Supplementary Information for

Hypoxia-inducible factor-dependent ADAM12 expression mediates breast cancer invasion and metastasis

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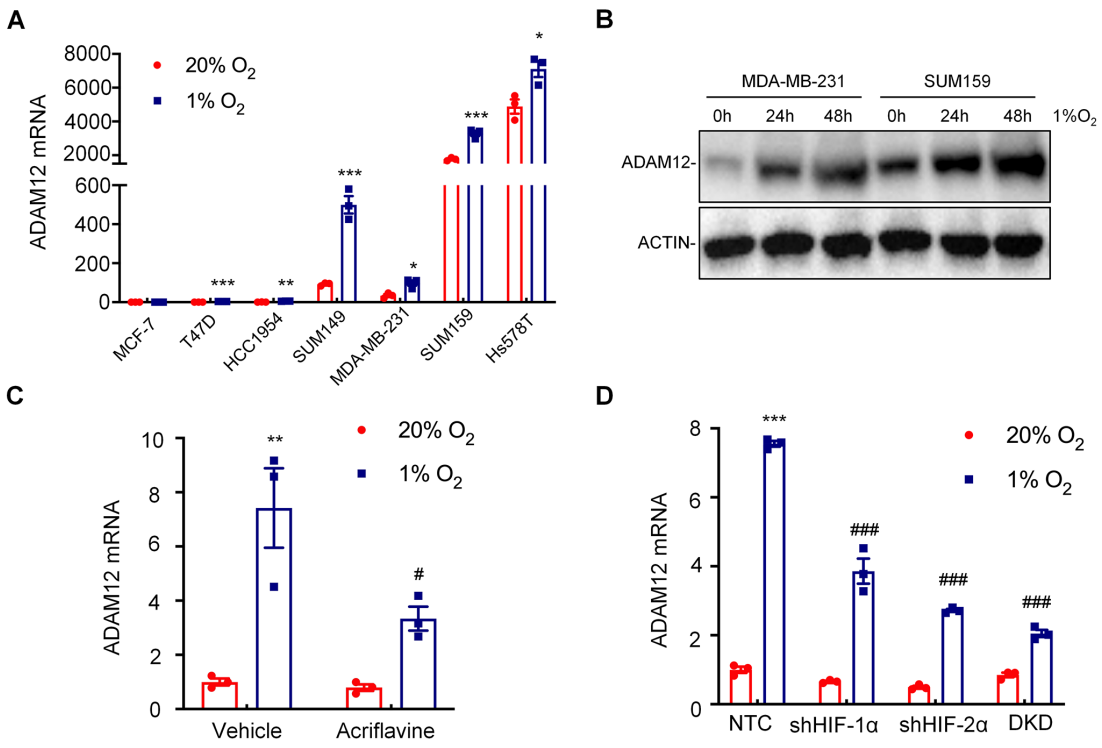

Fig. S1. ADAM12 expression in breast cancer is induced by hypoxia in a HIF-dependent manner. (*A*) RT-qPCR was performed to quantify ADAM12 mRNA levels in breast cancer cell lines following exposure to 20% or 1% O_2 for 24 h. For each cell line, the expression of ADAM12 mRNA was quantified relative to 18S rRNA and then normalized to the result obtained from HCC1954 cells at 20% O_2 (mean ± SEM; $n = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus 20% O_2 (Student's *t* test). (*B*) Immunoblot assays were performed to determine ADAM12 protein levels in breast cancer cell lines following exposure to 20% O_2 or to 1% O_2 for 24 or 48 h. (*C*) SUM149 cells were exposed to 20% or 1% O_2, in the presence of vehicle or acriflavine (2.5 μM) for 24 h, and the expression of ADAM12 mRNA was assayed by RT-qPCR. **$P < 0.01$ versus vehicle at 20% O_2; $^{\#}P < 0.05$ versus vehicle at 1% O_2 (two-way ANOVA with Tukey's post-test). (*D*) RT-qPCR was performed to quantify ADAM12 mRNA levels in SUM149 HIF-knockdown subclones exposed to 20% or 1% O_2 for 24 h. Data were normalized to NTC at 20% O_2 (mean ± SEM; $n = 3$). ***$P <$ 0.001 versus NTC at 20% O_2; $^{\#\#\#}P < 0.001$, versus NTC at 1% O_2 (two-way ANOVA with Tukey's post-test).

