

**DUOX2 BIDIRECTIONAL PROMOTER POLYMORPHISMS CONFER
DIFFERENTIAL IMMUNE RESPONSES IN AIRWAY EPITHELIA**

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ONLINE DATA SUPPLEMENT

SUPPLEMENTAL DATA

Supplemental Table 1: Distribution of SNP genotypes based on population data obtained from the 1000 Genomes project (<http://www.1000genomes.org/>). Number of individuals in each group (N) and percent of individuals carrying the specific genotype are shown.

Observed Genotype	African (246)	Asian (286)	European (381)
AA and TT	201 (81.71%)	286 (100%)	381 (100%)
AG and CT	44 (17.89%)	0	0
AG and TT	1 (0.41%)	0	0

Supplemental Table 2: Distribution of two-marker haplotypes (rs269855-rs2576089) in an African population. Overall haplotype frequencies are maximum likelihood estimates (42) based on the observed genotype frequencies (see Supplemental Table 1).

Haplotype	Overall frequency (%)
A-T	90.85
A-C	0
G-T	0.2
G-C	8.94

Supplemental Figure 1: Variability in harvested plasmid levels for each time point in HBE1 cells after transfection. HBE1 cells in submerged cell culture conditions were transfected at 60-70% confluence with the bidirectional reporter plasmid and total cellular DNA was prepared at various time points after transfection. Cells were counted and underwent lysis with equal buffer volumes for each sample to ensure similar input total DNA prior to PCR amplification. Mean \pm SD of threshold cycles (Ct; inversely proportional to the log₂ of plasmid DNA) measured by qRT-PCR of three separate samples per time point. Data from all experiments (three replicates for three different experiments) are shown.

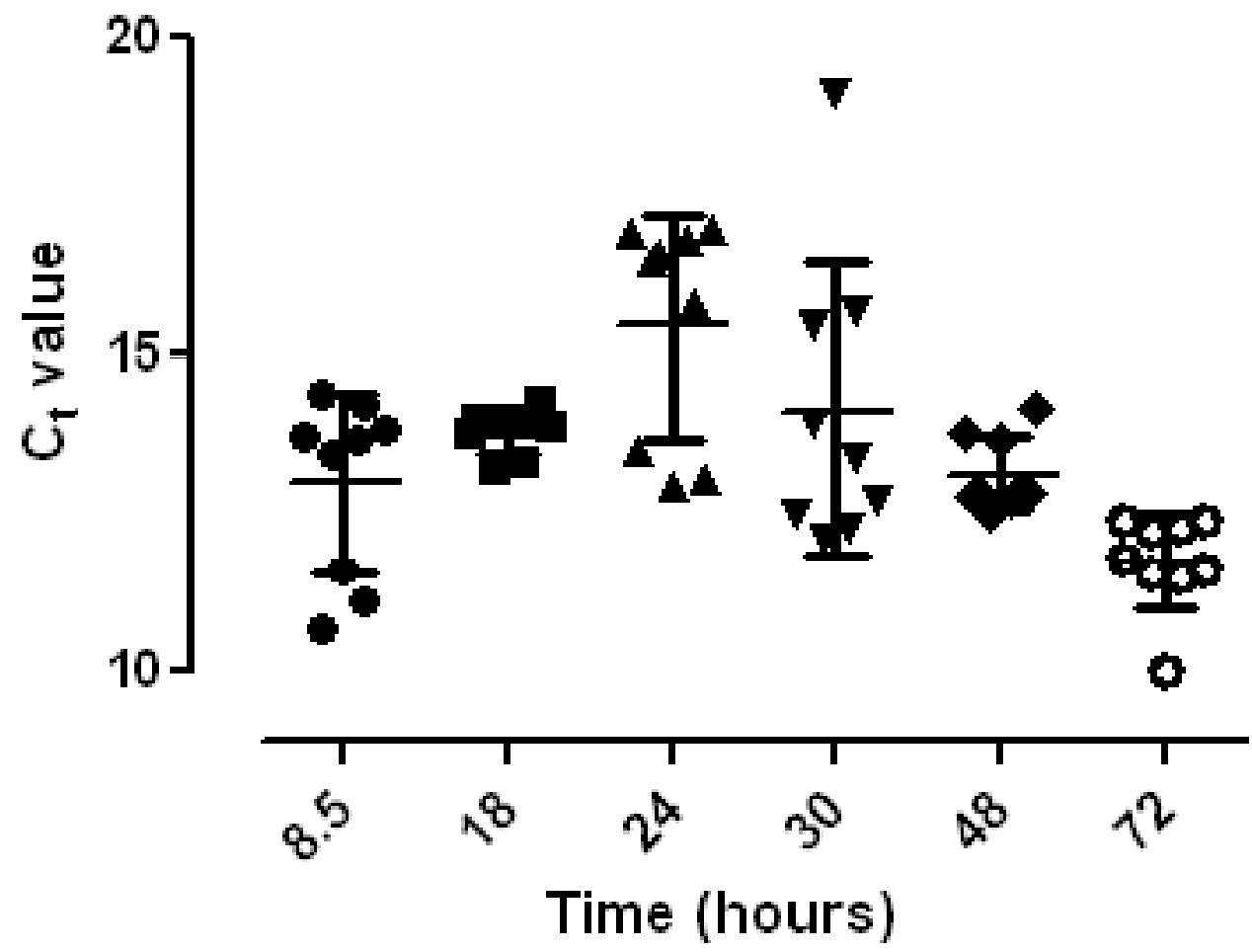
Supplemental Figure 2: Chromosome 15 sequence encompassing the translation start codons (boxed) for both *DUOX2* (opposite strand) and *DUOXA2*. This region includes exon 1 and 2 of *DUOX2* and exon 1 of *DUOXA2* (black upper case) and the intervening intronic DNA (gray lower case). Various transcription start sites (TSS) for *DUOX2* (D2) or *DUOXA2* (DA2) are shown including the canonical sequence available in GenBank (www.ncbi.nlm.nih.gov/genbank) (bold arrows) or published by Puchucki or Grasberger (19, 34) (thin arrows). A CpG island region was identified using highly restrictive criteria including GC content > 60%, observed CpG/Expected CpG ratio > 0.7, and CpG island length > 200 bp. Two different CpG island prediction programs were used (31, 32) and both programs predicted highly similar CpG island regions (underline).

