	1051.15632	311.2667412	Igkv3-2
ENSMUSG000000096833	3.208484861	0.004128622	0.02736411	10.96891109	124.3166056	23.87540627	3.196504737	4.175397498	9.788262303	Igkv4-55
ENSMUSG000000096910	1.124675965	0.006036	0.035819378	64.81629279	67.01442023	72.54450368	34.09605053	10.43849374	48.94131151	Zfp955b
ENSMUSG000000096954	1.206343759	0.003120612	0.022529545	33.903907	87.41011335	81.72735225	33.03054895	17.74543937	37.19539675	Gdap10
ENSMUSG000000097124	2.16107918	3.03E-13	7.50E-11	133.6212805	103.9209125	130.3964497	23.44103474	33.40317998	25.44948199	A530020G20Rik
ENSMUSG000000097221	1.550011559	0.000809985	0.008457711	46.86716556	44.67628015	42.24110341	8.524012632	9.39464437	27.40713445	1810049J17Rik
ENSMUSG000000097312	1.433041605	0.000236458	0.003456142	49.85868677	34.96404534	33.9765397	114.0086689	161.796653	45.02600659	Gm26870
ENSMUSG000000097536	1.179506676	0.003789911	0.025815692	36.89542821	55.35973845	40.40453369	13.85152053	26.09623468	18.59769837	2610037D02Rik
ENSMUSG000000097615	1.278009058	7.24E-05	0.001413729	33.903907	25.25181052	25.71197599	69.25760263	65.76251059	70.47548858	Gm2061
ENSMUSG000000097660	2.932543755	3.83E-06	0.00131806	4.985868677	4.856117408	6.427993997	60.73359	53.2363181	10.76708853	Gm26762
ENSMUSG000000097691	1.274551418	4.53E-08	3.23E-06	235.3330015	259.3166696	175.3924076	75.65061211	98.1218412	102.7767542	9030616G12Rik
ENSMUSG000000097743	1.078516143	0.000185309	0.002900208	55.84172918	55.35973845	50.5066712	155.5632305	98.1218412	88.09436072	Gm16973
ENSMUSG000000097908	1.156232879	5.54E-05	0.001144881	154.561929	95.1799012	98.25647967	52.20957737	45.92937248	57.75074759	4933404012Rik
ENSMUSG000000097971	1.77227667	3.92E-13	9.56E-11	2017.282467	2103.670061	1313.147345	8345.008366	6440.550641	3776.311596	Gm26917
ENSMUSG000000097994	1.156597047	0.008206265	0.04482173	14.95760603	25.25181052	11.93770314	29.83404421	48.01707123	38.17422298	Gm26982
ENSMUSG000000098041	1.469996023	0.000332181	0.004498467	22.93499591	20.39569311	17.44741228	68.19210105	69.93790809	30.34361314	Gm26981
ENSMUSG000000098661	1.627002493	0.000277688	0.003923434	19.94347471	6.798564371	14.69255771	43.68556474	48.01707123	36.21657052	Mir7052
ENSMUSG000000098814	4.705165719	0.001128634	0.010794205	2.991521206	1.942446963	1.836569713	13.85152053	157.6212555	4.894131151	Igkv19-93
ENSMUSG000000098882	1.905068974	0.000139968	0.002360908	128.6354119	27.19425749	93.66055538	37.29255526	18.78928874	10.76708853	Mir6392
ENSMUSG000000099568	2.251364762	0.000320113	0.004365382	1.994347471	4.856117408	19.28398199	18.11352684	59.4994135	47.96248537	Gm28513
ENSMUSG000000099858	1.088333588	4.80E-05	0.001024996	154.561929	202.0144842	168.0461288	56.47158369	75.15715496	114.5226689	Gm6652
ENSMUSG000000100094	1.758512646	5.46E-23	6.38E-10	3175.001173	2305.684545	3195.631301	1017.554008	731.7384115	815.3622498	181000818Rik
ENSMUSG000000100468	1.995883006	0.000292618	0.004105376	17.94912724	36.9064923	34.89482455	6.393009474	6.263096247	9.788262303	Tmem167-ps1
ENSMUSG000000101939	1.264629649	0.000784437	0.008279693	14.95760603	28.16548097	21.1205517	52.20957737	56.36786622	46.00483282	Gm28438
ENSMUSG000000102275	1.950030022	0.000225327	0.003321924	4.985868677	13.59712874	14.69255771	56.47158369	54.2801677	18.59769837	Gm37144
ENSMUSG000000102577	1.649520189	1.70E-08	1.42E-06	40.88412315	64.10074979	82.6456371	137.4497037	252.6115486	199.680551	Gm37969
ENSMUSG000000102719	1.730894623	0.006769906	0.039102589	12.96325856	5.82734089	4.591424283	38.35806684	30.27163126	8.809436072	Gm37760
ENSMUSG000000102869	1.362067771	5.85E-06	0.000185032	292.1719044	335.0721012	361.8042335	86.3056279	81.42025121	216.3205969	2900097C17Rik
ENSMUSG000000102882	2.19227753	3.68E-07	1.87E-05	88.74846344	63.12952631	216.7152262	26.63753947	38.62246896	15.66121968	Gm2065
ENSMUSG000000102918	1.264206677	0.002814898	0.021114835	30.91238579	47.5899506	66.11650968	18.11352684	14.61389124	27.40713445	Pcchgc3
ENSMUSG000000103285	1.692405475	0.00296955	0.020438322	9.971737353	5.82734089	8.26456371	27.70304105	35.49087873	14.68239345	Gm37274
ENSMUSG000000103546	1.97976669	0.000414043	0.005267075	6.980216147	6.798564371	6.427993997	28.76854263	33.40317998	17.61887214	Gm37666
ENSMUSG000000104030	3.028455317	2.18E-07	1.20E-05	3.988694941	4.856117408	5.50970914	56.47158369	39.66627623	21.53417707	5330406M23Rik
ENSMUSG000000104388	2.61061807	5.15E-05	0.001078975	6.980216147	1.942446963	6.427993997	46.88206947	34.4702936	12.72474099	Gm37033
ENSMUSG000000104399	1.177892321	0.00272173	0.020574411	23.93216965	29.13670445	18.36569713	77.78161526	41.75397498	42.0895279	Gm37963
ENSMUSG000000104445	1.012908858	0.003001159	0.022015494	68.80498774	51.47484453	89.99191596	38.35805684	40.71012561	25.44948199	Rhbg
ENSMUSG000000104973	2.007621104	9.36E-05	0.001719883	6.980216147	13.59712874	4.591424283	36.22705368	32.35933061	32.3012656	A530041M06Rik
ENSMUSG000000105161	1.279752452	0.00691512	0.039789659	21.93782218	8.741011335	13.77427285	44.75106632	39.66627623	23.49182953	Gm42595
ENSMUSG000000105434	2.02241612	0.000742647	0.007975668	5.983042412	7.769787853	6.427993997	26.63753947	42.79782435	12.72474099	Gm43359
ENSMUSG000000105547	2.707072316	4.65E-06	0.000153843	5.983042412	2.913670445	8.26456371	23.44103474	27.14008374	61.66605251	Igfc3
ENSMUSG000000105556	1.504518371	0.00309422	0.022420764	21.93782218	29.13670445	33.05825484	7.458511053	9.39464437	12.72474099	Gm43080
ENSMUSG000000105703	1.801332207	3.38E-12	6.40E-10	242.3132177	122.3741587	160.6998499	712.8205563	708.7737253	409.1493642	Gm43305
ENSMUSG000000105881	1.259909682	7.19E-06	0.001410701	247.2990864	245.7195409	252.5283356	43.68556474	106.4726362	160.5275018	4932422M17Rik
ENSMUSG000000105906	2.671616891	0.003078263	0.022366209	6.980216147	20.39569311	14.69255771	182.20077	62.63096247	23.49182953	Igfc1
ENSMUSG000000106030	1.145150269	5.54E-05	0.001144881	109.6891109	147.6259692	198.349529	62.86459316	88.72719683	54.81426889	Gm43611
ENSMUSG000000106664	1.606191029	0.000152255	0.002513686	9.971737353	17.48202267	19.28398199	54.34058053	55.32401685	33.28009183	Gm17936
ENSMUSG000000106705	2.05446715	2.41E-09	2.41E-07	18.94630097	32.05037489	31.22168513	87.37112948	163.8843518	91.03083941	Gm2602
ENSMUSG000000106706	1.806072384	0.002749571	0.020715964	9.971737353	8.741011335	4.591424283	31.96504737	38.62246896	10.76708853	C530043K16Rik
ENSMUSG000000106943	1.230168101	0.009404129	0.049324655	9.971737353	13.59712874	13.77427285	30.89954579	34.4702936	22.5130033	Dancr
ENSMUSG000000107168	2.535260536	5.76E-05	0.00118059	1.994347471	9.712234816	5.50970914	46.88206947	39.66627623	13.70356722	Gm42507
ENSMUSG000000107225	3.314781204	7.31E-09	6.61E-07	3.988694941	1.942446963	6.427993997	46.88206947	43.84167373	33.28009183	Gm43637
ENSMUSG000000107304	1.772473058	0.001145745	0.010886341	7.977389882	10.6834583	11.93770314	28.76854263	59.49941435	16.64004591	Gm43775
ENSMUSG000000107390	1.580957843	0.000625578	0.007061263	12.96325856	23.30936356	11.93770314	46.88206947	70.98175747	26.42830822	Gm43323
ENSMUSG000000107624	2.865902972	2.57E-10	3.34E-08	8.974563618	13.59712874	11.93770314	103.3536532	111.6918831	37.19539675	Gm44005
ENSMUSG000000108368	1.782724603	5.57E-05	0.001147878	20.94064844	11.65468178	7.346278854	46.88206947	41.75397498	47.96248537	Gm45053
ENSMUSG000000108633	1.682864395	0.001365295	0.012400579	8.974563618	12.62590526	9.182848567	49.01307263	33.40317998	16.64004591	Gm44694
ENSMUSG000000108820	1.905612833	0.000888445								

Table 3 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRt15-TGFRs (TGFRt15-TGFRs group) in aged mice liver

ENSMUSG00000109536	-1.039899156	0.001827878	0.015463254	63.81911906	37.87771578	29.38511541	109.7466626	90.81489558	68.51783612	9330162G02Rik
ENSMUSG00000109555	-1.255499819	0.007385438	0.041635066	10.96891109	14.56835222	15.61084256	43.68556474	33.40317998	21.53417707	Gm44891
ENSMUSG00000109807	-2.757236067	2.47E-09	2.44E-07	11.96608482	5.82734089	12.85598799	75.65061211	94.99029308	37.19539675	Gm45244
ENSMUSG00000109836	1.535086391	0.000730915	0.007885666	46.86716556	68.9569672	89.07363111	12.78601895	12.52619249	45.02600659	Gm45884
ENSMUSG00000109841	1.421032277	0.000627493	0.007061263	46.86716556	57.30218542	33.05825484	13.85152053	15.65774062	21.53417707	E330011021Rik
ENSMUSG00000110588	-5.501119532	2.73E-06	0.000102051	31.90955953	14.56835222	3.673139427	1747.42259	289.1462767	231.9818166	Gm45774
ENSMUSG00000110613	1.168711997	0.002147567	0.017360028	64.81629279	58.2734089	38.56796398	26.63753947	16.70158999	28.38596068	Ln cbate1
ENSMUSG00000110702	-1.389252147	0.004080864	0.027194141	18.94630097	11.65468178	8.26456371	45.8165679	30.27163186	25.44948199	Gm45767
ENSMUSG00000110755	1.852337964	4.42E-06	0.000147317	194.4488784	92.26623075	176.3106925	79.91261842	28.18393311	20.55535084	BC049987
ENSMUSG00000111282	-1.321989153	0.002140143	0.017319005	21.93782218	18.45324615	30.30340027	87.37112948	62.63096247	27.40713445	Gm47528
ENSMUSG00000111312	1.254957582	0.001977069	0.016387107	45.86999182	33.99282186	56.93366111	13.85152053	20.87698749	22.5130033	Gm47205
ENSMUSG00000111631	-1.948317608	1.41E-05	0.000376102	11.96608482	15.53957571	11.01941828	28.76854263	66.80635997	52.85661643	Gm32017
ENSMUSG00000111709	2.018073354	0.00211263	0.017179449	31.90955953	69.92809068	7.346278854	12.78601895	8.350794996	5.872957382	Gm3776
ENSMUSG00000111774	2.335615682	0.000501244	0.006070575	17.94912724	15.53957571	39.48624884	5.327507895	7.306945621	1.957652461	AC166078.1

Table 4. RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRt15-TGFRs (TGFRt15-TGFRs group) in aged mice liver

