Supporting Information

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SI Materials and Methods

In Vitro Eosinophil Differentiation Culture. Bone marrow from a single mouse was harvested in Iscove's modified Dulbecco's media (IMDM) (Gibco; 31980), 10% (vol/vol) FBS (HyClone; Thermo Scientific), 1% Pen Strep (Gibco; 15140), 2 mM glutamine (Gibco; 25030), and 55 μM 2-mercaptoethanol (Sigma; M6250), using a modification of previously described methods (1). During the 14-d culture, the culture media was supplemented with FMS-like tyrosine kinase 3 ligand and stem cell factor at 100 ng/mL (Peprotech) for the first 4 d and IL-5 at 10 ng/mL (Peprotech) for the following 8 d. Results from H&E staining and FACS analysis indicated that the purity of eosinophils was consistently >90–95% at day 14.

Flow Cytometry. Freshly extracted total bronchoalveolar lavage fluid (BALF) cells were resuspended with FACS buffer (0.5% BSA with 0.01% sodium azide in PBS) and stained with one of two panels of fluorescent antibodies. Panel A was used for the 4get system (for BALB/c transfer): CD11c, eGFP (no staining required), sialic acid-binding Ig-like lectin F (Siglec F) and CD11b. Panel B was used for the CD45.1 system (for C57BL/6 transfer): CD11c, CD45.2, Siglec-F, CD11b, and CD45.1. The antibodies (clone number, manufacturer) were as follows: CD11c Pacific Blue (N418; Biolegend), CD11b PE-Cy7 tandem dye (M1/70; BD Biosciences), CD45.1 Alexa Fluor 647 (A20; Biolegend), CD45.2 Alexa Fluor 488 (104; Biolegend), and Siglec-F PE (E50-2440; BD Biosciences). All staining was freshly performed on ice for 20 min, and samples were then washed with FACS buffer and subjected to a flow cytometer (FACS CANTO II; BD). FACS data were analyzed by the FlowJo software (Tree Star).

Transwell Assay. For each genotype, one million bone-marrow–derived eosinophils were washed with PBS and placed in the upper chamber (24-well plate with 3.0-μm pore polycarbonate membrane insert) (Corning; 3402) with 10 ng/mL IL-5; mouse recombinant eotaxin-1 (Peprotech; 250-01) of different concentrations was added to the lower chamber. The Transwell was incubated at 37 °C with 5% CO_2 for 3 h followed by FACS enumeration.

Eosinophil Adhesion Assay. Mouse epithelial cell line mouse aortic endothelial cells (MAEC) (2) were plated on 96 wells at passage 3 and treated with 20 ng/mL mTNF-α for 24 h upon 100% confluency. Fifty thousand calcein AM (Invitrogen; C-1429)-labeled eosinophils were plated in each well in 100 μL media with 10 ng/mL IL-5. After a 1-h incubation, wells were washed with PBS three times and fluorescence was read to assess the adhering percentage after baseline subtraction in the form of percentage remaining.

1. Dyer KD, et al. (2008) Functionally competent eosinophils differentiated ex vivo in high purity from normal mouse bone marrow. *J Immunol* 181(6):4004–4009.

2. Nishiyama T, et al. (2007) Functional analysis of an established mouse vascular endothelial cell line. *J Vasc Res* 44(2):138–148.