Supplemental Figure 3: Alignment of rat, mouse and human genomic sequence in two separate segments of the *DUOX2/A2* promoter demonstrating the conserved TATA box motif (box) for



DUOXA2, and the Inr (#)/DPE (*) sites for *DUOX2* in all three species. Of note, two Inr sites were conserved in all three species. Several other DPE sites were identified within this genomic sequence (not shown), but did not align in all three species. Nucleotides are numbered starting with the ATG site of *DUOX2* and the transcription start site for each gene is bold underlined.

Supplemental Figure 4: Alignment of rat, mouse and human genomic sequence in two separate segments of the *DUOX2/A2* promoter surrounding the two identified SNPs in the human genome (bold underline). Nucleotides preserved in all three species are denoted by (^). Nucleotides are numbered starting with the ATG site of *DUOX2*. Alignment of putative transcription factor binding sites for each SNP are shown (www.gene-regulation.com).

Supplemental Figure 5: Effect of rhinovirus (RV) infection or interferon-gamma (γ) treatment on basal *DUOX2/DUOXA2* promoter activity. HBE1 cells were grown in submerged cell culture conditions and transfected with the full length promoter construct (FL) followed by infection with RV, interferon- γ , or both for sequential measurements of *Renilla* and firefly luciferase activity. A β -galactosidase expression plasmid was co-transfected in all cells and used to normalize luciferase activity between experiments. (A) Cells were infected or treated for 4 hours prior to harvest at 12 hours after transfection. (B) Cells were infected or treated for 16 hours prior to harvest at 24 hours after transfection. Normalized data are presented as fold increase compared to the FL construct (mean \pm SEM; n = 3).



