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Supplemental Information

Nanoparticle Delivery of Anti-inflammatory LNA Oligonucleotides Prevents Airway Inflammation in a HDM Model of Asthma

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Supplemental Data

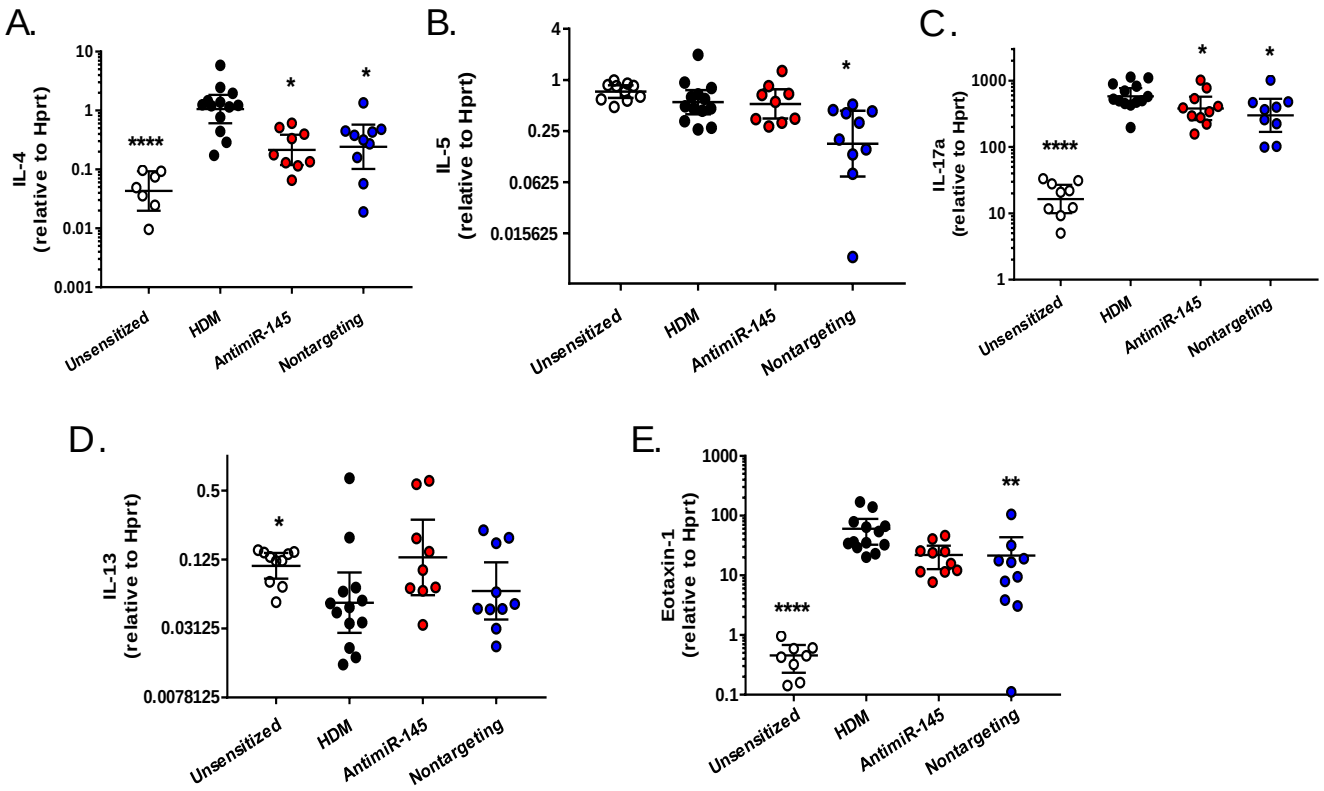

Figure S1. AntimiR-145 reduces expression of IL-4. qRT-PCR of isolated RNA of mouse lungs to determine the differences between treatment groups of (A) IL-4, (B) IL-5, (C) IL-13, (D) IL-17a, and (E) Eotaxin-1. Data are shown as means and 95% confidence intervals. Statistical analyses was performed by Kruskal-Wallis ANOVA with Dunn's post hoc test, * $p \leq 0.05$, *** $p \leq 0.01$, n = 9-13.

