

NF1-mutated tumors exhibit increased sensitivity to autophagy inhibition

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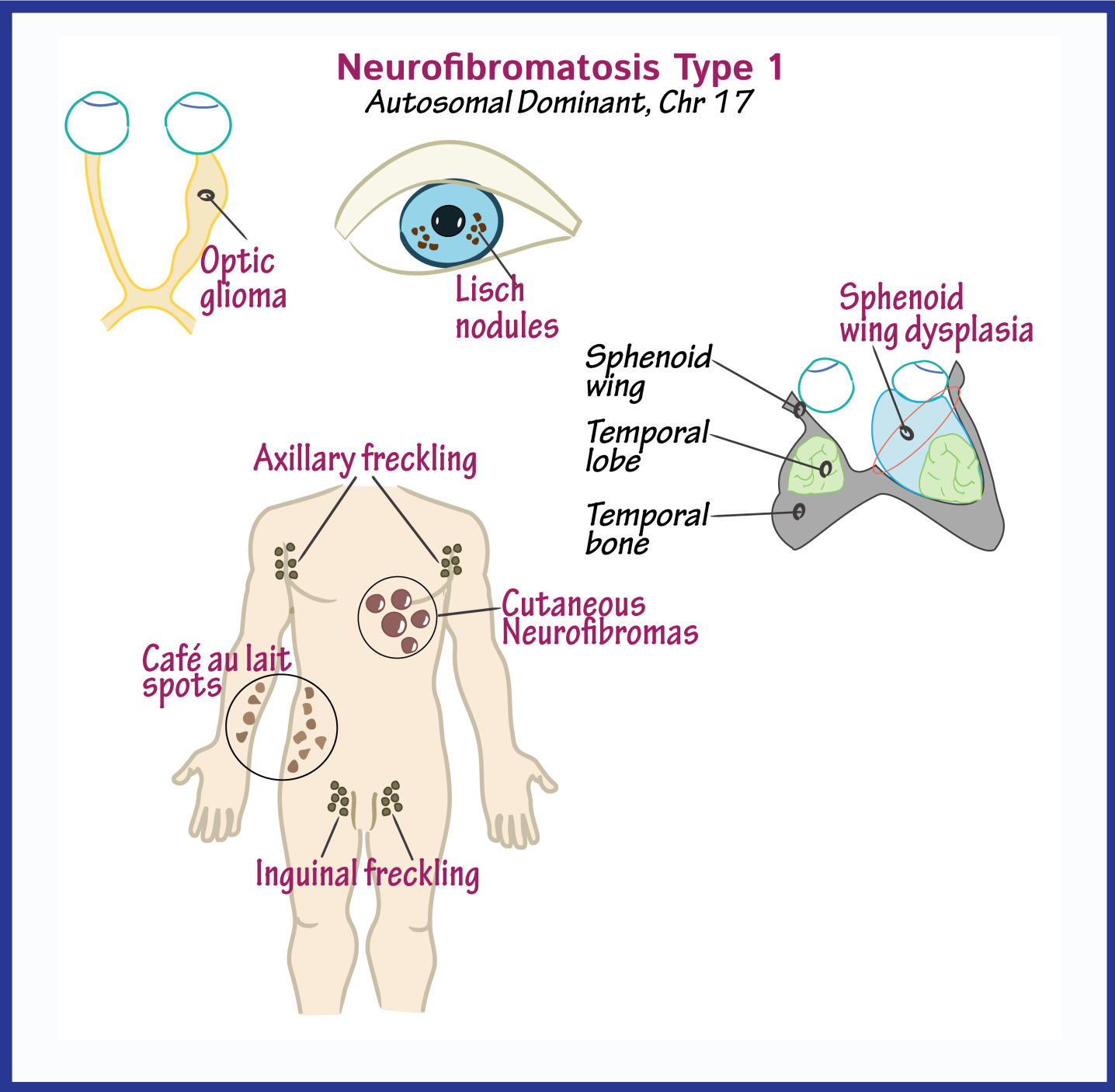
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Background

