

Supplementary information

***AHRR* hypomethylation, lung function, lung function decline, and respiratory symptoms**

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Methods

Spirometry

Forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) were determined using a dry wedge spirometer (Vitalograph; Maids Moreton, Buckinghamshire, UK) at the 1991-1994 and 2001-2003 examinations. Instruments were calibrated daily against a 1 L syringe. For each individual, spirometry was performed in triplicate, and results were only accepted if variation between the two best-performing of these was less than 5%. Spirometry measurements were available for 98.7% of individuals with available methylation measurements. FEV₁, FVC, and FEV₁/FVC z-scores were calculated according to the global lung function initiative 2012 equations (GLI-2012) [20]. Cross-sectional analyses were based on spirometry measurements from the 1991-1994 examination, whereas prospective analyses also included spirometry measurements from the 2001-2003 examination.

Covariates

Information on exposure to passive smoking, exposure to occupational dust and fumes, and highest completed education level were self-reported. Body mass index (BMI) was calculated as measured weight in kilograms divided by measured height in meters squared.

Information on smoking status was self-reported at the 1991-1994 and 2001-2003 examinations. Self-reported smoking of cigarettes, cheroots, cigars, and pipe tobacco were recalculated into daily grams of tobacco consumption. Cumulative smoking was calculated for former and current smokers in pack-years; a pack-year was defined as 20 cigarettes or equivalent per day smoked for one year. We did not have information on traffic air pollution exposure.

Information on COPD hospitalizations (ICD8: 491-492, ICD10:J41-J44) and death was obtained from the national Danish Patient Registry and the national Danish Civil Registration System.

Respiratory symptoms

All included respiratory symptoms were self-reported from the 1991-1994 examination. Chronic bronchitis was defined as coughing up sputum (in the morning or during the day) for three consecutive months every year. Exercise induced cough was defined as occasional cough during exercise. Sputum was defined as coughing up sputum in the morning and/or during the day. Dyspnea was defined according to the modified Medical Research Council dyspnea scale (mMRC) ≥ 2 , that is, experiencing more shortness of breath than peers, the need to stop to recover breath when walking in one's own pace, and/or experiencing shortness of breath while bathing or getting dressed. Wheezing was defined as whistling or wheezing while breathing, and any respiratory symptom was defined as answering affirmative to any of the above questions.

Supplementary Table S1: Association between *AHRR* methylation extent and age, sex and height according to smoking status.

	<i>AHRR</i> methylation extent			
	N	β	95%CI	R ²
Never smokers				
Age, per 10 year	2331	-0.01	-0.03; 0.002	0.001
Sex, men versus women	2331	-0.96	-1.54; -0.38	0.005
Height, cm	2331	-0.019	-0.47; 0.01	0.001
Age+ sex+ height multivariate model				0.08
Age, per 10 year	2331	-0.02	-0.04; -0.004	
Sex, men versus women	2331	-1.21	-2.01; -0.41	
Height, cm	2331	0.002	-0.04; 0.04	
Current smokers				
Age, per 10 year	4426	0.02	0.01; 0.04	0.003
Sex, men versus women	4426	-0.62	-1.0; -0.24	0.002
Height, cm	4426	-0.002	-0.02; 0.02	0.000
Age+ sex+ height multivariate model				0.009
Age, per 10 year		0.04	0.02; 0.05	
Sex, men versus women		-1.4	-1.9; -0.9	
Height, cm		0.07	0.04; 0.10	
Former smokers				
Age, per 10 year	2356	-0.03	-0.05; -0.008	0.003
Sex, men versus women	2356	-3.1	-3.7; -2.5	0.04
Height, cm	2356	-0.09	-0.1; -0.06	0.01
Age+ sex+ height multivariate model				0.05
Age, per 10 year		-0.02	-0.04; 0.0003	
Sex, men versus women		-3.3	-4.1; -2.5	
Height, cm		0.02	-0.03; 0.07	

Associations are all based on linear models.

CI: confidence interval; N: number of individuals; R²: coefficient of determination; β : Regression coefficient.

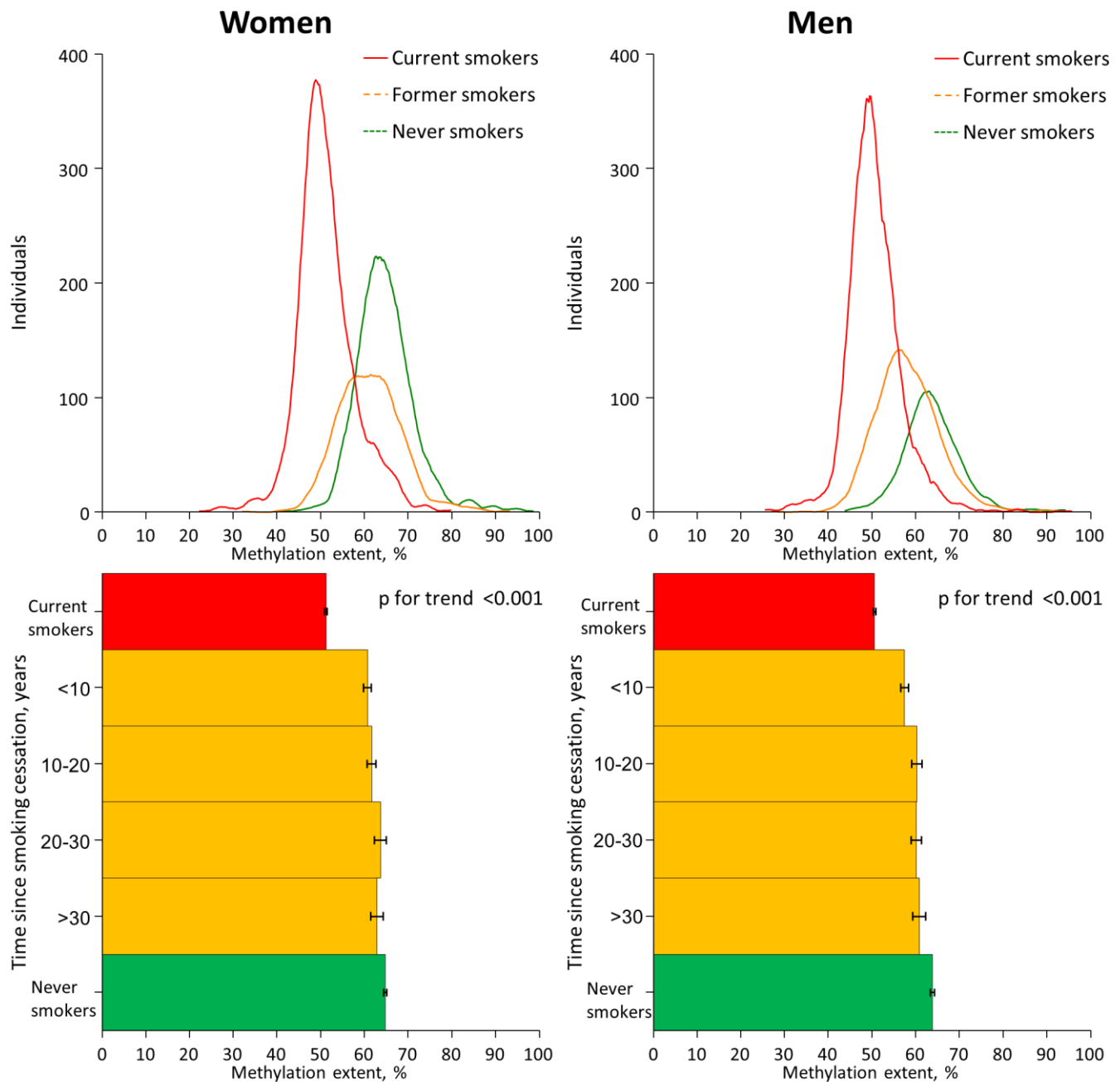
Supplementary Table S2. Characteristics of individuals according to examination attendance.

