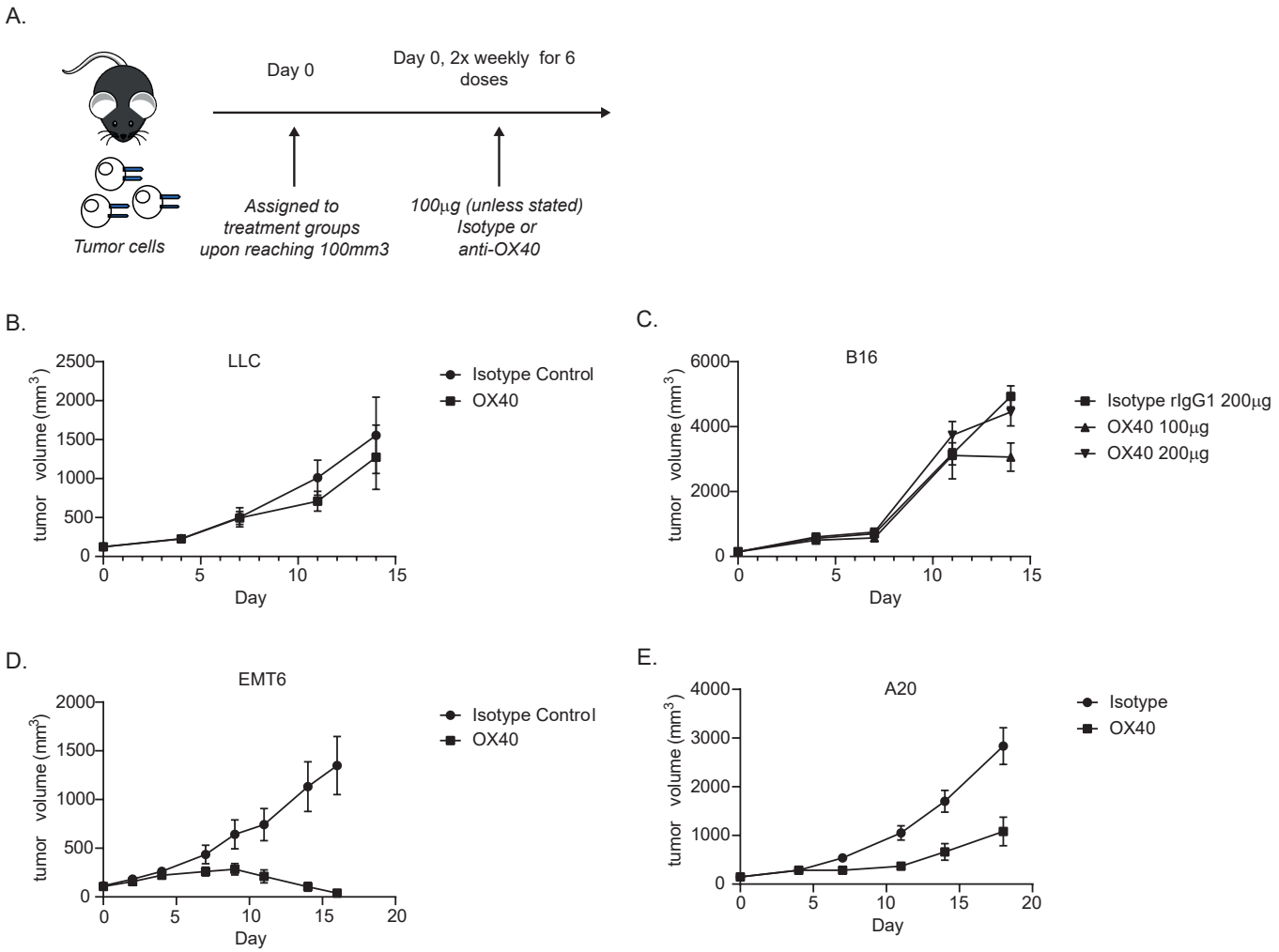
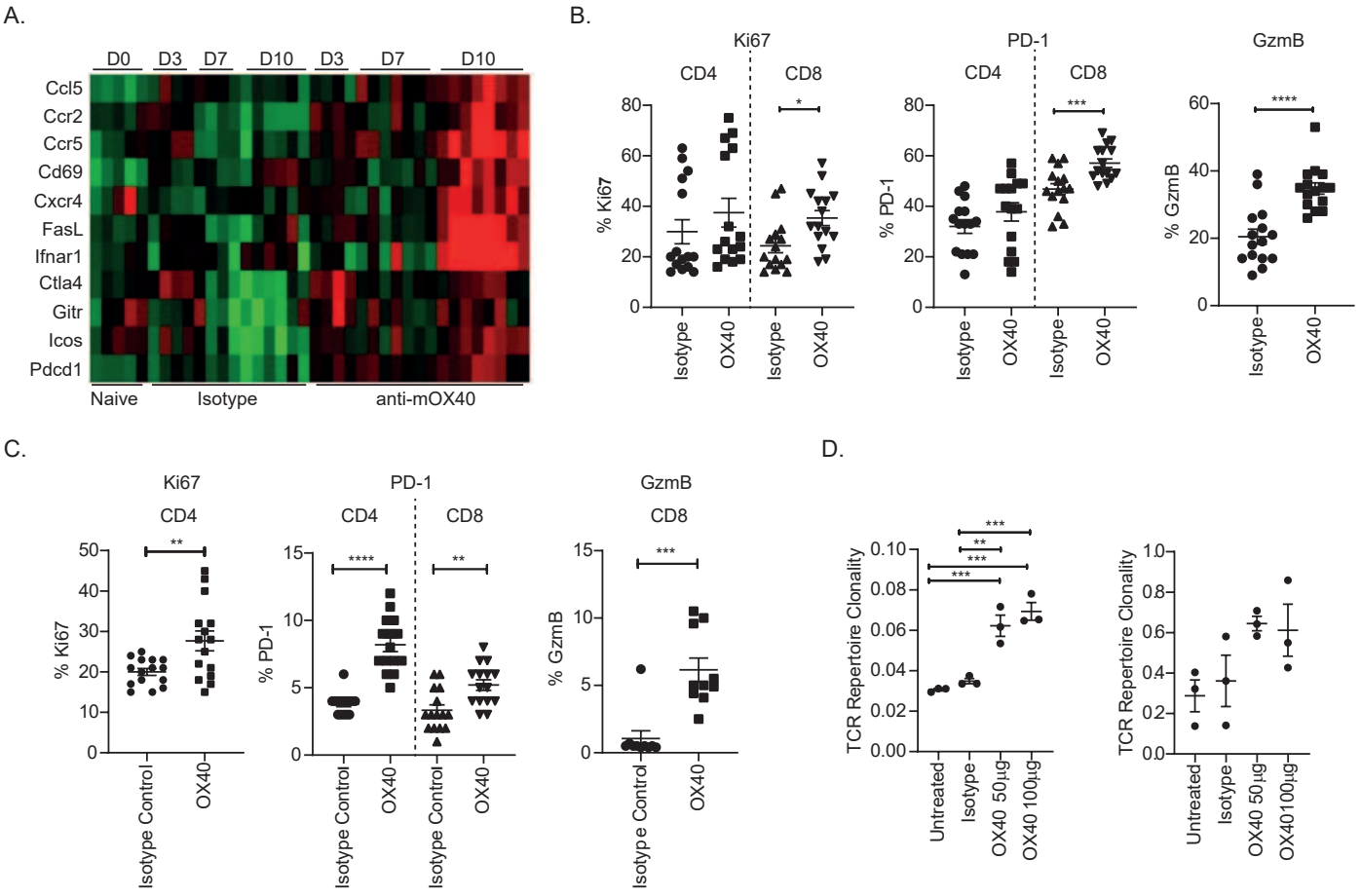
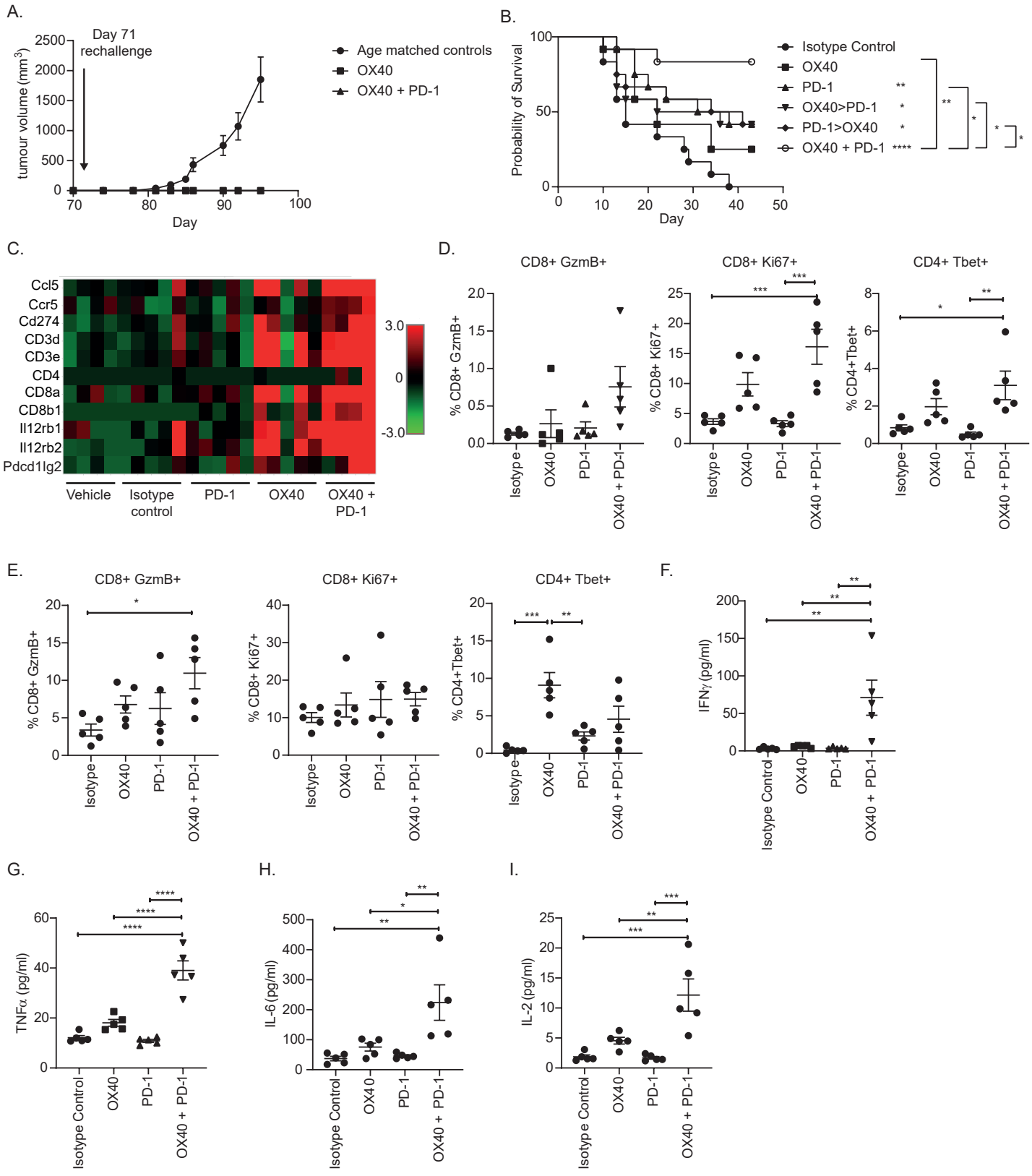


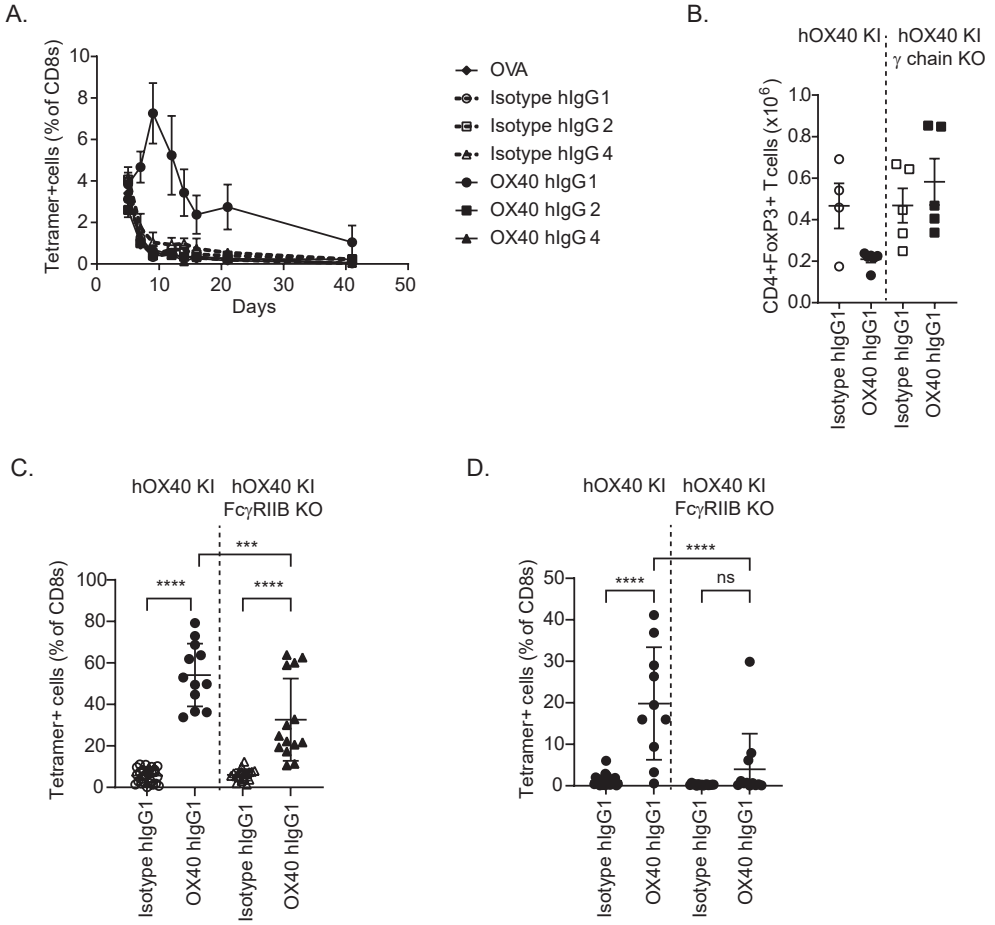
Supplemental Fig 1. A. Schematic for experiments shown in B-F. Mice were challenegd with tumour cells, assigned to treatment groups upon reaching 100mm^3 and treated with either isotype control or anti-OX40 mAb. B. Growth curves for mice challenged with LLC (1x10^5 cells) and treated with 100 μg n= 7, one experiment. C. Growth curves of mice challenged with B16F10 (2.5x10^4 cells) and treated with either 100 μg or 200 μg anti-OX40 mAb. n=6 for Isotype rIgG1 and n=7 for both anti-OX40 mAb groups, one experiment. D. Growth curves of EMT6 (1x10^5) and treated with 100 μg anti-OX40 mAb or isotype control. n=10 one experiment. E. Growth curves of mice challenged with A20 (1x10^6) and treated with 100 μg anti-OX40 mAb. n=25 isotype control and n=26 anti-OX40 one experiment.