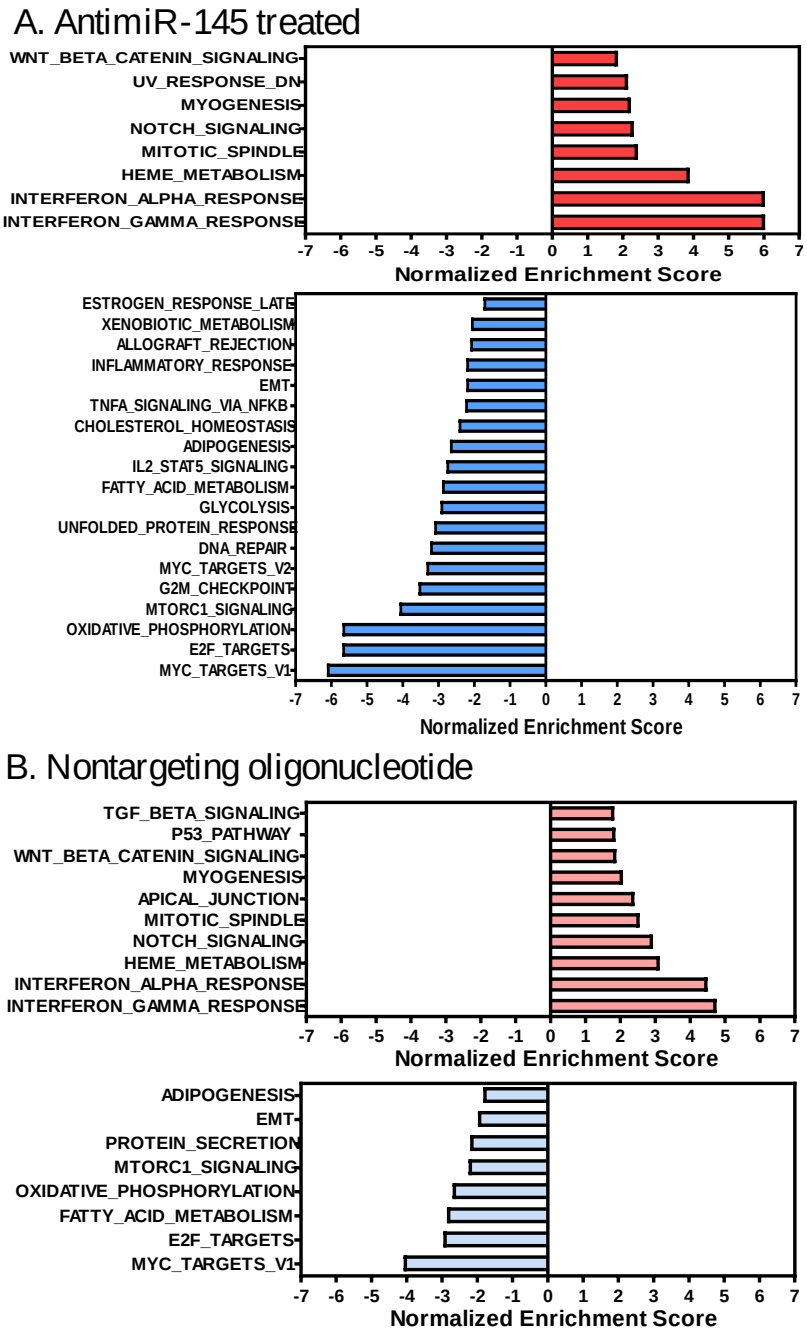

Figure S2. HDM-enhanced expression of Hallmark pathways is differentially affected by antimiR-145 compared to nontargeting oligonucleotide. Gene set enrichment analysis (GSEA) was performed using whole lung poly A RNA-seq data from HDM-sensitized mice treated with dextrose as the reference group. (A) Changes in signaling and metabolic pathways genes in the Hallmarks gene set of the MSigDB collection in HDM-sensitized mice treated with antimiR-145 (2 mg/kg, iv); FDR < 0.05. (B) Changes in signaling and metabolic pathways genes in HDM-sensitized mice treated with nontargeting oligonucleotide (2 mg/kg, iv); FDR < 0.05.