Supp. Figure 1

1 cgctagagga gcctgatacT TGCCCGATGG ACCCAGGGAT CCAGTCAGAA GAGCTCCCAG GAGCATCAGT GCCTCTGGTC TTGCACGGAG
91 **CAT**GCCAACC CTGCAGCctg cggggtgagg gtgggggtgg taggtggtat gcgaaagcca ctggttagggc gtcctctatg cctcccctct
181 tgttctctaca gctagtactg gaggaggagc accagctggt tccacttctg gaaggtagct gttagaagca tcaccgagga ctttcatcca
271 aacgccactc tttccaggac aattggcagc tctggaggca ttgacactgt tccccatccg ccaccccatc agagagttaa cccccgacca
361 taggaacca ctgggcagga gtcttctggg gcatgtcagt ccagggcagg actggtcaag cctcccaggg tgtgccaat gtctccaatg
451 tttcacctcc acccgcccc Aatccgtcag gctccgcttc tctccagga ggcaggggaag ggaaaaaggt tactgacctg ggagtgaggg
541 actgcagcac cttccacaa tgaatcccc cttcccaat aaactccct tctgcaatga acgcctgtgc atgatgggcg agggctaggg
631 tcagatccca aactctggtc taac**CTGTGG** TTTAGGGTGG TGTGGGTTC AGATGTCTTC TTTCCTCTTA AAATCTTTGC TTCTGTGCTC
721 TACTTCTTGC CTTACCCCTC ACTCTTCCAG CTCCGCCGAT CCTCAGCCTC CCCGGCTGCA CTCTCACCTT TCTCTCTGGG TCCTTGGTCT
811  CGCCACTGTG CAGGTGTCGG CTCAGGACAG AC**Ctgcgcca** gtgtgagcat ctggacctag ggctcaccct cctgcccgtg aggtggggcc
901 cttatattgca taacctcttc cagctcagac cagcccctgg gctgggacac ccgtgtggca cgtcgcccac gctg**ctataa** aaggggtccc
991  gcgcgacttc caaact**CAGC** GCCAACCCGC AGAACCAGGA AAGTAACGGC TACAGACAGT GAGAAATAGT TTCGCTCGCC GGCTAGAAAA
1081 ACTCTGTCGG TACCAACCCC AGAGCGTTGA GAGCAGCCCA CCTCCACGCT TCCTTAACGG AGAGGTGCAG GACTCAGACT TCACCAGCCC
1171 ACTCGGTCCC AGCCTTGAC GCAAAGAGAC GCCAAGGACG CGCTCTCCCG CGTCCAGGCA GCCCCAGCTT GCTGGCTTGC CTGCCCGCCT
1261 GCGTGCAGCA CTCGGCCGGC GTGCAGC**ATG** ACCCTGTGGA ACGGCCTACT GCCTTTTTTAC CCCCAGCCCC GGCATGCCGC AGGCTTCAGC
1351 GTTCCACTGC TCATCGTTAT TCTAGTGTTC TTGGCTCTAG CAGCAAGCTT CCTGCTCATC TTGCCGGGGA TCCGTGGCCA CTCGgtaagg

