Supplementary Figure 1. TIMP2 was differentially expressed across a panel of human HCC cell lines. Immunoblot showing differential expression of TIMP2 in a panel of HCC cell lines. α-Tubulin was used as the loading control.

Supplementary Figure 2. **Knockdown of TIMP1 did not enhance cell invasion in HCC cells.** Western blot analysis showed successful knockdown of TIMP1. Knockdown of TIMP1 did not result in increased cell invasive abilities. Transwell cell invasion assays of SMMC7721 and PLC/PRF/5 were performed 48 hours after transient transfection of siRNA targeting TIMP1 (siTIMP1) or the non-targeted control siRNA (siNTC). Quantitative data are presented as mean ± SD. Statistical significance was determined by t-test. Results were from three independent experiments.

Supplementary Figure 3. (A) Schematic diagram for invadopodia assay (B) Representative photo of SMMC-7721 with the F-actin rich region colocalized with gelatin degradation. The main panel shows the en face image generated from a series of xy plane confocal images along the z-axis. The upper and left panels show the x-z and y-z cross-sectional images, respectively. Green: Gelatin; Red: Phallodin staining for F-actin.

Supplementary Figure 4. miR-210 was the most consistently upregulated microRNA in hypoxic HCC cells. (A) qPCR-based miRNA profiling was performed on SMMC-7721, Huh7, and MHCC-97L subject to normoxia (20% O_2) or hypoxia (0.1% O_2) using Low Density Array which consisted of 664 unique miRNA molecules (Applied Biosystems). The Venn diagram shows the number of miRNAs ≥ 2-fold differentially expressed between these 3 HCC cell lines under hypoxic condition. The small table lists the miRNAs commonly upregulated in the 3 HCC cell lines under hypoxic condition. (B) miR-210 was overexpressed

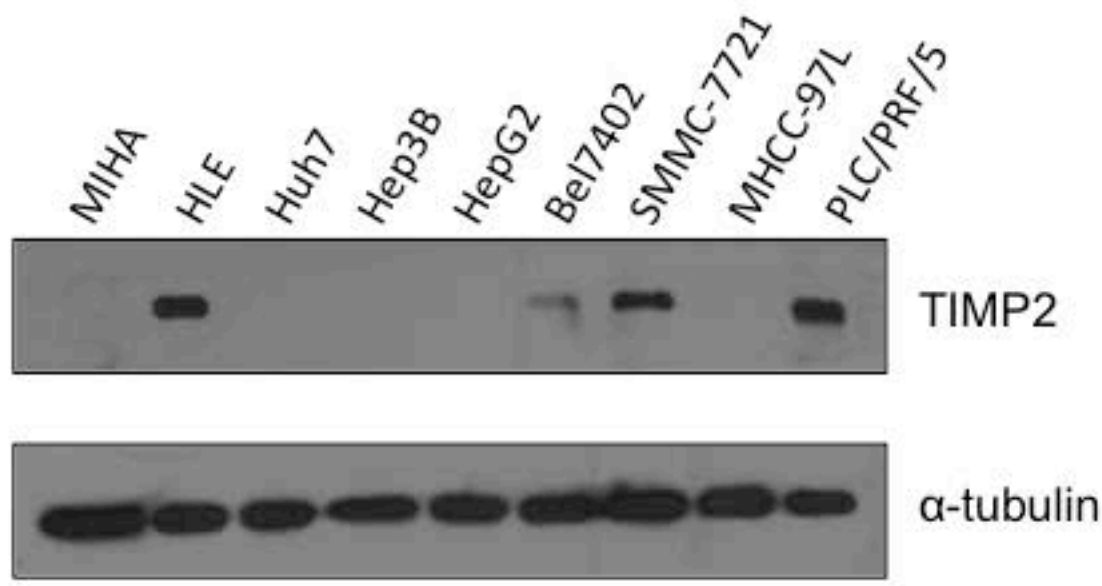
in hypoxic human HCC cell lines. Individual qPCR analysis of miR-210 was performed on 7 different HCC cell lines subject to normoxia and hypoxia. U6 was used as housekeeping gene for data normalization (*P<0.05, **P<0.01; ***P<0.001; t-test; mean ± S.D.; n=3). (C) miR-210 was overexpressed in hypoxic human HCC tissues. H&E section of a representing case consisting of peri-necrotic, CA9-positive regions of primary HCC used for microdissection (N = necrotic region; T = tumorous tissue; HT = hypoxic tumorous livers). U6 was used as housekeeping gene for data normalization. (***P<0.001, t-test; mean ± S.D.; n=3 individual cases).

Supplementary Figure 5. Induction of miR-210 and suppression of TIMP2 mediated by hypoxia were HIF-1α-dependent. (A) The protein levels of HIF-1α, HIF-2α, TIMP2 and α-tubulin in shNTC, shHIF-1α, and shHIF-2α SMMC-7721 clones are shown. All clones were cultured in 20% O_2 or 0.1% O_2 for 24 hours. B) The miR-210 levels of shNTC, shHIF1α, and shHIF2α SMMC-7721 clones were determined by real-time qPCR and normalized to U6. (P<0.001, t-test; mean ± S.D.; n=3). C) Protein levels of TIMP2 in SMMC-7721 and PLC/PRF/5 following transfection of negative control (NC) or miR-210 LNA were shown. D) Ectopic expression of flag-tagged HIF3 ORF in SMMC-7721 was demonstrated by Western blot. α-Tubulin was used as the loading control.

