# Targeting chemotherapy-induced PTX3 in tumor stroma to prevent the progression of drug-resistant cancers

**Supplementary Material**

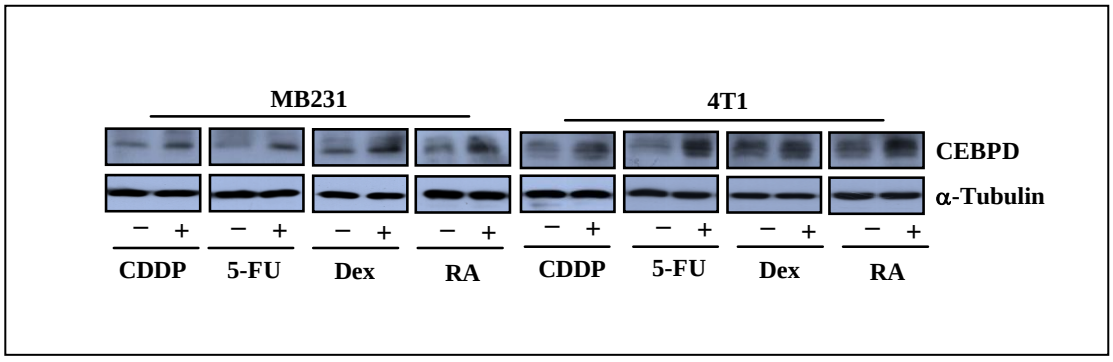


**Figure S1: Anticancer drugs induce CEBPD expression in breast cancer cells.** MDA-MB231 (MB231) and 4T1 cells were treated with CDDP (30 μM), 5-FU (10 μg/ml), dexamethasone (Dex; 1 μM) or retinoic acid (RA; 10 μM) for 6 h. Total lysates were harvested for Western blot analysis using CEBPD and α-tubulin antibodies.

