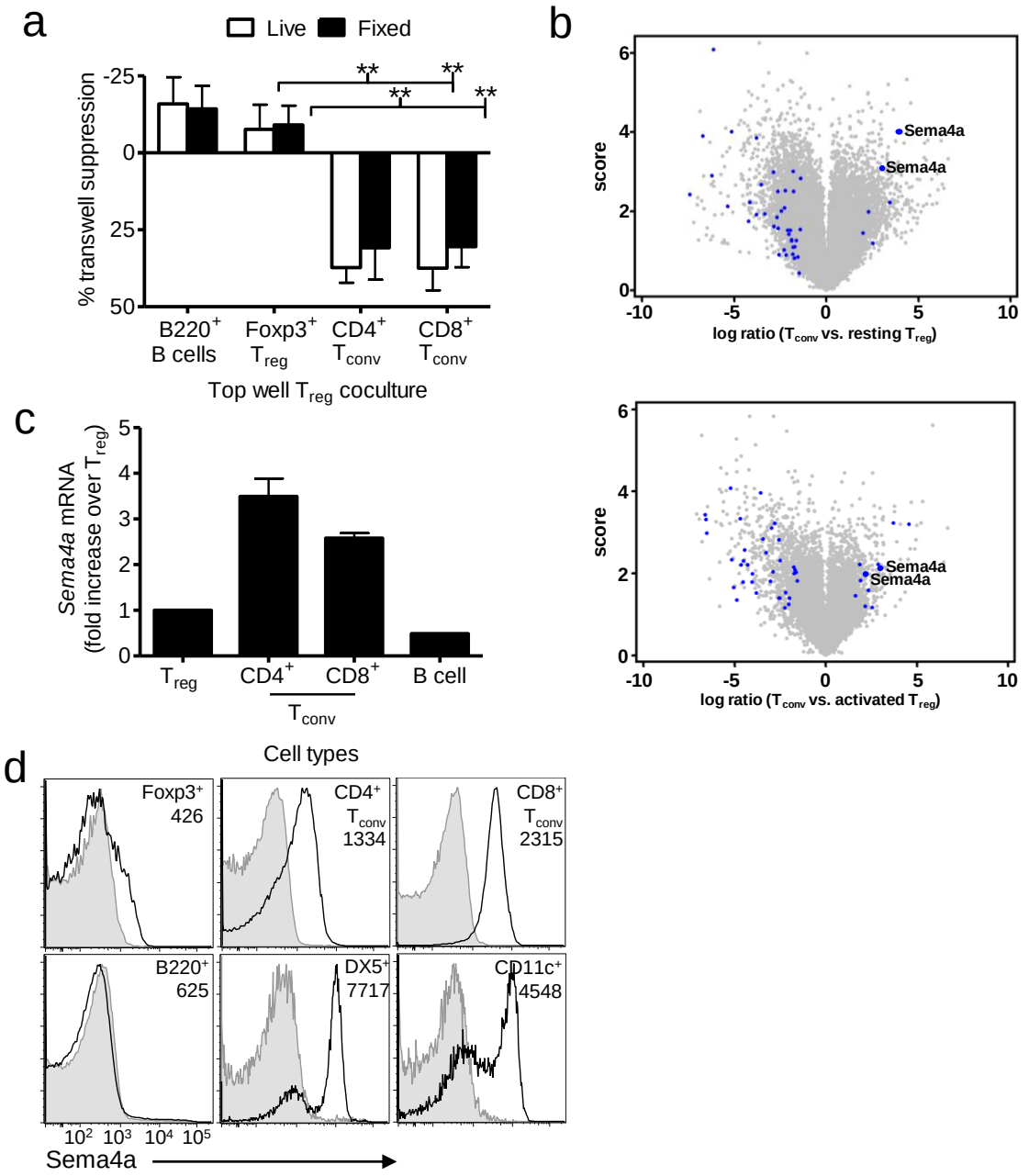
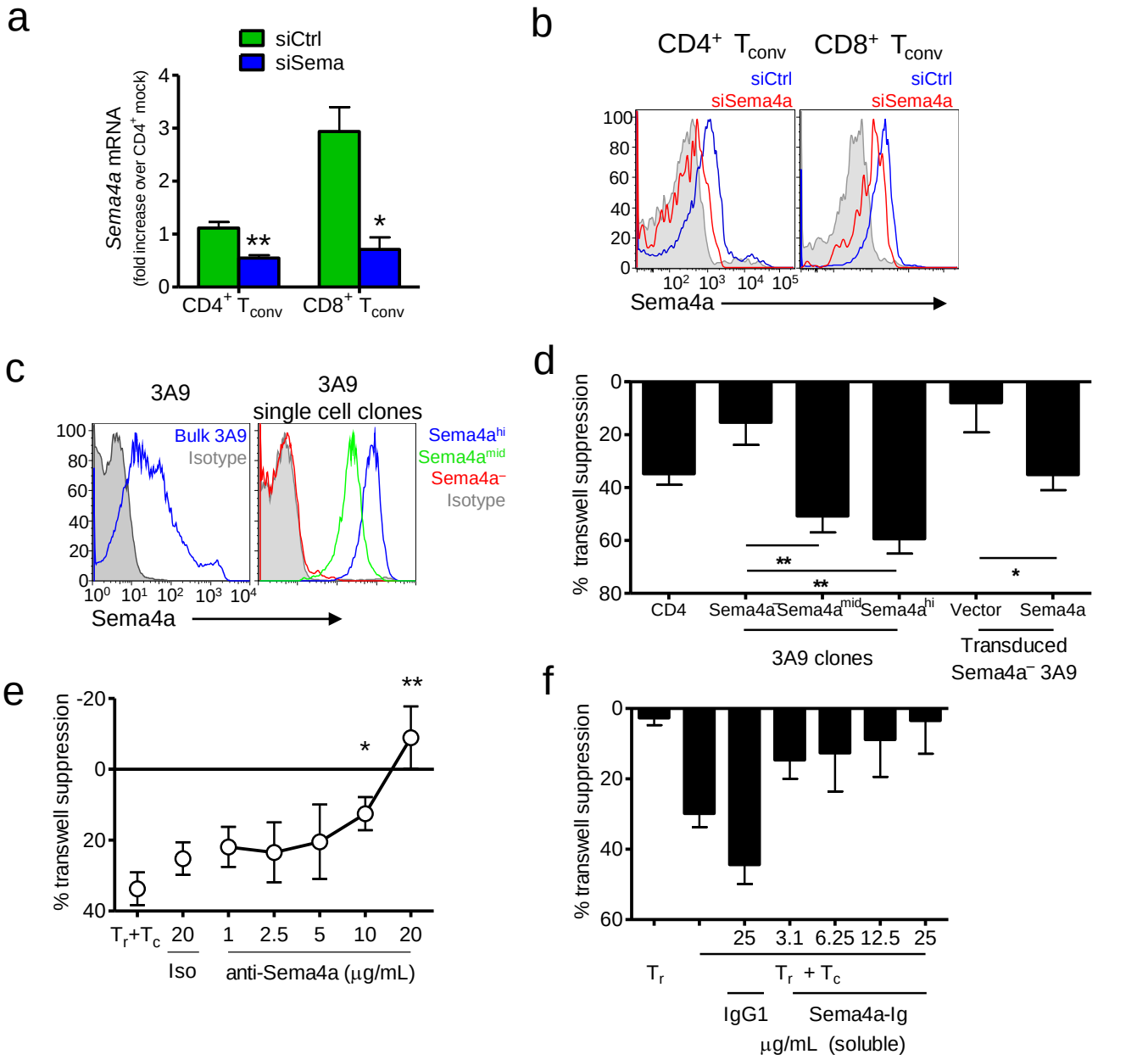
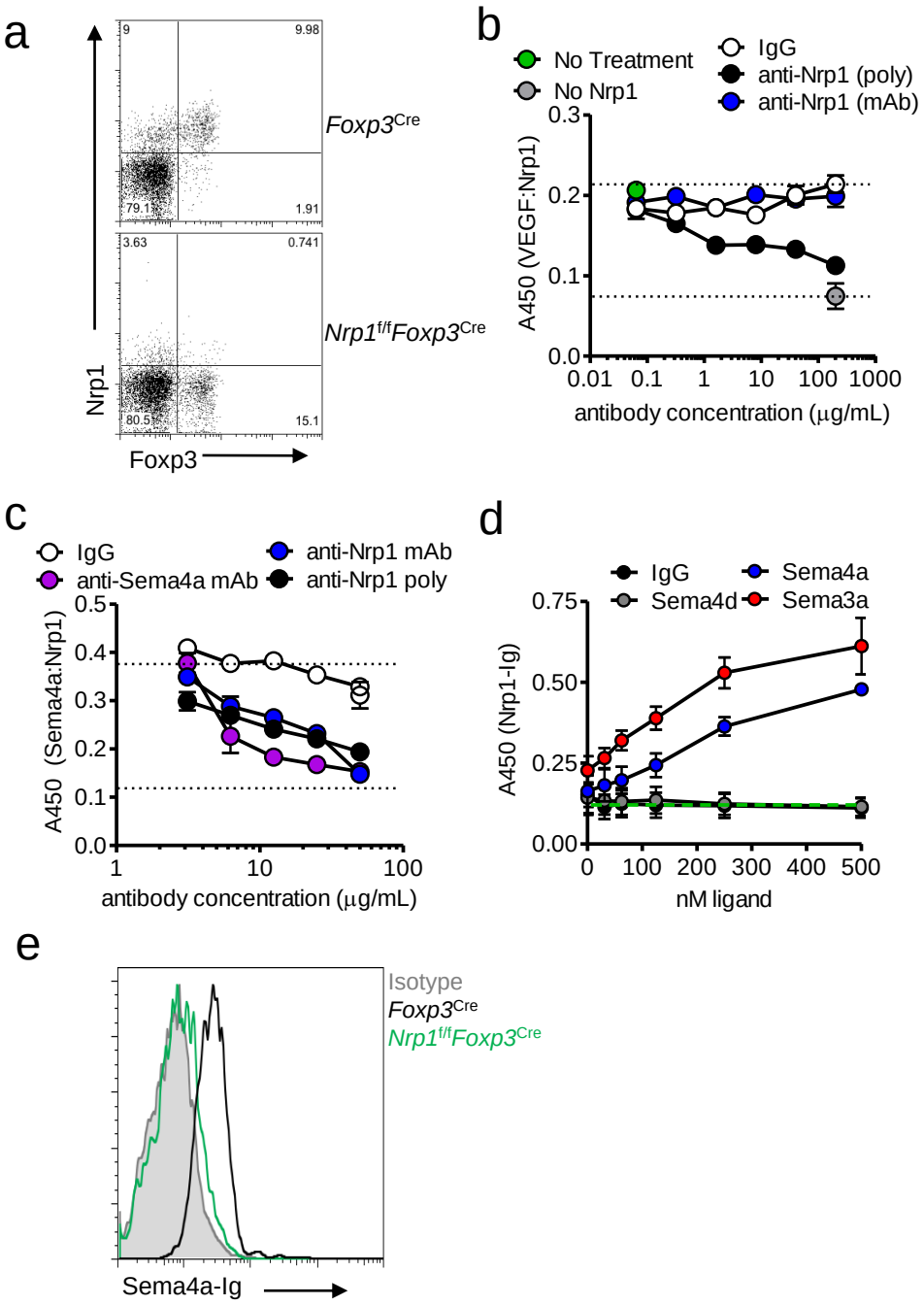


