Pridopidine restores mitochondrial function, ER connectivity and decreases ER stress, mediated through the S1R

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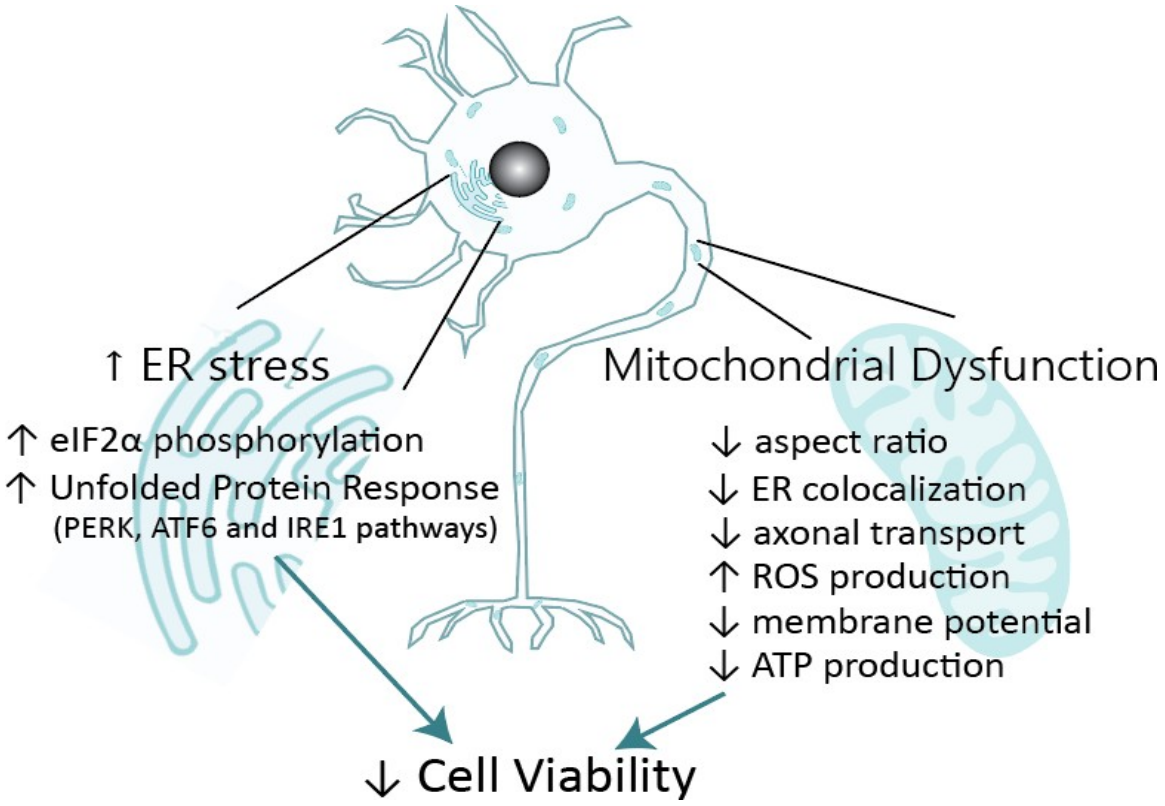
Background

- Pridopidine is a **selective and potent Sigma-1 receptor (S1R) agonist** in clinical development for HD and ALS.
- The S1R is a chaperone protein localized in the mitochondria-associated ER membranes (MAM). It **regulates ER signaling, generation of reactive oxygen species (ROS) and mitochondrial function.**
- Pridopidine exerts **neuroprotective functions** in several preclinical models of neurodegenerative disorders, including HD and ALS, via activation of the S1R.
  - Pridopidine enhances spine density, neuronal plasticity, axonal transport, and upregulation of neurotrophic factors.
- ER stress and mitochondrial dysfunction are both **contributors to the neurodegenerative process**, and **cellular hallmarks** of HD and ALS.

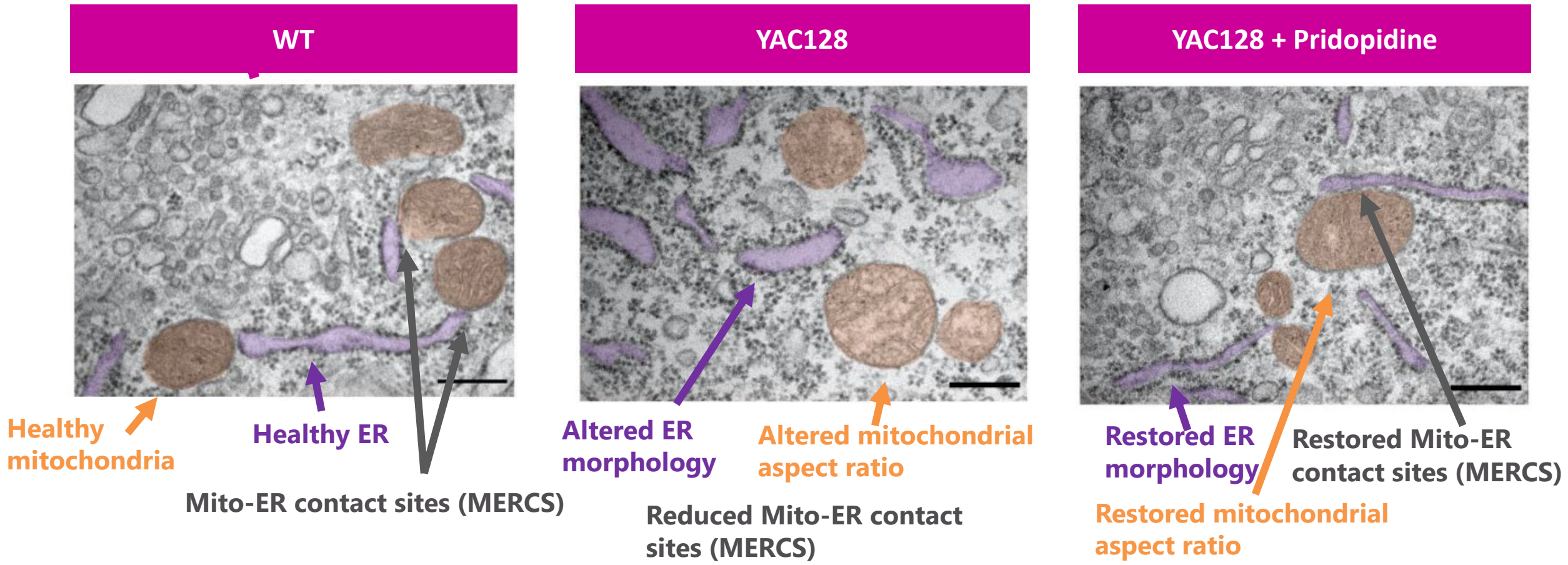
In HD neurons:



1. Tesei et al, Frontiers in Pharmacology. 2018; 2. Hayashi and Su, Cell. 2007; 3. Tsai et al, PNAS. 2009; 4. Ryskamp et al, Front. Neurosci. 2019; 5. Smith-Dijak et al.,Front Cell Neurosci 2019,. 6. Ryskamp et al, NBD. 2017, 7. Ionescu et al., Cell Death Dis 2019, 8. Geva et al., HMG 2016

Aspect ratio: ratio between major and minor axes of the mitochondria, ROS: reactive oxygen species

Pridopidine restores mitochondria and ER structure and connectivity, which are disrupted in HD



