

Supplementary Table S1. Characteristics at exam 3 (1986-1987) of twin pairs discordant for cardiovascular death¹

Characteristics	CVD-dMZ (n=19 pairs)				CVD-dDZ (n=19 pairs)			
	Total	Case Twins	Control Twins	P value ²	Total	Case Twins	Control Twins	P Value ²
Discordant pairs, n	19	19	19	–	19	19	19	–
Age at exam 3 (y)	65.4 (2.5)	65.4 (2.5)	65.4 (2.5)	1.00	64.9 (3.2)	64.9 (3.2)	64.9 (3.2)	1.00
Age at death (y)	79.0 (7.9)	74.3 (5.7)	83.7 (7.1)	<0.0001	80.6 (7.1)	76.7 (5.2)	84.5 (6.7)	<0.0001
Cigarette smoking, n (%)				0.21				0.96
Never smokers	9 (24)	3 (16)	6 (32)		7 (19)	3 (16)	4 (22)	
Former smokers	24 (63)	13 (68)	11 (58)		23 (62)	11 (58)	12 (67)	
Current smokers	5 (13)	3 (16)	2 (10)		7 (19)	5 (26)	2 (11)	
Education, ³ (y)	13.9 (2.6)	14.2 (3.1)	13.6 (2.2)	0.36	12.2 (3.0)	11.9 (2.8)	12.5 (3.1)	0.23
Body mass index (kg/m ²)	28.2 (3.6)	28.0 (3.4)	28.4 (3.7)	0.39	26.8 (3.8)	26.9 (4.1)	26.7 (3.7)	0.83
Obesity, n (%)	9 (24)	5 (26)	4 (21)	0.66	6 (16)	3 (16)	3 (16)	0.97
Systolic blood pressure (mm Hg)	137 (14)	139 (13)	135 (14)	0.20	136 (12)	138 (14)	134 (8)	0.24
Diastolic blood pressure (mm Hg)	83 (7)	82 (7)	85 (6)	0.13	84 (8)	85 (8)	83 (8)	0.40
Plasma biochemistry								
Total cholesterol (mg/dL) ⁴	232 (37)	223 (35)	241 (38)	0.04	214 (39)	215 (35)	213 (43)	0.87
Triglyceride (mg/dL) ⁵	163 (75)	172 (90)	153 (56)	0.26	148 (83)	157 (84)	193 (83)	0.35
HDL-C (mg/dL) ⁴	43 (11)	42 (11)	44 (10)	0.44	44 (13)	44 (14)	44 (11)	0.86
LDL-C (mg/dL) ⁴	157 (33)	146 (29)	166 (36)	0.013	145 (35)	143 (32)	147 (37)	0.69
Postload glucose (mg/dL) ⁶	186 (33)	190 (27)	182 (38)	0.50	179 (60)	186 (65)	173 (55)	0.46
Diabetes, n (%)	8 (21)	5 (26)	3 (16)	0.41	9 (24)	6 (32)	3 (16)	0.22
Use of cholesterol-lowering medicine, n (%)	0 (0)	0 (0)	0 (0)	1.00	1 (0.3)	1 (0.5)	0 (0)	1.00
Use of antihypertensives, n (%)	16 (42)	9 (47)	7 (37)	0.35	14 (37)	10 (53)	4 (21)	0.08
Peripheral blood leukocyte composition, ⁷ %					N/A	N/A	N/A	
CD8+ T-cells	2.6 (3.2)	3.0 (3.7)	2.2 (2.7)	0.44	–	–	–	
CD4+ T-cells	13.9 (5.7)	13.0 (5.4)	14.8 (5.9)	0.11	–	–	–	
Natural killer cells	5.6 (4.1)	5.7 (4.6)	5.6 (3.8)	0.87	–	–	–	
B-cells	4.1 (1.8)	4.1 (2.1)	4.1 (1.6)	0.94	–	–	–	
Monocytes	7.1 (3.2)	7.0 (2.9)	7.1 (3.4)	0.91	–	–	–	
Granulocytes	68.3 (7.9)	68.7 (8.8)	67.9 (7.0)	0.69	–	–	–	

Abbreviations: CVD-dMZ= monozygotic twin pairs discordant for CVD death; CVD-dDZ= dizygotic twin pairs discordant for CVD death; HDL-C=high-density lipoprotein cholesterol; LDL-C= low-density lipoprotein cholesterol

¹data at exam 3 are presented as mean (SD) unless otherwise specified. Raw values for continuous variables are presented.

