**Supplemental Figure captions:**

**Figure S1. Characterization of anti-PVRIG COM701 binding to PVRIG**

A) Binding of anti-PVRIG (COM701) or IgG isotype control to PVRIG expressing HEK293 cells or parental HEK293 cells is shown. B) A high density anti-human polyclonal antibody capture surface was prepared over all horizontal spots and interspots of a GLC ProteOn chip (Bio Rad) using standard amine coupling. For each cycle, COM701 antibody was captured for 3 minutes at a concentration of 0.5µg/mL and six concentrations of his-tagged monomeric human PVRIG extracellular domain at a range of 482 pM – 117 nM in a 3-fold dilution series were simultaneously injected over the captured COM701 antibody for one minute, followed by 15 minutes of dissociation at a flow rate of 100µL/min. All sensorgram data were zeroed, aligned, and double-referenced using Scrubber P43 software (BioLogic Ltd). Each replicate data set was globally fit with a simple bimolecular kinetic model that included a term for mass transport using Scrubber P43 to estimate the association rate constant, ka, and the dissociation rate constant, kd. The equilibrium dissociation constant KD was calculated with the ratio kd/ka. The average and the 95% confidence interval were calculated for each binding constant from the three replicate measurements.

**Figure S2: Receptor expression kinetics and the effect of CD96, DNAM, TIGIT, and PVRIG blockade on pp65 reactive CD8+ T cells in co-culture with cancer cell lines.**

PBMCs were activated with pp65(495 – 503) peptide, IL-2, and IL-7 for 11 days. A) Co-expression of PVRIG with TIGIT or PD-1 on day 11 activated pp65(495 – 503) specific CD8+ T cells from a representative donor is shown. B) Expression of PVRIG,TIGIT, PD-1, CD96, and DNAM-1 on pp65(495 – 503) specific CD8+ T at day 0, day 3, day 5, day 7 and day 11 of activation is shown. Additionally, co-expression kinetics of PVRIG with TIGIT or PD-1 is depicted. Data shown are average + SEM of 3 donors. C) Day 11 activated pp65(495 – 503) specific CD8+ T cells were co-cultured with 0.03 g/ml pp65(495 – 503) peptide loaded Mel-624 and Panc.05.04 cells for 18hr in the presence of 10 g/ml of the indicated antibodies. D) Expression of PVRIG on activated pp65(495 – 503) specific CD8+ T cells from 10 donors is shown. pp65(495 – 503) reactive T cells from 3 donors of varying PVRIG expression (high, medium and low) were co-cultured with 0.03 g/ml pp65(495 – 503) peptide loaded Panc.05.04 cells for 18hr in the presence of 10 g/ml anti-PVRIG or isotype. E) pp65(495 – 503) reactive T cells were co-cultured with 0.03 g/ml pp65(495 – 503) peptide loaded Panc.05.04 cells for 18hr in the presence of 10 µg/ml anti-PVRIG, anti-TIGIT, anti-PVRIG and anti-TIGIT, or isotype control. F) Expression of DNAM-1 (filled) relative to IgG (line) on activated pp65(495 – 503) specific CD8+ T cells is shown. G, H) pp65(495 – 503) reactive T cells were co-cultured with 0.03 µg/ml pp65(495 – 503) peptide loaded Panc.05.04 cells for 18hr in the presence of 10 g/ml anti-PVRIG, anti-DNAM-1, anti-PVRIG and anti-DNAM-1 in combination, or isotype control. I) 10 g/ml anti-CD3 (OKT3) and 10 g/ml of anti-PVRIG was coated overnight at 4°C on non-tissue culture treated flat bottom plates. 100,000 resting CFSE labeled CD3+ T cells were added to each well and incubated for 3 days. After 3 days co-culture, CD4+ and CD8+ T cells were gated and % CFSElo proliferated cells were determined. For C, D, E, G, H, bar graphs show the average + standard deviation for cytokine levels.

**Figure S3:**

A) Surface plasmon resonance measurement of human PVRL2 binding to TIGIT captured to the biosensor chip surface is shown. B) pp65(495 – 503) specific T cells from 2 donors were co-cultured with pp65(495 – 503) peptide loaded Mel-624 cells for 18 hours in the presence of 10 µg/ml anti-PVRIG, anti-TIGIT, anti-PVR, anti-PVRL2 or isotype control either alone or in combination. Levels of IFN- were assessed and average + SD for each donor shown. C) pp65(495 – 503) specific T cells from 2 donors were co-cultured with pp65(495 – 503) peptide loaded Panc.05.04 cells for 18 hours in the presence of 10 µg/ml anti-PVRIG, anti-TIGIT, anti-PD-1 or isotype control either in dual or triple combination.  After 18hr, conditioned media were assayed for IFN- secretion. D-G) pp65(495 – 503) reactive T cells were co-cultured with 0.03 g/ml pp65(495 – 503) peptide loaded Panc05.04 cells for 18hr in the presence of 10 g/ml of the indicated antibody. After co-culture, non-adherent cells were removed and stained for TIM3 (D), LAG-3(E), PD-1 (F), and PVRIG (G). Solid line represents isotype condition, dotted line represents anti-PVRIG, and dashed line/dashed-dot line represents anti-TIGIT or anti-PD-1. Data are representative of two donors and n > 3 experiments. H) Surface plasmon resonance imaging analysis was performed using a Continuous Flow Microspotter with an IBIS SPR instrument. A premix assay format was utilized. Briefly, antigen was pre-mixed with a molar excess of each mAb in solution (20nM PVRIG fusion protein mixed with 400nM each anti-PVRIG mAb) and then injected over each mAb covalently immobilized to unique spots in the array. The binding responses of the Ag/mAb analytes were compared to the response for antigen alone injected over each immobilized mAb. A response equal to or greater than the antigen alone response characterized the mAb in solution and the immobilized mAb as binding to two different epitopes on the PVRIG, or an “unblocking” pair of mAbs. No response or a response significantly lower than an antigen only injection characterized the mAb in solution as blocking the immobilized the mAb from binding PVRIG. Bidirectional pair-wise blocking patterns for all 30 mAbs were assessed to determine “bins” (identical blocking patterns) or “communities” (closely related blocking patterns). Hierarchical clustering dendrogram of the binding patterns of anti-PVRIG mAbs as analytes where the scrutiny level is indicated by the dotted blue line. Among the 30 PVRIG antibodies in the epitope binning study, there were 8 apparent “communities” of PVRIG pairwise blocking patterns.

