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(54) **Titre : ANTICORPS ANTI-RSPO ET LEURS METHODES D'UTILISATION**
(54) **Title: ANTI-RSPO ANTIBODIES AND METHODS OF USE**

(57) **Abrégé/Abstract:**

Provided herein are anti-RSPO antibodies, in particular anti-RSPO2 antibodies and/or anti-RSPO3 antibodies, and methods of using the same.



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(54) **Title**: ANTI-RSP02 AND/OR ANTI-RSP03 ANTIBODIES AND THEIR USES

(57) **Abstract**: Provided herein are anti-RSPO antibodies, in particular anti-RSPO2 antibodies and/or anti-RSPO3 antibodies, and methods of using the same.



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ANTI-RSPO ANTIBODIES AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. §119 to provisional U.S. Application Nos. 61/893,141, filed October 18, 2013 and 62/056,324, filed September 26, 2014, the contents of which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 14, 2014, is named P5719R1-WO_SequenceListing.txt and is 124950 bytes in size.

FIELD

[0003] Provided herein are anti-RSPO antibodies, in particular anti-RSPO2 antibodies and/or anti-RSPO3 antibodies, and methods of using the same.

BACKGROUND

[0004] The R-spondin (RSPO) family is a small group of four secreted proteins (RSPO1–RSPO4) that are widely expressed in vertebrate embryos and the adult. RSPOs have pleiotropic functions in development and stem cell growth by strongly enhancing Wnt pathway activation (Kazanskaya et al. *Dev. Cell* 7:525-534 (2004); Kim et al., *Cell Cycle* 5:23-26 (2006); WO 2005/040418). Mammalian RSPO1–RSPO4 share 40%–60% amino acid sequence identities and consist of a signal peptide, two adjacent furin-like cysteine-rich domains (FU-CRDs) followed by a thrombospondin type I repeat (TSR) domain, and a positively charged C-terminal region. The two FU-CRDs are essential and sufficient to promote Wnt/ β -catenin signaling (Kazanskaya et al., *Dev. Cell* 7:525-534 (2004); WO 2005/040418).

[0005] LGR4 (leucine-rich repeat [LRR]-containing G-protein-coupled receptor [GPCR] 4), LGR5, and LGR6 (Hsu et al., *Mol. Endocrinol.* 12:1830-1845 (1998) and Hsu et al., *Mol. Endocrinol.* 14:1257-1271 (2000)) are receptors for RSPOs. A common feature of the LGR4/5/6 receptors is their expression in distinct types of adult stem cells. LGR5 has already been described as a marker for resident stem cells in Wnt-dependent compartments, including the small intestine, colon, stomach, and hair follicle (Barker and Clevers *Gastroenterology* 138:1681-1696 (2010); Seshagiri et al., *Nature* 488:660-664 (2012)). LGR6 also serves as a marker of multipotent stem cells in the epidermis (Snippert et al., *Science* 327:1385-1389 (2010)). LGR4 is widely expressed in proliferating cells (Van Schoore et al., *Histochem Cell Biol.* 124:35-50 (2005)), and its knockout mice show developmental defects in many organs, including bone, kidney, testis, skin, and gall bladder (Mustata et al., *EMBO Rep* 12:558-564 (2011)). LGR4/5/6 receptors have a central array of 17 LRRs flanked by cysteine-rich

helices, and the extracellular domain is essential and sufficient for high-affinity binding with RSPOs (de Lau et al., *Genome Biol.* 13:242 (2011) and Wang et al., *Genes & Dev.* 27:1339-1344 (2013)).

[0006] LGR4/5/6 receptors may physically interact with low-density lipoprotein receptor-related protein 5/6 (LRP5/6) after RSPO recognition, and thereby RSPOs and Wnt ligands work together to activate Wnt/ β -catenin signaling (de Lau et al., *Genome Biol.* 13:242 (2011); Carmon et al., *Proc Natl Acad Sci* 108:11452-11457 (2012)). RSPOs are also able to promote Wnt/ β -catenin signaling by stabilizing the Frizzled and LRP5/6 receptors (Hao et al., *Nature* 485:195-200 (2012)). Zinc and RING finger 3 (ZNRF3) and its homolog, RING finger 43 (RNF43), are transmembrane E3 ubiquitin ligases that promote turnover of the Frizzled and LRP6 receptors on the cell surface (Hao et al., *Nature* 485:195-200 (2012); Koo et al., *Nature* 488:665-669 (2012)). RSPOs may induce clearance of ZNRF3 from the membrane by interacting with the extracellular domains of LGR4/5/6 and ZNRF3/RNF43, which stabilizes the Frizzled and LRP6 receptors to enhance Wnt/ β -catenin signaling (Hao et al., *Nature* 485:195-200 (2012)).

[0007] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0008] The invention provides anti-RSPO antibodies, in particular antibodies that bind RSPO2, RSPO3, and/or both RSPO2 and RSPO3, and methods of using the same.

[0009] Provided herein are isolated antibodies that bind to RSPO2, wherein the antibody inhibits the interaction of RSPO2 with a transmembrane E3 ubiquitinase. In some embodiments, the transmembrane E3 ubiquitinase is ZNRF3 and/or RNF43.

[0010] In some embodiments, the antibody does not inhibit the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6). For example, provided herein are isolated antibodies that bind to RSPO2, wherein the antibody comprises (a) a light chain variable domain (VL) comprising (i) hyper variable region-L1 (HVR-L1) comprising the amino acid sequence of SEQ ID NO:53, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:54, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:55, and (b) a heavy chain variable domain (VH) comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:56, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:57, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:58. In some embodiments, the antibody comprises (a) a VL sequence of SEQ ID NO:105 and a VH sequence of SEQ ID NO:106.

[0011] In some embodiments, the antibody inhibits the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6. For example, provided herein are isolated antibodies that bind to RSPO2, wherein the antibody comprises: (a) a VL comprising (i) HVR-L1 comprising the amino acid

(iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:61; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:62, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:63, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:64; (b) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:65, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:66, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:67; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:68, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:69, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:70; or (c) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:71, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:72, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:73; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:74, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:75, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:76. In some embodiments, the isolated antibody comprises (a) a VL sequence of SEQ ID NO:107 and a VH sequence of SEQ ID NO:108; (b) a VL sequence of SEQ ID NO:109 and a VH sequence of SEQ ID NO:110; or (c) a VL sequence of SEQ ID NO:111 and a VH sequence of SEQ ID NO:112.

[0012] Provided herein are also isolated antibodies that bind to RSPO3, wherein the antibody inhibits the interaction of RSPO3 with a transmembrane E3 ubiquitinase. In some embodiments, the transmembrane E3 ubiquitinase is ZNRF3 and/or RNF43.

[0013] In some embodiments, the antibody does not inhibit the interaction of RSPO3 with one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances binding of RSPO3 to one or more of LGR4, LGR5, and/or LGR6).

[0014] In some embodiments, the antibody inhibits the interaction of RSPO3 with one or more of LGR4, LGR5, and/or LGR6. For example, provided herein are isolated antibodies that bind to RSPO3, wherein the antibody comprises: (a) VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:9, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (b) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:11, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:12, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:13; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:14, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:15, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:16; (c) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:17, (ii) HVR-L2 comprising the amino acid

NO:19; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:21, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:22; (d) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28; (e) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:29, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:30, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:31; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:32, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:34; (f) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:35, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:36, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:37; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:38, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:40; or (g) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:41, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:42, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:43; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:44, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:45, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:46. In some embodiments, the antibody comprises a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28. In some embodiments, the antibody comprises a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:29, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:30, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:31; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:32, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:34. Further, provided herein are isolated antibodies that bind to RSPO3, wherein the antibody comprises (a) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID

HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:188; or (b) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:189. In some embodiments, the isolated antibody that binds to RSPO comprises a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28. In some embodiments, the isolated antibody that binds to RSPO comprises a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:188. In some embodiments, the isolated antibody that binds to RSPO comprises a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:189.

[0015] In some embodiments, the antibody comprises (a) a VL sequence of SEQ ID NO:89 and a VH sequence of SEQ ID NO:90; (b) a VL sequence of SEQ ID NO:91 and a VH sequence of SEQ ID NO:92; (c) a VL sequence of SEQ ID NO:93 and a VH sequence of SEQ ID NO:94; (d) a VL sequence of SEQ ID NO:95 and a VH sequence of SEQ ID NO:96; (e) a VL sequence of SEQ ID NO:97 and a VH sequence of SEQ ID NO:98; (f) a VL sequence of SEQ ID NO:99 and a VH sequence of SEQ ID NO:100; or (g) a VL sequence of SEQ ID NO:101 and a VH sequence of SEQ ID NO:102. In some embodiments, the isolated antibody that binds to RSPO3 comprises (a) a VL sequence of SEQ ID NO:208 and a VH sequence of SEQ ID NO:209, (b) a VL sequence of SEQ ID NO:212 and a VH sequence of SEQ ID NO:213, or (c) a VL sequence of SEQ ID NO:214 and a VH sequence of SEQ ID NO:205. In some embodiments, the isolated antibody that binds to RSPO3 comprises (a) a VL sequence of SEQ ID NO:208 and a VH sequence of SEQ ID NO:209. In some embodiments, the isolated antibody that binds to RSPO3 comprises (a) a VL sequence of SEQ ID

binds to RSPO3 comprises (a) a VL sequence of SEQ ID NO:214 and a VH sequence of SEQ ID NO:215.

[0016] Provided herein are isolated antibodies that bind to RSPO2 and RSPO3 (anti-RSPO2/3 antibody). In some embodiments, the antibody inhibits the interaction of RSPO2 and RSPO3 with a transmembrane E3 ubiquitinase. In some embodiments, the transmembrane E3 ubiquitinase is ZNRF3 and/or RNF43. In some embodiments, the antibody inhibits the interaction of RSPO3 with one or more of LGR4, LGR5, and/or LGR6. In some embodiments, the antibody does not inhibit the interaction of RSPO3 with one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances binding of RSPO3 to one or more of LGR4, LGR5, and/or LGR6). In some embodiments, the antibody inhibits the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6. In some embodiments, the antibody does not inhibit the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances binding of RSPO2 to one or more of LGR4, LGR5, and/or LGR6).

[0017] For example, provided herein are antibodies that bind to RSPO2 and RSPO3, wherein the antibody comprises: (a) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:47, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:48, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:49; and (b) a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:50, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:51, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:52. In some embodiments, the antibody comprises (a) a VL sequence of SEQ ID NO:103 and a VH sequence of SEQ ID NO:104.

[0018] In some embodiments of any of the anti-RSPO2/3 antibodies, the antibody comprises a first variable domain and a second variable domain, wherein the first variable domain comprises a first set of six HVRs and the second variable domain comprises a second set of six HVRs, and wherein the first and second set of six HVRs are identical. In some embodiments, the first set of six HVRs and the second set of six HVRs are the six HVRs of 26E11.

[0019] In some embodiments of any of the anti-RSPO2/3 antibodies, the antibody comprises the antibody comprises a first variable domain and a second variable domain, wherein the first variable domain comprises a first set of six HVRs and the second variable domain comprises a second set of six HVRs, and wherein the first and second set of six HVRs are different. In some embodiments, the first set of six HVRs are the six HVRs of any one of 4H1, 4D4, 5C2, 5D6, 5E11, 6E9, and 21C2 and the second set of six HVRs are the six HVRs of any one of 1A1, 11F11, 36D2, and 49G5. In some embodiments, the first set of six HVRs are the six HVRs of any one of 4H1, 4D4, 5C2, 5D6, 5E11, 6E9, and 21C2 and the second set of six HVRs are the six HVRs of 1A1.

[0020] Provided herein are also isolated antibodies that bind to RSPO3, wherein the antibody comprises: (a) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:77,

amino acid sequence of SEQ ID NO:79; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:80, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:81, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:82; or (b) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:83, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:84, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:85; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:86, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:87, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:88. Provided herein are also isolated antibodies that bind to RSPO3, wherein the antibody comprises: (a) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:77, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:78, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:79; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:80, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:81, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:216.

[0021] Provided herein are also isolated antibodies that bind to RSPO3, wherein the antibody binds to a region within amino acids 47-108 (*e.g.*, 49-108) of RSPO3.

[0022] Provided herein are also isolated antibodies that bind to an RSPO3 epitope and in some embodiments of any of the antibodies, wherein the RSPO3 epitope comprises amino acid residues of RSPO3: Gln72, Pro90, Asp91, and Lys94. In some embodiments, the RSPO3 epitope comprises amino acids of RSPO3: Asn 52, Leu55, Phe63, Gln72, Tyr89, Pro90, Asp91, Lys94, and Lys97. In some embodiments, the RSPO3 epitope comprises amino acid residues of RSPO3: Ser49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, Lys97, and Lys108.

[0023] Provided herein are also isolated antibodies that bind to an RSPO3 epitope and in some embodiments of any of the antibodies, wherein the RSPO3 epitope comprises amino acids of RSPO3: Thr47, Leu55, Gln72, Pro90, Asp91, and Lys94. In some embodiments, the RSPO3 epitope comprises amino acids of RSPO3: Thr47, Asn52, Leu55, Phe63, Gln72, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys97. In some embodiments, the RSPO3 epitope comprises amino acid residues of RSPO3: Thr47, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Tyr84, Tyr89, Pro90, Asp91, Ile92, Asn93, Lys94, Lys97, and Lys108.

[0024] Provided herein are also isolated antibodies that bind to an RSPO3 epitope and in some embodiments of any of the antibodies, wherein the RSPO3 epitope comprises one or more amino acids selected from Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys108 of RSPO3. In some embodiments, the RSPO3 epitope

Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys108.

[0025] Provided herein are also isolated antibodies that bind to an RSPO3 epitope and in some embodiments of any of the antibodies, wherein the RSPO3 epitope comprises one or more amino acids selected from Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, Lys97, and Lys108 of RSPO3. In some embodiments, the RSPO3 epitope comprises amino acid residues of RSPO3: Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, Lys97, and Lys108.

[0026] Provided herein are also isolated antibodies that bind to an RSPO3 epitope, wherein the RSPO3 epitope comprises one or more amino acids selected from Thr47, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Tyr84, Tyr89, Pro90, Asp91, Ile92, Asn93, Lys94, Lys97, and Lys108 of RSPO3. In some embodiments, the RSPO3 epitope comprises amino acid residues of RSPO3: Thr47, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Tyr84, Tyr89, Pro90, Asp91, Ile92, Asn93, Lys94, Lys97, and Lys108.

[0027] In some embodiments of any of the antibodies, the antibody inhibits the interaction of RSPO2 and RSPO3 with a transmembrane E3 ubiquitinase. In some embodiments, the transmembrane E3 ubiquitinase is ZNRF3 and/or RNF43. In some embodiments, the antibody inhibits the interaction of RSPO3 with one or more of LGR4, LGR5, and/or LGR6.

[0028] In some embodiments of any of the anti-RSPO antibodies, the antibody inhibits RSPO2 and/or RSPO3 mediated wnt signaling. In some embodiments of any of the anti-RSPO antibodies, the antibody is an antibody fragment that binds RSPO2 and/or RSPO3. In some embodiments of any of the anti-RSPO antibodies, the antibody fragment inhibits RSPO2 and/or RSPO3 mediated wnt signaling. In some embodiments of any of the anti-RSPO antibodies, the antibody inhibits cancer stem cell growth. In some embodiments of any of the anti-RSPO antibodies, the antibody induces and/or promotes cancer cell (*e.g.*, cancer stem cell) differentiation (*e.g.*, terminal differentiation and/or differentiation into progenitor cell).

[0029] In some embodiments of any of the anti-RSPO antibodies, the antibody is a monoclonal antibody. In some embodiments of any of the anti-RSPO antibodies, the antibody is a human, humanized, or chimeric antibody. In some embodiments of any of the anti-RSPO antibodies, the antibody is a full length IgG1 antibody. In some embodiments of any of the anti-RSPO antibodies, the antibody has reduced or depleted effector function. In some embodiments of any of the anti-RSPO antibodies, the anti-RSPO antibody comprises an engineered alanine at amino acid position 297 according to EU numbering convention. In some embodiments of any of the anti-RSPO antibodies, the anti-RSPO antibody comprises an engineered alanine at amino acid position 265 according to EU numbering convention.

medicament. In some embodiments of any of the anti-RSPO antibodies, the antibody is for use in treating cancer. In some embodiments, the cancer is gastrointestinal cancer, stomach cancer, colon cancer, colorectal cancer, or rectal cancer. In some embodiments, the cancer is characterized by increased expression of one or more RSPO (*e.g.*, RSPO2 and/or RSPO3) compared to a reference. In some embodiments, the cancer is characterized by a RSPO translocation (*e.g.*, RSPO2 translocation and/or RSPO3 translocation). In some embodiments of any of the anti-RSPO antibodies, the antibody is for use in inhibiting wnt signaling, inhibiting angiogenesis and/or vasculogenesis, and/or inhibiting cell proliferation.

[0031] Provided here are also isolated nucleic acids encoding an antibody described herein. Further provided herein are host cells comprising the nucleic acid of an antibody described herein. Provided here in are methods of producing an antibody described herein comprising culturing the host cell comprising the nucleic acid of an antibody described herein so that the antibody is produced. In some embodiments, the method of producing further comprising recovering the antibody from the host cell.

[0032] Provided here are immunoconjugates comprising an antibody described herein and a cytotoxic agent.

[0033] Further provided herein are pharmaceutical formulations comprising an antibody described herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical formulation further comprises an additional therapeutic agent. In some embodiments, the additional therapeutic agent is a taxane. In some embodiments, the taxane is paclitaxel or docetaxel. In some embodiments, the additional therapeutic agent is a platinum agent. In some embodiments, the platinum agent is carboplatin, oxaliplatin, and/or cisplatin. In some embodiments, the additional therapeutic agent is a topoisomerase inhibitor. In some embodiments, the topoisomerase inhibitor is irinotecan, topotecan, etoposide, and/or mitoxantrone. In some embodiments, the additional therapeutic agent is folinic acid (*e.g.*, Leucovorin). In some embodiments, the additional therapeutic agent is a nucleoside metabolic inhibitor. In some embodiments, the nucleoside metabolic inhibitor is fluorouracil, capecitabine, and/or gemcitabine. In some embodiments, the additional therapeutic agent is folinic acid, 5-fluorouracil, and/or oxaliplatin. In some embodiments, the additional therapeutic agent is 5-fluorouracil and irinotecan. In some embodiments, the additional therapeutic agent is a taxane and platinum agent. In some embodiments, the additional therapeutic agent is paclitaxel and carboplatin. In some embodiments, the additional therapeutic agent is pemetrexate. In some embodiments, the additional therapeutic agent is a hedgehog inhibitor (*e.g.*, vismodegib).

[0034] Provided herein are uses of an antibody described herein in the manufacture of a medicament for treatment of cancer. In some embodiments, the cancer is gastrointestinal cancer, stomach cancer, colon cancer, colorectal cancer, or rectal cancer. In some embodiments, the cancer is lung cancer. In some embodiments, the cancer is characterized by increased expression of one or more RSPO (*e.g.*,

a RSPO translocation (*e.g.*, RSPO2 translocation and/or RSPO3 translocation). Further, provided herein are uses of an antibody described herein in the manufacture of a medicament for inhibiting wnt signaling, inhibiting angiogenesis and/or vasculogenesis, and/or inhibiting cell proliferation. In some embodiments, the anti-RSPO antibody is used in combination with an additional therapeutic agent (*e.g.*, administered sequentially or concurrently). In some embodiments, the additional therapeutic agent is a taxane. In some embodiments, the taxane is paclitaxel or docetaxel. In some embodiments, the additional therapeutic agent is a platinum agent. In some embodiments, the platinum agent is carboplatin, oxaliplatin, and/or cisplatin. In some embodiments, the additional therapeutic agent is a topoisomerase inhibitor. In some embodiments, the topoisomerase inhibitor is irinotecan, topotecan, etoposide, and/or mitoxantrone. In some embodiments, the additional therapeutic agent is folinic acid (*e.g.*, Leucovorin). In some embodiments, the additional therapeutic agent is a nucleoside metabolic inhibitor. In some embodiments, the nucleoside metabolic inhibitor is fluorouracil, capecitabine, and/or gemcitabine. In some embodiments, the additional therapeutic agent is folinic acid, 5-fluorouracil, and/or oxaliplatin. In some embodiments, the additional therapeutic agent is 5-fluorouracil and irinotecan. In some embodiments, the additional therapeutic agent is a taxane and platinum agent. In some embodiments, the additional therapeutic agent is paclitaxel and carboplatin. In some embodiments, the additional therapeutic agent is pemetrexate. In some embodiments, the additional therapeutic agent is a hedgehog inhibitor (*e.g.*, vismodegib).

[0035] Provided herein are methods of treating an individual having cancer comprising administering to the individual an effective amount of an antibody described herein. In some embodiments, the cancer is gastrointestinal cancer, stomach cancer, colon cancer, colorectal cancer, or rectal cancer. In some embodiments, the cancer is lung cancer. In some embodiments, the method further comprises administering an additional therapeutic agent to the individual. In some embodiments, the cancer is characterized by increased expression of one or more RSPO (*e.g.*, RSPO2 and/or RSPO3) compared to a reference. In some embodiments, the cancer is characterized by a RSPO translocation (*e.g.*, RSPO2 translocation and/or RSPO3 translocation). Also provided herein are methods of inhibiting wnt signaling, inhibiting angiogenesis and/or vasculogenesis, and/or inhibiting cell proliferation in an individual comprising administering to the individual an effective amount of an antibody described herein to inhibit wnt signaling, inhibit angiogenesis and/or vasculogenesis, and/or inhibit cell proliferation. In some embodiments, the method comprises administering an additional therapeutic agent. In some embodiments, the additional therapeutic agent is a taxane. In some embodiments, the taxane is paclitaxel or docetaxel. In some embodiments, the additional therapeutic agent is a platinum agent. In some embodiments, the platinum agent is carboplatin, oxaliplatin, and/or cisplatin. In some embodiments, the additional therapeutic agent is a topoisomerase inhibitor. In some embodiments, the topoisomerase inhibitor is irinotecan, topotecan, etoposide, and/or mitoxantrone. In some

embodiments, the additional therapeutic agent is a nucleoside metabolic inhibitor. In some embodiments, the nucleoside metabolic inhibitor is fluorouracil, capecitabine, and/or gemcitabine. In some embodiments, the additional therapeutic agent is folinic acid, 5-fluorouracil, and/or oxaliplatin. In some embodiments, the additional therapeutic agent is 5-fluorouracil and irinotecan. In some embodiments, the additional therapeutic agent is a taxane and platinum agent. In some embodiments, the additional therapeutic agent is paclitaxel and carboplatin. In some embodiments, the additional therapeutic agent is pemetrexate. In some embodiments, the additional therapeutic agent is a hedgehog inhibitor (*e.g.*, vismodegib).

BRIEF DESCRIPTION OF THE FIGURES

[0036] Figure 1A-B. A panel of anti-RSPO2 and anti-RSPO3 antibodies were tested for ability to block recombinant human (rh) RSPO2-stimulated (A) and/or rhRSPO3-stimulated (B) WNT reporter activity. A subset of the antibodies block rhRSPO2- and/or rhRSPO3-stimulated WNT reporter activity. WNT reporter cells were stimulated with 10 ng/ml recombinant mouse (rm)Wnt3a, 50 pM rhRSPO2 (A) or rhRSPO3 (B), and increasing concentrations of the indicated antibody clones. Data were normalized to the amount of stimulation present in the absence of antibody.

[0037] Figure 2A-I. A panel of anti-RSPO2 and anti-RSPO3 antibodies were tested for IHC reactive to RSPO3-expressing cell pellets (A-C), RSPO2-expressing cell pellets (D-F), RSPO1-expressing cell pellet (G), RSPO4-expressing cell pellet (H), and non-RSPO1-4 expressing cells (293 cells) (I). As shown in Figure 2, the antibody 49G5 recognized as determined by IHC reactivity to RSPO2-expressing cell pellets, while not recognizing RSPO3, RSPO1, RSPO4, and non-RSPO1-4 expressing cell pellets. A complete table of antibodies tested for IHC reactivity is shown in Table 4. All tested antibodies in Table 4 did not recognize as determined by IHC reactivity RSPO1, RSPO4, and non-RSPO1-4 expressing cell pellets.

[0038] Figure 3A-D. A panel of anti-RSPO2 and anti-RSPO3 antibodies were tested for ability to inhibit rhRSPO2 (A), recombinant cynomolgus (rcyno) RSPO2 (B), mouse (m) RSPO2 (C), and rhRSPO2 L186P variant (D) stimulation of wnt reporter activity. WNT reporter cells were stimulated with 10 ng/ml rmWnt3a, either 50 pM rhRSPO2 (A), 8 pM rcynoRSPO2 (Genentech) (B), 90 pM mRSPO2 (R&D Systems) (C), or 38 pM rhRSPO2 L186P (Genentech) (D) and increasing concentrations of the indicated antibody clones.

[0039] Figure 4A-D. A panel of anti-RSPO2 and anti-RSPO3 antibodies were tested for ability to inhibit WNT reporter activity stimulated by rhRSPO3 (A), rcynoRSPO3 (B), mRSPO3 (C), and PTPRK fusion-RSPO3 (D). WNT reporter cells were stimulated with 10 ng/ml rmWnt3a, either 50 pM rhRSPO3 (A), 13 pM cynoRSPO3 (Genentech) (B), or 17 pM mRSPO3 (R&D Systems) (C) and increasing concentrations of the indicated antibody clones. In Figure 4D, WNT reporter cells were

indicated DNA, in the absence or presence of anti-RSPO3 at 5 ug/ml.

[0040] Figure 5. Affinities and IC50 measurements of nine anti-RSPO2 and anti-RSPO3 clones. The affinity of the Fab of the indicated clones for the indicated recombinant (r) RSPO2 and rRSPO3 was determined by Surface Plasmon Resonance. The IC50 measurements of the indicated clone was determined by stimulating a WNT reporter assay with the EC50 of the indicated rRSPO and increasing concentrations of each antibody. H, human; C, cynomolgus; M, mouse; -, no binding or IC50 > 500 nM.

[0041] Figure 6A-B. A panel of anti-RSPO2 and anti-RSPO3 antibodies were tested for their ability to inhibit LGR4 binding to rhRSPO2 (A) and rhRSPO3 (B). Individual antibody clones were tested for the ability to inhibit the binding of either LGR4-ECD to rhRSPO2 (A) or rhRSPO3 (B) by competitive binding ELISA. Similar results were seen with LGR5 (data not shown). *See Table 5 for a summary of the results.*

[0042] Figure 7A-B. A panel of anti-RSPO2 and anti-RSPO3 antibodies were tested for their ability to inhibit RNF43 binding to rhRSPO2 (A) and rhRSPO3 (B). Individual antibody clones were tested for the ability to inhibit the binding of RNF43-ECD to rhRSPO2 (A) or rhRSPO3 (B) by competitive binding ELISA. Similar results were seen with LGR5 (data not shown). *See Table 5 for a summary of the results.*

[0043] Figure 8A-B. Model of crystallized RSPO3 (33-210) in complex with Fab 26E11 (A). An enlargement of the Fab26E11/RSPO3 interaction is shown in (B).

[0044] Figure 9A-B. Alignment of variable light chain region sequences (A) and variable heavy chain region sequences (B) of 5D6, 26E11, 4H1, 5C2, 5E11, 4D4, 6E9 and 21C2. CDR sequences according to Kabat definition are underlined.

[0045] Figure 10A-B. Alignment of variable light chain region sequences (A) and variable heavy chain region sequences (B) of 11F11, 1A1, 36D2, and 49G5. CDR sequences according to Kabat definition are underlined.

[0046] Figure 11A-D. Change in mean tumor volume (mm³) of four colorectal cancer patient derived models (A-D) upon treatment with anti-RSPO3 antibody (5D6) at 30 mg/kg or anti-Ragweed antibody (control). Figure 11D also shows change in mean tumor volume (mm³) of CRCD colorectal cancer patient derived model upon treatment with anti-RSPO3 antibody (5D6) in combination with Irinotecan (100 mg/kg, day 0 and day 3) or anti-Ragweed antibody (control) and Irinotecan (100 mg/kg, day 0 and day 3).

[0047] Figure 12A-D. Staining of anti-Ragweed antibody (control) or anti-RSPO3 antibody (5D6) at 30 mg/kg treated colorectal cancer patient derived model tumors 2-3 weeks after last dose with hematoxylin and eosin stain (H&E stain) (A and C) and Alcian Blue stain (B and D). anti-RSPO3

indicated by Alcian Blue staining compared to the anti-Ragweed antibody control.

[0048] Figure 13A-C. (A) shows mean tumor volume (mm³) of CRCC colorectal cancer patient derived models upon treatment with (i) anti-RSPO3 antibody (5D6), (ii) anti-Ragweed antibody (control), (iii) anti-RSPO3 antibody (5D6) in combination with Irinotecan (100 mg/kg, Day 0), or anti-Ragweed antibody (control) and Irinotecan (100 mg/kg, Day 0). (B-C) shows serial transplant experiments in which colorectal cancer patient derived models were (a) treated with either anti-RSPO3 antibody (5D6) or anti-Ragweed antibody (control; 30 mg/kg) and (b) transplanted and treated with anti-RSPO3 antibody (5D6) or anti-Ragweed antibody (control). (B) shows a substantial reduction in percentage tumor (transplant) engraftment rate of serial transplanted tumors upon treatment with anti-RSPO3 antibody (5D6) either initially or at the time of serial transplant. (C) shows a significant decrease in change mean tumor volume of serial transplanted tumors upon treatment with anti-RSPO3 antibody (5D6) either initially or at the time of serial transplant.

[0049] Figure 14A-B. Alignment of variable light chain region sequences (A) and variable heavy chain region sequences (B) of 5D6, 5D6v1, 5D6v2.1, 5D6v2.2, 5D6v2.3, 5D6v2.4, 5D6v2.8, 5D6v2.10, 5D6v3.2, 5D6v3.3, 5D6v4.1, 5D6v4.3, 5D6v5.1, and 5D6v5.2. CDR sequences according to Kabat definition are underlined.

DETAILED DESCRIPTION

I. DEFINITIONS

[0050] The terms “R-spondin” and “RSPO,” as used herein, refer to any native RSPO (*e.g.*, RSPO1, RSPO2, RSPO3, and/or RSPO4) from any vertebrate source, including mammals such as primates (*e.g.*, humans) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed RSPO as well as any form of RSPO that results from processing in the cell. The term also encompasses naturally occurring variants of RSPO, *e.g.*, splice variants or allelic variants. In some embodiments, the amino acid sequence of an exemplary human RSPO is RSPO1, for example, as shown in SEQ ID NO:3. In some embodiments, the amino acid sequence of an exemplary human RSPO is RSPO2, for example, as shown in SEQ ID NO:1. In some embodiments, the amino acid sequence of an exemplary human RSPO is RSPO3, for example, as shown in SEQ ID NO:2. In some embodiments, the amino acid sequence of an exemplary human RSPO is RSPO4, for example, as shown in SEQ ID NO:4.

[0051] The terms “R-spondin 2” and “RSPO2,” as used herein, refers to any native RSPO2 from any vertebrate source, including mammals such as primates (*e.g.*, humans) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed RSPO2 as well as any form of RSPO2 that results from processing in the cell. The term also encompasses naturally occurring variants of RSPO2, *e.g.*, splice variants or allelic variants. In some embodiments, the amino acid sequence of an exemplary human RSPO2 is UNIPROT Q6UXX9-1 as of October 18, 2013. In

2 as of October 18, 2013. In some embodiments, the amino acid sequence of an exemplary human RSPO2 is UNIPROT Q6UXX9-3 as of October 18, 2013. In some embodiments, the amino acid sequence of an exemplary human RSPO2 is shown in SEQ ID NO:1.

[0052] The terms “R-spondin 3” and “RSPO3,” as used herein, refers to any native RSPO3 from any vertebrate source, including mammals such as primates (*e.g.*, humans) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed RSPO3 as well as any form of RSPO3 that results from processing in the cell. The term also encompasses naturally occurring variants of RSPO3, *e.g.*, splice variants or allelic variants. In some embodiments, the amino acid sequence of an exemplary human RSPO2 is UNIPROT Q9BXY4-1 as of October 18, 2013. In some embodiments, the amino acid sequence of an exemplary human RSPO2 is UNIPROT Q9BXY4-2 as of October 18, 2013. In some embodiments, the amino acid sequence of an exemplary human RSPO3 is shown in SEQ ID NO:2.

[0053] An “acceptor human framework” for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0054] “Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0055] An “affinity matured” antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[0056] The terms “anti-RSPO2 antibody” and “an antibody that binds to RSPO2” refer to an antibody that is capable of binding RSPO2 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting RSPO2. In one embodiment, the extent of binding of an anti-RSPO2 antibody to a non-RSPO2 protein is less than about 10% of the binding of the antibody

binds to RSPO2 has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (*e.g.*, 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-RSPO2 antibody binds to an epitope of RSPO2 that is conserved among RSPO2 from different species.

[0057] The terms “anti-RSPO3 antibody” and “an antibody that binds to RSPO3” refer to an antibody that is capable of binding RSPO3 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting RSPO3. In one embodiment, the extent of binding of an anti-RSPO3 antibody to a non-RSPO3 protein is less than about 10% of the binding of the antibody to RSPO3 as measured, *e.g.*, by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to RSPO3 has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (*e.g.*, 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-RSPO3 antibody binds to an epitope of RSPO3 that is conserved among RSPO3 from different species.

[0058] The terms “anti-RSPO2/3 antibody” and “an antibody that binds to RSPO2 and RSPO3” refer to an antibody that is capable of binding RSPO2 and RSPO3 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting RSPO2 and RSPO3. In one embodiment, the extent of binding of an anti-RSPO2/3 antibody to a non-RSPO2 or non-RSPO3 protein is less than about 10% of the binding of the antibody to RSPO2 and RSPO3 as measured, *e.g.*, by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to RSPO2 and RSPO3 has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (*e.g.*, 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-RSPO2/3 antibody binds to an epitope of RSPO2 and/or RSPO3 that is conserved among RSPO2 and/or RSPO3 from different species.

[0059] The term “antibody” herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[0060] An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (*e.g.*, scFv); and multispecific antibodies formed from antibody fragments.

[0061] An “antibody that competes for binding with” a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and

by 50% or more. An exemplary competition assay is provided herein.

[0062] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0063] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

[0064] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor); and B cell activation.

[0065] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

[0066] "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0067] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0068] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny

Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0069] A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0070] A “human consensus framework” is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

[0071] A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (*e.g.*, CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, *e.g.*, a non-human antibody, refers to an antibody that has undergone humanization.

[0072] The term “hypervariable region” or “HVR” as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence (“complementarity determining regions” or “CDRs”) and/or form structurally defined loops (“hypervariable loops”) and/or contain the antigen-contacting residues (“antigen contacts”). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

(a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));

(c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and

(L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

[0073] In one embodiment, HVR residues comprise those identified in Figures 9A-B and/or Figures 10A-B or elsewhere in the specification.

[0074] Unless otherwise indicated, HVR residues and other residues in the variable domain (*e.g.*, FR residues) are numbered herein according to Kabat et al., *supra*.

[0075] The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (*See, e.g.*, Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity.

Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. *See, e.g.*, Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

[0076] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

[0077] An “immunoconjugate” is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0078] An “isolated” antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, *see, e.g.*, Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

[0079] An “isolated” nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0080] “Isolated nucleic acid encoding an anti-RSPO2 antibody” refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic

one or more locations in a host cell.

[0081] “Isolated nucleic acid encoding an anti-RSPO3 antibody” refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0082] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, *e.g.*, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[0083] A “naked antibody” refers to an antibody that is not conjugated to a heterologous moiety (*e.g.*, a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

[0084] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0085] The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage,

therapeutic products.

[0086] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0087] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0088] The terms "R-spondin translocation" and "RSPO translocation" refer herein to an R-spondin wherein a portion of a broken chromosome including, for example, R-spondin, variant, or fragment thereof or a second gene, variant, or fragment thereof, reattaches in a different chromosome location,

location in and/or around the R-spondin native location which is different from the second gene's native location. The R-spondin translocation may be a RSPO1 translocation, RSPO2 translocation, RSPO3 translocation, and/or RSPO4 translocation.

