

Supplementary Figure 4. FER regulates cell morphology, integrin expression and anoikis resistance.

(A) FER inducible knockdown (iKD) in MDA-MB-231 induces cell spreading and actin stress fibre formation. MDA-MB-231 inducible knock-down (iKD) cells were cultured for 96 h in the absence (-) or presence (+) of doxycycline (dox; 2  $\mu$ g/ml) to induce shRNA expression. Control and FER iKD MDA-MB-231 cells were plated on collagen I-coated glass coverslips. F-actin (green) and phospho-paxillin (red) distribution was analysed in fixed cells by immunofluorescence microscopy. Scale bar = 50  $\mu$ m. (B) FER regulates  $\alpha_6$  and  $\beta_1$  integrin expression. MDA-MB-231 inducible knock-down (iKD) cells were cultured for 96 h in the absence (-) or presence (+) of doxycycline (dox; 2  $\mu$ g/ml) to induce shRNA expression. FER iKD cells were trypsinized and surface expression of the indicated integrin subunits was analysed by FACS.  $\Delta$ MFI represents the mean fluorescence intensity of dox-treated/untreated cells (corrected for background,  $\mu$ g). (C) FER controls anoikis resistance. Adherent cultures of MDA-MB-231 cells were transfected with the indicated siRNAs and cultured for 96 h. Cells were trypsinized and cultured in suspension conditions for 72 h, followed by labelling with Alexa 488-conjugated Annexin-V and propidium iodide (PI) and FACS analysis. Values represent the percentage of anoikis resistant cells (PI<sup>NEG</sup>/Annexin-V<sup>NEG</sup>)  $\pm$  SEM. \* indicates a significant difference compared to untreated cells (-dox); (p<0.05, one-way ANOVA). The results shown are representative of three independent experiments.