

Supplementary Figures:

A Novel Inhibitor of Carbonic Anhydrases Prevents Hypoxia-Induced TNBC Cell Plasticity

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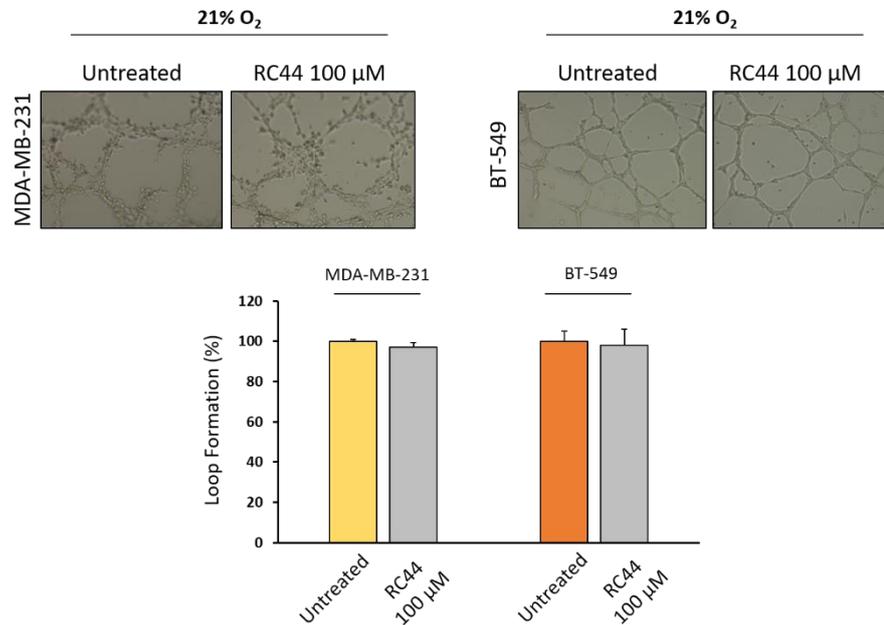


Figure S1. Effect of RC44 on TNBC vasculogenic mimicry in Normoxia. MDA-MB-231 and BT-549 were grown in normoxia (21% O₂) conditions and vascular loops were analyzed. TNBC cells were treated with RC44 (100 μM) and seeded into 24-well plates pre-coated with 80 μl/well Matrigel. Representative images were taken at 10× and average of the number of complete loops was calculated from 3–5 random fields by a macro made with ImageJ software. Bars depict mean ±SD of three independent experiments.

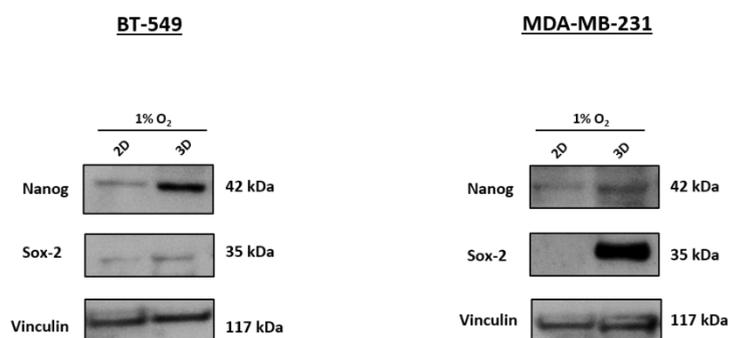


Figure S2. TNBC cells were grown in 2D and 3D under hypoxia (1% O₂). Nanog and Sox-2 protein levels were analyzed by western blot analysis. Vinculin was used as loading control.

