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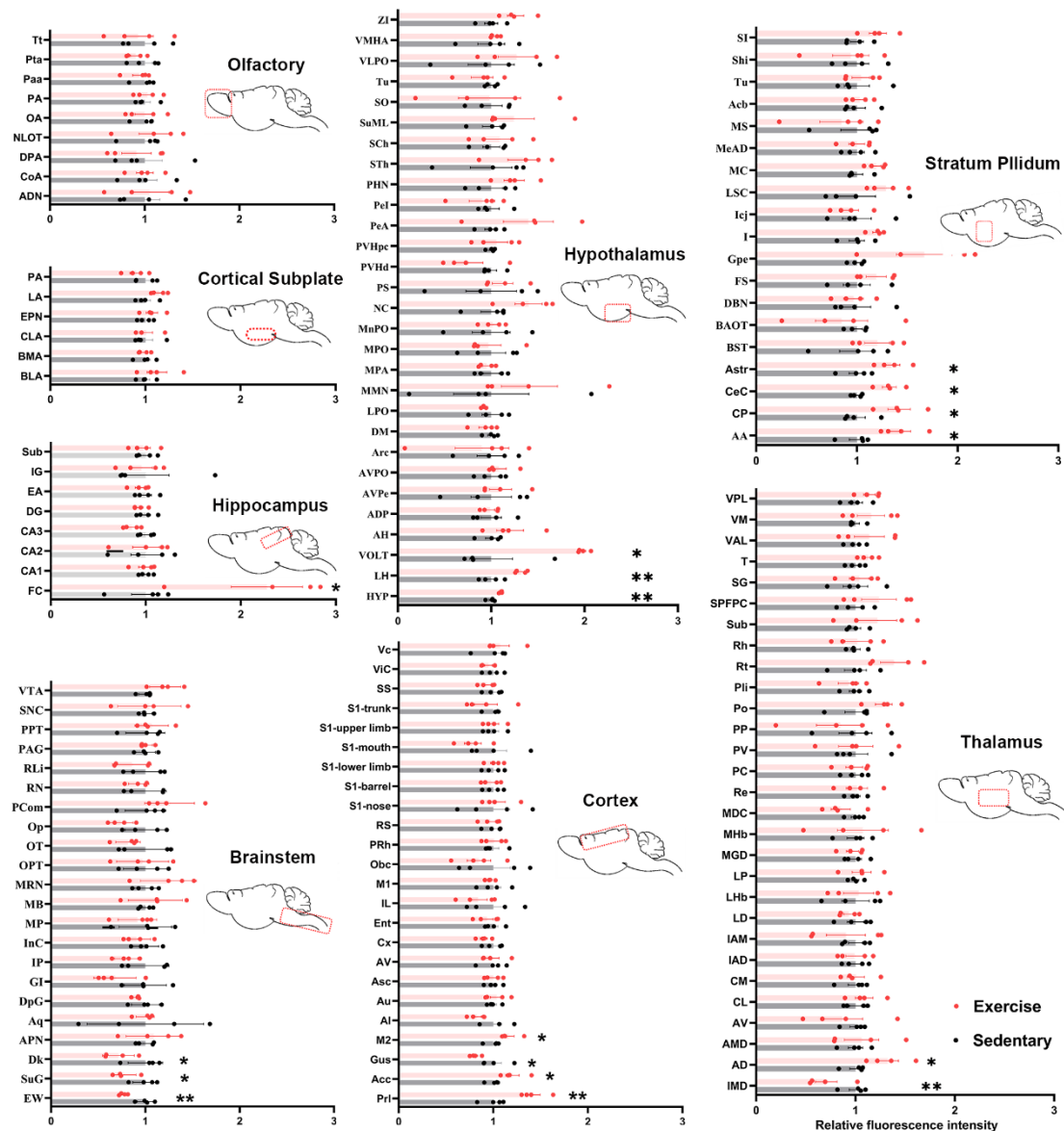


Fig. S1. Whole-brain analysis of c-Fos expression following single-bout physical exercise. Related to Figure 2. Relative fluorescence intensity of c-Fos protein in eight brain structures of sedentary and exercise mice (n = 4 mice per group). Unpaired t-test or Mann-Whitney test. Data were presented as mean $\pm$ SEM. *, P<0.05; **, P<0.01.