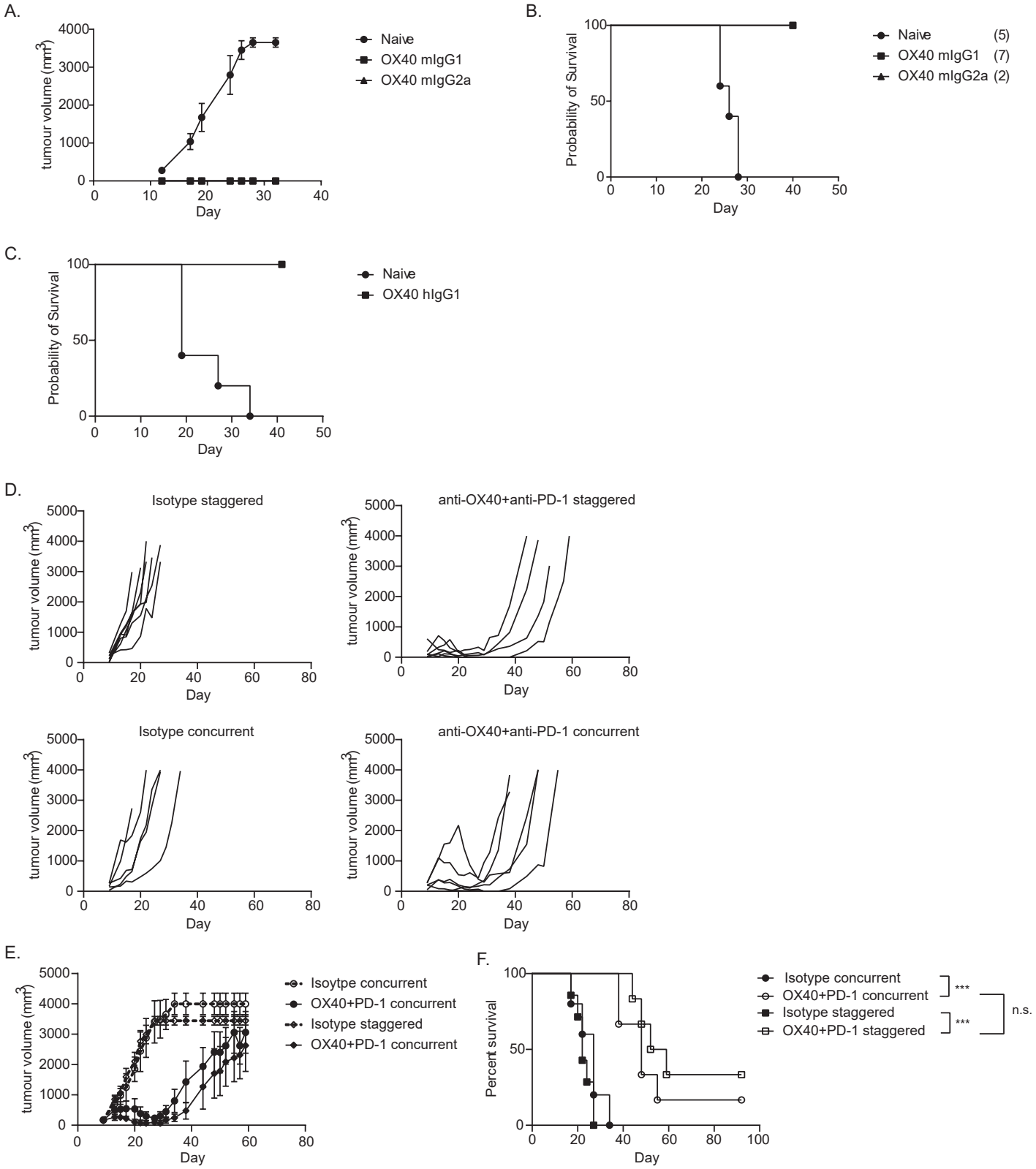
Supplemental Fig. 2. A. Gene expression analysis of CD8+ T cells isolated from A20 tumor bearing mice treated with 100 μg isotype control or anti-OX40 twice weekly. Mice treated as in schematic shown in Supplemental Fig. 1A. Tumor infiltrate (B) and Blood (C) taken from A20 tumor bearing mice on Day 10 post randomisation and treated with isotype control or anti-OX40 (200 μg) and analysed for Ki67 (left panel), PD-1 (middle panel) and GzmB (right panel). B. n = 15, pooled from 3 independent experiments C. n = 15 for Ki67 and PD-1, pooled from 3 independent experiments, n = 10 for GzmB, pooled from 2 independent experiments. D. Mice were challenged with $5x10^4$ CT26 tumor cells and treated with indicated doses of anti-OX40. Spleens (left panel) and tumor (right panel) were harvested 7 days post assignment to treatment groups and assessed for TCR repertoire clonality n=3, one experiment. **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05 Mean +/- sem B & C – unpaired T-Test, D - Tukey's mutliple comparison one way annova



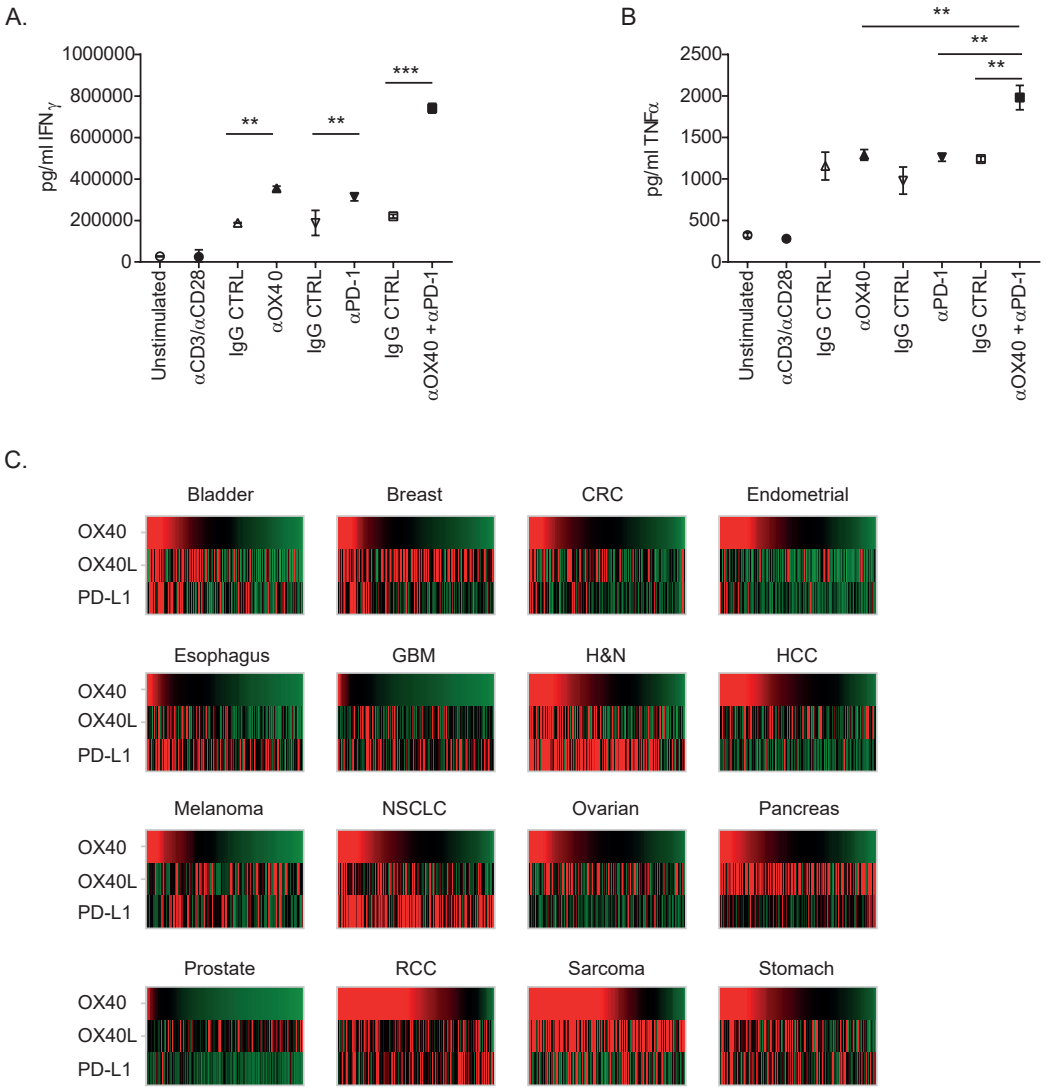
