

1 **Supplementary Information**

2

3 **Supplementary Figure 1. CoCl₂ induces HIF-1 α to interact with YAP, but not TAZ,**
4 **under high-cell density.**

5 **A** The quantification of the proportion of YAP localization in Figure 1A was shown.

6 Data are presented as mean \pm SEM. ** $p < 0.01$ vs. control group under high-cell density

7 conditions. **B** Quantified nuclear fluorescence intensities of HIF-1 α and YAP of Figure

8 1B were depicted. **C** MDCK cells at low density (5×10^4) and high density (3×10^5)

9 were treated with or without 400 μ M CoCl₂ for 8 h. Immunofluorescent images of TAZ

10 (red) and HIF-1 α (green) were taken using confocal microscope. Scale bar: 10 μ m.

11

12 **Supplementary Figure 2. Hypoxia also promotes HIF-1 α and YAP interaction in**
13 **HK-2 cells.**

14 **A–B** HK-2 cells at high density (3×10^5) were treated with or without 400 μ M CoCl₂

15 for 8 h. Immunofluorescent images of YAP (red) and HIF-1 α (green) were taken using

16 confocal microscope. Scale bar: 10 μ m. The proportion of YAP localization in

17 cytoplasmic, nuclear, or both cytoplasmic and nuclear at low and high density were

18 quantified in **(B)**. **C** HK-2 cells at low and high density were treated with or without

19 1% O₂ for 8 h. Protein levels of Hippo pathway in each group were determined by

1 Western blotting. The ratio of phospho-MST1/MST1, phospho-LATS1/LATS1, and
2 phospho-YAP/YAP were listed below the corresponding blots. **D** High-density HK-2
3 cells were transiently transfected with shHIF-1 α , and subjected to 1% O₂ for 24 h,
4 followed by immunostaining with anti- γ H2AX antibody. Scale bar: 10 μ m. **E** High-
5 density HK-2 cells were transiently transfected with shHIF-1 α , and subjected to 1% O₂
6 for 36 h, followed by Annexin V staining. Scale bar: 10 μ m. All immunofluorescence
7 images were obtained using confocal microscope.

8

9 **Supplementary Figure 3. Immunoprecipitations showed an increase of HIF-1 α**
10 **and YAP under CoCl₂-treated high-density condition.**

11 MDCK cells at low and high density were incubated with or without 400 μ M CoCl₂ for
12 8 h. The immunoprecipitations were performed and analyzed by Western blotting with
13 anti-HIF-1 α antibody.

14

15 **Supplementary Figure 4. HIF-1 α facilitates YAP downstream gene expression**
16 **under hypoxic condition.**

17 MDCK-Parental and MDCK-shHIF-1 α cells at high density were subjected to 1% O₂
18 for 0, 2, 4, and 6 h. Representative PCR results of gene expression for *yap* and the
19 downstream gene *cyr61* were shown. *gapdh* was used as an internal control.

1 **Supplementary Figure 5. Effects of Src and ERK inhibitors on YAP localization**
2 **under CoCl₂ treatment.**

3 MDCK cells at high cell density were treated with 1 μM dasatinib, 100 μM U0126, or
4 1 μM SCH772984 for 24 h, followed by 400 μM CoCl₂ for 8 h. **A** Representative
5 Western blots were shown. Quantification of **(B)** phospho-Src/Src, **(C)** phospho-
6 ERK2/ERK2, and **(D)** phospho-YAP/YAP is presented as mean ± SEM. **B** ***p* < 0.01
7 vs. the CoCl₂-alone group. **C** **p* < 0.05 vs. control group. ###*p* < 0.001 vs. control
8 group. ##*p* < 0.01 vs. CoCl₂-alone group. **D** ***p* < 0.01 and **p* < 0.05. **E**
9 Immunofluorescence images of YAP (red) and HIF-1α (green) were obtained using
10 confocal microscope. Scale bar: 10 μm. **F** Proportion of YAP localization in cytoplasm
11 and nucleus was quantified. Data are presented as mean ± SEM. ***p* < 0.01.

12

13 **Supplementary Figure 6. AKT inhibitor decreases YAP phosphorylation at serine**
14 **127 in MDCK-shHIF-1α cells under high-cell density conditions.**

15 High density MDCK-shHIF-1α cells were treated with or without 10 μM LY294002 for
16 24 h, followed by control or 400 μM CoCl₂ treatment for 8 h. **A–B** Immunofluorescence
17 images of YAP (red) and HIF-1α (green) obtained using confocal microscope. Scale bar:
18 10 μm. Proportion of YAP localization in cytoplasm, nucleus, or both after LY294002
19 treatment was quantified in **(B)**. Bars represent the mean ± SEM. ****p* < 0.001, ###*p* <

1 0.001. **C** Representative blots of HIF-1 α , phospho-AKT, AKT, phospho-YAP, and YAP
2 were shown. α -tubulin was used as a loading control.

3

4 **Supplementary Figure 7. DNA distribution of parental and shHIF-1 α cells under**
5 **normoxic and hypoxic conditions.**

6 MDCK-parental and MDCK-shHIF-1 α cells were treated with normoxia or 1% O₂ for
7 48 h and stained with Hoechst 33342. Nuclei morphology was observed under an
8 inverted fluorescence microscope. Pseudocolor shows distribution and intensity of
9 DNA by Hoechst 33342 staining in nucleus. Scale bar: 10 μ m.

10

11 **Supplementary Figure 8. Hypoxic mimetic CoCl₂ induced DNA damage in MDCK**
12 **cells.**

13 **A–B** MDCK-parental and MDCK-shHIF-1 α cells were treated with normoxia or 400
14 μ M CoCl₂ for 24 h, followed by immunostaining with anti- γ H2AX antibody. **A**
15 Representative confocal images were shown. Scale bar: 40 μ m. **B** γ H2AX intensity was
16 analyzed and presented as mean \pm SEM. *** $p < 0.001$.

