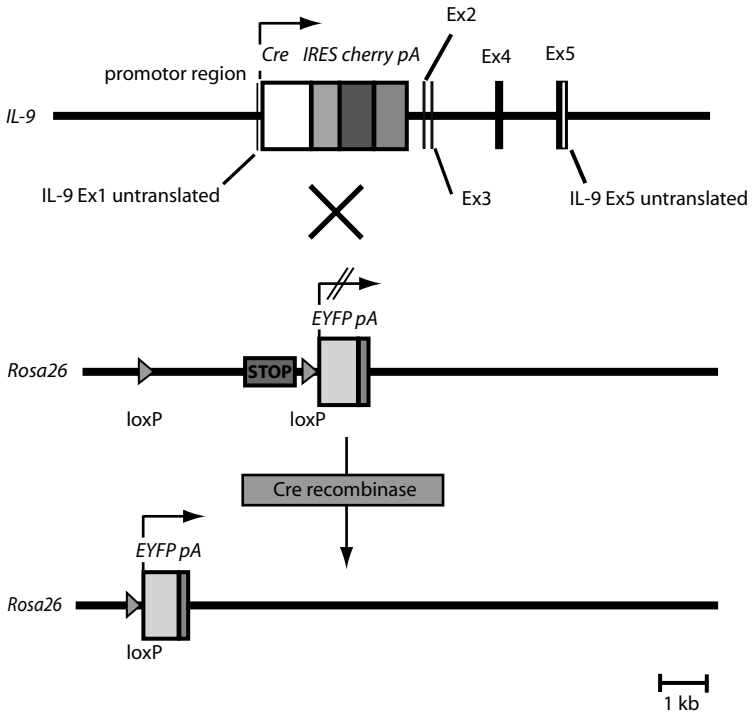
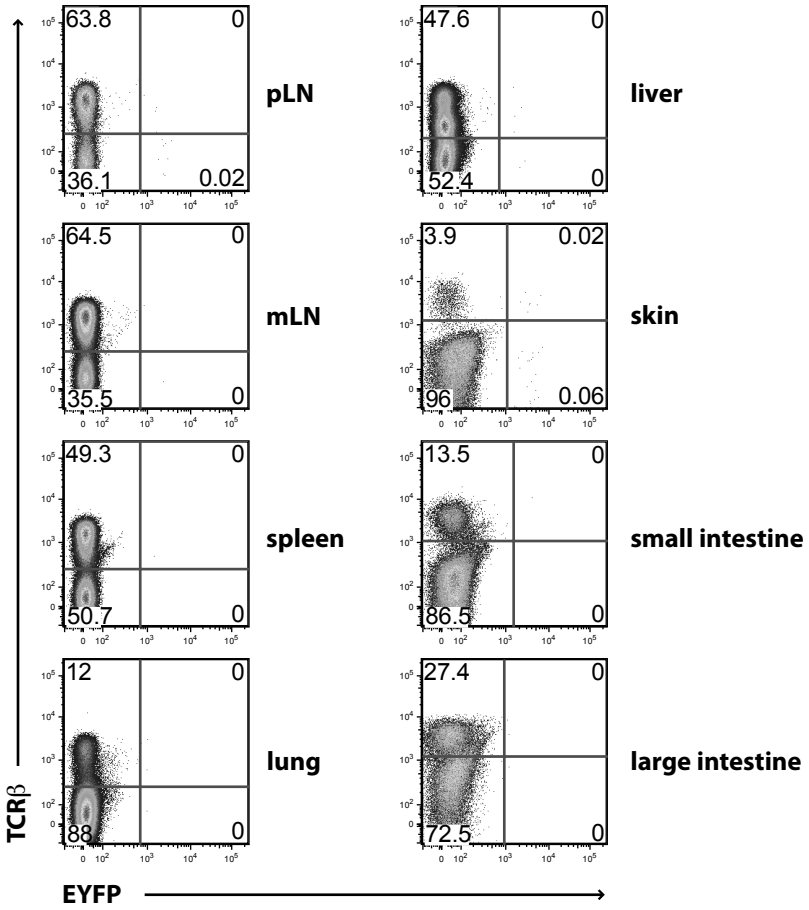
Supplementary Figure 1



