Online Repository Methods:

Virus and Mice. RSV strain 12/12-6 was isolated in 2012 from a hospitalized infant with severe lower respiratory tract infection and bronchiolitis as part of the INSPIRE study.[1] RSV was propagated and titrated in HEp-2 cells as previously described.[2] Mock inoculum was prepared by collecting cell culture supernatant from lysed, uninfected HEp-2 cells. 8 week old female BALB/cJ mice were purchased from Jackson Laboratories (Bar Harbor, ME). *Il33*$^{Citrine/+}$ reporter mice were generated by crossbreeding WT BALB/c mice and *Il33*$^{Citrine/Citrine}$ mice that were the kind gift of Dr. Andrew N.J. McKenzie.[3] Mice were maintained under specific pathogen free conditions and used in compliance with the revised 2011 Guide for the Care and Use of Laboratory Animals prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.[4] For infection, mice were anesthetized by intraperitoneal injection of ketamine/xylazine solution and inoculated via intranasal delivery with 9 x 10^5 PFU of RSV 12/12-6 or an equal volume of mock inoculum as previously described.[5] Weight loss was measured daily. The GLP-1R agonist liraglutide (Novo Nordisk, Plainsboro, NJ) was initiated at the same dose that is used in patients with Type II Diabetes. This was increased in two-fold increments daily until the final dose of 0.2 mg/kg weight was reached. The vehicle for liraglutide, 0.1% BSA in PBS, was used as a control. Treatment was given subcutaneously beginning 2 days prior to infection or beginning on the same day as infection and given twice daily until the mice were euthanized.

BAL and PAS Staining. 0.8 mL saline was instilled through a tracheostomy tube and withdrawn via syringe to obtain BAL fluid. Total cells were counted on a hemocytometer by using trypan blue exclusion. For cell differentials, 0.1 mL of BAL fluid was prepared via cytospin (Thermo Fisher Scientific, Waltham, MA) and subsequently fixed and stained using DiffQuik (American Scientific Products, Columbus, OH). Differential counts were based on counts of 200

cells, using standard morphological criteria to visualize neutrophils, eosinophils, lymphocytes, or macrophages. For PAS staining, lungs were perfused with PBS, inflated with 10% neutral buffered formalin, and fixed in 10% neutral buffered formalin for 24 hours at room temperature. Lungs were then paraffin embedded, sectioned (5 μm), and stained with periodic acid-Schiff (PAS) to visualize mucus. Small and medium sized airways were scored for mucus by a trained pathologist blinded to the experimental information using the following scoring scheme: (0) no PAS positive cells observed in cross sections of medium to small airways; (1) less than 10 PAS positive cells observed in cross sections of medium to small airways; (2) greater than 10 PAS positive cells observed in cross sections of medium to small airways; or (3) greater than 10 PAS positive cells observed in cross sections of medium to small airways with mucous strands observed in air spaces.

Airway Responsiveness. Airway responsiveness was measured as previously described.[6,7] Briefly, mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (85 mg/kg). A tracheostomy tube was inserted for ventilation. The internal jugular vein was cannulated for intravenous delivery of acetyl-β-methacholine. The mice were then placed in a whole body plethysmography chamber and mechanically ventilated. Precision glass microsyringes were used to deliver increasing doses of acetyl-β-methacholine. Baseline airway resistance measurements were collected followed by measurements with 137, 411, 1233, and 3700 μg/kg body weight of acetyl-β-methacholine (Sigma-Aldrich, Saint Louis, MO). Peak airway resistance measurements for each dose were recorded.