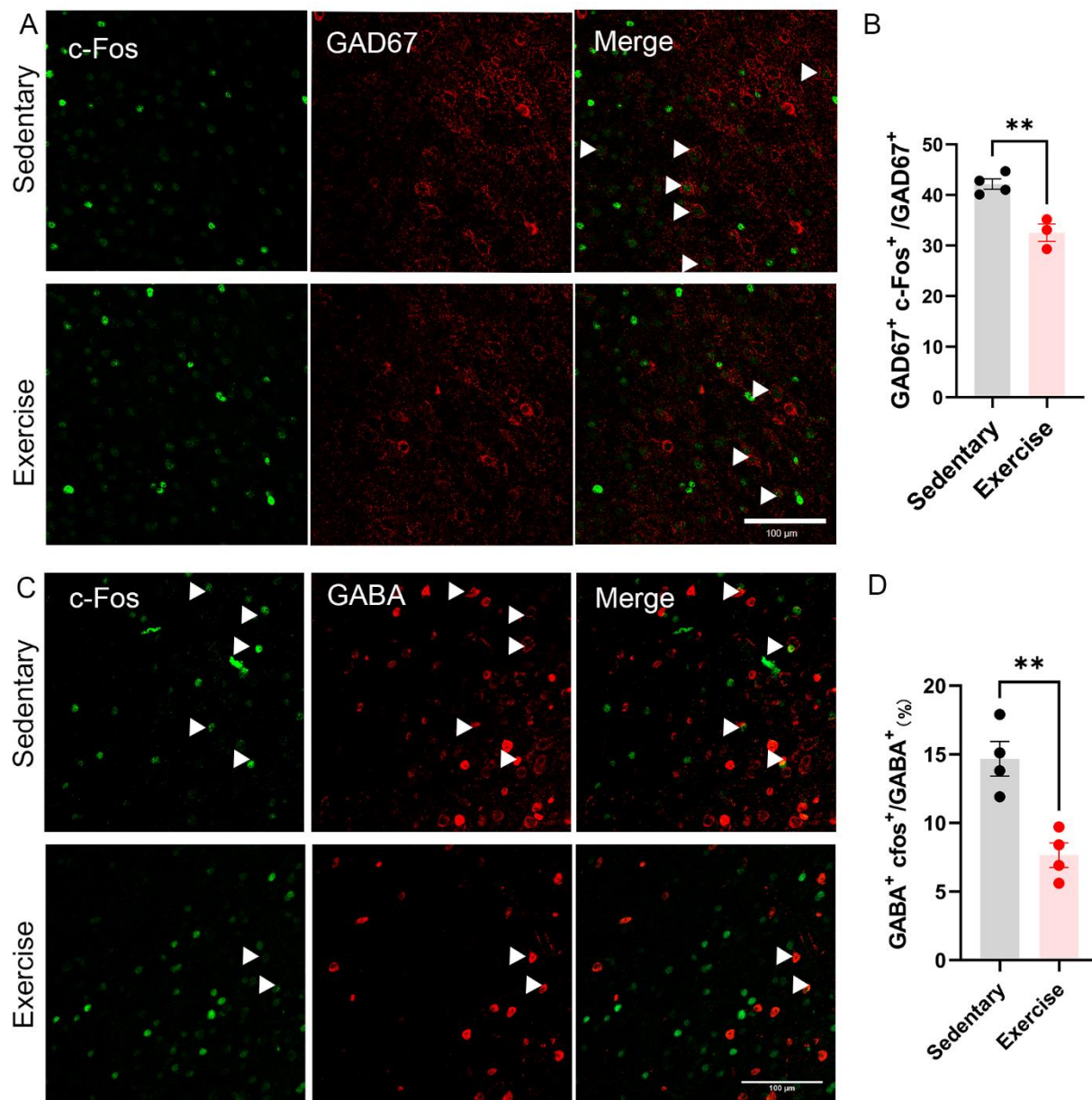


Fig. S2. Single-bout physical exercise reduces the activity of GABAergic neurons in the ACC.

Related to Figure 2. **(A)** Representative images of the GAD67⁺ (red) and c-Fos⁺ (green) cells of sedentary and exercised mice. Scale bar, 100 μ m. **(B)** Quantification of GAD67⁺ (red)/c-Fos⁺ (green) co-labeled neurons in GAD67⁺ neurons (n = 3-4 mice/group, 3-4 slices per mouse). Unpaired t-test. Data were presented as mean $\pm$ SEM. **P < 0.01. **(C)** Representative images of the GABA⁺ (red) and c-Fos⁺ (green) cells of sedentary and exercised mice. Scale bar, 100 μ m. **(D)** Quantification of

28 GABA⁺ (red)/c-Fos⁺ (green) co-labeled neurons in GABA⁺ neurons (n = 4 mice/group). Unpaired
29 t-test. Data were presented as mean $\pm$ SEM. **P < 0.01.

