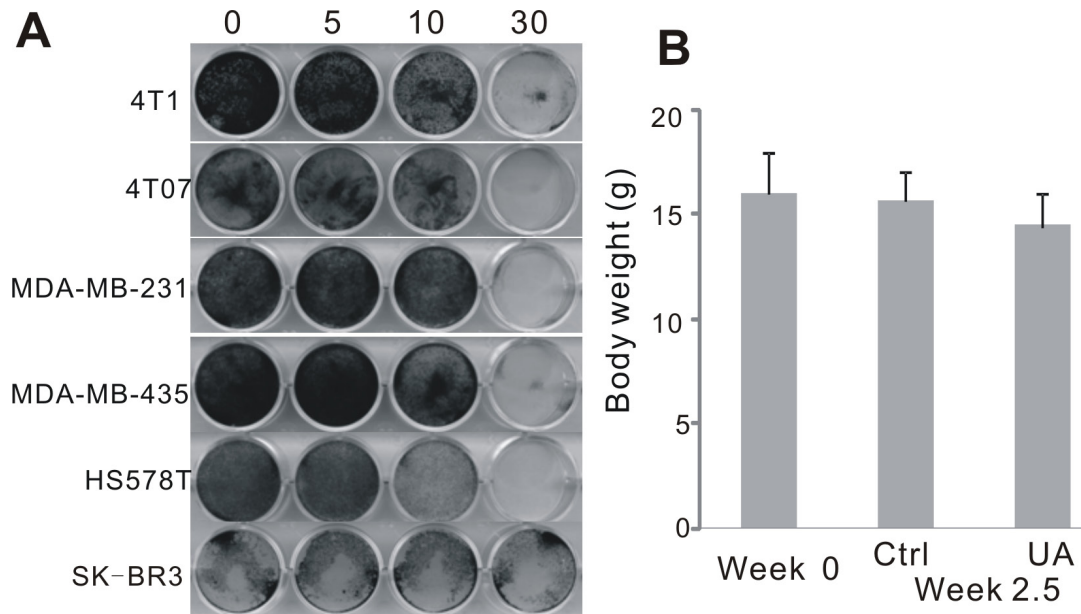
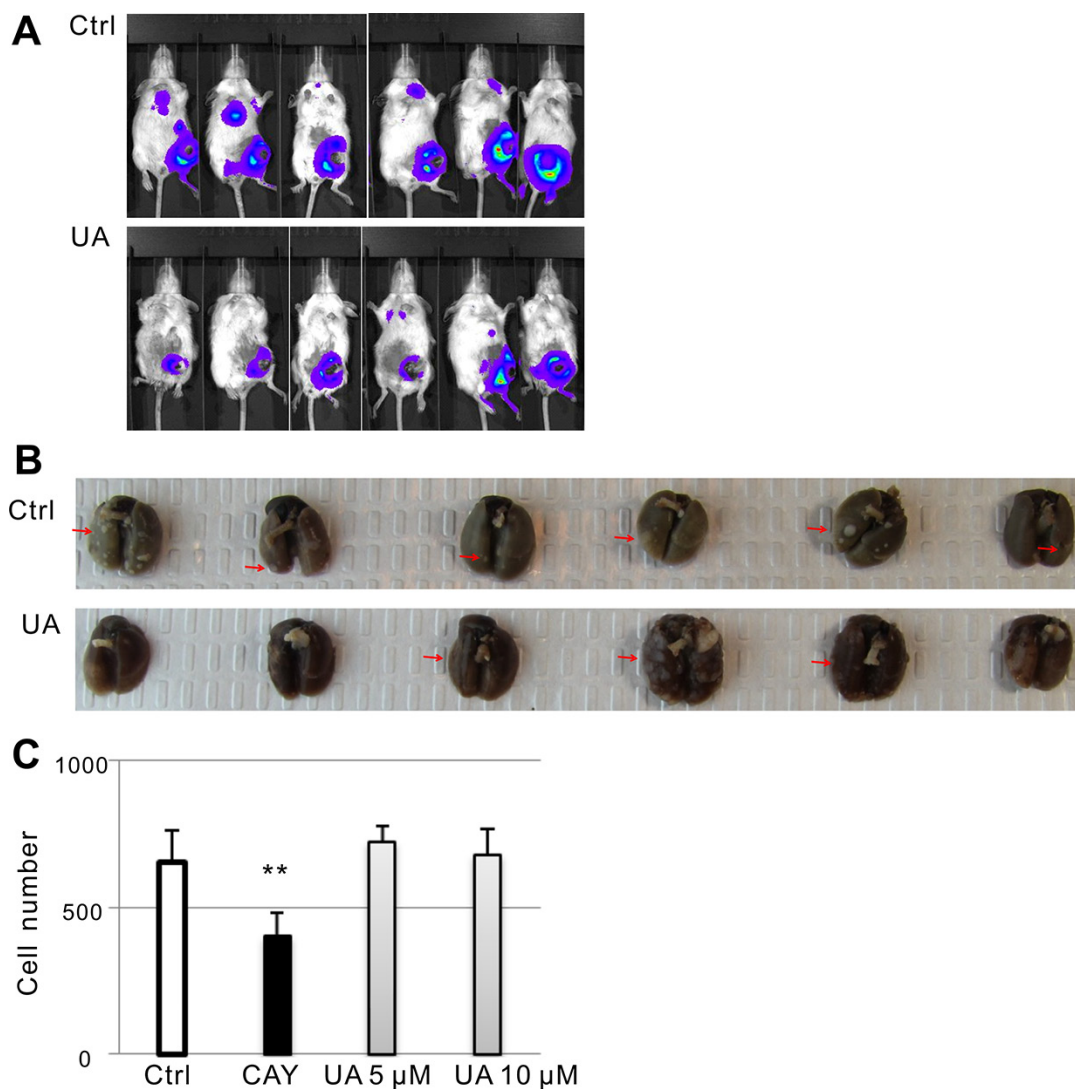


