Supplemental Information

Disrupted folate metabolism with anesthesia leads to myelination deficits mediated by epigenetic regulation of ERMN

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Supplemental Figures

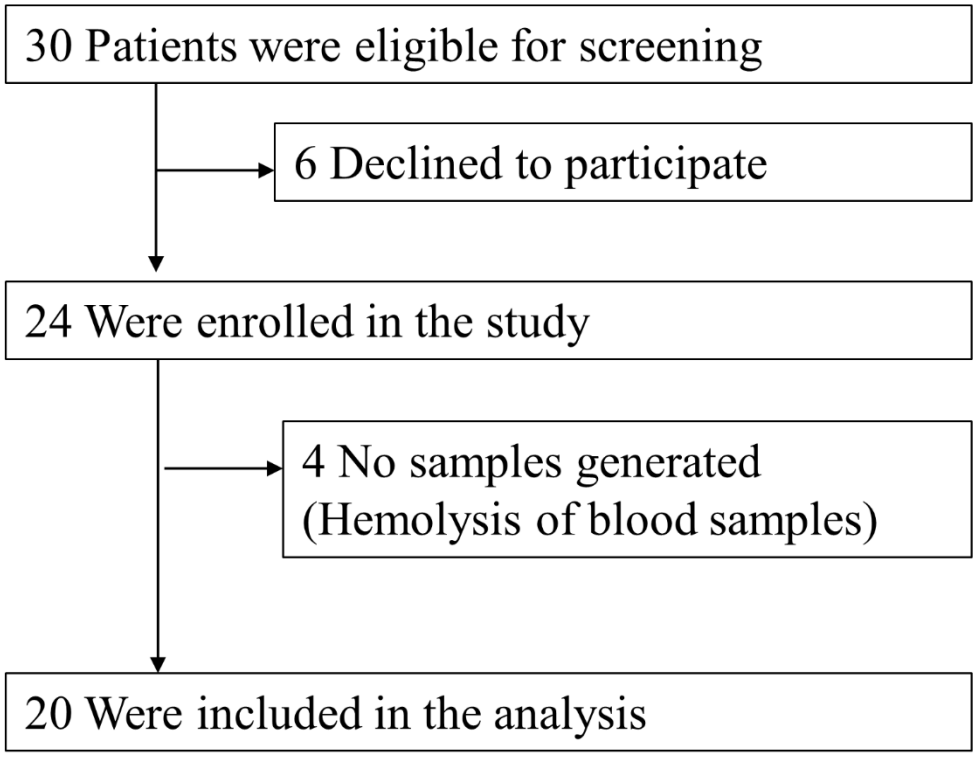

Figure S1. Flow diagram.

The flow diagram shows that 30 participants were initially screened for the studies and that ultimately 20 participants were included in the final data analysis.

