effective at preventing tissue binding, and may therefore be responsible for the greater ECM binding of the FGFR4 ECD Fc fusions observed *in vitro*.

[008] We have engineered FGFR4 ECDs, called FGFR4 ECD acidic region muteins, that have an increase in the total number of acidic residues within the D1-D2 linker and thus a "stronger" acid box region to reduce ECM binding and potentially reduce tissue binding *in vivo* and to increase the bioavailability of FGFR4 ECD fusion proteins. We have discovered that FGFR4 ECD acidic region muteins that contain an increased number of acidic residues within the D1-D2 linker exhibit decreased ECM binding. We have also discovered that certain FGFR4 ECD acidic region muteins exhibit decreased tissue binding and increased bioavailability. Thus, by increasing the total number of acidic residues within the D1-D2 linker, thus "strengthening" the FGFR4 acid box, we have engineered FGFR4 ECD acidic region muteins with improved properties, including decreased ECM binding and decreased tissue binding, which can in turn lead to increased bioavailability of FGFR4 ECD fusion proteins.

[008a] In a first aspect, the present invention refers to a polypeptide comprising an FGFR4 extracellular domain (ECD) acidic region mutein, wherein the FGFR4 ECD acidic region mutein is selected from an FGFR4 ECD acidic region chimera and an FGFR4 long acid box variant, and wherein the FGFR4 ECD acidic region mutein has a greater number of acidic residues in the D1-D2 linker region than wild-type FGFR4 ECD.

[008b] In a second aspect, the present invention refers to an FGFR4 ECD fusion molecule comprising a polypeptide as defined above and a fusion partner.

[008c] In a third aspect, the present invention refers to a polynucleotide comprising a nucleic acid sequence that encodes the polypeptide as defined above or an FGFR4 ECD fusion molecule as defined above.

[008d] In a fourth aspect, the present invention refers to a pharmaceutical composition comprising the polypeptide or FGFR4 fusion molecule as defined above, and a pharmaceutically acceptable carrier.

[008e] In a fifth aspect, the present invention refers to the use of the pharmaceutical composition as defined above in the manufacture of a medicament for treating an angiogenic disorder in a patient.

[008f] In a sixth aspect, the present invention refers to a polypeptide comprising an FGFR2 ECD acidic region mutein, wherein the FGFR2 ECD acidic region mutein has a greater number of acidic residues in the D1-D2 linker region than wild-type FGFR2 ECD.

[008g] In a seventh aspect, the present invention refers to a polypeptide comprising an FGFR3 ECD acidic region mutein, wherein the FGFR3 ECD acidic region mutein has a greater number of acidic residues in the D1-D2 linker region than wild –type FGFR3 ECD.

[009] In one approach for generating "stronger" FGFR4 ECD acidic region muteins, we have replaced certain non-acidic amino acid residues with acidic amino acid residues, such that the total number of acidic residues within the FGFR4 ECD long acid box is increased relative to the wild-type FGFR4 ECD long acid box. This class of FGFR4 ECD acidic region muteins is referred to herein as "FGFR4 ECD long acid box variants." We discovered that FGFR4 ECD long acid box variants that comprise two more acidic amino acid residues than the wild-type FGFR4 ECD long acid box exhibited decreased ECM binding. *See* **FIG. 14**. FGFR4 ECD long acid box variants that contained four more acidic amino acid residues in the long acid box exhibited even further decreased ECM binding. *See id.*

[010] In another approach for generating "stronger" FGFR4 ECD acidic region muteins, we have replaced all or portions of the D1-D2 linker region of FGFR4 with all or portions of the D1-D2 linker region of FGFR1, FGFR2, or FGFR3 to generate polypeptides in a class of FGFR4 ECD acidic region muteins referred to herein as "FGFR4 ECD acidic region chimeras." FGFR4 ECD acidic region chimeras include FGFR4 ECD D1-D2 linker chimeras, FGFR4 ECD exon 4 chimeras, FGFR4 ECD acid box chimeras, FGFR4 ECD long acid box chimeras, and FGFR4 ECD short acid box chimeras. We found that at least certain FGFR4 ECD D1-D2 linker chimeras, FGFR4 ECD exon 4 chimeras, and FGFR4 ECD acid box chimeras, retained the ability to bind to