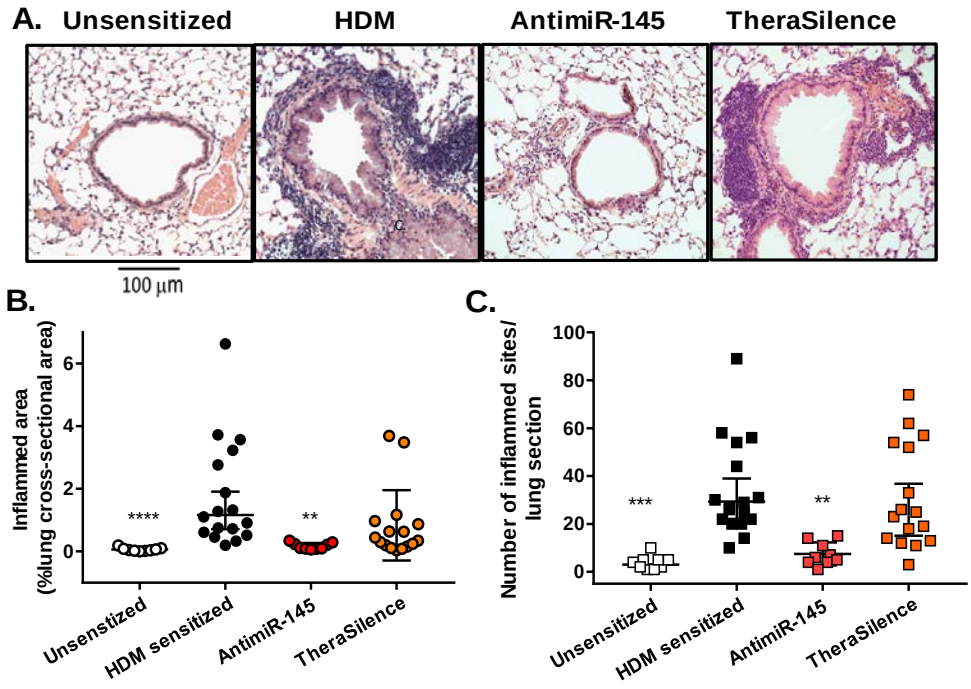

Figure S3. TheraSilence lipid nanoparticle does not have significant effects on airway inflammation. Paraffin embedded lung tissues were stained with (A) Hematoxylin and Eosin to identify inflammation (purple clusters). A representative micrograph for each treatment group was selected from all the airways within the treatment group. Images of Unsensitized , HDM-sensitized and antimiR145 treated lungs are duplicates of samples in Figure 6 used as reference images. (B) Volume density was quantified by inflammation area morphometry to determine differences in treatment groups. Data for Unsensitized, HDM and AntimiR-145 groups are reproduced from Figure 3A. (C) Number of inflamed sites in each treatment group. All numerical data are geometric mean ± 95% confidence interfavs. Hypothesis testing was conducted by Kruskal-Wallace ANOVA and Dunn's test using HD-sensitized mice as reference group; ** $p<0.005$, *** $p<0.0001$, N = 10-18.

