

Supplementary material

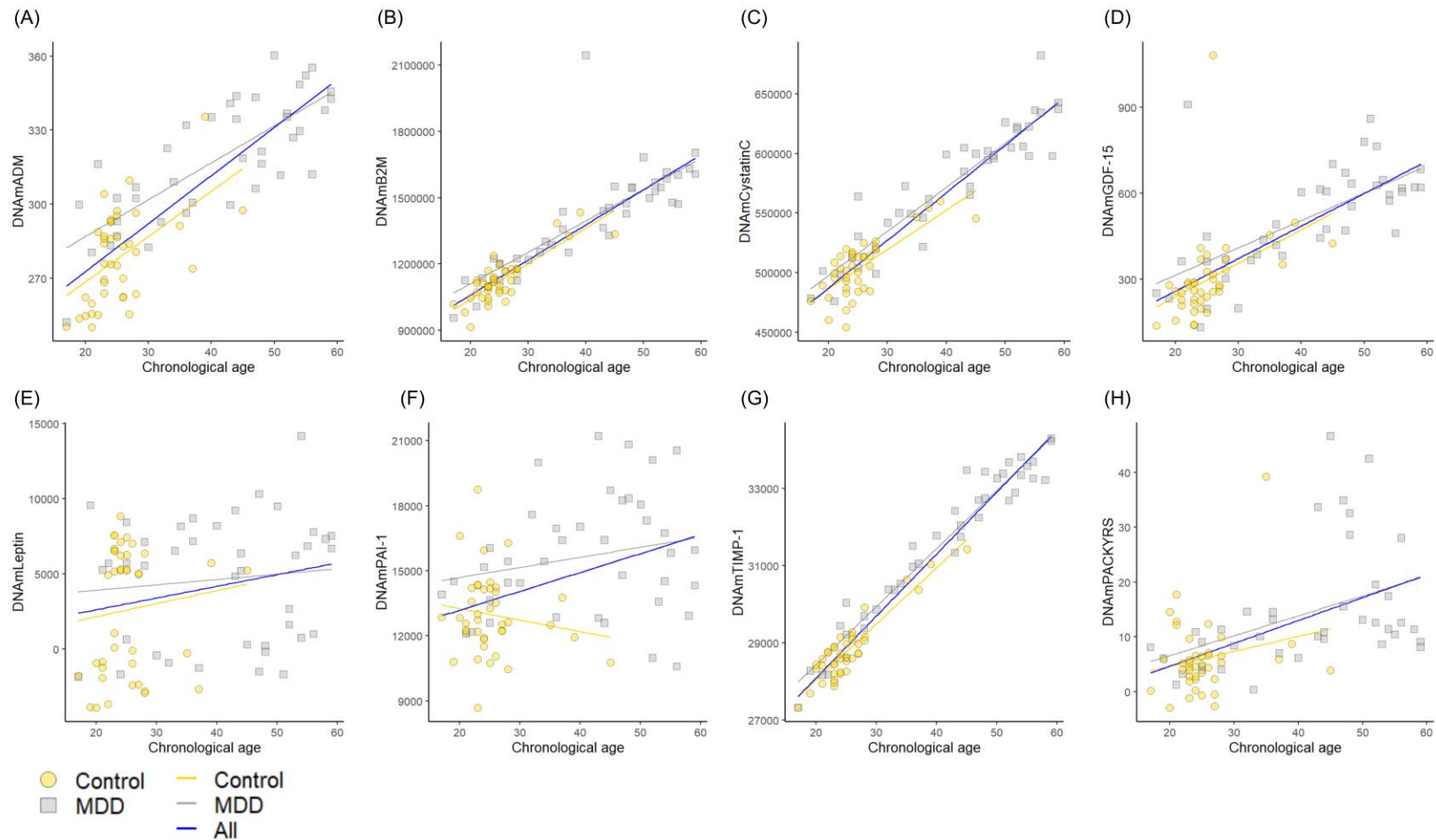
Accelerated epigenetic aging and decreased natural killer cells based on DNA methylation in untreated patients with major depressive disorder

