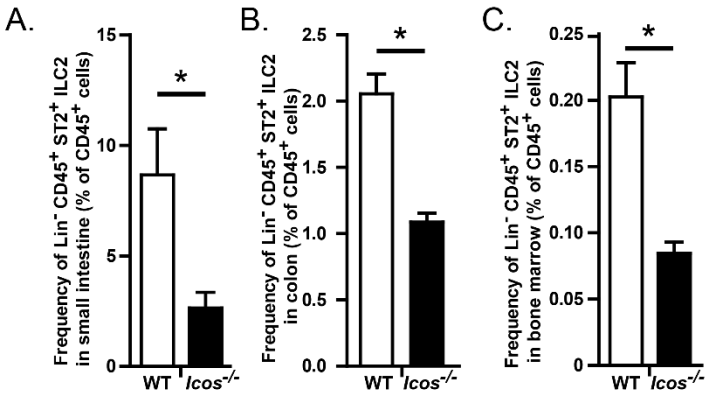
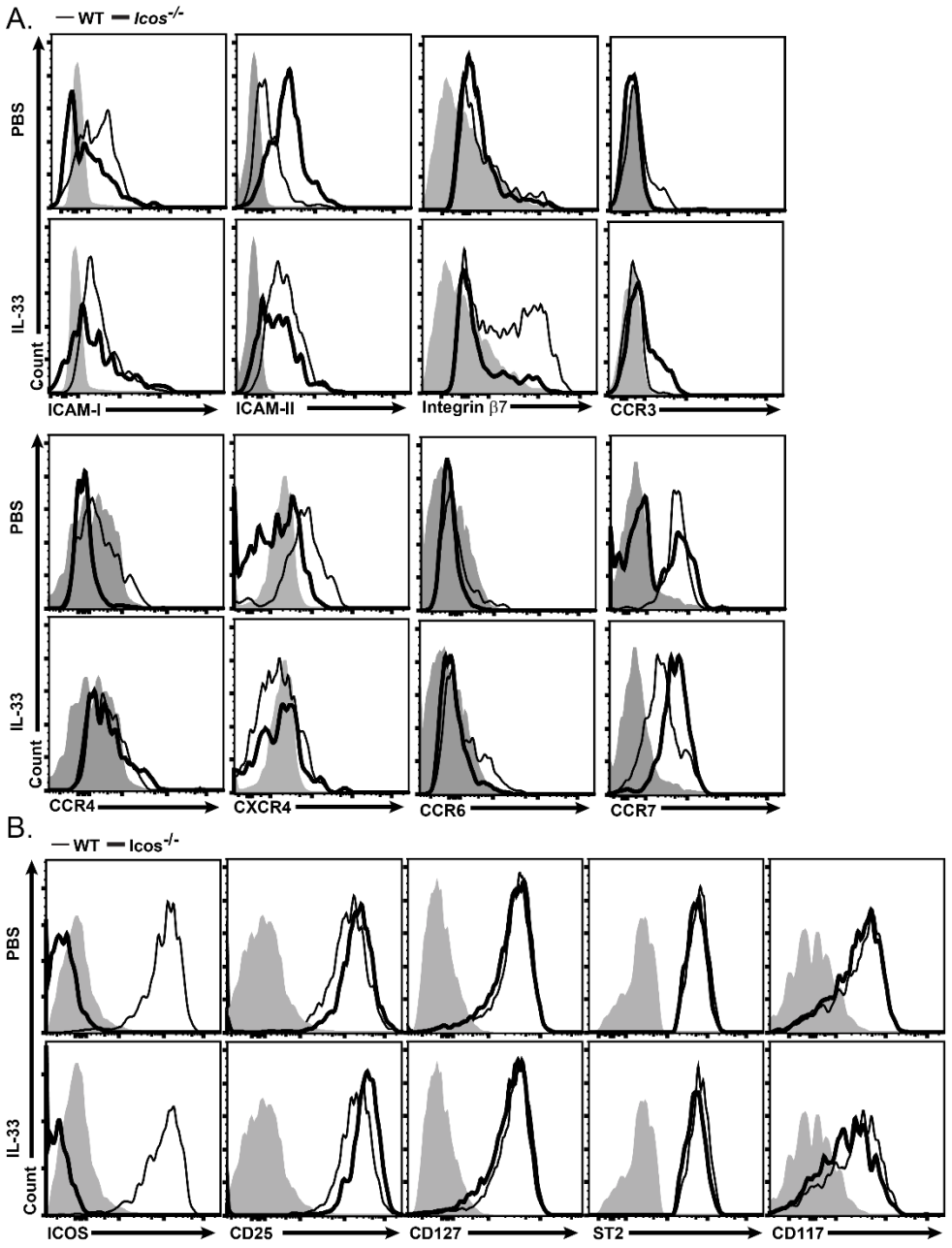
Supplementary Figures

