The Iron Chelators Dp44mT and DFO Inhibit TGF-β-Induced Epithelial-Mesenchymal Transition *via* Up-Regulation of N-myc Downstream Regulated Gene 1 (NDRG1)

Zhiqiang Chen[1, 2], Daohai Zhang[2], Fei Yue[1], Minhua Zheng[1,*], Zaklina Kovacevic[2] and Des R. Richardson[1, 2,*]

1. General Surgery Department of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
2. Iron Metabolism and Chelation Program, Department of Pathology, Bosch Institute, University of Sydney, New South Wales 2006, Australia

SUPPLEMENTARY FIGURES

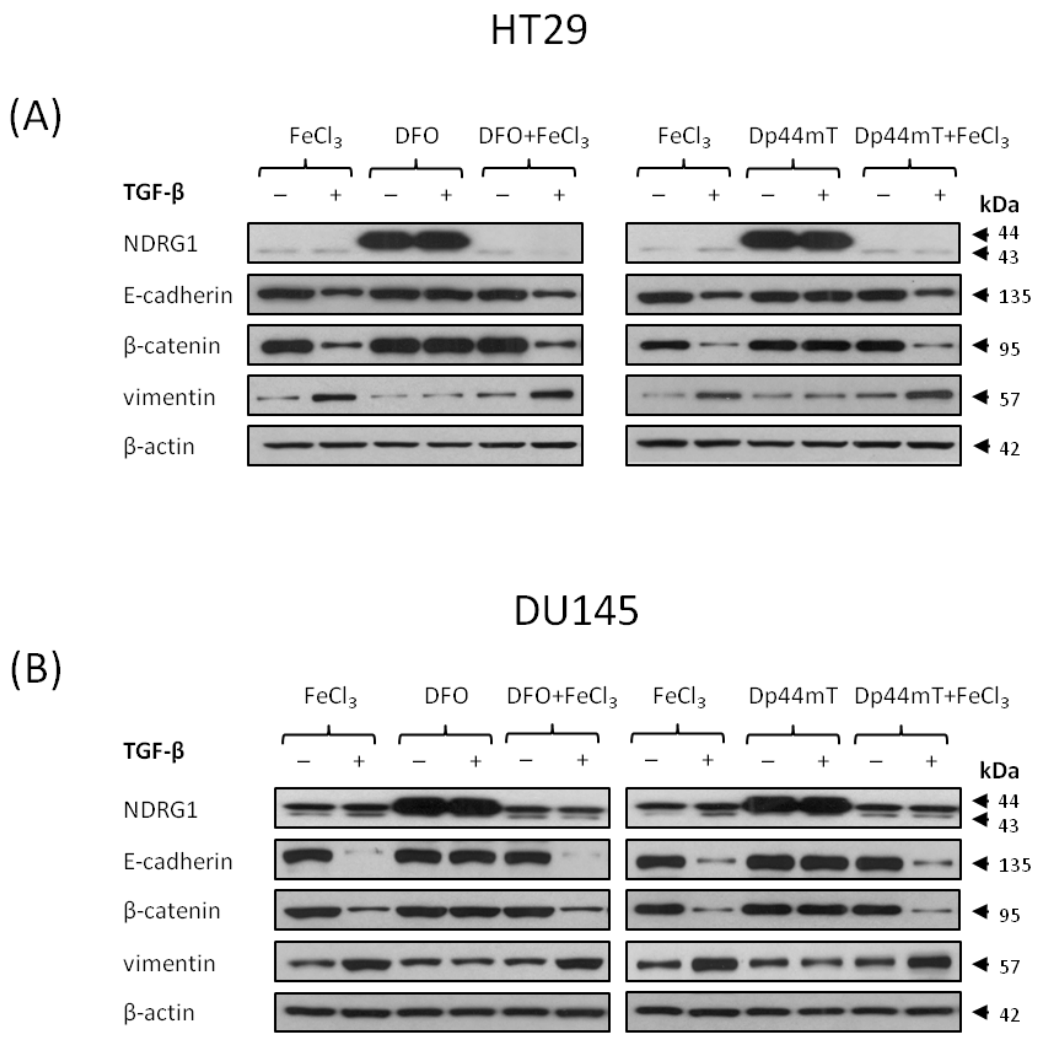

Fig. S1. Pre-treatment of iron chelators with iron ($FeCl_3$) to form their iron complexes inhibits their ability to attenuate the TGF-β-induced epithelial-mesenchymal transition (EMT). Iron chelators were pre-treated with $FeCl_3$ (1:1 molar ratio for DFO and 2:1 molar ratio for Dp44mT) before incubation with the cells. Then, HT29 and DU145 cells were pre-treated in the presence or absence of TGF-β (5 ng/mL) for 72 h (HT29) or 24 h (DU145) and followed by co-incubation with either: $FeCl_3$ (100 μM), DFO (100 μM), DFO + $FeCl_3$ (100 μM), $FeCl_3$ (10 μM), Dp44mT (10 μM) or Dp44mT + $FeCl_3$ (10 μM) for another 24 h. Western blotting analysis was used to probe the expression of NDRG1 and the EMT markers (E-cadherin, β-catenin and vimentin) in HT29 cells **(A)** and DU145 cells **(B).** Results are representative of 3 experiments.