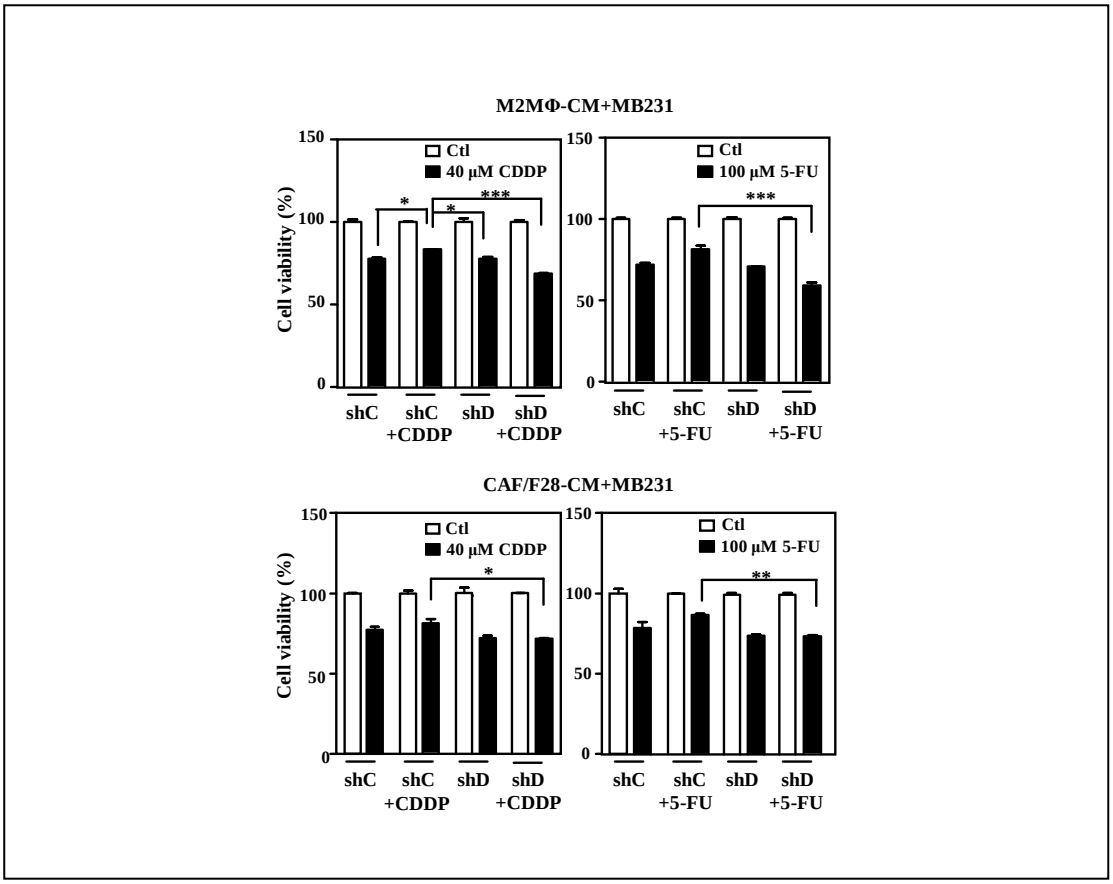


**Figure S2: CEBPD-expressing $M_2$-like macrophages or CAFs promote drug resistance of breast cancer cells after anticancer drug treatment.** The conditioned medium from THP-1/M2 or CAF/F28 cells lacking CEBPD significantly decreased the viability of MB231 after treatment with CDDP or 5-FU. The viability of MB231 cells was increased in the treatment with CDDP or 5-FU in the context of conditioned medium from THP-1/M2 or CAF/F28 cells infected with shC or shD lentiviruses with or without CDDP or 5-FU.

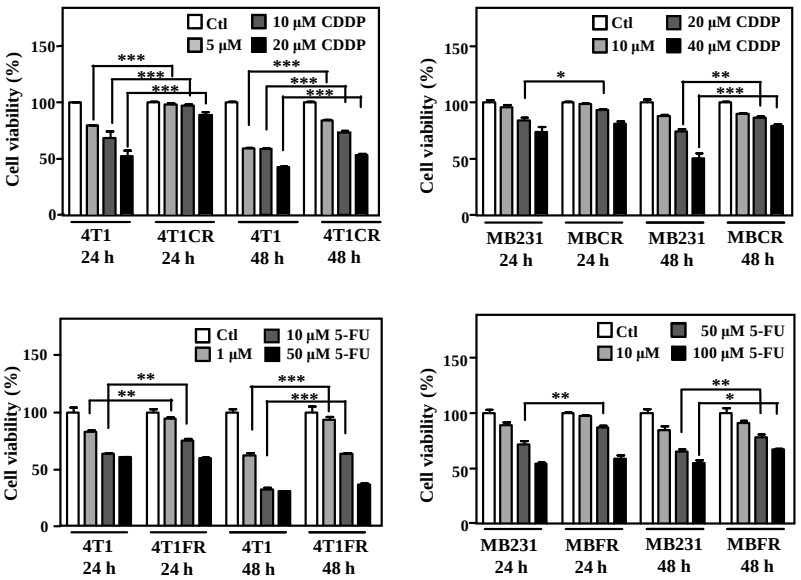


**Figure S3: The viability of parental mCherry fluorescent 4T1 (4T1) and mcMB231 (MB231) cells and drug-resistant mcCDR4T1 (4T1CR), mcFUR4T1 (4T1FR), mcCDRMB231 (MBCR) or mcFURMB231 (MBFR) cells was measured using a cell viability assay.**