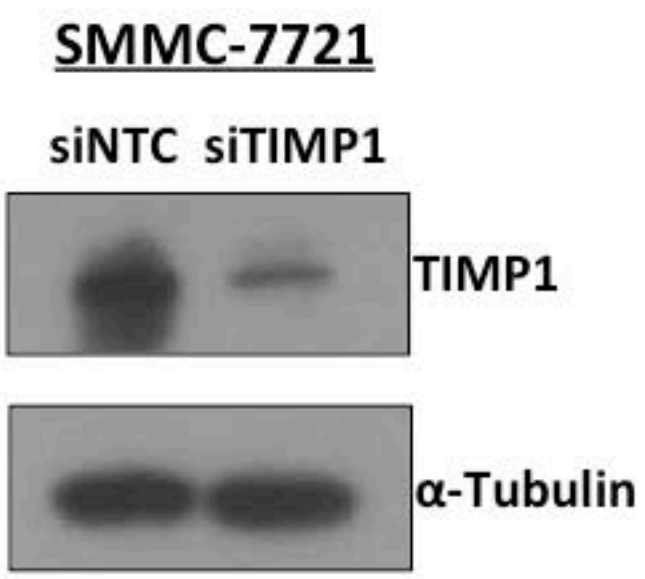
Supplementary Figure 6. The enhanced invadopodia formation conferred by TIMP2 suppression in hypoxia was perturbed by miR-210 inhibition. Invadopodia formation assay of SMMC7721 was performed following transfection with miR-210 LNA inhibitor or non-targeted (NTC) LNA inhibitor under normoxia (20% O_2) or hypoxia conditions (0.1% O_2). Cells were scored for gelatin degradation as described in “Experimental Procedures” and a minimum of 200 cells were scored for each experimental group. Quantitative data are

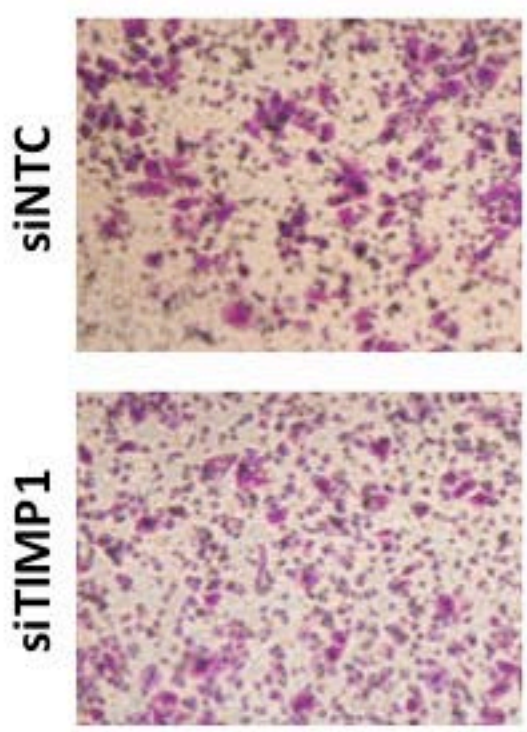
presented as mean ± SD and statistical significance was determined by t-test (*P<0.05; **P<0.01; ***P<0.001). Results were from three independent experiments.