ELISA. Lungs were snap-frozen in liquid nitrogen at the time of harvest. Lungs were mechanically disrupted using 1 mL of MEM media and homogenized via BeadBeater (BioSpec Products, Bartlesville, OK). Protein measurements were performed using either Duoset (IL-33), Quantikine (IL-13, IFN-γ, and IL-27), or Verikine (IFN-α and IFN-β) enzyme-linked

immunosorbent assay (ELISA) kits according to manufacturer instructions (R&D Systems, Minneapolis, MN). Serum RSV F-protein-specific antibody was measured as previously described.[2]

Flow Cytometry. Lungs were harvested, minced, and digested in RPMI media with 5% FBS, 1 mg/mL collagenase, and 0.02 mg/mL DNase I for 40 minutes at 37℃. The digestion was stopped with 100 µl of 0.5 M EDTA, and a single cell suspension was generated by straining these digestions through a 70 µm filter. RBC lysis (BioLegend, San Diego, CA) was performed according to manufacturer instructions. Cells were stimulated in IMDM media with 10% FBS, 0.01 mM non-essential amino acids, penicillin/streptomycin, 1 mM sodium pyruvate, 10 ng/mL PMA, 1 µM ionomycin, and 0.07% monensin for 4 hours at 37℃ . Cells were stained with Live/Dead Blue (Life Technologies, Carlsbad, CA) and combinations of the following surface markers: CD45 (30-F11), CD25 (PC61.5), FcεR1 (MAR-1), DX5 (DX5), FcεR1 (MAR-1), and NKG2D (CX5) from eBioscience (San Diego, CA); CD127 (SB/199), CD3 (17A2), CD146 (ME-9F1), and EpCAM (G8.8) from BioLegend (San Diego, CA); CD4 (H129.19) from BD Biosciences (San Jose, CA); and/ or a surface marker cocktail containing CD5, CD45R (B220), CD11b, Gr-1 (Ly-6G/C), 7-4, and Ter-119 from Miltenyi (Bergisch Gladbach, Germany). Cells were fixed/ permeabilized for 12 hours in Cytofix/ Cytoperm (BD Biosciences) and stained with combinations of the following intracellular markers: IL-13 (eBio13A) and IFN-γ (XMG1.2) from eBioscience. Anti-FcR antibody (BD Biosciences) was used to prevent nonspecific staining. Twelve hours of Cytofix/ Cyoperm incubation was performed consistently with our lab's previous methods (Newcomb DC et al., J Allergy Clin Immunol 136:1025-34, 2015). We are not concerned with potential epitope cross-linking that can result in overfixing with a longer fixation time, given that our FMO controls uniformly demonstrate a lack of staining. All samples were run on a BD LSR II Flow Cytometer and analyzed using FlowJo (Version 10; Treestar, Ashland, OR). ILC were defined as Lineage$^-$ CD45$^+$ CD25$^+$ CD127$^+$ cells where Lineage (Lin) includes

(CD3, CD5, CD45R [B220], CD11b, Gr-1 [Ly-6G/C], 7-4, and Ter-119). ILC2 were defined as ILC that expressed IL-13. Th cells were defined as $CD3^+$ $CD4^+$ cells, basophils were defined as $DX5^+$ $Fc\varepsilon R1^+$ cells, NK cells were defined as $CD3^-$ $DX5^+$ cells, and epithelial cells were defined as $CD45^-$ $CD146^+$ $EpCAM^+$ cells. MFI was determined as the geometric mean.

Viral Load. Lungs were snap-frozen in liquid nitrogen at the time of harvest. Thawed lungs were resuspended in 1 mL of sterile MEM media or TRIzol reagent (Invitrogen, Carlsbad, CA) and homogenized via BeadBeater (BioSpec Products). Total RNA was isolated using TRIzol reagent and cDNA was generated for qPCR analysis of RSV-M and GAPDH. Commercially available primers and probes for GAPDH were used (Applied Biosystems, Foster City, CA, catalog number 4331182, assay ID Mm99999915_g1). Custom primers were designed to measure RSV-M.[8] Gene expression was normalized to GAPDH before the fold change was calculated. The fold change in gene expression was calculated via the comparison of gene expression to that of lungs from RSV-infected, vehicle-treated mice. Primer sequences were as follows: RSV-M: forward, 5' -GGCAAATATGGAAACATACGTGAA-3', reverse, 5' -TCTTTTTCTAGGACATTGTAYTGAACAG-3'.

