**Supplemental Material**

# TARGETING THE HUMAN MUC1-C ONCOPROTEIN WITH AN ANTIBODY-DRUG CONJUGATE

**Supplemental Tables S1 – S3**

**and**

**Supplemental Figures S1 – S10**

**Supplemental Table S1.** PK Parameters for MAb-3D1-MMAE ADCs.

| **Dose** | **5 mg/kg** | **10 mg/kg** |
|---|---|---|
| Half-life (d) | 9.7 | 9.0 |
| $C_{max}$ (μg/ml) | 191.0 | 374.5 |
| ERC | 0.071 | 0.077 |
| $AUC_{o-t}$ | 905.6 | 1788.6 |

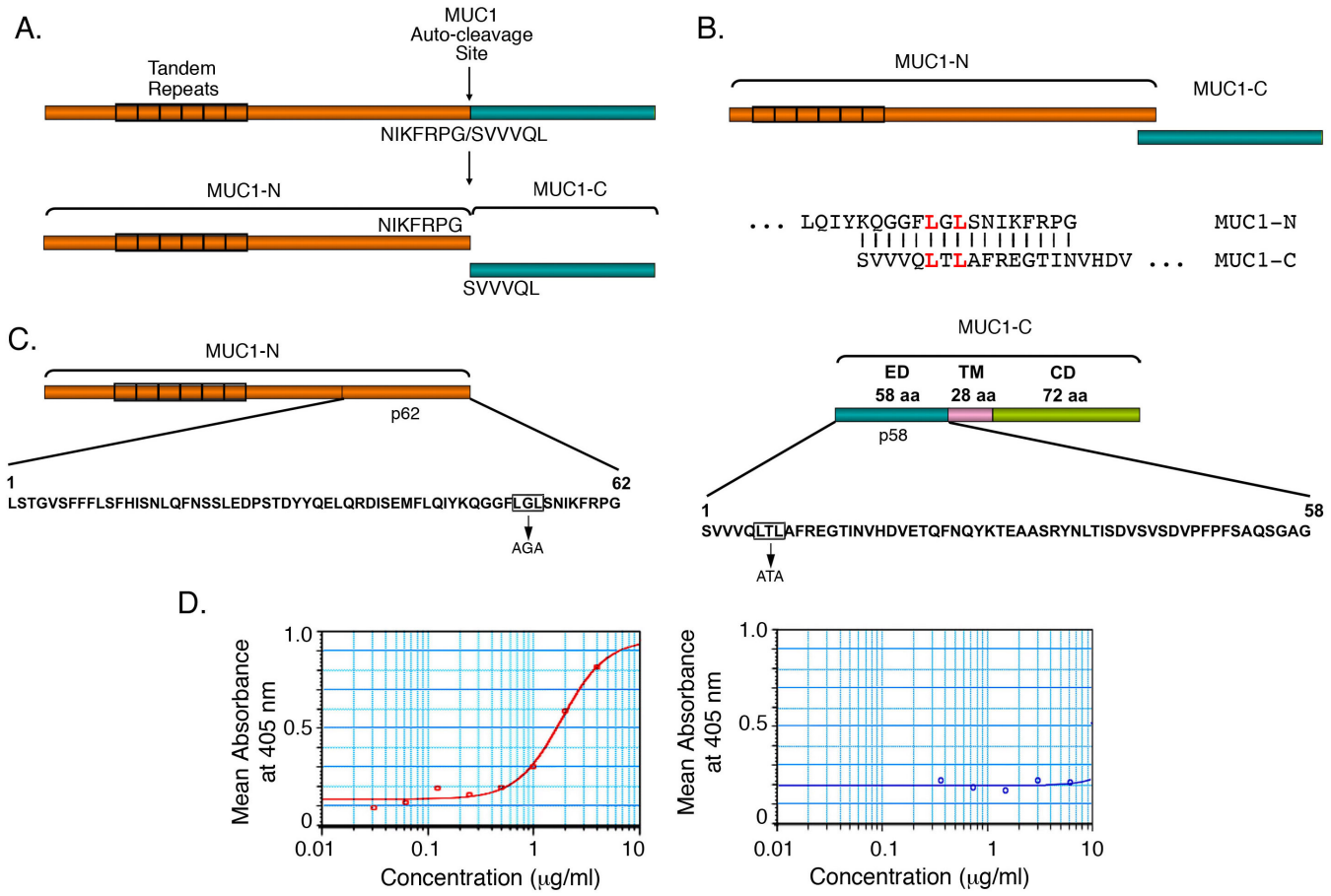
ERC Elimination Rate Constant

**Supplemental Table S2.** Blood Chemistry Analysis of HuMAb 3D1-MMAE ADCs.

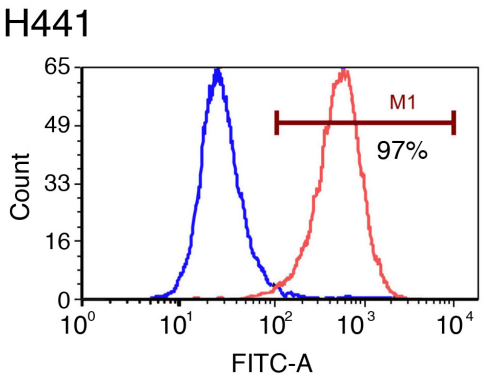
| | | Vehicle | | ADC | |
|---|---|---|---|---|---|
| **Assay** | **Units** | **Male** | **Female** | **Male** | **Female** |
