Supplementary Information:

Antibodies elicited by the first non-viral prophylactic cancer vaccine show tumor-specificity and immunotherapeutic potential

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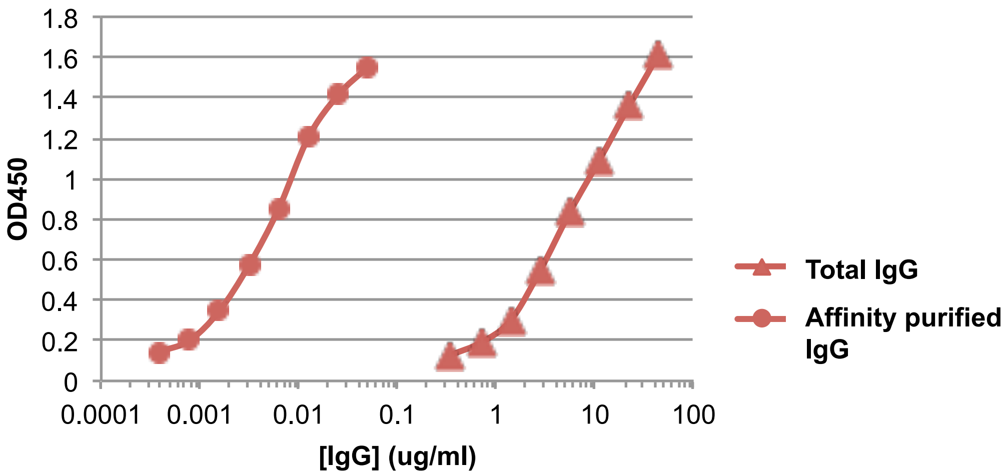


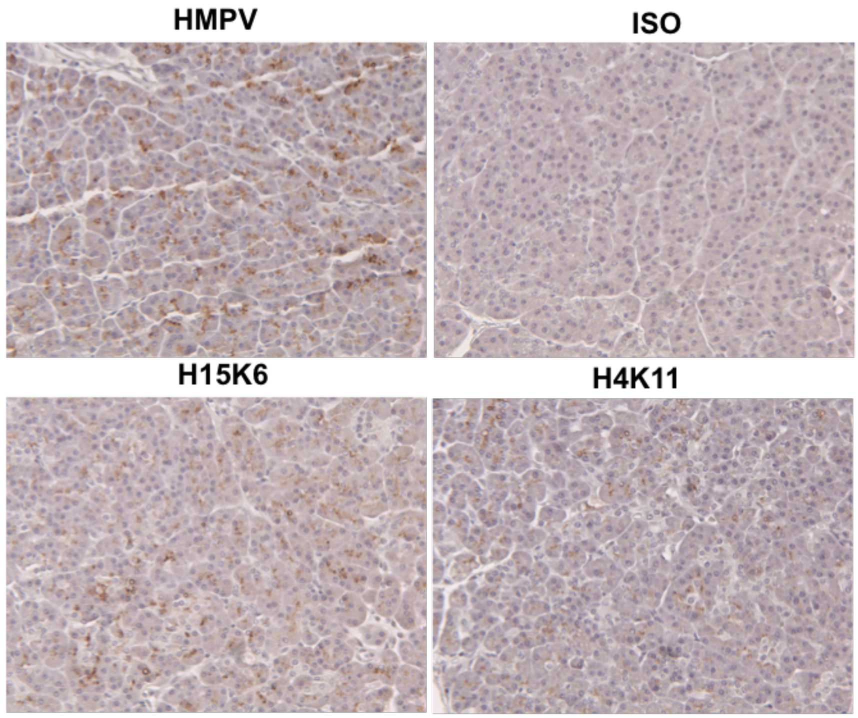
Supplementary Fig. 1. **Specific activity of total plasma IgG and affinity purified IgG from donor binding to 100mer VNTR peptide as determined by ELISA.** Anti-MUC1 vaccine peptide IgG in the plasma of the vaccinee was affinity purified first by protein A purification of total IgG then on 100mer VNTR peptide immobilized to magnetic beads. The specific binding activity of the eluted IgG after protein A purification (total IgG) and after peptide affinity purification (affinity purified IgG) was tested by ELISA.

