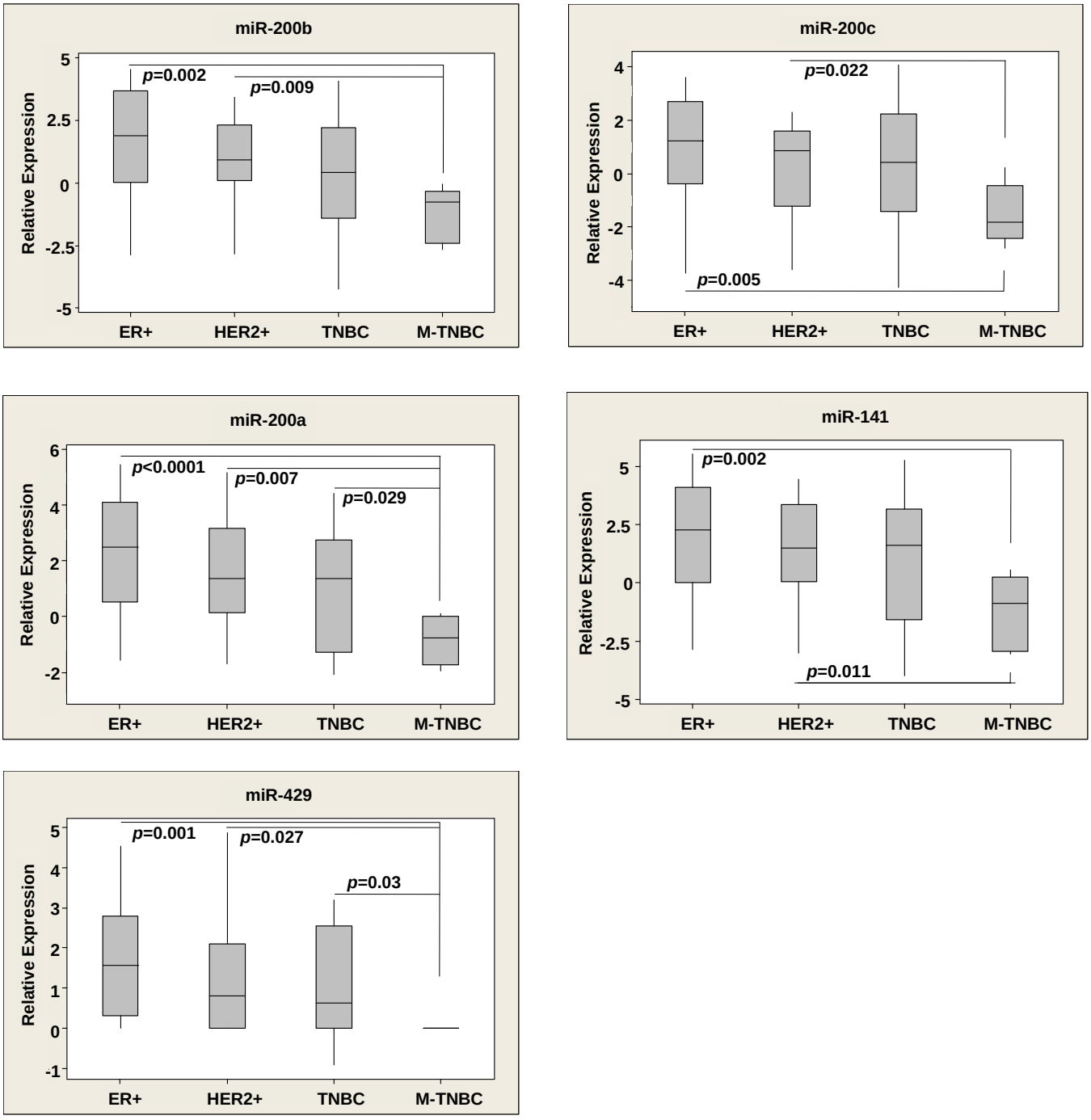