| **CHOL** | mg/dL | 94 | 86 | 88 | 77 |
| **TRIG** | mg/dL | 149 | 65 | 89 | 54 |
| **ALT** | U/L | 33 | 27 | 29 | 28 |
| **AST** | U/L | 114 | 62 | 114 | 75 |
| **ALP** | U/L | 53 | 83 | 55 | 101 |
| **GLU** | mg/dL | 145 | 137 | 165 | 143 |
| **PHOS** | mg/dL | 6.7 | 8.6 | 9.9 | 8.3 |
| **Ca** | mg/dL | 10.0 | 10.3 | 10.4 | 10.0 |
| **TBIL** | mg/dL | 0.14 | 0.15 | 0.13 | 0.14 |
| **TP** | g/dL | 5.1 | 5.2 | 4.8 | 5.4 |
| **ALB** | g/dL | 3.0 | 3.0 | 2.7 | 3.3 |
| **BUN** | mg/dL | 33 | 27 | 33 | 31 |
| **CREAT** | mg/dL | 0.3 | 0.3 | 0.2 | 0.3 |
| **Na** | mEq/L | 155 | 156 | 155 | 154 |
| **K** | mEq/L | 5.4 | 4.4 | 6.5 | 5.0 |
| **Cl** | mEq/L | 111 | 110 | 112 | 113 |

