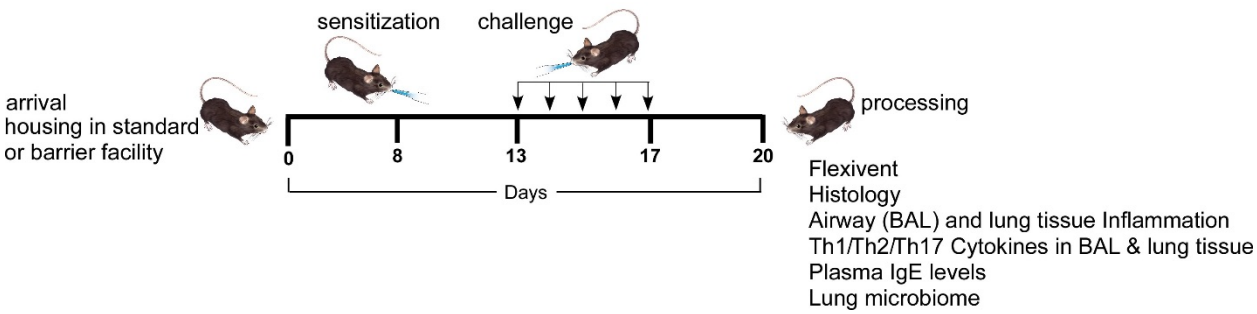
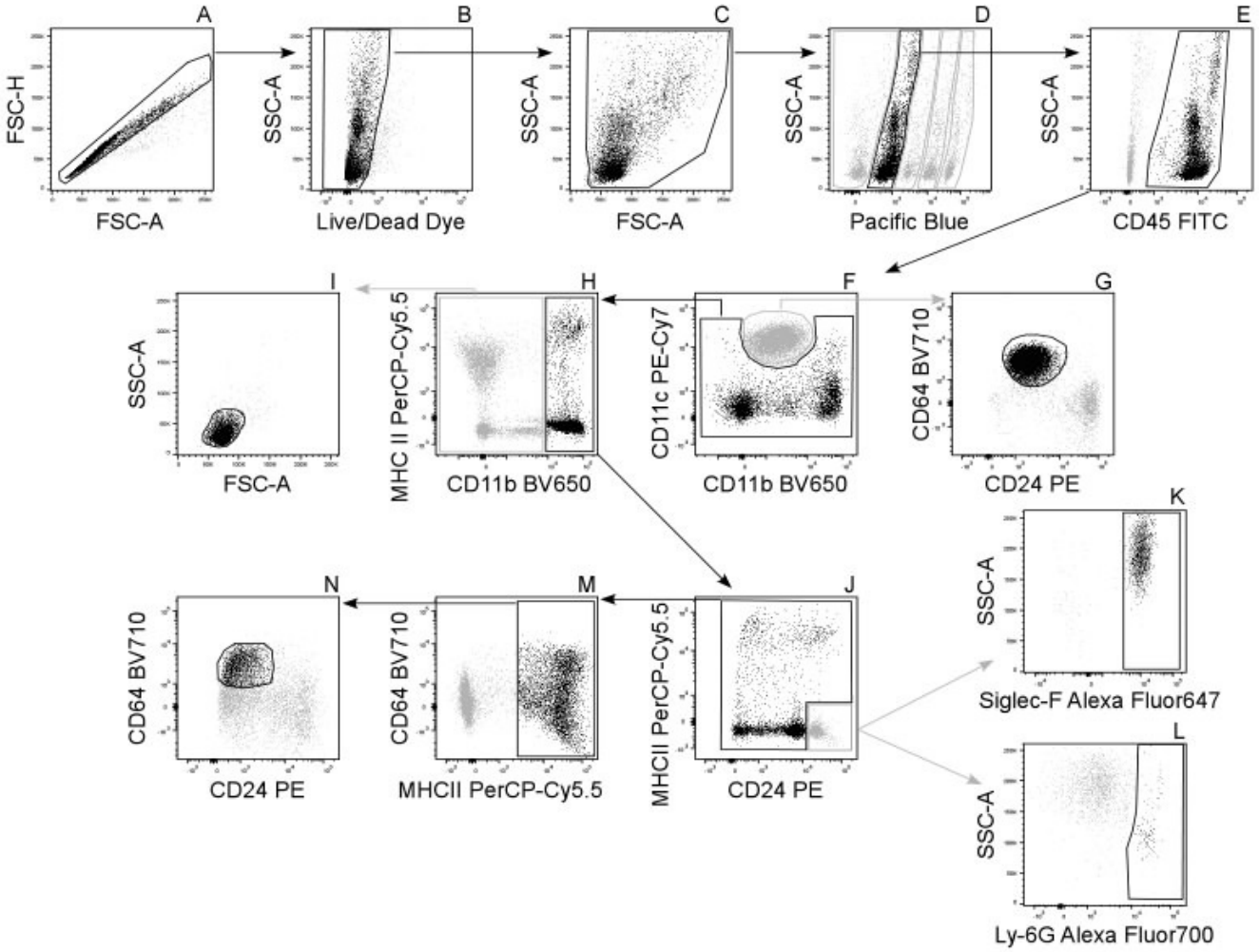
