

Supplementary file 1: Exposure to childhood maltreatment is associated with specific epigenetic patterns in sperm

DNAme validation analysis

Methods

Targeted bisulfite pyrosequencing was performed in the same samples used for RRBS to validate the reported DMRs. Specifically, the top significant DMR (CTRC1, FDR=0.035), as well as two additional nominally significant DMRs with FDR<0.16 (C8orf58/PDLIM2 and FSCN1/RNF216) were chosen as validation targets. In addition, we chose a single, non-significant DMR (MRPL36, FDR=0.74) to serve as a negative control. DNA sequences were retrieved from the GRCh38 assembly using the UCSC genome browser, and theoretical CpGs were therefore included in the primer design. Several primers targeting each region were designed using Qiagen PyroMark Assay Design Software 2.0. Ultimately, one primer pair was selected for each DMR, based on the best genomic overlap with the previously reported regions. Sequences for PCR primer pairs and sequencing primers can be viewed in supplementary table X. Briefly, 200 ng of genomic DNA was bisulfite-converted using the EZ DNA Methylation-Gold Kit (Zymo Research), and 10-15 ng of bisulfite-converted DNA was amplified with the Qiagen PyroMark PCR kit following manufacturer's instructions. Samples were sequenced on the Pyromark Q48 Autoprep, and data was analyzed with the PyroMark Q48 Autoprep software.

Statistical tests

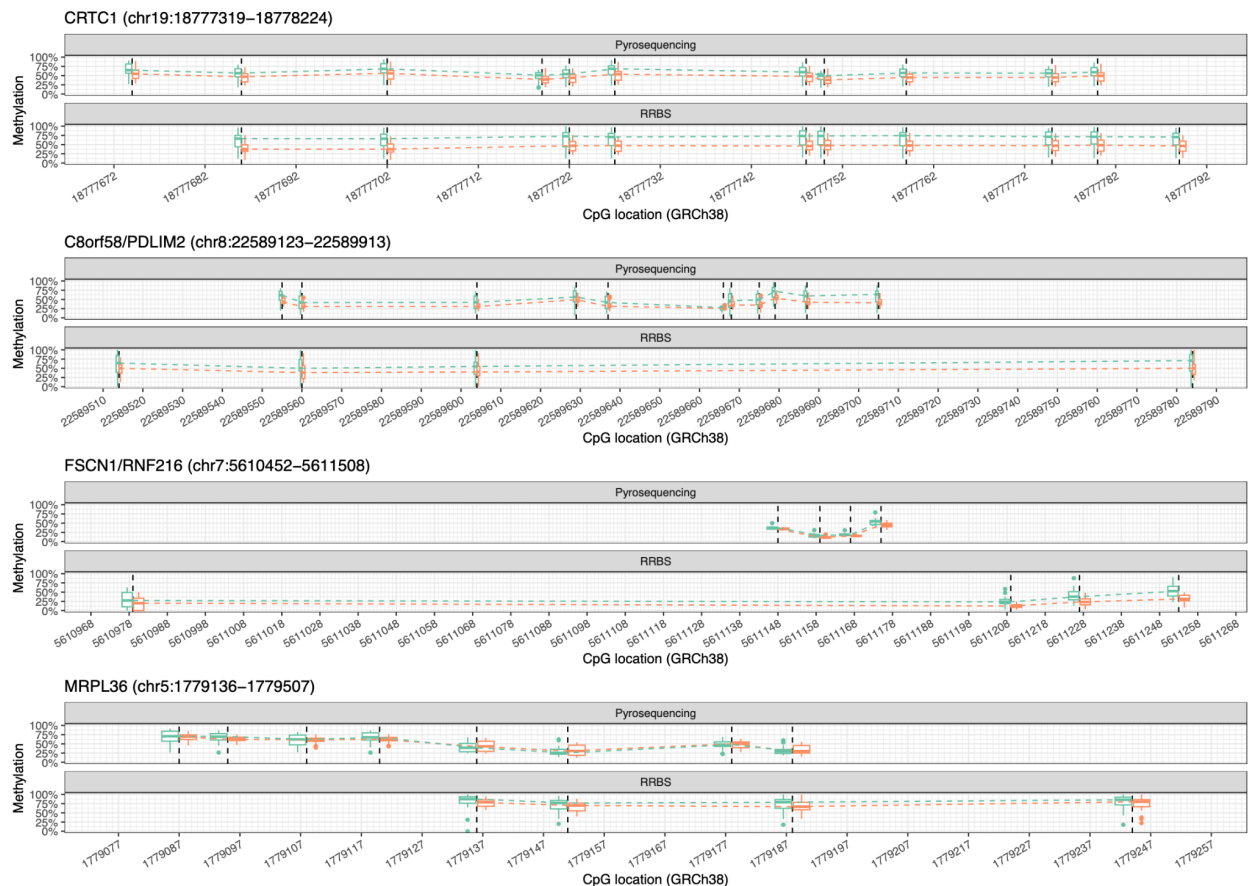
To test for significant differences in methylation levels between the high-TADS and the low-TADS group in the validation data, we modeled the methylation level at each CpG site independently. We used a simple beta-regression model framework (betareg R-package), that appropriately handles 0/100% methylation measurements. Specifically, we used the TADS-group indicator variable as the main explanatory variable, along with the following covariates: BMI, age, smoking (yes/no), daily alcohol consumption, summed SCL-90-R scores (assessment of current psychological symptoms), summed EPDS-scores (assessment of current depression symptoms), semen volume, and purified semen concentration.

To determine significance at the individual CpG sites, we extracted the P-values for the null hypothesis assuming no effect of the TADS-group variable, and used a significance threshold of $P < 0.05$.

Main findings

We find that the decreased methylation percentage in the high-TADS group compared to the low-TADS group is generally conserved in the validation data for all three DMRs (Supplementary Figure A).