**Supplemental Table S3.** Hematology Analysis of HuMAb 3D1-MMAE ADCs.

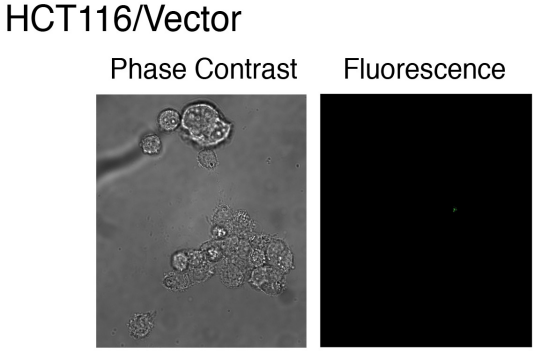
| | Vehicle | | ADC | |
|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** |
| WBC ($x10^3$ cells/μL) | 6.45 | 5.23 | 5.20 | 5.10 |
| #NE ($x10^3$ cells/μL) | 1.13 | 1.28 | 1.45 | 0.62 |
| #LY ($x10^3$ cells/μL) | 4.91 | 3.61 | 3.29 | 4.21 |
| #MO ($x10^3$ cells/μL) | 0.22 | 0.19 | 0.16 | 0.05 |
| #EO ($x10^3$ cells/μL) | 0.09 | 0.06 | 0.23 | 0.19 |
| RBC ($x10^6$ cells/μL) | 9.61 | 9.86 | 8.90 | 10.05 |
| HGB (g/dL) | 13.5 | 13.8 | 12.7 | 14.1 |
| HCT (%) | 48.3 | 49.8 | 46.7 | 51.1 |
| MCV (fL) | 50.3 | 50.5 | 52.5 | 50.8 |
| MCH (pg) | 14.1 | 14.0 | 14.3 | 14.0 |
| MCHC (g/dL) | 28.0 | 27.8 | 27.2 | 27.6 |
| PLT ($x10^3$ cells/μL) | 1018 | 1120 | 936 | 929 |



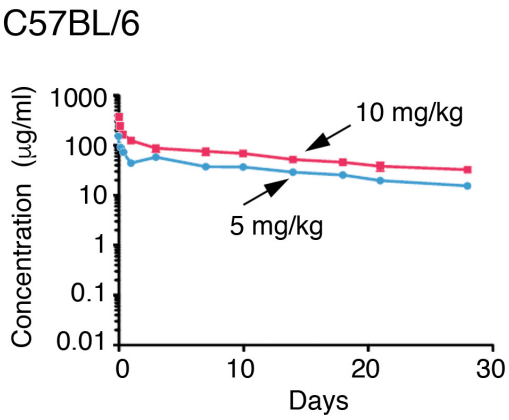

**Supplemental Figure S1. Formation of the p62/p58 heterodimer is attenuated by mutation of leucine residues in the junction.** (**A**) Schematic of the MUC1 protein and autocleavage at the G^SVVV site into the MUC1 N-terminal (MUC1-N) and C-terminal (MUC1-C) subunits. (**B**) Putative alignment of MUC1-N and MUC1-C at the junction. (**C**) Amino acid sequences of the MUC1-N p62 (left) and MUC1-C p58 (right) proteins with highlighting of the LA mutations. (**D**) The p62 protein (1 μg/ml) coated on a 96-well plate was incubated with the indicated concentrations of p58 (left). The p62(LGL→AGA) protein (1 μg/ml) coated on a 96-well plate was incubated with the indicated concentrations of p58(LTL→ATA) (right). Binding was determined by ELISA.