Antibody ELISA. Ectodomain F protein from RSV A2 was fused to a GCN4 trimerization domain and a His tag and expressed in mammalian cells, as previously described. [9] Immulon 2B (Thermo Scientific, Rochester, NY) plates were coated with 150 ng of RSV F protein in PBS overnight at 4℃. Plates were blocked with 1% BSA i n PBS for 1 hour at room temperature. Supernatants were serially diluted 1:2 starting at 1:80 over 6 total dilutions, and plates were incubated for 1 hour at room temperature. RSV-specific antibody was detected using horseradish peroxidase-conjugated goat anti-mouse antibody specific for mouse IgG (1:5,000), IgG1 (1:500), or IgG2a (1:500) for 1 hour at room temperature (Southern Biotech, Birmingham, AL). Plates were developed in Ultra-TMB (Pierce, Rockford, IL) and the reaction was stopped

with 1M HCl. Absorbance values (450 nm) were measured and assessed using Gen 5 software (BioTk, Vinooski, VT). The serum endpoint dilution at 0.2 absorbance units above background (PBS blank) was calculated for each antibody type.

Statistical Analysis. Groups were compared using unpaired t-test, one-way analysis of variance (ANOVA) with Bonferroni post test, or two-way ANOVA with Dunn's multiple comparison test, as appropriate with GraphPad Prism (Version 5; GraphPad Software, San Diego, CA). Measurements below the limit of detection were assigned half of the value of the limit of detection for statistical comparisons.

PheWAS Study. To investigate possible human associations with *THADA* variation, we used a population of 29,713 individuals of European ancestry who had genotyping on Illumina HumanExome BeadChip version 1.1 and available electronic medical record (EMR) data from the Vanderbilt BioVU DNA biobank.[10] This platform contained the *THADA* single nucleotide polymorphism (SNP) rs7578597, which corresponds to a threonine to alanine in multiple splice variants. The minor T allele of rs7578597 was present in 11% of individuals. We then evaluated all phenotypes defined using a PheWAS of this SNP using previously described methods.[11] Briefly, the method defines cases for 1,000 phenotypes by the presence of specific *International Classification of Diseases, Ninth Revision* (ICD9), codes on at least two different days. Controls for each phenotype are defined as individuals who lack case ICD9 codes and other codes that are related. For example, cases of the "acute bronchitis and bronchiolitis" phenotype are defined with the ICD9 code 483, while its controls are defined as absence of the 483 ICD9 codes. We used version 1.2 of the PheWAS code terminology system and the R PheWAS package to calculate the PheWAS and graph results,[12] both of which can be downloaded from http://phewascatalog.org. We used logistic regression for each phenotype with 40 cases or more, adjusted for age and sex, assuming an additive genetic model.

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166 **Online Repository Figure Legends**

167

168 **Figure E1: RSV 12/12-6 induces lung IL-13 and mucus production**. BALB/cJ mice were
169 infected with 9 x 10^5 PFU of RSV strain 12/12-6. ELISA for (A) IL-13 and (B) IFN-γ in the whole
170 lung homogenate (both lungs) 6 days after infection. (C) Representative PAS-stained section of
171 mucus-containing airway in the lungs 8 days after infection. Data plotted as mean + SEM. n = 5
172 mice per group. ***p < 0.001 by one-way ANOVA. Dashed line is the limit of detection of the
173 assay.

174

175 **Figure E2. Protocol for *in vivo* administration of GLP-1R agonist or vehicle and**
176 **subsequent infection with RSV or mock preparation.** Treatment with the GLP-1R agonist
177 liraglutide or vehicle (0.1% BSA in PBS) was initiated in BALB/cJ mice on day -2. Mice were
178 infected with 9 x 10^5 PFU of RSV strain 12/12-6 or mock inoculum on day 0. Treatment was
179 given twice daily until the mice were euthanized.

180

181 **Figure E3: GLP-1R agonist decreases IL-13-producing ILC2, Th2 cells, and basophils 6**
182 **days after RSV infection.** BALB/cJ mice were treated with GLP-1R agonist or vehicle and
183 infected with 9 x 10^5 PFU of RSV strain 12/12-6 or mock inoculum. (A) Percent of ILC that are
184 IL-13^+. MFI of (B) IL-13 and (C) CD127 staining in ILC2. Representative MFI of (D) IL-13 and
185 (E) CD127 staining in ILC2 by flow cytometry. (F) Total number of live lung cells, (G) $CD4^+$ Th2
186 cells, and (H) basophils. Data plotted as mean + SEM. n = 3-6 mice per group representative of
187 2 independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 by one-way ANOVA.

