

Summary

[0004] Provided herein are CD28-binding polypeptides having at least one single domain antibody (sdAb) that binds CD28, wherein the at least one CD28 sdAb contains a complementarity determining region 1 (CDR1) having an amino acid sequence selected from the group consisting of SEQ ID NO:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) having an amino acid sequence selected from the group consisting of SEQ ID NO:190, 193, 196, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, and 212; and a complementarity determining region 3 (CDR3) having an amino acid sequence selected from the group consisting of SEQ ID NO: 191, 194, and 197.

[0005] In some examples, the at least one CD28 sdAb contains: a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:189, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:191; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:192, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:194; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:198, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:199, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:200, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:202, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:203, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID

NO:204, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:205, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:207, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:208, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:209, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:210, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:211, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; or a CDR1 comprising an amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:212, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197.

[0006] Also provided herein are CD28-binding polypeptides having at least one single domain antibody (sdAb) that binds CD28, wherein the at least one CD28 sdAb comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:190, 193, 196, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, and 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 191, 194, and 197, 396, 397, and 398.

[0006a] In one aspect, the present disclosure refers to a CD28-binding polypeptide comprising at least one single domain antibody (sdAb) that binds CD28, wherein the at least one CD28 sdAb comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:195, 189, 192, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:206, 190, 193, 196, 202, 204, 205, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 197, 191, 194, 396, 397, and 398.

[0007] In some examples, the at least one CD28 sdAb contains: a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:189, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 comprising the amino acid sequence set

acid sequence set forth in SEQ ID NO:204, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:396; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:394, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:395, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:397; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:396; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:388, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:389, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:397; or a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398.

[0008] In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:189, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:191. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:192, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:194. In some examples, the at least one CD28 sdAb

acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:397. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:396. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:388, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:389, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:397; or a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398.

[0009] Also provided herein are CD28-binding polypeptides having at least one single domain antibody (sdAb) that binds CD28, wherein the at least one CD28 sdAb contains a complementarity determining region 1 (CDR1) having an amino acid sequence selected from the group consisting of SEQ ID NO:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) having an amino acid sequence selected from the group consisting of SEQ ID NO:190, 193, 196, 202, 204, 205, 206, 207, 208, 209, 210, 211, and 212; and a complementarity determining region 3 (CDR3) having an amino acid sequence selected from the group consisting of SEQ ID NO: 191, 194, and 197.

[0010] In some examples, the at least one CD28 sdAb contains: a CDR1 having an amino acid sequence set forth in SEQ ID NO:189, a CDR2 having the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:191; a CDR1 having an amino acid sequence set forth in SEQ ID NO:192, a CDR2 having the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:194; a CDR1 having an amino acid sequence set forth in SEQ

CDR2 having the amino acid sequence set forth in SEQ ID NO:212, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197.

[0011] Also provided herein are CD28-binding polypeptides containing at least one single domain antibody (sdAb) that binds CD28, wherein the at least one CD28 sdAb comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:190, 193, 196, 202, 204, 205, 206, 207, 208, 209, 210, 211, and 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 191, 194, and 197, 396, 397, and 398.

[0012] In some examples, the at least one CD28 sdAb contains: a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:189, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:191; a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:192, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:194; a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:198, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:199, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:200, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:202, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197; a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence

sequence set forth in SEQ ID NO:367; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:368; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:369; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:370; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:371; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:372; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:373; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:374; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:375; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:376; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:377; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:378; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:379; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:380; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:381; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:382; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:383; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:384; or a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:385.

[0014a] In one aspect, the present disclosure refers to a CD28-binding polypeptide comprising at least one single domain antibody (sdAb) that binds CD28, wherein the at least one CD28 sdAb (CD28 VHH) domain comprises: a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:342; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:186; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:187; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:188; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:213; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:214; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:215; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:216; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:217; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:218; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:219; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:221; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:222; a CDR1, a CDR2, and a

[0015] In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:186. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:187. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:188. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:213. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:214. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:215. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID

sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:371. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:372. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:373. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:374. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:375. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:376. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:377. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:378. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:379. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:380. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:381. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:382. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:383. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:384; or a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:385.

[0016] Also provided herein are CD28-binding polypeptides containing at least one single domain antibody (sdAb) that binds CD28 (CD28 VHH), wherein the CD28 VHH domain contains: a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:186; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:187; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:188; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:213; a CDR1, a CDR2, and a

forth in SEQ ID NO:378; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:379; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:380; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:381; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:382; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:383; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:384; or a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:385.

[0018] In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280.

[0019] In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385.

[0020] In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:186. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:187. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:188. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:213. In some examples, the at least one CD28 sdAb comprises the amino acid

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comprises the amino acid sequence set forth in SEQ ID NO:368. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:369. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:370. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:371. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:372. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:373. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:374. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:375. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:376. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:377. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:378. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:379. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:380. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:381. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:382. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:383. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:384. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO: 385.

[0021] In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280.

[0022] In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360,

361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385.

[0023] In some examples, the at least one CD28 sdAb contains the sequence set forth in (i) SEQ ID NO: 186, (ii) a humanized variant of SEQ ID NO:186, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 186. In some examples, the at least one CD28 sdAb contains the sequence set forth in SEQ ID NO: 186. In some examples, the at least one CD28 sdAb contains a humanized variant of SEQ ID NO:186. In some examples, the at least one CD28 sdAb contains a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 186. In some examples, the at least one CD28 sdAb contains a CDR1 having an amino acid sequence set forth in SEQ ID NO:189; a CDR2 having an amino acid sequence set forth in SEQ ID NO:190; and a CDR3 having an amino acid sequence set forth in SEQ ID NO:191.

[0024] In some examples, the at least one CD28 sdAb contains the sequence set forth in (i) SEQ ID NO: 187, (ii) a humanized variant of SEQ ID NO:187, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 187. In some examples, the at least one CD28 sdAb contains the sequence set forth in SEQ ID NO: 187. In some examples, the at least one CD28 sdAb contains a humanized variant of SEQ ID NO:187. In some examples, the at least one CD28 sdAb contains a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 187. In some examples, the at least one CD28 sdAb contains a CDR1 having an amino acid sequence set forth in SEQ ID NO:192; a CDR2 having an amino acid sequence set forth in SEQ ID NO:193; and a CDR3 having an amino acid sequence set forth in SEQ ID NO:194.

[0025] In some examples, the at least one CD28 sdAb contains the sequence set forth in (i) SEQ ID NO: 188, (ii) a humanized variant of SEQ ID NO:188, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 188. In some examples, the at least one CD28 sdAb contains the sequence set forth in SEQ ID NO: 188. In some examples, the at least one CD28 sdAb contains a humanized variant of SEQ ID NO:188. In some examples, the at least one CD28 sdAb contains a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 188.

[0026] In some examples, the at least one CD28 sdAb contains a CDR1 having an amino acid sequence set forth in any of SEQ ID NO:195, 198, 199, 200, and 201; a CDR2 having an amino acid sequence set forth in any of SEQ ID NO:196, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, and 212; and a CDR3 having an amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 containing the amino acid sequence set forth in any of SEQ ID NOS:195, 198, 199, 200, and 201; a CDR2 containing the amino acid sequence set forth in any of SEQ ID NOS:196, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a CDR3 containing the amino acid sequence set forth in any of SEQ ID NOS:197, 396, 397, and 398.

[0027] In some examples, the at least one CD28 sdAb contains a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 203, and 197, respectively; SEQ ID NOS: 195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; or SEQ ID NOS: 195, 212, and 197, respectively. In some examples, the at least one CD28 sdAb contains a CDR1, CDR2 and CDR3 set forth in: SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 203, and 197, respectively; SEQ ID NOS:

195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; SEQ ID NOS: 195, 212, and 197, respectively; SEQ ID NOS: 201, 202, and 197, respectively; SEQ ID NOS: 201, 202, and 396, respectively; SEQ ID NOS: 201, 386, and 197, respectively; SEQ ID NOS: 201, 387, and 197, respectively; SEQ ID NOS: 201, 202, and 397, respectively; SEQ ID NOS: 201, 202, and 398, respectively; SEQ ID NOS: 201, 206, and 197, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 388, and 197, respectively; SEQ ID NOS: 201, 389, and 197, respectively; SEQ ID NOS: 201, 206, and 397, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 203, and 197, respectively; SEQ ID NOS: 201, 203, and 396, respectively; SEQ ID NOS: 201, 390, and 197, respectively; SEQ ID NOS: 201, 391, and 197, respectively; SEQ ID NOS: 201, 203, and 397, respectively; SEQ ID NOS: 201, 203, and 398, respectively; SEQ ID NOS: 201, 204, and 197, respectively; SEQ ID NOS: 201, 204, and 396, respectively; SEQ ID NOS: 201, 392, and 197, respectively; SEQ ID NOS: 201, 393, and 197, respectively; SEQ ID NOS: 201, 204, and 397, respectively; SEQ ID NOS: 201, 204, and 398, respectively; SEQ ID NOS: 201, 196, and 396, respectively; SEQ ID NOS: 201, 394, and 197, respectively; SEQ ID NOS: 201, 395, and 197, respectively; SEQ ID NOS: 201, 196, and 397, respectively; SEQ ID NOS: 201, 196, and 398, respectively; SEQ ID NOS: 195, 206, and 396, respectively; SEQ ID NOS: 195, 388, and 197, respectively; SEQ ID NOS: 195, 389, and 197, respectively; SEQ ID NOS: 195, 206, and 397, respectively; or SEQ ID NOS: 195, 206, and 398, respectively.

[0028] In some examples, the at least one CD28 sdAb contains the sequence of amino acids set forth in any one of SEQ ID NOS:213-239 and 280, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NO:213-239 and 280. In some examples, the at least one CD28 sdAb contains the sequence of amino acids set forth in any one of SEQ ID NOS:213-239 and 280. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:213-239, 280, and 342-385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS:213-239, 280, and 342-385. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:213-239, 280, and 342-385.

[0029] In some examples, the at least one CD28 sdAb contains a CDR1 having an amino acid sequence set forth in any of SEQ ID NO:195, 198, 199, 200, and 201; a CDR2 having an amino acid sequence set forth in any of SEQ ID NO:196, 202, 204, 205, 206, 207, 208, 209, 210, 211, and 212; and a CDR3 having an amino acid sequence set forth in SEQ ID NO:197. In some examples, at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in any of SEQ ID NOS:195, 198, 199, 200, and 201; a CDR2 comprising the amino acid sequence set forth in any of SEQ ID NOS:196, 202, 204, 205, 206, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a CDR3 comprising the amino acid sequence set forth in any of SEQ ID NOS:197, 396, 397, and 398.

[0030] In some examples, the at least one CD28 sdAb contains a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; or SEQ ID NOS: 195, 212, and 197, respectively. In some examples, the at least one CD28 sdAb contains a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; SEQ ID NOS: 195, 212, and 197, respectively; SEQ ID NOS: 201, 202, and 197, respectively; SEQ ID NOS: 201, 202, and 396, respectively; SEQ ID NOS: 201, 386, and 197, respectively; SEQ ID NOS: 201, 387, and 197, respectively; SEQ ID NOS: 201, 202, and 397, respectively; SEQ ID NOS: 201, 202, and 398, respectively; SEQ ID NOS: 201, 206, and 197, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 388, and 197, respectively; SEQ ID NOS: 201, 389, and 197, respectively; SEQ ID NOS: 201, 206, and 397, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 203, and 197, respectively; SEQ ID NOS: 201, 203,

and 396, respectively; SEQ ID NOS: 201, 390, and 197, respectively; SEQ ID NOS: 201, 391, and 197, respectively; SEQ ID NOS: 201, 203, and 397, respectively; SEQ ID NOS: 201, 203, and 398, respectively; SEQ ID NOS: 201, 204, and 197, respectively; SEQ ID NOS: 201, 204, and 396, respectively; SEQ ID NOS: 201, 392, and 197, respectively; SEQ ID NOS: 201, 393, and 197, respectively; SEQ ID NOS: 201, 204, and 397, respectively; SEQ ID NOS: 201, 204, and 398, respectively; SEQ ID NOS: 201, 196, and 396, respectively; SEQ ID NOS: 201, 394, and 197, respectively; SEQ ID NOS: 201, 395, and 197, respectively; SEQ ID NOS: 201, 196, and 397, respectively; SEQ ID NOS: 201, 196, and 398, respectively; SEQ ID NOS: 195, 206, and 396, respectively; SEQ ID NOS: 195, 388, and 197, respectively; SEQ ID NOS: 195, 389, and 197, respectively; SEQ ID NOS: 195, 206, and 397, respectively; or SEQ ID NOS: 195, 206, and 398, respectively.

