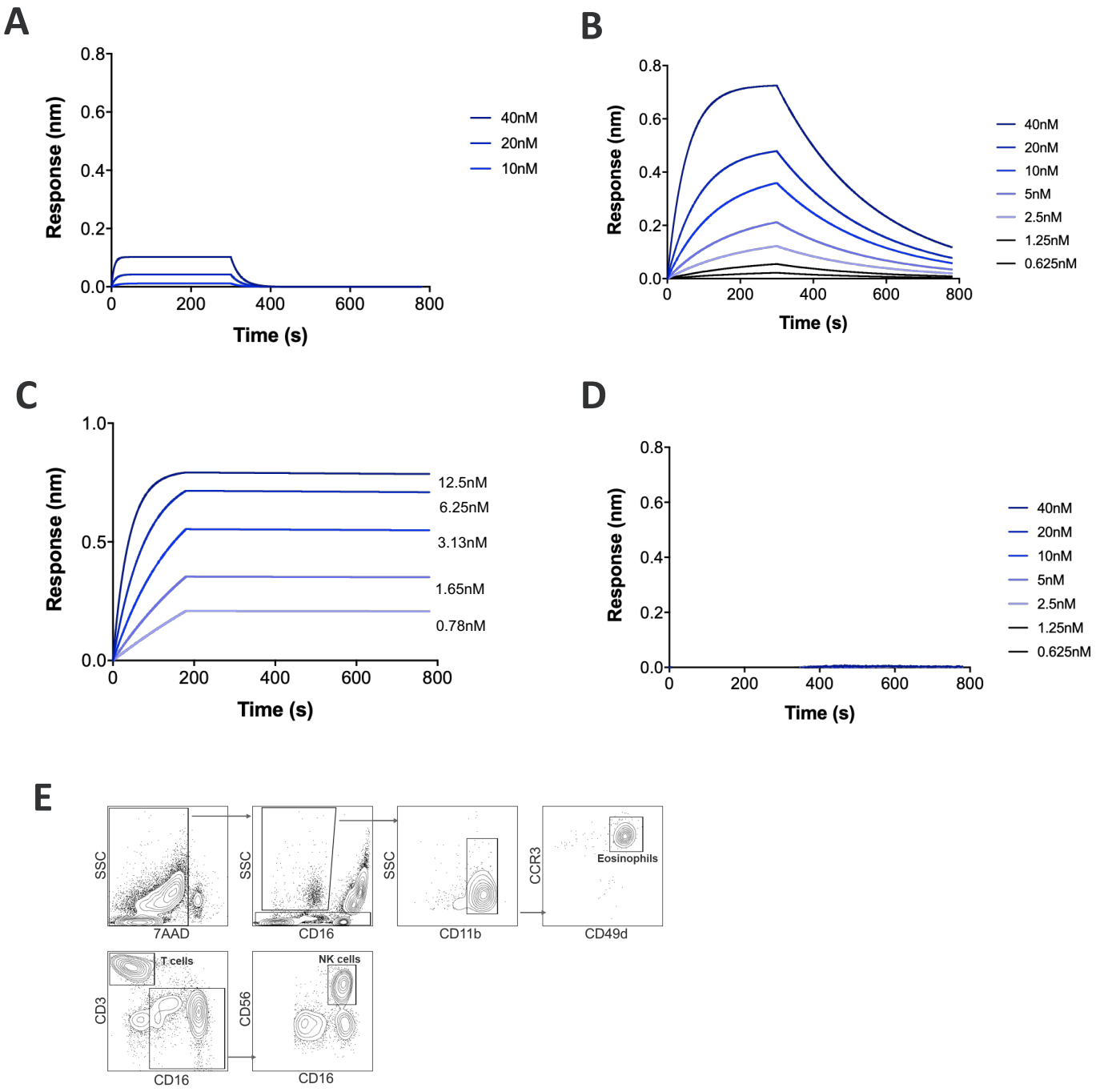

Figure S1. (A) Affinity to immobilized CD16b determined for AK002 (2-fold dilutions from 40 to 10nM; no significant binding observed). (B) Affinity to immobilized CD16a (158V variant) determined for AK002 (2-fold dilutions from 40 to 0.6 nM; k_{on}=3.84x10^{5}1/Ms, k_{off}=3.79x10^{-3}1/s, Kd=9.88 nM) (C) The bivalent avidity of AK002-G4 (2-fold dilutions from 12.5 to 0.8 nM) to the Siglec-8 ECD antigen was determined. The kinetic parameters were: k_{on}=1.72x10^{6}1/Ms, since no significant dissociation was observed for the measured time of 600 seconds the Kd was assigned to < 1pM. (D) Affinity to immobilized CD16a (158V variant) determined for AK002-G4 (2-fold dilutions from 40 to 0.6 nM; no significant binding observed). (E) Flow cytometry gating strategy used to identify eosinophils (7AAD-, SSCHi, CD16-, CD11b+, CD49d+, CCR3+), NK cells (7AAD-, SSCLo, CD3-, CD16+, CD56+) and T cells (7AAD-, SSCLo, CD16-, CD3+) among human peripheral blood leukocytes.

