Supplemetary information (SI)

Case Control Study

BDNF methylation and mRNA expression in brain and blood of completed suicides in Slovenia

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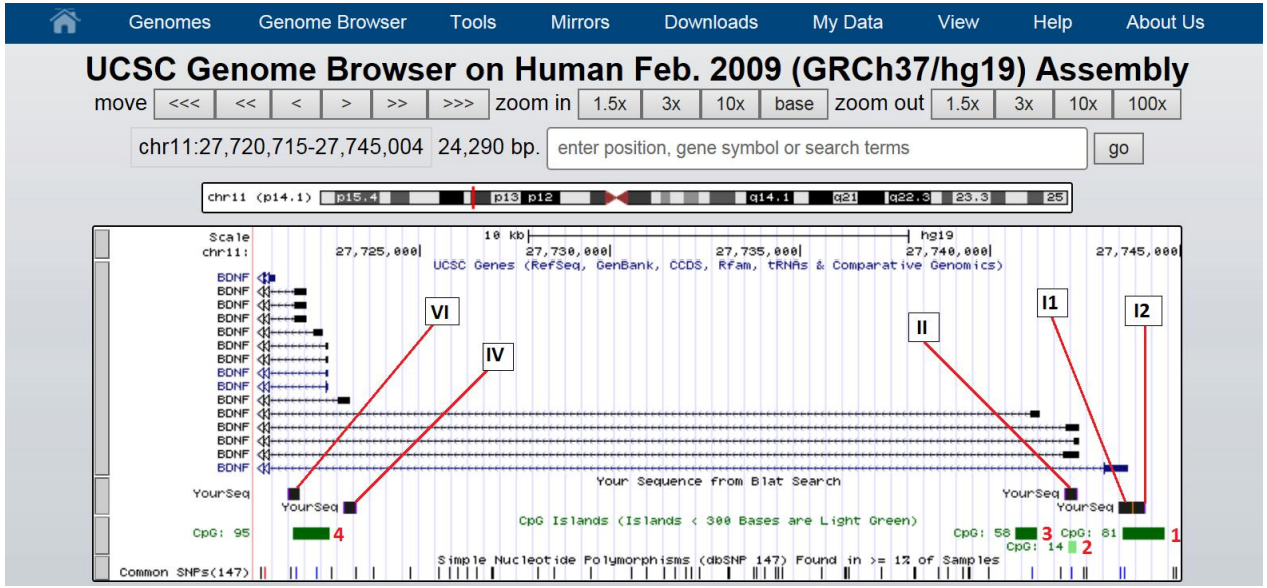


Supplementary Figure 1 **Representation of the positions of the *BDNF* regions according to the positions of** the ***BDNF* CpG islands and exons**. CpG islands are shown as green rectangles, and indicated as 1-4 at the bottom. Above the green rectangles of the CpG islands, the *BDNF* regions of interest are shown as black rectangles, and are labelled with roman numerals (I, II, IV and VI) according to the vicinity of the exons 1, 2, 4 or 6. The region preceding the first exon (I), is divided into two parts, I1 and I2 due to technical reason of maximum amplicon length recommendation for the 454 GS Junior sequencing system (400 bp/ region, including 454 GS Junior sequencing primers). The data on the CGI regions of the *BDNF* gene were obtained using the University of California Santa Cruz genome browser website (https://genome.ucsc.edu/) and the human genome, version GRCh37/Hg19 (February, 2009).

I1 >chr11:27743371-27743710 (+) [1,2]

agttcccagagaaagtgaggagtttcagggtacccctgtaaaaaaaaaactcagcctgcacacCG[1]ctcccttgcccact
CG[2]caggtcacacctggggctcCG[3]gCG[4]aagctgaattCG[5]gctCG[6]ggagatttccctgtCG[7]ccaggtaagaaa
accttCG[8]ccttgtcaggctagggCG[9]ggaagacCG[10]ctggggaacttgttgcttatcagCG[11]cCG[12]gcagactac
CG[13]ctttaataataataccagaaaagCG[14]cagcagggaggggtgggggCG[15]gcaaCG[16]gCG[17]aCG[18]act
aacctCG[19]ctgtttaCG[20]tgacCG[21]actcactgtgactctctgggtaaaaaaagg

I2 >chr11:27743690-27744027 (+) [1,2]

gactctctgggtaaaaaaaggaaacttcttagaaaagttCG[1]tgcccctcccctccccatcatgactaagggtctccagcC
G[2]atgaggtCG[3]tgagtgatgatcaaatggggactgggggggaggggggCG[4]agtaagtgacttgtccttgggaacatct
gcatgCG[5]tCG[6]aagCG[7]CG[8]aaccagcccaacaactttccctttcctcttaggtactgatgactaggCG[9]agaggcacc
aaggCG[10]agccactagttgcccacaggaacctgtgtaagcCG[11]agctctCG[12]agCG[13]gctgagttgaatgaagttat
gactgCG[14]tttgctctaatgggttatggcttc

II >chr11:27741923-27742250 (+)

tagatcctggagataaacacttgcatttcccaaagttaacccagtataccaaccCG[1]gagcttgccaagagtctattccagccta
cacCG[2]ctaggaagccaacttcagCG[3]agctcaatgaggggaccaaactggggctCG[4]ctttccaaaCG[5]ctcCG[6]ct
ccaaaatctgactctctctccagcccCG[7]atctcagtgtgagcCG[8]aacctcagaaaagaCG[9]ctttttaagggCG[10]aca
cagggttggctttacagCG[11]gggccaagaagactacctgggggtacCG[12]ccacctCG[13]gacaaatcCG[14]ttggctc
tgtccaaggtgctgaatggactc

IV >chr11:27723004-27723326 (+) [3]

