

Human Herpesvirus 8 ORF57 protein as a candidate therapeutic for TDP-43 pathology:

Network analysis Identifies Interacting Pathways

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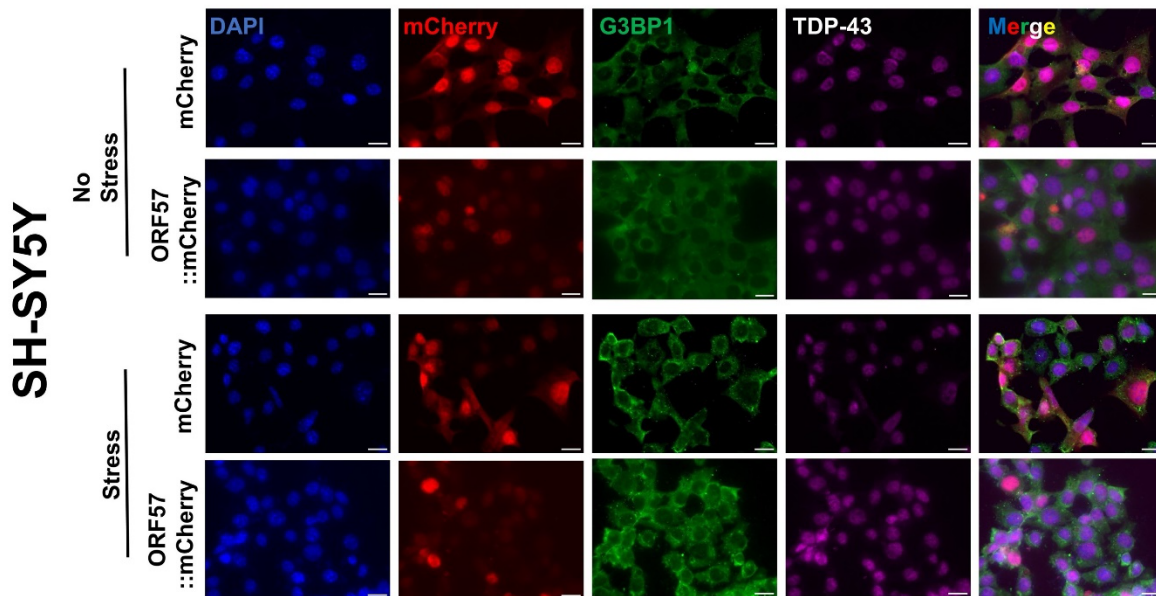