ID	log2FoldChange	pvalue	padj	agedliver92181tox	agedliver92182tox	agedliver92183tox	agedliverpbs1	agedliverpbs2	agedliverpbs3	Gene.name
ENSMUSG000000000204	-2.005879896	3.88E-05	0.000867087	8.974563618	11.65468178	11.93770314	61.79909158	46.97322185	22.5130033	Sifr4
ENSMUSG000000000317	-1.462090887	1.76E-06	7.11E-05	60.82759785	43.70505667	55.0970914	92.69863737	140.9196656	205.5535084	Bcl6b
ENSMUSG000000000686	1.151464201	2.84E-06	0.000104278	355.9910235	571.0794072	469.2435618	171.5457542	161.796653	294.6266953	Ahcl15
ENSMUSG000000001227	-1.099312542	1.49E-06	6.21E-05	100.7145473	67.98564371	92.74677053	171.5457542	203.550628	184.9981575	Sema6b
ENSMUSG000000001403	-1.612087212	0.002110809	0.017174878	16.9519535	6.798564371	11.01941828	51.14407579	37.57857748	17.61887214	Ube2c
ENSMUSG000000001983	1.013926416	1.20E-05	0.000332241	308.1266842	343.8131125	299.3608633	128.9256911	122.1303768	219.2570756	Taco1
ENSMUSG000000002233	-1.270303057	1.05E-10	1.46E-08	133.6212805	104.892136	141.4158679	330.3054895	288.1024274	298.5420002	Rhoc
ENSMUSG000000002250	-1.142106413	9.77E-06	0.000282853	303.1408155	156.3669805	415.9830401	627.58043	657.6251059	647.9829644	Ppard
ENSMUSG000000002289	-2.433615292	5.35E-35	1.82E-31	340.0362437	320.5037489	528.0137926	1865.693265	2130.496573	2428.467877	Angptl4
ENSMUSG000000002831	-2.428567233	6.40E-14	1.90E-11	67.807814	71.87053764	51.42395197	228.0173379	574.117156	226.1088592	Plin4
ENSMUSG000000003032	-1.814709673	6.30E-10	7.35E-08	33.903907	28.16548097	44.99595798	155.5632305	107.5164856	114.5226689	Klrf4
ENSMUSG000000003348	1.035062446	0.006673687	0.038727262	47.86433929	51.47484453	59.68851569	13.85152053	30.27163186	33.28009183	Mob3a
ENSMUSG000000003500	-1.169914769	9.39E-05	0.001721093	55.84172918	53.41729149	52.34223683	174.742259	99.16569058	90.05201318	Impch1
ENSMUSG000000003541	-2.579058324	2.05E-08	1.65E-06	9.971737353	11.65468178	11.01941828	105.4845653	45.92937248	44.04718036	Irb1
ENSMUSG000000003848	-1.114660438	1.53E-05	0.000400378	168.5223613	191.3310259	135.9061588	522.0957737	291.2339755	260.3677772	Nob1
ENSMUSG000000004100	-2.152000208	2.40E-05	0.000578522	177.4969249	181.6187911	165.2912742	1249.833352	729.6507128	351.3986167	Ppan
ENSMUSG000000004933	-1.661199536	0.00641801	0.037574902	11.96608482	8.741011335	3.673139427	42.62006316	14.61389124	19.57652461	Matk
ENSMUSG000000004951	-1.245761383	7.16E-05	0.001407433	177.4969249	194.2446963	114.7856071	237.6068521	636.7481184	278.9654756	Hspb1
ENSMUSG000000005148	-1.641216558	0.00407345	0.027163637	8.974563618	3.884893927	9.182848567	19.17902842	27.14008374	22.5130033	Klf5
ENSMUSG000000005547	1.316837452	9.30E-15	3.02E-12	9129.125547	13948.71164	12101.15784	5271.036311	4402.956662	4447.78639	Cyp2a5
ENSMUSG000000005580	1.4483211	2.75E-08	2.14E-06	248.2962601	371.00737	337.0105424	86.3056279	93.9464437	169.3369378	Acdy9
ENSMUSG000000006050	-1.042916307	2.32E-07	1.27E-05	992.1878666	1015.899762	869.6157953	2542.286767	2021.936238	1365.46591	Sra1
ENSMUSG000000006134	1.201348738	1.44E-09	1.52E-07	677.0809663	610.8995699	678.6125091	225.8863347	252.6115486	375.8692724	Crk1
ENSMUSG000000006517	1.182376585	1.39E-07	8.18E-06	2010.30225	1472.374798	1714.437827	1097.466626	579.3364028	613.7240464	Mwd
ENSMUSG000000006587	1.922052303	0.003206819	0.022932036	30.91238579	14.56835222	21.1205517	10.65501579	2.087698749	4.894131151	Sna13
ENSMUSG000000006711	1.246016282	4.07E-11	5.91E-09	435.7649223	410.8275327	410.4733309	210.9683126	141.9635149	177.1675477	D130043K22Rik
ENSMUSG000000006777	1.982388887	0.000106088	0.001901083	22.93499591	53.41729149	33.05825484	8.524012632	8.350794996	10.76708853	Krt23
ENSMUSG000000008153	1.444860417	1.72E-08	1.42E-06	278.2114721	339.9282186	185.4935411	96.96064369	120.0426781	78.30609842	C1stn3
ENSMUSG000000009013	-1.025154388	6.49E-07	3.04E-05	138.6071492	163.1655449	192.838199	329.2399879	396.6627623	281.9019543	Dynl11
ENSMUSG000000009633	-1.150697898	7.41E-07	3.38E-05	1177.662181	1721.008009	1060.619009	2971.683904	3862.242686	1956.673634	Gob2
ENSMUSG000000014547	1.47853344	5.88E-06	0.00018548	76.78237762	107.8058065	108.3576131	21.31003158	36.53472811	46.98365905	Wefy2
ENSMUSG000000014609	1.198939285	0.004891473	0.030823258	34.9018074	33.99282186	34.89482455	14.91702211	14.61389124	15.66121968	Chme
ENSMUSG000000015224	-1.47719251	7.85E-07	3.55E-05	57.83607665	125.2878291	79.80878253	318.5849721	255.7430968	158.5698493	Cyp2j9
ENSMUSG000000015312	-2.170653759	1.83E-07	1.04E-05	27.92086459	16.51079919	10.10113342	121.46718	59.4994435	63.62370497	Gadd45b
ENSMUSG000000016128	-1.194768611	1.81E-09	1.86E-07	486.6207828	535.1441384	631.7799814	199.2487953	218.1645193	304.4149576	Statd13
ENSMUSG000000016356	-1.303561468	6.94E-06	0.00021464	50.8558605	59.24463238	46.83252769	90.56763421	172.2351468	124.3109312	Col2a1
ENSMUSG000000017737	-1.495519922	0.005185903	0.032307064	19.94347471	7.769787853	5.50970914	38.35805684	32.35933051	22.5130033	Mmp9
ENSMUSG000000017868	1.520950393	2.42E-16	1.03E-13	1574.537328	1919.1376	1179.077756	543.4058053	586.6433485	498.2225512	Sgk2
ENSMUSG000000018486	1.561119026	0.006908525	0.039768921	26.92369085	21.3669166	27.5485457	3.196504737	15.65774062	6.851783612	Wnt5b
ENSMUSG000000019082	-1.21454588	1.93E-12	4.05E-10	2583.677148	3303.131061	2721.796315	7723.820946	7053.290223	5201.482588	Slc25a22
ENSMUSG000000019726	1.436923305	3.39E-10	4.31E-08	430.7790536	552.626161	627.1885571	179.0042653	147.1827618	268.193871	Lyst
ENSMUSG000000019737	-1.672592181	0.00414668	0.027410702	8.974563618	9.712234816	6.427993997	45.8165679	15.65774062	18.59769837	Syne4
ENSMUSG000000019883	1.383035463	1.73E-09	1.79E-07	790.7587721	685.683778	707.0793397	200.3142968	230.6907118	405.2340593	Enchc1
ENSMUSG000000020018	-1.224865075	5.55E-07	2.68E-05	76.78237762	100.0360186	117.5404617	268.5063979	250.5238499	170.3157641	Snrp1
ENSMUSG000000020027	-2.148540831	0.000201757	0.00309142	308.1266842	206.8706016	291.0962996	2138.461669	846.5618427	589.253906	Soes2
ENSMUSG000000020091	1.323362559	0.005934886	0.035409378	1482.797344	1694.784975	1814.530877	393.1700826	471.8199173	1129.56547	Eif4ebp2
ENSMUSG000000020122	-1.730853584	6.04E-21	5.49E-18	534.4851221	692.4823424	689.6319274	2670.146957	1807.947117	1885.219319	Egfr
ENSMUSG000000020335	1.099463556	0.002629472	0.020167667	41.88129688	88.38133683	80.80906739	24.50653632	41.75397498	32.3012656	Zfp354b
ENSMUSG000000020429	-1.914789163	0.005628577	0.034083881	1082.930677	219.495068	977.0550875	2643.509417	2014.629293	3936.839098	Igfbp1
ENSMUSG000000020441	-1.319584205	1.17E-07	7.17E-06	96.72585232	114.6043708	87.23706139	303.66795	273.4885361	168.3581116	2310033P09Rik
ENSMUSG000000020532	1.125678465	6.99E-06	0.000215157	6805.710743	2936.008585	5607.047335	2810.793165	1739.053058	2484.260972	Acaca
ENSMUSG000000020641	-1.035196027	2.26E-05	0.00055792	307.1295105	181.6187911	139.5792982	408.0871047	425.8905448	452.2177184	Statd13
ENSMUSG000000020656	-2.17958622	1.43E-08	1.21E-06	16.9519535	7.769787853	23.87540627	71.38860579	76.20100434	73.41196727	Grlh1
ENSMUSG000000020681	1.270736177	0.003452779	0.024158824	30.91238579	35.93526882	52.34223683	14.91702211	21.92083686	12.72474099	Ace
ENSMUSG000000020692	-1.373008874	7.68E-06	0.000232298	53.84738171	65.07197327	76.21764311	249.3273695	152.4020087	104.7344066	Nle1
ENSMUSG000000020812	-1.318469089	0.000692163	0.007515219	32.90673326	43.70505667	47.75081255	180.0697668	73.06945621	57.75074759	1810032008Rik
ENSMUSG000000020889	1.857219295	5.65E-19	3.50E-16	1005.151125	1714.209445	1803.511459	443.2486569	371.6103773	433.620002	Nrlc1
ENSMUSG000000020917	1.579449336	7.98E-12	1.40E-09	34685.69121	17873.42573	22782.64729	9135.610538	6014.660096	10059.39717	Acly
ENSMUSG000000020948	1.248898073	0.003935164	0.026500655	67.807814	53.41729149	56.93366111	9.589514211	20.87698749	44.04718036	Klf128
ENSMUSG000000020961	1.570215029	7.42E-07	3.38E-05	116.669327	116.5468178	141.4158679	25.5720379	35.4908773	64.6025312	Ston2
ENSMUSG000000021250	-3.196150425	1.40E-18	7.95E-16	14.95760603	8.741011335	25.71197599	204.5763032	115.8672806	135.0780198	Fos
ENSMUSG000000021260	1.563177947	0.002093122	0.017081929	31.90955953	29.13670445	23.87540627	6.393009474	11.48234312	10.76708853	Hhip11
ENSMUSG000000021416	1.171425747	1.31E-08	1.13E-06	910.4196203	675.9715432	629.0251268	404.8906	324.6371555	254.4948199	Eod3
ENSMUSG000000021453	-2.706074058	5.59E-23	6.28E-20	149.5760603	61.18707934	130.3964497	979.1959511	568.8979091	678.3265776	Gadd45g
ENSMUSG000000021611	1.027773708	1.21E-09	1.30E-07	416.8186214	423.453438	475.6715558	220.5588268	227.5591636	197.7228985	Tert
ENSMUSG000000021670	1.206582161	2.43E-07	1.30E-05	4843.272832	4623.994996	5239.733392	3051.596522	1273.496237	2047.704474	Hmgcr
ENSMUSG000000021684	-1.138260866	0.001182935	0.011115132	32.90673326	25.25181052	22.03883656	54.34058053	72.02560684	49.92013774	Pde8b
ENSMUSG000000021773	-1.294178405	0.002373717	0.01846284	17.94912724	12.62590526	22.03883656	44.75106632	55.32401685	29.36478691	Comt1
ENSMUSG000000021775	1.550962326	2.41E-10	3.16E-08	1168.687618	1405.360378	1119.38924	635.0389411	274.5323855	351.3986167	Nrlc2
ENSMUSG000000021804	1.339955479	0.00532128	0.032924815	26.92369085	35.935268					

Table 4 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRT15-TGFRs (TGFRT15-TGFRs group) in aged mice liver

ENSMUSG00000022528	-1.557702695	1.66E-19	1.13E-16	205.4177895	185.503685	197.4312442	643.5629537	588.7310472	500.1802037	Hes1
ENSMUSG00000022651	-4.171544663	2.34E-09	2.36E-07	3.988694941	2.913670445	0	52.20957737	37.57857748	32.3012656	Retnlg
ENSMUSG00000022704	-1.016808687	0.000177103	0.002808323	94.73150485	108.7770299	100.0930494	291.9474326	188.9367368	134.0991935	Qrt2
ENSMUSG00000022853	1.04479536	2.29E-11	3.59E-09	9525.00352	10073.52995	7460.146176	4279.054341	3944.706786	4891.194673	Hehach
ENSMUSG00000022883	1.330834067	1.00E-09	1.09E-07	280.2058196	336.0433246	355.3762395	166.2182463	116.9111299	103.7555804	Robo1
ENSMUSG00000022887	1.363326654	1.68E-07	9.74E-06	1196.608482	1241.22361	1538.127135	350.5500195	374.7419254	819.2775547	Satp1
ENSMUSG00000022911	1.024722285	3.96E-05	0.000880701	133.6212805	188.4173554	193.7581048	105.4846563	73.06945621	75.36961973	Ar13b
ENSMUSG00000023034	-2.875895339	0.000200077	0.003084527	58.83325038	34.96404534	22.95712142	572.1743479	134.6556593	149.7604132	Nr4a1
ENSMUSG00000023044	-1.350199698	7.38E-12	1.31E-09	1875.683796	2461.080302	2315.914409	4109.63959	5560.585618	7290.297763	Csad
ENSMUSG00000023052	-1.334599319	0.004914148	0.031082668	13.96043229	11.65468178	17.44741228	36.22705368	52.19246872	20.55535084	Npff
ENSMUSG00000023067	-3.022867976	2.66E-57	3.63E-53	136.6128017	95.1799012	134.9878739	1114.514652	1009.402345	858.4306039	Cckn1a
ENSMUSG00000023072	1.225822592	2.02E-05	0.00050363	443.7423122	525.4319036	592.2937326	369.7290479	140.9196656	157.5910231	Slc10a2
ENSMUSG00000023341	-1.14606456	1.95E-06	7.71E-05	89.74563618	99.06479513	85.40049167	264.2443916	178.488243	164.4428067	Mx2
ENSMUSG00000023571	-1.317554169	0.00212852	0.017761424	18.94630097	21.3669166	14.69255771	53.27507895	58.45556497	25.44848191	C1qtnf12
ENSMUSG00000023800	1.312092825	2.45E-12	4.99E-10	459.697092	410.8275327	456.3875738	160.8907384	217.1206699	156.6121968	Tiam2
ENSMUSG00000023905	-1.475845141	9.44E-06	0.000275028	54.84455544	95.1799012	81.72735225	328.1744863	207.7260255	109.6285378	Tnfrsf12a
ENSMUSG00000023927	-1.218211099	0.003077602	0.022366209	27.20864599	26.223034	12.85598799	61.79909158	60.54326372	33.28009183	Map1b
ENSMUSG00000023968	-1.267291327	0.008148531	0.04468095	10.96891109	11.65468178	15.61084256	20.24453	38.62242686	33.28009183	Crip3
ENSMUSG00000024118	-1.757053931	5.31E-18	2.79E-15	442.7451385	592.4463238	352.621385	1463.99917	1896.674313	1329.246021	Tec2
ENSMUSG00000024130	1.042830636	1.30E-05	0.000354612	2989.526858	2745.484783	3530.806274	1006.898992	1228.610714	2261.088592	Abca3
ENSMUSG00000024136	-1.225506878	0.002002269	0.016518413	17.92086459	19.42446963	17.44741228	64.99559632	52.19246872	34.25891806	Dnase12
ENSMUSG00000024190	-1.579039564	2.17E-13	5.48E-11	281.2029934	502.12254	460.0607132	1247.702349	1464.520672	1004.275712	Dusp1
ENSMUSG00000024236	-1.389584652	9.49E-13	2.12E-10	1344.190195	1041.151572	1299.373072	484.8032184	345.514143	575.5498234	Psip1
ENSMUSG00000024411	1.68012077	5.07E-05	0.001068968	63.81911906	48.56117408	35.81310941	14.91702211	13.57004187	17.61887214	Acp4
ENSMUSG00000024440	1.007637941	0.000827422	0.008613399	107.6947634	103.9209125	147.8438619	35.16155211	70.98175747	72.4314104	Pcsh12
ENSMUSG00000024665	1.18951247	3.11E-12	6.05E-10	9244.7977	8635.147975	9906.457034	3625.901873	3398.773563	5157.435407	Fads2
ENSMUSG00000024843	-1.175648724	2.45E-11	3.79E-09	1421.969747	1016.870985	1263.559963	3171.998201	2942.611387	2249.342677	Chka
ENSMUSG00000024887	1.067323547	1.55E-06	6.39E-05	398.8694941	436.0793433	565.6634717	191.7902842	179.5420924	296.5843478	Asah2
ENSMUSG00000024924	1.265415535	2.60E-12	5.20E-10	642.1798891	816.7989481	589.538878	269.5718995	262.006193	320.0761773	Vldlr
ENSMUSG00000024970	-1.174479585	0.000322102	0.004379373	31.90955953	39.82016275	25.71197599	90.56763421	63.67481184	65.58135743	AlbA6148
ENSMUSG00000024978	2.527869097	4.51E-08	3.23E-06	10405.50793	5475.757899	8311.396238	1324.418463	791.2378259	2079.026913	Gpm
ENSMUSG00000025003	1.045766059	5.74E-07	2.75E-05	301.1464681	286.5109271	410.4733309	152.3667258	144.0512137	186.95581	Cyp2c39
ENSMUSG00000025006	1.229102652	2.44E-06	9.41E-05	288.1832095	294.2807149	416.9013249	107.6156595	110.6480337	207.5111608	Sorbs1
ENSMUSG00000025153	1.113433094	9.44E-07	4.18E-05	85604.37365	41744.15646	57005.28733	35454.56504	23677.63536	26074.95195	Fasn
ENSMUSG00000025161	-1.470478397	0.001962044	0.016285081	18.94630097	9.712234816	14.69255771	61.79909158	26.09623436	32.3012656	Slc16a3
ENSMUSG00000025240	1.333245056	1.72E-06	6.99E-05	553.4314231	682.7701076	696.0599214	183.2662716	163.8843518	418.9376266	Sacm1l
ENSMUSG00000025233	1.252220218	7.14E-07	3.28E-05	138.6071492	127.2302761	172.6375531	71.38860579	52.19246872	60.68722628	Sp4
ENSMUSG00000025402	-1.340941683	6.59E-15	2.25E-12	217.3838743	245.7195409	214.8786565	646.7594584	568.8979091	502.1378561	Nab2
ENSMUSG00000025429	1.693186246	2.17E-13	5.48E-11	624.2307583	540.0002558	651.9822483	188.5937795	118.9988287	253.5159936	Ptprtp2
ENSMUSG00000025450	-1.257708301	2.83E-05	0.000669372	30.91238579	62.15830282	55.0970914	145.9737163	103.3410881	105.7132329	Gm9752
ENSMUSG00000025997	1.284615532	0.009041141	0.048049118	26.92369085	9.712234816	12.85598799	39.42355842	58.4556497	22.5130033	Ikzf2
ENSMUSG00000026020	-1.156643623	9.59E-08	6.11E-06	250.2906076	294.2807149	269.9757479	828.9602284	520.8808379	466.9001118	Nop58
ENSMUSG00000026249	-1.326755053	0.000451208	0.005060296	36.89542821	50.50362104	26.63026084	45.8165679	122.1303768	117.4591476	Serpine2
ENSMUSG00000026358	-2.793399328	5.66E-13	1.33E-10	12.96325856	11.65468178	12.85598799	104.4191547	58.4556497	96.9037968	Rgs1
ENSMUSG00000026398	1.137826301	6.48E-12	1.16E-09	1403.023446	1667.590718	1539.04542	615.8599126	631.5288716	846.6846892	Nr5a2
ENSMUSG00000026471	1.000583106	1.46E-05	0.000384933	269.2369085	237.949753	315.8899907	181.1352684	104.3849374	126.2685837	Mr1
ENSMUSG00000026475	-1.56210025	0.004964709	0.031300593	735.9142167	388.4893927	994.5024998	1039.929541	2826.744106	2390.293654	Rgs16
ENSMUSG00000026525	-1.790734538	0.001947167	0.0161813	7.977389882	8.741011335	6.427993997	43.68556474	19.8313812	16.64004591	Opr3
ENSMUSG00000026822	1.02651459	5.14E-06	0.00016724	122.6523694	119.4604882	110.1941828	304.734516	223.3837661	189.8922887	Lcn2
ENSMUSG00000026826	-1.483020728	0.008821676	0.047306802	15.95477976	10.6834583	11.93770314	69.25760263	25.05238499	13.70356722	Nr4a2
ENSMUSG00000026832	-1.174113684	0.001522033	0.013425591	30.91238579	34.96404534	25.71197599	101.22265	42.79782435	62.64487874	Cyp11b
ENSMUSG00000027360	-1.979964656	1.70E-12	3.69E-10	54.84455544	93.23745424	72.54450368	370.7945495	319.4179086	181.0828526	Hic
ENSMUSG00000027398	-1.057691022	0.001937845	0.016123489	78.77672509	37.87771578	32.13996998	98.02614527	82.46410059	128.2262362	Il1b
ENSMUSG00000027405	-1.241634744	7.40E-06	0.000226395	225.3612642	254.4605522	248.8551962	520.5933642	450.9429298	352.3774429	Nop56
ENSMUSG00000027496	-1.007831872	0.004782077	0.030501955	38.88977568	37.87771578	17.44741228	55.40608211	66.80635997	66.56018366	Aurka
ENSMUSG00000027513	-1.115800194	1.04E-10	1.46E-08	16805.36896	18645.5484	18901.97549	48173.45739	29794.5927	39823.54518	Pck1
ENSMUSG00000027605	1.86697993	9.67E-16	3.77E-13	27327.54622	18714.50527	21561.32844	8380.169919	3799.611723	6353.561061	Acs2
ENSMUSG00000027762	1.17286147	2.98E-15	1.13E-12	1319.260852	1520.935972	1404.975831	637.1699442	555.3278672	690.0724923	Sunr1
ENSMUSG00000027907	-1.140239221	0.000303295	0.004203282	47.86433929	56.33096193	106.5210434	207.7728079	117.9549793	139.9721509	S100a11
ENSMUSG00000027947	-1.05537492	2.15E-06	8.45E-05	434.7677486	468.1297181	461.8972829	713.8860579	763.0538928	1358.610808	Il6ra
ENSMUSG00000028008	1.245889	0.000553467	0.00652119	54.84455544	81.58277246	55.0970914	20.24453	24.00853561	36.21657052	Asic5
ENSMUSG00000028339	1.011959422	2.14E-08	1.71E-06	706.961783	505.0362104	521.5857986	295.1439374	274.5323855	289.7325642	Col15a1
ENSMUSG00000028445	-1.767301879	0.003925136	0.026446185	70.79933521	71.87053764	91.82848567	91.63313579	280.7954817	425.7894102	Enho
ENSMUSG00000028630	1.331166045	1.46E-07	8.59E-06	373.9401507	358.3814647	406.8001915	124.6636847	101.2533893	226.1088592	Dyrk2
ENSMUSG00000028838	1.693729472	3.36E-09	3.22E-07	304.1379893	151.5108631	198.349529	74.58511053	49.0692096	78.30609842	Extl1
ENSMUSG00000028859	-1.001034699	0.004539011	0.029294129	74.78803015	57.30218542	29.38511541	152.3667258	96.03414245	74.3907935	Csf3r
ENSMUSG00000028862	-2.278242573	1.62E-07	9.45E-06	8.974563618	9.712234816	17.44741228	77.78161526	40.71012561	57.75074759	Map3k6
ENSMUSG00000028864	1.08474786	0.000792949	0.008337279	131.6269331	134.0228405	203.8592382	39.42355842	69.93790809	111.586002	Hgf
ENSMUSG00000028957	2.966674755	1.69E-27	3.84E-24	314.1097266	609.9283465	341.6019667	62.96459316	53.2363181	46.00483282	Per3
ENSMUSG00000028976	1.532387058	3.09E-07	1.58E-05	157.5534502	94.20867772	101.9296191	42.62006316	34.44702936	45.02600659	Slc2a5
ENSMUSG00000029086	1.631659452	1.01E-18	6.00E-16	713.9763945	617.6981343	593.2120174	210			

