Supplemental Materials

Molecular Biology of the Cell

Wang et al.

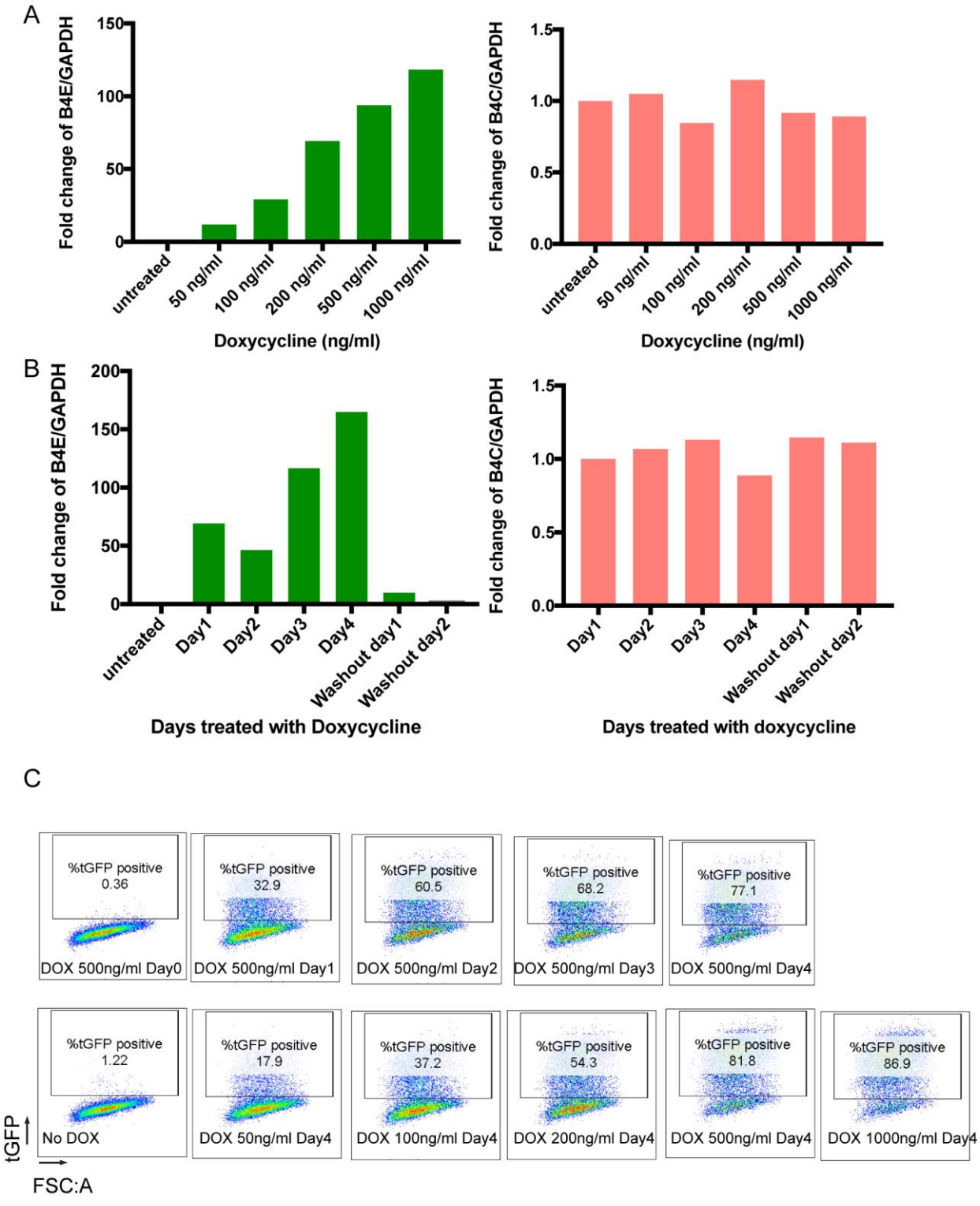
SUPPLEMENTAL FIGURES

SFIGURE 1: Integrin β4E-tGFP expression can be induced by doxycycline by dose and time without changing integrin β4C expression. (A) Increasing dose of doxycycline results in increasing β4E mRNA as determined by q-RT-PCR using β4E specific primers (A, left panel). The induction of β4E does not change β4C mRNA as determined by qPCR using β4C specific primers (A, right panel). (B) β4E mRNA increases with longer doxycycline treatment and the induction is reversible and determined by q-RT-PCR (B, left panel). The induction and reversal of β4E mRNA does not change β4C mRNA (B, right panel). (C) GAPDH was used as internal control in all samples. β4E-tGFP protein increases with increasing doxycycline treatment time (C, top panels) and dose (C, bottom panels) as determined by flow cytometry analysis.