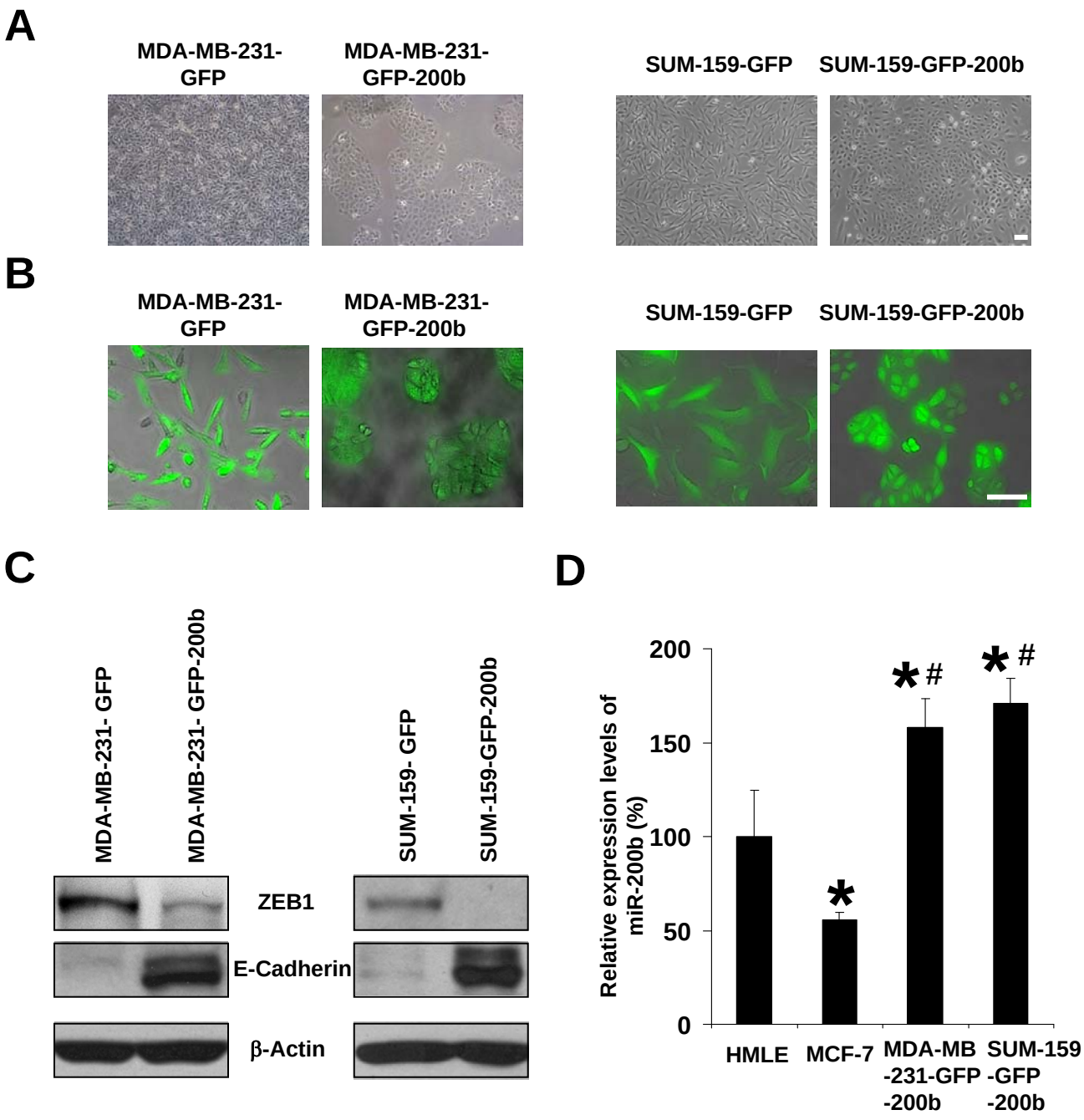
Supplementary Figure 1

