Supplemental material

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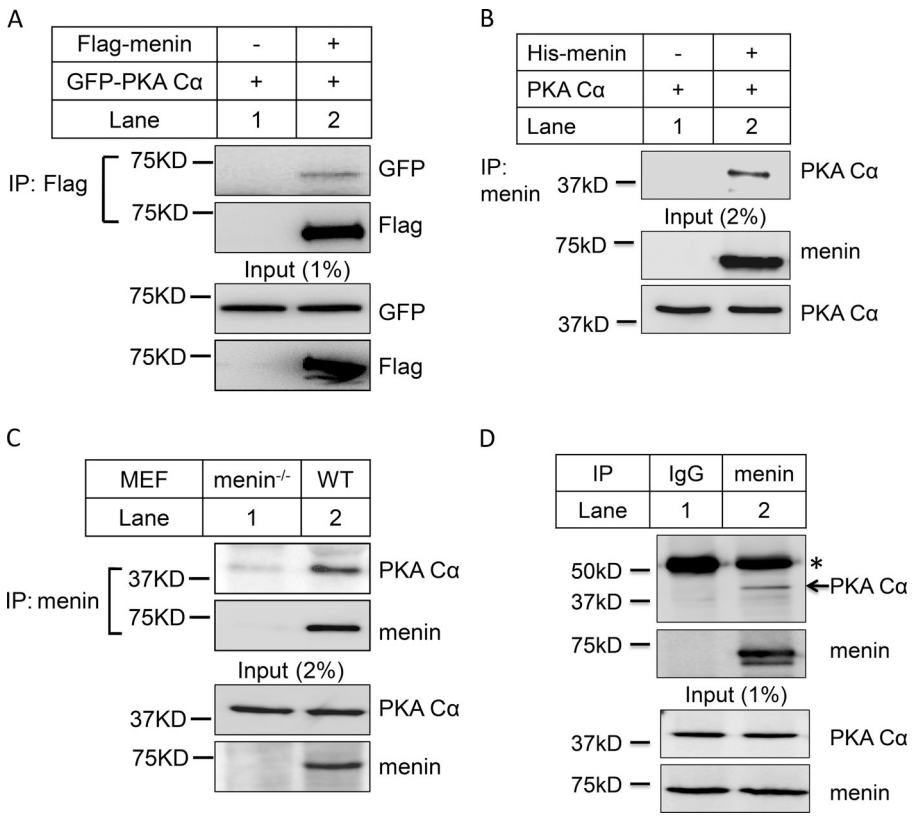

Figure S1. **PKA interacts with menin. (A)** HEK293 cells were cotransfected with Flag-menin and GFP-PKA Cα, followed by coIP and Western blot with the indicated antibodies. **(B)** Recombinant His-menin proteins were incubated with commercial PKA Cα and pulled down with anti-menin antibody attached to Protein A agarose beads, followed by Western blot using the indicated antibodies. **(C and D)** Endogenous PKA Cα and menin interaction was detected in MEF (C) and INS-1 (D) cells using anti-menin antibody for IP and the indicated antibodies for Western blot. Asterisk (*) indicates IgG heavy chain.