Interleukin-9 fate reporter mice (*IL9^{Cre}*) crossed to Rosa26eYFP mice (*R26R^{eYFP}*) allows fate reporting of past IL-9 producing cells. In the absence of Cre expression the premature stop sequence in front of the eYFP gene in Rosa26eYFP mice prevents eYFP expression. Upon Cre expression, which is regulated by the IL-9 locus the stop sequence flanked by loxp sites in front of the eYFP gene is excised, turning on permanent eYFP expression in cells, which previously have expressed the IL-9 gene.

Generation of *IL9^{Cre}* mice: A plasmid for BAC-targeted recombineering, which contained 5′ and 3′ IL-9 homology arms flanking the Cre-IRES-cherry sequence was generated using vectors containing the Cre-IRES-pA and cherry sequence (a gift from Dr. Tim Sparwasser from the Technical University in Munich). Cre was placed upstream of the ATG translation initiation site. The targeting construct was inserted via homologous recombination into a BAC clone from the Children's Hospital of Oakland Research Institute (CHORI) BACPAC Resource Center, clone 282A18 from the RPCI 24 Mouse BAC Library (RP24-282A18), which contained the IL-9 gene and approximately 140 kb of flanking sequence upstream and 50 kb downstream. BAC identity was verified by PCR and restriction digestion. Prior to recombining with the target construct the SacB gene in the BAC backbone (vector pTARBAC1) was removed by targeted homologous recombination. Resulting recombined BAC clones were checked for site directed integration by southern blotting and the inserted modification including flanking regions was sequenced. Purified BAC DNA was injected into fertilized oocytes prepared from the F1 generation of CBAxB10 mice for IL9Cre reporter mouse generation.

Supplementary Figure 2

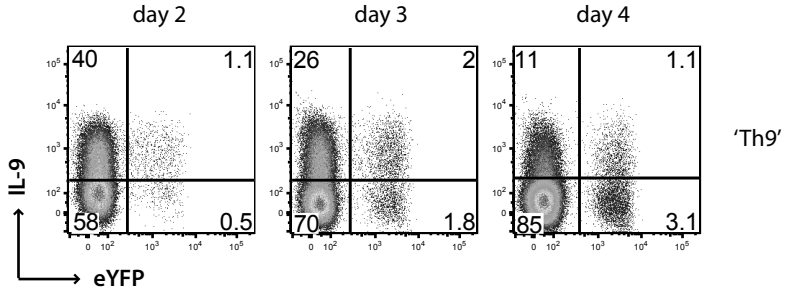


Un-challenged *IL9^{Cre}R26R^{eYFP}* reporter mice do not display eYFP^{+} cells

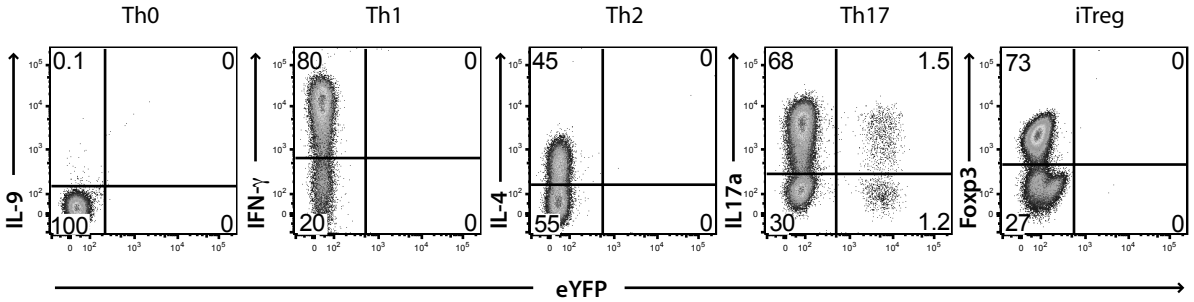
Flow cytometry of cells isolated from peripheral and mesenteric lymph nodes, spleen, lung liver, skin, small and large intestine of un-challenged naïve *IL9^{Cre}R26R^{eYFP}* mice, stained for TCR-β expression and assessed for eYFP expression. pLN: peripheral lymph nodes; mLN: mesenteric lymph nodes. Numbers in quadrants indicate percent cells in each. Data represents two independent experiments with 2 mice in each experimental group.

