Supplementary Data

EpCAM activates the ERK-EGR1 signaling axis and promotes TNF-α-induced the progression of anaplastic thyroid cancer

By Lee et al.

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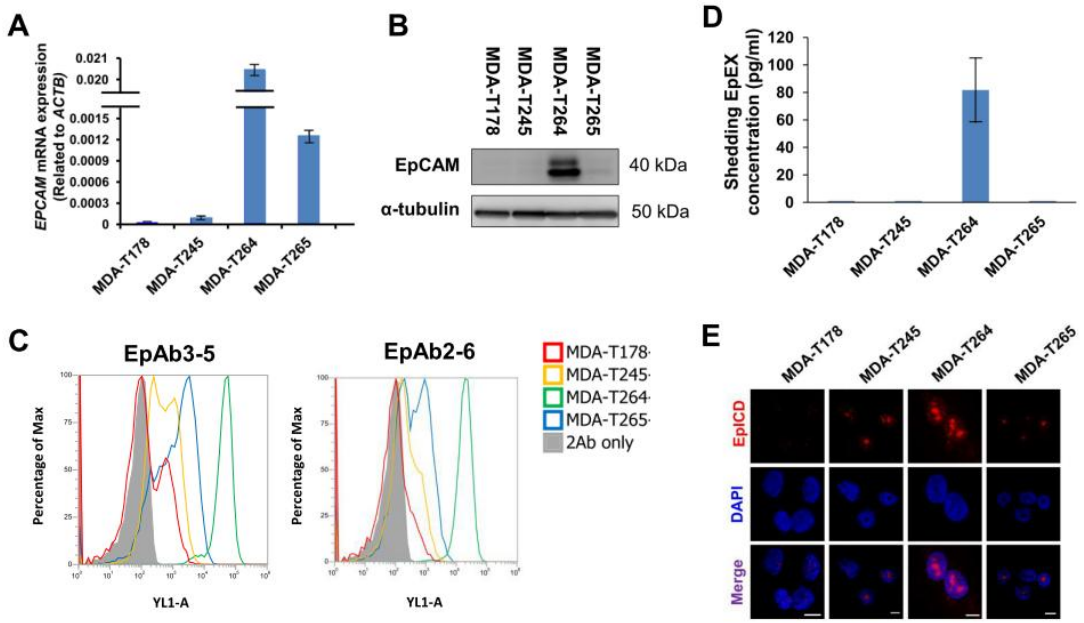
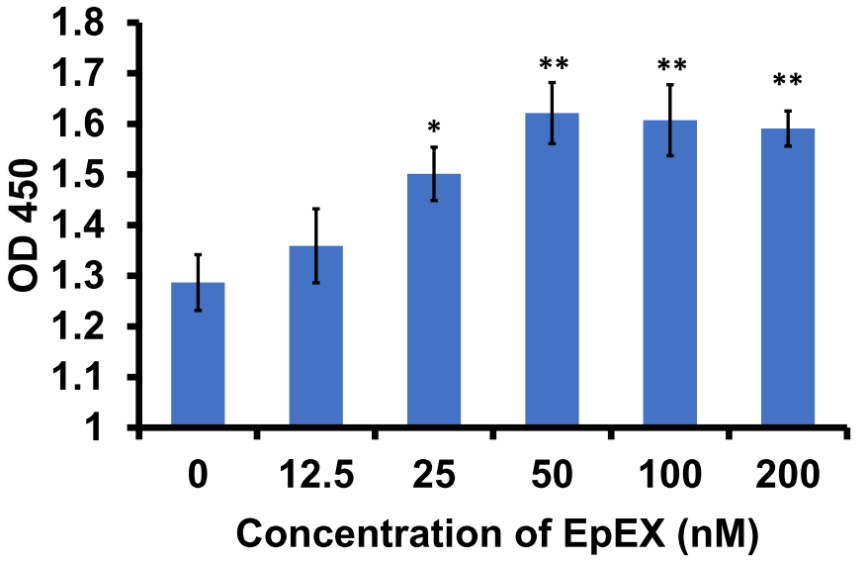
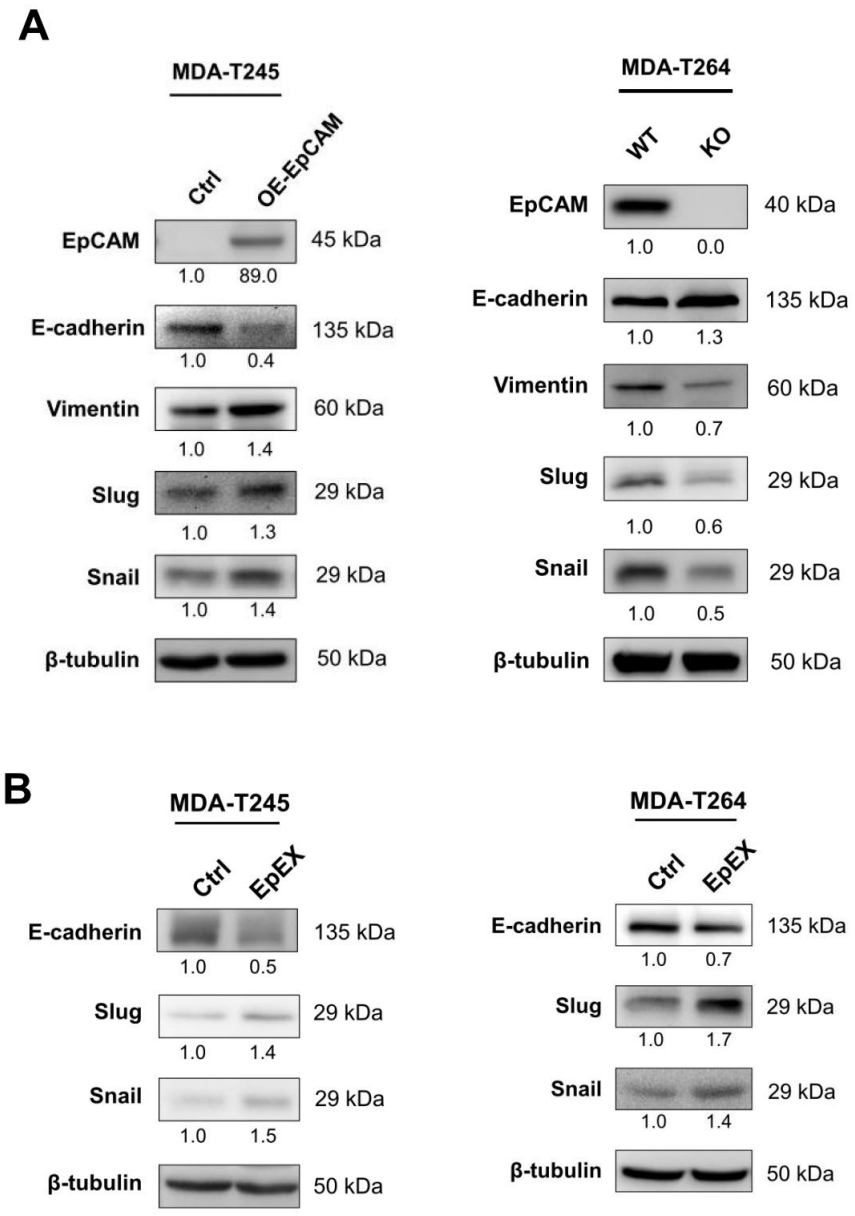


