

Supplemental material

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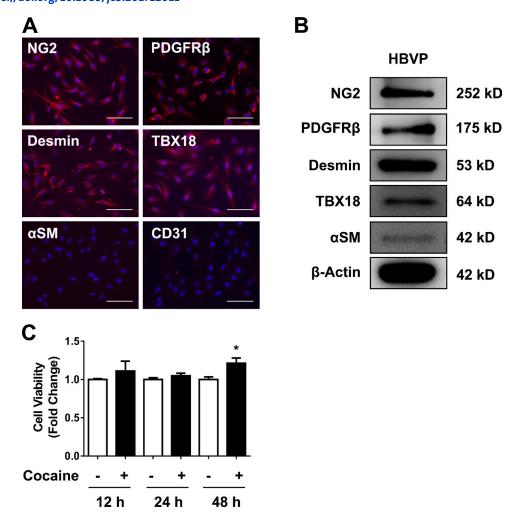


Figure S1. Immunostaining of different cell markers in HBVPs and MTS cell-viability assay of HBVPs. (A) Immunostaining of HBVPs using various cell-specific markers: pericyte markers, NG2, PDGFR- β , Desmin, TBX18; smooth muscle cell marker, α SM; endothelial cell marker, CD31. Bars, 50 μ m. (B) Representative Western blot of expression levels of various cell-specific markers in HBVPs: pericyte markers, NG2, PDGFR- β , Desmin, TBX18; smooth muscle cell marker, α SM. (C) Cell viability of HBVPs exposed to cocaine for 12, 24, and 48 h was determined by the MTS colorimetric assay. Two-tailed Student's t test. All data are presented as means \pm SD of four individual experiments (biological replicates). *, P < 0.05 versus control group.



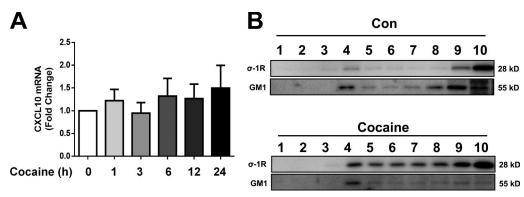


Figure S2. **CXCL10 mRNA expression levels in HEK293 cells and \sigma-1R expression levels in lipid raft isolation fractions of HBVPs. (A)** CXCL10 mRNA expression levels in HEK293 cells exposed to cocaine were examined by real-time PCR. One-way ANOVA followed by Bonferroni's post hoc test was used to determine the statistical significance among multiple groups. **(B)** Representative Western blot of σ -1R and GM1 in 1–10 lipid raft isolation fractions of HBVPs exposed to cocaine. One-way ANOVA followed by Bonferroni's post hoc test was used to determine the statistical significance among multiple groups. Data are presented as means \pm SD of three or four individual experiments (biological replicates).



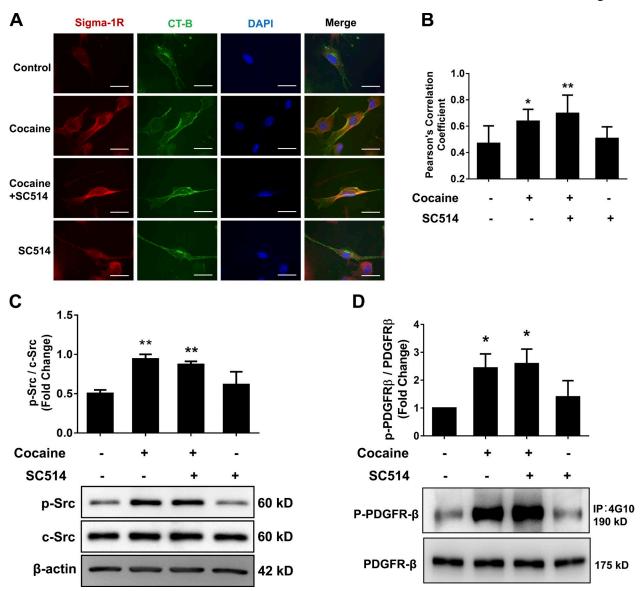


Figure S3. Pretreatment of HBVPs with the IKK-2/NF-κB inhibitor SC514 failed to abrogate cocaine-mediated lipid raft translocation of σ -1R, Src phosphorylation, and PDGFR- β phosphorylation. (A) Representative images of HBVPs stained with anti– σ -1R antibody and CT-B conjugated Alexa Fluor 488 specific for the ganglioside GM1-lipid raft marker. Bar, 20 μm. (B) Quantification of colocalization of σ -1R and CT-B. One-way ANOVA followed by Bonferroni's post hoc test was used to determine the statistical significance among multiple groups. (C) Representative Western blot and quantification of p-Src levels in HBVPs pretreated with SC514 for 1 h, followed by cocaine exposure for an additional 15 min. One-way ANOVA followed by Bonferroni's post hoc test was used to determine the statistical significance among multiple groups. (D) Representative Western blot and quantification of p-PDGFR- β levels in HBVPs pretreated with SC514 for 1 h, followed by cocaine exposure for an additional 15 min. One-way ANOVA followed by Bonferroni's post hoc test was used to determine the statistical significance among multiple groups. All data are presented as means \pm SD of three or four individual experiments (biological replicates). *, P < 0.05, **, P < 0.01 versus control group.



