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Supplemental Material

Association of Glyphosate Exposure with Blood DNA Methylation in a Cross-Sectional Study of Postmenopausal Women

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Figure S4. Correlation of epigenetic age from the Hannum (A), Horvath (B), and Levine (C) epigenetic clocks, epigenetic age acceleration from each clock (E-G), and DunedinPoAm (H) compared to chronologic age in 392 postmenopausal California women. Also shown is a histogram of standardized (z-score) DunedinPoAm (D). Epigenetic age acceleration was calculated by forming residuals from a model regressing chronologic age on epigenetic age from each clock. Dotted lines show where chronologic age equals epigenetic age (panels A-C) or where epigenetic age acceleration or epigenetic pace of aging (PoA) equals zero (panels D-H).

Figure S5. Performance of methylation index using 14 CpG sites on the 450K methylation array to predict urinary glyphosate concentration in the training set (A) and the validation set (B, C). Panel D shows the classification performance of the methylation index in the validation set for classifying the highest vs. the lowest tertile of urinary glyphosate. The index was developed using elastic net regression on methylation β values of differentially methylated probes associated with glyphosate and found on the Illumina 450K methylation array in the training set, a population of 332 postmenopausal California women.

Figure S6. Performance of methylation index using 2 CpG sites to predict urinary AMPA concentration in the training set (A) and the validation set (B, C). Panel D shows the classification performance of the methylation index in the validation set for classifying the highest vs. the lowest tertile of urinary AMPA. The index was developed using elastic net regression on methylation β values of differentially methylated probes associated with AMPA in the training set, a population of 332 postmenopausal California women. AMPA: aminomethylphosphonic acid.

Table S1. Linearity, limits, and precision of glyphosate and aminomethylphosphonic acid (AMPA) measurement by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in urine.

Analyte	Coefficient of determination (R^2)	LOD (ng/mL)	LOQ (ng/mL)	Precision (%)				
				At LOD	At LOQ	Quality control		
						Low (0.25 ng/mL)	Medium (0.75 ng/mL)	High (2.5 ng/mL)
Glyphosate	0.9921	0.014	0.041	13.7 (19.6)	5.3 (7.4)	12.2 (7.2)	6.9 (5.3)	2.9 (1.2)
AMPA	0.9951	0.013	0.040	6.3 (4.5)	6.9 (9.7)	9.4 (9.2)	6.8 (3.4)	4.4 (4.2)

Precision is shown as intraday (interday) precision.

Table S2. Distribution of estimated cell type proportions from the Houseman method, presented as mean (SD), by tertile of urinary glyphosate and AMPA in 392 postmenopausal California women.

	Proportions, Mean (SD)				Proportions, Mean (SD)			
	Glyphosate Tertile 1	Glyphosate Tertile 2	Glyphosate Tertile 3	p	AMPA Tertile 1	AMPA Tertile 2	AMPA Tertile 3	p
CD8T	10.7% (4.2%)	10.7% (4.0%)	10.3% (3.8%)	0.62	10.4% (4.3%)	11.0% (4.0%)	10.3% (3.7%)	0.27
CD4T	15.4% (6.0%)	14.7% (5.1%)	15.3% (5.6%)	0.65	15.3% (5.9%)	15.3% (5.7%)	14.7% (5.3%)	0.35
NK	5.8% (2.4%)	5.4% (1.9%)	5.6% (2.4%)	0.56	5.6% (2.5%)	5.7% (2.0%)	5.5% (2.3%)	0.47
B Cell	6.4% (2.5%)	6.4% (2.7%)	6.4% (2.2%)	0.73	6.5% (3.0%)	6.5% (2.1%)	6.2% (2.1%)	0.50
Monocyte	8.9% (1.8%)	8.9% (2.2%)	9.1% (2.1%)	0.95	9.0% (1.8%)	8.8% (1.9%)	9.1% (2.3%)	0.18
Neutrophil	57.8% (9.6%)	58.7% (8.8%)	58.4% (8.6%)	0.69	58.1% (9.6%)	57.6% (8.3%)	59.2% (9.0%)	0.28

P-values are for each cell type in a linear regression model including all cell types. Results were consistent when glyphosate and AMPA were analyzed as continuous variables. AMPA: aminomethylphosphonic acid

Table S3. Cohort characteristics stratified by randomized set assignment (training or validation).