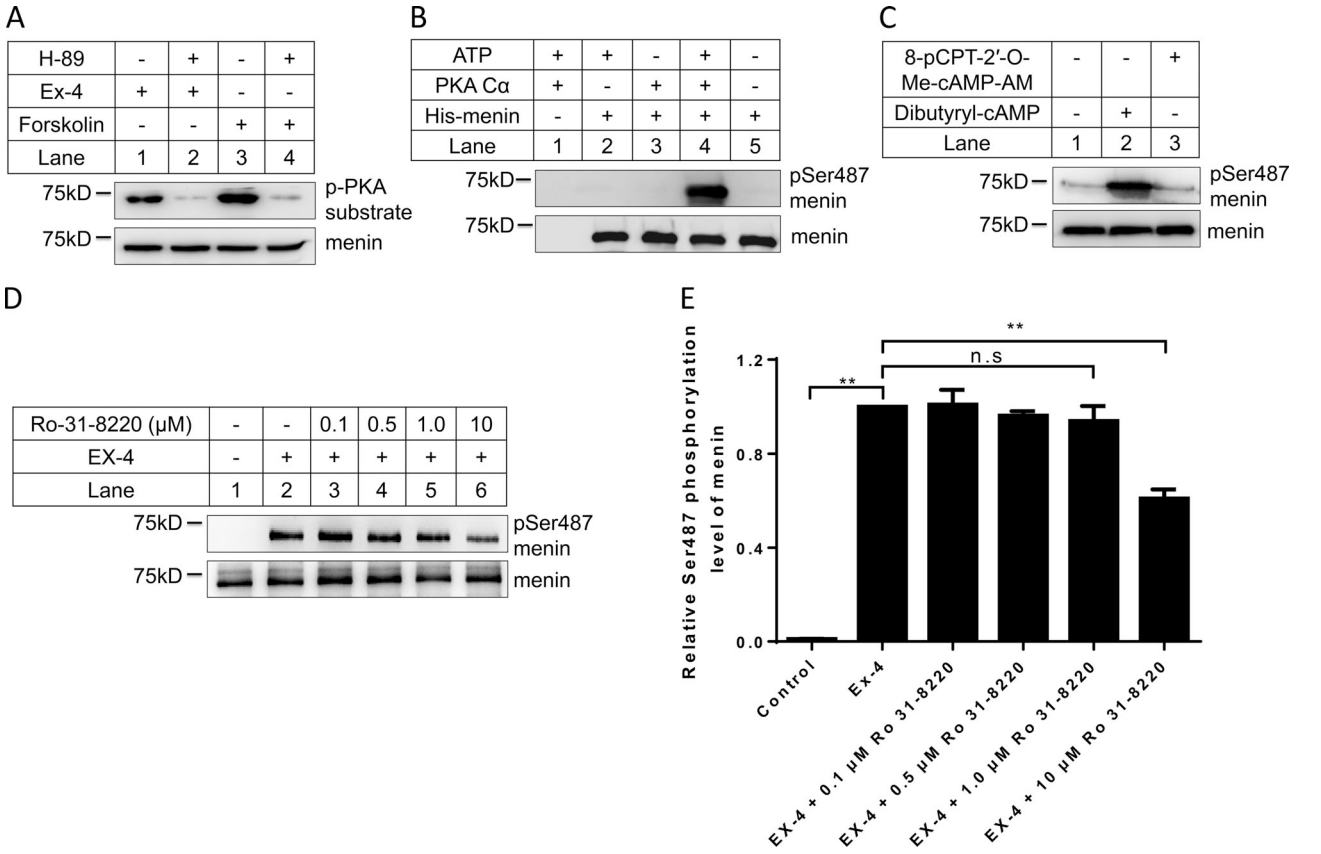

Figure S2. **GLP-1/PKA phosphorylates menin at the Ser487 residue. (A)** After overnight starvation, INS-1 cells were treated with 10 nM Ex-4 and 10 μM forskolin for 30 min and then lysed for IP with anti-menin antibody. Proteins retained on sepharose were blotted with the indicated antibodies. 2 h before Ex-4 or forskolin treatment, 10 μM H-89 was added to the starvation medium. **(B)** Samples from in vitro kinase assay conducted with purified recombinant His-menin and active PKA Cα were tested with anti-pSer487-menin and total menin antibodies. **(C)** INS-1 cells, treated with 100 μM dibutyryl-cAMP or 10 μM 8-pCPT-2′-O-Me-cAMP-AM for 30 min after overnight starvation, were lysed for IP with anti-menin antibody, followed by Western blot with the indicated antibodies. **(D)** INS-1 cells, treated with 10 nM Ex-4 for 30 min after overnight starvation, were lysed for IP with anti-menin antibody, followed by Western blot with the indicated antibodies. Ro-31-8220 was added to the starvation medium 2 h before treatment. **(E)** Band density analysis was executed with image J. Ser487 phosphorylated menin protein levels were quantified and normalized to total menin ($n = 3$). Significance was compared with the control group using a one-way ANOVA with LSD post hoc test. n.s, no significance; **, $P < 0.01$. Error bars indicate SD.

