



Supplemental Figure S1. Rgenf signaling and NF- κ B reporter activation. (a) Human 293T transfection with mCherry-tagged Rgenf wildtype, Rgenf Y1003A (GDP exchange defective) and an IL-6 luciferase reporter with or without mutated NF- κ B binding sites. (b and c) Adherent ID8-IP GFP-Rgenf cells were serum starved overnight, pre-treated with DMSO (vehicle), 10 μ M U0126, or 1 μ M wortmannin for 2h, and then stimulated with 5% fetal bovine serum plus 5% insulin-transferrin-selenium supplement for the indicated times prior to cell lysis. (b) Immunoblotting for phosphorylated ERK1/2 (T185/Y187) or total ERK1/2. (c) Immunoblotting for phosphorylated Akt (S473) or total Akt. (d) Human 293T cells were transfected as above and incubated with 10 μ M Bay 11-7082 or 10 μ M TPCA-1 for 24 h prior to analysis of luciferase activity. (a and d) Mean luciferase values are normalized from three independent analyses (** P < 0.001 and **** P < 0.0001, unpaired T test).