Supplementary Figure 3

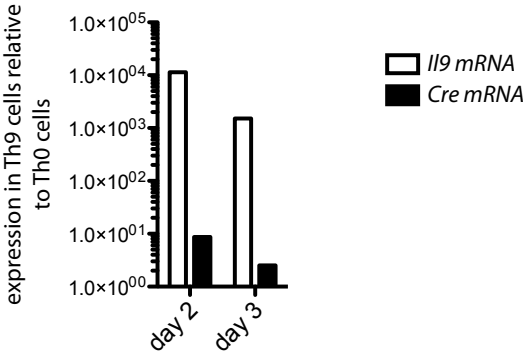
a



b



c



Induction of fate reporter eYFP$^+$ cells among IL-9-producing cells
a) Flow cytometry of naïve CD4+ CD44low CD25- T cells from *IL9^{Cre}R26R^{eYFP}* mice cultured under 'Th9' conditions for the indicated days and stained for intracellular IL-9 **b)** Flow cytometry of naive CD4$^+$ CD44^{low} CD25$^-$ T cells from *IL9^{Cre}R26R^{eYFP}* cultured for 4 days under Th0, Th1, Th2, Th17 or inducible regulatory T (iTreg) conditions and stained for intracellular cytokines or Foxp3. **c)** FACS sorted naïve T cells were cultured under T_H9 or T_H0 conditions for 2 and 3 days. IL9 and Cre specific transcripts were measured by qPCR. Figure shows fold induction over T_H0 cells. Numbers in quadrants indicate percent cells in each. Data represents at least three independent experiments.

In vitro T cell differentiation and cytokine determination: FACS sorted naïve T cells (CD4$^+$, CD25$^-$, CD44^{low}) were cultured in Iscove's modified Dulbecco medium (IMDM Sigma) supplemented with 5% FCS, 2x10-3M L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 5x10-5 M mercaptoethanol (all Sigma) in the presence of anti- CD3 (1µg/ml) and 10µg/ml anti-CD28 (both plate bound). Cytokines for effector cell differentiation were: 3ng/ml IL-12 for TH1, 10ng/ml IL-4 for TH2, 20ng/ml TGFβ for iTreg, 1ng/ml TGFβ, 20ng/ml IL-6 and 20ng/ml IL-1β for TH17 and 10ng/ml IL-4, 10ng/ml IL-6, 25ng/ml IL-1β and 5ng/ml TGFβ for 'TH9'. Cells were cultured for 4 days and then restimulated for 4h with PdBU/ionomycin (both at 500 ng/ml) in the presence of brefeldin A (1µg/ml) before intracellular staining for cytokines. In some cases cultures were analysed on day 2 to 4 after culture initiation. Antibodies for surface and intracellular staining were purchased from Biolegend.