Supplementary Figure 1. Expression of EpCAM in various ATC cell lines. (A) Gene and (B) protein expression were assayed for the indicated ATC cell lines by qRT-PCR and Western blot, respectively. (C) Flow cytometry analysis showing EpCAM expression on the cell surface of different ATC cell lines. (D) Detection of EpEX shed from ATC cell lines in conditioned medium, as assayed by capture ELISA. (E) Nuclear translocation of EpICD in ATC cell lines was assayed by immunofluorescence staining.

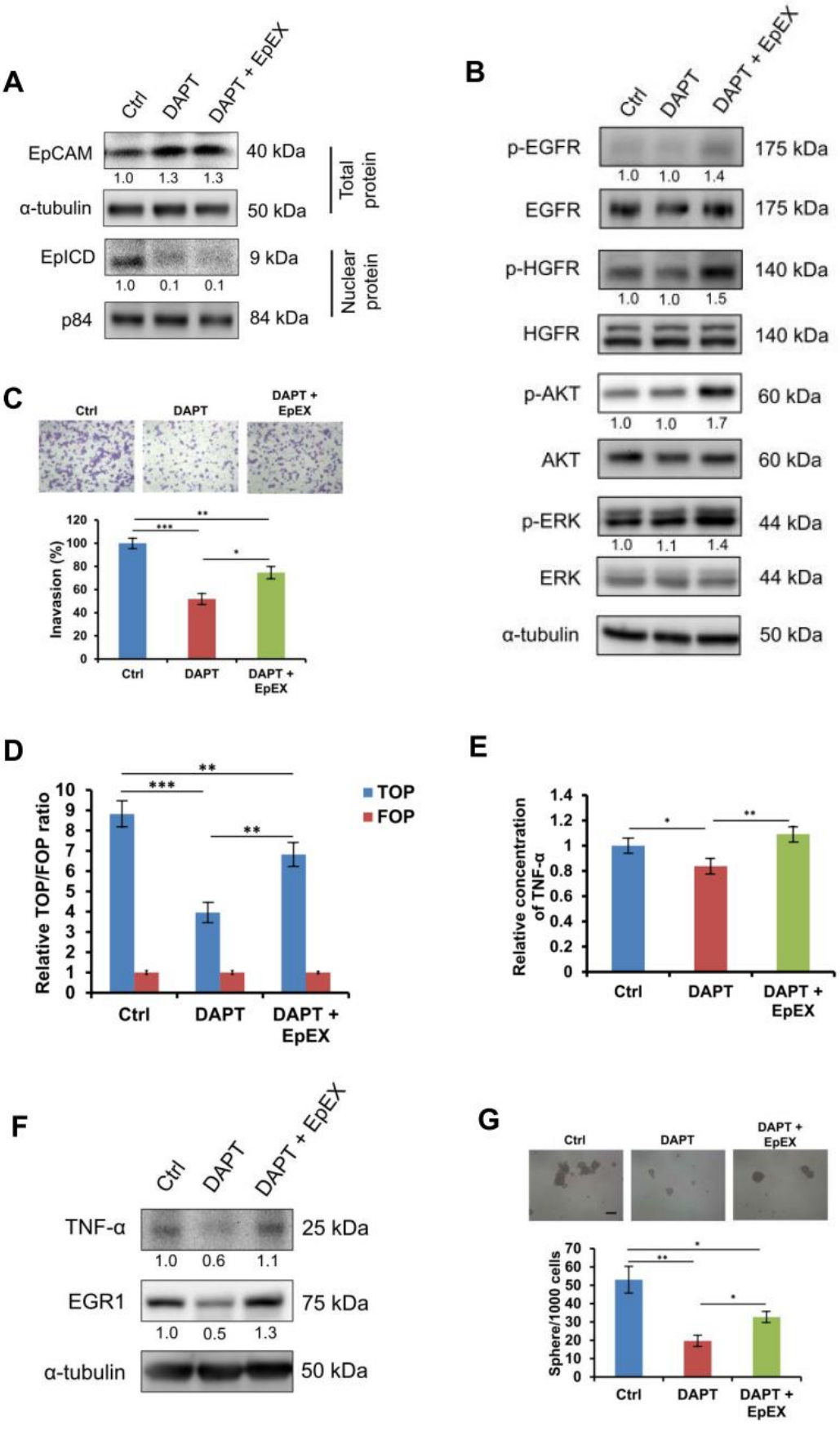


Supplementary Figure 2. EpEX induces cell viability on doses dependent. MDA-T264 cells were treated with indicated doses of EpEX for 48 h, the cell viability was assayed using WST-1.



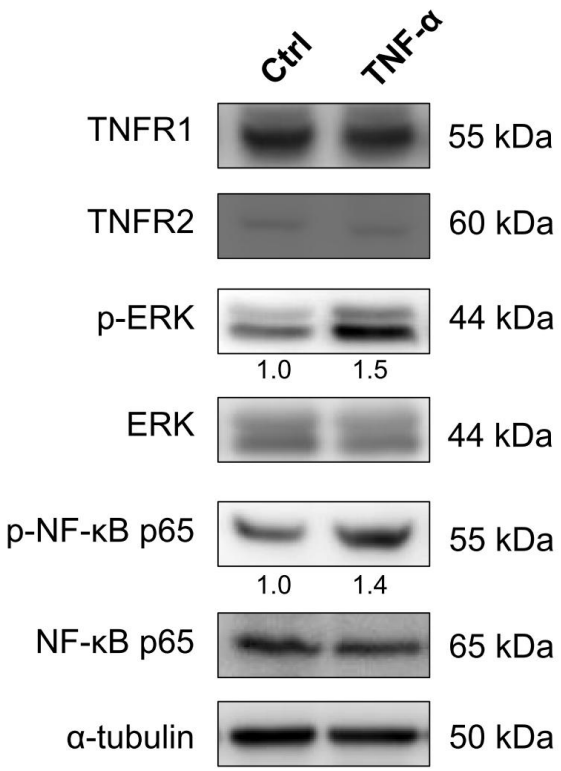
Supplementary Figure 3. EpCAM and EpEX induces signs of EMT in ATC cells. (A) The protein expression levels of EMT markers E-cadherin, Slug, and Snail were assayed by Western blotting in EpCAM-overexpressing MDA-T245 cells and EpCAM-knockout T264 cells. (B) ATC cell lines MDA-T245 and MDA-T264 were treated with 50 nM EpEX for 24 h before the levels of EMT markers, E-cadherin,

Slug, and Snail, were assayed by Western blotting. Statistically significant differences were identified by two-tailed Student's t-test. N = 3 independent experiments. All data are presented as mean ± SD. *$p < 0.05$; **$p < 0.01$.

