

ARTIFICIAL IMMUNE RECEPTORS

Background

Regulatory T cells (Tregs) are a pivotal T cell population with various functions in the body. Tregs foster tolerance against self-antigens, allergens and commensals, thereby limiting self-reactivity of immune cells and excessive inflammation. Mutations in the Treg master transcription factor Foxp3 lead to lethal multiorgan autoinflammation, both in mice and humans (Nat Genet, 2001. 27(1): 18-20; Nat Genet, 2001. 27(1): 68-73). In the last years, it became evident that Tregs exert additional functions in safeguarding tissue homeostasis and tissue regeneration (Immunology, 2020. 161(1): 4-17). On basis of these facts, Tregs can be considered as promising living drugs for autoimmune disorders and clinical trials have already proven the safety and efficacy of Treg-based cellular therapies (Front Immunol, 2019. 10: 43; Science, 2018. 362(6411): 154-155).

Innovative concepts applying the Chimeric Antigen Receptor (CAR) technology to Tregs are now the next step to enhance the potency of adoptive Treg cell therapy (Nat Rev Drug Discov, 2019. 18(10): 749-769.). Preclinical studies have already demonstrated the superiority of engineered Tregs with a CAR guided (auto-) antigen-specificity over Tregs with only a natural polyclonal TCR repertoire in diminishing alloimmune reactions in graft versus host disease (GvHD) and graft rejection after transplantation (Sci Transl Med, 2020. 12(557); Am J Transplant, 2020. 20(6): 1562-1573; J Clin Invest, 2016. 126(4): 1413-24; Am J Transplant, 2017. 17(4): 917-930; Am J Transplant, 2017. 17(4): p. 931-943). CAR-Tregs were also effectively used for treatment of asthma, hemophilia A, Type 1 diabetes, experimental autoimmune encephalitis (EAE) and inflammatory bowel disease (IBD) in preclinical models (J Neuroinflammation, 2012. 9: 112; J Immunol Methods, 2021. 488: 112931; Mol Ther, 2014. 22(5): 1018-28; Front Immunol, 2017. 8: p. 1125; J Autoimmun, 2019. 103: p. 102289).

However, in human autoimmune diseases the implicated autoantigens, which could serve as potential targets for CAR-Tregs, are in many cases not known, or multiple organs and tissues are affected without a uniform antigen. In contrast, the mediators of inflammation show a high redundancy in many inflammatory diseases, as well as a functional importance for the development of such diseases. Especially, cytokines of the Tumor-Necrosis-Factor (TNF) family are involved in many different inflammatory and autoimmune diseases, and therapeutic intervention of TNF receptor (TNFR) activation is an important treatment option for several inflammatory diseases (Trends Immunol, 2012. 33(3): 144-52). These considerations directed us to develop a new concept for engineered Treg cell therapy and to generate artificial immune receptors (AIRs) that target these inflammatory mediators instead of tissue specific antigens.

We focused on targets of the TNF family and chose receptors of the ligands LIGHT and $\text{L}\alpha 1\beta 2$, $\text{TNF}\alpha$ and TL1A, as these cytokines have pleiotropic roles in numerous autoimmune disease. Preclinical disease models and patient data indicate that LIGHT and $\text{L}\alpha 1\beta 2$ signaling through corresponding receptors HVEM and LTBR enhance pathology in IBD, autoimmune hepatitis, asthma, rheumatoid arthritis, multiple sclerosis and GvHD (reviewed in Immunol Rev, 2008. 223: 202-20.). A Role for TL1A and its receptor DR3 has been described in a variety of inflammatory diseases. Studies with blocking antibodies or TL1A- and DR3-deficient mice demonstrated a critical involvement for this receptor-ligand system in induction and maintenance of chronic inflammation in IBD, arthritis and EAE. Moreover, patient data and genome wide association studies point to a fundamental impact of TL1A-DR3 in human disease (reviewed in FEBS Lett, 2017. 591(17): 2543-2555.). The most prominent member of the TNF family, $\text{TNF}\alpha$ itself, has been studied extensively since its discovery in 1984. Its pro-inflammatory function was manifested in multiple diseases, among them ankylosing spondylitis, IBD, rheumatoid arthritis, psoriasis, systemic lupus erythematosus and juvenile idiopathic arthritis (reviewed in Crit Rev Immunol, 2019. 39(6): 439-479).

AIRs as a new synthetic tool for cellular therapy allow Tregs to sense these environmental signals and translate these into a Treg TCR-like activating program, enabling Treg cells to fulfill their suppressive and tissue protective functions, independent of a specific TCR- or CAR- antigen and independent of endogenous TCR-MHC restriction.

Certain constructs are known in the art that share certain features with the constructs of the present invention. WO2021/051195 for examples discloses constructs that are devoid of a CD3zeta TCR signaling domain. The additional CD3zeta TCR signaling domain however provides several advantages which are beyond the common functionality of a CD3 T cell signaling domain. Due to the presence of the CD3zeta domain, the AIRs of the present invention exert their function directly at the main signal of the signaling cascade. As shown herein, AIRs constructs without the CD3zeta domain are not functional. The constructs of WO2021/051195 are only able to modulate an ancillary signal, and not a TCR-like activation of the cell.

Summary of the invention

The present disclosure provides novel artificial immune receptors capable of activating regulatory T cells into a Treg TCR-like activating program. This program is independent of a specific TCR- or a CAR- antigen and also independent of endogenous TCR-MHC restriction.

Disclosed herein are artificial immune receptors, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily,
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

The general concept is exemplified utilizing the extracellular domains of four different tumor necrosis factor receptor superfamily members, namely TNFR2, DR3, LTBR and CD40. In certain embodiments the transmembrane domain is derived from the same protein as the extracellular domain.

The artificial immune receptors also comprise a cytoplasmic costimulatory signaling domain and a cytoplasmic T cell receptor signaling domain. Any commonly known costimulatory signaling domain and T cell receptor signaling domain can be used in the context of the AIRs of the present disclosure. Exemplified is the cytoplasmic costimulatory signaling domain of CD28 and the cytoplasmic T cell receptor signaling domain from CD3 zeta.

Particularly preferred artificial immune receptors comprising an extracellular domain and a transmembrane domain of TNFR2, a cytoplasmic costimulatory signaling domain from CD28 and said cytoplasmic T cell receptor signaling domain from CD3 zeta. Yet other particularly preferred artificial immune receptors comprising an extracellular domain and a transmembrane domain of DR3, a cytoplasmic costimulatory signaling domain from CD28 and said cytoplasmic T cell receptor signaling domain from CD3 zeta. Yet other particularly preferred artificial immune receptors comprising an extracellular domain and a transmembrane domain of LTBR, a cytoplasmic costimulatory signaling domain from CD28 and said cytoplasmic T cell receptor signaling domain from CD3 zeta.

Also preferred are artificial immune receptors that comprise the amino acid sequences of SEQ ID No.: 39, SEQ ID No.: 35, and SEQ ID No.: 37.

Also preferred are artificial immune receptors that comprise the amino acid sequences of SEQ ID No.: 41, SEQ ID No.: 35, and SEQ ID No.: 37.

Also preferred are artificial immune receptors that comprise the amino acid sequences of SEQ ID No.: 33, SEQ ID No.: 35, and SEQ ID No.: 37.

Also preferred are artificial immune receptors that comprise the amino acid sequences of SEQ ID No.: 47, SEQ ID No.: 35, and SEQ ID No.: 37. The present disclosure also provides nucleic acids encoding aforementioned artificial immune receptor. The present disclosure also provides vectors comprising

said nucleic acids. The present disclosure also provides host cell comprising said nucleic acids, said vectors, as well as host cells expressing the artificial immune receptors of the present disclosure.

The present disclosure also provides the artificial immune receptor for use in medicine. In certain embodiments said use in medicine is the treatment of cancer or inflammation. In other embodiments said use in medicine is the treatment of autoimmunity. In yet other embodiments said use in medicine is the treatment of graft versus host disease or in solid organ transplantation.

Definitions

The term "**cell**" as used herein includes a single cell as well as a plurality of cells.

The term "**host cell**" as used herein refers to a cell comprising a nucleic acid and/or a vector. In the context of the artificial immune receptors of the present disclosure, the term host cell refers to a cell comprising a nucleic acid and/or a vector encoding for an AIR. Such host cell will express the AIR on the cell surface and is suitable to be used as medicine. Preferred host cells of the present invention are eukaryotic host cells, such as immune cells.

The term "**T cell**" as used herein refers to a type of lymphocyte that plays a central role in cell-mediated immunity. T cells, also referred to as T lymphocytes, can be distinguished from other lymphocytes, such as B cells and natural killer cells, by the presence of a T-cell receptor (TCR) on the cell surface. There are several subsets of T cells with distinct functions, including but not limited to, T helper cells, cytotoxic T cells, memory T cells, regulatory T cells and natural killer T cells. In some embodiments, the T cell is an engineered T cell.

The terms "**regulatory T cell**" or "**Treg**" as used herein refer to a subpopulation of T cells which modulate the immune system, maintain tolerance to self-antigens, and abrogate autoimmune diseases. These cells generally suppress or downregulate induction and proliferation of effector T cells. Treg cells are long-lived cells that suppress excessive or uncontrolled immune responses in vivo in a dominant and antigen-specific manner. Genetic mutations in the forkhead box protein 3 (FoxP3), a key transcription factor required for differentiation of Treg cells, lead to severe autoimmunity. Indeed, research in a variety of animal models has demonstrated that Tregs can be used to treat many autoimmune diseases such as type 1 diabetes, inflammatory bowel disease, systemic lupus erythematosus, multiple sclerosis (MS), rheumatoid arthritis, and auto-immune gastritis. Treg cell therapy can also be used in controlling alloimmune responses in the context of GVHD, as well as organ and cell transplantation.

The term "**engineered TCR**" or "**engineered T-cell receptor**" means any TCR that has been modified from its naturally-occurring form. An engineered TCR may have modifications to the alpha and/or beta chains, or the gamma and/or delta chains (including replacement of any of the aforementioned chains) that enable the TCR to recognize a specific antigen (for example, a neoantigen). The engineered TCR may have modifications to any CD3 subunit (for example, CD3a, as in the case of TRuC receptors), including the addition of an antigen recognition domain (e.g., an antibody, an scFv, a DARPin). The engineered TCR may have an antigen recognition domain (e.g., an antibody, an scFv, a DARPin) joined to a transmembrane domain of the alpha and/or beta chains, or the gamma and/or delta chains.

The terms "**polynucleotide**" and/or "**nucleic acid sequence**" and/or "**nucleic acid**" as used herein refer to a sequence of nucleoside or nucleotide monomers consisting of bases, sugars and intersugar (backbone) linkages. The term includes DNA and RNA and can be either double stranded or single stranded, and represents the sense or antisense strand. The term also includes modified or substituted sequences comprising non- naturally occurring monomers or portions thereof. The nucleic acid sequences of the present application may be deoxyribonucleic acid sequences (DNA) or ribonucleic acid sequences (RNA) and may include naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil. The sequences may also contain modified bases. Examples of such modified bases include aza and deaza adenine, guanine, cytosine, thymidine and uracil; and xanthine and hypoxanthine. The nucleic acids of the present disclosure may be isolated from biological organisms, formed by laboratory methods of genetic recombination or obtained by chemical synthesis or other known protocols for creating nucleic acids.

The terms "**isolated polynucleotide**" or "**isolated nucleic acid sequence**" as used herein refer to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or other chemicals when chemically synthesized.

The terms "**recombinant nucleic acid**" or "**engineered nucleic acid**" as used herein refer to a nucleic acid or polynucleotide that is not found in a biological organism. For example, recombinant nucleic acids may be formed by laboratory methods of genetic recombination (such as molecular cloning) to create sequences that would not otherwise be found in nature. Recombinant nucleic acids may also be created by chemical synthesis or other known protocols for creating nucleic acids. Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the present application herein described for which they are suitable as would be understood by a person skilled in the art.

The term "**polypeptide**" or "**protein**" as used herein describes a chain of amino acids. A polypeptide or protein of this disclosure can be a peptide, which usually describes a chain of amino acids of from

two to about 30 amino acids. The term protein as used herein also describes a chain of amino acids having more than 30 amino acids and can be a fragment or domain of a protein or a full length protein. Furthermore, as used herein, the term protein can refer to a linear chain of amino acids or it can refer to a chain of amino acids that has been processed and folded into a functional protein. It is understood, however, that 30 is an arbitrary number with regard to distinguishing peptides and proteins and the terms can be used interchangeably for a chain of amino acids. The proteins of the present disclosure can be obtained by isolation and purification of the proteins from cells where they are produced naturally, by enzymatic (e.g., proteolytic) cleavage, and/or recombinantly by expression of nucleic acid encoding the proteins or fragments of this disclosure. The proteins and/or fragments of this disclosure can also be obtained by chemical synthesis or other known protocols for producing proteins and fragments.

The term "**isolated polypeptide**" refers to a polypeptide substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized.

The term "**vector**" as used herein refers to a polynucleotide that can be used to deliver a nucleic acid to the inside of a cell. In one embodiment, a vector is an expression vector comprising expression control sequences (for example, a promoter) operatively linked to a nucleic acid to be expressed in a cell. Vectors known in the art include, but are not limited to, plasmids, phages, cosmids and viruses.

The terms "**recipient**", "**individual**", "**subject**", "**host**", and "**patient**", are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans.

As used herein, the terms "**treatment**," "**treating**," and the like, in some embodiments, refer to administering an agent, or carrying out a procedure, for the purposes of obtaining an effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of affecting a partial or complete cure for a disease and/or symptoms of the disease. The terms include treatment of a disease or disorder (e.g. inflammation) in a mammal, particularly in a human, and includes: (a) preventing the disease or a symptom of a disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it (e.g, including diseases that may be associated with or caused by a primary disease; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease. The treatment or amelioration of symptoms is based on one or more objective or subjective parameters; including the results of an examination by a physician. Accordingly, the term "treating" includes the administration of the compounds or agents of the present invention

to prevent, delay, alleviate, arrest or inhibit development of the symptoms or conditions associated with diseases (e.g. inflammation).

The term "**therapeutic effect**" refers to the reduction, elimination, or prevention of the disease, symptoms of the disease, or side effects of the disease in the subject.

The terms "**tumor necrosis factor receptor superfamily**", "**TNFR superfamily**" or "**TNFRSF**" as used herein refers the protein superfamily of cytokine receptors characterized by the ability to bind ligands of tumor necrosis factor super family members (TNFSF) via an extracellular domain. There are numerous members of the TNFR Superfamily, including TNFR1, TNFR2, Fas, DR4, DR5, DR3, DR6, EDAR, XEDAR, TROY, LTBR or NGFR. Other members includes lymphotoxin beta receptor (CD18), Ox40 (CD134), CD40 (TNFRSF5), decoy receptor 3 (TR6, M68), CD27 (S152, Tp55), CD30 (Ki-1, TNFR8), 4-1BB (CD137), decoy receptor 1 (TRAILR3), decoy receptor 2 (TRAILR4), RANK (CD265), osteoprotegerin (OCIF, TR1), TWEAK receptor (Fn14, CD266), TACI (IGAD2, CD267), BAFF receptor (CD268), BAFF, APRIL, herpesvirus entry mediator (HVEM, CD270), B cell maturation antigen (TNFRSF13A, CD269), and glucocorticoid-induced TNFR-related protein (GITR, CD357).

Tumor necrosis factor receptor 1 (NCBI Entrez Gene: 7132; also known as TNFR1, tumor necrosis factor receptor superfamily member 1A, TNFRSF1A or CD 120a) is a membrane-bound receptor that binds tumor necrosis factor-alpha (TNFα). TNFR1 activates the transcription factor NF-κB, mediates apoptosis, and functions as a regulator of inflammation.

Tumor necrosis factor receptor 2 (NCBI Entrez Gene: 7133; also known as TNFR2, tumor necrosis factor receptor superfamily member 1B, TNFRSF1B or CD120b9, is a membrane-bound receptor that binds tumor necrosis factor-alpha (TNFα).

The Fas receptor (NCBI Entrez Gene: 355, also known as Fas, FasR, apoptosis antigen 1, APO-1, APT, CD95, tumor necrosis factor receptor superfamily member 6 or TNFRSF6), is a protein that in humans is encoded by the FAS gene. Multiple splice variants of Fas have been identified, which are translated into seven isoforms of the protein. Apoptosis inducing Fas receptor is referred to as isoform 1 and is a type 1 transmembrane protein. Many of the other isoforms are rare haplotypes that are usually associated with a state of disease. Any suitable isoform of Fas is contemplated for use with the embodiments disclosed herein.

Death receptor 4 (NCBI Entrez Gene: 8797, also known as death domain 4, DR4, TRAIL receptor 1, TRAILR1, tumor necrosis factor receptor superfamily member 10A or TNFRSF 10A), is a cell surface receptor of the TNF-receptor superfamily that binds TRAIL and mediates apoptosis.

Death domain 5 (NCBI Entrez Gene: 8795, also known as death receptor 5, DR5, TRAIL receptor 2, TRAILR2, tumor necrosis factor receptor superfamily member 10B or TNFRSF10B) is a cell surface receptor of the TNF-receptor superfamily that binds TRAIL and mediates apoptosis.

Death domain 3 (NCBI Entrez Gene: 8718, also known as death receptor 3, DR3, tumor necrosis factor receptor superfamily member 25 or TNFRSF25) is a cell surface receptor of the tumor necrosis factor receptor superfamily which mediates apoptotic signaling and differentiation. Its only known TNFSF ligand is TNF-like protein 1A (TL1A).

Death domain 6 (NCBI Entrez Gene: 27242, also known as death receptor 6, DR6, tumor necrosis factor receptor superfamily member 21 or TNFRSF21), is a cell surface receptor of the tumor necrosis factor receptor superfamily which activates the JNK and NF- κ B pathways.

Ectodysplasin receptor A (NCBI Entrez Gene: 10913, also known as ectodermal dysplasia receptor, EDA-A1 or EDAR) is a member of the TNF-receptor superfamily. It plays a key role in the process of ectodermal differentiation.

Ectodysplasin A2 receptor (NCBI Entrez Gene: 60401, also known as XEDAR, EDAR2, EDA-A2 or Tumor necrosis factor receptor superfamily member 27) is a protein that in humans is encoded by the EDA2R gene. EDA-A1 and EDA-A2 are two isoforms of ectodysplasin that are encoded by the anhidrotic ectodermal dysplasia (EDA) gene.

TROY (NCBI Entrez Gene: 55504, also known as Tumor necrosis factor receptor superfamily member 19 or TNFRSF19) is a member of the TNF-receptor superfamily. This receptor is highly expressed during embryonic development. It has been shown to interact with TNF receptor associated factor (TRAF) family members, and to activate c-Jun N-terminal kinases (JNK) signaling pathway when overexpressed in cells. This receptor is capable of inducing apoptosis by a caspase-independent mechanism, and it is thought to play an essential role in embryonic development.

NGFR (NCBI Entrez Gene: 4804, also known as nerve growth factor receptor, TNFR superfamily member 16, TNFRSF16, LNGFR or p75 neurotrophin receptor) is a member of the tumor necrosis factor receptor (TNF receptor) superfamily. It is one of the two receptor types for the neurotrophins, a family of protein growth factors that stimulate neuronal cells to survive and differentiate.

LTBR (NCBI Entrez Gene: 4055, also known as lymphotoxin beta receptor, TNFRSF3, TNFCR, Tumor Necrosis Factor C Receptor or TNFR3) plays a role in signaling during the development of lymphoid and other organs, lipid metabolism, immune response, and programmed cell death.

CD40 (NCBI Entrez Gene: 958, also known as Bp50, Tumor Necrosis Factor Receptor Superfamily Member 5 or TNFRSF5) is a costimulatory protein found on antigen-presenting cells and is required for

their activation. CD40 binds CD154 (CD40L) on T_H cells, thereby activating antigen presenting cells and inducing a variety of downstream effects.