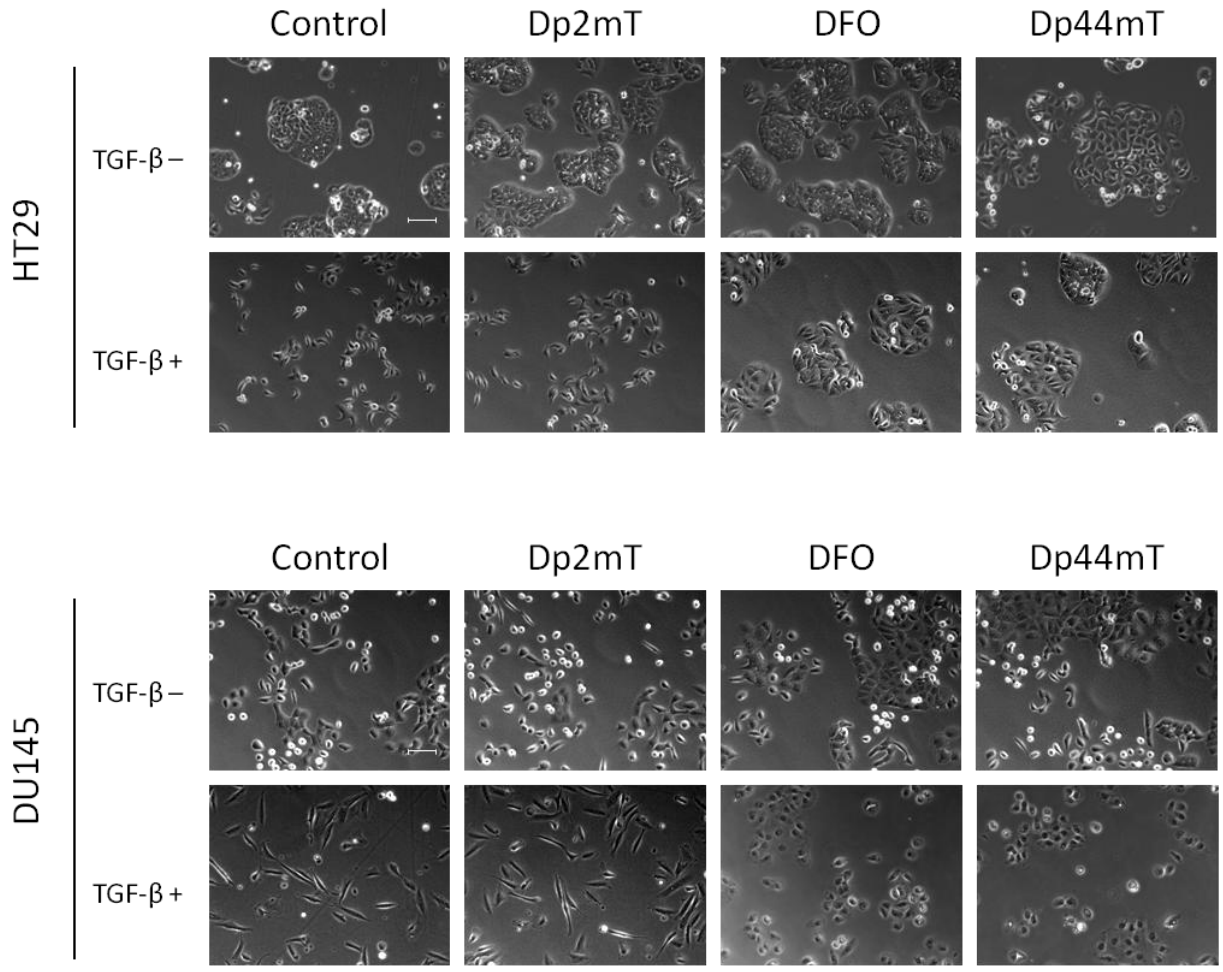

Fig. S2. Morphological changes in the HT29 and DU145 parental cell lines after incubation with iron chelators (DFO and Dp44mT) and the negative control (Dp2mT) in the presence and absence of TGF-β. Cells were pre-incubated with TGF-β (5 ng/mL) for 72 h (HT29) and 24 h (DU145) and followed by co-incubation with either DFO (100 μM), Dp2mT (10 μM) or Dp44mT (10 μM) for another 24 h. Bright field images were taken to show cell morphological changes. The cells incubated with DFO or Dp44mT maintained their epithelial phenotypes and still clustered