Supplemental material

Webb et al., https://doi.org/10.1084/jem.20180131

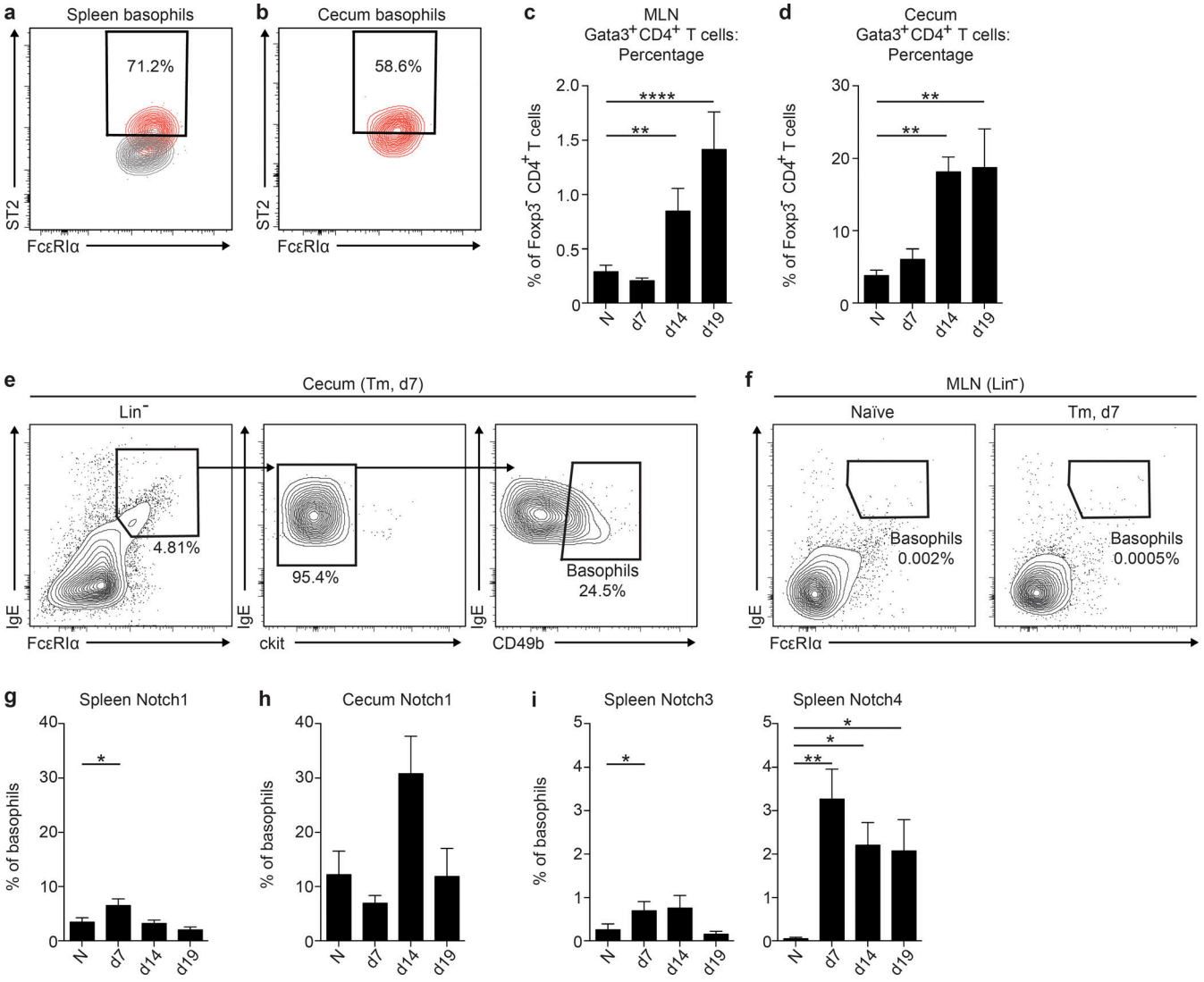

Figure S1. **Basophil phenotype. (a and b)** Representative plots of spleen (a) and cecum (b) basophil (gated as in Fig. 1 a) ST2 expression (gray, isotype; red, fully stained). **(c and d)** MLN (c) and cecum (d) percentage of Gata3$^+$ Th2 cells of TCRβ$^+$CD4$^+$Foxp3$^-$ cells in naive (N) and *T. muris*–infected (Tm) C57BL/6 mice on day (d) 7, 14, and 19 p.i. **(e and f)** Representative plots of cecum basophils (e; CD45$^+$Lineage(Lin)$^-$FcεRIα$^+$IgE$^+$CD49b$^+$ckit$^-$) and percentage of basophils of Lin$^-$ cells (f; percentage is FcεRIα$^+$IgE$^+$CD49b$^+$ckit$^-$ of Lin$^-$) in the MLN in day 7 *T. muris*–infected C57BL/6 mice. **(g–i)** Percentage of spleen (g) and cecum (h) basophils that expressed Notch1 and spleen basophils (i) that expressed Notch3 or 4 in N- and *T. muris*–infected C57BL/6 mice on day 7, 14, and 19 p.i. Mean ± SEM; *, P < 0.05; **, P < 0.01; ****, P < 0.0001; analyzed using (c, d, g, and i) a linear fixed-effect model with pairwise comparison; (a and b) representative of three experiments; (c and d) three experiments pooled (N, *n* = 18; Tm, *n* = 7–13/time point); (e and f) representative of three experiments; (g–i) three experiments pooled (N, *n* = 18; Tm, *n* = 7–13/time point).

