

Supporting Information

for *Adv. Sci.*, DOI 10.1002/adv.202306294

Prenatal 1-Nitropyrene Exposure Causes Autism-Like Behavior Partially by Altering DNA Hydroxymethylation in Developing Brain

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Supplementary data

Method

Measurement of 5hmC

APOBEC-coupled epigenetic sequencing (ACE-Seq) was used to detect 5hmC content in specific genes. Multiplex PCR of *Nrg1*, *ErbB4* and *Sema3F* genes was performed. The primers were listed in supplemental Table S1. PCR products were then performed with T4 Phage β -glucosyltransferase and APOBEC3A (A3A). Following deamination, the products were sequenced using the Accel Methyl-NGS kit (Swift Biosciences, Inc). Bismark (v0.14.3) was used to map the trimmed reads to the reference genomes. For each cytosine within CG dinucleotides, “C” bases from ACE-Seq were considered to be 5hmC. By contrast, “T” bases were considered to be methylated or unmodified cytosines.

Figures

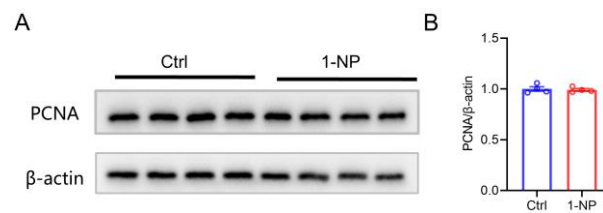


Fig.S1. Influence of gestational 1-NP exposure on PCNA protein in fetal forebrain. PCNA, a maker for cell proliferation, was determined by Western blotting. N=4. $t=0.317$, $df=6$, $P=0.762$.

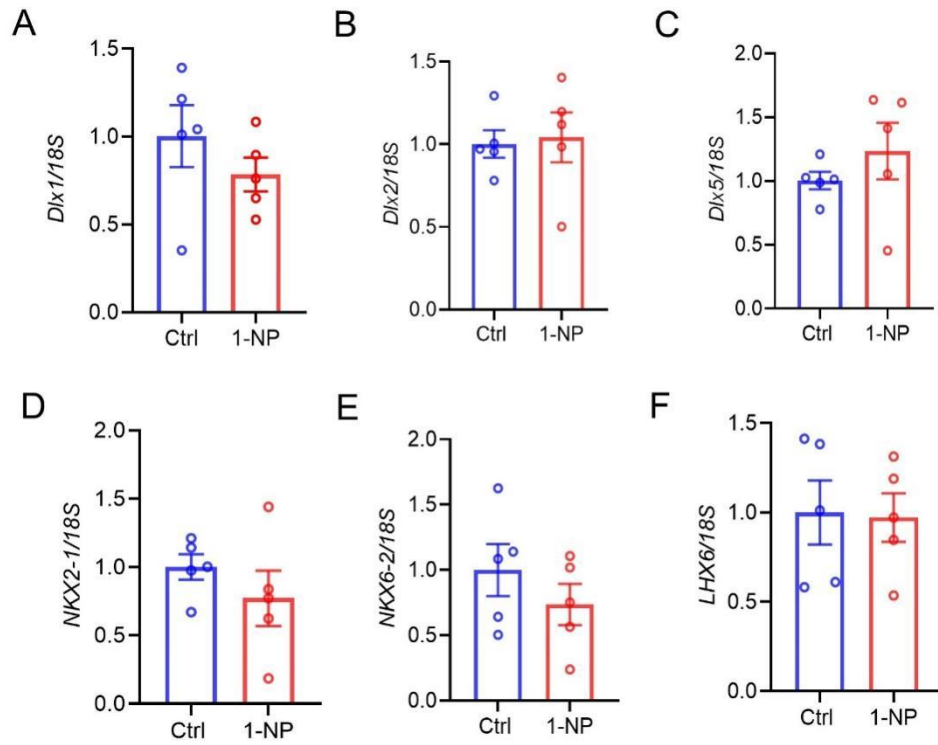


Fig.S2. Influence of gestational 1-NP exposure on the expression of differentiation related genes in forebrain interneuron. Differentiation-related genes in forebrain interneuron were detected in RT-PCR on GD14. N=5. (A) Dlx1. $t=1.087$, $df=8$, $P=0.308$. (B) Dlx2. $t=0.232$, $df=8$, $P=0.822$. (C) Dlx5. $t=0.999$, $df=8$, $P=0.347$. (D) NKX2-1. $t=1.027$, $df=8$, $P=0.334$. (E) NKX6-2. $t=1.037$, $df=8$, $P=0.330$. (F) LHX6. $t=0.126$, $df=8$, $P=0.903$.