[0089] The terms "R-spondin-translocation fusion polynucleotide" and "RSPO-translocation fusion polynucleotide" refer herein to the nucleic acid sequence of an R-spondin translocation gene product or fusion polynucleotide. The R-spondin-translocation fusion polynucleotide may be a RSPO1-translocation fusion polynucleotide, RSPO2-translocation fusion polynucleotide, RSPO3-translocation fusion polynucleotide, and/or RSPO4-translocation fusion polynucleotide. The terms "R-spondin-translocation fusion polypeptide" and "RSPO-translocation fusion polypeptide" refer herein to the amino acid sequence of an R-spondin translocation gene product or fusion polynucleotide. The R-spondin-translocation fusion polypeptide may be a RSPO1-translocation fusion polypeptide, RSPO2-translocation fusion polypeptide, RSPO3-translocation fusion polypeptide, and/or RSPO4-translocation fusion polypeptide.

[0090] The term "detection" includes any means of detecting, including direct and indirect detection.

[0091] The term "biomarker" as used herein refers to an indicator, *e.g.*, a predictive, diagnostic, and/or prognostic indicator, which can be detected in a sample. The biomarker may serve as an indicator of a particular subtype of a disease or disorder (*e.g.*, cancer) characterized by certain, molecular, pathological, histological, and/or clinical features. In some embodiments, the biomarker is a gene. In some embodiments, the biomarker is a variation (*e.g.*, mutation and/or polymorphism) of a gene. In some embodiments, the biomarker is a translocation. Biomarkers include, but are not limited to, polynucleotides (*e.g.*, DNA, and/or RNA), polypeptides, polypeptide and polynucleotide modifications (*e.g.*, posttranslational modifications), carbohydrates, and/or glycolipid-based molecular markers.

[0092] The "presence," "amount," or "level" of a biomarker associated with an increased clinical benefit to an individual is a detectable level in a sample. These can be measured by methods known to one skilled in the art and also disclosed herein. The expression level or amount of biomarker assessed can be used to determine the response to the treatment.

[0093] The terms "level of expression" or "expression level" in general are used interchangeably and generally refer to the amount of a biomarker in a sample. "Expression" generally refers to the process by which information (*e.g.*, gene-encoded and/or epigenetic) is converted into the structures present and operating in the cell. Therefore, as used herein, "expression" may refer to transcription into a polynucleotide, translation into a polypeptide, or even polynucleotide and/or polypeptide modifications (*e.g.*, posttranslational modification of a polypeptide). Fragments of the transcribed polynucleotide, the translated polypeptide, or polynucleotide and/or polypeptide modifications (*e.g.*, posttranslational modification of a polypeptide) shall also be regarded as expressed whether they

translational processing of the polypeptide, *e.g.*, by proteolysis. "Expressed genes" include those that are transcribed into a polynucleotide as mRNA and then translated into a polypeptide, and also those that are transcribed into RNA but not translated into a polypeptide (for example, transfer and ribosomal RNAs).

[0094] "Elevated expression," "elevated expression levels," or "elevated levels" refers to increased expression or increased levels of a biomarker in an individual relative to a control, such as an individual or individuals who are not suffering from the disease or disorder (*e.g.*, cancer) or an internal control (*e.g.*, housekeeping biomarker).

[0095] "Reduced expression," "reduced expression levels," or "reduced levels" refers to decrease expression or decreased levels of a biomarker in an individual relative to a control, such as an individual or individuals who are not suffering from the disease or disorder (*e.g.*, cancer) or an internal control (*e.g.*, housekeeping biomarker).

[0096] The term "housekeeping biomarker" refers to a biomarker or group of biomarkers (*e.g.*, polynucleotides and/or polypeptides) which are typically similarly present in all cell types. In some embodiments, the housekeeping biomarker is a "housekeeping gene." A "housekeeping gene" refers herein to a gene or group of genes which encode proteins whose activities are essential for the maintenance of cell function and which are typically similarly present in all cell types.

[0097] "Amplification," as used herein generally refers to the process of producing multiple copies of a desired sequence. "Multiple copies" mean at least two copies. A "copy" does not necessarily mean perfect sequence complementarity or identity to the template sequence. For example, copies can include nucleotide analogs such as deoxyinosine, intentional sequence alterations (such as sequence alterations introduced through a primer comprising a sequence that is hybridizable, but not complementary, to the template), and/or sequence errors that occur during amplification.

[0098] The term "diagnosis" is used herein to refer to the identification or classification of a molecular or pathological state, disease or condition (*e.g.*, cancer). For example, "diagnosis" may refer to identification of a particular type of cancer. "Diagnosis" may also refer to the classification of a particular subtype of cancer, *e.g.*, by histopathological criteria, or by molecular features (*e.g.*, a subtype characterized by expression of one or a combination of biomarkers (*e.g.*, particular genes or proteins encoded by said genes)).

[0099] Samples include, but are not limited to, primary or cultured cells or cell lines, cell supernatants, cell lysates, platelets, serum, plasma, vitreous fluid, lymph fluid, synovial fluid, follicular fluid, seminal fluid, amniotic fluid, milk, whole blood, blood-derived cells, urine, cerebrospinal fluid, saliva, sputum, tears, perspiration, mucus, tumor lysates, and tissue culture medium, tissue extracts such as homogenized tissue, tumor tissue, cellular extracts, and combinations thereof.

“control tissue”, as used herein, refers to a sample, cell, tissue, standard, or level that is used for comparison purposes. In one embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (*e.g.*, tissue or cells) of the same subject or individual. For example, healthy and/or non-diseased cells or tissue adjacent to the diseased cells or tissue (*e.g.*, cells or tissue adjacent to a tumor). In another embodiment, a reference sample is obtained from an untreated tissue and/or cell of the body of the same subject or individual. In yet another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (*e.g.*, tissues or cells) of an individual who is not the subject or individual. In even another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from an untreated tissue and/or cell of the body of an individual who is not the subject or individual.

[0101] The phrase “substantially similar,” as used herein, refers to a sufficiently high degree of similarity between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to not be of statistical significance within the context of the biological characteristic measured by said values (*e.g.*, K_d values). The difference between said two values may be, for example, less than about 20%, less than about 10%, and/or less than about 5% as a function of the reference/comparator value.

[0102] The phrase “substantially different,” refers to a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (*e.g.*, K_d values). The difference between said two values may be, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

[0103] The term “cytotoxic agent” as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu); chemotherapeutic agents or drugs (*e.g.*, methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylmelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scoplectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, e.g., Nicolaou *et al.*, *Angew. Chem Intl. Ed. Engl.*, 33: 183-186 (1994))); CDP323, an oral alpha-4 integrin inhibitor; dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®), liposomal doxorubicin TLC D-99 (MYOCET®), pegylated liposomal doxorubicin (CAELYX®), and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone;

edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2'-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoid, *e.g.*, paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and docetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; platinum agents such as cisplatin, oxaliplatin (*e.g.*, ELOXATIN®), and carboplatin; vincas, which prevent tubulin polymerization from forming microtubules, including vinblastine (VELBAN®), vincristine (ONCOVIN®), vindesine (ELDISINE®, FILDESIN®), and vinorelbine (NAVELBINE®); etoposide (VP-16); ifosfamide; mitoxantrone; leucovorin; novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid, including bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1 inhibitor (*e.g.*, LURTOTECAN®); rmRH (*e.g.*, ABARELIX®); BAY439006 (sorafenib; Bayer); SU-11248 (sunitinib, SUTENT®, Pfizer); perifosine, COX-2 inhibitor (*e.g.* celecoxib or etoricoxib), proteasome inhibitor (*e.g.* PS341); bortezomib (VELCADE®); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENA-SENSE®); pixantrone; EGFR inhibitors (see definition below); tyrosine kinase inhibitors (see definition below); serine-threonine kinase inhibitors such as rapamycin (sirolimus, RAPAMUNE®); farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

therapeutics” which act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer. They may be hormones themselves, including, but not limited to: anti-estrogens with mixed agonist/antagonist profile, including, tamoxifen (NOLVADEX®), 4-hydroxytamoxifen, toremifene (FARESTON®), idoxifene, droloxifene, raloxifene (EVISTA®), trioxifene, keoxifene, and selective estrogen receptor modulators (SERMs) such as SERM3; pure anti-estrogens without agonist properties, such as fulvestrant (FASLODEX®), and EM800 (such agents may block estrogen receptor (ER) dimerization, inhibit DNA binding, increase ER turnover, and/or suppress ER levels); aromatase inhibitors, including steroidal aromatase inhibitors such as formestane and exemestane (AROMASIN®), and nonsteroidal aromatase inhibitors such as anastrozole (ARIMIDEX®), letrozole (FEMARA®) and aminoglutethimide, and other aromatase inhibitors include vorozole (RIVISOR®), megestrol acetate (MEGASE®), fadrozole, and 4(5)-imidazoles; lutenizing hormone-releasing hormone agonists, including leuprolide (LUPRON® and ELIGARD®), goserelin, buserelin, and triptorelin; sex steroids, including progestins such as megestrol acetate and medroxyprogesterone acetate, estrogens such as diethylstilbestrol and premarin, and androgens/retinoids such as fluoxymesterone, all transretinoic acid and fenretinide; onapristone; anti-progesterones; estrogen receptor down-regulators (ERDs); anti-androgens such as flutamide, nilutamide and bicalutamide; and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above.

[0106] The term "cytostatic agent" refers to a compound or composition which arrests growth of a cell either in vitro or in vivo. Thus, a cytostatic agent may be one which significantly reduces the percentage of cells in S phase. Further examples of cytostatic agents include agents that block cell cycle progression by inducing G0/G1 arrest or M-phase arrest. The humanized anti-Her2 antibody trastuzumab (HERCEPTIN®) is an example of a cytostatic agent that induces G0/G1 arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Certain agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., *The Molecular Basis of Cancer*, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W.B. Saunders, Philadelphia, 1995), *e.g.*, p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

interact directly with EGFR and prevent or reduce its signaling activity, and is alternatively referred to as an “EGFR antagonist.” Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US Patent No. 4,943, 533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX[®]) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF or Panitumumab (see WO98/50433, Abgenix/Amgen); EMD 55900 (Stragliotto *et al. Eur. J. Cancer* 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF- α for EGFR binding (EMD/Merck); human EGFR antibody, HuMax-EGFR (GenMab); fully human antibodies known as E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6. 3 and E7.6.3 and described in US 6,235,883; MDX-447 (Medarex Inc); and mAb 806 or humanized mAb 806 (Johns *et al., J. Biol. Chem.* 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, e.g., EP659,439A2, Merck Patent GmbH). EGFR antagonists include small molecules such as compounds described in US Patent Nos: 5,616,582, 5,457,105, 5,475,001, 5,654,307, 5,679,683, 6,084,095, 6,265,410, 6,455,534, 6,521,620, 6,596,726, 6,713,484, 5,770,599, 6,140,332, 5,866,572, 6,399,602, 6,344,459, 6,602,863, 6,391,874, 6,344,455, 5,760,041, 6,002,008, and 5,747,498, as well as the following PCT publications: WO98/14451, WO98/50038, WO99/09016, and WO99/24037. Particular small molecule EGFR antagonists include OSI-774 (CP-358774, erlotinib, TARCEVA[®] Genentech/OSI Pharmaceuticals); PD 183805 (CI 1033, 2-propenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl]-, dihydrochloride, Pfizer Inc.); ZD1839, gefitinib (IRESSA[®]) 4-(3'-Chloro-4'-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline, AstraZeneca); ZM 105180 ((6-amino-4-(3-methylphenyl-amino)-quinazoline, Zeneca); BIBX-1382 (N8-(3-chloro-4-fluoro-phenyl)-N2-(1-methyl-piperidin-4-yl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, Boehringer Ingelheim); PKI-166 ((R)-4-[4-[(1-phenylethyl)amino]-1H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol); (R)-6-(4-hydroxyphenyl)-4-[(1-phenylethyl)amino]-7H-pyrrolo[2,3-d]pyrimidine); CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide); EKB-569 (N-[4-[(3-chloro-4-fluorophenyl)amino]-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide) (Wyeth); AG1478 (Pfizer); AG1571 (SU 5271; Pfizer); dual EGFR/HER2 tyrosine kinase inhibitors such as lapatinib (TYKERB[®], GSK572016 or N-[3-

quinazolinamine; Glaxo-SmithKline).

[0108] The term “tumor” refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms “cancer,” “cancerous,” “cell proliferative disorder,” “proliferative disorder” and “tumor” are not mutually exclusive as referred to herein.

[0109] The terms “cell proliferative disorder” and “proliferative disorder” refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

[0110] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer include, but are not limited to, carcinoma, lymphoma (*e.g.*, Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

[0111] The term “colon tumor” or “colon cancer” refers to any tumor or cancer of the colon (the large intestine from the cecum to the rectum).

[0112] The term “colorectal tumor” or “colorectal cancer” refers to any tumor or cancer of the large bowel, which includes the colon (the large intestine from the cecum to the rectum) and the rectum, including, *e.g.*, adenocarcinomas and less prevalent forms, such as lymphomas and squamous cell carcinomas.

[0113] An "effective amount" of an agent, *e.g.*, a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0114] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0115] A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats). In certain embodiments, the individual or subject is a human.

[0117] As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

[0118] By “reduce” or “inhibit” is meant the ability to cause an overall decrease of 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or greater. In some embodiments, reduce or inhibit can refer to a relative reduction compared to a reference (*e.g.*, reference level of biological activity (*e.g.*, wnt signaling) or binding). In some embodiments, reduce or inhibit can refer to the symptoms of the disorder being treated, the presence or size of metastases, or the size of the primary tumor.

[0119] As is understood by one skilled in the art, reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X”.

[0120] It is understood that aspect and embodiments of the invention described herein include “consisting” and/or “consisting essentially of” aspects and embodiments. As used herein, the singular form “a”, “an”, and “the” includes plural references unless indicated otherwise.

II. COMPOSITIONS AND METHODS

[0121] Provided herein are anti-RSPO antibodies and uses thereof. In certain embodiments, antibodies that bind to RSPO2 and/or RSPO3 are provided. Antibodies provided are useful, *e.g.*, for the diagnosis or treatment of cancer, such as colorectal cancer.

[0122] In some aspects, provided herein are a panel of anti-RSPO antibodies. The panel of antibodies where characterized for multiple properties, including but not limited to, based upon the ability to bind to RSPO2 and/or RSPO3, the ability to detect RSPO2 and/or RSPO3 by IHC, the ability to inhibit the interaction of RSPO2 and/or RSPO3 and an LGR polypeptide, for example LGR4 and/or LGR5, the ability to inhibit the interaction of RSPO2 and/or RSPO3 and an E3 ubiquitinase polypeptide, for example RNF43 and/or ZNRF3, and the ability to inhibit wnt signaling stimulated by RSPO2, RSPO3, RSPO2 polymorphisms, and/or RSPO2 translocation products, and subsets were identified.

some embodiments, the antibody binds to RSPO2. In some embodiments, the antibody binds to RSPO2 and do not significantly bind to RSPO3. In some embodiments, the antibody binds to RSPO3. In some embodiments, the antibody binds to RSPO3 and does not significantly bind to RSPO2. In some embodiments, the antibody binds to both RSPO2 and RSPO3. In some embodiments, the antibody is a multispecific antibody. In some embodiments, the multispecific antibody is a bispecific antibody. In some embodiments, the bispecific antibody comprises a first variable domain which binds to RSPO2 and a second variable domain which binds to RSPO3.

[0124] In certain embodiments, the antibody that binds to RSPO2 and/or RSPO3 is an antibody that binds RSPO2. In some embodiments, the anti-RSPO2 antibody binds RSPO2, wherein the RSPO2 has the sequence set forth in SEQ ID NO:1. In some embodiments, the anti-RSPO2 antibody binds RSPO2, wherein the RSPO2 lacks the signaling peptide sequence (*e.g.*, binds to amino acids within amino acids 22-243 of SEQ ID NO:1). In some embodiments, the anti-RSPO2 antibody binds to one or more furin-like cysteine-rich domains of RSPO2. In some embodiments, the anti-RSPO2 antibody binds a region within amino acids 34 to 134 of SEQ ID NO:1. In some embodiments, the anti-RSPO2 antibody binds a region within amino acids 39 to 134 of SEQ ID NO:1. In some embodiments, the anti-RSPO2 antibody binds a region within amino acids 34 to 84 of SEQ ID NO:1. In some embodiments, the anti-RSPO2 antibody binds a region within amino acids 90 to 134 of SEQ ID NO:1. In some embodiments, the anti-RSPO2 antibody does not bind to the thrombospondin type 1 domain of RSPO2 (*e.g.*, does not bind a region within amino acids 144-204 of SEQ ID NO:1). In some embodiments, the anti-RSPO2 antibody binds to the thrombospondin type 1 domain of RSPO2. In some embodiments, the anti-RSPO2 antibody binds a region within amino acids 144-204 of SEQ ID NO:1. In some embodiments, the anti-RSPO2 antibody inhibits wnt signaling. In some embodiments, the anti-RSPO2 antibody inhibits wnt signaling in an individual and/or cancer with an RSPO2 polymorphism (*e.g.*, RSPO2 L186P polymorphism). In some embodiments, the anti-RSPO2 antibody inhibits the interaction of RSPO2 and one or more of LGR4, LGR5, and/or LGR6. In some embodiments, the anti-RSPO2 antibody does not inhibit the interaction of RSPO2 and one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances binding of RSPO2 to one or more of LGR4, LGR5, and/or LGR6). In some embodiments, the anti-RSPO2 antibody inhibits the interaction of RSPO2 and a transmembrane E3 ubiquitinase (*e.g.*, one or more of ZNRF3 and/or RNF43). In some embodiments, the anti-RSPO2 antibody inhibits the interaction of RSPO2 with a syndecan (*e.g.*, Sdc4). In some embodiments, the anti-RSPO2 antibody inhibits the interaction of RSPO2 and one or more of LGR4, LGR5, and/or LGR6 and inhibits the interaction of RSPO2 and a transmembrane E3 ubiquitinase (*e.g.*, one or more of ZNRF3 and/or RNF43) (*e.g.*, 11F11, 36D2, 49G5, and/or 26E11). In some embodiments, the anti-RSPO2 antibody inhibits the interaction of RSPO2 and a transmembrane E3 ubiquitinase (*e.g.*, one or more of ZNRF3 and/or RNF43) and does not inhibit the interaction of

more of LGR4, LGR5, and/or LGR6) (*e.g.*, 1A1). In some embodiments, the anti-RSPO2 antibody inhibits cancer stem cell growth. In some embodiments, the anti-RSPO2 antibody induces and/or promotes cancer cell (*e.g.*, cancer stem cell) differentiation (*e.g.*, terminal differentiation and/or differentiation into progenitor cell). In some embodiments, the anti-RSPO3 antibody induces and/or promotes cancer cell (*e.g.*, cancer stem cell) differentiation into enterocyte, goblet cell, and/or enteroendocrine cell.

[0125] In certain embodiments, the antibody that binds to RSPO2 and/or RSPO3 is an antibody that binds RSPO3. In some embodiments, the anti-RSPO3 antibody binds RSPO3, wherein the RSPO3 has the sequence set forth in SEQ ID NO:2. In some embodiments, the anti-RSPO3 antibody binds RSPO3, wherein the RSPO3 lacks the signaling peptide sequence (*e.g.*, binds to amino acids within amino acids 22-272 of SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody binds to one or more furin-like cysteine-rich domains of RSPO3. In some embodiments, the anti-RSPO3 antibody binds a region within amino acids 35 to 135 of SEQ ID NO:2. In some embodiments, the anti-RSPO3 antibody binds a region within amino acids 35 to 86 of SEQ ID NO:2. In some embodiments, the anti-RSPO3 antibody binds to a region within amino acids 92 to 135 of SEQ ID NO:2. In some embodiments, the anti-RSPO3 antibody does not bind to the thrombospondin type 1 domain of RSPO3 (*e.g.*, does not bind amino acids within amino acids 147-207 of SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody binds to the thrombospondin type 1 domain of RSPO3. In some embodiments, the anti-RSPO3 antibody binds a region within amino acids 147-207 of SEQ ID NO:2. In some embodiments, the anti-RSPO3 antibody inhibits wnt signaling. In some embodiments, the anti-RSPO3 antibody inhibits the interaction of RSPO3 and one or more of LGR4, LGR5, and/or LGR6. In some embodiments, the anti-RSPO3 antibody does not inhibit the interaction of RSPO3 and one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances binding of RSPO3 to one or more of LGR4, LGR5, and/or LGR6). In some embodiments, the anti-RSPO3 antibody inhibits the interaction of RSPO3 and a transmembrane E3 ubiquitinase (*e.g.*, one or more of ZNRF3 and/or RNF43). In some embodiments, the anti-RSPO3 antibody inhibits the interaction of RSPO3 with a syndecan (*e.g.*, Sdc4). In some embodiments, the anti-RSPO3 antibody inhibits the interaction of RSPO3 and one or more of LGR4, LGR5, and/or LGR6 and inhibits the interaction of RSPO3 and a transmembrane E3 ubiquitinase (*e.g.*, one or more of ZNRF3 and/or RNF43). In some embodiments, the anti-RSPO3 antibody inhibits cancer stem cell growth. In some embodiments, the anti-RSPO3 antibody induces and/or promotes cancer cell (*e.g.*, cancer stem cell) differentiation (*e.g.*, terminal differentiation and/or differentiation into progenitor cell). In some embodiments, the anti-RSPO3 antibody induces and/or promotes cancer cell (*e.g.*, cancer stem cell) differentiation into a transit-amplifying cell. In some embodiments, the anti-RSPO3 antibody induces and/or promotes cancer cell (*e.g.*, cancer stem cell) differentiation into enterocyte, goblet cell, and/or enteroendocrine cell.

108 of SEQ ID NO:2. In some embodiments, the anti-RSPO3 antibody binds to an epitope comprising one or more amino acids selected from Ser49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys108 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody binds to an epitope comprising amino acids Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys108 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody binds to an epitope comprising amino acids residues of RSPO3 (*e.g.*, SEQ ID NO:2): Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys108. In some embodiments, the anti-RSPO3 antibody binds to an epitope comprising one or more amino acids selected from Ser49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, Lys97, and Lys108 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody binds to an epitope comprising amino acids Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, Lys97, and Lys108 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 4 angstroms or less from one or more amino acids Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys108 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 4 angstroms or less from amino acids residues of RSPO3 (*e.g.*, SEQ ID NO:2): Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys108. In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 4 angstroms or less from one or more amino acids Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, Lys97, and Lys108 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 4 angstroms or less from amino acids residues of RSPO3 (*e.g.*, SEQ ID NO:2): Ser 49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, Lys97, and Lys108. In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 3.5 angstroms or less from one or more amino acids Asn 52, Leu55, Phe63, Gln72, Tyr89, Pro90, Asp91, Lys94, and Lys97 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 3.5 angstroms or less from Asn 52, Leu55, Phe63, Gln72, Tyr89, Pro90, Asp91, Lys94, and Lys97 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 3 angstroms or less from one or more amino acids Gln72, Pro90, Asp91, and Lys94 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 3 angstroms or less from Gln72, Pro90, Asp91, and Lys94 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned about

embodiments, the one or more amino acids and/or the one or more amino acid residues is about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 amino acids and/or amino acid residues. In some embodiments, the epitope is determined by crystallography (*e.g.*, crystallography methods described in the Examples).

[0127] In some embodiments, the anti-RSPO3 antibody binds to amino acids within amino acids 47 to 108 of SEQ ID NO:2. In some embodiments, the anti-RSPO3 antibody binds to an epitope comprising one or more amino acids selected from Thr47, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Tyr84, Tyr89, Pro90, Asp91, Ile92, Asn93, Lys94, Lys97, and Lys108 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody binds to an epitope comprising amino acids Thr47, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Tyr84, Tyr89, Pro90, Asp91, Ile92, Asn93, Lys94, Lys97, and Lys108 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody binds to an epitope comprising amino acids residues of RSPO3 (*e.g.*, SEQ ID NO:2): Thr47, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Tyr84, Tyr89, Pro90, Asp91, Ile92, Asn93, Lys94, Lys97, and Lys108. In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 4 angstroms or less from one or more amino Thr47, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Tyr84, Tyr89, Pro90, Asp91, Ile92, Asn93, Lys94, Lys97, and Lys108 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 4 angstroms or less from amino acids residues of RSPO3 (*e.g.*, SEQ ID NO:2): Thr47, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Tyr84, Tyr89, Pro90, Asp91, Ile92, Asn93, Lys94, Lys97, and Lys108. In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 3.5 angstroms or less from one or more amino acids Thr47, Asn52, Leu55, Phe63, Gln72, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys97 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 3.5 angstroms or less from amino acids Thr47, Asn52, Leu55, Phe63, Gln72, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys97 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 3 angstroms or less from one or more amino acids Thr47, Leu55, Gln72, Pro90, Asp91, and Lys94 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned 3 angstroms or less from amino acids Thr47, Leu55, Gln72, Pro90, Asp91, and Lys94 of RSPO3 (*e.g.*, SEQ ID NO:2). In some embodiments, the anti-RSPO3 antibody when bound to RSPO3 is positioned about any of 4, 3.75, 3.5, 3.25, or 3 angstroms from one or more amino acids provided above. In some embodiments, the one or more amino acids and/or the one or more amino acid residues is about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 amino acids and/or amino acid residues. In some embodiments, the anti-RSPO3 antibody also binds RSPO2. In some embodiments, the epitope is determined by crystallography (*e.g.*, crystallography methods described in the Examples).

acids M33-E210 of RSPO3. In some embodiments, the epitope as determined by crystallography is performed by using an Labcyte Echo liquid handler to set several sparse matrix crystal screens using 100 nL sitting drops. Screens were stored at 18°C. In some embodiments, crystals may be obtained in a drop containing 100 mM MIB pH 9 and 25% PEG 1500 as the mother liquor. In some embodiments, crystals may be obtained in a drop containing 200 mM Sodium formate and 20% (w/v) PEG 3,350 as the mother liquor. In some embodiments, the crystal may be harvested and soaked in cryoprotectant solution for 10 seconds and flash-frozen in liquid nitrogen. In some embodiments, the cryoprotectant solution may be made by mixing 1 µL 70% glycerol with 1.8µL reservoir solution. In some embodiments, the crystals may be grown in PEG-based conditions, for example, about 20-25% PEG 3,350. In some embodiments, the crystals may be grown in about 20% PEG 6,000, about 20-25% PEG 4,000, and about 25% PEG 1,500. In some embodiments, the pH may range from about 3.5 – 9, for example, between about 7 and about 8. In some embodiments, the salt concentration is about 200 mM.

[0129] In certain embodiments, the antibody that binds to RSPO2 and/or RSPO3 is an antibody that binds RSPO2 and RSPO3 (*e.g.*, anti-RSPO2/3 antibody). In some embodiments, the anti-RSPO2/3 antibody binds RSPO2, wherein the RSPO2 has the sequence set forth in SEQ ID NO:1 and binds RSPO3, wherein the RSPO3 has the sequence set forth in SEQ ID NO:2. In some embodiments, the anti-RSPO2/3 antibody inhibits wnt signaling.

[0130] In some aspects, antibodies were identified that were able to cross react with both RSPO2 and RSPO3. A nonlimiting example of activities of these anti-RSPO2/3 antibodies may include the ability to bind to RSPO2 and RSPO3, detect RSPO2 and RSPO3 by IHC, inhibit the interaction of RSPO2 and RSPO3 and an LGR polypeptide, for example LGR4 and/or LGR5, inhibit the interaction of RSPO2 and RSPO3 and an E3 ubiquitinase polypeptide, for example RNF43 and/or ZNRF3, and/or inhibit wnt signaling stimulated by RSPO2, RSPO3, RSPO2 polymorphisms, and RSPO2 translocation products.

[0131] One skilled in the art would further appreciate that in some embodiments, any anti-RSPO2 antibody and/or anti-RSPO3 antibody could be engineered into an antibody format, in particular bispecific format, which would allow reactivity with both RSPO2 and RSPO3. These anti-RSPO2/3 bispecific antibodies be able to may include the ability to bind to RSPO2 and RSPO3, detect RSPO2 and RSPO3 by IHC, inhibit the interaction of RSPO2 and RSPO3 and an LGR polypeptide, for example LGR4 and/or LGR5, inhibit the interaction of RSPO2 and RSPO3 and an E3 ubiquitinase polypeptide, for example, RNF43 and/or ZNRF3, and/or inhibit wnt signaling stimulated by RSPO2, RSPO3, RSPO2 polymorphisms, and RSPO2 translocation products.

[0132] In some embodiments of any of the anti-RSPO2/3 antibodies, the anti-RSPO2/3 antibody inhibits the interaction of RSPO2 and RSPO3 with one or more of LGR4, LGR5, and/or LGR6. In some embodiments, the antibody is a dual arm antibody. In some embodiments, the anti-RSPO2/3

HVRs of 26E11. In some embodiments, the antibody is a bispecific antibody. In some embodiments, the anti-RSPO2/3 antibody comprises a first variable domain comprising the six HVRs of 5D6 or 5E11 and a second variable domain comprising the six HVRs of 36D2.

[0133] In some embodiments of any of the anti-RSPO2/3 antibodies, the anti-RSPO2/3 antibody inhibits the interaction of RSPO3 and one or more of LGR4, LGR5, and/or LGR6 and does not inhibit the interaction of RSPO2 and one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances binding of RSPO2 to one or more of LGR4, LGR5, and/or LGR6). In some embodiments, the antibody is a bispecific antibody. In some embodiments, the anti-RSPO2/3 antibody comprises a first variable domain comprising the six HVRs of 5D6 or 5E11 and a second variable domain comprising the six HVRs of 1A1.

[0134] In some embodiments of any of the anti-RSPO2/3 antibodies, the anti-RSPO2/3 antibody inhibits the interaction of RSPO2 and RSPO3 with a transmembrane E3 ubiquitinase (*e.g.*, one or more of ZNRF3 and/or RNF43). In some embodiments, the antibody is a dual arm antibody. In some embodiments, the anti-RSPO2/3 antibody comprises a first and second variable domain comprising on each variable domain the six HVRs of 26E11. In some embodiments, the antibody is a bispecific antibody. In some embodiments, the anti-RSPO2/3 antibody comprises a first variable domain comprising the six HVRs of 5D6 or 5E11 and a second variable domain comprising the six HVRs of 36D2 or 1A1.

[0135] In some embodiments of any of the anti-RSPO2/3 antibodies, the anti-RSPO2/3 antibody inhibits the interaction of RSPO2 and RSPO3 with one or more of LGR4, LGR5, and/or LGR6 and RSPO2 and inhibits the interaction of RSPO2 and RSPO3 with a transmembrane E3 ubiquitinase (*e.g.*, one or more of ZNRF3 and/or RNF43). In some embodiments, the antibody is a dual arm antibody. In some embodiments, the anti-RSPO2/3 antibody comprises a first and second variable domain comprising on each variable domain the six HVRs of 26E11. In some embodiments, the antibody is a bispecific antibody. In some embodiments, the anti-RSPO2/3 antibody comprises a first variable domain comprising the six HVRs of 5D6 or 5E11 and a second variable domain comprising the six HVRs of 36D2.

[0136] In some embodiments of any of the anti-RSPO2/3 antibodies, the anti-RSPO2/3 antibody inhibits the interaction of RSPO3 and one or more of LGR4, LGR5, and/or LGR6 and RSPO2 and inhibits the interaction of RSPO2 and RSPO3 with a transmembrane E3 ubiquitinase (*e.g.*, one or more of ZNRF3 and/or RNF43) and does not inhibit the interaction of RSPO2 and one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances binding of RSPO2 to one or more of LGR4, LGR5, and/or LGR6). In some embodiments, the antibody is a bispecific antibody. In some embodiments, the anti-RSPO2/3 antibody comprises a first variable domain comprising the six HVRs of 5D6 or 5E11 and a second variable domain comprising the six HVRs of 1A1.

epitope as one or more of the antibodies 4H1, 4D4, 5C2, 5D6, 5E11, 6E9, 21C2, and/or 26E11. Further, in one aspect provided herein are anti-RSPO3 antibodies that compete for binding to RSPO3 with one or more of the antibodies 4H1, 4D4, 5C2, 5D6, 5E11, 6E9, 21C2, and/or 26E11. In one aspect provided herein are anti-RSPO2 antibodies that bind the same or overlapping epitope as one or more of antibodies 1A1, 11F11, 26E11, 36D2, and/or 49G5. Further, in one aspect provided herein are anti-RSPO2 antibodies that compete for binding to RSPO2 with one or more of antibodies 1A1, 11F11, 26E11, 36D2, and/or 49G5. In one aspect provided herein are anti-RSPO2 antibodies that bind the same or overlapping epitope as 1A1. Further, in one aspect provided herein are anti-RSPO2 antibodies that compete for binding to RSPO2 with 1A1. In some embodiments, the antibody competes for binding with another antibody by BIACORE, competitive ELISA, and/or any other methods described herein and known in the art. Methods of determining epitopes are known in the art and described herein. In some embodiments, the epitope is a linear epitope. In some embodiments, the epitope is a conformational epitope. In some embodiments, the epitope is determined by antibody binding to peptide fragments. In some embodiments, the epitope is determined by mass spectrometry. In some embodiments, the epitope is determined by crystallography (*e.g.*, analysis of crystal structure).

Monoclonal Antibody 4H1 and Certain Other Antibody Embodiments

[0138] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:9; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7.

[0139] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:9; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:10. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:10 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:7. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:10, HVR-L3 comprising the amino acid sequence of SEQ ID NO:7, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:9. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:9; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10.

all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7.

[0141] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:9, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:10; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7.

[0142] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:9; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:7.

[0143] In another aspect, an anti-RSPO3 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:89 and SEQ ID NO:90, respectively, including post-translational modifications of those sequences.

Monoclonal Antibody 4D4 and Certain Other Antibody Embodiments

[0144] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:14; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:16; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:11; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:13.

[0145] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:14; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:16. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:16. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:16 and HVR-L3

comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:16, HVR-L3 comprising the amino acid sequence of SEQ ID NO:13, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:15. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:14; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:16.

[0146] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:11; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:13. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:11; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:13.

[0147] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:14, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:15, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:16; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:11, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:12, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:13.

[0148] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:14; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:16; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:11; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:13.

[0149] In another aspect, an anti-RSPO3 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:91 and SEQ ID NO:92, respectively, including post-translational modifications of those sequences.

Monoclonal Antibody 5C2 and Certain Other Antibody Embodiments

[0150] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:21; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:22; (d) HVR-L1 comprising the amino acid

(f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

[0151] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:21; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:22. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:22. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:22 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:19. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:22, HVR-L3 comprising the amino acid sequence of SEQ ID NO:19, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:21. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:21; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:22.

[0152] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:17; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:18; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:17; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:18; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

[0153] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:21, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:22; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:17, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:18, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19.

[0154] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:21; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:22; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:17; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:18; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:19.

[0155] In another aspect, an anti-RSPO3 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above.

NO:94, respectively, including post-translational modifications of those sequences.

Monoclonal Antibody 5D6 and Certain Other Antibody Embodiments

[0156] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25.

[0157] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:28. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:28 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:25. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:28, HVR-L3 comprising the amino acid sequence of SEQ ID NO:25, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:27. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28.

[0158] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25.

[0159] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:28; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25.

amino acid sequence of SEQ ID NO:26; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:25.

[0161] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:188 or SEQ ID NO:189; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, HVR-H3 comprises the amino acid sequence of SEQ ID NO:188. In some embodiments, HVR-H3 comprises the amino acid sequence of SEQ ID NO:189.

[0162] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:188 or SEQ ID NO:189. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:188 or SEQ ID NO:189. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:188 or SEQ ID NO:189 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:25. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:188 or SEQ ID NO:189, HVR-L3 comprising the amino acid sequence of SEQ ID NO:25, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:27. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:188 or SEQ ID NO:189. In some embodiments, HVR-H3 comprises the amino acid sequence of SEQ ID NO:188. In some embodiments, HVR-H3 comprises the amino acid sequence of SEQ ID NO:189.

[0163] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25.

one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:188 or SEQ ID NO:189; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, HVR-H3 comprises the amino acid sequence of SEQ ID NO:188. In some embodiments, HVR-H3 comprises the amino acid sequence of SEQ ID NO:189.

[0165] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:188 or SEQ ID NO:189; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:25. In some embodiments, HVR-H3 comprises the amino acid sequence of SEQ ID NO:188. In some embodiments, HVR-H3 comprises the amino acid sequence of SEQ ID NO:189.

[0166] In another aspect, an anti-RSPO3 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:95 and SEQ ID NO:96, respectively, including post-translational modifications of those sequences.

[0167] In any of the above embodiments, an anti-RSPO3 antibody is humanized. In one embodiment, an anti-RSPO3 antibody comprises HVRs as in any of the above embodiments, and further comprises a human acceptor framework, *e.g.* a human immunoglobulin framework or a human consensus framework. In certain embodiments, the human acceptor framework is the human VL kappa I consensus (VL_{KI}) framework and/or the VH framework VH_I. In certain embodiments, the human acceptor framework is the human VL kappa I consensus (VL_{KI}) framework and/or the VH framework VH_I comprising any one of the following mutations.