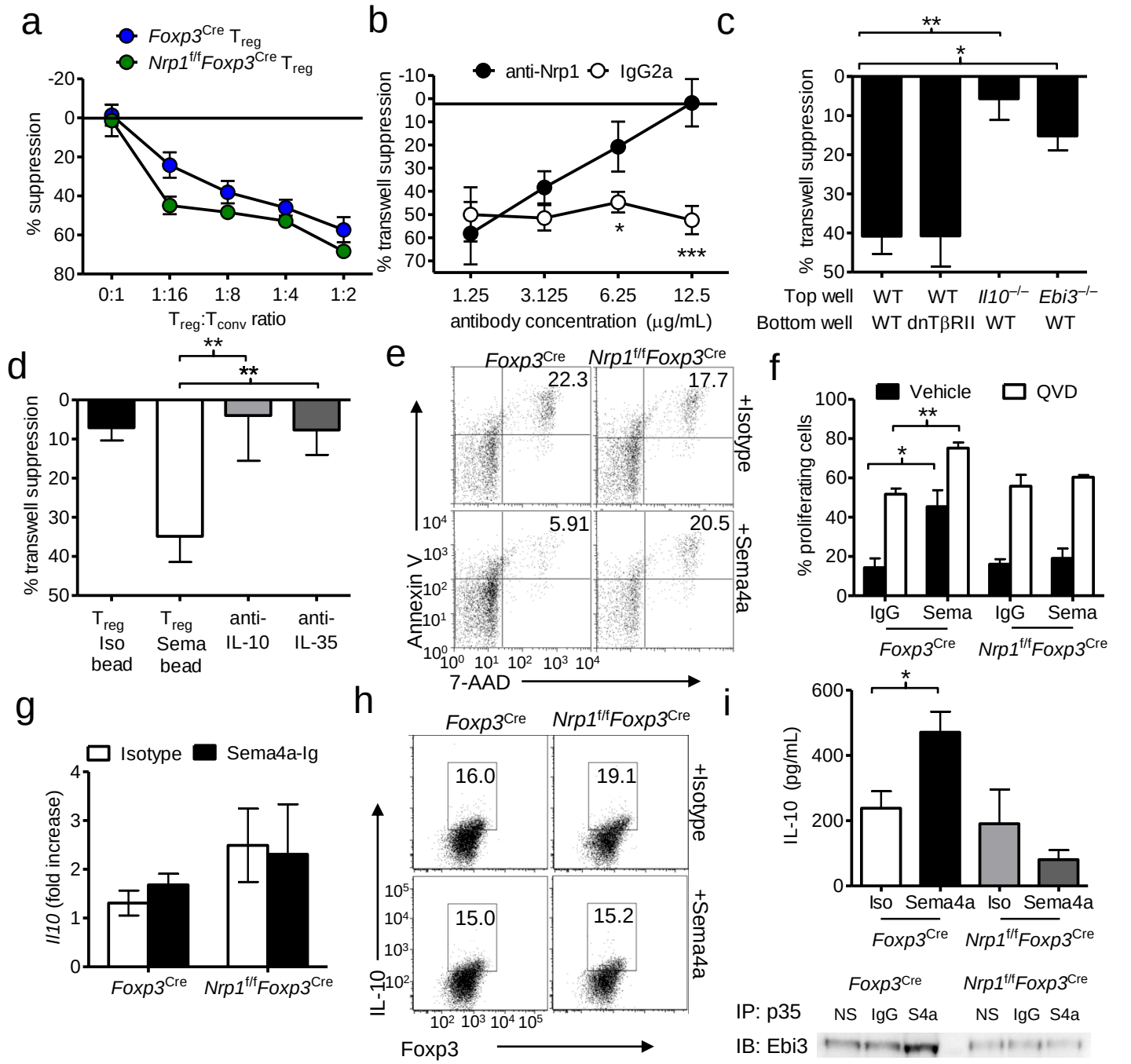

**Supplementary Figure 1. Sema4a is a putative ligand that potentiates $T_{reg}$ cell function upon $T_{conv}$ cell contact.** **a**, Transwell suppression assay of $T_{conv}$ stimulated with anti-CD3/anti-CD28 coated beads in the bottom well when regulatory T cells ($T_{regs}$) are stimulated in the top well in the presence of the indicated cell types. For some conditions, the coculture cell population was fixed prior to $T_{reg}$ stimulation. **b,** Volcano plot depicting genes in grey uniformly regulated in the three groups of $T_{conv}$ cells when compared to either resting (left) or activated $T_{reg}$ (right). $T_{conv}$ cells and $T_{reg}$ cells were sorted from Foxp3.GFP mice and incubated with irradiated APC in the presence or absence of anti-CD3. After 48h RNA extracted from cells re-sorted based on CD4 and GFP expression was subjected to Affymetrix analysis. Three replicates were analyzed of each cell type. Genes in blue exhibit at least a 3 fold difference and were found to contain signal sequences and transmembrane domains using SignalP 3.0 software. **c,** Validation of Sema4a expression by qPCR from RNA extracted from indicated cell types sorted flow cytometrically direct *ex vivo*. **d**, Peripheral spleen/lymph node preparations were stained with anti-Sema4a and analyzed flow cytometrically. MFI is reported. Data represent the mean of five [**a**] or three [**c**], or are representative of [**b, d**] experiments. **, $p < 0.01$. Error bars indicate s.e.m.



