

MULTIFUNCTIONAL MOLECULES THAT BIND TO CALRETICULIN AND USES THEREOF

RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application 62/808,779 filed on February 21, 2019, U.S. Provisional Application 62/818,427 filed on March 14, 2019, and U.S. Provisional Application 62/956,866 filed on January 3, 2020, the entire contents of each of which are hereby incorporated by reference.

SEQUENCE LISTING

10 The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 19, 2020, is named E2070-7021WO_SL.txt and is 1,760,247 bytes in size.

BACKGROUND

15 Myeloproliferative neoplasms (MPNs) are a group of conditions that cause blood cells to grow abnormally in the bone marrow. Common myeloproliferative neoplasms include primary or idiopathic myelofibrosis (MF), essential thrombocytosis (ET), polycythemia vera (PV), and
20 chronic myelogenous leukemia (CML). Primary myelofibrosis is a chronic blood cancer in which excessive scar tissue forms in the bone marrow and impairs its ability to produce normal blood cells. Given the ongoing need for improved treatment of myeloproliferative neoplasms such as myelofibrosis, new compositions and treatments targeting myeloproliferative neoplasms are highly desirable.

SUMMARY

25 In one aspect, the present disclosure refers to a multifunctional molecule comprising:

 (i) a first antigen binding domain that binds to a calreticulin protein,

 and

30 (ii) a second antigen binding domain that binds to a T cell, an NK cell or a tumor antigen.

In another aspect, the present disclosure refers to a polynucleotide comprising a sequence encoding the multifunctional molecule as described herein.

In another aspect, the present disclosure refers to a medicament comprising the multifunctional molecule as described herein, and a pharmaceutically acceptable carrier, excipient, or stabilizer.

In yet another aspect, the present disclosure refers to a use of a pharmaceutical composition comprising the multifunctional molecule as described herein in the manufacture of a medicament for treating cancer in a subject in need thereof,

optionally, wherein the cancer is a hematological cancer or a solid tumor cancer.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the multifunctional molecule as described herein for use in therapy.

The disclosure relates, inter alia, to novel multispecific or multifunctional molecules that include (i) an antigen binding domain that binds to a calreticulin protein (e.g., a wild-type or mutant calreticulin protein); and one, two or all of: (ii) an immune cell engager (e.g., chosen - from an NK cell engager, a T cell engager, a B cell engager, a dendritic cell engager, or a

macrophage cell engager); (iii) a cytokine molecule; and/or (iv) a stromal modifying moiety. The terms “multispecific” or “multifunctional” are used interchangeably herein.

Without wishing to be bound by theory, the multispecific or multifunctional molecules disclosed herein are expected to target (e.g., localize, bridge and/or activate) an immune cell (e.g., an immune effector cell chosen from an NK cell, a T cell, a B cell, a dendritic cell or a macrophage), at a target cell, e.g., a cancer cell, expressing a calreticulin protein (e.g., a wild-type or mutant calreticulin protein), and/or alter the tumor stroma, e.g., alter the tumor microenvironment near the cancer site. Increasing the proximity and/or activity of the immune cell using the multispecific molecules described herein is expected to enhance an immune response against the target cell (e.g., the cancer cell), thereby providing a more effective therapy (e.g., a more effective cancer therapy). Without being bound by theory, a targeted, localized immune response against the target cell (e.g., the cancer cell) is believed to reduce the effects of systemic toxicity of the multispecific molecules described herein.

Accordingly, provided herein are, *inter alia*, multispecific molecules (e.g., multispecific or multifunctional antibody molecules) that include the aforesaid moieties, nucleic acids encoding the same, methods of producing the aforesaid molecules, and methods of treating a cancer using the aforesaid molecules.

Accordingly, in one example, the disclosure features a multifunctional molecule that includes:

(i) a first antigen binding domain that binds to a calreticulin protein (e.g., a wild-type or mutant calreticulin protein),

and

(ii) a second antigen binding domain that binds to TCR β V, e.g., an anti-TCR β V antigen binding domain disclosed in any one of Table 1A, Table 2A, Table 3A, Table 10A, Table 11A, Table 12A, or Table 13A, or a second antigen binding domain that binds to NKp30, e.g., an anti-NKp30 antigen binding domain disclosed in Tables 7-10 or 18.

In some examples, the second antigen binding domain binds to TCR β V.

In some examples, the second antigen binding domain activates a T cell or the second antigen binding domain does not activate a T cell.

In some examples, the second antigen binding domain binds to TCR β V12 or TCR β V6 (e.g., comprising the amino acid sequence of SEQ ID NO: 1044).

In some examples, the second antigen binding domain comprises one or more amino acid sequences as listed in Table 1A, Table 2A, Table 3A, Table 10A, Table 11A, Table 12A, or Table 13A.

In some examples, the second antigen binding domain comprises:

5 (a) a heavy chain variable region (VH) and/or a light chain variable region (VL),
wherein:

(i) the VH comprises a heavy chain complementarity determining region 1 (VHCDR1) having an amino acid sequence of a VHCDR1 in Table 1A, Table 2A, Table 10A, Table 11A, Table 12A, or Table 13A (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 having an amino acid sequence of a VHCDR2 in Table 1A, Table 2A, Table 10A, Table 11A, Table 12A, or Table 13A (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 having an amino acid sequence of a VHCDR3 in Table 1A, Table 2A, Table 10A, Table 11A, Table 12A, or Table 13A (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions),

(ii) the VL comprises a light chain complementarity determining region 1 (VLCDR1) having an amino acid sequence of a VLCDR1 in Table 1A, Table 2A, Table 10A, Table 11A, Table 12A, or Table 13A (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 having an amino acid sequence of a VLCDR2 in Table 1A, Table 2A, Table 10A, Table 11A, Table 12A, or Table 13A (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 having an amino acid sequence of a VLCDR3 in Table 1A, Table 2A, Table 10A, Table 11A, Table 12A, or Table 13A (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions);

25 (b) a heavy chain variable region (VH) and/or a light chain variable region (VL),
wherein:

(i) the VH comprises a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 3 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 4 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g.,

(i) the VH comprises a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 48 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 49 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 50 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or

(ii) the VL comprises a light chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 54 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 55 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 56 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

In some examples, the second antigen binding domain comprises:

(a) a heavy chain variable region (VH) and/or a light chain variable region (VL),

wherein:

(i) the VH comprises the amino acid sequence of a VH in Table 1A, Table 2A, Table 10A, Table 11A, Table 12A, or Table 13A (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and/or

(ii) the VL comprises the amino acid sequence of a VL in Table 1A, Table 2A, Table 10A, Table 11A, Table 12A, or Table 13A (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto)

(iii) the VH comprises the amino acid sequence of SEQ ID NO: 9 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and/or

(iv) the VL comprises the amino acid sequence of SEQ ID NO: 10 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto);

(b) a heavy chain variable region (VH) and/or a light chain variable region (VL), wherein:

(i) the VH comprises the amino acid sequence of SEQ ID NO: 9 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and/or

(ii) the VL comprises the amino acid sequence of SEQ ID NO: 11 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto); and/or

(c) a heavy chain variable region (VH) and/or a light chain variable region (VL),

wherein:

(i) the VH comprises the amino acid sequence of SEQ ID NO: 1312 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and/or

(ii) the VL comprises the amino acid sequence of SEQ ID NO: 1314 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

In some examples, the second antigen binding domain comprises:

(a) a heavy chain variable region (VH) and/or a light chain variable region (VL),

wherein:

(i) the VH comprises a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 17 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 19 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or

(ii) the VL comprises a light chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 20 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 21 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 22 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions);

e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 68 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

5 In some examples, the second antigen binding domain comprises:

(a) a heavy chain variable region (VH) and/or a light chain variable region (VL),

wherein:

(i) the VH comprises the amino acid sequence of SEQ ID NO: 15 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and/or

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(ii) the VL comprises the amino acid sequence of SEQ ID NO: 16 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto); and/or

15 (b) a heavy chain variable region (VH) and/or a light chain variable region (VL),

wherein:

(i) the VH comprises:

the amino acid sequence of SEQ ID NO: 23 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto),

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the amino acid sequence of SEQ ID NO: 24 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), or

the amino acid sequence of SEQ ID NO: 25 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto); and/or

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(ii) the VL comprises:

the amino acid sequence of SEQ ID NO: 26 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto),

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the amino acid sequence of SEQ ID NO: 27 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto),

the amino acid sequence of SEQ ID NO: 28 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto),

the amino acid sequence of SEQ ID NO: 29 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), or

the amino acid sequence of SEQ ID NO: 30 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

In some examples, the multifunctional molecule comprises:

a first polypeptide comprising, e.g., from N-terminus to C-terminus, a first VL and a first CL,

a second polypeptide comprising, e.g., from N-terminus to C-terminus, a first VH, a first CH1, a first dimerization domain (e.g., a first Fc), and a first moiety that binds to TCR (e.g., TCRV β) (e.g., a first scFv that binds to TCR (e.g., TCRV β)),

a third polypeptide comprising, e.g., from N-terminus to C-terminus, a second VH, a second CH1, a second dimerization domain (e.g., a second Fc), and optionally a second moiety that binds to TCR (e.g., TCRV β) (e.g., a second scFv that binds to TCR (e.g., TCRV β)),

a fourth polypeptide comprising, e.g., from N-terminus to C-terminus, a second VL and a second CL,

wherein:

the first VL and the first VH form a first antigen binding domain that binds to a first calreticulin protein, and the second VL and the second VH form a third antigen binding domain that binds to a second calreticulin protein,

optionally wherein the first and second calreticulin proteins comprise the amino acid sequence of SEQ ID NO: 6285 or 6286,

optionally wherein the first and second calreticulin mutant proteins are each independently chosen from: a molecule comprising the amino acid sequence of SEQ ID NO: 6313, or a molecule comprising the amino acid sequence of SEQ ID NO: 6314,

optionally wherein the multifunctional molecule comprises the configuration of FIG. 3A or 3B.

In some examples, the second antigen binding domain binds to NKp30.

In some examples, the second antigen binding domain is chosen from an antibody molecule, e.g., an antigen binding domain, or ligand that binds to (e.g., activates) NKp30, e.g., the second antigen binding domain is an antibody molecule or ligand that binds to (e.g., activates) NKp30.

