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Supplemental Information

Increased Hematopoietic Extracellular RNAs and Vesicles in the Lung during Allergic Airway Responses

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0.5 fmol of calibrator cocktail was spiked into each library prep

$$\frac{\text{fmol miRNA sequenced}}{\text{ug total RNA}} = \frac{\text{raw miRNA reads} * \frac{0.5\ \text{fmol}}{\text{raw calibrator reads}}}{\text{ug total RNA input}}$$

Figure S1. Calibrator calculation of sequenced miRNA (related to Figure 1). Formula using spiked-in calibrators to calculate fmol of miRNAs per input of total RNA.

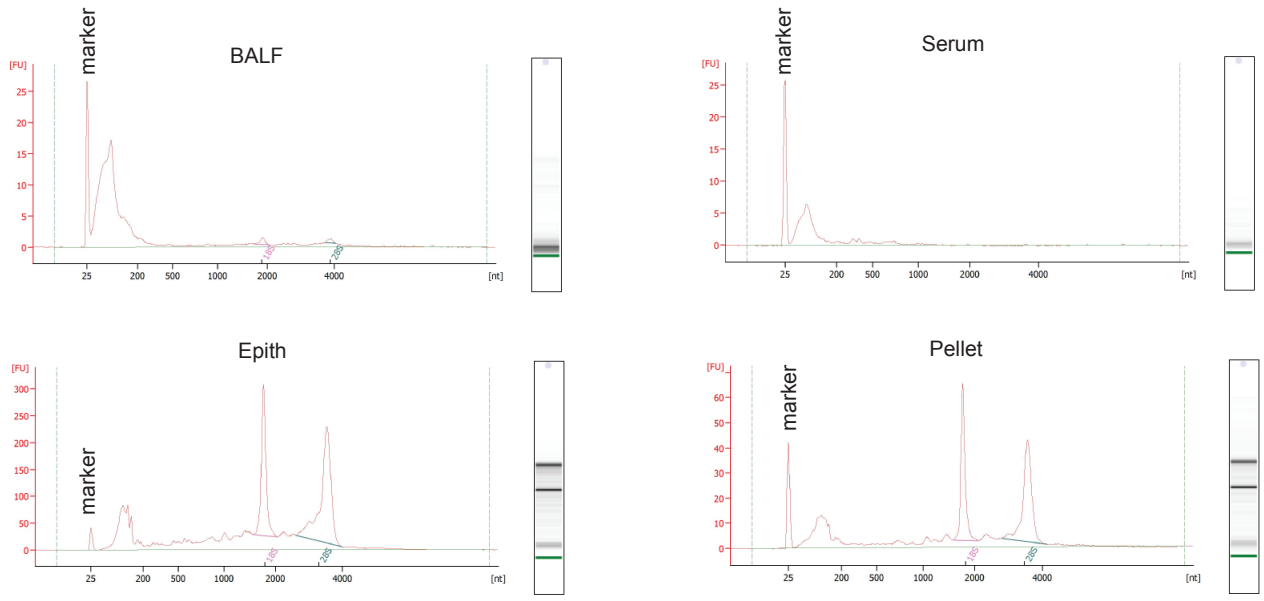

Figure S2. Biofluids contain predominantly small RNA species (related to Figure 1)**.** Example Bioanalyer tracings for BALF, epithelial brushings, hematopoietic rich cell pellets from bronchial washings, and serum used for generating sequencing libraries.