- Loss of functional NF1 gene leads to constitutive activation of the Ras/MAPK pathway which induces cellular proliferation and tumorigenesis.
- The NF1 phenotype involves large plexiform neurofibromas that often cannot be treated surgically as the lesions are likely to be extensively involved with the effected nerve and have a propensity to recur after resection
- Despite the recent expansion of kinase inhibitors (including MEK inhibition) for treatment of NF1 mutated tumors there remains a risk of chemo-resistance in kinase targeted therapies
- Autophagy, a heavily regulated process by which cellular waste is transferred to lysosomes for degradation and processing, is an integral part of tumor cell survival under stressful conditions including nutrient deprivation and chemotherapy.
- Autophagy has been demonstrated to play an important role in chemo-evasion in other tumor types with MAPK pathway dysregulation but has yet to be explored in NF1-mutated tumors



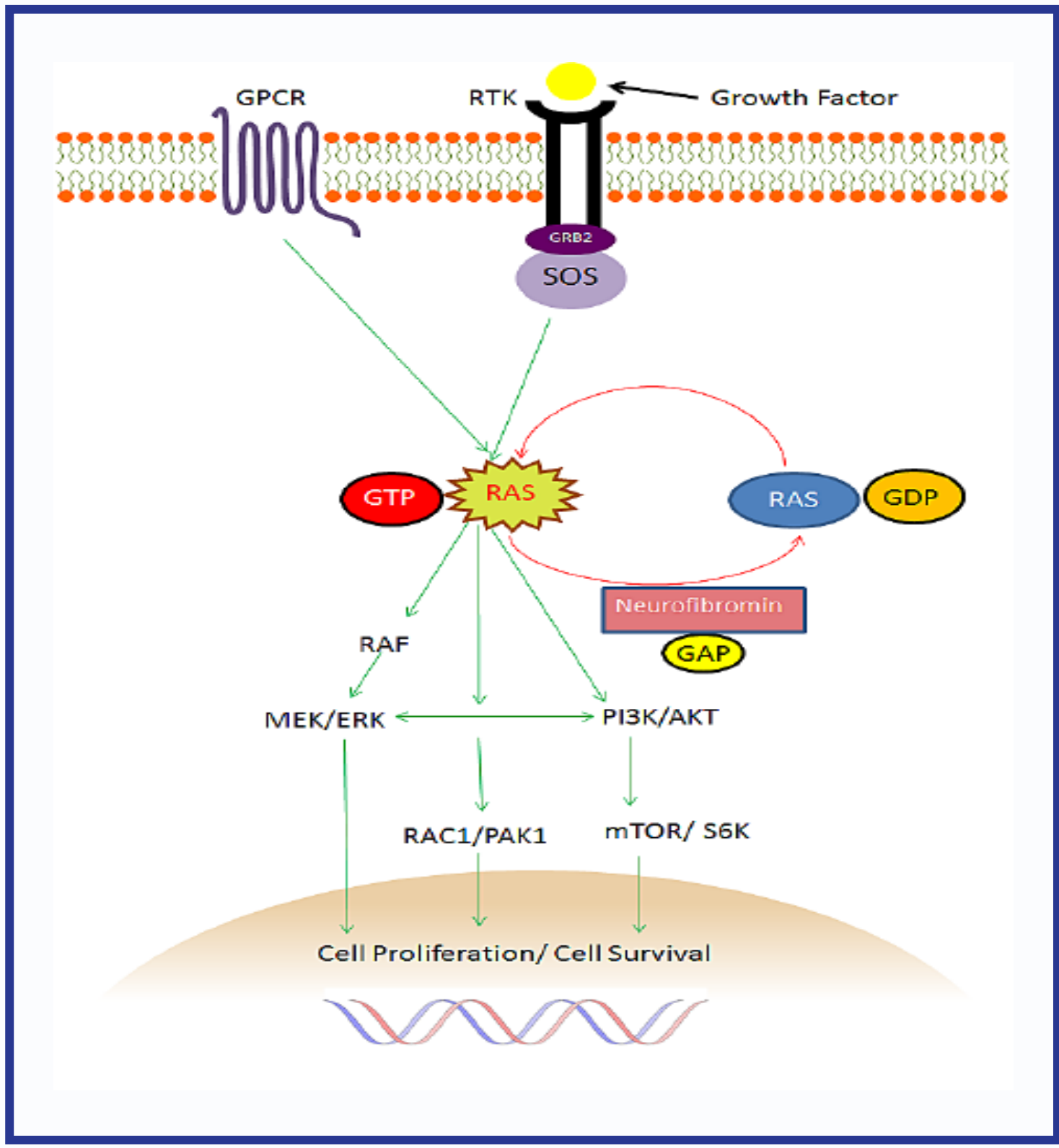
Rationale

NF1, neurofibromin1 has been shown to negatively regulate RAS proteins and therefore mutations in NF1 result in the dysregulation of the RAS/MAPK pathway. This in turn will increase cell autophagy dependence and survival. Based on our previous studies regarding tumors with the BRAF^{V600E} mutation in the MAPK pathway, we hypothesize that inhibition of autophagy in NF1 mutated tumors using Chloroquine (CQ) in combination with MEK inhibition will sensitize tumor cells to death thereby enhancing treatment efficacy.

Methods

- A CRISPR/Cas9 mediated NF1 KO was derived from human immortalized Schwann cells and utilized as a tumor model.
- Autophagy inhibition was achieved pharmacologically by chloroquine (CQ) and genetically via shRNAi of ATG5 and ATG7
- Trametinib was used for MEK inhibition.
- In vitro viability assays:
 - CellTiter-Glo® Luminescent Cell Viability Assay
 - Long term survival colony formation assay
- Incucyte growth measurement assay was used to assess cellular growth
- Western blot analysis was used for protein expression analysis

