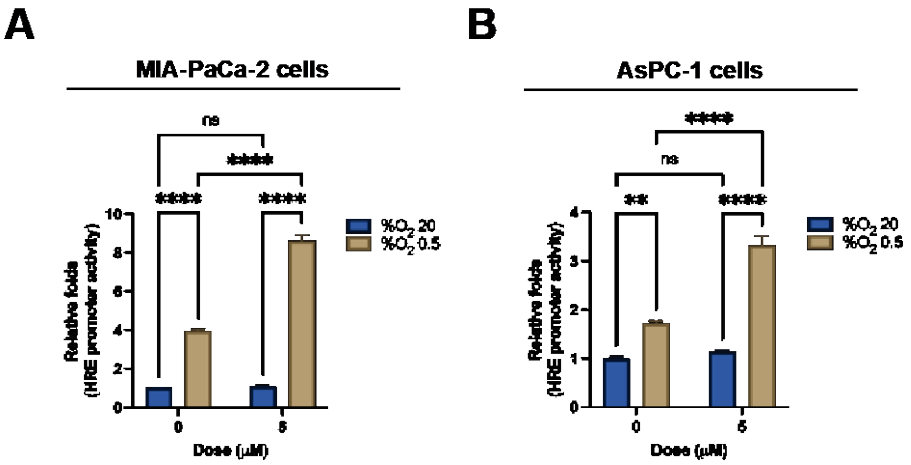
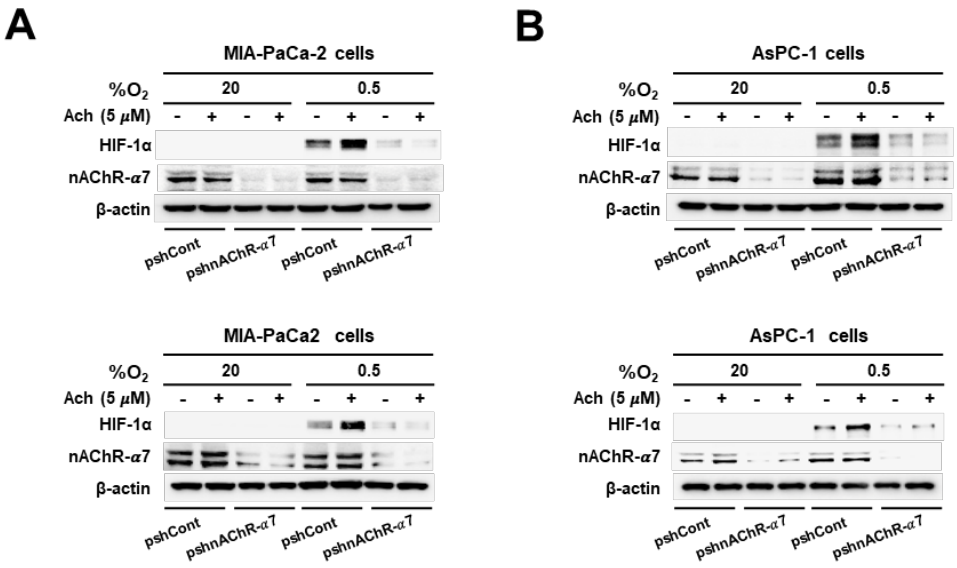
Acetylcholine enhances HIF-1α signaling in pancreatic cancer cells under hypoxia through the nAChR-α7/PDPK1/YAP pathway

Cho et al.,

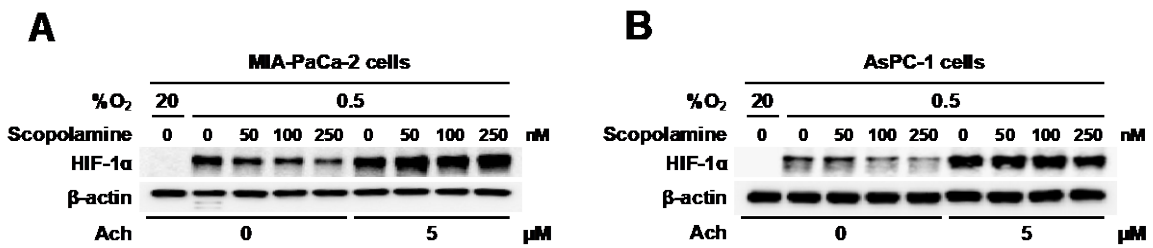
Supplementary Figures



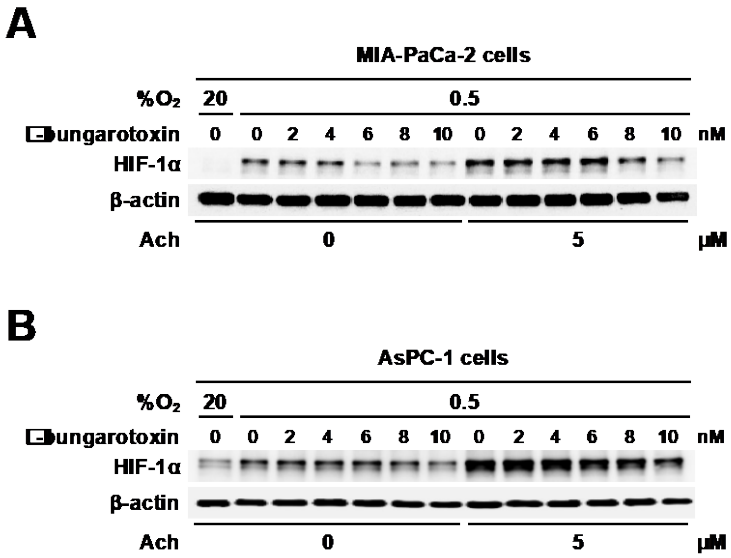
Supplementary Figure S1. Acetylcholine enhances the expression of HIF-1α target genes in pancreatic cancer cells under hypoxia. **(A-B)** MIA-PaCa-2 **(A)** and AsPC-1 **(B)** cells were co-transfected with p3×HRE-*luc* and pRL-*luc* and incubated with or without 5 μM acetylcholine for 1 h. Cells were subsequently exposed to 20% or 5% O_2 for 24 h. Luciferase activity in whole-cell lysates was normalized to that of *Renilla* luciferase. Data are presented as means ± SEM (**$P < 0.01$, ****$P < 0.0001$; ANOVA). Ns indicates no significance.



