

Supplementary Materials

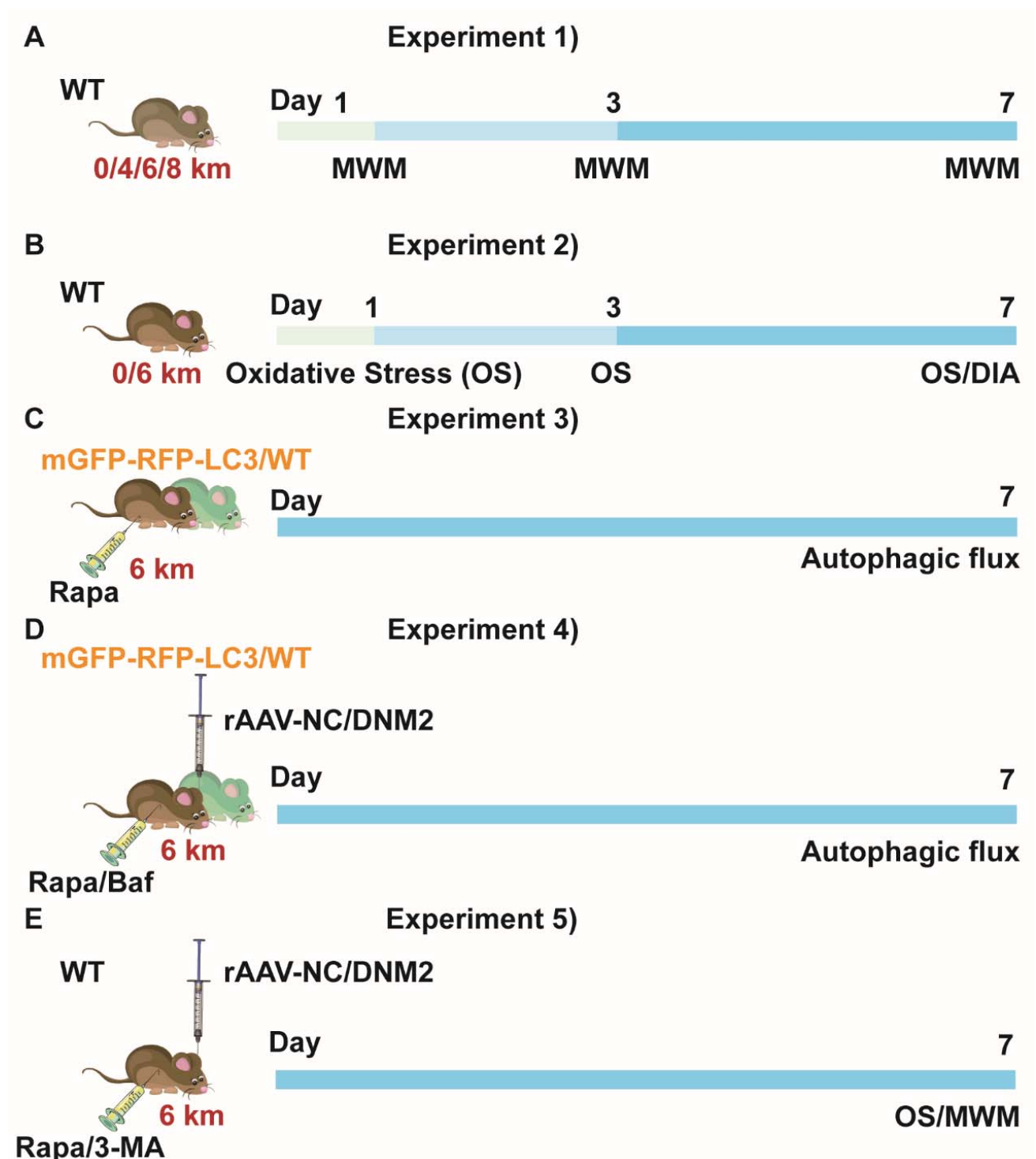


Fig. S1 Schedules of the experimental design and group assignment. A C57BL/6J mice ($n = 59$) were randomly divided into 4 groups: the normoxia group (NG, $n = 9$) and hypobaric hypoxia (HH) groups

