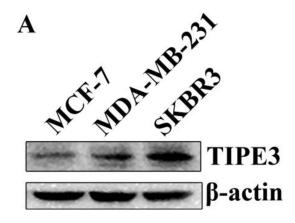
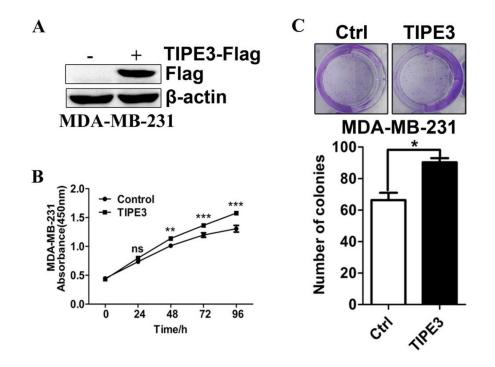
TIPE3 protein promotes breast cancer metastasis through activating AKT and NF-κB signaling pathways

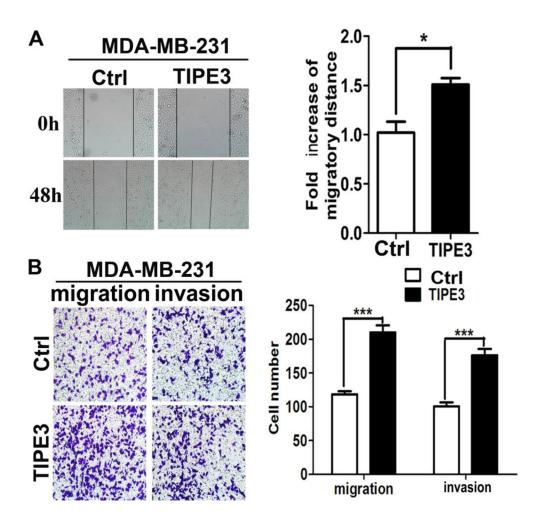
Supplementary Materials



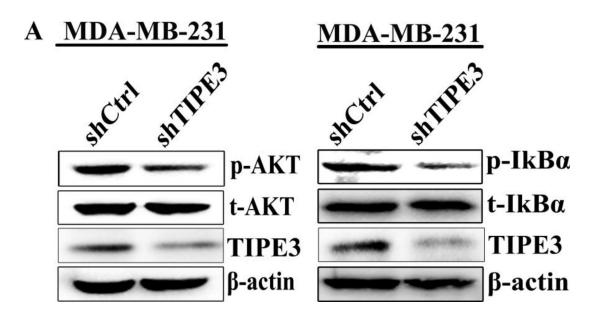
Supplementary Figure 1: TIPE3 expression in cell lines. The level of TIPE3 protein in HER2-positive breast cancer cells (SKBR3) was much higher than that in HER2-negative breast cancer cells (MCF-7 and MDA-MB-231) by Western blot. All of experiments were repeated at least three times with similar results and representative data are shown.



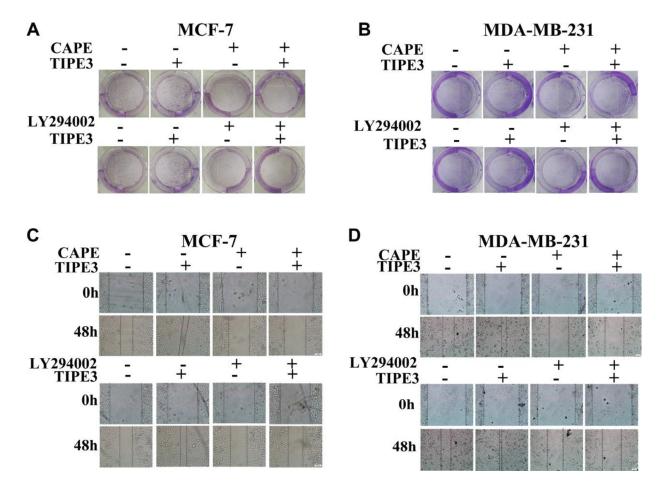
Supplementary Figure 2: Overexpression of TIPE3 promoted proliferation and colony formation of MDA-MB-231 cells. Overexpressed TIPE3 in MDA-MB-231 cells were examined using western blot (A), and promoted cells proliferation (B) and colony formation (C). All of the experiments were repeated at least three times with similar results and representative data are shown. (*P < 0.05, **P < 0.01 and ***P < 0.001).



Supplementary Figure 3: TIPE3 promoted migration and invasion of MDA-MB-231 cells. MDA-MB-231-shTIPE3 cells or Ctrl cells were subjected to wound healing assay (A), transwell migration (B) and matrigel invasion assays (B). All of the experiments were repeated at least three times with similar results and representative data are shown. (*P < 0.05, **P < 0.01 and ***P < 0.001).



Supplementary Figure 4: Knocking down TIPE3 in MDA-MB-231 cells prevented the activation of p-AKT and p-IκBα. The levels of p-AKT and p-IκBα were decreased in MDA-MB-231-shTIPE3 cells.



Supplementary Figure 5: The enhanced colony formation and migration ability induced by overexpressed TIPE3 in breast cancer cells were abolished through blocking the NF-kB and AKT signaling with CAPE or LY294002. The enhanced clonogenicity induced by overexpressed TIPE3 in MCF-7 cells (A) or MDA-MB-231 cells (B) were blocked using CAPE or LY294002. The enhanced migration ability induced by overexpressed TIPE3 in MCF-7 cells (C) or MDA-MB-231 cells (D) were blocked using CAPE or LY294002. All of the experiments were repeated at least three times with similar results and representative data are shown.

Clinical pathological	Number of		Expression scores of TIPE3								
features	cases	0	2	3	4	5	6	7	8		
Adjacent tissues*	25	10	5	6	3	1	0	0	0	< 0.0001*	
DCIS*	34	1	2	7	9	6	4	5	0	0.0033	
Invasive cancer	86	1	4	8	14	13	15	17	14		

Supplementary Table 1: Expression scores of TIPE3 protein in breast cancers

Note: P-value*: unpaired *t*-test.

 $P < 0.0001^*$, TIPE3 protein in Adjacent tissues vs TIPE3 in Cancer tissues.

P = 0.0033, TIPE3 protein in DCIS tissues vs TIPE3 in invasive tissues.

Supplementary Table 2: Comparison of TIPE3 expression scores between invasive breast cancer and lymph node metastatic tissues from same patients

Clinical pathological	l pathological Number of Expression scores of TIPE3								<i>P</i> -value*	
features	cases	0	2	3	4	5	6	7	8	
Invasive cancer*	36	0	2	4	6	5	5	7	7	0.0276*
Lymphatic met cancer*	36	0	1	1	4	3	4	10	13	

Note: P-value*: unpaired *t*-test.

Invasive cancer*: invasive cancer with lymphnode metastasis.

 $P = 0.0276^*$, TIPE3 protein in lymphatic metastasis carcinoma vs in invasive ductal carcinoma.