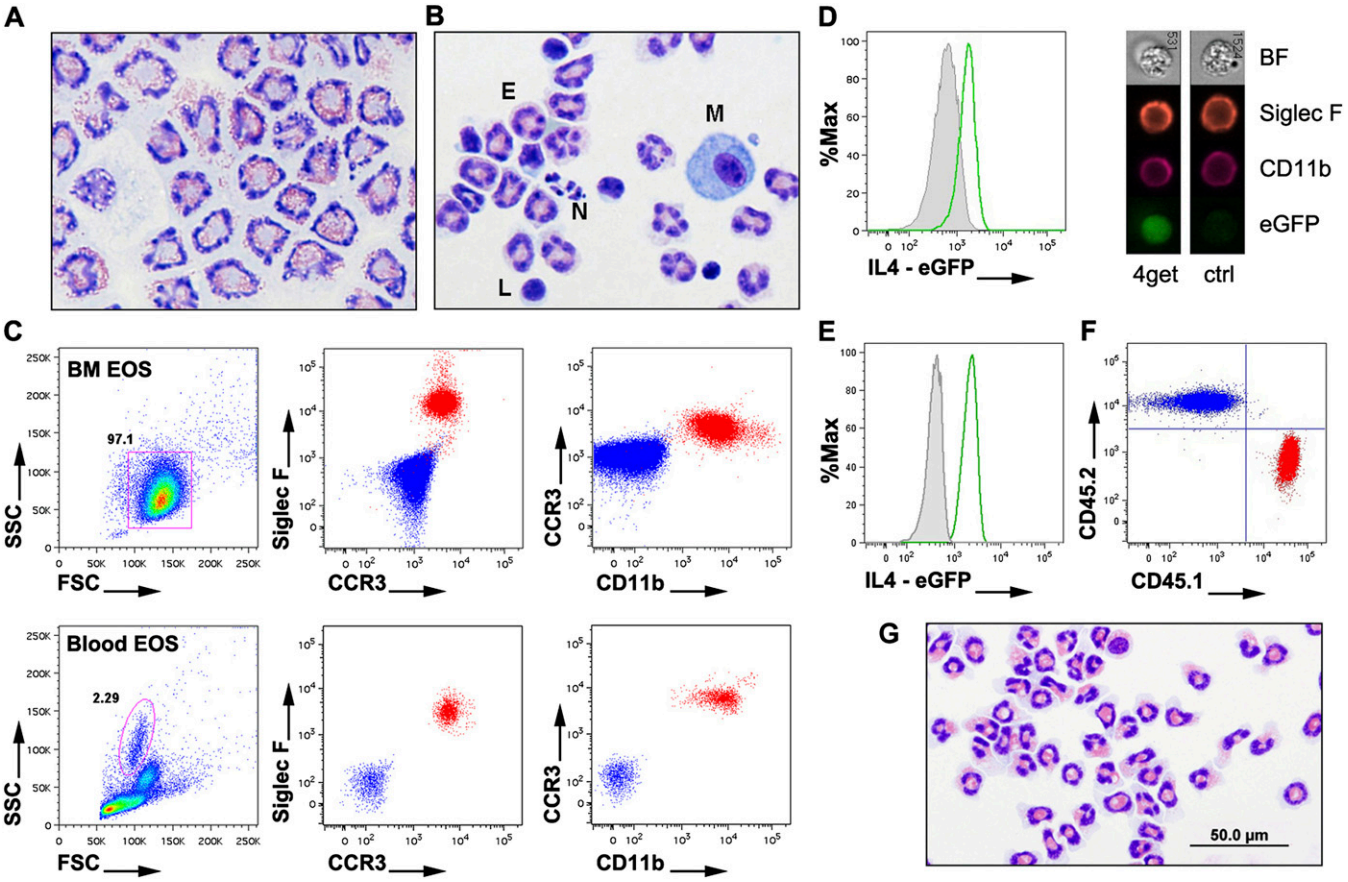

Fig. S1. Characteristics of bone-marrow–derived eosinophil donor cells. (*A*) Representative cytospin photomicrograph of day 14 bone-marrow–derived eosinophil culture. (*B*) Representative photomicrograph of a cytospin preparation from allergen-challenged BALF cells in experimental asthma models with ~80% eosinophils. E, eosinophils; N, neutrophils; L, lymphocytes; M, macrophages. (*C*) Blood and day-14 bone-marrow–cultured eosinophils (BM EOS) were identified by SSC^{high}, CCR3-CD11b-Siglec F triple-positive events (red) with blank staining as control (blue). (*D*) Bone-marrow–cultured wild-type and IL4-eGFP (4get) eosinophils were subjected to FACS for IL4-eGFP expression at day 14. The histogram together with the flow imaging micrograph indicates intracellular IL-4 expression by 4get eosinophils (green histogram) compared with wild-type control eosinophils (solid gray, ctrl; BF, bright field). (*E*) Allergen-induced experimental asthma was established in wild-type and 4get BALB/c mice. The superimposed histogram shows the IL4-eGFP expression levels of wild-type (solid gray) and 4get (green) mice after a serial gating. (*F*) Day 14 eosinophils cultured from bone marrow harvested from CD45.1 (red) and CD45.2 (blue) C57BL/6 mice were analyzed by FACS (superimposed plot). (*G*) The universal eosinophil