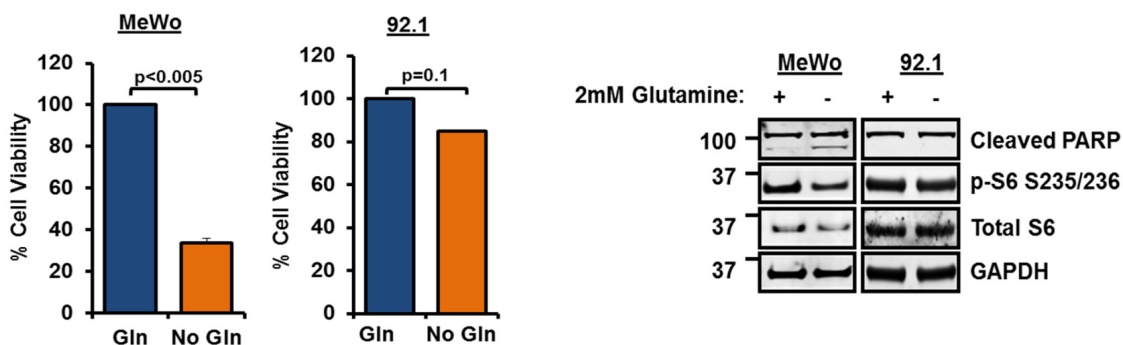


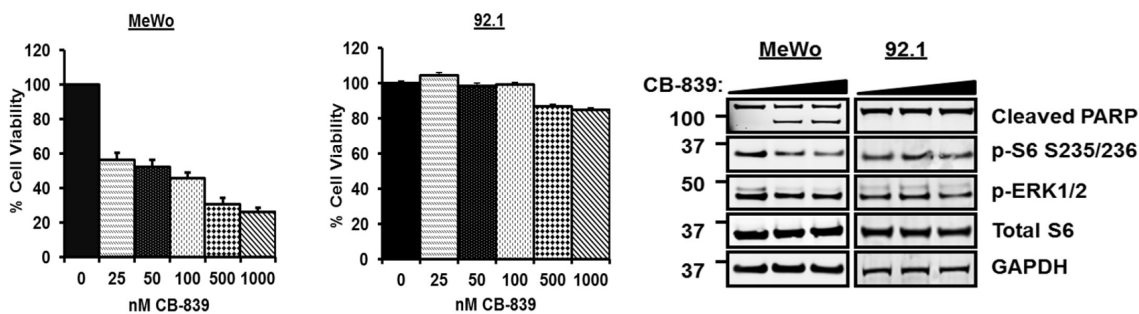
Targeted inhibition of glutaminase as a potential new approach for the treatment of *NF1* associated soft tissue malignancies

SUPPLEMENTARY MATERIALS

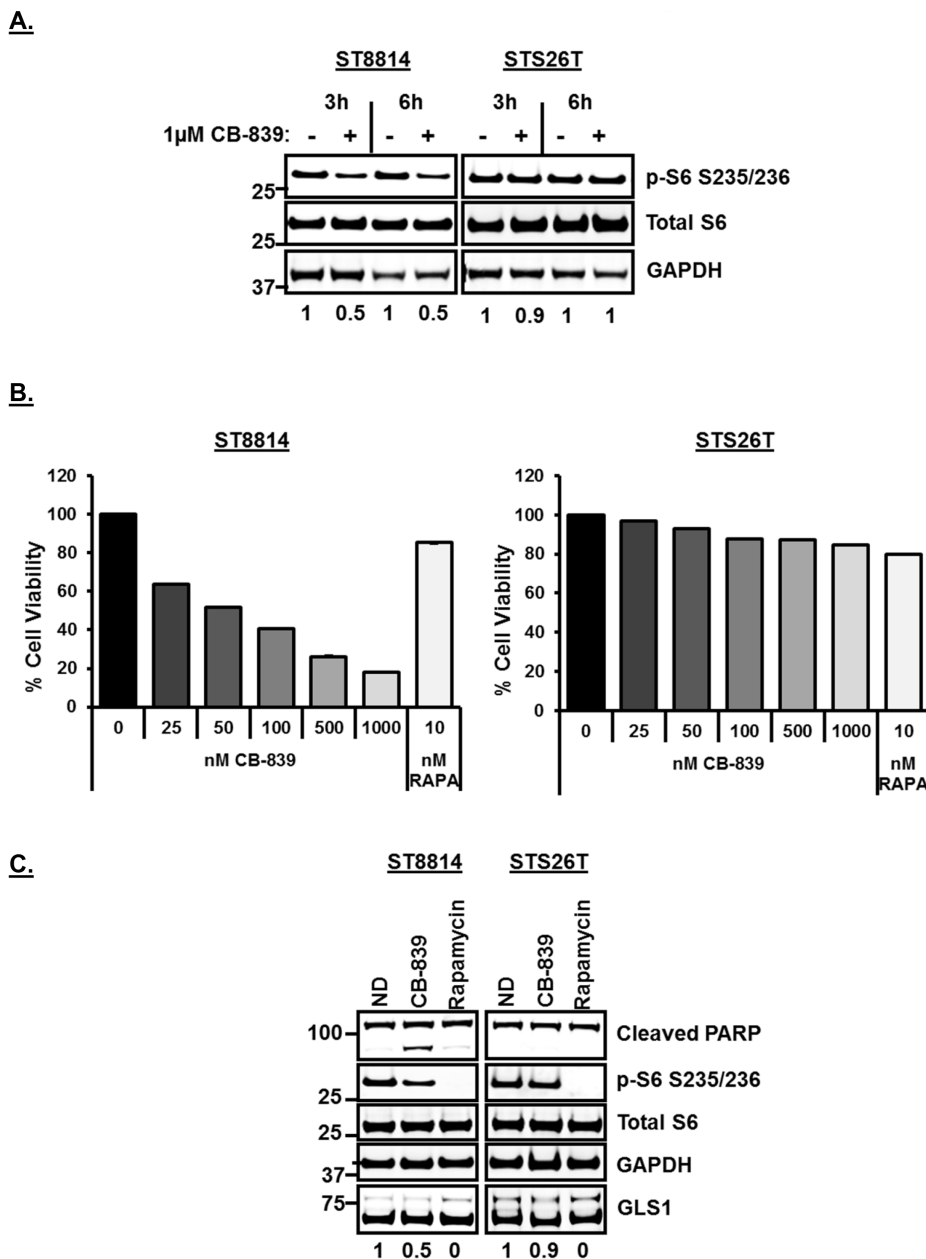
A.