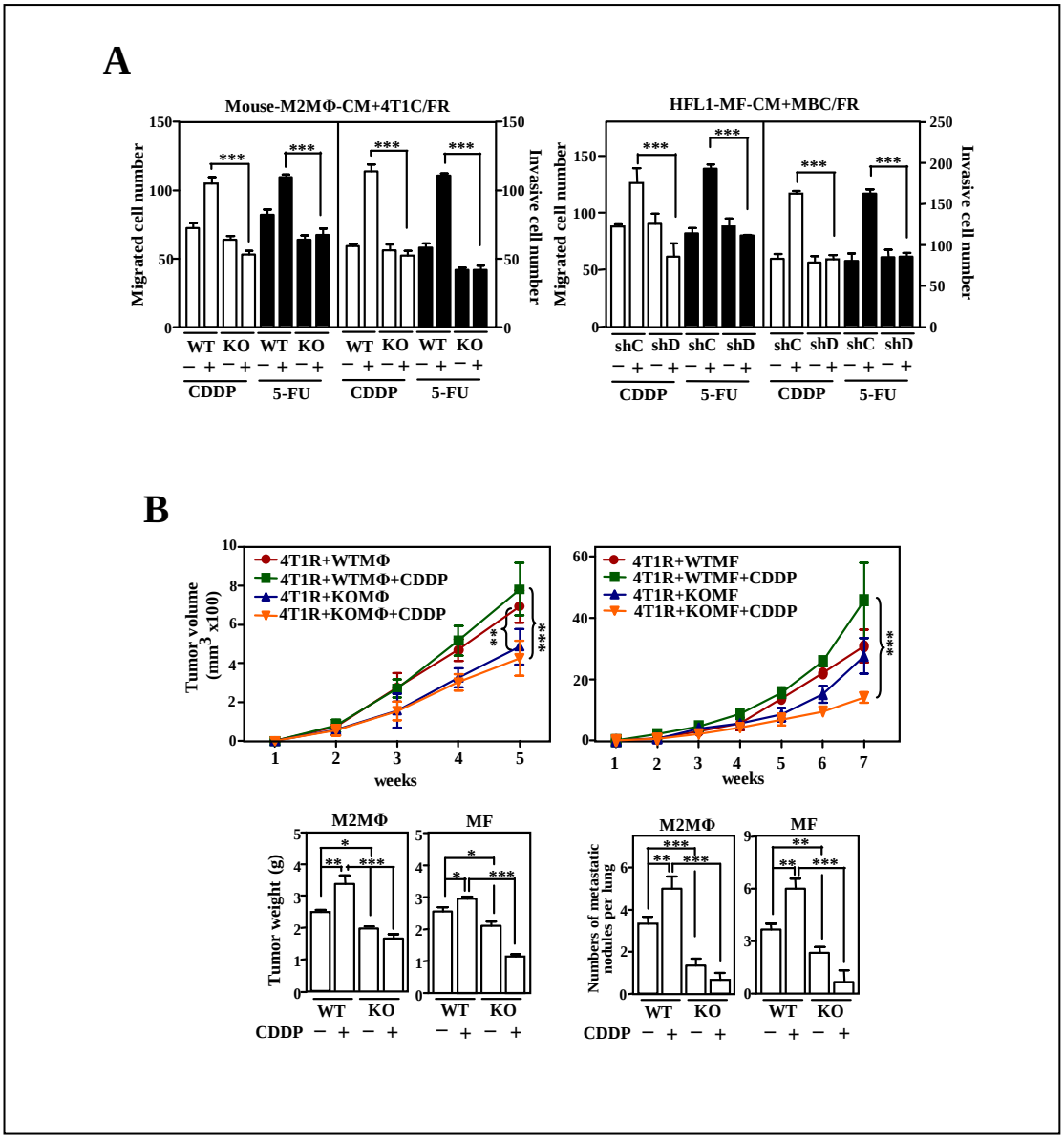


**Figure S4: CEBPD-expressing $M_2$ macrophages or myofibroblasts promote cancer growth and metastasis/invasion after anticancer drug treatment. (A)** The migration and invasion of drug-resistant cancer cells were assessed using a Boyden chamber assay in which the cells were cultured in conditioned medium from M-CSF-differentiated *Cebpd*$^{+/+}$ or *Cebpd*$^{-/-}$ M2 macrophages (mouse M2MΦ) or TGF-β-differentiated HFL myofibroblasts (HFL-MF) infected with shC or shD lentiviruses with or without CDDP or 5-FU treatment. **(B)** M-CSF-differentiated *Cebpd*$^{+/+}$ or *Cebpd*$^{-/-}$ M2 macrophages or TGF-β-differentiated immortalized 7V7 or KO5 myofibroblasts were subcutaneously co-inoculated with mcCDR4T1 (4T1R)

cells into NOD-SCID mice that were treated with or without CDDP (three times per week). Tumor size and metastatic tumors were assessed as described above (n=6 in per group).

