

Technical Data Sheet: MDA-MB-231 VIM RFP

ATCC® Number	HTB-26MET™
Organism	<i>Homo sapiens</i>
Tissue/Disease Source	Breast/adenocarcinoma
Product Description	MDA-MB-231 VIM RFP (ATCC® HTB-26MET™) is a reporter line designed to enable the real-time monitoring of the changing status of cells from mesenchymal to epithelial (MET), via the concurrent reduced expression of red fluorescent protein (RFP)-tagged vimentin (VIM).
Application	This cell line is not only an aid in dissecting the EMT/MET pathway in the research field, but also is a robust platform for anti-EMT drug screening, metastatic breast cancer drug screening, and vimentin intermediate filament dynamics.

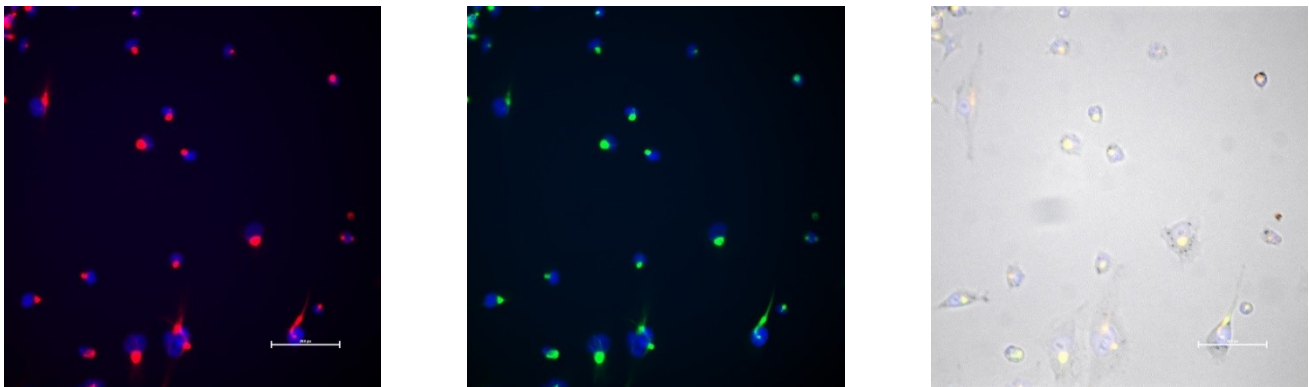


Figure 1: Clonal expression of VIM and RFP. MDA-MB-231 VIM RFP cell line expresses RFP (red; left panel) fused to VIM (green; middle panel). The nuclei of cells were counterstained with NucBlue® Live ReadyProbes® Reagent (blue; Thermo Fisher Scientific). A merged image (right panel) shows colocalization of RFP with VIM indicating clonal expression of the VIM-RFP fusion protein.

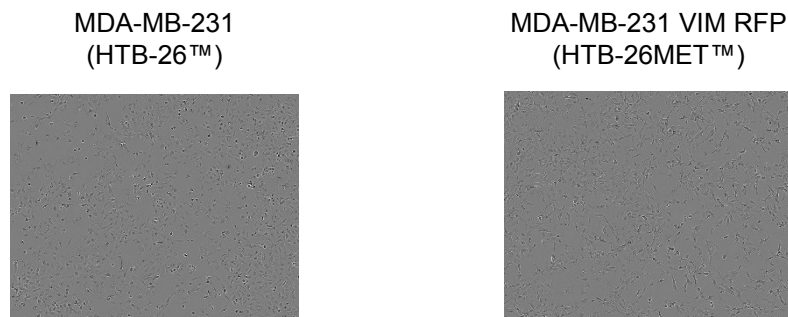


Figure 2: Cell Morphology of MDA-MB-231 parental and MDA-MB-231 VIM RFP. Photomicrographs of parental and derivative cell lines indicate similar morphology. Cells were maintained in ATCC recommended culture conditions. Cell morphology was monitored using an IncuCyte Live Cell Analysis System (Essen BioScience).