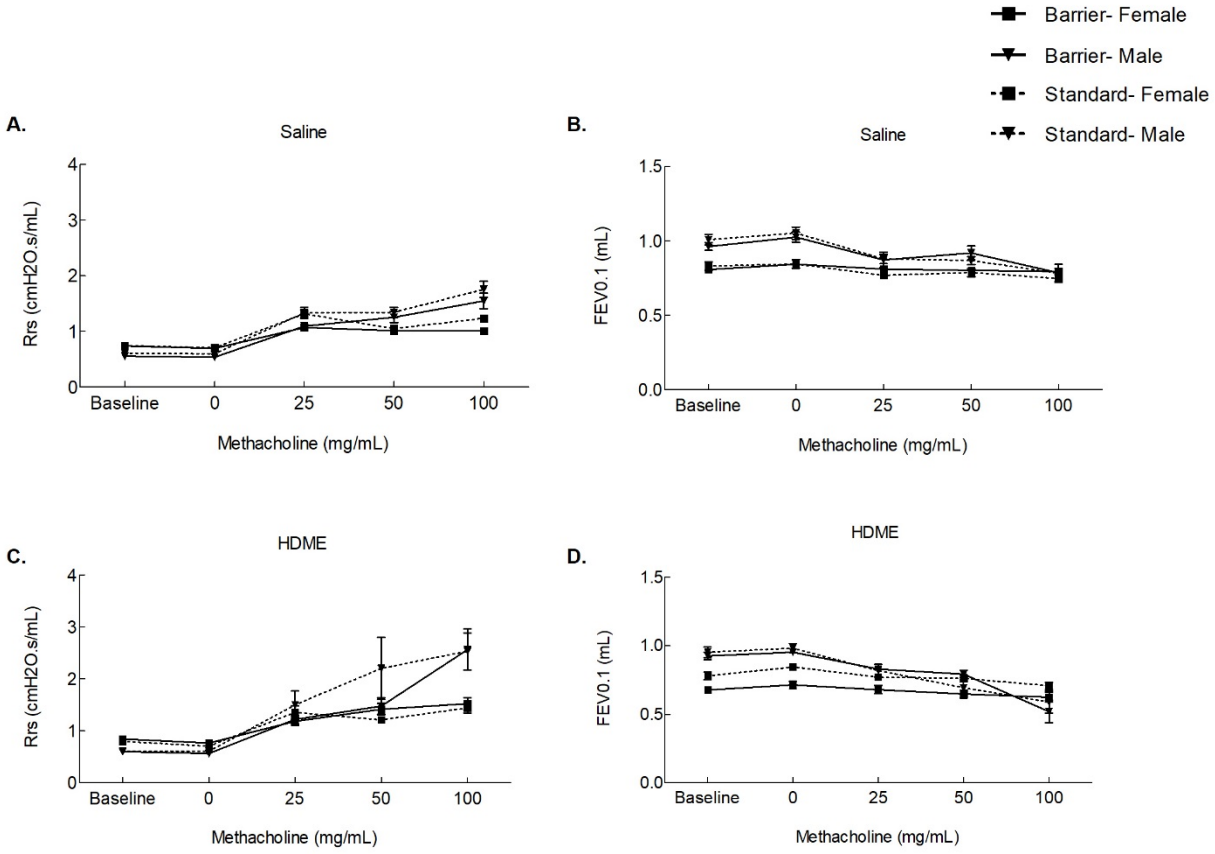