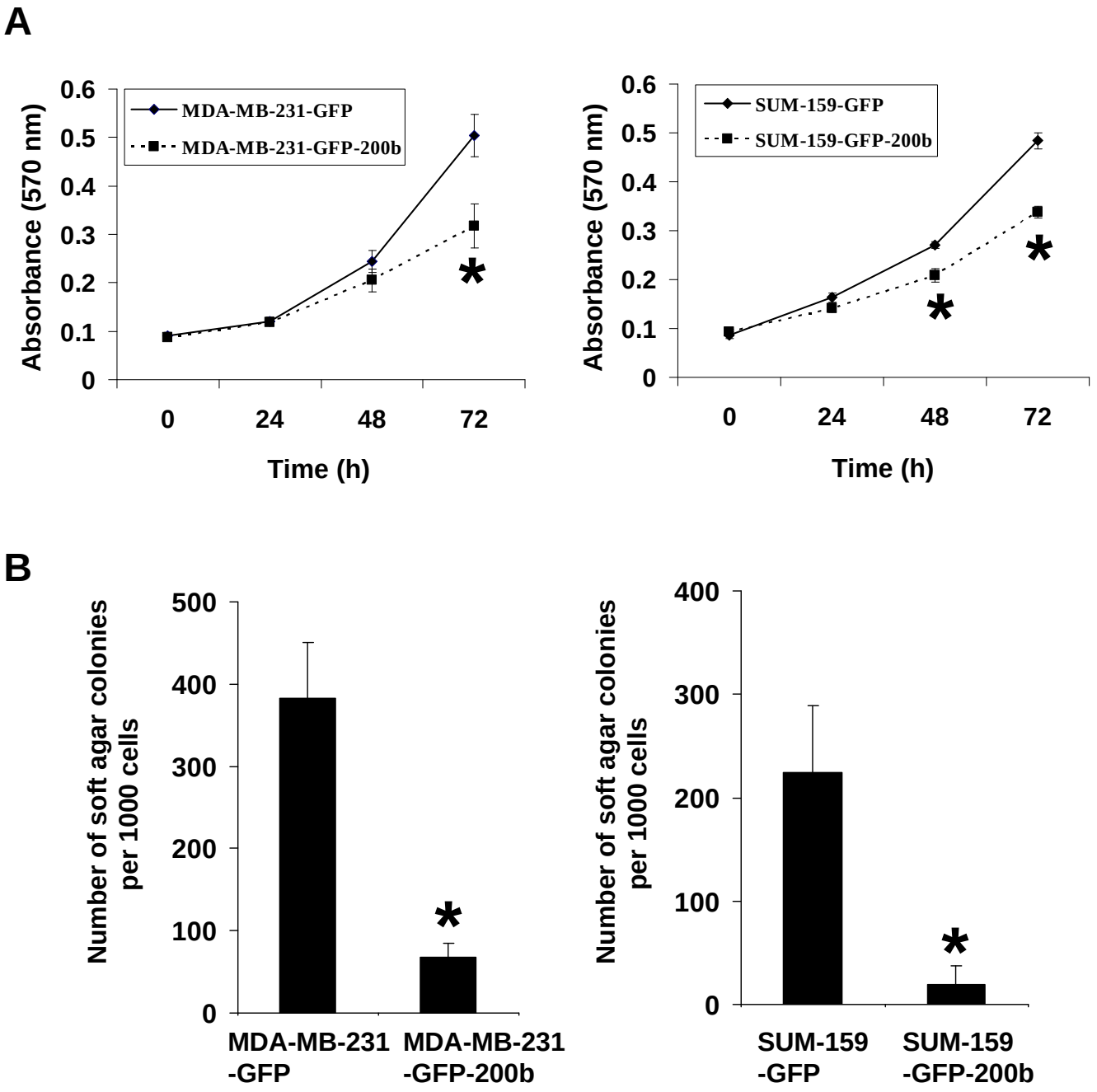


Supplementary Fig. 1. MiR-200 expression levels are significantly lower in metastatic TNBC (M-TNBC) tumors than other subtypes of breast cancer. The miR-200 expression values from a breast cancer tissue miRNA microarray data set in the Gene Expression Omnibus (accession number GSE39543) were log2 (Hy3/Hy5) transformed. A two sample t-test assuming unequal variance was applied to compare levels between different groups. A side-by-side boxplot was done to show the distribution of the log2 transformed relative expression values for different groups. ER+: n=14, HER2+: n=10, TNBC: n=10, M-TNBC: n=8.