In some examples, the second antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) having an amino acid sequence of a VHCDR1 of Table 7, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 having an amino acid sequence of a VHCDR2 of Table 7, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 having an amino acid sequence of a VHCDR3 of Table 7, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) having an amino acid sequence of a VLCDR1 of Table 8, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 having an amino acid sequence of a VLCDR2 of Table 8, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 having an amino acid sequence of a VLCDR3 of Table 8, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

In some examples, the second antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 7313 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2

amino acid sequence of SEQ ID NO: 6001 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 7315 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions; and/or

- 5 (ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 7326 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 7327 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of
10 SEQ ID NO: 7329 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

In some examples, the second antigen binding domain comprises:

- (i) a VH comprising the amino acid sequence of any of SEQ ID NOs: 7298 or 7300-7304 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence
15 identity to any of SEQ ID NOs: 7298 or 7300-7304); and/or
- (ii) a VL comprising the amino acid sequence of any of SEQ ID NOs: 7299 or 7305-7309 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to any of SEQ ID NOs: 7299 or 7305-7309).

In some examples, the second antigen binding domain comprises:

- 20 (i) a VH comprising the amino acid sequence of SEQ ID NO: 7302 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to 7302), and a VL comprising the amino acid sequence of SEQ ID NO: 7305 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to 7305); or
- (ii) a VH comprising the amino acid sequence of SEQ ID NO: 7302 (or an amino acid
25 sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to 7302), and a VL comprising the amino acid sequence of SEQ ID NO: 7309 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to 7309).

In some examples, the second antigen binding domain comprises:

- (i) an amino acid sequence of SEQ ID NO: 7310 (or an amino acid sequence having at
30 least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to 7310); or

(ii) an amino acid sequence of SEQ ID NO: 7311 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to 7311).

In some examples, the second antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6000, a VHCDR2 amino acid sequence of SEQ ID NO: 6001, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6002, and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6063, a VLCDR2 amino acid sequence of SEQ ID NO: 6064, and/or a VLCDR3 amino acid sequence of SEQ ID NO: 7293.

In some examples, the second antigen binding domain comprises:

(1) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) having an amino acid sequence of a VHFWR1 of Table 7, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR2 having an amino acid sequence of a VHFWR2 of Table 7, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VHFWR3 having an amino acid sequence of a VHFWR3 of Table 7, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a VHFWR4 having an amino acid sequence of a VHFWR4 of Table 7, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), and/or

(2) a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) having an amino acid sequence of a VLFWR1 of Table 8, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR2 having an amino acid sequence of a VLFWR2 of Table 8, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), a VLFWR3 having an amino acid sequence of a VLFWR3 of Table 8, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom), or a

VLFWR4 having an amino acid sequence of a VLFWR4 of Table 8, Table 9, Table 10, or Table 18 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions, therefrom).

In some examples, the second antigen binding domain comprises:

5 (1) a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6003, a VHFWR2 amino acid sequence of SEQ ID NO: 6004, a VHFWR3 amino acid sequence of SEQ ID NO: 6005, or a VHFWR4 amino acid sequence of SEQ ID NO: 6006, and

(3) a light chain variable region (VL) comprising a light chain framework region 1
10 (VLFWR1) amino acid sequence of SEQ ID NO: 6066, a VLFWR2 amino acid sequence of SEQ ID NO: 6067, a VLFWR3 amino acid sequence of SEQ ID NO: 7292, or a VLFWR4 amino acid sequence of SEQ ID NO: 6069.

In some examples, the second antigen binding domain comprises:

(i) a VH comprising the amino acid sequence of a VH of Table 7, Table 9, Table 10, or
15 Table 18 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and/or

(ii) a VL comprising the amino acid sequence of a VL of Table 8, Table 9, Table 10, or Table 18 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto).

20 In some examples, the second antigen binding domain comprises a heavy chain comprising the amino acid sequence of a heavy chain of Table 10 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

In some examples, the second antigen binding domain comprises a light chain comprising the amino acid sequence of a light chain of Table 10 (or an amino acid sequence having at least
25 about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

In some examples, the second antigen binding domain comprises a heavy chain comprising the amino acid sequence of a heavy chain of Table 10 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto), and a light chain comprising the amino acid sequence of a light chain of Table 10 (or an amino acid
30 sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto).

In some examples, the multispecific molecule comprises:

a first polypeptide comprising, e.g., from N-terminus to C-terminus, a first VL and a first CL,

a second polypeptide comprising, e.g., from N-terminus to C-terminus, a first VH, a first CH1, a first dimerization domain (e.g., a first Fc), and a first moiety that binds to NKp30 (e.g., a first antibody molecule or ligand that binds to NKp30),

a third polypeptide comprising, e.g., from N-terminus to C-terminus, a second VH, a second CH1, a second dimerization domain (e.g., a second Fc), and optionally a second moiety that binds to NKp30 (e.g., a second antibody molecule or ligand that binds to NKp30),

a fourth polypeptide comprising, e.g., from N-terminus to C-terminus, a second VL and a second CL,

wherein:

the first VL and the first VH form a first antigen binding domain that binds to a first calreticulin protein, and the second VL and the second VH form a third antigen binding domain that binds to a second calreticulin protein,

optionally wherein the first and second calreticulin proteins comprise the amino acid sequence of SEQ ID NO: 6285 or 6286,

optionally wherein the first and second calreticulin mutant proteins are each independently chosen from: a molecule comprising the amino acid sequence of SEQ ID NO: 6313, or a molecule comprising the amino acid sequence of SEQ ID NO: 6314,

optionally wherein the multifunctional molecule comprises the configuration of FIG. 3A or 3B.

In some examples, the calreticulin protein comprises an amino acid sequence chosen from SEQ ID NOs: 6285-6312, optionally wherein the calreticulin protein comprises an amino acid sequence chosen from SEQ ID NOs: 6313-6346.

In some examples, the calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6285.

In some examples, the calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286.

In some examples, the first antigen binding domain binds to an epitope located within the C-terminus of the calreticulin protein, optionally wherein the first antigen binding domain binds to an epitope located within the amino acid sequence of SEQ ID NO: 6285 or 6286.

In some examples, the multispecific molecule further comprises:

a third antigen binding domain that binds to a second calreticulin protein, e.g., wherein the second calreticulin mutant protein comprises the amino acid sequence of SEQ ID NO: 6285 or 6286, optionally wherein:

- 5 (i) the third antigen binding domain is different from the first antigen binding domain, or
 (ii) the third antigen binding domain is the same as the first antigen binding domain.

In some examples, the second calreticulin molecule is the same as the calreticulin molecule bound by the first antigen binding domain.

10 In some examples, the second calreticulin molecule is different from the calreticulin molecule bound by the first antigen binding domain.

In some examples, the second calreticulin protein comprises an amino acid sequence chosen from SEQ ID NOs: 6285-6312, optionally wherein the second calreticulin protein comprises an amino acid sequence chosen from SEQ ID NOs: 6313-6346.

15 In some examples, the calreticulin protein bound by the first antigen binding domain comprises the amino acid sequence of SEQ ID NO: 6285, and the second calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286.

20 In some examples, the third antigen binding domain binds to an epitope located within the C-terminus of the second calreticulin protein, optionally wherein the third antigen binding domain binds to an epitope located within the amino acid sequence of SEQ ID NO: 6285 or 6286.

In some examples, the first antigen binding domain comprises:

25 (i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) having an amino acid sequence of a VHCDR1 in Table 4, Table 7A, or Table 17 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 having an amino acid sequence of a VHCDR2 in Table 4, Table 7A, or Table 17 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 having an amino acid sequence of a VHCDR3 in Table 4, Table 7A, or Table 17 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions);

30 (ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VHCDR1) having an amino acid sequence of a VLCDR1 in Table 5,

Table 7A, or Table 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 having an amino acid sequence of a VLCDR2 in Table 5, Table 7A, or Table 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 having an amino acid sequence of a VLCDR3 in Table 5, Table 7A, or Table 18 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions);

(iii) a VH comprising the amino acid sequence of a VH in Table 7A or Table 16 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto);

(iv) a VL comprising the amino acid sequence of a VL in Table 7A or Table 16 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity thereto);

(v) a VH comprising a heavy chain framework region 1 (VHFWR1) having an amino acid sequence of a VHFWR1 in Table 4 or Table 6 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions), a VHFWR2 having an amino acid sequence of a VHFWR2 in Table 4 or Table 6 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions), a VHFWR3 having an amino acid sequence of a VHFWR3 in Table 4 or Table 6 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 having an amino acid sequence of a VHFWR4 in Table 4 or Table 6 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions), and/or

(vi) a VL comprising a light chain framework region 1 (VLFWR1) having an amino acid sequence of a VLFWR1 in Table 5 or Table 6 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 having an amino acid sequence of a VLFWR2 in Table 5 or Table 6 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions), a VLFWR3 having an amino acid sequence of a VLFWR3 in Table 5 or Table 6 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions), and/or a VLFWR4 having an amino acid sequence of a VLFWR4 in Table 5 or Table 6 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions).

In some examples, the multifunctional molecule further comprises a tumor-targeting moiety.

In some examples, the tumor-targeting moiety binds to a tumor antigen.

In some examples, the tumor antigen is selected from G6B, CD34, CD41, P-selectin, Clec2, cKIT, FLT3, MPL, ITGB3, ITGB2, GP5, GP6, GP9, GP1BA, DSC2, FCGR2A, TNFRSF10A, TNFRSF10B, or TM4SF1.

5 In some examples, the tumor-targeting moiety comprises an antibody molecule, e.g., that binds to a tumor antigen selected from G6B, CD34, CD41, P-selectin, Clec2, cKIT, FLT3, MPL, ITGB3, ITGB2, GP5, GP6, GP9, GP1BA, DSC2, FCGR2A, TNFRSF10A, TNFRSF10B, or TM4SF1.

10 In some examples, the tumor-targeting moiety comprises a VH and/or VL sequence, e.g., as listed in Table A or Table 20.

In some examples, the multifunctional molecule preferentially binds to a myeloproliferative neoplasm cell over a non-tumor cell, optionally wherein the binding between the multifunctional molecule and the myeloproliferative neoplasm cell is more than 10, 20, 30, 40, 50-fold greater than the binding between the multifunctional molecule and a non-tumor cell.

15 In some examples, the myeloproliferative neoplasm cell is chosen from a myelofibrosis cell, an essential thrombocythemia cell, a polycythemia vera cell, or a chronic myeloid cancer cell, optionally wherein:

the myeloproliferative neoplasm cell does not comprise a JAK2 V617F mutation, or the myeloproliferative neoplasm cell does not comprise a MPL mutation.

20 In some examples, the multispecific molecule further comprises a linker, e.g., a linker between the first antigen binding domain and the second antigen binding domain.

