

Supplemental Methods - Inclusion and Exclusion Criteria

Healthy nonsmokers

Inclusion criteria

- Males and females, at least 18 years old
- Provide informed consent
- Normal physical examination
- HIV1 negative
- Not pregnant (females)
- Good health without history of chronic lung disease, including asthma, and without recurrent or recent (within 3 months) acute pulmonary disease
- Normal routine laboratory evaluation, including general hematologic studies, general serologic/immunologic studies, general biochemical analyses, and urine analysis
- Normal PA and lateral chest X-ray
- Normal electrocardiogram (sinus bradycardia, premature atrial contractions are permissible)
- No history of allergies to medications used in the bronchoscopy procedure
- Not taking any medications relevant to lung disease or having an effect on the airway epithelium
- Willingness to participate in the study
- Self-reported nonsmokers, with smoking status validated by the absence of nicotine and cotinine in urine (nicotine<2 ng/ml, cotinine<5 ng/ml)

Exclusion criteria

- Unable to meet the inclusion criteria
- Current active infection or acute illness of any kind
- Evidence of malignancy within the past 5 years
- Alcohol or drug abuse within the past 6 months

Healthy active smokers

Inclusion criteria

- Males and females, at least 18 years old
- Provide informed consent
- Normal physical examination
- HIV1 negative
- Not pregnant (females)
- Good health without history of chronic lung disease, including asthma, and without recurrent or recent (within 3 months) acute pulmonary disease
- Normal routine laboratory evaluation, including general hematologic studies, general serologic/immunologic studies, general biochemical analyses, and urine analysis
- Normal PA and lateral chest X-ray
- Normal electrocardiogram (sinus bradycardia, premature atrial contractions are permissible)
- No history of allergies to medications used in the bronchoscopy procedure
- Not taking any medications relevant to lung disease or having an effect on the airway epithelium

- Willingness to participate in the study
- Self-reported current daily smokers with any number of pack-yr, validated by urine nicotine ≥ 30 ng/ml and cotinine ≥ 50 ng/ml

Exclusion criteria

- Unable to meet the inclusion criteria
- Current active infection or acute illness of any kind
- Evidence of malignancy within the past 5 years
- Alcohol or drug abuse within the past 6 months