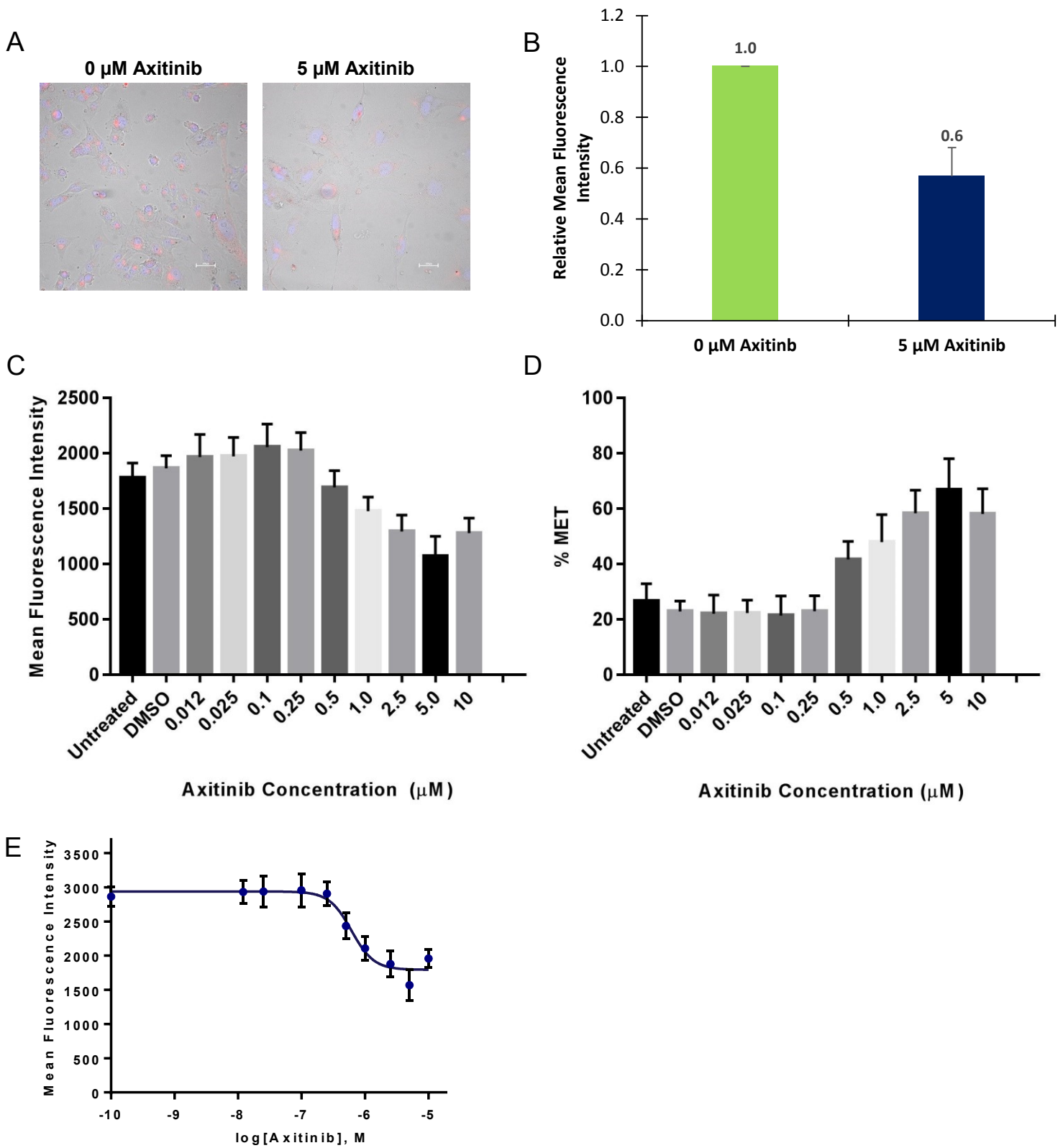


Figure 3. MET induction via tyrosine kinase inhibition. A) MDA-MB-231 VIM RFP cells were treated with vehicle (left panel) or 5 μM Axitinib (right panel) for 3 days, counterstained with NucBlue Live ReadyProbes Reagent (blue) and fixed. The left panel shows a visible reduction in the intensity of VIM RFP compared to the right panel, indicating a reduction in vimentin expression. B-E) MDA-MB-231 VIM RFP cells were treated with the indicated concentrations of Axitinib for 3 days, counterstained with NucBlue Live ReadyProbes Reagent and fixed as above. Mean fluorescence intensity was monitored via high-content imaging. Axitinib treatment of MDA-MB-231 cells resulted in a reduced intensity of VIM RFP staining in a concentration-dependent manner, demonstrating that this compound can induce an epithelial phenotype into the cells; $IC_{50} = 6.126e-007$.

