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(54) Title: COMPOSITIONS AND METHODS FOR TREATING PULMONARY HYPERTENSION

(57) Abrégé/Abstract:
In some aspects, the invention teaches pharmaceutical compositions that include a TGF-beta ligand trap, and methods of using a TGF-beta ligand trap to treat, prevent, or reduce the progression rate of pulmonary hypertension (PH). The invention also provides methods of using a TGF-beta ligand trap to treat, prevent, or reduce the progression rate of a variety of conditions including, but not limited to, pulmonary vascular remodeling, pulmonary fibrosis, right ventricular hypertrophy, diseases associated with excessive TGF-beta signaling, diseases associated with excessive GDF15 signaling, and diseases associated with excessive PAI-1 signaling. The invention further provides methods of using a TGF-beta ligand trap to reduce right ventricular systolic pressure in a subject.

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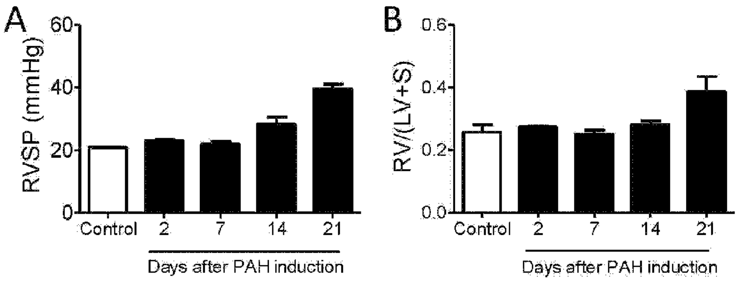
[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR TREATING PULMONARY HYPERTENSION

Figure 1A to 1B



(57) Abstract: In some aspects, the invention teaches pharmaceutical compositions that include a TGF-beta ligand trap, and methods of using a TGF-beta ligand trap to treat, prevent, or reduce the progression rate of pulmonary hypertension (PH). The invention also provides methods of using a TGF-beta ligand trap to treat, prevent, or reduce the progression rate of a variety of conditions including, but not limited to, pulmonary vascular remodeling, pulmonary fibrosis, right ventricular hypertrophy, diseases associated with excessive TGF-beta signaling, diseases associated with excessive GDF15 signaling, and diseases associated with excessive PAI-1 signaling. The invention further provides methods of using a TGF-beta ligand trap to reduce right ventricular systolic pressure in a subject.

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COMPOSITIONS AND METHODS FOR TREATING PULMONARY HYPERTENSION

[0001]

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH

[0002] This invention was made with government support under 5R01AR057374 awarded by the NIH. The government has certain rights in the invention.

FIELD OF INVENTION

[0003] The present invention generally relates to the field of medicine and cardiovascular and pulmonary diseases.

BACKGROUND

[0004]

The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0005] Many pathological processes and undesirable biological processes occur via ligand binding to cell surface receptors and excessive/overactive signaling. Thus, compositions and methods aimed at reducing or otherwise favorably modulating such binding and signaling can be useful.

[0006] The TGF-β superfamily includes a number of ligands of biological significance. TGF-β and Activin play important pathogenic roles in many diseases, including the progression of cancer and uncontrolled fibrosis, such as kidney, lung and liver fibrotic diseases. Myostatin/GDF8 is another important ligand, which is related to Activin, and which shares binding to the same Type II receptor

(ActivinRIIb). Myostatin is a powerful inhibitor of skeletal muscle growth and is a validated therapeutic target for muscle wasting diseases such as muscular dystrophy. Additional ligands in the TGF-β family include bone morphogenetic proteins (BMP), which have been implicated in cardiovascular diseases. For example, high levels of both BMP2 and BMP4 have been found in calcified atherosclerotic plaques and diseased aortic valves.

[0007] Methods have been developed to reduce ligand binding by trapping a ligand and preventing its interaction with cell surface receptors. Principal agents that target these ligands are ligand traps/antagonists that bind and sequester ligand. Two examples are: (1) anti-ligand antibodies and (2) soluble receptor ectodomains.

[0008] Inhibition of certain ligands has been reported using anti-ligand antibodies that trap and neutralize the ligand directly. Soluble versions of receptor ectodomains antagonize ligands directly by binding to them and preventing them from interacting with cell surface receptors. In the case of TGF-β, in animal models, expression of a TGF-β receptor type II (TβRII) ectodomain (ED) partially restored host immunity and promoted tumor clearance, indicating that receptor ectodomain-mediated neutralization of TGF-β inhibits tumor progression. Unfortunately, it has been demonstrated that monovalent TβRII-ED has less than optimal efficacy with respect to antagonizing TGF-β. Attempts to overcome this issue led to the production of bivalent artificially dimerized versions of TβRII-ED, which are dimerized via fusion to either coiled-coil domains or the Fc domain of IgG. This dimerization improved the antagonist effect. It has been demonstrated that non-covalent dimerization of TβRII-ED (for example, via fusion to heterodimerizing coil strands (coiled-coil TβRII-ED)), greatly enhances the antagonist potency of TβRII-ED (De Crescenzo et al., 2004, J. Biol, Chem. 279: 26013). A significant disadvantage of the coiled-coil fused dimer is that the non-covalent nature of the dimerization domain limits its potency, *i.e.* it dissociates at low concentrations such that a large portion of the coil-fused receptor ectodomain will be acting as a monomer rather than a dimer. Use of the Fc domain of IgG provides a covalent interaction, but at the cost of large size.

[0009] Importantly, among the obstacles to the clinical deployment of the TGFβRI inhibitors developed so far for treating PH has been toxicity, including hemorrhagic valve necrosis.

[00010] In view of the shortcomings of the therapeutic approaches attempted thus far, there is clearly a need in the art for receptor-based traps/neutralizers that can antagonize ligand activity and have the potential to act as therapeutic or diagnostic (imaging or non-imaging) agents for diseases/disorders caused by over-production/activity of the target ligands described herein.

SUMMARY OF THE INVENTION

[00011] The following embodiments and aspects thereof are described and illustrated in conjunction with systems, compositions and methods which are meant to be exemplary and illustrative, not limiting in scope.

[00012] Various embodiments of the present invention describe a pharmaceutical composition including a TGF-β ligand trap. In some embodiments, the TGF-β ligand trap is a soluble recombinant TGF-β type II receptor Fc-fusion protein (TGFBRII-Fc). In certain embodiments, the TGFBRII-Fc fusion protein comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:1.

[00013] Various embodiments of the present invention describe a method for treating, preventing, or reducing the progression rate of pulmonary hypertension (PH) in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of PH in the subject.

[00014] Various embodiments of the present invention describe a method of treating, preventing, or reducing the progression rate of pulmonary vascular remodeling in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of pulmonary vascular remodeling in the subject.

[00015] Various embodiments of the present invention describe a method of treating, preventing, or reducing the progression rate of pulmonary fibrosis in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of pulmonary fibrosis in the subject.

[00016] Various embodiments of the present invention describe a method of treating, preventing, or reducing the progression rate of right ventricular hypertrophy in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of right ventricular hypertrophy in the subject.

[00017] Various embodiments of the present invention describe a method of treating, preventing, or reducing the progression rate of a disease associated with excessive TGF-β signaling in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of the disease in the subject.

[00018] Various embodiments of the present invention describe a method of treating, preventing, or reducing the progression rate of a disease associated with excessive GDF15 signaling in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of the disease in the subject.

[00019] Various embodiments of the present invention describe a method of treating, preventing, or reducing the progression rate of a disease associated with excessive PAI-1 signaling in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of the disease in the subject.

[00020] Various embodiments of the present invention describe a method of reducing right ventricular systolic pressure in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby reducing right ventricular systolic pressure in the subject.

[00021] Various embodiments of the present invention describe a method of imaging/detecting TGF-β ligand in a subject, including administering a quantity of a TGF-β ligand trap linked to an imaging molecule to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[00022] Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

[00023] Figures 1A to 1E are graphs that demonstrate, in accordance with an embodiment of the invention, monocrotaline (MCT) induced pulmonary hypertension in rats is associated with increased PAI-1 and decreased Id1 transcriptional activity. Changes in right ventricular systolic pressure (RVSP, Fig. 1A) and right ventricular hypertrophy (RVH, Fig. 1B) were measured at various intervals after treatment of

Sprague Dawley rats with MCT (40 mg/kg SC). RVSP was measured by right ventricular catheterization, and RVH was determined by the ratio of the weight of the right ventricular (RV) free wall to the sum of the left ventricular and septal (LV+S) walls (n=3 per time point). Quantitative RT-PCR of lungs of MCT-treated rats revealed elevated PAI-1 transcription reflecting increased TGF-β signaling (Fig. 1C), the levels of which correlated directly with the degree of PH based on RVSP (Fig. 1D & Fig. 1E). In contrast, decreased expression of Bmpr2 and its transcriptional target Id1 were observed, with levels which both correlated inversely with RVSP. (n = 5-6, * $p<0.05$ and ** $p<0.01$ compared to control rats).

[00024] Figures 2A to 2F show immunoblots and graphs. Fig. 2A -2C demonstrate, in accordance with an embodiment of the invention, TGFBRII-Fc selectively inhibits the signaling of TGFβ1, TGFβ3, and GDF15 in human pulmonary artery smooth muscle cells (PASMC). Cultured PASMC were deprived of serum and incubated with BMP4, TGFβ1, TGFβ2, TGFβ3, and GDF15 ligands at various concentrations for 30 minutes. Western blot and qPCR were performed to assess the ability of TGFBRII-Fc to modulate signaling activity in vitro. Figure 2D-2F demonstrate, in accordance with an embodiment of the invention, TGFBRII-Fc selectively inhibits TGFβ1 and GDF15 signaling in vascular smooth muscle cells. (Fig. 2D) Human aortic smooth muscle cells were deprived of serum overnight, and then incubated with BMP4, TGFβ1, TGFβ2, or GDF15 at indicated concentrations for 30 min, and analyzed by immunoblot for phosphorylation of Smads 1, 2, 3 and 5 as shown. TGFβ1, TGFβ2, and GDF15 elicited activation of Smad2 and Smad3 in a dose dependent fashion, and Smads 1 and 5 to a lesser extent, whereas BMP4 only activated Smads 1 and 5. (Fig. 2E-Fig. 2F) HASMCs were deprived of serum, pretreated with TGFBRII-Fc (2000 ng/ml) or vehicle followed by incubation with TGFβ1 (1 ng/ml), TGFβ2 (1 ng/ml), or GDF15 (30 ng/ml) for 2 hours. Analysis of gene expression by qRT-PCR revealed potent inhibition of GDF15 and TGFβ1-induced PAI-1 and Id1 mRNA expression, but not that of TGFβ2 (n=3-5 samples each, * $p<0.05$, ** $p< 0.01$ compared to vehicle).

[00025] Figures 3A to 3D are graphs that demonstrate, in accordance with an embodiment of the invention, low dose TGFBRII-Fc treatment causes a trend towards reduced right ventricular systolic pressure (RVSP), a trend towards reduced right ventricular hypertrophy, and significantly reduced pulmonary vascular remodeling. Three weeks following treatment with MCT with or without TGFBRII-Fc (5 mg/kg, twice weekly), rats were analyzed in a blinded fashion by catheterization under anesthesia with pentobarbital and intratracheal intubation to determine RVSP (Fig. 3A), systemic arterial pressures (not shown), and euthanized. The degree of RVH was assessed in a blinded fashion based on measurement of Fulton's ratio (RV/(LV+S) (Fig. 3B). Values are represented as mean±SEM, n=6-8, *p<0.05 and **p<0.01 compared to control rats. Lung tissue sections were stained with alpha smooth muscle actin and von willebrand factor to identify vascular smooth muscle vessels and endothelium, respectively. Muscularization of distal intra-acinar vessels (10-50 μm diameter) was quantified, and the percentage of nonmuscular, partially muscularized, and fully (circumferentially) muscularized vessels was calculated (Fig. 3C). Medial wall thickness was calculated for all fully muscularized intra-acinar vessels (10-50 μm diameter, Fig. 3D). Wall thickness index was calculated as: index= (external diameter - internal diameter) / external diameter x 100. TGFBRII-Fc treatment (5 mg/kg, twice weekly) caused a trend towards reduced percentage of fully muscularized vessel and significantly reduced medial wall thickness index. Values are represented as mean±SEM, n=100-150 vessels per treatment group from 6-8 rats each, p values as shown.

[00026] Figures 4A to 4D show graphs that demonstrate, in accordance with an embodiment of the invention, high dose TGFBRII-Fc treatment attenuates right ventricular systolic pressure (RVSP), right ventricular hypertrophy, and prevents pulmonary vascular remodeling. Three weeks following treatment with MCT with or without TGFBRII-Fc (15 mg/kg, twice weekly), rats were analyzed in a blinded fashion to determine RVSP (Fig. 4A). The degree of RVH was assessed in a blinded fashion based on measurement of Fulton's ratio (Fig. 4B). Values are represented as mean±SEM, n=6-8. Muscularization of distal intra-acinar vessels (10-50 μm diameter) was quantified (Fig. 4C). Medial wall thickness was calculated for all fully muscularized intra-acinar vessels (10-50 μm diameter, Fig. 4D). TGFBRII-Fc treatment (15 mg/kg twice weekly) significantly reduced the percentage of fully muscularized vessels, and reduced medial wall thickness index. Values are represented as mean±SEM, n=89-127 vessels per treatment group from 6-8 rats each, *p<0.05 and ***p<0.001 compared to control rats.

[00027] Figures 5A to 5D show graphs that demonstrate, in accordance with an embodiment of the invention, TGFBRII-Fc attenuates echocardiographic RV hypertrophy. Following MCT (40 mg/kg SC) treatment, rats were treated with vehicle or TGFBRII-Fc (15 mg/kg, twice per week) starting 24 hours after MCT. Two weeks following MCT, rats were analyzed under anesthesia with 1.5% isoflurane by small animal ultrasonography to measure right ventricular thickness and diastolic dimension (Fig. 5A & Fig. 5B), pulmonary flow acceleration time (PAT, Fig. 5C), and pulmonary ejection time (PET, Fig. 5D). Values are represented as mean±SEM, n=6-8, *$p<0.05$ and ***$p<0.001$ compared to control rats.

[00028] Figures 6A to 6F are graphs that demonstrate, in accordance with an embodiment of the invention, TGFBRII-Fc inhibited TGFβ-mediated transcription in PH lung tissues. MCT-induced PH was correlated with a modest increase in TGFβ1 and a significant decrease in TGFβ2 mRNA expression (Fig. 6A-Fig. 6C). Suppression of Bmpr2 and Id1 expression following MCT treatment was not affected by TGFBRII-Fc (15 mg/kg twice weekly, Fig. 6D-Fig. 6E), whereas treatment with TGFBRII-Fc resulted in significant decreases in TGFβ1 and its transcriptional target PAI-1 (Fig. 6F). Values are represented as mean±SEM, n=3-5, *$p<0.05$ and ** $p<0.01$ compared to control.

