SUPPLEMENTARY INFORMATION OF MATERIALS AND METHODS

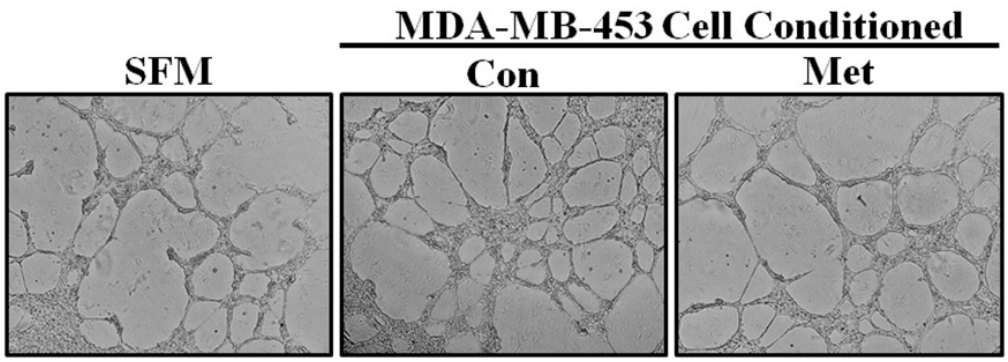
RNA Interference

To perform the RNA interference experiment, we purchased the siRNA for HIF-1α from GenePharm (Shanghai, China). 3x105 MDA-MB-453 cells were seeded in a six-well plate and transfected with 100 nM siRNA by using lipofectamine RNAi MAX reagent (Invitrogen, USA) according to the manufacturer's instructions. The related sequences were listed as following:

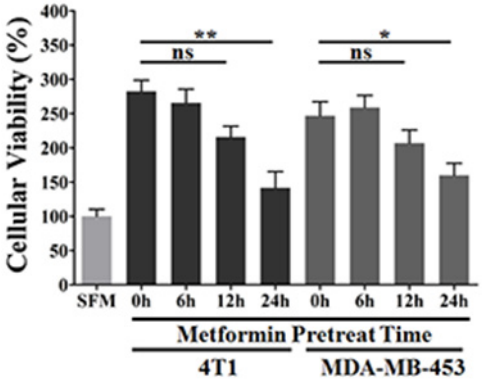
SiRNA for HIF-1α: 5′-CCACCACUGAUGAAUU AAATT-3′, 5′-UUUAAUUCAUCAGUGGUGGTT-3′

Negative Control siRNA: 5′-UUCUCCGAACGUG UCACGUTT-3′, 5′-ACGUGACACGUUCGGAGAA TT-3′)

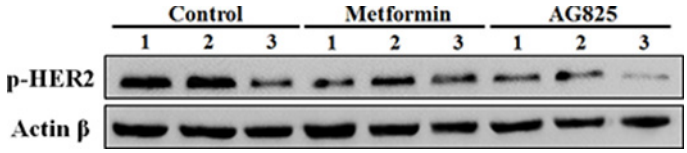
SUPPLEMENTARY FIGURES



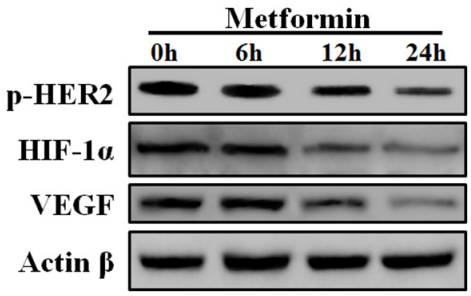
Supplementary Figure S1: Representative images of HMEC-1-mediated tube formation. HMEC-1 was cultured in the presence of SFM or 75% TCM of MDA-MB-453 cells either, with or without the pretreatment of metformin. 200X.



