Data supplement for Punzi et al., Genetics and Brain Transcriptomics of Completed Suicide. Am J Psychiatry (doi: 10.1176/appi.ajp.2021.21030299)

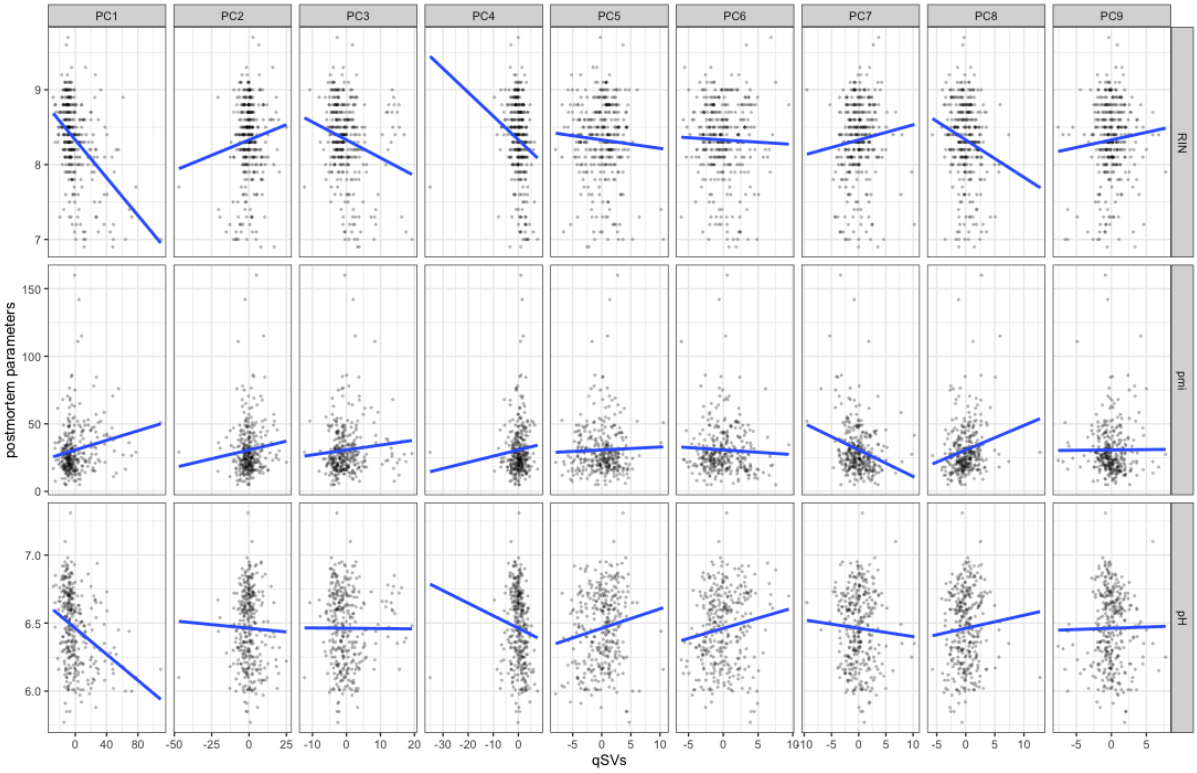
TABLE S1. Demographics

I. GRS sample (RNAseq sample)	**total**	**non-suicide unaffected**	**non-suicide patient**	**non-violent suicide patient**	**violent suicide patient**
total	**895(329)**	250(103)	369(99)	120(50)	156(77)
neurotypical	250(103)	250(103)	0	0	0
schizophrenia	149(77)	0	102(50)	20(11)	27(16)
major depression	311(106)	0	170(35)	53(24)	88(47)
bipolar disorder	185(43)	0	97(14)	47(15)	41(14)
Sex: F	292(106)	55(23)	133(39)	67(25)	37(19)
Age: Mean	46.29(43.09)	47.58(38.35)	47.88(48.58)	44.82(44.25)	41.56(41.62)
Age: SD	16.12(15.79)	19.71(17.75)	13.65(13.99)	14.44(13.52)	15.48(14.47)
II. RNAseq sample	**total**	**non-suicide unaffected**	**non-suicide patient**	**non-violent suicide patient**	**violent suicide patient**
total	**329**	103	99	50	77
known past suicide attempt	94	0	16	32	46
length of illness: Mean	14.10	0	25.44	17.54	16.14
length of illness: SD	14.42	0	13.45	12.11	10.98
lifetime antipsychotic	105	0	55	21	29
lifetime antidepressant	116	0	47	33	36
lifetime anticonvulsant	75	1	31	20	23
lifetime lithium	45	0	22	12	11
positive to alcohol	41	6	13	11	11
positive to anticholinergic	9	1	4	4	0
positive to antidepressant	87	0	39	29	19
positive to antihistaminic	35	6	10	12	7
positive to anti-inflammatory	33	1	12	11	9
positive to antipsychotic	59	0	34	17	8
positive to cannabis	7	0	3	1	3
positive to CNS depressant	36	1	14	16	5

positive to CNS stimulant	17	0	7	2	8
positive to hallucinogen	0	0	0	0	0
positive to lithium	39	1	15	14	9
positive to nicotine	85	24	20	19	22
positive to opioid	49	3	27	16	3
pH: Mean	6.46	6.55	6.43	6.31	6.49
pH: SD	0.28	0.29	0.24	0.21	0.29
pmi: Mean	30.67	26.06	31.91	29.15	36.23
pmi: SD	18.94	13.24	16.74	15.34	27.12
RIN: Mean	8.33	8.49	8.23	8.18	8.34
RIN: SD	0.52	0.46	0.6	0.54	0.43
III. RNAseq sample (Sex by Diagnosis)	**total**	**neurotypical**	**schizophrenia**	**major depression**	**bipolar disorder**
Sex: F	106	23	26	35	22
Sex: M	223	80	51	71	21

I. Total sample in the GRS study (N=895, before outliers' removal), a subset of which (N=329) is included in the RNAseq study. The numbers between brackets refer to the subsample in the RNAseq study. **II.** Additional information about the RNAseq sample on variables that may affect gene-expression (see additional sensitivity analysis). **III.** RNAseq sample with sex divided by diagnosis. Note that all donors were Caucasian, and all the analyses included sex and age among the covariates, and also qSVs in the gene-expression analysis (see also **Figure S1** and **Table S2** for relationship between postmortem parameters and qSVs). The large majority of neurotypicals died by natural death.

FIGURE S1. Relationship between qSVs and postmortem parameters



Scatterplots of the correlation between qSVs (y-axis) and RIN (top row), pmi (middle row) and pH (bottom row) (x-axis). Among the strongest relationships, qSV1 is highly correlated with RIN and pH; qSV7 is highly correlated with pmi. See **Table S2** for detailed statistics.

TABLE S2. Statistics of the relationship between qSVs and postmortem parameters

parameter	qSV	estimate	std.error	statistic	p.value	p.fdr
RIN	PC1	-0.013	0.001	-8.866	5.02E-17	1.35E-15
	PC2	0.008	0.004	1.897	0.059	0.132
	PC3	-0.024	0.006	-4.009	7.57E-05	0.0005
	PC4	-0.032	0.009	-3.584	0.0004	0.0021
	PC5	-0.011	0.010	-1.075	0.283	0.403
	PC6	-0.006	0.011	-0.523	0.601	0.706
	PC7	0.020	0.012	1.635	0.103	0.185
	PC8	-0.050	0.014	-3.484	0.001	0.002
	PC9	0.020	0.015	1.371	0.171	0.272
pmi	PC1	0.177	0.056	3.146	0.002	0.006
	PC2	0.260	0.156	1.666	0.097	0.185
	PC3	0.362	0.220	1.647	0.101	0.185
	PC4	0.458	0.326	1.406	0.161	0.271
	PC5	0.217	0.380	0.572	0.568	0.697
	PC6	-0.346	0.410	-0.844	0.399	0.513
	PC7	-1.962	0.433	-4.530	8.27E-06	7.44E-05
	PC8	1.812	0.517	3.504	0.0005	0.0022
	PC9	0.050	0.535	0.094	0.926	0.945
pH	PC1	-0.005	0.001	-6.069	3.56E-09	4.81E-08
	PC2	-0.001	0.002	-0.474	0.636	0.716
	PC3	0.000	0.003	-0.069	0.945	0.945
	PC4	-0.009	0.005	-1.954	0.052	0.127
	PC5	0.014	0.005	2.611	0.009	0.028
	PC6	0.015	0.006	2.524	0.012	0.033
	PC7	-0.006	0.006	-0.943	0.346	0.467
	PC8	0.010	0.008	1.250	0.212	0.318
	PC9	0.002	0.008	0.230	0.818	0.883

Additional Statistical Analyses

All data processing was implemented in the 'R' statistical language (Version 4.0.0)(1). In addition to the DEG analyses detailed above, we used the 'R' environment to calculate correlations between statistics of the DE of interest and between different GRSs using the function *Pearson* cor.test; to fit linear models (i.e. linear regressions) that compare GRSs between groups, using 10 ancestry-based principal components (accounting for population stratification), age and sex as covariates; to fit linear models that compare MEs between groups; to calculate; and to plot volcano, box plots and scatterplots (*ggplot2*). *Geneset* tests were performed using the function "geneSetTest" in the *limma* package(2). Grubbs' tests and 3x interquartile range method were used to identify outliers. To analyze the association of each GRS with case-control status, we used multiple logistic regressions (Diagnosis ~ GRS + covariates) adjusting for sex, age, and 10 ancestry-based principal components. To evaluate goodness of fit of the logistic models, we calculated the Nagelkerke R^2, by comparison of a full model (covariates + GRS) with a reduced model (covariates only). For each diagnostic group, we performed a first analysis including all the patients in one group, and further analyses on only non-suicide patients, only non-violent suicide patients, and only violent suicide patients.

Caption for Table S3 (separate excel file)