Supplemental Figure 1. Schematic overview of the house dust mite mouse model of allergic airway inflammation. Mice were housed in a pathogen-free barrier facility or pathogen accessible standard facility. On day 8 animals were anesthetized by isoflurane inhalation and sensitized, intranasally, with 100 µg HDME in 50 µl saline. The control group were sensitized with 50 µl of saline. On day 13, animals were anesthetized by isoflurane inhalation and intranasally challenged daily with 10 µg HDME in 50 µl saline for 5 consecutive days. The control group were challenged with 50 µl of saline. On day 20 animals were processed for AHR, NPFE, BAL, histology, flow cytometry, and cytokines.



Supplemental Figure 2. Gating strategy for inflammatory cell subsets. Lung single cell suspensions were used to quantify inflammatory cells**.** First, live single cells were identified while excluding artifacts. Single cells were gated from doublets (A). Live cells will take up less of the live/dead dye and were gated from dead cells with compromised membranes that results in brighter fluorescent staining (B). Cell debris has very low forward scatter and was excluded from analysis (C). Multiple samples were stained with antibodies and analyzed on the cytometer in a single tube by first staining with varying and unique concentrations of Pacific Blue succinimidyl ester before combining. Then individual samples were identified. In This figure the sample stained with 20ng/mL of Pacific Blue succinimidyl ester was selected for further gating (D). Differentiated hematopoietic cells were gated as $CD45^+$ (E). Sequential gating was used to identify specific cell subsets starting with expression of CD11c and CD11b (F). A $CD11c^+$ $CD11b^-$ population was gated, and based on expression of CD64 and CD24 alveolar macrophages were gated ($CD64^+$ $CD24^-$) (G). The remaining cells from Plot F were gated, then from this population $CD11b^{HI+}$ cells were gated from other cells (H). The side scatter and forward scatter of the $CD11b^{LOW+}$ and $CD11b^-$ cells was used to identify lymphocytes (I). Expression of MHCII and CD24 of the $CD11b^{HI+}$ cells is used to identify granulocytes ($MHCII^-$ $CD24^+$) (J). From the granulocytes population: $Siglec\text{-}F^+$ cells were gated as eosinophils (K), and $Ly\text{-}6G^+$ cells were gated as neutrophils (L). The non-granulocytes from plot J were gated then $MCHII^+$ cells were gated (M). CD64 and CD24 expression was used to gate interstitial macrophages ($CD64^+CD24^-$) (N). Gating of end subsets were confirmed by backgating.



Supplemental Figure 3. Airway Hyperresponsiveness (AHR) and Negative Pressure-Driven Forced Expiration (NPFE). Comparison between genders and facilities separated by Saline **(A - B)** and HDME **(C - D)** exposure in response to increasing doses of methacholine.

Table 1. Antibodies and Dyes for Flow Cytometry

Antibody or Dye	Vendor	Isotype - Clone	Dilution	Laser	Dichroic	Filter
Live/ Dead Blue Stain	Thermo Fisher	N/A	1/1000	355nm	-	450/50
Pacific Blue™ succinimidyl ester	Thermo Fisher	N/A	See Methods	407nm	-	450/50
Inflammatory Cell Panel						
CD45 FITC	eBioscience	Rat IgG2b, κ - 30-F11	1/200	488nm	505LP	515/20
Ly-6G Alexa Fluor 700	BD Bioscience	Rat IgG2a, κ - 1A8	1/100	641nm	685LP	730/45
Siglec-F Alexa Fluor 647	BD Bioscience	Rat IgG2a, κ - E50-2440	1/50	641nm	-	670/30
CD11c PE-Cy7	BD Bioscience	Armenian Hamster IgG1, λ2 - HL3	1/200	561nm	750LP	780/60
CD24 PE	eBioscience	Rat IgG2b, κ - M1/69	1/1600	561nm	-	582/15
CD64 BV711	Biolegend	Mouse IgG1, κ - X54-5/7.1	1/25	407nm	685LP	710/50
MHC II PerCP-Cy5.5	BD Bioscience	Rat BN x LEW IgG2b, κ - M5/114.15.2	1/200	515nm	685LP	710/50
CD11b BV650	BD Bioscience	DA/HA IgG2b, κ - M1/70	1/400	407nm	635LP	670/30