²case twins versus their co-twin brothers (controls).

³from baseline exam (exam 1)

⁴to convert mg/dl of total cholesterol, HDL or LDL cholesterol to mmol/l, divide by 38.67

⁵to convert mg/dl of triglycerides to mmol/l, divided by 88.5.

⁶to convert mg/dl of glucose to mmol/l, divided by 18.02.

⁷estimated from methylation data

Supplementary Table S2. Characterization of differentially hydroxymethylated region among the discovery sample of monozygotic twin pairs discordant for cardiovascular death (N=12 pairs)

	Total DhMRs ⁴ (n)	DhMR Features				DhMR Coverage, bp		
		Consistent DhMRs ⁵ (n)	Hyper-DhMRs (n)	Hypo-DhMRs (n)	Size, Mean (range), bp	Total DhMRs ⁴	Hyper-DhMRs	Hypo-DhMRs
Model 1¹	16,232		12,262	3,970	262 (150 -950)	4,264,650	3,209,400	1,055,250
Model 2²	6,438	1,893	5,264	1,174	232 (150-950)	1,497,950	1,229,500	268,450
Model 3³	7,864		6,411	1,453	228 (150-950)	1,789,550	1,463,950	325,600

Abbreviations: DhMRs=differentially hydroxymethylated regions; hyper-DhMRs = differentially hyper-hydroxymethylated regions (case twins vs. control twins); hypo-DhMRs = differentially hypo-hydroxymethylated regions (case twins vs. control twins)

¹does not adjust for any covariates while naturally adjust for germline and environmental factors shared between monozygotic co-twins

²adjust for blood leukocyte subtypes, including CD4+ T-cells, CD8+ T-cells, natural killer cells, B-cells, and monocytes, in addition to germline and environmental factors shared between monozygotic co-twins

³adjust for blood leukocyte subtypes and age, including CD4+ T-cells, CD8+ T-cells, natural killer cells, B-cells, and monocytes, in addition to germline and environmental factors shared between monozygotic co-twins