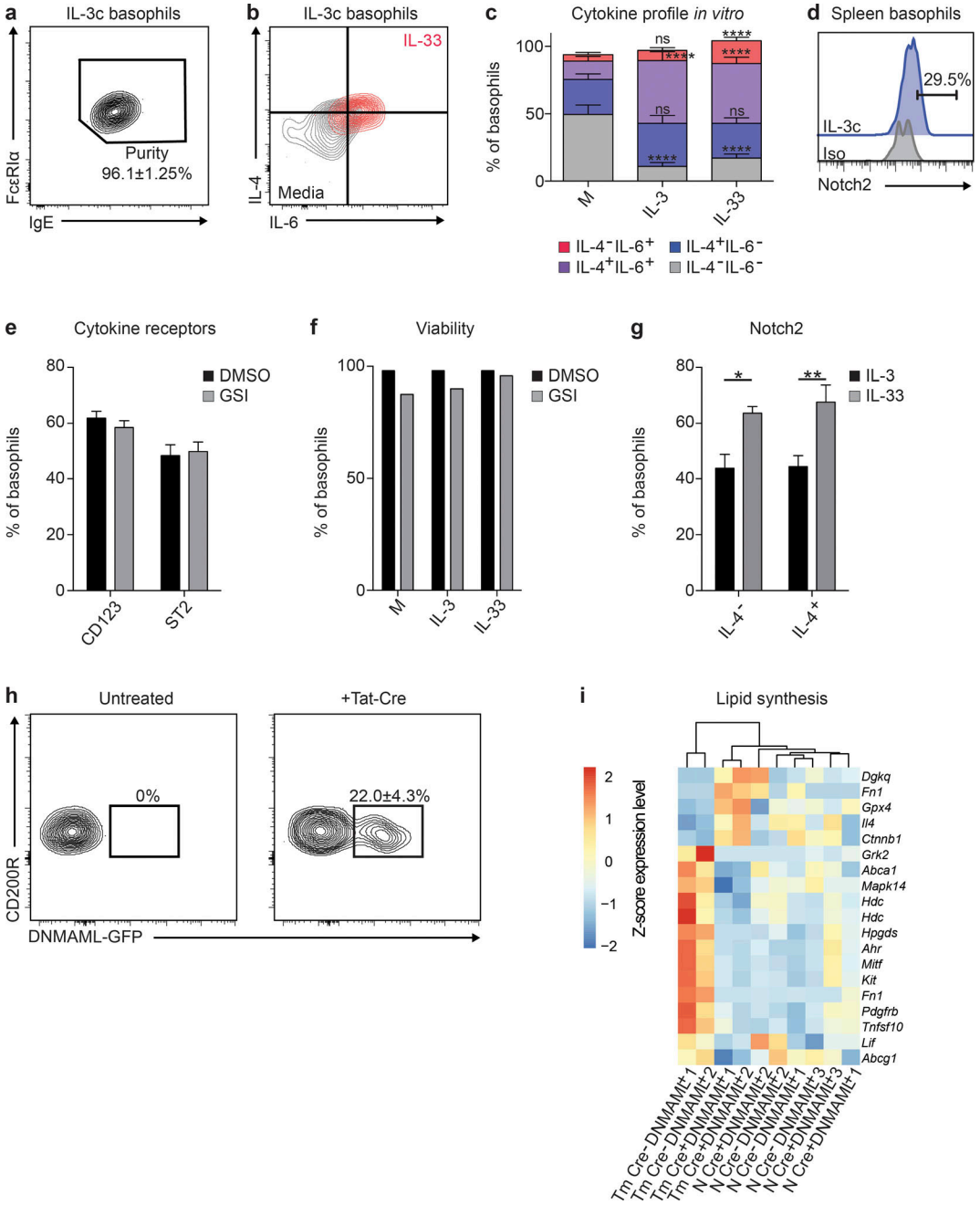

Figure S2. **Basophil in vitro cultures and RNaseq. (a)** Representative plot of splenic basophils sort-purified from IL-3c–treated mice. Mean ± SEM purity calculated as proportion of singlet, live CD45⁺Lin⁻IgE⁺FcεRIα⁺ in the lymphocyte gate (forward scatter area/side scatter area [FSC-A/SSC-A]). **(b)** Representative plot of IL-3c–elicited splenic basophil IL-4 and IL-6 intracellular staining. **(c)** Percentage of basophils staining positive for IL-4 and/or IL-6 following 18 h culture in media alone or with 10 ng/ml rmIL-33 or rmIL-3. **(d)** Representative histogram showing Notch2 staining on IL-3c–elicited splenic basophils (gray, isotype; blue, fully stained). **(e)** Percentage of IL-3c–elicited splenic basophils expressing CD123 or ST2 following 18-h culture in media alone or with 10 ng/ml rmIL-33 or rmIL-3. **(f)** Viability of IL-3c–elicited splenic basophils cultured in the presence or absence of 10 ng/ml IL-3 or IL-33 with DAPT (GSI) or a vehicle control (DMSO) for 3 d. **(g)** Percentage of IL-4⁺ and IL-4⁻ IL-3c–elicited splenic basophils expressing Notch2 following 18 h culture in media alone or with 10 ng/ml rmIL-33 or rmIL-3. **(h)** Representative plots of expression