Supplementary materials:
DNA methylation changes in a pharmaco-epigenomic EWAS in depression: comparing fixed and response-guided dosing paradigms for ketamine in the KADS trial

1. Sample description

Figure S1: description of the sample during the RCT. Cohort 1 = fixed doses; Cohort 2 = flexible doses.

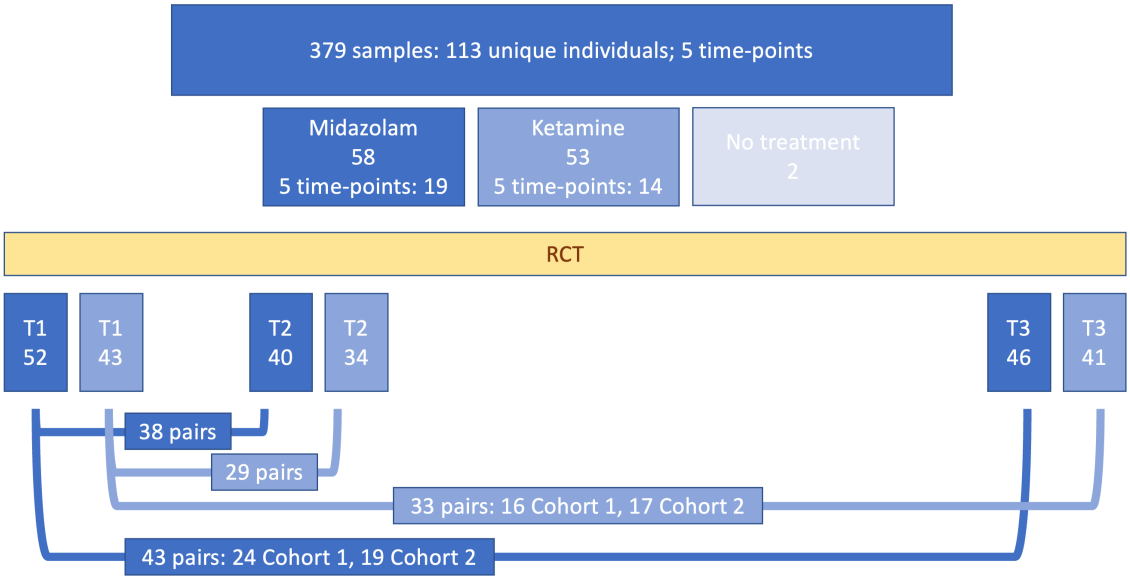
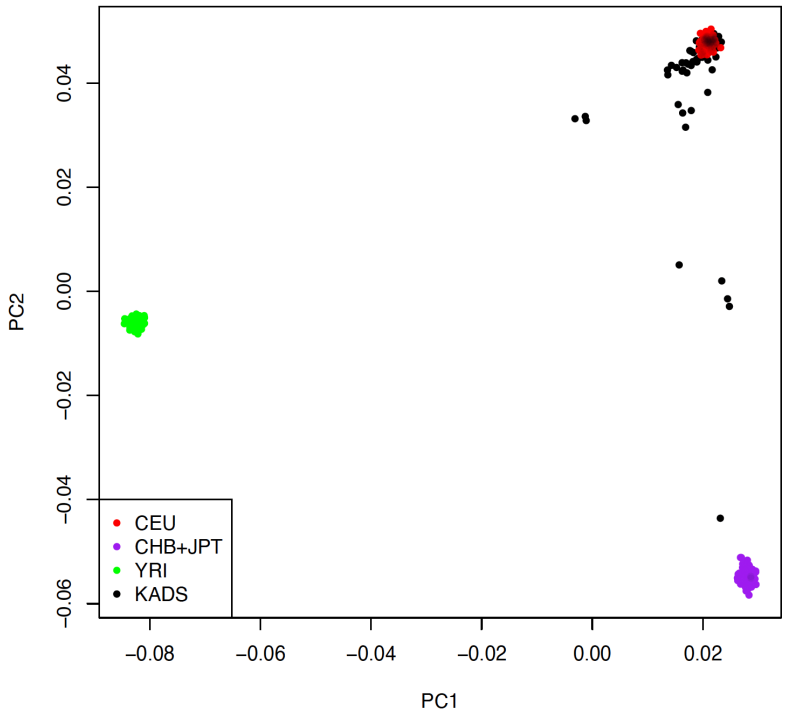


Figure S2: Genomic ancestry structure of the KADS-Sample. Ancestry was estimated using available SNP data. Data-preparation and principal component composition were performed using PLINK(1) and R according to the protocol by Anderson et al.(2).



2. QC of DNA methylation

- 388 samples available (5 Time-points)
- 4 Samples were removed due to overall DNA quality.
- Another 5 samples were removed in the DNA-methylation specific quality control steps.

Settings for preprocessing:

Normalisation method: dasen normalisation (WateRmelon)
Greedycut: detection p-value cut-off 0.05
Removal of: SNPrelated CGs, Sexrelated CGs, crossreactive probes

Table S1: A detailed description of the QC steps.

	Probes at start	**Samples at start**
Total at start preprocessing	**866 895**	**379**

Step (Filtering I)	**Probes removed**	**Samples removed**
Removal of SNP-enriched Probes	*139 721*	*0*
Removal of Cross-reactive Probes	*34 264*	*0*
Greedycut	*8 529*	*0*
Total removed	*182 514*	*0*

	Probes retained	**Samples retained**
Total retained	**684 381**	**379**

Step (Filtering II)	**Probes removed**	**Samples removed**
Context-specific Probes	*1 048*	*0*
Removal of Probes on Sex Chromosomes	*16 148*	*0*
Probes with Missing values > 90%	*158*	*0*
Total removed	*17 354*	*0*

	Probes retained	**Samples retained**
Total retained	**667 027**	**379**

3. Confounding variables

Cell type deconvolution
Method: both the target dataset (KADS) and the dataset with the DNA methylation reference for the estimate of cell-type fractions were analysed from IDAT-files and parallel, but uniformly prepared and pre-processed with the same settings.