[00029] Figures 7A to 7C are graphs that demonstrate, in accordance with an embodiment of the invention, treatment with TGFBRII-Fc following establishment of PH is associated with partial rescue of PH and mortality in accordance with various embodiments of the present invention. After treatment with MCT (40 mg/kg SC), rats were treated in a delayed fashion starting on day 17 after the establishment of PH with TGFBRII-FC (15 mg/kg three times weekly). Kaplan-Meier analysis (Fig. 7A) revealed a trend towards improved survival in the TGFBRII-Fc-treated group as compared to rats treated with vehicle (n=12 per group, p=0.10). Among surviving animals at 35 days, there was significantly decreased RVSP among animals treated with TGFBRII-Fc (Fig. 7B). Among surviving animals, however, there was no significant difference in RVH (**C**). Values shown are mean±SEM, n=8-11 per group, ** $p<0.01$ compared to control.

[00030] Figures 8A and 8B show the amino acid sequences of human IgG1, IgG2, IgG3 and IgG4 hinge (Fig. 8A) and Fc (Fig. 8B) domains. (IgG1 hinge domain (SEQ ID NO:65); IgG2 hinge domain (SEQ ID NO:66); IgG3 hinge domain (SEQ ID NO: 67); IgG4 hinge domain (SEQ ID NO: 68); Fig. 8B is shown in same order as Fig. 8A: IgG1 Fc domain (SEQ ID NO:69), i.e. first line of aa's; IgG2 Fc domain (SEQ ID NO:70) i.e. second line of aa's; IgG3 Fc domain (SEQ ID NO: 71) i.e. third line of aa's; IgG4 Fc domain (SEQ ID NO: 72), i.e. fourth line of aa's. The amino acid residues shown in Fig.8A and Fig.8B are numbered according to the numbering system of Kabat EU. Isotype sequences are aligned with the IgG1 sequence by placing the first and last cysteine residues of the respective hinge regions, which form the inter-heavy chain S--S bonds, in the same positions. For Fig.8B, residues in the CH2 domain are indicated by a plus sign (+), while residues in the CH3 domain are indicated by a squiggly line. Any Fc domain can be used in methods of the invention, all antibody sequences can be aligned as described in Figure 8 providing guidance for the various FC domains.

[00031] Figures 9A to 9B show tissue sections demonstrating the lack of mitral valve remodeling, degeneration or abnormalities in response to TGFBRII-Fc treatment. Figure 9A control. Figure 9B TGFBRII-Fc-treated.

DETAILED DESCRIPTION OF THE INVENTION

[00032]

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al., Dictionary of Microbiology and Molecular Biology 3rd ed.*, J. Wiley & Sons (New York, NY 2001); March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure 5th ed.*, J. Wiley & Sons (New York, NY 2001); and Sambrook and Russel, *Molecular Cloning: A Laboratory Manual 3rd ed.*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2001), provide one skilled in the art with a general guide to many of the terms used in the present application.

[00033] For references on how to prepare antibodies, see for example D. Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Press, Cold Spring Harbor NY, 1988); Kohler and Milstein, (1976) Eur. J. Immunol. 6: 511; Queen et al. U. S. Patent No. 5,585,089; and Riechmann et al., Nature 332: 323 (1988). The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, nucleic acid chemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, *Current Protocols in Immunology* (J. E. Coligan et al., eds., 1999, including supplements through 2011); *Current Protocols in Molecular Biology* (F. M. Ausubel et al., eds., 1987, including supplements through 2011); Short Protocols in Molecular Biology, F. M. Ausubel et al., eds., fifth edition 2002, including supplements through

2011; *Molecular Cloning: A Laboratory Manual,* third edition (Sambrook and Russel, 2001); *PCR: The Polymerase Chain Reaction, (*Mullis et al., eds., 1994); *The Immunoassay Handbook* (D. Wild, ed., Stockton Press NY, 1994); Bioconjugate Techniques (Greg T. Hermanson, ed., Academic Press, 1996); *Methods of Immunological Analysis* (R. Masseyeff, W. H. Albert, and N. A. Staines, eds., Weinheim: VCH Verlags gesellschaft mbH, 1993), Harlow and Lane *Using Antibodies: A Laboratory Manual* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999; and Beaucage et al. eds., Current Protocols in Nucleic Acid Chemistry John Wiley & Sons, Inc., New York, 2000).

[00034] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Other features and advantages of the invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, various features of embodiments of the invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, certain terms are defined below.

[00035] "Beneficial results" may include, but are in no way limited to, lessening or alleviating the severity of the disease condition, preventing the disease condition from worsening, curing the disease condition, preventing the disease condition from developing, lowering the chances of a patient developing the disease condition and prolonging a patient's life or life expectancy. In various embodiments, the disease condition is pulmonary hypertension, pulmonary vascular remodeling, pulmonary fibrosis, right ventricular hypertrophy, diseases associated with excessive TGF-β signaling, diseases associated with excessive GDF15 signaling, and diseases associated with excessive PAI-1 signaling.

[00036] "Treatment" and "treating", as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to slow down (lessen) the targeted pathologic condition, prevent the pathologic condition, pursue or obtain beneficial results, or lower the chances of the individual developing the condition even if the treatment is ultimately unsuccessful. Those in need of treatment include those already with the condition as well as those prone to have the condition or those in whom the condition is to be prevented.

[00037] "Pulmonary hypertension" (PH) as used herein can include an increase of blood pressure in the pulmonary artery (pulmonary arterial hypertension), pulmonary vein, or pulmonary capillaries, together known as the lung vasculature, leading to shortness of breath, dizziness, fainting, leg swelling and other symptoms. PH can be a severe disease with a markedly decreased exercise tolerance and heart failure. PH can be one of at least five different possible types, including: arterial, venous, hypoxic, thromboembolic or miscellaneous.

[00038] A "TGF-β ligand trap" as used herein refers to a protein that is capable of trapping a TGF-β ligand, even if only transiently, thereby modulating the ligand's ability to interact with one or more additional molecules.

[00039] In some embodiments, the TGF-β ligand can mean a ligand selected from among TGF-β1, TGF-β2, TGF-β3, and GDF 15.

[00040] An example of a TGF-β ligand trap includes, but is in no way limited to, a soluble recombinant TGF-β receptor Fc-fusion protein, which includes the TGF-β ligand binding domain of a TGF-β receptor and the Fc domain of an immunoglobin.

[00041] Accordingly, in one embodiment a method of treating, preventing, or reducing the progression rate of a pulmonary hypertension (PH) in a subject is provided. The method comprises administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of a PH in the subject, wherein the TGF-β ligand trap comprises 1) a TGF-β ligand binding domain of a TGF β receptor and 2) a Fc domain of an immunoglobulin, and 3) optionally a linker (an immunoglobulin linker or other linker) between the ligand binding domain and the Fc domain.

[00042] In one embodiment, the TGF-β ligand binding domain of a TGF β receptor comprises SEQ ID NO: 63, or portion thereof, or variant thereof: TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPD (SEQ ID NO: 63).

[00043] In one embodiment, the TGF-β ligand binding domain of a TGF β receptor comprises SEQ ID NO: 3, or SEQ ID NO; 4, or SEQ ID NO: 5, or portion thereof, or variant thereof:

[00044] In one embodiment, the Fc domain comprises SEQ ID NO: 64, or fragment/portion of SEQ ID NO: 64, or variant thereof.

SEQ ID NO: 64: ECPPCPAP PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTFRVV SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE KTISKTKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPMLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO: 64)

[00045] Further, exemplary Fc domains are described in Fig 1B, e.g. SEQ ID NO:'s 69, 70, 71 and 72. In certain embodiments the Fc domain comprises SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, or SEQ ID NO: 72, or comprise a fragment of SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, or SEQ ID NO: 72, or a variant of SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, or SEQ ID NO: 72.

[00046] It is within the capacity of one of ordinary skill in the art to select suitable binding domains in light of the disclosure herein. In some instances, the binding domains may be selected from the ectodomains of the TGF-β type II and TGF-β type I receptors. One non-limiting example is a soluble recombinant TGF-β type II receptor Fc-fusion protein (TGFBRII-Fc).

[00047] In a further example, the natural receptors from which the polypeptide binding domain is designed may be TβR-I-ED or TβR-II-ED.

[00048] In one embodiment the TGF-β ligand binding domain of a TGF β receptor comprises a sequence of the TGF-β type I receptor ectodomain, or portion of ectodomain, for example SEQ ID NO: 73, or portion thereof. 1 GVQVETISPG DGRTFPKRGQ TCVVHYTGML EDGKKFDSSR DRNKPFKFML GKQEVIRGWE EGVAQMSVGQ RAKLTISPDY AYGATGHPGI IPPHATLVFD VELLKLE (SEQ ID NO: 73), or e.g. SEQ ID NO: 74, or fragment/portion thereof, EDPSLDRPFI SEGTTLKDLI YDMTTSGSGS GLPLLVQRTI ARTIVLQESI GKGRFGEVWR GKWRGEEVAV KIFSSREERS WFREAEIYQT VMLRHENILG FIAADNKDNG TWTQLWLVSD YHEHGSLFDY LNRYTVTVEG MIKLALSTAS GLAHLHMEIV GTQGKPAIAH RDLKSKNILV KKNGTCCIAD LGLAVRHDSA TDTIDIAPNH RVGTKRYMAP EVLDDSINMK HFESFKRADI YAMGLVFWEI ARRCSIGGIH EDYQLPYYDL VPSDPSVEEM RKVVCEQKLR PNIPNRWQSC EALRVMAKIM RECWYANGAA RLTALRIKKT LSQLSQQEGI KM (SEQ ID NO: 74)(Chain A, Cytoplasmic Domain Of Unphosphorylated Type I Tgf-Beta

Receptor Crystallized Without Fkbp12 GeneBankACCESSION 1IAS_A GI:15988007.

[00049] In one embodiment the TGF-β ligand binding domain of a TGF β receptor comprises a sequence of the TβR-III-ED, or portion of SEQ ID NO: 75; 1 MTSHYVIAIF ALMSSCLATA GPEPGALCEL SPVSASHPVQ ALMESFTVLS GCASRGTTGL PQEVHVLNLR TAGQGPGQLQ REVTLHLNPI SSVHIHHKSV VFLLNSPHPL VWHLKTERLA TGVSRLFLVS EGSVVQFSSA NFSLTAETEE RNFPHGNEHL LNWARKEYGA VTSFTELKIA RNIYIKVGED QVFPPKCNIG KNFLSLNYLA EYLQPKAAEG CVMSSQPQNE EVHIIELITP NSNPYSAFQV DITIDIRPSQ EDLEVVKNLI LILKCKKSVN WVIKSFDVKG SLKIIAPNSI GFGKESERSM TMTKSIRDDI PSTQGNLVKW ALDNGYSPIT SYTMAPVANR FHLRLENNEE MGDEEVHTIP PELRILLDPG ALPALQNPPI RGGEGQNGGL PFPFPDISRR VWNEEGEDGL PRPKDPVIPS IQLFPGLREP EEVQGSVDIA LSVKCDNEKM IVAVEKDSFQ ASGYSGMDVT LLDPTCKAKM NGTHFVLESP LNGCGTRPRW SALDGVVYYN SIVIQVPALG DSSGWPDGYE DLESGDNGFP GDMDEGDASL FTRPEIVVFN CSLQQVRNPS SFQEQPHGNI TFNMELYNTD LFLVPSQGVF SVPENGHVYV EVSVTKAEQE LGFAIQTCFI SPYSNPDRMS HYTIIENICP KDESVKFYSP KRVHFPIPQA DMDKKRFSFV FKPVFNTSLL FLQCELTLCT KMEKHPQKLP KCVPPDEACT SLDASIIWAM MQNKKTFTKP LAVIHHEAES KEKGPSMKEP NPISPPIFHG LDTLT (SEQ ID NO: 75), (also known as soluble TGF-β receptor III, for example human recombinant soluble TGF-βsRIII is described in Moren A, *et al.* Molecular cloning and characterization of the human and porcine transforming growth factor-beta type III receptors, 1992, J. Biochem. Biophys. Res. Commun. 189 (1), 356-362).

[00050] Recombinant soluble TGF-βR type II cDNA is described in Melissa A. Rowland-Goldsmith *et al.* Soluble Type II Transforming Growth Factor-β (TGF-β) Receptor Inhibits TGF-β Signaling in COLO-357 Pancreatic Cancer Cells in Vitro and Attenuates Tumor Formation1, 2001, Clin Cancer Res, 7: 2931. The complete cDNA of human TβRII was used as the template for PCR amplification of the coding sequence of the extracellular domain of TβRII (nucleotides 1–477 including the signal sequence). PCR was performed using the sense primer, 5′-AAGCTTGCCGCCGCCATGGGTCG (SEQ ID NO: 76), and antisense primer, 5′-CTGGAATTCGTCAGGATTGCTGG (SEQ ID NO: 77). SEQ ID NO: 78 is an example of Type II Transforming Growth Factor-β (TGF-β) Receptor extracellular domain: MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL LLVIFQVTGI SLLPPLGVAI SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAIIL EDDRSDISST CANNINHNTE LLPIELDTLV GKGRFAEVYK AKLKQNTSEQ FETVAVKIFP YEEYASWKTE KDIFSDINLK HENILQFLTA EERKTELGKQ YWLITAFHAK GNLQEYLTRH VISWEDLRKL GSSLARGIAH LHSDHTPCGR PKMPIVHRDL KSSNILVKND LTCCLCDFGL SLRLDPTLSV DDLANSGQVG TARYMAPEVL ESRMNLENVE SFKQTDVYSM ALVLWEMTSR CNAVGEVKDY EPPFGSK (SEQ ID NO 78).

[00051] The complete extracellular portion of the TGF beta receptors typically includes unstructured segments flanking their folded ligand-binding domain. These unstructured extracellular portions are apparent from the experimentally determined 3D structures available from the PDB database (Berman *et al.*, 2000, Nucl. Acid Res. 28: 235), *e.g.*, crystal structures for type II TGF-β receptor ectodomain (Hart *et al.*, 2002 Nat. Struct. Biol. 9: 203; Boesen *et al.*, 2002, Structure 10: 913; Groppe *et al.*, 2008, Mol. Cell 29: 157), type I TGF-β receptor ectodomain (Groppe *et al.*, 2008, Mol. Cell 29:157), or the NMR structure of the type II TGF-β receptor ectodomain (Deep *et al.*, 2003, Biochemistry 42: 10126. One of skill in the art is well versed in identifying ligand binding domains of the TGF beta receptors. For the TGF beta traps, the binding of ligand can be confirmed using standard ligand binding assays, well known to those of skill in the art, e.g. radio ligand binding assays (See e.g. Sittampalam, G. S.; Kahl, S. D.; Janzen, W. P. High-throughput screening: Advances in assay technologies, 1997, Current Opinion in Chemical Biology 1 (3): 384–391; and De Jong, L. A. A.; et al. Receptor–ligand binding assays: Technologies and Applications, 2005, Journal of Chromatography B 829 (1–2): 1–25).

