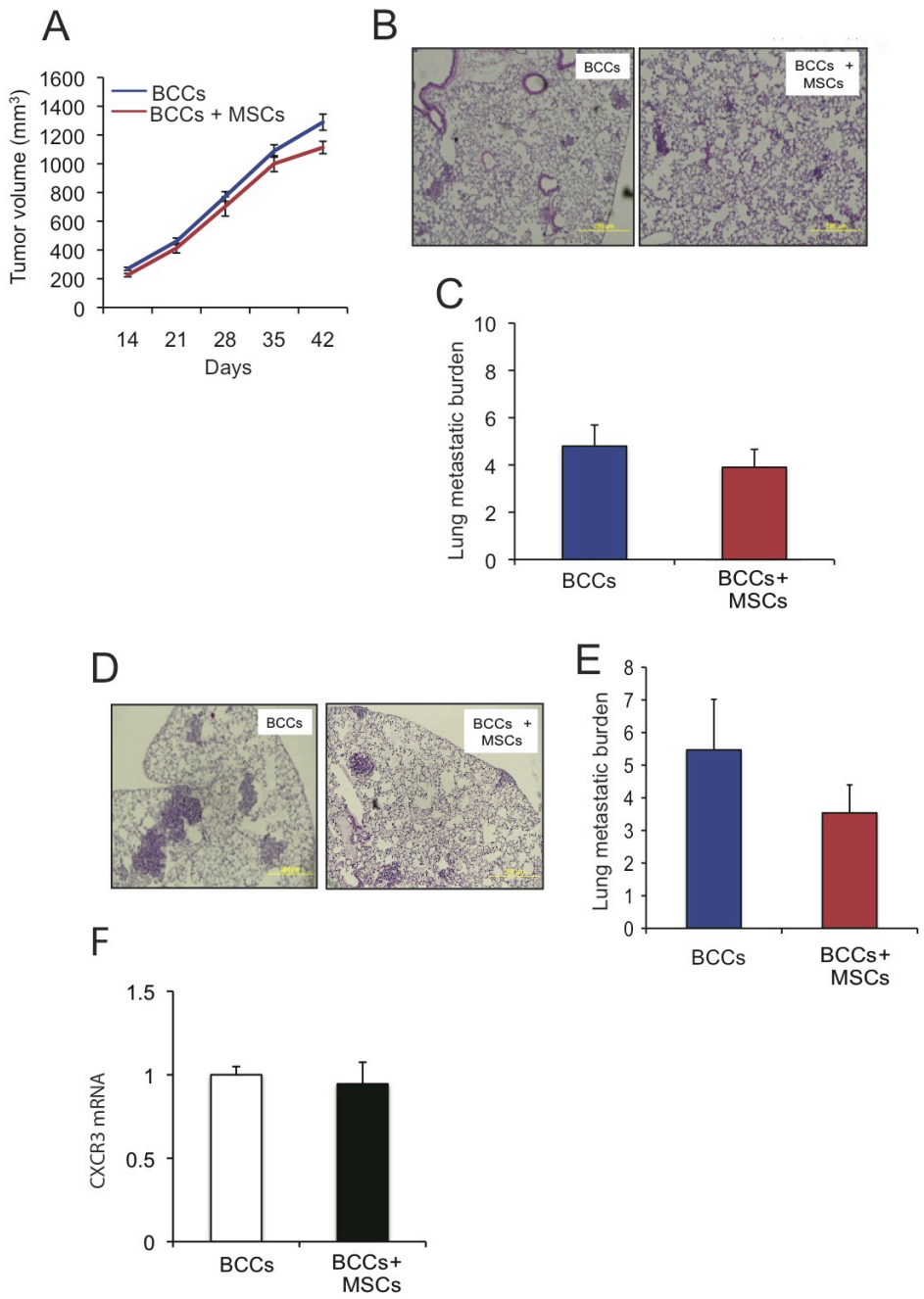
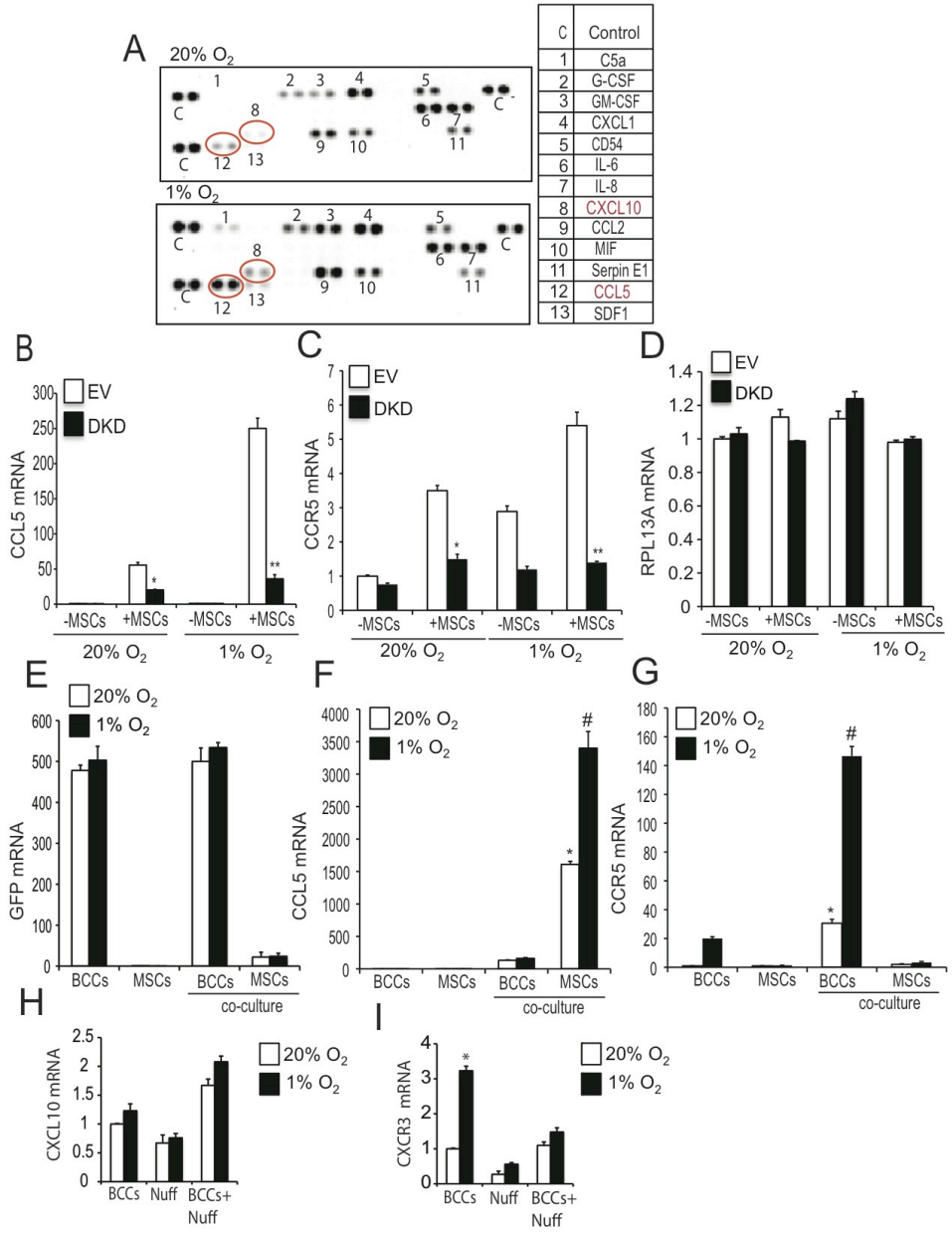
Supplemental Figure 1

