Supplementary information

HIF-2-induced long non-coding RNA RAB11B-AS1 promotes hypoxia-mediated angiogenesis and breast cancer metastasis

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1 70
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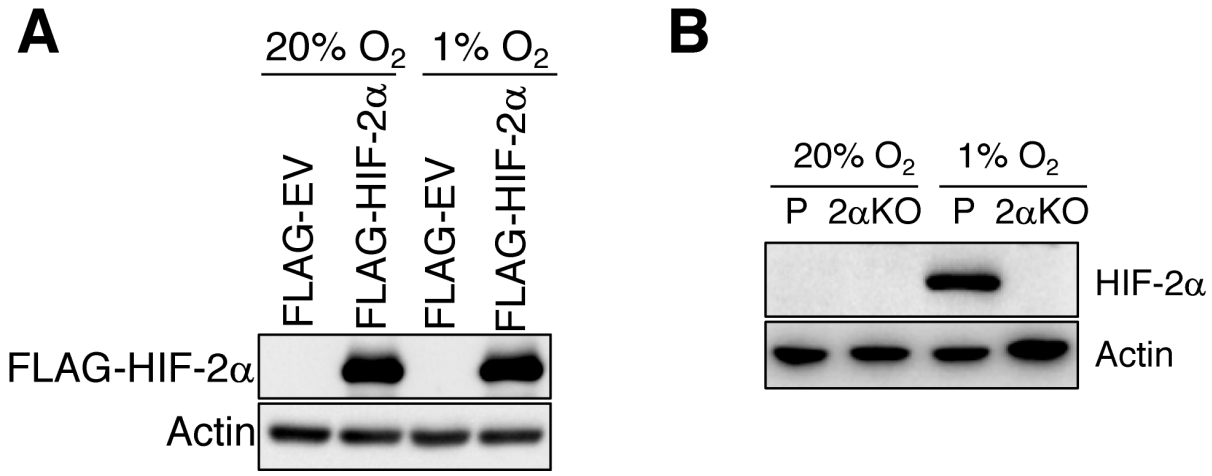
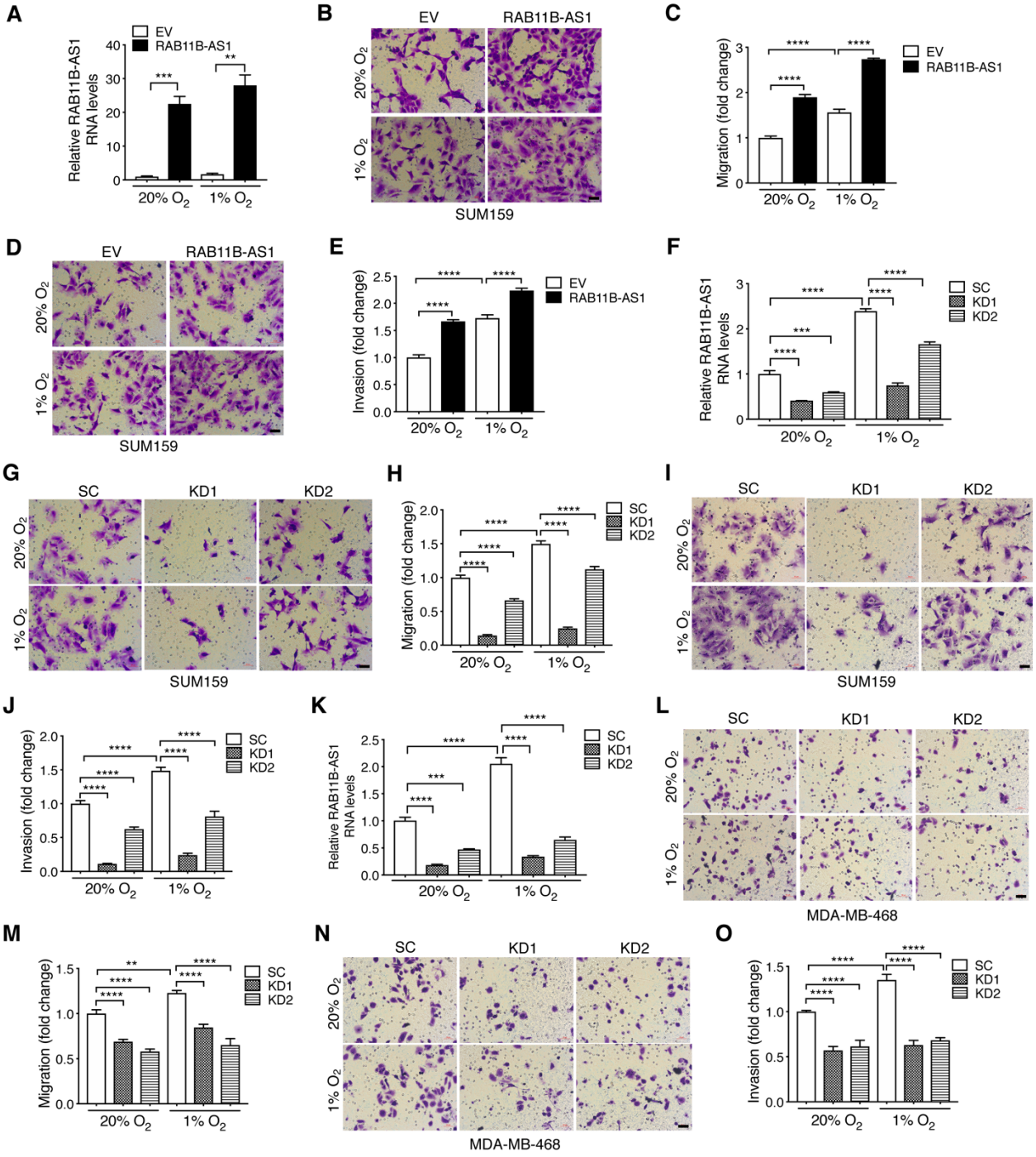
Supplementary Fig. S1. The nucleotide sequence of RAB11B-AS1 identified by 5'- and 3'-RACE in human MDA-MB-231 cells. The RAB11B-AS1 sequence from 5'- and 3'-RACE is shown in orange and the mutated nucleotides are marked in blue as compared with the reference sequence in the GenBank (NR_038237.1), which is shown in black. The nucleotide sequence of RAB11B-AS1 has been deposited in the GenBank with the accession number MK855053.