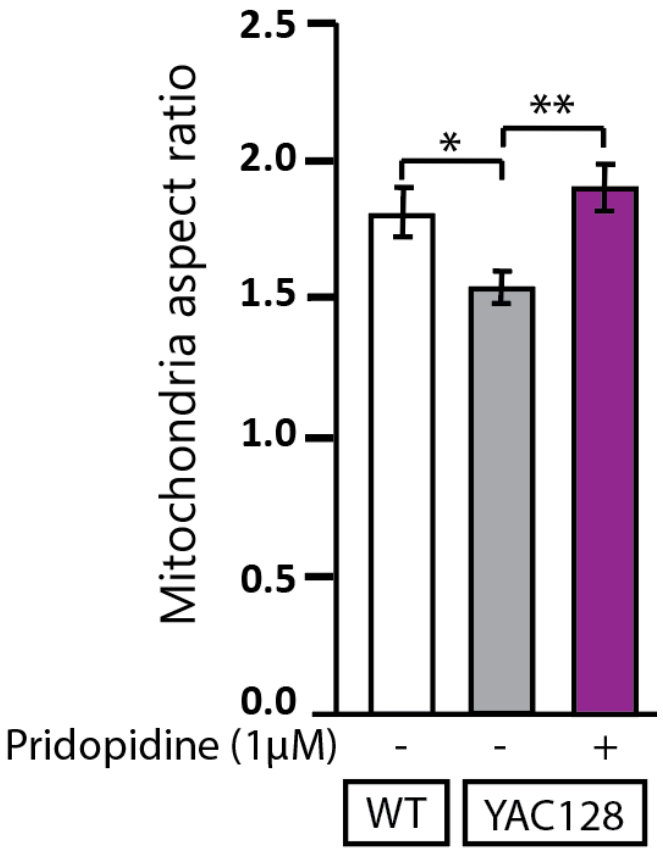
mitochondria ER YAC128 and WT striatal neurons, 24h pridopidine (1µM) treatment; 3 independent primary cultures. Scale bar= 300 nm;

YAC128 – HD model neurons , aspect ratio: ratio between major and minor axes of the mitochondria, indicative of mitochondrial health,

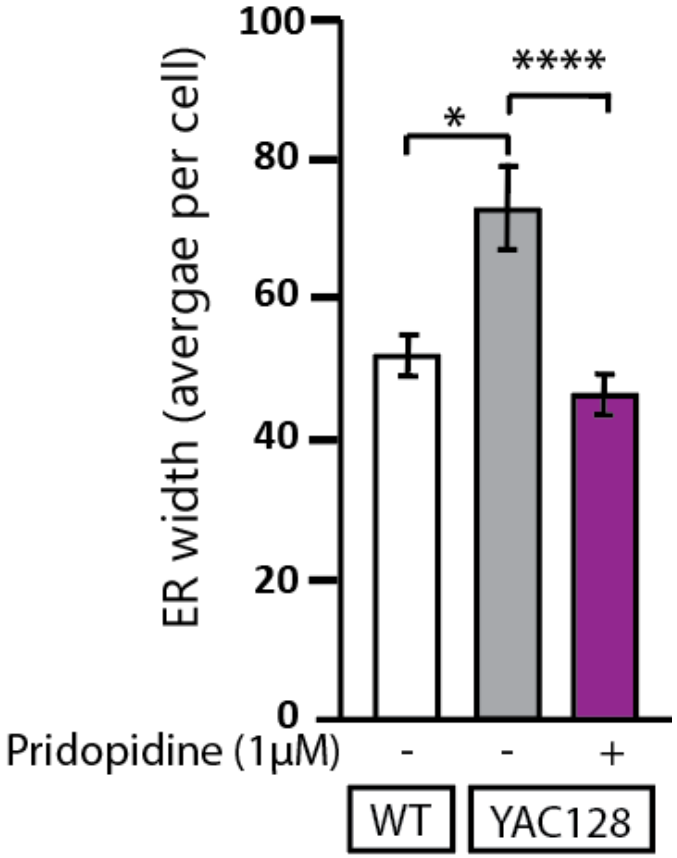
Naia et al., Neurotherapeutics. 2021

Pridopidine restores mitochondria and ER structure and connectivity, which are disrupted in HD

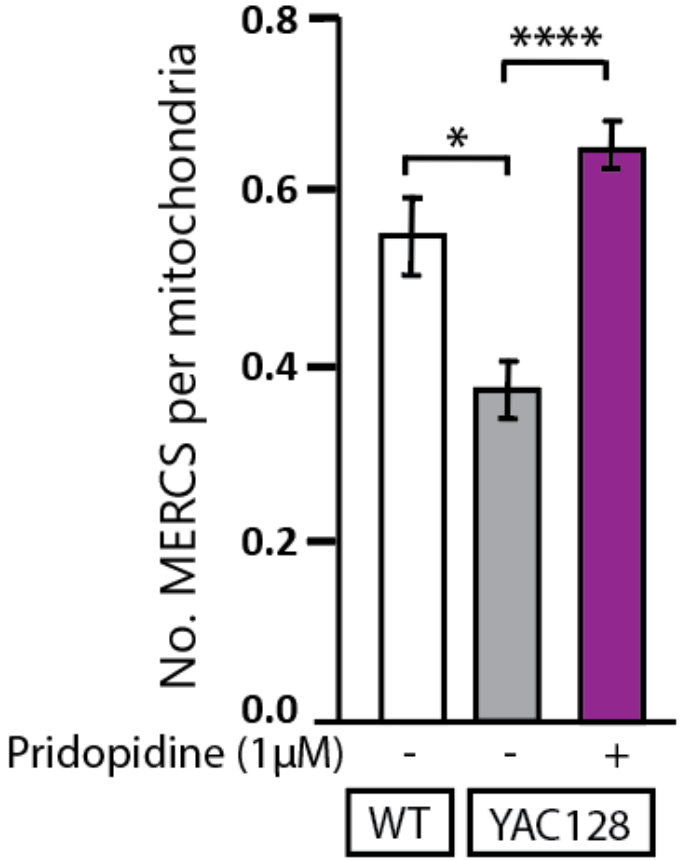
Mitochondrial morphology



ER morphology



Mitochondria-ER contact sites (MERCS)



WT HD untreated Pridopidine 1µM

YAC128 and WT striatal neurons, 24h pridopidine (1µM) treatment; 3 independent primary cultures.; *p<0.05, ****p<0.0001 by Kruskal Wallis test followed by Dunn multiple comparison test. ns = non-significant

YAC128 – HD model neurons , aspect ratio: ratio between major and minor axes of the mitochondria, indicative of mitochondrial health,

