CLAIMS:

What is claimed is:

1. An anti-glyco-MUC4 antibody or antigen binding fragment that specifically binds to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with GalNAc on the serine and threonine residues shown with bold and underlined text ("the MUC4 glycopeptide").

2. The anti-glyco-MUC4 antibody or antigen binding fragment of claim 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence and a light chain variable (VL) sequence of:

(a) SEQ ID NO:1 and SEQ ID NO:2, respectively;

(b) SEQ ID NO:23 and SEQ ID NO:24, respectively; or

(c) SEQ ID NO:45 and SEQ ID NO:46, respectively,

for binding to the MUC4 glycopeptide.

3. The anti-glyco-MUC4 antibody or antigen binding fragment of claim 1, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of any one of SEQ ID NOs:133-144 and a light chain variable (VL) sequence of any one of SEQ ID NOs:145-153 for binding to the MUC4 glycopeptide.

4. The anti-glyco-MUC4 antibody or antigen binding fragment of any one of claims 1 to 3, which specifically binds to COSMC knock-out T3M4 cells.

5. The anti-glyco-MUC4 antibody or antigen binding fragment of claim 4, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence and a light chain variable (VL) sequence of:

(a) SEQ ID NO:1 and SEQ ID NO:2, respectively;

(b) SEQ ID NO:23 and SEQ ID NO:24, respectively; or

(c) SEQ ID NO:45 and SEQ ID NO:46, respectively,

for binding to COSMC knock-out T3M4 cells.

6. The anti-glyco-MUC4 antibody or antigen binding fragment of claim 4, wherein the anti-glyco-MUC4 antibody or antigen binding fragment competes with an antibody or antigen binding fragment comprising a heavy chain variable (VH) sequence of any one of SEQ

ID NOs:133-144 and a light chain variable (VL) sequence of any one of SEQ ID NOs:145-153 for binding to COSMC knock-out T3M4 cells.

7. An anti-glyco-MUC4 antibody or antigen-binding fragment, which is optionally an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of claims 1 to 6, comprising:

(a) a complementarity determining region (CDR) H1 comprising the amino acid sequence of SEQ ID NO:67, SEQ ID NO:73, SEQ ID NO:79, SEQ ID NO:103, or SEQ ID NO:127;

(b) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:68, SEQ ID NO:74, SEQ ID NO:80, SEQ ID NO:104, or SEQ ID NO:128;

(c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81, SEQ ID NO:105, or SEQ ID NO:129;

(d) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:70, SEQ ID NO:76, SEQ ID NO:82, SEQ ID NO:106, or SEQ ID NO:130;

(e) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:71, SEQ ID NO:77, SEQ ID NO:83, SEQ ID NO:107, or SEQ ID NO:131; and

(f) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:72, SEQ ID NO:78, SEQ ID NO:84, SEQ ID NO:108, or SEQ ID NO:132.

8. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 7, which comprises:

(a) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:3-5, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:6-8, respectively;

(b) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:9-11, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:12-14, respectively; or

(c) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:15-17, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:18-20, respectively.

9. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 7, which comprises:

(a) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:25-27, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:28-30, respectively;

(b) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:31-33, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:34-36, respectively; or

(c) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:37-39, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:40-42, respectively.

10. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 7, which comprises:

(a) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:47-49, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:50-52, respectively;

(b) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:53-55, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:56-58, respectively; or

(c) a VH comprising CDR-H1, CDR-H2, and CDR-H3 having the amino sequences of SEQ ID NOs:59-61, respectively, and a VL comprising CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs:62-64, respectively.

11. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 10, which is a chimeric or humanized antibody or antigen-binding fragment of a chimeric or humanized antibody.

12. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 11, which comprises:

(a) a VH comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:1 and a VL comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:2;

(b) a VH comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:23 and a VL comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:24.

(c) a VH comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:45 and a VL comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:46.

13. An anti-glyco-MUC4 antibody or antigen-binding fragment that competes with a reference antibody or antigen binding fragment comprising:

(a) a heavy chain variable (VH) sequence of SEQ ID NO:1 and a light chain variable (VL) sequence of SEQ ID NO:2;

(b) a heavy chain variable (VH) sequence of SEQ ID NO:23 and a light chain variable (VL) sequence of SEQ ID NO:24;

(c) a heavy chain variable (VH) sequence of SEQ ID NO:45 and a light chain variable (VL) sequence of SEQ ID NO:46; or

(d) a heavy chain variable (VH) sequence of any one of SEQ ID NOs:133-144 and a light chain variable (VL) sequence of any one of SEQ ID NOs:145-153,

for binding to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with GalNAc on the serine and threonine residues shown with bold and underlined text ("the MUC4 glycopeptide"), the anti-glyco-MUC4 antibody or antigen-binding fragment comprising:

(a) a VH sequence with first, second and third CDR means within the VH sequence; and

(b) a VL sequence with fourth, fifth and sixth CDR means within the VL sequence,

wherein the first, second, third, fourth, fifth, and sixth CDR means cooperate to effect binding of the anti-glyco-MUC4 antibody or antigen-binding fragment to the MUC4 glycopeptide.

14. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 13, which preferentially binds to a glyco-MUC4 epitope that is overexpressed on cancer cells as compared to normal cells.

15. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 14, which specifically binds to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with STn on the serine and threonine residues shown with bold and underlined text.

16. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 1 to 14, which does not specifically bind to a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been glycosylated with STn on the serine and threonine residues shown with bold and underlined text.

17. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 16, which binds to the MUC4 glycopeptide with a binding affinity (KD) of:

(a) 1 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry;

(b) 1 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry;

(c) 1 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(d) 1 nM to 50 nM as measured by surface plasmon resonance or bio-layer interferometry;

(e) 5 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry;

(f) 5 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(g) 5 nM to 50 nM as measured by surface plasmon resonance or bio-layer interferometry;

(h) 5 nM to 25 nM as measured by surface plasmon resonance or bio-layer interferometry;

(i) 5 nM to 10 nM as measured by surface plasmon resonance or bio-layer interferometry;

(j) 10 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry;

(k) 10 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(l) 10 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry;

(m) 10 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(n) 10 nM to 50 nM as measured by surface plasmon resonance or bio-layer interferometry;

(o) 10 nM to 25 nM as measured by surface plasmon resonance or bio-layer interferometry;

(p) 50 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry;

(q) 50 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry;

(r) 50 nM to 100 nM as measured by surface plasmon resonance or bio-layer interferometry;

(s) 100 nM to 200 nM as measured by surface plasmon resonance or bio-layer interferometry; or

(t) 100 nM to 150 nM as measured by surface plasmon resonance or bio-layer interferometry.

18. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 17, which does not specifically bind to the unglycosylated MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) (the “unglycosylated MUC4 peptide”).

19. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 18, which does not specifically bind to the MUC1 tandem repeat $(VTSAPDTRPAPGSTAPPAHG)_3$ (SEQ ID NO:201) that has been glycosylated in vitro using purified recombinant human glycosyltransferases GalNAc-T1, GalNAc-T2, and GalNAc-T4 (“the first MUC1 glycopeptide”).

20. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 19, which does not specifically bind to the MUC1 peptide TAPPAHGV**TS**APD**T**RPAPG**ST**APPAHGVT (SEQ ID NO:202) that has been glycosylated in vitro with GalNAc on the serine and threonine residues shown with bold and underlined text (the “second MUC1 glycopeptide”).

21. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 20, which does not specifically bind to the CD44v6 peptide GYRQ**T**PKEDSH**S**TTGTAAA (SEQ ID NO:218) that has been glycosylated in vitro with GalNAc on the threonine and serine residues shown with bold and underlined text (the “CD44v6 glycopeptide”).

22. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 21, which does not specifically bind to the LAMP1 peptide CEQDRP**S**P**TT**APPAPPSPSP (SEQ ID NO:219) that has been glycosylated in vitro with GalNAc on the serine and threonine residues shown with bold and underlined text (the “LAMP1 glycopeptide”).

23. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 22, which does not specifically bind to the cMET peptide PTKSFISGG**ST**ITGVGKNLN (SEQ ID NO:220) that has been glycosylated in vitro with GalNAc on the serine and threonine residues shown with bold and underlined text (the “cMET glycopeptide”).

24. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 23, which is multivalent.

25. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 24, which is an antigen-binding fragment.

26. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 25, wherein the antigen-binding fragment is in the form of a single-chain variable fragment (scFv).

27. The anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 24, which is in the form of a multispecific antibody.

28. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 27, wherein the multispecific antibody is a bispecific antibody that binds to a second epitope that is different from the first epitope.

29. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 28, wherein the bispecific antibody is a bottle opener, mAb-Fv, mAb-scFv, central-scFv, one-armed central-scFv, or dual scFv format bispecific antibody.

30. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 28, wherein the bispecific antibody is a bispecific domain-exchanged antibody (*e.g.*, a CrossMab), a Fab-arm exchange antibody, a bispecific T-cell engager (BiTE), or a dual-affinity retargeting molecule (DART).

31. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 28 to 30, wherein the second epitope is a MUC4 epitope.

32. The anti-glyco-MUC4 antibody of antigen-binding fragment of any one of claims 28 to 30, wherein the second epitope is a MUC4 epitope that is overexpressed on cancer cells as compared to normal cells.