both FGF2 and FGF19, but showed decreased levels of ECM binding *in vitro* when compared to a parental FGFR4 ECD Fc fusion. *See* **Tables 3 and 4**, and **FIGs. 6 and 12**. Further, the FGFR4 ECD acidic region chimeras showed decreased binding to the surface of hepatocytes when compared to a parental FGFR4 ECD Fc fusion. *See* **FIG. 7**. *In vitro* ECM binding by an FGFR4 acidic region chimera was further reduced by introducing an N-glycan mutation either adjacent to the amino-terminus of the FGFR4 ECD D1-D2 linker or in the D2 heparin binding site. *See* **FIG. 15**.

[011] In *in vivo* studies, an FGFR4 ECD acidic region mutein exhibited substantially improved bioavailability and serum half-life relative to a parental FGFR4 ECD Fc fusion. *See* **FIG. 8**. Mice injected with the FGFR4 ECD acidic region mutein showed a statistically significant reduction in tumor burden in certain tumor models when compared to a control group, showing that the FGFR4 ECD acidic region muteins possess a similar anti-tumor activity as FGFR4 ECDs administered *in vivo*. *See* **FIG. 9**. The FGFR4 ECD acidic region muteins may therefore be used, e.g., to treat proliferative disorders, including cancer, disorders of angiogenesis, and macular degeneration.

[012] Both the FGFR2 ECD-Fc and FGFR3 ECD-Fc fusion proteins showed significantly lower levels of ECM binding *in vitro* than a parental FGFR4 ECD-Fc, however they showed slightly higher levels of ECM binding than an FGFR1 ECD-Fc fusion protein at higher protein concentrations. *See* **FIG. 5**. Although the FGFR2 and FGFR3 acid boxes contain a greater number of acidic amino acid residues than the FGFR4 acid box, they both contain fewer acidic amino acid residues than the FGFR1 acid box. *See* **FIGs. 11D** and **11E**, respectively. *In vitro* ECM binding experiments showed that FGFR2 and FGFR3 ECD acidic region muteins in which the total number of acidic residues within the FGFR2 and FGFR3 long acid boxes was increased exhibited decreased ECM binding relative to the parental FGFR2 and FGFR3 ECDs. *See* **FIGs. 17A and 17B**, respectively.

[013] In certain example~~embodiments~~, FGFR1, FGFR2, and FGFR3 ECDs may be engineered to have a decrease in the total number of acidic residues within the D1-D2 linker, and thus a "weaker" acid box to increase tissue binding *in vivo* and to decrease the bioavailability of the FGFR1, FGFR2, and FGFR3 ECD fusion proteins. Such "weakened" FGFR1, FGFR2, and FGFR3 ECDs may be useful, for example, when delivered locally, to prevent toxicity and/or side effects that might occur with systemic administration.

[014] In certain ~~embodiments~~examples, a polypeptide comprising an FGFR4 ECD acidic region mutein is provided. In certain ~~embodiments~~examples, an isolated polypeptide comprising an FGFR4 ECD acidic region mutein is provided. In certain ~~embodiments~~examples, an FGFR4 ECD acidic region mutein is an FGFR4 ECD D1-D2 linker chimera. In certain ~~embodiments~~examples, the FGFR4 ECD D1-D2 linker chimera comprises a D1-D2 linker selected from an FGFR1 D1-D2 linker, an FGFR2 D1-D2 linker, and an FGFR3 D1-D2 linker, in place of the FGFR4 D1-D2 linker. In certain ~~embodiments~~examples, the FGFR4 ECD D1-D2 linker chimera comprises an amino acid sequence selected from SEQ ID NOs: 22, 26, 28, and 32, in place of an FGFR4 D1-D2 linker selected from SEQ ID NOs: 16 and 17. In certain ~~embodiments~~examples, the FGFR4 ECD D1-D2 linker chimera comprises an amino acid sequence selected from SEQ ID NOs: 35 to 38.