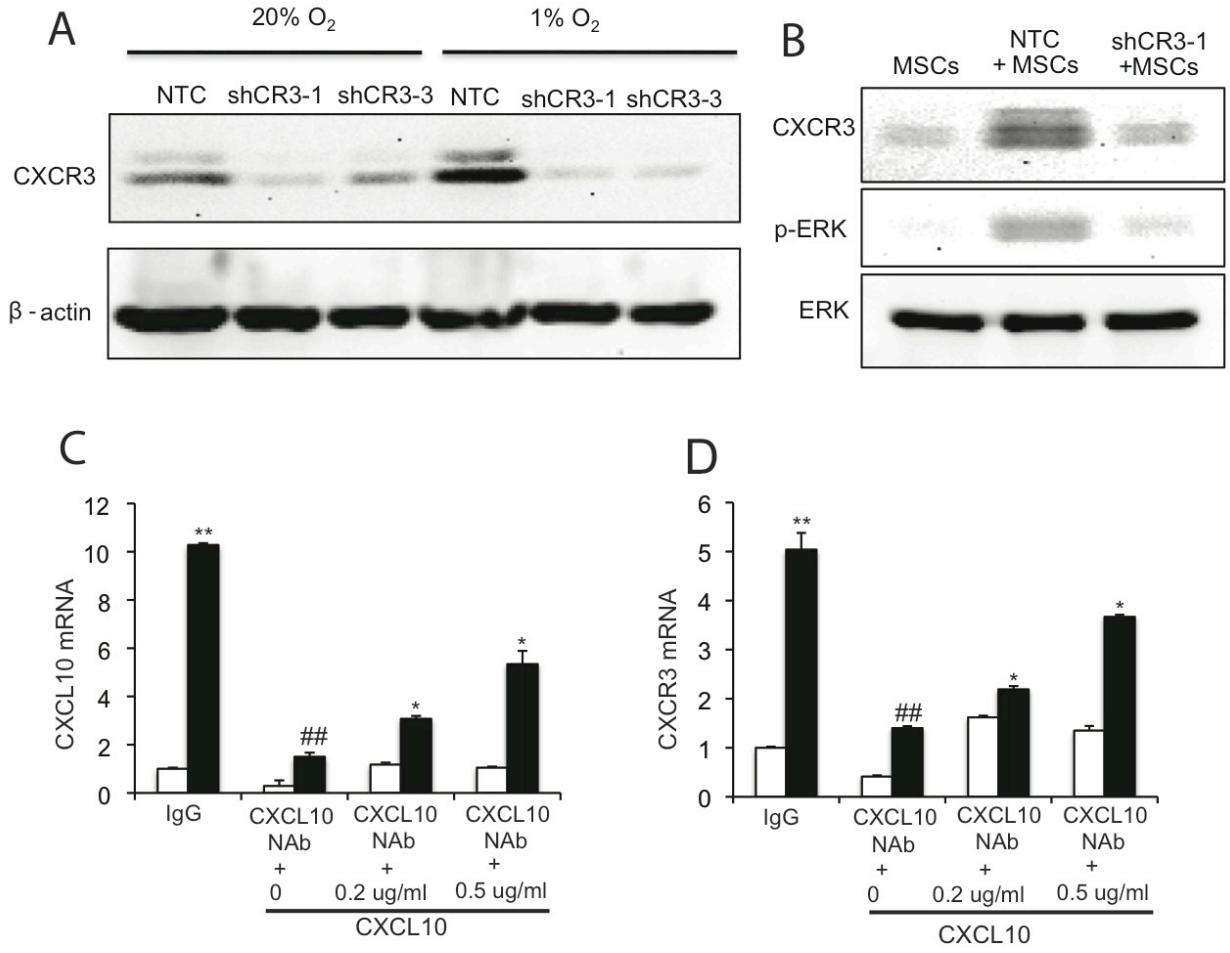


MFP injection of BCCs mixed with MSCs without prior co-culture or intravenous injection of MSCs co-cultured with BCCs does not significantly affect lung metastasis. **(A-C)** $0.5x10^6$ GFP-expressing BCCs were mixed with $0.5x10^6$ MSCs and immediately injected into the MFP of SCID mice. **(A)** Tumors were measured with calipers and tumor volume (mean ± SEM; $n = 5$) was plotted against time. **(B)** Lung sections were analyzed for metastases by H&E staining. Scale bar, 200 µm. **(C)** Lung DNA was used to quantify metastatic burden by qPCR using GFP primers. **(D-E)** BCCs+MSCs were co-cultured for 48 hours prior to intravenous injection. **(D)** Photomicrographs of H&E-stained lung sections are shown. Scale bar, 200 µm. **(E)** Lung DNA was used to quantify metastatic burden by qPCR using GFP primers. (**F**) CXCR3 mRNA levels in primary tumors were determined by RT-qPCR.