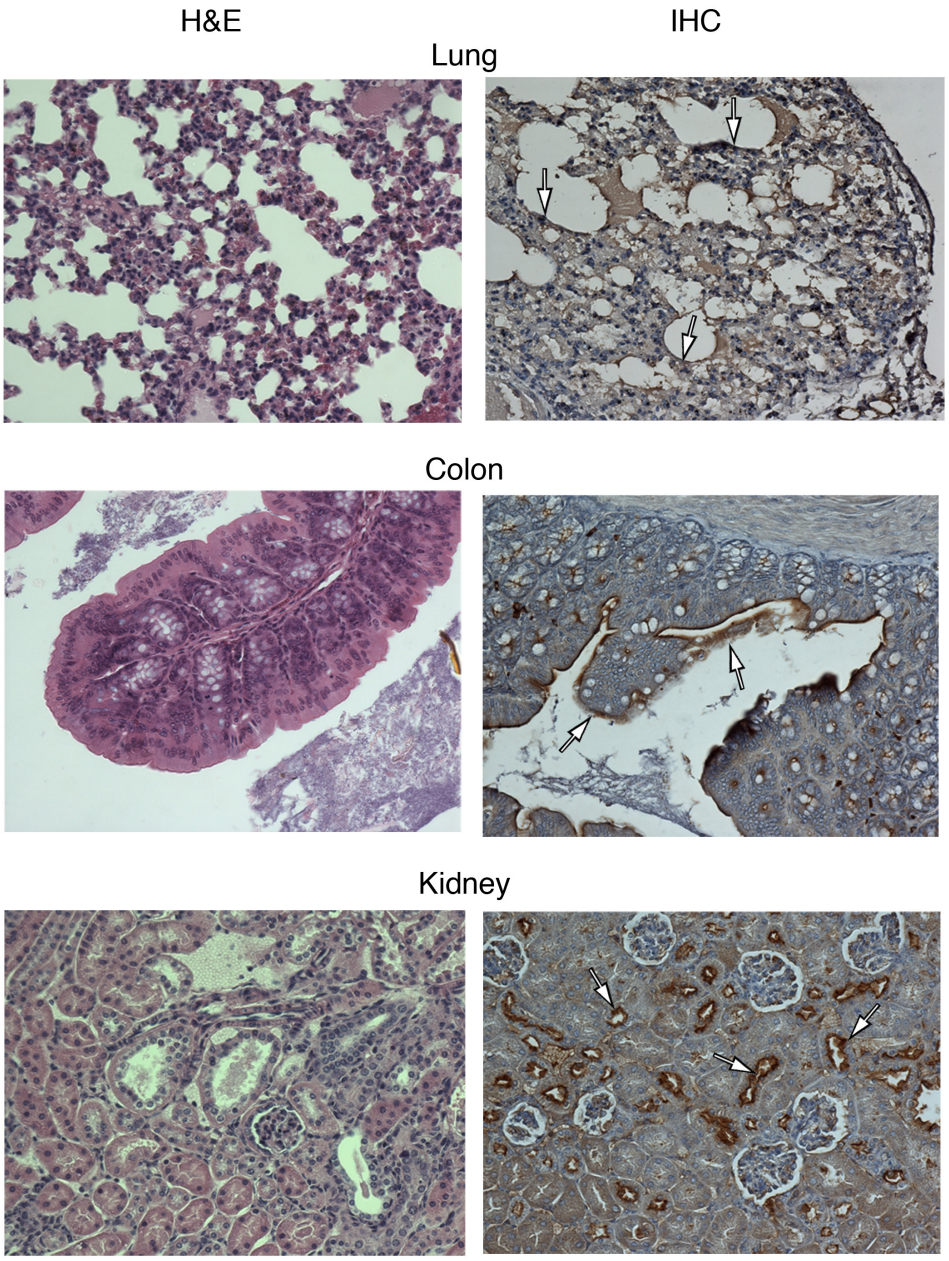

**Supplemental Figure S2. Binding of MAb 3D1 to NSCLC cells. (A-B)** H441 cells (A) and primary NSCLC cells from a resected specimen (B) were incubated with MAb 3D1 (red profile) or control MAb CD1 (blue profile) and analyzed by flow cytometry.



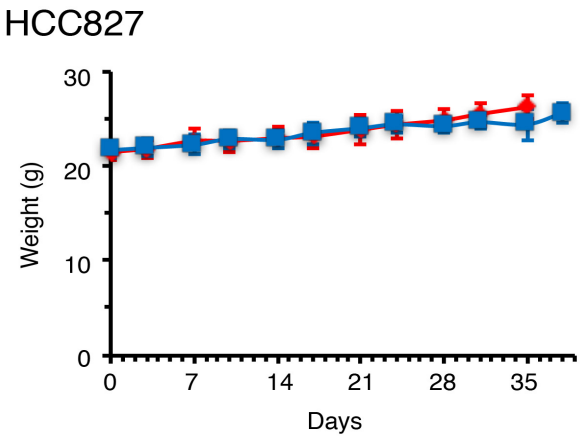

**Supplemental Figure S3. Absence of MAb 3D1 internalization with HCT116/vector cells.** MAb 3D1 labeled with Alexa Fluor-488 was incubated with HCT116/vector cells for 3 h at 37°C. Cells were visualized by phase contrast (left) and fluorescence (right) microscopy.