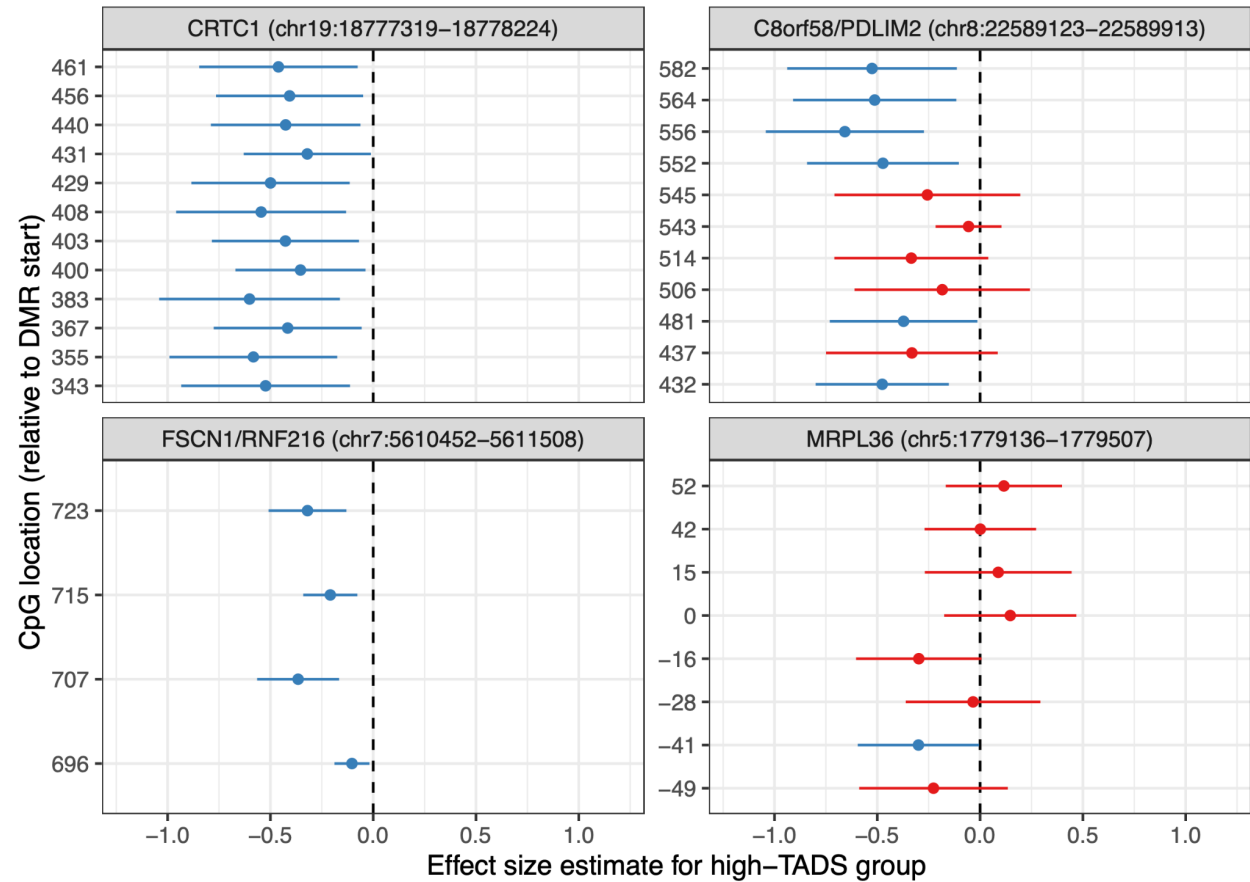
Group  low-TADS  high-TADS



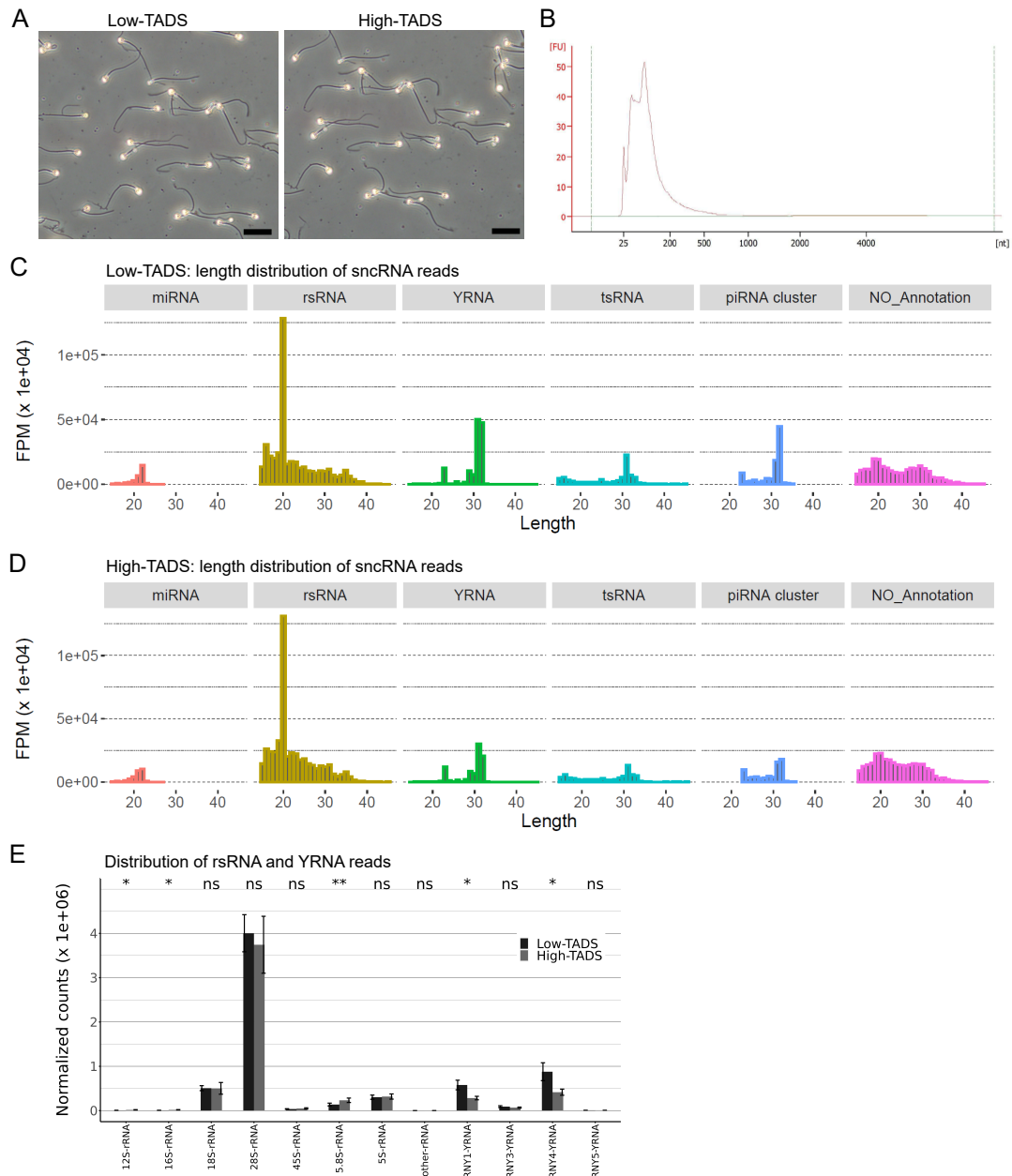
Supplementary figure A: Methylation results from pyrosequencing-based validation of RRBS data. Methylation levels are shown for each CpG site at the four DMRs: CRT1 (FDR=0.035), C8orf58/PDLIM2 (FDR=0.11), FSCN1/RNF216 (FDR=0.16), and MRPL36 (FDR=0.74). The experimentally observed CpG sites from either pyrosequencing or RRBS are indicated by dashed vertical lines. The median methylation levels between neighboring CpG sites across the low and high TADS groups are connected by green and orange dashed lines respectively.

Notably, there is a significant ($P < 0.05$) effect of high TADS at all CpG sites within two of the three tested DMRs: CRT1/ and FSCN1/RNF216 (Supplementary Figure B). While the use of more complex statistical models that account for correlation between neighboring CpGs would