[00052] "TGFBRII-Fc" as used herein refers to a fusion protein including the TGF-β ligand binding domain of a TGF-β type II receptor or a variant or biologically active portion thereof and the Fc domain of an immunoglobin. In various embodiments, between the TGF-β ligand binding domain and the Fc domain, a linker can be included. Also in accordance with the present invention, a fusion protein can include the entire extracellular portion of a TGF-β type II receptor or a variant thereof and the Fc domain of an immunoglobin. In some embodiments, a fusion protein can include part of the extracellular portion of a TGF-β type II receptor or a variant thereof and the Fc domain of an immunoglobin. Examples of variants can include, but are not limited to, those that include conservative amino acid mutations, SNP variants, and splicing variants. One non-limiting example is the IIb splicing variant of the TGF-β type II receptor. In various embodiments, the TGF-β ligand binding domain and/or the Fc domain may be modified, for example, to facilitate purification, so long as such modifications do not reduce the functions of these domains to unacceptable level.

[00053] The basic technology of Fc-fusions has been generally described in the art, for example, in Czajkowsky *et al.* Fc-fusion proteins: new developments and future perspectives, EMBO Mol Med. 2012 Oct;4(10):1015-28.

The TGF-β type II receptor can be from a mammal. In some examples the receptor is from a human, monkey, ape, dog, cat, cow, horse, goat, sheep, pig, rabbit, mouse, or rat. The immunoglobin can be from a mammal. Merely by way of example, it can be from a human, monkey, ape, dog, cat, cow, horse, goat, sheep, pig, rabbit, mouse, or rat.

[00054] When referring to the antibody domains, the assignment of amino acids to each domain is in accordance with the definitions of Kabat (*See, "Sequences of Proteins of Immunological Interest"* by Elvin A. Kabat, Tai Te Wu, Kay S. Gottesman, Carl Foeller 5th edition, Publication no. 91 3242. *National Institutes of Health,* Bethesda, Md., 1991, and earlier editions). Amino acids from the variable regions of the mature heavy and light chains of immunoglobulins are designated by the position of an amino acid in the chain. Kabat described numerous amino acid sequences for antibodies, identified an amino acid consensus sequence for each subgroup, and assigned a residue number to each amino acid. Kabat's numbering scheme is extendible to antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. This method for assigning residue numbers has become standard in the field and readily identifies amino acids at equivalent positions in different antibodies, including chimeric or humanized variants. For example, an amino acid at position 50 of a human antibody light chain occupies the equivalent position to an amino acid at position 50 of a mouse antibody light chain.

[00055] As used herein, the term "Fc region," "Fc domain" or analogous terms are used to define CH2/CH3 C-terminal region of an IgG heavy chain. An example of the amino acid sequence containing the human IgG1 is shown in FIG. 8B. Although boundaries may vary slightly, as numbered according to the Kabat system, the Fc domain extends from amino acid 231 to amino acid 447 (amino acid residues in FIG. 8B are numbered according to the Kabat system: *See* Kabat et al., "Sequences of Proteins of Immunological Interest", 5th Ed. Public Health Service, NIH, MD (1991)). FIG. 8B also provides examples of the amino acid sequences of the Fc regions of IgG isotypes IgG1, IgG2, IgG3, and IgG4.

[00056] The Fc region of an IgG comprises two constant domains, CH2 and CH3. The CH2 domain of a human IgG Fc region usually extends from amino acids 231 to amino acid 341 according to the numbering system of Kabat (FIG. 8B). The CH3 domain of a human IgG Fc region usually extends from amino acids 342 to 447 according to the numbering system of Kabat (FIG. 8B). The CH2 domain of a human IgG Fc region (also referred to as "Cγ2" domain) is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG.

[00057] Examples of TGFBRII-Fc include, but are not limited to, a protein having the sequence set forth in SEQ ID NO:1 or a variant thereof. In one embodiment, a variant of SEQ ID NO:1 includes a sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO:1.

TIPPHVQKSV NNDMIVTDNN GAVKFPQLCK FCDVRFSTCD NQKSCMSNCS ITSICEKPQE VCVAVWRKND ENITLETVCH DPKLPYHDFI LEDAASPKCI MKEKKKPGET FFMCSCSSDE CNDNIIFSEE YNTSNPDTGG GVECPPCPAP PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTFRVV SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE KTISKTKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPMLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK (SEQ ID NO:1).

[00058] In SEQ ID NO:1, amino acids 1-137 are a TGF-β ligand binding domain, amino acids 138-141 are a linker and amino acids 142 - 364 are an Fc domain. This exemplar TGFBRII-Fc can be expressed by a nucleic acid that includes a nucleotide sequence set forth in SEQ ID NO:2, or a degenerate variant thereof. A "degenerate variant" as used herein refers to a variant that has a mutated nucleotide sequence, but still encodes the same polypeptide due to the redundancy of the genetic code.

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC

51 AGTCTTCGTT TCGCCCGGCG CCACGATCCC ACCGCACGTT CAGAAGTCGG
101 TTAATAACGA CATGATAGTC ACTGACAACA ACGGTGCAGT CAAGTTTCCA
151 CAACTGTGTA AATTTTGTGA TGTGAGATTT TCCACCTGTG ACAACCAGAA
201 ATCCTGCATG AGCAACTGCA GCATCACCTC CATCTGTGAG AAGCCACAGG
251 AAGTCTGTGT GGCTGTATGG AGAAAGAATG ACGAGAACAT AACACTAGAG
301 ACAGTTTGCC ATGACCCCAA GCTCCCCTAC CATGACTTTA TTCTGGAAGA
351 TGCTGCTTCT CCAAAGTGCA TTATGAAGGA AAAAAAAAAG CCTGGTGAGA
401 CTTTCTTCAT GTGTTCCTGT AGCTCTGATG AGTGCAATGA CAACATCATC
451 TTCTCAGAAG AATATAACAC CAGCAATCCT GACACCGGTG GTGGAGTCGA
501 GTGCCCACCG TGCCCAGCAC CACCTGTGGC AGGACCGTCA GTCTTCCTCT
551 TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
601 ACGTGCGTGG TGGTGGACGT GAGCCACGAA GACCCCGAGG TCCAGTTCAA
651 CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCACGGG
701 AGGAGCAGTT CAACAGCACG TTCCGTGTGG TCAGCGTCCT CACCGTCGTG
751 CACCAGGACT GGCTGAACGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA
801 AGGCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAACC AAAGGGCAGC
851 CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA GGAGATGACC
901 AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ACCCCAGCGA
951 CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA
1001 CCACACCTCC CATGCTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG
1051 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC
1101 CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC
1151 TGTCTCCGGG TAAA (SEQ ID NO: 2)

[00059] The "hinge region" or "hinge domain" of a heavy chain IgG is generally defined as stretching from Glu216 to Pro230 of human IgG1 using Kabat numbering. An example of the amino acid sequence of the human IgG1 hinge region is shown in **FIG. 8A** (amino acid residues in FIG. 8A are numbered according to the Kabat system). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S--S binds in the same positions as shown in **FIG. 8A.** In certain embodiments, the linker between the ligand binding domain and the Fc domain comprises a hinge region, e.g. any of SEQ ID NO: 65-68 (See Figure 8A). In one embodiment, the linker comprises TGG G (SEQ ID NO: 79). In certain embodiments the linker comprises any of SEQ ID NO's: 6-48 (See Example 3).

[00060] One of skill in the art would readily appreciate that substantially identical peptides to those specifically described herein are contemplated and may include one or more conservative amino acid mutations. It is known in the art that one or more conservative amino acid mutations to a reference peptide may yield a mutant peptide with no substantial change in physiological, chemical, or functional properties, compared to the reference peptide; and in such a case, the reference and mutant peptides would be considered "substantially identical" polypeptides.

[00061] A conservative amino acid mutation may include the addition, deletion, or substitution of an amino acid. A conservative amino acid substitution is defined herein as the substitution of an amino acid residue for another amino acid residue with similar chemical properties (*e.g.* size, charge, or polarity). In a non-limiting example, a conservative mutation may be an amino acid substitution. Such a conservative amino acid substitution may substitute a basic, neutral, hydrophobic, or acidic amino acid for another of the same group.

[00062] As used herein, "basic amino acid" includes hydrophilic amino acids having a side chain pKa value of greater than 7, which are typically positively charged at physiological pH. Basic amino acids include histidine (His or H), arginine (Arg or R), and lysine (Lys or K). As used herein, "neutral amino acid" (also "polar amino acid") means hydrophilic amino acids having a side chain that is uncharged at physiological pH, but in which at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Polar amino acids include serine (Ser or S), threonine (Thr or T), cysteine (Cys or C), tyrosine (Tyr or Y), asparagine (Asn or N), and glutamine (Gin or Q). The term "hydrophobic amino acid" (also "non-polar amino acid") is meant to include amino acids exhibiting a hydrophobicity of greater than zero according to the normalized consensus hydrophobicity scale of Eisenberg (1984). Hydrophobic amino acids include proline (Pro or P), isoleucine (Ile or I), phenylalanine (Phe or F), valine (Val or V), leucine (Leu or L), tryptophan (Trp or W), methionine (Met or M), alanine (Ala or A), and glycine (Gly or G). "Acidic amino acid" refers to hydrophilic amino acids having a side chain pKa value of less than 7, which are typically negatively charged at physiological pH. Acidic amino acids include glutamate (Glu or E), and aspartate (Asp or D).

[00063] Sequence identity is used to evaluate the similarity of two sequences; it is determined by calculating the percent of residues that are the same when the two sequences are aligned for maximum correspondence between residue positions. Any known method may be used to calculate sequence identity; for example, computer software is available to calculate sequence identity. By way of non-limiting example, sequence identity can be calculated by software such as BLAST-P, Blast-N, or FASTA-N, or any other appropriate software that is known in the art. The substantially identical sequences of the present invention may be at least 80% identical. In other examples, the substantially identical sequences may be at least 80%, 85%, 90%, 95%, or 100% identical at the amino acid level to sequences described herein.

[00064] As indicated above, in various embodiments, between the TGF-β ligand binding domain and the Fc domain, there may be a linker. Provided herein are sequences of such linkers. In one embodiment, the linker is an unstructured and flexible polypeptide sequence. The linker region provides a segment that is distinct from the structured ligand binding and Fc domains and thus can be used for conjugation to accessory molecules (for example, molecules useful in increasing stability such as PEGylation moieties) or cargo molecules such as contrast agents for imaging and toxins without having to chemically modify the ligand binding and Fc domains. Conjugation methodologies are somewhat diverse, but typically can be performed using commercial kits that enable conjugation via common reactive groups such as primary amines, succinimidyl (NHS) esters and sulfhydral-reactive groups. Some non-limiting examples are: Alexa Fluor 488 protein labeling kit (Molecular Probes, Invitrogen detection technologies) and PEGylation kits (Pierce Biotechnology Inc.).

[00065] The linker may include an unstructured amino acid sequence that may be either the same as or derived from conservative modifications to the sequence of a natural unstructured region in the extracellular portion of the receptor for the ligand of interest or another receptor in the TGF-β superfamily. In other instances, such linkers may be entirely artificial in composition and origin but will contain amino acids selected to provide an unstructured flexible linker with a low likelihood of encountering electrostatic or steric hindrance complications when brought into close proximity to the ligand of interest.

[00066] The length of the linker is considered to be the number of amino acids between: (a) the C-terminal main chain carbon atom of the binding domain located at the linker's N-terminal end; and (b) the N-terminal main-chain nitrogen atom of binding domain located at the linker's C-terminal end. Linker length will be considered acceptable when it permits binding domains to bind their natural binding sites on their natural ligand. Examples of natural and artificial linker sequences of varying length are given in Table 2. For example, and without wishing to be limiting in any manner, the linker length may be between about 18-80 amino acids, 25-60 amino acids, 35-45 amino acids, or any other suitable length.

[00067] In some instances, it may be desirable to subject the polypeptide-based linking design of the ligand binding agents disclosed herein to optimization of characteristics desired for a particular application. For example, the linker may be modified in length and composition based on atomic-level simulations and knowledge-based design in order to improve binding affinity, specificity, immunogenicity and stability. This is applicable to a wide range of molecular systems exhibiting homomeric, heteromeric, dimeric and multimeric ligand-receptor structural characteristics. Additional different binding domains can be incorporated to generate multivalent traps with even higher binding potency.

[00068] Linkers may be designed to facilitate purification of the linker and/or ligand binding trap. The exact purification scheme chosen will determine what modifications are needed, for example and without wishing to be limiting, additions of purification "tags" such as His tags is contemplated; in other examples, the linker may include regions to facilitate the addition of cargo or accessory molecules. When such additions affect the unstructured nature of the linker or introduce potential electrostatic or steric concerns, appropriate increases to the linker length will be made to ensure that the binding domains are able to bind their sites on the ligand. In light of the methods and teachings herein, such determinations could be made routinely by one skilled in the art.

[00069] In an embodiment of the invention in which the ligand-binding domains and the linker contain primarily natural sequences they would not ordinarily be expected to be severely immunogenic or toxic in a typical patient.

[00070] Polypeptides of the invention can be useful as therapeutic agents that neutralize the action of disease-associated covalently-stabilized dimeric ligands such as growth factors. They may also have commercial potential for use as diagnostic agents to detect the presence of disease-associated covalently-stabilized dimeric ligands such as growth factors in imaging and non-imaging diagnostic applications.

[00071] The present invention also encompasses nucleotide sequences encoding polypeptides of the invention. These nucleotide sequences can be cloned and inserted into any suitable vector (including expression vector) and therefore are very amenable to production of polypeptides of the invention.

[00072] The term "vector," as used herein, refers to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be "exogenous," which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g., YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques.

Additionally, the techniques described herein and demonstrated in the referenced figures are also instructive with regard to effective vector construction.

[00073] The term "expression vector" refers to any type of genetic construct comprising a nucleic acid coding for a RNA capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules or ribozymes. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host cell. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described infra.

[00074] The term "polypeptide" or "protein," as used herein, means a polymer of amino acids joined in a specific sequence by peptide bonds. As used herein, the term "amino acid" refers to either the D or L stereoisomer form of the amino acid, unless otherwise specifically designated.

[00075] A "biologically active" portion of a molecule, as used herein, refers to a portion of a larger molecule that can perform a similar function as the larger molecule. Merely by way of a non-limiting example, a biologically active portion of a protein is any portion of a protein which retains the ability to perform one or more biological functions of the full-length protein (*e.g.* binding with another molecule, phosphorylation, etc.), even if only slightly. As a non-limiting example, the ligand binding domain is a biological portion of a TGFβ receptor.

