Electronic Supplementary Material (ESI) for Biomaterials Science.

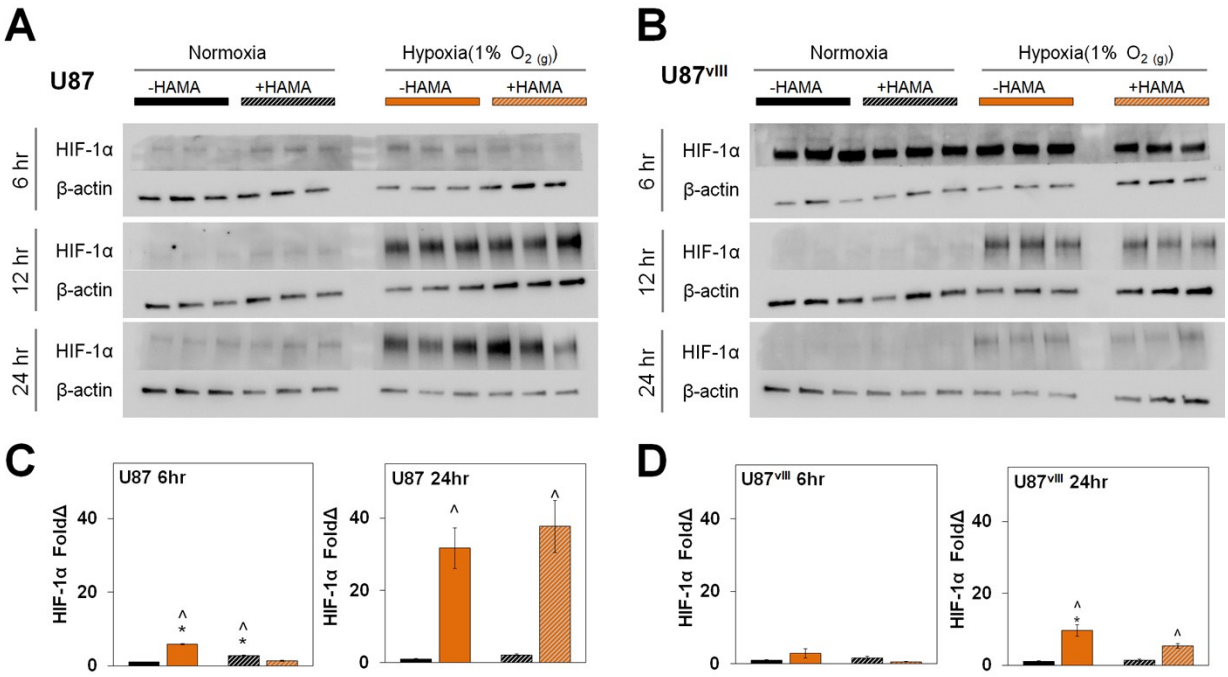
Supplementary Information

Hypoxia activates enhanced invasive potential and endogenous hyaluronic acid production by glioblastoma cells

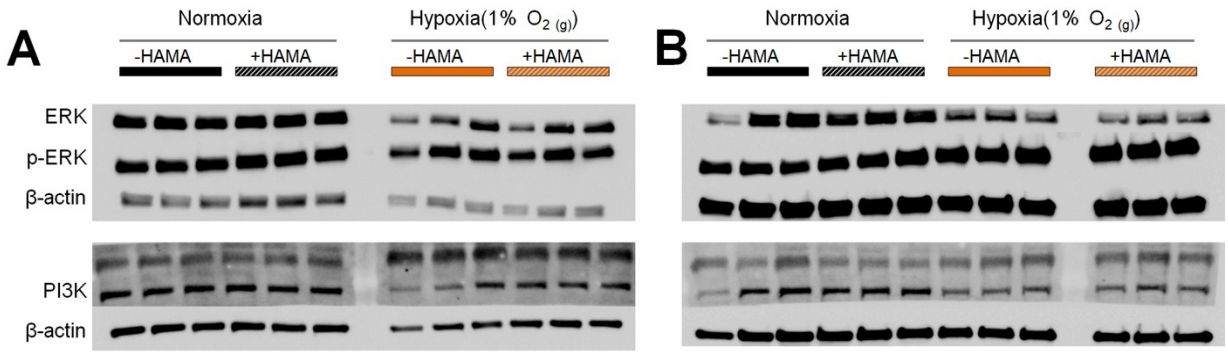
Jee-Wei Emily Chen[1,4], Jan Lumibao[2,4], Audrey Blazek[3], H. Rex Gaskins[2,4], Brendan Harley[1,4*]