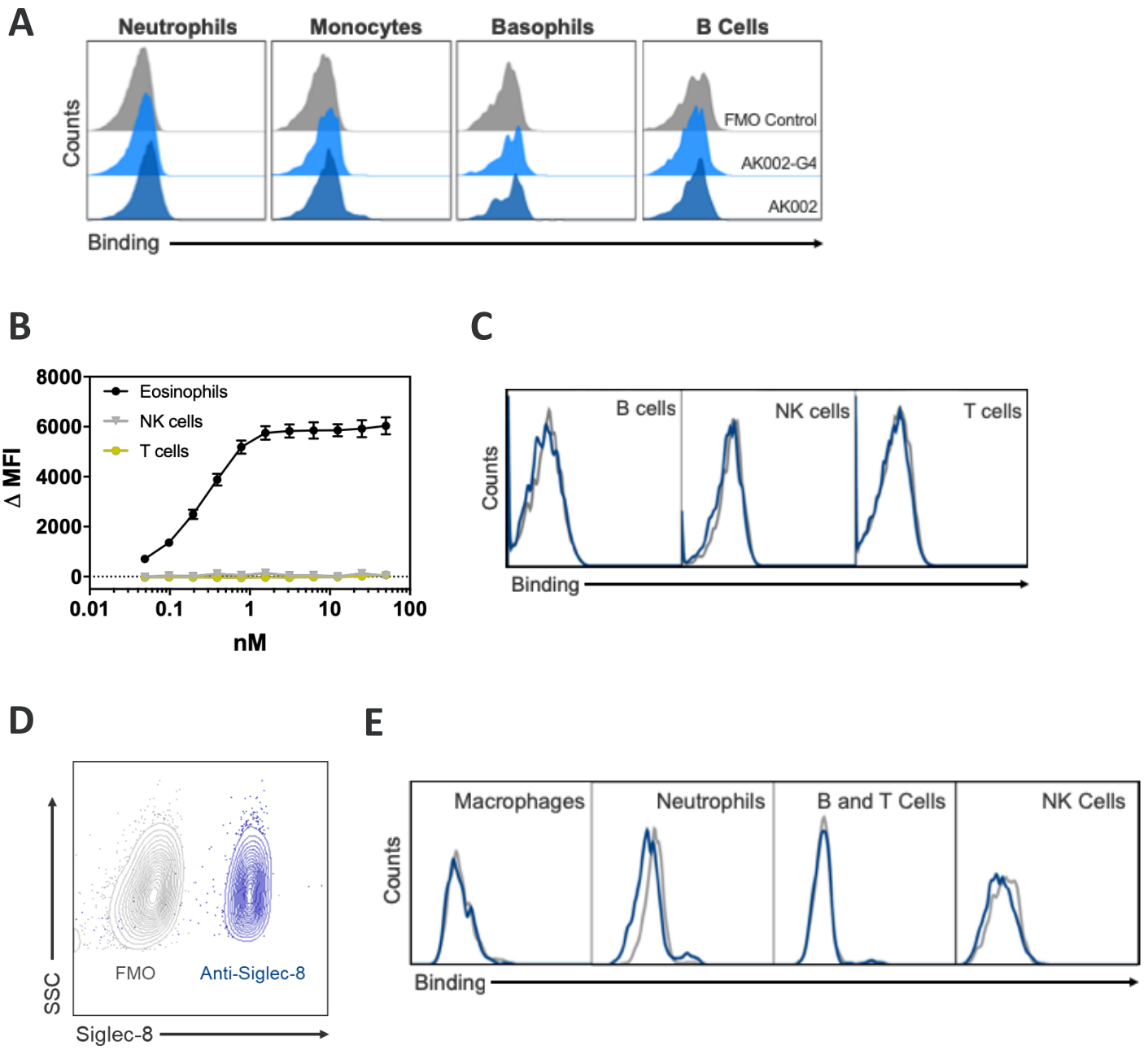

Figure S2. (A) Binding of 25 nM AK002-G4 (light blue) and AK002 (dark blue) to neutrophils, monocytes, basophils, and B cells in human peripheral blood by flow cytometry, identified via the gating strategy shown in Figure 2A. (B) Titration of AK002-F(ab')$_2$ binding in human peripheral blood to eosinophils, NK cells, and T cells gated as shown in panel S1E by flow cytometry. (C) Representative histograms for peripheral blood B cells, NK cells and T cells stained with an FMO control (gray) or AK002-G4 (blue) by flow cytometry, identified via the gating strategy shown in Figure 2A. (D) Live peripheral blood eosinophils were identified by flow cytometry and stained with either a conjugated anti-Siglec-8 antibody (blue) (837535; R&D Systems) or a fluorescence minus one (FMO) negative control (gray) identified via the gating strategy shown in Figure 2A. (E) Representative histograms for lung tissue macrophages, neutrophils, B and T cells, and NK cells stained with an FMO control (gray) or AK002-G4 (blue) by flow cytometry, identified via the gating strategy shown in Figure 3A.