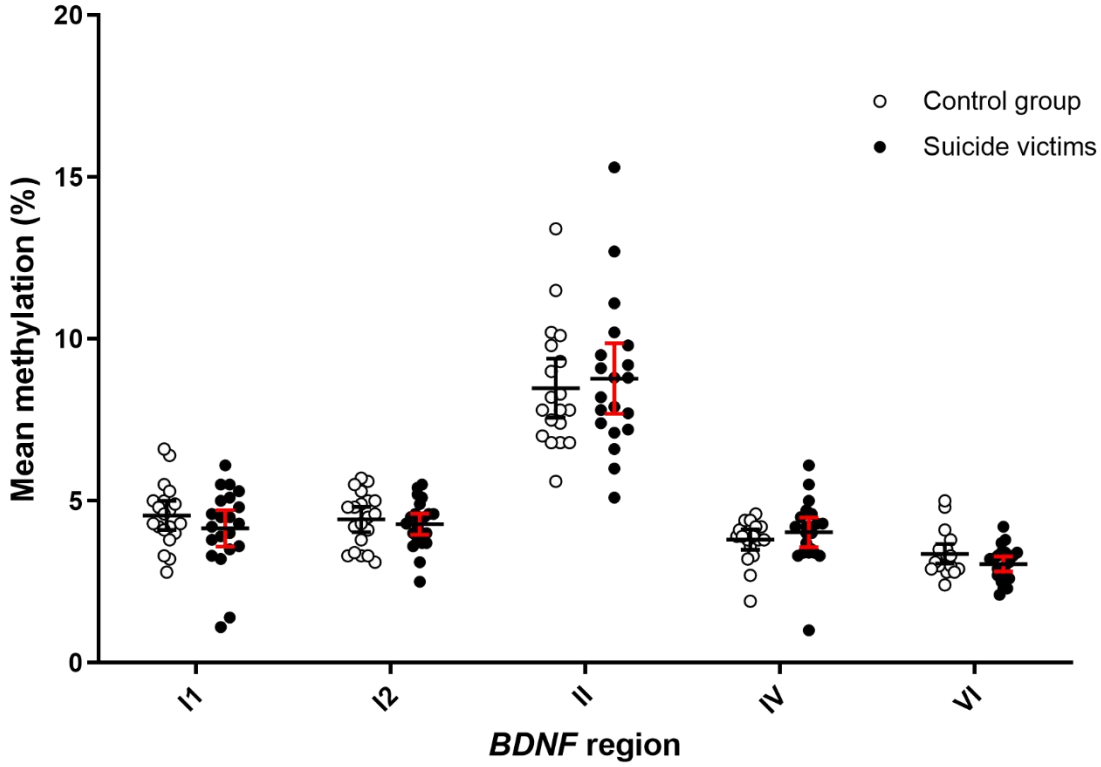
ttctgcagcaaagaagttaaattattgatagtggaaattgcatggCG[1]gaggtaatactCG[2]caccccatcagCG[3]agaag
ctccatttgatctCG[4]gcagaggcagggagatttcatgctagttCG[5]cCG[6]gggggagCG[7]gcagCG[8]agagcagcc
ctctcCG[9]CG[10]gtgaatgggaaagtgggtgggagtccaCG[11]agagggctccaCG[12]gtgccttgaCG[13]tgCG[14]ct
gtcatatgatacctcCG[15]ctgcctCG[16]aaatagacactctagtgcaCG[17]aattaccagaatcaaaattcagCG[18]catttа
aaatgatacatctttattagaagagttc

VI >chr11:27721544-27721854 (+) [4]

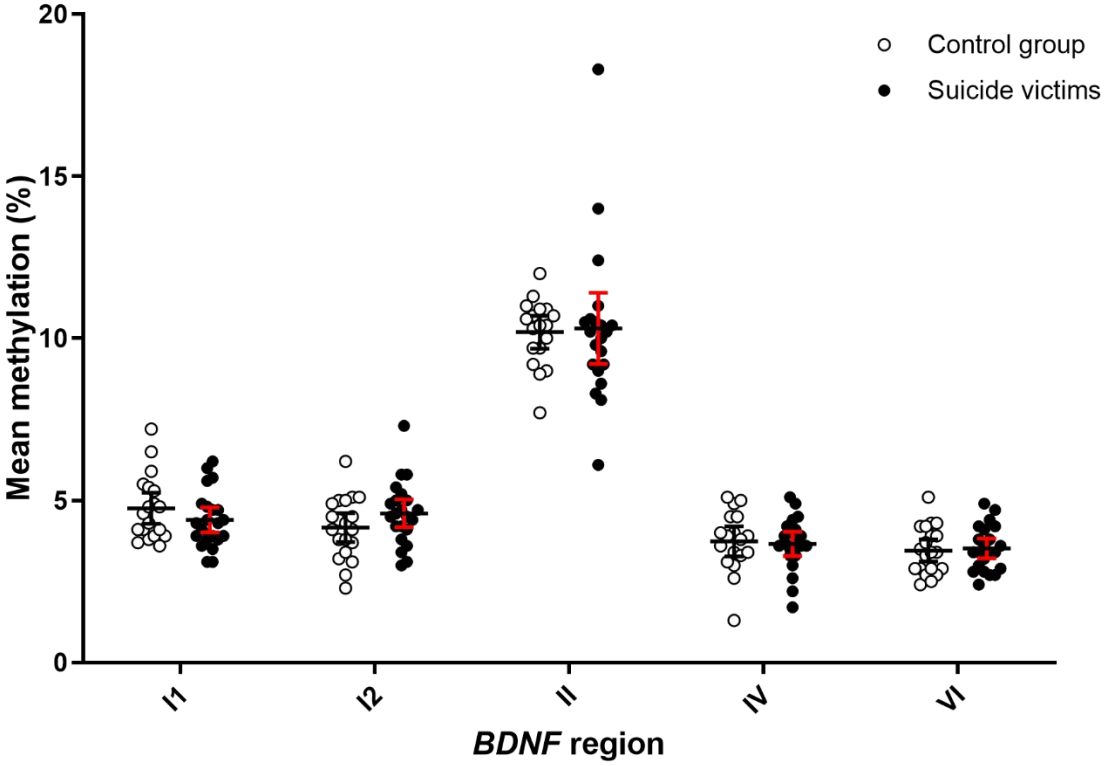
ggggagaaaactccccaagagtaactccaaatCG[1]tcccttctacCG[2]gaggggaggaaagaaggagactggcctCG[3]t
cccacaactttggggtgggggatccccagtcaactctctccCG[4]CG[5]gaCG[6]ggcagctcctgcaccaagccccattccca
gCG[7]cttgcctacctCG[8]gggtccacacaaacctcaCG[9]ggtcccCG[10]gCG[11]gCG[12]gagtcacatCG[13]tggttc
CG[14]attctggctccagCG[15]cccagcccCG[16]gtcccCG[17]tCG[18]CG[19]gtgctgctcccCG[20]cCG[21]gccccaca
gcagCG[22]gtgggtgtctcattaaagcc

Supplementary Figure 2 Nucleotide sequences of the five *BDNF* regions of interest before bisulfite conversion. The studied regions are labelled with roman numerals (I, II, IV and VI) according to the vicinity of the exons 1, 2, 4 or 6. The region preceding the