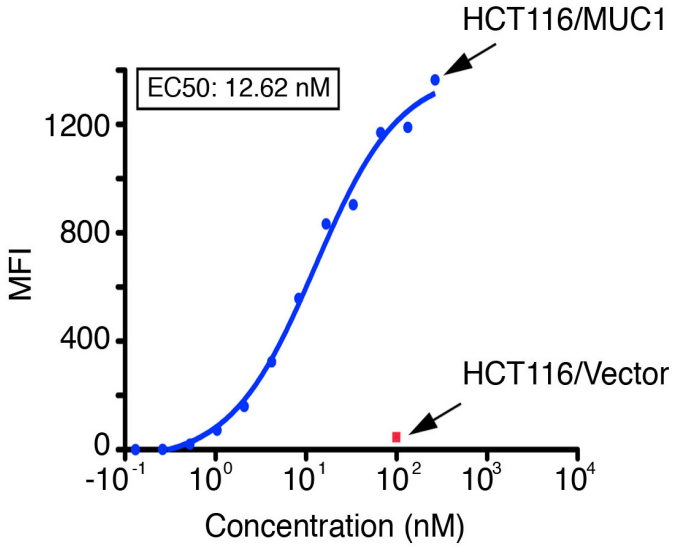

**Supplemental Figure S4. Pharmacokinetics of MAb 3D1.** MAb 3D1 was injected IV to C57BL/6 mice at doses of 5 (blue circles) or 10 (red squares) mg/kg. Blood samples obtained at the indicated times were analyzed for MAb 3D1 levels by ELISA.



