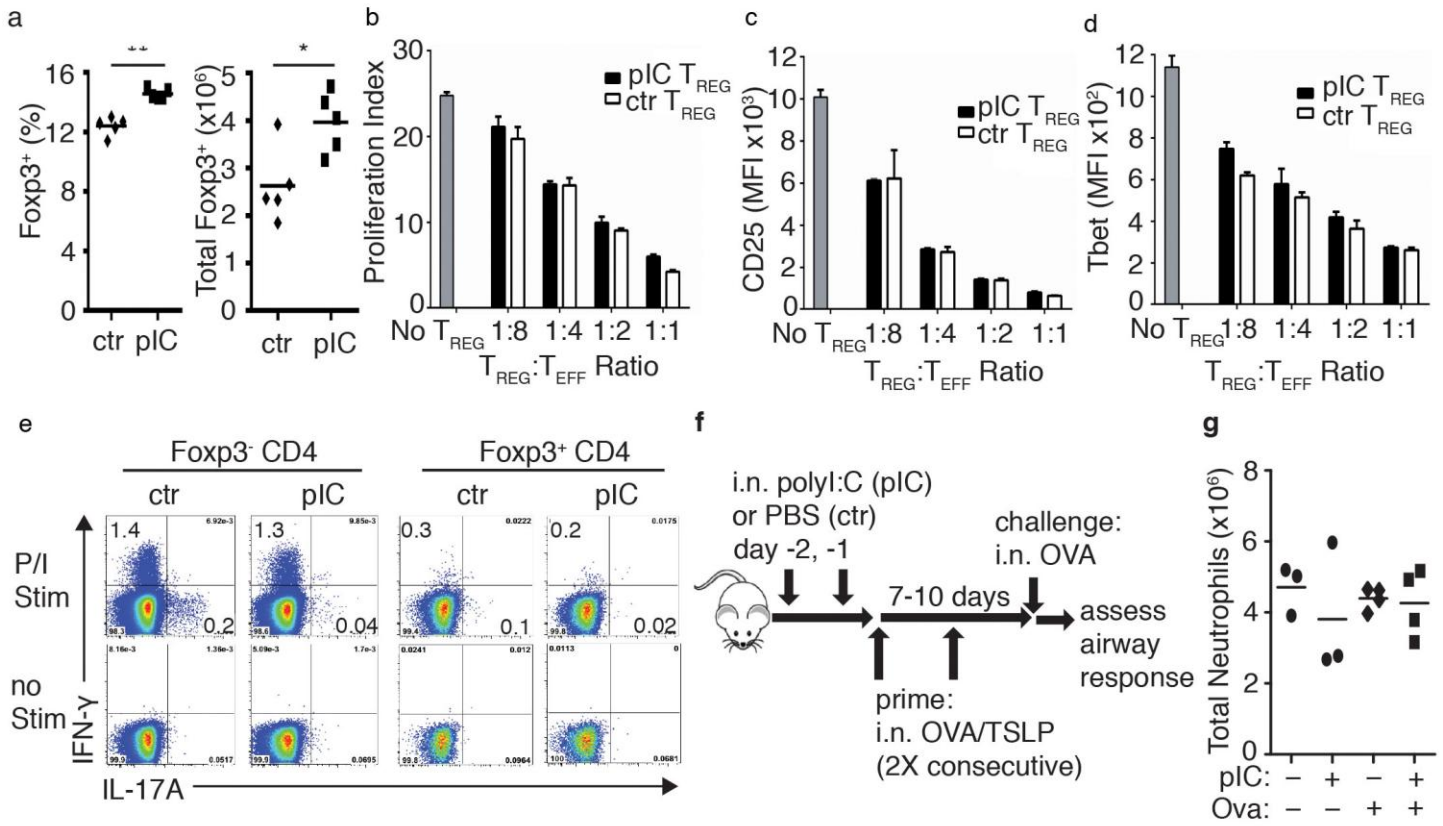
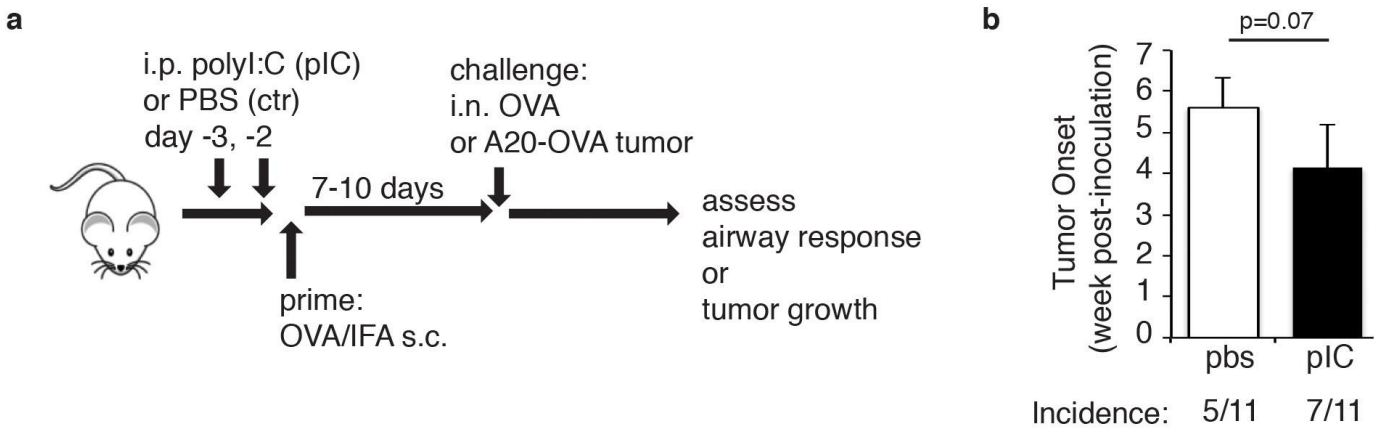