188

189 **Figure E4: Flow gating for ILC, CD4+ T cells, NK cells, basophils, and epithelial cells.** (A)
190 ILC were defined as viable Lin^- $CD45^+$ $CD25^+$ $CD127^+$ cells. (B) $CD4^+$ T cells were defined as
191 viable $CD3^+$ $CD4^+$ cells. (C) Basophils were defined as viable $FcεRI^+$ $DX5^+$ cells. (D) NK cells

were defined as viable $DX5^+$ $CD3^-$ cells. (E) Epithelial cells were defined as viable $CD45^-$ $CD146^+$ $EpCAM^+$ cells.

Figure E5: GLP-1R agonist decreases RSV-induced whole lung IL-13 accumulation and airway mucus production. BALB/cJ mice were treated with GLP-1R agonist or vehicle and infected with 9 x 10^5 PFU of RSV strain 12/12-6 or mock inoculum. (A) Protocol for *in vivo* administration of GLP-1R agonist or vehicle and simultaneous infection with RSV or mock preparation. (B) ELISA for IL-13 in whole lung homogenate 6 days after infection. (C) Quantification of airway mucus in the lungs 8 days post-infection. Data plotted as mean + SEM. n = 5 mice per group. *p < 0.05 by unpaired t-test.

Figure E6: GLP-1R agonist decreases whole lung IL-33 protein expression and IL-33-expressing epithelial cells 12 hours after RSV infection. *Il33*$^{Citrine/+}$ reporter mice were treated with GLP-1R agonist or vehicle and infected with 9 x 10^5 PFU of RSV strain 12/12-6 or mock inoculum. (A) Total number of live lung cells and (B) percent of epithelial cells that are IL-33^+.Data plotted as mean + SEM. n = 3-6 mice per group representative of 2 independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 by one-way ANOVA.

Figure E7: GLP-1R agonist does not decrease interferon responses during RSV infection. BALB/cJ mice were treated with GLP-1R agonist or vehicle and infected with 9 x 10^5 PFU of RSV strain 12/12-6 or mock inoculum. (A) Total number of IFN-γ^+ Th1 and (B) IFN-γ^+ NK cells 6 days after infection. ELISAs for (C) IFN-α, (D) IFN-β, and (E) IL-27 in whole lung homogenate 12 hours after infection. Serum (F) insulin and (G) glucose 6 days after infection. Data plotted as mean + SEM. n = 3-6 mice per group representative of 3 (A-E) or 1 (F-G) experiments. *p < 0.05 and **p < 0.01 by one-way (A-B & F-G) or two-way (C-E) ANOVA. NS = not significant.

Figure E8: Protocol for administration of GLP-1R agonist or vehicle, primary RSV or mock infection, and subsequent secondary RSV or mock infection. BALB/cJ mice were treated with GLP-1R agonist or vehicle beginning on day -2 and infected with 9 x 10^5 PFU of RSV strain 12/12-6 or mock inoculum on day 0. Treatment was given twice daily until day 8. On day 30, mice were infected a second time with 9 x 10^5 PFU of RSV strain 12/12-6 or mock inoculum.