The IDAT-files from the 6 celltypes as discussed by Salas et al. (3), reference dataset GSE110554. For our estimates we discarded the artificial mixes and only used the six isolated cell-types for a total of 37 samples: Neutrophils (N=6), Monocytes (N=6), B lymphocytes (N=6), CD4+ T-cells (N=7), CD8+ T-cells (N=6), and natural killer cells (N=6)

Both datasets were combined for the estimate of cell type fractions using the RnBeads built-in function 'rnb.execute.ct.estimation()'.

Celltype estimates were added to the phenotype and included as confounding variables in the cross-sectional analyses, along with biological sex, age, ancestry (PC1 and PC2), and collection site of blood samples.
Surrogate variables as estimated by the RnBeads package were added as well:

- Cross-sectional analysis overall midazolam vs. ketamine: 18 surrogate variables
- Cross-sectional analysis flexible doses midazolam vs. ketamine: 7 surrogate variables
- Cross-sectional analysis fixed doses midazolam vs. ketamine: 9 surrogate variables
- Cross-sectional analysis fixed doses vs. flexible doses: 11 surrogate variables

4. Tables of cross-sectional analyses at T3 (Analyses 1, 3 and 4)

Table S2: Top 10 of CpGs in the overall cross-sectional analysis at end of RCT comparing midazolam and ketamine. CpGs are ranked by p-value.

CpG	Position	Genomic context (UCSC)	Mean Mid.	Mean Ket.	Methylation difference	*p*-value	Fdr. *p*-value
cg11159519	Chr1: 210857380 South Shore	KCNH1 gene body	0.943	0.938	0.0041; Ketamine Hypomethylated	$2.38x10^{-6}$	0.70
cg04677410	Chr11: 95846117 Open Sea	MAML2 gene body	0.79	0.77	0.022; Ketamine Hypomethylated	$2.58x10^{-6}$	0.70
cg27376817	Chr7: 75931888 Island	close to HSBP1 promotor/ regulatory elements	0.06	0.08	-0.015; Ketamine Hypermethylated	$4.42x10^{-6}$	0.70
cg10930169	Chr15: 91072047 North Shore	regulatory elements/closest gene: CRTC3	0.89	0.88	0.010; Ketamine Hypomethylated	$4.86x10^{-6}$	0.70
cg26536813	Chr4: 56502469 Island	Proximity of NMU promotor	0.134	0.126	0.0080; Ketamine Hypomethylated	$5.37x10^{-6}$	0.70
cg08898192	Chr17: 8042067 Open Sea	Regulatory elements/closest gene: PER1	0.30	0.28	0.019; Ketamine Hypomethylated	$6.32x10^{-6}$	0.70
cg01778908	Chr16: 70759095 Open Sea	VAC14 gene body	0.87	0.86	0.0079; Ketamine Hypomethylated	$1.41x10^{-5}$	1.00
cg13296394	Chr6: 119823058 Open Sea	ENSG00000287100 RNA gene	0.64	0.67	-0.032; Ketamine Hypermethylated	$1.51x10^{-5}$	1.00
cg14560803	Chr10: 123551439 Open Sea	ATE1 Gene body/regulatory elements (histones)	0.928	0.933	-0.0059; Ketamine Hypermethylated	$1.57x10^{-5}$	1.00
cg16498879	Chr2: 134149472 Open Sea	NCKAP5 gene body/ close to RN7SKP154 pseudogene	0.86	0.87	-0.012; Ketamine Hypermethylated	$1.87x10^{-5}$	1.00

Table S3: Top 10 of CpGs comparing both fixed dosing cohorts.

CpG	Position	Genomic context (UCSC)	Mean Mid.	Mean Ket.	Methylation difference	*p*-value	Fdr. *p*-value
cg04584009	Chr19: 1826957 Island	*REXO1* gene body	0.86	0.88	-0.013; Ketamine Hypermethylated	$3.09x10^{-6}$	0.83
cg01821429	Chr3: 193922273 Island	*LINC00887/LINC02036/ENSG00000303507* body (*)	0.20	0.22	-0.024; Ketamine Hypermethylated	$3.35x10^{-6}$	0.83
cg24863347	Chr7: 116416245 Open Sea	*MET* gene body	0.87	0.84	0.030; Ketamine Hypomethylated	$6.05x10^{-6}$	0.83
cg10727879	Chr4: 139846096 Open Sea	ENSG00000250195/LOC105377448	0.90	0.91	-0.010; Ketamine Hypermethylated	$6.74x10^{-6}$	0.83
cg01539483	Chr4: 147164948 Open Sea	ENSG00000251010/regulatory elements	0.44	0.38	0.058; Ketamine Hypomethylated	$7.02x10^{-6}$	0.83
cg00604425	Chr3: 193922370 Island	*LINC00887/LINC02036/ENSG00000303507* body (*)	0.28	0.32	-0.033; Ketamine Hypermethylated	$9.73x10^{-6}$	0.83
cg20280170	Chr1: 29101609 North Shore	Vicinity of *YTHDF2*	0.53	0.50	0.025; Ketamine Hypomethylated	$1.19\ x10^{-5}$	0.83
cg15031763	Chr19: 49631660 North Shore	*PPFIA3* gene body	0.12	0.11	0.0094; Ketamine Hypomethylated	$1.30\ x10^{-5}$	0.83
cg27427514	Chr3: 193922037 Island	*LINC00887/LINC02036/ENSG00000303507* body (*)	0.10	0.15	-0.042; Ketamine Hypermethylated	$1.34x10^{-5}$	0.83
cg20749769	Chr11: 130029961 Island	*ST14* promotor-linked	0.068	0.072	-0.0046; Ketamine Hypermethylated	$1.36\ x10^{-5}$	0.83

Three CpGs from the top 10, marked (*), are part of the same region and linked to *LINC00887/LINC02036/ ENSG00000303507* body; ENCODE Candidate Cis-Regulatory Elements (cCREs): EH38E2270808. This region is expected to have a primarily regulatory function (see also Fig. S11).