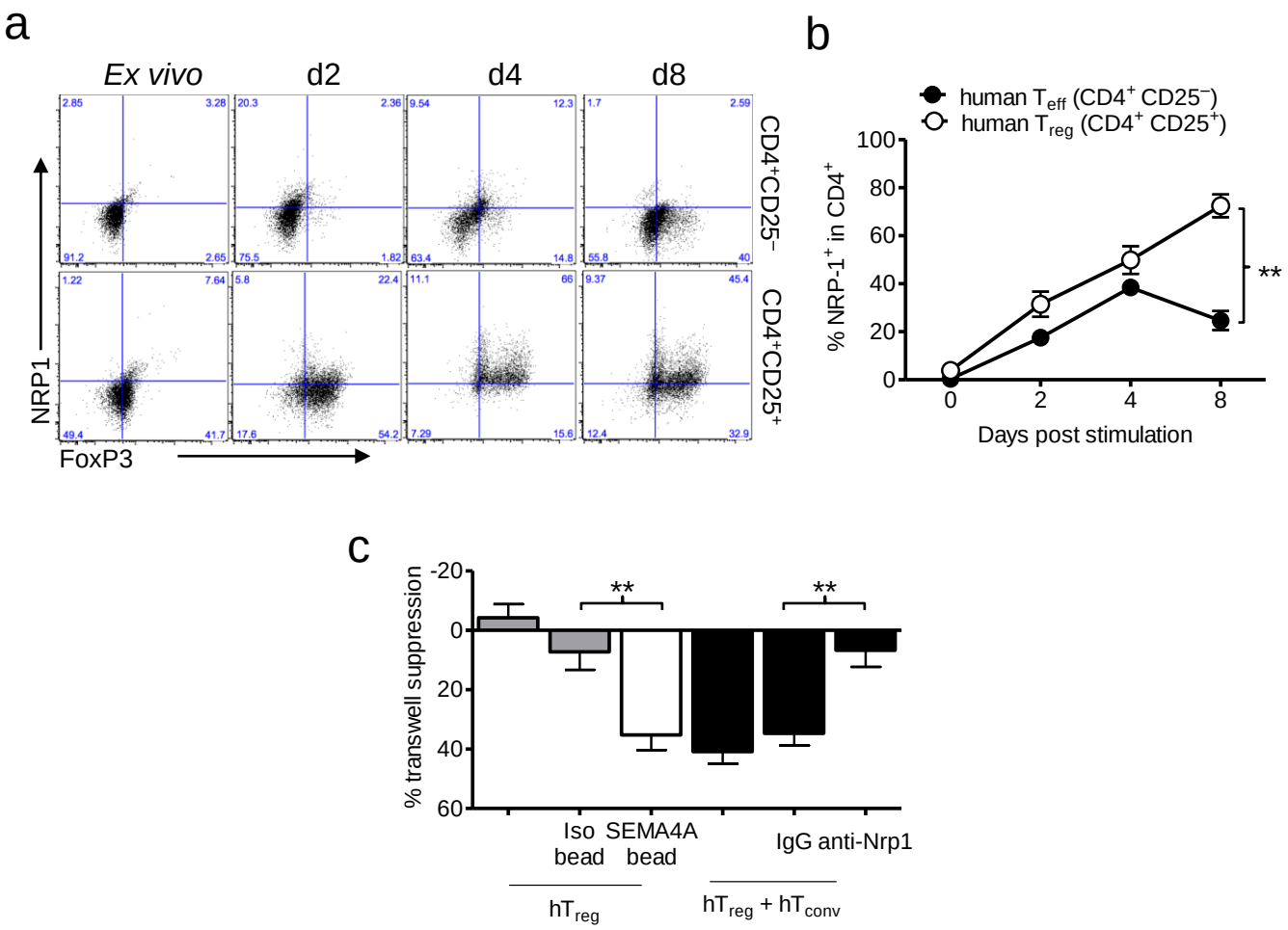

**Supplementary Figure 2. Sema4a is an immune mediator of $T_{reg}$ function. a,** Lymphocytes were nucleofected with 100pmol scrambled (siControl) or a pool of 3 *Sema4a*-targeting (siSema4a) siRNA. 16 h after transfection, mock and transfected cells were sorted and stimulated with anti-CD3 and anti-CD28 for 24 h. RNA was probed for *Sema4a* (left). Results are normalized to *Actb* and scaled to the signal from $CD4^+$ mock transfected cells. **b,** As in **a**, but some cells were stained with anti-Sema4a. Shaded histogram indicates isotype control. **c**, Flow cytometric analysis of 3A9 hybridomas stained with anti-Sema4a. Negative, mid, and hi, single cells were subcloned. **d,** Transwell suppression assay of 3A9 clones from **c**, as well as $Sema4a^-$ clones transduced with retrovirus expressing an empty vector (Vector) or a Sema4a overexpression construct (Sema4a) fixed, washed, and cocultured with $T_{reg}$ (4:1) in the top chamber of a transwell plate. **e**, Transwell suppression assay of $T_{reg}$ cells stimulated in the top well with $T_{conv}$ cells and varying doses of anti-Sema4a or isotype control. **f**, Transwell suppression assay using $T_{reg}$ cocultured with $T_{conv}$ cells in the presence of soluble Sema4a-Ig or its isotype control. Results are represent the mean of three [**a, d-f**] or are representative of five [**b**] or three [**c**] experiments. * $p < 0.05$, ** $p < 0.01$ by unpaired t-test. Error bars indicate s.e.m.



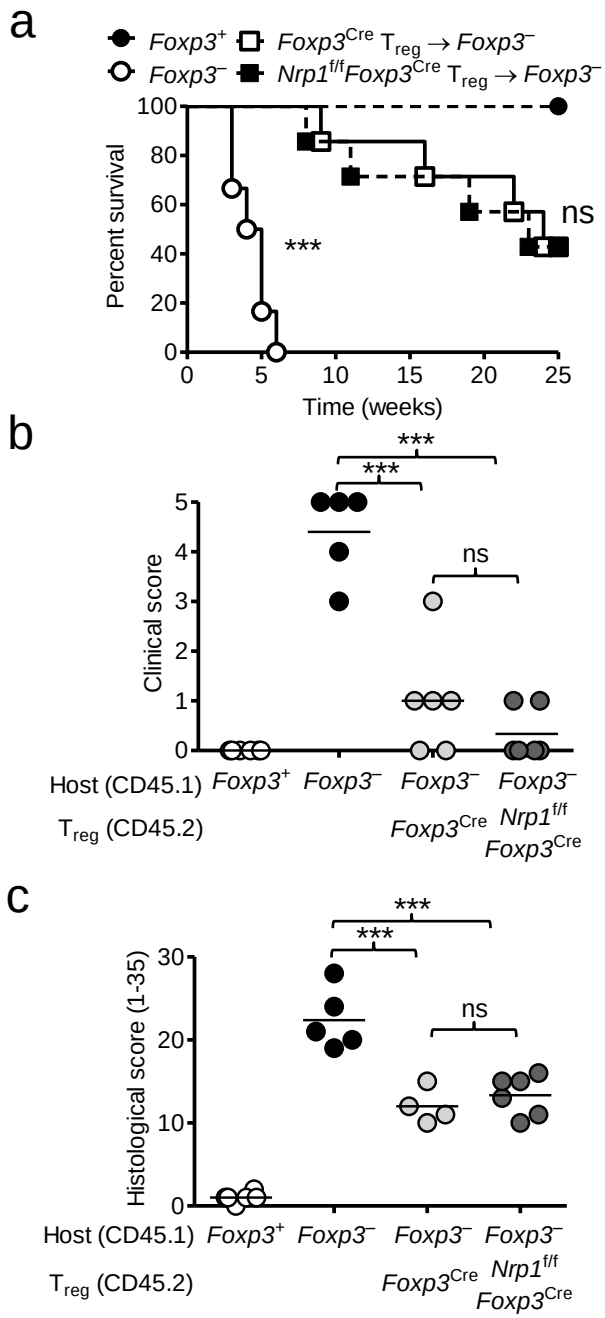

**Supplementary Figure 3. Semaphorin-4a binds neuropilin-1 . a**, Analysis of Foxp3 and neuropilin expression in CD4$^+$ T cells in *Foxp3*$^{Cre}$ and *Nrp1*$^{f/f}$*Foxp3*$^{Cre}$ mice. **b**, ELISA-based binding assay using plates coated with 500 ng/mL rmNrp1, then incubated with various antibodies in the presence of 50 ng/mL $VEGF_{165}$. VEGF was then detected using anti-VEGF biotin. **c**, ELISA-based assay as in Fig. **1d**, except 50 nM Sema4a-Ig was used and antibodies were titrated. **d**, ELISA-based assay as in **c**, except plates were coated with Sema4a-Ig, Sema3a-Fc, and Sema4d-Fc, and biotinylated Nrp1-Ig was used to detect binding. Dotted line indicates A450 value of biotinylated IgG1. **e,** Flow cytometric analysis *Foxp3*$^{Cre}$ and *Nrp1*$^{f/f}$*Foxp3*$^{Cre}$ $T_{reg}$ stained with Sema4a-Ig conjugated to Alexa Fluor 647. Results are representative of [**a, d**] five independent experiments or represent the mean [**b-d**] of three experiments. Error bars indicate s.e.m.