	Training	Validation	
	N (%) or Mean (SD)		p
Race/Ethnicity			0.44
Asian	37 (11.3%)	6 (10.2%)	
Hispanic	54 (16.5%)	15 (25.4%)	
Other	18 (5.5%)	2 (3.4%)	
White	218 (66.7%)	36 (61.0%)	
Age (years)	56.9 (4.6)	56.8 (4.3)	0.81
BMI (kg/m^2)	26.7 (6.2)	27.4 (8.1)	0.39
Smoking Status			0.029
Never	250 (75.3%)	35 (59.3%)	
Former	68 (20.5%)	21 (35.6%)	
Current	14 (4.2%)	3 (5.1%)	
Alcohol (drinks/week)			0.99
None	92 (27.7%)	15 (25.4%)	
1 or fewer	136 (41.0%)	25 (42.4%)	
2-6	61 (18.4%)	11 (18.6%)	
7 or more	43 (13.0%)	8 (13.6%)	
Organic Eating			0.23
Seldom/Never	109 (32.8%)	15 (25.4%)	
Sometimes	99 (29.8%)	15 (25.4%)	
Often/Always	124 (37.3%)	29 (49.2%)	
HEI	63.7 (12.3)	62.5 (11.2)	0.47
Physical Activity			0.88
≥150 minutes/week	129 (40.4%)	24 (42.1%)	
<150 minutes/week	190 (59.6%)	33 (57.9%)	
Herbicide Use (past week)			0.22
Yes	20 (9.7%)	1 (2.4%)	
No	186 (90.3%)	41 (97.6%)	
Glyphosate Tertile			1.0
Tertile 1	111 (33.4%)	20 (33.3%)	
Tertile 2	110 (33.1%)	20 (33.3%)	
Tertile 3	111 (33.4%)	20 (33.3%)	
AMPA Tertile			0.79
Tertile 1	109 (32.8%)	22 (36.7%)	
Tertile 2	110 (33.1%)	20 (33.3%)	
Tertile 3	113 (34.0%)	18 (30.0%)	

Randomization was stratified by glyphosate tertile. P-values are from ANOVA (continuous variables) or Fisher's exact test (categorical variables). BMI: body mass index; HEI: Healthy Eating Index; AMPA: aminomethylphosphonic acid

Table S4. Final coefficients for the glyphosate methylation index.

Probe	Coefficient (Main Index)	Coefficient (Probes on 450k Array Only)
(Intercept)	37.592	20.864
cg26483235	-11.943	-8.784
cg04915788	-6.849	-8.541
cg01430385	-7.175	-6.456
cg26787244	-7.146	-5.493
cg00355690	0.806	-4.898
cg20531550	-2.470	-3.612
cg02519806	-6.608	-3.144
cg13170005	-2.549	-2.701
cg13310154	-1.069	-0.942
cg18722557	2.785	1.348
cg05730283	7.747	4.327
cg11062848	6.206	5.956
cg20540608	5.693	6.934
cg25597976	6.881	6.935
cg06993862	-9.407	N/A
cg07261978	-5.128	N/A
cg07318309	1.379	N/A
cg07509511	-0.236	N/A
cg13499896	-4.945	N/A
cg16601151	-0.409	N/A
cg19029576	-1.952	N/A
cg24576174	-4.708	N/A
cg25629796	2.915	N/A
cg26833395	-1.108	N/A

Probes exclusive to the EPIC array are marked N/A for the 450k index. To obtain the predicted glyphosate level (natural log) for a sample, multiply the β percentage values for each probe by the coefficient, add all values, and add the intercept. The index was developed using elastic net regression on methylation β values of differentially methylated probes associated with glyphosate in the training set, a population of 332 postmenopausal California women.