Table S4: Top 10 of CpGs comparing fixed doses with higher, flexible doses (midazolam and ketamine combined).

CpG	Position	Genomic context (UCSC)	Mean Mid.	Mean Ket.	Methylation difference	*p*-value	Fdr. *p*-value
cg20023762	**Chr6: 11224219 Open Sea**	***NEDD9* gene body**	**0.92**	**0.91**	**0.0087; flexible hypomethylated**	$\mathbf{1.11x10^{-7}}$	**0.074**
cg14548284	Chr4: 10458049 North Shore	*ZNF518B* promotor-related island	0.66	0.69	-0.022; flexible hypermethylated	$2.28x10^{-6}$	0.68
cg08919846	Chr1: 3664586 South Shore	*GFOD3P/TP73-AS1* promotor-related island	0.93	0.94	-0.0091; flexible hypermethylated	$3.39x10^{-6}$	0.68
cg11572381	Chr19: 10827337 North Shore	*DNM2* promotor-related island	0.76	0.77	-0.0082; flexible hypermethylated	$4.88x10^{-6}$	0.68
cg25725823	Chr1: 1142254 South Shore	*TNFRSF18* promotor-related island	0.35	0.34	0.012; flexible hypomethylated	$6.85x10^{-6}$	0.68
cg01494075	Chr17: 70103034 Open Sea	*SOX9-AS1* proximity	0.74	0.71	0.028; flexible hypomethylated	$8.10x10^{-6}$	0.68
cg09776772	Chr7: 2150534 Open Sea	*MAD1L1* gene-body	0.89	0.88	0.010; flexible hypomethylated	$8.70x10^{-6}$	0.68
cg07891953	Chr18: 76544456 South Shelf	gene desert	0.89	0.90	-0.0086; hypermethylated	$1.01x10^{-5}$	0.68

cg12800939	Chr3: 112734980 North Shelf	*NEPRO/NEPRO-AS1* promotor-linked island	0.94	0.94	0.0035; flexible hypomethylated	$1.05x10^{-5}$	0.68
cg21472506	Chr2: 63283967 Island	*OTX1* gene body/regulatory elements	0.060	0,065	-0.0051; flexible hypermethylated	$1.12x10^{-5}$	0.68

5. QQ-plots cross-sectional analyses

Figure S3: Overall cross-sectional analysis Ketamine vs. Midazolam (λ=0.97).

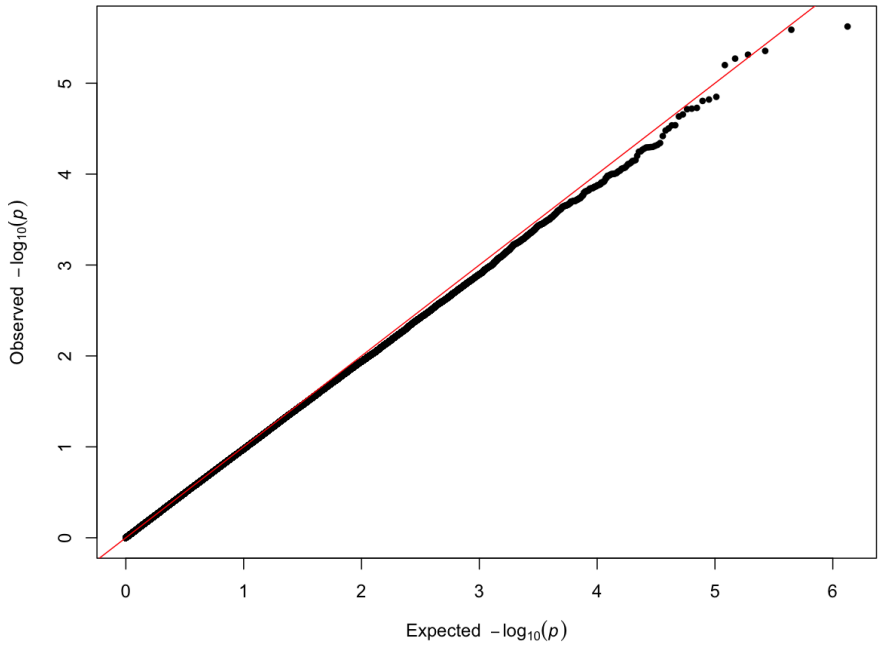


Figure S4: Cross-sectional analysis: flexible dose midazolam vs. flexible dose ketamine (λ=0.99).

