

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

16 December 2021 (16.12.2021)



WIPO | PCT



(10) International Publication Number

WO 2021/252327 A2

(51) International Patent Classification:

A61P 35/00 (2006.01) C07K 14/435 (2006.01)

A61K 9/51 (2006.01) C07K 16/28 (2006.01)

C07K 14/32 (2006.01)

(21) International Application Number:

PCT/US2021/036117

(22) International Filing Date:

07 June 2021 (07.06.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/036,062 08 June 2020 (08.06.2020) US

63/085,351 30 September 2020 (30.09.2020) US

63/088,586 07 October 2020 (07.10.2020) US

63/088,576 07 October 2020 (07.10.2020) US

(71) Applicant: UNIVERSITY OF WASHINGTON

[US/US]; 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

(72) Inventors: UEDA, George; c/o University of Washington,

4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

LAZAROVITS, James; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle,

WA 98105-4721 (US).

FALLAS, Jorge; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle,

WA 98105-4721 (US).

BAKER, David; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle,

WA 98105-4721 (US).

RUOHOLA-BAKER, Hannele; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

DIVINE, Robert; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

ZHAO, Yan (Blair), Ting; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

MATHIEU, Julie; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

KING, Neil, P.; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

TOOLEY, Marti, Rae; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

(74) Agent: HARPER, David, S.; McDonnell Boehnen Hulbert

& Berghoff LLP, 300 South Wacker Drive, Chicago, IL 60606 (US).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH,

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,

KM, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished

upon receipt of that report (Rule 48.2(g))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: ANTIBODY-BOUND NANOPARTICLES

(57) Abstract: Antibody particles are disclosed comprising polypeptides comprising an (Fc) binding domain, a helical polypeptide monomer, and an oligomer domain, and either Tie2 antibodies or dimers, or tumor necrosis factor receptor superfamily antibodies, and uses thereof.



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Antibody-bound nanoparticles**Cross Reference**

This application claims priority to U.S. Provisional Patent Application Serial Nos. 63/036,062 filed June 8, 2020; 63/085,351 filed September 30, 2020; 63/088,586 filed
10 October 7, 2020, and 63/088,576 filed October 7, 2020, each incorporated by reference herein in its entirety.

Sequence Listing Statement:

A computer readable form of the Sequence Listing is filed with this application by
15 electronic submission and is incorporated into this application by reference in its entirety. The Sequence Listing is contained in the file created on June 3, 2021 having the file name "20-1330-WO-SeqList_ST25.txt" and is 178 kb in size.

Background

20 Antibodies are very widely used in therapeutics and diagnostics applications. While there have been some efforts to oligomerize antibodies to enhance avidity and receptor clustering, there are no current methods to precisely form ordered and structurally homogeneous antibody-bound nanoparticle structures.

Summary

In a first aspect, the disclosure provides particles, comprising:

- (a) a plurality of polypeptide polymers, wherein
 - (i) each monomer in the polymers comprises an amino acid sequence at
least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
30 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:1;
 - (ii) each monomer in the polymers comprises an amino acid sequence at
least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:2;
 - (iii) each monomer in the polymers comprises an amino acid sequence at
35 least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:3;

(iv) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:4;

5 (v) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5;

(vi) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:6;

10 (vii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7;

(viii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
15 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:8; or

(ix) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:9;

20 wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement); and

(b) a plurality of (i) Tie2 receptor antibodies comprising Fc domains, and/or (ii) dimers of fibrinogen-like domain derived from angiopoietin (F domain)fused to an Fc domain;

wherein

25 (i) each Tie2 antibody or dimer comprises a first Fc domain and a second Fc domain;

(ii) each Tie2 antibody or dimer in the plurality is (A) non-covalently bound via the first Fc domain to one polypeptide monomer chain of a first polymer, and (B) non-covalently bound via the second Fc domain to one polypeptide monomer of a second
30 polymer; and

(iii) each polypeptide monomer chain of each polymer is non-covalently bound to one Fc domain;

wherein the particle comprises dihedral, tetrahedral, octahedral, or icosahedral symmetry.

In one embodiment, the Tie2 antibodies or dimers comprise Tie 2 antibodies, wherein the Tie-2 antibodies comprise an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs selected from the group consisting of:

SEQ ID NOS:11-12,

SEQ ID NOS:13-14, and

SEQ ID NOS:15-16.

In another embodiment, the dimers comprise an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of the amino acid sequence of SEQ ID NO:17 or 18, wherein residues in parentheses are optional.

In another embodiment, the particles or compositions thereof are used for treating comprising treating or limiting development of diseases or syndromes resulting from vascular dysfunction, including but not limited to bacterial or viral infections, sepsis, acute respiratory distress syndrome (ARDS), acute lung injury, acute kidney injury, wet-age related macular degeneration, open angle glaucoma, diabetic retinopathy, and diabetic nephropathy.

In another embodiment, the disclosure comprises polypeptides comprising an amino acid sequence comprising or consisting of the amino acid sequence of any one of SEQ ID NOS: 17-18 and 47, nucleic acids encoding such polypeptides, expression vectors comprising such nucleic acids operatively linked to control sequence, and host cells comprising such polypeptides, nucleic acids, and/or expression vectors.

In other embodiments, the disclosure provides kits comprising

(a) a polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homopolymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in embodiment disclosed herein; and

(b) Tie2 antibodies comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs selected from the

group consisting of SEQ ID NOS:11-12; SEQ ID NOS:13-14; and SEQ ID NOS:15-16, and/or a fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain optionally comprising the amino acid sequence selected from the group consisting of SEQ ID NOS: 17-18 and 47.

5 In further embodiments, the disclosure provides kits comprising:

(a) host cells capable of expressing a polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not
10 considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in embodiment disclosed herein; and

(b) host cells capable of expressing Tie2 antibodies amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
15 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs selected from the group consisting of SEQ ID NOS:11-12; SEQ ID NOS:13-14; and SEQ ID NOS:15-16, and/or a fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain optionally comprising the amino acid sequence selected from the group consisting of SEQ ID NOS: 17-18 and 47.

20 In another aspect, the disclosure provides particles, comprising:

(a) a plurality of polypeptide polymers, wherein
(i) each monomer in the -polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:1;

25 (ii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:2;

(iii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
30 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:3;

(iv) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:4;

(v) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5;

5 (vi) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:6;

(vii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7;

10 (viii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:8; or

(ix) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 15 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:9; wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement); and

(b) a plurality of α -TNFRSF (tumor necrosis factor receptor superfamily) antibodies comprising Fc domains;

20 wherein

(i) each α -TNFRSF antibody in the plurality of antibodies comprises a first Fc domain and a second Fc domain;

(ii) each α -TNFRSF antibody in the plurality of antibodies is (A) non-covalently bound via the first Fc domain to one polypeptide monomer chain of a first 25 polymer, and (B) non-covalently bound via the second Fc domain to one polypeptide monomer of a second polymer; and

(iii) each polypeptide monomer chain of each polymer is non-covalently bound to one Fc domain;

30 wherein the particle comprises dihedral, tetrahedral, octahedral, or icosahedral symmetry.

In one embodiment, the α -TNFRSF antibody targets one or more of DR5/TRAIL-R2/TNFRSF10B/CD262, CD40, 4-1BB, and TWEAKR (Tumor Necrosis Factor-like Weak Inducer of Apoptosis Receptor)/TNFRSF12A/CD266. In another embodiment, the α -

TNFRSF antibodies comprise an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs (when both heavy and light chain are needed) selected from the group consisting of:

- 5 SEQ ID NO: 19 and 20;
 SEQ ID NO: 21 and 22;
 SEQ ID NO: 23 and 24;
 SEQ ID NO: 25 and 26;
 SEQ ID NO: 27 and 28;
- 10 SEQ ID NO: 29;
 SEQ ID NO: 30;
 SEQ ID NO: 31 and 32;
 SEQ ID NO: 33;
 SEQ ID NO: 34 and 35;
- 15 SEQ ID NO: 36 and 37;
 SEQ ID NO: 38 and 39;
 SEQ ID NO: 40 and 41;
 SEQ ID NO: 42 and 43;
 SEQ ID NO: 44 and 45;
- 20 SEQ ID NO: 44 and 46;
 SEQ ID NO: 48 and 49;
 SEQ ID NO: 50 and 51;
 SEQ ID NO: 52 and 53;
 SEQ ID NO: 54 and 55;
- 25 SEQ ID NO: 56;
 Lob 7/6 heavy and light chains as disclosed in published US patent application US
 US20090074711; and
 Heavy and light chain pairs disclosed in 2018094300.
 The disclosure also provides methods for using such particles to treat tumors.
- 30 In another embodiment, the disclosure provides kits comprising:
 (a) one or more polypeptide comprising an amino acid sequence at least 50%,
 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 99%, or 100% identical to the amino acid sequence selected from the group consisting of
 SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the

percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in embodiment herein; and

- (b) α -TNFRSF antibodies comprising an antibody selected from the group consisting of: Lob 7/6, Lucatumumab, Dacetuzumab, Selicrelumab, Bleselumab, Urelumab, Utomilumab, Drozitumab, scTRAIL-Fc, KMTR2, 16E2, and Conatumumab (also referred to as AMG 655); optionally as further limited herein.

In another embodiment, the disclosure provides kits comprising:

- (a) host cells capable of expressing one or more polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in any embodiment herein; and

(b) host cells capable of expressing α -TNFRSF antibodies comprising an antibody selected from the group consisting of: Lob 7/6, Lucatumumab, Dacetuzumab, Selicrelumab, Bleselumab, Urelumab, Utomilumab, Drozitumab, scTRAIL-Fc, KMTR2, 16E2, and Conatumumab (also referred to as AMG 655); optionally as further limited herein.

Description of the Figures

Figure 1(A-F). Antibody nanocage (AbC) design. **A**, Polyhedral geometry is specified. **B**, An antibody Fc model from hIgG1 is aligned to one of the C2 axes (in this case, a D2 dihedron is shown). **C**, Antibody Fc-binders are fused to helical repeat proteins that are then fused to the monomeric subunit of helical cyclic oligomers. All combinations of building blocks and building block junctions are sampled (below inset). **D-E**, Tripartite fusions that successfully place the cyclic oligomer axis in the orientation required for the desired polyhedral geometry (**D**) move forward for sidechain redesign (**E**). **F**, Designed AbC-forming oligomers are bacterially expressed, purified, and assembled with antibody Fc or IgG.

Figure 2(A-F). Structural characterization of AbCs. **A**, Design models, with antibody Fc and designed AbC-forming oligomers. **B**, Overlay of SEC traces of assembly formed by mixing design and Fc with those of the single components. **C**, EM images with 2D averages in inset; all data is from negative-stain EM with the exception of designs o42.1 and

i52.3 (cryo-EM). **D-E**, SEC (**D**) and NS-EM representative micrographs with 2D class averages (**E**) of the same designed antibody cages assembled with full human IgG1 (with the 2 Fab regions intact).

Figure 3. 3D reconstructions of AbCs formed with Fc. Computational design models (cartoon representation) of each AbC are fit into the experimentally-determined 3D density from EM. Each nanocage is viewed along an unoccupied symmetry axis (left), and after rotation to look down one of the C2 axes of symmetry occupied by the Fc (right). 3D reconstructions from o42.1 and i52.3 are from cryo-EM analysis; all others, from NS-EM.

Figure 4(A-K). AbCs activate apoptosis and angiogenesis signaling pathways. (**A and B**) Caspase-3/7 is activated by AbCs formed with α -DR5 antibody (**A**), but not the free antibody, in RCC4 renal cancer cells (**B**). (**C and D**) α -DR5 AbCs (**C**), but not Fc AbC controls (**D**), reduce cell viability 4 days after treatment. (**E**) -DR5 AbCs reduce viability 6 days after treatment. (**F and G**) o42.1 α -DR5 AbCs enhance PARP cleavage, a marker of apoptotic signaling; (**G**) is a quantification of (**F**) relative to PBS control. (**H**) The F-domain from angiopoietin-1 was fused to Fc (A1F-Fc) and assembled into octahedral (o42.1) and icosahedral (i52.3) AbCs. (**I**) Representative Western blots show that A1F-Fc AbCs, but not controls, increase pAKT and pERK1/2 signals. (**J**) Quantification of (**I**): pAKT quantification is normalized to o42.1 A1F-Fc signaling (no pAKT signal in the PBS control); pERK1/2 is normalized to PBS. (**K**) A1F-Fc AbCs increase vascular stability after 72 hours. (Left) Quantification of vascular stability compared with PBS. (Right) Representative images; scale bars, 100 μ m. All error bars represent means $\pm$ SEM; means were compared using analysis of variance and Dunnett post-hoc tests (tables 11 and 12). * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$.

Figure 5(A-E). α -CD40 AbCs activate CD40 signaling over uncaged IgGs.

A-D, Octahedral AbCs produced with α -CD40 (**A**) form AbCs of the expected size and shape according to SEC (**B**), DLS (**C**), and NS-EM (**D**). **E**, CD40 pathways are activated by LOB7/6 α -CD40 octahedral nanocages but not by free LOB7/6. Scale bars represent means $\pm$ SD, n=3; EC50s reported in Table 7.

Figure 6(A-C). Designed Fc-binding designed helical repeat. **A**, Model of the helical repeat protein DHR79 docked against antibody Fc (PDB ID: 1DEE). Residues from protein A (PDB ID: 1L6X) are grafted at the interface between the Fc and the helical repeat protein. **B**, SEC trace of the Fc-binding helical repeat monomer. **C**, Biolayer interferometry

(BLI) of the Fc-binding helical repeat design with Fc (left) or with hIgG1 (right), with summary statistics (below).

Figure 7(A-F). Additional α -DR5 AbC experiments. **A**, α -DR5 AbCs and TRAIL activate caspase-3,7 in Colo205 colorectal cancer cell lines. **B-C**, AbCs formed with Fc from hIgG1 do not activate caspase-3,7 (**B**) or reduce viability (**C**) in RCC4 cells. **D**, α -DR5 AbCs do not greatly activate caspase-3,7 after 2 d (**D**) or reduce viability (**E**) in a primary tubular kidney cell line (RAM009). **F**, Cleaved PARP is activated by α -DR5 in RCC4 cells, but not by TRAIL, α -DR5, or Fc AbCs.

Figure 8(A-E). Additional A1F-Fc AbC experiments. **A-B**, o42.1 and i52.3 AbCs formed with A1F-Fc are monodisperse and of the expected size per SEC on a Superose 6 column (**A**) and DLS (**B**). SEC shows the assembly trace in black, the relevant AbC design component in light grey, and the A1F-Fc in dark grey. **C**, A control assembly displaying 8 A1F ligands ("H8-A1F") produced similar levels of pAKT and pERK1/2 activation to A1F-Fc AbCs along with a comparable increase in vascular stability; data for all other conditions besides H8-A1F are replotted for convenience from Fig. 4i-k. **D**, Representative images of o42.1, i52.3 AbCs, and H8-A1F formed with Fc in the vascular stability assays; scale bars are 100 μ m. **E**, o42.1 A1F-Fc AbCs were incubated with 100% human serum (HS) for 24 hours at 4 °C or 37 °C and applied to HUVEC cells at 150 nM. pAKT signal showed no decrease from o42.1 A1F-Fc particles incubated with serum. Statistical analyses are reported in Table

Detailed Description

All references cited are herein incorporated by reference in their entirety. Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: Molecular Cloning: A Laboratory Manual (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), Gene Expression Technology (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991, Academic Press, San Diego, CA), "Guide to Protein Purification" in Methods in Enzymology (M.P. Deutscher, ed., (1990) Academic Press, Inc.); PCR Protocols: A Guide to Methods and Applications (Innis, et al. 1990, Academic Press, San Diego, CA), Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed. (R.I. Freshney, 1987, Liss, Inc. New York, NY), Gene Transfer and Expression Protocols, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX).

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

As used herein, the amino acid residues are abbreviated as follows: alanine (Ala; A), asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I),
5 leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

In all embodiments of polypeptides disclosed herein, any N-terminal methionine residues are optional (i.e.: the N-terminal methionine residue may be present or may be absent).
10

All embodiments of any aspect of the disclosure can be used in combination, unless the context clearly dictates otherwise.

Unless the context clearly requires otherwise, throughout the description and the claims, the words 'comprise', 'comprising', and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to". Words using the singular or plural number also include the plural and singular number, respectively. Additionally, the words "herein," "above," and "below" and words of similar import, when used in this application, shall refer to this
15 application as a whole and not to any particular portions of the application.

In a first aspect, the disclosure provides particles, comprising:

(a) a plurality of polypeptide polymers, wherein

(i) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
25 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:1;

(ii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:2;

(iii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:3;
30

(iv) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:4;

(v) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5;

5 (vi) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:6;

(vii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7;

10 (viii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:8; or

(ix) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
15 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:9;

wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement); and

(b) a plurality of (i) Tie2 receptor antibodies comprising Fc domains, and/or (ii) dimers of fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc
20 domain;

wherein

(i) each Tie2 antibody or dimer comprises a first Fc domain and a second Fc domain;

(ii) each Tie2 antibody or dimer in the plurality is (A) non-covalently
25 bound via the first Fc domain to one polypeptide monomer chain of a first polymer, and (B) non-covalently bound via the second Fc domain to one polypeptide monomer of a second polymer; and

(iii) each polypeptide monomer chain of each polymer is non-covalently bound to one Fc domain;

30 wherein the particle comprises dihedral, tetrahedral, octahedral, or icosahedral symmetry.

As shown in the examples that follow, the particles and compositions of the disclosure Tie2 receptor antibodies comprising Fc domains, and/or dimers of fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain significantly increased AKT and

ERK1/2 phosphorylation above baseline and enhanced cell migration and vascular stability, and thus are useful for treating pathological symptoms that arise from bacterial and viral infections. For example, the ability to induce phosphorylation of AKT and ERK, can serve to enhance cell migration and tube formation, improve, wound healing after injury, and thus are useful in treating infections (such as bacterial and viral infections), as well as conditions characterized by diseases or syndromes resulting from vascular dysfunction, including but not limited to sepsis, acute respiratory distress syndrome (ARDS), acute lung injury, acute kidney injury, wet-age related macular degeneration, open angle glaucoma, diabetic retinopathy, and diabetic nephropathy.

- 5 The monomers in the plurality of polypeptide polymers comprise an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a polymer, including but not limited to a homo-polymer, and (b) binding to a constant region of an IgG antibody.

Table 1: Sequences and original building blocks used for all designs.

Name	Fc binder (underlined)	Monomer (standard font)	Oligomer (bold font)	Sequence
d2.3 SEQ ID NO:1	DHR79	EXT6-DHR62	bex2c2_G2	(M) <u>SDPEK</u> ENELIKRIEAAQRAREAAERTGDPVRELARELARIAQIAFYLVLF DPSSSEVNEALKAVVKAIELAVRALEAAEKTGDPVRELAREVVRLAVEVATATA ASENDTLKKVAEKALKLKEAAKRGDAKAAKQAAKIAKLAAANAGDEGVKKVEL VRLAIELVEIVVENAKRKGDDSKAEAAEAALAFRIVLAAQLAGIASLEVLAL RLIKEVVENAQREGYDIAVAAIAAAVAFVAVVAVAAAAADITSSEVLELAIRLIKE VVENAQREGYVILLAALAAAAAFVVVAAAAKAGITSSSETLKRAIEEIRKRVEEA QREGNDISEAARQAAEEFRKKAELK (GSLEHHHHHH)
d2.4 SEQ ID NO:2	DHR79	EXT6-DHR62	bex2c2_G2	(M) <u>SDPEK</u> ENELIKRIEAAQRAREAAERTGDPVRELARELARIAQIAFYLVLF DPSSSEVNEALKAVVKAIELAVRALEAAEKTGDPVRELAREVVKAADVAAEAQ AGLNKKLKEVAEKALKLKEAAKRGDSTAELAAEIAKLAAKLAGDEDVKKVKL VLEATKIVKIVVENAKRKGDDSKAEAAEAALAFRIVLAAAKLAGIASSEVLELAA RLIKEVVENAQREGYDIAVAAIAAAVAFVAVVAVAAAAADITSSEVLELAIRLIKE VVENAQREGYVILLAALAAAAAFVVVAAAAKAGITSSSETLKRAIEEIRKRVEEA QREGNDISEAARQAAEEFRKKAELK (GSLEHHHHHH)
d2.7 SEQ ID NO:3	DHR79	DHR76	bex2c2_G3	(M) <u>EDPEK</u> ENELIKRIEAAQRAREAAERTGDPVRELARELAKLAQIAFYLVLF DFCAKEVNLALELIVKAIELAVRALEAAEKTGDPHARELAREIVRLAVELARAVA EAAEEAKKQGNSELAEQVAAAAQVALEVIKAAITAQKQDRKAFAALELVLEVI YALIEAVVQGNPKKVAEVALKAEIRIVVQNAANKGDDADEAVEAARAAFEIVLA AAQLAGIDSEVLELAARLIKEVVENAQREGYDIAVAAIAAAVAFVAVVAVAAAA DITSSEVLELAIRLIKEVVENAVREGYVILLAALAAAAAFVVVAAAAKAGITSS ETLKRAIEEIRKRVEEAQREGNDISEAARQAAEEFRKKAELK (GSLEHHHHHH)
t32.4 old SEQ ID NO:4	Protein A	EXT6-DHR79	tj04c3_in t5v2	(M) <u>FNFSQ</u> QAFYLIINMPNLNEAQKNGFIQSLKDDPAKSEVVAGEAAIEAARNA SKKGFETAKEAVRLALELVQEAARVARKTGSTELLIAAAKLAIEVARVALKVG PEAAREAVRAALELVQELIRAARKTGSKVEVLEAAKLALAEVALVAAAVGSSSEAA KAVATAVEALKEAGASEDEIAEIVARVISEVIRILKENGSEYKVICVSVAKIVAE IVEALKRSGTSEDEIAEIVARVISEVIRILKESGSDYLIICVCAIIVAEIVEAL