17

18 **Supplementary Figure 9. Constitutively active YAP reduced DNA damage under**
19 **hypoxic conditions.**

1 **A–B** MDCK-shHIF-1 α -YAP-WT and MDCK-shHIF-1 α -YAP-S127A cells were
2 exposed to 1% O₂ for 24 h, followed by immunostaining with anti- γ H2AX antibody. **A**
3 Representative confocal images were shown. Scale bar: 40 μ m. **B** γ H2AX intensity was
4 analyzed and presented as mean \pm SEM. * p < 0.05.

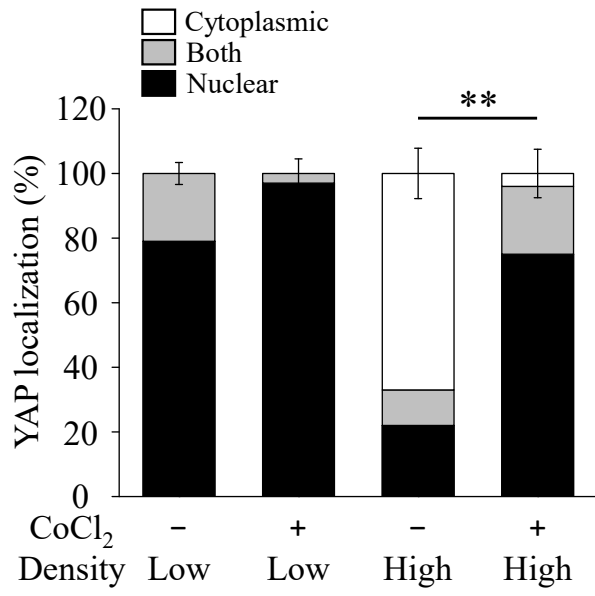
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6 **Supplementary Figure 10. MDCK cells with either HIF-1 α or YAP deficiency were**
7 **sensitive to hypoxic insults.**

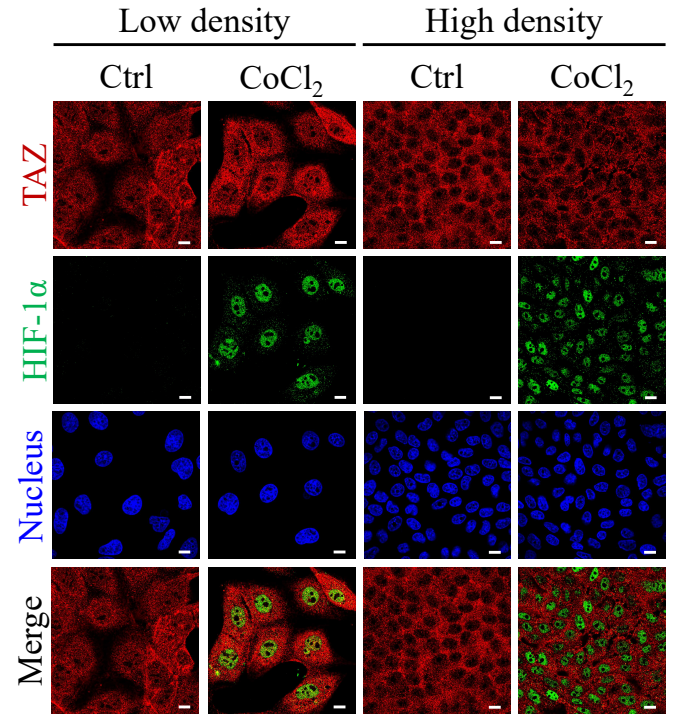
8 **A** High-density MDCK-Parental, MDCK-shHIF-1 α , and MDCK-shYAP cells were
9 subjected to 1% O₂ for 24 h, followed by immunostaining with anti-Rad51 antibody.
10 Scale bar: 10 μ m. **B** MDCK-Parental, MDCK-shHIF-1 α , and MDCK-shYAP cells were
11 seeded in high density and were treated with 1% O₂ for 36 h. The expression of PARP
12 and caspase 3 were determined by Western blotting. α -tubulin was used as a loading
13 control.

