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(54) Title: TGFB1-BINDING IMMUNOGLOBULINS AND USE THEREOF

(57) Abstract: Disclosed herein are immunoglobulins, such as antibodies, and antigen binding portions thereof, that specifically bind complexes of GARP-TGFβ1, LTBP1-TGFβ1, LTBP3- TGFβ1, and/or LRRC33-TGFβ1 The application also provides methods of use of these immunoglobulins for, for example, inhibiting TGFβ1activity, and treating subjects suffering from TGFβ1-related disorders, such as cancer and fibrosis.

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TGFβ1-BINDING IMMUNOGLOBULINS AND USE THEREOF

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/307,353, filed on March 11, 2016; U.S. Provisional Application No. 62/443,615, filed on January 6, 2017; and U.S. Provisional Application No. 62/452,866, filed on January 31, 2017; the entire contents of each of which are expressly incorporated herein by reference in their entireties.

BACKGROUND

Transforming growth factor β (TGFβ) superfamily of growth factors are involved in a number of signaling cascades that regulate diverse biological processes including, but not limited to: inhibition of cell growth, tissue homeostasis, extracellular matrix (ECM) remodeling, endothelial to mesenchymal transition (EMT), cell migration and invasion, and immune modulation/suppression, as well as mesenchymal to epithelial transition. In relation to ECM remodeling, TGFβ signaling may increase fibroblast populations and ECM deposition (*e.g.,* collagen). In the immune system, TGFβ ligand modulates T regulatory cell function and maintenance of immune precursor cell growth and homeostasis. In normal epithelial cells, TGFβ is a potent growth inhibitor and promoter of cellular differentiation. However, as tumors develop and progress, they frequently lose their negative growth response to TGFβ. In this setting, TGFβ may become a promoter of tumor development due to its ability to stimulate angiogenesis, alter the stromal environment, and induce local and systemic immunosuppression. For these and other reasons, TGFβ has been a therapeutic target for a number of clinical indications. Despite much effort made to date by a number of groups, clinical development of a TGFβ therapeutic has been challenging.

Observations from preclinical studies, including in rats and dogs, have revealed certain toxicities associated with inhibition of TGFβ *in vivo*. Moreover, although several TGFβ inhibitors have been developed to date, most clinical programs targeting TGFβ have been discontinued due to side effects.

For example, Anderton *et al.* (Toxicology Pathology, 39: 916-24, 2011) reported that small molecule inhibitors of TGFβ type I (ALK5) receptor induced heart valve lesions characterized by hemorrhage, inflammation, degeneration and proliferation of valvular interstitial cells in a preclinical animal model. The toxicity was observed in all heart valves at all doses tested. Frazier *et al.* (Toxicology Pathology, 35: 284-295, 2007) reported that

administration of the small molecule inhibitor of TGFβ type I (ALK5) receptor GW788388 induced physeal dysplasia in rats.

Stauber *et al.* (J. Clin. Practice 4:3, 2014) reported that a chronic (≥ 3 months) administration of the inhibitor of TGFβ receptor I kinase, LY2157299, which is being investigated for certain cancer treatments, caused multiple organ toxicities involving the cardiovascular, gastrointestinal, immune, bone/cartilage, reproductive, and renal systems, in rats and dogs.

Fresolimumab (GC1008), a "pan" TGFβ antibody capable of neutralizing all human isoforms of TGFβ, has been reported to induce an epithelial hyperplasia of the gingiva, bladder, and of the nasal turbinate epithelium after multiple adminstrations in studies with cynomolgus macaques (Lonning *et al.*, Current Pharmaceutical Biotechnology 12: 2176-89, 2011). Similarly, a variety of skin rashes/lesions, gingival bleeding and fatigue have been reported in clinical trials after administration of multiple doses of the drug. The most notable adverse reaction to fresolimumab includes the induction of cutaneous keratoacanthomas and/or squamous cell carcinomas in human cancer patients (see, for example: Lacouture *et al.,* 2015, Cancer Immunol Immunother, 64: 437-46; Stevenson *et al.,* 2013, OncoImmunology, 2:8, e26218; and Lonning *et al.*, 2011). Additional evidence from a clinical trial suggests that in some cases this antibody may accelerate tumor progression (Stevenson *et al.,* 2013, OncoImmunology, 2:8, e26218).

Thus, new methods and compositions for modulating TGFβ signaling are necessary that can be used to effectively and safely treat diseases and disorders involving TGFβ, including, for example, cancer, fibrosis and inflammation.

SUMMARY OF THE INVENTION

The present disclosure relates to selective modulation of a *subset* of TGFβ effects. The invention is based at least in part on the notion that lack of isoform-specificity of known TGFβ antagonists to date may underlie the source of toxicities associated with TGFβ inhibition. Indeed, the inventors of the present disclosure have found that most TGFβ inhibitors described to date antagonize multiple or all of the TGFβ isoforms. Moreover, the general trend described in the art has been that inhibitors (such as anti-TGFβ antibodies) that antagonize multiple isoforms of TGFβ are favored, on the account that neutralization of multiple isoforms of TGFβ would be necessary or advantageous to achieve "maximum therapeutic efficacy" (*see*, for example, Bedinger *et al.* (2016) MABS 8(2): 389-404).

Contrary to this general teaching, the inventors of the present invention instead sought to develop agents that enable isoform-specific inhibition of TGFβ1, as opposed to inhibition that also affects TGFβ2 and/or TGFβ3, with the aim of eliminating or markedly reducing toxicities (*e.g.,* adverse events, side effects) observed with known TGFβ antagonists *in vivo*. This novel approach is based, at least in part, on the notion that the clinical utility of a TGFβ inhibitor may rest not only on its efficacy, but also on its safety. It was reasoned that the ability to fine-tune the target with an unprecedented degree of specificity may achieve both efficacy and safety/tolerability in a clinical setting.

Accordingly, the invention encompasses the recognition that pharmaceutical agents that inhibit TGFβ signaling in an isoform-specific manner may provide improved safety profiles over agents that affect multiple TGFβ isoforms. By contrast, a number of known TGFβ antagonists in the art produce unacceptable levels of toxicity at a dose that is shown to be efficacious *in vivo*. In such instances, lower dosing may be employed to avoid the toxicity but it may no longer produce sufficient *in vivo* efficacy at the reduced dose. Without wishing to be bound by particular theory, it is contemplated that such toxicity at least in part stems from lack of isoform specificity/selectivity of the agent.

Thus, in one aspect, the invention provides methods for reducing toxicities (*e.g.*, adverse events, unwanted side effects) associated with TGFβ inhibition in a subject. According to the invention, TGFβ1-specific inhibitors, such as those described herein, have a superior safety-efficacy profile, as compared to agents that elicit activities towards broader target (*e.g.*, more than one isoforms of TGFβ). Such TGFβ1-specific inhibitors can therefore be administered to subjects in need thereof at a therapeutically effective dose without causing adverse effects. Such approach would therefore broaden the dosage range in which both efficacy and safety/tolerability can be achieved in patients. Thus, the invention provides methods for treating a disease associated with TGFβ1 signaling, by administering to a subject an effective amount of a TGFβ inhibitor that is specific or highly selective for the TGFβ1 isoform. In some embodiments, such TGFβ1 isoform-selective or TGFβ1 isoform-specific inhibitors may be small molecule agents or biologics (*e.g.*, antibodies). Use of any such inhibitors for reducing toxicities (*e.g.,* adverse events or side effects) associated with TGFβ inhibition in a subject is encompassed by the present invention. According to the invention, the effective amount is within a dosage range that enables both: i) efficacy (*e.g.*, therapeutically beneficial effects); and, ii) safety (*e.g.*, within acceptable levels of adverse effects or side effects). In some embodiments, adverse effects may include cardiovascular toxicities, gastrointestinal toxicities, immune toxicities, bone/cartilage toxicities, reproductive

toxicities, and renal toxicities. In some embodiments, cardiovascular toxicities include, but are not limited to: heart valve lesions, *e.g.*, hemorrhage, inflammation, degeneration and proliferation of valvular interstitial cells. In some embodiments, adverse effects may include bleeding. In some embodiments, adverse effects may include skin lesions or tumors. In some embodiments, adverse effects may include tumor progression.

Accordingly, in some embodiments, the invention provides isoform-specific TGFβ1 inhibitor antibodies or antigen-binding fragments thereof, characterized in that they selectively inhibit the step of TGFβ1 activation *in vivo* but do not inhibit the step of TGFβ2 and/or TGFβ3 activation. Such antibodies or fragments thereof can be administered to subjects who benefit from TGFβ1 inhibition, in an amount effective to achieve clinical efficacy without causing unacceptable or intolerable levels of adverse effects. Thus, the present invention teaches that TGFβ1 isoform-specific inhibitors be specifically selected to meet both the efficacy and safety criteria for the treatment of a disease or condition associated with TGFβ signaling in human patients.

In a related aspect, the invention provides production methods for isoform-specific TGFβ modulators with an improved safety profile (*e.g.*, reduced *in vivo* toxicity). Such methods require that candidate agents be tested and selected for isoform specificity. In some embodiments, candidate agents are selected for specific activities against TGFβ1 signaling, and not TGFβ2 and/or TGFβ3 signaling. In some embodiments, such agents are TGFβ1 isoform-specific inhibitors. In some embodiments, such agents are antibodies or antigen-binding fragments thereof that specifically bind and block activation of TGFβ1, but not TGFβ2 and/or TGFβ3. In some embodiments, such antibodies or antigen-binding fragments thereof do not bind free mature TGFβ1 growth factor that is not associated with a pro/latent complex.

In another aspect, the present invention provides compositions and related methods for achieving further fine tuning of TGFβ signaling by modulating TGFβ activation in a context-dependent manner.

TGFβ is involved in conferring a number of cellular/tissue effects, and each such effect is mediated in part by its interaction with so-called "presenting molecules." Because expression of various presenting molecules is cell type- or tissue-specific it is contemplated that TGFβ confers a cellular effect, depending on its interaction with a particular presenting molecule (*i.e.*, "context"). Thus, among other things, the present disclosure provides monoclonal antibodies that selectively bind TGFβ present in a particular *context* (*i.e.*, a complex comprising TGFβ and a presenting molecule). In some embodiments, such

monoclonal antibodies specifically bind at least one, at least two, or, at least three of the following complexes: i) TGFβ1-GARP; ii) TGFβ1-LRRC33; iii) TGFβ1-LTBP1; and, iv) TGFβ1-LTBP3. In some embodiments, such monoclonal antibodies specifically bind one of the following complexes: i) TGFβ1-GARP; ii) TGFβ1-LRRC33; iii) TGFβ1-LTBP1; and, iv) TGFβ1-LTBP3. In some embodiments, such monoclonal antibodies specifically bind two of the following complexes: i) TGFβ1-GARP; ii) TGFβ1-LRRC33; iii) TGFβ1-LTBP1; and, iv) TGFβ1-LTBP3. In some embodiments, such monoclonal antibodies specifically bind three of the following complexes: i) TGFβ1-GARP; ii) TGFβ1-LRRC33; iii) TGFβ1-LTBP1; and, iv) TGFβ1-LTBP3. In some embodiments, such monoclonal antibodies specifically bind all of the following complexes: i) TGFβ1-GARP; ii) TGFβ1-LRRC33; iii) TGFβ1-LTBP1; and, iv) TGFβ1-LTBP3. In some embodiments, such monoclonal antibodies do not bind mature TGFβ1 that is free TGFβ1 (*e.g.*, not complexed with a presenting molecule).

The present disclosure includes monoclonal antibodies that bind to the small latent complex (*e.g.,* "C4S") of TGFβ1.

The disclosure further provides monoclonal antibodies that selectively target and modulate TGFβ in a particular *context.* In some embodiments, such monoclonal antibodies either inhibit or activate TGFβ in a particular *context.*

Accordingly, the invention provides compositions and methods for modulating (activating or inhibiting) a subset of TGFβ activities. Thus, the invention includes monoclonal antibodies which can selectively modulate a subset of TGFβ-mediated signaling pathways without affecting the other TGFβ-mediated signaling pathways. In some embodiments, the subset of TGFβ-mediated signaling pathways includes at least one, at least two, or, at least three of: i) GARP-mediated TGFβ effects, ii) LRRC33-mediated TGFβ effects, iii) LTBP1-mediated TGFβ effects, and iv) LTBP3-mediated TGFβ effects. In some embodiments, such monoclonal antibodies specifically modulate one of the following TGFβ-mediated signaling pathways, without modulating the other three pathways: i) GARP-mediated TGFβ effects, ii) LRRC33-mediated TGFβ effects, iii) LTBP1-mediated TGFβ effects, and iv) LTBP3-mediated TGFβ effects. In some embodiments, such monoclonal antibodies specifically modulate two of the following TGFβ-mediated signaling pathways, without modulating the other two pathways: i) GARP-mediated TGFβ effects, ii) LRRC33-mediated TGFβ effects, iii) LTBP1-mediated TGFβ effects, and iv) LTBP3-mediated TGFβ effects. In some embodiments, such monoclonal antibodies specifically modulate three of the following TGFβ-mediated signaling pathways, without modulating the other one pathway: i) GARP-mediated TGFβ effects, ii) LRRC33-mediated TGFβ effects, iii) LTBP1-mediated

TGFβ effects, and iv) LTBP3-mediated TGFβ effects. In some embodiments, such monoclonal antibodies specifically modulate all of the following TGFβ-mediated signaling pathways: i) GARP-mediated TGFβ effects, ii) LRRC33-mediated TGFβ effects, iii) LTBP1-mediated TGFβ effects, and iv) LTBP3-mediated TGFβ effects. In some embodiments, such monoclonal antibodies specifically modulate GARP-mediated TGFβ effects. In some embodiments, such monoclonal antibodies specifically modulate LRRC33-mediated TGFβ effects. In some embodiments, such monoclonal antibodies specifically modulate LTBP1-mediated TGFβ effects. In some embodiments, such monoclonal antibodies specifically modulate LTBP3-mediated TGFβ effects. Thus, the present invention provides methods for selectively targeting TGFβ activities in a context-dependent manner.

Aspects of the present invention include pharmaceutical compositions and methods for treating a disease or disorder in a human subject. In some embodiments, such disease or disorder includes conditions associated with immune regulation/dysregulation, conditions associated with T cell regulation/dysregulation; conditions associated with a fibrotic feature (fibrosis); and/or conditions associated with tumor.

Because the antibodies or fragments thereof that specifically target the pro/latent complex of TGFβ described herein modulate the *activation step* (*i.e.*, release of a free, mature TGFβ growth factor from the inactive, latent preform complex), as opposed to targeting already released, free, mature growth factor, the mode of action of these modulatory agents depends on the *source* of the TGFβ growth factor in the tissue. It is therefore contemplated that identification of the source of TGFβ involved in a disease context may help select an agent that can effectively modulate TGFβ in the correct *context*. For example, to treat a disease phenotype that involves GARP-mediated TGFβ1 effects, it is desirable to select antibodies or fragments thereof that specifically target a GARP-proTGFβ1 complex. To treat a disease phenotype that involves LRRC33-mediated TGFβ1 effects, it is desirable to select antibodies or fragments thereof that specifically target a LRRC33-proTGFβ1 complex. To treat a disease phenotype that involves LTBP1-mediated TGFβ1 effects, it is desirable to select antibodies or fragments thereof that specifically target a LTBP1-proTGFβ1 complex. To treat a disease phenotype that involves LTBP2-mediated TGFβ1 effects, it is desirable to select antibodies or fragments thereof that specifically target a LTBP2-proTGFβ1 complex. To treat a disease phenotype that involves LTBP3-mediated TGFβ1 effects, it is desirable to select antibodies or fragments thereof that specifically target a LTBP3-proTGFβ1 complex. To treat a disease phenotype that involves LTBP4-mediated TGFβ1 effects, it is desirable to select antibodies or fragments thereof that specifically target a LTBP4-proTGFβ1 complex.

To treat a disease phenotype that involves TGFβ1 effects mediated by multiple (*e.g.*, two or more) contexts, it is desirable to select antibodies or fragments thereof that can target the corresponding multiple contexts of TGFβ1 presentation.

Certain diseases are associated with multiple biological roles of TGFβ signaling that are not limited to a single context of TGFβ function. In such situations, it may be beneficial to modulate TGFβ effects across multiple contexts. Thus, in some embodiments, the invention provides methods for targeting and modulating TGFβ1 in an isoform-specific manner, rather than in a context-specific manner. Such agents may be referred to as "*isoform-specific, context-permissive*" TGFβ1 modulators. In some embodiments, context-permissive TGFβ1 modulators target multiple contexts (*e.g.,* multiple types of pro/latent-TGFβ1 complexes). In some embodiments, context-permissive TGFβ1 modulators target all types of pro/latent TGFβ1 complexes (*e.g.*, GARP-associated, LRRC33-associated, LTBP-associated, etc.) so as to encompass all contexts.

Whilst context-permissive TGFβ1 modulators are capable of targeting more than one types of pro/latent-TGFβ1 complexes (*i.e.,* with different presenting molecules), in some embodiments, such modulators may favor one or more context over the other. Thus, in some embodiments, a context-permissive antibody that inhibits the activation of TGFβ1 may preferentially inhibit TGFβ1 activation mediated by one presenting molecule over another presenting molecule, even if such antibody is capable of binding to both types of pro/latent complexes. In some embodiments, such antibody is a monoclonal antibody that binds and inhibits activation of LTBP-associated TGFβ1, GARP-associated TGFβ1, and LRRC33-associated TGFβ1, but with preferential inhibitory activities toward LTBP-associated TGFβ1. In some embodiments, such antibody is a monoclonal antibody that binds and inhibits activation of LTBP1-associated TGFβ1, LTBP3-associated TGFβ1, GARP-associated TGFβ1, and LRRC33-associated TGFβ1, but with preferential inhibitory activities toward LTBP1- and LTBP-3-associated TGFβ1. In some embodiments, such antibody is a monoclonal antibody that binds and inhibits activation of LTBP1-associated TGFβ1, LTBP3-associated TGFβ1, GARP-associated TGFβ1, and LRRC33-associated TGFβ1, but with preferential inhibitory activities toward GARP-associated TGFβ1 and LRRC33-associated TGFβ1. In some embodiments, such antibody is a monoclonal antibody that binds and inhibits activation of GARP-associated TGFβ1 and LRRC33-associated TGFβ1, but with preferential inhibitory activities toward GARP-associated TGFβ1. In some embodiments, such antibody is a monoclonal antibody that binds and inhibits activation of GARP-

associated TGFβ1 and LRRC33-associated TGFβ1, but with preferential inhibitory activities toward LRRC33-associated TGFβ1.

Thus, according to the invention, varying degrees of selectivity may be generated in order to target subset of TGFβ effects. *Isoform-specific* modulators of TGFβ (which target a single isoform of TGFβ) provide greater selectivity than *pan*-TGFβ modulators (which target multiple or all isoforms of TGFβ). *Isoform-specific, context-permissive* modulators of TGFβ (which target multiple contexts of a single isoform of TGFβ) provide greater selectivity than isoform-specific modulators. *Isoform-specific, context specific* modulators of TGFβ (which target single context of a single isoform of TGFβ) provide even greater selectivity than isoform-specific, context-permissive modulators.

Thus, in some embodiments, the invention includes methods for treating a disease associated with TGFβ signaling which comprise first identifying or confirming the source and/or context of disease-associated TGFβ, then selecting an agent that specifically targets the particular sub-pool of TGFβ in the tissue. In this way, it is contemplated that such approach may preserve normal function of TGFβ while preferentially modulating disease-associated function of TGFβ. In some embodiments, to identify the source/context of disease-associated TGFβ, expression of TGFβ presenting molecule(s) present in the diseased tissue may be assessed. To give but one example, within a disease tissue, there may be both LTBP-associated TGFβ1 latent complex, and GARP-associated TGFβ1 complex, and only the latter of the two may be expressed as a disease phenotype. In that scenario, it is desirable to inhibit GARP-mediated TGFβ1 signaling, while maintaining LTBP-mediated TGFβ1 signaling intact. Determination of the source/context of disease-associated TGFβ1 may be carried out with the use of antibodies that specifically bind TGFβ1 latent complex that includes a particular presenting molecule (*e.g.*, GARP, LRRC33, LTBPs, etc.). For the treatment of conditions associated with T cell regulation/dysregulation, some embodiments of the invention comprise administration of a composition comprising an effective amount of a monoclonal antibody that modulates GARP-mediated TGFβ effects in the subject.

For the treatment of conditions associated with immune regulation/dysregulation, some embodiments of the invention comprise administration of a composition comprising an effective amount of a monoclonal antibody that modulates GARP-mediated TGFβ effects and/or LRRC33-mediated TGFβ effects in the subject.

For the treatment of conditions associated with fibrosis, some embodiments of the invention comprise administration of a composition comprising an effective amount of a

monoclonal antibody that modulates LTBP1-mediated TGFβ effects and/or LTBP3-mediated TGFβ effects in the subject.

For the treatment of conditions associated with certain types of cancer, some embodiments of the invention comprise administration of a composition comprising an effective amount of a monoclonal antibody that modulates GARP-mediated TGFβ effects, LTBP1-mediated TGFβ effects and/or LTBP3-mediated TGFβ effects in the subject.

Aspects of the present disclosure relate to immunoglobulins, such as antibodies, or antigen binding portions thereof, that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex and/or a LRRC33-TGFβ1 complex. The antibodies, or antigen binding portions thereof, described herein, specifically bind to an epitope of TGFβ1 that is available for binding by the antibodies, or antigen binding portions thereof, when the TGFβ1 is present in a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex and/or a LRRC33-TGFβ1.

In one aspect, provided herein is an isolated antibody, or antigen binding portion thereof, that specifically binds to an epitope of TGFβ1, wherein the epitope is available for binding by the antibody when the TGFβ1 is present in two or more of the following protein complexes: a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and a LRRC33-TGFβ1 complex; and wherein the antibody does not bind free mature TGFβ1.

In some embodiments, the TGFβ1 is latent TGFβ1. In some embodiments, the TGFβ1 is proTGFβ1.

In some embodiments, the antibody, or antigen binding portion thereof, does not bind to TGFβ2. In some embodiments, the antibody, or antigen binding portion thereof, does not bind to TGFβ3. In some embodiments, the antibody, or antigen binding portion thereof, does not prevent the ability of TGFβ1 to bind to integrin.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a complementarity determining region 3 (CDR3) having the amino acid sequence of SEQ ID NO: 5 and a light chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 11. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a complementarity determining region 2 (CDR2) having the amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 9. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a complementarity determining region 1 (CDR1) having the amino acid sequence of SEQ ID

NO: 1 and a light chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 7.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 13 and a light chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 14.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 13 and a light chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 14.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 6 and a light chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 12. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 4 and a light chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 10. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 2 and a light chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 8.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 15 and a light chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 16.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 15 and a light chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 16.

In some embodiments, the antibody, or antigen binding portion thereof, inhibits TGFβ1 activation.

In some embodiments, the antibody, or antigen binding portion thereof, inhibits the release of mature TGFβ1 from the GARP-TGFβ1 complex, the LTBP1-TGFβ1 complex, the LTBP3-TGFβ1 complex, or the LRRC33-TGFβ1 complex.

In some embodiments, the antibody, or antigen binding portion thereof, has a dissociation constant (K_D) to the epitope of TGFβ1 selected from the group consisting of: at least about 10^{-8} M; at least about 10^{-9} M; at least about 10^{-10} M; at least about 10^{-11} M; at least about 10^{-12} M; and at least about 10^{-13} M.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain immunoglobulin constant domain of a human IgM constant domain, a human IgG constant domain, a human IgG_1 constant domain, a human IgG_2 constant domain, a human IgG_2A constant domain, a human IgG_2B constant domain, a human IgG_2 constant domain, a human IgG_3 constant domain, a human IgG_3 constant domain, a human IgG_4 constant domain, a human IgA constant domain, a human IgA_1 constant domain, a human IgA_2 constant domain, a human IgD constant domain, or a human IgE constant domain. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain immunoglobulin constant domain of a human IgG_1 constant domain or a human IgG_4 constant domain. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain immunoglobulin constant domain of a human IgG_4 constant domain. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain immunoglobulin constant domain of a human IgG_4 constant domain having a backbone substitution of Ser to Pro that produces an IgG_1-like hinge and permits formation of inter-chain disulfide bonds.

In some embodiments, the antibody or antigen binding portion thereof, further comprises a light chain immunoglobulin constant domain comprising a human Ig lambda constant domain or a human Ig kappa constant domain.

In some embodiments, the antibody is an IgG having four polypeptide chains which are two heavy chains and two light chains.

In some embodiments, wherein the antibody is a humanized antibody, a diabody, or a chimeric antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a human antibody. In some embodiments, the antibody comprises a framework having a human germline amino acid sequence.

In some embodiments, the antigen binding portion is a Fab fragment, a F(ab')2 fragment, a scFab fragment, or an scFv fragment.

In one aspect, provided herein is an anti-TGFβ1 antibody, or antigen binding portion thereof, that competes for binding with an antibody, or an antigen binding portion thereof, as described herein.

In another aspect, provided herein is an anti-TGFβ1 antibody, or antigen binding portion thereof, that binds to the same epitope as an antibody, or antigen binding portion thereof, as described herein.

In some embodiments, the antibody, or antigen binding portion thereof, is conjugated to a drug or a detectable moiety. In some embodiments, the antibody, or antigen binding portion thereof, is conjugated to the drug or to the detectable moiety via a linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the detectable moiety is selected from the group consisting of a fluorescent agent, a luminescent agent, an enzymatic agent, and a radioactive agent.

In one aspect, provided herein is a pharmaceutical composition comprising an antibody, or antigen binding portion thereof, as described herein, and a pharmaceutically acceptable carrier.

In another aspect, provided herein is a method for inhibiting TGFβ1 activation, the method comprising exposing a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, or a LRRC33-TGFβ1 complex to an antibody, an antigen binding portion thereof, or a pharmaceutical composition described herein.

In some embodiments, the antibody, or antigen binding portion thereof, inhibits the release of mature TGFβ1 from the GARP-TGFβ1 complex, the LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, or the LRRC33-TGFβ1 complex.

In some embodiments, the method is performed *in vitro*. In some embodiments, the method is performed *in vivo*.

In some embodiments, the GARP-TGFβ1 complex or the LRRC33-TGFβ1 complex is present at the outer surface of a cell.

In some embodiments, the cell is a T-cell, a fibroblast, a macrophage, a monocyte, a dendritic cell, an antigen presenting cell, or a microglia.

In some embodiments, the LTBP1-TGFβ1 complex or the LTBP3-TGFβ1 complex is bound to an extracellular matrix. In some embodiments, the extracellular matrix comprises fibrillin. In some embodiments, the extracellular matrix comprises a protein comprising an RGD motif.

In another aspect, provided herein is a method for reducing TGFβ1 activation in a subject, the method comprising administering to the subject an effective amount of an

antibody, an antigen binding portion thereof, or a pharmaceutical composition, as described herein, thereby reducing TGFβ1 activation in the subject.

In some embodiments, the subject has or is at risk of having fibrosis. In some embodiments, the subject has a muscular dystrophy. In some embodiments, the subject has Duchenne muscular dystrophy (DMD). In some embodiments, the subject has or is at risk of having liver fibrosis, kidney fibrosis, or lung fibrosis (*e.g.*, idiopathic pulmonary fibrosis). In some embodiments, the subject has or is at risk of having cancer. In some embodiments, the subject has or is at risk of having dementia. In some embodiments, the subject has or is at risk of developing myelofibrosis.

In some embodiments, the subject further receives an additional therapy. In some embodiments, the additional therapy is selected from the group consisting of a myostatin inhibitor, a VEGF agonist, an IGF1 agonist, an FXR agonist, a CCR2 inhibitor, a CCR5 inhibitor, a dual CCR2/CCR5 inhibitor, a lysyl oxidase-like-2 inhibitor, an ASK1 inhibitor, an Acetyl-CoA Carboxylase (ACC) inhibitor, a p38 kinase inhibitor, Pirfenidone, Nintedanib, a GDF11 inhibitor, or any combination thereof.

In some embodiments, the antibody, or the antigen binding portion thereof, reduces the suppressive activity of regulatory T cells.

In some embodiments, the antibody, or the antigen binding portion thereof, does not induce organ toxicity in the subject. In some embodiments, the organ toxicity comprises cardiovascular toxicity, gastrointestinal toxicity, immunotoxicity, bone toxicity, cartilage toxicity, reproductive system toxicity, or renal toxicity.

In one aspect, provided herein is a method for treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of an antibody, an antigen binding portion thereof, or a pharmaceutical composition, as described herein, thereby treating cancer in the subject.

In another aspect, provided herein is a method of reducing tumor growth in a subject in need thereof, the method comprising administering to the subject an effective amount of an antibody, an antigen binding portion thereof, or a pharmaceutical composition, as described herein, thereby reducing tumor growth in the subject.

In some embodiments, the antibody, or antigen binding portion thereof, is administered in combination with an additional agent or an additional therapy. In some embodiments, the additional agent is a checkpoint inhibitor. In some embodiments, the additional agent is selected from the group consisting of a PD-1 antagonist, a PDL1

antagonist, a PD-L1 or PDL2 fusion protein, a CTLA4 antagonist, a GITR agonist, an anti-ICOS antibody, an anti-ICOSL antibody, an anti-B7H3 antibody, an anti-B7H4 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-OX40 antibody, an anti-CD27 antibody, an anti-CD70 antibody, an anti-CD47 antibody, an anti-41BB antibody, an anti-PD-1 antibody, an oncolytic virus, and a PARP inhibitor. In some embodiments, the additional therapy is radiation, a chemotherapeutic, or a combination thereof. In some embodiments, the additional therapy is radiation. In some embodiments, the additional agent is a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is Taxol. In some embodiments, the additional agent is an anti-inflammatory agent. In some embodiments, the additional agent inhibits the process of monocyte/macrophage recruitment and/or tissue infiltration. In some embodiments, the additional agent is an inhibitor of hepatic stellate cell activation. In some embodiments, the additional agent is a chemokine receptor antagonist, *e.g.*, CCR2 antagonists and CCR5 antagonists. In some embodiments, such chemokine receptor antagonist is a dual specific antagonist, such as a CCR2/CCR5 antagonist. In some embodiments, the additional agent to be administered as combination therapy is or comprises a member of the TGFβ superfamily of growth factors or regulators thereof. In some embodiments, such agent is selected from modulators (*e.g.*, inhibitors and activators) of GDF8/myostatin and GDF11. In some embodiments, such agent is an inhibitor of GDF8/myostatin signaling. In some embodiments, such agent is a monoclonal antibody that specifically binds a pro/latent myostatin complex and blocks activation of myostatin. In some embodiments, the monoclonal antibody that specifically binds a pro/latent myostatin complex and blocks activation of myostatin does not bind free, mature myostatin.

In yet another aspect, provided herein is a method of treating a renal disorder in a subject in need thereof, the method comprising administering to the subject an effective amount of an antibody, an antigen binding portion thereof, or a pharmaceutical composition, as described herein, thereby treating the renal disorder in the subject.

In one aspect, provided herein is a nucleic acid encoding an antibody, or an antigen binding portion thereof, as described herein. Vectors comprising the nucleic acid are also provided.

In another aspect, provided herein is a cell comprising a nucleic acid encoding an antibody, or an antigen binding portion thereof, as described herein. Also provided is a cell comprising a vector comprising a nucleic acid encoding an antibody, or an antigen binding portion thereof, as described herein.

In yet another aspect, provided herein is a kit comprising an antibody, an antigen binding portion thereof, or a pharmaceutical composition, as described herein, and instructions for use thereof.

In another aspect, provided herein is a method for treating a myofiber damage, the method comprising a step of: administering to a subject having a myofiber damage an agent that selectively inhibits TGFβ1 over TGFβ2/3 in an amount effective to i) promote myofiber repair; ii) protect from contraction-induced injury; iii) reduce inflammation in muscle; and/or, iv) reduce fibrosis in muscle. In some embodiments, the amount does not cause unacceptable level of adverse effects in the subject.

In some embodiments, the myofiber damage is i) associated with a muscular dystrophy; or, ii) associated with an acute muscle injury. In some embodiments, the agent blocks activation of TGFβ1 but not TGFβ2 or TGFβ3. In some embodiments, the agent is a monoclonal antibody. In some embodiments, the monoclonal antibody binds a GARP-proTGFβ1 latent complex, an LRRC33-proTGFβ1 latent complex, an LTBP1-proTGFβ1 latent complex, an LTBP2-proTGFβ1 latent complex, an LTBP3-proTGFβ1 latent complex, and/or an LTBP4-proTGFβ1 latent complex. In some embodiments, the subject further receives a myostatin inhibitor.

In some embodiments, the method further comprises a step of: identifying a source or context of disease-associated TGFβ1.

In yet another aspect, provided herein is a method for producing a pharmaceutical composition that modulates TGFβ signaling, the method comprising steps of: providing one or more agents that modulate signaling of at least one isoform of TGFβ; measuring activities of the one or more agents towards all isoforms of TGFβ; selecting an agent that is specific to a single isoform of TGFβ; formulating into a pharmaceutical composition comprising an isoform-specific TGFβ modulator and a pharmaceutically acceptable excipient. Also provided is a pharmaceutical composition produced by this method.

In some embodiments, the isoform-specific TGFβ modulator is a TGFβ1-specific modulator. In some embodiments, the TGFβ1-specific modulator is an inhibitor of TGFβ1. In some embodiments, the isoform-specific TGFβ modulator is an antibody or a fragment thereof. In some embodiments, the antibody or fragment thereof specifically binds a pro/latent complex of TGFβ1. In some embodiments, the antibody or fragment thereof does not bind free mature TGFβ1 which is not in the pro/latent complex. In some embodiments, the pro/latent complex comprises GARP, LRRC33, LTBP1, LTBP2, LTBP3 or LTBP4.

In another aspect, provided herein is a method for treating a disease associated with TGFβ signaling, the method comprising a step of: administering to a subject in need thereof a pharmaceutical composition provided herein, in an amount effective to treat the disease, wherein the amount achieves statistically significant clinical efficacy and safety when administered to a patient population having the disease.

In yet another aspect, provided herein is a TGFβ inhibitor for use in reducing adverse effects in a subject, wherein the TGFβ inhibitor is isoform-selective. In some embodiments, the TGFβ inhibitor is an antibody that specifically inhibits TGFβ1.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depicting TGFβ bound in a latent complex in the tissue microenvironment.

FIGs. 2A and 2B are schematics depicting niche modulation in the microenvironment. FIG. 2A shows latent-transforming growth factor beta-binding proteins (LTBPs) presenting TGFβ in a fibrotic disease niche and a wound healing niche. FIG. 2B illustrates that glycoprotein-A repetitions predominant protein (GARP) modulates TGFβ activation in the inflammatory niche.

FIG. 3 illustrates a protein expression platform for making a GARP-TGFβ1 complex and a LTBP-TGFβ1 complex. The HEK293-based expression system uses NiNTA affinity purification and gel filtration to obtain multimilligram quantities of purified protein. Schematics of wild-type proTGFβ1, LTPB1, sGARP, and proTGF β1 C4S are shown.

FIGs. 4A and 4B show the purification of the sGARP-proTGFβ1 complex. FIG. 4A is a chromatogram of the sample and FIG. 4B shows a blot of the products and a schematic illustrating the complex.

FIGs. 5A and 5B show the purification of the sGARP-TGFβ1 LAP complex. FIG. 5A is a chromatogram of the sample and FIG. 5B shows a blot of the products and a schematic illustrating the complex.

FIGs. 6A and 6B show the purification of the LTBP1 complexed with proTGFβ1. FIG. 6A is a chromatogram of the sample and FIG. 6B shows a blot of the products and a schematic illustrating the complex. NR stands for non-reducing and R stands for reducing.

FIG. 7 is a graph showing that Ab1 and Ab2 block activation of TGFβ1 activity.

FIG. 8 shows an initial dose-response analysis of Ab1 in human cells.

FIG. 9 shows CAGA12 reporter cell assays illustrating TGFβ1 inhibition by Ab1 in human cells.

FIG. 10 is a graph showing that inhibition of the GARP complex blocks suppressive activity of T regulatory (Treg) cells in T cells isolated from healthy donor blood.

FIGs. 11A-11C show inhibition of integrin-mediated TGFβ1 release from fibroblasts. FIGs. 11A and 11B are graphs depicting the inhibition of endogenous TGFβ1 in either normal human dermal fibroblasts (circles), normal human lung fibroblasts (squares), murine C57BL/6J lung fibroblasts (inverted triangles), or murine DBA2/J muscle fibroblasts (circles) using increasing concentrations of either Ab1 (FIG. 11A) or Ab2 (FIG. 11B). FIG. 11C provides a schematic of the co-culture assay system.

FIGs. 12A and 12B depict the binding of either Ab1 (FIG. 12A) or Ab2 (FIG. 12B) to the LRRC33-proTGFβ1 complex.

FIG. 13A and 13B are graphs depicting the inhibition of GARP-TGFβ1 complex (FIG 13A) or LRRC33-TGFβ1 complex (FIG. 13B) in SW480/β6 cell transfectants using increasing concentrations of either Ab1 ("Ab1"), Ab2 ("Ab2"), isotype control IgG1 antibody ("Isotype control"), or vehicle control ("Vehicle").

FIG. 14 is a bar graph depicting the levels of hydroxyproline in kidney tissue from mice that underwent permanent right unilateral UUO surgery and were administered either PBS (control), 30 mg/kg of murine IgG1 control antibody, 3 mg/kg of Ab2, or 30 mg/kg of Ab2 intraperitoneally (i.p.) prior to surgical intervention (second to fifth bars); or mice that were administered PBS and underwent a laparotomy (sham control; first bar).

FIGs. 15A-15H are bar graphs depicting the relative mRNA levels of either plasminogen activator inhibitor-1 (PAI-1; FIG. 15A), connective tissue growth factor (CTGF; FIG. 15B), TGFβ1 (FIG. 15C), fibronectin-1 (FIG. 15D), α-smooth muscle actin (α-SMA; FIG. 15E), monocyte chemotactic protein 1 (MCP-1; FIG. 15F), collagen type I alpha 1 (Col1a1; FIG. 15G), or collagen type III alpha 1 chain (Col3a1; FIG. 15H) in kidney tissue from mice that underwent permanent right unilateral UUO surgery and were administered either PBS (control), 30 mg/kg of murine IgG1 control antibody, 3 mg/kg of Ab2, or 30 mg/kg of Ab2 intraperitoneally (i.p.) the day prior to surgical intervention, and then 1 and 3 days after the surgery (second to fifth bars of each graph); or mice that were administered PBS and underwent a laparotomy (sham control; first bar of each graph). This data is representative of multiple experiments.

FIG. 16 depicts the composite cortical collagen volume fraction (CVF) from three serial sections of the harvested right kidney from mice that were stained with picrosirius red and subjected to quantitative histological analyses using color spectrum segmentation. CVF from mice that underwent permanent right unilateral UUO surgery and were administered

either PBS ("Veh"), 30 mg/kg of murine IgG1 control antibody ("IgG Ctrl"), 3 mg/kg of Ab2 ("3 Ab2"), or 30 mg/kg of Ab2 ("30 Ab2") intraperitoneally (i.p.) prior to surgical intervention (second to fifth bars of each graph); or mice that were administered PBS and underwent a laparotomy ("Sham"; first bar of each graph).

FIG. 17 depicts the median tumor volume of MC38 murine colon carcinoma syngeneic model C57/BL/6 mice that were administered either murine IgG1 isotype control antibody in combination with rat IgG2a control antibody (group 1; control); Ab1 in combination with rat IgG2a control antibody (group 2); Ab2 in combination with rat IgG2a control antibody (group 3); murine IgG1 control antibody in combination with anti-PD-1 antibody (group 4); Ab1 in combination with anti-PD-1 antibody (group 5); Ab2 in combination with anti-PD-1 antibody (group 6).

FIG. 18A-18C show binding specificity of exemplary monoclonal antibodies. FIG. 18A depicts that Ab1 and Ab2 specifically bind proTGFβ1 as measured by ELISA, but not proTGFβ2, proTGFβ3, or mature TGFβ1. FIG. 18B depicts an example of purified LTBP-proTGFβ1 expressed and purified for use as antigens to determine antibody binding specificity. FIG. 18C depicts an example of an antibody which binds (as measured by ELISA) specifically to the LTBP1- proTGFβ1 complex.

FIG. 19 depicts a survival curve of rats treated with either vehicle control (PBS; "Control"); 200 mg/kg of LY2109761; 300 mg/kg of LY2109761; 100 mg/kg of a pan-TGFβ antibody ("Pan-TGFβ Ab"); or 100 mg/kg of Ab2.

FIG. 20 depicts the body weight (mean/standard deviation) of rats treated with either vehicle control (PBS; "Control"); 200 mg/kg of LY2109761; 300 mg/kg of LY2109761; 100 mg/kg of a pan-TGFβ antibody ("Pan-TGFβ Ab"); or 100 mg/kg of Ab2.

FIGs. 21A-21C depicts the body weight of individual rats treated with vehicle control (PBS; "Control"), 200 mg/kg of LY2109761, or 300 mg/kg of LY2109761 (FIG. 21A); vehicle control (PBS; "Control"), or 100 mg/kg of a pan-TGFβ antibody ("Pan-TGFβ Ab"); or vehicle control (PBS; "Control"), or 100 mg/kg of Ab2.

FIGs. 22A and 22B are graphs depicting the inhibition of GARP-proTGFβ1 complex or LRRC33-proTGFβ1 complex in SW480/β6 cells transiently transfectanted with plasmids to express proTGFβ1 and a presenting molecule (*i.e.*, GARP or LRRC33) using increasing concentrations of either Ab1 (FIG. 22A) or Ab2 (FIG. 22B). The IC50 (μg/mL) of Ab1 for the GARP-TGFβ1 complex was 0.445, and the IC50 (μg/mL) of Ab1 for the LRRC33-TGFβ1 complex was 1.325.

FIG. 23 depicts microscopy images of hematoxylin and eosin-stained sections from the heart valves of rats treated with either 200 mg/kg of LY2109761 (upper right panel); 100 mg/kg of a pan-TGFβ antibody ("Pan-TGFβ Ab", lower left panel); 100 mg/kg of Ab2 (lower right panel), or untreated control (upper left panel). Note: The lower right panel (Ab2) is an oblique section which stained darker due to uneven thickness.

DETAILED DESCRIPTION

In mammals, the transforming growth factor-beta (TGFβ) superfamily is comprised of at least 33 gene products. These include the bone morphogenic proteins (BMPs), activins, growth and differentiation factors (GDFs), and the three isoforms of the TGFβ family: TGFβ1, TGFβ2, and TGFβ3. The TGFβs are thought to play key roles in diverse processes, such as inhibition of cell proliferation, extracellular matrix (ECM) remodeling, and immune homeostasis. The importance of TGFβ1 for T cell homeostasis is demonstrated by the observation that TGFβ1-/- mice survive only 3-4 weeks, succumbing to multiorgan failure due to massive immune activation (Kulkarni, A.B., *et al.*, Proc Natl Acad Sci U S A, 1993. 90(2): p. 770-4; Shull, M.M., *et al.*, Nature, 1992. 359(6397): p. 693-9). The roles of TGFβ2 and TGFβ3 are less clear. Whilst the three TGFβ isoforms have distinct temporal and spatial expression patterns, they signal through the same receptors, TGFβRI and TGFβRII, although in some cases, for example for TGFβ2 signaling, type III receptors such as betaglycan are also required (Feng, X.H. and R. Derynck, Annu Rev Cell Dev Biol, 2005. 21: p. 659-93; Massague, J., Annu Rev Biochem, 1998. 67: p. 753-91). Ligand-induced oligomerization of TGFβRI/II triggers the phosphorylation of SMAD transcription factors, resulting in the transcription of target genes, such as Col1a1, Col3a1, ACTA2, and SERPINE1 (Massague, J., J. Seoane, and D. Wotton, Genes Dev, 2005. 19(23): p. 2783-810). SMAD-independent TGFβ signaling pathways have also been described, for example in cancer or in the aortic lesions of Marfan mice (Derynck, R. and Y.E. Zhang, Nature, 2003. 425(6958): p. 577-84; Holm, T.M., *et al.*, Science, 2011. 332(6027): p. 358-61).

The biological importance of the TGFβ pathway in humans has been validated by genetic diseases. Camurati-Engelman disease results in bone dysplasia due to an autosomal dominant mutation in the TGFB1 gene, leading to constitutive activation of TGFβ1 signaling (Janssens, K., *et al.*, J Med Genet, 2006. 43(1): p. 1-11). Patients with Loeys/Dietz syndrome carry autosomal dominant mutations in components of the TGFβ signaling pathway, which cause aortic aneurism, hypertelorism, and bifid uvula (Van Laer, L., H. Dietz, and B. Loeys, Adv Exp Med Biol, 2014. 802: p. 95-105). As TGFβ pathway dysregulation has been

implicated in multiple diseases, several drugs that target the TGFβ pathway have been developed and tested in patients, but with limited success.

The inventors of the present disclosure reasoned that, while all three isoforms of TGFβ are capable of signaling through the same receptors and transducing the downstream effectors in cells expressing the receptors, each TGFβ isoform may generate distinct biological effects *in vivo*. Moreover, the inventors contemplate that, at least in some circumstances, the mode by which the growth factor-receptor interaction is triggered may further provide signaling specificity *in vivo*. In light of this recognition, it is noted that TGFβ inhibitors described in the literature to date lack specificity as briefly summarized below.

Fresolimumab, a humanized monoclonal antibody that binds and inhibits all three isoforms of TGFβ has been tested clinically in patients with focal segmental glomerulosclerosis, malignant melanoma, renal cell carcinoma, and systemic sclerosis (Rice, L.M., *et al.,* J Clin Invest, 2015. 125(7): p. 2795-807; Trachtman, H., *et al.,* Kidney Int, 2011. 79(11): p. 1236-43; Morris, J.C., *et al.,* PLoS One, 2014. 9(3): p. e90353). Additional companies have developed monoclonal antibodies against the TGFβ growth factors with varying degrees of selectivity for TGFβ isoforms. Such agents likely elicit toxicities *in vivo* through residual activity against other TGFβ family members besides TGFβ1. To the best of the knowledge of the inventors of the present disclosure, complete specificity for a single isoform has not been achieved by targeting the mature growth factor, due to the high degree of sequence identity between isoforms.

Other approaches to target the TGFβ pathway include ACE-1332, a soluble TGFβRII-Fc ligand trap from Acceleron (Yung, L.M., *et al.*, A Am J Respir Crit Care Med, 2016. 194(9): p. 1140-1151), or small molecule inhibitors of the ALK5 kinase, such as Lilly's galunisertib. While ACE-1332 binds TGFβ1 and TGFβ3 with equally high affinity (Yung, L.M., *et al.,* Am J Respir Crit Care Med, 2016. 194(9): p. 1140-1151). ALK5 inhibitors block the activity of all growth factors that signal through TGFR1. Substantial toxicities have been found in preclinical studies using ALK5 inhibitors (Anderton, M.J., *et al.,* Toxicol Pathol, 2011. 39(6): p. 916-24; Stauber, A., *et al.*, Clinical Toxicology, 2014. 4(3): p. 1-10), and sophisticated clinical dosing schemes are required to maintain efficacy while reducing adverse events (Herbertz, S., *et al.,* Drug Des Devel Ther, 2015. 9: p. 4479-99). In fact, the question of TGFβ signaling specificity and its possible effect on toxicity observed with the known TGFβ inhibitors has not been raised in most, if not all, of the candidate drugs that attempted to block TGFβ. For example, how much of the toxicities are due to inhibition of TGFβ1 versus TGFβ2 and/or TGFβ3 has not been addressed. Similarly, modes of TGFβ

activation have not been taken into account in designing or developing ways to antagonize TGFβ signaling.

Recent structural insights into the activation mechanism of TGFβ1 have enabled the present inventors to take novel, more specific, approaches to TGFβ inhibition (Shi, M., *et al.*, Nature, 2011. 474(7351): p. 343-9). Unlike other cytokines, TGFβ superfamily members are not secreted as active growth factors, but as dimeric pro-proteins which consist of an N-terminal prodomain and a C-terminal growth factor domain. Cleavage of proTGFβ1 by furin proteases separates the homodimeric growth factor domain from its prodomain, also referred to as latency associated peptide (LAP). However, the growth factor and LAP remain noncovalently associated, forming a latent complex which is unable to bind its receptors and induce signaling (FIG. 1). During translation latent TGFβ1, also called the small latent complex (SLC), becomes linked to "presenting molecules" via disulfide bridges, forming the large latent complex (LLC). These molecules allow proTGFβ1 to be presented in specific cellular or tissue *contexts*. Two cysteines near the N-terminus of the latent TGFβ1 link to appropriately positioned cysteines on the presenting molecule. The identity of the presenting molecule depends on the environment and cell type producing latent TGF β1. For example, fibroblasts secrete latent TGF β1 tethered to latent TGFβ-binding proteins (LTBPs), which then associates with proteins in the extracellular matrix (ECM) (*i.e.*, fibronectin, fibrillin-1) to link latent TGFβ to the ECM (Robertson *et al.* Matrix Biol 47: 44-53 (2015) (FIG. 2A). On the surface of activated regulatory T cells latent TGFβ1 is covalently linked to the transmembrane protein GARP (FIG. 2B), and recently a protein closely related to GARP, LRRC33, was identified as a presenting molecule for TGFβ1 on the surface of monocytes, macrophages and microglia (Wang, R., *et al.*, Mol Biol Cell, 2012. 23(6): p. 1129-39 and T.A. Springer, Int. BMP Conference 2016).