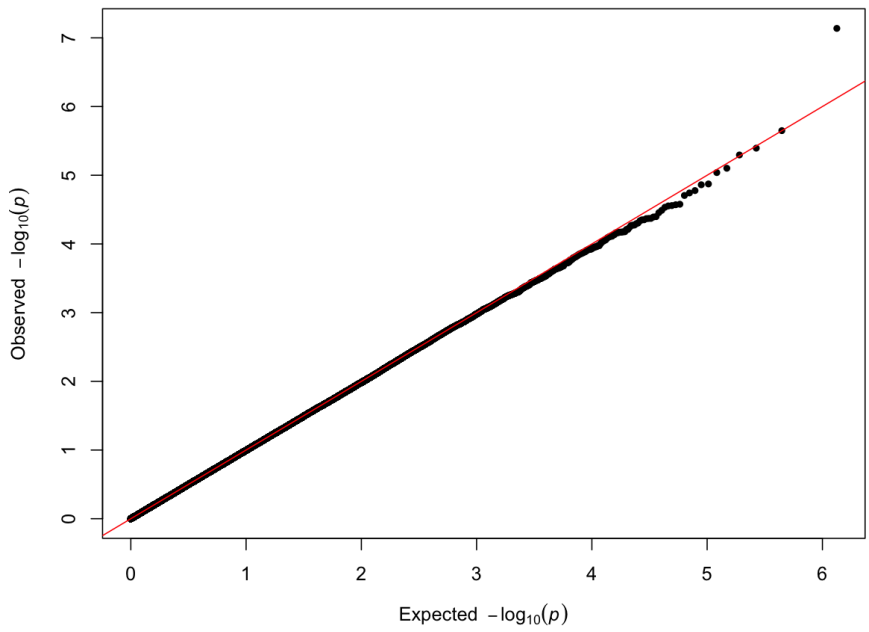


Figure S5: Cross-sectional analysis: fixed dose midazolam vs. fixed dose ketamine (λ=0.98).

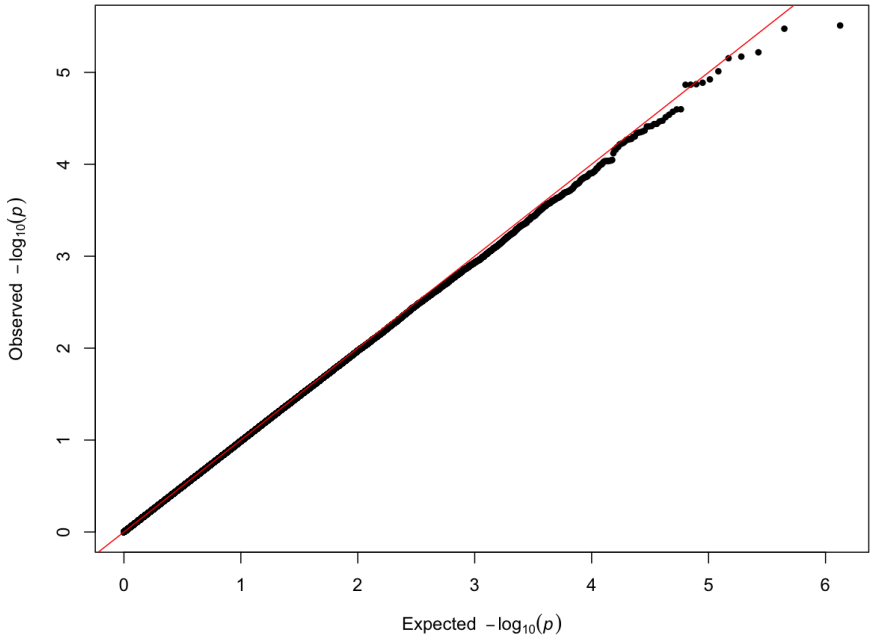
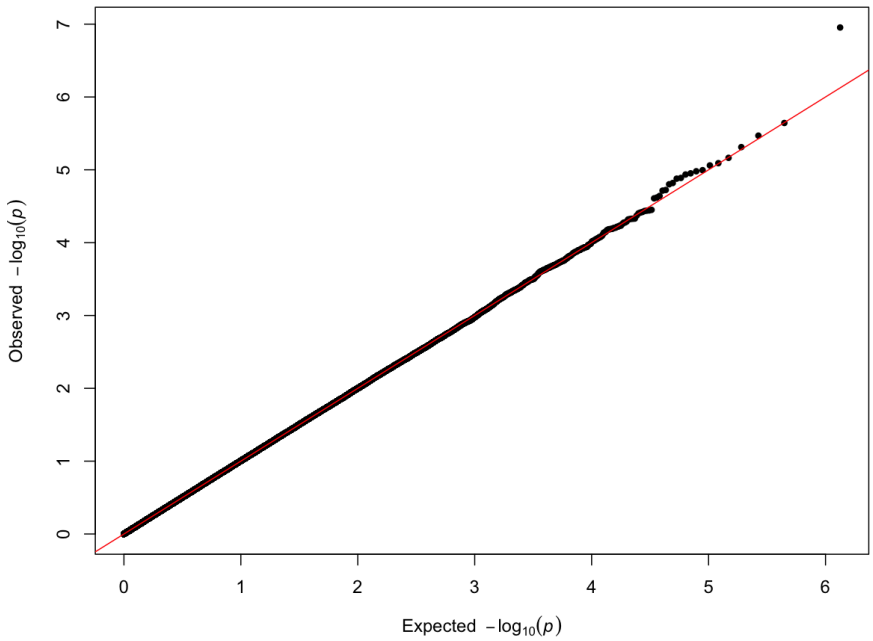


Figure S6: Cross-sectional analysis: Fixed doses vs. flexible doses; both therapies combined (λ=1.01).



6. QQ-plots paired longitudinal analyses.

Figure S7: Paired longitudinal analysis: all patients (λ=0.93).