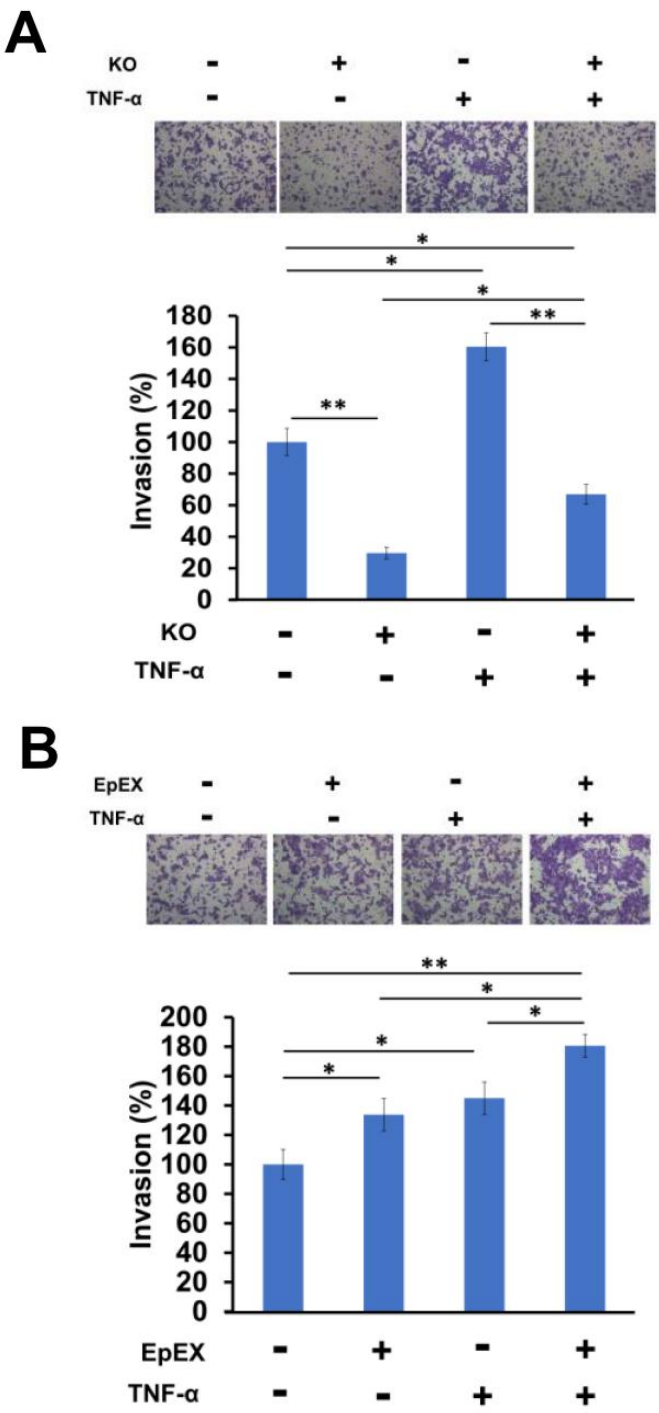


Supplementary Figure 4. EpEX induces signaling activation and cellular changes

independent of EpICD nuclear translocation. (A) MDA-T264 cells were pretreated with 50 μM DAPT, and treated with 50 nM EpEX for 6 h. The levels of cleaved EpICD in nuclear fraction was determined by Western blot analysis. (B) MDA-T264 cells were pretreated with 50 μM DAPT, and treated with EpEX for 30 min. The EGFR and HGFR signaling events were examined by Western blotting. (C) MDA-T264 cells were pretreated with 50 μM DAPT and 50 nM EpEX for 24 h. The invasion activity was assayed by transwell migration assays with matrigel. (D) MDA-T264 cells transfected with TOP-flash and FOP-flash vectors. The ratio of signals from these two reporters (each normalized to Renilla luciferase as an internal control) reflects β-catenin-specific canonical Wnt signaling. At 72 h, the TOP/FOP ratio was also measured in MDA-T264 cells pretreated with 50 μM DAPT, and treated with EpEX for 8 h. (E) MDA-T264 cells were pretreated with 50 μM DAPT, and treated with EpEX for 24 h. The shedding TNF-α in conditional medium was assay by capture ELISA. (F) MDA-T264 cells were pretreated with 50 μM DAPT, and treated with EpEX for 8 h. The TNF-α and EGR1 protein expressions were examined by Western blotting. (G) Tumor sphere formation and cell counts of MDA-T264 cells were treated with 50 μM DAPT and EpEX for 7 days (Scale bar: 50 μM).

