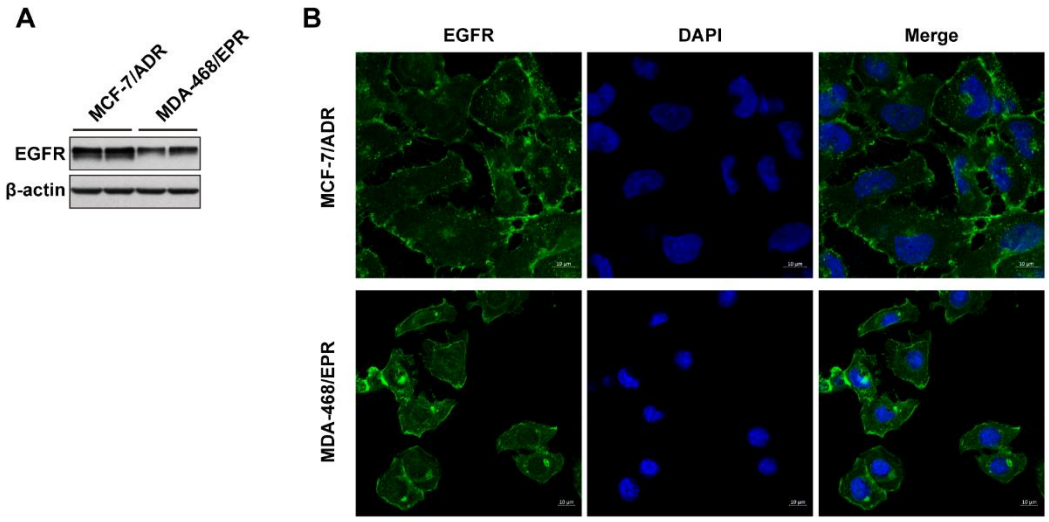

Figure S1. The expression of EGFR was higher in MCF-7/ADR cells than in MDA-MB-468/EPR cells. (A) Western blotting analysis showed that the expression of EGFR in MCF-7/ADR cells were higher than that in MDA-MB-468/EPR cells. (B) Confocal immunofluorescence microscopy analysis showed that the expression of EGFR in two drug-resistant cells mainly located in the cell membrane.

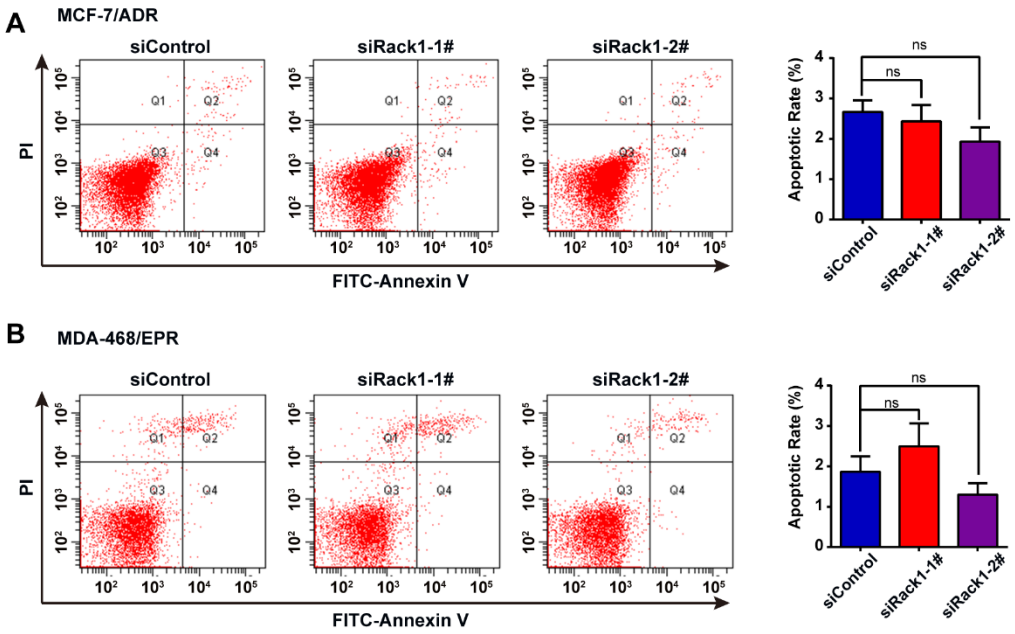

Figure S2. Silencing the expression of Rack1 had no significant effect on apoptosis in drug-resistant cancer cells. (A and B) Flow cytometry based apoptotic assay showed that the knockdown of Rack1 in two drug-resistant cells had no significant effect on cell death compared with that in control cells. The proportion of apoptotic cells at early stage (PI^-/Annexin V^+) and late stage (PI^+/Annexin V^+) was shown; mean ± SD, n = 3, $^{ns}P > 0.05$ versus siControl, ns means no statistical difference.