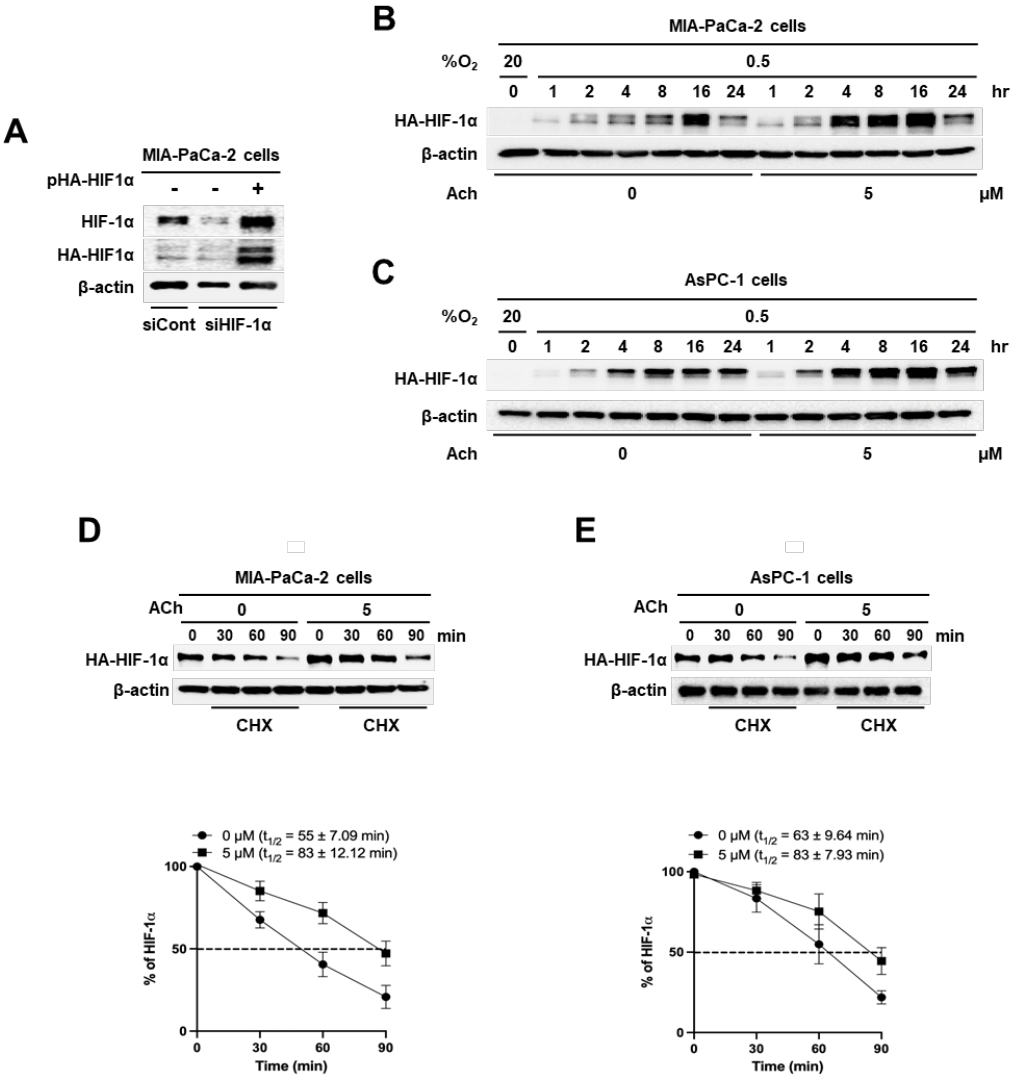
Supplementary Figure S2. Acetylcholine enhances HIF-1α expression via nAChR-α7 in pancreatic cancer cells under hypoxia. **(A-B)** pshCont- or pshnAChR-α7-transfected MIA-PaCa-2 **(A)** and AsPC-1 **(B**) cells were treated with 5 μM acetylcholine or left untreated for 1 h, exposed to 20% or 0.5% O_2 for 24 h, and harvested. The indicated proteins in whole-cell lysates were analyzed by immunoblotting.



