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Supplemental Information

MicroRNA-4500 Inhibits Migration, Invasion, and Angiogenesis of Breast Cancer Cells via RRM2-Dependent MAPK Signaling Pathway

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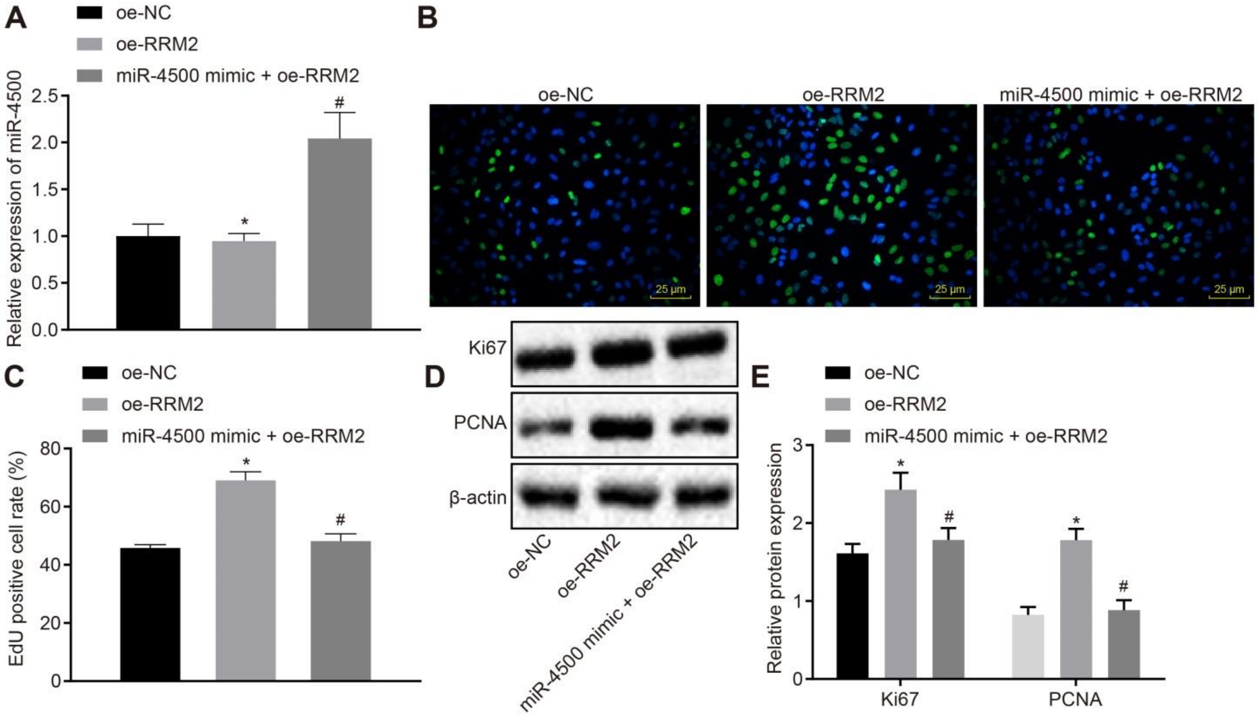
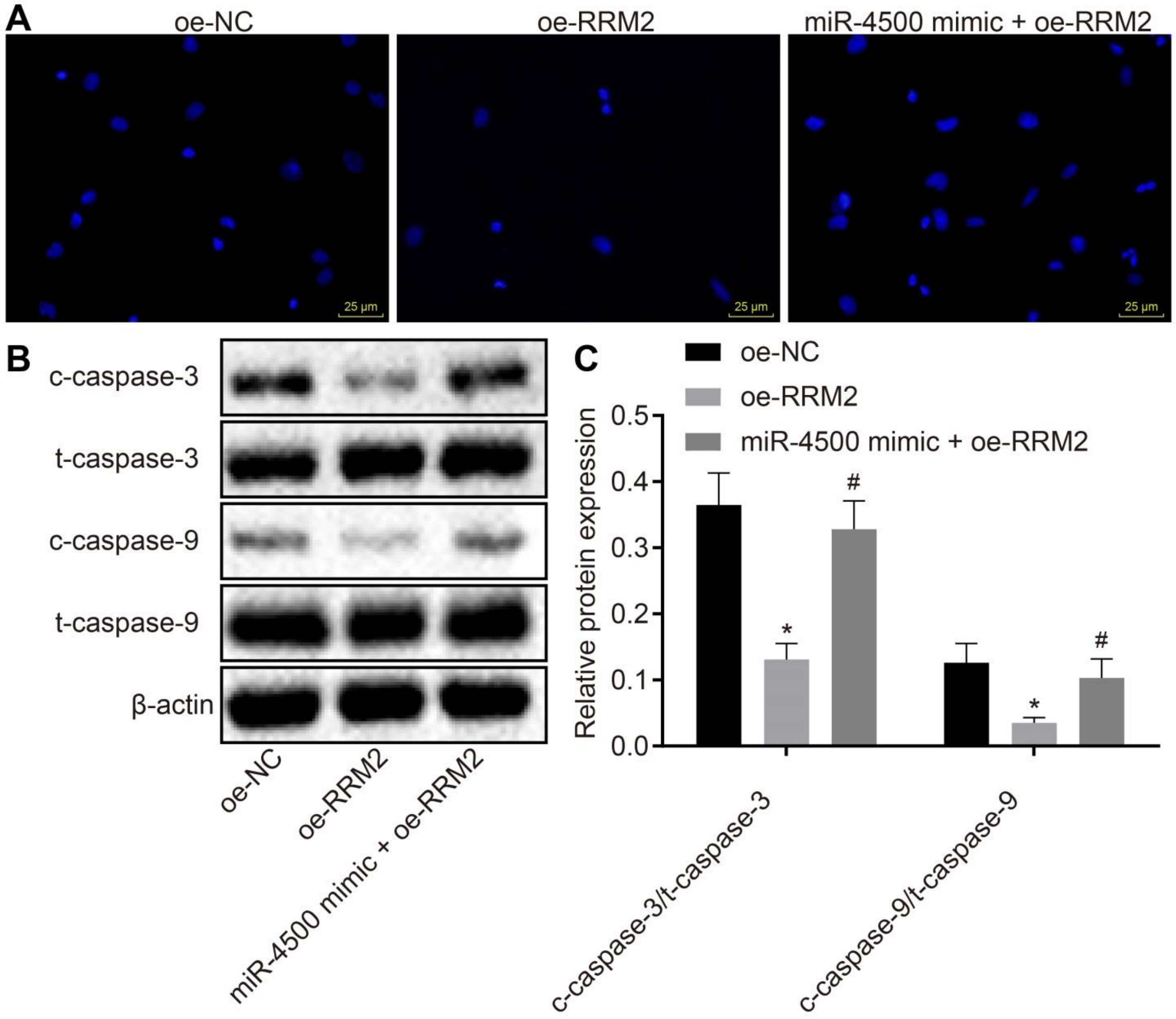