Supplemental Figure 2

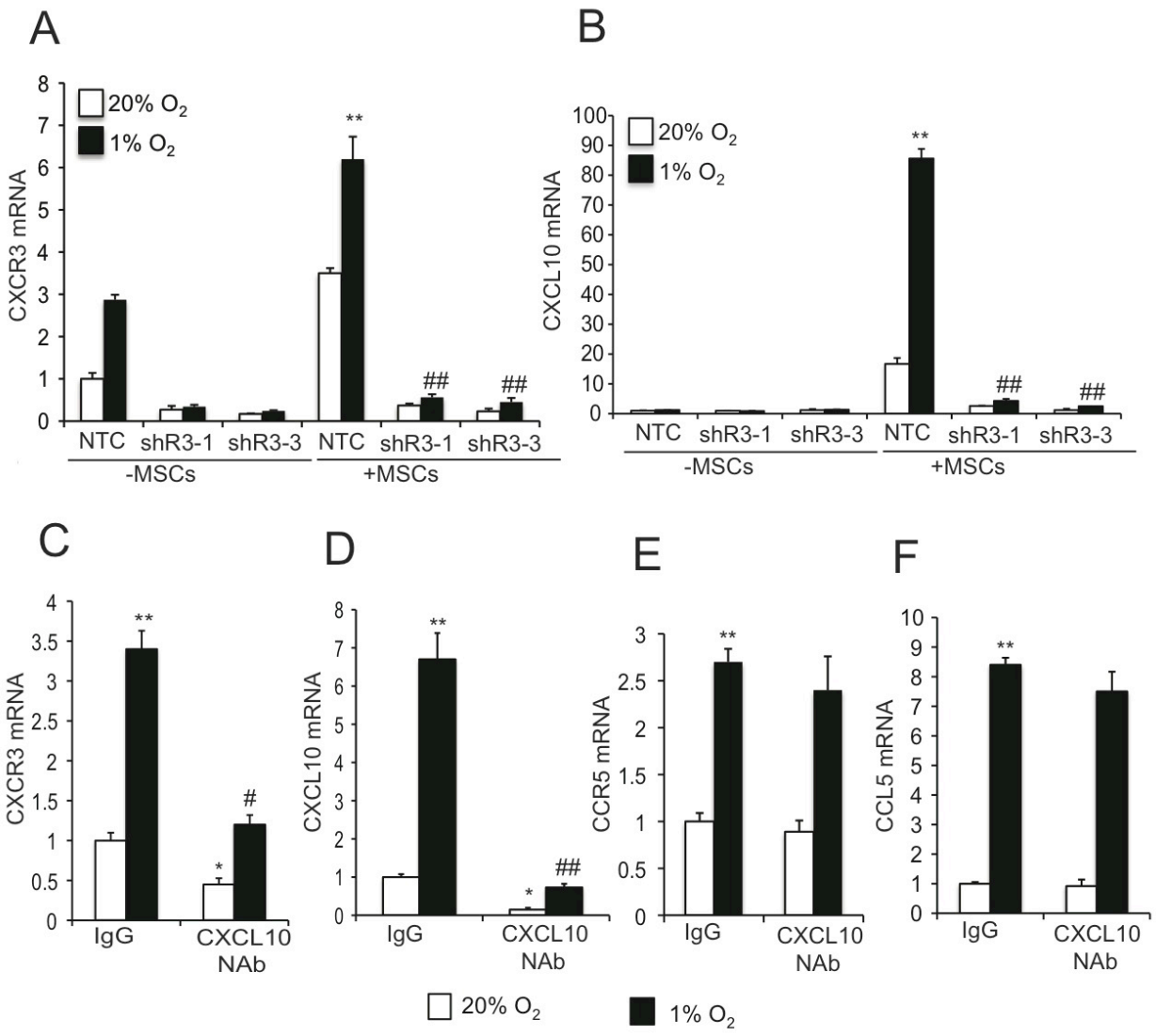


Co-culture and hypoxia-induced expression of chemokines. **(A)** A chemokine and cytokine antibody array was incubated with CM isolated from co-culture of MSCs and BCCs at 20% or 1% O_2 for 48 hours. Out of 33 cytokines and chemokines analyzed, levels of 13 proteins (identified in the table) in the CM were altered in response to hypoxia. **(B-D)** CCL5, CCR5 and RPL13A mRNA levels (mean ± SEM; $n = 3$) were analyzed in MDA-231-EV and MDA-231-DKD BCCs that were either cultured alone or with MSCs at 20% or 1% O_2. *P<0.05 *vs* EV+MSCs at 20% O_2 and **P<0.001 *vs* EV+MSCs at 1% O_2 by one-way ANOVA. **(E-G)** The co-cultured cells were subjected to FACS based on GFP fluorescence for BCCs and CD105 immunofluorescence for MSCs. Total RNA was extracted from flow-sorted BCCs and MSCs for analysis of GFP, CCL5 and CCR5 mRNA levels. BCCs and MSCs cultured alone were used as controls. Levels were normalized to those observed in BCCs at 20% O_2. *P<0.01 *vs* MSCs or BCCs alone at 20% O_2 and $^{\#}P$<0.001 *vs* MSCs or BCCs alone at 1% O_2 by one-way ANOVA. (**H-I**) BCCs, MSCs, or BCCs+Nuff (normal human foreskin fibroblasts) were cultured under 20% or 1% O_2 for 48 hours for RT-qPCR analysis of CXCL10 and CXCR3 mRNA levels, which were normalized to those observed in BCCs at 20% O_2. *P<0.05 *vs* BCCs at 20% O_2.



Supplemental Figure 3

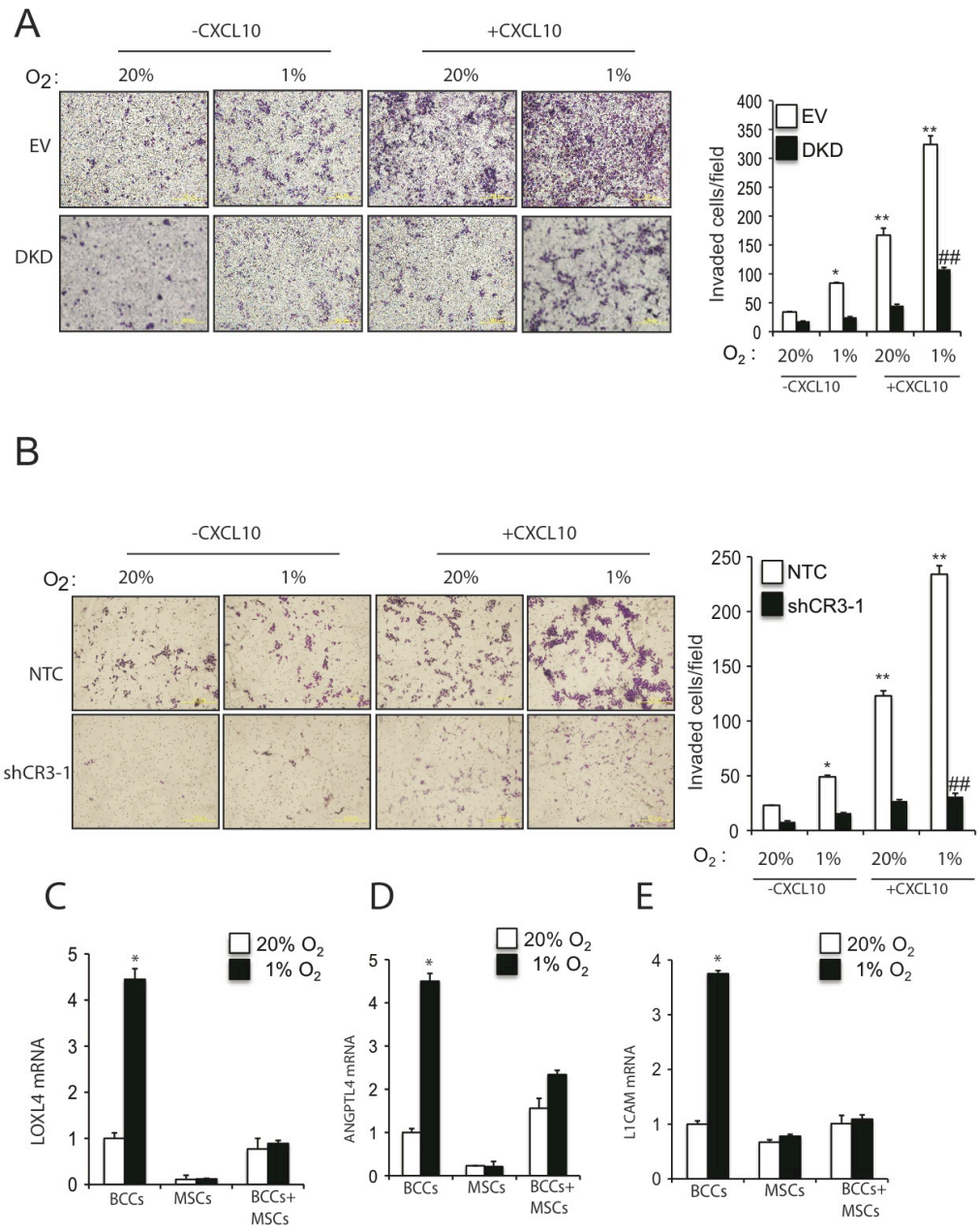
(A) CXCR3 protein levels were analyzed in MDA-231 subclones cultured at 20% or 1% O_2 for 48 hours. (**B**) Levels of phosphorylated (pERK) and total (ERK) ERK1/2 MAP kinases were analyzed in protein lysates isolated from NTC and shCR3-1 cells cultured alone or with MSCs at 20% or 1% O_2 for 48 hours. (**C-D**) CXCL10 and CXCR3 mRNA levels (mean ± SEM; $n = 3$) were analyzed in MDA-231 BCCs co-cultured with MSCs in the presence of IgG or CXCL10 neutralizing antibody (NAb) and different concentrations of recombinant CXCL10 at 20% or 1% O_2 for 48 hours. $^{**}P<0.001$ *vs* IgG at 20% O_2; $^{\#\#}P<0.005$ *vs* IgG at 1% O_2; $^{*}P<0.05$ *vs* CXCL10 NAb at 1% O_2.