FACS gating strategy used in this study guarantees >99% purity as shown by FACS sorting on total asthmatic BALF cells by Siglec $F^{+}CD11b^{+}CD11c^{-}SSC^{high}$. All data are representative of at least two experiments.

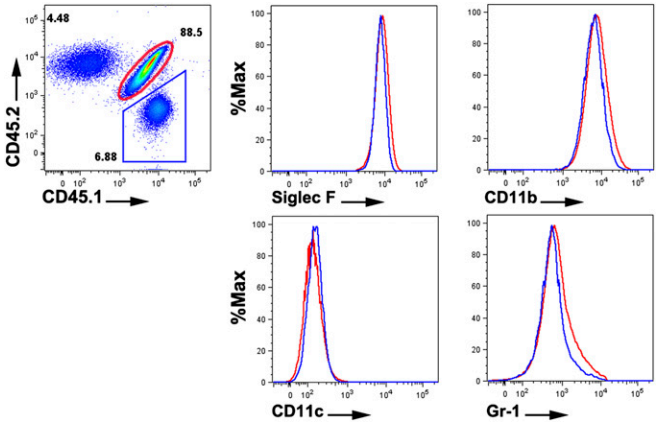

Fig. S2. Comparable properties between donor and recipient eosinophils after airway migration. From a representative cotransfer experiment, airway-migrated CD45.1 wild-type donor eosinophils' (blue gate and histogram) surface marker expression was compared with the heterozygous recipient's native ones (red gate and histogram) following serial eosinophil gating (data representative of at least three experiments).

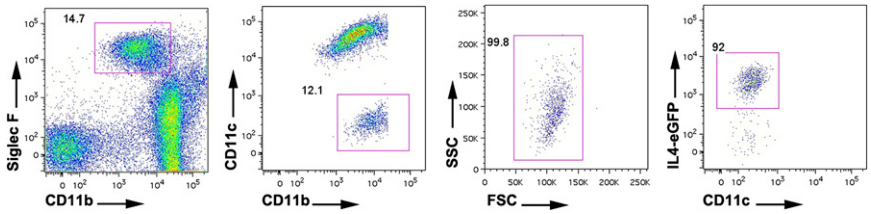

Fig. S3. Specific eosinophil gating strategy for eosinophil lineage deficient (ΔdblGATA-1) recipient mice. The 4get donor eosinophils were i.v. transferred into allergen-challenged ΔdblGATA-1 mice and constitute >90% of the airway eosinophil pool.

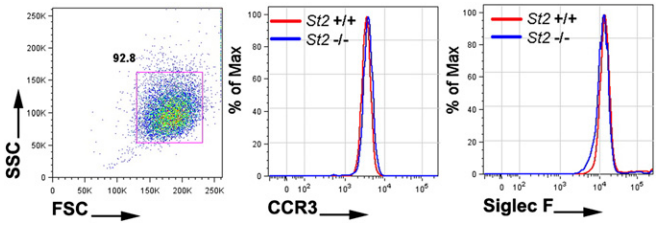

Fig. S4. Surface marker expression of $St2^{+/+}$ and $^{-/-}$ bone-marrow–derived eosinophils. Following the bone marrow culture, compared with $St2^{+/+}$ controls, $St2^{-/-}$ eosinophils exhibit similar levels of eosinophil surface marker expression, namely the chemotactic receptor CCR3 and specific surface inhibitory receptor Siglec-F.