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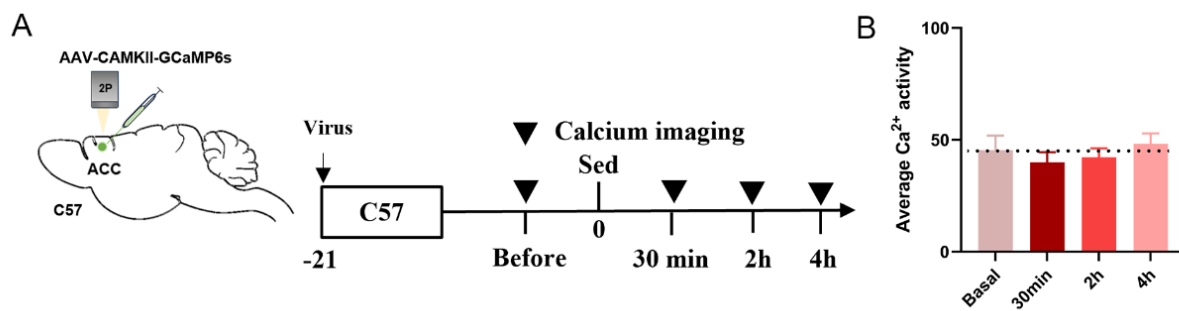
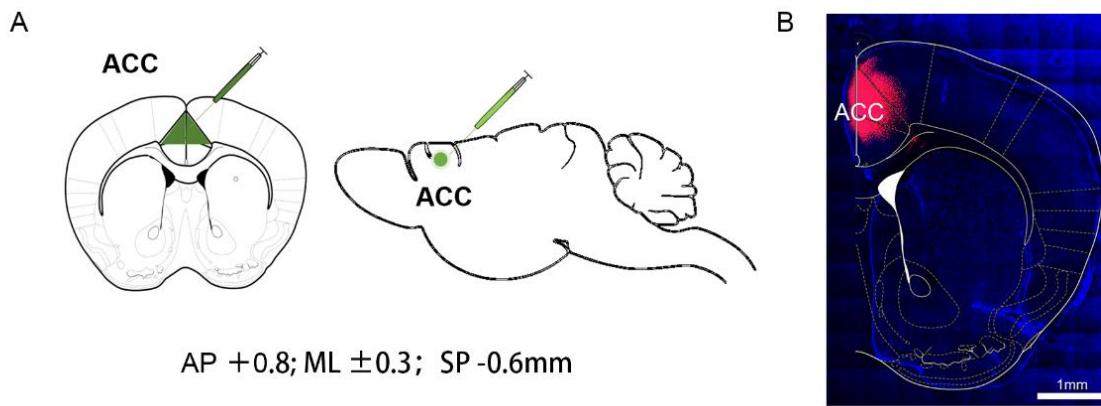


Fig. S3. Calcium activity in sedentary mice. Related to Figure 2. **(A)** Experimental design for in vivo calcium imaging of ACC-glutamatergic neurons in sedentary mice. **(B)** Average calcium activity of ACC-glutamatergic neurons in the sedentary mice detected pre-exercise (basal) and 30 min, 2 hours, and 4 hours post-exercise (n = 3 mice for recording). Friedman's test followed by Dunn's test for multiple comparisons.



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41 **Fig. S4. Virus injection site in the ACC.** (A) Coronal and sagittal representative views showing
42 the injection site in the ACC. Coordinates: anteroposterior (AP) 0.8 mm, medial lateral (ML) 0.3
43 mm, and subpial (SP) 0.6 mm. (B) Confocal image of a coronal section demonstrating the viral
44 expression predominantly localized in the ACC region. Scale bar, 1 mm.