[0168] In another aspect, an anti-RSPO3 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, or 215. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO:191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, or 215 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-RSPO3 antibody comprising that sequence retains the ability to bind to RSPO3. In certain embodiments, a total of 1 to 10 amino acids

207, 209, 211, 213, or 215. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, or 215. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Optionally, the anti-RSPO3 antibody comprises the VH sequence of SEQ ID NO:191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, or 215, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28, SEQ ID NO:188, or SEQ ID NO:189.

[0169] In another aspect, an anti-RSPO3 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, or 214. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO:190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, or 214 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-RSPO3 antibody comprising that sequence retains the ability to bind to RSPO3. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, or 214. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, or 214. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Optionally, the anti-RSPO3 antibody comprises the VL sequence of SEQ ID NO:190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, or 214, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25.

[0170] In another aspect, an anti-RSPO3 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above.

[0171] In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:190 and SEQ ID NO:191, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:192 and SEQ ID NO:193, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:194 and SEQ ID NO:195, respectively, including post-translational modifications of those sequences. In one

NO:197, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:198 and SEQ ID NO:199, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:200 and SEQ ID NO:201, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:202 and SEQ ID NO:203, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:204 and SEQ ID NO:205, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:206 and SEQ ID NO:207, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:208 and SEQ ID NO:209, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:210 and SEQ ID NO:211, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:212 and SEQ ID NO:213, respectively, including post-translational modifications of those sequences. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:214 and SEQ ID NO:215, respectively, including post-translational modifications of those sequences.

[0172] In a further aspect, provided are herein are antibodies that bind to the same epitope as an anti-RSPO3 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-RSPO3 antibody comprising a VH sequence of SEQ ID NO: 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, or 215 and a VL sequence of SEQ ID NO:190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, or 214, respectively. In some embodiments, the epitope is determined by crystallography.

[0173] In a further aspect of the invention, an anti-RSPO3 antibody according to any of the above embodiments is a monoclonal antibody, including a human antibody. In one embodiment, an anti-RSPO3 antibody is an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody, or F(ab')₂ fragment. In another embodiment, the antibody is a substantially full length antibody, *e.g.*, an IgG1 antibody, IgG2a antibody or other antibody class or isotype as defined herein.

Monoclonal Antibody 5E11 and Certain Other Antibody Embodiments

[0174] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:32; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:34; (d) HVR-L1 comprising the amino acid

(f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:31.

[0175] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:32; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:34. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:34. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:34 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:31. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:34, HVR-L3 comprising the amino acid sequence of SEQ ID NO:31, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:33. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:32; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:34.

[0176] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:29; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:30; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:31. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:29; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:30; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:31.

[0177] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:32, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:34; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:29, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:30, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:31.

[0178] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:32; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:34; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:29; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:30; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:31.

[0179] In another aspect, an anti-RSPO3 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above.

NO:98, respectively, including post-translational modifications of those sequences.

Monoclonal Antibody 6E9 and Certain Other Antibody Embodiments

[0180] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:38; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:40; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:35; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:36; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:37.

[0181] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:38; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:40. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:40. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:40 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:37. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:40, HVR-L3 comprising the amino acid sequence of SEQ ID NO:37, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:39. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:38; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:40.

[0182] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:35; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:36; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:37. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:35; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:36; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:37.

[0183] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:38, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:40; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:35, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:36, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:37.

amino acid sequence of SEQ ID NO:38; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:40; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:35; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:36; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:37.

[0185] In another aspect, an anti-RSPO3 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:99 and SEQ ID NO:100, respectively, including post-translational modifications of those sequences.

Monoclonal Antibody 21C2 and Certain Other Antibody Embodiments

[0186] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:44; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:45; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:46; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:41; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:42; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:43.

[0187] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:44; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:45; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:46. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:46. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:46 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:43. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:46, HVR-L3 comprising the amino acid sequence of SEQ ID NO:43, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:45. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:44; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:45; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:46.

[0188] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:41; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:42; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:43. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:41; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:42; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:43.

one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:44, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:45, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:46; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:41, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:42, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:43.

[0190] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:44; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:45; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:46; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:41; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:42; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:43.

[0191] In another aspect, an anti-RSPO3 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:101 and SEQ ID NO:102, respectively, including post-translational modifications of those sequences.

Monoclonal Antibody 26E11 and Certain Other Antibody Embodiments

[0192] In one aspect, the invention provides an anti-RSPO2/3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:52; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:47; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:48; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:49.

[0193] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:51; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:52. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:52. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:52 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:49. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:52, HVR-L3 comprising the amino acid sequence of SEQ ID NO:49, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:51. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:51; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:52.

all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:47; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:48; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:49. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:47; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:48; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:49.

[0195] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:50, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:51, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:52; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:47, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:48, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:49.

[0196] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:52; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:47; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:48; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:49.

[0197] In another aspect, an anti-RSPO2/3 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:103 and SEQ ID NO:104, respectively, including post-translational modifications of those sequences.

Anti-RSPO3 Monoclonal Antibodies and Certain Other Antibody Embodiments

[0198] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:80; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:81; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:82; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:77; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:78; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:79.

[0199] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:80; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:81; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:82. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:82. In another embodiment, the

comprising the amino acid sequence of SEQ ID NO:79. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:82, HVR-L3 comprising the amino acid sequence of SEQ ID NO:79, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:81. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:80; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:81; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:82.

[0200] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:77; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:78; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:79. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:77; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:78; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:79.

[0201] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:80, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:81, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:82; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:77, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:78, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:79.

[0202] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:80; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:81; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:82; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:77; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:78; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:79.

[0203] In one aspect, the invention provides an anti-RSPO3 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:86; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:87; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:88; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:83; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:84; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:85.

[0204] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:86; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:87; and (c) HVR-H3

HVR-H3 comprising the amino acid sequence of SEQ ID NO:88. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:88 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:85. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:88, HVR-L3 comprising the amino acid sequence of SEQ ID NO:85, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:87. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:86; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:87; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:88.

[0205] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:83; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:84; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:85. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:83; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:84; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:85.

[0206] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:86, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:87, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:88; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:83, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:84, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:85.

[0207] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:86; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:87; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:88; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:83; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:84; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:85.

Monoclonal Antibody 1A1 and Certain Other Antibody Embodiments

[0208] In one aspect, the invention provides an anti-RSPO2 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:56; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:57; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:58; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:53; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:54; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:55.

three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:56; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:57; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:58. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:58. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:58 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:55. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:58, HVR-L3 comprising the amino acid sequence of SEQ ID NO:55, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:57. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:56; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:57; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:58.

[0210] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:55. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:53; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:55.

[0211] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:56, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:57, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:58; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:53, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:54, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:55.

[0212] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:56; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:57; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:58; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:53; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:54; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:55.

[0213] In another aspect, an anti-RSPO2 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:105 and SEQ ID NO:106, respectively, including post-translational modifications of those sequences.

[0214] In one aspect, the invention provides an anti-RSPO2 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:62; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:63; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:64; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:59; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:60; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:61.

[0215] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:62; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:63; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:64. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:64. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:64 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:61. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:64, HVR-L3 comprising the amino acid sequence of SEQ ID NO:61, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:63. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:62; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:63; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:64.

[0216] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:59; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:60; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:61. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:59; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:60; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:61.

[0217] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:62, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:63, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:64; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:59, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:60, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:61.

[0218] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:62; (b) HVR-H2 comprising the amino acid sequence of SEQ ID

the amino acid sequence of SEQ ID NO:59; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:60; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:61.

[0219] In another aspect, an anti-RSPO2 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:107 and SEQ ID NO:108, respectively, including post-translational modifications of those sequences.

Monoclonal Antibody 36D2 and Certain Other Antibody Embodiments

[0220] In one aspect, the invention provides an anti-RSPO2 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:68; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:69; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:70; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:65; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:66; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:67.

[0221] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:68; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:69; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:70. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:70. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:70 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:67. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:70, HVR-L3 comprising the amino acid sequence of SEQ ID NO:67, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:69. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:68; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:69; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:70.

[0222] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:65; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:66; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:67. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:65; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:66; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:67.

[0223] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:68, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID

VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:65, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:66, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:67.

[0224] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:68; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:69; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:70; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:65; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:66; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:67.

[0225] In another aspect, an anti-RSPO2 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:109 and SEQ ID NO:110, respectively, including post-translational modifications of those sequences.

Monoclonal Antibody 49G5 and Certain Other Antibody Embodiments

[0226] In one aspect, the invention provides an anti-RSPO2 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:74; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:75; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:76; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:71; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:72; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:73.

[0227] In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:74; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:75; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:76. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:76. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:76 and HVR-L3 comprising the amino acid sequence of SEQ ID NO:73. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO:76, HVR-L3 comprising the amino acid sequence of SEQ ID NO:73, and HVR-H2 comprising the amino acid sequence of SEQ ID NO:75. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:74; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:75; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:76.

[0228] In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:71; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:72; and (c) HVR-L3

(a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:71; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:72; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:73.

[0229] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:74, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:75, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:76; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:71, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:72, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:73.

[0230] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:74; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:75; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:76; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:71; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:72; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:73.

[0231] In another aspect, an anti-RSPO2 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:111 and SEQ ID NO:112, respectively, including post-translational modifications of those sequences.

[0232] In any of the above embodiments, an anti-RSPO antibody is humanized. For example, humanized forms of any of the above anti-RSPO antibodies. In one embodiment, an anti-RSPO antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, *e.g.* a human immunoglobulin framework or a human consensus framework.

[0233] In a further aspect of the invention, an anti-RSPO antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-RSPO antibody is an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody, or F(ab')₂ fragment. In another embodiment, the antibody is a full length antibody, *e.g.*, an intact IgG1 or IgG2a antibody or other antibody class or isotype as defined herein.

[0234] In a further aspect, an anti-RSPO antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

1. Antibody Affinity

[0235] In certain embodiments, an antibody provided herein has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (*e.g.* 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M).

embodiment, an RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (*see, e.g.,* Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER[®] multi-well plates (Thermo Scientific) are coated overnight with 5 $\mu\text{g}/\text{ml}$ of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.,* consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.,* about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.,* for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20[®]) in PBS. When the plates have dried, 150 μl /well of scintillant (MICROSCINT-20[™]; Packard) is added, and the plates are counted on a TOPCOUNT[™] gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0237] According to another embodiment, K_d is measured using a BIACORE[®] surface plasmon resonance assay. For example, an assay using a BIACORE[®]-2000 or a BIACORE[®]-3000 (BIAcore, Inc., Piscataway, NJ) is performed at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). In one embodiment, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 $\mu\text{g}/\text{ml}$ (~0.2 μM) before injection at a flow rate of 5 $\mu\text{l}/\text{minute}$ to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20[™]) surfactant (PBST) at 25°C at a flow rate of approximately 25 $\mu\text{l}/\text{min}$. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE[®] Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) is calculated as the ratio $k_{\text{off}}/k_{\text{on}}$. *See, e.g.,* Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds $10^6 \text{ M}^{-1} \text{ s}^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295

PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. *Antibody Fragments*

[0238] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')₂, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, *see* Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, *see, e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); *see also* WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, *see* U.S. Patent No. 5,869,046.

[0239] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. *See*, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0240] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see, e.g.*, U.S. Patent No. 6,248,516).

[0241] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g.* *E. coli* or phage), as described herein.

3. *Chimeric and Humanized Antibodies*

[0242] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0243] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human

antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0244] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0245] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (*see, e.g.*, Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (*see, e.g.*, Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (*see, e.g.*, Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (*see, e.g.*, Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

[0246] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[0247] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, *see* Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). *See also, e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSETM technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870

2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.*, by combining with a different human constant region.

[0248] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, *e.g.*, Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0249] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

[0250] Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0251] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as

without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J*, 12: 725-734 (1993).

Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0252] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0253] In certain embodiments, an antibody provided herein is a multispecific antibody, *e.g.* a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is RSPO (*e.g.*, RSPO2 and/or RSPO3). and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of RSPO. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express RSPO (*e.g.*, RSPO2 and/or RSPO3). In some embodiments, the multispecific antibody (*e.g.*, bispecific antibody) binds to RSPO2 and RSPO3. In some embodiments, the multispecific antibody (*e.g.*, bispecific antibody) comprises a first variable domain comprising the HVRs of 5E11 and a second variable domain comprising the HVRs of 36D2. In some embodiments, the multispecific antibody (*e.g.*, bispecific antibody) comprises a first variable domain comprising the HVRs of 5D6 and a second variable domain comprising the HVRs of 36D2. In some embodiments, the multispecific antibody (*e.g.*, bispecific antibody) comprises a first variable domain comprising the HVRs of 5E11 and a second variable domain comprising the HVRs of 1A1. In some embodiments, the multispecific antibody (*e.g.*, bispecific antibody) comprises a first variable domain comprising the HVRs of 5D6 and a second variable domain comprising the HVRs of 1A1. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0254] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (*see* Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J*. 10: 3655 (1991)), and “knob-in-hole” engineering (*see, e.g.*, U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine

(1992)); using "diabody" technology for making bispecific antibody fragments (*see, e.g.*, Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (*see, e.g.* Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt et al. *J. Immunol.* 147: 60 (1991).

[0255] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (*see, e.g.* US 2006/0025576).

[0256] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to multiple RSPOs (*e.g.*, RSPO2 and/or RSPO3) (*see*, US 2008/0069820, for example).

7. Antibody Variants

[0257] In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

a) Substitution, Insertion, and Deletion Variants

[0258] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn

Residue	Substitutions	Substitutions
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0259] Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

[0260] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0261] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[0262] Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR “hotspots,” *i.e.*, residues encoded by codons that undergo mutation at

207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0263] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may, for example, be outside of antigen contacting residues in the HVRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0264] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0265] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

[0266] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0267] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. *See, e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0268] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about $\pm$ 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See, e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (*see, e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[0270] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.*, a substitution) at one or more amino acid positions.

[0271] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII) only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see, for example*, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo, e.g.*, in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see, for*

101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (*see, e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0272] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called “DANA” Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581). In some embodiments, the antibody comprises an engineered alanine at amino acid position 265 according to EU numbering convention. In some embodiments, the antibody comprises an engineered alanine at amino acid position 297 according to EU numbering convention.

[0273] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0274] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0275] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[0276] Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826). *See also* Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

[0277] In certain embodiments, it may be desirable to create cysteine engineered antibodies, *e.g.*, “thioMAbs,” in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug

embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

[0278] In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(*n*-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0279] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

[0280] Antibodies may be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-RSPO antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (*e.g.*, the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (*e.g.*, expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (*e.g.*, has been

comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, *e.g.* a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-RSPO antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0281] For recombinant production of an anti-RSPO antibody, nucleic acid encoding an antibody, *e.g.*, as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0282] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, *see, e.g.*, U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (*See also* Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0283] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized,” resulting in the production of an antibody with a partially or fully human glycosylation pattern. *See* Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

[0284] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

[0285] Plant cell cultures can also be utilized as hosts. *See, e.g.*, US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic plants).

to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.*, in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.*, in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, *e.g.*, in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR⁻ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, *see, e.g.*, Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C. Assays

Anti-RSPO antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

[0287] In one aspect, an antibody of the invention is tested for its antigen binding activity, *e.g.*, by known methods such as ELISA, Western blot, etc.

[0288] Methods of determining binding affinity are known in the art. In some embodiments, the binding affinity may be determined according to a BIAcore® assay as described herein in Example 1. Specifically, in some embodiments, K_d may be measured using surface plasmon resonance assays using a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ).

[0289] Methods of determining the ability of an anti-RSPO antibody to disrupt and/or inhibit the binding of an RSPO to LGR (*e.g.*, LGR4, 5, and/or 6), syndecan (*e.g.*, SDC4), and/or an E3 ubiquitinase (*e.g.*, ZNRF3 and/or RNF43) are known in the art. *See e.g.*, WO2011/076932, WO2013012747, Lau et al. *Nature* 476:293-297 (2011), Hao et al. *Nature* 485:195-200 (2012), which are hereby incorporated by reference in their entirety. In some embodiments, the ability of an anti-RSPO antibody to significantly disrupt the binding of an R-spondon (RSPO) to an LGR, syndecan and/or E3 ubiquitinase may be determined by flow cytometry, BIAcore assay, and/or ELISA (*e.g.*, Competitive Binding ELISA). In some embodiments, the ability of an anti-RSPO antibody to disrupt and/or inhibit the binding of an RSPO to LGR (*e.g.*, LGR4, 5, and/or 6), syndecan (SDC4), and/or an E3 ubiquitinase (*e.g.*, ZNRF3 and/or RNF43) may be determined according to a Competitive Binding ELISA as described herein in Example 1.

4H1, 4D4, 5C2, 5D6, 5E11, 6E9, 21C2, 26E11, 1A1, 11F11, 36D2, and/or 49G5 for binding to RSPO (*e.g.*, RSPO2 and/or RSPO3).

[0291] Methods of determining antibody competition are known in the art. In an exemplary competition assay, immobilized RSPO (*e.g.*, RSPO2 and/or RSPO3) is incubated in a solution comprising a first labeled antibody that binds to RSPO (*e.g.*, RSPO2 and/or RSPO3) (*e.g.*, 4H1, 4D4, 5C2, 5D6, 5E11, 6E9, 21C2, 26E11, 1A1, 11F11, 36D2, and/or 49G5) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to RSPO (*e.g.*, RSPO2 and/or RSPO3). The second antibody may be present in a hybridoma supernatant. As a control, immobilized RSPO (*e.g.*, RSPO2 and/or RSPO3) is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to RSPO (*e.g.*, RSPO2 and/or RSPO3), excess unbound antibody is removed, and the amount of label associated with immobilized RSPO (*e.g.*, RSPO2 and/or RSPO3) is measured. If the amount of label associated with immobilized RSPO (*e.g.*, RSPO2 and/or RSPO3) is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to RSPO (*e.g.*, RSPO2 and/or RSPO3). See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[0292] Another exemplar competition assay is described in the Example 1 useful for epitope binning and/or determining whether two antibodies compete for binding. In some embodiments, epitope binning and/or determining whether two antibodies compete for binding may be determined according to a Octet® assay as described herein in Example 1.

[0293] In certain embodiments, an antibody binds to the same epitope (*e.g.*, a linear or a conformational epitope) that is bound 4H1, 4D4, 5C2, 5D6, 5E11, 6E9, 21C2, 26E11, 1A1, 11F11, 36D2, and/or 49G5. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ). In some embodiments, the epitope is determined by peptide competition. In some embodiments, the epitope is determined by mass spectrometry. In some embodiments, the epitope is determined by crystallography. An exemplary method of crystallography is described in Example 1.

2. Activity assays

[0294] In one aspect, assays are provided for identifying anti-RSPO antibodies thereof having biological activity. Biological activity may include, *e.g.*, inhibit wnt signaling, inhibit angiogenesis, inhibit cell proliferation, inhibit cancer stem cell proliferation, and/or deplete cancer stem cells. Antibodies having such biological activity in vivo and/or in vitro are also provided.

are known in the art. *See e.g.*, WO2005/040418 and WO2013/012747, which is hereby incorporated by reference in its entirety. In some embodiments, the ability of an anti-RSPO antibody to significantly disrupt wnt/beta-catenin signaling may be determined using a reporter gene assay. In some embodiments, for example, a reporter construct comprising a reporter gene (such as, for example, a luciferase gene) under the control of a wnt/beta-catenin responsive promoter (such as, for example, a promoter comprising multimerized TCF/LEF DNA-binding sites) may be transfected into cells. The cells are then contacted with a Wnt ligand, such as Wnt3a, and an RSPO, such as RSPO1, RSPO2, RSPO3, and/or RSPO4, in the presence and absence of an RSPO antibody, and luciferase expression is measured.

[0296] Methods of determining ability of an anti-RSPO antibody inhibiting angiogenesis and/or vasculogenesis are known in the art. *See e.g.*, WO2008/046649, which is hereby incorporated by reference in its entirety. Examples of assays include the in vivo Matrigel plug and corneal neovascularization assays, the in vivo/in vitro chick chorioallantoic membrane (CAM) assay, the in vitro cellular (proliferation, migration, tube formation) and organotypic (aortic ring) assays, the chick aortic arch assays, and the Matrigel sponge assays.

[0297] Methods of determining the ability of an anti-RSPO antibody to induce stem cell differentiation and/or cancer stem cell depletion are known in the art. *See e.g.*, WO2013/036867, which is hereby incorporated by reference in its entirety. In some embodiments, stem cell differentiation may be assayed by determining ability to differentiation of crypt base columnar cells (CBCs), which are fast-cycling stem cells in the small intestine, into, for example, enterocytes, goblet cells, and/or enteroendocrine cells, in the presence and absence of an anti-RSPO antibody.

[0298] In certain embodiments, an antibody of the invention is tested for such biological activity and/or binding interactions by the assays described herein and in WO2005/040418, WO2008/046649, WO2011/076932, WO2013/012747, WO2013/054307, Lau et al. *Nature* 476:293-297 (2011), Hao et al. *Nature* 485:195-200 (2012), which are hereby incorporated by reference in their entirety.

[0299] In some embodiment, the epitope is determined by crystallography. In some embodiments, the epitope as determined by crystallography is determined using amino acids M33-E210 of RSPO3. In some embodiments, the epitope as determined by crystallography is performed by using an Labcyte Echo liquid handler to set several sparse matrix crystal screens using 100 nL sitting drops. Screens were stored at 18°C. In some embodiments, crystals may be obtained in a drop containing 100 mM MIB pH 9 and 25% PEG 1500 as the mother liquor. In some embodiments, crystals may be obtained in a drop containing 200 mM Sodium formate and 20% (w/v) PEG 3,350 as the mother liquor. In some embodiments, the crystal may be harvested and soaked in cryoprotectant solution for 10 seconds and flash-frozen in liquid nitrogen. In some embodiments, the cryoprotectant solution may be made by mixing 1 µL 70% glycerol with 1.8µL reservoir solution. In some embodiments, the crystals may be

crystals may be grown in about 20% PEG 6,000, about 20-25% PEG 4,000, and about 25% PEG 1,500. In some embodiments, the pH may range from about 3.5 – 9, for example, between about 7 and about 8. In some embodiments, the salt concentration is about 200 mM.

D. Immunoconjugates

[0300] The invention also provides immunoconjugates comprising an anti-RSPO antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0301] In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (*see* U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (*see* U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (*see* U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (*see* Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0302] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0303] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging,

oxygen-17, gadolinium, manganese or iron.

[0304] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a “cleavable linker” facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0305] The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

E. Methods and Compositions for Diagnostics and Detection

[0306] In certain embodiments, any of the anti-RSPO antibodies provided herein is useful for detecting the presence of RSPO in a sample. The term “detecting” as used herein encompasses quantitative or qualitative detection. In certain embodiments, a sample comprises a cell or tissue, such as gastrointestinal, stomach, esophageal, colon, rectal, and/or colorectal tissue. In some embodiments, a sample comprises a cell or tissue, such as adrenal, bladder, brain, breast, cervix, colon, head and neck, kidney, leukemia, liver, lung, lymphoid, ovarian, pancreas, prostate, rectum, skin, stomach, thyroid, and/or uterus tissue. In some embodiments, a sample comprises a cell or tissue, such as lung, ovarian, breast, liver, or multiple myeloma tissue.

[0307] In one embodiment, an anti-RSPO antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of RSPO in a sample is provided. In certain embodiments, the method comprises contacting the sample with an anti-RSPO antibody as described herein under conditions permissive for binding of the anti-RSPO antibody to RSPO and detecting whether a complex is formed between the anti-RSPO antibody and RSPO. Such method

subjects eligible for therapy with an anti-RSPO antibody, *e.g.* where RSPO is a biomarker for selection of patients. In some embodiments, the RSPO is RSPO2. In some embodiments, the RSPO is RSPO3. In some embodiments, the RSPO is RSPO2 and RSPO3. In some embodiments, the individual and/or cancer has increased expression of one or more stem cell biomarkers. In some embodiments, the stem cell biomarker comprises Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, the individual and/or cancer has decreased expression of one or more biomarker of differentiation. In some embodiments, the biomarker of differentiation comprises CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20.

[0308] For example, provided herein are methods of treating cancer in an individual, wherein the cancer comprises one or more biomarkers, comprising administering to the individual an effective amount of an anti-RSPO antibody. Also provided herein are methods of treating cancer in an individual comprising administering to the individual an effective amount of an anti-RSPO antibody, wherein treatment is based upon the individual having cancer comprising one or more biomarkers.

[0309] Translocation are exceptionally powerful cancer mutations, as they often have multiple effects on a target gene: in a single ‘mutation’ they can dramatically change expression, remove regulatory domains, force oligomerization, change the subcellular location of a protein or join it to novel binding domains. This is reflected clinically in the fact that some neoplasms are classified or managed according to the presence of a particular fusion gene. In some embodiments of any of the methods, the one or more biomarkers comprise a translocation (*e.g.*, intrachromosomal translocation, interchromosomal translocation, rearrangement and/or fusion) of one or more genes listed in Table 2.

[0310] In some embodiments of any of the methods, the translocation is a PVT1. In some embodiments, the PVT1 translocation comprises *PVT1* and *MYC*. In some embodiments, the RSPO2 translocation comprises *PVT1* and *IncDNA*. In some embodiments of any of the methods, the translocation is an R-spondin translocation. In some embodiments, the R-spondin translocation is a RSPO1 translocation. In some embodiments, the R-spondin translocation is a RSPO2. In some embodiments, the RSPO2 translocation comprises *EMC2* and *RSPO2*. In some embodiments, the RSPO2 translocation comprises *EIF3E* and *RSPO2*. In some embodiments, the RSPO2 translocation comprises *EIF3E* exon 1 and *RSPO2* exon 2. In some embodiments, the RSPO2 translocation comprises *EIF3E* exon 1 and *RSPO2* exon 3. In some embodiments, the RSPO2 translocation comprises SEQ ID NO:71. In some embodiments, the RSPO2 translocation is detectable by primers which include SEQ ID NO:114, 143, and/or 145. In some embodiments, the RSPO2 translocation is driven by the *EIF3E* promoter. In some embodiments, the RSPO2 translocation is driven by the *RSPO2* promoter. In some embodiments, the R-spondin translocation is a RSPO3 translocation. In some embodiments, the RSPO3 translocation comprises *PTPRK* and *RSPO3*. In some embodiments, the RSPO3 translocation comprises *PTPRK* exon 1 and *RSPO3* exon 2. In some embodiments, the

translocation comprises SEQ ID NO:171 and/or SEQ ID NO:172. In some embodiments, the RSPO3 translocation is detectable by primers which include SEQ ID NO:115, 116, 145, and/or 146. In some embodiments, the RSPO3 translocation is driven by the *PTPRK* promoter. In some embodiments, the RSPO3 translocation is driven by the *RSPO3* promoter. In some embodiments, the RSPO3 translocation) comprises the PTPRK secretion signal sequence (and/or does not comprise the RSPO3 secretion signal sequence).

Table 2 – Gene Fusions

5' GeneName	3' GeneName	Type	Genomic position	5' PCR primer	3' PCR primer	bp
PVT1	ENST00000502082	intrachrom.	8:128806980-8:128433074	CTTGCGAAAGGATGTTGG (SEQ ID NO:113)	TGGTGATCCAGAGAAGAAGC (SEQ ID NO:142)	150
EMC2	RSPO2		8:109455927-8:109095035	CACCCCGCTGCCTCTAGGTTCTGGGAAGATGGCG AAGGTCACAGAGCTTTACGATGTCACCTGGGAAG (SEQ ID NO:179)	GTTCGTGGCGGAGAGATGCTGATCGCGCTGAACCTGACCGGTGCGGCCCGGGGGTGAAGTG GCGAGTCTCC (SEQ ID NO:180)	
EIF3E(e1)	RSPO2(e2)	Deletion	8:109260842-8:109095035	ACTACTCGCATCGCGCACT (SEQ ID NO:114)	GGGAGGACTCAGAGGGAGAC (SEQ ID NO:143)	155
EIF3E(e1)	RSPO2(e2)	Deletion	8:109260842-8:109095035	ACTACTCGCATCGCGCACT (SEQ ID NO:114)	GGGAGGACTCAGAGGGAGAC (SEQ ID NO:143)	155
EIF3E(e1)	RSPO2(e3)	Deletion	8:109260842-8:109001472	ACTACTCGCATCGCGCACT (SEQ ID NO:114)	TGCAGGCACCTCCTCATACTG (SEQ ID NO:144)	205
EIF3E(e1)	RSPO2(e3)	Deletion	8:109260842-8:109001472	ACTACTCGCATCGCGCACT (SEQ ID NO:114)	TGCAGGCACCTCCTCATACTG (SEQ ID NO:144)	205
PTPRK(e1)	RSPO3(e2)	Inversion	6:128841404-6:127469793	AAACTCGGCATGGATACGAC (SEQ ID NO:115)	GCTTCATGCCAATTCTTTCC (SEQ ID NO:145)	226
PTPRK(e1)	RSPO3(e2)	Inversion	6:128841404-6:127469793	AAACTCGGCATGGATACGAC (SEQ ID NO:115)	GCTTCATGCCAATTCTTTCC (SEQ ID NO:145)	226
PTPRK(e1)	RSPO3(e2)	Inversion	6:128841404-6:127469793	AAACTCGGCATGGATACGAC (SEQ ID NO:115)	GCTTCATGCCAATTCTTTCC (SEQ ID NO:145)	226
PTPRK(e1)	RSPO3(e2)	Inversion	6:128841404-6:127469793	AAACTCGGCATGGATACGAC (SEQ ID NO:115)	GCTTCATGCCAATTCTTTCC (SEQ ID NO:145)	226
PTPRK(e7)	RSPO3(e2)	Inversion	6:128505577-6:127469793	TGCAGTCAATGCTCCAAC TT (SEQ ID NO:116)	GCCAATTCTTTCAGAGCAA (SEQ ID NO:146)	250
ETV6	NTRK3	Interchrom	12:12022903-15:88483984	AAGCCCATCAACCTCTCTCA (SEQ ID NO:117)	GGGCTGAGGTTGTAGCACTC (SEQ ID NO:147)	206
ANXA2	RORA	intrachrom.	15:60674541-15:60824050	CTCTACACCCCAAGTGCA T (SEQ ID NO:118)	TGACACCATAATGGATTCTCTG (SEQ ID NO:148)	164
TUBGCP3	PDS5B	Inversion	13:113200013-13:33327470	AACAGGAGACCCCGTACATGC (SEQ ID NO:119)	AAAGGGCACAGATTGCCATA (SEQ ID NO:149)	221
ARHGEF18	NCRNA00157	Interchrom	19:7460133-21:19212970	CCAGCTGCTAGCTACTGTGGA (SEQ ID NO:120)	ACTAGGTGGTCCAGGGTGTG (SEQ ID NO:150)	186
NT5C2	ASAH2	Deletion	10:104899163-10:51978390	TGAACCGAAGTTAGCAATGG (SEQ ID NO:121)	TGCTCAAGCAGGTAAGATGC (SEQ ID NO:151)	156
NRBP2	VPS28	intrachrom.	8:144919211-8:145649651	TGATGAAC TTGCAGCCACT (SEQ ID NO:122)	ATGGTCTCCATCAGCTCTCG (SEQ ID NO:152)	208
CDC42SE2	KIAA0146	Interchrom	5:130651837-8:48612965	AGGGCCAGATTTGAGTGTG T (SEQ ID NO:123)	AAACTGAAAA TCCCCGCTGT (SEQ ID NO:153)	188
MED13L	LAG3	Inversion	12:116675273-12:6886957	GTGTATGGCGTCGTGATGTC (SEQ ID NO:124)	GCTCCAGTCACCAAAAGGAG (SEQ ID NO:154)	205
PEX5	LOC389634	Inversion	12:7362838-12:8509737	CATGTGCGAGAACATCTGGA (SEQ ID NO:125)	TGTGGAGTCTCTTGC GTGTC (SEQ ID NO:155)	230
PLCE1	CYP2C19	Deletion	10:95792009-10:96602594	CCTTACTGCCTGTGGGAGA (SEQ ID NO:126)	TGGGATGAGGTCGATGTAT (SEQ ID NO:156)	224
TPM3	NTRK1	Inversion	1:154142876-1:156844363	CAGAGACCCG TGCTGAGTTT (SEQ ID NO:127)	CCAAAAGGTGTTTGC TCC TT (SEQ ID NO:157)	124
PAN3	RFC3	Deletion	13:28752072-13:34395269	GAC TTGTGCGCC TCAACAT (SEQ ID NO:128)	CAATTTTCCACTCCAACACC (SEQ ID NO:158)	150
CWC27	RNF180	intrachrom.	5:64181373-5:63865442	AACGGGAAC TCTTAGCAGCA (SEQ ID NO:129)	CATGTCAAACCACCATCCAC (SEQ ID NO:159)	182
CAPN1	SPDYC	intrachrom.	11:64956217-11:64939414	GAGACTTCATCGCGGAGTTG (SEQ ID NO:130)	ATCTGGAAGCAGGGGTCTTT (SEQ ID NO:160)	199
COG8	TERF2	intrachrom.	16:69373079-16:69391464	TGGCCTTCGCTAACTACAAGA (SEQ ID NO:131)	TCCCCATATTTCTGC ACTCC (SEQ ID NO:161)	233
TADA2A	MEF2B	Interchrom	17:35767040-19:19299492	GCTCTTTGGCGGGATTA (SEQ ID NO:132)	GGAGCTACCTGTGGCCCT (SEQ ID NO:162)	152
STRBP	DENND1A	intrachrom.	9:125935956-9:126220176	GTTGCAAAAGGCTTGCTGAT (SEQ ID NO:133)	ACGAAGGCTTCCTCACAGAA (SEQ ID NO:163)	155
CXorf56	UBE2A	Inversion	X:118694231-X:118717090	TGATTGATGCTGCCAAACAT (SEQ ID NO:134)	CACGCTTTTCATATATCCCGT (SEQ ID NO:164)	161
MED13L	CD4	Inversion	12:116675273-12:6923308	GTGTATGGCGTCGTGATGTC (SEQ ID NO:124)	TCCCAAAGGCTTCTTCTTGA (SEQ ID NO:165)	151
PRR12	PRRG2	intrachrom.	19:50097872-19:50093157	ATGAACCTTATCTCGGCCCT (SEQ ID NO:135)	GTCGTGTACCCCAAGAGGCT (SEQ ID NO:166)	227
ATP9A	ARFGEF2	Inversion	20:50307278-20:47601266	ATGTGTACGCAGAAAGACCCA (SEQ ID NO:136)	GTGCAGGAA TTGGGCTATGT (SEQ ID NO:167)	150
ANKRD17	HS3ST1	Deletion	4:73956384-4:11401737	GGAAAATCCTCATATATTGCCA (SEQ ID NO:137)	AGCAGGGAAGCCTCCTAGTC (SEQ ID NO:168)	158
RBM47	ATP8A1	intrachrom.	4:40517884-4:42829126	AGACCCAGGAGGAGTGAGGT (SEQ ID NO:138)	GGTCAGCCAGTGAGGTCTTC (SEQ ID NO:169)	151
FRS2	RAP1B	intrachrom.	12:69924740-12:69042479	AGATCCCCAGATGCAAAAGT (SEQ ID NO:139)	CAAAGCAGACTTTC AACCGC (SEQ ID NO:170)	161
CHEK2	PARVB	Inversion	22:29137757-22:44553862	GGCTGAGGGTGGAGTTTGTA (SEQ ID NO:140)	CTTCTGATCGAAGCTTTCCG (SEQ ID NO:171)	191
SFI1	TPST2	Inversion	22:31904362-22:26940641	CCCCAGTTAGAAGGGGAAGA (SEQ ID NO:141)	CAC TCTCATCTCTGGGCTCC (SEQ ID NO:172)	190

[0311] In some embodiments, the R-spondin translocation is a RSPO4 translocation. In some embodiments, the R-spondin translocation results in elevated expression levels of R-spondin (*e.g.*, compared to a reference without the R-spondin translocation). In some embodiments, the R-spondin translocation results in elevated activity and/or activation of R-spondin (*e.g.*, compared to a reference without the R-spondin translocation). In some embodiments, the presence of one or more biomarkers comprises an R-spondin translocation), such as a translocation in Table 2, and *KRAS* and/or *BRAF*. In some embodiments, the presence of one or more biomarkers is presence of an R-spondin translocation (*e.g.*, rearrangement and/or fusion), such as a translocation in Table 2, and a variation (*e.g.*, polymorphism or mutation) *KRAS* and/or *BRAF*. In some embodiments, the individual and/or cancer comprises a variation (polymorphism or mutation) in *KRAS* and/or *BRAF*. In some embodiments, the presence of one or more biomarkers is presence of an R-spondin translocation, such as a translocation in Table 2, and the absence of one or more biomarkers is absence of a variation (*e.g.*, polymorphism or mutation) *CTNNB1* and/or *APC*.

