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Technical Data Sheet: MCF 10A Ecadherin EmGFP

ATCC® Number	CRL-10317EMT™
Organism	*Homo sapiens*
Tissue/Disease Source	Fibrocystic disease
Product Description	Cells undergoing EMT often display downregulation of epithelial markers (such as E-cadherin; ECAD) and upregulation of mesenchymal markers (such as vimentin; VIM). Here, we created an ECAD-EmGFP reporter cell line using the CRISPR/Cas9 gene editing platform and the parental MCF 10A (ATCC® CRL-10317™). The created CRL-10317EMT cell line harbors a C-terminal green fluorescent protein (EmGFP) tag on the E-cadherin gene. This will enable the tracking of the EMT status of cells in vitro by monitoring GFP expression. The integrity of the ECAD-EmGFP knock-in has been verified at the genomic, mRNA and protein level for sequence and expression. The MCF 10A ECAD-EmGFP reporter cell line provides a convenient and sensitive platform for research on the mechanisms of metastasis in vitro and the development of new antitumor drugs for metastatic breast cancer.
Application	Epithelial to mesenchymal transition (EMT), anti-EMT drug screening, breast cancer drug screening, E-cadherin expression dynamics.

Design and validation of expression

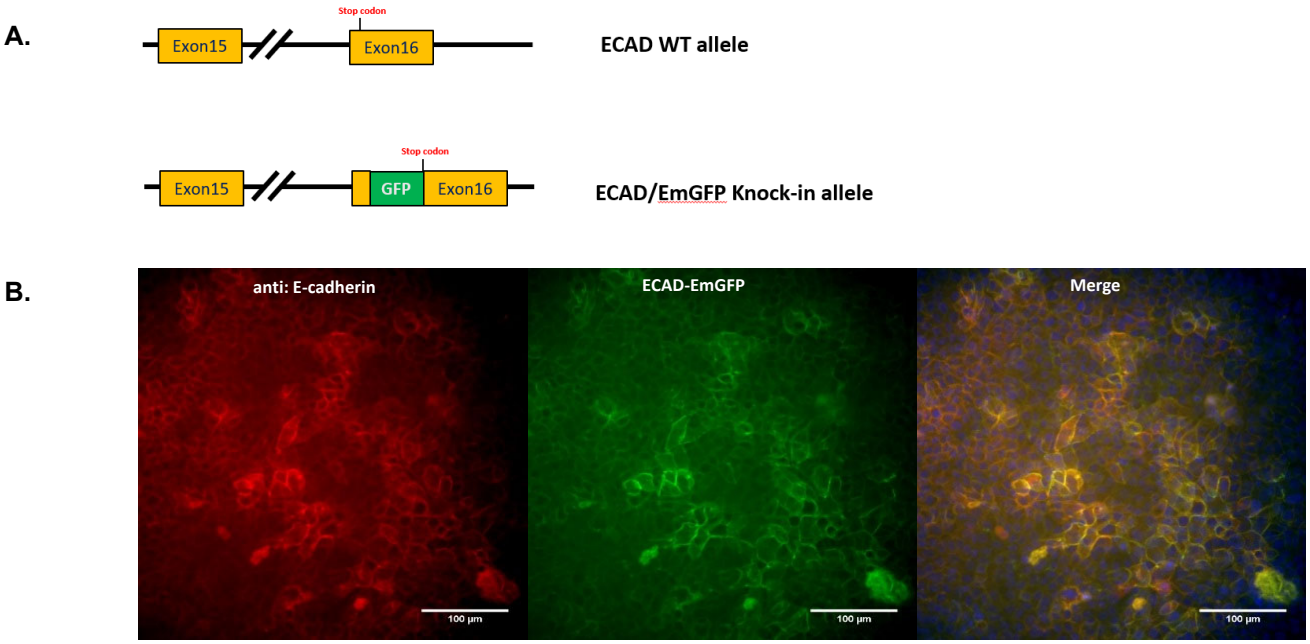

Figure 1: EmGFP activities accurately report E-cadherin gene expression in MCF 10A ECAD-EmGFP cells. (A) Partial schematic diagram of the E-cadherin wild type allele and ECAD-EmGFP knock-in allele, in which EmGFP is incorporated adjacent to endogenous E-cadherin in Exon 16. (B) Endogenous EmGFP (middle, green) co-localized with E-cadherin detected by immunofluorescence assay (left, red) as shown in the merged image (right).