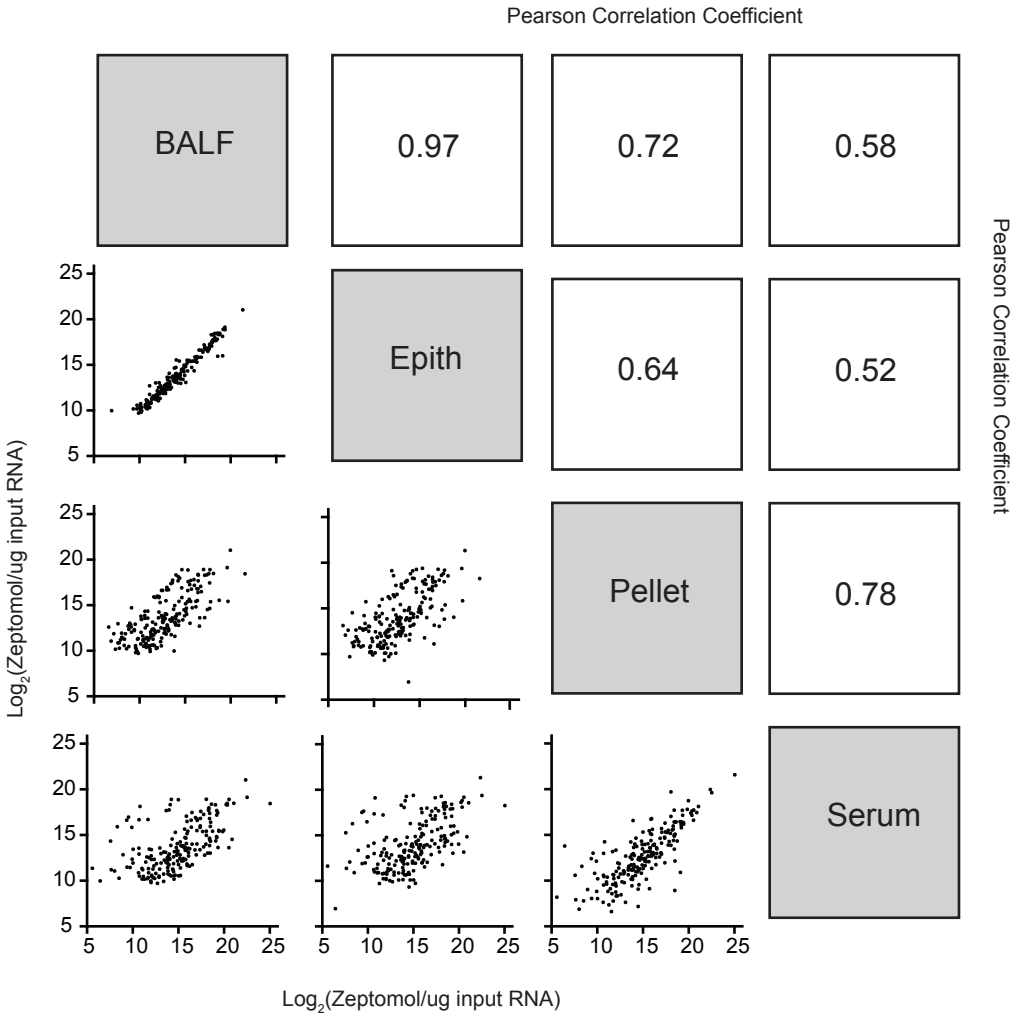

Figure S3. Correlation of ex-miRNA and miRNA expression by sample type (related to Figure 1)**.** Pearson correlation coefficient matrix of top 200 expressed ex-miRNAs in BALF and serum and cellular miRNA in epithelial brushings and hematopoietic cell rich cell pellets from bronchial washes. The log2 value of calibrator normalized miRNA per input of total miRNAs is depicted.