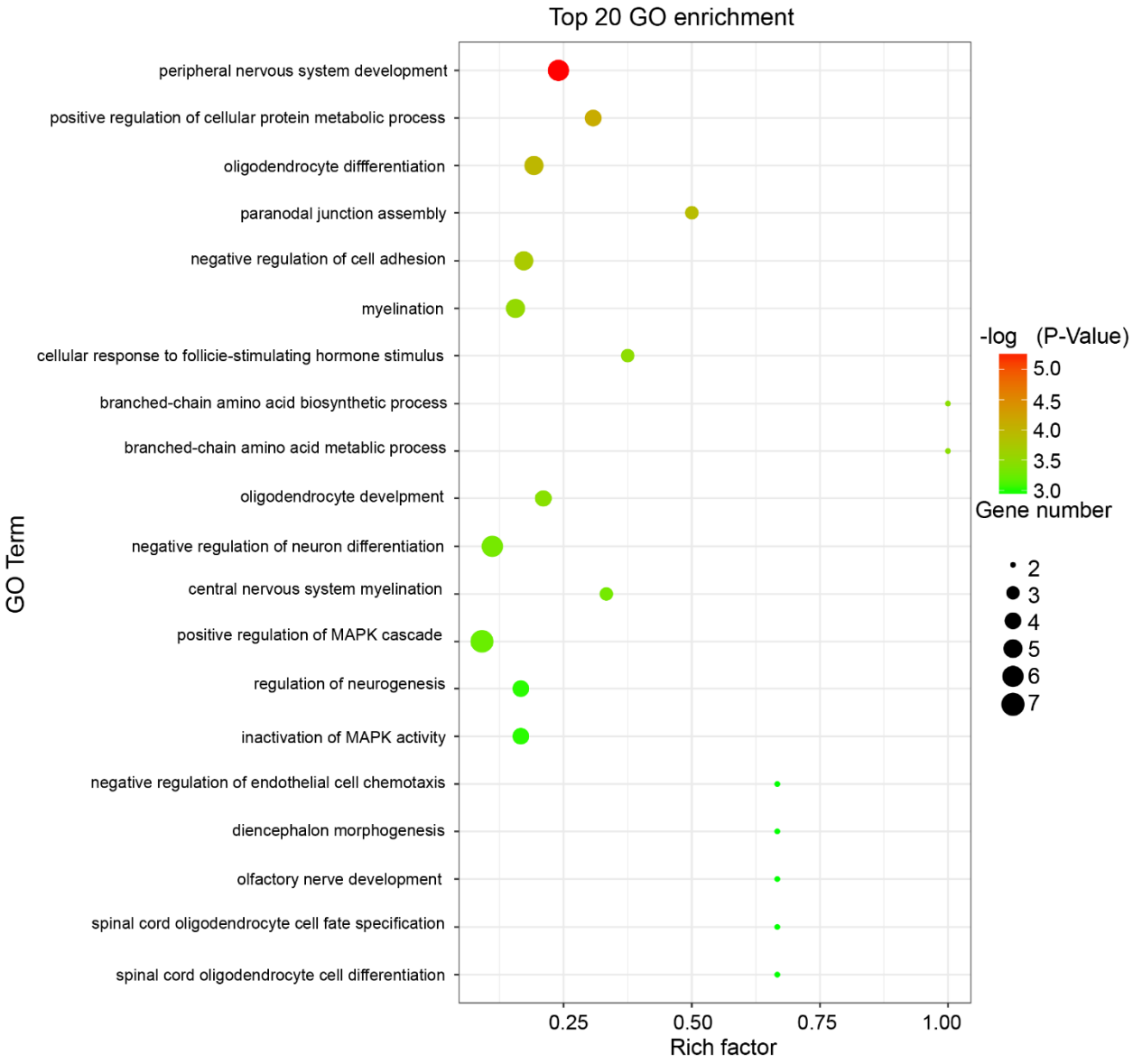

Fig. S2. Gene Ontology enrichment analysis of the RNA-seq data obtained from the prefrontal cortex of the rhesus macaque treated with the sevoflurane anesthesia. The graph displays the top 20 of the Gene Ontology enrichment in the prefrontal cortex of the rhesus macaque after the sevoflurane anesthesia. We found a significant ($p < 0.0001$, cumulative hypergeometric distribution) enrichment of the 20 different Gene Ontology (GO) terms among the differentially expressed genes (DEGs) detected in the RNA-Seq experiment. The most

significantly enriched GO was the “peripheral nervous system development,” which suggested that the sevoflurane anesthesia has an effect on the neurodevelopment of young macaques. Moreover, it should be noted that oligodendrocyte differentiation and myelination ranked 3rd and 6th respectively. These data suggest that sevoflurane may inhibit the developmental process of myelination. These findings are consistent with the phenotype of the damage of myelination which was induced by the sevoflurane anesthesia in mice.