[0312] In some embodiments of any of the translocation (*e.g.*, intrachromosomal translocation, interchromosomal translocation, rearrangement and/or fusion), the translocation (*e.g.*, intrachromosomal translocation, interchromosomal translocation, rearrangement and/or fusion) is a somatic translocation (*e.g.*, intrachromosomal translocation, interchromosomal translocation, rearrangement and/or fusion). In some embodiments, the translocation is an intrachromosomal translocation. In some embodiments, the translocation is an interchromosomal. In some embodiments, the translocation is an inversion. In some embodiments, the translocation is a deletion. In some embodiments, the translocation is a functional translocation fusion polynucleotide (*e.g.*, functional R-spondin-translocation fusion polynucleotide) and/or functional translocation fusion polypeptide (*e.g.*, functional R-spondin-translocation fusion polypeptide). In some embodiments, the functional translocation fusion polypeptide (*e.g.*, functional R-spondin-translocation fusion polypeptide) activates a pathway known to be modulated by one of the translocated genes (*e.g.*, wnt signaling pathway). In some embodiments, the pathway is canonical wnt signaling pathway. In some embodiments, the pathway is noncanonical wnt signaling pathway. In some embodiments, the Methods of determining pathway activation are known in the art and include luciferase reporter assays as described herein. In some embodiments, the method is one or more methods described in Seshagiri et al., *Nature* 488:660-664 (2012) and/or WO 2013/120056, which are incorporated by reference in their entirety.

[0313] Exemplary disorders that may be diagnosed using an antibody of the invention include tumors, cell proliferative disorders, cancer, gastrointestinal cancer, stomach cancer, colorectal cancer, colon

cancer, and/or rectal cancer. Exemplary disorders that may be diagnosed using an antibody of the invention further include adrenal cancer, bladder cancer, brain cancer, breast cancer, cervix cancer, colon cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer (*e.g.*, NSCLC), lymphoid cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectum cancer, skin cancer (*e.g.*, melanoma), stomach cancer, thyroid cancer, and/or uterine cancer. Exemplary disorders that may be diagnosed using an antibody of the invention also include lung cancer (*e.g.*, NSCLC), ovarian cancer, breast cancer, liver cancer, or multiple myeloma.

[0314] Samples include, but are not limited to, primary or cultured cells or cell lines, cell supernatants, cell lysates, platelets, serum, plasma, vitreous fluid, lymph fluid, synovial fluid, follicular fluid, seminal fluid, amniotic fluid, milk, whole blood, blood-derived cells, urine, cerebro-spinal fluid, saliva, sputum, tears, perspiration, mucus, tumor lysates, and tissue culture medium, tissue extracts such as homogenized tissue, tumor tissue, cellular extracts, and combinations thereof. In some embodiments, the sample is a sample from gastrointestinal, stomach, esophageal, colon, rectal, and/or colorectal tissue. In some embodiments, the sample is a sample from adrenal, bladder, brain, breast, cervix, colon, head and neck, kidney, leukemia, liver, lung, lymphoid, ovarian, pancreas, prostate, rectum, skin, stomach, thyroid, and/or uterus tissue. In some embodiments, the sample is a sample from lung, ovarian, breast, liver, or multiple myeloma tissue.

[0315] In certain embodiments, labeled anti-RSPO antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H , and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, *e.g.*, firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases, *e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

[0316] In some embodiments of any of the methods, elevated expression refers to an overall increase of about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of biomarker (*e.g.*, protein or nucleic acid (*e.g.*, gene or mRNA)), detected by standard art known methods such as those described herein, as compared to a reference sample,

reference cell, reference tissue, control sample, control cell, or control tissue. In certain embodiments, the elevated expression refers to the increase in expression level/amount of a biomarker in the sample wherein the increase is at least about any of 1.5X, 1.75X, 2X, 3X, 4X, 5X, 6X, 7X, 8X, 9X, 10X, 25X, 50X, 75X, or 100X the expression level/amount of the respective biomarker in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In some embodiments, elevated expression refers to an overall increase of greater than about 1.5 fold, about 1.75 fold, about 2 fold, about 2.25 fold, about 2.5 fold, about 2.75 fold, about 3.0 fold, or about 3.25 fold as compared to a reference sample, reference cell, reference tissue, control sample, control cell, control tissue, or internal control (*e.g.*, housekeeping gene).

[0317] In some embodiments of any of the methods, reduced expression refers to an overall reduction of about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of biomarker (*e.g.*, protein or nucleic acid (*e.g.*, gene or mRNA)), detected by standard art known methods such as those described herein, as compared to a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In certain embodiments, reduced expression refers to the decrease in expression level/amount of a biomarker in the sample wherein the decrease is at least about any of 0.9X, 0.8X, 0.7X, 0.6X, 0.5X, 0.4X, 0.3X, 0.2X, 0.1X, 0.05X, or 0.01X the expression level/amount of the respective biomarker in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue.

[0318] Presence and/or expression level/amount of various biomarkers in a sample can be analyzed by a number of methodologies, many of which are known in the art and understood by the skilled artisan, including, but not limited to, immunohistochemical (“IHC”), Western blot analysis, immunoprecipitation, molecular binding assays, ELISA, ELIFA, fluorescence activated cell sorting (“FACS”), MassARRAY, proteomics, quantitative blood based assays (as for example Serum ELISA), biochemical enzymatic activity assays, in situ hybridization, Southern analysis, Northern analysis, whole genome sequencing, polymerase chain reaction (“PCR”) including quantitative real time PCR (“qRT-PCR”) and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like), RNA-Seq, FISH, microarray analysis, gene expression profiling, and/or serial analysis of gene expression (“SAGE”), as well as any one of the wide variety of assays that can be performed by protein, gene, and/or tissue array analysis. Typical protocols for evaluating the status of genes and gene products are found, for example in Ausubel *et al.*, eds., 1995, Current Protocols In Molecular Biology, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting) and 18 (PCR Analysis). Multiplexed immunoassays such as those available from Rules Based Medicine or Meso Scale Discovery (“MSD”) may also be used.

[0319] In some embodiments, presence and/or expression level/amount of a biomarker is determined using a method comprising: (a) performing gene expression profiling, PCR (such as rtPCR), RNA-seq, microarray analysis, SAGE, MassARRAY technique, or FISH on a sample (such as a subject cancer sample); and b) determining presence and/or expression level/amount of a biomarker in the sample. In some embodiments, the microarray method comprises the use of a microarray chip having one or more nucleic acid molecules that can hybridize under stringent conditions to a nucleic acid molecule encoding a gene mentioned above or having one or more polypeptides (such as peptides or antibodies) that can bind to one or more of the proteins encoded by the genes mentioned above. In one embodiment, the PCR method is qRT-PCR. In one embodiment, the PCR method is multiplex-PCR. In some embodiments, gene expression is measured by microarray. In some embodiments, gene expression is measured by qRT-PCR. In some embodiments, expression is measured by multiplex-PCR.

F. Pharmaceutical Formulations

[0320] Pharmaceutical formulations of an anti-RSPO antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX[®], Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and

2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0321] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958.

Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0322] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0323] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0324] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

[0325] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

G. Therapeutic Methods and Compositions

[0326] Any of the anti-RSPO antibodies provided herein may be used in therapeutic methods.

[0327] In one aspect, an anti-RSPO antibody for use as a medicament is provided. In further aspects, an anti-RSPO antibody for use in treating tumor, cell proliferative disorder, and/or cancer is provided. In some embodiments, an anti-RSPO antibody is provided for use in promoting differentiation of cells including terminal differentiation of cancer cells. In certain embodiments, an anti-RSPO antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-RSPO antibody for use in a method of treating an individual having tumor, cell proliferative disorder, and/or cancer comprising administering to the individual an effective amount of the anti-RSPO antibody. In some embodiments, the cancer is colorectal cancer. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described below. In some embodiments, the RSPO is RSPO2. In some embodiments, the RSPO is RSPO3. In some embodiments, the RSPO is RSPO2 and RSPO3. In further embodiments, the

invention provides an anti-RSPO antibody for use in inhibiting wnt signaling, inhibiting angiogenesis, inhibiting cell proliferation, inhibiting cancer stem cell proliferation, and/or depleting cancer stem cells. In certain embodiments, the invention provides an anti-RSPO antibody for use in a method of inhibiting wnt signaling, inhibiting angiogenesis, inhibiting cell proliferation, inhibiting cancer stem cell proliferation, and/or depleting cancer stem cells in an individual comprising administering to the individual an effective of the anti-RSPO antibody to inhibit wnt signaling, inhibit angiogenesis, inhibit cell proliferation, inhibit cancer stem cell proliferation, and/or deplete cancer stem cells. An “individual” according to any of the above embodiments is preferably a human. In some embodiments, the individual and/or cancer has one or more biomarker. In some embodiments, the one or more biomarkers comprises an RSPO translocation. In some embodiments, the RSPO translocation comprises and RSPO2 and/or RSPO3 translocation. In some embodiments, the individual and/or cancer has increased expression of one or more biomarker. In some embodiments, the one or more biomarker comprises RSPO, *e.g.*, RSPO2 and/or RSPO3. In some embodiments, the one or more biomarker comprises a stem cell biomarker. In some embodiments, the stem cell biomarker comprises Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, the individual and/or cancer has decreased expression of one or more biomarker of differentiation. In some embodiments, the biomarker of differentiation comprises CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, treatment with the anti-RSPO antibody reduces expression of one or more stem cell biomarker, *e.g.*, Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, treatment with the anti-RSPO antibody increases expression of one or more biomarker of differentiation, *e.g.*, CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, the cancer is adrenal cancer, bladder cancer, brain cancer, breast cancer, cervix cancer, colon cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer (*e.g.*, NSCLC), lymphoid cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectum cancer, skin cancer (*e.g.*, melanoma), stomach cancer, thyroid cancer, and/or uterine cancer. In some embodiments, the cancer is lung cancer (*e.g.*, NSCLC), ovarian cancer, breast cancer, liver cancer, or multiple myeloma. In some embodiments, the cancer is colorectal cancer.

[0328] In a further aspect, the invention provides for the use of an anti-RSPO antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of tumor, cell proliferative disorder, and/or cancer. In a further embodiment, the medicament is for use in a method of treating tumor, cell proliferative disorder, and/or cancer comprising administering to an individual having tumor, cell proliferative disorder, and/or cancer an effective amount of the medicament. In some embodiments, the cancer is colorectal cancer. In one such embodiment, the

method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described below. In a further embodiment, the medicament is for inhibiting wnt signaling, inhibiting angiogenesis, inhibiting cell proliferation, inhibiting cancer stem cell proliferation, and/or depleting cancer stem cells. In a further embodiment, the medicament is for use in a method of inhibiting wnt signaling, inhibiting angiogenesis, inhibiting cell proliferation, inhibiting cancer stem cell proliferation, and/or depleting cancer stem cells in an individual comprising administering to the individual an amount effective of the medicament to inhibit wnt signaling, inhibit angiogenesis, inhibit cell proliferation, inhibit cancer stem cell proliferation, and/or deplete cancer stem cells. An “individual” according to any of the above embodiments may be a human. In some embodiments, the individual and/or cancer has one or more biomarker. In some embodiments, the one or more biomarkers comprises an RSPO translocation. In some embodiments, the RSPO translocation comprises RSPO2 and/or RSPO3 translocation. In some embodiments, the individual and/or cancer has increased expression of one or more biomarker. In some embodiments, the one or more biomarker comprises RSPO, *e.g.*, RSPO2 and/or RSPO3. In some embodiments, the one or more biomarker comprises a stem cell biomarker. In some embodiments, the stem cell biomarker comprises Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, the individual and/or cancer has decreased expression of one or more biomarker of differentiation. In some embodiments, the biomarker of differentiation comprises CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, treatment with the anti-RSPO antibody reduces expression of one or more stem cell biomarker, *e.g.*, Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, treatment with the anti-RSPO antibody increases expression of one or more biomarker of differentiation, *e.g.*, CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, the cancer is adrenal cancer, bladder cancer, brain cancer, breast cancer, cervix cancer, colon cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer (*e.g.*, NSCLC), lymphoid cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectum cancer, skin cancer (*e.g.*, melanoma), stomach cancer, thyroid cancer, and/or uterine cancer. In some embodiments, the cancer is lung cancer (*e.g.*, NSCLC), ovarian cancer, breast cancer, liver cancer, or multiple myeloma.

[0329] In a further aspect, the invention provides a method for treating a tumor, cell proliferative disorder, and/or cancer. In one embodiment, the method comprises administering to an individual having such tumor, cell proliferative disorder, and/or cancer an effective amount of an anti-RSPO antibody. In some embodiments, the cancer is colorectal cancer. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An “individual” according to any of the above embodiments

may be a human. In some embodiments, the individual and/or cancer has one or more biomarker. In some embodiments, the one or more biomarkers comprises an RSPO translocation. In some embodiments, the RSPO translocation comprises and RSPO2 and/or RSPO3 translocation. In some embodiments, the individual and/or cancer has increased expression of one or more biomarker. In some embodiments, the one or more biomarker comprises RSPO, *e.g.*, RSPO2 and/or RSPO3. In some embodiments, the one or more biomarker comprises a stem cell biomarker. In some embodiments, the stem cell biomarker comprises Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, the individual and/or cancer has decreased expression of one or more biomarker of differentiation. In some embodiments, the biomarker of differentiation comprises CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, treatment with the anti-RSPO antibody reduces expression of one or more stem cell biomarker, *e.g.*, Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, treatment with the anti-RSPO antibody increases expression of one or more biomarker of differentiation, *e.g.*, CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, the cancer is adrenal cancer, bladder cancer, brain cancer, breast cancer, cervix cancer, colon cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer (*e.g.*, NSCLC), lymphoid cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectum cancer, skin cancer (*e.g.*, melanoma), stomach cancer, thyroid cancer, and/or uterine cancer. In some embodiments, the cancer is lung cancer (*e.g.*, NSCLC), ovarian cancer, breast cancer, liver cancer, or multiple myeloma.

[0330] In a further aspect, the invention provides a method inhibiting wnt signaling, inhibiting angiogenesis, inhibiting cell proliferation, inhibiting cancer stem cell proliferation, and/or depleting cancer stem cells in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-RSPO antibody to inhibit wnt signaling, inhibit angiogenesis, inhibit cell proliferation, inhibit cancer stem cell proliferation, and/or deplete cancer stem cells. In one embodiment, an “individual” is a human. In some embodiments, the individual and/or cancer has one or more biomarker. In some embodiments, the one or more biomarkers comprises an RSPO translocation. In some embodiments, the RSPO translocation comprises and RSPO2 and/or RSPO3 translocation. In some embodiments, the individual and/or cancer has increased expression of one or more biomarker. In some embodiments, the one or more biomarker comprises RSPO, *e.g.*, RSPO2 and/or RSPO3. In some embodiments, the one or more biomarker comprises a stem cell biomarker. In some embodiments, the stem cell biomarker comprises Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, the individual and/or cancer has decreased expression of one or more biomarker of differentiation. In some embodiments, the biomarker of differentiation comprises

CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, treatment with the anti-RSPO antibody reduces expression of one or more stem cell biomarker, *e.g.*, Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, treatment with the anti-RSPO antibody increases expression of one or more biomarker of differentiation, *e.g.*, CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, the cancer is adrenal cancer, bladder cancer, brain cancer, breast cancer, cervix cancer, colon cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer (*e.g.*, NSCLC), lymphoid cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectum cancer, skin cancer (*e.g.*, melanoma), stomach cancer, thyroid cancer, and/or uterine cancer. In some embodiments, the cancer is lung cancer (*e.g.*, NSCLC), ovarian cancer, breast cancer, liver cancer, or multiple myeloma. In some embodiments, the cancer is colorectal cancer.

[0331] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-RSPO antibodies provided herein, *e.g.*, for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-RSPO antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-RSPO antibodies provided herein and at least one additional therapeutic agent, *e.g.*, as described below. In some embodiments, the RSPO is RSPO2. In some embodiments, the RSPO is RSPO3. In some embodiments, the RSPO is RSPO2 and RSPO3. In some embodiments, the individual and/or cancer has increased expression of one or more biomarker. In some embodiments, the one or more biomarker comprises RSPO, *e.g.*, RSPO2 and/or RSPO3. In some embodiments, the one or more biomarker comprises a stem cell biomarker. In some embodiments, the stem cell biomarker comprises Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, the individual and/or cancer has decreased expression of one or more biomarker of differentiation. In some embodiments, the biomarker of differentiation comprises CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, treatment with the anti-RSPO antibody reduces expression of one or more stem cell biomarker, *e.g.*, Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2. In some embodiments, treatment with the anti-RSPO antibody increases expression of one or more biomarker of differentiation, *e.g.*, CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and/or KRT20. In some embodiments, the cancer is adrenal cancer, bladder cancer, brain cancer, breast cancer, cervix cancer, colon cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer (*e.g.*, NSCLC), lymphoid cancer, ovarian cancer, pancreatic cancer, prostate cancer, rectum cancer, skin cancer (*e.g.*, melanoma), stomach cancer, thyroid cancer, and/or uterine cancer. In some embodiments,

the cancer is lung cancer (*e.g.*, NSCLC), ovarian cancer, breast cancer, liver cancer, or multiple myeloma. In some embodiments, the cancer is colorectal cancer.

[0332] Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is a cytotoxic agent, chemotherapeutic agent, cytostatic agent, anti-hormonal agent, and/or EGFR inhibitor.

[0333] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of the anti-RSPO antibody and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other. Antibodies of the invention can also be used in combination with radiation therapy.

[0334] An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, *e.g.* by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0335] Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0336] For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (*e.g.* 0.1 mg/kg-10 mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, *e.g.* every week or every three weeks (*e.g.* such that the patient receives from about two to about twenty, or *e.g.* about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0337] It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-RSPO antibody.

H. Articles of Manufacture

[0338] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the

composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0339] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-RSPO antibody.

III. EXAMPLES

[0340] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

METHODS

[0341] **Cloning and Purification:** FLAG-tagged RNF43 was purified by anti-FLAG affinity chromatography (Genentech), followed by size-exclusion chromatography (Superdex 75, GE Healthcare). FLAG-tagged R-spondins (hRSPO2, hRSPO2 L186, cynoRSPO2, hRSPO3, and cynoRSPO3FLAG) were purified by anti-FLAG affinity chromatography, followed by cation exchange chromatography (Mono S, GE Healthcare). Human IgG1 Fc-tagged LGR extracellular domains were purified by affinity chromatography (MabSelect SuRe, GE Healthcare), followed by size-exclusion chromatography (Superdex 200, GE Healthcare).

[0342] **WNT reporter assays:** In 96 well plates, 4,500 293T cells per well were plated in 90 ul of DMEM supplemented with 2.5% fetal bovine serum. Following 16-20 hours of culture, cells were co-transfected with 0.04 ug Topbrite 25 and 0.02 ug SV40 Renilla DNA in 10 ul of transfection mix using Fugene 6 (Promega, Madison, WI). Following an additional 16-20 hours of culture, cells were stimulated with 25 ul of a 5X solution for 6 hours at 37 degrees Celsius. For supernatant screens, cells were stimulated with hybridoma supernatant supplemented with 50 ng/ml rmWNT3a (R&D Systems, Minneapolis, MN) and 250 pM rhRSPO2 or rhRSPO3 (R&D Systems, Minneapolis, MN). For assays using cloned antibodies, DMEM supplemented with 10% fetal bovine serum, 50 ng/ml rmWNT3a, 250 pM or 5X calculated EC50 rRSPO (as indicated) and increasing concentrations of antibody were added. For assays testing conditioned media, media were prepared by transfecting 293T with the indicated genes using Fugene 6 according to manufacturer's instructions (Promega, Madison, WI).

Conditioned media were collected 3 days following transfection, supplemented with 50 ng/ml rmWNT3a +/- anti-RSPO antibodies and added to reporter cells. Following stimulation for 6 hours, luciferase activity was detected using the Promega Dual-Glo system (Promega, Madison, WI). Data were analyzed as either a ratio of Firefly/Renilla (RLU WNT reporter), or normalized values in absence of antibodies (RLU with antibody/RLU no antibody). IC50 measurements were determined by stimulating cells with the EC50 of rRSPO with increasing concentrations of antibody. Log transformed data were fit with a four-parameter dose-response equation using GraphPad Prism.

[0343] *Generation of RSPO-expressing cell pellets:* pGCIG is a HIV-based self-inactivating lentiviral vector that was created by replacing the Zeo^R-CMV_{ie}-tGFP-IRES-Puro^R-shRNA-WRE content of pGIPZ (Open Biosystems) with a fragment containing the CMV_{ie} promoter, a multiple cloning site (MCS), an internal ribosome entry site (IRES) and enhanced green fluorescent protein (eGFP). The human R-spondin 1-4 open reading frames (ORFs) were tagged with an HA epitope (YPYDVPDYA) at the C-terminus by PCR and inserted into the MCS of pGCIG. HEK-293 cells were plated on 15-cm dishes at 15x10⁶ cells/plate in DMEM High Glucose with 10% heat inactivated FBS 24 h prior to transfection. Lentiviral supernatants were prepared by cotransfection using 6 ug of pGCIG-hRSPO, 12 ug of the packaging vector Δ8.9 (Zufferey et al., 1997), 3 ug of the envelope vector pVSV-G (Clontech) and the transfection reagent Genejuice (Novagen). The culture medium was replaced 12 h after transfection and viral supernatant was collected 24 h later, filtered through a 0.45 μm PES filter (Nalgene) and stored at 4 °C until further processing. HEK-293 cells were plated on 10-cm dishes at 1x10⁶ cells/plate in DMEM High Glucose with 10% heat inactivated FBS. The cells were allowed to adhere for 12 h, after which the medium was replaced with 10 ml of viral supernatant. Viral supernatants remained on the cells for 60 h, after which the cells were harvested and analyzed for fluorescent protein expression by FACS. Gates were set to sort out 2x10⁵ low, medium and high eGFP expressing cells for each viral construct. Cell lines were expanded and tested for the absence of replication competent virus (RCV) production using the HIV-1 p24 Antigen ELISA 2.0 kit (ZeptoMetrix Corporation). Expression and secretion of human R-spondins was confirmed by anti-HA Western blotting of concentrated cell culture supernatants and correlated well with the eGFP expression levels.

[0344] *IHC reactivity screening:* Formalin-fixed paraffin-embedded cell pellets were sectioned at 4 μm. Slides were pre-treated with citrate-based pH 6.0 buffer (Dako cat no. S1699, Carpinteria, CA) at 99 degrees Celsius for 20 minutes. After a 10% serum block, anti-RSPO sera were used at 1:250 and hybridoma supernatants were run. Pre-immune sera at 1:250 or naïve mouse IgG1, 2a, and 2b at a total concentration of 10 ug/ml was used as the negative control. Biotinylated donkey anti-mouse secondary

(Jackson Immuno cat no 715-065-151, West Grove, PA) was used at 5ug/ml. VECTASTAIN Elite ABC Kit (Standard*) (Vector Labs cat no PK-6100) was used as detection and signal was visualized with Pierce Metal Enhanced DAB (Thermo cat no 34065, Rockford, IL).

[0345] *Epitope Binning:* Epitope binning of anti-RSPO antibodies was performed using the Octet RED384 instrument (ForteBio). Recombinant RSPO (R&D Systems, Minneapolis, MN) was biotinylated and captured onto Streptavidin biosensors at 10 µg/ml for 120 seconds. Binding of the first antibody to saturation was achieved by adding 10 µg/ml for 600 seconds. The same biosensors were dipped into the competing antibodies at 5 µg/ml and binding was measured for 300 seconds. The failure of the second antibody to bind in the presence of saturating quantities of the first antibody indicated the two antibodies were in the same epitope bin.

[0346] *Affinity Measurements:* Binding affinities of anti-RSPO antibodies were measured by Surface Plasmon Resonance (SPR) using a BIAcore™-2000 instrument. The CM5 biosensor chip was activated with *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) reagents according to the supplier's (GE Healthcare Biosciences, Piscataway, NJ) instructions. RSPO antigens were immobilized onto the biosensor chip to achieve approximately 250 response unit (RU), followed by blocking with 1M ethanolamine.

[0347] For kinetic measurements, two-fold serial dilutions of anti-RSPO Fabs were injected in HBS-P buffer (0.01M HEPES pH 7.4, 0.15M NaCl, 0.005% surfactant P20) at 25°C with a flow rate of 30µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (BIAcore Evaluation Software version 3.2). The equilibrium dissociation constant (KD) was calculated as the ratio k_{off}/k_{on} .

[0348] *Competitive Binding ELISA:* To measure the activity of anti-RSPO antibodies in blocking the binding of LGR4 and -5 ECDs to RSPOs, MaxiSorp 384-well microwell plates (Thermo Scientific Nunc, Roskilde, Denmark) were coated with 25 ul/well of 0.5 µg/ml hRSPO2 or hRSPO3 (Genentech) in 50 mM carbonate buffer, pH 9.6, overnight at 4°C. Plates were blocked with at 80 ul/well of 0.5% bovine serum albumin, 15 parts per million Proclin 300 in phosphate buffered saline (PBS), pH 7.4, for 1 hour. Serially diluted anti-RSPO antibodies (0.078-10 ng/ml in 3-fold serial dilution plus buffer blank) containing 0.1 µg/ml LGR4-Fc or 0.015 µg/ml LGR5-Fc in assay buffer (0.5% BSA, 0.05% polysorbate 20, 15 parts per million Proclin 300 in PBS) were added to the plates at 25 ul/well. After a 2-hour incubation, LGR4-Fc and LGR5-Fc bound to the plates were detected using peroxidase labeled goat F(ab')₂ anti-human Fc (Jackson ImmunoResearch, West Grove, PA). After a 1 hour incubation, the substrate 3,3',5,5'-tetramethyl benzidine (Moss Inc., Pasadena, Maryland) was added to the plates and the reaction was stopped by adding 1 M phosphoric acid. Plates were washed with PBS, pH 7.4,

containing 0.05% tween 20, between steps and all the incubation steps following the coating step were performed at room temperature on an orbital shaker. Absorbance was read at 450 nm on a multiskan Ascent reader (Thermo Scientific, Hudson, NH).

[0349] The activities of anti-RSPO antibodies in blocking binding of RNF43 to RSPOs were measured similarly using 0.5 ng/ml biotinylated RNF43-Flag (on RSPO2 coated plates) or 20 ng/ml biotinylated RNF43-Flag (on RSPO3 coated plates). Bound biotinylated RNF43-Flag was detected using peroxidase labeled streptavidin (GE Healthcare, Piscataway, NJ) followed by the substrate as described above.

[0350] *Humanization of anti-RSPO3 Antibodies:* Monoclonal antibody 5D6 was humanized as described below. Residue numbers are according to Kabat et al., Sequences of proteins of immunological interest, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, Md. (1991).

[0351] Variants constructed during the humanization of 5D6 were assessed in the form of Fab. The VL and VH domains from murine 5D6 were aligned with the human VL kappa I (VLKI) and human VH subgroup IV (VH4) consensus sequences. Hypervariable regions from the murine antibodies were engineered into VLKI and VHI acceptor frameworks. Specifically, from the mu5D6 VL domain, positions 24-34 (L1), 50-56 (L2) and 89-97 (L3) were grafted into VLKI and from the mu5D6 VH domain; positions 26-35 (H1), 50-65 (H2) and 95-102 (H3) were grafted into VHI. All VL and VH vernier positions from mu5D6 were also grafted to the VLK1 and VH4, respectively. This graft is referred to as v1.

[0352] The binding affinity of the antibodies in this section was determined by BIAcore™ T200 Format. Briefly, BIAcore™ research grade CM5 chips were activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) reagents according to the supplier's instructions. huRSPO3 was immobilized to achieve approximately 50 response units (RU) in each flow cell. Unreacted coupling groups were blocked with 1M ethanolamine. For kinetics measurements, Four-fold serial dilutions of variant antibody was injected in HBS-P buffer (0.01M HEPES pH7.4, 0.15M NaCl, 0.005% surfactant P20) at 25° C. with a flow rate of 30 µl/min. Association rates (kon) and dissociation rates (koff) were calculated using a 1:1 Langmuir binding model (BIAcore™ T200 Evaluation Software version 2.0). The equilibrium dissociation constant (Kd) was calculated as the ratio koff/kon.

[0353] *Crystallography RSPO3 (M33-E210) Purification:* RSPO3 (M33-E210) containing an N-terminal His-MBP tag was co-expressed with untagged EndoH in SF9 cells grown in medium treated with Kifunensine. Cell supernatants were harvested and passed over a 10 mL Nickel-NTA agarose

column that had been pre-equilibrated in Wash Buffer (25 mM Tris-HCl pH 7.5, 500 mM NaCl, 20 mM imidazole, 5% glycerol). The column was then washed with 10 column volumes of Wash Buffer. Protein was eluted from the column using 5 column volumes of Elution Buffer (25 mM Tris-HCl pH 7.5, 500 mM NaCl, 300 mM imidazole, 10% glycerol) and concentrated to less than 30 mL. TEV protease was added and the sample was dialyzed overnight against Dialysis Buffer (25 mM Tris-HCl pH 7.5, 500 mM NaCl, 10 mM imidazole, 10% glycerol) at 4°C. Following dialysis, the sample was passed through a 5 mL HisTrap column that had been pre-equilibrated with Wash Buffer. The sample was then concentrated to less than 2 mL and applied to a Superdex 75 16/60 column that had been pre-equilibrated with Gel Filtration Buffer (25 mM Tris-HCl pH 7.5, 300 mM NaCl, 5% glycerol). Fractions containing RSPO3 (M33-E210) were pooled and concentrated. Aliquots were stored at -80°C.

[0354] *Crystallography Fab Purification:* Fabs 5D6 and 26E11 were expressed in *E. coli* cells. Cell paste was resuspended in Lysis Buffer (PBS supplemented with 25 mM EDTA and 1 mM PMSF) and cells were lysed by three passages through a microfluidizer. Lysate was then spun at 12,000 rpm for one hour and the cleared lysates were filtered through a 0.8 µm filter. Cleared lysates were applied directly to a 25 mL Protein G column that had been pre-equilibrated with PBS supplemented with 25 mM EDTA. The column was washed with 10 column volumes of PBS and protein was eluted with 0.58% acetic acid. Eluates were then loaded onto a HiTrap SP HP column that had been pre-equilibrated with Buffer A (20 mM MES pH 5.5). The column was washed with 10 column volumes of Buffer A and protein was eluted over a 20 column volume linear gradient from Buffer A to Buffer B (20 mM MES pH 5.5, 500 mM NaCl). Fractions containing Fab were pooled and concentrated to less than 2 mL and applied to a Superdex 75 26/60 column that had been pre-equilibrated with Gel Filtration Buffer. Fractions containing Fab were pooled and concentrated. Aliquots were stored at -80°C.

[0355] *Crystallography RSPO3/Fab Complex Purification:* To form a complex, a 1.25-fold molar excess of RSPO3 (M33-E210) was added to 150 nmol of either Fab in an 800 µL binding reaction containing Gel Filtration Buffer. Binding reactions were incubated for one hour on ice. Reactions were then spun at 13,000 rpm at 4°C and loaded onto a Superdex 75 16/60 column that had been pre-equilibrated with Gel Filtration Buffer. Fractions containing complex were pooled and concentrated to 20 mg/mL. Aliquots were stored at -80°C.

[0356] *Crystallography:* For RSPO3 (M33-E210)/Fab 5D6, Labcyte Echo liquid handler was used to set several sparse matrix crystal screens using 100 nL sitting drops. Screens were stored at 18°C. Crystals were obtained in a drop containing 100 mM MIB pH 9 and 25% PEG 1500 as the mother

liquor. A cryoprotectant solution was made by mixing 1 μ L 70% glycerol with 1.8 μ L reservoir solution. A single crystal was harvested and soaked in cryoprotectant solution for 10 seconds and flash-frozen in liquid nitrogen.

[0357] For RSPO3 (M33-E210)/Fab 26E11, a Labcyte Echo liquid handler was used to set several sparse matrix crystal screens using 100 nL sitting drops. Screens were stored at 18°C. Crystals were obtained in a drop containing 200 mM Sodium formate and 20% (w/v) PEG 3,350 as the mother liquor. A cryoprotectant solution was made by mixing 1 μ L 70% ethylene glycol with 1.8 μ L reservoir solution. A single crystal was harvested and soaked in cryoprotectant solution for 10 seconds and flash-frozen in liquid nitrogen.

[0358] Both complexes crystallized in a wide range of similar conditions. Nearly all crystals grew in PEG-based conditions, with the most common being 20-25% PEG 3,350. Other successful precipitants included 20% PEG 6,000, 20-25% PEG 4,000, and 25% PEG 1,500. pH ranged from 3.5 – 9, with the majority of crystal growth seen between 7 and 8. Various salts at 200 mM concentration aided crystal growth

[0359] *Crystal Structure Determination and Refinement:* Diffraction data for the two RSPO3/Fab complexes was collected at the synchrotron. The data was indexed, integrated and scaled using XDS and SCALA. The crystal structure of RSPO3/5D6 and RSPO3/26E11 were solved by molecular replacement using the Fab structure as the search model. The phases for the initial molecular replacement solution were improved by solvent flattening and density modification using PHENIX. Iterative round of refinement and rebuilding were used to build the RSPO3 structure. The crystallographic statistics for the RSPO3/5D6 and RSPO/26E11 are below.

Table 1. Data collection and refinement statistics.

	RSPO3/5D6	RSPO3/26E11
Wavelength (Å)	1.0	0.976
Resolution range (Å)	46.94 - 2.15 (2.22 - 2.15)	48.0 - 2.51 (2.6 - 2.51)
Space group	P 1 21 1	P 1 21 1
Unit cell	87.6 52.1 91.8 90 116.87 90	95.7 47.7 96.4 90 114.7 90
Unique reflections	40700 (3821)	27615 (2713)
Multiplicity	3.4 (3.6)	3.7 (3.7)
Completeness (%)	99.40 (95.05)	99.87 (99.67)
Mean I/sigma(I)	10.92 (2.52)	13.2 (2.8)
Wilson B-factor	37.38	42.12
R-merge	0.046 (0.4)	0.083 (0.388)
R/Rfree	0.191/0.237 (0.276/0.314)	0.185/0.241 (0.249/0.296)
Number of non-hydrogen atoms	4888	4731
Protein residues	596	594
RMS(bonds)	0.029	0.004
RMS(angles)	1.06	0.83
Ramachandran F/A/D	86.2/13.8/0	81.6/18.3/0

Statistics for the highest-resolution shell are shown in parentheses.

[0360] *In Vivo Efficacy Experiments:* RSPO3-fusion positive patient-derived tumors were grown subcutaneously in Balbc/Nude mice. Once tumors reached a size of approximately 200 mm³, mice were treated either with control antibody or anti-RSPO3 antibody (5D6) at 30 mg/kg, twice a week for 3-4 weeks. For experiments in which anti-RSPO3 antibody (5D6) was used in combination with irinotecan, the anti-RSPO3 antibody was dosed as described above, and irinotecan was dosed at 100 mg/kg on day 0 or on day 0 and day 3.

[0361] For the serial transplantation study, mice implanted with RSPO3-fusion positive patient-derived tumors were treated with control antibody or anti-RSPO3 antibody as described above. One the growth curves began to separate, tumor fragments were removed and transplanted into naïve

Balbc/Nude mice. Mice with transplanted tumor fragments were then treated with either control or anti-RSPO3 antibody as described above.

RESULTS

Generation of function blocking and IHC reactive anti-RSPO antibodies

[0362] In effort to generate anti-RSPO antibodies, mice and hamsters were immunized with recombinant human RSPO2 and/or human RSPO3 and hybridoma cell lines were produced. Supernatants from these cells were first screened for binding to hRSPO1, hRSPO2, hRSPO3 and hRSPO4 by ELISA. Supernatants showing hRSPO2 and/or hRSPO3 binding were then tested for the ability to block hRSPO2 and hRSPO3 stimulation of WNT reporter activity. Candidates were subsequently cloned, expressed and purified. As shown in Figure 1, a subset of the purified clones potently inhibited rhRSPO2 stimulated WNT reporter activity (Figure 1A) and/or rhRSPO3 stimulated WNT reporter activity (Figure 1B).