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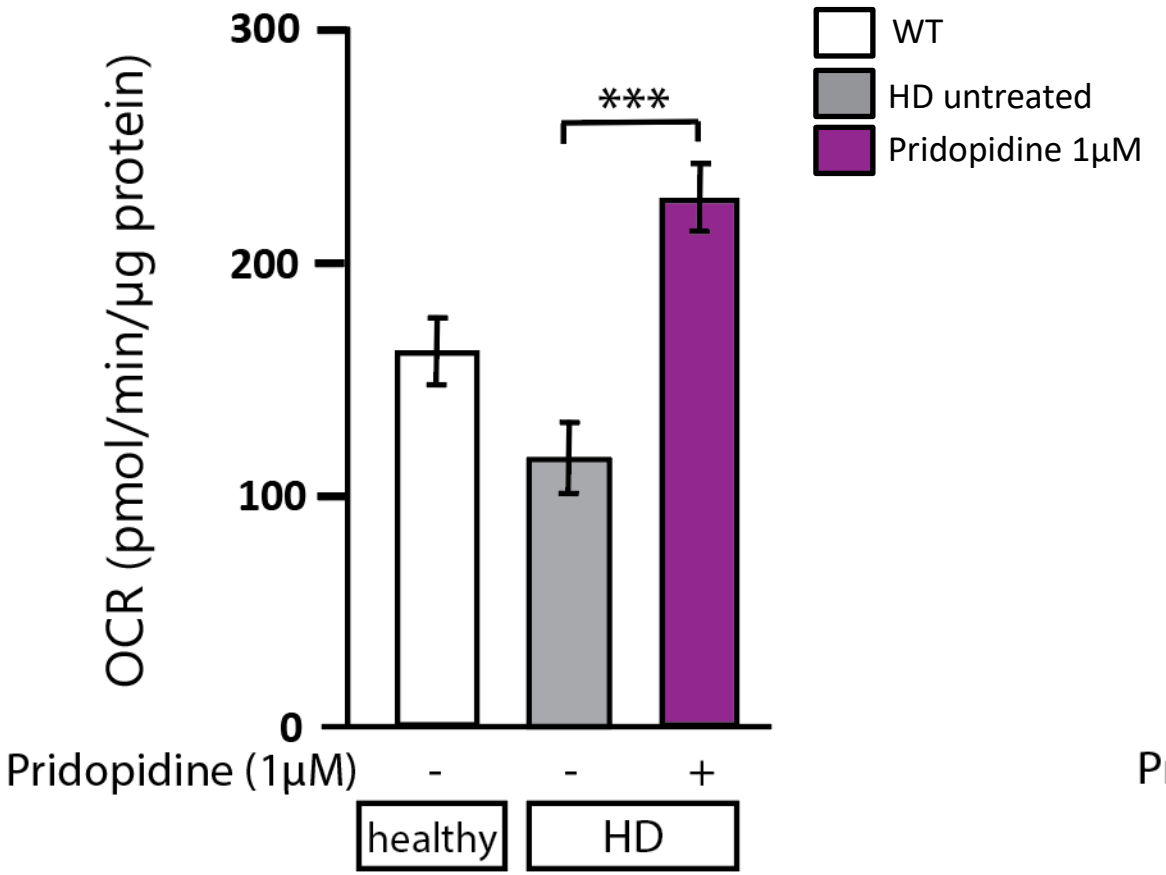
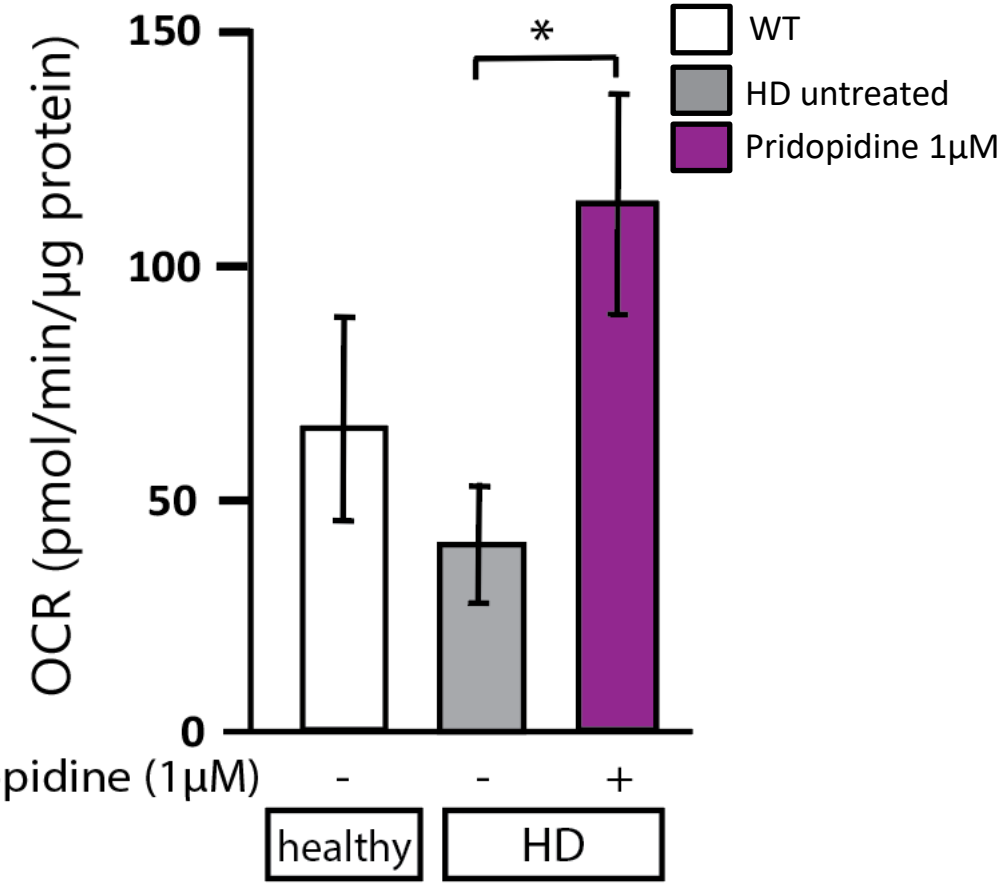
Pridopidine ↑ basal and maximal respiration and ATP production in HD neural stem cells

Basal Respiration



Maximal respiration



Oxygen consumption evaluated in neural stem cells (NSCs) treated with 1µM pridopidine for 24h using the Seahorse flux analyzer;

n=3-4, *p<0.05, ***p<0.001 by Kruskal Wallis test followed by Dunn multiple comparison test

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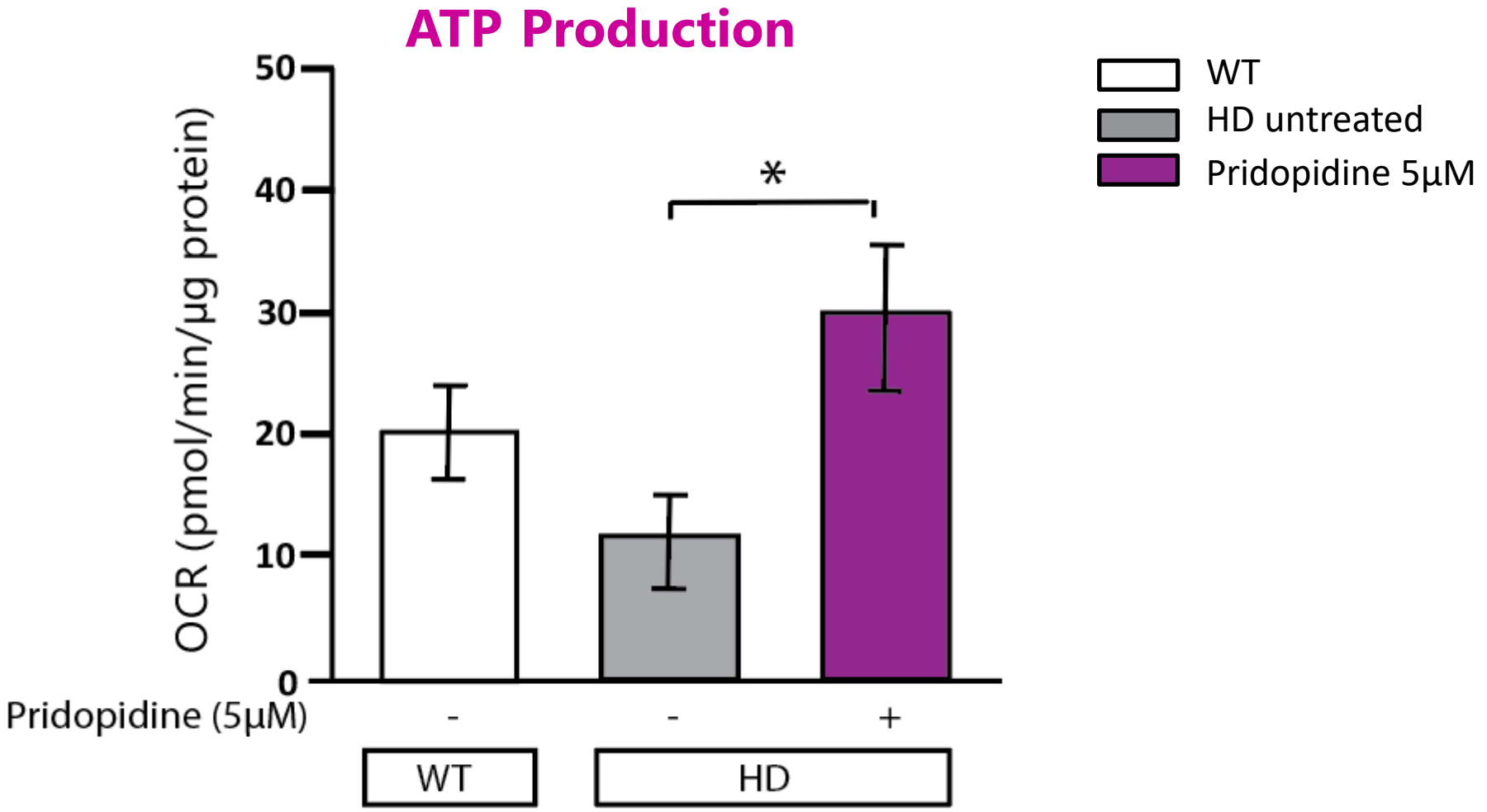
Pridopidine ↑ ATP production in YAC128 primary cortical/striatal neurons