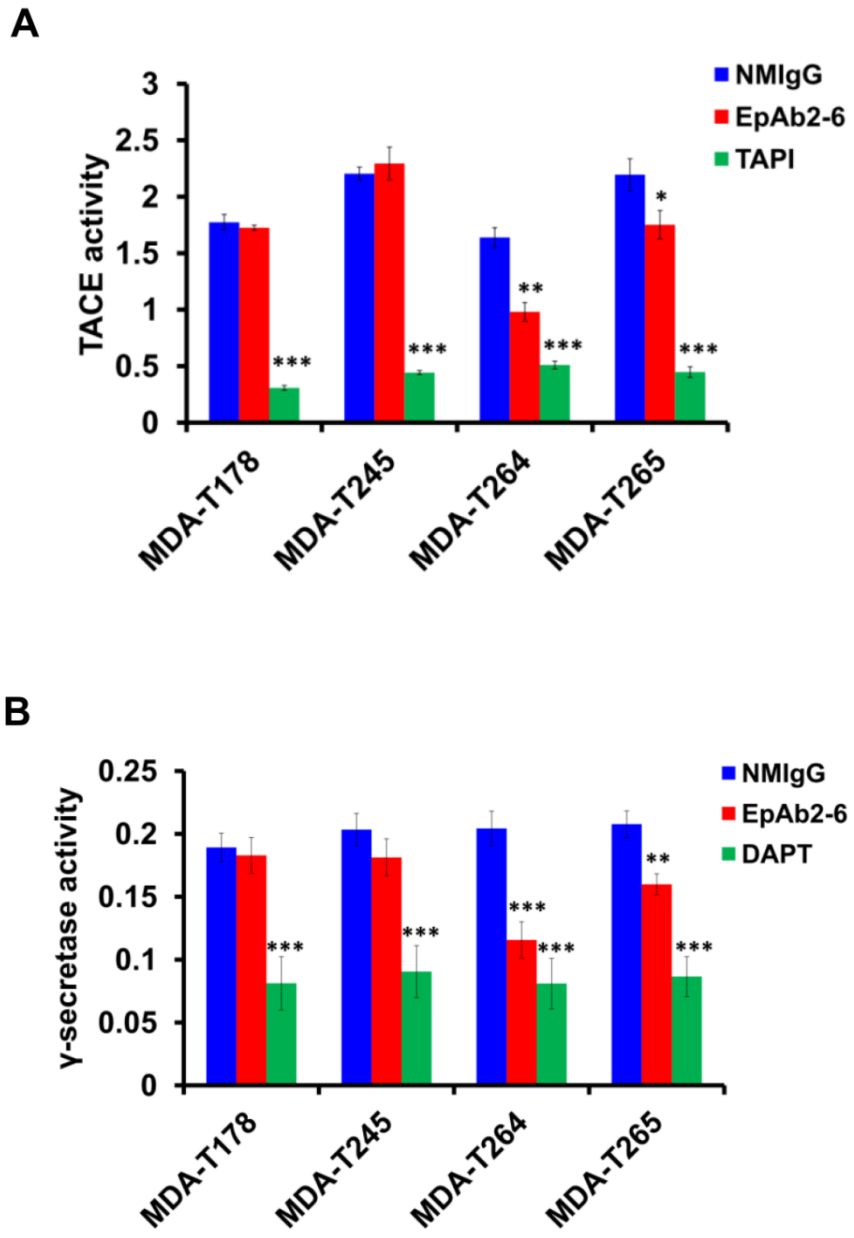


Supplementary Figure 5. TNF-α strongly induced ERK and NF-κB p65 phosphorylation. MDA-T264 cells treated with 20 ng/ml TNF-α for 30 min, The protein expression of TNFR1, TNFR2, phosphorated ERK, and NF-κB p65 was assayed by Western blotting.