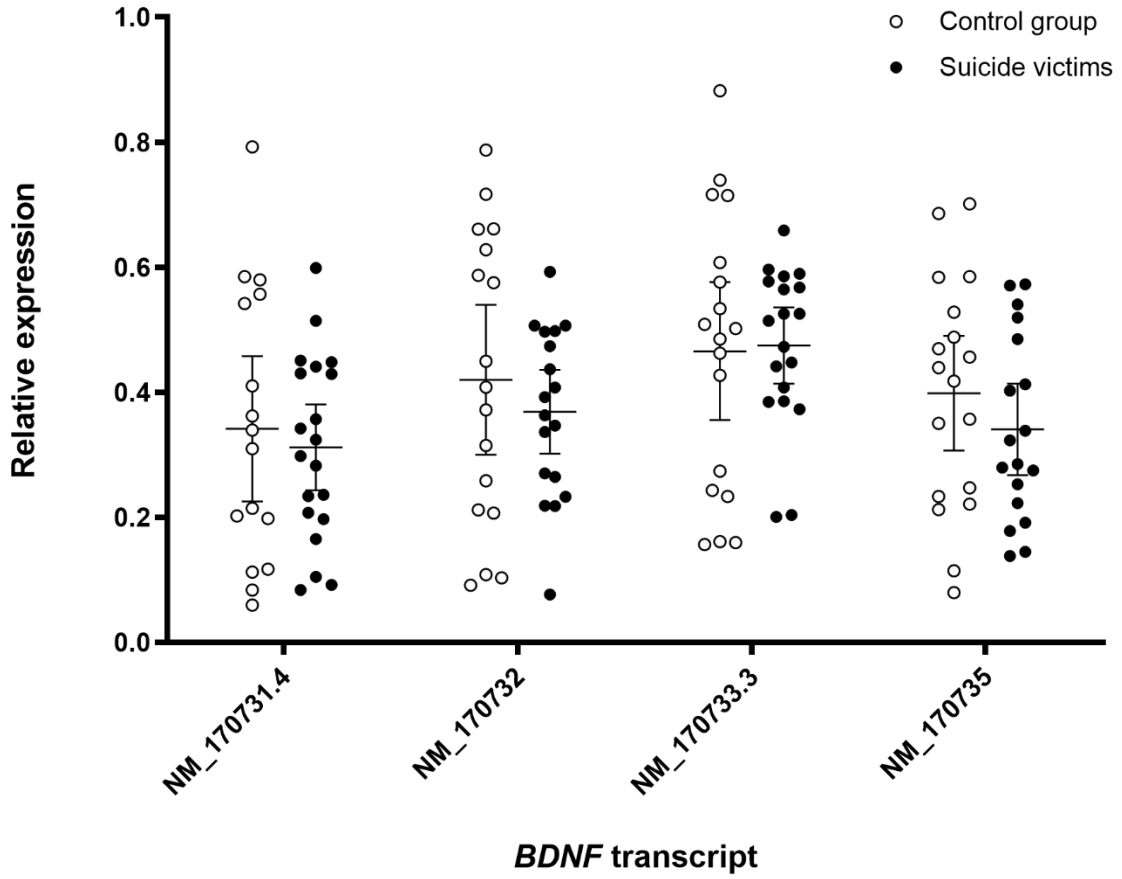
first exon (I), is divided into two parts, I1 and I2 due to technical reason of maximum amplicon length recommendation for the 454 GS Junior sequencing system (400 bp/region, including 454 GS Junior sequencing primers). CG#: Consecutive numbers of the CpG dinucleotides in each amplicon; red, single nucleotide polymorphisms; underlined, CpG island (or part of); Bold: Exon (or part of); Blue: Transcription factor binding sites; turquoise, CpGs already studied for association of methylation status with suicide or suicidal behavior [see references]; Yellow: Forward primer binding site (first round PCR); Green: Reverse primer binding site (first round PCR).



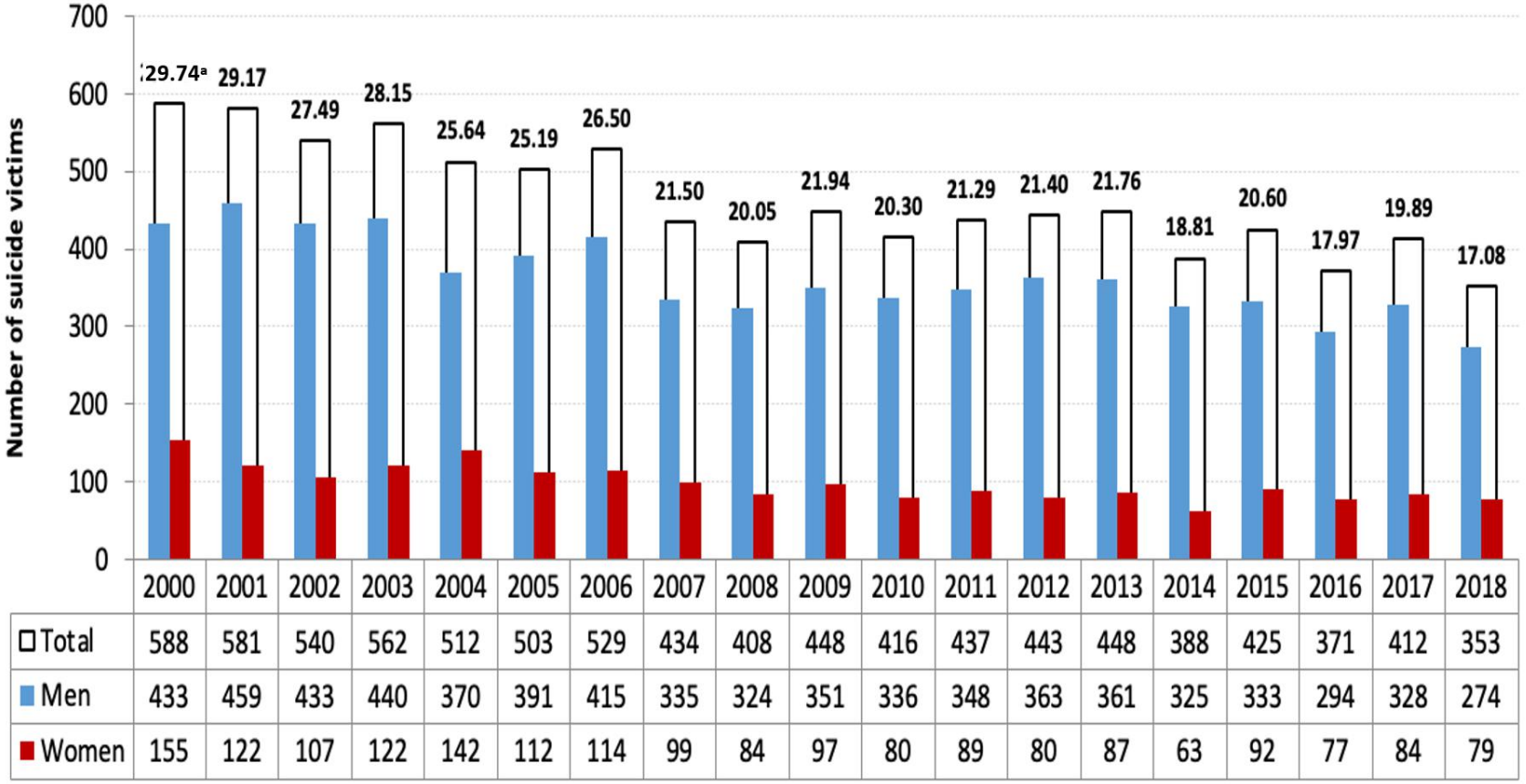
Supplementary Figure 3 Methylation levels across the five *BDNF* regions of interest in tissue from Brodmann area 9 of the brain of the control subjects and suicide victims. The studied regions are labelled with roman numerals (I, II, IV and VI) according to the vicinity of the exons 1, 2, 4 or 6. The region preceding the first exon (I), is divided into two parts, I1 and I2 due to technical reason of maximum amplicon length recommendation for the 454 GS Junior sequencing system (400 bp/region, including 454 GS Junior sequencing primers). Each circle symbol represents an individual study subject. Data are medians (horizontal bars) of the mean methylation levels across all the subjects shown for each *BDNF* regions ± 95% confidence interval; [a]*P* < 0.05 (two-tailed Student's t-tests for two independent samples), between groups.



