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, nsP > 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 Anxa2WT or Anxa2Y23D in Rack1-silenced cells recovered cell migration ability. (A and B) Wound healing assay showed that the overexpression of Anxa2WT or Anxa2Y23D, not Anxa2Y23A, 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.