



**Additional file 3 – IEC transformation induced by oncogenic Met, Grb2, and Shc signaling requires MEK, but not PI3K, activity.** (A) Cells were treated with vehicle (DMSO) or 10  $\mu$ M selective inhibitors of MEK1/2 (U0126, PD184352, or AZD6244) or PI3K (LY294002). Photographs depict typical cell morphologies following 24 hours of treatment. Cell morphology alterations resulting from 48 hours of treatment are shown in Figure 5. (B) E-cadherin and actin protein levels in TM-Grb2, TM-Shc1, and TM-Shc2 cells were assessed by IB analyses following 48 hours of treatments with DMSO or the indicated inhibitors. (C) Relative E-cadherin and actin protein levels were determined by densitometry analyses. The bar graph presents the mean fold-changes ( $\pm$  S.D.) in E-cadherin protein levels normalized to that of actin, relative to DMSO treated cells. Values were calculated from at least three independent experiments. The analysis of E-cadherin protein levels in Tpr-Met and control IEC-6 cells are shown in Figure 5. (D-G) A constitutively active cell-surface Met receptor mutant promotes morphological transformation of IEC-6 cells by MEK1/2-dependent pathways. As Tpr-Met is a cytosolic oncoprotein, we sought to determine whether oncogenic activation of Met signaling at the cell surface could induce morphological transformation of IECs in a similar manner. Stable IEC-6 cell lines expressing the CSF-Met<sup>mt</sup> were generated. This cell-surface chimeric Met receptor mutant is composed of the extracellular domain of the human colony stimulating factor-1 receptor fused to the Met transmembrane and intracellular domains. The Y1003F and M1268T substitutions in the Met intracellular domain reduce, respectively, Met degradation by preventing c-Cbl E3 ubiquitin ligase binding and enhance receptor kinase activity. (D) Much like Tpr-Met, CSF-Met<sup>mt</sup> is expressed as a tyrosine phosphorylated protein in IEC-6 cells, as determined by IB analyses of TCLs prepared from serum-starved cells. The phosphorylation levels of neither Erk1/2 nor Akt were elevated in the CSF-Met<sup>mt</sup>-expressing IEC-6 cells, relative to control cells. Total levels of these proteins were also comparable. (E) Photographs demonstrate that the CSF-Met<sup>mt</sup>-expressing IEC-6 cells were morphologically transformed, and that treatment with the MEK1/2 inhibitor (U0126), but not with a PI3K inhibitor (LY294002), promoted reversion of the transformed morphological features. (F) Restoration of epithelioid-like morphological features by the MEK inhibition in CSF-Met<sup>mt</sup> IEC-6 cells was concurrent with an increase in E-cadherin protein levels, evaluated by IB analyses. (G) Densitometric analyses of E-cadherin protein levels were performed. The bar graph presents the mean fold-change ( $\pm$  S.D.) in E-cadherin protein levels normalized to that of actin, relative to DMSO treated cells. The values were calculated from at least three independent experiments.