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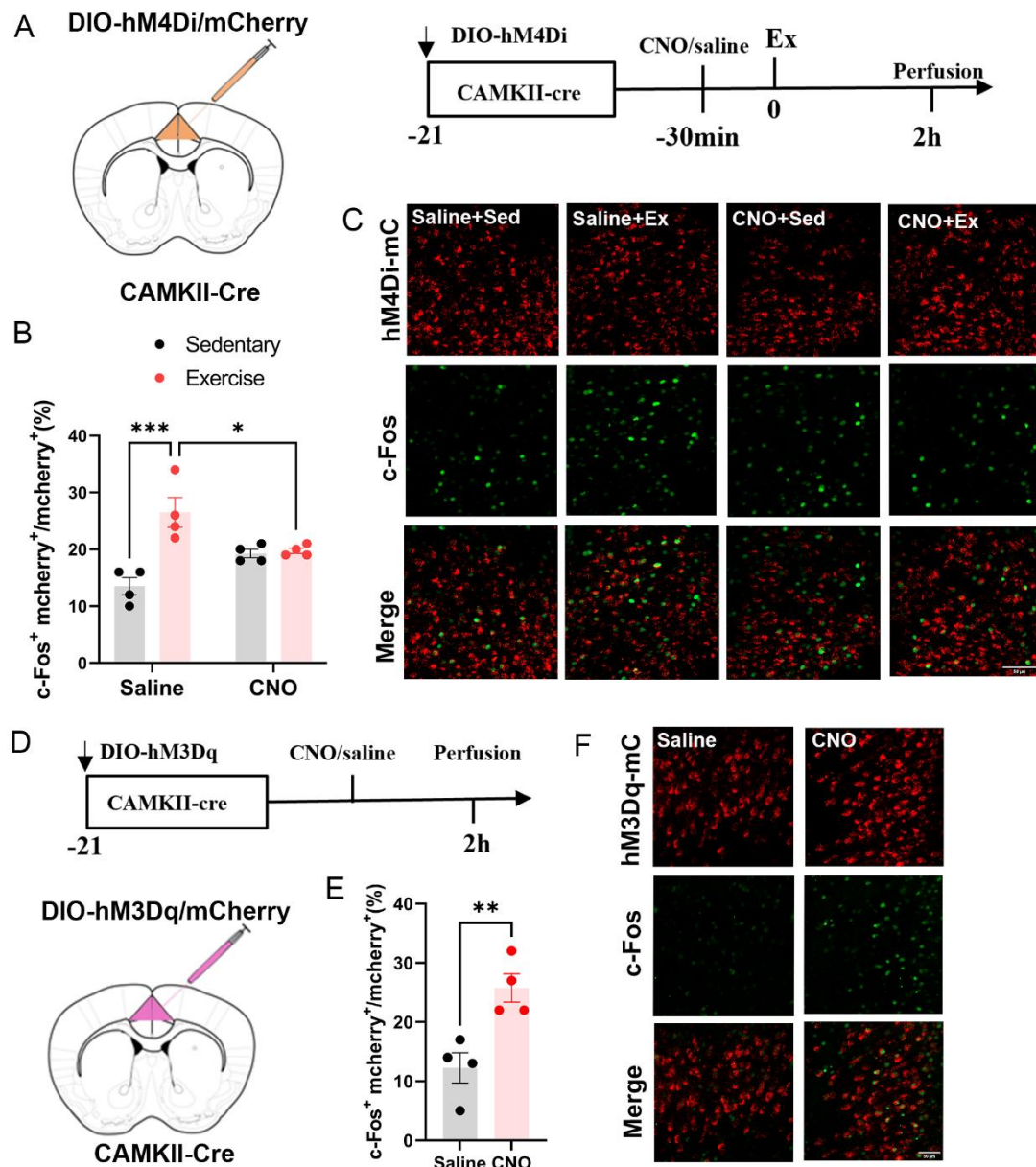
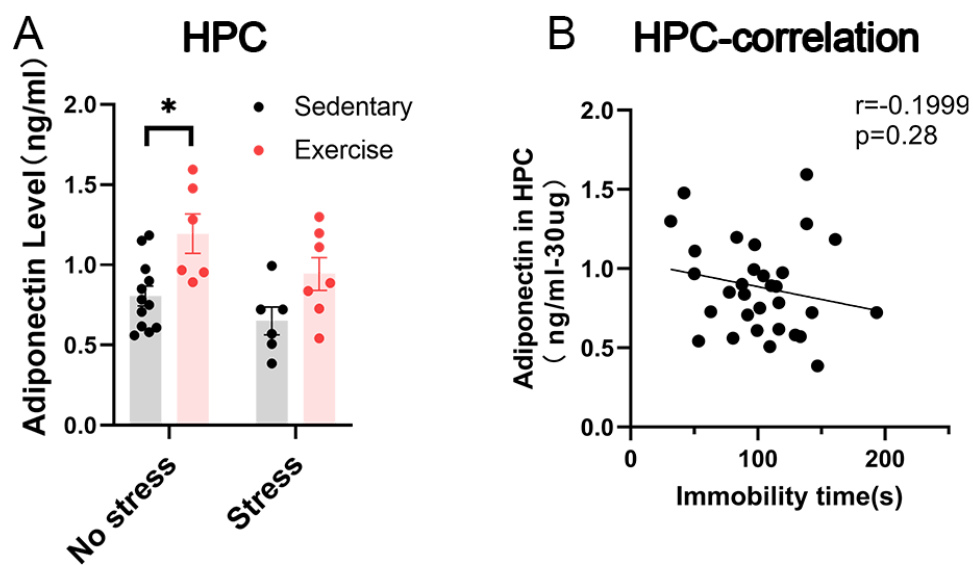