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[00076] As used herein, the term "therapeutically effective amount" means the amount of a TGF-β ligand trap that attenuates or inhibits excessive TGF-β signaling and hence results in treating, preventing or slowing the progression rate of a disease condition described herein. An effective amount will vary, depending upon the pathology or condition to be treated, by the patient and his or her status, and other factors well known to those of skill in the art. Effective amounts are easily determined by those of skill in the art. In some embodiments a therapeutic dose is administered at an interval from every day to every month via the subcutaneous, intrathecal, convection-enhanced, intravenous or intra-arterial route at a dose ranging from 0.05 mg to 50mg/kg of body weight, and optionally 1.0 mg to 10 mg/kg of body weight or 0.3 mg to 3.0 mg/kg of body weight. In various embodiments, the TGF-β ligand trap is administered to the subject 1-7 times per week or once weekly, or once every two, three or four weeks. In various embodiments, the TGF-β ligand trap is administered to the subject for 1-5 days, 1-5 weeks, 1-5 months, or 1-5 years.

[00077] Although certain exemplar routes of administration are provided according to the invention, any suitable route of administration of a TGF-β ligand trap may be adapted, and therefore the routes of administration described herein are not intended to be limiting. Routes of administration may including but are not limited to, intravenous, oral, buccal, intranasal, inhalation, topical application to a mucosal membrane or injection, including intradermal, intrathecal, intracisternal, intralesional or any other type of injection. Administration can be effected continuously or intermittently and will vary with the subject and the condition to be treated. One of skill in the art would readily appreciate that the various routes of administration described herein would allow for a TGF-β ligand trap or compositions to be delivered on, in, or near the pulmonary disease locations or targeted cells. One of skill in the art would also readily appreciate that various routes of administration described herein will allow for a TGF-β ligand trap and compositions described herein to be delivered to a region in the vicinity of diseased tissues, organs, or individual cells to be treated. "In the vicinity" can include any tissue or bodily fluid in the subject that is in sufficiently close proximity to or in sufficient communication with diseased tissues, organs, or individual cells such that at least a portion of the TGF-β ligand trap or compositions administered to the subject reach their intended targets and exert their therapeutic effects.

[00078] *Pharmaceutical Compositions*

[00079] In various embodiments, the present invention provides a pharmaceutical composition that includes a TGF-β ligand trap described herein. In various embodiments, the pharmaceutical composition is formulated for modified release, sustained release, or controlled release, or a combination thereof. In various embodiments, the pharmaceutical composition is formulated for oral, via inhalation, nasal, sublingual, buccal, subcutaneous, intradermal, intramuscular, intravenous, intraperitoneal, or parenteral administration.

[00080] In various embodiments, the pharmaceutical composition further includes at least one pharmaceutically acceptable excipient. Examples of excipients include but are not limited to starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, wetting agents, emulsifiers, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservatives, antioxidants, plasticizers, gelling agents, thickeners, hardeners, setting agents, suspending agents, surfactants, humectants, carriers, stabilizers, and combinations thereof.

[00081] In various embodiments, the pharmaceutical composition further includes at least one pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include aqueous solutions such as physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, vegetable oils (e.g., olive oil) or injectable organic esters. A pharmaceutically acceptable carrier can be used to administer the compositions of the invention to a cell in vitro or to a subject *in vivo*. A pharmaceutically acceptable carrier can contain a physiologically acceptable compound that acts, for example, to stabilize the composition or to increase the absorption of the agent. A physiologically acceptable compound can include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives, which are particularly useful for preventing the growth or action of microorganisms. Various preservatives are well known and include, for example, phenol and ascorbic acid. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the polypeptide. For example, a physiologically acceptable compound such as aluminum monosterate or gelatin is particularly useful as a delaying agent, which prolongs the rate of absorption of a pharmaceutical composition administered to a subject. Further examples of carriers, stabilizers or adjutants can be found in Martin, Remington's Pharm. Sci., 15th Ed. (Mack Publ. Co., Easton, 1975).

Other examples of carriers include, but are not limited to, a nanoparticle-based carrier (e.g. a polymer N-(2-hydorxylpropyl)methacrylamide (HPMA), glutamic acid, PEG, dextran) and a nanocarrier (e.g., nanoshell, liposome, nanoliposome).

[00082] *Treatment Methods*

[00083] In various embodiments, the present invention provides a method of treating, preventing, or reducing the progression rate of pulmonary hypertension (PH) in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of PH in the subject. In some embodiments, the method can further include mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject. Pulmonary arterial hypertension is a type of pulmonary hypertension that may be particularly amenable to treatment with a TGF-β ligand trap. Accordingly, in some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of pulmonary arterial hypertension in the subject, including any of the following subcategories of pulmonary arterial hypertension. Pulmonary arterial hypertension can arise secondary to other conditions or a as a primary or idiopathic pulmonary arterial hypertension. Of particular interest, certain types of familial pulmonary arterial hypertension are associated with decreased expression or function of the bone morphogenetic protein receptor type II (BMPRII), which is thought to result in excessive signaling by TGF-β.

[00084] Pulmonary hypertension can be of five major types, thus a series of tests is performed to distinguish pulmonary arterial hypertension from venous, hypoxic, thromboembolic, or miscellaneous varieties. These generally include pulmonary function tests; blood tests to exclude HIV, autoimmune diseases, and liver disease; electrocardiography (ECG); arterial blood gas measurements; X-rays of the chest (followed by high-resolution CT scanning if interstitial lung disease is suspected); and ventilation-perfusion or V/Q scanning to exclude chronic thromboembolic pulmonary hypertension. Diagnosis of PAH requires the presence of pulmonary hypertension. Although pulmonary arterial pressure can be estimated on the basis of echocardiography, pressure measurements with a Swan-Ganz catheter through the right side of the heart provides the most definite assessment for diagnosis.

[00085] On of skilled in the art is well versed in monitoring improvement in pulmonary hypertension, e.g. Clinical improvement is often measured by a "six-minute walk test", i.e. the distance a patient can walk in six minutes. Stability and improvement in this measurement correlate with better survival. Blood BNP level is also being used now to follow progress of patients with pulmonary hypertension. Improvement of symptoms can also be monitored by assaying arterial pressure. For example, normal pulmonary arterial pressure in a person living at sea level has a mean value of 8–20 mm Hg (1066–2666 Pa) at rest. Pulmonary hypertension is present when mean pulmonary artery pressure exceeds 25 mm Hg (3300 Pa) at rest. Mean pulmonary artery pressure (mPAP) should not be confused with systolic pulmonary artery pressure (sPAP), which is often reported on echocardiogram reports. A systolic pressure of 40 mm Hg typically implies a mean pressure of more than 25 mm Hg. Roughly, mPAP = 0.61•sPAP + 2.

[00086] In various embodiments, the present invention provides a method of treating, preventing, or reducing the progression rate of pulmonary vascular remodeling in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of pulmonary vascular remodeling in the subject. In some embodiments, the method can further include mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject.

[00087] In various embodiments, the present invention provides a method of treating, preventing, or reducing the progression rate of vascular remodeling in the heart of a subject. In some embodiments, the method can further include mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject. In certain embodiments, the methods of the invention reduce mitral valve degeneration, or e.g. mitral valve prolapse. Beneficial effects can be monitored by echocardiography.

[00088] In various embodiments, the present invention provides a method of treating, preventing, or reducing the progression rate of pulmonary fibrosis in a subject. In certain embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of pulmonary fibrosis in the subject.In various embodiments, the method can further include mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject.

[00089] In various embodiments, the present invention provides a method of treating, preventing, or reducing the progression rate of right ventricular hypertrophy in a subject. In various embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of right ventricular hypertrophy in the subject. In some embodiments, the method can further include mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject.

[00090] In various embodiments, the present invention provides a method of treating, preventing, or reducing the progression rate of a pulmonary disease associated with excessive TGF-β signaling in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of the disease in the subject.In some embodiments, the method can further include mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject.

[00091] In various embodiments, the TGF-β can be TGF-β1, TGF-β3, or a combination thereof.

[00092] In various embodiments, the present invention provides a method of treating, preventing, or reducing the progression rate of a pulmonary disease associated with excessive GDF15 signaling in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of the disease in the subject. In some embodiments, the method can further include mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject.

[00093] In various embodiments, the present invention provides a method of treating, preventing, or reducing the progression rate of a pulmonary disease associated with excessive PAI-1 signaling in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of the disease in the subject.In some embodiments, the method can further include mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject.

[00094] In various embodiments, the present invention provides a method of reducing right ventricular systolic pressure in a subject. In some embodiments, the method includes administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby reducing right ventricular systolic pressure in the subject.In certain embodiments, the method can further include mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject.

[00095] In various embodiments, the subjects in the examples described above are mammals. In some embodiments, the subject is a human, monkey, ape, dog, cat, cow, horse, goat, sheep, pig, rabbit, mouse, or rat. In various embodiments, the TGF-β is TGF-β1, TGF-β3, or a combination thereof.

[00096] In various embodiments, the amount of TGF-β ligand trap administered to the subject is 0.05 mg to 50mg/kg of body weight, and optionally 1.0 mg to 10 mg/kg of body weight or 0.3 mg to 3.0 mg/kg of body weight. In various embodiments, the TGF-β ligand trap is administered to the subject 1-7 times per week or once weekly, or once every two, three or four weeks. In various embodiments, the TGF-β ligand trap is administered to the subject for 1-5 days, 1-5 weeks, 1-5 months, or 1-5 years.TGF-β ligand trap may be administered by any route used for protein therapeutics, including but not limited to subcutaneous, intravenous or intramuscular administration.

[00097] As indicated above, in various embodiments, the TGF-β ligand trap is administered to the subject orally, via inhalation, nasally, sublingually, buccally, subcutaneously, intradermally, intramuscularly, intravenously, intraperitoneally, or parenterally. In various embodiments, the TGF-β ligand trap is administered before, during, or after the subject develops a disease condition, including but not limited to pulmonary hypertension, pulmonary vascular remodeling, pulmonary fibrosis, right

ventricular hypertrophy, pulmonary diseases associated with excessive TGF-β signaling, pulmonary diseases associated with excessive GDF15 signaling, and pulmonary diseases associated with excessive PAI-1 signaling.

[00098] In various embodiments, the TGF-β ligand trap is part of a pharmaceutical composition. In various embodiments, the pharmaceutical composition is formulated for modified release, sustained release, or controlled release, or a combination thereof. In various embodiments, the pharmaceutical composition is formulated for oral, via inhalation, nasal, sublingual, buccal, subcutaneous, intradermal, intramuscular, intravenous, intraperitoneal, or parenteral administration.

[00099] In various embodiments, the pharmaceutical composition further includes at least one pharmaceutically acceptable excipient. Examples of excipients include but are not limited to starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, wetting agents, emulsifiers, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservatives, antioxidants, plasticizers, gelling agents, thickeners, hardeners, setting agents, suspending agents, surfactants, humectants, carriers, stabilizers, and combinations thereof.

[000100] In some embodiments of the present invention may be defined in any of the following numbered paragraphs:

[000101] Paragraph 1. A method of treating, preventing, or reducing the progression rate of pulmonary hypertension (PH) in a subject, comprising: administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of PH in the subject.

[000102] Paragraph 2. The method of paragraph 1, wherein PH is mediated by excessive TGF-β signaling.

[000103] Paragraph 3. The method of any of paragraphs 1-2, wherein the subject is a human.

[000104] Paragraph 4. The method of any of paragraphs 1-3, wherein the TGF-β ligand trap comprises 1) a TGF-β ligand binding domain of a TGF receptor and 2) a Fc domain of an immunoglobulin.

[000105] Paragraph 5. The method of paragraph 4, wherein the TGF-β ligand trap further comprises a linker between the TGF-β ligand binding domain of a TGF receptorand the Fc domain.

[000106] Paragraph 6. The of any of paragraphs 1-5, wherein the TGF-β ligand trap is a soluble recombinant TGF-β type II receptor Fc-fusion protein (TGFBRII-Fc).

[000107] Paragraph 7. The method of any of paragraphs 1-6, wherein the TGFBRII-Fc consists of the sequence set forth in SEQ ID NO:1 or a variant thereof.

[000108] Paragraph 8. The method of any of paragraphs 1-6, wherein the TGFBRII-Fc comprises the sequence set forth in SEQ ID NO:1 or a variant thereof.

[000109] Paragraph 9. The method of any of paragraphs 1-6, wherein the TGFBRII-Fc comprises one or more biologically active portions of the sequence set forth in SEQ ID NO:1.

[000110] Paragraph 10. The method of any of paragraphs 1-6, wherein the TGFBRII-Fc is encoded by a nucleic acid comprising a nucleotide sequence set forth in SEQ ID NO:2 or a degenerate variant thereof.

[000111] Paragraph 11. The method of any of paragraphs 1-10, wherein the amount of TGF-β ligand trap administered to the subject is 0.1-10 mg/kg of body weight.

[000112] Paragraph 12. The method of any of paragraphs 1-11, wherein the TGF-β ligand trap is administered to the subject 1-7 times per month.

[000113] Paragraph 13. The method of any of paragraphs 1-12, wherein the TGF-β ligand trap is administered to the subject for 1-5 days, 1-5 weeks, 1-5 months, or 1-5 years.

[000114] Paragraph 14. The method of any of claims 1-13, wherein the TGF-β ligand trap is administered to the subject orally, via inhalation, nasally, sublingually, buccally, subcutaneously, intradermally, intramuscularly, intravenously, intraperitoneally, or parenterally.

[000115] Paragraph 15. The method of any of paragraphs 1-14, wherein the TGF-β ligand trap is administered before, during, or after the subject develops PH.

[000116] Paragraph 16. The method of any of paragraphs 1-15, further comprising mixing a pharmaceutically acceptable carrier with the TGF-β ligand trap prior to administering a therapeutically effective amount of the TGF-β ligand trap to the subject.

[000117] Paragraph 17. The method of any of paragraphs 1-16, wherein the TGF-β ligand trap is part of a pharmaceutical composition.

[000118] Paragraph 18. The method of paragraph 17, wherein the pharmaceutical composition is formulated for modified release, sustained release, or controlled release, or a combination thereof.

[000119] Paragraph 19. The method of paragraph 17, wherein the pharmaceutical composition is formulated for oral, via inhalation, nasal, sublingual, buccal, subcutaneous, intradermal, intramuscular, intravenous, intraperitoneal, or parenteral administration.

[000120] Paragraph 20. The method of paragraph 17, wherein the pharmaceutical composition further comprises at least one pharmaceutically acceptable excipient.

[000121] Paragraph 21. The method of paragraph 17, wherein the pharmaceutical composition further comprises at least one pharmaceutically acceptable carrier.