exposed to 4 ($n = 15$), 6 ($n = 15$), and 8 ($n = 20$) km altitude. Behavioral tests were performed on days 1, 3, and 7 of HH exposure. **B** The mice exposed to 6 km were used for the oxidative stress analysis on days 1, 3, and 7, and proteomics analysis was performed on day 7. **C** Tandem mGFP-RFP-LC3 transgenic mice ($n = 6$) and C57BL/6J mice ($n = 24$) were exposed to 6 km altitude for 7 days and 6 mGFP-RFP-LC3 mice were assigned to the NG group. C57BL/6J mice were divided into two groups each receiving blank solvent (HH+Veh) or rapamycin (HH+Rapa). All samples were used for the autophagic flux test. **D** mGFP-RFP-LC3 mice ($n = 12$) were divided into two groups and received AAV2/9-hSyn-NC or AAV2/9-hSyn-DNM2 transfection before hypobaric hypoxia exposure. C57BL/6J mice ($n = 54$) were divided into two groups that received AAV2/9-hSyn-NC or AAV2/9-hSyn-DNM2 transfection and in each group, 27 mice were divided into 3 subgroups that received rapamycin and/or bafilomycin A1 injection to measure the autophagic flux. **E** C57BL/6J genetically recombined mice ($n = 18$) were randomly and evenly assigned into AAV2/9-hSyn-NC or AAV2/9-hSyn-DNM2 groups. In each group, mice were injected with blank solvent, rapamycin, or 3-methyladenine (3-MA). All the mice were treated under 6 km hypobaric hypoxia exposure for 7 days.

