CLAIMS

~~What is claimed is:~~

1. A chimeric protein comprising:

(a) at least one recombinant heavy-chain-only antibody (VHH) or a single-chain antibody (scFv) targeting moiety that is a fibroblast activation protein (FAP) binding agent and

(b) a modified human interferon alpha 2 (IFNα2) signaling agent comprising an amino acid sequence having at least 95% identity with SEQ ID NO: 176 or 177 and having one or more mutations selected from K133A, L153A, R149A, and M148A that confer ~~improved safety~~lower toxicity, lessened or eliminated side effects, increased tolerability, lessened or eliminated adverse events, reduced or eliminated off-target effects, and/or increased therapeutic window, as compared to a wild type IFNα2 having an amino acid sequence of SEQ ID NO: 176 or 177;

wherein the one or more mutations confer reduced or ablated binding affinity and/or activity to the modified IFNα2 signaling agent that is restorable by the targeting moiety,

wherein the targeting moiety targets ~~disease-associated~~F2 fibroblasts, and

wherein the chimeric protein ~~directly or indirectly~~ alters the ~~disease-associated~~F2 fibroblast's function and/or a disease microenvironment comprising the ~~disease-associated~~F2 fibroblast.

2. The chimeric protein of claim 1, wherein the FAP binding agent ~~directly or indirectly~~ polarizes F2 fibroblasts.

3. The chimeric protein of claim 1, wherein the FAP binding agent recruits cytotoxic T cells to tumor cells or to a tumor microenvironment.

4. The chimeric protein of claim 1, wherein the FAP binding agent recognizes or binds FAP without ~~substantially functionally~~ modulating its activity.

~~5. The chimeric protein of claim 1, wherein the targeting moiety is a full-length antibody, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein, a darpin, an anticalin, an adnectin, a Fv, a Fab, a Fab', or a F(ab')2.~~

~~65~~. The chimeric protein of claim 1, wherein the FAP binding agent comprises three complementarity determining regions (CDR1, CDR2, and CDR3), wherein

~~(a)~~ CDR1 comprises an amino acid sequence of SEQ ID NO: 95; CDR2 comprises an amino acid sequence of SEQ ID NO: 121; CDR3 comprises an amino acid sequence of SEQ ID NO: 152;

CDR1 comprises an amino acid sequence of SEQ ID NO: 87; CDR2 comprises an amino acid sequence of SEQ ID NO: 116; CDR3 comprises an amino acid sequence of SEQ ID NO: 145;

CDR1 comprises an amino acid sequence of SEQ ID NO: 89; CDR2 comprises an amino acid sequence of SEQ ID NO: 117; CDR3 comprises an amino acid sequence of SEQ ID NO: 146;

CDR1 comprises an amino acid sequence of SEQ ID NO: 90; CDR2 comprises an amino acid sequence of SEQ ID NO: 117; CDR3 comprises an amino acid sequence of SEQ ID NO: 147;

CDR1 comprises an amino acid sequence of SEQ ID NO: 91; CDR2 comprises an amino acid sequence of SEQ ID NO: 118; CDR3 comprises an amino acid sequence of SEQ ID NO: 148;

CDR1 comprises an amino acid sequence of SEQ ID NO: 92; CDR2 comprises an amino acid sequence of SEQ ID NO: 118; CDR3 comprises an amino acid sequence of SEQ ID NO: 149;

CDR1 comprises an amino acid sequence of SEQ ID NO: 93; CDR2 comprises an amino acid sequence of SEQ ID NO: 119; CDR3 comprises an amino acid sequence of SEQ ID NO: 146;

CDR1 comprises an amino acid sequence of SEQ ID NO: 94; CDR2 comprises an amino acid sequence of SEQ ID NO: 120; CDR3 comprises an amino acid sequence of SEQ ID NO: 150;

CDR1 comprises an amino acid sequence of SEQ ID NO: 94; CDR2 comprises an amino acid sequence of SEQ ID NO: 120; CDR3 comprises an amino acid sequence of SEQ ID NO: 151;

CDR1 comprises an amino acid sequence of SEQ ID NO: 96; CDR2 comprises an amino acid sequence of SEQ ID NO: 122; CDR3 comprises an amino acid sequence of SEQ ID NO: 153;

CDR1 comprises an amino acid sequence of SEQ ID NO: 97; CDR2 comprises an amino acid sequence of SEQ ID NO: 123; CDR3 comprises an amino acid sequence of SEQ ID NO: 154;

CDR1 comprises an amino acid sequence of SEQ ID NO: 98; CDR2 comprises an amino acid sequence of SEQ ID NO: 124; CDR3 comprises an amino acid sequence of SEQ ID NO:155;

CDR1 comprises an amino acid sequence of SEQ ID NO: 99; CDR2 comprises an amino acid sequence of SEQ ID NO: 125; CDR3 comprises an amino acid sequence of SEQ ID NO: 156;

CDR1 comprises an amino acid sequence of SEQ ID NO: 100; CDR2 comprises an amino acid sequence of SEQ ID NO: 126; CDR3 comprises an amino acid sequence of SEQ ID NO: 157;