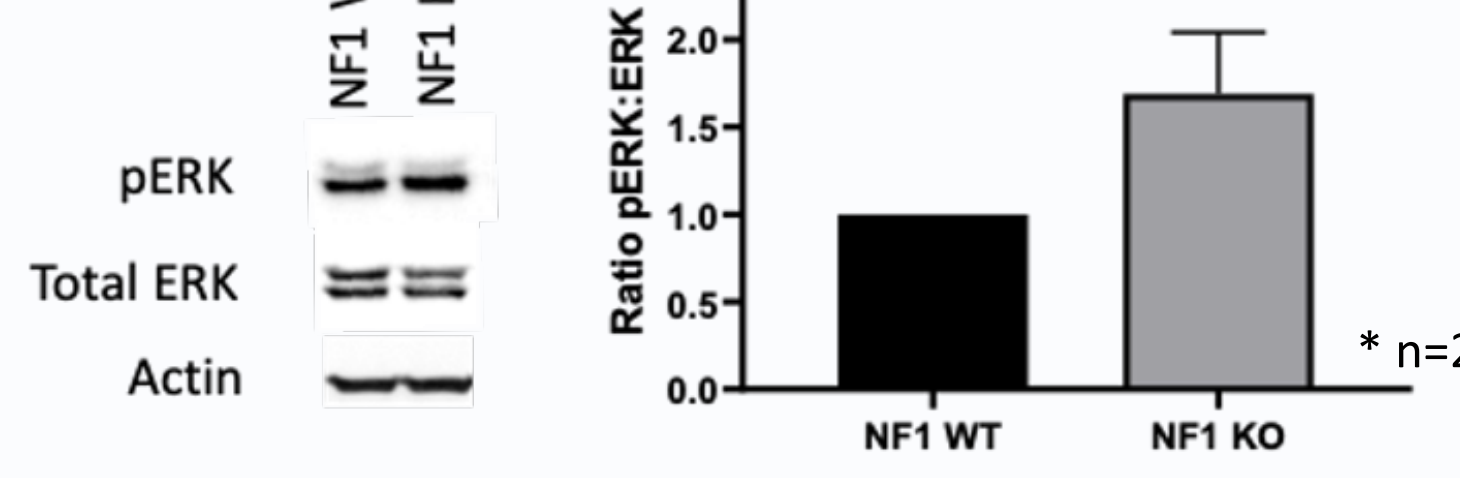
MAPK Pathway



A. NF1 WT NF1 KO



B. NF1 WT NF1 KO



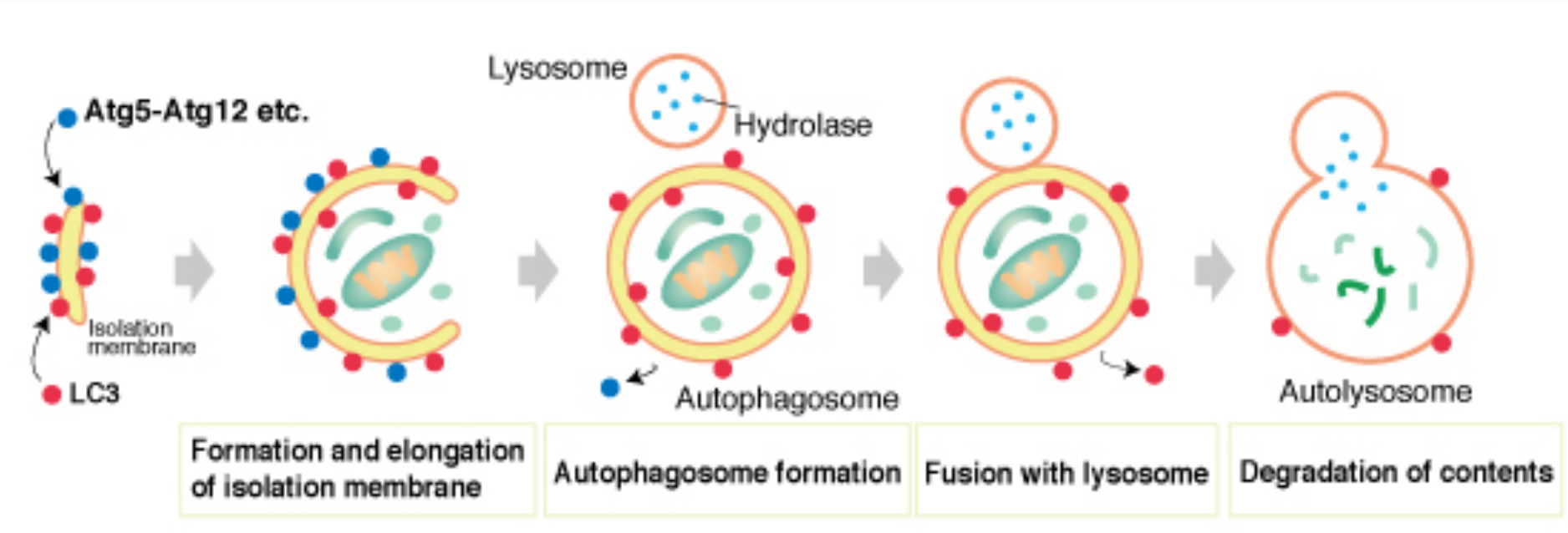
C. Cell Line IC50 (nM) Trametinib

Cell Line	IC50 (nM) Trametinib
NF1 WT	46.39
NF1 KO	24.54

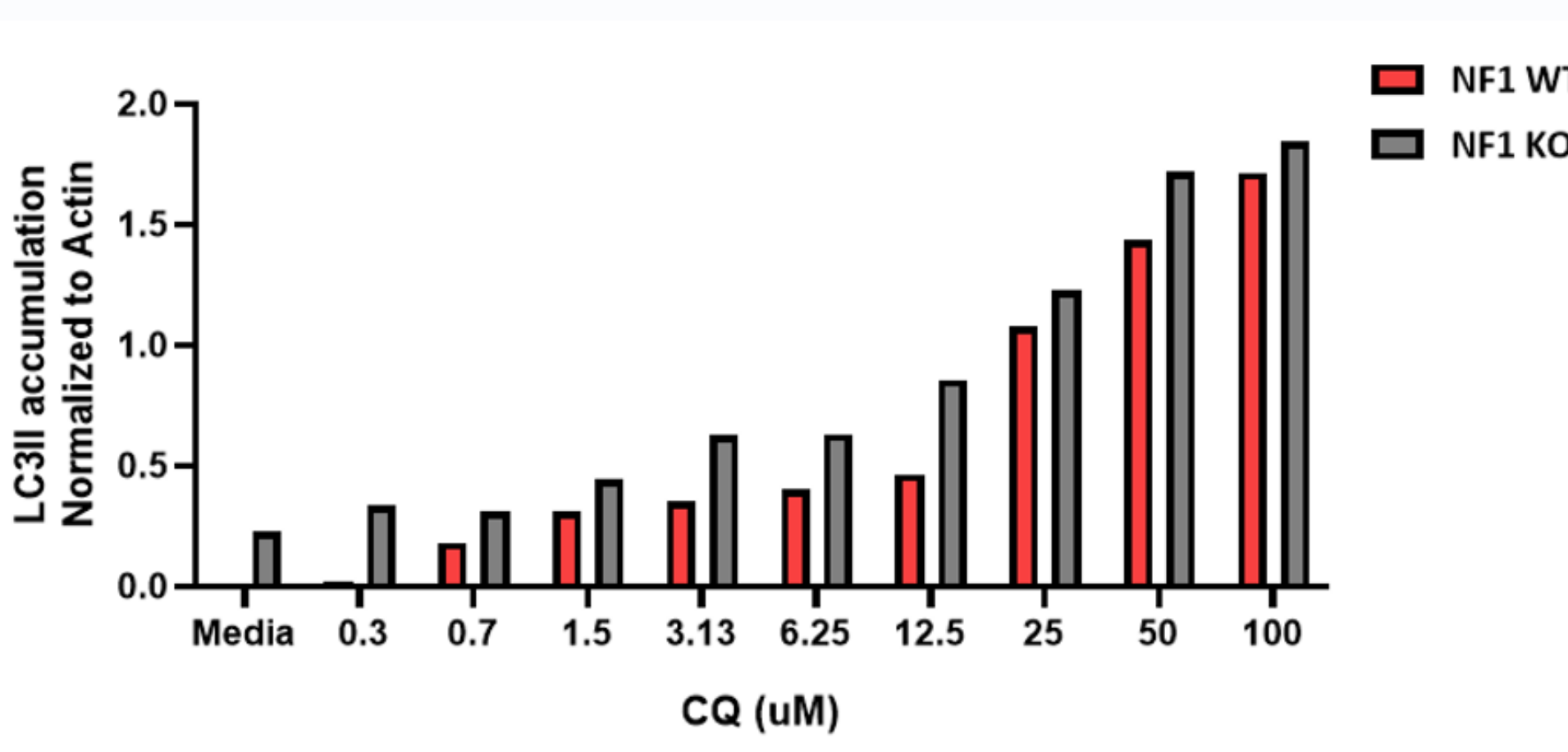
Figure 1. MAPK pathway is upregulated in NF1 KO and NF1 KO demonstrates increased sensitivity to MEK inhibition. **A.** Representative western blot demonstrating increased ras activity in NF1 KO line compared to WT. **B.** Left: representative western blot demonstrating increased phosphorylation of ERK in the NF1 KO compared to wild type. Quantified in bar graph at right. **C.** Comparison of IC50 of trametinib MEKi for NF1 WT and KO lines.

Results

A.



B.



C.

