Supplementary Figure 1 Amino acid sequences of the scFv of the MUC16CAR used in this study. The sequences for (A) 4H11, (B) K93, (C) K95, and (D) K101 are shown in scFv format. Sequences derived from hybridomas are in bold. The 2nd Generation CAR used throughout the study is in (E).

Supplementary Figure 2 Functional assay against MUC16neg lymphoma cell line BL41. A – CAR-transduced T cells were co-cultured with BL41 at E: T = 1:2 overnight, in the presence of anti-CD107a fluorescent antibody. T cells were gated out and analysed by flow cytometry (Mean ± S.D., One-way ANOVA with multiple comparisons, n=1-2 donors, N=4-5 independent experiments. * $p < 0.05$).

Supplementary Figure 3 CA125 staining of cells grown in 2D and 3D (spheroids). HeLa and OVCAR3 cells were grown in flat bottom wells, or in agarose-coated wells, and allowed to grow in 2- or 3-dimensions, respectively. Cells were then stained with X75 antibody, or isotype controls, and analysed on an IncuCyte. Total integrated intensity was then calculated. Quantification of 2-3 frames per condition from N=1 experiment.

Supplementary Figure 4 Categorisation of patient samples. Patient samples, including samples from debulking surgery, samples of patient ascites ('Effusion s') from **Figure 2B**, and the PDX26 sample used in **Figure 5**, were processed, and stained for CA125, then analysed by flow cytometry. Samples were categorised as positive or negative on the basis of the percentage of cells positive for X75 (minus isotype control). The distribution of samples within each group was plotted and the percentage of positive cells in PDX26 was used as a control.

Supplementary Figure 5 Detection of cleaved CA125. Recombinant CA125 was bound to a nitrocellulose membrane (2 µL drops of the indicated Units/mL). Purified K101 was compared with an equivalent amount of X75 (1/200 dilutions of both). K101 hybridoma supernatant was compared with an irrelevant hybridoma (1/25 dilutions of both). Original blots are shown in (A) and relative staining intensity is also plotted (B). Shedding of CA125 by cell lines was examined using a clinical-grade assay. (C) CA125 was detected in the cell culture media of HeLa and OVCAR3 cells, but not HEK cells. (D) OVCAR3 cells and PDX26 cells, when engrafted into NSG mice, resulted in detectable levels

of CA125 in the serum. (E) Engrafted OVCAR3 cells also produced detectable levels of CA125 in peritoneal washes.

Supplementary Figure 6 Quality control of the T cell prepared for *in vivo* experiments. A – Following transduction, PBMCs were cultured *in vitro*. Shown here is the fold expansion (relative to starting cell number) over 10 days, prior to injection into mice. B – Same cells as in (A) tested for CAR presence. After ten days of expansion, expression of CAR constructs was confirmed by staining with anti-mFab. N=3 donors, colour code indicates the transduced construct.

Supplementary Figure 7 PDX26 are MUC16^{pos}. Staining using anti-MUC16 X75 antibody. Background control was set using the secondary antibody only.