Supplementary Figure 2

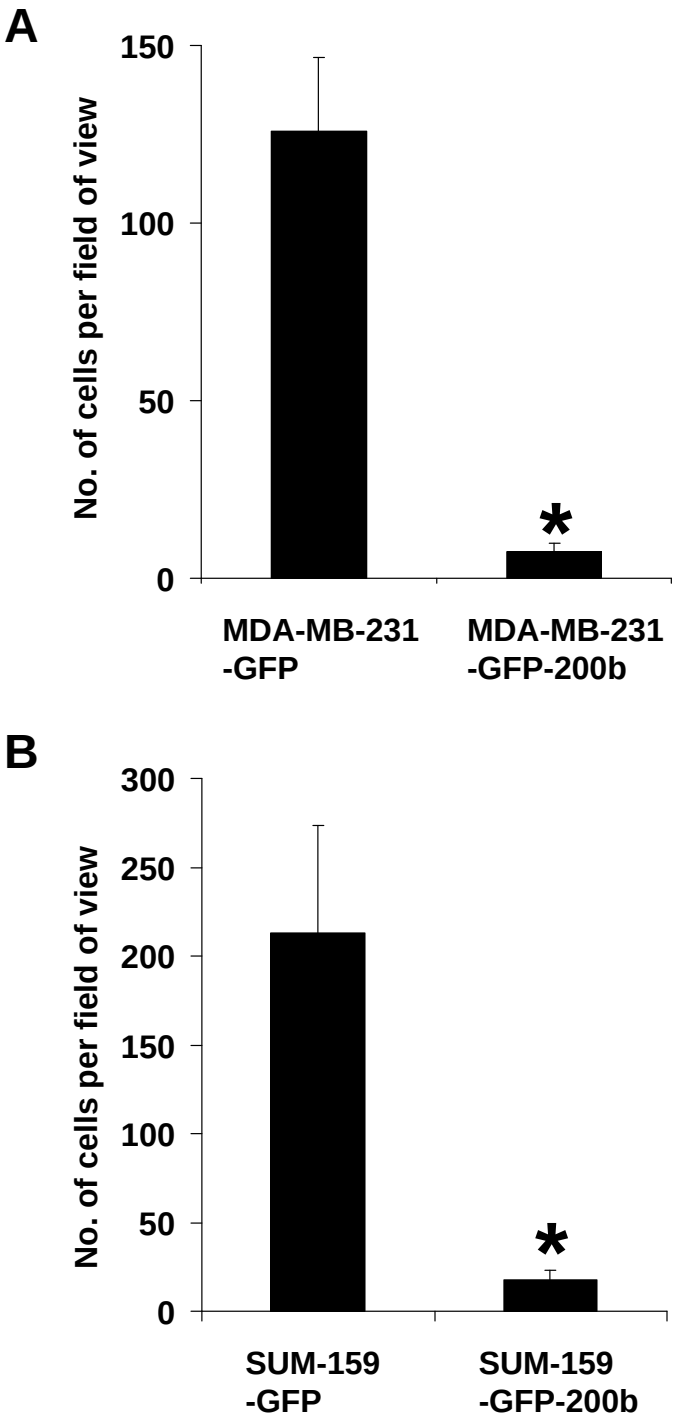


Supplementary Fig. 2. Effect of stably expressing miR-200b on TNBC cell morphology and the comparison of miR-200b expression levels among immortalized human mammary epithelial cells (HMLE), breast cancer MCF-7 cells, and miR-200b stably expressing cells. ***A.*** Representative bright field images of GFP control and miR-200b stably expressing cells. ***B.*** Representative overlaid bright field and fluorescent field images of GFP control and miR-200b stably expressing cells, showing all cells express GFP. ***C***. Western blot analysis of ZEB1 and E-Cadherin protein levels in GFP control and miR-200b stably expressing breast cancer cells. ***D.*** Cellular levels of miR-200b were determined by Q-PCR and are expressed relative to that of HMLE cells. Data are presented as means ± standard deviations (n=3). * $p<0.05$, compared to HMLE cells; # $p<0.05$, compared to MCF-7 cells.