[000122] Paragraph 22. A method of treating, preventing, or reducing the progression rate of pulmonary vascular remodeling in a subject, comprising: administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of pulmonary vascular remodeling in the subject.

[000123] Paragraph 23. A method of treating, preventing, or reducing the progression rate of pulmonary fibrosis in a subject, comprising: administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of pulmonary fibrosis in the subject.

[000124] Paragraph 24. The method of claim 23, using the TGF-β ligand trap of any of paragraphs 4-10.

[000125] Paragraph 25. A method of treating, preventing, or reducing the progression rate of right ventricular hypertrophy in a subject, comprising: administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of right ventricular hypertrophy in the subject.

[000126] Paragraph 26. The method of claim 25, using the TGF-β ligand trap of any of paragraphs 4-10.

[000127] Paragraph 27. A method of treating, preventing, or reducing the progression rate of a pulmonary disease associated with excessive TGF-β signaling in a subject, comprising: administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of the disease in the subject.

[000128] Paragraph 28. The method of claim 27, using the TGF-β ligand trap of any of paragraphs 4-10.

[000129] Paragraph 29. The method of any of claims 1-28, wherein the TGF-β is TGF-β1, TGF-β3, or a combination thereof.

[000130] Paragraph 30. A method of treating, preventing, or reducing the progression rate of a pulmonary disease associated with excessive GDF15 signaling in a subject, comprising: administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of the disease in the subject.

[000131] Paragraph 31. The method of claim 30, using the TGF-β ligand trap of any of paragraphs 4-10.

[000132] Paragraph 32. A method of treating, preventing, or reducing the progression rate of a pulmonary disease associated with excessive PAI-1 signaling in a subject, comprising: administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby treating, preventing, or reducing the progression rate of the disease in the subject.

[000133] Paragraph 33. The method of claim 32, using the TGF-β ligand trap of any of paragraphs 4-10

[000134] Paragraph 34. A method of reducing right ventricular systolic pressure in a subject, comprising: administering a therapeutically effective amount of a TGF-β ligand trap to the subject, thereby reducing right ventricular systolic pressure in the subject.

[000135] Paragraph 35. The method of claim 34, using the TGF-β ligand trap of any of paragraphs 4-10.

[000136] EXAMPLES

[000137] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

[000138] *Example 1: Additional Background and Brief Summary of Results*

[000139] As indicated above, Transforming Growth Factor- (TGF-β) ligands coordinate important processes in development, and regulate fibrosis and tissue remodeling in disease. An excess of TGF-β signaling has been implicated in the arterial remodeling of pulmonary hypertension (PH), based in part on the ability of TGFβ type I receptor (ALK5) kinase inhibitors to improve experimental PH in animal models. However, clinical deployment of ALK5 inhibitors has been limited by cardiovascular toxicity. The experiments and results disclosed herein demonstrate that a soluble recombinant TGFβ type II receptor Fc-fusion protein (TGFBRII-Fc) inhibits TGFβ signaling in rat monocrotaline (MCT)-induced PH. When administered prophylactically following MCT, TGFBRII-Fc treatment reduced right ventricular systolic pressure, right ventricular hypertrophy, and attenuated pulmonary vascular remodeling. Elevated mRNA levels of TGFβ transcriptional target PAI-1 in lungs of MCT rats were corrected by TGFBRII-Fc, consistent with attenuating of TGFβ signaling. When administered 2.5 weeks after MCT, TGFβRII-Fc partially rescued established PH with a trend towards improved survival at 5 weeks. Of note, no cardiac structural or valvular abnormalities were found in association with treatment with TGFBRII-Fc at any dose. Collectively, the data disclosed herein supports the conclusion that a TGFβ ligand trap could be an effective and acceptably safe strategy for correcting TGFβ-mediated pulmonary vascular remodeling and PH.

[000140] *Example 2*

[000141]

[000142] Table 1. Non-limiting Exemplar TGFβ Ligand Binding Domains

TGFβ Receptor	Ligand-Binding Domains	SEQ ID NO:
Human TGFβ receptor type II	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVA VWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIM KEKKKPGETFFMCSCSSDECNDNIIF	3
Human TGFβ receptor type IIb	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVA VWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIM KEKKKPGETFFMCSCSSDECNDNIIF	4
Human TGFβ receptor type I	ALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNS MCIAEIDLIPRDRPFVCAPSSKTGSVTTTYCCNQDHCN KIEL	5

[000143] Example 3

[000144] *Table 2. Non-limiting Exemplar Linkers*

Linker	SEQ ID NO:
COOH-IPPHVQKSVNNDMIVTDNNGAVKFP-NH2	6
COOH-SEEYNTSNPD-NH2	7
COOH-IPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFP-NH2	8
COOH-AALLPGAT-NH2	9
COOH-PTTVKSSPGLGPVE-NH2	10
COOH-AILGRSE-NH2	11
COOH-EMEVTQPTSNPVTPKPPYYNI-NH2	12
COOH-SGRGEAET-NH2	13
COOH-EAGGPEVTYEPPPTAPT-NH2	14
COOH-QNLDSMLHGTGMKSDSDQKKSENGVTLAPED-NH2	15
COOH-PVVIGPFFDGSIR-NH2	16
COOH-QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIF-NH2	17
COOH-QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIF-NH2	18
COOH-ALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVIHNSMCIAEIDLIPRDRPFVCAPSSKTGSVTTTYCCNQDHCNKIEL-NH2	19
COOH-TQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFP-NH2	20
COOH-RECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT-NH2	21
COOH-	22

TLPFLKCYCSGHCPDDAINNTCITNGHCFAIIEEDDQGETTLASGCMKYE GSDFQCKDSPKAQLRRTIECCRTNLCNQYLQPTLPPVVIGPFFDGSIR-NH2	
COOH-SEEYNTSNPDIPPHVQKSVNNDMIVTDNNGAVKFP-NH2	23
COOH-SEEYNTSNPDIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFP-NH2	24
COOH-EAGGPEVTYEPPPTAPTSGRGEAET-NH2	25
COOH-PVVIGPFFDGSIRQNLDSMLHGTGMKSDSDQKKSENGVTLAPED-NH2	26
COOH-PVVIGPFFDGSIRGNLDSMLHGTGMKSDSDQKKSENGVTLAPED-NH2	27
COOH-SEEYNTSNPDGPPHVQKSVNNDMIVTDNNGAVKFP-NH2	28
COOH-EAGGPEVTGEPPPTAPTSGRGEAET-NH2	29
COOH-SEEYNTSNPDGGRHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFP-NH2	30
COOH-SEEYNTSNPDGGPHVQKSVNNDMIVTDNNGAVKFP-NH2	31
COOH-SEEYNTSNPDGGRHVQKSVNNDMIVTDNNGAVKFP-NH2-	32
COOH-SEEYNTSNPSGGGSGGGSGGGMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFP-NH2	33
COOH-SEEYNTSNPSGGGSGGKSVNNDMIVTDNNGAVKFP-NH2	34
COOH-SEEYNTSNPSGGGSGGGSGGGDMIVTDNNGAVKFP-NH2	35
COOH-SEEYNTSNPDIPPHVQKSGGGSGGGSGGGSGGGSGGGSGGGSGGNNDMIVTDNNGAVKFP-NH2	36
COOH-SEEYNTSNPDGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGNNDMIVTDNNGAVKFP-NH2	37
COOH-SEEYNTSNPDIPPHVQKSVNNDMIVTDNNGAVKFP-NH2	38
COOH-SEEYNTSNPDIPPHVQKSVNNDMIPPHVQKSVNNDMIVIDNNGAVKFP-	39

NH2	
COOH-SEEYNTSNPPHVQKSVNNDMIVTDNNGAVKFP-NH2	40
COOH-SEEYNTSNPDGGGGGGGGGIPPHVQKSVNNDMIVIDNNGAVKFP-NH2	41
COOH-SEEYNTSNPDGGGSGGGSGGGSIPPHVQKSVNNDMIVTDNNGAVKFP-NH2	42
COOH-SEEYNTSNPDIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFP-NH2	43
COOH-SEEYNTSNPDIPPHVQKSDVEMEAQKDERTAHPLRHINNDMIVTDNNGAVKFP-NH2	44
COOH-EAGGPEVTYEPPPTAPTSGRGEAET-NH2	45
COOH-EAGGPEVTYEPPPTAPTGGGGGGGGGSGRGEAET-NH2	46
COOH-PVVIGPFFDGSIRQNLDSMLHGTGMKSDSDQKKSENGVTLAPED-NH2	47
COOH-PVVIGPDGSIRQNLDSHGTGMKSDSDQKKSENGVTLAPED-NH2	48

[000145] Also contemplated are nucleic acid sequences encoding each of the above linkers and binding domains.

[000146] Example 4

[000147] *Materials and Methods*

[000148] *Rat model of PAH*

[000149] Male Sprague-Dawley rats (6-8 weeks old, weight 150 to 170 g) were purchased from Charles River Laboratory. All protocols and surgical procedures were approved by the local animal care committee. Animals were housed at 24°C in a 12-hour light-dark cycle. Food and water were accessible *ad libitum*. To induce PAH, rats received a single subcutaneous injection of monocrotaline (MCT, 40 mg/kg). Mortality and total number of rats included in the present study are summarized in Table 3.

[000150] Table 3

	Experimental group	Starting n number	Data excluded due to mortality or low heart rate (<350 bpm) during right ventricular catheterization	Final n number included
Fig. 3	Control	6	0	6
	TGFBRII-Fc 5 mg/kg, twice per week	6	0	6
	MCT	8	0	8
	MCT + TGFBRII-Fc 5 mg/kg, twice per week	8	0	8
Fig. 4 and 5	Control	6	0	6
	TGFBRII-Fc 15 mg/kg, twice per week	6	0	6
	MCT	8	1	7
	MCT + TGFBRII-Fc 15 mg/kg, twice per week	8	0	8
Fig. 7	MCT	12	4	8
	MCT + TGFBRII-Fc 15 mg/kg, three time per week	12	1	11

[000151] Drug treatment

[000152] Prophylaxis protocol - At 24 hours after PAH induction, rats were randomized into TGFBRII-Fc (5 or 15 mg/kg, twice per week) or vehicle groups. Rats were treated for 21 days. At day 14, ventricular function and RV remodeling were examined by echocardiogram. At day 21, rats were subjected to hemodynamics and right ventricular hypertrophy measurements.

[000153] <u>Rescue protocol</u> - In another cohort, the ability of TGFBRII-Fc to reverse the progression of PAH was examined. At day 18, rats were injected with MCT and randomized for TGFBRII-Fc (15 mg/kg, three times per week) or vehicle. Hemodynamics and right ventricular hypertrophy (RVH) were examined on day 35.

[000154] *Echocardiographic assessment of LV and RV function*

[000155] At day *14* after PAH induction, rats were anesthetized with 1.5% isoflurane and held in a supine position. A VisualSonics small animal high-frequency ultrasound probe was used to detect pulmonary flow acceleration, right ventricular function and hypertrophy, and left ventricular function. Doppler across the mitral and tricuspid valves to determine if TGFBRII-Fc treatment induce any obvious regurgitation or lesions.

[000156] *Hemodynamic and RVH measurement*

[000157] At specific time points, rats were anesthetized with pentobarbital and intubated through the trachea. Rats were mechanically ventilated using a rodent ventilator and hemodynamic assessment using a fluid-filled catheter through the right ventricular (RV) apex, as described previously (Megalou, A. J., Glava, C., Vilaeti, A. D., Oikonomidis, D. L., Baltogiannis, G. G., Papalois, A., Vlahos, A. P., and Kolettis, T. M. (2012) Pulm Circ 2, 461-469). Lungs were perfused with PBS and one right lobe was excised and snap frozen for RNA and protein extraction. Lungs were further perfused with 1% paraformaldehyde (PFA) into the pulmonary artery, followed by trachea for 1 minute. Left lobes were embedded in paraffin. To access degree of RVH, the heart was removed and the RV free wall dissected from the left ventricle plus septum (LV+S) and weighted separately. Degree of RVH was determined from the ration RV/(LV+S).

[000158] Quantification of vascular remodeling

[000159] To determine the degree of pulmonary vascular remodeling, lung tissue sections were stained with alpha smooth muscle actin and von willebrand factor. Muscularization of distal intra-acinar vessels (10-50 μm diameter) was quantified and percentage of nonmuscular, partially muscular, and fully muscular vessels was calculated.

[000160] Medial wall thickness was calculated for all fully muscularized intra-acinar vessels (10-50 μm diameter). Wall thickness index was calculated as: index= (external diameter - internal diameter) / external diameter x 100.

[000161] *Expression studies*

[000162] Frozen lung samples were homogenized and total RNA extraction using TRIZOL reagent performed as previously described (Long, L., Crosby, A., Yang, X., Southwood, M., Upton, P. D., Kim, D. K., and Morrell, N. W. (2009) Circulation 119, 566-576). Reverse transcription and quantitative PCR were performed as described (Long, L., Crosby, A., Yang, X., Southwood, M., Upton, P. D., Kim, D. K., and Morrell, N. W. (2009) Circulation 119, 566-576). The ratio of a specific gene to β-actin was calculated and expressed as fold change. Sequences of rat-specific are summarized in Table 4.

[000163] Table 4

Gene of interest	Forward primer	SEQ ID NO:	Reverse primer	SEQ ID NO:
tgfb1	TGAGTGGCTGTCTTTT GACG	49	TTCTCTGTGGAGCTGAAG CA	50
tgfb2	CACGCCTCTCTTGTTT CCTC	51	TTTTCCAAGGGCAATGAA AG	52
tgfb3	GAAGGCTGCACTCAG GAAAC	53	GCTGCTTGGCTATGTGTTC A	54
pai1	CTTTATCCTGGGTCTC CCTG	55	TGATGCCTCCCTGACATA CA	56
bmpr2	AATAATCTGGGTAAGG CC	57	GCAGAACGAACGCAACCT ATCA	58
id1	TGGACGAACAGCAGG TGAACG	59	GCACTGATCTCGCCGTTC AGG	60
β-actin	TGTCACCAACTGGGAC GATA	61	ACCCTCATAGATGGGCAC AG	62

[000164] *Reagents*

[000165] Monocrotaline was purchased from Oakwood Products, Inc. Recombinant human BMP4, TGFβ1, TGFβ2 and GDF15 were obtained from R&D Systems. Primary antibody specific to phospho-Smad 3 was purchased from Abcam, while other primary antibodies against phospho-Smad 2, phospho-Smad 1/5, and total Smad 3 were obtained from Cell Signaling.

[000166] *Statistical analysis*

[000167] All the analysis of hemodynamic and RVH measurement and pulmonary vascular remodeling quantification were performed in a blinded manner. Data were presented as mean ± SEM and compared between group using t test. $P<0.05$ was considered statistically significant.