Supplementary Table 1. Antibody VDJ and VJ usage and the number of somatic hypermutations for the heavy (Hc SHM) and light (Lc SHM) chains of the human anti-MUC1 antibodies.

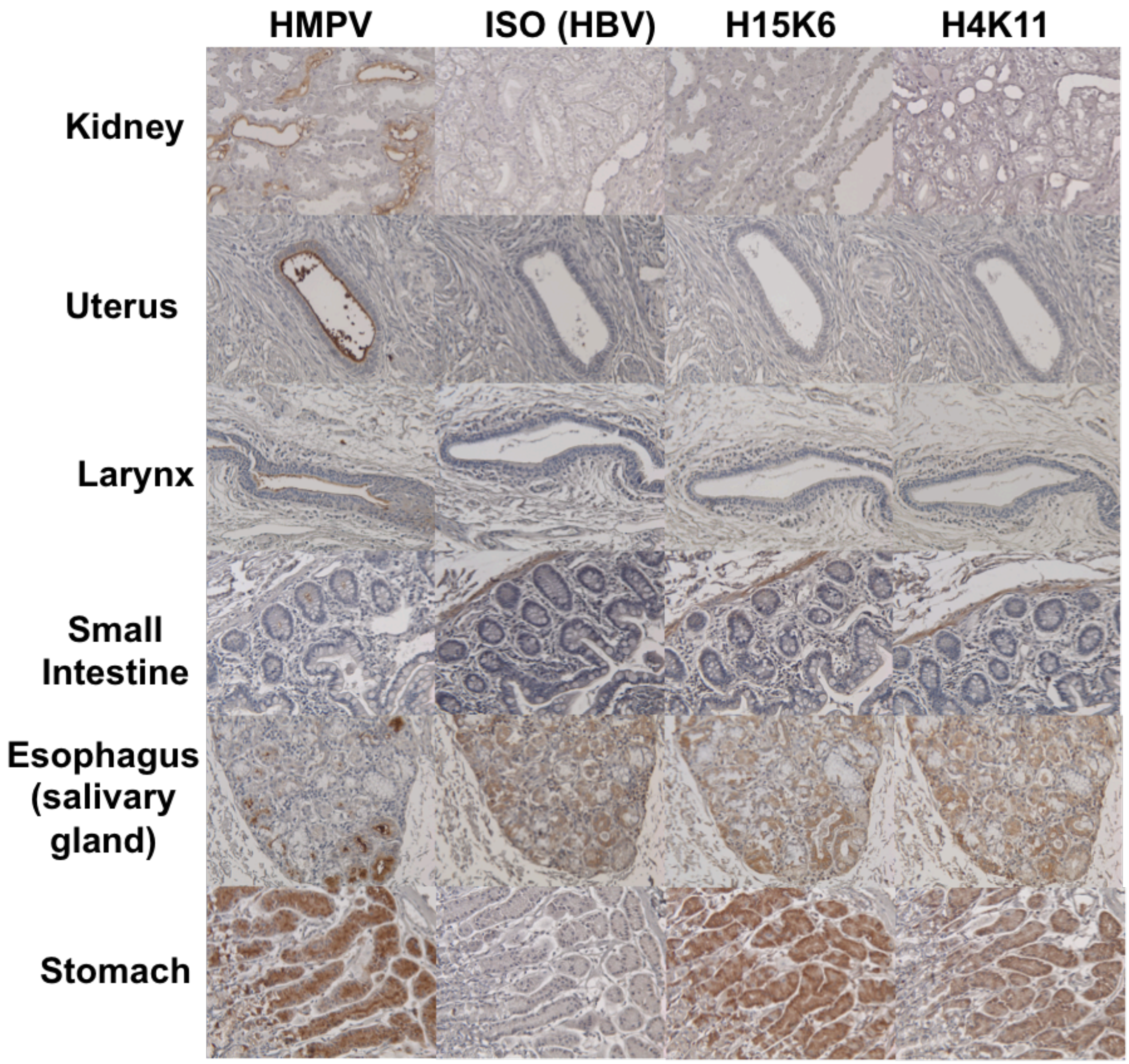
Mab	VDJ	Hc SHM	VJ	Lc SHM
H4/K10	VH4-39;DH1-26;JH5	3	VK4-1;JK3	1
H4/K11	VH4-39;DH1-26;JH5	3	VK4-1;JK2	0
H7/L1	VH4-39;DH3-10;JH6	5	VL1-40;JL2	1
H9/K4	VH3-66;DH3-22;JH3	0	VK2-28;JK2	7
H14/K6	VH3-11;DH2-15;JH6	3	VK2-24;JK4	0
H15/K6	VH3-11;DH2-8;JH6	3	VK2-24;JK4	0
H16/K6	VH3-11;DH2-15;JH6	3	VK2-24;JK4	0
H16/K16	VH3-11;DH2-15;JH6	3	VK2-24;JK4	1
H17/K7	VH3-74;DH6-6;JH4	1	VK2-30;JK1	3
H19/K6	VH3-11;DH2-15;JH6	2	VK2-24;JK4	0
H19/K15	VH3-11;DH2-15;JH6	2	VK2-24;JK3	1
H21/K7	VH3-66;DH3-22;JH4	1	VK2-30;JK1	3
H22/K7	VH3-53;DH3-16;JH6	1	VK2-30;JK1	3