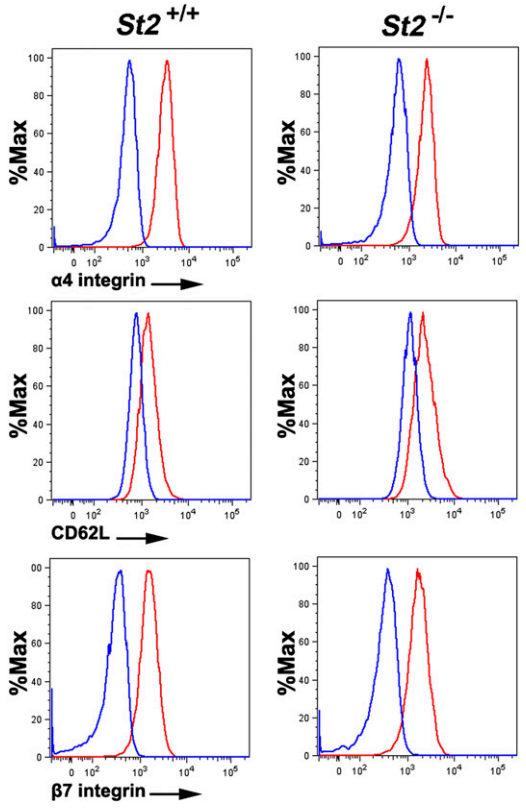

Fig. S5. Adhesion molecule expressions of $St2^{+/+}$ and $^{-/-}$ bone-marrow–derived eosinophils. $St2^{-/-}$ cultured eosinophils develop similar levels of major eosinophil adhesion molecules compared with $St2^{+/+}$ ones, including α4 integrin, β7 integrin and CD62L/L-selectin (blue histogram, isotype staining; red histogram, molecule of interest staining).

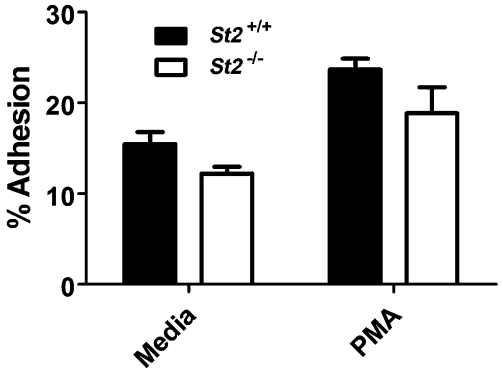

Fig. S6. In vitro eosinophil endothelial adhesion assay. Fluorescence-labeled $St2^{+/+}$ and $St2^{-/-}$ eosinophils' adhesion to mouse endothelial cells was assessed in the presence of 10 ng/mL IL-5 alone or together with 10 ng/mL phorbol 12-myristate 13-acetate (PMA).

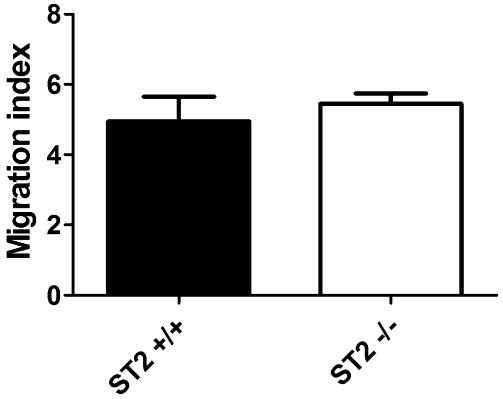

Fig. S7. Comparable chemotaxis toward eotaxin-2 between $St2^{+/+}$ and $^{-/-}$ bone-marrow–derived eosinophils. $St2^{+/+}$ and $St2^{-/-}$ cultured eosinophils were subjected to an eotaxin-2 (25 ng/mL)-driven transwell assay in the presence of 10 ng/mL IL-5. Migration index was calculated by (migration events − baseline events)/baseline events.