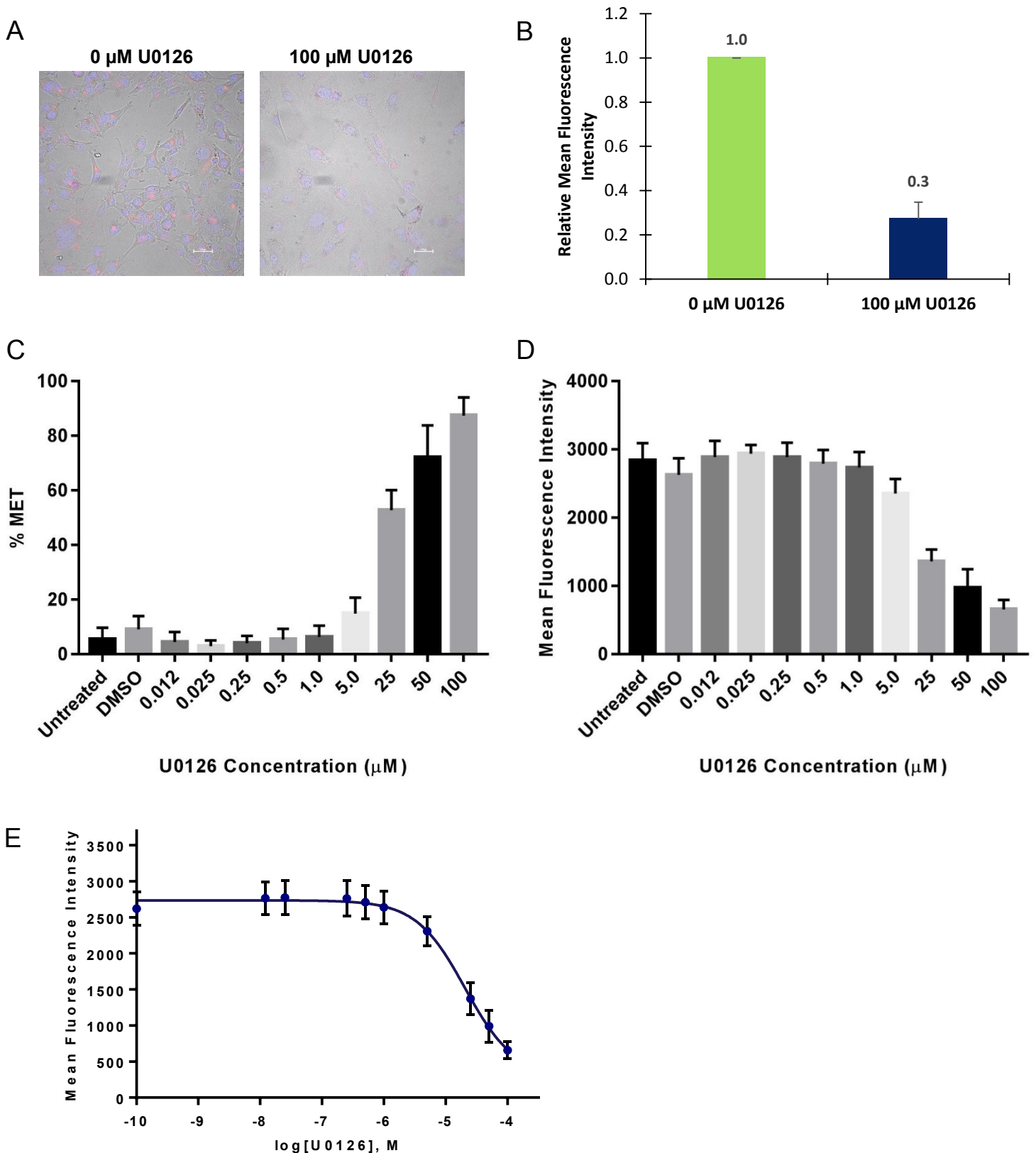


Figure 4. MET induction via MEK1/2 inhibition. A) MDA-MB-231 VIM RFP cells were treated with vehicle (left panel) or 100 μM U0126 (right panel) for 3 days, counterstained with NucBlue Live ReadyProbes Reagent (blue) and fixed. The left panel shows a visible reduction in the intensity of VIM RFP compared to the right panel, indicating a reduction in vimentin expression. B-E) MDA-MB-231 VIM RFP cells were treated with the indicated concentrations of U0126 for 3 days, counterstained with NucBlue Live ReadyProbes Reagent and fixed as above. Mean fluorescence intensity was monitored via high-content imaging. U0126 treatment of MDA-MB-231 cells resulted in a reduced intensity of RFP staining in a concentration-dependent manner, demonstrating that this compound can induce an epithelial phenotype into the cells; $IC_{50} = 2.044e-005$.