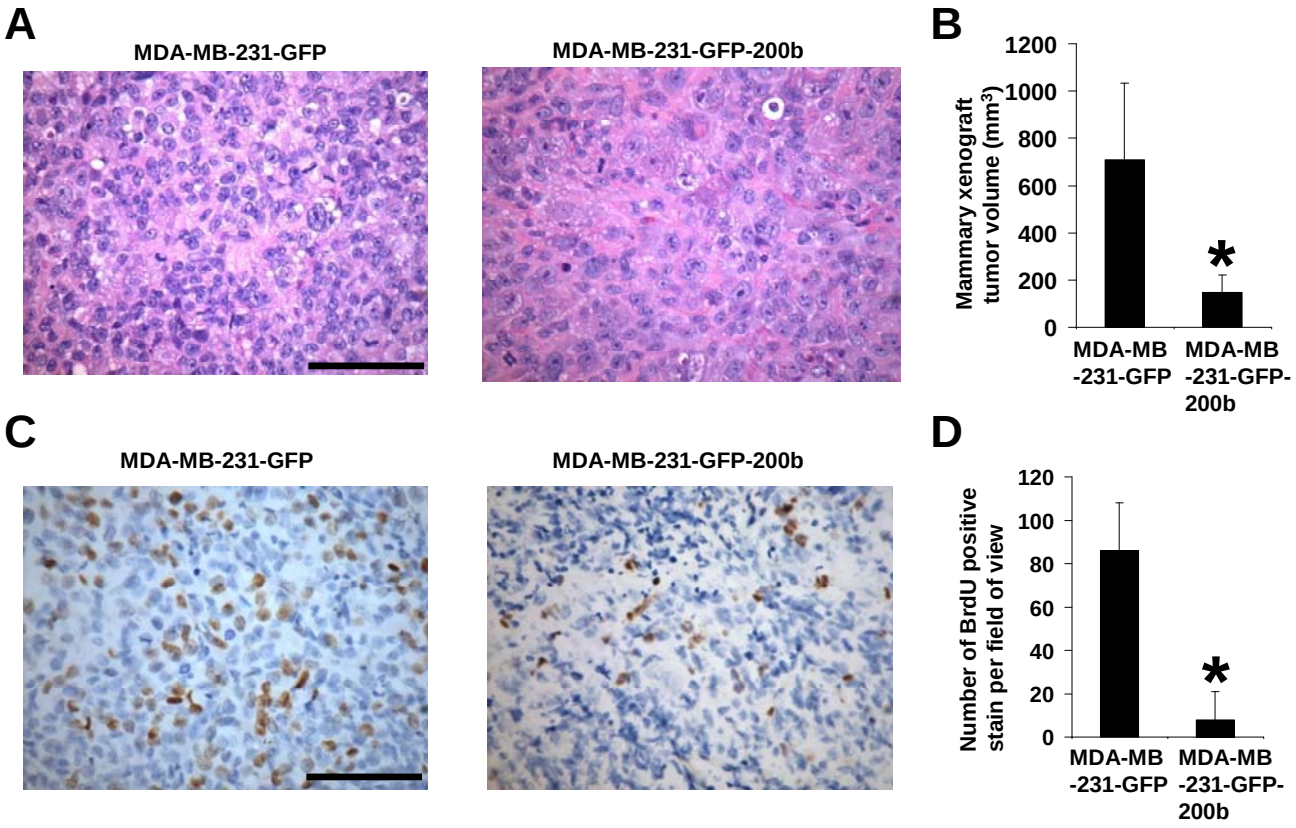
Supplementary Fig. 3. Stably expressing miR-200b reduces TNBC cell proliferation and colony formation in soft agar. ***A.*** Three thousands cells were seeded into each well of 96-well plates for MTT assay to indirectly monitor cell proliferation up to 72 h. Data are presented as mean ± SD (n=8). *p< 0.05, compared to the GFP control cells. ***B***. One thousand cells were used for soft agar colony formation assay as described in Materials and Methods. Data are presented as means ± standard deviations (n=3). * p<0.05, compared to the GFP control cells.