In some examples, the linker is chosen from: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker.

In some examples, the linker is a peptide linker.

25 In some examples, the peptide linker comprises Gly and Ser.

In some examples, the peptide linker comprises an amino acid sequence chosen from SEQ ID NOs: 6214-6217 or 6220-6221 and 77-78.

In another example, the disclosure provides a nucleic acid molecule encoding the multifunctional molecule as described herein.

30 In another example, the disclosure provides a vector, e.g., an expression vector, comprising the nucleic acid molecule as described herein.

In another example, the disclosure provides a host cell comprising the nucleic acid molecule or a vector as described herein.

In another example, the disclosure provides a method of making, e.g., producing, the multifunctional molecule as described herein, comprising culturing the host cell described
5 herein, under suitable conditions, e.g., conditions suitable for gene expression and/or homo- or heterodimerization.

In another example, the disclosure provides a pharmaceutical composition comprising the multifunctional molecule as described herein and a pharmaceutically acceptable carrier, excipient, or stabilizer.

10 In another example, the disclosure provides a method of treating a cancer, comprising administering to a subject in need thereof the multifunctional molecule as disclosed herein, wherein the multifunctional molecule is administered in an amount effective to treat the cancer.

In another example, the disclosure provides a use of the multifunctional molecule as described herein in treating a cancer. In another example, the disclosure provides a
15 multifunctional molecule disclosed herein for use in treating a cancer.

In some examples, the subject has cancer cells that express the first and/or second calreticulin protein.

In some examples, wherein the subject has the JAK2 V617F mutation.

In some examples, the subject does not have the JAK2 V617F mutation.

20 In some examples, the subject has a MPL mutation.

In some examples, the subject does not have a MPL mutation.

In some examples, the cancer is a hematological cancer, optionally wherein the cancer is a myeloproliferative neoplasm, e.g., primary or idiopathic myelofibrosis (MF), essential thrombocytosis (ET), polycythemia vera (PV), or chronic myelogenous leukemia (CML),
25 optionally wherein the cancer is myelofibrosis.

In some examples, the cancer is a solid tumor cancer.

In some examples, the method or use further comprises administering a second therapeutic treatment.

In some examples, the second therapeutic treatment comprises a therapeutic agent (e.g., a
30 chemotherapeutic agent, a biologic agent, hormonal therapy), radiation, or surgery.

In some examples, the therapeutic agent is selected from: a chemotherapeutic agent, or a biologic agent.

In another example, the disclosure features a multifunctional molecule (e.g., polypeptide or nucleic acid encoding the same) that includes:

(i) a first antigen binding domain that binds to a calreticulin protein (e.g., a wild-type or mutant calreticulin protein),

and

(ii) one, two, or all of:

(a) an immune cell engager chosen from a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager;

(b) a cytokine molecule;

(c) a stromal modifying moiety; or

(d) a tumor-targeting moiety that binds to a tumor antigen, e.g., chosen from:

G6B, CD34, CD41, P-selectin, Clec2, cKIT, FLT3, MPL, ITGB3, ITGB2, GP5, GP6, GP9, GP1BA, DSC2, FCGR2A, TNFRSF10A, TNFRSF10B, or TM4SF1.

In an example, the disclosure features a multifunctional molecule (e.g., polypeptide or nucleic acid encoding the same) that includes:

(i) a first antigen binding domain that binds to a calreticulin protein (e.g., a wild-type or mutant calreticulin protein), and

(ii) a second antigen binding domain comprising an immune cell engager (e.g., a T cell engager, e.g., an antigen binding domain that binds to TCR β V, e.g., as described herein).

In an example, the disclosure features a multifunctional molecule (e.g., polypeptide or nucleic acid encoding the same) that includes:

(i) a first antigen binding domain that binds to a calreticulin protein (e.g., a wild-type or mutant calreticulin protein), and

(ii) a second antigen binding domain comprising a tumor-targeting moiety, e.g., that binds to a tumor antigen chosen from: G6B, CD34, CD41, P-selectin, Clec2, cKIT, FLT3, MPL, ITGB3, ITGB2, GP5, GP6, GP9, GP1BA, DSC2, FCGR2A, TNFRSF10A, TNFRSF10B, or TM4SF1.

In an example, the disclosure features a multifunctional molecule (e.g., polypeptide or nucleic acid encoding the same) that includes:

(i) a first antigen binding domain that binds to a calreticulin protein (e.g., a wild-type or mutant calreticulin protein),

5 (ii) a second antigen binding domain comprising an immune cell engager (e.g., a T cell engager, e.g., an antigen binding domain that binds to TCR β V, e.g., as described herein, e.g., an anti-TCR β V antibody molecule described herein), and

(iii) a third antigen binding domain comprising a tumor-targeting moiety, e.g., that binds to a tumor antigen chosen from: G6B, CD34, CD41, P-selectin, Clec2, cKIT, FLT3, MPL, ITGB3, ITGB2, GP5, GP6, GP9, GP1BA, DSC2, FCGR2A, TNFRSF10A, TNFRSF10B, or TM4SF1.

In some examples, the multifunctional molecule further comprises a cytokine molecule or a modulator of a cytokine molecule, e.g., a TGF- β inhibitor, e.g., as described herein.

15 In some examples, the multifunctional molecule further comprises an NK cell engager, e.g., an antigen binding domain that binds to Nkp30, e.g., as described herein.

In some examples, the calreticulin protein (e.g., the wild-type or mutant calreticulin protein) comprises the amino acid sequence of SEQ ID NO: 6285 or 6286. In some examples, the wild type calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6285. In some examples, the calreticulin mutant protein comprises the amino acid sequence of SEQ ID NO: 6286.

In some examples, the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6224, a VHFWR2 amino acid sequence of SEQ ID NO: 6226, a VHFWR3 amino acid sequence of SEQ ID NO: 6228, or a VHFWR4 amino acid sequence of SEQ ID NO: 6230.

25 In some examples, the first antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6232, a VHFWR2 amino acid sequence of SEQ ID NO: 6234, a VHFWR3 amino acid sequence of SEQ ID NO: 6236, or a VHFWR4 amino acid sequence of SEQ ID NO: 6230. In some examples, the first antigen binding domain comprises a light chain variable region (VL) comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID

NO: 6238, a VLFWR2 amino acid sequence of SEQ ID NO: 6240, a VLFWR3 amino acid sequence of SEQ ID NO: 6242, or a VLFWR4 amino acid sequence of SEQ ID NO: 6244.

In some examples, the calreticulin protein (e.g., a wild-type or mutant calreticulin protein) comprises an amino acid sequence chosen from SEQ ID NOs: 6285-6312. In some examples, the calreticulin protein (e.g., a wild-type or mutant calreticulin protein) comprises an amino acid sequence chosen from SEQ ID NOs: 6313-6346. In some examples, the calreticulin protein (e.g., a wild-type or mutant calreticulin protein) is a calreticulin protein (e.g., a wild-type or mutant calreticulin protein) disclosed in Table 2 or 3. In some examples, the calreticulin protein (e.g., a wild-type or mutant calreticulin protein) comprises the amino acid sequence of SEQ ID NO: 6287. In some examples, the calreticulin protein (e.g., a wild-type or mutant calreticulin protein) comprises the amino acid sequence of SEQ ID NO: 6313. In some examples, the calreticulin protein (e.g., a wild-type or mutant calreticulin protein) comprises the amino acid sequence of SEQ ID NO: 6288. In some examples, the calreticulin protein (e.g., a wild-type or mutant calreticulin protein) comprises the amino acid sequence of SEQ ID NO: 6314.

In some examples, the multifunctional molecule further comprising a second antigen binding domain that preferentially binds to a second calreticulin protein (e.g., a wild-type or mutant calreticulin protein). In some examples, the second calreticulin protein (e.g., a wild-type or mutant calreticulin protein) comprises the amino acid sequence of SEQ ID NO: 6286. In some examples, the second antigen binding domain is different from the first antigen binding domain. In some examples, the second antigen binding domain is the same as the first antigen binding domain. In some examples, the second calreticulin protein (e.g., a wild-type or mutant calreticulin protein) comprises an amino acid sequence chosen from SEQ ID NOs: 6287-6312. In some examples, the second calreticulin protein (e.g., a wild-type or mutant calreticulin protein) comprises an amino acid sequence chosen from SEQ ID NOs: 6313-6346. In some examples, the second calreticulin protein (e.g., a wild-type or mutant calreticulin protein) is a calreticulin protein (e.g., a wild-type or mutant calreticulin protein) disclosed in Table 2 or 3. In some examples, the second calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6287. In some examples, the second calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6313. In some examples, the second calreticulin

protein comprises the amino acid sequence of SEQ ID NO: 6288. In some examples, the second calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6314.

In some examples, the first calreticulin protein (e.g., a wild-type or mutant calreticulin protein) is a Type 1 calreticulin protein (e.g., a wild-type or mutant calreticulin protein), and the second calreticulin protein (e.g., a wild-type or mutant calreticulin protein) is a Type 2 calreticulin protein (e.g., a wild-type or mutant calreticulin protein). In some examples, the first calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6287, and the second calreticulin protein the amino acid sequence of SEQ ID NO: 6288. In some examples, the first calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6313, and the first calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6314.

In some examples, the wild type calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6285.

In some examples, the first antigen binding domain has about the same affinity (e.g., equal affinity) for the first calreticulin protein (e.g., a mutant calreticulin protein) and for a wild-type calreticulin protein.

In some examples, the second antigen binding domain has about the same affinity (e.g., equal affinity) for the second calreticulin protein (e.g., a mutant calreticulin protein) and for a wild-type calreticulin protein.

In some examples, the first antigen binding domain has a higher affinity for a first calreticulin mutant protein than for the wild type calreticulin protein. In some examples, the K_D for the binding between the first antigen binding domain and the first calreticulin mutant protein is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the first antigen binding domain and the wild type calreticulin protein. In some examples, the first antigen binding domain binds to an epitope located within the C-terminus of the first calreticulin mutant protein. In some examples, the first antigen binding domain binds to an epitope located within the amino acid sequence of SEQ ID NO: 6286. In some examples, the first antigen binding domain does not bind to the wild type calreticulin protein. In some examples, the wild type calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6285.

