

Supplementary Figure 9. Loss of IFITM1 expression is associated with increased p21 expression, which mediates cell cycle arrest and is controlled by JAK/STAT signaling. (a) MCF-7:5C cells were transiently transfected with siCon or silFITM1C while MCF-7:5C/shIF cells were treated with vehicle or 1µM doxycycline (Dox). Cells were harvested after 48 and 72 hours respectively and then lysates were immunoblotted for IFITM1 and p21 expression. (b) MCF-7:5C, MCF-7:5C/shCon and MCF-7:5C/shIF cells were treated with vehicle or control over 72 hours. Samples were harvested and fixed at 24, 48 and 72 hours for cell cycle analysis. The percent of cells in G0/G1 phase is displayed and represents means from two experiments conducted in duplicate \pm standard deviation. (c) The percent of viable cells after siCon or silFITM1C transfection with and without 10µM Rux was determined by cell titer blue cell viability assay after 24, 48 and 72 hours of treatment. Values are means \pm SD of two independent experiments conducted in triplicate. (d) MCF-7:5C cells transfected with either siCon or silFITM1 were also treated with 10µM Rux or vehicle. Whole cell lysates after 24 and 48 hours of treatment were immunoblotted for phospho-STAT1 (p-STAT1), STAT1, IFITM1 and p21 expression. (e) MCF-7:5C cells were transfected with siCon, silFITM1C and/or treated with 10µM ruxolitinib (Rux). Dual annexin/PI staining was used to quantify cell death in each transfection group as compared to untreated siCon. Data represent means \pm SD from two experiments conducted in triplicate. ** p< 0.01