Fig. S5

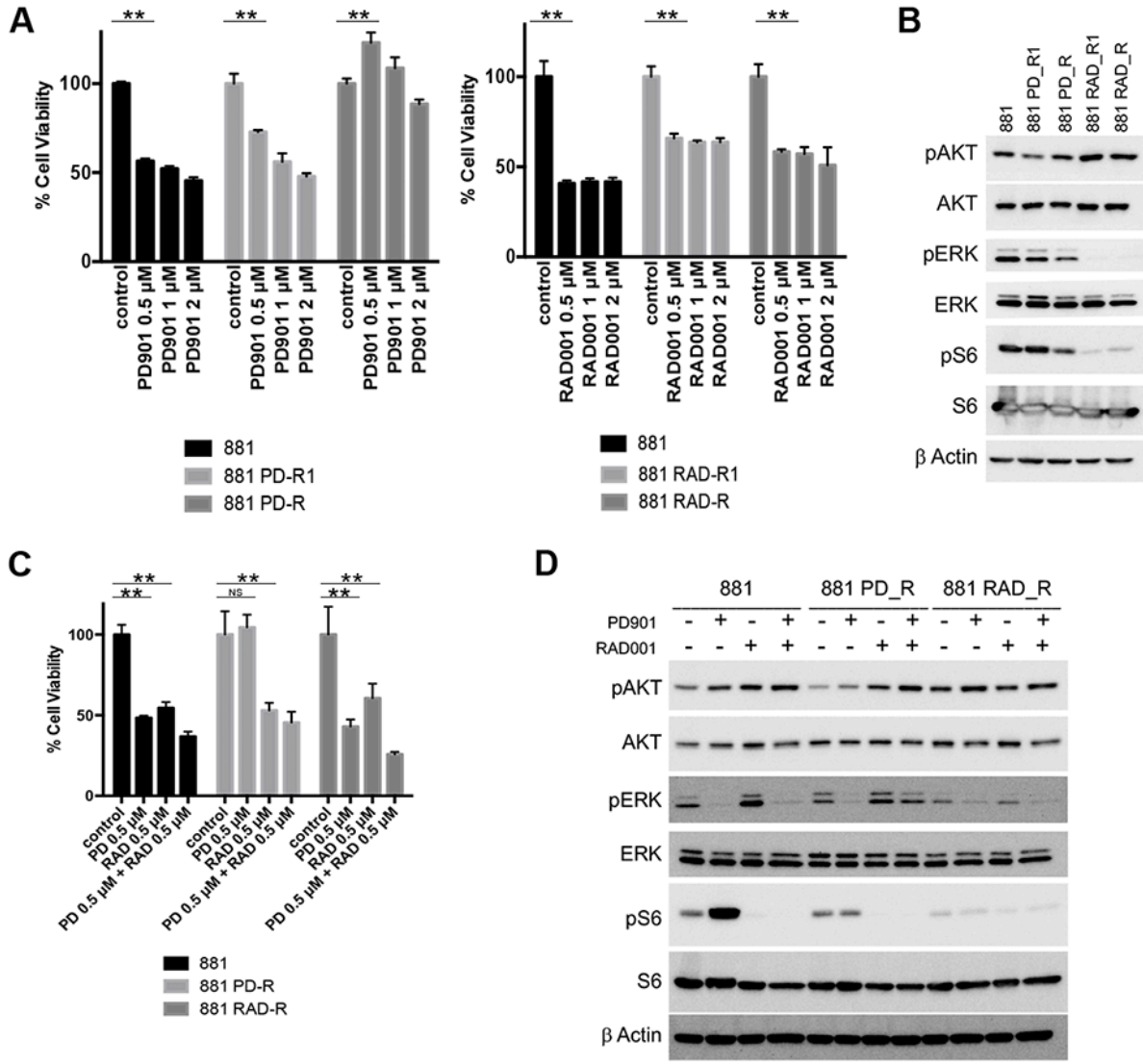

Fig. S5 Drug resistant 881 *Nf1* mutant cells. A. 881 parental cells were continuously exposed to increasing concentrations (from 25 nM to 500 nM) of PD901 or RAD001. Two sets of resistant cell lines were established for each drug, one resistant to 100 nM (881 PD_R1, 881 RAD_R1) and one resistant to 500 nM (881 PD_R, 881 RAD_R). Acquired resistance was confirmed by MTS proliferation assay by comparing the growth of parental and resistant cell lines to increasing drug concentrations for 72 hours (*P<0.05; **P<0.01; ns, not significant). B. Ras pathway activation in drug resistant cell lines under basal conditions was evaluated by Western blot analysis. Total and phosphorylated AKT (S473), S6 Ribosomal Protein (Ser235/236) and p44/42 MAPK (ERK1/2) (Thr202/Tyr204) were assessed. β actin was used as loading control. C. Sensitivity to 0.5 µM PD901, 0.5 µM RAD001 or the combination was assessed in parental and resistant cell lines by MTS proliferation assay after 72 hours of drug exposure (*P<0.05; **P<0.01; ns, not significant). D. Western blot analysis was performed to evaluate phosphorylation responses of Ras pathway effectors to single or combinatorial drug treatment.