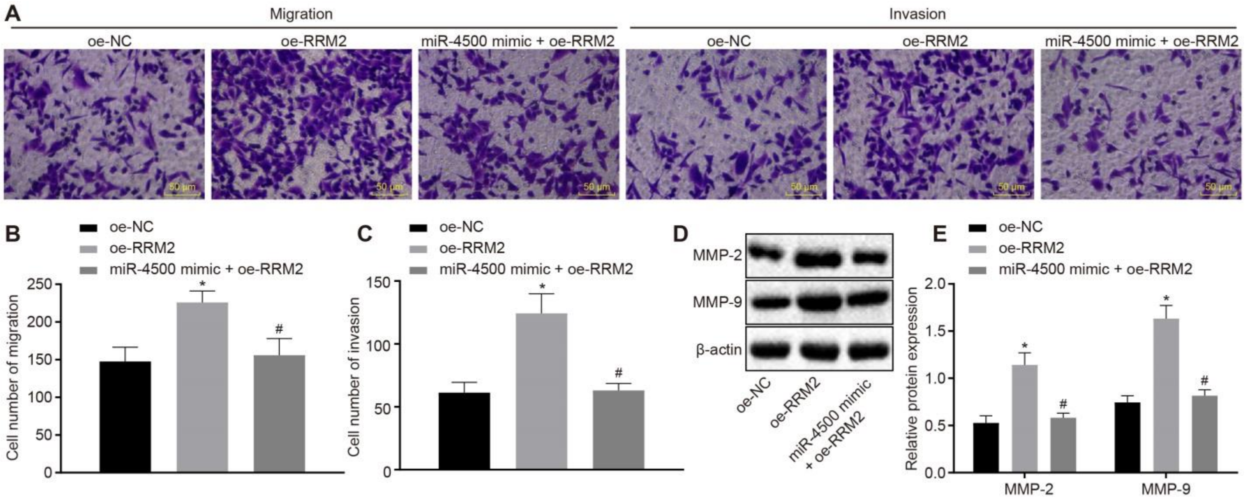
Supplementary Figure 1. Over-expressed RRM2 promotes proliferation of breast cancer cells, which is abrogated by up-regulated miR-4500.

A, The expression patterns of miR-4500 determined by RT-qPCR. B, The cell proliferation detected by EdU proliferation assay (400 ×). C, The quantitative analysis for EdU positive breast cancer cells detected by EdU proliferation assay. D, The protein bands of RRM2 and cell proliferation-related factors Ki67 and PCNA in each group detected by Western blot analysis. D, The RRM2 and cell proliferation-related factors Ki67 and PCNA protein expression patterns in each group detected by Western blot analysis. *, # respectively indicate that $p < 0.05$ *vs*. the oe-NC group and oe-RRM2 group. The data comparisons were analyzed by one-way ANOVA and the experiment was repeated three times independently.