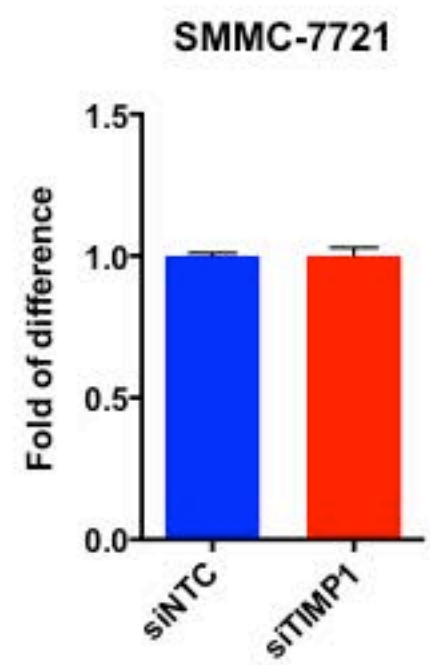
Supporting Figure 1

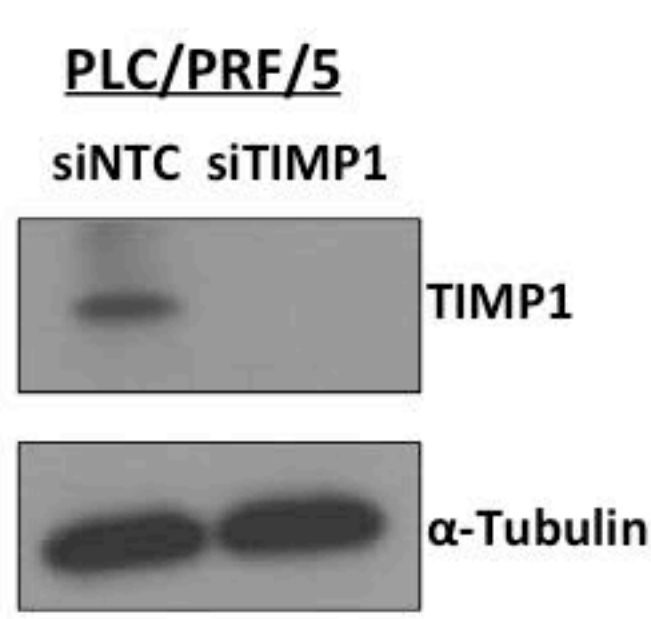


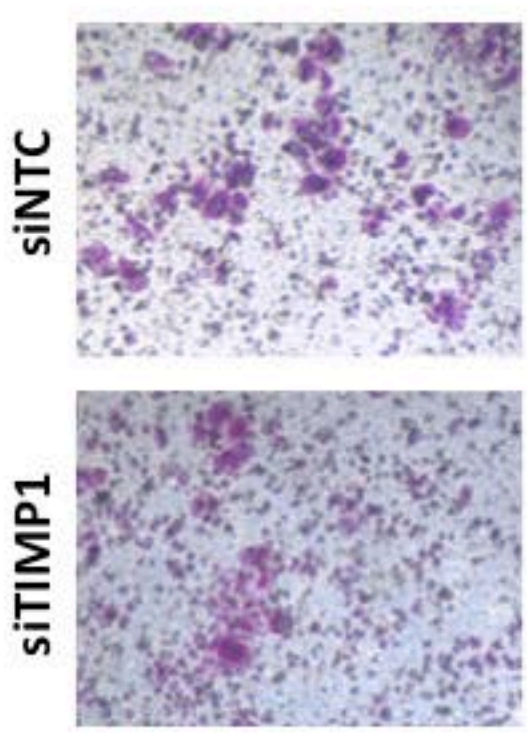
Supporting Figure 2

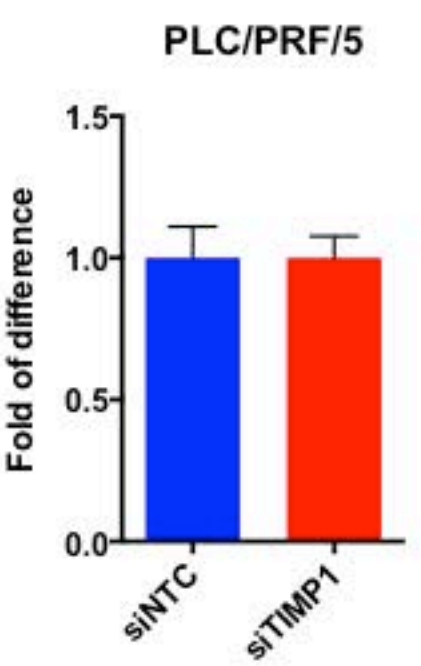






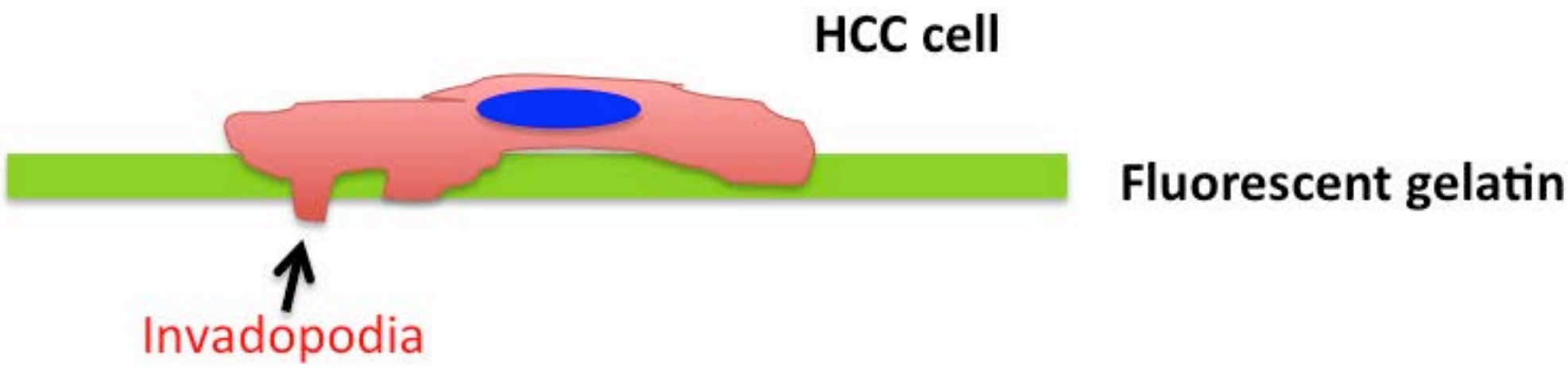