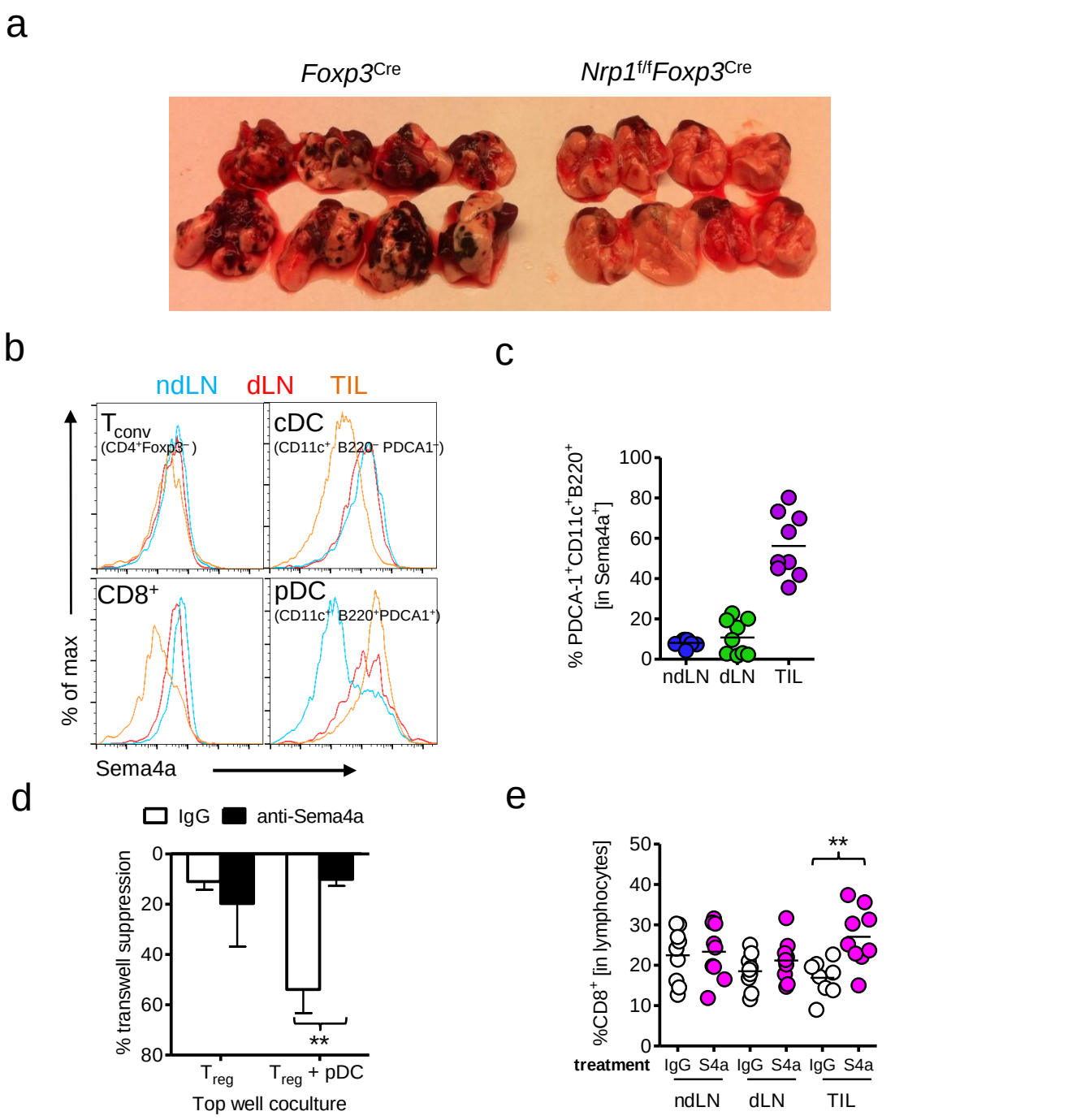

**Supplementary Figure 4. Nrp1 potentiates $T_{reg}$ function and survival. a**, Classical suppression assay in which *Foxp3*$^{Cre}$ or *Nrp1*$^{f/f}$*Foxp3*$^{Cre}$ $T_{reg}$ were cocultured with $T_{conv}$ at the indicated concentrations. **b**, Transwell suppression assay in which $T_{conv}$:$T_{reg}$ cocultures were stimulated in the presence of an neutralizing anti-Nrp1 antibody or its isotype control. **c,** Transwell suppression assay using WT, *Il10*$^{-/-}$, *Ebi3*$^{-/-}$ $T_{reg}$ cells cultured in the top well with anti-CD3/anti-CD28 coated beads and Sema4a-Ig coated beads, and WT or dnTGFβRII cells were stimulated in the bottom well. **d**, Transwell suppression assay of $T_{regs}$ cultured with Sema4a-Ig beads in the presence or absence of neutralizing antibodies to IL-10 and IL-35. **e**, Representative dot plot of $T_{regs}$ stimulated in the presence of isotype or Sema4a-Ig for 72 h with anti-CD3, anti-CD28, and IL-2. **f**, Proliferation dye dilution of $T_{reg}$ stimulated as in **e**. **g**, qPCR analysis of *Foxp3*$^{Cre}$ or*Nrp1*$^{f/f}$*Foxp3*$^{Cre}$ $T_{reg}$ cultured as in **e**. **h** Intracellular cytokine staining for IL-10 of cells stimulated as in **e**. Brefeldin A was added for the last 8 h of stimulation. **i,** IL-10 ELISA (top) and IL-35 IP/IB (bottom) from supernatants of cells stimulated as in **e**. Data represent the mean [**a-d, f, g, i**] or are representative of [**e, h, i**] 3-5 independent experiments. Error bars indicate s.e.m.



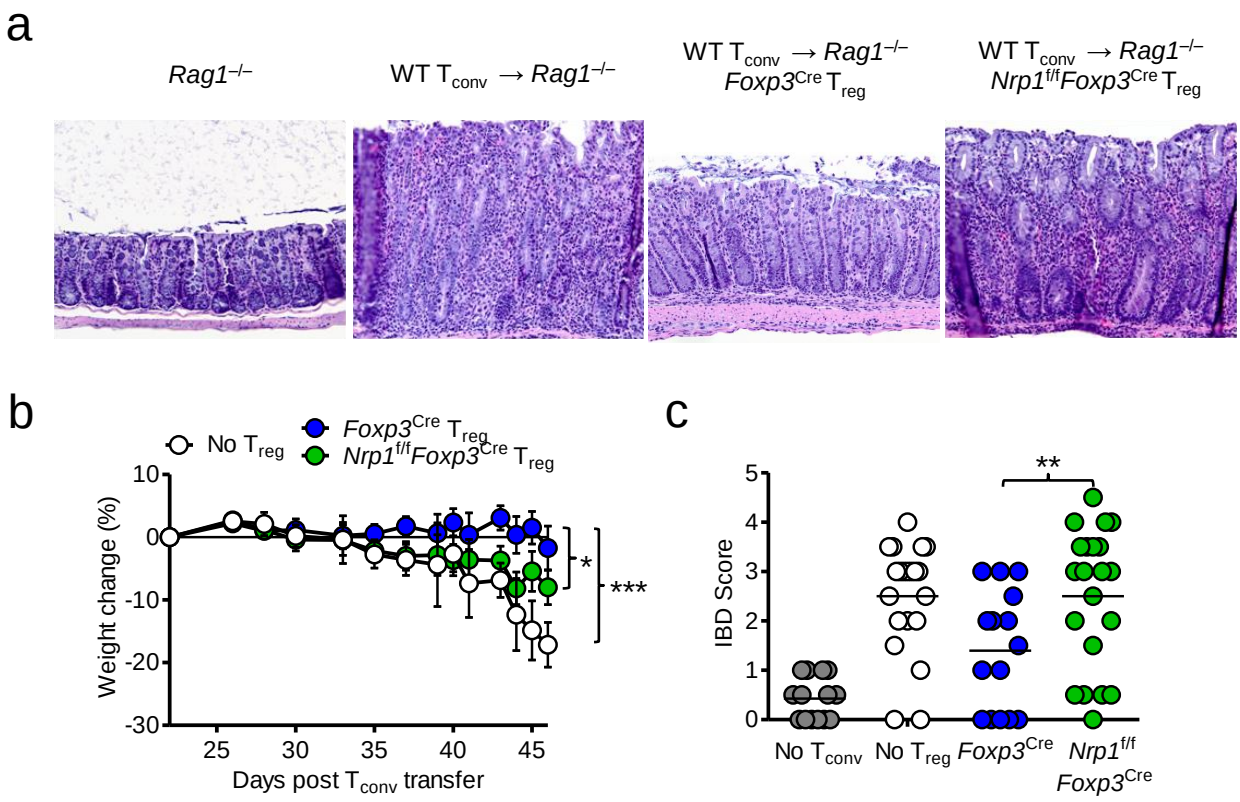

**Supplementary Figure 5. The SEMA4A-NRP1 axis is active in optimally suppressive human $T_{reg}$ cells. a**, NRP1 expression on $CD4^+$ $CD25^-$ and $CD4^+$ $CD25^+$ cell from umbilical cord blood activated for indicated times. **b**, Tabulation of **a**. **c**, Transwell suppression assay in which 8-day-expanded human $T_{reg}$ were cultured with either IgG or hSema4a-Ig coated beads, or with fixed autologous human $T_{eff}$ in the presence or absence of blocking antibodies to NRP1. Results are representative of [**a**] or represent the mean [**b, c**] of five independent experiments. **, $p < 0.01$ by unpaired t-test. Error bars indicate s.e.m.