				KRSGTSEDEIAEIVARVISEVIRTLKESGSSYEVIKECVQIIIVLAIILALMKSGT EVEEILLILLRVKTEVRRTLKESGS (GSLEHHHHHH)
t32.4 (aka t.4_r1)				(M) FNKQQSAFYILNMPNLNEAQRNGFIQSLKDDPSKSEVVAGEAAIEAARNA LKKQSPETAREAVRLALELVQEAEPQARKTGSTERLIAAAKLAIEVARVALKVGS PETAREAVPTALELVQELIRQARKTGSKEVLEEAAKLALEVAKVAEEVGSPEATAA RAVATAVEALKEAGASEDEIAEIVARVISEVIRILKESGSEYKVICRAVARIVAE IVEALKRSGTSEDEIAEIVARVISEVIRTLKESGSDYLIICVCVAIIIVAEIVEAL KRSQTSEDEIAEIVARVISEVIRTLKESGSSYEVIKECVQIIIVLAIILALMKSGT EVEEILLILLRVKTEVRRTLKES (GSLEHHHHHH)
SEQ ID NO:5	Protel n A	EXT6- DNR79	t304c3_in t5v2	
t32.8				(M) FNKQQSAFYEVNMPNLNEAQRNGFIQSLKDDPSQSLKILIKAAAGGDSSEL ESVAKRIVRELAEPGRSEKEAAKEAAELIERITRAAGGNSDLIELAVRIVKILEE QQRSPSSAAKEAVEAIEAIVRAAGGDEAIVKVAEEIAKTIITQKESGSEYKEICR TVARIVAEIVEKLKRGASEDEIAEIVAAIIAAVILTLKSGSDYLIICVCVAII VAEIVEALKRSGTSEDEIAEIVARVISAVIRVLKESGSSYEVIKECVQIIIVLAIIL LALMKSGTEVEEILLILLRVKTEVRRTLKES (GSLEHHHHHH)
SEQ ID NO:6	Protel n A	EXT6- DNR39	t304c3_in t5v2	
o42.1				(M) FNKQQSAFYEILNMPNLNEALRNGFIQLLKDDPSKSTVILTAAKVAELSE KIRTLKESGSSYEQIAETVAKAVAKLVEKLKRGVSEDEIALAVALIISAVIQTL KESGSSYEVIAEIVARIVAEIVEALKRSGTSEDEIAEIVARVISEVIRTLKESGS SYEVIAIVARIVAEIVEALKRSGTSEDEIAKIVARVIAEVLRTLKESGSSSEVI KSIVARIITEIKEALKRSGTSEDEIELITLMIEAALEIAKLKSSGSEYEICEDV ARRIAELVEKLKRDGTSAVEIAKIVAAIIISAVIAMLKASGSSYEVICCVARIVA EIVEALKRSGTSAIIIALIVALVISEVIRTLKESGSSFEVILECVIRIVLEIIEA LKRSQTSEQDVMLIVMAVLLVVLATLQLS (GSLEHHHHHH)
SEQ ID NO:7	Protel n A	EXT6- DNR9	t310c4_G1	
152.3				(M) SDEEERNELIKRIEAAQRAREAAERTGDPVRRELARELARLAQRAFYLV LH DPSSSDVNEALKLIVEAIEAAVRALEAAERAGDPPELPEDAREAVRLAVEAAEEVQ RNPSSSTANLLKAIIVALAEALAAANGDKEKFKKAAESALEIAKRVVEVASKEG DPEAVLEAAKVALRVAELAAKNGDKEVFKKAAESALEVAKRIVEVASKEGDPPELV LEAAKVALRVAELAAKNGDKEVFQKAAASAVEVALRLTEVASKEGDSSELETEAAK VITRVRELASKQGDAAVAILAETAEVKLEIEESKKRPQSESAKNLILIMQLLINQ IRLLVLQIRMLDEQORQE (GSLEHHHHHH)
SEQ ID NO:8	DNR79	EXT6- DNR71	5H2LD- 10.5	
152.6				(M) EDEEEKNELIKRIEAAQRAREAAERTGDPVRRELARELARLAQRAFYLV LH DFNSSDVNEALKLIVEAIEAAVRALEAAERTGDPKVRREARELVRAVEAAEEVQ UNPSSSEVNEKLKAIIVVEIEVKVASLEAKEVTDPPDKALKIAKVLIELALEAVKEN PSTEALKA9LEAVRLASEVAKRVTDPPDKALKIAKVLIELALEAVKEDPSTDALRA VLEAVRLASEVAKRVTDPPDKALKIAKVLIELALEAVKEDPSTDALPAAKEAERLA TEVAKRVTDPPKAREIEMLVKLQMEAILAETEEVKKEIEESKKRPQSESAKNLI LIMQLLINQIRLLALQIRMLALQLOE (GSLEHHHHHH)
SEQ ID NO:9	DNR79	EXT6- DNR57	5H2LD- 10.5	

As detailed in the examples that follow, the monomers comprise 3 domains (as reflected in the columns of Table 1):

- (1) An (Fc) binding domain;
- (2) A helical polypeptide (monomer) that helps position the Fc-binder domain and oligomer domain at the correct orientation to promote higher order structures (sometimes referred to as cages, or nanoparticles); and
- (3) An oligomer domain that can associate via non-covalent interactions to form polymers (including but not limited to homo-polymers), such as dimers, trimers, tetramers, or pentamers (C2, C3, C4, or C5 cyclic symmetry, respectively).

In some embodiments, the oligomer domain can self-associate via non-covalent interactions to form a homo-polymer with an identical polypeptide. In another embodiment, the oligomer domain can associate via non-covalent interactions to form a pseudo-polymer

with similar polypeptide that has some amino acid sequence differences, so long as each monomer has the required amino acid sequence identity to the reference polypeptide.

The polypeptide monomers fuse these domains at an orientation that when in oligomeric form and combined with IgG, forms the desired higher order structures as detailed
5 herein.

Each polypeptide monomer has two interfaces: (1) A Fc-binding interface (defined for each polypeptide in Table 3); and (2) An oligomerization domain interface (defined for each polypeptide in Table 2). The polypeptides of the disclosure, when expressed, will form a cyclic oligomer with C2, C3, C4, or C5 symmetry via the oligomerization domain. When
10 combined with antibody or dimer, a higher order, cage-like, polyhedral structure spontaneously assembles via interaction of the antibodies with Fc binding interfaces. The resulting higher order structures have C2 cyclic symmetry at the Fc position and cyclic 2, 3, 4, or 5-symmetry at each oligomerization domain interface. The resulting particles form precisely ordered and structurally homogeneous antibody-bound nanoparticle structures.

As used herein, a Tie-2 antibody "antibody" includes reference to full length and any functional antibody fragments (i.e.: that selectively bind to the Tie 2 receptor) including the Fc domain. In some embodiments, the antibody includes heavy and light chains. In other
15 embodiments, the antibody may comprise a fusion protein comprising a protein that selectively bind to the Tie 2 receptor and an Fc domain, that dimerizes since the Fc domains naturally dimerizes. In other embodiments, the antibody may comprise an Fc fragment chemically modified to a protein that selectively bind to the Tie 2 receptor, which dimerizes since the Fc domains naturally dimerizes.
20

The Tie-2 dimers include two monomers of the fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain. The two monomers dimerize since the Fc
25 domain naturally dimerizes. The F domain amino acid sequence present in each monomer comprises or consists of the amino acid sequence of SEQ ID NO:10:

KAEELASEKPFPRDCADVQAGFNKSGIYTIYINNMPEPKKVFECNMDVNGGGWTVIQHREDGSLDFQRGWKEYKMGF
GNPSGEYWLGNFIFAITSQRQYMLRIELMDWEGNRAYSQYDRFHIGNEKQNYRLYLKGHTGTAGKQSSLLHGA
DFSTKDADNDNCMKCALMLTGGWWEDACGPSNLNGMEYTAGQNHGKLNIGIKWHYFKGPSYSLRSTTMMIRPLDF
30 (SEQ ID NO:10).

When combined with Tie2 antibodies or the dimers, a higher order, cage-like, polyhedral structure spontaneously assembles via interaction of the antibodies or dimers with Fc binding interfaces. The resulting higher order structures have cyclic symmetry at each Fc-binding interface and each oligomerization domain interface. For example, the Tie2 antibody

heavy and light chains can be co-expressed in cells to produce the Tie2 antibody, which can then be mixed with the polymers to form the particles of the disclosure. Alternatively, the Tie2-binding domain fused to an Fc domain can be expressed in cells, which associate to form the dimer, which can then be mixed with the polymers to form the particles of the

5 disclosure

In one embodiment, amino acid residues that would be present at a polymeric interface (as defined in Table 2) in a polymer of the polypeptide monomer of any one of SEQ ID NOS:1-9 are conserved (i.e.: identical to the amino acid residue at the same position in the reference polypeptide).

10 Table 2: Predicted interface residues at oligomeric interface (i.e., not the Fc/Fc-binder interface) by residue position

Name	Interface residue positions
d2.3 SEQ ID NO:1	122, 185, 188, 189, 192, 195, 196, 199, 200, 202, 203, 204, 234, 235, 238, 239, 242, 245, 246, 249, 250, 252, 253, 280, 281, 282, 284, 285, 288, 292, 295, 296, 299, 303, 338
d2.4 SEQ ID NO:2	185, 188, 189, 192, 195, 196, 199, 200, 202, 203, 235, 238, 239, 242, 245, 246, 249, 250, 252, 253, 280, 281, 282, 284, 285, 288, 292, 295, 296, 299, 303, 338
d2.7 SEQ ID NO:3	202, 203, 204, 207, 208, 213, 216, 217, 219, 220, 221, 251, 252, 255, 256, 259, 262, 263, 266, 267, 269, 270, 297, 298, 299, 301, 302, 305, 309, 312, 313, 316, 320, 355
t32.4 old SEQ ID NO:4	202, 203, 204, 207, 208, 252, 254, 255, 258, 261, 262, 265, 266, 269, 278, 308, 312, 315, 316, 318, 319, 320, 322, 323, 325, 326, 327, 328, 329, 330, 331, 332, 333, 335, 336, 337, 339, 340, 343
t32.4 (aka t.4_r1) SEQ ID NO:5	202, 203, 204, 207, 208, 252, 254, 255, 258, 261, 262, 265, 266, 269, 278, 308, 312, 315, 316, 318, 319, 320, 322, 323, 325, 326, 327, 328, 329, 330, 331, 332, 333, 335, 336, 337, 339, 340, 343
t32.8 SEQ ID NO:6	155, 156, 157, 160, 161, 203, 207, 208, 211, 214, 215, 218, 219, 222, 231, 232, 261, 265, 268, 269, 271, 272, 273, 275, 276, 278, 279, 280, 281, 282, 283, 284, 285, 286, 288, 289, 290, 292, 293, 296
o42.1 SEQ ID NO:7	289, 289, 290, 294, 338, 339, 340, 341, 343, 344, 348, 364, 368, 369, 372, 373, 375, 376, 378, 383, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 400, 401, 402, 404, 405, 408, 409, 412
152.3 SEQ ID NO:8	201, 205, 209, 236, 248, 252, 255, 277, 281, 282, 284, 286, 287, 289, 290, 293, 294, 297, 300, 301, 304, 305, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 343
152.6 SEQ ID NO:9	203, 206, 278, 282, 285, 288, 289, 292, 293, 295, 296, 299, 302, 303, 306, 309, 310, 313, 314, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352

In another embodiment, amino acid residues in the monomers present at a Fc binding interface as defined in Table 3 are conserved.

15

Table 3

Name	Fc binder used in design	Interface residue positions
d2.3 SEQ ID NO:1	DHR79	4, 7, 8, 11, 12, 14, 15, 18, 19, 22, 35, 41, 42, 44, 45, 46, 48, 49, 50, 52, 53

d2.4 SEQ ID NO:2	DHR79	4, 7, 8, 11, 12, 14, 15, 18, 19, 22, 35, 41, 42, 44, 45, 46, 48, 49, 50, 52, 53
d2.7 SEQ ID NO:3	DHR79	4, 7, 8, 11, 12, 14, 15, 18, 19, 22, 35, 41, 42, 44, 45, 46, 48, 49, 50, 52, 53
i32.4, old SEQ ID NO:4	Protein A	2, 3, 4, 6, 7, 8, 10, 11, 14, 21, 24, 25, 28, 32
i32.4 (aka i4, r1) SEQ ID NO:5	Protein A	2, 3, 4, 6, 7, 8, 10, 11, 14, 21, 24, 25, 28, 32
i32.8 SEQ IDNO:6	Protein A	2, 3, 4, 6, 7, 8, 10, 11, 14, 21, 24, 25, 28, 32
o42.1 SEQ ID NO:7	Protein A	2, 3, 4, 6, 7, 8, 10, 11, 14, 21, 24, 25, 28, 32
i52.3 SEQ ID NO:8	DHR79	4, 7, 8, 11, 12, 14, 15, 18, 19, 22, 35, 41, 42, 44, 45, 46, 48, 49, 50, 52, 53
i52.6 SEQ ID NO:9	DHR79	4, 7, 8, 11, 12, 14, 15, 18, 19, 22, 35, 41, 42, 44, 45, 46, 48, 49, 50, 52, 53

In a further embodiment, amino acid substitutions relative to the reference monomer amino acid sequence comprise, consist essentially of, or consist of substitutions at polar residues in the reference polypeptide. In other embodiments, polar residues on the surface of the polypeptide monomer that are not at the Fc or oligomeric interfaces may be substituted with other polar residues while maintaining folding and assembly properties of the designs.

As used herein, "polar" residues are C, D, E, H, K, N, Q, R, S, T, and Y. "Non-polar" residues are defined as A, G, I, L, M, F, P, W, and V.

In one embodiment, amino acid substitutions relative to the reference monomer amino acid sequence comprise, consist essentially of, or consist of substitutions at polar residues at non-Gly/Pro residues in loop positions, as defined in Table 4, in the reference polypeptide monomer.

Table 4: Predicted secondary structure for all listed designs, using pyrosetta's display secstruct() function. L = Loop, H = Helix

[illegible]

[illegible]

In a further embodiment of any of these embodiments, amino acid changes from the reference polypeptide monomer are conservative amino acid substitutions. As used here, “conservative amino acid substitution” means that:

- hydrophobic amino acids (Ala, Cys, Gly, Pro, Met, Sec, Ser, Val, Ile, Leu) can only be substituted with other hydrophobic amino acids;
- hydrophobic amino acids with bulky side chains (Phe, Tyr, Trp) can only be substituted with other hydrophobic amino acids with bulky side chains;
- amino acids with positively charged side chains (Arg, His, Lys) can only be substituted with other amino acids with positively charged side chains;
- amino acids with negatively charged side chains (Asp, Glu) can only be substituted with other amino acids with negatively charged side chains; and
- amino acids with polar uncharged side chains (Ser, Thr, Asn, Gln) can only be substituted with other amino acids with polar uncharged side chains.

In all embodiments disclosed herein, the polypeptides may comprise one or more additional functional groups or residues as deemed appropriate for an intended use. The polypeptides of the disclosure may include additional residues at the N-terminus or C-terminus, or a combination thereof; these additional residues are not included in determining the percent identity of the polypeptides of the invention relative to the reference polypeptide. Such residues may be any residues suitable for an intended use, including but not limited to detectable proteins or fragments thereof (also referred to as "tags"). As used herein, "tags" include general detectable moieties (i.e.: fluorescent proteins, antibody epitope tags, etc.), therapeutic agents, purification tags (His tags, etc.), linkers, ligands suitable for purposes of purification, ligands to drive localization of the polypeptide, peptide domains that add functionality to the polypeptides. In non-limiting embodiments, such functional groups may comprise one or more polypeptide antigens, polypeptide therapeutics, enzymes, detectable domains (ex: fluorescent proteins or fragments thereof), DNA binding proteins, transcription factors, etc. In one embodiment, the polypeptides may further comprise a functional polypeptide covalently linked to the amino-terminus and/or the carboxy-terminus. In other embodiments, the functional polypeptide may include, but is not limited to, a detectable polypeptide such as a fluorescent or luminescent polypeptide, receptor binding domains, etc.

In one embodiment, the plurality of homo-polymers comprises homo-dimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-3. In these embodiments, adding the recited polypeptides with Tie2 antibodies or dimers results in spontaneous assembly into a D2 dihedral structure containing two antibodies per particle.

In another embodiment, the plurality of homo-polymers comprises homo-trimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:4-6. In these embodiments, adding the recited polypeptides with Tie2 antibodies or dimers results in spontaneous assembly into a T32 tetrahedral structure containing six antibodies per particle.

In a further embodiment, the plurality of homo-polymers comprises homo-tetramers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7. In these embodiments, adding the recited

polypeptides with Tie2 antibodies or dimers results in spontaneous assembly into an O42 octahedral structure containing twelve antibodies per particle.

In a still further embodiment, the plurality of homo-polymers comprises homo-pentamers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:8-9. In these embodiments, adding the recited polypeptides with Tie2 antibodies or dimers results in spontaneous assembly into an I52 icosahedral structure containing thirty antibodies per particle.

In one embodiment of all of these embodiments, the Tie2 antibodies or dimers comprise Tie 2 antibodies, wherein the Tie-2 antibodies comprise an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs selected from the group consisting of:

SEQ ID NOS:11-12,
SEQ ID NOS:13-14, and
SEQ ID NOS:15-16.

12H8

heavy chain (Fc domain underlined)

EVQLVESGGGLVQPGGSLKLSCAASGFTFSUYGMHWVRQAPKGLEWVAYINSGSSTITYADTVKGRFTISRDN
KNTLFLQMTSLRSEDTAIYYCARGYYGPYYFDYWGQGTALTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKKVEPKSSDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVMEALHNHYTQKSLSLSPGK
(SEQ ID NO:11)

light chain

DIVMTQSHKFMSTSVGDRVSFTCKASQNVGTAVAWYQQKPGQSPKLLIYWASSRHTGVPDRFTGSGSGTDFTLT
ITVQSEDLADYFCQEIYSSYPLTFGVGTELEKPTVAAPSVPFIFFPSDEQLKSGTASVVCLLNFPYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID
NO:12)

13H10

heavy chain (Fc domain underlined)

- QVQLQQSGPELKKPGETVKISCKASGYTFTDFSIHWVKQAPGKGLKWMGWINTETGETTYAEDFKGRFAFSLETS
ASTAYLQINNLLKNETATYFCSTRRYDYDTWFAYWGQGLTVTVSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
5 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYF
SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO:13)
- light chain
- 10 DIVMTQAALSVFVTFGESVSISCRSSSSLLHENGNTYYLWFLQRPQGSPQLLIYRMSNLASGVDPDRFSGSGSGTA
FTLRISRVEAEDVGVYYCMQRLEYPTFTGGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ
ID NO:14)
- 15 **15B8**
heavy chain (Fc domain underlined)
QVQLQQPGVVLVMPGASVKLSCKASGYTFASYWMHWVKQRPQGGLWIGEIDPSDSYRNYNQKFKGKATLTVDKS
SSTVNMQLSSLTSEDSAVYYCAKTSGLIGRANDYWGQETSVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKT
20 HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO:15)
- 25 light chain
DIQMIQSPASLSASVGETVTITCRASENIYSFVTWYQQKQKGKSPQLLVFNAKNLVEGVPSSFSFGSGSGTQFSLKI
DSLQPEDFGTYTCQHHYSIPYTFGGGKLEMRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
NO:16)
- 30

In another embodiment of all of these embodiments, the Tie2 antibodies or dimers comprise dimers, wherein the dimers comprise monomers comprising the amino acid sequence of SEQ ID NO:47, wherein (X) is optional and when present comprises an amino acid linker of any suitable length and amino acid content. As noted above, the Tie-2 dimers include two monomers of the fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain. The two monomers dimerize since the Fc domain naturally dimerizes. The F domain amino acid sequence present in each monomer comprises or consists of the amino acid sequence of SEQ ID NO:10:

Human Ang1 F domain (Tie2 receptor binding domain; SEQ ID NO:10): Bold font

Human IgG1 Fc: Underlined

5 KAE**LASEKPF**RD**CADVYQAGFNKSGIY**TIYINN**MP**EP**KKVFCNMDVN**GGG**WTVIQHREDGSLDFQ**RGWKEYKM**GF**
 GNPSGEYWLGN**EFIFAITSQRQYMLRIELMDWEGN**RAY**SQYDRFHIGNEKQNYRLYLK**GHTGTAG**KQSS**LILHGA
 DFSTKDADNDNC**MCKCALMLTGGW**WFDACG**PSNLNGMFYTAGQNHGKLN**GIKW**HYFKGPSYS**LRSTTMMIRPLDF
 (X)EPKSSDK**TH**TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
 KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK**TISKAKGP**REPQVYTLPPSRDELTKNQ
 VSLTCLVKG**FYP**SDIAVEWESNGQPENNYK**TT**PPVLDSDGSFFLYSKLTVDKSRWQQGNV**FSCSV**MHEALHNHYT
 10 QKSLSLSPGK (SEQ ID NO: 47)

In one embodiment, the dimers comprise monomers comprising an amino acid
 sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%,
 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of the amino acid
 15 sequence of SEQ ID NO:17 or 18, wherein residues in parentheses are optional. The residues
 in parentheses are either amino acid linkers (in these examples, GS-rich linkers), His-tags, or
 secretion signals (italicized—these may be absent, present, or replaced with any other
 secretion signal)

20 (METDTLLLWVLLLWVPGSTG) (HHHHHH) (GGSGGS) KAE**LASEKPF**RD**CADVYQAGFNKSGIY**TIYINN**MP**EP**KKVFCNMDVN**GGG**WTVIQHREDGSLDFQ**RGWKEYKM**GF**GNPSGEYWLGN**EFIFAITSQRQYMLRIELMDWEGN**RAY**SQYDRFHIGNEKQNYRLYLK**GHTGTAG**KQSS**LILHGA**DFSTKDADNDNCMCKCALMLTGGW**WFDACG**PSNLNGMFYTAGQNHGKLN**GIKW**HYFKGPSYS**LRSTTMMIRPLDF (GGSGGS) EPKSSDK**TH**TCPPCPAPELLGGPSVFLFPPKPK
 KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
 25 VSNKALPAPIEK**TISKAKGP**REPQVYTLPPSRDELTKNQVSLTCLVKG**FYP**SDIAVEWESNGQPENNYK**TT**PPV
 LDSDGSFFLYSKLTVDKSRWQQGNV**FSCSV**MHEALHNHYT**QKSLSLSPGK** (SEQ ID NO:17)

(METDTLLLWVLLLWVPGSTG) KAE**LASEKPF**RD**CADVYQAGFNKSGIY**TIYINN**MP**EP**KKVFCNMDVN**GGG**WTVIQHREDGSLDFQ**RGWKEYKM**GF**GNPSGEYWLGN**EFIFAITSQRQYMLRIELMDWEGN**RAY**SQYDRFHIGNEKQNYRLYLK**GHTGTAG**KQSS**LILHGA**DFSTKDADNDNCMCKCALMLTGGW**WFDACG**PSNLNGMFYTAGQNHGKLN**GIKW
 30 **HYFKGPSYS**LRSTTMMIRPLDF (GGSGGS) EPKSSDK**TH**TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK**TISKAKGP**REPQVYTLPPSRDELTKNQVSLTCLVKG**FYP**SDIAVEWESNGQPENNYK**TT**PPVLDSDGSFFLYSKLTVDKSRWQQGNV**FSCSV**MHEALHNHYT**QKSLSLSPGK** (GGSHHHHHH) (SEQ ID NO:18)

35

In one specific embodiment of any of the above embodiments, the plurality of homo-
 polymers comprises homo-tetramers of the polypeptide comprising an amino acid sequence
 at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7. In another specific embodiment of any of the above embodiments, the plurality of homo-polymers comprises homo-trimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
5 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5.