Supplemental Figure 4

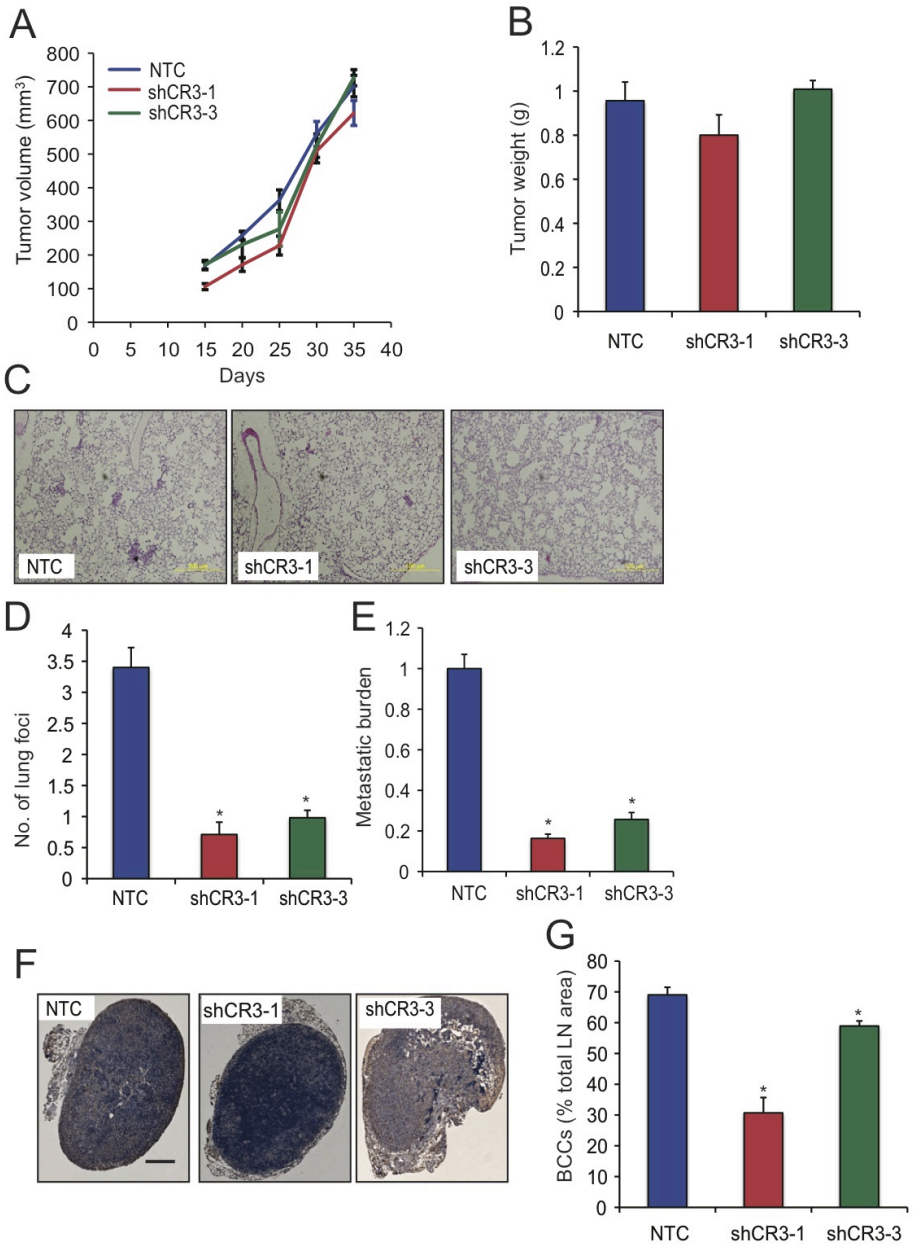
Hypoxia augments crosstalk between MDA-435 BCCs and MSCs by promoting CXCL10 → CXCR3 signaling. **(A-B)** CXCR3 and CXCL10 mRNA levels were analyzed in MDA-435 cells stably transfected with a lentiviral vector encoding a non-targeted control shRNA (NTC) or an shRNA targeted against CXCR3 (shCR3-1 or shCR3-3), either cultured alone or with MSCs at 20% or 1% O_2 for 48 hours. $^{**}P<0.001$ *vs* NTC at 20% O_2; $^{\#\#}P<0.001$ *vs* NTC+MSC at 1% O_2. **(C-F)** CXCL10, CXCR3, CCL5 and CCR5 mRNA levels (mean ± SEM; $n = 3$) were analyzed in co-cultures of MDA-435 BCCs with MSCs treated with CXCL10 NAb (2 μg) or IgG control for 48 hours at 20% or 1% O_2. $^{*}P<0.01$ and $^{**}P<0.001$ *vs* IgG at 20% O_2; $^{\#}P<0.05$ and $^{\#\#}P<0.001$ *vs* IgG at 1% O_2 by one-way ANOVA.