Characteristic	All	Individuals attending both examinations	Individuals only attending the 1991-1994 examination	p-value
Individuals	9113	4532	4581	<0.001
AHRR Methylation extent, %	56 (50 to 63)	57 (50 to 64)	54 (49 to 61)	<0.001
Men	4065 (45)	1924 (42)	2141 (47)	<0.001
Age	60 (47 to 70)	54 (43 to 64)	66 (54 to 73)	<0.001
Sputum	1231 (14)	429 (9.5)	802 (18)	<0.001
Any respiratory symptoms	4032 (44)	1618 (36)	2350 (51)	<0.001
COPD related hospital contact ^a	678 (8.0)	235 (5.2)	443 (9.7)	<0.001
Dead within 15 years ^b	3143 (35)	462 (10)	2681 (59)	<0.001
Never smokers	2331 (26)	1359 (30)	972 (21)	<0.001
Current smokers	4426 (49)	1961 (43)	2465 (54)	<0.001
Former smokers	2356 (26)	1212 (27)	1144 (25)	0.05
Exposed to passive smoking	3283 (36)	1661 (37)	1622 (35)	0.22
Dust and fumes exposure	1689 (19)	712 (16)	977 (21)	<0.001
Pack years ^c	18 (0 to 35)	25 (22 to 27)	25 (23 to 28)	<0.001
FEV ₁ z-score	-0.55 (-1.4 to 0.26)	-0.27 (-1.0 to 0.46)	-0.85 (-1.8 to -0.035)	<0.001
FEV ₁ /FVC	0.79 (0.73 to 0.83)	0.80 (0.75 to 0.84)	0.77 (0.70 to 0.82)	<0.001

Data are expressed as number and percentage for categorical values and median and interquartile range for continuous values. P-values were calculated using Cuzick's test for trend for continuous values and Pearson's chi-squared test for dichotomous values.

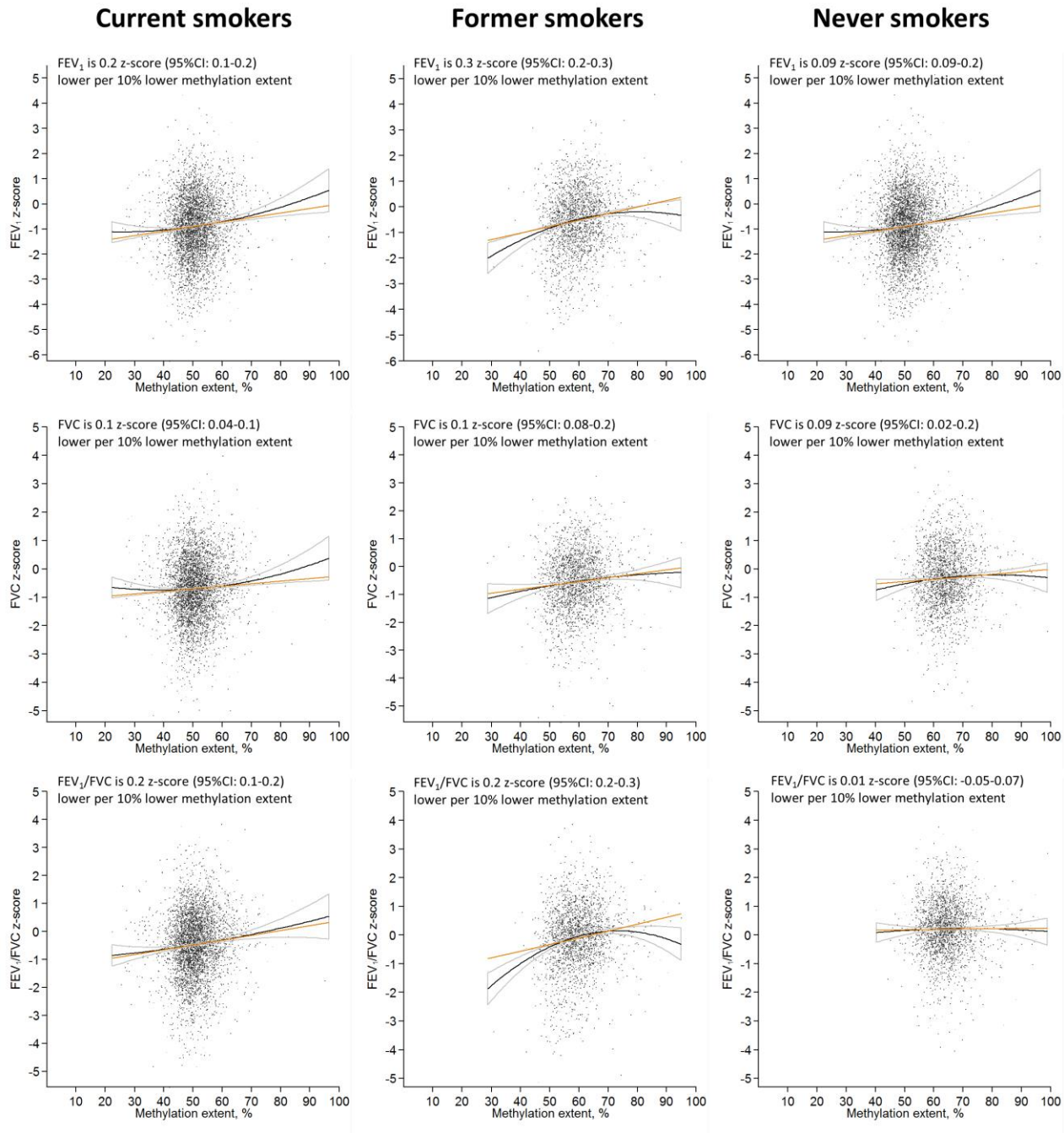
^a individuals receiving a COPD diagnosis at a hospital before November 6 2014. ^b individuals who died within 15 years of first examination attendance. ^a and ^b are based on data from national registers with complete follow-up. ^c Calculated for current and former smokers only.

FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity.