In another embodiment, the disclosure provides composition comprising a plurality of the particles of any embodiment herein comprising Tie2 receptor antibodies comprising Fc domains, and/or dimers of fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain. The compositions may be used, for example, in the methods and uses
10 of the disclosure. In one embodiment, all antibodies or dimers in the composition are identical. In another embodiment, the antibodies or dimers in the composition are, in total, not identical. For example, the composition may comprise particles comprising Tie2 antibodies and particles comprising F domain dimers. In another embodiment, the composition may comprise particles comprising different Tie2 antibodies and/or F domain
15 dimers having different amino acid sequences.

In another embodiment, the disclosure comprises pharmaceutical compositions comprising the Tie2 particle or composition of any embodiment herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions may be used, for example, in the methods and uses of the disclosure.

In another embodiment, the disclosure provides uses of the Tie 2 particles, compositions or pharmaceutical compositions for any suitable use, including but not limited to those described in the examples. In one embodiment, the disclosure provides methods for treating complications from bacterial or viral infections or any disease or syndrome resulting from vascular dysfunction, comprising administering to a subject having a bacterial or viral
25 infection or any disease or syndrome resulting from vascular dysfunction an amount of the particles, compositions, or pharmaceutical compositions or any embodiment or combination of embodiments herein effective to treat the bacterial or viral infection. The methods may be used to treat any bacterial or viral infection, or any disease or syndrome resulting from vascular dysfunction as deemed appropriate by attending medical personnel. In one
30 embodiment, the treating comprising treating or limiting development of diseases or syndromes resulting from vascular dysfunction, including but are not limited to sepsis, acute respiratory distress syndrome (ARDS), acute lung injury, acute kidney injury, wet-age related macular degeneration, open angle glaucoma, diabetic retinopathy, and diabetic nephropathy.

In another embodiment, the disclosure provides kits for generating the particles and compositions of the disclosure. In one embodiment, the kits comprise:

- (a) a polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally wherein the polypeptides are as disclosed in any embodiment disclosed herein; and
- (b) Tie2 antibodies comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs selected from the group consisting of SEQ ID NOS:11-12; SEQ ID NOS:13-14; and SEQ ID NOS:15-16, and/or a fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain optionally comprising the amino acid sequence selected from the group consisting of SEQ ID NOS:17-18 and 47.

In this embodiment, when the two components are combined the particles spontaneously assemble via interaction of the antibodies or dimers with Fc binding interfaces.

In another embodiment, the kits comprise:

- (a) host cells capable of expressing a polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally wherein the polypeptides are as disclosed for any embodiment herein; and
- (b) host cells capable of expressing Tie2 antibodies amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs selected from the group consisting of SEQ ID NOS:11-12; SEQ ID NOS:13-14; and SEQ ID NOS:15-16, and/or a fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain optionally comprising the amino acid sequence selected from the group consisting of SEQ ID NOS: 17-18 and 47.

In this embodiment, the two components can be produced by the host cells and then combined so that the particles spontaneously assemble via interaction of the antibodies or dimers with Fc binding interfaces.

5 In another embodiment, the disclosure provides polypeptides comprising an amino acid sequence comprising or consisting of the amino acid sequence of any one of SEQ ID NOS: 17-18 and 47. The polypeptides may be used in producing the Tie 2 particles disclosed herein.

10 In another aspect, the disclosure provides nucleic acids encoding the polypeptide comprising or consisting of the amino acid sequence of any one of SEQ ID NOS: 17-18 and 47. The nucleic acid sequence may comprise single stranded or double stranded RNA or DNA in genomic or cDNA form, or DNA-RNA hybrids, each of which may include chemically or biochemically modified, non-natural, or derivatized nucleotide bases. Such nucleic acid sequences may comprise additional sequences useful for promoting expression and/or purification of the encoded polypeptide, including but not limited to polyA sequences, 15 modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals. It will be apparent to those of skill in the art, based on the teachings herein, what nucleic acid sequences will encode the polypeptides of the disclosure.

20 In another aspect, the disclosure provides expression vectors comprising the nucleic acids of the disclosure operatively linked to control sequence. "Expression vector" includes vectors that operatively link a nucleic acid coding region or gene to any control sequences capable of effecting expression of the gene product. "Control sequences" operatively linked to the nucleic acid sequences of the disclosure are nucleic acid sequences capable of effecting the expression of the nucleic acid molecules. The control sequences need not be contiguous 25 with the nucleic acid sequences, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding 30 sites. Such expression vectors can be of any type, including but not limited plasmid and viral-based expression vectors. The control sequence used to drive expression of the disclosed nucleic acid sequences in a mammalian system may be constitutive (driven by any of a variety of promoters, including but not limited to, CMV, SV40, RSV, actin, EF) or inducible (driven by any of a number of inducible promoters including, but not limited to, tetracycline,

ecdysone, steroid-responsive). The expression vector must be replicable in the host organisms either as an episome or by integration into host chromosomal DNA. In various embodiments, the expression vector may comprise a plasmid, viral-based vector, or any other suitable expression vector.

5 In a further embodiment, the disclosure provides host cells comprising the polypeptide, nucleic acid, and/or expression vector of any embodiment disclosed herein. In various embodiments, the host cells can be either prokaryotic or eukaryotic.

In another aspect, the disclosure provides particles, comprising:

- (a) a plurality of polypeptide polymers, wherein
 - 10 (i) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:1;
 - (ii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
15 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:2;
 - (iii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:3;
 - (iv) each monomer in the polymers comprises an amino acid sequence at
20 least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:4;
 - (v) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5;
 - 25 (vi) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:6;
 - (vii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
30 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7;
 - (viii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:8; or

(ix) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:9; wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement);

5 and

(b) a plurality of α -TNFRSF (tumor necrosis factor receptor superfamily) antibodies comprising Fc domains;

wherein

(i) each α -TNFRSF antibody in the plurality of antibodies comprises a first Fc domain and a second Fc domain;

(ii) each α -TNFRSF antibody in the plurality of antibodies is (A) non-covalently bound via the first Fc domain to one polypeptide monomer chain of a first polymer, and (B) non-covalently bound via the second Fc domain to one polypeptide monomer of a second polymer; and

(iii) each polypeptide monomer chain of each polymer is non-covalently bound to one Fc domain;

wherein the particle comprises dihedral, tetrahedral, octahedral, or icosahedral symmetry.

As shown in the examples that following, the particles of the disclosure targeting cell-surface TNFRSF receptors enhance signaling compared to free antibodies or Fc-fusions in DR5-mediated apoptosis, and were shown to induce tumor cell apoptosis. Thus, the compositions may be used to treat tumors.

In this aspect, "antibody" includes reference to full length and any functional antibody fragments that selectively bind a TNFRSF including the Fc domain; fusion proteins comprising a protein that binds a TNFRSF and an Fc domain, that dimerizes since the Fc domains naturally dimerizes; and an Fc fragment chemically modified to a protein that binds a TNFRSF, which dimerizes since the Fc domains naturally dimerizes.

When combined with α -TNFRSF antibody, a higher order, cage-like, polyhedral structure spontaneously assembles via interaction of the antibodies with Fc binding interfaces. The resulting higher order structures have C2 cyclic symmetry at the Fc position and cyclic 2, 3, 4, or 5-symmetry at each homo-oligomerization domain interface.

All embodiments of the polypeptide monomers disclosed herein are equally applicable to this aspect of the disclosure. Thus, in various non-limiting embodiments,

residues present at a polymeric interface, as defined in Table 2, in a polymer of the polypeptide of any one of SEQ ID NOS:1-9 may be conserved; residues present at a Fc binding interface of any one of SEQ ID NOS:1-9 as defined in Table 3 may be conserved; substitutions relative to the reference sequence of any one of SEQ ID NOS:1-9 may

5 comprise, consist essentially of, or consist of substitutions at polar residues in the reference polypeptide; substitutions relative to the reference sequence of any one of SEQ ID NOS:1-9 may comprise, consist essentially of, or consist of substitutions at polar residues at non-Gly/Pro residues in loop positions, as defined in Table 4, in the reference polypeptide; and/or amino acid changes from the reference polypeptide of any one of SEQ ID NOS:1-9 may be

10 conservative amino acid substitutions. In all embodiments, the polypeptide monomers may further comprise a functional polypeptide covalently linked to the amino-terminus and/or the carboxy-terminus. In various non-limiting embodiments, the functional polypeptide may include, but is not limited to, a detectable polypeptide such as a fluorescent or luminescent polypeptide, receptor binding domains, etc.

15 In one embodiment, the α -TNFRSF antibody heavy and light chains can be co-expressed in cells to produce the α -TNFRSF antibody, which can then be mixed with the polymers to form the particles of the disclosure.

In some embodiments, the polypeptide monomers in each polymer are 100% identical, and the polymers are homo-oligomers. In other embodiments, the polymers may

20 comprise monomers with some amino acid differences, so long as each monomer has the required amino acid sequence identity to the reference polypeptide. In these embodiments, the polymers are not necessarily homo-oligomers. In light of this, as will be understood by those of skill in the art, the plurality of polymers in a given particle may comprise all homo-oligomers, the particle may comprise polymers that are not homo-oligomers, or a

25 combination thereof. Similarly, the particle may comprise all homo-oligomers, and each homo-oligomer may be identical, or the plurality of homo-oligomers may comprise 2 or more different homo-oligomers.

In one embodiment, the plurality of polymers comprises dimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%,

30 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-3. In these embodiments, adding the recited polypeptides with to α -TNFRSF antibodies results in spontaneous assembly into a D2 dihedral structure containing two antibodies per particle.

In another embodiment, the plurality of polymers comprises trimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:4-6. In these
 5 embodiments, adding the recited polypeptides with α -TNFRSF antibodies results in spontaneous assembly into a T32 tetrahedral structure containing six antibodies per particle.

In a further embodiment, the plurality of polymers comprises tetramers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the
 10 amino acid sequence of SEQ ID NO:7. In these embodiments, adding the recited polypeptides with α -TNFRSF antibodies results in spontaneous assembly into an O42 octahedral structure containing twelve antibodies per particle.

In one embodiment, the plurality of polymers comprises pentamers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%,
 15 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:8-9. In these embodiments, adding the recited polypeptides with α -TNFRSF antibodies results in spontaneous assembly into an I52 icosahedral structure containing thirty antibodies per particle.

Any TNFRSF may be targeted as appropriate for an intended use of the particles and
 20 compositions thereof. In various embodiments, the α -TNFRSF antibody targets one or more of DR5/TRAIL-R2/TNFRSF10B/CD262, CD40, 4-1BB, and TWEAKR (Tumor Necrosis Factor-like Weak Inducer of Apoptosis Receptor)/TNFRSF12A/CD266. In various further embodiments, the α -TNFRSF antibodies comprise an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 25 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs (when both heavy and light chain are needed) selected from the group consisting of:

SEQ ID NO: 19 and 20;
 SEQ ID NO: 21 and 22;
 SEQ ID NO: 23 and 24;
 30 SEQ ID NO: 25 and 26;
 SEQ ID NO: 27 and 28;
 SEQ ID NO: 29;
 SEQ ID NO: 30;

- SEQ ID NO: 31 and 32;
 SEQ ID NO: 33;
 SEQ ID NO: 34 and 35;
 SEQ ID NO: 36 and 37;
 5 SEQ ID NO: 38 and 39;
 SEQ ID NO: 40 and 41;
 SEQ ID NO: 42 and 43;
 SEQ ID NO: 44 and 45;
 SEQ ID NO: 44 and 46;
 10 SEQ ID NO: 48 and 49;
 SEQ ID NO: 50 and 51;
 SEQ ID NO: 52 and 53
 SEQ ID NO: 54 and 55;
 SEQ ID NO: 56;
 15 Lob 7/6 heavy and light chains as disclosed in published US patent application US
 US20090074711 (incorporated by reference herein in its entirety); and
 Heavy and light chain pairs disclosed in 2018094300 (incorporated by reference
 herein in its entirety).

20 **Table 5. Antibody sequences**
CD40 Targeting antibodies

<u>Selicrelumab</u>	
(Heavy chain)	
25	QVQLVQSGAE VKKFGASVKV SCKASGYTFT GYMHWVRQA PGQGLEWMGW INPDSSGGTNY AQKFQGRVTM TRDTSISTAY MELNRLRSDD TAVYYCARDQ PLGYCTNGVC SYFDYWGQGT LVTVSSASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS NFGTQTYTCN VDHKPSNTKV DKTVERKCCV ECPPCPAPPV AGPSVFLFPF KPKDTLMISR TFEVTCVVVD VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ
30	FNSTRFVSV LTVVHQDWLN GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSGGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALNHN YTKSLSLSP GK (SEQ ID NO:19)
(Light chain)	
35	DIQMTQSPSS VSASVGDRVT ITCRASQGIY SWLAWYQOKP GKAPNLLIYT ASTLQSGVPS RFSGSGSGTD PTLTISSLQP EDPATYYCQQ ANIFPLTFGG GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYERHK VYACEVTHQG LSSPVTRSFN RGEC (SEQ ID NO:20)
<u>Eleselumab</u>	
(Heavy chain)	
40	QLQLQESGPG LLEPSETLSL TCTVSGGSIS SPGYVGGWIR QPPGKLEWI GSIYKSGSTY HNPSTLKSRTV ISVDTSKNQF SLKLSSVTAA DTAVYYCTRP VVRYFGWFDG WGQGTILVTS SASTKGFSVF PLAPCSRSTS ESTAALGCLV KDYFPEPPTV SWNSGALTSG VHTFPAVLQS SGLYSLSSV TVPSSSLGK TYTCNVDHKP SNTKVDKRYE SKYGPPCPFC PAFEFEGGPS
45	VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAFT KPREEQFNST

YRVVSVLTIVL HQDQLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPOVY TLPPSQEEMT
 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE
 GNVFSCSVMH EALHNHYTQK SLSLSLGGK (SEQ ID NO:21)

(Light chain)

- 5 AIQLTQSPSS LSASVGDRVT ITCRASQGIS SALAWYQQKP GKAPKLLIYD ASNLESGVPS
 RFSGSGSGTD FTLTISSLQF EDPATYYCQQ FNSYPTFGQG TKVEIKRTVA APSVFIFPPS
 DEQLKSGTAS VVCLLNHFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT
 SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC (SEQ ID NO:22)

10 **4-1BB Targeting antibodies**

Urelumab

(Heavy chain)

- 15 QVQLQWAGG LLKPSSETLSL TCAVYGGSFSS GYVSWIRQS PEKGLEWIGE INHGGYVTYN
 PSLESRTIS VDTSKNQFSL KLSSVTAADT AVYYCARDYG PGNYDWYFDL WGRGTLVTVS
 SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS
 SGLYSLSVV TVPSSSLGK TYTCNVDPK SNTKVDKRV SKYGPPCPPC PAPEFLGGPS
 VFLFPKPKD TLMISRTPEV TCVVVDVSGE DPEVQFNWYV DGVEVHNAKT KPREEQFNST
 YRVVSVLTIVL HQDQLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPOVY TLPPSQEEMT
 20 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE
 GNVFSCSVMH EALHNHYTQK SLSLSLGGK (SEQ ID NO:23)

(Light chain)

- EIVLTQSEAT LSLSEGERAT LSCRASQSVS SYLAWYQQKP GQAPRLIYD ASNRATGIPA
 RFSGSGSGTD FTLTISLLEF EDPATYYCQQ RSNWPPALTF CGGKVEIKR TVAAPSVFIF
 25 PPSDEQLKSG TASVVCLLN PYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYLSST
 LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC (SEQ ID NO: 24)

Utomilumab

(Heavy chain)

- 30 EVQLVQSGAE VKKPGESLRL SCXGSGYSFS TYWISWVRQM PGKGLEWMGK IYPGDSYNTY
 SPSPQGGVTI SADKSLSTAY LQWSSLKASD TAMYVCARGY GIFDYWGQGT LVTVSSASTK
 GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS
 LSSVTVTPSS NFGTQTYTCN VDHKPSNTKV DKTVERKCCV ECPPCPAPPV AGPSVFLPPP
 KPKDTLMISR TPEVTCVVVD VSHEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTFRVVS
 35 LTVVHQDWLN GKEYKCKVSN KGLPAPIEKT ISKTKGQPRE PQVYTLPPSR EEMTKNQVSL
 TCLVKGFYPS DIAVEWESNG QPENNYKTTP PMLDSGDSFF LYSKLTVDKS RWQQGNVFSC
 SVMHEALHNH YTKSLSLSP GK (SEQ ID NO:25)

(Light chain)

- SYELTQPPSV SVSEGTASI TCSGDNIGDQ YANWYQQKPG QSPVLVIYQD KNRPSGIPER
 40 FSGNSGNTA TLTISGTQAM DEADYYCATY TGFGLAVFG GGTCLTVLQ PKAAPSVTLF
 PPSSEELQAN KATLVCLISD FYPGAVTVAW KADSSPVKAG VETTTPSKQS NNKYAASSYL
 SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP TECS (SEQ ID NO:26)

45 **DR5/TRAILR2 Targeting antibodies**

Drozitumab

(Heavy chain, Fc region underlined)

- 50 EVQLVQSGGG VEREGGSLRL SCAASGFTFD DYAMSWVRQA PGKGLEWVSG INWQGGSTGY
 ADSVKGRVTI SRDANKSLY LQMNLSRAED TAVYYCAKIL GAGRGWYFDY WKGKTTVTVS
 SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS
 SGLYSLSVV TVPSSSLGK TYICNVNPKP SNTKVDKKVE PKSCDKTHTC PPCPAPELLG
 GPSVFLFPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRE
 55 EMTKNQVSLT CLVKGFPYSD IAVEWESNGQ PENNYKTTPP VLDSGDSFFL YSKLTVDKSR
 WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K (SEQ ID NO:27)

(Light chain)

- SELTQDPAVS VALGQTVRIT CSGDSLRSYY ASWYQQKPGQ APVLVIYGAN NRPSGIPDRF
 SGGSSGNTAS LTITGAQED EADYYCNSAD SSGNHVVFVG GTKLTVLQPK KAAPSVTLFP
 60 PPSSEELQANK ATLVCISDF YPGAVTVAW ADSSPVKAGV ETFTPSKQSN NKYAASSYLS
 LTPEQWKSHK SYSCQVTHEG STVEKTVAPT ECS (SEQ ID NO:28)

scTRAIL-Fc

Heavy chain only needed, Fc region underlined

5 QPVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFSLSNLHLRNGELVIEHEKGFYIYSQTYFRFQE
 EIKENTKNDKQMVQYIYKYTSYFDPILLMKSARNSCWSKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMD
 HEASFFGAFLVGGSGSGNGSRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFSLSNLHLRNGELV
 IHEKGFYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYFDPILLMKSARNSCWSKDAEYGLYSIYQGGIFEL
 KENDRIFVSVTNEHLIDMDHEASFFGAFLVGGSGSGNGSRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWES
 10 SRSGHSFSLSNLHLRNGELVIEHEKGFYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYFDPILLMKSARNSCW
 SKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVGGPGSSSSSSSGCDKHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYSSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHTQKSLSLSPGK (SEQ ID
 NO:29)

KMTR2

HiscFv heavy chain (no light chain needed, Fc region underlined)

20 QVQLQQSGPGGLVKPSQTLSTCAISGDSVSSTTVANDWIRQSPSRGLEWLGRTYYRSKWYNEYAVSVKSRITINV
 DTSKNQISLQLNSVTPEDTAVYYCAREPDAGRGAFDIWGQGTITVTSPLRWGRFGWRGLGRGWLRSPVTQSPGTL
 LSPGERATLSCRASQSVSSSHLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISSELPEDFAVY
 YCQQRSNWPFRAVFGQGTTRLEIKGGSGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 25 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFCSCVMHEALHNNHTQKSLSLSPGK (SEQ ID NO:30)

H1 Heavy chain (pairs with L1, Fc region underlined)

30 QVQLQQSGPGGLVKPSQTLSTCAISGDSVSSTTVANDWIRQSPSRGLEWLGRTYYRSKWYNEYAVSVKSRITINV
 DTSKNQISLQLNSVTPEDTAVYYCAREPDAGRGAFDIWGQGTITVTSPTSTKGPSVFPLAPSSKSTSGGTAALGCL
 VKDYFFPEFVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSS
 DKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHTQKSLSLSP
GK (SEQ ID NO:31)

L1 Light chain (pairs with H1)

35 LRSPTVQSPGTLSPGERATLSCRASQSVSSSHLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLT
 TISSELPEDFAVYYCQQRSNWPFRAVFGQGTTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKV
 QWYVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTFLSKADYEKKHVKYACEVTHQGLSSPVTKSFNRGEC (SEQ
 40 ID NO:32)

H6scFv heavy chain (no light chain needed, Fc region underlined)