MUC1 VNTR Glycopeptides	Antibodies													
	H4K10	H4K11	H7L1	H9K4	H14K6	H15K6	H16K6	H16K16	H17K7	H19K15	H21K7	H22K7	3C6	4H5
(HGVTSAPDTRPAPGSTAPPA)x2	3.2	3.4	3.1	3.5	3.0	2.9	2.9	3.3	2.8	3.3	3.0	3.1	2.3	2.5
HGVTSAPDTRPAPGSTAPPA	0.1	0.1	0.1	0.1	2.1	2.0	2.1	2.2	1.9	2.4	2.1	2.1	1.5	1.9
HGVTSAPDTRPAPGSTAPPA	0.1	0.1	0.1	0.1	1.6	1.7	1.6	0.1	2.0	1.5	2.1	2.2	2.0	1.8
HGVTSAPDTRPAPGSTAPPA	0.1	0.1	0.1	0.1	2.1	2.4	2.1	1.3	2.1	2.7	2.1	2.2	2.0	1.8
HGVTSAPDTRPAPGSTAPPA	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	1.9	0.1	2.1	2.3	2.4	0.1
HGVTSAPDTRPAPGSTAPPA	0.1	0.1	0.1	0.1	2.5	2.5	2.4	2.2	0.1	2.5	0.1	0.1	1.5	1.9
HGVTSAPDTRPAPGSTAPPA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	1.9	0.1	2.2	2.3	2.4	0.1
HGVTSAPDTRPAPGSTAPPA	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	2.4	0.1
Bovine Serum Albumin (BSA)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1

Supplementary Fig. 2. Binding of antibodies to MUC1 glycopeptides. ELISAs were performed on plates coated with various MUC1 glycopeptides or BSA for each anti-MUC1 antibody. Glycosylated residues on the glycopeptides are highlighted in red. Absorbance values for each peptide/antibody combination are displayed in the heatmap. Two mouse antibodies that bind to either all MUC1 glycoforms (3C6) or only the tumor specific glycoforms (4H5) were used as controls.



Supplementary Fig. 3. Immunohistochemical staining of pancreatic acinar cells with human anti-MUC1 antibodies. Paraffin-embedded normal pancreas tissue sections were stained with HMPV antibody for total MUC1 expression, human IgG Isotype control antibody as a negative control, and human anti-MUC1 antibodies H15K6, and H4K11. Punctate staining is indicative of subcellular compartment staining. Images taken at 20X magnification.