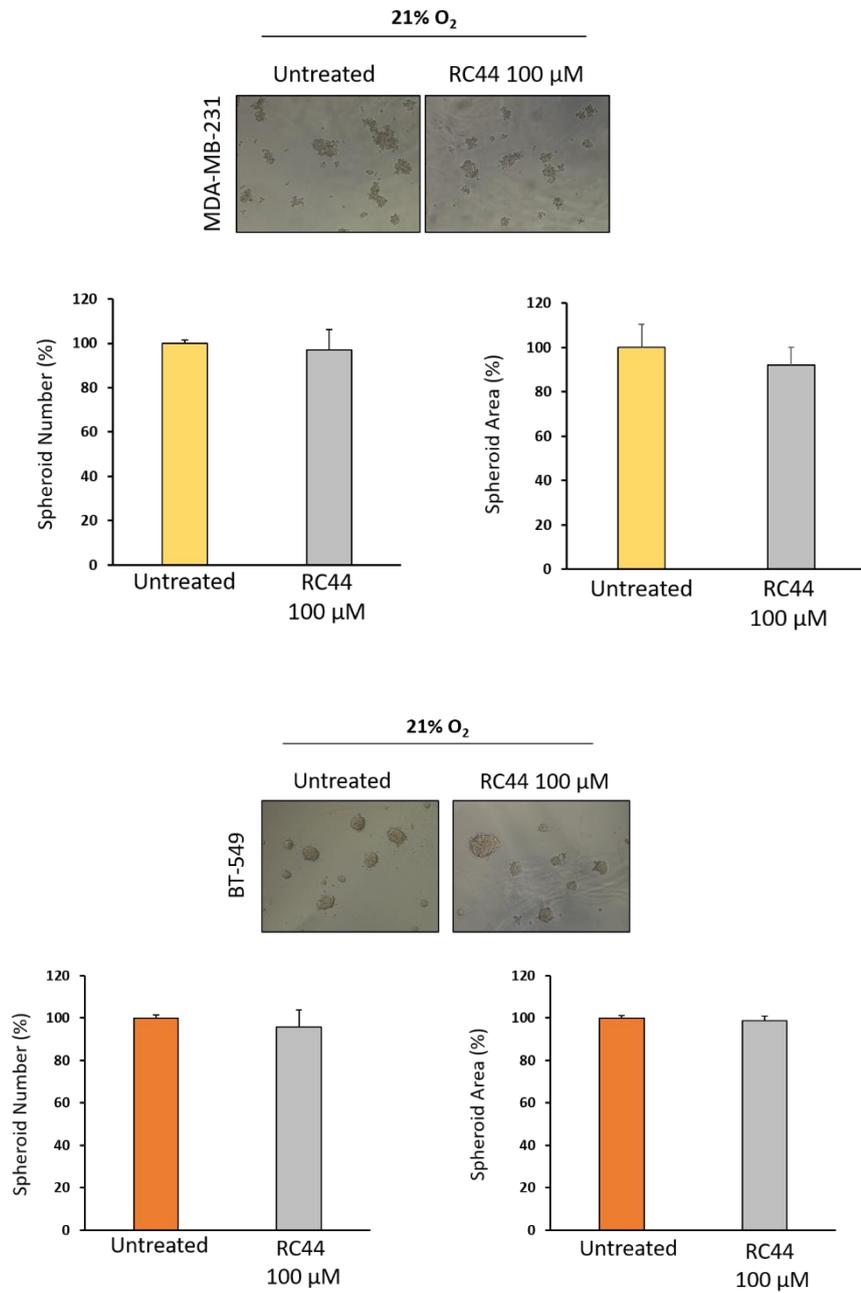


Figure S3. Effect of RC44 on TNBC spheroid formation in Normoxia (21% O₂). BT-549 and MDA-MB-231 cells were seeded in Ultra-Low attachment multiwell-plates and grown in serum free DMEM supplemented with B27, bFGF (20 ng/mL) and EGF (10 ng/mL) in normoxic conditions for 7 days. TNBC cells were treated with RC44 (100 μM) and grown as described above. Spheroid formation was analyzed under a phase-contrast microscopy and size and number of formed spheroids was calculated using ImageJ. Bars depict mean ±SD of three independent experiments.