**Supplementary materials and methods**

*Cell Line Maintenance*

HEK293T-17 cells (ATCC, CRL-11268) were maintained in DMEM (Invitrogen) plus 10% v/v heat inactivated fetal bovine serum (HI FBS, HyClone) and 1 X non-essential amino acids (NEAA, Invitrogen). Jurkat cells (ATCC, TIB-152) were maintained in RPMI 1640 (Invitrogen) plus 10% v/v HI FBS. Jurkat mGITR NFκB cells were maintained in RPMI 1640 supplemented with 10% HI FBS, 5 µg/mL blasticidin (Invitrogen) and 5 µg/mL puromycin (Invitrogen).

*Generation of mGITRL-FP and anti-PD-L1 mIgG1*

DNA fragments encoding a haemagglutinin signal peptide sequence, a mIgG1 or mIgG2a Fc domain, the yeast-derived coiled coil GCN4 pII, and mouse GITRL ECD were synthesized (Geneart) and cloned into pDest12.2oriP (Thermo Fisher). The full amino acid sequence of the mIgG2a version of mGITRL-FP is provided in Fig. S1.

CHO cells, grown in a proprietary chemically defined media similar to CD-CHO™ (Life Technologies Ltd, Paisley, UK), were transfected with the resulting plasmids and cultured for 8 days in shake flasks. Proteins were purified using MabSelect SuRe affinity chromatography (GE Healthcare) followed by size-exclusion chromatography (SEC).

To generate the PD-L1 mIgG1 antibody, rats were immunized with recombinant mPDL1 Fc (R&D systems 1019-B7). Rat lymph node samples were prepared and hybridomas established. Hybridoma supernatants were screened for binding to mPD-L1 protein using a Homogeneous Time Resolved Fluorescence (HTRF) assay and the clone was selected based on its desired specificity. Antibody variable genes were sequenced, the constant domain of the antibody exchanged to mouse IgG1 and expressed using a mammalian cell based system.

*ELISA*

Recombinant mouse GITR-Fc and OX40-Fc (R&D Systems) glycoproteins were coated overnight at 1 µg/mL in PBS onto 96-well plates (Greiner). Plates were washed with PBST (PBS + 0.01 % Tween-20), blocked for 1 hour at room temperature with PBS containing 1 % (w/v) BSA, and washed in PBST again.Twenty-five microlitres of 1 µg/mL mouse GITRL or OX40L-FPs diluted in assay buffer [PBS + 1 % bovine serum albumin (BSA)] was added to wells, and plates were incubated for 2 hours at room temperature. After 3 washes, 25 µL of 1 µg/mL horseradish peroxidase–conjugated anti-mouse Fc antibody (Sigma) diluted in assay buffer was added to each well, and plates were incubated for 1 hour. After incubation, plates were washed 3 times in PBST, 25 µL of tetramethylbenzidine substrate solution (KPL) was added to each well, and plates incubated for 5 minutes. After incubation 15 µL of 0.5 M sulphuric acid stop solution was added to all wells. The optical density at 450 nm was measured using an EnVision plate reader (PerkinElmer).

**Supplementary Figure Legends**

**Fig. S1 - Primary amino acid sequence of mGITRL-FP mIgG2a.**  The key domains (mIgG2a Fc, GCN4 pII and mGITRL ECD domain) are separated by glycine/glycine-serine linkers (underlined).

**Fig. S2 – Intratumoral T-reg depletion**

CT26 tumor bearing mice were injected with either saline control, mGITRL-FP mIgG1 (10 mg/kg) or mGITRL-FP mIgG2a (10 mg/kg) once i.p. at 6 days post CT26 implantation. Flow cytometric plots showing the proportion of CD4+ Foxp3+ T-regs in the tumor 4 days after treatment. CD4+ Foxp3+ flow cytometry analysis gate is positioned based on Foxp3 fluorescence minus one (FMO) control. N = 8 mice per group. Data is one representative of at least three independent experiments.

**Fig. S3 - Binding and potency profile of a mouse OX40 ligand fusion protein.**

(A) Binding ELISA showing that mGITRL-FP mIgG1 and mIgG2a each binds specifically to recombinant mouse GITR-Fc (black bars) and not to recombinant mouse OX40-Fc and that mOX40L-FP mIgG1 and mIgG2a each binds specifically to recombinant mouse OX40 (grey bars) and not recombinant mouse GITR. mOX40L-FP Y182A isotype control binds minimally to recombinant mouse OX40-Fc. N = 8 samples per group. Data is representative of two independent experiments.

(B) Binding of mOX40L-FP mIgG1 (black circles) or Y182A isotype control (open circles) to human OX40 on Jurkat human OX40 NF-κB reporter cell line. Mouse OX40L-FP mIgG1 induced NF‑κB signalling in the reporter assay but this was not evident for the mOX40L-FP Y182A isotype control. Data is one representative of two independent experiments.

**Fig. S4 – Combination of mGITRL-FP mIgG2a and mOX40L-FP mIgG1 induces increased survival of mice bearing B16F10-Luc2 tumors compared to monotherapy treatment.**

B16F10-Luc2 tumor bearing mice were dosed i.p. with saline, 25 mg/kg mGITRL-FP mIgG2a biweekly for two weeks, 15 mg/kg mOX40L-FP mIgG1 bi weekly for three weeks or a combination of both molecules and survival measured. Log Rank test, where \*\*\* indicates a P-value <0.001, \*\* indicates a P-value <0.01 and \* indicates a P-value of <0.05. N=8 mice per group. Data is one representative of two independent experiments.

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