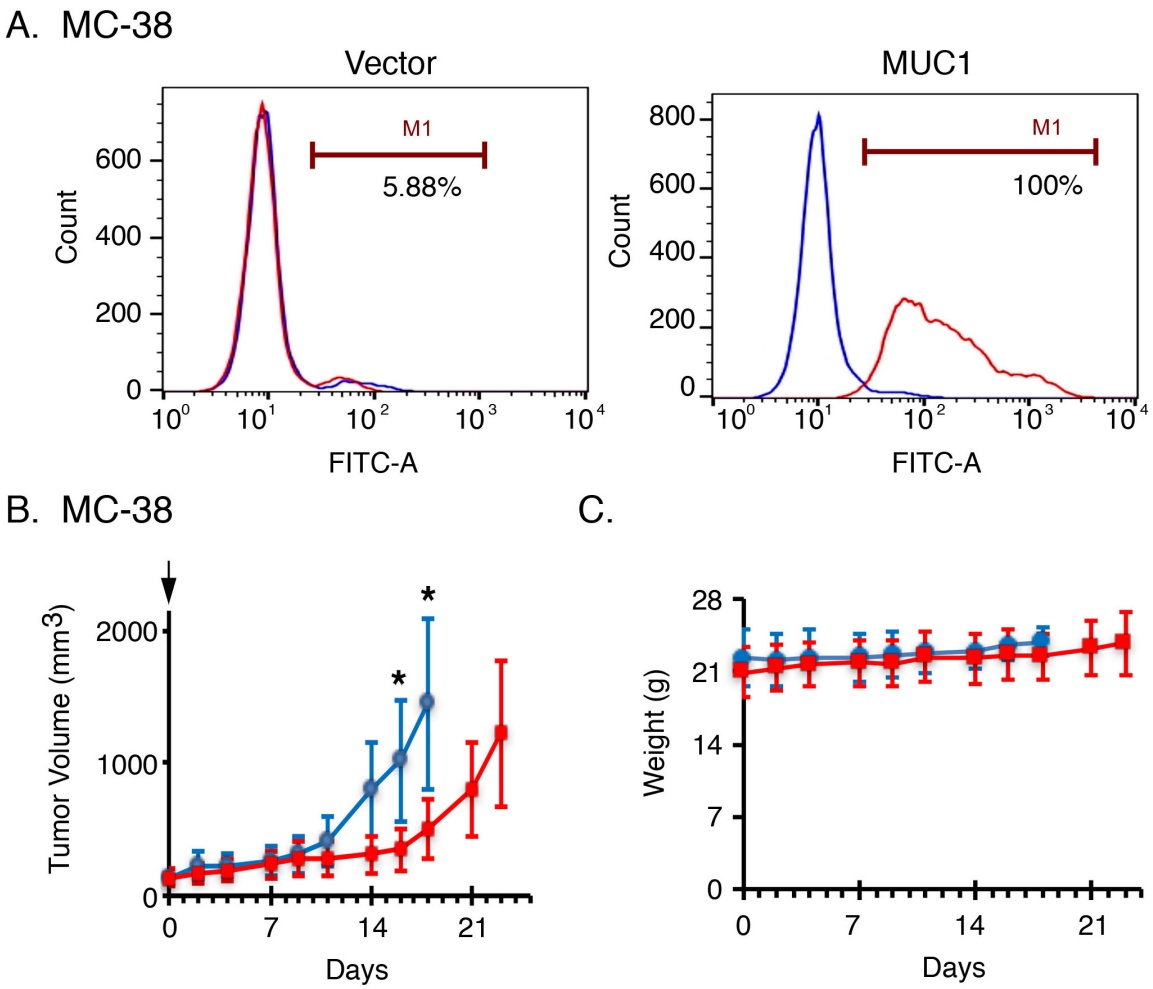

**Supplemental Figure S5. Analysis of tissues from MUC1.Tg mice treated with MAb 3D1-MMAE ADCs.** MAb 3D1-MMAE ADCs were injected IV to MUC1.Tg mice at a dose of 15 mg/kg. On day 7, lung, trachea, heart, thymus, thyroid, stomach, small intestine, colon, liver, pancreas, spleen, ovary, prostate, brain, spinal cord and leg muscle were fixed in Bouin's solution. Tissue sections were stained with H&E and examined by microscopy. There was no observed toxicity to these tissues. Representative H&E sections of the lung, colon and kidney are shown (left). The tissue sections were also evaluated for MUC1-C expression by immunohistochemical staining (IHC) with MAb CD1 reactive with the MUC1-C cytoplasmic domain (right). Arrows denote localization of MUC1-C to the apical borders of epithelial cells lining the lung, colon and kidney collecting ducts.



