

Figure S1

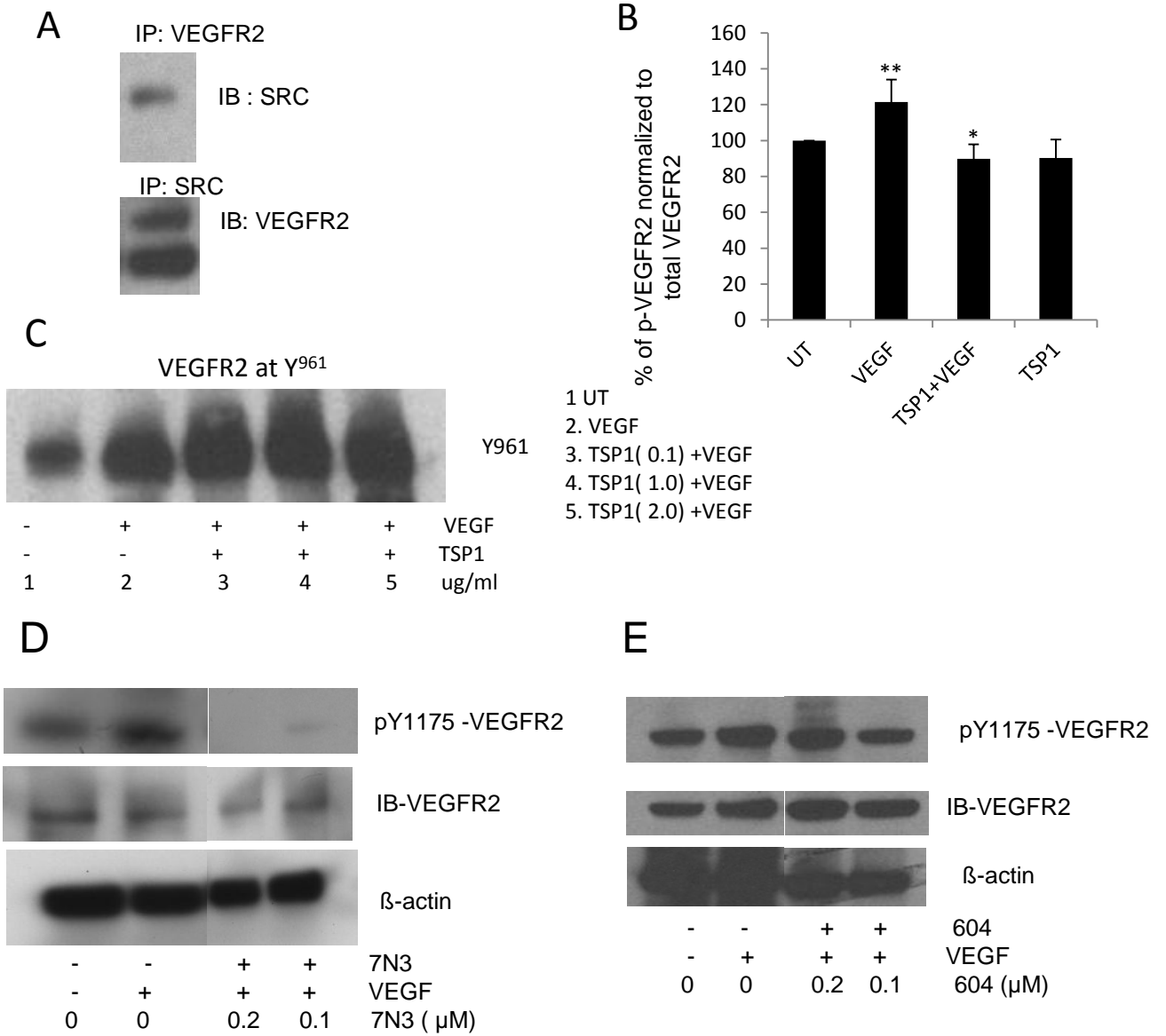


Fig S1. A) Immunoprecipitation of VEGFR2 and SRC was performed and western blot was done using either SRC or VEGFR2 antibodies. B) Quantification of p-VEGFR2 normalized to total VEGFR2 and analyzed using two sample t-Test assuming equal variances. C) Analysis of VEGFR2 phosphorylation at Y-961. D and E), VEGFR2 phosphorylation using the TSP1 derived CD47-binding peptide 7N3 (FIRVVMYEGKK) and the inactive control peptide 604 (FIRGGMYEGKK).