CD30 (NCBI Entrez Gene: 943, also known as D1S166E, Tumor Necrosis Factor Receptor Superfamily Member 8, TNFRSF8, CD30L Receptor or KI-1) is expressed by activated, but not by resting, T and B cells and interacts with TRAF2 and TRAF5.

BAFF (NCBI Entrez Gene: 10673, also known as TALL-1, Tumor Necrosis Factor Receptor Superfamily Member 13B, TNFRSF13B, THANK or CD257) is a ligand for various receptors. BAFF is expressed in B cell lineage cells, and acts as a B cell activator. It has been also shown to play an important role in the proliferation and differentiation of B cells.

APRIL (NCBI Entrez Gene: 8741, also known as CD256, Tumor Necrosis Factor Receptor Superfamily Member 13, TNFRSF13, TALL-2, TRDL-2 or ZNTF2) is recognized by the cell surface receptor TACI, and, together with its receptor, plays an important for B cell development.

The terms **"costimulatory molecule"** or **"costimulatory receptor"** as used herein refers to a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response in the T cell, such as, but not limited to, activation or proliferation. A costimulatory receptor may be expressed on cells other than T cells, such as NK cells or macrophages. A costimulatory molecule can be represented in the following protein families: TNF receptor proteins, Immunoglobulin like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), Toll-like receptors and NK cell receptors. Costimulatory molecules include, but are not limited to 4-1BB (CD137), BAFFR, OX40, CD27, CD28, CD40, ICOS, 2B4, GITR, HVEM, OX40, RELT, TACI, TROY, TWEAK, KIR receptors, TLR1 to TLR9 receptors, IL-2, IL-7 and IL-15 receptors.

The terms **"costimulatory signaling domain"** or **"costimulatory domain"** as used herein refer to the domain of a costimulatory molecule or costimulatory receptor responsible for mediating a costimulatory response by the T cell. The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment or derivative thereof.

The terms **"T cell receptor signaling domain"** or **"TCR signaling domain"** as used herein refers to cytoplasmic signaling sequence that acts in a stimulatory manner to induce immune effector functions. In some embodiments, the TCR signaling domain contains a signaling motif known as Immunoreceptor Tyrosine-based Activation Motif, or ITAM. In some embodiments, the primary intracellular signaling domain comprises a functional signaling domain of a protein selected from the group consisting of CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, common FcR gamma (FCER1G), FcR beta (Fc Epsilon Rib), CD79a, CD79b, Fcgamma RIIa, DAP10, and DAP12. A preferred TCR signaling domain is a TCR signaling

domain selected from CD3 zeta, CD3 gamma, CD3 delta and CD3 epsilon. A particularly preferred TCR signaling domain CD3 zeta, CD3 gamma, CD3 delta and CD3 epsilon.

The terms “**artificial immune receptor**” or “**AIR**” refer to the novel molecules of the present disclosure. AIRs comprise an extracellular domain of a member of the tumor necrosis factor receptor superfamily, a transmembrane domain, a cytoplasmic costimulatory signaling domain, and a cytoplasmic T cell receptor signaling domain.

The terms “**is**”, “**are**”, “**is derived from**” and “**are derived from**” in the context of a polypeptide or domain of a polypeptide refers to the amino acid sequence of said polypeptide or domain of a polypeptide and indicates that the amino acid sequence is either identical to the native version of said polypeptide or domain of a polypeptide, or a variant of said polypeptide or domain of a polypeptide which is functionally indistinguishable from the native version of said polypeptide or domain of a polypeptide.

The term “**P2A**” as used herein refers to a peptide sequence which is capable of self cleavage. P2A belongs to the family of 2A peptides, which all are capable of self cleavage. “A” peptides share a common core sequence motif. Any 2A peptide may be used instead of P2A. The amino acid sequence of P2A is shown in SEQ ID No. 13. Other commonly used 2A peptides are T2A, E2A and F2A.

The term “**CD90.1**” as used herein refers to an allelic isoform of mouse CD90. The amino acid sequence of mouse CD90.1 is shown in SEQ ID No. 15. CD90.1 is commonly used genetic marker.

The terms “**GVHD**”, “**GvHD**” and “**Graft versus Host Disease**” refers to a disease commonly associated with bone marrow transplants and stem cell transplants, characterized by inflammation. White blood cells of the donor’s immune system remain within the donated tissue or the donated cells (the graft) and are recognized by the recipient (the host) as non-self.

Figure legends

Figure 1 is a schematic representation of the AIR design. The construct on the top (A) shows an AIR according to the present disclosure. It consists of an extracellular binding domain of a Tumor-Necrosis-Factor (TNF) superfamily member, a transmembrane domain, and two intracellular domains – one co-stimulatory domain and one T cell receptor signaling domain. The AIR fusion proteins are linked via a P2A peptide cleavage site to modified CD90.1, which serves as reporter gene for transduction efficacy. P2A and CD90.1 are not part of the AIRs. These constructs are cloned into a Murine Stem Cell Leukemia Virus (MSCV), which serves as vector and mediates stable integration of genes of interest into T cell

DNA. Constructs designated as B, C, D and G are exemplary AIRs according to the present disclosure. Constructs E and F are control constructs. Construct E lacks an extracellular binding domain, construct F contains an extracellular anti-CD19 single chain antibody (scFv) instead of a binding domain of a Tumor-Necrosis-Factor (TNF) superfamily member.

Figure 2 depicts the mode of action of the AIRs according to the present disclosure. Binding of membrane bound TNF family members via AIRs leads to translation of the cytokine signal into a T cell receptor (TCR)-like signal. This initiates T cell activation independent of its endogenous TCR-MHC restriction in an environment/inflammation dependent manner.

Figure 3 demonstrates efficient transduction and expression of the AIRs according to the present disclosure. Murine regulatory T cells (Treg) were isolated from spleen and lymph nodes of C57/BL6 mice, sorted (naïve CD4+CD25+CD62L+ Treg or Foxp3+ reporter (hCD2+) positive) and expanded via anti-CD3 and anti-CD28 antibody coupled beads plus IL-2 (2000 U/ml) stimulation for 6 days. Purity of Treg cultures was determined with flow cytometry. Treg cultures contained about 90-99% Foxp3+ T cells. On day two after isolation Treg were transduced with AIR constructs or an anti-CD19 Chimeric Antigen Receptor (α -CD19 CAR) as control. Three days later transduction efficacy via CD90.1 staining and surface expression of AIRs was checked with flow cytometry.

Figure 4 demonstrates the surface expression of the AIRs according to the present disclosure. Panels A, B and C show the results with AIRs carrying the extracellular domain of LTBR, DR3 and TNFR2, respectively. In each panel the AIRs are compared to untransduced cells and to cells carrying an extracellular anti-CD19 single chain antibody (scFv).

Figure 5 depicts the scientific rationale for the experiment of Example 6. Nr4a1 is a well-known target gene of T-cell receptor signaling. It was investigated if the AIRs of the present disclosure can induce Nr4a1 upregulation in a similar way as TCR signaling. Treg from Nr4a1-eGFP reporter mice were used. As a control, Treg were stimulated with anti-CD3/CD28 antibody-coupled beads.

Figure 6 AIRs expressing Treg were stimulated in over night co-culture with HEK cells expressing murine Light (mLight), mTL1A or mTNF. As positive control Treg were stimulated with anti-CD3/CD28 antibody-coupled beads to mimic TCR signaling. Nr4a1-eGFP expression of AIR positive (CD90.1+) Treg is shown in the left, Nr4a1-eGFP expression of AIR negative (CD90.1-) Treg in the right panel.

Figure 7 demonstrates the expression of Nr4a1 upon administration of an AIR according to the present disclosure (exemplified with an AIR carrying the extracellular domain of LTBR). Tregs expressing an LTBR AIR according to the present disclosure or an irrelevant CAR carrying extracellularly an anti-CD19 single chain antibody (scFv) were co-cultured with murine EL4 cells, which are known to express

Light, in absence or presence of a mLight blocking LTBR-Ig fusion protein (50 ug/ml) and afterwards stained for intracellular Nr4a1 expression.

Figure 8 shows the pre-gating strategy for CD90.1+ cells of LTBR AIR's according to the present disclosure and an irrelevant CAR carrying extracellularly an anti-CD19 single chain antibody (scFv). Tregs expressing the constructs were rested for 24 h and then co-cultured for 18 h with HEK cells or HEK cells expressing mLight. Shown is the pregating for CD90.1+ cells.

Figure 9 shows the sorting of Tregs that were pregated as shown in Figure 8. Cells were sorted on CD4+CD25+CD90.1+ (left and middle) or CD4+CD25+CD90.1+Nr4a1+LAP+ (right).

Figure 10 depicts an RNA expression analysis differentially expressed transcripts after stimulation with LTBR AIR's according to the present disclosure (comparison of LTBR AIR Treg's stimulated with HEK-mLight vs. α -CD19 CAR Tregs stimulated with HEK-mLight). Cells were sorted as outlined in Figures 8 and 9 and in Example 7. Afterwards RNA was isolated and RNA sequencing was performed. Shown on the left is a summary of up and down regulated genes comparing α -CD19 CAR Treg stimulated with HEK-mLight vs. LTBR AIR Treg stimulated with HEK cells as control (endogenous mLight signaling) and LTBR AIR Treg stimulated with HEK-mLight vs. α -CD19 CAR Treg stimulated with HEK-mLight (specific LTBR AIR signaling). Shown on the right is a Volcano plot showing differentially expressed transcripts.

Figure 11 is analogous to Figure 10 with DR3 AIR's according to the present disclosure. DR3 AIR Tregs were stimulated with HEK cells (endogenous mTL1A signaling). DR3 AIR Treg were stimulated with HEK-mTL1A vs. α -CD19 CAR Treg stimulated with HEK-mTL1A (specific DR3 AIR signaling). Differentially expressed gene are shown on the left, a Volcano plot is shown on the right.

Figure 12 is also analogous to Figure 10 comparing α -CD19 CAR Treg stimulated with anti-CD3/CD28 beads vs. unstimulated Treg. Differentially expressed gene are shown on the left, a Volcano plot is shown on the right.

Figure 13 shows RNA expression data from LTBR AIR or control CAR expressing Treg for *Tnfrsf9*, *Tigit*, *Tgfb1*, *Cd69*, *Ccr8* and *Nr4a1* after 18 h co-culture with HEK +/-mLight (Deseq2, n = 3).

Figure 14 shows a representative flow cytometry analysis from LTBR AIR or control CAR expressing Treg after 18 h co-culture with HEK +/-mLight. Protein expression of CD137 and Tigit is shown in the upper, CD69 and LAP (membrane-bound Tgf β 1) in the lower panel. Data is representative for three independent experiments.

Figure 15 shows RNA expression data from DR3 AIR or control CAR expressing Treg for *Tnfrsf9*, *Tigit*, *Tgfb1* after 18 h co-culture with HEK +/-mLight (Deseq2, n = 3).

Figure 16 shows a representative flow cytometry analysis from DR3 AIR or control CAR expressing Treg after 18 h co-culture with HEK +/-mTL1A. Protein expression of CD137 and Tigit is shown in the upper, CD69 and LAP (Tgfβ1) in the lower panel. Data is representative for three independent experiments.

Figure 17 shows RNA expression data (upper left, Deseq2, n = 3) and protein expression (right) from LTBR AIR or control CAR expressing Treg for *Irf8* after 18 h co-culture with HEK +/-mLight. RNA expression data (lower left, Deseq2, n = 3) and protein expression (right) from DR3 AIR or control CAR expressing Treg for *Irf8* after 18 h co-culture with HEK +/-mTL1A.

Figure 18 demonstrates that the AIRs of the present disclosure mediate Treg activation and proliferation. LTBR AIR or control CAR expressing Treg were rested for 24 h and labeled with CFDA-SE proliferation dye. Engineered and labeled Treg were co-cultured with HEK +/- mLight for 72 h in presence of IL2 and afterwards analyzed with flow cytometry for proliferation. Representative dot plots are shown left. Summarized data from three experiments performed in three technical replicates are shown right (n=3).

Figure 19 shown a schematic overview of the (major MHC mismatch) Graft versus Host Diseases (GvHD) model. Briefly, FACS sorted Treg from Foxp3-hCD2 reporter mice were expanded and transduced with LTBR AIR or a truncated construct (lacking an extracellular binding domain, shown in Figure 1 a) as control. 2.5×10^5 engineered Treg were transplanted together with 2.5×10^6 bone marrow cells and 2.5×10^5 spleen cells from C57/BL6 mice into lethally irradiated Balb/c mice. As transplant control, one group received only bone marrow cells (BM) and as disease developing control, one group received bone marrow cell plus spleen cells. After transplantation, animals were monitored for 47 days.

Figure 20 shows the result of the quality control of the engineered Tregs. Tregs were stained for CD4, hCD2 (reporter for Foxp3) as well as CD90.1 and LTBR before transfer into mice.

Figure 21 shows a Kaplan-Meier curve showing survival of transplanted Balb/c mice treated with a LTBR AIR and respective control constructs. Graph contains data set from two independent experiments (log rank test, n=11-12).

Figure 22 shows the mean GvHD score per group, animals reaching a score of 40 had to be euthanized (dead animals kept the highest score over following time points for statistical analysis, two-way ANOVA).

Figure 23 shows on the left a representative FACS plot of a spleen of a surviving LTBR AIR Treg receiving animal. Shown on the right is the percentage of Foxp3+ Tregs in spleen of surviving mice at day47.

Figure 24 shows the analysis of Klr1+ Treg in spleens. Left: representative FACS plot of discriminated CD45.1+ (BM derived) and CD45.2+ (transferred AIR Treg) Treg cells. Expression of Klr1 (marker for tissue specific phenotype) and hCD2 (Foxp3 reporter) is shown. Middle: Klr1+ expression of engineered CD45.2+ or BM derived CD45.1+ Treg in surviving mice. Right: frequency of Klr1+ hCD2+ Treg in mice receiving LTBR AIR Treg (Mann-Whitney-U, survivors n=8 vs. dead n=4).

Figure 25 shows a representative FACS analysis of a digested colon of a surviving LTBR AIR Treg receiving animal. Shown is discrimination between CD45.1 and CD45.2 cells within the CD4+ population on the left. CD45.2 Treg are further analyzed for Klr1 and hCD2 expression on the right.

Figure 26 shows on the left the percentage of hCD2+ (indicating Foxp3+) cells among transferred engineered CD45.2+ cells demonstrating phenotypic stability over 47 day *in vivo*. Shown on the right is the cell count of transferred CD45.2 Treg in colon at day 47.

Figure 27 shows that human LTBR AIRs are efficiently transduced and express the AIR on the cell surface. Human T cells from healthy blood donors were sorted on the typical Treg protein markers (TCRb+CD4+CD25+CD127-CD45RA+) and expanded with anti-CD3/CD28 stimulation (TransACT, Miltenyi Biotec, Bergisch Gladbach, Germany) and IL-2 (500 U/ml) for 7 days. On day 2 after isolation Treg were transduced with the human LTBR AIR construct (using an amphotropic version of the retroviral expression system MSCV). 5 days later cultured Treg were stained for intracellular Foxp3+ to check for Treg purity and for CD90.1 as well as hLTBR to analyze transduction efficacy and surface expression of the AIR protein.

Figure 28 shows that human LTBR AIRs have the expected signaling capacity. Tregs expressing hLTBR AIR or irrelevant CAR (an anti-CEA, Carcinoembryonic Antigen) were co-cultured for 18 h with parental HEK cells or HEK cells expressing human Light protein on the surface. Expression of Ccr8, Glycoprotein A repetitions predominant (GARP), CD137 (4-1BB) and Tigit is shown in CD90.1+Foxp3+ Tregs.

Figure 29 shows on the top a LTBR "AIR" lacking the CD3zeta-chain. On the bottom, flow cytometry data are shown which confirm surface expression of the construct in Tregs.

Figure 30 shows a representative flow cytometry analysis of Tregs expressing a full length LTBR AIR with a LTBR "AIR" lacking the CD3zeta-chain after 18 h co-culture with HEK +/-mLight. Protein expression of CD137 and Tigit is shown in the upper, CD69 and LAP (membrane-bound Tgfβ1) in the lower panel.

Figure 31 shows the expression of TIGIT and CD137. CD40 AIR-expressing or a construct containing an anti-CD19 CAR expressing Treg cells were cocultured for 18 h with HEK cells or HEK cells expressing CD40L (CD154). Expression of TIGIT and CD137 was measured by flow cytometry.

Figure 32 summarizes three independent experiments as shown in Figure 31. Differences are statistically significant as calculated via a one-way Anova test.

Figure 33 shows the expression of LAP (TGFB1) and CD69. CD40 AIR-expressing or a construct containing an anti-CD19 CAR expressing Treg cells were cocultured for 18 h with HEK cells or HEK cells expressing CD40L (CD154). Expression of LAP (TGFB1) and CD69 was measured by flow cytometry.

Figure 34 summarizes three independent experiments as shown in Figure 33. Differences are statistically significant as calculated via a one-way Anova test.

Figure 35 shows that Treg cells from Nr4a1-eGFP reporter mice expressing a CD40 AIR trigger the expression of Nr4a1, an early response gene of TCR signaling.

Figure 36 shows a schematic representation of additional AIRs generated and tested in the present disclosure. Figure 36A shows a CD40 AIR with an ICOS costimulatory domain, Figure 36B a CD40 AIR with a 4-1BB costimulatory domain.

Figure 37 shows that CD40 AIRs with an ICOS or a 4-1BB costimulatory domain are efficiently expressed on the surface of regulatory T cells.

Figure 38 shows that all CD40 AIRs tested, i.e. AIRs with a CD28, an ICOS and a 4-1BB costimulatory domain, have the capacity to induce Treg cell activation.

Figure 39 shows the quality control of Treg cells prior to transplantation. Both construct, the CD40 AIR and the control construct, showed a high transduction efficiency, but only the cells transduced with the CD40 AIR exhibited binding to CD40 through its CD40 receptor extracellular domain.

Figure 40 shows a Kaplan-Meier curve showing survival of transplanted Balb/c mice treated with a CD40 AIR and respective control constructs. Graph contains data set from two independent experiments (log rank test, n=10-12).

Figure 41 demonstrates the importance of the CD3 for the constructs of the present invention. Only the full length LTBR AIR containing the CD3zeta domain, but not the other two constructs tested, is capable to induce Nr4a1.eGFP upregulation in response to the corresponding ligand, mLIGHT.

Embodiments of the invention

Artificial immune receptors

The artificial immune receptors disclosed herein are novel and superior tools that enable regulatory T cells to fulfill their suppressive and tissue protective functions, independent of a specific TCR- or CAR- antigen and independent of endogenous TCR- pMHC (peptide loaded MHC) restriction.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

In certain embodiments the present disclosure relates to an artificial immune receptor, consisting of

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said member of the tumor necrosis factor receptor superfamily is selected from TNFR1, TNFR2, Fas, DR4, DR5, DR3, DR6, EDAR, XEDAR, TROY, LTBR, NGFR, CD18, CD134, CD40, CD27, CD30, CD137, TRAILR3, TRAILR4, CD265, osteoprotegerin, CD266, TACI, BAFF, BAFF receptor, APRIL, CD270, CD269 and CD357.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said member of the tumor necrosis factor receptor superfamily is selected from TNFR2, DR3, LTBR and CD40.

In certain embodiments the present disclosure relates to an artificial immune receptor, consisting of

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said member of the tumor necrosis factor receptor superfamily is selected from TNFR1, TNFR2, Fas, DR4, DR5, DR3, DR6, EDAR, XEDAR, TROY, LTBR, NGFR, CD18, CD134, CD40, CD27, CD30, CD137, TRAILR3, TRAILR4, CD265, osteoprotegerin, CD266, TACI, BAFF, BAFF receptor, APRIL, CD270, CD269 and CD357.