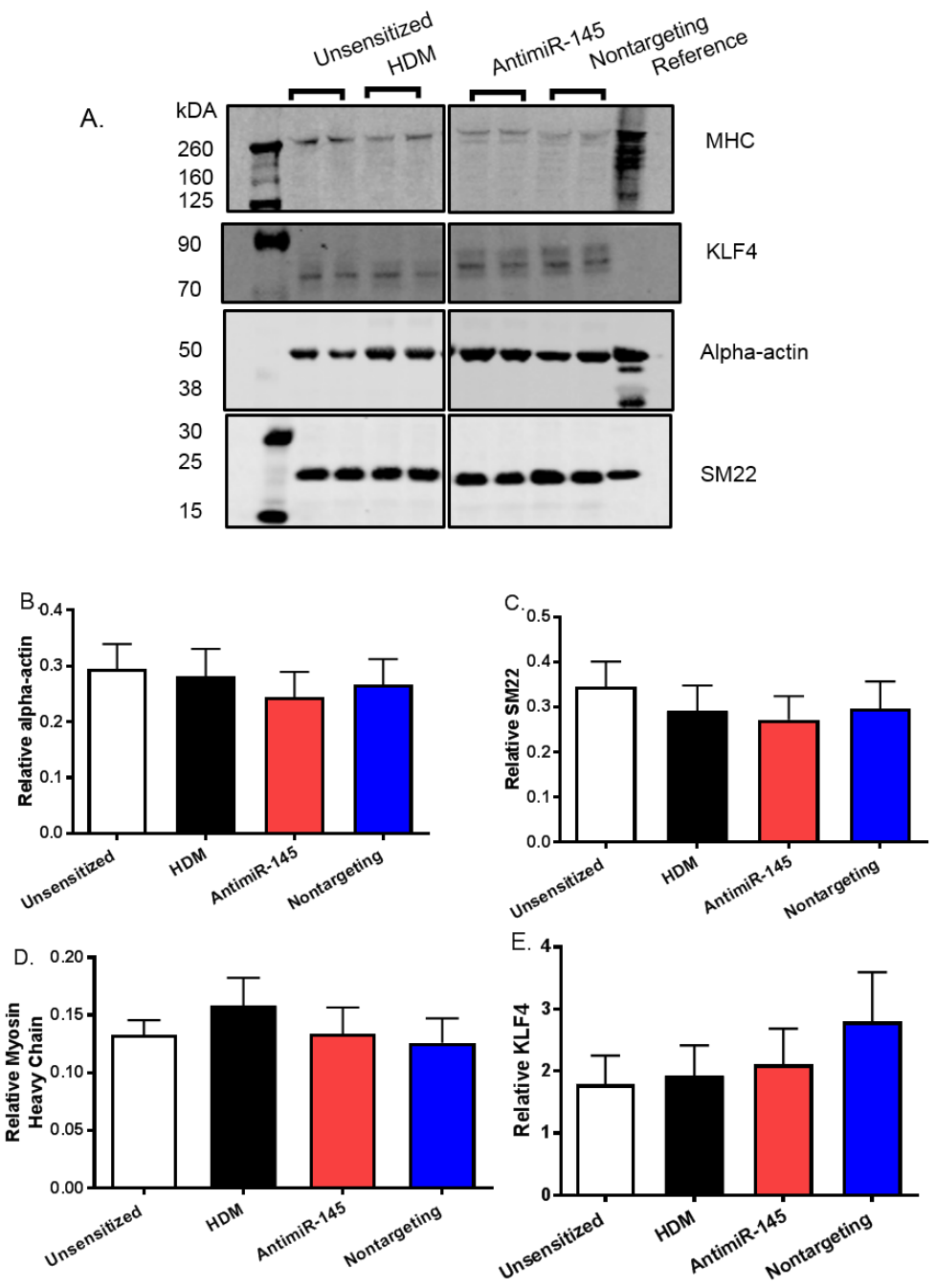

Figure S4. AntimiR-145 does not affect smooth muscle contractile protein abundance in the lung. Representative Immunoblots of protein from the lungs of mice were probed for contractile proteins (A). Quantitation of immunoblots for contractile proteins in all mice: (B) alpha-actin, (C), SM22, (D), myosin heavy chain, MHC and (E) KLF4. Human tracheal smooth muscle extract was used as a reference for quantitation. Results are mean ± SEM. Statistical analyses was performed by One-way ANOVA with Dunnett's post hoc test, * $p \leq 0.05$ comparing HDM vs antimiR-145 and HDM vs saline. N 10-11.