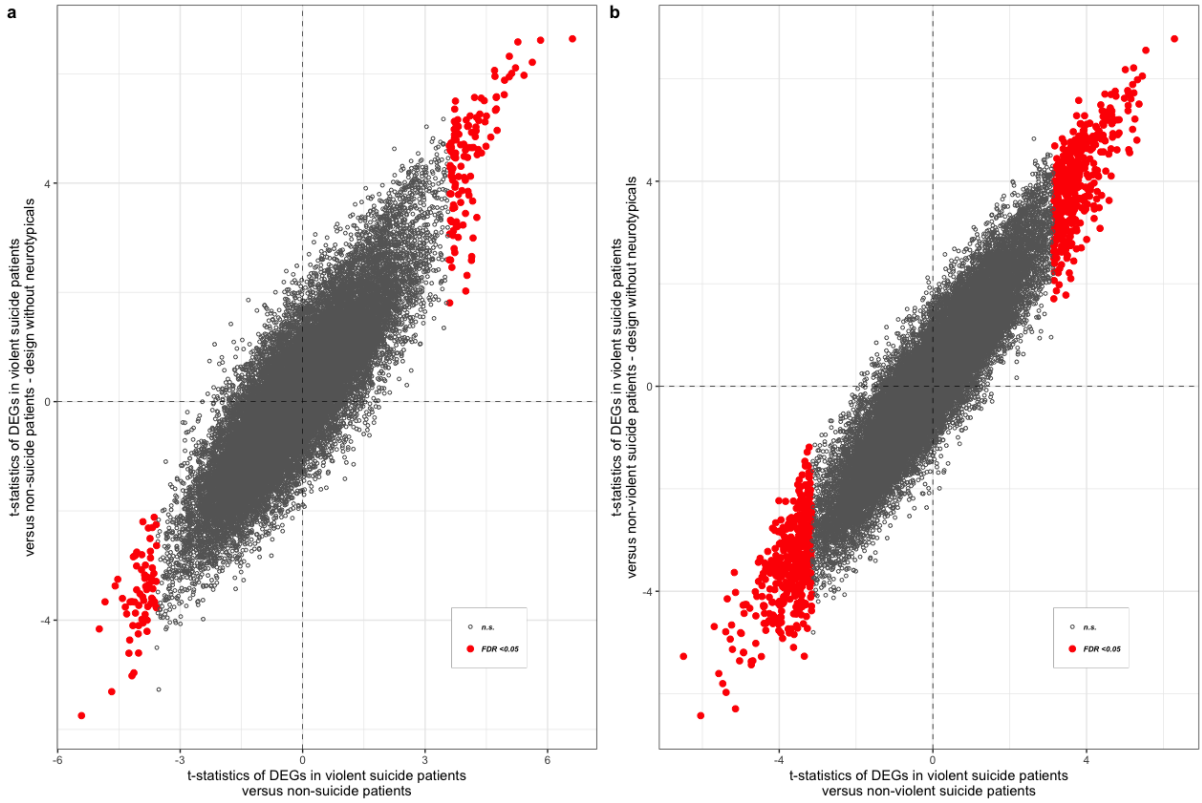
TABLE S3. DEGs and Gene Ontology results. Gene Ontology (GO) enrichment results on listed DEGs at FDR<0.05 from the following comparisons: DE of non-suicide patients compared with non-suicide controls (neurotypicals) (**Table S3a**), DE of non-violent suicide patients compared with non-suicide controls (neurotypicals) (**Table S3b**), DE of violent suicide patients compared with non-suicide controls (neurotypicals) (**Table S3c**), DE of non-violent suicide patients compared with non-suicide patients (**Table S3d**), DE of violent suicide patients compared with non-suicide patients (**Table S3e**), DE of violent suicide patients compared with non-violent suicide patients (**Table S3f**), DE of linear model design (**Table S3g**); DE statistics of top hits and enrichment of purinergic terms in each sensitivity analysis for the contrast of violent suicide patients compared with non-suicide patients, and compared with non-violent suicide patients (**Table S3h**); GO on aggression candidate genes (human ortholog) identified in GWAS of aggressive behavior in flies (**Table S3i**).

Sensitivity analysis on DE adjusting also for diagnosis

Since the three diagnoses were differently distributed within groups of patients, we performed a sensitivity analysis in patients only, using non-suicide patients as the baseline condition and covarying also by diagnosis. The analysis was necessary as the design of the main model (including neurotypicals) does not allow to covary by diagnosis because of collinearity between diagnosis and manner of death.

In this sensitivity analysis, only 17 genes were DE in non-violent suicide patients compared to non-suicide patients, suggesting that part of the signal in the full model may be driven by diagnosis rather than the suicidal phenotype. Further, the expression differences in violent suicide patients compared to non-suicide patients were not lessened by the sensitivity analysis, indeed in the model covarying for diagnosis there was a total of 549 DEGs (124 down, 425 up) and the results from the two models were highly correlated (**Figure S1a**). Finally, the expression differences in violent suicide patients compared to non-violent suicide patients increased to 1466 DEGs, with consistent directionality, confirming more than 83% of DEGs (**Figure S1b**).

FIGURE S2. DE sensitivity analysis, covarying by diagnosis



Scatterplots of the correlation between the t-statistics of DEGs in **a.** violent suicide patients compared with non-suicide patients in the main model (x-axis) and in the model excluding neurotypicals (y-axis); and **b.** violent suicide patients compared with non-violent suicide patients in the main model (x-axis) and in the model excluding neurotypicals (y-axis). The t-statistics were obtained from the DE analysis using a linear model adjusting for sex, age, and quality

surrogate variables accounting for RNA quality; and, in the sensitivity analysis, adjusting also for diagnosis.

Caption for Table S4 (separate excel file)

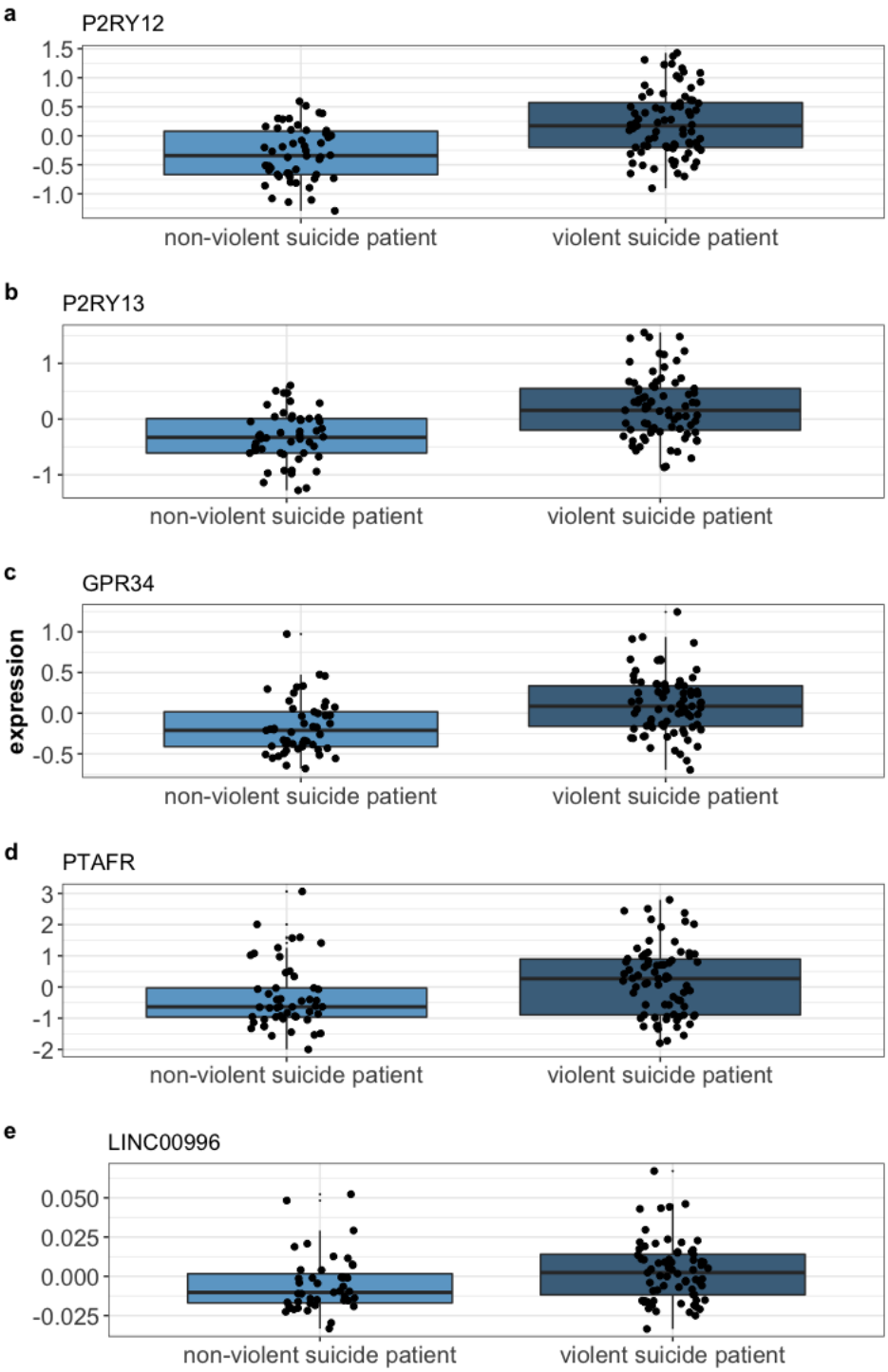
TABLE S4. DE Statistics for each contrast: DE of non-suicide patients compared with non-suicide controls (neurotypicals) (**Table S4a**), DE of non-violent suicide patients compared with non-suicide controls (neurotypicals) (**Table S4b**), DE of violent suicide patients compared with non-suicide controls (neurotypicals) (**Table S4c**), DE of non-violent suicide patients compared with non-suicide patients (**Table S4d**), DE of violent suicide patients compared with non-suicide patients (**Table S4e**), DE of violent suicide patients compared with non-violent suicide patients (**Table S4f**)**.**

Technical validation of the DE of purinergic genes and LINC00996

We performed qPCR to validate the DE of the most relevant DEGs. Total RNA from postmortem DLPFC tissue was extracted from 30mg of pulverized tissue with the RNeasy Lipid Tissue Mini Kit (QIAGEN). The yield of total RNA was determined by Qubit RNA BR Assay Kit and Qubit Fluorometer (ThermoFisher Scientific). RNA quality was assessed by RNA integrity number (RIN) on an Agilent Bioanalyzer 2100 (Agilent Technologies). Complementary DNA (cDNA) was created from 1ug total RNA using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) in 20ul reaction. mRNA expression levels of P2RY12 (Hs00224470_m1), P2RY13 (Hs01090437_g1), GPR34 (Hs00271105_s1), PTAFR (Hs00982700_s1), LINC00996 (Hs01377121_m1) were measured in 127 postmortem DLPFC samples (50 non-violent suicide and 77 violent suicide) by quantitative real-time PCR (RT-PCR), using Roche LightCycler 480 II with 384-well format. Samples were quantified in triplicate. The quantitative analyses by qPCR were carried out by the delta-delta method(3). mRNA expression levels of the genes were normalized to geometric means of two constitutively-expressed genes: β-actin (ACTB) and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). The DE of each candidate was confirmed by qPCR (**Figure S2**), even for low-expressed genes such as *PTAFR* and *LINC00996*, for which qPCR is a less sensitive method of quantification, compared with RNA sequencing:

P2RY12: t=5.754, p=6.69e-08;
P2RY13: t=5.799, p=5.45e-08;
GPR34: t=4.409, p=2.28e-05;
PTAFR: t=2.616, p=0.0101;
LINC00996: t=2.296, p=0.02365.

FIGURE S3. qPCR



Technical validation of the DE of purinergic genes (a-d) and LINC00996 (e); box plot of gene expression (y-axis, $2^{-\Delta\Delta Ct}$) in violent suicide patients against non-violent suicide (x-axis).

Additional sensitivity analyses

We repeated the DE analysis in the design of only patients, additionally including length of illness among the covariates, to account for disease severity. To this purpose, we used various relevant information (age of onset of symptoms / received diagnosis, age of earliest contact with healthcare / first outpatient treatment / inpatient hospitalization) to obtain, for each sample, a maximum length of illness. Addition of this variable to the covariates yielded the same DEGs, including the purinergic receptors genes (**Table S3h**), with an overlap of 93.49% significant DEGs in the contrast of violent suicide against non-suicide and of 99.02% in violent suicide against non-violent suicide.

We also repeated the analysis after removing samples < 17-year-old (only one suicide was < 14-year-old), again with consistent results as shown in **Table S3h**.

Because suicide by violent means is more likely to involve physical damage, and because adenosine, a purine nucleoside, as well as related metabolites, may be released in the brain following traumatic injury(4), we performed a sensitivity analysis to exclude that the results were affected by different levels of brain trauma in violent suicide patients. Of the 329 brains with available microscopic neuropathology, 198 had possible signs of brain trauma reported, including vascular congestion, tissue edema, contusion, hematoma, brain tissue hemorrhage, and hemorrhage in the meningeal space (**Table S5**). None of these parameters significantly differ between the three groups of non-suicide (patients and neurotypicals), non-violent suicide patients and violent suicide patients, except for hemorrhage in the meningeal space, which was more represented in violent suicide patients (Fisher p=0.03, **Table S5**). Bleeding in this area may suggest indirectly some degree of trauma to the brain that is not evident otherwise. We thus repeated the analysis removing samples showing meningeal hemorrhage and obtained similar results (as shown in **Figure S4a-b, Table S3h**). Hence, based on the existing data, brain trauma possibly resulting from the violent action of suicide does not *in itself* account for the DEGs in suicide by violent method.