14

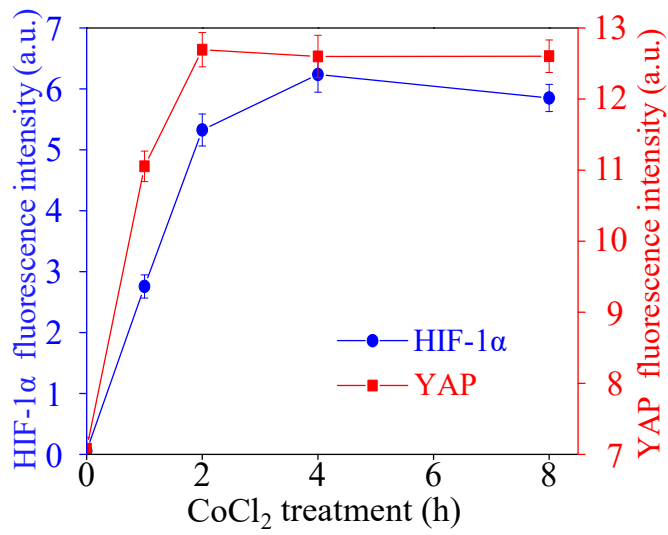
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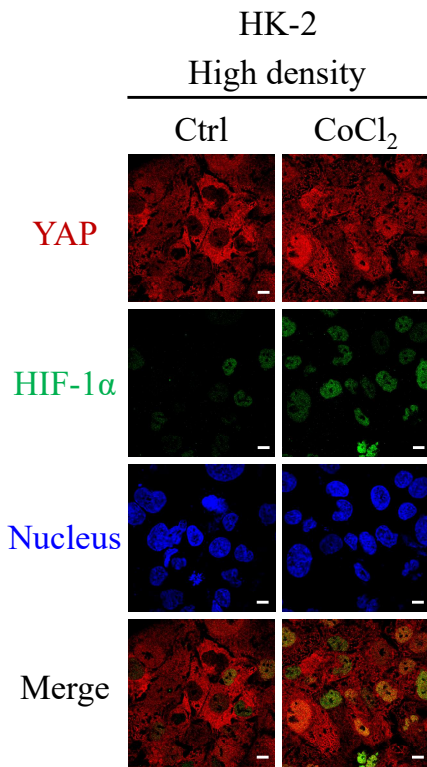
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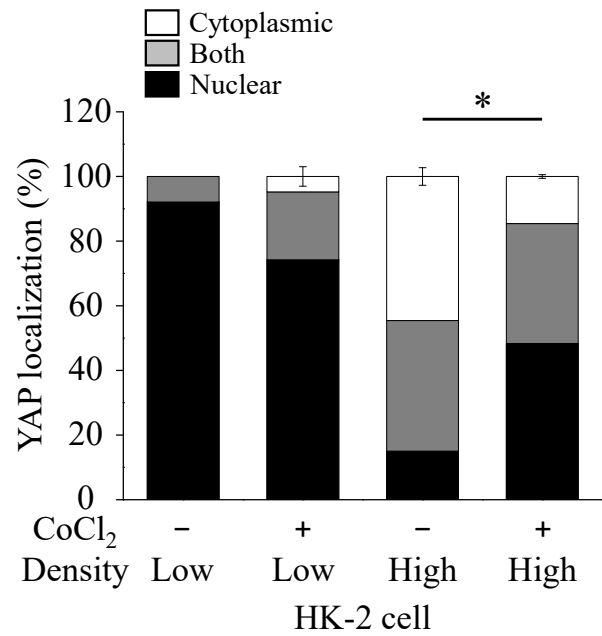
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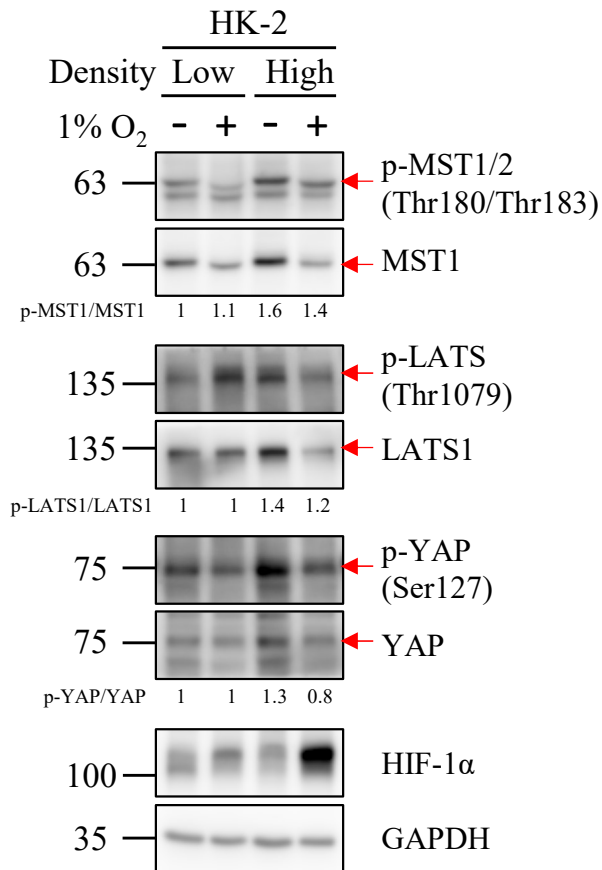
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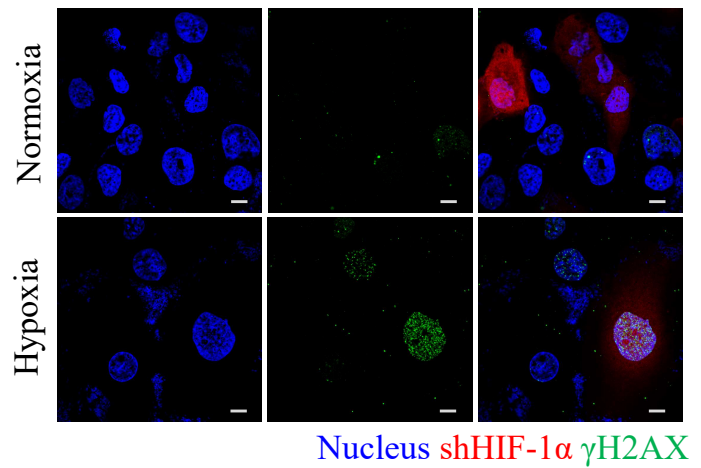
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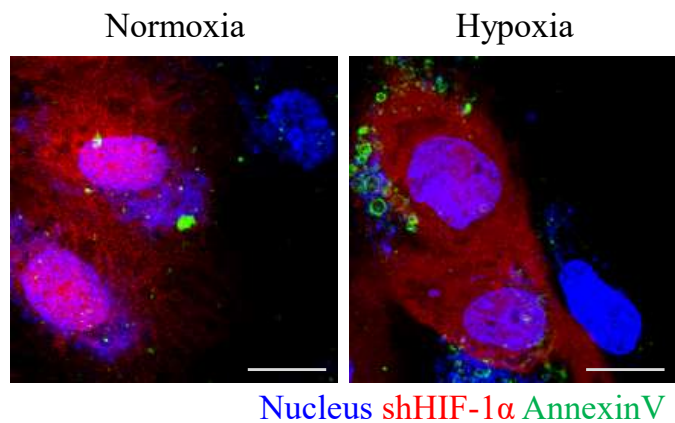
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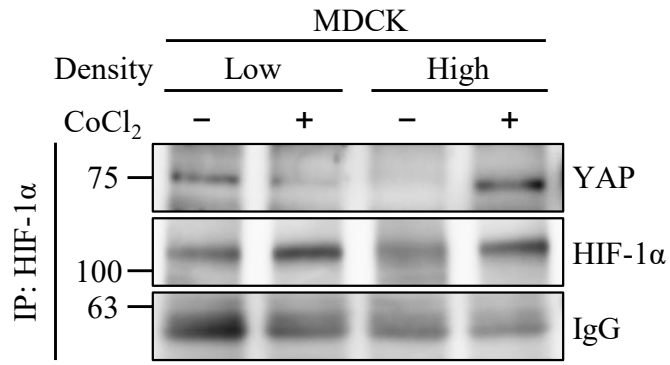