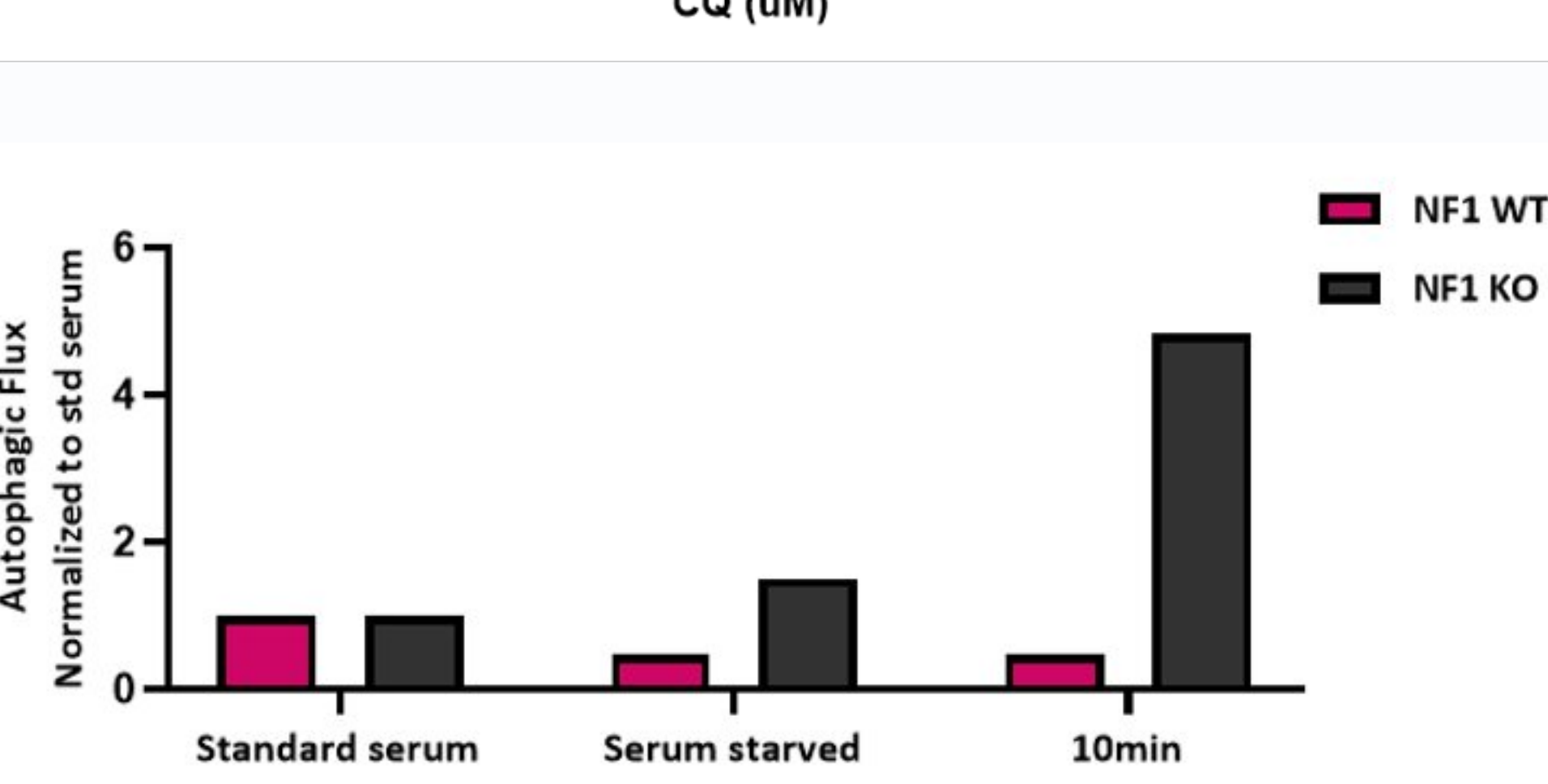