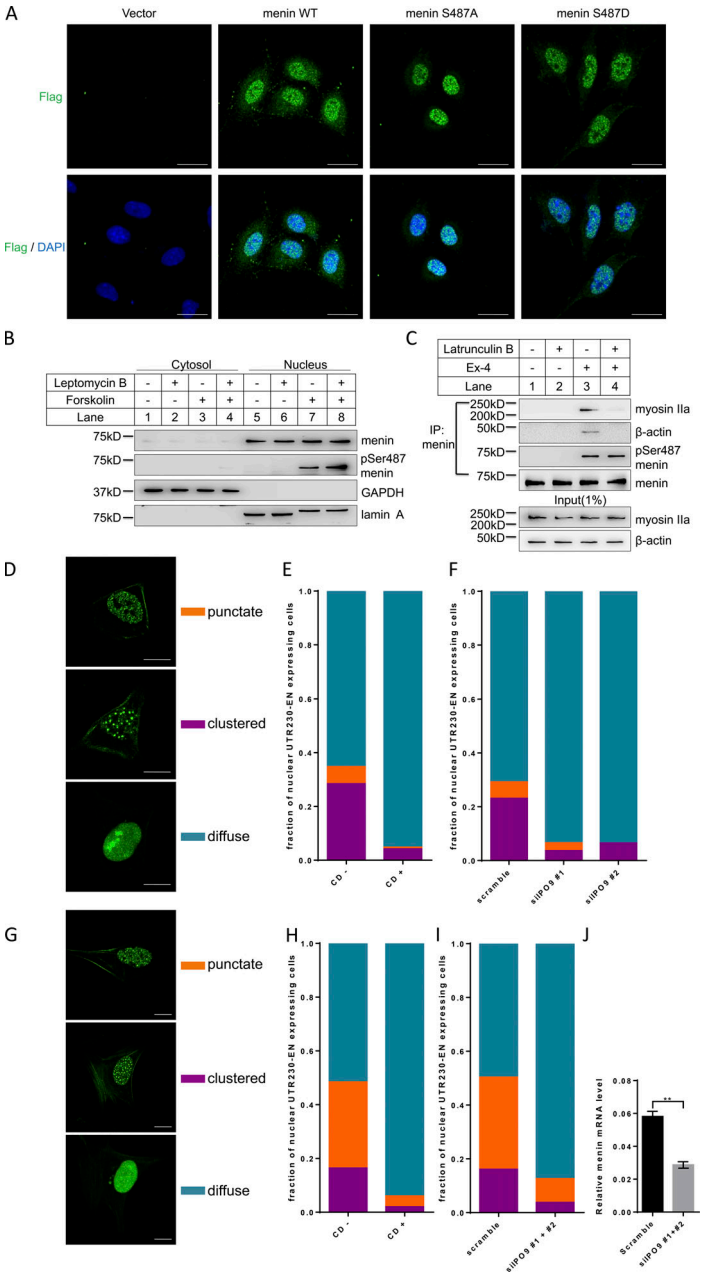

Figure S3. **Nuclear-localized pSer487 menin binds to F-actin, which could be disassembled by cytochalasin D or IPO9 knockdown. (A)** Menin-null MEF cells complemented with vector, Flag-tagged WT, or S487A or S487D menin were fixed and stained with anti-Flag antibody (green, Alexa Fluor 488 as the fluorochrome); nuclei were stained with DAPI (blue). Scale bars, 25 µm. **(B)** INS-1 cells, treated with 10 µM forskolin for 4 h after overnight starvation, were subjected to nuclear cytoplasmic separation, followed by Western blot with the indicated antibodies. 20 nM leptomycin B, which has been identified to inhibit nuclear export of proteins and RNA containing a nuclear export sequence, was added to the starvation medium 3 h before treatment, if applicable. **(C)** After overnight starvation, INS-1 cells were treated with 10 nM Ex-4 in the absence or presence of 10 µM latrunculin B for 1 h. Nuclear extract was isolated for coIP using anti-menin antibody. Proteins were separated by SDS-PAGE, followed by Western blot with the indicated antibodies. **(D)** INS-1 cells, transfected with Utr230-EN plasmid for 24 h, were fixed for imaging using confocal microscopy. The distribution of nuclear Utr230-EN exhibits three patterns in INS-1 cells: (1) punctate, (2) clustered, and (3) diffuse. Scale bars, 10 µm. **(E)** INS-1 cells were transfected with Utr230-EN plasmid for 12 h and then cultured for another 12 h with or without the treatment of 0.05 µM cytochalasin