Supporting Figure 3

A



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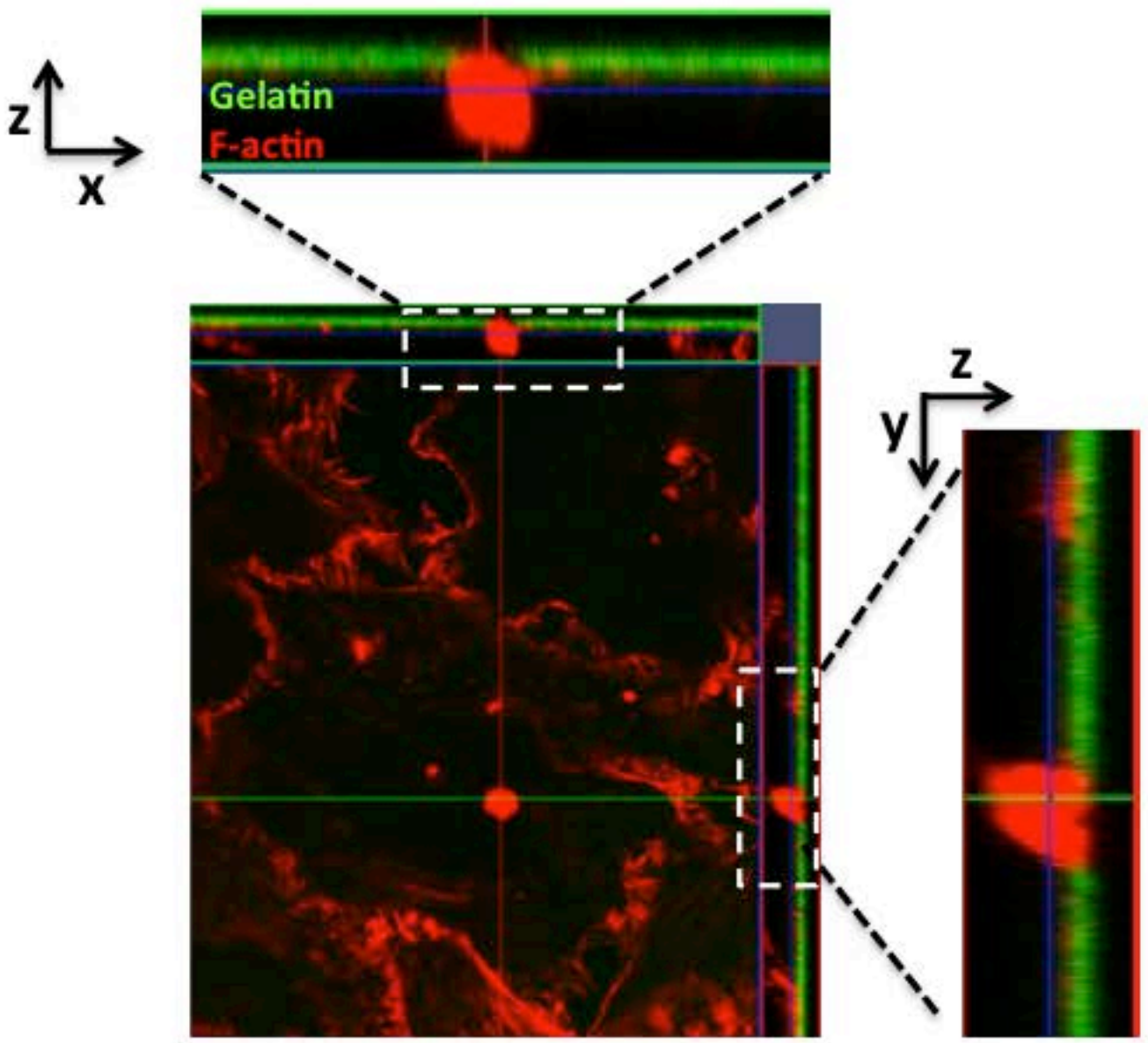

Supporting Figure 4

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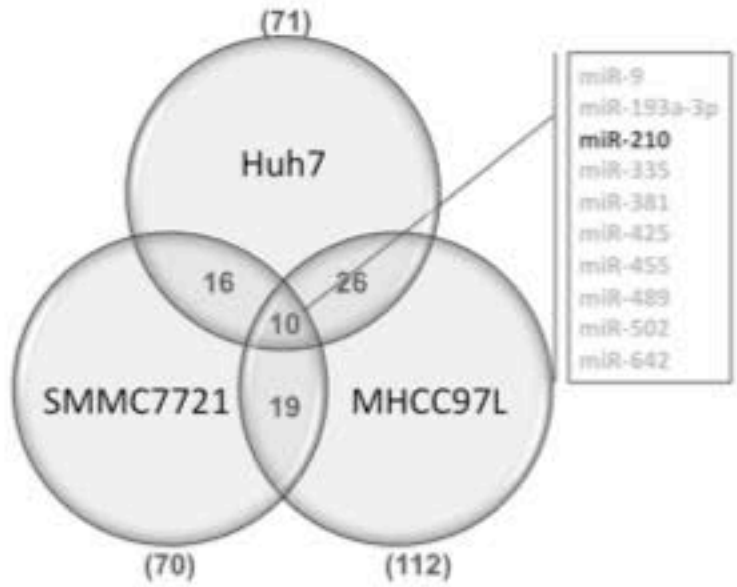