Supplementary Figure 1

Bystander inflammation conditioned T_{reg} cells have normal functional suppressive activity and *ex vivo* phenotype.

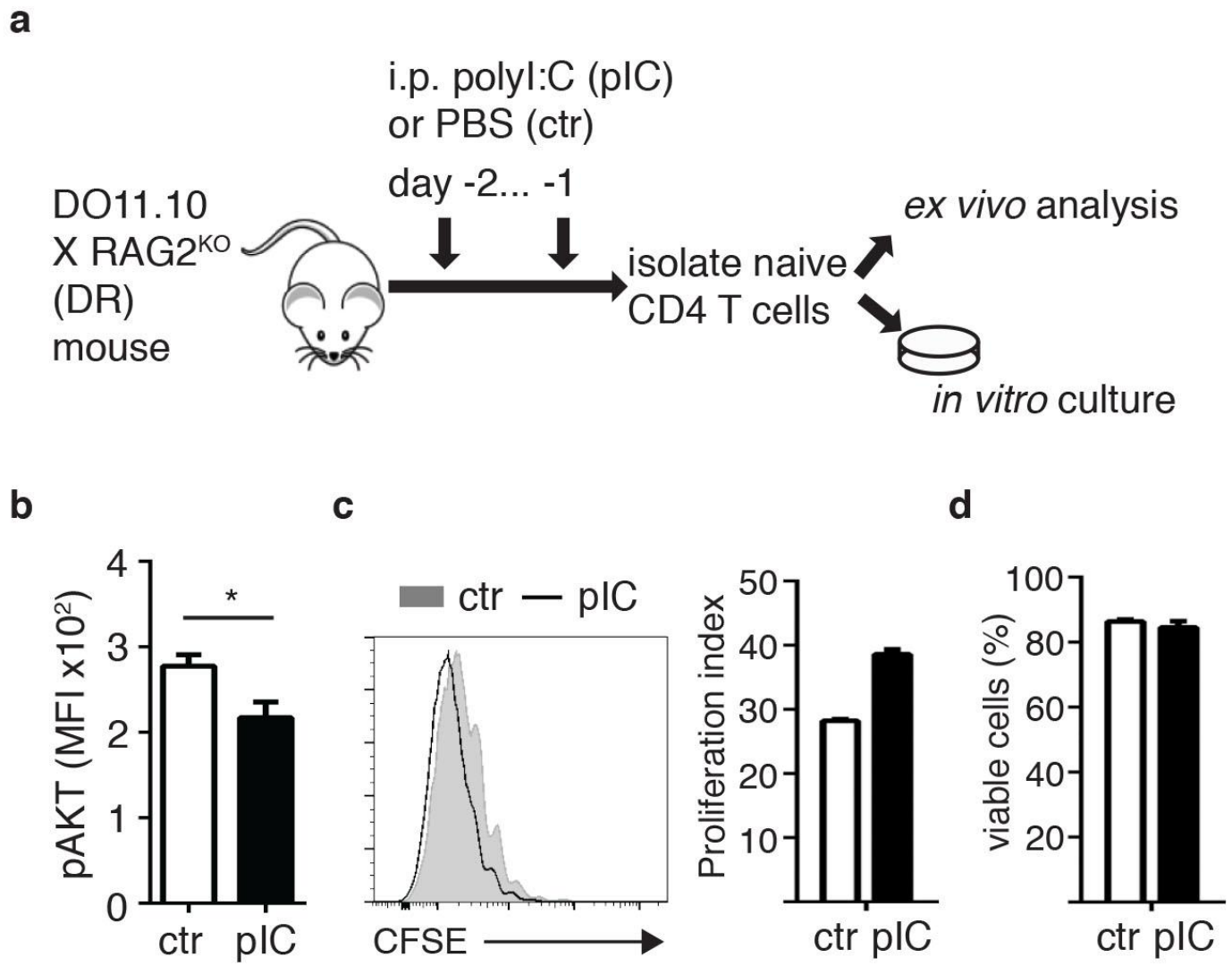
WT Balb/c mice were treated with polyI:C (pIC) or PBS (ctr) via i.p. injection for two consecutive days. (**a**) Splenocytes were assessed for frequency (left panel) and total number (right panel) of intracellular $Foxp3^+$ CD4 T cells seven days after the first injection. Each symbol represents an individual mouse. P-values by student's two-tailed t-test, *p<0.05, **p<0.01. (**b-d**) *In vitro* T cell suppression assay with T_{REG} isolated from ctr and pIC-treated mice. Activation of effector $CD4^+$ T cells as shown by (**b**) proliferation, (**c**) CD25 expression (mean florescence intensity, MFI), and (**d**) intracellular T-bet expression. Bar graph depicts mean plus SEM, data representative of two independent experiments, n=2 mice per group. (**e**) Splenocytes from pbs- and pIC-treated mice were stimulated *ex vivo* and intracellular cytokine expression was assessed. (**f**) Diagram depicting experimental approach for acute primary airway inflammation. Following intranasal treatment with pIC or PBS, mice were primed intranasally with TSLP and OVA, then challenged with OVA one week later, see Materials and Methods for detailed description. (**g**) Total pulmonary neutrophil counts from mice treated as described in main figure 1, each symbol represents an individual mouse.



Supplementary Figure 2

Diminished efficacy of immunization that follows nonspecific inflammation.

(**a**) Experimental schematic for data described in Figure 2. Briefly, Balb/c mice were treated with pbs or pIC for 2 consecutive days, then on the fourth day after the first treatment, mice were immunized with subcutaneous Ova/IFA. Approximately one week later mice were challenged with Ova either in the in the context of direct intranasal challenge or A20 lymphoma expressing Ova (A20-tGO). See Materials and Methods for detailed description. (**b**) Mean time of tumor onset, as determined by initial identification of measurable tumor mass following subcutaneous inoculation. Incidence of tumor-bearing mice as a fraction of the total mice in each group is shown below. P-value by one-tailed Mann-Whitney U-test.