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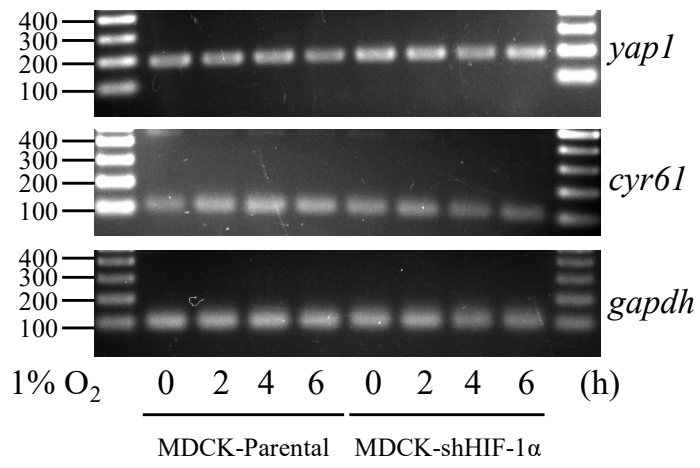


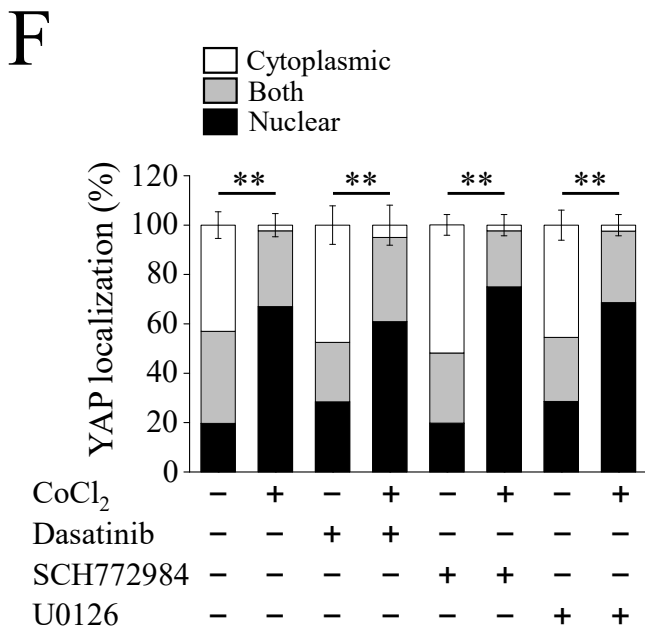
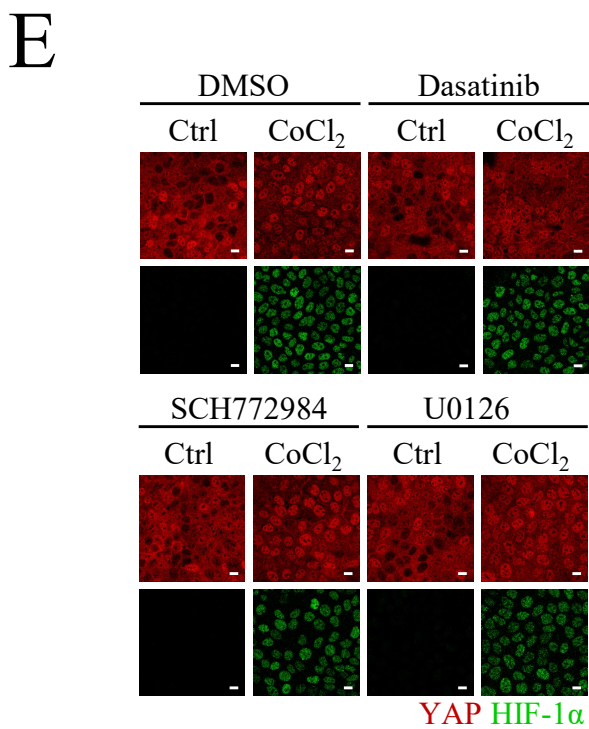
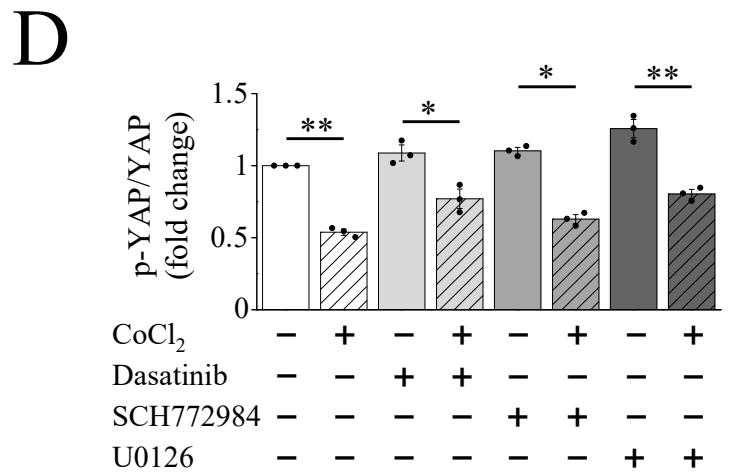