[1] Dept. of Chemical and Biomolecular Engineering
[2] Division of Nutritional Sciences
[3] Dept. of Bioengineering
[4] Carl R. Woese Institute for Genomic Biology
University of Illinois at Urbana-Champaign
Urbana, IL 61801

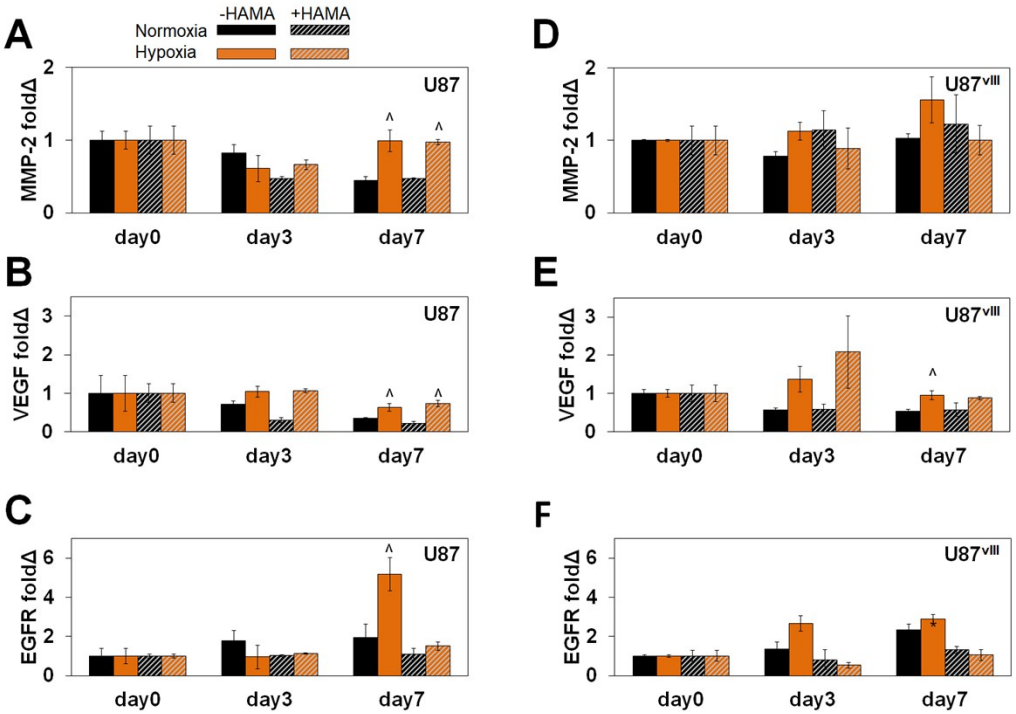
Supplementary Figures



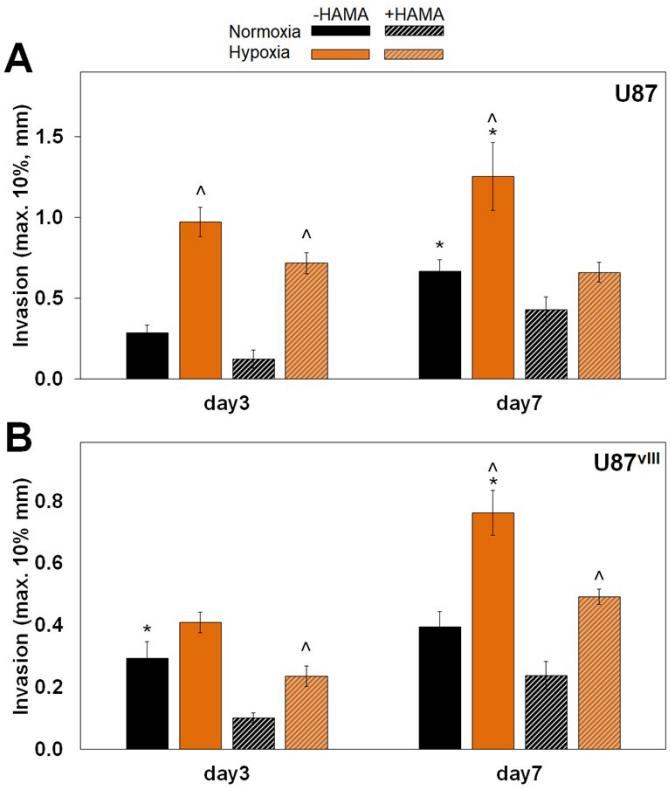
Supplemental Figure 1. Western blot results and quantified bands regarding HIF-1α expression profiles for **(A,C)** U87 and **(B,D)** U87^{vIII} GBM specimens. The early activation of HIF was observed as early as 6hr for -HAMA group and continued for all groups up to 24hr. ^ significant ($p < 0.05$) between +/- hypoxia; * significant ($p < 0.05$) between +/- HAMA.