Oxygen consumption evaluated in primary neurons treated with 5µM pridopidine for 24h

n=3-4, *p<0.05 by Kruskal Wallis test followed by Dunn multiple comparison test.

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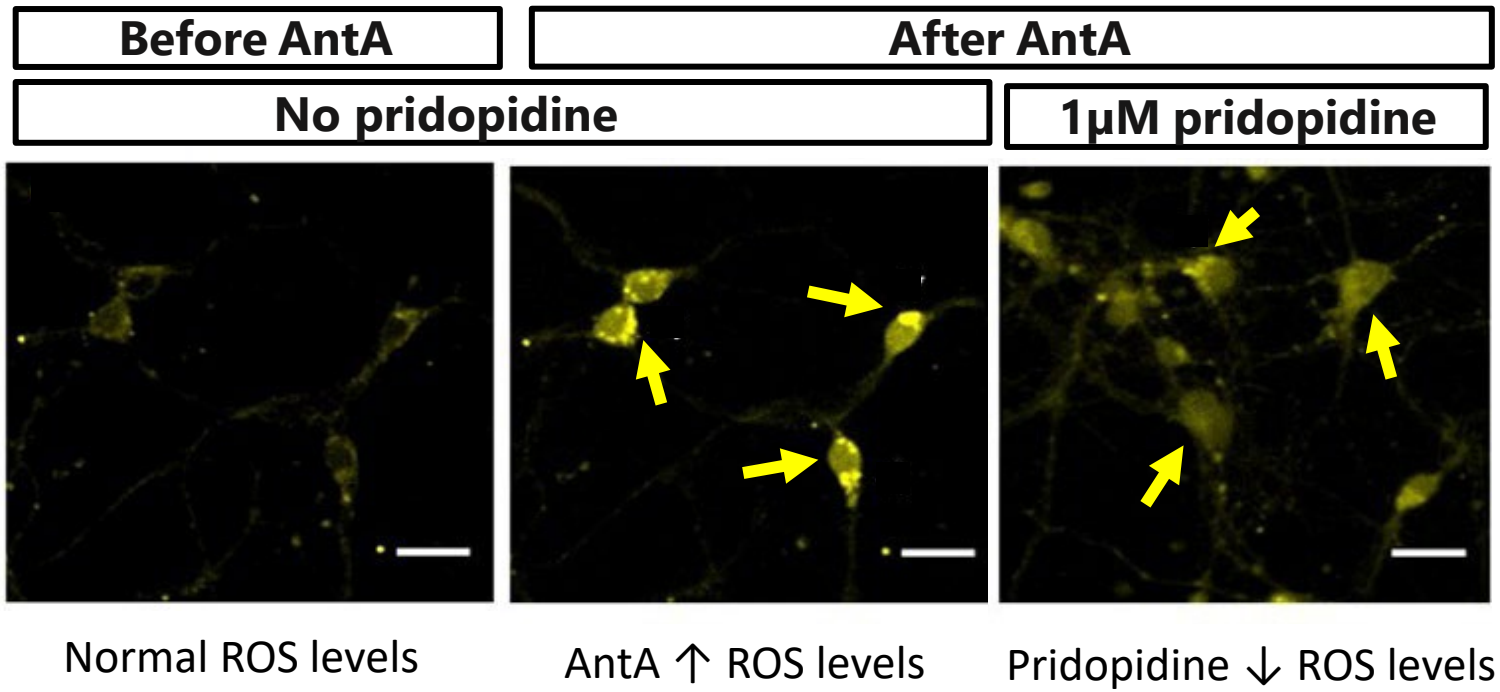
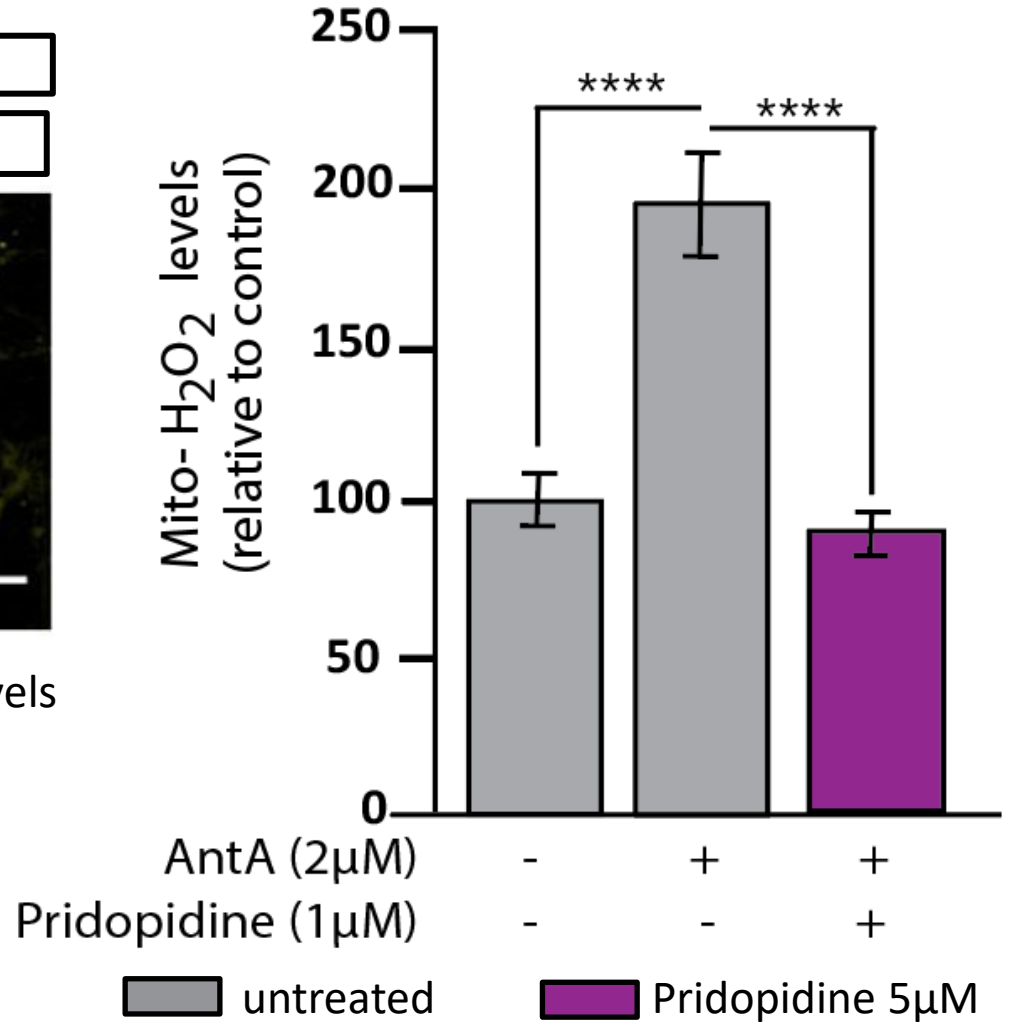
Pridopidine ↓ reactive oxygen species (ROS) levels in HD striatal neurons