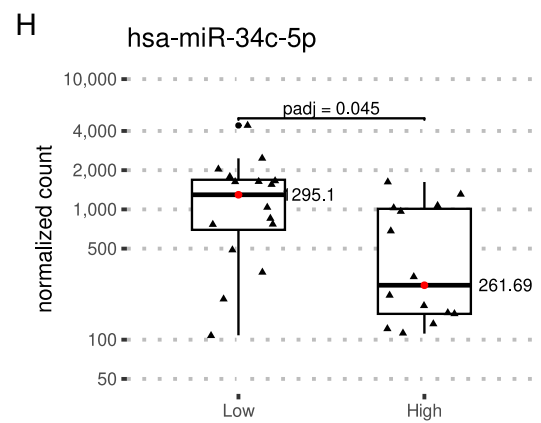
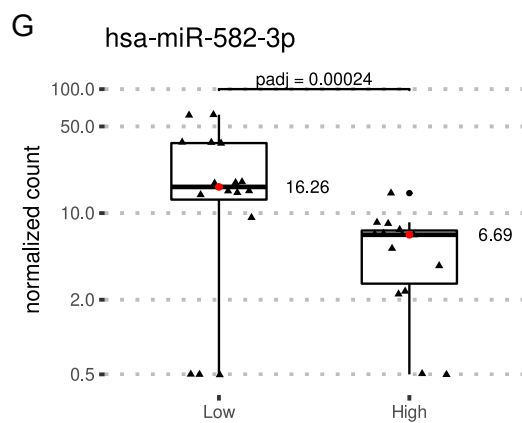
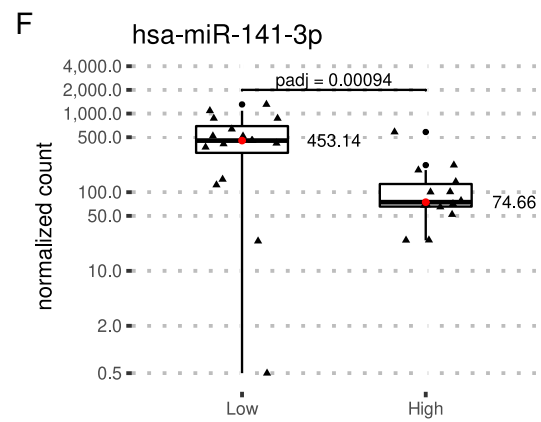
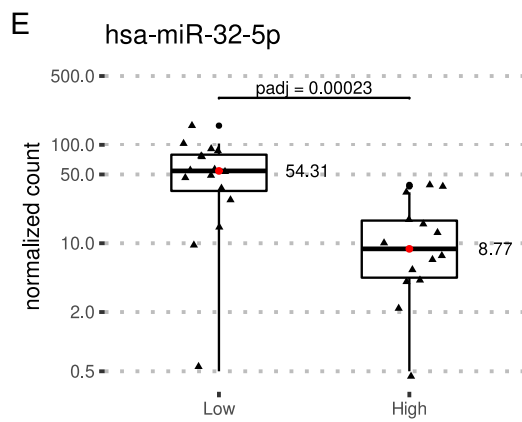
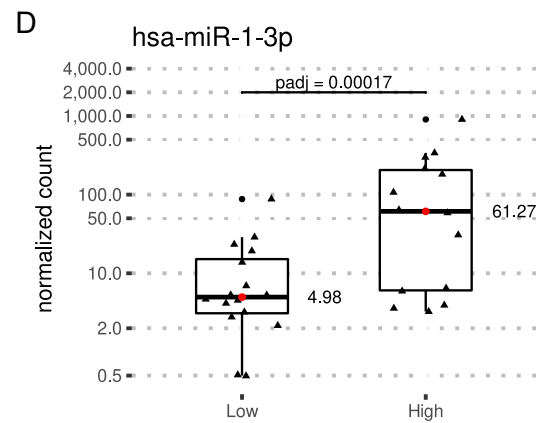
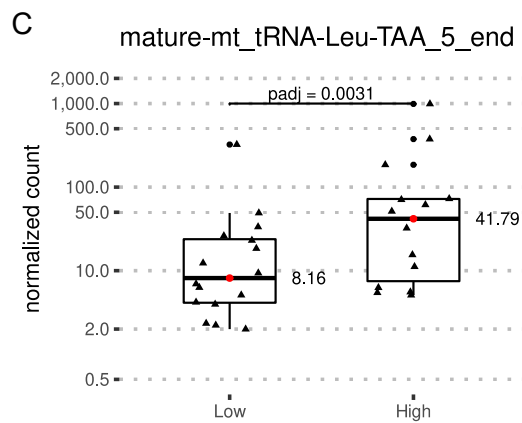
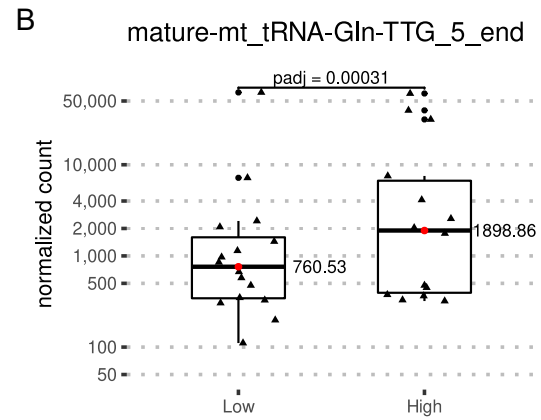
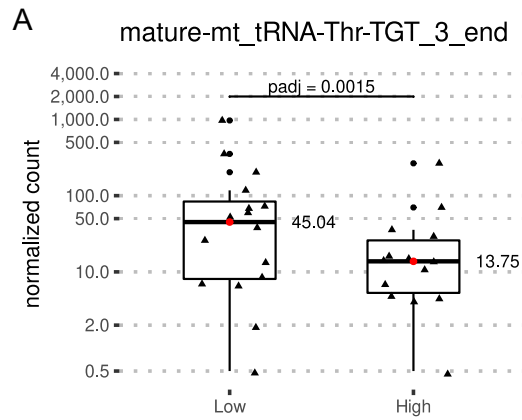
be preferred, we argue that this approach is sufficient validation of the reported results at the current stage. However, it should be noted that the performed pyrosequencing is only a technical validation of the reported results, and a biological replication by others would therefore be greatly informative.



Supplementary figure B: Statistical evaluation of the TADS-group effect in the pyrosequencing validation data. Beta regression effect size estimates with 95% confidence are given on the untransformed scale. A blue color indicates significance (null hypothesis $B=0$, $P<0.05$) of the TADS-group variable at the given CpG.

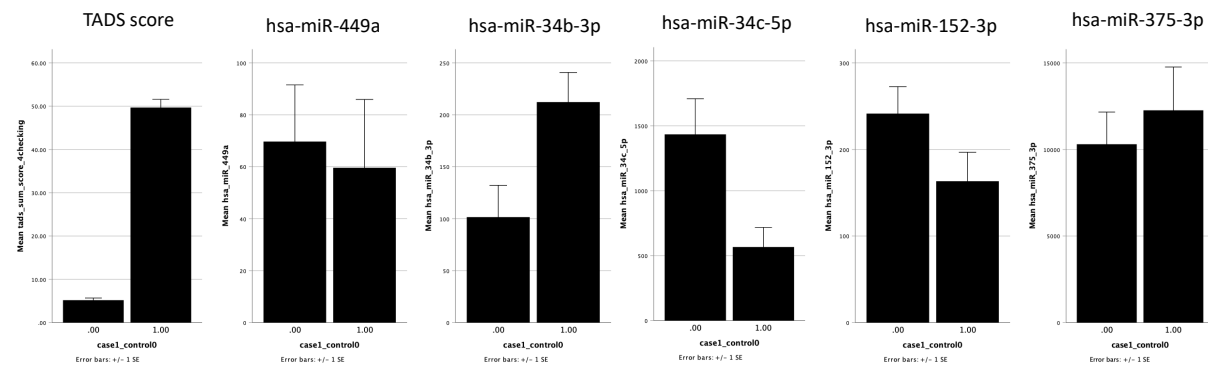


Supplementary Figure 1. Quality control of sperm samples. **(A)** Phase contrast images of representative unstained sperm samples from low-TADS and high-TADS individuals. Scale bars: 20 μ m. **(B)** Representative bioanalyzer profile of sperm total RNA sample (low-TADS). **(C, D)** Size distribution of sperm sncRNA reads, representative examples of low-TADS (C) and high-TADS (D) samples are shown. FPM: average fragments per million. Size of sncRNA reads is indicated in nucleotides on the x-axis (Length). **(E)** Distribution of rsRNA and YRNA reads in low- and high-TADS sperm samples. The bars represent the means $\pm$ standard error of the normalized reads (FPM, fragment per million) mapping to different types of rsRNAs and YRNAs in low-TADS (n = 16) and high-TADS (n = 14) samples. Wilcoxon-rank exact test indicated significant differences in the abundance of 12S-rsRNA (W=162, p=0.03827), 16S-rsRNA (W=170, p=0.0152), and 5.8-rsRNA (W=177, p=0.005991), as well as for RNY1 (W=58, p=0.02454) and RNY4 (W=57, p=0.02184).