In certain embodiments the present disclosure relates to an artificial immune receptor, consisting of

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said member of the tumor necrosis factor receptor superfamily is selected from TNFR2, DR3, LTBR and CD40.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain is from the same protein as the extracellular domain.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain and said extracellular domain are derived from the same protein.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from TNFR2.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor comprises SEQ ID No.: 39.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor comprises SEQ ID No.: 38.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor consists of SEQ ID No.: 38.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from DR3.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor comprises SEQ ID No.: 41.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor comprises SEQ ID No.: 40.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor consists of SEQ ID No.: 40.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from LTBR.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor comprises SEQ ID No.: 33.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor comprises SEQ ID No.: 31.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor consists of SEQ ID No.: 31.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from CD40.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor comprises SEQ ID No.: 46.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor comprises SEQ ID No.: 47.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein artificial immune receptor consists of SEQ ID No.: 47.

Costimulatory signaling domain

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

Wherein said cytoplasmic costimulatory signaling domain is selected from 4-1BB (CD137), BAFFR, OX40, CD27, CD28, ICOS, CD40, 2B4, GITR, HVEM, OX40, RELT, TACI, TROY, and TWEAK.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

Wherein said cytoplasmic costimulatory signaling domain is CD28.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

Wherein said cytoplasmic costimulatory signaling domain is derived from CD28.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,

- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

Wherein said cytoplasmic costimulatory signaling domain comprises SEQ ID No.: 35.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

Wherein said cytoplasmic costimulatory signaling domain consists of SEQ ID No.: 35.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain is from the same protein as the extracellular domain, and

wherein said cytoplasmic costimulatory signaling domain is selected from CD137, BAFFR, OX40, CD27, CD28, CD40, ICOS, 2B4, GITR, HVEM, OX40, RELT, TACI, TROY, and TWEAK.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain is from the same protein as the extracellular domain, and

wherein said cytoplasmic costimulatory signaling domain is CD28.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain is from the same protein as the extracellular domain, and

wherein said cytoplasmic costimulatory signaling domain comprises SEQ ID No.: 35.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain is from the same protein as the extracellular domain, and
wherein said cytoplasmic costimulatory signaling domain consists of SEQ ID No.: 35.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain and said extracellular domain are from TNFR2, and
wherein said cytoplasmic costimulatory signaling domain is CD28.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain and said extracellular domain are from DR3, and
wherein said cytoplasmic costimulatory signaling domain is CD28.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain and said extracellular domain are from LTBR, and
wherein said cytoplasmic costimulatory signaling domain is CD28.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain,

wherein said transmembrane domain and said extracellular domain are from CD40, and
wherein said cytoplasmic costimulatory signaling domain is CD28.

T cell receptor signaling domain

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said cytoplasmic T cell receptor signaling domain is selected from CD3 zeta, CD3 gamma and CD3 epsilon.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said cytoplasmic T cell receptor signaling domain is derived from CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said cytoplasmic T cell receptor signaling domain comprises SEQ ID No.: 37.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said cytoplasmic T cell receptor signaling domain consists of SEQ ID No.: 37.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said transmembrane domain is from the same protein as the extracellular domain, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from TNFR2, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from DR3, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and

- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from LTBR, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from CD40, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said transmembrane domain is from the same protein as the extracellular domain,

wherein said cytoplasmic costimulatory signaling domain is CD28, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

Preferred AIRs

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from TNFR2,

wherein said cytoplasmic costimulatory signaling domain is CD28, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from DR3,

wherein said cytoplasmic costimulatory signaling domain is CD28, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from LTBR,

wherein said cytoplasmic costimulatory signaling domain is CD28, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from CD40,

wherein said cytoplasmic costimulatory signaling domain is CD28, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from TNFR2 and comprise SEQ ID No.: 39,

wherein said cytoplasmic costimulatory signaling domain is CD28 and comprise SEQ ID No.: 35, and wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and comprise SEQ ID No.: 37.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from TNFR2 and consist of SEQ ID No.: 39,

wherein said cytoplasmic costimulatory signaling domain is CD28 and consists of SEQ ID No.: 35, and wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and consists of SEQ ID No.: 37.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor comprises SEQ ID No.: 38.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor consist of SEQ ID No.: 38.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from DR3 and consist of SEQ ID No.: 41,

wherein said cytoplasmic costimulatory signaling domain is CD28 and consists of SEQ ID No.: 35, and wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and consists of SEQ ID No.: 37.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor comprises SEQ ID No.: 40.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor consist of SEQ ID No.: 40.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from LTBR and consist of SEQ ID No.: 33,

wherein said cytoplasmic costimulatory signaling domain is CD28 and consists of SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and consists of SEQ ID No.: 37.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor comprises SEQ ID No.: 31.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor consist of SEQ ID No.: 31.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from CD40 and consist of SEQ ID No.: 46,

wherein said cytoplasmic costimulatory signaling domain is CD28 and consists of SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and consists of SEQ ID No.: 37.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor comprises SEQ ID No.: 47.

In certain embodiments the present disclosure relates to an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor consist of SEQ ID No.: 47.

Nucleic acids, vectors and host cells

The artificial immune receptors of the present disclosure are encoded by nucleic acids. Therefore, the in certain embodiments the present disclosure relates to nucleic acids encoding the artificial immune receptors.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;

- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from TNFR2 and comprise SEQ ID No.: 39,

wherein said cytoplasmic costimulatory signaling domain is CD28 and comprise SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and comprise SEQ ID No.: 37.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from TNFR2 and consist of SEQ ID No.: 39,

wherein said cytoplasmic costimulatory signaling domain is CD28 and consists of SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and consists of SEQ ID No.: 37.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor comprises SEQ ID No.: 38.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;

- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor consist of SEQ ID No.: 38.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from DR3 and comprise SEQ ID No.: 41,

wherein said cytoplasmic costimulatory signaling domain is CD28 and comprise SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and comprise SEQ ID No.: 37.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from DR3 and consist of SEQ ID No.: 41,

wherein said cytoplasmic costimulatory signaling domain is CD28 and consists of SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and consists of SEQ ID No.: 37.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor comprises SEQ ID No.: 40.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor consist of SEQ ID No.: 40.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from LTBR and comprise SEQ ID No.: 33,

wherein said cytoplasmic costimulatory signaling domain is CD28 and comprise SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and comprise SEQ ID No.: 37.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from LTBR and consist of SEQ ID No.: 33,

wherein said cytoplasmic costimulatory signaling domain is CD28 and consists of SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and consists of SEQ ID No.: 37.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor comprises SEQ ID No.: 31.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor consist of SEQ ID No.: 31.

In certain embodiments the present disclosure relates to a nucleic acid comprising SEQ ID No.: 30.

In certain embodiments the present disclosure relates to a nucleic acid consisting of SEQ ID No.: 30.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain,
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from CD40 and comprise SEQ ID No.: 46,

wherein said cytoplasmic costimulatory signaling domain is CD28 and comprise SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and comprise SEQ ID No.: 37.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from CD40 and consist of SEQ ID No.: 46,

wherein said cytoplasmic costimulatory signaling domain is CD28 and consists of SEQ ID No.: 35, and

wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta and consists of SEQ ID No.: 37.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,

- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor comprises SEQ ID No.: 47.

In certain embodiments the present disclosure relates to a nucleic acid encoding an artificial immune receptor, comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain, and

wherein said artificial immune receptor consist of SEQ ID No.: 47.

In certain embodiments the present disclosure relates to a nucleic acid encoding a protein comprising SEQ ID No.: 30.

In certain embodiments the present disclosure relates to a nucleic acid encoding a protein consisting of SEQ ID No.: 30.

In certain embodiments the present disclosure relates a vector comprising a nucleic acid encoding an artificial immune receptor of the present disclosure.

In certain embodiments the present disclosure relates a vector comprising a nucleic acid encoding an artificial immune receptor comprising

- a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily;
- b) a transmembrane domain,
- c) a cytoplasmic costimulatory signaling domain, and
- d) a cytoplasmic T cell receptor signaling domain.

In certain embodiments the present disclosure relates to a host cell comprising a nucleic acid or a vector encoding an artificial immune receptor according to the present disclosure.

In certain embodiments the present disclosure relates to a host cell expressing an artificial immune receptor of to the present disclosure.

In certain embodiments the present disclosure relates to a host cell expressing on the cell surface an artificial immune receptor of to the present disclosure.

In preferred embodiments, said host cell is a eukaryotic host cell. Therefore, in certain embodiments the present disclosure relates to a eukaryotic host cell comprising a nucleic acid or a vector encoding an artificial immune receptor according to the present disclosure. In other embodiments the present disclosure relates to a eukaryotic host cell expressing an artificial immune receptor of to the present

disclosure. In yet other embodiments the present disclosure relates to a eukaryotic host cell expressing on the cell surface an artificial immune receptor of to the present disclosure.

Therapeutic use

The artificial immune receptors of the present disclosure can be used in therapeutically for the prevention and treatment of diseases and disorders.

In certain embodiments the present disclosure relates to artificial immune receptors as disclosed herein for use in medicine. In certain embodiments the present disclosure relates to artificial immune receptors as disclosed herein for use in the prevention of treatment of a disease or disorder. In certain embodiments said disease or disorder is an inflammatory disorder or cancer. In preferred embodiments said disease or disorder is an autoimmune disorder. In certain embodiments said disease or disorder is the treatment of graft versus host disease or the use in solid organ transplantation.

Certain specific embodiments

1. An artificial immune receptor, comprising
 - a) an extracellular domain of a member of the tumor necrosis factor receptor superfamily,
 - b) a transmembrane domain,
 - c) a cytoplasmic costimulatory signaling domain, and
 - d) a cytoplasmic T cell receptor signaling domain.
2. An artificial immune receptor according to claim 1, wherein said member of the tumor necrosis factor receptor superfamily is selected from TNFR1, TNFR2, Fas, DR4, DR5, DR3, DR6, EDAR, XEDAR, TROY, LTBR, NGFR, CD18, CD134, CD40, CD27, CD30, CD137, TRAILR3, TRAILR4, CD265, osteoprotegerin, CD266, TACI, BAFF, BAFF receptor, APRIL, CD270, CD269 and CD357.
3. An artificial immune receptor according to claim 1 or 2, wherein said member of the tumor necrosis factor receptor superfamily is selected from TNFR2, DR3, LTBR and CD40.
4. An artificial immune receptor according to any one of claims 1-3, wherein said transmembrane domain is from the same protein as the extracellular domain.

5. An artificial immune receptor according to any one of claims 1-4, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from TNFR2.
6. The artificial immune receptor according to claim 5, wherein artificial immune receptor comprises SEQ ID No.: 39.
7. The artificial immune receptor according to claim 5 or 6, wherein artificial immune receptor comprises SEQ ID No.: 38.
8. An artificial immune receptor according to any one of claims 1-4, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from DR3.
9. The artificial immune receptor according to claim 8, wherein artificial immune receptor comprises SEQ ID No.: 41.
10. The artificial immune receptor according to claim 8 or 9, wherein artificial immune receptor comprises SEQ ID No.: 40.
11. An artificial immune receptor according to any one of claims 1-4, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from LTBR.
12. The artificial immune receptor according to claim 11, wherein artificial immune receptor comprises SEQ ID No.: 33.
13. The artificial immune receptor according to claim 11 or 12, wherein artificial immune receptor comprises SEQ ID No.: 31.
14. An artificial immune receptor according to any one of claims 1-4, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are derived from CD40.
15. The artificial immune receptor according to claim 11, wherein artificial immune receptor comprises SEQ ID No.: 46.
16. The artificial immune receptor according to claim 11 or 12, wherein artificial immune receptor comprises SEQ ID No.: 47.
17. An artificial immune receptor according to any one of claims 1-16, wherein said cytoplasmic costimulatory signaling domain is selected from 4-1BB (CD137), BAFFR, OX40, CD27, CD28, CD40,

2B4, GITR, HVEM, OX40, RELT, TACI, TROY, TWEAK, KIR receptors, TLR1 to TLR9 receptors, IL-2, IL-7 and IL-15 receptors.

18. An artificial immune receptor according to any one of claims 1-17, wherein said cytoplasmic costimulatory signaling domain is CD28.
19. An artificial immune receptor according to any one of claims 1-18, wherein said cytoplasmic costimulatory signaling domain comprises SEQ ID No.: 35.
20. An artificial immune receptor according to any one of claims 1-19, wherein said cytoplasmic T cell receptor signaling domain is selected from CD3 zeta, CD3 gamma and CD3 epsilon.
21. An artificial immune receptor according to any one of claims 1-20, wherein said cytoplasmic T cell receptor signaling domain is CD3 zeta.
22. An artificial immune receptor according to any one of claims 1-21, wherein said cytoplasmic T cell receptor signaling domain comprises SEQ ID No.: 37.
23. An artificial immune receptor according to any one of claims 1-7 or 17-22, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from TNFR2, said cytoplasmic costimulatory signaling domain is CD28 and said cytoplasmic T cell receptor signaling domain is CD3 zeta.
24. An artificial immune receptor according to any one of claims 1-7 or 17-23, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from TNFR2 and comprise SEQ ID No.: 39, said cytoplasmic costimulatory signaling domain is CD28 and comprise SEQ ID No.: 35, and said cytoplasmic T cell receptor signaling domain is CD3 zeta and comprise SEQ ID No.: 37.
25. An artificial immune receptor according to any one of claims 1-4, 8-10 or 17-22, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from DR3, said cytoplasmic costimulatory signaling domain is CD28 and said cytoplasmic T cell receptor signaling domain is CD3 zeta.
26. An artificial immune receptor according to any one of claims 1-4, 8-10, 17-22 or 25, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from DR3 and comprise SEQ ID No.: 41, said cytoplasmic costimulatory signaling domain is CD28 and comprise SEQ ID No.: 35, and said cytoplasmic T cell receptor signaling domain is CD3 zeta and comprise SEQ ID No.: 37.

27. An artificial immune receptor according to any one of claims 1-4, 11-13 or 17-22, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from LTBR, said cytoplasmic costimulatory signaling domain is CD28 and said cytoplasmic T cell receptor signaling domain is CD3 zeta.
28. An artificial immune receptor according to any one of claims 1-4, 11-13, 17-22 or 27, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from LTBR and comprise SEQ ID No.: 33, said cytoplasmic costimulatory signaling domain is CD28 and comprise SEQ ID No.: 35, and said cytoplasmic T cell receptor signaling domain is CD3 zeta and comprise SEQ ID No.: 37.
29. An artificial immune receptor according to any one of claims 1-4, 14-16 or 17-22, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from CD40, said cytoplasmic costimulatory signaling domain is CD28 and said cytoplasmic T cell receptor signaling domain is CD3 zeta.
30. An artificial immune receptor according to any one of claims 1-4, 14-16, 17-22 or 29, wherein said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from CD40 and comprise SEQ ID No.: 46, said cytoplasmic costimulatory signaling domain is CD28 and comprise SEQ ID No.: 35, and said cytoplasmic T cell receptor signaling domain is CD3 zeta and comprise SEQ ID No.: 37.
31. A nucleic acid encoding an artificial immune receptor according to any one of claims 1-30.
32. A vector comprising a nucleic acid according to claim 31.
33. A host cell comprising a nucleic acid according to claim 31 or a vector according to claim 32, or expressing an artificial immune receptor according to any one of claims 1-30.
34. An artificial immune receptor according to any one of claims 1-30 or a host cell of claim 33 for use in medicine.
35. An artificial immune receptor according to claims 34, wherein said use is use in the treatment of an inflammatory disorder.
36. An artificial immune receptor according to claims 35, wherein inflammatory disorder is graft versus host disease.

Examples

Example 1: Materials

Ethics statement

Peripheral blood mononuclear cells for T-cell enrichment were isolated from leukocyte reduction chambers from healthy donors donating thrombocytes. Collection of immune cells from those donors was performed in compliance with the Helsinki Declaration after ethical approval by the local ethical committee and signed informed consent.

Peripheral blood mononuclear cell isolation and pre-enrichment of blood lymphocytes

To isolate T cells from human blood, leukocyte reduction chambers (provided by Transfusion Medicine, University Clinics Regensburg) were used. Leukocytes were first diluted with PBS and the resulting blood and PBS mixture was split into four fractions and underlayered with an equal amount of Pancoll (PAN Biotech, Aidenbach, Germany). Samples were centrifuged at 1.000xg for 20 minutes at RT, with acceleration set to four and brake to zero. The PBMC layer was isolated and washed twice by centrifugation steps. Cells were pre-enriched with biotinylated anti-human CD25-PE (clone 2A3; BD Biosciences RRID: AB_341011) followed by column-based magnetic separation with anti-PE ultrapure microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) following manufacturer's protocol.

Mice

Female BALB/c mice, C57BL/6 Nr4a1-eGFP mice (JAX stock #016617, C57BL/6-Tg(Nr4a1-eGFP/cre)820Khog/J) and C57BL/6 CD45.1+ mice (JAX stock #002014, B6.SJL-PtprcaPepcb/BoyCr) were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA) or the Jackson Laboratory (Bar Harbor, ME, USA). B6N.129(Cg)-Foxp3tm3Ayr mice (Foxp3.IRES-DTR/GFP) were bred to C57BL/6 CD45.1+ mice and served as bone marrow donors for GvHD experiments.

C57BL/6 Foxp3-hCD2 (Foxp3tm1(CD2/CD52)Shori) mice were a gift from S. Hori (P Natl Acad Sci USA. 2009;106(6):1903-8). Mice were held under specific pathogen-free conditions and studies were approved by the Committee on Ethics of Animal Experiments at the Bavarian Government.

Cell lines

Phoenix-Eco and Phoenix-Ampho cell lines were purchased from ATCC (Cat#CRL-3214 and #CRL-3213). These are second-generation retrovirus producer cell lines for the generation of ecotropic and amphotropic retroviruses. EL4 lymphoblast cell line was purchased from ATCC (Cat#TIB-39).

Additional resources are listed in Tables 1, 2, 3 and 4.