[0363] In addition, supernatants were screened to identify anti-RSPO antibodies that could be used as IHC reagents. Formalin-fixed paraffin embedded cell pellets were prepared from 293 cells stably expressing high, medium, or low levels of hRSPO2 or hRSPO3. In addition, cell pellets were prepared from 293 cells and 293 cells stably expressing hRSPO1 or hRSPO4. Hybridoma supernatants and antibody clones were tested for IHC reactivity on the prepared cell pellets. As shown in Figure 2, the antibody 49G5 recognized by IHC reactivity high, medium, and low levels of hRSPO2 expression (D-F) while not recognizing hRSPO3 (A-C), hRSPO1 (G), hRSPO4 (H), or non-hRSPO1-4 (I). A summary of the results for all antibodies tested are shown below in Table 4. Antibodies 4H1, 4D4, 5C2, 5D6, 5E11, 21C2 specifically recognize hRSPO3. Antibodies 1A1, 36D2, 49G5 specifically recognize hRSPO2. Antibodies 6E9 and 26E11 recognize hRSPO2 and hRSPO3.

Table 4-IHC Reactivity of panel of anti-RSPO antibodies

Antibody	RSPO2 Hi	RSPO2 Med	RSPO2 Low	293 none	RSPO3 Hi	RSPO3 Med	RSPO3 Low	RSPO1 Hi	RSPO4 Hi
4H1	+	-	-	-	++ 90%	++ 60%	+	-	-
4D4	+	-	-	-	++ > 95%	++ 70%	++ 40%	-	-
5C2	+	-	-	-	++ > 95%	++ 60%	++ 40%	-	-
5D6	-	-	-	-	++ > 95%	++ 70%	++ 30%	-	-
5E11	-	-	-	-	+++ 90%	++ 80%	++ 20%	-	-
6E9	++ 50%	+	-	-	++ > 95%	++ 70%	+	-	-

Antibody	RSPO2 Hi	RSPO2 Med	RSPO2 Low	293 none	RSPO3 Hi	RSPO3 Med	RSPO3 Low	RSPO1 Hi	RSPO4 Hi
21C2	+	-	-	-	++ 95%	++ 60%	+ 10%	-	-
26E11	++ 70%	+ 40%	+ 10%	-	+++ 90%	++ 70%	++ 50%	-	-
1A1	++ 80%	+ 40%	+ 30%	-	-	-	-	-	-
11F11	-	-	-	-	-	-	-	-	-
36D2	++ > 95%	++ 50%	+ <5%	-	-	-	-	-	-
49G5	++ 95%	++ 60%	+ 40%	-	-	-	-	-	-
IgG	-	-	-	-	-	-	-	-	-
naïve mouse IgG2a	-	-	-	-	-	-	-	-	-

-, +, ++, +++ indicates relative intensity with – being not significant staining and +++ being highest level of staining;
Percentage indicates relative percentage of cells staining

Epitope binning of anti-RSPO antibodies

[0364] To further characterize anti-RSPO antibodies, the number of unique epitope bins the antibodies fell into was determined using an OCTET RED assay. Antibodies were first affinity ranked. The antibody with the highest affinity was bound to saturation to a hRSPO2 or hRSPO3 bound biosensor. Binding by a second antibody was then assessed. The anti-RSPO2 antibodies tested fell into two unique epitope bins defined by the ability to compete with either 1A1 or 11F11. The first unique epitope bin included 1A1, 49G5, and 36D2 while the second unique epitope bin included 11F11. The anti-RSPO3 antibodies tested fell into three unique epitope bins defined by the ability to compete with 26E11, 4H1, or 21C2. The first unique epitope bin included 26E11, 5D6, 5E11, and 6E9, the second unique epitope bin included 4H1, and the third unique epitope bin included 5C2 and 21C2.

Binding specificities and affinities of anti-RSPO antibodies

[0365] To further characterize the anti-RSPO antibodies, their function blocking activities were tested against mouse RSPO2 (R&D Systems) and cynomolgus RSPO2 (Genentech). A subset of antibody clones could block hRSPO2, cynoRSPO2, and mRSPO2 stimulation of WNT reporter cells (Figure 3A-C). A polymorphism at position 186 in RSPO2 was identified in the human population. To assess the functional blocking activity of anti-RSPO antibodies to this polymorphism and potential usefulness in this patient population, hRSPO2 L186P protein was first purified and then used to stimulate WNT reporter cells. A subset of anti-RSPO antibodies could block the function hRSPO2 L186P (Figure 3D).

[0366] In addition, anti-RSPO antibodies were tested for their ability to block the function of mouse RSPO3 (R&D Systems) and cynomolgus RSPO3 (Genentech). A subset of antibodies could inhibit the WNT reporter cell stimulation of hRSPO3, cynoRSPO3, mRSPO3 (Figure 4A-C). Anti-RSPO antibodies were additionally tested for their ability to inhibit RSPO3-fusion genes recently identified in colorectal tumors (Seshagiri et al., *Nature* 488:660-664 (2012)). Conditioned media was prepared by transfecting constructs encoding the two PTPRK-RSPO3 fusions genes identified (SEQ ID NO:176 and 178). Conditioned media containing RSPO3 or RSPO3-fusion genes could stimulate WNT reporter activity. An anti-RSPO3 antibody could inhibit RSPO3-fusion gene stimulation of reporter cells (Figure 4D). This result indicates that the anti-RSPO3 antibodies could inhibit RPSO translocation-mediated wnt signaling.

[0367] Surface plasmon resonance was used to confirm binding specificities and affinities to human, mouse, and cynomolgus RSPOs. Fab fragments from antibody clones were digested, purified, and then assayed using a BIAcore™-2000 instrument for binding to recombinant proteins. Antibodies fell into three groups: those specific to RSPO2, those specific to RSPO3, and those with some degree of cross-reactivity (Figure 5). Binding affinities were in the sub to low nanomolar range (range 0.073 – 80 nM).

Binding characteristics of anti-RSPO antibodies

[0368] It has previously been shown that RSPO proteins can bind to two different classes of transmembrane proteins: the E3-ligases (RNF43 and ZNRF3) and the LGRs (LGR4 and LGR5) (Hao et al., *Nature* 485(7397):195-200 (2012)). To test whether the anti-RSPO antibodies could inhibit binding with these two classes of proteins, a competitive binding ELISA assays was developed. Anti-RSPO antibodies fell into three categories when tested for the ability to inhibit LGR4 or LGR5 binding to hRSPO2 and hRSPO3: those that could inhibit, those that did not inhibit, and one that promoted the interaction of LGR4 and LGR5 (Figure 6A-B and data not shown). Likewise, a subset of the panel of anti-RSPO antibodies inhibited RNF43 binding to hRSPO2 or hRSPO3 (Figure 7A-B). A summary of anti-RSPO results is shown below (Table 5).

Humanization of anti-RSPO antibodies

[0369] The binding affinity of humanized 5D6v1(referred to as hu5D6v1) antibody was compared to chimeric 5D6. Murine vernier positions of hu5D6v1 were converted back to human residues to evaluate the contribution of murine vernier positions to binding to hRSPO3. Four additional light chains (L1: v1 + Y36(referred to as v2.1), v1+L46 (referred to as v2.2), v1+T69 (referred to as v2.3), v1+F71 (referred to as v2.4)) and four additional heavy chains (v1+V71(referred to as v2.8), v1+R94(referred to as v2.10), v1+W47+I48+F78(referred to as v3.2), v1+W47+I48+v67+F78(referred to as v3.3)). F36 and T46 on the light chain were the key mouse vernier residues, and V71 and R94 on

the heavy chain were determined to be the key mouse vernier residues based on binding affinity evaluation of the variant antibodies described above (data not shown). Chimeric 5D6 bound with a KD of $3.3\text{E-}11$ M, while v1+T69 (LC) + (W47+I48+V67+F78 (HC) (referred to as hu5D6v4.1), v1+T69 (LC) + (W47+I48+F78 (HC) (referred to as hu5D6v4.3), bound with a KD of $6.3\text{E-}11$ M, and $7.0\text{E-}11$ M, respectively.

[0370] The hu5D6v4.1 and the chimeric 5D6 were tested for their ability to bind cyno and mouse RSPO3 as described above except that cyno or murine RSPO3 replaced huRSPO3 in the binding assay. Binding properties for the humanized antibodies are shown below in Table 5.

Antibody	huKD (M)	huka (1/Ms)	hukd (1/s)	cynoKD (M)	cynoka (1/Ms)	cynokd (1/s)	muKD (M)	muka (1/Ms)	mukd (1/s)
5D6 chimera	3.31 E-11	3.51 E+06	1.16 E-04	4.53 E-11	4.96 E+06	2.24 E-04	5.78 E-11	4.10 E+06	2.37 E-04
Hu5D6v4.1	6.35 E-11	3.60 E+06	2.29E- 04	8.11 E-11	5.35 E+06	4.34 E-04	9.93 E-11	4.26 E+06	4.23 E-04

[0371] The humanized antibodies hu5D6v4.1 was tested under thermal stress (40°C , pH 5.5, 2 weeks) and 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH) Analysis. Then sample was thermally stressed to mimic stability over the shelf life of the product. The sample was buffer exchanged into 20mM His Acetate, 240mM sucrose, pH 5.5 and diluted to a concentration of 1 mg/mL. One mL of sample was stressed at 40°C for 2 weeks and a second was stored at -70°C as a control. Both samples were then digested using trypsin to create peptides that could be analyzed using liquid chromatography(LC) - mass spectrometry(MS) analysis. For each peptide in the sample retention time, from the LC as well as high resolution accurate mass and peptide ion fragmentation information (amino acid sequence information) were acquired in the MS. Extracted ion chromatograms (XIC) were taken for peptides of interest (native and modified peptide ions) from the data sets at a window of ± 10 ppm and peaks were integrated to determine area. Relative percentages of modification were calculated for each sample by taking the (area of the modified peptide) divided by (area of the modified peptide plus the area of the native peptide) multiplied by 100.

[0372] As determined by the thermal stress test, hu5D6v4.1 has $\text{W}^{100\text{b}}$ in CDR-H3, which is susceptible to oxidation (11.5% increase in Tryptophan oxidation. From 24.1% in control to 35.6% after AAPH stress). F100b (referred to as hu5D6v5.1) and W100bH (referred to as hu5D6v5.2) variants were constructed to reduce potential oxidation.

Epitope Mapping By Crystallography

[0373] To further characterize the anti-RSPO antibodies, crystals of RSPO3/Fab Complex (5D6 and 26E11) were prepared as described above and the crystal structure determined. See Figure 8. Table 6

contains a list of contacts between the heavy chain (HC) and light chain (LC) of 5D6 and RSPO3 (F chain). The cutoff for Table 6 is 4 angstroms. Table 7 contains a list of contacts between the heavy chain (HC) and light chain (LC) of 26E11 and RSPO3 (F chain). The cutoff for Table 7 is 4 angstroms. Most of the contacts of both 5D6 and 26E11 are with the Furin 1 domain of RSPO3.

Table 5-Summary of anti-RSPO antibody Results.

	Inhibit hRSPO2 wnt signaling	Inhibit hRSPO3 wnt signaling	Inhibit L186P wnt signaling	Epitope Bin 26F11	Epitope Bin 4H1	Epitope Bin 21C2	Epitope Bin 1A1	Epitope Bin 11F11	Inhibit LGR4/ RSPO2	Inhibit LGR4/ RSPO3	Inhibit LGR5/ RSPO2	Inhibit LGR5/ RSPO3	Inhibit RNF43/ RSPO2	Inhibit RNF43/ RSPO3
4H1	+	++	+	No	Yes	No	ND	ND	-	+++	-	++	-	+++
4D4	-	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5C2	-	+++	-	No	No	Yes	ND	ND	+	+++	-	+++	-	++
5D6	-	+++	-	Yes	No	No	ND	ND	+	+++	-	+++	-	+++
5E11	-	+++	-	Yes	No	No	ND	ND	+	+++	-	+++	-	+++
6E9	+	++	-	Yes	No	No	ND	ND	+++	+++	+	+++	-	+++
21C2	-	ND	ND	No	No	Yes	ND	ND	-	+++	-	+++	ND	ND
26E11	++	+++	++	Yes	No	No	ND	ND	+++	+++	++	+++	+++	+++
1A1	+++	-	+++	ND	ND	ND	Yes	No	- (enhanced binding)	-	- (enhanced binding)	-	+++	-
11F11	++	-	++	ND	ND	ND	No	Yes	-	-	-	-	-	-
36D2	+++	-	+++	ND	ND	ND	Yes	No	+++	-	+++	-	+++	-
49G5	-	-	ND	ND	ND	ND	Yes	No	++	-	+	-	+	-

Table 6-Contact residues of RSPO3 and 5D6 heavy and light chain

Antibody Chain	Residue	Atom name	Atom	RSPO3	Residue	Atom name	Atom	Distance
/H/	316(GLY)	O	O]:	/F/	49(SER)	OG	O]:	3.94
/H/	316(GLY)	CA	C]:	/F/	54(CYS)	O	O]:	3.59
				/F/	55(LEU)	CA	C]:	3.9
/H/	314(GLY)	CA	C]:	/F/	55(LEU)	O	O]:	3.51
/H/	314(GLY)	C	C]:	/F/	55(LEU)	O	O]:	3.56
/H/	315(TYR)	N	N]:	/F/	55(LEU)	O	O]:	3.53
/H/	316(GLY)	N	N]:	/F/	55(LEU)	O	O]:	3.03
/H/	316(GLY)	CA	C]:	/F/	55(LEU)	O	O]:	3.55
/H/	317(GLY)	N	N]:	/F/	55(LEU)	CD1	C]:	3.34
/H/	314(GLY)	N	N]:	/F/	55(LEU)	CD1	C]:	4
/H/	316(GLY)	C	C]:	/F/	55(LEU)	CD1	C]:	3.92
/H/	317(GLY)	CA	C]:	/F/	55(LEU)	CD1	C]:	3.46
/H/	313(TYR)	O	O]:	/F/	56(SER)	CB	C]:	3.62
/H/	315(TYR)	CD2	C]:	/F/	63(PHE)	CB	C]:	3.92
/H/	315(TYR)	CB	C]:	/F/	63(PHE)	CG	C]:	3.56
				/F/	63(PHE)	CD1	C]:	3.41
				/F/	63(PHE)	CD2	C]:	3.89
				/F/	63(PHE)	CE1	C]:	3.6
/H/	316(GLY)	N	N]:	/F/	63(PHE)	CE2	C]:	3.59
/H/	316(GLY)	CA	C]:	/F/	63(PHE)	CE2	C]:	3.77
/H/	315(TYR)	C	C]:	/F/	63(PHE)	CZ	C]:	3.83
/H/	315(TYR)	O	O]:	/F/	63(PHE)	CZ	C]:	3.97
/H/	315(TYR)	CB	C]:	/F/	63(PHE)	CZ	C]:	3.92
/H/	316(GLY)	N	N]:	/F/	63(PHE)	CZ	C]:	3.71
/H/	316(GLY)	CA	C]:	/F/	63(PHE)	CZ	C]:	3.76
/H/	264(TYR)	OH	O]:	/F/	89(TYR)	CB	C]:	3.79
/H/	315(TYR)	OH	O]:	/F/	89(TYR)	CD1	C]:	3.8
				/F/	89(TYR)	CE1	C]:	3.95
/H/	264(TYR)	OH	O]:	/F/	90(PRO)	N	N]:	3.5
				/F/	90(PRO)	CA	C]:	3.74
				/F/	90(PRO)	C	C]:	3.58
/H/	264(TYR)	CE1	C]:	/F/	90(PRO)	O	O]:	3.62
/H/	264(TYR)	CZ	C]:	/F/	90(PRO)	O	O]:	3.61
/H/	264(TYR)	OH	O]:	/F/	90(PRO)	O	O]:	2.72
/H/	271(THR)	O	O]:	/F/	90(PRO)	CB	C]:	3.81
/H/	264(TYR)	OH	O]:	/F/	90(PRO)	CB	C]:	3.5
/H/	271(THR)	O	O]:	/F/	90(PRO)	CG	C]:	3.33
/H/	264(TYR)	OH	O]:	/F/	90(PRO)	CG	C]:	3.91

				/F/	90(PRO)	CD	C]:	3.55
/H/	247(TYR)	OH	O]:	/F/	91(ASP)	C	C]:	3.66
/H/	247(TYR)	CE2	C]:	/F/	91(ASP)	O	O]:	3.55
/H/	247(TYR)	CZ	C]:	/F/	91(ASP)	O	O]:	3.52
/H/	247(TYR)	OH	O]:	/F/	91(ASP)	O	O]:	2.62
/H/	267(TYR)	CE2	C]:	/F/	91(ASP)	O	O]:	3.7
/H/	267(TYR)	OH	O]:	/F/	91(ASP)	O	O]:	3.7
/H/	266(SER)	CB	C]:	/F/	91(ASP)	CB	C]:	3.65
/H/	266(SER)	OG	O]:	/F/	91(ASP)	CB	C]:	3.56
/H/	267(TYR)	CE2	C]:	/F/	91(ASP)	CB	C]:	3.74
/H/	267(TYR)	CZ	C]:	/F/	91(ASP)	CB	C]:	3.93
/H/	267(TYR)	OH	O]:	/F/	91(ASP)	CB	C]:	3.88
/H/	268(SER)	OG	O]:	/F/	91(ASP)	CG	C]:	3.45
/H/	266(SER)	CB	C]:	/F/	91(ASP)	CG	C]:	4
/H/	266(SER)	OG	O]:	/F/	91(ASP)	CG	C]:	3.34
/H/	270(LYS)	NZ	N]:	/F/	91(ASP)	CG	C]:	3.97
/H/	267(TYR)	CE2	C]:	/F/	91(ASP)	CG	C]:	3.97
/H/	267(TYR)	CZ	C]:	/F/	91(ASP)	CG	C]:	3.71
/H/	267(TYR)	OH	O]:	/F/	91(ASP)	CG	C]:	3.68
/H/	268(SER)	CB	C]:	/F/	91(ASP)	OD1	O]:	3.47
/H/	268(SER)	OG	O]:	/F/	91(ASP)	OD1	O]:	2.47
/H/	270(LYS)	CG	C]:	/F/	91(ASP)	OD1	O]:	3.83
/H/	266(SER)	CB	C]:	/F/	91(ASP)	OD1	O]:	3.39
/H/	266(SER)	OG	O]:	/F/	91(ASP)	OD1	O]:	2.4
/H/	268(SER)	N	N]:	/F/	91(ASP)	OD1	O]:	3.78
/H/	270(LYS)	CB	C]:	/F/	91(ASP)	OD1	O]:	3.92
/H/	270(LYS)	NZ	N]:	/F/	91(ASP)	OD1	O]:	3.99
/H/	267(TYR)	CZ	C]:	/F/	91(ASP)	OD1	O]:	3.94
/H/	268(SER)	OG	O]:	/F/	91(ASP)	OD2	O]:	3.71
/H/	270(LYS)	NZ	N]:	/F/	91(ASP)	OD2	O]:	3.35
/H/	267(TYR)	CZ	C]:	/F/	91(ASP)	OD2	O]:	3.96
/H/	267(TYR)	OH	O]:	/F/	91(ASP)	OD2	O]:	3.54
/H/	270(LYS)	CD	C]:	/F/	91(ASP)	OD2	O]:	3.95
/H/	247(TYR)	CE2	C]:	/F/	92(ILE)	CB	C]:	3.92
/H/	247(TYR)	CZ	C]:	/F/	92(ILE)	CB	C]:	3.94
/H/	247(TYR)	OH	O]:	/F/	92(ILE)	CB	C]:	3.57
				/F/	92(ILE)	CG1	C]:	3.77
/H/	315(TYR)	CE2	C]:	/F/	92(ILE)	CG1	C]:	3.59
/H/	264(TYR)	OH	O]:	/F/	92(ILE)	CG2	C]:	3.8
/H/	247(TYR)	CZ	C]:	/F/	92(ILE)	CD1	C]:	3.92
/H/	247(TYR)	OH	O]:	/F/	92(ILE)	CD1	C]:	3.86

/H/	312(TYR)	CE1	C]:	/F/	92(ILE)	CD1	C]:	3.99
/H/	315(TYR)	CD2	C]:	/F/	92(ILE)	CD1	C]:	3.78
/H/	315(TYR)	CE2	C]:	/F/	92(ILE)	CD1	C]:	3.51
/H/	315(TYR)	CZ	C]:	/F/	94(LYS)	CB	C]:	3.95
/H/	315(TYR)	CD2	C]:	/F/	94(LYS)	CB	C]:	3.96
/H/	315(TYR)	CE2	C]:	/F/	94(LYS)	CB	C]:	3.56
/H/	315(TYR)	OH	O]:	/F/	94(LYS)	CG	C]:	3.98
/H/	315(TYR)	CE1	C]:	/F/	94(LYS)	CD	C]:	3.59
/H/	315(TYR)	CZ	C]:	/F/	94(LYS)	CD	C]:	3.33
/H/	315(TYR)	OH	O]:	/F/	94(LYS)	CD	C]:	3.28
/H/	315(TYR)	CE2	C]:	/F/	94(LYS)	CD	C]:	3.86
/L/	53(ARG)	NH2	N]:	/F/	52(ASN)	O	O]:	3.58
/L/	53(ARG)	NE	N]:	/F/	52(ASN)	CB	C]:	3.98
/L/	53(ARG)	CZ	C]:	/F/	52(ASN)	CB	C]:	3.29
/L/	53(ARG)	NH1	N]:	/F/	52(ASN)	CB	C]:	3.24
/L/	53(ARG)	NH2	N]:	/F/	52(ASN)	CB	C]:	3.37
/L/	53(ARG)	CD	C]:	/F/	52(ASN)	CG	C]:	3.99
/L/	53(ARG)	NE	N]:	/F/	52(ASN)	CG	C]:	3.54
/L/	53(ARG)	CZ	C]:	/F/	52(ASN)	CG	C]:	3.39
/L/	53(ARG)	NH1	N]:	/F/	52(ASN)	CG	C]:	3.62
/L/	53(ARG)	NH2	N]:	/F/	52(ASN)	CG	C]:	3.75
/L/	53(ARG)	CD	C]:	/F/	52(ASN)	ND2	N]:	3.41
/L/	53(ARG)	NE	N]:	/F/	52(ASN)	ND2	N]:	3.47
/L/	53(ARG)	CZ	C]:	/F/	52(ASN)	ND2	N]:	3.56
/L/	53(ARG)	NH1	N]:	/F/	52(ASN)	ND2	N]:	3.53
/L/	53(ARG)	NE	N]:	/F/	52(ASN)	OD1	O]:	3.9
/L/	53(ARG)	CZ	C]:	/F/	52(ASN)	OD1	O]:	3.96
/L/	32(TYR)	OH	O]:	/F/	63(PHE)	CD1	C]:	3.43
/L/	32(TYR)	CE1	C]:	/F/	63(PHE)	CE1	C]:	3.84
/L/	32(TYR)	OH	O]:	/F/	63(PHE)	CE1	C]:	3.49
				/F/	65(LEU)	CG	C]:	3.61
/L/	30(ASP)	O	O]:	/F/	65(LEU)	CD1	C]:	3.64
/L/	32(TYR)	CZ	C]:	/F/	65(LEU)	CD1	C]:	3.98
/L/	32(TYR)	OH	O]:	/F/	65(LEU)	CD1	C]:	3.94
/L/	50(LEU)	CD1	C]:	/F/	65(LEU)	CD2	C]:	3.81
/L/	53(ARG)	NH2	N]:	/F/	72(GLN)	C	C]:	3.55
/L/	53(ARG)	NE	N]:	/F/	72(GLN)	O	O]:	3.85
/L/	53(ARG)	CZ	C]:	/F/	72(GLN)	O	O]:	3.57
/L/	53(ARG)	NH2	N]:	/F/	72(GLN)	O	O]:	2.47
/L/	31(SER)	CB	C]:	/F/	72(GLN)	NE2	N]:	3.62
/L/	31(SER)	OG	O]:	/F/	72(GLN)	NE2	N]:	3.34

/L/	53(ARG)	NH2	N]:	/F/	73(ILE)	CA	C]:	3.66
				/F/	74(GLY)	N	N]:	3.93
/L/	30(ASP)	OD2	O]:	/F/	84(TYR)	CE2	C]:	3.77
/L/	94(PHE)	CD2	C]:	/F/	89(TYR)	CG	C]:	3.72
				/F/	89(TYR)	CD1	C]:	3.66
				/F/	89(TYR)	CD2	C]:	3.85
				/F/	89(TYR)	CE1	C]:	3.74
/L/	94(PHE)	CB	C]:	/F/	89(TYR)	CE2	C]:	3.89
/L/	94(PHE)	CD2	C]:	/F/	89(TYR)	CE2	C]:	3.93
/L/	94(PHE)	N	N]:	/F/	89(TYR)	CZ	C]:	3.57
/L/	94(PHE)	CD2	C]:	/F/	89(TYR)	CZ	C]:	3.87
/L/	93(GLU)	C	C]:	/F/	89(TYR)	OH	O]:	3.74
/L/	94(PHE)	N	N]:	/F/	89(TYR)	OH	O]:	3.14
/L/	93(GLU)	CA	C]:	/F/	89(TYR)	OH	O]:	3.35
/L/	93(GLU)	CB	C]:	/F/	89(TYR)	OH	O]:	3.39
/L/	92(ASP)	O	O]:	/F/	94(LYS)	CD	C]:	3.68
				/F/	94(LYS)	CE	C]:	3.55
/L/	32(TYR)	CE2	C]:	/F/	94(LYS)	CE	C]:	3.63
/L/	32(TYR)	CZ	C]:	/F/	94(LYS)	CE	C]:	3.66
/L/	92(ASP)	OD1	O]:	/F/	94(LYS)	CE	C]:	3.96
/L/	92(ASP)	CA	C]:	/F/	94(LYS)	NZ	N]:	3.95
/L/	92(ASP)	C	C]:	/F/	94(LYS)	NZ	N]:	3.76
/L/	92(ASP)	O	O]:	/F/	94(LYS)	NZ	N]:	2.89
/L/	32(TYR)	CE2	C]:	/F/	94(LYS)	NZ	N]:	3.74
/L/	92(ASP)	CG	C]:	/F/	94(LYS)	NZ	N]:	3.85
/L/	92(ASP)	OD1	O]:	/F/	94(LYS)	NZ	N]:	2.81
/L/	28(ASP)	OD2	O]:	/F/	97(LYS)	CD	C]:	3.7
				/F/	97(LYS)	CE	C]:	3.96
/L/	30(ASP)	OD1	O]:	/F/	97(LYS)	CE	C]:	4
/L/	28(ASP)	CG	C]:	/F/	97(LYS)	NZ	N]:	3.82
/L/	28(ASP)	OD1	O]:	/F/	97(LYS)	NZ	N]:	3.85
/L/	28(ASP)	OD2	O]:	/F/	97(LYS)	NZ	N]:	3.11
/L/	27(GLN)	NE2	N]:	/F/	108(LYS)	NZ	N]:	3.9

Table 7-Contact residues of RSPO3 and 26E11 heavy and light chain

Antibody Chain	Residue name	Atom name	Atom	RSPO3	Residue name	Atom name	Atom	Distance
/H/	313(HIS)	CE1	C	/F/	47(THR)	CB	C	3.72
/H/	313(HIS)	NE2	N	/F/	47(THR)	CB	C	3.73
/H/	313(HIS)	CE1	C	/F/	47(THR)	CG2	C	3.82
/H/	313(HIS)	NE2	N	/F/	47(THR)	CG2	C	3.3
/H/	313(HIS)	CE1	C	/F/	47(THR)	OG1	O	2.92
/H/	313(HIS)	NE2	N	/F/	47(THR)	OG1	O	3.25
/H/	316(GLY)	CA	C	/F/	54(CYS)	O	O	3.93
/H/	316(GLY)	CA	C	/F/	55(LEU)	CA	C	3.89
/H/	316(GLY)	C	C	/F/	55(LEU)	CA	C	3.86
/H/	316(GLY)	N	N	/F/	55(LEU)	C	C	3.92
/H/	316(GLY)	CA	C	/F/	55(LEU)	C	C	4
/H/	314(GLY)	CA	C	/F/	55(LEU)	O	O	3.72
/H/	314(GLY)	C	C	/F/	55(LEU)	O	O	3.63
/H/	315(TYR)	N	N	/F/	55(LEU)	O	O	3.66
/H/	316(GLY)	N	N	/F/	55(LEU)	O	O	2.92
/H/	316(GLY)	CA	C	/F/	55(LEU)	O	O	3.34
/H/	316(GLY)	C	C	/F/	55(LEU)	O	O	3.7
/H/	317(GLY)	N	N	/F/	55(LEU)	O	O	3.63
/H/	313(HIS)	ND1	N	/F/	55(LEU)	CB	C	3.96
/H/	313(HIS)	CE1	C	/F/	55(LEU)	CB	C	3.45
/H/	316(GLY)	C	C	/F/	55(LEU)	CD2	C	3.96
/H/	316(GLY)	O	O	/F/	55(LEU)	CD2	C	3.29
/H/	313(HIS)	CE1	C	/F/	56(SER)	CB	C	3.92
/H/	313(HIS)	CE1	C	/F/	56(SER)	OG	O	3.74
/H/	313(HIS)	NE2	N	/F/	56(SER)	OG	O	3.86
/H/	315(TYR)	CB	C	/F/	63(PHE)	CG	C	3.69
/H/	315(TYR)	CB	C	/F/	63(PHE)	CD2	C	3.48
/H/	315(TYR)	O	O	/F/	63(PHE)	CE2	C	3.86
/H/	315(TYR)	CB	C	/F/	63(PHE)	CE2	C	3.89
/H/	316(GLY)	N	N	/F/	63(PHE)	CE1	C	3.9
/H/	316(GLY)	CA	C	/F/	63(PHE)	CE1	C	3.82
/H/	315(TYR)	O	O	/F/	63(PHE)	CZ	C	3.95
/H/	315(TYR)	C	C	/F/	63(PHE)	CZ	C	3.98
/H/	316(GLY)	N	N	/F/	63(PHE)	CZ	C	3.87
/H/	316(GLY)	CA	C	/F/	63(PHE)	CZ	C	3.67
/H/	264(TYR)	OH	O	/F/	89(TYR)	CB	C	3.67
/H/	315(TYR)	OH	O	/F/	89(TYR)	CD2	C	3.53
/H/	315(TYR)	OH	O	/F/	89(TYR)	CE2	C	3.65

/H/	264(TYR)	OH	O	/F/	90(PRO)	N	N	3.57
/H/	264(TYR)	OH	O	/F/	90(PRO)	CA	C	3.96
/H/	264(TYR)	OH	O	/F/	90(PRO)	C	C	3.76
/H/	264(TYR)	CE1	C	/F/	90(PRO)	O	O	3.73
/H/	264(TYR)	CZ	C	/F/	90(PRO)	O	O	3.74
/H/	264(TYR)	OH	O	/F/	90(PRO)	O	O	2.86
/H/	264(TYR)	OH	O	/F/	90(PRO)	CB	C	3.87
/H/	271(THR)	O	O	/F/	90(PRO)	CG	C	3.48
/H/	264(TYR)	OH	O	/F/	90(PRO)	CD	C	3.51
/H/	247(TYR)	CE2	C	/F/	91(ASP)	C	C	3.95
/H/	247(TYR)	OH	O	/F/	91(ASP)	C	C	3.46
/H/	247(TYR)	CE2	C	/F/	91(ASP)	O	O	3.35
/H/	247(TYR)	CZ	C	/F/	91(ASP)	O	O	3.28
/H/	247(TYR)	OH	O	/F/	91(ASP)	O	O	2.37
/H/	267(PHE)	CE2	C	/F/	91(ASP)	O	O	3.8
/H/	266(SER)	CB	C	/F/	91(ASP)	CB	C	3.71
/H/	266(SER)	OG	O	/F/	91(ASP)	CB	C	3.73
/H/	267(PHE)	CE2	C	/F/	91(ASP)	CB	C	3.83
/H/	267(PHE)	CZ	C	/F/	91(ASP)	CB	C	3.81
/H/	266(SER)	CB	C	/F/	91(ASP)	CG	C	3.91
/H/	266(SER)	OG	O	/F/	91(ASP)	CG	C	3.36
/H/	270(LYS)	CD	C	/F/	91(ASP)	CG	C	3.75
/H/	268(SER)	OG	O	/F/	91(ASP)	CG	C	3.4
/H/	267(PHE)	CZ	C	/F/	91(ASP)	CG	C	3.84
/H/	266(SER)	CB	C	/F/	91(ASP)	OD2	O	3.35
/H/	266(SER)	OG	O	/F/	91(ASP)	OD2	O	2.48
/H/	270(LYS)	CB	C	/F/	91(ASP)	OD2	O	3.25
/H/	270(LYS)	CG	C	/F/	91(ASP)	OD2	O	3.29
/H/	270(LYS)	CD	C	/F/	91(ASP)	OD2	O	3.16
/H/	268(SER)	OG	O	/F/	91(ASP)	OD2	O	2.77
/H/	270(LYS)	NZ	N	/F/	91(ASP)	OD2	O	3.91
/H/	270(LYS)	CD	C	/F/	91(ASP)	OD1	O	3.97
/H/	267(PHE)	CE1	C	/F/	91(ASP)	OD1	O	3.83
/H/	268(SER)	OG	O	/F/	91(ASP)	OD1	O	3.41
/H/	270(LYS)	NZ	N	/F/	91(ASP)	OD1	O	3.97
/H/	267(PHE)	CZ	C	/F/	91(ASP)	OD1	O	3.61
/H/	247(TYR)	OH	O	/F/	92(ILE)	CA	C	3.82
/H/	247(TYR)	CE2	C	/F/	92(ILE)	CB	C	3.92
/H/	247(TYR)	CZ	C	/F/	92(ILE)	CB	C	3.72
/H/	247(TYR)	OH	O	/F/	92(ILE)	CB	C	3.26
/H/	315(TYR)	CD2	C	/F/	92(ILE)	CG1	C	3.8

/H/	315(TYR)	CE2	C	/F/	92(ILE)	CG1	C	3.61
/H/	247(TYR)	OH	O	/F/	92(ILE)	CG1	C	3.48
/H/	264(TYR)	OH	O	/F/	92(ILE)	CG2	C	3.72
/H/	315(TYR)	CD2	C	/F/	92(ILE)	CD1	C	3.65
/H/	315(TYR)	CE2	C	/F/	92(ILE)	CD1	C	3.62
/H/	247(TYR)	CE1	C	/F/	92(ILE)	CD1	C	3.88
/H/	247(TYR)	CZ	C	/F/	92(ILE)	CD1	C	3.81
/H/	247(TYR)	OH	O	/F/	92(ILE)	CD1	C	3.73
/H/	315(TYR)	CD2	C	/F/	93(ASN)	O	O	3.79
/H/	315(TYR)	CE2	C	/F/	93(ASN)	O	O	3.86
/H/	315(TYR)	CD2	C	/F/	94(LYS)	CB	C	3.57
/H/	315(TYR)	CE2	C	/F/	94(LYS)	CB	C	3.46
/H/	315(TYR)	CE2	C	/F/	94(LYS)	CG	C	3.73
/H/	315(TYR)	CZ	C	/F/	94(LYS)	CG	C	3.84
/H/	315(TYR)	CD1	C	/F/	94(LYS)	CD	C	3.83
/H/	315(TYR)	CE1	C	/F/	94(LYS)	CD	C	3.45
/H/	315(TYR)	CD2	C	/F/	94(LYS)	CD	C	3.99
/H/	315(TYR)	CE2	C	/F/	94(LYS)	CD	C	3.62
/H/	315(TYR)	CZ	C	/F/	94(LYS)	CD	C	3.33
/H/	315(TYR)	OH	O	/F/	94(LYS)	CD	C	3.73
/L/	53(ARG)	NH2	N	/F/	52(ASN)	O	O	3.44
/L/	53(ARG)	CZ	C	/F/	52(ASN)	CB	C	3.48
/L/	53(ARG)	NH1	N	/F/	52(ASN)	CB	C	3.31
/L/	53(ARG)	NH2	N	/F/	52(ASN)	CB	C	3.72
/L/	53(ARG)	CD	C	/F/	52(ASN)	CG	C	3.8
/L/	53(ARG)	CZ	C	/F/	52(ASN)	CG	C	3.44
/L/	53(ARG)	NH1	N	/F/	52(ASN)	CG	C	3.54
/L/	53(ARG)	NH2	N	/F/	52(ASN)	CG	C	3.96
/L/	53(ARG)	NE	N	/F/	52(ASN)	CG	C	3.53
/L/	53(ARG)	CD	C	/F/	52(ASN)	ND2	N	3.82
/L/	53(ARG)	NH1	N	/F/	52(ASN)	ND2	N	3.99
/L/	53(ARG)	CD	C	/F/	52(ASN)	OD1	O	3.72
/L/	53(ARG)	CZ	C	/F/	52(ASN)	OD1	O	3.42
/L/	53(ARG)	NH1	N	/F/	52(ASN)	OD1	O	3.96
/L/	53(ARG)	NH2	N	/F/	52(ASN)	OD1	O	3.76
/L/	53(ARG)	NE	N	/F/	52(ASN)	OD1	O	3.25
/L/	32(TYR)	OH	O	/F/	63(PHE)	CD2	C	3.3
/L/	32(TYR)	CE1	C	/F/	63(PHE)	CE2	C	3.9
/L/	32(TYR)	OH	O	/F/	63(PHE)	CE2	C	3.47
/L/	32(TYR)	OH	O	/F/	65(LEU)	CG	C	3.91
/L/	30(ASP)	O	O	/F/	65(LEU)	CD1	C	3.62