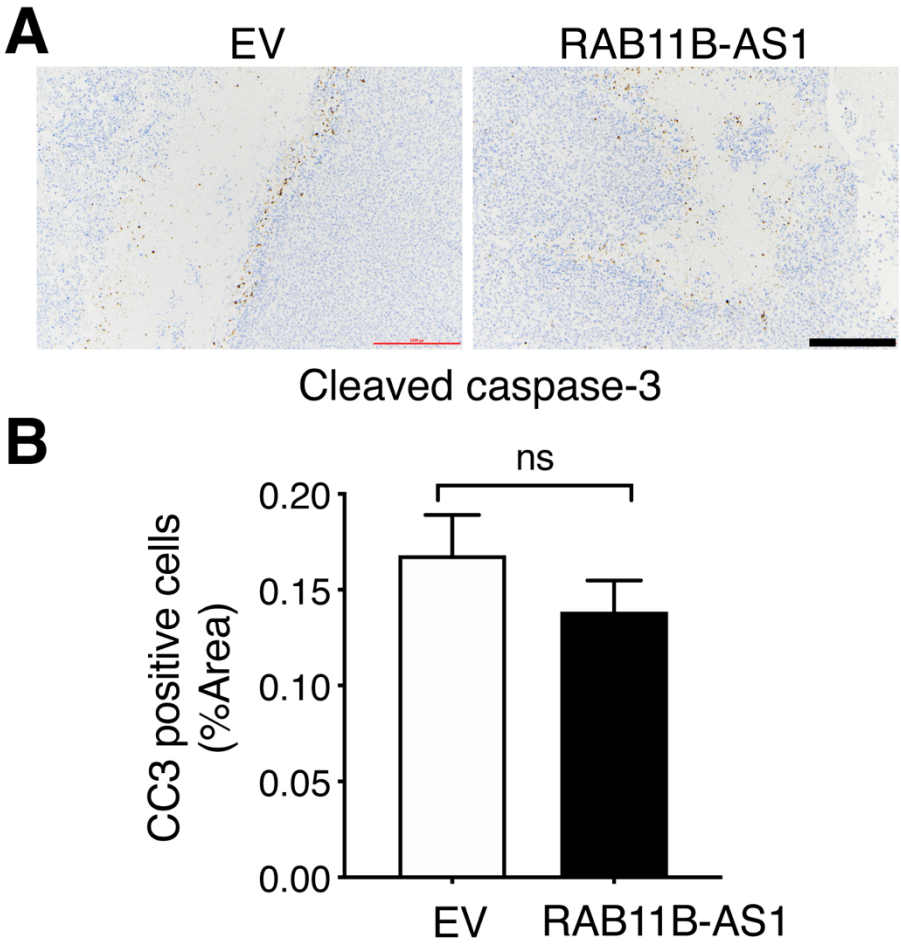


Supplementary Fig. S2. Validation of HIF-2α protein levels in overexpressed and knockout cells. **A**, Immunoblot analysis of FLAG-HIF-2α and actin in HEK293T cells exposed to 20% or 1% O_2 for 24 hours. Representative blots from three experiments are shown. **B**, Immunoblot analysis of HIF-2α and actin in parental and HIF-2α KO HeLa cells exposed to 20% or 1% O_2 for 24 hours. Representative blots from three experiments are shown.