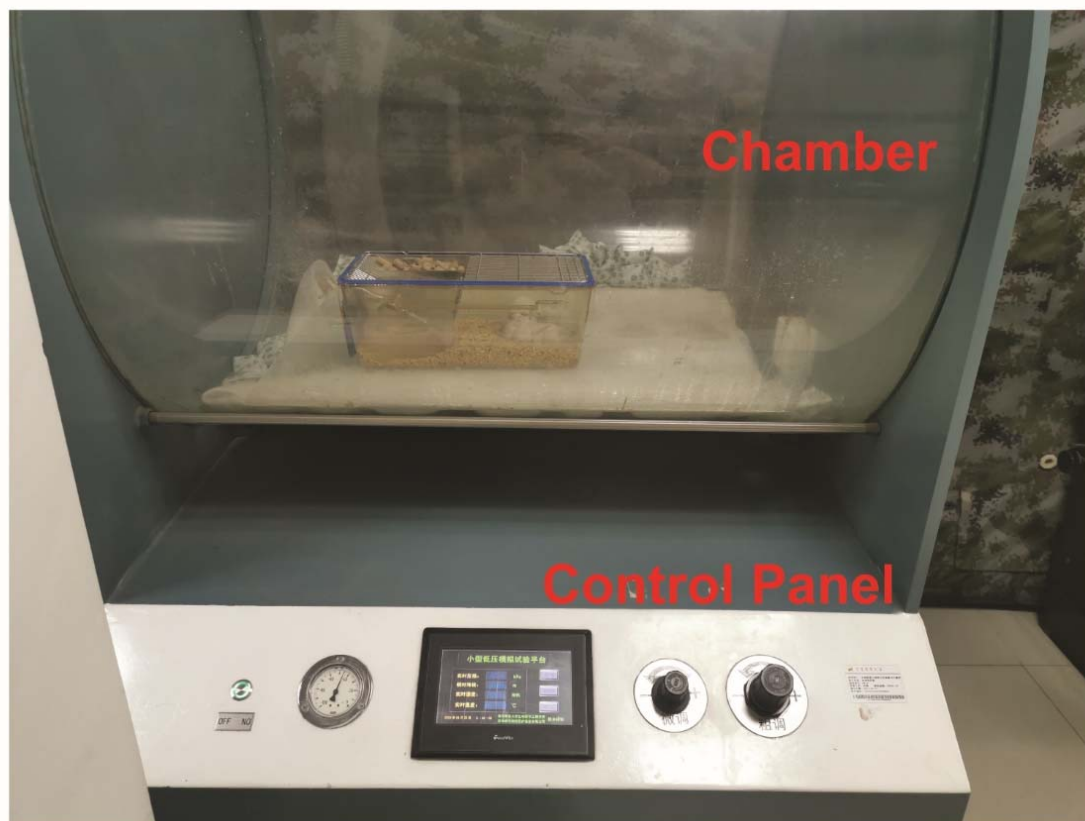


Fig. S2 The hypobaric hypoxia chamber used in the present study.

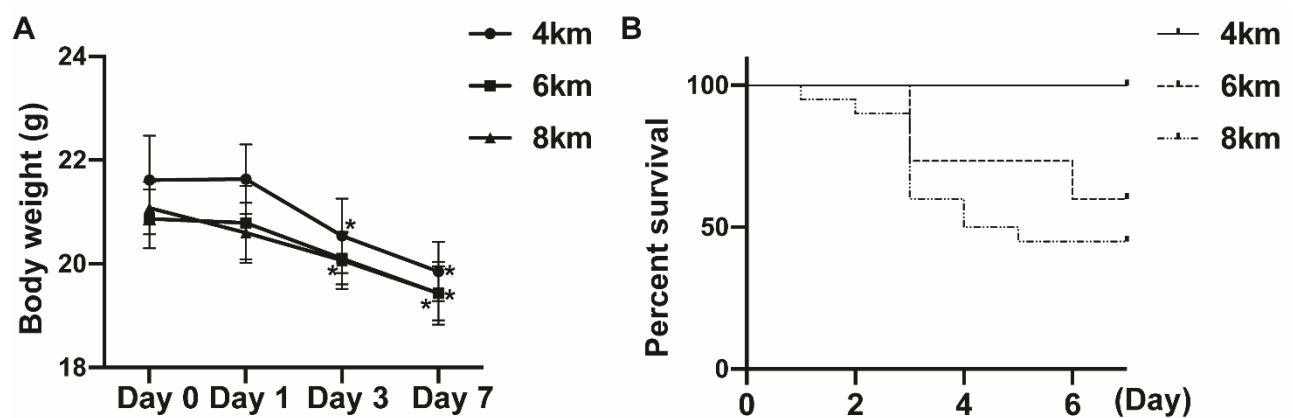


Fig. S3 The health condition of mice after hypobaric hypoxia. **A** The body weight of mice exposed to hypobaric hypoxia. **B** Survival analysis of mice exposed to HH.