ROS levels in mitochondria



AntA-Antamycin A → induces ROS production by inhibiting complex III of the electron transport chain

Striatal HD YAC128 neurons ; n=4, 20 cells/condition
Mito PY1 fluorescence, ****p<0.0001 by two-way ANOVA

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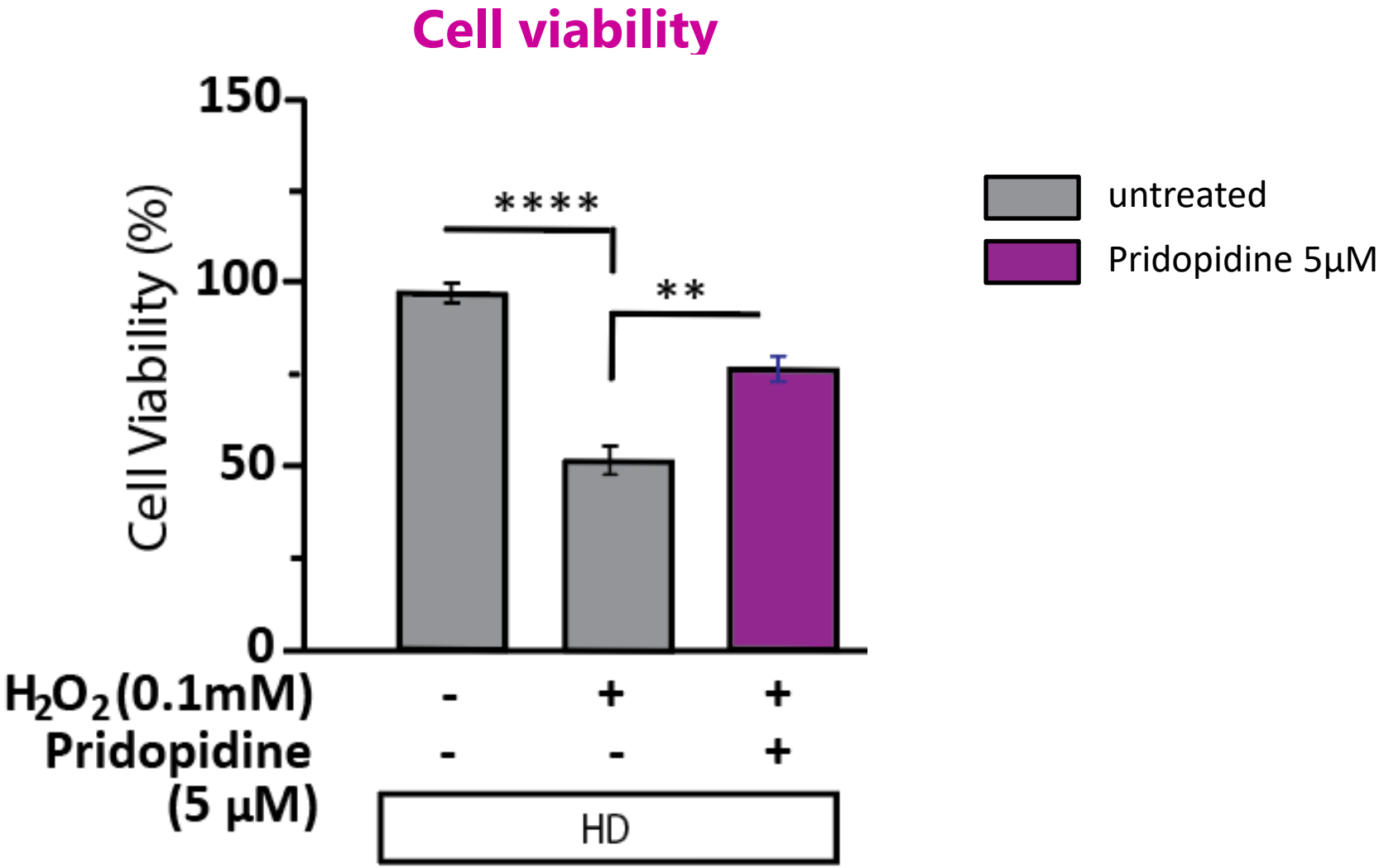
Pridopidine ↑ cell viability in human HD lymphoblasts (CAG 67/15)



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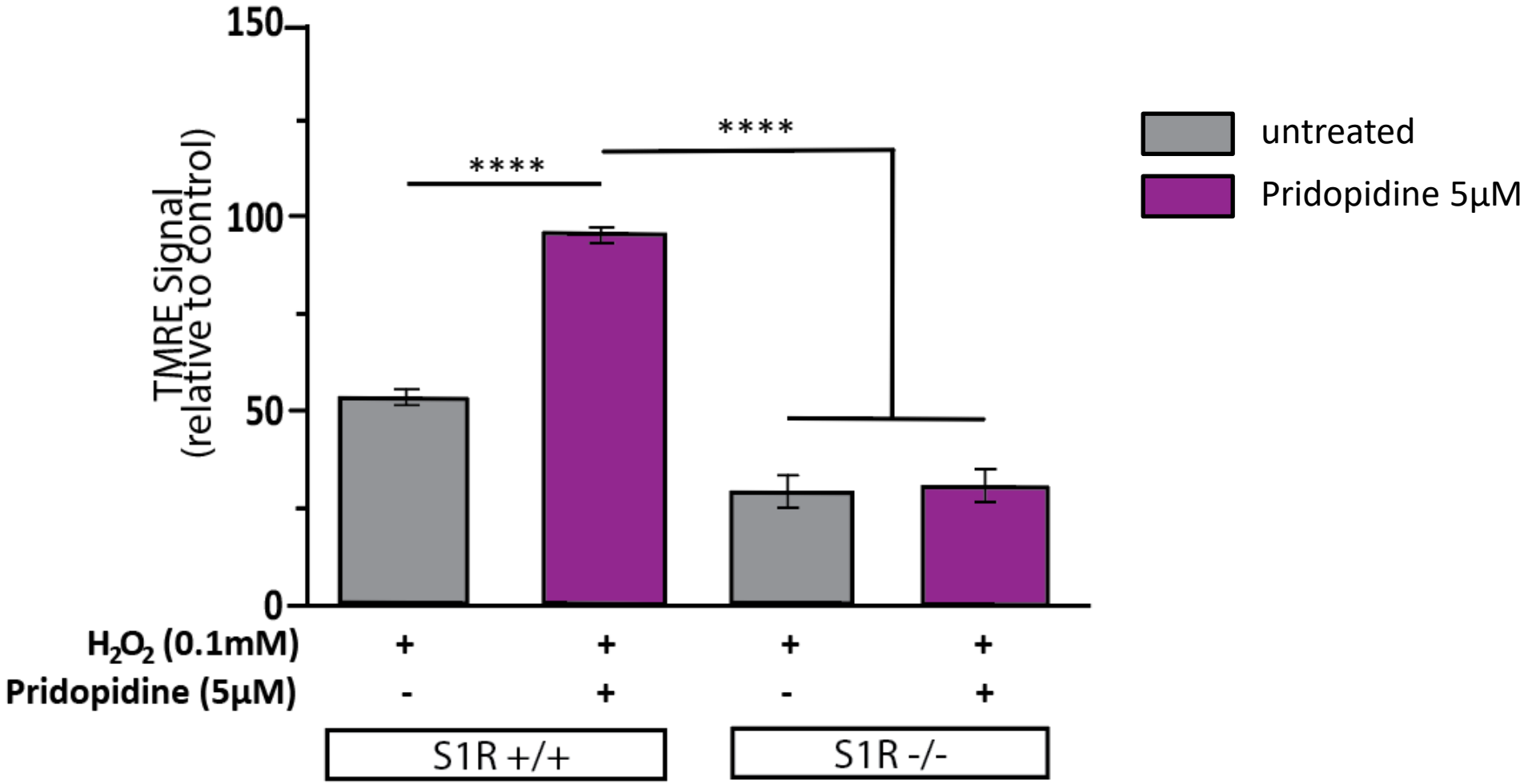
Human lymphoblasts HD patient NA04724, CAG repeat 67/15