[000168] *Vascular remodeling of mitral valve.*

[000169] Figures 9A to 9B show heart tissue sections demonstrating the lack of mitral valve remodeling, degeneration or abnormalities in response to TGFBRII-Fc treatment. Figure 9A control. Figure 9B TGFBRII-Fc-treated.

[000170] The various methods and techniques described above provide a number of ways to carry out the application. Of course, it is to be understood that not necessarily all objectives or advantages described can be achieved in accordance with any particular embodiment described herein. Thus, for example, those skilled in the art

will recognize that the methods can be performed in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objectives or advantages as taught or suggested herein. A variety of alternatives are mentioned herein. It is to be understood that some preferred embodiments specifically include one, another, or several features, while others specifically exclude one, another, or several features, while still others mitigate a particular feature by inclusion of one, another, or several advantageous features.

[000171] Furthermore, the skilled artisan will recognize the applicability of various features from different embodiments. Similarly, the various elements, features and steps discussed above, as well as other known equivalents for each such element, feature or step, can be employed in various combinations by one of ordinary skill in this art to perform methods in accordance with the principles described herein. Among the various elements, features, and steps some will be specifically included and others specifically excluded in diverse embodiments.

[000172] Although the application has been disclosed in the context of certain embodiments and examples, it will be understood by those skilled in the art that the embodiments of the application extend beyond the specifically disclosed embodiments to other alternative embodiments and/or uses and modifications and equivalents thereof.

[000173] In some embodiments, the terms "a" and "an" and "the" and similar references used in the context of describing a particular embodiment of the application (especially in the context of certain of the following claims) can be construed to cover both the singular and the plural. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (for example, "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the application and does not pose a limitation on the scope of the application otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the application.

[000174] Preferred embodiments of this application are described herein, including the best mode known to the inventors for carrying out the application. Variations on those preferred embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. It is contemplated that skilled artisans can employ such variations as appropriate, and the application can be practiced otherwise than specifically described herein. Accordingly, many embodiments of this application include all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the application unless otherwise indicated herein or otherwise clearly contradicted by context.

[000175]

[000176] It is to be understood that the embodiments of the application disclosed herein are illustrative of the principles of the embodiments of the application. Other modifications that can be employed can be within the scope of the application. Thus, by way of example, but not of limitation, alternative configurations of the embodiments of the application can be utilized in accordance with the teachings herein. Accordingly, embodiments of the present application are not limited to that precisely as shown and described.

[000177] Various embodiments of the invention are described above in the Detailed Description. While these descriptions directly describe the above embodiments, it is understood that those skilled in the art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included

therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

[000178] The foregoing description of various embodiments of the invention known to the applicant at this time of filing the application has been presented and is intended for the purposes of illustration and description. The present description is not intended to be exhaustive nor limit the invention to the precise form disclosed and many modifications and variations are possible in the light of the above teachings. The embodiments described serve to explain the principles of the invention and its practical application and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed for carrying out the invention.

[000179] While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that, based upon the teachings herein, changes and modifications may be made without departing from this invention and its broader aspects and, therefore, the appended claims are to encompass within their scope all such changes and modifications as are within the true spirit and scope of this invention. It will be understood by those within the art that, in general, terms used herein are generally intended as "open" terms (*e.g.*, the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.)

What is claimed is:

1. Use of a polypeptide comprising a TGF-β ligand binding domain of a TGF-β type II receptor and an Fc domain of an immunoglobulin in the manufacture of a medicament for treating, preventing, or reducing the progression rate of pulmonary hypertension (PH) in a subject.

2. The use of claim 1, wherein the pulmonary hypertension is selected from the group consisting of venous, hypoxic, thromboembolic, arterial, and miscellaneous.

3. The use of any one of claims 1 to 2, wherein the PH is pulmonary arterial hypertension (PAH).

4. Use of a polypeptide comprising a TGF-β ligand binding domain of a TGF-β type II receptor and an Fc domain of an immunoglobulin in the manufacture of a medicament for treating, preventing, or reducing the progression rate of PAH in a subject.

5. The use of any one of claims 1 to 4, wherein PH and/or PAH is mediated by excessive TGF-β signaling.

6. The use of any one of claims 1 to 5, wherein the TGF-β ligand binding domain comprises the sequence as set forth in SEQ ID NO: 3.

7. The use of any one of claims 1 to 6, wherein the polypeptide further comprises a linker between the TGF-β ligand binding domain of the TGF-β type II receptor and the Fc domain.

8. The use of any one of claims 1 to 7, wherein the polypeptide is a soluble recombinant TGF-β type II receptor Fc-fusion protein (TGFBRII-Fc).

9. The use of claim 7 or 8, wherein the TGFBRII-Fc comprises a sequence that is at least 80% identical to the sequence as set forth in SEQ ID NO: 1.

10. The use of any one of claims 1 to 9, wherein the amount of polypeptide for administration to the subject is between about 0.3 and about 3.0 mg/kg of body weight.

11. The use of any one of claims 1 to 10, wherein the polypeptide is for administration to the subject once per two weeks.

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12. The use of any one of claims 1 to 10, wherein the polypeptide is for administration to the subject once per three weeks.

13. The use of any one of claims 1 to 12, wherein the polypeptide is for administration to the subject subcutaneously.

14. The use of any one of claims 1 to 13, wherein the polypeptide is part of a pharmaceutical composition.

15. The use of claim 14, wherein the pharmaceutical composition is formulated for modified release, sustained release, controlled release, or a combination thereof.

16. The use of any one of claims 1 to 15, wherein the polypeptide binds TGF-β1 or TGF-β3.

17. The use of any one of claims 1 to 16, wherein the treatment improves the distance the subject can walk in six minutes.

18. The use of any one of claims 1 to 17, wherein the treatment improves the subject's blood brain natriuretic peptide (BNP) levels.

19. The use of any one of claims 1 to 18, wherein the treatment improves the subject's arterial pressure.

20. The use of any one of claims 1 to 19, wherein the treatment improves the subject's mean arterial pressure.

21. Use of a polypeptide comprising a TGF-β ligand binding domain of a TGF-β type II receptor and an Fc domain of an immunoglobulin in the manufacture of a medicament for treating, preventing, or reducing the progression rate of pulmonary vascular remodeling in a subject that has PH, wherein the polypeptide comprises the polypeptide of any one of claims 4 to 21.

22. Use of a polypeptide comprising a TGF-β ligand binding domain of a TGF-β type II receptor and an Fc domain of an immunoglobulin in the manufacture of a medicament for treating, preventing, or reducing the progression rate of pulmonary fibrosis in a subject that has PH, wherein the polypeptide comprises the polypeptide of any one of claims 4 to 21.

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23. Use of a polypeptide comprising a TGF-β ligand binding domain of a TGF-β type II receptor and an Fc domain of an immunoglobulin in the manufacture of a medicament for treating, preventing, or reducing the progression rate of right ventricular hypertrophy in a subject that has PH, wherein the polypeptide comprises the polypeptide of any one of claims 4 to 21.

24. Use of a polypeptide comprising a TGF-β ligand binding domain of a TGF-β type II receptor and an Fc domain of an immunoglobulin in the manufacture of a medicament for treating, preventing, or reducing the progression rate of a pulmonary disease associated with excessive TGF-β signaling and/or excessive PAI-1 signaling in a subject, wherein the polypeptide comprises the polypeptide of any one of claims 4 to 21.

25. Use of a polypeptide comprising a TGF-β ligand binding domain of a TGF-β type II receptor and an Fc domain of an immunoglobulin in the manufacture of a medicament for reducing right ventricular systolic pressure in a subject that has PH, wherein the polypeptide comprises the polypeptide of any one of claims 4 to 21.