Supplementary Figure 4



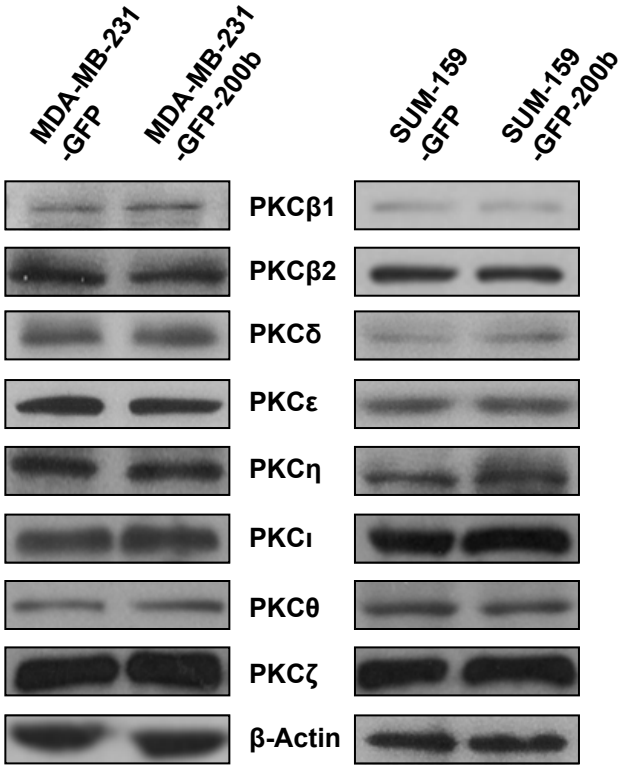
Supplementary Fig. 4. Stably expressing miR-200b significantly reduces MDA-MB-231 and SUM-159 breast cancer cell migration determined by Transwell cell migration assay. After 48 h culture, cells with 70-80% confluence were collected for Transwell cell migration assay as described in reference 36. Ten percent fetal bovine serum was used as the chemoattractant. The quantification of cell migration is presented as number of cells per field of view (means ± standard deviations, n=3). * $p<0.05$, compared to GFP Control cells.