Additionally, we have re-run the main DE analysis by adding to the original set of covariates also the several substances that have been tested in our sample per toxicological screening, including the following classes: opioid, alcohol, CNS stimulant, CNS depressant, antihistamine, anticonvulsant and lithium, antidepressant, antipsychotic, anticholinergic, anti-inflammatory, nicotine, cannabinoid, hallucinogen. We once again obtained similar results (**Figure S5a-b, Table S3h**)

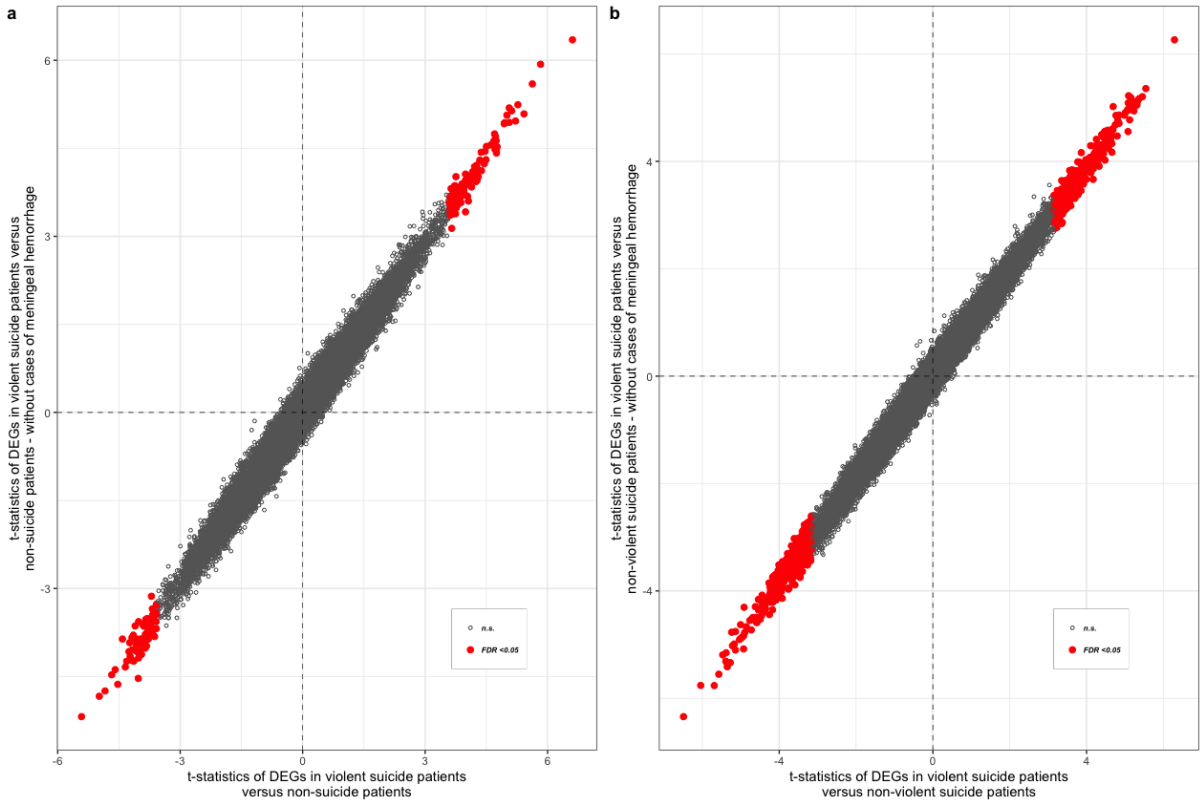
Finally, we observe that one of the top genes up-regulated in violent suicide patients is the target of a widely used drug, the antiaggregant clopidogrel, a P2RY12 antagonist(5). Data on recent treatment with clopidogrel was available only for a few donors, with possible false-negatives. We thus looked at any signs of cerebral vascular sclerosis, as reported in the microscopic pathology, as a potential (albeit limited) indicator of therapy with this agent, and we found that *P2RY12* brain expression is not affected by cerebral vascular sclerosis and so, by proxy, by antiaggregant medication (t=0.917, p=0.360192).

TABLE S5. Available microscopic neuropathology, potentially indicating trauma to the brain

		manner of death			
		nS	**nv-S**	**v-S**	
VASCULAR CONGESTION	no	9	2	2	
	yes	109	33	44	not sign.
TISSUE EDEMA	no	17	7	7	
	yes	101	28	39	not sign.
CONTUSION	no	117	35	46	
	yes	0	0	0	not sign.
HEMATOMA	no	117	35	46	
	yes	0	0	0	not sign.
INTRACEREBRAL HEMORRHAGE	no	114	34	44	
	yes	3	1	2	not sign.
MENINGEAL space HEMORRHAGE	no	113	35	40	
	yes	4	0	6	*Fisher P=0.03

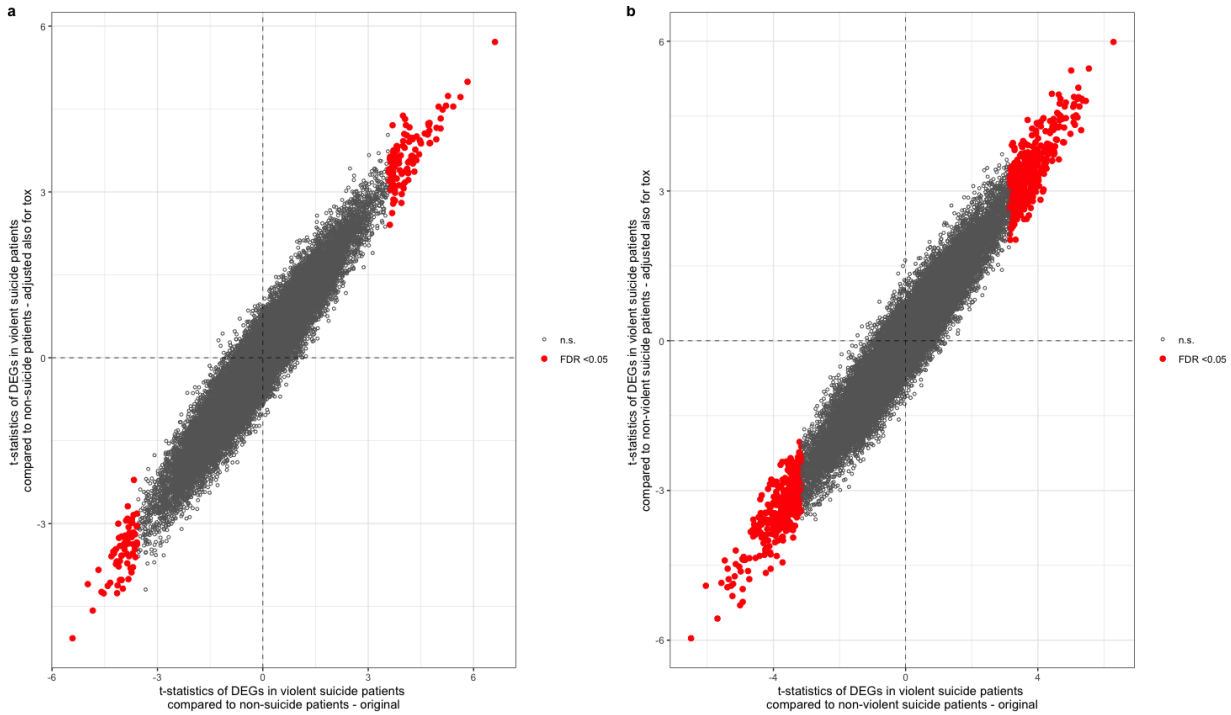
nS = non-suicide (controls and patients); nv-S = non-violent suicide patients; vS = violent suicide patients.

FIGURE S4. DE sensitivity analysis, removal of cases with meningeal hemorrhage



Scatterplots of the correlation between the t-statistics of DEGs in **a.** violent suicide patients compared with non-suicide patients in the whole sample (x-axis) and in the sample excluding meningeal hemorrhages (y-axis); and **b.** violent suicide patients compared with non-violent suicide patients in the whole sample (x-axis) and in the sample excluding meningeal hemorrhages (y-axis). The t-statistics were obtained from the DE analysis using a linear model adjusting for sex, age, and quality surrogate variables accounting for RNA quality.

FIGURE S5. DE sensitivity analysis, correction for medication and drugs exposure



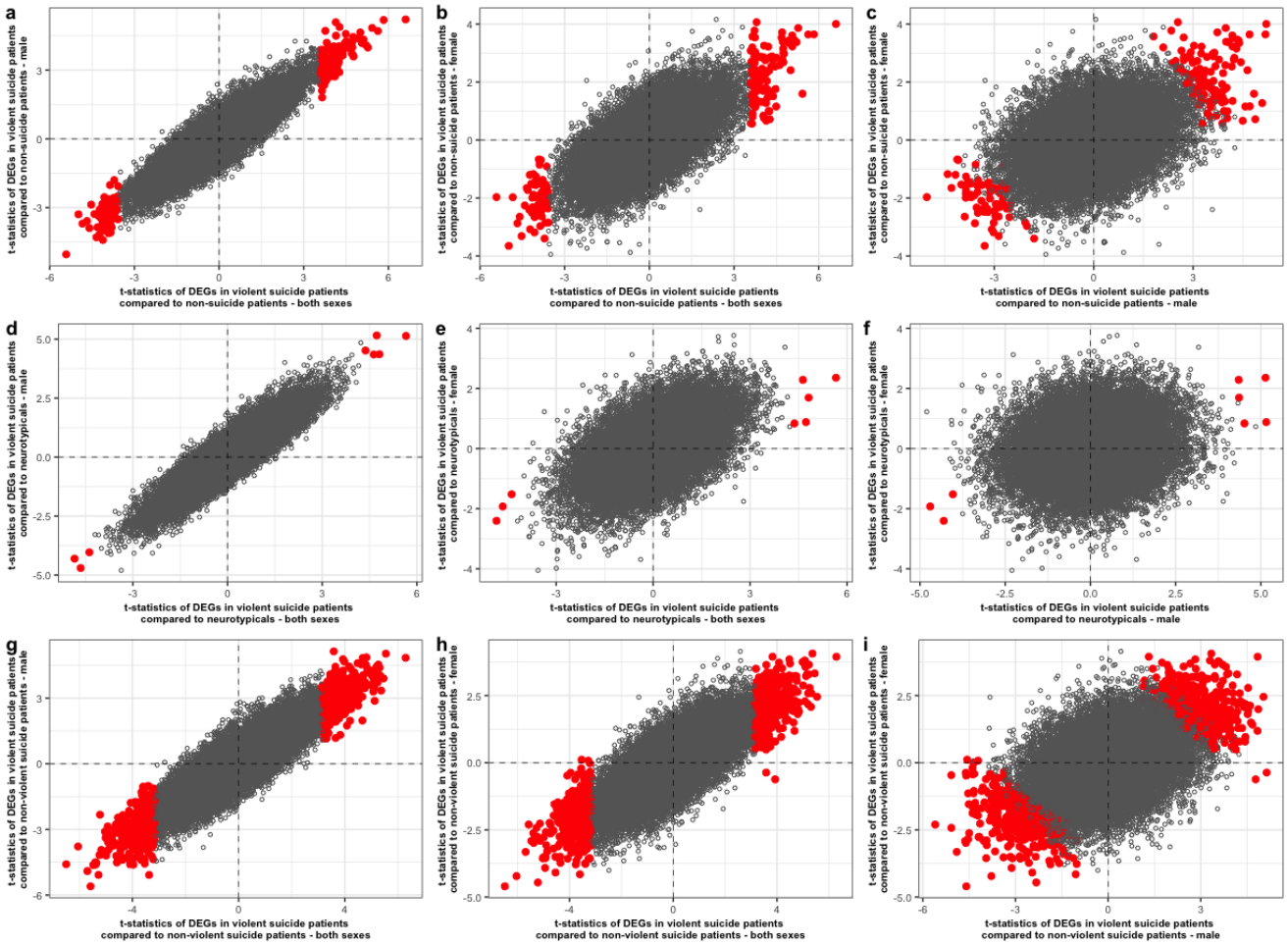
Scatterplots of the correlation between the t-statistics of DEGs in **a.** violent suicide patients compared with non-suicide patients in the original analysis (x-axis) and in the analysis correcting also for medications and drug exposure (y-axis); and **b.** violent suicide patients compared with non-violent suicide patients in the original analysis (x-axis) and in the analysis correcting also for medications and drug exposure (y-axis).