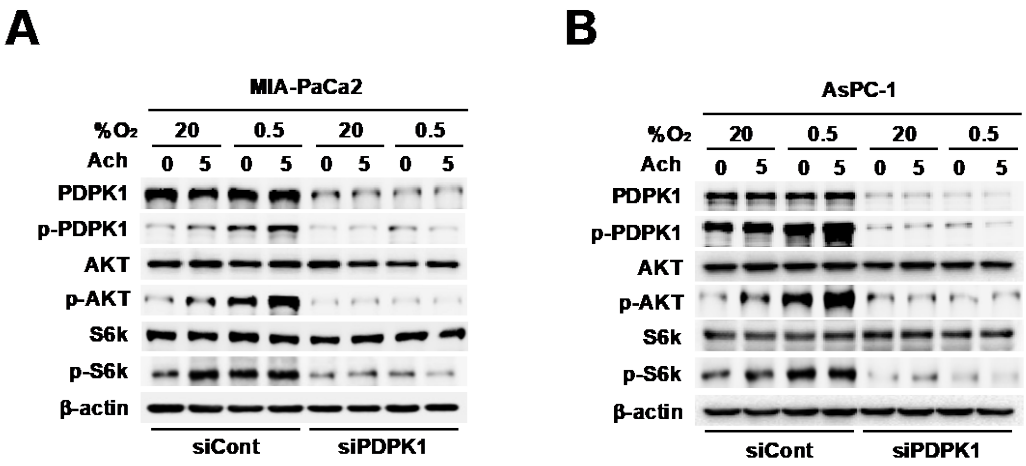
Supplementary Figure S3. Effects of scopolamine on acetylcholine-mediated enhancement of HIF-1α expression in pancreatic cancer cells under hypoxia. **(A-B)** MIA-PaCa-2 **(A)** and AsPC-1 **(B)** cells were treated with a range of concentrations of scopolamine (0–250 nM) for 1 h, followed by 5 μM acetylcholine for 1 h, and exposed to 20% or 0.5% O_2. After a 24 h incubation period, cells were harvested and whole-cell lysates analyzed via immunoblotting for the indicated proteins.



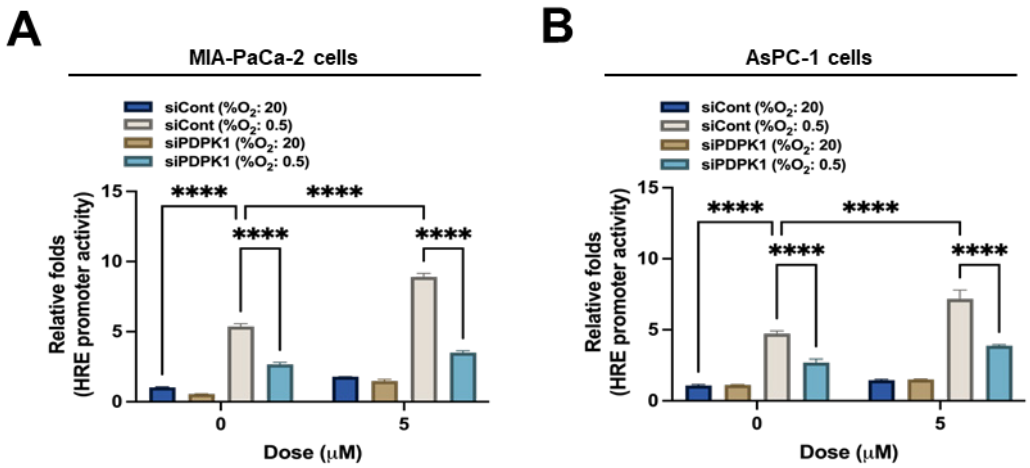
Supplementary Figure S4. Effect of α-bungarotoxin on acetylcholine-mediated enhancement of HIF-1α expression in pancreatic cancer cells under hypoxia. **(A-B)** MIA-PaCa-2 **(A)** and AsPC-1 **(B)** cells were treated with different concentrations of α-bungarotoxin (0–10 μM) for 1 h, followed by 5 μM acetylcholine for 1 h, and exposed to 20% or 0.5% O_2. After a 24 h incubation period, cells were harvested, and the indicated proteins analyzed via immunoblotting in whole-cell lysates.



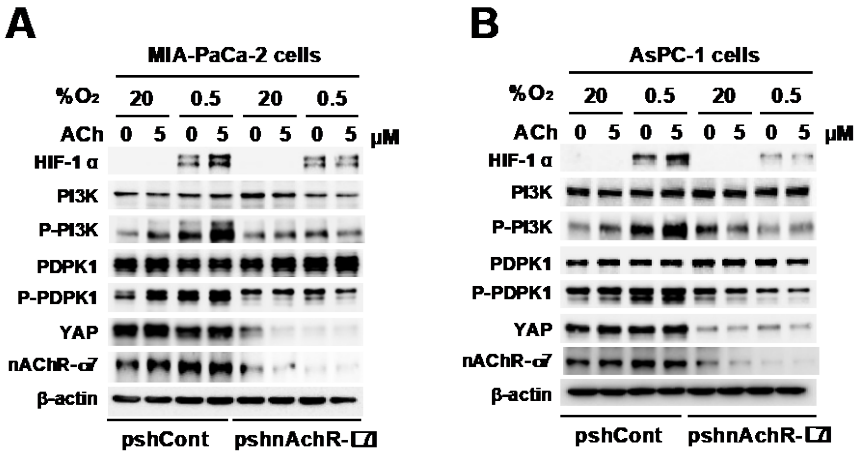
Supplementary Figure S5. Acetylcholine increases HIF-1α protein stability in pancreatic cancer cells under hypoxia**. (A)** MIA-PaCa-2 cells were transfected with siCont, siHIF-1α, or siHIF-1α along with pHA-HIF-1α for 24 h and subsequently exposed to 0.5% O_2. After a 24 h incubation, cells were harvested. HIF-1α protein levels were examined via immunoblot analysis using β-actin as an internal control. **(B-C)** MIA-PaCa-2 **(B)** and AsPC-1 **(C)** cells were transfected with siHIF-1α and pHA-HIF-1α for 24 h, treated with or without 5 μM acetylcholine, and exposed to normoxia (20% O2) or hypoxia (0.5% O_2) for the indicated times. Following harvest of cells at the indicated times, the HA-HIF-1α expression and β-actin was analyzed using immunoblot analysis. **(D-E)** MIA-PaCa-2 **(D)** and AsPC-1 **(E)** cells were transfected with siHIF-1α and pHA-HIF-1α for 24 h, treated with or without 5 μM acetylcholine for 1 h, and exposed to hypoxia (0.5% O_2) for 16 h. Then the cells were treated with 10 μg/mL CHX or left untreated and harvested at the indicated times. HIF-1α protein levels were examined via immunoblot analysis using β-actin as an internal control. Upper panel: Representative images of immunoblot analysis. Lower panel: HIF-1α protein levels were quantified using Image J and band intensities normalized to that of β-actin (the band intensity at t_0 is defined as 100%).