in groups compared to the medium control and the negative control, Dp2mT (10 μM). Scale bars: 100 μm. All the images are representatives of 3 different experiments.

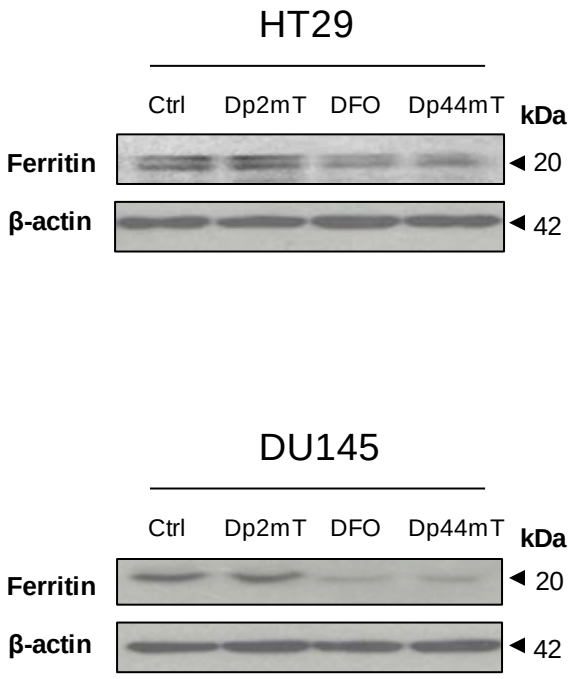

Fig. S3. The iron chelators, DFO and Dp44mT, lead to cellular iron depletion resulting in the down-regulation of ferritin in HT29 and DU145 cells. Cells were incubated with either control medium, Dp2mT (10 μM), DFO (100 μM) or Dp44mT (10 μM) for 24 h and western analysis performed. Data are typical of 3 experiments and the histogram values are mean ± SD (3 experiments).