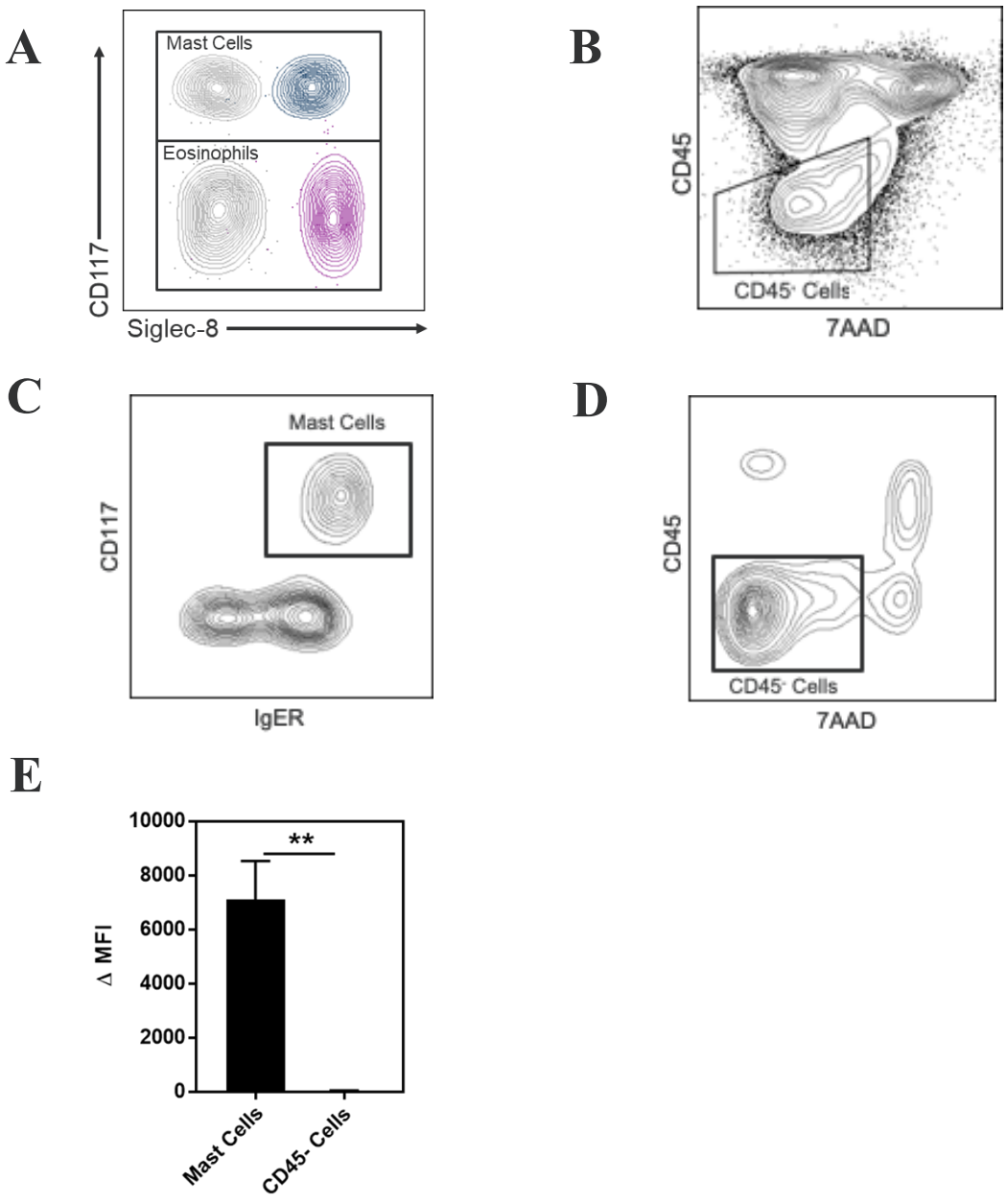

Figure S3. (A) Lung tissue-derived eosinophils and mast cells identified by flow cytometry and stained with either a conjugated anti-Siglec-8 antibody (blue or purple, respectively) or FMO negative control (gray). Eosinophils (CD117-) and mast cells (CD117+) are plotted to distinguish cell populations. (B) Representative contour plots of live, CD45 negative cells identified by flow cytometry in dissociated lung tissue. Representative contour plots of (C) mast cells (CD45+ 7AAD- CD117+ IgER+) or (D) live, CD45 negative cells identified by flow cytometry in dissociated skin tissue. (E) Surface expression of Siglec-8 plotted as ΔMFI on skin mast cells (black) and CD45 negative cells (gray) (Mean ± SD of 4 donors). ΔMFI was determined by subtracting the MFI from a FMO control sample from the MFI for cells stained with the conjugated antibody. ΔMFI values that were negative after subtracting the FMO were given a value of zero. **P<0.01

