

DESCRIPTION

TITLE OF INVENTION: FUSION PROTEIN SPECIFICALLY BINDING TO EXTRADOMAIN B OF FIBRONECTIN (EDB-FN) AND TRANSFORMING GROWTH FACTOR B (TGFB), AND USE THEREOF

5 **TECHNICAL FIELD**

[1] The present invention relates to a fusion protein that specifically binds to extradomain B of fibronectin (EDB-FN) and transforming growth factor β (TGF β) and use thereof, and more specifically to a fusion protein including a polypeptide that specifically binds to EDB-FN and a polypeptide that specifically binds to TGF β and
10 use thereof.

[2]

BACKGROUND ART

[3] One of the primary functions of TGF β is to suppress the activation and infiltration of immune cells, particularly CD8⁺ cytotoxic T cells, which are critical for
15 effective antitumor immune responses. TGF β performs this immunosuppressive function by promoting the differentiation of regulatory T cells (Tregs), which enhance immune tolerance. It also sustains the immunosuppressive activity of tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs), both of which contribute to the immunosuppressive and desmoplastic (stromal-rich) properties of the
20 tumor microenvironment (TME). In addition to suppressing immune responses, these stromal cells also contribute to the formation of a physical barrier that further inhibits immune cell infiltration by increasing the stiffness and density of the extracellular matrix (ECM). TGF β signaling also plays a pivotal role in promoting epithelial-to-mesenchymal transition (EMT), which is a process by which tumor cells acquire
25 invasiveness and metastatic potential. TGF β -driven EMT is known to promote

metastasis and cancer progression by increasing tumor cell motility, resisting apoptosis and evading immune detection (Derynck R, et al., *Nat. Rev. Clin. Oncol.* Vol. 18(1), pp. 9-34, 2021; Hao Y, et al., *Int. J. Mol. Sci.* Vol. 20(11):2767, 2019).

[4] Considering these functions, the inhibition of TGF β within the TME is
5 essential to counteract its tumor-promoting and immunosuppressive effects while
minimizing systemic toxicity. In particular, tumor-specific TGF β inhibition targeting
the ECM using fusion proteins or antibody conjugates can effectively reprogram the
TME by reducing matrix stiffness, increasing immune cell infiltration and enhancing
the efficacy of immune-based therapies such as checkpoint inhibitors. Therefore, the
10 inhibition of TGF β within the TME offers the dual benefit of reversing the
immunosuppressive state that protects tumors from immune attack and suppressing the
metastatic and invasive properties of cancer cells. These effects make tumor-specific
TGF β inhibitors a promising therapeutic strategy in cancer, particularly in solid tumors
where the TME plays a critical role in disease progression (Karin E. de Visser et al.,
15 Vol. 41(3), pp. 374-403, 2023).

[5] Meanwhile, pancreatic ductal adenocarcinoma (PDAC) is one of the most
lethal cancers, and is characterized by desmoplasia that forms a dense fibrous barrier
within the pancreatic duct, thereby causing physical and immunosuppressive problems.
This dense matrix impedes the efficacy of existing treatments, including immune
20 checkpoint inhibitors (ICIs), which have shown success in other cancers but are mostly
ineffective in PDAC due to the rigid nature of the ECM. Therefore, targeting the ECM
has become very important to improve the treatment outcomes of PDAC patients.

[6] Fibronectin, which is a glycoprotein found in the ECM, plays a key role in
cancer progression, particularly in PDAC. Among its isoforms, extradomain B (EDB-
25 FN) is re-expressed in malignant tissues and supports tumor growth, angiogenesis and

immune evasion. EDB-FN is overexpressed in a variety of tumors, including pancreatic, lung and prostate cancers, while it is rarely present in normal adult tissues, making it an attractive target for tumor-specific therapies. Targeting EDB-FN within the ECM offers several advantages, including the ability to localize therapies to the TME, thereby minimizing off-target effects and improving tumor penetration. In addition, the stiffness of the ECM due to fibronectin and collagen cross-linking creates a physical barrier that impedes the infiltration of immune cells, and this stiffness traps cytokines such as TGF β , which exacerbates immunosuppressive effects. Studies have shown that a stiff ECM insulates tumors from immune attack, thereby reducing the therapeutic efficacy of immune checkpoint inhibitors such as anti-PD-1 in cancers where they should be effective (Principe DR. et al., *Cancers*. Vol. 13(20):5086, 2021; Perez VM. et al., *Front. Oncol.* Vol. 11:751-311, 2021). Therefore, a dual-targeting strategy that reduces ECM stiffness while inhibiting TGF β is important to realize the potential of immune-based therapy in fibrotic tumors such as PDAC.

[7] Structures that combine an EDB-FN targeting motif (e.g., L19 scFv or anti-EDB antibody) with a TGF β -Trap are expected to localize TGF β inhibition directly within the TME by anchoring TGF β -Trap to the ECM via EDB-FN binding, thereby ensuring local rather than systemic effects. Therefore, they may enhance anti-tumor immune responses while minimizing side effects. Such fusion proteins could overcome ECM resistance to immunotherapy, converting “cold” tumors (immunologically inert) into “hot” tumors (immunologically active), making them more susceptible to immune checkpoint blockade.

[8] However, to date, no fusion protein or bispecific antibody with the above structure has been known, and only L19 (EDB Ab)-IL12, L19-IL9 (Aliyah BS et al., *Trends in Pharmacological Sciences*, Vol. 42(12), pp. 1064-81, 2021), LTBR-EDB

multispecific antibody (KR 2022-0130687) and TGFβ-Trap-PD-L1 (Lind H, et al., *J. Immunother. Cancer*. Vol. 8(1):e000433, 2020) are known.

[9] Under this technical background, the inventors of the present invention have made efforts to develop a fusion protein having excellent anticancer efficacy by simultaneously binding to EDF-FN targeting motif and TGFβ, and as a result, the inventors of the present invention confirmed that an excellent anticancer effect is exhibited when a polypeptide that specifically binds to TGFβ and a polypeptide that specifically binds to EDF-FN are fused with Fc as the center, thereby completing the present invention.

[10] The above information set forth in this Background Art section is intended solely to enhance understanding of the background of the present invention and may not include information that constitutes prior art already known to a person skilled in the art to which the present invention pertains.

[11]

[12] SUMMARY OF THE INVENTION

[13] An object of the present invention is to provide a fusion protein that specifically binds to extradomain B of fibronectin (EDB-FN) and transforming growth factor β (TGFβ).

[14] Another object of the present invention is to provide a nucleic acid encoding the fusion protein.

[15] Still another object of the present invention is to provide a recombinant expression vector including the nucleic acid or a cell into which the nucleic acid or expression vector is introduced.

[16] Still another object of the present invention is to provide a method for producing the fusion protein.

[17] Still another object of the present invention is to provide a composition for preventing or treating cancer, including a fusion protein.

[18] In order to achieve the above objects, the present invention provides a fusion protein, including (a) a polypeptide that specifically binds to extradomain B of fibronectin (EDB-FN); and (b) a polypeptide that specifically binds to transforming growth factor β (TGF β).

[19] The present invention also provides a nucleic acid encoding the fusion protein.

[20] The present invention also provides a recombinant expression vector, including the nucleic acid.

10 [21] The present invention also provides a host cell transfected with the recombinant expression vector.

[22] The present invention also provides a method for producing the fusion protein, including culturing the host cell to produce the fusion protein; and isolating and purifying the produced fusion protein.

15 [23] The present invention also provides a composition for preventing or treating cancer, including the fusion protein.

[24]

DESCRIPTION OF DRAWINGS

[25] FIG. 1A shows the results of analyzing the Fibronectin transcript in the human genome region.

[26] FIG. 1B shows the results of analyzing the expression of the EDB exon and the entire FN1 gene in cancer patients and normal people using the TCGA and GTEx databases.

[27] FIGS. 2A to F show the results of analyzing the association between FN1 EDB+ gene expression and prognosis in the TCGA database. ACC means adenoid

cystic carcinoma, BLCA means bladder urothelial carcinoma, BRCA means breast
invasive carcinoma, CHOL means cholangiocarcinoma, COAD means colon
adenocarcinoma, DLBC means lymphoid neoplasm diffuse large B-cell lymphoma,
ESCA means esophageal carcinoma, GBM means glioblastoma multiforme, HNSC
5 means head and neck squamous cell carcinoma, KICH means kidney chromophobe,
KIRC means kidney renal clear cell carcinoma, KIRP means kidney renal papillary
cell carcinoma, LGG means brain lower grade glioma, LIHC means liver
hepatocellular carcinoma, LUAD means lung adenocarcinoma, LUSC means lung
squamous cell carcinoma, MESO means mesothelioma, OV means ovarian serous
10 cystadenocarcinoma, PAAD means pancreatic adenocarcinoma, SARC means
sarcoma, STAD means stomach adenocarcinoma, and UVM means uveal melanoma.

[28] FIG. 3 shows the sequence alignment results of human, monkey, rat and
mouse of the FN1 EDB domain.

[29] FIG. 4 shows the results comparing the expression levels of FN1-EDB+
15 transcripts in 11 orthotopically transplanted tumor tissues and normal tissues.

[30] FIGS. 5A and B show the purification results of a dual-specific Fc fusion
protein including FEBM and TGF β Trap produced according to one embodiment of
the present invention, wherein the portions indicated by the marker and lane 1 are the
SDS-PAGE results, and the portions indicated by the graph are the size exclusion
20 chromatography (SEC) results.

[31] FIG. 6 shows the results of evaluating the simultaneous binding level of EDB-
FN and TGF- β 1 of a dual-specific Fc fusion protein including FEBM and TGF β Trap
produced according to one embodiment of the present invention.

[32] FIG. 7 shows the results of confirming the tissue-specific distribution of a dual-specific Fc fusion protein including FEBM and TGF β Trap produced according to one embodiment of the present invention in a 4T-1 orthotopic mouse model.

[33] FIG. 8 shows the results of analyzing the tumor growth rate in a breast cancer
5 orthotopic transplantation model by the administration of a dual-specific Fc fusion protein including FEBM and TGF β Trap according to one embodiment of the present invention. FIG. 8A shows the results of measuring the tumor volume over time for each fusion protein, and FIG. 8B shows the results of measuring the growth rate on day 25 for each fusion protein. Each data represents $\pm$ s.e.m.; *P < 0.05, **P < 0.01,
10 ***P < 0.001, ****P < 0.0001; NS represents not significant; and the results were analyzed by two-way ANOVA with post hoc Bonferroni t-test.

[34] FIG. 9 shows the results of analyzing the lung metastasis rate in a breast cancer orthotopic transplantation model by the administration of a dual-specific Fc fusion protein including FEBM and TGF β Trap according to one embodiment of the present
15 invention. **P < 0.01, ***P < 0.001; the results are analyzed by two-way ANOVA with post hoc Bonferroni t-test.

[35] FIG. 10 shows the results of measuring the survival rate in a breast cancer orthotopic transplantation model by the administration of a dual-specific Fc fusion protein including FEBM and TGF β Trap according to one embodiment of the present
20 invention. **P < 0.01, ****P < 0.0001; the results are analyzed by two-way ANOVA with log-rank test.

[36] FIG. 11 shows the results of measuring the expression levels of α -SMA in the tumor margin and center in a breast cancer orthotopic transplantation model administered with a dual-specific Fc fusion protein including FEBM and TGF β Trap
25 according to one embodiment of the present invention. The left panel shows the results

of quantifying α -SMA staining in the tumor margin and the right panel shows the results of quantifying α -SMA staining in the tumor center. Each data represents $\pm$ sem; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; NS represents not significant; and the results were analyzed by two-way ANOVA with post hoc Bonferroni t -test.

5 [37] FIG. 12 shows the results of measuring the Smad2 phosphorylation level and CD8 expression level in a breast cancer orthotopic transplantation model administered with a dual-specific Fc fusion protein including FEBM and TGF β Trap according to one embodiment of the present invention, wherein A represents the Smad2 phosphorylation level, and B represents the results of measuring the CD8 expression
10 level. Each data represents $\pm$ sem; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; NS represents not significant; and the results were analyzed by two-way ANOVA with post hoc Bonferroni t -test.

[38] FIGS. 13A and B show the results of purification of a fusion protein including Anti-EDB-FN antibody, TGF β Trap and hFc produced according to one embodiment
15 of the present invention, wherein the portions indicated by the marker and lane 1 are the results of SDS-PAGE, and the portions indicated by the graph are the results of size exclusion chromatography (SEC).

[39] FIG. 14 shows the results of evaluating the simultaneous binding level of EDB-FN and TGF- β 1 of a dual-specific Fc fusion protein including an anti-EDB-FN
20 antibody and a TGF β Trap produced according to one embodiment of the present invention.

[40] FIG. 15 shows the results of analyzing the tumor growth rate in a breast cancer orthotopic transplantation model by the administration of a dual-specific Fc fusion protein including Anti-EDB-FN antibody and TGF β Trap according to one
25 embodiment of the present invention. FIG. 15A shows the results of measuring the

tumor volume over time for each fusion protein, and FIG. 15B shows the results of measuring the growth rate over time for each fusion protein. Each data represents $\pm$ s.e.m; *** represents $P < 0.001$; NS represents not significant; and is the result of analysis by two-way ANOVA with post hoc Bonferroni t-test.

5 [41] FIG. 16 shows the results of analyzing the lung metastasis rate in a breast cancer orthotopic transplantation model by the administration of a dual-specific Fc fusion protein including an Anti-EDB-FN antibody and TGF β Trap produced according to one embodiment of the present invention. ** $P < 0.01$; the results are analyzed by two-way ANOVA with post hoc Bonferroni t-test.

10 [42] FIG. 17 shows the results of measuring the survival rate in a breast cancer orthotopic transplantation model by the administration of a dual-specific Fc fusion protein including an Anti-EDB-FN antibody and TGF β Trap produced according to one embodiment of the present invention. ** $P < 0.01$; the results are analyzed by two-way ANOVA with log-rank test.

15 [43] FIGS. 18A to 18C show the results of analyzing tumor growth in a pancreatic cancer orthotopic transplantation model administered with a dual-specific Fc fusion protein including FEBM and TGF β Trap according to one embodiment of the present invention, where a represents tumor weight, b represents growth rate, and c represents survival rate. Each data represents $\pm$ s.e.m.; * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$; NS represents not significant; and the results were analyzed by two-way ANOVA with post hoc Bonferroni t-test.

[44] FIGS. 18D to 18F show the results of analyzing tumor growth in a pancreatic cancer orthotopic transplantation model by the administration of a dual-specific Fc fusion protein including Anti-EDB-FN antibody and TGF β Trap produced according to one embodiment of the present invention, where a represents tumor weight, b

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represents growth rate, and c represents survival rate. Each data represents $\pm$ s.e.m.; * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$; NS represents not significant; and the results were analyzed by two-way ANOVA with post hoc Bonferroni t -test.

[45] FIG. 19 shows the results of measuring the Smad2 phosphorylation level and
5 CD8 expression level in a pancreatic cancer orthotopic transplantation model administered with a dual-specific Fc fusion protein including FEBM and TGF β Trap according to one embodiment of the present invention. FIG. 19A shows the results of measuring the Smad2 phosphorylation level, and FIG. 19B shows the results of measuring the CD8 expression level. Each data means $\pm$ sem; * $P < 0.05$, ** $P < 0.01$,
10 *** $P < 0.001$, **** $P < 0.0001$; NS means not significant; and the results were analyzed by two-way ANOVA with post hoc Bonferroni t -test.

[46] FIG. 20 is the results of confirming the tumor growth inhibitory effect in a melanoma orthotopic transplantation -> lung metastasis tumor growth model by the administration of a dual-specific Fc fusion protein including FEBM and TGF β Trap
15 and a dual-specific Fc fusion protein including Anti-EDB-FN antibody and TGF β Trap, produced according to an embodiment of the present invention. FIG. 20A is the result of measuring the tumor growth rate over time, and FIG. 20B is the tumor growth rate measured on day 25. Each data means $\pm$ sem; * $P < 0.05$, ** $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$; NS means not significant; and the result is analyzed by two-way
20 ANOVA with post hoc Bonferroni t -test.

[47] FIG. 21A shows the results of analyzing the tumor growth rate in a breast cancer orthotopic transplantation model by the administration of a trispecific Fc fusion protein including Anti-EDB-FN antibody, FEBM and TGF β Trap produced according to one embodiment of the present invention. Each data means $\pm$ sem; * $P < 0.01$, ** P

< 0.05, ****P* < 0.001; NS means not significant; and the results were analyzed by two-way ANOVA with post hoc Bonferroni *t*-test.

[48] FIG. 21B shows the results of analyzing the tumor growth rate in a pancreatic cancer orthotopic transplantation model by the administration of a trispecific Fc fusion protein including Anti-EDB-FN antibody, FEBM and TGFβ Trap produced according to one embodiment of the present invention. Each data means ± sem; **P* < 0.01, ***P* < 0.05, ****P* < 0.001; NS means not significant; and the results were analyzed by two-way ANOVA with post hoc Bonferroni *t*-test.

[49]

10 [50] **DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS**

[51] Unless otherwise defined, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention pertains. In general, the nomenclature used herein and the experimental methods described below are well known and commonly used in the art.

[52] In the present invention, it was attempted to confirm that a high anticancer effect is exhibited in various cancer types when a fusion protein that simultaneously binds to EDB-FN and TGFβ is used.

[53] That is, in one embodiment of the present invention, when a fusion protein (Table 2) that simultaneously binds to EDB-FN and TGFβ is used, it was confirmed that excellent anticancer effects were observed in tumors with high (pancreatic cancer), low (melanoma) and intermediate (breast cancer) expression of EDB-FN (FIGS. 10 to 20).

[54] Therefore, in one aspect,

25 [55] the present invention relates to a fusion protein, including

- [56] (a) a polypeptide that specifically binds to extradomain B of fibronectin (EDB-FN); and
- [57] (b) a polypeptide that specifically binds to transforming growth factor β (TGF β).
- 5 [58]
- [59] In the present invention, the fusion protein may further include (c) an antibody constant region.
- [60] In the present invention, the fusion protein including (a), (b) and (c) may include, from the N-terminus to the C-terminus,
- 10 [61] one or more structures selected from the group consisting of:
- [62] (i) a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to TGF β ;
- [63] (ii) a polypeptide that specifically binds to TGF β ; and a polypeptide that specifically binds to EDB-FN;
- 15 [64] (iii) one or more polypeptides that specifically bind to EDB-FN; an antibody constant region; and one or more polypeptides that specifically bind to TGF β ;
- [65] (iv) one or more polypeptides that specifically bind to TGF β ; an antibody constant region; and one or more polypeptides that specifically bind to EDB-FN;
- [66] (v) one or more polypeptides that specifically bind to EDB-FN; one or more
- 20 polypeptides that specifically bind to TGF β ; and an antibody constant region;
- [67] (vi) one or more polypeptides that specifically bind to EDB-FN; one or more polypeptides that specifically bind to TGF β ; an antibody constant region; and one or more polypeptides that specifically bind to EDB-FN;

[68] (vii) one or more polypeptides that specifically bind to TGF β ; one or more polypeptides that specifically bind to EDB-FN; an antibody constant region; and one or more polypeptides that specifically bind to EDB-FN;

[69] (viii) an antibody constant region; one or more polypeptides that specifically bind to EDB-FN; and one or more polypeptides that specifically bind to TGF β ;

[70] (ix) an antibody constant region; one or more polypeptides that specifically bind to TGF β ; and one or more polypeptides that specifically bind to EDB-FN; and

[71] (x) one or more polypeptides that specifically bind to EDB-FN; an antibody constant region; one or more polypeptides that specifically bind to TGF β ; and one or more polypeptides that specifically bind to EDB-FN;

[72] In the present invention, the fusion protein includes, from the N-terminus to the C-terminus,

[73] a structure selected from the group consisting of:

[74] (7-1) an antibody constant region; a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to TGF β ; and

[75] (7-2) an antibody constant region; a polypeptide that specifically binds to TGF β ; and a polypeptide that specifically binds to EDB-FN.

[76]

[77] In the present invention, the fusion protein may further include a polypeptide that specifically binds to (d) a tumor antigen.

[78] In the present invention, the term "tumor antigen" means an antigenic (poly-)peptide or protein derived from or associated with a (preferably malignant) tumor or cancer disease. The terms "cancer" and "tumor" as used herein are used interchangeably to refer to a neoplasm characterized by uncontrolled and usually rapid proliferation of cells that tend to invade surrounding tissues and metastasize to distant

body sites. The term includes benign and malignant neoplasms. Malignant neoplasms of cancer are typically characterized by anaplasia, invasiveness and metastasis; and benign neoplasms typically do not have these characteristics. The terms "cancer" and "tumor" refer in particular to neoplasms characterized by tumor growth, as well as
5 cancers of the blood and lymphatic system. A "tumor antigen" is typically derived from a tumor/cancer cell, preferably a mammalian tumor/cancer cell, and may be located within or on a tumor cell, for example a systemic or solid tumor, derived from a mammal, preferably a mammalian, preferably a human. "Tumor antigens" generally include tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs). TSAs
10 are usually due to tumor-specific mutations and are specifically expressed by tumor cells. The more common TAAs are generally presented by tumor and "normal" (healthy, non-tumor) cells.

