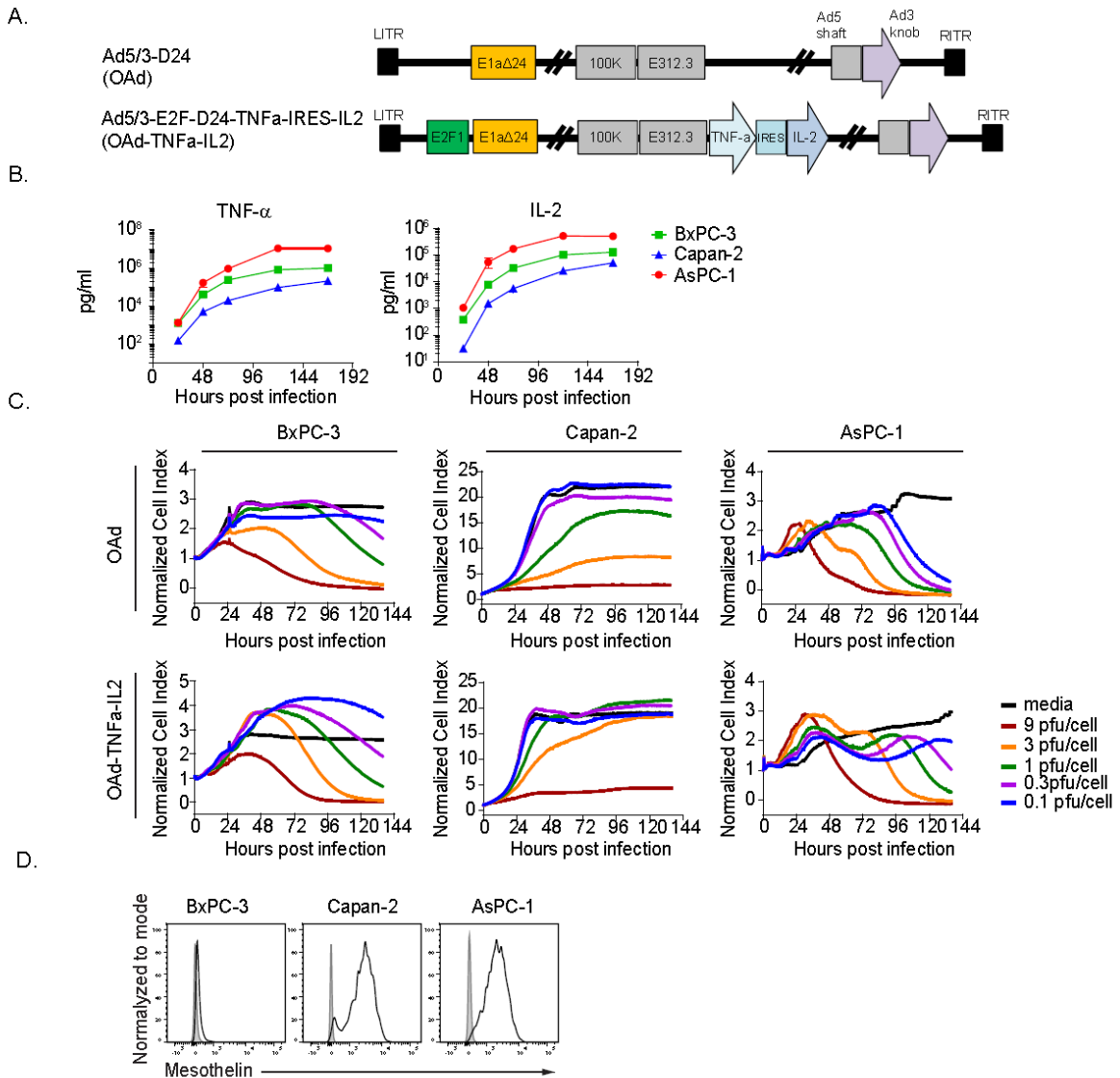


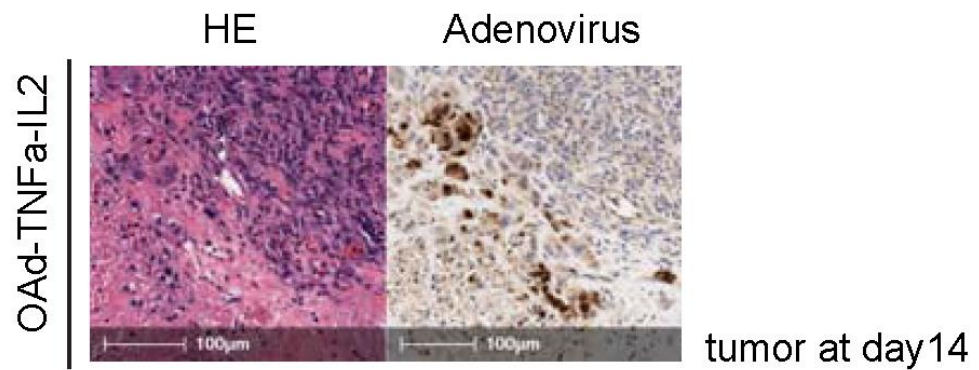
Supplementary figure S1



Supplementary Figure S1. Oncolytic adenovirus (OAd) delivers cytokine genes to pancreatic ductal adenocarcinoma (PDA) tumor cell lines and directly lyses target cells. (A) Schematic representation of oncolytic adenovirus expressing TNF- α and IL-2 (Ad5/3-E2F-D24-TNF α -IRES-IL2 or OAd-TNF α -IL2 for short) and its parental virus (Ad5/3-D24 or OAd for short). LITR, left inverted terminal repeats; RITR, right inverted terminal repeats; 100K, adenovirus 100K assembly protein; IRES, internal ribosome entry site; IL-2, IL-2 transgene; TNF- α , TNF- α transgene. (B) Time course analysis of cytokine production by pancreatic tumor cell lines infected with OAd-TNF α -IL2. Twenty thousand tumor cells were infected with 30 virus particle (vp)/cell of OAd-TNF α -IL2 (total 250 μ l media) and culture supernatant was

harvested at intervals from day 1 to day 7 after virus infection. Concentrations of TNF- α and IL-2 were analyzed by ELISA. Data are representative of two experiments. Means and SD from triplicate wells are shown. (C) Kinetics of tumor cell lysis by oncolytic adenoviruses. Ten thousand PDA targets were infected either with OAd (upper panels) or OAd-TNF α -IL2 (lower panels) at the indicated titers. Cell index over six days was collected with xCELLigence real time cell analyzer. Means of values from triplicate wells are plotted. pfu, plaque forming unit. Data are representative from three experiments. (D) Mesothelin expression by pancreatic cancer cell lines, BxPC-3, Capan-2 and AsPC-1 was analyzed by FCM.