Table 4 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRT15-TGFRs (TGFRT15-TGFRs group) in aged mice liver

ENSMUSG00000029373	-1.650047987	0.002725238	0.020577678	7.977389882	5.82734089	9.182848567	25.5720379	28.18393311	18.59769837	Pf4
ENSMUSG00000029380	-2.803403219	8.38E-26	1.43E-22	53.84738171	128.2014996	71.62621882	615.8599126	454.0744779	700.8395809	Cxcl1
ENSMUSG00000029580	-1.162469269	9.11E-12	1.55E-09	8321.414821	7155.974613	6927.540959	16369.30076	20515.81561	13266.0319	Actb
ENSMUSG00000029591	-1.593385799	3.01E-08	2.29E-06	43.87564435	60.21585586	35.81310941	177.9387637	131.5250212	112.5650165	Ung
ENSMUSG00000029656	-1.019716092	0.000134204	0.002299171	189.4630097	409.8563092	189.1666805	429.3971363	614.8272816	554.0156463	C8b
ENSMUSG00000030032	-1.061169015	0.007631272	0.042668167	23.93216965	14.56835222	25.71197599	43.68556474	55.32401685	35.23774429	Wdr54
ENSMUSG00000030055	1.305889925	4.54E-06	0.000151075	239.3216965	263.2015635	292.9328693	75.65061211	74.11330559	171.2945903	Rab43
ENSMUSG00000030691	1.035229834	1.01E-08	8.79E-07	365.9627609	443.8491311	415.0647552	189.6592811	180.5859418	227.0876854	Fchs2
ENSMUSG00000030782	-1.087204324	0.00053618	0.006369234	38.88977568	39.82016275	40.40453369	115.0741705	67.85020934	70.47548858	Tgfb11
ENSMUSG00000030814	-1.357189589	8.78E-10	9.81E-08	261.2595186	283.5972566	291.0962996	814.0432063	869.526529	459.069502	Bcl7c
ENSMUSG00000030827	-1.631143551	1.45E-05	0.000383464	26.92369085	20.39569311	33.05825484	120.4016784	80.37640184	48.94131151	Fgf21
ENSMUSG00000030934	1.434845811	0.000702548	0.007652274	13671.25191	24780.76713	9134.17947	5865.586192	5683.759844	6052.082582	Oat
ENSMUSG00000030968	-1.140744433	0.000133867	0.002296287	34.90108074	72.84176112	54.17880654	31.0566942	132.5688706	93.9673181	Polr1
ENSMUSG00000031010	1.281779593	3.26E-07	1.66E-05	953.2980909	1388.561814	1420.586673	414.4801142	361.1718836	775.2303744	Actp9k
ENSMUSG00000031271	-1.719181951	2.91E-23	3.97E-20	339.03907	470.0721651	364.5590881	1418.182602	1115.874981	1330.224847	Serpina7
ENSMUSG00000031378	1.154152634	6.32E-15	2.21E-12	1014.125689	912.9500727	885.2266019	429.3971363	392.4873648	441.4506298	Abcc1
ENSMUSG00000031465	-1.254345174	0.0029265	0.021654196	21.93782218	11.65468178	20.2026685	50.07857421	30.27163186	47.96248528	Angpt2
ENSMUSG00000031762	-4.701992063	1.08E-05	0.000305681	384.9090618	188.4173554	32.13996998	9477.636545	4672.2698	1607.23267	M2
ENSMUSG00000031765	-4.008275951	6.98E-08	4.64E-06	1129.797842	691.5111189	337.0105424	20312.7221	10174.39985	4243.211708	Mt1
ENSMUSG00000032009	1.096135097	4.98E-08	3.50E-06	816.6852892	873.12991	1044.089882	377.187559	338.2071973	562.8250824	Sesn3
ENSMUSG00000032064	1.076164714	3.75E-10	4.69E-08	659.131839	690.5398954	661.1650968	268.5063979	302.7163186	381.7422289	Idc1
ENSMUSG00000032083	-1.056710751	2.91E-09	2.86E-07	57416.2665	56826.28591	68671.17815	163165.5843	112907.9676	104422.1611	Apoa1
ENSMUSG00000032091	2.476316256	2.69E-06	0.000101175	102.7088947	50.5062104	32.13996998	20.24453	8.350794996	4.894131151	Tmprss4
ENSMUSG00000032285	1.014640865	0.008899206	0.047498424	51.85303424	70.89931416	52.34223683	26.63753947	15.65774062	44.04718036	Dnajb4
ENSMUSG00000032417	1.412452049	0.000231229	0.003394264	102.7088947	50.5062104	80.80906739	26.63753947	43.84167373	17.61887214	Rwdd2a
ENSMUSG00000032418	1.488424738	1.96E-09	2.00E-07	17569.20404	7712.485668	13799.06654	6053.11447	3739.068459	4136.519649	Me1
ENSMUSG00000032500	2.09734091	1.65E-20	1.25E-17	413.8271001	367.1224761	372.8236518	75.65061211	69.93790809	123.332105	Dcl3
ENSMUSG00000032561	1.808480512	0.000942567	0.009458265	1361.142149	660.4319675	1176.322901	453.9036726	141.9635149	317.1396986	Acpp
ENSMUSG00000032702	1.072973429	8.45E-08	5.48E-06	1328.235415	1308.23803	1494.049462	468.8206947	629.4411728	864.3035613	Kank1
ENSMUSG00000032724	1.34458697	3.25E-10	4.18E-08	647.1657542	540.0002558	680.4490788	234.4103474	176.4105443	323.9914822	Abtb2
ENSMUSG00000032735	-1.26252931	1.21E-08	1.04E-06	191.4573572	257.3742226	276.7949439	436.8556474	741.1330559	606.8728628	Abilim3
ENSMUSG00000032786	1.26794906	3.89E-08	2.81E-06	1778.957944	2029.857077	2206.638511	7150.581096	4035.521682	3301.580875	Alas1
ENSMUSG00000032849	1.170479352	7.00E-10	8.09E-08	403.8553628	338.9569951	399.4539127	168.3492495	143.0073643	195.7652461	Abcc4
ENSMUSG00000032860	1.046181292	6.20E-08	4.19E-06	339.03907	263.2015635	290.1780147	155.5632305	134.6556693	141.9298034	P2ry2
ENSMUSG00000032883	1.139189345	1.88E-11	3.09E-09	1754.0286	1475.288469	1528.944286	586.6632695	580.3802522	723.3525842	Acsl3
ENSMUSG00000033105	1.210153299	1.43E-10	1.97E-08	3593.814142	2741.763889	3240.627259	1574.811334	996.8761526	1567.100795	Lss
ENSMUSG00000033594	-1.045179866	5.30E-08	3.67E-06	379.9231931	342.841889	388.9482455	852.4012632	820.4656084	539.3332529	Scpt2l
ENSMUSG00000033624	1.545958738	3.43E-10	4.33E-08	290.177557	397.230404	428.8390281	105.4846563	98.1218412	178.1463739	Popa1
ENSMUSG00000033792	1.183185841	0.000561984	0.006589659	63.81911906	67.98564371	74.38107339	21.31003158	26.09623436	43.06835413	Actn7a
ENSMUSG00000033855	1.291796081	0.000477464	0.005865923	117.6665008	69.92809068	198.349529	37.29255526	42.79782435	77.32727219	Ston1
ENSMUSG00000033967	-2.822221219	4.36E-05	0.000952114	4.985868677	3.884893927	1.836569713	15.98252368	19.8313812	39.15304921	Rnf225
ENSMUSG00000034066	1.2566902	2.38E-07	1.29E-05	275.2195099	346.7267829	310.3802816	96.90664369	107.5164856	184.9981575	Farp2
ENSMUSG00000034110	1.131917427	3.20E-05	0.000736514	99.71737353	110.7194769	115.7038919	55.40608211	37.57857748	55.79309512	Kctd7
ENSMUSG00000034271	-1.155793079	0.004733656	0.030334993	36.89542821	29.13670445	40.40453369	135.3187005	37.57857748	64.6025312	Jcp2
ENSMUSG00000034755	-1.51005032	0.006510973	0.037993212	7.977389882	5.82734089	12.85598799	19.17902842	36.5347281	20.55535084	Pcchl1x
ENSMUSG00000034765	-2.457798355	3.78E-06	0.000131464	23.93216965	12.62950526	9.182848567	166.2182463	53.2363181	31.32243937	Dusp5
ENSMUSG00000034853	1.437062013	9.64E-14	2.63E-11	450.7225284	335.0721012	434.3487372	165.1527447	137.7881174	147.8027608	Acot11
ENSMUSG00000034926	1.093495822	1.31E-06	5.56E-05	17902.26007	19066.08817	17627.39611	6118.110066	6691.074491	12774.66113	Dhcr2
ENSMUSG00000035078	1.209517873	6.16E-05	0.001249206	322.0871165	384.6044987	346.193391	94.82964053	101.2533893	258.4101248	Mtmr9
ENSMUSG00000035112	-1.146398835	0.004042609	0.02701081	23.93216965	14.56835222	18.36569713	43.68556474	45.92937248	36.21657052	Wnk4
ENSMUSG00000035164	1.06213892	8.60E-06	0.000256206	131.6269331	166.0792154	141.4158679	61.79909158	70.98175747	77.32727219	Zc3h12c
ENSMUSG00000035165	-1.798528938	1.18E-06	5.05E-05	32.90673326	22.33814008	28.46683056	144.9082147	55.32401685	91.03083941	Kone3
ENSMUSG00000035284	1.432291641	4.22E-09	3.95E-07	725.9424793	940.1443302	1017.459621	293.0129342	218.1645193	482.5613315	Vpsl3c
ENSMUSG00000035900	1.041020638	2.29E-08	1.81E-06	343.0277649	318.561302	367.3139427	147.0392179	160.7528037	191.8499411	Gramd4
ENSMUSG00000035933	1.189062904	7.63E-07	3.47E-05	188.465836	221.4389538	244.2637719	77.78161526	81.42025121	127.2474099	Cog5
ENSMUSG00000035948	1.741049509	1.78E-10	2.38E-08	816.6852892	893.5256031	857.6780562	193.9212874	154.4897074	418.9376266	Acsc3
ENSMUSG00000036062	-1.708320866	0.004715534	0.030261525	7.977389882	9.712234816	3.673139427	27.70304105	29.22778249	12.72474099	Pf24
ENSMUSG00000036120	-1.340248384	3.85E-09	3.64E-07	263.2538661	219.4965068	224.9797899	773.5541463	626.3096247	392.5093183	Rfxank
ENSMUSG00000036611	1.04665245	4.08E-07	2.03E-05	846.6005013	616.7269108	585.8657386	415.5456158	267.2254399	309.3090888	Eepd1
ENSMUSG00000037035	-1.785829601	2.14E-10	2.84E-08	59.83042412	30.10792793	50.50566712	141.71171	149.2704066	192.8287674	Inhbb
ENSMUSG00000037071	1.600161226	3.89E-11	5.71E-09	372268.8876	280319.3774	340820.5063	157237.1335	63623.66322	106805.6029	Scd1
ENSMUSG00000037095	-1.027049761	7.90E-10	8.97E-08	3492.102421	3177.843232	2991.772063	5413.813523	8132.630477	6142.134595	Lrg1
ENSMUSG00000037157	1.742314913	8.42E-06	0.000251859	51.85303424	60.21585586	58.77023083	11.72051737	14.61389124	24.47065576	Il22ra1
ENSMUSG00000037336	-1.65964734	0.000936801	0.009424872	14.95760603	7.769787853	8.26456371	23.44103474	42.79782435	31.32243937	Mfsd2b
ENSMUSG00000037443	-1.349128808	2.89E-12	5.72E-10	750.8718227	1078.058065	1089.08584	3091.020081	2288.117829	2056.51391	Cep85
ENSMUSG00000037447	-1.380120078	0.000135203	0.002310483	30.91238579	23.30936356	25.71197599	99.09164684	54.28016747	54.81426889	Arid5a
ENSMUSG00000037465	-1.250556349	2.44E-12	4.99E-10	342.0305912	272.9137983	297.5242936	592.4188779	817.3340602	760.5479809	Klf10
ENSMUSG00000037583	-1.178511493	2.71E-06	0.000101645	181.4856198	270.0001279	213.9603716	304.7334516	631.5288716	569.676866	Nr0b2
ENSMUSG00000037709	1.621036788	2.49E-19	1.62E-16	850.5891962	1019.784656	857.6780562	239.7378553	289.1462767	357.271574	Fam13a
ENSMUSG00000037887	-1.887921138	0.000434734	0.005464027	15.95477976	8.741011335	11.01941828	80.97812	20.87698749	30.34361314	Dusp8
ENSMUSG00000038217	1.023783748	9.74E-10	1.07E-07							

Table 4 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGF α 15-TGFRs (TGF α 15-TGFRs group) in aged mice liver