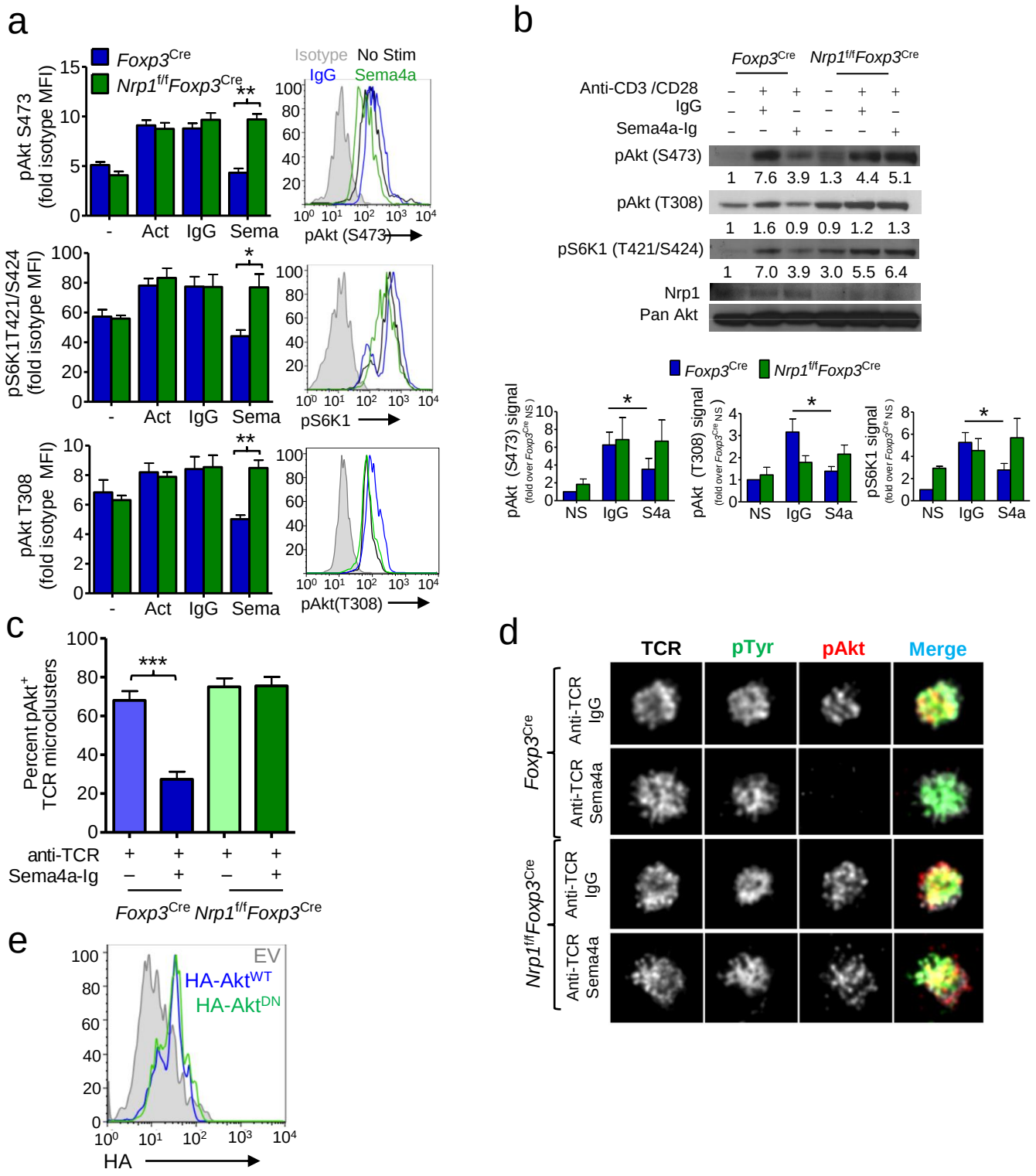

**Supplementary Figure 6. Nrp1-deficient $T_{regs}$ prevent the autoimmune disease of *Foxp3*-deficient animals. a,** Survival curve of *Foxp3*$^{-}$ male mice that received no injection or 1 x $10^6$ *Foxp3*$^{Cre}$ or *Nrp1*$^{f/f}$*Foxp3*$^{Cre}$ $T_{reg}$ at 1-2d of age. **b,** Clinical scores at 5 weeks of mice treated as in **a**. **c**, Histological scores of of liver, lung, and ear pinna (combined) from mice treated as in **a**. Results represent three independent experiments. **, $p < 0.01$ by one-way ANOVA (n = 7-8) [**a**], **, $p < 0.001$ by unpaired t-test [**b**-**c**], ns, not significant, $p > 0.05$.



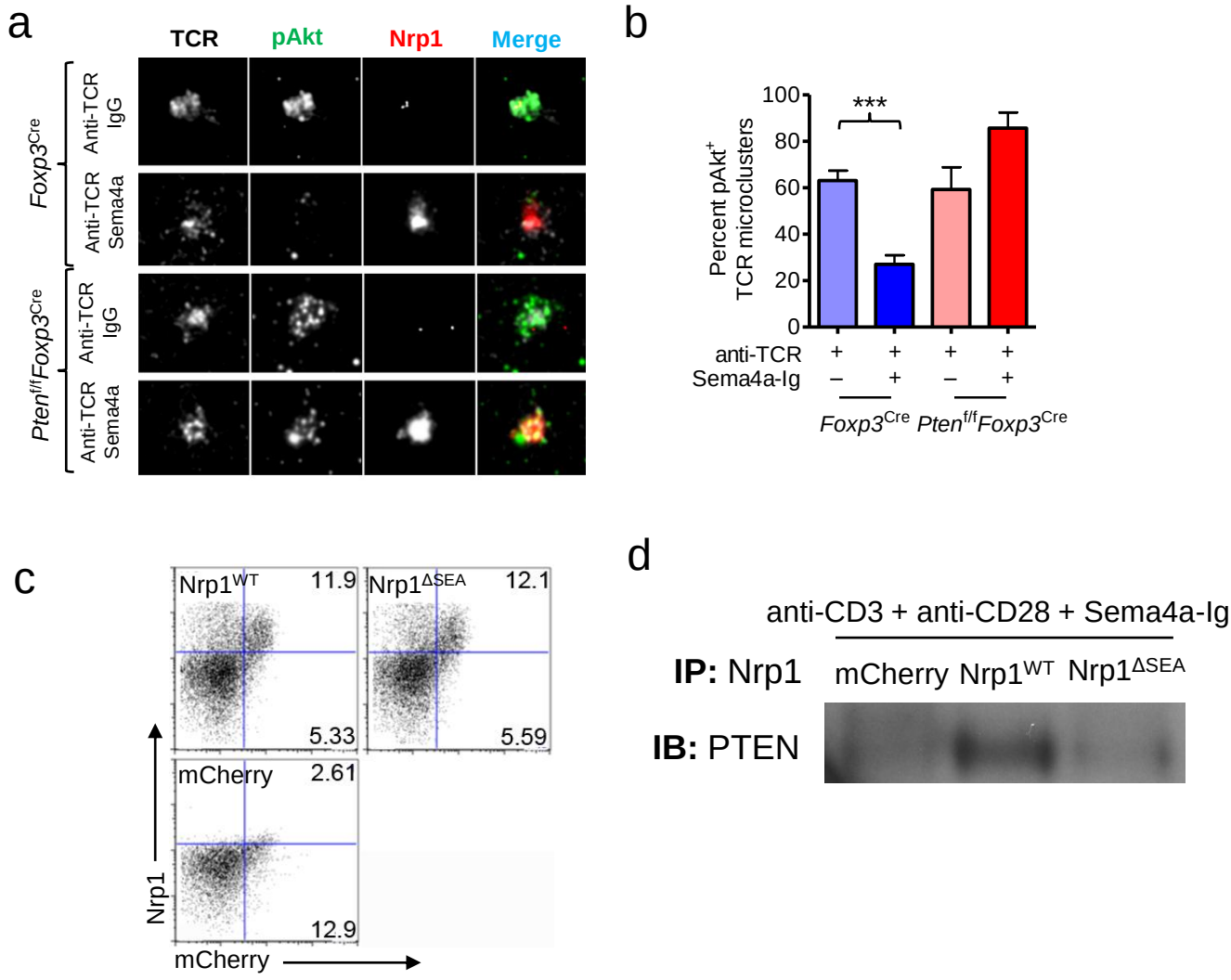

**Supplementary Figure 7. Sema4a-Nrp1 interaction on $T_{reg}$ suppresses antitumor immunity.** **a,** Gross evaluation of lung metastases from *Foxp3*$^{Cre}$ or *Nrp1*$^{f/f}$ *Foxp3*$^{Cre}$ mice that had received 1.25 x $10^5$ B16 melanoma i.v. 24 d earlier. **b,** Flow cytometric analysis of Sema4a expression in various immune cell compartments in ndLN, dLN, and TIL of mice receiving 1.25 x $10^5$ B16 melanoma cells i.d. 10 days earlier. **c,** Tabulation of pDC representation in ndLN, dLN, and TIL of tumor bearing mice. **d**, Transwell suppression assay using $T_{reg}$ cocultured with pDCs sorted from spleen and lymph node preparations, activated overnight with CpG oligonucleotides, and fixed briefly in 1% PFA followed by extensive washing. **e**, Tumor infiltrating $CD8^+$ cells in tumor bearing wild-type mice treated with either IgG or Sema4a-Ig. Results are representative of three [**a, b**] or represent the mean of [**c-e**] 3 independent experiments. Error bars indicate s.e.m.