D (CD). The fraction of nuclear Utr230-EN–expressing cells is presented (CD–, *n* = 158 cells; CD+, *n* = 165 cells). **(F)** INS-1 cells were transfected with IPO9 siRNAs (siIPO9 #1 or siIPO9 #2) for 48 h, and then Utr230-EN was expressed by a second round of transfection. 24 h later, cells were fixed for imaging. The fraction of nuclear Utr230-EN–expressing cells is presented (scramble, *n* = 147 cells; siIPO9 #1, *n* = 136 cells; siIPO9 #2, *n* = 137 cells). **(G)** MEF cells, transfected with Utr230-EN plasmid for 24 h, were fixed for imaging using confocal microscopy. The distribution of nuclear Utr230-EN also exhibits three patterns in MEF cells: (i) punctate, (ii) clustered, and (iii) diffuse. Scale bars, 10 µm. **(H)** MEF cells were transfected with Utr230-EN plasmid for 24 h and then cultured for another 12 h with or without the treatment of 0.05 µM cytochalasin D. The fraction of nuclear Utr230-EN–expressing cells is presented (CD–, *n* = 231 cells; CD+, *n* = 198 cells). **(I)** MEF cells were cotransfected with IPO9 siRNAs (mixture of siIPO9 #1 and siIPO9 #2) and Utr230-EN plasmid for 72 h, followed by fixation and imaging. The fraction of nuclear Utr230-EN–expressing cells is presented (scramble, *n* = 260 cells; siIPO9 #1 + #2, *n* = 237 cells). **(J)** The knockdown efficiency of IPO9 in MEF cells was confirmed with quantitative real-time PCR (*n* = 4). Data were normalized to the level of GAPDH. Statistics: two-tailed *t* tests. **, P < 0.01. Error bars indicate SD.