Hypoxia pathway and hypoxia-mediated extensive extramedullary hematopoiesis are involved in ursolic acid's anti-metastatic effect in 4T1 tumor bearing mice

Supplementary Materials



Supplementary Figure S1: (A) Effect of UA on the proliferation of breast cancer cells. Different kinds of breast cancer cells were treated with designed concentration for 48 hours and stained with crystal violet. (B) The effect of UA on mice body weight after 18 days treatment.



Supplementary Figure S2: Effect of UA on tumor growth and metastasis *in vivo*. (A) The Xenogen images of control group and UA treated group mice after 4 weeks UA treatment. UA suppressed the lung metastasis (top panel, control; bottom panel, UA group). Briefly, luciferase-tagged 4T1 cells were collected and injected into the mammary fat pad of BALB/c mice (0.5×10^5 cells/injection; six animals per assay condition). Animals were treated with 20 mg/kg UA and subjected to Xenogen imaging weekly. (B) The effect of UA on lung metastasis (control, top panel; UA group, bottom panel). Red arrow indicated the lung with metastasis; one representative animal from each group are shown. (C) UA didn't affect the cell invasion in MDA-MB-231 cells *in vitro*.

Supplementary Table S1: Primer sequences of target genes and housekeeping gene for RT-PCR

Gene	Forward Primer	Reverse Primer
human HIF1alpha	TGCATCTCCATCTCCTACCC	CGTTAGGGCTTCTTGATGA
human VEGF a	CCCACTGAGGAGTCCAACAT	TTTCTTGCGCTTTCGTTTTT
human VHL	GGTGGCATTGCTTCCTA	CAGTCTTCCCAAAGCAGGAG
human p53	GGCCCACTTCACCGTACTAA	GTGGTTTCAAGGCCAGATGT
human Cyclin D1	TGTTTGCAAGCAGGACTTTG	TGGCACCAAAGGATTCCTAA
human E2F1	ATGTTTTCTGTGCCCTGAG	ATCTGTGGTGAGGGATGAGG
human Jun	CAGGTGGCACAGCTTAAACA	TTTTTCTCTCCGTCGCAACT
human MDM2	CCGAATAAGGTTGCCTGAA	CAAATTGCAAAAGGCACTGA
human AKT1	AGAAGCAGGAGGAGGAGGAG	TCTCCTTCACCAGGATCACC
human ERK1	CCAGACCATGATCACACAGG	CTGGAAAGATGGGCCTGTTA
human GAPDH	CAGCGACACCCACTCCTC	TGAGGTCCACCACCCTGT

HIF1alpha, hypoxia-inducible factor 1 alpha; VEGF a, vascular endothelial growth factor a; VHL, von Hippel-Lindau; p53, protein 53; MDM2, murine double minute 2; AKT1, thymoma viral proto-oncogene 1; ERK1, extracellular regulated protein kinases 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.