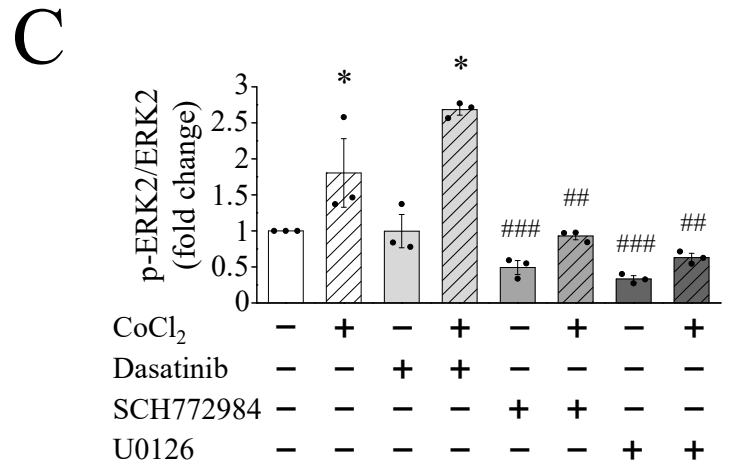
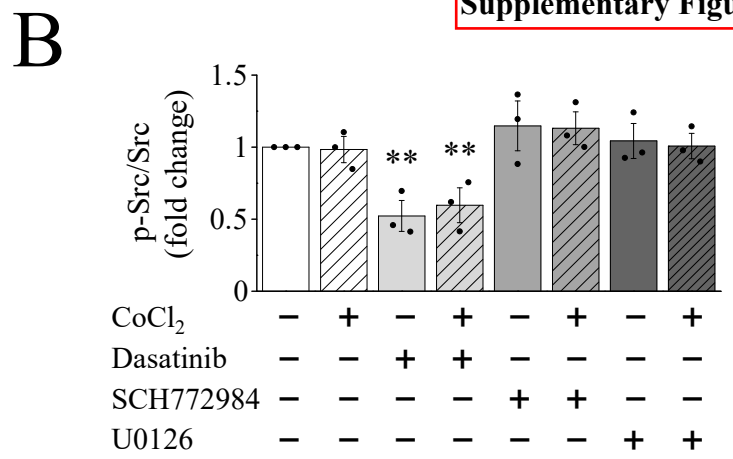
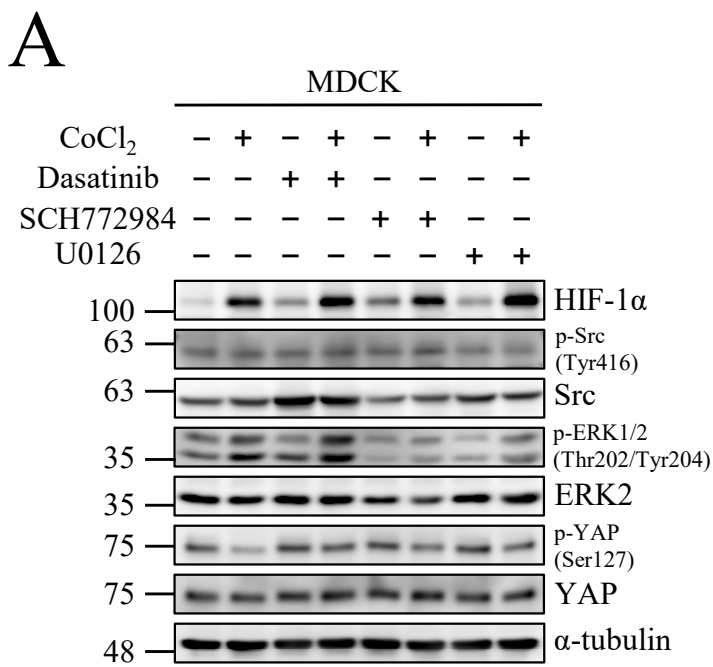
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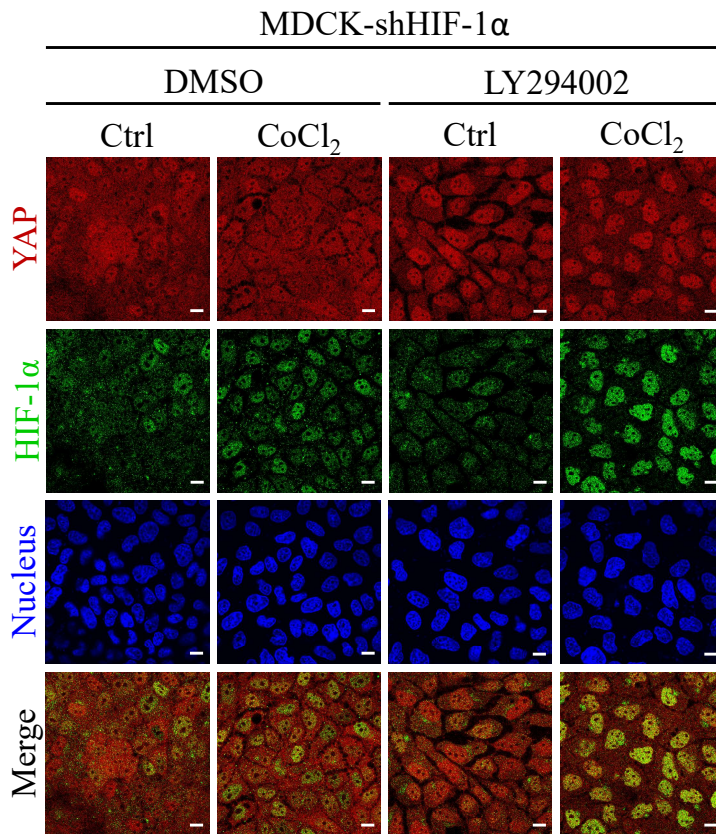