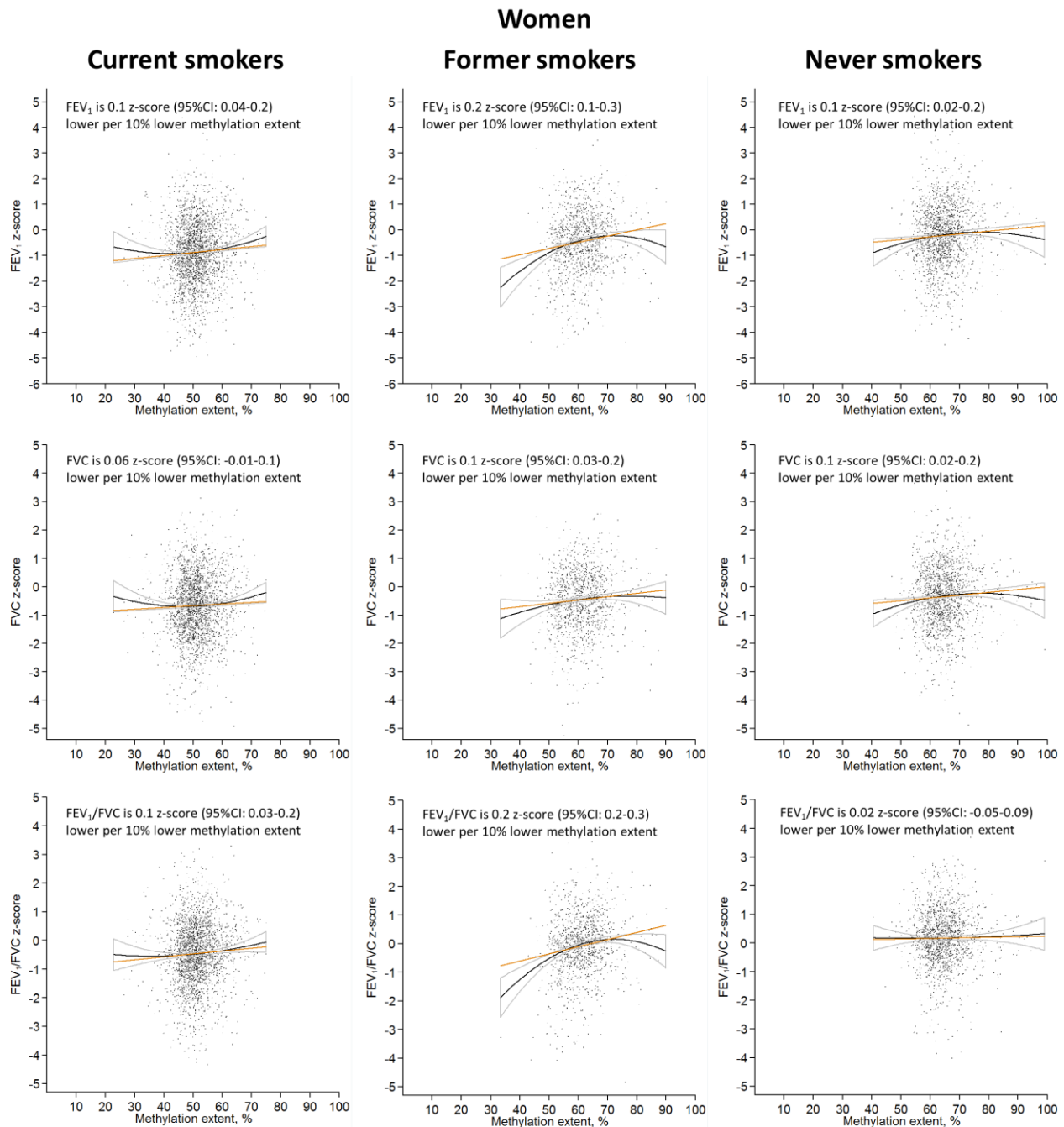
Supplementary Figure S1. Distribution of *AHR* methylation extent in women and men.

The upper panels show the distribution of methylation extent among current smokers, former smokers, and never smokers for each sex separately. The lower panels show the mean values of methylation extent with 95% confidence intervals for subgroups of former smokers according to years since smoking cessation. P-values for linear trends are reported. Current and never smokers are shown for reference. The black bars represent the 95% confidence interval.



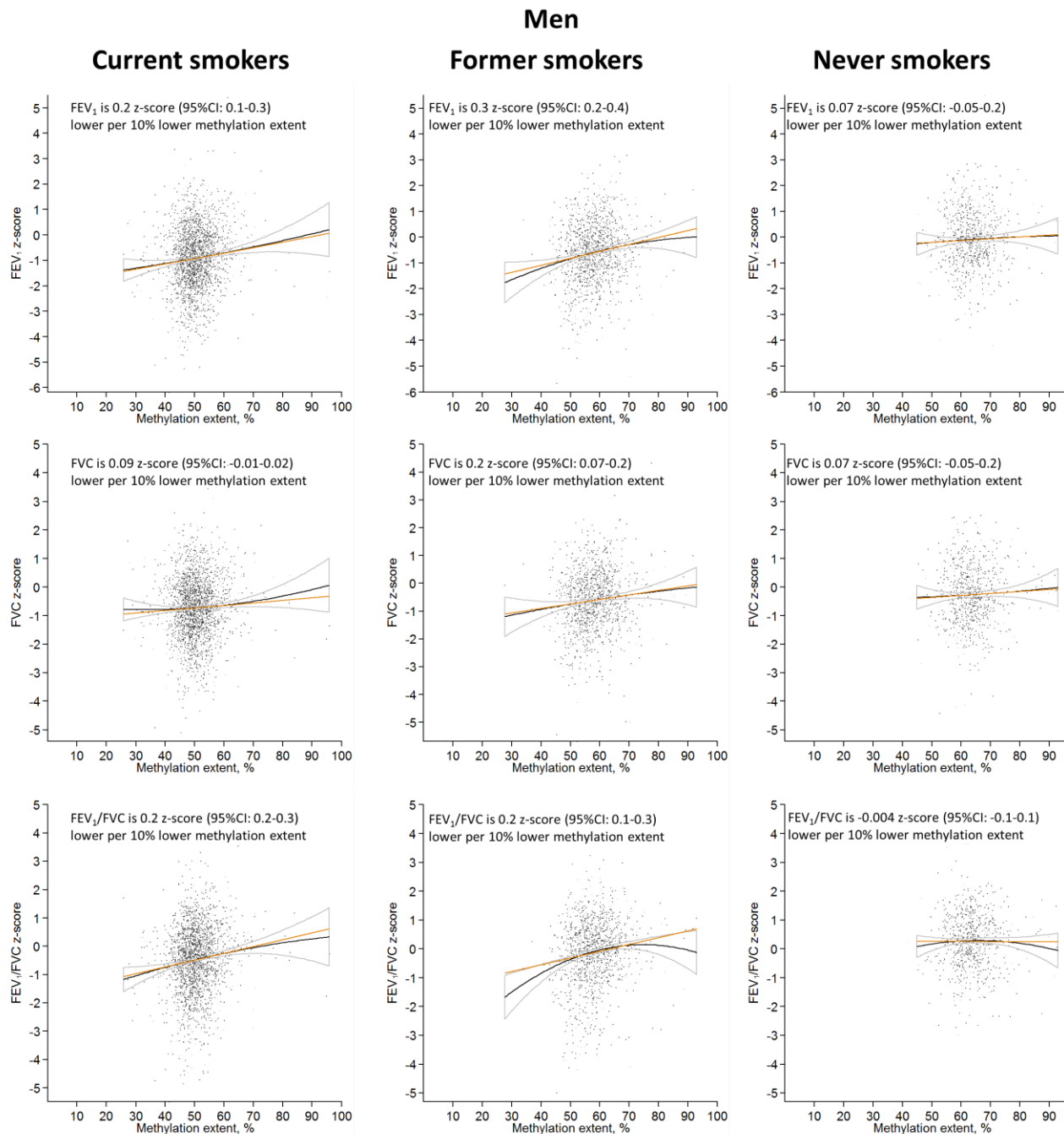
Supplementary Figure S2. Associations of AHRR methylation extent with FEV₁ z-score, FVC z-score, and FEV₁/FVC z-score stratified according to smoking status.