Supplemental Figure 5

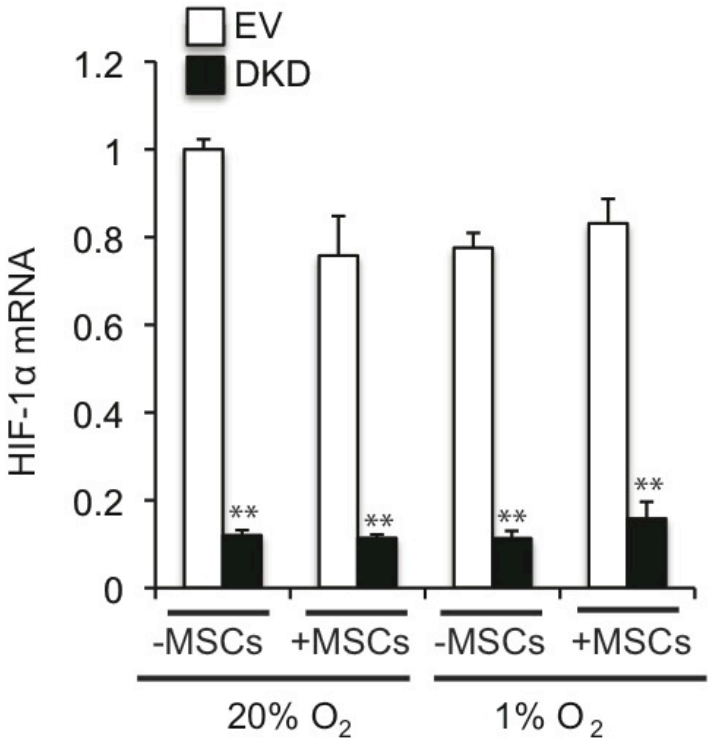


HIF or CXCR3 knockdown BCCs fail to invade in response to CXCL10. **(A-B)** Invasion of EV, DKD, NTC or shCR3-1 subclone of MDA-231 BCCs in response to CXCL10 at 20% or 1% O_2 was determined using a modified Boyden chamber assay. 0.5x 10^6 EV or DKD BCCs were seeded on top of matrigel-coated chamber inserts. The number of cells that invaded through the matrigel in response to CXCL10 (10 ng/ml) was counted under light microscopy after staining with crystal violet (mean ± SEM; n = 3). *P<0.05 and **P<0.001 *vs* control (EV or NTC) at 20% O_2; $^{\#\#}P$<0.001 *vs* EV or NTC at 1% O_2 by one-way ANOVA.