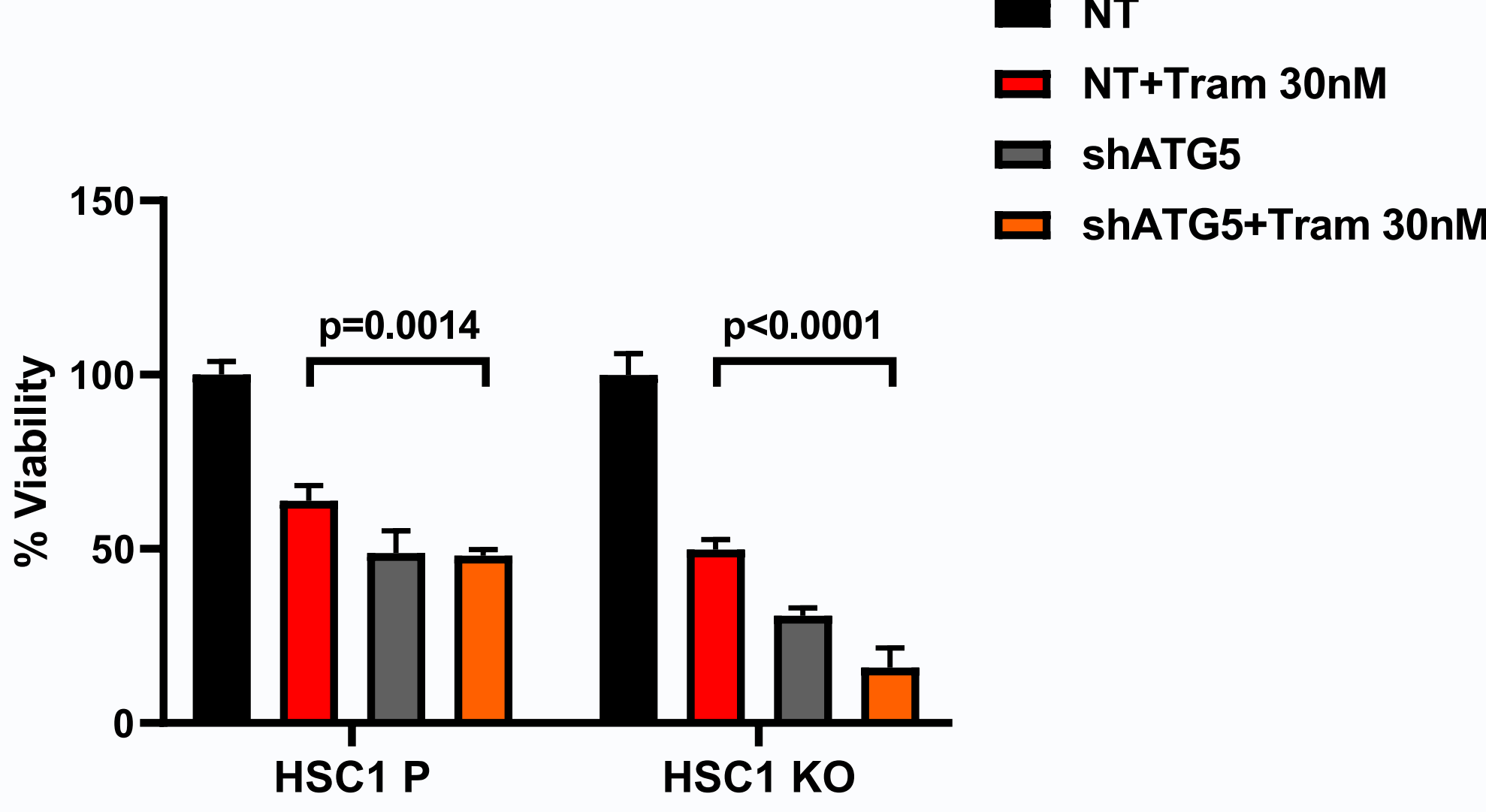