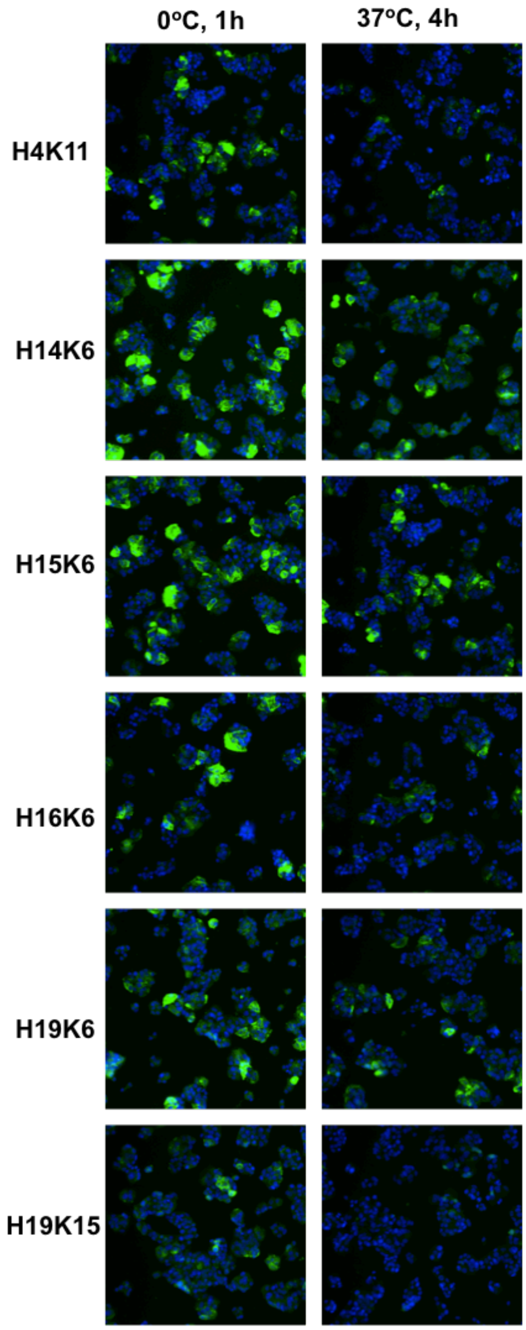
Supplementary Fig. 4. Immunohistochemical staining of paraffin-embedded normal tissue sections with human anti-MUC1 antibodies H15K6 and H4K11. HMPV is a mouse antibody that stains all MUC1 glycoforms (positive control). ISO (HBV) is a human anti-HBV IgG used as an isotype (negative) control. Images taken at 20X magnification.

Supplementary Table 2. Tumor and normal tissue sample information for stained tissue samples.