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 two-way ANOVA with a Tukey's multiple comparisons post hoc test.

Pridopidine ↑ mitochondrial membrane potential in human HD lymphoblasts (CAG 67/15) in a S1R-dependent mechanism

Mitochondria membrane potential



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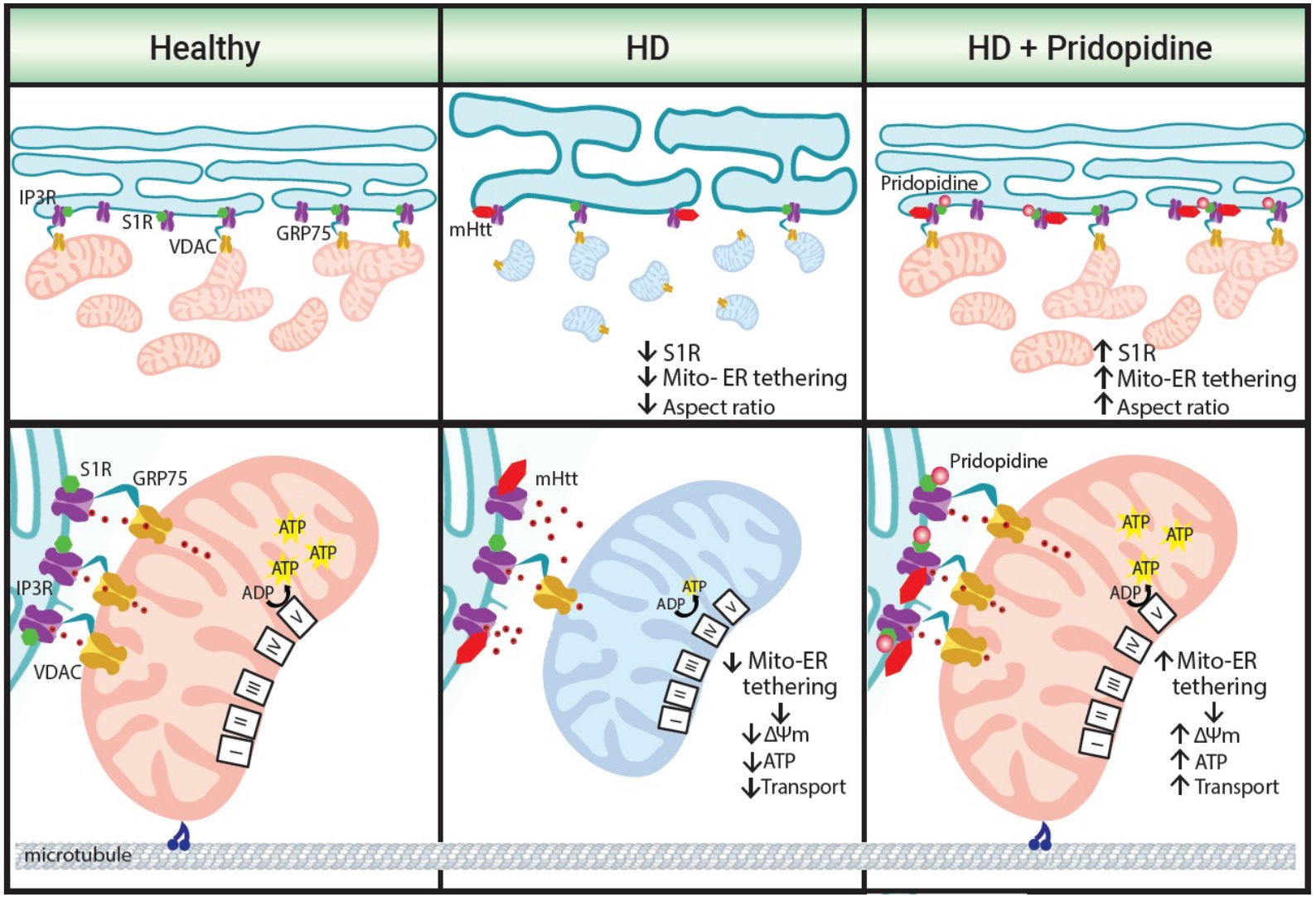
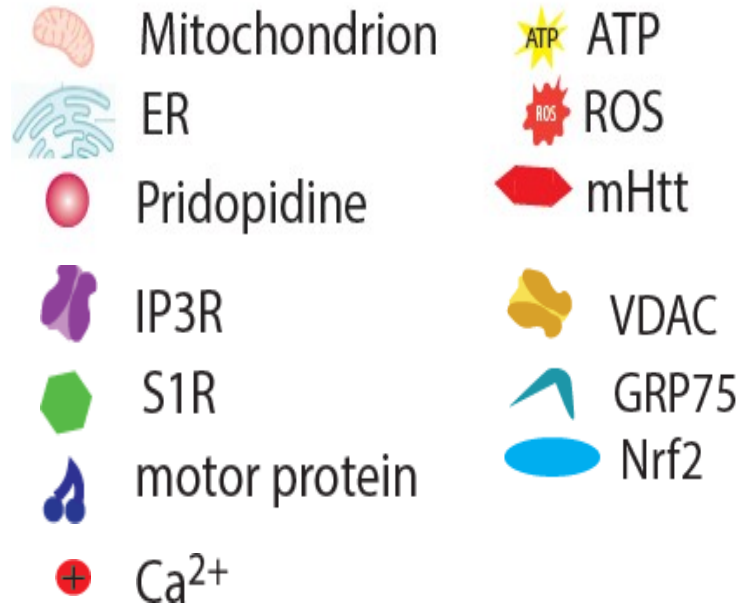
Human lymphoblasts: healthy control GM02174, CAG repeat 15/15, HD patient NA04724, CAG repeat 67/15
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 two-way ANOVA with a Tukey's multiple comparisons post hoc test.

