Figure 8

TGFBR2 dependent apoptosis is suppressed by *CDKN1A/p21* siRNA. A. siRNA for *CDKN1A/p21* suppresses *CDKN1A/p21* reporter luciferase activity in the V-400R2 cell line. A dose related increase in luciferase reporter activity in the siRNA control treated cell line is demonstrated. **B.** Apoptosis in V-400R2 was measured using the Cell Death ELISA (Roche) after 48 hours of treatment with TGF-ß (10ng/ml). Treatment with the *CDKN1A/p21* siRNA inhibits the TGFBR2 dependent induction of apoptosis, which is seen in the siRNA control treated cells. **C.** TGF-ß mediated apoptosis is inhibited by SMAD7. Apoptosis was induced by TGF-ß in the V-400R2 cell line after transfection with SMAD7. SMAD7 abrogates the increased apoptosis observed in the cells V-400R2 cells treated with increasing concentrations of RG-115819.

Supplemental Figure 1: Immunostaining for p21 in the V-400R2 cell line. The V-400R2 cell line was grown with increasing concentrations of RG-115819 and then immunostained with an anti-p21 antibody (OP#68, EMD) and detected with a FITC-tagged secondary antibody (SC2012 in a 1:1000 dilution). Predominantly cytoplasmic p21 is observed in the cells treated with 0 or 0.5 μM RG-115819 whereas nuclear p21 can be seen in the cells treated with 1μM RG-115819.

These studies were conducted as follows. The V-400R2 cells were seeded in a chambered slide and grown for 48 hr with RG-115819 and TGF-β (10ng/ml). Then cells were fixed in –10ªC methanol for 5 minutes and washed 3 times with 1X PBS. The slides were blocked with 10% normal blocking serum diluted in PBS (20 minute incubation at room

temperature) and the washed with 1X PBS. The slides were then incubated with the primary antibody for 60 minutes at room temperature, washed in 1XPBS (three times), incubated in the secondary antibody (30 minutes at room temperature), and washed in 1X PBS (three times). The slides were then subjected to fluorescence microscopy.