In mammals there are four known LTBPs, LTBP1-4, each with multiple splice variants (Robertson, I.B., *et al.*, Matrix Biol, 2015. 47: p. 44-53). LTBP2 is the only LTBP that does not associate with latent TGFβ (Saharinen, J. and J. Keski-Oja, Mol Biol Cell, 2000. 11(8): p. 2691-704). While the association between LTBP1 or LTBP3 and latent TGFβ1 has been well validated, the role of LTBP4 in TGFβ presentation is less clear. The complex with LTBP4 and latent TGFβ1 appears to form much less efficiently, potentially due to the absence of several negatively charged residues in the TGFβ-binding domain of LTBP4 (Saharinen, J. and J. Keski-Oja, Mol Biol Cell, 2000. 11(8): p. 2691-704; Chen, Y., *et al.,* J Mol Biol, 2005. 345(1): p. 175-86). Both LTBP4S-/- mice and Urban-Rifkin-Davis syndrome patients, who have null mutations in LTBP4, suffer from disrupted elastic fiber assembly

(Urban, Z., *et al.,* Am J Hum Genet, 2009. 85(5): p. 593-605; Dabovic, B., *et al.,* J Cell Physiol, 2015. 230(1): p. 226-36). Additionally, while LTBP4S-/- mice have a lung septation and an elastogenesis defect, transgenic mice with an LTBP4 that cannot form a complex with latent TGFβ1 have no obvious phenotype (Dabovic, B., *et al.,* J Cell Physiol, 2015. 230(1): p. 226-36). Whether LTBP4 is directly involved in regulation of latent TGFβ1 by functioning as a presenting molecule is unclear; LTBP4 may instead be required for proper formation of elastic fibrils in the ECM and its loss indirectly affect latent TGFβ1 activation through defects in the ECM.

A number of studies have shed light on the mechanisms of TGFβ1 activation. Three integrins, αVβ6, αVβ8, and αVβ1 have been demonstrated to be key activators of latent TGFβ1 (Reed, N.I., *et al.,* Sci Transl Med, 2015. 7(288): p. 288ra79; Travis, M.A. and D. Sheppard, Annu Rev Immunol, 2014. 32: p. 51-82; Munger, J.S., *et al.,* Cell, 1999. 96(3): p. 319-28). αV integrins bind the RGD sequence present in TGFβ1 and TGFβ1 LAPs with high affinity (Dong, X., *et al.,* Nat Struct Mol Biol, 2014. 21(12): p. 1091-6). Transgenic mice with a mutation in the TGFβ1 RGD site that prevents integrin binding, but not secretion, phenocopy the TGFβ1-/- mouse (Yang, Z., *et al.,* J Cell Biol, 2007. 176(6): p. 787-93). Mice that lack both β6 and β8 integrins recapitulate all essential phenotypes of TGFβ1 and TGFβ3 knockout mice, including multiorgan inflammation and cleft palate, confirming the essential role of these two integrins for TGFβ1 activation in development and homeostasis (Aluwihare, P., *et al.,* J Cell Sci, 2009. 122(Pt 2): p. 227-32). Key for integrin-dependent activation of latent TGFβ1 is the covalent tether to presenting molecules; disruption of the disulfide bonds between GARP and TGFβ1 LAP by mutagenesis does not impair complex formation, but completely abolishes TGFβ1 activation by αVβ6 (Wang, R., *et al.,* Mol Biol Cell, 2012. 23(6): p. 1129-39). The recent structure of latent TGFβ1 illuminates how integrins enable release of active TGFβ1 from the latent complex: the covalent link of latent TGFβ1 to its presenting molecule anchors latent TGFβ1, either to the ECM through LTBPs, or to the cytoskeleton through GARP or LRRC33. Integrin binding to the RGD sequence results in a force-dependent change in the structure of LAP, allowing active TGFβ1 to be released and bind nearby receptors (Shi, M., *et al.*, Nature, 2011. 474(7351): p. 343-9). The importance of integrin-dependent TGFβ1 activation in disease has also been well validated. A small molecular inhibitor of αVβ1 protects against bleomycin-induced lung fibrosis and carbon tetrachloride-induced liver fibrosis (Reed, N.I., *et al.,* Sci Transl Med, 2015. 7(288): p. 288ra79), and αVβ6 blockade with an antibody or loss of integrin β6 expression suppresses bleomycin-induced lung fibrosis and radiation-induced fibrosis (Munger, J.S., *et al.,* Cell,

1999. 96(3): p. 319-28); Horan, G.S., *et al.*, Am J Respir Crit Care Med, 2008. 177(1): p. 56-65). In addition to integrins, other mechanisms of TGFβ1 activation have been implicated, including thrombospondin-1 and activation by proteases such as matrix metalloproteinases (MMPs), cathepsin D or kallikrein. However, the majority of these studies were performed *in vitro* using purified proteins; there is less evidence for the role of these molecules from *in vivo* studies. Knockout of thrombospondin-1 recapitulates some aspects of the TGFβ1-/- phenotype in some tissues, but is not protective in bleomycin-induced lung fibrosis, known to be TGFβ-dependent (Ezzie, M.E., *et al.,* Am J Respir Cell Mol Biol, 2011. 44(4): p. 556-61). Additionally, knockout of candidate proteases did not result in a TGFβ1 phenotype (Worthington, J.J., J.E. Klementowicz, and M.A. Travis, Trends Biochem Sci, 2011. 36(1): p. 47-54). This could be explained by redundancies or by these mechanisms being critical in specific diseases rather than development and homeostasis.

TGFβ has been implicated in a number of biological processes, including fibrosis, immune-modulation and cancer progression. TGFβ1 was the first identified member of the TGFβ superfamily of proteins. Like other members of the TGFβ superfamily, TGFβ1 and the isoforms TGFβ2 and TGFβ3, are initially expressed as inactive precursor pro-protein forms (termed proTGFβ). TGFβ proteins (*e.g.*, TGFβ1, TGFβ2 and TGFβ3) are proteolytically cleaved by proprotein convertases (*e.g.*, furin) to yield the latent form (termed latent TGFβ). In some embodiments, a pro-protein form or latent form of a TGFβ protein (*e.g.*, TGFβ1, TGFβ2 and TGFβ3) may be referred to as "pro/latent TGFβ protein". TGFβ1 may be presented to other molecules in complex with multiple molecules including, for example, GARP (to form a GARP-TGFβ1 complex), LRRC33 (to form a LRRC33-TGFβ1 complex), LTBP1 (to form a LTBP1-TGFβ1 complex), and/or LTBP3 (to form a LTBP3-TGFβ1 complex). The TGFβ1 present in these complexes may be in either latent form (latent TGFβ1) or in precursor form (proTGFβ1).

The present invention is directed to immunoglobulins, *e.g.*, antibodies, or antigen binding portions thereof, that specifically bind to (1) a TGFβ protein (*e.g.*, pro/latent TGFβ1, pro/latent TGFβ2, and pro/latent TGFβ3) in complex with a GARP protein, (2) a TGFβ protein in complex with a LTBP protein (*e.g.*, LTBP1 or LTBP3), and/or (3) a TGFβ protein in complex with a LRRC33 protein. In some embodiments, the antibodies, or antigen binding portions thereof, disclosed herein bind to an epitope of TGFβ1 that is available for binding by the antibody, or antigen binding portions thereof, when the TGFβ1 is present in a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex. Without wishing to be bound by any particular theory, the ability

of the antibodies, and antigen binding portions thereof, disclosed herein to bind to TGFβ protein (*e.g.*, TGFβ1) that is in complex with either a GARP protein, a LTBP protein, and/or a LRRC33 protein allows for the targeting of TGFβ protein in a context-independent manner, and may be particularly suitable for therapeutic applications.

Definitions

In order that the disclosure may be more readily understood, certain terms are first defined. These definitions should be read in light of the remainder of the disclosure and as understood by a person of ordinary skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. Additional definitions are set forth throughout the detailed description.

As used herein, the term "specific binding" or "specifically binds" means that the interaction of the antibody, or antigen binding portion thereof, with an antigen is dependent upon the presence of a particular structure (*e.g.*, an antigenic determinant or epitope). For example, the antibody, or antigen binding portion thereof, binds to a specific protein rather than to proteins generally. In some embodiments, an antibody, or antigen binding portion thereof, specifically binds to a target, *e.g.*, TGFβ1, if the antibody has a K_D for the target of at least about 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M, or less. In some embodiments, the term "specific binding to an epitope of TGFβ1", "specifically binds to an epitope of TGFβ1", "specific binding to TGFβ1", or "specifically binds to TGFβ1" as used herein, refers to an antibody, or antigen binding portion thereof, that binds to TGFβ1 and has a dissociation constant (K_D) of 1.0×10^{-7} M or less, as determined by surface plasmon resonance. In one embodiment, an antibody, or antigen binding portion thereof, can specifically bind to both human and a non-human (*e.g.*, mouse) orthologues of TGFβ1.

In some embodiments, the binding affinity of an antibody, or antigen binding portion thereof, to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex is determined using an Octet assay. In some embodiments, an Octet assay is an assay that determines one or more a kinetic parameters indicative of binding between an antibody and antigen. In some embodiments, an Octet® system (ForteBio, Menlo Park, CA) is used to determine the binding affinity of an antibody, or antigen binding portion thereof, to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex. For example, binding affinities

of antibodies may be determined using the *forté*Bio Octet QK^e dip and read label free assay system utilizing bio-layer interferometry. In some embodiments, antigens are immobilized to biosensors (*e.g.,* streptavidin-coated biosensors) and the antibodies and complexes (*e.g.*, biotinylated GARP-TGFβ1 complexes and biotinylated LTBP-TGFβ1 complexes) are presented in solution at high concentration (50 μg/mL) to measure binding interactions. In some embodiments, the binding affinity of an antibody, or antigen binding portion thereof, to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex is determined using the protocol outlined in Table 6.

As used herein, the term "GARP-TGFβ1 complex" refers to a protein complex comprising a pro-protein form or latent form of a transforming growth factor-β1 (TGFβ1) protein and a glycoprotein-A repetitions predominant protein (GARP). In some embodiments, a pro-protein form or latent form of TGFβ1 protein may be referred to as "pro/latent TGFβ1 protein". In some embodiments, a GARP-TGFβ1 complex comprises GARP covalently linked with pro/latent TGFβ1 *via* one or more disulfide bonds. In other embodiments, a GARP-TGFβ1 complex comprises GARP non-covalently linked with pro/latent TGFβ1. In some embodiments, a GARP-TGFβ1 complex is a naturally-occurring complex, for example a GARP-TGFβ1 complex in a cell. An exemplary GARP-TGFβ1 complex is shown in FIG. 3.

As used herein, the term "LTBP1-TGFβ1 complex" refers to a protein complex comprising a pro-protein form or latent form of transforming growth factor-β1 (TGFβ1) protein and a latent TGF-beta binding protein 1 (LTBP1). In some embodiments, a LTBP1-TGFβ1 complex comprises LTBP1 covalently linked with pro/latent TGFβ1 via one or more disulfide bonds. In other embodiments, a LTBP1-TGFβ1 complex comprises LTBP1 non-covalently linked with pro/latent TGFβ1. In some embodiments, a LTBP1-TGFβ1 complex is a naturally-occurring complex, for example a LTBP1-TGFβ1 complex in a cell. An exemplary LTBP1-TGFβ1 complex is shown in FIG. 3.

As used herein, the term "LTBP3-TGFβ1 complex" refers to a protein complex comprising a pro-protein form or latent form of transforming growth factor-β1 (TGFβ1) protein and a latent TGF-beta binding protein 1 (LTBP3). In some embodiments, a LTBP3-TGFβ1 complex comprises LTBP3 covalently linked with pro/latent TGFβ1 via one or more disulfide bonds. In other embodiments, a LTBP3-TGFβ1 complex comprises LTBP1 non-covalently linked with pro/latent TGFβ1. In some embodiments, a LTBP3-TGFβ1 complex is a naturally-occurring complex, for example a LTBP3-TGFβ1 complex in a cell. An exemplary LTBP3-TGFβ1 complex is shown in FIG. 3.

As used herein, the term "LRRC33-TGFβ1 complex" refers to a complex between a pro-protein form or latent form of transforming growth factor-β1 (TGFβ1) protein and a Leucine-Rich Repeat-Containing Protein 33 (LRRC33; also known as Negative Regulator Of Reactive Oxygen Species or NRROS). In some embodiments, a LRRC33-TGFβ1 complex comprises LRRC33 covalently linked with pro/latent TGFβ1 via one or more disulfide bonds. In other embodiments, a LRRC33-TGFβ1 complex comprises LRRC33 non-covalently linked with pro/latent TGFβ1. In some embodiments, a LRRC33-TGFβ1 complex is a naturally-occurring complex, for example a LRRC33-TGFβ1 complex in a cell.

The term "antibody" refers to an immunoglobulin molecule that specifically binds to a target antigen, and includes, for instance, chimeric, humanized, fully human, and bispecific antibodies. An intact antibody will generally comprise at least two full-length heavy chains and two full-length light chains, but in some instances can include fewer chains such as antibodies naturally occurring in camelids which can comprise only heavy chains. Antibodies can be derived solely from a single source, or can be "chimeric," that is, different portions of the antibody can be derived from two different antibodies. Antibodies, or antigen binding portions thereof, can be produced in hybridomas, by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. The term antibodies, as used herein, includes monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, human antibodies, antibody fusions (sometimes referred to herein as "antibody conjugates"), respectively. In some embodiments, the term also encompasses peptibodies.

Naturally-occurring antibody structural units typically comprise a tetramer. Each such tetramer typically is composed of two identical pairs of polypeptide chains, each pair having one full-length "light" (in certain embodiments, about 25 kDa) and one full-length "heavy" chain (in certain embodiments, about 50-70 kDa). The amino-terminal portion of each chain typically includes a variable region of about 100 to 110 or more amino acids that typically is responsible for antigen recognition. The carboxy-terminal portion of each chain typically defines a constant region that can be responsible for effector function. Human antibody light chains are typically classified as kappa and lambda light chains. Heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the isotype of the antibody. An antibody can be of any type (*e.g.*, IgM, IgD, IgG, IgA, IgY, and IgE) and class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgM1, IgM2, IgA1, and IgA2). Within full-length light and heavy chains, typically, the variable and constant regions are joined by a "J" region of about

12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids (see, *e.g.*, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety)). The variable regions of each light/heavy chain pair typically form the antigen binding site.

The variable regions typically exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair typically are aligned by the framework regions, which can enable binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chain variable regions typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is typically in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk (1987) *J. Mol. Biol.* 196: 901-917; Chothia *et al.* (1989) *Nature* 342: 878-883. The CDRs of a light chain can also be referred to as CDR-L1, CDR-L2, and CDR-L3, and the CDRs of a heavy chain can also be referred to as CDR-H1, CDR-H2, and CDR-H3. In some embodiments, an antibody can comprise a small number of amino acid deletions from the carboxy end of the heavy chain(s). In some embodiments, an antibody comprises a heavy chain having 1-5 amino acid deletions in the carboxy end of the heavy chain. In certain embodiments, definitive delineation of a CDR and identification of residues comprising the binding site of an antibody is accomplished by solving the structure of the antibody and/or solving the structure of the antibody-ligand complex. In certain embodiments, that can be accomplished by any of a variety of techniques known to those skilled in the art, such as X-ray crystallography. In some embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. Examples of such methods include, but are not limited to, the Kabat definition, the Chothia definition, the AbM definition, and the contact definition.

A "functional antigen binding site" of a binding protein is one that that can bind to a target, antigen, or ligand. The antigen binding affinity of the antigen binding site is not necessarily as strong as the parent binding protein from which the antigen binding site is derived, but the ability to bind antigen must be measurable using any one of a variety of methods known for evaluating binding protein binding to an antigen. Moreover, the antigen binding affinity of each of the antigen binding sites of a multispecific binding protein herein need not be quantitatively the same.

The term "variable region" or "variable domain" refers to a portion of the light and/or heavy chains of an antibody, typically including approximately the amino-terminal 120 to 130 amino acids in the heavy chain and about 100 to 110 amino terminal amino acids in the light chain. In certain embodiments, variable regions of different antibodies differ extensively in amino acid sequence even among antibodies of the same species. The variable region of an antibody typically determines specificity of a particular antibody for its target.

An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art.

The term "compete" when used in the context of antigen binding proteins (*e.g.*, an antibody or antigen binding portion thereof) that compete for the same epitope means competition between antigen binding proteins as determined by an assay in which the antigen binding protein being tested prevents or inhibits (*e.g.*, reduces) specific binding of a reference antigen binding protein to a common antigen (*e.g.*, TGFβ1 or a fragment thereof). Numerous types of competitive binding assays can be used to determine if one antigen binding protein competes with another, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay; solid phase direct biotin-avidin EIA; solid phase direct labeled assay, and solid phase direct labeled sandwich assay. Usually, when a competing antigen binding protein is present in excess, it will inhibit (*e.g.*, reduce) specific binding of a reference antigen binding protein to a common antigen by at least 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75% or 75% or more. In some instances, binding is inhibited by at least 80-85%, 85-90%, 90-95%, 95-97%, or 97% or more.

The term "antigen" refers to a molecular structure that provides an epitope, *e.g.,* a molecule or a portion of a molecule, or a complex of molecules or portions of molecules, capable of being bound by a selective binding agent, such as an antigen binding protein (including, *e.g.*, an antibody). Thus, a selective binding agent may specifically bind to an antigen that is formed by two or more components in a complex. In some embodiments, the antigen is capable of being used in an animal to produce antibodies capable of binding to that antigen. An antigen can possess one or more epitopes that are capable of interacting with different antigen binding proteins, *e.g.*, antibodies.

As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each

of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region that can bind the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat *et al.* (1987; 1991) Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md.) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia & Lesk (1987) J. Mol. Biol. 196: 901-917; and Chothia *et al.* (1989) Nature 342: 877-883) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3, where the "L" and the "H" designate the light chain and the heavy chain regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (1995) FASEB J. 9: 133-139 and MacCallum (1996) J. Mol. Biol. 262(5): 732-45. Still other CDR boundary definitions may not strictly follow one of the herein systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs.

The terms "crystal" and "crystallized" as used herein, refer to a binding protein (*e.g.*, an antibody), or antigen binding portion thereof, that exists in the form of a crystal. Crystals are one form of the solid state of matter, which is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (*e.g.*, proteins such as antibodies), or molecular assemblies (*e.g.*, antigen/antibody complexes). These three-dimensional arrays are arranged according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit cell by regular translations in all three dimensions provides the crystal. See Giege, R. and Ducruix, A. Barrett, Crystallization of Nucleic Acids and Proteins,

a Practical Approach, 2nd ea., pp. 201-16, Oxford University Press, New York, New York, (1999).

The term "epitope" includes any molecular determinant (*e.g.,* polypeptide determinant) that can specifically bind to a binding agent, immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules, such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three- dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by a binding protein. An epitope thus consists of the amino acid residues of a region of an antigen (or fragment thereof) known to bind to the complementary site on the specific binding partner. An antigenic fragment can contain more than one epitope. In certain embodiments, an antibody is the to specifically bind an antigen when it recognizes its target antigen in a complex mixture of proteins and/or macromolecules. For example, antibodies are said to "bind to the same epitope" if the antibodies cross-compete (one prevents the binding or modulating effect of the other). In addition, structural definitions of epitopes (overlapping, similar, identical) are informative, but functional definitions are often more relevant as they encompass structural (binding) and functional (modulation, competition) parameters.

The term "treat" and "treatment" includes therapeutic treatments, prophylactic treatments, and applications in which one reduces the risk that a subject will develop a disorder or other risk factor. Treatment does not require the complete curing of a disorder and encompasses embodiments in which one reduces symptoms or underlying risk factors.

Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques can be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures can be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, *e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose. Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and

commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

The terms "antigen binding portion" or "antigen binding fragment" of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, TGFβ1). Antigen binding portions include, but are not limited to, any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. In some embodiments, an antigen-binding portion of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Non-limiting examples of antigen-binding portions include: (i) Fab fragments, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) F(ab')2 fragments, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) Fd fragments consisting of the VH and CH1 domains;; (iv) Fv fragments consisting of the VL and VH domains of a single arm of an antibody; (v) single-chain Fv (scFv) molecules (see, *e.g.*, Bird *et al.* (1988) SCIENCE 242:423-426; and Huston *et al.* (1988) PROC. NAT'L. ACAD. SCI. USA 85:5879-5883); (vi) dAb fragments (see, *e.g.*, Ward *et al.* (1989) NATURE 341: 544-546); and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR)). Other forms of single chain antibodies, such as diabodies are also encompassed. The term antigen binding portion of an antibody includes a "single chain Fab fragment" otherwise known as an "scFab," comprising an antibody heavy chain variable domain (VH), an antibody constant domain 1 (CH1), an antibody light chain variable domain (VL), an antibody light chain constant domain (CL) and a linker, wherein said antibody domains and said linker have one of the following orders in N-terminal to C-terminal direction: a) VH-CH1-linker-VL-CL, b) VL-CL-linker-VH-CH1, c) VH-CL-linker-VL-CH1 or d) VL-CH1-linker-VH-CL; and wherein said linker is a polypeptide of at least 30 amino acids, preferably between 32 and 50 amino acids.

An "isolated antibody", as used herein, refers to an antibody that is substantially free of other antibodies having different antigenic specificities. In some embodiments, an isolated antibody is substantially free of other cellular material and/or chemicals.

An "affinity matured" antibody is an antibody with one or more alterations in one or more CDRs thereof, which result an improvement in the affinity of the antibody for antigen

compared to a parent antibody, which does not possess those alteration(s). Exemplary affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks *et al.* (1992) Bio/Technology 10: 779-783 describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas, *et al.* (1994) Proc Nat. Acad. Sci. USA 91: 3809-3813; Schier *et al.* (1995) Gene 169: 147-155; Yelton *et al.*, (1995) J. Immunol. 155: 1994-2004; Jackson *et al.* (1995) J. Immunol. 154(7): 3310-9; and Hawkins *et al.* (1992) J. Mol. Biol. 226: 889-896; and selective mutation at selective mutagenesis positions, contact or hypermutation positions with an activity enhancing amino acid residue is described in U.S. Patent No. 6,914,128.

The term "CDR-grafted antibody" refers to antibodies, which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (*e.g.*, CDR3) has been replaced with human CDR sequences.

The term "chimeric antibody" refers to antibodies, which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "human antibody," as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the present disclosure may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "humanized antibody" refers to antibodies, which comprise heavy and light chain variable region sequences from a non-human species (*e.g.*, a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like," *i.e.*, more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences. Also

"humanized antibody" is an antibody, or a variant, derivative, analog or fragment thereof, which immunospecifically binds to an antigen of interest and which comprises an FR region having substantially the amino acid sequence of a human antibody and a CDR region having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab')2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (*i.e.,* donor antibody) and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. In an embodiment a humanized antibody also comprises at least a portion of an immunoglobulin Fc region, typically that of a human immunoglobulin. In some embodiments a humanized antibody contains the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments a humanized antibody only contains a humanized light chain. In some embodiments a humanized antibody only contains a humanized heavy chain. In specific embodiments a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub-regions constituting a framework region.

As used herein, the term "germline antibody gene" or "gene fragment" refers to an immunoglobulin sequence encoded by non-lymphoid cells that have not undergone the maturation process that leads to genetic rearrangement and mutation for expression of a

particular immunoglobulin (see, *e.g.*, Shapiro *et al.* (2002) Crit. Rev. Immunol. 22(3): 183-200; Marchalonis *et al.* (2001) Adv. Exp. Med. Biol. 484: 13-30). One of the advantages provided by various embodiments of the present disclosure stems from the recognition that germline antibody genes are more likely than mature antibody genes to conserve essential amino acid sequence structures characteristic of individuals in the species, hence less likely to be recognized as from a foreign source when used therapeutically in that species.

As used herein, the term "neutralizing" refers to counteracting the biological activity of an antigen when a binding protein specifically binds to the antigen. In an embodiment, the neutralizing binding protein binds to the antigen/ target, *e.g.*, cytokine, kinase, growth factor, cell surface protein, soluble protein, phosphatase, or receptor ligand, and reduces its biologically activity by at least about 20%, 40%, 60%, 80%, 85%, 90%, 95%. 96%, 97%. 98%, 99% or more.

The term "binding protein" as used herein includes any polypeptide that specifically binds to an antigen (*e.g.*, TGFβ1), including, but not limited to, an antibody, or antigen binding portions thereof, a DVD-IgTM, a TVD-Ig, a RAb-Ig, a bispecific antibody and a dual specific antibody.

The term "monoclonal antibody" or "mAb" when used in a context of a composition comprising the same may refer to an antibody preparation obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method.

The term "recombinant human antibody," as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further in Section II C, below), antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom, H.R. (1997) TIB Tech. 15: 62-70; Azzazy, H. and Highsmith, W.E. (2002) Clin. Biochem. 35: 425-445; Gavilondo, J.V. and Larrick, J.W. (2002) BioTechniques 29: 128-145; Hoogenboom, H. and Chames, P. (2000) Immunol. Today 21: 371-378, incorporated herein by reference), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see, Taylor, L. D.

et al. (1992) Nucl. Acids Res. 20: 6287-6295; Kellermann, S-A. and Green, L.L. (2002) Cur. Opin. in Biotechnol. 13: 593-597; Little, M. *et al.* (2000) Immunol. Today 21: 364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

As used herein, "Dual Variable Domain Immunoglobulin" or "DVD-IgTM" and the like include binding proteins comprising a paired heavy chain DVD polypeptide and a light chain DVD polypeptide with each paired heavy and light chain providing two antigen binding sites. Each binding site includes a total of 6 CDRs involved in antigen binding per antigen binding site. A DVD-IgTM is typically has two arms bound to each other at least in part by dimerization of the CH3 domains, with each arm of the DVD being bispecific, providing an immunoglobulin with four binding sites. DVD-IgTM are provided in US Patent Publication Nos. 2010/0260668 and 2009/0304693, each of which are incorporated herein by reference including sequence listings.

As used herein, "Triple Variable Domain Immunoglobulin" or "TVD-Ig" and the like are binding proteins comprising a paired heavy chain TVD binding protein polypeptide and a light chain TVD binding protein polypeptide with each paired heavy and light chain providing three antigen binding sites. Each binding site includes a total of 6 CDRs involved in antigen binding per antigen binding site. A TVD binding protein may have two arms bound to each other at least in part by dimerization of the CH3 domains, with each arm of the TVD binding protein being trispecific, providing a binding protein with six binding sites.

As used herein, "Receptor-Antibody Immunoglobulin" or "RAb-Ig" and the like are binding proteins comprising a heavy chain RAb polypeptide, and a light chain RAb polypeptide, which together form three antigen binding sites in total. One antigen binding site is formed by the pairing of the heavy and light antibody variable domains present in each of the heavy chain RAb polypeptide and the light chain RAb polypeptide to form a single binding site with a total of 6 CDRs providing a first antigen binding site. Each the heavy chain RAb polypeptide and the light chain RAb polypeptide include a receptor sequence that

independently binds a ligand providing the second and third "antigen" binding sites. A RAb-Ig is typically has two arms bound to each other at least in part by dimerization of the CH3 domains, with each arm of the RAb-Ig being trispecific, providing an immunoglobulin with six binding sites. RAb-Igs are described in US Patent Application Publication No. 2002/0127231, the entire contents of which including sequence listings are incorporated herein by reference).

The term "bispecific antibody," as used herein, and as differentiated from a "bispecific half-Ig binding protein" or "bispecific (half-Ig) binding protein", refers to full-length antibodies that are generated by quadroma technology (see Milstein, C. and Cuello, A.C. (1983) Nature 305(5934): p. 537-540), by chemical conjugation of two different monoclonal antibodies (see Staerz, U.D. *et al.* (1985) Nature 314(6012): 628-631), or by knob-into-hole or similar approaches, which introduce mutations in the Fc region that do not inhibit CH3-CH3 dimerization (see Holliger, P. *et al.* (1993) Proc. Natl. Acad. Sci USA 90(14): 6444-6448), resulting in multiple different immunoglobulin species of which only one is the functional bispecific antibody. By molecular function, a bispecific antibody binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different antigen (or epitope) on its second arm (a different pair of HC/LC). By this definition, a bispecific antibody has two distinct antigen binding arms (in both specificity and CDR sequences), and is monovalent for each antigen it binds to.

The term "dual-specific antibody," as used herein, and as differentiated from a bispecific half-Ig binding protein or bispecific binding protein, refers to full-length antibodies that can bind two different antigens (or epitopes) in each of its two binding arms (a pair of HC/LC) (see PCT Publication No. WO 02/02773). Accordingly, a dual-specific binding protein has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bivalent for each antigen to which it binds.

The term "pan-TGFβ antibody" refers to any antibody that is capable of binding to more than one isoform of TGFβ, for example, at least two of TGFβ1, TGFβ2, and TGFβ3. In some embodiments, a pan-TGFβ antibody binds all three isoforms, *i.e.,* TGFβ1, TGFβ2, and TGFβ3. In some embodiments, a pan-TGFβ antibody binds and neutralizes all three isoforms, *i.e.,* TGFβ1, TGFβ2, and TGFβ3.

The term "K_{on}," as used herein, is intended to refer to the on rate constant for association of a binding protein (*e.g.*, an antibody) to the antigen to form the, *e.g.*, antibody/antigen complex as is known in the art. The "K_{on}" also is known by the terms "association rate constant," or "k_a," as used interchangeably herein. This value indicating the

binding rate of an antibody to its target antigen or the rate of complex formation between an antibody and antigen also is shown by the equation: Antibody ("Ab") + Antigen ("Ag")→Ab-Ag.

The term "K_{off}," as used herein, is intended to refer to the off rate constant for dissociation of a binding protein (*e.g.*, an antibody) from the, *e.g.*, antibody/antigen complex as is known in the art. The "K_{off}" also is known by the terms "dissociation rate constant" or "k_d" as used interchangeably herein. This value indicates the dissociation rate of an antibody from its target antigen or separation of Ab-Ag complex over time into free antibody and antigen as shown by the equation: Ab + Ag←Ab-Ag.

The terms "equilibrium dissociation constant" or "K_D," as used interchangeably herein, refer to the value obtained in a titration measurement at equilibrium, or by dividing the dissociation rate constant (k_{off}) by the association rate constant (k_{on}). The association rate constant, the dissociation rate constant, and the equilibrium dissociation constant are used to represent the binding affinity of a binding protein, *e.g.,* antibody, to an antigen. Methods for determining association and dissociation rate constants are well known in the art. Using fluorescence–based techniques offers high sensitivity and the ability to examine samples in physiological buffers at equilibrium. Other experimental approaches and instruments, such as a BIAcore® (biomolecular interaction analysis) assay, can be used (*e.g.*, instrument available from BIAcore International AB, a GE Healthcare company, Uppsala, Sweden). Additionally, a KinExA® (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, Idaho), can also be used.

The term "linker" is used to denote polypeptides comprising two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well known in the art (see, *e.g.*, Holliger, P. *et al.* (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448; Poljak, R.J. *et al.* (1994) Structure 2:1121-1123). Exemplary linkers include, but are not limited to, ASTKGPSVFPLAP (SEQ ID NO: 55), ASTKGP (SEQ ID NO: 56); TVAAPSVFIFPP (SEQ ID NO: 57); TVAAP (SEQ ID NO: 58); AKTTPKLEEGEFSEAR (SEQ ID NO: 59); AKTTPKLEEGEFSEARV (SEQ ID NO: 60); AKTTPKLGG (SEQ ID NO: 61); SAKTTPKLGG (SEQ ID NO: 62); SAKTTP (SEQ ID NO: 63); RADAAP (SEQ ID NO: 64); RADAAPTVS (SEQ ID NO: 65); RADAAAAGGPGS (SEQ ID NO: 66); RADAAAA$(G4S)_4$ (SEQ ID NO: 67); SAKTTPKLEEGEFSEARV (SEQ ID NO: 68); ADAAP (SEQ ID NO: 69); ADAAPTVSIFPP (SEQ ID NO: 70); QPKAAP (SEQ ID NO: 71); QPKAAPSVTLFPP (SEQ ID NO: 72); AKTTPP (SEQ ID NO: 73); AKTTPPSVTPLAP (SEQ ID NO: 74);

AKTTAP (SEQ ID NO: 75); AKTTAPSVYPLAP (SEQ ID NO: 76); GGGGSGGGGSGGGGS (SEQ ID NO: 77); GENKVEYAPALMALS (SEQ ID NO: 78); GPAKELTPLKEAKVS (SEQ ID NO: 79); GHEAAAVMQVQYPAS (SEQ ID NO: 80); TVAAPSVFIFPPTVAAPSVFIFPP (SEQ ID NO: 81); and ASTKGPSVFPLAPASTKGPSVFPLAP (SEQ ID NO: 82).

The term "cancer" as used herein refers to the physiological condition in multicellular eukaryotes that is typically characterized by unregulated cell proliferation.

"Label" and "detectable label" or "detectable moiety" mean a moiety attached to a specific binding partner, such as an antibody or an analyte, *e.g.*, to render the reaction between members of a specific binding pair, such as an antibody and an analyte, detectable, and the specific binding partner, *e.g.*, antibody or analyte, so labeled is referred to as "detectably labeled." Thus, the term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. In an embodiment, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, *e.g.*, incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm); chromogens; fluorescent labels (*e.g.*, FITC, rhodamine, and lanthanide phosphors); enzymatic labels (*e.g.*, horseradish peroxidase, luciferase, and alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, and epitope tags); and magnetic agents, such as gadolinium chelates. Representative examples of labels commonly employed for immunoassays include moieties that produce light, *e.g.*, acridinium compounds, and moieties that produce fluorescence, *e.g.*, fluorescein. Other labels are described herein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety. Use of "detectably labeled" is intended to encompass the latter type of detectable labeling.

The term "surface plasmon resonance," as used herein, refers to an optical phenomenon that allows for the analysis of real-time bispecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example, using the BIAcore® system (BIAcore International AB, a GE Healthcare company, Uppsala, Sweden

and Piscataway, NJ). For further descriptions, see Jönsson, U. *et al.* (1993) Ann. Biol. Clin. 51: 19-26; Jönsson, U. *et al.* (1991) Biotechniques 11: 620-627; Johnsson, B. *et al.* (1995) J. Mol. Recognit. 8: 125-131; and Johnnson, B. *et al.* (1991) Anal. Biochem. 198: 268-277.

A "plasmid" or "vector" includes a nucleic acid construct designed for delivery to a host cell or transfer between different host cell. An "expression plasmid" or "expression vector" can be a plasmid that has the ability to incorporate and express heterologous nucleic acid fragments in a cell. An expression plasmid may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms. The nucleic acid incorporated into the plasmid can be operatively linked to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that polynucleotide sequence.

A "nucleic acid" or "nucleic acid sequence" may be any molecule, preferably a polymeric molecule, incorporating units of ribonucleic acid, deoxyribonucleic acid or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one nucleic acid strand of a denatured double- stranded DNA. Alternatively, it can be a single-stranded nucleic acid not derived from any double-stranded DNA. In one aspect, the nucleic acid can be DNA. In another aspect, the nucleic acid can be RNA. Suitable nucleic acid molecules are DNA, including genomic DNA or cDNA. Other suitable nucleic acid molecules are RNA, including mRNA.

Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages can mean ±1%.

While several embodiments of the present disclosure have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present disclosure. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present disclosure is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. It is, therefore, to

be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the disclosure may be practiced otherwise than as specifically described and claimed. The present disclosure is directed to each individual feature, system, article, material, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, and/or methods, if such features, systems, articles, materials, and/or methods are not mutually inconsistent, is included within the scope of the present disclosure.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, *i.e.*, elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to "A and/or B," when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

Use of ordinal terms such as "first," "second," "third," *etc.*, in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, *e.g.*, 10-20, 1-10, 30-40, etc.

Antibodies, and Antigen-binding Portions Thereof, that Specifically Bind to a GARP-TGFβ1 Complex, a LTBP1-TGFβ1 Complex, a LTBP3-TGFβ1 Complex, and/or a LRRC33-TGFβ1 Complex

The present invention is based, at least in part, on the discovery of antibodies, and antigen binding portions thereof, that bind TGFβ1 that is present in a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex. Accordingly, some aspects of the invention relate to antibodies, or antigen binding portions thereof, that specifically bind to an epitope of TGFβ1, wherein the epitope is available for binding by the antibody, or antigen binding portions thereof, when the TGFβ1 is present in a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex. In some embodiments, the epitope is available due to a conformational change in TGFβ1 when in complex with GARP, LTBP1, LTBP3, and/or LRRC33. In some embodiments, the epitope in TGFβ1 to which the antibodies, or antigen binding portions thereof, bind is not available when TGFβ1 is not in complex with GARP, LTBP1, LTBP3, and/or LRRC33. In some embodiments, the antibodies, or antigen binding portions thereof, do not specifically bind to TGFβ2. In some embodiments, the antibodies, or antigen binding portions thereof, do not specifically bind to TGFβ3. In some embodiments, the antibodies, or antigen binding portions thereof, do not prevent TGFβ1 from binding to integrin. For example, in some embodiments, the antibodies, or antigen binding portions thereof, do not mask the integrin-binding site of TGFβ1. In some embodiments, the antibodies, or antigen binding portions thereof, inhibit the activation of TGFβ1. In some embodiments, the antibodies, or antigen binding portions thereof, inhibit the release of

mature TGFβ1 from a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex.

Antibodies, or antigen binding portions thereof, provided herein specifically bind to an epitope of TGFβ1, wherein the epitope is available for binding by the antibody, or antigen binding portions thereof, when the TGFβ1 is present in a GARP-TGFβ1, a LTBP1-TGFβ1 complex, a LTBP2-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex. In some embodiments, the TGFβ1 comprises a naturally occurring mammalian amino acid sequence. In some embodiment, the TGFβ1 comprises a naturally occurring human amino acid sequence. In some embodiments, the TGFβ1 comprises a human, a monkey, a rat or a mouse amino acid sequence. In some embodiments, an antibody, or antigen binding portion thereof, described herein does not specifically bind to TGFβ2. In some embodiments, an antibody, or antigen binding portion thereof, described herein does not specifically bind to TGFβ3. In some embodiments, an antibody, or antigen binding portion thereof, described herein does not specifically bind to TGFβ2 or TGFβ3. In some embodiments, an antibody, or antigen binding portion thereof, described herein specifically binds to a TGFβ1 comprising the amino acid sequence set forth in SEQ ID NO: 21. The amino acid sequences of TGFβ2, and TGFβ3 amino acid sequence are set forth in SEQ ID NOs: 22 and 23, respectively. In some embodiments, an antibody, or antigen binding portion thereof, described herein specifically binds to a TGFβ1 comprising a non-naturally-occurring amino acid sequence (otherwise referred to herein as a non-naturally-occurring TGFβ1). For example, a non-naturally-occurring TGFβ1 may comprise one or more recombinantly generated mutations relative to a naturally-occurring TGFβ1 amino acid sequence. In some embodiments, a TGFβ1, TGFβ2, or TGFβ3 amino acid sequence comprises the amino acid sequence as set forth in SEQ ID NOs: 24-35, as shown in Table 1. In some embodiments, a TGFβ1, TGFβ2, or TGFβ3 amino acid sequence comprises the amino acid sequence as set forth in SEQ ID NOs: 36-43, as shown in Table 2.

TGFβ1

LSTCKTIDMELVKRKRIEAIRGQILSKLRLASPPSQGEVPPGPLPEAVLALYNSTRDRV
AGESAEPEPEPEADYYAKEVTRVLMVETHNEIYDKFKQSTHSIYMFFNTSELREAVPE
PVLLSRAELRLLRLKLKVEQHVELYQKYSNNSWRYLSNRLLAPSDSPEWLSFDVTGV
VRQWLSRGGEIEGFRLSAHCSCDSRDNTLQVDINGFTTGRRGDLATIHGMNRPFLLL
MATPLERAQHLQSSRHRRALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKG

YHANFCLGPCPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEPLPIVYYVGR KPKVEQLSNMIVRSCKCS (SEQ ID NO: 21)

TGFβ2

SLSTCSTLDMDQFMRKRIEAIRGQILSKLKLTSPPEDYPEPEEVPPEVISIYNSTRDLLQ EKASRRAAACERERSDEEYYAKEVYKIDMPPFFPSENAIPPTFYRPYFRIVRFDVSAM EKNASNLVKAEFRVFRLQNPKARVPEQRIELYQILKSKDLTSPTQRYIDSKVVKTRAE GEWLSFDVTDAVHEWLHHKDRNLGFKISLHCPCCTFVPSNNYIIPNKSEELEARFAGI DGTSTYTSGDQKTIKSTRKKNSGKTPHLLLMLLPSYRLESQQTNRRKKRALDAAYCF RNVQDNCCLRPLYIDFKRDLGWKWIHEPKGYNANFCAGACPYLWSSDTQHSRVLSL YNTINPEASASPCCVSQDLEPLTILYYIGKTPKIEQLSNMIVKSCKCS (SEQ ID NO: 22)

TGFβ3

SLSLSTCTTLDFGHIKKKRVEAIRGQILSKLRLTSPPEPTVMTHVPYQVLALYNSTREL LEEMHGEREEGCTQENTESEYYAKEIHKFDMIQGLAEHNELAVCPKGITSKVFRFNV SSVEKNRTNLFRAEFRVLRVPNPSSKRNEQRIELFQILRPDEHIAKQRYIGGKNLPTRG TAEWLSFDVTDTVREWLLRRESNLGLEISIHCPCHTFQPNGDILENIHEVMEIKFKGV DNEDDHGRGDLGRLKKQKDHHNPHLILMMIPPHRLDNPGQGGQRKKRALDTNYCF RNLEENCCVRPLYIDFRQDLGWKWVHEPKGYYANFCSGPCPYLRSADTTHSTVLGL YNTLNPEASASPCCVPQDLEPLTILYYVGRTPKVEQLSNMVVKSCKCS (SEQ ID NO: 23)

Table 1. Exemplary TGFβ1, TGFβ2, and TGFβ3 amino acid sequences

Protein	Sequence	SEQ ID NO
proTGFβ1	LSTCKTIDMELVKRKRIEAIRGQILSKLRLASPPSQGEV PPGPLPEAVLALYNSTRDRVAGESAEPEPEPEADYYA KEVTRVLMVETHNEIYDKFKQSTHSIYMFFNTSELRE AVPEPVLLSRAELRLLRLKLKVEQHVELYQKYSNNS WRYLSNRLLAPSDSPEWLSFDVTGVVRQWLSRGGEIE GFRLSAHCSCDSRDNTLQVDINGFTTGRRGDLATIHG MNRPFLLLMATPLERAQHLQSSRHRRALDTNYCFSST EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGP CPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALE PLPIVYYVGRKPKVEQLSNMIVRSCKCS	24
proTGFβ1 C4S	LSTSKTIDMELVKRKRIEAIRGQILSKLRLASPPSQGEV	25

	PPGPLPEAVLALYNSTRDRVAGESAEPEPEPEADYYA KEVTRVLMVETHNEIYDKFKQSTHSIYMFFNTSELRE AVPEPVLLSRAELRLLRLKLKVEQHVELYQKYSNNS WRYLSNRLLAPSDSPEWLSFDVTGVVRQWLSRGGEIE GFRLSAHCSCDSRDNTLQVDINGFTTGRRGDLATIHG MNRPFLLLMATPLERAQHLQSSRHRRALDTNYCFSST EKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGP CPYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALE PLPIVYYVGRKPKVEQLSNMIVRSCKCS	
proTGFβ1 D2G	LSTCKTIDMELVKRKRIEAIRGQILSKLRLASPPSQGEV PPGPLPEAVLALYNSTRDRVAGESAEPEPEPEADYYA KEVTRVLMVETHNEIYDKFKQSTHSIYMFFNTSELRE AVPEPVLLSRAELRLLRLKLKVEQHVELYQKYSNNS WRYLSNRLLAPSDSPEWLSFDVTGVVRQWLSRGGEIE GFRLSAHCSCDSRDNTLQVDINGFTTGRRGDLATIHG MNRPFLLLMATPLERAQHLQSSRHGALDTNYCFSSTE KNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPC PYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEP LPIVYYVGRKPKVEQLSNMIVRSCKCS	26
proTGFβ1 C4S D2G	LSTSKTIDMELVKRKRIEAIRGQILSKLRLASPPSQGEV PPGPLPEAVLALYNSTRDRVAGESAEPEPEPEADYYA KEVTRVLMVETHNEIYDKFKQSTHSIYMFFNTSELRE AVPEPVLLSRAELRLLRLKLKVEQHVELYQKYSNNS WRYLSNRLLAPSDSPEWLSFDVTGVVRQWLSRGGEIE GFRLSAHCSCDSRDNTLQVDINGFTTGRRGDLATIHG MNRPFLLLMATPLERAQHLQSSRHGALDTNYCFSSTE KNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPC PYIWSLDTQYSKVLALYNQHNPGASAAPCCVPQALEP LPIVYYVGRKPKVEQLSNMIVRSCKCS	27
proTGFβ2	SLSTCSTLDMDQFMRKRIEAIRGQILSKLKLTSPPEDYP EPEEVPPEVISIYNSTRDLLQEKASRRAAACERERSDEE YYAKEVYKIDMPPFFPSENAIPPTFYRPYFRIVRFDVSA MEKNASNLVKAEFRVFRLQNPKARVPEQRIELYQILK SKDLTSPTQRYIDSKVVKTRAEGEWLSFDVTDAVHE WLHHKDRNLGFKISLHCPCCTFVPSNNYIIPNKSEELE ARFAGIDGTSTYTSGDQKTIKSTRKKNSGKTPHLLLM LLPSYRLESQQTNRRKKRALDAAYCFRNVQDNCCLR PLYIDFKRDLGWKWIHEPKGYNANFCAGACPYLWSS DTQHSRVLSLYNTINPEASASPCCVSQDLEPLTILYYIG KTPKIEQLSNMIVKSCKCS	28
proTGFβ2 C5S	SLSTSSTLDMDQFMRKRIEAIRGQILSKLKLTSPPEDYP EPEEVPPEVISIYNSTRDLLQEKASRRAAACERERSDEE YYAKEVYKIDMPPFFPSENAIPPTFYRPYFRIVRFDVSA MEKNASNLVKAEFRVFRLQNPKARVPEQRIELYQILK SKDLTSPTQRYIDSKVVKTRAEGEWLSFDVTDAVHE WLHHKDRNLGFKISLHCPCCTFVPSNNYIIPNKSEELE ARFAGIDGTSTYTSGDQKTIKSTRKKNSGKTPHLLLM LLPSYRLESQQTNRRKKRALDAAYCFRNVQDNCCLR PLYIDFKRDLGWKWIHEPKGYNANFCAGACPYLWSS DTQHSRVLSLYNTINPEASASPCCVSQDLEPLTILYYIG	29

	KTPKIEQLSNMIVKSCKCS	
proTGFβ2 C5S D2G	SLSTSSTLDMDQFMRKRIEAIRGQILSKLKLTSPPEDYP EPEEVPPEVISIYNSTRDLLQEKASRRAAACERERSDEE YYAKEVYKIDMPPFFPSENAIPPTFYRPYFRIVRFDVSA MEKNASNLVKAEFRVFRLQNPKARVPEQRIELYQILK SKDLTSPTQRYIDSKVVKTRAEGEWLSFDVTDAVHE WLHHKDRNLGFKISLHCPCCTFVPSNNYIIPNKSEELE ARFAGIDGTSTYTSGDQKTIKSTRKKNSGKTPHLLLM LLPSYRLESQQTNRRKGALDAAYCFRNVQDNCCLRPL YIDFKRDLGWKWIHEPKGYNANFCAGACPYLWSSDT QHSRVLSLYNTINPEASASPCCVSQDLEPLTILYYIGKT PKIEQLSNMIVKSCKCS	30
proTGFβ2 D2G	SLSTCSTLDMDQFMRKRIEAIRGQILSKLKLTSPPEDYP EPEEVPPEVISIYNSTRDLLQEKASRRAAACERERSDEE YYAKEVYKIDMPPFFPSENAIPPTFYRPYFRIVRFDVSA MEKNASNLVKAEFRVFRLQNPKARVPEQRIELYQILK SKDLTSPTQRYIDSKVVKTRAEGEWLSFDVTDAVHE WLHHKDRNLGFKISLHCPCCTFVPSNNYIIPNKSEELE ARFAGIDGTSTYTSGDQKTIKSTRKKNSGKTPHLLLM LLPSYRLESQQTNRRKGALDAAYCFRNVQDNCCLRPL YIDFKRDLGWKWIHEPKGYNANFCAGACPYLWSSDT QHSRVLSLYNTINPEASASPCCVSQDLEPLTILYYIGKT PKIEQLSNMIVKSCKCS	31
proTGFβ3	SLSLSTCTTLDFGHIKKKRVEAIRGQILSKLRLTSPPEPT VMTHVPYQVLALYNSTRELLEEMHGEREEGCTQENT ESEYYAKEIHKFDMIQGLAEHNELAVCPKGITSKVFRF NVSSVEKNRTNLFRAEFRVLRVPNPSSKRNEQRIELFQ ILRPDEHIAKQRYIGGKNLPTRGTAEWLSFDVTDTVRE WLLRRESNLGLEISIHCPCHTFQPNGDILENIHEVMEIK FKGVDNEDDHGRGDLGRLKKQKDHHNPHLILMMIPP HRLDNPGQGGQRKKRALDTNYCFRNLEENCCVRPLY IDFRQDLGWKWVHEPKGYYANFCSGPCPYLRSADTT HSTVLGLYNTLNPEASASPCCVPQDLEPLTILYYVGRT PKVEQLSNMVVKSCKCS	32
proTGFβ3 C7S	SLSLSTSTTLDFGHIKKKRVEAIRGQILSKLRLTSPPEPT VMTHVPYQVLALYNSTRELLEEMHGEREEGCTQENT ESEYYAKEIHKFDMIQGLAEHNELAVCPKGITSKVFRF NVSSVEKNRTNLFRAEFRVLRVPNPSSKRNEQRIELFQ ILRPDEHIAKQRYIGGKNLPTRGTAEWLSFDVTDTVRE WLLRRESNLGLEISIHCPCHTFQPNGDILENIHEVMEIK FKGVDNEDDHGRGDLGRLKKQKDHHNPHLILMMIPP HRLDNPGQGGQRKKRALDTNYCFRNLEENCCVRPLY IDFRQDLGWKWVHEPKGYYANFCSGPCPYLRSADTT HSTVLGLYNTLNPEASASPCCVPQDLEPLTILYYVGRT PKVEQLSNMVVKSCKCS	33
proTGFβ3 C7S D2G	SLSLSTSTTLDFGHIKKKRVEAIRGQILSKLRLTSPPEPT VMTHVPYQVLALYNSTRELLEEMHGEREEGCTQENT ESEYYAKEIHKFDMIQGLAEHNELAVCPKGITSKVFRF NVSSVEKNRTNLFRAEFRVLRVPNPSSKRNEQRIELFQ ILRPDEHIAKQRYIGGKNLPTRGTAEWLSFDVTDTVRE	34