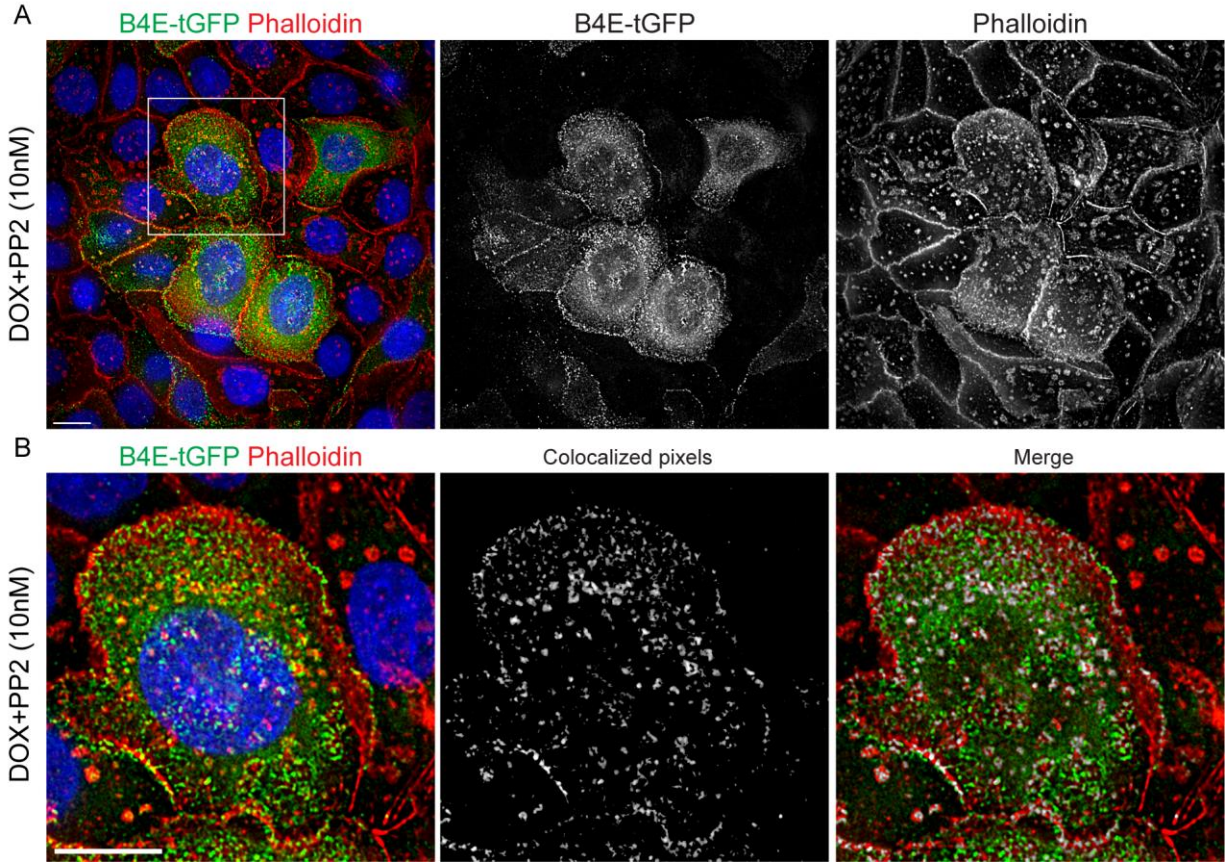
SFIGURE 2: β4E-tGFP localized at actin associated ring-like patterns independent of Src activation. (A) β4E-tGFP expressing cells (DOX) cultured in doxycycline (500 ng/ml) for 5 days in confluent culture conditions and were treated with PP2 (10 nM) for the final 2 days. Left panel: Epifluorescence images at the cell-ECM show the distribution of β4E-tGFP (green, B4E-tGTP), F-actin (Phalloidin, red), and nuclei (blue). Scale bar: 10 microns. Middle panel: β4E-tGFP channel only. Right panel: F-actin (Phalloidin) channel only. (B) Boxed region in (A) at higher magnification) shows the distribution of β4E-tGFP (green, B4E-tGTP), F-actin (Phalloidin, red), and nuclei (blue), with the corresponding co-localization pixel map (middle panel, white pixels) and a merged image (far right panel). Scale bar: 10 microns

SFIGURE 3: Integrin β4E-tGFP expression did not change biophysical properties of cell-cell and cell-ECM in sub-confluent cells during cell-ECM attachment. (A, B) Different cell densities of 6250 cells per well (A) or 12,500 cells per well (B) were compared. Untreated cells (No DOX) and β4E-tGFP expressing cells (DOX) cultured in doxycycline (500 ng/ml) were allowed to adhere for 30 mins and ECIS measurements were collected for up to 50 hours, using 40,000 Hz (left panels) or 400 Hz (right panels) under conditions of β4E-tGFP (DOX) and β4C (No DOX).

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3