Fig. S5. The validation experiments for chemogenetic regulation. Related to Figure 2. (A) Experimental design for hM4Di-mCherry-mediated modulation of neuronal activity in CAMKII-Cre mice. (B) Quantification of the mCherry⁺ (red)/c-Fos⁺ (green) co-labeled neurons in mCherry⁺ neurons (n = 4 mice per group). Two-way ANOVA with Tukey's test for multiple comparisons. (C) Immunostaining showing c-Fos and hM4Di-mCherry⁺ cells. Scale bar, 50 μ m. (D) Experimental design for hM3Dq-mCherry-mediated modulation of neuronal activity in CAMKII-Cre mice. (E) Quantification of the mCherry⁺ (red)/c-Fos⁺ (green) co-labeled neurons in the mCherry⁺ neurons (n

55 = 4 mice per group). Unpaired t-test. (F) Immunostaining showing c-Fos and hM3Dq-mCherry⁺
56 cells. Scale bar, 50 μ m. Data were presented as mean $\pm$ SEM. *P < 0.05, **P < 0.01, and ***P <
57 0.001.

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61 **Fig. S6. Adiponectin level in the hippocampus does not correlate with immobility time.** Related

62 to Figure 3. (A) Single-bout exercise increased adiponectin levels in the hippocampus in the non-

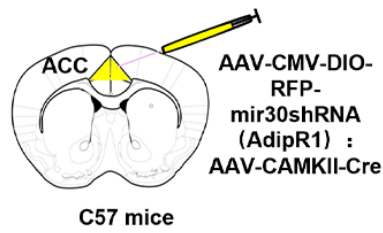
63 stressed mice. Two-way ANOVA with Tukey's test for multiple comparisons. (B) There was no

64 correlation between hippocampal adiponectin levels and immobility time in the FST. $r = -0.1999$;

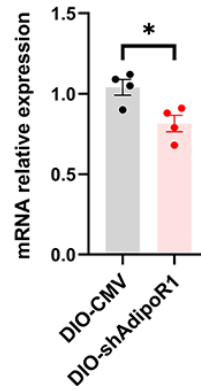
65 $P = 0.2808$. Data were presented as mean $\pm$ SEM. * $P < 0.05$.

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68 **Fig. S7. Verify the virus efficacy using qPCR.** Related to Figure 3. (A) Experimental design for

69 the knockdown of the AdipoR1 using AAV in ACC-glutamatergic neurons. (B) RT-qPCR results

70 showed a significant reduction in AdipoR1 mRNA 1 expression in the knockdown group compared

71 with those injected with a control virus (n = 4 mice per group). Unpaired t-test. Data were presented

72 as mean ± SEM. *P < 0.05.

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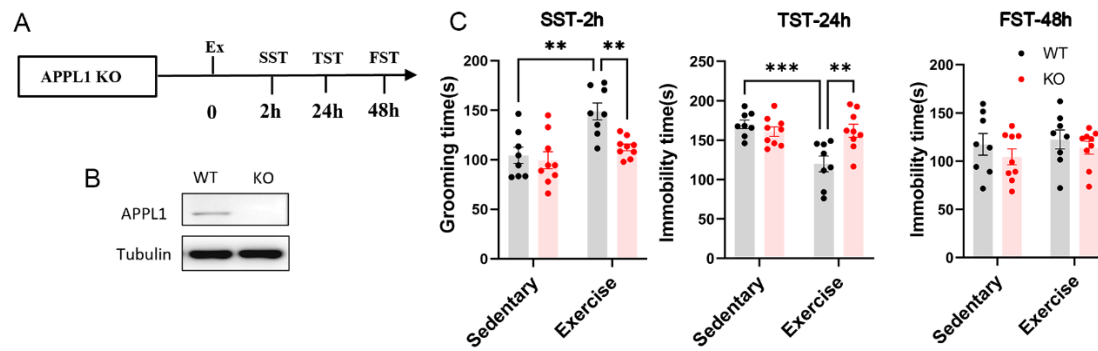


Fig. S8. APPL1 knockout abolishes single-bout exercise-elicited antidepressant behaviors.

Related to Figure 4. (A) Experimental timeline. (B) Western blotting confirmation of APPL1 knockout. (C) Global APPL1 knockout abolished the rapid antidepressant effect elicited by exercise in SST (2h) and TST (24h) (n = 8 to 9 mice per group). Two-way ANOVA with Tukey's test for multiple comparisons. Data were presented as mean $\pm$ SEM. **P < 0.01, and ***P < 0.001.