[0031] In some examples, the at least one CD28 sdAb contains the sequence of amino acids set forth in any one of SEQ ID NOS:213-219, 221-239, and 280, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NO:213-219, 221-239, and 280. In some examples, the at least one CD28 sdAb contains the sequence of amino acids set forth in any one of SEQ ID NOS:213-219, 221-239, and 280.

[0032] In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:213-219, 221-239, 280, and 342-385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS:213-219, 221-239, 280, and 342-385. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:213-239, 280, and 342-385.

[0033] In some examples, the at least one sdAb is one CD28 sdAb and/or the CD28-binding polypeptide is monovalent for CD28. In some examples, the at least one sdAb is one CD28 sdAb. In some examples, the CD28-binding polypeptide is monovalent for CD28. In some examples, the at least one sdAb is one CD28 sdAb and the CD28-binding polypeptide is monovalent for CD28.

[0034] In some examples, the at least one sdAb is two, three, or four CD28 sdAbs and/or the CD28-binding polypeptide is multivalent for CD28. In some examples, the at least one sdAb is two CD28 sdAbs and/or the CD28-binding polypeptide is bivalent for CD28. In some examples, the at least one sdAb is two CD28 sdAbs. In some examples, the CD28-binding polypeptide is bivalent for CD28. In some examples, the at least one sdAb is

two CD28 sdAbs and the CD28-binding polypeptide is bivalent for CD28. In some examples, the two CD28 sdAbs are the same CD28 sdAb. In some examples, both of the CD28 sdAbs contain the amino acid sequence set forth in SEQ ID NO:188. In some examples, each of the two CD28 sdAbs contain the amino acid sequence set forth in SEQ ID NO:188. In some examples, both of the CD28 sdAbs contain the amino acid sequence set forth in SEQ ID NO:220. In some examples, each of the two CD28 sdAbs contain the amino acid sequence set forth in SEQ ID NO:220. In some examples, both of the CD28 sdAbs contain the amino acid sequence set forth in SEQ ID NO:280. In some examples, each of the two CD28 sdAbs contain the amino acid sequence set forth in SEQ ID NO:280. In some examples, the two CD28 sdAbs are different CD28 sdAbs. In some examples, one of the two CD28 sdAbs contains the amino acid sequence set forth in SEQ ID NO:188 and the other of the two CD28 sdAbs contains the amino acid sequence set forth in SEQ ID NOS:220. In some examples, one of the two CD28 sdAbs contains the amino acid sequence set forth in SEQ ID NO:188 and the other of the two CD28 sdAbs contains the amino acid sequence set forth in SEQ ID NOS:280. In some examples, one of the two CD28 sdAbs contains the amino acid sequence set forth in SEQ ID NO:220 and the other of the two CD28 sdAbs contains the amino acid sequence set forth in SEQ ID NOS:280.

[0035] In some examples, the CD28-binding polypeptide includes a moiety that binds protein A. In some examples, the at least one CD28 sdAb contains an amino acid modification that reduces binding to protein A. In some examples, the amino acid modification of G65D by Kabat in framework region 3 (FR3).

[0036] In some examples, the CD28-binding polypeptide contains an immunoglobulin Fc region.

[0037] Also provide herein are anti-CD28 sdAb-Fc fusion proteins containing any of the CD28-binding polypeptide described herein and an immunoglobulin Fc region.

[0037a] In one aspect, the present disclosure refers to an anti-CD28 sdAb-Fc fusion protein, comprising a CD28-binding polypeptide disclosed herein and an immunoglobulin Fc region.

[0038] In some examples, the Fc region is linked by a linking peptide (LP) to at least one of the at least one CD28 sdAb. In some examples, the at least one CD28 sdAb is linked by a linking peptide (LP) to the Fc region at the N-terminal of the Fc region. In some examples, the LP is a non-cleavable linker. In some examples, the LP is a peptide of about 1 to 20 amino acids in length. In some examples, wherein the CD28-binding polypeptide contains from N-terminal to C-terminal: (CD28 sdAb)-LP-Fc. In some examples, the CD28-binding polypeptide contains from N-terminal to C-terminal: (CD28 sdAb)-LP-(CD28 sdAb)-LP-Fc.

[0039] In some examples, the C28-binding polypeptide is a dimer.

[0040] In some examples, the Fc region is a homodimeric Fc region. In some examples, the Fc region contains a sequence of amino acids selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOS: 8, 10, 11, 12 and 13. In some examples, the Fc region consists of a sequence of amino acids selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13. In some examples, the Fc region is a human IgG1. In some examples, the Fc region is a human IgG1. In some examples, the Fc region contains the sequence of amino acids set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8. In some examples, the Fc region consists of the sequence of amino acids set forth in SEQ ID NO: 8.

[0041] In some examples, the Fc region is a heterodimeric Fc region.

[0042] In some examples, the Fc region exhibits effector function.

[0043] In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces effector function. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to an effector molecule. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to an effector molecule selected from an Fc gamma receptor and C1q. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to Fc gamma receptor . In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to C1q. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235. In some examples, the one or more amino acid modification is deletion of Glu233. In some examples, the one or more amino acid modification is deletion of Leu234. In some examples, the one or more amino acid modification is deletion of Leu235. In some examples, the Fc region contains the sequence of amino acids set forth in SEQ ID NO: 9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9. In some examples, the Fc region consists of the sequence of amino acids set forth in SEQ ID NO: 9.

[0044] Provided herein are homodimeric Fc fusion proteins, containing two identical copies of any of the anti-CD28 sdAb-Fc fusion proteins described herein.

[0044a] In one aspect, the present disclosure refers to a homodimeric Fc fusion protein, comprising two identical copies of the anti-CD28 sdAb-Fc fusion protein disclosed herein.

[0045] Also provided herein are heterodimeric Fc fusion proteins, containing any two of the anti-CD28 sdAb-Fc fusion proteins described herein.

[0045a] In one aspect, the present disclosure refers to a heterodimeric fusion protein, comprising two of the anti-CD28 sdAb-Fc fusion proteins disclosed herein.

[0046] In some examples, the CD28-binding polypeptide contains one or more binding domain that binds to a target antigen other than CD28. In some examples, the one or more binding domain is one or more single domain antibody (sdAb) that binds a tumor associated antigen (TAA).

[0047] In some examples, the CD28-binding polypeptide includes a moiety that binds protein A.

[0048] Also provided herein are multi-specific binding polypeptides comprising any of the provided anti-CD28 sdAb-Fc fusion proteins and one or more binding domain (BD) that binds to a target antigen other than CD28.

[0048a] In one aspect, the present disclosure refers to a multi-specific binding polypeptide comprising the anti-CD28 sdAb-Fc fusion protein disclosed herein and one or more binding domain (BD) that binds to a target antigen other than CD28, optionally wherein the one or more BD binds a tumor associated antigen (TAA), the one or more BD binds a T cell surface marker that is a T cell activation marker or a T cell exhaustion marker, or the one or more BD binds a cell surface marker that is a tumor microenvironment marker.

[0049] In some examples, the one or more BD is one BD. In some examples, the one or more BD is two, three, four, or more BDs. In some examples, the one or more BDs is two BDs. In some examples, the one or more BDs is three BDs. In some examples, the one or more BDs is four BDs.

[0050] In some examples, the one or more binding domain (BD) binds a tumor associated antigen (TAA). In some examples, the one or more binding domain (BD) binds a T cell activation marker. In some examples, the one or more binding domain (BD) binds a T cell exhaustion marker. In some examples, the one or more binding domain (BD) binds a tumor microenvironment (TME) marker. In some examples, the one or more binding domain (BD) is a single domain antibody. In some examples, the one or more binding domain (BD) is a single domain antibody that binds to a TAA.

[0051] In some examples, the multi-specific binding polypeptide is an Fc fusion protein, wherein at least one of the one or more BD and/or the at least one CD28 sdAb is linked to an immunoglobulin Fc region.

[0052] Provided herein is a multi-specific Fc fusion protein comprising any of the provided multi-specific binding polypeptide and an immunoglobulin Fc region. In some examples, the multi-specific binding polypeptide is an Fc fusion protein, wherein at least one of the one or more BD and/or the at least one CD28 sdAb is linked to an immunoglobulin Fc region.

[0053] In some examples, the Fc fusion protein contains from N-terminus to C-terminus: the one or more BD that binds a target antigen other than CD28; the at least one CD28-binding domain comprising a sdAb that binds CD28; and the Fc region. In some examples, at least one of the one or more BD is joined by a linking peptide (LP) to at least one of the at least one CD28 sdAb. In some examples, the CD28-binding polypeptide contains from N-terminus to C-terminus: (BD)-LP-(CD28 sdAb)-LP-Fc.

[0054] In some examples, the multi-specific binding polypeptide is a dimer.

[0055] In some examples, the Fc region is a homodimeric Fc region. In some examples, the Fc region contains an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 8, 10, 11, 12 and 13. In some examples, the Fc region is a human IgG1. In some examples, the Fc region is a human IgG1. In some examples, the Fc region contains the amino acid sequence set forth in SEQ ID NO: 8. In some examples, the Fc region contains a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8.

[0056] In some examples, the Fc region is a heterodimeric Fc region.

[0057] In some examples, the Fc region exhibits effector function.

[0058] In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces effector function. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to an effector molecule selected from an Fc gamma receptor or C1q. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to Fc gamma receptor. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to C1q. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235. In some examples, the one or more amino acid modification is deletion of Glu233. In some examples, the one or more amino acid modification is deletion of Leu234. In some examples, the one or more amino acid modification is deletion of Leu235. In some examples, the Fc region contains the sequence of amino acids set forth in SEQ ID NO: 9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9. In some examples, the Fc region consists of the sequence of amino acids set forth in SEQ ID NO: 9.