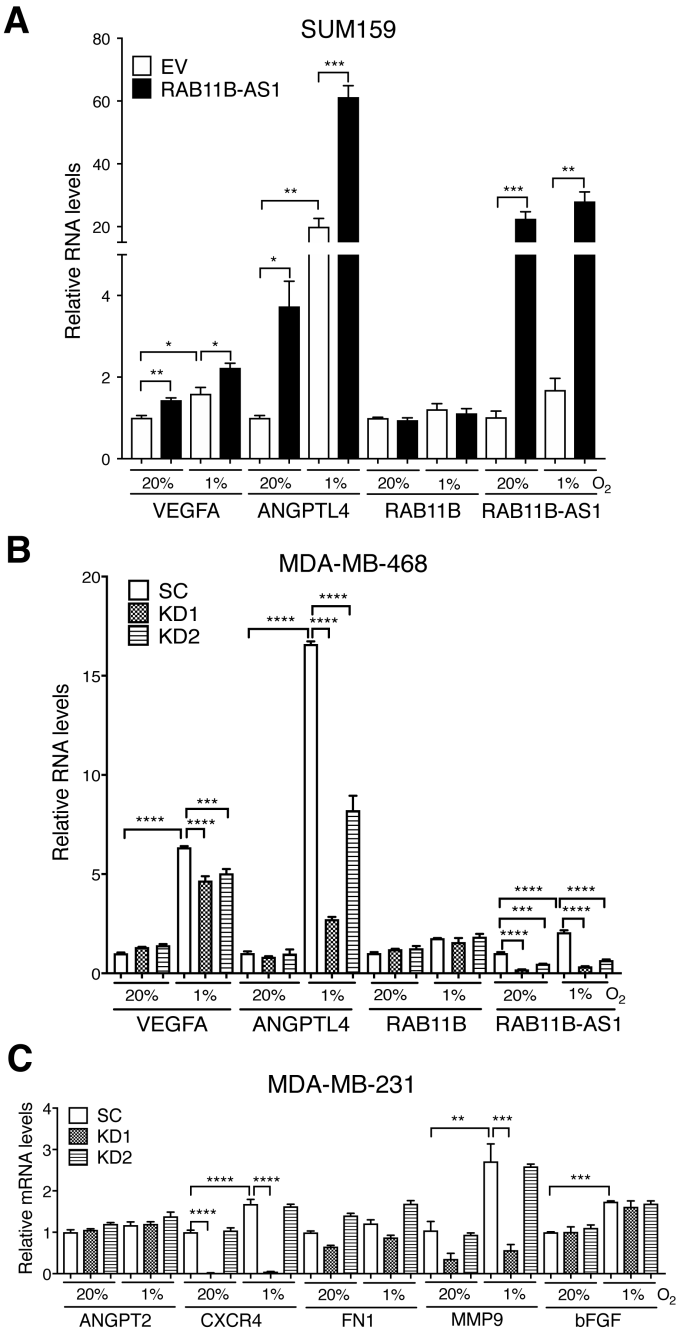


Supplementary Fig. S3. RAB11B-AS1 increases breast cancer cell migration and invasion in vitro. **A**, RT-qPCR analysis of RAB11B-AS1 RNA levels in SUM159 cells stably transfected with RAB11B-AS1 expression vector or EV and exposed to 20% or 1% O_2 for 24 hours. RAB11B-AS1 RNA/18*S* rRNA was normalized to EV at 20% O_2 (mean ± SEM, n = 3). $^{**}p < 0.01$; $^{***}p < 0.001$. **B** and **C**, Transwell migration of SUM159-EV and -RAB11B-AS1 cells exposed to 20% or 1% O_2 for 16 hours. Representative images from three independent experiments are shown in **B**. Scale bar, 50 μm. The number of migrated cells were counted and normalized to EV at 20% O_2 (**C**, mean

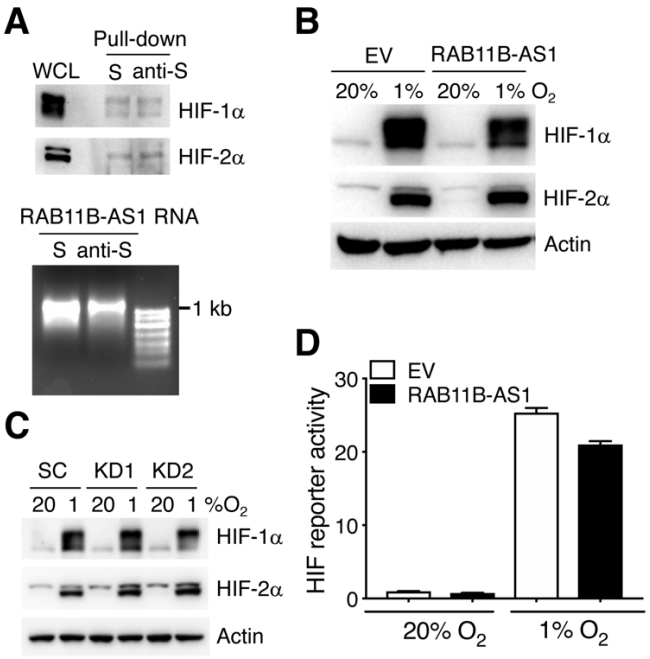
± SEM, n = 3). $^{****}p < 0.0001$. **D** and **E**, Transwell invasion of SUM159-EV and -RAB11B-AS1 cells exposed to 20% or 1% O_2 for 24 hours. Representative images from three independent experiments are shown in **D**. Scale bar, 50 μm. The number of invaded cells were counted and normalized to EV at 20% O_2 (**E**, mean ± SEM, n = 3). $^{****}p < 0.0001$. **F**, RT-qPCR analysis of RAB11B-AS1 RNA levels in SC and RAB11B-AS1 KD SUM159 cells exposed to 20% or 1% O_2 for 24 hours. RAB11B-AS1 RNA/18*S* rRNA was normalized to SC at 20% O_2 (mean ± SEM, n = 3). $^{***}p < 0.001$; $^{****}p < 0.0001$. **G** and **H**, Transwell migration of SC and RAB11B-AS1 KD SUM159 cells exposed to 20% or 1% O_2 for 16 