45 QVQLQQSGPGRVQPSQTLSTCAISGDSVSNNNAWYIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITISP
 DTSKNQFSLQLNSVTPEDTAVYYCARRGDGNSYFDYWGQGTITVTVSSGILRWGRFGWRGLGRGWLEIVLTQSPGT
 LSLSPGERATLSCRASQSVSSGYVSWYRQKPGQAPRLLIYGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFA
 VYYCHQYGSSEPTNYGQGTQVGIKGGSGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 50 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFCSCVMHEALHNNHTQKSLSLSPGK (SEQ ID NO:33)

H6 Heavy chain (pairs with L6, Fc region underlined)

55 QVQLQQSGPGRVQPSQTLSTCAISGDSVSNNNAWYIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITISP
 DTSKNQFSLQLNSVTPEDTAVYYCARRGDGNSYFDYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
 VKDYFFPEFVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSS
DKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHTQKSLSLSP
GK (SEQ ID NO:34)

L6 Light chain (pairs with H6)

LEIVLTQSPGTLISLSPGERATLSCRASQSVSSGVSWYRQKPGQAPRLLIYGASTRATGIPDRFSGSGSGTDFTLT
TISRLEPEDFAVYYCHQYGGSSPNTYGGGTKVGIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
NO:35)

1E62

Heavy chain, Fc region underlined

EVQLVQSGGSGVERPQGGSLRLSCAASGFTFDDYGMNWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRVTISRDN
KNSLYLQMNLSRAEDTAVYYCAKILGAGRGWYFELWGKGTITVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPYSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG
K (SEQ ID NO:36)

Light chain, Fc region underlined

SELTQDPAVSVALGQTVRITCQGDLSRSYYASWYQQKPGQAPVLIYGKNNRPSGIPDRFSGSSSGNTASLTITG
AQAEDEADYYCNSRDSSGNHVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK
ADSSPVKAGVETTTFSKQSNKYAASSYLSLTPEQWKS HKSYSCQVTHEGSTVEKTVAPTEC (SEQ ID
NO:37)

Conatumumab/AMG 655

Conatumumab // AMG 655 heavy chain (Fc region underlined)

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDFWWSIRQLPGKGLEWIGHIHNSTGYTYPNPSLKSRT
ISVDTSKKQFSLRLSSVTAADTAVYYCARDRGDYYGMDVWVGQGTITVTSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVDKYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHK
PSNTKVDKRVPEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDLSRWQQGNVFCSCVMHE
ALHNHYTQKSLSLSPGK (SEQ ID NO:38)

Conatumumab/AMG 655 light chain

EIVLTQSPGTLISLSPGERATLSCRASQGGISRSYLAWYQQKPGQAPSLLIYGASSRATGIPDRFSGSGSGTDFTLT
ISRLEPEDFAVYYCQFGSSPNTYGGGTKVGIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
NO:39)

TWEAKR Targeting antibodies**Enavatuzumab**

(Heavy chain, Fc region underlined)

EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYWMSWVRQA PGKGLEWVAE IRLKSDNYAT
HYAESVKGRF TISRDDSKNS LYLQMNLSRA EDTAVYYCTG YYADAMDYWG QGTLTVTSSA
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPVAVLQSSG
LYSLSSVTVTP PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK SCDKTHCTPP CPAPPELLGGP
SVFLFPPKPK DTLMISRTPE VTCVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSREEM
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVLD SDGSFFLYS KLTVDKSRWQ
QGNVFCSCVM HEALHNHYTQ KSLSLSPGK (SEQ ID NO:40)

(Light chain)

DIQMTQSPSS LSASVGRVYITCRASQSVS TSSYSYMHYQ QKPGKAPKL LIKYASNLES
GVPSRFSGSG SGTDFTLTIS SIQPEDFATY YCQHSWEIPY TFGGGTKVEI KRTVAAPSVF
IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYLS
STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC (SEQ ID NO:41)

Dacetuzumab (WO2016069919)

h32C6 heavy chain (SEQ ID NO:42)

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
 20 25 30
 5 Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Arg Val Ile Pro Asn Ala Gly Gly Thr Ser Tyr Asn Gln Lys Phe
 50 55 60
 10 Lys Gly Arg Phe Thr Leu Ser Val Asp Asn Ser Lys Asn Thr Ala Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Glu Gly Ile Tyr Trp Trp Gly Gln Gly Thr Leu Val Thr Val
 100 105 110
 15 Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
 115 120 125
 Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
 130 135 140
 20 Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
 145 150 155 160
 Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu
 165 170 175
 Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr
 180 185 190
 25 Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val
 195 200 205
 Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro
 210 215 220
 30 Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 225 230 235 240
 Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 245 250 255
 Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 260 265 270
 35 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 275 280 285
 Arg Glu Gln Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 290 295 300
 Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 305 310 315 320
 40 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 325 330 335
 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 340 345 350
 45 Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 355 360 365
 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 370 375 380
 50 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 385 390 395 400
 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 405 410 415
 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 420 425 430
 55 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 MS2C6 light chain (SEQ ID NO:43)
 60 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Leu Val His Ser

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                20                25                30
Asn Gly Asn Thr Phe Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala
   35   40   45
5  Pro Lys Leu Leu Ile Tyr Thr Val Ser Asn Arg Phe Ser Gly Val Pro
   50   55   60
   Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
   65   70   75   80
   Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Phe Cys Ser Gln Thr
   85   90   95
10  Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
   100  105  110
   Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
   115  120  125
15  Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
   130  135  140
   Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
   145  150  155  160
   Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
   165  170  175
20  Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
   180  185  190
   Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
   195  200  205
   Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
25  210  215

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Lucatumumab: WO2012075111)

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30  Light chain of WCD122 human anti-CD40 antibody (SEQ ID NO:44)
Met Ala Leu Pro Ala Gln Leu Leu Gly Leu Leu Met Leu Trp Val Ser
   1   5   10   15
35  Gly Ser Ser Gly Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Thr
   20  25  30
   Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
   35  40  45
40  Leu Leu Tyr Ser Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys
   50  55  60
   Pro Gly Gln Ser Pro Gln Val Leu Ile Ser Leu Gly Ser Asn Arg Ala
   65  70  75  80
   Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
   85  90  95
45  Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
   100 105 110
   Cys Met Gln Ala Arg Gln Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys
   115 120 125
   Val Asp Ile Arg Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
   130 135 140
50  Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
   145 150 155 160
   Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
   165 170 175
55  Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
   180 185 190
   Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
   195 200 205
60  Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
   210 215 220
   Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys

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225 230 235

Heavy chain of HCD122 human anti-CD40 antibody (SEQ ID NO:45)

5 Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Arg Gly
1 5 10 15
Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
20 25 30
10 Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45
Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60
Glu Trp Val Ala Val Ile Ser Tyr Glu Glu Ser Asn Arg Tyr His Ala
65 70 75 80
15 Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ile
85 90 95
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val
100 105 110
20 Tyr Tyr Cys Ala Arg Asp Gly Gly Ile Ala Ala Pro Gly Pro Asp Tyr
115 120 125
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
130 135 140
Pro Ser Val Phe Pro Leu Ala Pro Ala Ser Lys Ser Thr Ser Gly Gly
145 150 155 160
25 Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
165 170 175
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
180 185 190
30 Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
195 200 205
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
210 215 220
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
225 230 235 240
35 Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
245 250 255
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
260 265 270
40 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
275 280 285
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
290 295 300
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305 310 315 320
45 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
325 330 335
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
340 345 350
50 Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
355 360 365
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
370 375 380
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385 390 395 400
55 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
405 410 415
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
420 425 430
60 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
435 440 445
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser

450 455 460
 Leu Ser Pro Gly Lys
 465

5
 Heavy chain of variant of HCD122 human anti-CD40 antibody (SEQ ID NO:46)

10 Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Arg Gly
 1 5 10 15
 Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
 20 25 30
 Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 35 40 45
 Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 15 50 55 60
 Glu Trp Val Ala Val Ile Ser Tyr Glu Glu Ser Asn Arg Tyr His Ala
 65 70 75 80
 Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ile
 85 90 95
 Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val
 100 105 110
 Tyr Tyr Cys Ala Arg Asp Gly Gly Ile Ala Ala Pro Gly Pro Asp Tyr
 115 120 125
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 130 135 140
 Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
 145 150 155 160
 Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 165 170 175
 Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 180 185 190
 Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 195 200 205
 Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
 210 215 220
 Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
 225 230 235 240
 Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
 245 250 255
 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 260 265 270
 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 275 280 285
 Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
 290 295 300
 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
 305 310 315 320
 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 325 330 335
 Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
 340 345 350
 Pro Ile Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 355 360 365
 Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
 370 375 380
 Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 385 390 395 400
 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 405 410 415
 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 420 425 430

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
 435 440 445
 Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 450 455 460
 5 Leu Ser Pro Gly Lys
 465

GITR Targeting antibodies

10 AMG228 heavy chain (Fc region underlined)
 QVQLVESGGGVVQPGRSLRLSCVASGFTFSSYGMHWIRQAPGKGLEWVAVIWEYSNKKYAESVKGRFTISRDN
 KNTLYLQMNSLPAEDTAVYYCARGGPLGLDYSGMDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
 LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS
CDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 15 QYNSTYRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMH
ALHNHYTQKSLSLSPGK (SEQ ID NO:48)

AMG228 light chain
 20 DIQMTQSPSSLSASVGDRVTITCRASQGIKNDLGWYQQKPGKAPKRLIYATSSSLQSGVPSRFSGSGSGTEFTLT
 SSLQPEDFATYYCLQHNTYPTWTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
 DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
 NO:49)

25 TRX518 heavy chain (Fc region underlined)
 QVTLKESGPGILKPSQTLSTLTCFSGFSLSTSGMGVWIRQPSGKGLEWLAHIWDDDKYYNPSLKSQLTISKDT
 SRNQVFLKITSVDTADAATYYCARTRRYFPFAYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
 YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKT
 30 HTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMH
ALHNHYTQKSLSLSPGK (SEQ ID NO:50)

35 TRX518 light chain
 DIVMTQSQKFMSTSVGDRVSTCKASQNVGTNVAWYQQKPGQSPKALIYSASYRYSVGPDRFTGSGSGTDFTLTI
 MNVHSEDLAEYFCQQYNTDPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
 DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
 NO:51)

40 MK-4166 heavy chain (Fc region underlined)
 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVASISSGGTTYYPDSVKGRFTISRDN
 SKNTLYLQMNSLRAEDTAVYYCARVGGYYDSMDYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD

- 5 (SEQ ID NO:52)
- MK-4166 light chain
- 10 QWVVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:53)
- BMS-986156 heavy chain (Fc region underlined)
- 15 QVTLKESGPGILKPSQTLSTLCSFSGFSLSTSGMGVGVIRQPSGKGLEWLAHIWDDDKYYNPSLKSQLTISKDT
SRNQVFLKITSVDTADAATYYCARTRRYFFPAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
- 20 (SEQ ID NO:54)
- BMS-986156 light chain
- 25 DIVMTQSQKFMSTSVGDRVSVCKASQNVGTNAVWYQQKPGQSPKALIYSASYRYSQVDRFTGSGSGTDFTLTINNVHSEDLAEYFCQQYNTDPLTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:55)
- scGITRL-Fc (Heavy chain only needed, Fc region underlined)
- 30 ETAKEPCMAKFGPLPSKWQMASSEPPCVNKVSDWKLEILQNGLYLIYGQVAPNANYNDVAPFEVRLYKNKDMIQT
LTNKSQIQNVGGTYELHVGDTIDLIFNSEHQVLKNNNTYWGIIILLANPQFISGSGSGNGSETAKEPCMAKFGPLPS
KWQMASSEPPCVNKVSDWKLEILQNGLYLIYGQVAPNANYNDVAPFEVRLYKNKDMIQTLTNKSQIQNVGGTYEL
HVGDTIDLIFNSEHQVLKNNNTYWGIIILLANPQFISGSGSGNGSETAKEPCMAKFGPLPSKWQMASSEPPCVNKVS
DWKLEILQNGLYLIYGQVAPNANYNDVAPFEVRLYKNKDMIQTLTNKSQIQNVGGTYELHVGDTIDLIFNSEHQV
LKNNNTYWGIIILLANPQFISGSGSGSGSGSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYESTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDEETNNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
SRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:56)

- 40 In one specific embodiment of any of the above embodiments, the plurality of homo-polymers comprises homo-tetramers of the polypeptide comprising an amino acid sequence

at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7. In another specific embodiment of any of the above embodiments, the plurality of homo-polymers comprises homo-trimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5.

In another embodiment, the disclosure provides compositions comprising a plurality of the particles of any embodiment herein comprising α -TNFRSF antibodies. The compositions may be used, for example, in the methods and uses of the disclosure. In one embodiment, all antibodies in the composition are identical. In another embodiment, the antibodies are, in total, not identical.

In another embodiment, the disclosure comprises pharmaceutical compositions comprising the α -TNFRSF antibody particles or compositions of any embodiment herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions may be used, for example, in the methods and uses of the disclosure.

In another embodiment, the disclosure provides uses of the α -TNFRSF antibody particles, compositions or pharmaceutical compositions for any suitable use, including but not limited to those described in the examples. In one embodiment, the disclosure provides methods for treating method for treating a tumor, comprising administering to a subject having a tumor an amount of the particles, compositions, or pharmaceutical composition or any embodiment or combination of embodiments herein effective to induce tumor cell apoptosis. In one embodiment, the tumor overexpresses DR5 relative to a control tumor or a threshold DR5 expression level. As shown in the examples that following, the particles of the disclosure targeting cell-surface TNFRSF receptors enhance signaling compared to free antibodies or Fc-fusions in DR5-mediated apoptosis, and were shown to induce tumor cell apoptosis. Thus, the compositions may be used to treat tumors.

In another embodiment, the disclosure provides kits for generating the α -TNFRSF antibody particles and compositions of the disclosure. In one embodiment, the kits comprise:

(a) one or more polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a

homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in embodiment herein; and

- (b) α -TNFRSF antibodies comprising an antibody selected from the group consisting of: Lob 7/6, Lucatumumab, Dacetuzumab, Selicrelumab, Bleselumab, Urelumab, 5 Utomilumab, Drozitumab, scTRAIL-Fc, KMTR2, 16E2, and Conatumumab (also referred to as AMG 655); optionally as further specified by the heavy and light chain amino acid sequences described above.

In this embodiment, when the two components are combined the particles spontaneously assemble via interaction of the antibodies or dimers with Fc binding interfaces.

- 10 In another embodiment, the kits comprise:

- (a) host cells capable of expressing one or more polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional 15 (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in any embodiment herein; and

- (b) host cells capable of expressing α -TNFRSF antibodies comprising an antibody selected from the group consisting of: Lob 7/6, Lucatumumab, Dacetuzumab, Selicrelumab, 20 Bleselumab, Urelumab, Utomilumab, Drozitumab, scTRAIL-Fc, KMTR2, 16E2, and Conatumumab (also referred to as AMG 655); optionally as further specified by the heavy and light chain amino acid sequences described above.

- In this embodiment, the two components can be produced by the host cells and then combined so that the particles spontaneously assemble via interaction of the antibodies or 25 dimers with Fc binding interfaces.

- As described in the examples, the particles have substantial internal volume that can be used to package nucleic acid or protein cargo. Thus, in another embodiment that can be combined with any other embodiment, the particles comprise a cargo within the particle internal volume. Any suitable cargo may be packaged within the particles, including but not 30 limited to nucleic acids or polypeptides useful for an intended purpose.

The polypeptides described herein may be chemically synthesized or recombinantly expressed. The particles, polypeptides polymers, monomers, antibodies, and/or dimers may be linked to other compounds to promote an increased half-life in vivo or promote increased stability or activity in blood or at an injection site. Such linkage can be covalent or non-

covalent as is understood by those of skill in the art, and may be accomplished, by way of non-limiting example, by methods including but not limited to chemical crosslinking, PEGylation, HESylation, PASylation, and/or glycosylation.

In another embodiment, one or more monomer in the polypeptide polymer may be
5 linked covalently to either the antibody or dimer, in order to increase half-life in vivo or promote increased stability or activity in blood or injection site.

The pharmaceutical compositions of the disclosure may comprise (a) the particles, or compositions of any embodiment or combination of embodiments herein, and (b) a pharmaceutically acceptable carrier. The pharmaceutical compositions may further comprise
10 (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The composition may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the composition includes a preservative e.g.
15 benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the composition includes a bulking agent, like glycine. In yet other embodiments, the composition includes a surfactant e.g., polysorbate-20, polysorbate-40,
20 polysorbate-60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The composition may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents
25 include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the composition additionally includes a stabilizer, e.g., a molecule which substantially prevents or reduces chemical and/or physical instability of the nanostructure, in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and
30 arginine hydrochloride.

The particles, or compositions may be the sole active agent in the composition, or the composition may further comprise one or more other agents suitable for an intended use.

As used herein, "treat" or "treating" means accomplishing one or more of the following: (a) reducing severity of symptoms of the disorder in the subject; (b) limiting

increase in symptoms in the subject; (c) increasing survival; (d) decreasing the duration of symptoms; (e) limiting or preventing development of symptoms; and (f) decreasing the need for hospitalization and/or the length of hospitalization for treating the disorder.

As used herein, "limiting" means to limit development of the disorder in subjects at risk of such disorder.

As used herein, an "amount effective" refers to an amount of the particle, composition, or pharmaceutical composition that is effective for treating and/or limiting development of the disorder. The particle, composition, or pharmaceutical composition of any embodiment herein are typically formulated as a pharmaceutical composition, such as those disclosed above, and can be administered via any suitable route, including orally, parentally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intra-arterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. Polypeptide compositions may also be administered via microspheres, liposomes, immune-stimulating complexes (ISCOMs), or other microparticulate delivery systems or sustained release formulations introduced into suitable tissues (such as blood). Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). A suitable dosage range may, for instance, be 0.1 µg/kg-100 mg/kg body weight of the particle, composition, or pharmaceutical composition thereof. The composition can be delivered in a single bolus, or may be administered more than once (e.g., 2, 3, 4, 5, or more times) as determined by attending medical personnel.

Examples

We set out to design proteins that drive the assembly of arbitrary antibodies into symmetric assemblies with well-defined structures. We reasoned that symmetric protein assemblies could be built out of IgG antibodies, which are two-fold symmetric proteins, by placing the symmetry axes of the antibodies on the two-fold axes of the target architecture and designing a second protein to hold the antibodies in the correct orientation. As we aimed for a format that would work for many different antibodies, we chose as the nanoparticle interface the interaction between the constant fragment crystallizable (Fc) domain of IgG and the Fc-binding helical bundle protein A.

A general computational method for antibody cage design

To design a homo-oligomer terminating with an Fc-binding interface that has the correct geometry to hold the IgGs in the correct relative orientation for the desired architecture, we computationally fused three protein building blocks together: Fc-binders, monomers, and homo-oligomers. The Fc-binder forms the first nanocage interface between the antibody and the nanocage-forming design, the homo-oligomer forms the second nanocage interface between designed protein chains, and the monomer links the two interfaces together in the correct orientation to generate the desired nanomaterial.

To generate usable Fc-binding building blocks beyond protein A itself, we designed a second Fc-binding building block by grafting the protein A interface residues onto a designed helical repeat protein (Fig. 6). To create designs predicted to form antibody nanocages (hereafter AbCs, for Antibody Cage), we used a library consisting of these 2 Fc-binding proteins, 42 de novo designed helical repeat protein monomers, and between 1-3 homo-oligomers (2 C2s, 3 C3s, 1 C4, and 1 C5). An average of roughly 150 residues were available for fusion per protein building block, avoiding all positions involved in any protein-protein interface, leading to on the order of 10⁷ possible tripartite (Fc-binder/monomer/homo-oligomer) fusions. For each of these tripartite fusions, the rigid body transform between the internal homo-oligomeric interface and the Fc-binding interface is determined by the shapes of each of its three building blocks and the locations and geometry of the “junctions” that link them into a single subunit.

We used a recently described computational protocol (WORMS) that rapidly samples all possible fusions from our building block library to identify those with the net rigid body transforms required to generate dihedral, tetrahedral, octahedral, and icosahedral AbCs (20, 21). To describe the final nanocage architectures, we follow a naming convention which summarizes the point group symmetry and the cyclic symmetries of the building blocks. For example, a T32 assembly has tetrahedral point group symmetry and is built out of a C3 cyclic symmetric antibody-binding designed oligomer, and the C2 cyclic symmetric antibody Fc. While the antibody dimer aligns along the two-fold axis in all architectures, the designed component is a second homodimer in D2 dihedral structures; a homotrimer in T32 tetrahedral structures, O32 octahedral structures, and I32 icosahedral structures; a homotetramer in O42 octahedral structures; and a homopentamer in I52 icosahedral structures.

To make the fusions, the protocol first aligns the model of the Fc and Fc-binder protein along the C2 axis of the specified architecture (Fig. 1a-b). The Fc-binder is then fused to a monomer, which is in turn fused to a homo-oligomer. Rigid helical fusions are made by superimposing residues in alpha helical secondary structure from each building block; in the

resulting fused structure one building block chain ends and the other begins at the fusion point, forming a new, continuous alpha helix (Fig. 1c). For proper nanocage assembly to occur, fusions are made so that the antibody two-fold axis and the symmetry axis of the homo-oligomer intersect at precise angles at the center of the architecture (Fig. 1d). To
 5 generate D2 dihedral, T32 tetrahedral, O32 or O42 octahedral, and I32 or I52 icosahedral nanocages, the required respective intersection angles are 90.0°, 54.7°, 35.3°, 45.0°, 20.9°, and 31.7°. We allowed angular and distance deviations from the ideal architecture of at most 5.7° and 0.5 Å, respectively (see Methods). Candidate fusion models were further filtered
 10 based on the number of contacts around the fusion junction (to gauge structural rigidity) and clashes between backbone atoms. Next, the amino acid identities and conformations around the newly formed building block junction were optimized using the SymPackRotamersMover in RosettaTM to maintain the rigid fusion geometry required for assembly (Fig. 1e). Following sequence design, we selected for experimental characterization six D2 dihedral, eleven T32 tetrahedral, four O32 octahedral, two O42 octahedral, fourteen I32 icosahedral, and eleven
 15 I52 icosahedral designs predicted to form AbCs (Fig. 1f).