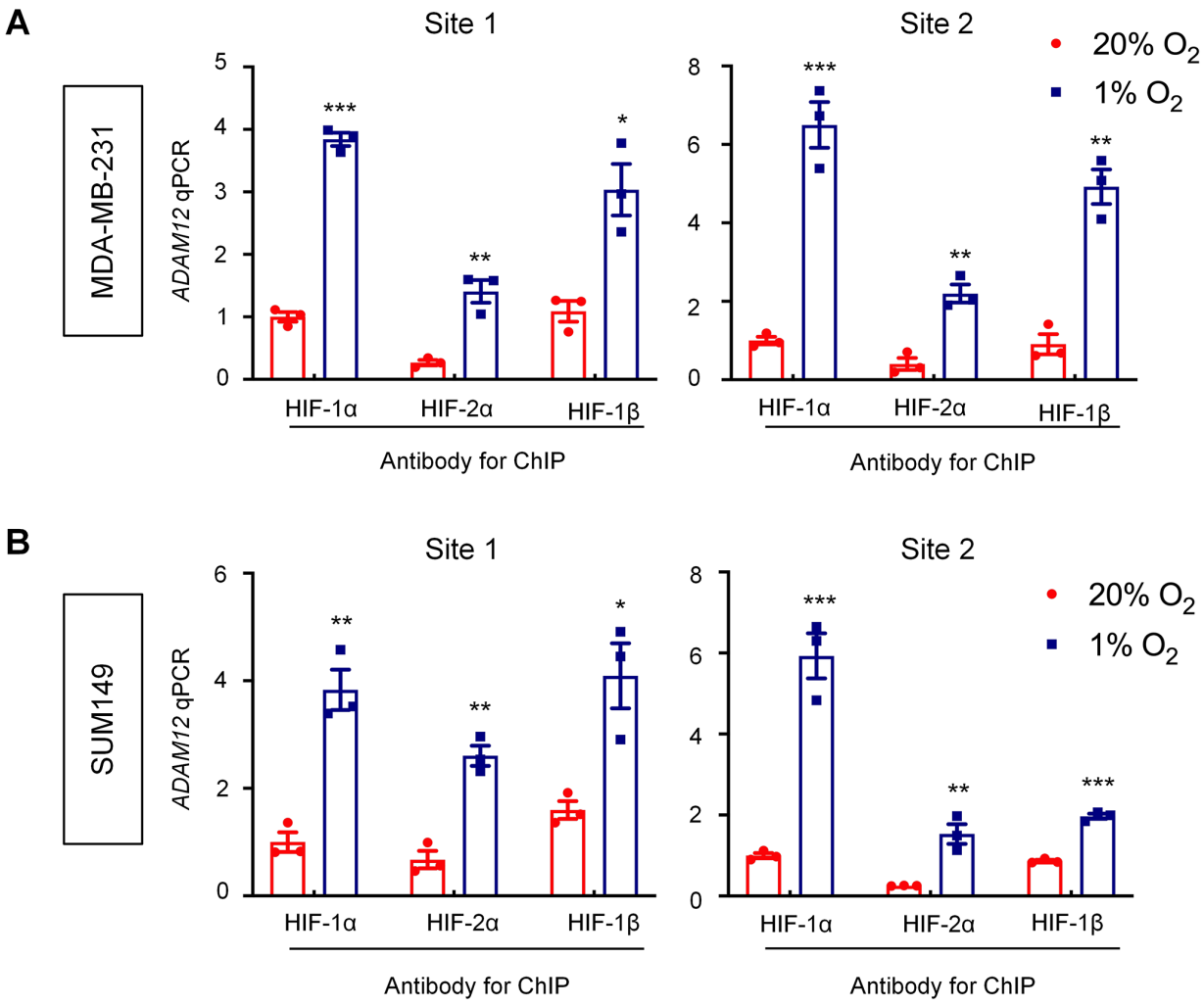

Fig. S2. *ADAM12* is a direct HIF target gene in human breast cancer cell lines. (*A* and *B*) MDA-MB-231 (*A*) and SUM149 (*B*) cells were incubated at 20% or 1% O_2 for 16 h, and ChIP was performed using antibodies against HIF-1α, HIF-2α, or HIF-1β. Primers flanking HIF binding sites were used for qPCR, and results were normalized to HIF-1α at 20% O_2 (mean ± SEM; $n = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus 20% O_2 (Student's *t* test).

