

CLAIMS

1. A multispecific binding molecule comprising:
 - (i) a first binding domain that specifically binds to a lymphotoxin beta receptor (LTBR), and
 - (ii) a second binding domain that specifically binds to extra domain B (EDB) of fibronectin,wherein the multispecific binding molecule activates LTBR upon binding of the EDB.
2. The multispecific binding molecule of claim 1, wherein the multispecific binding molecule activates LTBR in a tumor specific manner.
3. The multispecific binding molecule of claim 1 or 2, wherein the multispecific binding molecule is a bispecific antibody.
4. The multispecific binding molecule of any one of claims 1 to 3, wherein the multispecific binding molecule comprises two antigen binding domains.
5. The multispecific binding molecule of any one of claims 1 to 3, wherein the multispecific binding molecule comprises three antigen binding domains.
6. The multispecific binding molecule of claim 5, wherein the three antigen binding domains comprise one binding domain that specifically binds to LTBR.
7. The multispecific binding molecule of claim 5 or 6, wherein the three antigen binding domains comprise two binding domains that specifically bind EDB.
8. The multispecific binding molecule of any one of claims 5 to 7, wherein the binding domain that specifically binds to LTBR comprises a single chain variable domain of an antibody.
9. The multispecific binding molecule of any one of claims 1 to 8, wherein the first binding domain that specifically binds to LTBR comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and the VL comprises a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, wherein said VH and VL comprise any of the following:
 - (i) HCDR1, HCDR2 and HCDR3 comprising the amino acid sequences of SEQ ID NO:60, SEQ ID NO:61, and SEQ ID NO:62, respectively, and LCDR1, LCDR2 and LCDR3 comprising the amino acid sequences of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, respectively; or
 - (ii) HCDR1, HCDR2 and HCDR3 comprising the amino acid sequences of SEQ ID NO:83, SEQ

ID NO:61, and SEQ ID NO:62, respectively, and LCDR1, LCDR2 and LCDR3 comprising the amino acid sequences of SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, respectively; or
 (iii) HCDR1, HCDR2 and HCDR3 comprising the amino acid sequences of SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, respectively, and LCDR1, LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:69, SEQ ID NO:70, and SEQ ID NO:71, respectively; or
 (iv) VH comprises an amino acid sequence having at least 95%, 96%, 97%, 98%, 99% identity or 100% identity to the amino acid sequence of SEQ ID NO:43, and VL comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:44; or
 (v) VH comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:47, and VL comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:48: or
 (vi) SEQ ID NO: 22; or
 (vii) SEQ ID NO: 23; or
 (viii) SEQ ID NO: 25.

10. The multispecific binding molecule of any one of claims 1 to 9, wherein the second binding domain that specifically binds to EDB comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and a HCDR3, and the VL comprises a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, wherein said VH and VL comprise any of the following:

(i) HCDR1, HCDR2 and HCDR3 comprising the amino acid sequences of SEQ ID NO:72, SEQ ID NO:73, and SEQ ID NO:74, respectively, and LCDR1, LCDR2 and LCDR3 comprising the amino acid sequences of SEQ ID NO:75, SEQ ID NO:76, and SEQ ID NO:77, respectively; or
 (ii) VH comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:45, and VL comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:46.

11. The multispecific binding molecule of any one of claims 1 to 10, comprising any of:
 (a) (i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 1 forming a binding domain with a first light chain comprising the amino acid sequence of SEQ ID NO: 2, and
 (ii) a second heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a second light chain comprising the amino acid sequence of SEQ ID NO: 5;

or

(b) (i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 9 forming a binding domain with a first light chain comprising the amino acid sequence of SEQ ID NO: 10, and (ii) a second heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a second light chain comprising the amino acid sequence of SEQ ID NO: 5.

12. The multispecific binding molecule of any one of claims 1 to 3 or 5 to 10, comprising any of:

(c) (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 30, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5; or

(d) (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 31, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5; or

(e) (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 32, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5; or

(f) (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 33, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5; or

(g) (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 34, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of

SEQ ID NO: 5; or

(h) (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 35, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5; or

(j) (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 38, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5; or

(k) (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 39, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5; or

(l) (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 56, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5.

13. The multispecific binding molecule of any one of claims 1 to 3 or 5 to 10, comprising (i) an scFv-heavy chain fusion comprising the amino acid sequence of SEQ ID NO: 38, the heavy chain part thereof forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5, and (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 forming a binding domain with a light chain comprising the amino acid sequence of SEQ ID NO: 5.

14. One or more nucleic acid molecules encoding the multispecific binding molecule of any one of claims 1 to 13.

15. One or more vectors comprising the one or more nucleic acid molecules of claim 14.

16. An isolated host cell comprising the one or more nucleic acid molecules of claim 14 or the one or more vectors of claim 15.
17. A pharmaceutical composition comprising the multispecific binding molecule of any one of claims 1 to 13 and a pharmaceutically acceptable carrier.
18. ~~A method of treating cancer in a subject in need thereof, comprising administering to the subject~~ The multispecific binding molecule of any one of claims 1 to 13, the one or more nucleic acid molecules of claim 14, the one or more vectors of claim 15, or the pharmaceutical composition of claim 17 for use in the treatment of cancer, wherein the multispecific binding molecule, nucleic acid molecules, vectors, or pharmaceutical composition is to be administered to a subject in need thereof.
19. Use of the multispecific binding molecule of any one of claims 1 to 13, the one or more nucleic acid molecules of claim 14, the one or more vectors of claim 15, or the pharmaceutical composition of claim 17, in the manufacture of a medicament for activating LTBR in tumor tissue.
20. A method of producing a multispecific binding molecule of any one of claims 1 to 13, the method comprising expressing the one or more nucleic acid molecules of claim 14 or the one or more vectors of claim 15 in a host cell and harvesting the multispecific binding molecule.