

Chalcomoracin prevents vitreous-induced activation of AKT and migration of retinal pigment epithelial cells

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Fig. S1

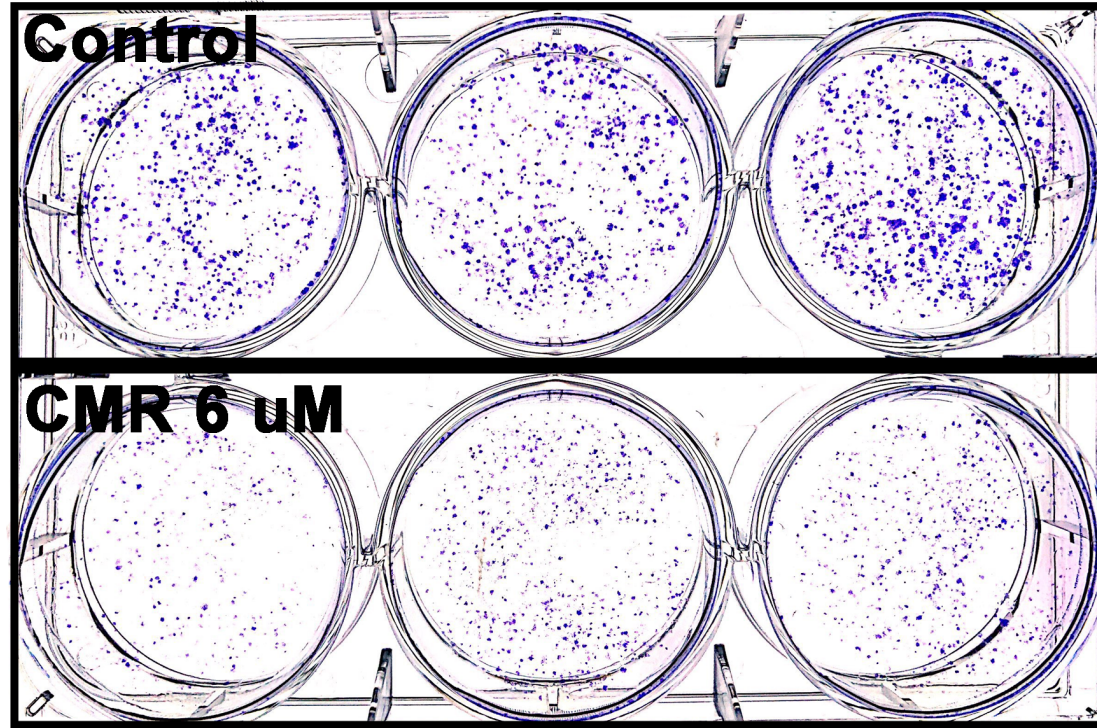


Fig. S1 CMR can inhibit proliferation of MDA-MB-231 cell.

Clonogenic assay. MDA-MB-231 cells were cultured overnight in a 6-well plate (1×10^4 cells per well) and then treated with CMR 6 μM for 9 days to form colonies. Then culture medium was removed and the cell monolayer was fixed with solvent contained methanol and glacial acetic acid (3:1) for 15 min and stained with crystal violet (0.2%) for 30 min to visualize cell colonies. Each group was repeated in triplicate.