In some examples, the second antigen binding domain has a higher affinity for a second calreticulin mutant protein than for the wild type calreticulin protein. In some examples, the K_D for the binding between the second antigen binding domain and the second calreticulin mutant protein is no more than 40%, 30%, 20%, 10%, 1%, 0.1%, or 0.01% of the K_D for the binding between the second antigen binding domain and the wild type calreticulin protein. In some examples, the second antigen binding domain binds to an epitope located within the C-terminus of the second calreticulin mutant protein. In some examples, the second antigen binding domain binds to an epitope located within the amino acid sequence of SEQ ID NO: 6286. In some examples, the second antigen binding domain does not bind to the wild type calreticulin protein. In some examples, the wild type calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6285.

In some examples, the multifunctional molecule preferentially binds to a myeloproliferative neoplasm cell over a non-tumor cell. In some examples, the binding between the multifunctional molecule and the myeloproliferative neoplasm cell is more than 10, 20, 30, 40, 50-fold greater than the binding between the multifunctional molecule and a non-tumor cell. In some examples, the myeloproliferative neoplasm cell is chosen from a myelofibrosis cell, an essential thrombocythemia cell, a polycythemia vera cell, or a chronic myeloid cancer cell. In some examples, the myeloproliferative neoplasm cell does not comprise a JAK2 V617F mutation. In some examples, the myeloproliferative neoplasm cell does not comprise a MPL mutation.

In some examples, the first and/or second antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6253 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6254 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6255 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions). In some examples, the first and/or second antigen binding domain comprises a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6259 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ

ID NO: 6260 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 6261 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

In some examples, the first and/or second antigen binding domain comprises:

5 (i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6253 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6254 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of
10 SEQ ID NO: 6255 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6259 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2
15 amino acid sequence of SEQ ID NO: 6260 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 6261 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

In some examples, the first and/or second antigen binding domain comprises a VH
20 comprising a VHCDR1 amino acid sequence of SEQ ID NO: 6253, a VHCDR2 amino acid sequence of SEQ ID NO: 6254, and a VHCDR3 amino acid sequence of SEQ ID NO: 6255. In some examples, the first and/or second antigen binding domain comprises a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6259, a VLCDR2 amino acid sequence of SEQ ID NO: 6260, and a VLCDR3 amino acid sequence of SEQ ID NO: 6261.

25 In some examples, the first and/or second antigen binding domain comprises:

(i) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 6253, a VHCDR2 amino acid sequence of SEQ ID NO: 6254, and a VHCDR3 amino acid sequence of SEQ ID NO: 6255, and

(ii) a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6259, a VLCDR2
30 amino acid sequence of SEQ ID NO: 6260, and a VLCDR3 amino acid sequence of SEQ ID NO: 6261.

In some examples, the first and/or second antigen binding domain comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6224, a VHFWR2 amino acid sequence of SEQ ID NO: 6226, a VHFWR3 amino acid sequence of SEQ ID NO: 6228, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6230. In some examples, the first and/or second antigen binding domain comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6238, a VLFWR2 amino acid sequence of SEQ ID NO: 6240, a VLFWR3 amino acid sequence of SEQ ID NO: 6242, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6244.

In some examples, the first and/or second antigen binding domain comprises:

(i) a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6224, a VHFWR2 amino acid sequence of SEQ ID NO: 6226, a VHFWR3 amino acid sequence of SEQ ID NO: 6228, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6230, and

(ii) a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6238, a VLFWR2 amino acid sequence of SEQ ID NO: 6240, a VLFWR3 amino acid sequence of SEQ ID NO: 6242, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6244.

In some examples, the first and/or second antigen binding domain comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6263 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions), a VHFWR2 amino acid sequence of SEQ ID NO: 6264 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions), a VHFWR3 amino acid sequence of SEQ ID NO: 6265 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 228. In some examples, the first and/or second antigen binding domain comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6277 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6278 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6279 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6280.

In some examples, the first and/or second antigen binding domain comprises:

(i) a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6263 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions), a VHFWR2 amino acid sequence of SEQ ID NO: 6264 (or a sequence with no more than 1, 2, 3, 4, 5, or 6 mutations, e.g., substitutions, additions, or deletions), a VHFWR3 amino acid sequence of SEQ ID NO: 6265 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 228, and

(ii) a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6277 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6278 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6279 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6280.

In some examples, the first and/or second antigen binding domain comprises a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6263, a VHFWR2 amino acid sequence of SEQ ID NO: 6264, a VHFWR3 amino acid sequence of SEQ ID NO: 6265, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 228. In some examples, the first and/or second antigen binding domain comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6277, a VLFWR2 amino acid sequence of SEQ ID NO: 6278, a VLFWR3 amino acid sequence of SEQ ID NO: 6279, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6280.

In some examples, the first and/or second antigen binding domain comprises:

(i) a VH comprising a VHFWR1 amino acid sequence of SEQ ID NO: 6263, a VHFWR2 amino acid sequence of SEQ ID NO: 6264, a VHFWR3 amino acid sequence of SEQ ID NO: 6265, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 228, and

(ii) a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6277, a VLFWR2 amino acid sequence of SEQ ID NO: 6278, a VLFWR3 amino acid sequence of SEQ ID NO: 6279, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6280.

In some examples, the first and/or second antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 6247 (or an amino acid sequence having at

least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6247). In some examples, the first and/or second antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 6249 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6249).

5 In some examples, the first and/or second antigen binding domain comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 6247 (or an amino acid sequence having at least about 77%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6247), and

10 (ii) a VL comprising the amino acid sequence of SEQ ID NO: 6249 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6249).

In some examples, the first and/or second antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 6247. In some examples, the first and/or second antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 6249. In some examples, the first and/or second antigen binding domain comprises (i) a VH
15 comprising the amino acid sequence of SEQ ID NO: 6247, and (ii) a VL comprising the amino acid sequence of SEQ ID NO: 6249.

In some examples, the first and/or second antigen binding domain comprises a VH comprising an amino acid sequence of at least 70% or 75% sequence identity to SEQ ID NO: 6250. In some examples, the first and/or second antigen binding domain comprises a VL
20 comprising an amino acid sequence of at least 85% or 90% sequence identity to SEQ ID NO: 6252. In some examples, the first and/or second antigen binding domain comprises (i) a VH comprising an amino acid sequence of at least 70% or 75% sequence identity to SEQ ID NO: 6250, and (ii) a VL comprising an amino acid sequence of at least 85% or 90% sequence identity to SEQ ID NO: 6252.

25 In some examples, the first and/or second antigen binding domain comprises a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6256 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6257 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions,
30 additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6258 or 116 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

In some examples, the first and/or second antigen binding domain comprises a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6259 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 6260 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 6261 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

In some examples, the first and/or second antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6256 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6257 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VHCDR3 amino acid sequence of SEQ ID NO: 6258 or 116 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6259 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VLCDR2 amino acid sequence of SEQ ID NO: 6260 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), and/or a VLCDR3 amino acid sequence of SEQ ID NO: 6261 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions).

In some examples, the first and/or second antigen binding domain comprises a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6232, a VHFWR2 amino acid sequence of SEQ ID NO: 6234, a VHFWR3 amino acid sequence of SEQ ID NO: 6236, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6230. In some examples, the first and/or second antigen binding domain comprises a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6238, a VLFWR2 amino acid sequence of SEQ ID NO: 6240, a VLFWR3 amino acid sequence of SEQ ID NO: 6242, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6244.

In some examples, the first and/or second antigen binding domain comprises:

(i) a VH comprising a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6232, a VHFWR2 amino acid sequence of SEQ ID NO: 6234, a VHFWR3 amino acid sequence of SEQ ID NO: 6236, and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6230, and

5 (ii) a VL comprising a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6238, a VLFWR2 amino acid sequence of SEQ ID NO: 6240, a VLFWR3 amino acid sequence of SEQ ID NO: 6242, and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6244.

In some examples, the first and/or second antigen binding domain comprises a VH
10 comprising a heavy chain framework 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6266 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions), a VHFWR2 amino acid sequence of SEQ ID NO: 6267 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHFWR3 amino acid sequence of SEQ ID NO: 6268 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8,
15 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a VHFWR4 amino acid sequence of SEQ ID NO: 6269. In some examples, the first and/or second antigen binding domain comprises a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6277 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6278 (or a sequence with no more than 1
20 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6279 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6280.

In some examples, the first and/or second antigen binding domain comprises:

(i) a VH comprising a heavy chain framework 1 (VHFWR1) amino acid sequence of
25 SEQ ID NO: 6266 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, or 9 mutations, e.g., substitutions, additions, or deletions), a VHFWR2 amino acid sequence of SEQ ID NO: 6267 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHFWR3 amino acid sequence of SEQ ID NO: 6268 (or a sequence with no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 mutations, e.g., substitutions, additions, or deletions), and/or a
30 VHFWR4 amino acid sequence of SEQ ID NO: 6269, and

(ii) a VL comprising a VLFWR1 amino acid sequence of SEQ ID NO: 6277 (or a sequence with no more than 1, 2, or 3 mutations, e.g., substitutions, additions, or deletions), a VLFWR2 amino acid sequence of SEQ ID NO: 6278 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), a VLFWR3 amino acid sequence of SEQ ID NO: 6279 (or a sequence with no more than 1 mutation, e.g., substitution, addition, or deletion), and/or a VLFWR4 amino acid sequence of SEQ ID NO: 6280.

In some examples, the first and/or second antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 6248 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6248). In some examples, the first and/or second antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 6249 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6249).

In some examples, the first and/or second antigen binding domain comprises

(i) a VH comprising the amino acid sequence of SEQ ID NO: 6248 (or an amino acid sequence having at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to SEQ ID NO: 6248), and

(ii) a VL comprising the amino acid sequence of SEQ ID NO: 6249 (or an amino acid sequence having at least about 93%, 95%, or 99% sequence identity to SEQ ID NO: 6249).

In some examples, the first and/or second antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 6248. In some examples, the first and/or second antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 6249. In some examples, the first and/or second antigen binding domain comprises (i) a VH comprising the amino acid sequence of SEQ ID NO: 6248, and (ii) a VL comprising the amino acid sequence of SEQ ID NO: 6249.

In some examples, the first and/or second antigen binding domain comprises a VH comprising an amino acid sequence of at least 70% or 74% sequence identity to SEQ ID NO: 6251. In some examples, the first and/or second antigen binding domain comprises a VL comprising an amino acid sequence of at least 85% or 90% sequence identity to SEQ ID NO: 6252. In some examples, the first and/or second antigen binding domain comprises (i) a VH comprising an amino acid sequence of at least 70% or 74% sequence identity to SEQ ID NO:

6251, and/or (ii) a VL comprising an amino acid sequence of at least 85% or 90% sequence identity to SEQ ID NO: 6252.