[79]

[80] In the present invention, the tumor antigen may be 4-1BB, 5T4, integrin,
15 Activin, Angiopoietin, angiopoietin-like 3, B cell maturation antigen (BCMA), B-cell activating factor (BAFF), BAGE-1, BCL-2, B7-H3, bcr/abl, beta-catenin/m, BING-4, BRCA1/m, BRCA2/m, CA 15-3/CA 27-29, CA 19-9, CA72-4, CA125, calreticulin, CAMEL, CASP-8/m, cathepsin B, cathepsin L, CCR4, CCR5, CCL11, CD11a, CD16A, CD19, CD2, CD20, CD22, CD25, CD3, CDE30, CD33, CD4, CD40, CD45,
20 CD46, CD47, CD52, CD55, CD56, CD6, CD80, CD86, CTLA4, CD105, CD123, CD154, CD166, CD262, CD278, CD319, CD326, CDC27/m, CDK4/m, CDKN2A/m, CEA, CLCA2, CML28, CML66, COA-1/m, c-met, coactosin-like protein, collagen XXIII, COX-2, CT-9/BRD6, Cten, cyclin B1, cyclin D1, cyp-B, CYPB1, DAM-10, DAM-6, DEK-CAN, EFTUD2/m, EGFR, ELF2/m, EMMPRIN, EpCam, EphA2,
25 EphA3, ErbB3, ETV6-AML1, EZH2, FGF-5, FN, Frau-1, G250, GAGE-1, GAGE-2,

GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE7b, GAGE-8, GDEP, GnT-V, gp100,
 GPC3, GPNMB/m, HAGE, HAST-2, hepsin, HER2, HER3, HERVK-MEL, HLA-
 A*0201 - R1 7I, HLA-A1 1/m, HLA-A2/m, HNE, homeobox NKX3.1, HOM-TES-
 14/SCP-1, HOM-TES-85, HPV-E6, HPV-E7, HSP70-2M, HST-2, hTERT, iCE, IGF-
 5 1 R, IL-13Ra2, IL-2R, IL-5, immature laminin receptor, kallikrein-2, kallikrein-4, i67,
 KIAA0205, KIAA0205/m, KK-LC- 1, K-Ras/m, LAGE-A1, LDLR-FUT, MAGE-A1,
 MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-A10, MAGE-
 A12, MAGE-B1, MAGE-B2, MAGE-B3, MAGE-B4, MAGE-B5, MAGE-B6,
 MAGE-B10, MAGE-B1 6, MAGE-B1 7, MAGE-C1, MAGE-C2, MAGE-C3,
 10 MAGE- D1, MAGED2, MAGE-D4, MAGE-E1, MAGE-E2, MAGE-F1, MAGE-H I,
 MAGEL2, mammaglobin A, MART-1/melan-A, MART-2, MART-2/m, matrix
 protein 22, MC1 R, M-CSF, ME 1/m, mesothelin, MG50/PXDN, MMP1 1, MN/CA
 IX-antigen, MRP-3, MUC-1, MUC-2, MUM-1/m, MUM-2/m, MUM-3/m, myosin
 class I/m, NA88-A, N-acetylglucosaminyltransferase- V, neo-PAP, neo-PAP/m,
 15 NFYC/m, NGEP, NMP22, NPM/ALK, N-Ras/m, NSE, NY-ESO-1, NY-ESOB, OA1,
 OFA-iLRP, OGT, OGT/m, OS-9, OS- 9/m, osteocalcin, osteopontin, pi 5, p190 minor
 bcr-abl, p53, p53/m, PAGE-4, PAI-1, PAI-2, PAP, PART-1, PATE, PDEF, PD-1, PD-
 L1, Pim-1 -Kinase, Pin-1, Pml/PARalpha, POTE, PRAME, PRDX5/m, prostein,
 proteinase-3, PSA, PSCA, PSGR, PSM, PSMA, PTPRK/m, RAGE-1, RBAF600/m,
 20 RHAMM/CD1 68, RU1, RU2, S-100, SAGE, SART-1, SART-2, SART-3, SCC,
 SIRT2/m, Sp1 7, SSX-1, SSX-2/HOM-MEL-40, SSX-4, STAMP-1, STEAP-1,
 survivin, survivin-2B, SYT-SSX-1, SYT-SSX-2, TA-90, TAG-72, TARP, TEL-
 AML1, TGM-4, TPI/m, TRAG-3, TRG, TRP-1, TRP-2/6b, TRP/INT2, TRP-p8, T cell
 immunoglobulin and mucin-domain containing-3 (Tim-3), tissue factor, tissue factor
 25 pathway inhibitor (TFPI), tumor necrosis factor (TNF), tyrosinase, UPA, vascular

endothelial growth factor (VEGF), VEGF receptor, vWF (von Willebrand Factor), VEGFR-2/FLK-1, WT1 and immunoglobulin genotypes of lymphocytes or T cell receptors of lymphocytes or homologs, fragments, variants or derivatives of the tumor antigen, and more preferably, it may be any one or more selected from the group
5 consisting of PD-1, PD-L1, CTLA-4, CD80, CD86 and VEGF, and most preferably, it may be PD-1.

[81]

[82] In the present invention, the polypeptide that specifically binds to (d) a tumor antigen may be a polypeptide that specifically binds to PD-1.

10 [83]

[84] In the present invention, when the polypeptide that specifically binds to the (d) tumor antigen is an antibody, the antibody may be one or more selected from the group consisting of Urelumab, Utomilumab, Bebtelovimab, Aducanumab, Bapinezumab, Crenezumab, Donanemab, Gantenerumab, Lecanemab, Solanezumab, Nesvacumab, Evinacumab, Enoblituzumab, Omburtamab, Belimumab, Ianalumab, Tabalumab, Bertilimumab, Mogamulizumab, Leronlimab, Siplizumab, Foralumab, Muromonab-CD3, Otelixizumab, Teplizumab, Ibalizumab, Tregalizumab, Zanolimumab, Itolizumab, Efalizumab, Inebilizumab, Tafasitamab, Tositumomab, Ocrelizumab, Ofatumumab, Rituximab, Ublituximab, Veltuzumab, Epratuzumab, Basiliximab, Daclizumab, Varlilumab, Lulizumab, Iratumumab, Lintuzumab, Daratumumab, Felzartamab, Isatuximab, Mezagitamab, Bleselumab, Dacetuzumab, Iscalimab, Lucatumumab, Mitazalimab, Sotigalimab, Tegoprubart, Dapirolizumab, Apamistamab, Ligufalimab, Magrolimab, Alemtuzumab, Crizanlizumab, Inclacumab, Cusatumumab, Oleclumab, Milatuzumab, Galiximab, Carotuximab, Adecatumumab, Eptinezumab, Erenumab, Fremanezumab, Galcanezumab, Zolbetuximab,
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Onartuzumab, Eculizumab, Pozelimab, Ravulizumab, Lacnotuzumab, Axatilimab,
Cabiralizumab, Emactuzumab, Ipilimumab, Quavonlimab, Tremelimumab,
Zalifrelimab, Cetuximab, Depatuxizumab, Futuximab, Imgatuzumab, Matuzumab,
Modotuximab, Necitumumab, Nimotuzumab, Panitumumab, Tomuzotuximab,
5 Zalutumumab, Batoclimab, Nipocalimab, Rozanolixizumab, Burosumab,
Farletuzumab, Dinutuximab, Dinutuximab beta, Naxitamab, Ragifilimab, Gimsilumab,
Lenzilumab, Mavrilimumab, Namilumab, Otilimab, Plonmarlimab, Codrituzumab,
Margetuximab, Pertuzumab, Trastuzumab, Datopotamab, Patritumab, Seribantumab,
Duligotuzumab, Ficlatuzumab, Rilotumumab, Alomfilimab, Anifrolumab,
10 Emapalumab, Ligelizumab, Omalizumab, Cixutumumab, Dalotuzumab, Figitumumab,
Ganitumab, Teprotumumab, Bermekimab, Canakinumab, Gevokizumab,
Briakinumab, Ustekinumab, Anrukinzumab, Cendakimab, Lebrikizumab,
Tralokinumab, Brodalumab, Bimekizumab, Ixekizumab, Secukinumab, Brazikumab,
Guselkumab, Mirikizumab, Risankizumab, Tildrakizumab, Nemolizumab,
15 Imsidolimab, Spesolimab, Pascolizumab, Dupilumab, Depemokimab, Mepolizumab,
Reslizumab, Benralizumab, Clazakizumab, Olokizumab, Siltuximab, Sirukumab,
Ziltivekimab, Levilimab, Sarilumab, Satralizumab, Tocilizumab, Abituzumab,
Fabezelimab, Fianlimab, Ieramilimab, Relatlimab, Simtuzumab, Abagovomab,
Oregovomab, Tanezumab, Ivuxolimab, Rocatinlimab, Tavalimab, Telazorlimab,
20 Vonlerolizumab, Alirocumab, Bococizumab, Ebronucimab, Evolocumab, Frovocimab,
Ongericimab, Tafolecimab, Dostarlimab, Balstilimab, Camrelizumab, Cemiplimab,
Geptanolimab, Nivolumab, Pembrolizumab, Penpulimab, Pidilizumab, Prolgolimab,
Retifanlimab, Sasanlimab, Serplulimab, Sintilimab, Spartalizumab, Tislelizumab,
Toripalimab, Ezabenlimab, Zimberelimab, Atezolizumab, Avelumab, Cosibelimab,
25 Sugemalimab, Durvalumab, Envafohimab, Suvratoxumab, Denosumab, Zilovetamab,

Elotuzumab, Domvanalimab, Etigilimab, Ociferlimab, Tiragolumab, Vibostolimab, Surzebiclimab, Cobolimab, Sabatolimab, Concizumab, Marstacimab, Adalimumab, Golimumab, Infliximab, Certolizumab, Conatumumab, Tigatuzumab, Tezepelumab, Gatipotuzumab, Cabiralizumab, Bevacizumab, Brolucizumab, Ranibizumab, Olinvacimab, Icrucumab, Ramucirumab, Caplacizumab, Abrilumab, Etrolizumab, Vedolizumab, Intetumumab, Natalizumab, Cinrebafusp alfa, Rozibafusp alfa, Obrindatamab, Elranatamab, Linvoseltamab, Teclistamab, Epcoritamab, Glofitamab, Mosunetuzumab, Odronextamab, Flotetuzumab, Vibecotamab, Catumaxomab, Cibisatamab, Talquetamab, Ubamatamab, Emfizatamab, Blinatumomab, Amivantamab, Emicizumab, Zenocutuzumab, Zanidatamab, Tibulizumab, Naptumomab, Belantamab, Pivekimab, Praluzatamab, Coltuximab, Denintuzumab, Loncastuximab, Ibritumomab, Inotuzumab, Epratuzumab, Moxetumomab, Brentuximab, Gemtuzumab, Vadastuximab, Lorvotuzumab, Polatuzumab, Tusamitamab, Telisotuzumab, Rovalpituzumab, Depatuxizumab, Farletuzumab, Mirvetuximab, Disitamab, Anetumab, Enfortumab, Sacituzumab, Vobarilizumab, Cadonilimab, Vudalimab, Tebotelimab, Ivonescimab, Erfonrilimab, Ozoralizumab, Faricimab, Vanucizumab and Navicixizumab.

[85]

[86] In the present invention, the fusion protein including (a), (b), (c) and (d) may include, from the N-terminus to the C-terminus,

[87] one or more structures selected from the group consisting of:

[88] (A) a polypeptide that specifically binds to PD-1; a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to TGF β ;

[89] (B) a polypeptide that specifically binds to PD-1; a polypeptide that specifically binds to TGF β ; and a polypeptide that specifically binds to EDB-FN;

[90] (C) a polypeptide that specifically binds to TGF β ; a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to PD-1;

[91] (D) a polypeptide that specifically binds to PD-1; an antibody constant region; a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to TGF β ;

[92] (E) a polypeptide that specifically binds to PD-1; an antibody constant region; a polypeptide that specifically binds to TGF β ; and a polypeptide that specifically binds to EDB-FN;

[93] (F) a polypeptide that specifically binds to TGF β ; a polypeptide that specifically binds to EDB-FN; a polypeptide that specifically binds to PD-1; an antibody constant region; and

[94] (G) a polypeptide that specifically binds to EDB-FN; a polypeptide that specifically binds to TGF β ; a polypeptide that specifically binds to PD-1; and an antibody constant region.

[95]

[96] In the present invention, each component of the fusion protein may be linked via a linker.

[97] In the present invention, the linker may be a peptide linker and may have a length of about 1 to 25 aa. For example, it may include a hydrophilic amino acid such as glycine and/or serine, but is not limited thereto.

[98] Specifically, the linker may include, for example, GGG, (GS)_n, (GGS)_n, (GGGS)_n, (SSSSG)_n or (G_nS)_m (where n and m are each 1 to 10), which impart structural flexibility without being cleaved by proteolytic enzymes, and more specifically, the linker may be, for example, GGG, (GGGS)_n or (SSSG)_n (where n and m are each 1 to 10).

[99]

[100] In the present invention, the polypeptide that specifically binds to EDB-FN may be a fibronectin EDB binding motif (FEBM), an antibody that specifically binds to EDB-FN or a fragment thereof.

5 [101]

[102] The term "antibody" as used herein means an antibody that specifically binds to a target substance (e.g., EDB-FN, TGF β and PD-1).

[103] A complete antibody is a structure having two full-length light chains and two full-length heavy chains, and each light chain is linked to a heavy chain by a disulfide
10 bond.

[104] As used herein, the term "heavy chain" refers to both a full-length heavy chain and fragments thereof, including a variable region domain VH including an amino acid sequence having sufficient variable region sequence to confer specificity to an antigen, and three constant region domains CH1, CH2 and CH3. In addition, the term "light
15 chain" as used herein refers to both a full-length light chain and fragments thereof, including a variable region domain VL including an amino acid sequence having sufficient variable region sequence to confer specificity to an antigen, and a constant region domain CL.

[105] The whole antibodies include subtypes of IgA, IgD, IgE, IgM and IgG, and in
20 particular, IgG includes IgG1, IgG2, IgG3 and IgG4. The heavy-chain constant region has gamma (γ), mu (μ), alpha (α), delta (δ) and epsilon (ϵ) types, and has gamma 1 (γ 1), gamma 2 (γ 2), gamma 3 (γ 3), gamma 4 (γ 4), alpha 1 (α 1) and alpha 2 (α 2) as subclasses. The light-chain constant region has kappa (κ) and lambda (λ) types.

[106] An antigen-binding fragment of an antibody, or antibody fragment, refers to a
25 fragment that has an antigen-binding function, and includes Fab, F(ab'), F(ab')₂ and Fv.

Among antibody fragments, Fab has a structure having a variable region of the light chain and heavy chain, a constant region of the light chain, and a first constant region (CH1) of the heavy chain, and has one antigen-binding site. Fab' differs from Fab in that it has a hinge region including one or more cysteine residues at the C-terminus of the heavy chain CH1 domain. F(ab')₂ is generated when the cysteine residues in the hinge region of Fab' form a disulfide bond.

[107] Fv corresponds to the smallest antibody fragment that has only a heavy chain variable region and a light chain variable region. A two-chain Fv has a heavy chain variable region and a light chain variable region non-covalently linked, and a single-chain Fv (scFv) has a heavy chain variable region and a light chain variable region covalently linked, usually via a peptide linker or directly linked at the C-terminus, such that it can form a dimer-like structure like a two-chain Fv. These antibody fragments may be produced using proteolytic enzymes (e.g., Fab can be obtained by restriction digestion of a complete antibody with papain, and F(ab')₂ can be obtained by digestion with pepsin) or using genetic recombination technology.

[108] The "Fv" fragment is an antibody fragment that contains a complete antibody recognition and binding site. This region is a dimer of one heavy chain variable domain and one light chain variable domain.

[109] The "Fab" fragment includes the variable and constant domains of the light chain, and the variable and first constant domains (CH1) of the heavy chain. An F(ab')₂ antibody fragment typically includes a pair of Fab' fragments covalently linked by cysteine in the hinge region at the C-terminus of the Fab' fragment.

[110] A "single-chain Fv (scFv)" antibody fragment is a construct consisting of a single polypeptide chain including the VH and VL domains of an antibody. The scFv

may further include a polypeptide linker between the VH and VL domains that enables it to form the desired structure for antigen binding.

[111] In one embodiment, the antibody of the present invention includes, but are not limited to, monoclonal antibodies, multispecific antibodies, human antibodies, 5 humanized antibodies, chimeric antibodies, scFv, Fab fragments, F(ab')₂ fragments, disulfide-linked Fvs (sdFv) and anti-idiotypic (anti-Id) antibodies, or epitope-binding fragments of the antibodies.

[112] The heavy-chain constant region may be selected from any one of the isotypes of gamma (γ), mu (μ), alpha (α), delta (δ) or epsilon (ϵ). For example, the constant 10 region is gamma 1 (IgG1), gamma 2 (IgG2), gamma 3 (IgG3) or gamma 4 (IgG4). The light-chain constant region may be kappa or lambda type.

[113]

[114] In the present invention, the polypeptide that specifically binds to TGF β may be a TGF β receptor type 2 ectodomain (TGF β RII ectodomain, TGF β Trap), an 15 antibody that specifically binds to TGF β or a fragment thereof.

[115] In the present invention, the polypeptide that specifically binds to TGF β may be any one or more of polypeptides represented by amino acid sequences of SEQ ID NOs: 5 to 8.

[116]

20 [117] In the present invention, the antibody constant region may be a heavy-chain constant region or a light-chain constant region.

[118] In the present invention, the antibody constant region may any one or more of polypeptides represented by amino acid sequences of SEQ ID NOs: 12 to 15.

[119]

[120] In the present invention, the polypeptide that specifically binds to PD-1 may be an antibody that specifically binds to PD-1 or a fragment thereof.

[121] In the present invention, the polypeptide that specifically binds to PD-1 may be any one or more of polypeptides represented by amino acid sequences of SEQ ID
5 NOs: 9 to 11.

[122]

[123] In the present invention, the fusion protein including (a), (b) and (c) may include, from the N-terminus to the C-terminus, any one or more polypeptides selected from the group consisting of:

10 [124] (4-1) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 12 and a polypeptide of SEQ ID NO: 1;

[125] (4-2) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 12;

[126] (4-3) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; and a
15 polypeptide of SEQ ID NO: 12;

[127] (4-4) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 5;

[128] (4-5) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5;

20 [129] (4-6) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

[130] (4-7) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 12;

[131] (4-8) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; a
25 polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

- [132] (4-9) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 1;
- [133] (4-10) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;
- [134] (4-11) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 13;
- [135] (4-12) a polypeptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5;
- [136] (4-13) a polypeptide of SEQ ID NO: 2; a polypeptide of SEQ ID NO: 14; and a polypeptide of SEQ ID NO: 5;
- [137] (4-14) a polypeptide of SEQ ID NO: 3; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 5;
- [138] (4-15) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 2; and a polypeptide of SEQ ID NO: 13;
- [139] (4-16) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 5;
- [140] (4-17) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;
- [141] (4-18) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 12;
- [142] (4-19) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 4;
- [143] (4-20) a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;

- [144] (4-21) a polypeptide of SEQ ID NO: 6; a polypeptide of SEQ ID NO: 14; and a polypeptide of SEQ ID NO: 1;
- [145] (4-22) a polypeptide of SEQ ID NO: 7; a polypeptide of SEQ ID NO: 15; and a polypeptide of SEQ ID NO: 1;
- 5 [146] (4-23) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 6; and a polypeptide of SEQ ID NO: 14;
- [147] (4-24) a polypeptide of SEQ ID NO: 2; a polypeptide of SEQ ID NO: 14; and a polypeptide of SEQ ID NO: 5;
- [148] (4-25) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 8; and
10 a polypeptide of SEQ ID NO: 12;
- [149] (4-26) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;
- [150] (4-27) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;
- 15 [151] (4-28) a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;
- [152] (4-29) a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;
- [153] (4-30) a polypeptide of SEQ ID NO: 2; a polypeptide of SEQ ID NO: 14; and a polypeptide of SEQ ID NO: 8;
- [154] (4-31) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 14; and
20 a polypeptide of SEQ ID NO: 4;
- [155] (4-32) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 2; and a polypeptide of SEQ ID NO: 14;
- [156] (4-33) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 6; and a polypeptide of SEQ ID NO: 14;

[157] (4-34) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 12;

[158] (4-35) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 12;

5 [159] (4-36) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;

[160] (4-37) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4;

[161] (4-38) a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;

10 and

[162] (4-39) a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4, and

[163] more preferably, it may include (4-5) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5; or (4-6) a

15 polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

[164] In the present invention, the fusion protein including (a), (b) and (c) may be any one or more selected from the group consisting of SEQ ID NOs: 16 to 56, and more preferably, it may be represented by an amino acid sequence of SEQ ID NO: 20,

20 or represented by an amino acid sequence of SEQ ID NO: 21.