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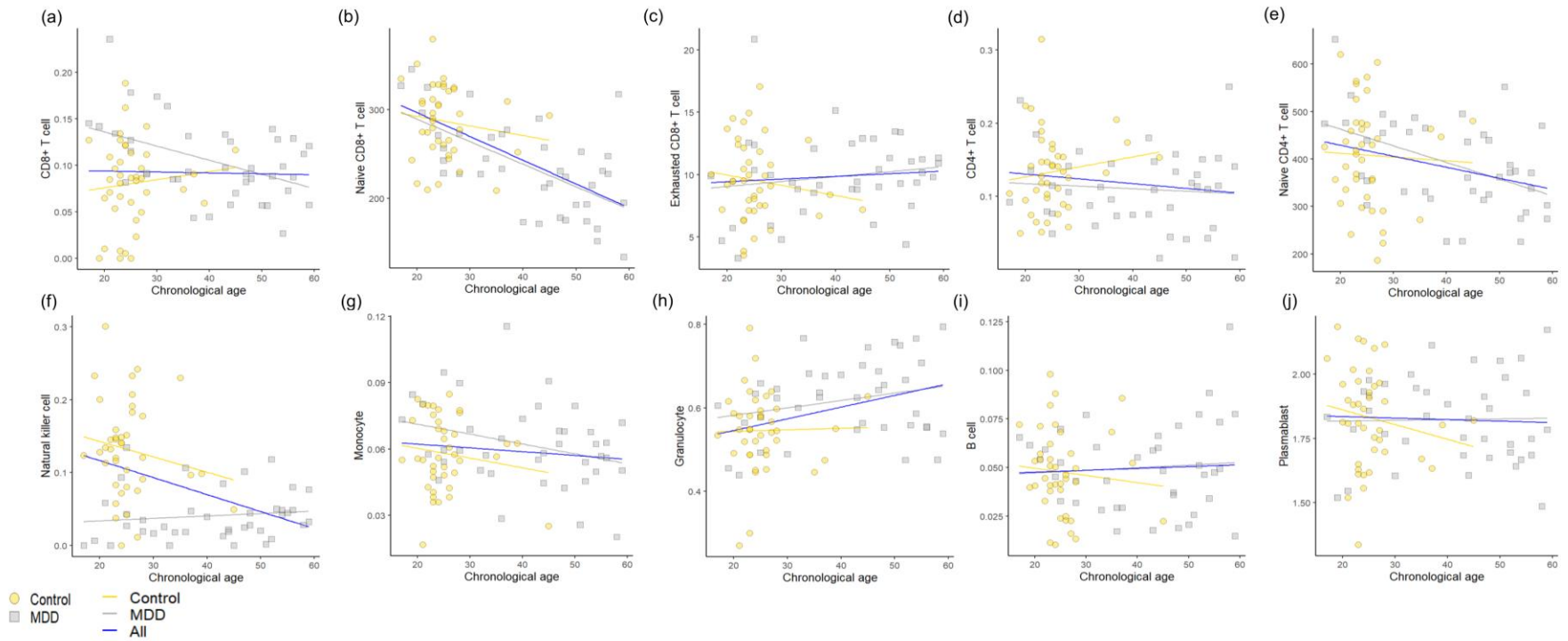
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Supplementary Figure 1. Correlation between GrimAge components and chorological age among patients with untreated MDD and controls.

(A) ADM, (B) B2M, (C) Cystatin C, (D) GDF-15, (E) Leptin, (F) PAI-1, (G) TIMP-1, and (H) PACKYRS. The scatter plots indicate GrimAge components on the y-axis vs. the chronological age on the x-axis.

ADM, adrenomedullin; BMI, body mass index; B2M, beta-2-microglobulin; CTL, control; DNAm, DNA methylation; GDF-15, growth differentiation factor 15; MDD, major depressive disorder; PACKYRS, smoking pack-years; PAI-1, plasminogen activator inhibitor-1; TIMP-1, tissue inhibitor of metalloproteinases-1.



Supplementary Figure 2. DNAm-based white blood cell composition correlated with the chronological age among patients with untreated MDD and controls.

(a) cytotoxic CD8+ T cells, (b) naive CD8+ T cells, (c) exhausted CD8+ T cells, (d) helper CD4+ T cells, (e) naive CD4+ T cells, (f) natural killer cells, (g) monocytes, (h) granulocytes, (i) B cells, and (j) plasma blasts.

White blood cell counts were estimated using DNAm. Scatter plots indicate DNAm-based white blood cell composition versus chronological age (p -values were evaluated using Spearman's rank correlation coefficient).

CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CTL, control; DNAm, DNA methylation; MDD, Major depressive disorder.

Supplementary Table 1. Correlation between DNAm-based Age and chorological age in patients with untreated MDD and controls

	Phenotype	ρ	p -value
HorvathAge	CTL	0.709	<0.001
	MDD	0.873	<0.001
	ALL	0.896	<0.001
HannumAge	CTL	0.602	<0.001
	MDD	0.877	<0.001
	ALL	0.876	<0.001
SkinBloodAge	CTL	0.818	<0.001
	MDD	0.893	<0.001
	ALL	0.924	<0.001
PhenoAge	CTL	0.519	0.0006
	MDD	0.879	<0.001
	ALL	0.857	<0.001
GrimAge	CTL	0.668	<0.001
	MDD	0.883	<0.001
	ALL	0.908	<0.001
DNAmTL	CTL	– 0.0332	0.839
	MDD	– 0.816	<0.001
	ALL	– 0.721	<0.001

We assessed p -value using the Spearman's rank correlation coefficient.

