ATCC® HCT-116 VIM RFP Epithelial-mesenchymal Transition (EMT) Reporter Cell line Validation Data

HCT-116 VIM RFP reporter cell line (ATCC® CCL-247EMT™) was created by introducing a red fluorescent protein (RFP) reporter gene into the endogenous vimentin gene using CRISPR/Cas9 gene editing technology. We confirmed that RFP activity accurately reports vimentin gene expression, and demonstrated that vimentin-RFP expression can be induced by miR-200 family inhibitors, indicating the cells' transition to mesenchymal status. This cell line is not only a useful *in vitro* model for dissecting the molecular switches underlying EMT, but could also be used for screening compounds targeting EMT in colorectal cancer.

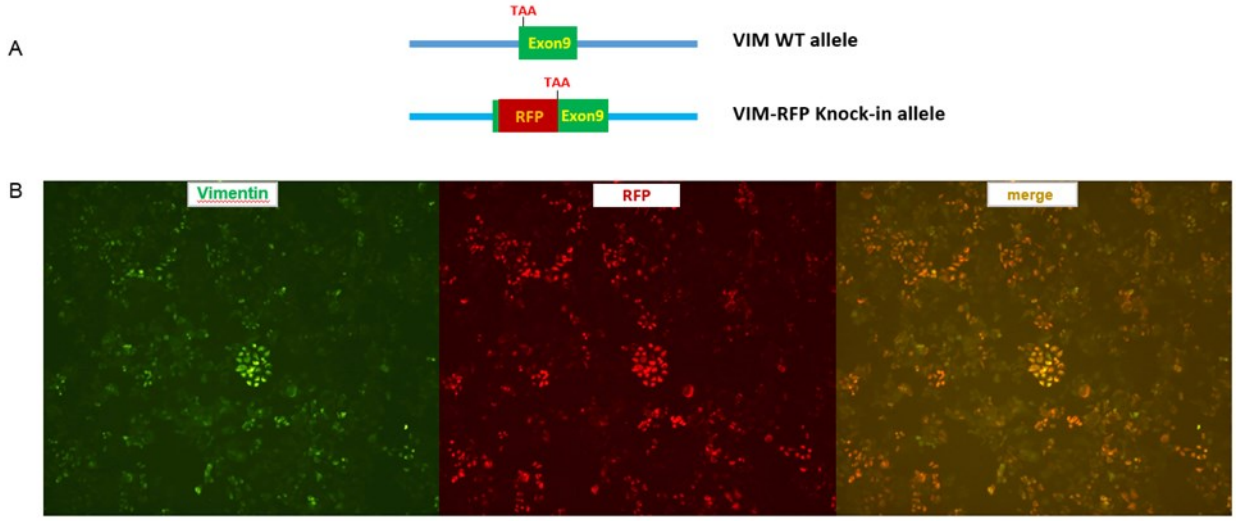

Figure 1. RFP activity accurately reports vimentin protein expression in HCT-116 VIM RFP cells (ATCC® CCL-247EMT™). (A). Partial schematic diagram of the vimentin wild type allele and RFP knock-in allele, in which the RFP gene is incorporated before the stop codon of endogenous vimentin gene at the last exon. (B). Vimentin protein detected by Immunofluorescence analysis (left, green) was co-localized with RFP (middle, red) as shown in the merged image (right).