of DNMAML-GFP in Mcpt8Cre⁻ DNMAML⁺ basophils sort-purified from IL-3c–treated mice following 18 h in culture with media alone or Tat-Cre. **(i)** Heatmap showing expression of genes depicted as z-score associated with IPA-designated groups of genes associated with lipid synthesis in basophils from naive, IL-3–treated (N), and day 14 *T. muris*–infected, IL-3c–treated (Tm) Mcpt8Cre⁺ DNMAML⁺ and Mcpt8Cre⁻ DNMAML⁺ mice (genes filtered on differences between day 14 *T. muris*–infected Mcpt8Cre⁺ DNMAML⁺ and Mcpt8Cre⁻ DNMAML⁺ basophils (−log10(q value) > 1), samples arranged by hierarchal clustering). Mean ± SEM; *, P < 0.05; **, P < 0.01; ****, P < 0.0001; analyzed by using (c and g) a linear fixed-effect model with pairwise comparison; (a) representative of all sorting experiments depicted; (b) representative of five experiments; (c) five experiments pooled (fifteen to sixteen wells/condition total); (d) representative of three experiments; (e) three experiments pooled (five wells/condition total); (f) representative of two experiments (one well/condition shown); (g) three experiments pooled (IL-3, *n* = 14; IL-33, *n* = 12); (h) representative of two experiments; and (i) samples included cells from three to four pooled mice.