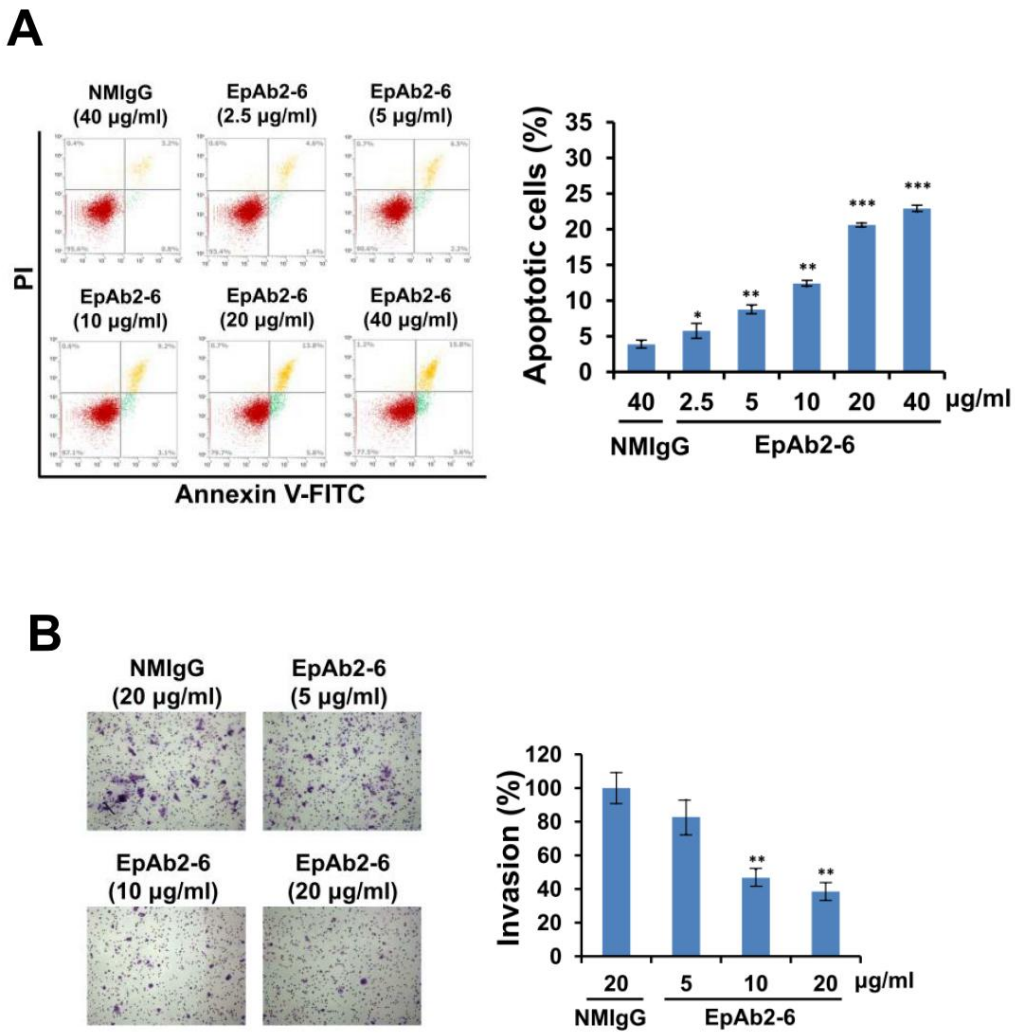
Supplementary Figure 6. EpCAM signaling and TNF activation may cooperatively regulate cancer cell invasion. (A) WT or KO MDA-T264 cells were treated with TNF-α (1 nM) for 24 h. Invasion of MDA-T264 cells was examined using a Transwell

chamber assay with matrigel. (B) MDA-T264 cells were treated with EpEX (50 nM) or/and TNF-α (1 nM) for 24 h. Cell invasion was assessed by a Transwell assay with matrigel. Cell invasion was examined using a Transwell chamber assay with matrigel.