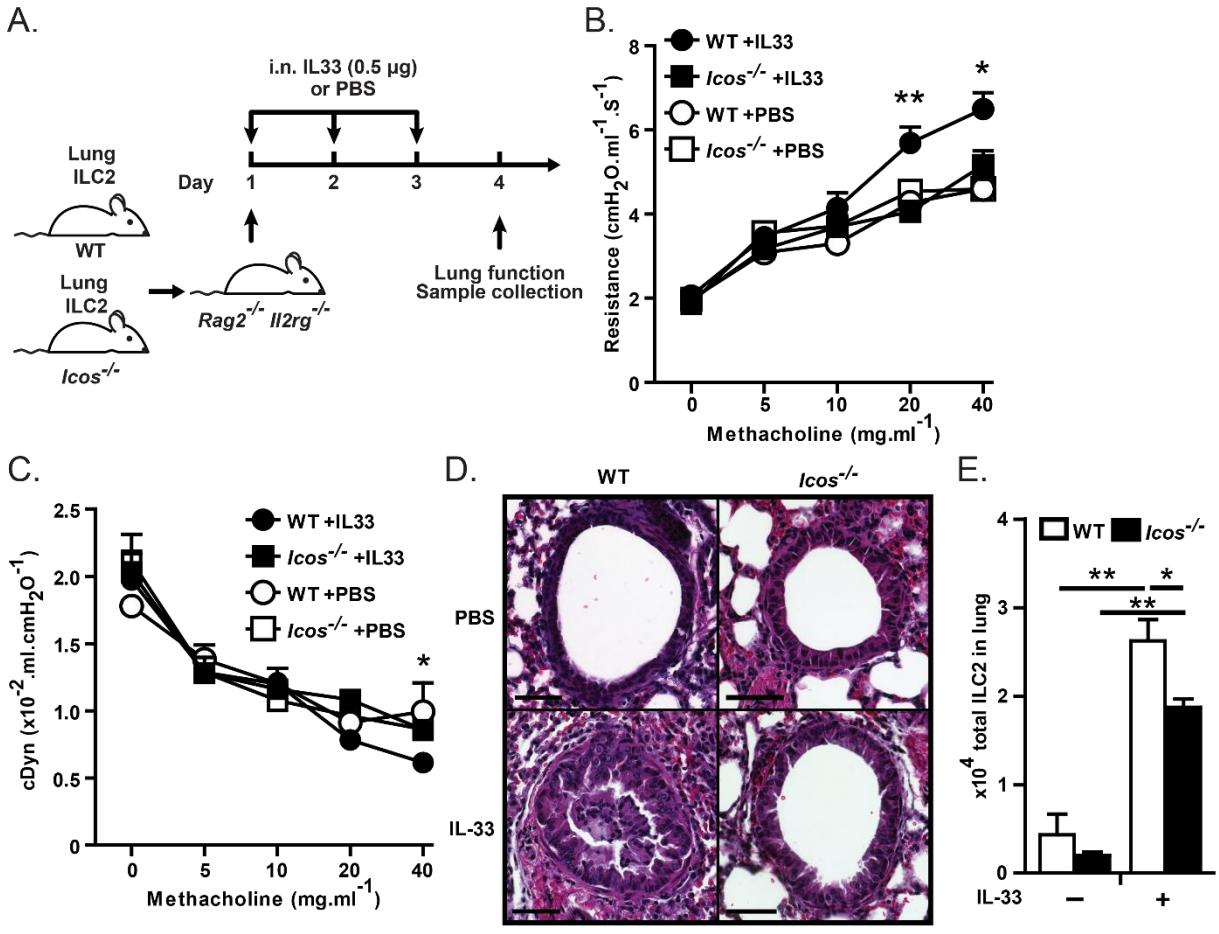
Supplementary figure 1 (relates to figure 1). Frequency of $CD45^+$ Lin^- $ST2^+$ ILC2s in A) small intestine, B) Colon and C) bone marrow. ILC2s from Bone marrow, small intestine and colon were isolated using Collagenase D, stained and quantified by flow cytometry as described elsewhere (Nussbaum et al., 2013) (*: $P<0.05$, n=5, 2 independent experiments).