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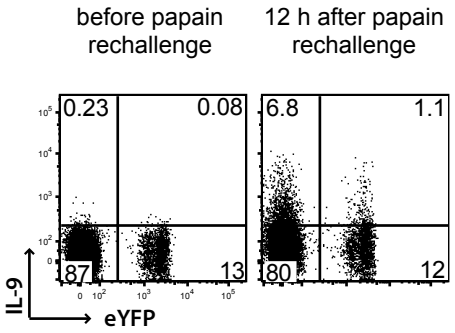
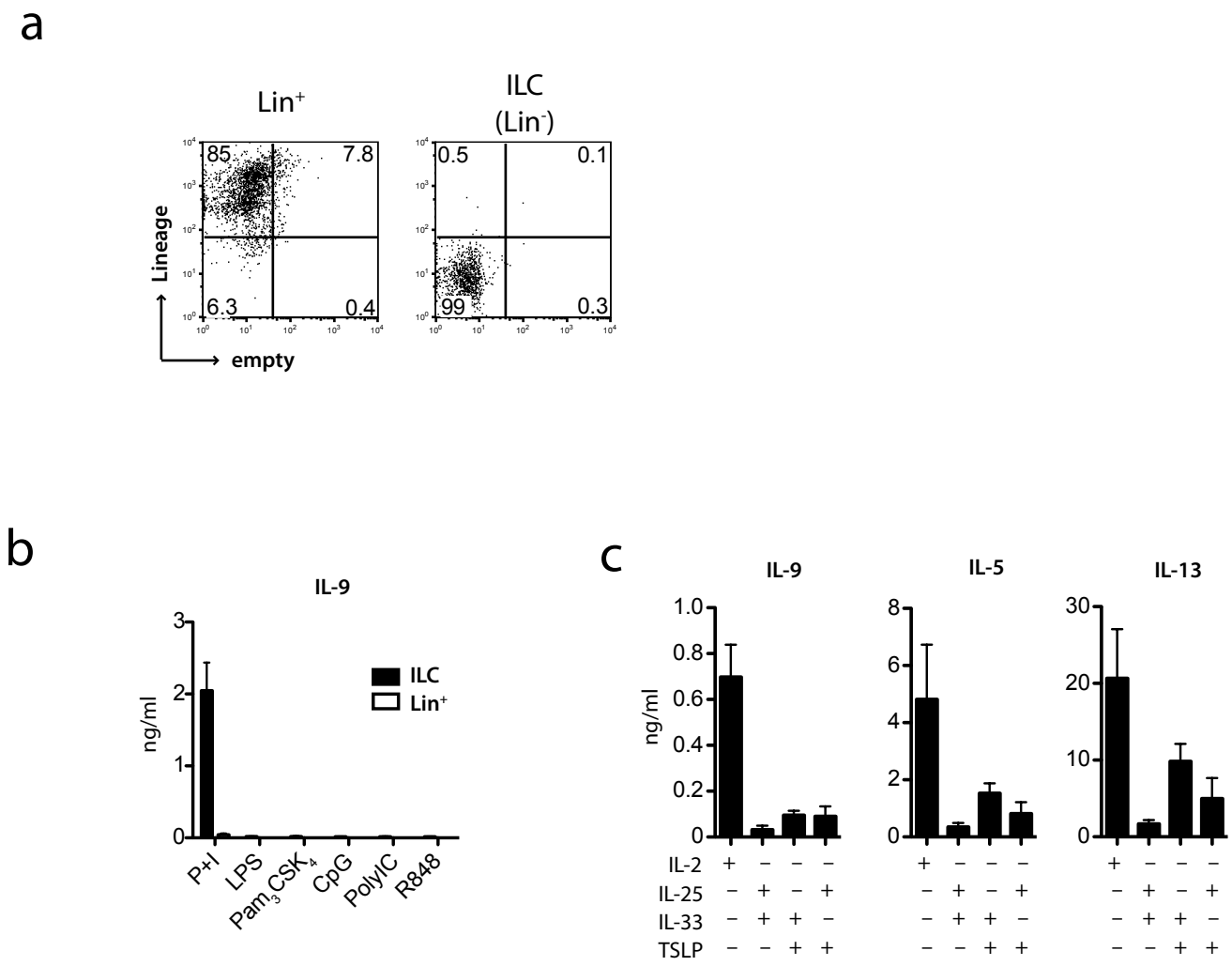
Supplementary Figure 4



