



Additional file 2 – Oncogenic Met, Grb2, and Shc signaling pathways alter the expression of critical E-cadherin transcriptional repressors. E-cadherin mRNA and protein levels were demonstrated to be further reduced in IEC-6 cells expressing the Grb2 or Shc docking-specific oncoproteins, compared to those transformed by Tpr-Met (Figure 1). Thus, the ability of these oncoproteins to alter the expression of E-cadherin transcriptional repressors was evaluated. **(A)** The mRNA levels of *Snail1*, *Snail2*, *Zeb1*, *Twist1*, and *Twist2* were analyzed by semi-quantitative RT-PCR assays performed with total RNA prepared from the indicated serum-starved cells. The *S18* mRNA level is shown as a loading control. **(B)** Relative expression levels of *Snail1*, *Snail2*, and *Zeb1* mRNAs were evaluated by quantitative RT-PCR analyses. The bar graph presents the mean fold-change (\pm S.E.M.) of the indicated mRNA levels relative to Control-IEC-6 cells, from three independent experiments performed in duplicate. The *TATA-binding protein (TBP)*, *pumilio RNA-binding family member 1 (Pum1)*, and *ribosomal protein L19 (Rpl19)* mRNA levels were used for normalization. The levels of *Snail1* mRNA were significantly reduced in all transformed IEC-6 cells (>4-fold relative to Control-IEC-6 cells). The expression of *Snail2* was slightly, but significantly, elevated in Tpr-Met and TM-Shc2-IEC-6 cells, but not in cells transformed by the TM-Grb2 or TM-Shc1 oncoproteins. A slight increase in *Zeb1* mRNA was observed in all transformed IECs when compared to control cells but did reach significance only in Tpr-Met and TM-Shc2 IEC-cells. The basal mRNA levels of *Twist1* and *Twist2* were too low in the Control-IEC-6 cells for reliable quantification by real-time RT-PCR methods. Thus, relative expression levels of *Twist1* and *Twist2* mRNAs were determined by performing semi-quantitative RT-PCR assays, with gel-based densitometric quantification of amplification products. The bar graph shows the mean fold-change (\pm S.D.) in *Twist1* or *Twist2* mRNA levels relative to Control-IEC-6 cells normalized to *S18* mRNA levels, from three independent experiments. *Twist1* and *Twist2* mRNA levels were noticeably elevated in cells transformed by Tpr-Met, and the Grb2 and Shc docking-specific oncoproteins, but the extent of up-regulation of these two genes was highly variable between multiple assays. **(D)** Primer sequences for these RT-PCR analyses are listed. Overall, these data suggest that a combined up-regulation of *Snail2*, *Twist1* or *Twist2* mRNAs, but not of *Snail1* and *Zeb1*, underlies the down-regulation of E-cadherin induced by Met oncogenic signaling pathways and those of Grb2 and Shc in IECs.