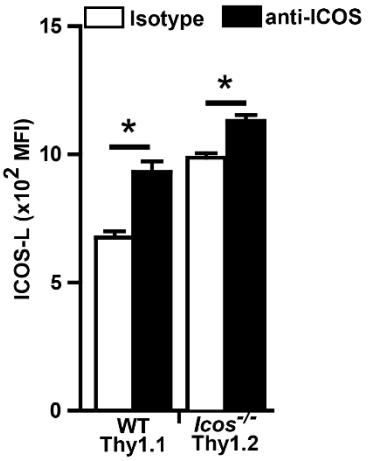
Supplementary figure 2 (relates to figure 2). Expression of A) adhesion molecules and chemokine receptors and B) cytokine receptors in WT (thin line) and *Icos*$^{-/-}$ (thick line) 24 hours after intranasal administration of PBS (upper panels) or IL-33 (0.5 μg/mouse, Lower panels). The level of isotype-matched stain control is shown as gray filled histogram.



Supplementary figure 3 (relates to figure 3). *Icos*$^{-/-}$ ILC2s fail to induce AHR and lung inflammation. A) ILC2s were purified from BALB/cBYJ and *Icos*$^{-/-}$ mice using FACS then injected into *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice intravenously (1.5 x 10^4 cells / mouse) followed by 3 intranasal challenges with rm-IL-33 or PBS on 3 consecutive days. One day after the last challenge lung function was measured and samples were collected. B) Lung resistance and C) dynamic compliance. D) Lung histology after PBS or IL-33 treatment. E) Total ILC2s in lungs after PBS or IL-33 treatment. Data are representative of at least 3 independent experiments and shown as mean ± SEM (n=3). (**: P<0.01 *Icos*$^{-/-}$+IL33 compared to WT+IL33 and WT-IL33 compared to WT-PBS, *: P<0.05 *Icos*$^{-/-}$+IL33 compared to WT+IL33 and WT-IL33 compared to WT-PBS).

