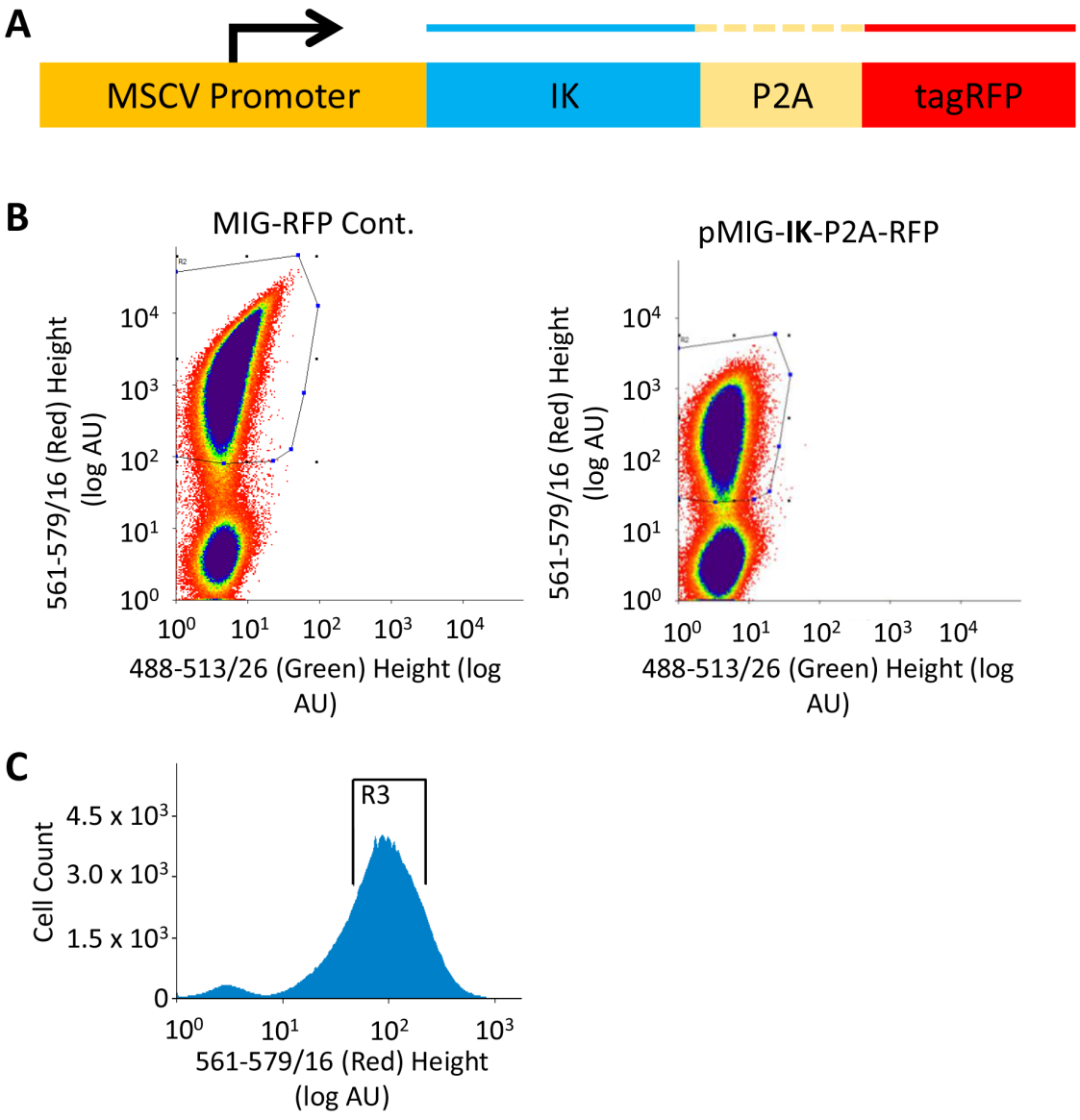
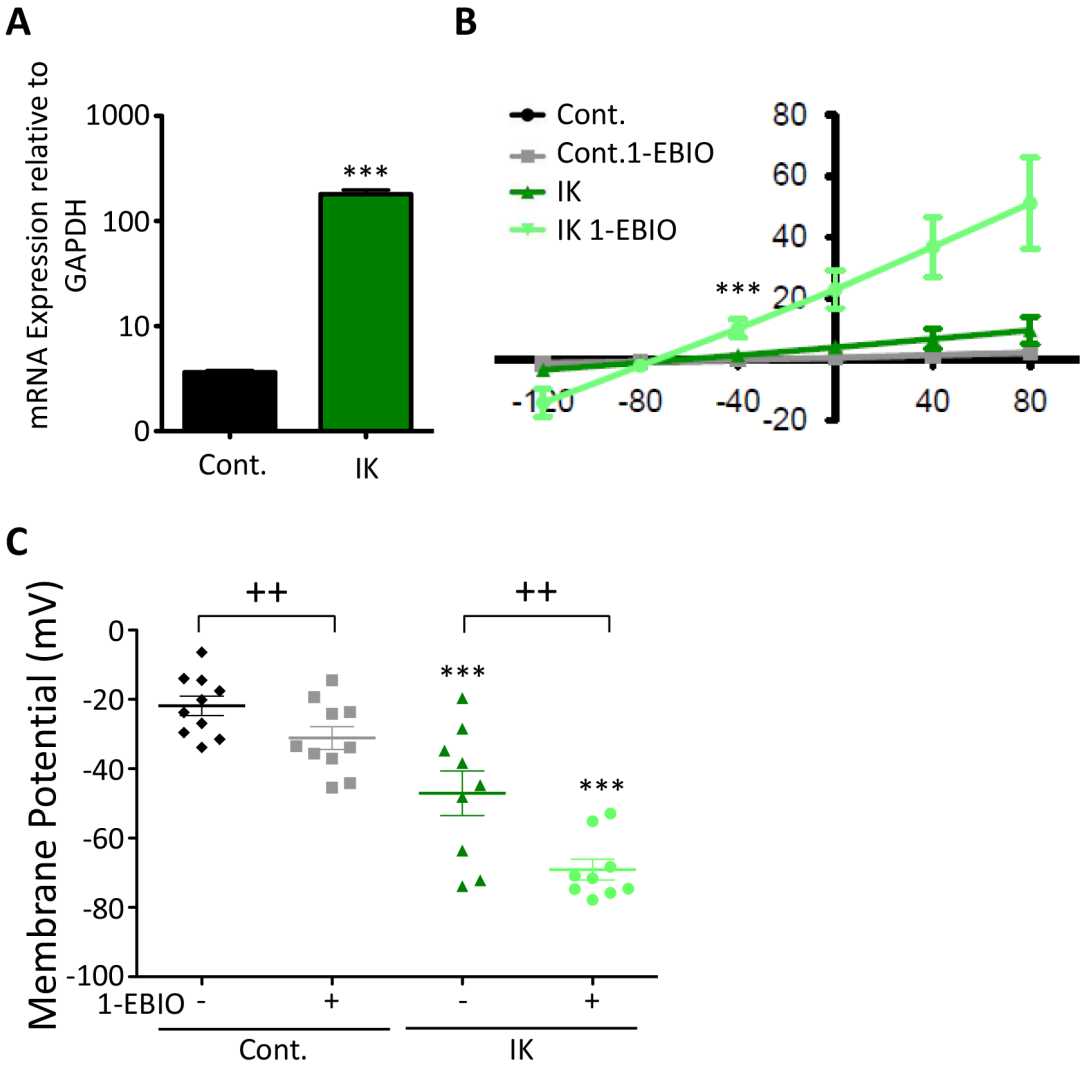
IK channel activation increases tumor growth and induces differential behavioral responses in two breast epithelial cell lines