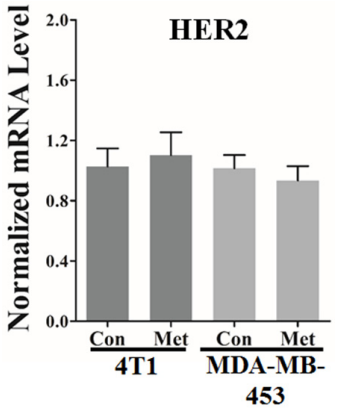
Supplementary Figure S2: Representative images of HUVEC proliferation promoted by TCM of MDA-MB-453 cells that were pretreated with metformin over multiple time periods (*n* = 6).



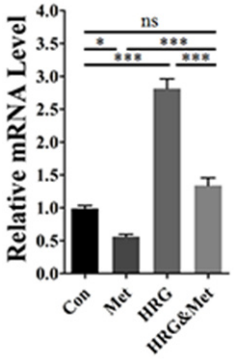
Supplementary Figure S3: Immunoblotting for p-HER2 (Tyr 1221/1222) and actin-β in 4T1 tumors from mice treated with metformin (200 mg/kg day), AG825 (10 mg/kg day), or feeding with water (control). Equal amounts of total proteins (80 μg/lane) were loaded.



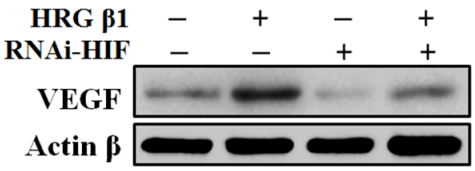
Supplementary Figure S4: Immunoblotting for detection of protein levels of phospho-HER2, HIF-1α, and VEGF in MDA-MB-453 cells treated with metformin over multiple time periods.



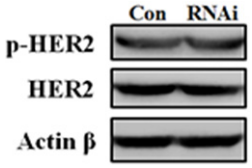
Supplementary Figure S5: Representative images of the mRNA levels of HER2 in 4T1 and MDA-MB-453 cancer cells treated with metformin or not (*n* = 5, *n* = 5, respectively).



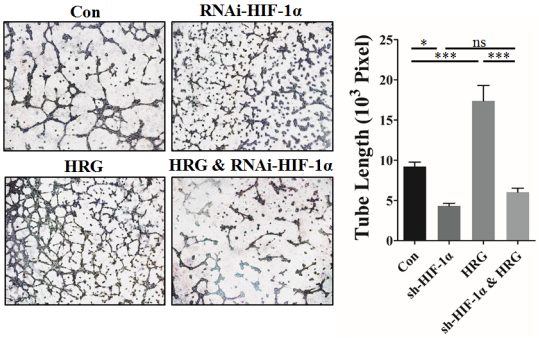
Supplementary Figure S6: Representative images showing the mRNA level of VEGF in MDA-MB-453 cells (*n* = 5).



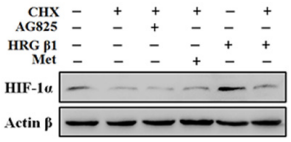
Supplementary Figure S7: Immunoblotting for detecting protein levels of $VEGF_{165}$ in MDA-MB-453 cells.



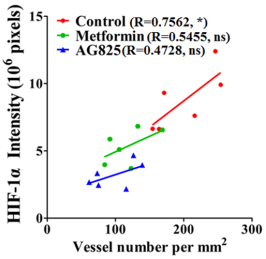
Supplementary Figure S8: Immunoblotting for total level and phosphorylation level of HER2 protein in MDA-MB-453 cells.



Supplementary Figure S9: Representative images showing HUVEC-mediated tube formation. HMEC-1 was cultured in the presence of SFM or 75% TCM from MDA-MB-453 cells transduced or not transduced with RNAi targeting HIF-1α in the absence or presence of Heregulin (HRG)-β1 pretreatment. 200X (Left). Quantification of the tube length produced by HUVECs (Right).



Supplementary Figure S10: Immunoblotting for the detection of the protein level of HIF-1α in MDA-MB-453 cells.



Supplementary Figure S11: Representative images showing the linear correlation between HIF-1α fluorescent intensity and microvessel density in 4T1 tumor. Sperman analysis was used to determine the significance of the linear correlation. *$P < 0.05$.