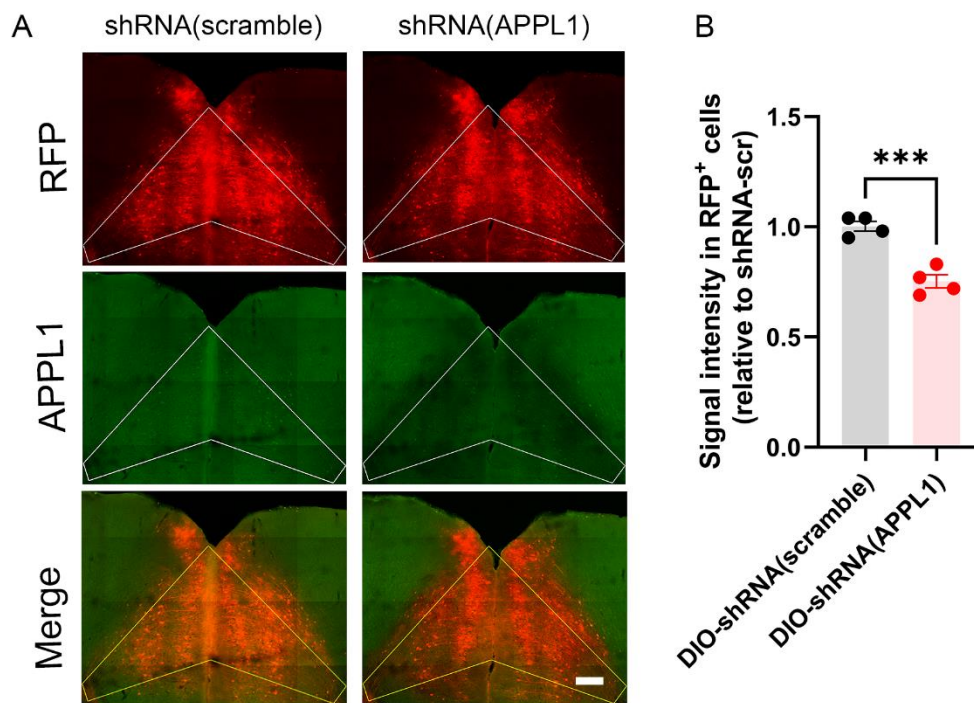


Fig. S9. Verify the APPL1 knockdown virus efficacy using immunofluorescence. Related to Figure 4. **(A)** Representative images of the APPL1 (green) and virus-affected RFP cells (red). Scale bar 200 μ m. **(B)** The quantification of APPL1 signal intensity in mice expressing shRNA(scramble) or shRNA(APPL1) virus, $n = 4$ mice per group. Immunofluorescence results showed a significant reduction in APPL1 in the shRNA(APPL1) knockdown group compared with the shRNA(scramble) virus group. Unpaired t-test. Data were presented as mean $\pm$ SEM. *** $P < 0.001$.