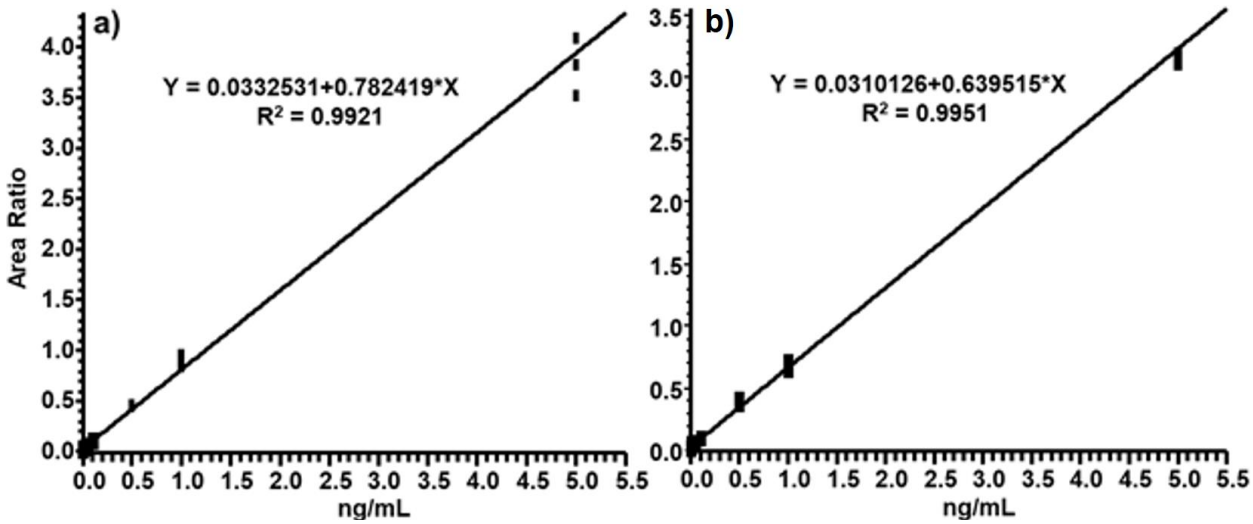


Figure S1. Calibration curve for a) glyphosate and b) AMPA ranging from 0 to 5 ng/mL in urine. The area ratio represents the ratio of variable concentration of glyphosate and AMPA (0 to 5ng/mL) to their respective internal standards ($^{13}C_2{}^{15}N$-Glyphosate and $D_2{}^{13}C^{15}N$-AMPA) with a constant concentration of 6.25 ng/mL spiked in urine. AMPA: aminomethylphosphonic acid