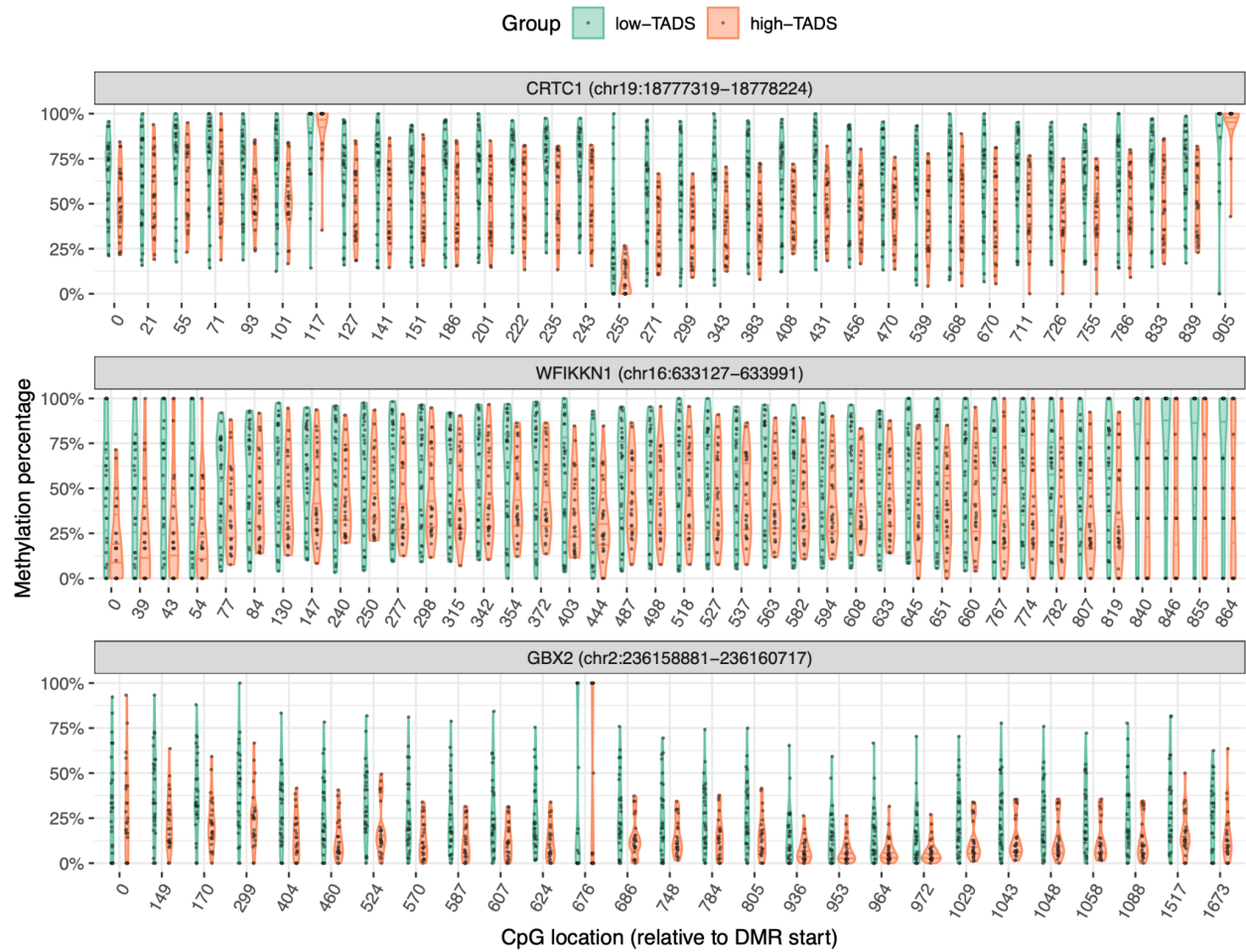


Supplementary Figure 2. Expression levels of a selection of individual sncRNAs in high-TADS and low-TADS sperm samples. (A-G) Box plots show the normalized expression levels of selected sncRNAs with an absolute log2 fold change > 2.0, Padj < 0.01 and baseMean > 10. **(A)** mature-mt_tRNA-Thr-TGT_3_end; **(B)** mature-mt_tRNA-Gln-TTG_5_end; **(C)** mature-mt_tRNA-Leu-TAA_5_end; **(D)** hsa-miR-1-3p; **(E)** hsa-miR-32-5p; **(F)** hsa-miR-141-3p; **(G)** hsa-miR-582-3p. **(H)** Box plot show the normalized expression level of hsa-miR-34c-5p (log2 fold change = -1.2, Padj = 0.045 and baseMean = 992). P-adjusted value was calculated by DESeq2 during differential expression analysis.

Supplementary Figure 3. Replication of Dickson et al. 2018 [replicating plots in Figure 1].



Supplementary figure 3. The bar plots depict mean values and error bars standard deviations to and possible between group differences between low and high ACE exposure and miRNA expression levels that were identified in prior work (Dickson et al. 2018). We replicated the lower expression levels in ACE exposed group for hsa-miR-34c-5p ($W = 140$, $p = 0.017$, rank biserial correlation [rbc] = 0.538) and that there were no differences in expression levels of hsa-miR-152-3p and hsa-miR-375-3p. Our analyses did not replicate group differences for has-miR-449a. Finally, we found the opposite group difference for hsa-miR-34b-3p, higher expression levels in the ACE exposed group ($W = 24$, $p < 0.001$, $rbc = -0.736$).



Supplementary figure 4. Raw methylation percentages per individual by TADS-group. Raw methylation levels are unadjusted for covariates. To avoid overplotting, only every second CpG site is visualized at the three DMRs.

Supplementary Table 1. Zero order correlations of the variables presented in Table 1.

Supplement 1. Full correlation matrix for the demographics data; including the TADS case control division and the availability of the sncRNA samples																
Variable																
1. Age	Spearman's rho	—														
	p-value	—														
2. BMI	Spearman's rho	-0.183	—													
	p-value	0.182	—													
3. waist circum.	Spearman's rho	-0.038	0.876	—												
	p-value	0.785	< .001	—												
4. EPDS	Spearman's rho	0.101	-0.206	-0.144	—											
	p-value	0.464	0.131	0.295	—											
5. SCL	Spearman's rho	-0.013	0.016	0.036	0.631	—										
	p-value	0.927	0.908	0.796	< .001	—										
6. TADS factor sum	Spearman's rho	0.252	0.099	-0.003	0.445	0.371	—									
	p-value	0.063	0.472	0.984	< .001	0.005	—									
7. TADS direct sum	Spearman's rho	0.210	0.072	-0.028	0.385	0.331	0.933	—								
	p-value	0.123	0.602	0.838	0.004	0.013	< .001	—								
8. Education	Spearman's rho	0.288	-0.283	-0.174	0.036	-0.082	-0.267	-0.186	—							
	p-value	0.033	0.036	0.204	0.794	0.551	0.049	0.174	—							
9. Relationship status	Spearman's rho	0.265	-0.300	-0.198	0.028	-0.003	0.052	-0.003	0.032	—						
	p-value	0.051	0.026	0.148	0.841	0.982	0.708	0.984	0.816	—						
10. Smoking	Spearman's rho	-0.182	0.026	0.167	-0.028	0.036	-0.010	0.033	0.000	0.162	—					
	p-value	0.183	0.848	0.222	0.839	0.792	0.944	0.813	1.000	0.239	—					
11. Semen volume	Spearman's rho	0.050	-0.097	-0.123	0.067	0.024	-0.165	-0.176	0.202	0.027	-0.112	—				
	p-value	0.717	0.483	0.370	0.626	0.860	0.229	0.198	0.140	0.843	0.415	—				
12. Sperm concentration	Spearman's rho	-0.085	-0.005	-0.095	-0.115	-0.099	-0.003	0.031	-0.224	-0.251	-0.192	-0.344	—			
	p-value	0.535	0.969	0.492	0.402	0.473	0.981	0.822	0.100	0.065	0.161	0.010	—			
13. Purified sperm conc.	Spearman's rho	-0.028	-0.102	-0.205	-0.178	-0.168	-0.074	-0.028	-0.147	-0.186	-0.261	0.009	0.866	—		
	p-value	0.838	0.458	0.133	0.193	0.220	0.590	0.840	0.283	0.173	0.054	0.951	< .001	—		
14. low / high CME	Spearman's rho	0.268	0.077	-0.045	0.393	0.429	0.870	0.864	-0.152	-0.045	-0.048	-0.169	-0.030	-0.066	—	
	p-value	0.052	0.585	0.751	0.004	0.001	< .001	< .001	0.277	0.747	0.734	0.227	0.833	0.640	—	
15. sncRNA data (yes/no)	Spearman's rho	-0.015	0.085	0.104	-0.050	-0.028	-0.072	-0.070	-0.037	-0.136	0.128	-0.036	-0.105	-0.033	-0.017	—
	p-value	0.914	0.538	0.449	0.718	0.841	0.603	0.611	0.791	0.322	0.352	0.797	0.444	0.810	0.903	—
	Variable	1. Age	2. BMI	3. waist circum.	4. EPDS	5. SCL	6. TADS factor sum	7. TADS direct sum	8. Education	9. Relationship status	10. Smoking	11. Semen volume	12. Sperm concentration	13. Purified sperm conc.	14. low / high CME	15. sncRNA data (yes/no)

Supplementary Table 4. Differentially expressed piRNA clusters in high vs. low-TAD5 sperm samples. Misregulated piRNA clusters ($\log_2$ FC < -1.0 or > 1.0, P_{adj} < 0.05) are indicated in red.