/L/	53(ARG)	NH2	N	/F/	72(GLN)	O	O	2.93
/L/	31(SER)	OG	O	/F/	72(GLN)	CD	C	3.94
/L/	31(SER)	OG	O	/F/	72(GLN)	NE2	N	3.81
/L/	31(SER)	CB	C	/F/	72(GLN)	OE1	O	3.48
/L/	31(SER)	OG	O	/F/	72(GLN)	OE1	O	3.24
/L/	30(ASP)	OD2	O	/F/	84(TYR)	CE2	C	3.77
/L/	94(PHE)	CD2	C	/F/	89(TYR)	CG	C	3.57
/L/	94(PHE)	CE2	C	/F/	89(TYR)	CG	C	4
/L/	94(PHE)	CD2	C	/F/	89(TYR)	CD1	C	3.69
/L/	94(PHE)	CE2	C	/F/	89(TYR)	CD2	C	3.49
/L/	94(PHE)	CE2	C	/F/	89(TYR)	CD2	C	3.83
/L/	94(PHE)	CB	C	/F/	89(TYR)	CE1	C	3.79
/L/	94(PHE)	CD2	C	/F/	89(TYR)	CE1	C	3.74
/L/	94(PHE)	CD2	C	/F/	89(TYR)	CE2	C	3.54
/L/	94(PHE)	CB	C	/F/	89(TYR)	CZ	C	3.96
/L/	94(PHE)	N	N	/F/	89(TYR)	CZ	C	3.59
/L/	94(PHE)	CD2	C	/F/	89(TYR)	CZ	C	3.66
/L/	94(PHE)	O	O	/F/	89(TYR)	OH	O	3.79
/L/	93(GLU)	CA	C	/F/	89(TYR)	OH	O	3.67
/L/	93(GLU)	C	C	/F/	89(TYR)	OH	O	3.85
/L/	94(PHE)	N	N	/F/	89(TYR)	OH	O	3.05
/L/	93(GLU)	CB	C	/F/	89(TYR)	OH	O	3.56
/L/	92(ASP)	O	O	/F/	94(LYS)	CD	C	3.91
/L/	92(ASP)	O	O	/F/	94(LYS)	CE C		3.31
/L/	32(TYR)	CD2	C	/F/	94(LYS)	CE	C	3.85
/L/	32(TYR)	CE2	C	/F/	94(LYS)	CE	C	3.35
/L/	32(TYR)	CZ	C	/F/	94(LYS)	CE	C	3.57
/L/	32(TYR)	OH	O	/F/	94(LYS)	CE	C	3.85
/L/	92(ASP)	OD1	O	/F/	94(LYS)	CE	C	3.54
/L/	92(ASP)	C	C	/F/	94(LYS)	NZ	N	3.72
/L/	92(ASP)	O	O	/F/	94(LYS)	NZ	N	2.61
/L/	92(ASP)	OD1	O	/F/	94(LYS)	NZ	N	3.14
/L/	30(ASP)	CG	C	/F/	97(LYS)	CG	C	3.96
/L/	30(ASP)	OD2	O	/F/	97(LYS)	CG	C	3.81
/L/	28(ASP)	CG	C	/F/	97(LYS)	CD	C	3.72
/L/	28(ASP)	OD1	O	/F/	97(LYS)	CD	C	3.81
/L/	28(ASP)	OD2	O	/F/	97(LYS)	CD	C	3.53
/L/	28(ASP)	OD2	O	/F/	97(LYS)	CE	C	3.96
/L/	30(ASP)	CG	C	/F/	97(LYS)	CE	C	3.71
/L/	30(ASP)	OD1	O	/F/	97(LYS)	CE	C	3.47
/L/	28(ASP)	CG	C	/F/	97(LYS)	NZ	N	3.74

/L/	28(ASP)	OD1	O	/F/	97(LYS)	NZ	N	3.59
/L/	28(ASP)	OD2	O	/F/	97(LYS)	NZ	N	3.2
/L/	27(GLN)	NE2	N	/F/	108(LYS)	CD	C	4

In Vivo Efficacy

[0374] Efficacy of anti-RSPO3 antibodies were tested in colorectal cancer PTPRK-RSPO fusion patient derived tumor models. In PTPRK-RSPO fusion patient derived tumor models and/or NSCLC tissue, anti-RSPO3 antibody (5D6) significantly reduced gene expression of markers of intestinal stem cell markers: Myc, Axin2, LGR5, TERT, BIRC5, and/or Ascl2, whereas gene expression of markers of differentiation were increased, *e.g.*, CEACAM7, SLC26A3, CA1, SYTI5, CA4, TFF1, and KRT20 compared to expression levels prior to treatment with the anti-RSPO3 antibody (data not shown).

While not wanting to be bound by any particular theory, these results suggest that the anti-RSPO3 antibody (5D6) is capable of promoting a transition, as determined by gene expression markers, from a stem cell-like marker profile to a differentiation marker profile.

[0375] The effect on tumor volume over time (*e.g.*, tumor growth inhibition) was also tested in the colorectal cancer PTPRK-RSPO fusion patient derived tumor models upon treatment with the anti-RSPO3 antibody (5D6) is shown in Figures 11A-D. Treatment of the models with anti-RSPO3 antibody (5D6) showed significant reduction in tumor growth or stasis of tumor growth. In the models, the onset of regression and/or stasis was not immediate upon treatment with the anti-RSPO3 antibody (5D6); there was a delay in the onset of regression or stasis after initiation of treatment. Further, when the colorectal cancer patient derived model tumors treated with anti-Ragweed antibody or anti-RSPO3 antibody (5D6) were stained with H&E stain and Alcian Blue stain, there was a striking difference in histopathology as shown in Figure 12A-D. In anti-RSPO3 (5D6)-treated tumors, there was a significant reduction in the number of tumor cells. The histology of most of the remaining cells was consistent with differentiated, mature non-proliferating goblet cells. In addition, there was a significant increase in mucous as indicated by Alcian Blue staining compared to the anti-Ragweed antibody control. Accordingly, the measured tumor volume may actually have been occupied in significant part by mucous, and not by tumor cells, and therefore, the effect on tumor growth inhibition may actually have been underestimated. While not wanting to be bound by any particular theory, these efficacy data are consistent with a hierarchical organization of RSPO3 fusion positive tumors: the proliferation of the cancer stem cells is dependent upon RSPO proteins, and upon treatment with anti-RSPO3 antibody (5D6), the cancer stem cells die or differentiate into transit-amplifying (TA) cell. In the absence of a stem cell source to ensure their replenishment, the latter undergo a limited number of cell divisions, after which they terminally differentiate, leading to their exhaustion. Therefore, the

kinetics and the overall size of the TA cell population may determine the onset of tumor growth inhibition.

[0376] Again while not wanting to be bound by any particular theory, based on the theory of hierarchical organization of RSPO3 fusion positive tumors described, combination treatment with a chemotherapeutic agent should reduce the delay in onset of regression and/or stasis by killing the TA cell population and increase efficacy compared to treatment with the chemotherapeutic agent alone in the PTPRK-RSPO fusion patient derived tumor models. Consistent with this theory and as shown in Figures 11D and 13A, the anti-RSPO3 antibody (5D6) in combination with Irinotecan significantly reduced the delay in onset of regression and/or stasis and a decreased tumor growth when compared to treatment with irinotecan alone in CRCD and CRCC colorectal cancer PTPRK-RSPO fusion patient derived tumor models. By administering an anti-RSPO3 antibody in combination with chemotherapy, both cancer stem cells and TA cells are targeted for earlier regression or stasis of tumor growth.

[0377] Further, while not wanting to be bound by any particular theory, based on the theory of hierarchical organization of RSPO3 fusion positive tumors described above as well as the idea that the stem cell compartment is responsible to tumor initiation as measured by a tumor transplantation assay, transplanted PTPRK-RSPO fusion patient derived tumor models treated with an anti-RSPO3 antibody should have a reduced cancer stem cell population, which should reduce the establishment and tumor growth of serial PTPRK-RSPO fusion patient derived tumors. Again, consistently with this theory and as shown Figure 13B-C, in serial transplant experiments, treatment with anti-RSPO3 antibodies (5D6) results in fewer tumors being established and growing from anti-RSPO3 treated fragments following serial transplantation.

[0378] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

SEQ ID NO: 1 >sp|Q6UXX9|RSP02_HUMAN R-spondin-2 OS=Homo sapiens GN=RSP02
 MQFRLFSFALIILNCMDYSHCQGNRWRRSKRASYVSNPICKGCLSCSKDNGCSRCQQKLF
 FFLRREGMRQYGECLHSCPSGYYGHRAPDMNRCARCRIENCDSFCSKDFCTKCKVGFYLH
 RGRCFDECPDGFAPLEETMECVEGCEVGHWSEWGTCSRNNRTC GFKWGLETRTRQIVKKP
 VKDTILCPTIAESRRCKMTMRHCPGGKRTPKAKEKRNKKKKRKLIERAQEQHSVFLATDR
 ANQ

SEQ ID NO:2 >sp|Q9BXY4|RSP03_HUMAN R-spondin-3 OS=Homo sapiens GN=RSP03
 MHLRLISWLFIIILNFMEYIGSQNASRGRRQRRMHPNVSQGCQGGCATCSDYNGCLSCCKPR
 LFFALERIGMKQIGVCLSSCPSGYYGTRYPDINKCTKCKADCDTCFNKNFCTKCKSGFYL
 HLGKCLDNCPEGLEANNHTMECVSIVHCEVSEWNPWSPCTKKGKTCGFKRGTETRVREII
 QHPSAKGNLCPPTNETRKCTVQRKKCQKGERGKKGRERKRKKPNKGESKEAIPDSKSLES
 SKEIPEQRENKQQQKKRKVQDKQKSVSVSTVH

SEQ ID NO:3 >sp|Q2MKA7|RSP01_HUMAN R-spondin-1 OS=Homo sapiens GN=RSP01
 MRGLCVVALVLSWTHLTISSRGIKGRQRRISAEGSQACAKGCELCSEVNGCLKCSPKL
 FILLERNDIRQVGVCLPSCPPGYFDARNPDMNCKICKIEHCEACFSHNFCTKCKEGLYL
 HKGRCYPACPEGSSAANGTMECSPAQCEMSEWSPWGPCSKKQQLCGFRRGSEERTRRVL
 HAPVGDAACSDTKETRRTVRRVPCPEGQKRRKGGQGRRENANRNLARKE SKEAGAGSR
 RRKGQQQQQQQGTVGPLTSAGPA

SEQ ID NO:4 >sp|Q2I0M5|RSP04_HUMAN R-spondin-4 OS=Homo sapiens GN=RSP04
 MRAPLCLLLLVAHAVDMLALNRRKKQVGTGLGGNCTGCIICSEENGCSCTCQQRLFLFIRR
 EGIRQYGKCLHDCPPGYFGIRGQEVNRCKKCGATCESCFSDFCIRCKRQFYLYKGKCLP
 TCPPGTLAHQNTRECQGECELGPWGGWSPCTHNGKTCGSAWGLESRVREAGRAGHEEAAT
 CQVLSESRKCPHQRPCPGRSPGQKKGRKDRRPRKDRKLDRLDVRPRQPGLQP

NAME	SEQUENCE	SEQ ID NO
4H1-HVR L1	RSSQSIVHSNGNTYLE	5
4H1-HVR L2	RISNRFS	6
4H1-HVR L3	FQGSHVPYT	7
4H1-HVR H1	NFAMS	8
4H1-HVR H2	EINNGGNYAYYQDTV TG	9
4H1-HVR H3	EDYVNYEAYFAY	10
4D4-HVR L1	RSSQSIVHSNGNTYLE	11
4D4-HVR L2	RISNRFS	12
4D4-HVR L3	FQGSHVPYT	13
4D4-HVR H1	NFAMS	14
4D4-HVR H2	EINNGGNYAYYQDTV TG	15
4D4-HVR H3	EDYVNYEAYFAY	16
5C2-HVR L1	RASQDISNYLN	17
5C2-HVR L2	YTSRLHS	18
5C2-HVR L3	QQGDTLPPT	19
5C2-HVR H1	SYGVH	20
5C2-HVR H2	VIWTGGSTNYNSALMS	21
5C2-HVR H3	VDGYYYFDY	22
5D6-HVR L1	KASQDIDSYLS	23
5D6-HVR L2	LTNRLVD	24
5D6-HVR L3	LHYDEFPLT	25
5D6-HVR H1	SGYWN	26
5D6-HVR H2	YISYSGKTYQNPSLKS	27
5D6-HVR H3	YYGYGGPWFA Y	28
5E11-HVR L1	RASQDISNYLN	29
5E11-HVR L2	YTSRLHS	30
5E11-HVR L3	QHGD TLPPT	31

5E11-HVR H1	SYAVH	32
5E11-HVR H2	VIWSGGSTDYNAAFIS	33
5E11-HVR H3	NDGYYYFDY	34
6E9-HVR L1	RASQDISNYLN	35
6E9-HVR L2	YTSRLHS	36
6E9-HVR L3	QQGDTLPPA	37
6E9-HVR H1	SYGVH	38
6E9-HVR H2	VIWSGGSTDYNAAFIS	39
6E9-HVR H3	NDGYYYFDY	40
21C2-HVR L1	RASESVDSYGNTFMH	41
21C2-HVR L2	LASNLES	42
21C2-HVR L3	QQNNEDPYT	43
21C2-HVR H1	DYVIH	44
21C2-HVR H2	VITTYYGDA SYNQKFKG	45
21C2-HVR H3	GAYGNSPSYWYFDV	46
26E11-HVR L1	KASQDIDSYLS	47
26E11-HVR L2	LTNRLID	48
26E11-HVR L3	LQYDEF PVT	49
26E11-HVR H1	SGYWS	50
26E11-HVR H2	YISFSGKTYI PSLKS	51
26E11-HVR H3	YHGYGGPWFAY	52
1A1-HVR L1	TLSSQHSTNYIE	53
1A1-HVR L2	VRDGSHSKGD	54
1A1-HVR L3	GLSDVSLYL	55
1A1-HVR H1	DYFMS	56
1A1-HVR H2	HIYTKTYNYATYYSGSVKG	57
1A1-HVR H3	DEDWYFDF	58
11F11-HVR L1	TLSSQHSSYGIT	59
11F11-HVR L2	LRSDGSHSKGD	60
11F11-HVR L3	VTYDSTVGV	61
11F11-HVR H1	EYYVT	62
11F11-HVR H2	DIDPENGD TDYNQKFQG	63
11F11-HVR H3	GYDYAFDS	64
36D2-HVR L1	TRSSGNIGS NYVS	65
36D2-HVR L2	KFDQRPS	66
36D2-HVR L3	LSGYDKYV	67
36D2-HVR H1	SSDWS	68
36D2-HVR H2	YMNYGGGTYYNPSLEN	69
36D2-HVR H3	ERHPYAYFDV	70
49G5-HVR L1	TLSSQYNTYYIE	71
49G5-HVR L2	LSDGSHSKGD	72
49G5-HVR L3	GVSDVSLYV	73
49G5-HVR H1	SYNIH	74
49G5-HVR H2	AVWRGGGTYYNSNLKS	75
49G5-HVR H3	EELRYVYFDV	76
COMP1-HVR L1	KASQDIDSYLS	77
COMP1-HVR L2	LTNRLX ₁ D wherein X ₁ is V or I	78
COMP1-HVR L3	LX ₁ YDEF PX ₂ T wherein X ₁ is H or Q and X ₂ is L or V	79
COMP1-HVR H1	SGYWX ₁ wherein X ₁ is N or S	80

COMP1-HVR H2	YISX ₁ SGKTYX ₂ X ₃ PSLKS wherein X ₁ is Y or F, X ₂ is Q or Y, and X ₃ is N or I	81
COMP1-HVR H3	YX ₁ GYGGPWFAY wherein X ₁ is Y or H	82
COMP2-HVR L1	RASQDISNYLN	83
COMP2-HVR L2	YTSRLHS	84
COMP2-HVR L3	QX ₁ GDTLPPX ₂ wherein X ₁ is Q or H and X ₂ is T or A	85
COMP2-HVR H1	SYX ₁ VH wherein X ₁ is A or G	86
COMP2-HVR H2	VIWX ₁ GGSTX ₂ YNX ₃ AX ₄ X ₅ S wherein X ₁ is S or T, X ₂ is D or N, X ₃ is A or S, X ₄ is L or F, X ₅ is M or I	87
COMP2-HVR H3	X ₁ DGYYYFDY wherein X ₁ is N or V	88
4H1 V _L	SIVMTQTPLSLPVS LGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLI YRISNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPTYFGGG TKLEIK	89
4H1 V _H	EVKLVESGGGFVKPGGSLKLSAASGFTFSNFAMSWVRQSPPEKRLEWVAEINN GGNYAYYQDVTVTGRFTISRDNANTLYLEMSSLRSEDAMTYFCAREDYVNYEA YFAYWGQGTTLTVSS	90
4D4 V _L	DIQMNQSHKFMSTSVGDRVSITWKASQDVGTA VAWYQQKPGQSPKLLIYWAST RHTGVDPDRFTGSGSGTDFTLTISNVQSEDLADYFCQQYSSSITFGAGTKLELK	91
4D4 V _H	QVQLQQSGPELVRPGESVKISCKGSGYSFTDYAMHWVKQSHAKSLEWIGIISI YYDNTNYNQKFKGRATMTVDKSSSTAYMELARLTSEDSAIYYCARGGNGYYYV MDYWGQGTSTVTVSS	92
5C2 V _L	DIVMTQSTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSR LHSGVPSRFSGSGSGTDYSLTISNLEPEDIATYFCQQQGD TLPPTFGGGTKLEI K	93
5C2 V _H	EVQLQESGPGLVAPSQSL SITCTVSGFSLTSYGVHWVRQPPGKGLEWLGVIWT GGSTNYSALMSRLSISKDNSKSQVFLKMNSLQTD DTA MYCARVDGYYYFDY WGQGTTLTVSS	94
5D6 V _L	DIVLTQSPSSMYASLGERVTITCKASQDIDSYLSWFQQKPGKSPKTLIYLTNR LVDGVPSRFSGSGSGQDYSLTISSLEYEDMGIYYCLHYDEFPLTFGAGTKLEI K	95
5D6 V _H	EVQLQESGPSLVKPSQTL SLTCSVTGDSITSGYWNWIRKFPGNKFEYMGYISY SGKTYQNPSLKSRI SITRDTSKNQYHLQLNSVT TTDATATYYCATYYGYGGPWF AYWGQGTTLTVSA	96
5E11 V _L	DIVMTQSTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSR LHSGVPSRFSGSGSGTDYSLTISNLEKEDVATYFCQHGD TLPPTFGGGTKLEI K	97
5E11 V _H	QVQLKQSGPGLVQPSQSL SITCTVSGFSLSSYAVHWVRQSPGEGLEWLGVIWS GGSTDYNAAFISRMSITKDNSKSQVFFKMNSLQADDTAIYFCARNDGYYYFDY WGQGTTLTVSS	98
6E9 V _L	DIKMTQSTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSR LHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGD TLP PAFGGG TKLEI K	99
6E9 V _H	QVQLKESGPGLVQPSQSL SITCTVSGFSLTSYGVHWVRQSPGKGLEWLGVIWS GGSTDYNAAFISRLSISKDNSKSQVFFKMNSLQANDTAIYYCARNDGYYYFDY WGQGTTLTVSS	100
21C2 V _L	DIVLTQSPASLT VSLGQRATISCRASESVDSYGN TFMHWYQQKPGQPPKLLIY LASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQ QNNEDPYTFGGGT KLEIK	101
21C2 V _H	QVQLQQSGAELVRPGVSVKISCKGSGYTFTDYVIHWVKQSHAKSLEWIGVITT YYGDASYNQKFKGKATMTVDKSSSTAYMELARLTSEDSAIYYCARGAYGNSPS YWYFDVWGAGTSVTVSS	102
26E11 V _L	DIKMTQSPSSMYASLGERVTITCKASQDIDSYLSWFQQKPGKSPKTLIYLTNR LIDGVPSRFSGSGSGQDYSLTINSLEYEDMGIYYCLQYDEF PVTFGAGTRLEI K	103
26E11 V _H	EVQLQESGPSLVKPSQTL SLTCSVTGDSITSGYWSWIRKFPGNKLEFMGYISF SGKTYIIPSLKS RVSITRDTSKNQYYLQLNSVT TTDATATYYCATYHGYGGPWF AYWGQGTTLTVSS	104
1A1 V _L	QPVL TQSPSVSASLGASVKLTCTLS SQHSTNYIEWYQQHPDKSPKFLMQVRDG SHSKGDGTPDRFSGSSSGAHRYLSISNLQLEDEAIYYCGLSDVSLYLFSGSGTQ	105

	LTLL	
1A1 V _H	EVQLVESGGGLVKPEGSLKLSCVASGFTFSDYFMSWVRQAPGQGLEWVAHIYT KTYNYATYYSGSVKGRFSISRDDSRNMVYLQMNRLRTEDTATYYCTTDEDWYF DFWGQGTQVTVSS	106
11F11 V _L	QPVLTSQSPSASASLGASVKLTCTLSSQHSSYGITWLQQHPDKAPKCVMYLRSD GSHSKGDGIPDRFSGSSSGAHRYLSISNVQPEDEAIYFCVTYDSTVGVFGSGT QLTVP	107
11F11 V _H	QVQLQQSGPQLVKPGFSVKFSCKASGITFTEYYVTWVKQRAGQGLEWVGIDIP ENGDTDYNQKFQ GKATITADKSSSTAYMELSSLTSEDSAVYYCATGYDYAFDS WGQGTLLVTVSS	108
36D2 V _L	ELVFTQPQSVSGSLGQEISISCTRSSGNIGSNYVSWYQQQSSNKPRLLIYKFD QRPSGVPDRFSGSTDSSSNSGILTISRLOPEDEGDYYCLSGYDKYVFGSGTQL TLL	109
36D2 V _H	QIQQLQESGPGLVKPSQSLSLTCSVTGNSITSSDWSWIRQFPGKKLEWMGYMNY GGGTYYNPSLENRISITRDTSKNQFFLHLKSVTTEDTATYYCARERPHPYAYF DVWGQGIQVTVSS	110
49G5 V _L	QPLLTQSPSVSASLGASVKLTCTLSSQYNTYYIEWYQQHPDKSPKFLMQLSDG SHSKGDGIPDRFSGSSSGAHRYLSISNLQLEDEAIYYCGVSDVSLYVFGSGTQ LTVL	111
49G5 V _H	QVQLKESGPGLVQPSQTLSLTCTVSGFSLTSYNIHWVRQPPGKGLEWMGAVWR GGGTYYNSNLKSRVITRDTSKQVLLKLNNLQHEDTAMYYCAREELRYVYFD VWGQGIQVTVSS	112
5D6v5.1-HVR-H3	YYGYGGPFFAY	188
5D6v5.2-HVR-H3	YYGYGGPHFAY	189
5D6v1 V _L	DIQMTQSPSSLSASVGDRVITITCKASQDIDSYLSWFQQKPGKAPKTLIYLTNR LVDGVPSRFSGSGSGQDYTLTISLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	190
5D6v1 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEYMGYISY SGKTYQNPSLKSRLTISRDTSKNQYSLKLSSVTAADTAVYYCATYYGYGGPWF AYWGQGTLLVTVSS	191
5D6v2.1 V _L	DIQMTQSPSSLSASVGDRVITITCKASQDIDSYLSWFQQKPGKAPKTLIYLTNR LVDGVPSRFSGSGSGQDYTLTISLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	192
5D6v2.1 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEYMGYISY SGKTYQNPSLKSRLTISRDTSKNQYSLKLSSVTAADTAVYYCATYYGYGGPWF AYWGQGTLLVTVSS	193
5D6v2.2 V _L	DIQMTQSPSSLSASVGDRVITITCKASQDIDSYLSWFQQKPGKAPKLLIYLTNR LVDGVPSRFSGSGSGQDYTLTISLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	194
5D6v2.2 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEYMGYISY SGKTYQNPSLKSRLTISRDTSKNQYSLKLSSVTAADTAVYYCATYYGYGGPWF AYWGQGTLLVTVSS	195
5D6v2.3 V _L	DIQMTQSPSSLSASVGDRVITITCKASQDIDSYLSWFQQKPGKAPKTLIYLTNR LVDGVPSRFSGSGSGTDYTLTISLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	196
5D6v2.3 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEYMGYISY SGKTYQNPSLKSRLTISRDTSKNQYSLKLSSVTAADTAVYYCATYYGYGGPWF AYWGQGTLLVTVSS	197
5D6v2.4 V _L	DIQMTQSPSSLSASVGDRVITITCKASQDIDSYLSWFQQKPGKAPKTLIYLTNR LVDGVPSRFSGSGSGQDYTLTISLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	198
5D6v2.4 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEYMGYISY SGKTYQNPSLKSRLTISRDTSKNQYSLKLSSVTAADTAVYYCATYYGYGGPWF AYWGQGTLLVTVSS	199
5D6v2.8 V _L	DIQMTQSPSSLSASVGDRVITITCKASQDIDSYLSWFQQKPGKAPKTLIYLTNR LVDGVPSRFSGSGSGQDYTLTISLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	200
5D6v2.8 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEYMGYISY SGKTYQNPSLKSRLTISVDTSKNQYSLKLSSVTAADTAVYYCATYYGYGGPWF	201

	AYWGQGTLVTVSS	
5D6v2.10 V _L	DIQMTQSPSSLSASVGDRVTITCKASQDIDSYLSWFQQKPGKAPKTLIYLNR LVDGVPSRFSGSGSGQDYTLTISSSLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	202
5D6v2.10 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEVMGYISY SGKTYQNPSLKSRLTISRDTSKNQYSLKLSSVTAADTAVYYCARYYGYGGPWF AYWGQGTLVTVSS	203
5D6v3.2 V _L	DIQMTQSPSSLSASVGDRVTITCKASQDIDSYLSWFQQKPGKAPKTLIYLNR LVDGVPSRFSGSGSGQDYTLTISSSLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	204
5D6v3.2 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEWIGYISY SGKTYQNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCATYYGYGGPWF AYWGQGTLVTVSS	205
5D6v3.3 V _L	DIQMTQSPSSLSASVGDRVTITCKASQDIDSYLSWFQQKPGKAPKTLIYLNR LVDGVPSRFSGSGSGQDYTLTISSSLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	206
5D6v3.3 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEWIGYISY SGKTYQNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCATYYGYGGPWF AYWGQGTLVTVSS	207
5D6v4.1 V _L	DIQMTQSPSSLSASVGDRVTITCKASQDIDSYLSWFQQKPGKAPKTLIYLNR LVDGVPSRFSGSGSGTDYTLTISSSLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	208
5D6v4.1 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEWIGYISY SGKTYQNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCATYYGYGGPWF AYWGQGTLVTVSS	209
5D6v4.3 V _L	DIQMTQSPSSLSASVGDRVTITCKASQDIDSYLSWFQQKPGKAPKTLIYLNR LVDGVPSRFSGSGSGTDYTLTISSSLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	210
5D6v4.3 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEWIGYISY SGKTYQNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCATYYGYGGPWF AYWGQGTLVTVSS	211
5D6v5.1 V _L	DIQMTQSPSSLSASVGDRVTITCKASQDIDSYLSWFQQKPGKAPKTLIYLNR LVDGVPSRFSGSGSGTDYTLTISSSLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	212
5D6v5.1 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEWIGYISY SGKTYQNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCATYYGYGGPFF AYWGQGTLVTVSS	213
5D6v5.2 V _L	DIQMTQSPSSLSASVGDRVTITCKASQDIDSYLSWFQQKPGKAPKTLIYLNR LVDGVPSRFSGSGSGTDYTLTISSSLQPEDFATYYCLHYDEFPLTFGQGTKVEI K	214
5D6v5.2 V _H	EVQLVESGPGLVKPSETLSLTCTVSGDSITSGYWNWIRQPPGKGLEWIGYISY SGKTYQNPSLKSRLTISRDTSKNQFSLKLSSVTAADTAVYYCATYYGYGGPHF AYWGQGTLVTVSS	215
COMP1-HVR H3	YX ₁ GYGGPX ₂ FAY wherein X ₁ is Y or H and X ₂ is W, F, or H	216

EIF3E(e1)-RSP02(e2) translocation fusion polynucleotide (SEQ ID NO:173)

GAGCACAGACTCCCTTTTCTTTGGCAAGATGGCGGAGTACGACTTGACTACTCGCATCGCGCACTTTTGGATCGGCAT
CTAGTCTTTCCGCTTCTTGAATTTCTCTCTGTAAAGGAGGTTTCGTGGCGGAGAGATGCTGATCGCGCTGAAGTACCGG
TGCGGCCCGGGGTGAGTGGCGAGTCTCCCTCTGAGTCCTCCCCAGCAGCGCGGCCCGCGCCGGCTCTTTGGGCGAACC
CTCCAGTTCCTAGACTTTGAGAGGCGTCTCTCCCCGCCCCGACCGCCCAGATGCAGTTTCGCCTTTTCTCCTTTGCCCT
CATCATTTCTGAAGTGCATGGATTACAGCCACTGCCAAGGCAACCGATGGAGACGCAGTAAGCGAGCTAGTTATGTATCA
AATCCCATTTGCAAGGGTTGTTTGTCTTGTTCAAAGGACAATGGGTGTAGCCGATGTCAACAGAAGTTGTTCTTCTTCC
TTCGAAGAGAAGGGATGCGCCAGTATGGAGAGTGCCTGCATTCTGCCCATCCGGGTACTATGGACACCGAGCCCCAGA
TATGAACAGATGTGCAAGATGCAGAATAGAAAAGTGTGATTCTTGCTTTAGCAAAGACTTTTGTACCAAGTGCAAAGTA
GGCTTTTATTTGCATAGAGGCCGTTGCTTTGATGAATGTCCAGATGGTTTTGCACCATTAGAAGAAACCATGGAATGTG
TGGAAGGATGTGAAGTTGGTCATTGGAGCGAATGGGGAAGTTGTAGCAGAAATAATCGCACATGTGGATTTAAATGGGG
TCTGGAAACCAGAACACGGCAAATTTGTTAAAAAGCCAGTGAAAGACACAATACTGTGTCCAACCATTTGCTGAATCCAGG
AGATGCAAGATGACAATGAGGCATTGTCCAGGAGGGAAGAGAACACCAAAGGCGAAGGAGAAGAGGAACAAGAAAAAGA
AAAGGAAGCTGATAGAAAGGGCCCAGGAGCAACACAGCGTCTTCCTAGCTACAGACAGAGCTAACCAATAA

EIF3E(e1)-RSP02(e2) translocation fusion polypeptide sequence (SEQ ID NO:174)

MAEYDLTTRIAHFLDRHLVFPLLEFLSVKEVRGGEMLIALNMQFRLFSFALIILNCMDYSHCQGNRWRRSKRASYSVSNP
ICKGCLSCSKDNGCSRCQQKLFFFLRREGMRQYGECLHSCPSGYYGHRAPDMNRCARCRIENCDSFCSKDFCTKCKVGF
YLHRGRCFDECPDGFAPLEETMECEVEGVHSEWGTCSRNNRTC GFKWGLETRTRQIVKKPVKDTILCPTIAESRRC
KMTMRHCPGGKRTPKAKEKRNKKKKRKLIERAQEQHSVFLATDRANQ

PTPRK(e1)-RSP03(e2) translocation fusion polynucleotide sequence (SEQ ID NO:175)

ATGGATACGACTGCGGCGGCGGCGCTGCCTGCTTTTGTGGCGCTCTTGCTCCTCTCTCCTTGGCCTCTCCTGGGATCGG
CCCAAGGCCAGTTCTCCGCAGTGCATCCTAACGTTAGTCAAGGCTGCCAAGGAGGCTGTGCAACATGCTCAGATTACAA
TGGATGTTTGTTCATGTAAGCCCAGACTATTTTTTGTCTCTGGAAAGAATTGGCATGAAGCAGATTGGAGTATGTCTCTCT
TCATGTCCAAGTGGATATTATGGAACCTCGATATCCAGATATAAATAAGTGTACAAAATGCAAAGCTGACTGTGATACCT
GTTTCAACAAAAATTTCTGCACAAAATGTAAAAGTGGATTTTACTTACACCTTGAAAGTGCCTTGACAATTGCCCAGA
AGGGTTGGAAGCCAACAACCATACTATGGAGTGTGTCAGTATTGTGCACTGTGAGGTCAGTGAATGGAATCCTTGGAGT
CCATGCACGAAGAAGGGAAAAACATGTGGCTTCAAAAGAGGGACTGAAACACGGGTCCGAGAAATAATACAGCATCCTT
CAGCAAAGGGTAACCTGTGTCCCCCAACAAATGAGACAAGAAAGTGTACAGTGCAAAGGAAGAAGTGTGAGAAGGGAGA
ACGAGGAAAAAAGGAAGGGAGAGGAAAAAGAAAAAACCTAATAAAGGAGAAAGTAAAGAAGCAATACCTGACAGCAAA
AGTCTGGAATCCAGCAAAGAAATCCCAGAGCAACGAGAAAAACAAACAGCAGCAGAAGAAGCGAAAAAGTCCAAGATAAAC
AGAAATCGGTATCAGTCAGCACTGTACACTAG

PTPRK(e1)-RSP03(e2) translocation fusion polypeptide sequence (SEQ ID NO:176)
MDTTAAALPAFVALLLLSPWPLLGSAQGQFSVHPNVVSQGCQGGCATCSDYNGCLSCKPRLFFALERIGMKQIGVCL
SCPSGYYGTRYPDINKCTKCKADCDTCFNKNFCTKCKSGFYHLHLGKCLDNCPEGLEANNHTMECVSIVHCEVSEWNPWS
PCTKKGKTCGFKRGTETRVREIIQHPSAKGNLCPPTNETRKCTVQRKKCQKGERGKKGR

PTPRK(e7)-RSP03(e2) translocation fusion polynucleotide sequence (SEQ ID NO:177)

ATGGATACGACTGCGGCGGCGGCGCTGCCTGCTTTTGTGGCGCTCTTGCTCCTCTCTCCTTGGCCTCTCCTGGGATCGG
CCCAAGGCCAGTTCTCCGCAGGTGGCTGTACTTTTGATGATGGTCCAGGGGCTGTGATTACCACCAGGATCTGTATGA
TGACTTTGAATGGGTGCATGTTAGTGCTCAAGAGCCTCATTATCTACCACCCGAGATGCCCCAAGGTTCTATATGATA
GTGGACTCTTCAGATCACGACCCTGGAGAAAAAGCCAGACTTCAGCTGCCTACAATGAAGGAGAACGACACTCACTGCA
TTGATTTTCAATTACCTATTATATAGCCAGAAAGGACTGAATCCTGGCACTTTGAACATATTAGTTAGGGTGAATAAAGG
ACCTCTTGCCAATCCAATTTGGAATGTGACTGGATTACAGGGTAGAGATTGGCTTCGGGCTGAGCTAGCAGTGAGCACC
TTTTGGCCCAATGAATATCAGGTAATATTTGAAGCTGAAGTCTCAGGAGGGAGAAGTGGTTATATTGCCATTGATGACA
TCCAAGTACTGAGTTATCCTTGTGATAAATCTCCTCATTTCCTCCGTCTAGGGGATGTAGAGGTGAATGCAGGGCAAAA
CGCTACATTTTCACTGTCATTGCCACAGGGAGAGATGCTGTGCATAACAAGTTATGGCTCCAGAGACGAAATGGAGAAGAT
ATACCAGTAGCCCAGACTAAGAACATCAATCATAGAAGGTTTGCCGCTTCCTTCAGATTGCAAGAAGTGACAAAACTG
ACCAGGATTTGTATCGCTGTGTAACCTCAGTCAGAACGAGGTTCCGGTGTGTCCAATTTTGCTCAACTTATTGTGAGAGA
ACCGCCAAGACCCATTGCTCCTCCTCAGCTTCTTGGTGTGGGGCTACATATTTGCTGATCCAATAAATGCCAACTCG
ATCATTGGCGATGGTCCTATCATCCTGAAAGAAGTAGAGTACCGAATGACATCAGGATCCTGGACAGAAACCCATGCAG
TCAATGCTCCAACCTACAAATTATGGCATTTAGATCCAGATACCGAATATGAGATCCGAGTTCTACTTACAAGACCTGG
TGAAGGTGGAACGGGGCTCCCAGGACCTCCACTAATCACCAGAACAAAATGTGCAGTGCATCCTAACGTTAGTCAAGGC
TGCCAAGGAGGCTGTGCAACATGCTCAGATTACAATGGATGTTTGTTCATGTAAGCCCAGACTATTTTTTGTCTCTGGAAA
GAATTGGCATGAAGCAGATTGGAGTATGTCTCTTTCATGTCCAAGTGGATATTATGGAACCTCGATATCCAGATATAAA
TAAGTGTACAAAATGCAAAGCTGACTGTGATACCTGTTTCAACAAAAATTTCTGCACAAAATGTAAAAGTGGATTTTAC
TTACACCTTGAAAGTGCCTTGACAATTGCCCAGAAAGGGTTGGAAGCCAACAACCATACTATGGAGTGTGTCAGTATTG
TGCACTGTGAGGTGAGTGAATGGAATCCTTGGAGTCCATGCACGAAGAAGGGAAAAACATGTGGCTTCAAAAGAGGGAC
TGAAACACGGGTCCGAGAAATAATACAGCATCCTTCAGCAAAGGGTAACCTGTGTCCCCCAACAAATGAGACAAGAAAG
TGTACAGTGCAAAGGAAGAAGTGTGAGAAGGGAGAACGAGGAAAAAAGGAAGGGAGAGGAAAAAGAAAAAACCTAATA
AAGGAGAAAGTAAAGAAGCAATACCTGACAGCAAAAGTCTGGAATCCAGCAAAGAAATCCCAGAGCAACGAGAAAAACA
ACAGCAGCAGAAGAAGCGAAAAAGTCCAAGATAAACAGAAATCGGTATCAGTCAGCACTGTACACTAG

PTPRK(e7)-RSP03(e2) translocation fusion polypeptide sequence (SEQ ID NO:178)
MDTTAAALPAFVALLLLSPWPLLGSAQGQFSAGGCTFDDGPGACDYHQDLYDDFEWVHVSAQEPHYLPPEMPQGSYMI
VDSSDHPGEKARLQLPTMKENDTHCIDFSYLLYSQKGLNPGLNLIVRVNKGPLANPIWNVTGFTGRDWLRAELAVST
FWPNEYQVIFEAEVSGGRSGYIAIDDIQVLSYPCDKSPHFLRLGDVEVNAGQNATFQCIATGRDAVHNKLWLQRRNGED
IPVAQTKNINHRRFAASFRLQEVTKTDQDLYRCVTQSERGSGVSNFAQLIVREPPRPIAPPQLLGVGPTYLLIQLNANS
IIGDGPIILKEVEYRMTSGSWTETHAVNAPTYKLWHLDPDEYEIRVLLTRPGEAGTGLPGPPLITRTKCAVHPNVVSQ
CQGGCATCSDYNGCLSCKPRLFFALERIGMKQIGVCLSSCPSGYYGTRYPDINKCTKCKADCDTCFNKNFCTKCKSGFY
LHLGKCLDNCPEGLEANNHTMECVSIVHCEVSEWNPWSPTKKGKTCGFKRGTETRVREIIQHPSAKGNLCPPTNETRK
CTVQRKKCQKGERGKKGRERKRKKPNKGESKEAIPDSKSLESSKEIPEQRENKQQQKKRKVQDKQKSVSVSTVH

WHAT IS CLAIMED IS:

1. An isolated antibody that binds to RSPO2, wherein the antibody inhibits the interaction of RSPO2 with a transmembrane E3 ubiquitinase.
2. The isolated antibody of claim 1, wherein the antibody does not inhibit the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6).
3. An isolated antibody that binds to RSPO2, wherein the antibody comprises (a) a light chain variable domain (VL) comprising (i) hyper variable region-L1 (HVR-L1) comprising the amino acid sequence of SEQ ID NO:53, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:54, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:55, and (b) a heavy chain variable domain (VH) comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:56, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:57, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:58.
4. The isolated antibody of claim 3, wherein the antibody comprises (a) a VL sequence of SEQ ID NO:105 and a VH sequence of SEQ ID NO:106.
5. The isolated antibody of claim 1, wherein the antibody inhibits the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6.
6. An isolated antibody that binds to RSPO2, wherein the antibody comprises:
 - (a) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:59, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:60, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:61; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:62, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:63, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:64;
 - (b) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:65, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:66, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:67; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:68, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:69, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:70; or
 - (c) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:71, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:72, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:73; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:74, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:75, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:76.