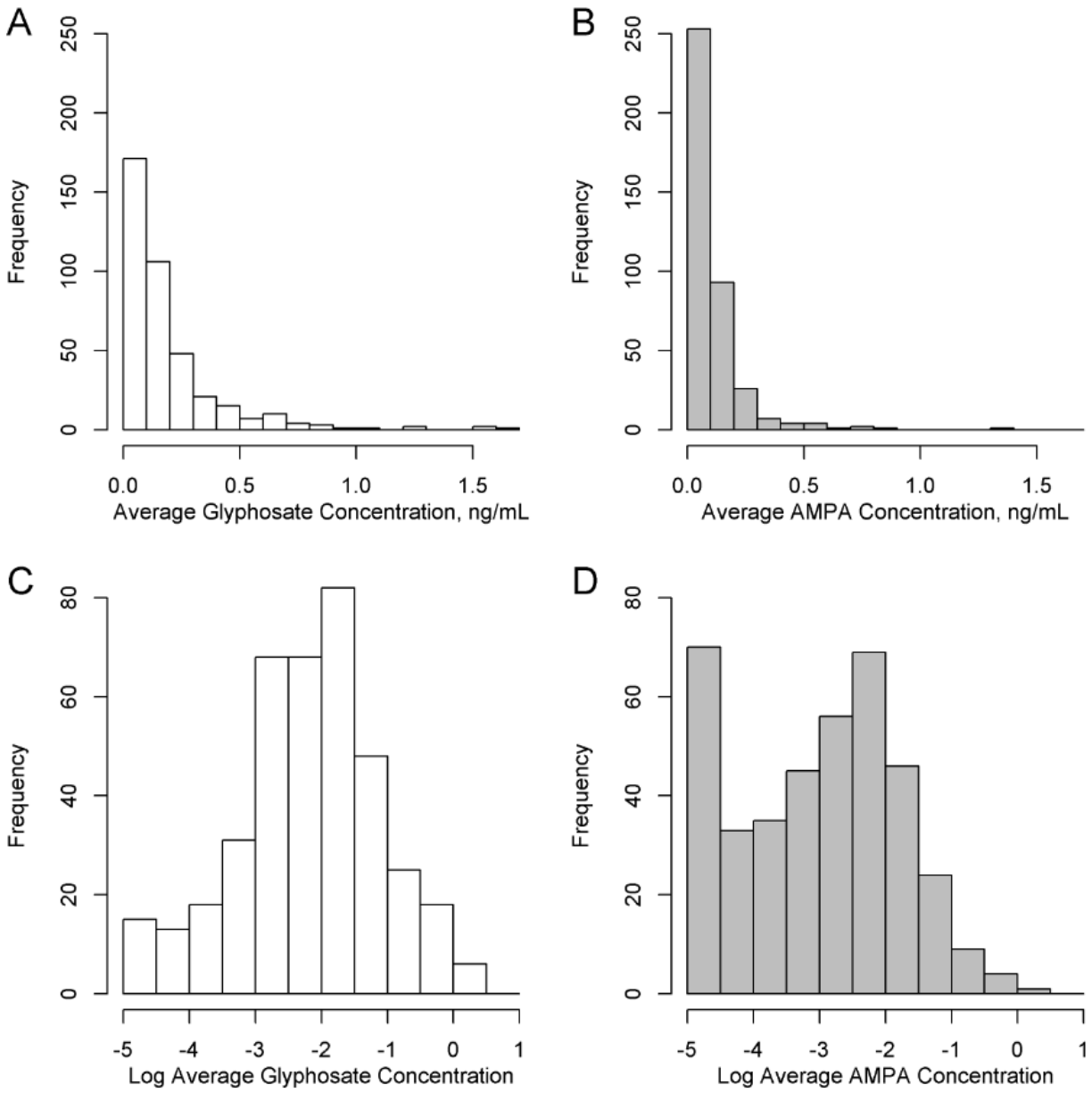
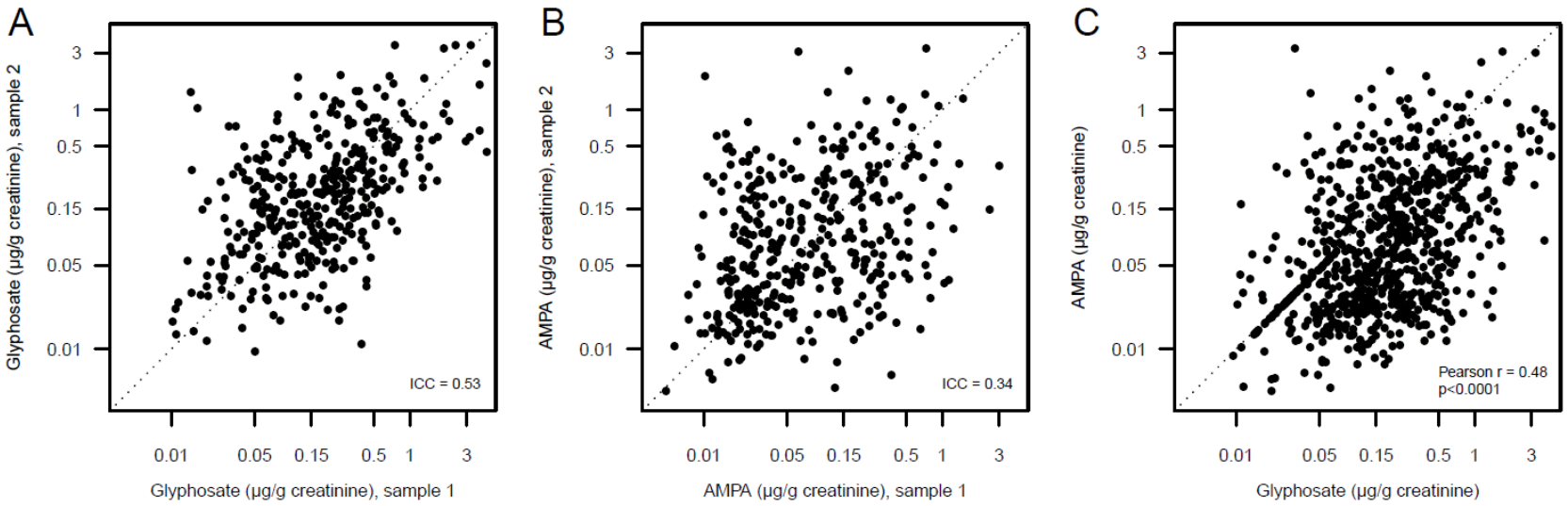