ENSMUSG00000038418	-2.528561862	1.50E-17	7.58E-15	95.72867859	79.64032549	131.3147345	515.7027642	342.3825948	912.2660466	Egr1
ENSMUSG00000038473	1.076370271	0.000625033	0.007061263	117.6665008	63.12952631	89.99191596	45.8165679	35.49087873	46.98365905	Nos1ap
ENSMUSG00000038530	-1.722746304	4.58E-05	0.000989869	17.94912724	16.51079919	13.77427285	64.9959632	30.27163186	63.62370497	Rgs4
ENSMUSG00000038583	-1.702074711	0.00088397	0.009002758	12.96325856	13.59712874	11.93770314	67.12659947	41.75397498	16.64004591	Pln
ENSMUSG00000038587	-1.31455808	3.12E-06	0.000113043	97.72302606	73.8129846	141.4158679	349.4845179	176.4105443	253.5159936	Akap12
ENSMUSG00000038751	1.774904606	6.46E-12	1.16E-09	167.5251875	125.2878291	171.7192682	46.88206947	44.8855231	44.04718036	Ptk6
ENSMUSG00000038768	1.297337324	1.64E-05	0.000422782	329.0673326	229.2087417	124.8867405	106.5501579	77.24485371	93.9673181	9130409123Rik
ENSMUSG00000038774	1.007283954	3.14E-06	0.00011311	450.7225284	497.2664226	488.5275438	194.986789	189.9805862	328.8856134	Ascc3
ENSMUSG00000038844	1.003328537	1.78E-06	7.13E-05	364.9655871	380.7196048	386.5979247	169.4147511	146.1389124	248.6218625	Kif16b
ENSMUSG00000038895	-1.113485639	6.66E-08	4.45E-06	100.7145473	103.9209125	94.58334024	230.1483411	226.5153143	190.8711149	Zfp653
ENSMUSG00000039103	-1.109845105	0.002277587	0.018174053	37.89260194	21.3669166	26.63026084	68.19210105	74.11330559	43.06835413	Nexn
ENSMUSG00000039304	1.09876453	1.10E-06	4.76E-05	197.4403996	176.7626737	181.8204016	105.4846563	73.06945621	81.2425771	Trnfst10
ENSMUSG00000039533	1.597388363	4.59E-08	3.26E-06	367.9571083	378.7771578	293.8511541	167.2837479	54.2801677	122.3532788	Mmd2
ENSMUSG00000039601	1.185885146	6.77E-14	1.97E-11	707.933521	644.8923918	732.7913156	337.7640005	298.5409211	280.9231281	Rcan2
ENSMUSG00000039704	1.942291849	0.00175377	0.014994461	267.2425611	391.4030631	463.7338526	59.66808842	50.10476998	182.0616788	Lmbre2
ENSMUSG00000039741	1.087450189	5.12E-08	3.58E-06	264.2510399	270.0001279	292.0145844	105.4846563	146.1389124	137.0356722	Bahcc1
ENSMUSG00000039853	1.374596414	3.52E-09	3.36E-07	477.6462192	438.0217902	491.2823983	126.7946879	156.5774062	258.4101248	Slc16a6
ENSMUSG00000039981	1.024216498	5.34E-05	0.001113305	116.669327	128.2014996	147.8438619	53.27507895	77.24485371	62.64487874	Zc3h12a
ENSMUSG00000040093	1.487860932	2.33E-07	1.27E-05	234.358278	188.4173554	357.2128093	75.6061211	69.9379009	132.1415411	Bmf
ENSMUSG00000040128	-1.050165381	1.95E-11	3.16E-09	1378.094102	1310.180477	1381.100424	3368.050491	2671.210549	2388.336002	Pnrc1
ENSMUSG00000040152	-1.254644006	0.001007039	0.00915514	35.89825447	19.42446963	35.81310941	91.63313579	40.71012561	85.15788203	Thbs1
ENSMUSG00000040435	-1.205190782	4.71E-06	0.000155637	119.6608482	131.11517	127.6415951	430.4626379	241.1292055	201.632034	Ppp1r15a
ENSMUSG00000040584	1.185136822	2.65E-06	9.97E-05	302.1436418	375.8634874	406.8001915	118.2706753	124.2180756	233.939469	Abcb1a
ENSMUSG00000040855	1.071792937	4.60E-07	2.25E-05	584.3438089	647.8060622	645.5542543	259.9823853	223.3873661	409.1493642	Reps2
ENSMUSG00000040891	-2.088765162	3.05E-41	2.00E-37	406.846884	431.2232258	360.889487	1650.461946	1922.770548	1525.990093	Foxa3
ENSMUSG00000041134	1.050155799	0.006573499	0.03826156	41.88129688	57.30218542	84.48220682	18.11352684	31.31548123	39.15304921	Cyp11
ENSMUSG00000041372	2.023614953	1.33E-05	0.000359411	51.85303424	49.53239756	28.46683056	9.580514211	12.52619249	9.788262303	Bapalnt3
ENSMUSG00000041695	-1.532466883	9.09E-05	0.001683702	33.903907	18.45324615	23.87540627	101.22265	78.28870309	41.11070167	Kcnj2
ENSMUSG00000041702	1.159708962	1.79E-05	0.000451095	125.6438906	178.7051206	179.0655471	59.66808842	58.45556497	97.88262303	Btbd7
ENSMUSG00000041920	-1.612549242	5.78E-11	8.29E-09	130.6297593	116.5468178	162.5364196	586.0258684	355.9526367	312.2455675	Slc16a6
ENSMUSG00000041930	-2.069670842	7.78E-10	8.92E-08	29.91521206	25.25181052	29.38511541	157.6942337	126.3057743	71.45431481	Fam22a
ENSMUSG00000041945	2.034435558	0.000645414	0.007184911	35.89825447	23.30936356	20.20226685	3.196504737	5.219246872	10.76708853	Msf9
ENSMUSG00000042010	1.335313237	1.72E-08	1.42E-06	9652.641758	5265.973717	7079.05796	4018.006454	2149.285862	2550.821156	Acacb
ENSMUSG00000042115	1.249312452	6.34E-07	3.01E-05	57.83607665	47.5899506	51.42395197	117.2051737	124.2180756	131.1627149	Klhlcc8a
ENSMUSG00000042246	-1.458803387	0.000333225	0.004503661	16.9519535	19.42446963	23.87540627	77.78161526	33.40317998	54.81462889	Tmc7
ENSMUSG00000042333	1.17659167	0.000212792	0.003210009	83.76259377	76.72665505	64.27993997	30.89954579	27.14008374	41.11070167	Trnfstf14
ENSMUSG00000042354	-1.004338102	0.000195353	0.003017019	312.1153791	284.5684801	269.057463	922.7243674	424.8466954	389.5728396	Gm13
ENSMUSG00000042379	-1.128744357	0.001182685	0.011115132	63.81911906	39.82016275	89.99191596	140.6462084	79.33255246	203.5958559	Em1
ENSMUSG00000042444	1.165704626	4.84E-10	5.85E-08	592.3211988	520.5757862	674.0210848	255.720379	221.2960674	319.0973511	Mindy2
ENSMUSG00000042510	-1.154445757	0.002024484	0.016651324	28.91803832	29.13670445	26.63026084	94.82964053	45.92937248	47.96248528	A996860
ENSMUSG00000042607	-1.584091426	0.001074406	0.010435667	12.96325856	6.798564371	14.69255771	37.29255526	24.00853561	42.0895279	Asb4
ENSMUSG00000042622	-1.343729967	0.00016867	0.001912519	29.91521206	22.33814008	31.22168513	89.50213263	50.10476998	72.43314104	Meff
ENSMUSG00000042680	1.086909564	0.000262276	0.00374505	262.2566924	328.2735368	358.1310941	87.37112948	114.8234312	243.7277313	Grem1
ENSMUSG00000042743	1.143476031	0.005459928	0.033421057	39.88694941	35.93526882	50.5066712	15.98252368	14.61389124	26.42830822	Sgltb
ENSMUSG00000042745	-1.243661764	2.94E-08	2.25E-06	425.793185	215.6116129	317.7265604	726.6720769	889.3596671	654.834748	lcl1
ENSMUSG00000043165	-1.65740279	0.002445867	0.019147761	6.980216147	13.59712874	9.182848567	13.85152053	41.75397498	38.17422298	Lor
ENSMUSG00000043421	-1.515224307	3.07E-05	0.000711553	31.90955953	28.16548097	33.9765397	138.5152053	55.32401685	75.36961973	Ilp12a
ENSMUSG00000043639	1.042566706	0.00209562	0.017092091	83.76259377	70.89931416	87.23706139	20.24453	46.97322185	49.92013774	Rbm20
ENSMUSG00000043681	-1.124534949	2.43E-09	2.42E-07	201.4290945	238.9209765	202.0226685	543.4058053	474.9514654	382.721056	Fam25c
ENSMUSG00000044042	1.154045613	3.06E-05	0.000711225	258.2679974	274.8562453	353.5396698	108.681161	85.59564871	203.5958559	Fmn1
ENSMUSG00000044186	1.148916053	0.009243418	0.048746497	35.89825447	33.99282186	30.30340027	17.04802526	10.43849473	17.61887214	Nkx2-6
ENSMUSG00000044339	-1.202694639	0.000240284	0.0035008	44.87281809	27.19425749	28.46683056	61.79909158	89.77104621	79.28492465	Alkbh2
ENSMUSG00000044349	1.020247724	0.000864482	0.008883909	70.5167087	218.5252834	182.7386865	148.1047195	83.50794996	50.89869397	Snhg11
ENSMUSG00000044359	1.419594491	2.01E-07	1.12E-05	221.3725692	303.9929497	263.5477579	69.25760263	76.20100434	148.781587	P2y2
ENSMUSG00000044676	-1.235532178	3.06E-06	0.000111153	173.5082299	206.8706016	146.0072922	609.4669032	360.1280342	270.1560396	Zfp612
ENSMUSG00000044749	1.260739468	0.003720434	0.025546608	2581.682801	3424.533996	3631.816608	1050.584557	874.7457758	2096.645785	Abca6
ENSMUSG00000044948	-1.380324526	9.28E-05	0.001711935	28.91803832	18.45324615	23.87540627	73.51960895	52.19246872	59.70840005	Cfap43
ENSMUSG00000045045	1.563883127	9.68E-08	6.14E-06	106.6975897	117.5180413	171.7192682	38.35805684	38.62242686	56.77192136	Lrn4
ENSMUSG00000045294	1.216661032	2.49E-17	1.21E-14	16820.32657	15644.46784	18334.47545	8335.418852	7066.860265	6456.337815	Insig1
ENSMUSG00000045348	-1.535176629	0.002140436	0.017319005	17.94912724	8.741011335	9.182848567	38.35805684	44.8855231	20.55535084	Nyap1
ENSMUSG00000045382	-1.705364762	8.30E-05	0.001575106	17.94912724	15.53957571	23.87540627	89.50213263	68.89405872	29.36478691	Cxcr4
ENSMUSG00000045411	-1.265704961	6.98E-06	0.000215157	217.3838743	180.6475676	227.7346445	798.0606826	403.9697079	303.4361314	2410002F23Rik
ENSMUSG00000045776	1.435732199	4.30E-15	1.59E-12	1029.083295	1166.439401	1214.890865	514.6372626	321.5056073	424.8105839	Lrtm1
ENSMUSG00000045875	1.205673082	0.000172777	0.002749319	91.73998365	131.11517	117.5404617	28.76854263	45.92937248	72.43314104	Aclra1a
ENSMUSG00000046541	1.523461987	5.84E-06	0.000185032	69.80216147	95.1799012	84.48220682	22.37553316	22.96468624	41.11070167	Zfp526
ENSMUSG00000046721	-1.016110295	0.006334733	0.037187581	17.94912724	26.223034	22.95712142	46.88206947	46.97322185	42.0895279	Rpl14-ps1
ENSMUSG00000046908	-1.354651164	0.005396039	0.033236578	15.95477976	8.741011335	10.10113342	36.22705368	28.18393311	24.47065576	Lrb41
ENSMUSG00000047496	1.093888518	1.32E-05	0.00035939	433.7705749	520.5757862	451.7961495	124.6636847	234.8661093	298.542002	Rnf152
ENSMUSG00000047649	-1.227032883	5.45E-06	0.000175137	72.79368268	67.01442023	64.27993997	120.9683126	155.5335568	111.5861902	Cd3eap
ENSMUSG00000047875	1.019701978	0.000156296	0.002567963	101.711721	104.892136	85.40049				

Table 4 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRt15-TGFRs (TGFRt15-TGFRs group) in aged mice liver

ENSMUSG00000049580	-1.860685334	2.05E-30	5.59E-27	548.4455544	407.9138623	479.3446952	1707.999031	1943.647535	1562.206663	Tsku
ENSMUSG00000049791	1.116973741	1.01E-06	4.43E-05	636.1968431	678.8852137	759.4215765	215.231319	290.1901261	450.2600659	Fzc4
ENSMUSG00000049950	-1.164244145	0.007174451	0.040867998	18.94630097	37.87771578	21.1205517	94.82964053	44.8855231	35.23774429	Rpp38
ENSMUSG00000050390	1.148547066	4.80E-15	1.72E-12	1574.537328	1571.439593	1292.026793	692.5760263	634.6604197	674.4112726	C7080
ENSMUSG00000050503	-1.747508686	0.001612805	0.014098606	6.980216147	9.712234816	7.346278854	25.5720379	38.6224686	16.64004591	Fbxl22
ENSMUSG00000050663	1.366962359	0.003061462	0.022321797	20.94064844	51.47484453	44.07767312	15.98252368	13.57004187	15.66121968	Trhee
ENSMUSG00000050737	-1.262831751	0.008424555	0.045643887	26.92369085	13.59712874	44.99595798	119.3361768	55.32401685	31.32243937	Ptges
ENSMUSG00000050914	-2.867341906	0.000724516	0.007829023	13.96043229	23.30936356	14.68255771	273.8339058	58.45556497	46.98365905	Ankrd37
ENSMUSG00000051149	1.736018564	0.001879809	0.015833824	31.90955953	33.02159838	39.48624884	6.393009474	3.131548123	21.53417707	Adnp
ENSMUSG00000051339	1.051356464	2.17E-16	9.53E-14	1923.548135	1976.439785	1850.343986	917.3968595	914.4120521	942.6096597	2900026A02Rik
ENSMUSG00000051452	1.412708477	1.55E-10	2.11E-08	276.2171247	240.8634234	283.7500027	92.69863737	80.37640184	127.2474099	Gm11437
ENSMUSG00000051674	1.054513908	9.53E-09	8.39E-07	515.5388212	743.9571869	608.82286	295.1439374	270.356988	333.7797445	Dcn1143
ENSMUSG00000051998	-1.5364341	0.002646663	0.020230437	9.971737353	19.42446963	6.427993997	42.62006316	39.66627623	21.53417707	Lax1
ENSMUSG00000052085	1.272820714	3.30E-11	4.99E-09	423.7988375	546.7988202	446.2864404	197.1177921	161.796653	227.0876854	Dock8
ENSMUSG00000052595	1.233431784	0.003777269	0.025781088	1666.277312	2467.878867	2847.601341	821.5017174	661.8005034	1485.852118	Aclf
ENSMUSG00000052656	1.063492715	4.09E-12	7.63E-10	4427.451385	3448.814583	3382.043127	1950.933391	1650.325861	1785.379044	Rnf103
ENSMUSG00000052684	-1.26752533	1.09E-07	6.78E-06	161.5421451	161.223098	181.8204016	547.6678116	268.2692892	399.3611019	Jun
ENSMUSG00000052713	1.631077965	2.29E-06	8.94E-05	74.78803015	84.4964429	102.8479039	23.44103474	18.78928874	42.0895279	Zfp608
ENSMUSG00000052837	-1.91589408	1.98E-18	1.08E-15	205.4177895	302.0505028	276.4037419	1233.850828	697.2913822	1027.767542	Junb
ENSMUSG00000053560	-1.145431158	4.41E-10	5.44E-08	324.0814664	268.0576809	242.4272022	725.6065753	569.9417585	550.10034414	Ier2
ENSMUSG00000053964	-1.860738134	2.78E-14	8.62E-12	110.7145473	119.4604882	153.3535711	348.4190163	621.0903778	388.5940134	Lgals4
ENSMUSG00000053977	-1.428502316	0.007562752	0.042337131	8.974563618	28.16548097	7.346278854	57.53708526	21.92083686	40.13187544	Col8a
ENSMUSG00000054008	1.359856014	0.005424595	0.033352211	3190.955953	3047.699285	3392.144261	753.3096163	842.3864451	2156.354185	Nest1
ENSMUSG00000054150	1.583267643	1.23E-07	7.44E-06	98.72019979	105.8633595	78.97249768	33.03054895	29.22778249	32.3012656	Syne3
ENSMUSG00000054422	-1.006699851	5.17E-07	2.51E-05	21378.40771	26089.97639	27702.81756	57173.74923	60050.56682	33819.42508	Fabp1
ENSMUSG00000054453	1.090734081	0.000462815	0.005711685	93.73433112	87.41011335	126.7233102	69.25760263	36.53472811	39.15304021	Sytl5
ENSMUSG00000054659	1.244126007	0.002259781	0.018053098	79.77389882	73.8129846	100.0930494	15.98252368	25.05238499	65.58135743	Pm20x2
ENSMUSG00000054932	1.219377704	9.42E-05	0.001724207	87.75128871	202.0144842	233.2443536	72.45410737	72.02560684	80.26375088	Afp
ENSMUSG00000055148	-1.210391107	1.04E-07	6.56E-06	210.4036581	153.4533101	137.7427285	476.2792058	390.3996661	293.6478691	Klf2
ENSMUSG00000055254	1.702350158	1.36E-13	3.56E-11	1260.427601	1100.396205	2139.603716	409.1526063	418.5835992	554.9944726	Ntrk2
ENSMUSG00000055491	-1.429157403	5.36E-08	3.69E-06	191.4573572	205.8993781	144.1707225	706.4275469	442.5921348	309.3090888	Pprc1
ENSMUSG00000055660	1.215391205	9.47E-05	0.001725945	86.75411497	97.12234816	112.0307525	29.83404421	36.53472811	60.68722628	Mett14
ENSMUSG00000055692	-1.507668302	6.91E-05	0.001367589	23.93216965	24.28068704	19.28398199	90.56763421	57.417156	44.04718036	Tmem191c
ENSMUSG00000055980	1.007478258	8.74E-05	0.001638705	336.0475488	377.8059344	381.0882155	114.0086689	159.7089543	270.1560396	Inr1
ENSMUSG00000056054	-4.00774487	1.39E-20	1.11E-17	6.980216147	6.798564371	4.591424283	100.1571484	98.1218412	95.92497057	S100a8
ENSMUSG00000056071	-3.639131514	1.15E-24	1.74E-21	15.95477976	8.741011335	12.85598799	140.6462084	203.350628	123.332105	S100a9
ENSMUSG00000056091	-1.031452251	1.62E-09	1.68E-07	1990.358776	1481.115809	2190.109383	3671.718441	3506.290049	4394.929774	St3gal5
ENSMUSG00000056148	1.3596028	0.000119043	0.002094688	75.78520388	1088.770299	71.62621882	23.44103474	50.89896397	30.9496193	Rch9
ENSMUSG00000056313	-1.726808914	2.85E-11	4.37E-09	435.7649223	244.7483174	415.0647552	1762.339612	990.6130564	874.0918236	Tcm
ENSMUSG00000057342	1.09304789	8.77E-15	2.92E-12	1269.402165	1384.964685	1236.011417	564.7158369	644.0550641	614.7028726	Sphk2
ENSMUSG00000057604	-1.272122676	0.008168023	0.044709526	10.96891109	10.6834583	11.93770314	25.5720379	28.18393311	27.40713445	Lmnc1
ENSMUSG00000057722	-1.840565518	1.78E-07	1.02E-05	45.86999182	35.93526882	110.1941828	323.91248	199.3752305	166.4045951	Lepr
ENSMUSG00000057969	1.066228289	0.000219235	0.003267437	95.7867859	74.78420809	99.17476452	49.01307263	42.79782435	37.19539675	Sema3b
ENSMUSG00000058207	-1.338541988	1.08E-13	2.88E-11	23432.58561	23413.28447	28330.92444	48372.70618	79528.79614	62218.1105	Serpina3k
ENSMUSG00000058503	-1.136818503	6.67E-07	3.10E-05	130.6297593	164.1367684	153.3535711	376.1220574	383.0927204	227.0876854	Fam133b
ENSMUSG00000058793	1.056167061	4.57E-10	5.57E-08	3494.096768	3071.008649	3164.409616	1397.938072	1320.469459	1959.610113	Cds2
ENSMUSG00000058794	1.61977926	2.23E-06	8.73E-05	144.5901916	102.9466891	144.1707225	71.38860579	26.09623436	30.34361314	Nfe2
ENSMUSG00000058921	1.372756574	4.51E-07	2.22E-05	911.4167941	1126.619239	1263.559963	261.0478868	329.8564023	683.2207087	Sic10a5
ENSMUSG00000059149	1.084964814	0.00015449	0.002547498	343.0277649	296.2231619	348.9482455	147.0392179	80.37640184	237.854774	Mfsd4a
ENSMUSG00000059824	4.510710868	5.51E-13	1.32E-10	248.2962601	730.3600582	248.8551962	24.50653632	16.70158999	12.72474099	Dbp
ENSMUSG00000060429	1.717674566	1.22E-12	2.68E-10	734.9170429	831.3673003	944.9151175	201.3797984	180.5859418	380.7634036	Snrnb1
ENSMUSG00000061175	1.152852085	3.29E-14	9.98E-12	1100.879804	976.079599	989.9110755	495.4852342	405.0135573	478.6406266	Frip2
ENSMUSG00000061292	1.112980568	1.04E-05	0.000296719	1260.427601	1520.935972	1350.797024	523.1612753	399.7943104	986.6568401	Cyp3a59
ENSMUSG00000061436	1.042732241	2.40E-05	0.000578522	326.0758114	431.2232258	663.9199514	228.0173379	186.849038	275.0501707	Hipk2
ENSMUSG00000061536	1.045389319	1.00E-05	0.000287463	219.3782218	260.2878931	199.2678139	88.43663105	94.99029308	144.8662821	Sec22c
ENSMUSG00000061825	1.826944112	9.41E-14	2.63E-11	718.9622632	690.5398954	678.6125091	298.3404421	160.7528037	130.1838886	Ces2c
ENSMUSG00000062901	1.319446488	6.22E-09	5.70E-07	788.7644246	893.5256031	992.6659301	297.2749405	259.9184942	513.8837709	Klhl24
ENSMUSG00000063535	1.201850675	0.000151738	0.002508185	62.82194532	71.87053764	70.70793397	27.70304105	29.22778249	32.3012656	Zfp773
ENSMUSG00000063704	-1.127471271	2.47E-07	1.32E-05	294.1662519	311.7627376	224.061505	581.7638621	784.9747296	446.3447611	Mapk15
ENSMUSG00000063929	-1.198836957	5.83E-06	0.000185032	50.8558605	105.8633595	82.6456371	208.8383095	167.0158999	174.231069	Cyp4a32
ENSMUSG00000065126	-1.713055356	1.38E-07	8.18E-06	29.91521206	48.56117408	40.40453369	154.497729	158.6651049	77.32727219	Snrnd104
ENSMUSG00000065147	-1.294541943	0.007255788	0.041125179	14.95760603	12.62590526	15.61084256	54.3058053	32.35933061	19.57652461	Snrora31
ENSMUSG00000065952	1.57267976	1.62E-10	2.19E-08	199.4347471	157.338204	188.2483956	51.14407579	55.32401685	76.34844596	C30021F23Rik
ENSMUSG00000066456	1.032022635	0.005405213	0.033278057	76.78237762	64.10074979	150.5987165	70.32310421	34.44702936	38.17422298	Hmgn3
ENSMUSG00000066477	-1.128543552	2.29E-05	0.000561201	57.83607665	44.67628015	49.58738226	93.76413895	118.9988287	119.4168001	Gm16551
ENSMUSG00000066687	-2.591322343	5.98E-23	6.28E-20	139.6043229	122.3741587	161.6181348	472.0171995	943.698345	1137.39608	Zrb16
ENSMUSG00000066944	1.544707207	0.000412457	0.005261624	43.87564435	49.53239756	40.40453369	10.65501579	10.43849374	24.47065576	NA
ENSMUSG00000067149	-1.843573184	0.000282252	0.003980428	113.6778058	169.9641093	162.5364196	437.921149	878.9211733	284.838433	Jchain
ENSMUSG00000068463	2.382320454	0.000472976	0.005821273	4.985868677	3.884893927	5.50970914	46.88206947	15.65774062	12.72474099	B630019A10Rik
ENSMUSG00000068742	1.190690012	3.19E-12	6.14E-10	611.2674997	770.1802209	691.4684971	312.1919626	260.9623436	334.7585707	Cry2
ENSMUSG00000068877	-1.095647878	6.50E-07	3.04E-05	98.						