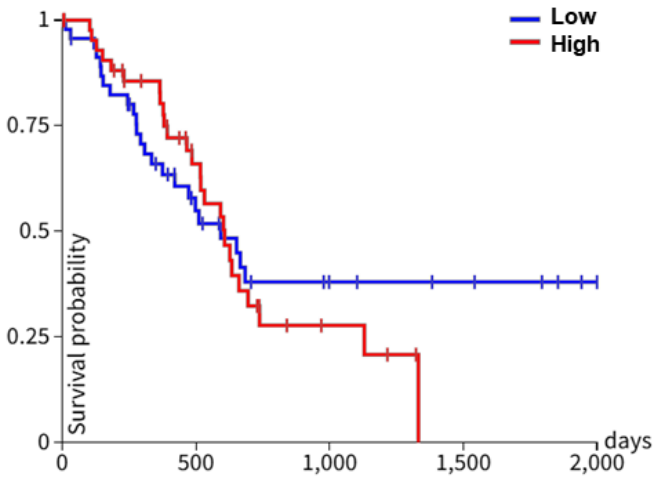
Supplementary Figure S6. Acetylcholine enhances activation of PDPK1 and its target proteins in pancreatic cancer cells under hypoxia. **(A-B)** siCont- or siPDPK1-transfected MIA-PaCa-2 **(A)** and AsPC-1 **(B)** cells were treated with or without 5 μM acetylcholine for 1 h and exposed to 20% or 0.5% O_2. After a 24 h incubation period, cells were harvested, and the indicated proteins analyzed in whole-cell lysates via immunoblotting.



Supplementary Figure S7. Acetylcholine enhances HIF-1α signaling pathway through regulating PDPK1 activation in pancreatic cancer cells under hypoxia. **(A-B)** siCont- or siPDPK1-transfected MIA-PaCa-2 **(A)** and AsPC-1 **(B)** cells were co-transfected with p3×HRE-*luc* and pRL-*luc*, followed by treatment with or without 5 μM acetylcholine for 1 h. Cells were subsequently exposed to 20% or 5% O_2 for 24 h. Luciferase activity measured in whole-cell lysates was normalized to that of *Renilla* luciferase. Data are presented as means ± SEM (****$P < 0.0001$; ANOVA).

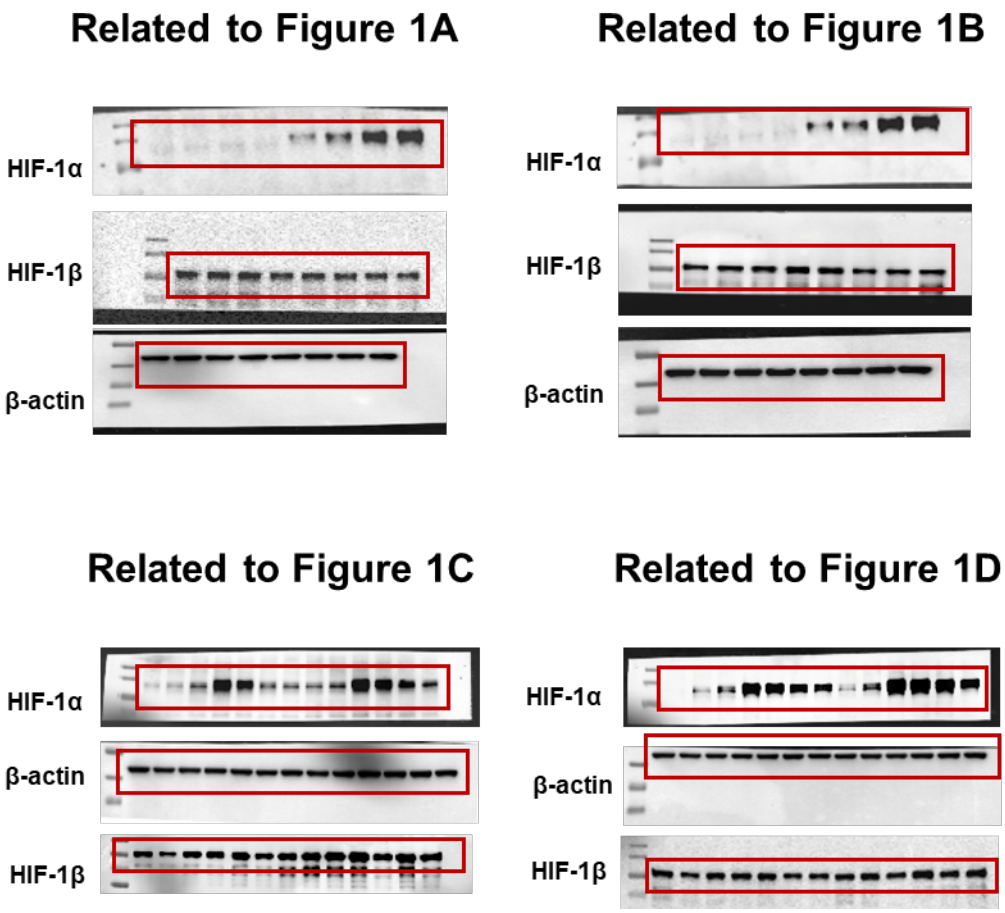


Supplementary Figure S8. Acetylcholine enhances HIF-1α signaling pathway through regulating nAChR-α7/PI3K/PDPK1/YAP signaling pathway in pancreatic cancer cells under hypoxia. **(A-B)** pshCont- or pshAChR-α7-transfected MIA-PaCa-2 **(A)** and AsPC-1 **(B)** cells were treated with or without 5 μM acetylcholine for 1 h and exposed to 20% or 0.5% O_2. After a 24 h incubation period, cells were harvested and the indicated proteins analyzed in whole-cell lysates via immunoblotting.