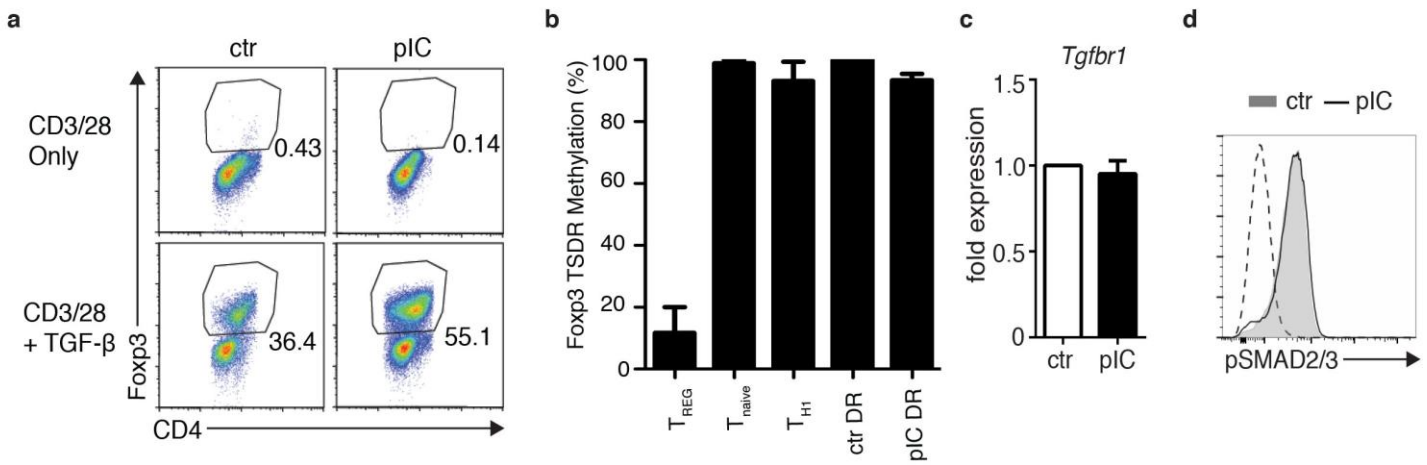


Supplementary Figure 3

Nonspecific bystander inflammation produces $_{IC}T_N$ cells that have altered molecular response but retain survival and proliferative ability.

(**a**) Experimental Schematic; DR mice were given intraperitoneal injection of pbs (ctr) or pIC for two consecutive days, then on the third day spleen and lymph nodes were harvested and purified by magnetic bead enrichment. Cells were then analyzed directly *ex vivo* or manipulated as described. (**b**) DR cells were cultured for 16-20 hours with Ova-pulsed APC, cells were fixed and stained for intracellular phospho-AKT (pAKT). For controls, DR cells were cultured with APC with no antigen (not shown). Bar graphs (right panels) depict mean plus SEM of triplicate values of mean florescence intensity (mfi) of pAKT. Data are representative of three independent experiments, n=3 mice per group. (**c-d**) CFSE-labeled DR cells were cultured for 5 days with OVA-peptide-pulsed APC and cells were assessed by FACS for proliferation by CFSE dilution and survival by viability marker. (**b**) CFSE dilution of DR cells; representative FACS histogram (left), bar graph depicting mean plus SEM of triplicate values for proliferation index of DR cells as calculated based on CFSE dilution (right). (c) Frequency of viable DR cells by fluorescent viability marker, bar graph depicts mean plus SEM of triplicate values. Data are representative of three independent experiments, n=3 mice per group. P-