[0059] In any of the provided examples, the binding domain (BD) binds to an antigen selected from the group consisting of: 1-92-LFA-3, 2B4, 5T4, Alpha-4 integrin, Alpha-V integrin, alpha4beta1 integrin, alpha4beta7 integrin, alpha-SMA, AGR2, Anti-Lewis-Y, Apelin J receptor, APRIL, B7-H3, B7-H4, BAFF, BTLA, C5 complement, C-242, CA9, CA19-9, (Lewis a), Carbonic anhydrase 9, CD2, CD3, CD6, CD9, CD11a, CD19, CD20, CD22, CD24, CD25, CD27, CD28, CD30, CD33, CD38, CD40, CD40L, CD41, CD44, CD44v6, CD47, CD51, CD52, CD56, CD64, CD69, CD70, CD71, CD74, CD80, CD81, CD86, CD95, CD107a, CD117, CD123, CD125, CD132, (IL-2RG), CD133, CD137, CD138, CD160, CD166, CD172A, CD248, CDH6, CEACAM5 (CEA), CEACAM6 (NCA-90), CLAUDIN-3, CLAUDIN-4, cMet, Collagen, Cripto, CSFR, CSFR-1, CTLA-4, CTGF, CXCL10, CXCL13, CXCR1, CXCR2, CXCR4, CYR61, DL44, DLK1, DLL3, DLL4, DPP-4, DSG1, EDA, EDB, EGFR, EGFRviii, Endothelin B receptor (ETBR), ENPP3, EpCAM, EPHA2, EPHB2, ERBB3, F protein of RSV, FAP, FGF-2, FGF8, FGFR1, FGFR2, FGFR3, FGFR4, FLT-3, Folate receptor alpha (FRalpha), FSP-1, GAL3ST1, G-CSF, G-CSFR, GD2, GITR, GLUT1, GLUT4, GM-CSF, GM-CSFR, GP IIb/IIIa receptors, Gp130, GPIIB/IIIA, GPNMB, GRP78, HER2/neu, HER3, HER4, HGF, hGH, HLA-DR, HVEM, Hyaluronidase, ICOS, IFNalpha, IFNbeta, IFNgamma, IgE, IgE Receptor (FceRI), IGF, IGF1R, IL1B, IL1R, IL2, IL11, IL12, IL12p40, IL-12R, IL-12Rbeta1, IL13, IL13R, IL13Ra2, IL15, IL17, IL18, IL21, IL23, IL23R, IL27/IL27R (wsx1), IL29, IL-31R, IL31/IL31R, IL2R, IL4, IL4R, IL6, IL6R, IL1 receptor accessory protein (IL1RAP), Insulin Receptor, Jagged Ligands, Jagged 1, Jagged 2, KISS1-R, KLRG1, LAG-3, LIF-R, Lewis X, LIGHT, LRP4, LRRC26, Ly6G6D, LyPD1, MCSP, Mesothelin, MRP4, MUC1, Mucin-16 (MUC16, CA-125), Na/K ATPase, NGF, Nicastrin, Notch Receptors, Notch 1, Notch 2, Notch 3, Notch 4, NOV, OSM-R, OX-40, PAR2, PDGF-AA, PDGF-BB, PDGFRalpha, PDGFRbeta, PD-1, PD-L1, PD-L2, Phosphatidyl-serine, P1GF, PSCA, PSMA, PSGR, RAAG12, RAGE, SLC44A4, Siglec15, Sphingosine 1 Phosphate, STEAP1, STEAP2, TAG-72, TAPA1, TEM-8, TGFbeta, TIGIT, TIM-3, TLR2, TLR4, TLR6, TLR7, TLR8, TLR9, TMEM31, TNFalpha, TNFR, TNFRS12A, TRAIL-R1, TRAIL-R2, Transferrin, Transferrin receptor, TRK-A, TRK-B, uPAR, VAP1, VCAM-1, VEGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGFR1, VEGFR2, VEGFR3, VISTA, WISP-1, WISP-2, and WISP-3.

[0060] In some examples, the T cell activation marker is selected from CD25, CD44, CD69, CD71, CD107a, CD137, HLA-DR, and/or KLRG1. In some examples, the T cell exhaustion marker is selected from 2B4, CD160, LAG3, PD-1, and/or TIGIT. In some embodiments, the tumor microenvironment marker is selected from alpha-SMA, EDB, FAP, FSP-1, PDGFRalpha, and/or PDGFRbeta.

[0061] In some examples, the one or more TAA BD is one TAA BD and/or the multi-specific binding polypeptide is monovalent for the TAA. In some examples, the one or more TAA BD is two, three, or four TAA BDs and/or the multi-specific binding polypeptide is multivalent for one or more TAA. In some examples, the one or more TAA BD is two TAA BDs and/or the multi-specific binding polypeptide is bivalent for one or more TAA. In some examples, the two TAA BDs are single domain antibodies (sdAbs), and the two sdAbs are the same TAA sdAbs. In some examples, the two TAA BDs are single domain antibodies (sdAbs), and the two sdAbs are different TAA sdAbs that bind the same TAA. In some examples, the different TAA sdAbs bind different epitopes of the same TAA. In some examples, the two TAA BDs are single domain antibodies (sdAbs) and the two sdAbs are different TAA sdAbs that bind different TAAs.

[0062] Also provided herein are homodimeric multi-specific Fc fusion proteins containing two identical copies of any of the provided multi-specific polypeptides.

[0062a] In one aspect, the present disclosure refers to a homodimeric multi-specific Fc fusion protein, comprising two identical copies of the multi-specific binding polypeptide disclosed herein.

[0063] Also provided herein are heterodimeric multi-specific Fc fusion proteins containing any two of the provided multi-specific polypeptides.

[0063a] In one aspect, the present disclosure refers to a heterodimeric multi-specific Fc fusion protein, comprising two of the multi-specific binding polypeptides disclosed herein.

[0064] In any of the provided examples, the Fc fusion protein contains a linking peptide between at least one of the at least one CD28 sdAb and the Fc region. In some examples, the Fc fusion protein contains a linking peptide (LP) between at least one of the one or more BD and the Fc region. In some examples, the linker is non-cleavable. In some examples, the linker is a peptide of about 1 to 20 amino acids in length. In some embodiments, contains the amino acid sequence set forth in any of SEQ ID NOS: 1-7, 89, 90, 123-129, 244, and 249. In some embodiments, consists of the amino acid sequence set forth in any of SEQ ID NOS: 1-7, 89, 90, 123-129, 244, and 249.

[0065] In some examples, the at least one CD28 sdAb is not able to, or is not substantially able to, bind or engage CD28 unless at least one of the one or more BD is bound to its antigen. In some examples, the at least one CD28 sdAb not able to, or is not substantially able to, bind or engage CD28 unless each of the one or more BD is bound to its antigen.

[0066] Also provided herein are CD28-binding polypeptides containing from N-terminal to C-terminal: one or more antigen binding domain containing a single domain antibody (sdAb) that binds a tumor associated antigen (TAA); at least one CD28-binding domain containing a sdAb that binds CD28; and an Fc region.

[0067] In any of the provided examples, at least one of the one or more TAA sdAb is joined by a linking peptide (LP) to at least one of the at least one CD28 sdAb. In some examples, the CD28-binding polypeptide contains from N-terminal to C-terminal: (TAA sdAb)-LP-(CD28 sdAb)-LP-Fc.

[0068] In some examples, the CD28-binding polypeptide is a dimer. In some examples, the Fc region is a homodimeric Fc region.

[0069] Also provided herein are CD28-binding polypeptides containing, from the N-terminal to the C-terminal: one or more antigen binding domain having a single domain antibody (sdAb) that binds a tumor associated antigen (TAA); at least one CD28-binding domain having a sdAb that binds CD28; and an Fc region, wherein the CD28-binding polypeptide is a dimer containing a homodimeric Fc region.

[0070] In some examples, the Fc region contains a sequence of amino acids selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOS: 8, 10, 11, 12 and 13. In some examples, the Fc region consists of a sequence of amino acids selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13. In some examples, the Fc region is a human IgG1. In some examples, the Fc region is a human IgG1. In some examples, the Fc region contains the sequence of amino acids set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8. In some examples, the Fc region consists of the sequence of amino acids set forth in SEQ ID NO: 8.

[0071] In some examples, the Fc region is a heterodimeric Fc region.

[0072] In some examples, the Fc region exhibits effector function.

[0073] In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces effector function. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to an effector molecule selected from an Fc gamma receptor or C1q. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding

to Fc gamma receptor. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to C1q. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235. In some examples, the one or more amino acid modification is deletion of Glu233. In some examples, the one or more amino acid modification is deletion of Leu234. In some examples, the one or more amino acid modification is deletion of Leu235. In some examples, the Fc region contains the sequence of amino acids set forth in SEQ ID NO: 9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9. In some examples, the Fc region consists of the sequence of amino acids set forth in SEQ ID NO: 9.

[0074] In any of the provided examples, the TAA is selected from the group consisting of: 1-92-LFA-3, 5T4, Alpha-4 integrin, Alpha-V integrin, alpha4beta1 integrin, alpha4beta7 integrin, AGR2, Anti-Lewis-Y, Apelin J receptor, APRIL, B7-H3, B7-H4, BAFF, BTLA, C5 complement, C-242, CA9, CA19-9, (Lewis a), Carbonic anhydrase 9, CD2, CD3, CD6, CD9, CD11a, CD19, CD20, CD22, CD24, CD25, CD27, CD28, CD30, CD33, CD38, CD40, CD40L, CD41, CD44, CD44v6, CD47, CD51, CD52, CD56, CD64, CD70, CD71, CD74, CD80, CD81, CD86, CD95, CD117, CD123, CD125, CD132, (IL-2RG), CD133, CD137, CD138, CD166, CD172A, CD248, CDH6, CEACAM5 (CEA), CEACAM6 (NCA-90), CLAUDIN-3, CLAUDIN-4, cMet, Collagen, Cripto, CSFR, CSFR-1, CTLA-4, CTGF, CXCL10, CXCL13, CXCR1, CXCR2, CXCR4, CYR61, DL44, DLK1, DLL3, DLL4, DPP-4, DSG1, EDA, EDB, EGFR, EGFRviii, Endothelin B receptor (ETBR), ENPP3, EpCAM, EPHA2, EPHB2, ERBB3, F protein of RSV, FAP, FGF-2, FGF8, FGFR1, FGFR2, FGFR3, FGFR4, FLT-3, Folate receptor alpha (FRalpha), GAL3ST1, G-CSF, G-CSFR, GD2, GITR, GLUT1, GLUT4, GM-CSF, GM-CSFR, GP IIb/IIIa receptors, Gp130, GPIIB/IIIA, GPNMB, GRP78, HER2/neu, HER3, HER4, HGF, hGH, HVEM, Hyaluronidase, ICOS, IFNalpha, IFNbeta, IFNgamma, IgE, IgE Receptor (FcεRI), IGF, IGF1R, IL1B, IL1R, IL2, IL11, IL12, IL12p40, IL-12R, IL-12Rbeta1, IL13, IL13R, IL13Ra2, IL15, IL17, IL18, IL21, IL23, IL23R, IL27/IL27R (wsx1), IL29, IL-31R, IL31/IL31R, IL2R, IL4, IL4R, IL6, IL6R, IL1 receptor accessory protein (IL1RAP), Insulin Receptor, Jagged Ligands, Jagged 1, Jagged 2, KISS1-R, LAG-3, LIF-R, Lewis X, LIGHT, LRP4, LRRC26, Ly6G6D, LyPD1, MCSP, Mesothelin, MRP4, MUC1, Mucin-16 (MUC16, CA-125), Na/K ATPase, NGF, Nicastrin, Notch Receptors,

Notch 1, Notch 2, Notch 3, Notch 4, NOV, OSM-R, OX-40, PAR2, PDGF-AA, PDGF-BB, PDGFRalpha, PDGFRbeta, PD-1, PD-L1, PD-L2, Phosphatidyl-serine, P1GF, PSCA, PSMA, PSGR, RAAG12, RAGE, SLC44A4, Siglec15, Sphingosine 1 Phosphate, STEAP1, STEAP2, TAG-72, TAPA1, TEM-8, TGFbeta, TIGIT, TIM-3, TLR2, TLR4, TLR6, TLR7, TLR8, TLR9, TMEM31, TNFalpha, TNFR, TNFRS12A, TRAIL-R1, TRAIL-R2, Transferrin, Transferrin receptor, TRK-A, TRK-B, uPAR, VAP1, VCAM-1, VEGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGFR1, VEGFR2, VEGFR3, VISTA, WISP-1, WISP-2, and WISP-3.