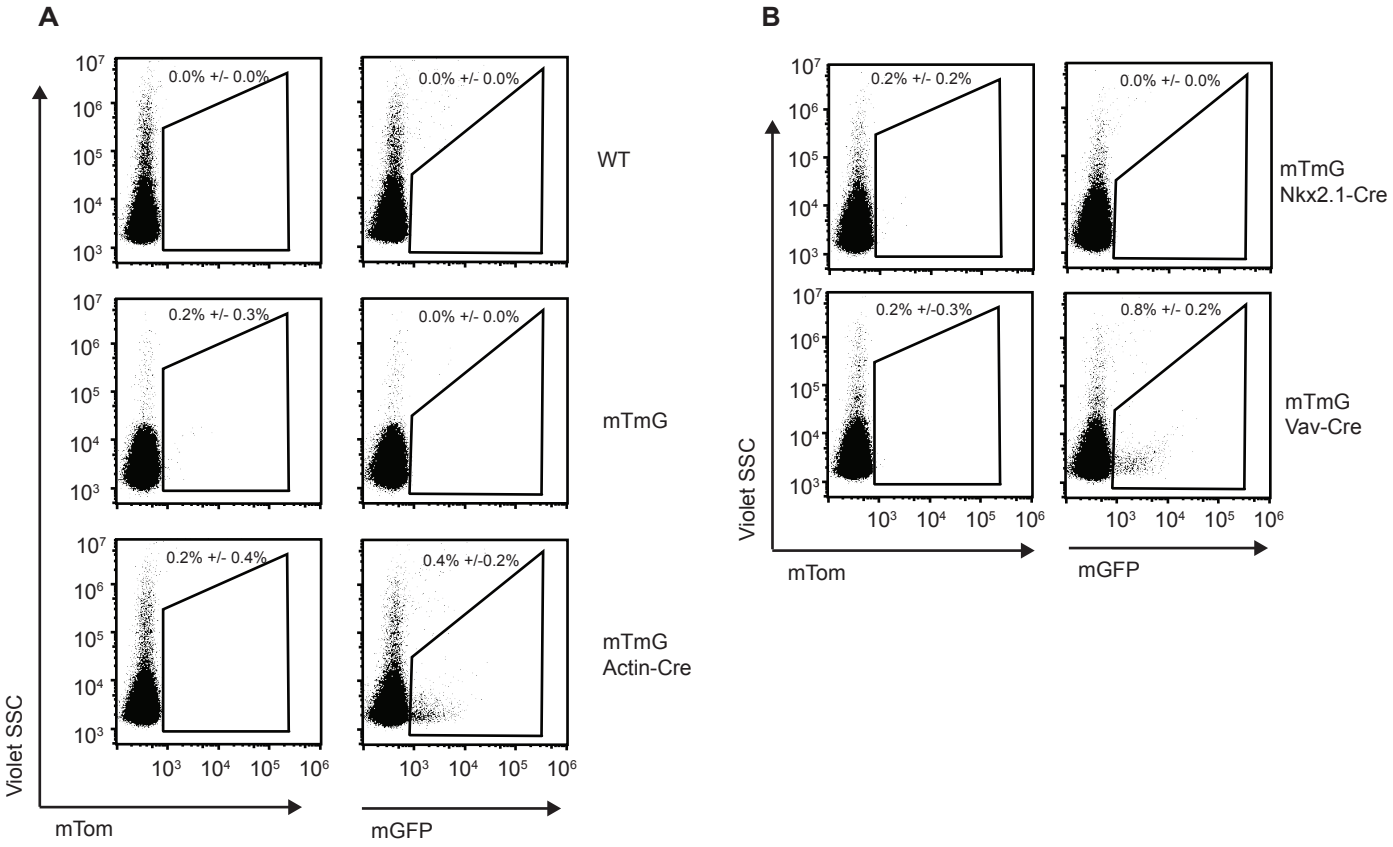

Figure S4. EV flow in serum (related to Figures 5 and 6). EV Flow of serum from (A) wild type control (WT), mTmG, and mTmG Actin-Cre and (B) mTmG Nkx2.1-Cre and mTmG Vav-Cre mice for detection of vesicles positive for membrane-bound Tomato (mTom) and membrane-bound Green Fluorescent Protein (mGFP).