Supplementary Figure 5



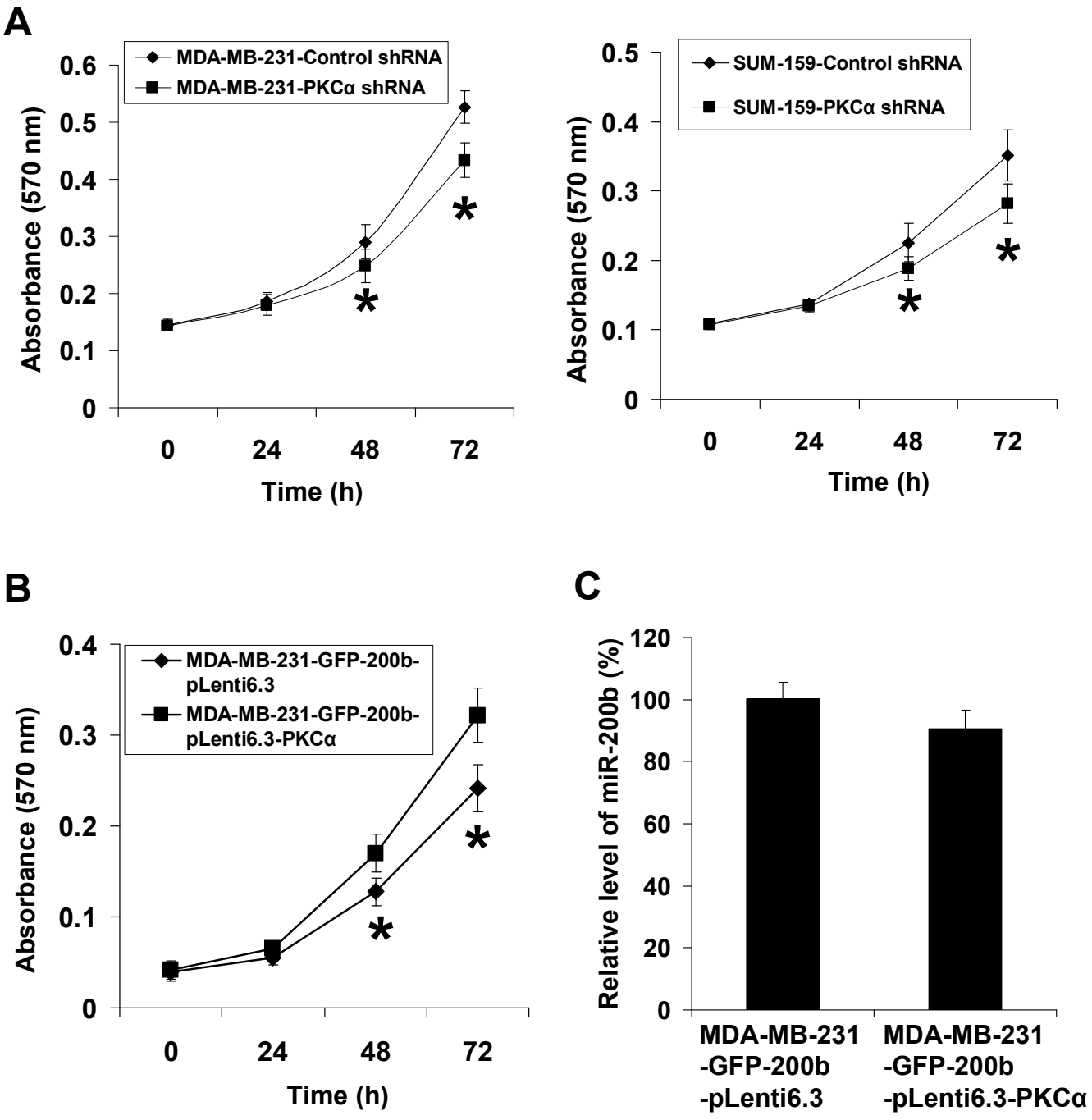
Supplementary Fig. 5. Effect of stably expressing miR-200b on mouse mammary xenograft tumor histology and growth. ***A.*** and ***B***. Representative images of H&E staining (***A***) and the average volumes (***B***) of mouse mammary tumors resulting from injection of MDA-MB-231-GFP or MDA-MB-231-GFP-200b cells. ***C.*** and ***D***. Representative images of BrdU immunohistochemistry staining (***C***) and the quantification of the staining (***D***) of mouse mammary tumor sections. Quantitative data are presented as means ± SD (n=7-8). *p< 0.05, compared to the GFP control cell tumor group. Scale bar=100 μm.