hours. Representative images from three independent experiments are shown in **G**. Scale bar, 50 μm. The number of migrated cells were counted and normalized to SC at 20% O_2 (**H**, mean ± SEM, n = 3). $^{****}p < 0.0001$. **I** and **J**, Transwell invasion of SC and RAB11B-AS1 KD SUM159 cells exposed to 20% or 1% O_2 for 24 hours. Representative images from three independent experiments are shown in **I**. Scale bar, 50 μm. The number of invaded cells were counted and normalized to SC at 20% O_2 (**J**, mean ± SEM, n = 3). $^{****}p < 0.0001$. **K**, RT-qPCR analysis of RAB11B-AS1 RNA levels in SC and RAB11B-AS1 KD MDA-MB-468 cells exposed to 20% or 1% O_2 for 24 hours. RAB11B-AS1 RNA/18*S* rRNA was normalized to SC at 20% O_2 (mean ± SEM, n = 3). $^{***}p < 0.001$; $^{****}p < 0.0001$. **L** and **M**, Transwell migration of SC and RAB11B-AS1 KD MDA-MB-468 cells exposed to 20% or 1% O_2 for 16 hours. Representative images from three independent experiments are shown in **L**. Scale bar, 50 μm. The number of migrated cells were counted and normalized to SC at 20% O_2 (**M**, mean ± SEM, n = 3). $^{**}p < 0.01$; $^{****}p < 0.0001$. **N** and **O**, Transwell invasion of SC and RAB11B-AS1 KD MDA-MB-468 cells exposed to 20% or 1% O_2 for 24 hours. Representative images from three independent experiments are shown in **N**. Scale bar, 50 μm. The number of invaded cells were counted and normalized to SC at 20% O_2 (**O**, mean ± SEM, n = 3). $^{****}p < 0.0001$.