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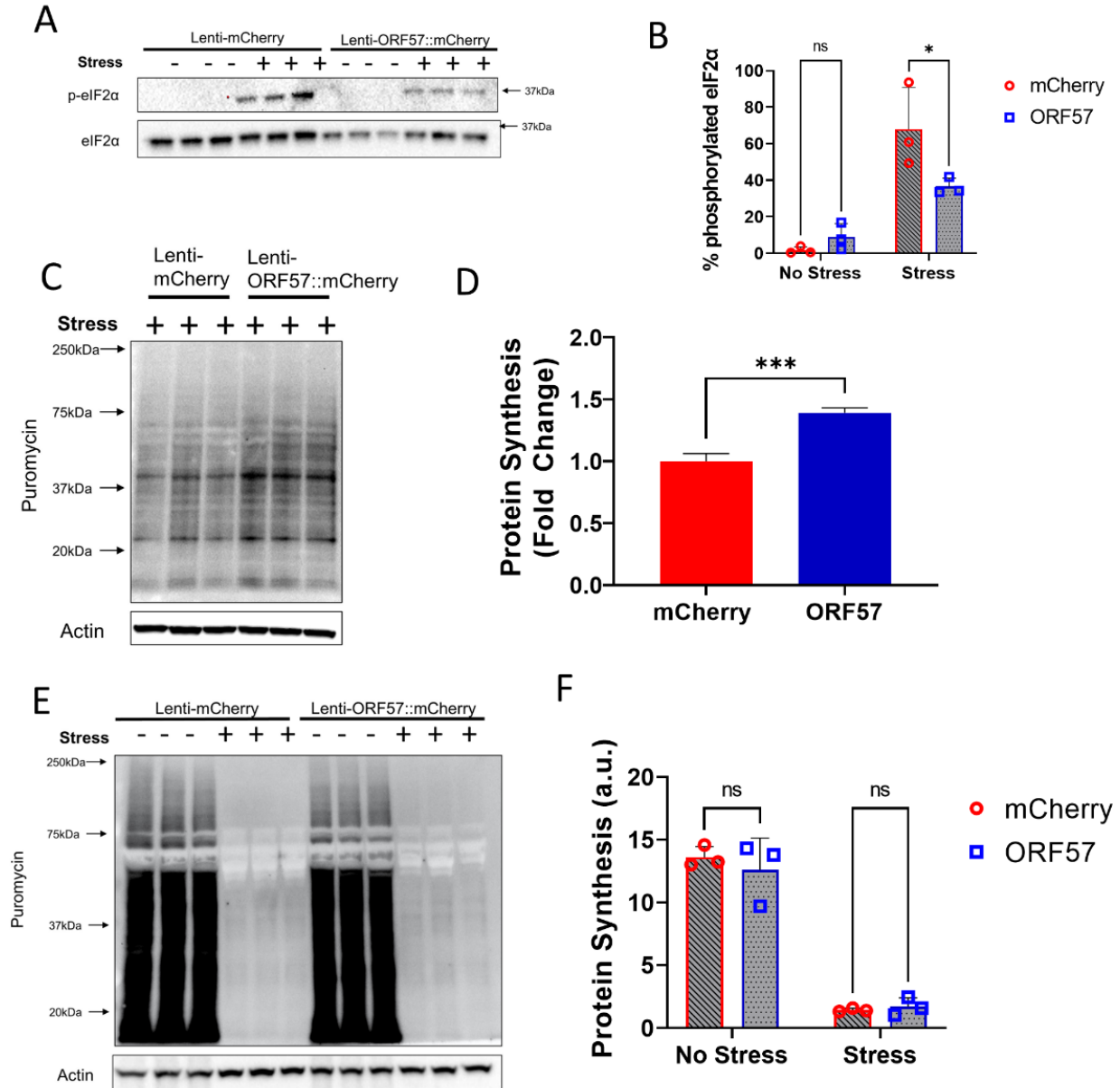
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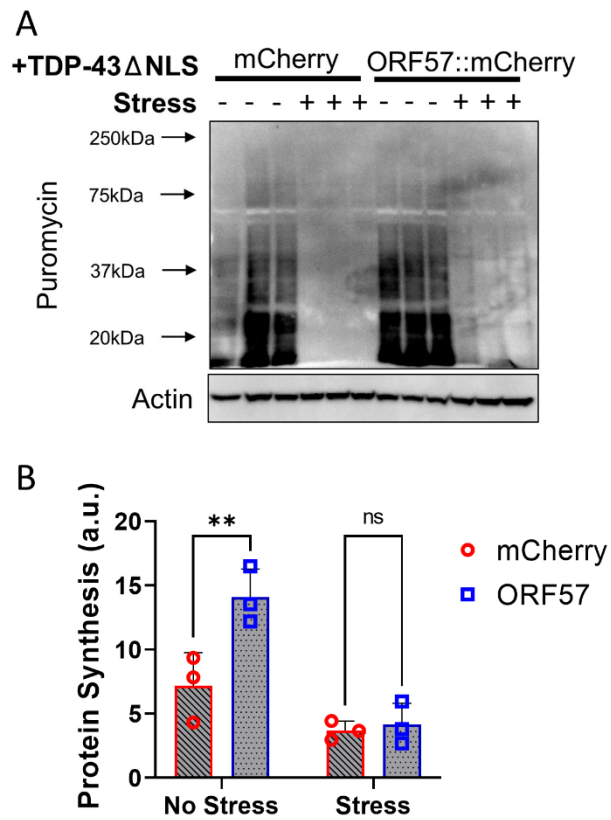