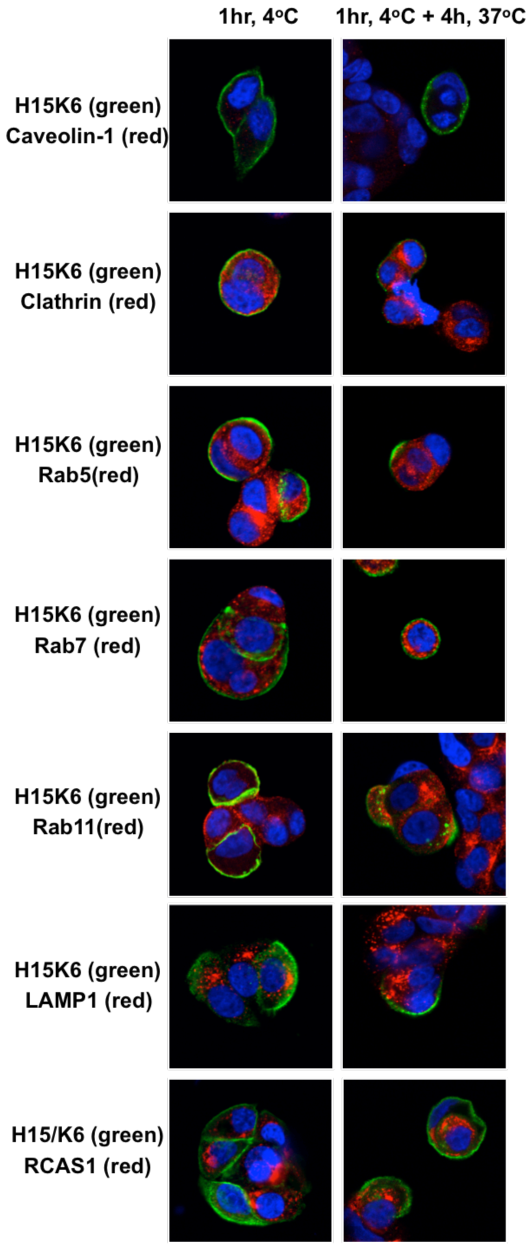
Catalog ID#	Vendor	Tissue type	Age	Sex
HUCAT171	US BIOMAX	Adenocarcinoma (grade II) of pancreas	54	F
HUCAT171	US BIOMAX	Adenocarcinoma (grade II) of pancreas	54	F
HUCAT226	US BIOMAX	Adenocarcinoma (grade II) of right lung	42	M
HUCAT226	US BIOMAX	Adenocarcinoma (grade II) of right lung	42	M
HUCAT300	US BIOMAX	Human breast invasive ductal carcinoma	47	F
HUCAT300	US BIOMAX	Human breast invasive ductal carcinoma	47	F
HUCAT391	US BIOMAX	Human Kidney Cancer	57	F
HUCAT391	US BIOMAX	Human Kidney Cancer	57	F
HUFPT041	US BIOMAX	Normal Human Esophagus	21	F
HUFPT041	US BIOMAX	Normal Human Esophagus	21	F
HUFPT073	US BIOMAX	Normal Human Kidney Medulla	59	M
HUFPT073	US BIOMAX	Normal Human Kidney Medulla	59	M
HUFPT078	US BIOMAX	Normal Human Body Of Pancreas	63	M
HUFPT078	US BIOMAX	Normal Human Body Of Pancreas	63	M
HUFPT081	US BIOMAX	Normal Human Small Intestine	2	F
HUFPT081	US BIOMAX	Normal Human Small Intestine	2	F
HUFPT096	US BIOMAX	Normal Human Uterus	21	F
HUFPT096	US BIOMAX	Normal Human Uterus	21	F
HUFPT101	US BIOMAX	Normal Human Tongue	21	F
HUFPT101	US BIOMAX	Normal Human Tongue	21	F
HUFPT116	US BIOMAX	Normal Human Larynx	21	F
HUFPT116	US BIOMAX	Normal Human Larynx	21	F
HUFPT130	US BIOMAX	Normal Breast Tissue	35	F
HUFPT130	US BIOMAX	Normal Breast Tissue	35	F
HUFPT178	US BIOMAX	Normal Lung	47	M
HUFPT178	US BIOMAX	Normal Lung	47	M
Colon N.434	CST	Normal Colon	#N/A	#N/A
Colon N.434	CST	Normal Colon	#N/A	#N/A
Colon N.434	CST	Normal Colon	#N/A	#N/A
Colon N.434	CST	Normal Colon	#N/A	#N/A
Colon 433	CST	Colon Adenocarcinoma	#N/A	#N/A
Colon 433	CST	Colon Adenocarcinoma	#N/A	#N/A
Colon 433	CST	Colon Adenocarcinoma	#N/A	#N/A