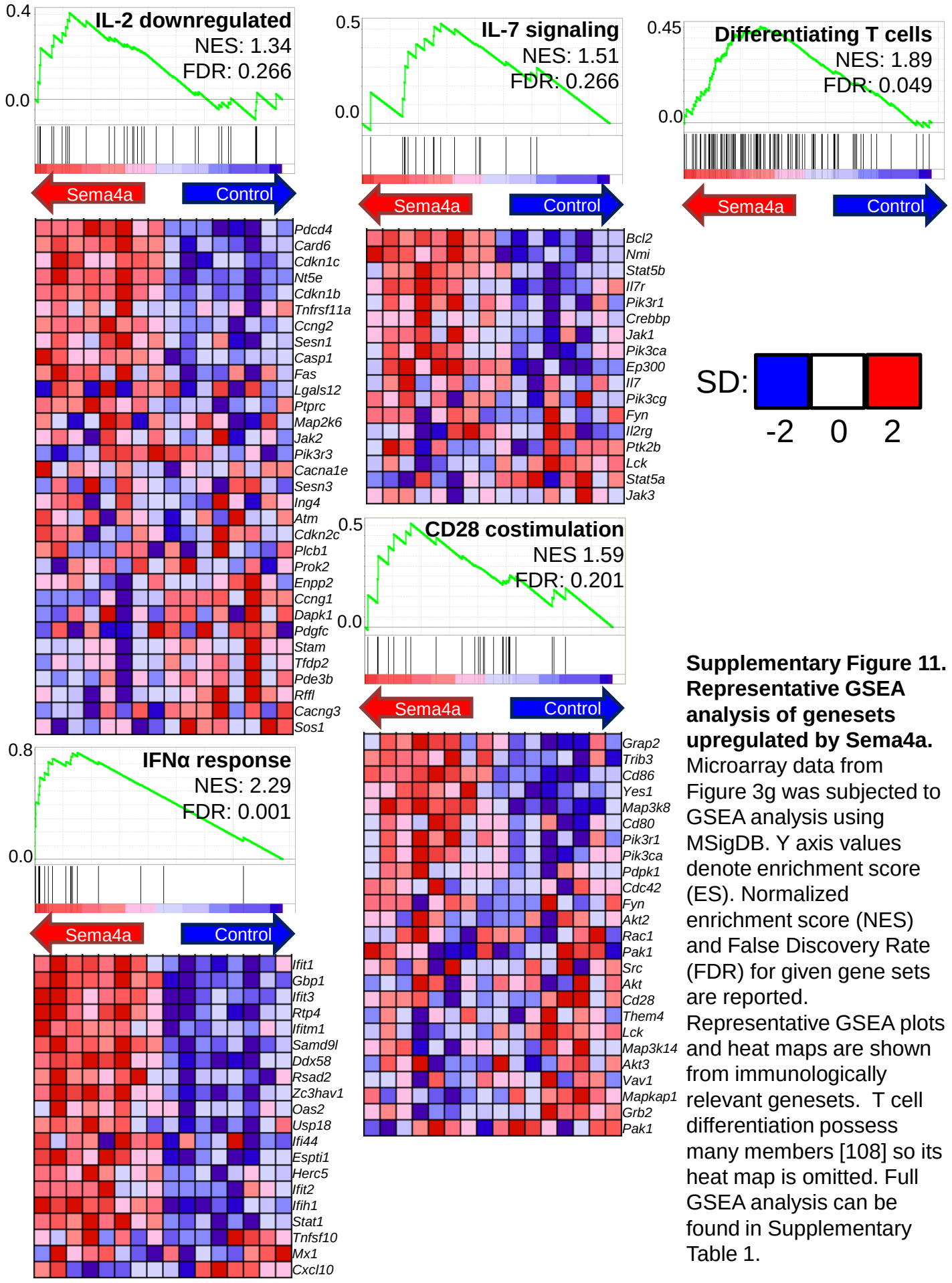

**Supplementary Figure 8. Nrp1-deficient $T_{reg}$ cells fail to cure experimental colitis.** **a,** Representative histology of large intenstines from *Rag1*$^{-/-}$ mice having received an adoptive transfer of $T_{conv}$ cells to induce colitis and a second adoptive transfer of $T_{reg}$ to rescue. **b,** Weight change among populations of mice as in **a**. **c,** Histological analysis of mice as in **a**. Plots represent the mean of four independent experiments (n=17-21 per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by (**a**) one-way ANOVA with repeated measures and (**b**) unpaired t-test. Animals that did not lose 10% of their weight after $T_{conv}$ cells (did not develop colitis) were excluded from analysis and did not receive $T_{reg}$ cells. Error bars indicate s.e.m.