Supplementary Figure 4 Methylation levels across the five *BDNF* regions of interest in hippocampal tissue of the control subjects and suicide victims. The studied regions are labelled with roman numerals (I, II, IV and VI) according to the vicinity of the exons 1, 2, 4 or 6. The region preceding the first exon (I), is divided into two parts, I1 and I2 due to technical reason of maximum amplicon length recommendation for the 454 GS Junior sequencing system (400 bp/region, including 454 GS Junior sequencing primers). Each circle symbol represents an individual study subject. Data are medians (horizontal bars) of the mean methylation levels across all the subjects shown for each *BDNF* regions ± 95% confidence interval; $^{a}P < 0.05$ (two-tailed Student's t-tests for two independent samples), between groups.



Supplementary Figure 5 Relative expression of alternative *BDNF* transcripts in hippocampal tissue for the control subjects and suicide victims. Data are medians (horizontal bars) of the mean methylation levels across all the subjects shown for each *BDNF* regions ± 95% confidence interval; $^{a}P < 0.05$ (two-tailed Student's t-tests for two independent samples), between groups.



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Total	588	581	540	562	512	503	529	434	408	448	416	437	443	448	388	425	371	412	353
Men	433	459	433	440	370	391	415	335	324	351	336	348	363	361	325	333	294	328	274
Women	155	122	107	122	142	112	114	99	84	97	80	89	80	87	63	92	77	84	79

Supplementary Figure 6 Numbers of suicide victims and annual suicide rates (above columns) from 2000 to 2018 in Slovenia. [a]Annual suicide rates per 100000 citizens in Slovenia. Data source: SI-NIJZ Data Portal.

Table 1 Template-specific parts (*i.e.*, *BDNF* regions of interest) for first round polymerase chain reaction fusion primers, and length of amplified *BDNF* region of interest

BDNF region	First round PCR fusion primers		Length of *BDNF* region of interest (bp)
	Forward (5' → 3')	Reverse (5' → 3')	
I1	agtttttagagaaagtgaggagttttag	ccttttttacccaaaaaatcacaata	340
I2	gatttttgggtaaaaaaaggaaattt	aaaaccataacccattaaaacaaac	338
II	tagattttggagataaatatttgtattt	aaatccattcaacaccttaaa	328
IV	ttttgtagtaaagaagttaaattattga	aaactcttctaataaaaaatatatcatttt	323
VI	ggggagaaaatttttttaagagta	aactttaataaaacacccacc	311

The studied regions are labelled with roman numerals (I, II, IV and VI) according to the vicinity of the exons 1, 2, 4 or 6. The region preceding the first exon (I), is divided into two parts, I1 and I2 due to technical reason of maximum amplicon length recommendation for the 454 GS Junior sequencing system (400 bp/region, including 454 GS Junior sequencing primers). PCR: Polymerase chain reaction.

Supplementary Table 2 Reaction mixtures for the first and second rounds of polymerase chain reaction

Reagent	Supplier	Stock concentration	Volume *per* sample (μL)	Final concentration
First round PCR (final volume, 25 μL)				
ddH_2O	Life Technologies, United States	-	17.5	-
$MgCl_2$	Roche, Germany	18 mM	2.5	1.8 mM
Dimethylsulfoxide	Roche, Germany	-	1.25	5% (v/v)
Polymerase[1]	Roche, Germany	-	0.25	-
dNTP mix	Applied Biosystems, United States	10 mM	0.5	0.2 mM
Forward primer	Sigma, Germany	20 μM	0.5	0.4 μM
Reverse primer	Sigma, Germany	20 μM	0.5	0.4 μM
Sample	(bisulfite converted DNA)	-	2	20-40 ng
Second round PCR (final volume, 30 μL)				
ddH_2O	Life Technologies, United States	-	25.5	-
$MgCl_2$	Roche, Germany	18 mM	1.5	0.9 mM
Dimethylsulfoxide	Roche, Germany	-	0.75	2.5% (v/v)
Polymerase[1]	Roche, Germany	-	0.15	-
dNTP mix	Applied Biosystems, United States	10 mM	0.30	0.1 mM
Forward primer	Eurogentec, Belgium	20 μM	0.4	0.27 μM
Reverse primer	Eurogentec, Belgium	20 μM	0.4	0.27 μM
Sample (template)	100× diluted amplicon	-	1	-

[1]Polymerase: Fast Start High Fidelity. PCR: Polymerase chain reaction.

Table 3 Cycling conditions of the first and second rounds of polymerase chain reaction

Step	Temperature (°C)	Time	Number of cycles
First round PCR (thermocycler: SimpliAmp; Applied Biosystems, United States)			
Initial denaturation	94	5 min	1
Denaturation	94	45 s	1
Annealing	I1: 63	45 s	I1: 40
	I2: 60	45 s	I2: 37
	II: 59	45 s	II: 40
	IV: 58	45 s	IV: 37
	VI: 60	45 s	VI: 37
Extension	72	45 s	1
Final extension	72	7 min	1
Cooling	4	∞	1
Second round PCR (thermocycler: ABI 9600 (Applied Biosystems, United States)			
Initial denaturation	94	5 min	1
Denaturation	94	45 s	1
Annealing (all)	69	45 s	29
Extension	72	45 s	1
Final extension	72	7 min	1
Cooling	4	∞	1

PCR: Polymerase chain reaction.

Supplementary Table 4 Details of the primer sequences used for the studied transcripts

Transcript	NM (Refseq)	Primer	Sequence (5′→3′)	Exon-exon junction spanning	Efficiency	Product length (bp)
BECN1 (RG)	NM_003766.4[1]	Fw	TTCAAGATCCTGGACCGTGTCAC C	No	1.84	61
		Rev	GGGCTGTGGTAAGTAATGGAGC TG	Yes		
DCTN2 (RG)	NM_006400.4[1]	Fw	AGTTCTCTCAAGCTGCCAAAGTC G	Yes	1.94	65
		Rev	TACAGCTGTCTCCAGCTCTGTC	No		
BDNF I-IX	NM_170731.4[2]	Fw	TGTTGGGGAGACGAGATTTT	No	2.13	90
		Rev	AAGGATGGTCATCACTCTTCTCA	No		
BDNF IIc-IX	NM_170732.4[1]	Fw	TCCGGGTTGGTATACTGGGT	No	2.07	97
		Rev	CTGGTGGAACTTCTTTGCGG	Yes		
BDNF IV-IX	NM_170733.3[2]	Fw	GCTGCCTTGATGGTTACTTTG	No	1.98	73
		Rev	AAGGATGGTCATCACTCTTCTCA	No		
BDNF IXabcd	NM_170735[1]	Fw	GTCTGGTGCAGCTGGAGTTT	No	2.04	106
		Rev	TTTCTTTCACCGGGATGCCA	No		

[1]Primers were designed using the on-line tool: Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/); [2]Predesigned primers were used, available on https://lifescience.roche.com/global_en/brands/universal-probe-library.html#assay-design-centre. RG: Reference gene; Fw: Forward; Rev: Reverse.