Supplementary Fig. S4. RAB11B-AS1 has no effect on caspase-3-dependent cell death in tumors. **A**, Representative immunohistochemical staining of cleaved caspase-3 (CC3) in MDA-MB-231-EV or -RAB11B-AS1-overexpressed tumors. Scale bar, 1 mm. **B**, The CC3-positive area was quantified and normalized to the total area (mean ± SEM, n = 5). ns, not significant.



Supplementary Fig. S5. RAB11B-AS1 increases hypoxia-induced expression of angiogenic factors in breast cancer cells. **A**, RT-qPCR analysis of VEGFA mRNA, ANGPTL4 mRNA, RAB11B mRNA, and RAB11B-AS1 RNA in SUM159-EV and -RAB11B-AS1 cells exposed to 20% or 1% O_2 for 24 hours (mean ± SEM, n = 3). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. **B**, RT-qPCR analysis of VEGFA mRNA, ANGPTL4 mRNA, RAB11B mRNA, and RAB11B-AS1 RNA in SC and RAB11B-AS1 KD MDA-MB-468 cells exposed to 20% or 1% O_2 for 24 hours (mean ± SEM, n = 3). $^{***}p < 0.001$; $^{****}p < 0.0001$. **C**, RT-qPCR analysis of ANGPT2, CXCR4, FN1, MMP9, and bFGF mRNAs in SC and RAB11B-AS1 KD MDA-MB-231 cells exposed to 20% or 1% O_2 for 24 hours (mean ± SEM, n = 3). $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.



Supplementary Fig. S6. RAB11B-AS1 fails to regulate HIF transcriptional activity. **A**, Sense and antisense of biotin-labeled RAB11B-AS1 RNA were transcribed *in vitro* and incubated in the presence of streptavidin magnetic beads for 2 hours at 4°C with lysates from MDA-MB-231 cells exposed to 1% O_2 for 24 hours, followed by immunoblot analysis with anti-HIF-1α or anti-HIF-2α antibody. **B** and **C**, Immunoblot analysis of HIF-1α and HIF-2α in RAB11B-AS1 overexpressed (**B**) or knockdown (**C**) MDA-MB-231 cells exposed to 20% or 1% O_2 for 6 hours. β-actin was used as an internal control. Representative blots from three experiments are shown. **D**, HEK293T cells were co-transfected with a HIF luciferase reporter plasmid, pSV40-Renilla, and EV or RAB11B-AS1 expression vector, and exposed to 20% or 1% O_2 for 24 hours. Firefly/*Renilla* luciferase activities were normalized to EV at 20% O_2 (mean ± SEM, n = 3).