Fig. S2

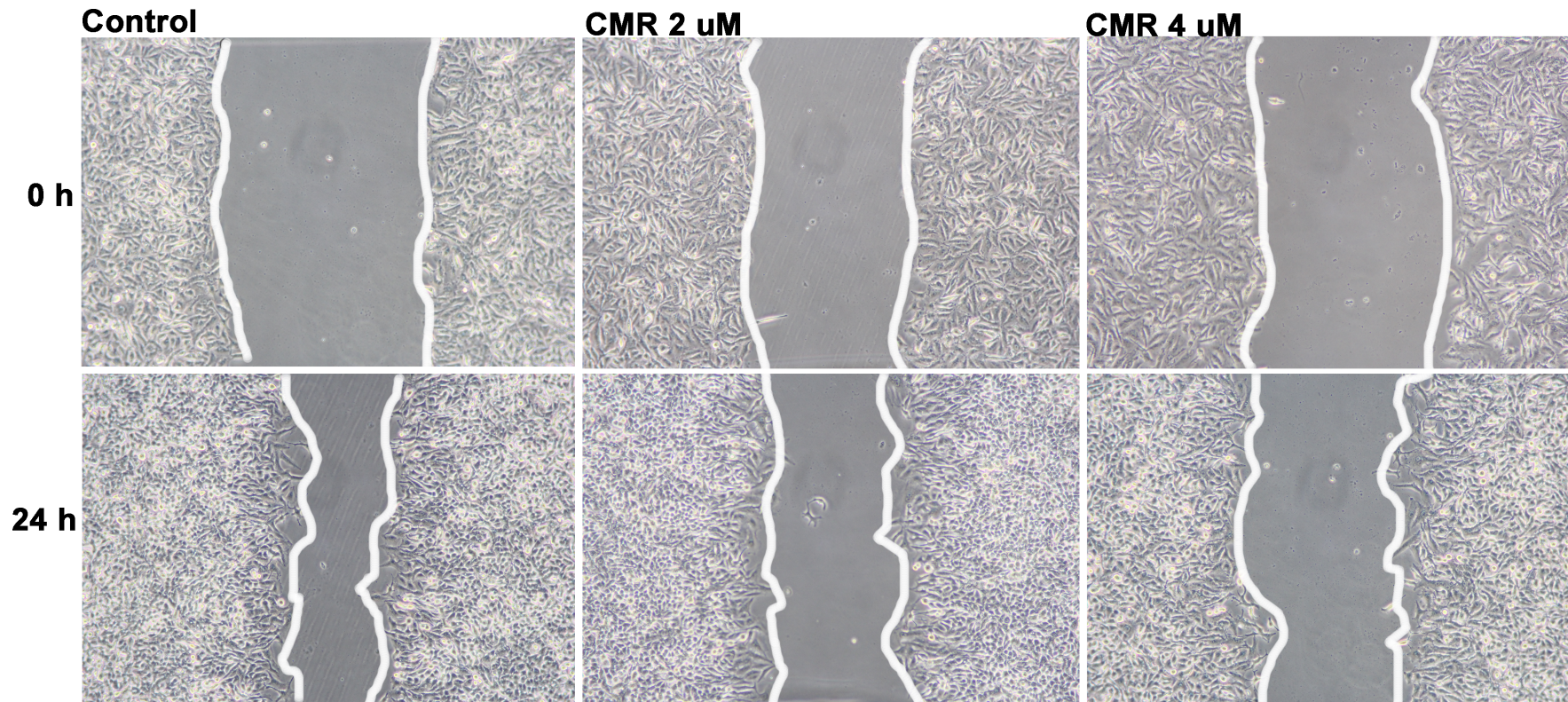


Fig. S2 CMR can inhibit migration of MDA-MB-231 cell.

When MDA-MB-231 cells were grown to confluence in 6-well plates, autoclaved 200 μ l pipet tips were used to scratch the cells across the wells. The detached cells were aspirated out in PBS, and the rest cells were treated with plus or minus CMR (2 μ M and 4 μ M). The scratched areas were photographed to capture the initial width and photographed again 24 h later.

Fig. S3

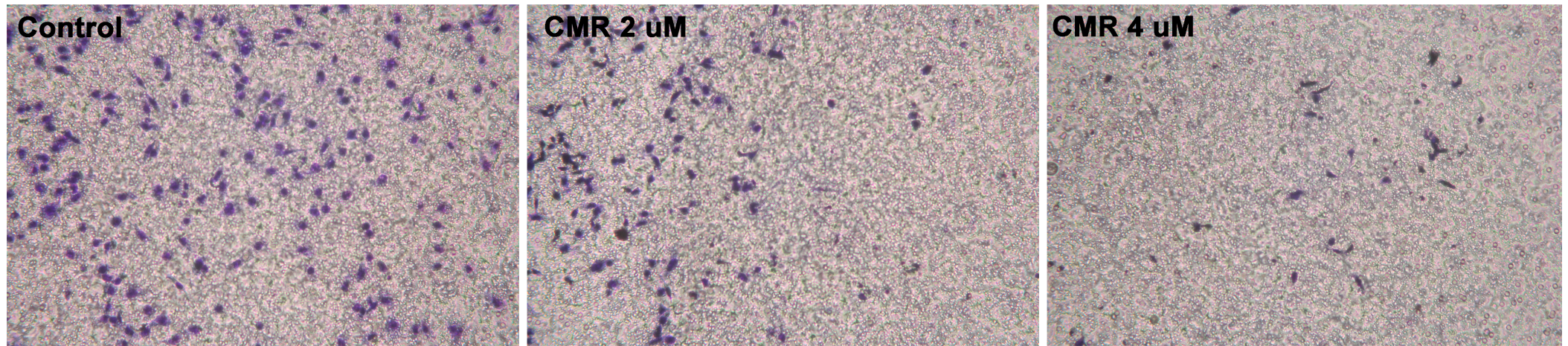


Fig. S3 CMR can inhibit invasion of MDA-MB-231 cell.

Transwell assay was performed to estimate the effects of CMR on the invasion of MDA-MB-231 cells. Matrigel (5 mg/ml to 1 mg/ml) was diluted in serum free-cold DMEM medium and put 100 μ l of the diluted matrigel into upper chamber of 24-well transwell. Incubated the transwell at 37 °C for 4 h for gelling. Cells were harvested by Trypsin/EDTA and resuspended in media containing 1% FBS at a density of 100 cells/ml. Gently wash gelled matrigel with warmed serum free media. Put 100 μ l of the cell suspension onto the matrigel. 600 μ l of DMEM containing 2 uM or 4 uM CMR was filled in the upper chamber. After 24 h of incubation, transwells were removed from the 24-well and cells were fixed with solvent contained methanol and glacial acetic acid (3:1) for 15 min and stained with crystal violet (0.2%) for 30 min. The noninvaded cells on the top of the transwell were scraped off using a cotton swab and the invaded cells were taken pictures under light microscope.