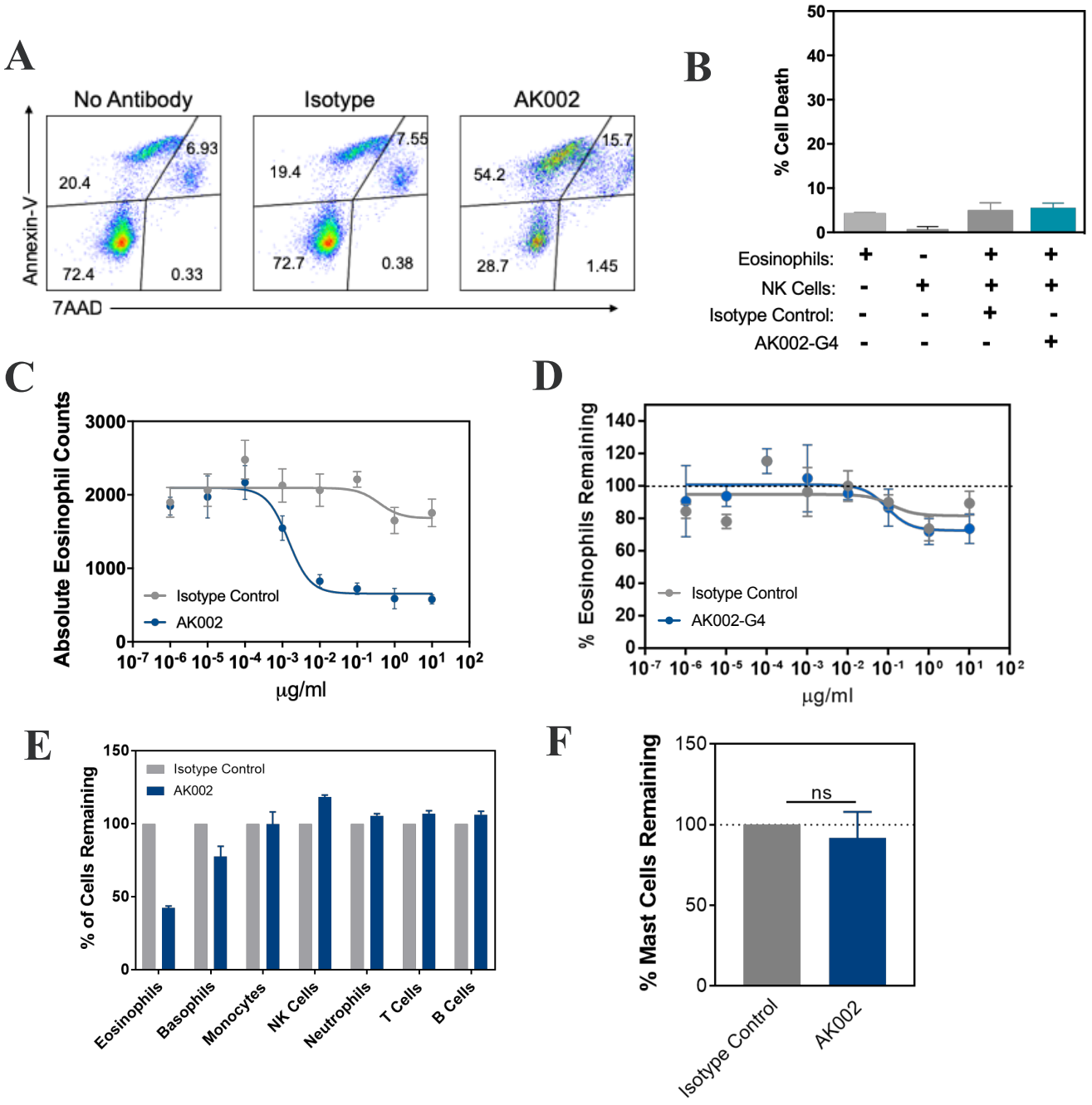

Figure S4. (A) Representative dotplots of AnnexinV staining of purified human eosinophils cultured for 18hr with either 10µg/ml isotype control, AK002 or no antibody in the presence of IL-5 (50ng/ml). (B) Purified human eosinophils were cultured with purified NK cells from an allogeneic donor at an E:T ratio of 9:1 in the presence of 10 µg/mL isotype control or AK002-G4. Cell supernatants were analyzed for LDH levels 4 hours after initiation of culture and amounts of LDH release were compared with maximal release to determine % cell death. (C) PBLs from healthy donor were incubated for 16 hours with the indicated concentrations of AK002 (blue) or isotype control antibody (gray). The absolute number of eosinophils was determined by flow cytometry (CCR3+SSCHigh). (Mean±SD of 3 technical replicates and representative of 3 different donors). (D) PBL from a healthy donor were incubated for 16 hours with the indicated concentrations of AK002-G4 or isotype control antibody. Residual