Statistical analysis in the figure legends

Fig. 2. Influence of maternal 1-NP exposure on autism-like behavior in offspring.

	Groups		<i>t</i> value	df	<i>P</i> value
Figure 2B	1-NP (0 µg/kg)	S1 vs E	2.135	16	0.049
	1-NP (10 µg/kg)	S1 vs E	0.286	20	0.778
	1-NP (100 µg/kg)	S1 vs E	2.790	8.947	0.021
Figure 2C	1-NP (0 µg/kg)	S1 vs E	4.591	16	<0.01
	1-NP (10 µg/kg)	S1 vs E	2.395	13.52	0.032
	1-NP (100 µg/kg)	S1 vs E	0.416	20	0.682
Figure 2E	1-NP (0 µg/kg)	S1 vs S2	-2.296	16	0.036
	1-NP (10 µg/kg)	S1 vs S2	-2.017	15.057	0.052
	1-NP (100 µg/kg)	S1 vs S2	0.100	16	0.921
Figure 2F	1-NP (0 µg/kg)	S1 vs S2	-4.173	20	<0.01
	1-NP (10 µg/kg)	S1 vs S2	2.395	13.52	0.032
	1-NP (100 µg/kg)	S1 vs S2	-0.877	20	0.391
Figure 2H	1-NP (0 µg/kg)	S1 vs E	1.979	16	0.065
	1-NP (10 µg/kg)	S1 vs E	-0.045	20	0.965
	1-NP (100 µg/kg)	S1 vs E	-1.569	20	0.132
Figure 2I	1-NP (0 µg/kg)	S1 vs E	2.481	16	0.025
	1-NP (10 µg/kg)	S1 vs E	0.921	18	0.369
	1-NP (100 µg/kg)	S1 vs E	3.872	20	<0.01
Figure 2K	1-NP (0 µg/kg)	S1 vs S2	-2.275	16	0.037
	1-NP (10 µg/kg)	S1 vs S2	-0.676	20	0.507
	1-NP (100 µg/kg)	S1 vs S2	-0.358	20	0.724
Figure 2L	1-NP (0 µg/kg)	S1 vs S2	-4.221	16	<0.01
	1-NP (10 µg/kg)	S1 vs S2	-3.280	18	<0.01
	1-NP (100 µg/kg)	S1 vs S2	0.003	20	0.998

Fig. 3. Influence of maternal 1-NP exposure on mIPSC in offspring.

	Groups	<i>t</i> value	df	<i>P</i> value
Figure 3B	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	4.181	22	<0.01
Figure 3C	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	1.069	22	0.297
Figure 3E	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	0.145	22	0.887
Figure 3F	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.481	22	0.0212
Figure 3H	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	8.575	22	<0.01
Figure 3I	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.514	22	0.02
Figure 3K	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	0.815	22	0.424
Figure 3L	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	4.118	22	<0.01

Fig. 4. Influence of maternal 1-NP exposure on GAD67+ interneurons in weaning offspring.

	Groups	<i>t</i> value	df	<i>P</i> value
Figure 4B	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.558	6	0.043

Figure 4D	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)		0.075	6	0.943
	Cg1	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	6.985	4.517	<0.01
Figure 4F	PrL	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	4.796	6	<0.01
	IL	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	6.245	3.576	<0.01
	Cg1	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-0.872	6	0.417
Figure 4G	PrL	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-0.264	6	0.8
	IL	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	0.115	6	0.912
Figure 4I	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)		2.558	6	0.043
Figure 4K	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)		0.075	6	0.943
	Cg1	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	6.735	3.859	<0.01
Figure 4M	PrL	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	3.202	3.685	0.037
	IL	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.728	4	0.052
	Cg1	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-1.500	6	0.184
Figure 4N	PrL	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-2.477	6	0.048
	IL	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-0.647	6	0.541

Fig. 5. Influence of gestational 1-NP exposure on migration of interneurons in fetal brain.