DE analysis by sex

We also repeated the main (on all groups) DE analysis separately by sex. DE analysis in the female group failed to produce significant DEGs, likely due to smaller sample size (106 females vs 223 males). However, comparison of violent suicide DEGs of each sex with the total sample (**Figure S6 a, d, g,** for males and **b, e, h** for females) and between sexes (**Figure S6 c, f, i**) shows that the results are highly correlated. Consistently, we failed to find any significant interaction of sex on DEGs.

Because our cohort is skewed towards males, we run 500 analyses in male random subsamples (N = 106, equal to the female N) to further explore whether the DE results are driven by the male sample; a higher N of DEGs in the male random subsamples would support that the result for the full cohort is driven by males. We obtained an average t-statistic for each gene in each contrast; by comparing the t-statistics in the DE on all samples, only females, and only randomized males, we detected a stronger correlation between results from males and all samples (r=0.892 in violent suicide against non-suicide, and r=0.874 in violent suicide against non-violent suicide) than between females and all samples (r=0.674 in violent suicide against non-suicide, and r=0.771 in violent suicide against non-violent suicide), suggesting again that the data for the full cohort may be driven by males. This is not surprising, given that the full cohort contains a higher N of males than females. Further, we calculated the average number of DEGs in each contrast on the male random subsamples: males have a higher number of significant DEGs compared with females (i.e., for violent suicide patients against non-suicide patients, an average of 4.888 DEGs at FDR < 0.05 [*vs* none in only females], 1659.112 DEGs at p-value < 0.05 [*vs* 1035 in only females]). However, we note that the purinergic signaling is still at the top of the list of nominally DEGs in the female subsample and that the t-statistics of the DE in randomized males and in females show some degree of correlation, especially in the comparison of violent suicide against non-violent suicide (r = 0.425, p-value < 2.2e-16) and in the comparison of violent suicide against non-suicide (r = 0.383, p-value < 2.2e-16). We reason that the results may be indeed driven by males, although we note that the purinergic receptors and *LINC00996* are still nominally differentially expressed also in females, and an increased female sample would be necessary for a definitive conclusion.

FIGURE S6. DE sensitivity analysis by sex



Scatterplots of the correlation between the log fold-change (logFC) of DEGs in – first row: violent suicide patients compared with non-suicide patients **a.** in both sexes (x-axis) and in only males (y-axis); **b.** in both sexes (x-axis) and in only females (y-axis); **c.** in only males (x-axis) and in only females (y-axis); second row: violent suicide patients compared with neurotypicals **d.** in both sexes (x-axis) and in only males (y-axis); **e.** in both sexes (x-axis) and in only females (y-axis); **f.** in only males (x-axis) and in only females (y-axis); third row: violent suicide patients compared with non-violent suicide patients **g.** in both sexes (x-axis) and in only males (y-axis); **h.** in both sexes (x-axis) and in only females (y-axis); **i.** in only males (x-axis) and in only females (y-axis). The logFCs were obtained from the DE analysis adjusting for sex (except in samples separated by sex), age, and quality surrogate variables accounting for RNA quality.

Violent suicide patients are less differentiated from neurotypicals than from other patients in DEGs

We further explored the transcriptomic divergence between violent suicide patients, non-violent suicide patients, non-suicide patients, and neurotypicals by analyzing how differences in genes expression between these four groups were related. First, we found a significant correlation between the moderated t-statistics of the DE analyses that compared i) violent suicide patients with non-suicide patients and ii) violent suicide patients with neurotypicals, (r= 0.3583226, p-value < 2.2e-16; **Figure S7a**). These finding suggests that, although only a few genes reach genome-wide significance in DE when comparing violent suicide patients with neurotypicals (**Figure 1d**), genes tend to be DE with consistent directionality when comparing violent suicide patients with non-suicide patients, and with neurotypicals. Indeed, a *geneset* test revealed that the set of genes up-regulated in violent suicide patients compared with non-suicide patients was highly ranked among the genes up-regulated in violent suicide patients compared with neurotypicals (p= 4.274914e-36; **Figure S7b**); and the same was true for the genes down-regulated in violent suicide patients (p= 3.557036e-14; **Figure S7b**).

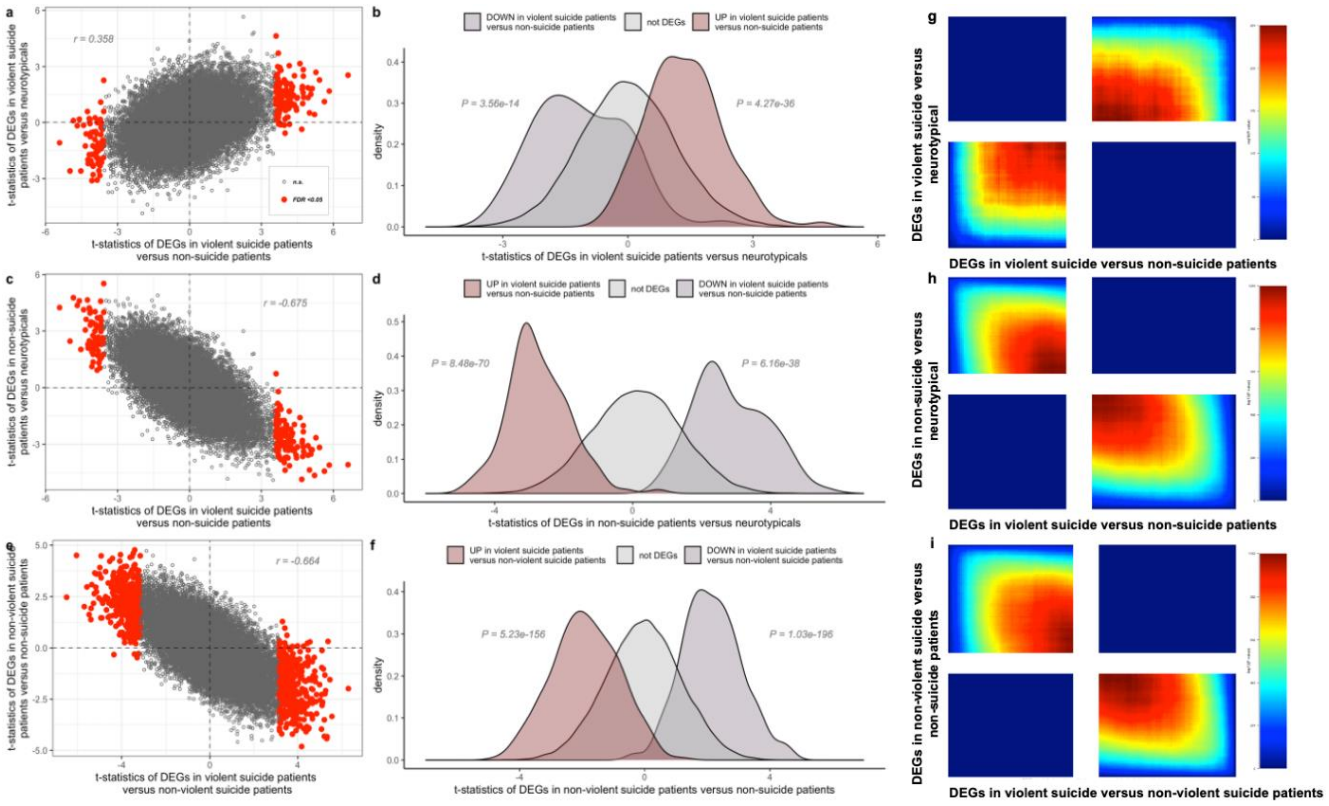
We then analyzed the correlation between the moderated t-statistics from the DE analyses that compared i) violent suicide patients with non-suicide patients and ii) non-suicide patients with neurotypicals. Surprisingly, we detected a strong negative correlation (r= -0.6753079, p-value < 2.2e-16; **Figure S7c**), suggesting that genes tend to be DE with opposite directionality when comparing violent suicide patients with non-suicide patients, and non-suicide patients with neurotypicals. Indeed, a *geneset* test confirmed that the set of genes up-regulated in violent suicide patients compared with non-suicide patients were enriched among the genes down-regulated in non-suicide patients compared with neurotypicals (p= 8.4767e-70; **Figure S7d**); and, vice versa, the same was true for the genes down-regulated in violent suicide patients compared with non-suicide patients, which were highly ranked among the genes up-regulated in non-suicide patients compared with neurotypicals (p= 6.163535e-38; **Figure S7d**).

Overall, these analyses indicate that the genes directionally up-regulated in violent suicide patients compared with non-suicide patients tended also to be up-regulated in violent suicide patients compared with neurotypicals, while they were up-regulated in neurotypicals compared with non-suicide patients. And the genes down-regulated in violent suicide patients compared with non-suicide patients also tended to be down-regulated in violent suicide patients compared with neurotypicals, while they were down-regulated in neurotypicals compared with non-suicide patients. In other words, our results suggest the intriguing possibility that, at a transcriptional level, the neurotypical condition lies *in-between* non-suicide patients and violent suicide patients.

Finally, to better position non-violent suicide patients in respect to the other groups, we analyzed the correlations between the moderated t-statistics of the DE analyses that compared i) violent suicide patients with non-violent suicide patients and ii) non-violent suicide patients with non-suicide patients. Here, we found again a negative correlation (r= -0.6642933; **Figure S7e**), suggesting that genes tended to be DE with opposite directionality when comparing violent suicide patients with non-violent suicide patients, and non-violent suicide patients with non-suicide patients. Again, a *geneset* test confirmed that the set of genes up-regulated in violent suicide patients compared with non-violent suicide patients were highly ranked among the genes

down-regulated in non-violent suicide patients compared with non-suicide patients (p= 5.227671e-156; **Figure S7f**); and, vice versa, the same was true for the genes down-regulated in violent suicide patients compared with non-violent suicide patients, which were enriched among the genes up-regulated in non-violent suicide patients compared with non-suicide patients (p= 1.033003e-196; **Figure S7f**). In other words, our results suggest not only that the neurotypical condition lies in-between non-suicide patients and violent suicide patients on these measures, but that the non-violent suicide patient and the violent suicide patient conditions represent the relative tails of a potential continuum. These results were further supported by an analysis using the rank-rank hypergeometric overlap (RRHO) method in its updated version (stratified RRHO method(6)). RRHO is a threshold-free algorithm for detecting and visualizing overlap trends between two complete, continuous gene-expression profiles(7). The modified algorithm improves its efficiency by overcoming limitations in revealing discordant signatures and providing more intuitive representations(6). We used the updated RRHO to re-analyze how the transcriptomic signatures in the various contrasts compare with each other. The RRHO analysis confirms our data, notably the existence of the linear continuum of expression, while offering a more intuitive visualization (**Figure S7g-i**); particularly, the RRHO analysis confirms the data that neurotypicals' gene-expression lies in between that of violent suicide and other patients.