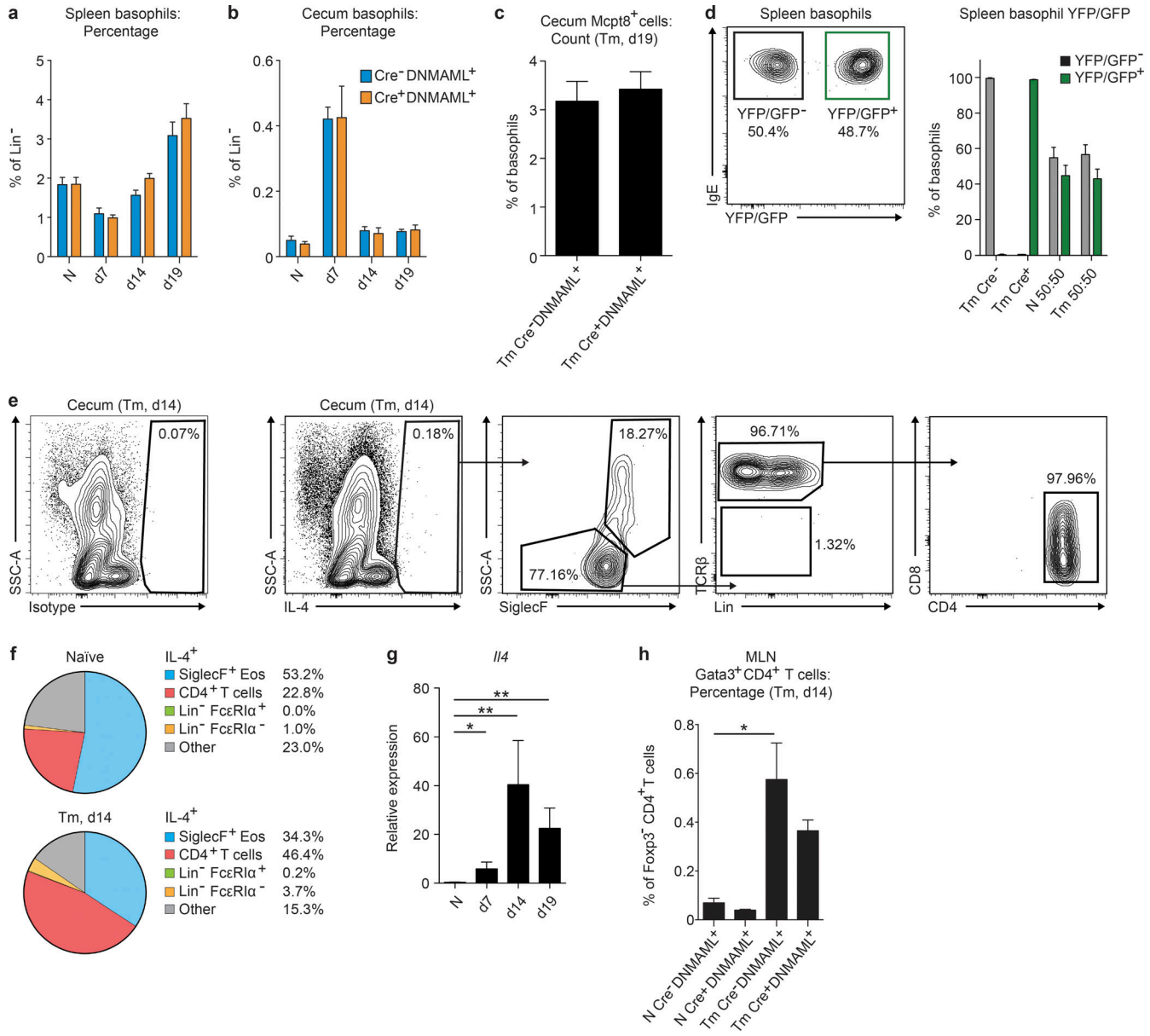

Figure S3. **Immune activation in the MLN, spleen, and cecum of *T. muris*–infected mice lacking Notch signaling in basophils. (a and b)** Spleen (a) and cecum (b) basophil percentage of Lin⁻ cells in naive (N) and *T. muris*–infected (Tm) Mcpt8Cre⁻ DNMAML⁺ and Mcpt8Cre⁻ DNMAML⁺ mice on day (d) 7, 14, and 19 p.i. **(c)** Average number of Mcpt8⁺ basophils per high-powered field from Mcpt8Cre⁻ DNMAML⁺ and Mcpt8Cre⁺ DNMAML⁺ mice day 19 after *T. muris* infection quantified from immunofluorescent staining. **(d)** Distribution of Mcpt8Cre⁻ DNMAML⁺ and Mcpt8Cre⁺ DNMAML⁺ basophils in 50:50 and single genotype transfer chimeric mice that were N or on day 19 p.i. with *T. muris* in the spleen. **(e and f)** Representative gating (e) and quantification (f) of IL-4–expressing cell types in the cecum of N or day 14 *T. muris*–infected C57BL/6 mice following 5 h incubation with Brefeldin A. **(g)** *Il4* gene expression (relative to *Actβ* and normalized to N) measured by real-time PCR in colon homogenates in N and *T. muris*–infected C57BL/6 mice on day 7, 14, and 19 p.i. **(h)** Percentage of TCRβ⁺CD4⁺CD8⁻Foxp3⁻ cells that expressed Gata3 in the MLN in N or day 14 *T. muris*–infected Mcpt8Cre⁻ DNMAML⁺ and Mcpt8Cre⁺ DNMAML⁺ mice. Mean ± SEM; *, $P < 0.05$; **, $P < 0.01$; analyzed (g and h) using a linear fixed-effect model with pairwise comparison; (a, b, and g) three experiments pooled (N, $n = 6$; Tm, $n = 5$–7/time point); (c) two experiments pooled ($n = 8$); (d) representative of two experiments (Tm single transfer controls, $n = 2$–3; 50:50 N, $n = 4$; 50:50 Tm, $n = 7$); (e) representative of two experiments; (f) two experiments pooled (N, $n = 4$; Tm, $n = 7$); and (h) three experiments pooled (N, Tm $n = 6$).

Table S1 is provided online as a separate Excel file, and lists gene information for all differentially expressed genes (with q value <0.1, β value >|1.5|) between Mcpt8Cre⁻ DNMAML⁺ and Mcpt8Cre⁺ DNMAML⁺ basophils from mice on day 14 of *T. muris* infection following IL-3c treatment.