Table 1: Antibodies

Reagent or Resource	Source	Identifier
Antibodies		
BV711 anti-mouse CD4	Biolegend	RRID: AB_2562099
PerCP/Cy5.5 anti-mouse CD4	Biolegend	RRID: AB_100540
BV605 anti-mouse CD4	Biolegend	RRID: AB_100548
BUV395 anti-mouse CD4	BD Biosciences	RRID: AB_563790
BUV737 anti-mouse CD4	BD Biosciences	RRID: AB_564933
FITC anti-mouse CD19	Biolegend	RRID: AB_115506
BV421 anti-mouse CD25	Biolegend	RRID: AB_102034
BV711 anti-mouse CD25	Biolegend	RRID: AB_102049
PerCP/Cy5.5 anti-mouse CD25	Biolegend	RRID: AB_102030
APC anti-mouse CD90.1	Biolegend	RRID: AB_202526
BV421 anti-mouse CD90.1	Biolegend	RRID: AB_202529
PerCp/Cy5.5 anti-mouse CD90.1	Biolegend	RRID: AB_202516
PE anti-mouse LT β R	Biolegend	RRID: AB_134403
PE/Cy7 anti-mouse LT β R	Biolegend	RRID: AB_134409
FITC anti-mouse CD69	BD Biosciences	RRID: AB_553236
PE anti-mouse TNFR2	Biolegend	RRID: AB_113406
PE anti-mouse DR3	Biolegend	RRID: AB_144405
BV421 anti-mouse LAP	Biolegend	RRID: AB_141408
PE/Cy7 anti-mouse Tigit	Biolegend	RRID: AB_142108
PE anti-mouse CD137	Biolegend	RRID: AB_106106
APC/Cy7 anti-mouse CD8a	Biolegend	RRID: AB_100714
BV605 anti-mouse CD8a	Biolegend	RRID: AB_100738
AF700 anti-mouse CD8a	Biolegend	RRID: AB_557959

BV785 anti-mouse Klrg1	Biolegend	RRID: AB_138429
BV711 anti-mouse TCR- β -chain	Biolegend	RRID: AB_109243
BUV737 anti-mouse CD45.1	BD Biosciences	RRID: AB_612811
BV605 anti-mouse CD45.1	Biolegend	RRID: AB_110738
BUV737 anti-mouse CD45.2	BD Biosciences	RRID: AB_612778
BUV496 anti-mouse H2Kb	BD Biosciences	RRID: AB_750257
PE/Cy7 anti mouse H2Kd	Biolegend	RRID: AB_116622
PE anti-mouse Nr4a1	eBioscience	RRID: AB_12-5965-80
PE anti-mouse Irf8	eBioscience	RRID: AB_12-9852-82
AF488 anti-mouse Foxp3	eBioscience	RRID: AB_53-5773-82
APC anti-mouse Foxp3	eBioscience	RRID: AB_17-5773-82
APC anti human CD2	Biolegend	RRID: AB_300213
PE anti-human CD2	Biolegend	RRID: AB_300207/300208
BV605 anti-human CD45RA	Biolegend	RRID: AB_304134
BV711 anti-human CD4	Biolegend	RRID: AB_317440
BUV737 anti-human CD4	BD Biosciences	RRID: AB_612749
PE anti-human CD25	BD Biosciences	RRID: AB_341011
PE/Cy7 anti-human TCR- β -chain	Biolegend	RRID: AB_306720
PerCP/Cy5.5 anti-human CD45RO	Biolegend	RRID: AB_304222
APC anti-human CD127	Biolegend	RRID: AB_351342
BUV395 anti-human CCR8	BD Biosciences	RRID: AB_747573
BV711 anti-human CD137	Biolegend	RRID: AB_309832
PE/Cy7 anti-human GARP	Biolegend	RRID: AB_352507
PE anti-human LTBR	Biolegend	RRID: AB_322008
AF647 anti-human Foxp3	Biolegend	RRID: AB_320114

Table 2: Chemicals, Plasmids and Recombinant Proteins

Reagent or Resource	Source	Identifier
Antibodies		
Recombinant human IL-2	Novartis	Proleukin S® 18 Mio U

TexMACS medium	Miltenyi Biotec	Cat#130-097-196
Proteinase K	Carl Roth	Cat#7528.5
HBSS	Gibco	Cat#14025092
PBS	Gibco	Cat#10010023
DPBS	Gibco	Cat#14190-094
DMEM	Gibco	Cat#41965
OptiMEM	ThermoFisherScientific	Cat#11058021
Bovine Serum Albumin	Sigma	Cat#A4503
Dnase I	Roche	Cat#11284932001
TransIT-293	MoBiTec	Cat#Mir2700
Pancoll	PAN biotech	#P04-601000
Non Essential Amino Acid Solution (100x)	PAN biotech	#P08-32100
β -Mercaptoethanol	PAN biotech	#P07-05100
L-Glutamin (100x)	PAN biotech	#P04-82050
HEPES Buffer 1M	PAN biotech	#P05-011000
Penicillin-Streptomycin (10,000 U/mL)	Gibco	#15140122
soluble mLTBR-Ig fusion protein	T.Hehlgers	
pCL-Eco Retrovirus Packaging Vector	Novus Biologicals	NBP2-29540
pCL-Ampho Retrovirus Packaging Vector	Novus Biologicals	NBP2-29541
pMSCV-IRES-Thy1.1 DEST	Addgene (gift from Anjana Rao)	Cat#17442

Table 3: Commercial Assays

Reagent or Resource	Source	Identifier
Antibodies		
Lamina Propria Dissociation Kit	Miltenyi Biotec	Cat#130-097-410
GentleMACS C tube	Miltenyi	Cat#130-096-334
Anti-CD2 microbeads, human	Miltenyi Biotec	Cat#130-091-114
Anti-PE microbeads, ultrapure	Miltenyi Biotec	Cat#130-105-639
LS column	Miltenyi Biotec	Cat#130-042-401

MS column	Miltenyi Biotec	Cat#130-042-201
ACK lysis buffer	Gibco	Cat#A1049201
Foxp3 / Transcription Factor Buffer Set	eBiosciences	Cat#00-5523-00
RNEasy Plus Mini Kit	Qiagen	Cat#74134
round bottom 96 well plate tc treated	Corning	Cat#3799
Mouse T cell activation and expansion kit	Miltenyi Biotec	Cat#130-093-627
T Cell TransAct™, human	Miltenyi Biotec	Cat#130-111-160
CellTrace™ CFSE Cell Proliferation Kit	Thermo Fisher / Life Technologies	Cat#V12883
Qiagen Rneasy Micro Kit	Qiagen	Cat#74004
High-Sensitivity RNA screentape	Agilent	Cat#5067-5579
SMART-seq Stranded Kit	Takara	Cat# 634444

Table 4: Software and algorithms

Software	Source	Identifier
Flowjo (V10.6.2)	BD Biosciences	https://www.flowjo.com/solutions/flowjo/downloads/
FACS Diva (V8.0.2)	BD Biosciences	http://www.bdbiosciences.com/en-us/instruments/research-instruments/research-software/flow-cytometry-acquisition/facsdiva-software
GraphPad Prism (V8.4.3)	GraphPad Software Inc	https://www.graphpad.com/
SnapGene (V1.4.1)	GSL Biotech LLC	https://www.snapgene.com/

Statistical analysis

Data were analyzed with Prism software or algorithm. Statistical details are indicated in the figure legend. For survival differences Kaplan–Meier analysis was performed and the log-rank test was used. P values < 0.05 were considered significant (*p < 0.05; **p < 0.01; ***p < 0.001).

Example 2: Methods

Digestion of murine tissues for flow cytometric analysis, FACS sorting of cells and transplantation

To isolate cells from colon tissue, colons were isolated, cleared of feces and prepared according to manufacturer's instructions with a lamina propria dissociation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and a gentleMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany, program 37C_mLPDK_1). More detailed protocols about T cell isolation from murine tissues are published (Eur J Immunol. 2019;49(10):1457-973.).

To isolate cells from blood, peripheral blood was collected and incubated in heparin buffer. Blood samples were centrifuged and red blood cells were lysed using ACK lysis buffer (ThermoFisher/GIBCO, Waltham, MA, USA, #A1049201).

To isolate cells from spleen and lymph nodes, tissues were harvested and mechanically disintegrated. Samples were centrifuged and red blood cells were lysed using ACK lysis buffer. Samples were pre-enriched with anti-human CD2 (RPA-2.10) microbeads (for Foxp3-hCD2 reporter mice) or anti-CD25 (PC61) antibody staining and anti-PE microbeads.

To isolate cells from bone marrow for transplantation, femurs were collected and femoral head and femoral medial and lateral epicondyle were removed. Bone shafts were flushed with PBS and bone marrow collected by centrifugation followed by red blood cell lysis.

Preparation of samples for flow cytometry

Single cells suspensions were prepared and pre-enriched as described previously (Eur J Immunol. 2019; 49(10):1457-973). Samples were stained either in 1.5 mL Eppendorf tubes or 96-well plates in FACS buffer (1%FCS in PBS). Surface staining was performed at 4°C for 20 minutes in 50-100 µL staining volume. Antibodies were used, if not indicated otherwise, as recommended by the manufacturer. Following antibodies were used for surface staining of murine samples: TCR-β-chain (H57-597), CD4 (RM4-5), CD8α (53-6.7), CD19 (6D5), CD25 (PC61), CD45.1 (A20), CD45.2 (104), CD90.1 (OX-7), CD120b (TR79-89), I-A/I-E/MHCII (M5/114.15.2), Klrg1 (2F1), Tigit (1G9), DR3 (4C12), CD62L (MEL-14), CD137 (17B5), CD69 (H1.2F3), LTBR (5G11), LAP (TW7-16B4), H2Kb (AF6-88.5), H2Kd (SF1-1.1), hCD2 (RPA-2.10).

The following antibodies were used for surface staining of human samples: CD4 (OKT4), CD25 (BC96, 2A3), CD127 (A019D5), CD45RO (UCHL1), CD45RA (HI100), CD137 (41BB), TCR- β chain (IP26), CCR8 (433H RUO), and GARP (7B11).

Intracellular staining was performed with the Foxp3 / Transcription Factor Buffer Set (Thermo Fisher/Biosciences, Waltham, MA, USA) according to manufacturer's protocol with the following adaptations: intracellular staining steps were performed for 60 minutes at room temperature. Antibodies for intracellular staining include Foxp3 (JFK-16S), Nr4a1 (12.14), Irf8 (V3GYWCH) for mouse and Foxp3 (206D) for human samples. Dead cells were excluded with a fixable live/dead dye (eBioscience Fixable Viability Dye eFluor780). All antibodies are listed in the resource table.

Flow cytometry and FACS sorting of T cells from blood, tissues and cell cultures

Cells were isolated, pre-enriched and stained as described previously (Eur J Immunol. 2019; 49(10): 1457-973). Afterwards, samples were filtered with a 40 μ M filter unit and acquired on a BD FACSymphony™, a BD FACSCelesta™ or a BD FACSFusion™ flow cytometer (all from Becton Dickinson, Franklin Lakes/NJ, USA). BD CS&T beads were used to validate machine functionality. Fluorescence spillover compensation was performed with lymphocytes stained with anti-CD4 antibodies in the respective colors. Flow cytometry data were analyzed using BD FlowJo™ (Version 10.6.2). Sorting was performed with a BD FACSAria™ or BD FACSFusion™ cell sorter with 70 μ m nozzle. Post-sort quality controls were performed as applicable. For murine Treg cultures, anti-CD25 enriched cells were sorted for naïve CD4+CD25+CD62L+ Treg (Leukemia 2020; 34(3):895-908). hCD2 pre-enriched cells were sorted for CD4+CD25+hCD2+. For cultivation, cells were sorted directly into cell culture medium. All procedures were performed in DNA low-bind tubes (Eppendorf, Hamburg, Germany, #0030108051) or 15ml tubes.

For bulk RNA sequencing 4 x 10⁴ – 8 x 10⁴ cells were sorted on live CD4+CD25+CD90.1+GFP(Nr4a1)+LAP+ or CD4+CD25+CD90.1 directly into 500 μ L RLT+ lysis buffer (Qiagen, Hilden, Germany, RNEasy Plus Micro Kit #74034). For human Treg cultures, CD25 pre-enriched cells from human blood were sorted on TCR- β -chain+CD4+CD25+CD45RA+ directly into TexMACS medium.

Murine and human Treg cell cultures

For murine Treg cultures, sorted Treg cells were seeded at 3 x 10⁴ – 4 x 10⁴ cells per well in a 96 well round-bottom plate (Corning, Wiesbaden, Germany) with anti-CD3/CD28 beads (Miltenyi Biotec,

Bergisch Gladbach, Germany, 4 beads per cell) and 2000U/ml rhIL-2 (Proleukin S®, Novartis, Basel, Switzerland). Culture medium was DMEM (Gibco/Invitrogen) supplemented with 10% FCS, 2 mM L-glutamine, 5×10^{-5} M 2-ME, 10 mM HEPES, 1% NEAA (PAN Biotech, Aidenbach, Germany), 100 U/ml penicillin-streptomycin (Gibco/Sigma-Aldrich, St.Louis/MO, USA). Murine Treg cells were transduced 48 h after isolation. Human sorted Treg cells were cultured in TexMACS medium supplemented with 500 U/ml rhIL-2 and TransAct (1:100 diluted). Human Treg cells were transduced 48 h after isolation.

Retroviral transduction of Treg cells

Retrovirus in the pMSCV-Thy1.1 system can be manufactured in Phoenix-Eco cells, a pCLEco (packaging plasmid) carrying variant of HEK293 cells. Therefore, Phoenix-Eco cells were seeded on a gelatin matrix at 1.8×10^6 cells per well in a six well plate 6 hours before lipofection. To produce liposomal particles containing the viral transgene, 3 µg of vector DNA and 1 µg of additional pCL-Eco packaging plasmid were coincubated with 12 µL of TransIT-293 transfection reagent (MoBiTec, Göttingen, Germany) for 20 minutes in OptiMEM medium at RT. Liposomes were added to Phoenix-Eco cells and incubated for an additional 16 hours. Afterwards medium was exchanged and production of viral particles could proceed for 24 h. Then supernatant with produced pMSCV retrovirus was added to Treg cell cultures and mixed gently. Treg cells were transduced for 6.5 hours incubation at 37°C. Afterwards, viral supernatant was removed and cells were incubated with fresh medium supplemented with IL-2 (2000 U/ml) for another 72 to 96 hours. Then, cells were harvested and anti-CD3/CD28 antibody coupled beads were removed by using a MACSiMAG separator magnet (Miltenyi Biotec, Bergisch Gladbach, Germany). For transduction of human Tregs an amphotrophic Phoenix cell line was used. Phoenix-Ampho cells were seeded at 2×10^6 cells per well in a six well plate 6 hours before lipofection. For production of liposomal particles containing the viral transgene, 3 µg of vector DNA and 1 µg of additional pCL-Ampho packaging plasmid were coincubated with 12 µL of TransIT-293 transfection reagent (MoBiTec, Göttingen, Germany) for 20 minutes in OptiMEM medium at RT. Liposomes were added to Phoenix-Ampho cells and incubated for an additional 16 hours. Afterwards medium was exchanged and production of viral particles could proceed for 24 h. Then supernatant with produced pMSCV retrovirus was added to Treg cell cultures and mixed gently. Treg cells were transduced for 6.5 hours incubation at 37°C. Afterwards, viral supernatant was removed and cells were incubated with fresh medium supplemented with IL-2 (500 U/ml), 100 U/ml penicillin-streptomycin and fresh TransAct (1:100 diluted) for another 72 to 96 hours.

Transient Transfection of HEK293 cells with ligands of the TNFSF

For production of liposomal particles, 2µg of plasmid DNA was incubated with 6µl TransIT-293 transfection reagent in 100µl OptiMEM medium for 20 minutes at room temperature. Then liposomes were gently added to 5 x 10⁵ HEK293 cells resuspended in 500µl DMEM. After shaking for 1 minute 230µl DMEM were added and 100µl of the cell-liposome suspension (containing approximately 6x10⁴ cells) were added per well into a 96 well flat bottom plate. 18 hours after lipofection medium was changed and transfected HEK293 cells could be used for coculture experiments.

Treg Proliferation Assay

Anti-CD3/CD28 beads were removed from transduced Treg cultures via a MACSiMAG separator magnet. Tregs were rested for 18 hours in fresh medium supplemented with 100 U/ml rhIL-2. Then Tregs were labeled with CellTrace™ CFSE Cell Proliferation dye (1µM) and added onto a HEK293 cells expressing mTNF, mTL1A or mLIGHT on the cell surface. rhIL-2 (2000 U/ml) was added to the cell cultures. After 72 h of cocubation proliferation of transduced CFSE-labeled Tregs was analyzed via flow cytometry.

RNA-Sequencing

Total RNA was isolated using the Qiagen Rneasy Micro Kit (Qiagen, Hilden, Germany) and RNA was eluted in 14 µL RNase-free water. RNA quality was assessed using the TapeStation system 4200 and High-Sensitivity RNA screentape (Agilent). 7 µl of the RNA was used for generating RNA-seq libraries using the SMART-seq Stranded Kit from Takara (Mountain View/CA, USA). Indexed libraries were pooled in an equimolar ratio and sequenced on an Illumina NextSeq 550 machine with NextSeq 500/550 High Output Kit v2.5 (75 cycles).

GvHD model

BALB/c (H2Kd) recipients were irradiated (8Gy) and transplanted retrobulbar with 2.5 × 10⁶ BM cells with or without (BM control) 5 × 10⁵ splenocytes from C57BL/6 donors (H2Kb, congenic CD45.1). The animals in the therapy groups received 2.5 × 10⁵ in vitro expanded and transduced Treg (C57BL/6, Foxp3-hCD2, congenic CD45.2). Recipients were monitored daily, body weight and GvHD symptoms assessed two or three times weekly by nonblinded investigators applying standardized scoring.

Example 3: Recombinant proteins**Murine TNF**

For the production of murine TNF the expression plasmid BCMGS-L6 coding for a non-cleavable mouse TNF protein was used (JBC 1999, 274(53):38112-8).

Murine TL1A

To generate a non-cleavable version of TL1A (mTL1A), amino acids 69-93 were deleted from wildtype sequence as described in J Immunol 2018; 200(4):1360-9. cDNA encoding the non-cleavable mTL1A was cloned into pIRES2-DsRed via NheI/Sall. The nucleotide sequence of mTL1A is depicted below:

```
ATGGGGGGCTCTCTGGTCAGAAGGGATCAGAAGTCTCTCCAAGACAGCAGAAGGATGGCAGAGGAGCT
GGGGTTGGGCTTCGGAGAAGGAGTCCCAGTGGAAGTGCTGCCGGAAGGCTGTAGACACAGGCCAGAGG
CCAGGGCCGGGCTAGCTGCCAGGAGCAAAGCCTGCCTGGCTCTCACCTGCTGCCTGTTGTTCATTTCCC
ATCCTCGCAGGACTTAGCACCTCCTAATGGCTGGCCAGCTCCGGGTCCCCGGAGGCAAGCCGAGAGC
ACACCTGACAATTAAGAAACAAACCCAGCACCACATCTGAAAAATCAGCTCTCTGCTCTACACTGGG
AACATGACCTAGGGATGGCCTTCACCAAGAACGGGATGAAGTACATCAACAAATCCCTGGTGATCCCA
GAGTCAGGAGACTATTTTCATCTACTCCCAGATCACATTCCGAGGGACCACATCTGTGTGTGGTGACAT
CAGTCGGGGGAGACGACCAAAACAAGCCAGACTCCATCACCATGGTTATCACCAAGGTAGCAGACAGCT
ACCTTGAGCCTGCCCGCTACTAACAGGGTCCAAGTCTGTGTGTGAAATAAGCAACAACCTGGTTCCAG
TCCCTCTACCTTGGGGCCACGTTCTCCTTGGAAGAAGGAGACAGACTAATGGTAAACGTCAGTGACAT
CTCCTTGGTGATTACACAAAAGAAGATAAAACTTTCTTTGGAGCTTTCTTGCTATAA (SEQ ID
NO.: 1)
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Human LIGHT

cDNA encoding human LIGHT was cloned into pcDNA3.1 neo via BamHI /EcoRV. The nucleotide sequence of hLIGHT is depicted below:

```
ATGGAGGAGAGTGTCTGACGGCCCTCAGTGTGTTGTGGTGGATGGACAGACCGACATCCCATTACAGAG
GCTGGGACGAAGCCACCGGAGACAGTCGTGCAGTGTGGCCCGGTGGGTCTGGGTCTCTTGCTGTTGC
TGATGGGGGCCGGGCTGGCCGTCCAAGGCTGGTTCCTCCTGCAGCTGCACTGGCGTCTAGGAGAGATG
GTCACCCGCTGCTGACGGACCTGCAGGCTCCTGGGAGCAGCTGATACAAGAGCGAAGGTCTCACGA
GGTCAACCCAGCAGCGCATCTCACAGGGGCCAACTCCAGCTTGACCGGCAGCGGGGGCCGCTGTTAT
GGGAGACCCAGCTGGGCCTGGCCTTCCTGAGGGGCCCTCAGCTACCACGATGGGGCCCTTGTGGTCACC
```

AAAGCTGGCTACTACTACATCTACTCCAAGGTGCAGCTGGGCGGTGTGGGCTGCCCGCTGGGCCTGGC
CAGCACCATCACCCACGGCCTCTACAAGCGCACACCCCGCTACCCCGAGGAGCTGGAGCTGTTGGTCA
GCCAGCAGTCACCCCTGCGGACGGGCCACCAGCAGCTCCCGGGTCTGGTGGGACAGCAGCTTCCTGGGT
GGTGTGGTACACCTGGAGGCTGGGGAGGAGGTGGTCCGTGTGCTGGATGAACGCCTGGTTCGACT
GCGTGATGGTACCCGGTCTTACTTCGGGGCTTTCATGGTGTGA (SEQ ID NO.: 2)

Murine LIGHT

cDNA encoding mouse LIGHT was cloned into pIRES2-DsRed via NheI / SalI. Amino acids 63-84 were deleted in order to generate an uncleavable form of mouse LIGHT. The nucleotide sequence of mLIGHT is depicted below:

ATGGAGAGTGTGGTACAGCCTTCAGTGTTCAGTGGTGGATGGACAGACGGACATCCCATTCAGGCGGCT
GGAACAGAACCACCGGAGACGGCGCTGTGGCACTGTCCAGGTCAGCCTGGCCCTGGTGCTGCTGCTAG
GTGCTGGGCTGGCCACTCAGGGCTGGTTTCTCCTGAGACTGCATCAACGTCAACGATCTCACCAGGCC
AACCAGCAGCACATCTTACAGGAGCCAACGCCAGCTTGATAGGTATTGGTGGACCTCTGTTATGGGA
GACACGACTTGGCCTGGCCTTCTTGAGGGGCTTGACGTATCATGATGGGGCCCTGGTGACCATGGAGC
CCGGTTACTACTATGTGTACTCCAAAGTGCAGCTGAGCGGCGTGGGCTGCCCCAGGGGCTGGCCAAT
GGCCTCCCCATCACCCATGGACTATACAAGCGCACATCCCGCTACCCGAAGGAGTTAGAACTGCTGGT
CAGTCGGCGGTACCCCTGTGGCCGGGCCAACAGCTCCCGAGTCTGGTGGGACAGCAGCTTCCTGGGCG
GCGTGGTACATCTGGAGGCTGGGGAAGAGGTGGTGGTCCGCGTGCCCTGGAAACCGCCTGGTCAGACCA
CGTGACGGCACCAGGTCTTATTTTCGGAGCTTTCATGGTCTGA (SEQ ID NO.: 3)

Example 4: Design of Artificial Immune Receptors (AIRs)

Murine and human AIRs were designed based on published nucleotide sequences of the individual parts and domains (<https://www.ensembl.org/>). Extracellular and transmembrane domains of LTbR, DR3, TNFR2 and CD40 were fused to the intracellular signaling domains of CD28 and the CD3-zeta-chain. Two point mutations were introduced into murine CD3-zeta-chain as they were shown to increase expression of chimeric antigen receptors (Blood 2003; 102(13):4320-5). As negative control, an ORF was used coding for transmembrane and signaling domain of CD28 and the signaling domain of the CD3-zeta-chain. The anti-CD19 CAR as well as the anti-CEA CAR have been described earlier (Blood 2010; 116(19):3875-86; Mol Ther 2019; 27(10):1825-35). The cDNAs coding AIRs as well as the anti-CD19 CAR and the anti-CEA CAR were fused to the congenic marker CD90.1 by ligating to a self cleaving P2A sequence. cDNAs were synthesized by ThermoFisher/Life Technologies (Waltham, MA, USA) and cloned into the pMSCV-Thy1.1 retroviral backbone (Addgene, cat#17442) via NotI/MluI. An

overview of the constructs is shown in Figure 1. Sequences of the constructs and their parts are shown in Table 5 (N = nucleic acid, P = polypeptide). The CD90.1 marker gene is cut off via the P2A cleavage site and is not part of the AIR.

Table 5:

SEQ ID No.	Description	N / P	Sequence
4	TNFR2, complete AIR, mouse	N	ATGGCGCCCGCCGCCCTCTGGGTGCGCTGGTCTTCGAACT GCAGCTGTGGGCCACCGGGCACACAGTGCCCGCCAGGTTG TCTTGACACCTTACAAACCGGAACCTGGGTACGAGTGCCAG ATCTCACAGGAATACTATGACAGGAAGGCTCAGATGTGCTG TGCTAAGTGTCTCTGGCCAATATGTGAAACATTTCTGCA ACAAGACCTCGGACACCGTGTGTGCGGACTGTGAGGCAAGC ATGTATACCCAGGTCTGGAACCAGTTTCGTACATGTTTGAG CTGCAGTTCTTCTGTACCACTGACCAGGTGGAGATCCGCG CCTGCACTAAACAGCAGAACCGAGTGTGTGCTTGCGAAGCT GGCAGGTACTGCGCCTTGAAAACCCATTCTGGCAGCTGTG ACAGTGCATGAGGCTGAGCAAGTGCGGCCCTGGCTTCGGAG TGGCCAGTTCAAGAGCCCCAAATGGAAATGTGCTATGCAAG GCCTGTGCCCCAGGGACGTTCTCTGACACCACATCATCCAC TGATGTGTGCAGGCCCCACCGCATCTGTAGCATCCTGGCTA TTCCCGGAAATGCAAGCACAGATGCAGTCTGTGCGCCGAG TCCCCAACTCTAAGTGCCATCCCAAGGACACTCTACGTATC TCAGCCAGAGCCCACAAGATCCCAACCCCTGGATCAAGAGC CAGGGCCAGCCAAACTCCAAGCATCCTTACATCGTTGGGT TCAACCCCATTTATTGAACAAAGTACCAAGGGTGGCATCTC TCTTCCAATTGGTCTGATTGTTGGAGTGACATCACTGGGTC TGCTGATGTTAGGACTGGTGAAGTGCATCATCCTGGTGCAG AGGAACAGCAGAAGAAACAGAGGCGGCCAGAGCGACTACAT GAACATGACCCCCAGAAGGCCAGGCCTGACCAGAAAGCCCT ACCAGCCCTACGCCCCCTGCCAGAGACTTCGCCGCTACAGA CCCAGAGCCAAGTTCAGCAGATCCGCCGAGACAGCCGCCAA CCTGCAGGATCCCAACCAGCTGTACAACGAGCTGAACCTGG GCAGACGGGAGGAATACGACGTGCTGGAAAAGAAGAGAGCC AGGGACCCCGAGATGGGCGGCAAGCAGCAGAGAAGAAGAAA CCCTCAGGAAGGCGTCTACAACGCCCTGCAGAAAGACAAGA TGGCCGAGGCCCTACAGCGAGATCGGCACCAAGGGCGAGAGA AGAAGGGGCAAGGGCCACGATGGCCTGTACCAGGGCCTGTC CACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGA CCCTGGCCCCCAGAGCCACGAACTTCTCTCTGTTAAAGCAA GCAGGAGACGTGGAAGAAAACCCCGGTCTATGAACCCAGC CATCAGCGTCGCTCTCTGCTCTCAGTCTTGCCAGGTGTCCC GAGGGCAGAAGGTGACCAGCCTGACAGCCTGCCTGGTGAAC

			CAAAACCTTCGCCTGGACTGCCGCCATGAGAATAACACCAA GGATAACTCCATCCAGCATGAGTTCAGCCTGACCCGAGAGA AGAGGAAGCACGTGCTCTCAGGCACCCTTGGGATACCCGAG CACACGTACCGCTCCCGCGTCACCCTCTCCAACCAGCCCTA TATCAAGGTCCCTTACCCTAGCCAACCTTACCACCAAGGATG AGGGCGACTACTTTTGTGAGCTTCGCGTCTCGGGCGCGAAT CCCATGAGCTCCAATAAAAGTATCAGTGTGTATAGAGACAA GCTGGTCAAGTGTGGCGGCATAAGCCTGCTGGTTCAGAACA CATCCTGGATGCTGCTGCTGCTGCTTTCCCTCTCCCTCCTC CAAGCCCTGGACTTCATTTCTCTGTGA
5	TNFR2, complete AIR, mouse	P	MAPAALWVALVFELQLWATGHTVPAQVVLTPYKPEPGYECQ ISQEYYDRKAQMCCAACPPGQYVKHFCNKTSDTVACDEAS MYTQVWNQFRTCLSCSSSCTTDQVEIRACTKQQNRVCACEA GRYCALKTHSGSCRQCMRLSKCGPGFGVASSRAPNGNVLCCK ACAPGTFSDTTSSTDVCRPHRISILAIIPGNASTDAVCAPE SPTLSAIPRTLYVSQPEPTRSQPLDQEPGPSQTPSILTSLG STPIIEQSTKGGISLPIGLIVGVTSLGLLMLGLVNCIILVQ RNSRRNRGGQSDYMNMTPRRPGLTRKPYQPYAPARDFAAAYR PRAKFSRSAETAANLQDPNQLYNELNLGRREEYDVLEKKRA RDPENMGKQORRRNPQEGVYNALQKDKMAEAYSEIGTKGER RRGKHDGLYQGLSTATKDTYDALHMQTLAPRATNFSLLKQ AGDVEENPGPMNPAISVALLLSVLQVSRGQKVTSLTACLVN QNLRLDRCRHHNNTKDNSIQHEFSLTREKRKHVLSGTLGIPE HTYRSRVTLNQPYIKVLTLANFTTKDEGDYFCELRVSGAN PMSSNKSISVYRDKLVKCGGISLLVQNTSWMLLLLLLSLL QALDFISL
6	TNFR2, extracellular and transmembrane domain, mouse	N	ATGGCGCCCGCCGCCCTCTGGGTCGCGCTGGTCTTCGAACT GCAGCTGTGGGCCACCGGGCACACAGTGCCCGCCAGGTTG TCTTGACACCCACAAACCGGAACCTGGGTACGAGTGCCAG ATCTCACAGGAATACTATGACAGGAAGGCTCAGATGTGCTG TGCTAAGTGTCTCTGCGCAATATGTGAAACATTTCTGCA ACAAGACCTCGGACACCGTGTGTGCGGACTGTGAGGCAAGC ATGTATACCCAGGTCTGGAACAGTTTCGTACATGTTTGAG CTGCAGTTCTTCTGTACCACTGACCAGGTGGAGATCCGCG CCTGCACTAAACAGCAGAACCGAGTGTGTGCTTGCGAAGCT GGCAGGTACTGCGCCTTGAAAACCCATTCTGGCAGCTGTGCG ACAGTGCGATGAGGCTGAGCAAGTGCGGCCCTGGCTTCGGAG TGGCCAGTTCAAGAGCCCCAAATGGAATGTGCTATGCAAG GCCTGTGCCCCAGGGACGTTCTCTGACACCACATCATCCAC TGATGTGTGCAGGCCCCACCGCATCTGTAGCATCTGGCTA TTCCCGGAAATGCAAGCACAGATGCAGTCTGTGCGCCCGAG TCCCCAACTCTAAGTGCCATCCCAAGGACACTCTACGTATC TCAGCCAGAGCCCACAAGATCCCAACCCCTGGATCAAGAGC CAGGGCCAGCCAAACTCCAAGCATCCTTACATCGTTGGGT

			TCAACCCCCATTATTGAACAAAGTACCAAGGGTGGCATCTC TCTTCCAATTGGTCTGATTGTTGGAGTGACATCACTGGGTC TGCTGATGTTAGGACTGGTGAACTGCATCATCCTGGTGCAG AGG
7	TNFR2, extracellular and transmembrane domain, mouse	P	MAPAALWVALVFELQLWATGHTVPAQVVLT PYKPEPGYECQ ISQEYYDRKAQMCCAKCPPGQYVKHFCNKTS DTCADCEAS MYTQVWNQFRTCLSCSSSCTTDQVEIRACTKQ QNRVCACEA GRYCALKTHSGSCRQCMRLSKCGPGFGVASSR APNGNVLCK ACAPGTFSDTTSSTDVCRPHRISILAI PGNASTD AVCAPE SPTLSAIPRTLYVSQPEPTRSQPLDQEPGPSQ TPSILTSLG STPIIEQSTKGGISLPIGLIVGVTS LGLMLGLVNCIILVQ R
8	CD28 signaling domain, mouse	N	AACAGCAGAAGAAACAGAGGCGGCCAGAGCGA CTACATGAACATGACCCCCAGAAGGCCAGGCCT GACCAGAAAGCCCTACAGCCCTACGCCCCCT GCCAGAGACTTCGCCGCCTACAGACCC
9	CD28 signaling domain, mouse	P	NSRRNRGGQSDYMNMTPRRPLTRKPYQPYA PARDFAAYRP
10	CD3 zeta domain, mouse	N	AGAGCCAAGTTCAGCAGATCCGCCGAGACAGCC GCAACCTGCAGGATCCCAACCAGCTGTACAACG AGCTGAACCTGGGCA GACGGGAGGAATACGACGTGCTGGAAAAGAAG AGAGCCAGG GACCCCGAGATGGGCGGCAAGCAGCAGAGAAGA AGAAACCC TCAGGAAGGCGTCTACAACGCCCTGCAGAAAG ACAAGATGG CCGAGGCCTACAGCGAGATCGGCACCAAGGGCG AGAGAAGA AGGGGCAAGGGCCACGATGGCCTGTACCAGGGC CTGTCCAC CGCCACCAAGGACACCTACGACGCCCTGCACAT GCAGACCC TGGCCCCCAGA
11	CD3 zeta domain, mouse	P	RAKFSSRAETAANLQDPNQLYNELNLGRREEYD VLEKKRAR DPEMGGKQQRNRNPQEGVYNALQKDKMAEAY SEIGTKGERR RGKGHDGLYQGLSTATKDTYDALHMQTLAPR
12	P2A cleavage site	N	GCCACGAACTTCTCTCTGTTAAAGCAAGCAGG AGACGTGGAGAAAACCCCGGTCTCT
13	P2A cleavage site	P	ATNFSLLKQAGDVEENPGP
14	CD90.1 marker, mouse	N	ATGAACCCAGCCATCAGCGTCGCTCTCCTGCTC TCAGTCTT GCAGGTGTCCCAGGGCAGAAGGTGACCAGCCTG ACAGCCT GCCTGGTGAACCAAAACCTTCGCCTGGACTGCC GCCATGAG AATAACACCAAGGATAACTCCATCCAGCATGAG TTCAGCCT GACCCGAGAGAAGAGGAAGCACGTGCTCTCAGG CACCCCTTG

			GGATACCCGAGCACACGTACCGCTCCCGCTCACCCCTCTCC AACCAGCCCTATATCAAGGTCTTACCCTAGCCAACTTCAC CACCAAGGATGAGGGCGACTACTTTTGTGAGCTTCGCGTCT CGGGCGCGAATCCCATGAGCTCCAATAAAAGTATCAGTGTG TATAGAGACAAGCTGGTCAAGTGTGGCGGCATAAGCCTGCT GGTTCAGAACACATCCTGGATGCTGCTGCTGCTGCTTTCCC TCTCCCTCCTCCAAGCCCTGGACTTCATTTCTCTGTGA
15	CD90.1 marker, mouse	P	MNPAISVALLLSVLQVSRGQKVTSLTACLVNQNLRLDCRHE NNTKDNSIQHEFSLTREKRKHVLSGTLGIPEHTYRSRVTL NQPYIKVLTLANFTTKDEGDYFCELRVSGANPMSSNKSISV YRDKLVKCGGISLLVQNTSWMLLLLLLSLLQALDFISL
16	LTBR, complete AIR, mouse	N	ATGCGCCTGCCCCGGGCCTCCTCTCCCTGCGGCCTGGCCTG GGGGCCACTCCTGCTGGGACTCAGCGGGCTTCTGGTGGCCT CTCAGCCCCAGCTGGTGCCCCCTTATCGCATAGAAAACCAG ACTTGCTGGGACCAGGACAAGGAATACTACGAGCCCATGCA CGACGTCTGCTGCTCCCGCTGTCCCCCAGGCGAGTTTGTCT TTGCGGTATGCAGCCGCAGCCAAGACACGGTTTGCAAGACT TGCCCCCATAATTCTATAATGAACACTGGAACCATCTCTC CACCTGCCAGCTGTGCCGCCCCTGTGACATTGTGCTGGGCT TTGAGGAGGTTGCCCCCTGCACCAGCGATCGGAAAGCCGAG TGCCGCTGTGAGCCGGGGATGTCTGTGTGTATCTGGACAA TGAGTGTGTGCACTGTGAGGAGGAGCGGCTTGACTCTGCC AGCCTGGCACAGAAGCCGAGGTCACAGATGAAATTATGGAT ACTGACGTCAACTGTGTCCCCTGTAAGCCGGGACACTTCCA GAACACTTCCTCCCCCTCGAGCCCGCTGTCAACCCCATACCA GATGTGAGATCCAGGGCCTGGTGGAGGCAGCTCCAGGTACC TCCTACTCGGATACCATCTGTAAAAATCCCCCAGAGCCAGG AGCAATGCTACTGCTGGCCATCCTGCTGTGCTGGTCTCTCT TTCTGCTCTTCACCACTGTCTGGCCTGTGCCTGGGCGGCC GCGAACAGCAGAAGAAACAGAGGCGGCCAGAGCGACTACAT GAACATGACCCCCAGAAGGCCAGGCCTGACCAGAAAGCCCT ACCAGCCCTACGCCCCCTGCCAGAGACTTCGCCGCTACAGA CCCAGAGCCAAGTTCAGCAGATCCGCCGAGACAGCCGCCAA CCTGCAGGATCCCAACCAGCTGTACAACGAGCTGAACCTGG GCAGACGGGAGGAATACGACGTGCTGGAAGAAGAGAGCC AGGGACCCCCGAGATGGGCGGCAAGCAGCAGAGAAGAAGAAA CCCTCAGGAAGGCGTCTACAACGCCCTGCAGAAAGACAAGA TGGCCGAGGCCTACAGCGAGATCGGCACCAAGGGCGAGAGA AGAAGGGGCAAGGGCCACGATGGCTGTACCAGGGCTGTCTC CACCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGA CCCTGGCCCCCAGAGCCACGAACTTCTCTCTGTTAAAGCAA GCAGGAGACGTGGAAGAAAACCCCGGTCTATGAACCCAGC CATCAGCGTCGCTCTCCTGCTCTCAGTCTTGAGGTGTCCC GAGGGCAGAAGGTGACCAGCCTGACAGCCTGCCTGGTGAAC

			CAAAACCTTCGCTGGACTGCCGCCATGAGAATAACACCAA GGATAACTCCATCCAGCATGAGTTCAGCCTGACCCGAGAGA AGAGGAAGCACGTGCTCTCAGGCACCCTTGGGATACCCGAG CACACGTACCGCTCCCGCGTCACCCTCTCCAACCAGCCCTA TATCAAGGTCCCTTACCCTAGCCAACCTTACCACCAAGGATG AGGGCGACTACTTTTGTGAGCTTCGCGTCTCGGGCGCGAAT CCCATGAGCTCCAATAAAAGTATCAGTGTGTATAGAGACAA GCTGGTCAAGTGTGGCGGCATAAGCCTGCTGGTTCAGAACA CATCCTGGATGCTGCTGCTGCTGCTTTCCCTCTCCCTCCTC CAAGCCCTGGACTTCATTTCTCTGTGA
17	LTBR, complete AIR, mouse	P	MRLPRASSPCGLAWGPLLLGLSGLLVASQPQLVPPYRIENQ TCWDQDKEYEPMHDVCCSRCPPEFVFAVCSRSQDTVCKT CPHNSYNEHWNHLSTCQLCRPCDIVLGFEVAPCTSDRKAE CRCQPGMSCVYLDNECVHCEEERLVLCPGTEAEVTDEIMD TDVNCVPCKPGHFQNTSSPRARCQPHTRCEIQGLVEAAPGT SYSDTICKNPPEPGAMLLLAILLSLVLFLLFTTVLACAWAA ANSRNRGGQSDYMNMTPRRPGLTRKPYQPYAPARDFAAAYR PRAKFSRSAETAANLQDPNQLYNELNLGRREEYDVLEKKRA RDPENGGKQORRRNPQEGVYNALQKDKMAEAYSEIGTKGER RRGKGDGLYQGLSTATKDTYDALHMQTLAPRATNFSLLKQ AGDVEENPGPMNPAISVALLLSVLQVSRGQKVTSLTACLVN QNLRLDRCRHNNTKDNSIQHEFSLTREKRKHVLSGTLGIPE HTYRSRVTLNQPYIKVLTLANFTTKDEGDYFCELRVSGAN PMSSNKSISVYRDKLVKCGGISLLVQNTSWMLLLLLLSLSLL QALDFISL
18	LTBR, extracellular and transmembrane domain, mouse	N	ATGCGCCTGCCCCGGGCCTCCTCTCCCTGCGGCCTGGCCTG GGGGCCACTCCTGCTGGGACTCAGCGGGCTTCTGGTGGCCT CTCAGCCCCAGCTGGTGCCCCCTTATCGCATAGAAAACCAG ACTTGCTGGGACCAGGACAAGGAATACTACGAGCCCATGCA CGACGTCTGCTGCTCCCGCTGTCCCCAGGCGAGTTTGTCT TTGCGGTATGCAGCCGCAGCCAAGACACGGTTTGCAAGACT TGCCCCCATAATTCTATAATGAACACTGGAACCATCTCTC CACCTGCCAGCTGTGCCGCCCTGTGACATTGTGCTGGGCT TTGAGGAGGTTGCCCTTGACACGCGATCGGAAAGCCGAG TGCCGCTGTGAGCCGGGGATGTCCTGTGTGTATCTGGACAA TGAGTGTGTGCACTGTGAGGAGGAGCGGCTTGTACTCTGCC AGCCTGGCACAGAAAGCCGAGGTACAGATGAAATTATGGAT ACTGACGTCAACTGTGTCCCCTGTAAGCCGGGACACTTCCA GAACACTTCTCCCTCGAGCCCGCTGTCAACCCCATACCA GATGTGAGATCCAGGGCCTGGTGGAGGCAGCTCCAGGTACC TCCTACTCGGATACCATCTGTAAAAATCCCCAGAGCCAGG AGCAATGCTACTGCTGGCCATCCTGCTGTGCTGGTCTCTCT

			TTCTGCTCTTCACCACTGTCCTGGCCTGTGCCTGGGCGGCC GCG
19	LTBR, extracellular and transmembrane domain, mouse	P	MRLPRASSPCGLAWGPLLLGLSGLLVASQPQLVPPYRIENQ TCWDQDKEYEPMHDVCCSRCPPEFVFAVCSRSQDTVCKT CPHNSYNEHWNHLSTCQLCRPCDIVLGFEVAPCTSDRKAE CRCQPGMSCVYLDNECVHCEEERLVLCQPGTEAEVTDEIMD TDVNCVPCKPGHFQNTSSPRARCQPHTRCEIQGLVEAAPGT SYSDTICKNPPEPGAMLLAILLSLVLFLLFTTVLACAWAA A
20	DR3, complete AIR, mouse	N	ATGGAGGCACGGCTGCTGCGGGGCTGCGTGGTGGAGCCTCT GTTCTTACCACTGCTGCTGCTGCTGCTGCTGCTGCTTGGTG GCCAGGGCCAGGGCGGCATGTCTGGCAGGTGTGACTGTGCC AGTGAGTCCCAGAAGAGGTATGGCCCGTTTTGTTGCAGGGG CTGCCCCAAAGGGACACTACATGAAGGCCCCCTGCGCAGAAC CCTGTGGCAACTCCACCTGCCTTCCCTGTCCCTCGGACACC TTCTTGACCAGAGACAACCACTTTAAGACTGACTGTACCCG CTGCCAAGTCTGTGATGAAGAGGCCCTTCAAGTGACCCTTG AGAACTGCTCGGCAAAGTCGGACACCCACTGTGGCTGCCAG TCAGGCTGGTGTGTTGACTGCTCCACCGAGCCATGTGGGAA AAGCTCACCTTTCTCTTGTGTCCCATGCGGGGCTACAACAC CAGTCCATGAGGCTCCAACCCCCCGGCCCTGCCTGCCGTCG TTCTATATACGTGGCAATGACTGCACGTCCTGCCCCACGGG CTTCAGCAGCGTTTGCCCTAAGGCTTGCACTGCTGTCTGTG GCTGGAAGCAGATGTTTTGGGTCCAGGTGCTTCTAGGAGTC GCGTTCCTTTTTGGGGCTATCCTGATCTGTGCATATTGTAA CAGCAGAAGAAACAGAGGCGGCCAGAGCGACTACATGAACA TGACCCCCAGAAGGCCAGGCCTGACCAGAAAGCCCTACCAG CCCTACGCCCCTGCCAGAGACTTCGCCGCCTACAGACCCAG AGCCAAGTTCAGCAGATCCGCCGAGACAGCCGCCAACCTGC AGGATCCCAACCAGCTGTACAACGAGCTGAACCTGGGCAGA CGGGAGGAATACGACGTGCTGGAAAAGAAGAGAGCCAGGGA CCCCGAGATGGGCGGCAAGCAGCAGAGAAGAAGAAACCCCTC AGGAAGGCGTCTACAACGCCCTGCAGAAAGACAAGATGGCC GAGGCCTACAGCGAGATCGGCACCAAGGGCGAGAGAAGAAG GGGCAAGGGCCACGATGGCCTGTACCAGGGCCTGTCCACCG CCACCAAGGACACCTACGACGCCCTGCACATGCAGACCCCTG GCCCCCAGAGCCACGAACCTTCTCTCTGTTAAAGCAAGCAGG AGACGTGGAAGAAAACCCCGGTCCTATGAACCCAGCCATCA GCGTCGCTCTCCTGCTCTCAGTCTTGACGGTGTCCCGAGGG CAGAAGGTGACCAGCCTGACAGCCTGCCTGGTGAACCAAAA CCTTCGCTTGGACTGCCGCCATGAGAATAACACCAAGGATA ACTCCATCCAGCATGAGTTCAGCCTGACCCGAGAGAAGAGG AAGCACGTGCTCTCAGGCACCCCTGGGATAACCCGAGCACAC GTACCGCTCCCGCGTCACCCCTCTCCAACCAGCCCTATATCA