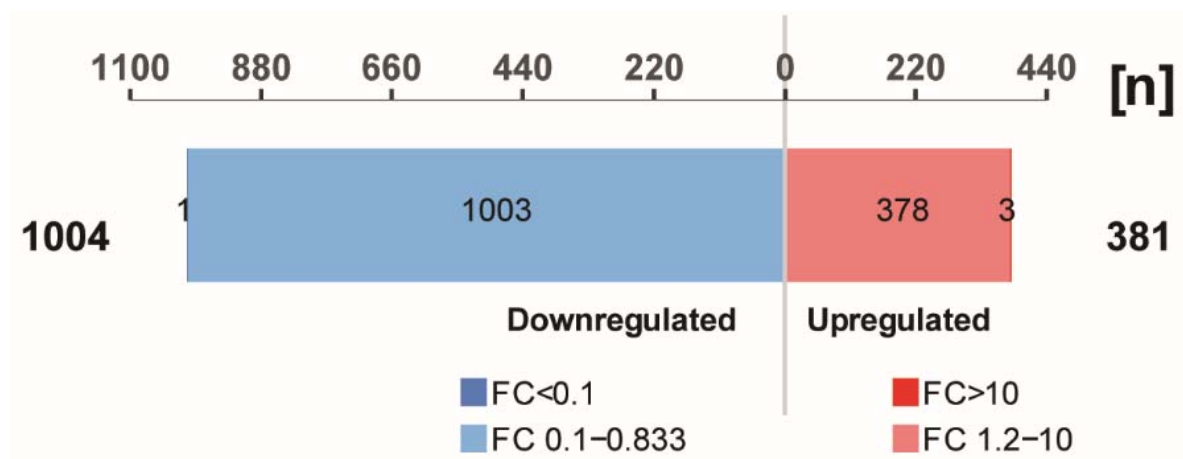


Fig. S4 The numbers of differentially-expressed proteins in the hypobaric hypoxia group compared with the NG group.

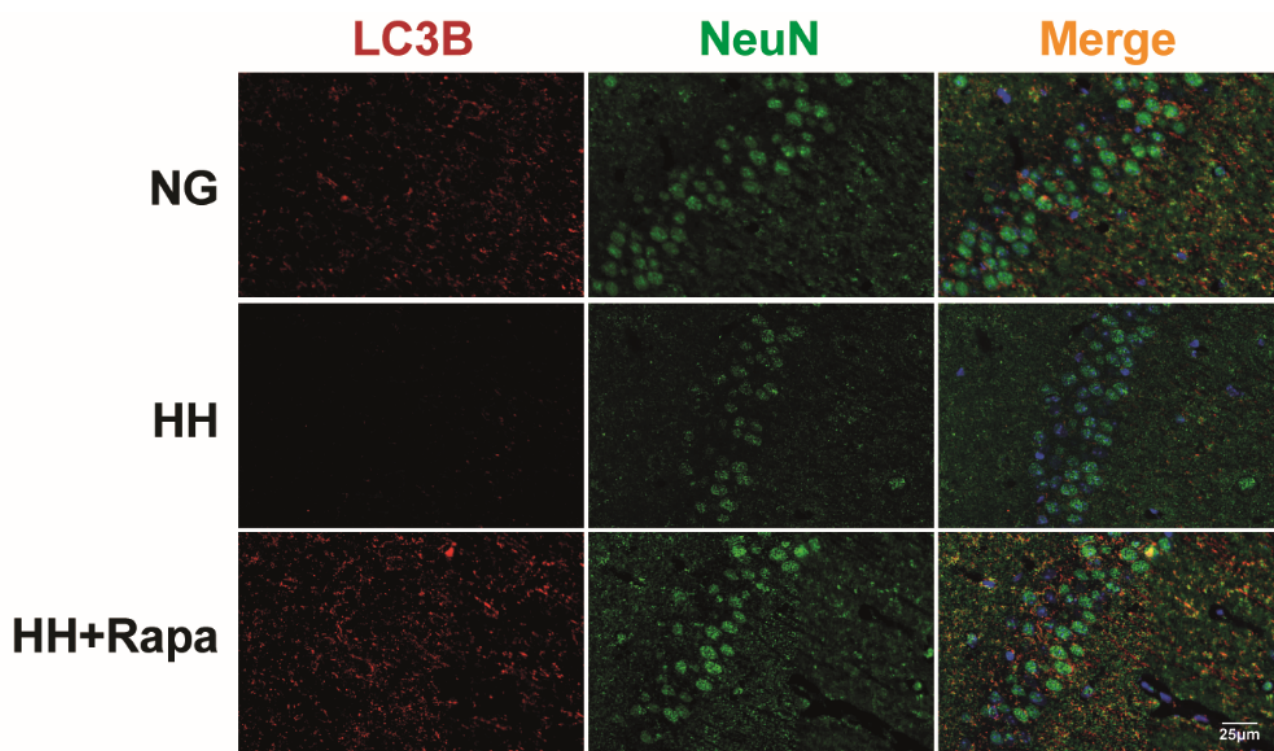


Fig. S5 Double-immunofluorescence staining indicates LC3B expression in hippocampal neurons after hypobaric hypoxia exposure of 6 km for 7 days. LC3B is marked as red puncta and neurons are marked green. The cells co-expressing LC3B and NeuN are merged as yellow puncta. Scale bar, 25 μm .