Supporting Figure 1. Sodium arsenite stress in SH-SY5Y cells expressing wildtype TDP-43. SH-SY5Y cells were stressed with 300 μ m sodium arsenite stress for 90 minutes and compared to nonstressed cells. Immunocytochemistry of these cells was performed with DAPI (405), mCherry or ORF57::mCherry (594), G3BP1 (488), and TDP-43 (647).

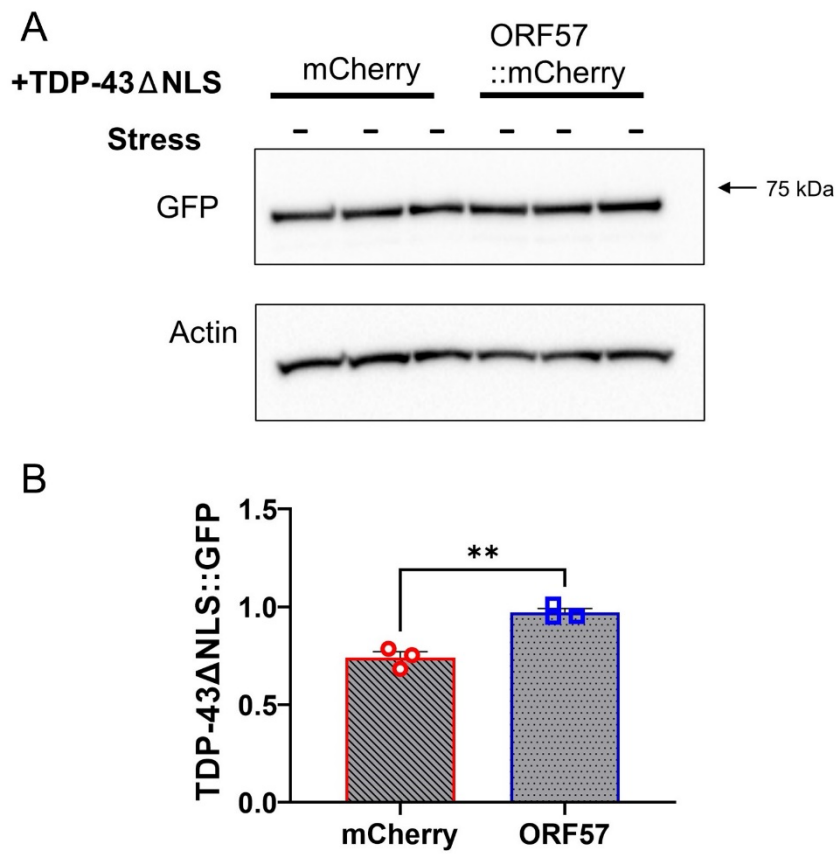


Supporting Figure 2. ORF57 protects against oxidative stress in human neuroblastoma cells.

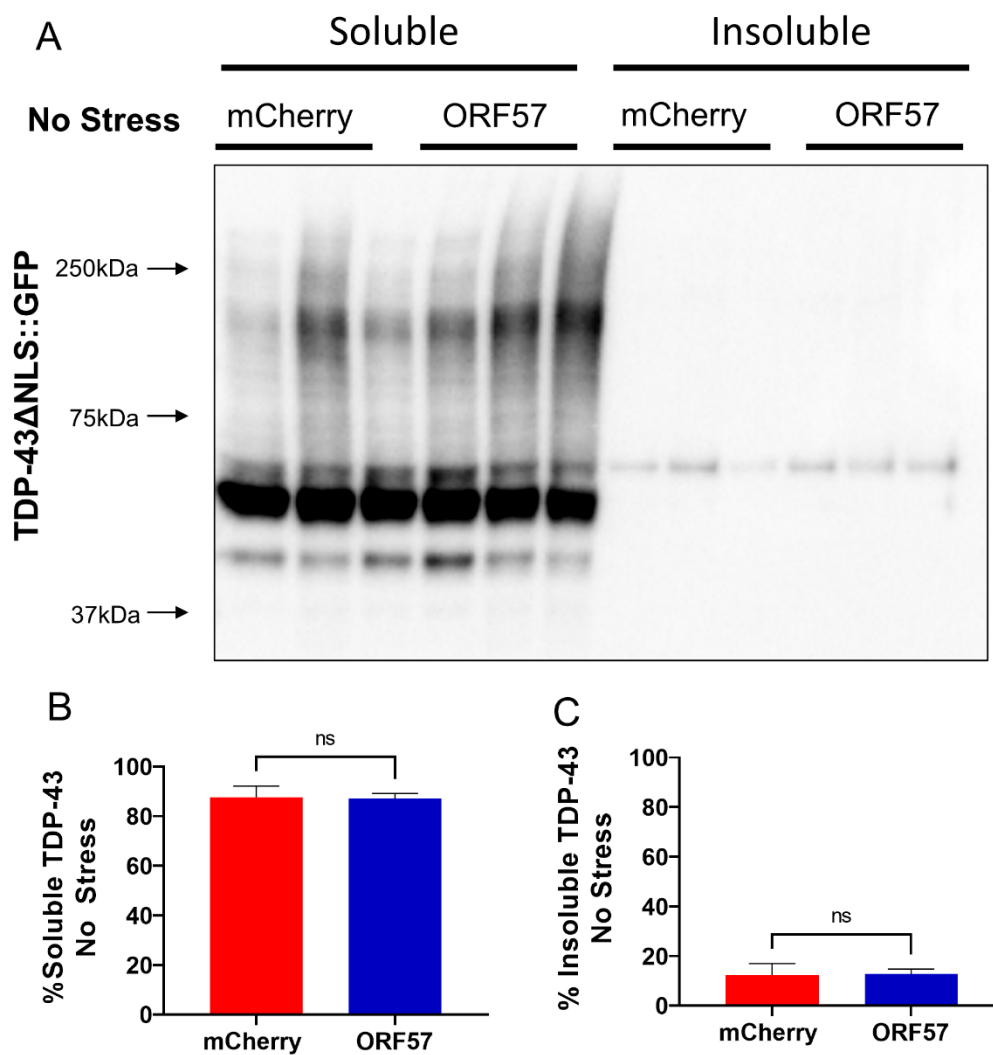
Experiments conducted in mCherry and ORF57 mCherry expressing SH-SY5Y cells with and without SA stress. **A)** Immunoblot for phosphorylated eIF2 α and total eIF2 α . **B)** Quantification of A. **C)** SUnSET of mCherry and ORF57 expressing cells with sodium arsenite stress. **D)** Quantification of protein synthesis in stressed cells. **E)** SUnSET of mCherry and ORF57 expressing cells with and without sodium arsenite stress **F)** Quantification of protein synthesis in E. Two-way ANOVA with Bonferroni post hoc comparison, *P < 0.05, **P < 0.01, ***P < 0.0001. Scale bars 20 μ m.