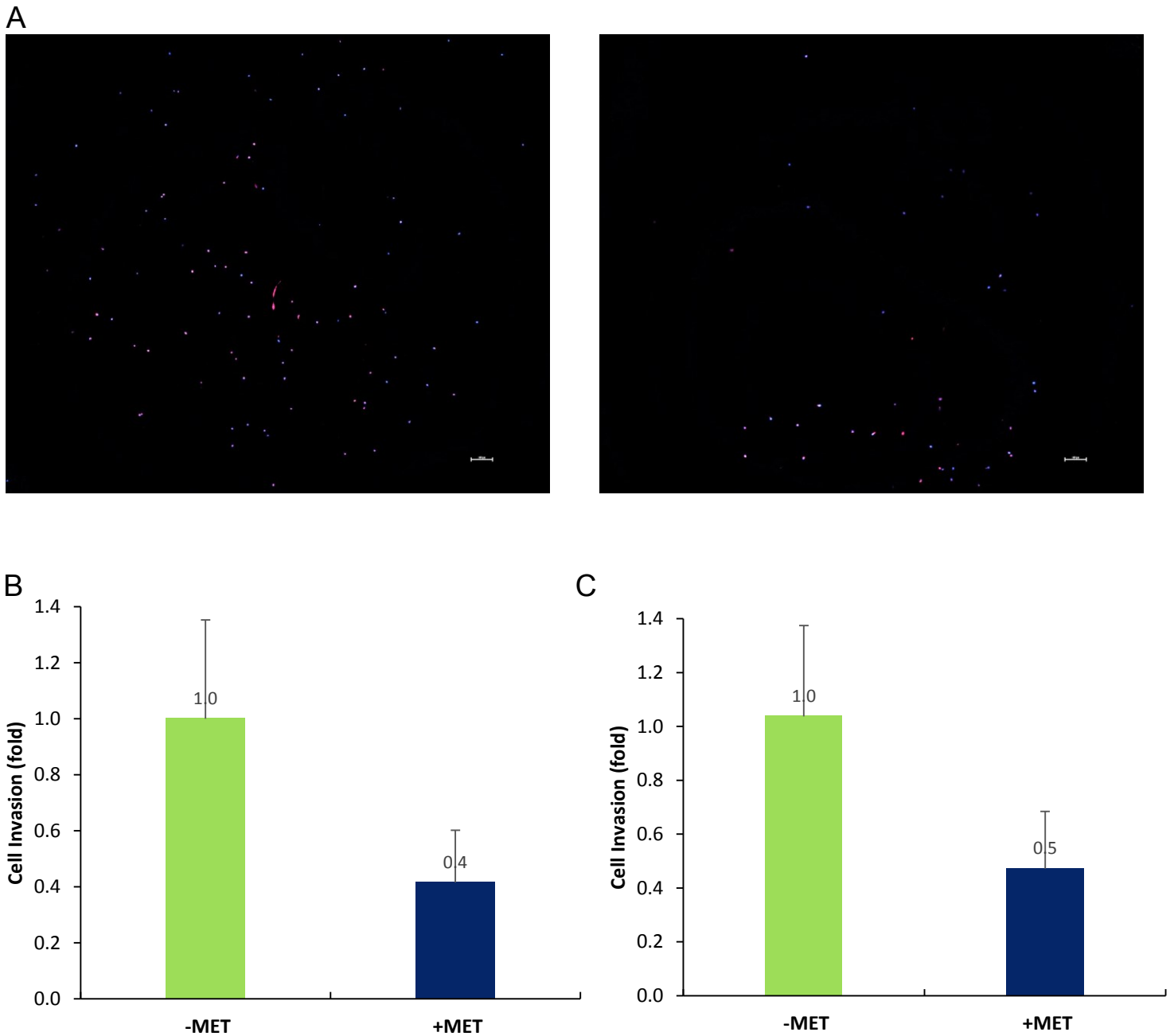


Figure 5. EMT-induced cells have decreased invasion capacities. A) MDA-MB-231 VIM RFP cells were treated with vehicle (left panel) or 100 μ M U0126 (right panel) for 3 days. The NucBlue counterstained cells (blue) were subjected to an invasion assay, which consisted of monitoring the migration of cells through the 8 μ m pore filter of a Fluoroblok (Corning) basement membrane after incubation for 24 hours. Migrated cells were visualized on an inverted fluorescence microscope and counted via B) NucBlue nuclear stain and C) RFP expression. Treatment of MDA-MB-231 RFP cells with U0126 resulted in a reduced number of migrated cells, signifying an increase in the presence of epithelial-like cells and a reduction in mesenchymal-like cells.

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