[015] In certain ~~embodiments~~examples, an FGFR4 ECD acidic region mutein is an FGFR4 ECD exon 4 chimera. In certain ~~embodiments~~examples, the FGFR4 ECD exon 4 chimera comprises an exon 4 selected from an FGFR1 exon 4, an FGFR2 exon 4, and an FGFR3 exon 4, in place of the FGFR4 exon 4. In certain ~~embodiments~~examples, the FGFR4 ECD exon 4 chimera comprises an amino acid sequence selected from SEQ ID NOs: 23, 92, 29, and 33, in place of an FGFR4 D1-D2 linker selected from SEQ ID NOs: 18 and 19. In certain ~~embodiments~~examples, the FGFR4 ECD exon 4 chimera comprises an amino acid sequence selected from SEQ ID NOs: 39 to 42.

[016] In certain ~~embodiments~~examples, an FGFR4 ECD acidic region mutein is an FGFR4 ECD acid box chimera. In certain ~~embodiments~~examples, the FGFR4 ECD acid box chimera comprises an acid box selected from the FGFR1 acid box, the FGFR2 acid box, and the FGFR3 acid box, in place of the FGFR4 acid box. In certain ~~embodiments~~examples, the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 24, 30, and 34 in place of the FGFR4 acid box having an amino acid sequence of SEQ ID NO: 20. In certain ~~embodiments~~examples, an FGFR4 ECD acid box chimera comprises an acid box region selected from an FGFR1 acid box region, an FGFR2 acid box region, and an FGFR3 acid box region, in place of the FGFR4 acid box. In certain ~~embodiments~~examples, the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 56 to 65, in place of the FGFR4 acid box having an amino acid sequence of SEQ ID NO: 20. In certain ~~embodiments~~examples, the FGFR4 ECD acid box chimera comprises an acid box region selected from an FGFR1 acid box region, an FGFR2 acid box region, and an FGFR3 acid box region, in place of an FGFR4 acid box region. In certain ~~embodiments~~examples,

the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 56 to 65, in place of an FGFR4 acid box region having an amino acid sequence selected from SEQ ID NOs: 46 to 55. In certain ~~embodiments~~examples, the FGFR4 ECD acid box chimera comprises an FGFR1 acid box region having the amino acid sequence of SEQ ID NO: 56 in place of an FGFR4 acid box region having the amino acid sequence of SEQ ID NO: 51. In certain ~~embodiments~~examples, the FGFR4 ECD acid box chimera comprises an acid box selected from the FGFR1 acid box, the FGFR2 acid box, and the FGFR3 acid box, in place of an FGFR4 acid box region. In certain ~~embodiments~~examples, the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 24, 30, and 34 in place of an FGFR4 acid box region having an amino acid sequence selected from SEQ ID NOs: 46 to 55. In certain ~~embodiments~~examples, the FGFR4 ECD acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 43 to 45 and 157.

[017] In certain ~~embodiments~~examples, an FGFR4 ECD acidic region chimera is an FGFR4 ECD long acid box chimera. In certain ~~embodiments~~examples, the FGFR4 ECD long acid box chimera comprises a long acid box selected from an FGFR1 long acid box, an FGFR2 long acid box, and an FGFR3 long acid box, in place of the FGFR4 long acid box. In certain ~~embodiments~~examples, the FGFR4 long acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 98 to 100, in place of an FGFR4 long acid box selected from SEQ ID NOs: 96 and 97. In certain ~~embodiments~~examples, the FGFR4 long acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 105 to 107.

[018] In certain ~~embodiments~~examples, an FGFR4 ECD acidic region mutein is an FGFR4 ECD short acid box chimera. In certain ~~embodiments~~examples, the FGFR4 ECD short acid box chimera comprises a short acid box selected from an FGFR1 short acid box, an FGFR2 short acid box, and an FGFR3 short acid box, in place of the FGFR4 short acid box. In certain ~~embodiments~~examples, the FGFR4 ECD short acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 102 to 104, in place of an FGFR4 short acid box having an amino acid sequence of SEQ ID NO: 101. In certain ~~embodiments~~examples, the FGFR4 ECD short acid box chimera comprises an amino acid sequence selected from SEQ ID NOs: 108 to 110.