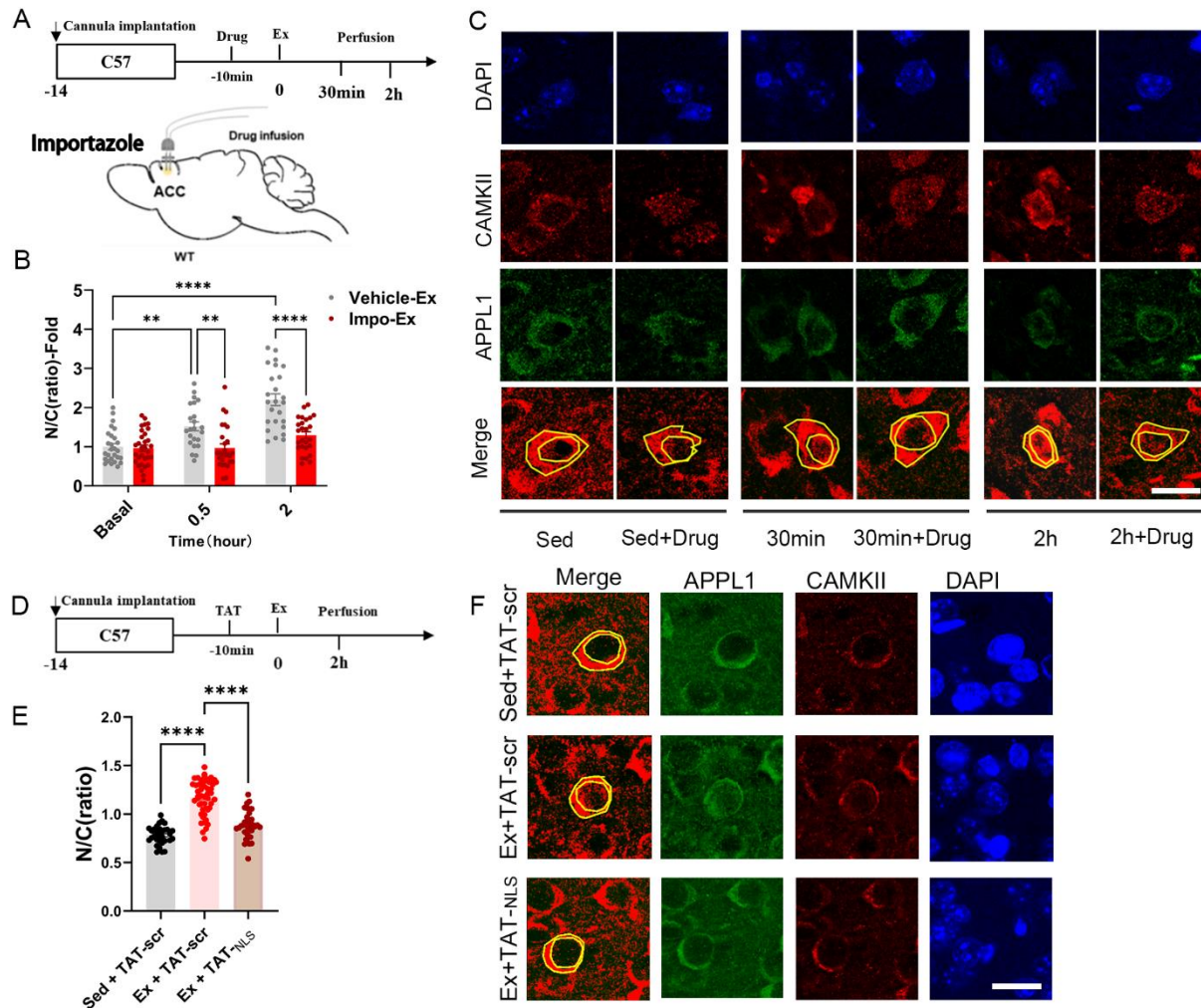


Fig. S10. Administration of importazole in the ACC effectively inhibits the exercise-induced nuclear translocation of APPL1. Related to Figure 5. **(A)** Experimental timeline. **(B)** Effects of importazole on APPL1 nuclear translocation at different time points following single-bout exercise. Statistical analysis of the nuclear/cytoplasmic ratio of APPL1. Each point represents a ROI, whereas each ROI contains 3-5 neurons. N = 3-4 animals per timepoint. Two-way ANOVA with Tukey's test for multiple comparisons. **(C)** Immunofluorescence staining showing temporal changes of APPL1 distribution for two groups after single-bout exercise. Scale bar, 10 μ m. Data were presented as mean $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. **(D)** Experimental timeline. **(E)** Effects of TAT-APPL1_{NLS} and TAT-scr on APPL1 nuclear translocation at the 2-hour timepoint following single-bout exercise. Statistical analysis of the

103 nuclear/cytoplasmic ratio of APPL1. Each point represents a ROI, whereas each ROI contains 2-3
104 neurons. N = 3-4 animals per timepoint. (F) Immunofluorescence staining showing the changes of
105 APPL1 distribution of three groups 2 hours after single-bout exercise. Scale bar, 10 μ m. One-way
106 ANOVA with Tukey's test for the multiple comparisons test. Data were presented as mean $\pm$ SEM.
107 **P < 0.01, and ****P < 0.0001.

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118 **Table S1. Clinical characteristics of participants.** Related to Figure 1.

	HADS Symptoms (n=20)	HADS no symptoms (n=20)
Depression (HADS score)	7.35 ± 2.72	1.95 ± 1.79
Anxiety (HADS score)	10.75 ± 3.57	3.45 ± 2.11
Age (years)	27.1 ± 4.91	26.95 ± 4.81
Biological sex	10 male /10 female	6 male /14 female
Systolic blood pressure (mmHg)	115.5 ± 14.25	117.75 ± 13.08
Diastolic blood pressure (mmHg)	69.45 ± 2.02	67.4 ± 9.09
Height (cm)	170.05 ± 9.08	167.75 ± 9.88
Weight - Kg	63.39 ± 11.14	58.19 ± 7.54
BMI – Body Mass Index	21.76 ± 2.17	20.67 ± 1.82
Hip circumference (cm)	94.24 ± 8.99	90.83 ± 8.78
Waist circumference (cm)	77.10 ± 8.21	75.57 ± 5.01
Waist Hip ratio	0.82 ± 0.12	0.84 ± 0.11

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120 Data were shown as means ± SD. Data normality was assessed using the D’Agostino and Pearson
121 test. For the normally distributed data, an unpaired t-test was used. For non-parametric data, the
122 Mann-Whitney test was employed.

123 **Table S2. Chronic unpredictable stress protocol.** Related to Figure 1.