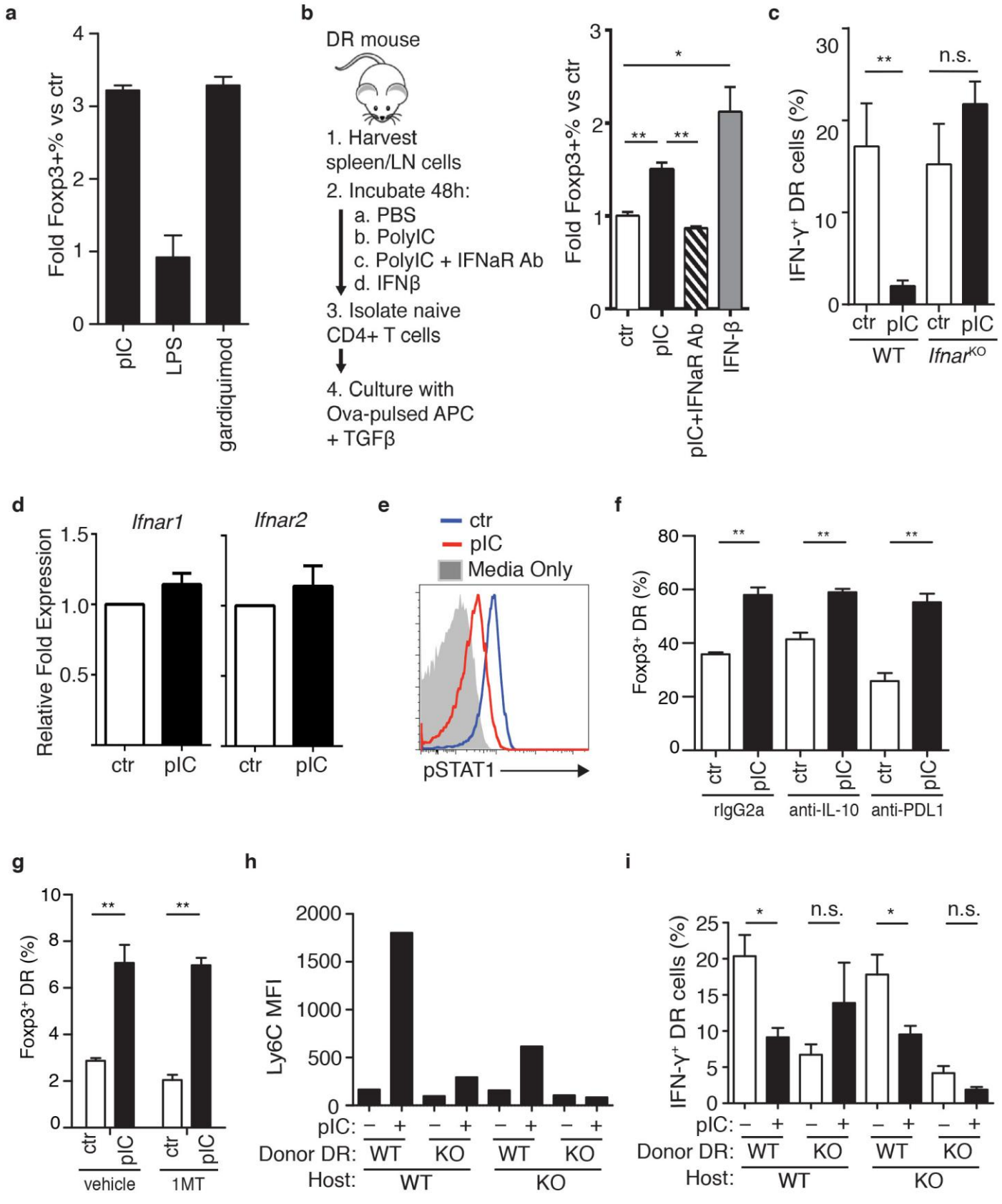
value by student’s two-tailed t-test, *p<0.01.



Supplementary Figure 4

Enhanced *de novo* Foxp3$^+$ cell induction from $_{IC}T_N$ cells is not dependent on APCs in the microenvironment.

(**a**) CD4$^+$ T cells from ctr or pIC-treated DR mice were isolated then stimulated for 5 days with plate-bound anti-CD3 and anti-CD28 with no APC. Foxp3 expression was assessed by intracellular staining, representative FACS plot from 3 independent experiments is shown, n=3 mice per group. (**b**) CD4$^+$ T cells from PBS-treated (ctr) or polyI:C-treated (pIC) DR mice were assessed for methylation of the *Foxp3* promoter by bisulfite sequencing analysis. Control cells were obtained by FACS sorting of CD4$^+$ T cells; regulatory T cells, (T_{REG}) were isolated by positive expression of CD25 and GITR, naïve T cells (T_{naive}) were CD25-negative, CD62L$^+$, CD44^{low}. Effector T_H1 cells (T_H1) were obtained by culturing T_{naive} cells with plate-bound anti-CD3/CD28 with IL-12 for six days. Bar graph depicts mean plus SEM of values obtained by analysis of samples from three independent experiments, n=3 mice per group. (**c**) mRNA expression of *Tgfbr1* from purified DR cells from control and polyI:C-treated donors. Bar graphs depict fold change in expression of indicated mRNA from polyI:C-treated cells vs controls (set to 1) after normalization to *Gapdh* mRNA. Mean plus SEM of fold change ratios from three independent experiments, n=3 mice per group. (**d**) DR cells from ctr (open histogram) or pIC-treated mice (shaded histogram) were cultured for 24 hours with OVA-peptide-pulsed APC and TGFβ then assessed for intracellular phospho-SMAD2/3. FACS histogram representative of two independent experiments, n=2 mice per group.