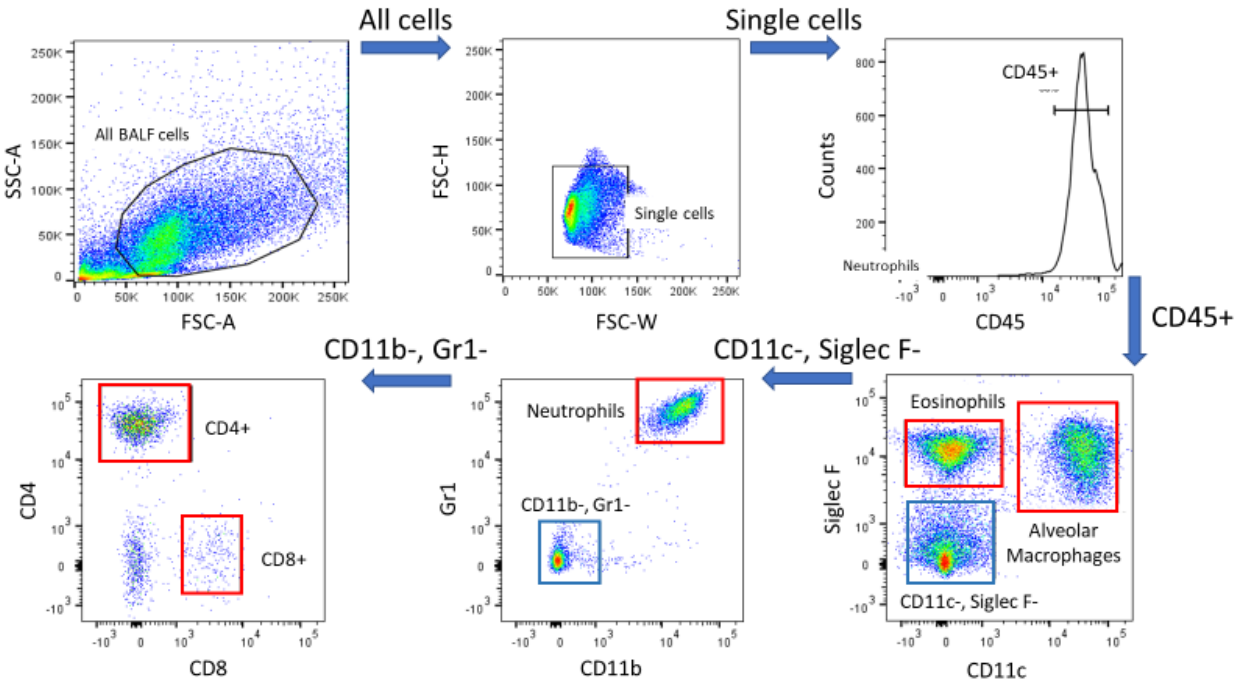

Figure S5. Gating scheme to identify cells in the bronchoalveolar lavage. Red gates indicate a terminal gate (cell population). Blue gates indicate non-terminal populations further gated as indicated by the labeled arrow of corresponding color.

Table S1. Antibodies and conjugates used in the BAL cell staining cocktail.

Target	Clone	Conjugate	Company
Fc Receptor	2.4G2	----	Bio X Cell, Lebanon, NH, USA
CD11b	M1/70	Pac Blue	BD Biosciences, San Jose, CA, USA
CD11c	N418	Brilliant Violet	Biolegend, San Diego, CA, USA
Gr1	RB6-8C5	FITC	BD PHarmigen, BD Biosciences
CD45	30-F11	PE	BD Biosciences
CD4	Gk1.5	PE-Cy7	eBiosciences, San Diego, CA, USA
CD8	53-6.7	APC-Cy7	BD Biosciences
CD68	FA-11	PerCP-Cy5.5	Biolegend
Siglec F	E50-2440	APC	BD Biosciences

Table S2. Antibodies and conjugates used in the compensation controls.

Target	Clone	Conjugate	Company
B220	R43-6B2	Pac Blue	BD Pharmigen
B220	R43-6B2	SA-Amcyan	BD Biosciences
B220	R43-6B2	FITC	eBiosciences
B220	R43-6B2	PE	BD Pharmigen
CD19	eBio 103	PE-Cy7	eBiosciences
CD19	1D3	APC-Cy7	BD pharmigen
B220	R43-6B2	PerCP-Cy5.5	eBiosciences
B220	----	APC	BD Pharmigen

Table S3. Primers used in RT-PCR and accession numbers of RT-PCR targets

mRNA	Forward (F)	Tm (°C)	Reverse (R)	Tm (°C)
Muc5ac	CAA CCT CCT CTC TTG ACA AC	54.81	GGC GAC TGG TGC TAT T	55.06
Muc5b	CCT CAA GAC CTC ATA CC	55.13	GTC CAA AGT CCA CAT TAC	55.13
IL-4	GAC GGC ACA GAG CTA TTG AT	54.78	CTC ACT CTC TGT GGT CTT C	62
IL-5	TGA AGT GCT GGA GAT GGA AC	54.72	GCT AGG AGA AAG GAT GCT AAG G	54.78
IL-13	GGA TTC CCT GAC CAA CAT CTC	55.19	AGG GAT GGT CTC TCC TCA TT	54.87
IL-17a	CAA CCT CCT CTC TTG ACA CTA AC	54.81	GGC GAC TGG TGC TGA TAT T	55.06
Eotaxin-1	GCA GCA TGG TAT GGA GTG T	55.08	TAT CCT CTG GGT CCT GTA GAT G	54.96
Hprt	CCC CAA AAT GGT TAA GGT TGC	54.76	AAC AAA GTC TGG CCT GTA TCC	54.86
	Ensembl Gene ID			
Muc5ac	ENSMUSG00000037974			
Muc5b	ENSMUSG00000066108			
IL-4	ENSMUSG00000000869			
IL-5	ENSMUSG00000036117			
IL-13	ENSMUSG00000020383			
IL-17a	ENSMUSG00000025929			
Eotaxin-1	ENSMUSG00000020676			
Hprt	ENSMUSG00000025630			

Table S4. Validated miR-145 targets and functions relevant to lung remodeling

Cell type	Targets	Functional effect	Reference
Smooth muscle	KLF4/5 Fascin	↑ contractile proteins ↓ proliferation and motility	46 66 67
Endothelial cells	JAM-A	↓atherosclerotic lesions	68
Epithelial cells	CFTR	↓CFTR protein	69 70
Immune cells	PDE8A (macrophages) PBMCs CTLA-4 (Treg)	Modifies HIV replication ↑cell content of miR-145 Undefined	71 72 73
Stem cells	OCT4, SOX2, KLF4 Dab2	↓pluripotency ↓Wnt/β catenin activity	74, 75