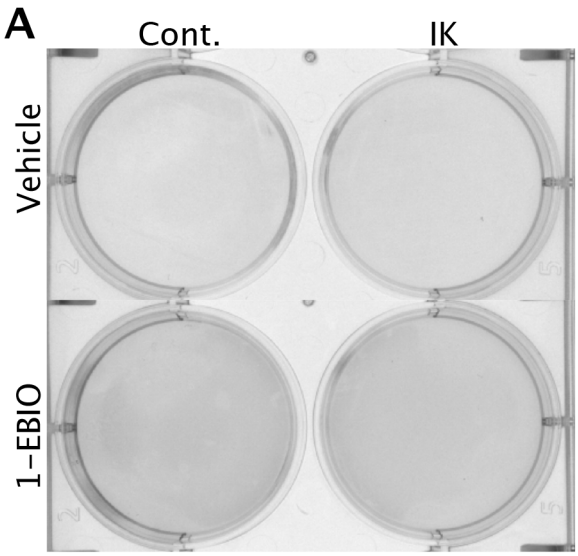
SUPPLEMENTARY FIGURES



Supplementary Figure 1: IK expression strategy and selection of RFP positive cells. (A) Schematic of IK expression plasmid. IK was inserted in tandem with the P2A peptide bond skip sequence and RFP causing translation to produce a separate IK and RFP protein. Expression was driven by the MSCV promoter giving high constitutive expression. A control plasmid was also created that lacked the IK sequence. **(B)** Plots of FACS sort of infected MDA-MB-231 cells. Cells infected with pMIG-RFP control vector (left) and pMIG-IK (middle) sorted based on RFP fluorescence and cells within the polygon were selected. **(C)** In order to create a more homogenously expressing population, the sorted MDA-MB-231-IKpopulation was expanded and sorted a second time selecting for a narrower range of highly expressing cells (cells within R3 selected).



Supplementary Figure 2: Functional contribution of IK over-expression to MCF-10A current density and V_{mem}. (A) IK mRNA expression levels relative to GAPDH in total RNA collected from MCF-10A infected with pMIG-RFP (Cont.) or pMIG-IK (IK) and selected for RFP fluorescence by FACS. Data are presented as mean with SEM of 3 independent replicates, (*** $p < 0.01$, 2 sample t-test). **(B)** Endogenous and 1-EBIO induced current-voltage relationship in control and IK-expressing cells recorded in the cell attached perforated patch configuration from MCF-10A cells. Data are presented as mean with SEM from a minimum of 7 cell recordings. The current density was significantly increased in MCF-10A-IK 1-EBIO treated cells compared to control vehicle treated, control 1-EBIO treated, and MCF-10A-IK vehicle treated cells (*** $p < 0.001$, 1-way ANOVA of -40 mV current density) **(C)** V_{mem} averaged over 20 seconds from recordings of same cells as B. Data points represent individual cells, bars show mean with SEM (*** $p < 0.001$, 1-way ANOVA significantly different than control vehicle treated; ++ $p < 0.01$, paired t-test of same cell before and after 1-EBIO treatment).



Supplementary Figure 3: Neither IK over-expression or activation are sufficient to induce MCF-10A colony formation in soft agarose. (A) Bright field images of crystal violet stained MCF-10A control and MCF-10A-IK cells grown in soft agarose and treated with vehicle control or 1-EBIO for 28 days. There was no colony formation in any condition.