	WLLRRESNLGLEISIHCPCHTFQPNGDILENIHEVMEIK FKGVDNEDDHGRGDLGRLKKQKDHHNPHLILMMIPP HRLDNPGQGGQRKGALDTNYCFRNLEENCCVRPLYI DFRQDLGWKWVHEPKGYYANFCSGPCPYLRSADTTH STVLGLYNTLNPEASASPCCVPQDLEPLTILYYVGRTP KVEQLSNMVVKSCKCS	
proTGFβ3 D2G	SLSLSTCTTLDFGHIKKKRVEAIRGQILSKLRLTSPPEPT VMTHVPYQVLALYNSTRELLEEMHGEREEGCTQENT ESEYYAKEIHKFDMIQGLAEHNELAVCPKGITSKVFRF NVSSVEKNRTNLFRAEFRVLRVPNPSSKRNEQRIELFQ ILRPDEHIAKQRYIGGKNLPTRGTAEWLSFDVTDTVRE WLLRRESNLGLEISIHCPCHTFQPNGDILENIHEVMEIK FKGVDNEDDHGRGDLGRLKKQKDHHNPHLILMMIPP HRLDNPGQGGQRKGALDTNYCFRNLEENCCVRPLYI DFRQDLGWKWVHEPKGYYANFCSGPCPYLRSADTTH STVLGLYNTLNPEASASPCCVPQDLEPLTILYYVGRTP KVEQLSNMVVKSCKCS	35

Table 2. Exemplary non-human amino acid sequences

Protein	Species	Sequence	SEQ ID NO
proTGFβ1	Mouse	LSTCKTIDMELVKRKRIEAIRGQILSKLRLASPPSQG EVPPGPLPEAVLALYNSTRDRVAGESADPEPEPEAD YYAKEVTRVLMVDRNNAIYEKTKDISHSIYMFFNT SDIREAVPEPPLLSRAELRLQRLKSSVEQHVELYQK YSNNSWRYLGNRLLTPTDTPEWLSFDVTGVVRQW LNQGDGIQGFRFSAHCSCDSKDNKLHVEINGISPKR RGDLGTIHDMNRPFLLLMATPLERAQHLHSSRHRR ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHE PKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNP GASASPCCVPQALEPLPIVYYVGRKPKVEQLSNMIV RSCKCS	36
proTGFβ1	Cyno	LSTCKTIDMELVKRKRIEAIRGQILSKLRLASPPSQG EVPPGPLPEAVLALYNSTRDRVAGESAEPEPEPEAD YYAKEVTRVLMVETHNEIYDKFKQSTHSIYMFFNT SELREAVPEPVLLSRAELRLLRLKLKVEQHVELYQK YSNNSWRYLSNRLLAPSDSPEWLSFDVTGVVRQW LSRGGEIEGFRLSAHCSCDSKDNTLQVDINGFTTGR RGDLATIHGMNRPFLLLMATPLERAQHLQSSRHRR ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHE PKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMI VRSCKCS	37
TGFβ1 LAP C4S	Mouse	LSTSKTIDMELVKRKRIEAIRGQILSKLRLASPPSQG EVPPGPLPEAVLALYNSTRDRVAGESADPEPEPEAD YYAKEVTRVLMVDRNNAIYEKTKDISHSIYMFFNT	38

		SDIREAVPEPPLLSRAELRLQRLKSSVEQHVELYQK YSNNSWRYLGNRLLTPTDTPEWLSFDVTGVVRQW LNQGDGIQGFRFSAHCSCDSKDNKLHVEINGISPKR RGDLGTIHDMNRPFLLLMATPLERAQHLHSSRHRR	
TGFβ1 LAP C4S	Cyno	LSTSKTIDMELVKRKRIEAIRGQILSKLRLASPPSQG EVPPGPLPEAVLALYNSTRDRVAGESAEPEPEPEAD YYAKEVTRVLMVETHNEIYDKFKQSTHSIYMFFNT SELREAVPEPVLLSRAELRLLRLKLKVEQHVELYQK YSNNSWRYLSNRLLAPSDSPEWLSFDVTGVVRQW LSRGGEIEGFRLSAHCSCDSKDNTLQVDINGFTTGR RGDLATIHGMNRPFLLLMATPLERAQHLQSSRHRR	39
proTGFβ1 C4S D2G	Mouse	LSTSKTIDMELVKRKRIEAIRGQILSKLRLASPPSQG EVPPGPLPEAVLALYNSTRDRVAGESADPEPEPEAD YYAKEVTRVLMVDRNNAIYEKTKDISHSIYMFFNT SDIREAVPEPPLLSRAELRLQRLKSSVEQHVELYQK YSNNSWRYLGNRLLTPTDTPEWLSFDVTGVVRQW LNQGDGIQGFRFSAHCSCDSKDNKLHVEINGISPKR RGDLGTIHDMNRPFLLLMATPLERAQHLHSSRHGA LDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEP KGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNPG ASASPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVR SCKCS	40
proTGFβ1 C4S	Mouse	LSTSKTIDMELVKRKRIEAIRGQILSKLRLASPPSQG EVPPGPLPEAVLALYNSTRDRVAGESADPEPEPEAD YYAKEVTRVLMVDRNNAIYEKTKDISHSIYMFFNT SDIREAVPEPPLLSRAELRLQRLKSSVEQHVELYQK YSNNSWRYLGNRLLTPTDTPEWLSFDVTGVVRQW LNQGDGIQGFRFSAHCSCDSKDNKLHVEINGISPKR RGDLGTIHDMNRPFLLLMATPLERAQHLHSSRHRR ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHE PKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNP GASASPCCVPQALEPLPIVYYVGRKPKVEQLSNMIV RSCKCS	41
proTGFβ1 C4S	Cyno	LSTSKTIDMELVKRKRIEAIRGQILSKLRLASPPSQG EVPPGPLPEAVLALYNSTRDRVAGESAEPEPEPEAD YYAKEVTRVLMVETHNEIYDKFKQSTHSIYMFFNT SELREAVPEPVLLSRAELRLLRLKLKVEQHVELYQK YSNNSWRYLSNRLLAPSDSPEWLSFDVTGVVRQW LSRGGEIEGFRLSAHCSCDSKDNTLQVDINGFTTGR RGDLATIHGMNRPFLLLMATPLERAQHLQSSRHRR ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHE PKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMI VRSCKCS	42
proTGFβ1 C4S D2G	Cyno	LSTSKTIDMELVKRKRIEAIRGQILSKLRLASPPSQG EVPPGPLPEAVLALYNSTRDRVAGESAEPEPEPEAD YYAKEVTRVLMVETHNEIYDKFKQSTHSIYMFFNT SELREAVPEPVLLSRAELRLLRLKLKVEQHVELYQK YSNNSWRYLSNRLLAPSDSPEWLSFDVTGVVRQW LSRGGEIEGFRLSAHCSCDSKDNTLQVDINGFTTGR	43

		RGDLATIHGMNRPFLLLMATPLERAQHLQSSRHGA LDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEP KGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNPG ASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVR SCKCS	
LTBP3	CYNO	GPAGERGAGGGGALARERFKVVFAPVICKRTCLKG QCRDSCQQGSNMTLIGENGHSTDTLTGSGFRVVVC PLPCMNGGQCSSRNQCLCPPDFTGRFCQVPAGGAG GGTGGSGPGLSRAGALSTGALPPLAPEGDSVASKH AIYAVQVIADPPGPGEGPPAQHAAFLVPLGPGQISA EVQAPPPVVNVRVHHPPEASVQVHRIESSNAEGAA PSQHLLPHPKPSHPRPPTQKPLGRCFQDTLPKQPCG SNPLPGLTKQEDCCGSIGTAWGQSKCHKCPQLQYT GVQKPGPVRGEVGADCPQGYKRLNSTHCQDINEC AMPGVCRHGDCLNNPGSYRCVCPPGHSLGPSRTQC IADKPEEKSLCFRLVSPEHQCQHPLTTRLTRQLCCC SVGKAWGARCQRCPADGTAAFKEICPAGKGYHILT SHQTLTIQGESDFSLFLHPDGPPKPQQLPESPSQAPP PEDTEEERGVTTDSPVSEERSVQQSHPTATTSPARP YPELISRPSPPTMRWFLPDLPPSRSAVEIAPTQVTET DECRLNQNICGHGECVPGPPDYSCHCNPGYRSHPQ HRYCVDVNECEAEPCGPGRGICMNTGGSYNCHCN RGYRLHVGAGGRSCVDLNECAKPHLCGDGGFCINF PGHYKCNCYPGYRLKASRPPVCEDIDECRDPSSCPD GKCENKPGSFKCIACQPGYRSQGGGACRDVNECAE GSPCSPGWCENLPGSFRCTCAQGYAPAPDGRSCVD VDECEAGDVCDNGICTNTPGSFQCQCLSGYHLSRD RSHCEDIDECDFPAACIGGDCINTNGSYRCLCPQGH RLVGGRKCQDIDECTQDPGLCLPHGACKNLQGSYV CVCDEGFTPTQDQHGCEEVEQPHHKKECYLNFDDT VFCDSVLATNVTQQECCCSLGAGWGDHCEIYPCPV YSSAEFHSLCPDGKGYTQDNNIVNYGIPAHRDIDEC MLFGAEICKEGKCVNTQPGYECYCKQGFYYDGNL LECVDVDECLDESNCRNGVCENTRGGYRCACTPPA EYSPAQRQCLSPEEMDVDECQDPAACRPGRCVNLP GSYRCECRPPWVPGPSGRDCQLPESPAERAPERRD VCWSQRGEDGMCAGPQAGPALTFDDCCCRQGRG WGAQCRPCPPRGAGSQCPTSQSESNSFWDTSPLLL GKPRRDEDSSEEDSDECRCVSGRCVPRPGGAVCEC PGGFQLDASRARCVDIDECRELNQRGLLCKSERCV NTSGSFRCVCKAGFARSRPHGACVPQRRR	44
LTBP3	Mouse	GPAGERGTGGGGALARERFKVVFAPVICKRTCLKG QCRDSCQQGSNMTLIGENGHSTDTLTGSAFRVVVC PLPCMNGGQCSSRNQCLCPPDFTGRFCQVPAAGTG AGTGSSGPGLARTGAMSTGPLPPLAPEGESVASKH AIYAVQVIADPPGPGEGPPAQHAAFLVPLGPGQISA EVQAPPPVVNVRVHHPPEASVQVHRIEGPNAEGPA SSQHLLPHPKPPHPRPPTQKPLGRCFQDTLPKQPCG SNPLPGLTKQEDCCGSIGTAWGQSKCHKCPQLQYT GVQKPVPVRGEVGADCPQGYKRLNSTHCQDINEC	45

		AMPGNVCHGDCLNNPGSYRCVCPPGHSLGPLAAQ CIADKPEEKSLCFRLVSTEHQCQHPLTTRLTRQLCC CSVGKAWGARCQRCPADGTAAFKEICPGKGYHILT SHQTLTIQGESDFSLFLHPDGPPKPQQLPESPSRAPP LEDTEEERGVTMDPPVSEERSVQQSHPTTTTSPPRP YPELISRPSPPTFHRFLPDLPPSRSAVEIAPTQVTETD ECRLNQNICGHGQCVPGPSDYSCHCNAGYRSHPQH RYCVDVNECEAEPCGPGKGICMNTGGSYNCHCNR GYRLHVGAGGRSCVDLNECAKPHLCGDGGFCINFP GHYKCNCYPGYRLKASRPPICEDIDECRDPSTCPDG KCENKPGSFKCIACQPGYRSQGGGACRDVNECSEG TPCSPGWCENLPGSYRCTCAQYEPAQDGLSCIDVD ECEAGKVCQDGICTNTPGSFQCQCLSGYHLSRDRS RCEDIDECDFPAACIGGDCINTNGSYRCLCPLGHRL VGGRKCKKDIDECSQDPGLCLPHACENLQGSYVCV CDEGFTLTQDQHGCEEVEQPHHKKECYLNFDDTVF CDSVLATNVTQQECCCSLGAGWGDHCEIYPCPVYS SAEFHSLVPDGKRLHSGQQHCELCIPAHRDIDECILF GAEICKEGKCVNTQPGYECYCKQGFYYDGNLLEC VDVDECLDESNCRNGVCENTRGGYRCACTPPAEYS PAQAQCLIPERWSTPQRDVKCAGASEERTACVWGP WAGPALTFDDCCCRQPRLGTQCRPCPPRGTGSQCP TSQSESNSFWDTSPLLLGKSPRDEDSSEEDSDECRC VSGRCVPRPGGAVCECPGGFQLDASRARCVDIDEC RELNQRGLLCKSERCVNTSGSFRCVCKAGFTRSRP HGPACLSAAADDAAIAHTSVIDHRGYFH	
LTBP1S	Cyno	NHTGRIKVVFTPSICKVTCTKGSCQNSCEKGNTTTLI SENGHAADTLTATNFRVVLCHLPCMNGGQCSSRD KCQCPPNFTGKLCQIPVHGASVPKLYQHSQQPGKA LGTHVIHSTHTLPLTVTSQQGVKVKFPPNIVNIHVK HPPEASVQIHQVSRIDGPTGQKTKEAQPGQSQVSYQ GLPVQKTQTIHSTYSHQQVIPHVYPVAAKTQLGRCF QETIGSQCGKALPGLSKQEDCCGTVGTSWGFNKCQ KCPKKPSYHGYNQMMECLPGYKRVNNTFCQDINE CQLQGVCPNGECLNTMGSYRCTCKIGFGPDPTFSSC VPDPPVISEEKGPCYRLVSSGRQCMHPLSVHLTKQL CCCSVGKAWGPHCEKCPLPGTAAFKEICPGGMGYT VSGVHRRRPIHHHVGKGPVFVKPKNTQPVAKSTHP PPLPAKEEPVEALTFSREHGPGVAEPEVATAPPEKEI PSLDQEKTKLEPGQPQLSPGISTIHLHPQFPVVIEKTS PPVPVEVAPEASTSSASQVIAPTQVTEINECTVNPDI CGAGHCINLPVRYTCICYEGYKFSEQQRKCVDIDEC TQVQHLCSQGRCENTEGSFLCICPAGFMASEEGTNC IDVDECLRPDVCGEGHCVNTVGAFRCEYCDSGYR MTQRGRCEDIDECLNPSTCPDEQCVNSPGSYQCVP CTEGFRGWNGQCLDVDECLEPNVCTNGDCSNLEG SYMCSCHKGYTRTPDHKHCKDIDECQQGNLCVNG QCKNTEGSFRCTCGQGYQLSAAKDQCEDIDECQHH HLCAHGQCRNTEGSFQCVCDQGYRASGLGDHCEDI NECLEDKSVCQRGDCINTAGSYDCTCPDGFQLDDN	46

		KTCQDINECEHPGLCGPQGECLNTEGSFHCVCQQG FSISADGRTCEDIDECVNNTVCDSHGFCDNTAGSFR CLCYQGFQAPQDGQGCVDVNECELLSGVCGEAFC ENVEGSFLCVCADENQEYSPMTGQCRSRTSTDLDV EQPKEEKKECYYNLNDASLCDNVLAPNVTKQECC CTSGAGWGDNCEIFPCPVLGTAEFTEMCPKGKGFV PAGESSSEAGGENYKDADECLLFGQEICKNGFCLNT RPGYECYCKQGTYYDPVKLQCFDMDECQDPSSCID GQCVNTEGSYNCFCTHPMVLDASEKRCIRPAESNE QIEETDVYQDLCWEHLSDEYVCSRPLVGKQTTYTE CCCLYGEAWGMQCALCPMKDSDDYAQLCNIPVTG RRQPYGRDALVDFSEQYAPEADPYFIQDRFLNSFEE LQAEECGILNGCENGRCVRVQEGYTCDCFDGYHLD TAKMTCVDVNECDELNNRMSLCKNAKCINTEGSY KCLCLPGYVPSDKPNYCTPLNTALNLEKDSDLE	
LTBP1S	mouse	NHTGRIKVVFTPSICKVTCTKGNCQNSCQKGNTTTL ISENGHAADTLTATNFRVVICHLPCMNGGQCSSRD KCQCPPNFTGKLCQIPVLGASMPKLYQHAQQQGKA LGSHVIHSTHTLPLTMTSQQGVKVKFPPNIVNIHVK HPPEASVQIHQVSRIDSPGGQKVKEAQPGQSQVSYQ GLPVQKTQTVHSTYSHQQLIPHVYPVAAKTQLGRC FQETIGSQCGKALPGLSKQEDCCGTVGTSWGFNKC QKCPKKQSYHGYTQMMECLQGYKRVNNTFCQDIN ECQLQGVCPNGECLNTMGSYRCSCKMGFGPDPTFS SCVPDPPVISEEKGPCYRLVSPGRHCMHPLSVHLTK QICCCSVGKAWGPHCEKCPLPGTAAFKEICPGGMG YTVSGVHRRRPIHQHIGKEAVYVKPKNTQPVAKST HPPPLPAKEEPVEALTSSWEHGPRGAEPEVVTAPPE KEIPSLDQEKTRLEPGQPQLSPGVSTIHLHPQFPVVV EKTSPPVPVEVAPEASTSSASQVIAPTQVTEINECTV NPDICGAGHCINLPVRYTCICYEGYKFSEQLRKCVD IDECAQVRHLCSQGRCENTEGSFLCVCPAGFMASE EGTNCIDVDECLRPDMCRDGRCINTAGAFRCEYCD SGYRMSRRGYCEDIDECLKPSTCPEEQCVNTPGSYQ CVPCTEGFRGWNGQCLDVDECLQPKVCTNGSCTN LEGSYMCSCHRGYSPTPDHRHCQDIDECQQGNLCM NGQCRNTDGSFRCTCGQGYQLSAAKDQCEDIDECE HHHLCSHGQCRNTEGSFQCVCNQGYRASVLGDHC EDINECLEDSSVCQGGDCINTAGSYDCTCPDGFQLN DNKGCQDINECAQPGLCGSHGECLNTQGSFHCVCE QGFSISADGRTCEDIDECVNNTVCDSHGFCDNTAGS FRCLCYQGFQAPQDGQGCVDVNECELLSGVCGEAF CENVEGSFLCVCADENQEYSPMTGQCRSRVTEDSG VDRQPREEKKECYYNLNDASLCDNVLAPNVTKQE CCCTSGAGWGDNCEIFPCPVQGTAEFTEMCPRGKG LVPAGESSYDTGGENYKDADECLLFGEEICKNGYC LNTQPGYECYCKQGTYYDPVKLQCFDMDECQDPN SCIDGQCVNTEGSYNCFCTHPMVLDASEKRCVQPT ESNEQIEETDVYQDLCWEHLSEEYVCSRPLVGKQT TYTECCCLYGEAWGMQCALCPMKDSDDYAQLCNI	47

		PVTGRRRPYGRDALVDFSEQYGPETDPYFIQDRFLN SFEELQAEECGILNGCENGRCVRVQEGYTCDCFDG YHLDMAKMTCVDVNECSELNNRMSLCKNAKCINT EGSYKCLCLPGYIPSDKPNYCTPLNSALNLDKESDL E	
GARP	mouse	ISQRREQVPCRTVNKEALCHGLGLLQVPSVLSLDIQ ALYLSGNQLQSILVSPLGFYTALRHLDLSDNQISFLQ AGVFQALPYLEHLNLAHNRLATGMALNSGGLGRL PLLVSLDLSGNSLHGNLVERLLGETPRLRTLSLAEN SLTRLARHTFWGMPAVEQLDLHSNVLMDIEDGAFE ALPHLTHLNLSRNSLTCISDFSLQQLQVLDLSCNSIE AFQTAPEPQAQFQLAWLDLRENKLLHFPDLAVFPR LIYLNVSNNLIQLPAGLPRGSEDLHAPSEGWSASPLS NPSRNASTHPLSQLLNLDLSYNEIELVPASFLEHLTS LRFLNLSRNCLRSFEARQVDSLPCLVLLDLSHNVLE ALELGTKVLGSLQTLLLQDNALQELPPYTFASLASL QRLNLQGNQVSPCGGPAEPGPPGCVDFSGIPTLHVL NMAGNSMGMLRAGSFLHTPLTELDLSTNPGLDVA TGALVGLEASLEVLELQGNGLTVLRVDLPCFLRLK RLNLAENQLSHLPAWTRAVSLEVLDLRNNSFSLLP GNAMGGLETSLRRLYLQGNPLSCCGNGWLAAQLH QGRVDVDATQDLICRFGSQEELSLSLVRPEDCEKG GLKNVNLILLLSFTLVSAIVLTTLATICFLRRQKLSQ QYKA	48
sGARP	mouse	ISQRREQVPCRTVNKEALCHGLGLLQVPSVLSLDIQ ALYLSGNQLQSILVSPLGFYTALRHLDLSDNQISFLQ AGVFQALPYLEHLNLAHNRLATGMALNSGGLGRL PLLVSLDLSGNSLHGNLVERLLGETPRLRTLSLAEN SLTRLARHTFWGMPAVEQLDLHSNVLMDIEDGAFE ALPHLTHLNLSRNSLTCISDFSLQQLQVLDLSCNSIE AFQTAPEPQAQFQLAWLDLRENKLLHFPDLAVFPR LIYLNVSNNLIQLPAGLPRGSEDLHAPSEGWSASPLS NPSRNASTHPLSQLLNLDLSYNEIELVPASFLEHLTS LRFLNLSRNCLRSFEARQVDSLPCLVLLDLSHNVLE ALELGTKVLGSLQTLLLQDNALQELPPYTFASLASL QRLNLQGNQVSPCGGPAEPGPPGCVDFSGIPTLHVL NMAGNSMGMLRAGSFLHTPLTELDLSTNPGLDVA TGALVGLEASLEVLELQGNGLTVLRVDLPCFLRLK RLNLAENQLSHLPAWTRAVSLEVLDLRNNSFSLLP GNAMGGLETSLRRLYLQGNPLSCCGNGWLAAQLH QGRVDVDATQDLICRFGSQEELSLSLVRPEDCEKG GLKNVN	49

In some embodiments, an antibody, or antigen binding portion thereof, as described herein, is capable of binding to a LTBP1-TGFβ1 complex. In some embodiments, antigenic protein complexes (*e.g.*, a LTBP-TGFβ1 complex) may comprise one or more LTBP proteins (*e.g.*, LTBP1, LTBP2, LTBP3, and LTBP4). In some embodiments, the LTBP1 protein is a

naturally-occurring protein. In some embodiments, the LTBP1 protein is a non-naturally occurring protein. In some embodiments, the LTBP1 protein is a recombinant protein. Such recombinant LTBP1 protein may comprise LTBP1, alternatively spliced variants thereof and/or fragments thereof. Recombinant LTBP1 proteins may also be modified to comprise one or more detectable labels. In some embodiments, the LTBP1 protein comprises a leader sequence (*e.g.*, a native or non-native leader sequence). In some embodiments, the LTBP1 protein does not comprise a leader sequence (*i.e.*, the leader sequence has been processed or cleaved). Such detectable labels may include, but are not limited to biotin labels, polyhistidine tags, myc tags, HA tags and/or fluorescent tags. In some embodiments, the LTBP1 protein is a mammalian LTBP1 protein. In some embodiments, the LTBP1 protein is a human, a monkey, a mouse, or a rat LTBP1 protein. In some embodiments, the LTBP1 protein comprises an amino acid sequence as set forth in SEQ ID NOs: 46 and 47 in Table 2. In some embodiments, the LTBP1 protein comprises an amino acid sequence as set forth in SEQ ID NO: 50 in Table 3.

In some embodiments, an antibody, or antigen binding portion thereof, as described herein, is capable of binding to a LTBP3-TGFβ1 complex. In some embodiments, the LTBP3 protein is a naturally-occurring protein. In some embodiments, the LTBP3 protein is a non-naturally occurring protein. In some embodiments, the LTBP3 protein is a recombinant protein. Such recombinant LTBP3 protein may comprise LTBP3, alternatively spliced variants thereof and/or fragments thereof. In some embodiments, the LTBP3 protein comprises a leader sequence (*e.g.*, a native or non-native leader sequence). In some embodiments, the LTBP3 protein does not comprise a leader sequence (*i.e.*, the leader sequence has been processed or cleaved). Recombinant LTBP3 proteins may also be modified to comprise one or more detectable labels. Such detectable labels may include, but are not limited to biotin labels, polyhistidine tags, myc tags, HA tags and/or fluorescent tags. In some embodiments, the LTBP3 protein is a mammalian LTBP3 protein. In some embodiments, the LTBP3 protein is a human, a monkey, a mouse, or a rat LTBP3 protein. In some embodiments, the LTBP3 protein comprises an amino acid sequence as set forth in SEQ ID NOs: 44 and 45 in Table 2. In some embodiments, the LTBP1 protein comprises an amino acid sequence as set forth in SEQ ID NO: 51 in Table 3.

In some embodiments, an antibody, or antigen binding portion thereof, as described herein, is capable of binding to a GARP-TGFβ1 complex. In some embodiments, the GARP protein is a naturally-occurring protein. In some embodiments, the GARP protein is a non-naturally occurring protein. In some embodiments, the GARP protein is a recombinant

protein. Such a GARP may be recombinant, referred to herein as recombinant GARP. Some recombinant GARPs may comprise one or more modifications, truncations and/or mutations as compared to wild type GARP. Recombinant GARPs may be modified to be soluble. In some embodiments, the GARP protein comprises a leader sequence (*e.g.*, a native or non-native leader sequence). In some embodiments, the GARP protein does not comprise a leader sequence (*i.e.*, the leader sequence has been processed or cleaved). In other embodiments, recombinant GARPs are modified to comprise one or more detectable labels. In further embodiments, such detectable labels may include, but are not limited to biotin labels, polyhistidine tags, flag tags, myc tags, HA tags and/or fluorescent tags. In some embodiments, the GARP protein is a mammalian GARP protein. In some embodiments, the GARP protein is a human, a monkey, a mouse, or a rat GARP protein. In some embodiments, the GARP protein comprises an amino acid sequence as set forth in SEQ ID NOs: 48-49 in Table 2. In some embodiments, the GARP protein comprises an amino acid sequence as set forth in SEQ ID NOs: 52 and 53 in Table 4. In some embodiments, the antibodies, or antigen binding portions thereof, described herein do not bind to TGFβ1 in a context-dependent manner, for example binding to TGFβ1 would only occur when the TGFβ1 molecule was complexed with a specific presenting molecule, such as GARP. Instead, the antibodies, and antigen-binding portions thereof, bind to TGFβ1 in a context-independent manner. In other words, the antibodies, or antigen-binding portions thereof, bind to TGFβ1 when bound to any presenting molecule: GARP, LTBP1, LTBP3, and/or LRCC33.

In some embodiments, an antibody, or antigen binding portion thereof, as described herein, is capable of binding to a LRRC33-TGFβ1 complex. In some embodiments, the LRRC33 protein is a naturally-occurring protein. In some embodiments, the LRRC33 protein is a non-naturally occurring protein. In some embodiments, the LRRC33 protein is a recombinant protein. Such a LRRC33 may be recombinant, referred to herein as recombinant LRRC33. Some recombinant LRRC33 proteins may comprise one or more modifications, truncations and/or mutations as compared to wild type LRRC33. Recombinant LRRC33 proteins may be modified to be soluble. For example, in some embodiments, the ectodomain of LRRC33 may be expressed with a C-terminal His-tag in order to express soluble LRRC33 protein (sLRRC33; see, *e.g.*, SEQ ID NO: 84). In some embodiments, the LRRC33 protein comprises a leader sequence (*e.g.*, a native or non-native leader sequence). In some embodiments, the LRRC33 protein does not comprise a leader sequence (*i.e.*, the leader sequence has been processed or cleaved). In other embodiments, recombinant LRRC33 proteins are modified to comprise one or more detectable labels. In further embodiments,

such detectable labels may include, but are not limited to biotin labels, polyhistidine tags, flag tags, myc tags, HA tags and/or fluorescent tags. In some embodiments, the LRRC33 protein is a mammalian LRRC33 protein. In some embodiments, the LRRC33 protein is a human, a monkey, a mouse, or a rat LRRC33 protein. In some embodiments, the LRRC33 protein comprises an amino acid sequence as set forth in SEQ ID NOs: 83, 84, and 85 in Table 4.

Table 3. Exemplary LTBP amino acid sequences.

Protein	Sequence	SEQ ID NO
LTBP1S	NHTGRIKVVFTPSICKVTCTKGSCQNSCEKGNTTTLI SENGHAADTLTATNFRVVICHLPCMNGGQCSSRDK CQCPPNFTGKLCQIPVHGASVPKLYQHSQQPGKALG THVIHSTHTLPLTVTSQQGVKVKFPPNIVNIHVKHPP EASVQIHQVSRIDGPTGQKTKEAQPGQSQVSYQGLP VQKTQTIHSTYSHQQVIPHVYPVAAKTQLGRCFQETI GSQCGKALPGLSKQEDCCGTVGTSWGFNKCQKCPK KPSYHGYNQMMECLPGYKRVNNTFCQDINECQLQG VCPNGECLNTMGSYRCTCKIGFGPDPTFSSCVPDPPV ISEEKGPCYRLVSSGRQCMHPLSVHLTKQLCCCSVG KAWGPHCEKCPLPGTAAFKEICPGGMGYTVSGVHR RRPIHHHVGKGPVFVKPKNTQPVAKSTHPPPLPAKE EPVEALTFSREHGPGVAEPEVATAPPEKEIPSLDQEK TKLEPGQPQLSPGISTIHLHPQFPVVIEKTSPPVPVEV APEASTSSASQVIAPTQVTEINECTVNPDICGAGHCIN LPVRYTCICYEGYRFSEQQRKCVDIDECTQVQHLCS QGRCENTEGSFLCICPAGFMASEEGTNCIDVDECLRP DVCGEGHCVNTVGAFRCEYCDSGYRMTQRGRCEDI DECLNPSTCPDEQCVNSPGSYQCVPCTEGFRGWNG QCLDVDECLEPNVCANGDCSNLEGSYMCSCHKGYT RTPDHKHCRDIDECQQGNLCVNGQCKNTEGSFRCT CGQGYQLSAAKDQCEDIDECQHRHLCAHGQCRNTE GSFQCVCDQGYRASGLGDHCEDINECLEDKSVCQR GDCINTAGSYDCTCPDGFQLDDNKTCQDINECEHPG LCGPQGECLNTEGSFHCVCQQGFSISADGRTCEDIDE CVNNTVCDSHGFCDNTAGSFRCLCYQGFQAPQDGQ GCVDVNECELLSGVCGEAFCENVEGSFLCVCADEN QEYSPMTGQCRSRTSTDLDVDVDQPKEEKKECYYN LNDASLCDNVLAPNVTKQECCCTSGVGWGDNCEIF PCPVLGTAEFTEMCPKGKGFVPAGESSSEAGGENYK DADECLLFGQEICKNGFCLNTRPGYECYCKQGTYY DPVKLQCFDMDECQDPSSCIDGQCVNTEGSYNCFCT HPMVLDASEKRCIRPAESNEQIEETDVYQDLCWEHL SDEYVCSRPLVGKQTTYTECCCLYGEAWGMQCALC PLKDSDDYAQLCNIPVTGRRQPYGRDALVDFSEQYT PEADPYFIQDRFLNSFEELQAEECGILNGCENGRCVR	50

	VQEGYTCDCFDGYHLDTAKMTCVDVNECDELNNR MSLCKNAKCINTDGSYKCLCLPGYVPSDKPNYCTPL NTALNLEKDSDLE	
LTBP3	GPAGERGAGGGGALARERFKVVFAPVICKRTCLKG QCRDSCQQGSNMTLIGENGHSTDTLTGSGFRVVVCP LPCMNGGQCSSRNQCLCPPDFTGRFCQVPAGGAGG GTGGSGPGLSRTGALSTGALPPLAPEGDSVASKHAI YAVQVIADPPGPGEGPPAQHAAFLVPLGPGQISAEV QAPPPVVNVRVHHPPEASVQVHRIESSNAESAAPSQ HLLPHPKPSHPRPPTQKPLGRCFQDTLPKQPCGSNPL PGLTKQEDCCGSIGTAWGQSKCHKCPQLQYTGVQK PGPVRGEVGADCPQGYKRLNSTHCQDINECAMPGV CRHGDCLNNPGSYRCVCPPGHSLGPSRTQCIADKPE EKSLCFRLVSPEHQCQHPLTTRLTRQLCCCSVGKAW GARCQRCPTDGTAAFKEICPAGKGYHILTSHQTLTIQ GESDFSLFLHPDGPPKPQQLPESPSQAPPPEDTEEERG VTTDSPVSEERSVQQSHPTATTTPARPYPELISRPSPP TMRWFLPDLPPSRSAVEIAPTQVTETDECRLNQNICG HGECVPGPPDYSCHCNPGYRSHPQHRYCVDVNECE AEPCGPGRGICMNTGGSYNCHCNRGYRLHVGAGGR SCVDLNECAKPHLCGDGGFCINFPGHYKCNCYPGY RLKASRPPVCEDIDECRDPSSCPDGKCENKPGSFKCI ACQPGYRSQGGGACRDVNECAEGSPCSPGWCENLP GSFRCTCAQGYAPAPDGRSCLDVDECEAGDVCDNG ICSNTPGSFQCQCLSGYHLSRDRSHCEDIDECDFPAA CIGGDCINTNGSYRCLCPQGHRLVGGRKCQDIDECS QDPSLCLPHGACKNLQGSYVCVCDEGFTPTQDQHG CEEVEQPHHKKECYLNFDDTVFCDSVLATNVTQQE CCCSLGAGWGDHCEIYPCPVYSSAEFHSLCPDGKGY TQDNNIVNYGIPAHRDIDECMLFGSEICKEGKCVNT QPGYECYCKQGFYYDGNLLECVDVDECLDESNCRN GVCENTRGGYRCACTPPAEYSPAQRQCLSPEEMDV DECQDPAACRPGRCVNLPGSYRCECRPPWVPGPSGR DCQLPESPAERAPERRDVCWSQRGEDGMCAGPLAG PALTFDDCCCRQGRGWGAQCRPCPPRGAGSHCPTS QSESNSFWDTSPLLLGKPPRDEDSSEEDSDECRCVSG RCVPRPGGAVCECPGGFQLDASRARCVDIDECRELN QRGLLCKSERCVNTSGSFRCVCKAGFARSRPHGACV PQRRR	51

Table 4 – Exemplary GARP and LRRC33 amino acid sequences.

Protein	Sequence	SEQ ID NO
GARP	AQHQDKVPCKMVDKKVSCQVLGLLQVPSVLPPDTET LDLSGNQLRSILASPLGFYTALRHLDLSTNEISFLQPGA FQALTHLEHLSLAHNRLAMATALSAGGLGPLPRVTSL DLSGNSLYSGLLERLLGEAPSLHTLSLAENSLTRLTRH TFRDMPALEQLDLHSNVLMDIEDGAFEGLPRLTHLNL	52

	SRNSLTCISDFSLQQLRVLDLSCNSIEAFQTASQPQAEF QLTWLDLRENKLLHFPDLAALPRLIYLNLSNNLIRLPT GPPQDSKGIHAPSEGWSALPLSAPSGNASGRPLSQLLN LDLSYNEIELIPDSFLEHLTSLCFLNLSRNCLRTFEARR LGSLPCLMLLDLSHNALETLELGARALGSLRTLLLQG NALRDLPPYTFANLASLQRLNLQGNRVSPCGGPDEPG PSGCVAFSGITSLRSLSLVDNEIELLRAGAFLHTPLTEL DLSSNPGLEVATGALGGLEASLEVLALQGNGLMVLQ VDLPCFICLKRLNLAENRLSHLPAWTQAVSLEVLDLR NNSFSLLPGSAMGGLETSLRRLYLQGNPLSCCGNGWL AAQLHQGRVDVDATQDLICRFSSQEEVSLSHVRPEDC EKGGLKNINLIIILTFILVSAILLTTLAACCCVRRQKFNQ QYKA	
sGARP	AQHQDKVPCKMVDKKVSCQVLGLLQVPSVLPPDTET LDLSGNQLRSILASPLGFYTALRHLDLSTNEISFLQPGA FQALTHLEHLSLAHNRLAMATALSAGGLGPLPRVTSL DLSGNSLYSGLLERLLGEAPSLHTLSLAENSLTRLTRH TFRDMPALEQLDLHSNVLMDIEDGAFEGLPRLTHLNL SRNSLTCISDFSLQQLRVLDLSCNSIEAFQTASQPQAEF QLTWLDLRENKLLHFPDLAALPRLIYLNLSNNLIRLPT GPPQDSKGIHAPSEGWSALPLSAPSGNASGRPLSQLLN LDLSYNEIELIPDSFLEHLTSLCFLNLSRNCLRTFEARR LGSLPCLMLLDLSHNALETLELGARALGSLRTLLLQG NALRDLPPYTFANLASLQRLNLQGNRVSPCGGPDEPG PSGCVAFSGITSLRSLSLVDNEIELLRAGAFLHTPLTEL DLSSNPGLEVATGALGGLEASLEVLALQGNGLMVLQ VDLPCFICLKRLNLAENRLSHLPAWTQAVSLEVLDLR NNSFSLLPGSAMGGLETSLRRLYLQGNPLSCCGNGWL AAQLHQGRVDVDATQDLICRFSSQEEVSLSHVRPEDC EKGGLKNIN	53
LRRC33 (also known as NRROS; Uniprot Accession No. Q86YC3)	**MELLPLWLCLGFHFLTVG**WRNRSGTATAASQGVC KLVGGAADCRGQSLASVPSSLPPHARMLTLDANPLKT LWNHSLQPYPLLESLSLHSCHLERISRGAFQEQGHLRS LVLGDNCLSENYEETAAALHALPGLRRLDLSGNALTE DMAALMLQNLSSLRSVSLAGNTIMRLDDSVFEGLERL RELDLQRNYIFEIEGGAFDGLAELRHLNLAFNNLPCIV DFGLTRLRVLNVSYNVLEWFLATGGEAAFELETLDLS HNQLLFFPLLPQYSKLRTLLLRDNNMGFYRDLYNTSS PREMVAQFLLVDGNVTNITTVSLWEEFSSSDLADLRF LDMSQNQFQYLPDGFLRKMPSLSHLNLHQNCLMTLHI REHEPPGALTELDLSHNQLSELHLAPGLASCLGSLRLF NLSSNQLLGVPPGLFANARNITTLDMSHNQISLCPLPA ASDRVGPPSCVDFRNMASLRSLSLEGCGLGALPDCPF QGTSLTYLDLSSNWGVLNGSLAPLQDVAPMLQVLSL RNMGLHSSFMALDFSGFGNLRDLDLSGNCLTTFPRFG GSLALETLDLRRNSLTALPQKAVSEQLSRGLRTIYLSQ NPYDCCGVDGWGALQHGQTVADWAMVTCNLSSKII RVTELPGGVPRDCKWERLDLGLLYLVLILPSCLTLLV ACTVIVLTFKKPLLQVIKSRCHWSSVY	83

	* Native signal peptide is depicted in bold font.	
soluble LRRC33 (sLRRC33)	**MDMRVPAQLLGLLLLWFSGVLG**WRNRSGTATAAS QGVCKLVGGAADCRGQSLASVPSSLPPHARMLTLDA NPLKTLWNHSLQPYPLLESLSLHSCHLERISRGAFQEQ GHLRSLVLGDNCLSENYEETAAALHALPGLRRLDLSG NALTEDMAALMLQNLSSLRSVSLAGNTIMRLDDSVFE GLERLRELDLQRNYIFEIEGGAFDGLAELRHLNLAFNN LPCIVDFGLTRLRVLNVSYNVLEWFLATGGEAAFELE TLDLSHNQLLFFPLLPQYSKLRTLLLRDNNMGFYRDL YNTSSPREMVAQFLLVDGNVTNITTVSLWEEFSSSDL ADLRFLDMSQNQFQYLPDGFLRKMPSLSHLNLHQNC LMTLHIREHEPPGALTELDLSHNQLSELHLAPGLASCL GSLRLFNLSSNQLLGVPPGLFANARNITTLDMSHNQIS LCPLPAASDRVGPPSCVDFRNMASLRSLSLEGCGLGA LPDCPFQGTSLTYLDLSSNWGVLNGSLAPLQDVAPML QVLSLRNMGLHSSFMALDFSGFGNLRDLDLSGNCLTT FPRFGGSLALETLDLRRNSLTALPQKAVSEQLSRGLRT IYLSQNPYDCCGVDGWGALQHGQTVADWAMVTCNL SSKIIRVTELPGGVPRDCKWERLDLGL<u>HHHHHH</u>	84
	* Modified human kappa light chain signal peptide is depicted in bold font. ** Histidine tag is underlined.	
Human LRRC33-GARP chimera	**MDMRVPAQLLGLLLLWFSGVLG**<u>WRNRSGTATAAS QGVCKLVGGAADCRGQSLASVPSSLPPHARMLTLDA NPLKTLWNHSLQPYPLLESLSLHSCHLERISRGAFQEQ GHLRSLVLGDNCLSENYEETAAALHALPGLRRLDLSG NALTEDMAALMLQNLSSLRSVSLAGNTIMRLDDSVFE GLERLRELDLQRNYIFEIEGGAFDGLAELRHLNLAFNN LPCIVDFGLTRLRVLNVSYNVLEWFLATGGEAAFELE TLDLSHNQLLFFPLLPQYSKLRTLLLRDNNMGFYRDL YNTSSPREMVAQFLLVDGNVTNITTVSLWEEFSSSDL ADLRFLDMSQNQFQYLPDGFLRKMPSLSHLNLHQNC LMTLHIREHEPPGALTELDLSHNQLSELHLAPGLASCL GSLRLFNLSSNQLLGVPPGLFANARNITTLDMSHNQIS LCPLPAASDRVGPPSCVDFRNMASLRSLSLEGCGLGA LPDCPFQGTSLTYLDLSSNWGVLNGSLAPLQDVAPML QVLSLRNMGLHSSFMALDFSGFGNLRDLDLSGNCLTT FPRFGGSLALETLDLRRNSLTALPQKAVSEQLSRGLRT IYLSQNPYDCCGVDGWGALQHGQTVADWAMVTCNL SSKIIRVTELPGGVPRDCKWERLDLGL*LIIILTFILVSAIL LTTLAACCC*VRRQKFNQQYKA</u> * Modified human kappa light chain signal peptide is depicted in bold font. ** LRRC33 ectodomain is underlined. # GARP transmembrane domain is italicized. ## GARP intracellular tail is double underlined.	85

In some embodiments, the antibodies, or antigen binding portions thereof, of the present invention that specifically bind to an epitope of TGFβ1 that is available for binding by the antibody when the TGFβ1 is present in a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex and the nucleic acid molecules of the present disclosure that encode the antibodies include one or more of the CDR amino acid sequences shown in Table 5.

Table 5. Complementary determining regions of the heavy chain (CDRHs) and the light chain (CDRLs) as determined using the Kabat numbering scheme are shown for antibodies Ab1 and Ab2.

Antibody	Ab1	Ab2
CDRH1 (SEQ ID NOs: 1-2)	SYGMH (SEQ ID NO: 1)	SDWIG (SEQ ID NO: 2)
CDRH2 (SEQ ID NOs: 3-4)	VISYDGSNKYYADSVKG (SEQ ID NO: 3)	VIYPGDSDTRYSASFQG (SEQ ID NO: 4)
CDRH3 (SEQ ID NOs: 5-6)	DIRPYGDYSAAFDI (SEQ ID NO: 5)	AAGIAAAGHVTAFDI (SEQ ID NO: 6)
CDRL1 (SEQ ID NOs: 7-8)	TGSSGSIASNYVQ (SEQ ID NO: 7)	KSSQSVLYSSNNKNYLA (SEQ ID NO: 8)
CDRL2 (SEQ ID NOs: 9-10)	EDNQRPS (SEQ ID NO: 9)	WASTRES (SEQ ID NO: 10)
CDRL3 (SEQ ID NOs: 11-12)	QSYDSSNHGGV (SEQ ID NO: 11)	QQYYSTPVT (SEQ ID NO: 12)

In some embodiments, antibodies of the present invention that specifically bind to GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex include any antibody, or antigen binding portion thereof, comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, or CDRL3, or combinations thereof, as provided for any one of the antibodies shown in Table 5. In some embodiments, antibodies that specifically bind to GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex include the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 of any one of the antibodies shown in Table 5. The present invention also provides any nucleic acid sequence that encodes a molecule comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, or CDRL3 as provided for any one of the antibodies shown in Table 5. Antibody heavy and light chain CDR3 domains may play a particularly important role in the binding specificity/affinity of an antibody for an

antigen. Accordingly, the antibodies that specifically bind to GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex of the disclosure, or the nucleic acid molecules encoding these antibodies, or antigen binding portions thereof, may include at least the heavy and/or light chain CDR3s of the antibodies as shown in Table 5.

Aspects of the invention relate to a monoclonal antibody, or antigen binding portion thereof, that binds specifically to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, and that comprises six complementarity determining regions (CDRs): CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3.

In some embodiments, CDRH1 comprises a sequence as set forth in any one of SEQ ID NOs: 1 and 2. In some embodiments, CDRH2 comprises a sequence as set forth in any one of SEQ ID NOs: 3 and 4. In some embodiments, CDRH3 comprises a sequence as set forth in any one of SEQ ID NOs: 5 and 6. CDRL1 comprises a sequence as set forth in any one of SEQ ID NOs: 7 and 8. In some embodiments, CDRL2 comprises a sequence as set forth in any one of SEQ ID NOs: 9 and 10. In some embodiments, CDRL3 comprises a sequence as set forth in any one of SEQ ID NOs: 11 and 12.

In some embodiments (*e.g.*, as for antibody Ab1, shown in Table 5), the antibody or antigen binding portion thereof, that specifically binds to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex comprises: a CDRH1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a CDRH2 comprising an amino acid sequence as set forth in SEQ ID NO: 3, a CDRH3 comprising an amino acid sequence as set forth in SEQ ID NO: 5 , a CDRL1 comprising an amino acid sequence as set forth in SEQ ID NO: 7, a CDRL2 comprising an amino acid sequence as set forth in SEQ ID NO: 9, and a CDRL3 comprising an amino acid sequence as set forth in SEQ ID NO: 11.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a complementarity determining region 3 (CDR3) having the amino acid sequence of SEQ ID NO: 5 and a light chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 11. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a complementarity determining region 2 (CDR2) having the amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 9. In some embodiments, the antibody, or

antigen binding portion thereof, comprises a heavy chain variable region comprising a complementarity determining region 1 (CDR1) having the amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 7.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 13 and a light chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 14. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 13 and a light chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 14.

In some embodiments, the antibody or antigen binding portion thereof, that specifically binds to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex comprises a heavy chain variable domain amino acid sequence encoded by a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the nucleic acid sequence set forth in SEQ ID NO: 91, and a light chain variable domain amino acid sequence encoded by a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the nucleic acid sequence set forth in SEQ ID NO: 92. In some embodiments, the antibody or antigen binding portion thereof, comprises a heavy chain variable domain amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO: 91, and a light chain variable domain amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO: 92.

In some embodiments (*e.g.*, as for antibody Ab2, shown in Table 5), the antibody or antigen binding portion thereof, that specifically binds to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex comprises a CDRH1 comprising an amino acid sequence as set forth in SEQ ID NO: 2, a CDRH2 comprising an amino acid sequence as set forth in SEQ ID NO: 3, a CDRH3 comprising an amino acid sequence as set forth in SEQ ID NO: 6, a CDRL1 comprising an amino acid sequence as set forth in SEQ ID NO: 8, a CDRL2 comprising an amino acid sequence as set forth in SEQ ID NO: 10, and a CDRL3 comprising an amino acid sequence as set forth in SEQ ID NO: 12.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 6 and a light chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 12. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 4 and a light chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 10. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 2 and a light chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 8.

In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 15 and a light chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 16. In some embodiments, the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 15 and a light chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 16.

In some embodiments, the antibody or antigen binding portion thereof, that specifically binds to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex comprises a heavy chain variable domain amino acid sequence encoded by a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the nucleic acid sequence set forth in SEQ ID NO: 93, and a light chain variable domain amino acid sequence encoded by a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the nucleic acid sequence set forth in SEQ ID NO: 94. In some embodiments, the antibody or antigen binding portion thereof, comprises a heavy chain variable domain amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO: 93, and a light chain variable domain amino acid sequence encoded by the nucleic acid sequence set forth in SEQ ID NO: 94.

In some examples, any of the antibodies of the disclosure that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex include any antibody (including antigen binding portions thereof)

having one or more CDR (*e.g.*, CDRH or CDRL) sequences substantially similar to CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and/or CDRL3. For example, the antibodies may include one or more CDR sequences as shown in Table 5 (SEQ ID NOs: 1-12) containing up to 5, 4, 3, 2, or 1 amino acid residue variations as compared to the corresponding CDR region in any one of SEQ ID NOs: 1-12. The complete amino acid sequences for the heavy chain variable region and light chain variable region of the antibodies listed in Table 5 (*e.g.*, Ab1 and Ab2), as well as nucleic acid sequences encoding the heavy chain variable region and light chain variable region of the antibodies are provided below.