Figure S2: Histograms of urinary glyphosate (A) and AMPA (B) concentration measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in 392 postmenopausal California women, averaged for two samples from each individual, and histograms of the natural log of urinary glyphosate (C) and AMPA (D). LOD: 0.014 ng/mL for glyphosate and 0.013 ng/mL for AMPA. AMPA: aminomethylphosphonic acid



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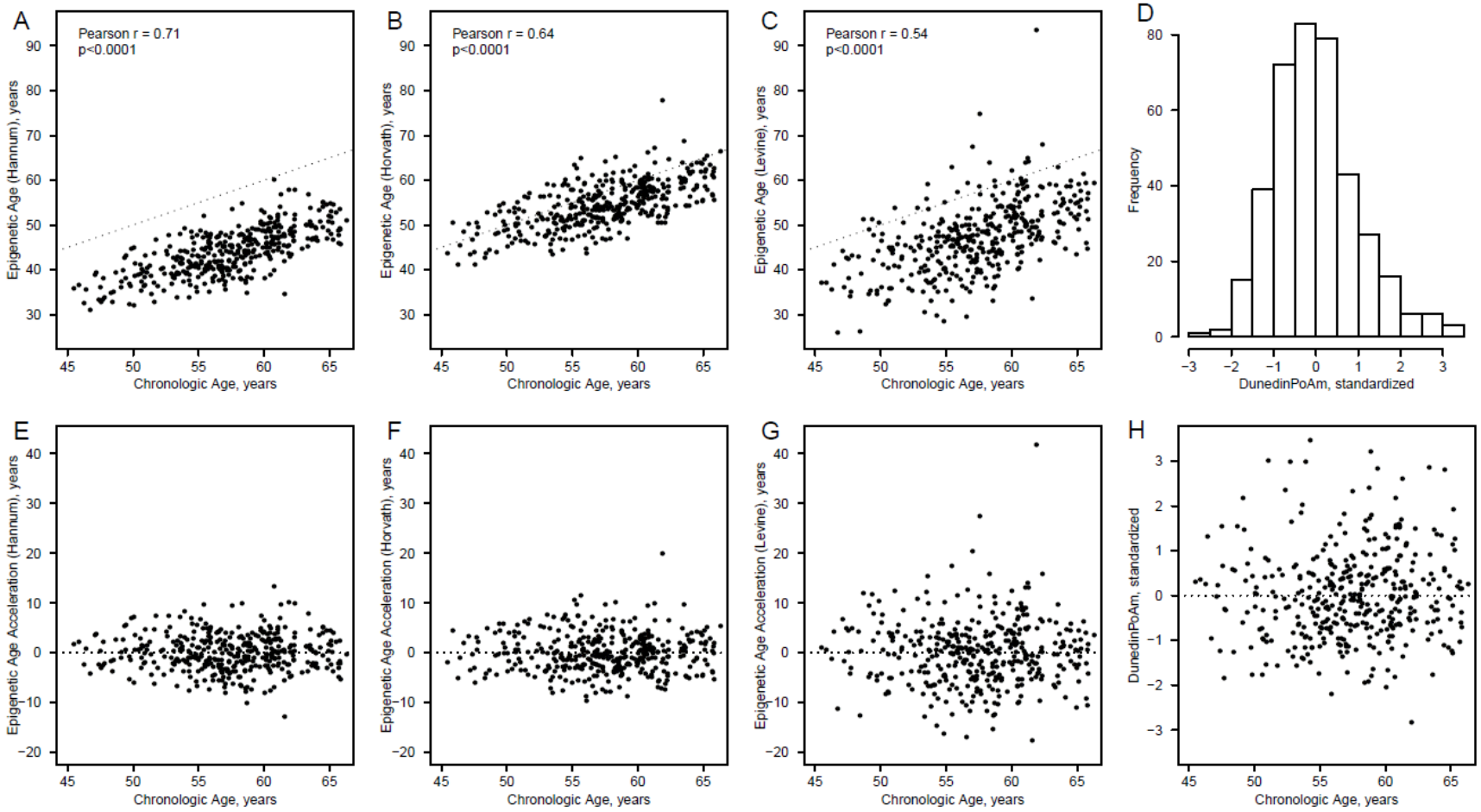