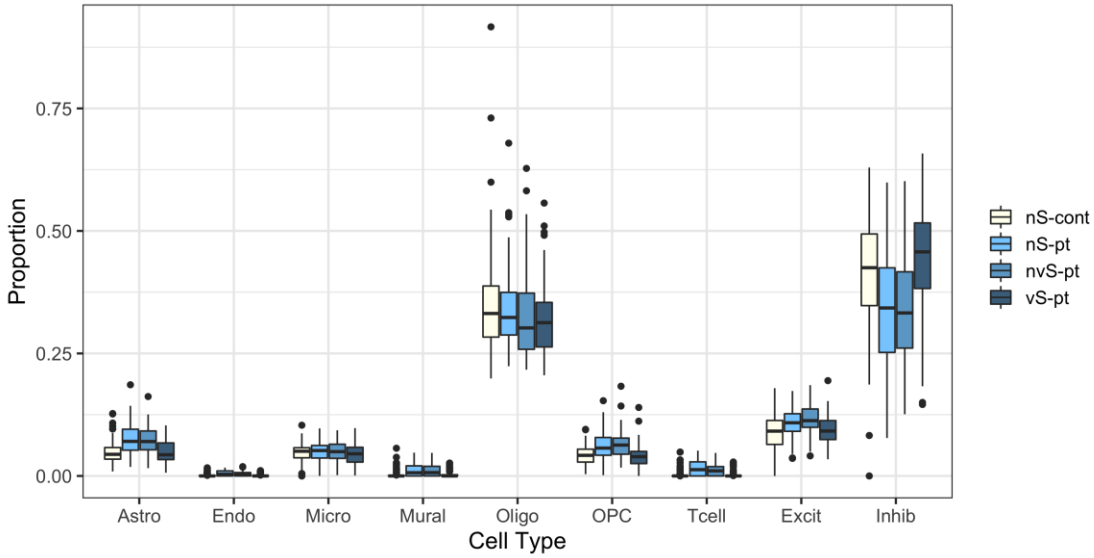
FIGURE S7. Relationship between DEGs in the four groups



a. Scatterplot of the moderated t-statistics of the DE analyses that compared i) violent suicide patients with non-suicide patients and ii) violent suicide patients with neurotypicals. **b.** Density plots of the t-statistics of the DEGs in violent suicide patients versus neurotypicals of the

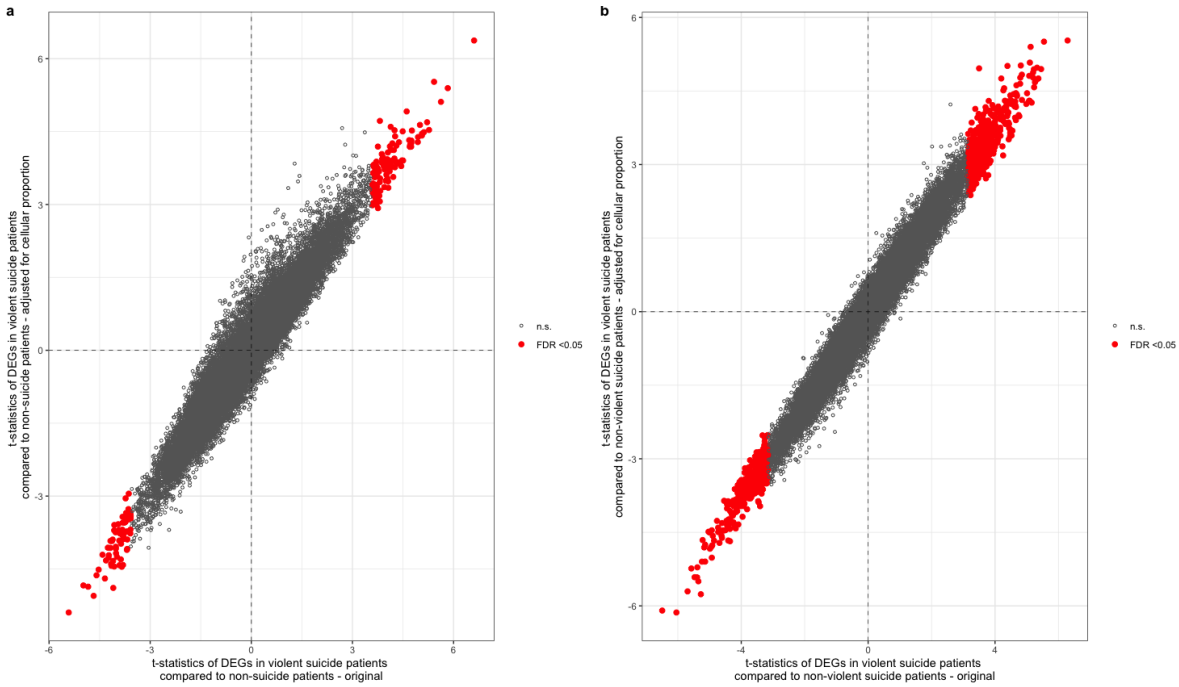
following gene-sets: genes up-regulated (FDR<0.05) in violent suicide patients versus non-suicide patients (light red), genes down-regulated (FDR<0.05) in violent suicide patients versus non-suicide patients (light purple), and remaining genes (grey). **c.** Scatterplot of the moderated t-statistics from the DE analyses that compared i) violent suicide patients with non-suicide patients and ii) non-suicide patients with neurotypicals. **d.** Density plots of the t-statistics of the DEGs in non-suicide patients versus neurotypicals of the following gene-sets: genes up-regulated (FDR<0.05) in violent suicide patients versus non-suicide patients (light red), genes down-regulated (FDR<0.05) in violent suicide patients versus non-suicide patients (light purple), and remaining genes (grey). **e.** Scatterplot of the moderated t-statistics of the DE analyses that compared i) violent suicide patients with non-violent suicide patients and ii) non-violent suicide patients with non-suicide patients. **f.** Density plots of the t-statistics of the DEGs in non-violent suicide patients versus non-suicide patients of the following gene-sets: genes up-regulated (FDR<0.05) in violent suicide patients versus non-suicide patients (light red), genes down-regulated (FDR<0.05) in violent suicide patients versus non-suicide patients (light purple), and remaining genes (grey). The moderated t-statistics were obtained from the DE analysis using a linear model adjusting for sex, age, and quality surrogate variables accounting for RNA quality; Pearson correlation coefficients at the top of the scatterplots (in **a**, **c**, **e**). $P_{\text{one-sided}}$ from the Wilcoxon 'geneSetTest' statistics at the top of the density plots (in **b**, **d**, **f**). **g-i.** The last column represents heatmaps obtained using the stratified RRHO method to compare the gene signatures associated with each DE. Each pixel in the stratified heatmap represents a $-\log10(p\text{-value})$ from the hypergeometric distribution. A hotspot in the top left quadrant indicates overlap in genes up-regulated in the analysis on the x-axis and down-regulated in the analysis on the y-axis. A hotspot in the top right quadrant indicates overlap in genes down-regulated in both analyses. A hotspot in bottom left quadrant indicates overlap in genes up-regulated in both analysis. A hotspot in the bottom right quadrant indicates overlap in genes down-regulated in analysis on the x-axis and up-regulated in the analysis on the y-axis. The RRHO visualization further shows the consistent transcriptional directionality in violent suicide versus non-suicide patients and violent suicide versus neurotypical (**g**); and the opposite transcriptional directionality in violent suicide versus non-suicide patients and non-suicide patients versus neurotypical (**h**); and the opposite transcriptional directionality in violent suicide versus non-violent suicide patients and non-violent suicide patients versus non-suicide patients (**i**).

FIGURE S8. Deconvolution



Box plots of the differences in cellular proportion between the main groups. The analysis shows a difference in the estimated proportion of OPCs between violent and non-violent suicide (t=-2.933, p=0.00360, p-corr=0.0324), all the other differences are not significant after correction for multiple comparisons.

FIGURE S9. Deconvolution

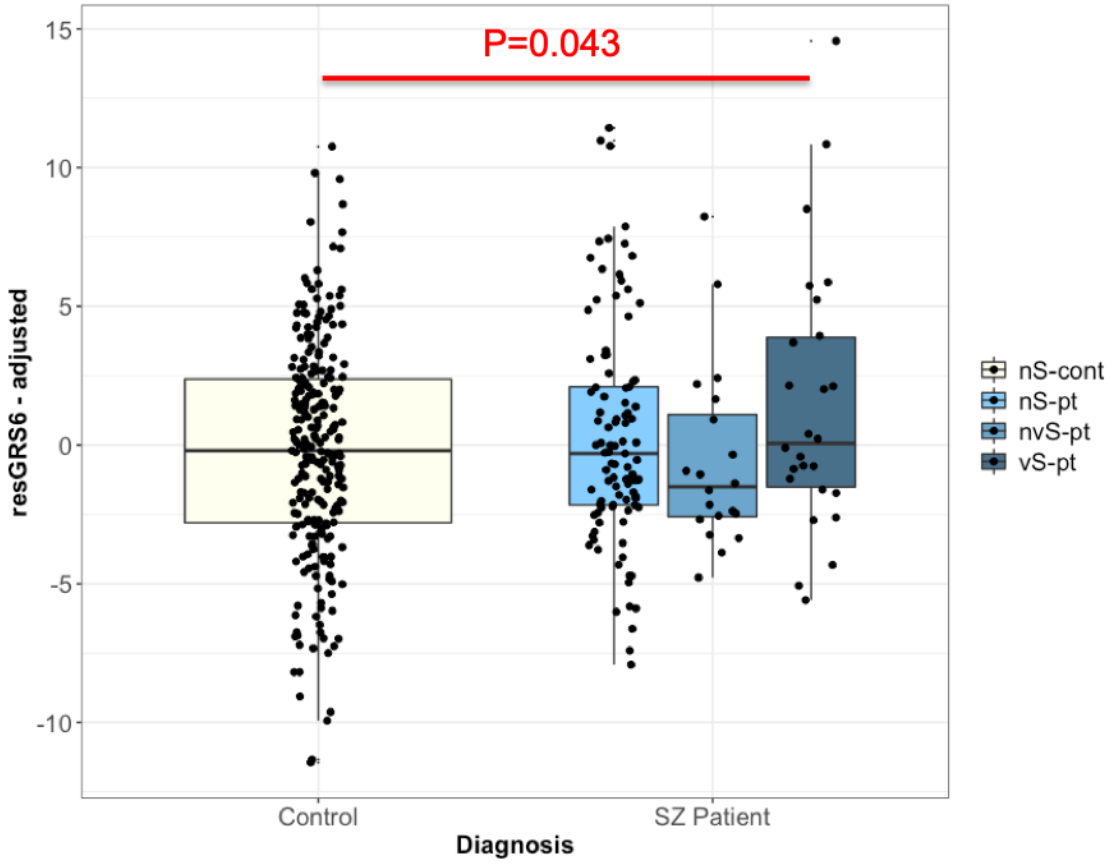


Scatterplots of the correlation between the t-statistics of DEGs in **a.** violent suicide patients compared with non-suicide patients in the original analysis (x-axis) and in the analysis correcting also for cellular composition (y-axis); and **b.** violent suicide patients compared with non-violent suicide patients in the original analysis (x-axis) and in the analysis correcting also for cellular composition (y-axis).