Supplementary Figure S9. Kaplan-Meier overall survival of TCGA-PAAD patients stratified by CHRNA7 mRNA expression (log-rank p = 0.7022; n = 91).

Supplementary Figure S10.



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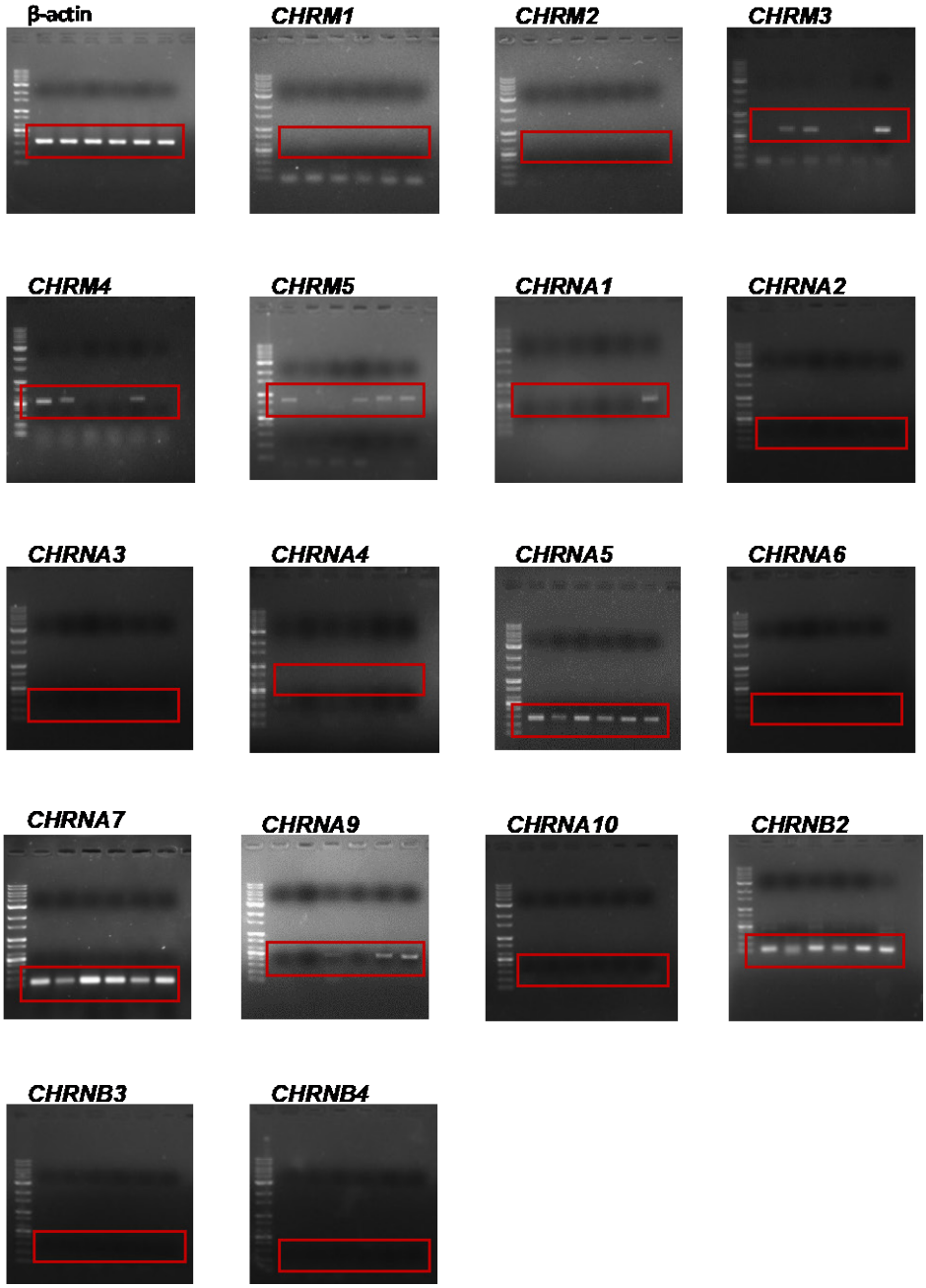

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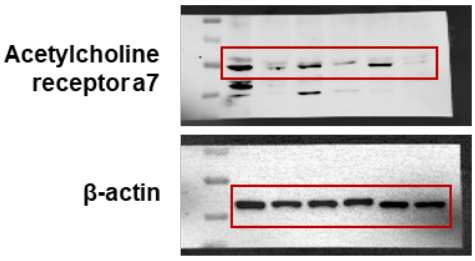

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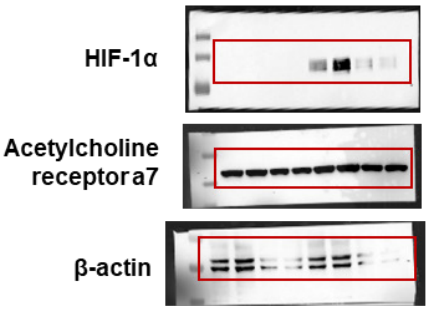