In certain ~~embodiments~~examples, the FGFR4 ECD acidic region mutein is an FGFR4 ECD long acid box variant. In certain ~~embodiments~~examples, the the FGFR4 ECD long acid box variant comprises a variant of the FGFR4 ECD that has an increased number of acidic amino acid residues in the long acid box relative to the FGFR4 wild-type long acid box. In certain

such ~~embodiments~~examples, at least two, three, or four non-acidic residues within the long acid box of the FGFR4 ECD are each independently replaced with an acidic residue selected from Glu (E) and Asp (D). In certain such ~~embodiments~~examples, at least one acidic residue is inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2. In certain ~~embodiments~~examples, two acidic residues are inserted between amino acids 103 and 104 of SEQ ID NOs: 1 and 2. In certain such ~~embodiments~~examples, the number of acidic residues in the FGFR4 long acid box is at least seven. In certain such ~~embodiments~~examples, FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 106 to 117 (SEQ ID NO: 149); FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 107 to 117 (SEQ ID NO: 150); FGFR4 ECD residues 104 to 110 (SEQ ID NO: 146) are replaced with FGFR1 ECD residues 105-113 (SEQ ID NO: 151); FGFR4 ECD residues 113 to 116 (SEQ ID NO: 147) are replaced with FGFR1 ECD residues 116-119 (SEQ ID NO: 152); or FGFR4 ECD residues 109 to 113 (SEQ ID NO: 148) are replaced with FGFR1 ECD residues 112-116 (SEQ ID NO: 153). In certain such ~~embodiments~~examples, FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 106 to 117 (SEQ ID NO: 149); FGFR4 ECD residues 104 to 114 (SEQ ID NO: 145) are replaced with FGFR1 ECD residues 107 to 117 (SEQ ID NO: 150); FGFR4 ECD residues 104 to 110 (SEQ ID NO: 146) are replaced with FGFR1 ECD residues 105-113 (SEQ ID NO: 151); FGFR4 ECD residues 113 to 116 (SEQ ID NO: 147) are replaced with FGFR1 ECD residues 116-119 (SEQ ID NO: 152); or FGFR4 ECD residues 109 to 113 (SEQ ID NO: 148) are replaced with FGFR1 ECD residues 112-116 (SEQ ID NO: 153).

[019] In certain ~~embodiments~~examples, an FGFR4 ECD fusion molecule comprising an FGFR4 ECD acidic region mutein and a fusion partner is provided. In certain ~~embodiments~~examples, an isolated FGFR4 ECD fusion molecule comprising an FGFR4 ECD acidic region mutein and a fusion partner is provided. In certain ~~embodiments~~examples, an FGFR4 ECD fusion molecule comprising an amino acid sequence selected from SEQ ID NOs: 35 to 45, 105 to 121, and 157 is provided. In certain ~~embodiments~~examples, an isolated FGFR4 ECD fusion molecule comprising an amino acid sequence selected from SEQ ID NOs: 35 to 45, 105 to 121, and 157 is provided. In certain ~~embodiments~~examples, an FGFR4 ECD fusion molecule comprising the amino acid sequence of SEQ ID NO: 35, is provided. In certain ~~embodiments~~examples, an isolated FGFR4 ECD fusion molecule comprising the amino acid sequence of SEQ ID NO: 35, is provided. In certain ~~embodiments~~examples, the fusion partner is selected from Fc, albumin, and polyethylene glycol. In certain ~~embodiments~~examples, the fusion

partner is Fc. In certain ~~embodiments~~examples, an FGFR4 ECD fusion molecule comprising an amino acid sequence selected from SEQ ID NOs: 86 to 88, 124 to 140, 143, 144, and 158 is provided. In certain ~~embodiments~~examples, an isolated FGFR4 ECD fusion molecule comprising an amino acid sequence selected from SEQ ID NOs: 86 to 88, 124 to 140, 143, 144, and 158 is provided. In certain ~~embodiments~~examples, an FGFR4 ECD fusion molecule comprising the amino acid sequence of SEQ ID NO: 86 is provided. In certain ~~embodiments~~examples, an isolated FGFR4 ECD fusion molecule comprising the amino acid sequence of SEQ ID NO: 86 is provided. In certain ~~embodiments~~examples, an FGFR4 ECD fusion molecule consisting of the amino acid sequence of SEQ ID NO: 86 is provided. In certain ~~embodiments~~examples, an isolated FGFR4 ECD fusion molecule consisting of the amino acid sequence of SEQ ID NO: 86 is provided.

[020] In certain ~~embodiments~~examples, a pharmaceutical composition comprising an FGFR4 ECD acidic region mutein and a pharmaceutically acceptable carrier is provided. In certain ~~embodiments~~examples, a polynucleotide comprising a nucleic acid sequence that encodes an FGFR4 ECD acidic region mutein is provided.