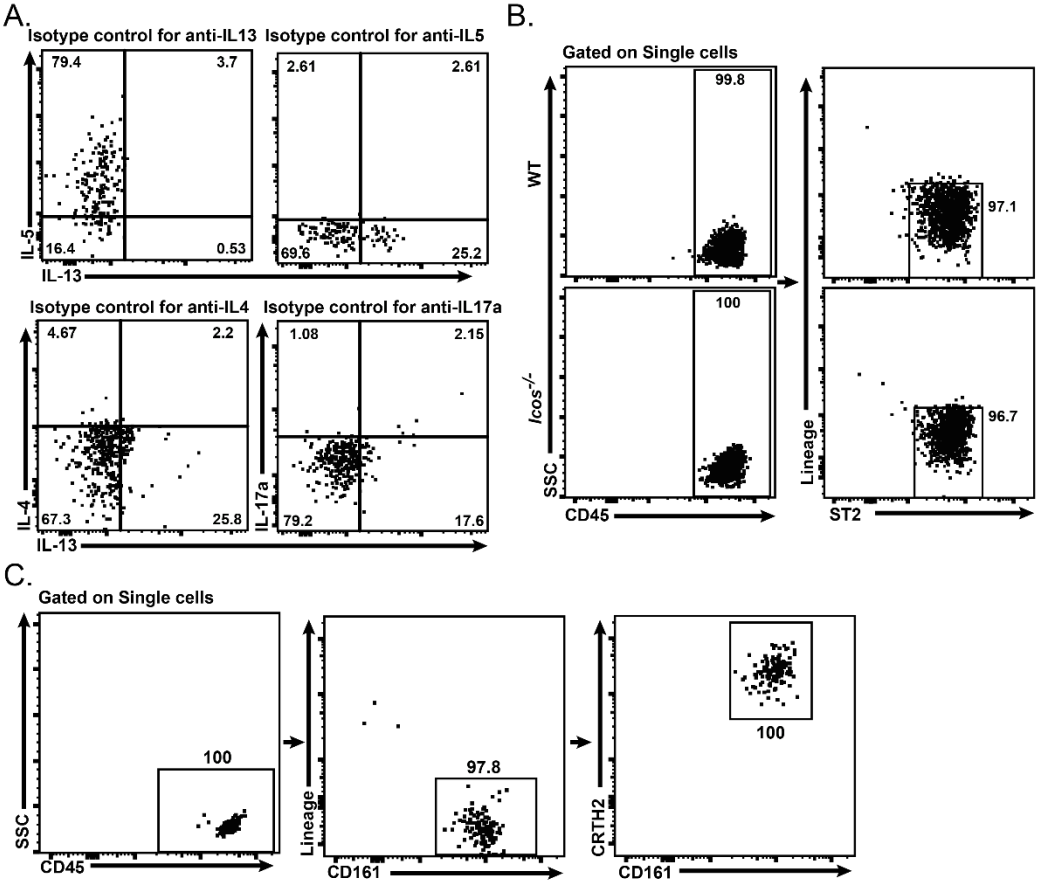


Supplementary figure 4 (relates to figure 6). Expression of ICOS-L in WT ILC2s co-cultured with *Icos*$^{-/-}$ ILC2s as an indication of ICOS:ICOS-L interaction in the presence of anti-ICOS blocking antibody or isotype-matched antibody (*: P<0.05, n=4).



Supplementary figure 5 (relates to figure 2). A) Isotype-matched stain controls for intracellular cytokine analyses.

Histogram demonstrations of isotype-matched stain controls for mouse IL-13, IL-5, IL-4 and IL-17a as mentioned in the figures. The corresponding data are demonstrated in figure 2. B) Purity of sorted mouse ILC2s. Single cells of mouse lungs were made by incubating the chopped lungs in collagenase D (400 U/ml) for 45 minutes at 37°C followed by washing, staining and sorting ILC2s lineage markers (CD3ε, CD45R, Gr-1, CD11c, CD11b, Ter119, NK1.1, TCR-γδ and FCεRI)-, CD45+ and ST2+ by BD FACS ARIA-III. Dot plots show gating and purity of sorted ILC2s**.** C) Purity of sorted human peripheral ILC2s. Human peripheral blood mononuclear cells were isolated from fresh blood using a Ficoll-based gradient isolation followed by staining and sorting ILC2s (CD3, CD14, CD16, CD19, CD20, CD56, CD235a, CD1a, CD123)$^-$, CRTH2$^+$, CD161$^+$, and CD45$^+$ by BD FACS ARIA-III. Dot plots show gating and purity of sorted ILC2s.



Supplementary Experimental Procedure

In vivo and In vitro stimulations of ILC2s

For *in vivo* stimulation studies described in figures 1, 3 and 4, carrier free recombinant mouse IL-33 (Biolegend, San Diego, CA, 0.5 μg/mouse in 50 μl) or PBS (50 μl) was administered intranasally to mice on three consecutive days. One day after the last intranasal stimulation lung function was measured, mice were euthanized and samples were taken. For *Alternaria* experiments described in figure 5, *Alternaria alternata* (Greerlabs, Lenoir, NC, 100 μg/mouse in 50 μl) or PBS (50 μl) was administered intranasally on four consecutive days followed by measurement of lung function and sample withdrawal one day after the last intranasal challenge. For *in vivo* inhibition of ICOS:ICOSL interaction mice received blocking anti-mouse ICOS (Clone: 7E.17G9, 250 μg/ml , BioXCell, West Lebanon, NH) or Rat IgG2b (Clone: LTF-2, 250 μg/ml, BioxCell, West Lebanon, NH) intraperitoneally. For experiments reported in figure 2, mouse ILC2s were purified from the lung of either BALB/cBYJ or *Icos*$^{-/-}$ mice based on the lack of expression of classical lineage markers (CD3e, CD45R, Gr-1, CD11c, CD11b, Ter119, NK1.1, TCR-γδ and FCεRI) and expression of CD45, ST2, and CD117, using BD FACSARIA III cell sorter with >95% purity (supplementary figure 2) then cells were injected into *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice intravenously followed by intranasal administration of IL-33 as described above.