In some examples, the multifunctional molecule comprises an immune cell engager chosen from a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager. In some examples, the immune cell engager binds to and activates an immune cell, e.g., an effector cell. In some examples, the immune cell engager binds to, but does not activate, an immune cell, e.g., an effector cell.

In some examples, the immune cell engager is a T cell engager, e.g., a T cell engager that mediates binding to and activation of a T cell, or a T cell engager that mediates binding to but not activation of a T cell. In some examples, the T cell engager binds to CD3, TCR α , TCR β , TCR γ , TCR ζ , ICOS, CD28, CD27, HVEM, LIGHT, CD40, 4-1BB, OX40, DR3, GITR, CD30, TIM1, SLAM, CD2, or CD226. In some examples, the T cell engager is an anti-CD3 antibody molecule. In some examples, the T cell engager is an anti-TCR β antibody molecule, e.g., an anti-TCR β V antibody molecule described herein.

In some examples, the immune cell engager is an NK cell engager, e.g., an NK cell engager that mediates binding to and activation of an NK cell, or an NK cell engager that mediates binding to but not activation of an NK cell. In some examples, the NK cell engager is chosen from an antibody molecule, e.g., an antigen binding domain, or ligand that binds to (e.g., activates): NKp30, NKp40, NKp44, NKp46, NKG2D, DNAM1, DAP10, CD16 (e.g., CD16a, CD16b, or both), CRTAM, CD27, PSGL1, CD96, CD100 (SEMA4D), NKp80, CD244 (also known as SLAMF4 or 2B4), SLAMF6, SLAMF7, KIR2DS2, KIR2DS4, KIR3DS1, KIR2DS3, KIR2DS5, KIR2DS1, CD94, NKG2C, NKG2E, or CD160. In some examples, the NK cell engager is an antibody molecule or ligand that binds to (e.g., activates) NKp30. In some examples, the NK cell engager is an antibody molecule, e.g., an antigen binding domain. In some examples, the NK cell engager is an antibody molecule, e.g., an antigen binding domain, that binds to NKp30 or NKp46. In some examples, the NK cell engager is a ligand, optionally, the ligand further comprises an immunoglobulin constant region, e.g., an Fc region. In some examples, the NK cell engager is a ligand of NKp44 or NKp46, e.g., a viral HA. In some examples, the NK cell engager is a ligand of DAP10, e.g., a coreceptor for NKG2D. In some examples, the NK cell engager is a ligand of CD16, e.g., a CD16a/b ligand, e.g., a CD16a/b ligand further comprising an antibody Fc region. In some examples,

the immune cell engager mediates binding to, or activation of, or both of, one or more of a B cell, a macrophage, and/or a dendritic cell.

In some examples, the immune cell engager comprises a B cell, macrophage, and/or dendritic cell engager chosen from one or more of CD40 ligand (CD40L) or a CD70 ligand; an antibody molecule that binds to CD40 or CD70; an antibody molecule to OX40; an OX40 ligand (OX40L); an agonist of a Toll-like receptor (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4) or a TLR9 agonist); a 41BB; a CD2 agonist; a CD47; or a STING agonist, or a combination thereof. In some examples, the immune cell engager is a B cell engager, e.g., a CD40L, an OX40L, or a CD70 ligand, or an antibody molecule that binds to OX40, CD40 or CD70. In some examples, the immune cell engager is a macrophage cell engager, e.g., a CD2 agonist; a CD40L; an OX40L; an antibody molecule that binds to OX40, CD40 or CD70; an agonist of a Toll-like receptor (TLR) (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4) or a TLR9 agonist); CD47; or a STING agonist. In some examples, the immune cell engager is a dendritic cell engager, e.g., a CD2 agonist, an OX40 antibody, an OX40L, 41BB agonist, a Toll-like receptor agonist or a fragment thereof (e.g., a TLR4, e.g., a constitutively active TLR4 (caTLR4)), CD47 agonist, or a STING agonist. In some examples, the STING agonist comprises a cyclic dinucleotide, e.g., a cyclic di-GMP (cdGMP), a cyclic di-AMP (cdAMP), or a combination thereof, optionally with 2',5' or 3',5' phosphate linkages, e.g., wherein the STING agonist is covalently coupled to the multifunctional molecule.

In some examples, the multifunctional molecule comprises a cytokine molecule or a modulator thereof. In some examples, the cytokine molecule is chosen from TGF- β , interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18), interleukin-21 (IL-21), or interferon gamma, or a fragment or variant thereof, or a combination of any of the aforesaid cytokines. In some examples, the cytokine molecule is a monomer or a dimer. In some examples, the cytokine molecule further comprises a receptor dimerizing domain, e.g., an IL15Ralpha dimerizing domain. In some examples, the cytokine molecule (e.g., IL-15) and the receptor dimerizing domain (e.g., an IL15Ralpha dimerizing domain) are not covalently linked, e.g., are non-covalently associated.

In some examples, the modulator of the cytokine molecule comprises a TGF- β inhibitor.

In some examples, the multifunctional molecule comprises a stromal modifying moiety. In some examples, the stromal modifying moiety causes one or more of: decreases the level or production of a stromal or extracellular matrix (ECM) component; decreases tumor fibrosis; increases interstitial tumor transport; improves tumor perfusion; expands the tumor

5 microvasculature; decreases interstitial fluid pressure (IFP) in a tumor; or decreases or enhances penetration or diffusion of an agent, e.g., a cancer therapeutic or a cellular therapy, into a tumor or tumor vasculature. In some examples, the stromal or ECM component decreased is chosen from a glycosaminoglycan or an extracellular protein, or a combination thereof. In some examples, the glycosaminoglycan is chosen from hyaluronan (also known as hyaluronic acid or
10 HA), chondroitin sulfate, chondroitin, dermatan sulfate, heparan sulfate, heparin, entactin, tenascin, aggrecan or keratin sulfate. In some examples, the extracellular protein is chosen from collagen, laminin, elastin, fibrinogen, fibronectin, or vitronectin. In some examples, the stromal modifying moiety comprises an enzyme molecule that degrades a tumor stroma or extracellular matrix (ECM). In some examples, the enzyme molecule is chosen from a hyaluronidase
15 molecule, a collagenase molecule, a chondroitinase molecule, a matrix metalloproteinase molecule (e.g., macrophage metalloelastase), or a variant (e.g., a fragment) of any of the aforesaid. In some examples, the stromal modifying moiety decreases the level or production of hyaluronic acid. In some examples, the stromal modifying moiety comprises a hyaluronan degrading enzyme, an agent that inhibits hyaluronan synthesis, or an antibody molecule against
20 hyaluronic acid. In some examples, the hyaluronan degrading enzyme is a hyaluronidase molecule or a variant (e.g., fragment thereof) thereof. In some examples, the hyaluronan degrading enzyme is active in neutral or acidic pH, e.g., pH of about 4-5. In some examples, the hyaluronidase molecule is a mammalian hyaluronidase molecule, e.g., a recombinant human hyaluronidase molecule, or a variant thereof (e.g., a truncated form thereof). In some examples,
25 the hyaluronidase molecule is chosen from HYAL1, HYAL2, or PH-20/SPAM1, or a variant thereof (e.g., a truncated form thereof). In some examples, the truncated form lacks a C-terminal glycosylphosphatidylinositol (GPI) attachment site or a portion of the GPI attachment site. In some examples, the hyaluronidase molecule is glycosylated, e.g., comprises at least one N-linked glycan. In some examples, the hyaluronidase molecule comprises the amino acid sequence of
30 SEQ ID NO:6213, or a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical

thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 6213). In some examples, the hyaluronidase molecule comprises the amino acid residues 36-464 of SEQ ID NO: 6213. In some examples, the

5 hyaluronidase molecule comprises the amino acid residues 36-481, 36-482, or 36-483 of PH20, wherein PH20 has the amino acid sequence of SEQ ID NO: 6213. In some examples, the hyaluronidase molecule comprises an amino acid sequence having at least 95% to 100 % sequence identity to the polypeptide or truncated form of the amino acid sequence of SEQ ID NO: 6213. In some examples, the hyaluronidase molecule comprises an amino acid sequence

10 having 30, 20, 10, 5 or fewer amino acid substitutions to the amino acid sequence of SEQ ID NO: 6213. In some examples, the hyaluronidase molecule comprises an amino acid sequence at least 95% (e.g., at least 95%, 96%, 97%, 98%, 99%, 100%) identical to the amino acid sequence of SEQ ID NO: 6213. In some examples, the hyaluronidase molecule is encoded by a nucleotide sequence at least 95% (e.g., at least 96%, 97%, 98%, 99%, 100%) identical to the nucleotide

15 sequence of SEQ ID NO: 6213. In some examples, the hyaluronidase molecule is PH20, e.g., rHuPH20. In some examples, the hyaluronidase molecule is HYAL1 and comprises the amino acid sequence of SEQ ID NO: 6218, or a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or

20 insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 6218). In some examples, the hyaluronan degrading enzyme, e.g., the hyaluronidase molecule, further comprises a polymer, e.g., is conjugated to a polymer, e.g., PEG. In some examples, the hyaluronan-degrading enzyme is a PEGylated PH20 enzyme (PEGPH20). In some examples, the hyaluronan degrading enzyme, e.g., the hyaluronidase molecule, further comprises an

25 immunoglobulin chain constant region (e.g., Fc region) chosen from, e.g., the heavy chain constant regions of IgG1, IgG2, IgG3, or IgG4, more particularly, the heavy chain constant region of human IgG1, IgG2, IgG3, or IgG4. In some examples, the immunoglobulin constant region (e.g., the Fc region) is linked, e.g., covalently linked to, the hyaluronan degrading enzyme, e.g., the hyaluronidase molecule. In some examples, the immunoglobulin chain

30 constant region (e.g., Fc region) is altered, e.g., mutated, to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the

number of cysteine residues, effector cell function, or complement function. In some examples, the hyaluronan degrading enzyme, e.g., the hyaluronidase molecule, forms a dimer. In some examples, the stromal modifying moiety comprises an inhibitor of the synthesis of hyaluronan, e.g., an HA synthase. In some examples, the inhibitor comprises a sense or an antisense nucleic acid molecule against an HA synthase or is a small molecule drug. In some examples, the inhibitor is 4- methylumbelliferone (MU) or a derivative thereof (e.g., 6,7-dihydroxy-4-methyl coumarin or 5,7-dihydroxy-4-methyl coumarin), or leflunomide or a derivative thereof. In some examples, the stromal modifying moiety comprises a collagenase molecule, e.g., a mammalian collagenase molecule, or a variant (e.g., fragment) thereof. In some examples, the collagenase molecule is collagenase molecule IV, e.g., comprising the amino acid sequence of SEQ ID NO: 6219, or a fragment thereof, or an amino acid sequence substantially identical thereto (e.g., 95% to 99.9% identical thereto, or having at least one amino acid alteration, but not more than five, ten or fifteen alterations (e.g., substitutions, deletions, or insertions, e.g., conservative substitutions) to the amino acid sequence of SEQ ID NO: 6219.