eosinophils (CCR3+SSCHigh) were detected by flow cytometry to determine the percentage remaining by comparing numbers in samples without antibody treatment. Mean ± SD of 3 technical replicates are shown. (E) PBL from a healthy donor were incubated for 16 hours with the indicated concentrations of AK002 or isotype control antibody. Residual immune cells shown were detected by FACS to determine the percentage remaining by comparing numbers in samples without antibody treatment. Mean ± SD of 3 technical replicates are shown. (F) Dissociated human tissue incubated overnight with 1 μg/mL isotype control (gray) or AK002 (blue) (Mean±SD of 4 donors). Mast cells were counted by flow cytometry (CD45+ 7AAD- CD117+ IgER+) and percent mast cells remaining calculated by normalizing to isotype control.

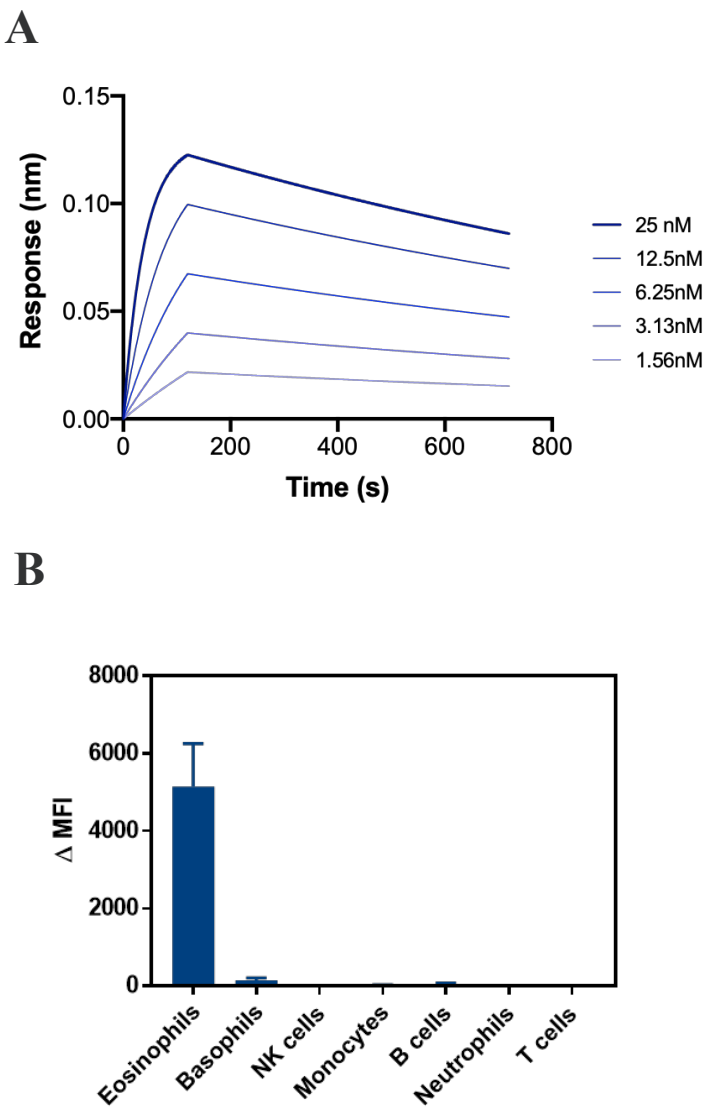

Figure S5. (A) The binding kinetics of monovalent mAK002 antigen-binding fragments (Fabs; 2-fold dilutions from 25 to 1.6 nM) to the Siglec-8 ECD antigen was measured using biolayer interferometry. The following kinetic parameters were determined: kon=1.00x1061/Ms, koff=5.91x10-41/s, Kd=590pM. (B) mAK002 binding plotted as ΔMFI on immune cells in human blood identified using gating strategy in Figure 2, panel A (Mean ± SD of 4 donors). ΔMFI was determined by subtracting the median fluorescence intensity (MFI) for a Fluorescence Minus One (FMO) control sample from the MFI for cells stained with the conjugated antibody. ΔMFI values that were negative after subtracting the FMO were given a value of zero.