Colon 433	CST	Colon Adenocarcinoma	#N/A	#N/A
Lung N.947	CST	Normal Lung	#N/A	#N/A
Lung N.947	CST	Normal Lung	#N/A	#N/A
Lung N.947	CST	Normal Lung	#N/A	#N/A
Lung N.947	CST	Normal Lung	#N/A	#N/A
Lung 946	CST	Lung Adenocarcinoma	#N/A	#N/A
Lung 946	CST	Lung Adenocarcinoma	#N/A	#N/A
Lung 946	CST	Lung Adenocarcinoma	#N/A	#N/A
Lung 946	CST	Lung Adenocarcinoma	#N/A	#N/A
Stomach	BioChain	Normal Stomach	#N/A	#N/A
Stomach	BioChain	Normal Stomach	#N/A	#N/A

Vendor catalogue number, vendor, tissue sample type, and patient age and sex are listed for each of the tissues stained. “#N/A” indicates that this information was not readily available for the particular sample.

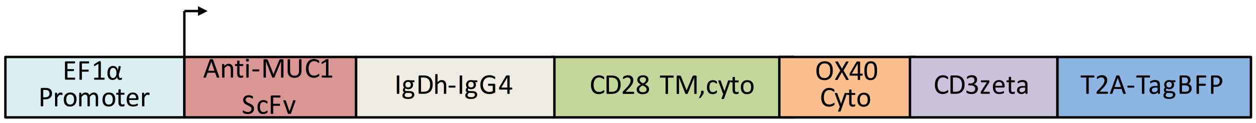


Supplementary Fig. 5. Immunofluorescence staining of MUC1 internalization following binding of human anti-MUC1 antibodies. Punctate staining is seen with H15K6 and not with the other antibodies. ZR-75-1 cells were incubated with various anti-MUC1 human antibodies for the indicated times and temperatures. Cells were then washed and fixed with paraformaldehyde followed by detection with secondary antibodies. MUC1: anti-human IgG-Alexa 488 complexes are shown in green. Nuclei were stained with DAPI (blue).

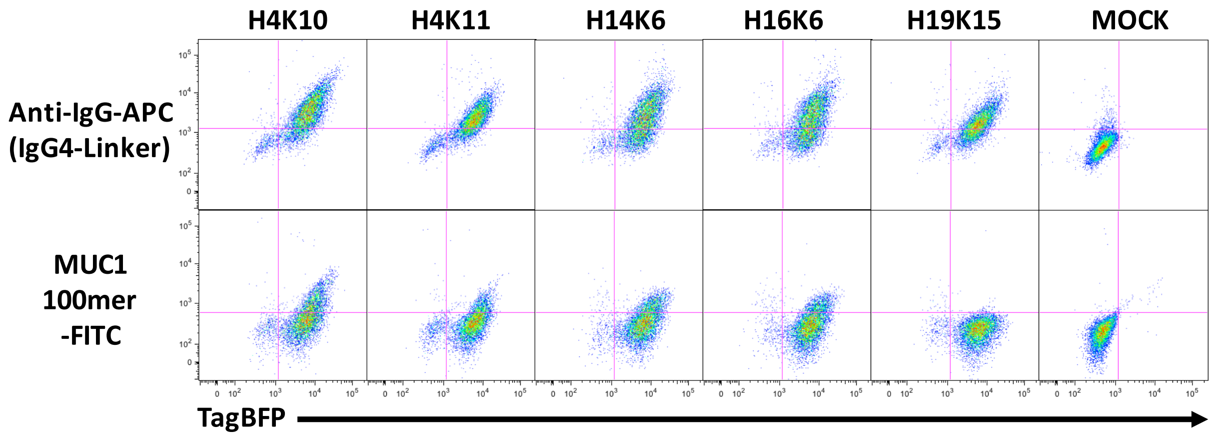


Supplementary Fig. 6. Confocal images of MUC1 internalization after binding of H15K6 and colocalization with indicated intracellular compartment markers. ZR-75-1 cells were incubated with anti-MUC1 human antibody H15K6 for the indicated times and temperatures. Cells were then washed and fixed with paraformaldehyde, then incubated with rabbit antibodies specific for molecules in the endocytic pathway (Caveolin1, Clathrin, Rab5, Rab7, Rab11), lysosome (LAMP1) or golgi (RCAS1), followed by detection with secondary antibodies (MUC1:

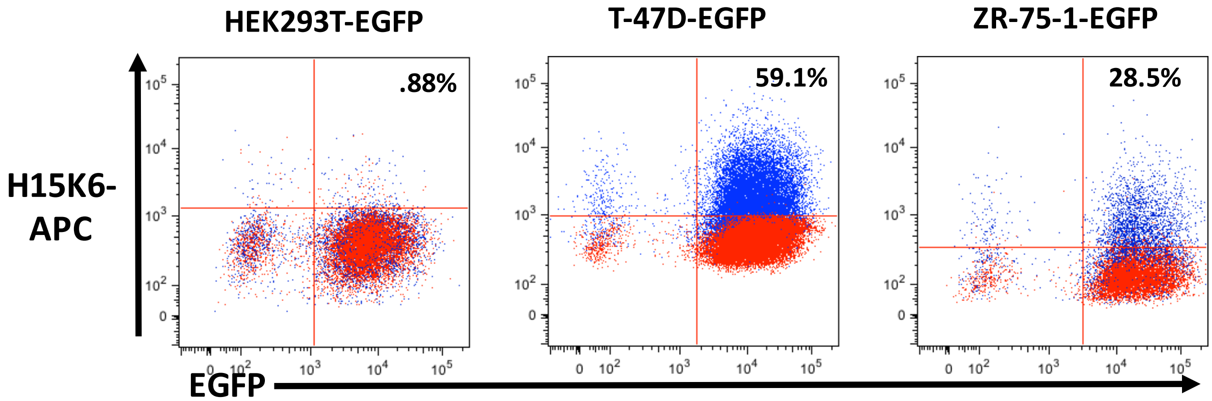
anti-human IgG-Alexa 488 complex is shown in green; organelle markers: anti-rabbit IgG-Alexa 555 complex is shown in red). Nuclei were stained with DAPI (blue). Signal was visualized by confocal immunofluorescence microscopy and captured images were overlaid to assess co-localization (yellow) of anti-MUC1 antibody with organelle marker antibody.



Supplementary Fig. 7. Design of CAR Lentiviral Expression Cassettes. CARs are expressed from the EF1α promoter, and consist of the scFv's of the H4K10, H4K11, H14K6, H16K6 or H19K15 antibodies fused to the IgDhinge and IgG4(CH_2,CH_3) spacer domains, the CD28 transmembrane and cytoplasmic domains, the OX40 cytoplasmic domain, CD3zeta and the T2A cleavage tag, and the TagBFP reporter gene.



Supplementary Fig. 8. Confirmation of CAR cell surface expression by Fc-APC staining of extracellular linker domain and FITC labeled MUC1-100mer peptide. Flow sorted CAR+ cells (TagBFP+) or MOCK transduced negative control cells were stained with an anti-human IgG APC-conjugated F(ab')$_2$ to identify IgG4-linker expressing cells or FITC-labeled MUC1-100mer peptide indicating scFv binding to MUC1 peptide and evaluated by flow cytometry.



Supplementary Fig. 9. Hypoglycosylated MUC1+ populations of HEK293T-EGFP, T-47D-EGFP, and ZR-75-1-EGFP target cells. Cell lines were stained with H15K6 antibody followed by anti-human IgG APC-conjugated $F(ab')_2$ to identify the hypoglycosylated MUC1 population of each EGFP+ cell line and underwent flow cytometry. Percentage of double positive populations for APC (H15K6) and EGFP are listed in this gate for each line.

Supplementary Table 3. DNA sequences of anti-MUC1 CARs and expression vector.

