Expanded View Figures

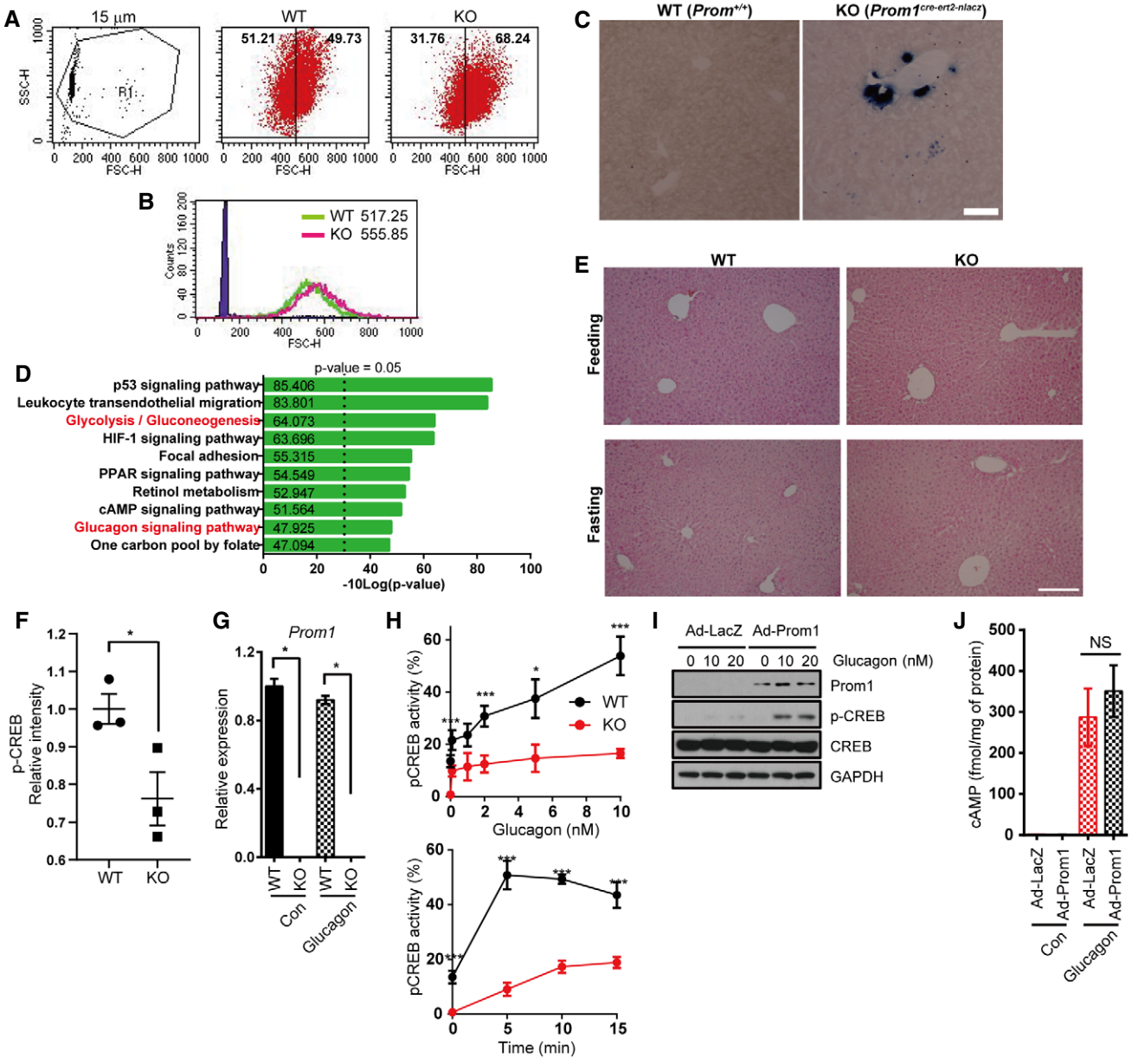


Figure EV1.

Figure EV1. Prom1 disruption prevents glucagon-induced gluconeogenesis in primary mouse hepatocytes.

A Typical plot of side scatter versus forward scatter for 15 μm beads, WT (*Prom1*$^{+/+}$), and KO (*Prom1*$^{-/-}$) primary hepatocytes.