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Figure 1A to 1B

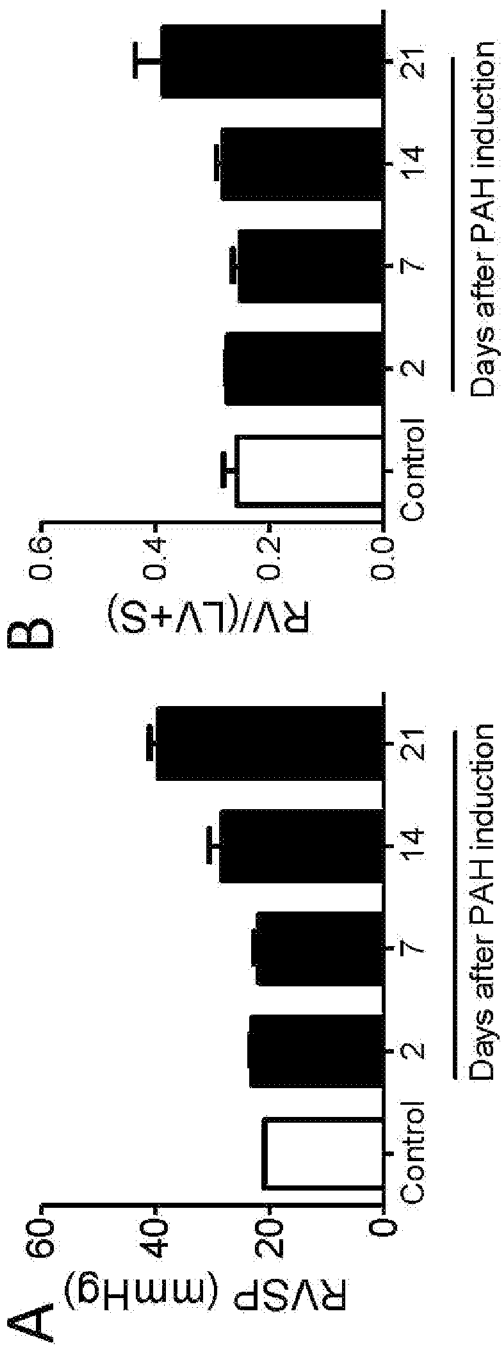


Figure 1C to 1E

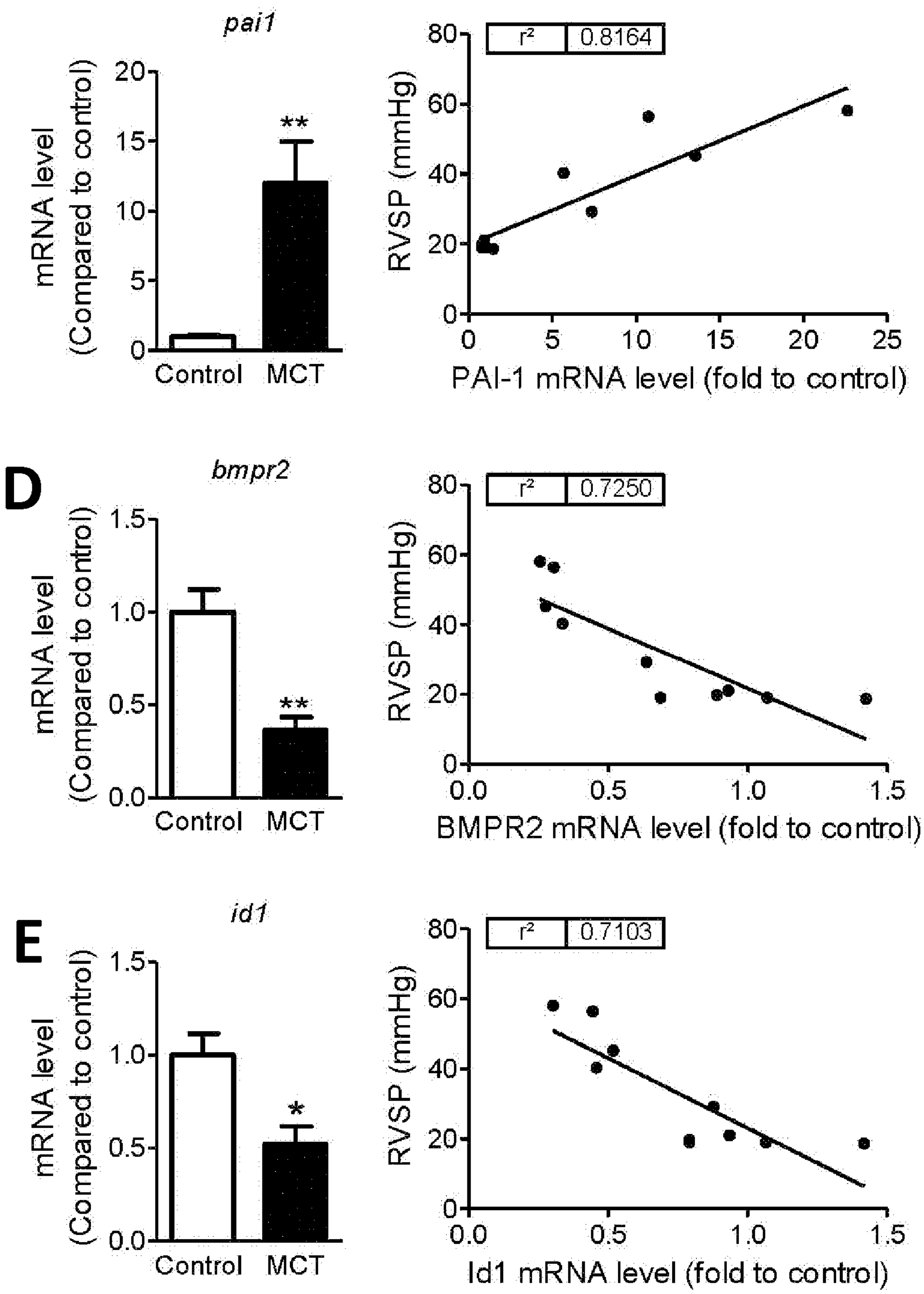


Figure 2

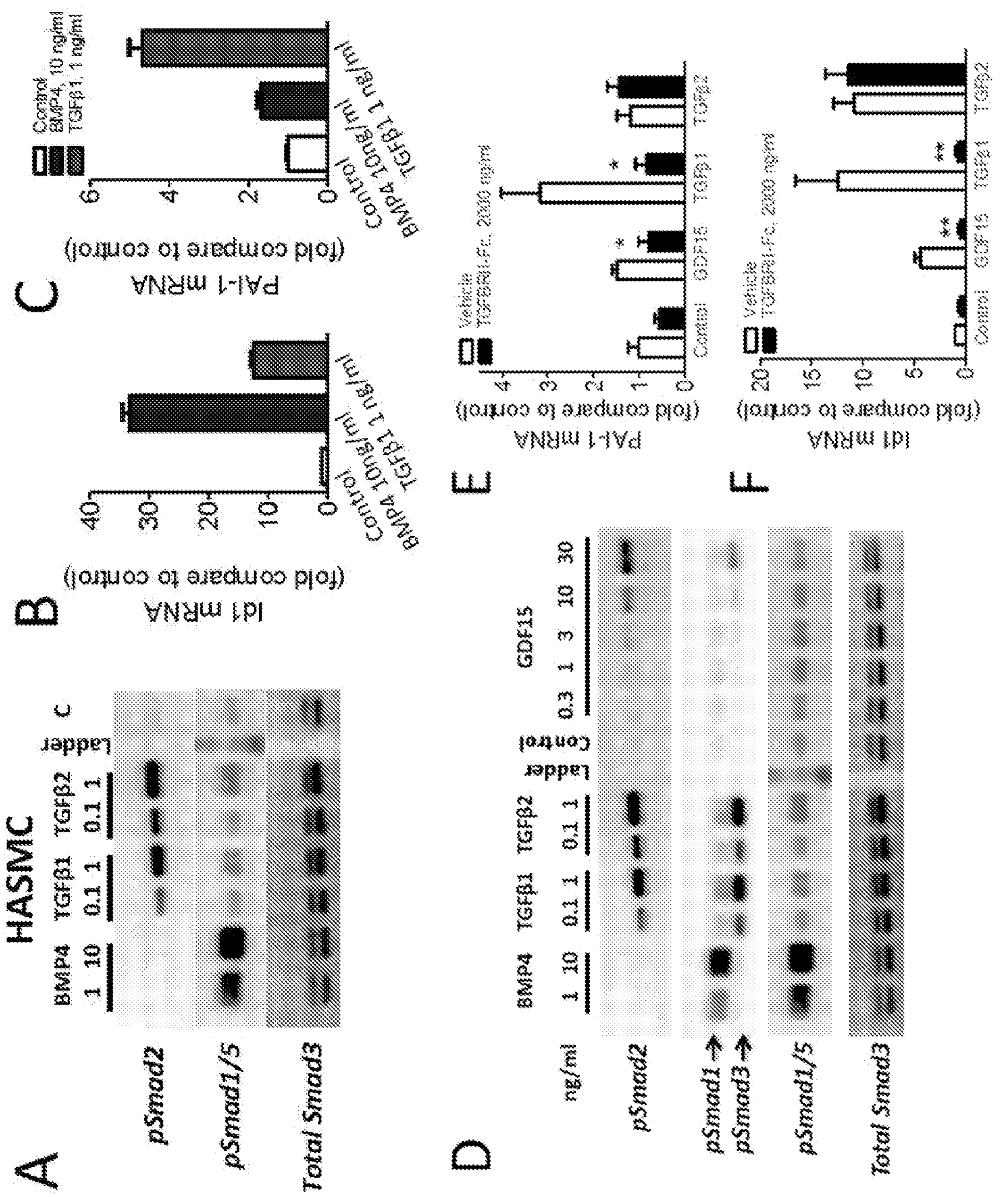

A
HASMC
BMP4
TGFβ1
TGFβ2
Ladder
pSmad2
pSmad1/5
Total Smad3
B
Id1 mRNA
(fold compare to control)
Control
BMP4 10ng/ml
TGFβ1 1 ng/ml
C
PAI-1 mRNA
(fold compare to control)
Control
BMP4, 10 ng/ml
TGFβ1, 1 ng/ml
D
ng/ml
GDF15
Control
pSmad1
pSmad3
E
PAI-1 mRNA
Vehicle
TGFBRII-Fc, 2000 ng/ml
GDF15
TGFβ2
F
Id1 mRNA

Figure 3

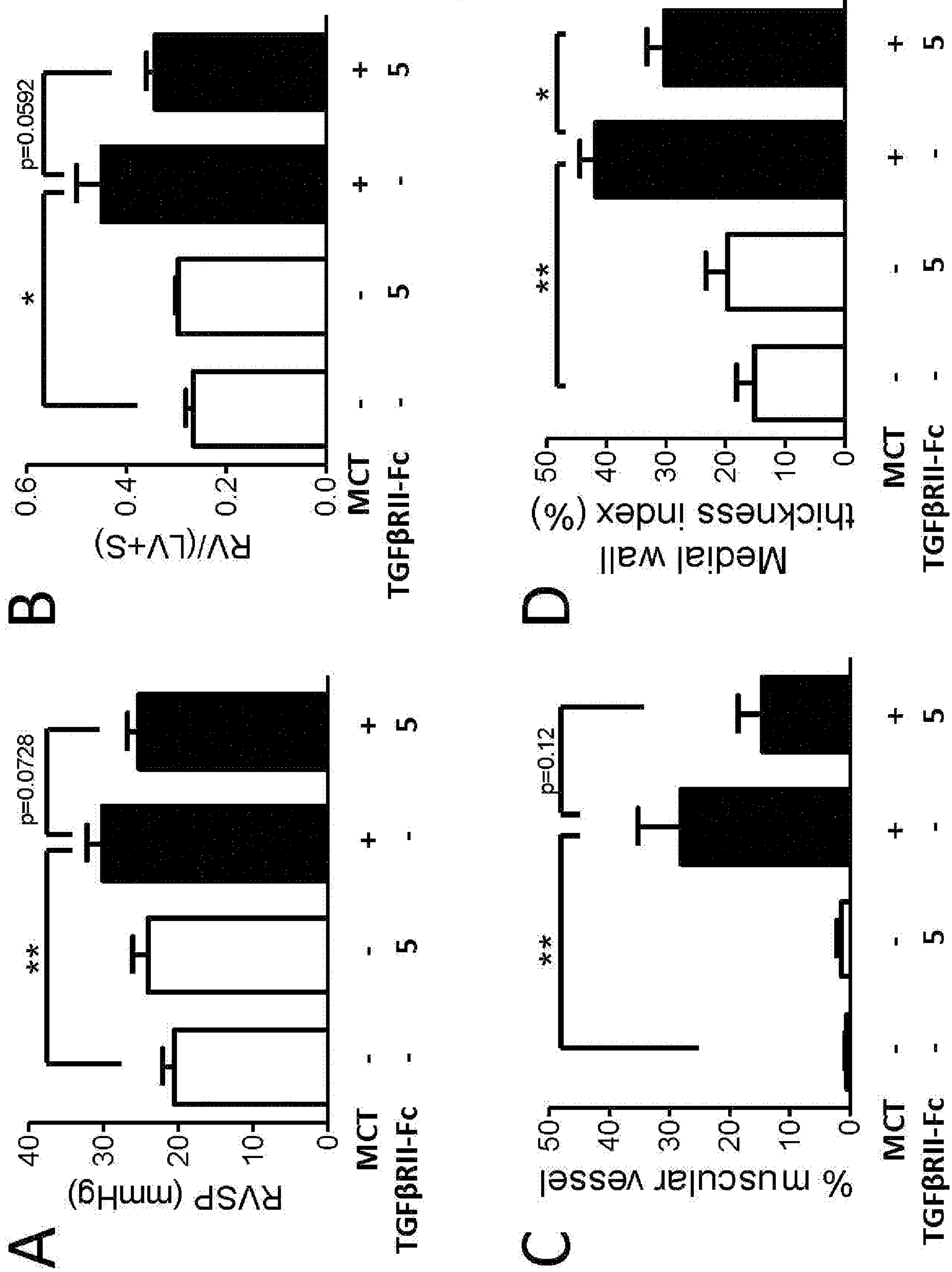

A
RVSP (mmHg)
40
30
20
10
0
**
p=0.0728
MCT - - + +
TGFβRII-Fc - 5 - 5
B
RV/(LV+S)
0.6
0.4
0.2
0.0
*
p=0.0592
MCT - - + +
TGFβRII-Fc - 5 - 5
C
% muscular vessel
50
40
30
20
10
0
**
p=0.12
MCT - - + +
TGFβRII-Fc - 5 - 5
D
Medial wall thickness index (%)
50
40
30
20
10
0
**
*
MCT - - + +
TGFβRII-Fc - 5 - 5

Figure 4

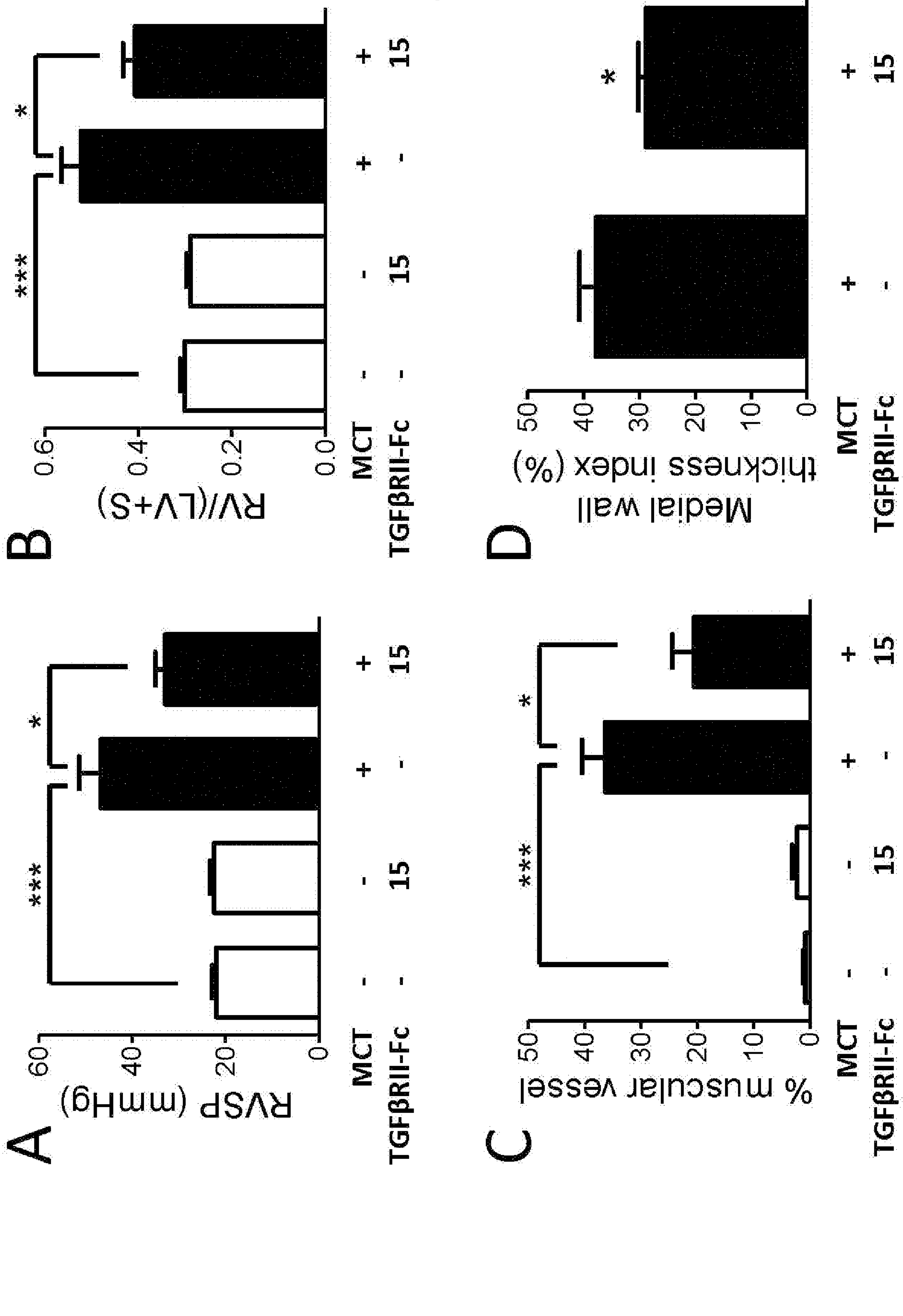

A
RVSP (mmHg)
60
40
20
0
***
*
MCT - - + +
TGFβRII-Fc - 15 - 15
B
RV/(LV+S)
0.6
0.4
0.2
0.0
***
*
MCT - - + +
TGFβRII-Fc - 15 - 15
C
% muscular vessel
50
40
30
20
10
0
***
*
MCT - - + +
TGFβRII-Fc - 15 - 15
D
Medial wall thickness index (%)
50
40
30
20
10
0
*
MCT + +
TGFβRII-Fc - 15

Figure 5

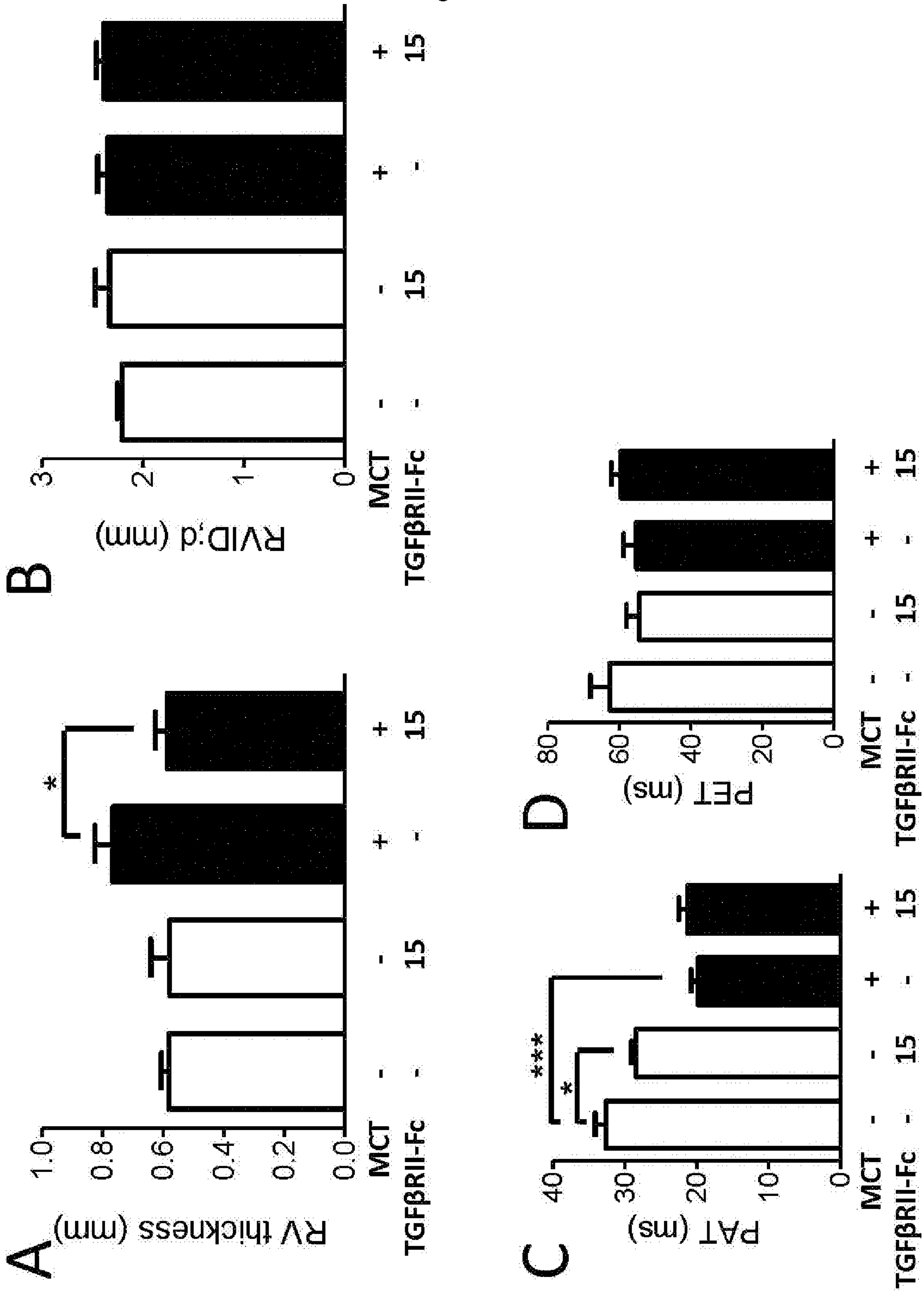

A
RV thickness (mm)
1.0
0.8
0.6
0.4
0.2
0.0
MCT
TGFβRII-Fc
*
B
RVID;d (mm)
3
2
1
0
C
PAT (ms)
40
30
20
10
0
*
***
D
PET (ms)
80
60
40
20
0
-
+
15