Ab1 – Heavy chain variable region amino acid sequence
EVQLVESGGGLVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS NKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDIRPYGDYSAAFDI WGQGTLVTVSS (SEQ ID NO: 13)

Ab1 – Heavy chain variable region nucleic acid sequence
GAGGTGCAACTCGTGGAGTCAGGCGGTGGACTTGTTCAGCCTGGGCGAAGTCTG AGACTCTCATGTGCAGCAAGTGGATTCACTTTCTCCAGTTACGGCATGCACTGGG TGAGACAGGCGCCTGGAAAGGGTTTGGAATGGGTCGCTGTGATCTCTTACGACG GGTCAAACAAATATTACGCGGATTCAGTGAAAGGGCGGTTCACTATTTCACGGG ATAACTCCAAGAACACCCTGTATCTGCAGATGAATAGCCTGAGGGCAGAGGACA CCGCTGTGTACTATTGTGCCCGGGACATAAGGCCTTACGGCGATTACAGCGCCGC ATTTGATATTTGGGGACAAGGCACCCTTGTGACAGTATCTTCT (SEQ ID NO: 91)

Ab1 – Light chain variable region amino acid sequence
NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPSIVIFEDNQRPSGA PDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSNHGGVFGGGTQLTVL (SEQ ID NO: 14)

Ab1 – Light chain variable region nucleic acid sequence
AATTTTATGCTTACCCAACCACATAGTGTGAGTGAGTCTCCCGGCAAGACTGTAA CAATTTCATGTACCGGCAGCAGTGGCTCCATCGCTAGCAATTATGTGCAATGGTA CCAACAGCGCCCCGGGAGCGCACCTTCAATAGTGATATTCGAGGATAACCAACG GCCTAGTGGGGCTCCCGATAGATTTAGTGGGAGTATAGATAGCTCCTCCAACTCT GCCTCTCTCACCATTAGCGGGCTGAAAACAGAGGATGAAGCCGACTATTACTGCC

AAAGCTATGATTCTAGCAACCACGGCGGAGTGTTTGGCGGAGGAACACAGCTGA CAGTCCTAGG (SEQ ID NO: 92)

Ab1 – Heavy chain amino acid sequence
EVQLVESGGGLVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGS NKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDIRPYGDYSAAFDI WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG PPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG (SEQ ID NO: 15)

Ab1 – Light chain amino acid sequence
NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPSIVIFEDNQRPSGA PDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSNHGGVFGGGTQLTVLGQPKA APSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSN NKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO: 16)

Ab2 – Heavy chain variable region amino acid sequence
EVQLVQSGAEMKKPGESLKISCKGSGYNFASDWIGWVRQTPGKGLEWMGVIYPGDS DTRYSASFQGQVTISADKSINTAYLQWSSLKASDTAMYYCASAAGIAAAGHVTAFDI WGQGTMVTVSS (SEQ ID NO: 17)

Ab2 – Heavy chain variable region nucleic acid sequence
GAGGTGCAACTGGTGCAATCCGGAGCCGAGATGAAAAAGCCAGGGGAGAGCCT GAAGATCTCTTGTAAGGGCTCTGGCTATAACTTCGCTAGTGATTGGATCGGATGG GTGAGGCAAACCCCCGGAAAGGGCCTCGAGTGGATGGGCGTGATCTACCCCGGC GACTCCGACACACGCTATAGCGCCTCATTCCAGGGCCAGGTCACCATAAGTGCTG ATAAATCAATAAATACAGCCTACTTGCAATGGTCAAGTCTGAAAGCCTCAGATAC TGCCATGTACTATTGTGCCTCTGCCGCCGGCATTGCCGCGGCCGGTCACGTCACC GCCTTCGACATTTGGGGTCAGGGCACTATGGTCACTGTAAGCTCC (SEQ ID NO: 93)

Ab2 – Light chain variable region amino acid sequence
DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWA
STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPVTFGQGTKLEIK
(SEQ ID NO: 18)

Ab2 – Light chain variable region nucleic acid sequence
GACATAGTCATGACCCAGTCACCTGACTCTTTGGCCGTGTCTCTGGGGGAGAGAG
CCACAATAAATTGCAAGTCATCACAGAGCGTCCTGTACTCCTCCAATAATAAAAA
TTACCTGGCCTGGTACCAGCAAAAGCCCGGGCAACCCCCCAAATTGTTGATTTAC
TGGGCTAGTACAAGGGAATCTGGAGTGCCAGACCGGTTTTCTGGTTCTGGATCTG
GTACTGACTTCACCCTGACAATCAGCTCCCTGCAGGCCGAAGACGTGGCTGTGTA
CTATTGTCAGCAGTACTATAGTACACCAGTTACTTTCGGCCAAGGCACTAAACTC
GAAATCAAG (SEQ ID NO: 94)

Ab2 – Heavy chain amino acid sequence
EVQLVQSGAEMKKPGESLKISCKGSGYNFASDWIGWVRQTPGKGLEWMGVIYPGDS
DTRYSASFQGQVTISADKSINTAYLQWSSLKASDTAMYYCASAAGIAAAGHVTAFDI
WGQGTMVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
PPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG
VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK
AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG (SEQ
ID NO: 19)

Ab2 – Light chain amino acid sequence
DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWA
STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPVTFGQGTKLEIKRT
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:
20)

In some embodiments, antibodies of the disclosure that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and a LRRC33-

TGFβ1 complex include any antibody that includes a heavy chain variable domain of SEQ ID NO: 13 or 17, or a light chain variable domain of SEQ ID NO: 14 or 18. In some embodiments, antibodies of the disclosure that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and a LRRC33-TGFβ1 complex include any antibody that includes the heavy chain variable and light chain variable pairs of SEQ ID NOs: 13 and 14; and 17 and 18.

Aspects of the disclosure provide antibodies that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and a LRRC33-TGFβ1 complex having a heavy chain variable and/or a light chain variable amino acid sequence homologous to any of those described herein. In some embodiments, the antibody that that specifically binds to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and a LRRC33-TGFβ1 complex comprises a heavy chain variable sequence or a light chain variable sequence that is at least 75% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the heavy chain variable amino acid sequence of SEQ ID NO: 13 or 17, or a light chain variable sequence of SEQ ID NO: 14 or 18. In some embodiments, the homologous heavy chain variable and/or a light chain variable amino acid sequences do not vary within any of the CDR sequences provided herein. For example, in some embodiments, the degree of sequence variation (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) may occur within a heavy chain variable and/or a light chain variable amino acid sequence excluding any of the CDR sequences provided herein.

In some embodiments, antibodies of the disclosure that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex include any antibody, or antigen binding portion thereof, that includes a heavy chain of SEQ ID NO: 15 or 19, or a light chain of SEQ ID NO: 16 or 20. In some embodiments, antibodies of the disclosure that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex include any antibody that includes the heavy chain and light chain pairs of SEQ ID NOs: 15 and 16; or 19 and 20.

Aspects of the disclosure provide antibodies that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex having a heavy chain and/or a light chain amino acid sequence homologous to any of those described herein. In some embodiments, the antibody that specifically binds to a

GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex comprises a heavy chain sequence or a light chain sequence that is at least 75% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the heavy chain sequence of SEQ ID NO: 15, or 19, or a light chain amino acid sequence of SEQ ID NO: 16, or 20. In some embodiments, the homologous heavy chain and/or a light chain amino acid sequences do not vary within any of the CDR sequences provided herein. For example, in some embodiments, the degree of sequence variation (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) may occur within a heavy chain and/or a light chain amino acid sequence excluding any of the CDR sequences provided herein.

In some embodiments, antibodies of the disclosure that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex include any antibody, or antigen binding portion thereof, that includes a heavy chain of SEQ ID NO: 15 or 19, or a light chain of SEQ ID NO: 16 or 20. In some embodiments, antibodies of the disclosure that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex include any antibody that includes the heavy chain and light chain pairs of SEQ ID NOs: 15 and 16; or 19 and 20.

Aspects of the disclosure provide antibodies that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex having a heavy chain and/or a light chain amino acid sequence homologous to any of those described herein. In some embodiments, the antibody that that specifically binds to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex comprises a heavy chain sequence or a light chain sequence that is at least 75% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the heavy chain sequence of SEQ ID NO: 15 or 19, or a light chain amino acid sequence of SEQ ID NO: 16 or 20. In some embodiments, the homologous heavy chain and/or a light chain amino acid sequences do not vary within any of the CDR sequences provided herein. For example, in some embodiments, the degree of sequence variation (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) may occur within a heavy chain and/or a light chain amino acid sequence excluding any of the CDR sequences provided herein.

In some embodiments, the "percent identity" of two amino acid sequences is determined using the algorithm of Karlin and Altschul Proc. Natl. Acad. Sci. USA 87:2264-68, 1990, modified as in Karlin and Altschul Proc. Natl. Acad. Sci. USA 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* J. Mol. Biol. 215:403-10, 1990. BLAST protein searches can be performed with the XBLAST program, score=50, word length=3 to obtain amino acid sequences homologous to the protein molecules of interest. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul *et al.*, Nucleic Acids Res. 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

In any of the antibodies or antigen-binding fragments described herein, one or more conservative mutations can be introduced into the CDRs or framework sequences at positions where the residues are not likely to be involved in an antibody-antigen interaction. In some embodiments, such conservative mutation(s) can be introduced into the CDRs or framework sequences at position(s) where the residues are not likely to be involved in interacting with a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and a LRRC33-TGFβ1 complex as determined based on the crystal structure. In some embodiments, likely interface (*e.g.*, residues involved in an antigen-antibody interaction) may be deduced from known structural information on another antigen sharing structural similarities.

As used herein, a "conservative amino acid substitution" refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, *e.g.,* Molecular Cloning: A Laboratory Manual, J. Sambrook, *et al.*, eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, *et al.*, eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

In some embodiments, the antibodies provided herein comprise mutations that confer desirable properties to the antibodies. For example, to avoid potential complications due to Fab-arm exchange, which is known to occur with native IgG4 mAbs, the antibodies provided herein may comprise a stabilizing 'Adair' mutation (Angal *et al.*, "A single amino acid

substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody," *Mol Immunol* 30, 105-108; 1993), where serine 228 (EU numbering; residue 241 Kabat numbering) is converted to proline resulting in an IgG1-like (CPPCP (SEQ ID NO: 54)) hinge sequence. Accordingly, any of the antibodies may include a stabilizing 'Adair' mutation or the amino acid sequence CPPCP (SEQ ID NO: 54).

Antibodies of this disclosure that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex may optionally comprise antibody constant regions or parts thereof. For example, a V_L domain may be attached at its C-terminal end to a light chain constant domain like Cκ or Cλ. Similarly, a V_H domain or portion thereof may be attached to all or part of a heavy chain like IgA, IgD, IgE, IgG, and IgM, and any isotype subclass. Antibodies may include suitable constant regions (see, for example, Kabat *et al.*, Sequences of Proteins of Immunological Interest, No. 91-3242, National Institutes of Health Publications, Bethesda, Md. (1991)). Therefore, antibodies within the scope of this may disclosure include V_H and V_L domains, or an antigen binding portion thereof, combined with any suitable constant regions.

In some embodiments, antibodies that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex may or may not include the framework region of the antibodies of SEQ ID NOs: 13-20. In some embodiments, antibodies that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex are murine antibodies and include murine framework region sequences.

In some embodiments, antibodies that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex of the disclosure can bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex with relatively high affinity, *e.g.*, with a K_D less than 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M or lower. For example, antibodies that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex can bind a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex with an affinity between 5 pM and 500 nM, *e.g.*, between 50 pM and 100 nM, *e.g.*, between 500 pM and 50 nM. The disclosure also includes antibodies or antigen binding fragments that compete with any of the antibodies described herein for binding to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex and that have an affinity of 50 nM or lower (*e.g.*, 20 nM or lower, 10 nM or

lower, 500 pM or lower, 50 pM or lower, or 5 pM or lower). The affinity and binding kinetics of the antibodies that specifically bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex can be tested using any suitable method including but not limited to biosensor technology (*e.g.*, OCTET or BIACORE).

Antibodies that Inhibit TGFβ

The present invention in one aspect provides *functional* antibodies. As used herein, "a functional antibody" confers one or more biological activities by virtue of its ability to bind an antigen. Functional antibodies may include inhibiting antibodies (or inhibitory antibodies) and activating antibodies. Thus, the present disclosure includes TGFβ antibodies which can modulate (*e.g.*, inhibit or activate) a biological process mediated by TGFβ signaling.

As used herein, the term "inhibiting antibody" refers to an antibody that inhibits mature growth factor release or reduces growth factor activity. Inhibiting antibodies include antibodies targeting any epitope that reduces growth factor release or activity when associated with such antibodies. Such epitopes may lie on prodomains of TGFβ proteins (*e.g.* TGFβ1), growth factors or other epitopes that lead to reduced growth factor activity when bound by antibody. Inhibiting antibodies of the present invention include, but are not limited to, TGFβ1-inhibiting antibodies.

Embodiments of the present disclosure include methods of using inhibiting antibodies in solution, in cell culture and/or in subjects to modify growth factor signaling.

Polypeptides

Some aspects of the disclosure relate to a polypeptide having a sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 15, and SEQ ID NO: 19. In some embodiments, the polypeptide is a variable heavy chain domain or a heavy chain domain. In some embodiments, the polypeptide is at least 75% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to any one of the amino acid sequences set forth in SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 15, and SEQ ID NO: 19.

Some aspects of the disclosure relate to a polypeptide having a sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 16, and SEQ ID NO: 20. In some embodiments, the polypeptide is a variable light chain domain or a light

chain domain. In some embodiments, the polypeptide is at least 75% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to any one of the amino acid sequences set forth in SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 16, and SEQ ID NO: 20.

Antibodies Competing with Antibodies that Specifically Bind to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex

Aspects of the disclosure relate to antibodies that compete or cross-compete with any of the antibodies provided herein. The term "compete", as used herein with regard to an antibody, means that a first antibody binds to an epitope (*e.g.*, an epitope of a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex) in a manner sufficiently similar to the binding of a second antibody, such that the result of binding of the first antibody with its epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are within the scope of this disclosure. Regardless of the mechanism by which such competition or cross-competition occurs (*e.g.*, steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods and/or compositions provided herein.

Aspects of the disclosure relate to antibodies that compete or cross-compete with any of the specific antibodies, or antigen binding portions thereof, as provided herein. In some embodiments, an antibody, or antigen binding portion thereof, binds at or near the same epitope as any of the antibodies provided herein. In some embodiments, an antibody, or antigen binding portion thereof, binds near an epitope if it binds within 15 or fewer amino acid residues of the epitope. In some embodiments, any of the antibody, or antigen binding portion thereof, as provided herein, binds within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues of an epitope that is bound by any of the antibodies provided herein.

In another embodiment, provided herein is an antibody, or antigen binding portion thereof, competes or cross-competes for binding to any of the antigens provided herein (*e.g.*, a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex) with an equilibrium dissociation constant, K_D, between the antibody and the protein of less than 10^{-6} M. In other embodiments, an antibody competes or cross-competes for binding to any of the antigens provided herein with a K_D in a range from 10^{-11} M to 10^{-6} M. In some embodiments, provided herein is an anti-TGFβ1 antibody, or antigen binding portion thereof, that competes for binding with an antibody, or antigen binding portion thereof, described herein. In some embodiments, provided herein is an anti-TGFβ1 antibody, or antigen binding portion thereof, that binds to the same epitope as an antibody, or antigen binding portion thereof, described herein.

Any of the antibodies provided herein can be characterized using any suitable methods. For example, one method is to identify the epitope to which the antigen binds, or "epitope mapping." There are many suitable methods for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999. In an additional example, epitope mapping can be used to determine the sequence to which an antibody binds. The epitope can be a linear epitope, *i.e.*, contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch (primary structure linear sequence). In some embodiments, the epitope is a TGFβ1 epitope that is only available for binding by the antibody, or antigen binding portion thereof, described herein, when the TGFβ1 is in a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex. Peptides of varying lengths (*e.g.*, at least 4-6 amino acids long) can be isolated or synthesized (*e.g.*, recombinantly) and used for binding assays with an antibody. In another example, the epitope to which the antibody binds can be determined in a systematic screen by using overlapping peptides derived from the target antigen sequence and determining binding by the antibody. According to the gene fragment expression assays, the open reading frame encoding the target antigen is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of the antigen with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein *in vitro*, in the

presence of radioactive amino acids. The binding of the antibody to the radioactively labeled antigen fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. In an additional example, mutagenesis of an antigen binding domain, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or necessary for epitope binding. For example, domain swapping experiments can be performed using a mutant of a target antigen in which various fragments of the GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex have been replaced (swapped) with sequences from a closely related, but antigenically distinct protein, such as another member of the TGFβ protein family (*e.g.*, GDF11). By assessing binding of the antibody to the mutant of the a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, the importance of the particular antigen fragment to antibody binding can be assessed.

Alternatively, competition assays can be performed using other antibodies known to bind to the same antigen to determine whether an antibody binds to the same epitope as the other antibodies. Competition assays are well known to those of skill in the art.

Further, the interaction of the any of the antibodies provided herein with one or more residues in aa GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex can be determined by routine technology. For example, a crystal structure can be determined, and the distances between the residues in a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex and one or more residues in the antibody can be determined accordingly. Based on such distance, whether a specific residue in a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex interacts with one or more residues in the antibody can be determined. Further, suitable methods, such as competition assays and target mutagenesis assays can be applied to determine the preferential binding of a candidate antibody.

Production of Antibodies that Bind a GARP-TGFβ1 Complex, a LTBP1-TGFβ1 Complex, a LTBP3-TGFβ1 Complex, and/or a LRRC33-TGFβ1 Complex

Numerous methods may be used for obtaining antibodies, or antigen binding fragments thereof, of the disclosure. For example, antibodies can be produced using recombinant DNA methods. Monoclonal antibodies may also be produced by generation of hybridomas (see *e.g.*, Kohler and Milstein (1975) Nature, 256: 495-499) in accordance with known methods. Hybridomas formed in this manner are then screened using standard methods, such as enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (*e.g.*, OCTET or BIACORE) analysis, to identify one or more hybridomas that produce an antibody that specifically binds to a specified antigen. Any form of the specified antigen may be used as the immunogen, *e.g.*, recombinant antigen, naturally occurring forms, any variants or fragments thereof, as well as antigenic peptide thereof (*e.g.*, any of the epitopes described herein as a linear epitope or within a scaffold as a conformational epitope). One exemplary method of making antibodies includes screening protein expression libraries that express antibodies or fragments thereof (*e.g.*, scFv), *e.g.*, phage or ribosome display libraries. Phage display is described, for example, in Ladner *et al.*, U.S. Pat. No. 5,223,409; Smith (1985) Science 228:1315-1317; Clackson *et al.* (1991) Nature, 352: 624-628; Marks *et al.* (1991) J. Mol. Biol., 222: 581-597; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; and WO 90/02809.

In addition to the use of display libraries, the specified antigen (*e.g.*, a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex) can be used to immunize a non-human animal, *e.g.*, a rodent, *e.g.*, a mouse, hamster, or rat. In one embodiment, the non-human animal is a mouse.

In another embodiment, a monoclonal antibody is obtained from the non-human animal, and then modified, *e.g.*, chimeric, using suitable recombinant DNA techniques. A variety of approaches for making chimeric antibodies have been described. See *e.g.*, Morrison *et al.*, Proc. Natl. Acad. Sci. U.S.A. 81:6851, 1985; Takeda *et al.*, Nature 314:452, 1985, Cabilly *et al.*, U.S. Pat. No. 4,816,567; Boss *et al.*, U.S. Pat. No. 4,816,397; Tanaguchi *et al.*, European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom Patent GB 2177096B.

For additional antibody production techniques, *see* Antibodies: A Laboratory Manual, eds. Harlow *et al.*, Cold Spring Harbor Laboratory, 1988. The present disclosure is not necessarily limited to any particular source, method of production, or other special characteristics of an antibody.

Some aspects of the present disclosure relate to host cells transformed with a polynucleotide or vector. Host cells may be a prokaryotic or eukaryotic cell. The polynucleotide or vector which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally. The host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. In some embodiments, fungal cells are, for example, those of the genus Saccharomyces, in particular those of the species S. cerevisiae. The term "prokaryotic" includes all bacteria which can be transformed or transfected with a DNA or RNA molecules for the expression of an antibody or the corresponding immunoglobulin chains. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. The term "eukaryotic" includes yeast, higher plants, insects and vertebrate cells, *e.g.*, mammalian cells, such as NSO and CHO cells. Depending upon the host employed in a recombinant production procedure, the antibodies or immunoglobulin chains encoded by the polynucleotide may be glycosylated or may be non-glycosylated. Antibodies or the corresponding immunoglobulin chains may also include an initial methionine amino acid residue.

In some embodiments, once a vector has been incorporated into an appropriate host, the host may be maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the immunoglobulin light chains, heavy chains, light/heavy chain dimers or intact antibodies, antigen binding fragments or other immunoglobulin forms may follow; see, Beychok, Cells of Immunoglobulin Synthesis, Academic Press, N.Y., (1979). Thus, polynucleotides or vectors are introduced into the cells which in turn produce the antibody or antigen binding fragments. Furthermore, transgenic animals, preferably mammals, comprising the aforementioned host cells may be used for the large scale production of the antibody or antibody fragments.

The transformed host cells can be grown in fermenters and cultured using any suitable techniques to achieve optimal cell growth. Once expressed, the whole antibodies, their dimers, individual light and heavy chains, other immunoglobulin forms, or antigen binding fragments, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; see, Scopes, "Protein Purification", Springer Verlag, N.Y. (1982). The antibody or antigen binding fragments can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, *e.g.*, microbially expressed antibodies or antigen binding fragments may be by any conventional means such

as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed, *e.g.*, against the constant region of the antibody.

Aspects of the disclosure relate to a hybridoma, which provides an indefinitely prolonged source of monoclonal antibodies. As an alternative to obtaining immunoglobulins directly from the culture of hybridomas, immortalized hybridoma cells can be used as a source of rearranged heavy chain and light chain loci for subsequent expression and/or genetic manipulation. Rearranged antibody genes can be reverse transcribed from appropriate mRNAs to produce cDNA. In some embodiments, heavy chain constant region can be exchanged for that of a different isotype or eliminated altogether. The variable regions can be linked to encode single chain Fv regions. Multiple Fv regions can be linked to confer binding ability to more than one target or chimeric heavy and light chain combinations can be employed. Any appropriate method may be used for cloning of antibody variable regions and generation of recombinant antibodies.

In some embodiments, an appropriate nucleic acid that encodes variable regions of a heavy and/or light chain is obtained and inserted into an expression vectors which can be transfected into standard recombinant host cells. A variety of such host cells may be used. In some embodiments, mammalian host cells may be advantageous for efficient processing and production. Typical mammalian cell lines useful for this purpose include CHO cells, 293 cells, or NSO cells. The production of the antibody or antigen binding fragment may be undertaken by culturing a modified recombinant host under culture conditions appropriate for the growth of the host cells and the expression of the coding sequences. The antibodies or antigen binding fragments may be recovered by isolating them from the culture. The expression systems may be designed to include signal peptides so that the resulting antibodies are secreted into the medium; however, intracellular production is also possible.

The disclosure also includes a polynucleotide encoding at least a variable region of an immunoglobulin chain of the antibodies described herein. In some embodiments, the variable region encoded by the polynucleotide comprises at least one complementarity determining region (CDR) of the VH and/or VL of the variable region of the antibody produced by any one of the above described hybridomas.

Polynucleotides encoding antibody or antigen binding fragments may be, *e.g.*, DNA, cDNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination. In some embodiments, a polynucleotide is part of a vector. Such vectors may

comprise further genes such as marker genes which allow for the selection of the vector in a suitable host cell and under suitable conditions.

In some embodiments, a polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells. Expression of the polynucleotide comprises transcription of the polynucleotide into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They may include regulatory sequences that facilitate initiation of transcription and optionally poly-A signals that facilitate termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally associated or heterologous promoter regions. Possible regulatory elements permitting expression in prokaryotic host cells include, *e.g.*, the PL, Lac, Trp or Tac promoter in E. coli, and examples of regulatory elements permitting expression in eukaryotic host cells are the AOX1 or GAL1 promoter in yeast or the CMV-promoter, SV40-promoter, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells.

Beside elements which are responsible for the initiation of transcription such regulatory elements may also include transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system employed, leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the polynucleotide and have been described previously. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into, for example, the extracellular medium. Optionally, a heterologous polynucleotide sequence can be used that encode a fusion protein including a C- or N-terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product.

In some embodiments, polynucleotides encoding at least the variable domain of the light and/or heavy chain may encode the variable domains of both immunoglobulin chains or only one. Likewise, polynucleotides may be under the control of the same promoter or may be separately controlled for expression. Furthermore, some aspects relate to vectors, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise a polynucleotide encoding a variable domain of an immunoglobulin chain of an antibody or antigen binding fragment; optionally in combination

with a polynucleotide that encodes the variable domain of the other immunoglobulin chain of the antibody.

In some embodiments, expression control sequences are provided as eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector into targeted cell population (*e.g.*, to engineer a cell to express an antibody or antigen binding fragment). A variety of appropriate methods can be used to construct recombinant viral vectors. In some embodiments, polynucleotides and vectors can be reconstituted into liposomes for delivery to target cells. The vectors containing the polynucleotides (*e.g.*, the heavy and/or light variable domain(s) of the immunoglobulin chains encoding sequences and expression control sequences) can be transferred into the host cell by suitable methods, which vary depending on the type of cellular host.

Modifications

Antibodies, or antigen binding portions thereof, of the disclosure may be modified with a detectable label or detectable moiety, including, but not limited to, an enzyme, prosthetic group, fluorescent material, luminescent material, bioluminescent material, radioactive material, positron emitting metal, nonradioactive paramagnetic metal ion, and affinity label for detection and isolation of a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex. The detectable substance or moiety may be coupled or conjugated either directly to the polypeptides of the disclosure or indirectly, through an intermediate (such as, for example, a linker (*e.g.*, a cleavable linker)) using suitable techniques. Non-limiting examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, glucose oxidase, or acetylcholinesterase; non-limiting examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; non-limiting examples of suitable fluorescent materials include biotin, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, or phycoerythrin; an example of a luminescent material includes luminol; non-limiting examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include a radioactive metal ion, *e.g.*, alpha-emitters or other radioisotopes such as, for

example, iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115}mIn, ^{113}mIn, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99}mTc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{86}R, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, and tin (^{113}Sn, ^{117}Sn). The detectable substance may be coupled or conjugated either directly to the antibodies of the disclosure that bind specifically to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, or indirectly, through an intermediate (such as, for example, a linker) using suitable techniques. Any of the antibodies provided herein that are conjugated to a detectable substance may be used for any suitable diagnostic assays, such as those described herein.

In addition, antibodies, or antigen binding portions thereof, of the disclosure may also be modified with a drug. The drug may be coupled or conjugated either directly to the polypeptides of the disclosure, or indirectly, through an intermediate (such as, for example, a linker (*e.g.*, a cleavable linker)) using suitable techniques.

Targeting Agents

In some embodiments methods of the present disclosure comprise the use of one or more targeting agents to target an antibody, or antigen binding portion thereof, as disclosed herein, to a particular site in a subject for purposes of modulating mature TGFβ release from a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex. For example, LTBP1-TGFβ1 and LTBP3-TGFβ1 complexes are typically localized to extracellular matrix. Thus, in some embodiments, antibodies disclosed herein can be conjugated to extracellular matrix targeting agents for purposes of localizing the antibodies to sites where LTBP1-TGFβ1 and LTBP3-TGFβ1 complexes reside. In such embodiments, selective targeting of antibodies leads to selective modulation of LTBP1-TGFβ1 and/or LTBP3-TGFβ1 complexes. In some embodiments, selective targeting of antibodies leads to selective inhibition of LTBP1-TGFβ1 and/or LTBP3-TGFβ1 complexes (*e.g.*, for purposes of treating fibrosis). In some embodiments, extracellular matrix targeting agents include heparin binding agents, matrix metalloproteinase binding agents, lysyl oxidase binding domains, fibrillin-binding agents, hyaluronic acid binding agents, and others.

Similarly, GARP-TGFβ1 complexes are typically localized to the surface of cells, *e.g.*, activated FOXP3^{+} regulatory T cells (Tregs). Thus, in some embodiments, antibodies disclosed herein can be conjugated to immune cell (*e.g.*, Treg cell) binding agents for

purposes of localizing antibodies to sites where GARP-TGFβ1 complexes reside. In such embodiments, selective targeting of antibodies leads to selective modulation of GARP-TGFβ1 complexes. In some embodiments, selective targeting of antibodies leads to selective inhibition of GARP-TGFβ1 complexes (*e.g.*, selective inhibition of the release of mature TGFβ1 for purposes of immune modulation, *e.g.*, in the treatment of cancer). In such embodiments, Treg cell targeting agents may include, for example, CCL22 and CXCL12 proteins or fragments thereof.

In some embodiments, bispecific antibodies may be used having a first portion that selectively binds GARP-TGFβ1 complex and a LTBP-TGFβ1 complex and a second portion that selectively binds a component of a target site, *e.g.*, a component of the ECM (*e.g.*, fibrillin) or a component of a Treg cell (*e.g.*, CTLA-4).

Pharmaceutical Compositions

The invention further provides pharmaceutical compositions used as a medicament suitable for administration in human and non-human subjects. One or more antibodies that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex can be formulated or admixed with a pharmaceutically acceptable carrier (excipient), including, for example, a buffer, to form a pharmaceutical composition. Such formulations may be used for the treatment of a disease or disorder that involves TGFβ signaling. In some embodiments, such disease or disorder associated with TGFβ signaling involves one or more contexts, *i.e.*, the TGFβ is associated with a particular type or types of presenting molecules. In some embodiments, such context occurs in a cell type-specific and/or tissue-specific manner. In some embodiments, for example, such context-dependent action of TGFβ signaling is mediated in part via GARP, LRRC33, LTBP1 and/or LTBP3.

In some embodiments, the antibody of the present invention binds specifically to two or more contexts of TGFβ, such that the antibody binds TGFβ in a complex with presenting molecules selected from two or more of: GARP, LRRC33, LTBP1 and LTBP3. Thus, such pharmaceutical compositions may be administered to patients for alleviating a TGFβ-related indication (*e.g.*, fibrosis, immune disorders, and/or cancer). "Acceptable" means that the carrier is compatible with the active ingredient of the composition (and preferably, capable of stabilizing the active ingredient) and not deleterious to the subject to be treated. Examples of pharmaceutically acceptable excipients (carriers), including buffers, would be apparent to the skilled artisan and have been described previously. See, *e.g.*, Remington: The Science and

Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover. In one example, a pharmaceutical composition described herein contains more than one antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex where the antibodies recognize different epitopes/residues of the a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex.

The pharmaceutical compositions to be used in the present methods can comprise pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formulations or aqueous solutions (Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used, and may comprise buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Pharmaceutically acceptable excipients are further described herein.

In some examples, the pharmaceutical composition described herein comprises liposomes containing an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, which can be prepared by any suitable method, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688 (1985); Hwang *et al. Proc. Natl. Acad. Sci. USA* 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

The antibodies that specifically bind a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Exemplary techniques have been described previously, see, *e.g.*, Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

In other examples, the pharmaceutical composition described herein can be formulated in sustained-release format. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

The pharmaceutical compositions to be used for *in vivo* administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. Therapeutic antibody compositions are generally placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The pharmaceutical compositions described herein can be in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or suppositories, for oral, parenteral or rectal administration, or administration by inhalation or insufflation.

For preparing solid compositions such as tablets, the principal active ingredient can be mixed with a pharmaceutical carrier, *e.g.*, conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, *e.g.*, water, to form a solid preformulation composition containing a homogeneous mixture of a compound of the present disclosure, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly

throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from 0.1 mg to about 500 mg of the active ingredient of the present disclosure. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate. Suitable surface-active agents include, in particular, non-ionic agents, such as polyoxyethylenesorbitans (*e.g.* Tween™ 20, 40, 60, 80 or 85) and other sorbitans (*e.g.* Span™ 20, 40, 60, 80 or 85). Compositions with a surface-active agent will conveniently comprise between 0.05 and 5% surface-active agent, and can be between 0.1 and 2.5%. It will be appreciated that other ingredients may be added, for example mannitol or other pharmaceutically acceptable vehicles, if necessary.

Suitable emulsions may be prepared using commercially available fat emulsions, such as Intralipid™, Liposyn™, Infonutrol™, Lipofundin™ and Lipiphysan™. The active ingredient may be either dissolved in a pre-mixed emulsion composition or alternatively it may be dissolved in an oil (*e.g.* soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil or almond oil) and an emulsion formed upon mixing with a phospholipid (*e.g.* egg phospholipids, soybean phospholipids or soybean lecithin) and water. It will be appreciated that other ingredients may be added, for example glycerol or glucose, to adjust the tonicity of the emulsion. Suitable emulsions will typically contain up to 20% oil, for example, between 5 and 20%.

The emulsion compositions can be those prepared by mixing an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex with Intralipid™ or the components thereof (soybean oil, egg phospholipids, glycerol and water).

Pharmaceutical compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically

acceptable excipients as set out above. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably sterile pharmaceutically acceptable solvents may be nebulised by use of gases. Nebulised solutions may be breathed directly from the nebulising device or the nebulising device may be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

Use of Antibodies, and Antigen-binding Portions Thereof, that Specifically Bind a GARP-TGFβ1 Complex, a LTBP1-TGFβ1 Complex, a LTBP3-TGFβ1 Complex, and/or a LRRC33-TGFβ1 Complex

In some embodiments, antibodies, antigen binding portions thereof, and compositions of the disclosure may be used to treat a wide variety of diseases, disorders and/or conditions. In some cases, such diseases, disorders and/or conditions may be TGFβ-related indications. As used herein, the term "TGFβ-related indication" refers to any disease, disorder and/or condition related to expression, activity and/or metabolism of a TGFβ family member protein or any disease, disorder and/or condition that may benefit from modulation of the activity and/or levels of one or more TGFβ family member protein. TGFβ-related indications may include, but are not limited to, fibrosis, cancer (including, but not limited to colon cancer, renal cancer, breast cancer, malignant melanoma and glioblastoma, facilitation of rapid hematopoiesis following chemotherapy, bone healing, wound healing, dementia, myelofibrosis, a renal disease, unilateral ureteral obstruction (UUO), tooth loss and/or degeneration, endothelial proliferation syndromes, asthma and allergy, gastrointestinal disorders, anemia of the aging, aortic aneurysm, orphan indications (such as Marfan's syndrome and Camurati-Engelmann disease,) obesity, diabetes, arthritis, multiple sclerosis, muscular dystrophy, amyotrophic lateral sclerosis (ALS) Parkinson's disease, osteoporosis, osteoarthritis, osteopenia, metabolic syndromes, nutritional disorders, organ atrophy, chronic obstructive pulmonary disease (COPD), and anorexia. Additional indications may include any of those disclosed in US Pub. No. 2013/0122007, US Pat. No. 8,415,459 or International Pub. No. WO 2011/151432, the contents of each of which are herein incorporated by reference in their entirety.

Fibrosis

In some embodiments, antibodies and/or compositions of the present disclosure may be useful for altering fibrosis. In some embodiments, such antibodies and/or compositions are antagonists of TGFβ (*e.g.*, TGFβ1). TGFβ1 is recognized as the central orchestrator of the fibrotic response. Antibodies targeting TGFβ1 decrease fibrosis in numerous preclinical models. Such antibodies and/or antibody-based compounds include LY2382770 (Eli Lilly, Indianapolis, IN). Also included are those described in U.S. Patent Numbers US 6,492,497, US 7,151,169, US 7,723,486 and U.S. Appl. Publ. No. 2011/0008364, the contents of each of which are herein incorporated by reference in their entirety.

Fibrotic indications for which antibodies and/or compositions of the present disclosure may be used therapeutically include, but are not limited to lung indications (*e.g.* idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disorder (COPD), allergic asthma, acute lung injury, eosinophilic esophagitis, pulmonary arterial hypertension and chemical gas-injury), kidney indications (*e.g.*, diabetic glomerulosclerosis, focal segmental glomeruloclerosis (FSGS), chronic kidney disease, fibrosis associated with kidney transplantation and chronic rejection, IgA nephropathy, and hemolytic uremic syndrome), liver fibrosis (*e.g.*, non-alcoholic steatohepatitis (NASH), chronic viral hepatitis, parasitemia, inborn errors of metabolism, toxin-mediated fibrosis, such as alcohol fibrosis, non-alcoholic steatohepatitis-hepatocellular carcinoma (NASH-HCC), primary biliary cirrhosis, and sclerosing cholangitis), cardiovascular fibrosis (*e.g.*, cardiomyopathy, hypertrophic cardiomyopathy, atherosclerosis and restenosis,) systemic sclerosis, skin fibrosis (*e.g.* skin fibrosis in systemic sclerosis, diffuse cutaneous systemic sclerosis, scleroderma, pathological skin scarring, keloid, post-surgical scarring, scar revision surgery, radiation-induced scarring and chronic wounds) and cancers or secondary fibrosis (*e.g.* myelofibrosis, head and neck cancer, M7 acute megakaryoblastic leukemia and mucositis). Other diseases, disorders or conditions related to fibrosis that may be treated using compounds and/or compositions of the present disclosure, include, but are not limited to Marfan's syndrome, stiff skin syndrome, scleroderma, rheumatoid arthritis, bone marrow fibrosis, Crohn's disease, ulcerative colitis, systemic lupus erythematosus, muscular dystrophy, (such as DMD), Dupuytren's contracture, Camurati-Engelmann disease, neural scarring, dementia, proliferative vitreoretinopathy, corneal injury, complications after glaucoma drainage surgery, and multiple sclerosis. Many such fibrotic indications are also associated with inflammation of the affected tissue(s), indicating involvement of an immune component.

The antibodies described herein may be used to treat fibrosis. In some embodiments, TGFβ1 isoform-specific agents are administered to a subject in an amount effective to treat the fibrosis. The effective amount of such an antibody is an amount effective to achieve both therapeutic efficacy and clinical safety in the subject. In some embodiments, such an antibody is a context-specific antibody that can block activation of TGFβ1 that is mediated by an LTBP-containing, ECM-associated TGFβ1. In some embodiments, the LTBP is LTBP1 and/or LTBP3. In some embodiments, antibody is a context-permissive antibody that can block activation of an LTBP-mediated TGFβ1 localized in the ECM and GARP-mediated TGFβ1 localized in immune cells. In some embodiments, antibody is a context-permissive antibody that can block activation of an LTBP-mediated TGFβ1 localized in the ECM and LRRC33-mediated TGFβ1 localized in monocytes/macrophages. In some embodiments, the LTBP is LTBP1 and/or LTBP3. In some embodiments, targeting and inhibiting TGFβ1 presented by LRRC33 on profibrotic, M2-like macrophages in the fibrotic microenvironment may be beneficial.

Assays useful in determining the efficacy of the antibodies and/or compositions of the present disclosure for the alteration of fibrosis include, but are not limited to, histological assays for counting fibroblasts and basic immunohistochemical analyses known in the art.

Cancer

Various cancers may be treated with antibodies and/or compositions of the present disclosure. As used herein, the term "cancer" refers to any of various malignant neoplasms characterized by the proliferation of anaplastic cells that tend to invade surrounding tissue and metastasize to new body sites and also refers to the pathological condition characterized by such malignant neoplastic growths. Cancers may be tumors or hematological malignancies, and include but are not limited to, all types of lymphomas/leukemias, carcinomas and sarcomas, such as those cancers or tumors found in the anus, bladder, bile duct, bone, brain, breast, cervix, colon/rectum, endometrium, esophagus, eye, gallbladder, head and neck, liver, kidney, larynx, lung, mediastinum (chest), mouth, ovaries, pancreas, penis, prostate, skin, small intestine, stomach, spinal marrow, tailbone, testicles, thyroid and uterus.

In cancer, TGFβ (*e.g.*, TGFβ1) may be either growth promoting or growth inhibitory. As an example, in pancreatic cancers, SMAD4 wild type tumors may experience inhibited growth in response to TGFβ, but as the disease progresses, constitutively activated type II receptor is typically present. Additionally, there are SMAD4-null pancreatic cancers. In

some embodiments, antibodies, antigen binding portions thereof, and/or compositions of the present disclosure are designed to selectively target components of TGFβ signaling pathways that function uniquely in one or more forms of cancer. Leukemias, or cancers of the blood or bone marrow that are characterized by an abnormal proliferation of white blood cells, *i.e.*, leukocytes, can be divided into four major classifications including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia or acute myeloid leukemia (AML) (AML with translocations between chromosome 10 and 11 [t(10, 11)], chromosome 8 and 21 [t(8;21)], chromosome 15 and 17 [t(15;17)], and inversions in chromosome 16 [inv(16)]; AML with multilineage dysplasia, which includes patients who have had a prior myelodysplastic syndrome (MDS) or myeloproliferative disease that transforms into AML; AML and myelodysplastic syndrome (MDS), therapy-related, which category includes patients who have had prior chemotherapy and/or radiation and subsequently develop AML or MDS; d) AML not otherwise categorized, which includes subtypes of AML that do not fall into the above categories; and e) acute leukemias of ambiguous lineage, which occur when the leukemic cells cannot be classified as either myeloid or lymphoid cells, or where both types of cells are present); and chronic myelogenous leukemia (CML).

The types of carcinomas include, but are not limited to, papilloma/carcinoma, choriocarcinoma, endodermal sinus tumor, teratoma, adenoma/adenocarcinoma, melanoma, fibroma, lipoma, leiomyoma, rhabdomyoma, mesothelioma, angioma, osteoma, chondroma, glioma, lymphoma/leukemia, squamous cell carcinoma, small cell carcinoma, large cell undifferentiated carcinomas, basal cell carcinoma and sinonasal undifferentiated carcinoma.

The types of sarcomas include, but are not limited to, soft tissue sarcoma such as alveolar soft part sarcoma, angiosarcoma, dermatofibrosarcoma, desmoid tumor, desmoplastic small round cell tumor, extraskeletal chondrosarcoma, extraskeletal osteosarcoma, fibrosarcoma, hemangiopericytoma, hemangiosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, lymphosarcoma, malignant fibrous histiocytoma, neurofibrosarcoma, rhabdomyosarcoma, synovial sarcoma, and Askin's tumor, Ewing's sarcoma (primitive neuroectodermal tumor), malignant hemangioendothelioma, malignant schwannoma, osteosarcoma, and chondrosarcoma.

In some embodiments, antibodies and methods of the disclosure may be used to treat one or more types of cancer or cancer-related conditions that may include, but are not limited to colon cancer, renal cancer, breast cancer, malignant melanoma and glioblastomas (Schlingensiepen *et al.*, 2008; Ouhtit *et al.*, 2013).

In some embodiments, the antibodies, or antigen binding portions thereof, that specifically bind a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, as described herein, may be used in methods for treating cancer in a subject in need thereof, said method comprising administering the antibody, or antigen binding portion thereof, to the subject such that the cancer is treated. In certain embodiments, the cancer is colon cancer.

In some embodiments, the antibodies, or antigen binding portions thereof, that specifically bind a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, as described herein, may be used in methods for treating solid tumors. In some embodiments, solid tumors may be desmoplastic tumors, which are typically dense and hard for therapeutic molecules to penetrate. By targeting the ECM component of such tumors, such antibodies may "loosen" the dense tumor tissue to disintegrate, facilitating therapeutic access to exert its anti-cancer effects. Thus, additional therapeutics, such as any known anti-tumor drugs, may be used in combination.

In some embodiments, the antibodies, or antigen binding portions thereof, that specifically bind a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, as described herein, may be used in methods for inhibiting or decreasing solid tumor growth in a subject having a solid tumor, said method comprising administering the antibody, or antigen binding portion thereof, to the subject such that the solid tumor growth is inhibited or decreased. In certain embodiments, the solid tumor is a colon carcinoma tumor. In some embodiments, the antibodies, or antigen binding portions thereof useful for treating a cancer is an isoform-specific, context-permissive inhibitor of TGFβ1 activation. In some embodiments, such antibodies target a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and a LRRC33-TGFβ1 complex. In some embodiments, such antibodies target a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, and a LTBP3-TGFβ1 complex. In some embodiments, such antibodies target a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and a LRRC33-TGFβ1 complex. In some embodiments, such antibodies target a GARP-TGFβ1 complex and a LRRC33-TGFβ1 complex.

In certain embodiments, the antibodies, or antigen binding portions thereof, that specifically bind a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, as described herein, are administered to a subject having cancer or a tumor, either alone or in combination with an additional agent, *e.g.*, an anti-PD-1 antibody (*e.g,* an anti-PD-1 antagonist). Other combination therapies

which are included in the invention are the administration of an antibody, or antigen binding portion thereof, described herein, with radiation, or a chemotherapeutic agent. Exemplary additional agents include, but are not limited to, a PD-1 antagonist, a PDL1 antagonist, a PD-L1 or PDL2 fusion protein, a CTLA4 antagonist, a GITR agonist, an anti-ICOS antibody, an anti-ICOSL antibody, an anti-B7H3 antibody, an anti-B7H4 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-OX40 antibody, an anti-CD27 antibody, an anti-CD70 antibody, an anti-CD47 antibody, an anti-41BB antibody, an anti-PD-1 antibody, an oncolytic virus, and a PARP inhibitor.

Role of TGFβ in Skeletal Muscle Conditions

In skeletal muscle TGFβ plays a variety of roles including inhibition of proliferation and differentiation, induction of atrophy, and development of fibrosis. TGFβ reduces satellite cell proliferation and prevents differentiation (via inhibition of MyoD and myogenin) (Allen, R.E. and L.K. J Cell Physiol, 1987. 133(3): p. 567-72; Brennan, T.J., *et al.,* Proc Natl Acad Sci U S A, 1991. 88(9): p. 3822-6; Massague, J., *et al.,* Proc Natl Acad Sci U S A, 1986. 83(21): p. 8206-10; Olson, E.N., *et al.*, J Cell Biol, 1986. 103(5): p. 1799-805). The isoform of TGFβ (*i.e.*, TGFβ1, 2, or 3) is not specified in these early papers, but is presumed to be TGFβ1. TGFβ also contributes to muscle fibrosis; direct injection of recombinant TGFβ1 results in skeletal muscle fibrosis, and pan-TGFβ inhibition decreases fibrosis in acute and chronically injured muscle (Li, Y., *et al.,* Am J Pathol, 2004. 164(3): p. 1007-19; Mendias, C.L., *et al.,* Muscle Nerve, 2012. 45(1): p. 55-9; Nelson, C.A., *et al.,* Am J Pathol, 2011. 178(6): p. 2611-21). TGFβ1 is expressed by myofibers, macrophages, regulatory T cells, fibroblasts, and fibrocytes within the skeletal muscle (Li, Y., *et al.,* Am J Pathol, 2004. 164(3): p. 1007-19; Lemos, D.R., *et al.*, Nat Med, 2015. 21(7): p. 786-94; Villalta, S.A., *et al.,* Sci Transl Med, 2014. 6(258): p. 258ra142; Wang, X., *et al.,* J Immunol, 2016. 197(12): p. 4750-4761); and expression is increased upon injury and in disease (Li, Y., *et al.,* Am J Pathol, 2004. 164(3): p. 1007-19; Nelson, C.A., *et al.,* Am J Pathol, 2011. 178(6): p. 2611-21; Bernasconi, P., *et al.,* J Clin Invest, 1995. 96(2): p. 1137-44; Ishitobi, M., *et al.,* Neuroreport, 2000. 11(18): p. 4033-5). TGFβ2 and TGFβ3 are also upregulated (at the mRNA level) in mdx muscle, although to a lesser extent than TGFβ1 (Nelson, C.A., *et al.,* Am J Pathol, 2011. 178(6): p. 2611-21; Zhou, L., *et al.,* Neuromuscul Disord, 2006. 16(1): p. 32-8). Pessina, *et al.*, recently used lineage tracing experiments to show that cells of multiple origins within dystrophic muscle adopt a fibrogenic fate via a TGFβ-dependent pathway (Pessina, P., *et al.,* Stem Cell Reports, 2015. 4(6): p. 1046-60).

TGFβ1 has been implicated in human muscular dystrophies. Duchenne muscular dystrophy (DMD) is a severe, progressive, and ultimately fatal disease caused by the absence of dystrophin (Bushby, K., *et al.*, Lancet Neurol, 2010. 9(1): p. 77-93). Lack of dystrophin results in increased susceptibility to contraction-induced injury, leading to continual muscle degeneration (Petrof, B.J., *et al.*, Proc Natl Acad Sci U S A, 1993. 90(8): p. 3710-4; Dellorusso, C., *et al.*, J Muscle Res Cell Motil, 2001. 22(5): p. 467-75; Pratt, S.J., *et al.*, Cell Mol Life Sci, 2015. 72(1): p. 153-64). Repeated rounds of repair contribute to chronic inflammation, fibrosis, exhaustion of the satellite cell pool, eventual loss of mobility and death (Bushby, K., *et al.*, Lancet Neurol, 2010. 9(1): p. 77-93; McDonald, C.M., *et al.*, Muscle Nerve, 2013. 48(3): p. 343-56). Expression of TGFβ1 is significantly increased in patients with DMD and correlates with the extent of fibrosis observed in these patients (Bernasconi, P., *et al.*, J Clin Invest, 1995. 96(2): p. 1137-44; Chen, Y.W., *et al.*, Neurology, 2005. 65(6): p. 826-34). Excessive ECM deposition has detrimental effects on the contractile properties of the muscle and can limit access to nutrition as the myofibers are isolated from their blood supply (Klingler, W., *et al.*, Acta Myol, 2012. 31(3): p. 184-95). Recently, additional data has further implicated TGFβ1 in muscular dystrophies. Variants in LTBP4 have been found to modify disease severity in mouse and human. In mouse, a variant of LTBP4 is protective in mice lacking dystrophin or γ-sarcoglycan (Coley, W.D., *et al.*, Hum Mol Genet, 2016. 25(1): p. 130-45; Heydemann, A., *et al.*, J Clin Invest, 2009. 119(12): p. 3703-12). In humans, two groups independently identified a variant of LTBP4 as protective in DMD, delaying loss of ambulation by several years (Flanigan, K.M., *et al.*, Ann Neurol, 2013. 73(4): p. 481-8; van den Bergen, J.C., *et al.*, J Neurol Neurosurg Psychiatry, 2015. 86(10): p. 1060-5). Although the nature of the genetic variants in mouse and human differs, in both species the protective variant results in decreased TGFβ signaling (Heydemann, A., *et al.*, J Clin Invest, 2009. 119(12): p. 3703-12); Ceco, E., *et al.*, Sci Transl Med, 2014. 6(259): p. 259ra144). Many of the functions of TGFβ1 in skeletal muscle biology have been inferred from experiments in which purified active growth factor is injected into animals or added to cells in culture (Massague, J., *et al.*, Proc Natl Acad Sci U S A, 1986. 83(21): p. 8206-10; Li, Y., *et al.*, Am J Pathol, 2004. 164(3): p. 1007-19; Mendias, C.L., *et al.*, Muscle Nerve, 2012. 45(1): p. 55-9). Given the importance of cellular context for specific functions of TGFβ1 (see, for example, Hinck *et al.*, Cold Spring Harb. Perspect. Biol, 2016. 8(12)) it is possible that some of the effects observed in these experiments do not reflect the endogenous role(s) of the cytokine *in vivo*. For example, treatment of human dermal fibroblasts with recombinant TGFβ1, myostatin, or GDF11 results in nearly identical changes in gene

expression in these cells, although *in vivo* the roles of these proteins are quite different (Tanner, J.W., Khalil, A., Hill, J., Franti, M., MacDonnell, S.M., Growth Differentiation Factor 11 Potentiates Myofibroblast Activation, in Fibrosis: From Basic Mechanisms to Targeted therapies. 2016: Keystone, CO).