B The forward scatter distribution of 15 μm beads, WT, and KO hepatocytes is presented as the superimposed histograms, and the means of forward scatter of WT and KO hepatocytes were labeled.

C β-galactosidase expression pattern in WT (*Prom1*$^{+/+}$) and KO (*Prom1*$^{cre\text{-}ert2\text{-}nlacz}$) liver using X-gal and nitroblue tetrazolium. Scale bar, 200 μm.

D Enriched KEGG pathway terms for differentially expressed genes. *P*-value was calculated by gProfileR in R package using g:SCS algorithm.

E H&E stain (Hematoxylin and eosin stain) of the liver of mouse in each condition. Scale bar, 500 μm.

F Relative level of p-CREB in WT and KO mouse livers after fasting (Fig 2B, n = 3 mice/group).

G Primary mouse hepatocytes were prepared from 12-week-old male WT and KO mice and serum-starved for 16 h. Relative expression levels of *Prom1* mRNAs were determined using qRT–PCR (n = 3/group).

H The percentage of cells with nuclear p-CREB staining among more than 300 cells from each group was statistically determined after stimulation with the indicated concentrations of glucagon for 10 min (left panel) or stimulation with 10 nM glucagon for the indicated times (right panel) (n = 3 replicates/group).

I, J *Prom1* overexpression in KO hepatocytes restores glucagon-induced CREB phosphorylation. Primary hepatocytes were isolated from 12-week-old male KO mice, infected with an adenovirus harboring *Prom1* for 24 h, serum-starved for 18 h, and then stimulated with glucagon (10 nM). (I) Levels of p-CREB and CREB were determined by immunoblotting 0, 10, and 20 min after glucagon (10 nM) stimulation. (J) The cAMP concentration was determined using the cAMP assay kit 10 min after glucagon stimulation (10 nM) in the presence of 10 μM IBMX (n = 3 mice/group).

Data information: Data are presented as mean values ± SEM. Two-tailed Student's *t*-test was used for statistical analysis. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and NS, not significant, $P > 0.05$.
Source data are available online for this figure.