Restoration of mitochondria-ER connectivity by pridopidine rescues multiple mitochondrial functions which are disrupted in HD





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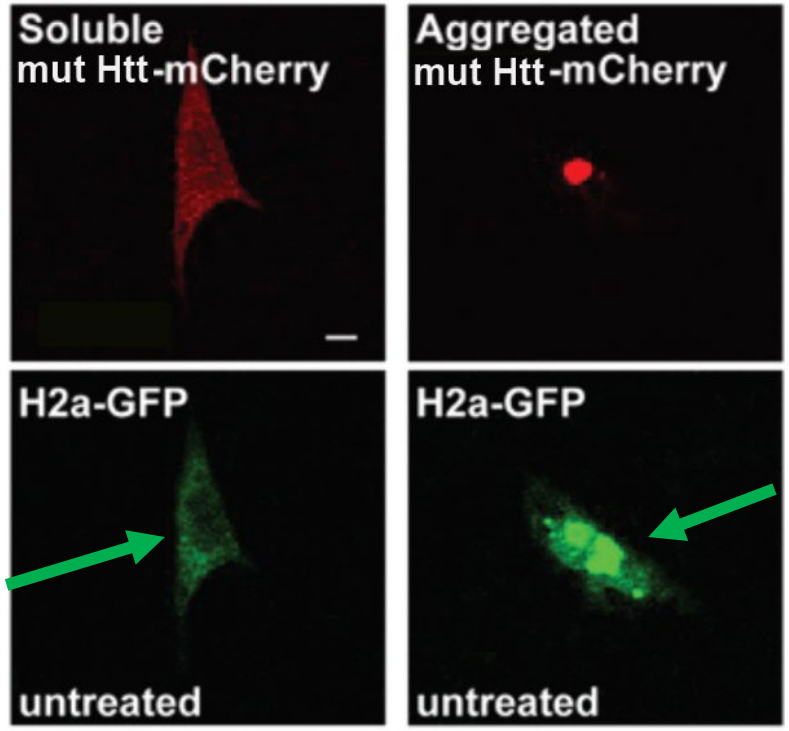
Pridopidine ↓ mHtt-induced ER stress

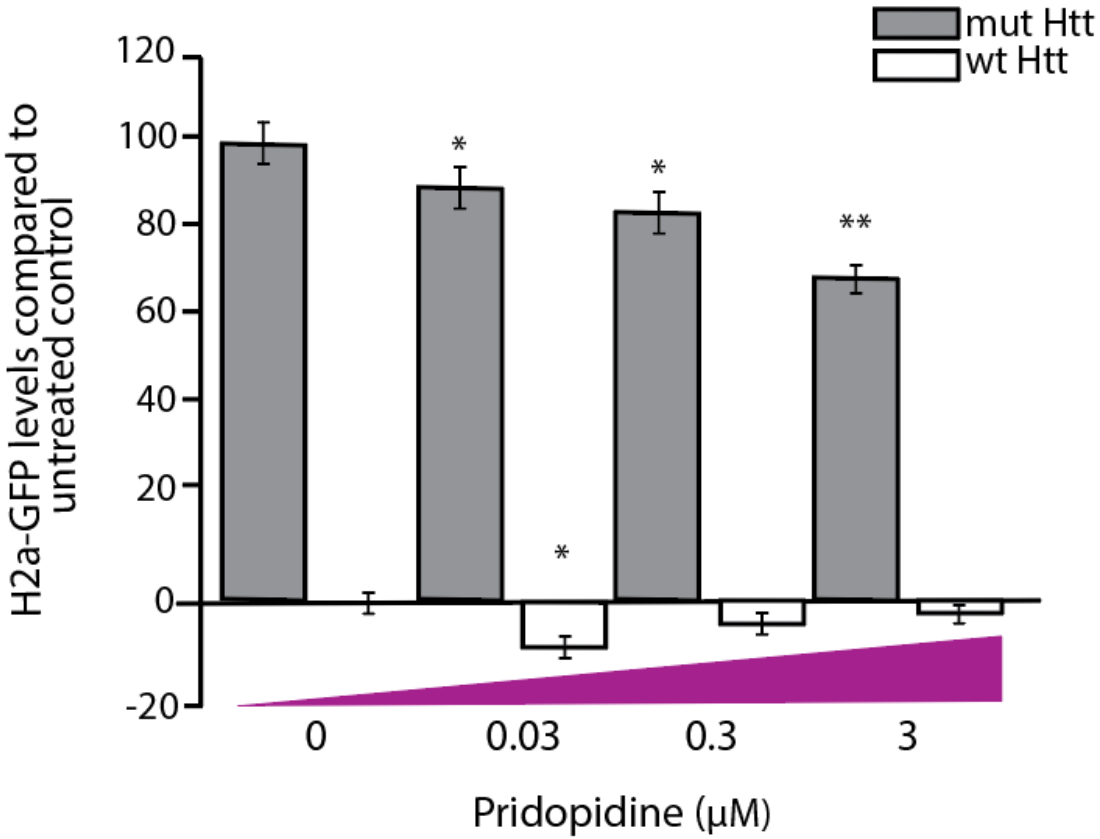
mHtt induces ER stress measured by aggregated H2a



Dispersed H2a =no ER stress

H2a aggregates indicate early ER stress

Pridopidine ↓ ER stress induced by mutant Htt



STHdhQ7/7 cells,
H2a-GFP coexpressed with Htt20Q-mCherry (wt) or Htt96Q-mCherry (mut Htt)
N=150 cells/experiment, 3 experiments.; *p<0.05; **p<0.01 compared to untreated mut Htt

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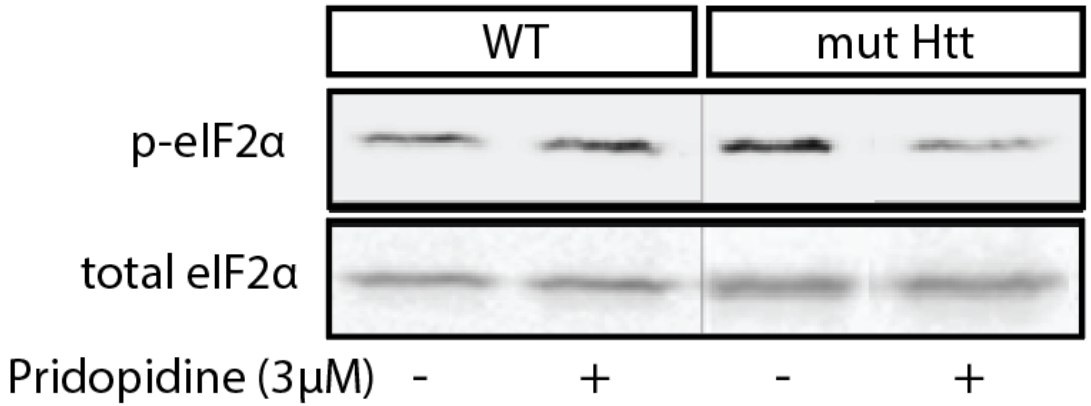
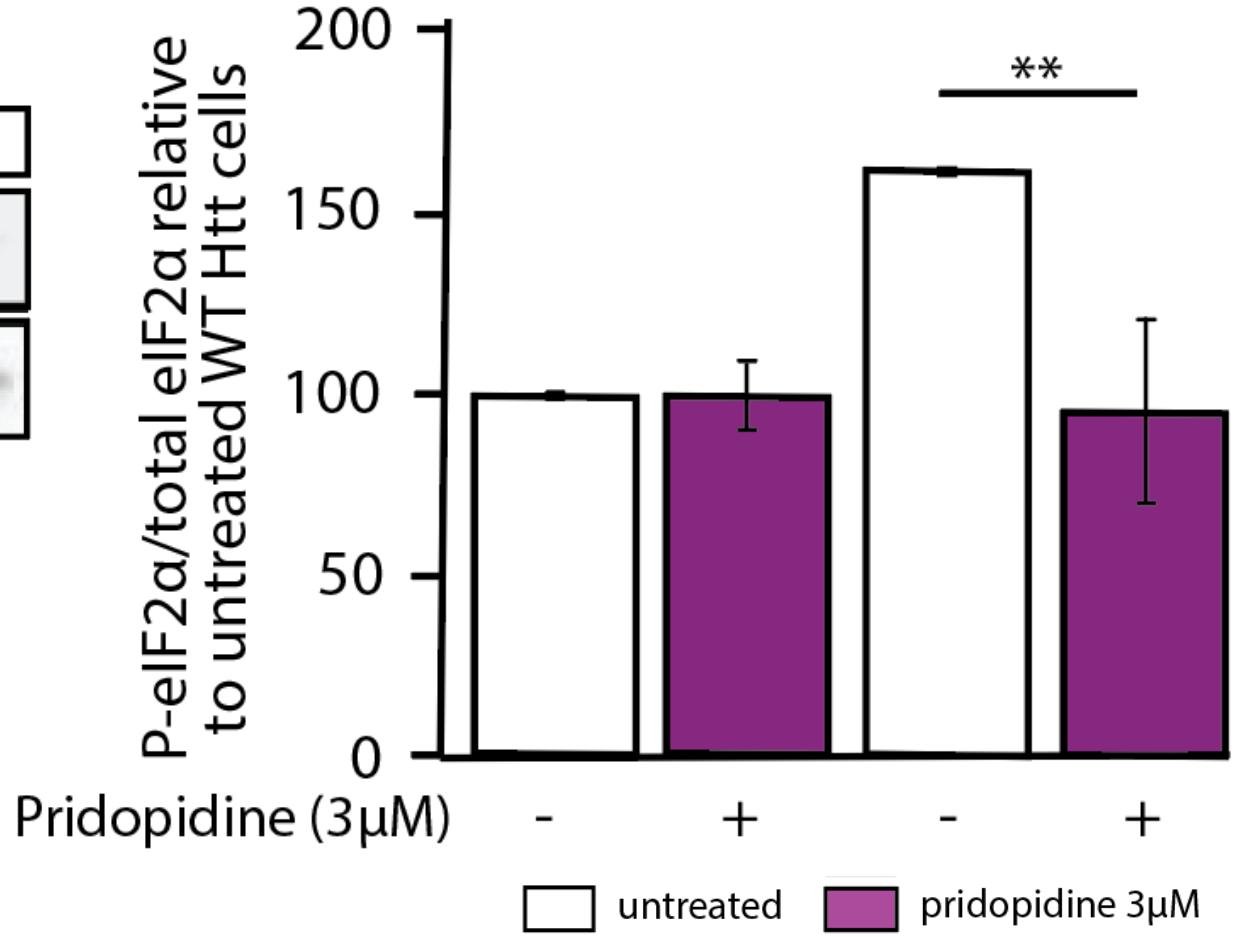
Pridopidine ↓ phosphorylated eIF2α, a marker of ER Stress in the PERK arm of the unfolded protein response (UPR) pathway