ID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
ID=1896	6.2637040777	-0.028461386	0.694953666	-5.796733773	6.76E-09	3.15E-05
ID=792	8.257139279	-1.304914545	0.136315464	-4.12535805	3.70E-05	0.045379705

47569	2338.850156	2.437354777	0.592496189	4.113705484	3.89E-05	0.045379705
47570	7.197205284	2.260230555	0.540971676	4.178084081	2.94E-05	0.045379705
47571	2393746048	-2.395793929	0.368692823	-3.779634055	0.000157059	0.146441828
47572	4273115372	2.647210011	0.713368313	3.619554269	0.000291751	0.223901131
47573	913021727	-2.829984079	0.797431601	-3.548873753	0.000386838	0.257663578
47574	5639369544	2.29158721	0.564100847	3.503415752	0.000495932	0.267657681
47575	1436217309	-1.527572215	0.456328717	-3.745729276	0.000815354	0.290158137
47576	1404067951	2.659027132	0.507120157	3.292015322	0.000994722	0.421581237
47577	1718295146	-2.305157589	0.603636026	-3.310329602	0.001291298	0.421581237
47578	2466617078	-1.421181195	0.465130825	-2.799921153	0.001451509	0.421581237
47579	2448681194	2.354864541	0.407518929	3.278097387	0.000554058	0.649846851
47580	1300247063	0.347279994	0.467379994	3.825877445	0.000554058	0.649846851
47581	299733106	-1.969233429	0.108398181	-2.80503081	0.000977241	0.649846851
47582	244366516	2.200734249	0.375102124	3.058187207	0.002276804	0.649846851
47583	1631052495	1.881661911	0.655989056	3.021146438	0.002515493	0.649846851
47584	773.1463645	1.065111418	0.365452534	2.291774525	0.003480424	0.649846851
47585	87.571897	-1.387974069	0.460283454	-2.015674781	0.002565756	0.649846851
47586	12.18494798	1.610943685	0.576001353	3.197676903	0.000165129	0.649846851
47587	9.611799439	1.354393936	0.589181153	2.951214809	0.003165767	0.649846851
47588	7.16938089	1.525370391	0.542033643	3.814777183	0.004811106	0.649846851
47589	88.2830362	-0.89289938	0.317519863	-2.186186203	0.004951825	0.649846851
47590	90.48619487	-0.882001426	0.307705459	-2.866390597	0.004151816	0.649846851
47591	92.19640976	-0.898359153	0.311108694	-2.887602172	0.003881898	0.649846851
47592	91.79302926	-0.885860546	0.314496445	-2.851245429	0.004874	0.649846851
47593	29.9586154	1.771021065	0.608291148	2.88051442	0.003698082	0.649846851
47594	54.7689668	-1.707476198	0.164963618	-2.806981587	0.00500081	0.649846851
47595	1.81662633	1.856026952	0.61398774	2.860214683	0.005017228	0.649846851
47596	25.9926244	1.748992477	0.627214411	2.788400717	0.005296907	0.649846851
47597	2.911661028	-1.69908282	0.551554475	-2.026998802	0.002407076	0.649846851
47598	2.95351386	1.4054133	0.468624505	2.868698957	0.002407076	0.649846851
47599	17.8251227	-1.463270787	0.451562193	-2.68910083	0.001812343	0.649846851
47600	2.448681194	2.354864541	0.407518929	3.278097387	0.000554058	0.649846851
47601	10.52192746	-1.073640916	0.400714365	-2.679312827	0.003714751	0.649846851
47602	68.5666405	2.099993023	0.778595476	3.466866299	0.002341816	0.649846851
47603	2283.47806	1.743356168	0.658162773	2.648822663	0.0080772	0.649846851
47604	7.7690176	-2.134066198	0.04892108	-2.651072833	0.00023654	0.649846851
47605	92.2494902	-2.668941873	1.005230398	-2.655054881	0.002595551	0.649846851
47606	1.92549446	1.115017853	0.423427535	3.361334464	0	

	DICKSON ET AL. 2018		TUULARI ET AL. 2023				ROBERTS ET AL. 2018		TUULARI ET AL. 2023	
SPERM EPIGENOME ANALYSIS	sncRNA		sncRNA				DNAm		DNAm	
SAMPLE SIZE	N = 28		N = 30				N = 34		N = 55	
SOURCE POPULATION	Infertility treatment clinic		Community sample				Community sample		Community sample	
MEAN AGE OF THE PARTICIPANTS	32.4 years		38.5 years				25.7 years		39.5 years	
DISCOVERY ANALYSIS	Low	High	Low	High			Low	High	Low	High
CONTROL VS. CASE (N)	5	5	16	14			12	17	30	25
MULTIVARIATE STATISTICS	No		Yes				Yes		Yes	
			A	B	C		A	B		
SEMEN SAMPLE VOLUME	X		*		*		*	*	*	
SPERM CONCENTRATION	X		*		*				*	
SPERM MORPHOLOGY	X									
AGE				*	*		*	*	*	
BMI	X			*	*			*	*	
SMOKING	X			*	*			*	*	
ALCOHOL USE	X			*	*				*	
DRUG USE	X							*		
DEPRESSIVE SYMPTOMS				*	*				*	
ANXIETY SYMPTOMS				*	*				*	
POST-TRAUMATIC STRESS								*		
ADULTHOOD TRAUMA								*		
MEASURE OF EARLY LIFE STRESS EXPOSURE	Adverse child experiences (ACE) questionnaire		Trauma and Distress Scale (TADS) questionnaire				Childhood Trauma Questionnaire (CTQ)		Trauma and Distress Scale (TADS) questionnaire	

MAIN FINDINGS	A negative correlation between levels of multiple miRNAs of the miR-449/34 family and ACE scores of Caucasian males.	A replication of the negative correlation between CME miR-34 but not with miR-449.	13 differentially methylated DNA regions associated with by childhood abuse (high abuse vs. no abuse); 3 negative associations and 10 positive associations.	3 differentially methylated DNA regions associated with by childhood abuse (high abuse vs. no abuse); all associations were negative.
	The authors also performed studies in mice and found the same sperm miRNA family are also reduced in mice exposed to adolescent chronic social instability.	Discovery analyses across a wider set of small non-coding RNAs revealed multiple associations with dozens of implicated sncRNAs.	The implicated sites included genes associated with neuronal function (<i>MAPT</i>, <i>CLU</i>), fat cell regulation (<i>PRDM16</i>), and immune function (<i>SDK1</i>).	The implicated sites included genes associated with neuronal function (<i>CRTC1</i>, <i>GBX2</i>).

Supplementary Table 5. Table comparing the data, sample sizes and statistical analyses in published studies on childhood trauma and sperm epigenome. (X = reported only in univariate linear regression model across the whole sample: miRNA (miR-449a / miR-34c) ~ X), (* = included in multivariate testing associations between early life stress and sperm epigenome that statistically adjusted for these potential confounders in the same model).

Footnotes:

Jawaid et al. report change in sperm miRNAs from men aged 21-50 years (n = 23, control n = 35) exposed to two or more significant traumatic events in childhood (Mann Whitney U test; no covariates reported). The traumatic events were defined from childhood trauma questionnaire (CTQ). The study was available as a preprint at the time of writing and did not perform discovery analyses, so the data are not included in the

table. **Reference:** Distinct microRNA signature in human serum and germline after childhood trauma; Ali Jawaid, et al 2020, medRxiv 2020.08.11.20168393; doi: <https://doi.org/10.1101/2020.08.11.20168393>

Methodological differences to prior work in sncRNA analyses: In contrast to the earlier study by Dickson et al. that used miRNA microarrays for initial screening of differentially expressed miRNAs (Dickson et al., 2018), we chose a genome-wide high throughput approach and used small RNA-seq to identify differentially expressed miRNAs in men exposed to high CME compared to men with low CME.

Methodological differences to prior work in DNA methylation analyses: The previous study by Roberts et al. (8) identified differentially methylated sperm DNA using methylation BeadChips, but we chose a sequencing-based approach and used RRBS. Of note, these methods have been shown to cover different CpG loci (33). Our approach was validated separately with pyrosequencing as detailed in the manuscript.