[165]

[166] In the present invention, the fusion protein including (a), (b), (c) and (d) may include, from the N-terminus to the C-terminus,

[167] any one or more polypeptides selected from the group consisting of:

- [168] (5-1) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5;
- [169] (5-2) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;
- 5 [170] (5-3) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5;
- [171] (5-4) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;
- [172] (5-5) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; a
10 polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;
- [173] (5-6) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;
- [174] (5-7) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5;
- 15 [175] (5-8) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;
- [176] (5-9) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 11;
- [177] (5-10) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 1; and
20 a polypeptide of SEQ ID NO: 5;
- [178] (5-11) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;
- [179] (5-12) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 11;

- [180] (5-13) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;
- [181] (5-14) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 4;
- 5 [182] (5-15) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;
- [183] (5-16) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 4;
- [184] (5-17) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 5; a
10 polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;
- [185] (5-18) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;
- [186] (5-19) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;
- 15 [187] (5-20) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 4;
- [188] (5-21) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 11;
- [189] (5-22) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 4; and
20 a polypeptide of SEQ ID NO: 5;
- [190] (5-23) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 4;
- [191] (5-24) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 11; and a polypeptide of SEQ ID NO: 4;

- [192] (5-25) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;
- [193] (5-26) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;
- 5 [194] (5-27) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;
- [195] (5-28) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;
- [196] (5-29) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 1; a
10 polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;
- [197] (5-30) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;
- [198] (5-31) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;
- 15 [199] (5-32) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;
- [200] (5-33) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 11;
- [201] (5-34) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 1; and
20 a polypeptide of SEQ ID NO: 8;
- [202] (5-35) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;
- [203] (5-36) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 11;

- [204] (5-37) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;
- [205] (5-38) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4;
- 5 [206] (5-39) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;
- [207] (5-40) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4;
- [208] (5-41) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 8; a
10 polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;
- [209] (5-42) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;
- [210] (5-43) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;
- 15 [211] (5-44) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4;
- [212] (5-45) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 11;
- [213] (5-46) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 4; and
20 a polypeptide of SEQ ID NO: 8;
- [214] (5-47) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4; and
- [215] (5-48) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 11.

[216] In the present invention, the fusion protein including (a), (b), (c) and (d) may be any one selected from the group consisting of SEQ ID NOs: 57 to 104.

[217]

[218] The fusion protein of the present invention may include not only the sequence
5 of the fusion protein of the present invention described herein, but also biological
equivalents thereof, within the scope of specifically recognizing EDB-FN and TGF β .
For example, additional changes may be made to the amino acid sequence of the
protein to further improve the binding affinity and/or other biological properties of the
protein. Such changes may include, for example, deletions, insertions and/or
10 substitutions of amino acid sequence residues of the protein. Such amino acid
mutations are made based on the relative similarity of the amino acid side chain
substituents, such as hydrophobicity, hydrophilicity, charge, size and the like. Analysis
of the size, shape and type of the amino acid side chain substituents reveals that
arginine, lysine and histidine are all positively charged residues; alanine, glycine and
15 serine have similar sizes; and phenylalanine, tryptophan and tyrosine have similar
shapes. Accordingly, based on these considerations, arginine, lysine and histidine;
alanine, glycine and serine; and phenylalanine, tryptophan and tyrosine are
biologically functional equivalents.

[219] Considering the mutation having the above-described biological equivalent
20 activity, the fusion protein of the present invention or the nucleic acid molecule
encoding the same is interpreted to also include a sequence showing substantial
identity with the sequence described in the sequence number. The substantial identity
means a sequence showing at least 90% homology, most preferably at least 95%
homology, 96% or more, 97% or more, 98% or more, or 99% or more homology when
25 the sequence of the present invention and any other sequence are aligned to the greatest

extent possible and the aligned sequence is analyzed using an algorithm commonly used in the art. Alignment methods for sequence comparison are known in the art. The NCBI Basic Local Alignment Search Tool (BLAST) is accessible from NCI, and the like, and may be used in conjunction with sequence analysis programs such as blastp, 5 blastm, blastx, tblastn and tblastx on the Internet. BLAST is accessible at www.ncbi.nlm.nih.gov/BLAST/. Methods for comparing sequence homology using this program can be found at www.ncbi.nlm.nih.gov/BLAST/blast_help.html.

[220] Based on this, the fusion protein of the present invention may have 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homology to the specified 10 sequences or all of them described in the specification. Such homology may be determined by sequence comparison and/or alignment by methods known in the art. For example, the percent sequence homology of the nucleic acids or proteins of the present invention may be determined using a sequence comparison algorithm (i.e., BLAST or BLAST 2.0), manual alignment, or visual inspection.

15 [221]

[222] In another aspect, the present invention relates to a nucleic acid encoding the fusion protein. The nucleic acid encoding the fusion protein of the present invention may be isolated to recombinantly produce the fusion protein.

[223] "Nucleic acid" has a comprehensive meaning including DNA (gDNA and 20 cDNA) and RNA molecules, and nucleotides, which are the basic structural units of nucleic acids, include not only natural nucleotides but also analogues in which sugar or base moieties are modified. The sequence of a nucleic acid encoding the fusion protein of the present invention may be modified. The modifications include addition, deletion or non-conservative or conservative substitution of nucleotides.

[224] The DNA encoding the above fusion protein may be readily isolated or synthesized using conventional molecular biological techniques (e.g., by using an oligonucleotide probe capable of specifically binding to DNA encoding the antibody and the heavy and light chains), and the nucleic acid may be isolated and further cloned
5 (amplification of the DNA) or further expressed by inserting the same into a replicable vector.

[225] Based on this, in still another aspect, the present invention relates to a recombinant expression vector including the nucleic acid.

[226] The term "vector" as used herein refers to a means for expressing a target gene
10 in a host cell, and includes viral vectors such as plasmid vectors, cosmid vectors, bacteriophage vectors, adenovirus vectors, retrovirus vectors, and adeno-associated virus vectors. Components of a vector typically include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more antibiotic resistance marker genes, an enhancer element, a promoter, and a
15 transcription termination sequence. A nucleic acid encoding a fusion protein is operably linked to the promoter and the transcription termination sequence.

[227] "Operatively linked" means a functional linkage between a nucleic acid expression regulatory sequence (e.g., a promoter, a signal sequence, or an array of transcription factor binding sites) and another nucleic acid sequence, whereby the
20 regulatory sequence regulates transcription and/or translation of the other nucleic acid sequence.

[228] In the case of a prokaryotic host, it usually includes a strong promoter capable of driving transcription (e.g., tac promoter, lac promoter, lacUV5 promoter, lpp promoter, pL λ promoter, pR λ promoter, rac5 promoter, amp promoter, recA promoter,
25 SP6 promoter, trp promoter, and T7 promoter), a ribosome binding site for translation

initiation and a transcription/translation termination sequence. In addition, for example, when a eukaryotic cell is used as a host, promoters derived from the genome of mammalian cells (e.g., metallothionine promoter, β -actin promoter, human hemoglobin promoter, and human muscle creatine promoter) or promoters derived
5 from mammalian viruses (e.g., adenovirus late promoter, vaccinia virus 7.5K promoter, SV40 promoter, cytomegalovirus (CMV) promoter, tk promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of Moloney virus, promoter of Epstein-Barr virus (EBV), and promoter of Rous sarcoma virus (RSV)) may be used, which generally have a polyadenylation sequence as a
10 transcription termination sequence.

[229] In some cases, the vector may be fused with other sequences to facilitate purification of the fusion protein expressed therefrom. Sequences to be fused include, for example, glutathione S-transferase (Pharmacia, USA), maltose binding protein (NEB, USA), FLAG (IBI, USA), and 6x His (hexahistidine) (Quiagen, Germany).

15 [230] The vector includes antibiotic resistance genes commonly used in the art as selectable markers, and for example, there are resistance genes for ampicillin, gentamicin, carbenicillin, chloramphenicol, streptomycin, kanamycin, geneticin, neomycin and tetracycline.

[231]

20 [232] In still another aspect, the present invention relates to a host cell transfected with the recombinant expression vector. The host cell used to produce the fusion protein of the present invention may be, but is not limited to, a prokaryotic, yeast or higher eukaryotic cell.

[233] Prokaryotic host cells may be used, such as strains of the genus *Bacillus*, such
25 as *Escherichia coli*, *Bacillus subtilis* and *Bacillus thuringiensis*, and *Streptomyces*,

Pseudomonas (e.g., *Pseudomonas putida*), *Proteus mirabilis* and *Staphylococcus* (e.g., *Staphylococcus carnosus*).

[234] However, animal cells are of greatest interest, and examples of useful host cell lines include, but are not limited to, COS-7, BHK, CHO, CHOK1, DXB-11, DG-44,
5 CHO/-DHFR, CV1, COS-7, HEK293, BHK, TM4, VERO, HELA, MDCK, BRL 3A, W138, Hep G2, SK-Hep, MMT, TRI, MRC 5, FS4, 3T3, RIN, A549, PC12, K562, PER.C6, SP2/0, NS-0, U20S or HT1080.

[235]

[236] In still another aspect, the present invention relates to a method for producing
10 a fusion protein, comprising culturing the host cell to produce a fusion protein; and isolating and purifying the produced fusion protein.

[237] The host cell may be cultured in various media. Any commercially available media may be used as a culture medium without limitation. Any other necessary supplements known to those skilled in the art may be included in appropriate
15 concentrations. Culture conditions, such as temperature, pH and the like, are already used with the host cell selected for expression and will be apparent to those skilled in the art.

[238] The fusion protein may be recovered by removing impurities, for example, by centrifugation or ultrafiltration, and the resultant product may be purified, for example,
20 using affinity chromatography. Additional purification techniques may be used, for example, anion or cation exchange chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography and the like.

[239]

[240] In still another aspect, the present invention relates to a composition for
25 preventing or treating cancer, including the fusion protein.

[241] In the present invention, the cancer may be selected from the group consisting of squamous cell carcinoma, small cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous cell carcinoma of the lung, peritoneal cancer, skin cancer, cutaneous or intraocular melanoma, rectal cancer, anal cancer, esophageal cancer, small intestinal cancer, endocrine cancer, parathyroid cancer, adrenal cancer, soft tissue sarcoma, urethral cancer, chronic or acute leukemia, lymphocytic lymphoma, hepatocellular carcinoma, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, large intestine cancer, endometrial or uterine cancer, salivary gland cancer, kidney cancer, liver cancer, prostate cancer, vulvar cancer, thyroid cancer, liver cancer, and head and neck cancer.

[242] The term "prevention" as used in the present invention means any act of suppressing or delaying the onset of an immune disease or an inflammatory disease by administering the pharmaceutical composition of the present invention.

[243] The term "treatment" as used in the present invention means any act in which the symptoms of an immune disease or an inflammatory disease are improved or beneficially changed by administration of the pharmaceutical composition of the present invention.

[244] In the present invention, the composition may include a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier included in the composition is one commonly used in formulation, and includes, but is not limited to, lactose, dextrose, sucrose, sorbitol, mannitol, starch, acacia gum, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate and mineral oil. In addition to the

above components, the pharmaceutical composition may further include a lubricant, a wetting agent, a sweetening agent, a flavoring agent, an emulsifier, a suspending agent, a preservative and the like.

[245] The appropriate dosage of the pharmaceutical composition for preventing or
5 treating cancer may be prescribed in various ways depending on factors such as the formulation method, administration method, patient's age, weight, gender, pathological condition, food, administration time, administration route, excretion rate and response sensitivity. The preferred dosage of the composition is within the range of 0.001-100 mg/kg for adults. The term "pharmaceutically effective amount" means
10 an amount sufficient to prevent or treat cancer, or prevent or treat a disease caused by angiogenesis.

[246] The composition may be prepared in a unit dose form or may be prepared by introducing the same into a multi-dose container by formulating the same using a pharmaceutically acceptable carrier and/or excipient according to a method that can
15 be easily performed by a person skilled in the art. In this case, the formulation may be in the form of a solution, suspension, syrup or emulsion in an oil or aqueous medium, or may be in the form of an extract, powder, granule, tablet or capsule, and may additionally contain a dispersant or a stabilizer. In addition, the composition may be administered as an individual therapeutic agent or in combination with another
20 therapeutic agent, and may be administered sequentially or simultaneously with conventional therapeutic agents. Meanwhile, since the composition includes an antibody or an antigen-binding fragment, it may be formulated as an immunoliposome. A liposome including an antibody may be prepared according to a method widely known in the art. The immunoliposome may be prepared by a reverse phase
25 evaporation method as a lipid composition including phosphatidylcholine, cholesterol

and polyethylene glycol-derivatized phosphatidylethanolamine. For example, the Fab' fragment of an antibody may be conjugated to liposomes via a disulfide-exchange reaction. A chemotherapeutic agent such as doxorubicin may be additionally included within the liposomes.

5 [247]

[248] In still another aspect, the present invention relates to a composition for treating cancer, including the fusion protein as an active ingredient.

[249] In still another aspect, the present invention relates to a method for treating cancer, including administering the fusion protein.

10 [250] In still another aspect, the present invention relates to the use of the fusion protein in the manufacture of a medicament for treating cancer.

[251] In still another aspect, the present invention relates to the use of a fusion protein including a) a polypeptide that specifically binds to extradomain B of fibronectin (EDB-FN); and (b) a polypeptide that specifically binds to transforming growth factor β (TGF β).

[252] In still another aspect, the present invention relates to the use of a fusion protein including a) a polypeptide that specifically binds to extradomain B of fibronectin (EDB-FN); and (b) a polypeptide that specifically binds to transforming growth factor β (TGF β).

20 [253] In still another aspect, the present invention relates to the combined therapeutic use of a fusion protein including a) a polypeptide that specifically binds to extradomain B of fibronectin (EDB-FN); and (b) a polypeptide that specifically binds to transforming growth factor β (TGF β).

[254]

25 [255] **Example**

[256] Hereinafter, the present invention will be described in more detail through examples. It will be apparent to those skilled in the art that these examples are intended only to illustrate the present invention, and the scope of the present invention is not to be construed as being limited by these examples.

5 [257]

[258] Example 1. Materials and methods

[259] 1-1. Fibronectin transcriptome expression and prognosis analysis

[260] TCGA or GTEx database analysis was performed by downloading the exon mRNA level including the EDB domain of FN1 (Fibronectin 1) through gepia2
10 ((<http://gepia2.cancer-pku.cn/#index>) to obtain data, and then analyzing the same by utilizing TCGA (human cancer genome/transcriptome and cancer patient information data resource) and GTEx (normal tissue genome/transcriptome information data resource) according to the user manual of gepia2.

[261]

15 [262] 1-2. Culture and expression analysis of orthotopically transplanted tumor tissue and normal cells

[263] The expression levels of FN1-EDB+ transcripts were compared in 11 different orthotopically transplanted tumor tissues and normal tissues.

[264] That is, Renca murine renal adenocarcinoma (RENCA, kidney
20 cancer), Colon 26 murine colorectal cancer (Colon26, colon cancer), 4T1-Luc murine breast cancer (4T-1, breast cancer), B16F10-Luc murine melanoma (B16F10, melanoma), Hepa6 murine hepatoma (Hepa6, liver cancer), and RM1 murine prostate cancer (RM1, prostate cancer) cell lines were purchased from the American Type Culture Collection (ATCC, USA). The GL26 murine glioblastoma (brain tumor) cell
25 line was obtained from Dr. Henry Brem of the Johns Hopkins Medical School. The

YTN-16 murine gastric cell line (YTN16, stomach cancer) was obtained from Professor Sachiyo Nomura of the University of Tokyo. The Panc02 murine pancreatic adenocarcinoma cell line (PANC02, pancreatic cancer) was obtained from Chungnam National University. KRasG12D/+TP53-/- PLC (Primary lung cancer cell lines
5 derived from spontaneous Kras/p53 GEMMs, lung cancer), KRasG12D/+TP53-/- PSC (Primary sarcoma cell lines derived from spontaneous Kras/p53 GEMMs, sarcoma) and TP53-/- PTLC (Primary T lymphoma cell lines derived from spontaneous p53 GEMMs, lymphoma) cell lines were obtained from primary tumors obtained from KRasG12D/+TP53-/- mice provided by the Howard Hughes Medical Institute (*Nature*.
10 2001, 26;410(6832):1111-6.). C57BL/6 mice and BALB/c mice (Orient Bio, Korea) were purchased for the recovery of normal tissues.

[265] 4T-1-Luc cells were transplanted into the mammary fat pad of BALB/c mice (OrientBio, Korea), and one month later, lung metastatic nodules were collected and digested into single cells. This process was repeated twice to isolate metastatic cancer
15 cells called 4T-1-LMT-2-Luc (a cancer cell line with high lung metastasis potential), which were then cultured *in vitro* and used to create an allograft mouse model.

[266] 4T1, 4T-1-Luc and 4T-1-LMT-2-Luc cell lines were cultured in R10 medium (RPMI1640 (Welgene, Korea) containing penicillin/streptomycin (Welgene, Korea) and 10% heat-inactivated fetal bovine serum (Welgene, Korea)). Panc02-luc, Panc02,
20 B16F10-luc, B16F10, colon 26, Hepa6, GL26, RM1 and Renca cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Welgene, Korea) containing 10% FBS. KRasG12D/+ TP53-/- PLC (primary lung cancer cells derived from a spontaneous KRas/p53 GEMM; LA1, *Nature*. 2001, 26;410(6832):1111-6), PSC (primary sarcoma cell line derived from a spontaneous KRas/p53 GEMM, sarcoma),
25 and TP53-/- PTLC (primary T lymphoma cell line derived from a spontaneous p53

GEMM, lymphoma) cells were generated from soft bone sarcoma, lung tumor, and thymic tumor of p53-deficient oncogenic KrasG12D mice, respectively. Their origin was confirmed by PCR of excised Kras and p53 alleles, and they were cultured in DMEM/F12 medium containing 10% FBS after collagen-coated tissue culture. All cell lines were cultured for 2 to 16 passages at 37°C in a 5% CO₂ concentration, and then harvested using TrypLE Express (Thermo fisher scientific, USA) or 0.25% trypsin before performing *in vivo* transplantation.

[267] The harvested cell lines were lysed using 700 µL QIAzol lysis reagent (QIAGEN, Germany) and then homogenized. Total RNA was extracted using RNeasy Micro Kit (Qiagen), reverse transcription was performed using SuperScript II Reverse Transcriptase (Thermo fisher Scientific, USA), and amplification was performed using SuperScript II Reverse Transcriptase (Thermo fisher Scientific, USA) in StepOnePlus Real-Time PCR System (Thermo fisher Scientific, USA). The expression level of the target gene was determined using the change-in-cycling-threshold (2- $\Delta\Delta C_t$) method and normalized using Gapdh.

[268]

[269] 1-3. Design of Fusion proteins

[270] Each component of the fusion protein to be used in the examples below was constructed as shown in Table 1 below, and then, the fusion protein was designed by connecting each component with a linker as described in Table 2.

