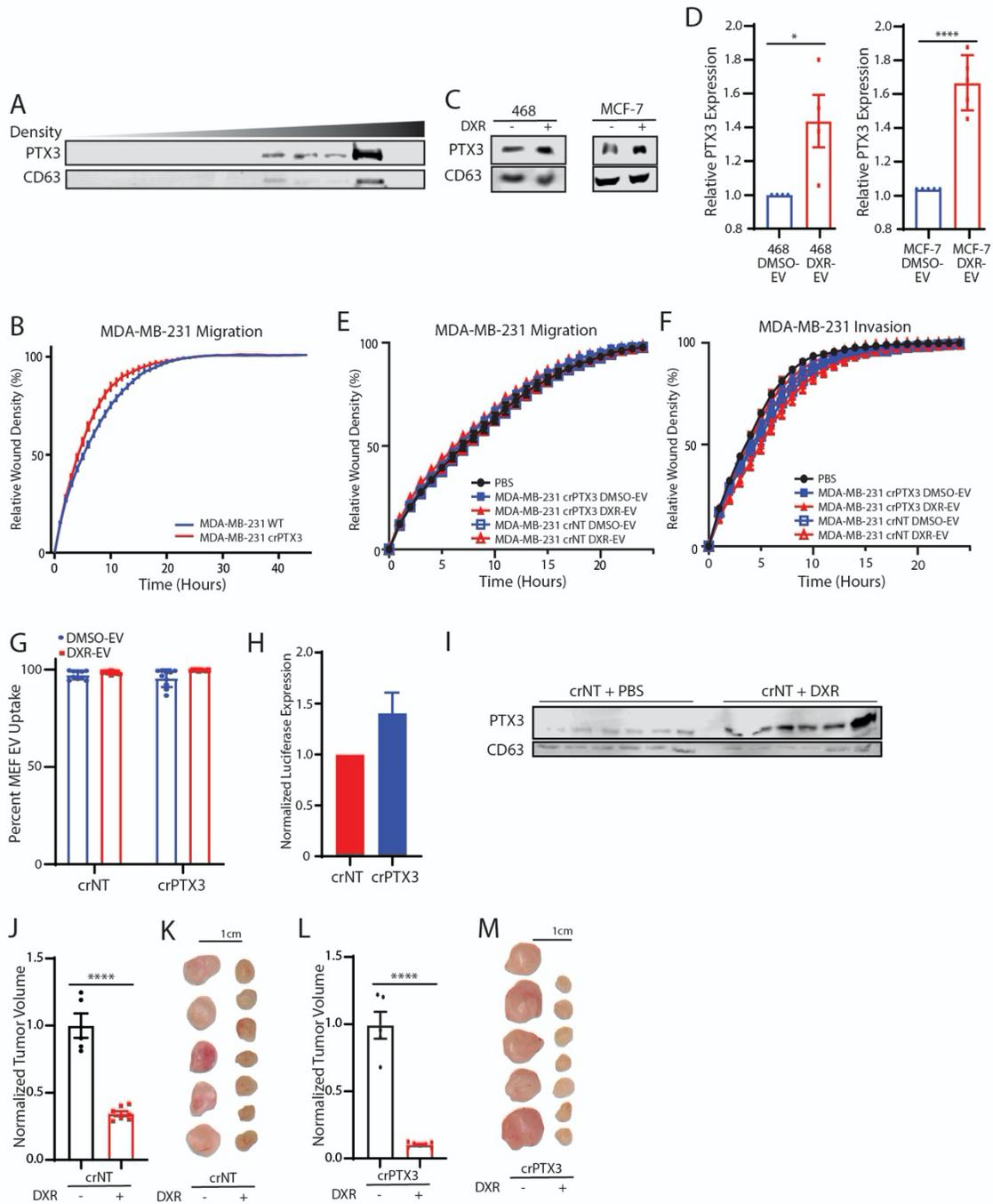


Supplementary Figure 6



Supplementary Figure 6. Chemotherapy upregulates small extracellular vesicle-associated PTX3 protein expression, which has no effect on cell migration and invasion *in vitro* or primary tumor growth *in vivo*.

(a) Immunoblot of sEVs separated using density gradient ultracentrifugation and probed for PTX3 and CD63. (b) MDA-MB-231 crPTX3 and MDA-MB-231 WT cell relative wound density. Mean \pm SEM, n=18 for all treatments. (c) Immunoblot of DMSO-EVs and DXR-EVs isolated from MDA-MB-468 cells treated with 40nM DXR and MCF-7 cells treated with 400nM DXR, probed for PTX3 and CD63. (d). Quantification of relative PTX3 expression in MDA-MB-468 and MCF-7 DMSO-EVs and DXR-EVs, n=4 for all treatment conditions. Student's t-test, *p<0.05, ****p<0.0001. (e) MDA-MB-231 cell relative wound density. Cells were treated with PBS, 10ug/mL MDA-MB-231 crPTX3 and crNT DMSO-EVs or DXR-EVs. Mean \pm SEM, n=6 for all treatments. (f) MDA-MB-231 cell relative wound density. Cells were plated in Matrigel and treated with PBS, 10ug/mL MDA-MB-231 crPTX3 and crNT DMSO-EVs or DXR-EVs. Mean \pm SEM, PBS n=4, MDA-MB-231 crNT DMSO-EV n=5, MDA-MB-231 crNT DXR-EV n=5, crPTX3 DMSO-EV n=4, MDA-MB-231 crPTX3 DXR-EV n=6. (g) Vybrant DiD-labeled crNT and crPTX3 EVs uptake in MEFs analyzed using flow cytometry. Combined results of two independent experiments, n=10 for all conditions. (h) Quantification of MDA-MB-231 crNT and crPTX3 luciferase expression normalized to MDA-MB-231 crNT. MDA-MB-231 crPTX3 luciferase expression is 1.41 times higher than MDA-MB-231 crNT. (i) Immunoblot of sEVs isolated from the plasma of crNT tumor-bearing mice treated with PBS or DXR as in Figure 5g probed for PTX3 and CD63 expression. (j) Quantification of primary MDA-MB-231 crNT tumor volume measured *ex vivo* normalized to the average crNT + PBS tumor volume. Data represents one experiment. crNT + PBS n=5, crNT + DXR n=7. Student's t-test, mean \pm SEM, p****<0.0001. (k) Representative images of primary MDA-MB-231 crNT *ex vivo*. (l) Quantification of primary MDA-MB-231 crPTX3 tumor volume measured *ex vivo* normalized to the average crPTX3 + PBS tumor volume. Data represents one experiment.

crPTX3 + PBS n=5, crPTX3 + DXR n=7. Student's t-test, mean \pm SEM, $p^{****}<0.0001$. (**m**)

Representative images of primary MDA-MB-231 crPTX3 *ex vivo*.