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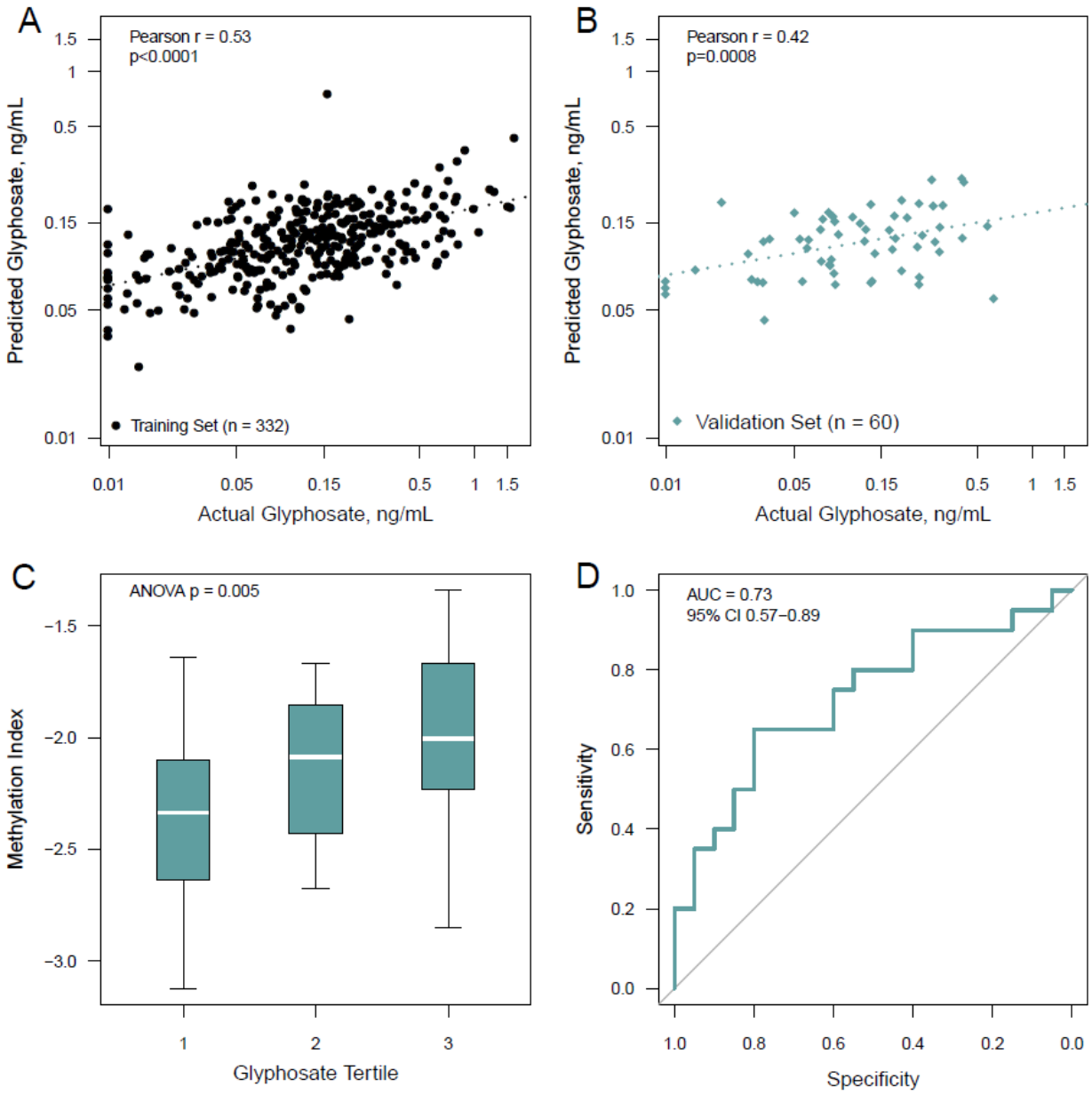