Structural characterization

Synthetic genes encoding designed protein sequences appended with a C-terminal 6×histidine tag were expressed in *E. coli*. Designs were purified from clarified lysates using
 20 immobilized metal affinity chromatography (IMAC), and size exclusion chromatography (SEC) was used as a final purification step. Across all geometries, 34 out of 48 AbC-forming designs had a peak on SEC that roughly corresponded to the expected size of the design model. Designs were then combined with human IgG1 Fc, and the assemblies were re-purified via SEC. Eight of these AbC-forming designs assembled with Fc into a species that
 25 eluted as a monodisperse peak at a volume consistent with the target nanoparticle molecular weight (Figure 2a-b; 3 D2 dihedral, 2 T32 tetrahedral, 1 O42 octahedral, and 2 I52 icosahedral AbCs). For the i52.6 design, adding 100 mM L-arginine to the assembly buffer prevented aggregation after combining with Fc; all other designs readily self-assembled in Tris-buffered saline. Most other designs still bound Fc, as evidenced by SEC, native gels, or
 30 by visibly precipitating with Fc after combination, but did not form monodisperse nanoparticles by SEC (Table 6), perhaps because of deviations from the target fusion geometry.

Geometry	# ordered	Soluble component	Good SEC component	Forms cage with Fc
D2 dihedron	6	5	4	3
T32 tetrahedron	11	8	7	2
O32 octahedron	4	3	3	0
O42 octahedron	2	1	1	1
I32 icosahedron	14	14	10	0
I52 icosahedron	11	11	10	2
Total	48	42	35	8

Table 6. Success rates of designed antibody-binding cage-forming oligomers.

Solubility (column 2) refers to the presence of protein in the post-lysis, post-centrifugation, pre-IMAC soluble fraction as read out by SDS gel. Good SEC component (column 3) refers to SEC traces with some peak corresponding to the approximate predicted size of the nanocage-forming design model. Data for cage formation with Fc are shown in Fig. 2 and 3.

NS-EM micrographs and two-dimensional class averages revealed nanocages with shapes and sizes corresponding to the design models (Fig. 2c). AbCs also formed when assembled with intact antibodies (IgG with Fc and Fab domains), again generating monodisperse nanocages as shown by SEC and NS-EM (Fig. 2d-e). There is considerably more evidence of flexibility in the electron micrographs of the IgG-AbCs than the Fc-AbCs, as expected given the flexibility of the Fc-Fab hinge. In all cases, 2D class averages collected from the NS-EM data of AbCs made with intact IgG were still able to resolve density corresponding to the non-flexible portion of the assembly (Fig. 2e).

Single-particle NS-EM and cryo-EM reconstructed 3D maps of the AbCs formed with Fc are in close agreement with the computational design models (Fig. 3). Negative-stain EM reconstructions for the dihedral (d2.3, d2.4, d2.7), tetrahedral (t32.4, t32.8), and one of the icosahedral (i52.6) nanocages clearly show dimeric “U”-shaped Fcs and longer designed protein regions that fit together as computationally predicted. A single-particle cryo-EM reconstruction for the o42.1 design has clear density for the six designed tetramers sitting at the C4 vertices, which twist along the edges of the octahedral architecture to bind twelve dimeric Fcs, leaving the eight C3 faces unoccupied. Cryo-EM density for i52.3 with Fc likewise recapitulates the 20-faced shape of a regular icosahedron, with 12 designed pentamers protruding outwards at the C5 vertices (due to the longer length of the C5 building block compared to the monomer or Fc-binder), binding to 30 dimeric Fcs at the center of the edge, with 20 unoccupied C3 faces. In all cases, the computationally designed models fit clearly into the EM densities

Enhancing cell signaling with AbCs

The designed AbCs provide a general platform for investigating the effect of associating cell surface receptors into clusters on signaling pathway activation. Binding of
 5 antibodies to cell surface receptors can result in antagonism of signaling as engagement of the natural ligand is blocked (25). While in some cases receptor clustering has been shown to result in activation (11, 26, 27), there have been no systematic approaches to varying the valency and geometry of receptor engagement that can be readily applied to many different signaling pathways. We took advantage of the fact that almost any receptor-binding antibody,
 10 of which there are many, can be readily assembled into a wide array of different architectures using our AbC-forming designs to investigate the effect of receptor clustering on signaling. We assembled antibodies and Fc-fusions targeting a variety of signaling pathways into nanoparticles and investigated their effects as described in the following paragraphs.

15 Designed Tie2 agonists for the treatment of sepsis and ARDS

This work focuses on the use of antibody fragment crystallizable (Fc)-fusions to angiopoietin-1 F-domain to enhance angiogenesis signaling.

Tie-2 pathway activation by Fc-Angiopoietin 1 nanocages

20 Certain receptor tyrosine kinases (RTKs), such as the Angiopoietin-1 receptor (Tie2), activate downstream signaling cascades when clustered (31, 32). Scaffolding the F-domain from angiopoietin-1 (A1F) onto nanoparticles induces phosphorylation of AKT and ERK, enhances cell migration and tube formation in vitro, and improves wound healing after injury in vivo (32). Therapeutics with these activities could be useful in treating conditions
 25 characterized by cell death and inflammation, such as sepsis and acute respiratory distress syndrome (ARDS). To determine whether the AbC platform could be used to generate such agonists, we assembled o42.1 and i52.3 AbCs with Fc fusions to A1F (Fig. 4g, Fig. 4a-b). The octahedral and icosahedral A1F-AbCs, but not Fc-only controls or free Fc-Ang1F, significantly increased AKT and ERK1/2 phosphorylation above baseline (Fig. 4h-i) and
 30 enhanced cell migration and vascular stability (Fig. 4j-k, Fig. 8c-d). These results show that the AbCs are more potent inducers of angiogenesis than free A1F-Fc, and as the components can be readily produced in large quantities, they are promising therapeutic candidates.

TNFR superfamily agonism by antibody cages

The designed AbCs provide a general platform for investigating the effect of associating cell surface receptors into clusters on signaling pathway activation. Binding of antibodies to cell surface receptors can result in antagonism of signaling as engagement of the natural ligand is blocked (6). There have been no systematic approaches to varying the valency and geometry of receptor engagement that can be readily applied to many different signaling pathways. We took advantage of the fact that almost any receptor-binding antibody, of which there are many, can be readily assembled into a wide array of different architectures using our AbC-forming designs to investigate the effect of receptor clustering on signaling. This work focuses on the use of antibodies targeting two tumor necrosis family receptor superfamily members: Death Receptor 5 (DR5) and CD40.

Induction of tumor cell apoptosis by α -DR5 nanocages

Death Receptor 5 (DR5) is a tumor necrosis factor receptor (TNFR) superfamily cell surface protein that initiates a caspase-mediated apoptotic signaling cascade terminating in cell death when cross-linked by its trimeric native ligand, TNF-related apoptosis-inducing ligand (TRAIL) (9, 10, 27–30). Like other members of the family, DR5 can also form alternative signaling complexes that activate non-apoptotic signaling pathways such as the NF- κ B pro-inflammatory pathway and pathways promoting proliferation and migration upon ligand binding (29). Because DR5 is overexpressed in some tumors, multiple therapeutic candidates have been developed to activate DR5, such as α -DR5 mAbs and recombinant TRAIL, but these have failed clinical trials due to low efficacy and the development of TRAIL resistance in tumor cell populations (29, 30). Combining trimeric TRAIL with bivalent α -DR5 IgG leads to a much stronger apoptotic response than either component by itself, likely due to induction of larger-scale DR5 clustering via the formation of two-dimensional arrays on the cell surface (27).

We investigated whether α -DR5 AbCs formed with the same IgG (conatumumab) could have a similar anti-tumor effect without the formation of unbounded arrays. Five designs across four geometries were chosen (d2.4, t32.4, t32.8, o42.1, and i52.3) to represent the range of valencies and shapes (Fig. 4a). All α -DR5 AbCs were found to form single peaks on SEC and yielded corresponding NS-EM micrographs that were consistent with the formation of assembled particles (Fig. 2d-e).

All five α -DR5 AbCs caused caspase 3/7-mediated apoptosis at similar levels to TRAIL in a colorectal tumor cell line, whereas the antibody alone or AbCs formed with bare Fe did not lead to caspase-3/7 activity or cell death, even at the highest concentrations tested (Fig. 4a, 7a-b). On the TRAIL-resistant renal cell carcinoma line RCC4, we found that all α -DR5 AbCs induced caspase-3,7 activity (Fig. 4b) and designs t32.4, t32.8, and o42.1 greatly reduced cell viability at 150 nM concentration (Fig. 4c). Designs t32.4 and o42.1 activated caspase at 100-fold lower concentrations (1.5 nM), and prolonged treatment of RCC4 with α -DR5 AbCs t32.4 and o42.1 at 150 nM resulted in the killing of nearly all cells after six days, suggesting that RCC4 cells do not acquire resistance to the nanocages (Fig. 4e). Free α -DR5 antibody, TRAIL, (Fig. 4b-c) or Fe-only AbCs (Fig. 4d, 7c) did not activate caspase or lead to cell death in RCC4 tumor cell lines.

We next investigated the downstream pathways activated by the α -DR5 AbCs by analyzing their effects on cleaved PARP, a measure of apoptotic activity, as well as the NF- κ B target cFLIP. Consistent with the caspase and cell viability data, o42.1 α -DR5 AbCs increased cleaved PARP, while free α -DR5 antibody, TRAIL or o42.1 Fe AbCs did not result in an increase in cleaved PARP over baseline (Fig. 4f-g). All other α -DR5 AbCs were also found to increase cleaved PARP (Fig. 7f). These results suggest that α -DR5 AbCs may overcome TRAIL resistance by inhibiting anti-apoptotic pathways, which enhances the apoptotic cascade induced by DR5 super-clustering. Finally, the α -DR5 AbCs did not induce apoptosis in healthy primary kidney tubular cells (Fig. 7d-e).

α -CD40 nanocages activate CD40-expressing CHO Cells

CD40, a TNFR superfamily member expressed on antigen presenting dendritic cells and B cells, is cross-linked by trimeric CD40 ligand (CD40L or CD154) on T cells, leading to signaling and cell proliferation (33, 34). We investigated whether assembling a non-agonist α -CD40 antibody (LOB7/6) into nanocages could substitute for the need for cell surface presentation. Octahedral AbCs were assembled with LOB7/6 IgG; SEC, dynamic light scattering (DLS), and NS-EM (Fig. 5a-d) characterization showed these to be monodisperse with the expected octahedral shape. The octahedral α -CD40 LOB7/6 AbCs were found to induce robust CD40 activation in CD40-expressing reporter CHO cells (J215A, Promega), at concentrations hundredfold less than a control activating α -CD40 antibody (Promega), while no activation was observed for the free LOB7/6 antibody or octahedral AbC formed with

non-CD40 binding IgG (Fig. 5e, Table 7). This demonstrates that nanocage assembly converts the non-agonist α -CD40 mAb into a CD40 pathway agonist.

	EC50 log(μ M)	95% CI log(μ M)
α 42.1 IgG control	-1.422	Not found
α -CD40	1.466	1.247 to 1.833
LOB7/6	-1.471	Not found
α 42.1 LOB7/6	0.1134	-0.001058 to 0.2037

5

Table 7. EC50s from CD40 activation experiments. EC50 values were interpolated from the response curves determined using the log(agonist) vs. response -- Variable slope (four parameters) fit using Graphpad PrismTM Software.

Ab reactivity	Ab subclass	Designs (validated by SEC at minimum)	Comments
α -CD4	mIgG2b	α 42.1	OKT4
α -CD40	mIgG2a or mIgG2b	α 42.1	LOB7/6 or 82111 (respectively)
α -DR5 (human)	hIgG1	d2.3, d2.4, d2.7, t32.4, t32.8, α 42.1, i52.3, i52.6	conatumumab
α -DR5 (mouse)	Armenian hamster IgG	t32.4, α 42.1	MD5-1
α -EGFR	hIgG1	mIgG2b	cetuximab
α -LRP6	hIgG1	t32.4, α 42.1	YW210.09
α -RSV F	hIgG1	d2.3, d2.4, d2.7, t32.4, t32.8, α 42.1, i52.3, i52.6	mpe8
Non-specific	Rabbit IgG	d2.4, α 42.1	Rabbit serum IgG

10

Table 8. List of antibodies formed into cages as verified by at minimum size exclusion chromatography. Successfully formed cages (by SEC) listed by the antibody target reactivity, antibody species and isotype, and designs used.

Fc-fusion ligand	Fc subclass	Designs (validated by SEC at minimum)	Comments
Angiopoietin-1 F-domain	hIgG1	d2.4, t32.4, t32.8, α 42.1, i52.3	
Angiotensin-converting enzyme 2 (ACE2)	hIgG1	α 42.1	

CD80	hIgG1	o42.1	
mRuby2	hIgG1	d2.4, t32.4, t32.8, o42.1, i52.3	
sfGFP	hIgG1	d2.4, t32.4, t32.8, o42.1, i52.3	
VEGF-a	hIgG1	t32.4, o42.1	
VEGF-c	hIgG1	t32.4, o42.1	

Table 9. List of Fc-fusions formed into cages as verified by at minimum size exclusion chromatography. Successfully formed cages (by SEC) listed by the ligand that was fused to Fc, the Fc sequence species and isotype, and designs used

Discussion

Our approach goes beyond previous computational design efforts to create functional nanomaterials by integrating form and function; our AbCs employ antibodies as both structural and functional components. By fashioning designed antibody-binding, cage-forming oligomers through rigid helical fusion, a wide range of geometries and orientations can be achieved. This design strategy can be generalized to incorporate other homo-oligomers of interest into cage-like architectures. For example, nanocages could be assembled with viral glycoprotein antigens using components terminating in helical antigen-binding proteins, or from symmetric enzymes with exposed helices available for fusion to maximize proximity of active sites working on successive reactions. The AbCs offer considerable advantages in modularity compared to previous fusion of functional domain approaches; any of the thousands of known antibodies with sufficient protein A binding can be simply mixed with the appropriate design to drive formation of the desired symmetric assembly, and we have demonstrated this principle using multiple different IgGs and Fc-fusions (Tables 8-9). EM and SEC demonstrate monodispersity comparable to IgM and not (to our knowledge) attained by any other antibody-protein nanoparticle formulations.

AbCs show considerable promise as signaling pathway agonists. Assembly of antibodies against RTK- and TNFR-family cell-surface receptors into AbCs led to activation of diverse downstream signaling pathways involved in cell death, proliferation, and differentiation. While antibody-mediated clustering has been previously found to activate signaling pathways (11, 27, 33), our approach has the advantage of much higher structural homogeneity, allowing more precise tuning of phenotypic effects and more controlled formulation. AbCs also enhanced antibody-mediated viral neutralization. There are exciting applications to targeted delivery, as the icosahedral AbCs have substantial internal volume (around 15,000 nm³, based on an estimated interior radius of 15.5 nm) that could be used to

package nucleic acid or protein cargo, and achieving different target specificity in principle is as simple as swapping one antibody for another. We anticipate that the AbCs developed here, coupled with the very large repertoire of existing antibodies, will be broadly useful across a wide range of applications in biomedicine.

5

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25 Materials and Methods

Computational design and testing of Fc-binder helical repeat protein (DHR79-FcB)

- The crystal structure of the B-domain from *S. aureus* protein A in complex with Fc fragment (PDB ID: 1L6X) was relaxed with structure factors using Phenix Rosetta™ (39, 40). Briefly, the RosettaScripts™ MotifGraft mover was used to assess suitable solutions to insertions of the protein A binding motif extracted from 1L6X into a previously reported designed helical repeat protein (DHR79) (17). Specifically, a minimal protein A binding motif was manually defined and extracted and used as a template for full backbone alignment of DHR79 while retaining user-specified hotspot residues that interact with the Fc domain in the crystal structure at the Fc/DHR interface and retaining native DHR residues in all other

positions. The MotifGraft alignment was followed by 5 iterations of FastDesign and 5 iterations of FastRelax in which the DHR side chain and backbone rotamers were allowed to move while the Fc context was completely fixed. The best designs were selected based on a list of heuristic filter values. See supplementary materials for the full XML file used during design. Fig 61a shows the design model of DHR79-FcB.

Designs were initially assessed via yeast surface display binding to biotinylated Fc protein. Upon confirmation of a qualitative binding signal, the design was closed into a pET29b expression vector with a C-terminal His-tag. The protein was expressed in BL21 DE3 in autoinduction medium (10 mL 50×M, 10 mL 50x5052, 480 mL almost TB, 1× chloramphenicol, 1× kanamycin) for 20 hours at 27°C at 225 rpm (41). Cells were resuspended in lysis buffer (20mM Tris, 300mM NaCl, 30mM imidazole, 1mM PMSF, 5% glycerol (v/v), pH 8.0) and lysed using a microfluidizer at 18000 PSI. Soluble fractions were separated via centrifugation at 24,000×g. IMAC with Ni-NTA batch resin was used for initial purification; briefly, nickel-nitrilotriacetic acid (Ni-NTA) resin was equilibrated with binding buffer (20mM Tris, 300mM NaCl, 30mM imidazole, pH 8.0), soluble lysate was poured over the columns, columns were washed with 20 column volumes (CVs) of binding buffer, and eluted with 5 CVs of elution buffer (20mM Tris, 300mM NaCl, 500mM imidazole, pH 8.0). Size exclusion chromatography (SEC) with a Superdex 200 column was used as the polishing step (Fig 6b). SEC buffer was 20 mM Tris/HCl pH 7.4, 150 mM NaCl.

Affinity of DHR79-FcB to biotinylated IgG1 and biotinylated Fc protein was assessed using Octet™ Biolayer Interferometry (BLI). DHR79-FcB exhibits a 71.7 nM affinity to IgG1 (full antibody) and a 113 nM affinity to the IgG1 Fc protein (Fig 6c).

Computational Design of Antibody Nanocages

Input pdb files were compiled to use as building blocks for the generation of antibody cages. For the protein A binder model, the Domain D from *Staphylococcus aureus* Protein A (PDB ID 1DEE) was aligned to the B-domain of protein A bound to Fc (PDB ID 1L6X) (16, 39). The other Fc-binding design structure, where protein A was grafted onto a helical repeat protein, was also modeled with Fc from 1L6X. PDB file models for monomeric helical repeat protein linkers (42) and cyclic oligomers (2 C2s, 3 C3s, 1 C4, and 2 C5s) that had at least been validated via SAXS were compiled from previous work from our lab (17–19). Building block models were manually inspected to determine which amino acids were suitable for making fusions without disrupting existing protein-protein interfaces.

These building blocks were used as inputs, along with the specified geometry and fusion orientation, into the alpha helical fusion software (Supplementary Text for a description on how to operate WORMS) (20, 21). Fusions were made by overlapping helical segments at all possible allowed amino acid sites. Fusions are then evaluated for deviation for which the cyclic symmetry axes intersect according to the geometric criteria: D2, T32, O32, O42, I32, and I52 intersection angles are 45.0°, 54.7°, 35.3°, 45.0°, 20.9°, and 31.7°, respectively (22) with angular and distance tolerances of at most 5.7° and 0.5 Å respectively. Post-fusion .pdb files were manually filtered to ensure that the N-termini of the Fc domains are facing outwards from the cage, so that the Fabs of an IgG would be external to the cage surface. Sequence design was performed using Rosetta™ symmetric sequence design (SymPackRotamersMover in RosettaScripts™) on residues at and around the fusion junctions (42), with a focus on maintaining as many of the native residues as possible. Residues were redesigned if they clashed with other residues, or if their chemical environment was changed after fusion (e.g. previously-core facing residues were now solvent-exposed). Index residue selectors were used to prevent design at Fc residue positions.

Structural characterization of antibody nanocages

Genes were codon optimized for bacterial expression of each designed antibody-nanocage forming oligomers, with a C-terminal glycine/serine linker and 6× C-terminal histidine tag appended. Synthetic genes were cloned into pct29b+ vectors between NdeI and XhoI restriction sites; the plasmid contains a kanamycin-resistant gene and T7 promoter for protein expression. Plasmids were transformed into chemically competent Lemo21(DE3) *E. coli* bacteria using a 15-second heat shock procedure as described by the manufacturer (New England Biolabs). Transformed cells were added to auto-induction expression media, as described above, and incubated for 16 hours at 37°C and 200 rpm shaking (41). Cells were pelleted by centrifugation at 4000×g and resuspended in lysis buffer (150 mM NaCl, 25 mM Tris-HCl, pH 8.0, added protease inhibitor and DNase). Sonication was used to lyse the cells at 85% amplitude, with 15 second on/off cycles for a total of 2 minutes of sonication time. Soluble material was separated by centrifugation at 16000×g. IMAC was used to separate out the His-tagged protein in the soluble fraction as described above. IMAC elutions were concentrated to approximately 1 mL using 10K MWCO spin concentrators, filtered through a 0.22 µm spin filter, and run over SEC as a final polishing step (SEC running buffer: 150 mM NaCl, 25 mM Tris-HCl, pH 8.0).

Designs that produced monodisperse SEC peaks around their expected retention volume were combined with Fc from human IgG1. Fc was produced recombinantly either using standard methods for expression in HEK293T cells or in *E. coli* (43). Cage components were incubated at 4°C for at minimum 30 minutes. 100 mM L-arginine was added during the assembly to AbCs formed with the i52.6 design, as this was observed to maximize the formation of the designed AbC i52.6 and minimize the formation of visible “crashed out” aggregates (23). Fc-binding and cage formation were confirmed via SEC; earlier shifts in retention time (compared to either component run alone) show the formation of a larger structure. NS-EM was used as previously described to confirm the structures of designs that passed these steps.

For confirming AbC structures with intact IgGs, human IgG1 (hIgG1) was combined with AbC-forming designs following the same protocol for making Fc cages. This assembly procedure was also followed for all IgG or Fc-fusion AbCs reported hereafter. The data in Figure 2d-e shows AbCs formed with the α -DR5 antibody AMG-655 (23) for the following designs: d2.3, d2.4, d2.7, t32.4, o42.1, and i52.3. The data for t32.8 and i52.6 designs shown in Figure 2d-e is from AbCs formed with the hIgG1 antibody mpe8 (44). Tables 8 and 9 show the list of IgGs and Fc fusions that have been formed into AbCs.

Dynamic light scattering measurements (DLS) were performed using the default Sizing and Polydispersity method on the UNcle™ (Unchained Labs). 8.8 μ L of AbCs were pipetted into the provided glass cuvettes. DLS measurements were run in triplicate at 25°C with an incubation time of 1 second; results were averaged across runs and plotted using Graphpad Prism. The estimated hydrodynamic diameter is listed next to all DLS peaks shown below.