IL-9 production from eYFP$^+$ ILC

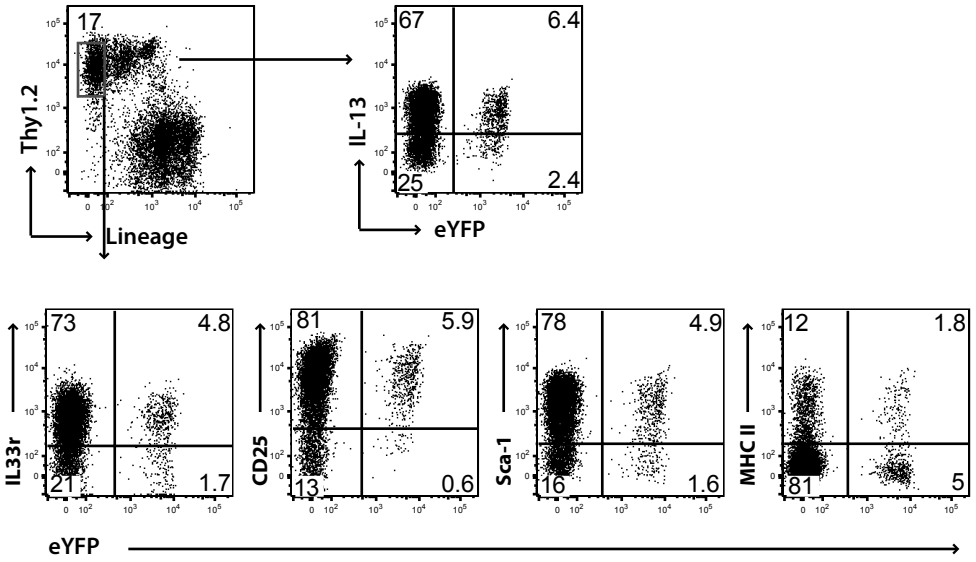
Flow cytometry of lung cells isolated from papain *IL9^{Cre}R26R^{eYFP}* mice before or 12 h after papain re-challenge, gated on ILC and assessed for intracellular IL-9 staining and eYFP. Numbers in gates or quadrants indicate percent cells in each. Data represents at least two independent experiments.

Supplementary Figure 5



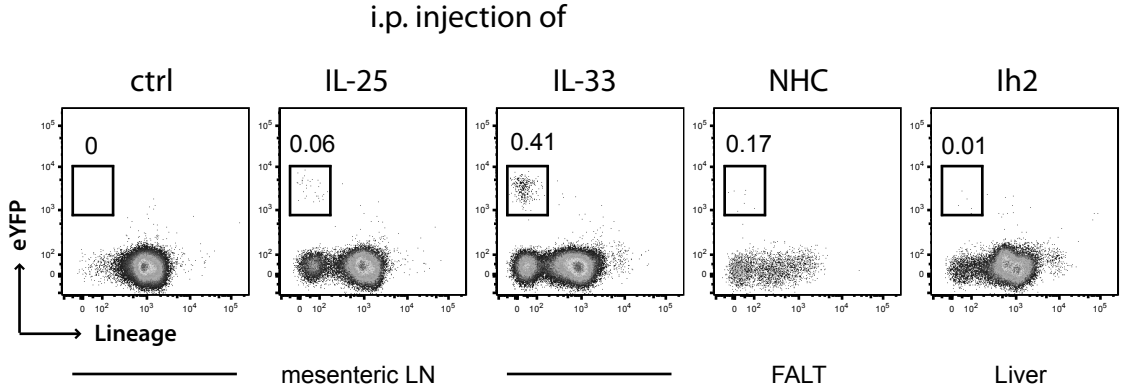
MACS sorted ILC do not produce IL-9 upon TLR or IL25, IL33 and TSL stimulation
a) Flow cytometry of ILC and Lin$^+$ cells stained for lineage marker after MACS separation Numbers in quadrants indicate percent cells in each. **b)** IL-9 concentration in the supernatant of MACS sorted ILC (black bars) and cells expressing lineage markers (Lin$^+$, white bars) isolated from the lungs of papain challenged mice and stimulated in vitro overnight with P+I, LPS, Pam_3CSK_4, CpG, Poly I:C and R848. **c)** IL-9, IL-5 and IL-13 concentration in the supernatant of MACS sorted ILC isolated from the lungs of papain challenged mice and stimulated in vitro overnight with IL-2 or a combination of IL-25, IL-33 and TSLP as indicated. Data represents three (a and b) or two (c) independent experiments (mean ±SEM). P+I, PdBU and ionomycin.