Figure 2. NF1 KO demonstrates increased autophagy at baseline and in response to cellular stress. **A.** Cartoon representing role of LC3 in autophagosome formation. LC3 accumulation is representative of autophagic flux. **B.** Representative western blot and bar graph demonstrating increased accumulation of LC3 at lower concentrations of CQ in NF1 KO. **C.** Bar graph demonstrating increased autophagic flux in the NF1 KO in response to nutrient deprivation at 0 and 10 min.

A.



B.

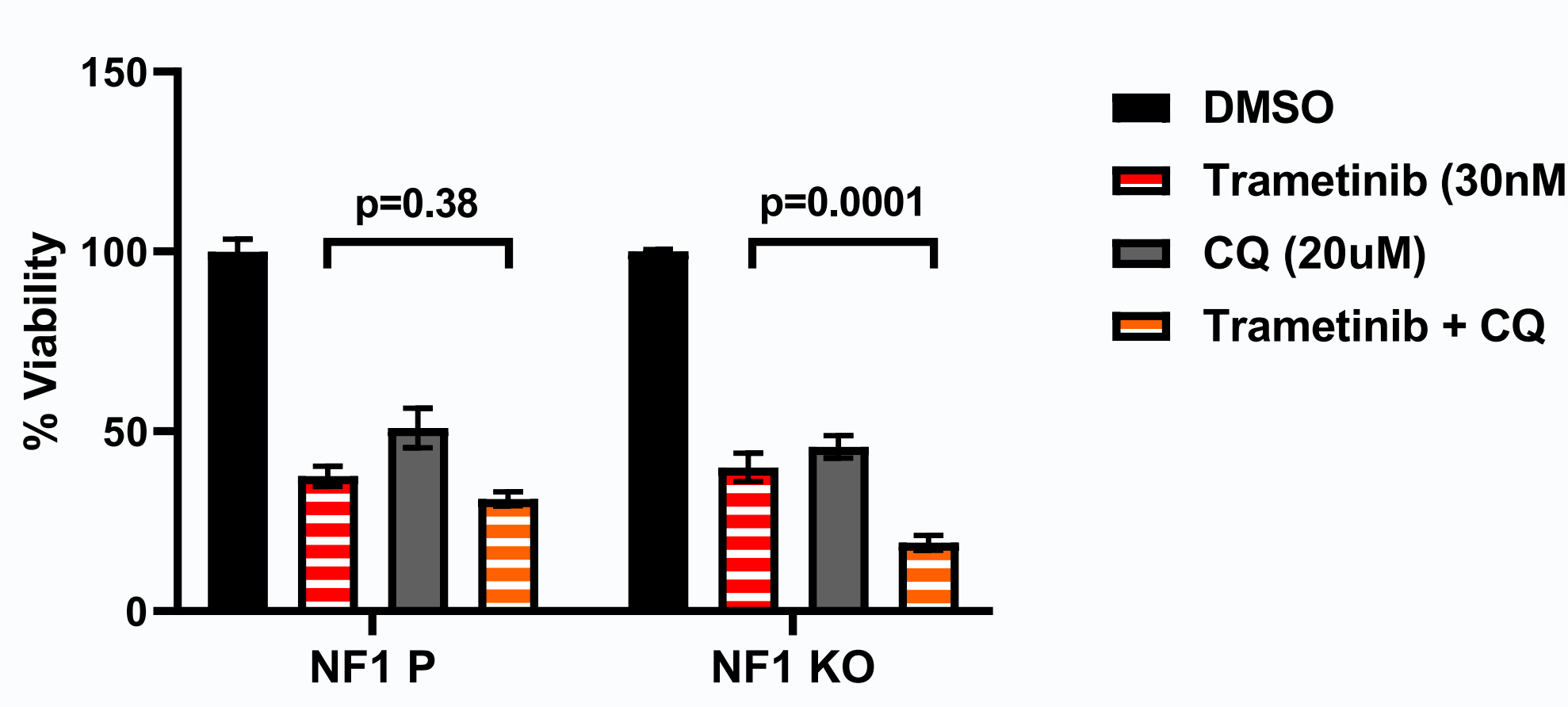


Figure 3. Pharmacologic and genetic autophagy inhibition increase efficacy of trametinib **A.** Bar graphs representing % viability of control and NF1 KO cell lines after 5 days pharmacologic treatment. Combination CQ and trametinib is significantly more effective than trametinib alone in the NF1 KO but not in control lines (n=3). **B.** Bar graphs representing decreased cellular viability when trametinib is combined genetic autophagic inhibition via shRNAi targeting of ATG5 than alone in NF1 KO line (n=3)

Conclusions

- Our NF1-KO cells exhibit upregulation of the MAPK pathway
- Non-BRAF dysregulation within the MAPK pathway yields increased autophagic activity
- Under serum starvation stress, NF1 KO cells upregulate autophagy to a greater extent than NF1 WT cells.
- Inhibition of autophagy via genetic inhibition or chloroquine increases sensitivity to MEK inhibition
- Autophagy inhibition via CQ may be an effective adjunctive treatment for NF1 mutated tumors and suggests that diverse CNS tumor types with MAPK pathway dysregulation are susceptible to autophagy inhibition

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