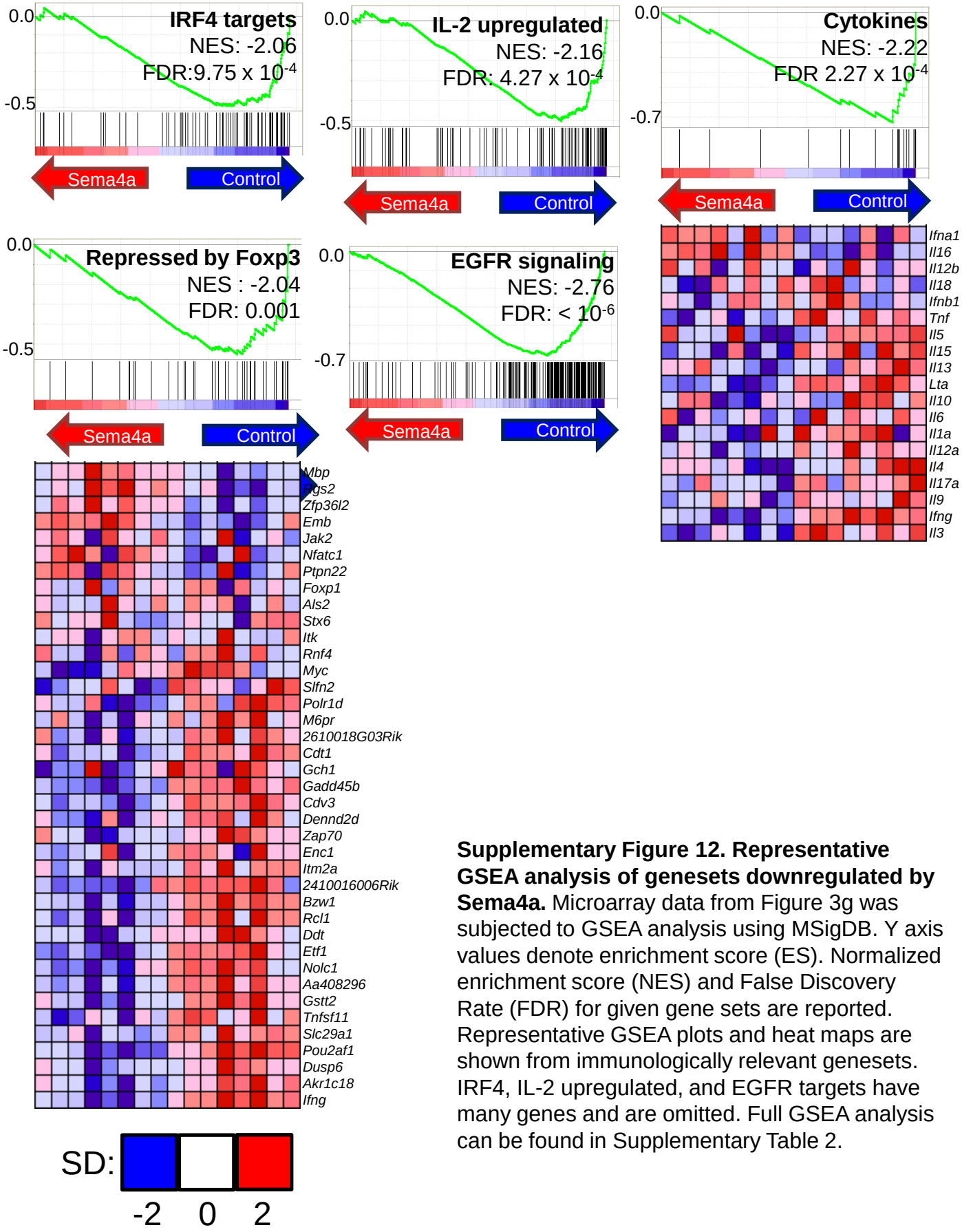

**Supplementary Figure 9. Nrp1 restrains Akt phosphorylation. a,** Flow cytometric analysis of whole-cell Akt-mTOR signaling in *Foxp3*$^{Cre}$ or *Nrp1*$^{f/f}$*Foxp3*$^{Cre}$ $T_{regs}$. Purified $T_{regs}$ were stimulated 16h with anti-CD3/CD28 beads and isotype or Sema4a-Ig beads. **b**, IB of whole-cell Akt-mTOR activation in IL-2-expanded *Foxp3*$^{Cre}$ or *Nrp1*$^{f/f}$ *Foxp3*$^{Cre}$ $T_{reg}$ stimulated as in **a**. Means of 3 experiments are tabulated below **c**, Tabulation of phosphorylation of IS Akt from Fig. **3a**. **d**, TIRF microscopy of IS phosphorylation of Akt and Tyr in *Foxp3*$^{Cre}$ or *Nrp1*$^{f/f}$ *Foxp3*$^{Cre}$ stimulated as in **3a**. **e,** HA staining of $T_{reg}$ cells retrovirally overexpressing HA-tagged Akt mutants 3 d after selection. Data represent the mean of [**a-c**] or are representative of [**b, d-e**] three independent experiments. *** $p < 0.001$ by one-way ANOVA, * $p < 0.05$, ** $p < 0.01$ by t-test. Error bars indicate s.e.m.



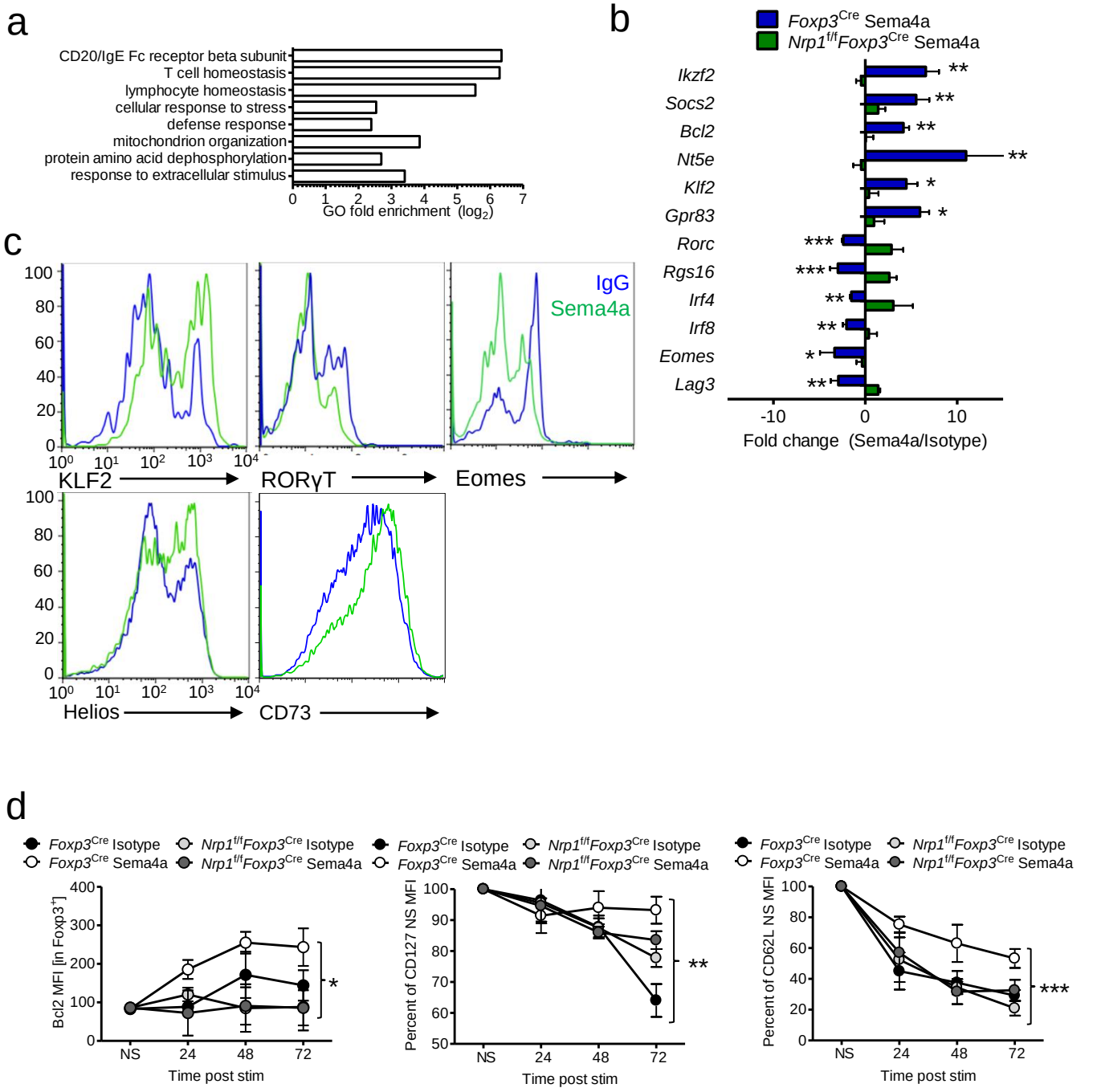

**Supplementary Figure 10. Nrp1 binds PTEN using its SEA domain. a**, TIRF microscopy of IS recruitment of Nrp1 and phosphorylation of IS Akt in *Foxp3*$^{Cre}$ or *Pten*$^{f/f}$*Foxp3*$^{Cre}$ $T_{reg}$ stimulated on as in **3a**. **b**, Tabulation of IS phosphorylation of Akt from **e**. Results represent 3 independent experiments. **c**, Nrp1 staining of *Nrp1*$^{f/f}$*Foxp3*$^{Cre}$ $T_{reg}$ retrovirally reconstituted with WT or ΔSEA Nrp1. **d**, IB of PTEN in Nrp1 IPs from expanded *Nrp1*$^{f/f}$*Foxp3*$^{Cre}$ $T_{reg}$ retrovirally reconstituted with WT or ΔSEA Nrp1, expanded again post flow cytometric sorting, and stimulated using anti-CD3, anti-CD28, and Sema4a-Ig 6 h. Results are representative of [**a, c, d**] or represent the mean of [**b**] three independent experiments. ***, $p < 0.001$ by unpaired t-test. Error bars indicate s.e.m.