NS-EM analysis of Fc and IgG AbCs

For all samples except o42.1 Fc and i52.3 Fc, 3.0 μ L of each SEC-purified sample between 0.008- 0.014 mg/mL in TBS pH 8.0 was applied onto a 400-mesh or 200-mesh Cu grid glow-discharged carbon-coated copper grids for 20 seconds, followed by 2 $\times$ application of 3.0 μ L 2% nano-W stain. Micrographs were recorded using Legikon software on a 120kV FEI Tecnai G2 Spirit™ with a Gatan Ultrascan™ 4000 4k $\times$ 4k CCD camera at 67,000 nominal magnification (pixel size 1.6 Å/pixel) or 52,000 nominal magnification (pixel size 2.07 Å) at a defocus range of 1.5 – 2.5 μ m. Particles were picked either with DoGPicker or cisTEM; both are reference-free pickers. Contrast-transfer function was estimated using GCTF or cisTEM. 2D class averages were generated in cryoSPARC or in cisTEM.

Reference-free *ab initio* 3D reconstruction of selected 2D class averages from each dataset was performed in cryoSPARC or in cisTEM (Table 10).

Sample name	Stain	Voltage (kV)	Magnification	Pixel size (Å/pixel)	Particle picking	CTF estimation	2D class averages	3D reconstruction
d2.3 Fe	UF	120	67,000	1.6	cisTEM	cisTEM	cisTEM	cisTEM
d2.4 Fe	UF	120	67,000	1.6	DoG picker	GCTF	cryoSPARC	cryoSPARC
d2.7 Fe	nano-W	120	67,000	1.6	cisTEM	cisTEM	cisTEM	cisTEM
i32.4 Fe	nano-W	120	67,000	1.6	cisTEM	cisTEM	cisTEM	cisTEM
i32.8 Fe	nano-W	120	67,000	1.6	cisTEM	cisTEM	cisTEM	cisTEM
o42.1 Fe	cryo	200	36,000	1.16	Manual picking	GCTF	cryoSPARC	cryoSPARC
i52.3 Fe	cryo	200	36,000	1.16	Manual picking	GCTF	cryoSPARC	cryoSPARC
i52.6 Fe	nano-W	120	52,000	2.07	cisTEM	cisTEM	cisTEM	cisTEM
d2.3 hlgG	UF	120	67,000	1.6	cisTEM	cisTEM	cisTEM	N/A
d2.4 hlgG	UF	120	67,000	1.6	cisTEM	cisTEM	cisTEM	N/A
d2.7 hlgG	nano-W	120	67,000	1.6	cisTEM	cisTEM	cisTEM	N/A
i32.4 hlgG	nano-W	120	67,000	1.6	cisTEM	cisTEM	cisTEM	N/A
i32.8 hlgG	nano-W	120	67,000	1.6	cisTEM	cisTEM	cisTEM	N/A
o42.1 hlgG	UF	120	67,000	1.6	DoG picker	GCTF	cryoSPARC	N/A
i52.3 hlgG	nano-W	120	52,000	2.07	cisTEM	cisTEM	cisTEM	N/A
i52.6 hlgG	nano-W	120	52,000	2.07	cisTEM	cisTEM	cryoSPARC	N/A
D3-08 Fe	nano-W	120	57,000	2.52	cisTEM	cisTEM	cisTEM	cisTEM
D3-36 Fe	nano-W	120	57,000	2.52	cisTEM	cisTEM	cisTEM	cisTEM

5 **Table 10.** Details on data acquisition and data processing of different nanocages samples.

Cryo-EM analysis of o42.1 and i52.3 AbCs

3.0 μ L of i52.3 Fe sample at 0.8 mg/mL in TBS pH 8.0 with 100mM Arginine was applied onto C-flat 1.2 μ m glow-discharged copper grids. Grids were then plunge-frozen in liquid ethane, cooled with liquid nitrogen using and FEI MK4 Vitrobot with a 6 second blotting time and 0 force. The blotting process took place inside the Vitrobot chamber at 20°C and 100% humidity. Data acquisition was performed with the Leginon data collection software on an FEI Talos electron microscope at 200kV and a Gatan K2 Summit camera. The nominal magnification was 36,000 $\times$ with a pixel size of 1.16 Å/pixel. The dose rate was adjusted to 8 counts/pixel/s. Each movie was acquired in counting mode fractionated in 50 frames of 200 ms/frame. Frame alignment was performed with MotionCorr2. Particles were manually picked within the Appion interface. Defocus parameters were estimated with

GCTF. Reference-free 2D classification with cryoSPARC was used to select a subset of particles for *Ab-Initio* 3D reconstruction function in cryoSPARC.

A summary of data acquisition and processing is provided in Table 11.

5 DR5 and A1F-Fc experiments

Cell culture

Colorectal adenocarcinoma cell line-Colo205, and renal cell carcinoma cell line RCC4 were obtained from ATCC. Primary kidney tubular epithelial cells RAM009 were a gift from Dr. Akilesh (University of Washington). Colo205 cells were grown in RPMI1640 medium with 10% Fetal Bovine Serum (FBS) and penicillin/streptomycin. RCC4 cells were grown in Dulbecco's Modified Eagle's Medium with 10% FBS and penicillin/streptomycin. RAM009 were grown in RPMI with 10% FBS, ITS-supplement, penicillin/streptomycin and Non Essential Amino Acids (NEAA). All cell lines were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

Human Umbilical Vein Endothelial Cells (HUVECs, Lonza, Germany, catalog # C2519AS) were grown on 0.1% gelatin-coated 35 mm cell culture dish in EGM2 media. Briefly, EGM2 consist of 20% Fetal Bovine Serum, 1% penicillin-streptomycin, 1% Glutamax (Gibco, catalog #35050061), 1% endothelial cell growth factor (31), 1mM sodium pyruvate, 7.5mM HEPES, 0.08mg/mL heparin, 0.01% amphotericin B, a mixture of 1× RPMI 1640 with and without glucose to reach 5.6 mM glucose concentration in the final volume. Media was filtered through a 0.45-micrometer filter. HUVECs at passage 7 were utilized in Tie2 signaling and cell migration experiments. HUVECs at passage 6 were used in tube formation assay.

25 Caspase 3/7 Glo assay

Cells were passaged using trypsin and 20,000 cells/well were plated onto a 96-well white tissue culture plate and grown in appropriate media. Medium was changed the next day (100 µL/well) and cells were treated with either uncaged α-DR5 AMG655 antibody (150nM), recombinant human TNF Related Apoptosis Inducing Ligand (rhTRAIL; 150nM), Fc-only AbCs or α-DR5 AbCs (150nM, 1.5nM, 15pM) and incubated at 37°C for 24 hours. The following day 100 µL/well of caspase GLOTM reagent (Promega, USA) was added on top of the media and incubated for 2 hours at 37°C. Luminescence was then recorded using Perkin EnVision microplate reader (Perkin Elmer). Statistical comparisons were performed using Graphpad PrismTM (see Table 11 for full detail).

Experiment (Fig.)	Condition	Concentration	n	Test	Mean compared to	α	Summary	Adjusted P value
Caspase-3,7 RCC4 (4b)	PBS	15 pM	6	2way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
		1.5 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9996
		150 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9994
	TRAHL	15 pM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9996
		1.5 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9996
		150 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9668
	α -DR5	15 pM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9997
		1.5 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	>0.9999
		150 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.2005
	d2.4 α -DR5	15 pM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9995
		1.5 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9988
		150 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
	132.4 α -DR5	15 pM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9998
		1.5 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
		150 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
	132.8 α -DR5	15 pM	3	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.984
		1.5 nM	3	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.6177

		150 nM	3	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
	o42.1 α -DR5	15 pM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9991
		1.5 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
		150 nM	6	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
	i52.3 α -DR5	15 pM	3	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9442
		1.5 nM	3	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.8227
		150 nM	3	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
Viability 4d RCC4 (4c)	PBS	150 nM	3	1way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
	TRAIL	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.5207
	α -DR5	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9996
	d2.4 α -DR5	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	*	0.0238
	i32.4 α -DR5	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
	i32.8 α -DR5	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
	o42.1 α -DR5	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
	i52.3 α -DR5	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	**	0.0079
Viability 4d RCC4 Fe cages (4d)	PBS	150 nM	3	1way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
	d2.4 Fe	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.7157
	i32.4 Fe	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9976
	i32.8 Fe	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.8556

	042.1 Fc	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.2309
	152.3 Fc	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9302
Viability 6d RCC4 (4e)	PBS	150 nM	6	1way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
	TRAIL	150 nM	6	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9996
	α -DR5	150 nM	6	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	>0.9999
	132.4 Fc	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9591
	042.1 Fc	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9593
	132.4 α -DR5	150 nM	6	1way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
	042.1 α -DR5	150 nM	6	1way ANOVA with post-hoc Dunnett	PBS	0.05	***	0.0001
c-PARP quant. (4g)	PBS	150 nM	4	1way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
	TRAIL	150 nM	4	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.0845
	α -DR5	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.4746
	042.1 Fc	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9979
	042.1 α -DR5	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
Caspase-3,7 Colo205	PBS	15 pM	2	2way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
		1.5 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	>0.9999
		150 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	>0.9999
	TRAIL	15 pM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	>0.9999
		1.5 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	***	0.0006

	150 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
α -DR5	15 pM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9997
	1.5 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	>0.9999
	150 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9996
d2.4 α -DR5	15 pM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.9996
	1.5 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
	150 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
i32.4 α -DR5	15 pM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.1074
	1.5 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
	150 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
i32.8 α -DR5	15 pM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	>0.9999
	1.5 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
	150 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
o42.1 α -DR5	15 pM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	0.6538
	1.5 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
	150 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
i52.3 α -DR5	15 pM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	ns	>0.9999
	1.5 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001
	150 nM	2	2way ANOVA with post-hoc Dunnett	15 pM PBS	0.05	****	<0.0001

Caspase-3,7 RCC4 Fe	PBS	150 nM	3	1way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
	d2.4 Fe	150 nM	6	1way ANOVA with post-hoc Dunnett	PBS	0.05	*	0.0129
	t32.4 Fe	150 nM	6	1way ANOVA with post-hoc Dunnett	PBS	0.05	*	0.046
	t32.8 Fe	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9198
	o42.1 Fe	150 nM	6	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.2112
	i52.3 Fe	150 nM	3	1way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9996
RAM009 Caspase-3,7	PBS	1.5 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	N/A	N/A
		150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.3848
	TRAIL	1.5 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9726
		150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.0525
	α -DR5	1.5 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9566
		150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.2752
	d2.4 α -DR5	1.5 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9677
		150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	**	0.0076
	t32.4 α -DR5	1.5 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9703
		150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	**	0.0028
	t32.8 α -DR5	1.5 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9996
		150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	**	0.0067
	o42.1 α -DR5	1.5 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9991

		150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	**	0.0038
	i52.3 α -DR5	1.5 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9994
		150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	***	0.0006
	d2.4 Fe	150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9966
	t32.4 Fe	150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9997
	t32.8 Fe	150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9992
	o42.1 Fe	150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9994
	i52.3 Fe	150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9995
RAM009 Viability	PBS	150 nM	6	2way ANOVA with post-hoc Dunnett	PBS	0.05		
	TRAIL	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9901
	α -DR5	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9995
	d2.4 α -DR5	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9996
	t32.4 α -DR5	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9212
	t32.8 α -DR5	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.7875
	o42.1 α -DR5	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.7485
	i52.3 α -DR5	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.1419
	d2.4 Fe	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9996
	t32.4 Fe	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9718
	t32.8 Fe	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.999

	α 42.1 Fe	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9913
	α 52.3 Fe	150 nM	3	2way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9837

Table 11. Statistical information for DR5 experiments.

5 Titer Glo cell viability assay (4 day viability)

Cells were plated onto a 96-well plate at 20,000 cells/well. The next day, cells were treated with 150nM of α -DR5 AbCs, rhTRAIL and α -DR5 antibody for 4 days. At day 4, 100 μ L of CellTiter-Glo reagent (Promega Corp. USA, #G7570) was added to the 100 μ L of media per well, incubated for 10 min at 37°C and luminescence was measured using a

10 Perkin-Elmer Envision plate reader.

Alamar Blue cell viability assay (6 day viability)

Cells were seeded onto a 12-well tissue culture plate at 50,000 cells/well. The next day, cells were treated with α -DR5 AbCs, rhTRAIL, or α -DR5 antibodies at 150 nM concentration. Three days later, cells were passaged at 30,000 cells/well and treated with 150 nM of α -DR5 cages, rhTRAIL and α -DR5 antibody for 3 days. At 6 days, the media was replaced with 450 μ L/well of fresh media and 50 μ L of AlamarTM blue reagent (ThermoFisher Scientific, USA, #DAL1025) was then added. After 4 hours of incubation at 37°C, 50 μ L of media was transferred into a 96-well opaque white plate and fluorescence intensity was

15 measured using plate reader according to manufacturer's instructions.

20

Protein analysis

Cells were passaged onto a 12-well plate at 40,000 cells/well and were grown until 80% confluency is reached. Before treatment, the media was replaced with 500 μ L of fresh media. For DR5 experiments, AMG-655 antibody and rhTRAIL were added at 150nM concentration and Fe-only nanocages or α -DR5 nanocages were added at 150nM, 1.5nM and 15pM concentration onto the media and incubated for 24 hours at 37°C prior to protein isolation.

Media containing dead cells was transferred to a 1.5ml Eppendorf tube, and the cells were gently rinsed with 1 $\times$ phosphate buffered saline. 1 $\times$ trypsin was added to the cells for 3

30

min. All the cells were collected into the 1.5mL Eppendorf containing the medium with dead cells. Cells were washed once in PBS 1× and lysed with 70 μL of lysis buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 15% Glycerol, 1% Triton, 3% SDS, 25 mM β-glycerophosphate, 50mM NaF, 10mM Sodium Pyrophosphate, 0.5% Orthovanadate, 1% PMSF (all chemicals were from Sigma-Aldrich, St. Louis, MO), 25 U Benzonase Nuclease (EMD Chemicals, Gibbstown, NJ), protease inhibitor cocktail (Pierce™ Protease Inhibitor Mini Tablets, Thermo Scientific, USA), and phosphatase inhibitor cocktail 2 (catalog#P5726), in their respective tubes. Total protein samples were then treated with 1 μL of Benzonase (Novagen, USA) and incubated at 37°C for 10 min. 21.6 μL of 4× Laemmli Sample buffer (Bio-Rad, USA) containing 10% beta-mercaptoethanol was added to the cell lysate and then heated at 95°C for 10 minutes. The boiled samples were either used for Western blot analysis or stored at -80°C.

Production of A1F-Fc

Synthetic genes were optimized for mammalian expression and subcloned into the CMV/R vector (VRC 8400; PMID:15994776). XbaI and AvrII restriction sites were used for insertion of A1F-Fc. Gene synthesis and cloning was performed by Genscript. Expi 293F cells were grown in suspension using Expi293 Expression Medium (Thermo Fisher Scientific) at 150 RPM, 5% CO₂, 70% humidity, 37°C. At confluency of $\sim 2.5 \times 10^6$ cells/mL, the cells were transfected with the vector encoding A1F-Fc (1000 μg per 1 L of cells) using PEI MAX (Polysciences) as a transfection reagent. Cells were incubated for 96 hours, after which they were spun down by centrifugation (4,000×g, 10 min, 4 °C) and the protein-containing supernatant was further clarified by vacuum-filtration (0.45 μm, Millipore Sigma). In preparation of nickel-affinity chromatography steps, 50 mM Tris, 350 mM NaCl, pH 8.0 was added to clarified supernatant. For each liter of supernatant, 4 mL of Ni Sepharose™ excel resin (GE) was added to the supernatant, followed by overnight shaking at 4 °C. After 16-24 hours, resin was collected and separated from the mixture and washed twice with 50 mM Tris, 500 mM NaCl, 30 mM imidazole, pH 8.0 prior to elution of desired protein with 50 mM Tris, 500 mM NaCl, 300 mM imidazole, pH 8.0. Eluates were purified by SEC using a Superdex™ 200 Increase column.

Western blotting

The protein samples were thawed and heated at 95 °C for 10 minutes. 10 μL of protein sample per well was loaded and separated on a 4-10% SDS-PAGE gel for 30 minutes

at 250 Volt. The proteins were then transferred onto a Nitrocellulose membrane for 12 minutes using the semi-dry turbo transfer western blot apparatus (Bio-Rad, USA). Post-transfer, the membrane was blocked in 5% nonfat dry milk for 1 hour. After 1 hour, the membrane was probed with the respective antibodies: cleaved-PARP (Cell Signaling, USA) at 1:2000 dilution; cFLIP (R&D systems, USA) at 1:1000 dilution; pERK1/2 (Cell Signaling) at 1:5000 dilution; pFAK (Cell Signaling) at 1:1000 dilution; p-AKT(S473) (Cell Signaling) at 1:2000 dilution; and actin (Cell Signaling, USA) at 1:10,000 dilution. Separately, for p-AKT(S473) the membrane was blocked in 5% BSA for 3 hours followed by primary antibody addition. Membranes with primary antibodies were incubated on a rocker at 4°C, overnight.

Next day, the membranes were washed with 1× TBST (3 times, 10 minutes interval) and the respective HRP-conjugated secondary antibody (Bio-Rad, USA) (1:10,000) was added and incubated at room temperature for 1 hour. For p-AKT(S473), following washes, the membrane was blocked in 5% milk at room temperature for 1 hour and then incubated in the respective HRP-conjugated secondary antibody (1:2000) prepared in 5% milk for 2 hours.

After secondary antibody incubation, all the membranes were washed with 1× TBST (3 times, 10 minutes interval) and developed using Luminol reagent and imaged using Bio-Rad ChemiDoc™ Imager. Data were quantified using the ImageJ™ software to analyze band intensity. Quantifications were done by calculating the peak area for each band. Each signal was normalized to the actin quantification from that lane of the same gel, to allow for cross-gel comparisons. Fold-changes were then calculated compared to PBS for all samples except for the pAKT reported for the A1F-Fc western blot (there was not enough pAKT signal for comparison, so o42.1 A1F-Fc was used for normalization). Statistical comparisons were performed using Graphpad Prism™ (see Tables 11, 12 for full detail).

25 Cell migration ("scratch") assay

Passage 7 HUVECs were seeded onto 35mm, 0.1% gelatin-coated plates and cultured in EGM-2. Once a monolayer of cells has been established, a scratch is made on the cell layer using a 200 µL pipette tip. Media is changed to DMEM Low glucose supplemented with 2% Fetal Bovine Serum. Scaffolds were added into the media at 18 nM A1F-Fc concentrations.

The imaging was performed in Leica Microscope at 10× magnification under phase contrast at 0 and 12 hours. The images are quantified using ImageJ software to calculate the level of cell migration as a ratio of change in wound area to initial wound area. Level of cell migration is normalized to PBS. Statistical comparisons were performed using Graphpad Prism (see Table 12 for full detail).

Experiment (Fig.)	Condition	n	Test	Mean compared to	α	Summary	Adjusted P value
pAKT (4j, 9c (H8))	PBS	13	1 way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
	A1F-Fc	3	1 way ANOVA with post-hoc Dunnett	PBS	0.05	ns	>0.9999
	α 42.1 Fc	4	1 way ANOVA with post-hoc Dunnett	PBS	0.05	ns	>0.9999
	i52.3 Fc	3	1 way ANOVA with post-hoc Dunnett	PBS	0.05	ns	>0.9999
	α 42.1 A1F-Fc	9	1 way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
	i52.3 A1F-Fc	8	1 way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
	H8-A1F	4	1 way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
pERK1-2 (4j, 9c (H8))	PBS	13	1 way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
	A1F-Fc	3	1 way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9997
	α 42.1 Fc	4	1 way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9957
	i52.3 Fc	3	1 way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9997
	α 42.1 A1F-Fc	9	1 way ANOVA with post-hoc Dunnett	PBS	0.05	**	0.0032
	i52.3 A1F-Fc	8	1 way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
	H8-A1F	6	1 way ANOVA with post-hoc Dunnett	PBS	0.05	*	0.0112
Vascular stability (4k, 9c (H8))	PBS	7	1 way ANOVA with post-hoc Dunnett	N/A	N/A	N/A	N/A
	A1F-Fc	6	1 way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.9932
	α 42.1 Fc	4	1 way ANOVA with post-hoc Dunnett	PBS	0.05	ns	>0.9999
	i52.3 Fc	3	1 way ANOVA with post-hoc Dunnett	PBS	0.05	ns	0.8699

	o42.1 A1F-Fc	6	1way ANOVA with post-hoc Dunnett	PBS	0.05	***	0.0006
	i52.3 A1F-Fc	5	1way ANOVA with post-hoc Dunnett	PBS	0.05	****	<0.0001
	H8-A1F	4	1way ANOVA with post-hoc Dunnett	PBS	0.05	*	0.0208
pAKT (HS exp.)	PBS	3	1way ANOVA with post-hoc Dunnett	o42.1 A1F-Fc	0.05	***	<0.0001
	o42.1 A1F-Fc	3	1way ANOVA with post-hoc Dunnett	o42.1 A1F-Fc	0.05	N/A	N/A
	10% HS	3	1way ANOVA with post-hoc Dunnett	o42.1 A1F-Fc	0.05	***	0.0002
	o42.1 A1F-Fc 10% HS 4°C	3	1way ANOVA with post-hoc Dunnett	o42.1 A1F-Fc	0.05	ns	0.9431
	o42.1 A1F-Fc 10% HS	3	1way ANOVA with post-hoc Dunnett	o42.1 A1F-Fc	0.05	ns	0.9998

Table 12. Statistical information for A1F-Fc experiments.