Table 4 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRT15-TGFRs (TGFRT15-TGFRs group) in aged mice liver

ENSMUSG000000071076	-1.243260003	3.56E-08	2.62E-06	1324.24672	1340.288405	938.4871235	3733.517533	2948.874483	1847.045097	Jund
ENSMUSG000000071456	1.345823726	0.001263422	0.011712633	45.86999182	70.89931416	48.6690974	12.78601895	16.70158999	35.23774429	Nt5ec2
ENSMUSG000000071547	-1.168738292	0.000250901	0.003706323	44.87281809	25.25181052	47.75081255	90.56763421	104.3849374	70.47548858	Cebpd
ENSMUSG000000071637	-1.028910519	0.000250761	0.0036106	107.6947634	64.10074979	89.99191596	239.7378553	123.1742262	171.2945903	Tut1
ENSMUSG000000071645	-1.14015686	5.54E-07	2.68E-05	206.4149632	273.8850218	188.2483956	605.2048969	525.0562354	343.5680068	Klf12
ENSMUSG000000072294	2.084673623	3.53E-08	2.62E-06	97.72302606	148.5971927	196.5129593	24.50653632	18.78928874	60.68722628	Tmem253
ENSMUSG000000072571	1.01455077	0.003907572	0.026379983	56.83890291	47.5899506	55.0970914	20.24453	29.22778249	29.36478691	Ugr3a1
ENSMUSG000000072664	1.275253331	1.57E-06	6.41E-05	951.3037435	1401.475484	1131.326943	273.8339058	440.504436	724.3314104	Rpl37r
ENSMUSG000000072692	-1.135811828	0.000365333	0.004818223	34.90108074	29.13670445	28.46683056	73.51960895	70.98175747	58.72957382	Serpina1e
ENSMUSG000000072849	-2.106422019	7.14E-21	6.08E-18	100.7145473	96.15112468	102.8479039	570.0433447	342.3825948	378.8057511	Gm15401
ENSMUSG000000072999	-1.145851135	1.86E-07	1.05E-05	116.669327	176.7626737	168.0461288	294.0784358	417.5397498	310.287915	Gm15401
ENSMUSG000000073460	-1.28577089	0.005211876	0.032439227	18.94630097	39.82016275	10.10113342	51.14407579	79.33255246	77.19537695	Prldc1
ENSMUSG000000073835	2.295483419	0.000631649	0.007089542	332.0588539	185.530685	325.0728393	85.24012632	69.93790809	16.64004591	Mup-ps12
ENSMUSG000000074024	1.08003527	4.28E-05	0.000939631	43.87564435	61.18707934	53.26052169	118.2706753	117.9549793	98.86144926	4632427E13Rik
ENSMUSG000000074063	-2.210318446	1.23E-07	7.44E-06	989.1963454	819.7126185	1493.131177	2843.823714	6567.900264	5870.020903	Osgin1
ENSMUSG000000074213	1.225081814	0.003513884	0.024460869	87.75128871	38.84893927	37.64967912	24.50653632	27.14008374	18.59769837	Gm10642
ENSMUSG000000074345	1.610694413	1.86E-06	7.43E-05	95.1799012	56.93366111	26.63753947	24.00853561	29.36478691	TrnfaiP8I3	
ENSMUSG000000074375	1.123374183	1.71E-16	7.76E-14	1739.070994	2059.965005	1850.343986	860.9252758	876.8334746	855.4941252	Sult2a3
ENSMUSG000000074876	1.013446872	0.000205306	0.003120937	101.711721	119.4604882	117.5404617	71.38860579	41.75397498	54.81426889	Spat51l
ENSMUSG000000075470	1.495450545	0.008033121	0.044154642	346.0192861	387.5181692	422.4110341	77.78161526	80.37640184	251.5583142	Deaf1
ENSMUSG000000075552	1.02486804	2.18E-07	1.20E-05	1632.373405	1558.813688	2640.068963	1023.947017	947.8152322	894.6471745	Cyp3a41b
ENSMUSG000000075590	-1.36961476	1.11E-14	3.51E-12	556.4229443	571.0794072	507.8115258	1682.426993	1456.169877	1087.475942	Nrbp2
ENSMUSG000000076490	-1.690670704	0.002848332	0.021272015	9.971737353	4.856117408	8.26456371	35.1615521	20.87688749	18.59769837	Trbc1
ENSMUSG000000076569	-1.340585852	0.000314538	0.004311858	26.92369085	70.89931416	46.83252769	72.45410737	110.6480337	183.0405051	Igkv3-19
ENSMUSG000000076596	-3.365323343	1.13E-07	6.96E-06	3.988694941	6.798564371	9.182848567	152.3667258	28.18393311	26.42830822	Igkv3-30
ENSMUSG000000076609	-2.421594655	3.22E-06	0.000115075	484.6264354	675.0003197	704.3244851	4135.211628	4467.675323	1384.06029	Igkc
ENSMUSG000000076613	-1.082440472	0.000712125	0.007817157	31.90955983	61.18707934	74.38107339	100.1571484	155.5335568	99.84027549	Ighg2b
ENSMUSG000000076617	-1.218011643	6.44E-08	4.32E-06	1755.025774	1885.144778	1859.526835	5037.691465	5276.658588	2480.345667	Ighm
ENSMUSG000000076934	-3.037010136	1.44E-09	1.52E-07	7.977389882	2.913670445	10.10113342	77.78161526	36.53472811	58.72957382	Igvl1
ENSMUSG000000077148	1.93809708	8.47E-06	0.000252998	48.86151303	38.84893927	69.7864911	9.589514211	19.8313812	11.74591476	Gm22935
ENSMUSG000000078193	-1.269506646	0.004582659	0.029506003	13.96043229	9.712234816	21.1205517	42.62006316	35.49087873	30.34361314	Gm2000
ENSMUSG000000078234	1.031251449	6.47E-07	3.04E-05	557.420118	503.0937635	599.6400114	197.1177921	263.0500424	351.3986167	Klhl7c7a
ENSMUSG000000078650	-1.017019231	3.99E-07	2.00E-05	5816.514398	3628.490927	4180.950953	6922.563759	10279.82864	10371.64274	G6pc
ENSMUSG000000078651	-2.099102297	6.20E-07	2.95E-05	8.974563618	10.6834583	16.52912742	55.40680211	57.4117156	43.06835413	Aoc2
ENSMUSG000000078672	-1.051061583	1.66E-05	0.000428362	108.6919371	197.1583668	152.4352862	337.7640005	374.7419254	237.854774	Mup20
ENSMUSG000000078688	1.787709931	3.76E-11	5.58E-09	119.6608482	143.7410753	173.5558379	49.01307263	38.6224686	39.15304921	Mup2
ENSMUSG000000078817	-1.628755428	1.37E-11	2.31E-09	107.6947634	79.64032549	80.8090639	204.5763032	280.7954817	342.5891806	Nlrp12
ENSMUSG000000079017	-1.001509959	0.000541038	0.006404635	74.78803015	60.21585586	44.07767312	145.9737163	124.2180756	88.09436072	Ifi272a
ENSMUSG000000079036	-1.378588057	0.008915895	0.0047568881	170.5167087	124.3166056	106.5210434	575.3708526	288.1024274	180.1040264	Alkbh1
ENSMUSG000000079065	-1.153356662	4.39E-06	0.00146637	123.6495432	164.1367684	125.8050254	371.8600511	353.864938	194.7864198	Bco005561
ENSMUSG000000079465	-1.030415111	0.006780187	0.03914539	18.94630097	27.19425749	22.03883656	46.8806947	56.36786622	36.21657052	Col4a3
ENSMUSG000000079470	1.222302248	2.09E-11	3.33E-09	498.5868677	463.2736007	474.7532709	201.3797984	169.103587	244.7065576	Utp14b
ENSMUSG000000080059	-1.546208951	0.008392591	0.045561211	7.977389882	9.712234816	6.427993997	37.29255526	13.57004187	19.57652197	Nr1p9-ps3
ENSMUSG000000081344	-1.007584184	0.007335419	0.041406148	22.93499591	19.42446963	38.56796398	64.9959632	55.32401685	43.06835413	Gm14303
ENSMUSG000000082065	2.441656125	0.003600075	0.02496215	179.4912724	141.7986283	255.2831902	60.73359	39.66627623	5.872957382	Mup-ps14
ENSMUSG000000082173	2.591199516	2.10E-11	3.33E-09	150.573234	114.6043708	224.061505	41.55456158	31.3154821	8.809436072	Mup-ps10
ENSMUSG000000082586	1.935822239	3.52E-06	0.000123572	53.84738171	55.35973845	47.75081255	15.98252368	7.306945621	17.61887214	Sult2a-ps1
ENSMUSG000000082658	-1.204694651	0.004700851	0.030195718	67.807814	23.30936356	23.87540627	85.24012632	129.4373224	49.92013774	Fau-ps2
ENSMUSG000000083327	1.031096879	0.000937166	0.009424872	102.7088947	96.15112468	82.6456371	36.22705368	34.44702936	66.56018366	Vcp-ps
ENSMUSG000000083621	-1.163358375	0.003315291	0.023547198	19.94347471	19.42446963	20.20226685	54.34058053	48.01707123	31.32243937	Gm14586
ENSMUSG000000083716	-1.168157812	0.001666635	0.01443041	25.92651712	25.25181052	35.81310941	70.32310421	86.63949808	39.15304921	Gm13436
ENSMUSG000000083813	-1.588176086	8.35E-05	0.001580916	11.96608482	53.41729149	22.03883656	86.3056279	84.55179933	92.00966564	Gm15502
ENSMUSG000000083863	-1.215047234	0.000627696	0.007061263	24.92934338	19.42446963	27.5485457	64.9959632	54.28016747	47.96248528	Gm13341
ENSMUSG000000083992	-1.283026783	0.003457083	0.024176532	11.96608482	12.62590526	20.20226685	39.42355842	40.71012551	29.36478691	Gm11478
ENSMUSG000000084822	-1.954137616	1.52E-07	8.88E-06	16.9519535	25.25181052	13.77427285	66.0610979	91.85874496	58.72957382	Myaaim2os
ENSMUSG000000084883	1.839966406	9.14E-09	8.09E-07	157.5534502	141.7986283	126.7233102	24.50653632	30.27163186	63.62370497	Cocdc8c
ENSMUSG000000085001	-2.14213554	3.42E-08	2.56E-06	29.91521206	16.51079919	21.1205517	156.6287321	70.98175747	70.47548858	Rapgef4os2
ENSMUSG000000085156	-1.793249778	0.00014396	0.002408808	24.2934338	31.07915141	16.52912742	157.6942337	59.49941435	34.25891806	Snhg15
ENSMUSG000000085445	-1.469856228	8.55E-08	5.52E-06	45.86999182	46.61872712	72.54450368	126.7946879	143.0073643	187.9343662	Gm16348
ENSMUSG000000085834	-3.692490083	4.07E-39	1.85E-35	50.8558605	56.33096193	71.62621882	1120.907661	744.264604	450.2600659	Gm15622
ENSMUSG000000085995	-1.230019641	9.11E-06	0.000268289	189.4630097	167.0504388	353.5396698	444.3141584	772.4485371	450.2600659	Gm2788
ENSMUSG000000086140	1.216209638	0.003436989	0.024085413	34.90108074	60.21585586	44.99595798	11.72051737	22.96468624	25.44948199	Hnf1aosa2
ENSMUSG000000086446	-2.158985376	0.000113634	0.002012508	17.94912724	6.798564371	9.182848567	27.70304105	97.07799183	26.42830822	Prkg2os1
ENSMUSG000000086529	2.155075918	4.95E-05	0.001053778	27.92086459	38.84893927	44.07767312	13.85152053	8.350794996	2.936478691	Acss2os
ENSMUSG000000086786	1.909493382	0.004138057	0.027366965	31.90955953	14.56835222	33.05825484	2.131003158	3.131548123	15.66121968	Gm15908
ENSMUSG000000086844	-1.797314635	1.20E-05	0.000331862	10.96891109	17.48202267	18.36569713	73.51960895	42.79782435	46.98365905	B230206H07Rik
ENSMUSG000000087342	-1.074896574	1.10E-05	0.000308734	161.5421451	285.5397036	136.8244436	414.4801142	369.5226786	445.3659348	Ctcfios
ENSMUSG000000087445	-1.629934605	4.98E-05	0.001058506	21.93782218	12.62590526	15.61084256	61.79909158	54.28016747	39.15304921	Gm14286
ENSMUSG000000087595	-1.356715257	0.000782501	0.008272069	15.95477976	21.3669166	17.44741228	41.55456158	62.63096247	36.21657052	1810012K08Rik
ENSMUSG000000087613	1.300460925	0.0026								

Table 4 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRt15-TGFRs (TGFRt15-TGFRs group) in aged mice liver