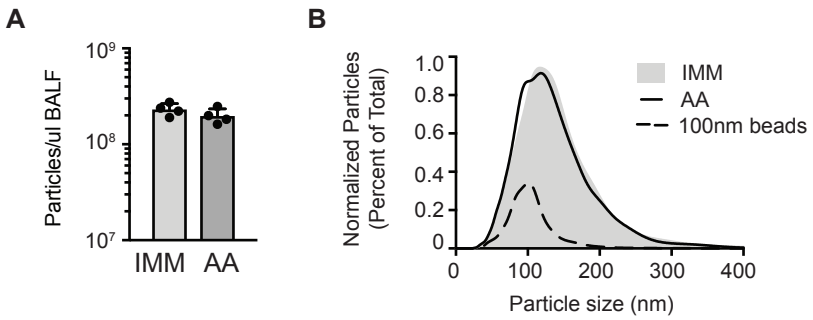

Figure S5. Nanoparticle tracking of immunized versus allergic airway BALF (related to Figure 6). (A) Concentration of particles in IMM and AA BALF by nanoparticle tracking assays. Each data point represents the average of three technical replicate readings for a single mouse (n=4, two-tailed t-test). (B) Size distribution of particles in BALF and serum in same mice as (A). 100nm beads are included as a positive control.