Supplemental Figure 6

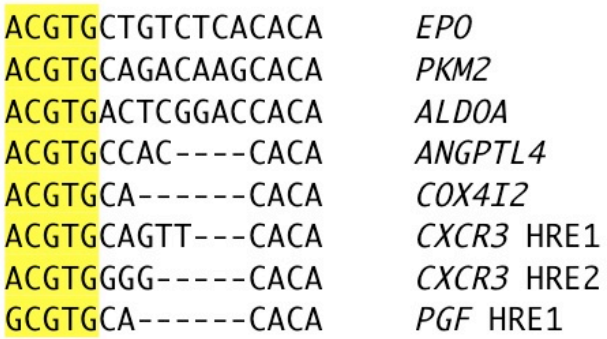


CXCR3 promotes lung and LN metastasis of MDA-231 cells. $1x10^6$ cells of MDA-231-NTC control subclone and two subclones stably expressing shRNA against CXCR3 (shCR3-1 and shCR3-3) were implanted in the MFP of SCID mice. **(A)** Primary tumor volumes were determined over the experimental time course. **(B)** Primary tumor weight was measured at the end of the experiment. **(C)** Photomicrographs of H&E-stained lung sections are shown. **(D)** Metastatic foci in lung sections were counted under 20x magnification. At least 3 random fields were counted per section. **(E)** Lung DNA was used to quantify BCCs by qPCR with human *HK2* primers. **(F)** LN sections were stained with a human-specific vimentin antibody. **(G)** LN metastasis was quantified by image analysis. **P*<0.05 *vs* NTC by one-way ANOVA.



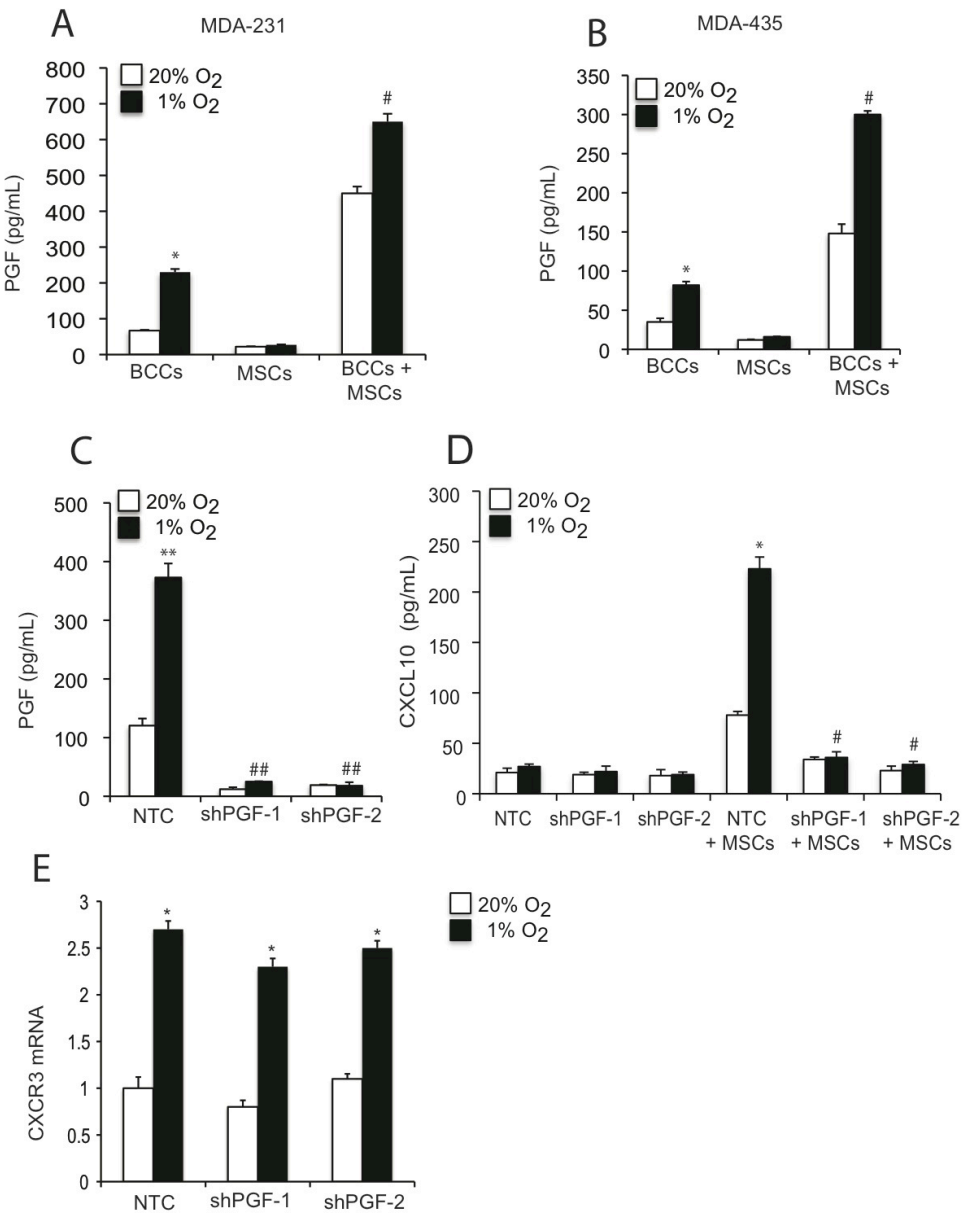
Supplemental Figure 7

HIF-1α mRNA levels (mean ± SEM; $n = 3$) were analyzed by RT-qPCR in MDA-231-EV and MDA-231-DKD BCCs cultured alone or with MSCs at 20% or 1% O_2. $^{**}P<0.001$ *vs* EV-MSCs or EV+MSCs at 20% O_2 by one-way ANOVA.



