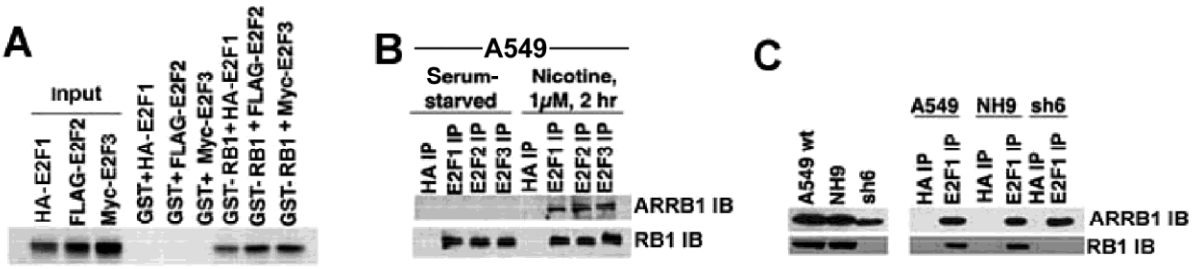

Supplementary Figure 2. In vitro GST Pull-down Assays to Detect Binding Between ARRB1 and E2F pathway proteins. A) The E2F family members namely E2F1, E2F2 and E2F3 bind to ARRB1 in vitro. The first three lanes (labeled as "input") represent 20% of the input ^{35}S-Methionine-labeled-HA-E2F1, ^{35}S-Methionine-labeled FLAG-E2F2, ^{35}S-Methionine-labeled Myc-E2F3 used in the GST pull-down assay. B) Immunoprecipitation and immunoblot experiments were done to assess the binding of ARRB1 to E2F1, 2 and 3 in vivo. Immunoprecipitation with mouse-anti-human HA antibody was used as the negative control. C) Immunoprecipitation and immunoblot experiments were performed to examine the role of RB1 in E2F1-ARRB1 interaction. The direct binding of ARRB1 to E2F1 was found to be independent of RB1 status in A549 cells. Lysates were made from quiescent or nicotine-treated untransfected A549, A549 cells transfected with control vector (NH9) and A549 cells transfected with RB1-shRNA (sh6). Subsequently, these lysates were immunoprecipitated with mouse-anti-human E2F1 antibody. Immunoblot analysis of the E2F1-immuneprecipates show that the ARRB1-E2F1 interaction is robustly detected in untransfected A549, NH9 and sh6 cells, All the immunoprecipitation and immunoblot experiments are representative of two independent experiments.