**Oudin et al, MENA confers resistance to paclitaxel in triple-negative breast cancer**

**Supplemental Figures**

**Figure S1:** **MENA expression in breast cancer and paclitaxel resistance**. RPKM for MENA (A) and MENAINV (B) levels in the TCGA breast cancer cohort (data from 1060 patients), as well as by subtype HER2+, ER+/HER2- or TNBC. (C) MENAINV expression, as measured by immunostaining from a 300 patient tumor microarray, where MENAINV expression was measured by fluorescence intensity in the tumor compartment in arbitrary units. (D) Whole western blot image from Fig 1A of lysate prepared from the panel of breast cancer cell lines MDA-MB 175IIV and T47D (Luminal A), MDA-MB 453 (HER2+), MDA-MB 436, BT-549, LM2, SUM159, MDA-MB 231 and BT-20 (TNBC), probed with anti-MENA and anti-Tubulin antibodies. Two lanes (3 and 4) containing lysates from cell lines that were not analyzed were removed from the blot. (E) Western Blot for MENA expression in T47D cells stably expressing shCtrl or shMENA, which shown 75% knockdown. Cell viability was assessed in 231-Control, 231-MENA or 231-MENAINV cells after 72h of treatment with doxorubicin (F), or cisplatin (G) using Prestoblue assay. The cell viability is expressed as a fraction relative to untreated cells. Data presented as mean± SEM for three independent experiments, each performed in duplicate. Statistics determined by unpaired t-test with Welch's correction, where \*\*\* p<0.001, \*\* p<0.01, \* p<0.05.

**Figure S2: Paclitaxel treatment decreases cell velocity *in vitro*** (A) Velocity of Control, MENA and MENAINV cells plated on glass bottom dishes coated with Collagen (0.1 mg/ml) and treated with different concentrations of paclitaxel. Data presented as mean± SEM for at least 50 cells tracked in two independent experiments. Statistics determined by unpaired t-test with Welch's correction, where \*\*\* p<0.001, \*\* p<0.01, \* p<0.05.

**Figure S3: MENA isoform driven taxane resistance is not due to drug efflux, FA signaling**

(A) Fraction of viable cells of 231-Control or 231-MENAINV cells treated during 72h by paclitaxel with or without the MDR1 inhibitor HM30181. Data presented as mean± SEM for three independent experiments, each performed in duplicate. (B) Fraction of viable cells after 72h of treatment with 100nM of Taxol of 231-Control, 231-MENA, 231-MENAINV, 231-MENAΔLERER or 231-MENAINVΔLERER. Data presented as mean± SEM for two independent experiments, each performed in duplicate. Statistics determined by unpaired t-test with Welch's correction, where \*\*\* p<0.001, \*\* p<0.01, \* p<0.05.

**Figure S4: MENA isoform driven taxane resistance affects progression through the cell cycle**

Cell cycle analysis of 231-Control (A), 231-MENA (B) and 231- MENAINV (C) cells treated with 10nM or 100nM paclitaxel during 16 hours. Data presented as mean± SEM of two independent experiments. Representative transmitted light images of 231-Control (D), 231-MENA (E) and 231- MENAINV (F) before, during and after successful cell division with vehicle or 10nM paclitaxel treatment. Scale bar is 2μm. G) Quantification of the time cells spent in cell division, from the time when mother cell rounds up, to when the daughter cells attach, with vehicle or 10nM paclitaxel treatment. H) Quantification of the percent of successful cell divisions that lead to two surviving daughter cells for 231-Control, 231-MENA and 231-MENAINV cells, treated with vehicle or 10nM paclitaxel treatment. Data presented as mean± SEM pooled from 3 independent experiments. Statistics determined by unpaired t-test with Welch's correction, where \*\*\* p<0.001, \*\* p<0.01, \* p<0.05.

**Figure S5: MENA expression alters MT length**

Representative images of 231-Control (A) and 231-MENAINV (B) cells treated with paclitaxel (10nM) for 24h, and immunostained for Tubulin (red) and DAPI (blue). Scale bar is 4μm, 0.5μm for inset. (C) Quantification of MT length in 231-Control, MENA or MENAINV cells treated with vehicle (0.01% DMSO) or 10nM paclitaxel for 24h. Data pooled from 3 separate experiments, with at least 5 cells analyzed per experiment. Data presented as mean± SEM. Statistics determined by one-way ANOVA, where \*\*\* p<0.001, \*\* p<0.01, \* p<0.05.

**Figure S6: MENA-driven resistance to paclitaxel does not involve Akt signaling**

(A) Representative Western Blot of lysates obtained 231-Control, 231-MENA and 231-MENAINV treated with 10 or 100nM paclitaxel for 72hrs immunostained for total ERK. (B) Quantification of Western Blot for ERK relative to GAPH. (C) Representative Western Blot of lysates obtained 231-Control, 231-MENA and 231-MENAINV treated with 10 or 100nM paclitaxel for 72hrs immunostained for pAkt473. (D) Quantification of Western Blot for pAkt473 relative to GAPH. (C) Representative Western Blot of lysates obtained 231-Control, 231-MENA and 231-MENAINV treated with 10 or 100nM paclitaxel for 72hrs immunostained for total Akt. (D) Quantification of Western Blot for total Akt relative to GAPH. Data pooled from at least 3 experiments, two technical replicates per experiment. Statistics determined by unpaired t-test, where \*\*\* p<0.001, \*\* p<0.01, \* p<0.05.