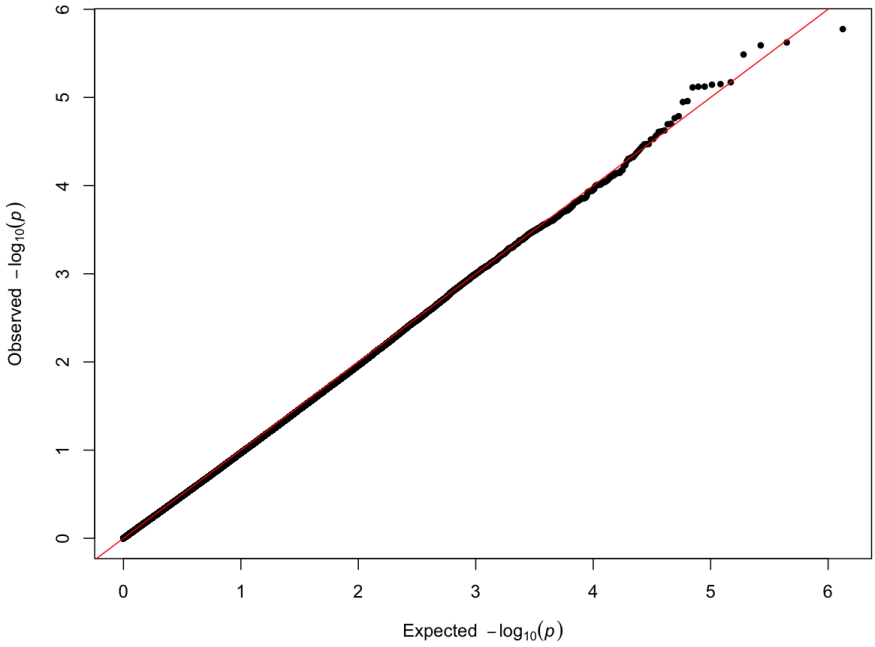


Figure S8: Paired longitudinal analysis: all Ketamine patients (λ=1.07)

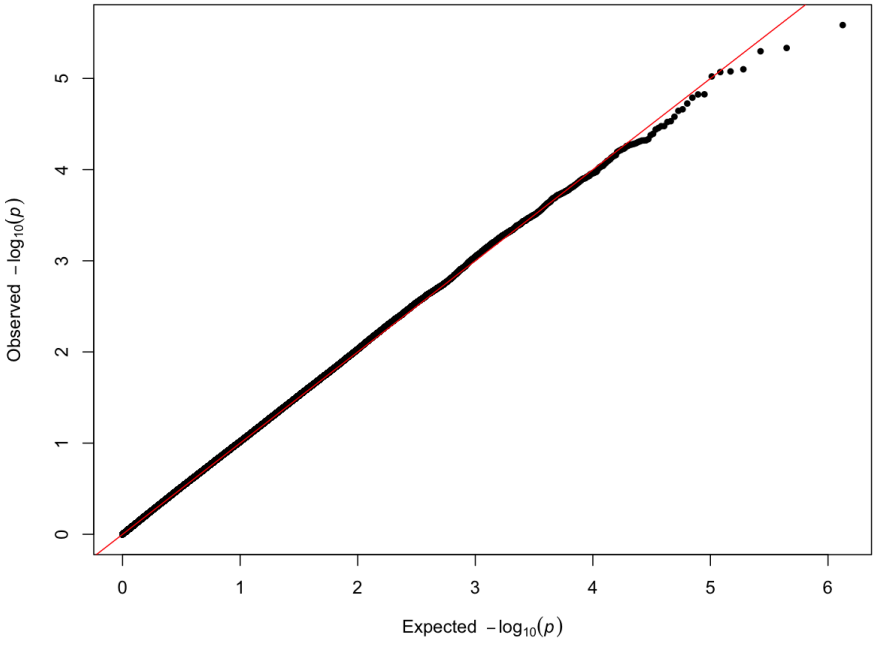


Figure S9: Paired longitudinal analysis: all Midazolam patients (λ=0.87)

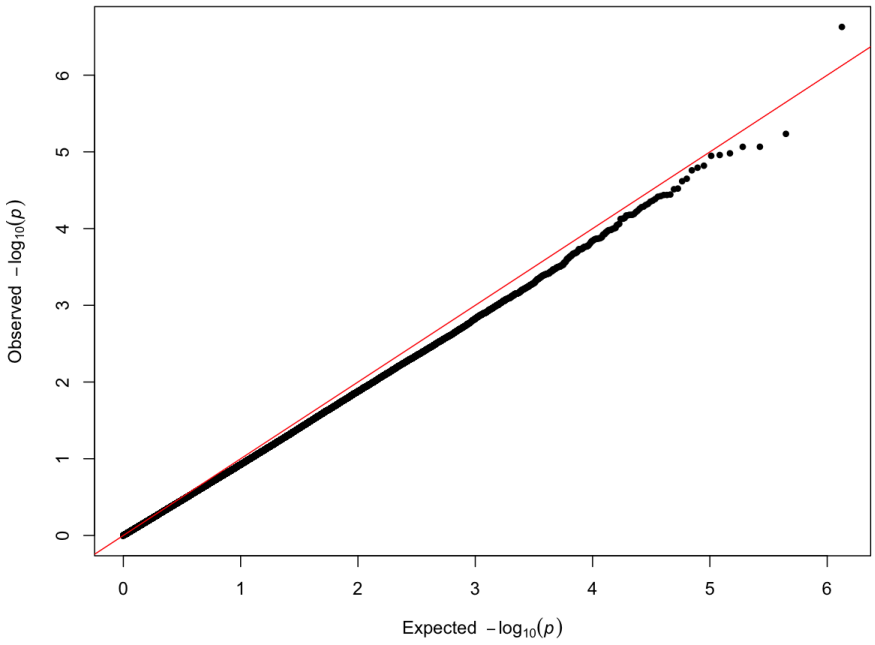


Figure S10: Paired longitudinal analysis: Ketamine flexible doses (λ=1.00).

