# **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**

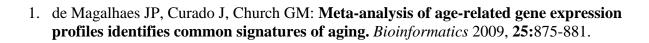
- Males and females, at least 18 years old
- Provide informed consent
- Normal physical examination
- HIV1 negative
- Not pregnant (females)
- Good health without history of chronic lung disease, including asthma, and without recurrent or recent (within 3 months) acute pulmonary disease
- Normal routine 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

# References



# Supplemental Table I. List of Aging Signature Genes Expressed in Small Airway Epithelium<sup>1,2</sup>

234312_s_at	Probeset ID <sup>3</sup>	Gene symbol	Gene title
200939_at ANXA3 annexin A3 201525_at APOD apolipoprotein D 207508_at APSOB_at APSOB_		ACSS2	acyl-CoA synthetase short-chain family member 2
2007508_at APOD apolipoprotein D 2007508_at ATP5G3 ATP synthase; H+ transporting; mitochondrial Fo complex; subunit C3 201891_s_at B2M beta-2-microglobulin 218232_at C1QA complement component 1; q subcomponent; A chain 202953_at C1QB complement component 1; q subcomponent; B chain 225353_s_at C1QC complement component 1; q subcomponent; C chain 217767_at C3 complement component 13 214428_x_at C4A complement component 4A (Rodgers blood group) 201310_s_at C5orf13 chromosome 5 open reading frame 13 206209_s_at CA4 carbonic anhydrase IV 20562_s_at CALB1 calbindin 1; 28kDa 201560_at CLU4 chloride intracellular channel 4 201560_s_at COLJA1 collagen; type II; alpha 1 215076_s_at COLJA1 collagen; type II; alpha 1 215076_s_at COLJA1 collagen; type IV; alpha 5 202002_s_at CX3CL chemokine (C-X3-C motif) ligand 1 213110_s_at COL4A5 collagen; type IV; alpha 5 202902_s_at EFEMP1 EGF-containing fibulin-like extracellular matrix protein 1 201842_s_at EFEMP1 EGF-containing fibulin-like extracellular matrix protein 1 207538_s_at FGGR2B Fe fragment of IgG; low affinity IIIs; receptor (CD32) 202748_at GBP2 guanylate binding protein 2; interferon-inducible 202949_s_at GHITM growth hormone inducible transmembrane protein 212335_at GNS glucosamine (N-acetyl)-6-sulfatase 201141_at GPNMB glycoprotein (Transmembrane) nmb 212350_s_at LAPT6 20388_s_at HBST1HIC histone-cluster I; HIC 211530_x_at LAPT6 212592_at IL33 interleukin 3 210776_s_at LAPTM5 lyosomal protein transmembrane 5 208949_s_at LGALS3 lectin; galactoside-binding; soluble; 3 210770_s_at LAPTM5 lyosomal protein transmembrane 5 208949_s_at LGALS3 lectin; galactoside-binding; soluble; 3 210760_at MSN meesin	201346_at	ADIPOR2	adiponectin receptor 2
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211530_x_at HLA-G major histocompatibility complex; class I; G 212592_at IGJ immunoglobulin J polypeptide 209821_at IL33 interleukin 33 201776_s_at KIAA0494 KIAA0494 201720_s_at LAPTM5 lysosomal protein transmembrane 5 208949_s_at LGALS3 lectin; galactoside-binding; soluble; 3 200704_at LITAF lipopolysaccharide-induced TNF factor 1555745_a_at LYZ lysozyme 224918_x_at MGST1 microsomal glutathione S-transferase 1 226818_at MPEG1 macrophage expressed 1 200600_at MSN moesin	223640_at	HCST	hematopoietic cell signal transducer
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201776_s_at KIAA0494 KIAA0494 201720_s_at LAPTM5 lysosomal protein transmembrane 5 208949_s_at LGALS3 lectin; galactoside-binding; soluble; 3 200704_at LITAF lipopolysaccharide-induced TNF factor 1555745_a_at LYZ lysozyme 224918_x_at MGST1 microsomal glutathione S-transferase 1 226818_at MPEG1 macrophage expressed 1 200600_at MSN moesin	212592_at	IGJ	immunoglobulin J polypeptide
201720_s_at LAPTM5 lysosomal protein transmembrane 5 208949_s_at LGALS3 lectin; galactoside-binding; soluble; 3 200704_at LITAF lipopolysaccharide-induced TNF factor 1555745_a_at LYZ lysozyme 224918_x_at MGST1 microsomal glutathione S-transferase 1 226818_at MPEG1 macrophage expressed 1 200600_at MSN moesin	209821_at	IL33	interleukin 33
208949_s_at LGALS3 lectin; galactoside-binding; soluble; 3 200704_at LITAF lipopolysaccharide-induced TNF factor 1555745_a_at LYZ lysozyme 224918_x_at MGST1 microsomal glutathione S-transferase 1 226818_at MPEG1 macrophage expressed 1 200600_at MSN moesin	201776_s_at	KIAA0494	KIAA0494
208949_s_at LGALS3 lectin; galactoside-binding; soluble; 3 200704_at LITAF lipopolysaccharide-induced TNF factor 1555745_a_at LYZ lysozyme 224918_x_at MGST1 microsomal glutathione S-transferase 1 226818_at MPEG1 macrophage expressed 1 200600_at MSN moesin	201720_s_at	LAPTM5	lysosomal protein transmembrane 5
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226818_at MPEG1 macrophage expressed 1 200600_at MSN moesin			
200600_at MSN moesin			
	<del>-</del>		
		MT1F	metallothionein 1F