All analyses were adjusted for age, sex, body mass index, dust and fume exposure, passive smoking, educational level, and cumulative smoking. The orange line represents a linear regression line. The black line represents a quadratic regression line. The area surrounding the quadratic line represents the 95% confidence interval of the regression line. The black dots represent individual measurements. The individual measurements are adjusted for covariates. CI: confidence interval; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity.



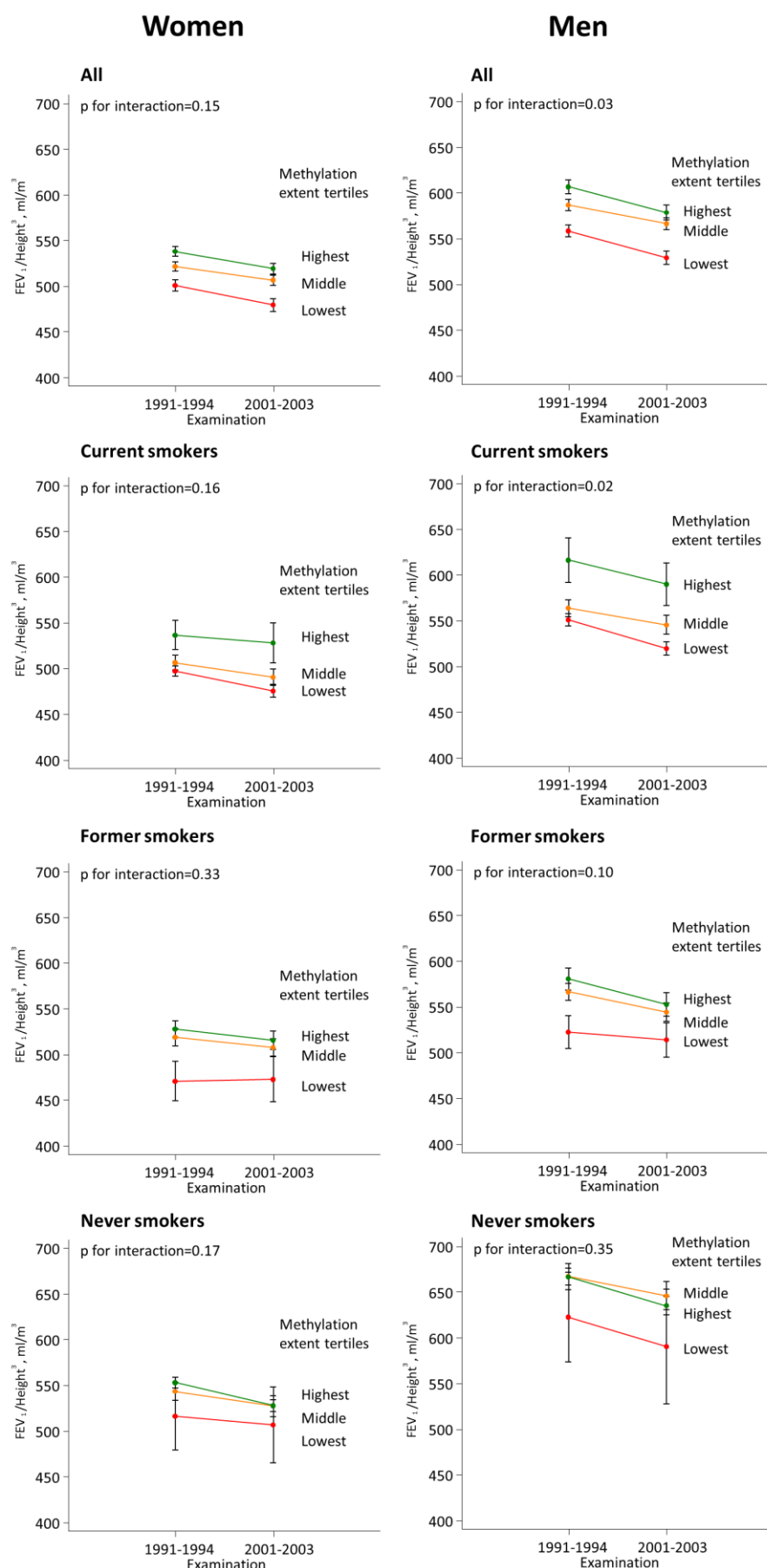
Supplementary Figure S3. Associations of *AHRR* methylation extent with FEV₁ z-score, FVC z-score, and FEV₁/FVC z-score stratified according to smoking status in women.

All analyses were adjusted for age, body mass index, dust and fume exposure, passive smoking, educational level, and cumulative smoking. The orange line represents a linear regression line. The black line represents a quadratic regression line. The area surrounding the quadratic line represents the 95% confidence interval of the regression line. The black dots represent individual measurements. The individual measurements are adjusted for covariates. CI: confidence interval; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity.