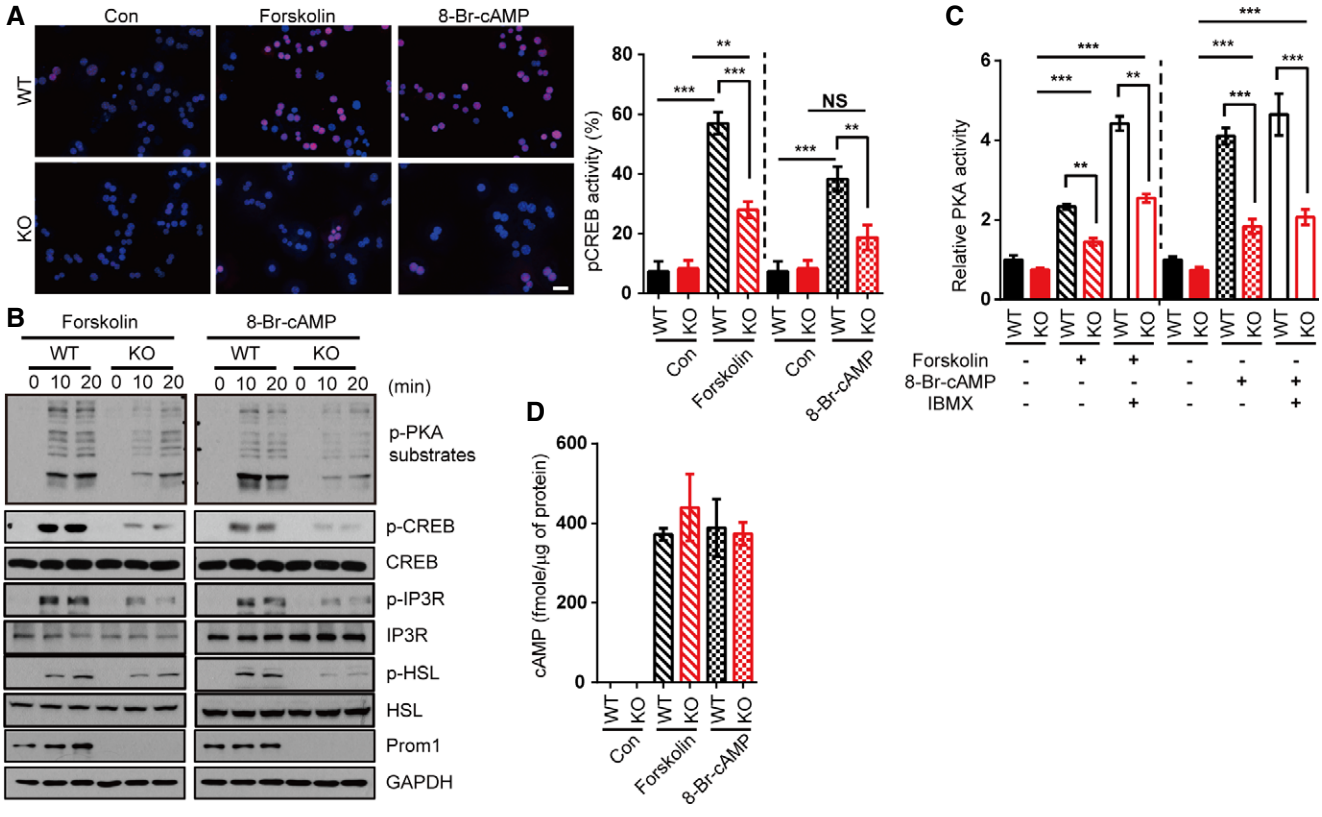


Figure EV2.

Figure EV2. Prom1 deficiency prevents glucagon-, forskolin-, or 8-Br-cAMP-induced PKA activation in primary mouse hepatocytes.

Primary mouse hepatocytes were prepared from 12-week-old male WT (*Prom1*$^{+/+}$) and KO (*Prom1*$^{-/-}$) mice and serum-starved for 16 h.

A Left panel, the nuclear localization of p-CREB was determined by immunofluorescence staining after a 10-min of forskolin (10 μM) or 8-Br-cAMP stimulation (10 μM). Right chart, the percentage of cells with nuclear p-CREB staining among more than 300 cells in each group was statistically determined in the images of immunofluorescence staining shown above. Scale bar, 20 μm.

B Levels of p-PKA substrates, p-CREB, CREB, p-IP3R, IP3R, p-HSL, HSL, and Prom1 after stimulation with forskolin (10 μM) or 8-Br-cAMP (10 μM) for the indicated times were determined by immunoblotting.

C Relative PKA activities were determined using the PKA assay kit after a 10-min stimulation with 8-Br-cAMP (10 μM) or forskolin (10 μM) in the presence of 10 μM IBMX (n = 3/group).

D The cAMP concentration was determined using the cAMP assay kit after a 10-min stimulation with 8-Br-cAMP (10 μM) or forskolin (10 μM) in the presence of 10 μM IBMX (n = 3/group).

Data information: Data are presented as mean values ± SEM. Two-tailed Student's t-test was used for statistical analysis. **$P < 0.01$ and ***$P < 0.001$.
Source data are available online for this figure.

Figure EV3. Radixin functions as an AKAP in primary hepatocytes.

A The relative expression levels of *Akap1*, *Akap10*, *Akap11*, *Akap12*, *Akap13*, *Akap14*, *Akap17b*, *Akap2*, *Akap3*, *Akap4*, *Akap5*, *Akap6*, *Akap7*, *Akap8*, *Akap9*, *ezrin*, *radixin*, and *moesin* mRNAs in hepatocytes isolated from 12-week-old male WT (*Prom1*$^{+/+}$) and KO (*Prom1*$^{-/-}$) mice and serum-starved for 16 h were determined using RNA-seq. AKAP, A kinase-anchored protein.

B The expression of ezrin, radixin, and moesin in mouse primary hepatocyte, liver, and kidney was determined by immunoblotting. Lv, liver; Kd, kidney; HC, hepatocyte; W, wild type; K, knockout.

C The expression of Prom1, radixin, AKAP7, AKAP8, AKAP8I, AKAP9, AKAP10, AKAP12, or AKAP13 was silenced in WT hepatocytes using siRNAs. Hepatocytes were then serum-starved for 18 h and stimulated with glucagon (10 nM) for 10 min. Levels of the *Prom1*, *radixin*, *Akap7*, *Akap8*, *Akap8l*, *Akap9*, *Akap10*, *Akap12*, and *Akap13* mRNA were determined using qRT–PCR (n = 3 replicated siRNA treatments/group).