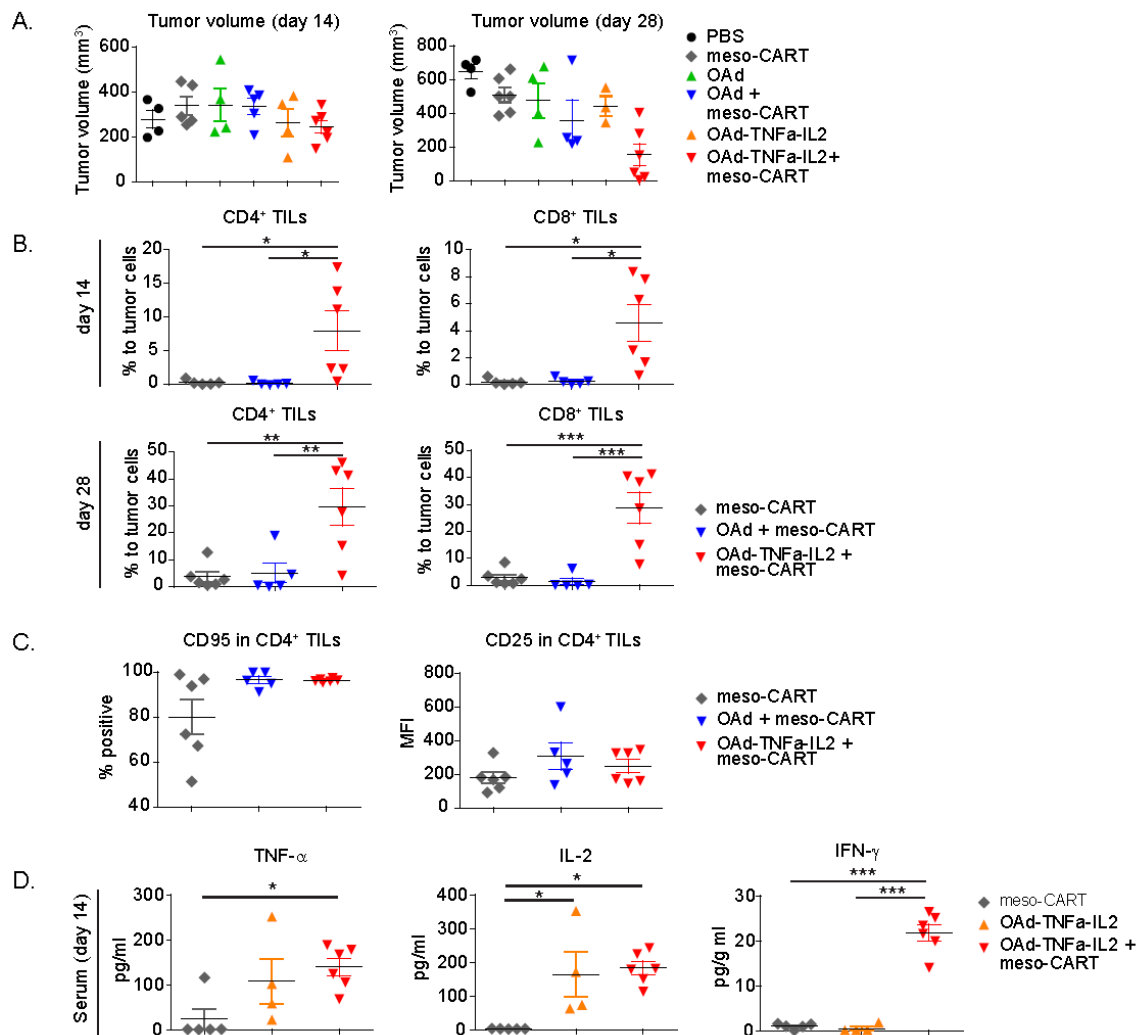
Supplementary figure S2



Supplementary Figure S2. Adenovirus infects AsPC-1 tumors and induces necrosis.

Adenovirus staining on tumors at day 14 after the injection of Oncolytic adenovirus (OAd) expressing TNF- α and IL-2, Ad5/3-E2F-D24-TNF α -IRES-IL2 (Ad5/3-OAd-TNF α -IL2) in an AsPC-1 xenograft NSG mouse model. A representative tumor treated with intratumoral injection of OAd-TNF α -IL2 using the same schedules and procedures as described in figure 2A is shown. Adenovirus positive cells are typically observed between intact tumor area and necrotic tumor area, which indicated that adenoviruses were gradually expanding while inducing tumor necrosis.