a
Fold Foxp3+% vs ctr
pIC
LPS
gardiquimod
b
DR mouse
1. Harvest spleen/LN cells
2. Incubate 48h:
a. PBS
b. PolyIC
c. PolyIC + IFNaR Ab
d. IFNβ
3. Isolate naive CD4+ T cells
4. Culture with Ova-pulsed APC + TGFβ
Fold Foxp3+% vs ctr
ctr
pIC
pIC+IFNaR Ab
IFN-β
c
IFN-γ+ DR cells (%)
n.s.
ctr pIC ctr pIC
WT
Ifnar^KO
d
Ifnar1
Ifnar2
Relative Fold Expression
ctr pIC ctr pIC
e
ctr
pIC
Media Only
pSTAT1
f
Foxp3+ DR (%)
ctr pIC ctr pIC ctr pIC
rIgG2a
anti-IL-10
anti-PDL1
g
Foxp3+ DR (%)
ctr pIC ctr pIC
vehicle
1MT
h
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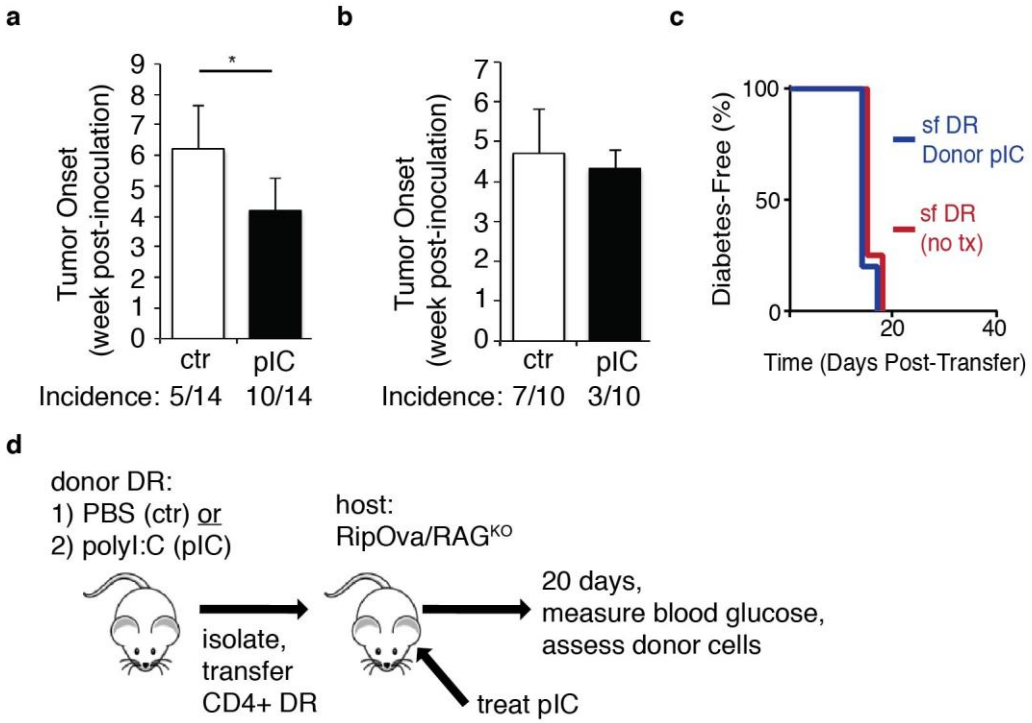
pIC: – + – + – + – +
Donor DR: WT KO WT KO
Host: WT KO
i
IFN-γ+ DR cells (%)
n.s.
pIC: – + – + – + – +
Donor DR: WT KO WT KO
Host: WT KO

Supplementary Figure 5

Enhanced *de novo* Foxp3$^+$ cell induction from $_{IC}T_N$ cells utilizes IFN-I pathways but does not involve IL-10, PDL-1 or IDO.