D Prom1 or radixin expression was silenced in WT hepatocytes, and radixin expression was silenced in KO hepatocytes. Hepatocytes were serum-starved for 18 h and stimulated with glucagon (10 nM) for 10 min. Levels of p-PKA substrates, p-CREB, CREB, radixin, Prom1, and Gapdh were determined by immunoblotting.

E Levels of p-PKA substrates, ezrin, and GAPDH in siRNA-treated primary hepatocytes were determined by immunoblotting after stimulation with glucagon (10 nM) for 10 min.

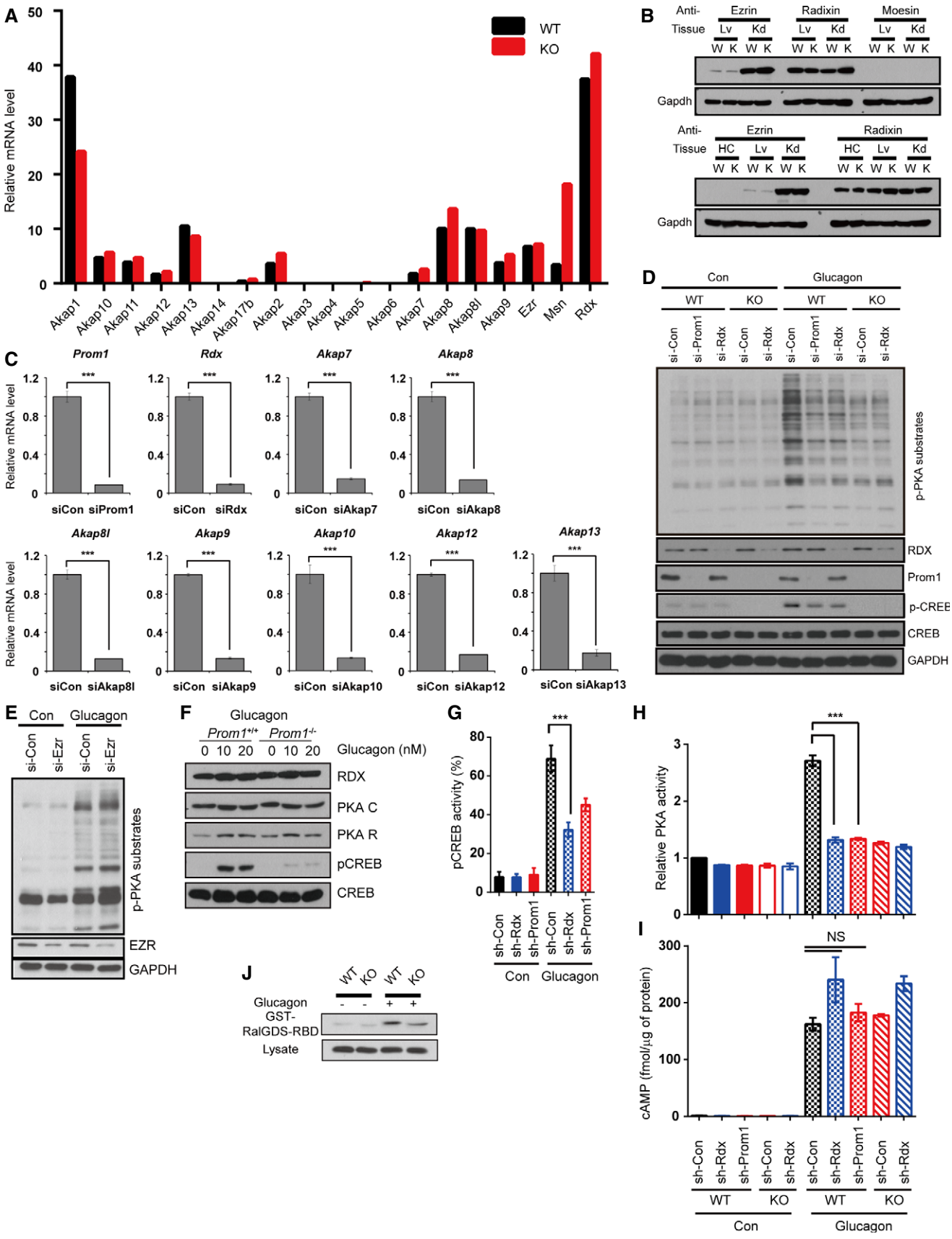
F Levels of radixin, PKA catalytic subunit, regulatory subunit, p-CREB, and CREB were determined by immunoblotting after glucagon stimulation (10 nM).