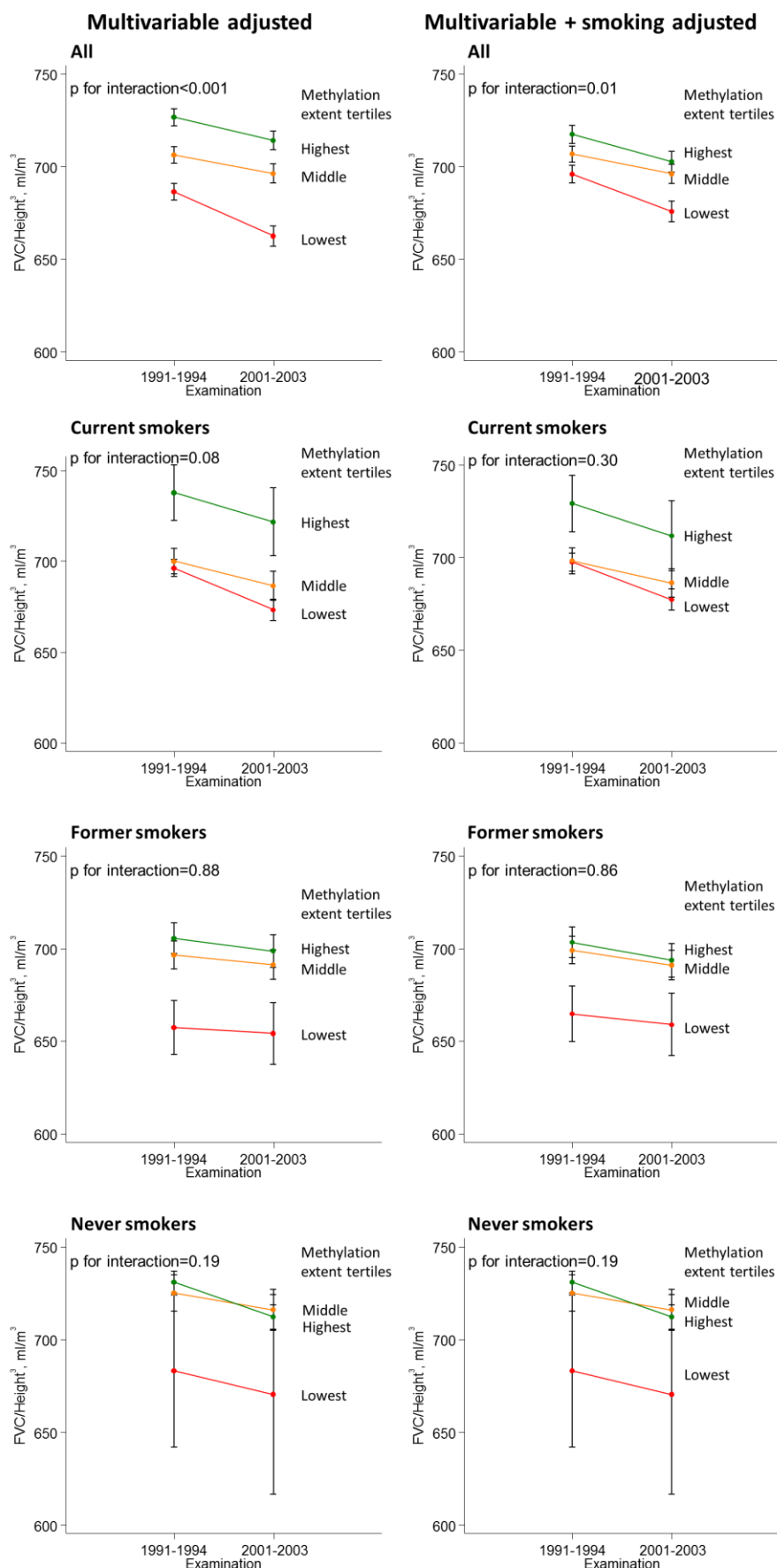
Supplementary Figure S4. Associations of *AHRR* methylation extent with FEV₁ z-score, FVC z-score, and FEV₁/FVC z-score stratified according to smoking status in men.

All analyses were adjusted for age, body mass index, dust and fume exposure, passive smoking, educational level, and cumulative smoking. The orange line represents a linear regression line. The black line represents a quadratic regression line. The area surrounding the quadratic line represents the 95% confidence interval of the regression line. The black dots represent individual measurements. The individual measurements are adjusted for covariates. CI: confidence interval; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity.



Supplementary Figure S5.
Relationship between changes in $FEV_1/height^3$ according to tertiles of *AHRM* methylation for each sex, overall and stratified according to smoking status.

Analyses were adjusted for cumulative smoking updated at each examination, age, and examination. Based on one to two spirometries for each of 9113 individuals, spanning up to 11.5 years; those individuals with only one measurement were included to increase precision of the baseline estimate. Identity of each individual was introduced as a random effect to specify the grouping structure, hereby accounting for within-subject correlation. Only baseline height was used. The black bars represent the 95% confidence interval. P-values for interaction of examination (1991-1994 versus 2001-2003) with tertiles of methylation extent are reported. FEV_1 : forced expiratory volume in 1 s.