[271] [Table 1]

SEQ ID NO:	Name	Sequence
1	FEBM	SSSPIQGSWTWENGKWTWKGIIRLEQ
2	Anti-EDB-FN-HC	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFPYFDYWGGQGLTVTVSS

3	Anti-EDB-FN-LC	EIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQQKPGQ APRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYY CQQTGRIPPTFGQGTKVEIK
4	anti-EDB-FN-scfv	EVQLLESQGLVQPGGSLRLSCAASGFTSSFSMSWVRQAPG KGLEWVSSISGSSGTTYADSVKGRFTISRDNKNTLYLQMN LRAEDTAVYYCAKPFYFDYWGQGLTVTVSSGGGGSGGGGS GGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQ QKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPED FAVYYCQQTGRIPPTFGQGTKVEIK
5	TGFβ Trap	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQK SCMSNCSITSICEKPEVCVAVWRKNDENITLETVCHDPKLPY HDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIFSEE YNTSNPD
6	Anti-TGFβ1-HC	QVQLVQSGAEVKKPGSSVKVSKASGFTSSYAISWVRQAPG QGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSS LRSEDVAVYYCARGLWEVRALPSVYWGQGLTVTVSS
7	Anti-TGFβ1-LC	SYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQA PVLVVSIEDIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYC QVWDRDSDQYVFGTGTKVTVL
8	Anti-TGFβ1-scfv	QVQLVQSGAEVKKPGSSVKVSKASGFTSSYAISWVRQAPG QGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSS LRSEDVAVYYCARGLWEVRALPSVYWGQGLTVTVSSSSSSGS SSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWY QQKAGQAPVLVVSIEDIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRDSDQYVFGTGTKVTVL
9	Anti-PD-1-HC	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQA PGQGLEWMGGINPSNGGTNFNEKFKNRVTLTDSSTTTAYME LKSLLQFDDTAVYYCARRDYRFDMGFDYWGQTTTVTVSS

[272]

10	Anti-PD-1-LC	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQK PGQAPRLLIYLASYLESQVPAFSGSGSGTDFTLTISSLEPEDFA VYYCQHSRDLPLTFGGGTKVEIK
11	Anti-PD-1-scfv	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQA PGQGLEWMGGINPSNGGTNFNEKFKNRVTLTDSSTTTAYME LKSLLQFDDTAVYYCARRDYRFDMGFDYWGQTTTVTVSSGG GGSGGGGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVST SGYSYLHWYQQKPGQAPRLLIYLASYLESQVPAFSGSGSGT DFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIK
12	hFc(=hIgG4-Fc)	ESKYGPPCPPCAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSGSEFLYSRLTVDKSRWQEGNVFSCSVMHE ALHNHYTQKSLSLSPGK
13	hIgG1-Fc	THTCPPCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGSEFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK
14	IGHG	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVD HKPSNTKVDKRVESKYGPPCPPCAPEFLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD

		IAVEWESNGQPENNYKTTTPVLDSDGSSFFLYSRLTVDKSRWQ EGNVFSCSVMEALHNHYTQKSLSLSLGK
15	IGKC	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGE

[273] [Table 2]

SEQ ID NO:	Name	Sequence
16	TGFβ Trap-hFc-FEBM	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPG ETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGKGGGGS GGGSSSSPIQGSWTWENGKWTWKGIIRLEQ
17	TGFβ Trap-FEBM-hFc	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPG ETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGSSSSPIQGSWTWENGKWTWK GIIRLEQGGGSGGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSSFFLYSRLTVDKSR WQEGNVFSCSVMEALHNHYTQKSLSLSLGK
18	FEBM-TGFβ Trap-hFc	SSSPIQGSWTWENGKWTWKGIIRLEQGGGGSIPPHVQKSVNNDMIVTDNNGA VKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLET TVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEY NTSNPDGGGSGGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSSFFLYSRLTVDKSR WQEGNVFSCSVMEALHNHYTQKSLSLSLGK
19	FEBM-hFc-TGFβ Trap	SSSPIQGSWTWENGKWTWKGIIRLEQESKYGPPCPPCPAPEFLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSFF LYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGKGGGIPPHVQK SVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV CAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMC SCSSDECNDNIIFSEEYNTSNPD

[274]

20	hFc-FEBM-TGFβ Trap	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSSFFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLSLGKGGGSSSPIQGSWTWENGKWTWKGIIRLEQGGGGSIPP HVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEK PQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD
21	hFc-TGFβ Trap-FEBM	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSSFFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLSLGKGGGIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDV

		RFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDGGGGS SSSPIQGSWTWENGKWTWKGIIRLEQ
22	FEbM- TGFβ Trap- FEbM- hFc	SSSPIQGSWTWENGKWTWKGIIRLEQGGGGSIPPHVQKSVNNDMIVTDNNGA VKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL TVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEY NTSNPDGGGGSSSSPIQGSWTWENGKWTWKGIIRLEQGGGGSGGGGSSESKYG PPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNH YTQKSLSLSLGK
23	hFc- FEbM- TGFβ Trap- FEbM	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEHA LHNHYTQKSLSLSLGKGGGSSSPIQGSWTWENGKWTWKGIIRLEQGGGGSIPP HVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEK PQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSSYSSDECNDNIIFSEYNTSNPDGGGGSGGGGSSSSPIQGSWTWENGKWT WKGIIRLEQ

[275]

24	FEbM- TGFβ Trap- FEbM- hFc- FEbM	SSSPIQGSWTWENGKWTWKGIIRLEQGGGGSIPPHVQKSVNNDMIVTD NNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKND NITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI FSEYNTSNPDGGGGSSSSPIQGSWTWENGKWTWKGIIRLEQGGGGSGGGGSSE SKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLSLGKGGGSSSPIQGSWTWENGKWTWKGIIRLEQ
25	FEbM- hFc- FEbM- TGFβ Trap- FEbM	SSSPIQGSWTWENGKWTWKGIIRLEQGGGGSIPPHVQKSVNNDMIVTD NNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKND NITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSSYSSDECNDNI FSEYNTSNPDGGGGSGGGGSSSSPIQGSWTWENGKWTWKGIIRLEQ
26	TGFβ Trap- FEbM- hIgG1-Fc	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSIC EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP GETFFMCSCSSDECNDNIIFSEYNTSNPD GGGSSSPIQGSWTWENGKWTWKGIIRLEQGGGGSGGGGSSTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG K

[276]

27	hIgG1-Fc-FEBM-TGFβ Trap	THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL HNHYTQKSLSLSPGKGGGSSSSPIQGSWTWENGKWTWKGIIRLEQGGGGSIP PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICE KPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGE TFFMCSCSSDECNDNIIFSEEYNTSNPD
28	hFc-soluble TGFβ Trap	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCFSVMHEA LHNHYTQKSLSLGLGKGGGGIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDF RSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAAASPKCIMKEKKKPGETTFFMCSCSSDECNDNIIFSEEYNTSNPD
29	Anti-EDB-FN-HC-IGHG-TGFβ Trap	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKPFYFDYW GQGTLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTKVDKR VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPE VQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCFSVMHE ALHNHYTQKSLSLGLGKGGGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFP QLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVC HDPKLPYHDFILEDAAASPKCIMKEKKKPGETTFFMCSYSSDECNDNIIFSEEYNTS NPD
30	Anti-EDB-FN-LC-IGKC-TGFβ Trap	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSR ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKRT VAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSSTYSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG GGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPK CIMKEKKKPGETTFFMCSCSSDECNDNIIFSEEYNTSNPD

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31	TGFβ Trap-anti-EDB-FN-hIgG	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSIC EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGE ETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSV KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGQGTLTVTS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPCP PCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCFSVMHEALHNHYTQ KSLSLSLGK
32	Anti-EDB-FN-scFv-hFc-TGFβ Trap	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKPFYFDYW GQGTLTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSV SSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIKSSSSGSSSSGSSSSGESKYGPPCPPCPAPEFL GGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCFSVMHEALHNHYTQKSLSLSLG KGGGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQ

		KSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS PKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
33	hFc-anti- EDB-FN- scFv- TGFβ Trap	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEHA LHNHYTQKSLSLGLKSSSSGSSSSGSSSSGEVQLLESAGGLVQPGGSLRLSCAA SGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNKNT LYLQMNSLRAEDTAVYYCAKPPFPYFDYWGGQTLTVTVSSGGGGSGGGGSGGG GSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKS SSSGSSSSGSSSSGIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDN QKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDA SPKIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

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34	Anti- EDB-FN- scFv- TGFβ Trap-hFc	EVQLLESAGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPPFPYFDYW GGQTLTVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSV SSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIKSSSSGSSSSGSSSSGIPPHVQKSVNNDMIVT DNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKND ENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND NIIFSEEYNTSNPDGGGGSGGGGSGGGGSESKYGPCCPPCPAPEFLGGPSVFLF PKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLK
35	hFc- TGFβ Trap- Anti- EDB-FN scFv	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEHA LHNHYTQKSLSLGLKSSSSGSSSSGSSSSGIPPHVQKSVNNDMIVTDNNGAVK FPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETV CHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNT SNPDSSSSGSSSSGSSSSGEVQLLESAGGLVQPGGSLRLSCAASGFTFSSFSMSW VRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNKNTLYLQMNSLRAE DTAVYYCAKPPFPYFDYWGGQTLTVTVSSGGGGSGGGGSGGGGSEIVLTQSPGT LSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSG SGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK

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36	Anti- EDB-FN scFv- TGFβ Trap	EVQLLESAGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPPFPYFDYW GGQTLTVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSV SSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIKSSSSGSSSSGSSSSGIPPHVQKSVNNDMIVT DNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKND ENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND NIIFSEEYNTSNPD
37	Anti- TGFβ1- HC- IGHG- FEBM	QVQLVQSGAEVKKPGSSVKVSCKASGCTFSSYAIWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGLWEVRAL PSVYWGQGTLLTVVSSASTKGPSVFPLAPCSRSTSESTAALGLVKDYFPEPVT SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNT KVDKRVESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK

		FYPSPDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFS CSVMHEALHNHYTQKSLSLSLGKGGGSSPIQGSWTWENGKWTWKGIIRLEQ
38	Anti- TGFβ1- LC- IGKC- FEBM	SYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSEDIIRP SGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRSDQYVFGTGTKVT LRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG ECGGGSSPIQGSWTWENGKWTWKGIIRLEQ
39	FEBM- Anti- TGFβ1- IGHG	SSSPIQGSWTWENGKWTWKGIIRLEQGGGGSGGGGSQVQLVQSGAEV KKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKF QGRVTITADESTSTAYMELSSLRSEDATVYYCARGLWEVRALPSVYWGQGT LTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSMHEAL HNHYTQKSLSLSLGK

[280]

40	Anti- TGFβ1 scFv- FEBM- hFc	QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDATVYYCARGLWEVRAL PSVYWGQGTLLTVSSSSSSSGSSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVVSEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRSDQYVFGTGTKVTVLGGGSSSPIQGSWTWENGKWTWK GIIRLEQGGGGSGGGGSGGGGSESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRL TVDKSRWQEGNVFSCSMHEALHNHYTQKSLSLSLGK
41	FEBM- Anti- TGFβ1 scFv-hFc	SSSPIQGSWTWENGKWTWKGIIRLEQGGGGSGGGGSQVQLVQSGAEV KKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKF QGRVTITADESTSTAYMELSSLRSEDATVYYCARGLWEVRALPSVYWGQGT LTVSSSSSSSGSSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWY QQKAGQAPVLVVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQV WDRSDQYVFGTGTKVTVLGGGSGGGGSGGGGSESKYGPCCPPCPAPEFLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSMHEALHNHYTQKSLSLSLGK
42	hFc- FEBM- anti- TGFβ1 scFv	ESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSMHEA LHNHYTQKSLSLSLGKGGGSSSPIQGSWTWENGKWTWKGIIRLEQGGGGSGG GGSGGGGSQVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQ GLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDATVYYCAR GLWEVRALPSVYWGQGTLLTVSSSSSSSGSSSSSGSSSSGSYELTQPPSVSVAPGQ TARITCGANDIGSKSVHWYQQKAGQAPVLVVSEDIIRPSGIPERISGSNSGNTAT LTISRVEAGDEADYYCQVWDRSDQYVFGTGTKVTVL

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43	hFc-anti- TGFβ1- scFv- FEBM	ESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSMHEA LHNHYTQKSLSLSLGKGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVS CKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADES
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		TSTAYMELSSLRSEDТАVYYCARGLWEVRALPSVYWGQGTЛTVTVSSSSSSSGSSSSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGKVTVLSSSSSGSSSSSGSSSSSGSSSPIQGSWTWENGKWTWKGIIRLEQ
44	Anti-TGFβ1-scFv-FEBM	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDТАVYYCARGLWEVRALPSVYWGQGTЛTVTVSSSSSSSGSSSSSGSSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGKVTVLGGGGSGGGGSGGGGSSSSSPIQGSWTWENGKWTWKGIIRLEQ
45	FEBM-anti-TGFβ1-scFv	SSSPIQGSWTWENGKWTWKGIIRLEQGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDТАVYYCARGLWEVRALPSVYWGQGTЛTVTVSSSSSSSGSSSSSGSSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGKVTVL
46	Anti-EDB-FN-HC-IGHG-anti-TGFβ1-scFv	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDТАVYYCAKPFYFDYWGGGTЛTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTKVKDRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHIALHNHYTQKSLSLGLKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDТАVYYCARGLWEVRALPSVYWGQGTЛTVTVSSSSSSSGSSSSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGKVTVL

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47	Anti-TGFβ1-hIgG-Anti-EDB-FN-scFv	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDТАVYYCARGLWEVRALPSVYWGQGTЛTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTKVKDRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHIALHNHYTQKSLSLGLKSSSSSGSSSSSGSSSSSGEVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDТАVYYCAKPFYFDYWGGGTЛTVTVSSGGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK
48	Anti-TGFβ1-scFv-anti-EDB-FN-hIgG	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDТАVYYCARGLWEVRALPSVYWGQGTЛTVTVSSSSSSSGSSSSSGSSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGKVTVLGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSNSAMTWVRQAPGKRLEWVASISGGGTТYYPDSVKGRFTISRDNKNTLYLQMNSLRAEDТАVYYCARSHYWGQGTЛTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTKVKDRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ

		PENNYKTTTPVLDSGSSFFLYSRLTVDKSRWQEGNVFSCSVMHLEALHNHYTQ KSLSLSLGK
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[283]

49	Anti-TGFβ1 scFv- anti- EDB-FN- hIgG	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARGLWEVRAL PSVYWGQGTLLTVSSSSSSSGSSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRSDQYVFGTGKVTVLGGGGSGGGGSGGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSNSAMTWVRQAPGKRLEWVASISGGGTYY PDSVKGRFTISRDN SKNTLYLQMNSLRAEDNAVYYCARSHYWGQGTLLTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDPHKPSNTKVDKRVESKYGPPCPP CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSGSSFFLYSRLTVDKSRWQEGNVFSCSVMHLEALHNHYTQ KSLSLSLGK
50	Anti- EDB-FN scFv- Anti- TGFβ1 hIgG	EVQLLES GGGLVQPGGSLRLSCAASGFTFSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDNAVYYCAKPFYFDYW GQGTLLTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSV SSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIKSSSSSGSSSSSGSSSSGQVQLVQSGAEVKKPG SSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPFGTANYAQKFQGRV TITADESTSTAYMELSSLRSEDNAVYYCARGLWEVRALPSVYWGQGTLLTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDPHKPSNTKVDKRVESKYGPPCPP CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSGSSFFLYSRLTVDKSRWQEGNVFSCSVMHLEALHNHYTQ KSLSLSLGK
51	Anti- EDB-FN scFv- anti- TGFβ1 scFv-hFc	EVQLLES GGGLVQPGGSLRLSCAASGFTFSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDNAVYYCAKPFYFDYW GQGTLLTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSV SSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIKSSSSSGSSSSSGSSSSGQVQLVQSGAEVKKPG SSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPFGTANYAQKFQGRV TITADESTSTAYMELSSLRSEDNAVYYCARGLWEVRALPSVYWGQGTLLTVSS GGGGSGGGGSGGGGSSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQ KAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWD RSDQYVFGTGKVTVLGGGGSGGGGSGGGGSESKYGPPCPPCPAPEFLGGP SVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGSSFFLYSRLTVDKSRWQEGNVFSCSVMHLEALHNHYTQKSLSLSLGK

[284]

52	Anti-TGFβ1 scFv- anti- EDB-FN scFv-hFc	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARGLWEVRAL PSVYWGQGTLLTVSSSSSSSGSSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRSDQYVFGTGKVTVLGGGGSGGGGSGGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSFSMSWVRQAPGKGLEWVSSISGSSGTTYA DSVKGRFTISRDN SKNTLYLQMNSLRAEDNAVYYCAKPFYFDYWGQGTLLTV VSSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAW YQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ
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		TGRIPPTFGQGTKVEIKGGGGSGGGSGGGGSESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD SDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK
53	hFc-Anti- EDB-FN scFv- Anti- TGFβ1 scfv	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLGLGKSSSSGSSSSGSSSSGEVQLLESGGGLVQPGGSLRLSCAA SGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNKNT LYLQMNSLRAEDTAVYYCAKPFYFDYWGGQTLTVSSGGGGSGGGGSGGG GSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYAS SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKS SSSGSSSSGSSSSGQVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAIWVRQA PGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVY YCARGLWEVRALPSVYWGQTLTVSSSSSSGSSSSGSSSSGSYELTQPPSVSV APGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSNSG NTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGTKVTVL

[285]

54	hFc- Anti- TGFβ1 scfv- Anti- EDB-FN scFv	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLGLGKGGGGSGGGSGGGGSQVQLVQSGAEVKKPGSSVKVS CKASGGTFSSYAIWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADES TSTAYMELSSLRSEDNAVYYCARGLWEVRALPSVYWGQTLTVSSSSSSGSS SSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVL VVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYV GTGKTVTVLGGGGSGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFT FSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNKNTLYLQ MNSLRAEDTAVYYCAKPFYFDYWGGQTLTVSSGGGGSGGGGSGGGGSEIV LTQSPGTLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK
55	Anti- EDB-FN scFv-Anti TGFβ scFv	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYW GGQTLTVSSGGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSV SSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIKSSSSGSSSSGSSSSGQVQLVQSGAEVKKPG SSVKVSKASGGTFSSYAIWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV TITADESTSTAYMELSSLRSEDNAVYYCARGLWEVRALPSVYWGQTLTVSS GGGGSGGGSGGGGSSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQ KAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVW DSDQYVFGTGTKVTVLG
56	Anti- TGFβ1 scFv- Anti- EDB-FN scFv	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAIWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARGLWEVRAL PSVYWGQTLTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRDSDQYVFGTGTKVTVLGGGGSGGGSGGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYA DSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGGQTLTV VSSGGGGSGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAW YQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQ TGRIPPTFGQGTKVEIK

[286]

57	Anti-PD-1-HC-IGHG-FEBM-TGFβ Trap	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDHKP SNTKVDKRVESKYGPPCPPAPAEFLGGPSVFLFPPKPKDTLMISRTPEVTCVNV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCSVMEALHNHYTQKSLSLGLKGGGSSSPIQGSGWTWENGKWTWKGIIRLE QGGGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQ KSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAS PKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
58	Anti-PD-1-HC-IGHG-TGFβ Trap-FEBM	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDHKP SNTKVDKRVESKYGPPCPPAPAEFLGGPSVFLFPPKPKDTLMISRTPEVTCVNV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCSVMEALHNHYTQKSLSLGLKGGGGSIPPHVQKSVNNDMIVTDNNGAV KFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLET VCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSSYSSDECNDNIIFSEEYN TSNPDGGGGSGGGGSSSPIQGSGWTWENGKWTWKGIIRLEQ

[287]

59	Anti-PD-1-LC-IGKC-FEBM-TGFβ Trap	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYL ASYLESQVPAARFSGSGSGTDFTLTISLEPEDFAVYYCQHSRDLPLTFGGGTKE IKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNR GECGGGSSSPIQGSGWTWENGKWTWKGIIRLEQGGGGSGGGGSIPPHVQKSVN NDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEVCAV VWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSS DECNDNIIFSEEYNTSNPD
60	Anti-PD-1-LC-IGKC-TGFβ trap-FEBM	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYL ASYLESQVPAARFSGSGSGTDFTLTISLEPEDFAVYYCQHSRDLPLTFGGGTKE IKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNR GECGGGSGGGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFR FSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDF ILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDSSSSGSS SGSSSSGSSSPIQGSGWTWENGKWTWKGIIRLEQ
61	TGFβ Trap-FEBM-anti-PD-1-HC-IGHG	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSIC EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPG ETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSSSPIQGSGWT WENGKWTWKGIIRLEQSSSSGSSSSGSSSSGQVQLVQSGVEVKKPGASVKVSC KASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTDT SSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTITVTVSSASTK GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVTPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPAP EFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLS LGK

62	FEBM-TGFβ Trap-anti-PD-1-HC-IGHG	SSSPIQGSWTWENGKWTWKGIIRLEQSSSSGSSSSGSSSSGIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFVFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTA VYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSL LGK
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[288]

63	Anti-PD-1 scFv-hFc-FEBM-TGFβ Trap	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTA VYYCARRDYRFDMGFDYWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVPARFSGSGSGTDFTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSGSSSSGSSSSGESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSL LGKGGGSSSPIQGSWTWENGKWTWKGIIRLEQGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFVFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
64	Anti-PD-1 scFv-hFc-TGFβ Trap-FEBM	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTA VYYCARRDYRFDMGFDYWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVPARFSGSGSGTDFTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSGSSSSGSSSSGESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSL LGKGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFVFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSSYSSDECNDNIIFSEEYNTSNPDGGGSGGGGSSSSSPIQGSWTWENGKWTWKGIIRLEQ

[289]

65	TGFβ Trap-FEBM-hFc-anti-PD1-scFv	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFVFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSSSSSPIQGSWTWENGKWTWKGIIRLEQSSSSGSSSSGSSSSGESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSL LGKGGGSGGGGSGGGGSQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTA VYYCARRDYRFDMGFDYWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVPARFSGSGSGTDFTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIK
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66	Anti-PD-1 scFv-FEBM-TGFβ Trap	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVGPARGSGSGSDT FTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSSGSSSSSGSSSSPI QGSWTWENGKWTWKGIIRLEQGGGGSGGGGSGGGGSIPPHVQKSVNNDMIV TDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKN DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND NIIFSEEYNTSNPD
67	Anti-PD-1 scFv-TGFβ Trap-FEBM	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVGPARGSGSGSDT FTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSSGSSSSSGSSSSIPPHV QKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQ EVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFF MCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSSSSPIQGSWTWENG KWTWKGIIRLEQ

[290]