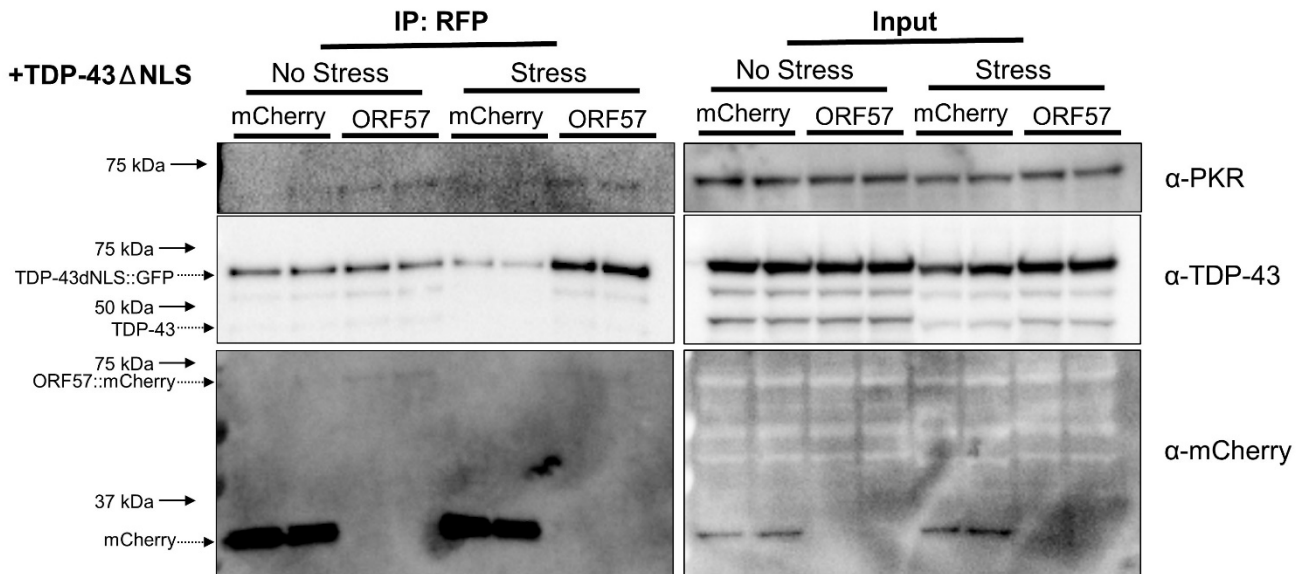
Supporting Figure 3. Protein synthesis in TDP-43 Δ NLS cells with and without sodium arsenite stress. A) SUnSET of mCherry and ORF57 expressing TDP-43 Δ NLS cells with and without sodium arsenite stress **B)** Quantification of protein synthesis in A.



Supporting Figure 4. TDP-43 Δ NLS transgene expression. A) Immunoblot of TDP-43 Δ NLS::GFP with mCherry or ORF57 overexpression **B)** Quantification of immunoblot in A.



Supporting Figure 5. Basal TDP-43 fractionation. Lysate from TDP43DNLS cells was fractionated in RIPA soluble and insoluble fractions and run on a semi non-denaturing immunoblot. A.) Immunoblot of soluble and insoluble TDP-43::GFP in unstressed cells. Quantification of the immunoblot in B.) and C.).



Supporting Figure 6. Immunoprecipitation of ORF57::mCherry and mCherry control. mCherry tag on ORF57::mCherry and mCherry control cells was immunoprecipitated with RFP nanobody. IP fraction and total lysate were probed for PKR, TDP-43, and mCherry.

Supporting Table 1: See Excel file.

Supporting Table 1: ORF57 interactors identified by IP-MS (in basal and stress conditions). 211 proteins that were 1) significant in mCherry vs ORF57 or mCherry-SA vs ORF57-SA comparisons and 2) showed a log₂ fold-change bigger than 0.58 are included in the table. Proteins that were significant but higher in the mCherry conditions or not significant in any of the two comparisons are not shown.