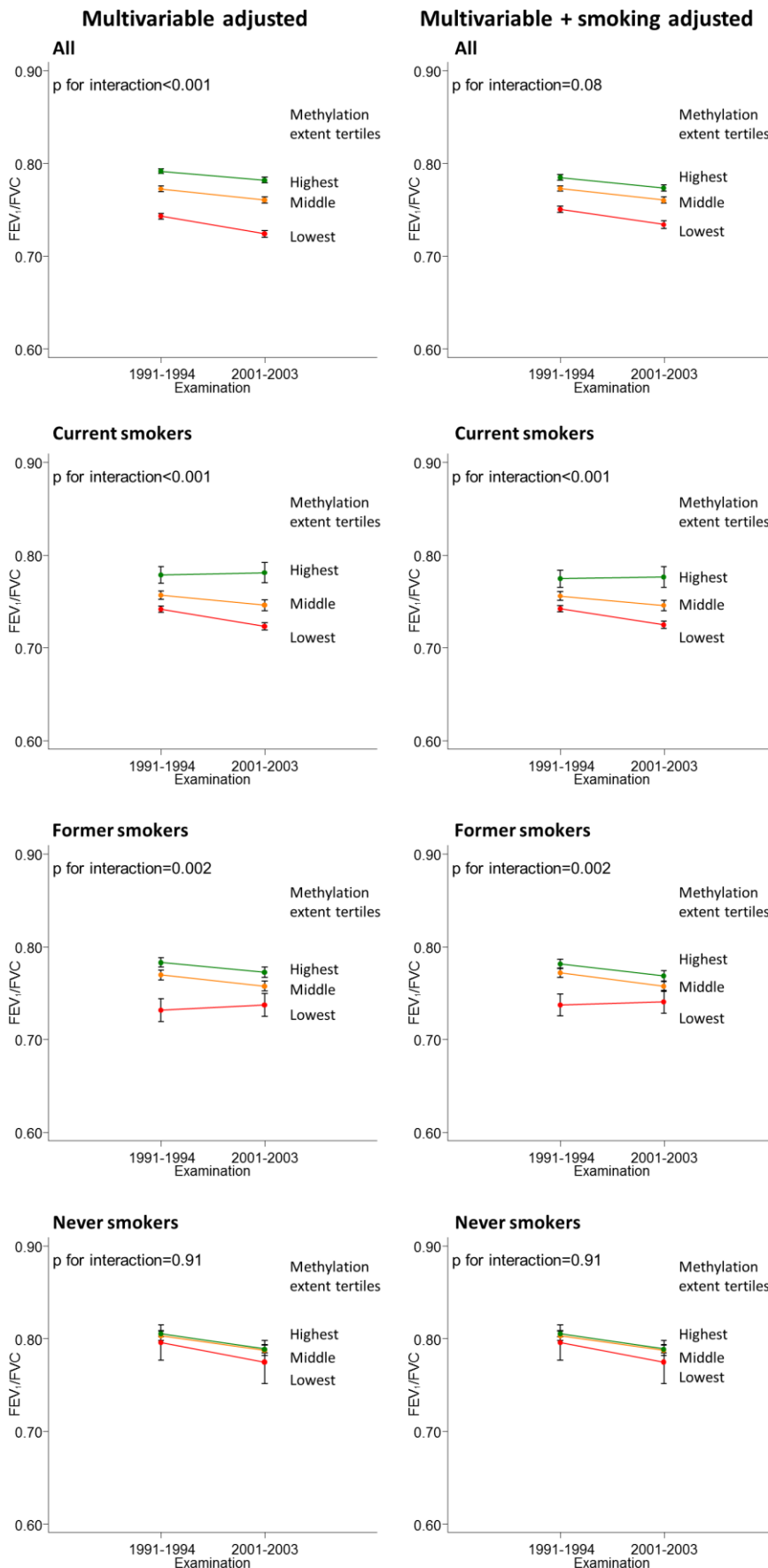


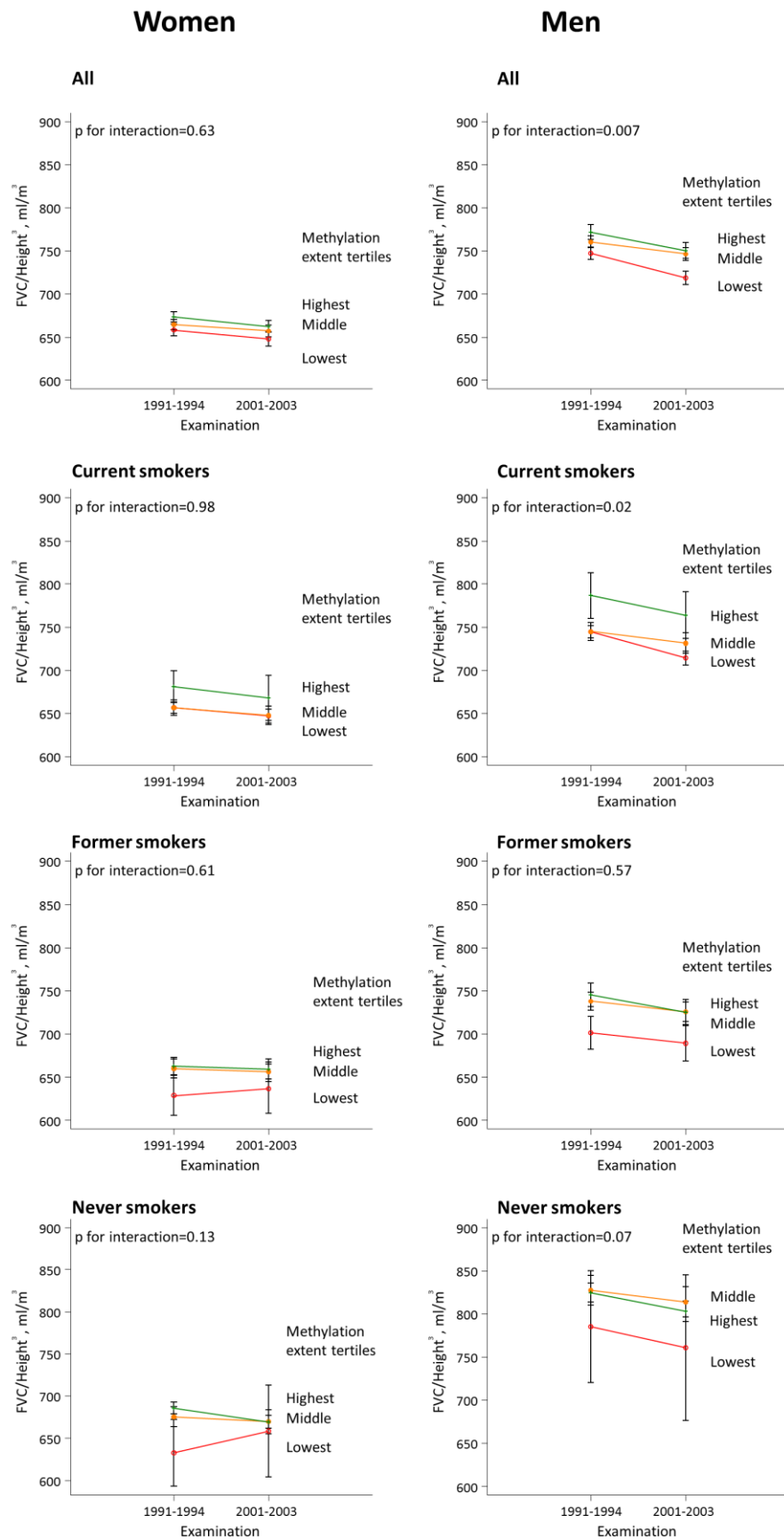
Supplementary Figure S6.
Relationship between changes in FVC/height³ according to tertiles of AHR methylation extent and stratified according to smoking status.

Analyses were adjusted for age, sex, and examination. Multivariable + smoking were further adjusted for cumulative smoking updated at each examination. Based on one to two spirometries for each of 9113 individuals, spanning up to 11.5 years; those individuals with only one measurement were included to increase precision of the baseline estimate. Identity of each individual was introduced as a random effect to specify the grouping structure, hereby accounting for within-subject correlation. The black bars represent the 95% confidence interval. P-values for interaction of examination (1991-1994 versus 2001-2003) with tertiles of methylation extent are reported. FVC: forced vital capacity.

**Supplementary Figure S7.
Relationship between changes
in FEV₁/FVC according to
tertiles of *AHRR* methylation
extent and stratified according
to smoking status.**

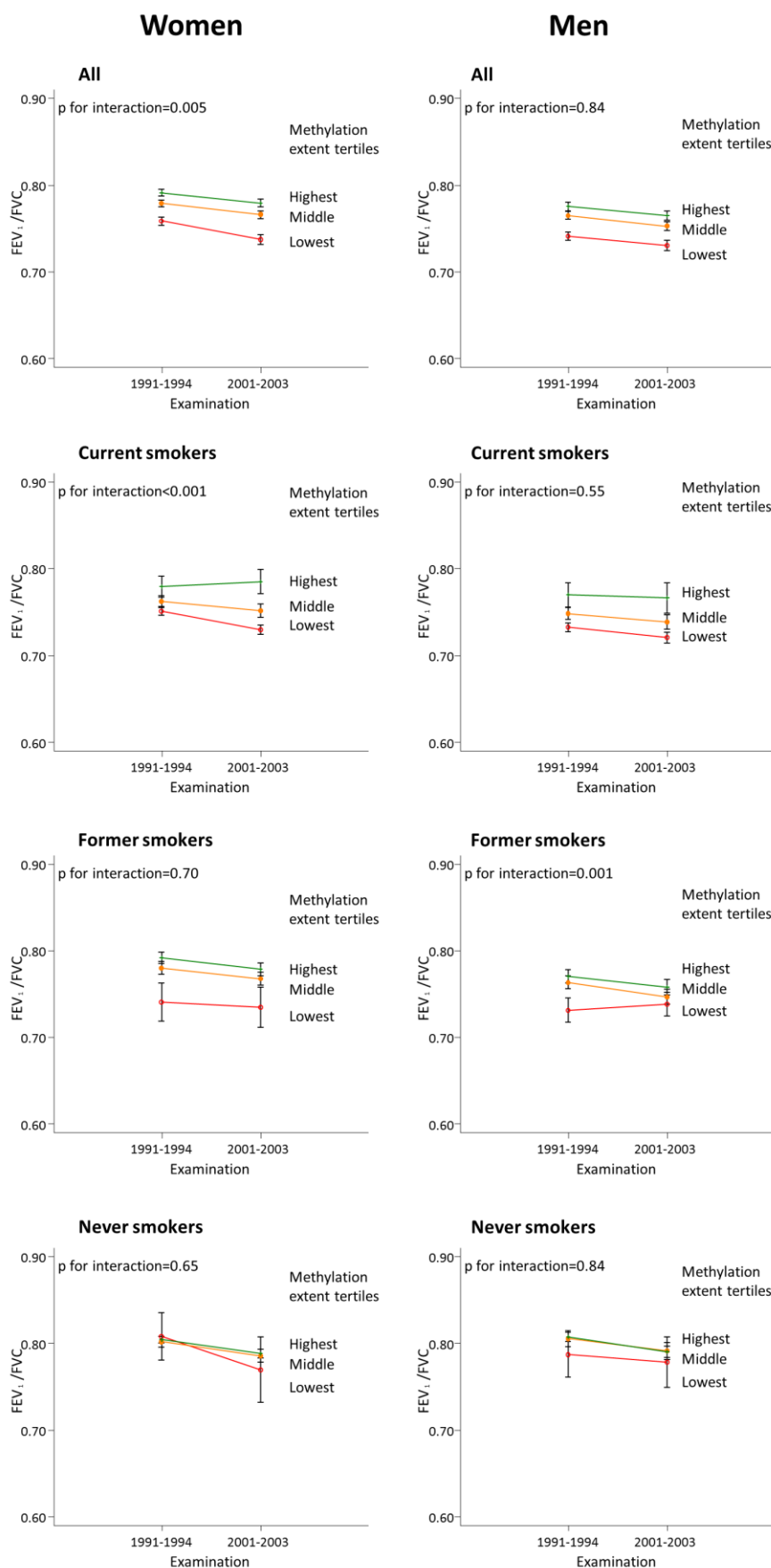
Analyses were adjusted for age, sex, and examination. Multivariable + smoking were further adjusted for cumulative smoking updated at each examination. Based on one to two spirometries for each of 9113 individuals, spanning up to 11.5 years; those individuals with only one measurement were included to increase precision of the baseline estimate. Identity of each individual was introduced as a random effect to specify the grouping structure, hereby accounting for within-subject correlation. The black bars represent the 95% confidence interval. P-values for interaction of examination (1991-1994 versus 2001-2003) with tertiles of methylation extent are reported. FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity.