Tube formation assay (vascular stability)

Tube formation was done with modified protocol from Liang et al., 2007. Briefly, passage 6 HUVECs were seeded onto 24-well plates precoated with 150 μ L of 100% cold Matrigel™ (Corning, USA) at 150,000 cells/well density along with scaffolds at 89 nM A1F-Fc concentrations or PBS in low glucose DMEM medium supplemented with 0.5% FBS for 24 hours. At the 24 hour time point, old media is aspirated and replaced with fresh media without scaffolds. The cells continue to be incubated up to 72 hours. Cells were imaged at 48-hour and 72-hour time points using Leica Microscope at 10X magnification under phase contrast. Thereafter, the tubular formations were quantified by calculating the number of nodes, meshes and tubes using Angiogenesis Analyzer plugin in Image J software. Vascular stability is calculated by averaging the number of nodes, meshes, and tubes then normalizing to PBS. Statistical comparisons were performed using Graphpad Prism™ (see Table 12 for full detail).

Immune cell activation materials and methods

CD40 luminescence assay

A non-agonistic antibody (clone LOB7/6, product code MCA1590T, BioRad), was combined with the octahedral o42.1 AbC-forming design as described above and the AbCs

were characterized by DLS and NS-EM (Figure 5). Negative control α 42.1 AbC was made using a non-CD40 binding IgG (mpe8), which binds to RSV spike protein (45). These two AbCs, along with uncaged LOB7/6 and a positive control CD40-activating IgG (Promega, catalog #K118A) were diluted to make a 10-point, threefold dilution series for triplicate technical repeats starting at 1.2 μ M. The positive control CD40-activating IgG (K118A) is a murine IgG1a antibody, and so it was not compatible for assembly with the α 42.1 design, likely due to the low binding interface between protein A and mIgG1a (data not shown).

To assay CD40 activation, we followed manufacturer's instructions for a bioluminescent cell-based assay that measures the potency of CD40 response to external stimuli such as IgGs (Promega, JA2151). Briefly, CD40 effector Chinese Hamster Ovary (CHO) cells were cultured and reagents were prepared according to the assay protocol. The antibodies and AbCs were incubated with the CD40 effector CHO cells for 8 hours at 37°C, 5% CO₂. Bio-Glo™ Luciferase Assay System (G7941) included in the assay kit was used to visualize the activation of CD40 from luminescence readout from a plate reader. The Bio-Glo™ Reagent was applied to the cells and luminescence was detected by a Synergy Neo2 plate reader every min for 30 minutes. Data were analyzed by averaging luminescence between replicates and subtracting plate background. The fold induction of CD40-binding response was determined by RLU of sample normalized to RLU of no antibody controls. Data curves were plotted and EC50 was calculated using GraphPad Prism™ using the log(agonist) vs. response -- Variable slope (four parameters); see Table 7 for EC50 values and 95% CI values.

We claim

1. A particle, comprising:
 - (a) a plurality of polypeptide polymers, wherein
 - (i) each monomer in the polymers comprises an amino acid sequence at
5 least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:1;
 - (ii) each monomer in the polymers comprises an amino acid sequence at
least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:2;
 - 10 (iii) each monomer in the polymers comprises an amino acid sequence at
least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:3;
 - (iv) each monomer in the polymers comprises an amino acid sequence at
least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
15 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:4;
 - (v) each monomer in the polymers comprises an amino acid sequence at
least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5;
 - (vi) each monomer in the polymers comprises an amino acid sequence at
20 least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:6;
 - (vii) each monomer in the polymers comprises an amino acid sequence at
least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7;
 - 25 (viii) each monomer in the polymers comprises an amino acid sequence at
least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:8; or
 - (ix) each monomer in the polymers comprises an amino acid sequence at
least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
30 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:9;

wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement); and

 - (b) a plurality of (i) Tie2 receptor antibodies comprising Fc domains, and/or (ii) dimers of fibrinogen-like domain derived from angiopoietin (F domain)fused to an Fc

domain;

wherein

(i) each Tie2 antibody or dimer comprises a first Fc domain and a second Fc domain;

5 (ii) each Tie2 antibody or dimer in the plurality is (A) non-covalently bound via the first Fc domain to one polypeptide monomer chain of a first polymer, and (B) non-covalently bound via the second Fc domain to one polypeptide monomer of a second polymer; and

(iii) each polypeptide monomer chain of each polymer is non-covalently
10 bound to one Fc domain;

wherein the particle comprises dihedral, tetrahedral, octahedral, or icosahedral symmetry.

2. The particle of claim 1, wherein the polymers comprise monomers with some amino
15 acid differences.

3. The particle of claim 1 or 2, wherein the particle comprises polymers that are not homo-oligomers.

20 4. The particle of any one of claims 1-3, wherein each polymer in the particle is identical.

5. The particle of claim 1, wherein each monomer in each polymer is identical and each polymer is a homo-polymer.

25 6. The particle of claim 5, wherein each homo-polymer in the particle is identical.

7. The particle of any one of claims 1-6, wherein the plurality of polymers comprises dimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%,
30 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-3.

8. The particle of any one of claims 1-6, wherein the plurality of polymers comprises trimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%,

70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:4-6.

9. The particle of any one of claims 1-6, wherein the plurality of polymers comprises
5 tetramers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7.

10. The particle of any one of claims 1-6, wherein the plurality of polymers comprises
10 pentamers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:8-9.

15 11. The particle of any one of claims 1-10, wherein residues present at a polymeric interface, as defined in Table 2, in a polymer of the polypeptide of any one of SEQ ID NOS:1-9 are conserved.

20 12. The particle of any one of claims 1-11, wherein residues present at a Fc binding interface of any one of SEQ ID NOS:1-9 as defined in Table 3 are conserved.

25 13. The particle of any one of claims 1-12, wherein substitutions relative to the reference sequence of any one of SEQ ID NOS:1-9 comprise, consist essentially of, or consist of substitutions at polar residues in the reference polypeptide.

30 14. The particle of any one of claims 1-13, wherein substitutions relative to the reference sequence of any one of SEQ ID NOS:1-9 comprise, consist essentially of, or consist of substitutions at polar residues at non-Gly/Pro residues in loop positions, as defined in Table 4, in the reference polypeptide.

15. The particle of any one of claims 1-14, wherein amino acid changes from the reference polypeptide of any one of SEQ ID NOS:1-9 are conservative amino acid substitutions.

16. The particle of any one of claims 1-15, wherein the Tie2 antibodies or dimers comprise Tie 2 antibodies, wherein the Tie-2 antibodies comprise an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs selected from the group consisting of:
- SEQ ID NOS:11-12,
SEQ ID NOS:13-14, and
SEQ ID NOS:15-16.
17. The particle of any one of claims 1-15, wherein the Tie2 antibodies or dimers comprise dimers, wherein the dimers comprise monomers comprising the amino acid sequence of SEQ ID NO:47, wherein (X) is optional and when present comprises an amino acid linker of any suitable length and amino acid content.
18. The particle of claim 17, wherein the dimers comprise monomers comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of the amino acid sequence of SEQ ID NO:17 or 18, wherein residues in parentheses are optional.
19. The particles of any one of claims 16-18, wherein the plurality of homo-polymers comprises homo-tetramers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7.
20. The particle of any one of claims 16-18, wherein the plurality of homo-polymers comprises homo-trimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5.
21. A composition comprising a plurality of the particles of any one of claims 1-20.
22. The composition of claim 21, wherein all antibodies or dimers in the composition are identical.

23. The composition of claim 17, wherein the antibodies or dimers in the composition are, in total, not identical.
24. A pharmaceutical composition comprising (a) the particle or composition of any one
5 of claims 1-23, and (b) a pharmaceutically acceptable carrier.
25. Use of the composition or pharmaceutical composition for any suitable use, including but not limited to those described in the examples.
- 10 26. A method for treating complications from bacterial or viral infections, or for treating or limiting development of diseases or syndromes resulting from vascular dysfunction, comprising administering to a subject having a bacterial or viral infection or a disease or syndromes resulting from vascular dysfunction an amount of the particle, composition or pharmaceutical composition or any embodiment or combination of embodiments herein
15 effective to treat the bacterial or viral infection, or to treat or limit development of the disease or syndrome resulting from vascular dysfunction.
27. The method of claim 26, wherein the treating comprising treating or limiting development of diseases or syndromes resulting from vascular dysfunction, including but not
20 limited to sepsis, acute respiratory distress syndrome (ARDS), acute lung injury, acute kidney injury, wet-age related macular degeneration, open angle glaucoma, diabetic retinopathy, and diabetic nephropathy.
28. A polypeptide comprising an amino acid sequence comprising or consisting of the
25 amino acid sequence of any one of SEQ ID NOS: 17-18 and 47.
29. A nucleic acid encoding the polypeptide of claim 28.
30. An expression vector comprising the nucleic acid of claim 29 operatively linked to
30 control sequence.
31. A host cell comprising the polypeptide, nucleic acid, and/or expression vector of any one of claims 28-30.

32. A kit comprising:

- (a) a polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in any one of claims 2-15; and
- (b) Tie2 antibodies comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs selected from the group consisting of SEQ ID NOS:11-12; SEQ ID NOS:13-14; and SEQ ID NOS:15-16, and/or a fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain optionally comprising the amino acid sequence selected from the group consisting of SEQ ID NOS: 17-18 and 47.

33. A kit comprising:

- (a) host cells capable of expressing a polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in any one of claims 2-15; and
- (b) host cells capable of expressing Tie2 antibodies amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of heavy and light chain pairs selected from the group consisting of SEQ ID NOS:11-12; SEQ ID NOS:13-14; and SEQ ID NOS:15-16, and/or a fibrinogen-like domain derived from angiopoietin (F domain) fused to an Fc domain optionally comprising the amino acid sequence selected from the group consisting of SEQ ID NOS: 17-18 and 47.

34. A particle, comprising:

- (a) a plurality of polypeptide polymers, wherein

- (i) each monomer in the -polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:1;
- (ii) each monomer in the polymers comprises an amino acid sequence at
5 least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:2;
- (iii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:3;
- 10 (iv) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:4;
- (v) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
15 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5;
- (vi) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:6;
- (vii) each monomer in the polymers comprises an amino acid sequence at
20 least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7;
- (viii) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:8; or
- 25 (ix) each monomer in the polymers comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:9; wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement); and
- 30 (b) a plurality of α -TNFRSF (tumor necrosis factor receptor superfamily) antibodies comprising Fc domains;
wherein
- (i) each α -TNFRSF antibody in the plurality of antibodies comprises a first Fc domain and a second Fc domain;

(ii) each α -TNFRSF antibody in the plurality of antibodies is (A) non-covalently bound via the first Fc domain to one polypeptide monomer chain of a first polymer, and (B) non-covalently bound via the second Fc domain to one polypeptide monomer of a second polymer; and

5 (iii) each polypeptide monomer chain of each polymer is non-covalently bound to one Fc domain;

wherein the particle comprises dihedral, tetrahedral, octahedral, or icosahedral symmetry.

10 35. The particle of claim 34, wherein the polymers comprise monomers with some amino acid differences.

36. The particle of claim 34 or 35, wherein the particle comprises polymers that are not homo-oligomers.

15 37. The particle of any one of claims 34-36, wherein each polymer in the particle is identical.

38. The particle of claim 34, wherein each monomer in each polymer is identical, and
20 each polymer is a homo-polymer.

39. The particle of claim 38, wherein each homo-polymer in the particle is identical.

40. The particle of any one of claims 34-39, wherein the plurality of polymers comprises
25 dimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-3.

41. The particle of any one of claims 34-39, wherein the plurality of polymers comprises
30 trimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:4-6.

42. The particle of any one of claims 34-39, wherein the plurality of polymers comprises tetramers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7.

5

43. The particle of any one of claims 34-39, wherein the plurality of polymers comprises pentamers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:8-9.

10

44. The particle of any one of claims 34-43, wherein residues present at a polymeric interface, as defined in Table 2, in a polymer of the polypeptide of any one of SEQ ID NOS:1-9 are conserved.

15

45. The particle of any one of claims 34-44, wherein residues present at a Fc binding interface of any one of SEQ ID NOS:1-9 as defined in Table 3 are conserved.

46. The particle of any one of claims 34-45, wherein substitutions relative to the reference sequence of any one of SEQ ID NOS:1-9 comprise, consist essentially of, or consist of substitutions at polar residues in the reference polypeptide.

20

47. The particle of any one of claims 34-46, wherein substitutions relative to the reference sequence of any one of SEQ ID NOS:1-9 comprise, consist essentially of, or consist of substitutions at polar residues at non-Gly/Pro residues in loop positions, as defined in Table 4, in the reference polypeptide.

25

48. The particle of any one of claims 34-47, wherein amino acid changes from the reference polypeptide of any one of SEQ ID NOS:1-9 are conservative amino acid substitutions.

30

49. The particle of any one of claims 34-48, wherein the α -TNFRSF antibody targets one or more of DR5/TRAIL-R2/TNFRSF10B/CD262, CD40, 4-1BB, and TWEAKR (Tumor Necrosis Factor-like Weak Inducer of Apoptosis Receptor)/TNFRSF12A/CD266.

50. The particle of any one of claims 34-48, wherein the α -TNFRSF antibodies comprise an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence
- 5 of heavy and light chain pairs (when both heavy and light chain are needed) selected from the group consisting of:
- SEQ ID NO: 19 and 20;
 - SEQ ID NO: 21 and 22;
 - SEQ ID NO: 23 and 24;
 - 10 SEQ ID NO: 25 and 26;
 - SEQ ID NO: 27 and 28;
 - SEQ ID NO: 29;
 - SEQ ID NO: 30;
 - SEQ ID NO: 31 and 32;
 - 15 SEQ ID NO: 33;
 - SEQ ID NO: 34 and 35;
 - SEQ ID NO: 36 and 37;
 - SEQ ID NO: 38 and 39;
 - SEQ ID NO: 40 and 41;
 - 20 SEQ ID NO: 42 and 43;
 - SEQ ID NO: 44 and 45;
 - SEQ ID NO: 44 and 46;
 - SEQ ID NO: 48 and 49;
 - SEQ ID NO: 50 and 51;
 - 25 SEQ ID NO: 52 and 53
 - SEQ ID NO: 54 and 55;
 - SEQ ID NO: 56;
- Lob 7/6 heavy and light chains as disclosed in published US patent application US US20090074711; and
- 30 Heavy and light chain pairs disclosed in 2018094300.

51. The particles of any one of claims 34-50, wherein the plurality of polymers comprises tetramers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%,

65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:7.

52. The particle of any one of claims 34-50, wherein the plurality of polymers comprises trimers of the polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:5.

53. A composition comprising a plurality of the particles of any one of claims 34-52.

54. The composition of claim 53, wherein all antibodies in the composition are identical.

55. The composition of claim 53, wherein the antibodies in the composition are, in total, not identical.

56. A pharmaceutical composition comprising (a) the particles or compositions of any one of claims 34-55, and (b) a pharmaceutically acceptable carrier.

57. Use of the particles, compositions, or pharmaceutical compositions of any one of claims 34-57 for any suitable use, including but not limited to those described in the examples.

58. A method for treating a tumor, comprising administering to a subject having a tumor an amount of the particles, compositions, or pharmaceutical composition of any one of claims 34-56 effective to induce tumor cell apoptosis.

59. The method of claim 58, wherein the tumor overexpresses DR5 relative to a control tumor or a threshold DR5 expression level.

60. A kit comprising:

(a) one or more polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the

percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in any one of claims 44-48; and

- (b) α -TNFRSF antibodies comprising an antibody selected from the group consisting of: Lob 7/6, Lucatumumab, Dacetuzumab, Selicrelumab, Bleselumab, Urelumab, Utomilumab, Drozitumab, scTRAIL-Fc, KMTR2, 16E2, and Conatumumab (also referred to as AMG 655); optionally as further limited in claim 50.

61. A kit comprising:

- (a) host cells capable of expressing one or more polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, wherein residues in parentheses are optional (i.e.: not considered in the percent identity requirement), wherein the polypeptide is capable of (a) assembling into a homo-polymer, and (b) binding to a constant region of an IgG antibody; optionally the polypeptides as further limited in any one of claims 44-48; and

- (b) host cells capable of expressing α -TNFRSF antibodies comprising an antibody selected from the group consisting of: Lob 7/6, Lucatumumab, Dacetuzumab, Selicrelumab, Bleselumab, Urelumab, Utomilumab, Drozitumab, scTRAIL-Fc, KMTR2, 16E2, and Conatumumab (also referred to as AMG 655); optionally as further limited in claim 50.

62. The particle of any preceding claim, wherein

- (a) the particle, polypeptide polymer, monomer, antibody, and/or dimer may be linked to other compounds to promote an increased half-life in vivo or promote increased stability or activity in blood or at an injection site, wherein such linkage may be covalent or non-covalent, and may be accomplished, by way of non-limiting example, by methods including but not limited to chemical crosslinking, PEGylation, HESylation, PASylation, and/or glycosylation; and/or

- (b) one or more monomer in the polypeptide polymer may be linked covalently to either the antibody or dimer, in order to increase half-life in vivo or promote increased stability or activity in blood or injection site.