	Groups		<i>t</i> value	df	<i>P</i> value
Figure 5B	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)		3.421	4	0.027
	SVZ/VZ	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-3.426	6	0.014
Figure 5D	IZ	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	0.110	6	0.916
	CP	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	3.279	6	0.017
	MZ	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	3.110	6	0.021
Figure 5F	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)		2.820	4	0.048
	SVZ/VZ	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-2.318	6	0.06
Figure 5H	IZ	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	0.460	6	0.662

	CP	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	6.401	6	<0.01
	MZ	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	4.267	6	<0.01
Figure 5J	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)		0.287	6	0.784
	Bin 1	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	1.082	6	0.321
Figure 5L	Bin 2	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.392	6	0.054
	Bin 3	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.591	6	0.041
Figure 5M	Cxcl12	Ctrl vs 1-NP	0.456	8	0.660
Figure 5N	Cxcr4	Ctrl vs 1-NP	0.194	8	0.851
Figure 5O	Cxcr7	Ctrl vs 1-NP	2.090	8	0.07
Figure 5P	Slit1	Ctrl vs 1-NP	0.6086	8	0.547
Figure 5Q	Efna5	Ctrl vs 1-NP	0.036	8	.9718
Figure 5R	Arx	Ctrl vs 1-NP	0.507	8	0.625
Figure 5S	Nrp1	Ctrl vs 1-NP	1.279	8	0.237
Figure 5T	Nrp2	Ctrl vs 1-NP	0.291	8	0.778
Figure 5U	Sema3A	Ctrl vs 1-NP	0.765	8	0.466
Figure 5V	Nrg1	Ctrl vs 1-NP	2.477	8	0.038
Figure 5W	ErbB4	Ctrl vs 1-NP	3.353	8	0.01
Figure 5X	Sema3F	Ctrl vs 1-NP	2.494	8	0.0373

Fig. 6. Influence of gestational 1-NP exposure on hydroxymethylation of interneuron migration-related genes in fetal forebrain.

	Groups	<i>t</i> value	df	<i>P</i> value	
	Nrg1_1	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-0.268	8	0.795
	Nrg1_2	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-0.679	8	0.516
Figure 6B	ErbB4	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-0.358	8	0.73
	Sema3F_1	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	3.264	8	0.011
	Sema3F_2	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-0.841	8	0.425
	Nrg1_2 (9246 site)	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.766	8	0.024
Figure 6C	ErbB4 (7743 site)	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.177	8	0.061
	ErbB4 (7866 site)	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.363	8	0.046

	Sema3F_1 (9269 site)	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.567	8	0.033
	Sema3F_1 (9348 site)	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	4.555	8	<0.01
	Sema3F_2 (0238 site)	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	2.577	8	0.033
Figure 6D	Tet1	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-2.634	4.330	0.053
	Tet2	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	0.86	8	0.415
	Tet3	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	-2.250	8	0.055
Figure 6F	Tet1	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	1.448	6	0.1978
	Tet2	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	0.791	6	0.459
	Tet3	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)	0.245	6	0.814
Figure 6G	1-NP (0 µg/kg) vs 1-NP (100 µg/kg)		3.400	8	<0.01

Fig. 7. Influence of gestational 1-NP exposure on mitochondrial function in fetal forebrain.

	Groups	<i>t</i> value	df	<i>P</i> value
Figure 7H	Ctrl vs 1-NP	0.263	6	0.802
Figure 7I	Ctrl vs 1-NP	2.890	6	0.027
Figure 7K	Ctrl vs 1-NP	4.133	6	<0.01
Figure 7L	Ctrl vs 1-NP	2.733	6	0.034
Figure 7N	Ctrl vs 1-NP	4.175	6	<0.01
Figure 7O	Ctrl vs 1-NP	2.791	6	0.024

Fig. 8. Effects of supplementation with α -KG on 1-NP-evoked hypohydroxymethylation of interneuron migration-related genes and interneuron migration inhibition in fetal forebrain.

	Groups	F value	df	<i>P</i> value
Figure 8A	α -KG main effect	0.587	1	0.455
	1-NP main effect	20.039	1	<0.01
	α -KG*1-NP interaction	4.355	1	0.053
	Ctrl vs 1-NP	-	-	<0.01
	1-NP vs 1-NP+ α -kG	-	-	0.061
	Figure 8B	α -KG main effect	0.075	1
1-NP main effect		1.671	1	0.214
α -KG*1-NP interaction		1.759	1	0.203
Ctrl vs 1-NP		-	-	0.083
1-NP vs 1-NP+ α -kG		-	-	0.274

