Supplemental Figures for:

Cdc42-Dependent Transfer of mir301 from Breast Cancer-Derived Extracellular Vesicles Regulates the Matrix Modulating Ability of Astrocytes at the Blood–Brain Barrier

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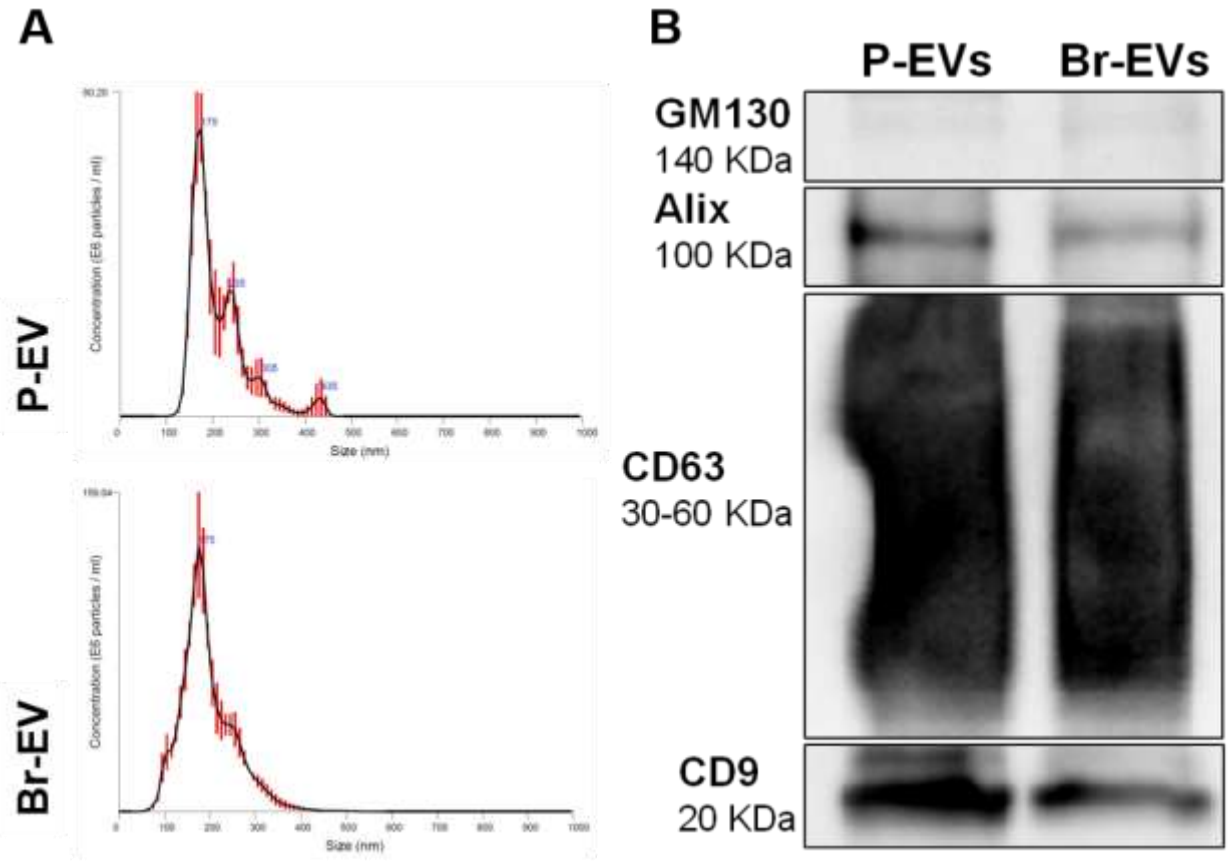

Figure S1. Characterization of breast cancer-derived EVs. (A) Nanoparticle tracking analysis of the size of P-EVs and Br-EVs. (B) Representative western blot images of EV markers CD9, CD63, Alix, and the golgi marker, GM130.