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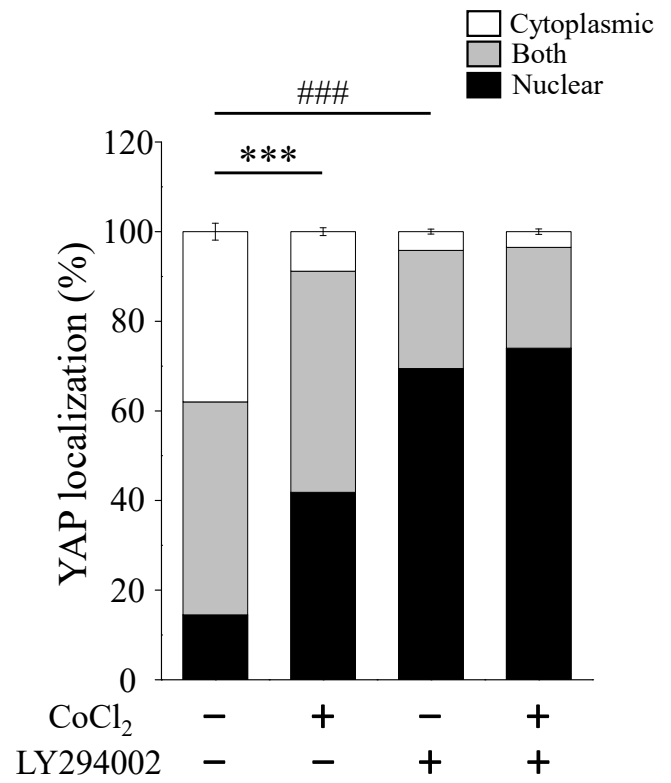




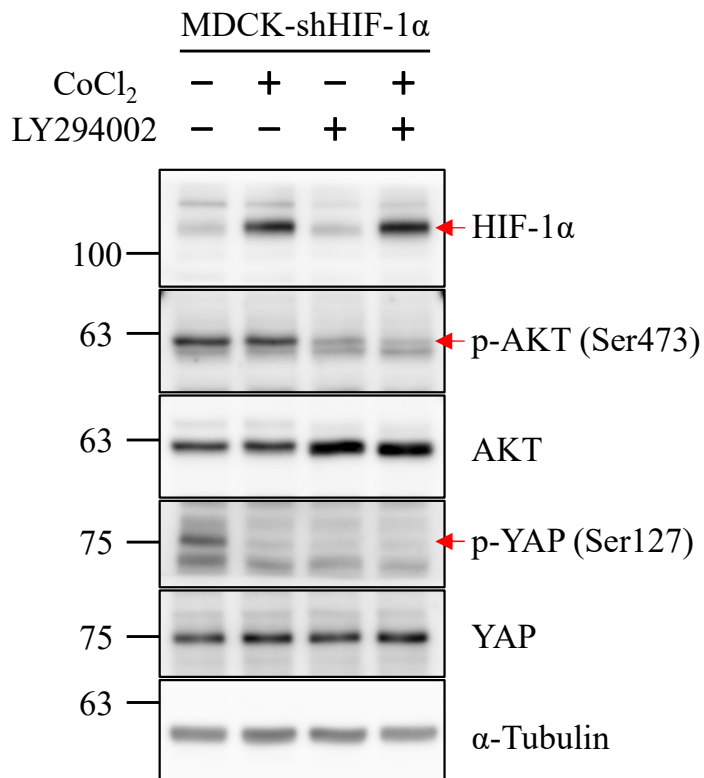
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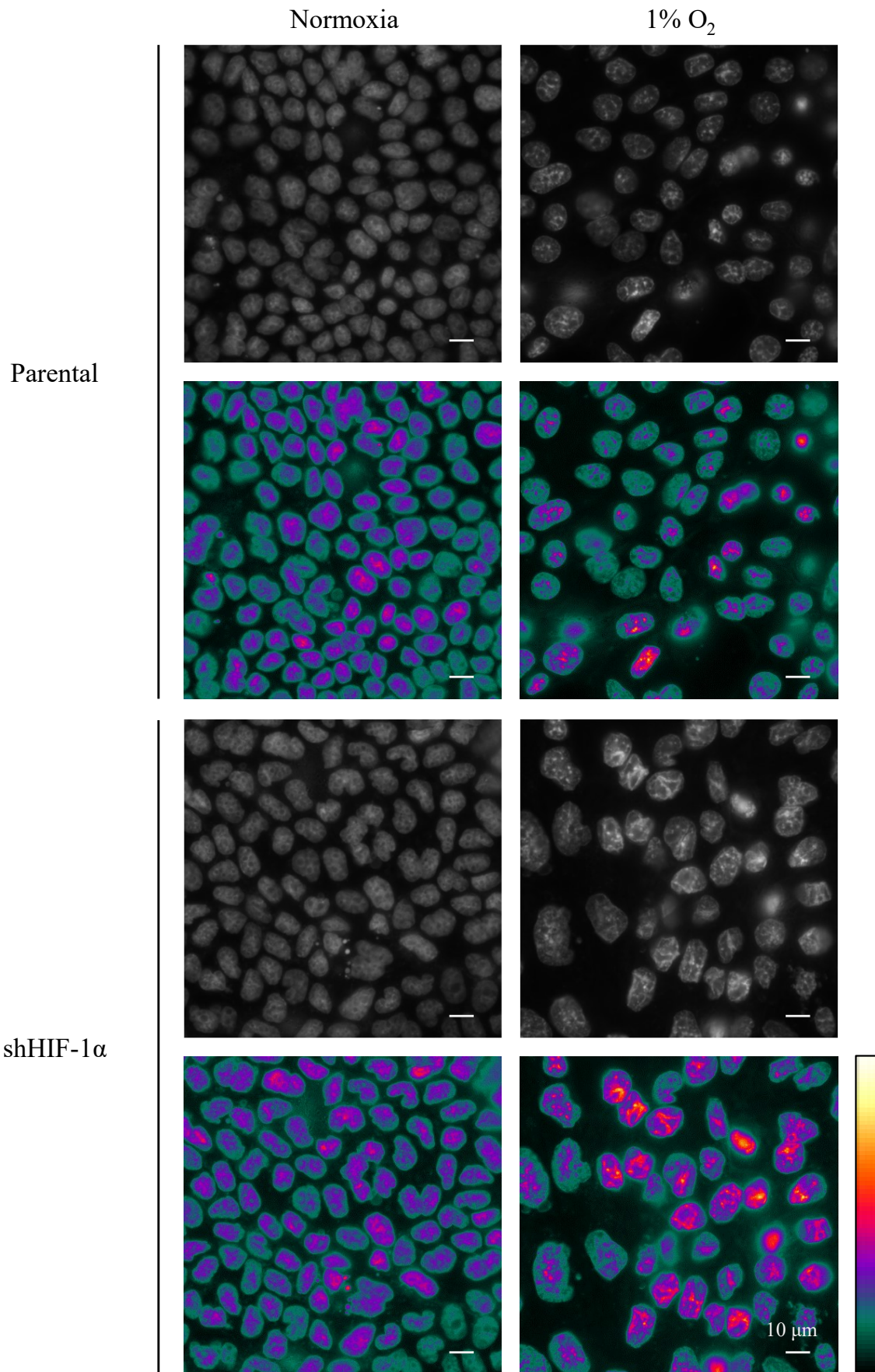