# Supplemental Table I. List of Aging Signature Genes Expressed in Small Airway Epithelium<sup>1,2</sup> (cont., page 2)

Probeset ID <sup>3</sup>	Gene symbol	Gene title
200632_s_at	NDRG1	N-myc downstream regulated 1
218320_s_at	NDUFB11	NADH dehydrogenase (ubiquinone) 1 beta subcomplex; 11
200701_at	NPC2	Niemann-Pick disease; type C2
207414_s_at	PCSK6	proprotein convertase subtilisin/kexin type 6
208777_s_at	PSMD11	proteasome (prosome; macropain) 26S subunit; non-ATPase; 11
200627_at	PTGES3	prostaglandin E synthase 3 (cytosolic)
225562_at	RASA3	RAS p21 protein activator 3
225931_s_at	RNF213	ring finger protein 213
203186_s_at	S100A4	S100 calcium binding protein A4
217728_at	S100A6	S100 calcium binding protein A6
200986_at	SERPING1	serpin peptidase inhibitor; clade G (C1 inhibitor); member 1
201739_at	SGK1	serum/glucocorticoid regulated kinase 1
209875_s_at	SPP1	secreted phosphoprotein 1
207332_s_at	TFRC	transferrin receptor (p90; CD71)
212352_s_at	TMED10	transmembrane emp24-like trafficking protein 10 (yeast)
201008_s_at	TXNIP	thioredoxin interacting protein
208909_at	UQCRFS1	ubiquinol-cytochrome c reductase; Rieske iron-sulfur polypeptide 1
201568_at	UQCRQ	ubiquinol-cytochrome c reductase; complex III subunit VII; 9.5kDa
208626_s_at	VAT1	vesicle amine transport protein 1 homolog (T. californica)

<sup>&</sup>lt;sup>1</sup> 73 gene aging signature was taken from de Magalhaes et al 2009.

<sup>2</sup> 67 genes of the published 73 gene aging signature contained U133 2.0 probesets and were expressed in the small airway epithelium of healthy nonsmokers (P call ≥10).

<sup>3</sup> Affymatrix U133 2.0 microarray.

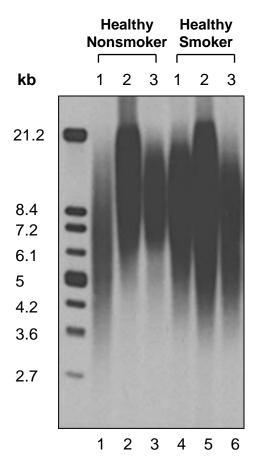
# **Supplemental Figure Legends**

Supplementary Figure 1. Southern analysis of telomere length in the small airway epithelium and peripheral blood leukocytes of nonsmokers and smokers. Telomere length [terminal restriction fragment (TRF)] of DNA isolated from the small airway epithelium and blood of male healthy nonsmokers and male healthy smokers was quantified by Southern blot analysis. A. Representative blot from small airway epithelium (SAE) samples. B. Representative blot from blood leukocyte samples. For both A and B lanes 1-3 represent 3 independent nonsmoker samples and lanes 4-6 represent 3 independent smoker samples.

Supplementary Figure 2. Telomere length in the small airway epithelium of nonsmokers and smokers from different racial categories. DNA was isolated from the small airway epithelium of male healthy nonsmokers (n=8 Black, n=6 White, and n=7 Other) and male healthy smokers (n=14 Black, n=3 White and n=5 Other) and telomere length [terminal restriction fragment (TRF)] quantified by Southern analysis. Within each phenotype the telomere length of subjects was separated based on racial group. Data shown is represented as the average ± standard deviation of the TRF length (kb). The difference in mean TRF length between phenotypes was calculated by 2-tailed Students t test.

**Supplementary Figure 3**. Telomere length in the small airway epithelium and peripheral blood leukocytes of nonsmokers and smokers. DNA was isolated from the small airway epithelium and peripheral blood leukocytes of male healthy nonsmokers (n=12) and male healthy smokers (n=16) and telomere length [terminal restriction fragment (TRF)] quantified by Southern analysis. Data shown is represented as the average  $\pm$  standard deviation of the TRF length (kb). The difference in mean TRF length between phenotypes was calculated by 2-tailed Students t test.

# A. Southern (SAE)



# B. Southern (blood leukocytes)