In some examples, the multifunctional molecule comprises an immune cell engager (e.g., a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager) and a cytokine molecule. In some examples, the multifunctional molecule comprises an immune cell engager (e.g., a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager) and a stromal modifying moiety. In some examples, the multifunctional molecule comprises a cytokine molecule and a stromal modifying moiety. In some examples, the multifunctional molecule comprises an immune cell engager (e.g., a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager), a cytokine molecule, and a stromal modifying moiety.

In some examples, the multifunctional molecule comprises at least two non-contiguous polypeptide chains.

In some examples, the multifunctional molecule comprises the following configuration:

A, B-[dimerization module]-C, -D

e.g., the configuration shown in FIGs. 1A, 1B, and 1C, wherein:

(1) the dimerization module comprises an immunoglobulin constant domain, *e.g.*, a heavy chain constant domain (*e.g.*, a homodimeric or heterodimeric heavy chain constant region, *e.g.*, an Fc region), or a constant domain of an immunoglobulin variable region (*e.g.*, a Fab region); and

5 (2) A, B, C, and D are independently absent; (i) an antigen binding domain that binds to a calreticulin protein (*e.g.*, a wild type calreticulin protein or a mutant calreticulin protein), wherein the calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286; (ii) an immune cell engager chosen from a T cell engager, an NK cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager; (iii) a cytokine molecule; or (iv) a stromal
10 modifying moiety, provided that:

at least one, two, or three of A, B, C, and D comprises an antigen binding domain that binds to a calreticulin protein (*e.g.*, a wild type calreticulin protein or a calreticulin mutant protein), wherein the calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, and

15 any of the remaining A, B, C, and D is absent or comprises one of an immune cell engager, a cytokine molecule, or a stromal modifying moiety.

In some examples,

(i) A comprises an antigen binding domain that binds to a calreticulin protein (*e.g.*, a wild type calreticulin protein or a calreticulin mutant protein), wherein the calreticulin protein
20 comprises the amino acid sequence of SEQ ID NO: 6286, and B, C, or D comprises an immune cell engager, *e.g.*, a T cell engager, *e.g.*, an anti-CD3 antibody molecule;

(ii) A comprises an antigen binding domain that binds to a calreticulin protein (*e.g.*, a wild type calreticulin protein or a calreticulin mutant protein), wherein the calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, and B, C, or D comprises a cytokine
25 molecule;

(iii) A comprises an antigen binding domain that binds to a calreticulin protein (*e.g.*, a wild type calreticulin protein or a calreticulin mutant protein), wherein the calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, and B, C, or D comprises a stromal modifying moiety;

30 (iv) A comprises a first antigen binding domain that binds to a first calreticulin protein (*e.g.*, a wild type calreticulin protein or a calreticulin mutant protein), wherein the first

(xviii) A comprises a first antigen binding domain that binds to a first calreticulin protein (e.g., a wild type calreticulin protein or a calreticulin mutant protein), wherein the first calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, C comprises a second antigen binding domain that binds to a second calreticulin protein (e.g., a wild type calreticulin protein or a calreticulin mutant protein), wherein the second calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, and B or D comprises (a) a cytokine molecule and (b) a stromal modifying moiety;

(xix) A comprises a first antigen binding domain that binds to a first calreticulin protein (e.g., a wild type calreticulin protein or a calreticulin mutant protein), wherein the first calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, and B, C, or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-CD3 antibody molecule, (b) a cytokine molecule, and (c) a stromal modifying moiety;

(xx) A comprises a first antigen binding domain that binds to a first calreticulin protein (e.g., a wild type calreticulin protein or a calreticulin mutant protein), wherein the first calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, B comprises a second antigen binding domain that binds to a second calreticulin protein (e.g., a wild type calreticulin protein or a calreticulin mutant protein), wherein the second calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, and C or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-CD3 antibody molecule, (b) a cytokine molecule, and (c) a stromal modifying moiety; or

(xxi) A comprises a first antigen binding domain that binds to a first calreticulin protein (e.g., a wild type calreticulin protein or a calreticulin mutant protein), wherein the first calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, C comprises a second antigen binding domain that binds to a second calreticulin protein (e.g., a wild type calreticulin protein or a calreticulin mutant protein), wherein the second calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, and B or D comprises (a) an immune cell engager, e.g., a T cell engager, e.g., an anti-CD3 antibody molecule, (b) a cytokine molecule, and (c) a stromal modifying moiety.

In some examples, the dimerization module comprises one or more immunoglobulin chain constant regions (e.g., Fc regions) comprising one or more of: a paired cavity-protuberance (“knob-in-a hole”), an electrostatic interaction, or a strand-exchange. In some examples, the

one or more immunoglobulin chain constant regions (e.g., Fc regions) comprise an amino acid substitution at a position chosen from one or more of 347, 349, 350, 351, 366, 368, 370, 392, 394, 395, 397, 398, 399, 405, 407, or 409, e.g., of the Fc region of human IgG1. In some examples, the one or more immunoglobulin chain constant regions (e.g., Fc regions) comprise an amino acid substitution chosen from: T366S, L368A, or Y407V (e.g., corresponding to a cavity or hole), or T366W (e.g., corresponding to a protuberance or knob), or a combination thereof.

In some examples, the multifunctional molecule further comprises a linker, e.g., a linker between one or more of: the antigen binding domain and the immune cell engager, the antigen binding domain and the cytokine molecule, the antigen binding domain and the stromal modifying moiety, the immune cell engager and the cytokine molecule, the immune cell engager and the stromal modifying moiety, the cytokine molecule and the stromal modifying moiety, the antigen binding domain and the dimerization module, the immune cell engager and the dimerization module, the cytokine molecule and the dimerization module, or the stromal modifying moiety and the dimerization module. In some examples, the linker is chosen from: a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, or a non-helical linker. In some examples, the linker is a peptide linker. In some examples, the peptide linker comprises Gly and Ser. In some examples, the peptide linker comprises an amino acid sequence chosen from SEQ ID NOs: 6214-6217 or 6220-6221 and 77-78.

In one example, the disclosure provides a multifunctional molecule, comprising:

(i) an antigen binding domain that binds to a calreticulin protein (e.g., a wild type calreticulin protein or a calreticulin mutant protein), e.g., wherein the calreticulin mutant protein comprises the amino acid sequence of SEQ ID NO: 6286, and

(ii) a moiety that binds to CD3, e.g., an antibody molecule that binds to CD3.

In some examples, the multifunctional molecule comprises:

a first polypeptide comprising, e.g., from N-terminus to C-terminus, a first VL and a first CL,

a second polypeptide comprising, e.g., from N-terminus to C-terminus, a first VH, a first CH1, a first dimerization domain (e.g., a first Fc), and a first moiety that binds to CD3 (e.g., a first scFv that binds to CD3),

5 a third polypeptide comprising, e.g., from N-terminus to C-terminus, a second VH, a second CH1, a second dimerization domain (e.g., a second Fc), and optionally a second moiety that binds to CD3 (e.g., a second scFv that binds to CD3),

a fourth polypeptide comprising, e.g., from N-terminus to C-terminus, a second VL and a second CL, wherein:

10 the first VL and the first VH form a first antigen binding domain that binds to a first calreticulin protein (e.g., a wild-type calreticulin protein or a calreticulin mutant protein), and the second VL and the second VH form a second antigen binding domain that binds to a second calreticulin protein (e.g., a wild-type calreticulin protein or a calreticulin mutant protein), wherein the first and second calreticulin proteins comprise the amino acid sequence of SEQ ID NO: 6286, optionally wherein the first and second calreticulin proteins are each independently
15 chosen from: a molecule comprising the amino acid sequence of SEQ ID NO: 6313, or a molecule comprising the amino acid sequence of SEQ ID NO: 6314.

In some examples, the multifunctional molecule comprises the configuration of FIG. 2A or 2B.

20 In one example, the disclosure provides a multifunctional molecule, comprising:

(i) an antigen binding domain that binds to a calreticulin protein (e.g., a wild type calreticulin protein or a calreticulin mutant protein), e.g., wherein the calreticulin mutant protein comprises the amino acid sequence of SEQ ID NO: 6286, and

(ii) a moiety that binds to TCR (e.g., TCR β), e.g., an antibody molecule that binds to
25 TCR (e.g., TCR β).

In some examples, the multifunctional molecule comprises:

a first polypeptide comprising, e.g., from N-terminus to C-terminus, a first VL and a first CL,

30 a second polypeptide comprising, e.g., from N-terminus to C-terminus, a first VH, a first CH1, a first dimerization domain (e.g., a first Fc), and a first moiety that binds to TCR (e.g., TCR β) (e.g., a first scFv that binds to TCR (e.g., TCR β)),

a third polypeptide comprising, e.g., from N-terminus to C-terminus, a second VH, a second CH1, a second dimerization domain (e.g., a second Fc), and optionally a second moiety that binds to TCR (e.g., TCR β) (e.g., a second scFv that binds to TCR (e.g., TCR β)),

a fourth polypeptide comprising, e.g., from N-terminus to C-terminus, a second VL and a second CL, wherein:

the first VL and the first VH form a first antigen binding domain that binds to a first calreticulin protein (e.g., a wild-type calreticulin protein or a calreticulin mutant protein), and the second VL and the second VH form a second antigen binding domain that binds to a second calreticulin protein (e.g., a wild-type calreticulin protein or a calreticulin mutant protein), wherein the first and second calreticulin proteins comprise the amino acid sequence of SEQ ID NO: 6286, optionally wherein the first and second calreticulin proteins are each independently chosen from: a molecule comprising the amino acid sequence of SEQ ID NO: 6313, or a molecule comprising the amino acid sequence of SEQ ID NO: 6314.

In some examples, the multifunctional molecule comprises the configuration of FIG. 3A or 3B.

In one example, the disclosure provides a multifunctional molecule, comprising:

(i) an antigen binding domain that binds to a calreticulin protein (e.g., a wild-type calreticulin protein or a calreticulin mutant protein), e.g., wherein the calreticulin protein comprises the amino acid sequence of SEQ ID NO: 6286, and

(ii) a moiety that binds to NKp30, e.g., an antibody molecule or ligand that binds to (e.g., activates) NKp30.