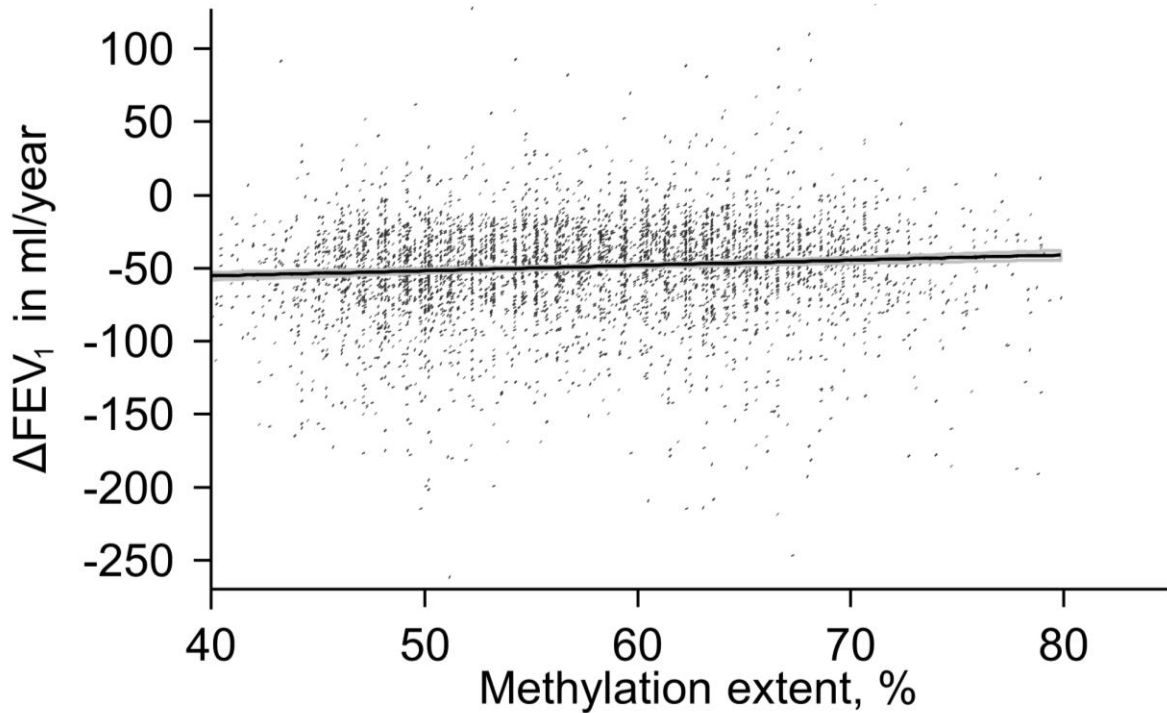
Supplementary Figure S8.
Relationship between changes in FVC/height³ according to tertiles of AHRH methylation for each sex, overall and stratified according to smoking status.

Analyses were adjusted for cumulative smoking updated at each examination, age, and examination. Based on one to two spirometries for each of 9113 individuals, spanning up to 11.5 years; those individuals with only one measurement were included to increase precision of the baseline estimate. Identity of each individual was introduced as a random effect to specify the grouping structure, hereby accounting for within-subject correlation. Only baseline height was used. The black bars represent the 95% confidence interval. P-values for interaction of examination (1991-1994 versus 2001-2003) with tertiles of methylation extent are reported. FVC: forced vital capacity.



Supplementary Figure S9.
Relationship between changes in FEV₁/FVC according to tertiles of *AHR* methylation for each sex, overall and stratified according to smoking status.
 Analyses were adjusted for cumulative smoking updated at each examination, age, and examination. Based on one to two spirometries for each of 9113 individuals, spanning up to 11.5 years; those individuals with only one measurement were included to increase precision of the baseline estimate. Identity of each individual was introduced as a random effect to specify the grouping structure, hereby accounting for within-subject correlation. Only baseline height was used. The black bars represent the 95% confidence interval. P-values for interaction of examination (1991-1994 versus 2001-2003) with tertiles of methylation extent are reported. FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity.