Multiple investigators have used inhibitors of TGFβ to clarify the role of the growth factor *in vivo*. Treatment of mdx mice with the pan-TGFβ neutralizing antibody 1D11 clearly results in reduced fibrosis (by histology and hydroxyproline content), reduced muscle damage (reduced serum creatine kinase and greater myofiber density), and improved muscle function (by plethysmography, force generation of isolated EDL muscles, and increased forelimb grip strength) (Nelson, C.A., *et al.*, Am J Pathol, 2011. 178(6): p. 2611-21; Andreetta, F., *et al.*, J Neuroimmunol, 2006. 175(1-2): p. 77-86; Gumucio, J.P., *et al.*, J Appl Physiol (1985), 2013. 115(4): p. 539-45). In addition, myofiber-specific expression of a dominant negative TGFβ type II receptor protects against muscle damage after cardiotoxin injury and in δ-sarcoglycan-/- mice (Accornero, F., *et al.*, Hum Mol Genet, 2014. 23(25): p. 6903-15). The proteoglycan decorin, which is abundant in skeletal muscle and inhibits TGFβ activity, decreases muscle fibrosis in mdx mice and following laceration injury (Li, Y., *et al.*, Mol Ther, 2007. 15(9): p. 1616-22; Gosselin, L.E., *et al.*, Muscle Nerve, 2004. 30(5): p. 645-53). Other molecules with TGFβ inhibitory activity, such as suramin (an anti-neoplastic agent) and losartan (an angiotensin receptor blocker) have been effective in improving muscle pathology and reducing fibrosis in mouse models of injury, Marfan's syndrome, and muscular dystrophy (Spurney, C.F., *et al.*, J Cardiovasc Pharmacol Ther, 2011. 16(1): p. 87-95; Taniguti, A.P., *et al.*, Muscle Nerve, 2011. 43(1): p. 82-7; Bedair, H.S., *et al.*, Am J Sports Med, 2008. 36(8): p. 1548-54; Cohn, R.D., *et al.*, Nat Med, 2007. 13(2): p. 204-10). While all of the therapeutic agents described above do inhibit TGFβ1 or its signaling, none of them is specific for the TGFβ1 isoform. For example, 1D11 binds to and inhibits the TGFβ1, 2, and 3 isoforms (Dasch, J.R., *et al.*, J Immunol, 1989. 142(5): p. 1536-41). Suramin inhibits the ability of multiple growth factors to bind to their receptors, including PDGF, FGF, and EGF, in addition to TGFβ1 (Hosang, M., J Cell Biochem, 1985. 29(3): p. 265-73; Olivier, S., *et al.*, Eur J Cancer, 1990. 26(8): p. 867-71; Scher, H.I. and W.D. Heston, Cancer Treat Res, 1992. 59: p. 131-51). Decorin also inhibits myostatin activity, both by direct binding and through upregulation of follistatin, a myostatin inhibitor (Miura, T., *et al.*, Biochem Biophys Res Commun, 2006. 340(2): p. 675-80; Brandan, E., C. Cabello-Verrugio, and C. Vial, Matrix Biol, 2008. 27(8): p. 700-8; Zhu, J., *et al.*, J Biol Chem, 2007. 282(35): p. 25852-63). Losartan affects additional signaling pathways through its effects on the renin-angiotensin-

aldosterone system, including the IGF-1/AKT/mTOR pathway (Burks, T.N., *et al.*, Sci Transl Med, 2011. 3(82): p. 82ra37; Sabharwal, R. and M.W. Chapleau, Exp Physiol, 2014. 99(4): p. 627-31; McIntyre, M., *et al.*, Pharmacol Ther, 1997. 74(2): p. 181-94). Therefore, all of these therapies inhibit additional molecules which may contribute to their therapeutic effects, as well as toxicities.

Considering the postulated role of TGFβ in muscle homeostasis, repair, and regeneration, agents, such as monoclonal antibodies described herein, that selectively modulate TGFβ1 signaling may be effective for treating damaged muscle fibers, such as in chronic/genetic muscular dystrophies and acute muscle injuries, without the toxicities associated with more broadly-acting TGFβ inhibitors developed to date.

Accordingly, the present invention provides methods for treating damaged muscle fibers using an agent that preferentially modulates a subset, but not all, of TGFβ effects *in vivo*. Such agents can selectively modulate TGFβ1 signaling ("isoform-specific modulation"). In some embodiments, such agents can further selectively modulate TGFβ1 in a particular *context* ("context-specific modulation").

Muscle Fiber Repair in Chronic Muscular Diseases

The invention encompasses methods to improve muscle quality and function in DMD patients, by limiting fibrosis and contributing to a normalization of muscle morphology and function. As TGFβ1 also inhibits myogenesis, TGFβ1 blockade may promote regeneration in dystrophic muscle, adding further therapeutic benefit. TGFβ1 inhibitors may be used in combination with dystrophin upregulating therapies, such as Exondys 51 (Eteplirsen). Given the potential therapeutic benefits of TGFβ1 inhibition in muscular dystrophy, it is critical to (1) differentiate the role(s) of TGFβ1 from those of TGFβ2 and TGFβ3, and (2) clarify in which molecular context(s) TGFβ1 inhibition would be most beneficial. As mentioned above, pan-TGFβ inhibitors have been associated with significant toxicities, limiting the clinical use of these compounds (Anderton, M.J., *et al.*, Toxicol Pathol, 2011. 39(6): p. 916-24; Stauber, A., *et al.*, Clinical Toxicology, 2014. 4(3): p. 1-10). It is unclear which of the TGFβ isoform(s) causes these toxicities. Some of the described toxicities may be due to TGFβ1 inhibition in the immune system. For example, while 1D11 significantly reduced levels of fibrosis in the diaphragm, treatment also increased numbers of CD4+ and CD8+ T cells in the muscle, suggesting an increased inflammatory response upon pan-TGFβ inhibition which could be detrimental with long-term treatment (Andreetta, F., *et al.*, J Neuroimmunol, 2006. 175(1-2): p. 77-86). Indeed, depletion of T cells from muscle improves the muscle pathology

of mdx mice, suggesting T-cell mediated inflammatory responses are detrimental to dystrophic muscle (Spencer, M.J., *et al.*, Clin Immunol, 2001. 98(2): p. 235-43). Increases in T cell numbers upon 1D11 administration are likely due to the effects of TGFβ1 on regulatory T (Treg) cells. Tregs present TGFβ1 on their cell surface via GARP, and release of TGFβ1 from this complex enhances Treg suppressive activity, thus limiting T cell mediated inflammation (Wang, R., *et al.*, Mol Biol Cell, 2012. 23(6): p. 1129-39; Edwards, J.P., A.M. Thornton, and E.M. Shevach, J Immunol, 2014. 193(6): p. 2843-9; Nakamura, K., *et al.*, J Immunol, 2004. 172(2): p. 834-42; Nakamura, K., A. Kitani, and W. Strober, J Exp Med, 2001. 194(5): p. 629-44). Indeed, depletion of Tregs using the PC61 antibody resulted in increased inflammation and muscle damage in the diaphragm of mdx mice, while augmentation of Treg numbers and activity reduced muscle damage (Villalta, S.A., *et al.*, Sci Transl Med, 2014. 6(258): p. 258ra142). Interestingly, an additional population of immunosuppressive T cells, Tr1 cells, has recently been identified. These cells produce large amounts of TGFβ3, which is required for their suppressive activity (Gagliani, N., *et al.*, Nat Med, 2013. 19(6): p. 739-46; Okamura, T., *et al.*, Proc Natl Acad Sci U S A, 2009. 106(33): p. 13974-9; Okamura, T., *et al.*, Nat Commun, 2015. 6: p. 6329). While the role of Tr1 cells in skeletal muscle is unknown, the possibility exists that inhibition of both TGFβ1 and TGFβ3 by 1D11 could have additive pro-inflammatory effects by inhibiting both Tregs and Tr1 cells.

The structural insights described above regarding TGFβ1 latency and activation allow for novel approaches to drugs discovery that specifically target activation of TGFβ1 (Shi, M., *et al.*, Nature, 2011. 474(7351): p. 343-9). The high degree of sequence identity shared between the three mature TGFβ growth factors is not shared by the latent complexes, allowing for the discovery of antibodies that are exquisitely specific to proTGFβ1. Using proprietary approaches to antibody discovery, the instant inventors have identified antibodies (Ab1 and Ab2) which specifically bind to proTGFβ1 (FIG. 18A). Using an *in vitro* co-culture system these antibodies were demonstrated to inhibit integrin-mediated release of TGFβ1. In this system, fibroblasts derived from human skin or mouse skeletal muscles are the source of latent TGFβ1, a cell line expressing αVβ6 allows for release of active TGFβ1, which is then measured using a third cell line expressing a SMAD2/3 responsive luciferase reporter (FIGs. 11A-C). One of these antibodies, Ab1, has been tested *in vivo* and shown efficacy in the UUO (unilateral ureteral obstruction) mouse model of kidney fibrosis. In this model, treatment of mice (n=10) with 9 mg/kg/week Ab1 prevented upregulation of TGFβ1-responsive genes (FIG. 15) and reduced the extent of fibrosis following injury (by picrosirius

red staining) (FIG. 16). TGFβ1 specific therapies may have improved efficacy and safety profiles compared to pan-TGFβ inhibitors, a critical aspect for a therapeutic which would be used long term as in the DMD population. TGFβ1 inhibitory antibodies can be used to determine if specific TGFβ1 inhibition has potential as a therapeutic for DMD or other muscle diseases, and to clarify the role of TGFβ1 in skeletal muscle regeneration.

Chronic vs. Acute Myofiber Injuries and Selection of Optimal Therapeutics

In normal, but regenerating muscle following an acute injury (such as traumatic injury to otherwise healthy muscles or motor neurons), it is believed that the initial infiltration of inflammatory macrophages is required to clear out the damaged tissue and to secrete factors (*e.g.*, cytokines) necessary for satellite cell activation. Subsequently, these cells switch to the M2 phenotype to drive wound resolution.

By contrast, in chronic conditions, such as diseases including DMD, the pro-inflammatory macrophages predominated at all time, and that switch to M2 does not happen (or at least not efficiently enough), and the pro-inflammatory macrophages continue to drive inflammation and muscle damage. In DMD, the NFkB pathway is perpetually active, resulting in constitutive inflammation. In some embodiments, therefore, an NFkB inhibitor may be administered to DMD patients in order to reduce the chronic inflammation.

Thus, in chronic conditions such as DMD, therapeutic focus may be on muscle repair as opposed to muscle regeneration. This is because DMD muscle fibers are defective but not destroyed – they are damaged by tears in the membrane, dysregulation of calcium transients, and ROS damage from the macrophages. In comparison, in cases of injuries to healthy muscles, therapeutic focus may be on regeneration. For example, in cardiotoxin models, muscle fibers are killed and have to be regenerated. This simulates the process of recovery after a traumatic injury, such as crush injury.

Evidence suggests that LRRC33 is expressed in thioglycollate-induced peritoneal macrophages, which have an M2-like phenotype (characterized in that they express high levels of Arginase, no iNOS, and high levels of CD206).

In situations where LRRC33 is expressed primarily on the M2 cells and where its presentation of TGFβ1 ("*context*") is important for the pro-wound healing effects of these cells, it may be beneficial to activate LRRC33-mediated TGFβ1 to promote repair and/or myogenesis. On the other hand, in situations where LRRC33 is also expressed on the pro-inflammatory M1 cells, then it may be beneficial to inhibit LRRC33-mediated TGFβ1, given that inflammation drives the fibrosis, especially in the dystrophic setting, such as DMD.

Thus, identifying the source/context of disease-associated TGFβ1 can be an important step in selecting the right modulator of the TGFβ signaling, which will inform what level of selectivity should be considered (*e.g.*, isoform-specific, context-permissive TGFβ1 modulators, or, context-specific TGFβ1 modulators; TGFβ1 inhibitors or activators, etc.).

Apart from chronic inflammation, the hallmark of DMD is excessive, and progressive, fibrosis. In advanced disease the fibrosis is so severe that it can actually isolate individual muscle fibers from their blood supply. It also alters the contractile properties of the muscle. In human patients, there is a strong correlation between the extent of TGFβ1 upregulation and fibrosis, and a strong link between the extent of fibrosis and negative mobility outcomes. Therefore, in some embodiments, LTBP-proTGFβ1 inhibitors may be administered to dystrophic patients for the prevention and/or reduction of fibrosis to selectively target the ECM-associated TGFβ1 effects in the disease. In some embodiments, various isoform- and/or context-selective agents described herein can be employed to achieve inhibition of TGFβ1 signaling to prevent fibrosis and promote myogenesis, but without having unwanted effects on the immune system (*e.g.*, through GARP or LRRC33).

Treatments

To practice the method disclosed herein, an effective amount of the pharmaceutical composition described above can be administered to a subject (*e.g.*, a human) in need of the treatment via a suitable route, such as intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, inhalation or topical routes. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, antibodies, or antigen binding portions thereof, that specifically bind a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

The subject to be treated by the methods described herein can be a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats. A human subject who needs the treatment may be a human patient having, at risk for, or suspected of having a TGFβ-related indication, such as those noted above. A subject having a TGFβ-related indication can be identified by

routine medical examination, *e.g.*, laboratory tests, organ functional tests, CT scans, or ultrasounds. A subject suspected of having any of such indication might show one or more symptoms of the indication. A subject at risk for the indication can be a subject having one or more of the risk factors for that indication.

As used herein, the terms "effective amount" and "effective dose" refer to any amount or dose of a compound or composition that is sufficient to fulfill its intended purpose(s), *i.e.*, a desired biological or medicinal response in a tissue or subject at an acceptable benefit/risk ratio. For example, in certain embodiments of the present invention, the intended purpose may be to inhibit TGFβ-1 activation *in vivo*, to achieve clinically meaningful outcome associated with the TGFβ-1 inhibition. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. For example, antibodies that are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a TGFβ-related indication. Alternatively, sustained continuous release formulations of an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex may be appropriate. Various formulations and devices for achieving sustained release would be apparent to the skilled artisan and are within the scope of this disclosure.

In one example, dosages for an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex as described herein may be determined empirically in individuals who have been

given one or more administration(s) of the antibody. Individuals are given incremental dosages of the antagonist. To assess efficacy, an indicator of the TGFβ-related indication can be followed. For example, methods for measuring for myofiber damage, myofiber repair, inflammation levels in muscle, and/or fibrosis levels in muscle are well known to one of ordinary skill in the art.

The present invention encompasses the recognition that agents capable of modulating the activation step of TGFβs in an isoform-specific manner may provide improved safety profiles when used as a medicament. Accordingly, the invention includes antibodies and antigen-binding fragments thereof that specifically bind and inhibit activation of TGFβ1, but not TGFβ2 or TGFβ3, thereby conferring specific inhibition of the TGFβ1 signaling *in vivo* while minimizing unwanted side effects from affecting TGFβ2 and/or TGFβ3 signaling.

In some embodiments, the antibodies, or antigen binding portions thereof, as described herein, are not toxic when administered to a subject. In some embodiments, the antibodies, or antigen binding portions thereof, as described herein, exhibit reduced toxicity when administered to a subject as compared to an antibody that specifically binds to both TGFβ1 and TGFβ2. In some embodiments, the antibodies, or antigen binding portions thereof, as described herein, exhibit reduced toxicity when administered to a subject as compared to an antibody that specifically binds to both TGFβ1 and TGFβ3. In some embodiments, the antibodies, or antigen binding portions thereof, as described herein, exhibit reduced toxicity when administered to a subject as compared to an antibody that specifically binds to TGFβ1, TGFβ2 and TGFβ3.

Generally, for administration of any of the antibodies described herein, an initial candidate dosage can be about 2 mg/kg. For the purpose of the present disclosure, a typical daily dosage might range from about any of 0.1 μg/kg to 3 μg/kg to 30 μg/kg to 300 μg/kg to 3 mg/kg, to 30 mg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient therapeutic levels are achieved to alleviate a TGFβ-related indication, or a symptom thereof. An exemplary dosing regimen comprises administering an initial dose of about 2 mg/kg, followed by a weekly maintenance dose of about 1 mg/kg of the antibody, or followed by a maintenance dose of about 1 mg/kg every other week. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. For example, dosing from one-four times a week is contemplated. In some embodiments, dosing ranging from about 3 μg/mg to about 2 mg/kg (such as about 3 μg/mg,

about 10 μg/mg, about 30 μg/mg, about 100 μg/mg, about 300 μg/mg, about 1 mg/kg, and about 2 mg/kg) may be used. Pharmacokinetics experiments have shown that the serum concentration of an antibody disclosed herein (*e.g.*, Ab2) remains stable for at least 7 days after administration to a preclinical animal model (*e.g.*, a mouse model). Without wishing to be bound by any particular theory, this stability post-administration may be advantageous since the antibody may be administered less frequently while maintaining a clinically effective serum concentration in the subject to whom the antibody is administered (*e.g.*, a human subject). In some embodiments, dosing frequency is once every week, every 2 weeks, every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, or every 10 weeks; or once every month, every 2 months, or every 3 months, or longer. The progress of this therapy is easily monitored by conventional techniques and assays. The dosing regimen (including the antibody used) can vary over time.

In some embodiments, for an adult patient of normal weight, doses ranging from about 0.3 to 5.00 mg/kg may be administered. The particular dosage regimen, *e.g.*., dose, timing and repetition, will depend on the particular individual and that individual's medical history, as well as the properties of the individual agents (such as the half-life of the agent, and other relevant considerations).

For the purpose of the present disclosure, the appropriate dosage of an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex will depend on the specific antibody (or compositions thereof) employed, the type and severity of the indication, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antagonist, and the discretion of the attending physician. In some embodiments, a clinician will administer an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, until a dosage is reached that achieves the desired result. Administration of an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex can be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex may be essentially continuous over a preselected period of time or may be in a series of spaced dose, *e.g.*, either before, during, or after developing a TGFβ-related indication.

As used herein, the term "treating" refers to the application or administration of a composition including one or more active agents to a subject, who has a TGFβ-related indication, a symptom of the indication, or a predisposition toward the indication, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the indication, the symptom of the indication, or the predisposition toward the indication.

Alleviating a TGFβ-related indication with an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex includes delaying the development or progression of the indication, or reducing indication's severity. Alleviating the indication does not necessarily require curative results. As used therein, "delaying" the development of an indication associated with a TGFβ-related indication means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the indication. This delay can be of varying lengths of time, depending on the history of the indication and/or individuals being treated. A method that "delays" or alleviates the development of an indication, or delays the onset of the indication, is a method that reduces probability of developing one or more symptoms of the indication in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

DBA2/J mice have a 40 bp deletion in the LTBP4 allele. Dysregulation of the ECM to which latent TGFb1 is associated may expose the epitope to which Ab1 binds. There may be diseases in which the epitope to which Ab1 binds gets exposed, and those diseases may be therapeutic opportunities for Ab1 if TGFb1 inhibition is indicated.

Combination Therapies

The disclosure further encompasses pharmaceutical compositions and related methods used as combination therapies for treating subjects who may benefit from TGFβ inhibition *in vivo*. In any of these embodiments, such subjects may receive combination therapies that include a first composition comprising at least one TGFβ inhibitor, *e.g.,* antibody or antigen-binding portion thereof, described herein, in conjunction with a second composition comprising at least one additional therapeutic intended to treat the same or overlapping disease or clinical condition. The first and second compositions may both act on the same cellular target, or discrete cellular targets. In some embodiments, the first and second compositions may treat or alleviate the same or overlapping set of symptoms or aspects of a disease or clinical condition. In some embodiments, the first and second compositions may

treat or alleviate a separate set of symptoms or aspects of a disease or clinical condition. To give but one example, the first composition may treat a disease or condition associated with TGFβ signaling, while the second composition may treat inflammation or fibrosis associated with the same disease, etc. Such combination therapies may be administered in conjunction with each other. The phrase "in conjunction with," in the context of combination therapies, means that therapeutic effects of a first therapy overlaps temporarily and/or spatially with therapeutic effects of a second therapy in the subject receiving the combination therapy. Thus, the combination therapies may be formulated as a single formulation for concurrent administration, or as separate formulations, for sequential administration of the therapies.

In preferred embodiments, combination therapies produce synergistic effects in the treatment of a disease. The term "synergistic" refers to effects that are greater than additive effects (*e.g.,* greater efficacy) of each monotherapy in aggregate.

In some embodiments, combination therapies comprising a pharmaceutical composition described herein produce efficacy that is overall equivalent to that produced by another therapy (such as monotherapy of a second agent) but are associated with fewer unwanted adverse effect or less severe toxicity associated with the second agent, as compared to the monotherapy of the second agent. In some embodiments, such combination therapies allow lower dosage of the second agent but maintain overall efficacy. Such combination therapies may be particularly suitable for patient populations where a long-term treatment is warranted and/or involving pediatric patients.

Accordingly, the invention provides pharmaceutical compositions and methods for use in combination therapies for the reduction of TGFβ1 protein activation and the treatment or prevention of diseases or conditions associated with TGFβ1 signaling, as described herein. Accordingly, the methods or the pharmaceutical compositions further comprise a second therapy. In some embodiments, the second therapy may be useful in treating or preventing diseases or conditions associated with TGFβ1 signaling. The second therapy may diminish or treat at least one symptom(s) associated with the targeted disease. The first and second therapies may exert their biological effects by similar or unrelated mechanisms of action; or either one or both of the first and second therapies may exert their biological effects by a multiplicity of mechanisms of action.

It should be understood that the pharmaceutical compositions described herein may have the first and second therapies in the same pharmaceutically acceptable carrier or in a different pharmaceutically acceptable carrier for each described embodiment. It further

should be understood that the first and second therapies may be administered simultaneously or sequentially within described embodiments.

The one or more anti-TGFβ antibodies, or antigen binding portions thereof, of the invention may be used in combination with one or more of additional therapeutic agents. Examples of the additional therapeutic agents which can be used with an anti-TGFβ antibody of the invention include, but are not limited to, a myostatin inhibitor, a VEGF agonist, an IGF1 agonist, an FXR agonist, a CCR2 inhibitor, a CCR5 inhibitor, a dual CCR2/CCR5 inhibitor, a lysyl oxidase-like-2 inhibitor, an ASK1 inhibitor, an Acetyl-CoA Carboxylase (ACC) inhibitor, a p38 kinase inhibitor, Pirfenidone, Nintedanib, a GDF11 inhibitor, and the like.

In some embodiments, the additional agent is a checkpoint inhibitor. In some embodiments, the additional agent is selected from the group consisting of a PD-1 antagonist, a PDL1 antagonist, a PD-L1 or PDL2 fusion protein, a CTLA4 antagonist, a GITR agonist, an anti-ICOS antibody, an anti-ICOSL antibody, an anti-B7H3 antibody, an anti-B7H4 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-OX40 antibody, an anti-CD27 antibody, an anti-CD70 antibody, an anti-CD47 antibody, an anti-41BB antibody, an anti-PD-1 antibody, an oncolytic virus, and a PARP inhibitor. In some embodiments, the additional therapy is radiation. In some embodiments, the additional agent is a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is Taxol. In some embodiments, the additional agent is an anti-inflammatory agent. In some embodiments, the additional agent inhibits the process of monocyte/macrophage recruitment and/or tissue infiltration. In some embodiments, the additional agent is an inhibitor of hepatic stellate cell activation. In some embodiments, the additional agent is a chemokine receptor antagonist, *e.g.*, CCR2 antagonists and CCR5 antagonists. In some embodiments, such chemokine receptor antagonist is a dual specific antagonist, such as a CCR2/CCR5 antagonist. In some embodiments, the additional agent to be administered as combination therapy is or comprises a member of the TGFβ superfamily of growth factors or regulators thereof. In some embodiments, such agent is selected from modulators (*e.g.*, inhibitors and activators) of GDF8/myostatin and GDF11. In some embodiments, such agent is an inhibitor of GDF8/myostatin signaling. In some embodiments, such agent is a monoclonal antibody that specifically binds a pro/latent myostatin complex and blocks activation of myostatin. In some embodiments, the monoclonal antibody that specifically binds a pro/latent myostatin complex and blocks activation of myostatin does not bind free, mature myostatin.

Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

Modulating TGFβ Activity

Methods of the present disclosure include methods of modulating growth factor activity in one or more biological system. Such methods may include contacting one or more biological system with an antibody and/or composition of the disclosure. In some cases, these methods include modifying the level of free growth factor in a biological system (*e.g.* in a cell niche or subject). Antibodies and/or compositions according to such methods may include, but are not limited to biomolecules, including, but not limited to recombinant proteins, protein complexes and/or antibodies, or antigen portions thereof, described herein.

In some embodiments, methods of the present disclosure may be used to reduce or eliminate growth factor activity, termed "inhibiting methods" herein. Some such methods may comprise mature growth factor retention in a TGFβ complex (*e.g.*, a TGFβ1 complexed with GARP, LTBP1, LTBP3 and/or LRRC33) and/or promotion of reassociation of growth factor into a TGFβ complex. In some cases, inhibiting methods may comprise the use of an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex. According to some inhibiting methods, one or more inhibiting antibody is provided.

In some embodiments, antibodies, antigen binding portions thereof, and compositions of the disclosure may be used for inhibiting TGFβ1 activation. In some embodiments, provided herein is a method for inhibiting TGFβ1 activation comprising exposing a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex to an antibody, an antigen binding portion thereof, or a pharmaceutical composition described herein. In some embodiments, the antibody, antigen binding portion thereof, or pharmaceutical composition, inhibits the release of mature TGFβ1 from the GARP-TGFβ1 complex, the LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or the LRRC33-TGFβ1 complex. In some embodiments, the method is performed *in vitro*. In some embodiments, the method is performed *in vivo*. In some embodiments, the method is performed *ex vivo*.

In some embodiments, the GARP-TGFβ1 complex, the LTBP1-TGFβ1 complex, the LTBP3-TGFβ1 complex, or the LRRC33-TGFβ1 complex is present at the outer surface of a

cell. In some embodiments, the cell is a T-cell, a fibroblast, a macrophage, a monocyte, or a microglia.

In some embodiments, the LRRC33-TGFβ1 complex is present at the outer surface of profibrotic (M2-like) macrophages. In some embodiments, the profibrotic (M2-like) macrophages are present in the fibrotic microenvironment. In some embodiments, targeting of the LRRC33-TGFβ1 complex at the outer surface of profibrotic (M2-like) macrophages provides a superior effect as compared to solely targeting LTBP1-TGFβ1 and/or LTBP1-TGFβ1 complexes.

In some embodiments, the GARP-TGFβ1 complex, the LTBP1-TGFβ1 complex, the LTBP3-TGFβ1 complex, and/or the LRRC33-TGFβ1 complex is bound to an extracellular matrix. In some embodiments, the extracellular matrix comprises fibrillin. In some embodiments, the extracellular matrix comprises a protein comprising an RGD motif.

In some embodiments, provided herein is a method for reducing TGFβ1 protein activation in a subject comprising administering an antibody, an antigen binding portion thereof, or a pharmaceutical composition described herein to the subject, thereby reducing TGFβ1 protein activation in the subject. In some embodiments, the subject has or is at risk of having fibrosis. In some embodiments, the subject has or is at risk of having cancer. In some embodiments, the subject has or is at risk of having dementia.

In some embodiments, the antibodies, or the antigen binding portions thereof, as described herein, reduce the suppressive activity of regulatory T cells (Tregs).

Kits For Use in Alleviating Diseases/Disorders Associated with a TGFβ-related Indication

The present disclosure also provides kits for use in alleviating diseases/disorders associated with a TGFβ-related indication. Such kits can include one or more containers comprising an antibody, or antigen binding portion thereof, that specifically binds to a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex, *e.g.*, any of those described herein.

In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of administration of the antibody, or antigen binding portion thereof, that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex to treat, delay the onset, or alleviate a target disease as those described herein. The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the target disease. In

still other embodiments, the instructions comprise a description of administering an antibody, or antigen binding portion thereof, to an individual at risk of the target disease.

The instructions relating to the use of antibodies, or antigen binding portions thereof, that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the disclosure are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable. The label or package insert indicates that the composition is used for treating, delaying the onset and/or alleviating a disease or disorder associated with a TGFβ-related indication. Instructions may be provided for practicing any of the methods described herein.

The kits of this disclosure are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (*e.g.*, an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody, or antigen binding portion thereof, that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex as those described herein.

Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiments, the disclosure provides articles of manufacture comprising contents of the kits described above.

Assays for Detecting a GARP-TGFβ1 Complex, a LTBP1-TGFβ1 Complex, a LTBP3-TGFβ1 Complex, and/or a LRRC33-TGFβ1 Complex

In some embodiments, methods and compositions provided herein relate to a method for detecting a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex in a sample obtained from a subject. As used

herein, a "subject" refers to an individual organism, for example, an individual mammal. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human mammal. In some embodiments, the subject is a non-human primate. In some embodiments, the subject is a rodent. In some embodiments, the subject is a sheep, a goat, a cattle, poultry, a cat, or a dog. In some embodiments, the subject is a vertebrate, an amphibian, a reptile, a fish, an insect, a fly, or a nematode. In some embodiments, the subject is a research animal. In some embodiments, the subject is genetically engineered, *e.g.*, a genetically engineered non-human subject. The subject may be of either sex and at any stage of development. In some embodiments, the subject is a patient or a healthy volunteer.

In some embodiments, a method for detecting a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex in a sample obtained from a subject involves (a) contacting the sample with an antibody that specifically binds a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or a LRRC33-TGFβ1 complex under conditions suitable for binding of the antibody to the antigen, if the antigen is present in the sample, thereby forming binding complexes; and (b) determining the level of the antibody bound to the antigen (*e.g.*, determining the level of the binding complexes).

In one embodiment, a screening assay that utilizes biotinylated latent TGFβ1 complexes immobilized onto a surface, which allows for the activation of latent TGFβ by integrins by providing tether. Other, non-integrin activators could also be tested in that system. Readout can be through reporter cells or other TGFβ-dependent cellular responses.

Cell-based assays for measuring TGFβ activation

Activation of TGFβ (and inhibition thereof by a TGFβ test inhibitor, such as an antibody) may be measured by any suitable method known in the art. For example, integrin-mediated activation of TGFβ can be utilized in a cell-based assay, such as the "CAGA12" luciferase assay, described in more detail herein. An exemplary embodiment of such assay is depicted in FIG. 11C for illustrative purposes. As shown, such an assay system may comprise the following components: i) a source of TGFβ (recombinant, endogenous or transfected); ii) a source of integrin (recombinant, endogenous, or transfected); and iii) a reporter system that responds to TGFβ activation, such as cells expressing TGFβ receptors capable of responding to TGFβ and translating the signal into a readable output (*e.g.*, luciferase activity in CAGA12 cells or other reporter cell lines). In some embodiments, the reporter cell line comprises a reporter gene (*e.g.*, a luciferase gene) under the control of a

TGFβ-responsive promoter (*e.g.*, a PAI-1 promoter). In some embodiments, certain promoter elements that confer sensitivity may be incorporated into the reporter system. In some embodiments, such promoter element is the CAGA12 element. Reporter cell lines that may be used in the assay have been described, for example, in Abe *et al.* (1994) *Anal Biochem.* 216(2): 276-84, incorporated herein by reference. In some embodiments, each of the aforementioned assay components are provided from the same source (*e.g.*, the same cell). In some embodiments, two of the aforementioned assay components are provided from the same source, and a third assay component is provided from a different source. In some embodiments, all three assay components are provided from different sources. For example, in some embodiments, the integrin and the latent TGFβ complex (proTGFβ and a presenting molecule) are provided for the assay from the same source (*e.g.*, the same transfected cell line). In some embodiments, the integrin and the TGF are provided for the assay from separate sources (*e.g.*, two different cell lines, a combination of purified integrin and a transfected cell). When cells are used as the source of one or more of the assay components, such components of the assay may be endogenous to the cell, stably expressed in the cell, transiently transfected, or any combination thereof. The results from a non-limiting exemplary embodiment of a cell-based assay for measuring TGFβ activation demonstrating the inhibition of either GARP-proTGFβ1 complex or LRRC33-proTGFβ1 complex using antibodies Ab1 and Ab2, disclosed herein, is shown at FIGs. 22A and FIG. 22B, respectively. In this exemplary assay, the IC50 (μg/mL) of Ab1 for the GARP-TGFβ1 complex was 0.445, and the IC50 (μg/mL) of Ab1 for the LRRC33-TGFβ1 complex was 1.325.

A skilled artisan could readily adapt such assays to various suitable configurations. For instance, a variety of sources of TGFβ may be considered. In some embodiments, the source of TGFβ is a cell that expresses and deposits TGFβ (*e.g.*, a primary cell, a propagated cell, an immortalized cell or cell line, etc.). In some embodiments, the source of TGFβ is purified and/or recombinant TGFβ immobilized in the assay system using suitable means. In some embodiments, TGFβ immobilized in the assay system is presented within an extracellular matrix (ECM) composition on the assay plate, with or without de-cellularization, which mimics fibroblast-originated TGFβ. In some embodiments, TGFβ is presented on the cell surface of a cell used in the assay. Additionally, a presenting molecule of choice may be included in the assay system to provide suitable latent-TGFβ complex. One of ordinary skill in the art can readily determine which presenting molecule(s) may be present or expressed in certain cells or cell types. Using such assay systems, relative changes in TGFβ activation in the presence or absence of a test agent (such as an antibody) may be

readily measured to evaluate the effects of the test agent on TGFβ activation *in vitro*. Data from exemplary cell-based assays are provided in the Example section below.

Such cell-based assays may be modified or tailored in a number of ways depending on the TGFβ isoform being studied, the type of latent complex (*e.g.*, presenting molecule), and the like. In some embodiments, a cell known to express integrin capable of activating TGFβ may be used as the source of integrin in the assay. Such cells include SW480/β6 cells (*e.g.*, clone 1E7). In some embodiments, integrin-expressing cells may be co-transfected with a plasmid encoding a presenting molecule of interest (such as GARP, LRRC33, LTBP (*e.g.*, LTBP1 or LTBP3), etc.) and a plasmid encoding a pro-form of the TGFβ isoform of interest (such as proTGFβ1). After transfection, the cells are incubated for sufficient time to allow for the expression of the transfected genes (*e.g.*, about 24 hours), cells are washed, and incubated with serial dilutions of a test agent (*e.g.*, an antibody). Then, a reporter cell line (*e.g.,* CAGA12 cells) is added to the assay system, followed by appropriate incubation time to allow TGFβ signaling. After an incubation period (*e.g.*, about 18-20 hours) following the addition of the test agent, signal/read-out (*e.g.,* luciferase activity) is detected using suitable means (*e.g.*, for luciferase-expressing reporter cell lines, the Bright-Glo reagent (Promega) can be used). In some embodiments, Luciferase fluorescence may be detected using a BioTek (Synergy H1) plate reader, with autogain settings.

Nucleic Acids

In some embodiments, antibodies, antigen binding portions thereof, and/or compositions of the present disclosure may be encoded by nucleic acid molecules. Such nucleic acid molecules include, without limitation, DNA molecules, RNA molecules, polynucleotides, oligonucleotides, mRNA molecules, vectors, plasmids and the like. In some embodiments, the present disclosure may comprise cells programmed or generated to express nucleic acid molecules encoding compounds and/or compositions of the present disclosure. In some cases, nucleic acids of the disclosure include codon-optimized nucleic acids. Methods of generating codon-optimized nucleic acids are known in the art and may include, but are not limited to those described in US Patent Nos. 5,786,464 and 6,114,148, the contents of each of which are herein incorporated by reference in their entirety.

The present invention is further illustrated by the following examples, which are not intended to be limiting in any way. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the Figures, are hereby incorporated herein by reference.

EXAMPLES

Example 1: Inhibition of TGFβ1

The TGFβ superfamily includes propeptides complexed with active growth factors (FIG. 1). Selection strategies to obtain antibodies that stabilize the complex, resulting in more selective and potent inhibition, were developed.

Using a HEK293-based expression system, NiNTA affinity and gel filtration were performed to obtain multimilligram quantities of purified protein, which were used to generate TGFβ1 complexed to LTBP (LTBP-TGFβ1 complex) and TGFβ1 complexed to GARP (GARP-TGFβ1 complex) (FIG. 3). The diversity of proteins manufactured enabled the testing of species cross-reactivity and epitope mapping. The purification of an sGARP-proTGFβ complex (FIG. 4A and 4B), an sGARP-TGFβ LAP complex (FIG. 5), and a LTBP1-proTGFβ1 complex (FIG. 6) are shown.

The candidate antibodies were tested using an *in vitro* luminescence assays (FIG. 8). In the screen, antibodies that inhibited growth factor release turned reporter cells "off" when faced with a stimulus for normal activation. Ab1 and Ab2 were shown to be inhibitors of activation of latent TGFβ1 complexes (FIG. 7) and were cross-reactive to mouse.

Initial dose-response analysis curves of Ab1 in cells expressing human TGFβ1 showed TGFβ1 activity inhibition (FIG. 8). Using a more sensitive CAGA12 reporter cell line, Ab1 showed similar inhibition of human proTGFβ1 activity (FIG. 9). Furthermore, the inhibition of a GARP complex was shown to block the suppressive activity of T regulatory cells (Tregs) as measured by the percent of dividing T effector cells (Teff) in T cells isolated from healthy donor blood (FIG. 10).

The affinity of GARP-proTGFβ1 inhibitors was measured by Octet assay on human GARP-proTGFβ1 cells, while activity was measured by CAGA12 reporter cells testing human GARP-proTGFβ1 inhibition. The protocol used to measure the affinity of antibodies Ab1 and Ab2 to the complexes provided herein is summarized in Table 6. The results are shown in Table 7.

Table 6: Protocol for performing Octet binding assay.

Materials:
- 96 well black polypropylene plates
- Streptavidin-coated tips for Octet

- 10x kinetics buffer (diluted 1:10 in PBS)

1. Soak required amount of streptavidin tips in 1X kinetics buffer; place in machine to equilibrate

2. Load sample plate:

- 200 µl of buffer or antibody dilution to each well

a. Column 1 – baseline (buffer)
b. Column 2 – biotinylated protein (*e.g.*, sGARP-proTGFβ1 or LTBP1-proTGFβ1); diluted to 5 µg/mL
c. Column 3 - baseline 2 (buffer)
d. Column 4 - antibody association for Ab1
e. Column 5 - antibody association for Ab2
f. Column 6 - dissociation Ab 1 (buffer)
g. Column 7 - dissociation Ab2 (buffer)

3. Make dilutions in the 96 well plate:

a. Dilute both antibodies to 50 µg/mL in 300 µl of 1x buffer in row A.
b. Add 200 µl of buffer to the rest of each column
c. Transfer 100 µl down the column to make 3-fold dilutions

4. Place the sample plate in the machine next to the tips plate

5. Set up the software

a. Indicate buffer, load, sample (one assay per antibody tested)
b. Indicate steps of the protocol (baseline, load, association, dissociation) for set amounts of time:
   - Baseline: 60 seconds
   - Loading: 300 seconds
   - Baseline 2: 60 seconds
   - Association: 300 seconds
   - Dissociation: 600 seconds

6. Analyze data

a. Subtract baseline from reference well
b. Set normalization to last five seconds of baseline
c. Align to dissociation
d. Analyze to association and dissociation (1:1 binding model, fit curves)
e. Determine the best R^2 values; include concentrations with best R^2 values
f. Select global fit
g. Set colors of samples by sensor type
h. Analyze
i. Save table and export

Table 7: Affinity and Activity of GARP-proTGFβ1 Inhibitors

Clone	Affinity for GARP-proTGFβ1 (nM ± SEM)	Inhibition (IC50) of GARP-proTGFβ1 (nM; 95% CI)	Max effect (% inhibition)
Ab1	0.046 ± 0.043	3.4 (2.1-5.4)	75%
Ab2	0.561 ± 0.014	3.9 (1.5-10.3)	50%

The clones were further screened for binding selectivity (Table 8) and species cross-reactivity (Table 9). Ab1 and Ab2 did not bind to TGFβ1, TGFβ2, or TGFβ3, but did bind the proTGFβ1 complexes and showed species cross-reactivity.

Table 8: Selectivity of GARP-proTGFβ1 Inhibitors

Clone	GARP-proTGFβ1	LTBP1-proTGFβ1	LTBP3-proTGFβ1
Ab1	+++	+++	+++
Ab2	+++	+++	+++

Table 9: Species Cross-Reactivity of GARP-proTGFβ1 Inhibitors

Clone	huGARP-proTGFβ1	muGARP-proTGFβ1	cyGARP-proTGFβ1
Ab1	+++	++	+++
Ab2	+++	+++	+++

+++ KD < 1 nM,
++ KD 1 – 10 nM
+ KD 10 – 100 nM
- No binding

Example 2: Ab1 and Ab2 Specifically Bind to proTGFβ1 Complexes from Multiple Species

To determine if Ab1 and Ab2 are capable of specifically binding to proTGFβ1 complexes from multiple species, Octet binding assays were performed as described in Table 6. As shown in Table 10 (below), both antibodies (*i.e.*, Ab1 and Ab2) specifically bound to human and murine LTBP1-proTGFβ1 complexes, human LTBP3-proTGFβ1 complexes, and human GARP-proTGFβ1 complexes. However, only Ab2 specifically bound to rat LTBP1-proTGFβ1 complexes.

Table 10. Affinity of Ab1 and Ab2 for proTGFβ1 Complexes from Multiple Species

	Ab1 (K_D)	Ab2 (K_D)
human LTBP1-proTGFβ1	16 ± 1.3	5.8 ± 0.6
human LTBP3-proTGFβ1	85 ± 5.0	122 ± 3.9
mouse LTBP1-proTGFβ1	203 ± 13	61 ± 4.0
rat LTBP1-proTGFβ1	No binding detected	38 ± 6.8
human GARP-proTGFβ1	293 ± 22	58 ± 6.2

Example 3: Ab1 and Ab2 inhibit endogenous TGFβ1 in human and murine fibroblasts

To determine if Ab1 and Ab2 were capable of inhibiting endogenous TGF-β1 secreted by primary cultured fibroblasts of different origin, a quantitative *in vitro* assay was performed in which the activity of secreted TGF-β1 was determined by measuring luciferase levels produced by mink lung epithelial cells that were stably transfected with a nucleic acid comprising a luciferase reporter gene fused to a CAGA12 synthetic promoter, and co-cultured with fibroblasts treated with either Ab1 or Ab2. As shown in FIGs. 11A and 11B, both Ab1 and Ab2 were inhibited endogenous TGF-β1 secreted by normal human dermal fibroblasts, murine C57BL.6J lung fibroblasts, and DBA2/J muscle fibroblasts. Differences in the maximal inhibition observed with each antibody were cell line-specific.

Example 4: Ab2 binds to LRRC33-proTGFβ1

To determine whether Ab1 and Ab2 bind to proTGFβ1 that is complexed with LRRC33, Octet binding assays were performed. As shown in Figures 12A and 12B, both Ab1 and Ab2 are capable of binding to the LRRC33-proTGFβ1 protein complex. However, Ab1 shows a slow on-rate for binding the LRRC33-proTGFβ1 protein complex. Binding of Ab1 and Ab2 to the LRRC33-proTGFβ1 protein complex was further confirmed using ELISA.

Example 5: Ab1 and Ab2 inhibit the activity of both GARP-proTGFβ1 and LRRC33-proTGFβ1

To determine whether Ab1 and Ab2 inhibit the activity of GARP-proTGF-β1 and/or LRRC33-proTGF-β1, an *in vitro* cell-based assay was performed. In this assay system, an engineered human colon cancer cell line (SW480/β6 cells) stably transfected with β6 integrin was co-transfected with a construct to express proTGF-β1 and a construct to express a

presenting molecule (*i.e.,* GARP or LRRC33). To express the presenting molecules, constructs encoding chimeric LRRC33-GARP (SEQ ID NO: 85) or GARP were employed. The transfected cells were incubated to allow for sufficient expression and deposition of the components (integrins and proTGFβ1 complexed with a respective presenting molecule). Activation of TGFβ1 in the presence or absence of Ab1 or Ab2 was assayed using reporter cells (CAGA12 cells) expressing TGFβ receptors coupled to its downstream signal transduction pathway, to measure the inhibitory activity of the antibody. As shown in Figures 13A and 13B, Ab1 and Ab2 inhibited both GARP-proTGF-β1 and LRRC33-proTGF-β1.

An additional cell-based assay was performed to detect inhibition of either GARP-proTGFβ1 complex or LRRC33-proTGFβ1 complex using antibodies Ab1 and Ab2. As shown in FIGs. 22A and FIG. 22B, Ab1 and Ab2 inhibited both GARP-proTGF-β1 and LRRC33-proTGF-β1. In this assay, the IC50 (μg/mL) of Ab1 for the GARP-TGFβ1 complex was 0.445, and the IC50 (μg/mL) of Ab1 for the LRRC33-TGFβ1 complex was 1.325.

Example 6: Effects of Ab2 on Renal Biomarkers and Fibrosis in a Unilateral Ureteral Occluded (UUO) Mouse Model

The unilateral ureteral occluded mouse model has been widely used to study interstitial fibrosis, a common pathological process which may lead to end-stage renal disease (*see* Isaka *et al.* (2008) *Contrib. Nephrol.* 159: 109-21, and Chevalier (1999) *Pediatr. Nephrol.* 13: 612-9). UUO mice are characterized by renal myofibroblast activation, tubular atrophy and interstitial fibrosis with minimal glomerular lesions (*see* Lian *et al.* (2011) *Acta Pharmacol. Sin.* 32: 1513-21). Increased expression of TGFβ1 is considered to play a role in the phenotype observed in UUO mice. To evaluate the effect of Ab2 on the presentation of interstitial fibrosis in the UUO mouse model, the following experiment was performed.

Briefly, 7-8 week old male CD-1 mice (Charles River Laboratories) were 4 groups of mice (n = 10) were administered either Ab2 (3 mg/kg or 30 mg/kg; dosing volume of 10 mL/kg), murine IgG1 control antibody (30 mg/kg; dosing volume of 10 mL/kg), or PBS, as vehicle control intraperitoneally (i.p.) prior to surgical intervention. Treatments were administered one day prior to surgery (d-1), one day after surgery (d1), and 3 days after surgery (d3). On day 0 (d0), mice were anesthetized with isoflurane anesthesia on a nosecone, and a laparotomy performed followed by permanent right unilateral UUO surgery. An additional control group of mice (n = 8) was administered PBS as described above, but solely underwent sham surgery (*i.e.*, laparotomy with no occlusion of the ureter).

Immediately following completion of the surgical procedure, all mice received one subcutaneous injection of 0.001 mg/kg buprenorphine. Mice were sacrificed five days after surgery and tissues were harvested for analysis. After harvest, both kidneys were placed in ice-cold 0.9% NaCl, de-encapsulated and weighed. Hydroxyproline levels to assess collagen content of the kidney tissue were assessed. As shown in FIG. 14, kidney hydroxyproline levels, a marker of tissue fibrosis and collagen deposition, were significantly increased in mice that received surgical intervention as compared to mice receiving sham surgery.

A mid traverse section of each right kidney was immersion fixed in 10% neutral buffered formalin for 48 hours, which was then transferred to 70% ethanol for histological processing and analysis. Fixed kidney sections were paraffin embedded, sectioned (three 5 μm serial sections acquired 200-250 μm apart per animal kidney to enable greater sampling and representation of kidney injury), stained with Picrosirius Red, and subjected to quantitative histological analyses using color spectrum segmentation to determine cortical collagen volume fraction (CVF). One composite CVF score was calculated for each animal by determining the average CVF score for each of the three serial sections. Statistical analyses were performed using the unpaired t-test. As shown in FIG. 16, renal cortical fibrosis, as determined by CVF, was increased in UUO obstructed kidneys as compared to control sham-treated mice. Mice receiving either 3 mg/kg or 30 mg/kg of Ab2 showed an significant attenuation in UUO-induced increases in CVF, as compared to mice receiving either vehicle control (PBS) or IgG control.