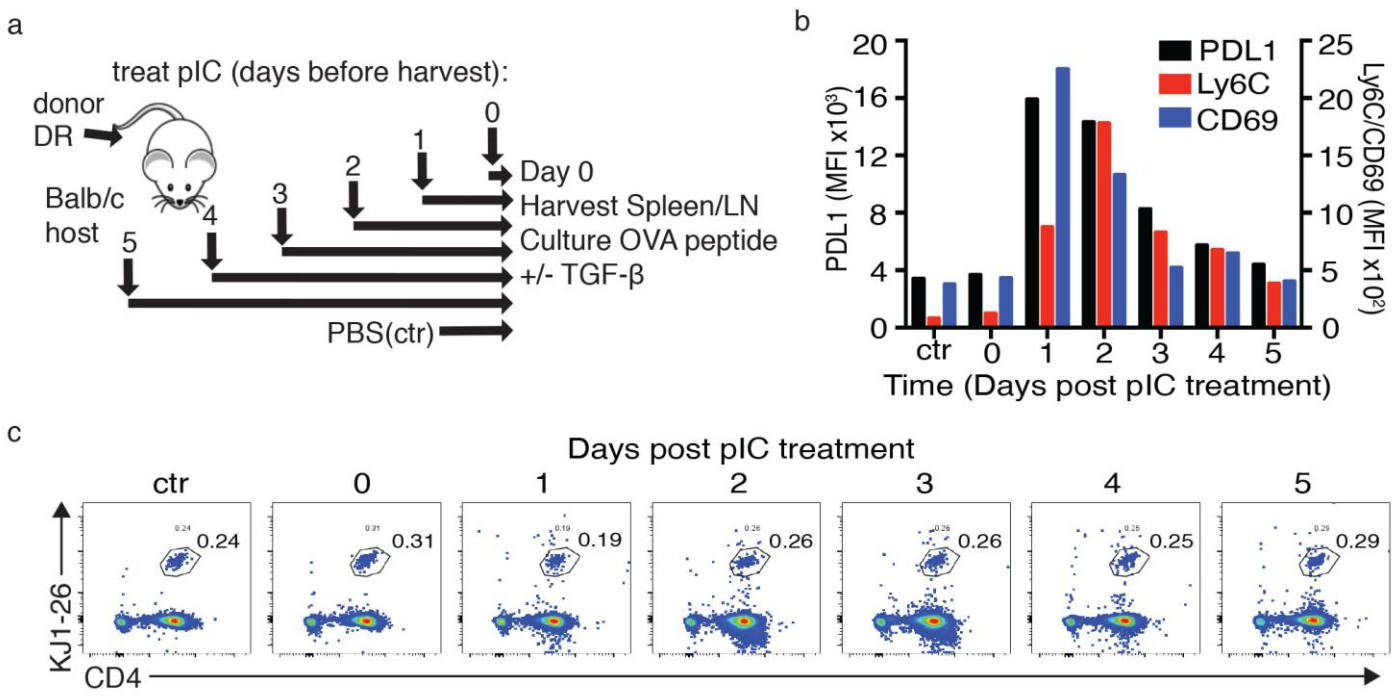
(**a**) DR cells from mice treated with pIC, LPS, or gardiquimod were cultured 5 days with Ova-pulsed APCs with or without TGFβ and intracellular Foxp3 was assessed. Bar graph depicts mean plus SEM of triplicate values of fold difference in % Foxp3$^+$ relative to average control % Foxp3$^+$ in PBS (ctr) DR cells. (**b**) Total spleen and lymph node cells were harvested from DR mice and incubated with either PBS, 3μg of polyI:C alone (pIC) or with blocking anti-IFNaR antibody (pIC + aIFNaR Ab), or recombinant IFNβ (IFNβ). After 48 hours, naïve CD4$^+$ T cells were purified by MACS and cultured with Ova-pulsed APCs and TGFβ and 3 days later stained for intracellular Foxp3. Experimental schematic shown (left). Bar graph depicts mean plus SEM of triplicate values of fold difference in % Foxp3$^+$ relative to average control % Foxp3$^+$ in PBS (ctr) DR cells. Data are representative of two independent experiments, n=2 mice per group. (**c**) WT and *Ifnar*^{KO} DR cells were cultured for 5 days in non-skewing conditions, then stimulated with PMA+Ionomycin and assessed for IFNγ production by intracellular cytokine staining. Bar graph depicts % IFNγ$^+$ DR cells, mean plus SEM for triplicate values. Data are representative of two independent experiments, n=2 mice per group. (**d**) mRNA expression of *Ifnar1* and *Ifnar2* from purified DR cells from control