			AGGTCCTTACCCTAGCCAACCTCACCACCAAGGATGAGGGC GACTACTTTTGTGAGCTTCGCGTCTCGGGCGGAATCCCAT GAGCTCCAATAAAAGTATCAGTGTGTATAGAGACAAGCTGG TCAAGTGTGGCGGCATAAGCCTGCTGGTTCAGAACACATCC TGGATGCTGCTGCTGCTGCTTTCCCTCTCCCTCCTCCAAGC CCTGGACTTCATTTCTCTGTGA
21	DR3, complete AIR, mouse	P	MEARLLRGCVVEPLFLPLLLLLLLLLLGGQGQGGMSGRCDCA SESQKRYGPFCCRGCPKGHYMKAPCAEPCGNSTCLPCPSDT FLTRDNHFKTDCTRCQVCDEEALQVTLENCsAKSDTHCGCQ SGWCVDCSTEPCKGSSPFSCVPCGATTVPHEAPTPRPCLPG FYIRGNDCTSCPTGFSSVCPKACTAVCGWKQMFVWQVLLGV AFLFGAILICAYCNSRRNRGGQSDYMNMTPRRPLTRKPYQ PYAPARDFAAAYRPRAKFSRSAETAANLQDPNQLYNELNLGR REEYDVLEKKRARDPEMGGKQORRRNPQEGVYNALQDKMA EAYSEIGTKGERRRGKGHDLQGLSTATKDTYDALHMQTL APRATNFSLLKQAGDVEENPGPMNPASVALLLSVLQVSRG QKVTSLTACLVNQNLRLDCRHENNTKDNSIQHEFSLTREKR KHVLSGTLGIPEHTYRSRVTLNQPPIKVLTLANFTTKDEG DYFCELRVSGANPMSSNKSISVYRDKLVKCGGISLLVQNTS WMLLLLLLSLSLLQALDFISL
22	DR3, extracellular and transmembrane domain, mouse	N	ATGGAGGCACGGCTGCTGCGGGGCTGCGTGGTGGAGCCTCT GTTCTTACCCTGCTGCTGCTGCTGCTGCTGCTGCTTGGTG GCCAGGGCCAGGGCGGCATGTCTGGCAGGTGTGACTGTGCC AGTGAGTCCCAGAAGAGGTATGGCCCGTTTTGTTGCAGGGG CTGCCCCAAAGGGACACTACATGAAGGCCCCCTGCGCAGAAC CCTGTGGCAACTCCACCTGCCTTCCCTGTCCCTCGGACACC TTCTTGACCAGAGACAACCACTTTAAGACTGACTGTACCCG CTGCCAAGTCTGTGATGAAGAGGCCCTTCAAGTGACCCTTG AGAACTGCTCGGCAAAGTCGGACACCCACTGTGGCTGCCAG TCAGGCTGGTGTGTTGACTGCTCCACCGAGCCATGTGGGAA AAGCTCACCTTTCTCTTGTGTCCCATGCGGGGCTACAACAC CAGTCCATGAGGCTCCAACCCCCGGCCCTGCCTGCCTGGC TTCTATATACGTGGCAATGACTGCACGTCCTGCCCCACGGG CTTCAGCAGCGTTTGCCCTAAGGCTTGCACTGCTGTCTGTG GCTGGAAGCAGATGTTTGGGTCCAGGTGCTTCTAGGAGTC GCGTTCCTTTTTGGGGCTATCCTGATCTGTGCATATTGT
23	DR3, extracellular and transmembrane domain, mouse	P	MEARLLRGCVVEPLFLPLLLLLLLLLLGGQGQGGMSGRCDCA SESQKRYGPFCCRGCPKGHYMKAPCAEPCGNSTCLPCPSDT FLTRDNHFKTDCTRCQVCDEEALQVTLENCsAKSDTHCGCQ SGWCVDCSTEPCKGSSPFSCVPCGATTVPHEAPTPRPCLPG

			FYIRGNDCTSCPTGFSSVCPKACTAVCGWKQMFVWQVLLGV AFLFGAILICAYC
24	Truncated control AIR, complete	N	ATGGCGCCCGCCGCCCTCTGGGTCGCGCTGGTCTTCGAACT GCAGCTGTGGGCCACCGGGCACACATTCTGGGCCCTGGTGG TGGTGGCCGGCGTGCTGTTCTGTTACGGCCTGCTGGTCACA GTGGCCCTGTGCGTGATCTGGACCAACAGCAGAAGAAACAG AGGCGGCCAGAGCGACTACATGAACATGACCCCAAGAAGGC CAGGCCTGACCAGAAAGCCCTACCAGCCCTACGCCCCTGCC AGAGACTTCGCCGCTACAGACCCAGAGCCAAGTTCAGCAG ATCCGCCGAGACAGCCGCCAACCTGCAGGATCCCAACCAGC TGTACAACGAGCTGAACCTGGGCAGACGGGAGGAATACGAC GTGCTGAAAAAGAAGAGAGCCAGGGACCCGAGATGGGCGG CAAGCAGCAGAGAAGAAGAAACCTCAGGAAGGCGTCTACA ACGCCCTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAG ATCGGCACCAAGGGCGAGAGAAGAAGGGGCAAGGGCCACGA TGGCCTGTACCAGGGCCTGTCCACCGCCACCAAGGACACCT ACGACGCCCTGCACATGCAGACCCTGGCCCCAGAGCCACG AACTTCTCTCTGTTAAAGCAAGCAGGAGACGTGGAAGAAAA CCCCGGTCCATGAACCCAGCCATCAGCGTCGCTCTCCTGC TCTCAGTCTTGCAAGGTGTCCCGAGGGCAGAAGGTGACCAGC CTGACAGCCTGCCTGGTGAACCAAAACCTTCGCCTGGACTG CCGCCATGAGAATAACACCAAGGATAACTCCATCCAGCATG AGTTCAGCCTGACCCGAGAGAAGAGGAAGCACGTGCTCTCA GGCACCCCTGGGATACCCGAGCACACGTACCGCTCCCGCGT CACCTCTCCAACCAGCCCTATATCAAGGTCCTTACCCTAG CCAACCTCACCAACCAAGGATGAGGGCGACTACTTTTGTGAG CTTCGCGTCTCGGGCGGAATCCCATGAGCTCCAATAAAAAG TATCAGTGTGTATAGAGACAAGCTGGTCAAGTGTGGCGGCA TAAGCCTGCTGGTTCAGAACACATCCTGGATGCTGCTGCTG CTGCTTTCCCTCTCCCTCCTCCAAGCCCTGGACTTCATTTT TCTGTGA
25	Truncated control AIR, complete	P	MAPAALWVALVFELQLWATGHTFWALVVVAGVLFYGLLVT VALCVIWTNSRRNRGGQSDYMNMTPRRPGLTRKPYQPYAPA RDFAAYRPRAKFSRSAETAANLQDPNQLYNELNLGRREEYD VLEKKRARDPEMGGKQRRRNPOEGVYNALQDKMAEAYSE IGTKGERRRGKGHDGLYQGLSTATKDTYDALHMQTLAPRAT NFSLLKQAGDVEENPGPMNPAISVALLSVLQVSRGQKVT LTACLVNQNLRLDCRHENNTKDNSIQHEFSLTREKRKHVLS GTLGIPEHTYRSRVTLNQPYIKVLTLANFTTKDEGDYFCE LRVSGANPMSSNKSISVYRDKLVKCGGISLLVQNTSWMLLL LLSLSLQALDFISL

26	TNFR2 signaling peptide, mouse	N	ATGGCGCCCGCCGCTCTGGGTCGCGCTGGTCTTCGAACTGCAGCTGTGGGCCACCGGGCACACA
27	TNFR2 signaling peptide, mouse	P	MAPAALWVALVFELQLWATGHT
28	CD28, transmembrane domain, mouse	N	TTCTGGGCCCTGGTGGTGGTGGCCGGCGTGCTGTTCTGTTA CGGCCTGCTGGTCACAGTGGCCCTGTGCGTGATCTGGACC
29	CD28, transmembrane domain, mouse	P	FWALVVVAGVLFYGLLVTVLALCVIWT
30	LTBR, complete AIR, human	N	ATGCTCCTGCCTTGGGCCACCTCTGCCCCGGCCTGGCCTG GGGGCCTCTGGTGCTGGGCCTCTTCGGGCTCCTGGCAGCAT CGCAGCCCCAGGCGGTGCTCCATATGCGTCGGAGAACCAG ACCTGCAGGGACCAGGAAAAGGAATACTATGAGCCCCAGCA CCGCATCTGCTGCTCCCCGCTGCCCCGCCAGGCACCTATGTCT CAGCTAAATGTAGCCGCATCCGGGACACAGTTTGTGCCACA TGTGCCGAGAAATTCCTACAACGAGCACTGGAACCTACCTGAC CATCTGCCAGCTGTGCCGCCCCCTGTGACCCAGTGATGGGCC TCGAGGAGATTGCCCCCTGCACAAGCAAACGGAAGACCCAG TGCCGCTGCCAGCCGGAATGTTCTGTGCTGCCTGGGCCCT CGAGTGACACACTGCGAGCTACTTTCTGACTGCCCGCCTG GCACTGAAGCCGAGCTCAAAGATGAAGTTGGGAAGGGTAAC AACCACTGCGTCCCCTGCAAGGCCGGGCACTTCAGAATAC CTCCTCCCCCAGCGCCCGCTGCCAGCCCCACACCAGGTGTG AGAACCAAGGTCTGGTGAGGCAGCTCCAGGCAGTGGCCAG TCCGACACAACCTGCAAAAATCCATTAGAGCCACTGCCCCC AGAGATGTCAGGAACCATGCTGATGCTGGCCGTTCTGCTGC CACTGGCCTTCTTTCTGCTCCTTGCCACCGTCTTCTCCTGC ATCTGGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTACTA CATGAACATGACTCCCCGCGCCCCGGGCCACCCGCAAGC ATTACCAGCCCTATGCCCCCCCACGCGACTTCGCAGCCTAT CGCTCCCTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCC CGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCA ATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGA CGTGGCCGGGACCTGAGATGGGGGGAAGCCGAGAAGGAA GAACCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATA AGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAG CGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCT CAGTACAGCCACCAAGGACACCTACGACGCCCTTACATGC AGGCCCTGCCCCCTCGCGCCACGAACTTCTCTCTGTTAAAG CAAGCAGGAGACGTGGAAGAAAACCCCGGTCTATGAACCC AGCCATCAGCGTCGCTCTCCTGCTCTCAGTCTTGACAGGTGT CCCGAGGGCAGAAGGTGACCAGCCTGACAGCCTGCCTGGTG

			AACC AAAACCTTCGCCTGGACTGCCGCCATGAGAATAACAC CAAGGATAACTCCATCCAGCATGAGTTCAGCCTGACCCGAG AGAAGAGGAAGCACGTGCTCTCAGGCACCCTTGGGATACCC GAGCACACGTACCGCTCCCGCGTCACCCTCTCCAACCAGCC CTATATCAAGGTCCTTACCCTAGCCAACCTTACCACCAAGG ATGAGGGCGACTACTTTTGTGAGCTTCGCGTCTCGGGCGCG AATCCCATGAGCTCCAATAAAAGTATCAGTGTGTATAGAGA CAAGCTGGTCAAGTGTGGCGGCATAAGCCTGCTGGTTCAGA ACACATCCTGGATGCTGCTGCTGCTGCTTTCCCTCTCCCTC CTCCAAGCCCTGGACTTCATTTCTCTGTGA
31	LTBR, complete AIR, human	P	MLLPWATSAPGLAWGPLVLGLFGLLAASQPQAVPPYASENQ TCRDQEKEYEPQHRICCSRCPPGTIVSAKCSRI RDTVCAT CAENSYNEHWNLTICQLCRPCDPVMGLEE IAPCTSKRK TQ CRCQPGMFCAAWALECTHCELLSDCPPGTEAELKDEVGKGN NHCVPCKAGHFQNTSSPSARCQPHTRCENQGLVEAAPGTAQ SDTTCKNPLEPLPPMSGTMLMLAVLLPLAFFLLLATV FSC IWRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDF AAY RSLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKGDGLYQGLSTATKDTYDALHMQALPPRATNFSL LK QAGDVEENPGPMNPAISVALLLSVLQVSRGQKVTSLTACL V NQNLRLDCRHENNTKDNSIQHEFSLTREKRKHVLSGTLG I P EHTYRSRVTL SNQPYIKVLTLANFTTKDEGDYFCELRVSGA NPMSSNKSISVYRDKLVKCGGISLLVQNTSWMLLLLLLSL S L LQALDFISL
32	LTBR, extracellular and transmembrane domain, human	N	ATGCTCCTGCCTTGGGCCACCTCTGCCCCGGCCTGGCCTG GGGGCCTCTGGTGCTGGGCCTCTTCGGGCTCCTGGCAGCAT CGCAGCCCCAGCGGTGCTCCATATGCGTCGGAGAACCAG ACCTGCAGGGACCAGGAAAAGGAATACTATGAGCCCCAGCA CCGCATCTGCTGCTCCCGCTGCCCGCCAGGCACCTATGTCT CAGCTAAATGTAGCCGCATCCGGGACACAGTTTGTGCCACA TGTGCCGAGAATTCTACAACGAGCACTGGAAC TACCTGAC CATCTGCCAGCTGTGCCGCCCTGTGACCCAGTGATGGGCC TCGAGGAGATTGCCCCCTGCACAAGCAAACGGAAGACCCAG TGCCGCTGCCAGCCGGGAATGTTCTGTGCTGCCTGGGCCCT CGAGTGACACACTGCGAGCTACTTTCTGACTGCCCGCCTG GCACTGAAGCCGAGCTCAAAGATGAAGTTGGGAAGGGTAAC AACCACTGCGTCCCCCTGCAAGGCCGGGCACTTCCAGAATAC CTCCTCCCCCAGCGCCCGCTGCCAGCCCCACACCAGGTGTG AGAACCAAGGTCTGGTGGAGGCAGCTCCAGGCACTGCCAG TCCGACACAACCTGCAAAAATCCATTAGAGCCACTGCCCC AGAGATGTCAGGAACCATGCTGATGCTGGCCGTTCTGCTGC

			CACTGGCCTTCTTTCTGCTCCTTGCCACCGTCTTCTCCTGC ATCTGG
33	LTBR, extracellular and transmembrane domain, human	P	MLLPWATSAPGLAWGPLVLGLFGLLAASQPQAVPPYASENQ TCRDQEKEYEYEPQHRICCSRCPPGTIVSAKCSRIRDTVCAT CAENSYNEHWNLTICQLCRPCDPVMGLEEIPCTSKRKTQ CRCQPGMFCAAWALECTHCELLSDCPPGTEAELKDEVGKGN NHCVPCKAGHFQNTSSPSARCQPHTRCENQGLVEAAPGTAQ SDTTCKNPLEPLPPEMSGTMLMLAVLLPLAFFLLLATVFSC IW
34	CD28 signaling domain, human	N	AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAA CATGACTCCCCGCGCCCGGGCCACCCGCAAGCATTACC AGCCCTATGCCCCCCCCACGCGACTTCGCAGCCTATCGCTCC
35	CD28 signaling domain, human	P	RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS
36	CD3 zeta domain, human	N	CTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCGCGTA CCAGCAGGGCCAGAACAGCTCTATAACGAGCTCAATCTAG GACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGC CGGGACCCTGAGATGGGGGAAAGCCGAGAAGGAAGAACCC TCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGG CGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGG AGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTAC AGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCC TGCCCCCTCGC
37	CD3 zeta domain, human	P	LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG RDPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR
38	TNFR2, complete AIR, human	P	MAPVAVWAALAVGLELWAAHALPAQVAFTPYAPEPGSTCR LREYYDQTAQMCCSKCSPGQHAKVFCTKSDTVCDSCEDST YTQLWNWVPECLSCGSRSSDQVETQACTREQNRICTRPG WYCALSKQEGCRLCAPLRKCRPGFGVARPGTETSDVVCPC APGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSP TRSMAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLPMG PSPPAEGSTGDFALPVGLIVGVTALGLLIIGVNCVIMTQV RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG RDPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR

39	TNFR2, extracellular and transmembrane domain, human	P	MAPVAVWAALAVGLELWAAHALPAQVAFTPYAPEGSTCR LREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDST YTQLWNWVPECLSCGSRCSDDQVETQACTREQNRICTRPG WYCALSKQEGCRLCAPLRKCRPGFGVARPGTETSDVVCPC APGTFSTNTSSTDICRPHQICNVVAIPGNASMDAVCTSTSP TRSMAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLPMG PSPPAEGSTGDFALPVGLIVGVTALGLLIIGVNCVIMTQV
40	DR3, complete AIR, human	P	MEQRPRGCAAVAAALLLVLLGARAQGGTRSPRCDGDFHK KIGLFCCRCGPAGHYLKAPCTEPCGNSTCLVCPQDTFLAWE NHHNSECARCQACDEQASQVALENC SAVADTRCGCKPGWFV ECQVSQCVSSSEFFYCQPCLDCGALHRHTRLLCSRRDTCGT CLPGFYEHDGCVSCPTSTLGSCPERCAAVCGWRQMFVWQV LLAGLVVPLLLGATLRSKRSRLHSDYMNMTPRRPGPTRKH YQPYAPPRDFAAYRSLRVKFSRSADAPAYQQGQNQLYNELN LGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQ ALPPR
41	DR3, extracellular and transmembrane domain, human	P	MEQRPRGCAAVAAALLLVLLGARAQGGTRSPRCDGDFHK KIGLFCCRCGPAGHYLKAPCTEPCGNSTCLVCPQDTFLAWE NHHNSECARCQACDEQASQVALENC SAVADTRCGCKPGWFV ECQVSQCVSSSEFFYCQPCLDCGALHRHTRLLCSRRDTCGT CLPGFYEHDGCVSCPTSTLGSCPERCAAVCGWRQMFVWQV LLAGLVVPLLLGATL
42	CD40, complete AIR, mouse	N	ATGGTGTCTTTGCCTCGGCTGTGCGCGCTATGGGGCTGCTT GTTGACAGCGGTCCATCTAGGGCAGTGTGTTACGTGCAGTG ACAAACAGTACCTCCACGATGGCCAGTGTGTGATTTGTGC CAGCCAGGAAGCCGACTGACAAGCCACTGCACAGCTCTTGA GAAGACCCAATGCCACCCATGTGACTCAGGCGAATTCTCAG CCCAGTGGAACAGGGAGATTGCTGTCAACAGCACAGACAC TGTGAACCCAATCAAGGGCTTCGGGTAAAGAAGGAGGGCAC CGCAGAATCAGACACTGTCTGTACCTGTAAGGAAGGACAAC ACTGCACCAGCAAGGATTGCGAGGCATGTGCTCAGCACACG CCCTGTATCCCTGGCTTTGGAGTTATGGAGATGGCCACTGA GACCACTGATACCGTCTGTCTATCCCTGCCAGTCGGCTTCT TCTCCAATCAGTCATCACTTTTCGAAAAGTGTTATCCCTGG ACAAGCTGTGAGGATAAGAACTTGAGGTCCTACAGAAAGG AACGAGTCAGACTAATGTCATCTGTGGTTTAAAGTCCCGGA TGCGAGCCCTGCTGGTCATTCTGTGCTGATGGGCATCCTC ATCACCATTTTCGGGGTGTTTCTCTATATCAACAGCAGAAG AAACAGAGGCGGCCAGAGCGACTACATGAACATGACCCCCA GAAGGCCAGGCCTGACCAGAAAGCCCTACCAGCCCTACGCC CCTGCCAGAGACTTCGCCGCTACAGACCCAGAGCCAAGTT CAGCAGATCCGCCGAGACAGCCGCCAACCTGCAGGATCCCA

			ACCAGCTGTACAACGAGCTGAACCTGGGCAGACGGGAGGAA TACGACGTGCTGAAAAAGAAGAGAGCCAGGGACCCCGAGAT GGGCGGCAAGCAGCAGAGAAGAAGAAACCCTCAGGAAGGCG TCTACAACGCCCTGCAGAAAGACAAGATGGCCGAGGCCTAC AGCGAGATCGGCACCAAGGGCGAGAGAAGAAGGGGCAAGGG CCACGATGGCCTGTACCAGGGCCTGTCCACCGCCACCAAGG ACACCTACGACGCCCTGCACATGCAGACCCTGGCCCCCAGA
43	CD40, complete AIR with CD28 costim, mouse	P	MVSLPRICALWGCLLTAVHLGQCVTCSDKQYLHDGQCCDLC QPGSRLTSHCTALEKTQCHPCDSGEFSAQWNREIRCHQHRH CEPNQGLRVKKEGTAEADTVCTCKEGQHCTSKDCEACAQHT PCIPGFGVMEMATETTTDTVCHPCPVGFFSNQSSLFKCYPW TSCEDKNLEVLQKGTSQTNVICGLKSRMRALLVIPVVMGIL ITIFGVFLYINSRRNRGGQSDYMNMTPRRPGLTRKPYQPYA PARDFAAAYRPAKFSRSAETAANLQDPNQLYNELNLGRREE YDVLEKKRARDPEMGGKQRRRNPOEGVYNALQDKMAEAY SEIGTKGERRRGKGGHDGLYQGLSTATKDTYDALHMQLAPR
44	CD40, extracellular and transmembrane domain, mouse	N	ATGGTGTCTTTGCCTCGGCTGTGCGCGCTATGGGGCTGCTT GTTGACAGCGGTCCATCTAGGGCAGTGTGTTACGTGCAGTG ACAAACAGTACCTCCACGATGGCCAGTGTGTGATTTGTGC CAGCCAGGAAGCCGACTGACAAGCCACTGCACAGCTCTTGA GAAGACCCAATGCCACCCATGTGACTCAGGCGAATTCTCAG CCCAGTGGAAACAGGGAGATTGCTGTGCTCACCAGCACAGACAC TGTGAACCCAATCAAGGGCTTCGGGTAAAGAAGGAGGGCAC CGCAGAATCAGACACTGTCTGTACCTGTAAGGAAGGACAAC ACTGCACCAGCAAGGATTGCGAGGCATGTGCTCAGCACACG CCCTGTATCCCTGGCTTTGGAGTTATGGAGATGGCCACTGA GACCACTGATACCGTCTGTCTATCCCTGCCCAGTCGGCTTCT TCTCCAATCAGTCATCACTTTTCGAAAAGTGTTATCCCTGG ACAAGCTGTGAGGATAAGAACTTGAGGTCCTACAGAAAGG AACGAGTCAGACTAATGTCATCTGTGGTTTAAAGTCCCGGA TGCAGGCCCTGCTGGTCATTCTGTGTCGTATGGGCATCCTC ATCACCATTTTCGGGGTGTTTCTCTATATC
45	CD40, extracellular and transmembrane domain, mouse	P	MVSLPRICALWGCLLTAVHLGQCVTCSDKQYLHDGQCCDLC QPGSRLTSHCTALEKTQCHPCDSGEFSAQWNREIRCHQHRH CEPNQGLRVKKEGTAEADTVCTCKEGQHCTSKDCEACAQHT PCIPGFGVMEMATETTTDTVCHPCPVGFFSNQSSLFKCYPW TSCEDKNLEVLQKGTSQTNVICGLKSRMRALLVIPVVMGIL ITIFGVFLYI
46	CD40, extracellular and	P	MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLC QPGQKLVSDCTEFTETECLPCGESEFLDTWNRETHCHQHKY CDPNLGLRVQQGTSETDTICTCEEGWHCTSEACESCVLHR

	transmembrane domain, human		SCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPW TSCETKDLVVQQAGTNKTDVVCGPQDRLRALVVIPIIFGIL FAILLVLVFI