ENSMUSG00000090145	1.252091278	1.05E-06	4.56E-05	514.5416474	669.1729788	606.0680054	154.497729	220.252218	375.8692724	Ugr1a6b
ENSMUSG00000090175	1.660039728	0.006419999	0.037574902	840.6174589	1098.453758	415.0647552	125.7291863	209.8137243	409.1493642	Ugr1a9
ENSMUSG00000090264	2.18334921	1.38E-07	8.18E-06	7.977389882	12.62590526	21.1205517	57.53708526	80.37640184	52.85661643	Eif4ebp3
ENSMUSG00000090369	1.713925914	3.56E-08	2.62E-06	82.76542003	126.2590526	106.5210434	27.70304105	27.14008374	41.11070167	4933411K16Rik
ENSMUSG00000090555	2.901973235	9.10E-10	1.01E-07	121.6551957	110.7194769	103.7661888	1396.87257	624.2219259	491.3707676	Gm8893
ENSMUSG00000090610	1.076721434	0.00270073	0.020438322	79.77389882	53.41729149	47.75081255	35.16155211	28.18393311	22.5130033	Gm3571
ENSMUSG00000090698	2.55687976	1.21E-08	1.04E-06	20.94064844	19.42446963	24.79369113	247.1963663	60.54326372	76.34844596	Apold1
ENSMUSG00000091021	1.527245218	0.003008341	0.022044462	10.96891109	16.51079919	18.36569713	82.04362158	27.14008374	23.49182953	Gm17300
ENSMUSG00000091509	1.002937779	0.000805178	0.008420412	69.80216147	64.10074979	68.87136425	143.8427132	183.7174899	79.28492465	Gm17066
ENSMUSG00000092075	5.716673555	4.92E-16	1.97E-13	2.991521206	2.913670445	0	83.10912316	163.8843518	57.75074759	Serpina4-ps1
ENSMUSG00000094410	1.220382244	0.001002234	0.009882478	53.84738171	132.0863935	116.6221768	47.94757105	25.05238499	56.77192136	Gm38394
ENSMUSG00000095290	1.650205335	4.03E-05	0.000893784	58.83325038	25.25181052	24.79369113	126.7946879	158.6651049	55.79309512	Gm21738
ENSMUSG00000095351	3.092804449	0.006028299	0.035789253	6.980216147	60.21585586	166.2095591	629.7114332	1051.15632	311.2667412	Igkv4-5
ENSMUSG00000096833	3.208484861	0.004128622	0.02736411	10.96891109	124.3166056	23.87540627	3.196504737	4.175397498	9.788262303	Igkv4-55
ENSMUSG00000096910	1.124675965	0.006036	0.035819378	64.81629279	67.01442023	72.54450368	34.09605053	10.43849374	48.94131151	Zfp955b
ENSMUSG00000096954	1.206343759	0.003120612	0.022529545	33.903907	87.41011335	81.72735225	33.03054895	17.74543937	37.19539675	Gdap10
ENSMUSG00000097124	2.16107918	3.03E-13	7.50E-11	133.6212805	103.9209125	130.3964497	23.44103474	33.40317998	25.44948199	A530020G20Rik
ENSMUSG00000097221	1.550011559	0.000809985	0.008457711	46.86716556	44.67628015	42.24110341	8.524012632	9.39464437	27.40713445	1810049J17Rik
ENSMUSG00000097312	1.433041605	0.000236458	0.003456142	49.85868677	34.96404534	33.9765397	114.0086689	161.796653	45.02600659	Gm26870
ENSMUSG00000097536	1.179506676	0.003789911	0.025815692	36.89542821	55.35973845	40.40453369	13.85152053	26.09623468	18.59769837	2610037D02Rik
ENSMUSG00000097615	1.278009058	7.24E-05	0.001413729	33.903907	25.25181052	25.71197599	69.25760263	65.76251059	70.47548858	Gm2061
ENSMUSG00000097660	2.932543755	3.83E-06	0.00131806	4.985868677	4.856117408	6.427993997	60.73359	53.2363181	10.76708853	Gm26762
ENSMUSG00000097691	1.274551418	4.53E-08	3.23E-06	235.3330015	259.3166696	175.3924076	75.65061211	98.1218412	102.7767542	9030616G12Rik
ENSMUSG00000097743	1.078516143	0.000185309	0.002900208	55.84172918	55.35973845	50.5066712	155.5632305	98.1218412	88.09436072	Gm16973
ENSMUSG00000097908	1.156232879	5.54E-05	0.001144881	154.561929	95.1799012	98.25647967	52.20957737	45.92937248	57.75074759	4933404012Rik
ENSMUSG00000097971	1.77227667	3.92E-13	9.56E-11	2017.282467	2103.670061	1313.147345	8345.008366	6440.550641	3776.311596	Gm26917
ENSMUSG00000097994	1.156597047	0.008206265	0.04482173	14.95760603	25.25181052	11.93770314	29.83404421	48.01707123	38.17422298	Gm26982
ENSMUSG00000098041	1.469996023	0.000332181	0.004498467	22.93499591	20.39569311	17.44741228	68.19210105	69.93790809	30.34361314	Gm26981
ENSMUSG00000098661	1.627002493	0.000277688	0.003923434	19.94347471	6.798564371	14.69255771	43.68556474	48.01707123	36.21657052	Mir7052
ENSMUSG00000098814	4.705165719	0.001128634	0.010794205	2.991521206	1.942446963	1.836569713	13.85152053	157.6212555	4.894131151	Igkv19-93
ENSMUSG00000098882	1.905068974	0.000139968	0.002360908	128.6354119	27.19425749	93.66505538	37.29255526	18.78928874	10.76708853	Mir6392
ENSMUSG00000099568	2.251364762	0.000320113	0.004365382	1.994347471	4.856117408	19.28398199	18.11352684	59.4994135	47.96248537	Gm28513
ENSMUSG00000099858	1.088333588	4.80E-05	0.001024996	154.561929	202.0144842	168.0461288	56.47158369	75.15715496	114.5226689	Gm6652
ENSMUSG00000100094	1.758512646	5.46E-23	6.28E-10	3175.001173	2305.684545	3195.631301	1017.554008	731.7384115	815.3622498	181000818Rik
ENSMUSG00000100468	1.995883006	0.000292618	0.004105376	17.94912724	36.9064923	34.89482455	6.393009474	6.263096247	9.788262303	Tmem167-ps1
ENSMUSG00000101939	1.264629649	0.000784437	0.008279693	14.95760603	28.16548097	21.1205517	52.20957737	56.36786622	46.00483282	Gm28438
ENSMUSG00000102275	1.950030022	0.000225327	0.003321924	4.985868677	13.59712874	14.69255771	56.47158369	54.2801677	18.59769837	Gm37144
ENSMUSG00000102577	1.649520189	1.70E-08	1.42E-06	40.88412315	64.10074979	82.6456371	137.4497037	252.6115486	199.680551	Gm37969
ENSMUSG00000102719	1.730894623	0.006769906	0.039102589	12.96325856	5.82734089	4.591424283	38.35806684	30.27163326	8.809436072	Gm37760
ENSMUSG00000102869	1.362067771	5.85E-06	0.000185032	292.1719044	335.0721012	361.8042335	86.3056279	81.4205121	216.3205969	2900097C17Rik
ENSMUSG00000102882	2.19227753	3.68E-07	1.87E-05	88.74846344	63.12952631	216.7152262	26.63753947	38.62246896	15.66121968	Gm2065
ENSMUSG00000102918	1.264206677	0.002814898	0.021114835	30.91238579	47.5899506	66.11650968	18.11352684	14.61389124	27.40713445	Pcchgc3
ENSMUSG00000103285	1.692405475	0.00296955	0.020438322	9.971737353	5.82734089	8.26456371	27.70304105	35.49087873	14.68239345	Gm37274
ENSMUSG00000103546	1.97976669	0.000414043	0.005267075	6.980216147	6.798564371	6.427993997	28.76854263	33.40317998	17.61887214	Gm37666
ENSMUSG00000104030	3.028455317	2.18E-07	1.20E-05	3.988694941	4.856117408	5.50970914	56.47158369	39.66627623	21.53417707	5330406M23Rik
ENSMUSG00000104388	2.61061807	5.15E-05	0.001078975	6.980216147	1.942446963	6.427993997	46.88206947	34.4702936	12.72474099	Gm37033
ENSMUSG00000104399	1.177892321	0.00272173	0.020574411	23.93216965	29.13670445	18.36569713	77.78161526	41.75397498	42.0895279	Gm37963
ENSMUSG00000104445	1.012908858	0.003001159	0.022015494	68.80498774	51.47484453	89.99191596	38.35805684	40.71012561	25.44948199	Rhbg
ENSMUSG00000104973	2.007621104	9.36E-05	0.001719883	6.980216147	13.59712874	4.591424283	36.22705368	32.35933061	32.3012656	A530041M06Rik
ENSMUSG00000105161	1.279752452	0.00691512	0.039789659	21.93782218	8.741011335	13.77427285	44.75106632	39.66627623	23.49182953	Gm42595
ENSMUSG00000105434	2.02241612	0.000742647	0.007975668	5.983042412	7.769787853	6.427993997	26.63753947	42.79782435	12.72474099	Gm43359
ENSMUSG00000105547	2.707072316	4.65E-06	0.000153843	5.983042412	2.913670445	8.26456371	23.44103474	27.14008374	61.66605251	Igfc3
ENSMUSG00000105556	1.504518371	0.00309422	0.022420764	21.93782218	29.13670445	33.05825484	7.458511053	9.39464437	12.72474099	Gm43080
ENSMUSG00000105703	1.801332207	3.38E-12	6.40E-10	242.3132177	122.3741587	160.6998499	712.8205563	708.7737253	409.1493642	Gm43305
ENSMUSG00000105881	1.259909682	7.19E-06	0.001410701	247.2990864	245.7195409	252.5283356	43.68556474	106.4726362	160.5275018	4932422M17Rik
ENSMUSG00000105906	2.671616891	0.003078263	0.022366209	6.980216147	20.39569311	14.69255771	182.20077	62.63096247	23.49182953	Igfc1
ENSMUSG00000106030	1.145150269	5.54E-05	0.001144881	109.6891109	147.6259692	198.349529	62.86459316	88.72719683	54.81426889	Gm43611
ENSMUSG00000106664	1.606191029	0.000152255	0.002513686	9.971737353	17.48202267	19.28398199	54.34058053	55.32401685	33.28009183	Gm17936
ENSMUSG00000106705	2.05446715	2.41E-09	2.41E-07	18.94630097	32.05037489	31.22168513	87.37112948	163.8843518	91.03083941	Gm2602
ENSMUSG00000106706	1.806072384	0.002749571	0.020715964	9.971737353	8.741011335	4.591424283	31.96504737	38.62246896	10.76708853	C530043K16Rik
ENSMUSG00000106943	1.230168101	0.009404129	0.049324655	9.971737353	13.59712874	13.77427285	30.89954579	34.4702936	22.5130033	Dancr
ENSMUSG00000107168	2.535260536	5.76E-05	0.00118059	1.994347471	9.712234816	5.50970914	46.88206947	39.66627623	13.70356722	Gm42507
ENSMUSG00000107225	3.314781204	7.31E-09	6.61E-07	3.988694941	1.942446963	6.427993997	46.88206947	43.84167373	33.28009183	Gm43637
ENSMUSG00000107304	1.772473058	0.001145745	0.010886341	7.977389882	10.6834583	11.93770314	28.76854263	59.49941435	16.64004591	Gm43775
ENSMUSG00000107390	1.580957843	0.000625578	0.007061263	12.96325856	23.30936356	11.93770314	46.88206947	70.98175747	26.42830822	Gm43323
ENSMUSG00000107624	2.865902972	2.57E-10	3.34E-08	8.974563618	13.59712874	11.93770314	103.3536532	111.6918831	37.19539675	Gm44005
ENSMUSG00000108368	1.782724603	5.57E-05	0.001147878	20.94064844	11.65468178	7.346278854	46.88206947	41.75397498	47.96248537	Gm45053
ENSMUSG00000108633	1.682864395	0.001365295	0.012400579	8.974563618	12.62590526	9.182848567	49.01307263	33.40317998	16.64004591	Gm44694
ENSMUSG00000108820	1.905612833	0.000888445	0.00904159	6.980216147	3.884893927	8.26456371	21.31			

Table 4 (cont'd). RNA-seq analysis of differentially expressed genes between the PBS (Control Group) or TGFRt15-TGFRs (TGFRt15-TGFRs group) in aged mice liver

ENSMUSG00000109536	-1.039899156	0.001827878	0.015463254	63.81911906	37.87771578	29.38511541	109.7466626	90.81489558	68.51783612	9330162G02Rik
ENSMUSG00000109555	-1.255499819	0.007385438	0.041635066	10.96891109	14.56835222	15.61084256	43.68556474	33.40317998	21.53417707	Gm44891
ENSMUSG00000109807	-2.757236067	2.47E-09	2.44E-07	11.96608482	5.82734089	12.85598799	75.65061211	94.99029308	37.19539675	Gm45244
ENSMUSG00000109836	1.535086391	0.000730915	0.007885666	46.86716556	68.9569672	89.07363111	12.78601895	12.52619249	45.02600659	Gm45884
ENSMUSG00000109841	1.421032277	0.000627493	0.007061263	46.86716556	57.30218542	33.05825484	13.85152053	15.65774062	21.53417707	E330011021Rik
ENSMUSG00000110588	-5.501119532	2.73E-06	0.000102051	31.90955953	14.56835222	3.673139427	1747.42259	289.1462767	231.9818166	Gm45774
ENSMUSG00000110613	1.168711997	0.002147567	0.017360028	64.81629279	58.2734089	38.56796398	26.63753947	16.70158999	28.38596068	Ln cbate1
ENSMUSG00000110702	-1.389252147	0.004080864	0.027194141	18.94630097	11.65468178	8.26456371	45.8165679	30.27163186	25.44948199	Gm45767
ENSMUSG00000110755	1.852337964	4.42E-06	0.000147317	194.4488784	92.26623075	176.3106925	79.91261842	28.18393311	20.55535084	BC049987
ENSMUSG00000111282	-1.321989153	0.002140143	0.017319005	21.93782218	18.45324615	30.30340027	87.37112948	62.63096247	27.40713445	Gm47528
ENSMUSG00000111312	1.254957582	0.001977069	0.016387107	45.86999182	33.99282186	56.93366111	13.85152053	20.87698749	22.5130033	Gm47205
ENSMUSG00000111631	-1.948317608	1.41E-05	0.000376102	11.96608482	15.53957571	11.01941828	28.76854263	66.80635997	52.85661643	Gm32017
ENSMUSG00000111709	2.018073354	0.00211263	0.017179449	31.90955953	69.92809068	7.346278854	12.78601895	8.350794996	5.872957382	Gm3776
ENSMUSG00000111774	2.335615682	0.000501244	0.006070575	17.94912724	15.53957571	39.48624884	5.327507895	7.306945621	1.957652461	AC166078.1

Example 10: TGFRt15-TGFRs treatment downregulates genes related to glucose metabolism, lipid metabolism, and amino acid metabolism in liver

In light of the fact that type-II diabetes (T2D) is a metabolic disease and the liver is a key metabolic organ governing body energy metabolism, RNA-seq analysis on the
 5 livers of db/db mice was performed following TGFRt15-TGFRs treatment. Differentially expressed liver genes were detected in treated db/db mice and untreated control db/db mice. One gene was upregulated and 32 genes were downregulated, which together were grouped into four clusters based on function, as shown in Figure 27. Expression of 8
 10 genes related to glucose, lipid, or amino acid metabolism were significantly reduced in the liver following TGFRt15-TGFRs treatment, as shown in Figure 28. For example, Resistin (Retn) has been shown to induce insulin resistance in mice partially through toll-like receptor 4 signaling pathway and downregulation of Retn after TGFRt15-TGFRs treatment can contribute to the reduction of insulin resistance. As shown in Figure 28,
 15 the expression of cellular senescence related genes, Cav1, Endod1, Pdk4, and Gadd45b, was also downregulated after TGFRt15-TGFRs treatment, indicating TGFRt15-TGFRs treatment can reduce senescent cell levels in livers. As shown in Figure 28, fourteen pro-inflammation genes were downregulated and one gene was upregulated (Cish) indicating that TGFRt15-TGFRs treatment reduced liver inflammation. As shown in Figure 28,
 20 expression of nine genes related to vascular regulation was also reduced. This result further indicates that the reduction of SNCs and SASP in db/db mice may favorably impact vascular health in diabetes. Taken together, these RNA-seq results indicate that TGFRt15-TGFRs treatment reduces the cellular senescence, SASP, and gluconeogenesis induced by metabolic dysfunction to improve glucose metabolism, metabolic homeostasis, and lower sterile inflammation in the livers of T2D db/db mice.

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Example 11: Senescence-associated genes are downregulated in livers of aged mice treated with TGFRt15-TGFRs

In order to investigate the effect of TGFRt15-TGFRs treatment on senescent cells (SNCs) and senescence-associated secretory phenotype (SASP) of peripheral organs,
 30 expression of inflammation and senescence-associated genes in aged mice (76 weeks) were interrogated by RNA-seq. Aged mice received either one or two subcutaneous doses

of TGFRt15-TGFRs (3 mg/kg) or PBS (negative control). RNA-seq analysis was performed on the liver isolated at 60 or 90 days after TGFRt15-TGFRs treatment to determine the global transcriptional changes. Significant differentially expressed genes were clustered by their gene ontology and the enrichment of gene ontology terms was tested using Fisher exact test (GeneSCF v1.1-p2). The livers of TGFRt15-TGFRs-treated aged mice showed significant changes in gene expression with a total of 539 differentially expressed mRNAs compared to PBS-treated mice. As shown in Figure 29, RNA-seq analysis indicated significant downregulation of genes including *Cdkn1a*, *Nle1*, *Jund*, *Sema3b*, *Bcl6*, *Bcl7c*, and *Gadd45β* and upregulation of senescence and inflammation associated genes (e.g., cytokines: *Il6α*, *Il1α*, *Il-6*, *Tnfa*, *S100a8*, *S100a9*, *S100a11*, *Lcn2*, *Retnlg*, *Inhbb*; chemokines: *Cxcl1*, *Cxcr4*, *Mt1*, and *Mt2*; metalloproteinases: *Mmp9*; gene expression and signaling pathways: e.g., *Cebpd*, *Klf12*, *Egr1*, *Egfr*, *Gadd45β*, *Gadd45g*, *Ppara*, *Pparδ*, *Fos*, *Fosl2*, *Jun*, *Junb*, *Mapk15*, *Adcy9*).

Example 12: Cellular senescence in peripheral organs of aged mice treated with TGFRt15-TGFRs

In order to further analyze the impacts of TGFRt15-TGFRs treatment on cellular senescence and senescence-associate secretory phenotype (SASP) in the peripheral organs of aged mice, qRT-PCR, ELISA, and immunofluorescence studies were performed for selected markers. As shown in Figure 30, either one or two doses of TGFRt15-TGFRs treatment was given to aged mice. As shown in Figure 31, qRT-PCR analysis of liver of aged mice either 10 days or 60 days after a single-dose TGFRt15-TGFRs treatment showed a significant reduction in gene expression for the cellular senescence and SASP signature genes, PAI-1, *Il1a*, *Il6*, *Il1β*, and *Tnfa* compared to the PBS control mice. As shown in Figure 32, two-dose TGFRt15-TGFR treatment also provided significant reduction in *Il1a*, *Cdkn1a*, PAI, *Il1b*, and *Il6* transcripts in the liver at 120 days post-treatment initiation versus the control group. As shown in Figure 33, reduction of liver IL-1α, IL-6 and IL-8 were also observed at protein levels by ELISA. As shown in Figure 34, in a two-dose treatment regimen, treatment with TGFRt15-TGFRs lowered biomarkers PAI-1 and fibronectin, indicating that treatment with TGFRt15-TGFRs can reduce liver fibrosis in aged mice, consistent with significant down-

regulation of Col4a3 and Col20a1 expression observed in the RNA-seq study of Example 10. As shown in Figure 35, immunofluorescence staining of aged mice liver sections confirmed accumulation of p21+ SNCs which were reduced with TGF β Rt15-TGFRs treatment.

5 To further investigate the durability of the senolytic and senomorphic activities of TGF β Rt15-TGFRs treatment on gene expression in the livers of aged mice, RNA-seq studies were performed. Significant downregulation (e.g., *Cdkn1a*) or upregulation (e.g., *Tert*) of senescence and inflammation associated (SASP) genes (e.g., cytokine: *Il7*, *Il15*, *Il18*, *S100g*, *S100a1*, *S100a4*, *S100a6*, *S100a10*, *S100a16*, *S100g*; chemokines: *Ccl2*,
 10 *Ccl4*, *Ccl6*, *Ccl7*, *Ccl8*, *Ccl9*, *Ccl24*, *Ccl25*, *Ccl27*, *Cxcl11*, *Cxcl10*, *Cxcl11*; metalloproteins: *Mmp12*, *Mmp13*, *Mmp27*; gene expression and signaling pathways: *Klf1*, *Klf3*, *Klf7*, *Klf9*, *Klf13*, *Egr1*, *Ppara*, *Jun*, *Fos12*; *Mapk3*, *Mapk6*, *Mapk7*, *Mapk9*, *Mapk12*, *Mapk15*, *Adcy1*, *Adcy3*, *Adcy5*, *Adcy6*, *Adcy9*, *Adcy10*), and gene associated liver functions (e.g., *Dbp*, *Tef*) and immune stimulation (e.g., *Lys1*, *Sesn2*, *Sesn3*) were
 15 observed following TGF β Rt15-TGFRs treatment. Results of these RNA-seq studies are shown as heatmaps in Figure 36.

Example 13: Senolytic and senomorphic function of TGF β Rt15-TGFRs in livers of young and aged mice

20 To further evaluate whether the TGF β Rt15 component of TGF β Rt15-TGFRs exhibited senolytic and senomorphic function, young and aged mice were treated with a single-dose of TGF β Rt15-TGFRs and RNA-seq analysis on livers were performed 10 days after treatment. TGF β Rt15-TGFRs treatment significantly lowered the expression of *Cdkn1a* and many circadian clock genes in the liver. A comparison of impacts of
 25 TGF β Rt15-TGFRs and TGF β Rt15*-TGFRs 120 days after treatment was also performed. RNA-seq analysis on liver from treated mice showed that TGF β Rt15-TGFRs, but not TGF β Rt15*-TGFRs, maintained the downregulation of *Cdkn1a* expression and both treatments continued to upregulate the *Tert* gene expression compared with PBS treatment as shown in Figure 36. Interestingly, TGF β Rt15*-TGFRs treatment
 30 significantly increased circadian molecular clock activator genes *Arntl* and *Npas2* compared to TGF β Rt15-TGFRs-treated or the control group. Since TGF β Rt15*-TGFRs did

not activate or promote proliferation of immune cells, this suggests that direct neutralization of TGF- β by the TGF β RII component of TGF β Rt15-TGFRs may contribute to the senolytic and senomorphic activities of TGF β Rt15-TGFRs. This also suggests that the IL-15 component of TGF β Rt15-TGFRs provides long lasting senolytic activity.

5 Taken together, these Examples indicates that TGF β Rt15-TGFRs treatment durably reduces genes associated with SNCs and SASP, and enhances the immune-cell activities in naturally aged mice. It also suggests that TGF β Rt15-TGFRs treatment improves the metabolic function, fibrosis, and circadian rhythms of liver cells of naturally aged mice.