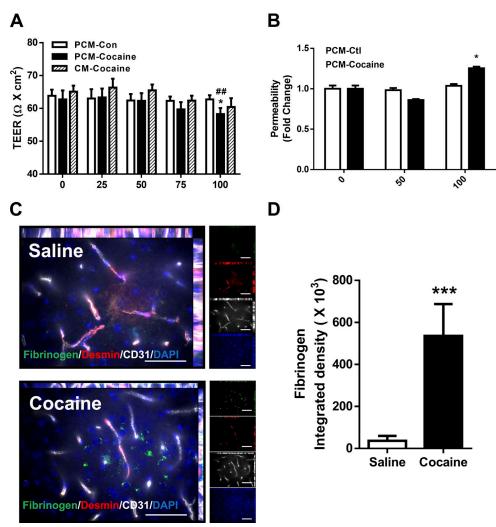


Figure S4. The effect of cocaine on permeability of BBB in vitro and in vivo. (A) TEER values of HBMECs cultured in the Transwell and exposed to different concentrations of PCM-Con, PCM-cocaine, and CM-cocaine (without HBVPs) were examined by the Millicell ERS-2 Voltohmmeter. One-way ANOVA followed by Bonferroni's post hoc test was used to determine the statistical significance among multiple groups. *, P < 0.05 versus 0% PCM group. ##, P < 0.01 versus PCM-Con group. (B) The permeability of HBMECs exposed to different concentrations of PCM-Con and PCM-cocaine. Two-tailed Student's t test. (C) Representative images of brain sections from saline or cocaine-administered mice and stained for anti-fibrinogen, anti-Desmin, and anti-CD31 antibodies. Bar, 50 µm. (D) Quantification of fibrinogen fluorescent intensity in the brains of mice administrated saline or cocaine. Two-tailed Student's t test. All data are presented as means ± SD of three or four individual experiments (biological replicates). *, P < 0.05, ***, P < 0.001 versus saline or control group.



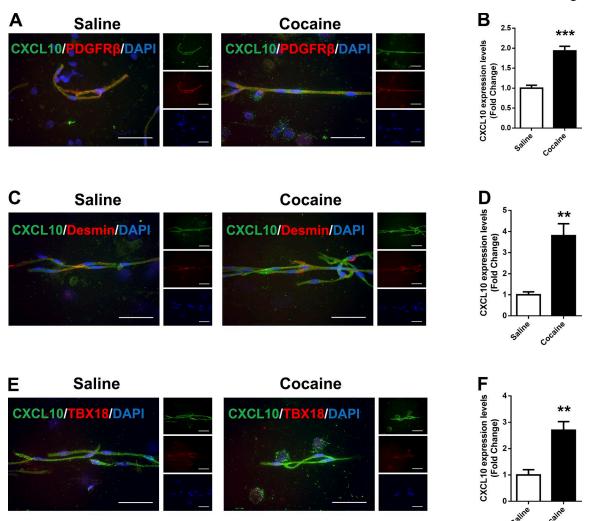


Figure S5. **CXCL10 expression levels in pericytes of MIVs isolated from brains of mice administrated with saline or cocaine. (A)** Representative images of MIVs, isolated from brains of mice administrated saline or cocaine and stained with anti-CXCL10 and anti-PDGFR-β. Bar, 50 μm. **(B)** Quantification of fluorescent intensities of CXCL10 staining in PDGFR-β+ cells. Two-tailed Student's *t* test. **(C)** Representative images of MIVs isolated from brains of mice administrated saline or cocaine and stained with anti-CXCL10 and anti-Desmin. Bars, 50 μm. **(D)** Quantification of fluorescent intensities of CXCL10 staining in Desmin+ cells. Two-tailed Student's *t* test. **(E)** Representative images of MIVs isolated from brains of mice administrated saline or cocaine and stained with anti-CXCL10 and anti-TBX18. Bar, 50 μm. **(F)** Quantification of fluorescent intensities of CXCL10 staining in TBX18+ cells. Two-tailed Student's *t* test. All data are presented as means ± SEM of three or four individual experiments (biological replicates). ***, P < 0.01, ****, P < 0.001 versus saline group.