B.

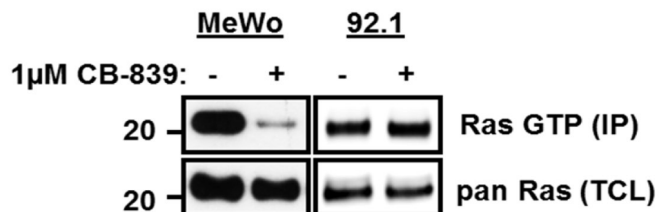


Supplementary Figure 1: (A and B). Glutamine dependency and effect of CB-839 in *NF1* null MeWo versus wild-type *NF1* carrying 92.1 melanoma cell line. Cell viability assay and western blot analysis after glutamine deprivation or CB-839 treatment was carried out as described in Figures 1 and 2.

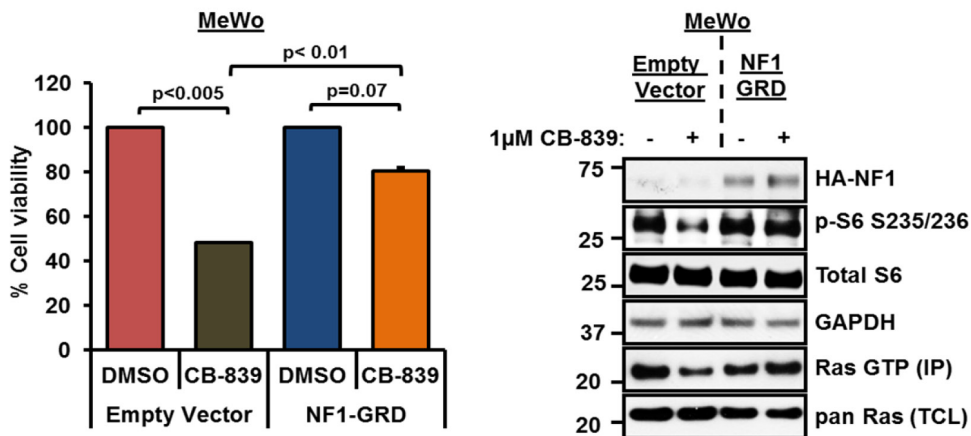


Supplementary Figure 2: (A). Downregulation of mTORC1 target p-S6 after 3h and 6h of CB-839 treatment in NF1 null (ST8814) versus wild-type NF1 cell line (STS26T). Cells were grown to 60% confluency in 60-mm plates for 24 hours and treated with DMSO or 1μM CB-839 for the indicated time points and harvested for western blot analysis. Western blot analysis was carried out as described in Figures 1 and 2. **(B).** Anti-proliferative efficacy of CB-839 versus Rapamycin in *NF1* null (ST8814) versus *NF1* wild-type (STS26T) cell line. 1000-2000 cells per well were plated in 96 well plates in triplicate in RPMI+10%FBS without Glutamine for 24 hours. Next day, cells were treated with increasing dose of CB-839 or 10nM Rapamycin in RPMI+10%FBS with 2mM Glutamine. After 72 hours, cell viability was measured using Dojindo CCK-8 kit using manufacturer’s instructions. Cell viability was calculated as percentage of growth compared to DMSO control. **(C).** Induction of apoptosis and downregulation of mTORC1 target p-S6 with the treatment of CB-839 and Rapamycin in *NF1* null versus wild-type *NF1* cell lines. Cells were grown to 60% confluency in 60-mm plates for 24 hours and treated with DMSO, 1μM CB-839 or 10nM Rapamycin for another 48 hours, harvested cell pellets were lysed in RIPA lysis buffer and 30μg of lysates were loaded on SDS/PAGE. Proteins were detected on western blot using indicated antibodies.

A.



B.



Supplementary Figure 3: (A). Detection of active Ras (Ras-GTP) in *NF1* null and *NF1* wild-type cell lines. **(B).** Rescue of cell proliferation and mTORC1 activity by wild-type *NF1*-GRD overexpression in metastatic melanoma MeWo cell line. Active Ras pull down as well as cell viability assay and western blot analysis after *NF1*-GRD overexpression was carried out as described in Figure 5.