Doc S1 (The details of transfection)

Total RNA was extracted from normal human beings colon epithelium cell line HCoEpic (Hongshun Biotechnology, Shanghai, China) at 80% confluence by TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.). 5 μg of total RNA was used for cDNA synthesis using BeyoRT cDNA synthesis kit (D7166; Beyotime Institute of Biotechnology, Haimen, China). The reverse transcription reaction mixture contained 5 μg total RNA, 1 μl random hexamer primer and diethyl pyrocarbonate -treated water to produce a final volume of 12 μl. After incubation in 70˚C for 5 min, the mixture was cool down on ice, the following agents were added: 1 μl RNase inhibitor, 2 μl dNTP mix, 1 μl reverse transcriptase and 4 μl reaction buffer. The mixture was gently mixed and centrifuged transiently at 6,000 ×g at 4˚C. The supernatant was incubated at 25˚C for 10 min and 42˚C for 60 min. The reaction was terminated by incubation at 70˚C for 10 min and the product was used for the following polymerase chain reaction (PCR). cDNA of the human Cx43 coding region was amplified by PCR using PCR kit with Taq (D7232; Beyotime Institute of Biotechnology) according to the manufacturer's instructions. The Cx43 primers used were as follows: forward, 5'-cacaattgagtggaatcttgatg-3'; reverse, 5'-caa cat ggg tga ctg gag c-3'. β-actin was used as the control. The β -actin primers used were as follows: forward, 5'-gtg ggg cgc ccc agg cac ca-3'; reverse, 5'-cttccttaatgtcacgcacgatttc-3'. The PCR reaction mixture contained 5 μl 10×PCR buffer, 4 μl 2.5 mM dNTP, 0.1 μg template, 2 μl primers, 0.25 μl Taq DNA polymerase, and was added to 50 μl of total volume by double distilled water. The PCR profile was 94˚C for 30 sec, 55˚C for 30 sec, and 72˚C for 60 sec for 30 cycles, followed by 72˚C for 10 min. The results were analyzed and quantified by Quantity One software (v4.62; Bio-Rad Laboratories, Inc., Hercules, CA, USA). A total of 45 ng purified PCR product was inserted into 140 ng pTARGET vector (A1410; Promega Corporation, Madison, WI, USA). Constructed expression vector (0.8 μg/10^6 cells) was transfected into the paclitaxel-resistant cells by lipofection using Lipofectamine reagent, according to the manufacturer's protocol. After transfection, cultures were selected with 400 μg/ml G418. Cells of subclones were diluted and seeded to obtain further clones.