Supplemental Fig 3. Combination treatment with anti-OX40 and anti-PD-1 leads to increase in Th1 cytokines in CT26 tumor model A.Growth curves of naive mice or long term survivors from set up as in Fig 1F. Mice challenged with CT26 cells ($5x10^4$) on Day 71 n=10 age matched controls, n=2 anti-OX40 mAb monotherapy and n=14 anti-OX40 + anti-PD-1 mAb, one experiment. B. Survival curves of mice challenged with CT26 ($5x10^4$) and treated with 6 doses of Isotype combination (rIgG1 100 μg + rIgG2a 200 μg), anti-OX40 mAb (100 μg), anti-PD-1 mAb (200 μg) or combination given concurrently (anti-OX40 + anti-PD-1) or sequentially either as anti-OX40 mAb first (anti-OX40>anti-PD-1 mAb) or anti-PD-1 mAb first (anti-PD-1>anti-OX40 mAb) n=12, one experiment. C Gene expression analysis of TILs harvested on Day 7 n= 4 for vehicle and anti-OX40 + anti-PD-1 mAb, n=5 for isotype, anti-OX40 mAb and anti-PD-1 mAb monotherapies. D & E. Blood (D) and tumour (E) samples taken on Day 10 immunophenotyped for CD8+GzmB+ (left panels), CD8+Ki67+ (middle panels) and CD4+Tbet+ (right panels). n=5, one experiment. F-I. Serum from mice challenged as in Fig. 1F were analysed for IFNγ (F), TNFα (G), IL-6 (H) and IL-2 (I). n=5 one experiment. **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05 Mean +/- sem B - Log rank test, D-I Tukeys one-way annova with multiple comparison.