Figure 8C		α -KG main effect	0.063	1	0.804
		1-NP main effect	12.403	1	<0.01
		α -KG*1-NP interaction	1.593	1	0.225
		Ctrl vs 1-NP	-	-	<0.01
		1-NP vs 1-NP+ α -kG	-	-	0.300
Figure 8D		α -KG main effect	0.588	1	0.454
		1-NP main effect	5.353	1	0.034
		α -KG*1-NP interaction	9.544	1	<0.01
		Ctrl vs 1-NP	-	-	<0.01
		1-NP vs 1-NP+ α -kG	-	-	0.120
Figure 8E		α -KG main effect	0.014	1	0.909
		1-NP main effect	1.785	1	0.200
		α -KG*1-NP interaction	1.512	1	0.237
		Ctrl vs 1-NP	-	-	0.088
		1-NP vs 1-NP+ α -kG	-	-	0.443
Figure 8F		α -KG main effect	5.145	1	0.038
		1-NP main effect	3.712	1	0.072
		α -KG*1-NP interaction	10.504	1	<0.01
		Ctrl vs 1-NP	-	-	<0.01
		1-NP vs 1-NP+ α -kG	-	-	<0.01
Figure 8G		α -KG main effect	0.199	1	0.662
		1-NP main effect	3.036	1	0.101
		α -KG*1-NP interaction	6.155	1	0.025
		Ctrl vs 1-NP	-	-	<0.01
		1-NP vs 1-NP+ α -kG	-	-	<0.01
Figure 8H		α -KG main effect	0.040	1	0.843
		1-NP main effect	14.699	1	<0.01
		α -KG*1-NP interaction	5.187	1	0.037
		Ctrl vs 1-NP	-	-	<0.01
		1-NP vs 1-NP+ α -kG	-	-	0.090
Figure 8I		α -KG main effect	0.634	1	0.438
		1-NP main effect	7.255	1	0.016
		α -KG*1-NP interaction	1.981	1	0.178
		Ctrl vs 1-NP	-	-	0.010
		1-NP vs 1-NP+ α -kG	-	-	0.069
Figure 8K	Bin 1	α -KG main effect	1.473	1	0.248
		1-NP main effect	0.829	1	0.381
		α -KG*1-NP interaction	0.655	1	0.434
		Ctrl vs 1-NP	-	-	0.247
		1-NP vs 1-NP+ α -kG	-	-	0.178
	Bin 2	α -KG main effect	13.963	1	<0.01
		1-NP main effect	2.509	1	0.139
	α -KG*1-NP interaction	2.258	1	0.159	

	Ctrl vs 1-NP	-	-	0.050
	1-NP vs 1-NP+ α -kG	-	-	<0.01
	α -KG main effect	25.004	1	<0.01
	1-NP main effect	25.814	1	<0.01
Bin 3	α -KG*1-NP interaction	2.033	1	0.179
	Ctrl vs 1-NP	-	-	<0.01
	1-NP vs 1-NP+ α -kG	-	-	<0.01

Fig. 9. Effects of gestational α -KG supplementation on interneurons in weaning offspring.

	Groups	F value	df	P value
Figure 9B	α -KG main effect	3.062	1	0.118
	1-NP main effect	15.657	1	<0.01
	α -KG*1-NP interaction	5.158	1	0.052
	Ctrl vs 1-NP	-	-	<0.01
	1-NP vs 1-NP+ α -kG	-	-	0.022
Cg1	α -KG main effect	13.905	1	<0.01
	1-NP main effect	20.137	1	<0.01
	α -KG*1-NP interaction	12.084	1	<0.01
	Ctrl vs 1-NP	-	-	<0.01
	1-NP vs 1-NP+ α -kG	-	-	<0.01
Figure 9D	α -KG main effect	4.842	1	0.048
	1-NP main effect	16.70	1	<0.01
	α -KG*1-NP interaction	3.798	1	0.075
	Ctrl vs 1-NP	-	-	<0.01
	1-NP vs 1-NP+ α -kG	-	-	0.013
IL	α -KG main effect	2.803	1	0.120
	1-NP main effect	11.212	1	<0.01
	α -KG*1-NP interaction	2.803	1	0.120
	Ctrl vs 1-NP	-	-	<0.01
	1-NP vs 1-NP+ α -kG	-	-	0.036
Figure 9E	α -KG main effect	0.004	1	0.953
	1-NP main effect	0.613	1	0.449
	α -KG*1-NP interaction	0.033	1	0.860
	Ctrl vs 1-NP	-	-	0.509
	1-NP vs 1-NP+ α -kG	-	-	0.934
PrL	α -KG main effect	1.738	1	0.212
	1-NP main effect	0.679	1	0.426
	α -KG*1-NP interaction	0.033	1	0.860
	Ctrl vs 1-NP	-	-	0.431
	1-NP vs 1-NP+ α -kG	-	-	0.498