and polyI:C-treated donors. Bar graphs depict fold change in expression of indicated mRNA from polyI:C-treated cells vs controls (set to 1) after normalization to *Gapdh* mRNA. Mean plus SEM of fold change ratios from three independent experiments, n=3 mice per group. (**e**) DR cells were rested overnight, then stimulated for 30 minutes with recombinant IFNβ and stained for intracellular phospho-STAT1. PBS added for 30 minutes as negative control. FACS histogram representative of two independent experiments. (**f-g**) DR cells from ctr or pIC-treated mice were cultured with Ova-peptide-pulsed APC and TGFβ in the presence of (**f**) rat IgG2a isotype control, anti-IL-10, or anti-PDL1 neutralizing antibodies or (**g**) NaOH vehicle control or 1-MT, and intracellular Foxp3 was assessed. (**h-i**) WT or *Ifnar*^{KO} DR cells were transferred into WT or *Ifnar*^{KO} Balb/c recipients, and host mice were treated with PBS (ctr) or pIC. Data are representative of two independent experiments, n=2 mice per group. (**h**) Expression of Ly6C on donor DR cells directly ex vivo. (**i**) DR cells were cultured with Ova-pulsed APCs in non-skewing conditions and at day 5 cultures were stimulated with PMA+Ionomycin and intracellular IFNγ was assessed. Mean plus SEM of fold change ratios from three independent experiments, n=3 mice per group. P-values by student's two-tailed t-test, *p<0.05, **p<0.01.