Relative mRNA expression levels of plasminogen activator inhibitor-1 (PAI-1), connective tissue growth factor (CTGF), TGFβ1, fibronectin-1, α-smooth muscle actin (α-SMA), monocyte chemotactic protein 1 (MCP-1), collagen type I alpha 1 (Col1a1), and collagen type III alpha 1 chain (Col3a1), in the harvested kidney tissue was determined (FIG. 15A-15H). mRNA levels were normalized using the housekeeping gene hypoxanthine phosphoribosyltransferase 1 (HPRT1) mRNA levels. Moreover, in mice receiving either 3 mg/kg or 30 mg/kg of Ab2 prior to surgical intervention, mRNA levels of PAI-1, CTGF, TGFβ1, fibronectin 1, Col1a1, and Col3a1 were significantly decreased, as compared to mice receiving 30 mg/kg IgG1 control. Mice receiving 3 mg/kg of Ab2 prior to surgical intervention, mRNA levels of α-SMA was significantly decreased, as compared to mice receiving 30 mg/kg IgG1 control. Further, mice receiving 30 mg/kg of Ab2 prior to surgical intervention, mRNA levels of MCP-1 was significantly decreased, as compared to mice receiving 30 mg/kg IgG1 control.

In summary, significant effects were observed in mice treated with Ab2 in the UUO mouse model, with the exception of hydroxyproline levels. As shown in FIGs. 15A-15H and 16, Ab2 treatment significantly attenuated UUO-induced increases in CVF, and significantly decreased gene expression of known fibrosis markers, such as PAI-1, CTGF, TGFβ1, fibronectin 1, Col1a1, and Col3a1. These data demonstrate that TGFβ1 is the major form of TGFβ playing a role in renal disease and that, surprisingly, TGFβ2 and TGFβ3 are likely not involved in pathogenesis.

Example 7: Effects of Ab1 and Ab2 Alone or in Combination with Anti-PD-1 Antibody on Tumor Progression in the MC38 Murine Colon Carcinoma Syngeneic Mouse Model

To evaluate the effects of Ab1 and Ab2, alone or in combination with an anti-PD-1 antibody to decrease colon carcinoma tumor progression, the MC38 murine colon carcinoma C57BL/6 mouse syngeneic model was used.

Tumor Cell Culture

MC38 murine colon carcinoma cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, 100 units/mL penicillin G sodium, 100 µg/mL streptomycin sulfate, 25 µg/mL gentamicin, and 2 mM glutamine. Cell cultures were maintained in tissue culture flasks in a humidified incubator at 37 °C, in an atmosphere of 5% CO2 and 95% air.

In vivo Implantation and Tumor Growth

The MC38 cells used for implantation were harvested during log phase growth and resuspended in phosphate buffered saline (PBS). On the day of tumor implant, each test mouse was injected subcutaneously in the right flank with 5 x 10^5 cells (0.1 mL cell suspension), and tumor growth was monitored as the average size approached the target range of 80 to 120 mm^3. Eleven days later, designated as Day 1 of the study, mice were sorted according to calculated tumor size into groups each consisting of twelve animals with individual tumor volumes ranging from 63 to 196 mm^3 and group mean tumor volumes of 95 to 98 mm^3. Tumors were measured in two dimensions using calipers, and volume was calculated using the formula:

$$\text{Tumor Volume } (mm^3) = \frac{w^2 \times l}{2}$$

where w = width and l = length, in mm, of the tumor. Tumor weight may be estimated with the assumption that 1 mg is equivalent to 1 mm^3 of tumor volume.

Treatment

Briefly eight week old female C57BL/6 mice (n=12) bearing subcutaneous MC38 tumors (63-172 mm^3) on Day 1 were administered intraperitoneally (i.p.) twice a week for four weeks either Ab1, Ab2, murine IgG1 control antibody (each at 30 mg/kg in a dosing volume of 10 mL/kg). When tumors reached 150 mm^3 (Day 6) in the control groups, mice were administered either rat anti-mouse PD-1 antibody (RMP1-14) or rat IgG2A control antibody i.p. twice a week for two weeks (each antibody at 5 mg/kg in a dosing volume of 10 mL/kg).

Group 1 served as tumor growth controls, and received murine IgG1 isotype control antibody in combination with rat IgG2a control antibody. Group 2 received Ab1 in combination with rat IgG2a control antibody. Group 3 received Ab2 in combination with rat IgG2a control antibody. Group 3 received murine IgG1 control antibody in combination with anti-PD-1 antibody. Group 4 received Ab1 in combination with anti-PD-1 antibody. Group 5 received Ab2 in combination with anti-PD-1 antibody. Group 6 (n=16) was not treated and served as a sampling control group.

Endpoint and Tumor Growth Delay (TGD) Analysis

Tumors were measured using calipers twice per week, and each animal was euthanized when its tumor reached the endpoint volume of 1,000 mm^3 or at the end of the study (Day 60), whichever happened earlier. Mice that exited the study for tumor volume endpoint were documented as euthanized for tumor progression (TP), with the date of euthanasia. The time to endpoint (TTE) for analysis was calculated for each mouse using the following equation:

$$TTE = \frac{\log10\ (\text{endpoint volume}) - b}{M}$$

where TTE is expressed in days, endpoint volume is expressed in mm^3, b is the intercept, and m is the slope of the line obtained by linear regression of a log-transformed tumor growth data set. The data set consisted of the first observation that exceeded the endpoint volume used in analysis and the three consecutive observations that immediately preceded the attainment of this endpoint volume. The calculated TTE is usually less than the TP date, the day on which the animal was euthanized for tumor size. Mice with tumors that did not reach the endpoint volume were assigned a TTE value equal to the last day of the study (Day 60). In instances in which the log-transformed calculated TTE preceded the day prior to reaching

endpoint or exceeded the day of reaching tumor volume endpoint, a linear interpolation was performed to approximate the TTE. Mice classified as having died from non-treatment-related (NTR) causes were excluded from TTE calculations (and all further analyses). Animals classified as TR (treatment-related) deaths or NTRm (non- treatment-related death due to metastasis) were assigned a TTE value equal to the day of death.

Treatment outcome was evaluated from tumor growth delay (TGD), defined as the increase in the median time to endpoint (TTE) in a treatment group compared to the control group:

$$TGD = T - C,$$

expressed in days, or as a percentage of the median TTE of the control group:

$$\%TGD = \frac{T - C}{C} \times 100$$

where:

T = median TTE for a treatment group, and

C = median TTE for the designated control group.

MTV and Criteria for Regression Responses

Treatment efficacy may be determined from the tumor volumes of animals remaining in the study on the last day. The MTV (n) was defined as the median tumor volume on the last day of the study in the number of animals remaining (n) whose tumors had not attained the endpoint volume.

Treatment efficacy may also be determined from the incidence and magnitude of regression responses observed during the study. Treatment may cause partial regression (PR) or complete regression (CR) of the tumor in an animal. In a PR response, the tumor volume was 50% or less of its Day 1 volume for three consecutive measurements during the course of the study, and equal to or greater than 13.5 mm^3 for one or more of these three measurements. In a CR response, the tumor volume was less than 13.5 mm^3 for three consecutive measurements during the course of the study. An animal with a CR response at the termination of a study was additionally classified as a tumor-free survivor (TFS). Animals were monitored for regression responses.

Tumor Growth Inhibition

Tumor growth inhibition (TGI) analysis evaluates the difference in median tumor volumes (MTVs) of treated and control mice. For this study, the endpoint for determining TGI was Day 29, which was the day control mice reached the mean tumor volume of 1500 mm^3. The MTV (n), the median tumor volume for the number of animals, n, on the day of

TGI analysis, was determined for each group. Percent tumor growth inhibition (%TGI) was defined as the difference between the MTV of the designated control group and the MTV of the drug-treated group, expressed as a percentage of the MTV of the control group:

$$\%\,TGI = \left(\frac{MTV_{control} - MTV_{drug\text{-}treated}}{MTV_{control}} \right) x\,100 = [1-(MTV_{drug\text{-}treated}/MTV_{control})]\,x\,100$$

The data set for TGI analysis included all mice in a group, except those that died due to treatment-related (TR) or non-treatment-related (NTR) causes prior to the day of TGI analysis.

In the present study, Ab1 and Ab2 were evaluated alone and in combination with anti-PD-1 in the MC38 murine colon carcinoma C57BL/6 mouse syngeneic model. Mice that were administered Ab2 in combination with anti-PD-1 resulted in significant Day 29 TGI ($P < 0.05$, Mann-Whitney U test), producing survival benefit that was statistically significantly different from vehicle-treated controls using logrank survival analyses ($P < 0.05$, logrank) (see FIG. 17). Mice receiving Ab1 or Ab2 in combination with rat IgG2a control antibody had regression responses of 1 CR and 1 PR respectively. In combination with anti-PD-1 the regressions responses of Ab1 and Ab2 were 1 PR and 1 CR, and 4 CRs, respectively. Ab2 in combination with anti-PD-1 produced significant short-term efficacy on Day 29 and produced overall survival benefit in this 60-day TGD study in the MC38 murine colon carcinoma C57BL/6 mouse syngeneic model.

Example 8: Role of TGFβ1 in Muscular Dystrophy

TGFβ plays multiple roles in skeletal muscle function, including inhibition of myogenesis, regulation of inflammation and muscle repair, and promotion of fibrosis. While there is considerable interest in TGFβ inhibition as a therapy for a wide range of diseases, including muscular dystrophies, these therapies inhibit TGFβ1, TGFβ2, and TGFβ3 regardless of molecular context. The lack of specificity/selectivity of these inhibitors may result in unwanted side effects leading to clinical doses with insufficient efficacy. While pan-TGFβ inhibitory molecules have been reported to improve muscle function and reduce fibrosis in the mdx mouse, whether those effects are due to inactivation of TGFβ1, β2, or β3 has yet to be addressed.

To that end, antibodies have been generated that specifically block the integrin-mediated activation of latent TGFβ1, while sparing TGFβ2 and β3. D2.mdx mice are treated with proTGFβ1 specific antibodies, so as to ascertain the role of TGFβ1 specifically in

muscle repair in dystrophic muscle. The functional effects of TGFβ1 inhibition on protection from contraction-induced injury are assessed, as well as on recovery from the same method of injury. Histological evaluation includes whether treatment affects muscle damage, fibrosis, and inflammation. Additionally, possible toxicities may be assessed to determine whether the observed negative effects reported with pan-TGFβ inhibition in muscle (*e.g.*, increased inflammation, long-term deficits in muscle function) are due to inhibition of TGFβ1 or TGFβ2/3. To understand whether inhibition of TGFβ1 in specific molecular contexts is more efficacious and/or has fewer negative effects (adverse effects), the efficacy of LTBP-proTGFβ1 inhibitors in this model may be assessed in order to deconvolute the role of immune cell presented TGFβ1 from that presented in the extracellular matrix (ECM), potentially leading to safer and/or more effective anti-fibrotic therapies.

Dystrophic muscle is highly susceptible to contraction-induced injury. Following injury, muscle from mdx mice shows a significant reduction in force generation and increased uptake of Evan's Blue dye, indicative of physical injury/damage to the muscle fiber, compared to WT (Lovering, R.M., *et al.*, Arch Phys Med Rehabil, 2007. 88(5): p. 617-25). Therapeutic agents which reduce the extent of contraction-induced injury, or improve recovery following injury, would be of significant clinical benefit to muscular dystrophy patients (Bushby, K., *et al.*, Lancet Neurol, 2010. 9(1): p. 77-93). Ab1 and Ab2 will be evaluated for their ability to i) prevent contraction-induced injury, as well as to ii) promote recovery from injury. The D2.mdx strain may be used for our experiments, as opposed to the traditional mdx strain on the B10 background. These mice, generated by crossing the mdx onto a DBA2/J background, have the non-protective variant of LTBP4 described above, and therefore exhibit disease pathology that is more severe, progressive, and similar to the human disease than the standard mdx strain (Coley, W.D., *et al.*, Hum Mol Genet, 2016. 25(1): p. 130-45). Since the D2.mdx mice are being used, DBA2/J mice can serve as wild-type controls. Since DMD affects primarily males, the studies may focus on male mice.

To examine the ability of Ab1 and Ab2 to prevent/limit contraction-induced injury, 6 week old male D2.mdx mice (n=10) are treated with 10 mg/kg/week of either IgG control, Ab1, or Ab2 for 6 weeks. To allow for comparison to published work using a pan-TGFβ inhibitor, a fourth group is dosed with 10mg/kg/week 1D11. All antibodies are mIgG1 isotype and this dose has previously been shown to be effective in the UUO model (FIGs. 15 & 16). A WT group dosed with the IgG control is also included. 24 hours prior to sacrifice, mice are administered 1% Evan's Blue dye (EBD) in PBS (volume 1% of body weight) to allow assessment of myofiber damage by fluorescence microscopy. At the end of treatment,

mice are subjected to an *in vivo* eccentric contraction protocol. Eccentric injury of the gastrocnemius muscle will be may be performed with a 305B muscle lever system (Aurora Scientific) as described (Khairallah, R.J., *et al.*, Sci Signal, 2012. 5(236): p. ra56). Briefly, 20 eccentric contractions with 1-minute pauses in between are performed, and the decrease in peak isometric force before the eccentric phase may be taken as an indication of muscle damage. The extent of force loss and the percent of EBD positive fibers may be determined. DBA2/J mice subjected to this protocol lose 30-40% of initial force after 20 eccentric contractions. In contrast, D2.mdx mice lose 80% of initial force following the same protocol, as previously described (Pratt, S.J., *et al.*, Cell Mol Life Sci, 2015. 72(1): p. 153-64; Khairallah, R.J., *et al.*, Sci Signal, 2012. 5(236): p. ra56). The ability of Ab1 and Ab2 to reduce force loss following injury may be assessed. Mice are sacrificed at the end of the experiment and both the injured and uninjured gastrocnemius muscles may be collected for histological analyses. EBD uptake may be assessed from both muscles. Myofiber cross-sectional area and the extent of fibrosis may be measured. For cross-sectional area determination, sections from the mid-belly of the muscle may be stained with wheat germ agglutinin conjugated to a fluorophore to visualize cell membranes. Sections may be digitized using fluorescent microscopy, cell boundary traced using predictive software and cross-sectional area determined via unbiased automated measurements. For analysis of fibrosis, sections may be stained with picrosirius red (PSR) and the area of PSR+ per slide computed.

The ability of Ab1 and Ab2 to accelerate recovery from contraction-induced injury is assessed. 12 week old DBA2/J and D2.mdx mice may undergo the same eccentric contraction protocol described above. Following injury, mice are divided into treatment groups (n=10) and administered either an IgG control (for WT and D2.mdx mice), 1D11, Ab1, or Ab2 (D2.mdx only). Antibodies may be dosed at 10mg/kg/week for the duration of the experiment. Seven and 14 days post injury, maximal peak isometric force, twitch-to-tetanic ratio, and force-frequency relationship may be measured to evaluate the effect of treatment on recovery from injury. While Ab1 and Ab2 inhibit release of TGFβ1 regardless of presenting molecule, selective release of TGFβ1 from the extracellular matrix (*i.e.*, LTBP-presented) could have greater benefit in DMD due to the preservation of TGFβ1 driven Treg activity. To address this question, specific LTBP-proTGFβ1 inhibitory antibodies may also be assessed for both the ability to prevent contraction-induced injury and to accelerate recovery from injury.

Example 9: Role of TGFβ1 in skeletal muscle regeneration following acute injury.

The role of TGFβ1 specifically in myofiber regeneration following muscle injury may be investigated. TGFβ1-specific antibodies may be employed in the cardiotoxin injury model to determine the role of TGFβ1 specifically during myofiber regeneration. Regeneration may be assessed histologically and functional assessments of muscle strength and quality may be conducted. Given the potential benefits of TGFβ1 inhibition for muscle regeneration, therapies which have beneficial effects without the toxicities observed with pan-TGFβ inhibition would be of great benefit. This allows an investigation of the effects of TGFβ1-specific inhibition on satellite cell function and may provide insights into satellite cell transplant studies.

As described above, TGFβ appears to have multiple effects on muscle biology, including inhibition of myoblast proliferation and differentiation, as well as promotion of atrophy and fibrosis (Allen, R.E. and L.K. Boxhorn, J Cell Physiol, 1987. 133(3): p. 567-72; Brennan, T.J., *et al.*, Proc Natl Acad Sci U S A, 1991. 88(9): p. 3822-6; Massague, J., *et al.*, Proc Natl Acad Sci U S A, 1986. 83(21): p. 8206-10; Olson, E.N., *et al.*, J Cell Biol, 1986. 103(5): p. 1799-805; Li, Y., *et al.*, Am J Pathol, 2004. 164(3): p. 1007-19; Mendias, C.L., *et al.*, Muscle Nerve, 2012. 45(1): p. 55-9; Nelson, C.A., *et al.*, Am J Pathol, 2011. 178(6): p. 2611-21). However, these studies either used recombinant TGFβ1 in culture or injected into mice which may have non-physiological results as the growth factor is removed from its molecular context. Alternatively, investigators used TGFβ inhibitors which are not selective for TGFβ1.

To evaluate isoform-specific effects of TGFβ1, multiple proTGFβ1 antibodies (*e.g.*, Ab1 and Ab2) may be examined for their ability to affect muscle regeneration following CTX-induced injury. These antibodies are "isoform-specific" and "context-permissive" inhibitors of TGFβ1 activation, such that they specifically inhibit release of TGFβ1 (as opposed to TGFβ2 or TGFβ3) from any presenting molecule and do not bind the mature growth factors (FIG. 18A).

Muscle regeneration may be induced in male DBA2/J mice (n=10) via CTX injection into the right gastrocnemius muscle. One day prior to injury, mice may be administered 10mg/kg IgG control, 1D11, Ab1, or Ab2. Antibodies are continued to be dosed weekly until end of study. At 7 and 14 days post injury, muscle force measurements may be measured *in vivo* with a 305C muscle lever system (Aurora Scientific Inc., Aurora, CAN). Briefly, for the plantarflexor muscle group, contractions are elicited by percutaneous electrical stimulation of the sciatic nerve in anaesthetized mice, and a series of stimulations is then performed at

increasing frequency of stimulation (0.2 ms pulse, 500 ms train duration): 1, 10, 20, 40, 60, 80, 100, 150 Hz, followed by a final stimulation at 1 Hz. Maximal peak isometric force, twitch-to-tetanic ratio, and force-frequency relationship will be determined. Following force measurements, the injured gastrocnemius and soleus muscles are collected and prepared for histology. Myofiber cross sectional area and %PSR+ area may be determined as described in Example 8 above.

Treatment with Ab1 and Ab2 may result in reduced fibrosis and improved muscle function. However, given the role of TGFβ1 in regulating immune activation, it is possible that we may observe increased inflammation with the antibodies, as has been reported with 1D11 treatment (Andreetta, F., *et al.*, J Neuroimmunol, 2006. 175(1-2): p. 77-86). In the event increased inflammation may limit the therapeutic effects of TGFβ1 inhibition, context-specific antibodies may be subsequently evaluated to provide further degree of specificity, which may limit toxicity. For example, antibodies that inhibit release of TGFβ1 from LTBPs only may be used, using the readouts and methods described above. These antibodies may limit release of TGFβ1 only from the ECM, without affecting release from Tregs or macrophages.

Example 10: Selection of Suitable TGFβ1 inhibitory agents.

Expression analysis of proTGFβ1 and its presenting molecules in healthy, regenerating, and diseased muscle may provide useful information to aid the selection of optimal therapeutic approach. Given the potential benefits of TGFβ1 inhibition in muscle regeneration and repair, understanding the context of proTGFβ1 presentation (*e.g.*, in the ECM or on immune cells) in skeletal muscle under different conditions (healthy, acutely injured, and chronically injured) can help inform the therapeutic utility of antibodies, and ultimately provide insight into the degree of specificity/selectivity required to achieve both clinical efficacy and safety. The nature of TGFβ1 presentation may vary depending on the health status of the muscle and over the course of disease, which could have implications for any TGFβ1 targeted therapies. Understanding the expression profiles of these molecules will also aid in selection of appropriate time of dosing for potential therapeutic molecules. Using western blot, immunohistochemistry, and immunoprecipitation, expression of proTGFβ1 and its presenting molecules may be assessed in normal, acutely injured (cardiotoxin injury), and chronically regenerating (D2.mdx mouse) muscle. Expression of these molecules may be investigated specifically in key cell types or subset of cell types (*e.g.*, satellite cells, macrophages, fibro-adipogenic progenitors, etc.) in the different conditions described above.

While expression of TGFβ isoforms has been examined in muscles from mdx mice, previous work focused on expression of the *mature* growth factors (Nelson, C.A., *et al.*, Am J Pathol, 2011. 178(6): p. 2611-21; Zhou, L., *et al.*, Neuromuscul Disord, 2006. 16(1): p. 32-8). Given the target specificity of the TGFβ1 antibodies described herein, it is essential that the expression patterns be examined not only for mature and proTGFβ1, but those of the presenting molecules as well, which should provide information as to the source and/or context of a pool of TGFβ1 of interest. Ideally, it is desirable to gain understanding of the expression patterns of the latent complexes, not merely of each component.

Antibodies are screened for western blot and IHC for targets of interest. Antibodies against mouse TGFβ1-LAP, LTBP1, LTBP3, and LTBP4 are commercially available. The antibody against TGFβ1-LAP (clone TW7-16B4) has been extensively characterized and is effective in both flow cytometry and western blot (Oida, T. and H.L. Weiner, PLoS One, 2010. 5(11): p. e15523). Antibodies against LTBP1 (ProteinTech # 22065-1-AP) and LTBP3 (Millipore #ABT316) have been validated internally using SW480 cells transfected with LTBP1-proTGFβ1 or LTBP3-proTGFβ1 and shown to be specific for their targets. The utility of these antibodies for IHC may be determined. Muscles from healthy and D2.mdx mice are sectioned and the antibodies tested on frozen and FFPE sections. Antibodies may be validated by including conditions with 100x excess of purified target protein or complex (made in house, see FIGs. 18B for example) to ensure that the signal observed is specific.

Previous work has identified antibodies which specifically bind a given latent complex but have no inhibitory activity. Antigen binding by these antibodies has been confirmed by ELISA (FIGs. 18B & 18C) and may also be evaluated for their utility in IHC (given the three-dimensional structure of these epitopes these antibodies are unlikely to be effective as western blot reagents). The presence of latent TGFβ1 complexes from bulk tissue may also be assessed by western blot or immunoprecipitation. Latent complexes can be identified by western blot by running the same sample under reducing and non-reducing conditions. Under reducing conditions, TGFβ1, LAP and the presenting molecule separate, and the three molecules can be identified on the same blot but using dual-color western blot methods. Under non-reducing conditions, the LAP:presenting molecule complex remains associated while TGFβ1 is released; the complex migrates slower than the empty presenting molecule and migrates together with TGFβ1-LAP (FIG. 18B). Various antibodies are also evaluated for their ability to immunoprecipitate latent complexes from muscle to demonstrate direct binding of TGFβ1 to specific presenting molecules.

Once appropriate antibodies have been identified, expression in healthy, regenerating, and dystrophic muscle is assessed, by western and/or IHC, depending on the antibodies available. Tibialis anterior (TA) and diaphragm muscles may be collected from DBA2/J and D2.mdx mice at 4, 8, and 12 weeks of age. For regenerating muscle, cardiotoxin may be injected into the TA of 12 week old DBA2/J mice, and muscles collected 3, 7, and 14 days post injury. Tissue from at least 4 mice may be used for each condition/time point. Co-staining experiments may also be conducted to identify cell populations expressing the various molecules (for example: CD11b for macrophages, FoxP3 for Tregs, MyoD for myogenic cells).

Example 11: Ab2 exhibits reduced toxicity as compared to the ALK5 kinase inhibitor LY2109761 and a Pan-TGFβ Antibody

To evaluate the toxicity of Ab2, as compared to the small molecule TGF-β type I receptor (ALK5) kinase inhibitor LY2109761 and to a pan-TGFβ antibody (hIgG4), toxicity studies were performed in rats. Briefly, female F344/NHsd rats were administered either Ab2 at 3 mg/kg (1 group, n=5), at 30 mg/kg (1 group, n=5), or at 100 mg/kg (1 group, n=5); a pan-TGFβ antibody at 3 mg/kg (1 group, n=5), at 30 mg/kg (1 group, n=5), or at 100 mg/kg (1 group, n=5); LY2109761 at 200 mg/kg (1 group, n=5) or 300 mg/kg (1 group, n=5); or PBS (pH 7.4) vehicle control (1 group, n=5). Animals receiving either Ab2, the pan-TGFβ antibody, or the vehicle control were dosed once intravenously (at day 1), and the rats receiving LY2109761 were dosed by oral gavage once daily during 7 days (7 doses). Animal body weight was determined at days 1, 3, and 7 of the dosing phase. Animals were sacrificed at day 8 and necropsies performed.

As shown in the survival data shown FIG. 19, Ab2 exhibited reduced toxicity as compared to the other treatment groups. All animals administered 300mg/kg of the ALK5 kinase inhibitor LY2109761 were sacrificed in a moribund condition or found dead on days 3, 6, or 7 of the study. Two of the animals administered 200 mg/kg of LY2109761 were found dead at day 7 of the study. One animal administered 100 mg/kg of the pan-TGFβ antibody was found dead at day 6 of the study. All animals administered up to 100 mg/kg of Ab 2 survived until terminal sacrifice.

Further, the toxicity of the treatments was assessed by monitoring the body weights of the animals during the dosing phase. As shown in FIGs. 20 and 21A-21C, animals receiving LY2109761 at either 200 mg/kg or 300 mg/kg exhibited decreased body weight during the course of the study.

Animal organ weight was also assessed post-mortem. As shown in Table 11, Increased heart weights were observed in animals administered ≥ 200 mg/kg of LY2109761. Increased heart weights were also observed in animals administered ≥ 30 mg/kg of the pan-TGFβ antibody. No effects on organ weight were observed in animals administered upto 100 mg/kg of Ab2.

Table 11. Organ Weight Changes in Treatment Groups

	Treatment Group					
	Vehicle Control[a]	**LY2109761**		**Pan-TGFβ Antibody**		
Dose Level (mg/kg/day)	0	200	300	3	30	100
Heart						
Absolute Weight (g)	0.4084	112	NE	99	123	119
Body Weight Ratio (%)	0.3952	132	NE	96	122	122
Brain Weight Ratio (%)	26.3420	113	NE	98	123	116

NE = not evaluated due to early mortality.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for each treatment groups expressed as percentage control mean value.
[a] Vehicle control = phosphate buffered saline (PBS), pH 7.4.

While no macroscopic findings were observed in animals administered up to 100 mg/kg of Ab2 or of the pan-TGFβ antibody, abnormally-shaped sternum was observed in four animals of each treatment group receiving either 200 mg/kg or 300 mg/kg of LY2109761. 2.5 mL of clear fluid in the thoracic cavity and an enlarged thymus due to excess fluid (*i.e.*, edema) was observed in one animal administered 300 mg/kg of LY2109761, which was found dead on Day 3 of the study.

As shown in Table 12, at the microscopic level, animals administered ≥ 200 mg/kg of LY2109761 exhibited heart valve findings (*i.e.*, valvulopathy). Valvulopathy was characterized by heart valve thickening due to hemorrhage, endothelial hyperplasia, mixed inflammatory cell infiltrates, and/or stromal hyperplasia (*see* FIG. 23, upper right panel). Most animals had multiple valves affected. Additionally, atrium findings were observed including minimal to slight mixed inflammatory cell infiltrates, minimal hemorrhage, and/or minimal endothelium (endocardium) hyperplasia resulting in increased basophilic staining of the atrium in hematoxylin and eosin-stained sections. Myocardium findings were also observed mostly in the base of the heart and consisted of minimal to slight degeneration/necrosis, slight hemorrhage, and/or slight mixed inflammatory cell infiltrates. One animal administered 300 mg/kg of LY2109761 had slight necrosis with inflammation of

a coronary artery. Further, two animals administered 200 mg/kg of LY2109761 had minimal mixed inflammatory cell infiltrates or hemorrhage in the aortic root.

Table 12. Microscopic Heart Findings in Animals Receiving LY2109761

		LY2109761		
Dose Level (mg/kg/day)		0	200	300
Heart				
Heart valves				
Valvulopathy				
	Minimal	0	1	2
	Slight	0	3	3
	Moderate	0	1	0
Atrium				
Infiltrate, mixed cell				
	Minimal	0	2	3
	Slight	0	0	1
Hyperplasia, endothelium				
	Minimal	0	1	3
Hemorrhage				
	Minimal	0	1	2
Myocardium				
Degeneration/necrosis				
	Minimal	0	0	1
	Slight	0	1	1
Hemorrhage				
	Slight	0	1	0
Infiltrate, mixed cell				
	Slight	0	0	1
Coronary artery				
Necrosis with inflammation				
	Slight	0	0	1
Aortic root				
Hemorrhage				
	Minimal	0	1	0
Infiltrate, mixed cell				
	Minimal	0	1	0

As shown in Table 13, animals administered ≥3 mg/kg of the pan-TGFβ antibody exhibited heart valve findings (*i.e.*, valvulopathy) similar to those described in the animals administered LY2109761, as described above (*see* also FIG. 23, lower left panel). Animals administered ≥30 mg/kg of the pan-TGFβ antibody exhibited atrium findings similar to those described in animals administered LY2109761. Animals administered 100 mg/kg of the pan-TGFβ antibody exhibited myocardium findings similar to those described in animals administered LY2109761, and animals administered 30 mg/kg of pan-TGFβ antibody had hemorrhage in the myocardium. One animal administered 100 mg/kg of the pan-TGFβ antibody had moderate intramural necrosis with hemorrhage in a coronary artery, which was associated with slight perivascular mixed inflammatory cell infiltrates.

Table 13. Microscopic Heart Findings in Animals Receiving the Pan-TGFβ Antibody

	Pan-TGFβ Antibody			
Dose Level (mg/kg/day)	0	3	30	100
Heart				
Heart valves				
Valvulopathy				
Minimal	0	2	0	0
Slight	0	2	4	5
Moderate	0	0	1	0
Atrium				
Infiltrate, mixed cell				
Minimal	0	0	1	2
Slight	0	0	1	1
Hyperplasia, endothelium				
Minimal	0	0	3	1
Hemorrhage				
Minimal	0	0	1	0
Myocardium				
Degeneration/necrosis				
Slight	0	0	0	2
Hemorrhage				
Minimal	0	0	2	1
Slight	0	0	1	1
Infiltrate, mixed cell, base				
Slight	0	0	0	1
Coronary artery				
Necrosis with hemorrhage				
Moderate	0	0	0	1
Infiltrate, mixed cell, perivascular				
Slight	0	0	0	1

In contrast, a single animal in the treatment group administered 100 mg/kg of Ab2 had minimal mixed inflammatory cell infiltrates in a single heart valve leaflet of left atrioventricular valve (*see* FIG. 23, lower right panel), which was consistent with a background finding due to the single incidence and lack of concurrent valve findings. Thus, treatment with Ab2 surprisingly resulted in reduced mortality and reduced cardiotoxicity as compared to treatment with either the ALK5 kinase inhibitor LY2109761 or to treatment with the pan-TGFβ antibody.

CLAIMS

What is claimed is:

1. An isolated antibody, or antigen binding portion thereof, that specifically binds to an epitope of TGFβ1, wherein the epitope is available for binding by the antibody when the TGFβ1 is present in two or more of the following protein complexes:

a GARP-TGFβ1 complex,

a LTBP1-TGFβ1 complex,

a LTBP3-TGFβ1 complex, and

a LRRC33-TGFβ1 complex; and

wherein the antibody does not bind free mature TGFβ1.

2. The antibody, or antigen binding portion thereof, of claim 1, wherein the TGFβ1 is latent TGFβ1.

3. The antibody, or antigen binding portion thereof, of claim 1, wherein the TGFβ1 is proTGFβ1.

4. The antibody, or antigen binding portion thereof, of any one of claims 1-3, wherein the antibody does not bind to TGFβ2.

5. The antibody, or antigen binding portion thereof, of any one of claims 1-4, wherein the antibody, or antigen binding portion thereof, does not bind to TGFβ3.

6. The antibody, or antigen binding portion thereof, of any one of claims 1-5, wherein the antibody, or antigen binding portion thereof, does not prevent the ability of TGFβ1 to bind to integrin.

7. The antibody, or antigen binding portion thereof, of any one of claims 1-6, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a complementarity determining region 3 (CDR3) having the amino acid sequence of SEQ ID NO: 5 and a light chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 11.

8. The antibody, or antigen binding portion thereof, of any one of claims 1-7, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a complementarity determining region 2 (CDR2) having the amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 9.

9. The antibody, or antigen binding portion thereof, of any one of claims 1-8, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a complementarity determining region 1 (CDR1) having the amino acid sequence of SEQ ID NO: 1 and a light chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 7.

10. The antibody, or antigen binding portion thereof, of any one of claims 1-9, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 13 and a light chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 14.

11. The antibody, or antigen binding portion thereof, of any one of claims 1-10, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 13 and a light chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 14.

12. The antibody, or antigen binding portion thereof, of any one of claims 1-6, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 6 and a light chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 12.

13. The antibody, or antigen binding portion thereof, of any one of claims 1-6 and 12, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 4 and a light

chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 10.

14. The antibody, or antigen binding portion thereof, of any one of claims 1-6, 12 and 13, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 2 and a light chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 8.

15. The antibody, or antigen binding portion thereof, of any one of claims 1-6, and 12-14, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 15 and a light chain variable domain comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence set forth in SEQ ID NO: 16.

16. The antibody, or antigen binding portion thereof, of any one of claims 1-6, and 12-15, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 15 and a light chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 16.

17. The antibody, or antigen binding portion thereof, or any one of claims 1-16, wherein the antibody, or antigen binding portion thereof, inhibits TGFβ1 activation.

18. The antibody, or antigen binding portion thereof, of any one of claims 1-17, wherein the antibody, or antigen binding portion thereof, inhibits the release of mature TGFβ1 from the GARP-TGFβ1 complex, the LTBP1-TGFβ1 complex, the LTBP3-TGFβ1 complex, and/or the LRRC33-TGFβ1 complex.

19. The antibody, or antigen binding portion thereof, of any one of claims 1-18, wherein the antibody, or antigen binding portion thereof, has a dissociation constant (K_D) to the epitope of TGFβ1 selected from the group consisting of: at least about 10^{-8} M; at least about 10^{-9} M; at least about 10^{-10} M; at least about 10^{-11} M; at least about 10^{-12} M; and at least about 10^{-13} M.

20. The antibody, or antigen binding portion thereof, of any one of claims 1-19, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain immunoglobulin constant domain of a human IgG_1 constant domain or a human IgG_4 constant domain.

21. The antibody, or antigen binding portion thereof, of any one of claims 1-20, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain immunoglobulin constant domain of a human IgG_4 constant domain.

22. The antibody, or antigen binding portion thereof, of claim 21, wherein the antibody, or antigen binding portion thereof, comprises a heavy chain immunoglobulin constant domain of a human IgG_4 constant domain having a backbone substitution of Ser to Pro that produces an IgG_1-like hinge and permits formation of inter-chain disulfide bonds.

23. The antibody, or antigen binding portion thereof, of any one of any one of claims 1-22, wherein the antibody or antigen binding portion thereof, further comprises a light chain immunoglobulin constant domain comprising a human Ig lambda constant domain or a human Ig kappa constant domain.

24. The antibody of any one of claims 1-23, wherein the antibody is an IgG having four polypeptide chains which are two heavy chains and two light chains.

25. The antibody of any one of claims 1-24, wherein the antibody is a humanized antibody, a diabody, or a chimeric antibody.

26. The antibody of any one of claims 1-25, wherein the antibody is a humanized antibody.

27. The antibody of any one of claims 1-26, wherein the antibody is a human antibody.

28. The antibody of any one of claims 1-27, wherein the antibody comprises a framework having a human germline amino acid sequence.

29. The antigen binding portion of any one claims 1-28, wherein the antigen binding portion is a Fab fragment, a F(ab')2 fragment, a scFab fragment, or an scFv fragment.

30. An anti-TGFβ1 antibody, or antigen binding portion thereof, that competes for binding with the antibody, or antigen binding portion thereof, or any one of claims 1-29.

31. An anti-TGFβ1 antibody, or antigen binding portion thereof, that binds to the same epitope as the antibody, or antigen binding portion thereof, or any one of claims 1-29.

32. A pharmaceutical composition comprising the antibody, or antigen binding portion thereof, of any one of claims 1-31, and a pharmaceutically acceptable carrier.

33. A method for inhibiting TGFβ1 activation, the method comprising exposing a GARP-TGFβ1 complex, a LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, or a LRRC33-TGFβ1 complex to the antibody, or an antigen binding portion thereof, of any one of claims 1-31, or the pharmaceutical composition of claim 32.

34. The method of claim 33, wherein the antibody, or antigen binding portion thereof, inhibits the release of mature TGFβ1 from the GARP-TGFβ1 complex, the LTBP1-TGFβ1 complex, a LTBP3-TGFβ1 complex, and/or the LRRC33-TGFβ1 complex.

35. The method of claim 33 or claim 34, wherein the method is performed *in vitro*.

36. The method of claim 33 or claim 34, wherein the method is performed *in vivo*.

37. The method of any one of claims 33-34, wherein the GARP-TGFβ1 complex or the LRRC33-TGFβ1 complex is present at the outer surface of a cell.

38. The method of claim 37, wherein the cell is a T-cell, a fibroblast, a macrophage, a monocyte, a dendritic cell, an antigen presenting cell, or a microglia.

39. The method of any one of claims 33-36, wherein the LTBP1-TGFβ1 complex or the LTBP3-TGFβ1 complex is bound to an extracellular matrix.

40. The method of claim 39, wherein the extracellular matrix comprises fibrillin.

41. The method of claim 39 or claim 40, wherein the extracellular matrix comprises a protein comprising an RGD motif.

42. A method for reducing TGFβ1 activation in a subject, the method comprising administering to the subject an effective amount of the antibody, or the antigen binding portion thereof, of any one of claims 1-31, or the pharmaceutical composition of claim 32, thereby reducing TGFβ1 activation in the subject.

43. The method of claim 42, wherein the subject has or is at risk of having a condition selected from the group consisting of fibrosis, muscular dystrophy, cancer, dementia, and myelofibrosis.

44. The method of claim 43, wherein the subject has or is at risk of having liver fibrosis , kidney fibrosis, or lung fibrosis (*e.g.*, idiopathic pulmonary fibrosis).

45. The method of claim 44, wherein the subject further receives a therapy comprising a myostatin inhibitor, a VEGF agonist, an IGF1 agonist, an FXR agonist, a CCR2 inhibitor, a CCR5 inhibitor, a dual CCR2/CCR5 inhibitor, a lysyl oxidase-like-2 inhibitor, an ASK1 inhibitor, an Acetyl-CoA Carboxylase (ACC) inhibitor, a p38 kinase inhibitor, Pirfenidone, Nintedanib, a GDF11 inhibitor, or any combination thereof.

46. The method of any one of claims 42-45, wherein the antibody, or the antigen binding portion thereof, reduces the suppressive activity of regulatory T cells.

47. The method of any one of claims 33-46, wherein the antibody, or the antigen binding portion thereof, does not induce organ toxicity in the subject.

48. The method of claim 47, wherein the organ toxicity comprises cardiovascular toxicity, gastrointestinal toxicity, immunotoxicity, bone toxicity, cartilage toxicity, reproductive system toxicity, or renal toxicity.

49. A method for treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of the antibody, or the antigen binding portion thereof, of any one of claims 1-31, or the pharmaceutical composition of claim 32, thereby treating cancer in the subject.

50. The method of claim 49, wherein the antibody, or antigen binding portion thereof, is administered in combination with an additional agent or an additional therapy.

51. The method of claim 49, wherein the additional agent is a checkpoint inhibitor.

52. The method of claim 49, wherein the additional agent is selected from the group consisting of a PD-1 antagonist, a PDL1 antagonist, a PD-L1 or PDL2 fusion protein, a CTLA4 antagonist, a GITR agonist, an anti-ICOS antibody, an anti-ICOSL antibody, an anti-B7H3 antibody, an anti-B7H4 antibody, an anti-TIM3 antibody, an anti-LAG3 antibody, an anti-OX40 antibody, an anti-CD27 antibody, an anti-CD70 antibody, an anti-CD47 antibody, an anti-41BB antibody, an anti-PD-1 antibody, an oncolytic virus, and a PARP inhibitor.

53. The method of claim 49, wherein the additional therapy is radiation, a chemotherapeutic agent, or a combination thereof.

54. A nucleic acid encoding the antibody, or the antigen binding portion thereof, of any one of claims 1-31.

55. A kit comprising the antibody, or the antigen binding portion thereof, of any one of claims 1-31, or the pharmaceutical composition of claim 32, and instructions for use thereof.

56. A method for treating a myofiber damage, the method comprising a step of:

administering to a subject having a myofiber damage an agent that selectively inhibits TGFβ1 over TGFβ2/3 in an amount effective to

i) promote myofiber repair;

ii) protect from contraction-induced injury;

iii) reduce inflammation in muscle; and/or,

iv) reduce fibrosis in muscle.

57. The method of claim 56, wherein the amount does not cause unacceptable level of adverse effects in the subject.

58. The method of claim 56 or 57, wherein the myofiber damage is
i) associated with a muscular dystrophy; or,
ii) associated with an acute muscle injury.

59. The method of any one of claims 56-58, wherein the agent blocks activation of TGFβ1 but not TGFβ2 or TGFβ3.

60. The method of any one of claim 56-59, wherein the agent is a monoclonal antibody.

61. The method of claim 60, wherein the monoclonal antibody binds a GARP-proTGFβ1 latent complex, an LRRC33-proTGFβ1 latent complex, an LTBP1-proTGFβ1 latent complex, an LTBP2-proTGFβ1 latent complex, an LTBP3-proTGFβ1 latent complex, and/or an LTBP4-proTGFβ1 latent complex.

62. The method of any one of claim 56-61, wherein the subject further receives a myostatin inhibitor.

63. The method of any one of claims 56-62, further comprising a step of:
identifying a source or context of disease-associated TGFβ1.

64. A method for producing a pharmaceutical composition that modulates TGFβ signaling, the method comprising steps of:
providing one or more agents that modulate signaling of at least one isoform of TGFβ;
measuring activities of the one or more agents towards all isoforms of TGFβ;
selecting an agent that is specific to a single isoform of TGFβ;
formulating into a pharmaceutical composition comprising an isoform-specific TGFβ modulator and a pharmaceutically acceptable excipient.

65. The method of claim 64, wherein the isoform-specific TGFβ modulator is a TGFβ1-specific modulator.

66. The method of claim 65, wherein the TGFβ1-specific modulator is an inhibitor of TGFβ1.

67. The method of claim 64, wherein the isoform-specific TGFβ modulator is an antibody or a fragment thereof.

68. The method of claim 67, wherein the antibody or fragment thereof specifically binds a pro/latent complex of TGFβ1.

69. The method of claim 68, wherein the antibody or fragment thereof does not bind free mature TGFβ1 which is not in the pro/latent complex.

70. The method of claim 68, wherein the pro/latent complex comprises GARP, LRRC33, LTBP1, LTBP2, LTBP3 or LTBP4.

71. A pharmaceutical composition produced by the method of claim 64.

72. A method for treating a disease associated with TGFβ signaling, the method comprising a step of:

administering to a subject in need thereof the pharmaceutical composition of claim 71 in an amount effective to treat the disease, wherein the amount achieves statistically significant clinical efficacy and safety when administered to a patient population having the disease.