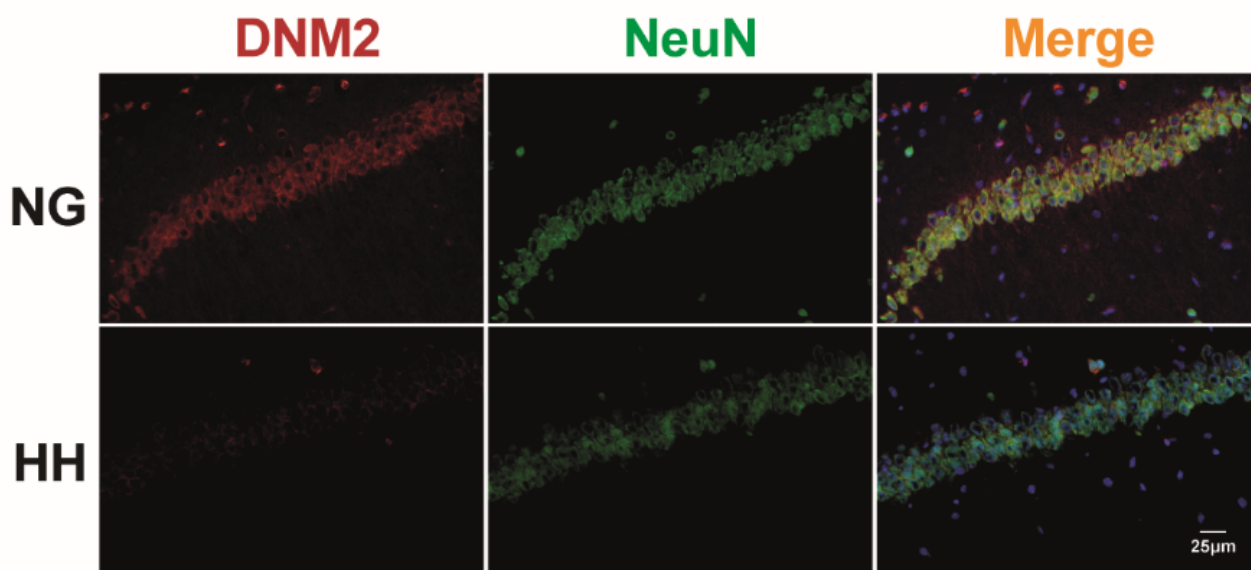


Fig. S6 Double-immunofluorescence staining indicates DNM2 expression (red) in hippocampal neurons (green) in mouse hippocampus after hypobaric hypoxia (6 km, 7 days). The cells co-expressing DNM2 and NeuN are merged as yellow puncta. Scale bar, 25 μ m.