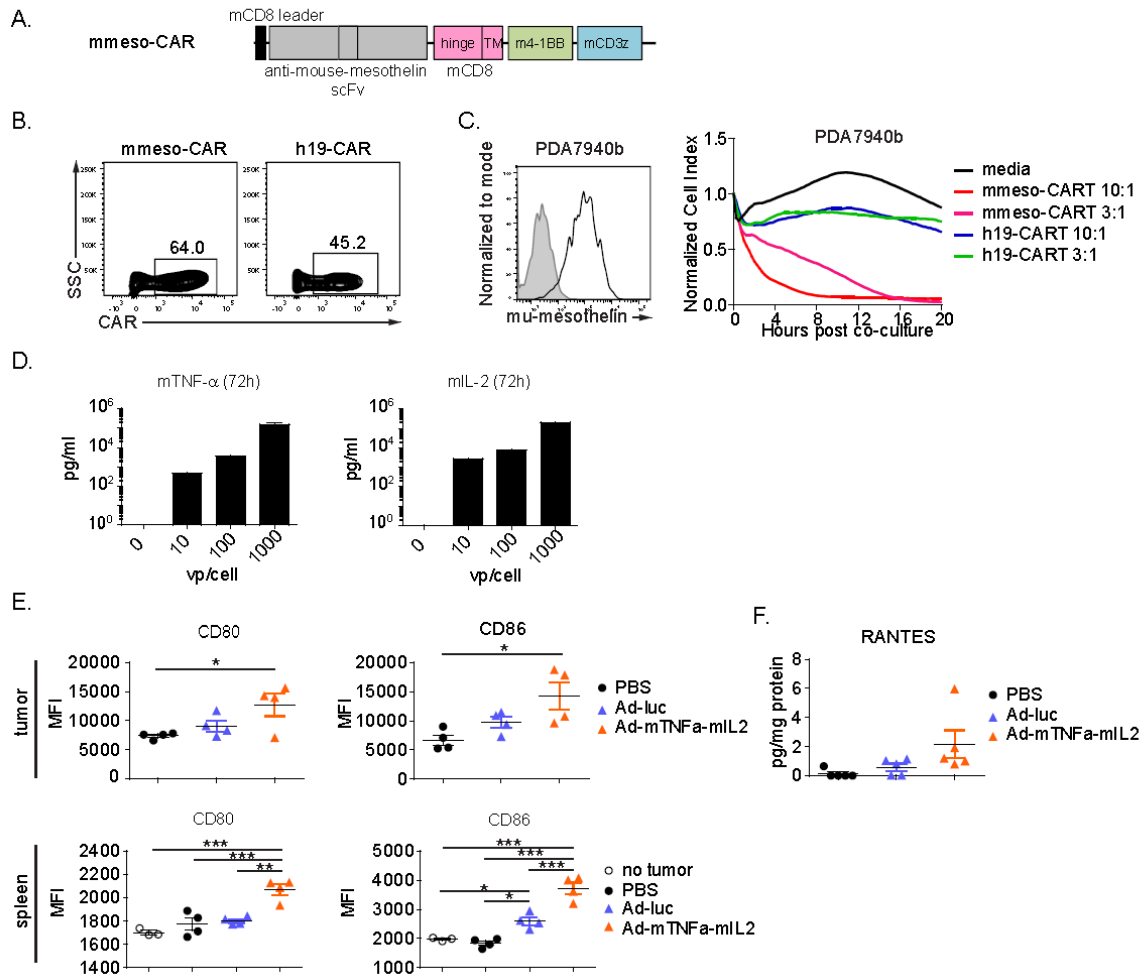
Supplementary figure S3



Supplementary Figure S3. Oncolytic adenovirus (OAd) expressing TNF- α and IL-2, Ad5/3-E2F-D24-TNF α -IRES-IL2 (Ad5/3-OAd-TNF α -IL2) induces robust T cell recruitment and infiltration to tumors and enhances T cell functions. Data are from the experiment shown in main figure 3. (A) Tumor volumes at day 14 and day 28. Tumor volumes by caliper measurements are shown. (B) Number of CD4⁺ and CD8⁺ tumor infiltrating lymphocytes (TILs) at day 14 and day 28. TILs were analyzed by FCM at day 14 and day 28. Number of TILs was normalized to percent CD4⁺ or CD8⁺ cells in total nucleated cells. *, p<0.05; **, p<0.01; ***, p<0.001 by one-way ANOVA with Tukey's post-hoc test. (C) Expression of activation markers on TILs at day 28. T cell activation markers, CD95 and CD25 on CD4⁺ TILs were analyzed by FCM. (D) Cytokine levels in serum at day 14. Indicated human

cytokines in mouse serum were analyzed by high-sensitivity LUMINEX assay. *, $p < 0.05$; ***, $p < 0.001$ by one-way ANOVA with Tukey's post-hoc test. For all scatter grams (A, B, C and D), each dot represents an individual mouse, and bars represent means and SEM.

Supplementary figure S4



Supplementary Figure S4. Development of new mouse mesothelin-redirected CAR T cells (mmeso-CAR T cells) and adenoviruses expressing mouse cytokines (Ad-mTNFa and Ad-mIL2) enabling assessment of the combination therapy of Ad-mTNFa-IL2 with CAR T cells in an immunocompetent setting. (A) Schematic representation of mmeso-CAR expressed using standard gamma retrovirus technology. (B) Surface expression of mmeso-CAR and control h19-CAR on mouse T cells. CAR expression by mouse splenic T cells was analyzed at day 5 after retroviral transduction to express CARs. Data are representative of at least four different T cell preparations. (C) Kinetics of target cell killing by mmeso-CAR T cells and control human CD19-redirected CAR T cells (h19-CAR T cells) by xCELLigence real time cell analyzer. PDA7940b cells expressed high levels of mesothelin (left panel). Five

thousand PDA7940b cells were seeded in the e-plate. After 24 hours incubation, either control media, control h19-CAR T cells or mmeso-CAR T cells were added at the indicated E:T ratio. Cell index was recorded every 20 minutes (right panel). Data are representative of at least four experiments from four different T cell preparations. Means of triplicate wells are shown. (D) Cytokine production of PDA7940b cells infected with Ad-mTNFa-mIL2. Five thousand PDA7940b tumor cells were seeded to a 96 well plate and infected with Ad-mTNFa-mIL2 at the indicated concentrations (total 250 μ l media). Supernatant was harvested at 72 hours after the infection and cytokine levels were analyzed by ELISA. Data are representative of two experiments. Means and SD of triplicate wells are shown. (E) CD80 and CD86 expression by DC at day 1 post intratumoral adenovirus injection. CD80 and CD86 expression on DCs from tumors and spleen were analyzed by FCM. Data are from the experiment shown.