Humanized mice and purification of humanILC2

For human peripheral ILC2, peripheral blood mononuclear cells (PBMCs) were first isolated from human fresh blood by diluting the blood 1:1 in PBS then adding to SepMate™-50 separation tubes (STEMCELL Technologies Inc, Vancuver, Canada) prefilled with 15-ml Lymphoprep™ each (Axis-Shield, Oslo, Norway) and centrifugation at 1200 xg for 15 minutes. Human PBMCs were then stained with antibodies against human lineage markers (CD3, CD14, CD16, CD19, CD20, CD56, CD235a, CD1a, CD123), CRTH2, CD161, CD127and CD45. Thereafter, ILC2s were defined as CD45^{+} lineage- CRTH2^{+} CD127^{+} CD161^{+} and purified by flow cytometry using BD FACS ARIA III (BD biosciences, San Jose, CA) with a purity of >95% (supplementary figure 5). Purified human ILC2s were cultured with rh-IL2 (20 ng/ml) and rh-IL-7 (20 ng/ml) for 3 days then adoptively transferred to *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice (2 x10^{4} cells/mouse) followed by *i.n.* administration of recombinant human IL-33 (0.5 μg/mouse) or PBS *i.n.* on day 1-3. On day 1 both groups received either anti-human (clone: 9F.8A4, 500 μg/mouse) + anti-mouse ICOS-L (clone: 16F.7E5, 500 μg/mouse) or isotype-matched control (500 μg/mouse). On day 4 lung function was measured and BAL was performed and analyzed. Anti-ICOS-L antibodies were generated by Dr. Gordon Freeman as described elsewhere (Akbari et al., 2002).

Flow cytometry antibodies and reagents

Biotinylated anti-mouse lineage (CD3e, CD45R, Gr-1, CD11c, CD11b, Ter119, NK1.1, TCR-γδ and FCεRI), Streptavidin-FITC, Streptavidin-BV510, BV421 anti-mouse CD25, BV510 anti-mouse CD90.2, PE Annexin V, Annexin V binding buffer were purchased from Biolegend (San Diego, CA). APC anti-mouse CD127, PerCP-

eFluor® 710 anti-Mouse ST2 (IL-33R), Streptavidin APC-eFluor® 780, PE anti-mouse ICOS (CD275), PE/Cy7 anti-mouse CD117 (c-kit), FITC anti-mouse Sca-1, PE/Cy7 anti-mouse CD45, FITC anti-mouse CD45, PE anti-mouse IL-5, PE/Cy7 anti-mouse IL-13, PE/Cy7 anti-mouse IL-4, APC anti-mouse IL-13, eFluor® 660 anti-mouse Ki-67, PE/Cy7 anti-mouse IL-17a, PE anti-mouse pSTAT5(Y694), PerCP/AF710 anti-mouse pSTAT6 (Y641), PE/Cy7 anti-mouse BCL-2, Fixation Permeabilization buffer set and Fixable Viability Dye eFluor® 780 were purchased from eBioscience (San Diego, CA). BV421 anti-mouse GATA3, BD Cytofix™Fixation Buffer and BD Phosflow™ Perm Buffer III were purchased from BD biosciences (San Jose, CA).

Analysis of BAL by Flow cytometry

After collecting BAL, cells were stained with PE-anti-SiglecF (BD biosciences), FITC-anti-CD19, PerCP/Cy5.5-anti-CD3e, APC-anti-Gr-1, PE/Cy7-anti-CD45, APC/Cy7-anti-CD11c (Biolegend) and eFluor450-anti-CD11b (eBioscience) in the presence of anti-mouse FC-block (BioXcell, West Lebanon, NH). Thereafter cells were washed twice with PBS + 1% BSA, and after adding countBright absolute count beads (Life Technologies, Grand Island, NY) at least $1x10^4$ $CD45^+$ cells were acquired on BD FACSCANTO-II (BD biosciences). Data were analyzed using FlowJo software (Treestar,Ashland, OR).

Gene expression analysis using Nanostring® nCounter technology

ILC2s were purified from a cohort of *Icos*$^{-/-}$ and naïve WT mice (30 and 20 mice respectively) and from 10 mice of each strain after 3 *i.n.* IL-33 administration using FACS. Total RNA was then isolated using MicroRNAeasy (Qiagen, Valencia, CA) and analyzed for gene expression by Nanostring® nCounter system (Seattle, WA) as described elsewhere (Geiss et al., 2008; Schmitt et al., 2014). Probes for the depicted genes were designed by Nanostring bio-informatics team and ordered from IDT-DNA technologies (Coralville, IA). The difference in gene expression were calculated between naïve *Icos*$^{-/-}$ and WT and IL-33 stimulated *Icos*$^{-/-}$ and WT mice and then ranged from -1 form the max decrease (by dividing the negative values by the least value) to +1 for the max increase (by dividing the positive values by the highest value) in each panel. Heat plots were generated using R statistical software.