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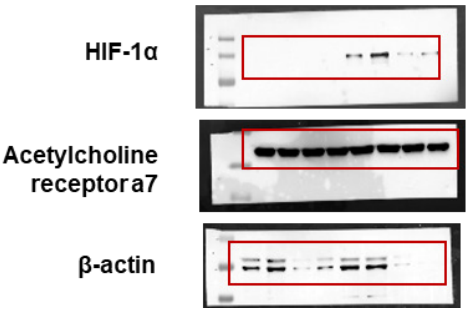

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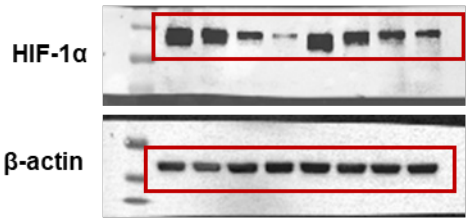

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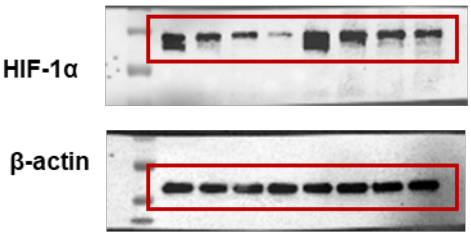

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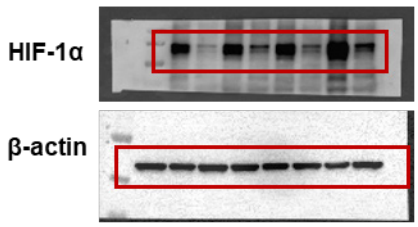

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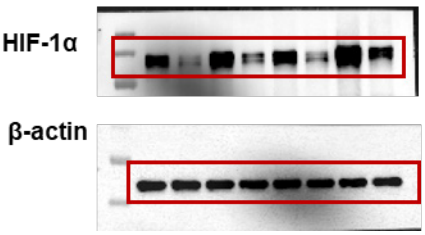

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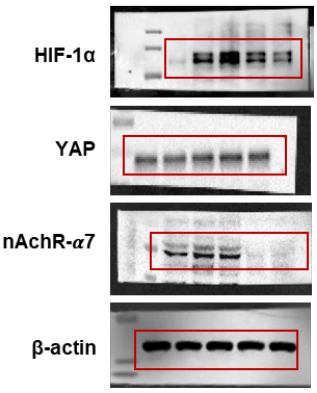

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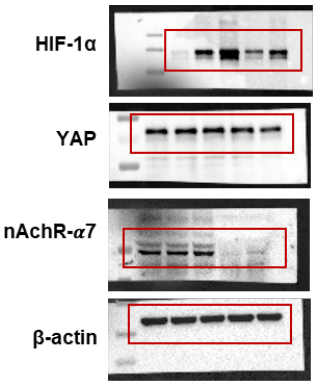

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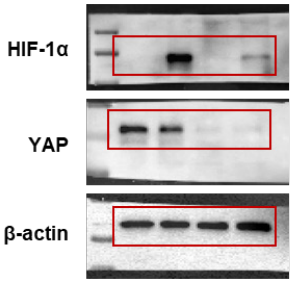

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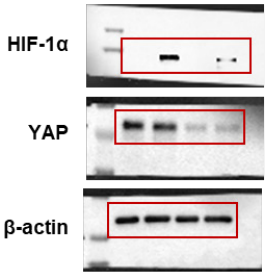

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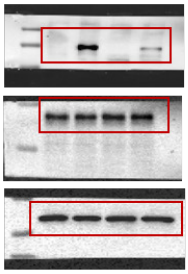

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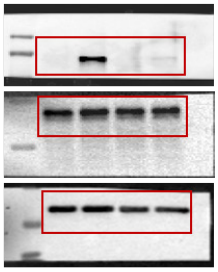

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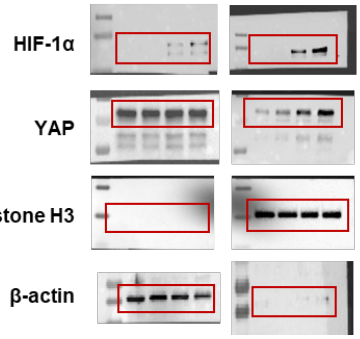

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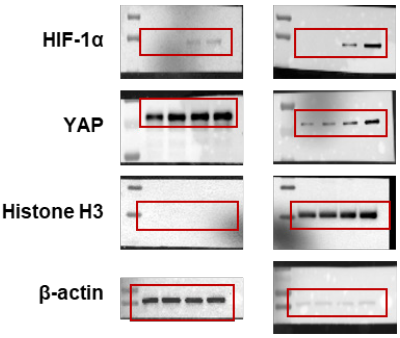

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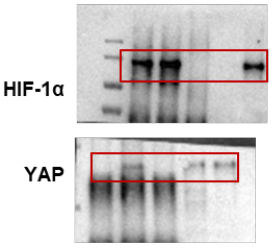

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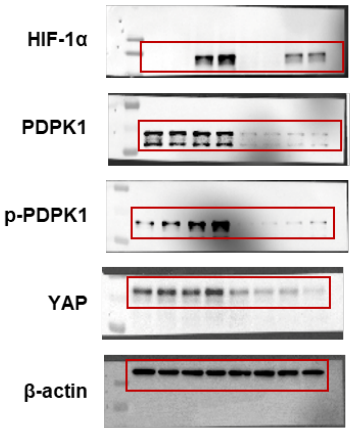

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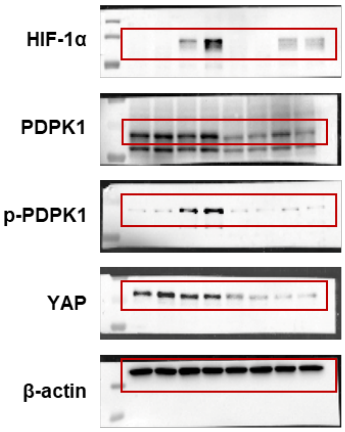