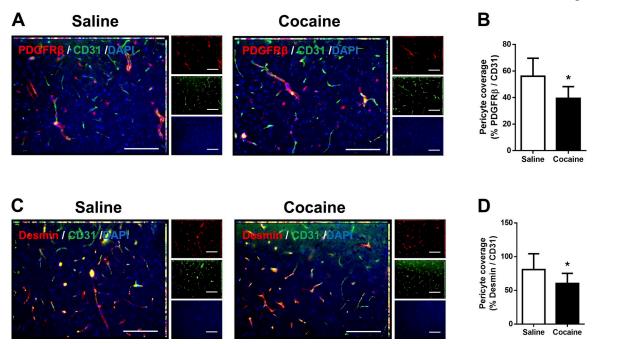


Figure S6. **Pericyte coverage in MIVs from brains of mice administrated either saline or cocaine.** (A) Representative images of MIVs in the brains of mice administrated either saline or cocaine and immunostained with anti-PDGFR- β and anti-CD31 antibodies. Bar, 100 μ m. (B) Percentage of PDGFR- β * surface area covering CD31* capillary surface area. Two-tailed Student's t test. (C) Representative images of MIVs in the brains of mice administrated either saline or cocaine and immunostained with anti-Desmin and anti-CD31 antibodies. Bar, 100 μ m. (D) Percentage of Desmin* surface area covering CD31* capillary surface area. Two-tailed Student's t test. All data are presented as means t SD of three or four individual experiments (biological replicates). *, P < 0.05 versus saline group.

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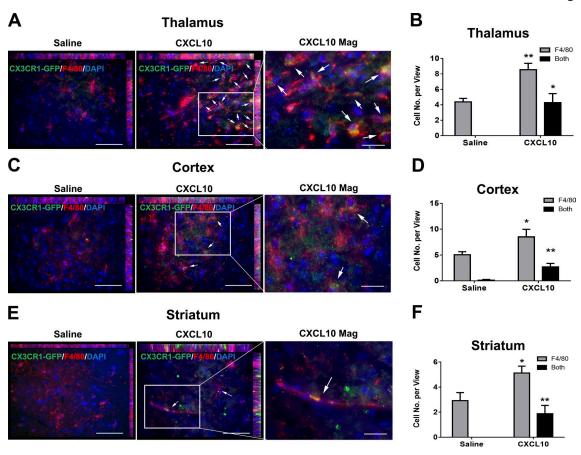


Figure S7. **CX3CR1-GFP* BMMs transmigration in the brain of mice administrated CXCL10. (A)** Representative images of F4/80* and GFP*/F4/80* cells in the thalamus of mice administrated saline or CXCL10. Arrow: GFP*/F4/80* cells. Bar, 50 μm; enlarged images, 20 μm. **(B)** Quantification of F4/80* and GFP*/F4/80* cells in the thalamus of mice administrated saline or CXCL10. Two-tailed Student's *t* test. **(C)** Representative images of F4/80* and GFP*/F4/80* cells in the cortex of mice administrated saline or CXCL10. Arrow: GFP*/F4/80* cells. Bar, 50 μm; enlarged images, 20 μm. **(D)** Quantification of F4/80* and GFP*/F4/80* cells in the cortex of mice administrated saline or CXCL10. Two-tailed Student's *t* test. **(E)** Representative images of F4/80* and GFP*/F4/80* cells in the striatum of mice administrated saline or CXCL10. Arrows: GFP*/F4/80* cells. Bar, 50 μm; enlarged images, 20 μm. **(F)** Quantification of F4/80* and GFP*/F4/80* cells in the striatum of mice administrated saline or CXCL10. Two-tailed Student's *t* test. All data are presented as means ± SD of three or four individual experiments (biological replicates). *, P < 0.05, **, P < 0.01 versus saline group.

Table S1. Clinical data for human brain tissue samples

Case number	Dependence, current	Age (years)	Gender	Race	Cause of death
1 (101)	Nil	64	Male	Caucasian	Natural
2 (102)	Nil	18	Male	Caucasian	Natural
3 (108)	Nil	61	Male	Caucasian	Accident
4 (110)	Nil	26	Male	Caucasian	Accident
5 (113)	Alcohol-cocaine	24	Male	Caucasian	Accident
6 (114)	Alcohol-cocaine	39	Male	Caucasian	Suicide
7 (118)	Cocaine	37	Male	Caucasian	Suicide
8 (119)	Cocaine	45	Male	Caucasian	Suicide