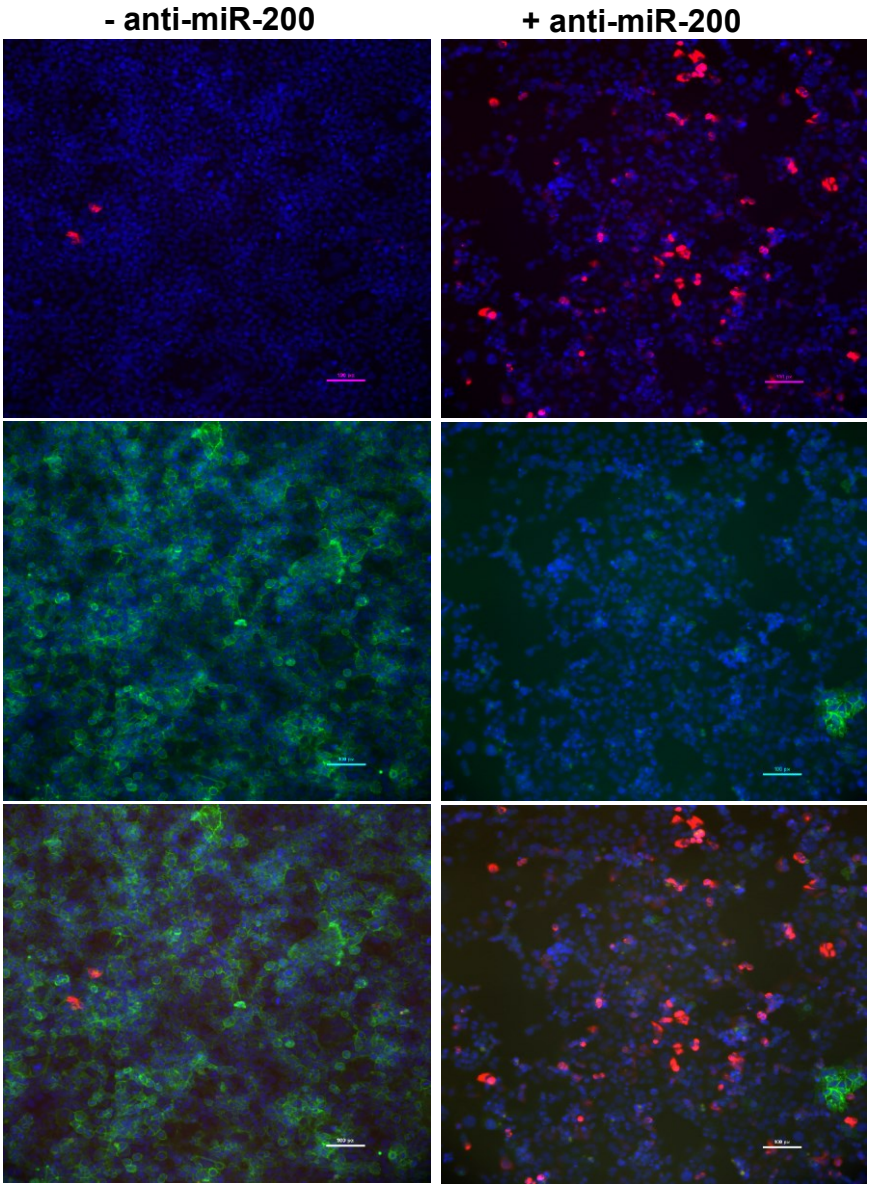

Figure 2. Induction of vimentin-RFP and E-cadherin (CDH1) protein expression in HCT-116 VIM RFP cells through introduction of exogenous miR-200 family inhibitors. HCT-116 VIM RFP cells received six cycles of transfection with either miR-200 family inhibitors (right column) or an equivalent volume of 1X Dulbecco's phosphate buffered saline (as a no treatment control, left column). miR-200 family inhibitor treatment induced a significant increase in vimentin-RFP protein expression level (red; top left and right), and decrease in E-cadherin protein expression level (green; middle left and right). The bottom panels are an overlay of the top and middle left and the top and middle right panels. The nuclei of cells were counterstained with DAPI (blue).