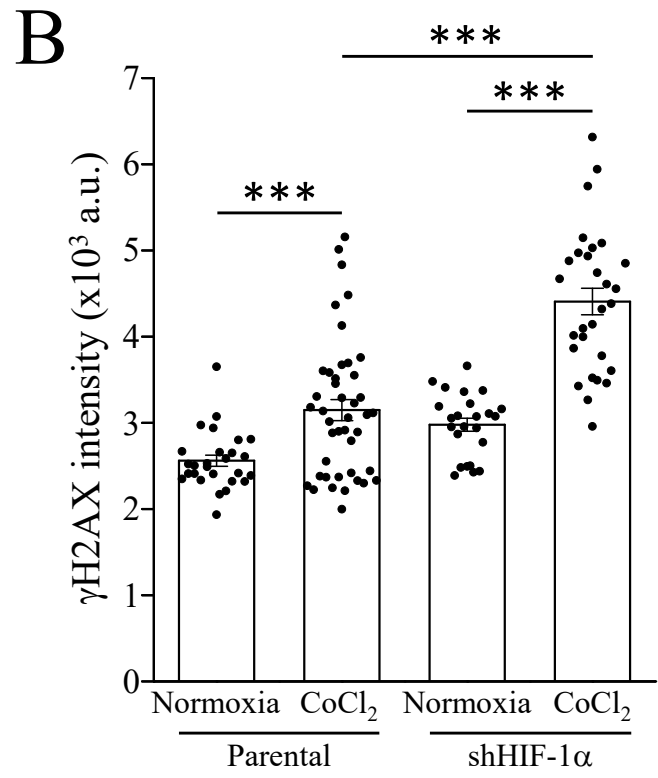
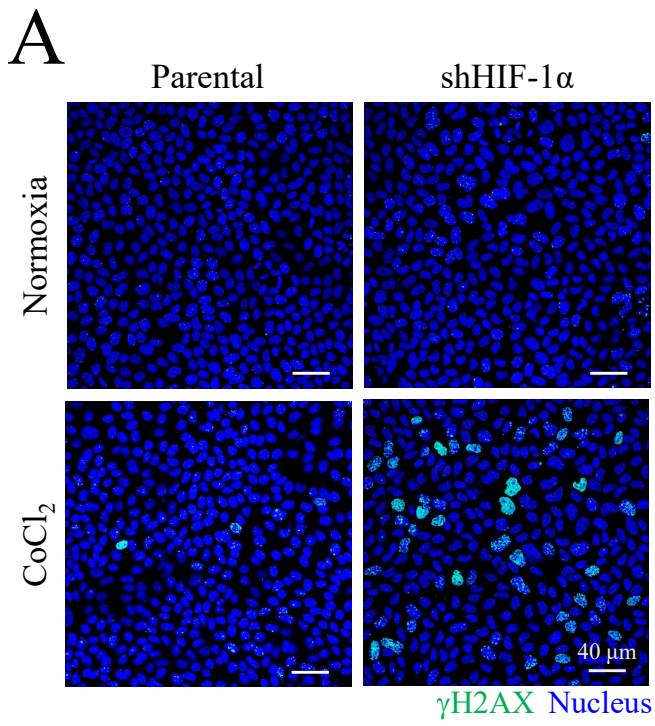
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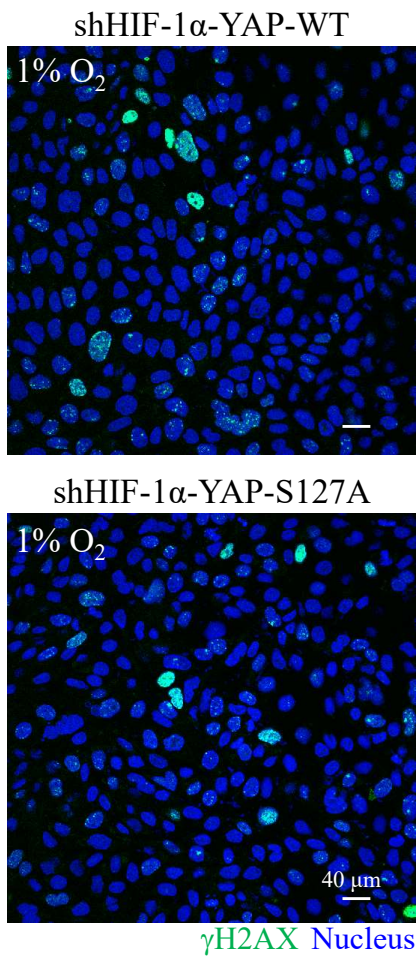
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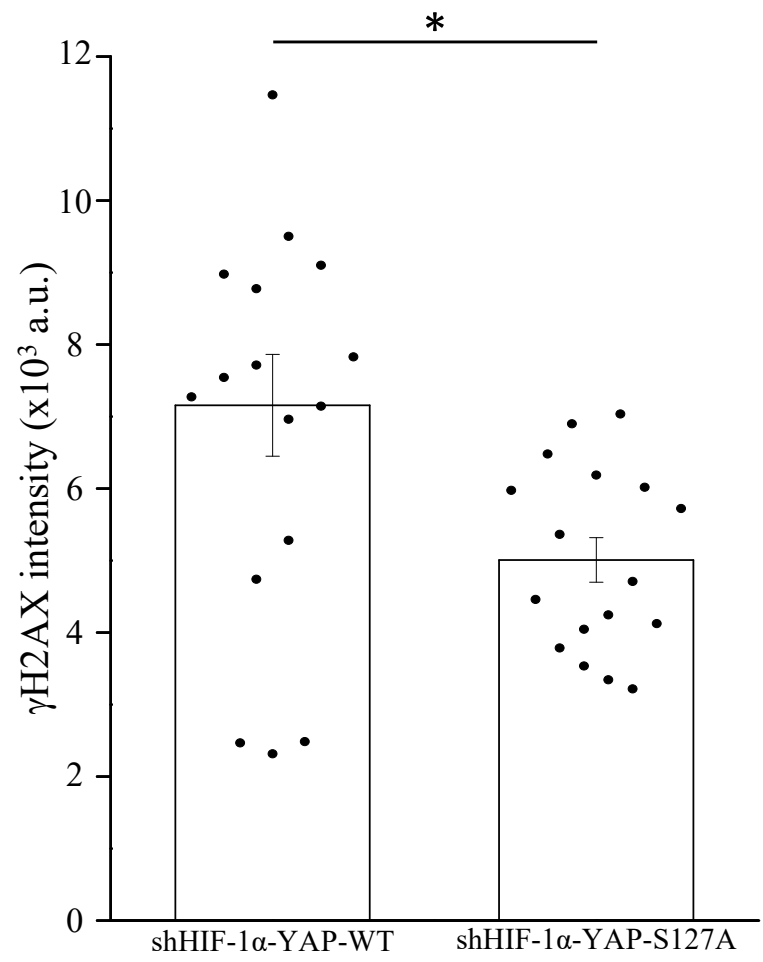