G The percentage of cells with nuclear p-CREB staining among more than 300 cells in each group was also statistically analyzed.

H, I Prom1 and radixin expression was silenced in WT (*Prom1*$^{+/+}$) and KO (*Prom1*$^{-/-}$) hepatocytes by infection with an adenovirus harboring sh-control (sh-Con), sh-radixin (sh-Rdx), or sh-Prom1 for 24 h. Hepatocytes were further serum-starved for 18 h and stimulated with glucagon (10 nM) for 10 min. (H) Relative PKA activities were determined using the PKA assay kit after stimulation with glucagon for 10 min (n = 3 replicates/group). (I) The cAMP concentration was determined using the cAMP assay kit after a 10-min glucagon stimulation in the presence of 10 μM IBMX (n = 3 replicates/group).

J Pull-down of activated Rap1 with GST-RalGDS-RBD from WT (*Prom1*$^{+/+}$) and KO (*Prom1*$^{-/-}$) primary mouse hepatocytes stimulated with glucagon (10 nM) for 10 min.

Data information: Data are presented as mean values ± SEM. Two-tailed Student's t-test was used for statistical analysis. ***$P < 0.001$ and NS, not significant, $P > 0.05$.
Source data are available online for this figure.


A
Relative mRNA level
WT
KO
Akap1
Akap10
Akap11
Akap12
Akap13
Akap14
Akap17b
Akap2
Akap3
Akap4
Akap5
Akap6
Akap7
Akap8
Akap8l
Akap9
Ezr
Msn
Rdx
B
Anti-
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Ezrin
Radixin
Moesin
Lv
Kd
W K
Gapdh
HC
C
Prom1
Rdx
Akap7
Akap8
siCon siProm1
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siCon siAkap8
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Akap9
Akap10
Akap12
Akap13
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siCon siAkap9
siCon siAkap10
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siCon siAkap13
D
Con
Glucagon
si-Con
si-Prom1
si-Rdx
p-PKA substrates
RDX
Prom1
p-CREB
CREB
GAPDH
E
si-Ezr
EZR
F
Prom1+/+
Prom1-/-
0 10 20
Glucagon (nM)
PKA C
PKA R
pCREB
G
pCREB activity (%)
sh-Con
sh-Rdx
sh-Prom1
H
Relative PKA activity
I
cAMP (fmol/μg of protein)
NS
J
Glucagon
GST-RalGDS-RBD
Lysate

Figure EV3.

Figure EV4. Radixin functions as an AKAP required for glucagon-induced PKA activation in primary hepatocytes and the liver.

A The transduction efficiency of adenoviral particles was confirmed by bicistronic expression of GFP. Confocal microscope images of WT mice livers infected with adenovirus harboring sh-Con or sh-Rdx linked with IRES-GFP. Scale bar, 100 μm.

B, C 12-week-old male wild-type mice were infected with an adenovirus harboring sh-control (sh-Con) or sh-radixin (sh-Rdx) for 3 days, fasted for 4 h, and intraperitoneally injected with glucagon. (B) Relative PKA activities in the liver were determined using the PKA assay kit 10 min after glucagon (2 mg/kg body weight) stimulation (n = 3/group). (C) The cAMP concentration in the liver 10 min after glucagon (2 mg/kg body weight) stimulation was determined using the cAMP assay kit (n = 3/group).

D Immunofluorescent staining of Prom1 or radixin using actin staining by phalloidin as a reference in cryosections of WT mouse liver. Scale bar, 100 μm.

E The cellular localization of radixin and actin in primary hepatocytes from 12-week-old male wild-type mice infected with an adenovirus harboring sh-control (sh-Con), sh-Prom1, or sh-radixin (sh-Rdx) for 3 days was determined by immunofluorescence staining for radixin and phalloidin staining. Scale bar, 20 μm.