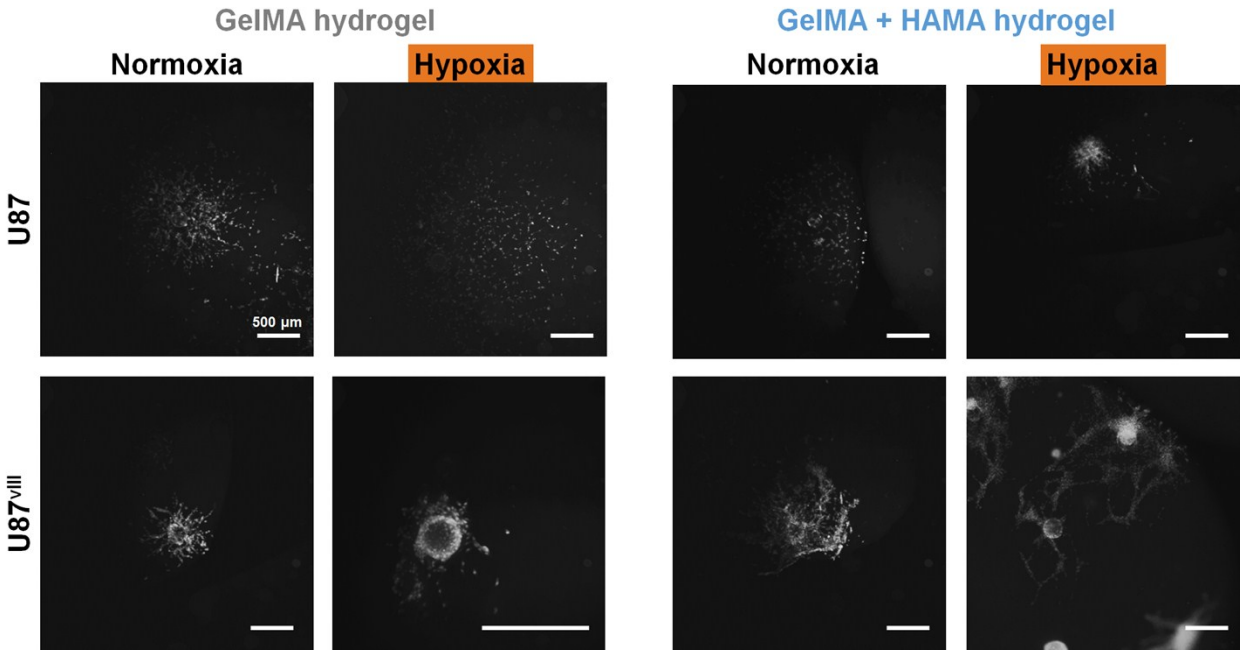
Supplemental Figure 2. Full Western blot results regarding activation of ERK and PI3K pathways for **(A)** U87 and **(B)** U87^{vIII} GBM specimens.



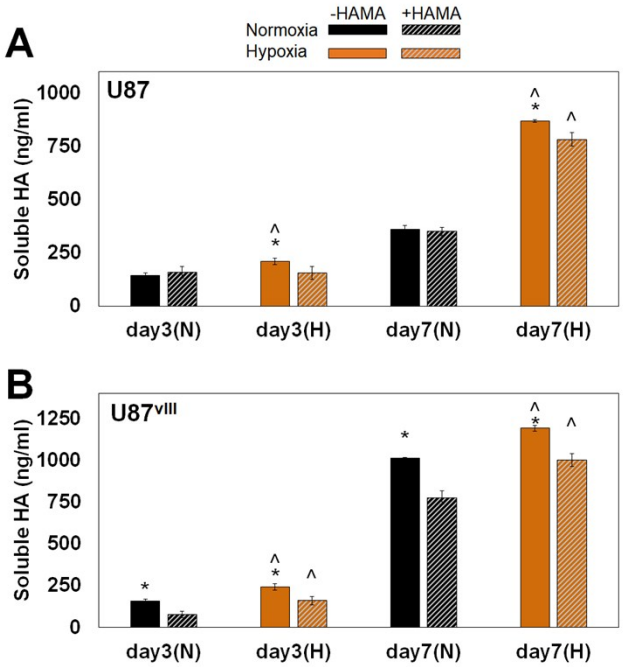
Supplemental Figure 3. Gene expression profiles for **(A, D)** *MMP-2*, **(B, E)** *VEGF*, and **(C, F)** *EGFR* across all time points for U87 and U87^{vIII} GBM specimens, respectively. ^ significant ($p < 0.05$) for +/- hypoxia; * significant ($p < 0.05$) for +/- HAMA