[021] In certain ~~embodiments~~examples, a method of treating an angiogenic disorder in a patient comprising administering to the patient a pharmaceutical composition comprising an FGFR4 ECD acidic region mutein is provided. In certain ~~embodiments~~examples, a method of treating cancer in a patient comprising administering to the patient a pharmaceutical composition comprising an FGFR4 ECD acidic region mutein is provided. In certain ~~embodiments~~examples, the cancer is selected from colon, liver, lung, breast, and prostate cancers. In certain ~~embodiments~~examples, a method of treating macular degeneration in a patient comprising administering to the patient a pharmaceutical composition comprising an FGFR4 ECD acidic region mutein is provided.

[022] In certain ~~embodiments~~examples, the FGFR4 acidic region mutein comprises at least one point mutation that inhibits glycosylation. In certain ~~embodiments~~examples, the at least one point mutation that inhibits glycosylation is selected from N91A, N156A, N237A, N269A, N290A, and N301A. In certain ~~embodiments~~examples, the FGFR4 acidic region mutein comprises an amino acid sequence selected from SEQ ID NOs: 120, 121, and 168.

[023] In certain ~~embodiments~~examples, an FGFR2 ECD acidic region mutein is provided. In certain ~~embodiments~~examples, the FGFR2 ECD acidic region mutein is an FGFR2 ECD short acid box chimera. In certain ~~embodiments~~examples, the FGFR2 ECD short acid box chimera comprises at least the FGFR1 short acid box in place of at least the FGFR2 short acid box.

In certain such ~~embodiments~~examples, FGFR2 ECD residues 111 to 118 (SEQ ID NO: 155) are replaced with FGFR1 ECD residues 105 to 112 (SEQ ID NO: 154). In certain ~~embodiments~~examples, the FGFR2 ECD short acid box chimera comprises the amino acid sequence of SEQ ID NO: 122.

[024] In certain ~~embodiments~~examples, an FGFR3 ECD acidic region mutein is provided. In certain ~~embodiments~~examples, the FGFR3 ECD acidic region mutein is an FGFR3 ECD short acid box chimera. In certain ~~embodiments~~examples, the FGFR3 ECD short acid box chimera comprises at least the FGFR1 short acid box in place of at least the FGFR3 short acid box. In certain such ~~embodiments~~examples, FGFR3 ECD residues 110 to 117 (SEQ ID NO: 156) are replaced with FGFR1 ECD residues 105 to 112 (SEQ ID NO: 154). In certain ~~embodiments~~examples, the FGFR3 ECD short acid box chimera comprises the amino acid sequence of SEQ ID NO: 123.

BRIEF DESCRIPTION OF THE DRAWINGS

[025] **FIG. 1** shows the extracellular domain (ECD) amino acid sequence of FGFR4 with a 17 amino acid C-terminal deletion, which was fused to an Fc domain in the parental FGFR4 ECD-Fc (also referred to herein as "R4Mut4"). The amino acid sequence in Figure 1 includes the signal peptide, which is cleaved in the mature fusion protein. The numbers refer to the amino acid position, and certain domains within the ECD are illustrated in gray above the amino acid numbers. The amino acid positions within the signal peptide are given negative values because they are cleaved in the mature fusion protein. The first amino acid residue of the mature fusion protein is designated as amino acid position 1. The linker between the first and second Ig domains (referred to herein interchangeably as the "linker domain," "linker region," "D1-D2 linker," and "D1-D2 linker region") is illustrated in a darker gray.

[026] **FIG. 2** shows a sequence alignment of the linker domains from FGFR1 and FGFR4 and the boundaries and sequence of the swapped regions in the three variants, called FGFR4ECD(ABMut1: delta17)-Fc (ABMut1), FGFR4ECD(ABMut2: delta17)-Fc (ABMut2), and FGFR4ECD(ABMut3: delta17)-Fc (ABMut3). Acidic residues within the D1-D2 linker are indicated with underlining and bold font.

[027] **FIG. 3** shows the binding of R1Mut4, three different preparations of R4Mut4, R4Mut4 plus heparin, and an IgG control to hepatocytes, detected by flow