68	TGFβ Trap-FEBM-anti-PD1-scFv	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPG ETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSSSSPIQGSWT WENGKWTWKGIIRLEQSSSSSGSSSSSGSSSSGQVQLVQSGVEVKKPGASVKVSC KASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTDT SSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSSGGGG SGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQ KPGQAPRLLIYLASYLESVGPARGSGSGSDTFTLTISSELEPEDFAVYYCQHSR LPLTFGGGTKVEIK
69	Anti-PD1-HC-IGHG-anti-EDB-FN scfv-TGFβ Trap	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKP SNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCSVMHEALHNHYTQKSLSLGLKSSSSSGSSSSSGSSSSGEVQLLESGLLVQP GGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGR FTISRDN SKNTLYLQMNSLR AEDTAVYYCAKFPYFDYWGQGLTVTVSSGGG GSGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQQKPG QAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPT FGQGTKVEIKSSSSSGSSSSSGSSSSGIPPHVQKSVNNDMIVTDNNGAVKFPQLCKF CDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLP YHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
70	Anti-PD1-HC-IGHG-TGFβ Trap-anti-EDB-FN scfv	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKP SNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCSVMHEALHNHYTQKSLSLGLKGGGSIPPHVQKSVNNDMIVTDNNGAV KFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLET VCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNT SNPDSSSSSGSSSSSGSSSSGEVQLLESGLLVQPGGSLRLSCAASGFTFSSFSMS WVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDN SKNTLYLQMNSLRA

		EDTAVYYCAKPFYFDYWGGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPG TSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFS SGSGGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK
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71	Anti- PD1-LC- IGKC- anti- EDB-FN scfv- TGFβ Trap	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYL ASYLESQVPAFSGSGGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECSSSSGSSSSSGSSSGEVQLLESGGGLVQPGGSLRLSCAASGFTFSFSMSWV RQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNKNTLYLQMNSLRAED TAVYYCAKPFYFDYWGGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTL LSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGS SGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKGGGGSGGGGSIPPH VQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKP QEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETF FMCSCSSDECNDNIIFSEEYNTSNPD
72	Anti- PD1-LC- IGKC- TGFβ Trap- anti- EDB-FN scfv	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYL ASYLESQVPAFSGSGGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAA APKCMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDSSSSGSSSSGSS SGEVQLLESGGGLVQPGGSLRLSCAASGFTFSFSMSWVRQAPGKGLEWVSSIS GSSGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDY WGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQ SVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSSTDFTLTISRLEPE DFAVYYCQQTGRIPPTFGQGTKVEIK

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73	Anti- EDB-FN scfv- TGFβ Trap- anti-PD1- HC- IGHG	EVQLLESQGGLVQPGGSLRLSCAASGFTFSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYW GGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSV SSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSSTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIKGGGGSGGGGSIPPHVQKSVNNDMIVTDN GAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENI TLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIF EEYNTSNPDSSSSGSSSSGSSSSGQVQLVQSGVEVKKPGASVKVSCASGYTFT NYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVLTITDSSTTTAYM ELKSLQFDDTAVYYCARRDYRFDMGFYWGQTTVTVSSASTKGPSVFLAP CSRSTSESTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV LFPPKPKDTLMISRTPEVTCVVVDVSDPEVFQFNWYVDGVEVHNAKTKPREE QFNSTYRVSVLTVTLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD DGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLK
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74	TGFβ Trap- anti- EDB-FN scfv-anti- PD1-HC- IGHG	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC EKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPG ETFFMCSCSSDECNDNIIFSEEYNTSNPDSSSSGSSSSGSSSSGEVQLLESGGGLV QPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGQGLTVTVSSG GGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQKQ PGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIP PTFGQGTKVEIKSSSSGSSSSGSSSSGQVQLVQSGVEVKKPGASVKVSCKASGY TFTNYYMYWVRQAPGQGLEWMGINPSNGGTNFKNEFKNRVTLTDSSTTTA YMEKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTITVTVSSASTKGPSVFPL APCSRSTSESTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVTVTPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPETCVVVDVVSQEDPEVQFNWYVDGVEVHNAKTKP REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLK
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75	Anti- PD1- scfv-hFc- anti- EDB-FN scfv- TGFβ Trap	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEFKNRVTLTDSSTTTAYMEKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTITVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQKPGQAPRLLIYLASYLESVGPARGSGSGSGTD FTLTISLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSGSSSSGSSSSGESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPETCVVVDVVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGGSFFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLGLKSSSSGSSSSGSSSSGEVQLLESGGGLVQPGGSLRLSCAAS GFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDN SKNTL YLQMNSLRAEDTAVYYCAKPFYFDYWGQGLTVTVSSGGGGSGGGGSGGGG SEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQKQPGQAPRLLIYYASS RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKG GGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPK CIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD
76	Anti- PD1- scfv-hFc- TGFβ Trap- anti- EDB-FN scfv	QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEFKNRVTLTDSSTTTAYMEKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTITVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQKPGQAPRLLIYLASYLESVGPARGSGSGSGTD FTLTISLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSGSSSSGSSSSGESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPETCVVVDVVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGGSFFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLGLKSSSSGSSSSGSSSSGEVQLLESGGGLVQPGGSLRLSCAAS GFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDN SKNTL YLQMNSLRAEDTAVYYCAKPFYFDYWGQGLTVTVSSGGGGSGGGGSGGGG SEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQKQPGQAPRLLIYYASS RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKG GGSGGGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPK CIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

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77	Anti- EDB-FN scfv- TGFβ Trap hFc- anti-PD1 scfv	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYW GQGT LVT VSSGGGGSGGGGSGGGGSEIVLTQSPGTL SLSPGERATL SCRASQSV SSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIKGGGGSGGGGSIPPHVQKSVNNDMIVTDNN GAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENI TLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS EEYNTSNPDSSSSSGSSSSSGSSSGESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLKSSSSSGSSSSSGSSSSG QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVLT TDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGT TTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATL SLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLA SYLESGV PARFSGSGSGTD FTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIK
78	Anti- PD1- scfv-anti- EDB-FN scfv- TGFβ Trap	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVLT TDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGT TTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATL SLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLA SYLESGV PARFSGSGSGTD FTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSSGSSSSSGEVQL LES GGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGQGT LVT VSSGGGGSGGGGSGGGGSEIVLTQSPGTL SLSPGERATL SCRASQSVSSSFL AWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYY CQQTGRIPPTFGQGTKVEIKSSSSSGSSSSSGSSSGIPPHVQKSVNNDMIVTDNNG AVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENIT LETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSE EYNTSNPD

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79	Anti- PD1- scfv- TGFβ Trap- anti- EDB-FN scfv	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVLT TDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGT TTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATL SLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLA SYLESGV PARFSGSGSGTD FTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSSGSSSSSGIPPHV QKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQ EVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFF MCSCSSDECNDNIIFSEEYNTSNPDEVQLLES GGGLVQPGGSLRLSCAASGFTFS SFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDNKNTLYLQM NSLRAEDTAVYYCAKPFYFDYWGQGT LVT VSSGGGGSGGGGSGGGGSEIVL TQSPGTL SLSPGERATL SCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK
80	TGFβ Trap- Anti- PD1- scfv-anti- EDB-FN scfv	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP GETFFMCSCSSDECNDNIIFSEEYNTSNPDSSSSSGSSSSSGSSSGQVQLVQSGVEVK KPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFKNEK FKNRVLT TDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGT TVTVSSGGGGSGGGGSGGGGSEIVLTQSPATL SLSPGERATL SCRASKGVSTSG YSYLHWYQQKPGQAPRLLIYLA SYLESGV PARFSGSGSGTDFTLTISSELEPEDFA VYYCQHSRDLPLTFGGGTKVEIKSSSSSGSSSSSGEVQLLES GGGLVQPGGS LRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGQGT LVT VSSGGGGSG GGGSGGGGSEIVLTQSPGTL SLSPGERATL SCRASQSVSSSFLAWYQQKPGQAP RLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQ GTKVEIK

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81	Anti-PD1-HC-IGHG-FEBM-anti-TGFβ1 scfv	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGGQTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTKYTCNVDHKP SNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVNV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCVMHEALHNHYTQKSLSLGLKSSSSGSSSSGSSSSGSSSPIQGSWTWENG KWTWKGIIRLEQGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCAS GGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTA YMESSLRSED TAVYYCARGLWEVRALPSVYWGQGLTVTVSSSSSSGSSSSGS SSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVSE DIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGT KVTVL
82	Anti-PD1-HC-IGHG-anti-TGFβ1 scfv-FEBM	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGGQTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTKYTCNVDHKP SNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVNV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCVMHEALHNHYTQKSLSLGLKGGGGSGGGGSGGGGSQVQLVQSGAEVK KPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQ GRVTITADESTSTAYMESSLRSED TAVYYCARGLWEVRALPSVYWGQGLTV TVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQ QKAGQAPVLVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVW DRDSDQYVFGTGT KVTVLGGGGSGGGGSGGGGSSSPIQGSWTWENGKWTW KGIIRLEQ
83	Anti-PD1-LC-IGKC-FEBM-anti-TGFβ1 scfv	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYL ASYLESQVPAFSGSGSGTDFTLTISLEPEDFAVYYCQHSRDLPLTFGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSPVTKSFNR GECGGGGSGGGGSGGGGSSSPIQGSWTWENGKWTWKGIIRLEQGGGGSGGG GSGGGGSQVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGL EWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMESSLRSED TAVYYCARG LWEVRALPSVYWGQGLTVTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQT ARITCGANDIGSKSVHWYQQKAGQAPVLVSEDIIRPSGIPERISGSNSGNTATL TISRVEAGDEADYYCQVWDRDSDQYVFGTGT KVTVL

[297]

84	Anti-PD1-LC-IGKC-anti-TGFβ1 scfv-FEBM	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYL ASYLESQVPAFSGSGSGTDFTLTISLEPEDFAVYYCQHSRDLPLTFGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSPVTKSFNR GECGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAI SWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMESSLR SED TAVYYCARGLWEVRALPSVYWGQGLTVTVSSSSSSGSSSSGSSSSGSYELT QPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVSEDIIRPSGIPE RISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGT KVTVLGGG SGGGGSSSPIQGSWTWENGKWTWKGIIRLEQ
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85	Anti-TGFβ1 scfv- FEBM- anti-PD1- HC- IGHG	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGLWEVRAL PSVYWGQGLTVTVSSSSSSSGSSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRSDQYVFGTGTKVTVLGGGSGGGGSSSSPIQGSWTWENG KWTWKGIIRLEQSSSSGSSSSSGSSSSGQVQLVQSGVEVKKPGASVKVSKASG YTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVLTITDSSTTT AYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTITVTVSSASTKGPSVF PLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVTPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGG PSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK
86	FEBM- anti- TGFβ1 scfv-anti- PD1-HC- IGHG	SSSPIQGSWTWENGKWTWKGIIRLEQGGGGSGGGGSGGGGSQVQLVQSGAEV KKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIIPFGTANYAQKF QGRVTITADESTSTAYMELSSLRSEDTAVYYCARGLWEVRALPSVYWGQGLT VTVSSSSSSSGSSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWY QQKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQV WDRSDQYVFGTGKTVTVLGGGSGGGGSGGGGSQVQLVQSGVEVKKPGAS VKVSKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRV LTITDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTITVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPP CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQ KSLSLSLGK

[298]

87	Anti- PD1- scfv-hFc- FEBM- anti- TGFβ1 scfv	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVLTITDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTITVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLIYLASYLESGVPARFSGSGSGTD FTLTISSELEPEDFAVYYCQHSRDLPLTFGGGKVEIKSSSSGSSSSGSSSSGESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLSLGKGGGGSGGGGSSSSPIQGSWTWENGKWTWKGIIRLEQG GGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVR QAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTA VYYCARGLWEVRALPSVYWGQGLTVTVSSSSSSSGSSSSGSYELTQPPSV SVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSN SGNTATLTISRVEAGDEADYYCQVWDRSDQYVFGTGKTVTVL
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88	Anti-PD1-scfv-hFc-anti-TGFβ1-scfv-FEBM	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTITVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVGPARGSGSGSGTD FTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSSGSSSSSGSSSGESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLGLKGGGGSGGGGSGGGGSGVQLVQSGAEVKKPGSSVKVSC KASGDTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYCARGLWEVRALPSVYWGQGTITVTVSSSSSSGSSS SGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLV VSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRSDQYVFGT GTKVTVLGGGGSGGGGSSSSPIQGSWTWENGKWTWKGIIRLEQ
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[299]

89	anti-TGFβ1-scfv-FEBM-hFc-anti-PD1-scfv	QVQLVQSGAEVKKPGSSVKVSCASGDTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGLWEVRAL PSVYWGQGTITVTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVSEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRSDQYVFGTGTKVTVLGGGGSGGGGSSSSPIQGSWTWEN GKWTWKGIIRLEQGGGGSGGGGSGGGGSESKYGPPCPPCPAPEFLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLKSSSSGSSSS GSSSSGQVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGL EWMGGINPSNGGTNFKNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCA RRDYRFDMGFDYWGQGTITVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSP GERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVGPARGSG SGSGTDFTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIK
90	Anti-PD1-scfv-FEBM-anti-TGFβ1-scfv	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTITVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVGPARGSGSGSGTD FTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIKGGGGSGGGGSSSSPIQGS WTWENGKWTWKGIIRLEQGGGGSGGGGSGGGGSGVQLVQSGAEVKKPGSSV KVSCASGDTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTIT ADESTSTAYMELSSLRSEDTAVYYCARGLWEVRALPSVYWGQGTITVTVSSSS SGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQA PVLVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRSDQY VFGTGTKVTVL

[300]

91	Anti-PD1-scfv-anti-TGFβ1-scfv-FEBM	QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTITVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVGPARGSGSGSGTD FTLTISSELEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSSGSSSSGSSSSGQVQL VQSGAEVKKPGSSVKVSCASGDTFSSYAISWVRQAPGQGLEWMGGIPIFGT ANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGLWEVRALPSV YWGQGTITVTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIG SKSVHWYQQKAGQAPVLVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEA DYYCQVWDRSDQYVFGTGTKVTVLGGGGSGGGGSSSSPIQGSWTWENGKW TWKGIIRLEQ
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92	Anti-TGFβ1 scfv- FEBM- anti-PD1 scfv	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARGLWEVRAL PSVYWGQGLTVTVSSSSSSSGSSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRSDQYVFGTGTKVTVLGGGGSGGGGSSSSPIQGSWTWEN GKWTWKGIIRLEQSSSSSGSSSSSGSSSSGQVQLVQSGVEVKKPGASVKVSKAS GYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFKNEKFKNRVTLTDSST TTAYMELKSLQFDDNAVYYCARRDYRFDMGFYWGQGTITVTVSSGGGGSGG GGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPG QAPRLLIYLAstyleSGVPARFSGSGSGTDFTLTISLEPEDFAVYYCQHSRDLP TFGGGKVEIK
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[301]

93	Anti-PD1-HC- IGHG- anti- EDB-FN scfv-anti- TGFβ1 scfv	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVTLTDSSTTTAYMELKSLQFDDNAVYYCARRDYRF DMGFYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTKYTCNVDP SNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCVMHEALHNHYTQKSLSLGLKSSSSSGSSSSSGSSSSGVEVQLLESGGGLVQP GGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGQGLTVTVSSGGG GGGGGGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQQKPG QAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPT FGQGTKVEIKSSSSSGSSSSSGSSSSGQVQLVQSGAEVKKPGSSVKVSKASGGTF SSYAISWVRQAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADESTSTAYMEL SSLRSEDNAVYYCARGLWEVRALPSVYWGQGLTVTVSSSSSSSGSSSSSGSSSSGS YELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSIEDIIRPS GIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRSDQYVFGTGKVTVL
94	Anti-PD1-HC- IGHG- anti- TGFβ1 scfv-anti- EDB-FN scfv	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFKNEKFKNRVTLTDSSTTTAYMELKSLQFDDNAVYYCARRDYRF DMGFYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTKYTCNVDP SNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCVMHEALHNHYTQKSLSLGLKGGGGSGGGGSGGGGSGVQLVQSGAEVK KPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIIPFGTANYAQKFQ GRVTITADESTSTAYMELSSLRSEDNAVYYCARGLWEVRALPSVYWGQGLTV TVSSSSSSSGSSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQ QKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVW DRSDQYVFGTGKVTVLSSSSSGSSSSSGSSSSGVEVQLLESGGGLVQPGSLRLS CAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGQGLTVTVSSGGGGSGGGGS GGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIY YASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKV EIK

[302]

95	Anti-PD1-LC-IGKC-anti-EDB-FN scfv-anti-TGFβ1 scfv	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYL ASYLESGVPARFSGSGSGTDFTLTISLLEPEDFAVYYCQHSRDLPLTFGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECSSSSGSSSSGSSSSGEVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWV RQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAED TAVYYCAKPFYFDYWGGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTL LSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSG SGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKSSSSGSSSSGSSSSG QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSED TAVYYCARGLWEVRAL PSVYWGQGT LVTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVVSEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRSDQYVFGTGTKVTVL
96	Anti-PD1-LC-IGKC-anti-TGFβ1 scfv-anti-EDB-FN scfv	EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYL ASYLESGVPARFSGSGSGTDFTLTISLLEPEDFAVYYCQHSRDLPLTFGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAI SWVRQAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADESTSTAYMELSSLR SEDTAVYYCARGLWEVRALPSVYWGQGT LVTVSSSSSSGSSSSGSSSSGSYELT QPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSEDIIRPSGIPE RISGSNSGNTATLTISRVEAGDEADYYCQVWDRSDQYVFGTGTKVTVLSSSS GSSSSGSSSSGEVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGK GLEWVSSISGSSGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC AKPFYFDYWGGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTL SLSPGER ATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK

[303]

97	Anti-EDB-FN scfv-anti-TGFβ1 scfv-anti-PD1-HC-IGHG	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKPFYFDYW GGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTL SLSPGERATLSCRASQSV SSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIKSSSSGSSSSGSSSSGQVQLVQSGAEVKKPG SSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIIPFGTANYAQKFQGRV TITADESTSTAYMELSSLRSED TAVYYCARGLWEVRALPSVYWGQGT LVTVSS SSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKA GQAPVLVVSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDS DQYVFGTGTKVTVLGGGGSGGGGSGGGGSQVQLVQSGVEVKKPGASVKVSC KASGYTFTNYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLTTD SSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGGQTTTVTVSSASTK GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGKTYTCNVDPKSNKVDKRVESKYGPPCPPCPAP EFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGSEFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLS LGK
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98	Anti-TGFβ1 scfv-anti-EDB-FN scfv-anti-PD1-HC-IGHG	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVYYCARGLWEVRAL PSVYWGQGTLLTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVSEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRSDQYVFGTGTKVTVLGGGGSGGGGSGGGGSEVQLLESG GGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYA DSVKGRFTISRDNKNTLYLQMNSLRAEDNAVYYCAKPFYFDYWGQGTLLTV VSSGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAW YQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ TGRIPPTFGQGTKVEIKSSSSGSSSSGSSSSGQVQLVQSGVEVKKPGASVKVSK ASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNEKFKNRVTLLTDS STTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTLTVSSASTKG PSVFLAPCSRSTSESTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQS SGLYSLSVVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPE FLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSL GK
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[304]

99	Anti-PD1- scfv-hFc- anti-EDB-FN scfv-anti-TGFβ1 scfv	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVTLLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTTLTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVGPARGSGSGSGTD FTLTISLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSGSSSSGSSSSGESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLSLGKGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSC AASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNK NTLYLQMNSLRAEDNAVYYCAKPFYFDYWGQGTLLTVSSGGGGSGGGGSG GGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAWYQKPGQAPRLLIYY ASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEI KGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISW VRQAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADESTSTAYMELSSLRSED NAVYYCARGLWEVRALPSVYWGQGTLLTVSSSSSSGSSSSGSSSSGSYELTQPP SVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVSEDIIRPSGIPERISG SNSGNTATLTISRVEAGDEADYYCQVWDRSDQYVFGTGKVTVL
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[305]

100	Anti-PD1- scfv-hFc- anti-TGFβ1 scfv-anti-EDB-FN scfv	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVTLLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRF DMGFDYWGQGTTLTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATL SCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVGPARGSGSGSGTD FTLTISLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSGSSSSGSSSSGESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLSLGKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSC KASGGTFSSYAISWVRQAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADEST STAYMELSSLRSEDNAVYYCARGLWEVRALPSVYWGQGTLLTVSSSSSSGSSS SGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLV VSEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRSDQYVFGT GKVTVLGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSS FMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNKNTLYLQMN
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		SLRAEDTAVYYCAKPFYFDYWGGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK
101	Anti-EDB-FN scfv-anti-TGFβ1 scfv-hFc-anti-PD1 scfv	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSGTTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKGGGGSGGGGSGGGGSGVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARGLWEVRALPSVYWGQTLVTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGKVTVLGGGGSGGGGSGGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLKSSSSGSSSSGSSSSGQVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFKNEKFKNRVLTLDSTSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGGQTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVPARFSGSGSGTDFTLTISRLEPEDFAVYYCQHSRDLPLTFGGGTKVEIK

[306]