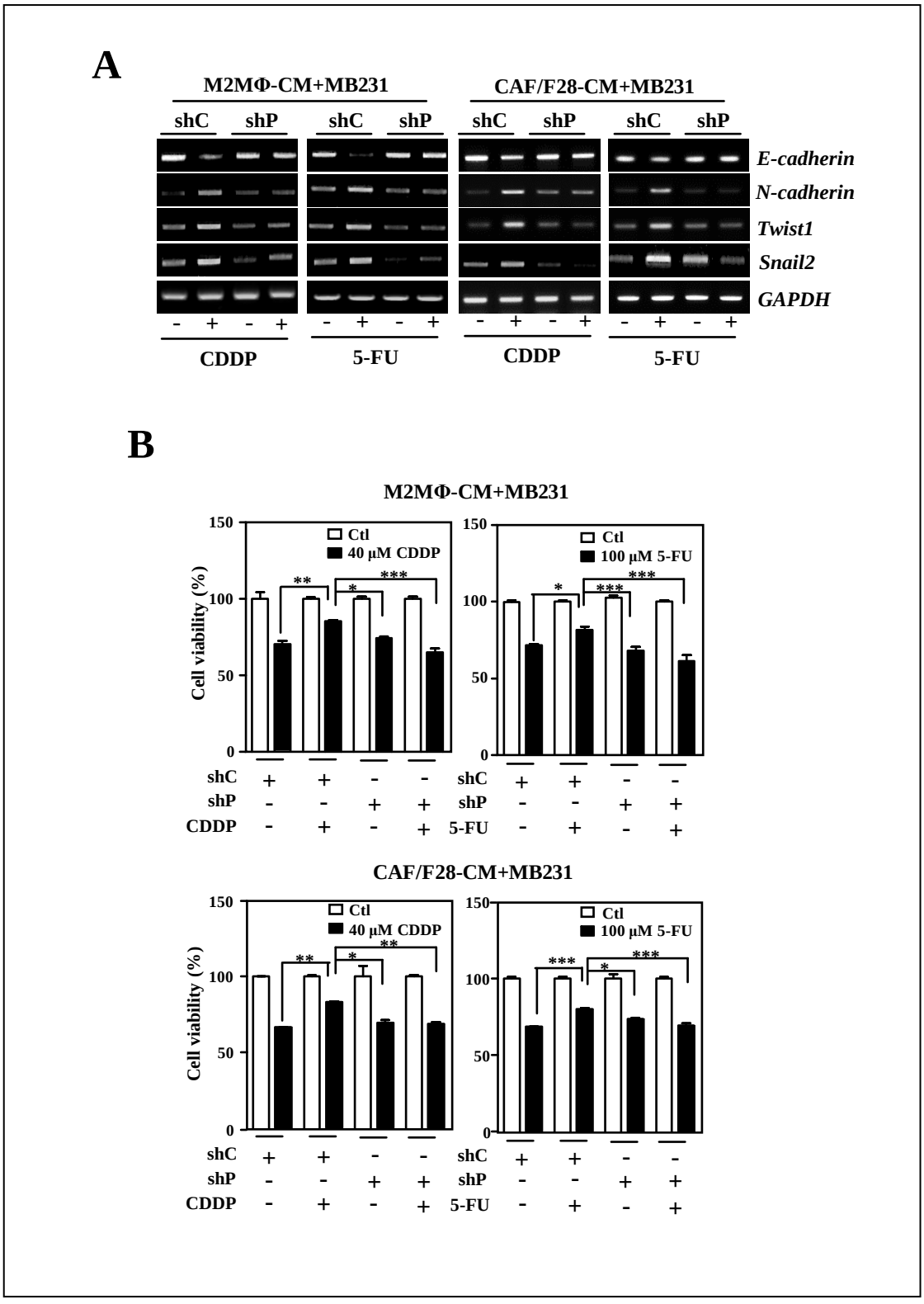


**Figure S5: PTX3-expressing $M_2$-like macrophages or CAFs promote the EMT and drug resistance of breast cancer cells upon anticancer drug treatment. (A)** PTX3-expressing THP-1/M2 or CAF/F28 cells regulated transcription of EMT (*E-cadherin* and *N-cadherin*) and cancer stem cell (*Twist1* and *Snail2*) markers in

MB231 cells. An RT-PCR assay was conducted on total RNA harvested from MB231 cells cultured with conditioned medium from THP-1/M2 or CAF/F28 cells infected with shC or shP lentiviruses and treated with or without CDDP or 5-FU. **(B)** The conditioned medium from THP-1/M2 or CAF/F28 cells lacking PTX3 significantly decreased the viability of MB231 after treatment with CDDP or 5-FU. The effect of viability of MB231 cells after treatment with CDDP or 5-FU in the context of conditioned medium from THP-1/M2 or CAF/F28 cells infected with shC or shP lentiviruses with or without CDDP or 5-FU.