3rd Generation CAR sequence for anti-MUC1 CARs
ATGGAGACAGACACACTCCTGCTATGGGTGCTGCTGCTCTGGGTTCCAGGTTCCACA GG-scFv here- AAAGACGGACAGCTACTACATGACAAGCAGCCAGCTCTCCACCCCCCTCCAGCAG TGGCGCCAAGGCGAGTACAAATGCGTGGTCCAGCACACCGCCAGCAAGAGTAAGAA GGAGATCTTCCGCTGGCCAGAGTCTCCAAAGGCACAGGCCTCCTCAGTGCCCACTGC ACAACCCCAAGCAGAGGGCAGCCTCGCCAAGGCAACCACAGCCCCAGCCACCACCC GTAACACAGGAAGAGGAGGAGAAGAGAAGAAGAAGGAGAAGGAGAAAGAGGAAC AAGAAGAGAGAGAGACAAAGACACCAGAGGAGAGCAAGTACGGCCCTCCCTGCCC CCCTTGCCCTGCCCCCGAGTTCCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAA GCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGTGACCTGCGTGGTGGTGG ACGTGAGCCAGGAAGATCCCGAGGTCCAGTTCAATTGGTACGTGGACGGCGTGGAA GTGCACAACGCCAAGACCAAGCCCAGAGAGGAACAGTTCAACAGCACCTACCGGGT GGTGTCTGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGT GCAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAAAAGACCATCAGCAAGGCC AAGGGCCAGCCTCGCGAGCCCCAGGTGTACACCCTGCCTCCCTCCCAGGAAGAGAT GACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGACA TCGCCGTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCT CCCGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAA GAGCCGGTGGCAGGAAGGCAACGTCTTTAGCTGCAGCGTGATGCACGAGGCCCTGC ACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCTGGGCAAGATGTTCTGGGTG CTGGTGGTGGTGGGCGGGGTGCTGGCCTGCTACAGCCTGCTGGTGACAGTGGCCTTC ATCATCTTTTGGGTGCGGAGCAAGCGGAGCAGAGGCGGCCACAGCGACTACATGAA CATGACCCCCAGACGGCCTGGCCCCACCCGGAAGCACTACCAGCCCTACGCCCCAC CCAGGGACTTTGCCGCCTACCGGTCCGGCGGAGGGCAGAGGCTGCCCCCCGATGCC CACAAGCCCCCTGGGGGAGGCAGTTTCCGGACCCCCATCCAAGAGGAGCAGGCCGA CGCCCACTCCACCCTGGCCAAGATCCGGGTGAAGTTCAGCAGAAGCGCCGACGCCC CTGCCTACCAGCAGGGCCAGAATCAGCTGTACAACGAGCTGAACCTGGGCAGAAGG GAAGAGTACGACGTCCTGGATAAGCGGAGAGGCCGGGACCCTGAGATGGGCGGCA AGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTATAACGAACTGCAGAAAGACAA GATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGAGGCGGGGCAAG GGCCACGACGGCCTGTATCAGGGCCTGTCCACCGCCACCAAGGATACCTACGACGC CCTGCACATGCAGGCCCTGCCCCCAAGGCTCGAGGGCGGCGGAGAGGGCAGAGGA AGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTCGCATGAGCGAGCT GATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGGACAACCATC ACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATG AGAATCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACT AGCTTCCTCTACGGCAGCAAGACCTTCATCAACCACACCCAGGGCATCCCCGACTTC TTCAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGA CGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCT ACAACGTCAAGATCAGAGGGGTGAACTTCACATCCAACGGCCCTGTGATGCAGAAG AAAACACTCGGCTGGGAGGCCTTCACCGAGACGCTGTACCCCGCTGACGGCGGCCT GGAAGGCAGAAACGACATGGCCCTGAAGCTCGTGGGCGGAGCCATCTGATCGCAA ACATCAAGACCACATATAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCTGGC GTCTACTATGTGGACTACAGACTGGAAAGAATCAAGGAGGCCAACAACGAAACATA

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scFv DNA sequences (V_H-GS Linker-V_L)
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pSICO-EF1 Lentiviral-expression vector
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