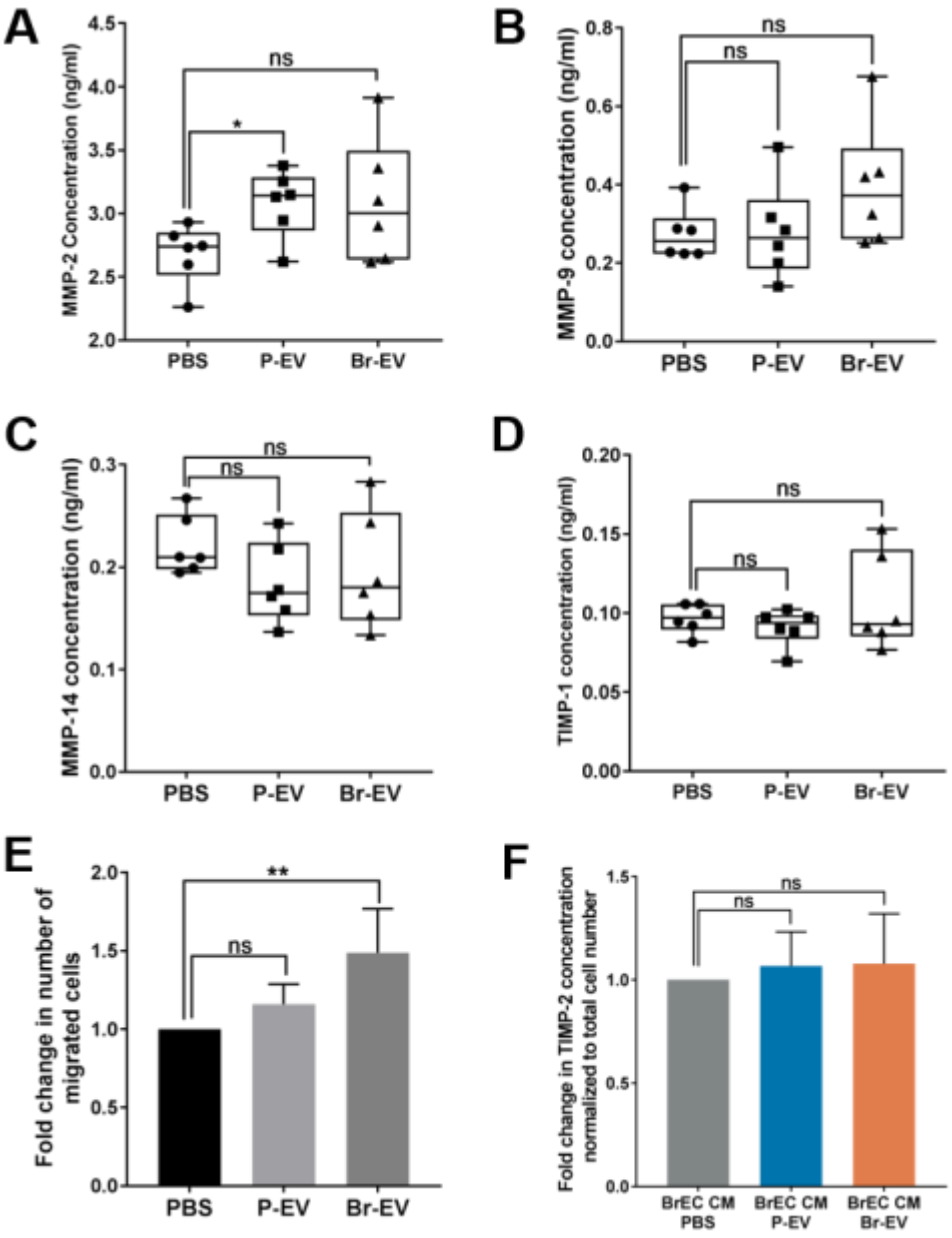

Figure S2. Effect of breast cancer-derived EVs on matrix metalloproteinases and their endogenous inhibitors. Average concentration of (**A**) MMP-2, (**B**) MMP-9, (**C**) MMP-14, and (**D**) TIMP-1 in brain tissue homogenates measured by ELISA (mean ± SD; n= 6 mice per group). Statistical analysis was performed using Mann-Whitney test. (**E**) Fold change in the number of migrated astrocytes in a transwell migration assay following pre-treatment with PBS, P- or Br-EVs (mean ± SD; 3 independent experiments). Statistical analysis was performed using one-way ANOVA with Tukey's test for multiple comparison. (**F**) Fold change in the concentration of TIMP-2 in astrocyte conditioned media following treatment with conditioned media from PBS-, P-EV, and Br-EV-treated endothelial cells (mean ± SD; 3 independent experiments). Statistical analysis was performed using one-way ANOVA. In all panels, ns, not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.