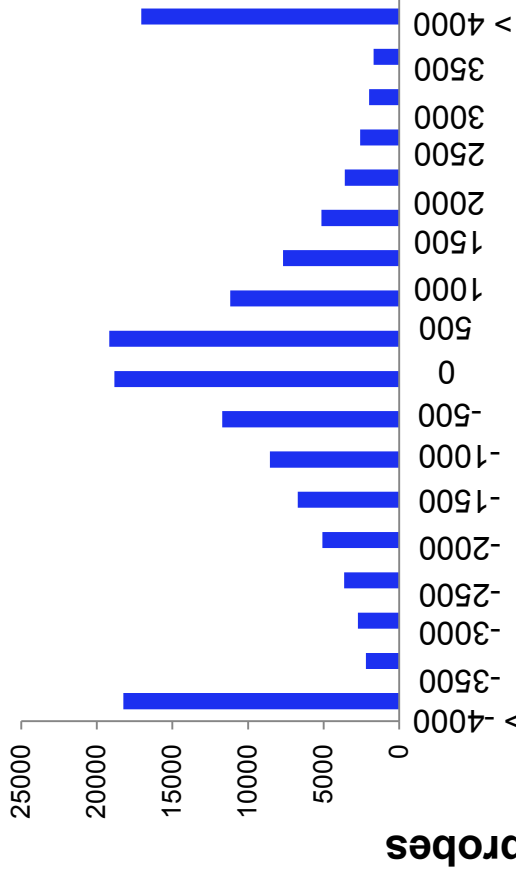
Table S1. Primers for EpiTyper Analysis

Primer name	Primer sequence (5'-3')
ALDH3A1_000_10F	aggaagagagGGAGAGGTTGTGTTAAAGGTAATTT
ALDH3A1_000_T7R	cagtaatacactcactatagggagaaggctCCTTCCTTCCAATCATAAAATCTAA
ALDH3A1_001_10F	aggaagagagTGTTTTTGGGATTATTATTTTTTGGGA
ALDH3A1_001_T7R	cagtaatacactcactatagggagaaggctTACAACATACACTAAAAACCTCCCC
ALDH3A1_002_10F	aggaagagagGGGGAGGTTTTTAGTGTATGTTGTA
ALDH3A1_002_T7R	cagtaatacactcactatagggagaaggctCTAACCCTAATCCCAAACCTTTCTA
ALDH3A1_003_10F	aggaagagagTTTTTTATTTGGGTTGTTTGTTTTG
ALDH3A1_003_T7R	cagtaatacactcactatagggagaaggctTCCATCAACTATCAAATACTACCCC
CYP1B1_000_10F	aggaagagagGAGTAGTTGGGATTATAGGTGTGTATT
CYP1B1_000_T7R	cagtaatacactcactatagggagaaggctTAAAAAACCCAAATACCTTCTTTCC
CYP1B1_001_10F	aggaagagagGTTTAAGTTTTTTAAAGTGTGGGA
CYP1B1_001_T7R	cagtaatacactcactatagggagaaggctTAATAAATTCACCAAAAACAACCCCT
CYP1B1_002_10F	aggaagagagAGGGTGTTTTTTGGTGAATTTATTA
CYP1B1_002_T7R	cagtaatacactcactatagggagaaggctAAACCCTCCCTTTATAAAAAAACCC
SFRP2_000_10F	aggaagagagAGATTATGAAGGAGGTGTTGGAGTA
SFRP2_000_T7R	cagtaatacactcactatagggagaaggctACACCCCAAACAAAATACACTAAA
SFRP2_001_10F	aggaagagagTTTTGTTTTTTTATTTTTTGTGGG
SFRP2_001_T7R	cagtaatacactcactatagggagaaggctACCTCTAACCAAAATACTCACCACA
SFRP2_002_10F	aggaagagagTTGTTAAAGATGTTAGTATTGGAGTTG
SFRP2_002_T7R	cagtaatacactcactatagggagaaggctAAAAAATAATTTCCAAAAATTCCC
RPL26_000_10F	aggaagagagTTTGTTTTAGTTTTTTGGAAAGAGG
RPL26_000_T7R	cagtaatacactcactatagggagaaggctAATCCAATCTTAATTTCCCTCAAACA
RPL26_001_10F	aggaagagagAATTTTTTAAGTAGTGTGTTGAGGAAA
RPL26_001_T7R	cagtaatacactcactatagggagaaggctCAAACCACACTTACCCACTAAAAAA
RPL26_002_10F	aggaagagagGGGTTTTAAAAGTTTTTTAGTGGGT
RPL26_002_T7R	cagtaatacactcactatagggagaaggctTTTATCTATTACTTTCTTAAATCAAAAACC
RPL26_003_10F	aggaagagagGGTTTTTGATTTAAGAAAGTAATAGATAAA
RPL26_003_T7R	cagtaatacactcactatagggagaaggctCTAAACAACCTTAACCAATAACCCC
RPL26_004_10F	aggaagagagGGTTATTGGTTAAGGTTGTTTAGGG
RPL26_004_T7R	cagtaatacactcactatagggagaaggctTAATCTAAACCAACACCTCCCAC
RPL26_005_10F	aggaagagagTTAGAGATTTAGTTTTGGGTGGTGA
RPL26_005_T7R	cagtaatacactcactatagggagaaggctCCTATAATCCCAACTACTCAAAAAA
RPL26_006_10F	aggaagagagTTGTTTTAGTTTTTTGAGTAGTTGGG
RPL26_006_T7R	cagtaatacactcactatagggagaaggctACCTCAAAAACCATTTACAAAAACC
RPL26_007_10F	aggaagagagGGGTTTATTATGTTGGTTAGGTTGG
RPL26_007_T7R	cagtaatacactcactatagggagaaggctTAAAATTCCCACAAATTAACCAT
RPL26_008_10F	aggaagagagGGTTTTTTTTGGGAATTGTATATGG
RPL26_008_T7R	cagtaatacactcactatagggagaaggctCCAATCAATACTAACCTCACCTCAA
RPL26_009_10F	aggaagagagGGAAGATTTTTGAGGTGAGGTTAGT
RPL26_009_T7R	cagtaatacactcactatagggagaaggctCAACTCCCTAAAACCTCCAAACC