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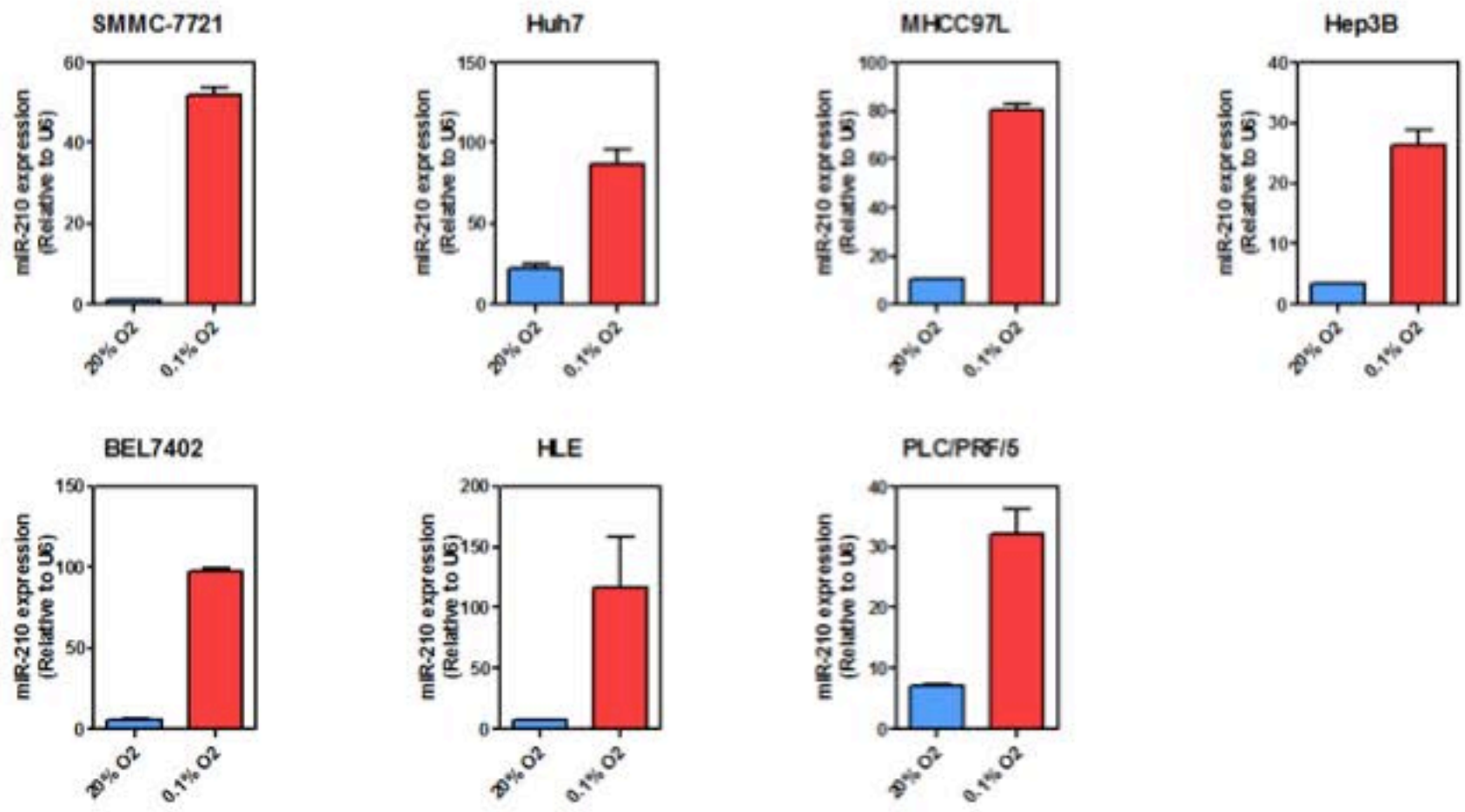