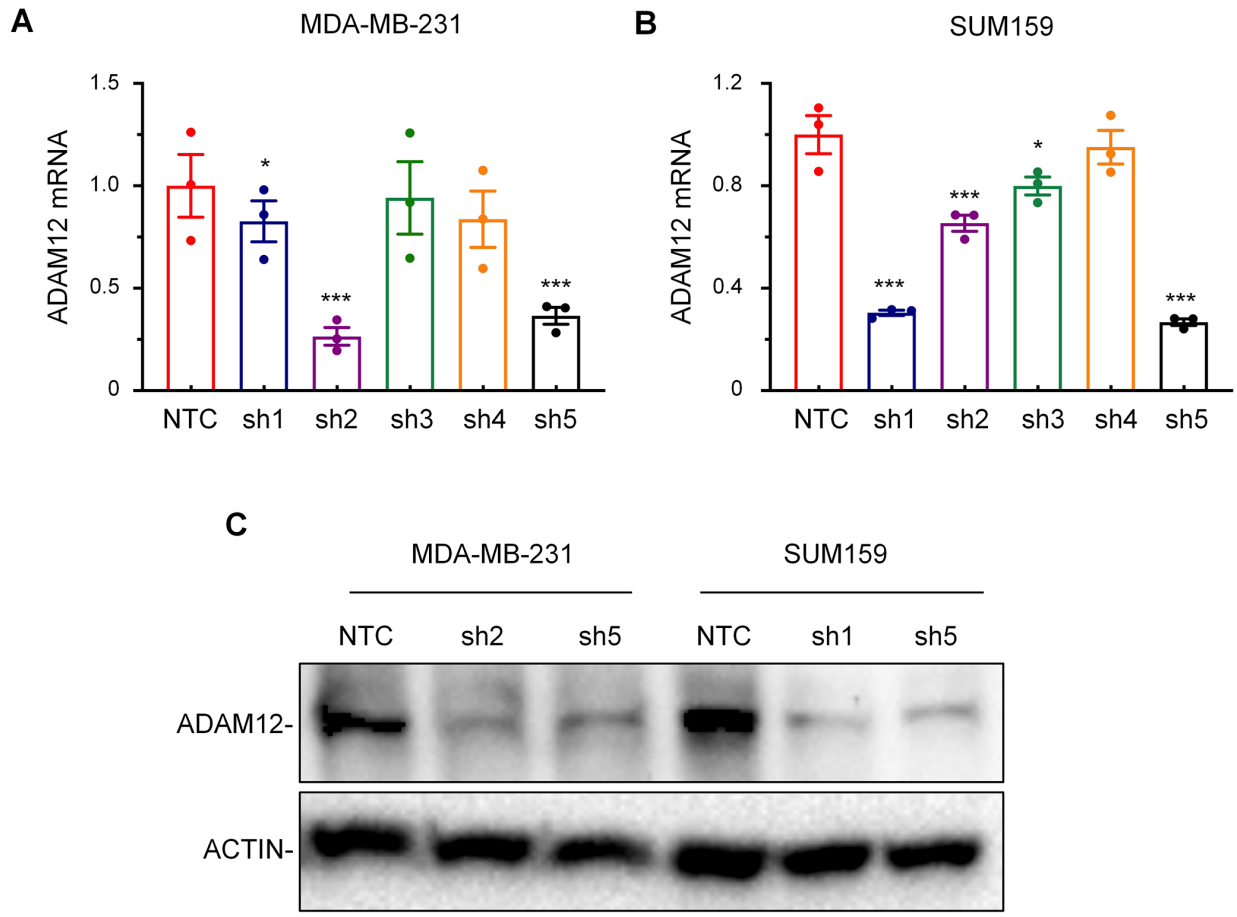

Fig. S3. Analysis of ADAM12 knockdown efficiency. (*A* and *B*) MDA-MB-231 (*A*) and SUM159 (*B*) cells were transduced with a non-targeting control (NTC) short hairpin RNA (shRNA) or one of five different shRNAs targeting ADAM12 (sh1, sh2, sh3, sh4, sh5), and RT-qPCR assays were performed to analyze ADAM12 mRNA expression (mean ± SEM; $n = 3$). *$P < 0.05$, ***$P < 0.001$ versus NTC (one-way ANOVA). (*C*) Immunoblot assays were performed to