[0075] In some examples, the one or more TAA sdAb is one TAA sdAb. In some examples, the CD28-binding polypeptide is monovalent for the TAA. In some examples, the one or more TAA sdAb is two, three, or four TAA sdAbs. In some examples, the one or more TAA sdAb is two TAA sdAb. In some examples, the one or more TAA sdAb is three TAA sdAb. In some examples, the one or more TAA sdAb is four TAA sdAb. In some examples, the CD28-binding polypeptide is multivalent for one or more TAA. In some examples, the one or more TAA sdAb is two TAA sdAbs. In some examples, the CD28-binding polypeptide is bivalent for a TAA. In some examples, the CD28-binding polypeptide is bivalent for one or more TAA. In some examples, the two TAA sdAbs are the same TAA sdAbs. In some examples, the two TAA sdAbs are different TAA sdAbs that bind the same TAA. In some examples, the TAA sdAbs bind different epitopes of the same TAA. In some examples, the two TAA sdAbs are different TAA sdAbs that bind different TAAs.

[0076] In some examples, the CD28-binding polypeptide contains a linking peptide (LP) between at least one of the at least one CD28 sdAb and the Fc region. In some examples, the CD28-binding polypeptide contains a linking peptide (LP) between at least one of the one or more TAA sdAb and the Fc region. In some examples, the LP is a non-cleavable linker. In some examples, the LP is a peptide of about 1 to 20 amino acids in length. In some examples, the LP is or contains a sequence set forth in any of SEQ ID NOS:1-7, 89, 90, 123-129, 244, and 249.

[0077] In any of the provided examples, the at least one CD28 sdAb is not able to, or is not substantially able to, bind or engage CD28 unless at least one of the one or more TAA sdAb is bound to its TAA. In any of the provided examples, the at least one CD28 sdAb is not able to bind or engage CD28 unless at least one of the one or more TAA sdAb is bound to its TAA. In any of the provided examples, the at least one CD28 sdAb is not able to substantially bind or engage CD28 unless at least one of the one or more TAA sdAb is bound to

its TAA. In some examples, the at least one CD28 sdAb is not able to, or is not substantially able to, bind or engage CD28 unless each of the one or more TAA sdAb is bound to its TAA. In some examples, the at least one CD28 sdAb is not able to bind or engage CD28 unless each of the one or more TAA sdAb is bound to its TAA. In some examples, the at least one CD28 sdAb is not substantially able to bind or engage CD28 unless each of the one or more TAA sdAb is bound to its TAA.

[0078] In any of the provided examples, the CD-28 binding polypeptide does not contain a CD3 binding region. In some examples, the anti-CD28 sdAb-Fc fusion protein does not contain a CD3 binding region. In some examples, the homodimeric Fc fusion protein does not contain a CD3 binding region. In some examples, the heterodimeric Fc fusion protein does not contain a CD3 binding region. In some examples, the multi-specific binding polypeptide does not contain a CD3 binding region. In some examples, the homodimeric multi-specific binding polypeptide does not contain a CD3 binding region. In some examples, the heterodimeric multi-specific binding polypeptide does not contain a CD3 binding region. In some examples, the fusion protein does not contain a CD3 binding region.

[0079] In some examples, the CD28-binding polypeptide includes a CD3 binding region.

[0080] Provided herein are CD28-binding polypeptides containing at least one CD28 sdAb and a CD3 binding region.

[0081] In some examples, the at least one CD28 sdAb contains a complementarity determining region 1 (CDR1) having an amino acid sequence selected from the group consisting of SEQ ID NO:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) having an amino acid sequence selected from the group consisting of SEQ ID NO:190, 193, 196, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, and 212; and a complementarity determining region 3 (CDR3) having an amino acid sequence selected from the group consisting of SEQ ID NO: 191, 194, and 197. In some examples, the at least one CD28 sdAb contains a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:190, 193, 196, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a

complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 191, 194, 197, 396, 397, and 398.

[0081a] In one aspect, the present disclosure refers to an anti-CD28 single domain antibody (sdAb) comprising: a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:195, 189, 192, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:206, 190, 193, 196, 202, 204, 205, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 197, 191, 194, 396, 397, and 398.

[0082] In some examples, the at least one CD28 sdAb contains: a CDR1 having an amino acid sequence set forth in SEQ ID NO:189, a CDR2 having the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:191; a CDR1 having an amino acid sequence set forth in SEQ ID NO:192, a CDR2 having the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:194; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:198, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:199, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:200, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:201, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:202, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:203, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:204, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:205, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:207, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ

ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:208, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:209, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:210, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:211, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; or a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:212, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197.

[0083] In some examples, the at least one CD28 sAb contains: a CDR1 containing an amino acid sequence set forth in SEQ ID NO:189, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:191; a CDR1 having an amino acid sequence set forth in SEQ ID NO:192, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:194; a CDR1 containing an amino acid sequence set forth in SEQ ID NO:195, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:197; a CDR1 containing an amino acid sequence set forth in SEQ ID NO:198, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:197; a CDR1 containing an amino acid sequence set forth in SEQ ID NO:199, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:197; a CDR1 containing an amino acid sequence set forth in SEQ ID NO:200, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:197; a CDR1 containing an amino acid sequence set forth in SEQ ID NO:201, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:197; a CDR1 containing an amino acid sequence set forth in SEQ ID NO:195, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:202, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:197; a CDR1 containing an amino acid sequence set forth in SEQ ID NO:195, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:203, and a CDR3 containing the amino acid sequence set

acid sequence set forth in SEQ ID NO:195, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:397; or a CDR1 containing the amino acid sequence set forth in SEQ ID NO:195, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:398.

[0084] In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:189, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:191. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:192, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:194. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:198, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:199, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:200, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:202, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:203, and a CDR3 comprising the amino acid sequence set

acid sequence set forth in SEQ ID NO:204, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:396. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:394, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:395, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:397. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:396. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:388, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:389, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:397; or a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398.

[0085] In some examples, the at least one CD28 sdAb contains a complementarity determining region 1 (CDR1) having an amino acid sequence selected from the group consisting

of SEQ ID NO:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) having an amino acid sequence selected from the group consisting of SEQ ID NO:190, 193, 196, 202, 204, 205, 206, 207, 208, 209, 210, 211, and 212; and a complementarity determining region 3 (CDR3) having an amino acid sequence selected from the group consisting of SEQ ID NO: 191, 194, and 197. In some examples, the at least one CD28 sdAb contains a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:190, 193, 196, 202, 204, 205, 206, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 191, 194, 197, 396, 397, and 398.

[0086] In some examples, the at least one CD28 sdAb contains: a CDR1 having an amino acid sequence set forth in SEQ ID NO:189, a CDR2 having the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:191; a CDR1 having an amino acid sequence set forth in SEQ ID NO:192, a CDR2 having the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:194; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:198, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:199, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:200, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:201, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:202, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:204, and a CDR3 having the amino acid sequence set

set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398.

[0087] In some examples the at least one CD28 sdAb contains: a CDR1 having an amino acid sequence set forth in SEQ ID NO:189, a CDR2 having the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:191; a CDR1 having an amino acid sequence set forth in SEQ ID NO:192, a CDR2 having the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:194; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:198, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:199, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:200, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:201, a CDR2 having the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:202, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:204, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:205, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:207, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:208, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ

ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:209, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:210, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:211, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197; or a CDR1 having an amino acid sequence set forth in SEQ ID NO:195, a CDR2 having the amino acid sequence set forth in SEQ ID NO:212, and a CDR3 having the amino acid sequence set forth in SEQ ID NO:197.

[0088] In some examples, the at least one CD28 sdAb (CD28 VHH) contains: a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:186; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:187; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:188; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:213; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:214; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:215; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:216; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:217; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:218; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:219; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:220; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:221; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:222; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:223; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:224; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:225; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:226; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:227; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:228; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:229; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:230; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:231; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:232; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:233; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:234; a CDR1, a CDR2, and a

CDR3 as contained in the VHH set forth in SEQ ID NO:235; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:236; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:237; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:238; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:239; or a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:280.

[0089] In some examples, the at least one CD28 sdAb (CD28 VHH) contains: a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:186; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:187; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:188; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:213; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:214; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:215; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:216; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:217; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:218; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:219; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:220; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:221; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:222; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:223; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:224; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:225; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:226; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:227; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:228; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:229; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:230; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:231; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:232; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:233; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:234; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:235; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:236; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:237; a CDR1, a CDR2, and a CDR3 as contained in the

CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:370; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:371; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:372; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:373; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:374; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:375; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:376; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:377; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:378; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:379; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:380; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:381; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:382; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:383; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:384; or a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:385.

[0089a] In one aspect, the present disclosure refers to an anti-CD28 single domain antibody (sdAb) comprising: a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:342; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:186; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:187; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:188; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:213; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:214; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:215; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:216; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:217; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:218; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:219; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:221; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:222; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:223; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:224; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:225; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:226; a CDR1, a CDR2,

domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:372. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:373. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:374. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:375. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:376. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:377. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:378. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:379. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:380. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:381. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:382. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:383. In some examples, the at least one CD28 sdAb domain comprises a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:384; or a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:385.

[0091] In some examples, the at least one CD28 sdAb (CD28 VHH) contains: a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:186; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:187; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:188; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:213; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:214; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:215; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:216; a CDR1, a CDR2, and a CDR3 as contained in the VHH set

forth in SEQ ID NO:217; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:218; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:219; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:221; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:222; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:223; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:224; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:225; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:226; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:227; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:228; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:229; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:230; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:231; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:232; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:233; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:234; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:235; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:236; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:237; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:238; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:239; or a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:280.

[0092] In some examples, the at least one CD28 sdAb (CD28 VHH) contains: a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:186; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:187; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:188; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:213; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:214; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:215; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:216; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:217; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:218; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:219; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:221; a CDR1, a CDR2, and a CDR3 as contained in the VHH set forth in SEQ ID NO:222; a CDR1, a

[0093] In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS: 186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS: 186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385.

[0094] In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:186. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:187. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:188. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:213. In some examples, the at least one CD28 sdAb comprises the amino acid

[illegible]

comprises the amino acid sequence set forth in SEQ ID NO:368. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:369. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:370. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:371. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:372. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:373. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:374. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:375. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:376. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:377. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:378. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:379. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:380. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:381. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:382. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:383. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:384. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in SEQ ID NO:385.

[0095] In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and 280. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS: 186, 187, 188,

213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS: 186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385.

[0096] In some examples, the at least one CD28 sdAb contains the sequence set forth in (i) SEQ ID NO: 186, (ii) a humanized variant of SEQ ID NO:186, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 186. In some examples, the at least one CD28 sdAb contains the sequence set forth in SEQ ID NO: 186. In some examples, the at least one CD28 sdAb contains a humanized variant of SEQ ID NO:186. In some examples, the at least one CD28 sdAb contains a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 186. In some examples, the at least one CD28 sdAb contains a CDR1 having an amino acid sequence set forth in SEQ ID NO:189; a CDR2 having an amino acid sequence set forth in SEQ ID NO:190; and a CDR3 having an amino acid sequence set forth in SEQ ID NO:191.

[0097] In some examples, the at least one CD28 sdAb contains the sequence set forth in (i) SEQ ID NO: 187, (ii) a humanized variant of SEQ ID NO:187, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 187. In some examples, the at least one CD28 sdAb contains the sequence set forth in SEQ ID NO: 187. In some examples, the at least one CD28 sdAb contains a humanized variant of SEQ ID NO:187. In

some examples, the at least one CD28 sdAb contains a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 187. In some examples, the at least one CD28 sdAb contains a CDR1 having an amino acid sequence set forth in SEQ ID NO:192; a CDR2 having an amino acid sequence set forth in SEQ ID NO:193; and a CDR3 having an amino acid sequence set forth in SEQ ID NO:194.

[0098] In some examples, the at least one CD28 sdAb contains the sequence set forth in (i) SEQ ID NO: 188, (ii) a humanized variant of SEQ ID NO:188, or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 188. In some examples, the at least one CD28 sdAb contains the sequence set forth in SEQ ID NO: 188. In some examples, the at least one CD28 sdAb contains a humanized variant of SEQ ID NO:188. In some examples, the at least one CD28 sdAb contains a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 188.