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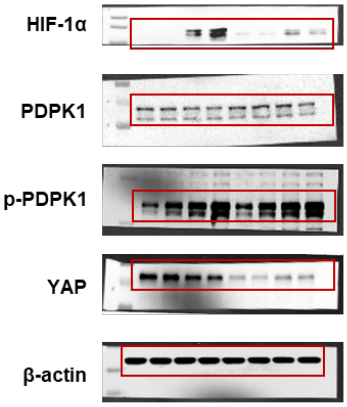

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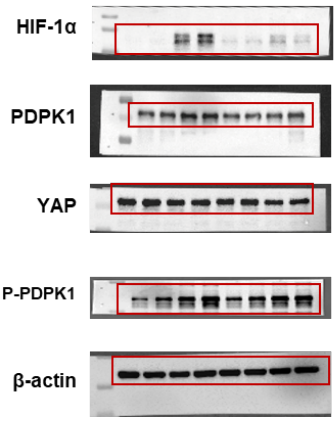

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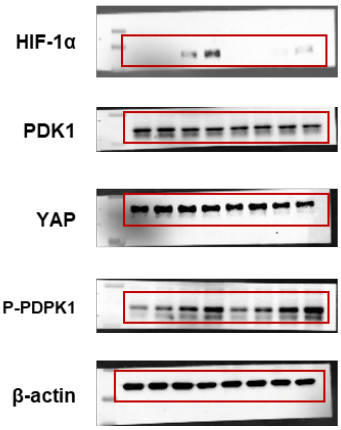

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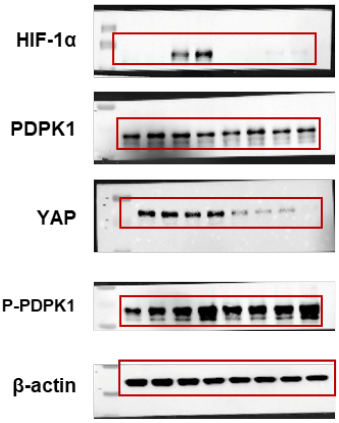

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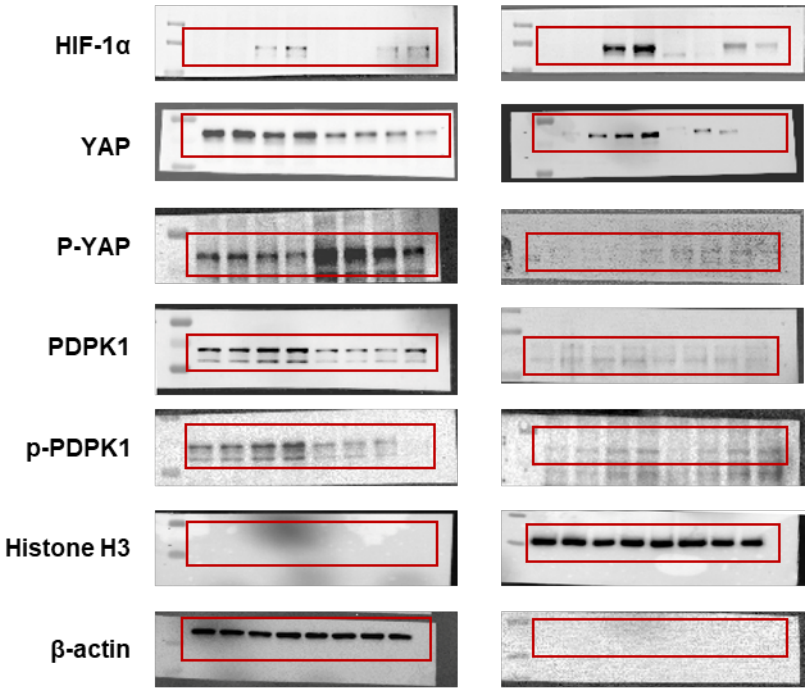

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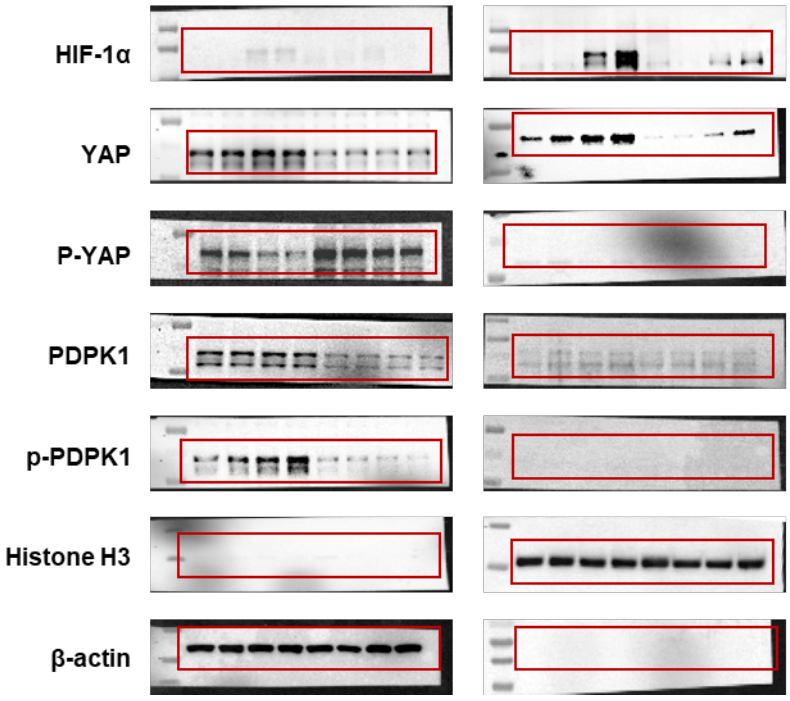