determine ADAM12 protein expression in ADAM12 knockdown subclones of MDA-MB-231 and SUM159 cells.

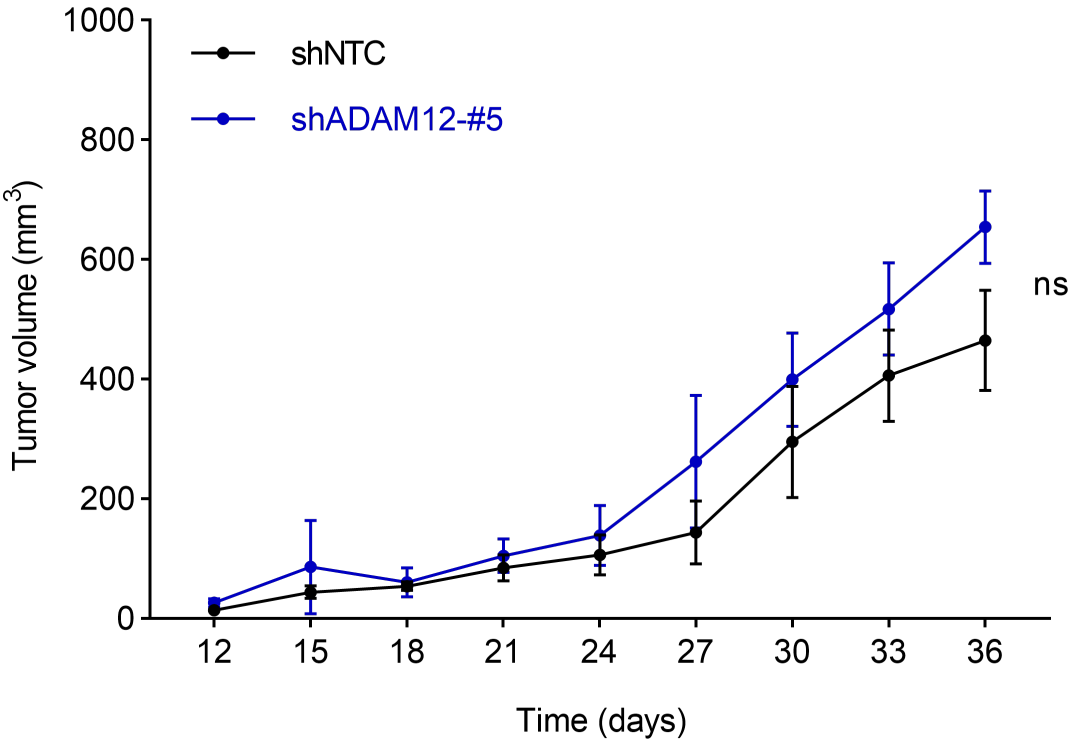

Fig. S4. Analysis of SUM159 tumor xenograft growth. SUM159 NTC and ADAM12 knockdown sh5 subclones (2×10^6 cells) were implanted into the mammary fat pad of 6-week-old female SCID mice. Primary tumor volume was determined every three days from day 12 to day 36 (mean + SEM, n = 5 mice each; Kruskal-Wallis test with Benjamini-Hochberg post-test).