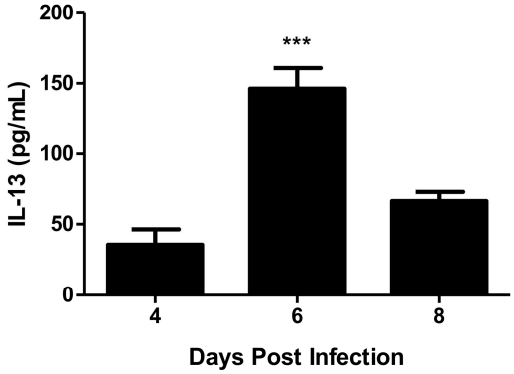
Figure E9: GLP-1R agonist treatment during primary infection prevents airway inflammation and does not reduce anti-RSV antibody responses or lung IFN-γ protein expression during secondary RSV infection. BALB/cJ mice were treated with GLP-1R agonist or vehicle and infected with 9 x 10^5 PFU of RSV strain 12/12-6 or mock inoculum. Mice were re-infected 30 days after primary infection, and serum was collected 6 days after secondary infection. (A) BAL cell counts 6 days post-infection. (B) ELISA for IFN-γ in whole lung homogenate (right lung only). ELISA for RSV F-protein-specific (C) IgG, (D) IgG1, and (E) IgG2a. Data plotted as mean + SEM. n = 6-12 mice per group combined from 2 independent experiments. One-way ANOVA. NS = not significant.

Table E1: PheWAS results for THADA rs7578597 in humans. All phenotypes with p<0.01 are reported below.

Table E1: PheWAS results for THADA rs7578597 in humans. All phenotypes with p<0.01 are reported below.

Phenotype	Cases	Controls	Odds ratio	p
Scleritis and episcleritis	67	25573	2.33	0.00006
Alkalosis	187	18873	1.67	0.00032
Acute pharyngitis	798	22135	1.31	0.00051
Acute bronchitis and bronchiolitis	1223	22347	1.24	0.00063
Postinflammatory pulmonary fibrosis	349	19926	1.43	0.00094
Diabetes mellitus	5032	21287	0.88	0.00136
Type 1 diabetes	468	21287	0.67	0.00157
Morbid obesity	972	24650	1.23	0.00276
Other forms of chronic heart disease	1095	21791	1.22	0.00359
Other disorders of male genital organs	243	9538	0.58	0.00374
Personality disorders	107	20065	0.41	0.00566
Other symptoms/disorders or the urinary system	4228	21638	1.11	0.00600
Anxiety, phobic and dissociative disorders	3229	20065	0.89	0.00884

A



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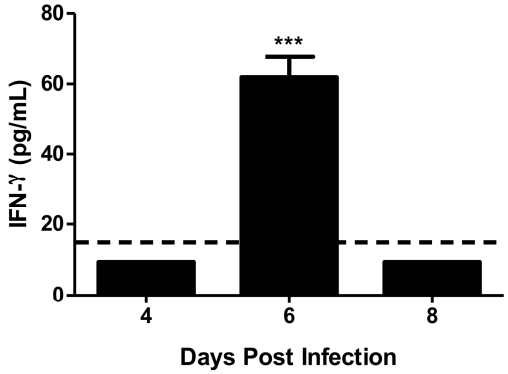

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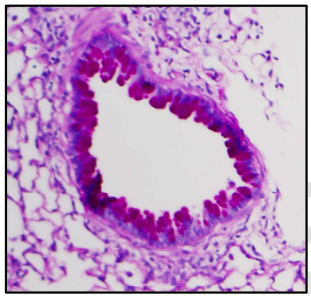