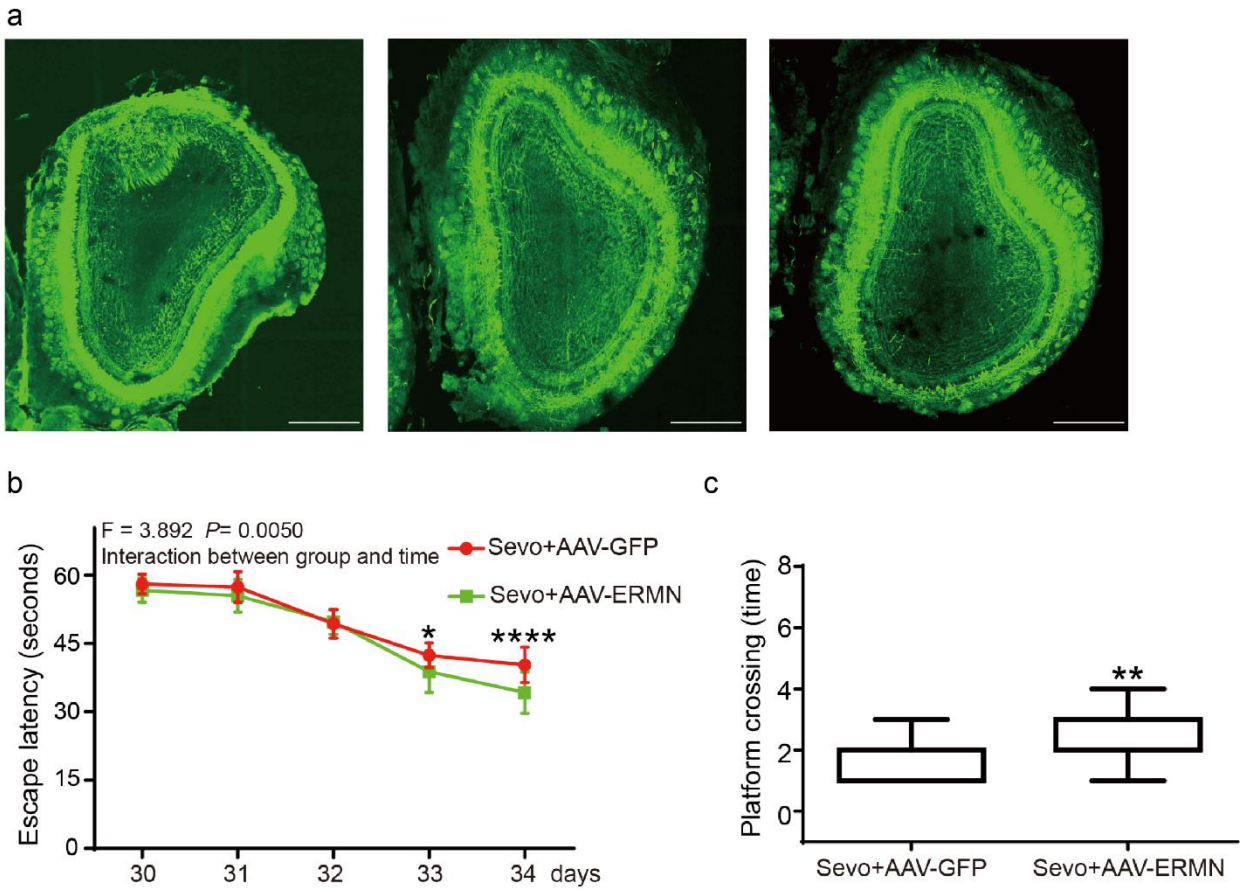

Fig. S3. The high efficiency of AAV-PHP.EB virus infection in brain of mice and the overexpression of ERMN mitigated the sevoflurane anesthesia-induced cognition impairment.

a. Fluorescence of GFP in three different parts of the olfactory bulb of mice brain that showed the higher infection efficiency of the AAV-PHP.EB virus in the mice brains at P14 after the injection

of the GFP-AAV-PHP.EB vectors into the hearts of the mice at P0. These data suggest that the AAV-PHP.EB vectors could cross the blood brain barrier of the mice and were also highly expressed in their brains.

b. A two-way ANOVA with repeated-measurement analysis showed a significant interaction between treatment (sevo + AAV-GFP *vs.* sevo + AAV-ERMN) and time (days 30 to 34) on escape latency of MWM (F = 3.892, $p = 0.005$**, N = 15, two-way ANOVA). A *post-hoc* test (Bonferroni) showed that the mice in the sevo. + AAV-ERMN group were able to locate the position of the platform of the MWM-pool in a shorter time (escape latency) as compared to the mice in the group of sevo. + AAV-GFP) at P33 ($p < 0.05$*) and P34 ($p < 0.0001$****).

c. The ERMN overexpression mitigated the sevoflurane anesthesia-induced decrease in the platform-crossing times (p=0.0085**, Mann–Whitney-*U*-test) (N = 15).

(Control: ctrl.; sevoflurane: sevo.; AAV-GFP: Adeno-associated virus- Green fluorescent protein; AAV-ERMN: Adeno-associated virus- ERM-like protein).

