# ABLATION OF GLUTAREDOXIN-1 MODULATES HOUSE DUST MITE-INDUCED ALLERGIC AIRWAYS DISEASE IN MICE

## ONLINE DATA SUPPLEMENT

## MATERIALS AND METHODS

*Serum IgG1 and IgE.* ELISAs were performed as follows: a 96-well plate was incubated with 1 µg/ml of HDM overnight, then blocked with 1% BSA/PBS for one hour at room temperature. Samples were diluted 1:10, loaded on the plate and incubated overnight at 4°C. As a control, samples were loaded on a plate not pre-incubated with HDM. All samples were incubated with biotinylated (rat anti-mouse) IgG1 and IgE antibodies (BD Biosciences, San Jose, CA) for 2 hours at room temperature. Streptavidin-HRP conjugated antibody was added to the wells for 30 minutes, washed, then substrate solution was added to each well per manufacturer's instructions (R&D Systems Inc., Minneapolis, MN). The reaction was terminated with 2 N sulfuric acid solution and the plate was read using a spectrophotometer at 450 nm. Control values were subtracted from sample plates pre-incubated with HDM and adjusted results were expressed as OD 450 values.

*qRT-PCR.* 1 µg of RNA was reverse transcribed to cDNA for TaqMan gene analysis using SYBR Green (Bio-Rad; Hercules, CA, USA) to assess expression of chemokine (C-C motif) ligand 20 (*Ccl20*), Interleukin-6 (*Il-6*), Interleukin-13 (*Il-13*) and Interleukin-17A (*Il-17a*), Regulated on Activation, Normal T cell Expressed and Secreted (RANTES, gene name *Ccl5*), Mucin 5ac (*Muc5ac*), F4/80 (gene name *Adgre1*), nitric oxide synthase 2 (*Nos2*), arginase-1 (*Arg-1*). Expression values were normalized to the house keeping gene cyclophilin. Primers were as follows: *Ccl20* (FW 5'-AAGACAGATGGCCGATGAAG-3', RV 5'-ACGCCTTTTCACCCAGTTCT-3'), *Il-13* (FW 5'-CCAGGCCCCTTCTAATGA-3', RV 5'-GCCTCTCCCCAGCAAAGTCT-3'), *Il-17a* (FW 5'-TTTAACTCCCTTGGCGCAAAA-3', RV 5'-CTTTCCCTCCGCATTGACAC-3'), *Ccl5* (FW 5'-ATATGGCTCGGACACCACTC-3', RV 5'-TCCTTCGAGTGACAAACACG-3'), *Muc5ac* (FW 5'-CAGTGAATTCTGGAGGCCAACAAGGTAGAG-3', RV 5'-AGCTAAGCTTAGATCTGGTTGGGACAGCAGC-3'), *Il-6* (FW 5'-CTGATGCTGGTGACAACCAC-3', RV 5'-CAGAATTGCCATTGCACAAC-3'), *Ifn-γ* (FW 5'-

GCGTCATTGAATCACACCTG-3', RV 5'-ACCTGTGGGTTGTTGACCTC-3'), *Adgre1* (FW 5'-CTGTAACCGGATGGCAAACT-3', RV 5'-ATGGCCAAGGCAAGACATAC-3'), *Nos2* (FW 5'-CCTTGTTCAGCTACGCCTTC-3', RV 5'-AAGGCCAAACACAGCATACC-3'), *Arg-1* (FW 5'-AAGCTGGTCTGCTGGAAAAA-3', RV 5'-GACATCAACAAAGGCCAGGT-3') and cyclophilin (FW 5'-TTCCTCCTTCACAGAATTATTCCA-3', RV 5'-CCAGTGCCATTATGG-3').

*Preparation of lung tissue for single-cell suspension.* Red blood cells were lysed with ACK buffer (8,024 mg/L $NH_4Cl$, 1,001 mg/L $KHCO_3$, 7.722 mg/L EDTA·$Na_2 2H_2O$). Suspensions were then spun and filtered through a 100 µm (BD Biosciences; San Jose, CA, USA) mesh filter. Cells were plated in $CD4^+$ complete medium (5% FBS (Life Technologies; Grand Island, NY, USA), pen/strep, L-glutamine, folic acid, glucose, and 2-Mercaptoethanol in RPMI-1640 (Life Technologies)) and either left untreated or restimulated with 15 µg/ml of HDM for 96 h prior to evaluation of IL-5 and IL-17A in supernatants.

*Preparation of splenocytes.* Single cell suspensions were generated by passing the tissues through a 70 µm nylon mesh filter (BD Biosciences) and lymphocytes were enriched by centrifugation through Lymphocyte Separation Medium (MP Biomedicals; Santa Ana, CA, USA). Cells were plated in $CD4^+$ complete medium and either left untreated or restimulated with 15 µg/ml of HDM for 96 h prior to evaluation of pro-infammatory mediators in supernatants.

*Antibodies.* The following antibodies were used in this study: mouse anti-GSH (ViroGen; Watertown, MA, USA), goat anti-Glrx1 (Jackson ImmunoResearch; West Grove, PA, USA) mouse anti β-actin (Sigma-Aldrich; St. Louis, MO. USA), secondary HRP-conjugated anti-mouse (GE; Pittsburgh, PA, USA), myeloperoxidase (Abcam; Cambridge, MA, USA) and secondary anti-goat (Jackson ImmunoResearch).

*Histopathology.* Following euthanization, left lung lobes were inflated to 25 cm $H_2O$ and fixed with 4% paraformaldehyde in PBS followed by paraffin imbedding. Paraffin blocks were cut into 5-µm sections and mounted to slides. Tissue histopathology and inflammation were assessed by hematoxylin and eosin (H&E) staining. Mucus metaplasia was assessed by periodic acid Schiff staining (PAS) staining, and quantified by scoring airway PAS reactivity using a scale of 0 to 3 (0 representing no positive staining and 3 being the highest intensity) by two independent, blinded observers. Data represented are averages of the cumulative score from each mouse according to treatment group.