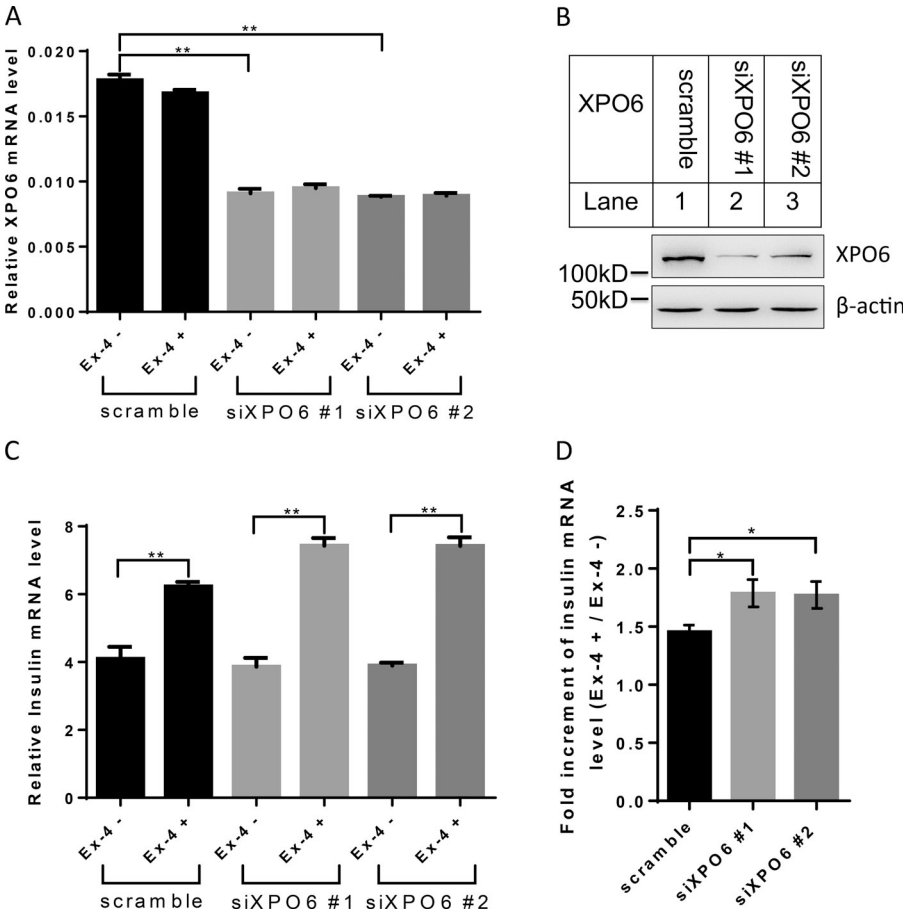

Figure S4. **Reduced expression of XPO6 renders INS-1 cells more sensitive to Ex-4–induced insulin transcription. (A)** INS-1 cells were transfected with XPO6 siRNAs for 48 h before serum was withdrawn from the culture medium. After overnight starvation, 10 nM Ex-4 was added to the starvation medium for 12 h, followed by quantitative RT-PCR analysis of XPO6 mRNA levels (n = 3). Data were normalized to the level of GAPDH. **(B)** The knockdown efficiency of XPO6 was confirmed with Western blot. **(C)** Samples from A were analyzed for insulin mRNA levels by quantitative RT-PCR (n = 3). Data were normalized to the level of GAPDH. **(D)** Fold increment of insulin mRNA level after Ex-4 treatment was calculated in scramble and siXPO6 INS-1 cells (n = 3). Statistics in A and C: one-way ANOVA with LSD post hoc test. Statistics in D: two-tailed t tests. *, P < 0.05; **, P < 0.01. Error bars indicate SD.

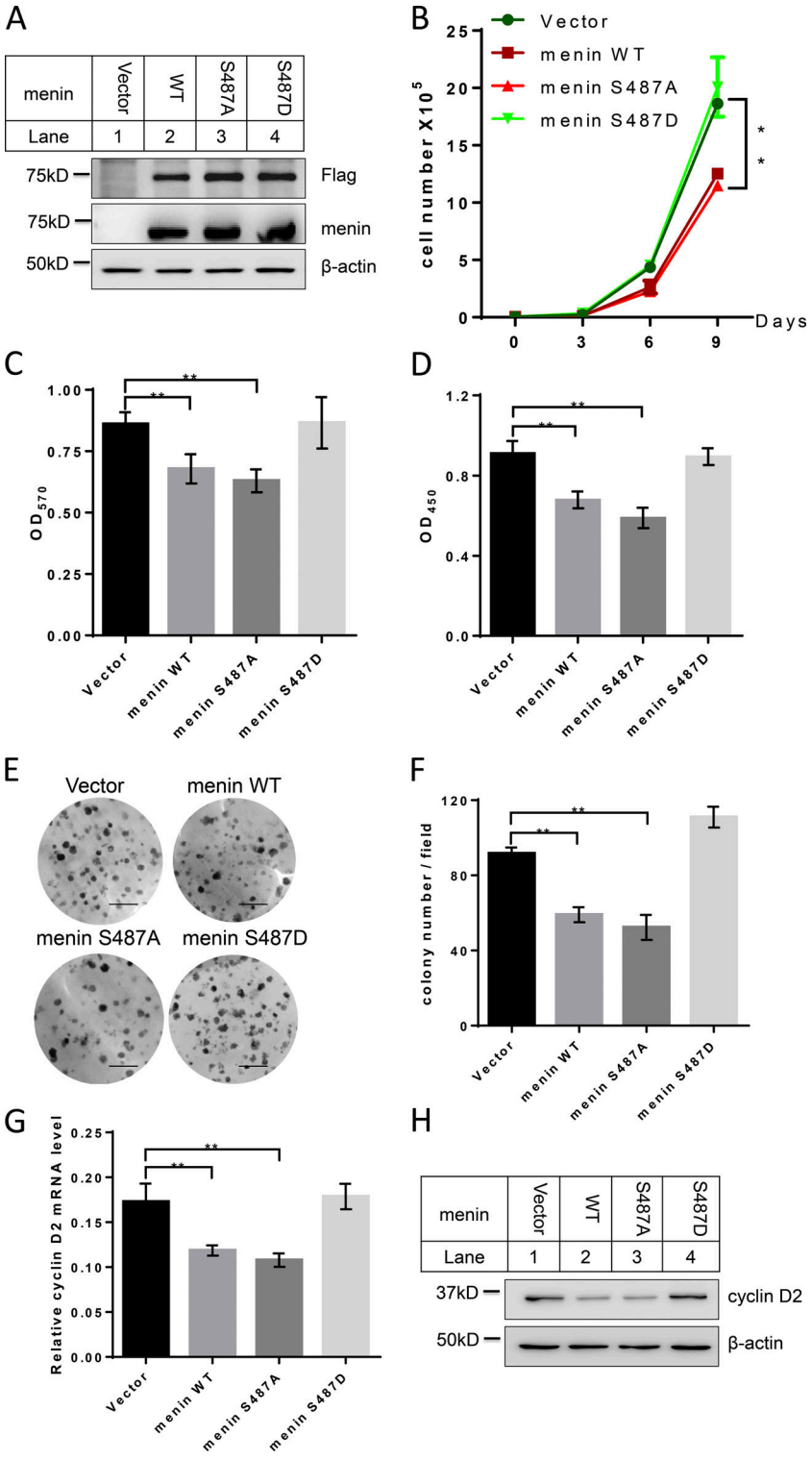

