Supplementary Figure 3. Phosphorylation of GSK-3 by active Akt is decreased with increasing concentrations of GRK2. The amount of ATP remaining in solution following an Akt kinase reaction with GRK2 was determined by using the kinase-Glo™ luminescent kinase assay kit (Promega, Madison, WI) as per the manufacturers directions. Briefly, reactions that contained 100 ng active Akt were preincubated with different amounts of GRK2 as indicated for 10 mins at room temperature; Akt kinase reactions were performed in 50 μl of 40 mM Tris (pH 7.5), 20 mM MgCl2 for 20 mins using 10 nM ATP with 1 μl GST-3 as the substrate. An equal volume of kinase-Glo™ reagent was added, mixed, and luminescence was measured. Values represent the mean of 3 replicates (* p < 0.05 compared to 50 ng GRK2, ** p < 0.01 compared to 50 ng GRK2). In addition, we found that when GRK2, Akt, and ATP were combined together in a kinase reaction and GSK-3 was used as a substrate, that incubation for longer periods of time (5-30 mins), led to reduced GSK-3 phosphorylation compared to control (not shown).