CDR1 comprises an amino acid sequence of SEQ ID NO: 101; CDR2 comprises an amino acid sequence of SEQ ID NO: 127; CDR3 comprises an amino acid sequence of SEQ ID NO: 158;

CDR1 comprises an amino acid sequence of SEQ ID NO: 102; CDR2 comprises an amino acid sequence of SEQ ID NO: 128; CDR3 comprises an amino acid sequence of SEQ ID NO: 159;

CDR1 comprises an amino acid sequence of SEQ ID NO: 103; CDR2 comprises an amino acid sequence of SEQ ID NO: 129; CDR3 comprises an amino acid sequence of SEQ ID NO: 160;

CDR1 comprises an amino acid sequence of SEQ ID NO: 104; CDR2 comprises an amino acid sequence of SEQ ID NO: 129; CDR3 comprises an amino acid sequence of SEQ ID NO: 160;

CDR1 comprises an amino acid sequence of SEQ ID NO: 105; CDR2 comprises an amino acid sequence of SEQ ID NO: 130; CDR3 comprises an amino acid sequence of SEQ ID NO: 160;

CDR1 comprises an amino acid sequence of SEQ ID NO: 105; CDR2 comprises an amino acid sequence of SEQ ID NO: 131; CDR3 comprises an amino acid sequence of SEQ ID NO: 161;

CDR1 comprises an amino acid sequence of SEQ ID NO: 106; CDR2 comprises an amino acid sequence of SEQ ID NO: 132; CDR3 comprises an amino acid sequence of SEQ ID NO: 162;

CDR1 comprises an amino acid sequence of SEQ ID NO: 107; CDR2 comprises an amino acid sequence of SEQ ID NO: 133; CDR3 comprises an amino acid sequence of SEQ ID NO: 163;

CDR1 comprises an amino acid sequence of SEQ ID NO: 108; CDR2 comprises an amino acid sequence of SEQ ID NO: 132; CDR3 comprises an amino acid sequence of SEQ ID NO: 164;

CDR1 comprises an amino acid sequence of SEQ ID NO: 106; CDR2 comprises an amino acid sequence of SEQ ID NO: 134; CDR3 comprises an amino acid sequence of SEQ ID NO: 165;

CDR1 comprises an amino acid sequence of SEQ ID NO: 106; CDR2 comprises an amino acid sequence of SEQ ID NO: 135; CDR3 comprises an amino acid sequence of SEQ ID NO: 166;

CDR1 comprises an amino acid sequence of SEQ ID NO: 106; CDR2 comprises an amino acid sequence of SEQ ID NO: 136; CDR3 comprises an amino acid sequence of SEQ ID NO: 167;

CDR1 comprises an amino acid sequence of SEQ ID NO: 109; CDR2 comprises an amino acid sequence of SEQ ID NO: 137; CDR3 comprises an amino acid sequence of SEQ ID NO: 168;

CDR1 comprises an amino acid sequence of SEQ ID NO: 106; CDR2 comprises an amino acid sequence of SEQ ID NO: 138; CDR3 comprises an amino acid sequence of SEQ ID NO: 169;

CDR1 comprises an amino acid sequence of SEQ ID NO: 110; CDR2 comprises an amino acid sequence of SEQ ID NO: 139; CDR3 comprises an amino acid sequence of SEQ ID NO: 170;

CDR1 comprises an amino acid sequence of SEQ ID NO: 111; CDR2 comprises an amino acid sequence of SEQ ID NO: 140; CDR3 comprises an amino acid sequence of SEQ ID NO: 171;

CDR1 comprises an amino acid sequence of SEQ ID NO: 112; CDR2 comprises an amino acid sequence of SEQ ID NO: 141; CDR3 comprises an amino acid sequence of SEQ ID NO: 172;

CDR1 comprises an amino acid sequence of SEQ ID NO: 113; CDR2 comprises an amino acid sequence of SEQ ID NO: 142; CDR3 comprises an amino acid sequence of SEQ ID NO: 173;

CDR1 comprises an amino acid sequence of SEQ ID NO: 114; CDR2 comprises an amino acid sequence of SEQ ID NO: 143; CDR3 comprises an amino acid sequence of SEQ ID NO: 174; or

CDR1 comprises an amino acid sequence of SEQ ID NO: 115; CDR2 comprises an amino acid sequence of SEQ ID NO: 144; CDR3 comprises an amino acid sequence of SEQ ID NO: 175

~~or having any one of SEQ ID NOS: 87 to 94 or SEQ ID NOS: 96 to 115;~~

~~(b) CDR2 comprises an amino acid sequence of SEQ ID NO: 121 or having any one of SEQ ID NOS: 116 to 120 or SEQ ID NOS: 122 to 144; and~~

~~(c) CDR3 comprises an amino acid sequence of SEQ ID NO: 152 or having any one of SEQ ID NOS: 145 to 151 or SEQ ID NOS: 153 to 175~~.

~~7~~6. The chimeric protein of claim 1, wherein the targeting moiety comprises ~~an~~ the amino acid sequence ~~having at least 95% sequence similarity with~~of SEQ ID NO: 58 or any one of SEQ ID NOS: 2 to 42, 46 to 57, or 59 to 86.