7. The isolated antibody of claim 6, wherein the antibody comprises
- (a) a VL sequence of SEQ ID NO:107 and a VH sequence of SEQ ID NO:108;
 - (b) a VL sequence of SEQ ID NO:109 and a VH sequence of SEQ ID NO:110; or
 - (c) a VL sequence of SEQ ID NO:111 and a VH sequence of SEQ ID NO:112.

8. An isolated antibody that binds to RSPO3, wherein the antibody inhibits the interaction of RSPO3 with a transmembrane E3 ubiquitinase.

9. The isolated antibody of claim 8, wherein the antibody inhibits the interaction of RSPO3 with one or more of LGR4, LGR5, and/or LGR6.

10. An isolated antibody that binds to RSPO3, wherein the antibody comprises:

(a) VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:9, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10;

(b) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:11, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:12, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:13; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:14, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:15, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:16;

(c) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:17, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:18, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:19; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:20, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:21, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:22;

(d) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28;

(e) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:29, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:30, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:31; and a VH comprising (i) HVR-H1 comprising the amino acid

sequence of SEQ ID NO:32, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:33, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:34;

(f) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:35, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:36, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:37; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:38, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:40;

(g) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:41, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:42, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:43; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:44, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:45, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:46

(h) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:188; or

(i) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:24, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:25; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:189.

11. The isolated antibody of claim 10, wherein the antibody comprises

- (a) a VL sequence of SEQ ID NO:89 and a VH sequence of SEQ ID NO:90;
- (b) a VL sequence of SEQ ID NO:91 and a VH sequence of SEQ ID NO:92;
- (c) a VL sequence of SEQ ID NO:93 and a VH sequence of SEQ ID NO:94;
- (d) a VL sequence of SEQ ID NO:95 and a VH sequence of SEQ ID NO:96;
- (e) a VL sequence of SEQ ID NO:97 and a VH sequence of SEQ ID NO:98;
- (f) a VL sequence of SEQ ID NO:99 and a VH sequence of SEQ ID NO:100;
- (g) a VL sequence of SEQ ID NO:101 and a VH sequence of SEQ ID NO:102;
- (h) a VL sequence of SEQ ID NO:190 and a VH sequence of SEQ ID NO:191;
- (i) a VL sequence of SEQ ID NO:192 and a VH sequence of SEQ ID NO:193;
- (j) a VL sequence of SEQ ID NO:194 and a VH sequence of SEQ ID NO:195;

- (k) a VL sequence of SEQ ID NO:196 and a VH sequence of SEQ ID NO:197;
- (l) a VL sequence of SEQ ID NO:198 and a VH sequence of SEQ ID NO:199;
- (m) a VL sequence of SEQ ID NO:200 and a VH sequence of SEQ ID NO:201;
- (n) a VL sequence of SEQ ID NO:202 and a VH sequence of SEQ ID NO:203;
- (o) a VL sequence of SEQ ID NO:204 and a VH sequence of SEQ ID NO:205;
- (p) a VL sequence of SEQ ID NO:206 and a VH sequence of SEQ ID NO:207;
- (q) a VL sequence of SEQ ID NO:208 and a VH sequence of SEQ ID NO:209;
- (r) a VL sequence of SEQ ID NO:210 and a VH sequence of SEQ ID NO:211;
- (w) a VL sequence of SEQ ID NO:212 and a VH sequence of SEQ ID NO:213; or
- (x) a VL sequence of SEQ ID NO:214 and a VH sequence of SEQ ID NO:215.

12. An isolated antibody that binds to RSPO2 and RSPO3.

13. The isolated antibody of claim 12, wherein the antibody inhibits the interaction of RSPO2 and RSPO3 with a transmembrane E3 ubiquitinase.

14. The isolated antibody of any one of claims 12-13, wherein the antibody inhibits the interaction of RSPO3 with one or more of LGR4, LGR5, and/or LGR6.

15. The isolated antibody of any one of claims 12-14, wherein the antibody inhibits the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6.

16. The isolated antibody of any one of claims 12-14, wherein the antibody does not inhibit the interaction of RSPO2 with one or more of LGR4, LGR5, and/or LGR6 (*e.g.*, enhances binding of RSPO2 to one or more of LGR4, LGR5, and/or LGR6).

17. The isolated antibody of any one of claims 12-16, wherein the antibody comprises a first variable domain and a second variable domain, wherein the first variable domain comprises a first set of six HVRs and the second variable domain comprises a second set of six HVRs, and wherein the first and second set of six HVRs are identical.

18. An isolated antibody that binds to RSPO2 and RSPO3, wherein the antibody comprises: (a) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:47, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:48, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:49; and (b) a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:50, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:51, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:52.

19. The isolated antibody of claim 18, the antibody comprises (a) a VL sequence of SEQ ID NO:103 and a VH sequence of SEQ ID NO:104.

20. The isolated antibody of any one of claims 12-19, wherein the antibody comprises a first variable domain and a second variable domain, wherein the first variable domain comprises a first set of six HVRs and the second variable domain comprises a second set of six HVRs, and wherein the first and second set of six HVRs are different.

21. The isolated antibody of any one of claims 12-16 and 20, wherein the first set of six HVRs are the six HVRs of any one of 4H1, 4D4, 5C2, 5D6, 5E11, 6E9, and 21C2 and the second set of six HVRs are the six HVRs of any one of 1A1, 11F11, 36D2, and 49G5.

22. The isolated antibody of claim 21, wherein the first set of six HVRs are the six HVRs of any one of 4H1, 4D4, 5C2, 5D6, 5E11, 6E9, and 21C2 and the second set of six HVRs are the six HVRs of 1A1.

23. The isolated antibody of any one of claims 1-2, 5, 8-9, 13-17, and 20-22, wherein the transmembrane E3 ubiquitinase is ZNRF3 and/or RNF43.

24. An isolated antibody that binds to RSPO3, wherein the antibody comprises:

(a) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:77, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:78, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:79; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:80, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:81, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:82;

(b) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:83, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:84, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:85; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:86, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:87, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:88; or

(c) a VL comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:77, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:78, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:79; and a VH comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:80, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:81, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:216.

25. An isolated antibody that binds to RSPO3, wherein the antibody binds to a region within amino acids 47-108 (*e.g.*, 49-108) of RSPO3.

26. An isolated antibody that binds to an RSPO3 epitope, wherein the RSPO3 epitope comprises amino acid residues of RSPO3: Gln72, Pro90, Asp91, and Lys94.

27. The isolated antibody of claim 26, wherein the RSPO3 epitope comprises amino acids of RSPO3: Asn 52, Leu55, Phe63, Gln72, Tyr89, Pro90, Asp91, Lys94, and Lys97.
28. The isolated antibody of claim 27, wherein the RSPO3 epitope comprises amino acid residues of RSPO3: Ser49, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Ile73, Gly74, Tyr84, Tyr89, Pro90, Asp91, Ile92, Lys94, Lys97, and Lys108.
29. An isolated antibody that binds to an RSPO3 epitope, wherein the RSPO3 epitope comprises amino acids of RSPO3: Thr47, Leu55, Gln72, Pro90, Asp91, and Lys94.
30. The antibody of claim 29, wherein the RSPO3 epitope comprises amino acids of RSPO3: Thr47, Asn52, Leu55, Phe63, Gln72, Tyr89, Pro90, Asp91, Ile92, Lys94, and Lys97.
31. The isolated antibody of claim 30, wherein the RSPO3 epitope comprises amino acid residues of RSPO3: Thr47, Asn52, Cys54, Leu55, Ser56, Phe63, Leu65, Gln72, Tyr84, Tyr89, Pro90, Asp91, Ile92, Asn93, Lys94, Lys97, and Lys108.
32. The isolated antibody of any one of claims 1-31, wherein the antibody inhibits RSPO2 and/or RSPO3 mediated wnt signaling.
33. The isolated antibody of claim 32, which is an antibody fragment that binds RSPO2 and/or RSPO3.
34. The antibody of any one of claims 1-33, which is a monoclonal antibody.
35. The antibody of any one of claims 1-34, which is a human, humanized, or chimeric antibody.
36. The antibody of any one of claims 1-35, which is a full length IgG1 or IgG2a antibody.
37. Isolated nucleic acid encoding the antibody of any one of claims 1-36.
38. A host cell comprising the nucleic acid of claim 37.
39. A method of producing an antibody comprising culturing the host cell of claim 38 so that the antibody is produced.
40. The method of claim 39, further comprising recovering the antibody from the host cell.
41. An immunoconjugate comprising the antibody of any one of claims 1-36 and a cytotoxic agent.
42. A pharmaceutical formulation comprising the antibody of any one of claims 1-36 and a pharmaceutically acceptable carrier.
43. The pharmaceutical formulation of claim 42, further comprising an additional therapeutic agent.
44. The antibody of any one of claims 1-36 for use as a medicament.
45. The antibody of any one of claims 1-36 for use in treating cancer.

46. The antibody of claim 45, wherein the cancer is gastrointestinal cancer, stomach cancer, colon cancer, colorectal cancer, lung cancer, or rectal cancer.

47. The antibody of any one of claims 1-36 for use in inhibiting wnt signaling, inhibiting angiogenesis and/or vasculogenesis, and/or inhibiting cell proliferation.

48. Use of the antibody of any one of claims 1-36 in the manufacture of a medicament for treatment of cancer.

49. Use of the antibody of any one of claims 1-36, wherein the cancer is gastrointestinal cancer, stomach cancer, colon cancer, colorectal cancer, lung cancer, or rectal cancer.

50. Use of the antibody of any one of claims 1-36 in the manufacture of a medicament for inhibiting wnt signaling, inhibiting angiogenesis and/or vasculogenesis, and/or inhibiting cell proliferation.

51. A method of treating an individual having cancer comprising administering to the individual an effective amount of the antibody of any one of claims 1-36.

52. The method of claim 51, wherein the cancer is gastrointestinal cancer, stomach cancer, colon cancer, colorectal cancer, lung cancer, or rectal cancer.

53. The method of any one of claims 51-52 further comprising administering an additional therapeutic agent to the individual.

54. A method of inhibiting wnt signaling, inhibiting angiogenesis and/or vasculogenesis, and/or inhibiting cell proliferation in an individual comprising administering to the individual an effective amount of the antibody of any one of claims 1-36 to inhibit wnt signaling, inhibit angiogenesis and/or vasculogenesis, and/or inhibit cell proliferation.

55. The antibody, use or method of any one of claims 45-46, 48-49, and 51-53, wherein the cancer is characterized by increased expression of one or more RSPO (*e.g.*, RSPO2 and/or RSPO3) compared to a reference.

56. The antibody, use or method of any one of claims 45-46, 48-49, and 51-53, wherein the cancer is characterized by a RSPO translocation (*e.g.*, RSPO2 translocation and/or RSPO3 translocation).

Figure 1

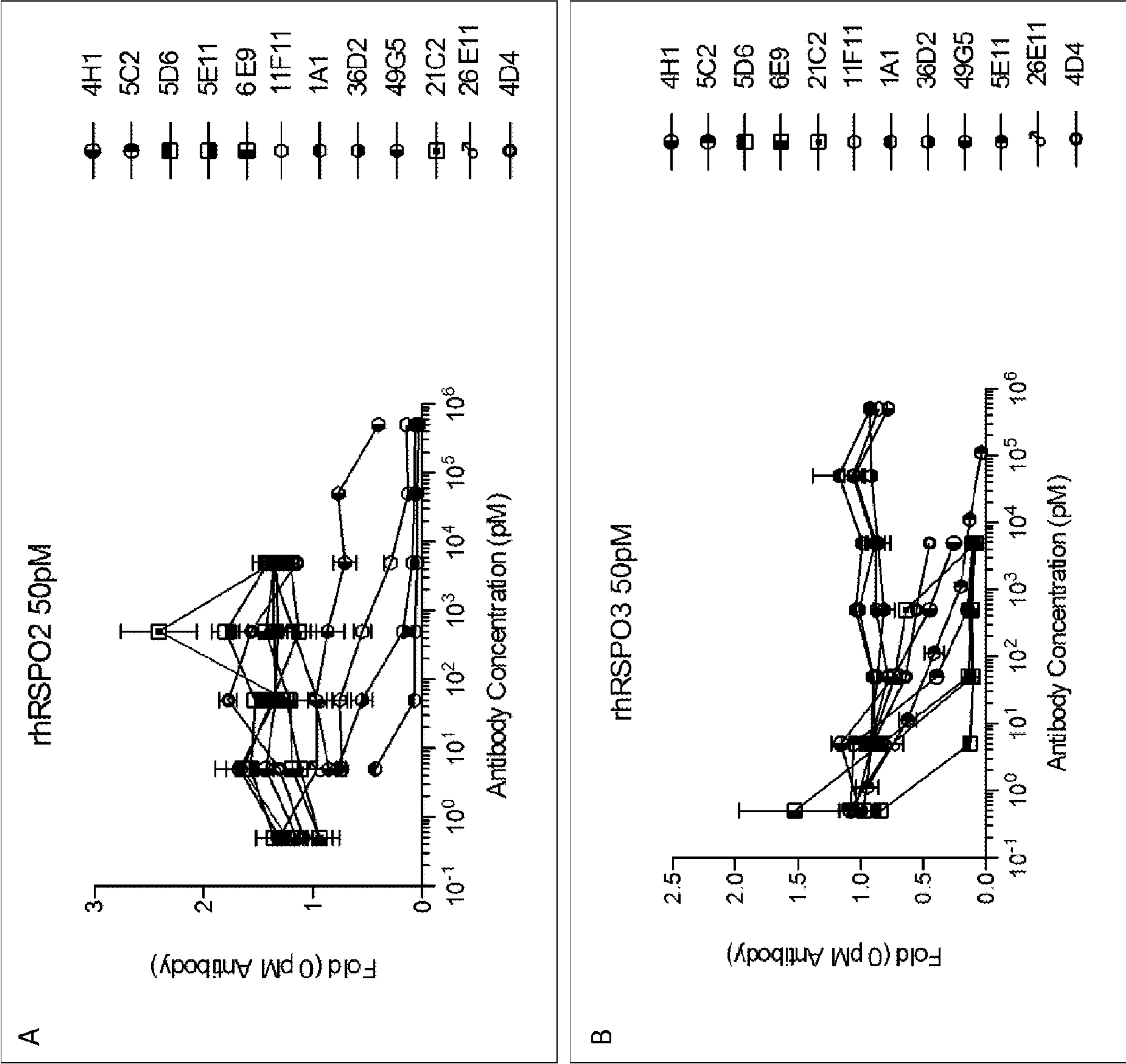


Figure 2

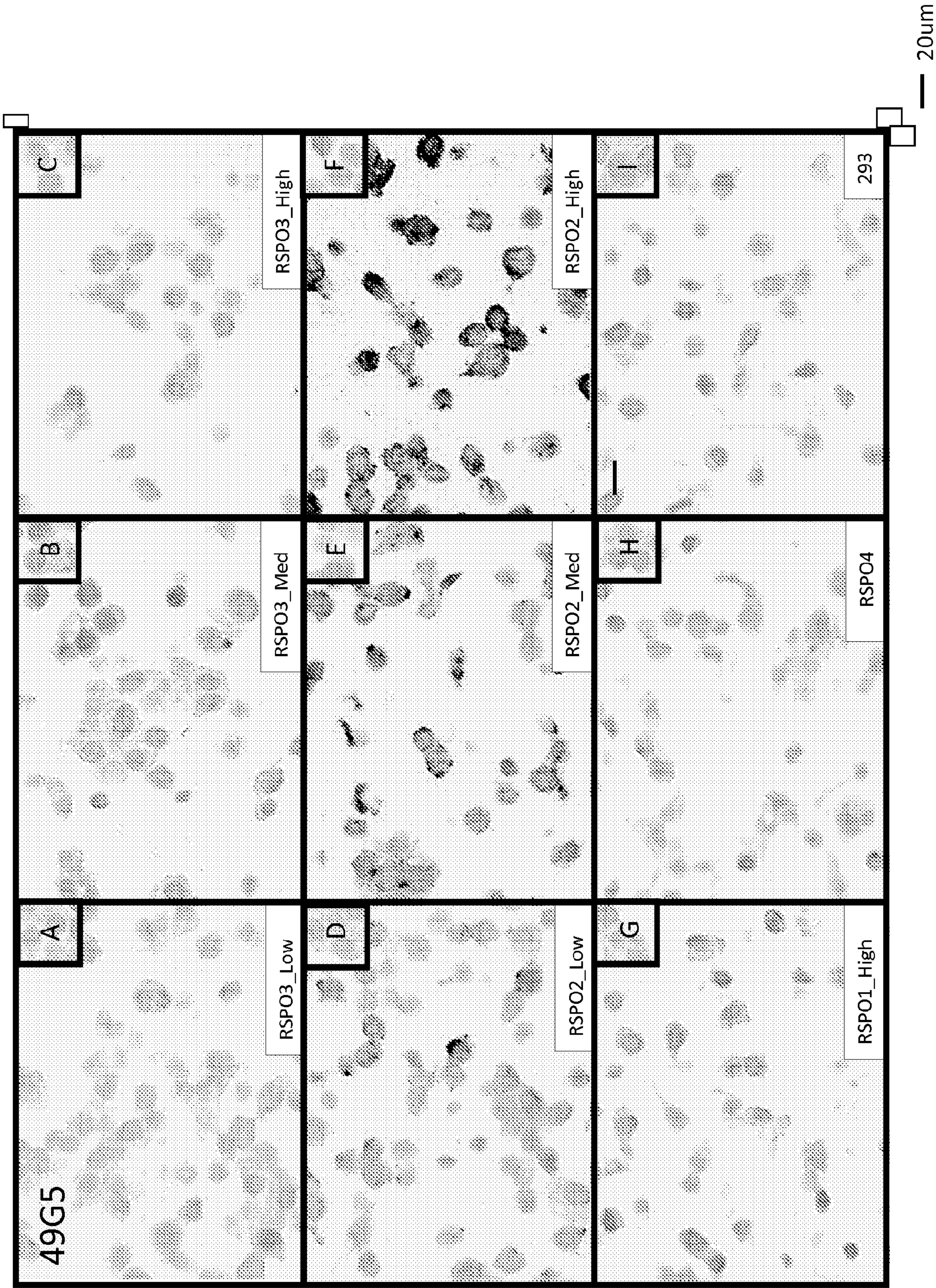


Figure 3

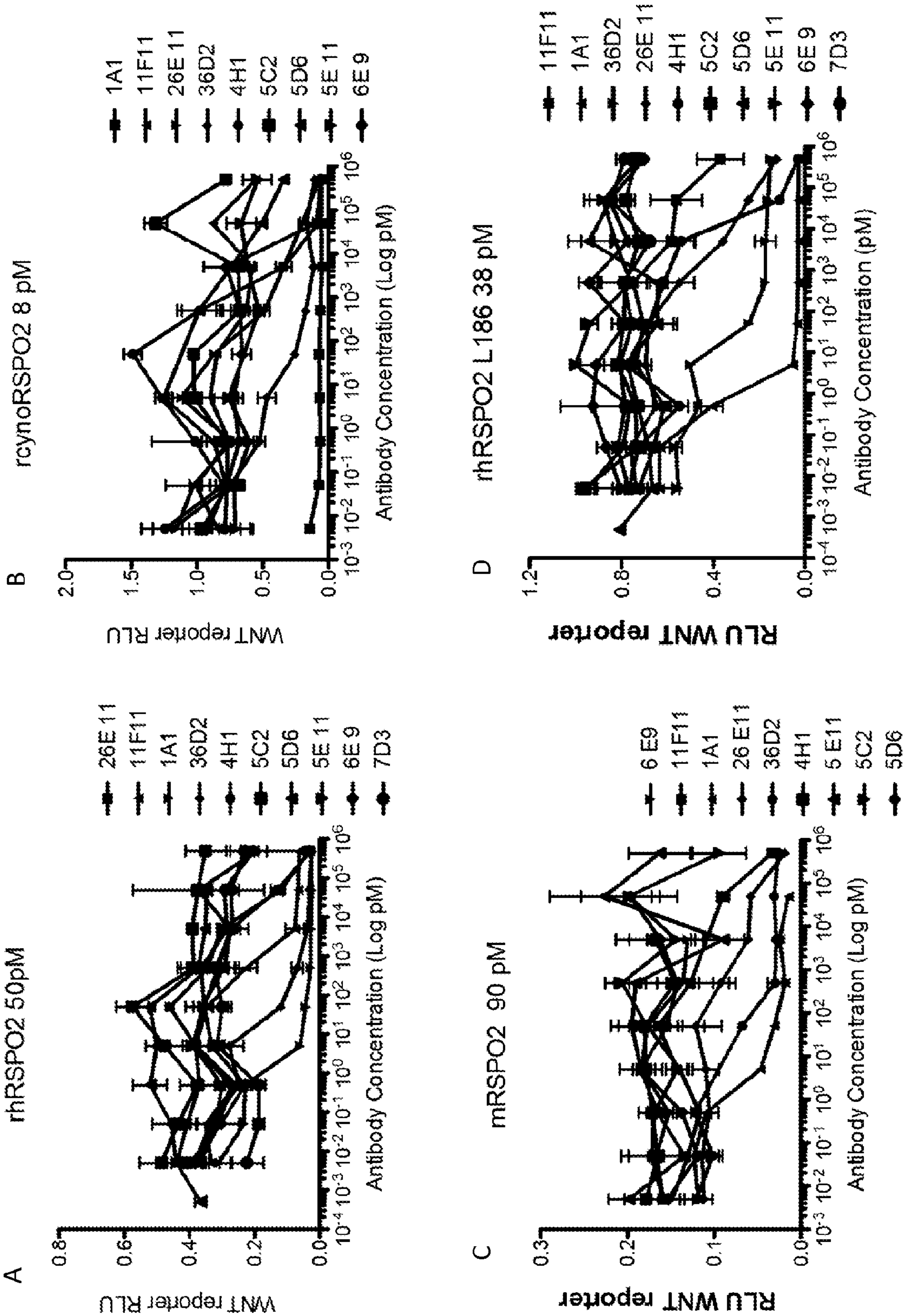


Figure 4

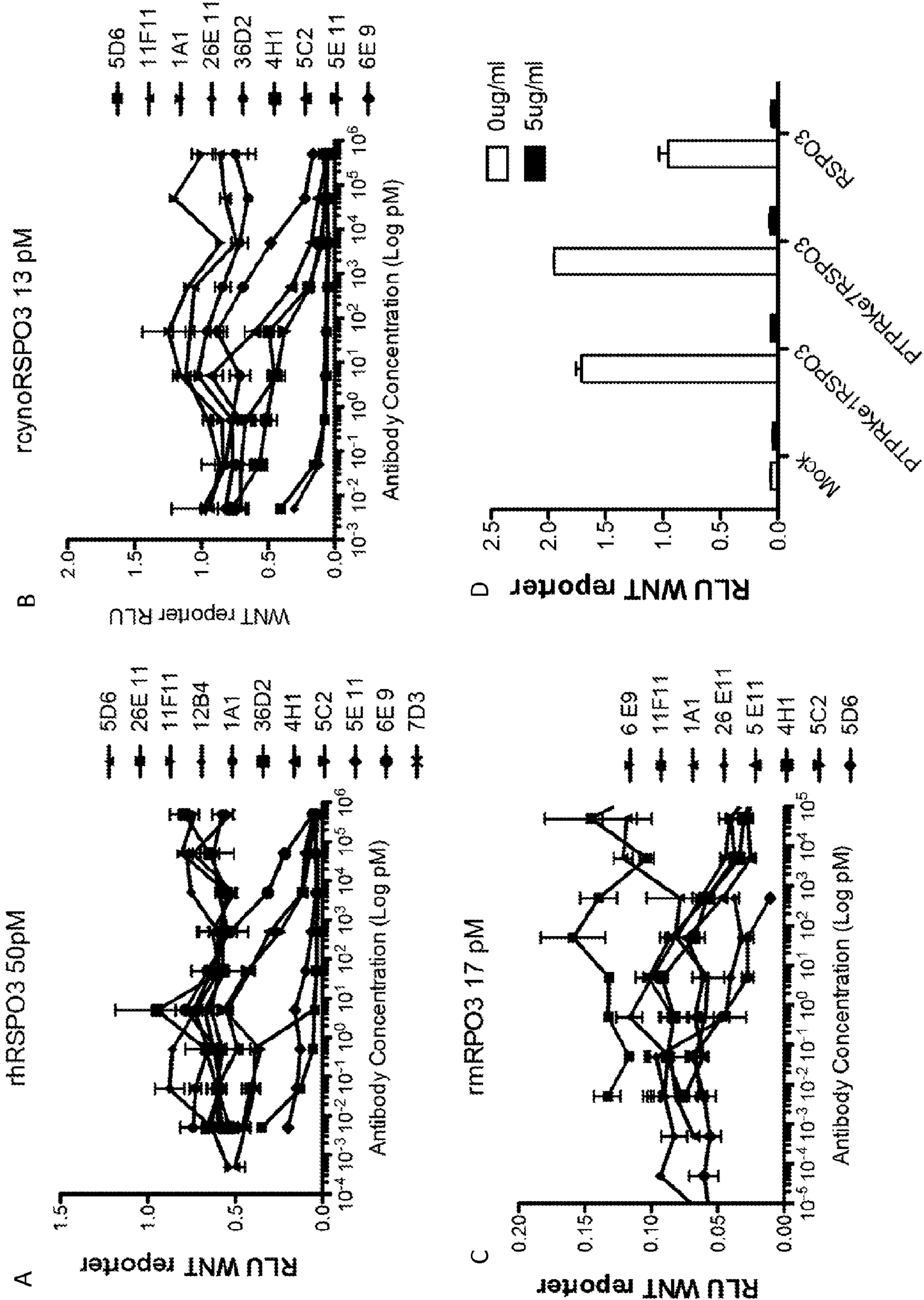


Figure 5

anti-RSPO2												anti-RSPO3				anti-RSPO2/3			
		1A1	36D2	11F11	5D6	5E11	5C2	26E11	4H1	6E9									
RSP02	Affinity ¹ (nM) Fab	H: 2.9 C: 1.4 M: 3.6	H: 6.3 C: 11 M: 5.5	H: 63 C: - M: -	H: - C: - M: -	H: - C: - M: -	H: - C: - M: -	H: 80 C: 62 M: 66	H: - C: - M: -	H: - C: - M: -	H: - C: - M: -								
	IC50 ² (nM) Bivalent	H: 0.0004 C: 0.0005 M: 0.0022	H: 0.012 C: 0.013 M: 0.044	H: 0.179 C: 1.9 M: -	H: - C: - M: -	H: - C: - M: -	H: - C: - M: -	H: 9.8 C: 2.7 M: 24	H: 6.4 C: 2.8 M: -	H: - C: - M: -	H: - C: - M: -								
RSP03	Affinity ¹ (nM) Fab	H: - C: - M: -	H: - C: - M: -	H: - C: - M: -	H: 0.073 C: 1.9 M: 3.36	H: 10.6 C: 39 M: 98	H: 17 C: 70 M: 63	H: 0.7 C: 2.43 M: 1.42	H: 9.5 C: 6.4 M: 6.8	H: 67 C: - M: -	H: 67 C: - M: -								
	IC50 ² (nM) Bivalent	H: - C: - M: -	H: - C: - M: -	H: - C: - M: -	H: 0.0003 C: 0.0003 M: 0.001	H: 0.09 C: 0.31 M: 0.5	H: 0.65 C: 1.3 M: 0.47	H: .00001 C: .0002 M: 0.0004	H: 0.8 C: 0.29 M: 0.69	H: 5.6 C: 6.5 M: 140	H: 5.6 C: 6.5 M: 140								

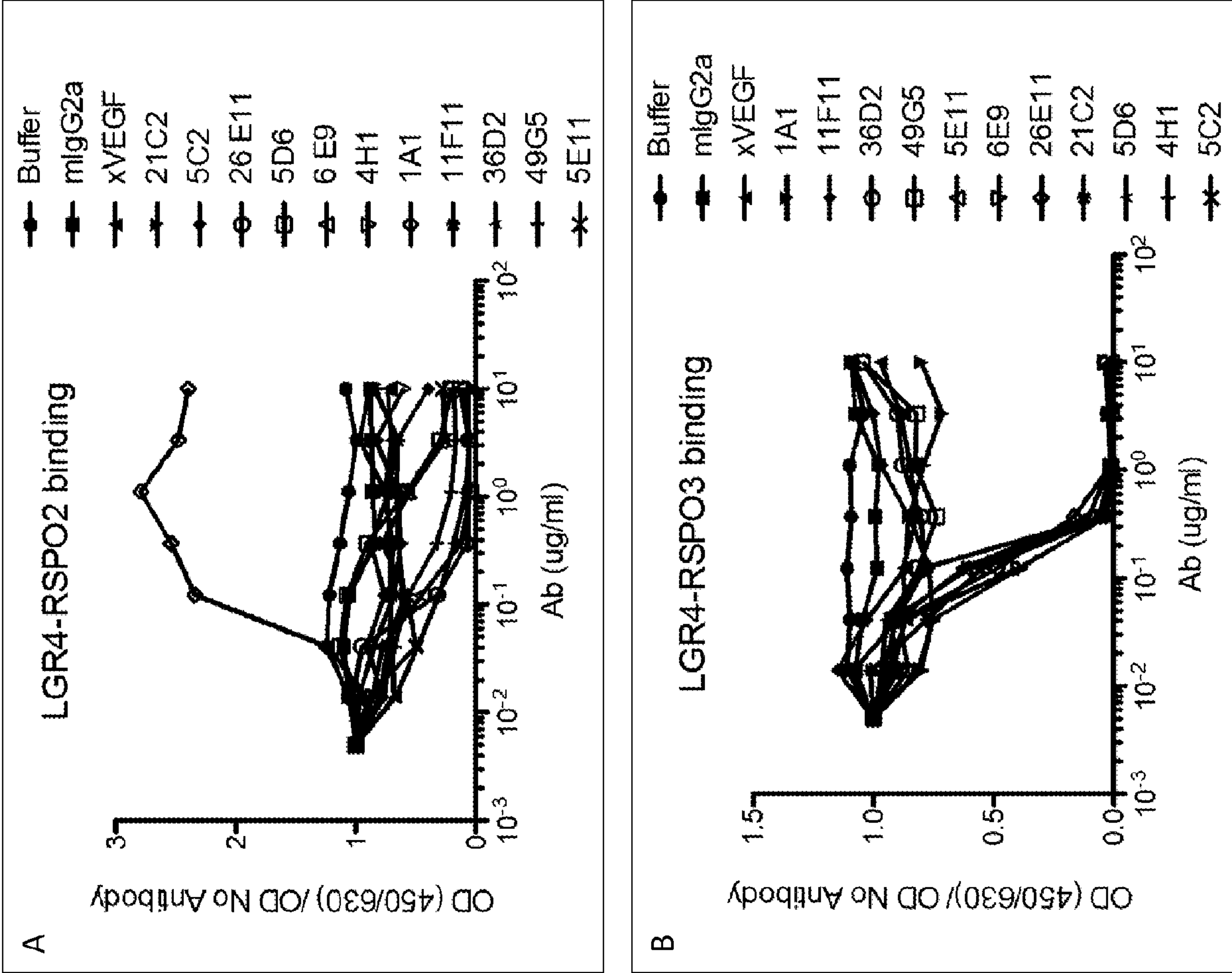
¹ Affinity was measured by Biacore using Fab of antibodies

² IC50 measurements were generated by stimulating a WNT luciferase reporter with the EC50 of RSPO.