Supplementary Table 6. Multivariate statistics of miRNA						
	Variables		Low / High CME	Low / High CME	Low / High CME	Low / High CME
A) Controlling for semen sample volume sperm concentration	1. Low [0] / High [1] CME	Spearman's rho p-value	No covariates	A	B	C
			— —	— —	— —	— —
B) Controlling for age, BMI, smoking (yes / no), average alcohol use / day depressive symptoms anxiety symptoms	2. TADS sum score	Spearman's rho p-value	0.864 < .001	0.861 < .001	0.819 < .001	0.812 < .001
			0.641 < .001	0.623 < .001	0.585 0.005	0.576 0.010
	3. hsa-miR-1-3p	Spearman's rho p-value	0.622 < .001	-0.611 0.001	-0.609 0.003	-0.605 0.006
			0.634 < .001	0.598 0.002	0.484 0.026	0.487 0.035
C) Controlling for covariates in A + B.	6. hsa-miR-1251-5p	Spearman's rho p-value	-0.373 0.055	-0.373 0.066	-0.604 0.004	-0.590 0.008
			-0.536 0.004	-0.543 0.005	-0.675 < .001	-0.684 0.001
	8. hsa-miR-1307-5p	Spearman's rho p-value	-0.479 0.012	-0.555 0.004	-0.605 0.004	-0.650 0.003
			0.479 0.011	0.450 0.024	0.162 0.483	0.161 0.510
	10. hsa-miR-141-3p	Spearman's rho p-value	-0.612 < .001	-0.611 0.001	-0.641 0.002	-0.642 0.003
			-0.609 < .001	-0.622 < .001	-0.659 0.001	-0.677 0.001
	12. hsa-miR-148a-3p	Spearman's rho p-value	-0.574 0.002	-0.543 0.005	-0.570 0.007	-0.556 0.013
			0.607 < .001	0.572 0.003	0.567 0.007	0.552 0.014
	14. hsa-miR-196b-5p	Spearman's rho p-value	-0.536 0.004	-0.534 0.006	-0.582 0.006	-0.585 0.009
			-0.569 0.002	-0.623 < .001	-0.588 0.005	-0.643 0.003
	16. hsa-miR-200a-3p	Spearman's rho p-value	-0.507 0.007	-0.509 0.009	-0.538 0.012	-0.536 0.018
			-0.670 < .001	-0.677 < .001	-0.656 0.001	-0.663 0.002
	18. hsa-miR-210-3p	Spearman's rho p-value	-0.335 0.088	-0.400 0.048	-0.459 0.036	-0.537 0.018
			-0.603 < .001	-0.656 < .001	-0.643 0.002	-0.685 0.001
	20. hsa-miR-29b-3p	Spearman's rho p-value	-0.421 0.029	-0.505 0.010	-0.660 0.001	-0.689 0.001
			-0.737 < .001	-0.757 < .001	-0.716 < .001	-0.739 < .001
	22. hsa-miR-30e-5p	Spearman's rho p-value	-0.651 < .001	-0.674 < .001	-0.622 0.003	-0.647 0.003
			-0.699 < .001	-0.718 < .001	-0.714 < .001	-0.736 < .001
	24. hsa-miR-323a-3p	Spearman's rho p-value	0.546 0.003	0.492 0.012	0.209 0.363	0.181 0.458
			0.557 0.003	0.484 0.014	0.402 0.071	0.366 0.123
	26. hsa-miR-34c-5p	Spearman's rho p-value	-0.469 0.014	-0.422 0.035	-0.443 0.044	-0.420 0.073
			0.224 0.261	0.101 0.631	0.131 0.572	0.030 0.904
	28. hsa-miR-362-3p	Spearman's rho p-value	-0.568 0.002	-0.656 < .001	-0.628 0.002	-0.693 0.001
			0.354 0.070	0.452 0.023	0.101 0.662	0.209 0.391
	30. hsa-miR-374a-5p	Spearman's rho p-value	-0.737 < .001	-0.760 < .001	-0.699 < .001	-0.733 < .001
			0.442 0.021	0.450 0.024	0.556 0.009	0.568 0.011
	32. hsa-miR-409-3p	Spearman's rho p-value	0.575 0.002	0.559 0.004	0.511 0.018	0.495 0.031
			-0.555 0.003	-0.520 0.008	-0.560 0.008	-0.543 0.016
	34. hsa-miR-433-3p	Spearman's rho p-value	0.732 < .001	0.792 < .001	0.726 < .001	0.769 < .001
			-0.502 0.008	-0.525 0.007	-0.533 0.013	-0.551 0.015
	36. hsa-miR-509-3p	Spearman's rho p-value	0.526 0.005	0.465 0.019	0.539 0.012	0.525 0.021
			0.447 0.019	0.385 0.057	0.295 0.194	0.269 0.266
	38. hsa-miR-532-3p	Spearman's rho p-value	0.440 0.022	0.396 0.050	0.310 0.172	0.285 0.237
			-0.550 0.003	-0.508 0.010	-0.496 0.022	-0.480 0.038
	40. hsa-miR-548ah-3p	Spearman's rho p-value	-0.579 0.002	-0.558 0.004	-0.534 0.013	-0.548 0.015
			0.544 0.003	0.559 0.004	0.561 0.008	0.587 0.008
	42. hsa-miR-582-3p	Spearman's rho p-value	-0.528 0.005	-0.501 0.011	-0.568 0.007	-0.565 0.012
			0.517 0.006	0.530 0.006	0.623 0.003	0.621 0.005
	44. hsa-miR-660-3p	Spearman's rho p-value	-0.761 < .001	-0.757 < .001	-0.727 < .001	-0.731 < .001
			-0.483 0.011	-0.512 0.009	-0.608 0.003	-0.641 0.003
	46. hsa-miR-760	Spearman's rho p-value	0.601 < .001	0.616 0.001	0.535 0.012	0.553 0.014
			-0.440 0.022	-0.471 0.017	-0.527 0.014	-0.546 0.016
	48. hsa-miR-891b	Spearman's rho p-value	-0.422 0.028	-0.424 0.035	-0.464 0.034	-0.473 0.041
			0.529 0.005	0.511 0.009	0.392 0.079	0.393 0.096