10

Example 14: TGF β Rt15-TGFRs treatment is safe and tolerated in mice and non-human primates

Short-term and long-term toxicity studies of TGF β Rt15-TGFRs treatment were performed in mice and non-human primates. Subcutaneous administration of TGF β Rt15-TGFRs at 5 to 100 mg/kg in two doses on days 1 and 15 was well tolerated in a GLP toxicity study in C57BL/6 mice with no observed mortality and no test article related changes in clinical signs or clinical pathology. In a GLP toxicology study in cynomolgus monkeys, subcutaneous administration of TGF β Rt15-TGFRs at 1 to 10 mg/kg in two doses on days 1 and 15 was also well tolerated. There was no test article related changes in clinical signs, body weight, ophthalmology, ECG, blood pressure, or gross pathology. Dose-dependent increases of MCP-1 and decreases of TGF β 1 and TGF β 2 in the serum were observed. Immunophenotyping indicated that TGF β Rt15-TGFRs induced dose-dependent increases in the percentage of Ki67+ cells and absolute cell numbers of CD4+, CD8+, Treg and CD16+ NK cells (Figures 37 and 38). There was no observed adverse effect of multidose subcutaneous TGF β Rt15-TGFRs administration in cynomolgus monkeys even at a dose level as high as 10 mg/kg.

Pharmacokinetic analysis showed a half-life of 12 to 21 hours for 1 mg/kg to 10 mg/kg subcutaneously administered TGF β Rt15-TGFRs in cynomolgus monkeys. The results also confirm that exposure to TGF β Rt15-TGFRs increased serum levels in a dose-dependent manner with no apparent accumulation of TGF β Rt15-TGFRs following repeated dosing at 14-day intervals.

30

The activity and tolerability of TGFRT15-TGFRs was also assessed in naturally aged C57BL/6 mice. 76-week-old mice treated subcutaneously with 3 mg/kg TGFRT15-TGFRs (N=20) or PBS (control; N=20) were observed weekly for changes in body weight and overall survival. In subsequent studies, 90-week-old mice were treated with two subcutaneous 3 mg/kg doses of TGFRT15-TGFRs 45 days apart. Blood was drawn at various time points to assess immune cell subset frequencies. As expected, TGFRT15-TGFRs treatment mediated significant increases in the percentage of CD8+ T cells and NK cells in the blood which returned to baseline 4 weeks post treatment.

TGFRT15-TGFRs treatment was well-tolerated by mice and non-human primates at dose levels significantly higher than the therapeutic dosage (3 mg/kg). There was also no long-term adverse effect of TGFRT15-TGFRs treatment observed on the health span of naturally aged mice.

Example 15. TGFRT15-TGFRs Treatment Enhances Immune Cell Populations in

db/db mice

Five-week-old male db/db mice [BKS.Cg-*Dock7^m* +/+ *Lepr^{db}*/J (Wildtype for *Dock7^m*, Homozygous for *Lepr^{db}*), strain#000642] from Jackson Lab (Bar Harbor, ME) were fed with standard chow diet (Irradiated 2018 Teklad global 18% protein rodent diet, Envigo) and received drinking water ad libitum. Mice were divided into three groups as follows: PBS control group (*n* =6), TGFRT15-TGFRs group (*n* =6) and TGFRT15*-TGFRs group (*n* =6). Mice were treated subcutaneously with PBS, TGFRT15-TGFRs (3 mg/kg) and TGFRT15*-TGFRs (3 mg/kg). The mice were euthanized, and spleen was harvested and processed to a single cell suspension. Single cells suspension was prepared in order to evaluate the different subsets of immune cells after treatment with TGFRT15-TGFRs. RBCs were lysed in ACK buffer for 5 minutes at room temperature. The remaining cells were washed in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% Sodium Azide (Sigma)). To assess the different types of immune cells in spleen, cells were stained with antibodies specific to cell-surface CD3, CD45, CD8 and NK1.1 (BioLegend) for 30 minutes at RT. After surface staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% Sodium Azide (Sigma)). After two washes, cells were resuspended in fixation buffer and analyzed by Flow Cytometry (Celesta-BD Bioscience). The results in Figure 39 indicate

that treatment with TGFRt15-TGFRs an increase total spleen cells and also increase in the percentages of CD3⁺CD8⁺, CD3⁺NK1.1⁺, and CD3⁺CD45⁺ immune cells in the spleen subsets, whereas treatment with TGFRt15*-TGFRs had no effect on the percentage of these cell populations. TGFRt15-TGFRs treatment also increase the central and effector memory cells population (Figure 39). These results suggest that IL-15 activity of TGFRt15-TGFRs plays a role in increasing CD8⁺ T cells and NK cells in the blood of db/db mice and able to proliferate CD3⁺CD8⁺, CD3⁺NK1.1⁺, and CD3⁺CD45⁺ immune cells.

Example 16. TGFRt15-TGFRs Treatment Enhances Cytotoxic Activity of

Splenocytes in *db/db* Mice after Day 4 Post-Treatment

Five-week-old male *db/db* mice were purchased from the Jackson Laboratory. Mice were housed in a controlled temperature and controlled light environment. Mice were divided into three groups as follows: Saline control group (*n* =5), TGFRt15-TGFRs group (*n* =5) and TGFRt15*-TGFRs group (*n* =6). Mice were treated subcutaneously with PBS, TGFRt15-TGFRs (3 mg/kg) and TGFRt15*-TGFRs (3 mg/kg). The mice were euthanized, and spleen was harvested and processed to a single cell suspension. Single cells suspension was prepared in order to evaluate cytotoxic activity of splenocytes against Yac-1 cells. Yac-1 cells were labelled with CellTrace Violet and mixed with splenocytes (E:T 20:1) and incubated for 20 hours. The cells were washed and resuspended in complete media containing propidium iodide (PI) solution (Sigma Aldrich, St. Louis, MO). The cytotoxicity was assessed by flow cytometry as previously described.

The results in Figure 40 indicate that treatment with TGFRt15-TGFRs significantly increase the cytotoxic activity of splenocytes compared to TGFRt15*-TGFRs treated splenocytes.

Example 17. TGFRt15-TGFRs Treatment Enhances IFN-gamma Production of Splenocytes in *db/db* Mice After Day 4 Post-Treatment and *In Vitro* αCD3/CD28 Stimulation Assays

Five-week-old male *db/db* mice were purchased from the Jackson Laboratory. Mice were housed in a controlled temperature and controlled light environment. Mice were divided into three groups as follows: Saline control group (*n* =5), TGFRt15-TGFRs group (*n* =5) and TGFRt15*-TGFRs group (*n* =6). Mice were treated subcutaneously with PBS, TGFRt15-

TGFRs (3 mg/kg) and TGFRt15*-TGFRs (3 mg/kg). The mice were euthanized, and spleen was harvested and processed to a single cell suspension. Single cells suspension were plated at 2×10^5 cells/well in 96 well U-bottom plate and stimulated with Miltenyi T Cell Activation/Expansion Kit at a 1:1 ratio of beads to cells. Cells were cultured for 4 days, and supernatant was collected to measure TNF- α or IFN- γ cytokine released by using Magpix multiplexing cytokine assay.

The data in Figure 41 show that both TGFRt15-TGFRs and TGFRt15*-TGFRs enhance interferon-gamma production of splenocytes in *db/db* mice after day 4 post-treatment and *in vitro* α CD3/CD28 stimulation assays.

Example 18. TGFRt15-TGFRs Treatment Enhances Glycolytic Activity of Splenocytes in *db/db* Mice After Day 4 Post-Treatment

Five-week-old male *db/db* mice were purchased from the Jackson Laboratory. Mice were housed in a controlled temperature and controlled light environment. Mice were divided into three groups as follows: Saline control group ($n=5$), TGFRt15-TGFRs group ($n=5$) and TGFRt15*-TGFRs group ($n=6$). Mice were treated subcutaneously with PBS, TGFRt15-TGFRs (3 mg/kg) and TGFRt15*-TGFRs (3 mg/kg). The mice were euthanized at day 4, and spleen was harvested and processed to a single cell suspension. Single cells suspension was prepared in order to measure the glycolytic activity of the splenocytes, the cells were washed and resuspended in seahorse media and resuspended in 4×10^6 cells/mL. Cells were seeded at 50 μ L/well in Cell-Tak-coated Seahorse Bioanalyzer XFe96 culture plates in Seahorse XF RPMI medium, pH 7.4 supplemented with 2 mM L-glutamine for glycolysis stress test. The cells were allowed to attach to the plate for 30 mins at 37°C. Additionally, 130 μ L of the assay medium was added to each well of the plate (also the background wells). The plate was incubated in 37°C, non-CO₂ incubator for 1 hr. For glycolysis stress test the calibration plate contained 10x solution of Glucose/oligomycin/2DG prepared in Seahorse assay media and 20 μ L of Glucose/oligomycin/2DG were added to each of the ports of the extracellular flux plate that was calibrated overnight. The glycolysis stress test is based on extracellular acidification rate (ECAR) and measures three key parameters of glycolytic function including glycolysis, glycolytic capacity and glycolytic reserve. Complete ECAR analysis consisted of four stages: non glycolytic acidification (without drugs), glycolysis

(10 mM glucose), maximal glycolysis induction/glycolytic capacity (2 μ M oligomycin), and glycolysis reserve (100 mM 2-DG). At the end of the experiment the data was exported as a Graph Pad Prism file. The XF glycolysis stress test report generator automatically calculated the XF cell glycolysis stress test parameters from the Wave data.

5 The data was analyzed using the Wave software (Agilent).

As shown in Figure 42, the splenocytes isolated from *db/db* mice at day 4 after TGF α 15-TGFRs therapy showed enhanced basal glycolysis, capacity, and reserve rate, when compared to splenocytes of the saline or TGF α 15*-TGFRs treatment groups.

10 **Example 19. TGF α 15-TGFRs Treatment Enhances Mitochondrial Respiration of Splenocytes in *db/db* Mice After Day 4 Post-Treatment**

Five-week-old male *db/db* mice were purchased from the Jackson Laboratory. Mice were housed in a controlled temperature and controlled light environment. Mice were divided into three groups as follows: Saline control group ($n=5$), TGF α 15-TGFRs group ($n=5$) and
 15 TGF α 15*-TGFRs group ($n=6$). Mice were treated subcutaneously with PBS, TGF α 15-TGFRs (3 mg/kg) and TGF α 15*-TGFRs (3 mg/kg). The mice were euthanized at day 4, and spleen was harvested and processed to a single cell suspension. Single cells suspension was prepared in order to measure the glycolytic activity of the splenocytes, the cells were washed and resuspended in seahorse media and resuspended in 4×10^6 cells/mL. Cells
 20 were seeded at 50 μ L/well in Cell-Tak-coated Seahorse Bioanalyzer XFe96 culture plates in Seahorse XF RPMI medium, pH 7.4 supplemented with 2 mM L-glutamine for glycolysis stress test. The cells were allowed to attach to the plate for 30 mins at 37°C. Additionally, 130 μ L of the assay medium was added to each well of the plate (also the background wells). The plate was incubated in 37°C, non-CO₂ incubator for 1 hr. For
 25 mitochondrial stress test, the Calibration plate contained 10x solution of oligomycin/FCCP/Rotenone prepared in Seahorse assay media and 20 μ L of oligomycin, FCCP and Rotenone was added to each of the ports of the extracellular flux plate that was calibrated overnight. Oxygen Consumption Rate (OCR) was measured using an XFe96 Extracellular Flux Analyzer. Complete OCR analysis consisted of four stages: basal
 30 respiration (without drugs), ATP-linked respiration/Proton leak (1.5 μ M mM Oligomycin), maximal respiration (2 μ M FCCP), and spare respiration (0.5 μ M

Rotenone). At the end of the experiment, the data was exported as a Graph Pad Prism file. The XF mitochondrial stress test report generator automatically calculates the XF mitochondrial stress test parameters from the Wave data that have been exported to Excel. The data was analyzed by using the Wave software (Agilent).

- 5 As shown in Figure 43, the splenocytes isolated from db/db mice at day 4 after TGF α 15-TGFRs therapy showed enhanced basal respiration, mitochondria respiration, capacity, and ATP production, when compared to splenocytes of the saline or TGF α 15*-TGFRs treatment groups.

10 **Example 20. TGF α 15-TGFRs Treatment Decreases Plasma TGF β 1 and TGF β 2 Levels in db/db Mice After Day Post-Treatment**

- Five-week-old male db/db mice were purchased from the Jackson Laboratory. Mice were housed in a controlled temperature and controlled light environment. Mice were divided into three groups as follows: Saline control group ($n=5$), TGF α 15-TGFRs group ($n=5$) and
 15 TGF α 15*-TGFRs group ($n=6$). Mice were treated subcutaneously with PBS, TGF α 15-TGFRs (3 mg/kg) and TGF α 15*-TGFRs (3 mg/kg). Blood was collected from the submandibular vein in tubes containing EDTA and plasma was isolated by centrifugation. The plasma TGF- β levels were analyzed by using cytokine array, TGF β 3-plex (TGF β 1-3) (Eve Technologies, Calgary, AL, Canada). As shown in Figure 44, plasma TGF β 1 and 2
 20 levels were decrease in db/db mice at day 4 after TGF α 15-TGFRs treatment compared to PBS or TGF α 15*-TGFRs treatment groups.

Example 21. Generation of TGF α 15*-TGFRs

- A fusion protein complex was generated comprising of TGF α /IL15R α Su and
 25 TGF α /TF/IL-15D8N fusion proteins. The human TGF-b receptor (TGFR), IL-15 alpha receptor sushi domain (IL15RaSu), tissue factor (TF) and IL-15 with D8N mutant (IL15D8N) sequences were obtained from the GenBank website and DNA fragments for these sequences were synthesized by Genewiz. Specifically, a construct was made linking the TGFR sequence to the N-terminus coding region of IL15RaSu and the TGFR
 30 sequence to the N-terminus of tissue factor 219 followed by the N-terminus coding region of IL-15D8N.

The nucleic acid sequence of the TGFR/IL15R α Su construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCTCTCCAGCGCCT
5 ACTCC

(Single chain Human TGF- β Receptor II homodimer)

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACGAGCATCAC
10 CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCTGGAGACCGTGTGTACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
15 GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
20 TGGAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Sushi domain of IL15 receptor alpha chain)

25 ATTACATGCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO:
61)

30

The nucleic acid sequence of the TGFR/TF/IL15D8N construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGGGAGTGAAAGTTCTTTTTGCCCTTATTTGTATTGCTGTGGCCGAGGCC

5 *(Single chain Human TGF-beta Receptor II homodimer)*

ATCCCACCGCACGTTTCAGAAAGTCGGTGAATAACGACATGATAGTCACT
GACAACAACGGTGCAGTCAAGTTTCCACAACCTGTGTAAATTTTGTGATGTGA
GATTTTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCAC
CTCCATCTGTGAGAAGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAAT
10 GACGAGAACATAAAGTAGAGACAGTTTGCCATGACCCCAAGCTCCCCTACC
ATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAAA
AAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCTGTAGCTCTGATGAGTGCA
ATGACAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACGGAGG
TGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCAC
15 GTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCC
TGAAATTTCCCCAGCTGTGCAAATTCCTGCGATGTGAGGTTTTCCACCTGCGAC
AACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGC
CTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCTT
GGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTTCATCCTGGAA
20 GACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAG
ACCTTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTT
TAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

TCAGGCACTACAAATACTGTGGCAGCATATAATTTAACTTGGAAATCA
25 ACTAATTTCAAGACAATTTTGGAGTGGGAACCCAAACCCGTCAATCAAGTCT
ACACTGTTCAAATAAGCACTAAGTCAGGAGATTGGAAAAGCAAATGCTTTTA
CACAACAGACACAGAGTGTGACCTACCGACGAGATTGTGAAGGATGTGAA
GCAGACGTACTTGGCACGGGTCTTCTCCTACCCGGCAGGGAATGTGGAGAGC
ACCGGTTCTGCTGGGGAGCCTCTGTATGAGAACTCCCCAGAGTTCACACCTTA
30 CCTGGAGACAAACCTCGGACAGCCAACAATTCAGAGTTTTGAACAGGTGGGA
ACAAAAGTGAATGTGACCGTAGAAGATGAACGGACTTTAGTCAGAAGGAAC

AACACTTTCCTAAGCCTCCGGGATGTTTTTGGCAAGGACTTAATTTATACACT
 TTATTATTGGAAATCTTCAAGTTCAGGAAAGAAAACAGCCAAAACAAACACT
 AATGAGTTTTTTGATTGATGTGGATAAAGGAGAAAACACTACTGTTTCAGTGTTCA
 AGCAGTGATTCCCTCCCGAACAGTTAACCGGAAGAGTACAGACAGCCCGGTA
 5 GAGTGTATGGGCCAGGAGAAAGGGGAATTCAGAGAA

(Human IL-15D8N)

AACTGGGTGAATGTAATAAGTAATTTGAAAAAAATTGAAGATCTTATT
 CAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTACCCCCAG
 TTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTATTTTAC
 10 TTGAGTCCGGAGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATCCT
 AGCAAACAACAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCAAA
 GAATGTGAGGAACTGGAGGAAAAAAATATTAAAGAATTTTGCAGAGTTTGT
 TACATATTGTCCAAATGTTTCATCAACACTTCT

15 The amino acid sequence of TGFR/IL15RaSu fusion protein (including signal
 peptide sequence) is as follows:

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Single chain Human TGF-beta Receptor II homodimer)

20 IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
 TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKK
 PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSIPPHVQKSVN
 NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAV
 WRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDE
 25 CNDNIIFSEEYNTSNPD

(Human IL-15 receptor α sushi domain)

ITCPPPMSEHADIWVKSYSLSRERYICNSGFKRKAGTSSLTECVLNKAT
 NVAHWTTPSLKCIR (SEQ ID NO: 8)

30 The amino acid sequence of TGFR/TF/IL15D8N fusion protein (including signal
 peptide sequence) is as follows:

(Signal peptide)

MGVKVLFALICIAVAEA

(Single chain Human TGF-beta Receptor II homodimer)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI

5 TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAV
WRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

10 (Tissue factor)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFQSVQAVIPSRTVNRKSTDSPVECMGQE
15 KGEFRE

(IL-15D8N)

NWVNVISNLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVIS
LESGDASIHDVTENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS (SEQ ID NO: 68)

20

The TGFR/IL15R α Su and TGFR/TF/IL-15D8N constructs were cloned into a
modified retrovirus expression vectors as described previously (Hughes MS, Yu YY,
Dudley ME, Zheng Z, Robbins PF, Li Y, et al). The expression vectors were transfected
into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for
25 formation and secretion of the soluble TGFR/IL15R α Su - TGFR/TF/IL-15D8N protein
complex (referred to as TGFRt15*-TGFRs), which can be purified by anti-TF antibody
affinity.