EMT induction, migration, and invasion

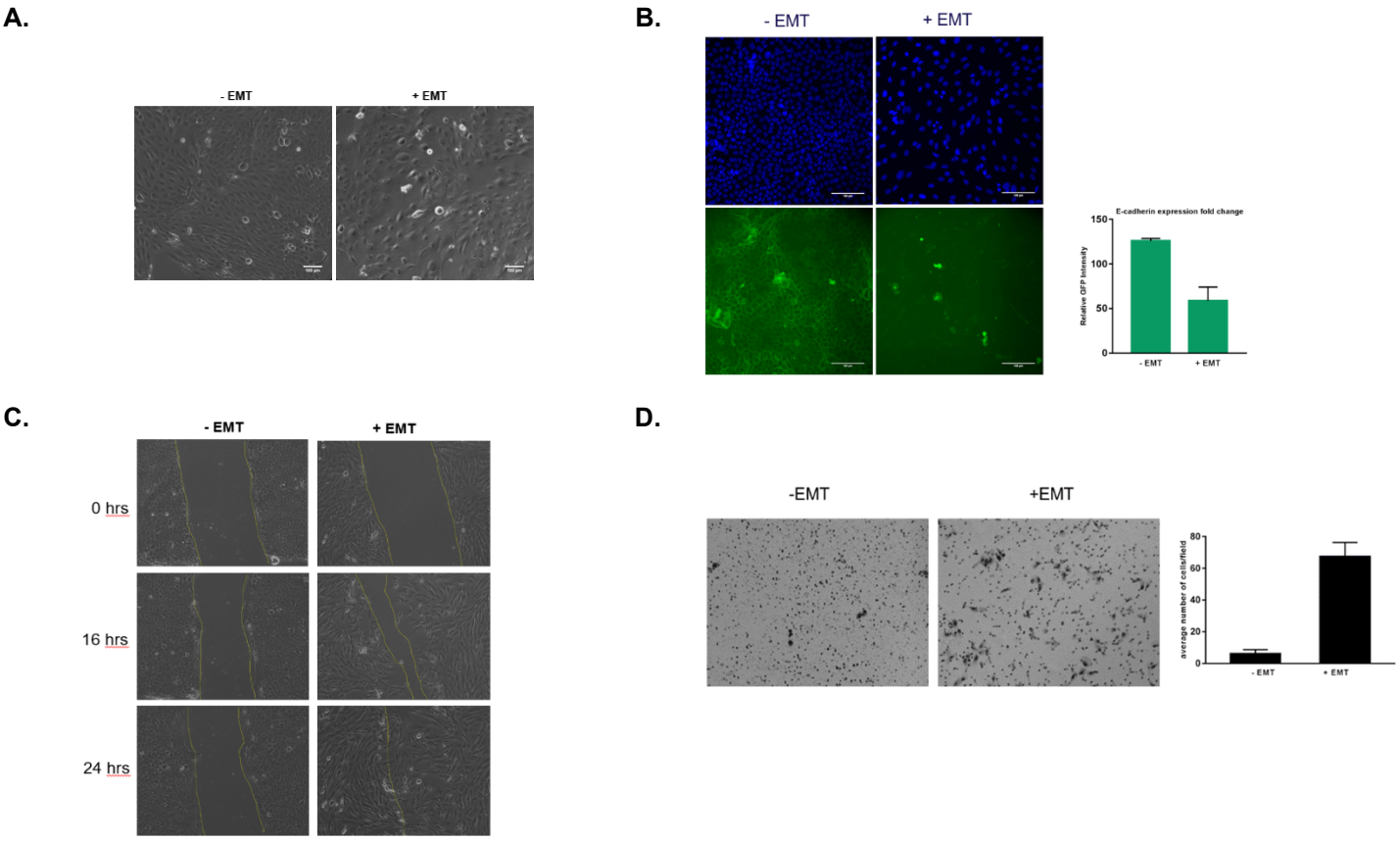

Figure 2: ECAD-EmGFP cells undergo EMT upon the induction. MCF10A ECAD-EmGFP cells were incubated in complete growth media supplemented with either 1X StemXVivo EMT Inducing Supplement (R&D Systems; + EMT) or an equivalent volume of 1X Dulbecco's Phosphate-Buffered Saline (- EMT as a no treatment control) for 5 days. (A) The morphology of ECAD-EmGFP cells changed from a cobblestone appearance to cells of a spindle-like shape upon EMT induction. (B) Induction media incubation induced a significant decrease in ECAD-EmGFP protein expression (green; bottom left and right). The nuclei of cells were counterstained with DAPI (blue, top left and right). (C) Control and EMT induced cells were kept in culture for an additional day in the complete media without EGF. A scratch was made after 6 days of induction on a confluent monolayer to assess the mobility of the cells. Images were taken at 0 hours, 16 hours, and 24 hours after scratching. EMT induced cells displayed a significant increase in motility. (D) Trans-well migration assay. Representative images were taken 48 hours after cells were seeded into inserts of chambers. The induced cells showed a significant increase in migration capacity.