Pridopidine ↓ eIF2α phosphorylation



Mutant huntingtin-induced ER stress causes phosphorylation of **eIF2α**.



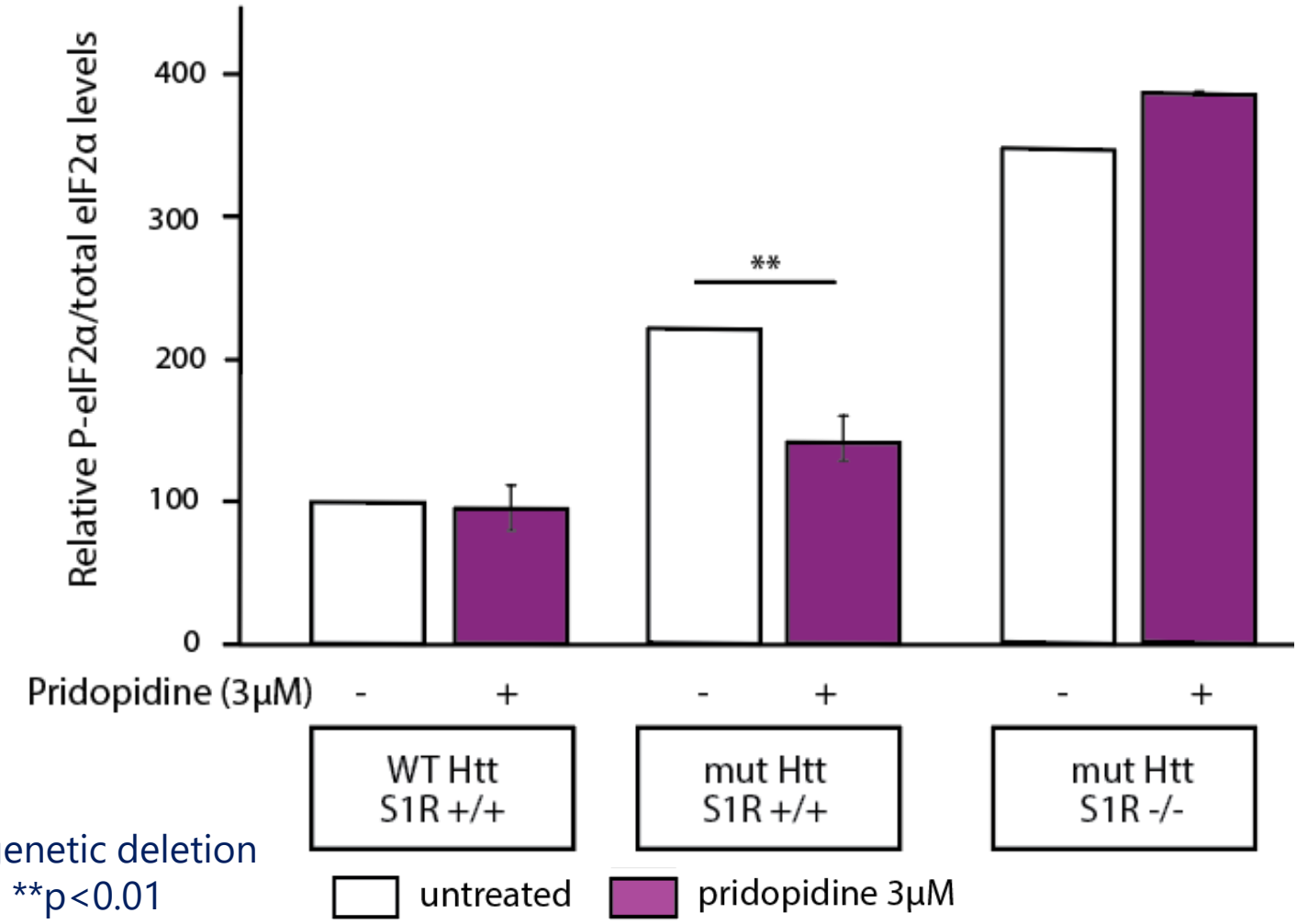
HEK 293 cells, mut Htt: Htt96Q, wt – Htt20Q, 8h pridopidine treatment; n=3, mean ±SD, **p<0.01

Shenkman et al., J Neurochem 2021

Pridopidine ↓ mutant Htt-induced ER stress in a S1R-dependent mechanism

- mHtt induces ER stress as measured by eIF2α-p
- Pridopidine ↓ eIF2α-p in S1R+/+ cells
- S1R -/- further ↑ eIF2α-p
- Pridopidine effect is abolished in S1R-/- cells

Pridopidine ↓ eIF2α phosphorylation in S1R -/- cells



HEK 293 cells, eIF2α-p: eIF2α phosphorylation; S1R genetic deletion (S1R-/-). 48h pridopidine treatment; N=3, mean ±SD, **p<0.01

Shenkman et al., J Neurochem 2021

Conclusions

- **Pridopidine rescues mitochondrial functions, mediated by the S1R:**
  - ↑ Mitochondrial-ER contacts
  - ↑ Respiration
  - ↑ ATP production
  - ↓ Production of toxic ROS
  - ↑ Membrane potential
  - ↑ Cell viability
- **Pridopidine reduces mHtt-induced ER stress, mediated by the S1R.**
  - ↓ ER stress
  - ↓ eIF2α-p

Pridopidine rescues mitochondrial function and reduces ER stress, which are hallmarks of HD.