47	CD40, complete AIR with CD28 costim, human	P	MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLC QPGQKLVS DCTEFTETECLPCGESEFLDTWNRETHCHQHKY CDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCVLHR SCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPW TSCETKDLVVQQAGTNKTDVVCGPQDRLRALVVIPIIFGIL FAILLVLVFIRSKRSRLHSDYMNMTPRRPGPTRKHYPYA PPRDFAAAYRSLRVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

The mode of action of the AIRs is shown in Figure 2. Binding of membrane bound TNF family members via AIRs leads to translation of the cytokine signal into a T cell receptor (TCR)-like signal. This initiates T cell activation independent of its endogenous TCR-MHC restriction in an environment/inflammation dependent manner.

Example 5: Transduction and surface expression of the AIRs

Transduction and expression of the AIRs according to the present disclosure was investigated in murine regulatory T cells (Treg). Tregs were isolated from spleen and lymph nodes of C57/BL6 mice, sorted (naïve CD4⁺CD25⁺CD62L⁺ Treg or Foxp3⁺ reporter (hCD2⁺) positive) and expanded via anti-CD3 and anti-CD28 antibody coupled beads plus IL-2 (2000 U/ml) stimulation for 6 days. Purity of Treg cultures was determined with flow cytometry. Treg cultures contained about 90-99% Foxp3⁺ T cells. On day two after isolation Treg were transduced with AIR constructs or an anti-CD19 Chimeric Antigen Receptor (anti-CD19 CAR) as control. Three days later transduction efficacy via CD90.1 staining and surface expression of AIRs was checked with flow cytometry. Results are shown in Figures 3 and 4. Similar results were obtained with a CD40 AIR (93.6% Foxp3⁺ T cells). It could be demonstrated that all AIRs are efficiently expressed on the surface of regulatory T cells.

Example 6: AIRs induce the expression of T-cell receptor signaling marker genes

To test if the AIRs of the present disclosure induce the T-cell receptor signaling, the induction of Nr4a1 was investigated. Nr4a1 is a well-known target gene of T-cell receptor signaling. Treg from Nr4a1-eGFP reporter mice were used. As a control, Treg were stimulated with anti-CD3/CD28 antibody-coupled beads. The outline of the experiment is shown in Figure 5.

Tregs expressing AIRs were stimulated in over-night co-culture with HEK cells expressing murine Light (mLight), mTL1A or mTNF. As a positive control, Tregs were stimulated with anti-CD3/CD28 antibody-coupled beads to mimic TCR signaling. Results are shown in Figure 6. Only AIR positive (CD90.1+) Tregs express Nr4a1-eGFP (left), but not AIR negative (CD90.1-) Tregs (right). Anti-CD19 CAR expressing Tregs, in contrast, did not show upregulation of Nr4a1-GFP (lower panel).

Figure 7 demonstrates the expression of Nr4a1 upon administration of an AIR according to the present disclosure, exemplified with an AIR carrying the extracellular domain of LTBR. Tregs expressing an LTBR AIR according to the present disclosure or an irrelevant CAR carrying extracellularly an anti-CD19 single chain antibody (scFv) were co-cultured with murine EL4 cells, which are known to express LIGHT, in absence or presence of a mLight blocking LTBR-Ig fusion protein (50 ug/ml) and afterwards stained for intracellular Nr4a1 expression.

Example 7: RNA and protein expression analysis of Tregs stimulated with AIRs

In this experiment it was tested if Tregs stimulated with AIRs according to the present disclosure show an expression pattern, as measured by RNA sequence expression analysis, that is consistent with the postulated TCR signaling effect.

First cells were sorted accordingly. Tregs expressing an AIR or an irrelevant CAR carrying extracellularly an anti-CD19 single chain antibody (scFv) were rested for 24 h and then co-cultured for 18 h with HEK cells or HEK cells expressing mLight. Figure 8 shows the pre-gating for CD90.1+ cells. Pre-gated cells were then sorted on CD4+CD25+CD90.1+ (left and middle) or CD4+CD25+CD90.1+Nr4a1+LAP+ (right). Results are shown in Figure 9.

After cell sorting, RNA was isolated and RNA sequencing was performed. Results are shown in Figures 10 (LTBR AIR), 11 (DR3 AIR) and 12 (α -CD19 control CAR).

Shown on the left in Figure 10 is a summary of up and down regulated genes comparing α -CD19 CAR Treg stimulated with HEK-mLight vs. LTBR AIR Treg stimulated with HEK cells as control (endogenous mLight signaling) and LTBR AIR Treg stimulated with HEK-mLight vs. α -CD19 CAR Treg stimulated with

HEK-mLight (specific LTBR AIR signaling). Shown on the right is a Volcano plot showing differentially expressed transcripts.

Similarly, in Figure 11 DR3 AIR Treg's were stimulated with HEK cells (endogenous mTL1A signaling). DR3 AIR Treg were stimulated with HEK-mTL1A vs. α -CD19 CAR Treg stimulated with HEK-mTL1A (specific DR3 AIR signaling). Differentially expressed gene are shown on the left, a Volcano plot is shown on the right.

Also similarly, Figure 12 compares an α -CD19 CAR Treg stimulated with anti-CD3/CD28 beads vs. unstimulated Treg. Differentially expressed gene are shown on the left, a Volcano plot is shown on the right.

The RNA and or protein expression levels of various specific marker genes were analyzed in more detail. Figure 13 shows the RNA expression data from LTBR AIR or control CAR expressing Treg for *Tnfrsf9*, *Tigit*, *Tgfb1*, *Cd69*, *Ccr8* and *Nr4a1* after 18 h co-culture with HEK +/-mLight (Deseq2, n = 3). LTBR AIRs (in each of the graphs shown on the very right) lead to a significant upregulation of all six marker genes tested. Figure 14 shows a representative flow cytometry analysis from LTBR AIR or control CAR expressing Tregs after 18 h co-culture with HEK +/-mLight. Protein expression of CD137 and *Tigit* is shown in the upper, CD69 and LAP (membrane-bound Tgf β 1) in the lower panel. Data is representative for three independent experiments. Again the LTBR AIR leads to a significant upregulation of the marker genes investigated.

Figure 15 shows RNA expression data from DR3 AIR or control CAR expressing Treg for *Tnfrsf9*, *Tigit*, *Tgfb1* after 18 h co-culture with HEK +/-mLight (Deseq2, n = 3). DR3 AIRs (in each of the graphs shown on the very right) lead to a significant upregulation of all three marker genes tested. Figure 16 shows a representative flow cytometry analysis from DR3 AIR or control CAR expressing Treg after 18 h co-culture with HEK +/-mTL1A. Protein expression of CD137 and *Tigit* is shown in the upper, CD69 and LAP (Tgf β 1) in the lower panel. Data is representative for three independent experiments. Again the DR3 AIR leads to a significant upregulation of the marker genes investigated.

Figure 17 shows RNA expression data (upper left, Deseq2, n = 3) and protein expression (right) from LTBR AIR or control CAR expressing Treg for *Irf8* after 18 h co-culture with HEK +/-mLight. RNA expression data (lower left, Deseq2, n = 3) and protein expression (right) from DR3 AIR or control CAR expressing Treg for *Irf8* after 18 h co-culture with HEK +/-mTL1A. Again, all marker genes tested are significantly upregulated.

Figure 18 demonstrates that the AIRs of the present disclosure mediate Treg activation and proliferation. LTBR AIR or control CAR expressing Treg were rested for 24 h and labeled with CFDA-SE proliferation dye. Engineered and labeled Treg were co-cultured with HEK +/- mLight for 72 h in

presence of IL2 and afterwards analyzed with flow cytometry for proliferation. Representative dot plots are shown left. Summarized data from three experiments performed in three technical replicates are shown right (n=3). Yet again, all marker genes tested are significantly upregulated.

In summary, the RNA and protein expression analyses clearly indicate that the AIRs of the present disclosure elicit a cellular response in line with the underlying hypothesis.

Example 8: LTBR AIR ameliorates GvHD pathology in mice

Usefulness of the AIRs of the present disclosure was tested in a Graft versus Host Diseases (GvHD) mouse model. FACS sorted Treg from Foxp3-hCD2 reporter mice were expanded and transduced with LTBR AIR or a truncated construct (lacking an extracellular binding domain, shown in Figure 1 a) as control. 2.5×10^5 engineered Treg were transplanted together with 2.5×10^6 bone marrow cells and 2.5×10^5 spleen cells from C57/BL6 mice into lethally irradiated (8 Gy) Balb/c mice. As transplant control, one group received only bone marrow cells (BM) and as disease developing control, one group received bone marrow cell plus spleen cells. After transplantation, animals were monitored for 47 days. An overview of the experiment is depicted in Figure 19. The quality of the engineered Tregs were checked prior to transfer into mice by staining for CD4, hCD2 (reporter for Foxp3) as well as CD90.1 and LTBR before transfer into mice. Results are shown in Figure 20. On day 20 a blood sample from each animal was taken to verify for donor derived (H2kB+) CD19+ B-cell recovery.

Figure 21 shows a Kaplan-Meier plot containing data set from two independent experiments (log rank test, n=11-12). As can be seen the LTBR AIR was highly efficient in the GvHD model, leading to a significantly increased survival of transplanted Balb/c mice. This can also be seen in Figure 22, which shows the mean GvHD score per treatment group (animals reaching a score of 40 had to be euthanized; dead animals kept the highest score over following time points for statistical analysis, two-way ANOVA). Figure 23 shows a representative FACS plot (left) of a spleen of a surviving LTBR AIR Treg receiving animal. Shown on the right is the percentage of Foxp3+ Tregs in spleen of surviving mice at day 47. Figure 24 shows the analysis of Klr1+ Tregs in spleens. Shown on the left is a representative FACS plot of discriminated CD45.1+ (BM derived) and CD45.2+ (transferred AIR Treg) Treg cells. Klr1 is a marker for the tissue specific phenotype; hCD2 is a Foxp3 reporter. Shown in the middle is the Klr1+ expression of engineered CD45.2+ and BM derived CD45.1+ Treg in surviving mice. Shown on the right frequency of Klr1+ hCD2+ Tregs in mice receiving LTBR AIR Treg (Mann-Whitney-U, survivors n=8 vs. dead n=4). Figure 25 shows a representative FACS analysis of a digested colon of a surviving LTBR AIR Treg receiving animal. Shown is discrimination between CD45.1 and CD45.2 cells within the

CD4⁺ population on the left. CD45.2 Treg are further analyzed for Klrp1 and hCD2 expression on the right. Figure 26 shows the percentage of hCD2⁺ (indicating Foxp3⁺) cells among transferred engineered CD45.2⁺ cells (left), demonstrating the phenotypic stability over 47 day *in vivo*. Shown on the right is the cell count of transferred CD45.2 Treg in colon at day 47.

Taken together, this example demonstrates the clinical usefulness of the AIRs of the present disclosure in an established clinical model for Graft versus Host Diseases.

Example 9: Human LTBR AIR

A human LTBR AIR construct was generated as similar to the murine LTBR construct. The human construct contains the same elements as the murine construct, except that all domains, i.e. the LTBR extracellular domain, the LTBR transmembrane domain, the cytoplasmic CD28 domain and the cytoplasmic CD3zeta domain are of human origin (SEQ ID No. 30 (nucleic acid); SEQ ID No. 31 (polypeptide)). Expression and the signaling capacity of the human LTBR AIR construct was tested.

Human T cells from healthy blood donors were sorted on Treg protein markers (TCRb⁺CD4⁺CD25⁺CD127⁺CD45RA⁺) and expanded with anti-CD3/CD28 stimulation (TransACT, Miltenyi Biotec, Bergisch Gladbach, Germany) and IL-2 (500 U/ml) for 7 days. On day 2 after isolation Treg were transduced with the human LTBR AIR construct (using an amphotropic version of the retroviral expression system MSCV). 5 days later cultured Treg were stained for intracellular Foxp3⁺ to check for Treg purity and for CD90.1 as well as hLTBR to analyze transduction efficacy and surface expression of the AIR protein. Results are shown in Figure 27. LTBR AIR expression on human Treg cells could be verified.

In another experiment, Tregs expressing the human LTBR AIR or an irrelevant CAR (an anti-CEA, Carcinoembryonic Antigen) were co-cultured for 18 h with parental HEK cells or HEK cells expressing human Light protein on the surface. Expression of Ccr8, Glycoprotein A repetitions predominant (GARP), CD137 (4-1BB) and Tigit is shown in CD90.1⁺Foxp3⁺ Tregs. Again, the expression levels of all marker genes confirm the postulated activity of the human LTBR AIR.

Example 10: TCR signaling is important for the activity of the AIRs

Control constructs were generated that are functional comparable to the AIRs of the present disclosure, with the important difference that they are devoid of the CD3zeta TCR signaling domain. Such constructs without a CD3zeta domain are known in the prior art (see WO2021/051195). Unlike

the constructs of WO2021/051195, the constructs provided by the present invention exert their function directly at the main signal of the signaling cascade and in a CD3 (TCR)-dependent manner.

The architecture of the construct is shown on the top of Figure 29. The amino acid sequence of this construct is depicted below:

```
MRLPRASSPCGLAWGPLLLGLSGLLVASQPQLVPPYRIENQTCWDQDKEYEYEPMDHVCCSRCPPGEFV
FAVCSRSQDTVCKTCPHNSYNEHWNHLSTCQLCRPCDIVLGFEEVAPCTSDRKAECRCQPGMSCVYLD
NECVHCEEERLVLCPGTEAEVTDEIMDTDVNCVPCKPGHFQNTSSPRARCQPHTRCEIQGLVEAAPG
TSYSDTICKNPPEPGAMLLLAILLSLVLFLLFTTVLACAWAAANSRRNRGGQSDYMNMTPRRPGLTRK
PYQPYAPARDFAAAYRPID (SEQ ID NO.: 48)
```

Surface expression of the construct could be demonstrated by flow cytometry. See Figure 29, bottom. However, unlike the AIRs of the present disclosure, the construct which is devoid of CD3zeta does not induce the marker genes. The AIRs of the present disclosure are hence functionally different from the molecules of the prior art. Figure 30 shows a representative flow cytometry analysis of Tregs expressing a full length LTBR AIR with a LTBR "AIR" lacking the CD3zeta-chain after 18 h co-culture with HEK +/-mLight. Protein expression of CD137 and Tigit is shown in the upper, CD69 and LAP (membrane-bound Tgfβ1) in the lower panel.

The importance of the CD3 zeta domain was further demonstrated with a LTBR-AIR, aLTBR-AIR without the CD3 zeta chain, and anti-CD19 CAR-expressing Treg cells from Nr4a1.eGFP reporter mice. Cells were rested for 24 h and then cocultured with HEK cells or HEK cells expressing mLIGHT for 18 h. Afterward cells were analyzed for eGFP and LAP expression via flow cytometry. Results of one of two independent experiments are shown in Figure 41. Only the full length LTBR AIR containing the CD3zeta domain, but not the other two constructs tested, is capable to induce Nr4a1.eGFP upregulation in response to the corresponding ligand, mLIGHT. This finding demonstrates again the importance of the CD3 domain for the AIR mediated TCR-like signaling and Treg cell activation.

Example 11: CD40 AIR leads to an expression functionally relevant marker genes

A similar experiment was also performed with a murine CD40 AIR of SEQ ID No. 43. This AIR was tested in comparison to a construct containing an extracellular anti-CD19 single chain antibody (scFv) instead of a binding domain of a Tumor-Necrosis-Factor (TNF) superfamily member (construct F of Figure 1). The CD40 AIR or the anti-CD19 construct expressing Treg cells were co-cultured for 18 h with HEK cells or HEK cells expressing CD40L (CD154). Afterwards cells were analyzed via flow cytometry

for expression of TIGIT and CD137 (Figures 31 and 32), as well as LAP (TGFB1) and CD69 (Figure 33 and 34).

Again, it could be shown that the AIRs of the present disclosure are functionally active and induce the expected marker genes (Figures 31 and 33). Results are summarized in Figures 32 and 34. The induction of the marker genes by the CD40 AIR is statistically significant as determined by a one-way Anova analysis.

Example 12: CD40 AIR triggers the expression of Nr4a1, an early response gene of TCR signaling

Treg cells from Nr4a1-eGFP reporter mice expressing the constructs of Example 11 were cocultured for 18 h with HEK cells or HEK cells expressing CD40L (CD154). Afterwards expression of Nr4a1-eGFP was measured by flow cytometry.

The expression of Nr4a1, an early response gene of TCR signaling, was induced by Treg cells expressing the CD40 AIR, but not by the construct expressing the anti-CD19 CAR (Figure 35), again confirming the TCR-like activation mediated by the AIRs of the present disclosure.

Example 13: Design of AIRs with alternative costimulatory domains

In order to test if alternative costimulatory domains are also functional, additional constructs were generated. To do so, the CD28 costimulatory domain of the construct shown in Figure 1G was replaced by the ICOS costimulatory domain and the 4-1BB costimulatory domain. These additional constructs are shown in Figure 36A (ICOS) and 36B (4-1BB). Sequences of the constructs and their parts are shown in Table 6 (N = nucleic acid, P = polypeptide). The CD90.1 marker gene is cut off via the P2A cleavage site and is not part of the AIR. Constructs were generated as outlined in Example 4.

Table 6:

SEQ ID No.	Description	N / P	Sequence
49	CD40, complete AIR with ICOS costim, mouse	N	ATGGTGTCTTTGCCTCGGCTGTGCGCGCTATGGGGCTGCTT GTTGACAGCGGTCCATCTAGGGCAGTGTGTTACGTGCAGTG ACAAACAGTACCTCCACGATGGCCAGTGCTGTGATTTGTGC CAGCCAGGAAGCCGACTGACAAGCCACTGCACAGCTCTTGA GAAGACCCAATGCCACCCATGTGACTCAGGCGAATTCTCAG

			CCCAGTGGAAACAGGGAGATTGCTGTACCAGCACAGACAC TGTGAACCCAATCAAGGGCTTCGGGTAAAGAAGGAGGGCAC CGCAGAATCAGACACTGTCTGTACCTGTAAGGAAGGACAAC ACTGCACCAGCAAGGATTGCGAGGCATGTGCTCAGCACACG CCCTGTATCCCTGGCTTTGGAGTTATGGAGATGGCCACTGA GACCACTGATACCGTCTGTCTATCCCTGCCAGTCGGCTTCT TCTCCAATCAGTCATCACTTTTCGAAAAGTGTATCCCTGG ACAAGCTGTGAGGATAAGAACTTGGAGGTCTACAGAAAGG AACGAGTCAGACTAATGTCATCTGTGGTTTAAAGTCCCGGA TGCAGGCCCTGCTGGTCATTCTGTCTGTATGGGCATCCTC ATCACCATTTTCGGGGTGTCTCTATATCTCAAAAAAGAA ATACGGATCTAGTGTGCATGACCCTAATAGTGAATACATGT TCATGGCGGCAGTCAACACAAAACAAAAGTCTAGACTTGCA GGTGTGACCTCAAGAGCCAAGTTCAGCAGATCCGCCGAGAC AGCCGCCAACCTGCAGGATCCCAACCAGCTGTACAACGAGC TGAACCTGGGCAGACGGGAGGAATACGACGTGCTGGAAAAG AAGAGAGCCAGGGACCCGAGATGGGCGGCAAGCAGCAGAG AAGAAGAAACCTCAGGAAGGCGTCTACAACGCCCTGCAGA AAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCACCAAG GGCGAGAGAAGAAGGGGCAAGGGCCACGATGGCCTGTACCA GGGCCTGTCCACCGCCACCAAGGACACCTACGACGCCCTGC ACATGCAGACCCTGGCCCCCAGA
50	CD40, complete AIR with ICOS costim, mouse	P	MVSLPRLCALWGCLLTAVHLGQCVTCSDKQYLHDGQCCDLC QPGSRLTSHCTALEKTQCHPCDSGEFSAQWNREIRCHQHRH CEPNQGLRVKKEGTAESDTVCTCKEGQHCTSKDCEACAQHT PCIPGFGVMEMATETTTDTVCHPCPVGFFSNQSSLFEKCPW TSCEDKNLEVLQKGSQTNVICGLKSRMRALLVIPVVMGIL ITIFGVFLYISKKKYGSSVHDPNSEYMFMAAVNTNKKSRILA GVTSTRAKFSRSAETAANLQDPNQLYNEINLGRREEYDVLEK KRARDPEMGGKQORRRNPQEGVYNALQKDKMAEAYSEIGTK GERRRGKGDGLYQGLSTATKDTYDALHMQLAPR
51	CD40, complete AIR with 4-1BB costim, mouse	N	ATGGTGTCTTTGCCTCGGCTGTGCGCGCTATGGGGCTGCTT GTTGACAGCGGTCCATCTAGGGCAGTGTGTTACGTGCAGTG ACAAACAGTACCTCCACGATGGCCAGTGTGTGATTTGTGC CAGCCAGGAAGCCGACTGACAAGCCACTGCACAGCTCTTGA GAAGACCCAATGCCACCCATGTGACTCAGGCGAATTCTCAG CCCAGTGGAAACAGGGAGATTGCTGTACCAGCACAGACAC TGTGAACCCAATCAAGGGCTTCGGGTAAAGAAGGAGGGCAC CGCAGAATCAGACACTGTCTGTACCTGTAAGGAAGGACAAC ACTGCACCAGCAAGGATTGCGAGGCATGTGCTCAGCACACG CCCTGTATCCCTGGCTTTGGAGTTATGGAGATGGCCACTGA GACCACTGATACCGTCTGTCTATCCCTGCCAGTCGGCTTCT TCTCCAATCAGTCATCACTTTTCGAAAAGTGTATCCCTGG ACAAGCTGTGAGGATAAGAACTTGGAGGTCTACAGAAAGG

			AACGAGTCAGACTAATGTCATCTGTGGTTTAAAGTCCCGGA TGCGAGCCCTGCTGGTCATTCTCTGTCTGATGGGCATCCTC ATCACCATTTTCGGGGTGTCTCTATATCAAATGGATCAG GAAAAAATCCCCACATATTCAAGCAACCATTAAAGAAGA CCACTGGAGCAGCTCAAGAGGAAGATGCTTGTAGCTGCCGA TGTCCACAGGAAGAAGAAGGAGGAGGAGGAGGCTATGAGCT GAGAGCCAAGTTCAGCAGATCCGCCGAGACAGCCGCCAACCC TGCAGGATCCCAACCAGCTGTACAACGAGCTGAACCTGGGC AGACGGGAGGAATACGACGTGCTGGAAGAAGAGAGCCAG GGACCCCGAGATGGGCGGCAAGCAGCAGAGAAGAAGAAACC CTCAGGAAGGCGTCTACAACGCCCTGCAGAAAGACAAGATG GCCGAGGCCTACAGCGAGATCGGCACCAAGGGCGAGAGAAG AAGGGCAAGGGCCACGATGGCCTGTACCAGGGCCTGTCCA CCGCCACCAAGGACACCTACGACGCCCTGCACATGCAGACC CTGGCCCCCAGA
52	CD40, complete AIR with 4-1BB costim, mouse	P	MVSLPRICALWGCLLTAVHLGQCVTCSDKQYLHDGQCCDLC QPGSRLTSHCTALEKTQCHPCDSGEFSAQWNREIRCHQHRH CEPNQGLRVKKEGTAESDTVCTCKEGQHCTSKDCEACAQHT PCIPGFGVMEMATETTDTVCHPCPVGFFSNQSSLFKCYPW TSCEDKNLEVLQKGTSTQTNVICGLKSRMRALLVIPVVGIL ITIFGVFLYIKWIRKKFPHIFKQPFKKTGAAQEEDACSCR CPQEEEEGGGGYELRAKFSRSAETAANLQDPNQLYNELNLG RREEYDVLEKKRARDPEMGGKQORRRNPQEGVYNALQDKM AEAYSEIGTKGERRRGKGDGLYQGLSTATKDTYDALHMQT LAPR
53	CD40, complete AIR with ICOS costim, human	P	MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLC QPGQKLVSDCTEFTETETECLPCGESEFLDTWNRETHCHQHKY CDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCVLHR SCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPW TSCETKDLVVQAGTNKTDVVCQPQDRLRALVVIPIIFGIL FAILLVLVFIKWLTKKKYSSSVHDPNGEYMFMRVNTAKKS RLTDVTLRVKFSRSADAPAYQQQONQLYNELNLGRREEYD VLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEI GMKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR
54	CD40, complete AIR with 4-1BB costim, human	P	MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLC QPGQKLVSDCTEFTETETECLPCGESEFLDTWNRETHCHQHKY CDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCVLHR SCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPW TSCETKDLVVQAGTNKTDVVCQPQDRLRALVVIPIIFGIL FAILLVLVFIKGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCELLRVKFSRSADAPAYQQQONQLYNELNLGRR EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEA

			YSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPP R
55	ICOS costimulatory domain, mouse	P	SKKKYGSSVHDPNSEYMFMAAVNTNKKSRLAGVTS
56	4-1BB costimulatory domain, mouse	P	KWIRKKFPHIFKQPFKKTGAAQEEDACSCRCPQEEEGGG GYEL
57	ICOS costimulatory domain, human	P	CWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL
58	4-1BB costimulatory domain, human	P	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE L

Example 14: Surface expression of CD40 AIR with ICOS and 41BB costimulatory domains

Transduction and surface expression of the AIRs with the ICOS and the 41BB costimulatory domains was tested as described in Example 5.

Transduction rates of the CD40 AIRs with different costimulatory domains (CD28, ICOS, 41BB) were comparable, as measured by flow cytometric analysis (data shown). Surface expression of the CD40 AIRs with the ICOS and the 41BB costimulatory domain are shown in Figure 37. It could be demonstrated that, like the CD40 AIR with a CD28 costimulatory domain, also the CD40 AIR with an ICOS costimulatory domain or a 41BB costimulatory domain are efficiently expressed on the surface of regulatory T cells.

Example 15: All tested costimulatory domains lead to signaling of the CD40 AIR

In this experiment the signaling capability of the CD40 AIRs with the three different costimulatory domains was compared. Treg cells expressing CD40-AIR versions with a CD28, an ICOS or a 41BB costimulatory domain were rested for 24 h, and then cocultured with HEK cells or HEK cells expressing

mCD40L for 18 h. Afterwards expression of CD69 and LAP was analyzed via flow cytometry (one way ANOVA).

Results are shown in Figure 38. All constructs tested show a similar capacity to induce Treg cell activation, confirming that any costimulatory domain can be used.

Example 16: CD40 ameliorates GvHD pathology in mice

Like the LTBR AIR (see Example 8), also the CD40 AIR was tested for its therapeutic usefulness in a Graft versus Host Diseases (GvHD) mouse model. Prior to transplantation Treg cells were quality controlled by flow cytometric analysis for transduction efficiency (CD90.1 expression) and CD40 AIR surface expression on Treg cells three days after transduction. As a control, a construct lacking the extracellular CD40-binding domain was used). Results are shown in Figure 39. Both construct, the CD40 AIR and the control construct, showed a high transduction efficiency, but only the cells transfected with the CD40 AIR exhibited binding to CD40.

Results of the experiment are shown in Figure 40. The Kaplan-Meier plot contains data set from two independent experiments (log rank test, n=10-12) and shows survival of transplanted BALB/c mice. As can be seen the CD40 AIR was highly efficient in the GvHD model, leading to a significantly increased survival of transplanted Balb/c mice. Taken together, this example validates the clinical usefulness of the AIRs of the present disclosure in an established clinical model for Graft versus Host Diseases.

Claims

1. An artificial immune receptor, comprising
 - b) an extracellular domain of a member of the tumor necrosis factor receptor superfamily,
 - c) a transmembrane domain,
 - d) a cytoplasmic costimulatory signaling domain, and
 - e) a cytoplasmic CD3 T cell receptor signaling domain.
2. An artificial immune receptor according to claim 1, wherein said member of the tumor necrosis factor receptor superfamily is selected from TNFR1, TNFR2, Fas, DR4, DR5, DR3, DR6, EDAR, XEDAR, TROY, LTBR, NGFR, CD18, CD134, CD40, CD27, CD30, CD137, TRAILR3, TRAILR4, CD265, osteoprotegerin, CD266, TACI, BAFF, BAFF receptor, APRIL, CD270, CD269 and CD357.
3. An artificial immune receptor according to claim 1 or 2, wherein said member of the tumor necrosis factor receptor superfamily is selected from TNFR2, DR3, LTBR and CD40.
4. An artificial immune receptor according to any one of claims 1-3, wherein said transmembrane domain is from the same protein as the extracellular domain.
5. The artificial immune receptor according to any one of claims 1-4, wherein said artificial immune receptor comprises the amino acid sequence of SEQ ID No.: 33, SEQ ID No.: 39, SEQ ID No.: 41 or SEQ ID No.: 46.
6. An artificial immune receptor according to any one of claims 1-5, wherein said cytoplasmic costimulatory signaling domain is selected from 4-1BB (CD137), BAFFR, OX40, CD27, CD28, ICOS, CD40, 2B4, GITR, HVEM, OX40, RELT, TACI, TROY, TWEAK, KIR receptors, TLR1 to TLR9 receptors, IL-2, IL-7 and IL-15 receptors.
7. An artificial immune receptor according to any one of claims 1-6, wherein said cytoplasmic costimulatory signaling domain is selected from 4-1BB (CD137), CD28 and ICOS.
8. An artificial immune receptor according to any one of claims 1-7, wherein said cytoplasmic costimulatory signaling domain comprises the amino acid sequence of SEQ ID No.: 35.
9. An artificial immune receptor according to any one of claims 1-8, wherein said cytoplasmic CD3 T cell receptor signaling domain is selected from CD3 zeta, CD3 gamma and CD3 epsilon, preferably CD3 zeta.

10. An artificial immune receptor according to any one of claims 1-9, wherein said cytoplasmic T cell receptor signaling domain comprises the amino acid sequence of SEQ ID No.: 37.
11. An artificial immune receptor according to any one of claims 1-10, wherein
 - a) said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from TNFR2, said cytoplasmic costimulatory signaling domain is CD28 and said cytoplasmic T cell receptor signaling domain is CD3 zeta,
 - b) said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from DR3, said cytoplasmic costimulatory signaling domain is CD28 and said cytoplasmic T cell receptor signaling domain is CD3 zeta,
 - c) said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from LTBR, said cytoplasmic costimulatory signaling domain is CD28 and said cytoplasmic T cell receptor signaling domain is CD3 zeta, or
 - d) said member of the tumor necrosis factor receptor superfamily and said transmembrane domain are from CD40, said cytoplasmic costimulatory signaling domain is CD28 and said cytoplasmic T cell receptor signaling domain is CD3 zeta.
12. A nucleic acid encoding an artificial immune receptor according to any one of claims 1-11.
13. A vector comprising a nucleic acid according to claim 12.
14. A host cell comprising a nucleic acid according to claim 12 or a vector according to claim 13, or expressing an artificial immune receptor according to any one of claims 1-11.
15. An artificial immune receptor according to any one of claims 1-11 or a host cell of claim 14 for use in medicine.