Supplementary Figure 6



IL-33 induces eYFP^{+} ILC resembling ILC generated in papain mediated lung inflammation
a) Flow cytometry of lung cells isolated from *IL9^{Cre}R26R^{eYFP}* mice challenged intranasally with IL-33, stained for surface lineage markers CD4, CD8α, TCRβ, TCRγδ, CD19, Nkp46, Gr-1, CD11c, Ter-119, CD11b (lineage) and Thy1.2 (upper left panel) and lung cells restimulated with PdBU and ionomycin for 2.5 h and assessed for intracellular IL-13 and eYFP in the ILC compartment (upper right panel). The lower panel shows flow cytometry of papain challenged *IL9^{Cre}R26R^{eYFP}* mice stained for IL33R, CD25, MHC II and Sca-1 gated on ILC and assessed for eYFP expression. Numbers in gates or quadrants indicate percent cells in each. Data represents at least two independent experiments.

Supplementary Figure 7

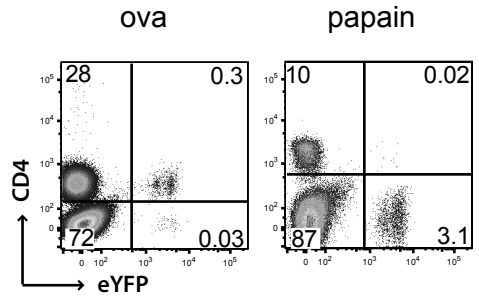


eYFP^{+} expression in nuocytes, natural helper cells and Ih2 cells
Flow cytometry of cells isolated from the mesenteric lymph node of unchallenged *IL9^{Cre}R26R^{eYFP}* control mice (ctrl), mice injected with IL-25 or IL-33 or cells isolated from the liver and fat associated tissue (FALT) of naïve mice. Cells were gated on lymphocytes, stained for surface lineage markers (lineage) and assessed for eYFP expression. Numbers in gates or quadrants indicate percent cells in each. Data represents at least two independent experiments. NHC, Natural helper cells; Ih2, Innate helper cells type 2.

Intraperitoneal injection of IL-25 and IL-33
IL9^{Cre}R26R^{eYFP} mice were intraperitoneal injected on three consecutive days with 500 ng IL-25 or IL-33 in PBS.

Isolation of cells from FALT and liver
Mesenteric fat tissue was separated from the intestine and mesenteric lymph nodes, minced and digested for 40 min at 37˚C with 1 mg/ml liberase TL (Roche) in serum free IMDM medium. Samples were passed through a 70 µm cell strainer and stained for flow cytometry analysis. To isolate liver lymphocytes, whole livers were passed trough a 70 µm cell strainer. Isolated cells were further purified using a 37.5% percoll gradient, followed by lysis of red blood cells and staining for flow cytometry analysis.