~~8. The chimeric protein of claim 1, wherein the mutant IFNα2 comprises an amino acid sequence having at least 95% identity with SEQ ID NO: 176 or 177 and has one or more mutations at positions 144 to 154 with respect to SEQ ID NO: 176 or 177 or one or more of positions L15, A19, R22, R23, L26, F27, L30, L30, K31, D32, R33, H34, D35, Q40, H57, E58, Q61, F64, N65, T69, L80, Y85, Y89, D114, L117, R120, R125, K133, K134, R144, A145, A145, M148, R149, S152, L153, and N156 with respect to SEQ ID NO: 176 or 177.~~

~~9. The chimeric protein of claim 8, wherein the mutant IFNα2 has one or more mutations at position R149, M148, or L153.~~

~~10~~7. The chimeric protein of claim ~~8~~1, wherein the mutation is one or more of ~~L15A, A19W, R22A, R23A, L26A, F27A, L30A, L30V, K31A, D32A, R33K, R33A, R33Q, H34A, D35A, Q40A, H57Y, E58N, Q61S, F64A, N65A, T69A, L80A, Y85A, Y89A, D114R, L117A, R120A, R125A, K133A, K134A, R144A, A145G, A145M, M148A, R149A, S152A, L153A, and N156A, optionally~~ K133A, M148A, R149A, or L153A with respect to SEQ ID NO: 176 or 177.

~~11~~8. The chimeric protein of claim 1, wherein the FAP binding agent comprises one or more additional targeting moieties that recognize ~~or functionally modulate~~ a tumor antigen or an antigen on an immune cell selected from a T cell, a B cell, a dendritic cell, a macrophage, neutrophil, and a NK cell.

~~12~~9. The chimeric protein of claim ~~11~~8, wherein the one or more additional targeting moieties recognize ~~or functionally modulate~~ PD-1, PD-L1, PD-L2, CTLA4, OX40L, OX40, CD20, XCR1, and Clec9A.

~~13~~10. The chimeric protein of claim 1, wherein the ~~disease-associated~~F2 fibroblast is associated with a disease selected from cancer, infections, immune disorders, autoimmune disease, fibrotic diseases, and cardiovascular disease.

~~14~~11. A recombinant nucleic acid encoding the chimeric protein of claim 1, or a Fc-based component thereof.

~~15~~12. A host cell comprising the nucleic acid of claim ~~14~~11.

~~16~~13. The chimeric protein of any one of claims 1-~~13~~ 10 for use in treating ~~or preventing~~ cancer, infections, immune disorders, fibrotic diseases, and/or autoimmune diseases that are related to the FAP protein, comprising administering an effective amount of the chimeric protein to a patient in need thereof.

~~17. A FAP binding agent comprising three complementarity determining regions (CDR1, CDR2, and CDR3), wherein~~

~~(a) CDR1 comprises an amino acid sequence of SEQ ID NO: 95 or having any one of SEQ ID NOS: 87 to 94 or SEQ ID NOS: 96 to 115;~~

~~(b) CDR2 comprises an amino acid sequence of SEQ ID NO: 121 or having any one of SEQ ID NOS: 116 to 120 or SEQ ID NOS: 122 to 144; and~~

~~(c) CDR3 comprises an amino acid sequence of SEQ ID NO: 152 or having any one of SEQ ID NOS: 145 to 151 or SEQ ID NOS: 153 to 175.~~

~~18. The FAP binding agent of claim 17, wherein the targeting moiety is a full-length antibody, a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a single-chain antibody (scFv), a shark heavy-chain-only antibody (VNAR), a microprotein, a darpin, an anticalin, an adnectin, a Fv, a Fab, a Fab', or a F(ab')2.~~

~~19. The FAP binding agent of claim 17, wherein the targeting moiety comprises an amino acid sequence having at least 90% sequence similarity with SEQ ID NO: 58 or any one of SEQ ID NOS: 2 to 42, 46 to 57, or 59 to 86.~~

~~20. A recombinant nucleic acid encoding the FAP binding agent of clam 17, or a component thereof.~~

~~21. A host cell comprising the nucleic acid of claim 20.~~

~~22. The FAP binding agent of any one of claims 17-19 for use in treating or preventing cancer, infections, immune disorders, fibrotic diseases, and/or autoimmune diseases, comprising administering an effective amount of the FAP binding agent to a patient in need thereof.~~

~~23~~14. The chimeric protein of any one of claims 1-~~13~~10, for use as a medicament.

~~24~~15. Use of the chimeric protein of any one of claims 1-~~13~~ 10 in the manufacture of a medicament for treating ~~or preventing~~ cancer, infections, immune disorders, fibrotic diseases, and/or autoimmune diseases that are related to the FAP protein.

~~25. The FAP binding agent of any one of claims 17-19, for use as a medicament.~~

~~26. Use of the FAP binding agent of any one of claims 17-19 in the manufacture of a medicament for treating or preventing cancer, infections, immune disorders, fibrotic diseases, and/or autoimmune diseases.~~