Variables		TADS sum score	TADS sum score	TADS sum score	TADS sum score
1. TADS sum score		No covariates	A	B	C
		— —	— —	— —	— —
2. Low [0] / High [1] CME	Spearman's rho p-value	0.864 < .001	0.861 < .001	0.819 < .001	0.812 < .001
		0.581 0.001	0.563 0.003	0.532 0.013	0.529 0.020
3. hsa-miR-1-3p	Spearman's rho p-value	-0.671 < .001	-0.664 < .001	-0.701 < .001	-0.692 0.001
		0.572 0.002	0.523 0.007	0.384 0.086	0.394 0.095
6. hsa-miR-1251-5p	Spearman's rho p-value	-0.531 0.004	-0.544 0.005	-0.796 < .001	-0.796 < .001
		-0.521 0.005	-0.545 0.005	-0.644 0.002	-0.677 0.001
7. hsa-miR-1296-5p	Spearman's rho p-value	-0.539 0.004	-0.617 0.001	-0.656 0.001	-0.702 < .001
		0.409 0.034	0.381 0.060	0.133 0.565	0.136 0.580
10. hsa-miR-141-3p	Spearman's rho p-value	-0.669 < .001	-0.663 < .001	-0.727 < .001	-0.717 < .001
		-0.672 < .001	-0.682 < .001	-0.743 < .001	-0.745 < .001
11. hsa-miR-141-5p	Spearman's rho p-value	-0.754 < .001	-0.736 < .001	-0.774 < .001	-0.762 < .001
		0.533 0.004	0.503 0.010	0.564 0.008	0.569 0.011
13. hsa-miR-18a-3p	Spearman's rho p-value	-0.617 < .001	-0.625 < .001	-0.671 < .001	-0.687 0.001
		-0.633 < .001	-0.671 < .001	-0.701 < .001	-0.729 < .001
16. hsa-miR-200a-3p	Spearman's rho p-value	-0.610 < .001	-0.609 0.001	-0.675 < .001	-0.665 0.002
		-0.646 < .001	-0.645 < .001	-0.686 < .001	-0.688 0.001
18. hsa-miR-210-3p	Spearman's rho p-value	-0.324 0.100	-0.394 0.051	-0.478 0.028	-0.596 0.007
		-0.524 0.005	-0.553 0.004	-0.602 0.004	-0.611 0.005
20. hsa-miR-29b-3p	Spearman's rho p-value	-0.410 0.034	-0.474 0.017	-0.682 < .001	-0.710 < .001
		-0.729 < .001	-0.755 < .001	-0.737 < .001	-0.775 < .001
22. hsa-miR-30e-5p	Spearman's rho p-value	-0.519 0.005	-0.509 0.009	-0.503 0.020	-0.495 0.031
		-0.662 < .001	-0.672 < .001	-0.723 < .001	-0.726 < .001
24. hsa-miR-323a-3p	Spearman's rho p-value	0.507 0.007	0.469 0.018	0.213 0.355	0.223 0.358
		0.545 0.003	0.460 0.021	0.435 0.049	0.374 0.115
26. hsa-miR-34c-5p	Spearman's rho p-value	-0.454 0.017	-0.398 0.049	-0.455 0.038	-0.409 0.082
		0.317 0.107	0.194 0.352	0.328 0.147	0.232 0.340
28. hsa-miR-362-3p	Spearman's rho p-value	-0.589 0.001	-0.658 < .001	-0.695 < .001	-0.734 < .001
		0.165 0.412	0.259 0.212	-0.100 0.665	-0.002 0.995
30. hsa-miR-374a-5p	Spearman's rho p-value	-0.665 < .001	-0.661 < .001	-0.644 0.002	-0.643 0.003
		0.466 0.014	0.455 0.022	0.653 0.001	0.639 0.003
32. hsa-miR-409-3p	Spearman's rho p-value	0.532 0.004	0.517 0.008	0.577 0.006	0.560 0.013
		-0.681 < .001	-0.657 < .001	-0.698 < .001	-0.686 0.001
34. hsa-miR-433-3p	Spearman's rho p-value	0.477 0.012	0.536 0.006	0.410 0.065	0.460 0.048
		-0.535 0.004	-0.569 0.003	-0.572 0.007	-0.602 0.006
36. hsa-miR-509-3p	Spearman's rho p-value	0.531 0.004	0.457 0.022	0.548 0.010	0.503 0.028
		0.486 0.010	0.417 0.038	0.354 0.115	0.322 0.179
37. hsa-miR-511-5p	Spearman's rho p-value	0.515 0.006	0.482 0.015	0.448 0.042	0.422 0.072
		-0.497 0.008	-0.442 0.027	-0.494 0.023	-0.450 0.053
40. hsa-miR-548ah-3p	Spearman's rho p-value	-0.503 0.007	-0.468 0.018	-0.466 0.033	-0.433 0.064
		0.731 < .001	0.752 < .001	0.772 < .001	0.787 < .001
42. hsa-miR-582-3p	Spearman's rho p-value	-0.683 < .001	-0.658 < .001	-0.762 < .001	-0.750 < .001
		0.559 0.002	0.572 0.003	0.688 < .001	0.688 0.001
44. hsa-miR-660-3p	Spearman's rho p-value	-0.824 < .001	-0.821 < .001	-0.814 < .001	-0.814 < .001
		-0.558 0.002	-0.580 0.002	-0.761 < .001	-0.775 < .001
46. hsa-miR-760	Spearman's rho p-value	0.510 0.007	0.521 0.008	0.432 0.051	0.444 0.057
		-0.535 0.004	-0.561 0.004	-0.718 < .001	-0.724 < .001
48. hsa-miR-891b	Spearman's rho p-value	-0.540 0.004	-0.534 0.006	-0.659 0.001	-0.653 0.002
		0.401 0.038	0.359 0.078	0.276 0.225	0.266 0.271

Supplementary Table 7. Multivariate statistics of tsRNA							
		Variables		Low / High CME	Low / High CME	Low / High CME	Low / High CME
A) Controlling for semen sample volume sperm concentration				No covariates	A	B	C
		1. Low [0] / High [1] CME	Spearman's rho	—	—	—	—
			p-value	—	—	—	—
B) Controlling for age, BMI, smoking (yes / no), average alcohol use / day depressive symptoms anxiety symptoms		2. TADS sum score	Spearman's rho	0.864	0.861	0.819	0.812
			p-value	< .001	< .001	< .001	< .001
		3. mature-mt_tRNA-Gln-TTG_5_end	Spearman's rho	0.182	0.187	0.098	0.135
			p-value	0.364	0.371	0.672	0.582
		4. mature-mt_tRNA-Gln-TTG_CCA_end	Spearman's rho	0.443	0.489	0.449	0.502
			p-value	0.021	0.013	0.041	0.029
		5. mature-mt_tRNA-Leu-TAA_5_end	Spearman's rho	0.345	0.365	0.379	0.416
C) Controlling for covariates in A + B.			p-value	0.078	0.073	0.090	0.077
		6. mature-mt_tRNA-Lys-TTT_CCA_end	Spearman's rho	0.208	0.268	0.136	0.228
			p-value	0.297	0.194	0.558	0.347
		7. mature-mt_tRNA-Thr-TGT_3_end	Spearman's rho	-0.330	-0.308	-0.477	-0.562
			p-value	0.093	0.134	0.029	0.012
		8. mature-mt_tRNA-Trp-TCA_CCA_end	Spearman's rho	0.048	0.118	0.059	0.151
			p-value	0.813	0.576	0.798	0.537
		9. mature-tRNA-Arg-CCG_3_end	Spearman's rho	-0.316	-0.177	-0.016	0.114
			p-value	0.109	0.398	0.944	0.641
		10. mature-tRNA-Arg-CCT_3_end	Spearman's rho	-0.507	-0.415	-0.373	-0.319
			p-value	0.007	0.039	0.096	0.182
		11. mature-tRNA-Asp-GTC	Spearman's rho	-0.622	-0.556	-0.588	-0.610
			p-value	< .001	0.004	0.005	0.006
		12. mature-tRNA-Asp-GTC_3_end	Spearman's rho	-0.402	-0.328	-0.434	-0.393
			p-value	0.038	0.109	0.049	0.096
		13. mature-tRNA-Asp-GTC_CCA_end	Spearman's rho	-0.737	-0.694	-0.713	-0.716
			p-value	< .001	< .001	< .001	< .001
		14. mature-tRNA-Glu-TTC_CCA_end	Spearman's rho	-0.756	-0.730	-0.724	-0.748
			p-value	< .001	< .001	< .001	< .001
		15. mature-tRNA-Gly-CCC_5_end	Spearman's rho	-0.478	-0.447	-0.525	-0.511
			p-value	0.012	0.025	0.014	0.025
		16. mature-tRNA-Gly-GCC_5_end	Spearman's rho	-0.545	-0.522	-0.528	-0.524
			p-value	0.003	0.007	0.014	0.021
		17. mature-tRNA-Leu-TAA_5_end	Spearman's rho	0.249	0.203	-0.040	-0.051
			p-value	0.211	0.329	0.862	0.835
		18. mature-tRNA-Lys-CTT	Spearman's rho	-0.345	-0.292	-0.070	-0.053
			p-value	0.078	0.157	0.762	0.828
		19. mature-tRNA-Lys-TTT_5_end	Spearman's rho	-0.373	-0.410	-0.570	-0.572
			p-value	0.055	0.042	0.007	0.011
		20. mature-tRNA-Met-CAT	Spearman's rho	-0.545	-0.593	-0.597	-0.638
			p-value	0.003	0.002	0.004	0.003
		21. mature-tRNA-Phe-GAA_5_end	Spearman's rho	-0.460	-0.468	-0.540	-0.552
			p-value	0.016	0.018	0.012	0.014
		22. mature-tRNA-SeC-TCA	Spearman's rho	-0.498	-0.481	-0.570	-0.564
			p-value	0.008	0.015	0.007	0.012
		23. mature-tRNA-Val-AAC	Spearman's rho	-0.373	-0.419	-0.434	-0.457
			p-value	0.055	0.037	0.049	0.049