If p -values are significant at <0.05, they are denoted in bold and in italics.

CTL, control; DNAmTL, DNA methylation-based telomere length MDD, Major depressive disorder

Supplementary Table 2. Correlation between GrimAge components and chorological age in patients with untreated MDD and controls

	Phenotype	<i>rho</i>	<i>p</i> -value
DNAmADM	CTL	0.507	<i>0.00085</i>
	MDD	0.763	<i><0.001</i>
	ALL	0.791	<i><0.001</i>
DNAmB2M	CTL	0.677	<i><0.001</i>
	MDD	0.886	<i><0.001</i>
	ALL	0.899	<i><0.001</i>
DNAmCystatinC	CTL	0.633	<i><0.001</i>
	MDD	0.917	<i><0.001</i>
	ALL	0.894	<i><0.001</i>
DNAmGDF-15	CTL	0.628	<i><0.001</i>
	MDD	0.688	<i><0.001</i>
	ALL	0.774	<i><0.001</i>
DNAmLeptin	CTL	0.112	0.491
	MDD	0.132	0.417
	ALL	0.244	<i>0.0293</i>
DNAmPAI-1	CTL	- 0.119	0.465
	MDD	0.198	0.220
	ALL	0.375	<i>0.0006</i>
DNAmTIMP-1	CTL	0.752	<i><0.001</i>
	MDD	0.949	<i><0.001</i>
	ALL	0.940	<i><0.001</i>
DNAmPACKYRS	CTL	0.0453	0.781
	MDD	0.509	<i>0.0008</i>
	ALL	0.537	<i><0.001</i>

We assessed *p* -value using the Spearman's rank correlation coefficient.

If *p*-values are significant at <0.05, they are denoted in bold and in italics.

ADM, adrenomedullin; B2M, beta-2-microglobulin; CTL, control; DNAm, DNA methylation; DNAmPACKYRS, DNA methylation-based smoking pack-years; GDF-15, growth differentiation factor 15; MDD, Major depression disorder; PAI-1, plasminogen activator inhibitor-1; TIMP-1, tissue inhibitor of metalloproteinases-1

Supplementary Table 3. DNAm-based white blood cell composition correlated with the chronological age in patients with untreated MDD and controls

	Phenotype	<i>rho</i>	<i>p</i> -value
CD8+ T cell	CTL	0.095	0.558
	MDD	− 0.394	<i>0.012</i>
	ALL	− 0.027	0.810
Naive CD8+ T cell	CTL	− 0.132	0.416
	MDD	− 0.588	<i><0.001</i>
	ALL	− 0.598	<i><0.001</i>
Exhausted CD8+ T cell	CTL	− 0.170	0.293
	MDD	0.216	0.181
	ALL	0.089	0.431
CD4+ T cell	CTL	0.089	0.586
	MDD	− 0.0464	0.776
	ALL	− 0.131	0.245
Naive CD4+ T cell	CTL	− 0.107	0.513
	MDD	− 0.432	<i>0.005</i>
	ALL	− 0.289	<i>0.009</i>
Natural killer cell	CTL	− 0.142	0.383
	MDD	0.259	0.107
	ALL	− 0.386	<i>0.0004</i>
Monocyte	CTL	− 0.016	0.921
	MDD	− 0.291	0.068
	ALL	− 0.092	0.417
Granulocyte	CTL	− 0.023	0.890
	MDD	0.162	0.318
	ALL	0.332	0.003
B cell	CTL	− 0.203	0.208
	MDD	0.041	0.801
	ALL	− 0.076	0.503
Plasmablast	CTL	− 0.141	0.385
	MDD	− 0.0358	0.827
	ALL	− 0.040	0.724

We assessed *p* -value using the Spearman's rank correlation coefficient.

If *p*-values are significant at <0.05, they are denoted in bold and in italics.

CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CTL, control; DNAm, DNA methylation; MDD, Major depressive disorder

Supplementary Table 4. Multiple regression analyses of DNAm-based Ages and TL acceleration, as well as GrimAge components, and DNAm-based white blood cell counts between patients with untreated MDD and controls younger than 40 years old

	Phenotype		Age		Sex		Adjusted R ²
	Estimate	P-value	Estimate	P-value	Estimate	P-value	
DNAmAge and DNAmTL acceleration ^a							
AgeAccelHorvath	− 0.94919606	0.500	0.07602254	0.546	− 1.97439809	0.119	0.007486
AgeAccelHannum	5.38458490	<0.001	− 0.02734922	0.835	− 2.41054733	0.069	0.1914
AgeAccelSkinBlood	0.9996374	0.461	0.1516941	0.214	− 1.0495608	0.386	0.004659
AgeAccelPheno	2.5288128	0.182	0.1213498	0.471	− 2.0435853	0.226	0.01807
AgeAccelGrim	2.295827438	0.0016	0.003072431	0.960	− 2.129536659	0.00106	0.2458
DNAmTLAdjAge	− 0.077913526	0.131	0.001547362	0.734	0.101123630	0.030	0.06086
GrimAge components ^b							
DNAmADM	10.702830	0.00287	1.908057	<0.001	22.206395	<0.001	0.7027
DNAmB2M	25007.25	0.159	17763.46	<0.001	15923.37	0.312	0.7335
DNAmCystatinC	11414.879	0.0344	3778.947	<0.001	− 2245.985	0.633	0.5968
DNAmGDF-15	39.393067	0.407	9.519754	0.028	15.606152	0.712	0.0792
DNAmLeptin	420.6267	0.442	28.8737	0.554	7574.7376	<0.001	0.8225
DNAmPAI-1	1798.56551	0.00304	71.92594	0.170	− 738.19550	0.158	0.1862
DNAmTIMP-1	381.0633	0.00089	165.3476	<0.001	− 178.9794	0.0687	0.8668
DNAmPACKYRS	0.9295317	0.611	0.4119869	0.0143	− 3.5397149	0.0334	0.1353
DNAm-based white blood cell composition ^c							
CD8+ T cell	0.04777	0.0012	− 0.00156	0.219	0.01117	0.375	0.1611
Naive CD8+ T cell	− 7.6774	0.525	− 2.5083	0.0234	7.4340	0.490	0.07248
Exhausted CD8+ T cell	− 0.36848	0.734	− 0.01344	0.890	− 0.25152	0.795	− 0.05201
CD4+ T cell	− 0.01841	0.268	− 0.00027	0.856	0.00234	0.873	− 0.02812
Naive CD4+ T cell	33.801	0.259	− 4.9206	0.0687	67.541	0.0134	0.1238
Natural killer cell	− 0.09080	<0.001	− 0.00017	0.915	− 0.02590	0.109	0.3614
Monocyte	0.00917	0.094	− 0.00008	0.862	− 0.00680	0.162	0.02481
Granulocyte	0.04360	0.131	0.00262	0.307	0.02301	0.367	0.05322
B cell	0.00152	0.806	− 0.00068	0.228	− 0.00453	0.414	− 0.01306
Plasmablast	0.00701	0.903	0.00135	0.794	− 0.01865	0.718	− 0.05333

Dummy variables: phenotype, CTL = 0, and MDD = 1; sex, male = 0 and female = 1.

If p -values are significant at <0.05 , they are denoted in bold and in italics.

R^2 is the coefficient of determination.

^a We defined epigenetic age acceleration (AgeAccelHorvath, AgeAccelHannum, AgeAccelSkinBlood, AgeAccelPheno, and AgeAccelGrim) as the residual from regressing each DNAm age on the chronological age. We defined DNAmTLadjAge as the residual from regressing DNAmTL on chronological age.

^b GrimAge components indicated DNAm-based age-predictive factors. For the GrimAge components, we corrected the significance level for multiple comparisons and the Bonferroni method defined the p -value as $0.05/8 = 0.00625$ (eight GrimAge components).

If p -values are significant at <0.00625 , they are denoted in bold and in italics.

^c These white blood cell counts were predicted using DNAm. For DNAm-based white blood cells, we adjusted the significance level for multiple comparisons and the Bonferroni method defined the p -value as $0.05/10 = 0.005$ (ten white blood cell compositions).

If p -values are significant at <0.005 , they are denoted in bold and in italics.

ADM, adrenomedullin; B2M, beta-2-microglobulin; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; CTL, control; DNAm, DNA methylation; DNAmPACKYRS, DNA methylation-based smoking pack-years; DNAmTL, DNA methylation-based telomere length; DNAmTLadjAge, age-adjusted estimate of DNA methylation-based telomere length; GDF-15, growth differentiation factor 15; MDD, Major depressive disorder; PAI-1, plasminogen activator inhibitor-1; TIMP-1, tissue inhibitor of metalloproteinases-1