Figure S5. **Ser487-phosphorylated menin loses its ability of suppressing cell proliferation. (A)** Menin-null MEFs complemented with vector, Flag-menin WT, or S487A or S487D mutant were examined for menin expression with the indicated antibodies. **(B)** Menin-null MEFs complemented with vector, Flag-menin WT, or S487A or S487D mutant were examined for cell number with standard cytometry (n = 3). **(C)** MTT assay for assessing metabolic activity of MEF cells (n = 6). **(D)** Menin-null MEFs complemented with vector, Flag-menin WT, or S487A or S487D mutant were examined for proliferation with a BrdU assay kit (n = 7). **(E)** The in vitro proliferative ability of MEF cells was tested with colony-formation assay. Scale bars, 5 mm. **(F)** Colony number per field for each MEF cell line was counted (n = 3). **(G)** Quantitative real-time PCR analysis of cyclin D2 mRNA levels in menin-null MEFs complemented with vector, Flag-menin WT, or S487A or S487D mutant (n = 3). Data were normalized to the level of GAPDH. **(H)** Menin-null MEFs complemented with vector, Flag-menin WT, or S487A or S487D mutant were examined for cyclin D2 expression by Western blot. All statistics: one-way ANOVA with LSD post hoc test. **, P < 0.01. Error bars indicate SD.