73. A TGFβ inhibitor for use in reducing adverse effects in a subject, wherein the TGFβ inhibitor is isoform-selective.

74. The use of claim 73, wherein the TGFβ inhibitor is an antibody that specifically inhibits TGFβ1.

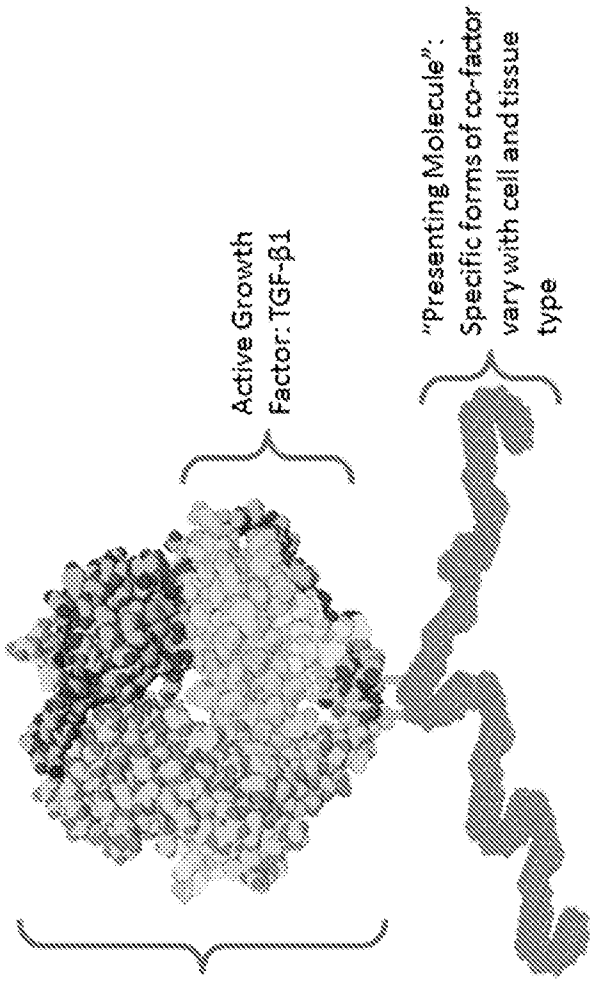

The "Cage": TGF-β1 Pro-peptide
Active Growth Factor: TGF-β1
"Presenting Molecule": Specific forms of co-factor vary with cell and tissue type

FIG. 1

FIG. 2A

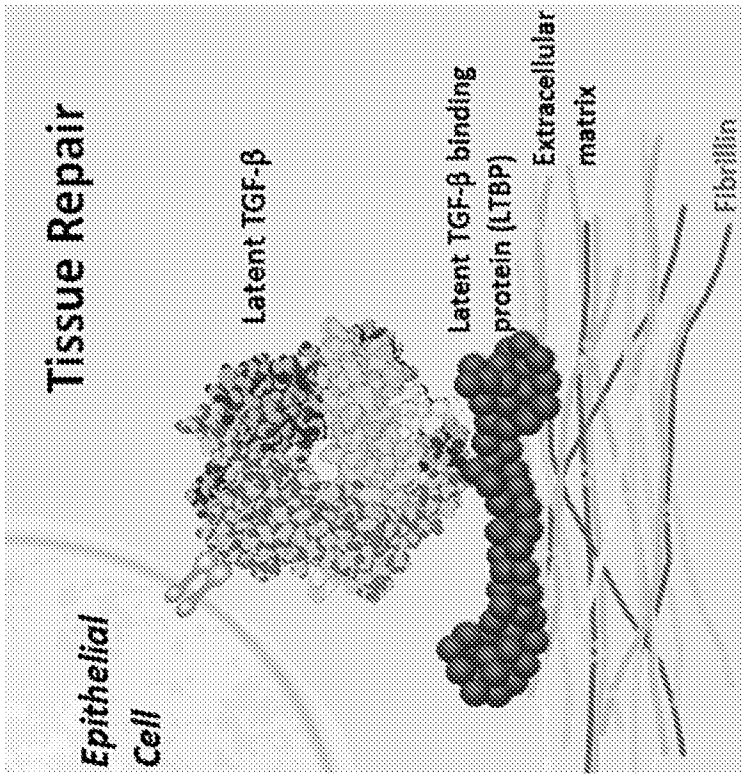

Tissue Repair
Epithelial Cell
Latent TGF-β
Latent TGF-β binding protein (LTBP)
Extracellular matrix
Fibrillin

FIG. 2B

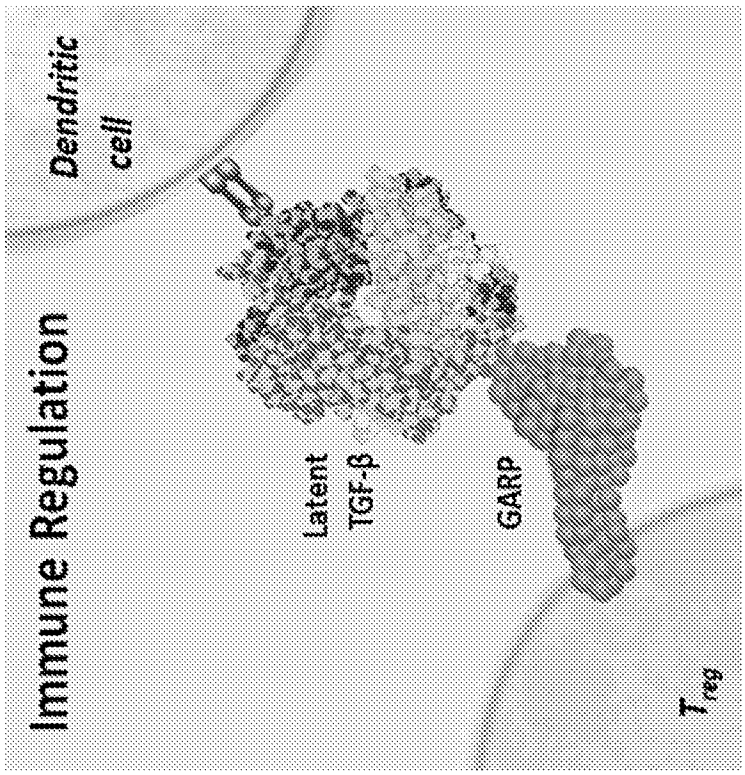

Immune Regulation
Dendritic cell
Latent TGF-β
GARP
T_{reg}

FIG. 3

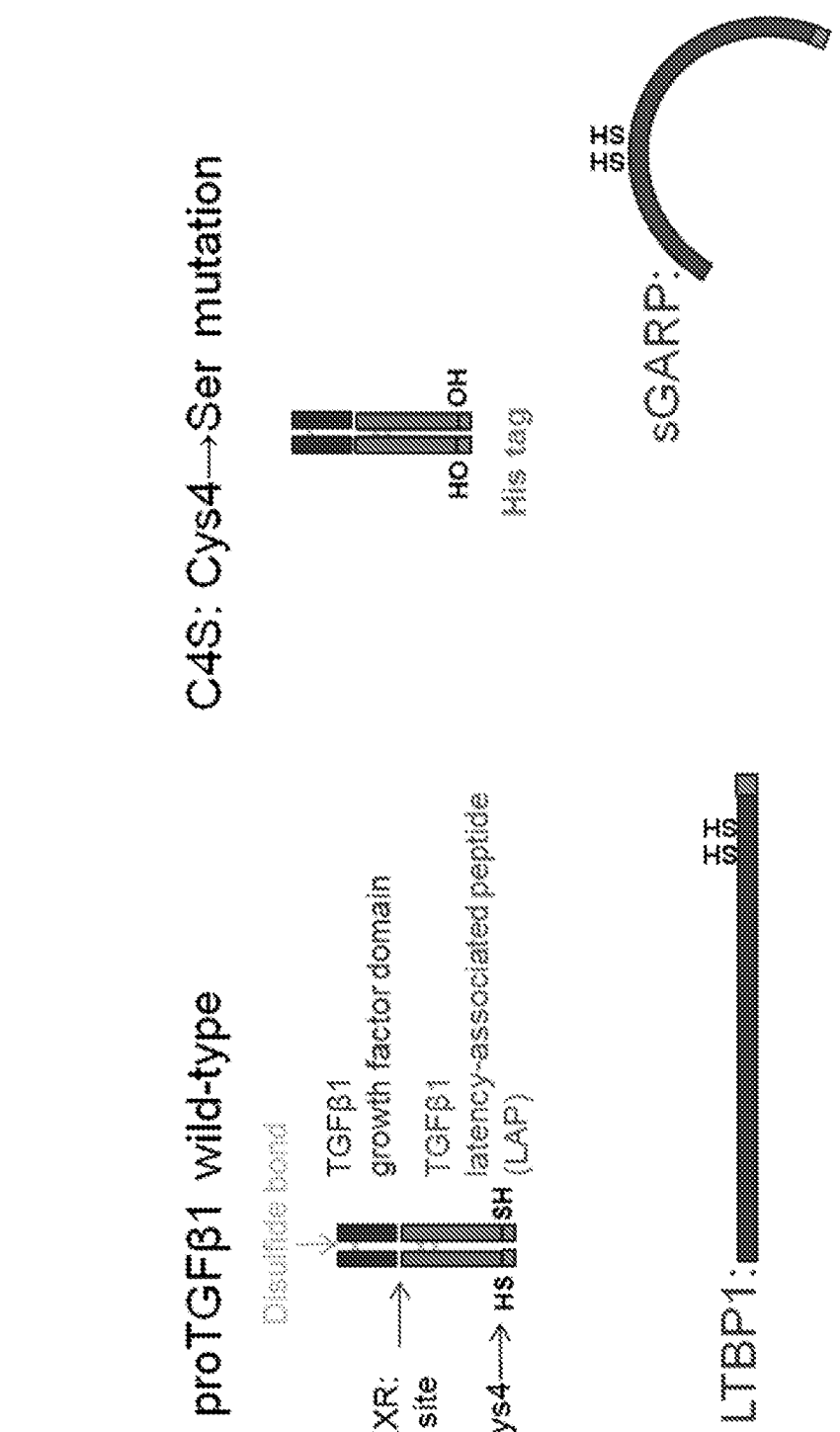
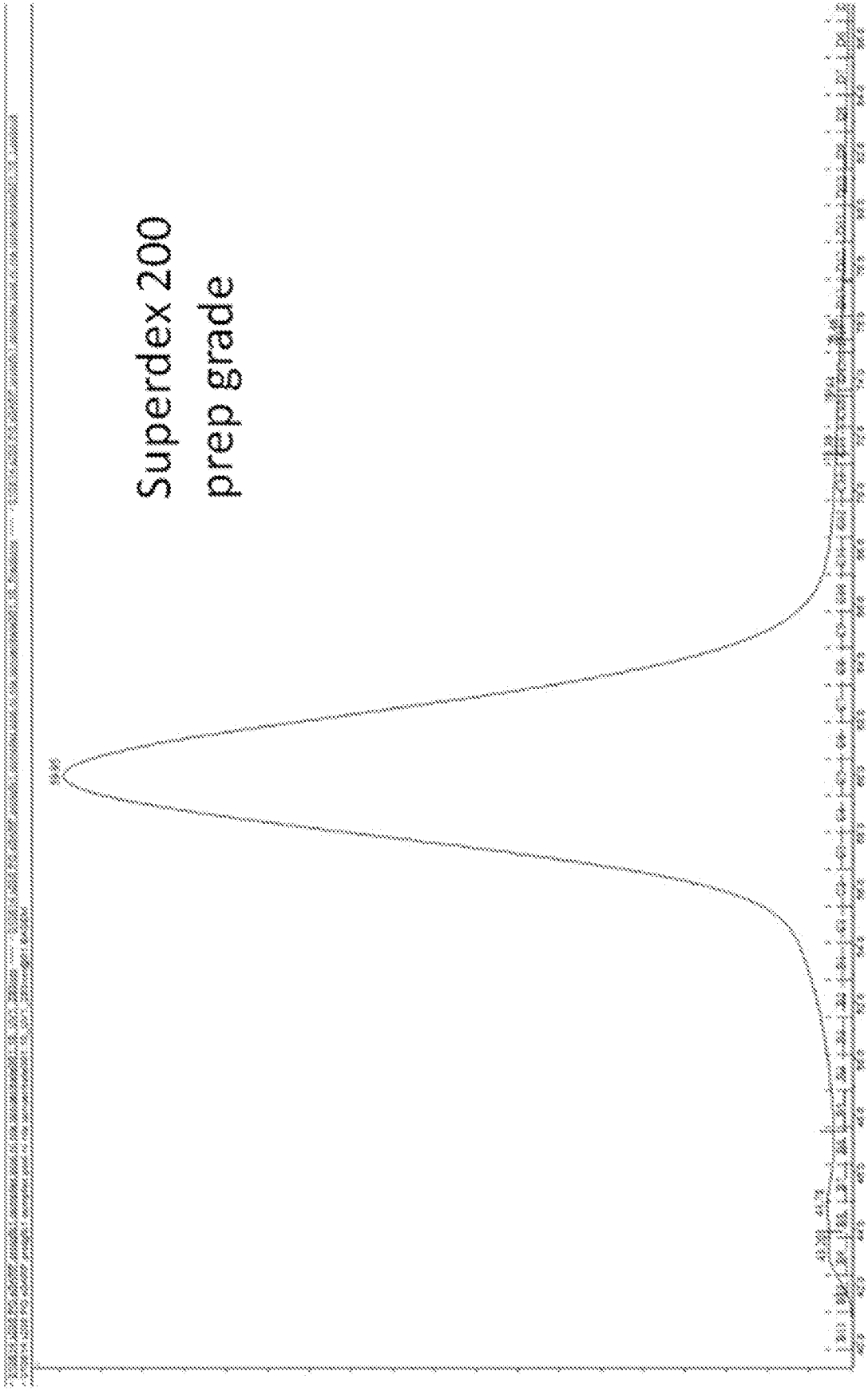

proTGFβ1 wild-type
C4S: Cys4→Ser mutation
Disulfide bond
TGFβ1
growth factor domain
TGFβ1
latency-associated peptide
(LAP)
RXXR:
furin cleavage site
Cys4→
HS
SH
HO
OH
His tag
sGARP:
HS
HS
LTBP1:
HS
HS

FIG. 4A



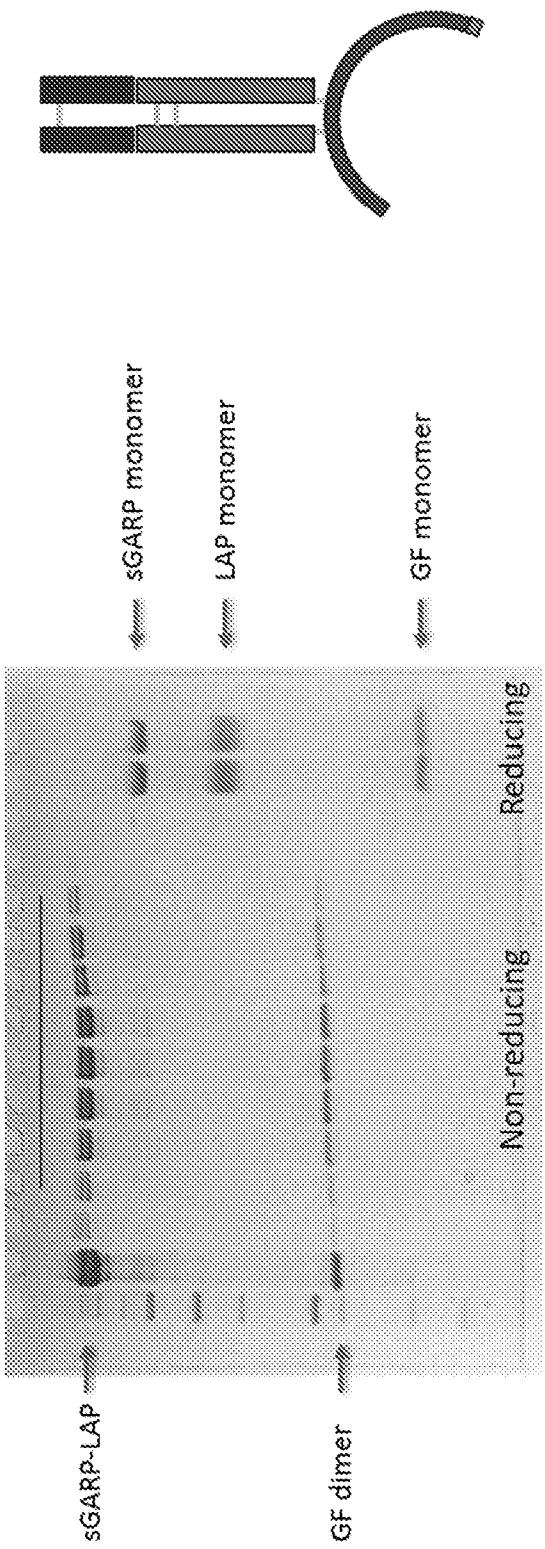

sGARP monomer
LAP monomer
GF monomer
sGARP-LAP
GF dimer
Non-reducing
Reducing

FIG. 4B

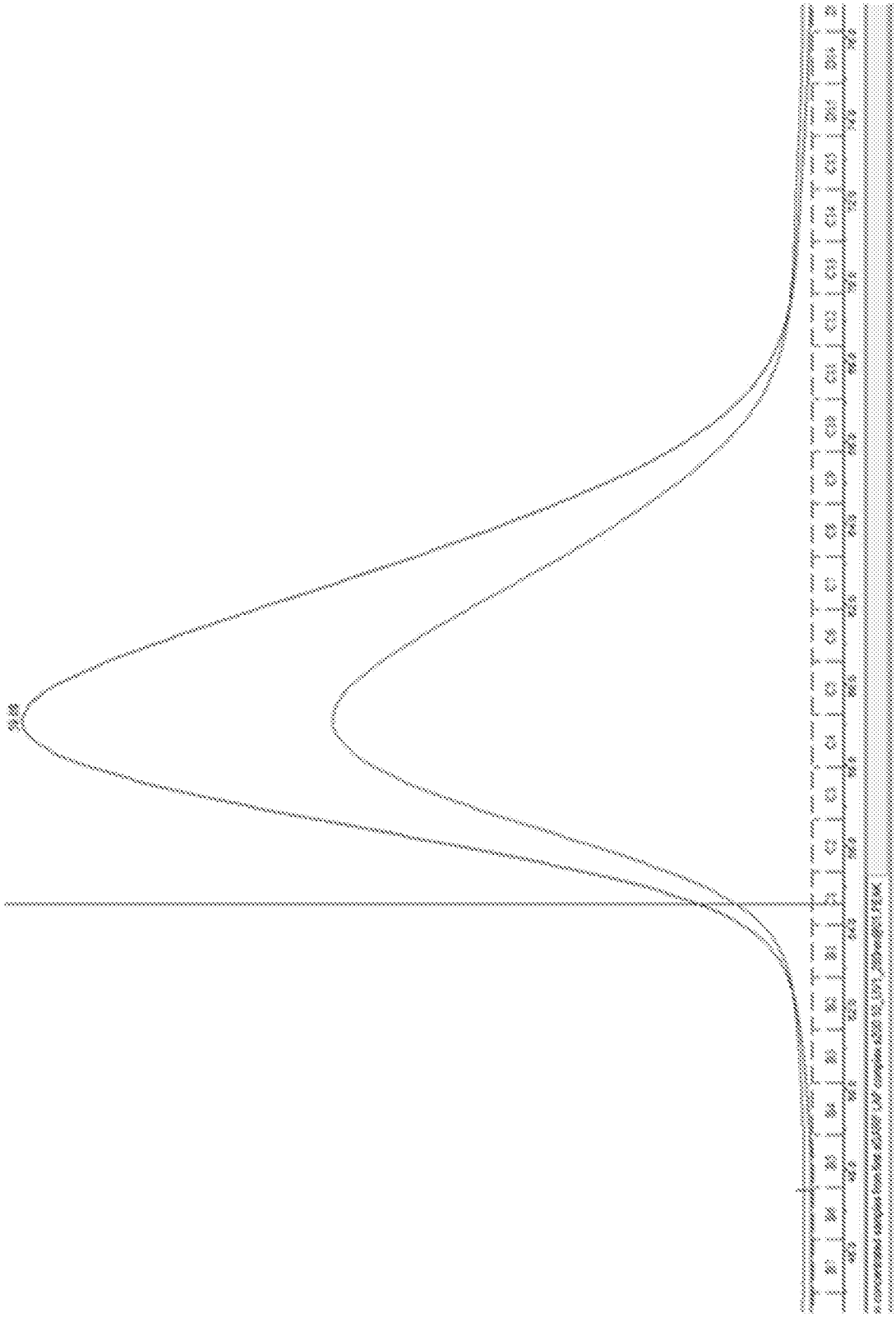


FIG. 5A

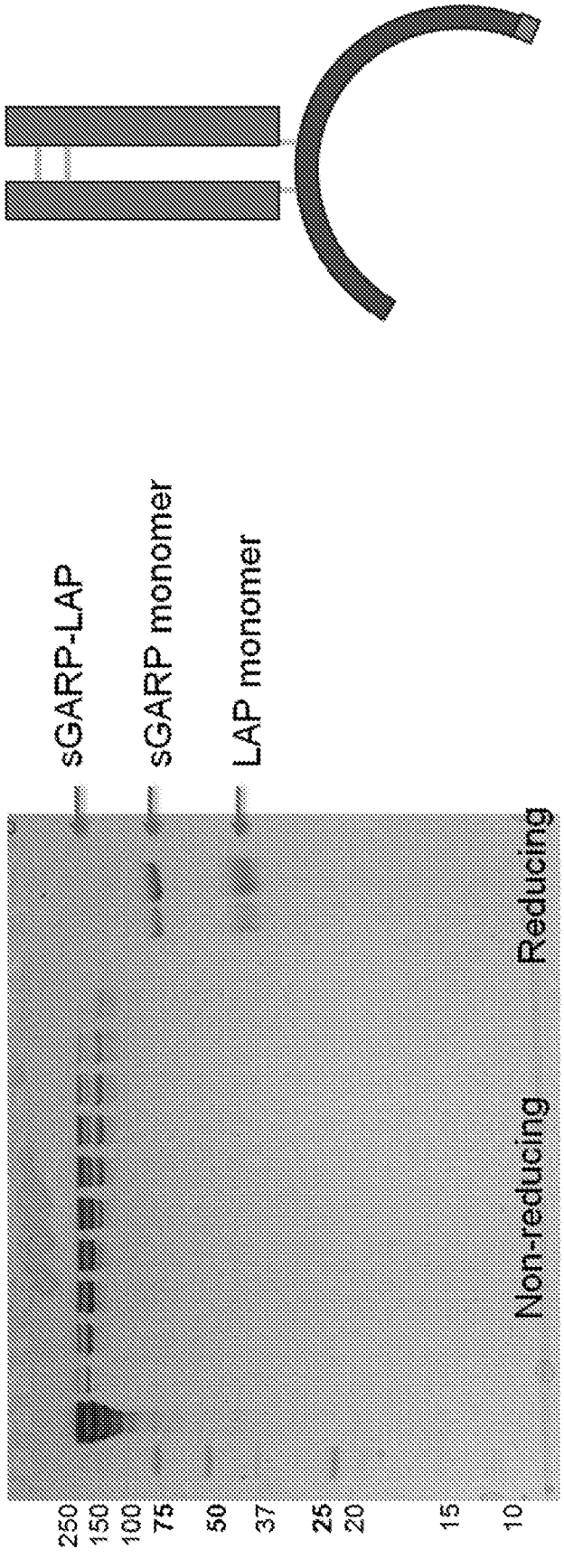

sGARP-LAP
sGARP monomer
LAP monomer
Reducing
Non-reducing
250
150
100
75
50
37
25
20
15
10

FIG. 5B

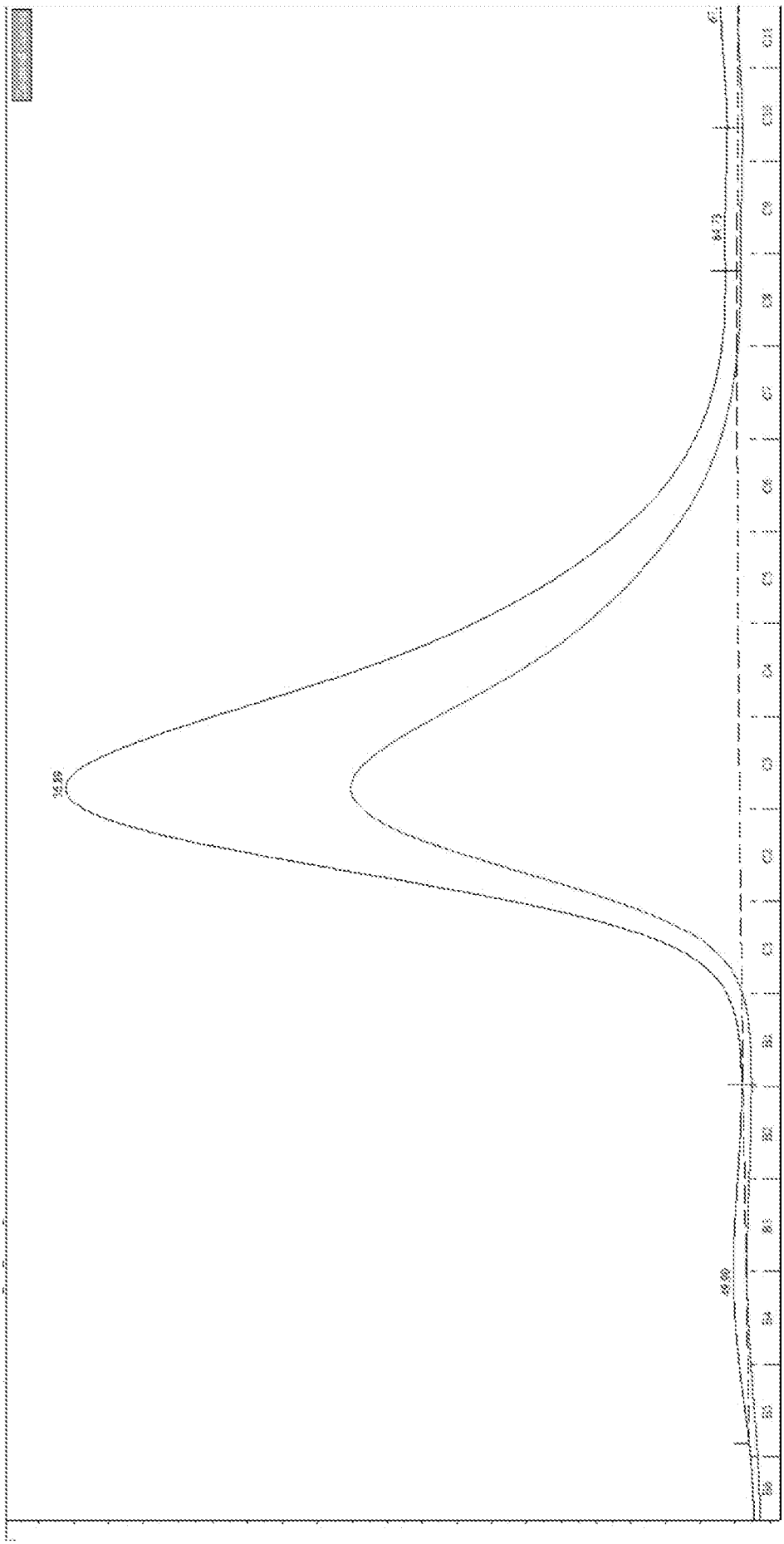


FIG. 6A

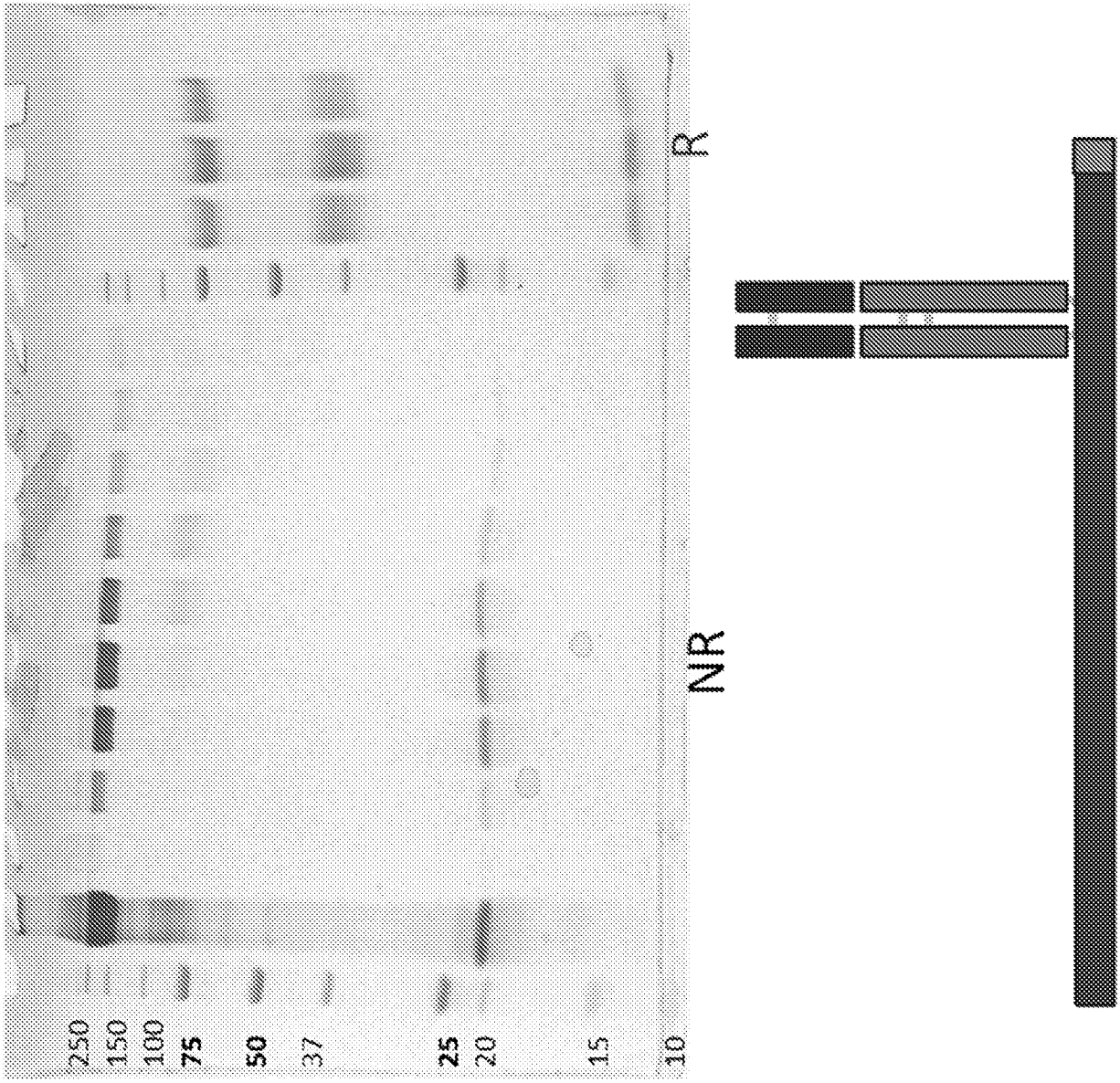


FIG. 6B

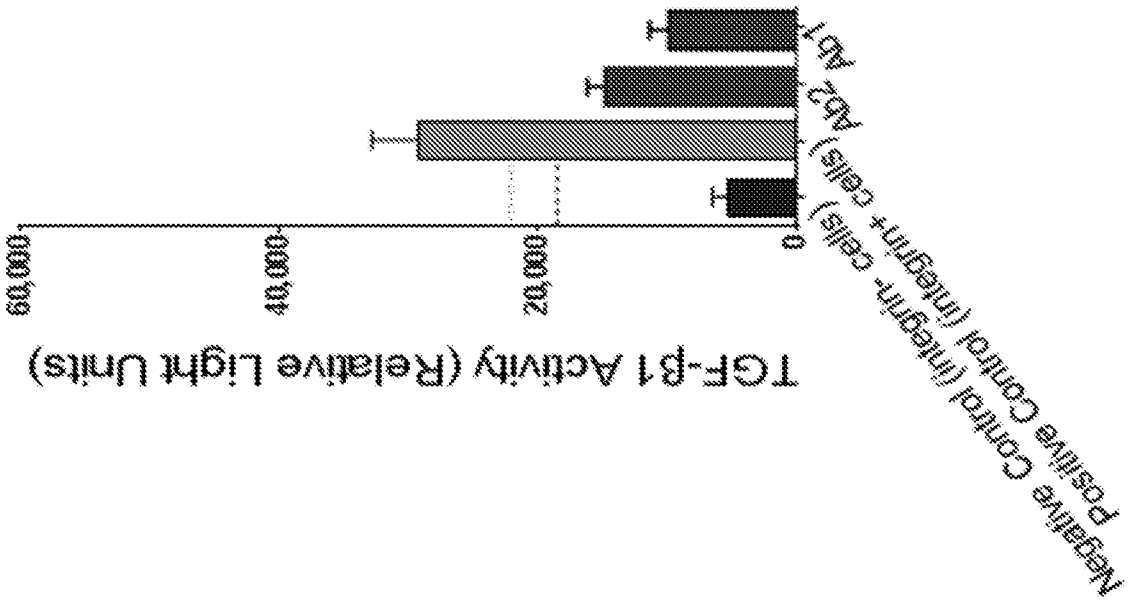

TGF-β1 Activity (Relative Light Units)
60,000
40,000
20,000
0
Negative Control (integrin- cells)
Positive Control (integrin+ cells)
Ab2
Ab1

FIG. 7

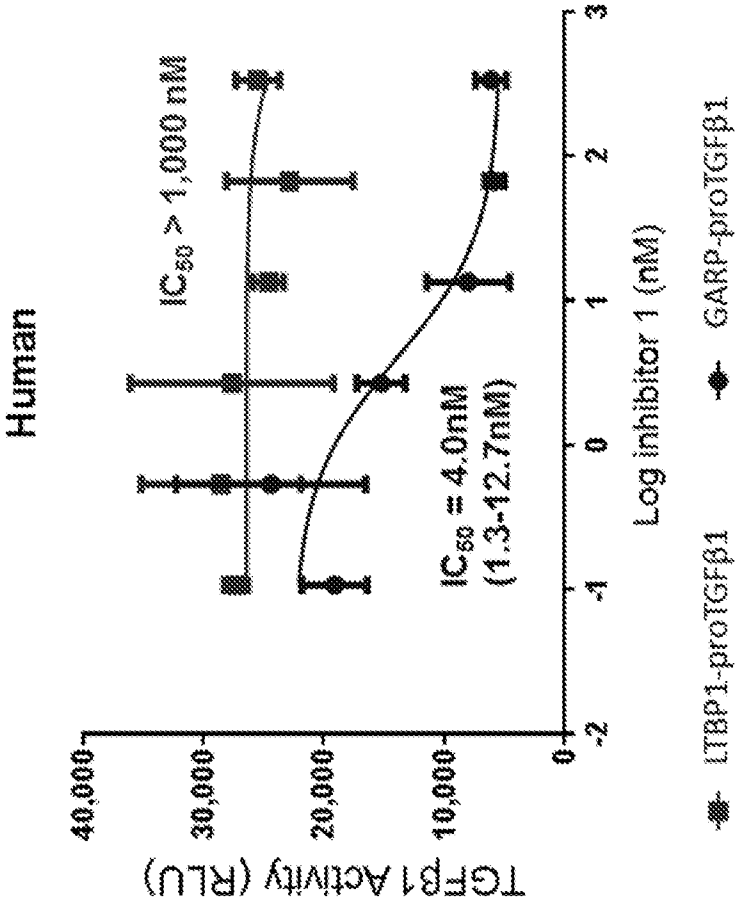

Human
TGFβ1 Activity (RLU)
40,000
30,000
20,000
10,000
0
-2
-1
0
1
2
3
Log inhibitor 1 (nM)
IC50 > 1,000 nM
IC50 = 4.0nM
(1.3-12.7nM)
LTBP1-proTGFβ1
GARP-proTGFβ1

FIG. 8

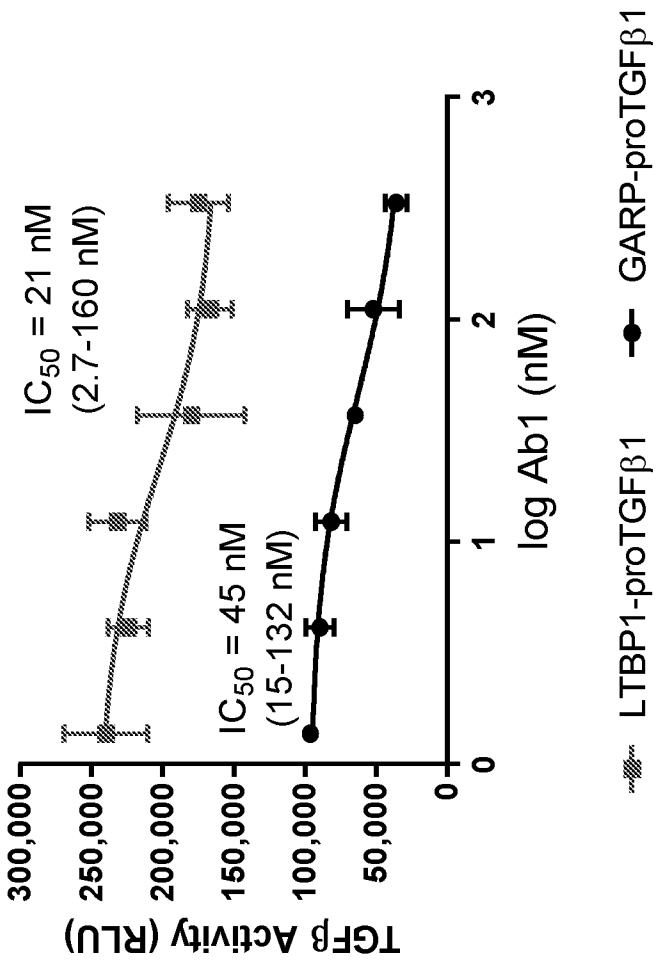

Human
IC50 = 21 nM
(2.7-160 nM)
IC50 = 45 nM
(15-132 nM)
TGFβ Activity (RLU)
300,000
250,000
200,000
150,000
100,000
50,000
0
0
1
2
3
log Ab1 (nM)
LTBP1-proTGFβ1
GARP-proTGFβ1

FIG. 9

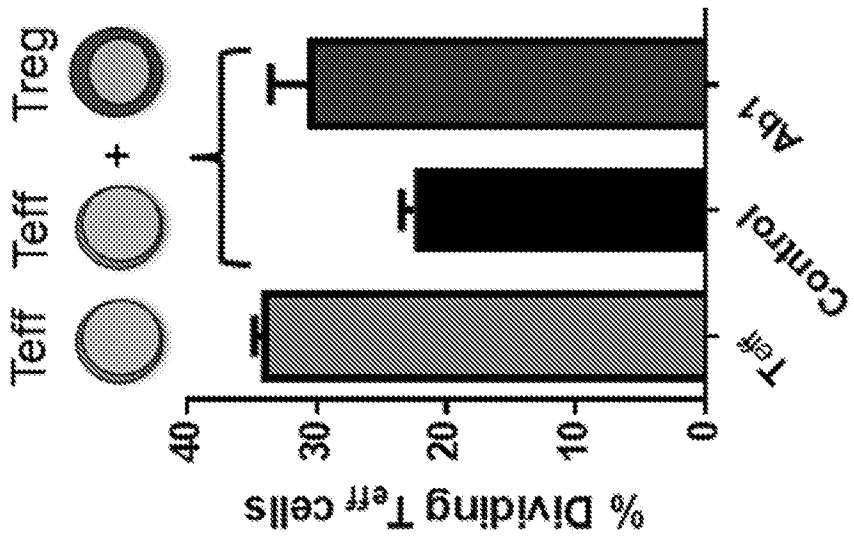

Teff
Teff
+
Treg
% Dividing Teff cells
0
10
20
30
40
Teff
Control
Ab1

FIG. 10

FIG. 11A

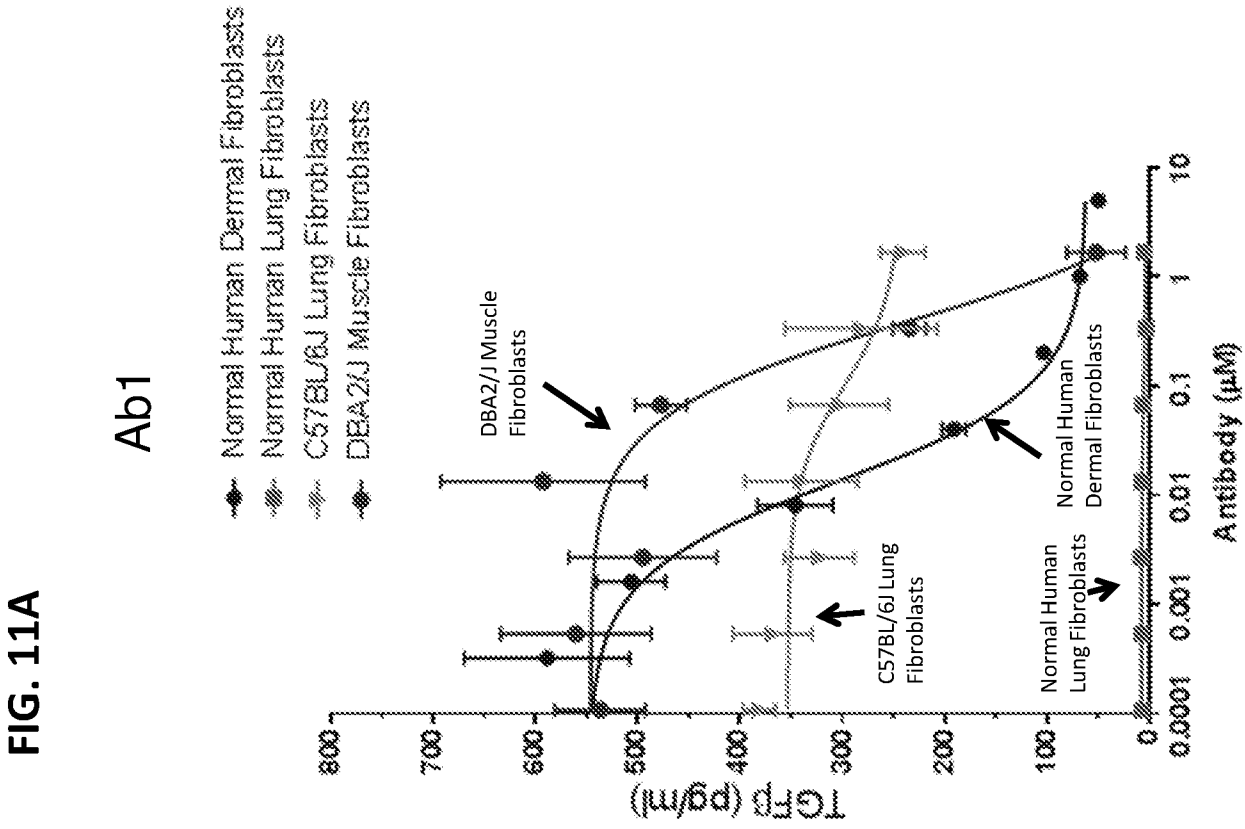
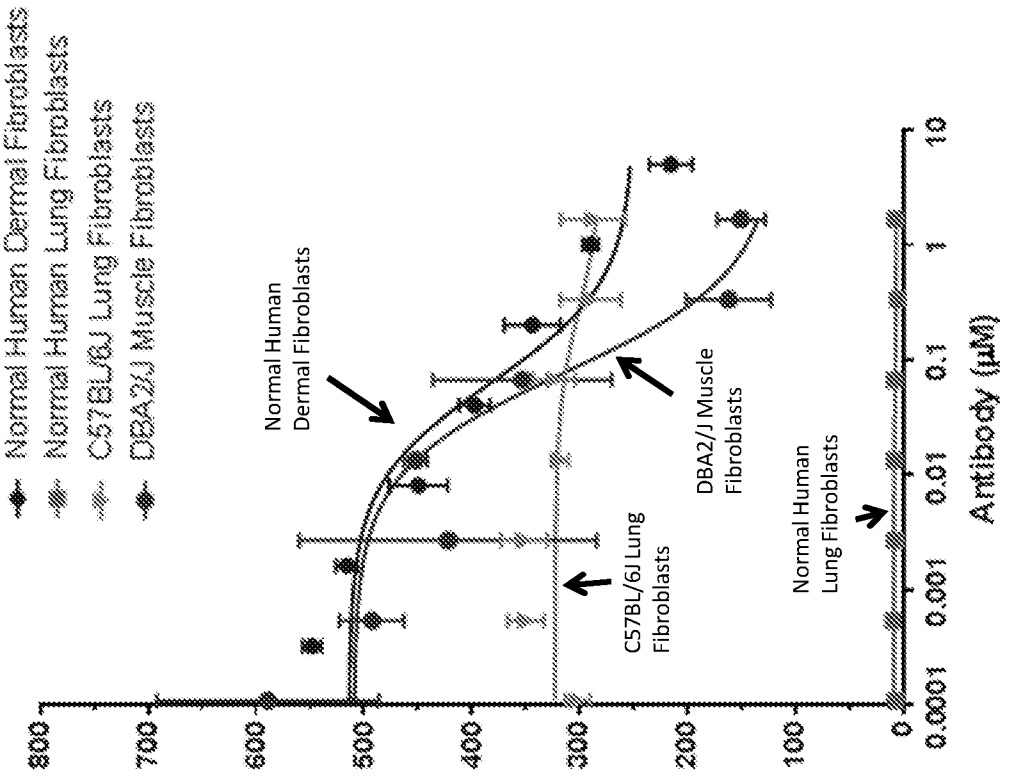


FIG. 11B



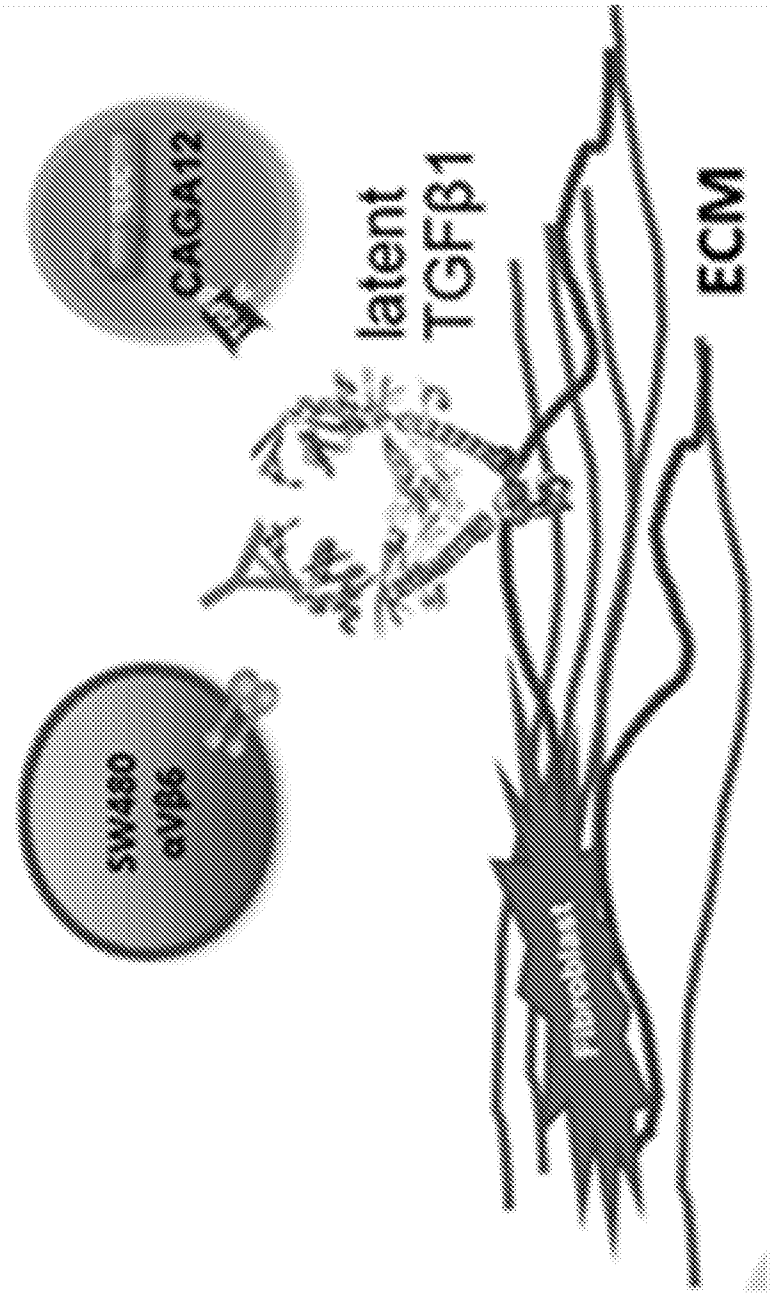


FIG. 11C

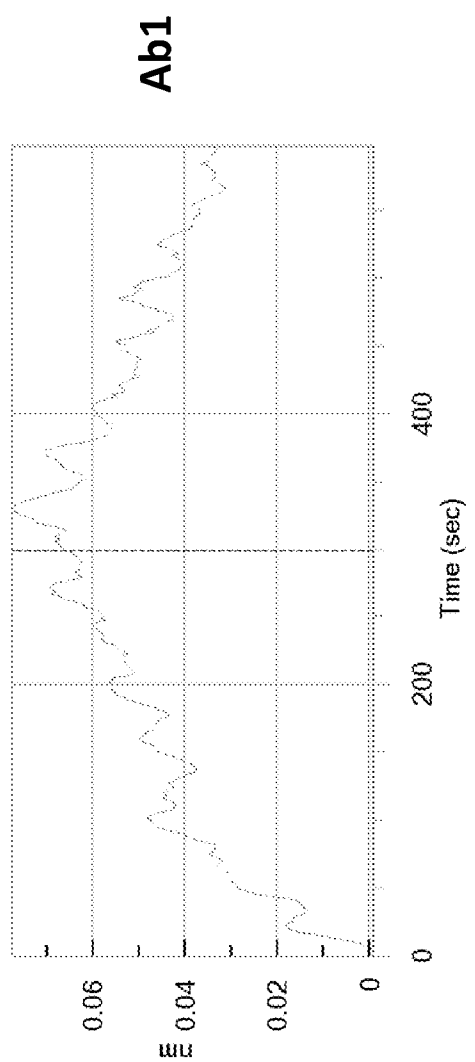


FIG. 12A

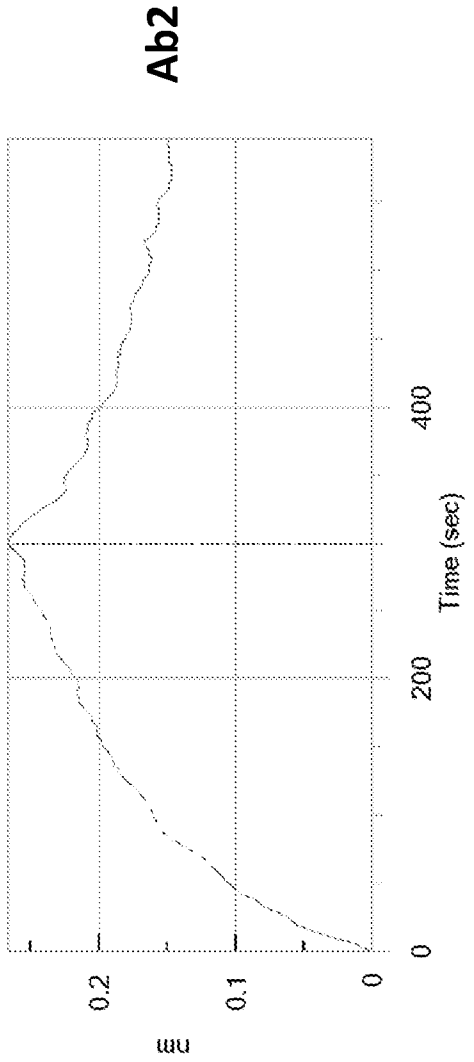
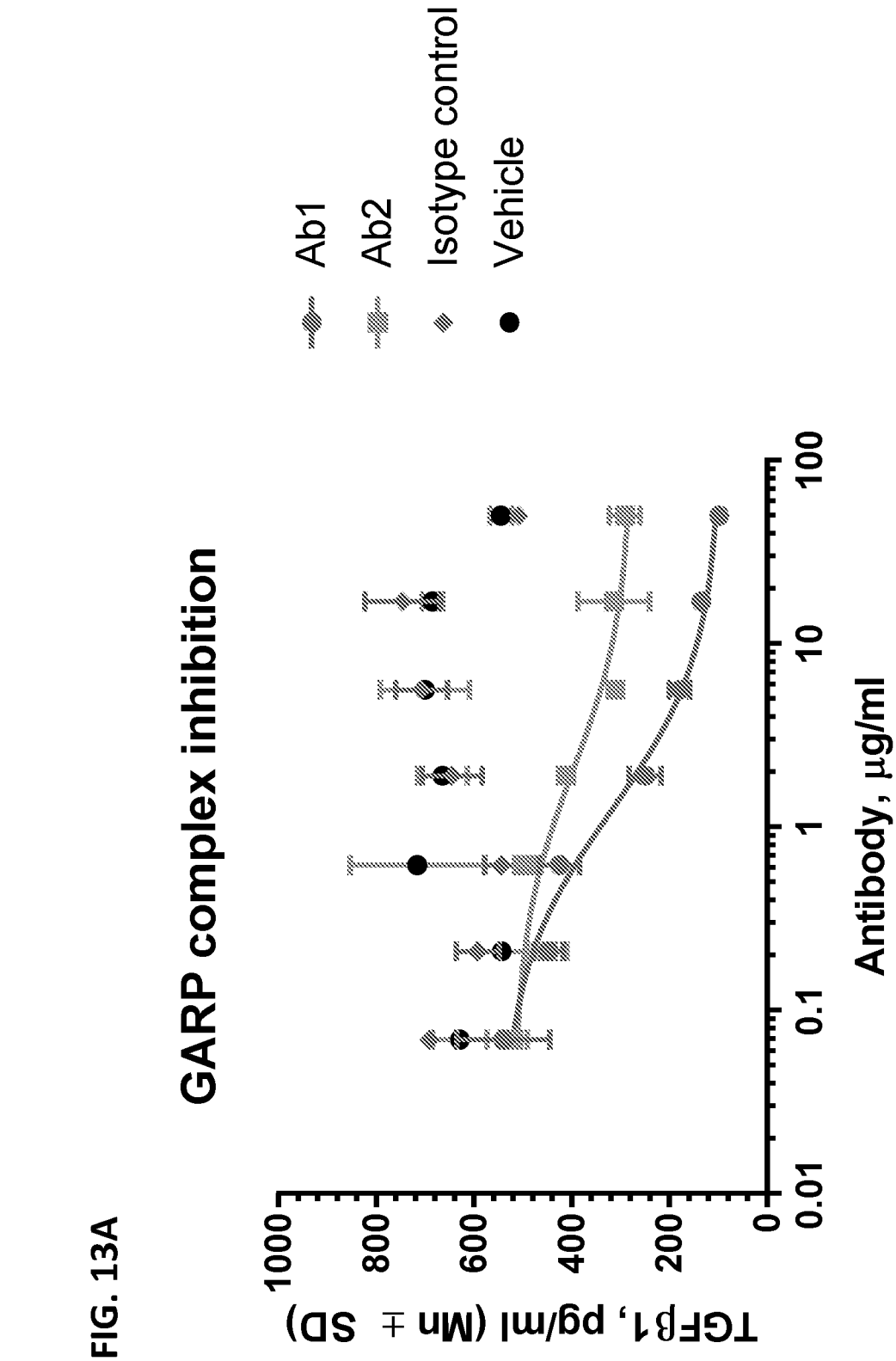


FIG. 12B

FIG. 13A


GARP complex inhibition
Ab1
Ab2
Isotype control
Vehicle
TGFβ1, pg/ml (Mn ± SD)
1000
800
600
400
200
0
0.01
0.1
1
10
100
Antibody, µg/ml