30

Example 22. Protection of TGF α 15-TGFRs from Chemical Induced Liver**Damages**

B6C3F1 male mice were purchased from The Jackson Laboratory. The mice were divided into two groups as follows: saline control group ($n=6$) and TGF α 15-TGFRs group ($n=6$). All mice (14-day-old) were peritoneally treated with DEN (1 mg/kg, diethylnitrosamine) on study day zero (SD0). CCl₄ (0.2 mL/kg, carbon tetrachloride) was peritoneally injected into the mice at 8 weeks of age (SD42) and continued to treat at twice a week for up to 14 additional weeks. TGF α 15-TGFRs was subcutaneously injected (3 mg/kg) on SD43 and SD71. The treated mice were euthanized on SD161 and the livers were harvested and embedded in 4% formalin. The liver sections were stained with hematoxylin and eosin. Tumor, steatosis and hepatocellular ballooning were examined under the light microscope. The severity of liver damage was expressed as mild (1), moderate (2) and extensive (3). Statistical analyses were performed using GraphPad Prism 9 by unpaired t test. For each test, a P value of less than 0.05 was considered statistically significant. As shown in Figure 45, TGF α 15-TGFRs significantly inhibited liver tumor development and growth, steatosis and hepatocellular ballooning induced by DEN and CCl₄ in B6C3F1 mice.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating a liver disease or a metabolic syndrome in a subject, wherein the method comprises administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

- (i) a first target-binding domain;
- (ii) soluble tissue factor domain; and
- (iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

- (i) a second domain of a pair of affinity domains; and
- (ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

2. The method of claim 1, wherein the liver disease is selected from the group consisting of: fatty liver disease, hepatic steatosis, acute hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease.

3. The method of claim 1, wherein the metabolic syndrome is selected from the group consisting of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial hypertension, stroke, and cataracts.

4. A method of reducing one or more of the rate of: progression from non-alcoholic fatty liver disease (NAFL) to non-alcoholic steatohepatitis (NASH), progression from NASH to cirrhosis, and progression from cirrhosis to hepatocellular carcinoma, comprising administering to a subject identified or diagnosed as having NAFL, NASH, or cirrhosis, a therapeutically effective amount of a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

- (i) a first target-binding domain;
- (ii) soluble tissue factor domain; and
- (iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

- (i) a second domain of a pair of affinity domains; and
- (ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

5. The method of claim 4, wherein the method results in a decreasing in the rate of progression from NAFL to NASH.

6. The method of claim 4, wherein the method results in decreasing the rate of progression of NASH to cirrhosis.

7. The method of claim 4, wherein the method results in decreasing the rate of progression from cirrhosis to hepatocellular carcinoma.

8. A method of reducing inflammation in a liver of a subject, wherein the method comprises administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

- (i) a first target-binding domain;
- (ii) soluble tissue factor domain; and
- (iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

- (i) a second domain of a pair of affinity domains; and
- (ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

9. A method of decreasing gluconeogenesis in a liver of a subject, wherein the method comprises administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

- (i) a first target-binding domain;
- (ii) soluble tissue factor domain; and
- (iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

- (i) a second domain of a pair of affinity domains; and
- (ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

10. A method of decreasing lipogenesis in a liver of a subject, wherein the method comprises administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

- (i) a first target-binding domain;
- (ii) soluble tissue factor domain; and
- (iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

- (i) a second domain of a pair of affinity domains; and
- (ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

11. A method of decreasing hepatocytic senescence in a liver of a subject, wherein the method comprises administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising:

- (a) a first chimeric polypeptide comprising:
 - (i) a first target-binding domain;
 - (ii) soluble tissue factor domain; and
 - (iii) a first domain of a pair of affinity domains;
- (b) a second chimeric polypeptide comprising:
 - (i) a second domain of a pair of affinity domains; and
 - (ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

12. A method of rebalancing metabolic function in a liver of a subject, wherein the method comprises administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising:

- (a) a first chimeric polypeptide comprising:
 - (i) a first target-binding domain;
 - (ii) soluble tissue factor domain; and
 - (iii) a first domain of a pair of affinity domains;
- (b) a second chimeric polypeptide comprising:
 - (i) a second domain of a pair of affinity domains; and
 - (ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

13. A method of modulating expression of one or more genes in Tables 1-4 in a liver of a subject, wherein the method comprises administering to the subject a therapeutically effective amount of a multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

- (i) a first target-binding domain;
- (ii) soluble tissue factor domain; and
- (iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

- (i) a second domain of a pair of affinity domains; and
- (ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain binds specifically to a ligand of TGF- β receptor II (TGF- β RII) and the second target-binding domain binds specifically to a ligand of TGF- β RII.

14. The method of claim 13, wherein the administering results in a decrease in the expression of one or more genes in the liver of the subject selected from the group consisting of: ACSS1, RETN, SLC2A4, PDK4, PNPLA3, GADD45B, PPARGC1A, CAV1, ENDOD1, REG3G, IGHG3, IGHG2B, SCGB3A1, GLYCAM1, IGHG2C, IGKC, LTF, MS4A1, JCHAIN, CD19, IGHM, IFI27L2A, ACKR3, LSP1, PMEPA1,

CORO1A, GPX3, MYH8, NPPA, TCAP, FLNC, SLC36A2, MYH6, ACTC1, ACTA2, and TPM2, as compared to the level of expression of the one or more genes in the subject prior to the administering.

15. The method of claim 13 or 14, wherein the administering results in an increase in the expression of one or more genes in the liver of the subject selected from the group consisting of: SLC34A2, and CISH, as compared to the level of expression of the one or more genes in the subject prior to the administering.

16. The method of claim 13, wherein the administering results in a decrease in the expression of one or more genes in the liver of the subject selected from the group consisting of: CSF3R, IFI27L2A, GM17066, GNL3, FABP1, GM14303, AURKA, RPL14-PS1, QTRT2, G6PC, C8B, DYNLL1, LCN2, LRG1, CEBPD, COL4A3, ST3GAL5, RSAD2, 9330162G02RIK, PINX1, SRA1, SPATA2L, PNRC1, MUP20, IL6RA, APOA1, IL1B, WDR54, CTCFLOS, GM16973, 4632427E13RIK, IGHG2B, TGFB11I, SELENBP2, SEMA6B, NEXN, ZFP653, NOB1, PCK1, FAM25C, MAPK15, GM16551, ESM1, RPL37RT, FAM133B, PDE8B, TUT1, S100A11, PDILT, PPAR, IER2, GM15401, MX2, WNK4, G0S2, BC005561, AA986860, JDP2, GM26982, NOP58, ACTB, GM14586, RPP38, GM13436, NT5DC2, IMPDH1, CYTIP, AI846148, CHKA, GM37963, NR0B2, CYP4A32, ALKBH2, FAU-PS2, PPP1R15A, KLF2, SLC25A22, GM13341, IGHM, SATB1, SNRPF, DNASE1L2, CD3EAP, GM2788, DANCER, ZFP612, NOP56, JUND, ID1, HSPB1, KLHDC8A, KLF10, ANGPT2, THBS1, GM44891, GM9752, ABLIM3, PTGES, GM28438, 2410002F23RIK, FOSL2, CRIP3, JUN, ALAS1, GM2000, RHOC, LMCD1, GM2061, GM42595, GM11478, IKZF2, PNLDC1, COMTD1, SNORA31, COL20A1, AKAP12, C1QTNF12, 1810032O08RIK, 2310033P09RIK, GM47528, SERPINE2, NPFF, SERPINA3K, RFXANK, IGKV5-39, NAB2, MAFF, CEP85, CSAD, LTB4R1, 1810012K08RIK, BCL7C, NRBP2, NLE1, ALKBH1, ARID5A, CFAP43, GM45767, CD8A, PPRC1, GM26870, TMC7, BCL6B, GM16348, GM26981, SLC16A3, TNFRSF12A, CYP2J9, NR4A2, MMP9, MIR17HG, TMEM191C, PCDH11X, HILPDA, RAPGEF4, GM17300, SLC25A47, KCNJ2, NYAP1, LAX1, RPS19-PS3, HES1, RGS16, DUSP1, GM43323,

ASB4, MUC6, GM15502, UNG, FOXQ1, GM17936, UBE2C, SLC16A6, MIR7052, NLRP12, GM14286, FGF21, KLF5, GM37969, PF4, GM21738, HOTAIRM1, GM6493, LOR, MFSD2B, MATK, SYNE4, GM44694, TRBC1, GM37274, PLN, CXCR4, PHF24, SNORD104, SERPINA7, RGS4, TCIM, EGFR, GM37760, FBXL22, TEDC2, ENHO, GM26917, GM43775, 4833411C07RIK, GM45053, INHBB, OPN3, SNHG15, B230206H07RIK, KCNE3, GM43305, C530043K16RIK, KLF4, LEPR, JCHAIN, TSKU, LGALS4, PCP4L1, GM44829, DUSP8, GM44620, IGFBP1, JUNB, GM32017, GM2814, GM37144, MYADML2OS, GM37666, HDC, SLFN4, A530041M06RIK, GM43359, GM2602, GM10277, FAM222A, FOXA3, AOC2, SERPINA1E, CTXN1, RAPGEF4OS2, SOCS2, PPAN, PRKAG2OS1, GADD45B, HOXA5, GRHL1, EIF4EBP3, OSGIN1, GM28513, MAP3K6, SLC34A2, B630019A10RIK, IGKC, PLIN4, ANGPTL4, DUSP5, EGR1, GM42507, GM14257, APOLD1, IER3, ZBTB16, GM37033, IGLC1, GADD45G, IGLC3, GM45244, RGS1, CXCL1, RNF225, GM44005, ANKRD37, NR4A1, GM8893, GM26762, CDKN1A, 5330406M23RIK, IGLV1, IGKV3-2, FOS, GM43637, IGKV3-10, S100A9, GM15622, S100A8, MT1, RETNLG, MT2, IGKV19-93, GM45774, and SERPINA4-PS1, as compared to the level of expression of the one or more genes in the subject prior to the administering.

17. The method of claim 13 or 16, wherein the administering results in an increase in the expression of one or more genes in the liver of the subject selected from the group consisting of: DBP, IGKV4-55, PER3, MUP-PS10, GPAM, TMPRSS4, MUP-PS14, AC166078.1, MUP-PS12, GM2065, A530020G20RIK, ACSS2OS, DCLK3, KLF12, GM44669, MFSD9, B4GALNT3, GM3776, TMEM167-PS1, KRT23, LMBRD2, GM22935, SULT2A-PS1, SNAI3, GM15908, MIR6392, ACSS2, NR1D1, BC049987, CCDC85C, CES2C, ACPP, MUP2, PTK6, UGT1A5, 1810008I18RIK, IL22RA1, ACSS3, ADNP, RDH16, SNTB1, 4933411K16RIK, NTRK2, EXTL1, PSTPIP2, RASSF6, AQP4, UGT1A9, PROM1, ZFP608, FAM13A, NFE2, TEF, TNFAIP8L3, SCD1, MMD2, SYNE3, ACLY, C330021F23RIK, STON2, LRFN4, HHIPL1, WNT9B, NR1D2, 1810049J17RIK, PDPR, NA, GM45884, SLC2A5, FAM83F, ZFP526, SGK2, GM43080, DEAF1, ME1, BMF, WDFY2, ADCY9, CLSTN3, ACOT11, LYST, LRTM1, OAT, VPS13C, E330011O21RIK, P2RY4, GM11437, RWDD2A, SVIL,

ECHDC1, TRIM14, SLC10A5, TRHDE, MASP1, 2900097C17RIK, NDST1, RDH9, 1110002L01RIK, ABTB2, RGR, ACACB, SACM1L, DYRK2, ROBO1, GM44744, EIF4EBP2, KLHL24, CYP2A5, TIAM2, RAB43, GM13855, 9130409I23RIK, STON1, USP9X, UGT3A1, 9030616G12RIK, DOCK8, KLB, ACE, VLDLR, PCDHGC3, ABCA6, 4932422M17RIK, GM45838, FARP2, GM47205, SP4, UGT1A6B, KLHL28, D130043K22RIK, ASIC5, PM20D2, A1CF, SORBS1, SLC10A2, GM10642, UTP14B, GM38394, AFP, INSIG1, HNF1AOS2, METTL4, LSS, MTMR9, HMGCR, GDAP10, ADRA1A, ZFP773, CRKL, CHRNE, STARD13, CRY2, FADS2, COG5, FV1, RCAN2, ABCB1A, PPARA, ATP7A, MVD, 2610037D02RIK, TNFRSF14, SUCNR1, ECI3, ABCC4, LNCBATE1, MINDY2, BTBD7, 4933404O12RIK, ABCD1, FMN1, FNIP2, ABHD15, NKX2-6, C77080, GM43611, SGTB, ACSL3, NR5A2, FAM198A, KCTD7, ACACA, ZFP955B, SULT2A3, FZD4, FASN, CYP3A59, ZFP354B, TNFSF10, SESN3, MN1, RNF152, DHCR24, SPHK2, SYTL5, GM6652, BAHCC1, GAREM1, MFSD4A, HGF, GM3571, NOS1AP, DIXDC1, KANK1, REPS2, ASAH2, SEMA3B, RNF103, ZC3H12C, CDS2, DCUN1D4, 2900026A02RIK, CYR1, EEPD1, P2RY2, CYP2C39, SEC22C, EHHADH, ABCA3, HIPK2, RBM20, GRAMD4, FCHSD2, MOB3A, HMGN3, KLHDC7A, VCP-RS, TERT, CYP3A41B, ARL13B, ZC3H12D, TLCD2, SNHG11, SORL1, GPR157, DNAJA4, TMEM253, TACO1, SPATA5L1, RHBG, COL15A1, PCDH12, IRS1, ASCC3, KIF16B, and MR1, as compared to the level of expression of the one or more genes in the subject prior to the administering.

18. The method of any one of claims 8-117, wherein the subject has been previously identified or diagnosed as having a liver disease or a metabolic syndrome.

19. The method of claim 18, wherein the subject has been previously identified or diagnosed as having a liver disease.

20. The method of claim 19, wherein the liver disease is selected from the group consisting of: fatty liver disease, hepatic steatosis, acute hepatic porphyria, Alagille syndrome, alcohol-related liver disease, alpha-1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, cholangiocarcinoma, biliary atresia, Budd-Chiari

syndrome, cirrhosis, Crigler-Najjar syndrome, galactosemia, Gilbert syndrome, hemochromatosis, hepatic encephalopathy, hepatitis A, hepatitis B, hepatitis C, hepatorenal syndrome, intrahepatic cholestasis of pregnancy (ICP), lysosomal acid lipase deficiency (LAL-D), liver cysts, liver cancer, newborn jaundice, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), progressive familial intrahepatic cholestasis (PFIC), Reye's syndrome, type 1 glycogen storage disease, and Wilson's disease.

21. The method of claim 18, wherein the subject has been previously identified or diagnosed as having a metabolic syndrome.

22. The method of claim 21, wherein the metabolic syndrome is selected from the group consisting of: coronary heart disease, pulmonary disease, gall bladder disease, dyslipidemia, hypertension, type 2 diabetes, dementia, cancer, gynecological abnormalities including polycystic ovarian syndrome, osteoarthritis, pancreatitis, idiopathic intracranial hypertension, stroke, and cataracts.

23. The method of any one of claims 1-22, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

24. The method of any one of claims 1-22, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

25. The method of any one of claims 1-24, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

26. The method of any one of claims 1-24, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

27. The method of any one of claims 1-26, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

28. The method of any one of claims 1-26, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

29. The method of any one of claims 1-28, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

30. The method of any one of claims 1-28, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

31. The method of any one of claims 1-30, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s).

32. The method of any one of claims 1-31, wherein the second chimeric polypeptide further comprises one or more additional target-binding domain(s).

33. The method of any one of claims 1-32, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

34. The method of claim 33, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 1.

35. The method of any one of claims 1-34, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15R α) and a soluble IL-15.

36. The method of any one of claims 1-28 and 30-35, wherein the first target-binding domain comprises a soluble TGF- β RII.

37. The method of claim 36, wherein the first target-binding domain comprises a first sequence that is at least 80% identical to SEQ ID NO: 2 and a second sequence that is at least 80% identical to SEQ ID NO: 2, wherein the first and second sequence are separated by a linker.

38. The method of claim 37, wherein the first target-binding domain comprises a first sequence that is at least 90% identical to SEQ ID NO: 2 and a second sequence that is at least 90% identical to SEQ ID NO: 2.

39. The method of claim 38, wherein the first target-binding domain comprises a first sequence of SEQ ID NO: 2 and a second sequence of SEQ ID NO: 2.

40. The method of any one of claims 37-39, wherein the linker comprises a sequence of SEQ ID NO: 3.

41. The method of claim 36, wherein the first target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4.

42. The method of claim 41, wherein the first target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4.

43. The method of claim 42, wherein the first target-binding domain comprises a sequence of SEQ ID NO: 4.

44. The method of claim 36, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 6.

45. The method of claim 44, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 6.

46. The method of claim 45, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 6.

47. The method of claim 46, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 7.

48. The method of any one of claims 1-28 and 30-47, wherein the second target-binding domain comprises a soluble TGF- β RII.

49. The method of claim 48, wherein the second target-binding domain comprises a first sequence that is at least 80% identical to SEQ ID NO: 2 and a second sequence that is at least 80% identical to SEQ ID NO: 2, wherein the first and second sequence are separated by a linker.

50. The method of claim 49, wherein the second target-binding domain comprises a first sequence that is at least 90% identical to SEQ ID NO: 2 and a second sequence that is at least 90% identical to SEQ ID NO: 2.

51. The method of claim 50, wherein the second target-binding domain comprises a first sequence of SEQ ID NO: 2 and a second sequence of SEQ ID NO: 2.

52. The method of any one of claims 49-51, wherein the linker comprises a sequence of SEQ ID NO: 3.

53. The method of claim 48, wherein the second target-binding domain comprises a sequence that is at least 80% identical to SEQ ID NO: 4.

54. The method of claim 53, wherein the second target-binding domain comprises a sequence that is at least 90% identical to SEQ ID NO: 4.

55. The method of claim 54, wherein the second target-binding domain comprises a sequence of SEQ ID NO: 4.

56. The method of claim 48, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 5.

57. The method of claim 56, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 6.

58. The method of claim 56, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 5.

59. The method of claim 58, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 5.

60. The method of claim 59, wherein the first chimeric polypeptide comprises a sequence of SEQ ID NO: 6.

61. The method of claim 59, wherein the second chimeric polypeptide comprises a sequence of SEQ ID NO: 8.