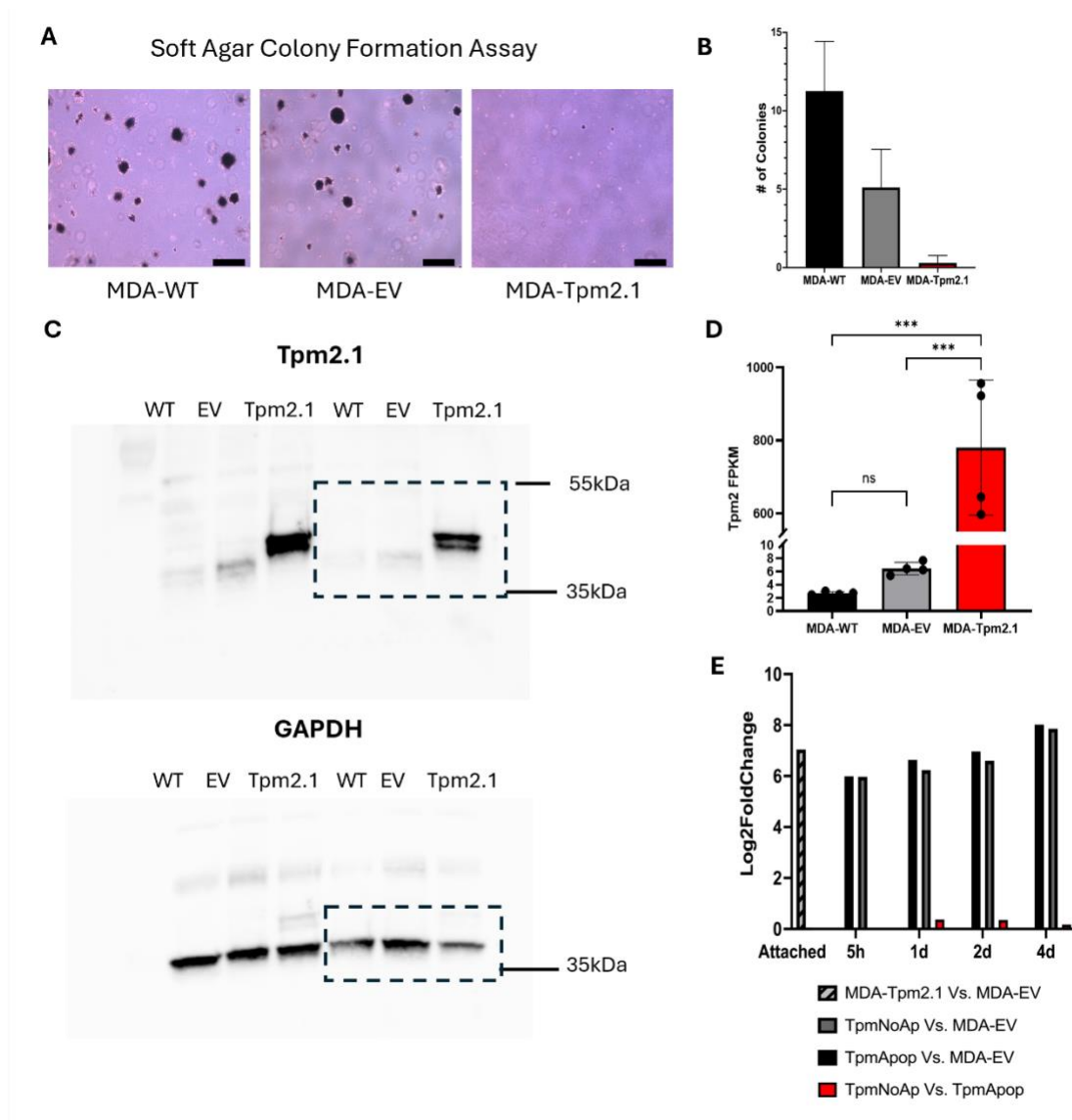


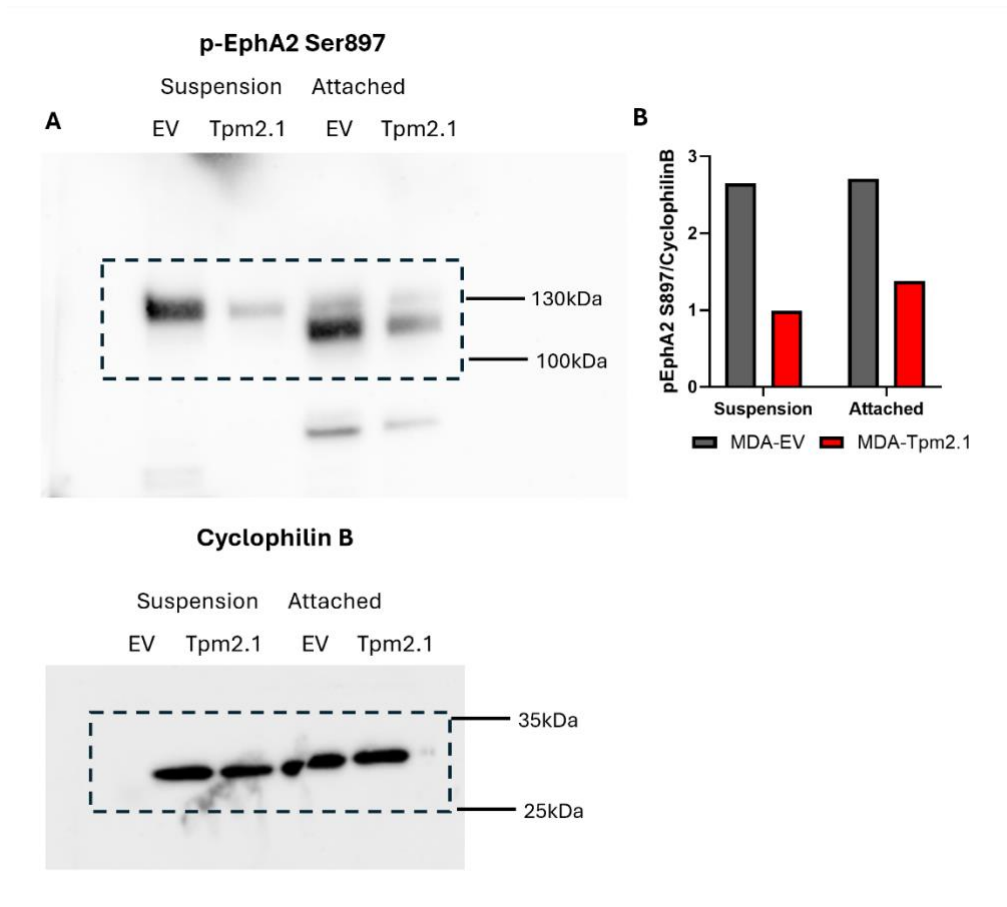
Supplementary Figure 1



Supplementary 1. Tpm2.1 expression enhances anoikis in MDA-MB-231 breast cancer cells - Control experiments.

(A) Soft agar colony formation assay to ensure that MDA-Tpm2.1 cells do not grow on soft surfaces. Representative phase-contrast images of MDA-WT, MDA-EV, and MDA-Tpm2.1 colonies in 3D soft agar. Scale bar: 100 μ m. (B) Quantification (mean $\pm$ SD) of colony number for MDA-WT, MDA-EV, and MDA-Tpm2.1 cells grown in soft agar. Colonies were stained with crystal violet, counted, and averaged from three biological replicates (n = 3). (C) Western blot analysis of Tpm2.1 protein expression in MDA-WT, MDA-EV, and MDA-Tpm2.1 cells. GAPDH was used as a loading control. Samples were run in duplicate using two different protein concentrations. The marked box highlights clear separation of Tpm2.1 bands. (D) RNA-seq analysis of Tpm2.1 gene expression in MDA-WT, MDA-EV, and MDA-Tpm2.1 cells cultured under attached conditions. (E) RNA-seq analysis of Tpm2.1 gene expression in MDA-EV and MDA-Tpm2.1 cells following apoptotic selection at various time points during suspension culture.