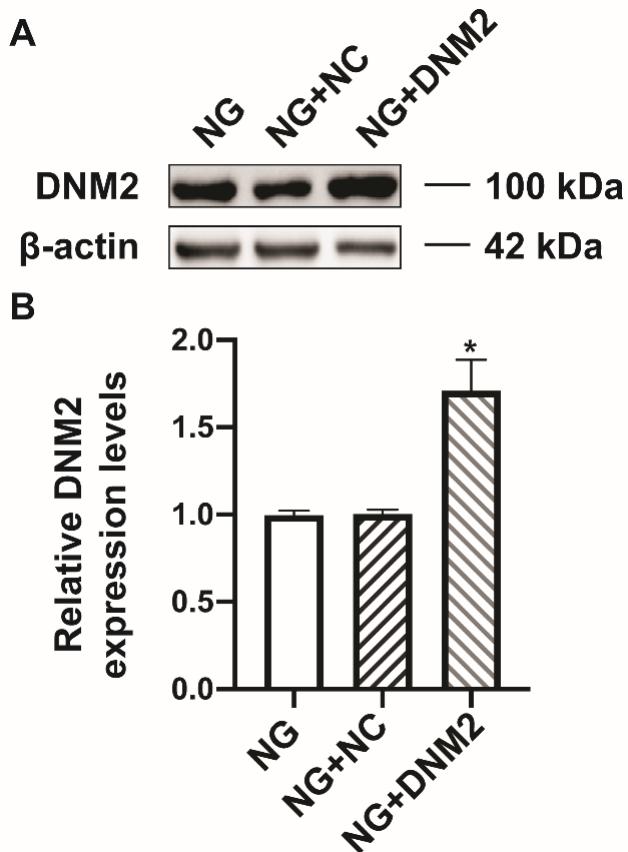


Fig. S7 The expression of DNM2 in the hippocampus of mice with AAV2/9 infection. **A** Western blots and analysis confirm the transfection effects of AAV2/9-hSyn-DNM2. **B** The relative integrated density of DNM2 to β -actin. Data are presented as the mean $\pm$ SD, $n = 6$, $^*P < 0.05$ vs NG+NC groups (one-way ANOVA followed by *post hoc* Tukey's test for multiple comparisons).

Table S1 Behavioral performance after HH exposure (data are shown as the mean $\pm$ SD)

Groups		Scores				
Altitude (km)	Time (day)	Escape latency (s)	Time in the target quadrant (s)	Visits to target quadrant (#)	Distance in the target quadrant (%)	Velocity (cm/s)
NG (0)	1	26.8 $\pm$ 5.8	34.9 $\pm$ 13.3	5.2 $\pm$ 1.3	53.8 $\pm$ 10.8	15.5 $\pm$ 4.6
	3	25.4 $\pm$ 6.2	37.7 $\pm$ 10.4	4.4 $\pm$ 1.3	57.0 $\pm$ 16.3	19.6 $\pm$ 5.4
	7	23.3 $\pm$ 4.3	44.5 $\pm$ 12.9	4.9 $\pm$ 1.5	62.1 $\pm$ 5.3	18.3 $\pm$ 3.7
4	1	30.0 $\pm$ 5.5	32.0 $\pm$ 6.5	4.4 $\pm$ 1.7	52.6 $\pm$ 5.2	15.6 $\pm$ 4.5
	3	33.7 $\pm$ 5.7	26.0 $\pm$ 4.8	3.3 $\pm$ 1.4	48.3 $\pm$ 7.1	10.6 $\pm$ 1.5
	7	48.3 $\pm$ 9.7	13.6 $\pm$ 9.0	1.8 $\pm$ 1.6	28.7 $\pm$ 17.1	9.0 $\pm$ 3.7
6	1	37.8 $\pm$ 9.0	30.1 $\pm$ 4.4	3.2 $\pm$ 1.6	47.0 $\pm$ 10.1	11.4 $\pm$ 3.2
	3	44.7 $\pm$ 8.2	17.7 $\pm$ 3.8	2.8 $\pm$ 1.3	40.6 $\pm$ 10.4	9.2 $\pm$ 1.7
	7	50.6 $\pm$ 11.3	8.8 $\pm$ 6.3	1.0 $\pm$ 1.4	22.1 $\pm$ 14.2	8.5 $\pm$ 1.3
8	1	44.0 $\pm$ 7.3	25.8 $\pm$ 9.3	2.6 $\pm$ 1.3	39.9 $\pm$ 8.1	8.6 $\pm$ 1.1
	3	51.8 $\pm$ 7.9	14.7 $\pm$ 11.7	1.3 $\pm$ 1.0	25.7 $\pm$ 16.6	7.2 $\pm$ 1.9
	7	59.1 $\pm$ 1.8	3.49 $\pm$ 3.8	0.2 $\pm$ 0.4	10.8 $\pm$ 11.7	5.8 $\pm$ 1.3