szGRS sensitivity analyses

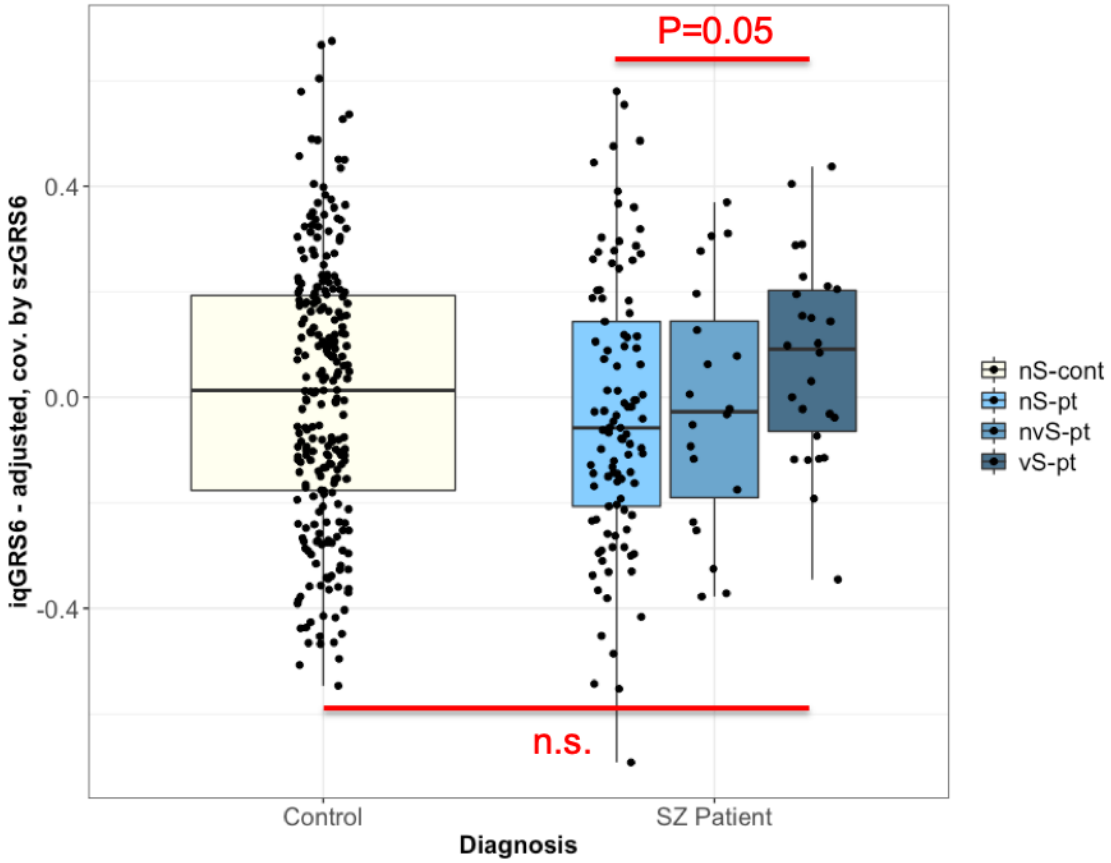
To exclude that different diagnostic subtypes would drive the results in violent suicide patients with SZ, we reviewed the sample and found a minority of cases (N= 17) meeting criteria for schizoaffective disorder rather than SZ. Although PGC2 GWAS sampling criteria include schizoaffective disorder cases as strongly related to SZ(8), we repeated the analysis after removing those samples, and confirmed all the results (prediction accuracy, i.e., Nalgelkerke R^2, of szGRS for non-suicide patients = 0.125; for non-violent suicide patients = 0.0236; for violent suicide patients= 0.007).

FIGURE S10. Schizophrenia resilience GRS and suicide



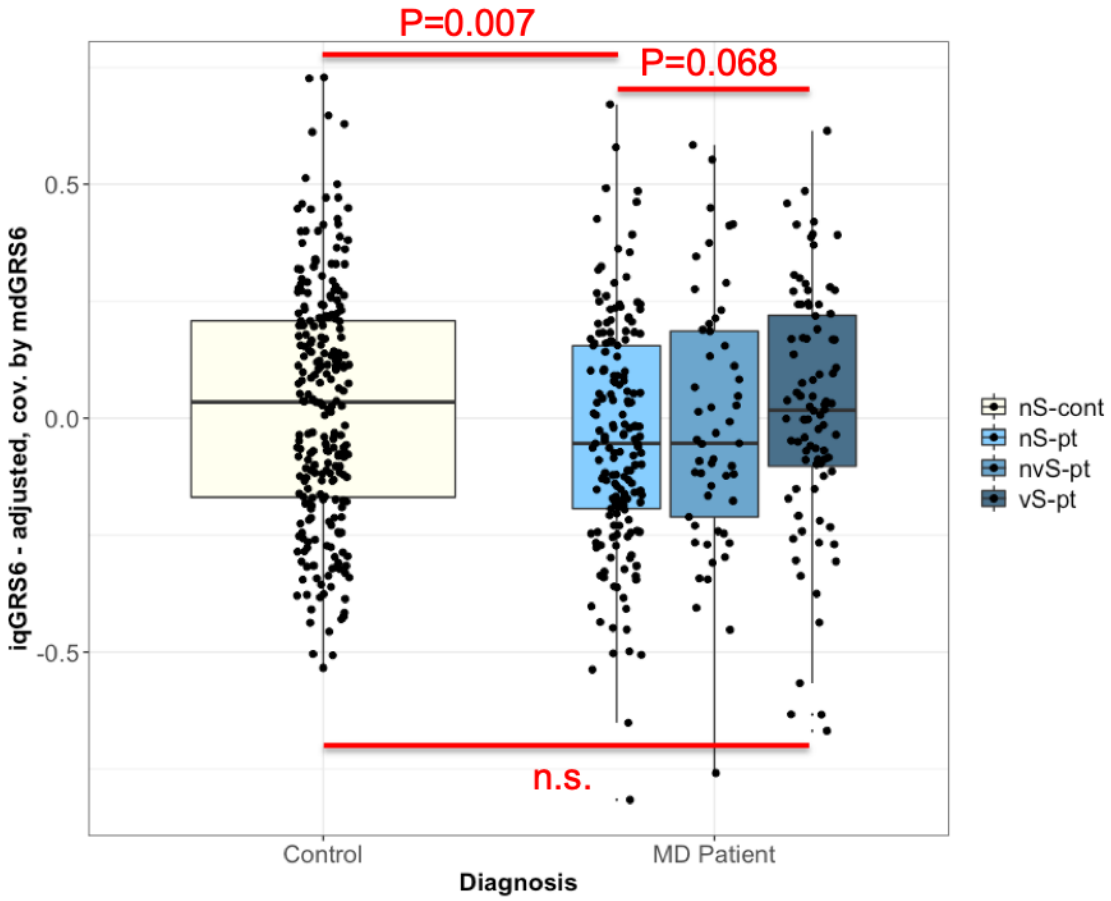
Box plots of the relationship between schizophrenia resilience GRS6 (resGRS6) and SZ (schizophrenia) case-control status in the subsample of neurotypicals (nS-cont) and SZ patients divided in: non-suicide patients (nS-pt), non-violent suicide patients (nvS-pt), violent suicide patients (vS-pt). Violent suicide patients have higher resGRS6 compared with neurotypicals (p-value at the top). All the statistics (reported in the main text) were generated using multiple regression, adjusting for population stratification (ten PCs), sex, and age. Box plot centers depict median; lower and upper hinges correspond to 25th and 75th percentiles; whiskers extend from hinges to smallest and larger values no further than 1.5*IQR from the 25th and 75th percentiles.

FIGURE S11. IQ GRS and suicide in schizophrenia, covaried by schizophrenia GRS



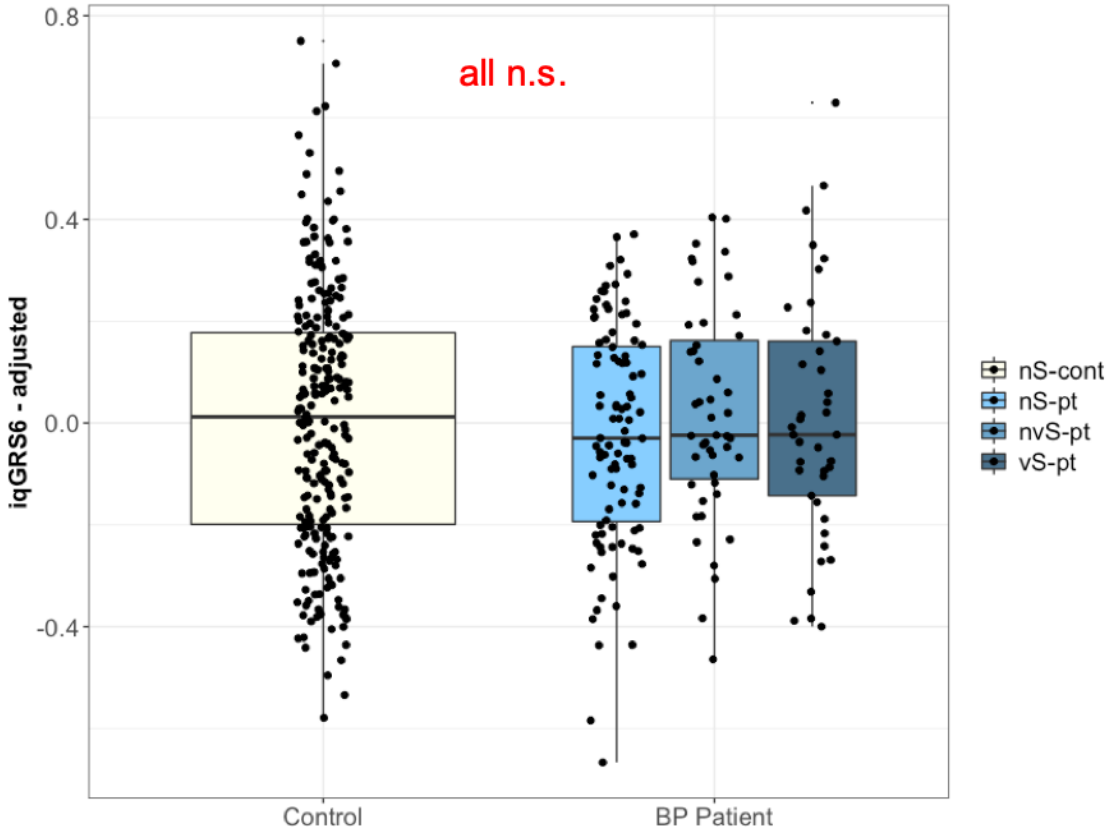
Box plots of the relationship between GRS6 for IQ (iqGRS6) covaried by schizophrenia GRS (szGRS6) and schizophrenia (SZ) case-control status in the subsample of neurotypicals (nS-cont) and SZ patients divided in: non-suicide patients (nS-pt), non-violent suicide patients (nvS-pt), violent suicide patients (vS-pt). After covarying for szGRS6, violent suicide patients have higher iqGRS6 compared with non-suicide patients (p-value at the top), similar to neurotypicals. All the statistics were generated using multiple regression, adjusting for population stratification (ten PCs), sex, age, and szGRS6. Box plot centers depict median; lower and upper hinges correspond to 25th and 75th percentiles; whiskers extend from hinges to smallest and larger values no further than 1.5*IQR from the 25th and 75th percentiles.