Supplementary Figure 6

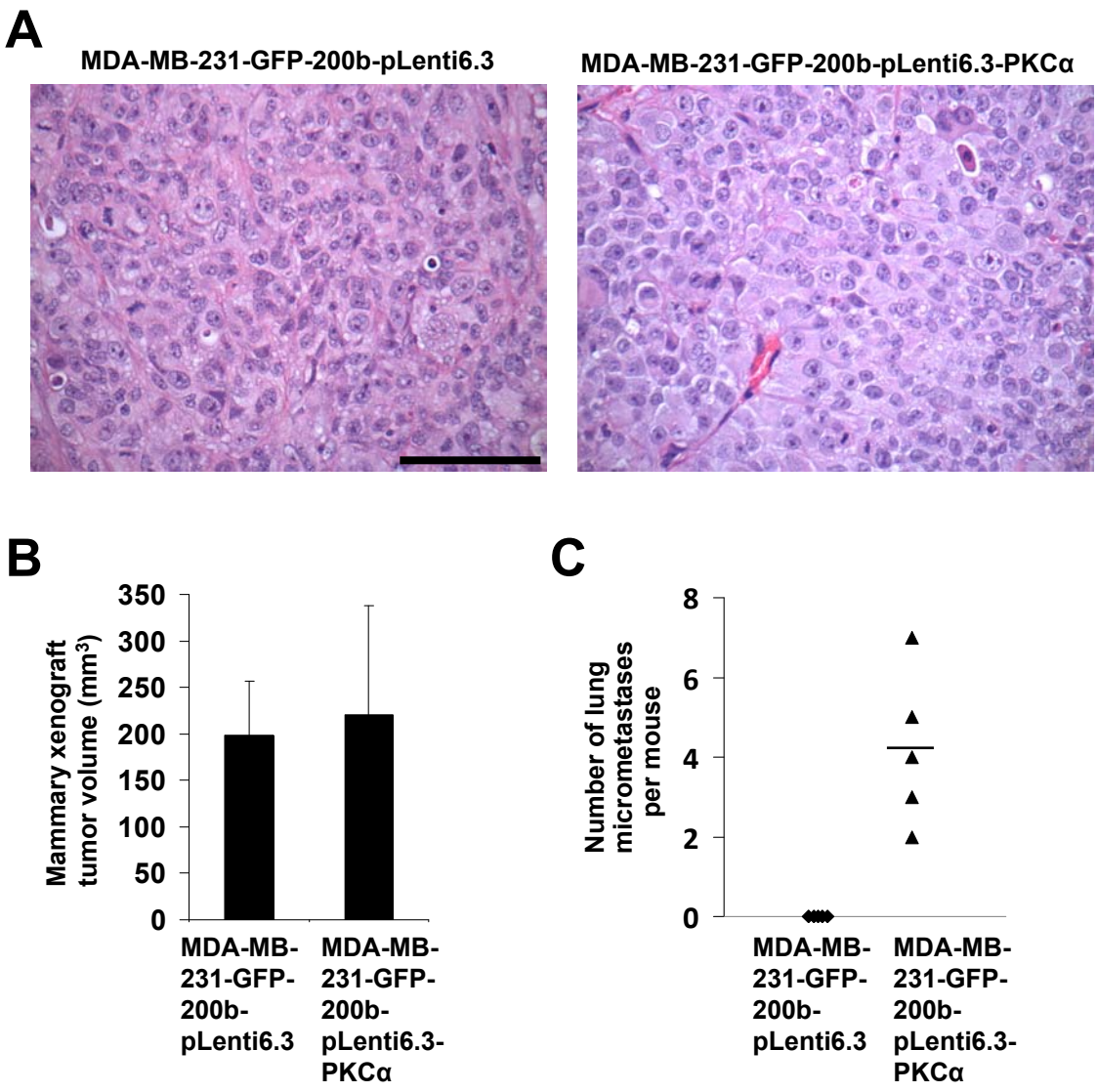


Supplementary Fig. 6. Stably expressing miR-200b has no significant effect on the protein levels of other PKC isozymes in MDA-MB-231 and SUM-159 breast cancer cells. After 48 h culture, cells with 70-80% confluence were harvested for Western blot analysis using specific primary antibodies for different PKC isozymes (Santa Cruz Biotechnology and BD Biosciences).

Supplementary Figure 7



Supplementary Fig. 7. Effect of knocking down PKCα expression or forced expression of PKCα on TNBC cell proliferation determined by the MTT assay. Three thousands Control shRNA or PKCα shRNA cells (***A***), and MDA-MB-231-GFP-200b-plenti6.3 or MDA-MB-231-GFP-200b-plenti6.3-PKCα cells (***B***) were seeded into each well of 96-well plates for MTT assay to indirectly monitor cell proliferation up to 72 h. Data are presented as mean ± SD (n=6-8). *p< 0.05, compared to the control shRNA cells or MDA-MB-231-GFP-200b-pLenti6.3 cells. ***C***. Forced expression of PKCα has no significant effect on miR-200b expression level determined by Q-PCR using ABI TaqMan miR-200b Q-PCR assay. Data are presented as mean ± SD (n=3).



Supplementary Fig. 8. Effect of forced expression of PKCα on mouse mammary xenograft tumor histology, growth and lung micrometastasis. ***A***. Representative images of H&E staining of mouse mammary tumors resulting from injection of MDA-MB-231-GFP-pLenti6.3 or MDA-MB-231-GFP-200b-pLenti6.3-PKCα cells. Scale bar=100 μm. ***B.*** and ***C***. The averages of mouse mammary xenograft tumor volumes (means ± standard deviations, n=5) (***B***) and the quantifications of GFP immunofluorescence positive staining foci in lung tissue sections (***C***) from mice with mammary fat pad injection of MDA-MB-231-GFP-pLenti6.3 or MDA-MB-231-GFP-200b-pLenti6.3-PKCα cells.