33. The anti-glyco-MUC4 antibody or antigen-binding fragment of any one of claims 28 to 30, wherein the second epitope is a T-cell epitope.

34. The anti-glyco-MUC4 antibody or antigen-binding fragment of claim 33, wherein the T-cell epitope comprises a CD3 epitope, a CD8 epitope, a CD16 epitope, a CD25 epitope, a CD28 epitope, or an NKG2D epitope.

35. A fusion protein comprising the amino acid sequence of the anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 34 operably linked to at least a second amino acid sequence.

36. A chimeric antigen receptor (CAR) comprising one or more antigen-binding fragments according to claim 25 or claim 26.

37. An antibody-drug conjugate comprising the anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 34 or the fusion protein of claim 35 conjugated to a cytotoxic agent.

38. A chimeric T cell receptor (TCR) comprising:

(a) an antigen-binding fragment according to claim 25 or claim 26;

(b) a first polypeptide chain comprising a first TCR domain comprising a first TCR transmembrane domain from a first TCR subunit; and

(c) a second polypeptide chain comprising a second TCR domain comprising a second TCR transmembrane domain from a second TCR subunit.

39. A nucleic acid comprising a coding region for an anti-glyco-MUC4 antibody or antigen-binding fragment of any of claims 1 to 34, the fusion protein of claim 35, the CAR of claim 36, or the chimeric TCR of claim 38.

40. A vector comprising the nucleic acid of claim 39.

41. A host cell engineered to express the nucleic acid of claim 39 or comprising the vector of claim 40.

42. A pharmaceutical composition comprising (a) the anti-glyco-MUC4 antibody or antigen binding fragment of any of claims 1 to 34, the fusion protein of claim 35, the CAR of claim 36, the antibody-drug conjugate of claim 37, the chimeric TCR of claim 38, the nucleic acid of claim 39, the vector of claim 40, or the host cell of claim 41, and (b) a physiologically suitable buffer, adjuvant, diluent, or combination thereof.

43. Use of the anti-glyco-MUC4 antibody or antigen binding fragment of any of claims 1 to 34, the fusion protein of claim 35, the CAR of claim 36, the antibody-drug conjugate of claim 37, the chimeric TCR of claim 38, the nucleic acid of claim 39, the vector of claim 40, the host cell of claim 41, or the pharmaceutical composition of claim 42 in the manufacture of a medicament for treating cancer.

44. The use of claim 43, wherein the cancer is pancreatic cancer, lung cancer, breast cancer, cancer of the gall bladder, salivary gland cancer, prostate cancer, biliary tract cancer, esophageal cancer, papillary thyroid carcinoma, low-grade fibromyxoid sarcoma, or ovarian cancer.

45. A method of detecting cancer in a biological sample, comprising contacting a sample (*e.g.*, a sample comprising or suspected of comprising cancer cells and/or cancer-derived extracellular vesicles) with an anti-glyco-MUC4 antibody or antigen-binding fragment according to any one of claims 1 to 34 and detecting binding of the anti-glyco-MUC4 antibody or antigen-binding fragment.

46. The method of claim 45, wherein the cancer is pancreatic cancer, lung cancer, breast cancer, cancer of the gall bladder, salivary gland cancer, prostate cancer, biliary tract cancer, esophageal cancer, papillary thyroid carcinoma, low-grade fibromyxoid sarcoma, or ovarian cancer.

47. A peptide of 13-30 amino acids in length comprising (a) amino acids 4-16 of a MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) or (b) an amino acid sequence corresponding to amino acids 4-16 of the MUC4 peptide CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) with one or two amino acid substitutions at positions other than the serine corresponding to position 12 of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155) and/or the threonine corresponding to position 13 of CTIPSTAMHTRSTAAPIPILP (SEQ ID NO:155).

48. A peptide of 13-30 amino acids in length comprising (a) amino acids 4-16 of a MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been O-glycosylated on the serine and threonine residues shown with bold and underlined text or (b) an amino acid sequence corresponding to amino acids 4-16 of the MUC4 peptide CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) that has been O-glycosylated on the serine and threonine residues shown with bold and underlined text with one or two amino acid substitutions at positions other than the serine corresponding to position 12 of CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154) and/or the threonine corresponding to position 13 of CTIPSTAMHTR**ST**AAPIPILP (SEQ ID NO:154).

49. A composition comprising the peptide of claim 47 or claim 48 and an adjuvant.

50. A method of generating antibodies against a tumor-associated form of MUC4, comprising administering to an animal:

(a) the peptide of claim 48; or

(b) the composition of claim 49, wherein the composition comprises the peptide of claim 48.

51. A method of eliciting an immune response against a tumor-associated form of MUC4, comprising administering to a subject:

(a) the peptide of claim 48; or

(b) The composition of claim 49, wherein the composition comprises the peptide of claim 48.