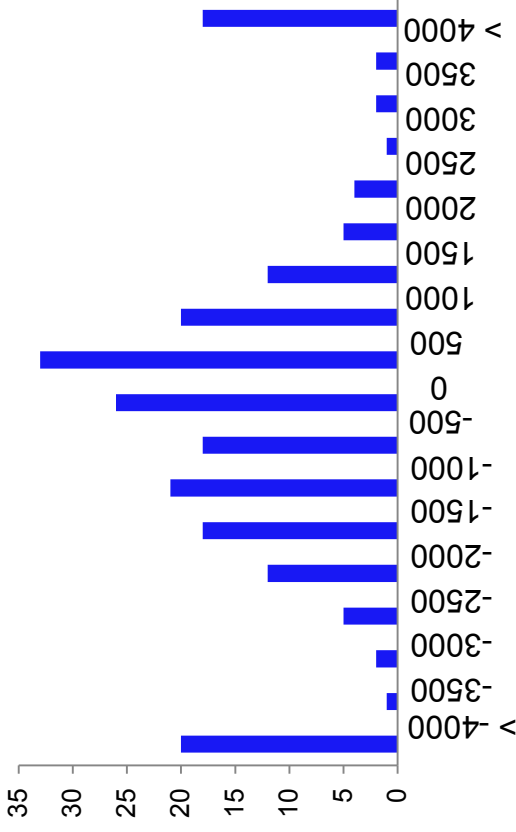
Supplemental Figure Legends

Supplemental Figure 1. HELP probe locations in relation to the transcriptional start site. Y-axis – number of probes; X-axis - the probe distance upstream (- numbers), or downstream (+ numbers) from the transcriptional start site. **A.** All probe locations from the HELP assay are represented regardless of differential methylation. **B.** Probe locations for the 220 differentially methylated probes ($p < 0.05$, fold-change $> \pm 1.5$), comparing smokers ($n=20$) to nonsmokers ($n=19$). **C.** Probe locations in relation to the transcriptional start site of the 56 hypermethylated probe sets ($p < 0.05$, fold-change $> \pm 1.5$), comparing smokers ($n=20$) to nonsmokers ($n=19$). **D.** Probe locations in relation to the transcriptional start site of the 164 hypomethylated probe sets ($p < 0.05$, fold-change $> \pm 1.5$), comparing smokers ($n=20$) to nonsmokers ($n=19$).

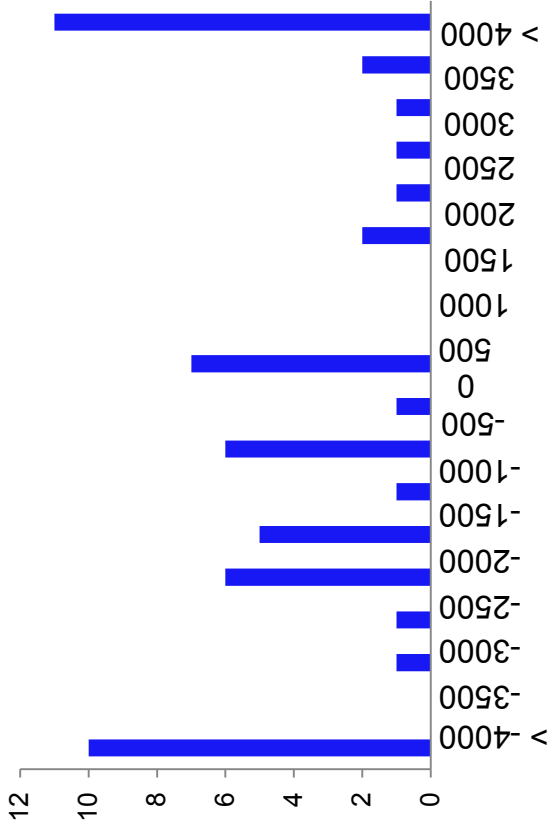
A. All probes



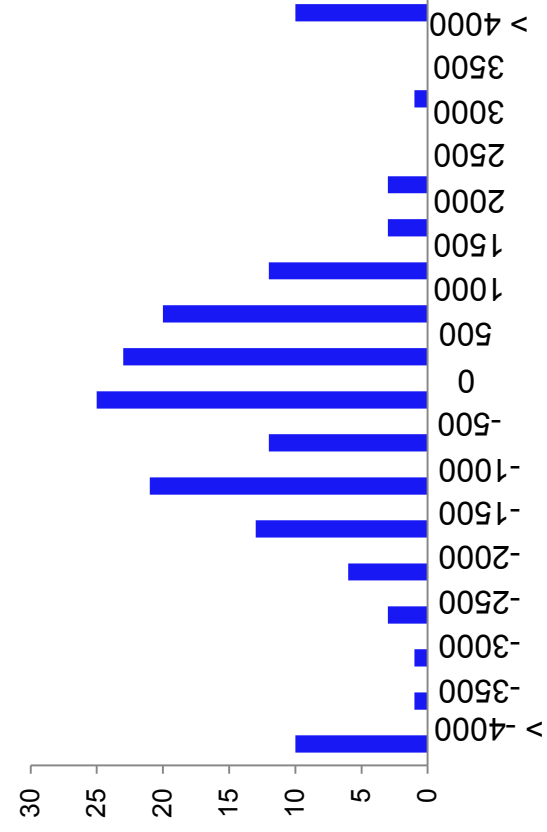
B. Significant probes



C. Hypermethylated probes



D. Hypomethylated probes



Distance to transcriptional start site