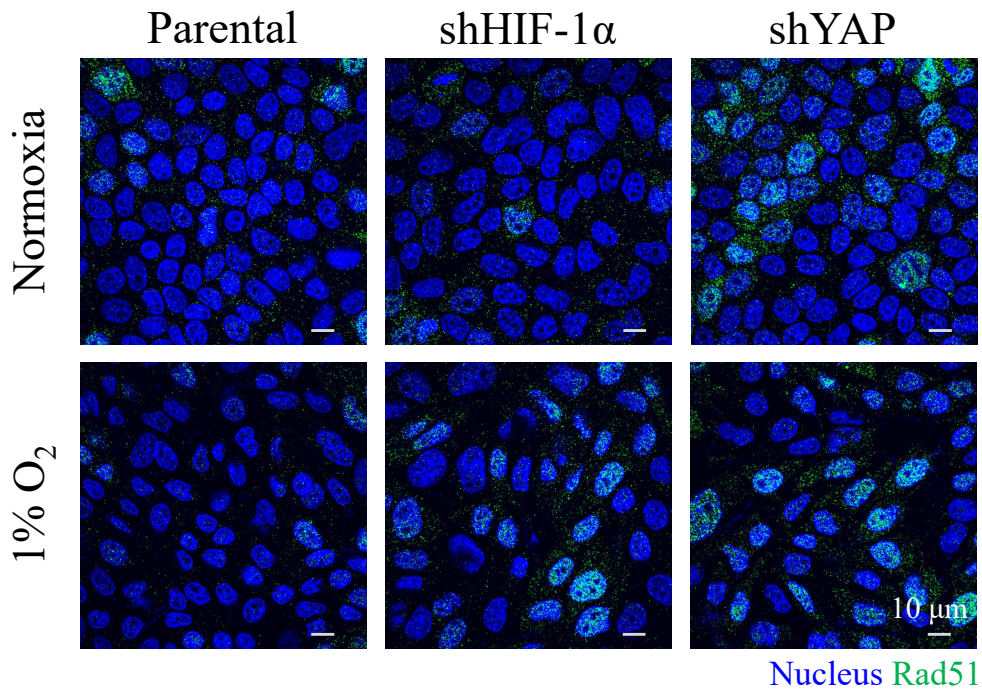
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