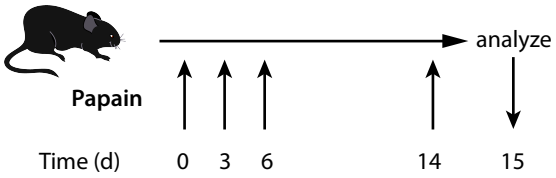
Supplementary Figure 8



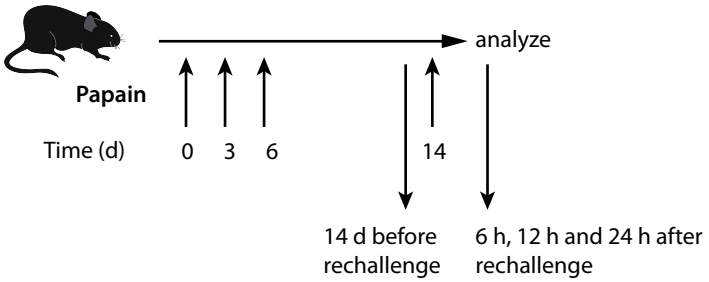
Ova induced lung inflammation results in eYFP$^+$ CD4$^+$ cells but not eYFP$^+$ ILC
Flow cytometry of lung cells isolated from ova (left panel) or papain (right panel) challenged *IL9^{Cre}R26R^{eYFP}* mice gated on lymphocytes, stained for CD4 and assessed for eYFP expression. Numbers in quadrants indicate percent cells in each. Data represents three independent experiments.

Ova induced airway inflammation
Mice were sensitized with 50 µg OVA in alum administered by intraperitoneal injection (day 0) followed by 5 intranasal challenges with 100 µg OVA on day 7, 10, 13, 16 and 21. 24 h after the last challenge mice were sacrificed and lung cells isolated as described in the Materials and Methods section.

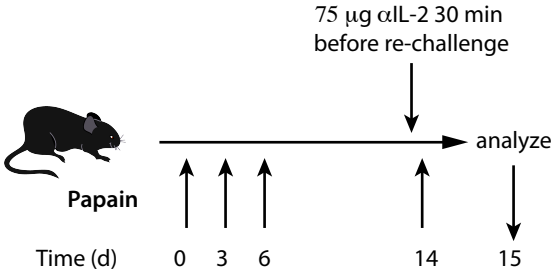
Supplementary Figure 9



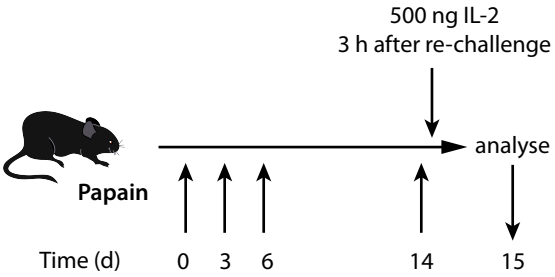
challenge protocol corresponding to Figure 1a



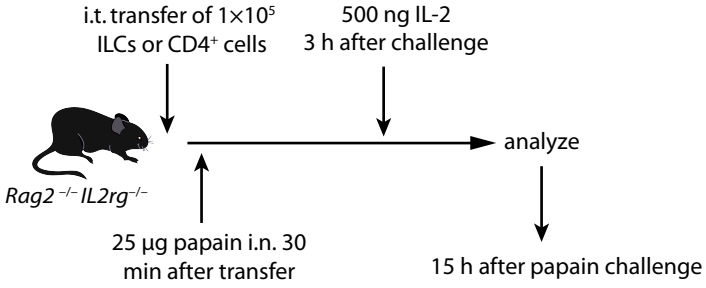
challenge protocol corresponding to Figure 2b



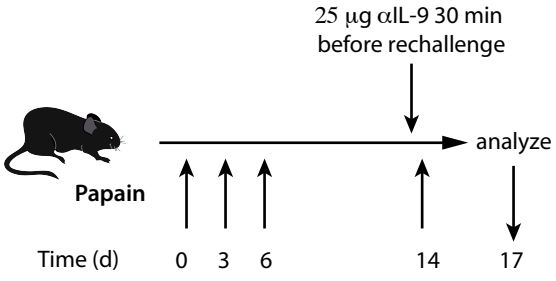
challenge protocol corresponding to Figure 5c



challenge protocol corresponding to Figure 5d



challenge protocol corresponding to Figure 6e



challenge protocol corresponding to Figure 7a and f