F, G The molecular interaction between catalytic subunit of PKA and CREB in WT (*Prom1*$^{+/+}$) and KO (*Prom1*$^{-/-}$) hepatocytes was determined by a proximity ligation assay using anti-PKA Cα and anti-CREB antibodies. These hepatocytes were stimulated with glucagon (10 nM) for the indicated time or treated with H-89, PKA inhibitor, prior to glucagon stimulation. Scale bar, 50 μm. (G) The portion of cells with nuclear PKA Cα-CREB interaction (F) in more than 5 to 8 imaging area from each group was statistically determined.

H Inhibition of ERK1/2 activity did not affect CREB phosphorylation. Levels of p-CREB, p-ERK1/2, and actin in mouse primary hepatocytes after glucagon stimulation (10 nM) or insulin stimulation (100 nM) for 15 min were determined by immunoblotting. PKA inhibitor, H-89 or ERK1/2 inhibitor, PD98059 were added prior to glucagon/insulin treatment at 30 and 50 μM, respectively.

Data information: Data are presented as mean values ± SEM. Two-tailed Student's t-test was used for statistical analysis. *$P < 0.05$ and NS, not significant, $P > 0.05$.
Source data are available online for this figure.

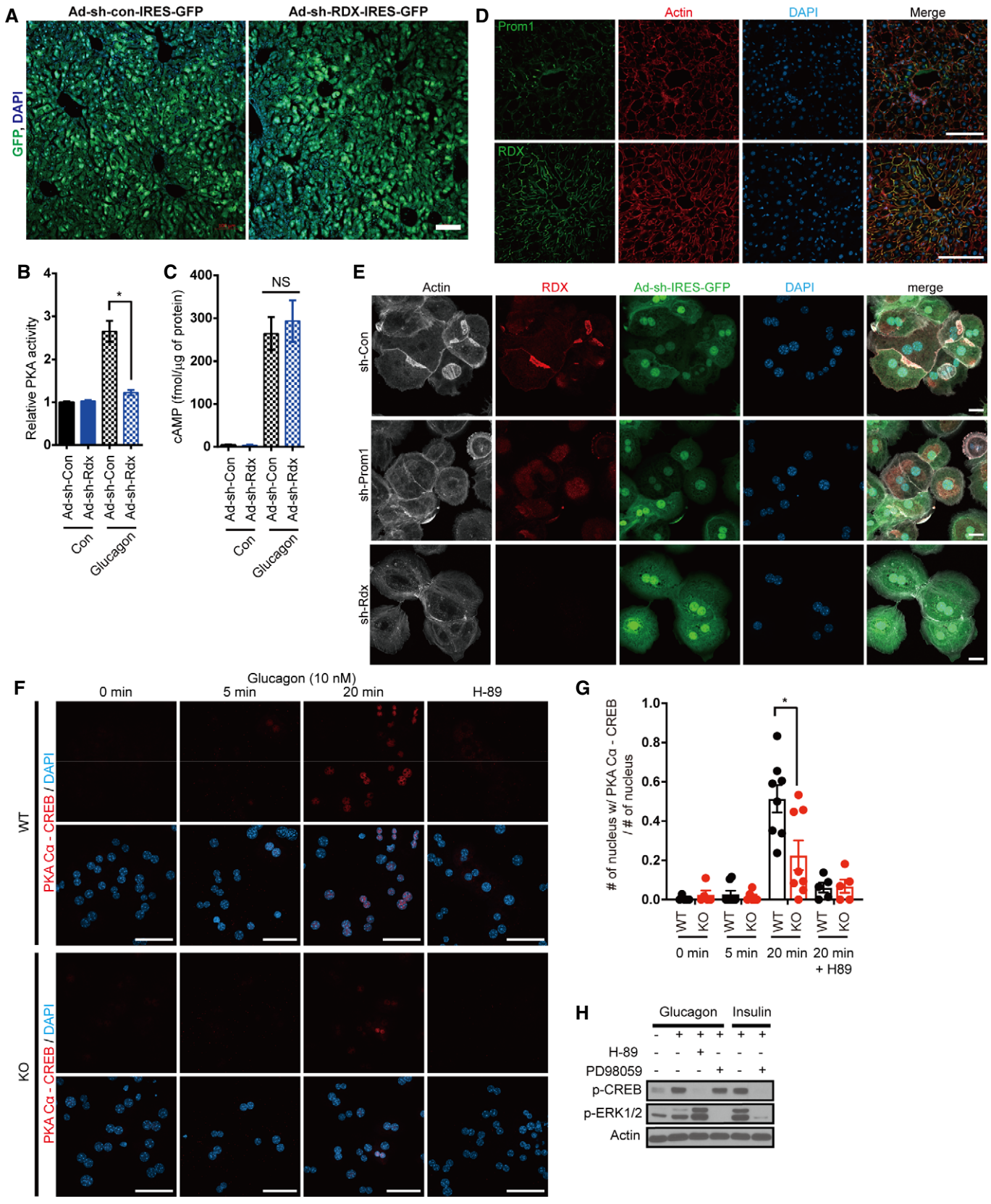

Figure EV4.