Figure S2:

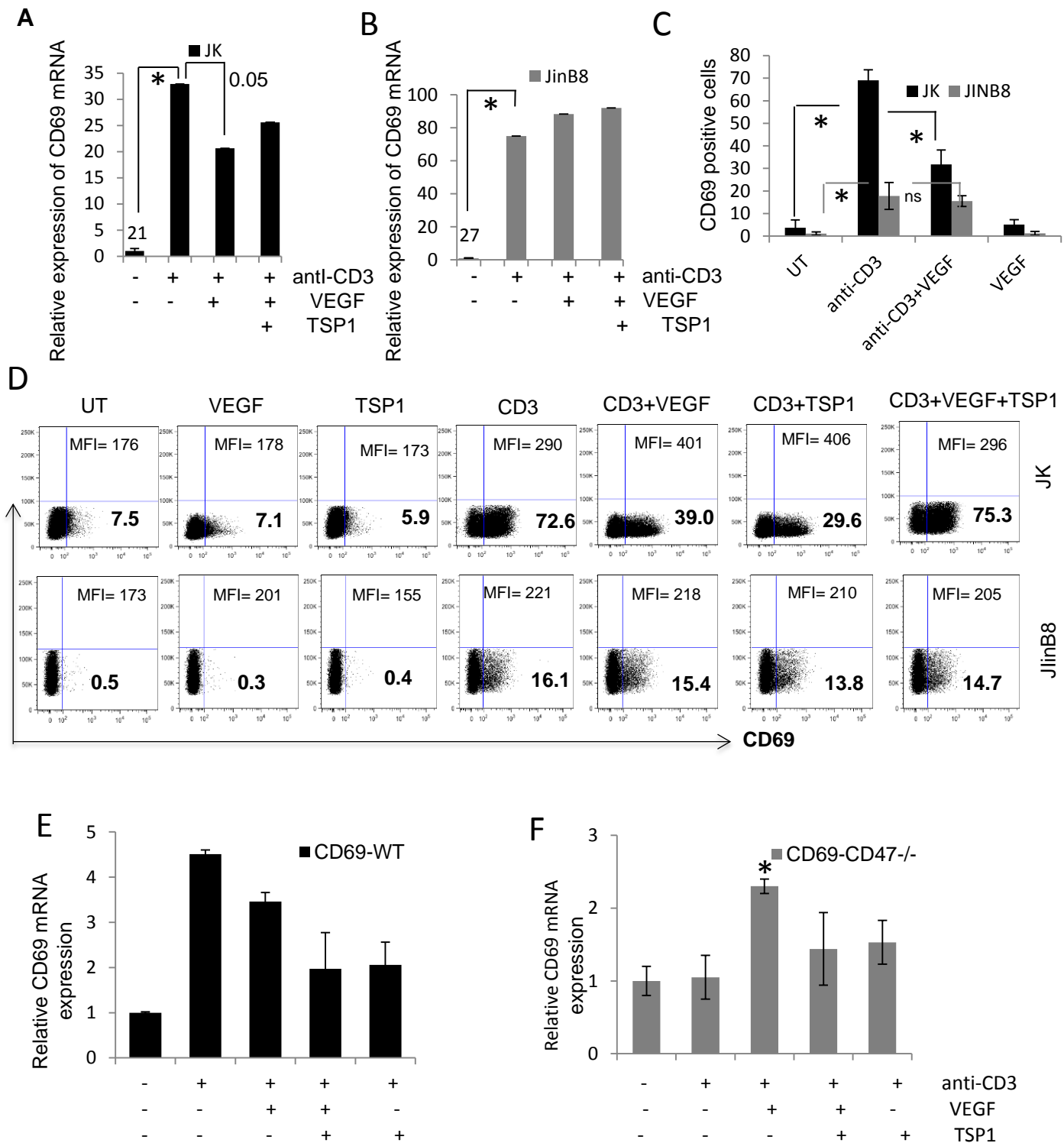


Fig. S2 . Induction of CD69 mRNA in Jurkat (A) and JinB8 (B) T cells plated on immobilized anti-CD3 in the presence of VEGF (30 ng/ml) and/or TSP1 (2.0 nM) for 6h. (C) Quantification of CD69 cell surface expression on WT or CD47-deficient Jurkat T cells plated on anti-CD3 antibody coated plates in the presence or absence of VEGF for 6 h. (D) CD69 cell surface expression on WT or CD47-deficient Jurkat T cells plated on anti-CD3 and anti-CD28 antibodies-coated plates in the presence or absence of VEGF and/or TSP1 for 6 h. (E and F) Induction of CD69 mRNA in WT and CD47-null CD4⁺ murine T cells plated on immobilized anti-CD3 in the presence of VEGF (30 ng/ml) and/or TSP1 (2.0 nM). Note that the Jurkat and JinB8 or WT and CD47^{-/-} T cells (UT) have different basal Ct levels. Two factor ANOVA with replication was used for statistical analysis.

Figure S3:

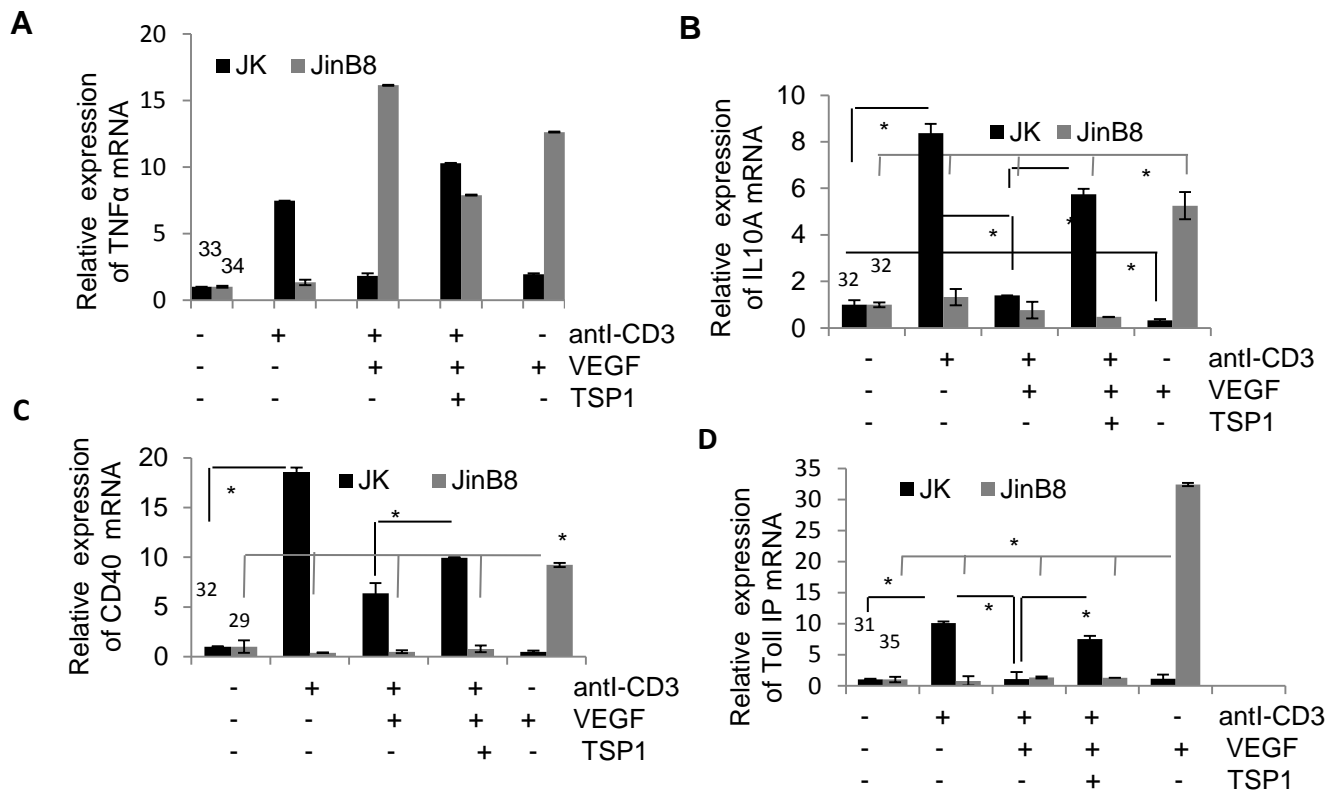


Fig. S3 . TNF α (A), IL10 A (B) , CD40 (C) and Toll IP (D) mRNA levels in cells incubated on anti-CD3 antibody-coated plates for 6 h in the presence or absence of VEGF and TSP1 as indicated. All mRNA expression is presented normalized to 1 for the respective untreated controls, and the raw Ct values for those controls are indicated in each panel. Two factor ANOVA with replication was used for statistical analysis

Figure S4:

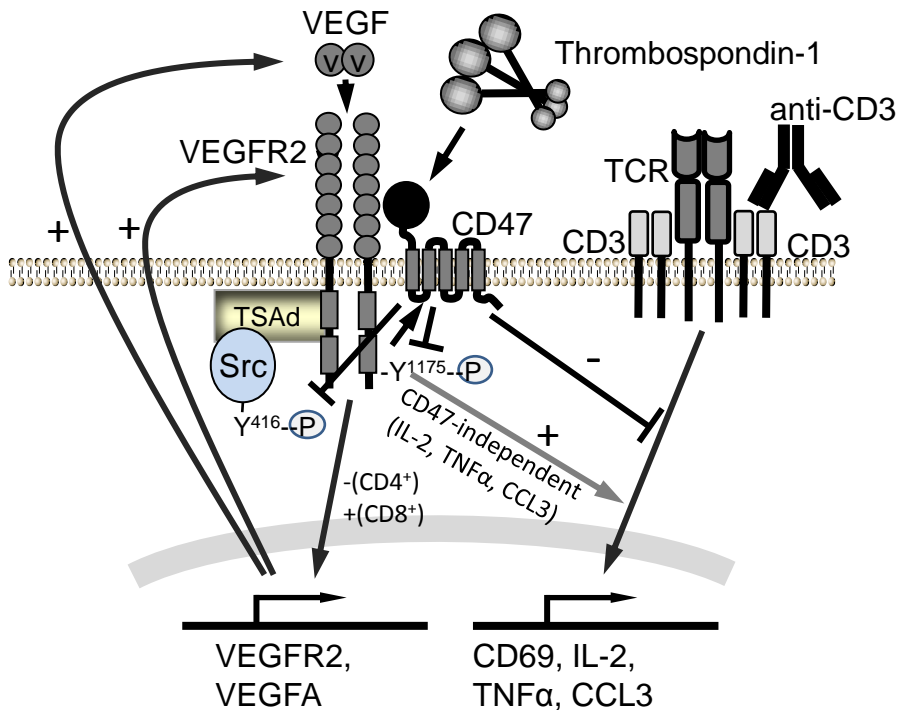


Fig.S4. Model for thrombospondin-1 and VEGF cross-talk with TCR signaling in CD4⁺ T cells. CD47 constitutively associates with VEGFR2 and limits phosphorylation of VEGFR2 and its downstream target Src in response to VEGF binding. Binding of TSP1 to CD47 further inhibits these signals. CD47 signaling also limits VEGF and VEGFR2 mRNA and protein expression, creating a feedback loop. The VEGFR2/CD47 complex inhibits TCR signaling to induce CD69, IL-2, TNF α , and CCL3. However, deletion of CD47 uncovers a CD47-independent pathway through which VEGFR2 signaling positively regulates some of the same T cell activation markers.