[0099] In some examples, the at least one CD28 sdAb contains a CDR1 having an amino acid sequence set forth in any of SEQ ID NO:195, 198, 199, 200, and 201; a CDR2 having an amino acid sequence set forth in any of SEQ ID NO:196, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, and 212; and a CDR3 having an amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an amino acid sequence set forth in any of SEQ ID NOS:195, 198, 199, 200, and 201; a CDR2 comprising an amino acid sequence set forth in any of SEQ ID NOS:196, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a CDR3 comprising an amino acid sequence set forth in any of SEQ ID NOS:197, 396, 397, and 398.

[0100] In some examples, the at least one CD28 sdAb contains CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 203, and 197, respectively; SEQ ID NOS: 195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and

197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; or SEQ ID NOS: 195, 212, and 197, respectively. In some examples, the at least one CD28 sdAb contains a CDR1, CDR2 and CDR3 set forth in: SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 203, and 197, respectively; SEQ ID NOS: 195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; SEQ ID NOS: 195, 212, and 197, respectively; SEQ ID NOS: 201, 202, and 197, respectively; SEQ ID NOS: 201, 202, and 396, respectively; SEQ ID NOS: 201, 386, and 197, respectively; SEQ ID NOS: 201, 387, and 197, respectively; SEQ ID NOS: 201, 202, and 397, respectively; SEQ ID NOS: 201, 202, and 398, respectively; SEQ ID NOS: 201, 206, and 197, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 388, and 197, respectively; SEQ ID NOS: 201, 389, and 197, respectively; SEQ ID NOS: 201, 206, and 397, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 203, and 197, respectively; SEQ ID NOS: 201, 203, and 396, respectively; SEQ ID NOS: 201, 390, and 197, respectively; SEQ ID NOS: 201, 391, and 197, respectively; SEQ ID NOS: 201, 203, and 397, respectively; SEQ ID NOS: 201, 203, and 398, respectively; SEQ ID NOS: 201, 204, and 197, respectively; SEQ ID NOS: 201, 204, and 396, respectively; SEQ ID NOS: 201, 392, and 197, respectively; SEQ ID NOS: 201, 393, and 197, respectively; SEQ ID NOS: 201, 204, and 397, respectively; SEQ ID NOS: 201, 204, and 398, respectively; SEQ ID NOS: 201, 196, and 396, respectively; SEQ ID NOS: 201, 394, and 197, respectively; SEQ ID NOS: 201, 395, and 197, respectively; SEQ ID NOS: 201, 196, and 397, respectively; SEQ ID NOS: 201, 196, and 398, respectively; SEQ ID NOS: 195, 206, and 396, respectively; SEQ ID NOS: 195, 388, and 197, respectively; SEQ ID NOS: 195, 389, and 197, respectively; SEQ ID NOS: 195, 206, and 397, respectively; or SEQ ID NOS: 195, 206, and 398, respectively.

[0101] In some examples, the at least one CD28 sdAb contains the sequence of amino acids set forth in any one of SEQ ID NOS:213-239 and 280, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NO:213-239 and 280. In some examples,

the at least one CD28 sdAb contains the sequence of amino acids set forth in any one of SEQ ID NOS:213-239 and 280.

[0102] In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:213-239, 280, and 342-385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NO:213-239, 280, and 342-385. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:213-239, 280, and 342-385.

[0103] In some examples, the at least one CD28 sdAb contains a CDR1 having an amino acid sequence set forth in any of SEQ ID NO:195, 198, 199, 200, and 201; a CDR2 having an amino acid sequence set forth in any of SEQ ID NO:196, 202, 204, 205, 206, 207, 208, 209, 210, 211, and 212; and a CDR3 having an amino acid sequence set forth in SEQ ID NO:197. In some examples, the at least one CD28 sdAb contains a CDR1 comprising an amino acid sequence set forth in any of SEQ ID NO:195, 198, 199, 200, and 201; a CDR2 comprising an amino acid sequence set forth in any of SEQ ID NO:196, 202, 204, 205, 206, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a CDR3 comprising an amino acid sequence set forth in any of SEQ ID NO:197, 396, 397, and 398.

[0104] In some examples, the at least one CD28 sdAb contains a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; or SEQ ID NOS: 195, 212, and 197, respectively. In some examples, the at least one CD28 sdAb contains a CDR1, CDR2 and CDR3 set forth in: SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; or SEQ ID NOS: 195, 212, and 197, respectively.

210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; SEQ ID NOS: 195, 212, and 197, respectively; SEQ ID NOS: 201, 202, and 197, respectively; SEQ ID NOS: 201, 202, and 396, respectively; SEQ ID NOS: 201, 386, and 197, respectively; SEQ ID NOS: 201, 387, and 197, respectively; SEQ ID NOS: 201, 202, and 397, respectively; SEQ ID NOS: 201, 202, and 398, respectively; SEQ ID NOS: 201, 206, and 197, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 388, and 197, respectively; SEQ ID NOS: 201, 389, and 197, respectively; SEQ ID NOS: 201, 206, and 397, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 203, and 197, respectively; SEQ ID NOS: 201, 203, and 396, respectively; SEQ ID NOS: 201, 390, and 197, respectively; SEQ ID NOS: 201, 391, and 197, respectively; SEQ ID NOS: 201, 203, and 397, respectively; SEQ ID NOS: 201, 203, and 398, respectively; SEQ ID NOS: 201, 204, and 197, respectively; SEQ ID NOS: 201, 204, and 396, respectively; SEQ ID NOS: 201, 392, and 197, respectively; SEQ ID NOS: 201, 393, and 197, respectively; SEQ ID NOS: 201, 204, and 397, respectively; SEQ ID NOS: 201, 204, and 398, respectively; SEQ ID NOS: 201, 196, and 396, respectively; SEQ ID NOS: 201, 394, and 197, respectively; SEQ ID NOS: 201, 395, and 197, respectively; SEQ ID NOS: 201, 196, and 397, respectively; SEQ ID NOS: 201, 196, and 398, respectively; SEQ ID NOS: 195, 206, and 396, respectively; SEQ ID NOS: 195, 388, and 197, respectively; SEQ ID NOS: 195, 389, and 197, respectively; SEQ ID NOS: 195, 206, and 397, respectively; or SEQ ID NOS: 195, 206, and 398, respectively.

[0105] In some examples, the at least one CD28 sdAb contains the sequence of amino acids set forth in any one of SEQ ID NOS:213-219, 221-239, and 280, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NO:213-219, 221-239, and 280. In some examples, the at least one CD28 sdAb contains the sequence of amino acids set forth in any one of SEQ ID NOS:213-219, 221-239, and 280. In some examples, the at least one CD28 sdAb contains the amino acid sequence set forth in any of SEQ ID NOS:213-239, 280, and 342-385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NO:213-239, 280, and 342-385. In some examples, the at least one CD28 sdAb comprises the amino acid sequence set forth in any of SEQ ID NOS:213-239, 280, and 342-385.

[0106] In any of the provided examples, the CD28-binding polypeptide contains a CD3 binding region. In some examples, the anti-CD28 sdAb-Fc fusion protein includes a CD3 binding region. In some examples, the homodimeric Fc fusion protein includes a CD3

binding region. In some examples, the heterodimeric Fc fusion protein includes a CD3 binding region. In some examples, the multi-specific binding polypeptide includes a CD3 binding region. In some examples, the homodimeric multi-specific binding polypeptide includes a CD3 binding region. In some examples, the heterodimeric multi-specific binding polypeptide includes a CD3 binding region. In some examples, the fusion protein includes a CD3 binding region.

[0107] Provided herein in a multi-specific construct containing at least one of the provided sdAbs, one or more binding domain (BD) that binds to an antigen other than CD28, and a CD3 binding region. In some examples, the BD binds to a tumor associated (antigen). In some examples, the BD is a single domain antibody.

[0107a] In one aspect, the present disclosure refers to a multi-specific construct comprising at least one anti-CD28 single domain antibody (sdAb) disclosed herein, one or more binding domain (BD) that binds an antigen other than CD28, and a CD3 binding region, optionally wherein the BD binds a TAA.

[0108] In some examples, the CD3-binding region binds CD3 (CD3ε). In some examples, the CD3 binding region is an anti-CD3 antibody or antigen-binding fragment. In some examples, the CD3 binding region is an anti-CD3 antibody. In some examples, the CD3 binding region is an anti-CD3 antigen-binding fragment. In some examples, the anti-CD3 antibody or antigen-binding fragment contains a variable heavy chain region (VH) and a variable light chain region (VL). In some examples, the CD3 binding region is monovalent. In some examples, the CD3 binding region is a variable fragment (Fv) comprising a variable heavy chain region (VH) and a variable light chain region (VL). In some examples, the anti-CD3 antibody or antigen-binding fragment is not a single chain antibody. In some examples, the anti-CD3 antibody or antigen-binding fragment is not a single chain variable fragment (scFv).

[0109] In any of the provided examples, the CD28-binding polypeptide contains an immunoglobulin Fc region. In some examples, the Fc region is a homodimeric Fc region. In some examples, the Fc region contains a sequence of amino acids selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOS: 8, 10, 11, 12 and 13. In some examples, the Fc region consists of a sequence of amino acids selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13. In some examples, the Fc region is a human IgG1. In some examples, the Fc region is a human IgG1. In some examples, the Fc region contains the sequence of amino acids set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%

sequence identity to SEQ ID NO: 8. In some examples, the Fc region consists of the sequence of amino acids set forth in SEQ ID NO: 8.

[0110] In some examples, the Fc region is a heterodimeric Fc region.

[0111] In some examples, the Fc region exhibits effector function.

[0112] In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces effector function. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to an effector molecule. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to an effector molecule selected from an Fc gamma receptor and C1q. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to Fc gamma receptor. In some examples, the Fc region contains a polypeptide having one or more amino acid modification that reduces binding to C1q. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235. In some examples, the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235. In some examples, the one or more amino acid modification is deletion of Glu233. In some examples, the one or more amino acid modification is deletion of Leu234. In some examples, the one or more amino acid modification is deletion of Leu235. In some examples, the Fc region contains the sequence of amino acids set forth in SEQ ID NO: 9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9. In some examples, the Fc region consists of the sequence of amino acids set forth in SEQ ID NO: 9.

[0113] In some examples, the CD28-binding polypeptide is a dimer. In some examples, the Fc is a heterodimeric Fc and the VH and VL that comprise the anti-CD3 antibody or antigen-binding fragment are linked to opposite polypeptides of the heterodimeric Fc.

[0114] In some examples, the CD3 binding region is not able to, or is not substantially able to, bind or engage CD3 unless at least one of the at least one CD28 sdAb is bound to CD28. In some examples, the CD3 binding region is not able to bind or engage CD3 unless at least one of the at least one CD28 sdAb is bound to CD28. In some examples, the CD3 binding region is not substantially able to bind or engage CD3 unless at least one of the at least one CD28 sdAb is bound to CD28. In some examples, the CD3 binding region is not able to, or is not substantially able, to bind or engage CD3 unless at least

one of the one or more TAA sdAb is bound to its TAA. In some examples, the CD3 binding region is not able to bind or engage CD3 unless at least one of the one or more TAA sdAb is bound to its TAA. In some examples, the CD3 binding region is not substantially able to bind or engage CD3 unless at least one of the one or more TAA sdAb is bound to its TAA.

[0115] In some examples, the CD28-binding polypeptide includes a moiety that binds protein A.

[0116] In some examples, the at least one CD28 sdAb comprises an amino acid modification that reduces binding to protein A. In some embodiments, the at least one CD28 sdAb comprises the amino acid modification G65D by Kabat in framework region 3 (FR3).