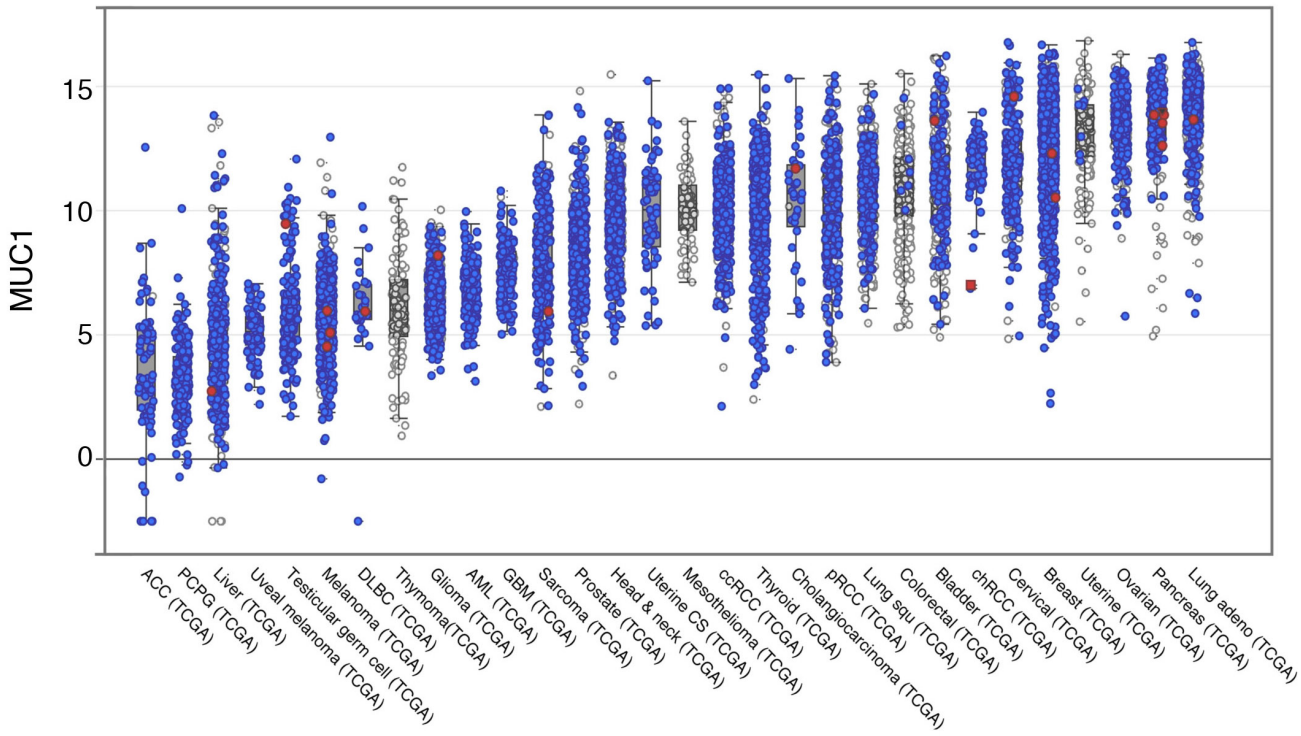

**Supplemental Figure S6. Administration of MAb 3D1-MMAE ADCs has no effect on mouse body weight.** Nude mice bearing HCC827 tumor xenografts (~150 $mm^3$) were pair-matched and then treated with vehicle control (blue squares) or the MAb 3D1-MMAE ADC (red diamonds) at a dose of 5 mg/kg/week x 3 weeks. The results are expressed as body weights (mean±SEM; 6 mice per group).




**Supplemental Figure S7. Selective binding of huMAb 3D1 to MUC1-C-expressing HCT116 cells.** The indicated concentrations of huMAb 3D1 were incubated with HCT116/vector or HCT116/MUC1 cells. Mean fluorescence intensity (MFI) was determined by flow cytometry.




**Supplemental Figure S8. Activity of huMAb 3D1-MMAE ADCs in MUC1.Tg mice harboring syngeneic MC-38/MUC1 tumors.** (**A**) Mouse MC-38/vector and MC-38/MUC1 cells were incubated with huMAb 3D1 (red profiles) or control MAb CD1 (blue profiles) and analyzed by flow cytometry. (**B-C**) MUC1.Tg mice bearing MC-38/MUC1 tumor xenografts (~150 $mm^3$) were pair-matched and then treated with vehicle control (blue circles) or huMAb 3D1-MMAE ADC (red squares) at a single dose of 10 mg/kg. The results (mean±SEM; 6 mice per group) are expressed as tumor volumes (B) body weights (C). Asterisk (*) denotes $p < 0.05$ comparing control and treated groups.




**Supplemental Figure S9. MUC1 overexpression in human cancer as obtained from the cBioPortal for Cancer Genomics database.** Blue dots, no mutations detected in the *MUC1* gene. Red dots, mutations detected in the *MUC1* gene. Gray dots, mutation status of *MUC1* gene is unknown.