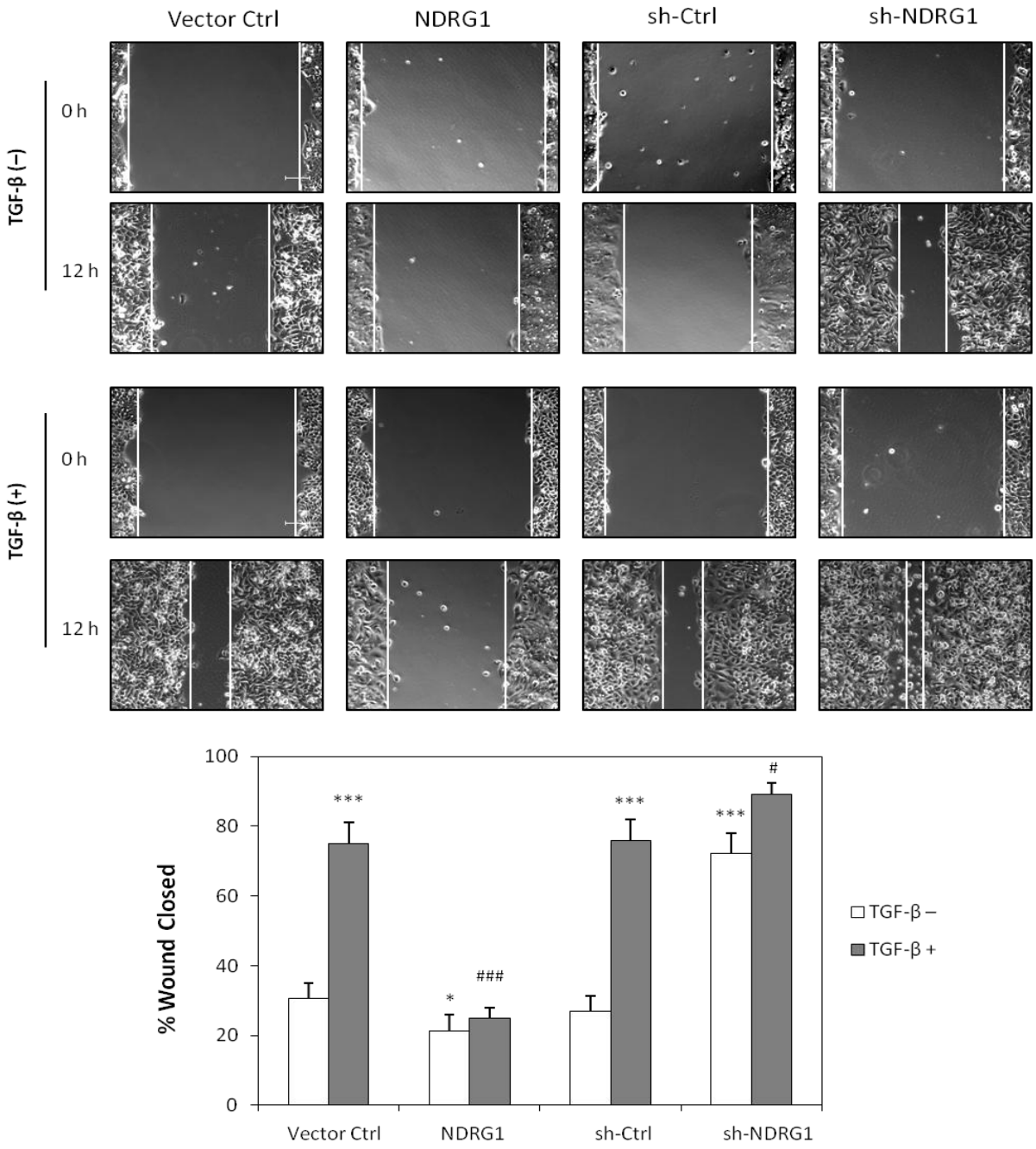

Fig. S4. Wound healing assays using NDRG1 over-expression and knock-down clones of HT29 cells incubated with TGF-β. HT29 cells were incubated with TGF-β (5 ng/mL) for 96 h to induce the EMT. Cell monolayers at 90-95% confluence were serum-starved overnight and then carefully scratched and washed to remove detached cells. The images were taken at 0 h and 12 h after scratching at the same position. Cellular migration was evaluated by measuring the percentage of the wound closed. The images shown are typical of 3 experiments and the histogram represents mean ± SD (3 experiments). Scale bars: 100 μm. * relative to vector or sh-control without TGF-β; # relative to vector or sh-control with TGF-β. ** $p<0.01$, *** $p<0.001$, # $p<0.05$, ### $p<0.001$.

