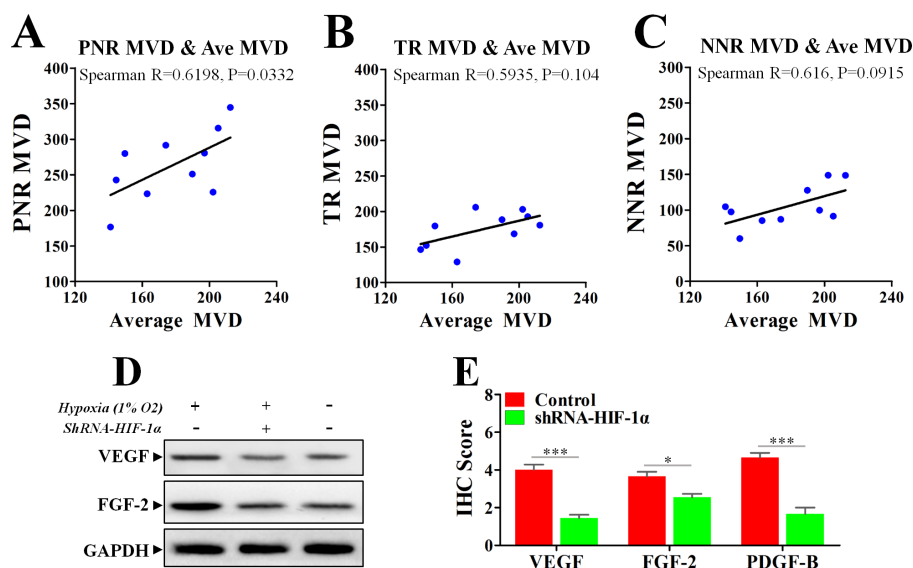


Suppression of hypoxia-induced excessive angiogenesis by metformin via elevating tumor blood perfusion

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: PNR but not NNR or TR MVD was linearly correlated with the Ave MVD, and knockdown of HIF-1 α restrained hypoxia-induced expression of angiogenesis-associated factors. Spearman analysis for evaluation of the linear correlation between Ave MVD and (A) PNR, (B) TR or (C) NNR MVD. Spearman R indicates the degree of correlation. Ave: average; MVD: microvessel density; NNR: non-necrotic region; PNR: peri-necrotic region; TR: transitional region. A two-sided $P < 0.05$ was considered statistically significant. (D) Immunoblotting for VEGF, FGF-2 and PDGF-B, showing reduced protein level of angiogenesis-associated factors in Lentivirus-shRNA-HIF-1 α 4T1 cancer cells cultured in hypoxic condition (1% O₂) *in vitro*. (E) Quantification of VEGF, FGF-2 and PDGF-B IHC score (addition of intensity score and positive signal area) in 4T1 tumors transfected with Lentivirus-shRNA-HIF-1 α or Lentivirus-shRNA-Con. $n = 9$. Quantitative data are indicated as mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Supplementary Table 1: Primers and antibodies

Primers		
Gene	Forward Primer	Reverse Primer
Act- β	GACATCCGTAAAGACCTCTATGCC	TCCACACAGAGTACTTGCGCTCA
Ang-1	CAAATGCGCTCTCATGCTA	GTAGTGCCACTTTATCCCAT
Ang-2	CGCTGTATGATCACTTCTACCTC	TCCTGAGAGCATCTGGGAAC
FGF-2	GGCTGCTGGCTTCTAAGTGT	TCTGTCCAGGTCCCGTTTTG
HIF-1 α	AGTGGTATTATTTCAGCACGAC	AAGGCAGCTTGTATCCTCT
PDGF-B	TCTCTGCTGCTACCTGCGTCT	CAGCCCCATCTTCATCTACGG
PIGF	GTCTCACATATTCAGTCCGTCC	TTGGCTGTCTTTATCGGCAC
VEGF	GTGCAGGCTGCTGTAACGAT	GGGATTTCTTGCGCTTTCGT

Antibodies for IHC		
Antibody	Company, Species	Concentration
Anti-CD31	Abcam, Rabbit	1 μ g/ml
Anti-HIF-1 α	Abcam, Rabbit	1.5 μ g/ml
Anti-PIGF	ProteinTech, Rabbit	1.5 μ g/ml
Anti-PDGF-B	Boster, Rabbit	1.5 μ g/ml
Anti-Ang-1	Boster, Rabbit	1.5 μ g/ml
Anti-Ang-2	Boster, Rabbit	1.5 μ g/ml
Anti-VEGF	ProteinTech, Rabbit	1.5 μ g/ml
Anti-FGF-2	ProteinTech, Rabbit	1.5 μ g/ml