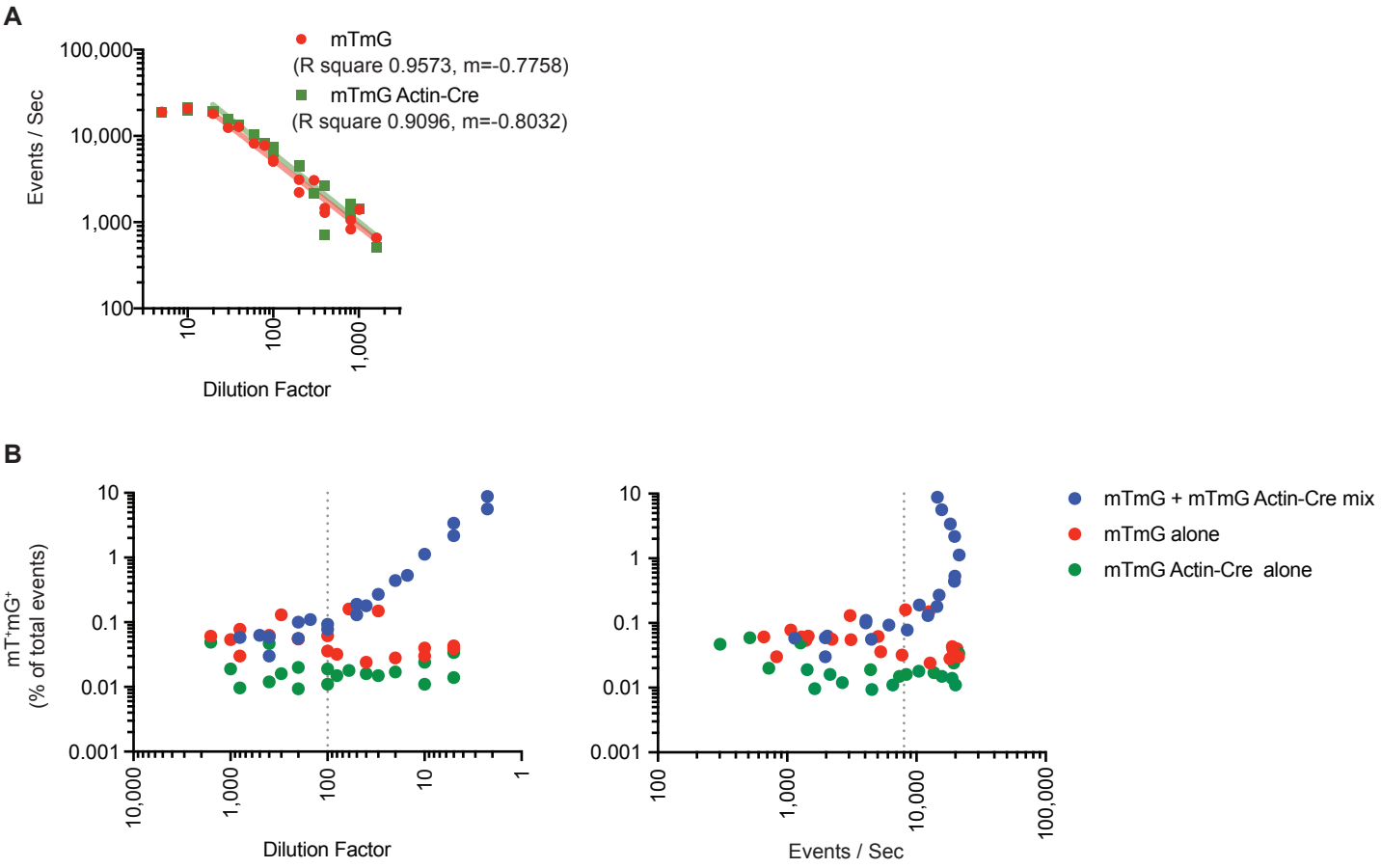

Figure S6. Measurement of flow rate and swarm effects in EV flow (related to Figure 5). (A) mTmG and mTmG Actin-Cre BALF samples were diluted 1:5 to 1:1600 in PBS and total events per second were determined. Linear regressions were calculated with exclusion of the two most concentrated specimens. (B) mTmG and mTmG Actin-Cre BALF samples were either run separately or mixed in equal ratio at different dilutions and the percent of detected Tomato positive (mT+) and GFP positive (mG+) particles used to measure coincident detection. Dotted lines at 1:100 dilution factor and 8,000 events/sec were used as cutoffs for collecting data in experiments in this study. Data plotted in both (A) and (B) is from four mice from two independent experiments.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
calibrator 1 pGUCCCACUCCGUAGAUCUGUUC	Williams et al., 2013	N/A
calibrator 2 pGAUGUAACGAGUUGGAAUGCAA	Williams et al., 2013	N/A
calibrator 3 pUAGCAUAUCGAGCCUGAGAACA	Williams et al., 2013	N/A
calibrator 4 pCAUCGGUCGAACUUAUGUGAAA	Williams et al., 2013	N/A
calibrator 7 pAGGUUCCGGAUAAGUAAGAGCC	Williams et al., 2013	N/A
Primer: miR-21a-5p: UAGCUUAUCAGACUGAUGUUGA	www.mirbase.org	N/A
Primer: miR-24-3p: UGGCUCAGUUCAGCAGGAACAG	www.mirbase.org	N/A
Primer: miR-34c-5p: AGGCAGUGUAGUUAGCUGAUUGC	www.mirbase.org	N/A
Primer: miR-142a-3p: UGUAGUGUUUCCUACUUUAUGGA	www.mirbase.org	N/A
Primer: miR-200b-3p: UAAUACUGCCUGGUAAUGAUGA	www.mirbase.org	N/A
Primer: miR-223-3p: UGUCAGUUUGUCAAAUACCCCA	www.mirbase.org	N/A
Primer: 5.8S ribosomal RNA Forward: ATCGTAGGCACCGCTACGCCTGTCTG	Bronevetsky et al., 2013	N/A
Primer: U7 small nuclear RNA Forward: GTTACAGCTCTTTTAGAATTTGTCTAGC	Bronevetsky et al., 2013	N/A
Small RNA sequencing: 3' oligodeoxynucleotide adapter: 5Phos/NNNNNXXXXXTGGAATTCTCGGGTGCCAAGG /3AmMO (where "N" are random nucleotides and "X" are pentamer indexing barcodes as outlined in the original protocol)	This paper and Williams et al., 2013	N/A
Small RNA sequencing: 5' oligoribonculeotide adapter: rGrUrUrCrArGrArGrUrUrCrUrArCrArGrUrCrCrGrArCrGr ArUrCrNrNrNrNrN (where "rN" are random nucleotides)	This paper and Williams et al., 2013	N/A
Small RNA sequencing: Library amplification 3' PCR primer:CAAGCAGAAGACGGCATACGAGATCGTGATG TGACTGGAGTTCCTTGGCACCCGAGAATTCCA	This paper and Williams et al., 2013	N/A
Small RNA sequencing: RT of library: GCCTTGGCACCCGAGAATTCCA	This paper and Williams et al., 2013	N/A

Table S5: Oligonucleotides (related to Key Resources Table in STAR METHODS)**.**