In some examples, the multifunctional molecule comprises:

a first polypeptide comprising, e.g., from N-terminus to C-terminus, a first VL and a first CL,

a second polypeptide comprising, e.g., from N-terminus to C-terminus, a first VH, a first CH1, a first dimerization domain (e.g., a first Fc), and a first moiety that binds to NKp30 (e.g., a first antibody molecule or ligand that binds to NKp30),

a third polypeptide comprising, e.g., from N-terminus to C-terminus, a second VH, a second CH1, a second dimerization domain (e.g., a second Fc), and optionally a second moiety that binds to NKp30 (e.g., a second antibody molecule or ligand that binds to NKp30),

a fourth polypeptide comprising, e.g., from N-terminus to C-terminus, a second VL and a second CL, wherein:

the first VL and the first VH form a first antigen binding domain that binds to a first calreticulin protein (e.g., a wild-type calreticulin protein or a calreticulin mutant protein), and the second VL and the second VH form a second antigen binding domain that binds to a second calreticulin protein (e.g., a wild-type calreticulin protein or a calreticulin mutant protein), wherein the first and second calreticulin proteins comprise the amino acid sequence of SEQ ID NO: 6286, optionally wherein the first and second calreticulin proteins are each independently chosen from: a molecule comprising the amino acid sequence of SEQ ID NO: 6313, or a molecule comprising the amino acid sequence of SEQ ID NO: 6314.

In some examples, the multifunctional molecule comprises the configuration of FIG. 4A or 4B.

In another example, the disclosure provides an isolated nucleic acid molecule encoding any multispecific or multifunctional molecule described herein. In another example, the disclosure provides an isolated nucleic acid molecule, which comprises the nucleotide sequence encoding any of the multispecific or multifunctional molecules described herein, or a nucleotide sequence substantially homologous thereto (e.g., at least 80%, 90%, 95%, or 99.9% identical thereto). In another example, the disclosure provides a host cell comprising a nucleic acid molecule or a vector described herein.

In another example, the disclosure provides a method of making, e.g., producing, a multispecific or multifunctional molecule polypeptide described herein, comprising culturing a host cell described herein, under suitable conditions, e.g., conditions suitable for gene expression and/or homo- or heterodimerization.

In another example, the disclosure provides a pharmaceutical composition comprising a multispecific or multifunctional molecule polypeptide described herein and a pharmaceutically acceptable carrier, excipient, or stabilizer.

In another example, the disclosure provides a method of treating a cancer, comprising administering to a subject in need thereof a multispecific or multifunctional molecule polypeptide described herein, wherein the multispecific antibody is administered in an amount effective to treat the cancer. In some examples, the subject has cancer cells that express the

first and/or second calreticulin mutant. In some examples, the subject has tumor cells that express the first, second, or third tumor antigen, e.g., the subject has tumor cells that express a tumor antigen chosen from G6B, CD34, CD41, P-selectin, Clec2, cKIT, FLT3, MPL, ITGB3, ITGB2, GP5, GP6, GP9, GP1BA, DSC2, FCGR2A, TNFRSF10A, TNFRSF10B, or TM4SF1.

5 In some examples, the subject has the JAK2 V617F mutation. In some examples, the subject does not have the JAK2 V617F mutation. In some examples, the subject has a MPL mutation. In some examples, the subject does not have a MPL mutation. In some examples, the cancer is a hematological cancer, optionally wherein the cancer is a myeloproliferative neoplasm, e.g., primary or idiopathic myelofibrosis (MF), essential thrombocytosis (ET), polycythemia vera
10 (PV), or chronic myelogenous leukemia (CML). In some examples, the cancer is myelofibrosis. In some examples, the cancer is a solid tumor cancer. In some examples, the solid tumor cancer is one or more of pancreatic (e.g., pancreatic adenocarcinoma), breast, colorectal, lung (e.g., small or non-small cell lung cancer), skin, ovarian, or liver cancer.

In some examples, the cancer cell comprises a myeloproliferative neoplasm cell. In
15 examples, the myeloproliferative neoplasm cell is chosen from a myelofibrosis cell, an essential thrombocythemia cell, a polycythemia vera cell, or a chronic myeloid cancer cell. In some examples, the myeloproliferative neoplasm cell is a myelofibrosis cell. In some examples, the myeloproliferative neoplasm cell is an essential thrombocythemia cell. In some examples, the myeloproliferative neoplasm cell is a polycythemia vera cell. In some examples, the
20 myeloproliferative neoplasm cell is a chronic myeloid cancer cell. In some examples, the myeloproliferative neoplasm cell comprises a JAK2 mutation (e.g., a JAK2 V617F mutation). In some examples, the myeloproliferative neoplasm cell comprises a calreticulin mutation. In some examples, the myeloproliferative neoplasm cell comprises a MPL mutation.

In some examples, the method further comprises administering a second therapeutic
25 treatment. In some examples, second therapeutic treatment comprises a therapeutic agent (e.g., a chemotherapeutic agent, a biologic agent, hormonal therapy), radiation, or surgery. In some examples, therapeutic agent is selected from: a chemotherapeutic agent, or a biologic agent.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Other features and advantages of the disclosure will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIGs. 1A-1B shows the alignment of the Antibody A source mouse VH and VL framework 1, CDR 1, framework 2, CDR 2, framework 3, CDR3, and framework 4 regions with their respective humanized sequences. Kabat CDRs are shown in bold, Chothia CDRs are shown in italics, and combined CDRs are shown in boxes. The framework positions that were back mutated are double underlined. **FIG. 1A** shows VH sequences for murine Antibody A (SEQ ID NO: 1) and humanized Antibody A-H (SEQ ID NO: 9). **FIG. 1B** shows VL sequences for murine Antibody A (SEQ ID NO: 2) and humanized Antibody A-H (SEQ ID NO: 10 and SEQ ID NO: 11).

FIGs. 2A-2B shows the alignment of the Antibody B source mouse VH and VL framework 1, CDR 1, framework 2, CDR 2, framework 3, CDR3, and framework 4 regions with their respective humanized sequences. Kabat CDRs are shown in bold, Chothia CDRs are shown in italics, and combined CDRs are shown in boxes. The framework positions that were back mutated are double underlined. **FIG. 2A** shows the VH sequence for murine Antibody B (SEQ ID NO: 15) and humanized VH sequences B-H.1A to B-H.1C (SEQ ID NOs: 23-25). **FIG. 2B** shows the VL sequence for murine Antibody B (SEQ ID NO: 16) and humanized VL sequences B-H.1D to B-H.1H (SEQ ID NOs: 26-30).

We claim:

1. A multifunctional molecule comprising:

(i) a first antigen binding domain that binds to a calreticulin protein,

and

(ii) a second antigen binding domain that binds to a T cell, an NK cell or a tumor antigen.

2. The multifunctional molecule of claim 1, further comprising a cytokine molecule.

3. The multifunctional molecule of claim 1 or 2, further comprising a stromal modifying moiety.

4. The multifunctional molecule of any one of claims 1-3, further comprising a TGF- β inhibitor.

5. The multifunctional molecule of any one of claims 1-4, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising:

(i) a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6358, a VHCDR2 amino acid sequence of SEQ ID NO: 6360, and a VHCDR3 amino acid sequence of SEQ ID NO: 227;

(ii) a VHCDR1 amino acid sequence of SEQ ID NO: 6358, a VHCDR2 amino acid sequence of SEQ ID NO: 6360, and a VHCDR3 amino acid sequence of SEQ ID NO: 227;

(iii) a VHCDR1 amino acid sequence of SEQ ID NO: 6253, a VHCDR2 amino acid sequence of SEQ ID NO: 6254, and a VHCDR3 amino acid sequence of SEQ ID NO: 6255;

(iv) a VHCDR1 amino acid sequence of SEQ ID NO: 6253, a VHCDR2 amino acid sequence of SEQ ID NO: 243, and a VHCDR3 amino acid sequence of SEQ ID NO: 6255;

(v) a VHCDR1 amino acid sequence of SEQ ID NO: 6356, a VHCDR2 amino acid sequence of SEQ ID NO: 6360, and a VHCDR3 amino acid sequence of SEQ ID NO: 227;

(vi) a VHCDR1 amino acid sequence of SEQ ID NO: 6256, a VHCDR2 amino acid sequence of SEQ ID NO: 6257, and a VHCDR3 amino acid sequence of SEQ ID NO: 6258; or

(vii) a VHCDR1 amino acid sequence of SEQ ID NO: 6256, a VHCDR2 amino acid sequence of SEQ ID NO: 6257, and a VHCDR3 amino acid sequence of SEQ ID NO: 6262.

6. The multifunctional molecule of any one of claims 1-5, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising:

(i) a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 251, a VHCDR2 amino acid sequence of SEQ ID NO: 246, and a VLCDR3 amino acid sequence of SEQ ID NO: 248;

(ii) a VLCDR1 amino acid sequence of SEQ ID NO: 251, a VHCDR2 amino acid sequence of SEQ ID NO: 253, and a VLCDR3 amino acid sequence of SEQ ID NO: 255;

(iii) a VLCDR1 amino acid sequence of SEQ ID NO: 258, a VLCDR2 amino acid sequence of SEQ ID NO: 260, and a VLCDR3 amino acid sequence of SEQ ID NO: 262;

(iv) a VLCDR1 amino acid sequence of SEQ ID NO: 265, a VLCDR2 amino acid sequence of SEQ ID NO: 267, and a VLCDR3 amino acid sequence of SEQ ID NO: 269;

(v) a VLCDR1 amino acid sequence of SEQ ID NO: 272, a VLCDR2 amino acid sequence of SEQ ID NO: 274, and a VLCDR3 amino acid sequence of SEQ ID NO: 276;

(vi) a VLCDR1 amino acid sequence of SEQ ID NO: 279, a VLCDR2 amino acid sequence of SEQ ID NO: 281, and a VLCDR3 amino acid sequence of SEQ ID NO: 283;

(vii) a VLCDR1 amino acid sequence of SEQ ID NO: 113, a VLCDR2 amino acid sequence of SEQ ID NO: 6260, and a VLCDR3 amino acid sequence of SEQ ID NO: 6261;

(viii) a VLCDR1 amino acid sequence of SEQ ID NO: 6259, a VLCDR2 amino acid sequence of SEQ ID NO: 6260, and a VLCDR3 amino acid sequence of SEQ ID NO: 6261; or

(ix) a VLCDR1 amino acid sequence of SEQ ID NO: 279, a VLCDR2 amino acid sequence of SEQ ID NO: 281, and a VLCDR3 amino acid sequence of SEQ ID NO: 283.