**Figure S4:** **Co-expression of PVRIG, PD-1 and TIGIT on CD8+ T cells.**

A) Analysis of PVRIG,TIGIT, CD96, and PD-1 expression on CD8+ naïve and memory subsets from healthy donor PBMCs are shown. Cell subsets are defined as CD45RA+CD197+(Naïve), CD8+CD45RA-CD197+(CM), CD8+CD45RA+CD197- cells (T EMRA) and CD8+CD45-CD197- cells (EM). On the graph, each dot represents an individual donor and data are average + SEM. Co-expression of PVRIG with TIGIT or PD-1 from a representative donor is shown. Fold expression is defined as the ratio of PVRIG MFI relative to isotype control MFI. B) Expression of PVRIG on NK TILs from dissociated human tumors of various cancer types is shown. Each dot represents a distinct tumor from an individual patient. Average + SEM is shown by the error bars. C) Co-expression of PVRIG with TIGIT or PD-1 is shown on CD4+ and CD8+ TILs from a lung cancer patient. D) Cell surface and intracellular PVRIG expression was assessed on ovarian CD8+ TILs. E) Percentage of CD8+ T cells expressing cell surface PVRIG is shown. F) Percentage of CD8+ T cells expressing intracellular PVRIG is shown. G) Expression of PVRIG on CD4+ and CD8+ T cells immediately after isolation from the tumor (ex vivo) and day 6 post activation with anti-CD3 + anti-CD28 beads. Representative histograms from lung and kidney TILs are shown. H) Gating strategy for receptor and ligand expression on myeloid and lymphoid cells. In order to identify lymphocyte populations, cells were gated based on forward (FSC) and side scatter (SSC) properties. Doublets and dead cells were excluded, using FSC height and amine reactive dye respectively. From this gate, CD3+lin−CD14- cells were gated as T cells, and then further divided into CD4+ and CD8+ T lymphocytes. NK cell populations were defined as CD56+CD3- cells.  CD14+ cells were identified as monocytes/macrophages.

**Figure S5: Characterization of anti-PVRL2 by western blot and FFPE cell pellets**. A) A western blot analysis of anti-PVRL2 was performed on lysates from CHO cells expressing either variant of PVRL2. B) HEK293 cells that endogenously express PVRL2 were transfected with a PVRL2 siRNA or a scrambled control siRNA and cells lysates generated. Anti-PVRL2 was tested on these cell lysates by western blot analysis. C) CHO-S PVRL2 and control CHO-S EV cells were fixed with formalin, paraffin embedded, and sections stained with anti-PVRL2. D) A panel of human cells lines was formalin fixed, paraffin embedded, and stained with anti-PVRL2. E) PVRL2 RNA transcript levels are shown in multiple cell lines. Data are from CCLE. F) Representative images for each PVRL2 score are shown. G) Hematoxylin and eosin staining and anti-PVRL2 staining on tumor and immune cells from a representative donor is shown.

**Figure S6: Co-expression of PVRL2 and PD-L1 in cancer tissues.** Based on PD-L1 staining, lung, colon, breast, ovarian and renal tumors were categorized as PD-L1- (no membranous staining of PD-L1 observed in either duplicate cores for each tumor) or PD-L1+ (positive staining observed in both duplicate cores for each tumor). A) Tumors were grouped by cancer type and by PD-L1 status. PVRL2 expression is shown on the y-axis. B) Expression of PD-L1, PVR and PVRL2 (shaded) on Panc.05.04 and HT-29 cells treated with IFN-y or media alone is shown. Line represents isotype. C) Freshly isolated CD3+ TILS from several tumor types obtained within 24 hours of surgical resection were cultured either alone (TILs), with Mel-624 (TILs+Mel-624) or with Mel-624 OKT3 (TILs+Mel-624 OKT3), a modified Mel-624 tumor cell line expressing surface bound anti-CD3 scFv (OKT3). TILs+Mel-624 OKT3 cells were cultured in the presence of isotype control antibody (IgG). After 24-48 hrs, conditioned media was assessed for IFN- and each line represents a single patient.

**Figure S7: Statistical tests for data in Figure 1, 2, and 6.**

For each donor, Student’s t-test was performed on data from Figure 1, 2, and 6. p values are reported in the tables.