		α -KG main effect	0.123	1	0.732
		1-NP main effect	0.123	1	0.732
	IL	α -KG*1-NP interaction	0.204	1	0.660
		Ctrl vs 1-NP	-	-	0.945
		1-NP vs 1-NP+ α -kG	-	-	0.581
Figure 9G		α -KG main effect	1.171	1	0.311
		1-NP main effect	6.809	1	0.031
		α -KG*1-NP interaction	3.973	1	0.081
		Ctrl vs 1-NP	-	-	0.012
		1-NP vs 1-NP+ α -kG	-	-	0.061
		α -KG main effect	4.757	1	0.050
		1-NP main effect	42.971	1	<0.01
		α -KG*1-NP interaction	1.255	1	0.285
		Ctrl vs 1-NP	-	-	<0.01
		1-NP vs 1-NP+ α -kG	-	-	0.038
Figure 9I		α -KG main effect	0.062	1	0.807
		1-NP main effect	17.266	1	<0.01
		α -KG*1-NP interaction	1.813	1	0.203
		Ctrl vs 1-NP	-	-	<0.01
		1-NP vs 1-NP+ α -kG	-	-	0.281
		α -KG main effect	3.286	1	0.095
		1-NP main effect	17.217	1	<0.01
		α -KG*1-NP interaction	2.199	1	0.164
		Ctrl vs 1-NP	-	-	<0.01
		1-NP vs 1-NP+ α -kG	-	-	0.038
		α -KG main effect	0.778	1	0.395
		1-NP main effect	1.130	1	0.309
		α -KG*1-NP interaction	0.001	1	0.982
		Ctrl vs 1-NP	-	-	0.476
		1-NP vs 1-NP+ α -kG	-	-	0.534
Figure 9J		α -KG main effect	0.046	1	0.834
		1-NP main effect	7.292	1	0.019
		α -KG*1-NP interaction	1.546	1	0.237
		Ctrl vs 1-NP	-	-	0.481
		1-NP vs 1-NP+ α -kG	-	-	0.323
		α -KG main effect	0.601	1	0.453
		1-NP main effect	0.082	1	0.779
		α -KG*1-NP interaction	0.082	1	0.779
		Ctrl vs 1-NP	-	-	0.692
		1-NP vs 1-NP+ α -kG	-	-	0.467

Fig. 10. Effects of gestational α -KG supplementation on 1-NP-induced mIPSC transmission disorder and autism-like behaviors.

	Groups		F value	df	P value
Figure 10B	α -KG main effect		5.135	1	0.028
	1-NP main effect		35.559	1	<0.01
	α -KG*1-NP interaction		3.084	1	0.085
	Ctrl vs 1-NP		-	-	<0.01
	1-NP vs 1-NP+ α -kG		-	-	<0.01
Figure 10C	α -KG main effect		1.611	1	0.210
	1-NP main effect		8.726	1	<0.01
	α -KG*1-NP interaction		0.012	1	0.913
	Ctrl vs 1-NP		-	-	0.050
	1-NP vs 1-NP+ α -kG		-	-	0.335
Figure 10E	α -KG main effect		0.140	1	0.710
	1-NP main effect		0.002	1	0.968
	α -KG*1-NP interaction		0.002	1	0.962
	Ctrl vs 1-NP		-	-	0.951
	1-NP vs 1-NP+ α -kG		-	-	0.767
Figure 10F	α -KG main effect		0.033	1	0.858
	1-NP main effect		9.319	1	<0.01
	α -KG*1-NP interaction		6.794	1	0.012
	Ctrl vs 1-NP		-	-	<0.01
	1-NP vs 1-NP+ α -kG		-	-	0.093
Figure 10G	Ctrl	S1 vs E	2.893	18	0.01
	α -kG	S1 vs E	0.147	20	0.044
	1-NP	S1 vs E	1.566	18	0.135
	1-NP+ α -kG	S1 vs E	2.356	16	0.032
Figure 10H	Ctrl	S1 vs S2	-3.900	18	<0.01
	α -kG	S1 vs S2	-3.787	20	<0.01
	1-NP	S1 vs S2	0.833	18	0.416
	1-NP+ α -kG	S1 vs S2	-6.918	16	<0.01
Figure 10I	Ctrl	S1 vs E	3.858	18	<0.01
	α -kG	S1 vs E	2.371	18	0.029
	1-NP	S1 vs E	1.292	16	0.215
	1-NP+ α -kG	S1 vs E	2.704	18	0.015
Figure 10J	Ctrl	S1 vs S2	-3.782	13.042	<0.01
	α -kG	S1 vs S2	-2.256	11.934	0.044
	1-NP	S1 vs S2	-1.280	16	0.219
	1-NP+ α -kG	S1 vs S2	-3.705	18	<0.01