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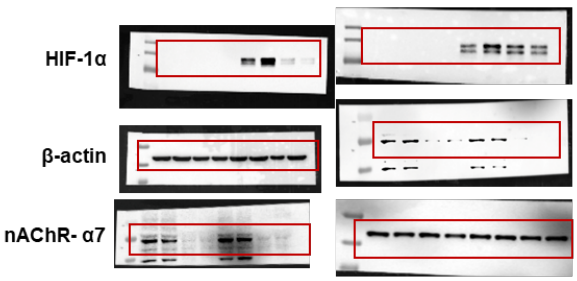

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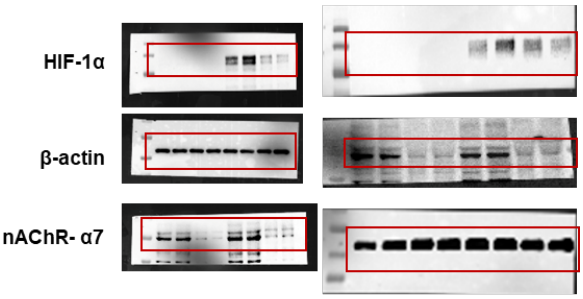

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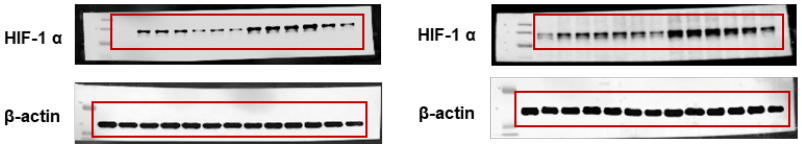

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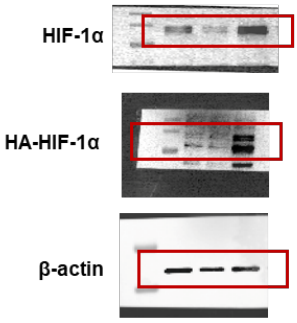

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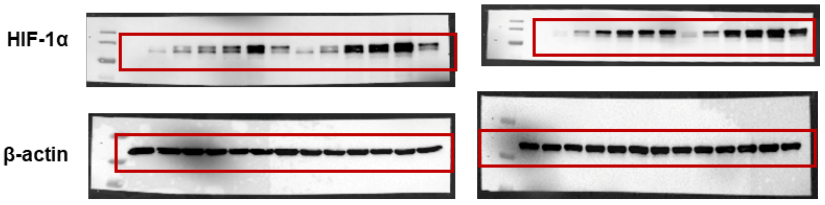

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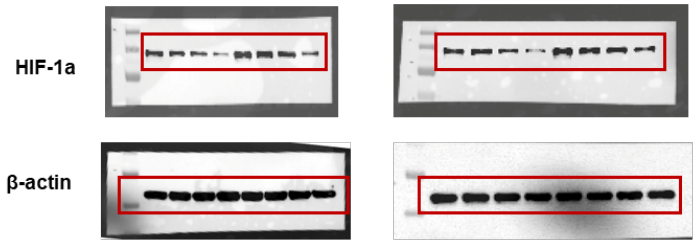

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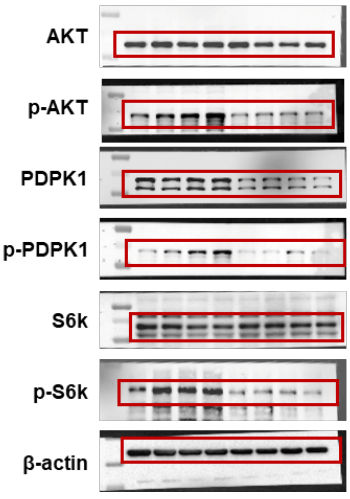

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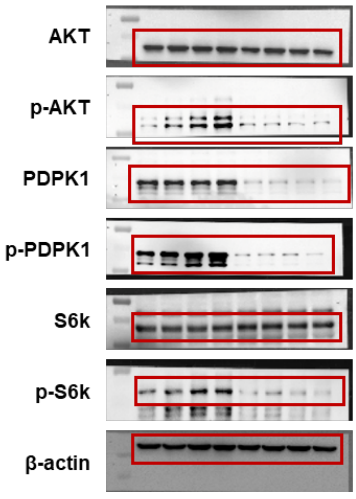

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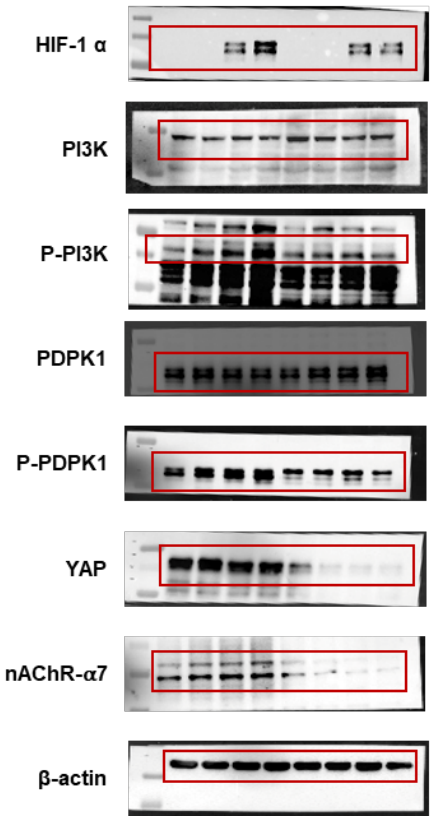

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