“-” = no binding, or an IC50 > 500 nM

H: human, C: cyno; M: mouse

Figure 6



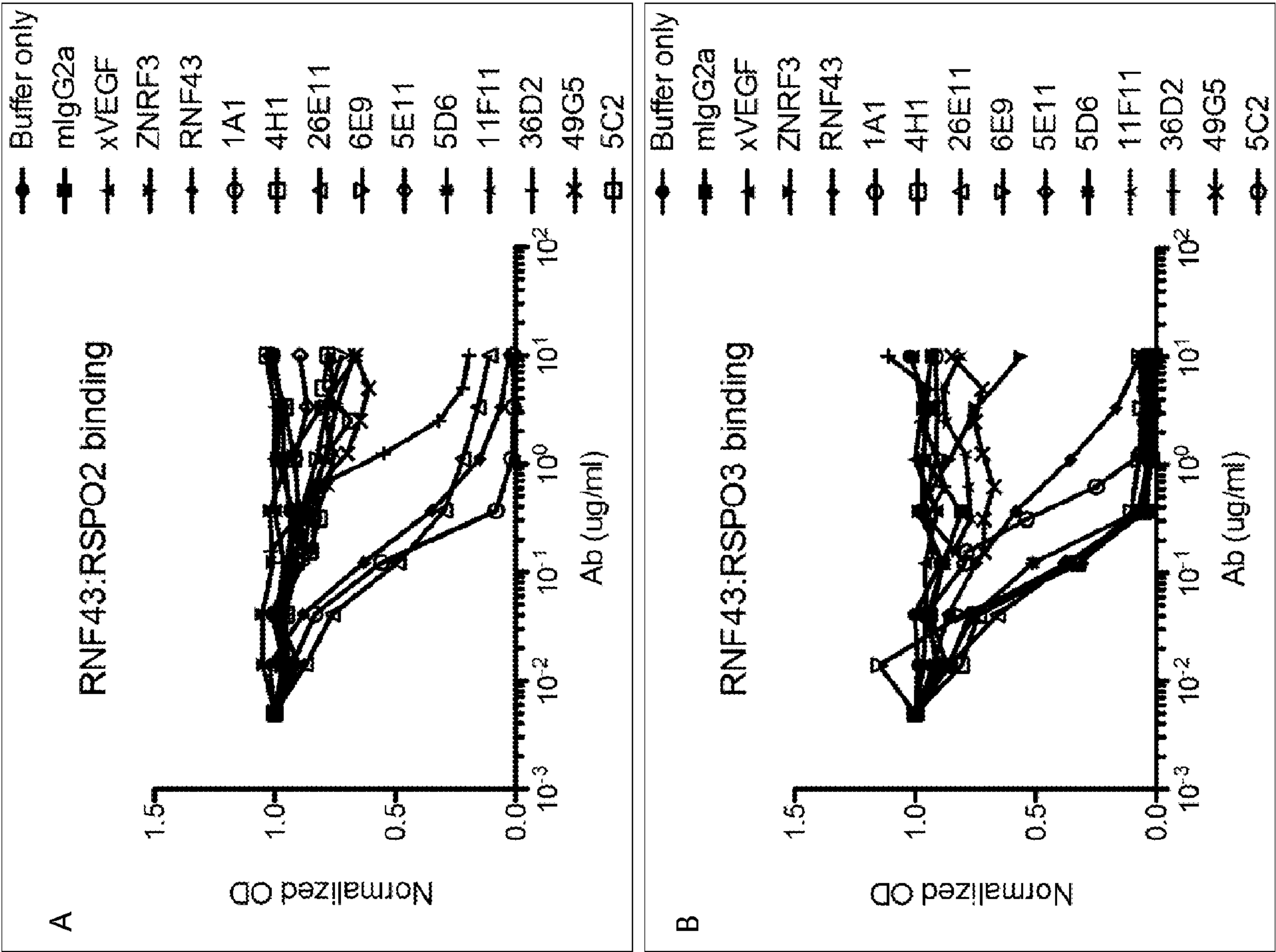


Figure 8

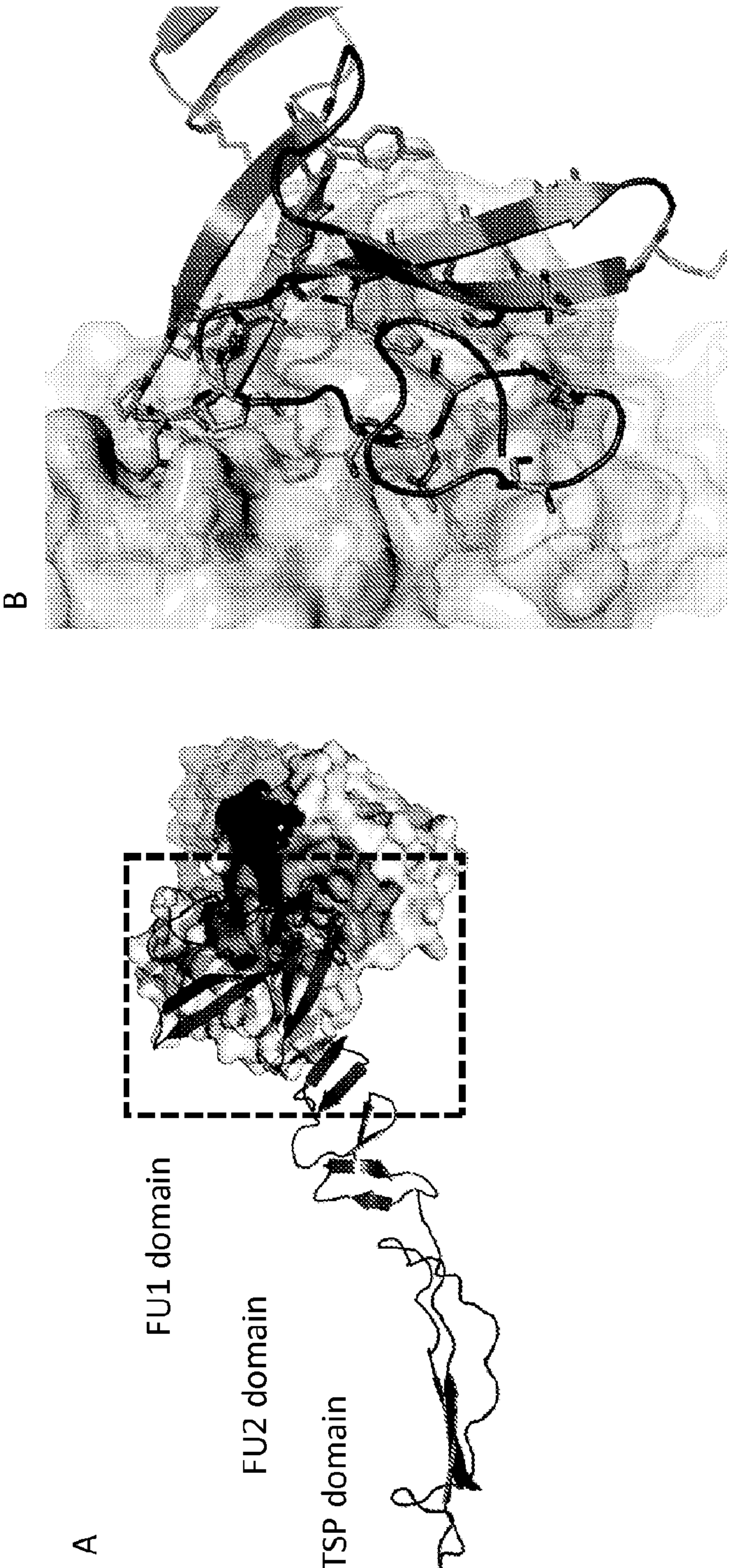


Figure 9A

Light chain variable region

Kabat number	CDR L1 - Contact																										
	CDR L1 - Kabat																										
5D6	D	I	V	L	T	Q	S	P	S	S	M	Y	A	S	L	G	E	R	V	T	I	T	C	K	A	S	Q
26 E11	D	I	K	M	T	Q	S	P	S	S	M	Y	A	S	L	G	E	R	V	T	I	T	C	K	A	S	Q
4H1	S	I	V	M	T	Q	T	P	L	S	L	P	V	S	L	G	D	Q	A	S	I	S	C	R	S	S	Q
5C2	D	I	V	M	T	Q	S	T	S	S	L	S	A	S	L	G	D	R	V	T	I	S	C	R	A	S	Q
5 E11	D	I	V	M	T	Q	S	T	S	S	L	S	A	S	L	G	D	R	V	T	I	S	C	R	A	S	Q
4D4	D	I	Q	M	N	Q	S	H	K	F	M	S	T	S	V	G	D	R	V	S	I	T	W	K	A	S	Q
6 E9	D	I	K	M	T	Q	S	T	S	S	L	S	A	S	L	G	D	R	V	T	I	S	C	R	A	S	Q
21C2	D	I	V	L	T	Q	S	P	A	S	L	T	V	S	L	G	Q	R	A	T	I	S	C	R	A	S	E
	CDR L2 - Contact																										
	CDR L2 - Kabat																										
5D6	Q	K	P	G	K	S	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G
26 E11	Q	K	P	G	K	S	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G
4H1	Q	K	P	G	Q	S	P	K	L	L	I	Y	R	I	S	N	R	F	S	G	V	P	S	R	F	S	G
5C2	Q	K	P	D	G	T	V	K	L	L	I	Y	T	S	R	L	H	S	G	V	P	S	R	F	S	G	
5 E11	Q	K	P	D	G	T	V	K	L	L	I	Y	T	S	R	L	H	S	G	V	P	S	R	F	S	G	
4D4	Q	K	P	G	Q	S	P	K	L	L	I	Y	W	A	S	T	R	H	T	G	V	P	S	R	F	S	
6 E9	Q	K	P	D	G	T	V	K	L	L	I	Y	T	S	R	L	H	S	G	V	P	S	R	F	S	G	
21C2	Q	K	P	G	Q	P	P	K	L	L	I	Y	L	A	S	N	L	S	G	V	P	A	R	F	S	G	
	CDR L3 - Contact																										
	CDR L3 - Kabat																										
5D6	Y	E	D	M	G	I	Y	Y	C	L	H	Y	D	E	F	F	P	L	T	F	F	G	A	G	T	K	L
26 E11	Y	E	D	M	G	I	Y	Y	C	L	H	Y	D	E	F	F	P	L	T	F	F	G	A	G	T	K	L
4H1	A	E	D	L	G	V	Y	Y	C	F	Q	Q	S	H	V	P	Y	T	T	F	F	G	G	T	K	L	
5C2	P	E	D	I	A	T	Y	F	C	Q	Q	G	D	T	L	P	P	T	T	F	F	G	G	T	K	L	
5 E11	K	E	D	V	A	T	Y	F	C	Q	Q	H	G	D	T	L	P	P	T	T	F	G	G	T	K	L	
4D4	S	E	D	L	A	D	Y	F	C	Q	Q	Y	S	S	S	I	T	F	F	G	A	G	T	K	L		
6 E9	Q	E	D	I	A	T	Y	F	C	Q	Q	G	D	T	L	P	P	A	F	F	G	G	T	K	L		
21C2	A	D	D	A	A	T	Y	C	Q	Q	N	N	E	D	P	Y	T	F	F	G	G	T	K	L			

5D6	SEQ ID NO: 95
26 E11	SEQ ID NO: 103
4H1	SEQ ID NO: 89
5C2	SEQ ID NO: 93
5 E11	SEQ ID NO: 97
4D4	SEQ ID NO: 91
6 E9	SEQ ID NO: 99
21C2	SEQ ID NO: 101

Figure 9B

Heavy chain variable region

		CDR H1 - Contact		CDR H1 - Kabat	
Kabat number					
5D6	EVQLQESGGPSLVKPKPSQTTLSLTCSVLTGDSITSGYWNWIRKFFPG				27
26 E11	EVQLQESGGPSLVKPKPSQTTLSLTCSVLTGDSITSGYWNWIRKFFPG				17
4H1	EVQLVESGGGFLVAPSGGSLKLTCTVSCAASGFTFSNFAMSWVRQSPPE				88
5C2	EVQLQESGGGFLVAPSGGSLKLTCTVSCAASGFTFSNFAMSWVRQSPPE				88
5E11	QVQLKQSGGPELVAPSGGSLKLTCTVSCAASGFTFSNFAMSWVRQSPPE				88
4D4	QVQLKQSGGPELVAPSGGSLKLTCTVSCAASGFTFSNFAMSWVRQSPPE				88
6E9	QVQLKQSGGPELVAPSGGSLKLTCTVSCAASGFTFSNFAMSWVRQSPPE				88
21C2	QVQLKQSGGPELVAPSGGSLKLTCTVSCAASGFTFSNFAMSWVRQSPPE				88
		CDR H2 - Contact		CDR H2 - Kabat	
Kabat number					
5D6	NKFEYMGYISYSGKTYQNPSSLKSRISITRDTSKNDTSKNQYHLLQLN				808
26 E11	NKFEYMGYISYSGKTYQNPSSLKSRISITRDTSKNDTSKNQYHLLQLN				28
4H1	KRLQEWLVGVISYSGKTYQNPSSLKSRISITRDTSKNDTSKNQYHLLQLN				88
5C2	KRLQEWLVGVISYSGKTYQNPSSLKSRISITRDTSKNDTSKNQYHLLQLN				88
5E11	EGLEWLVGVISYSGKTYQNPSSLKSRISITRDTSKNDTSKNQYHLLQLN				88
4D4	KGLEWLVGVISYSGKTYQNPSSLKSRISITRDTSKNDTSKNQYHLLQLN				88
6E9	KGLEWLVGVISYSGKTYQNPSSLKSRISITRDTSKNDTSKNQYHLLQLN				88
21C2	KGLEWLVGVISYSGKTYQNPSSLKSRISITRDTSKNDTSKNQYHLLQLN				88
		CDR H3 - Contact		CDR H3 - Kabat	
Kabat number					
5D6	SVTTEDTATYFCARVDDGYVYFAYWGGQGTLLVTVSSA SEQ ID NO: 96				211
26 E11	SVTTEDTATYFCARVDDGYVYFAYWGGQGTLLVTVSSA SEQ ID NO: 104				211
4H1	SLRSEDTATYFCARVDDGYVYFAYWGGQGTLLVTVSSA SEQ ID NO: 90				211
5C2	SLQTDDTATYFCARVDDGYVYFAYWGGQGTLLVTVSSA SEQ ID NO: 94				211
5E11	SLQADDATYFCARVDDGYVYFAYWGGQGTLLVTVSSA SEQ ID NO: 98				211
4D4	RLTSEDDATYFCARVDDGYVYFAYWGGQGTLLVTVSSA SEQ ID NO: 92				211
6E9	SLQANDATYFCARVDDGYVYFAYWGGQGTLLVTVSSA SEQ ID NO: 100				211
21C2	RLTSEDDATYFCARVDDGYVYFAYWGGQGTLLVTVSSA SEQ ID NO: 102				211

Figure 10A

Light chain variable region

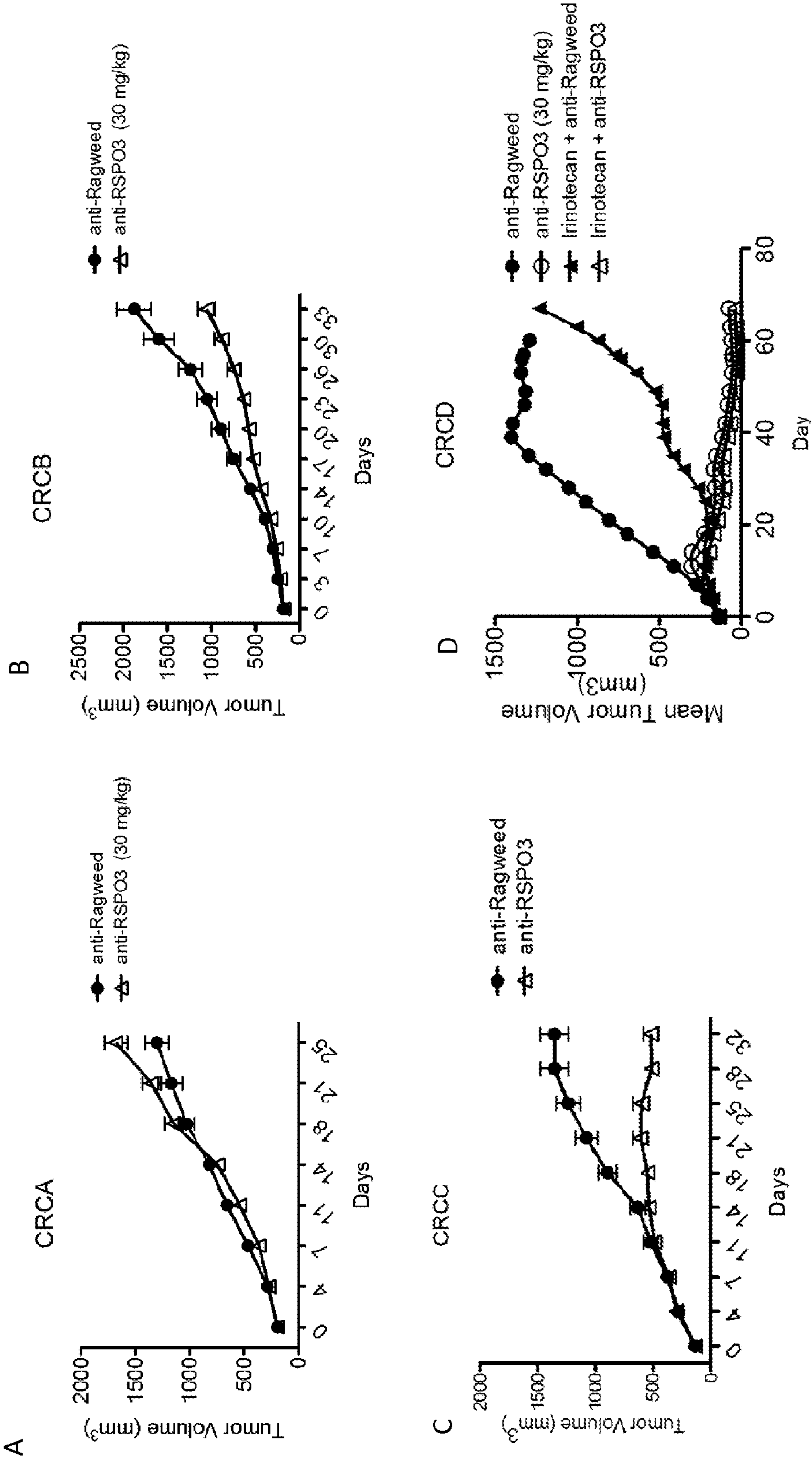
Kabat number	CDR L1 - Contact		CDR L1 - Kabat	
	256	257	258	259
11F11	Q P V L T Q S P S A S L G A S V K L T C T L S S Q	Q P V L T Q S P S A S L G A S V K L T C T L S S Q	Q P V L T Q S P S A S L G A S V K L T C T L S S Q	Q P V L T Q S P S A S L G A S V K L T C T L S S Q
1A1	Q P V L T Q S P S A S L G A S V K L T C T L S S Q	Q P V L T Q S P S A S L G A S V K L T C T L S S Q	Q P V L T Q S P S A S L G A S V K L T C T L S S Q	Q P V L T Q S P S A S L G A S V K L T C T L S S Q
36D2	E L V F T Q P Q S V S G S L G Q E I S I S C T R S S G N I G S N Y V S W Y Q Q Q S S	E L V F T Q P Q S V S G S L G Q E I S I S C T R S S G N I G S N Y V S W Y Q Q Q S S	E L V F T Q P Q S V S G S L G Q E I S I S C T R S S G N I G S N Y V S W Y Q Q Q S S	E L V F T Q P Q S V S G S L G Q E I S I S C T R S S G N I G S N Y V S W Y Q Q Q S S
49G5	Q P L L T Q S P S V S A S L G A S V K L T C T L S S Q	Q P L L T Q S P S V S A S L G A S V K L T C T L S S Q	Q P L L T Q S P S V S A S L G A S V K L T C T L S S Q	Q P L L T Q S P S V S A S L G A S V K L T C T L S S Q
Kabat number	CDR L2 - Contact		CDR L2 - Kabat	
	260	261	262	263
11F11	K A P K C V M Y L R S D G S H S K G D G I P D R F S G S S S G	K A P K C V M Y L R S D G S H S K G D G I P D R F S G S S S G	K A P K C V M Y L R S D G S H S K G D G I P D R F S G S S S G	K A P K C V M Y L R S D G S H S K G D G I P D R F S G S S S G
1A1	K S P K F L M Q V R D G S H S K G D G I P D R F S G S S S G	K S P K F L M Q V R D G S H S K G D G I P D R F S G S S S G	K S P K F L M Q V R D G S H S K G D G I P D R F S G S S S G	K S P K F L M Q V R D G S H S K G D G I P D R F S G S S S G
36D2	N K P R L L I Y K F D Q R . . . P S G V P D R F S G S S S G	N K P R L L I Y K F D Q R . . . P S G V P D R F S G S S S G	N K P R L L I Y K F D Q R . . . P S G V P D R F S G S S S G	N K P R L L I Y K F D Q R . . . P S G V P D R F S G S S S G
49G5	K S P K F L M Q L S D G S H S K G D G I P D R F S G S S S G	K S P K F L M Q L S D G S H S K G D G I P D R F S G S S S G	K S P K F L M Q L S D G S H S K G D G I P D R F S G S S S G	K S P K F L M Q L S D G S H S K G D G I P D R F S G S S S G
Kabat number	CDR L3 - Contact		CDR L3 - Kabat	
	264	265	266	267
11F11	V Q P E D E A I Y F C V T Y D S T V G V F G S G T Q L T V P	V Q P E D E A I Y F C V T Y D S T V G V F G S G T Q L T V P	V Q P E D E A I Y F C V T Y D S T V G V F G S G T Q L T V P	V Q P E D E A I Y F C V T Y D S T V G V F G S G T Q L T V P
1A1	L Q L E D E A I Y Y C G L S D V S L Y L F G S G T Q L T L L	L Q L E D E A I Y Y C G L S D V S L Y L F G S G T Q L T L L	L Q L E D E A I Y Y C G L S D V S L Y L F G S G T Q L T L L	L Q L E D E A I Y Y C G L S D V S L Y L F G S G T Q L T L L
36D2	L Q P E D E G D Y Y C L S G Y D K Y Y V F G S G T Q L T L L	L Q P E D E G D Y Y C L S G Y D K Y Y V F G S G T Q L T L L	L Q P E D E G D Y Y C L S G Y D K Y Y V F G S G T Q L T L L	L Q P E D E G D Y Y C L S G Y D K Y Y V F G S G T Q L T L L
49G5	L Q L E D E A I Y Y C G V S D V S L Y V F G S G T Q L T V L	L Q L E D E A I Y Y C G V S D V S L Y V F G S G T Q L T V L	L Q L E D E A I Y Y C G V S D V S L Y V F G S G T Q L T V L	L Q L E D E A I Y Y C G V S D V S L Y V F G S G T Q L T V L

Figure 10B

Heavy chain variable region

Kabat number	11F11	1A1	36D2	49G5
11F11	QVQLVQ	QVQLVQ	QVQLVQ	QVQLVQ
1A1	QVQLVQ	QVQLVQ	QVQLVQ	QVQLVQ
36D2	QVQLVQ	QVQLVQ	QVQLVQ	QVQLVQ
49G5	QVQLVQ	QVQLVQ	QVQLVQ	QVQLVQ

Figure 11



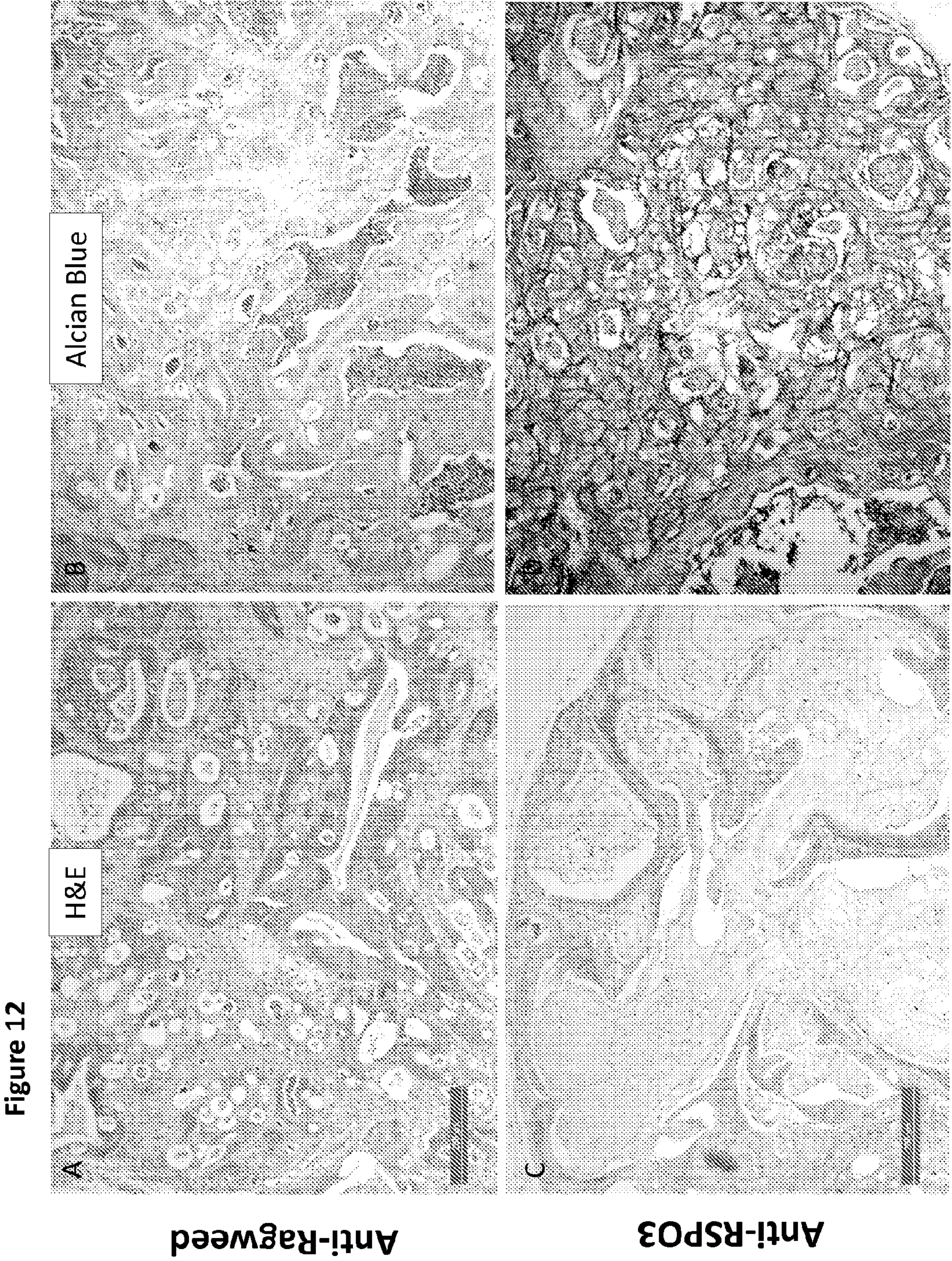


Figure 13

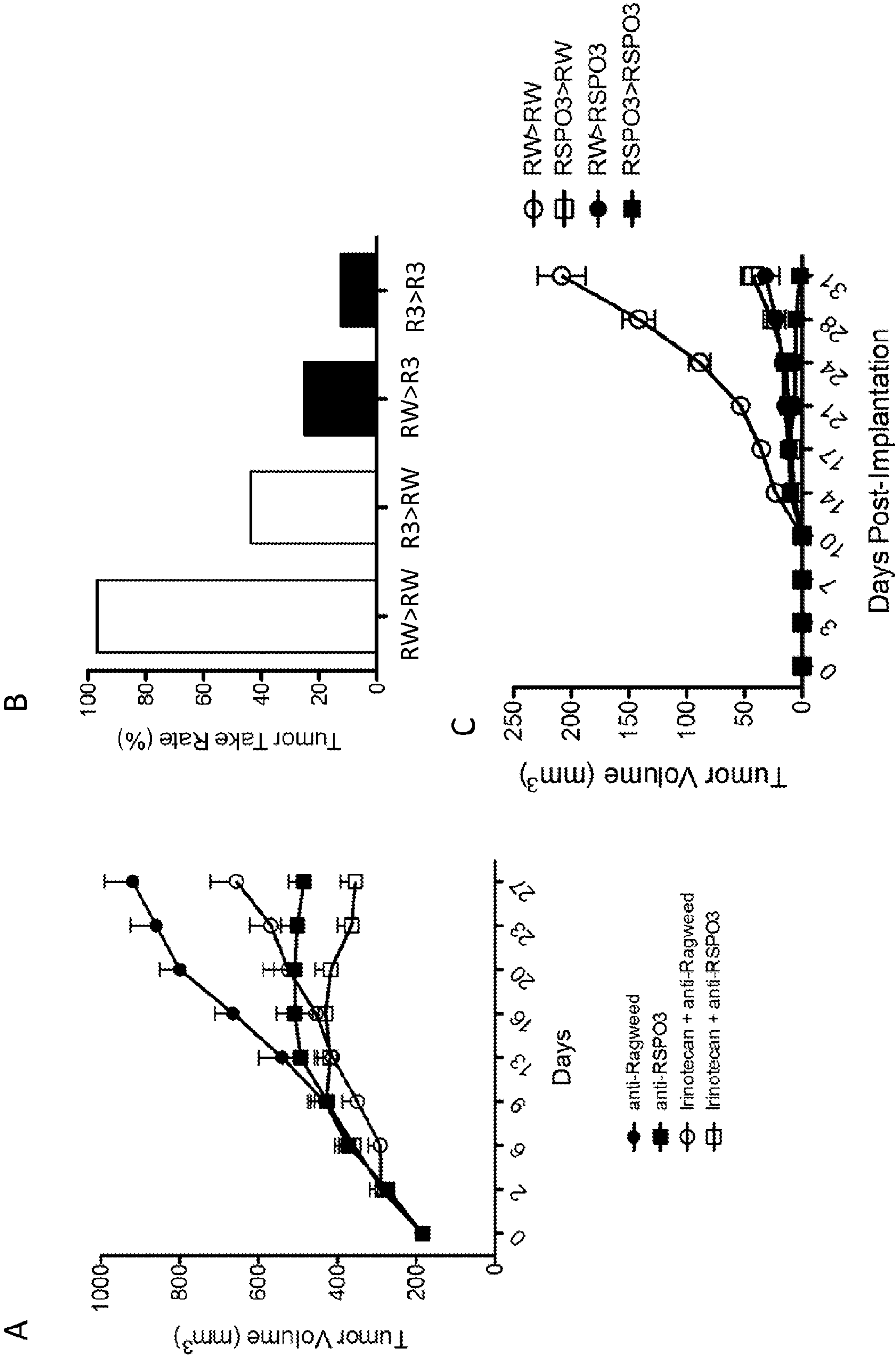


Figure 14A

Light chain variable region

CDR L1 - Contact																																										
Kabat number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
5D6	D	I	V	L	T	Q	S	P	S	S	M	Y	A	S	L	G	E	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v2.1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v2.2	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v2.3	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v2.4	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v2.8	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v2.10	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v3.2	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v3.3	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v4.1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v4.3	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v5.1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K
hu5D6v5.2	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	K	A	S	Q	D	I	D	S	Y	L	S	W	F	Q	Q	K	P	G	K

CDR L2 - Contact																																										
Kabat number	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84
5D6	S	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	Q	D	Y	S	L	T	I	S	S	L	E	Y	E	D	M	G
hu5D6v1	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	Q	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v2.1	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	Q	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v2.2	A	P	K	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	Q	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A	
hu5D6v2.3	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	T	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v2.4	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	Q	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v2.8	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	Q	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v2.10	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	Q	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v3.2	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	Q	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v3.3	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	Q	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v4.1	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	T	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v4.3	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	T	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v5.1	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	T	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A
hu5D6v5.2	A	P	K	T	L	I	Y	L	T	N	R	L	V	D	G	V	P	S	R	F	S	G	S	G	S	G	T	D	Y	T	L	T	I	S	S	L	Q	P	E	D	F	A

CDR L3 - Contact																								
Kabat number	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	
5D6	I	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	A	G	T	K	L	E	I	K	SEQ ID NO:95
hu5D6v1	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:190
hu5D6v2.1	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:192
hu5D6v2.2	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:194
hu5D6v2.3	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:196
hu5D6v2.4	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:198
hu5D6v2.8	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:200
hu5D6v2.10	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:202
hu5D6v3.2	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:204
hu5D6v3.3	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:206
hu5D6v4.1	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:208
hu5D6v4.3	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:210
hu5D6v5.1	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:212
hu5D6v5.2	T	Y	Y	C	L	H	Y	D	E	F	P	L	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:214

Figure 14B

Heavy chain variable region

CDR H1 - Contact

CDR H1 - Kabat

Kabat number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
5D6	E	V	Q	L	Q	E	S	G	P	S	L	V	K	P	S	Q	T	L	S	L	T	C	S	V	T	G	D	S	I	T	S	G	Y	W	N	W	I	R	K	F	P	G
hu5D6v1	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v2.1	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v2.2	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v2.3	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v2.4	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v2.8	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v2.10	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v3.2	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v3.3	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v4.1	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v4.3	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v5.1	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G
hu5D6v5.2	E	V	Q	L	V	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	D	S	I	T	S	G	Y	W	N	W	I	R	Q	P	P	G

CDR H2 - Contact

CDR H2 - Kabat

Kabat number	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	82a	82b
5D6	N	K	F	E	Y	M	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	I	S	I	T	R	D	T	S	K	N	Q	Y	H	L	Q	L	N	S
hu5D6v1	K	G	L	E	Y	M	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	I	T	I	S	R	D	T	S	K	N	Q	Y	S	L	K	L	S	S
hu5D6v2.1	K	G	L	E	Y	M	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	I	T	I	S	R	D	T	S	K	N	Q	Y	S	L	K	L	S	S
hu5D6v2.2	K	G	L	E	Y	M	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	I	T	I	S	R	D	T	S	K	N	Q	Y	S	L	K	L	S	S
hu5D6v2.3	K	G	L	E	Y	M	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	I	T	I	S	R	D	T	S	K	N	Q	Y	S	L	K	L	S	S
hu5D6v2.4	K	G	L	E	Y	M	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	I	T	I	S	R	D	T	S	K	N	Q	Y	S	L	K	L	S	S
hu5D6v2.8	K	G	L	E	Y	M	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	I	T	I	S	V	D	T	S	K	N	Q	Y	S	L	K	L	S	S
hu5D6v2.10	K	G	L	E	Y	M	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	I	T	I	S	R	D	T	S	K	N	Q	Y	S	L	K	L	S	S
hu5D6v3.2	K	G	L	E	W	I	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	I	T	I	S	R	D	T	S	K	N	Q	F	S	L	K	L	S	S
hu5D6v3.3	K	G	L	E	W	I	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	V	T	I	S	R	D	T	S	K	N	Q	F	S	L	K	L	S	S
hu5D6v4.1	K	G	L	E	W	I	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	V	T	I	S	R	D	T	S	K	N	Q	F	S	L	K	L	S	S
hu5D6v4.3	K	G	L	E	W	I	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	V	T	I	S	R	D	T	S	K	N	Q	F	S	L	K	L	S	S
hu5D6v5.1	K	G	L	E	W	I	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	V	T	I	S	R	D	T	S	K	N	Q	F	S	L	K	L	S	S
hu5D6v5.2	K	G	L	E	W	I	G	Y	I	S	Y	S	G	K	T	Y	Q	N	P	S	L	K	S	R	V	T	I	S	R	D	T	S	K	N	Q	F	S	L	K	L	S	S

CDR H3 - Contact

CDR H3 - Kabat

Kabat number	92c	93	94	95	96	97	98	99	100	100a	100b	100c	101	102	103	104	105	106	107	108	109	110	111	112	113											
5D6	V	T	T	E	D	T	A	T	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	A	SEQ ID NO:96
hu5D6v1	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:191
hu5D6v2.1	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:193
hu5D6v2.2	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:195
hu5D6v2.3	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:197
hu5D6v2.4	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:199
hu5D6v2.8	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:201
hu5D6v2.10	V	T	A	A	D	T	A	V	Y	Y	C	A	R	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:203
hu5D6v3.2	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:205
hu5D6v3.3	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:207
hu5D6v4.1	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:209
hu5D6v4.3	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	W	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:211
hu5D6v5.1	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	F	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:213
hu5D6v5.2	V	T	A	A	D	T	A	V	Y	Y	C	A	T	Y	Y	G	Y	G	G	P	H	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S	SEQ ID NO:215