Variables		TADS sum score	TADS sum score	TADS sum score	TADS sum score
		No covariates	A	B	C
1. TADS sum score	Spearman's rho	—	—	—	—
	p-value	—	—	—	—
2. Low [0] / High [1] CME	Spearman's rho	0.864	0.861	0.819	0.812
	p-value	< .001	< .001	< .001	< .001
3. mature-mt_tRNA-Gln-TTG_5_end	Spearman's rho	0.160	0.190	0.063	0.144
	p-value	0.425	0.363	0.785	0.558
4. mature-mt_tRNA-Gln-TTG_CCA_end	Spearman's rho	0.567	0.635	0.568	0.649
	p-value	0.002	< .001	0.007	0.003
5. mature-mt_tRNA-Leu-TAA_5_end	Spearman's rho	0.441	0.486	0.454	0.520
	p-value	0.021	0.014	0.039	0.022
6. mature-mt_tRNA-Lys-TTT_CCA_end	Spearman's rho	0.221	0.320	0.128	0.287
	p-value	0.269	0.119	0.579	0.233
7. mature-mt_tRNA-Thr-TGT_3_end	Spearman's rho	-0.367	-0.304	-0.534	-0.552
	p-value	0.060	0.140	0.013	0.014
8. mature-mt_tRNA-Trp-TCA_CCA_end	Spearman's rho	0.006	0.096	-0.031	0.105
	p-value	0.977	0.648	0.894	0.668
9. mature-tRNA-Arg-CCG_3_end	Spearman's rho	-0.268	-0.098	-0.005	0.164
	p-value	0.177	0.642	0.981	0.502
10. mature-tRNA-Arg-CCT_3_end	Spearman's rho	-0.465	-0.368	-0.300	-0.228
	p-value	0.015	0.070	0.186	0.347
11. mature-tRNA-Asp-GTC	Spearman's rho	-0.718	-0.665	-0.713	-0.747
	p-value	< .001	< .001	< .001	< .001
12. mature-tRNA-Asp-GTC_3_end	Spearman's rho	-0.381	-0.287	-0.431	-0.373
	p-value	0.050	0.165	0.051	0.116
13. mature-tRNA-Asp-GTC_CCA_end	Spearman's rho	-0.717	-0.660	-0.694	-0.680
	p-value	< .001	< .001	< .001	0.001
14. mature-tRNA-Glu-TTC_CCA_end	Spearman's rho	-0.613	-0.577	-0.545	-0.583
	p-value	< .001	0.003	0.011	0.009
15. mature-tRNA-Gly-CCC_5_end	Spearman's rho	-0.486	-0.438	-0.587	-0.551
	p-value	0.010	0.028	0.005	0.015
16. mature-tRNA-Gly-GCC_5_end	Spearman's rho	-0.565	-0.525	-0.604	-0.575
	p-value	0.002	0.007	0.004	0.010
17. mature-tRNA-Leu-TAA_5_end	Spearman's rho	0.053	0.003	-0.252	-0.246
	p-value	0.792	0.989	0.271	0.310
18. mature-tRNA-Lys-CTT	Spearman's rho	-0.330	-0.280	-0.076	-0.047
	p-value	0.093	0.175	0.744	0.847
19. mature-tRNA-Lys-TTT_5_end	Spearman's rho	-0.330	-0.351	-0.540	-0.523
	p-value	0.093	0.086	0.012	0.022
20. mature-tRNA-Met-CAT	Spearman's rho	-0.671	-0.723	-0.746	-0.775
	p-value	< .001	< .001	< .001	< .001
21. mature-tRNA-Phe-GAA_5_end	Spearman's rho	-0.609	-0.631	-0.663	-0.683
	p-value	< .001	< .001	0.001	0.001
22. mature-tRNA-SeC-TCA	Spearman's rho	-0.594	-0.585	-0.648	-0.646
	p-value	0.001	0.002	0.001	0.003
23. mature-tRNA-Val-AAC	Spearman's rho	-0.547	-0.599	-0.656	-0.675
	p-value	0.003	0.002	0.001	0.002

ID	chr	start	end	direction	h_diff_perct	coefficient	statistic	p.value	q.value	wic
19:187,719		18777319	18778224	Hypomethy	19	-0.59763	-21.845	3.62E-05	0.034391	

6331027	6339916	6340096	6340128	6340156	6340184	6340212	6340240	6340268	6340296	6340324	6340352	6340380	6340408	6340436	6340464	6340492	6340520	6340548	6340576	6340604	6340632	6340660	6340688	6340716	6340744	6340772	6340800	6340828	6340856	6340884	6340912	6340940	6340968	6340996	6341024	6341052	6341080	6341108	6341136	6341164	6341192	6341220	6341248	6341276	6341304	6341332	6341360	6341388	6341416	6341444	6341472	6341500	6341528	6341556	6341584	6341612	6341640	6341668	6341696	6341724	6341752	6341780	6341808	6341836	6341864	6341892	6341920	6341948	6341976	6342004	6342032	6342060	6342088	6342116	6342144	6342172	6342200	6342228	6342256	6342284	6342312	6342340	6342368	6342396	6342424	6342452	6342480	6342508	6342536	6342564	6342592	6342620	6342648	6342676	6342704	6342732	6342760	6342788	6342816	6342844	6342872	6342900	6342928	6342956	6342984	6343012	6343040	6343068	6343096	6343124	6343152	6343180	6343208	6343236	6343264	6343292	6343320	6343348	6343376	6343404	6343432	6343460	6343488	6343516	6343544	6343572	6343600	6343628	6343656	6343684	6343712	6343740	6343768	6343796	6343824	6343852	6343880	6343908	6343936	6343964	6343992	6344020	6344048	6344076	6344104	6344132	6344160	6344188	6344216	6344244	6344272	6344300	6344328	6344356	6344384	6344412	6344440	6344468	6344496	6344524	6344552	6344580	6344608	6344636	6344664	6344692	6344720	6344748	6344776	6344804	6344832	6344860	6344888	6344916	6344944	6344972	6345000	6345028	6345056	6345084	6345112	6345140	6345168	6345196	6345224	6345252	6345280	6345308	6345336	6345364	6345392	6345420	6345448	6345476	6345504	6345532	6345560	6345588	6345616	6345644	6345672	6345700	6345728	6345756	6345784	6345812	6345840	6345868	6345896	6345924	6345952	6345980	6346008	6346036	6346064	6346092	6346120	6346148	6346176	6346204	6346232	6346260	6346288	6346316	6346344	6346372	6346400	6346428	6346456	6346484	6346512	6346540	6346568	6346596	6346624	6346652	6346680	6346708	6346736	6346764	6346792	6346820	6346848	6346876	6346904	6346932	6346960	6346988	6347016	6347044	6347072	6347100	6347128	6347156	6347184	6347212	6347240	6347268	6347296	6347324	6347352	6347380	6347408	6347436	6347464	6347492	6347520	6347548	6347576	6347604	6347632	6347660	6347688	6347716	6347744	6347772	6347800	6347828	6347856	6347884	6347912	6347940	6347968	6347996	6348024	6348052	6348080	6348108	6348136	6348164	6348192	6348220	6348248	6348276	6348304	6348332	6348360	6348388	6348416	6348444	6348472	6348500	6348528	6348556	6348584	6348612	6348640	6348668	6348696	6348724	6348752	6348780	6348808	6348836	6348864	6348892	6348920	6348948	6348976	6349004	6349032	6349060	6349088	6349116	6349144	6349172	6349200	6349228	6349256	6349284	6349312	6349340	6349368	6349396	6349424	6349452	6349480	634950
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Supplementary table 9. Details of the pyrosequencing analyses

PCR primers for pyrosequencing

Name		Sequence (5' to 3', bio - biotinylated on 5' end)
<i>CTRC1</i> (3'UTR)	F	GAA GGT AGT GAG AGT TGG ATA AA
	R	bio – TAC AAA CCC TCC CTA CAC TAA C
<i>C8orf58/PDLIM2</i> (promoter)	F	bio – GTT TTT AGG GTT TTT TGT AAG AG
	R	CCA CCC CCT TAC TTA CCT
<i>FSCN1</i> (distal intergenic region)	F	TTT TTT TGG GAA GGA GTT TGG
	R	bio – CCC CTA TAA TCC CAA CTA CTC A
<i>MRPL36</i> (distal intergenic region)	F	GGA ATA TAT TGT ATA GGT TAA TTA TAA GT
	R	bio – TCC TAA TAA AAA AAA ACC CAA AAA AAC T

Sequencing primers for pyrosequencing

Name		Sequence (5' to 3')	Chromosomal region targeted (hg38)
<i>CTRC1</i>	F	GTG AGA GTT GGA TAA ATA G	chr19:18,777,661-18,777,792
<i>C8orf58</i>	F	CCC CCT TAC TTA CCT AA	chr8:22,589,551-22,589,731
<i>FSCN1</i>	F	GGA AGG AGT TTG GTT TT	chr7:5,611,146-5,611,178
<i>MRPL36</i>	F	AGT AAG TAG AAA TTA GAG TGT TG	chr5:1,779,081-1,779,213