Table S1. Nucleotide sequences of primers used in RT-qPCR assays.

RNA	**Primer**	**Nucleotide sequence** (5' to 3')
18S rRNA	Forward	CGGCGACGACCCATTCGAAC
	Reverse	GAATCGAACCCTGATTCCCCGTC
ADAM12 mRNA	Forward	CGAGGGGTGAGCTTATGGAAC
	Reverse	GCTTTCCCGTTGTAGTCGAATA

Table S2. Antibodies used for immunoblot (IB) and ChIP assays.

Antibody	**Vendor**	**Catalog number**
HIF-1α (IB)	BD Biosciences	610959
HIF-1α (ChIP)	Novus Biologicals	NB100-479
HIF-1β	Novus Biologicals	NB100-110
HIF-2α	Novus Biologicals	NB100-122
ADAM12	Abcam	ab223476
HB-EGF	Novus Biologicals	AF259
EGFR	Cell Signaling	#4267
p-EGFR (Y1173)	Novus Biologicals	AF1095
FAK	Cell Signaling	#3285
p-FAK (Y397)	Cell Signaling	#8556
β-ACTIN	Santa Cruz Biotechnology	SC-47778
Rabbit IgG	Novus Biologicals	NBP2-36463

Table S3. Nucleotide sequences of primers used for ChIP-qPCR assays.

***ADAM12* gene site**	**Primer**	**Nucleotide sequence** (5' to 3')
Site 1	Forward	CAACTCGGACAGTTTGCTCA
	Reverse	CGCTGAGCTCTTCTAGCCTTT
Site 2	Forward	CAGCAGTTCAGGTCACAGGA
	Reverse	ACAGGAATGCTGCAGGAAGT

Table S4. Wild-type (WT) and mutant (MUT) HRE1 and HRE2 oligonucleotides that were annealed and inserted into firefly luciferase reporter plasmid.

		Nucleotide sequence (5' to 3')
pA12-HRE1-WT	Sense	GATCAAAGTTTCCCCCCGTGTGTGTGCGTGCGTGCGCGCGCG CGCGCCGTTCTGGCACA
	Antisense	TCGATGTGCCAGAACGGCGCGCGCGCGCGCACGCACGCACA CACACGGGGGGAAACTTT
pA12-HRE1-MUT	Sense	GATCAAAGTTTCCCCCCGTGTGTGTGCGTGAAAGCGCGCGCG CGCGCCGTTCTGGCACA
	Antisense	TCGATGTGCCAGAACGGCGCGCGCGCGCGCTTTCACGCACAC ACACGGGGGGAAACTTT
pA12-HRE2-WT	Sense	GATCGGAGGCCGGGCACCTGGCCAGAATTCACGCCTCTGGCA GTGGGCAGAGCTCAGGC
	Antisense	TCGAGCCTGAGCTCTGCCCACTGCCAGAGGCGTGAATTCTGG CCAGGTGCCCGGCCTCC
pA12-HRE2-MUT	Sense	GATCGGAGGCCGGGCACCTGGCCAGAATTCTTTCCTCTGGCA GTGGGCAGAGCTCAGGC
	Antisense	TCGAGCCTGAGCTCTGCCCACTGCCAGAGGAAAGAATTCTGG CCAGGTGCCCGGCCTCC

Table S5. Clone ID number for ADAM12 shRNAs.

Gene	**Catalog number**
ADAM12-sh1	TRCN0000047033
ADAM12-sh2	TRCN0000047034
ADAM12-sh3	TRCN0000047035
ADAM12-sh4	TRCN0000047036
ADAM12-sh5	TRCN0000047037