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Day	Stressors	Day	Stressors
1	restraint stress (0.5h)	12	Empty bottle exposure (1h)
2	inescapable footshock (5 min – 5mA)	13	wet bedding (maximum 24h)
3	exposure to the shock apparatus (without shock 1h)	14	paired housing (1h)
4	paired housing (1h)	15	inescapable footshock (5 min 5mA)
5	cage tilt (maximum 24h)	16	exposure to the shock apparatus (without shock 1h)
6	restraint stress (1.5h)	17	wet bedding (maximum 24h)
7	paired housing (1h)	18	food and water deprivation (maximum 24h)
8	wet bedding (maximum 24h)	19	Empty bottle exposure (1h)
9	paired housing (1h)	20	cage tilt (maximum 24h)
10	restraint stress (2h)	21	restraint stress (2.5h)
11	food and water deprivation (maximum 24h)		

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127 **Table S3. Antibodies used in Western blotting and immunofluorescence staining.**

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Items	Antibody	Host	Company	Dilution	Catalog #
Western blotting - Primary antibody	APPL1	Rabbit	CST	1:1000	3858S
	GluR1	Rabbit	CST	1:1000	13185S
	PSD95	Rabbit	CST	1:1000	2507S
	Synapsin I	Rabbit	CST	1:1000	5297T
	Synaptophysin	Rabbit	CST	1:1000	5461S
	Histone 4	Mouse	CST	1:1000	2935
	Acetyl-Histone 4 (Lys12)	Rabbit	CST	1:1000	13944S
	Actin	Rabbit	CST	1:5000	4967S
	Tubulin	Rabbit	CST	1:5000	2144S
	GAPDH	Rabbit	CST	1:5000	5174
Western blotting - Second antibody	Goat anti-rabbit IgG	-	CST	1:2000	7074
	Goat anti-mouse IgG	-	CST	1:2000	7076
Immunofluorescence - Primary antibody	GABA	Rabbit	Sigma	1:200	A2052
	GAD67	Mouse	Millipore	1: 200	MAB5406
	CAMKII	Rabbit	Abcam	1: 200	ab5683
	CAMKII	Mouse	Abcam	1: 200	ab171095
	c-Fos	Mouse	Abcam	1:200	ab208942
	c-Fos	Rabbit	Abcam	1: 200	ab214672
	APPL1	Mouse	Santa Cruz	1:300	sc-271909
	AdipoR1	Rabbit	Abcam	1:200	Ab70362
	AdipoR1	Rabbit	Bioss	1:200	Bs-0610R
	Goat anti-mouse, 488	-	Abcam	1:200	ab150113
	Goat anti-mouse, 568	-	Abcam	1:200	ab175473

Immunofluorescence - Second antibody	Goat anti-mouse, 568	-	Thermofish er	1:200	A-11004
	Goat anti-rabbit, 568		Thermofish er	1:200	A-11011
	Goat anti-rabbit, 488	-	Thermofish er	1:200	A-11008

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132 **Table S4. Virus information**

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Name	Viral titers (vg/ml)	Supplier and Catalog
AAV2/9-CaMKIIa -GCaMP6s	2.7×10^{12}	Brain VTA
AAV2-Syn-DIO-hM3Dq-mCherry	2.7×10^{13} vg/ ml	Addgene plasmid #44361
AAV2-Syn-DIO-hM4Di-mCherry	2.5×10^{13} vg/ml	Addgene plasmid #44362
AAV2-hSyn-DIO-mCherry	1.5×10^{13} vg/ml	Addgene plasmid #50459
AAV9-CMV-DIO-RFP- mir30shRNA (AdipoR1)	$>1.0 \times 10^{13}$ vg/ml	WZ Biosciences Inc
AAV9-CMV-DIO-RFP- mir30shRNA (AdipoR2)	$>1.0 \times 10^{13}$ vg/ml	WZ Biosciences Inc
AAV2- CaMKIIa -Cre	1.5×10^{13} vg/ml	Addgene plasmid #105558
AAV9-CMV-DIO-RFP- mir30shRNA (APPL1)	$>1.0 \times 10^{13}$ vg/ml	WZ Biosciences Inc
AAV9-CMV-DIO-RFP- mir30shRNA (Control)	$>1.0 \times 10^{13}$ vg/ml	WZ Biosciences Inc
AAV9- CaMKII a -APPL1	1.0×10^{13} vg/ml	WZ Biosciences Inc
AAV9- CaMKII a -mCherry	1.0×10^{13} vg/ml	WZ Biosciences Inc

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137 **Table S5. Primer sequences for RT-PCR**

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Name	Sequence (5' to 3')
APPL1-F	GGTAGCCAGTGACCCTTTAT
APPL1-R	CTCCTGCCACATCTCCAC
Adiponectin R1-F	GAAAGACAACGACTACCTGCTAC
Adiponectin R1-R	TGAACAAAGGCACCAGCAA
GAPDH-F	CCCATCACCATCTTCCAGGAGC
GAPDH-R	CCAGTGAGCTTCCCGTTCAGC

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