Supplemental Fig 4. anti-OX40 hIgG1 expands OT-I T cells in WT recipients. A. $1x10^5$ hOX40KI$^{+/-}$ OT-I were transferred into WT C57BL/6 recipients and challenged with 5 mg Ova + 100 μg isotype or anti-OX40 mAb. Blood samples were analysed on the indicated days, n=3 representative of 2 independent experiments. B. Splenic analysis of CD4+Foxp3+ T cell numbers on Day 4 post Ova (5 mg) and antibody (100 μg) challenge. Isotype hIgG1 mAb n=4, anti-OX40 hIgG1 mAb n=6, γ chain KO Isotype hIgG1 mAb and anti-OX40 hIgG1 mAb n=5 representative of 2 independent experiments. C. Blood analysis of OT-I T cells on Day 7, Isotype hIgG1 mAb n=16, anti-OX40 hIgG1 mAb n=12 (4 mice excluded due to lack of response), FcγRIIB KO Isotype hIgG1 mAb n=19, FcγRIIB KO anti-OX40 hIgG1 mAb n= 15 (4 mice excluded due to lack of response). Data pooled from 4 independent experiments. D. Blood analysis of OT-I T cells on Day 61, Isotype hIgG1 mAb n=12, anti-OX40 hIgG1 mAb n=10 (2 mice excluded due to a lack of response), FcγRIIB KO Isotype hIgG1 mAb n=15 and anti-OX40 hIgG1 mAb n= 13 (2 mice excluded due to lack of response). Data pooled from 3 independent experiments. **** p<0.0001, *** p<0.01, Mean +/- sem C & D – Tukey's multiple comparison one way ANOVA



Supplemental Fig 5. Anti-OX40 mAb induces protective memory and is augmented with staggered or concurrent treatment with anti-PD-1 mAb. EG.7 Ova rechallenge experiments with mice previously challenged with EG.7 Ova and treated with anti-OX40 mIgG1 and mIgG2a (A & B) and anti-OX40 hIgG1 (C). Mice were re-challenged with $0.5x10^6$ EG.7 Ova, as were Naive mice to act as controls. Naive mice, n=5, mIgG1 n=7 (A & B), mIgG2a n=2 (A & B), hIgG1 n= 2. D-F Mice challenegd with $0.5x10^6$ EG.7 Ova were treated with anti-OX40 hIgG1 and anti-PD-1 mAb either concurrently or with a staggered dosing schedule. Individual growth curves are shown in (D), combined growth curves in (E) and Survival curves are shown in (F). Isotype concurrent n=5, Isotype staggered n=7, OX40+PD1 concurrent n=6 and OX40+PD-1 staggered n=6 *** p<0.001, F - Log rank test.



Supplemental Fig 6. OX40 and PD-1 boost Th1 cytokines in PBMCs from healthy donors. A & B. Treatment of healthy hPBMCs cultured in presence of anti-CD3/anti-CD28 expander beads (1:20 ratio) for 48 hours, then restimulated with anti-OX40, pembrolizumab or both (10μg/ml) in the presence of anti-CD3 beads at a 1:1 ratio for a further 4 days. Supernatants tested for IFNγ (A) and TNFα (B). Representative data shown from one of 5 donors. C. OX40, OX40L and PD-L1 abundance in various tumours (stage IV) using RNA-Seq data from TCGA. Expression is based on reads per Kb per million (RPKM). Mean +/- S.E.M. *** p<0.0003, ** p<0.005 A & B - Unpaired t test.