METHOD

Wound healing assay

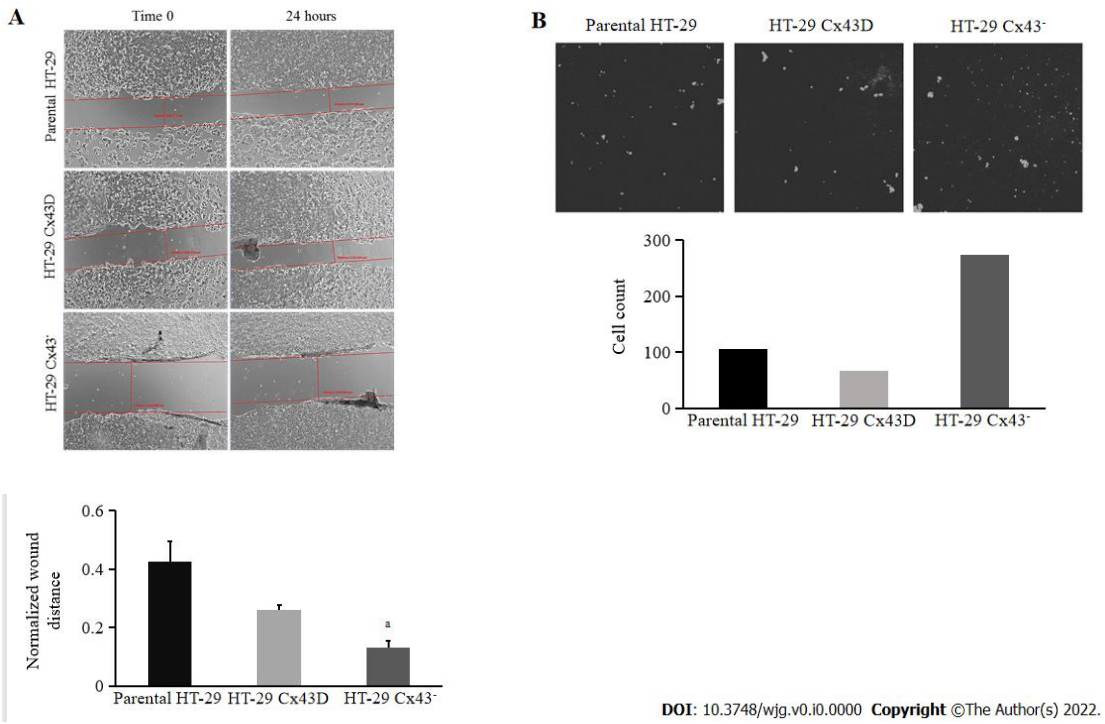
Parental HT-29, HT-29 Cx43D, and HT-29 Cx43- cells were seeded in 12-well plates and grown until a confluent monolayer formed. Using the 10 µL-pipette tip, a scratch was made across the cell culture well, and wells were washed twice with PBS to remove cellular debris. Fresh medium containing BCECF (2′,7′–bis-(carboxyethyl)-5-(and-6)-carboxyfluorescein; Molecular Probes, Inc., Oregon, United States) was added for 4 hours to inhibit cell proliferation, followed by replenishment of the wells with fresh cell culture medium. Cells were incubated at 37 ^{o}C and photomicrographs were taken at baseline (T0) and then at 24 h using the Zeiss microscope. The ZEN software was used to measure the width of the scratch at T0 and then at 24 h, which reflects the rate of gap closure, to evaluate cell migration.

Bar graphs are results of three independent experiments.

Invasion assay

8.0 µm pore size PET cell culture inserts (Corning Inc, NY, United States) were coated with a layer of Matrigel (Diluted 1:20) for 3 h at 37 ^{o}C. Complete media was then added to the bottom of the well and parental HT-29, HT-29 Cx43D, and HT-29 Cx43- were seeded on top of the Matrigel in incomplete media to create a gradient with the serum and incubated for 24 h. Inserts were then washed with PBS, fixed with PFA 4%, wiped from the top side, cut, and put upside down on microscopic slides. Cells were stained with DAPI for 10 min at room temperature. Inserts were then mounted with coverslips using Prolong Antifade. Images were acquired on the laser scanning confocal microscope LSM 710, 258 (Carl Zeiss, Germany). Invading cells were counted using the Zeiss LSM 710 software.

Bar graphs represent cell counts of tile scans obtained from a single experiment.



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Supplementary Figure 1 Micrographs. A: Micrographs show representative fields of cells in the wound healing assay. The scratch was performed at T0, and the gap was measured 24 h later. Average wound distance is displayed in the bar graph, showing narrower gap in the HT-29 Cx43- cell subset ($P < 0.05$); B: Micrographs showing invading cells stained with DAPI in parental HT-29, HT-29 Cx43D, and HT-29 Cx43- cells 24 h post-seeding. HT-29 Cx43- cells demonstrated high invasive properties as compared to parental HT-29 cells, while HT-29 Cx43D cells were the least invasive between all conditions.