FIGURE S12. IQ GRS and suicide in major depression, covaried by major depression GRS



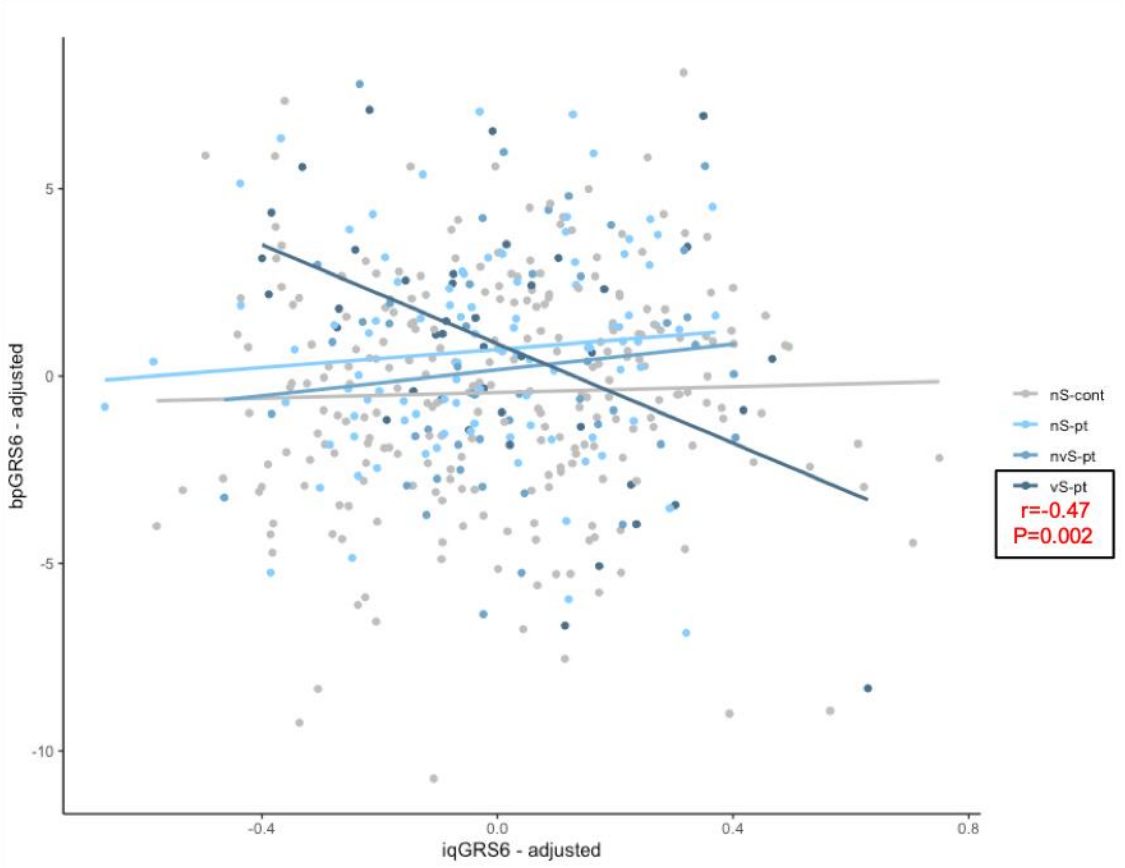
Box plots of the relationship between GRS6 for IQ (iqGRS6) covaried by major depression GRS (mdGRS6) and major depression (MD) case-control status in the subsample of neurotypicals (nS-cont) and MD patients divided in: non-suicide patients (nS-pt), non-violent suicide patients (nvS-pt), violent suicide patients (vS-pt). After covarying for mdGRS6, violent suicide patients have higher iqGRS6 compared with non-suicide patients, similar to neurotypicals (p-values at the top). All the statistics were generated using multiple regression, adjusting for population stratification (ten PCs), sex, age, and mdGRS6. Box plot centers depict median; lower and upper hinges correspond to 25th and 75th percentiles; whiskers extend from hinges to smallest and larger values no further than 1.5*IQR from the 25th and 75th percentiles.

FIGURE S13. IQ GRS and suicide in bipolar disorder



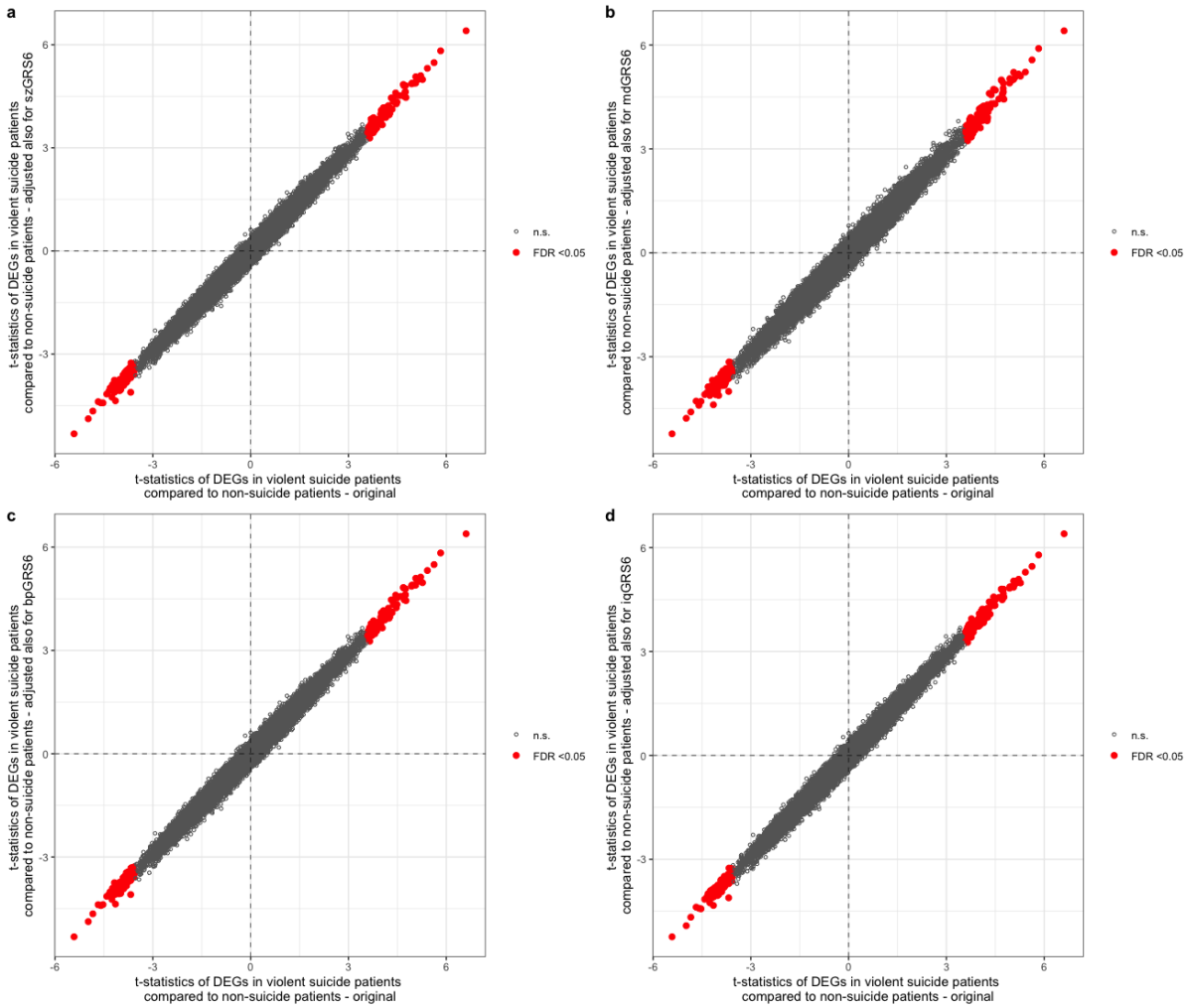
Box plots of the relationship between GRS6 for bipolar disorder (bpGRS6) and bipolar disorder (BP) case-control status in the subsample of neurotypicals and BP patients, divided in the three manner of death categories: non-suicide patients (nS-pt), non-violent suicide patients (nvS-pt), violent suicide patients (vS-pt). iqGRS6 was not significantly different across groups. All the statistics were generated using multiple regression, adjusting for population stratification (ten PCs), sex, age. Box plot centers depict median; lower and upper hinges correspond to 25th and 75th percentiles; whiskers extend from hinges to smallest and larger values no further than 1.5*IQR from the 25th and 75th percentiles.

FIGURE S14. Relationship between GRS for IQ and bipolar disorder (BP)



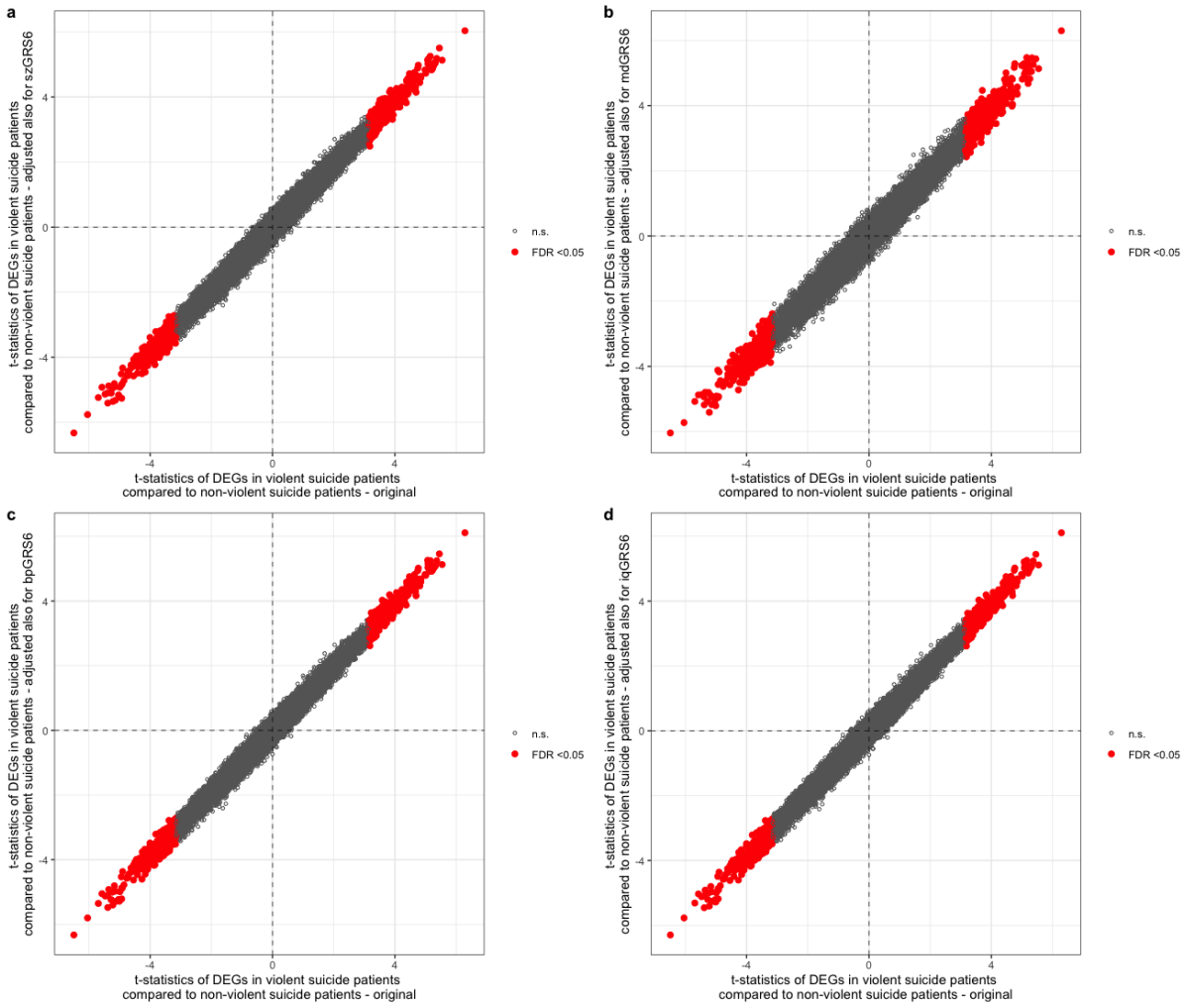
Scatterplot of the relationship between GRS6 for IQ (iqGRS6, x-axis) and GRS6 for bipolar disorder (bpGRS6, y-axis) in the subsample of neurotypicals (nS-cont) and BP patients divided in: non-suicide patients (nS-pt), non-violent suicide patients (nvS-pt), violent suicide patients (vS-pt). bpGRS6 and iqGRS6 show a significant negative correlation only in violent suicide patients (Pearson correlation and p-value from the correlation test in the caption).