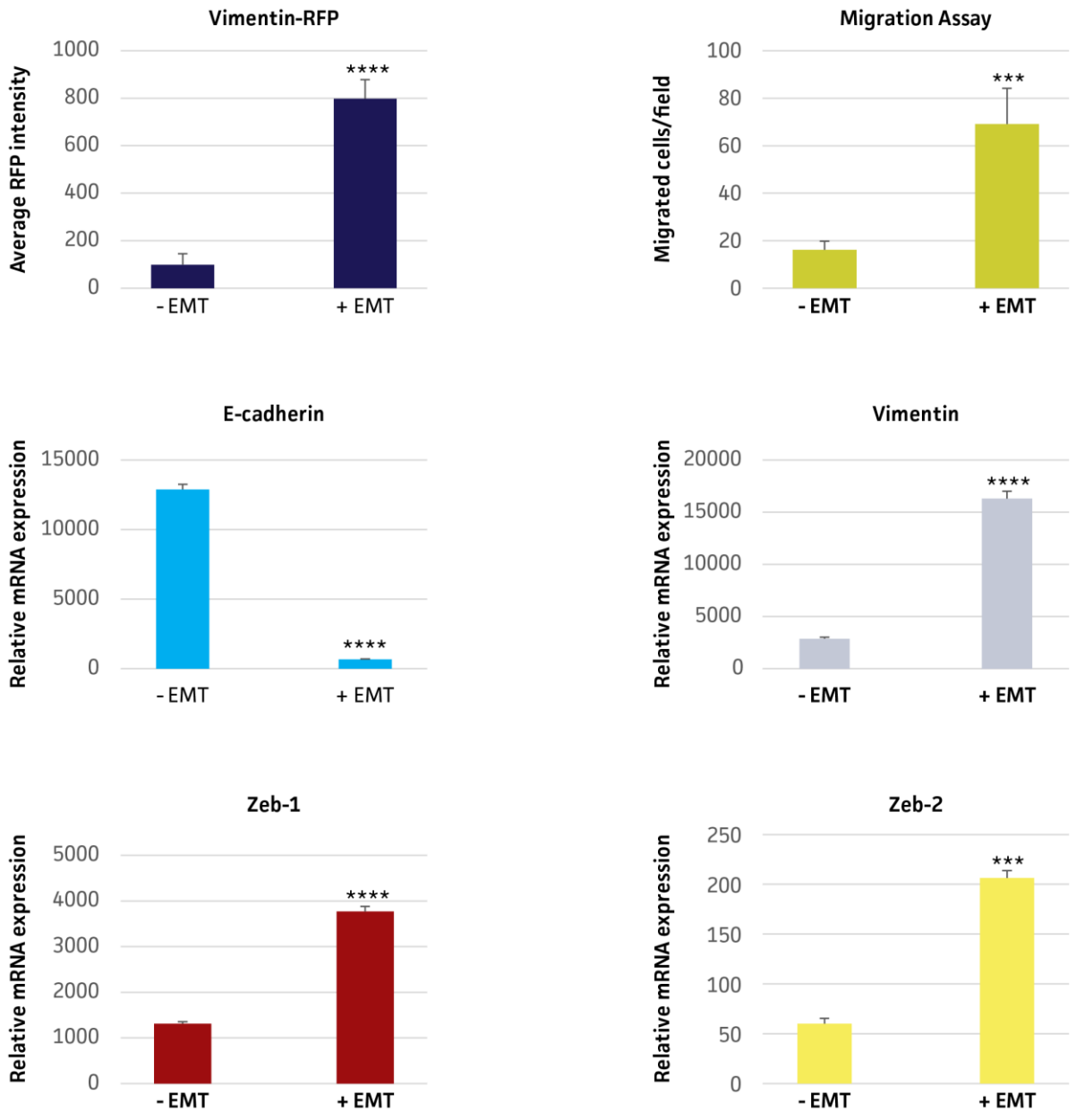

Figure 3. miR-200 family inhibitors induce EMT in HCT-116 VIM RFP cells. HCT-116 VIM RFP cells were transfected every 3-4 days with either miR-200 family inhibitors or an equivalent volume of 1X Dulbecco's phosphate buffered saline (as a no treatment control). (A) 22 days after the initial transfection, vimentin-RFP protein fluorescence intensities of the cells were measure by CellInsight™ CX7 High Content Imaging Analysis System. HCT-116 VIM RFP cells treated with miR-200 inhibitors, showed an approximately 8-fold increase in RFP fluorescence intensity compared to control. (B) HCT-116 VIM RFP cells treated with miR-200 inhibitors displayed increased migration capacities at day 18 after the initial transfection. (C) mRNA expression of EMT transcriptional factors ZEB1, ZEB2, as well as EMT markers E-cadherin and vimentin were quantified by digital droplet PCR at day 22 after the initial transfection. (Data are means ± SD. n=5 for A. n=3 for B and C. Student's t-test, ***p<0.001, ****p<0.0001).