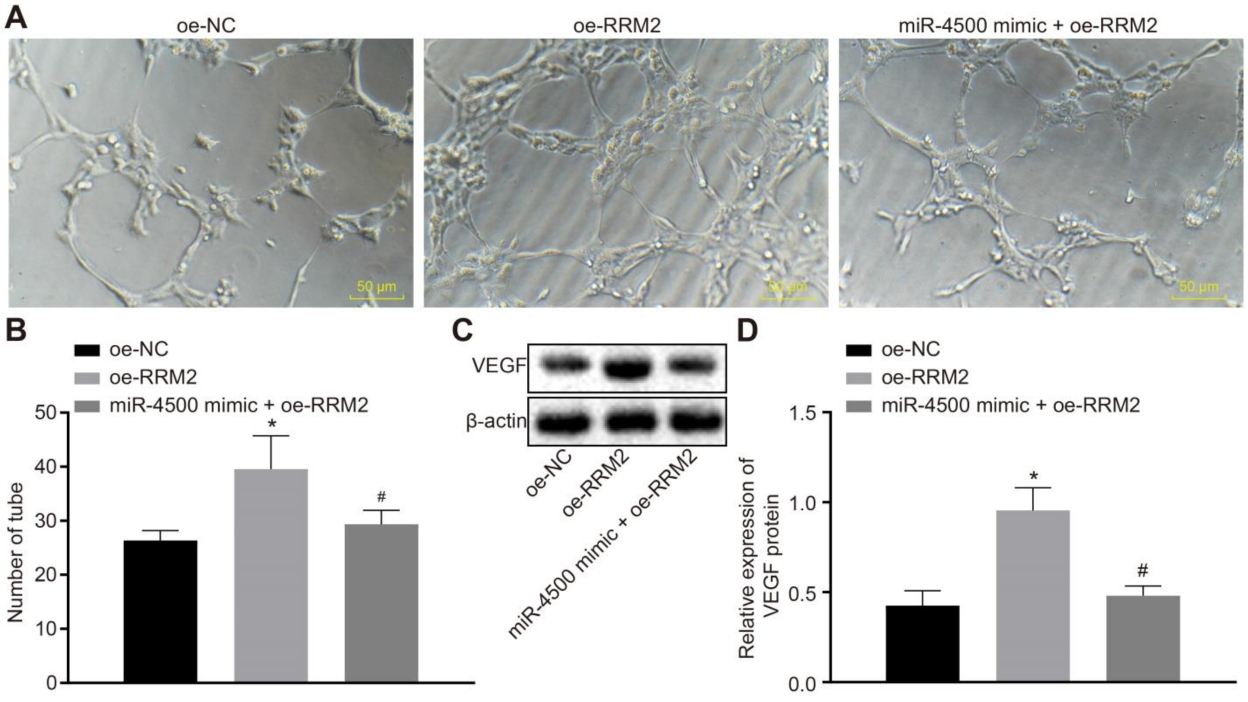
Supplementary Figure 2. Over-expressed RRM2 inhibits apoptosis of breast cancer cells, which is abrogated by up-regulated miR-4500.

A, The cell apoptosis detected by Hoechst staining (400 ×). B, The protein bands of cell apoptosis-related factors c-Caspase-3/t-Caspase-3 and c-Caspase-9/t-Caspase-9 detected by Western blot analysis. C, The protein expression patterns of cell apoptosis-related factors c-Caspase-3/t-Caspase-3 and c-Caspase-9/t-Caspase-9 in each group detected by Western blot analysis; *, # respectively indicate that $p < 0.05$ *vs.* the oe-NC group and oe-RRM2 group. The data comparisons were analyzed by one-way ANOVA and the experiment was repeated three times independently.



Supplementary Figure 3. Over-expressed RRM2 promotes migration and invasion of breast cancer cells, which is abrogated by up-regulated miR-4500.

A, The migration and invasion of breast cancer cells in each group under the microscope (200 ×). B, The cell migration in each group detected by Transwell assay. C, The cell invasion in each group detected by Transwell assay. D and E, The protein expression patterns of RRM2, MMP-2 and MMP-9 in each group detected by Western blot analysis. *, # respectively indicate that $p < 0.05$ *vs*. the oe-NC group and oe-RRM2 group. The data comparisons were analyzed by one-way ANOVA and the experiment was repeated three times independently.



Supplementary Figure 4. Over-expressed RRM2 promotes the capillary-like tube formation of endothelial cells and angiogenesis of breast cancer cells, which is abrogated by up-regulated miR-4500.

A and B, Capillary-like tube formation observed under a microscope by *in vitro* assay (200 ×). C and D, The protein expressions of tumor angiogenic factor VEGF detected by Western blot analysis. *, # indicate that $p < 0.05$ respectively *vs.* the oe-NC group and oe-RRM2 group. The data comparisons were analyzed by one-way ANOVA and the experiment was repeated three times independently.