[0117] Also provided herein are anti-CD28 single domain antibodies containing a complementarity determining region 1 (CDR1) having an amino acid sequence selected from the group consisting of SEQ ID NO:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) containing an amino acid sequence selected from the group consisting of SEQ ID NOS:190, 193, 196, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, and 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a complementarity determining region 3 (CDR3) containing an amino acid sequence selected from the group consisting of SEQ ID NOS: 191, 194, and 197, 396, 397, and 398.

[0118] Also provided herein are anti-CD28 sdAbs containing a complementarity determining region 1 (CDR1) containing an amino acid sequence selected from the group consisting of SEQ ID NOS:189, 192, 195, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) containing an amino acid sequence selected from the group consisting of SEQ ID NOS:190, 193, 196, 202, 204, 205, 206, 207, 208, 209, 210, 211, and 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a complementarity determining region 3 (CDR3) containing an amino acid sequence selected from the group consisting of SEQ ID NOS: 191, 194, and 197, 396, 397, and 398.

[0119] In some examples, the CD28 sdAb contains a CDR1 containing an the amino acid sequence set forth in SEQ ID NO:189, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:191. In some examples, the CD28 sdAb contains a CDR1 containing an the amino acid sequence set forth in SEQ ID NO:192, a CDR2 containing the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 containing the amino acid sequence set forth in SEQ ID NO:194. In some examples, the CD28 sdAb contains a CDR1 containing an the amino acid sequence set forth in SEQ ID NO:195, a CDR2 containing the amino acid sequence set forth in SEQ ID

[illegible]

[illegible]

forth in SEQ ID NO:384. In some examples, the CD28 sdAb contains the amino acid sequence set forth in SEQ ID NO:and 385.

[0121] In some examples, the anti-CD28 sdAb is isolated. In some examples, the anti-CD28 sdAb is purified.

[0122] In some examples, the CD28 sdAb comprises an amino acid modification that reduces binding to protein A. In some examples, the at least one CD28 sdAb comprises an amino acid modification that reduces binding to protein A. In some examples, the at least one CD28 sdAb comprises the amino acid modification G65D by Kabat in framework region 3 (FR3).

[0123] Provided herein is a polynucleotide(s) encoding any of the anti-CD28 sdAbs, CD28-binding polypeptides, fusion proteins, and multi-specific binding polypeptide, and multi-specific constructs described herein.

[0123a] In one aspect, the present disclosure refers to a polynucleotide(s) encoding: the CD28-binding polypeptide disclosed herein; the fusion protein disclosed herein; the multi-specific binding polypeptide disclosed herein; or the anti-CD28 sdAb disclosed herein.

[0124] Also provided herein is a polynucleotide, containing a first nucleic acid sequence encoding a first polypeptide of any of the CD28-binding polypeptides provided herein and a second nucleic acid sequence encoding a second polypeptide of the CD28-binding polypeptide. In some embodiments, the first and second nucleic acid sequence are separated by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping. Also provided herein is a polynucleotide, containing a first nucleic acid sequence encoding a first polypeptide of any of the CD28-binding polypeptides provided herein and a second nucleic acid sequence encoding a second polypeptide of the multispecific construct, wherein the first and second nucleic acid sequence are separated by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping.

[0125] Also provided herein is a polynucleotide, containing a first nucleic acid sequence encoding a first polypeptide of any of the fusion proteins provided herein and a second nucleic acid sequence encoding a second polypeptide of the fusion protein. Also provided herein is a polynucleotide, containing a first nucleic acid sequence encoding a first polypeptide of any of the multi-specific binding polypeptide provided herein and a second nucleic acid sequence encoding a second polypeptide of the multi-specific binding polypeptide. Also provided herein is a polynucleotide, containing a first nucleic acid sequence encoding a first polypeptide of any of the multi-specific construct provided herein and a second nucleic acid sequence encoding a second polypeptide of the multi-specific construct.

[0126] In some embodiments, the first and second nucleic acid sequence are separated by an internal ribosome entry site (IRES), or a nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping. In some examples, the first nucleic acid sequence and second nucleic acid sequence are operably linked to the same promoter. In some examples, the nucleic acid encoding a self-cleaving peptide or a peptide that causes ribosome skipping is selected from a T2A, a P2A, a E2A or a F2A.

[0127] Provided herein is a vector, comprising any of the polynucleotides described herein. In some examples, the vector is an expression vector. In some examples, the vector is a viral vector or a eukaryotic vector. In some examples, the eukaryotic vector is a mammalian vector.

[0127a] In one aspect, the present disclosure refers to a vector, comprising the polynucleotide disclosed herein, optionally wherein the vector is an expression vector; and/or a viral vector or a eukaryotic vector, optionally wherein the eukaryotic vector is a mammalian vector.

[0128] Provided herein is a cell containing any of the polynucleotide or polynucleotides described herein. In some examples, the cell is a lymphocyte. In some examples, the cell is a T cell or a natural killer (NK) cells.

[0128a] In one aspect, the present disclosure refers to a cell comprising the polynucleotide or polynucleotides disclosed herein or the vector disclosed herein.

[0129] Provided herein are methods of producing a polypeptide, the method including introducing into a cell any polynucleotide or polynucleotides provided herein or any vector or vectors provided herein and culturing the cell under conditions to produce the anti-CD28 sdAb, CD28-binding polypeptide, fusion protein, multi-specific binding polypeptide, or multi-specific construct. In some examples, the method includes isolating or purifying the polypeptide from the cell. Also provided herein is a polypeptide produced by any of the methods described herein.

[0129a] In one aspect, the present disclosure refers to a method of producing a polypeptide, the method comprising introducing into a cell a polynucleotide or polynucleotides disclosed herein or a vector disclosed herein and culturing the cell under conditions to produce the polypeptide, optionally comprising isolating or purifying the polypeptide from the cell.

[0129b] In one aspect, the present disclosure refers to a polypeptide produced by the method disclosed herein.

[0130] Provided herein are pharmaceutical compositions comprising any of the anti-CD28 sdAbs, CD28-binding polypeptides, fusion proteins, multi-specific binding polypeptides, and multi-specific constructs described. In some examples, the pharmaceutical composition includes a pharmaceutically acceptable carrier. In some examples, the pharmaceutical composition is sterile.

[0130a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising a CD28-binding polypeptide disclosed herein; a fusion protein disclosed herein; a multi-specific binding polypeptide disclosed herein; a multi-specific construct disclosed herein; or an anti-CD28 sdAb disclosed herein, optionally comprising a pharmaceutically acceptable carrier.

[0131] Provided herein are methods of stimulating an immune response in a subject, the methods including administering, to a subject in need thereof, any of the CD28 sdAbs, CD28-binding polypeptides, fusion proteins, multi-specific binding polypeptides, and multi-specific constructs or the pharmaceutical compositions described herein. In some examples, the immune response is increased against a tumor or cancer. In some examples, the method treats a disease or condition in the subject.

[0131a] In one aspect, the present disclosure refers to use of a CD28-binding polypeptide disclosed herein; a fusion protein disclosed herein; a multi-specific binding polypeptide disclosed herein; an anti-CD28 sdAb disclosed herein; a multi-specific construct disclosed herein; or a pharmaceutical composition disclosed herein, in the manufacture of a medicament for stimulating an immune response in a subject in need thereof, optionally wherein the immune response is increased against a tumor or cancer.

[0132] Also provided herein are methods of treating a disease or condition in a subject, the methods including administering, to a subject in need thereof, a therapeutically effective amount

of any of the anti-CD28 sdAbs, CD28-binding polypeptides, fusion proteins, multi-specific binding polypeptides, and multi-specific constructs or the pharmaceutical compositions described herein.

[0132a] In one aspect, the present disclosure refers to use of a composition comprising a CD28-binding polypeptide disclosed herein; a fusion protein disclosed herein; a multi-specific binding polypeptide disclosed herein; an anti-CD28 sdAb disclosed herein; a multi-specific construct disclosed herein; or a pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a disease or condition in a subject.

[0133] Also provided herein are uses of any of the anti-CD28 sdAbs, CD28-binding polypeptides, fusion proteins, multi-specific binding polypeptides, and multi-specific constructs, or the pharmaceutical compositions described herein. Provided herein are uses of any of the provided multi-specific binding polypeptides. Provided herein are uses of any of the provided multi-specific constructs. Provided herein are uses of any of the provided pharmaceutical compositions.

[0134] Also provided are compositions comprising any of the anti-CD28 sdAbs, CD28-binding polypeptides, fusion proteins, multi-specific binding polypeptides, and multi-specific constructs, or the pharmaceutical compositions described herein for use treating a disease or condition in a subject. Provided herein are compositions comprising the multi-specific binding polypeptides for use in treating a disease or condition in a subject. Provided herein are compositions comprising the multi-specific conjugates for use in treating a disease or condition in a subject.

[0134a] In one aspect, the present disclosure refers to a composition comprising a CD28-binding polypeptide disclosed herein; a fusion protein disclosed herein; a multi-specific binding polypeptide disclosed herein; or an anti-CD28 sdAb disclosed herein; a multi-specific construct disclosed herein; or a pharmaceutical composition disclosed herein for use in treating a disease or condition in a subject.

[0135] Also provided are any of the anti-CD28 sdAbs, CD28-binding polypeptides, fusion proteins, multi-specific binding polypeptides, and multi-specific constructs, or the pharmaceutical compositions described herein for use in the manufacture of a medicament for treating a disease or condition in a subject. Provided herein are compositions comprising the multi-specific binding polypeptides for use in the manufacture of a medicament for treating a disease or condition in a subject. Provided herein are compositions comprising the multi-specific conjugates for use in the manufacture of a medicament for treating a disease or condition in a subject.

[0136] In some examples, the disease or condition is a tumor or a cancer. In some examples, the disease or condition is a tumor. In some examples, the disease or condition is a cancer. In some examples, the subject is a human.

Brief Description of the Drawings

[0137] **FIG. 1** depicts the ability of CD28-targeting single domain antibodies formatted as sdAb-IgG1 to bind cell-surface CD28. Binding was assessed by flow cytometry using HEK293FS (293FS) cells transiently transfected with full-length human (hCD28) or cynomolgus (cyCD28) antigen (**FIGS. 1A** and **1B**, respectively). As a negative control, binding

Claims

1. A CD28-binding polypeptide comprising at least one single domain antibody (sdAb) that binds CD28, wherein the at least one CD28 sdAb comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:195, 189, 192, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:206, 190, 193, 196, 202, 204, 205, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 197, 191, 194, 396, 397, and 398.

2. The CD28-binding polypeptide of claim 1, wherein the at least one CD28 sdAb comprises:

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:189, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:191;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:192, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:194;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:198, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:199, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398.

3. A CD28-binding polypeptide comprising at least one single domain antibody (sdAb) that binds CD28, wherein the at least one CD28 sdAb (CD28 VHH) domain comprises:
a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:342; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:186; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:187; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:188; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:213; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:214; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:215; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:216; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:217; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:218; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:219; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:221; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:222; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:223; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:224; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:225; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:226; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:227; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:228; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:229; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:230; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:231; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:232; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:233; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set

CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:367; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:368; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:369; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:370; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:371; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:372; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:373; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:374; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:375; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:376; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:377; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:378; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:379; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:380; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:381; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:382; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:383; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:384; or a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:385.

4. The CD28-binding polypeptide of any of claims 1-3, wherein the at least one CD28 sdAb comprises the amino acid sequence set forth in any of SEQ ID NOS:342, 186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:342, 186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353,

354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385.

5. The CD28-binding polypeptide of any of claims 1-4, wherein the at least one CD28 sdAb comprises the amino acid sequence set forth in (i) SEQ ID NO: 186, (ii) a humanized variant of SEQ ID NO: 186, and/or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 186.