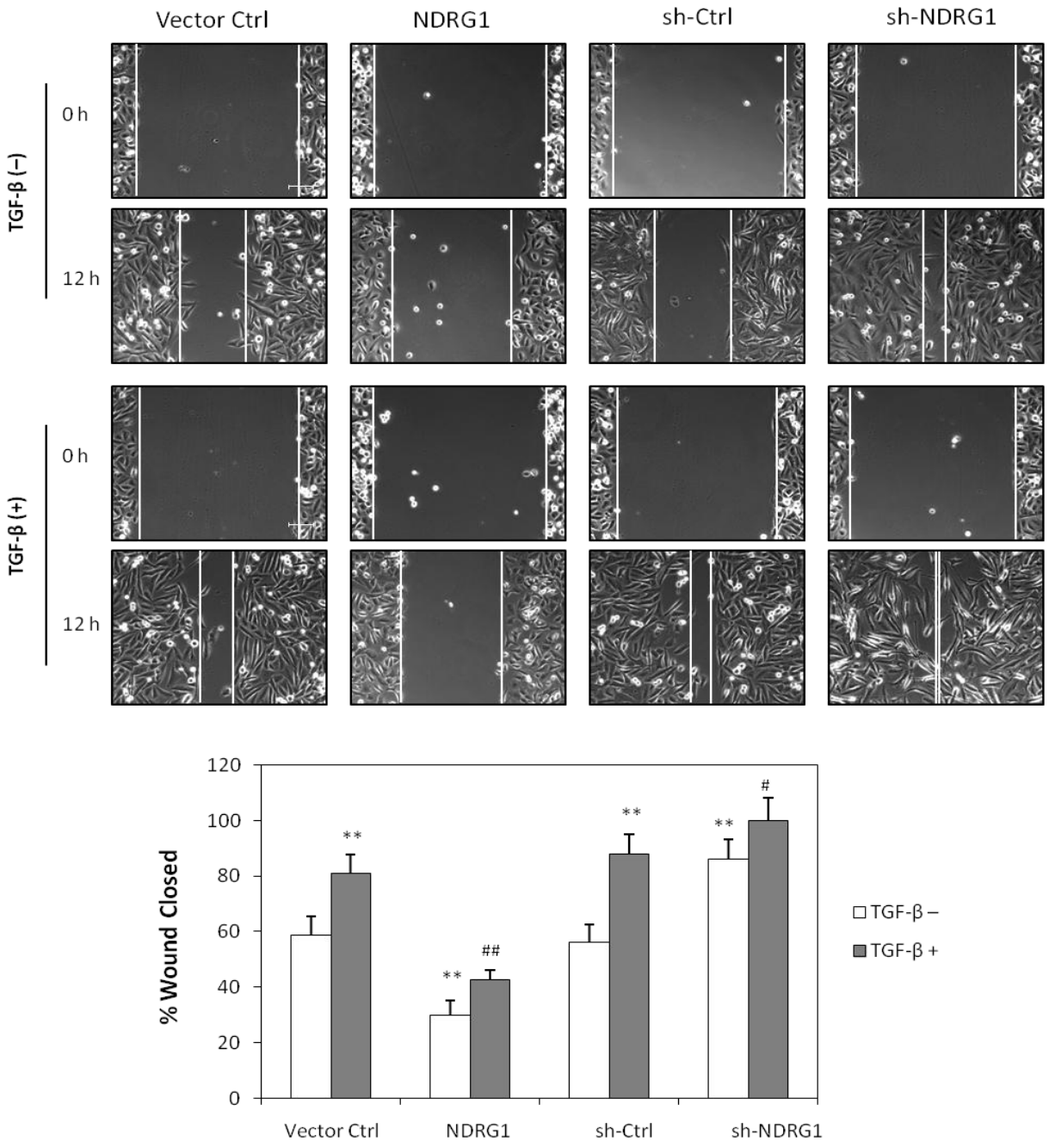

Fig. S5. Wound healing assays using NDRG1 over-expression and knock-down clones of DU145 cells incubated with TGF-β. DU145 cells were incubated with TGF-β (5 ng/mL) for 48 h to induce the EMT. Cell monolayers at 90-95% confluence were serum-starved overnight, carefully scratched and washed to remove detached cells. The images were taken at 0 h and 12 h after scratching at the same position. Cellular migration was evaluated by measuring the percentage of wound closed. The images shown are typical of 3 experiments and the histogram represents mean ± SD (3 experiments). Scale bars: 100 μm. * relative to vector or sh-control without TGF-β; # relative to vector or sh-control with TGF-β. ** $p<0.01$, # $p<0.05$, ## $p<0.01$.