Supplementary Table 5 Diagnostic and toxicology data for the control subjects and suicide victims

Subject group	Group ID#	Age	Diagnosis	Drug prescription	Toxicology					
					Blood			Urine		
					Ethanol	Psychoactive substances	Other	Ethanol	Psychoactive substances	Other
Controls	1	49	Schizophrenia	N/A	Yes	Biperiden, carbamazepine, levomepromazine, clopenthixol		Yes	Levomepromazine, amisulpride, clopenthixol	
	2	51	None	N/A		Sertraline			Sertraline	
	3	60	None	N/A	Yes			Yes	THC	
	4	54	None	N/A						Acetaminophen
	5	60	None	N/A						Perindopril
	6	60	None	N/A	Yes			Yes		
	7	50	None	N/A	Yes			Yes		
	8	63	None	N/A	Yes			Yes		
	9	63	None	N/A	Yes			Yes		

	10	63	None	N/A	Yes		Yes	
	11	45	None	N/A				
	12	50	None	N/A				
	13	33	None	N/A				
	14	59	None	N/A				
	15	64	None	N/A				
	16	47	None	N/A				
	17	57	None	N/A				
	18	55	None	N/A				
	19	57	None	N/A				
	20	52	None	N/A				
Suicide victims	1	46	Schizophrenia, previous suicide attempt	Antipsychotics				Mirtazapine, olanzapine
	2	51	Schizophrenia	Hypnotics, sedatives, antipsychotics	Yes	Tradozone, medazepam, diazepam	Yes	Tradozone, diazepam, nordazepam
	3	59	Major depression	Hypnotics,		Mirtazapin		Mirtazapin

		disorder	sedatives, antipsychotics				
4	29	Bipolar disorder			Olanzapine	Yes	Olanzapine
5	60	Adjustment disorder, symptoms of anxiety and depression	Antipsychotics, antidepressives		Mirtazapine, olanzapine, citalopram		Mirtazapine, olanzapine, citalopram
6	58	Alcohol dependence		Yes		Yes	
7	48	None			Bupropion		Bupropion
8	54	Tumors (brain, liver, kidney)					
9	35	None		Yes		Yes	
10	32	None		Yes		Yes	
11	39	None		Yes		Yes	
12	32	None		Yes		Yes	
13	46	None		Yes		Yes	
14	17	None		Yes		Yes	
15	54	None		Yes		Yes	
16	50	None		Yes		Yes	

17	58	None	Yes	Yes	
18	30	None	Yes	Yes	
19	38	None			Creatinine
20	33	None			Creatinine
21	60	None			
22	39	None			

N/A: Data not available

Supplementary Table 6 Number of subjects in each group (n; totals: 20 controls, 22 suicide victims) with a sufficient number of reads *per* amplicon[1], and mean bisulfite conversion rates (m.c.r.)

Tissue	Study	*BDNF* regions									
	group	I1		I2		II		IV		VI	
		n (%)	m.c.r. (%)	*n* (%)	m.c.r. (%)	*n* (%)	m.c.r. (%)	*n* (%)	m.c.r. (%)	*n* (%)	m.c.r. (%)
BA9	Control	20	95.92	20	90.57	19	95.70	19	96.21	20	95.92
	Suicide victim	21	95.70	22	90.88	20	95.71	21	96.48	22	95.70
Hippocampus	Control	19	96.02	20	90.63	18	95.84	18	95.85	20	95.94
	Suicide victim	22	95.62	22	90.72	21	95.53	21	96.37	22	95.74
Venous blood	Control	20	95.83	20	89.97	20	95.54	20	96.67	20	95.61
	Suicide victim	22	95.88	22	89.73	22	95.37	22	96.64	22	95.34

[1]*BDNF* regions of study subjects were excluded from further analysis if the number of reads for a particular *BDNF* region was ≤ 20. The studied regions are labelled with roman numerals (I, II, IV and VI) according to the vicinity of the exons 1, 2, 4 or 6. The region preceding the first exon (I), is divided into two parts, I1 and I2 due to technical reason of maximum amplicon length recommendation for the 454 GS Junior sequencing system (400 bp/region, including 454 GS Junior sequencing primers).

Supplementary Table 7 Number of reads obtained per sequencing run

Read filtration step	**Number of reads**	
	1st run (brain tissue)	**2nd run (blood)**
After quality filtering; settings customized for bisulfite-converted DNA	72896	123493
Imported into sequence alignment program (BiQ HT Analyzer)	46922	76805
Exported for statistical analysis	40237[1]	69021

[1]BA9: 21225 reads, hippocampus: 19012 reads.

Supplementary Table 8 Methylation of the CpG dinucleotides in the five *BDNF* regions of interest in Brodmann area 9 of the controls and suicide victims

CpG #	Mean CpG methylation rate per *BDNF* region of interest (%)																	
	I1			I2			II			IV			VI					
	Control	Suicide victim	*P* value	Control	Suicide victim	*P* value	Control	Suicide victim	*P* value	Control	Suicide victim	*P* value	Control	Suicide victim	*P* value			
1	5.76 ± 2.44	5.81 ± 1.84	0.974	2.96 ± 1.77	3.28 ± 1.80	0.791	4.56 ± 1.25	5.27 ± 2.13	0.556	5.18 ± 1.26	4.81 ± 1.48	0.701	4.84 ± 1.57	3.84 ± 1.12	0.276			
2	5.19 ± 3.23	3.77 ± 1.78	0.420	4.37 ± 1.46	4.34 ± 1.49	0.979	10.91 ± 1.98	10.09 ± 2.44	0.591	3.63 ± 1.22	3.04 ± 0.97	0.423	5.09 ± 1.48	4.33 ± 1.36	0.438			
3	8.21 ± 3.05	5.79 ± 1.64	0.147	3.20 ± 0.95	2.86 ± 1.12	0.632	13.36 ± 3.08	12.37 ± 3.50	0.661	3.24 ± 1.10	2.71 ± 1.31	0.522	1.56 ± 0.82	2.42 ± 0.89	0.152			
4	2.67 ± 1.09	2.94 ± 1.47	0.762	5.89 ± 1.33	5.79 ± 1.50	0.922	8.01 ± 2.52	7.99 ± 1.69	0.986	3.48 ± 1.12	2.56 ± 0.76	0.157	3.78 ± 1.33	2.77 ± 1.12	0.230			
5	4.24 ± 1.55	1.67 ± 0.90	0.004	4.19 ±1.08	5.23 ± 1.58	0.275	6.02 ± 1.93	7.90 ± 2.60	0.236	3.02 ± 0.91	3.53 ± 1.16	0.482	2.86 ± 1.17	2.25 ± 0.97	0.405			
6	2.73 ± 1.27	2.57 ± 1.28	0.857	4.05 ± 1.24	3.87 ± 1.58	0.854	12.19 ± 3.18	10.78 ± 3.19	0.517	4.79 ± 1.18	4.28 ± 1.16	0.522	3.39 ± 1.15	3.50 ± 1.58	0.903			
7	4.38 ± 3.12	0.23	4.59 ± 4.90	0.72	3.31 ±0.97	4.56 ± 0.139	4.81 ± 5.23	0.68	4.37 ± 2.77	0.02								