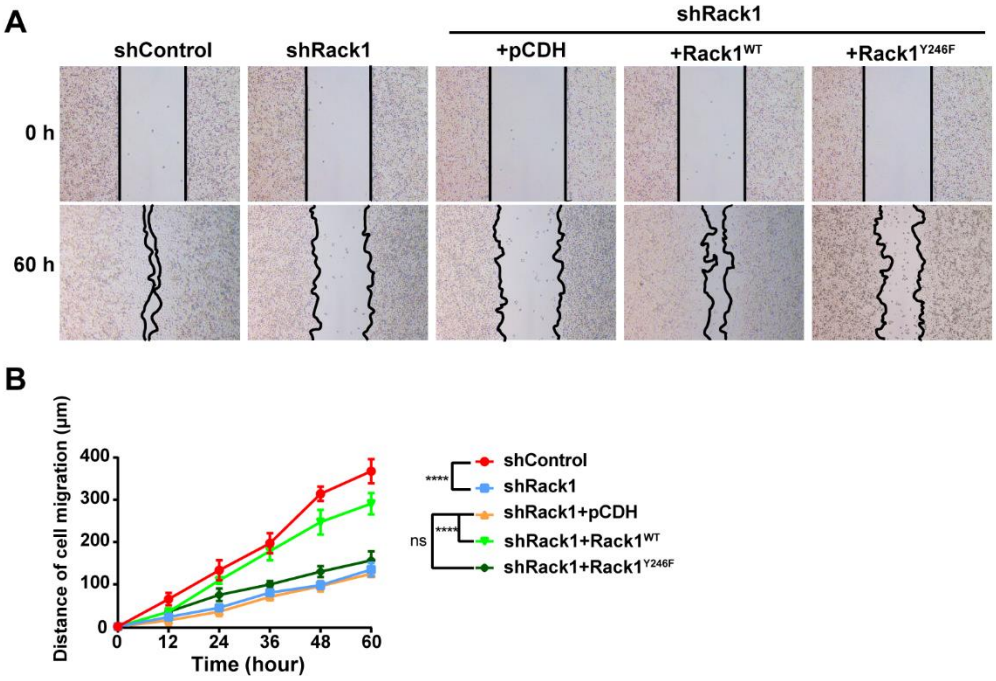

Figure S3. Re-expression of Rack1^{WT}, not Rack1^{Y246F}, rescued migration ability in drug-resistant cancer cells. (A) Wound healing assay showed that re-expression of Rack1^{WT}, but not Rack1^{Y246F} mutant, rescued cell migration ability in MCF-7/ADR cells. (B) The relative cell migration distance was quantified and plotted in the lower panel. Data are shown as mean ± SD; n = 6. Statistical analysis was performed by two-way ANOVA. ***$P < 0.001$ and ns $P > 0.05$ indicates no statistical significance.