Supp. Figure 2

← DUOX2

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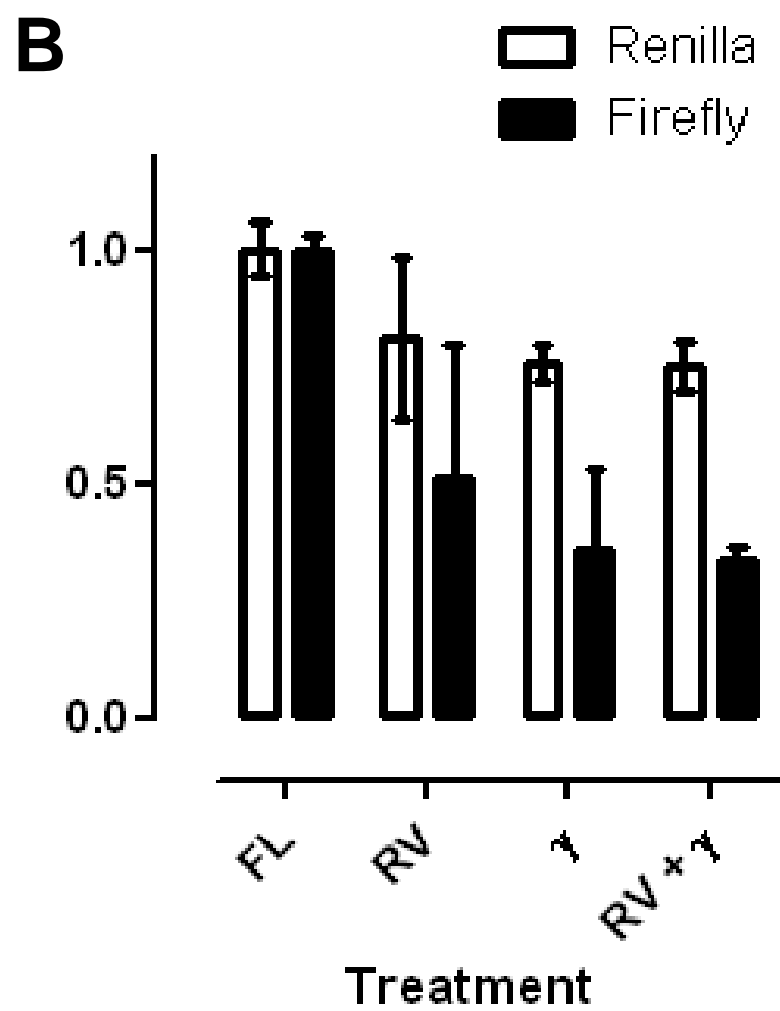
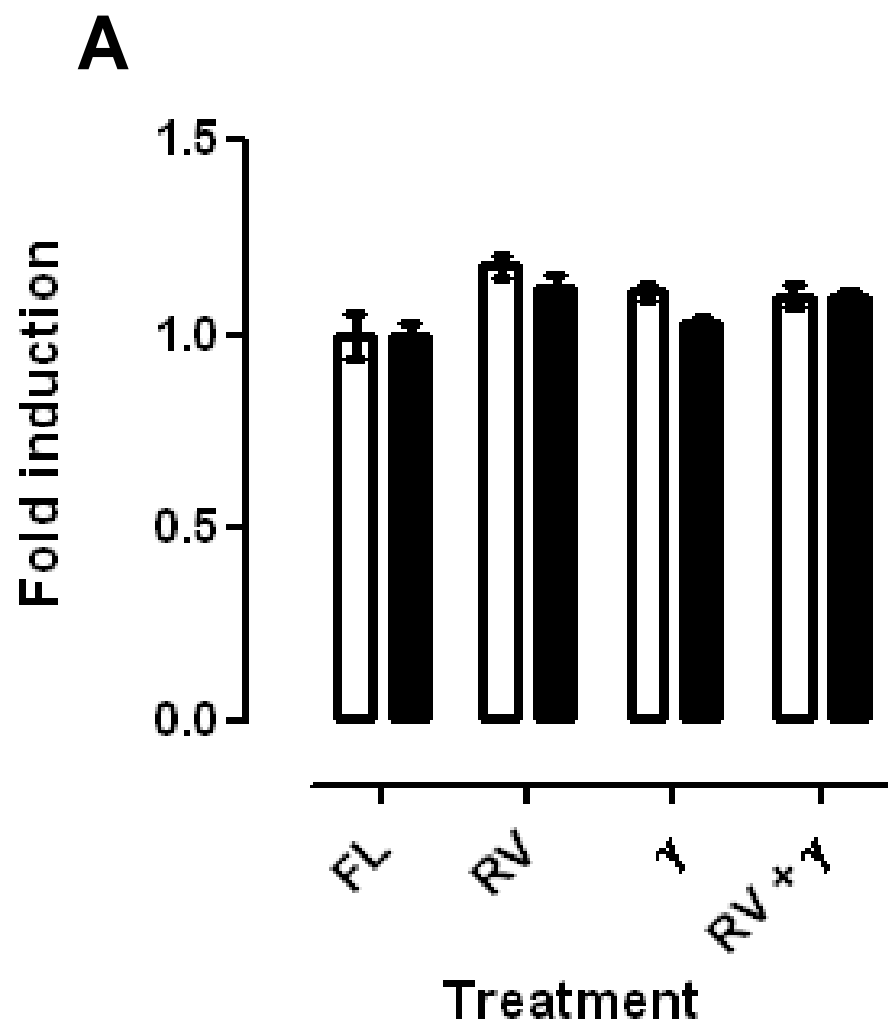
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RAT AGGTGTCAGCTCGCGCCA---CCGCT-GCAGGTGTAAATGTGTGAATCAGAGGCTAA 498
MOUSE AGGTGTC-GCTCGCGCCA---CCGCTAGCAGGTGTGAATGTGTGAATCAGAGGTTTG 584
HUMAN AGGTGTCGGCTCAGGACAGACCTGC--GCCAGTGTGAGCATCTGGACCTAGGGCTCA 785

RAT TCCCCTGCTTGCTGCCACGCTCCTATAAAAG---AGGGTCTGCTTCTTGTGAACGC 651
MOUSE GCCCCTGCTTGCTGCCACGCTCCTATAAAAGGAGGGGGTCTGCGTTTTGTGAACAC 740
HUMAN CGTGTGGCACGTCGCCACGCTGCTATAAAAG---GGGTCCCGCGCGTC-CAAACTC 913

DUOXA2 →

Supp. Figure 3



Supp. Figure 5