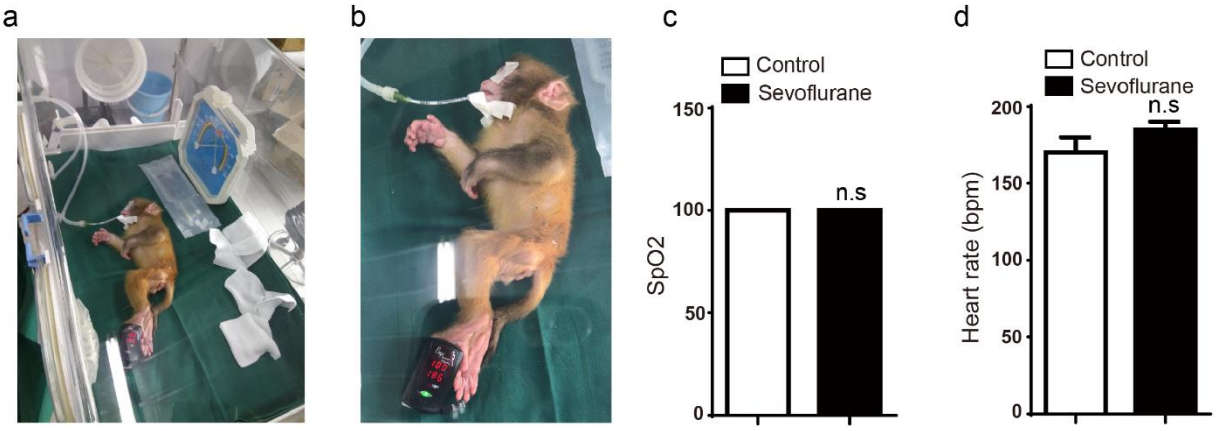

Figure S4. The oxygen levels and heart rate in the sevoflurane anesthetized rhesus macaques. The demonstration of the anesthesia in the rhesus macaque (**a**) and (b). There were no significant differences on oxygen levels (**c**) and heart rate (**d**) between the rhesus macaques in the anesthesia group and control group.

Table S1. Characteristics of the participants

	N = 20
Age (months)	
Mean±SD	21.35±10.35
0-12	7(35%)
13-24	8(40%)
25-36	5(25%)
Male sex-no.(%)	13(65%)
Body weight (kg) mean±SD	12.26±3.23
Length of surgery (minutes) mean±SD	59±21.13
Length of anesthesia (minutes) mean±SD	82.25±26.13
ASA class	
I	20
II	0
Preoperative folate (median and 25–75% percentile)	16.40 (9.32-17.75)（ng/ml)
Postoperative folate (median and 25–75% percentile)	14.50 (8.65-17.33)（ng/ml)

Table S1: The length of anesthesia was defined from the time anesthesiologists first administered the general anesthesia to the participants to the time when the participants were sent to the post-anesthesia care unit. The length of surgery was defined from the time of initial incision to the time of the closure of the skin. The values of folate in the serum of all 20 participants were obtained using a Folate Binding Protein assay (see text for details). ASA, American Society of Anesthesiologists; min, minute; kg, kilogram; mg, milligram.

Table S2. The details of anesthesia/surgery in each participant

Number	Age (months)	Weight (kg)	Gender	Preoperative folate (ng/ml)	Postoperative folate (ng/ml)	Name of surgery	Total time during surgery (minute)	Total time during anesthesia (minute)	Midazolam (mg)	Fentanyl (mg)
1	24	15	M	16	12.5	Mandibular bone cyst	65	75	1	0.04
2	36	16	M	8.6	8.1	Laser scarring	60	80	1	0.05
3	36	16	M	8.5	6.4	Correction of scar	75	90	1	0.04
4	24	13	F	19.1	17.5	Angioma	90	105	1	0.04
5	12	6	M	18.4	18.8	Lip scar repair	100	120	0.5	0.03
6	24	11	F	12.3	11.3	Correction of scar deformity	45	105	1	0.02
7	36	17.5	M	11.5	10.3	OSAS	25	45	1	0.06
8	36	15.7	F	17.1	15.7	Correction of scar deformity	40	60	1	0.04
9	36	16	M	8.6	7.7	Congenital associated fingers	60	80	1	0.06
10	24	10	M	17.8	17.1	Cleft palate	60	75	1	0.05
11	11	10	M	18.7	17.4	Cleft palate	75	120	1	0.06
12	12	10	M	17	14.3	Cleft palate	100	120	1	0.01
13	24	14	M	16.8	16	Accessory auricles	60	70	1	0.04
14	13	10	F	15.4	14.5	Sacral arch syndrome	60	110	1	0.2
15	6	9	M	7.2	6.8	Cleft palate	45	60	1	0.15
16	12	10	M	17.4	17.6	Cleft palate	50	70	1	0.03
17	12	10	F	18.8	17.8	Cleft palate	30	45	1	0.04
18	24	16	F	7	6.3	Cleft palate	45	60	1	0.03
19	13	10	F	15.4	14.5	Branchial arch syndrome	60	110	1	0.02
20	12	10	M	17.6	16.8	Cleft palate	35	45	1	0.04

The reference ranges for folic acid in young children is 6.7-15ng/ml[1]

Reference:

1. Vaish S, White M, Daly L, Molloy AM, Staines A, Sweeney MR. Synthetic folic acid intakes and status in children living in Ireland exposed to voluntary fortification. *Am J Clin Nutr* 2016; **103**(2): 512-8.