FIGURE S15. DE analysis adjusted for GRSs



Scatterplots of the correlation between the t-statistics of DEGs in violent suicide patients compared with non-suicide patients in the original analysis (x-axis) and in the analysis correcting also for szGRS (**a**), mdGRS (**b**), bpGRS (**c**) and iqGRS (**d**) (y-axis).

FIGURE S16. DE analysis adjusted for GRSs

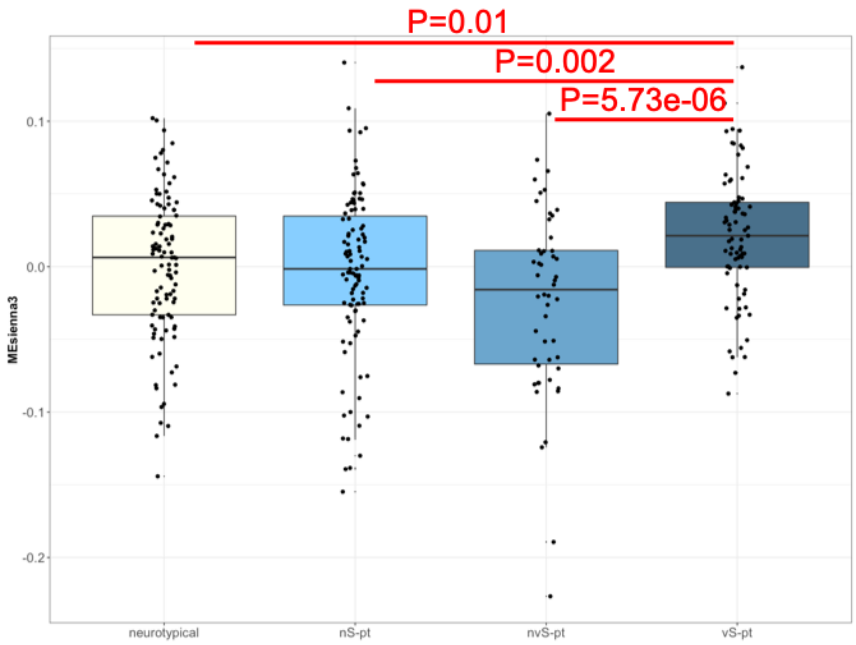


Scatterplots of the correlation between the t-statistics of DEGs in violent suicide patients compared with non-violent suicide patients in the original analysis (x-axis) and in the analysis correcting also for szGRS (**a**), mdGRS (**b**), bpGRS (**c**) and iqGRS (**d**) (y-axis).

Caption for Table S6 (separate excel file)

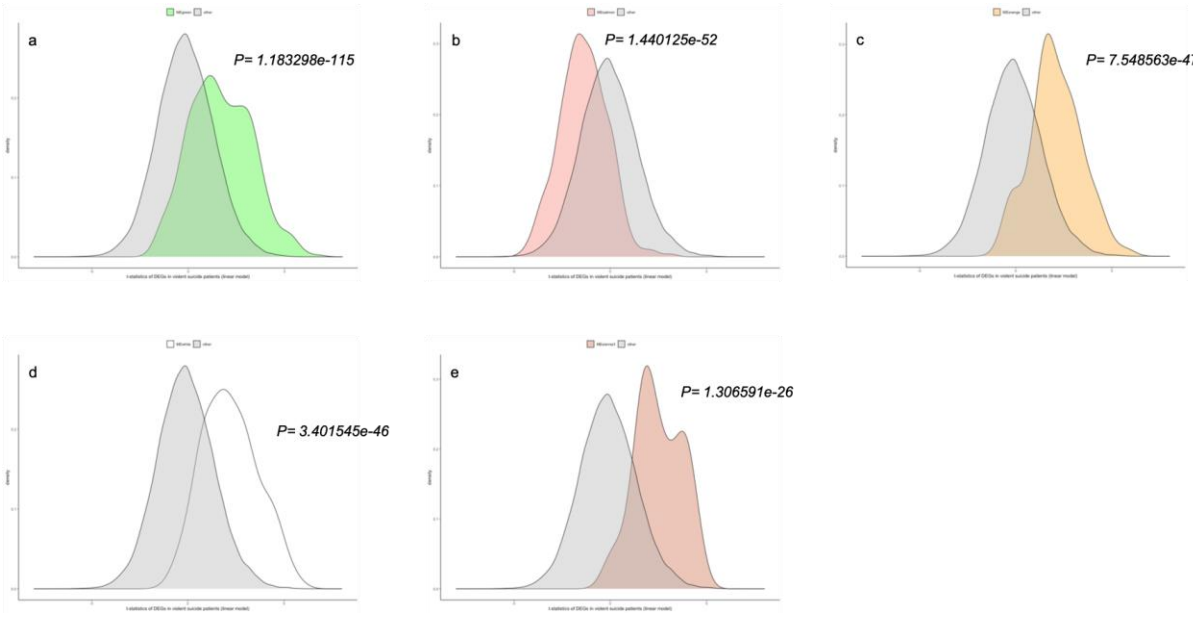
TABLE S6. WGCNA Gene Ontology results and hub genes. Gene Ontology (GO) enrichment results on WGCNA modules: *sienna3* (**Table S6a**), *green* (**Table S6b**), *salmon* (**Table S6c**), *orange* (**Table S6d**), *white* (**Table S6e**), *yellowgreen* (**Table S6f**), *lightcyan1* (**Table S6g**); gene hubs (**Table S6h**), and kMEs (the module membership i.e., the Pearson's correlation between a given gene's expression level and a given module eigengene, **Table S6i**).

FIGURE S17. WGCNA, association with violent suicide of gene modules constructed on the 329 samples combined



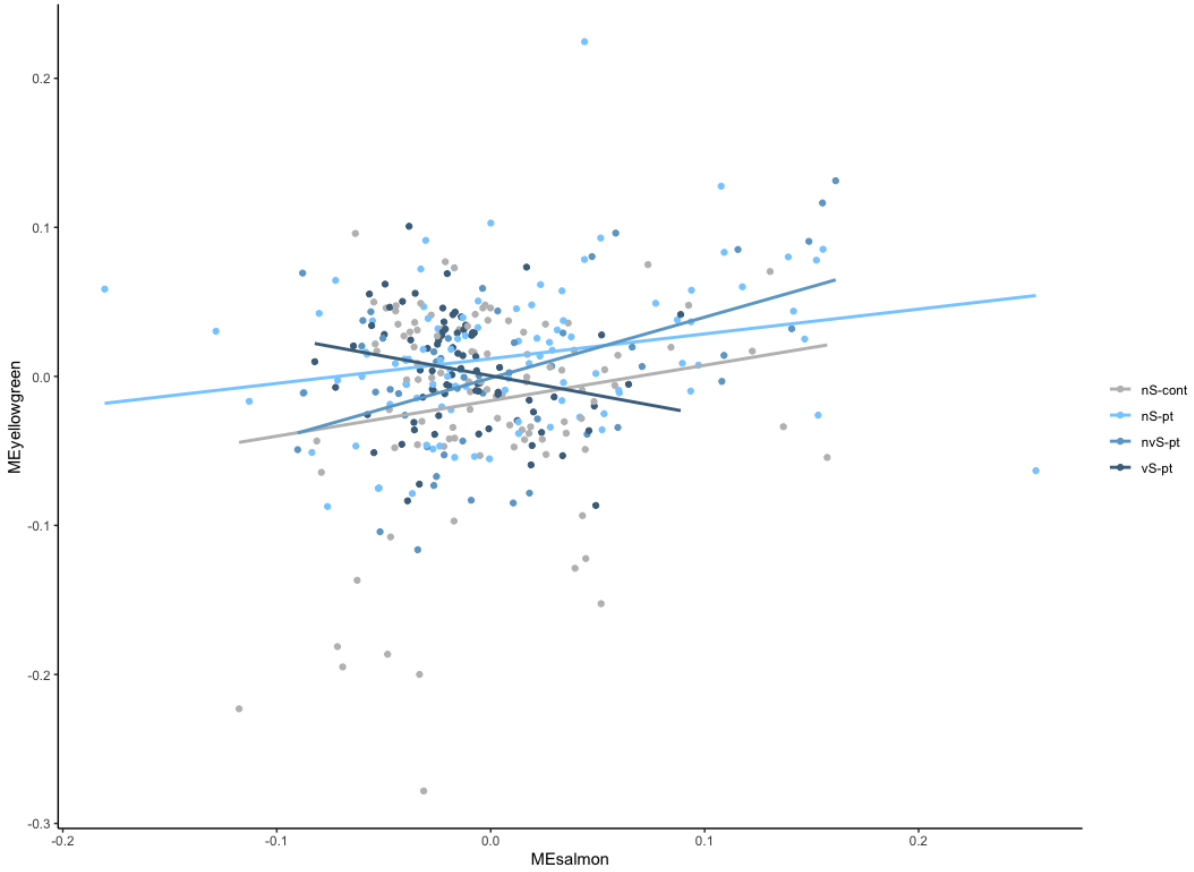
Box plots of the relationship between *sienna3* (i.e. GABA synthesis) module eigengenes (ME) and the 4 main groups. All the statistics (at the top of the picture) were generated using a linear model with MEs as dependent variable and group as predictor.

FIGURE S18. WGCNA, violent suicide DEGs' gene-set enrichment in gene modules constructed on the 329 samples combined



Density plots of the t-statistics of the genes associated with the violent suicide condition in the linear model of the gene-sets: **a.** *green* module eigengene and remaining genes, **b.** *salmon* module eigengene and remaining genes, **c.** *orange* module eigengene and remaining genes, **d.** *white* module eigengene and remaining genes, **e.** *sienna3* module eigengene and remaining genes. All the statistics were generated by Wilcoxon test, and also validated by Chi-Square test.

FIGURE S19. WGCNA, interaction between *salmon* ME and the four groups on *yellowgreen* ME



Box plots of the interaction between *salmon* (i.e. immune response) module eigengenes (ME) and the 4 main groups on *yellowgreen* (no significant enrichment) ME. Interaction statistics: neurotypicals (a.ka. nS-cont)*violent suicide patients t=2.259, p=0.02455; non-suicide patients*violent suicide patients t=2.070, p=0.03922; non-violent suicide patients*violent suicide patients t=2.987, p=0.00303. All the statistics were generated using using multiple regression with the interaction term ME*group.

Supplementary References

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