Table S1 Primers for Multiplex PCR in measurement of 5hmC

Genes	Sequences
Nrg1_1	Forward: AGATTTAGAGGATTYGGGAGGA
	Reverse: ACCCRAACCCAAATAAATACCA
Nrg1_2	Forward: GYGATAAGTTTGGTTTAAGGGTTTGTAG
	Reverse: CCCRAAACACCCCCAAAC
ErbB4	Forward: GGGTTTAYGGGTTTGGAAAGT
	Reverse: ACTCCCCCAAACCCAAAA
Sema3F_1	Forward: TTGYGGGGGTGGAAAAAG
	Reverse: CCTCCCRACCCCACTAAAA
Sema3F_2	Forward: GGGTAGGGTTTAGGGTTTTTAGG
	Reverse: CCAACCCTTCTACCCAAAA

Table S2 Primers for real-time RT-PCR

Genes	Sequences
<i>18S</i>	Forward: GTAACCCGTTGAACCCATT
	Reverse: CCATCCAATCGGTAGTAGCG
<i>Tet1</i>	Forward: ACACAGTGGTGCTAATGCAG
	Reverse: AGCATGAACGGGAGAATCGG
<i>Tet2</i>	Forward: AGAGAAGACAATCGAGAAGTCGG
	Reverse: CCTTCCGTACTCCCAAACCTCAT
<i>Tet3</i>	Forward: GTAACCCGTTGAACCCATT
	Reverse: CCATCCAATCGGTAGTAGCG
<i>ErbB4</i>	Forward: GGAACAGCAGTACCGAGCCTTG
	Reverse: GGATAGACCGCAGGAAGGAGAGG
<i>Nrg1</i>	Forward: GCTCATCACTCCACGACTGTCAC
	Reverse: CTGCTGTGCTGCTGTTCTCTAC
<i>Cxcl12</i>	Forward: ACCAGTCAGCCTGAGCTACCG
	Reverse: AAGGGCACAGTTTGGAGTGTTGAG
<i>Cxcr4</i>	Forward: GTCAACCTCTACAGCAGCGTTCTC
	Reverse: CTGACTGTTGGTGGCGTGGAC
<i>Sema3A</i>	Forward: TGGGACGGGACTTCGCTATCTTC
	Reverse: GGGATGAGATGGGCACTGATGAATC
<i>Sema3F</i>	Forward: GACCTGCATGACATCAACCGAGAG
	Reverse: CTCCATTGCCATCCTTGCTGAC
<i>Nrp1</i>	Forward: ACAGCATCCAATCAAGCCGACAG
	Reverse: TCTTCTCATCTCCCAGGTCCACTTG
<i>Nrp2</i>	Forward: ATCAGTGCCTCCTCCACCTTCTC
	Reverse: TGTCCAGCCATTGTCATCACCATG
<i>Slit1</i>	Forward: GCTACGCCTGCCTCTGTGTTG
	Reverse: GGTCTACGCAGTTGGCTCCATTC
<i>Efna5</i>	Forward: ATGAGTCAGCCGAGCCATCCC

Arx Reverse: AGCATCGCCAGGAGGAACAGTAG
Forward: AGGGCAAGGATGGTGAGGACAG
Reverse: GCTGGTAACTGGTGAACGTGGTG
Dlx1 Forward: ACCACCATGCCAGAAAGTCTCAAC
Reverse: GCCCGCCGAGTGTAACAGTG
Dlx2 Forward: CAACGAGCCGGACAAGGAAGAC
Reverse: CTGGAGTAGATGGTGCGTGGTTTC
Dlx5 Forward: GCGACTTCCAAGCTCCGTTCC
Reverse: AAGCAGAGGTAGGAGAGCAGTAGC
NKX2-1 Forward: CTCAGCCGACGCCGAATCATG
Reverse: GCCCTCCATGCCCACTTTCTTG
NKX6-2 Forward: AAGTGAAGGTGTGGTTCCAGAATCG
Reverse: CCGGTTGTATTCGTCATCGTCCTC
LHX6 Forward: TCTGGACAAGGACGAAGGTAGAGC
Reverse: CAGACCGCAACTGGAGCAGATATTC