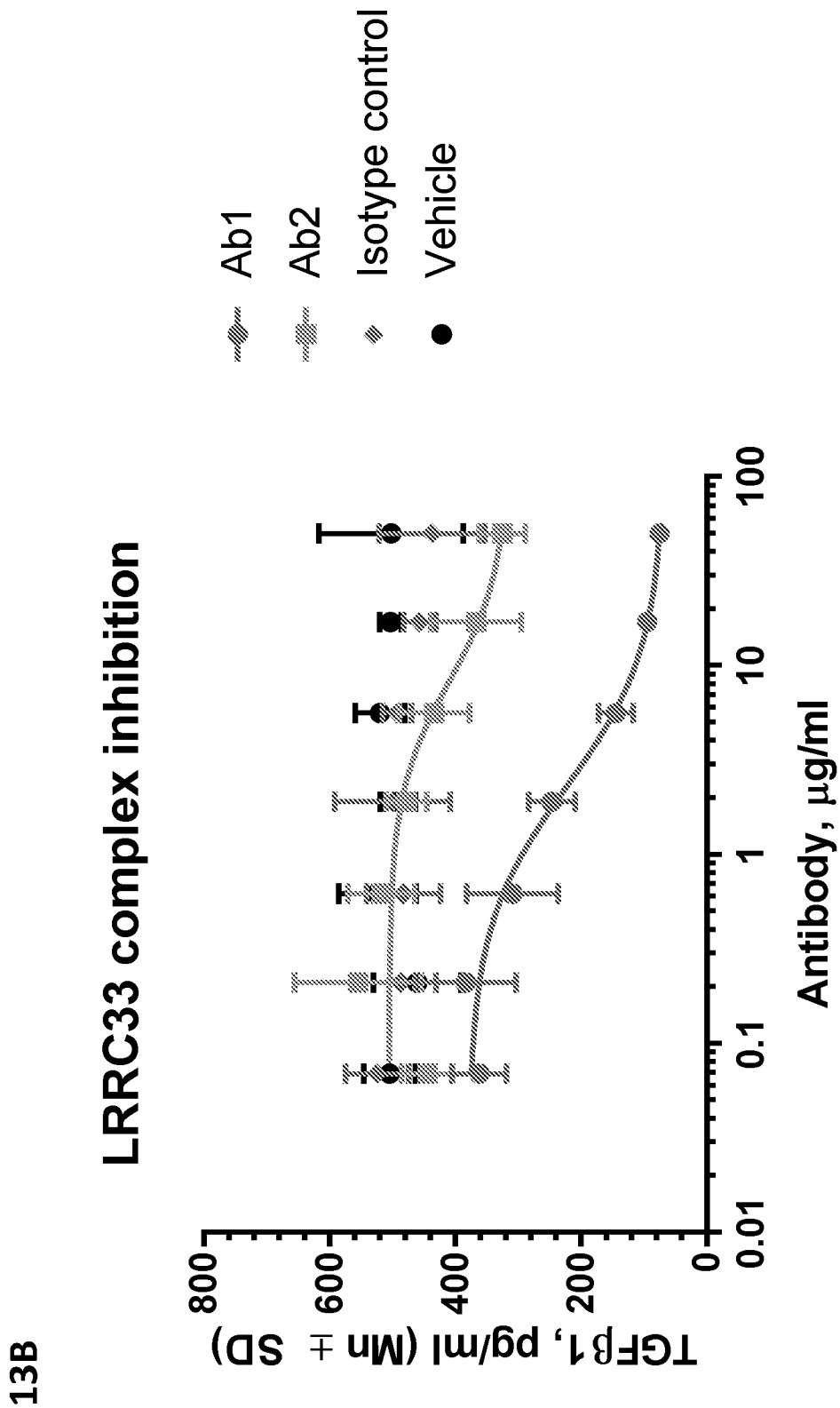

LRRC33 complex inhibition
Ab1
Ab2
Isotype control
Vehicle
TGFβ1, pg/ml (Mn ± SD)
800
600
400
200
0
0.01
0.1
1
10
100
Antibody, μg/ml

FIG. 13B

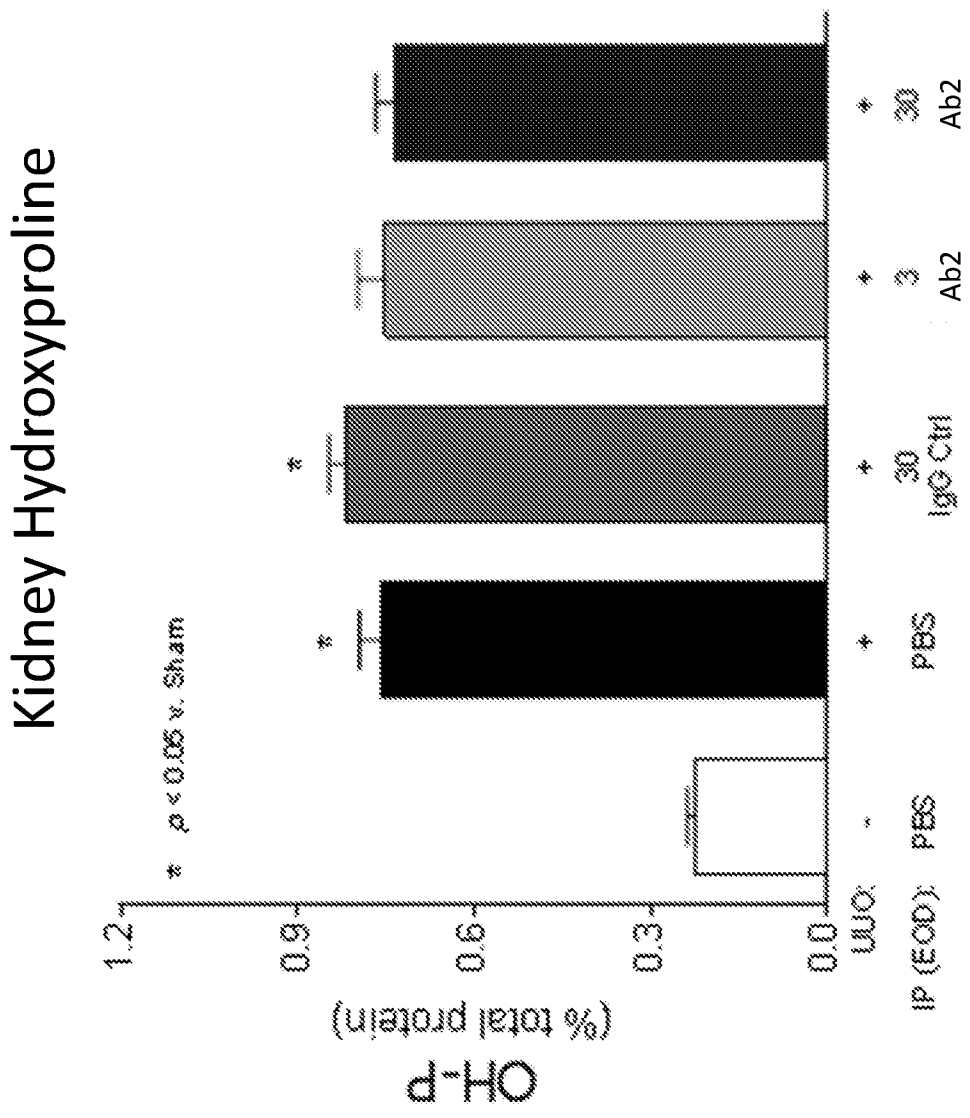

Kidney Hydroxyproline
* p < 0.05 v. Sham
OH-P
(% total protein)
1.2
0.9
0.6
0.3
0.0
UUO
-
PBS
PBS
30
IgG Ctrl
3
Ab2
30
Ab2
IP (EOD)

FIG. 14

FIG. 15A

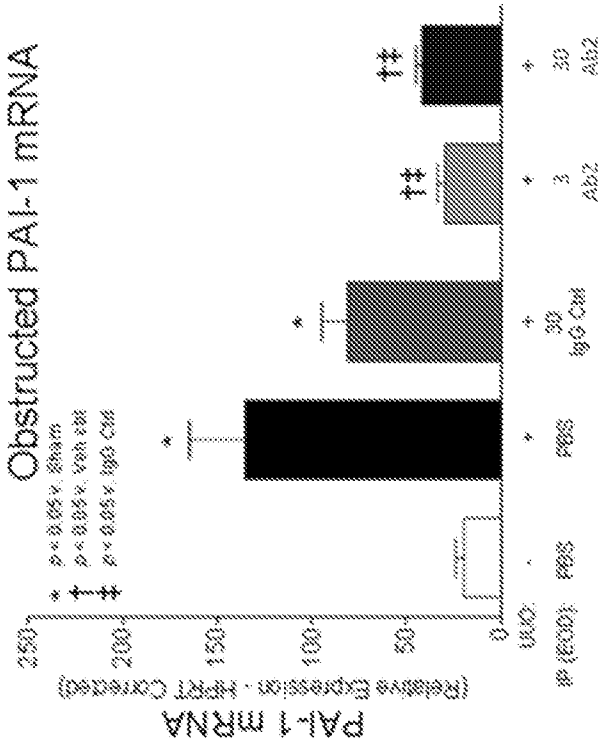


FIG. 15B

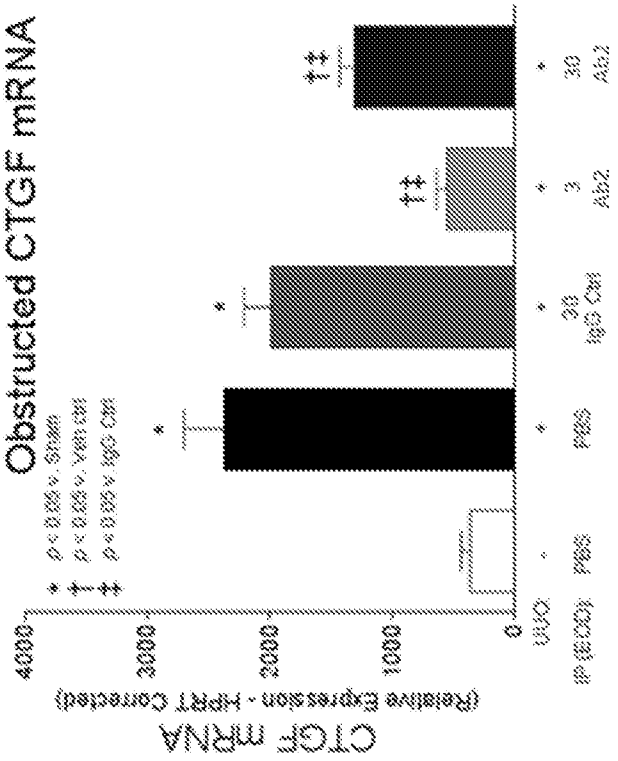


FIG. 15C

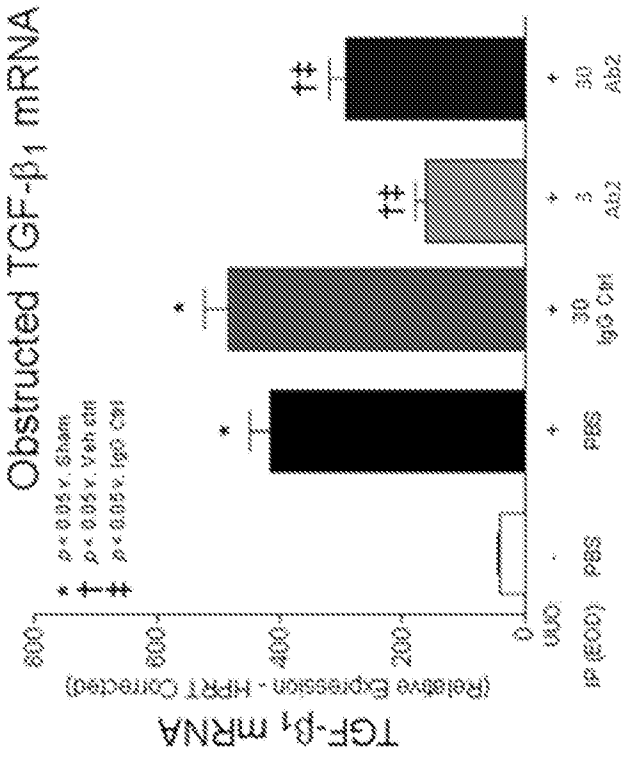


FIG. 15D

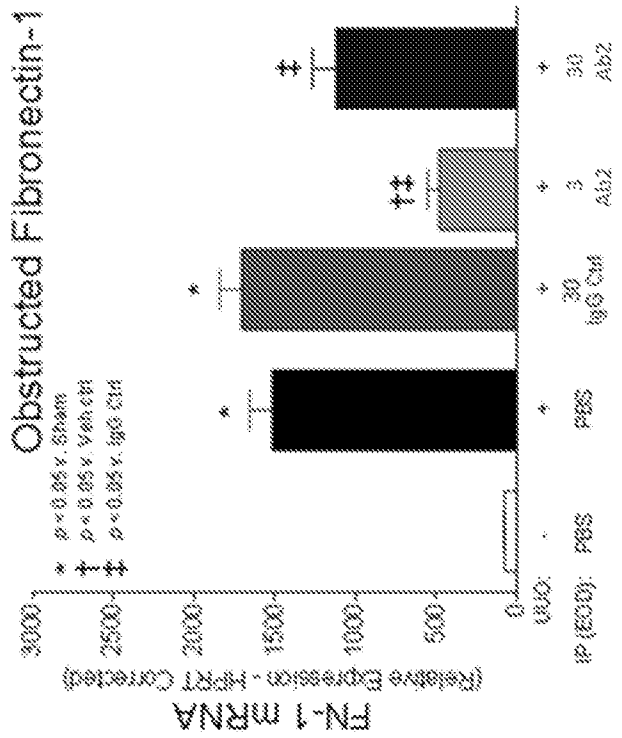


FIG. 15E

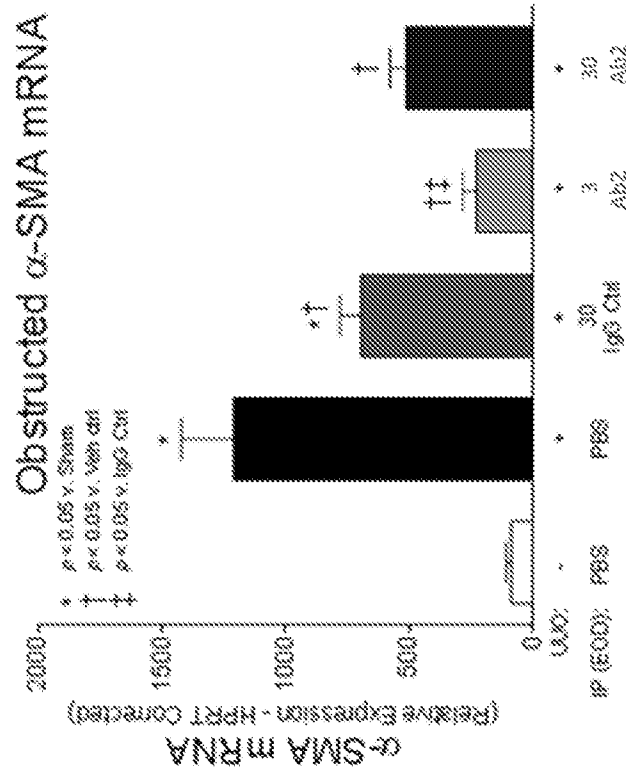


FIG. 15F

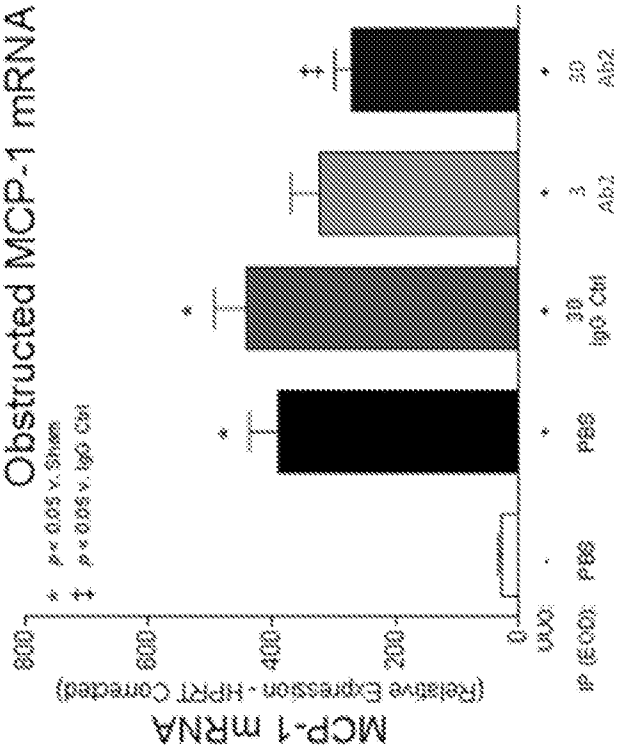


FIG. 15H

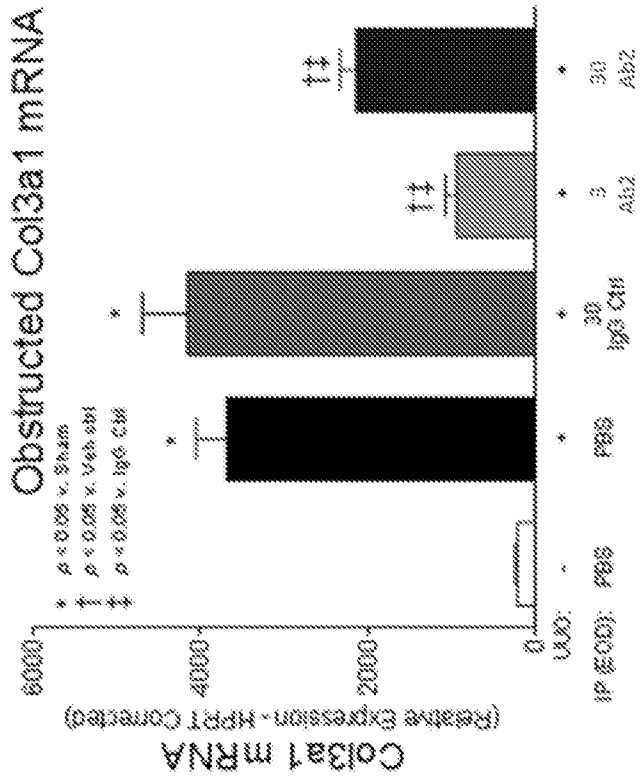
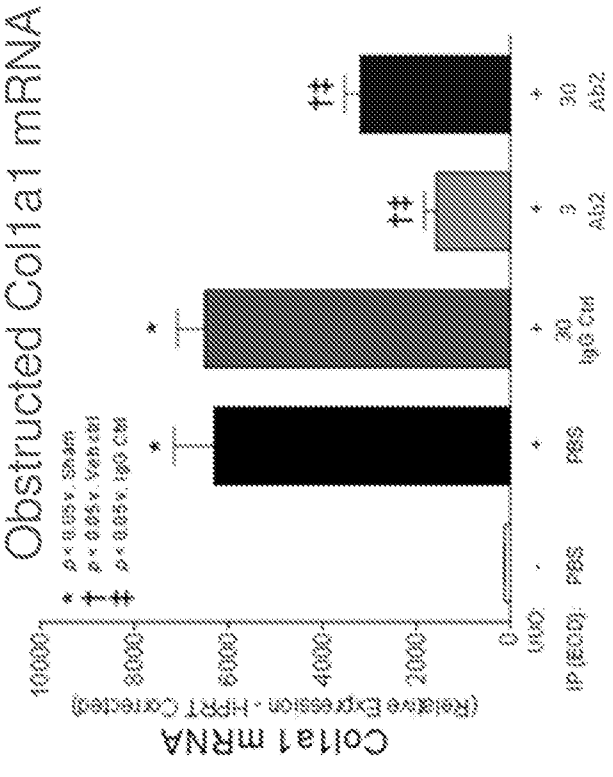


FIG. 15G



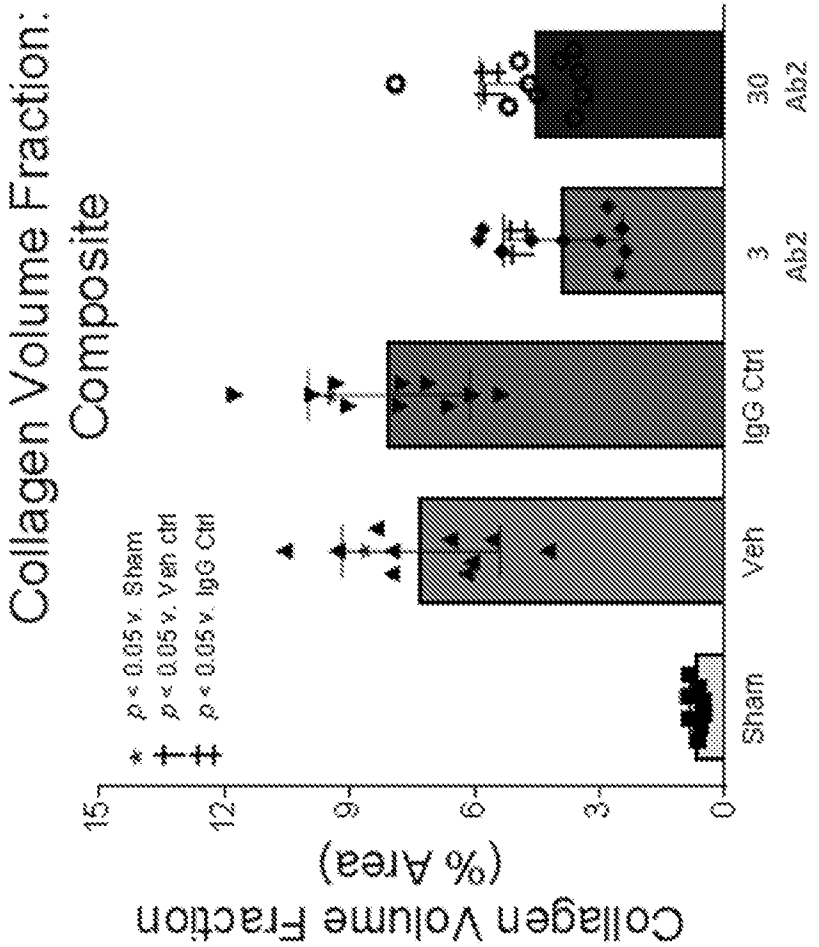

Collagen Volume Fraction: Composite
Collagen Volume Fraction (% Area)
15
12
9
6
3
0
* p < 0.05 v. Sham
† p < 0.05 v. Veh ctrl
‡ p < 0.05 v. IgG Ctrl
Sham
Veh
IgG Ctrl
3 Ab2
30 Ab2

FIG. 16

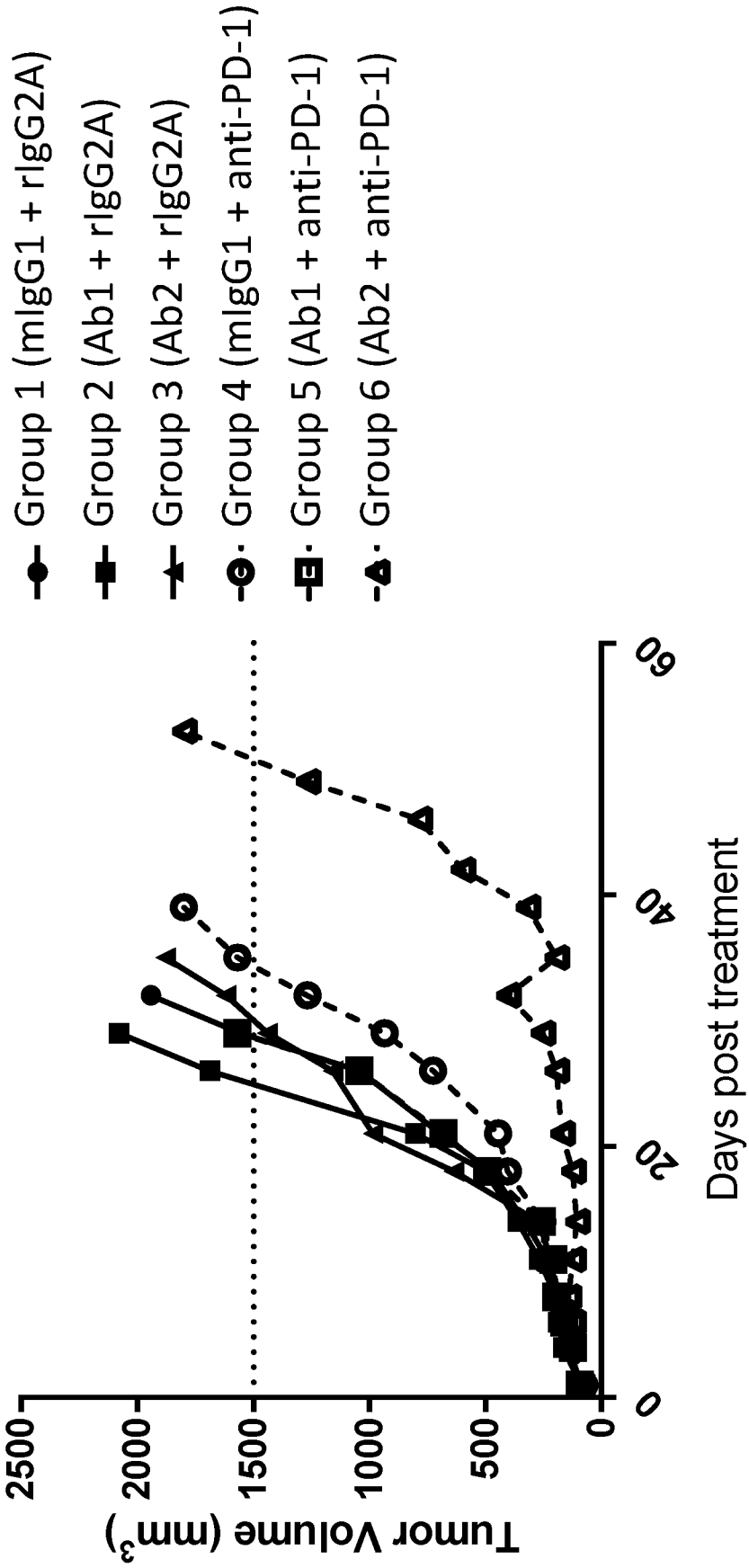

Group 1 (mIgG1 + rIgG2A)
Group 2 (Ab1 + rIgG2A)
Group 3 (Ab2 + rIgG2A)
Group 4 (mIgG1 + anti-PD-1)
Group 5 (Ab1 + anti-PD-1)
Group 6 (Ab2 + anti-PD-1)
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
20
40
60
Days post treatment

FIG. 17

FIG. 18A

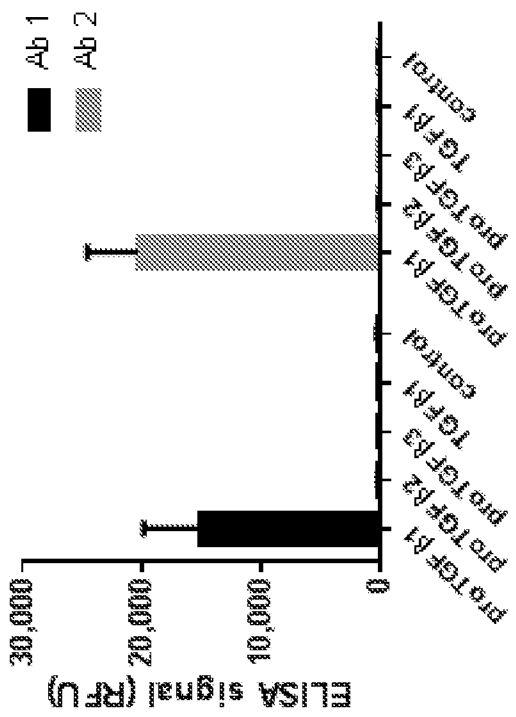


FIG. 18B

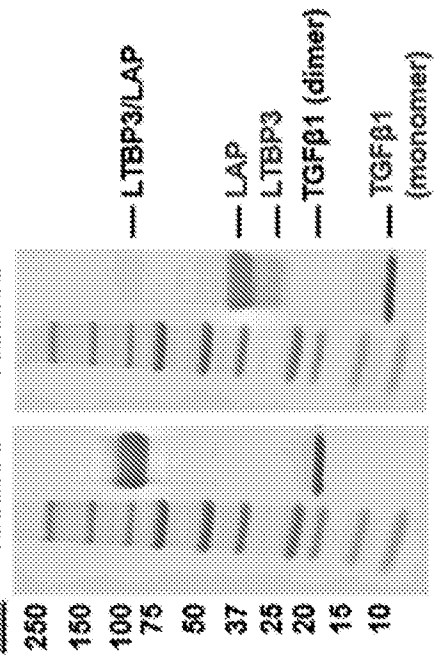
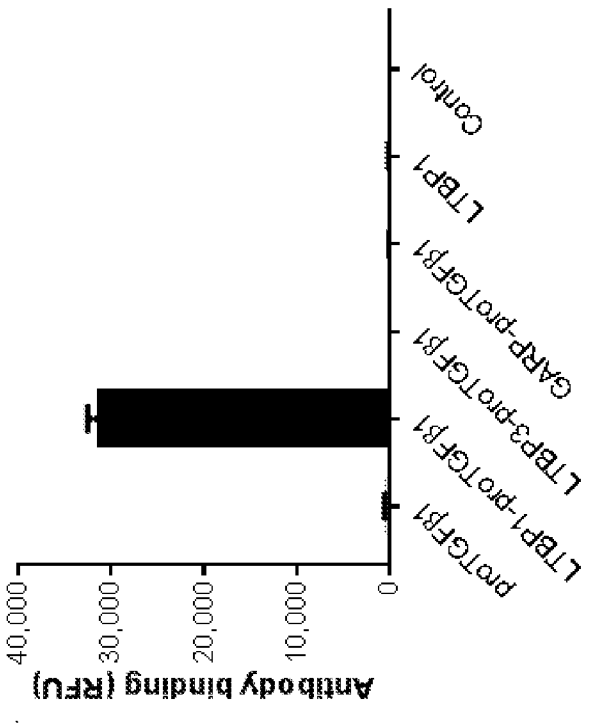


FIG. 18C



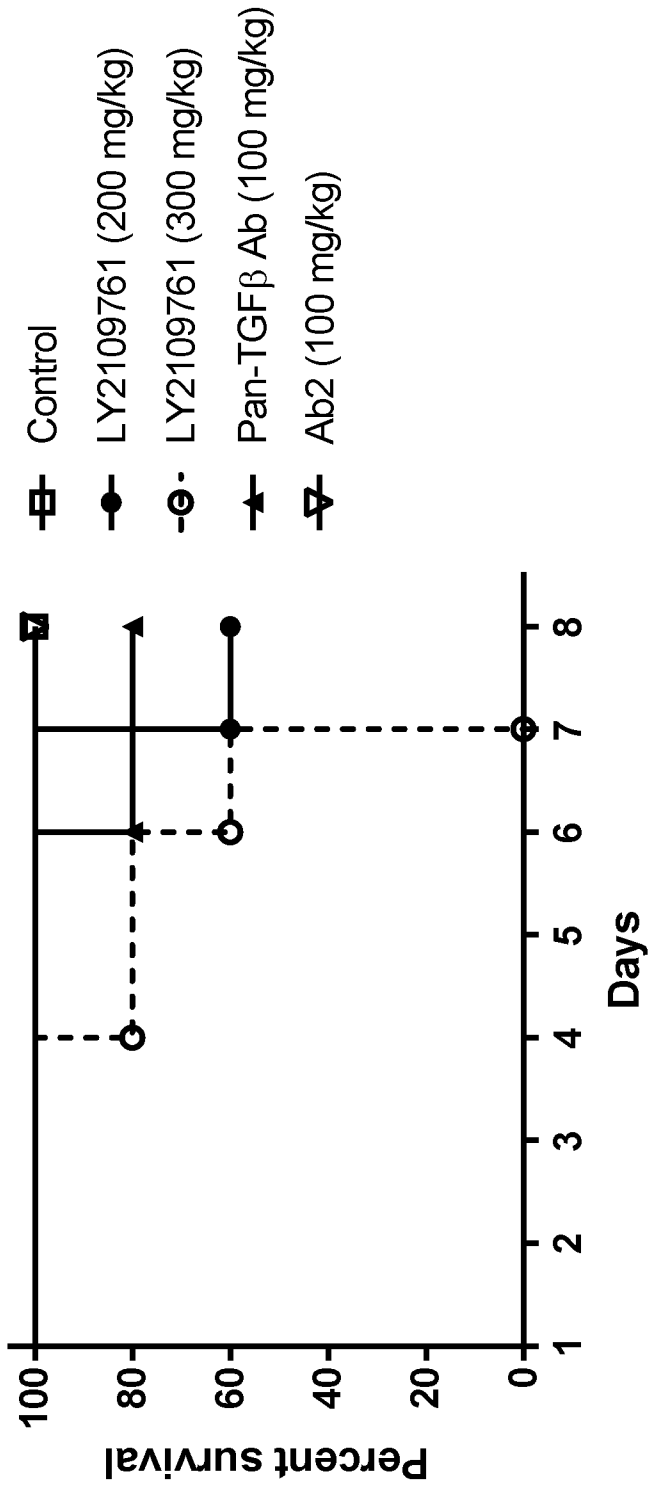

Control
LY2109761 (200 mg/kg)
LY2109761 (300 mg/kg)
Pan-TGFβ Ab (100 mg/kg)
Ab2 (100 mg/kg)
Percent survival
100
80
60
40
20
0
1
2
3
4
5
6
7
8
Days

FIG. 19

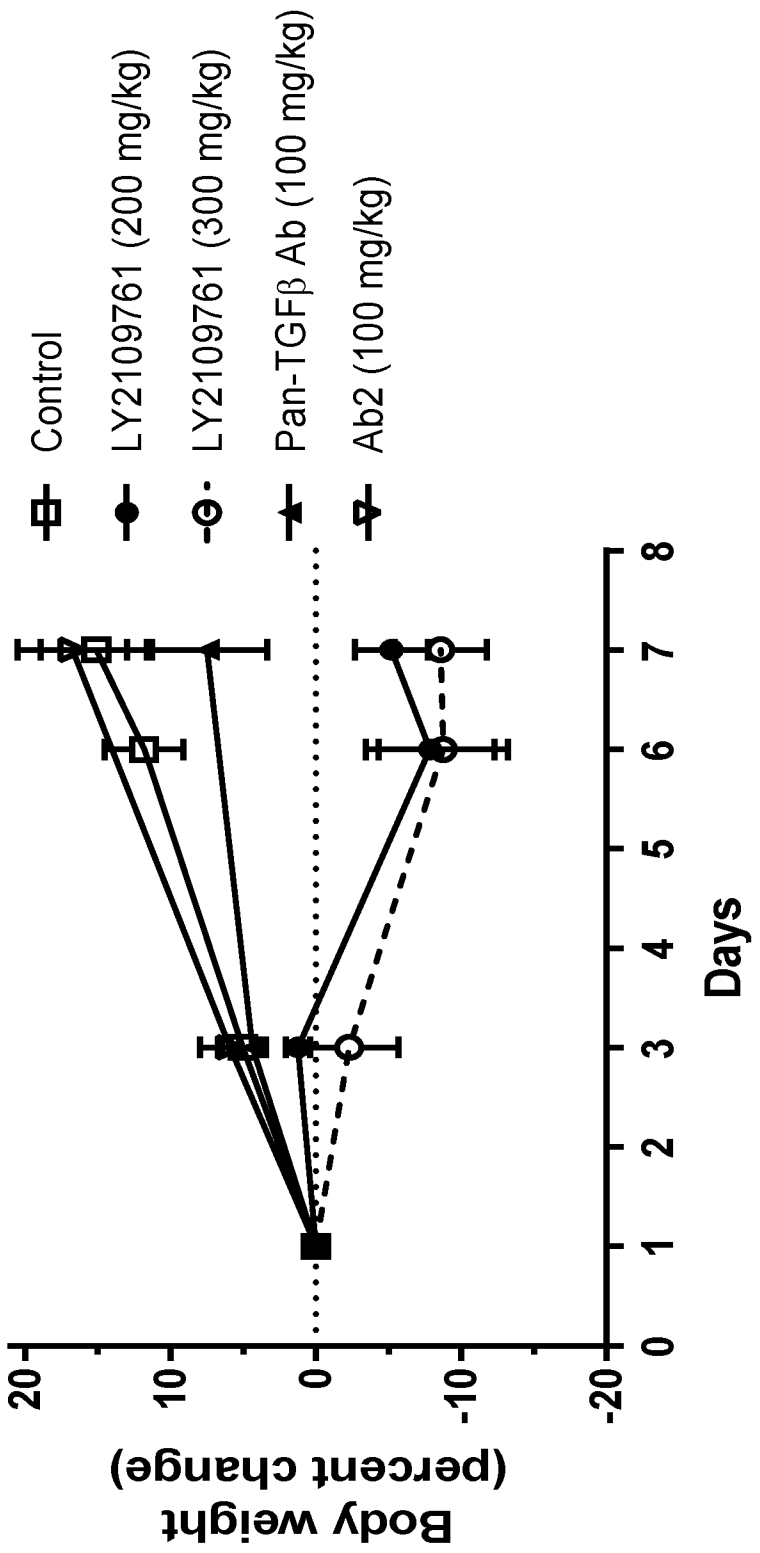

Control
LY2109761 (200 mg/kg)
LY2109761 (300 mg/kg)
Pan-TGFβ Ab (100 mg/kg)
Ab2 (100 mg/kg)
Body weight
(percent change)
20
10
0
-10
-20
0
1
2
3
4
5
6
7
8
Days

FIG. 20

FIG. 21A

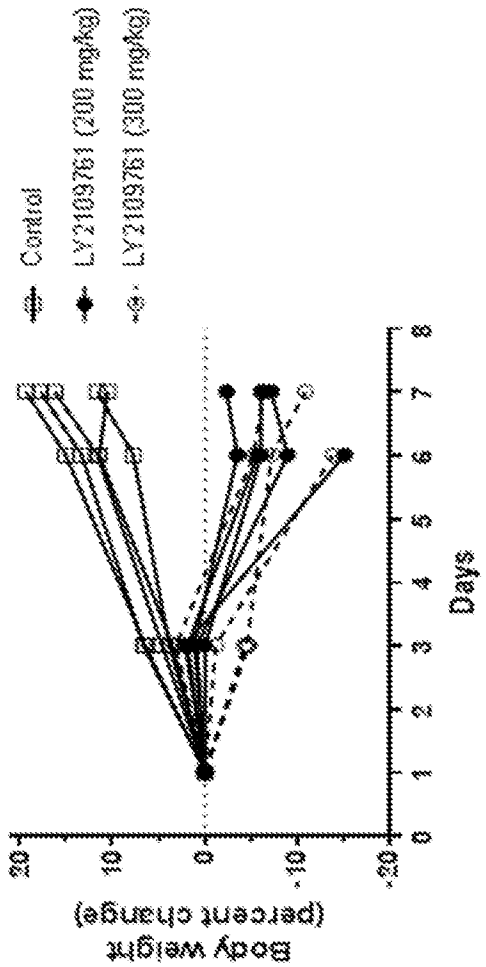


FIG. 21B

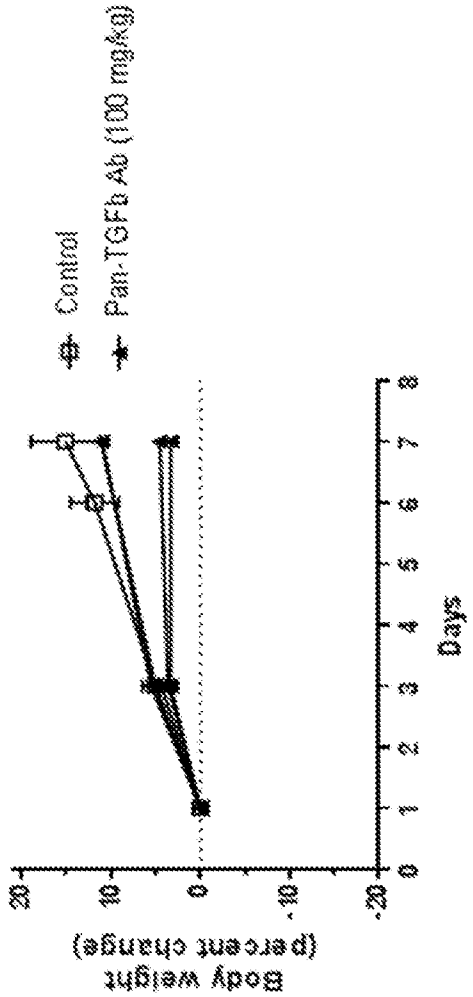


FIG. 21C

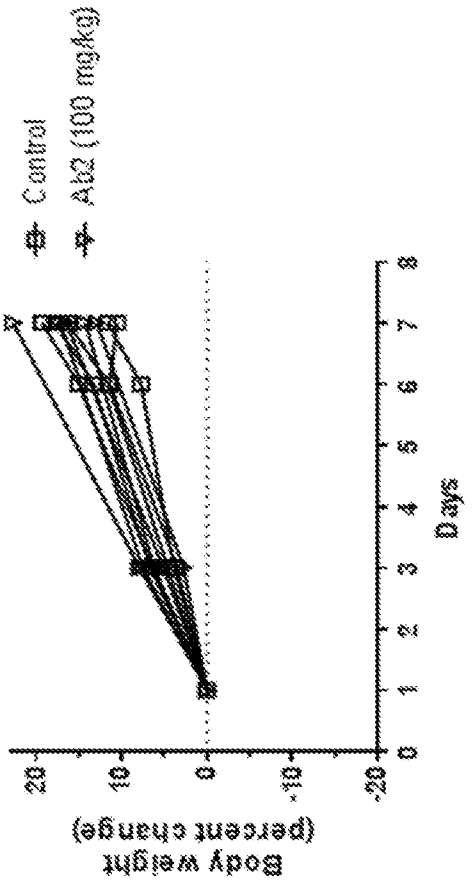


FIG. 22A

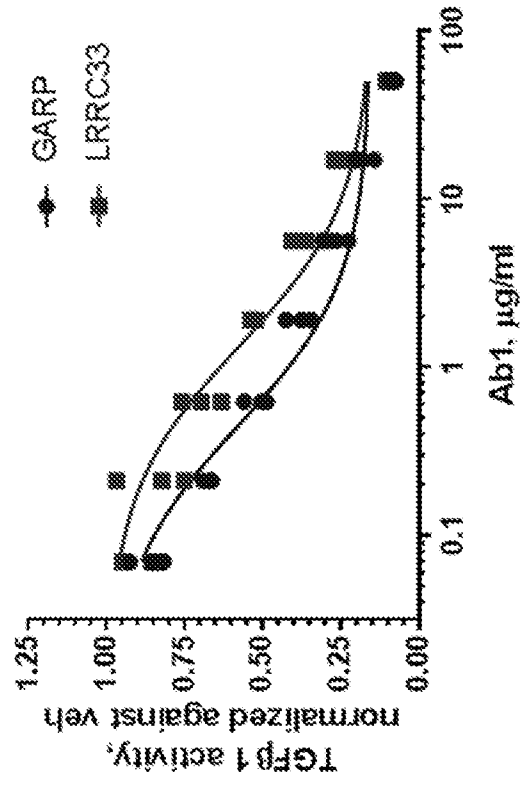


FIG. 22B

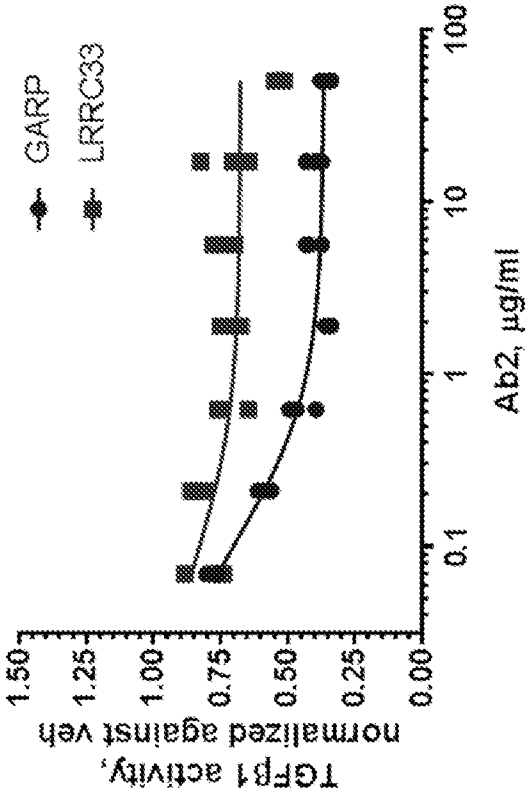
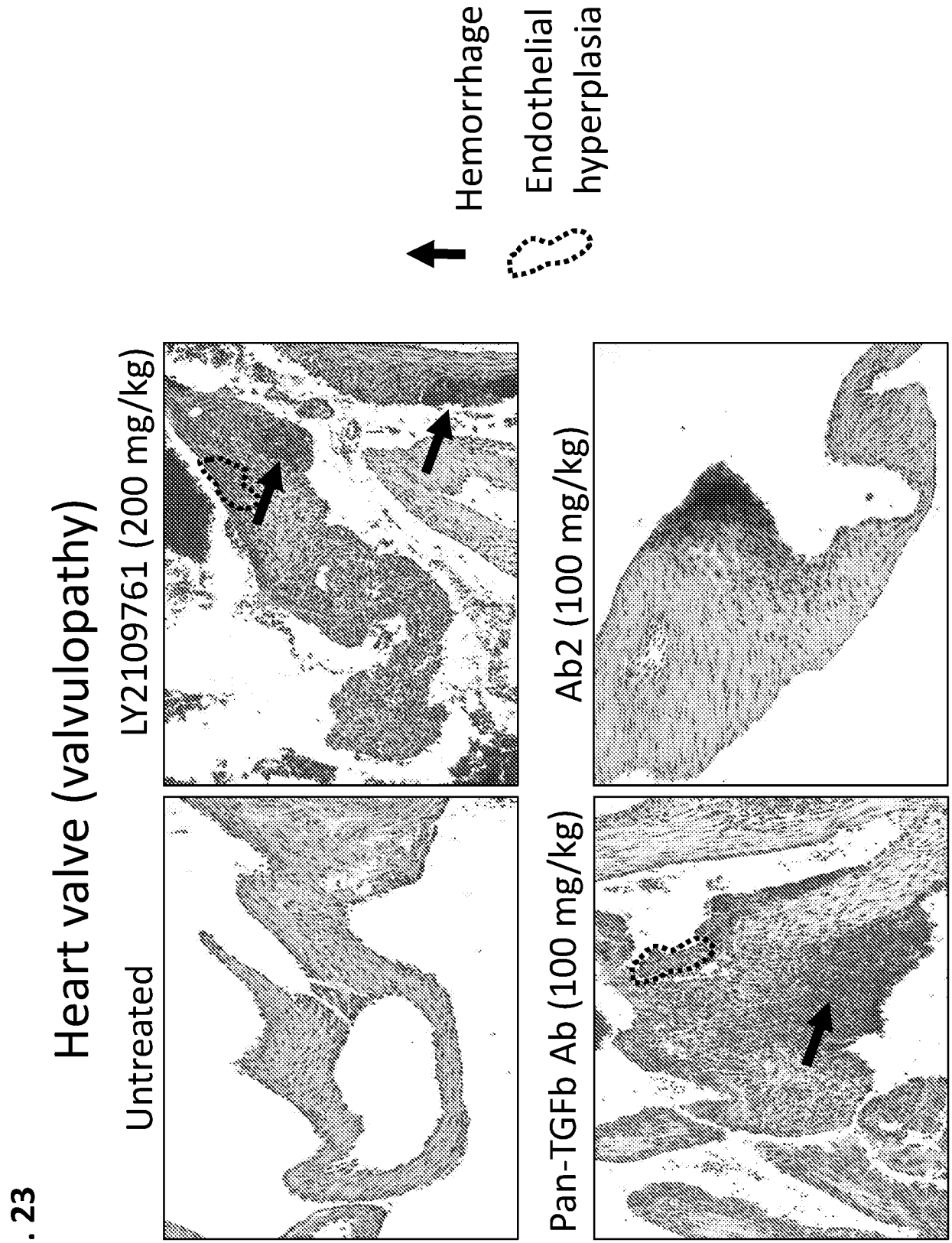


FIG. 23


Heart valve (valvulopathy)
Untreated
LY2109761 (200 mg/kg)
Pan-TGFb Ab (100 mg/kg)
Ab2 (100 mg/kg)
Hemorrhage
Endothelial hyperplasia

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/021972

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/22
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/102483 A1 (RIKEN [JP]; SUKENAGA YOSHIKAZU [JP]; KOJIMA SOICHI [JP]; HARA MITSUKO) 25 August 2011 (2011-08-25) paragraph [0071]; figure 6a paragraph [0081] paragraph [0076] -----	1-41,54, 55
X	N.N.: "Human LAP (TGF-[beta]1) Antibody", , 1 January 1985 (1985-01-01), XP055383034, Retrieved from the Internet: URL:https://resources.rndsystems.com/pdfs/datasheets/af-246-na.pdf [retrieved on 2017-06-20] page 1 - page 2 ----- -/--	1-6,54, 55

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2017	07/07/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Wagner, René

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 2013/134365 A1 (LUDWIG INST FOR CANCER RES LTD [US]) 12 September 2013 (2013-09-12) example 2 claim 28 example 3 example 4	64-67, 71-74
A	WO 2006/116002 A2 (LILLY CO ELI [US]; DAVIES JULIAN [US]; DICKINSON CRAIG DUANE [US]; HUA) 2 November 2006 (2006-11-02) the whole document	1-74
A	WO 2015/015003 A1 (UNIVERSITÉ CATHOLIQUE DE LOUVAIN [BE]; LUDWIG INST FOR CANCER RES LTD) 5 February 2015 (2015-02-05) the whole document	1-74
A	Rui Wang ET AL: "GARP regulates the bioavailability and activation of TGF[beta]", Molecular biology of the cell, 1 March 2012 (2012-03-01), pages 1129-1139, XP55094855, United States DOI: 10.1091/mbc.E11-12-1018 Retrieved from the Internet: URL:http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3302739&tool=pmcentrez&rendertype=abstract the whole document	1-74
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Information on patent family members

International application No
PCT/US2017/021972

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		WO 2006116002 A2	02-11-2006
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