	1.73	1.37	8	1.48	1.14	5		1.41		1.49	1.53	6	1.06	0.89	0
8	2.72	± 2.74	± 0.97	5.24	± 4.80	± 0.64	3.34	± 4.19	± 0.259	3.75	± 4.78	± 0.23	2.65	± 2.47	± 0.75
	1.38	1.23	6	1.56	1.17	0	0.97	1.22		1.22	1.28	0	0.90	0.84	1
9	3.68	± 4.90	± 0.42	6.58	± 6.72	± 0.90	8.60	± 10.53	± 0.257	3.37	± 4.24	± 0.33	3.42	± 2.65	± 0.33
	1.92	2.50	9	1.67	1.74	0	2.00	2.85		1.44	1.20	4	1.31	1.02	1
10	8.07	± 6.88	± 0.44	6.98	± 5.62	± 0.20	14.24	± 11.66	± 0.199	2.96	± 2.67	± 0.67	2.09	± 2.60	± 0.47
	2.22	2.37	6	1.70	1.40	2	3.42	2.39		1.23	0.80	7	1.11	0.97	4
11	4.62	± 4.92	± 0.83	3.52	± 3.18	± 0.63	7.03	± 9.63	± 0.103	2.87	± 4.67	± 0.05	3.48	± 3.11	± 0.60
	2.00	2.11	0	1.01	1.11	4	1.91	2.61		1.18	1.48	7	1.11	1.00	3
12	7.08	± 5.42	± 0.31	3.69	± 2.29	± 0.08	12.59	± 14.04	± 0.528	2.79	± 4.15	± 0.06	4.70	± 3.54	± 0.24
	2.18	2.59	4	1.57	0.66	3	3.10	3.59		0.94	1.12	1	1.43	1.47	9
13	4.43	± 2.87	± 0.03	3.37	± 3.66	± 0.71	8.44	± 7.36	± 0.552	2.25	± 3.18	± 0.24	2.98	± 2.39	± 0.39
	0.94	1.14	5	1.23	1.08	7	2.82	2.50		1.11	1.18	0	1.13	0.90	5
14	5.11	± 4.96	± 0.94	2.23	± 2.29	± 0.90	5.22	± 5.74	± 0.604	3.47	± 4.26	± 0.42	3.25	± 3.40	± 0.84
	3.67	2.29	2	0.90	0.63	0	1.47	1.45		1.60	1.36	8	1.13	1.10	5
15	1.51	± 1.15	±- 0.52							3.80	± 4.26	± 0.47	4.55	± 3.19	± 0.13
	1.03	0.65	9							1.06	0.82	2	1.69	0.90	6
16	4.60	± 3.83	± 0.54							2.64	± 3.36	± 0.24	2.45	± 2.17	± 0.71
	1.54	2.13	5							0.85	0.95	6	1.40	0.86	5
17	3.83	± 3.11	± 0.62							5.81	3.47	± 0.08	3.28	± 2.99	± 0.77

	2.74		1.42		6		±-2.51		1.35		6	1.68		1.30		1
18	2.95	±	3.91	±	0.44		6.14	±	7.02	±	0.51	2.54	±	3.55	±	0.18
	1.34		2.19		6		1.26		2.38		3	0.89		1.24		6
19	5.16	±	3.82	±	0.38							3.06	±	3.22	±	0.84
	2.11		2.40		5							1.14		1.21		2
20	4.59	±	4.95	±	0.82							2.68	±	1.99	±	0.26
	2.92		1.76		8							1.04		0.76		9
21	3.22	±	6.71	±	0.01							3.07	±	3.43	±	0.69
	1.28		2.39		2							1.19		1.46		3
22												3.07	±	3.45	±	0.71
												1.39		1.62		7

Data are mean methylation rates of individual CpG ± 95% confidence interval. *P* value: Nominal p-value (two-tailed Student's t-tests for two independent samples). The studied regions are labelled with roman numerals (I, II, IV and VI) according to the vicinity of the exons 1, 2, 4 or 6. The region preceding the first exon (I), is divided into two parts, I1 and I2 due to technical reason of maximum amplicon length recommendation for the 454 GS Junior sequencing system (400 bp/region, including 454 GS Junior sequencing primers).

Supplementary Table 9 Methylation of the CpG dinucleotides in the five studied *BDNF* regions of interest in the hippocampus of the controls and suicide victims

CpG #	Mean CpG methylation rate per *BDNF* region of interest (%)														
	I1			I2			II			IV			VI		
	Control	Suicide victim	*P* value	Control	Suicide victim	*P* value	Control	Suicide victim	*P* value	Control	Suicide victim	*P* value	Control	Suicide victim	*P* value
1	4.90 ± 1.83	6.53 ± 2.68	0.315	3.40 ± 1.61	3.23 ± 1.40	0.866	4.54 ± 2.25	6.44 ± 3.95	0.409	5.01 ± 1.28	4.54 ± 1.54	0.634	3.61 ± 1.62	4.40 ± 1.41	0.449
2	5.40 ± 1.74	5.44 ± 2.09	0.976	3.20 ± 1.38	4.66 ± 1.21	0.104	14.25 ± 3.90	10.97 ± 2.12	0.116	2.63 ± 1.14	2.02 ± 0.87	0.369	4.19 ± 1.48	4.37 ± 1.28	0.841
3	7.09 ± 2.28	5.41 ± 1.97	0.250	2.92 ± 0.85	2.94 ± 0.83	0.979	12.21 ± 3.27	16.04 ± 3.80	0.125	4.01 ± 1.53	3.10 ± 1.27	0.343	2.86 ± 1.17	2.15 ± 0.85	0.309
4	6.03 ± 2.24	3.91 ± 1.77	0.124	6.57 ± 2.17	5.58 ± 1.43	0.419	10.87 ± 2.14	11.78 ± 2.47	0.572	3.56 ± 1.36	3.85 ± 0.97	0.711	2.99 ± 1.45	2.26 ± 1.07	0.395
5	5.01 ± 2.48	2.59 ± 1.13	0.059	4.83 ± 1.43	5.56 ± 1.19	0.415	9.07 ± 2.79	9.00 ± 2.26	0.968	3.62 ± 1.36	3.41 ± 1.03	0.800	2.46 ± 1.26	3.86 ± 1.63	0.172
6	5.84 ± 4.52	2.14 ± 1.31	0.087	4.80 ± 1.28	5.15 ± 1.86	0.748	13.73 ± 2.77	14.61 ± 3.54	0.692	3.56 ± 1.23	3.37 ± 1.10	0.807	3.82 ± 1.82	4.03 ± 1.12	0.830
7	3.23 ±	3.71 ±	0.620	3.02 ±	4.07 ±	0.194	3.46 ±	4.07 ±	0.590	4.06 ±	5.00 ±	0.411	3.60 ±	3.59 ±	0.993