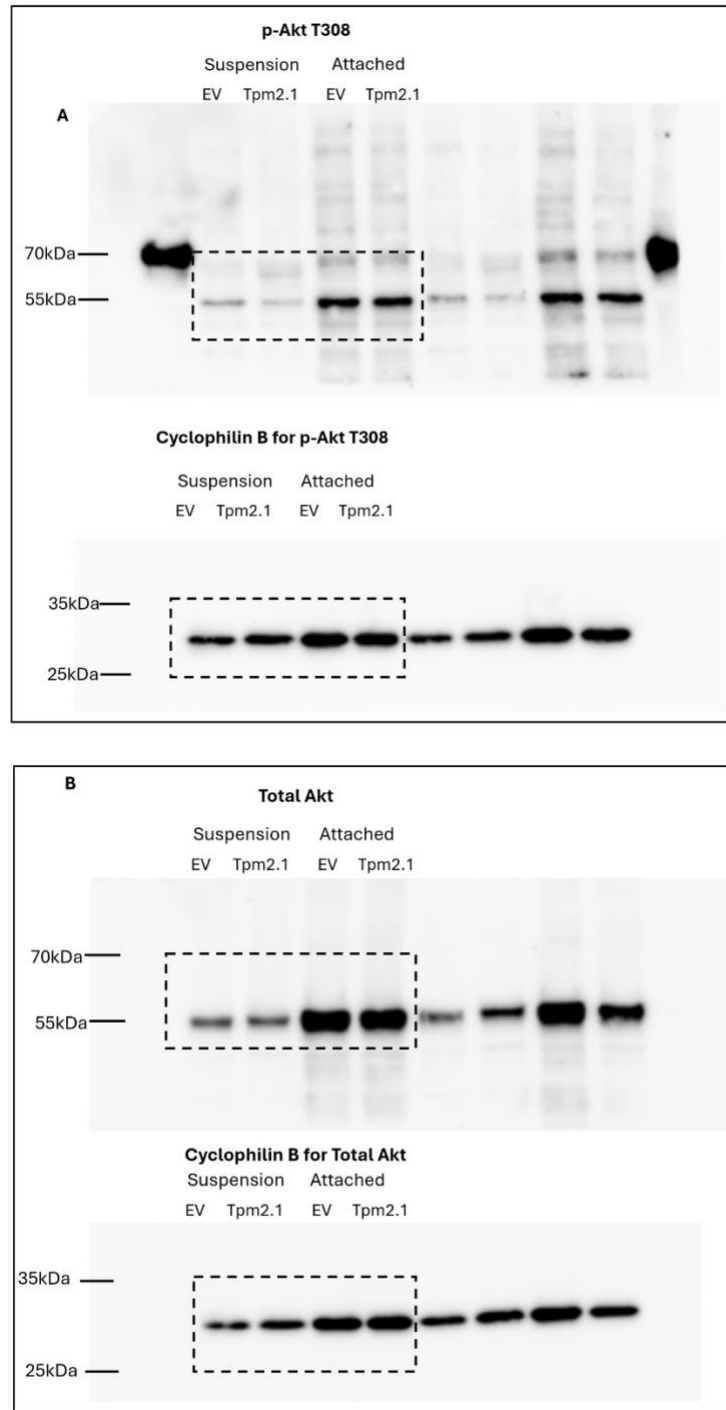
Supplementary Figure 2



Supplementary 2: Phospho-EpHA2 (Ser897) levels in MDA-EV and MDA-Tpm2.1 cells.

