



Supplemental Figure 6: Generation FR $\alpha$ -targeted FR-B BiTE for use in preclinical mouse models and therapeutic testing of FR-B T cells: **A**) Graphical depiction of FR-B retroviral construct configuration **B**) Specific binding of FR-B to IE9-mp1-hFR $\alpha$  cells and mouse CD8 $^+$  T cells. FR-Bh (which lacks mouse CD3 binding), Control Supernatant (Cont Sup) containing no BiTEs, and CD19 $^+$  B cells were included as staining controls. **C**) Experimental Design (left), tumor growth (middle), and survival of Pan02-hFR $\alpha$  tumor bearing mice treated locoregionally with  $3 \times 10^6$  FR-B T cells or Unarmed Control T cells (2 doses) by SQ injection ( $n=6$ /group). **D**) Mice were lymphodepleted by delivery of 5Gy TBI immediately prior to IE9-mp1-hFR $\alpha$  tumor implantation, with delivery of  $2.4 \times 10^6$  FR-B or Unarmed T cells 5 days later. Tumor progression was tracked based on accumulation of peritoneal ascites, measured as increased abdominal circumference ( $n=4-5$ /group). **E**) Representative FACs plots demonstrating limited persistence of FR-B CD8 $^+$  or CD4 $^+$  T cells in either the tumor or ascites at disease endpoint. Data presented as mean  $\pm$  SEM. Data in C) is from one experiment and data in D) is from one representative experiment (two independent studies). C) Two-way ANOVA (left) and Log-rank Test (right), D) Two-way ANOVA, \*\*\*\*  $p < 0.0001$