Figure 6

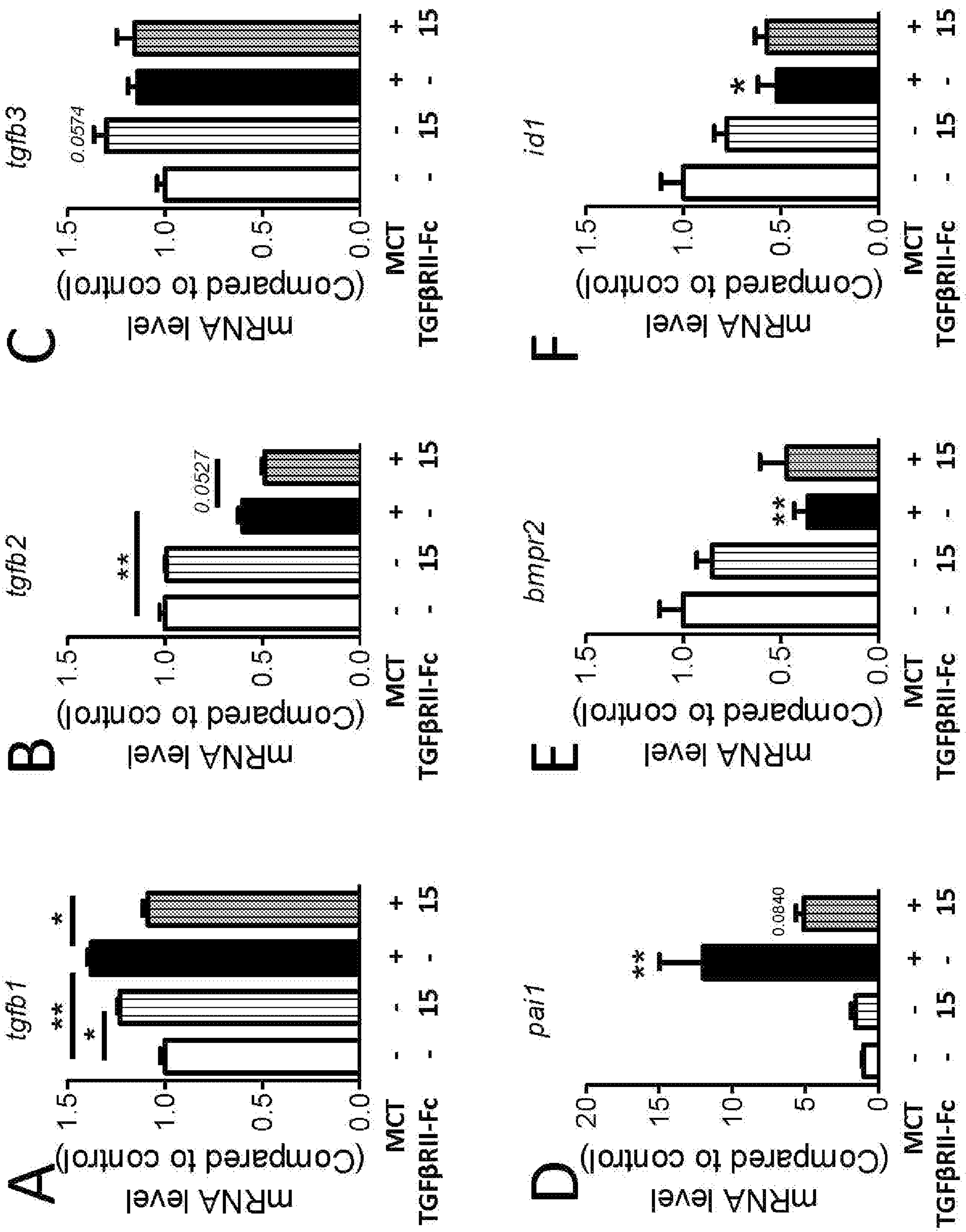

A
tgfb1
B
tgfb2
C
tgfb3
D
pai1
E
bmpr2
F
id1
mRNA level
(Compared to control)
MCT
TGFβRII-Fc
0.0574
0.0527
0.0840

Figure 7

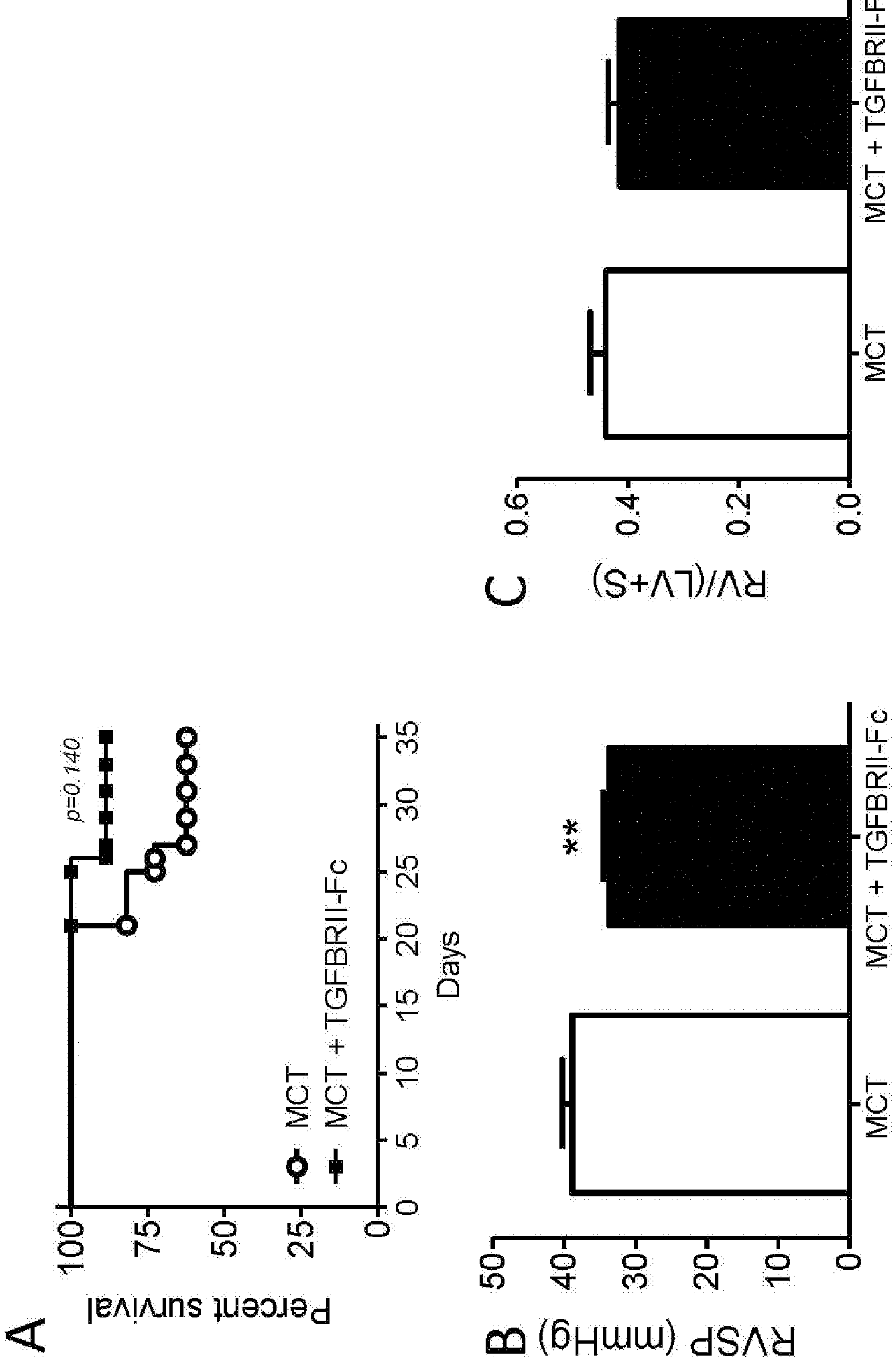

A
Percent survival
100
75
50
25
0
Days
0
5
10
15
20
25
30
35
MCT
MCT + TGFBRII-Fc
p=0.140
B
RVSP (mmHg)
50
40
30
20
10
0
**
MCT
MCT + TGFBRII-Fc
C
RV/(LV+S)
0.6
0.4
0.2
0.0
MCT
MCT + TGFBRII-Fc

Figure 8A

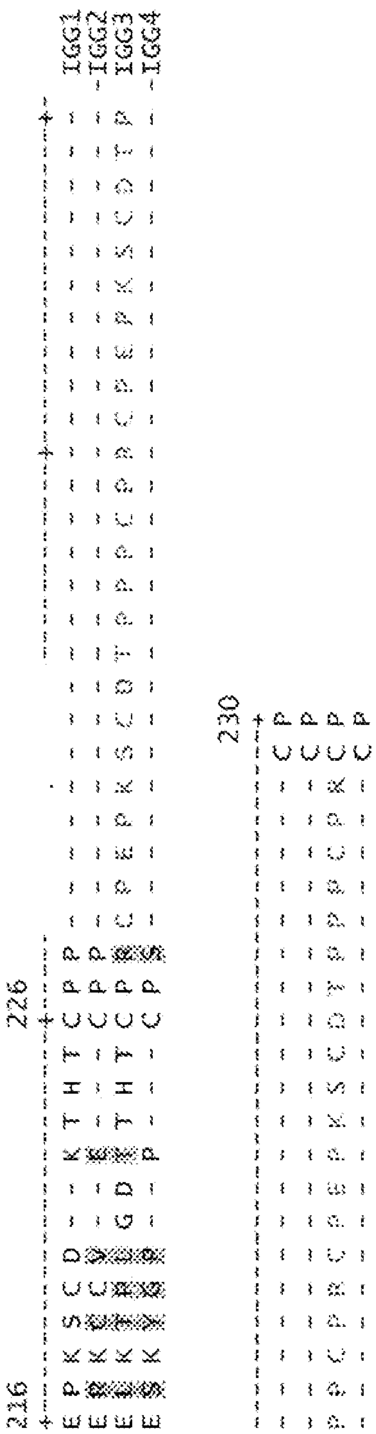
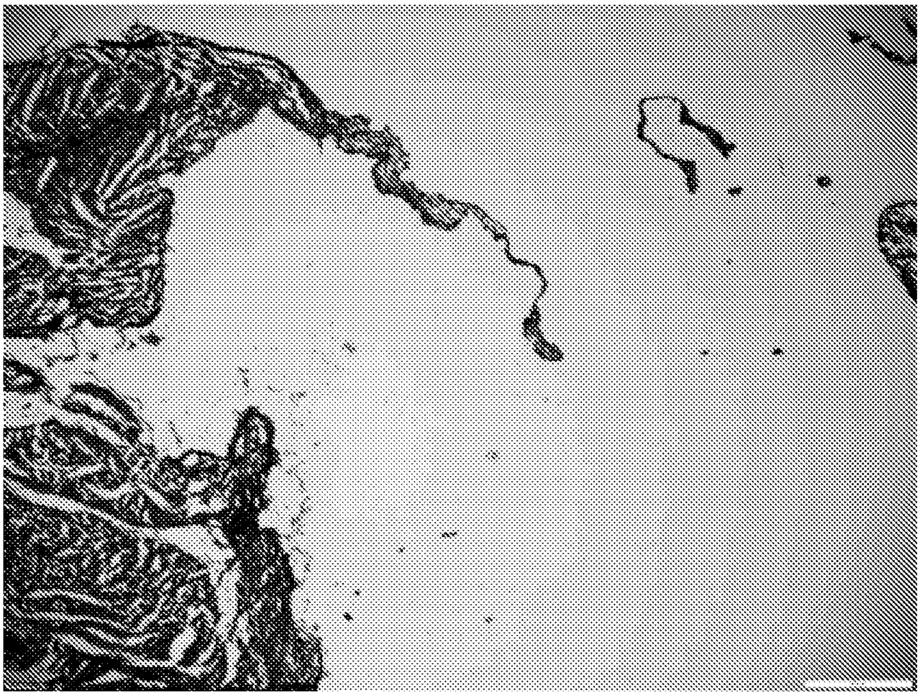


Figure 8B

```
231
A P E L L G G
A P P V A G -
A P E L L G G
A P E F L G G

240                                     260
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V Q F N W
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V Q F K W
P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S Q E D P E V Q F N W

280                                     300
Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K
Y V D G V E V H N A K T K P R E E Q F N S T F R V V S V L T V V H Q D W L N G K
Y V D G V [illegible] V H N A K T K P R E Q Q F N S T F R V V S V L T V L H Q [illegible] W L [illegible] G K
Y V D G V E V H N A K T K P R E E Q F N S T Y R V V S V L T V L H Q D W L N G K

320                                     340
E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R D E
E Y K C K V S N K G L P A P I E K T I S K T K G Q P R E P Q V Y T L P P S R E E
E Y K C K V S N K A L P A P I E K T I S K T K G Q P R E P Q V Y T L P P S R E E
E Y K C K V S N K G L P S S I E K T I S K A K G Q P R E P Q V Y T L P P S Q E E

360                                     380
L T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V
M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P M
M T K N Q V S L T C L V K G F Y P S D I A V E W E S S G Q P E N N Y N T T P P M
M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V

400                                     420
L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T
L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T
L D S D G S F F L Y S K L T V D K S R W Q Q G N I F S C S V M H E A L H N R F T
L D S D G S F F L Y S R L T V D K S R W Q E G N V F S C S V M H E A L H N H Y T

440
Q K S L S L S P G K
Q K S L S L S P G K
Q K S L S L S P G K
Q K S L S L S L G K
```

Figures 9A to 9B

Vehicle



TGFBRII-Fc

B