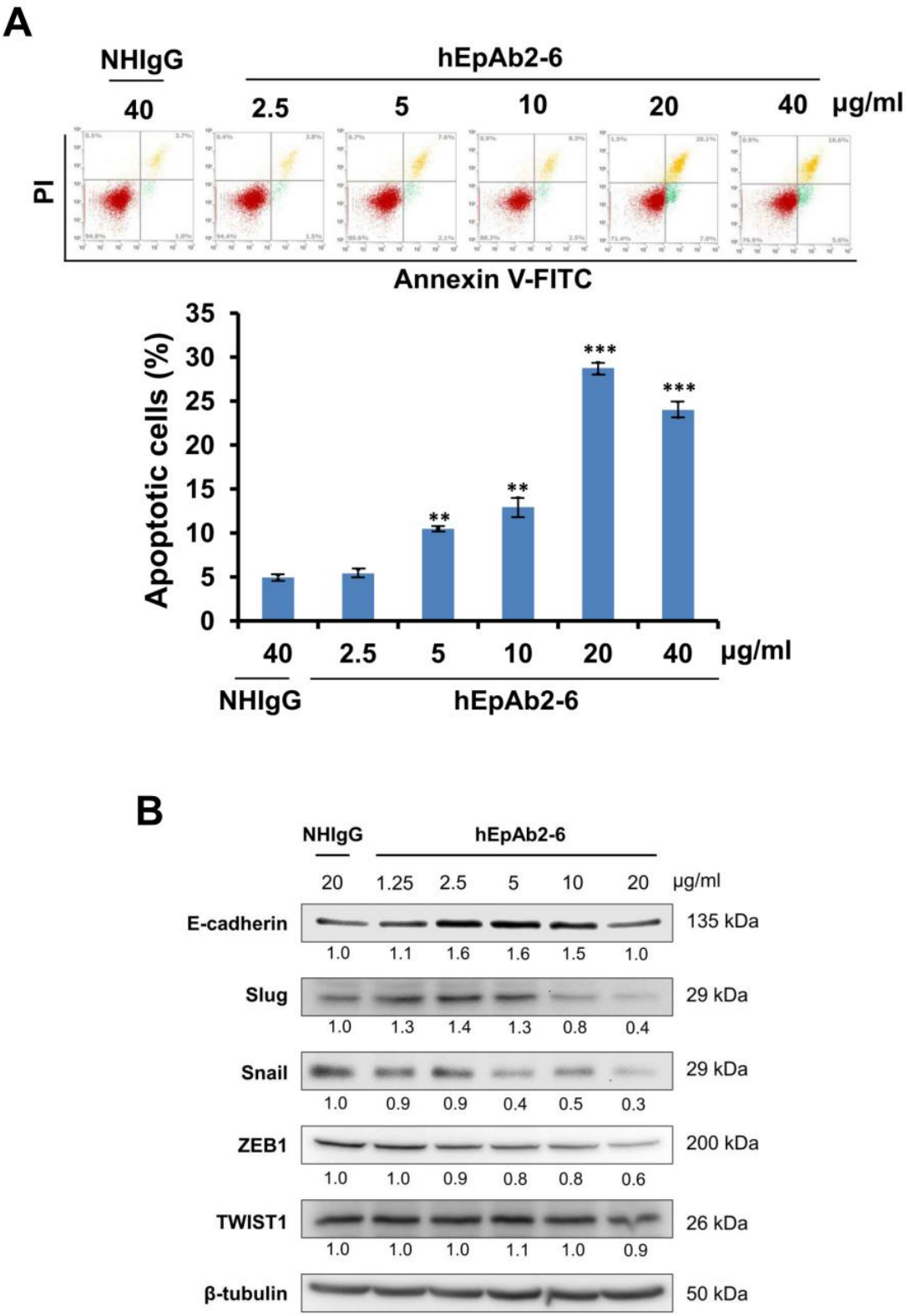


Supplementary figure 7. Effect of EpAb2-6 on TACE activity in ATC cell lines. (A) TACE activity in indicated ATC cell lines after treatment with control IgG (NMIgG), EpAb2-6 (20 μg/ml) or TAPI (TACE inhibitor, 10 μM) for 24 h. (B) γ-secretase activity was measured in indicated ATC cell lines after treatment with either NMIgG, EpAb2-6 (20 μg/ml) or DAPT (γ-secretase inhibitor, 10 μM) for 24 h. Statistically significant differences were identified by one-way ANOVA. N = 3 independent

experiments. All data are presented as mean ± SD. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