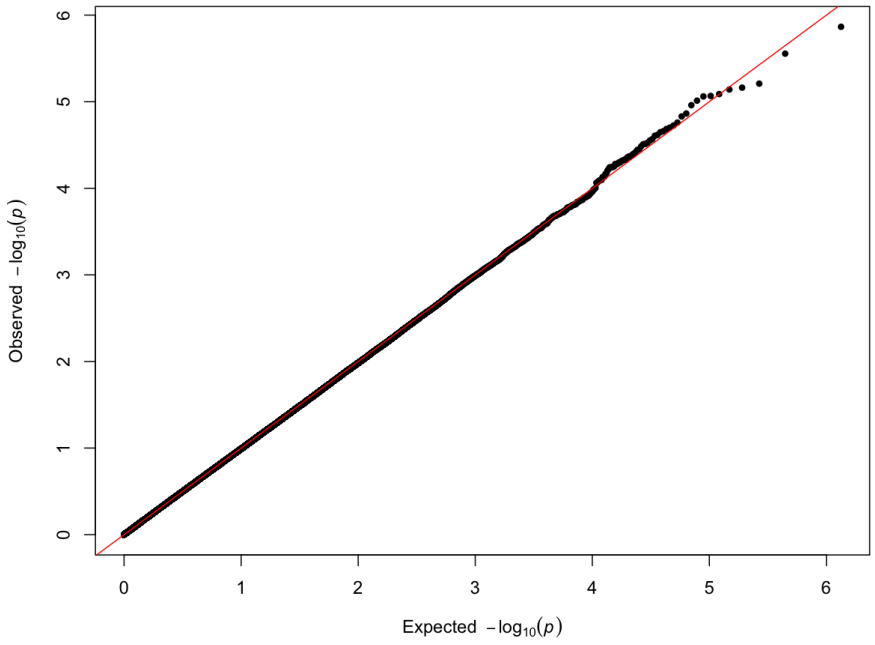


Figure S11: Paired longitudinal analysis: Ketamine fixed doses (λ=0.99).

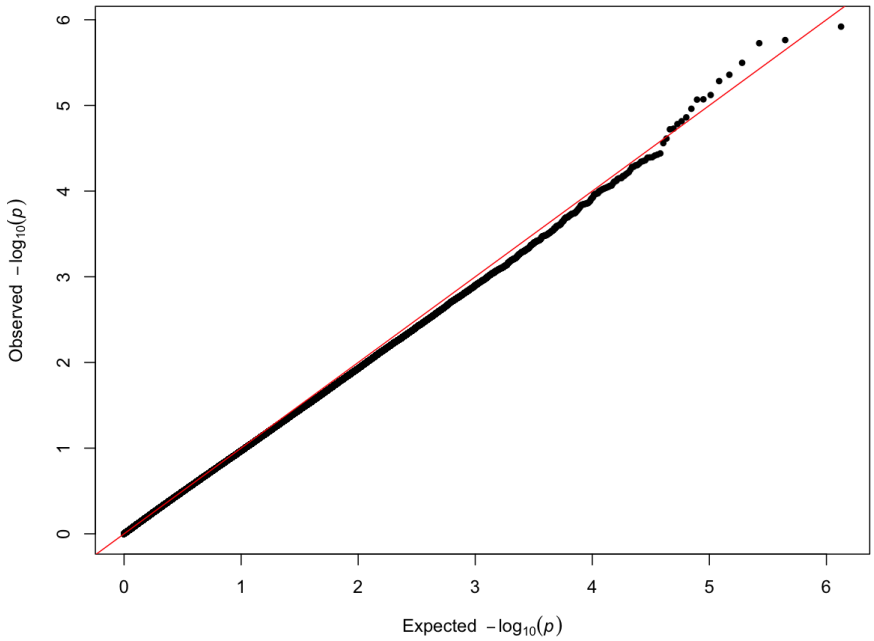


Figure S12: Paired longitudinal analysis: Midazolam flexible doses (λ=0.99).