	1.60		1.24			1.18		1.18			1.41		1.84		3	1.86		1.53			1.10		1.34	
8	5.06	±	3.81	±	0.327	6.14	±	5.29	±	0.476	4.18	±	5.74	±	0.33	4.53	±	3.52	±	0.294	2.86	±	3.50	±	0.410
	2.23		1.51			2.23		1.24			1.83		2.67		5	1.83		0.98			1.09		1.17	
9	4.48	±	2.58	±	0.269	6.48	±	7.87	±	0.269	11.05	±	10.04	±	0.59	3.60	±	3.30	±	0.725	2.57	±	4.66	±	0.053
	3.64		1.07			1.61		2.00			2.86		2.65		2	1.09		1.33			1.28		1.73	
10	5.33	±	7.10	±	0.272	4.79	±	6.00	±	0.241	16.73	±	14.66	±	0.30	3.05	±	2.12	±	0.193	4.25	±	2.37	±	0.052
	1.93		2.58			1.37		1.59			3.34		2.65		9	1.29		0.78			1.53		1.24	
11	6.29	±	5.39	±	0.631	2.88	±	3.68	±	0.269	9.47	±	8.40	±	0.58	2.79	±	3.72	±	0.294	3.26	±	4.27	±	0.310
	3.09		2.47			1.00		1.07			3.12		2.67		5	1.50		1.11			1.63		1.28	
12	6.64	±	5.00	±	0.245	2.57	±	2.61	±	0.946	17.07	±	16.78	±	0.88	2.61	±	3.04	±	0.498	4.23	±	2.72	±	0.130
	2.34		1.81			0.99		0.86			3.46		2.70		9	0.88		0.98			1.67		1.21	
13	3.75	±	3.40	±	0.737	3.47	±	3.87	±	0.668	8.23	±	8.18	±	0.96	4.29	±	2.91	±	0.156	4.25	±	3.82	±	0.709
	1.80		1.24			1.25		1.44			1.94		1.32		4	1.79		1.04			1.56		1.79	
14	4.94	±	5.37	±	0.749	1.97	±	2.65	±	0.328	6.07	±	5.73	±	0.77	3.21	±	3.21	±	0.992	4.89	±	2.79	±	0.031
	1.93		1.97			0.97		1.03			1.97		1.57		2	1.23		1.03			1.81		0.90	
15	2.07	±	4.30	±	0.218											4.46	±	4.57	±	0.892	4.66	±	4.50	±	0.905
	1.95		3.00													1.18		1.36			1.85		2.06	
16	4.36	±	3.92	±	0.741											2.77	±	2.88	±	0.884	3.54	±	3.16	±	0.671
	2.06		1.85													1.21		0.99			1.38		1.25	
17	2.42	±	3.75	±	0.335											3.92	±	3.69	±	0.756	2.62	±	3.10	±	0.496

	1.19		2.42			1.25		0.96			0.96		1.07	
18	2.52	±	3.17	±	0.635	5.32	±	7.14	±	0.185	2.17	±	2.87	± 0.345
	1.23		2.41			1.55		2.25			1.12		1.04	
19	4.05	±	2.68	±	0.263						3.44	±	5.11	± 0.045
	2.37		1.18								1.08		1.27	
20	5.69	±	5.15	±	0.775						2.00	±	3.30	± 0.198
	3.39		2.23								1.20		1.65	
21	3.86	±	5.88	±	0.073						2.84	±	2.09	± 0.364
	1.33		1.79								1.33		1.06	
22											3.70	±	2.96	± 0.413
											1.66		0.96	

Data are methylation rates of individual CpGs ± 95% confidence interval. *P* value: nominal p-value (two-tailed Student's t-tests for two independent samples). The studied regions are labelled with roman numerals (I, II, IV and VI) according to the vicinity of the exons 1, 2, 4 or 6. The region preceding the first exon (I), is divided into two parts, I1 and I2 due to technical reason of maximum amplicon length recommendation for the 454 GS Junior sequencing system (400 bp/region, including 454 GS Junior sequencing primers).

Supplementary Table 10 Methylation of the CpG dinucleotides in the five *BDNF* regions of interest in venous blood cells of the controls and suicide victims

CpG#	Mean CpG methylation rate per *BDNF* region of interest (%)														
	I1			I2			II			IV			VI		
	C	SV	*P* value	C	SV	*P* value	C	SV	*P* value	C	SV	*P* value	C	SV	*P* value
1	12.66 ± 3.00	11.79 ± 2.73	0.655	3.75 ± 0.79	3.86 ± 0.67	0.835	11.79 ± 4.13	12.89 ± 3.50	0.672	7.64 ± 0.98	6.85 ± 0.85	0.204	5.42 ± 1.46	5.77 ± 0.90	0.665
2	11.58 ± 1.56	10.88 ± 2.77	0.662	7.75 ± 0.96	5.93 ± 0.81	0.004[a]	23.28 ± 2.63	22.93 ± 2.08	0.826	4.15 ± 0.85	4.27 ± 0.83	0.840	9.42 ± 2.56	7.91 ± 1.32	0.266
3	13.65 ± 2.89	11.24 ± 2.35	0.180	4.20 ± 0.65	3.60 ± 0.72	0.211	29.91 ± 3.99	25.68 ± 3.00	0.081	4.42 ± 1.24	3.78 ± 0.85	0.368	6.17 ± 1.44	6.32 ± 0.74	0.838
4	6.41 ± 1.51	8.19 ± 1.92	0.142	7.95 ± 0.89	6.77 ± 1.35	0.144	20.36 ± 2.88	18.26 ± 3.39	0.336	3.90 ± 1.12	4.90 ± 0.81	0.135	2.98 ± 0.76	3.58 ± 0.91	0.296
5	4.92 ±1.47	5.09 ±1.44	0.863	9.32 ±1.14	7.43 ±1.14	0.019	12.82 ±2.15	11.74 ±1.77	0.422	5.34 ±0.80	4.25 ±0.80	0.051	4.13 ±1.25	3.34 ±0.84	0.271
6	4.78 ± 2.01	3.86 ±	0.390	10.38 ±	7.08 ±	0.0007	19.37 ±	16.23 ±	0.115	6.88 ±	5.03	0.018	3.23 ±	3.36 ±	0.776

		1.06		1.59	1.06	[a]	3.28	2.47		1.31	± 0.92		0.74	0.57					
7	5.43 ± 1.47	6.94 ± 2.18	0.248	6.25 ± 1.11	6.07 ± 1.06	0.805	9.81 ± 2.00	8.53 ± 2.19	0.379	6.50 ± 1.03	6.09 ± 1.32	0.611	4.61 ± 0.89	4.60 ± 0.98	0.978				
8	8.37 ± 1.82	6.94 ± 1.58	0.223	9.19 ± 1.70	7.39 ± 1.14	0.068	8.89 ± 2.29	7.71 ± 1.97	0.419	6.40 ± 1.34	5.54 ± 0.93	0.269	3.69 ± 0.82	2.99 ± 0.74	0.193				
9	5.22 ± 2.07	4.37 ±1.35	0.468	7.72 ± 1.69	7.44 ± 0.87	0.748	13.98 ± 1.64	13.01 ± 2.21	0.475	5.26 ± 0.88	5.48 ± 1.25	0.775	4.75 ± 1.54	3.61 ± 0.76	0.161				
10	11.58 ± 1.98	9.70 ± 1.54	0.121	5.58 ± 0.84	6.13 ± 1.24	0.456	16.46 ± 3.43	15.75 ± 2.44	0.723	3.61 ± 0.62	3.70 ± 0.86	0.863	4.21 ± 1.09	3.52 ± 0.75	0.276				
11	6.50 ± 2.13	5.62 ± 1.57	0.486	6.16 ± 0.89	4.43 ± 0.85	0.006[a]	14.12 ± 3.07	11.17 ± 2.20	0.105	3.50 ± 1.01	3.47 ± 0.77	0.961	5.86 ± 1.26	4.34 ± 0.93	0.047				
12	7.88 ± 2.04	6.12 ± 1.16	0.117	5.29 ± 0.58	3.64 ± 0.64	0.0003 [a]	21.29 ± 2.39	19.43 ± 2.56	0.278	4.32 ± 1.05	5.01 ± 0.85	0.292	7.64 ± 1.68	6.20 ± 1.10	0.135				