SUPPLEMENTAL METHODS

CD16a and CD16b Affinity Analysis

The binding affinities of AK002 antibodies (Allakos, Inc) for recombinant CD16a (FcγRIIIA) and CD16b (FcγRIIIB) were also measured by biolayer interferometry using a FortéBio Octet Red96 instrument at 25°C at 1000 RPM in FortéBio 1X kinetics buffer. Antibodies were diluted from 40 nM to 0.625 nM in assay buffer in a 2-fold dilution series. CD16a or CD16b his-tagged protein (R&D Systems) was immobilized on penta-His H1S1K kinetic sensors at 88 nM in 1X kinetics buffer for 5 minutes until a sensor change of approximately 1.0 nm was achieved. The association phase was 5 minutes followed by a 10-minute dissociation phase. An empty reference cell sensor was used as a blank control, and affinities were analyzed using FortéBio analysis software with 1:1 global fit parameters.

Antibodies and Gating Strategies

Antibodies used for identifying immune cell populations in blood included: CD45 APCH7, CD16 PECy7, HLADR APC (BD Biosciences), CD14 BV605, CD193 BV421, and CD11c PE (BioLegend). Antibodies used for identifying immune cell populations in human tissue included: CD45 BV785, CD16 PECy7, HLADR APC, CD123 BV421 (BD Biosciences), CD14 BV605, CD206 BV421, CD24 APCCy7, and CD11c PE. Live/dead cells were identified by 7AAD (BD Biosciences). Siglec-8 positive cells were identified using AK002-G4 conjugated to Alexa Fluor® 488 or Alexa Fluor® 647 (Allakos, Inc). In addition, a commercially available anti-Siglec-8 PE antibody (R&D Systems) was used for confirmation in some experiments. Expression of surface markers on blood and tissue eosinophils was analyzed as described above with the following antibodies: CD62L PE (BioLegend), IL5Rα PE (BD Biosciences), and Siglec-8 PE (R&D Systems). Surface marker expression was determined by subtracting the median fluorescence intensity (MFI) for a Fluorescence Minus One (FMO) control sample from the MFI for cells stained with a conjugated Siglec-8 antibody (ΔMFI). MFI values that were negative after subtracting the FMO were given a value of zero. The gating strategy used for analysis of peripheral blood was as follows: Eosinophils: SSChi CD16-; Neutrophils: SSChi CD16+; Monocytes: SSClo CD14+; Basophils: SSClo CD14- CCR3+ HLA-DR-; Dendritic cells: SSClo CD14- CCR3- HLADR+ CD11c+; B cells: SSClo CD14- CCR3- CD11c- HLA-DR+ CD16-; NK cells: SSClo CD14- CCR3- CD11c- HLA-DR- CD16+; T cells: SSClo CD14- CCR3- CD11c- HLA-DR- CD16-. Gating strategy for cells from human lung tissue was as follows: Macrophages: CD45+ CD206+; Eosinophils: CD45+ CD206- SSChi CD16- CD24+; Neutrophils: CD45+ CD206- SSChi CD16+ CD24+; Mast cells: CD45+ CD206- SSChi CD16- CD24+; Monocytes: CD45+ CD206- SSClo CD14+ CD24-; Basophils: CD45+ CD206- SSClo CD14- CD123+ HLADR-; pDCs: CD45+ CD206- SSClo CD14- CD123+ HLADR+; Dendritic cells: CD45+ CD206- SSClo CD14- CD123- HLADR+ CD11c+; B and T cells: CD45+ CD206- SSClo CD14- CD123- CD11c- CD16-; NK cells: CD45+ CD206- SSClo CD14- CD123- CD11c- CD16+.