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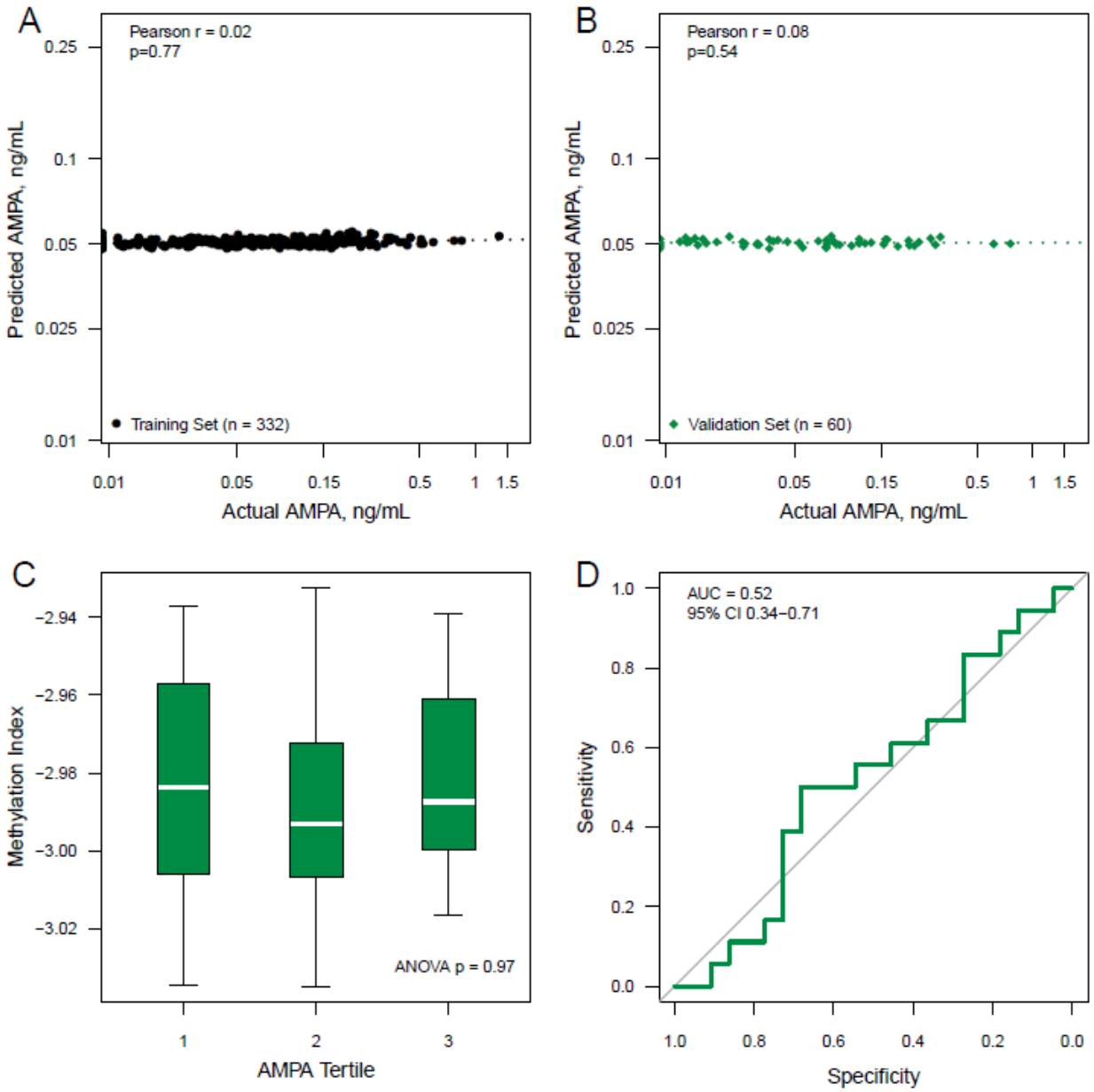

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