Table S2 Oxidative stress in the hippocampus after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Days	Oxidative stress-related proteins			
	ROS (fold change)	MDA (nmol/mg)	SOD (U/mg)	GSH-Px (mg/g)
NG (0)	1.0 $\pm$ 0.1	14.6 $\pm$ 2.1	7.6 $\pm$ 0.9	10.8 $\pm$ 1.0
1	2.6 $\pm$ 0.5	34.9 $\pm$ 5.2	3.9 $\pm$ 0.8	16.2 $\pm$ 1.9
3	3.4 $\pm$ 0.2	41.5 $\pm$ 2.1	2.5 $\pm$ 0.2	6.4 $\pm$ 0.7
7	4.0 $\pm$ 0.5	52.5 $\pm$ 2.1	2.5 $\pm$ 0.3	5.4 $\pm$ 0.8

Table S3 JC-1⁺ cell number in the hippocampus after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

number/mm ²	Days			
	NG (0)	1	3	7
FJC⁺ cell	45.7 $\pm$ 16.8	60.8 $\pm$ 11.4	110.0 $\pm$ 17.5	187.2 $\pm$ 34.2

Table S4 Bcl-2/Bax protein expression in the hippocampus after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Fold change	Days			
	NG (0)	1	3	7
Bcl-2/Bax	3.0 $\pm$ 0.7	1.7 $\pm$ 0.5	0.8 $\pm$ 0.2	0.3 $\pm$ 0.2

Table S5 Protein expression in the hippocampus after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Group	Fold change			
	p-mTOR	P62	LC3B-II/I	LC3B-II
NG	1.0 $\pm$ 0.0	1.0 $\pm$ 0.1	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
HH	1.9 $\pm$ 0.1	1.6 $\pm$ 0.5	0.8 $\pm$ 0.1	0.5 $\pm$ 0.1

Table S6 Autophagic structure in the hippocampus after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Group	Puncta/cell		
	AP	AL	AP+AL
NG	61.3 $\pm$ 10.5	47.2 $\pm$ 7.2	108.5 $\pm$ 9.5
HH	40.8 $\pm$ 10.3	29.2 $\pm$ 17.4	70.0 $\pm$ 23.7

Table S7 Protein expression in the hippocampus of mice treated with/without rapamycin after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Group	Fold change				
	p-mTOR	P62	LC3B-II/I	LC3B-II	DNM2
NG	1.0 $\pm$ 0.0	1.0 $\pm$ 0.1	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.1
HH+Veh	1.9 $\pm$ 0.2	1.9 $\pm$ 0.4	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.3 $\pm$ 0.2
HH+Rapa	1.3 $\pm$ 0.1	3.0 $\pm$ 0.8	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	0.3 $\pm$ 0.1

Table S8 Autophagic structure in the hippocampus of mice after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Group	Puncta/cell		
	AP	AL	AP+AL
HH+Veh	35.8 $\pm$ 7.9	22.8 $\pm$ 15.7	58.7 $\pm$ 12.7
HH+Rapa	68.0 $\pm$ 18.6	25.0 $\pm$ 4.7	93.0 $\pm$ 19.1
HH+Rapa+NC	73.8 $\pm$ 19.2	25.0 $\pm$ 5.8	98.8 $\pm$ 17.9
HH+Rapa+DNM2	92.5 $\pm$ 14.6	51.7 $\pm$ 7.9	144.2 $\pm$ 17.6