Human Eosinophil Purification

Human buffy coat preparations were obtained from healthy donors, and granulocytes were isolated by density gradient centrifugation using Ficoll (GE Healthcare). The buffy coat preparation was diluted with PBS and applied on top of an equal volume of Ficoll in a 50 mL tube; the tube was then centrifuged at room temperature 800 x g for 20 minutes with no brake. Plasma and mononuclear cells were discarded and the remaining cell pellet was suspended in 50 mL RBC lysis buffer. Granulocytes were washed with 50 mL PBS, centrifuged again, and suspended in 10 mL 0.5% BSA-PBS, passed through a 40 μm nylon

filter, and counted. Eosinophils were purified using either the Miltenyi Biotec Eosinophil Isolation Kit (#130-092-010) or the StemCell EasySep Human Eosinophil Isolation Kitt (#17956). For the Miltenyi kit, granulocytes were suspended in 40 μL buffer for every 1×10^7 cells in preparation for immunomagnetic negative selection. Eosinophil purifications were carried out using LS Columns (composed of ferromagnetic spheres and used for cell purification by depletion; Miltenyi Biotec) according to the manufacturer's instructions. For the StemCell kit, granulocytes were suspended at 5 x 10^7 cells per ml in preparation for immunomagnetic negative selection. Eosinophil purification were carried out using the EasySep magnets (StemCell) according to the manufacturer's instructions. Immediately after purification, cell purity was examined using flow cytometry. Only perparations with a purity of at least 90% eosinophils were used in subsequent experiments.

AK002 Binding Analysis in PBL

The level of AK002-specific binding to immune cell populations in PBL was determined by incubating PBL of healthy donors with varying concentrations (0.05 to 50 nM) of Alexa Fluor® 647-conjugated AK002 antibodies (Allakos, Inc) or fluorescence minus one (FMO) control for 10 minutes on ice. Cells were co-stained with fluorochrome-conjugated antibodies against individual cell types, and cell populations were identified as described above. ΔMFI was determined by subtracting the median fluorescence intensity (MFI) for a Fluorescence Minus One (FMO) control sample from the MFI for cells stained with the conjugated antibody. The saturation binding curve data were plotted and analyzed using GraphPad Prism 6 software.

Establishment of NSG-SGM3-BLT Humanized Mice

NSG-SGM3 BLT mice were established using a protocol for the generation of NSG-BLT mice that was previously described (Bryce et al JACI 2016; L Covassin et al, Clin Exp Immunology 2013). NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} (NSG, JAX stock number 05557) and NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ (NOD-*scid IL2rg*^{null} *SCF/GM-CSF/IL3* abbreviated as NSG-SGM3, JAX stock number 013062) were obtained from The Jackson Laboratory, Bar Harbor, ME. Human fetal thymus and fetal liver (gestational age between 16 and 20 weeks) specimens were provided by Advanced Bioscience Resources (Alameda, CA, USA) or StemExpress (Placerville, CA, USA). Upon receipt, tissues were washed with RPMI supplemented with penicillin G (100 U/ml), streptomycin (100 mg/ml), fungizone (0·25 μg/ml) and gentamycin (5 μg/ml) and then 1 mm^3 fragments were prepared from the thymus and liver for transplantation. When indicated 1 mm^3 fragments of fetal NSG mouse liver were co-implanted with the human tissues. The remaining human fetal liver was processed to recover human HSC. Indicated groups of recipient mice were irradiated with 200 cGy and then implanted with a fetal thymus and fetal liver fragment together in the renal subcapsular space or subcutaneously in the ventral area. At a minimum of 4 h after irradiation of recipient mice, CD3-depleted fetal liver cells were injected i.v. with 1 to 5×10^5 $CD34^+$ HSC per mouse. Following surgery, recipient mice received a subcutaneous injection of gentamycin (0.2 mg) and cefazolin (0.83 mg).

Statistics

P values were determined by the nonparametric Mann Whitney U test using GraphPad Prism (San Diego, CA).