102	Anti-PD1- scfv-anti-EDB-FN scfv-anti-TGFβ1 scfv	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFKNEKFKNRVLTLDSTSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGGQTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVPARFSGSGSGTDFTLTISRLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSGSSSSGSSSSGQVQLLESGLLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKGGGGSGGGGSGGGGSGVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARGLWEVRALPSVYWGQTLVTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGKVTVL
103	Anti-PD1- scfv-anti-TGFβ1 scfv-anti-EDB-FN scfv	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFKNEKFKNRVLTLDSTSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGGQTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESVPARFSGSGSGTDFTLTISRLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKSSSSGSSSSGSSSSGQVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARGLWEVRALPSVYWGQTLVTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGANDIGSKSVHWYQQKAGQAPVLVVSIEDIIRPSGIPERISGSNSGNTATLTISRVEAGDEADYYCQVWDRDSDQYVFGTGKVTVLGGGGSGGGGSGGGGSEVQLLESGLLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGGQTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK

[307]

104	Anti-TGFβ1 scfv-anti-EDB-FN scfv-anti-PD1 scfv	QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGLWEVRAL PSVYWGQGLTVTVSSSSSSGSSSSGSSSSGSYELTQPPSVSVAPGQTARITCGAN DIGSKSVHWYQQKAGQAPVLVSEDIIRPSGIPERISGSNSGNTATLTISRVEAG DEADYYCQVWDRSDQYVFGTGKVTVLGGGGSGGGGSGGGGSEVQLLES GGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYA DSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWGQGLTVT VSSGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVSSSFLAW YQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ TGRIPPTFGQGTKVEIKSSSSGSSSSGSSSSGQVQLVQSGVEVKKPGASVKVSC ASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFKNEKFKNRVTLTDS STTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTITVTVSSGGGGS GGGSGGGGSEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQK PGQAPRLLIYLAstyleGVPARFSGSGSGTDFTLTISLEPEDFAVYYCQHSRDL PLTFGGGGTKVEIK
105	Soluble TGFβ Trap-hFc	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC EKQPEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPG ETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGGSGGGGSGGGGSESKYGPPCP PCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSGSSFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSL SLSLGK

[308]

106	hFc- soluble TGFβ Trap	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSGSSFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLSLGKGGGGSGGGGSGGGGSIIPPHVQKSVNNDMIVTDNNGAV KFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKQPEVCVAVWRKNDENITLET VCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIFSEEYN TSNPD
107	hFc- FEBM	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSGSSFLYSRLTVDKSRWQEGNVFSCSVMEAL HNHYTQKSLSLSLGKGGGGSGGGGSSSSPIQGSWTWENGKWTWKGIIRLEQ
108	Anti- EDB-FN- HC- IGHG	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGS SGTTYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAKPFYFDYWG QGLTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDHKPSNTKVDKRV SKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE ESNGQPENNYKTTTPVLDSGSSFLYSRLTVDKSRWQEGNVFSCSVMEALHN HYTQKSLSLSLGK

109	Anti-PD-1-HC-IGHG	QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGG INPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFD MGFDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNT KVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVTLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS VMHEALHNHYTQKSLSLGLGK
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[309] 1-4. Production and purification of fusion proteins

[310] Transient CHO expression

[311] The signal sequence MGWSCIILFLVATATGAYA (SEQ ID NO: 112) was included at the N-terminus of the fusion protein of Example 1-2. The fusion protein
5 was cloned into the KpnI (5' end) and NotI (3' end) sites of the pCAG mammalian expression plasmid vector (FUJIFILM Wako, Japan), and then produced by transient transfection of Chinese hamster ovary (CHO) cells.

[312] That is, the ExpiCHO™ expression system kit (Thermo fisher Scientific, USA) was used. Plasmid DNA was transfected into 400 mL to 1,200 mL of ExpiCHO-S cells
10 in serum-free ExpiCHO™ expression medium (Thermo fisher Scientific, USA) containing GlutaMax (Thermo fisher Scientific, USA) and maintained at 37°C.

[313] Transfection conditions were performed according to the user guide of the ExpiCHO™ expression system. DNA 1µg/mL and ExpiFectamine™ CHO Reagent were diluted in OptiPRO SFM (Thermo fisher Scientific, USA) for 1L of culture flask
15 and treated. 18 to 22 hours after transfection, ExpiCHO™ Feed and ExpiCHO™ Enhancer were added, and the cells were harvested after maintaining them at 37°C for 7-11 days after transfection. The cell viability at the final harvest was 70% - 80%.

[314] Purification

[315] The supernatant harvested from CHO cells was filtered through a 0.22 µm
20 PES filter (Corning, NY, USA) and loaded onto a column XK16 (Cytiva, USA) packed with Protein A MebSelect Sure LX (Cytiva, USA) resin. The solution was washed

with 5 column volumes each of Wash 1 buffer and Wash 2 buffer, and the protein was eluted using 5 column volumes of 0.02 M sodium pH 4.0 and pH 3.8 buffers.

[316] The purified fraction samples of the eluted protein were subjected to size exclusion chromatography (SEC) analysis and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis under non-reducing conditions to select samples with high purity. In addition, the purified fraction samples with purity of 90% or less were re-loaded onto the protein A column for the purpose of reprocessing and eluted as described above to maximize the production yield. In addition, the eluted purified protein was analyzed by the same method.

[317] The purified fraction samples containing the fusion protein were pooled, and the eluted fractions were neutralized to approximately pH 7.0 using 1 M Tris. For SEC analysis, and 20 to 40 µg of protein was diluted in PBS (Enzygnomics, Korea) and injected onto a TSKgel G3000SWxL (Tosoh, Japan) SEC analytical column (5.0 µm, 7.8 X 300 mm) equipped with a TSKgel guard column (7.0 µm, 6.0 X 40 mm) using an Agilent (Agilent infinity II) system. Resolution was performed at a flow rate of 0.8 mL/min at room temperature for 30 minutes, and the target protein peak was detected at UV 280 nm/214 nm using an Agilent VWD detector (Agilent, USA). SDS-PAGE analysis was performed under non-reducing conditions with EzStainAqua (ATTO, Japan) staining, followed by destaining using triple-distilled water.

[318]

[319] 1-5. Evaluation of target binding level of fusion proteins

[320] The level of simultaneous binding of each fusion protein to its target was assessed using a sandwich ELISA method.

[321] That is, 96-well ELISA plates (Nunc, USA) coated with recombinant EDB-FN-6x His (Abcam) were blocked with PBS solution containing 1% BSA (Abcam,

USA) for 1 hour at room temperature, and then, various serially diluted (1:2) target proteins such as FEBM-, L19 hIgG-, L19 scfv-fusion TGFβ-Trap or soluble Trap control were reacted for 2 hours at room temperature, and washed X times with washing buffer, and then, biotinylated TGF-β1 (R&D Systems, Catalog #NFTG0, USA) (1:50 diluted in PBS) was reacted for 2 hours at room temperature, and then, 100 μL of ELISA substrate (streptavidin conjugated with horseradish peroxidase) was added to each well after 4 times of dilution, and then, 2N H₂SO₄ was added to stop the HRP reaction. Then, the level of binding was assessed by measuring the optical density (OD) at 450 nm.

[322]

[323] 1-6. Creation of breast cancer orthotopic transplantation mouse model and confirmation of fusion protein effects

[324] A breast cancer orthotopic transplantation mouse model was created by injecting 5×10^4 4T-1-LMT-2-Luc cell lines produced in Example 1-2 into BALB/c mice (Orient Bio, Korea).

[325] Confirmation of *in vivo* fusion protein distribution

[326] After intravenous injection of 20 mg/kg of Cy5-labeled various fusion proteins (e.g., hFc-soluble TGFβ Trap, FEBM-hFc-TGFβ Trap, hFc-FEBM-TGFβ Trap, and hFc-TGFβ Trap-FEBM, etc.) into orthotopic transplantation mouse models, mice were sacrificed 6 days later, and tissues were collected and analyzed for Cy5 fluorescence signals using IVIS imaging-Lumina S5 (PerkinElmer, USA).

[327] Tumor size analysis

[328] Orthotopic transplantation mouse models were injected subcutaneously with 20 mg/kg of various fusion proteins (e.g., hFc-soluble TGFβ Trap, FEBM-hFc-TGFβ Trap, hFc-FEBM-TGFβ Trap, hFc-TGFβ Trap-FEBM, etc.) and controls (e.g.,

control-hFc) at 4-day intervals from Days 5 to 25. Tumor sizes were measured using electronic calipers on Days 5, 9, 13, 17, 21 and 25.

[329] Transition rate analysis

[330] On day 7 of the growth of the orthotopic transplantation mouse model, D-luciferin was injected intraperitoneally to use the BLI (bioluminescence) signal as a background. The growth of primary fat pad tumors was monitored by BLI signal and separated into 5 groups with similar BLI signals. Various fusion proteins (e.g., hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap, hFc-TGF β Trap-FEBM, etc.) and control groups (e.g., control-hFc) at 20 mg/kg were injected subcutaneously at 4-day intervals from day 5 to 25.

[331] The metastasis rate was analyzed by BLI signals on Days 5, 11, 18, 21 and 25 using IVIS imaging-Lumina S5 (PerkinElmer, USA). The dead mice were dissected within 6 hours and analyzed for the presence of metastases.

[332] Survival rate analysis

[333] In the same way as the metastasis analysis, various fusion proteins were prepared in the mouse model, and the number of mice that survived up to 50 days was counted to create a survival curve.

[334] Anti-pSMAD and anti-CD8 immunohistochemical analysis

[335] The orthotopic transplantation mouse model was administered with 20 mg/kg of various fusion proteins (e.g., hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap, hFc-TGF β Trap-FEBM, etc.) and control groups (e.g., control-hFc) at 4-day intervals from day 5 to day 25, and then sacrificed 24 hours later. The 4T-1-L2-Luc tumor tissues were fixed in formalin and embedded in paraffin to create 5 μ m tumor sections, which were then mounted on SuperFrost Plus slides (Thermo fisher Scientific, USA) and stained according to the user guide of the Leica Bond autostainer.

[336] That is, the slides were baked, dewaxed and rehydrated, and then exposed to antigens for 20 minutes using ER2 at 95°C, blocked with 2.5% normal goat serum, and then reacted with primary SMAD2 phosphorylation antibody (Ser465/467, clone 138D4, NEB, 0.6 µg/mL, USA) and primary mCD8a antibody (clone 4SM15, eBioscience, 2.5 µg/mL, USA) for 60 minutes. Antigens were detected using secondary anti-rabbit and anti-rat secondary antibodies conjugated with HRP (Vector Labs, MP-7444, USA), and visualized using DAB substrate (Abcam, AB64238, USA). Signals were quantified using Definiens Tissue Studio software, and the total number of cells was calculated by counting the number of hematoxylin-stained nuclei. Positive signals were detected by setting the threshold for DAB chromogen above the background signal.

[337]

[338] 1-7. Creation of pancreatic cancer orthotopic transplantation mouse model and confirmation of fusion protein effects

[339] A pancreatic cancer orthotopic transplantation mouse model was created by injecting 2×10^6 PANC02-Luc cell lines produced in Example 1-2 into C57BL/6J mice (Orient Bio, Korea).

[340] Tumor weight analysis

[341] The orthotopic transplantation mouse model was subcutaneously injected with 30 mg/kg of various fusion proteins (e.g., hFc-soluble TGFβ Trap, FEBM-hFc-TGFβ Trap, hFc-FEBM-TGFβ Trap, hFc-TGFβ Trap-FEBM, etc.) and controls (e.g., control-hFc) at 5-day intervals from day 7 to day 57. Tumor weights were measured on day 63 after all mice were sacrificed and tumors were collected.

[342] Transition rate analysis

[343] The orthotopic transplantation mouse model was grown for 7 days, and then, D-luciferin was injected intraperitoneally to use the bioluminescence (BLI) signal as a background. The growth of primary fat pad tumors was monitored by BLI signal and separated into 7 groups with similar BLI signals. Various fusion proteins (e.g., hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap, hFc-TGF β Trap-FEBM, etc.) and controls (e.g., control-hFc) at 30 mg/kg were injected subcutaneously at 5-day intervals from day 7 to day 57.

[344] The cancer metastasis rate was analyzed by BLI signals on Days 7 and 62 using IVIS imaging -Lumina S5 (PerkinElmer, USA). The dead mice were dissected within 6 hours and analyzed for the presence of metastases. All mice were sacrificed on day 63.

[345] Survival rate analysis

[346] In the same way as the metastasis analysis, various fusion proteins were prepared in the mouse model, and the number of mice that survived up to 63 days was counted to create a survival curve.

[347] Anti-pSMAD and anti-CD8 immunohistochemical analysis

[348] Panc02-Luc tumor tissues were fixed in formalin and embedded in paraffin to create 5 μ m tumor sections, which were analyzed using the same method as the analysis of the breast cancer orthotopic transplantation mouse model.

[349] 1-8. Creation of mouse model of tumor growth after melanoma vein transplantation -> melanoma lung metastasis and confirmation of the effects of fusion proteins

[350] A melanoma transplantation mouse model was created by intravenously injecting 1×10^5 B16-F10-Luc cell line produced in Example 1-2 into C57BL/6 male mice (Orient Bio, Korea).

[351] Growth rate analysis

[352] Intravenous transplantation mouse models were injected intravenously with 30 mg/kg of various fusion proteins (e.g., hFc-soluble TGF β Trap, hFc-FEBM-TGF β Trap, hFc-TGF β Trap-FEBM, hFc-FEBM-anti-TGF β 1 scFv, Anti-EDB-FN-TGF β Trap-hFc, or hFc-anti-EDB-FN scFv-anti-TGF β 1 scFv) and controls (e.g., control-hFc) at 3-day intervals from day 5 to day 25. On day 5, D-luciferin was injected intraperitoneally to use the bioluminescence (BLI) signal as a background. The growth of primary fat pad tumors was monitored by BLI signal and separated into 5 groups with similar BLI signals. The growth rate of B16-F10-Luc-tumor in the lung was analyzed by BLI signals on Days 5, 11, 18, 21 and 28 using IVIS imaging-Lumina S5 (PerkinElmer, USA). The average radiant efficiency value is the value normalized to the overall value by each ROI size.

[353]

[354] **Example 2. Confirmation of cancer association of fibronectin gene FN1 and EDB domain**

[355] As a result of analyzing the expression of fibronectin EDB transcripts by the method of Example 1-1, it was confirmed that 4 transcripts among the fibronectin transcripts in the human genome region include EDB exons that are specifically expressed in cancer tissues, as described in FIG. 1A. As a result of analyzing the expressions of EDB exons and the entire FN1 gene by the expression analysis method of Example 1-1, it was confirmed that both EDB exons and FN1 gene showed higher expression in tumor tissues than in normal tissues, as described in FIG. 1B.

[356] In addition, when the expression of the FN1 EDB+ gene in various cancers was analyzed in TCGA data using the prognostic analysis method of Example 1-1, it was confirmed that in most cancers, the higher the expression of the FN1 EDB+ gene,

the worse the prognosis, as described in FIGS. 2A to 2F. In addition, when the FN1 EDB domain was analyzed in humans, monkeys, rats and mice using the sequence alignment method of Example 1-1, it was confirmed that it was 100% conserved in the four species, as described in FIG. 3.

5 [357] Moreover, as a result of comparing the expression levels of FN1-EDB+ transcripts in 11 different tumor tissues and normal tissues that were orthotopically transplanted by the method of Example 1-2, as described in FIG. 4, it was confirmed that FN1 EDB+ expression was significantly higher in all solid tumor tissues than in three normal tissues, including 4T-1 (breast cancer), Colon26 (colon cancer), PANC02
10 (pancreatic cancer), KRasG12D/+TP53-/- PLC (Primary lung cancer cell lines derived from spontaneous Kras/p53 GEMMs, lung cancer), B16F10 (melanoma), Hepa6 (liver cancer), GL26 (brain tumor), RM1 (prostate cancer), YTN16 (gastric cancer), RENCA (renal cancer) and KRasG12D/+TP53-/- PSC (Primary sarcoma cell lines derived from spontaneous Kras/p53 GEMMs, sarcoma). TP53-/- PTLC (Primary T lymphoma cell
15 lines derived from spontaneous p53 GEMMs, lymphoma) hematological cancers were confirmed to express similarly to normal tissues, and among the transplanted solid tumors, pancreatic cancer tumors showed the highest FN1 EDB+ expression, while the lowest expression was confirmed in transplanted melanoma tumors.

[358] Subsequently, tumor models of FN1-EDB high (pancreatic cancer model),
20 FN1-EDB intermediate (breast cancer model), and FN1-EDB low (melanoma model) levels were used to evaluate efficacy.

[359]

[360] **Example 3. Production of bispecific Fc fusion proteins including FEBM and TGFβ Trap and confirmation of binding capability**

[361] 3-1. Production and purification of bispecific Fc fusion proteins including FEBM and TGF β Trap

[362] The bispecific Fc fusion protein including FEBM and TGF β Trap designed by the method of Example 1-3 was purified by the method of Example 1-4, and as a result, as described in a and b of FIG. 5, it was confirmed that the monomer purity was 90% or more, and the endotoxin level was 0.1 EU/mL or less. In this example, among the bispecific Fc fusion proteins including FEBM and TGF β Trap of Table 2,

[363] Control-hFc (SEQ ID NO: 12): Human IgG4 heavy chain Fc region (hIgG4-Fc)

10 [364] hFc-FEBM (SEQ ID NO: 107): FEBM polypeptide is fused to the C-terminus of hIgG4-Fc (hIgG4-Fc-FEBM)

[365] Soluble TGF β Trap-hFc (SEQ ID NO: 105): TGF β RII ectodomain domain is fused to the N-terminus of hIgG4-Fc.

15 [366] TGF β Trap-hFc-FEBM (SEQ ID NO: 16): TGF β RII ectodomain domain is fused to the N-terminus of hIgG4-Fc, and FEBM polypeptide is fused to the C-terminus of Fc.

[367] TGF β Trap-FEBM-hFc (SEQ ID NO: 17): FEBM polypeptide is fused to the N-terminus of hIgG4-Fc, onto which the TGF β RII ectodomain domain is fused.

20 [368] FEBM-TGF β Trap-hFc (SEQ ID NO: 18): TGF β RII ectodomain domain is fused to the N-terminus of hIgG4-Fc, onto which FEBM polypeptide is fused.

[369] hFc-soluble TGF β Trap (SEQ ID NO: 106): TGF β RII ectodomain domain is fused to the C-terminus of hIgG4-Fc.

[370] FEBM-hFc-TGF β Trap (SEQ ID NO: 19): FEBM polypeptide is fused to the N-terminus of hIgG4-Fc, and TGF β RII ectodomain domain is fused to the C-terminus.

[371] hFc-FEBM-TGF β Trap (SEQ ID NO: 20): FEBM polypeptide is fused to the C-terminus of hIgG4-Fc, followed by TGF β RII ectodomain domain.

[372] hFc-TGF β Trap-FEBM (SEQ ID NO: 21): TGF β RII ectodomain domain fused to the C-terminus of hIgG4-Fc, followed by FEBM polypeptide fusion.

5 [373] Proteins such as these were produced and purified.

[374] 3-2. Evaluation of binding capability of dual-specific Fc fusion proteins including FEBM and TGF β Trap

[375] As a result of evaluating the binding capability of the fusion proteins purified in Example 3-1 by the method of Example 1-5, it was confirmed that consistent
10 binding affinity was formed in the range of 304.3 to 370 ng/mL for both targets despite structural changes at different positions of the FEBM-TGF β Trap structure, as described in FIG. 6.

[376] 3-3. Confirmation of binding capability of bispecific Fc fusion proteins including FEBM and TGF β Trap in breast cancer orthotopic transplantation model

15 [377] 4T1 breast cancer tumors are a commonly used mouse model to study triple-negative breast cancer (TNBC) due to their aggressive growth, metastatic potential, and ability to simulate immune responses seen in human cancers. These tumors often do not respond to anti-PD-1 or anti-PD-L1 therapy, either as monotherapy or in combination with other treatments, reflecting the immunosuppressive nature of the
20 tumor microenvironment (TME) in this model. Highly immunosuppressive tumor microenvironment: One of the characteristics of the 4T1 tumor model is the lack of infiltration of CD8⁺ cytotoxic T cells, which are essential for an effective anti-tumor immune response. This lack of immune cell infiltration is due in part to the dense extracellular matrix (ECM) and fibrous stroma surrounding the tumor, which
25 constitute a physical barrier.

[378] In previous studies, 4T1 breast cancer tumors showed activated TGF β signaling, and in recent studies, intermediate to high expression of EDB-FN was observed in solid tumors. Based on these results, the inventors of the present invention decided that it would be appropriate to evaluate a drug that dually targets FN1 EDB+,
5 which binds to tumor ECM and enables local TGF β trapping.

[379] As such, the *in vivo* distribution of tumors and organs collected from 4T-1 orthotopic transplantation mice produced by the method of Example 1-6 was confirmed, and as described in FIG. 7, relatively high fluorescence signal intensity was observed in the liver and kidneys because the probe was mainly excreted through these
10 organs, and despite the appearance of a high fluorescence background in the liver tissues of mice not administered Cy5, it was confirmed that the tumors treated with hFc-FEBM-TGF β Trap or hFc-TGF β Trap-FEBM dual fusion protein showed the highest fluorescence intensity compared to hFc-soluble TGF β Trap and FEBM-hFc-TGF β Trap based on the radiation efficiency excluding autofluorescence.