6. The CD28-binding polypeptide of any of claims 1-5, wherein the at least one CD28 sdAb comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 189; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 190; and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 191.

7. The CD28-binding polypeptide of any of claims 1-4, wherein the at least one CD28 sdAb comprises the amino acid sequence set forth in (i) SEQ ID NO: 187, (ii) a humanized variant of SEQ ID NO: 187, and/or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 187.

8. The CD28-binding polypeptide of any of claims 1-4 and 7, wherein the at least one CD28 sdAb comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 192; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 193; and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 194.

9. The CD28-binding polypeptide of any of claims 1-4, wherein the at least one CD28 sdAb comprises the amino acid sequence set forth in (i) SEQ ID NO: 188, (ii) a humanized variant of SEQ ID NO: 188, and/or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 188.

10. The CD28-binding polypeptide of any of claims 1-4 and 9, wherein the at least one CD28 sdAb comprises a CDR1 comprising the amino acid sequence set forth in any of SEQ ID NOS:195, 198, 199, 200, and 201; a CDR2 comprising the amino acid sequence set forth in any of SEQ ID NOS:206, 196, 202, 204, 205, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a CDR3 comprising the amino acid sequence set forth in any of SEQ ID NOS:197, 396, 397, and 398.

11. The CD28-binding polypeptide of any of claims 1-4, 9 and 10, wherein the at least one CD28 sdAb comprises a CDR1, CDR2 and CDR3 set forth in SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; SEQ ID NOS: 195, 212, and 197, respectively; SEQ ID NOS: 201, 202, and 197, respectively; SEQ ID NOS: 201, 202, and 396, respectively; SEQ ID NOS: 201, 386, and 197, respectively; SEQ ID NOS: 201, 387, and 197, respectively; SEQ ID NOS: 201, 202, and 397, respectively; SEQ ID NOS: 201, 202, and 398, respectively; SEQ ID NOS: 201, 206, and 197, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 388, and 197, respectively; SEQ ID NOS: 201, 389, and 197, respectively; SEQ ID NOS: 201, 206, and 397, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 203, and 197, respectively; SEQ ID NOS: 201, 203, and 396, respectively; SEQ ID NOS: 201, 390, and 197, respectively; SEQ ID NOS: 201, 391, and 197, respectively; SEQ ID NOS: 201, 203, and 397, respectively; SEQ ID NOS: 201, 203, and 398, respectively; SEQ ID NOS: 201, 204, and 197, respectively; SEQ ID NOS: 201, 204, and 396, respectively; SEQ ID NOS: 201, 392, and 197, respectively; SEQ ID NOS: 201, 393, and 197, respectively; SEQ ID NOS: 201, 204, and 397, respectively; SEQ ID NOS: 201, 204, and 398, respectively; SEQ ID NOS: 201, 196, and 396, respectively; SEQ ID NOS: 201, 394, and 197, respectively; SEQ ID NOS: 201, 395, and 197, respectively; SEQ ID NOS: 201, 196, and 397, respectively; SEQ ID NOS: 201, 196, and 398, respectively; SEQ ID NOS: 195, 206, and 396, respectively; SEQ ID NOS: 195, 388, and 197, respectively; SEQ ID NOS: 195, 389, and 197, respectively; SEQ ID NOS: 195, 206, and 397, respectively; or SEQ ID NOS: 195,

206, and 398, respectively; and/or the at least one CD28 sdAb comprises the amino acid sequence set forth in any of SEQ ID NOS:342, 213-219, 221-239, 280, and 343-385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS:342, 213-219, 221-239, 280, and 343-385.

12. The CD28-binding polypeptide of any of claims 1-11, wherein the at least one CD28 sdAb is one CD28 sdAb and/or the CD28-binding polypeptide is monovalent for CD28.

13. The CD28-binding polypeptide of any of claims 1-11, wherein the at least one CD28 sdAb is two, three, or four CD28 sdAbs and/or the CD28-binding polypeptide is multivalent for CD28.

14. The CD28-binding polypeptide of any of claims 1-11 and 13, wherein the at least one CD28 sdAb is two CD28 sdAbs and/or the CD28-binding polypeptide is bivalent for CD28, optionally wherein the two CD28 sdAbs are the same CD28 sdAb, or wherein the two CD28 sdAbs are different CD28 sdAbs.

15. The CD28-binding polypeptide of claim 14, wherein each of the two CD28 sdAbs comprise a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 342, 213-219, 221-239, 280, and 343-385.

16. The CD28-binding polypeptide of any of claims 1-15, wherein the CD28-binding polypeptide comprises a moiety that binds protein A.

17. The CD28-binding polypeptide of any of claims 1-16, wherein the at least one CD28 sdAb comprises an amino acid modification that reduces binding to protein A, optionally wherein the amino acid modification is G65D by Kabat in framework region 3 (FR3).

18. An anti-CD28 sdAb-Fc fusion protein, comprising a CD28-binding polypeptide of any of claims 1-17 and an immunoglobulin Fc region.

19. The anti-CD28 sdAb-Fc fusion protein of claim 18, wherein the Fc region is a homodimeric Fc region.

20. The anti-CD28 sdAb-Fc fusion protein of claim 18 or 19, wherein the Fc region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 8, 10, 11, 12 and 13.

21. The anti-CD28 sdAb-Fc fusion protein of any of claims 18-20, wherein the Fc region is a human IgG1, optionally wherein the Fc region comprises the amino acid sequence set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8.

22. The anti-CD28 sdAb-Fc fusion protein of claim 18, wherein the Fc region is a heterodimeric Fc region.

23. The anti-CD28 sdAb-Fc fusion protein of any of claims 18-22, wherein the Fc region exhibits effector function.

24. The anti-CD28 sdAb-Fc fusion protein of any of claims 18-22, wherein the Fc region comprises a polypeptide comprising one or more amino acid modification that reduces effector function and/or reduces binding to an effector molecule selected from an Fc gamma receptor and C1q, optionally wherein the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235, optionally wherein the Fc region comprises the amino acid sequence set forth in SEQ ID NO: 9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9.

25. A homodimeric Fc fusion protein, comprising two identical copies of the anti-CD28 sdAb-Fc fusion protein of any of claims 18, 21, 23, and 24.

26. A heterodimeric fusion protein, comprising two of the anti-CD28 sdAb-Fc fusion proteins of any of claims 18 and 20-24.

27. A multi-specific binding polypeptide comprising the anti-CD28 sdAb-Fc fusion protein of any of claims 18-24 and one or more binding domain (BD) that binds to a target antigen other than CD28, optionally wherein the one or more BD binds a tumor associated antigen (TAA), the one or more BD binds a T cell surface marker that is a T cell activation marker or a T cell exhaustion marker, or the one or more BD binds a cell surface marker that is a tumor microenvironment marker.

28. The multi-specific binding polypeptide of claim 27, wherein the one or more BD, optionally each of the one or more BD, in a single domain antibody (sdAb).

29. The multi-specific binding polypeptide of claim 27 or 28, wherein the multi-specific binding polypeptide is an Fc fusion protein, wherein at least one of the one or more BD and/or the at least one CD28 sdAb is linked to the immunoglobulin Fc region.

30. The multi-specific binding polypeptide of any of claims 27-29 that is a dimer.

31. The multi-specific binding polypeptide of any of claims 27-30, wherein the Fc region is a homodimeric Fc region.

32. The multi-specific binding polypeptide of any of claims 27-31, wherein the Fc region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 8, 10, 11, 12 and 13.

33. The multi-specific binding polypeptide of any of claims 27-32, wherein the Fc region is a human IgG1, optionally wherein the Fc region comprises the amino acid sequence set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8.

34. The multi-specific binding polypeptide of any of claims 27-30 and 32 and 33, wherein the Fc region is a heterodimeric Fc region.

35. The multi-specific binding polypeptide of any of claims 27-34, wherein the Fc region exhibits effector function.

36. The multi-specific binding polypeptide of any of claims 27-34, wherein the Fc region comprises a polypeptide comprising one or more amino acid modification that reduces effector function and/or reduces binding to an effector molecule selected from an Fc gamma receptor and C1q, optionally

wherein the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235, optionally wherein the Fc region comprises the amino acid sequence set forth in SEQ ID NO: 9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9.

37. The multi-specific binding polypeptide of any of claims 27-36, wherein the antigen to which the BD binds is selected from the group consisting of: 1-92-LFA-3, 2B4, 5T4, Alpha-4 integrin, Alpha-V integrin, alpha4beta1 integrin, alpha4beta7 integrin, alpha-SMA, AGR2, Anti-Lewis-Y, Apelin J receptor, APRIL, B7-H3, B7-H4, BAFF, BTLA, C5 complement, C-242, CA9, CA19-9, (Lewis a), Carbonic anhydrase 9, CD2, CD3, CD6, CD9, CD11a, CD19, CD20, CD22, CD24, CD25, CD27, CD28, CD30, CD33, CD38, CD40, CD40L, CD41, CD44, CD44v6, CD47, CD51, CD52, CD56, CD64, CD69, CD70, CD71, CD74, CD80, CD81, CD86, CD95, CD107a, CD117, CD123, CD125, CD132, (IL-2RG), CD133, CD137, CD138, CD160, CD166, CD172A, CD248, CDH6, CEACAM5 (CEA), CEACAM6 (NCA-90), CLAUDIN-3, CLAUDIN-4, cMet, Collagen, Cripto, CSFR, CSFR-1, CTLA-4, CTGF, CXCL10, CXCL13, CXCR1, CXCR2, CXCR4, CYR61, DL44, DLK1, DLL3, DLL4, DPP-4, DSG1, EDA, EDB, EGFR, EGFRviii, Endothelin B receptor (ETBR), ENPP3, EpCAM, EPHA2, EPHB2, ERBB3, F protein of RSV, FAP, FGF-2, FGF8, FGFR1, FGFR2, FGFR3, FGFR4, FLT-3, Folate receptor alpha (FRalpha), FSP-1, GAL3ST1, G-CSF, G-CSFR, GD2, GITR, GLUT1, GLUT4, GM-CSF, GM-CSFR, GP IIb/IIIa receptors, Gp130, GPIIB/IIIA, GPNMB, GRP78, HER2/neu, HER3, HER4, HGF, hGH, HLA-DR, HVEM, Hyaluronidase, ICOS, IFNalpha, IFNbeta, IFNgamma, IgE, IgE Receptor (FceRI), IGF, IGF1R, IL1B, IL1R,

IL2, IL11, IL12, IL12p40, IL-12R, IL-12Rbeta1, IL13, IL13R, IL13Ra2, IL15, IL17, IL18, IL21, IL23, IL23R, IL27/IL27R (wsx1), IL29, IL-31R, IL31/IL31R, IL2R, IL4, IL4R, IL6, IL6R, IL1 receptor accessory protein (IL1RAP), Insulin Receptor, Jagged Ligands, Jagged 1, Jagged 2, KISS1-R, KLRG1, LAG-3, LIF-R, Lewis X, LIGHT, LRP4, LRRC26, Ly6G6D, LyPD1, MCSP, Mesothelin, MRP4, MUC1, Mucin-16 (MUC16, CA-125), Na/K ATPase, NGF, Nicastrin, Notch Receptors, Notch 1, Notch 2, Notch 3, Notch 4, NOV, OSM-R, OX-40, PAR2, PDGF-AA, PDGF-BB, PDGFRalpha, PDGFRbeta, PD-1, PD-L1, PD-L2, Phosphatidyl-serine, P1GF, PSCA, PSMA, PSGR, RAAG12, RAGE, SLC44A4, Siglec15, Sphingosine 1 Phosphate, STEAP1, STEAP2, TAG-72, TAPA1, TEM-8, TGFbeta, TIGIT, TIM-3, TLR2, TLR4, TLR6, TLR7, TLR8, TLR9, TMEM31, TNFalpha, TNFR, TNFRS12A, TRAIL-R1, TRAIL-R2, Transferrin, Transferrin receptor, TRK-A, TRK-B, uPAR, VAP1, VCAM-1, VEGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGFR1, VEGFR2, VEGFR3, VISTA, WISP-1, WISP-2, and WISP-3.