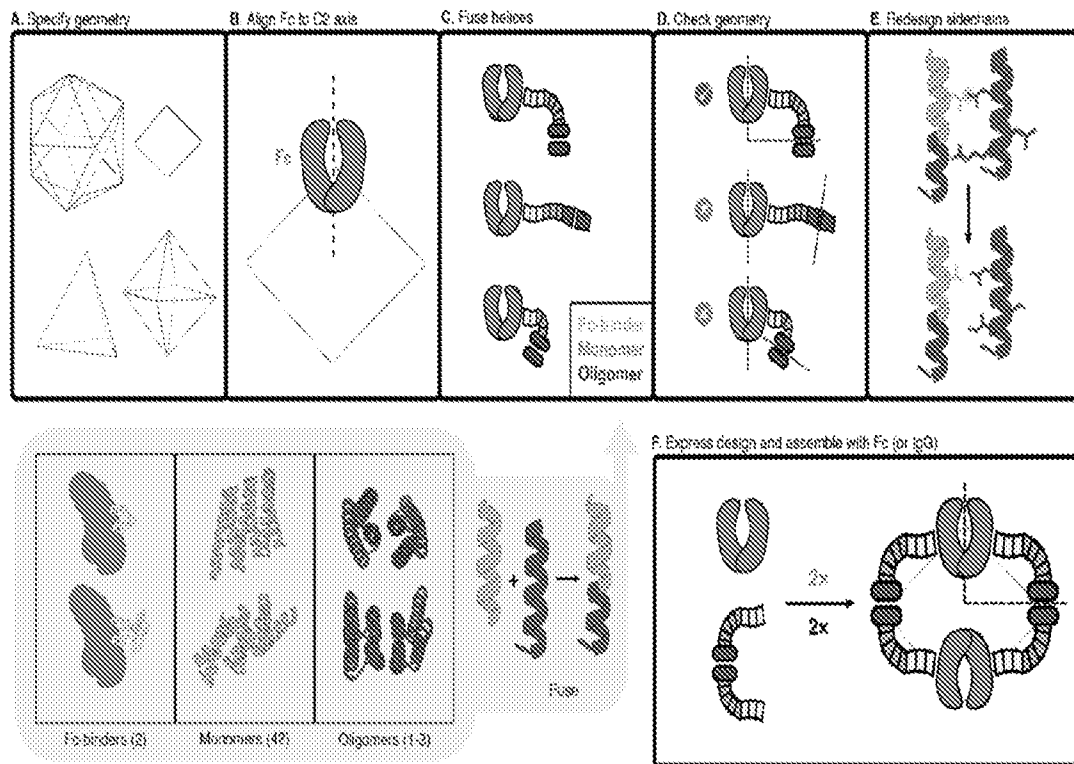


Figure 1

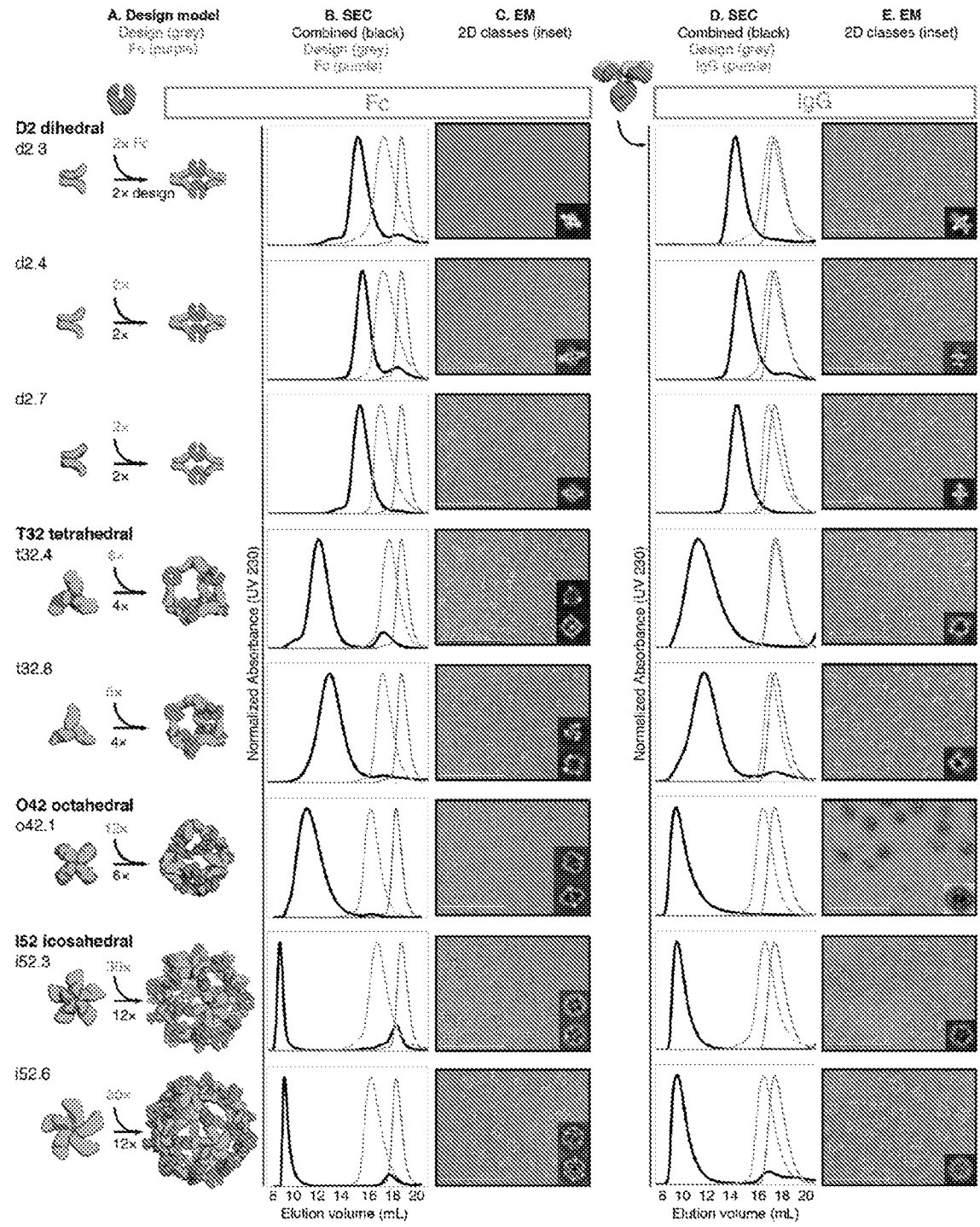


Figure 2

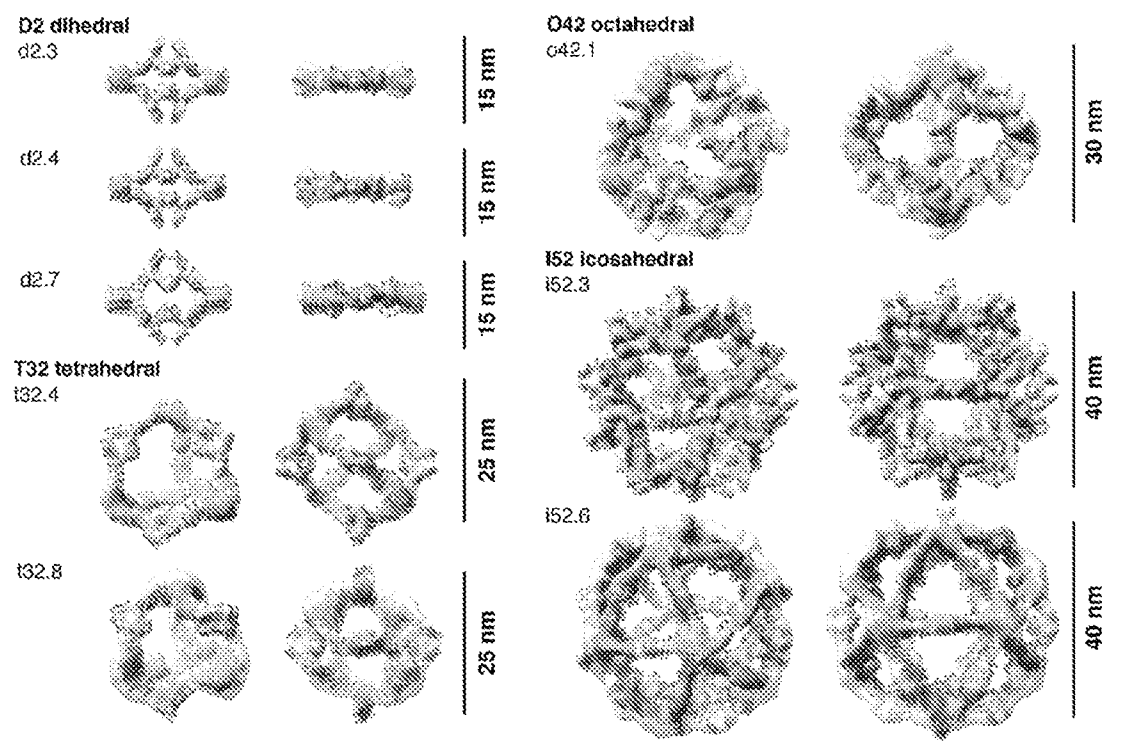


Figure 3

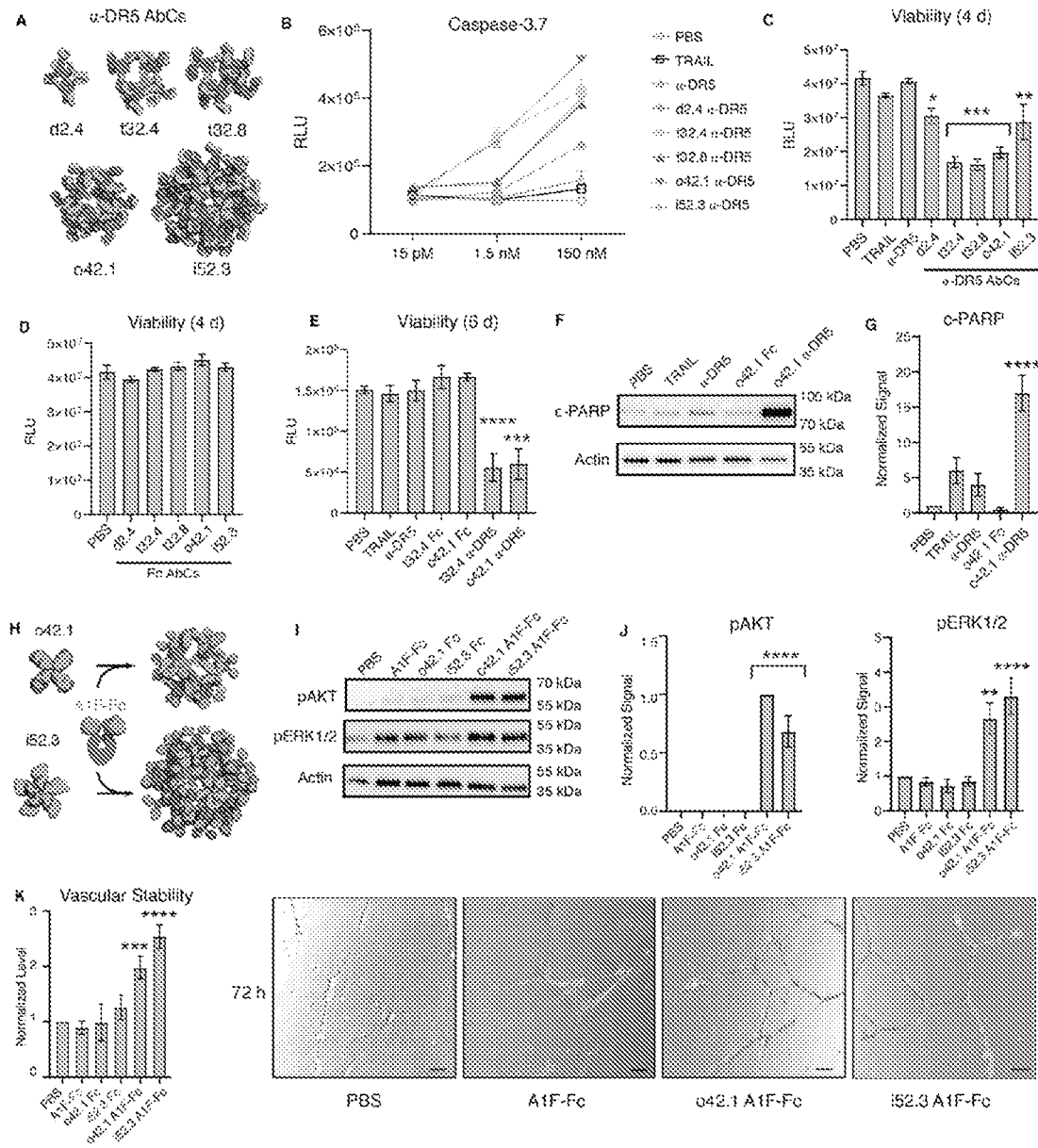


Figure 4

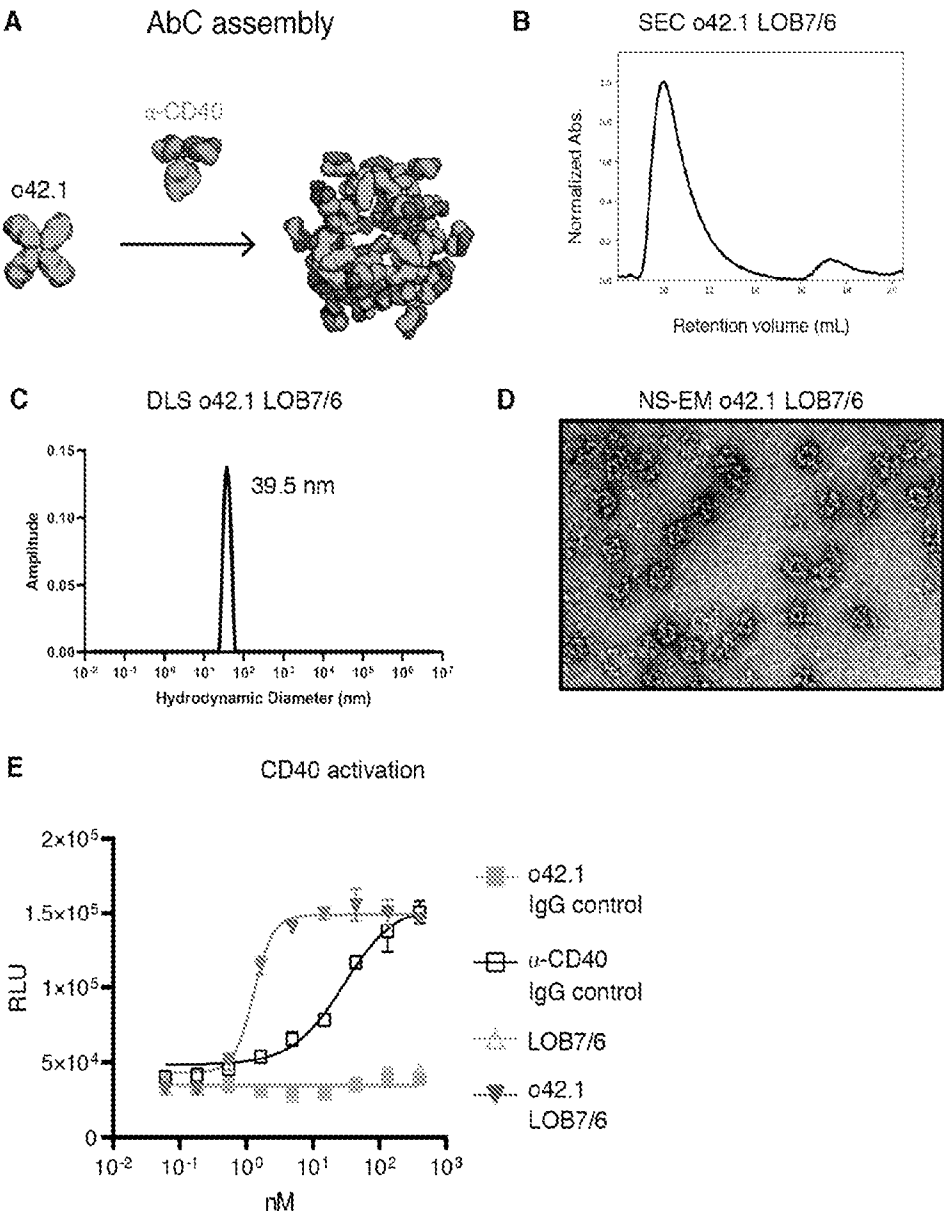


Figure 5

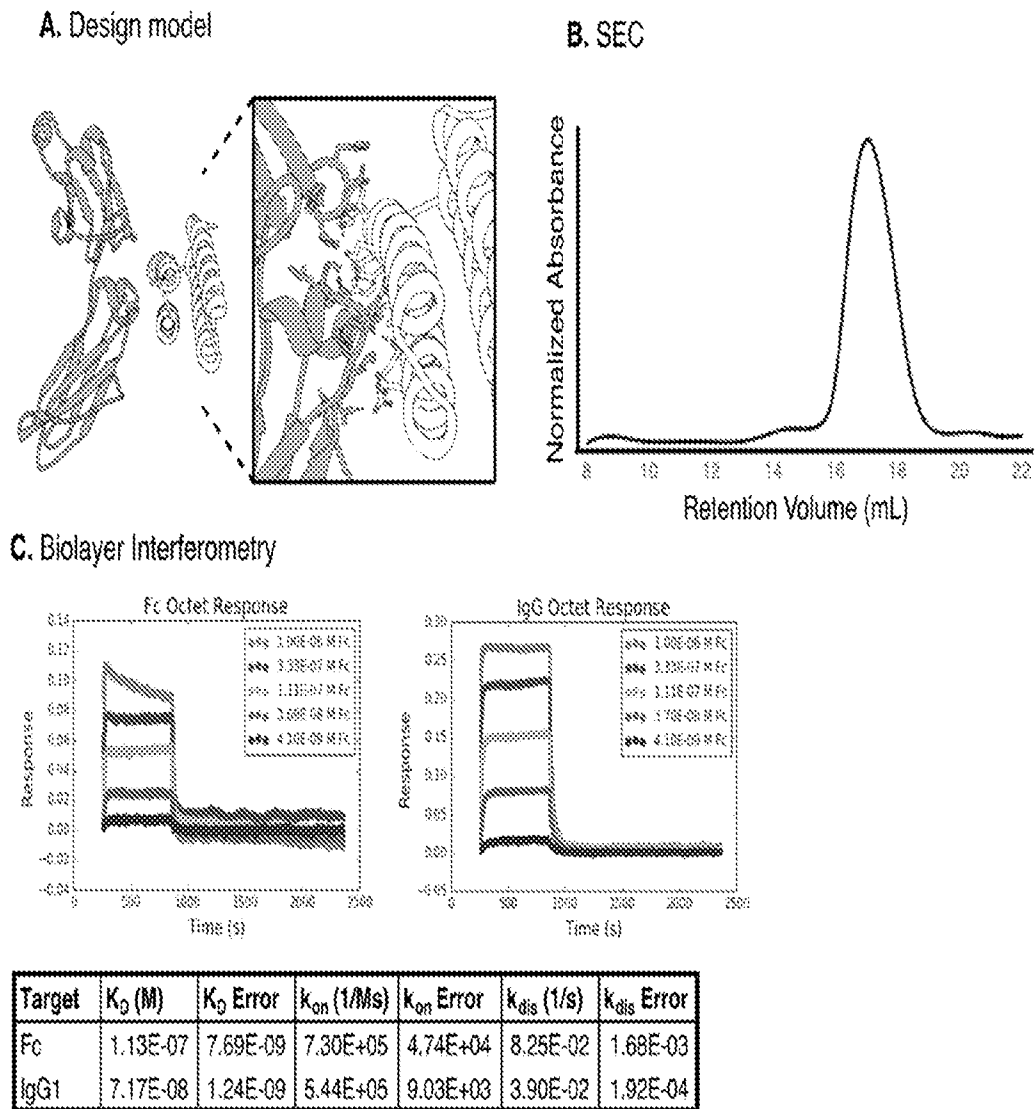


Figure 6

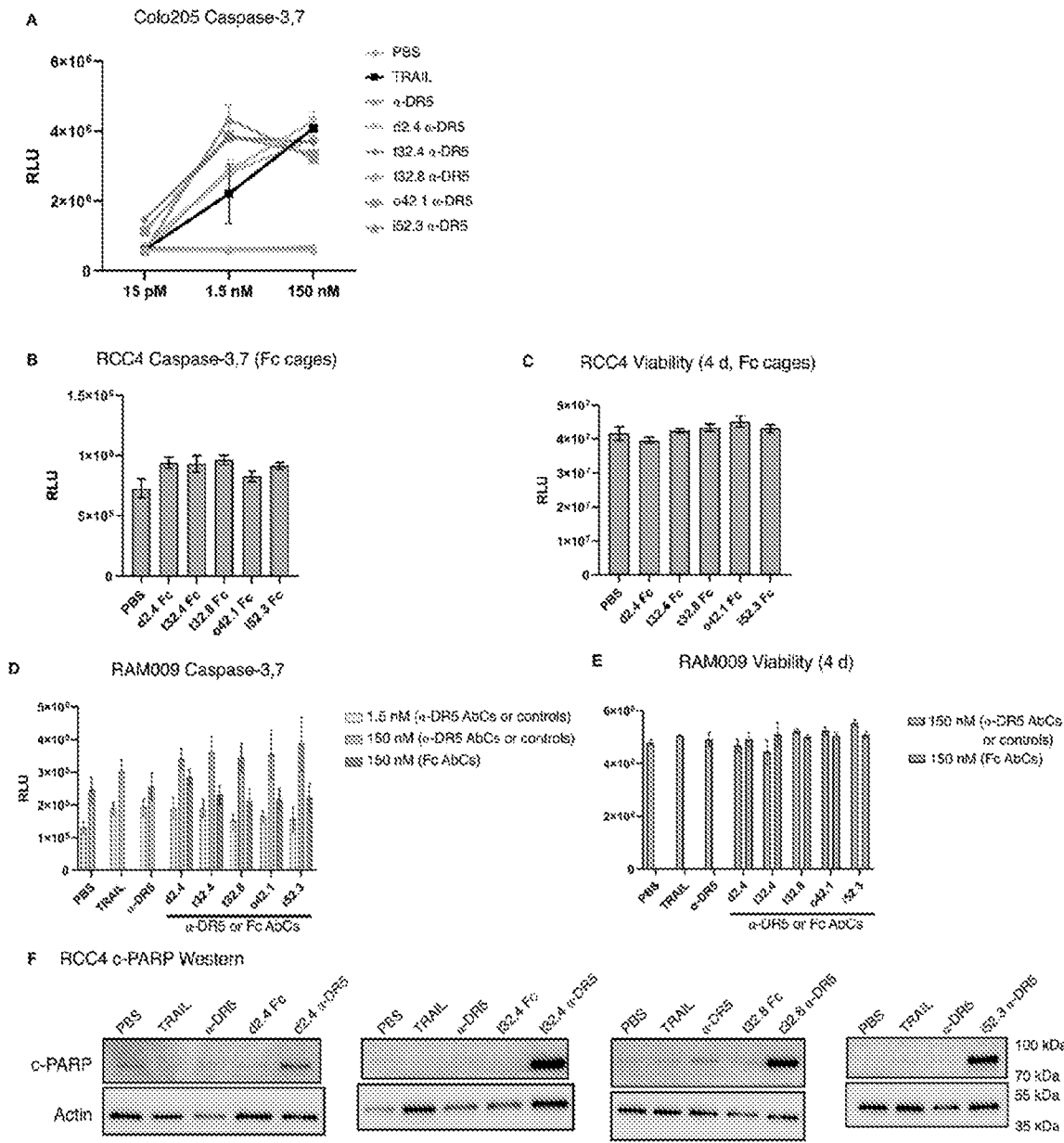


Figure7

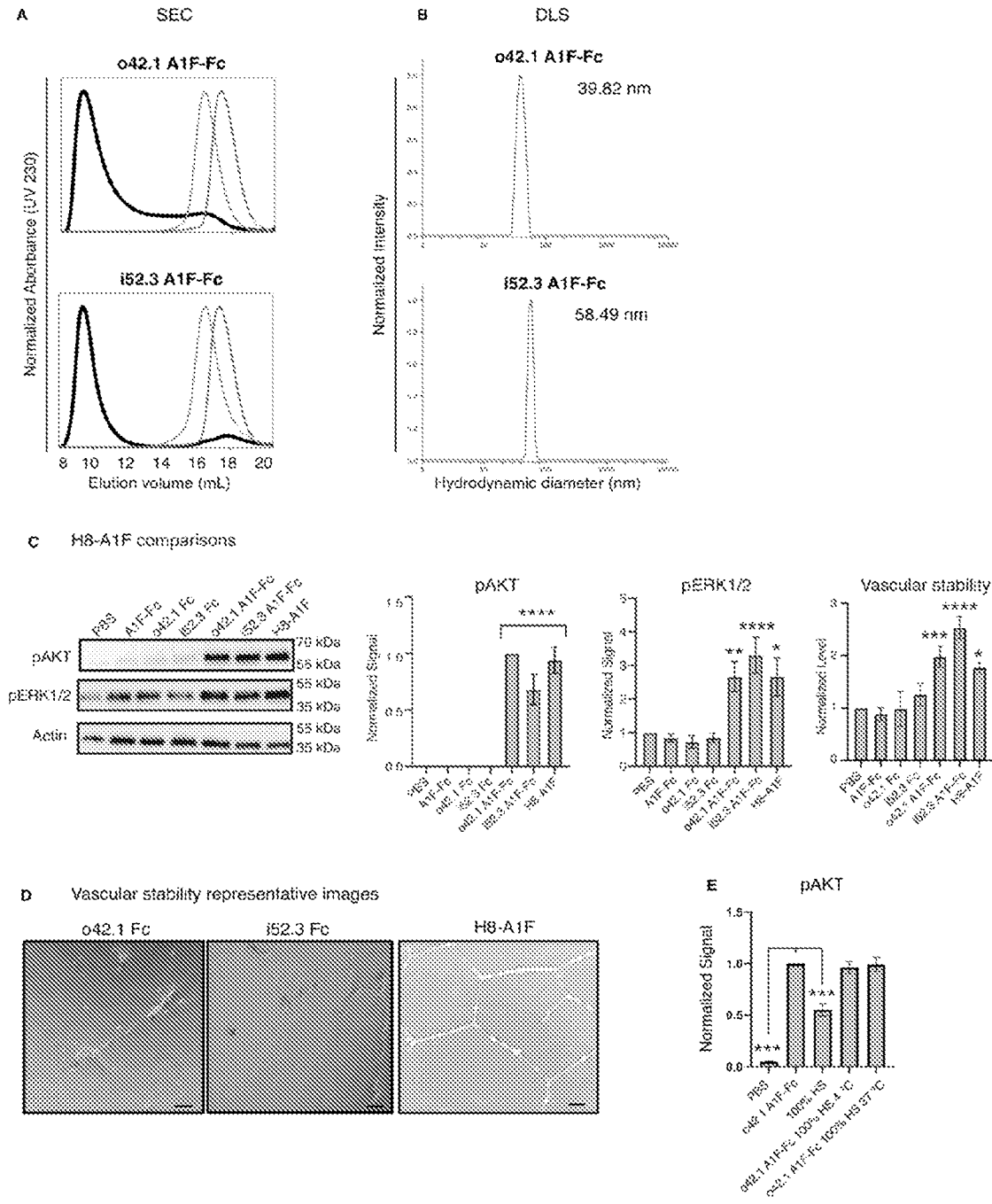


Figure 8