⁴Benjamini–Hochberg False Discovery Rate (B-H FDR) <0.01

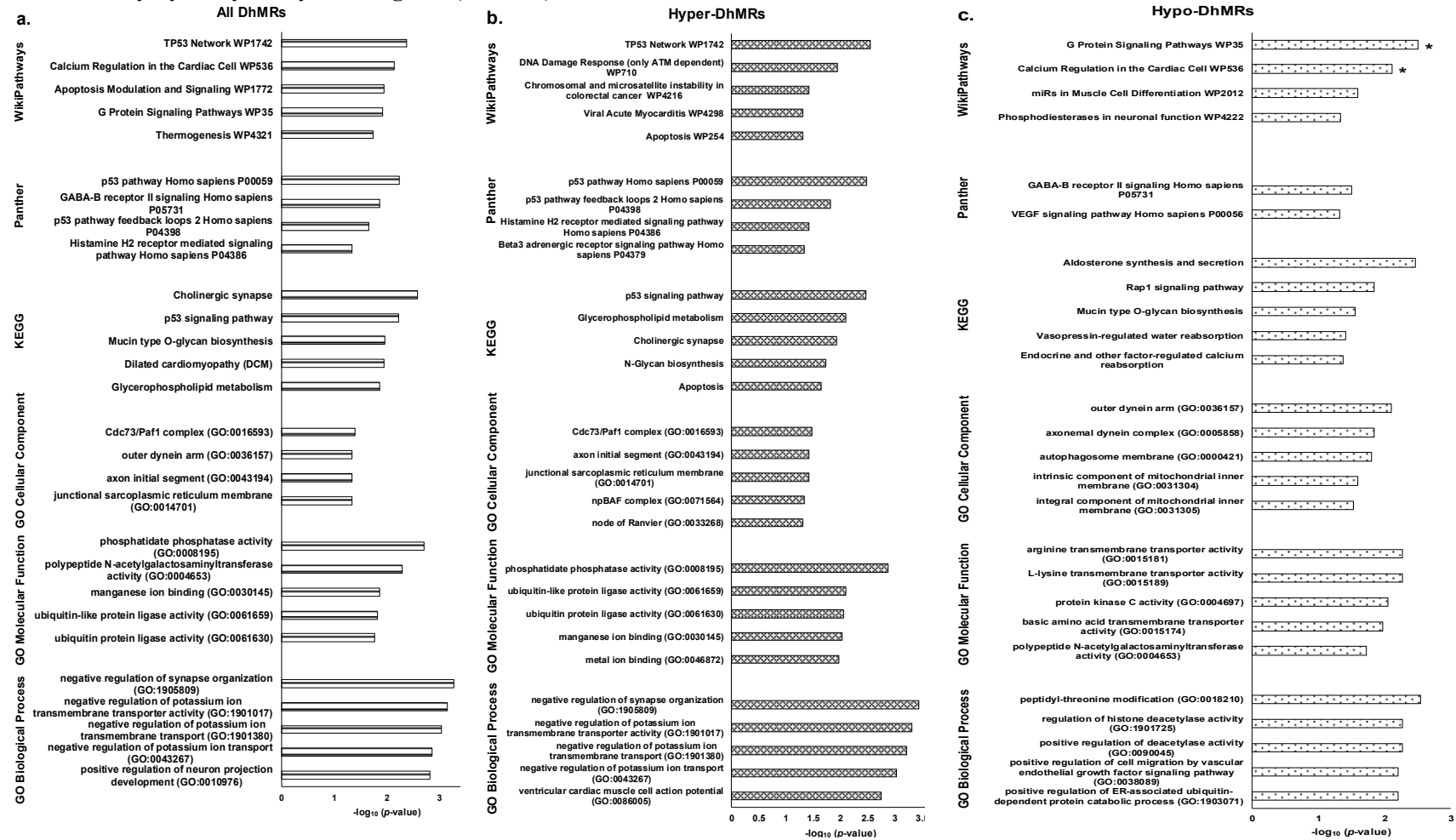
⁵the number of directionally consistent DhMRs among models 1, 2, and 3

Supplementary Table S4. Evaluation of the 102 differentially hydroxymethylated regions among dizygotic twin pairs discordant for cardiovascular death using the binary linear classifier

	N	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
CVD-dDZ	19 pairs	89.5%	63.2%	70.8%	85.7%	76.3%
CVD-dMZ	19 pairs	68.4%	52.6%	59.1%	62.5%	60.5%
CVD-dDZ and CVD-dMZ combined	38 pairs	78.9%	57.9%	65.2%	73.3%	68.4%

Abbreviations: CVD=cardiovascular death; CVD-dMZ= monozygotic twin pairs discordant for cardiovascular death; CVD-dDZ= dizygotic twin pairs discordant for cardiovascular death