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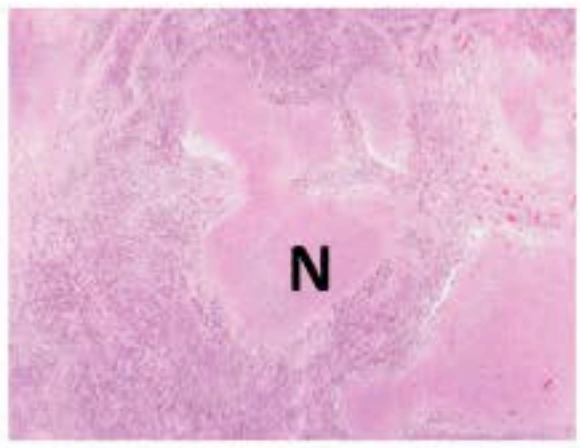
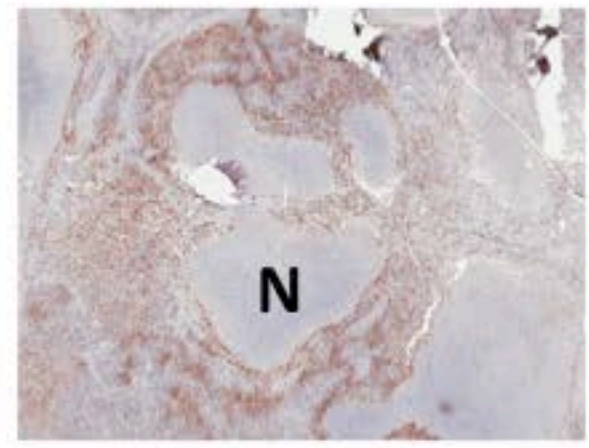
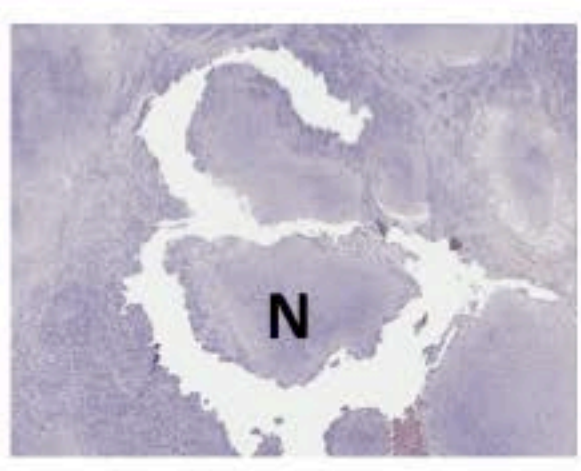
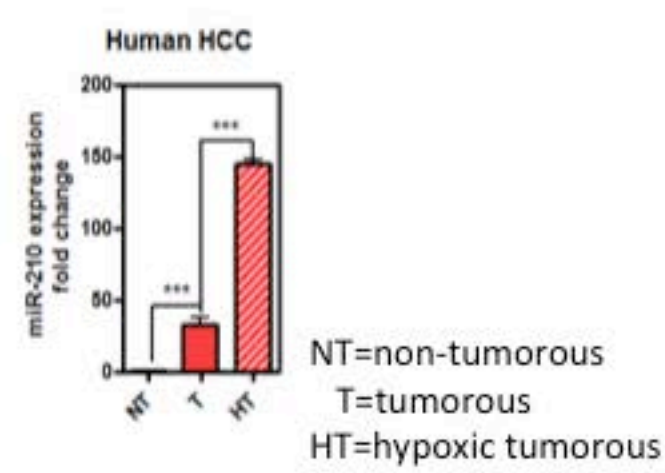
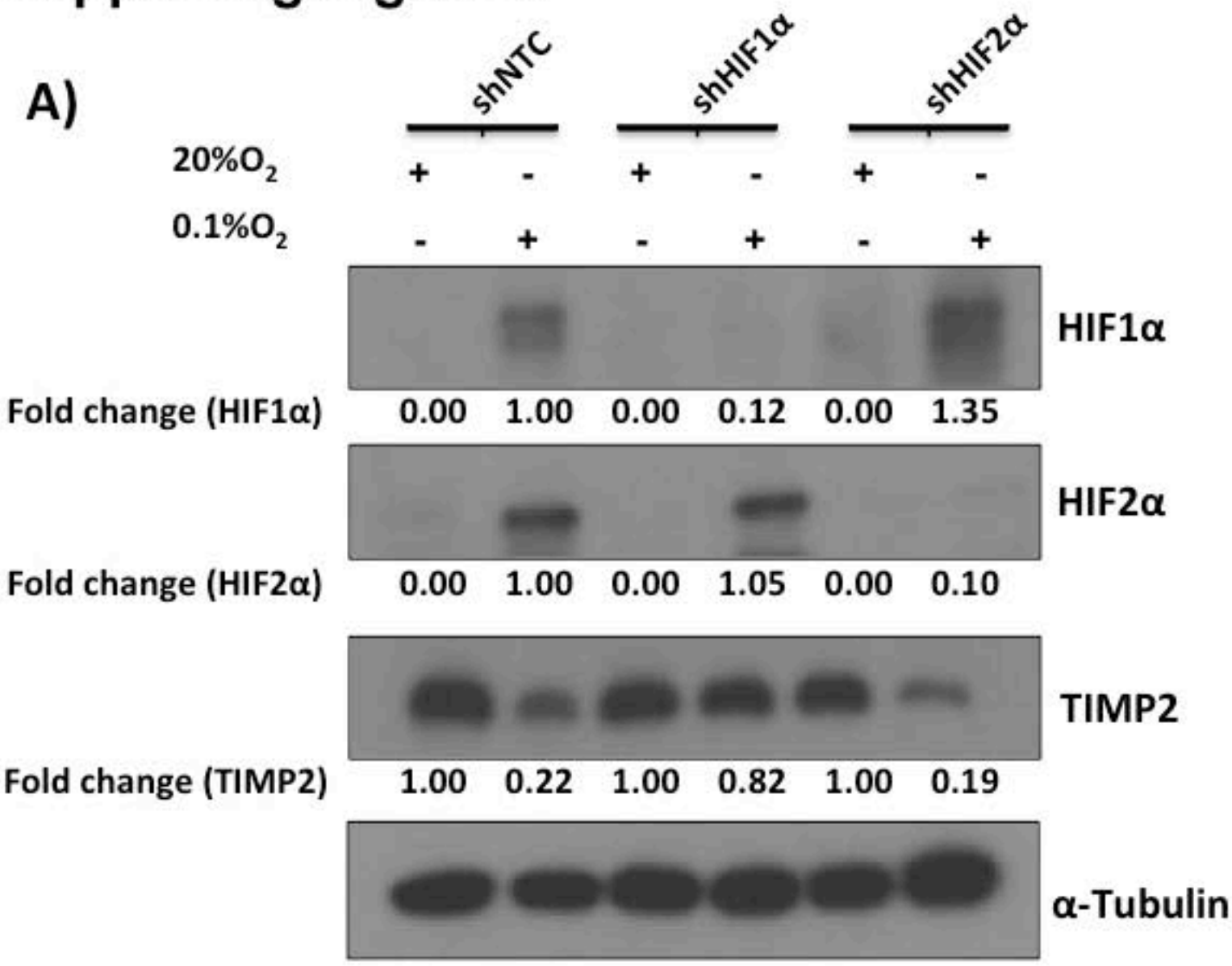