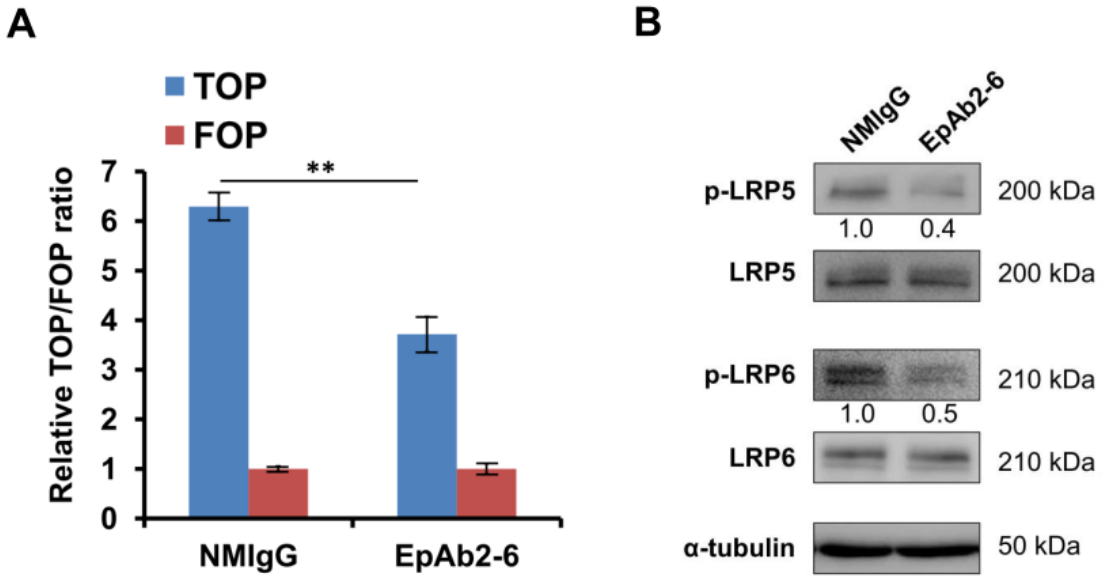


Supplementary figure 8. EpAb2-6 induces apoptosis and inhibits invasion activity in ATC cells. MDA-T264 cells were treated with indicated concentrations of EpAb2-6 for 24 h. (A) Proportions of apoptotic cells were quantified by fluorescein annexin V-FITC/PI double labeling. (B) Cell invasion activity was assayed by a transwell invasion assay with matrigel. Statistically significant differences were identified by one-way ANOVA. N = 3 independent experiments. All data are presented as mean ± SD. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.



Supplementary figure 9. Effect of hEpAb2-6 on apoptosis and EMT in MDA-T264 cells. (A) Cells were treated with different concentrations of hEpAb2-6 for 24 h, and apoptotic cells were identified by fluorescein annexin V-FITC/PI double labeling. (B)

Cells were treated with 20 μg/ml NHIgG or different concentrations of hEpAb2-6 in 2% FBS medium for 8 h. The levels of EMT-associated factors were assayed by Western blotting. Statistically significant differences were identified by one-way ANOVA. N = 3 independent experiments. All data are presented as mean ± SD. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.



Supplementary figure 10. Effect of EpAb2-6 on TOP/FOP ratio and phosphorylation of LRP5 and LRP6 in MDA-T264 cells. (A) The relative luciferase activity in cells transfected with TOP-flash and FOP-flash vectors in MDA-T264 cells after EpAb2-6 (20 μg/ml) treatment for 8 h. The ratio of signals from these two reporters, each normalized to Renilla luciferase as internal control, reflects β-catenin- specific canonical Wnt signaling. After 72 hours, TOP/FOP ratio was measured. (B) MDA-T264 cells were treated with EpAb2-6 (20 μg/ml) for 4 h. The phosphorylation of LRP5 and LRP6 was assayed by Western blotting. Statistically significant differences were identified by two-tailed Student's t-test. N = 3 independent experiments. All data are presented as mean ± SD. $^{**}p < 0.01$.

Supplementary Table 1: List of oligonucleotides for real-time PCR assay

Gene	Sequence
EPCAM	Forward: 5'- CTCCACGTGCTGGTGTGT R -3' Reverse: 5'- TGTTTTAGTTCAATGATGATCCAGTA -3'
SOX2	Forward: 5'-TATTTGAATCAGTCTGCCGAG-3' Reverse:5'-ATGTACCTGTTATAAGGATGATATTAGT-3'
OCT-4	Forward: 5'- AGCAAAACCCGGAGGAGT-3' Reverse: 5'-CCACATCGGCCTGTGTATATC-3'
NANOG	Forward: 5'-ATGCCTCACACGGAGACTGT-3' Reverse: 5'-AGGGCTGTCCTGAATAAGCA-3'
CSF2	Forward: 5'- ACCTGCCTACAGACCCGCCT -3' Reverse: 5'- GAAGTTTCCGGGGTTGGAGGGC -3'
CCL20	Forward: 5'- ACTGAGGAGACGCACAATATAT-3' Reverse: 5'- TGTACCAAGAGTTTGCTCCTGG-3'
CXCL3	Forward: 5'- CGCCCAAACCGAAGTCATAG-3' Reverse: 5'- GCTCCCCTTGTTCAGTATCTTTT-3'
CXCL5	Forward: 5'- AGCTGCGTTGCGTTTGTTTAC-3' Reverse: 5'- TGGCGAACACTTGCAGATTAC-3'
TNF	Forward: 5'- CCCTGGTATGAGCCCATCTATC-3'

	Reverse: 5'- AAAGTAGACCTGCCCAGACTCG-3'
EGR1	Forward: 5'- TGAAACAGCAGTCCCAGTATTC-3' Reverse: 5'- GAGAGTACGGTCAAGCAGTATTT-3'
ACTB	Forward: 5'- TTGTTACAGGAAGTCCCTTGCC -3' Reverse: 5'- ATGCTATCACCTCCCCTGTGTG-3'