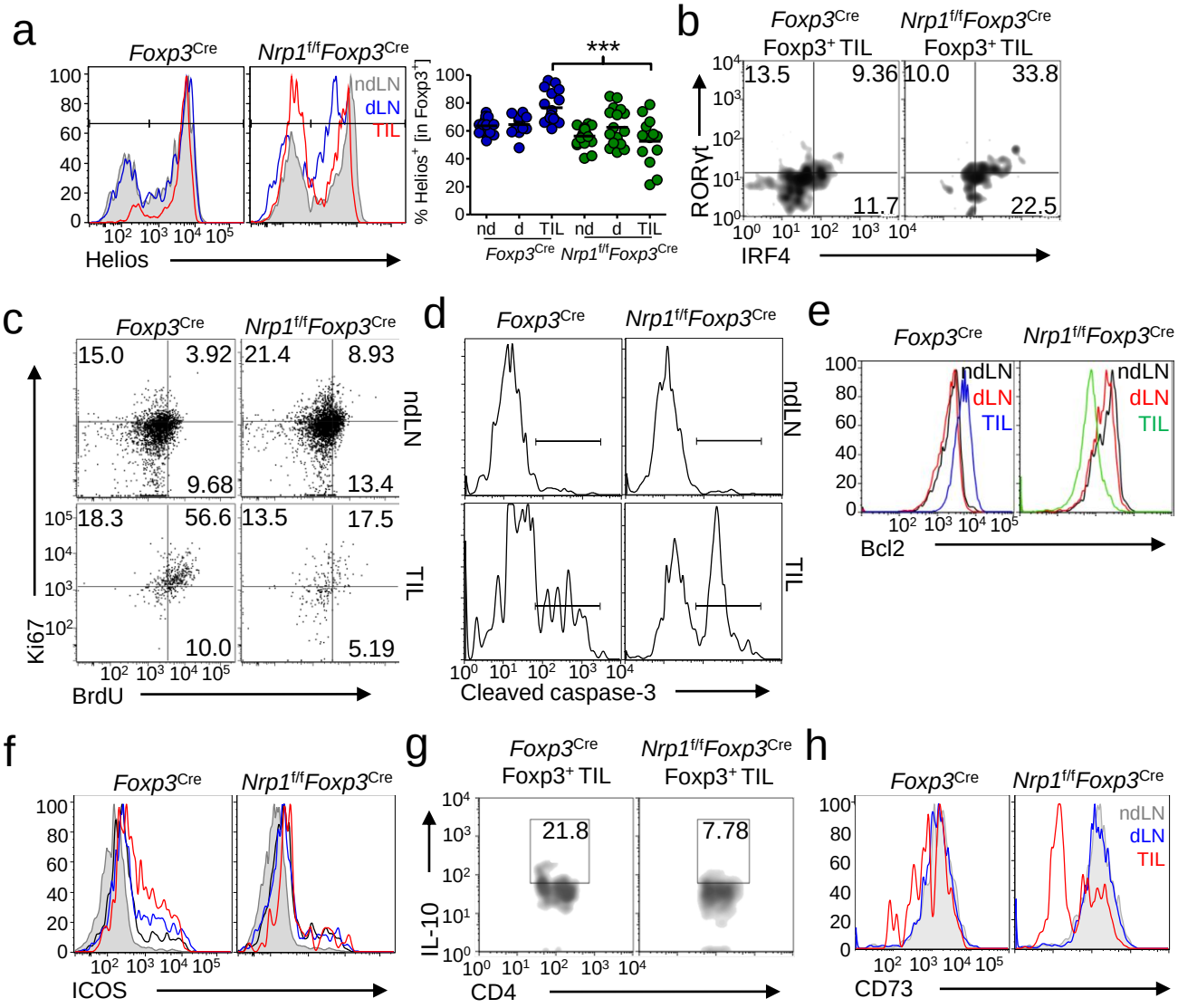

**Supplementary Figure 11. Representative GSEA analysis of genesets upregulated by Sema4a.** Microarray data from Figure 3g was subjected to GSEA analysis using MSigDB. Y axis values denote enrichment score (ES). Normalized enrichment score (NES) and False Discovery Rate (FDR) for given gene sets are reported. Representative GSEA plots and heat maps are shown from immunologically relevant genesets. T cell differentiation possess many members [108] so its heat map is omitted. Full GSEA analysis can be found in Supplementary Table 1.



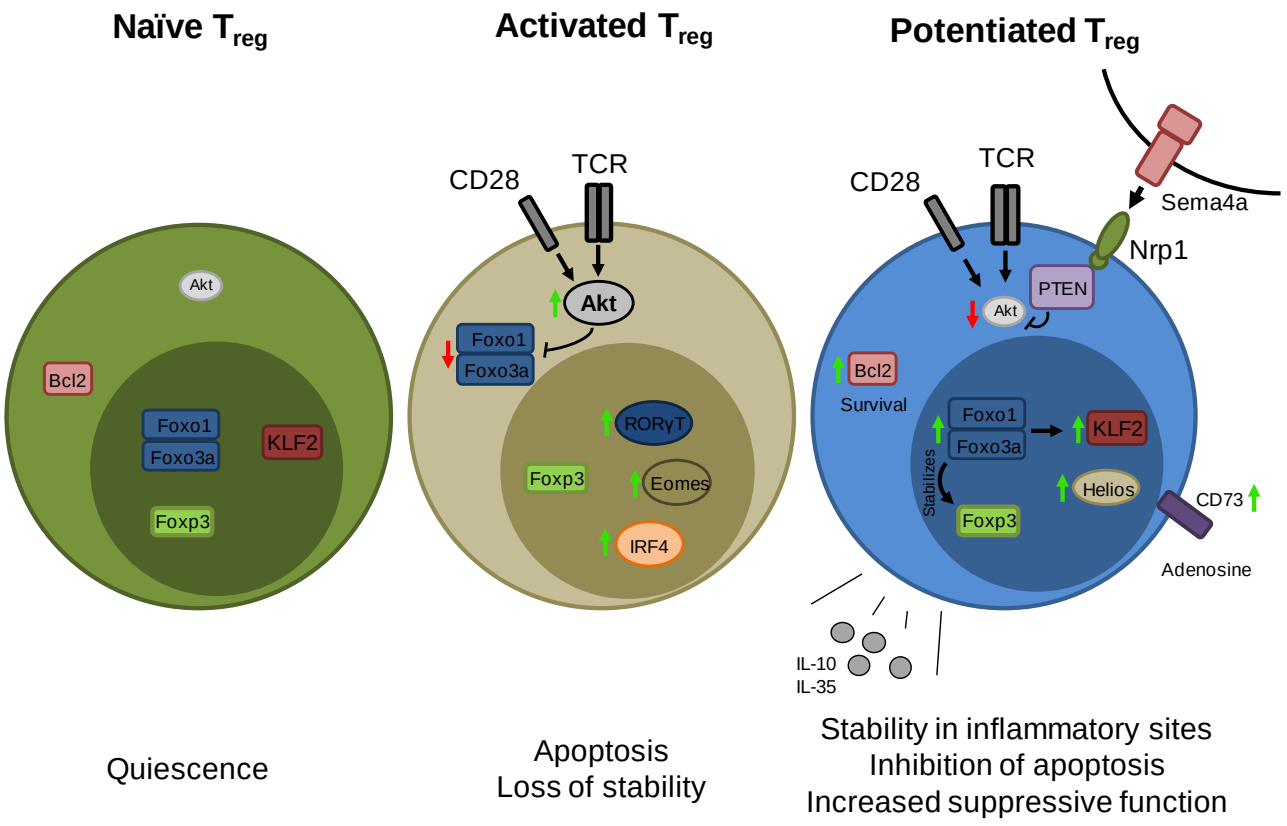

**Supplementary Figure 12. Representative GSEA analysis of genesets downregulated by Sema4a.** Microarray data from Figure 3g was subjected to GSEA analysis using MSigDB. Y axis values denote enrichment score (ES). Normalized enrichment score (NES) and False Discovery Rate (FDR) for given gene sets are reported. Representative GSEA plots and heat maps are shown from immunologically relevant genesets. IRF4, IL-2 upregulated, and EGFR targets have many genes and are omitted. Full GSEA analysis can be found in Supplementary Table 2.




**Supplementary Figure 13. Affymetrix profiling and validation of Nrp1-mediated gene targets. a,** Gene Ontology DAVID analysis of genes affected by Sema4a in *Foxp3*$^{Cre}$ $T_{reg}$ but not *Nrp1*$^{f/f}$ *Foxp3*$^{Cre}$ $T_{reg}$. **b,** Real-time PCR validation of selected genes identified by Affymetrix analysis. RNA was extracted from $T_{reg}$ stimulated with anti-CD3, anti-CD28, 100U/mL of IL-2, and either isotype control or Sema4a-Ig. **c,** Flow cytometric analysis of KLF2, Helios, RORγT, Eomes, and CD73 expression. $T_{reg}$ were stimulated for 48 h in the presence of isotype control or Sema4a-Ig. **d,** Flow cytometric analysis of Bcl2 and KLF2 target proteins over various times. Results are the mean of seven [**a**], three [**b**, **d**], or are representative of three [**c**] independent experiments. ** $p < 0.01$, *** $p < 0.001$ by [**d**] two-way ANOVA . Error bars indicate s.e.m.




**Supplementary Figure 14. Flow cytometric validation of protein targets of Nrp1 *in vivo*.** Representative flow cytometric plots of Foxp3$^+$ cells in tumors, nondraining, or draining lymph nodes from mice innoculated with 1.25 x $10^5$ B16 melanoma cells 14-17 days earlier that were stained for Helios (**a**), IRF4 and RORγt (**b**), Ki67 and BrdU (**c**), cleaved caspase-3 (**d**), Bcl2 (**e**), ICOS (**f**), IL-10 (**g**), or CD73 (**h**). For BrdU analysis mice were injected with 100 µg BrdU 8 h prior to harvest. For IL-10 staining, cells were stimulated with PMA and ionomycin 16 h in the presence of a protein transport inhibitor. Results are tabulated in Fig **4** or are present (**a**)**.** *** $p < 0.001$ by unpaired t-test [n=13-19 mice]




**Supplementary Figure 15. Neuropilin-1 maintains $T_{reg}$ stability.** Naïve $T_{reg}$ maintain low Akt activation, which promotes their quiescence through the activity of factors like Foxos and KLF2 (left). Upon activation, $T_{regs}$ stimulated in the absence of Sema4a:Nrp1 have high activation of Akt, which promotes the nuclear exclusion of Foxos, leading to loss of $T_{reg}$ stability (center). Nrp1 ligation via Sema4a restrains Akt activation via recrutiment of PTEN, inhibiting the nuclear exclusion of Foxos (right). This promotes a genetic program associated with stability and increased $T_{reg}$ function.