[380] In addition, both hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM proteins showed significantly higher fluorescence intensities in tumors than in 9 types of normal tissues and organs, and hFc-FEBM-TGF β Trap showed slightly higher tumor specificity than hFc-TGF β Trap-FEBM protein, hFc-soluble TGF β Trap did not show tumor specificity, and FEBM-hFc-TGF β Trap showed some tumor specificity
20 compared to all normal tissues except the liver, but the difference was not significant, and hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM proteins were confirmed to bind with high specificity to EDB-FN, which is abundantly expressed in 4T-1 orthotopic transplant tumors.

[381]

[382] Example 4. Confirmation of therapeutic effects of bispecific Fc fusion proteins including FEBM and TGFβ Trap

[383] 4-1. Tumor growth rate analysis

[384] An advance breast tumor model that is orthotopically transplanted in the breast
5 pad is clinically more relevant than subcutaneous tumor models due to the
establishment of organ-specific tumors. The method of Examples 1-6 was used to
establish highly metastatic cell lines, and as a result, hFc-FEBM-TGFβ Trap and hFc-
TGFβ Trap-FEBM exhibited statistically significant potent antitumor activity
compared to other experimental groups in an orthotopic transplantation mouse
10 advanced breast cancer model in which intermediate levels of EDB expression were
observed among all solid tumors, as described in FIG. 8A.

[385] In addition, as described in FIG. 8B, the tumor volumes of the hFc-soluble
TGFβ Trap, FEBM-hFc-TGFβ Trap, hFc-FEBM-TGFβ Trap, or hFc-TGFβ Trap-
FEBM administration groups on day 25 were confirmed to decrease by about 32.5%,
15 about 42.4%, about 92.8% and about 74.9%, respectively, compared to the tumor
volume of the isotype control group. That is, it was confirmed that the administration
of hFc-FEBM-TGFβ Trap or hFc-TGFβ Trap-FEBM inhibited tumor growth to a
greater extent (>90% and >50%, respectively) than hFc-soluble TGFβ Trap.

[386] 4-2. Analysis of lung metastasis rates

20 [387] As a result of analyzing the lung metastasis rates of the breast cancer
orthotopic transplantation model by the administration of hFc-soluble TGFβ Trap,
FEBM-hFc-TGFβ Trap, hFc-FEBM-TGFβ Trap and hFc-TGFβ Trap-FEBM fusion
proteins using the method of Example 1-6, as described in FIG. 9, on day 33, the lung
metastasis characteristics of the hFc-soluble TGFβ Trap, FEBM-hFc-TGFβ Trap, hFc-
25 FEBM-TGFβ Trap, or hFc-TGFβ Trap-FEBM administration groups were confirmed

to have decreased by an average of 0%, 0%, 100%, and 100%, respectively, compared to the syngeneic control group.

[388] In addition, when hFc-FEBM-TGF β Trap was administered, the primary tumor completely responded, whereas hFc-TGF β Trap-FEBM responded in 2 out of 5 mice, and hFc-FEBM-TGF β Trap showed a 0% lung metastasis rate. On day 43, the hFc-soluble TGF β Trap and FEBM-hFc-TGF β Trap administration groups showed a 100% lung metastasis rate, and hFc-TGF β Trap-FEBM showed a 40% lung metastasis rate.

[389] 4-3. Survival rate analysis

[390] As a result of analyzing the survival rate of breast cancer orthotopic transplantation models administered with hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM fusion proteins using the method of Example 1-6, as described in FIG. 10, it was confirmed that hFc-TGF β Trap-FEBM showed a statistically significant 40% increase in survival rate based on 50 days compared to the isotype control group, and more surprisingly, it was confirmed that all mice survived for 50 days even after drug administration was discontinued when hFc-FEBM-TGF β Trap was administered.

[391] 4-4. Analysis of α -SMA expression in tumor margin and tumor center

[392] Alpha-smooth muscle actin (α -SMA) is a key marker of myofibroblasts and cancer-associated fibroblasts (CAFs), which play a critical role in shaping the extracellular matrix (ECM) of the tumor microenvironment (TME). The expression thereof tends to differ between the tumor margin and center, and α -SMA-positive CAFs form a dense stroma that promotes the accumulation of ECM proteins, contributing to the creation of an immunosuppressive environment. This stroma acts as a physical barrier to restrict the infiltration of immune cells into the tumor core, and

high α -SMA expression is correlated with immune cell infiltration and low immune resistance.

[393] The 4T-1 tumor margin was defined as an ROI consisting of a tumor epithelium island that includes an area extending 200 to 300 μ m on both sides of boundary or multiple areas of tumor stroma larger than 50 μ m, and the tumor core was defined as the entire area within the tumor margin.

[394] As a result of quantifying alpha SMA staining in the tumor margin and tumor center of 4T-1 using the method of Example 1-6, as described in the left panel of FIG. 11, it was confirmed that α -SMA expression in the tumor margin of the hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap, or hFc-TGF β Trap-FEBM administration groups was reduced by about 21.0%, about 38.1%, about 83.7%, and about 61.7%, respectively, compared to the isotype control group. In particular, the groups administered with hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM suppressed α -SMA expression in the tumor margin to a greater extent (>80% and >50%, respectively) than hFc-soluble TGF β Trap.

[395] In addition, as described in the right panel of FIG. 11, it was confirmed that α -SMA expression in the tumor center of the hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap, or hFc-TGF β Trap-FEBM administration groups increased by about 2.5-fold, about 4.4-fold, about 20-fold, and about 9.7-fold, respectively, compared to the isotype control group. In particular, the groups administered with hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM showed a greater increase (more than 8-fold and 4-fold, respectively) in α -SMA expression in the tumor center than that of hFc-soluble TGF β Trap.

[396] 4-5. Confirmation of decreased phosphorylation of Smad2 and increased cytotoxic immune CD8⁺ T cells

[397] As a result of confirming the phosphorylation of Smad2 and the expression level of CD8 by the method of Example 1-6, as described in FIG. 12A, it was confirmed that the phosphorylation of Smad2 in the tumors of the hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap, or hFc-TGF β Trap-FEBM administration groups decreased by about 23.8%, about 39.2%, about 67.2%, and about 57.8%, respectively, compared to the isotype control group (control-hFc).

[398] In particular, the groups administered with FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM suppressed p-smad2 levels to a greater extent (>21%, >57%, and >43%) than hFc-soluble TGF β Trap, respectively. Although hFc-soluble TGF β Trap, characterized by systemic exposure, did not sustain Smad2 phosphorylation in tumor tissues of mice sacrificed 2 days after the last administration, the FEBM fusion protein suppressed Smad2 phosphorylation more effectively through TGF β Trap due to tumor specificity and higher persistence in the tumor microenvironment.

[399] In addition, as described in FIG. 12B, the expression of CD8, which is a memory marker effective in killing cancer cells in the hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap or hFc-TGF β Trap-FEBM administration groups, was confirmed to increase about 2.6-fold, about 4.2-fold, about 18.7-fold, and about 10.1-fold, respectively, compared to the isotype control group (Control-hFc). In particular, the tumor-specific CD8⁺ T cells in the groups administered with hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM were confirmed to increase about 8-fold and about 4-fold, respectively, compared to hFc-soluble TGF β Trap.

[400] CD8 activation by the administration of Fc-FEBM-TGF β Trap or hFc-TGF β Trap-FEBM could be achieved directly or indirectly by 1) a potent inhibitory effect of CD8⁺ immune cell deactivation due to stromal-immune evasion-related cell

interaction in the TME or 2) an effect of inducing stromal cell imbalance/reprogramming due to TME-specific targeted TGF β inhibition, leading to CD8⁺ immune cell infiltration into the immune-decentralized TME. Tumors administered with hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM showed
5 reduced p-Smad2 levels and increased CD8⁺ T cell abundance in the tumor center and tumor border, regardless of tumor epithelium and stroma.

[401]

[402] Example 5. Production of bispecific Fc fusion proteins including anti-EDB-FN antibody and TGF β Trap and confirmation of binding capability

10 [403] 5-1. Production and purification of bispecific Fc fusion proteins including anti-EDB-FN antibody and TGF β Trap

[404] The bispecific Fc fusion protein including Anti-EDB-FN antibody and TGF β Trap designed by the method of Example 1-3 was purified by the method of Example 1-4, and as a result, as described in a and b of FIG. 13, it was confirmed that the
15 monomer purity was 90% or more and the endotoxin level was 0.1 EU/mL or less. In this example, among the bispecific Fc fusion proteins including Anti-EDB-FN antibody and TGF β Trap of Table 2,

[405] Control-hIgG: RecombiMAb human IgG4 (S228P) isotype control, anti-hen egg lysozyme Cat: CP147, Bio X cell, USA)

20 [406] Anti-EDB-FN-hIgG (SEQ ID NO: 108): Anti-EDB-FN antibody (L19) hIgG4 form

[407] Soluble TGF β Trap-hFc (SEQ ID NO: 105): TGF β RII ectodomain domain is fused to the N-terminus of hIgG4-Fc.

[408] Anti-EDB-FN-hIgG-TGF β Trap (L19-hIgG-TGF β Trap, SEQ ID NO: 29):
25 TGF β RII ectodomain is fused to the C terminus of L19 hIgG4-Fc.

[409] Anti-EDB-FN-hIgG-LC-TGF β Trap (L19-hIgG-LC-TGF β Trap, SEQ ID NO: 30): The TGF β RII ectodomain is fused to the C-terminus of L19 hIgG4-light chain (LC).

[410] Anti-EDB-FN scFv-hFc-TGF β Trap (L19 scFv-hFc-TGF β Trap, SEQ ID NO: 32): The L19 scFv antibody fragment is fused to the N-terminus of the heavy chain Fc region, and the TGF β RII ectodomain is fused to the C-terminus of the heavy chain Fc region.

[411] Anti-EDB-FN scFv-TGF β Trap-hFc (L19 scFv-TGF β Trap-hFc, SEQ ID NO: 34): The L19 scFv antibody fragment and the TGF β RII ectodomain are fused at the N-terminus of IgG4-Fc.

[412] hFc-Anti-EDB-FN-TGF β Trap (SEQ ID NO: 33): The TGF β RII ectodomain is fused to the C terminus of the L19 scFv antibody fragment and linked beneath the heavy chain Fc region.

[413] hFc-TGF β Trap-Anti-EDB-FN (SEQ ID NO: 35): L19 scFv antibody fragment is fused to the C-terminus of the TGF β RII ectodomain and Trap below the heavy chain Fc region.

[414] Anti-EDB-FN scFv-TGF β Trap (SEQ ID NO: 36): L19 scFv antibody fragment is fused onto TGF β Trap without FC fusion

[415] Proteins such as these were produced and purified.

[416] 5-2. Evaluation of binding capability of dual-specific Fc fusion proteins including anti-EDB-FN antibody and TGF β Trap

[417] The binding capability of the fusion proteins purified in Example 5-1 was evaluated by the method of Example 1-5, and as described in FIG. 14, it was confirmed that consistent co-binding affinity was shown for both targets at a binding level in the range of 10 to 86 ng/mL despite structural changes at different positions of L19

antibody or L19 scfv and TGF β Trap. Compared to the Fc fusion form, Anti-EDB-FN scFv-TGF β Trap without Fc fusion was confirmed to have a lower simultaneous binding affinity for both targets.

[418]

5 **[419] Example 6. Confirmation of therapeutic effects of bispecific Fc fusion proteins including anti-EDB-FN antibody and TGF β Trap**

[420] 6-1. Tumor growth rate analysis

[421] An advanced breast tumor model that is transplanted orthotopically in the breast pad is clinically more relevant than a subcutaneous tumor model due to the
10 establishment of organ-specific tumors. The method of Examples 1-6 was used to establish highly metastatic cell lines, and as a result, as described in FIG. 15A, anti-EDB-FN scfv-TGF β Trap-hFc and hFc-anti-EDB-FN scFv-TGF β Trap exhibited statistically significant potent antitumor activity compared to other experimental groups in an orthotopic transplantation mouse advanced breast cancer model.

15 [422] In addition, as described in FIG. 15B, the tumor volumes of the anti-EDB-FN-hIgG, anti-EDB-FN-hIgG-TGF β Trap, anti-EDB-FN scfv-TGF β Trap-hFc, or hFc-anti-EDB-FN scFv-TGF β Trap administration groups on day 25 were confirmed to have decreased by about 1%, about 6%, about 57%, and about 51%, respectively, compared to the tumor volume of the isotype control group (control hIgG). In addition,
20 it was confirmed that only anti-EDB-FN scfv-TGF β Trap-hFc and hFc-anti-EDB-FN scFv-TGF β Trap administration inhibited tumor growth by more than 50% compared to anti-EDB-FN-hIgG-TGF β Trap, which was designed with a standard conceptual structure.

[423] 6-2. Analysis of lung metastasis rates

[424] As a result of analyzing the lung metastasis rates of breast cancer orthotopic transplantation models administered with a dual heavy-chain fusion protein consisting of L19 antibody-TGF β Trap or L19 scfv-TGF β Trap based on the binding between L19 antibody or L19 scfv of EDB FN by the method of Example 1-6, as described in

5 FIG. 16, on day 35, the lung metastasis rates of the anti-EDB-FN-hIgG, anti-EDB-FN-hIgG-TGF β Trap, anti-EDB-FN scfv-TGF β Trap-hFc, or hFc-anti-EDB-FN scFv-TGF β Trap administration groups were confirmed to have decreased by an average of 0%, 0%, 80%, and 60%, respectively, compared to the isotype control group (control hIgG).

10 [425] 6-3. Survival rate analysis

[426] The survival rate of breast cancer orthotopic transplantation models administered with dual heavy chain fusion proteins consisting of L19 antibody-TGF β Trap or L19 scfv-TGF β Trap based on the binding between L19 antibody or L19 scfv of EDB FN by the method of Example 1-6 was analyzed. As a result, as described in

15 FIG. 17, compared to the isotype control group and anti-EDB-FN-hIgG, anti-EDB-FN scFv-hFc-TGF β Trap designed as a standard bispecific molecule did not improve the survival rate at all up to 35 days, whereas the groups administered with anti-EDB-FN hIgG-TGF β Trap-hFc and hFc-anti-EDB-FN scFv-TGF β Trap showed statistically significant increases of 60% and 60%, respectively.

20 [427] The commonality between the therapeutic effects of the two substances, hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM, and anti-EDB-FN hIgG-TGF β Trap-hFc and hFc-anti-EDB-FN scFv-TGF β Trap, is determined to be that they have the structural characteristic of directly linking the EDB-FN target and the TGF β trap, reflecting the three-dimensional structure targeting the tumor ECM and the activity of

25 TGF β trapping.

[428]

[429] Example 7. Confirmation of therapeutic effects of pancreatic cancer orthotopic transplantation models by administration of bispecific Fc fusion protein consisting of FEBM-, L19 antibody-TGF β Trap or L19 scfv-TGF β Trap

5 [430] 7-1. Tumor growth rate analysis of bispecific Fc fusion protein consisting of FEBM-TGF β Trap

[431] It is known that the pancreatic cancer model, which is advanced Panc02 immune desert tumors orthotopically transplanted into the tail of the pancreas, is more clinically relevant than the subcutaneous tumor model due to the establishment of
10 organ-specific tumors. Accordingly, hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap, or hFc-TGF β Trap-FEBM and isotype control Fc were administered to pancreatic cancer orthotopic transplantation mice produced by the method of Example 1-7, and the therapeutic effects were confirmed by the method described in Example 1-7.

15 [432] As a result, as described in FIG. 18A, hFc-soluble TGF β Trap showed no statistical significance in reducing tumor weight compared to the isotype control group, whereas hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM showed statistically significant potent antitumor activity compared to other experimental groups in the orthotopic mouse advanced pancreatic cancer model in which the highest level of EDB
20 expression was observed among all solid tumors.

[433] As described in FIG. 18B, on day 63 after cell transplantation, the tumor weights of the hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap, or hFc-TGF β Trap-FEBM administration groups were confirmed to have decreased by about 22.1%, about 38.7%, about 90.1%, and about 76.4%, respectively,
25 compared to the isotype control group (control hIgG). It was confirmed that the

administration of hFc-FEBM-TGFβ Trap or hFc-TGFβ Trap-FEBM inhibited tumor growth to a greater extent (>85% and >70%, respectively) than hFc-soluble TGFβ Trap.

[434] In addition, as described in FIG. 18C, on day 63, the metastasis rates including liver, pleura, and diaphragm in the hFc-soluble TGFβ Trap, FEBM-hFc-TGFβ Trap, hFc-FEBM-TGFβ Trap, or hFc-TGFβ Trap-FEBM administration groups were confirmed to have decreased by an average of 20%, 40%, 100%, and 100%, respectively, compared to the homologous control group.

[435] 7-2. Confirmation of decreased phosphorylation of Smad2 and increased cytotoxic immune CD8⁺ T cells by dual heavy-chain fusion protein consisting of

FEBM-TGFβ Trap

[436] As a result of confirming the phosphorylation of Smad2 and the expression level of CD8 by the method of Example 1-7, as described in FIG. 19A, it was confirmed that the phosphorylation of Smad2 in the tumors of the hFc-soluble TGFβ Trap, FEBM-hFc-TGFβ Trap, hFc-FEBM-TGFβ Trap, or hFc-TGFβ Trap-FEBM administration groups was reduced by about 17.2%, about 33%, about 84.5%, and about 72.8%, respectively, compared to the isotype control group. In particular, it was confirmed that the FEBM-hFc-TGFβ Trap, hFc-FEBM-TGFβ Trap and hFc-TGFβ Trap-FEBM administration groups suppressed the p-smad2 level to a greater extent (>80% and >65%, respectively) than the hFc-soluble TGFβ Trap. Although hFc-soluble TGFβ Trap, characterized by systemic exposure, failed to sustain Smad2 phosphorylation in tumor tissues of mice sacrificed 2 days after the last administration, the FEBM fusion structure was determined to be more effective in inhibiting Smad2 phosphorylation via TGFβ Trap due to its tumor specificity and higher persistence in the tumor microenvironment.

[437] In addition, as described in FIG. 19B, the expression of CD8, which is a memory marker effective in killing cancer cells in the hFc-soluble TGF β Trap, FEBM-hFc-TGF β Trap, hFc-FEBM-TGF β Trap, or hFc-TGF β Trap-FEBM administration groups, was confirmed to increase about 3.4-fold, about 7.7-fold, about 25.4-fold, and
5 about 16.1-fold, respectively, compared to the isotype control group (Control-Fc). In particular, the tumor-specific CD8⁺ T cells in the hFc-FEBM-TGF β Trap and hFc-TGF β Trap-FEBM administration groups were confirmed to increase about 7.4-fold and about 4.7-fold, respectively, compared to the hFc-soluble TGF β Trap.

[438] Tumors treated with hFc-FEBM-TGF β Trap or hFc-TGF β Trap-FEBM
10 showed reduced p-Smad2 levels and an abundance of CD8⁺ T cells in the tumor center and tumor border, regardless of tumor epithelium and stroma.

[439] 7-3. Tumor growth rate analysis of bispecific Fc fusion proteins including anti-EDB-FN antibody and TGF β Trap

[440] 30 mg/kg anti-EDB-FN-hIgG, anti-EDB-FN-hIgG-TGF β Trap, or anti-EDB-FN scFv-TGF β Trap-hFc and isotype control Fc were administered to pancreatic cancer orthotopic transplantation mice produced by the method of Example 1-7, and
15 the therapeutic effect was confirmed by the method described in Example 1-7.

[441] As a result, as described in FIG. 18B, the anti-EDB-FN scfv-TGF β Trap-hFc administration group, which has L19 scfv in the form of an antibody platform in
20 addition to FEBM, a polypeptide that binds to EDB-FN of the tumor ECM, was confirmed to exhibit statistically significant potent antitumor activity compared to other experimental groups in the orthotopic transplanted mouse advanced pancreatic cancer model, and as described in FIG. 18E, on day 63 after cell transplantation, the tumor weights of the anti-EDB-FN-hIgG, anti-EDB-FN-hIgG-TGF β Trap, or anti-
25 EDB-FN scfv-TGF β Trap-hFc administration groups were confirmed to have

decreased by about 9.2%, about 31.2%, and about 60.4%, respectively, compared to the isotype control group. Compared to anti-EDB-FN-hIgG-TGF β Trap, which was designed with a standard conceptual structure, the administration of anti-EDB-FN scfv-TGF β Trap-hFc alone inhibited tumor growth by more than 50%.

5 [442] In addition, as described in FIG. 18F, on day 63, the metastasis rates including liver, pleura, and diaphragm in the anti-EDB-FN-hIgG, anti-EDB-FN-hIgG-TGF β Trap, or anti-EDB-FN scfv-TGF β Trap-hFc administration groups were confirmed to have decreased by an average of 20%, 40%, and 80%, respectively, compared to the homologous control group.