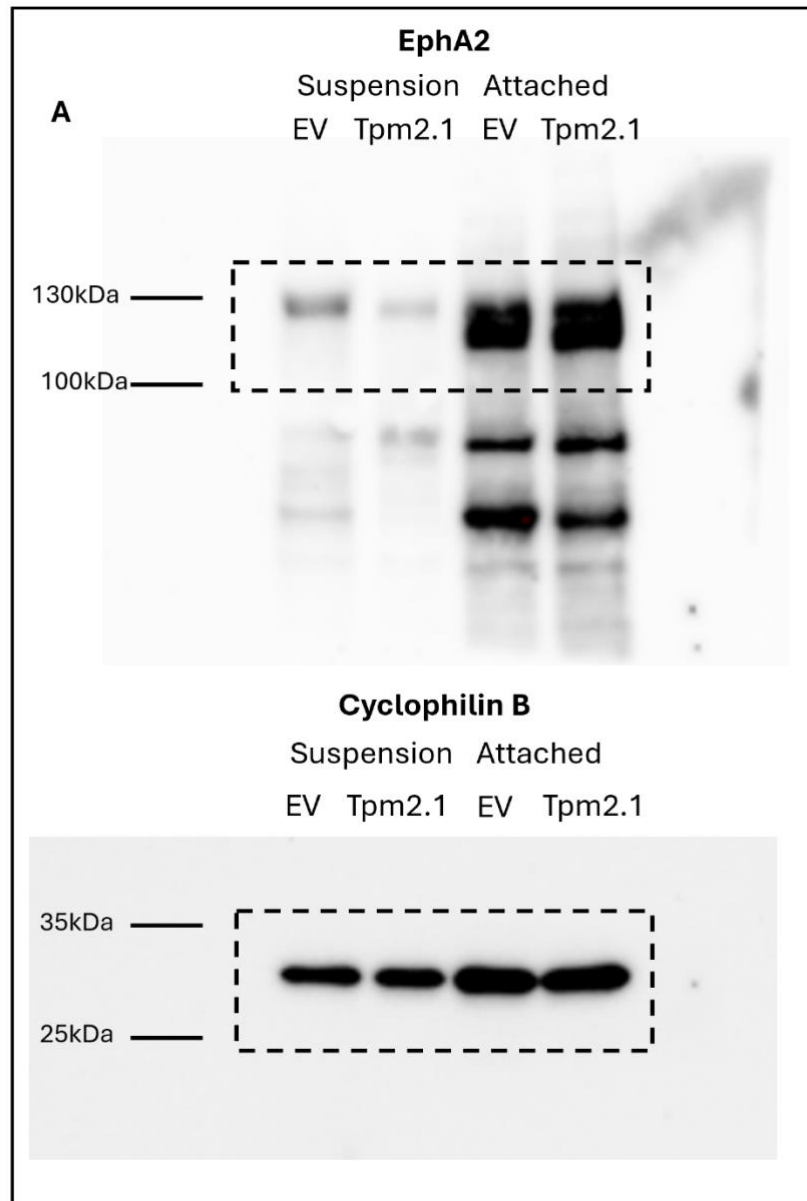
(A) Western blot showing the expression of phospho-EphA2(Ser897) in MDA-EV and MDA-Tpm2.1 cells cultured under suspension and attached conditions for 5 days. Cyclophilin B used as a loading control. (B) Quantification of band intensity for phospho-EphA2(Ser897), normalized to cyclophilin B (loading control) in MDA-EV and MDA-Tpm2.1 cells under suspension and attached conditions for 5 days.

Supplementary Figure 3



Supplementary 3. Uncropped western blot images of (A) phospho-Akt (T308) and (B) total Akt in MDA-EV and MDA-Tpm2.1 cells cultured under suspension and attached conditions for 5 days. Cyclophilin B used as a loading control. These images correspond to the blots shown in Figure 5A. Relevant lanes used in the main figure are indicated by dashed boxes.

Supplementary Figure 4



Supplementary 4. Uncropped western blot images of EphA2 in MDA-EV and MDA-Tpm2.1 cells cultured under suspension and attached conditions for 5 days. Cyclophilin B used as a loading control. These images correspond to the blots shown in Figure 5F. Relevant lanes used in the main figure are indicated by dashed boxes.