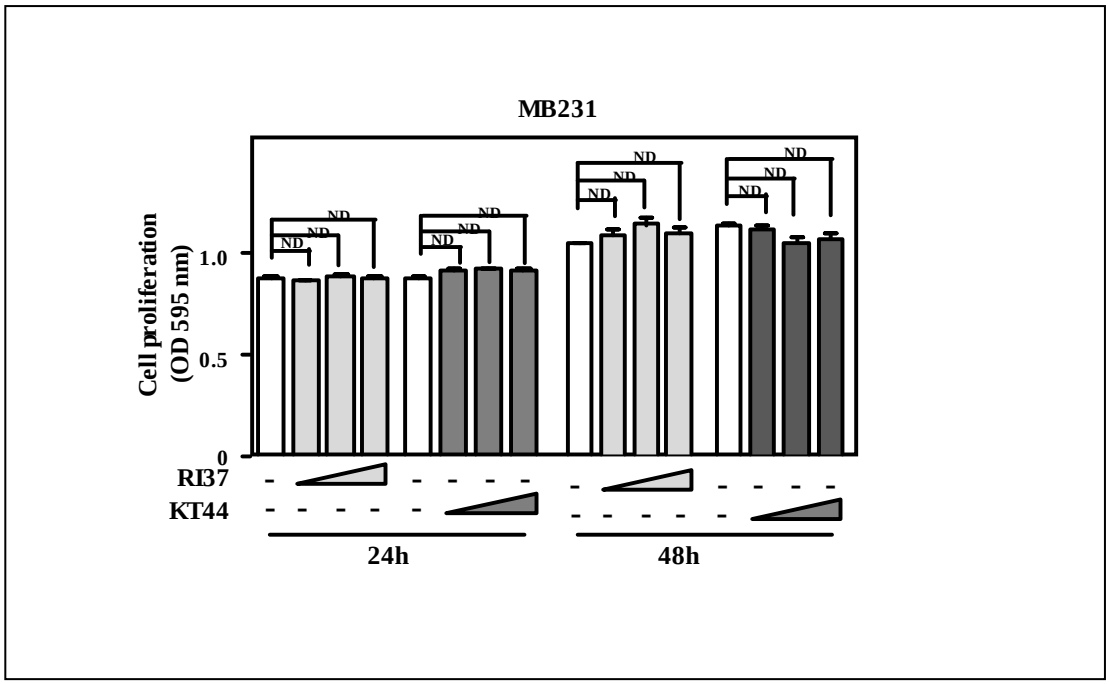


**Figure S6: PTX3 peptides have no effect on the growth of cancer cells.** The effects of the indicated peptides were tested in MB231 cells. The cell proliferation was conducted after the indicated cells were treated with RI37 or KT44 at 24$^{th}$ h and 48$^{th}$ h.

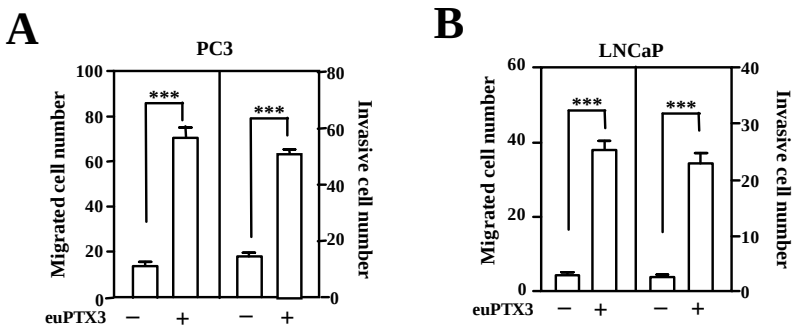


**Figure S7: Effects of euPTX3 on the migration and invasion of prostate cancer cells. (A)** PC3 and **(B)** LNCaP cells were seeded in the upper layer of a Boyden chamber. After 3 h, the culture medium was replaced with serum-free medium in the upper layer and the indicated amount of euPTX3 was added to serum-free medium in the bottom layer. After 16 h of incubation, the cells that migrated to the bottom layer were stained with DAPI and counted using a fluorescence microscope.