7. The multifunctional molecule of any one of claims 1-6, wherein the first antigen binding domain comprises a heavy chain variable region (VH) comprising a sequence with at least 75% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6247, 6248, 6250, 6251, 6347, 6349-6351, 6372, 234-237, and 244.

5 8. The multifunctional molecule of any one of claims 1-7, wherein the first antigen binding domain comprises a light chain variable region (VL) comprising a sequence with at least 75% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6249, 6252, 6348, 6352-6356, 238-242, and 245.

9. The multifunctional molecule of any one of claims 1-8, wherein the first antigen binding domain comprises:

(i) a heavy chain variable region (VH) comprising

(a) a heavy chain framework region 1 (VHFWR1) amino acid sequence of SEQ ID NO: 6232, a VHFWR2 amino acid sequence of SEQ ID NO: 6234, a VHFWR3 amino acid sequence of SEQ ID NO: 6236, and a VHFWR4 amino acid sequence of SEQ ID NO: 6230;

(b) a VHFWR1 amino acid sequence of SEQ ID NO: 6363, a VHFWR2 amino acid sequence of SEQ ID NO: 6363, a VHFWR3 amino acid sequence of SEQ ID NO: 226, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;

(c) a VHFWR1 amino acid sequence of SEQ ID NO: 229, a VHFWR2 amino acid sequence of SEQ ID NO: 6369, a VHFWR3 amino acid sequence of SEQ ID NO: 6371, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;

(d) a VHFWR1 amino acid sequence of SEQ ID NO: 6373, a VHFWR2 amino acid sequence of SEQ ID NO: 6369, a VHFWR3 amino acid sequence of SEQ ID NO: 6371, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;

(e) a VHFWR1 amino acid sequence of SEQ ID NO: 6263, a VHFWR2 amino acid sequence of SEQ ID NO: 6264, a VHFWR3 amino acid sequence of SEQ ID NO: 6265, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;

(f) a VHFWR1 amino acid sequence of SEQ ID NO: 6357, a VHFWR2 amino acid sequence of SEQ ID NO: 6359, a VHFWR3 amino acid sequence of SEQ ID NO: 6361, and a VHFWR4 amino acid sequence of SEQ ID NO: 6273;

- (g) a VHFWR1 amino acid sequence of SEQ ID NO: 6266, a VHFWR2 amino acid sequence of SEQ ID NO: 6267, a VHFWR3 amino acid sequence of SEQ ID NO: 6268, and a VHFWR4 amino acid sequence of SEQ ID NO: 6269;
- 5 (h) a VHFWR1 amino acid sequence of SEQ ID NO: 6270, a VHFWR2 amino acid sequence of SEQ ID NO: 6271, a VHFWR3 amino acid sequence of SEQ ID NO: 6272, and a VHFWR4 amino acid sequence of SEQ ID NO: 6273;
- (i) a VHFWR1 amino acid sequence of SEQ ID NO: 6274, a VHFWR2 amino acid sequence of SEQ ID NO: 6275, a VHFWR3 amino acid sequence of SEQ ID NO: 6276, and a VHFWR4 amino acid sequence of SEQ ID NO: 6273; or
- 10 (j) a VHFWR1 amino acid sequence of SEQ ID NO: 6362, a VHFWR2 amino acid sequence of SEQ ID NO: 6363, a VHFWR3 amino acid sequence of SEQ ID NO: 226, and a VHFWR4 amino acid sequence of SEQ ID NO: 228;
- and/or
- (ii) a light chain variable region (VL) comprising
- 15 (a) a light chain framework region 1 (VLFWR1) amino acid sequence of SEQ ID NO: 6238, a VLFWR2 amino acid sequence of SEQ ID NO: 6240, a VLFWR3 amino acid sequence of SEQ ID NO: 6242, and a VLFWR4 amino acid sequence of SEQ ID NO: 6244;
- (b) a VLFWR1 amino acid sequence of SEQ ID NO: 250, a VLFWR2 amino acid sequence of SEQ ID NO: 252, a VLFWR3 amino acid sequence of SEQ ID NO: 254, and a VLFWR4 amino acid sequence of SEQ ID NO: 256;
- 20 (c) a VLFWR1 amino acid sequence of SEQ ID NO: 257, a VLFWR2 amino acid sequence of SEQ ID NO: 259, a VLFWR3 amino acid sequence of SEQ ID NO: 261, and a VLFWR4 amino acid sequence of SEQ ID NO: 263;
- (d) a VLFWR1 amino acid sequence of SEQ ID NO: 264, a VLFWR2 amino acid sequence of SEQ ID NO: 266, a VLFWR3 amino acid sequence of SEQ ID NO: 268, and a VLFWR4 amino acid sequence of SEQ ID NO: 270;
- 25 (e) a VLFWR1 amino acid sequence of SEQ ID NO: 271, a VLFWR2 amino acid sequence of SEQ ID NO: 273, a VLFWR3 amino acid sequence of SEQ ID NO: 275, and a VLFWR4 amino acid sequence of SEQ ID NO: 277;
- 30

- (f) a VLFWR1 amino acid sequence of SEQ ID NO: 278, a VLFWR2 amino acid sequence of SEQ ID NO: 280, a VLFWR3 amino acid sequence of SEQ ID NO: 282, and a VLFWR4 amino acid sequence of SEQ ID NO: 284;
- (g) a VLFWR1 amino acid sequence of SEQ ID NO: 6277, a VLFWR2 amino acid sequence of SEQ ID NO: 6278, a VLFWR3 amino acid sequence of SEQ ID NO: 6279, and a VLFWR4 amino acid sequence of SEQ ID NO: 6280;
- (h) a VLFWR1 amino acid sequence of SEQ ID NO: 6374, a VLFWR2 amino acid sequence of SEQ ID NO: 6375, a VLFWR3 amino acid sequence of SEQ ID NO: 247, and a VLFWR4 amino acid sequence of SEQ ID NO: 249; or
- (i) a VLFWR1 amino acid sequence of SEQ ID NO: 6281, a VLFWR2 amino acid sequence of SEQ ID NO: 6282, a VLFWR3 amino acid sequence of SEQ ID NO: 6283, and a VLFWR4 amino acid sequence of SEQ ID NO: 6284.
10. The multifunctional molecule of any one of claims 1-9, wherein the second antigen binding domain binds to NKp30.
11. The multifunctional molecule of any one of claims 1-9, wherein the second antigen binding domain binds to TCR β V.
12. The multifunctional molecule of claim 11, wherein the antigen binding domain binds to TCR β V12, TCR β V6, or TCR β V5.
13. The multifunctional molecule of claim 12, wherein the second antigen binding domain comprises:
- (i) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 3, a VHCDR2 amino acid sequence of SEQ ID NO: 4, and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and
- (ii) a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6, a VLCDR2 amino acid sequence of SEQ ID NO: 7, and a VLCDR3 amino acid sequence of SEQ ID NO: 8.
14. The multifunctional molecule of claim 12, wherein the second antigen binding domain comprises:

(i) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 17, a VHCDR2 amino acid sequence of SEQ ID NO: 18, and a VHCDR3 amino acid sequence of SEQ ID NO: 19; and

5 (ii) a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 20, a VLCDR2 amino acid sequence of SEQ ID NO: 21, and a VLCDR3 amino acid sequence of SEQ ID NO: 22.

15. The multifunctional molecule of claim 12, wherein the second antigen binding domain comprises:

10 (i) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 1315, a VHCDR2 amino acid sequence of SEQ ID NO: 1316, and a VHCDR3 amino acid sequence of SEQ ID NO: 1317; and

a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 1321, a VLCDR2 amino acid sequence of SEQ ID NO: 1322, and a VLCDR3 amino acid sequence of SEQ ID NO: 1323.

15 16. The multifunctional molecule of claim 12, wherein the second antigen binding domain comprises:

(i) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 1298, a VHCDR2 amino acid sequence of SEQ ID NO: 1299, and a VHCDR3 amino acid sequence of SEQ ID NO: 1300; and

20 (ii) a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 1305, a VLCDR2 amino acid sequence of SEQ ID NO: 1306, and a VLCDR3 amino acid sequence of SEQ ID NO: 1307.

17. The multifunctional molecule of any one of claims 1-9, wherein the multifunctional molecule comprises:

25 (i) an antigen binding domain that binds to a mutant calreticulin protein, and
(ii) an antigen binding domain that binds to TCR β V.

18. The multifunctional molecule of any one of claims 1-9, wherein the multifunctional molecule comprises:

30 (i) an antigen binding domain that binds to a mutant calreticulin protein, and
(ii) an antigen binding domain that binds to NKp30.

19. The multifunctional molecule of any one of claims 1-18, wherein the first antigen binding domain binds to an epitope located within the C-terminus of the calreticulin protein, optionally, wherein the first antigen binding domain binds to an epitope located within the amino acid sequence of SEQ ID NO: 6285 or 6286.

5 20. The multifunctional molecule of any one of claims 1-19, wherein the multifunctional molecule comprises:

- (i) an antigen binding domain that binds to a calreticulin protein, and
- (ii) an antigen binding domain that binds to a tumor antigen.

10 21. The multifunctional molecule of claim 20, wherein the tumor antigen is an antigen from a protein selected from the group consisting of G6B, CD34, CD41, P-selectin, Clec2, cKIT, FLT3, MPL, ITGB3, ITGB2, GP5, GP6, GP9, GP1BA, DSC2, FCGR2A, TNFRSF10A, TNFRSF10B, and TM4SF1.

 22. A polynucleotide comprising a sequence encoding the multifunctional molecule of any one of claims 1-21.

15 23. A medicament comprising the multifunctional molecule of any one of claims 1-21, and a pharmaceutically acceptable carrier, excipient, or stabilizer.

 24. Use of a pharmaceutical composition comprising the multifunctional molecule of any one of claims 1-21 in the manufacture of a medicament for treating cancer in a subject in need thereof,

20 optionally, wherein the cancer is a hematological cancer or a solid tumor cancer.

 25. The use of claim 24, wherein the cancer is a myeloproliferative neoplasm, optionally, wherein the myeloproliferative neoplasm is primary or idiopathic myelofibrosis (MF), essential thrombocytosis (ET), polycythemia vera (PV), or chronic myelogenous leukemia (CML).

25 26. A pharmaceutical composition comprising the multifunctional molecule of any one of claims 1-21 for use in therapy.