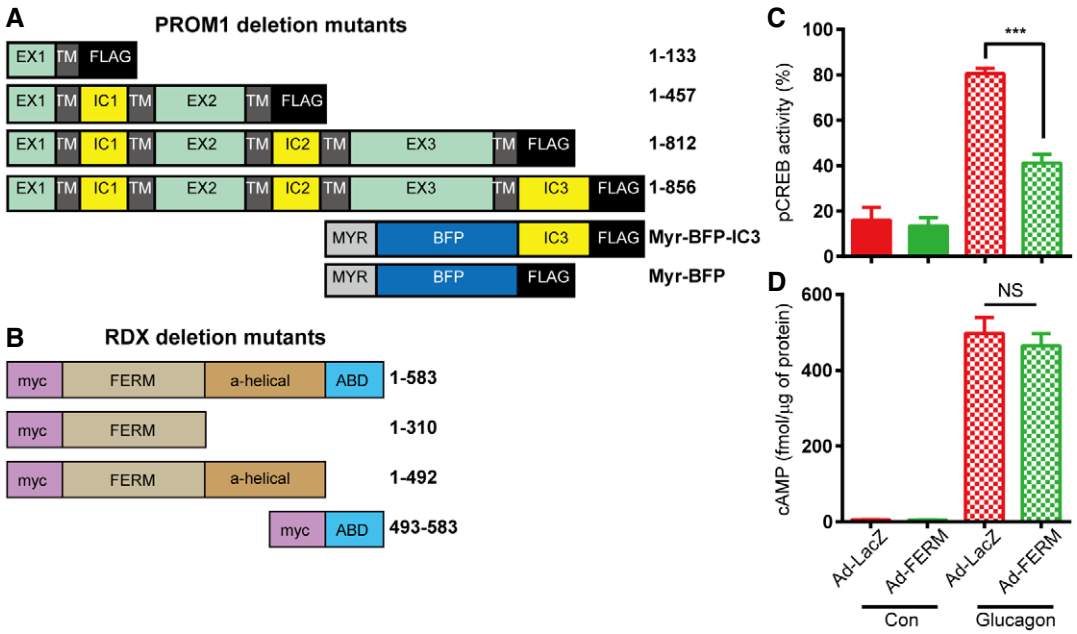

Figure EV5. Prom1 and radixin interact via C-terminal cytoplasmic domain of Prom1 and N-terminal FERM domain of radixin.

A, B Domain structures of deletion mutants of human Prom1 (A) and human radixin (B). EX, extracellular domain; TM, transmembrane domain; IC, intracellular domain; BFP, blue fluorescence protein; Myr, myristoylation site; FERM, 4.1 protein–ezrin–radixin–moesin domain; ABD, actin-binding domain.

C, D Overexpression of the FERM domain prevents glucagon-induced PKA activation. WT primary hepatocytes were infected with an adenovirus harboring the FERM domain for 48 h, serum-starved for 18 h, and stimulated with glucagon (10 nM). (C) The percentage of cells displaying nuclear p-CREB staining among more than 300 cells from each group was statistically determined by analyzing the images of immunofluorescence staining shown in Fig 6E. (D) The cAMP concentration was determined using the cAMP assay kit 10 min after stimulation with glucagon (n = 3 replicates/group).

Data information: Data are presented as mean values ± SEM. Two-tailed Student's t-test was used for statistical analysis. ***$P < 0.001$ and NS, not significant, $P > 0.05$.