Supplemental Methods

Cell Culture Conditions

Tumor cells and T cells were cultured in RPMI 1640 (Gibco 11875-085) supplemented with 10% heat inactivated fetal calf serum (FCS), 100U/ml penicillin, 100ug/ml streptomycin sulfate, and 1% L-glutamine.

Isolation, bead activation, transduction, and expansion of primary human T lymphocytes

Primary human $CD4^+$ T and $CD8^+$ T cells were isolated from healthy volunteer donors following leukapheresis by negative selection using RosetteSep kits (Stem Cell Technologies). All specimens were collected under a University Institutional Review Board-approved protocol. CD4+ and CD8+ T cells were mixed at a 1:1 ratio and were cultured in RPMI 1640 supplemented with 10% FCS, 100-U/ml penicillin, 100ug/ml streptomycin sulfate and stimulated with magnetic beads coated with anti-CD3/anti-CD28 at a 1:3 cell to bead ratio without the addition of exogenous IL-2. T cells were transduced with lentiviral vectors at an MOI of approximately 5. Cells were counted and fed with full RPMI media every 2 days and once T cells appeared to become quiescent, as determined by both decreased growth kinetics and cell size, they were used either for functional assays or cryopreserved.

T cell Effector Assays

Effector T cells were cocultured with firefly luciferase expressing tumor cells at different ratios for a specified period time. At the end of the co-culture incubation period,

supernatant was saved for IFNγ levels by ELISA (Biolegend #430106), wells were washed, and remaining tumor cells were lysed with 1X cell lysis buffer for 30 minutes. The luciferase activity in the lysates was analyzed using the Luciferase Assay System on a GloMax Multi Detection System (Promega.) Results are reported as percent killing based on luciferase activity in wells with tumor, but no T cells. (% killing = 100-((RLU from well with effector and target cell coculture)/(RLU from well with target cells)x100)). Effector-to-target ratios represent total T cells per target cell.

Antibodies

The following conjugated antibodies were purchased from BD Biosciences: mouse IgG1 isotype, goat anti-mouse Ig (PE Cy5.5), anti-CD45 (PE, PE-Cy5.5), anti-CD8 (PERCP, AF650), anti-IFNγ (APC), anti-IL2 (Pacific Blue), anti-PD1 (PE-Cy7), anti-2B4 (PE-Cy5), anti-Lag3 (APC). The following antibodies were purchased from Cell Signaling: anti-myc (AF647), anti-phosphorylated ERK (Thr202/Tyr204). The following antibodies were purchased from Novus Biologicals: anti-DGKα. The anti-human mesothelin antibody CAK1 was purchased from Signet Laboratories. The biotinylated $F(ab')_2$ fragment of goat anti-mouse IgG sera (specific for scFvs of murine origin) was purchased from Jackson ImmunoResearch. Streptavidin (PE/FITC) were purchased from BD Biosciences. The primary antibody for DGKa was purchased from Novus Biologicals (Littleton, CO). The primary antibody for DGKz was purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas.)

Intracellular IFNγ expression

TILs were incubated in 15 IU/ml IL-2 supplemented R10 with and without 0.1ug/ml phorbol 12-myristate-13-acetate/2ug/ml ionomycin (PMA/I) in the presence of 40ug/ml of brefeldin A for 4 hours at 37^{o}C, 5%CO2. After incubation, cells were washed once in PBS/1% FBS and centrifuged and stained for CD4 and CD8. The cells were then permeabilized and stained for intracellular IFNγ-APC (eBioscience # 17-7319) They were then analyzed gating on CD4 and/or CD8 expression and further analyzed for cytokine expression using a Boolean gate platform on a FACs Canto. Intracellular IFNγ expression was also measured in similar fashion on the TILs in response to EMMESO tumor and albumin vs. mesothelin labeled beads

Cytokine Production of Restimulated T Cells:

Cryopreserved T cells transduced with with mesoCAR or FAPCAR were thawed, washed, and placed in culture for around 8 to 10 hours. T cells (1x10^{5}) were cocultured with 5000 firefly luciferase expressing tumor cells and supernatants were harvested 18 h later. Concentrations of IFNγ were determined using standard ELISA protocol (Biolegend, 430106).

Mesothelin IHC

To visualize tumor mesothelin expression, animals were killed, and excised tumors were embedded in OCT medium and stained with the CAK1 antibody followed

by biotinylated secondary antibody (goat anti-mouse IgG; Vectastain Elite ABC kit). Signal was localized using 3,3’-diaminobenzidine tetrahydrochloride as the chromogen.