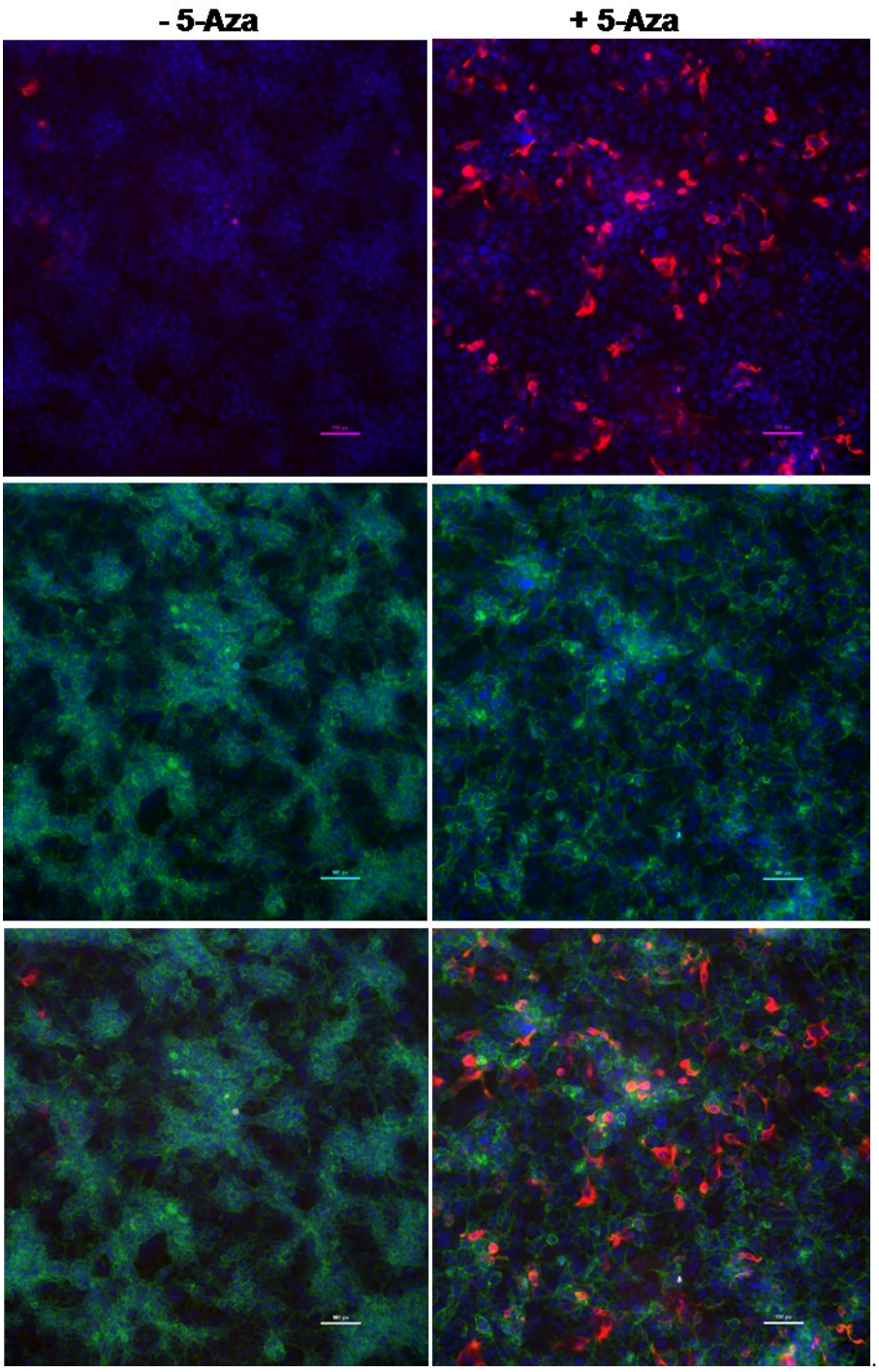

Figure 4. 5-aza-2'-deoxycytidine treatment induced an increase in vimentin-RFP protein expression, but did not significantly affect E-cadherin protein expression in HCT-116 VIM RFP cells. HCT-116 VIM RFP cells were incubated in complete growth media supplemented with either 2 µM 5-aza-2'-deoxycytidine (right column) or an equivalent volume of 1X Dulbecco's phosphate buffered saline (as a no treatment control; left column) for 2 days. Treatment of HCT-116 VIM RFP cells with 5-aza-2'-deoxycytidine induced an increase in vimentin-RFP protein expression (red; top left and right), however, E-cadherin protein expression (green; middle left and right) was not significantly affected by 5-aza-2'-deoxycytidine treatment. The bottom panels are an overlay of the top and middle left and the top and middle right panels. The nuclei of cells were counterstained with DAPI (blue).