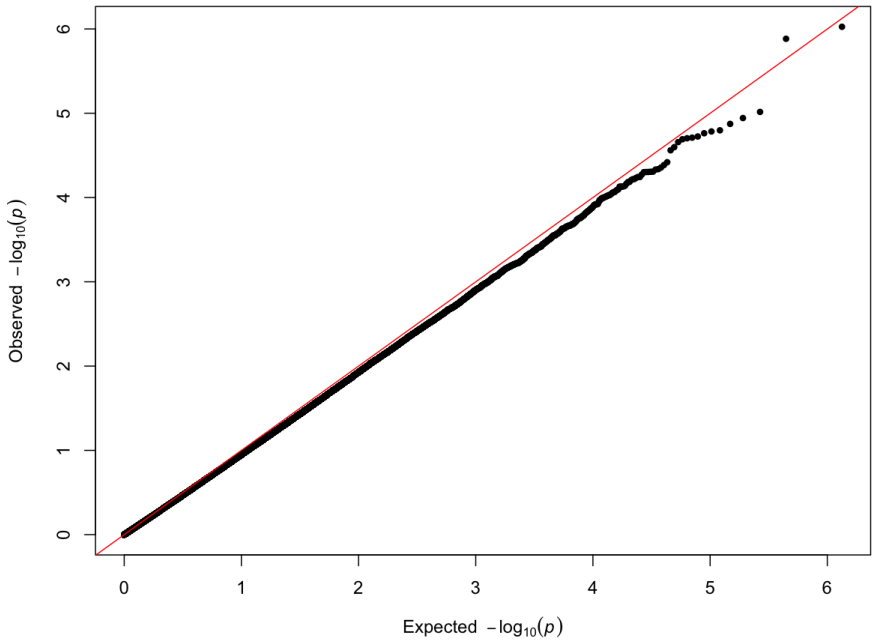
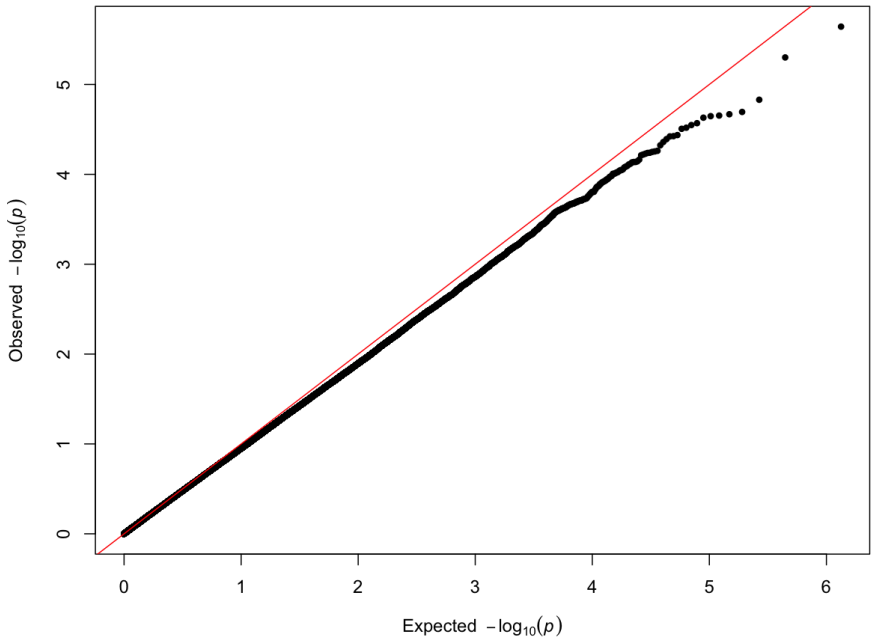
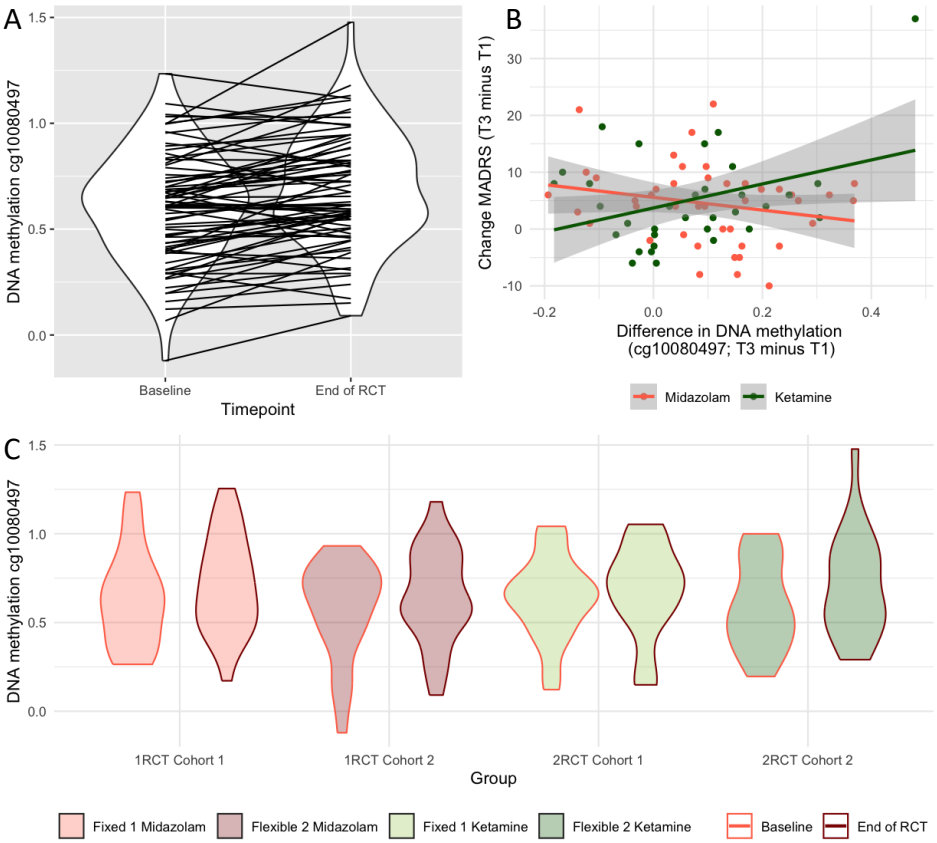


Figure S13: Paired longitudinal analysis: Midazolam fixed doses (λ=0.94).



7. Sensitivity analysis

Figure S14: Sensitivity analyses for the whole sample longitudinal analysis. Most significant CpG: cg10080497.