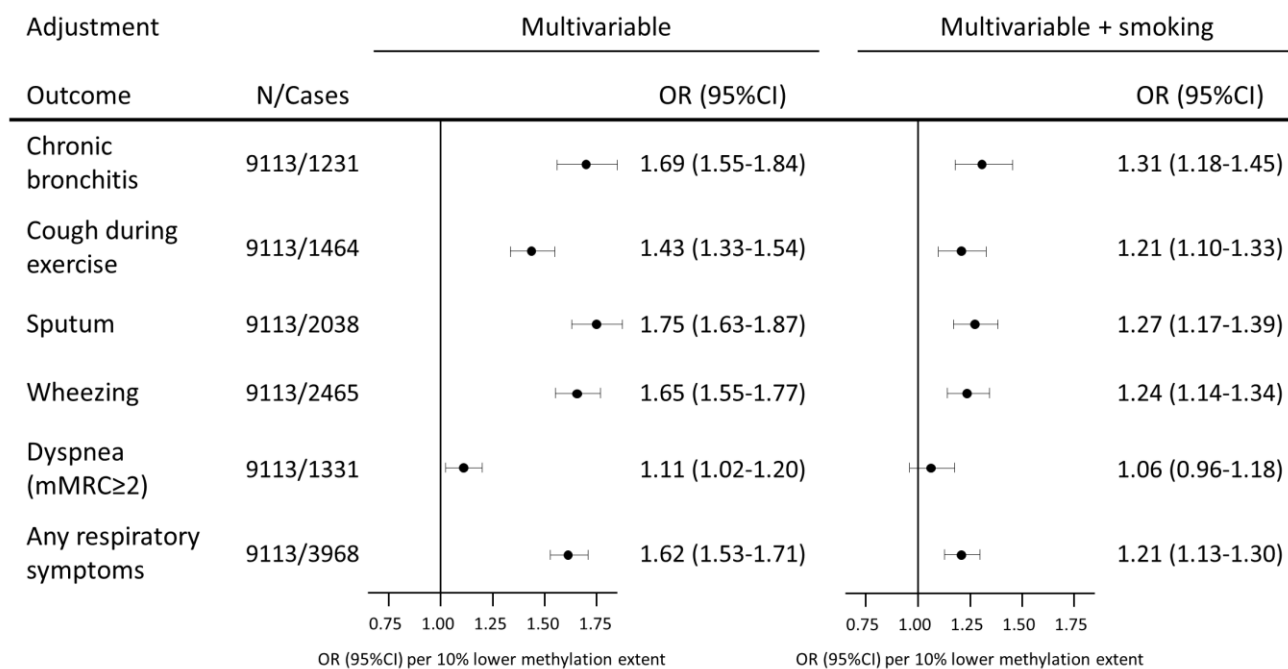
ΔFEV_1 is 3.6 ml/year (95%CI: 2.2-5.0) lower per 10% lower methylation extent



Supplementary Figure S10. FEV_1 change for individuals attending both examinations according to *AHRR* methylation extent.

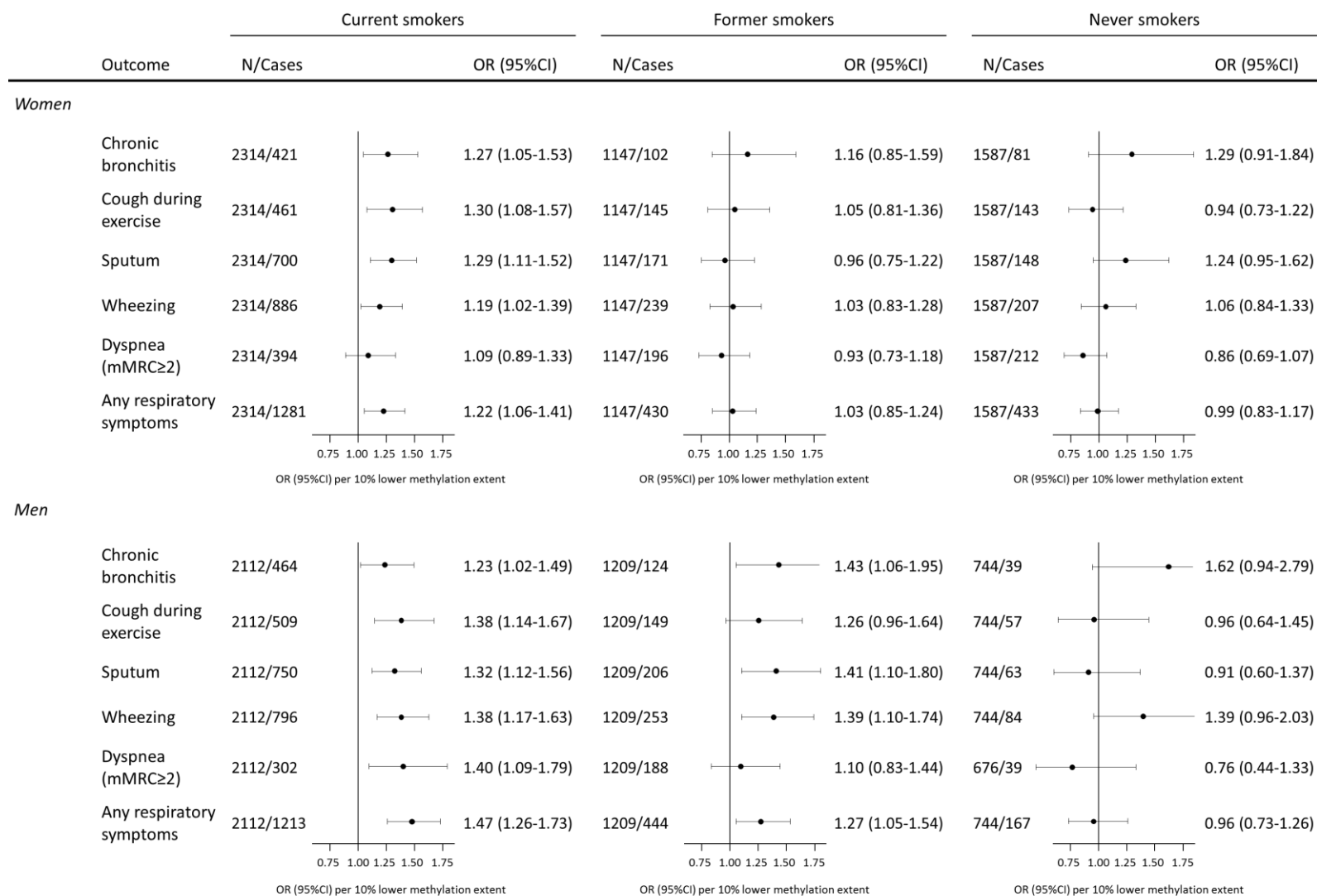
Only individuals participating in both the 1991-1994 and 2001-2003 examinations of the Copenhagen City Heart Study are included. The difference in FEV_1 was then divided by the time in years between each individual's examination dates.

The black line represents an unadjusted linear regression line. The grey area surrounding the regression line represents the 95% confidence interval of the regression line. The black dots represent individual values. CI: confidence interval; FEV_1 : forced expiratory volume in 1 s.



Supplementary Figure S11. Odds ratio for respiratory symptoms per 10 % lower *AHR* methylation extent at the 1991-1994 examination.

Analyses were multivariable adjusted for age, sex, body mass index, dust and fume exposure, passive smoking, educational level, and forced expiratory volume in 1 s z-score. Multivariable + smoking analyses were further adjusted for smoking status and cumulative smoking. CI: confidence interval; mMRC: modified Medical Research Council dyspnea scale; N: number of individuals; OR: odds ratio.



Supplementary Figure S12. Odds ratio for respiratory symptoms per 10 % lower *AHRR* methylation extent stratified by smoking status for each sex separately.

All analyses are adjusted for age, sex, body mass index, dust and fume exposure, passive smoking, educational level, forced expiratory volume in 1 s z-score, and cumulative smoking (former and current smokers only). CI: confidence interval; mMRC: modified Medical Research Council dyspnea scale; N: number of individuals. OR: odds ratio.