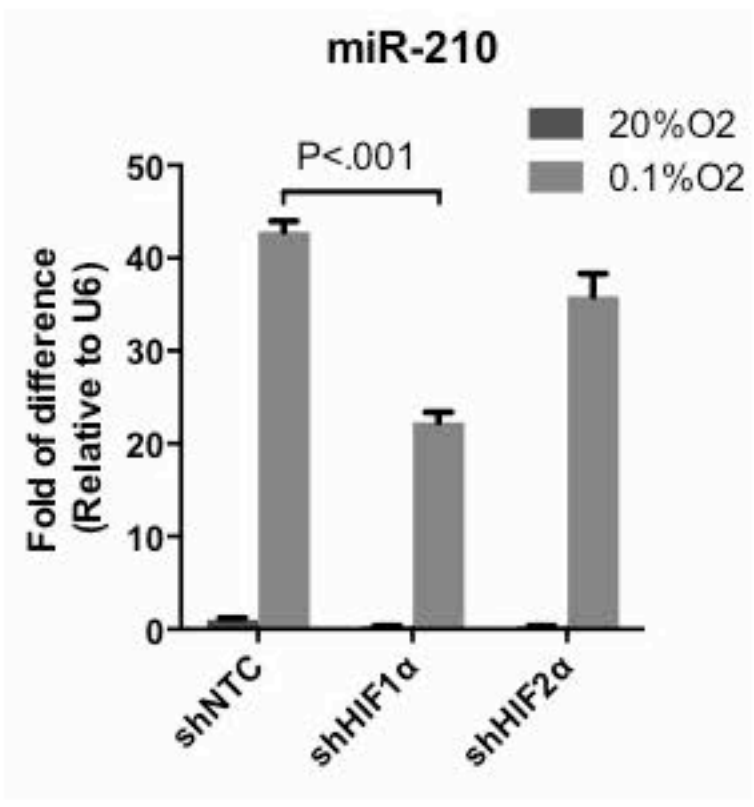


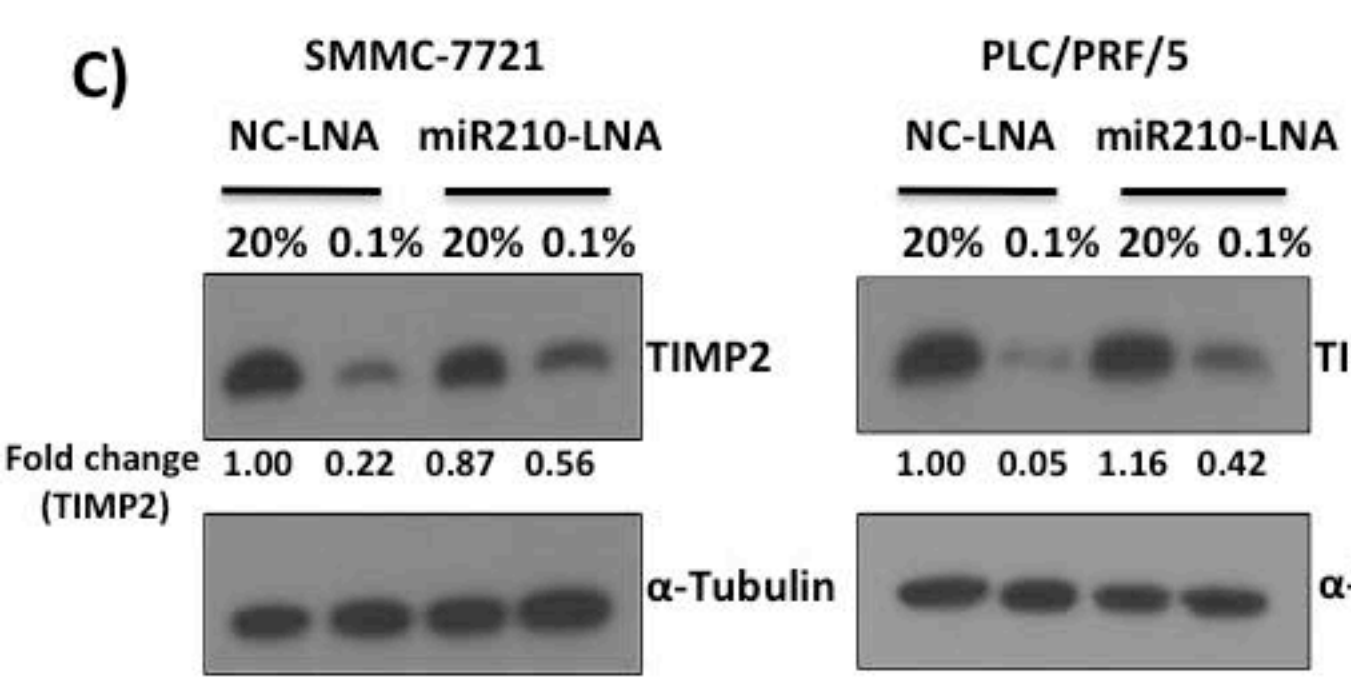


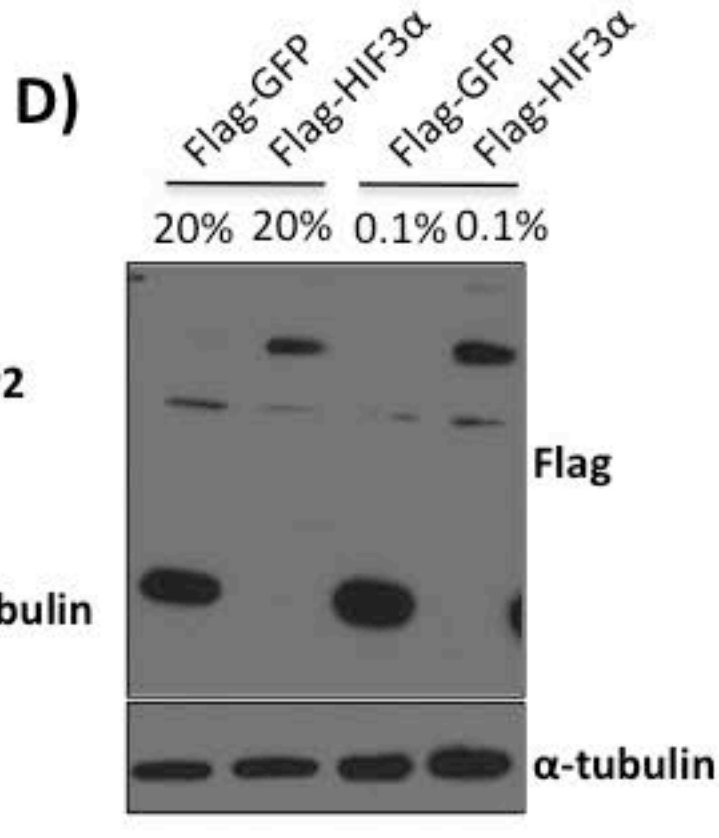


Supporting Figure 5