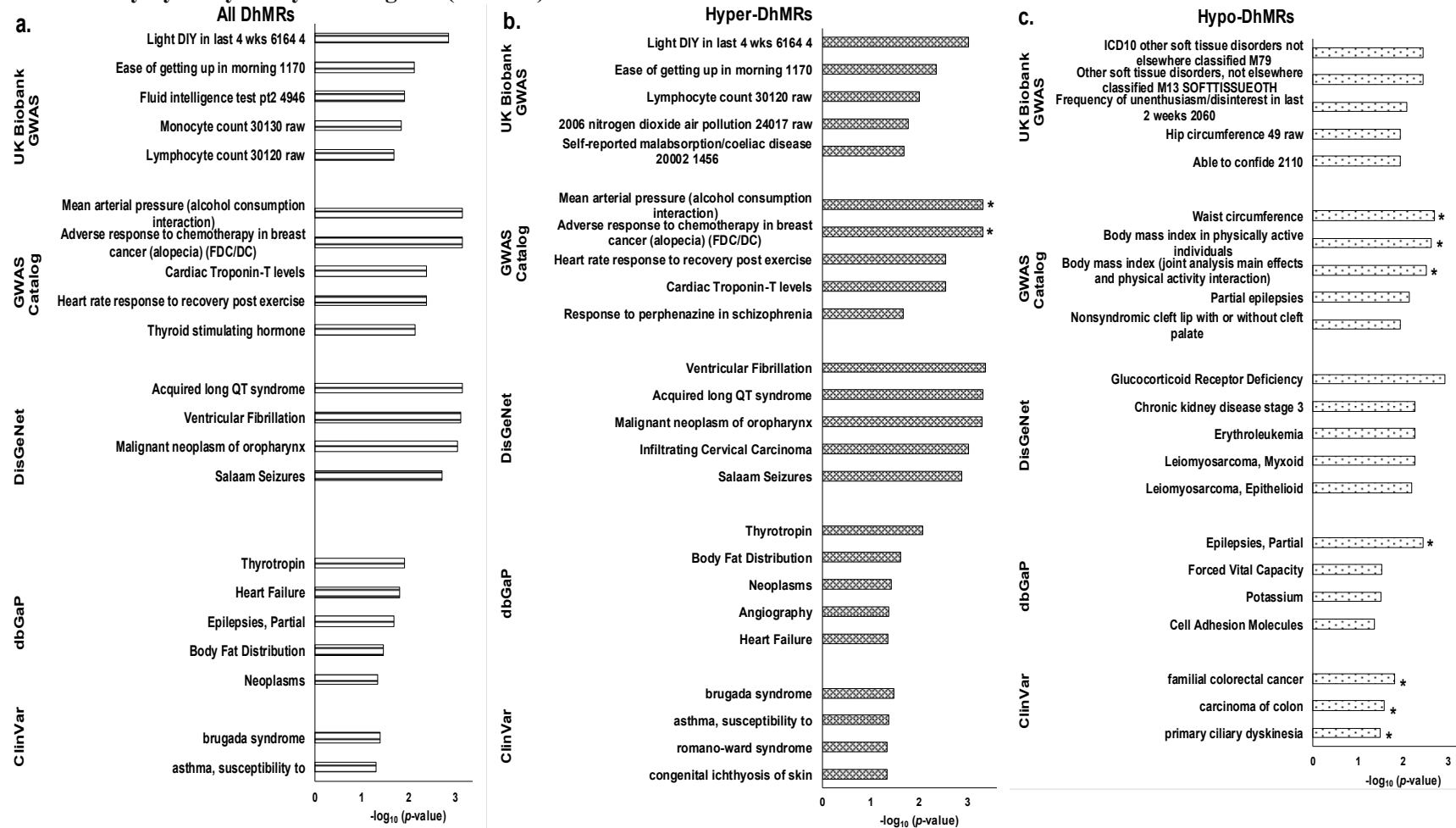
Supplementary Figure S1: Up to top five biological functions and pathways from bioinformatic analysis of genes mapped to differentially hydroxymethylated regions (DhMRs) with Enrichr¹



¹ up to top 5 enriched terms with the smallest nominal p -values < 0.05 . * $p_{adj} < 0.05$, multiple-testing adjusted p -value using the Benjamini-Hochberg False Discovery Rate (FDR) procedure

DhMRs= differentially hydroxymethylated regions; hyper-DhMRs= differentially hyper-hydroxymethylated regions (case vs. control twins); hypo-DhMRs= differentially hypo-hydroxymethylated regions (case vs. control twins)