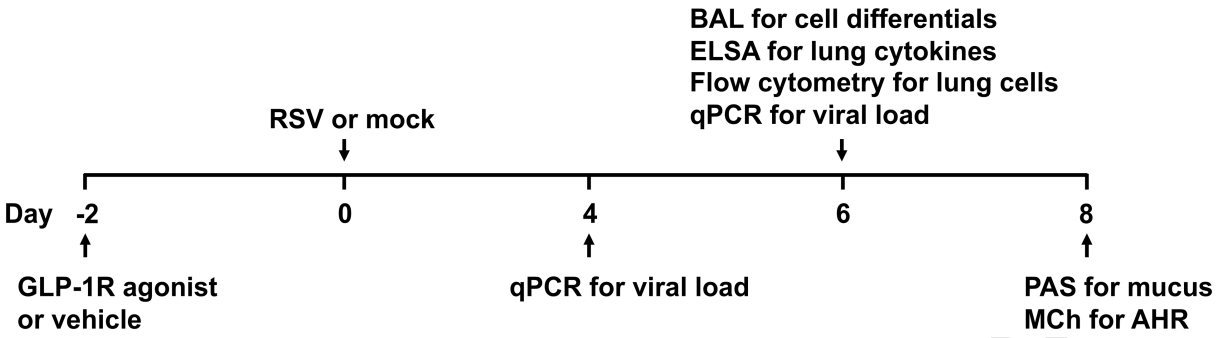

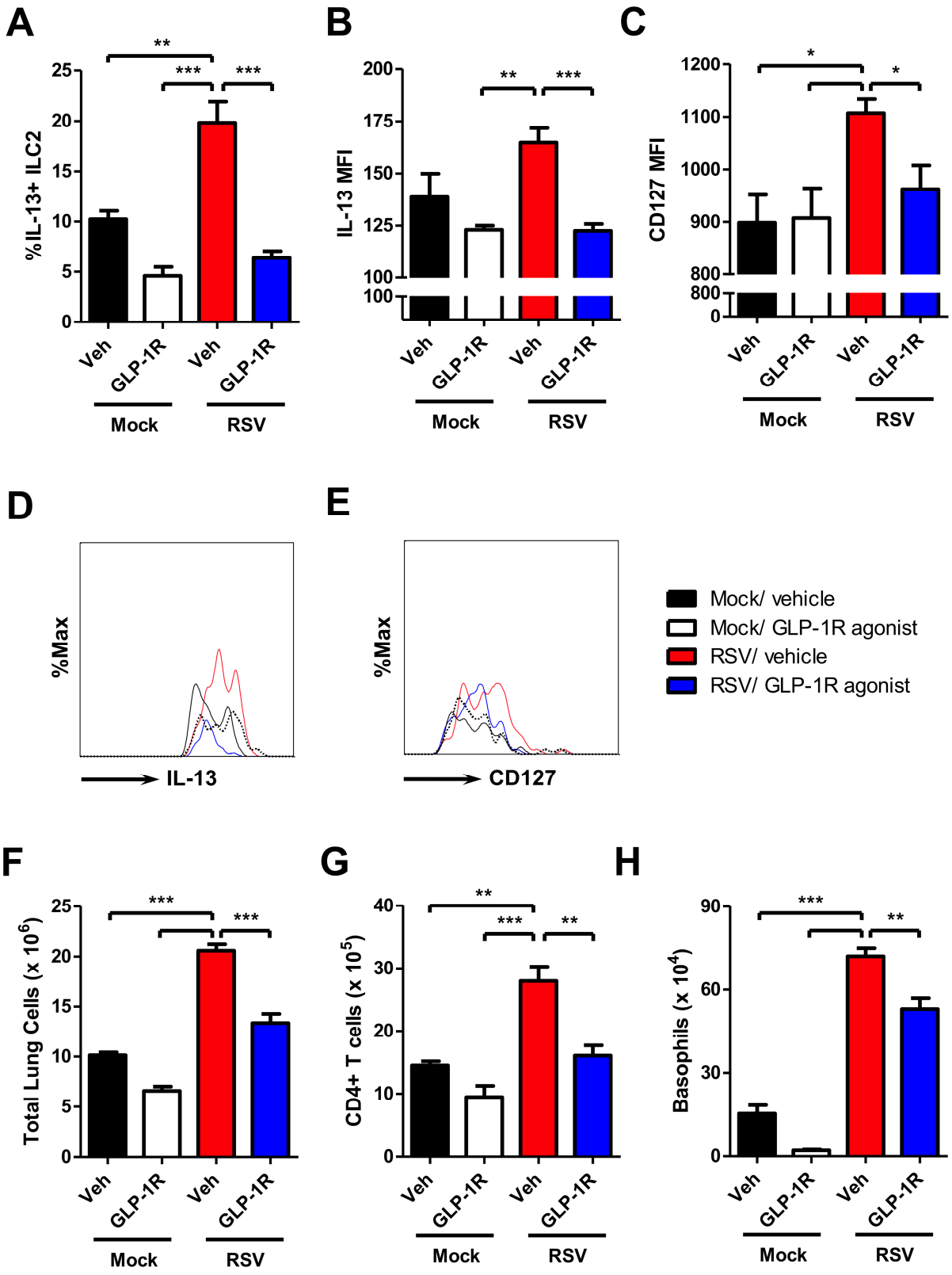
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Basophils (x 10^4)
Veh