Supplemental Figure 4. Mean invasion distance for the 10% of **(A)** U87 and **(B)** U87^{vIII} GBM cells exhibiting the greatest overall invasion into GelMA and GelMA-HAMA hydrogels. Observed trends regarding average invasion distance match those experienced by the entire complement of GBM specimens. ^ significant ($p < 0.05$) for +/- hypoxia; * significant ($p < 0.05$) for +/- HAMA



Supplemental Figure 5. Representative images of taken from analysis of U87/U87^{vIII} GBM cell invasion into the surrounding GelMA or GelMA + HAMA hydrogel environments in the presence of continuous normoxia or hypoxia (day 7). Cell nuclei are stained with Hoechst dye. Scale bar: 500 µm.



Supplemental Figure 6. Quantifying soluble HA production by **(A)** U87 and **(B)** U87^{vIII} GBM specimens in GelMA and GelMA-HAMA hydrogels over 7 days in culture as a function of hypoxia vs. normoxia. ^ significant ($p < 0.05$) for +/- hypoxia; * significant ($p < 0.05$) for +/- HAMA.

Supplemental Table 1. Mechanical characterization of GelMA hydrogel variants with (+HAMA) and without (-HAMA) HA functionalization. Total GelMA wt% was adjusted so that all hydrogel variants containing the identical total wt% of polymer (4 wt%).

Hydrogel	GelMA	HAMA	LAP	Elastic Modulus
-HAMA	4 wt%	0 wt%	0.1 wt%	2.758 ± 0.24 kPa
+HAMA	3.4 wt%	0.6 wt%	0.02 wt%	2.785 ± 0.14 kPa

Supplemental Table 2. Primers used for gene expression.

Gene	Primer Sequence (5'-xxx-3')	Citation
VEGF	Forward: AAGCCCATTCCCTCTTTAGC Reverse: GGCAAAGTGAGTGACCTGCT	[1]
MMP-2	Forward: ATAACCTGGATGCCGTCGT Reverse: AGGCACCCTTGAAGAAGTAGC	[2]
EGFR	Forward: GCAACCAGCAACAATTCC Reverse: AGAGGCTGATTGTGATAGAC	[3]
HIF-1α	Forward: CGTTCCTTCGATCAGTTGTC Reverse: TCAGTGGTGGCAGTGGTAGT	[4]
GAPDH	Forward: CCTTCCACGATACCAAAGTTG Reverse: CCATGAGAAGTATGACAACAGCC	[5]

Supplemental Table 3. Antibodies and concentration for Western blot analyses.

Protein	Blocking	Primary antibody	Secondary antibody
ERK 1/2 (42-44 kDa)	5% BSA	1:1000 in 5% BSA (Cell Signaling, Rabbit mAb 9102S)	Anti-rabbit IgG, HRP-linked antibody (Cell Signaling, 7074S) 1:2500 in TBST
p-ERK 1/2 (42-44 kDa)	5% BSA	1:1000 in 5% BSA (Cell Signaling, Rabbit mAb 4370S)	
PI3K (85 kDa)	5% NFDM	1:1000 in 2% NFDM (Cell Signaling, Rabbit mAb 4292S)	
HIF-1α (93 kDa)	5% NFDM	1:2000 in 5% NFDM (Abcam, ab51608)	
β-actin (45 kDa)	5% BSA	1:1000 in 5% BSA (Cell Signaling, Rabbit mAb 4967L)	

Supplemental References

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2. C. Blázquez, M. Salazar, A. Carracedo, M. Lorente, A. Egia, L. González-Feria, A. Haro, G. Velasco and M. Guzmán, *Cancer Research*, 2008, **68**, 1945.
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