Table S9 Protein expression in the hippocampus of mice with/without DNM2 overexpression after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Group	Fold change		
	P62	LC3B-II/I	LC3B-II
NC	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
NC+Rapa+Baf	3.6 $\pm$ 0.5	3.1 $\pm$ 0.1	1.9 $\pm$ 0.2
NC+Rapa	2.3 $\pm$ 0.4	2.8 $\pm$ 0.2	1.8 $\pm$ 0.1
DNM2	0.6 $\pm$ 0.1	0.9 $\pm$ 0.1	1.1 $\pm$ 0.0
DNM2+Rapa	1.4 $\pm$ 0.3	1.5 $\pm$ 0.2	1.5 $\pm$ 0.1
DNM2+Rapa+Baf	3.9 $\pm$ 0.4	3.0 $\pm$ 0.1	1.7 $\pm$ 0.1

Table S10 Oxidative stress in the hippocampus of mice with/without DNM2 overexpression after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Group	OS			
	ROS (fold change)	MDA (nmol/mg)	SOD (U/mg)	GSH-Px (mg/g)
NC+Veh	1.0 $\pm$ 0.0	49.5 $\pm$ 4.5	2.4 $\pm$ 0.3	4.7 $\pm$ 0.5
NC+Rapa	0.6 $\pm$ 0.1	29.6 $\pm$ 5.5	4.1 $\pm$ 0.7	6.8 $\pm$ 1.2
NC+3-MA	0.9 $\pm$ 0.2	38.2 $\pm$ 3.7	2.1 $\pm$ 0.1	4.3 $\pm$ 0.7
DNM2+Veh	0.6 $\pm$ 0.1	41.7 $\pm$ 2.2	2.6 $\pm$ 0.3	5.4 $\pm$ 0.5
DNM2+Rapa	0.4 $\pm$ 0.1	19.8 $\pm$ 2.8	5.0 $\pm$ 0.7	8.4 $\pm$ 1.0
DNM2+ 3-MA	0.6 $\pm$ 0.1	30.0 $\pm$ 4.1	2.8 $\pm$ 0.4	6.1 $\pm$ 0.7

Table S11 JC-1⁺ cell number in the hippocampus of mice with DNM2 overexpression after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

number/mm ²	Group		
	Veh	Rapa	3-MA
FJC ⁺ cell	151.3 $\pm$ 22.5	85.0 $\pm$ 7.9	134.0 $\pm$ 7.0

Table S12 Bcl-2/Bax protein expression in the hippocampus of mice with DNM2 overexpression after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Fold change	Group		
	Veh	Rapa	3-MA
Bcl-2/Bax	0.4 $\pm$ 0.1	2.0 $\pm$ 0.2	1.1 $\pm$ 0.1

Table S13 Behavioral performance with/without DNM2 overexpression after 6 km altitude exposure (data are shown as the mean $\pm$ SD)

Groups	Scores				
	Escape latency (s)	Time in the target quadrant (s)	Visits to target quadrant (#)	Distance in the target quadrant (%)	Velocity (cm/s)
NC+Veh	48.2 $\pm$ 13.0	6.8 $\pm$ 6.0	0.8 $\pm$ 1.0	15.9 $\pm$ 8.4	8.8 $\pm$ 1.2
NC+Rapa	35.3 $\pm$ 13.0	15.7 $\pm$ 8.0	1.5 $\pm$ 1.0	24.0 $\pm$ 7.8	14.1 $\pm$ 2.1
NC+3-MA	55.3 $\pm$ 8.8	7.1 $\pm$ 3.8	0.3 $\pm$ 0.5	21.0 $\pm$ 4.2	8.6 $\pm$ 1.4
DNM2+Veh	45.2 $\pm$ 12.1	8.7 $\pm$ 4.8	0.8 $\pm$ 0.8	19.4 $\pm$ 10.3	8.6 $\pm$ 1.3
DNM2+Rapa	25.2 $\pm$ 5.2	32.2 $\pm$ 11.2	3.2 $\pm$ 1.2	38.7 $\pm$ 4.9	15.6 $\pm$ 1.2
DNM2+3-MA	44.7 $\pm$ 8.5	15.5 $\pm$ 3.5	1.3 $\pm$ 0.8	24.2 $\pm$ 4.1	8.5 $\pm$ 1.6