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RSV/ vehicle
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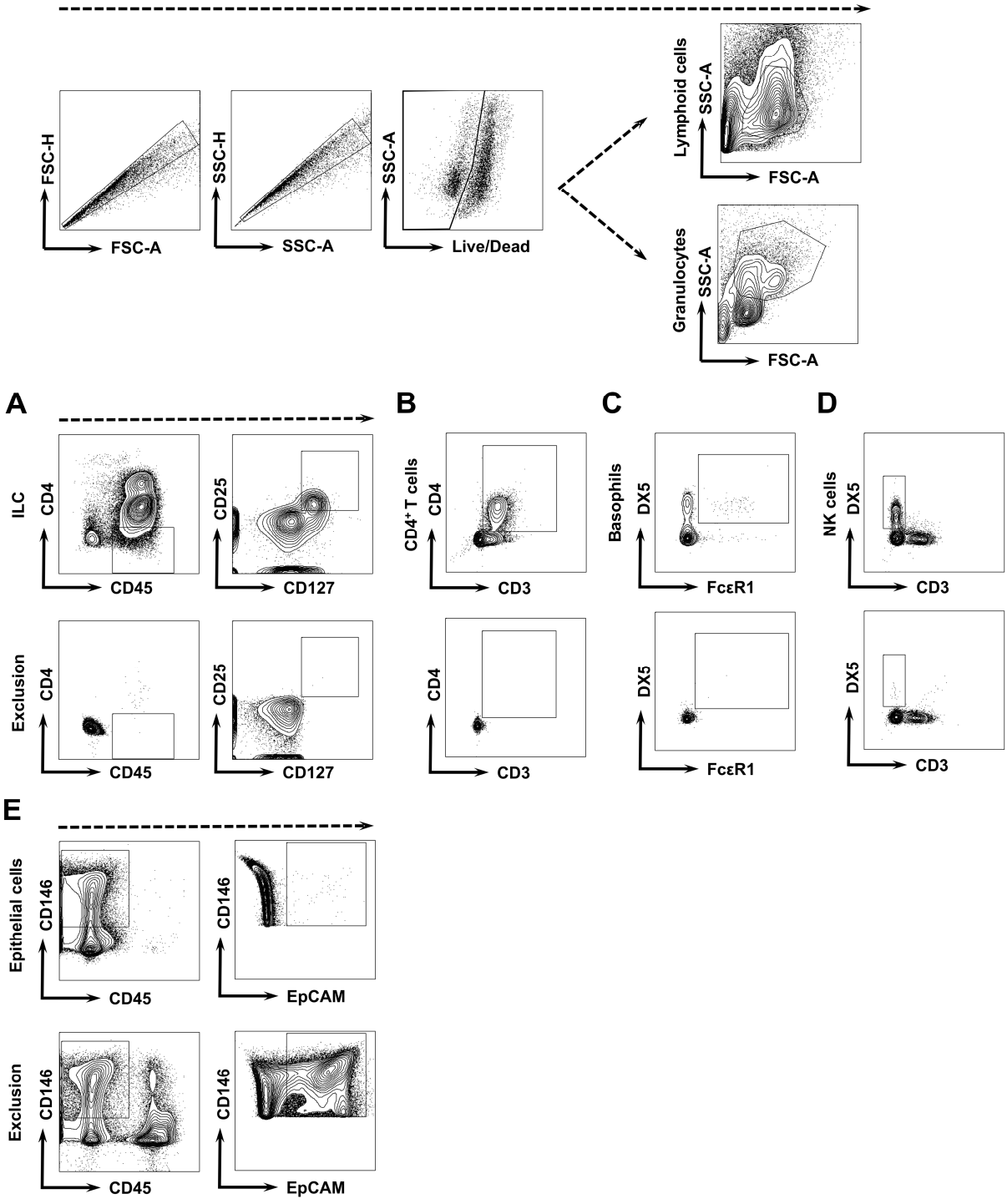
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