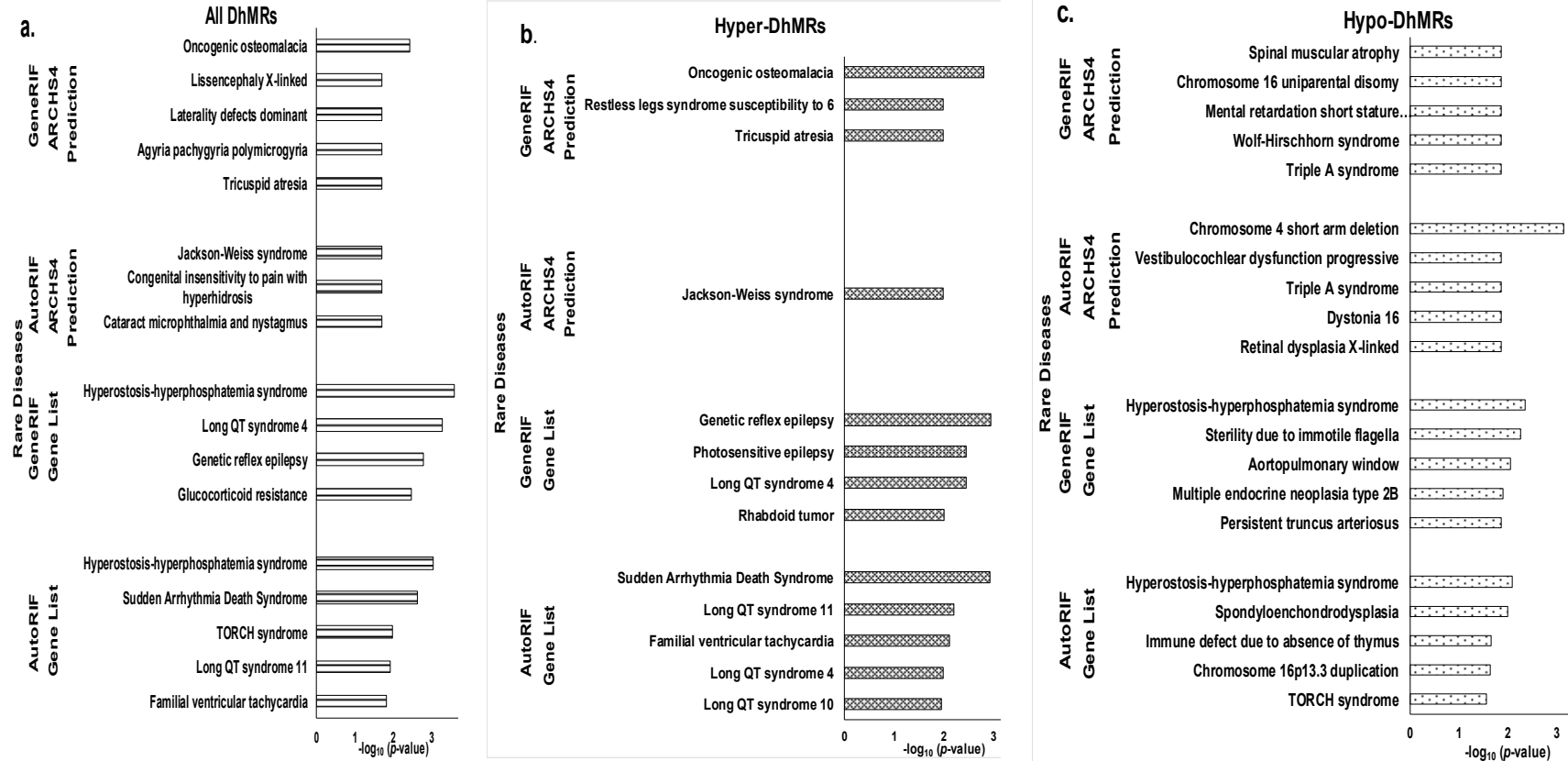
Supplementary Figure S2: Up to top five phenotypes, disorders, and diseases from bioinformatic analysis of genes mapped to differentially hydroxymethylated regions (DhMRs) with Enrichr¹



¹ up to top 5 enriched terms with the smallest nominal p -values < 0.05 . * $p_{adj} < 0.05$, multiple-testing adjusted p -value using the Benjamini-Hochberg False Discovery Rate (FDR) procedure

DhMRs= differentially hydroxymethylated regions; hyper-DhMRs= differentially hyper-hydroxymethylated regions (case vs. control twins); hypo-DhMRs= differentially hypo-hydroxymethylated regions (case vs. control twins)

Supplementary Figure S3: Up to top five rare diseases/disorders from bioinformatic analysis of genes mapped to differentially hydroxymethylated regions (DhMRs) with Enrichr¹



¹ up to top 5 enriched terms with the smallest nominal p -values < 0.05.

DhMRs= differentially hydroxymethylated regions; hyper-DhMRs= differentially hyper-hydroxymethylated regions (case vs. control twins); hypo-DhMRs= differentially hypo-hydroxymethylated regions (case vs. control twins)