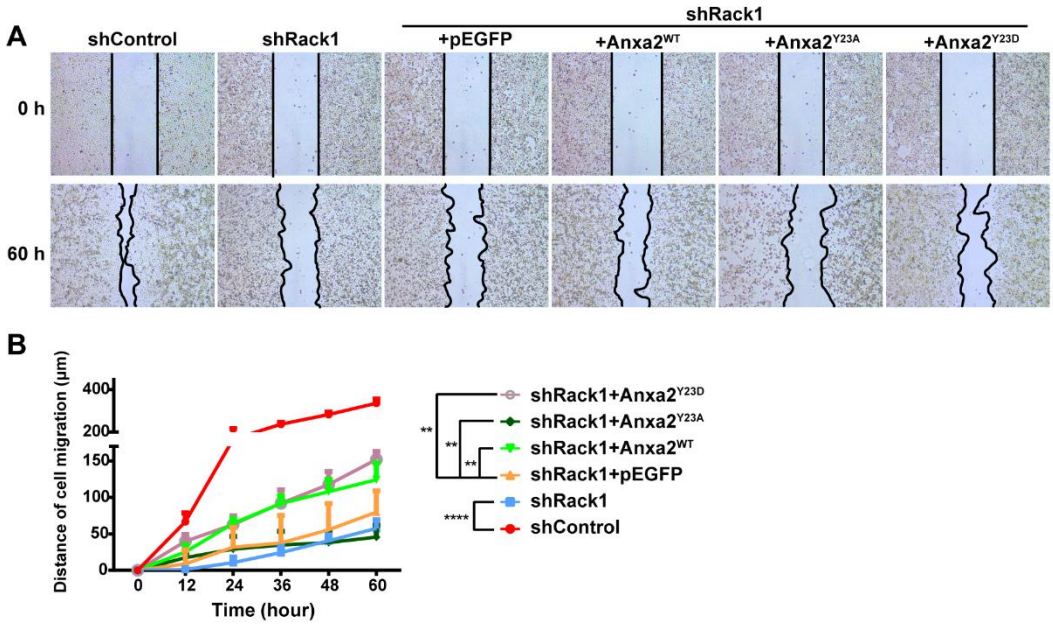

Figure S4. Increased expression of Anxa2^{WT} or Anxa2^{Y23D} in Rack1-silenced cells recovered cell migration ability. (A and B) Wound healing assay showed that the overexpression of Anxa2^{WT} or Anxa2^{Y23D}, not Anxa2^{Y23A}, partially rescued the cell migration ability in Rack1 silenced MCF-7/ADR cells. Data are shown as mean ± SD; n = 6. Statistical analysis was performed by two-way ANOVA. ****$P < 0.0001$ and **$P < 0.01$.

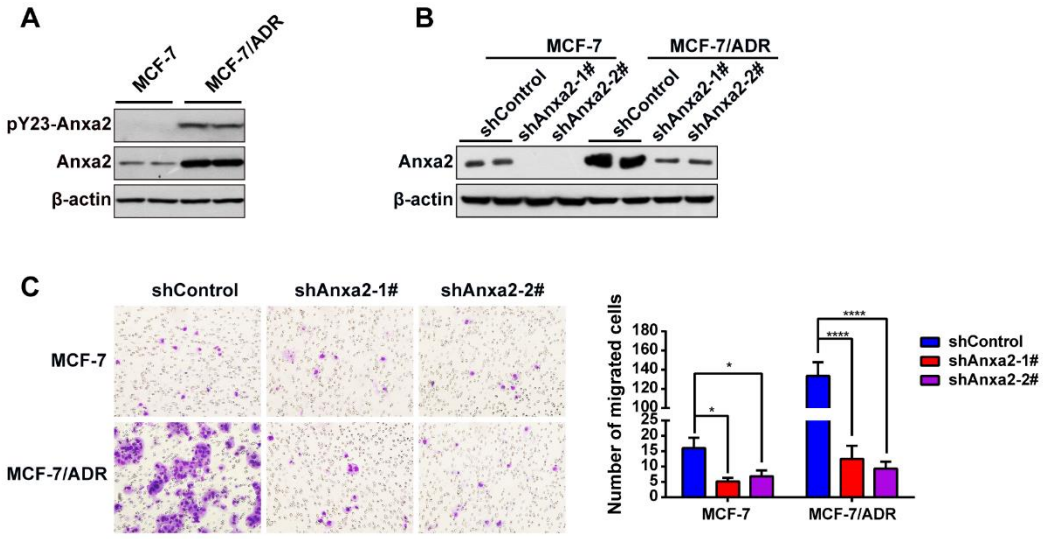

Figure S5. Silencing of Anxa2 expression attenuates migration ability in breast cancer cells. (A) Western blotting analysis showed that the expression of Anxa2 and pY23-Anxa2 in drug resistant MCF-7/ADR cells were elevated compared with that in MCF-7 cells. (B and C) Silencing the expression of Anxa2 in MCF-7 and MCF-7/ADR cells decreased migration ability as measured by transwell assay. Data were displayed as mean ± SD; n = 6. Statistical analysis was performed by one-way ANOVA. *P < 0.05, ****P < 0.0001 versus shControl.