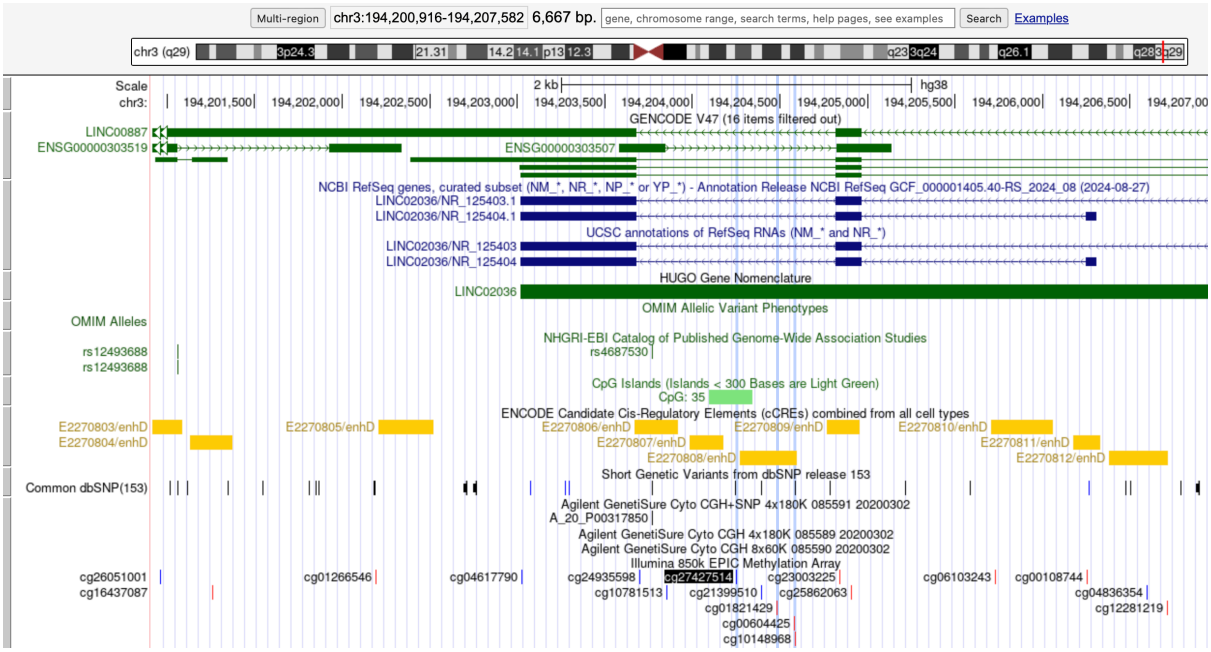


(A) Longitudinal whole-group effect (paired t-test: $t(75)= -5.14$; p-value = 2.15×10^{-6}; Cohen's D= 0.59); (B) DNA methylation difference and MADRS difference between baseline and End of RCT. For ketamine patients: $r(31)=0.36$,p=0.042. For midazolam patients: n.s.; (C) DNA

methylation per treatment group and per time-point: Fixed doses combined: $t(39)= -3.33$; $p < 0.005$, Cohen's D = 0.53 (estimated mean difference = -0.068); Flexible doses combined: $t(35)= -3.91$; $p < 0.001$; Cohen's D = 0.65 (estimated mean difference = -0.098).

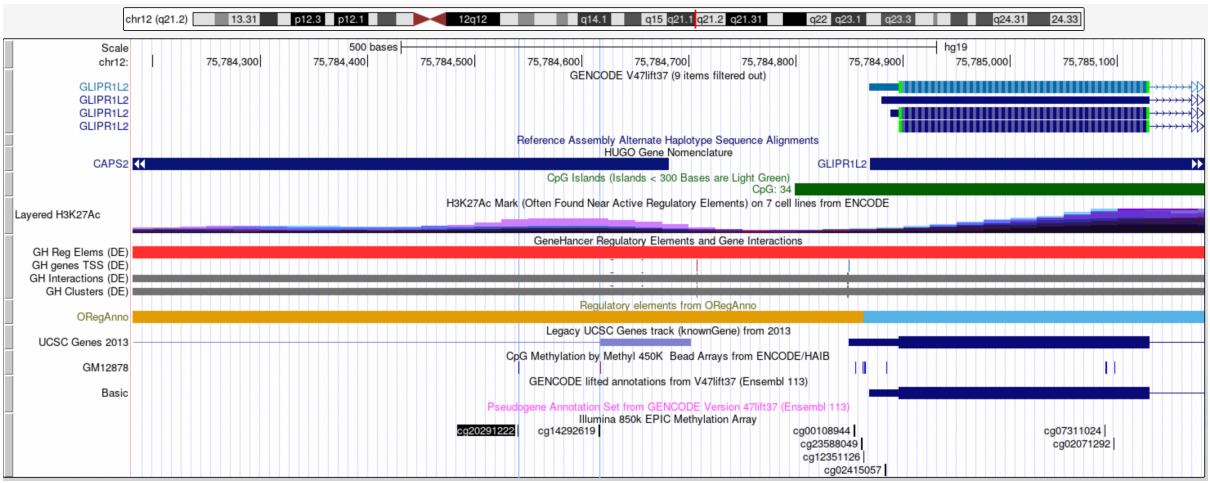
8. Genomic plots of results

Figure S15: Location of three CpGs from the top 10 of the cross-sectional fixed doses comparison between midazolam and ketamine. The region is linked to long non-coding and other regulatory genomic elements.



The three vertical blue lines represent the three CpGs within the 10 most statistically significant CpGs of the analysis (see also table S3). Source: UCSC Genome Browser

Figure S16: CAPS 2 and GLIPR1L2. Significant DMR for the flexible dosed ketamine paired longitudinal analysis (two CpGs).



The two vertical blue lines represent the two CpGs from the significant DMR for the longitudinal analysis of flexibly dosed ketamine. Source: UCSC Genome Browser

References

1. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. The American Journal of Human Genetics. 2007;81(3):559–75.

2. Anderson C a, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. Nature protocols. 2010 Sep;5(9):1564–73.

3. Salas LA, Koestler DC, Butler RA, Hansen HM, Wiencke JK, Kelsey KT, et al. An optimized library for reference-based deconvolution of whole-blood biospecimens assayed using the Illumina HumanMethylationEPIC BeadArray. Genome Biol. 2018 May 29;19(1):64.