13	7.98 ± 2.62	7.11 ± 1.53	0.541	5.86 ± 0.97	5.02 ± 0.91	0.196	12.39 ± 2.35	9.26 ± 1.75	0.029	3.34 ± 0.70	3.60 ± 0.98	0.654	6.34 ± 1.96	5.35 ± 1.02	0.341
14	10.49 ± 2.37	10.10 ± 3.39	0.848	5.29 ± 0.94	4.22 ± 0.78	0.074	8.14 ± 1.63	6.24 ± 1.24	0.057	6.89 ± 1.25	7.63 ± 1.15	0.366	8.75 ± 2.55	6.83 ± 1.15	0.146
15	3.96 ± 1.50	2.29 ± 0.62	0.032							10.06 ± 1.56	8.76 ± 1.15	0.165	5.99 ± 1.14	5.71 ± 1.18	0.724
16	6.82 ± 1.74	5.36 ± 1.36	0.172							5.51 ± 1.36	5.46 ± 1.06	0.959	5.95 ± 1.17	5.62 ± 1.16	0.681
17	4.84 ± 1.59	3.92 ± 1.21	0.337							5.88 ± 1.57	6.08 ± 0.94	0.813	4.60 ± 1.24	4.31 ± 0.97	0.700
18	6.06 ± 1.39	4.87 ± 1.36	0.208							8.91 ± 1.52	9.61 ± 1.21	0.454	5.09 ± 1.22	4.77 ± 0.91	0.661
19	4.73 ± 2.02	5.59 ± 2.05	0.537										3.69 ± 1.07	4.50 ± 0.91	0.229

20	9.46 ± 2.13		8.19	±	0.384				5.53	±	4.01	±	0.038
			2.12						1.26		0.84		
21	10.50	±	8.42	±	0.223				4.61	±	3.62	±	0.091
	3.03		1.90						0.94		0.75		
22									4.88	±	4.74	±	0.841
									1.04		0.97		

[a]*P*-value that remained statistically significant after correction for multiple comparisons ($P_{corr} < 0.05$; two-tailed Student's t-tests for two independent samples). Data are methylation rate of individual CpGs ± 95% confidence interval. *P* value: Nominal p-value. The studied regions are labelled with roman numerals (I, II, IV and VI) according to the vicinity of the exons 1, 2, 4 or 6. The region preceding the first exon (I), is divided into two parts, I1 and I2 due to technical reason of maximum amplicon length recommendation for the 454 GS Junior sequencing system (400 bp/region, including 454 GS Junior sequencing primers).

Supplementary Table 11 RNA quality data

Tissue	Group	Subjects (n)	Concentration (ng/μL)	$A_{260/280}$ (AU)	$A_{260/230}$ (AU)	RNA integrity number
Brodmann area 9	Controls	20	449.9 ± 179.1	1.85 ± 0.05	2.30 ± 0.15	6.7 ± 0.9
	Suicide victims	21	380.5 ± 196.9	1.86 ± 0.06	2.29 ± 0.14	6.5 ± 1.9
Hippocampus	Controls	20	545.8 ± 135.5	1.90 ± 0.03	2.22 ± 0.17	6.0 ± 1.1
	Suicide victims	21	501.5 ± 188.2	1.89 ± 0.03	2.26 ± 0.22	6.6 ± 1.4
Venous blood	Controls	19		1.77	1.79	2.4
	Suicide victims	20		1.84	1.92	2.5

Data are means ± standard deviation.

Supplementary Table 12 Mean quantification cycles (Cq) for each of the primer pairs used for gene expression study for Brodmann area 9, hippocampus, and venous blood of the control subjects and suicide victims

Tissue	Group	Mean Cq					
		Reference genes		*BDNF* transcript			
		BECN1	*DCTN2*	I	IIc	IV	IXabcd
Brodmann area 9	Controls	25.7 ± 0.7	25.3 ± 0.7	30.5 ± 0.9	31.0 ± 1.0	29.1 ± 1.2	31.1 ± 0.9
	Suicide victims	26.3 ± 1.9	25.7 ± 1.7	29.7 ± 1.2	31.1 ± 1.9	29.3 ± 2.1	31.0 ± 1.2
	Combined	26.0 ± 1.5	25.5 ± 1.3	30.1 ± 1.1	31.1 ± 1.4	29.2 ± 1.7	31.1 ± 1.0
Hippocampus	Controls	26.5 ± 1.8	26.4 ± 2.3	28.7 ± 1.7	30.1 ± 1.1	28.3 ± 1.9	29.8 ± 1.4
	Suicide victims	26.3 ± 1.7	26.0 ± 1.8	28.5 ± 1.4	30.0 ± 1.1	28.0 ± 1.7	29.6 ± 1.1
	Combined	26.4 ± 1.7	26.2 ± 2.1	28.6 ± 1.5	30.0 ± 1.1	28.2 ± 1.8	29.7 ± 1.3
Venous blood	Controls	30.5 ± 1.0	32.3 ± 1.0	N/A	N/A	N/A	N/A
	Suicide victims	30.7 ± 0.8	32.3 ± 1.4	N/A	N/A	N/A	N/A
	Combined	30.6 ± 0.9	32. 3± 1.2	N/A	N/A	N/A	N/A

N/A: Not applicable, due to experimental failure.

References

1 **Fuchikami M,** Morinobu S, Segawa M, Okamoto Y, Yamawaki S, Ozaki N, Inoue T, Kusumi I, Koyama T, Tsuchiyama K, Terao T. DNA Methylation Profiles of the Brain-Derived Neurotrophic Factor (BDNF) Gene as a Potent Diagnostic Biomarker in Major Depression. *PLoS One* 2011; **6:** e23881 [PMID: 21912609 DOI: 10.1371/journal.pone.0023881]

2 **Ikegame T,** Bundo M, Sunaga F, Asai T, Nishimura F, Yoshikawa A, Kawamura Y, Hibino H, Tochigi M, Kakiuchi C, Sasaki T, Kato T, Kasai K, Iwamoto K. DNA methylation analysis of BDNF gene promoters in peripheral blood cells of schizophrenia patients. *Neurosci Res* 2013; **77:** 208–14 [PMID: 23973796 DOI: 10.1016/j.neures.2013.08.004]

3 **Keller S,** Sarchiapone M, Zarrilli F, Videtič A, Ferraro A, Carli V, Sacchetti S, Lembo F, Angiolillo A, Jovanovic N, Pisanti F, Tomaiuolo R, Monticelli A, Balazic J, Roy A, Marusic A, Cocozza S, Fusco A, Bruni CB, Castaldo G, Chiariotti L. Increased BDNF Promoter Methylation in the Wernicke Area of Suicide Subjects. *Arch Gen Psychiatry* 2010; **67:** 258 [PMID: 20194826 DOI: 10.1001/archgenpsychiatry.2010.9]

4 **Kang HJ,** Kim JM, Lee JY, Kim SY, Bae KY, Kim SW, Shin IS, Kim HR, Shin MG, Yoon JS. BDNF promoter methylation and suicidal behavior in depressive patients. *J Affect Disord* 2013; **151:** 679–85 [PMID: 23992681 DOI: 10.1016/j.jad.2013.08.001]