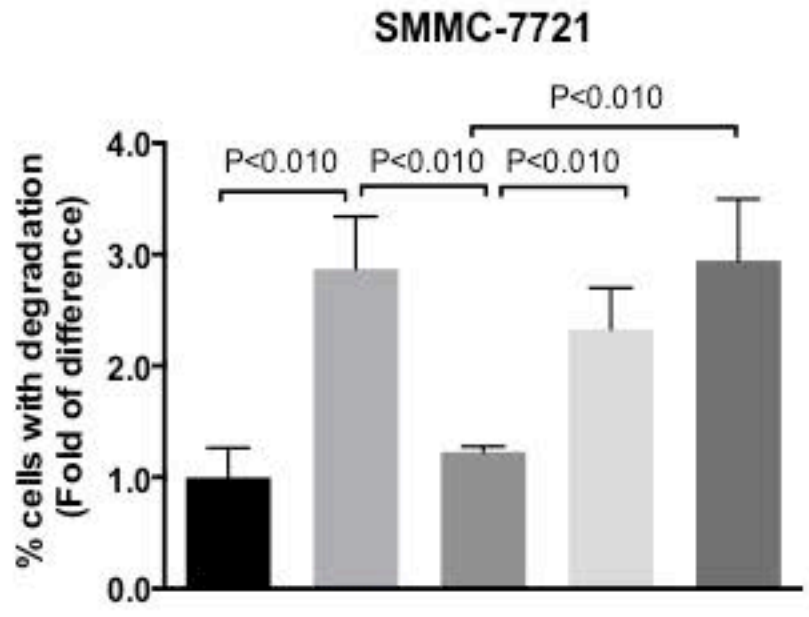








Supporting Figure 6



20%O_2	+	-	-	-	-
0.1%O_2	-	+	+	+	+
miR210 LNA	-	-	+	+	-
shTIMP2	-	-	-	+	+