Supplemental Figure 8
Nucleotide sequences matching the consensus core hypoxia-response element (HRE) sequence from five known HIF target genes [5'-RCGTG$(N)_{1-8}$CACA-3'] are present in the 5'-flanking region (HRE-1) and 3'- flanking region (HRE-2) of the human *CXCR3* gene. HRE-1 in the 5'-flanking region of the *PGF* gene also matched the consensus HRE sequence. Core HIF binding site is highlighted.

Supplemental Figure 9



Co-culture and hypoxia-induced PGF expression in BCCs. (**A-B**) Conditioned medium (CM) was isolated from MDA-231 (**A**) or MDA-435 (**B**) BCCs that were cultured alone or with MSCs for 48 hours at 20% or 1% O_2. ELISA was performed to determine PGF protein levels in CM (mean ± SEM; $n = 3$). *$P<0.05$ *vs* BCCs at 20% O_2; $^{\#}P<0.01$ *vs* all other conditions by ANOVA. (**C**) CM was isolated from MDA-231 cells stably transfected with lentiviral vector encoding NTC shRNA or PGF shRNA (shPGF-1 or shPGF-2) that were cultured at 20% or 1% O_2. ELISA was performed to determine PGF protein levels in CM (mean ± SEM; $n = 3$). **$P<0.005$ *vs* NTC at 20% O_2; $^{\#\#}P<0.001$ *vs* NTC at 1% O_2 by one-way ANOVA. (**D**) CM was isolated from MDA-231 BCCs stably transfected with lentiviral vector encoding NTC shRNA or PGF shRNA (shPGF-1 or shPGF-2) that were cultured alone or with MSCs for 48 hours at 20% or 1% O_2. ELISA was performed to determine PGF protein levels in CM (mean ± SEM; $n = 3$). $^{*}P<0.05$ *vs* NTC+MSCs at 20% O_2; $^{\#}P<0.005$ *vs* NTC+MSCs at 1% O_2 by ANOVA. (**E**) MDA-231 BCCs stably transfected with lentiviral vector encoding NTC shRNA or PGF shRNA (shPGF-1 or shPGF-2) were cultured alone or with MSCs at 20% or 1% O_2 for 48 hours. CXCR3 mRNA levels were analyzed by RT-qPCR (mean ± SEM; $n = 3$). *$P<0.05$ *vs* NTC at 20% O_2 by one-way ANOVA.

Supplemental Table 1

Primers	Sequence
Hs-HK2-FWD	CCAGTTCATTCACATCATCAG
Hs-HK2-REV	CTTACACGAGGTCACATAGC
EGFP-FWD	CAAGGACGACGGCAACTACAAGAC
EGFP-REV	GTGGCTGTTGTAGTTGTACTCCAGC
CXCR3-FWD	AAGTACGGCCCTGGAAGACT
CXCR3-REV	GGCGTCATTTAGCACTTGGT
CXCL10-FWD	CCCACGTGTTGAGATCATTG
CXCL10-REV	CACTGGGTAAAGGGGAGTGA
CCR5-FWD	GGCAAAGACAGAAGCCTCAC
CCR5-REV	AACCTTCTGCAACACCAACC
CCL5-FWD	TGCAGAGGATCAAGACAGCA
CCL5-REV	GAGCACTTGCCACTGGTGTA
PGF-FWD	GCCTGGATGAGAAACAGCTC
PGF-REV	GAGAATCTGGCTTGGCAGTC
VEGFR1-FWD	AATCATTCCGAAGCAAGGTG
VEGFR1-REV	TTTCTTCCCACAGTCCCAAC
MMP9-FWD	GGGACGCAGACATCGTCATC
MMP9-REV	TCGTCATCGTCGAAATGGGC
LOX-FWD	GTTCCAAGCTGGCTACTC
LOX-REV	GGGTTGTCGTCAGAGTAC
HIF-1α-FWD	CGTTGTGAGTGGTATTATTCAGCA
HIF-1α-REV	CAGTTTCTGTGTCGTTGCTGCC
RPL13A-FWD	GAGGCGAGGGTGATAGAG
RPL13A-REV	ACACACAAGGGTCCAATTC
Hs-SRY-FWD	GCTGGGATACCAGTGGAAAA
Hs-SRY-REV	CCTTCCGACGAGGTCGATACT
Hs 18S-FWD	GAGGATGAGGTGGAACGTGT
Hs 18S-REV	AGAAGTGACGCAGCCCTCTA
CXCR3-HRE1 chip FWD	GAGGGAGCATTACTGCCTGA
CXCR3-HRE1 chip REV	AAACAATGCACAACCTAGATCC
CXCR3-HRE2 chip FWD	AAGGCTAATCCTAGCCATCTCC
CXCR3-HRE2 chip REV	TCAGAAAGATGGGACCCGTA
PGF-HRE1 chip FWD	GCGGGTCTCGAACTCCTAAT
PGF-HRE1 chip REV	TGGTAGCAATTGATCACGATT
PGF-HRE2 chip FWD	AGGGAGGGCACACACAAAC
PGF-HRE2 chip REV	TGTTCGTGTCCGCTGTGTAT