10 [443]

[444] **Example 8. Therapeutic effect of administration of bispecific Fc fusion proteins consisting of FEBM-, L19 antibody-TGF β Trap or L19 scfv-TGF β Trap in tumor growth model after orthotopic transplantation or lung metastasis of melanoma**

15 [445] B16-F10 is a tumor model that is cytokine-deficient and MHC-I suppressed, and is invisible to the immune system and therefore does not significantly infiltrate immune cells. Therefore, despite its moderately high mutation burden, this tumor is often classified as an immunologically cold tumor. B16-F10 is a malignant melanoma cell whose most notable characteristic is its strong metastatic potential to spread
20 throughout the body. Since the lung metastasis model through the tail vein is known to represent the clinical response, the metastatic melanoma tumor model prepared by the method of Example 1-8 was administered with Control hFc, hFc-soluble TGF β Trap, hFc-FEBM-TGF β Trap, hFc-TGF β Trap-FEBM, hFc-FEBM-anti-TGF β 1 scfv, Anti-EDB-FN-TGF β Trap-hFc, and hFc-anti-EDB-FN scfv-anti-TGF β 1 scfv, and the
25 tumor growth was analyzed.

[446] Since melanoma can arise from any organ with melanocytes, primary tumors can also arise in the lungs, and lung metastases are the most common site of distant metastatic disease in malignant melanoma. Therefore, it is important to observe whether tumor growth can be suppressed in the lungs, which are the most common
5 clinical therapeutic site for melanoma. After confirming that cells metastasized to the lungs were settled on day 5 after transplantation, tumor growth was evaluated.

[447]

[448] As a result, as described in FIG. 20A, despite relatively low expression of fibronectin EDB compared to other solid cancers, the administration of well-structured
10 hFc-FEBM-TGF β Trap, hFc-TGF β Trap-FEBM, hFc-FEBM-anti-TGF β 1 scfv, Anti-EDB-FN-TGF β Trap-hFc, or hFc-anti-EDB-FN scfv-anti-TGF β 1 scfv for ECM fused TGF β trapping was confirmed to exhibit potent anticancer and anti-metastatic activities in the metastatic B16F10 melanoma model after 10 days.

[449] In addition, as described in FIG. 20B, on day 25 after tumor transplantation,
15 the average luminescence activity within the tumors of the hFc-soluble TGF β Trap (lane 2), hFc-FEBM-TGF β Trap (lane 3), hFc-TGF β Trap-FEBM (lane 4), hFc-FEBM-anti-TGF β 1 scfv (lane 5), Anti-EDB-FN-TGF β Trap-hFc (lane 6), and hFc-anti-EDB-FN scfv-anti-TGF β 1 scfv (lane 7) administration groups was confirmed to have decreased by about 28.5%, about 96.1%, about 88.8%, about 72.2%, about 76.9%,
20 and about 81.7%, respectively, compared to the luminescence activity of the isotype control group (Control-Fc). hFc-FEBM-TGF β Trap (lane 3), hFc-TGF β Trap-FEBM (lane 4), hFc-FEBM-anti-TGF β 1 scfv (lane 5), Anti-EDB-FN-TGF β Trap-hFc (lane 6) and hFc-anti-EDB-FN scfv-anti-TGF β 1 scfv (lane 7) groups showed statistically significant tumor growth inhibition to a greater extent (>94%, >84%, >60.6%, >67.2%,
25 and >74.1%, respectively) than hFc-soluble TGF β Trap (lane 2).

[450] That is, although the mRNA expression of EDB-FN1 in tumors of B16-F10 transplanted mice was higher than that in normal tissues, it was the lowest level compared to other solid tumors, and the optimal structural combination for EDB-FN targeting/TGF β Trapping was confirmed to have sufficient effectiveness.

5 [451]

[452] Example 9. Confirmation of cancer therapeutic effect by administration of triple-specific Fc fusion protein consisting of FEBM or L19 scfv, TGF β Trap and PD-1 antibody or scFv

[453] Most solid tumor patients with high-frequency microsatellite instability-H
10 (MSI-H) have the opportunity to receive combination therapy with anti-PD-1 antibodies, and for cancers with low or no defects in gene replication, numerous trials and thousands of clinical trials are underway related to the fusion of anti-PD-1 antibodies or scfv. Therefore, in the present invention, the tumor suppression efficacy of the fusion of anti-PD-1 antibodies or scfv to the optimal structure targeting EDB-
15 FN/TGF β was compared with that of the existing clinical trial Anti-PD1 / TGF β Trap in metastatic breast cancer and pancreatic cancer models.

[454] 9-1. Construction and production of triple-specific fusion proteins

[455] The construction and production of a triple fusion protein consisting of FEBM-, L19 scfv structure for EDB-FN targeting and TGF β Trap for inhibiting TGF β
20 signaling based on anti-PD-1 antibody or anti-PD-1 scfv were performed by the methods of Examples 1-3 and 1-4, and among the fusion proteins in Table 2, Anti-PD-1-hIgG-FEBM-TGF β Trap (SEQ ID NO: 57) and Anti-EDB-FN scfv-TGF β Trap-hFc-anti-PD1 scfv (SEQ ID NO: 77) fusion proteins were constructed and produced.

[456] In addition, control hIgG (RecombiMAB human IgG4(S228P) isotype control,
25 BioXcell, USA) was used as a control, Anti-PD-1 (SEQ ID NO: 109) was constructed

and produced, and Bs anti-PD-1 / TGF β Trap (Bintrafusp alfa, Cat. No.: HY-P99480, MedChemExpress, China), which is in clinical trial, was used in later examples.

[457] 9-2. Analysis of tumor growth rates in breast cancer orthotopic transplantation model

5 [458] A breast cancer orthotopic transplantation model was produced by the method of Example 1-6, and then, 15 mg/kg of Anti-PD-1 hIgG4, Bs anti-PD-1-hIgG4/TGF β Trap, anti-PD-1-hIgG-FEBM-TGF β Trap and anti-EDB-FN scFv-TGF β Trap-hFc-anti-PD-1 scFv and control-hFc were administered, and the tumor growth rate was analyzed by the method of Example 1-6.

10 [459] As a result, as described in FIG. 21, on day 25, the tumor volumes of the anti-PD-1 hIgG4, Bs anti-PD-1-hIgG4/TGF β Trap, anti-PD-1-hIgG-FEBM-TGF β Trap, and anti-EDB-FN scfv-TGF β Trap-hFc-anti-PD-1 scfv administration groups were confirmed to have decreased by about 2.2%, about 3.1%, about 88%, and about 75.5%, respectively, compared to the tumor volume of the isotype control group (control
15 hIgG).

[460] 9-3. Analysis of tumor growth rates in pancreatic cancer orthotopic transplantation model

[461] A pancreatic cancer orthotopic transplantation model was created by the method of Example 1-7, and then, 20 mg/kg of Anti-PD-1 hIgG4, Bs anti-PD-1-
20 hIgG4/TGF β Trap, anti-PD-1-hIgG-FEBM-TGF β Trap and anti-EDB-FN scFv-TGF β Trap-hFc-anti-PD-1 scFv and control-hFc were administered, and the tumor growth rates were analyzed by the method of Example 1-7.

[462] As a result, as described in FIG. 21B, on day 57, the tumor volumes of the anti-PD-1 hIgG4, Bs anti-PD-1-hIgG4/TGF β Trap, anti-PD-1-hIgG-FEBM-TGF β
25 Trap, and anti-EDB-FN scfv-TGF β Trap-hFc-anti-PD-1 scfv administration groups

were confirmed to have decreased by about 16.0%, about 20.1%, about 89.2%, and about 74.8%, respectively, compared to the tumor volume of the isotype control group (control hIgG).

[463] That is, the administration of anti-PD-1 alone and soluble-TGF β Trap dual
5 fusion substances in breast cancer and pancreatic cancer models did not observe statistically significant antitumor activity compared to the isotype control group (control hIgG), but the administration of anti-PD-1-hIgG-FEBM-TGF β Trap and anti-EDB-FN scfv-TGF β Trap-hFc-anti-PD-1 scfv proteins was confirmed to exhibit potent antitumor effects, and it was confirmed that the anti-PD-1 fused triple protein
10 under the dual structure of linked EDB-FN targeting/TGF β Trap structure exhibited potent anticancer efficacy in cancer types that did not respond to anti-PD-1 alone or in combination.

[464]

[465] While the specific parts of the present invention have been described in detail
15 above, it will be apparent to those skilled in the art that such specific descriptions are merely preferred embodiments and that the scope of the present invention is not limited thereby. Accordingly, the actual scope of the present invention will be defined by the appended claims and their equivalents.

[466]

20 **INDUSTRIAL APPLICABILITY**

[467] The fusion protein according to the present invention secures a local effect rather than a systemic effect by directly localizing TGF β inhibition within the cancer microenvironment by fixing TGF β , which is important for the antitumor immune response, to the extracellular matrix, thereby exhibiting excellent anticancer effects in
25 all cancer types, and can be advantageously used for the prevention or treatment of

cancer by exhibiting excellent anticancer effects even in pancreatic cancer, where general TGF β inhibitors do not work due to the rigidity of the extracellular matrix.

[468]

SEQUENCE LIST

- 5 [469] An electronic file is attached.

CLAIMS

[Claim 1]

A fusion protein, comprising:

(a) a polypeptide that specifically binds to extr domain B of fibronectin
5 (EDB-FN); and

(b) a polypeptide that specifically binds to transforming growth factor β
(TGF β).

[Claim 2]

10 The fusion protein of claim 1, wherein the fusion protein further comprises (c)
an antibody constant region.

[Claim 3]

The fusion protein of claim 2, wherein the fusion protein further comprises (d)
15 a polypeptide that specifically binds to a tumor antigen.

[Claim 4]

The fusion protein of claim 3, wherein the tumor antigen is any one or more
selected from the group consisting of PD-1, PD-L1, CTLA-4, CD80, CD86 and VEGF.

20

[Claim 5]

The fusion protein of claim 3, wherein the fusion protein further comprises a
polypeptide that specifically binds to PD-1.

[Claim 6]

The fusion protein of claim 2, wherein the fusion protein comprises, from the N-terminus to the C-terminus, one or more structures selected from the group consisting of:

5 (i) a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to TGF β ;

(ii) a polypeptide that specifically binds to TGF β ; and a polypeptide that specifically binds to EDB-FN;

10 (iii) one or more polypeptides that specifically bind to EDB-FN; an antibody constant region; and one or more polypeptides that specifically bind to TGF β ;

(iv) one or more polypeptides that specifically bind to TGF β ; an antibody constant region; and one or more polypeptides that specifically bind to EDB-FN;

(v) one or more polypeptides that specifically bind to EDB-FN; one or more polypeptides that specifically bind to TGF β ; and an antibody constant region;

15 (vi) one or more polypeptides that specifically bind to EDB-FN; one or more polypeptides that specifically bind to TGF β ; an antibody constant region; and one or more polypeptides that specifically bind to EDB-FN;

(vii) one or more polypeptides that specifically bind to TGF β ; one or more polypeptides that specifically bind to EDB-FN; an antibody constant region; and one
20 or more polypeptides that specifically bind to EDB-FN;

(viii) an antibody constant region; one or more polypeptides that specifically bind to EDB-FN; and one or more polypeptides that specifically bind to TGF β ;

(ix) an antibody constant region; one or more polypeptides that specifically bind to TGF β ; and one or more polypeptides that specifically bind to EDB-FN; and

25 (x) one or more polypeptides that specifically bind to EDB-FN; an antibody

constant region; one or more polypeptides that specifically bind to TGF β ; and one or more polypeptides that specifically bind to EDB-FN.

[Claim 7]

5 The fusion protein of claim 6, wherein the fusion protein comprises, from the N-terminus to the C-terminus, a structure selected from the group consisting of:

(7-1) an antibody constant region; a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to TGF β ; and

(7-2) an antibody constant region; a polypeptide that specifically binds to
10 TGF β ; and a polypeptide that specifically binds to EDB-FN.

[Claim 8]

The fusion protein of claim 5, wherein the fusion protein comprises, from the N-terminus to the C-terminus, one or more structures selected from the group
15 consisting of:

(A) a polypeptide that specifically binds to PD-1; a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to TGF β ;

(B) a polypeptide that specifically binds to PD-1; a polypeptide that specifically binds to TGF β ; and a polypeptide that specifically binds to EDB-FN;

20 (C) a polypeptide that specifically binds to TGF β ; a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to PD-1;

(D) a polypeptide that specifically binds to PD-1; an antibody constant region; a polypeptide that specifically binds to EDB-FN; and a polypeptide that specifically binds to TGF β ;

25 (E) a polypeptide that specifically binds to PD-1; an antibody constant region;

a polypeptide that specifically binds to TGF β ; and a polypeptide that specifically binds to EDB-FN;

(F) a polypeptide that specifically binds to TGF β ; a polypeptide that specifically binds to EDB-FN; a polypeptide that specifically binds to PD-1; and an
5 antibody constant region; and

(G) a polypeptide that specifically binds to EDB-FN; a polypeptide that specifically binds to TGF β ; a polypeptide that specifically binds to PD-1; and an antibody constant region.

10 [Claim 9]

The fusion protein of claim 1, wherein the polypeptide that specifically binds to EDB-FN is a fibronectin EDB binding motif (FEBM), an antibody that specifically binds to EDB-FN or a fragment thereof.

15 [Claim 10]

The fusion protein of claim 1, wherein the polypeptide that specifically binds to TGF β is a TGF β receptor type 2 ectodomain (TGF β RII ectodomain, TGF β Trap), an antibody that specifically binds to TGF β or a fragment thereof.

20 [Claim 11]

The fusion protein of claim 2, wherein the antibody constant region is a heavy-chain constant region or a light-chain constant region.

[Claim 12]

25 The fusion protein of claim 5, wherein the polypeptide that specifically binds

to PD-1 is an antibody that specifically binds to PD-1 or a fragment thereof.

[Claim 13]

The fusion protein of claim 9, wherein the polypeptide that specifically binds
5 to EDB-FN is any one or more of polypeptides represented by amino acid sequences
of SEQ ID NOs: 1 to 4.

[Claim 14]

The fusion protein of claim 10, wherein the polypeptide that specifically binds
10 to TGF β is any one or more of polypeptides represented by amino acid sequences of
SEQ ID NOs: 5 to 8.

[Claim 15]

The fusion protein of claim 11, wherein the antibody constant region is any
15 one or more of polypeptides represented by amino acid sequences of SEQ ID NOs: 12
to 15.

[Claim 16]

The fusion protein of claim 12, wherein the polypeptide that specifically binds
20 to PD-1 is any one or more of polypeptides represented by amino acid sequences of
SEQ ID NOs: 9 to 11.

[Claim 17]

The fusion protein of claim 6, wherein the fusion protein comprises, from the
25 N-terminus to the C-terminus, any one or more polypeptides selected from the group

consisting of:

(4-1) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 12 and a polypeptide of SEQ ID NO: 1;

(4-2) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; and a
5 polypeptide of SEQ ID NO: 12;

(4-3) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 12;

(4-4) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 5;

10 (4-5) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5;

(4-6) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

(4-7) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; a
15 polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 12;

(4-8) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

(4-9) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; and a polypeptide of
20 SEQ ID NO: 1;

(4-10) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

(4-11) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; and
25 a polypeptide of SEQ ID NO: 13;

- (4-12) a polypeptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 1; and
a polypeptide of SEQ ID NO: 5;
- (4-13) a polypeptide of SEQ ID NO: 2; a polypeptide of SEQ ID NO: 14; and
a polypeptide of SEQ ID NO: 5;
- 5 (4-14) a polypeptide of SEQ ID NO: 3; a polypeptide of SEQ ID NO: 15; a
polypeptide of SEQ ID NO: 5;
- (4-15) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 2; and
a polypeptide of SEQ ID NO: 13;
- (4-16) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 12; and
10 a polypeptide of SEQ ID NO: 5;
- (4-17) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 4; and
a polypeptide of SEQ ID NO: 5;
- (4-18) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 5; and
a polypeptide of SEQ ID NO: 12;
- 15 (4-19) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 5; and
a polypeptide of SEQ ID NO: 4;
- (4-20) a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;
- (4-21) a polypeptide of SEQ ID NO: 6; a polypeptide of SEQ ID NO: 14; and
a polypeptide of SEQ ID NO: 1;
- 20 (4-22) a polypeptide of SEQ ID NO: 7; a polypeptide of SEQ ID NO: 15; and
a polypeptide of SEQ ID NO: 1;
- (4-23) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 6; and
a polypeptide of SEQ ID NO: 14;
- (4-24) a polypeptide of SEQ ID NO: 2; a polypeptide of SEQ ID NO: 14; and
25 a polypeptide of SEQ ID NO: 5;

- (4-25) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 8; and
a polypeptide of SEQ ID NO: 12;
- (4-26) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and
a polypeptide of SEQ ID NO: 8;
- 5 (4-27) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 8; and
a polypeptide of SEQ ID NO: 1;
- (4-28) a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;
- (4-29) a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;
- (4-30) a polypeptide of SEQ ID NO: 2; a polypeptide of SEQ ID NO: 14; and
10 a polypeptide of SEQ ID NO: 8;
- (4-31) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 14; and
a polypeptide of SEQ ID NO: 4;
- (4-32) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 2; and
a polypeptide of SEQ ID NO: 14;
- 15 (4-33) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 6; and
a polypeptide of SEQ ID NO: 14;
- (4-34) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 8; and
a polypeptide of SEQ ID NO: 12;
- (4-35) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 4; and
20 a polypeptide of SEQ ID NO: 12;
- (4-36) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 4; and
a polypeptide of SEQ ID NO: 8;
- (4-37) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 8; and
a polypeptide of SEQ ID NO: 4;
- 25 (4-38) a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;

and

(4-39) a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4.

[Claim 18]

5 The fusion protein of claim 6, wherein the fusion protein comprises, from the N-terminus to the C-terminus:

(4-5) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5; or

(4-6) a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 5; and
10 a polypeptide of SEQ ID NO: 1.

[Claim 19]

The fusion protein of claim 8, wherein the fusion protein comprises, from the N-terminus to the C-terminus, any one or more polypeptides selected from the group
15 consisting of:

(5-1) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5;

(5-2) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

20 (5-3) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5;

(5-4) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

(5-5) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; a
25 polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;

(5-6) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;

(5-7) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 5;

5 (5-8) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

(5-9) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 11;

(5-10) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 1; and
10 a polypeptide of SEQ ID NO: 5;

(5-11) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 1;

(5-12) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 11;

15 (5-13) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;

(5-14) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 4;

(5-15) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a
20 polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;

(5-16) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 4;

(5-17) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;

25 (5-18) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 4; a

polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;

(5-19) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;

(5-20) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a
5 polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 4;

(5-21) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 11;

(5-22) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 5;

10 (5-23) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 5; and a polypeptide of SEQ ID NO: 4;

(5-24) a polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 11; and a polypeptide of SEQ ID NO: 4;

(5-25) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a
15 polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;

(5-26) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;

(5-27) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;

20 (5-28) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;

(5-29) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;

(5-30) a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 8; a
25 polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;

(5-31) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;

(5-32) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 1;

5 (5-33) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 1; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 11;

(5-34) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 8;

(5-35) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 8; and
10 a polypeptide of SEQ ID NO: 1;

(5-36) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 1; and a polypeptide of SEQ ID NO: 11;

(5-37) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;

15 (5-38) a polypeptide of SEQ ID NO: 9; a polypeptide of SEQ ID NO: 14; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4;

(5-39) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;

(5-40) a polypeptide of SEQ ID NO: 10; a polypeptide of SEQ ID NO: 15; a
20 polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4;

(5-41) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;

(5-42) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 9; and a polypeptide of SEQ ID NO: 14;

25 (5-43) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a

polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;

(5-44) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 12; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4;

(5-45) a polypeptide of SEQ ID NO: 4; a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 12; and a polypeptide of SEQ ID NO: 11;

(5-46) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 8;

(5-47) a polypeptide of SEQ ID NO: 11; a polypeptide of SEQ ID NO: 8; and a polypeptide of SEQ ID NO: 4; and

(5-48) a polypeptide of SEQ ID NO: 8; a polypeptide of SEQ ID NO: 4; and a polypeptide of SEQ ID NO: 11.

[Claim 20]

The fusion protein of claim 17, wherein the fusion protein is any one selected from the group consisting of SEQ ID NOs: 16 to 56.

[Claim 21]

The fusion protein of claim 18, wherein the fusion protein is represented by an amino acid sequence of SEQ ID NO: 20, or represented by an amino acid sequence of SEQ ID NO: 21.

[Claim 22]

The fusion protein of claim 19, wherein the fusion protein is any one selected from the group consisting of SEQ ID NOs: 57 to 104.

[Claim 23]

A nucleic acid, encoding the fusion protein according to any one of claims 1 to 22.

5 [Claim 24]

A recombinant expression vector, comprising the nucleic acid of claim 23.

[Claim 25]

A host cell, transfected with the recombinant expression vector of claim 24.

10

[Claim 26]

A method for producing a fusion protein, comprising:

culturing the host cell of claim 25 to produce a fusion protein; and isolating and purifying the produced fusion protein.

15

[Claim 27]

A composition for preventing or treating cancer, comprising the fusion protein according to any one of claims 1 to 22.