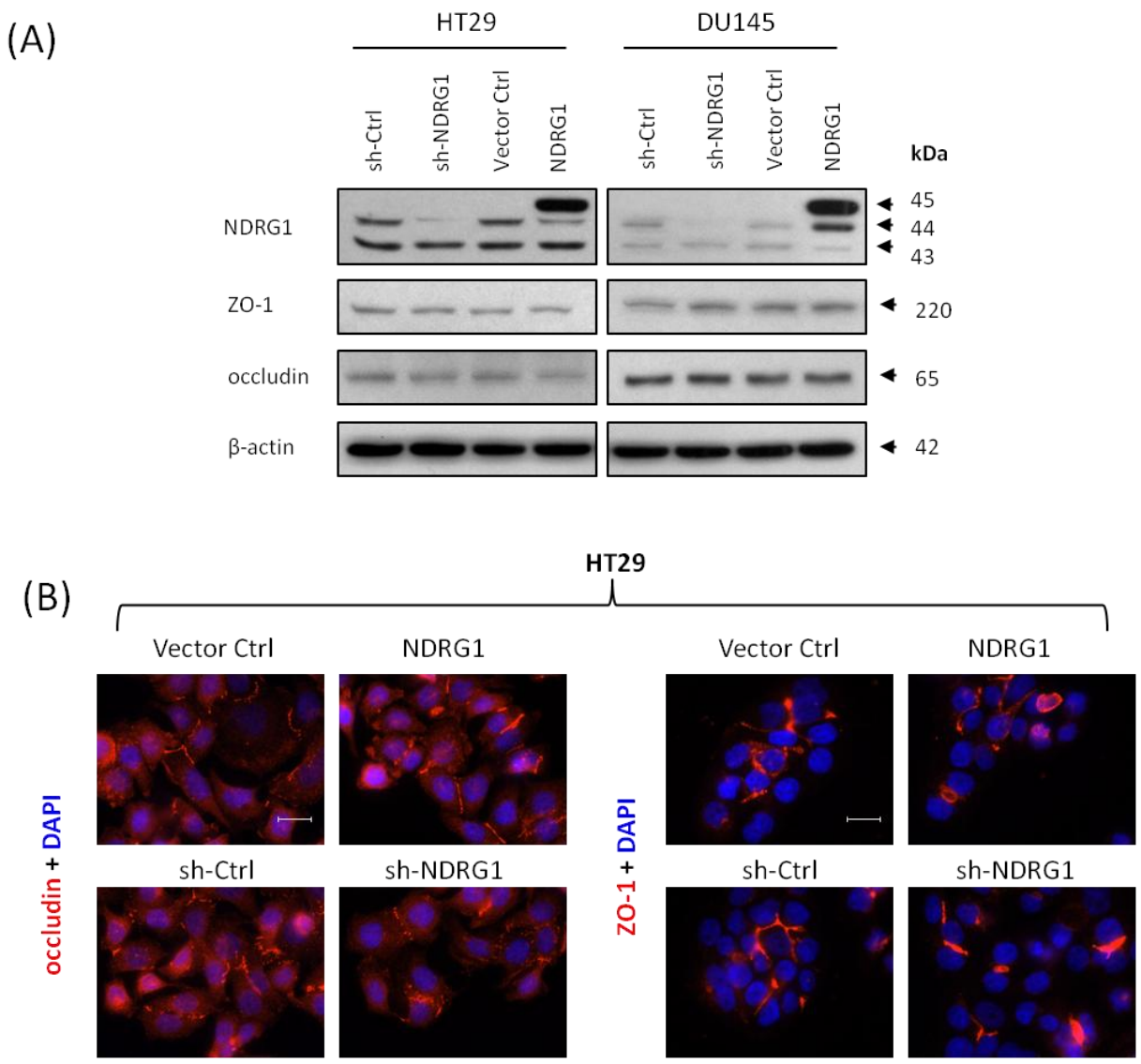

Fig. S6. Expression of the cell tight junction (TJ) components, zonula occludin-1 (ZO-1) or occludin, are not affected by modulation of NDRG1 expression. (A) Western blots showing there is no significant difference in the expression of the tight junction markers, ZO-1 or occludin, after NDRG1 over-expression or knock-down in HT29 and DU145 cells. Results are representative of 3 experiments. **(B)** Merged immunofluorescence staining showing that ZO-1 and occludin expression were not affected after NDRG1 over-expression or knock-down in HT29 cells. These observations are in agreement with the western blotting results in **(A)**. The images shown were typical of 3 separate experiments. Scale bars: 100 μm.