Supplementary Figure 6

Functional Foxp3 is required for $_{IC}T_N$ cells to regulate antigen-specific responses.

(**a,b**) Mean time of tumor onset for data described in (main **Fig. 6a,b**), respectively. Incidence of tumor-bearing mice as a fraction of the total mice in each group is shown below. (**c**) RIP-mOva/RAG^{KO} host mice received pIC-primed sf DR cells (sf DR, Donor pIC), or received ctr sf DR cells (sf DR, no tx), and blood glucose was monitored. Graph shows diabetes-free survival. P-value by one-tailed Mann-Whitney U-test, $p<0.05$. (**d**) Experimental setup for data described in **Fig. 6d-e**. Donor DR mice were treated with pIC or PBS as described previously, then CD4$^+$ T cells were isolated and transferred into Rip-mOva/RAG^{KO} host mice. One day after transfer, all host mice were treated with pIC, and assessments conducted as described on day 20.



Supplementary Figure 7

Phenotype and stability of DR cells during transient bystander inflammation.

(**a**) Experimental schematic; purified DR cells were transferred into wt Balb/c recipients. Host mice were treated with pIC at indicated days prior to harvest of lymphoid tissues, for two consecutive daily treatments. Mice treated on day 0 were treated 6 and 3 hours prior to harvest (first day of treatment indicated for simplicity). Control mice were given PBS injections. (**b**) Profile of bystander activation markers from donor DR cells from mice treated with pIC at indicated timepoints. (**c**) Frequency of donor DR cells among total TCRb+ T cells in mice at indicated timepoints after pIC treatment. One of two independent experiments shown.