38. A homodimeric multi-specific Fc fusion protein, comprising two identical copies of the multi-specific binding polypeptide of any of claims 27-33 and 35-37.

39. A heterodimeric multi-specific Fc fusion protein, comprising two of the multi-specific binding polypeptides of any of claims 27-30 and 32-37.

40. The multi-specific binding polypeptide of any of claims 27-37 or the multi-specific Fc fusion protein of claim 38 or 39, wherein the at least one CD28 sdAb is not able to, or is not substantially able to, bind or engage CD28 unless at least one of the one or more BD is bound to its antigen.

41. An anti-CD28 single domain antibody (sdAb) comprising: a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:195, 189, 192, 198, 199, 200, and 201; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:206, 190, 193, 196, 202, 204, 205, 207, 208, 209, 210, 211, 212, 386, 387, 388, 389, 390, 391, 392, 393, 394, and 395; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 197, 191, 194, 396, 397, and 398.

42. The anti-CD28 sdAb of claim 41, wherein the anti-CD28 sdAb comprises:

- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;
- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:189, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:190, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:191;
- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:192, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:193, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:194;
- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;
- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:198, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;
- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:199, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;
- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:200, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;
- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;
- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:202, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;
- a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:204, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:395, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:397;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:201, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:196, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:396;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:388, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:389, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:197;

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:397; or

a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:195, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:206, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:398.

43. An anti-CD28 single domain antibody (sdAb) comprising:

a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:342; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:186; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:187; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:188; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:213; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:214; a CDR1, a CDR2, and a CDR3 as contained

NO:378; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:379; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:380; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:381; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:382; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:383; a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:384; or a CDR1, a CDR2, and a CDR3 as contained in the amino acid sequence set forth in SEQ ID NO:385.

44. The anti-CD28 sdAb of any of claims 41-43, wherein the anti-CD28 sdAb comprises the amino acid sequence set forth in any of SEQ ID NOS:342, 186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of any of SEQ ID NOS:342, 186, 187, 188, 213, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 280, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, and 385.

45. The anti-CD28 sdAb of any of claims 41-44, wherein the anti-CD28 sdAb comprises the amino acid sequence set forth in (i) SEQ ID NO: 186, (ii) a humanized variant of SEQ ID NO:186, and/or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 186.

46. The anti-CD28 sdAb of any of claims 41-45, wherein the anti-CD28 sdAb comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:189; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:190; and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:191.

47. The anti-CD28 sdAb of any of claims 41-44, wherein the anti-CD28 sdAb comprises the amino acid sequence set forth in (i) SEQ ID NO: 187, (ii) a humanized variant of SEQ ID NO:187, and/or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 187.

48. The anti-CD28 sdAb of any of claims 41-44 and 47, wherein the anti-CD28 sdAb comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:192; a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:193; and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:194.

49. The anti-CD28 sdAb of any of claims 41-44, wherein the anti-CD28 sdAb comprises the amino acid sequence set forth in (i) SEQ ID NO: 188, (ii) a humanized variant of SEQ ID NO:188, and/or (iii) a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 188.

50. The anti-CD28 sdAb of any of claims 41-44 and 49, wherein the anti- CD28 sdAb comprises a CDR1, CDR2 and CDR3 set forth in: SEQ ID NOS: 195, 206, and 197, respectively; SEQ ID NOS: 195, 196, and 197, respectively; SEQ ID NOS: 198, 196, and 197, respectively; SEQ ID NOS: 199, 196, and 197, respectively; SEQ ID NOS: 200, 196, and 197, respectively; SEQ ID NOS: 201, 196, and 197, respectively; SEQ ID NOS: 195, 202, and 197, respectively; SEQ ID NOS: 195, 204, and 197, respectively; SEQ ID NOS: 195, 205, and 197, respectively; SEQ ID NOS: 195, 207, and 197, respectively; SEQ ID NOS: 195, 208, and 197, respectively; SEQ ID NOS: 195, 209, and 197, respectively; SEQ ID NOS: 195, 210, and 197, respectively; SEQ ID NOS: 195, 211, and 197, respectively; SEQ ID NOS: 195, 212, and 197, respectively; SEQ ID NOS: 201, 202, and 197, respectively; SEQ ID NOS: 201, 202, and 396, respectively; SEQ ID NOS: 201, 386, and 197, respectively; SEQ ID NOS: 201, 387, and 197, respectively; SEQ ID NOS: 201, 202, and 397, respectively; SEQ ID NOS: 201, 202, and 398, respectively; SEQ ID NOS: 201, 206, and 197, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 388, and 197, respectively; SEQ ID NOS: 201, 389, and 197, respectively; SEQ ID NOS: 201, 206, and 397, respectively; SEQ ID NOS: 201, 206, and 398, respectively; SEQ ID NOS: 201, 203, and 197, respectively; SEQ ID NOS: 201, 203, and 396,

respectively; SEQ ID NOS: 201, 390, and 197, respectively; SEQ ID NOS: 201, 391, and 197, respectively; SEQ ID NOS: 201, 203, and 397, respectively; SEQ ID NOS: 201, 203, and 398, respectively; SEQ ID NOS: 201, 204, and 197, respectively; SEQ ID NOS: 201, 204, and 396, respectively; SEQ ID NOS: 201, 392, and 197, respectively; SEQ ID NOS: 201, 393, and 197, respectively; SEQ ID NOS: 201, 204, and 397, respectively; SEQ ID NOS: 201, 204, and 398, respectively; SEQ ID NOS: 201, 196, and 396, respectively; SEQ ID NOS: 201, 394, and 197, respectively; SEQ ID NOS: 201, 395, and 197, respectively; SEQ ID NOS: 201, 196, and 397, respectively; SEQ ID NOS: 201, 196, and 398, respectively; SEQ ID NOS: 195, 206, and 396, respectively; SEQ ID NOS: 195, 388, and 197, respectively; SEQ ID NOS: 195, 389, and 197, respectively; SEQ ID NOS: 195, 206, and 397, respectively; or SEQ ID NOS: 195, 206, and 398, respectively.

51. The anti-CD28 sdAb of any of claims 41-44 and 49 and 50, wherein the anti-CD28 sdAb comprises the amino acid sequence set forth in any of SEQ ID NOS:342, 213-219, 221-239, 280, and 343-385, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 342, 213-219, 221-239, 280, and 343-385.

52. The anti-CD28 sdAb of any of claims 41-51, wherein the anti-CD28 sdAb comprises an amino acid modification that reduces binding to protein A, optionally wherein the amino acid modification is G65D by Kabat in framework region 3 (FR3).

53. A multi-specific construct comprising at least one anti-CD28 single domain antibody (sdAb) of any of claims 41-52, one or more binding domain (BD) that binds an antigen other than CD28, and a CD3 binding region, optionally wherein the BD binds a TAA.

54. The multi-specific construct of claim 53, wherein the BD is a single domain antibody (sdAb).

55. The multi-specific construct of claim 53 or 54, wherein the CD3-binding region binds CD3 (CD3 ϵ).

56. The multi-specific construct of any of claims 53-55, wherein the CD3 binding region is an anti-CD3 antibody or antigen-binding fragment.

57. The multi-specific construct of any of claims 53-56, wherein the CD3 binding region is monovalent.

58. The multi-specific construct of any of claims 53-57, wherein the CD3 binding region is an variable fragment (Fv) comprising a variable heavy chain region (VH) and a variable light chain region (VL).

59. The multi-specific construct of any of claims 53-58, wherein the CD28-binding polypeptide comprises an immunoglobulin Fc region.

60. The multi-specific construct of claim 59, wherein the Fc region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 8, 10, 11, 12 and 13, or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS: 8, 10, 11, 12 and 13.

61. The multi-specific construct of claim 59 or 60, wherein the Fc region is a human IgG1, optionally wherein the Fc region comprises the amino acid sequence set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8.

62. The multi-specific construct of any of claims 59-61, wherein the Fc region is a heterodimeric Fc region.

63. The multi-specific construct of any of claims 59-62, wherein the Fc region exhibits effector function.

64. The multi-specific construct of any of claims 59-62, wherein the Fc region comprises a polypeptide comprising one or more amino acid modification that reduces effector function and/or reduces binding to an effector molecule selected from an Fc gamma receptor and

C1q, optionally wherein the one or more amino acid modification is deletion of one or more of Glu233, Leu234 or Leu235, optionally wherein the Fc region comprises the amino acid sequence set forth in SEQ ID NO: 9 or a sequence of amino acids that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9.

65. The multi-specific construct of any of claims 59-64, wherein the Fc is a heterodimeric Fc and the VH and VL that comprise the anti-CD3 antibody or antigen-binding fragment are linked to opposite polypeptides of the heterodimeric Fc.

66. The multi-specific construct of any of claims 53-65, wherein the CD3 binding region is not able to, or is not substantially able to, bind or engage CD3 unless at least one of the one or more BD is bound to its antigen.

67. A polynucleotide(s) encoding: the CD28-binding polypeptide of any of claims 1-17; the fusion protein of any of claims 18-26, 38, and 39; the multi-specific binding polypeptide of any of claims 27-37, and 40; or the anti-CD28 sdAb of any of claims 41-52.

68. A vector, comprising the polynucleotide of claim 67, optionally wherein the vector is an expression vector; and/or a viral vector or a eukaryotic vector, optionally wherein the eukaryotic vector is a mammalian vector.

69. A cell comprising the polynucleotide or polynucleotides of claim 67 or the vector of claim 68.

70. A method of producing a polypeptide, the method comprising introducing into a cell a polynucleotide or polynucleotides of claim 67 or a vector of claim 68 and culturing the cell under conditions to produce the polypeptide, optionally comprising isolating or purifying the polypeptide from the cell.

71. A polypeptide produced by the method of claim 70.

72. A pharmaceutical composition comprising a CD28-binding polypeptide of any of claims 1-17; a fusion protein of any of claims 18-26, 38, and 39; a multi-specific binding polypeptide of any of claims 27-37, and 40; a multi-specific construct of any of claims 53-66; or an anti-CD28 sdAb of any of claims 41-52, optionally comprising a pharmaceutically acceptable carrier.

73. Use of a CD28-binding polypeptide of any of claims 1-17; a fusion protein of any of claims 18-26, 38, and 39; a multi-specific binding polypeptide of any of claims 27-37, and 40; an anti-CD28 sdAb of any of claims 41-52; a multi-specific construct of any of claims 53-66; or a pharmaceutical composition of claim 72, in the manufacture of a medicament for stimulating an immune response in a subject in need thereof, optionally wherein the immune response is increased against a tumor or cancer.

74. Use of a composition comprising a CD28-binding polypeptide of any of claims 1-17; a fusion protein of any of claims 18-26, 38, and 39; a multi-specific binding polypeptide of any of claims 27-37, and 40; an anti-CD28 sdAb of any of claims 41-52; a multi-specific construct of any of claims 53-66; or a pharmaceutical composition of claim 72 in the manufacture of a medicament for treating a disease or condition in a subject.

75. A composition comprising a CD28-binding polypeptide of any of claims 1-17; a fusion protein of any of claims 18-26, 38, and 39; a multi-specific binding polypeptide of any of claims 27-37, and 40; or an anti-CD28 sdAb of any of claims 41-52; a multi-specific construct of any of claims 53-66; or a pharmaceutical composition of claim 72 for use in treating a disease or condition in a subject.

76. The use of claim 74, or the composition of claim 75, wherein the disease or condition is a tumor or a cancer.