Lowering Glutamate-Dehydrogenase in Neurons induces the autophagic-degradation of mHTT toxic aggregates and ameliorates motility defects in a *Drosophila* model for HD

Chiara Londero*, Stefania Santarelli*, and Paola Bellosta
CIBIO, University of Trento ITALY (*equally contributed-presenters)

EUROPEAN HUNTINGTON'S DISEASE NETWORK
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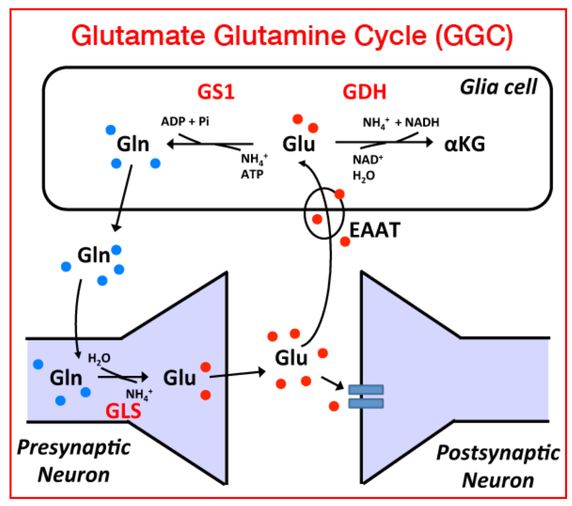
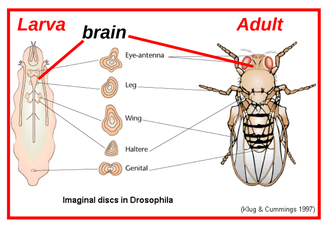
The pathogenesis of many neurodegenerative diseases, including Huntington's disease (HD), depends on metabolic changes controlled by glutamate, which is maintained at physiological level by a non-autonomous cycle between glia and neurons called *glutamate-glutamine cycle* (GGC). Key enzymes of this cycle are Glutamate Dehydrogenase (GDH) and Glutamine synthetase (GS1) that converts glutamate (Glu) to α-keto glutarate (αKG) and glutamine (Gln) respectively, and Glutaminase (GLS) that in neurons converts glutamine in glutamate, toxic to neurons. Few reports showed that some of these enzymes had an abnormal activity in patients with HD, so we decided to analyze their function in HD using Drosophila.

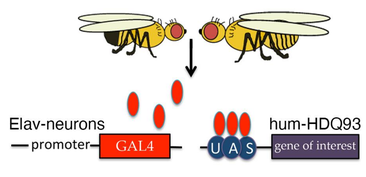


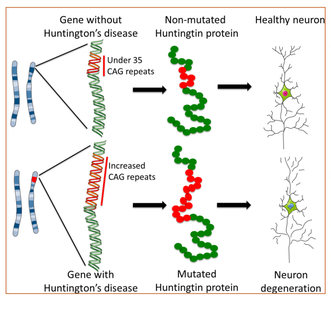
Drosophila model to study Huntington's diseases

- *Most basic biological, physiological, and neurological properties are conserved between mammals and Drosophila.*
- *Excellent to analyze therapeutic approaches of compounds and genes.*
- *Easy to manipulate and inexpensive to culture in laboratory.*

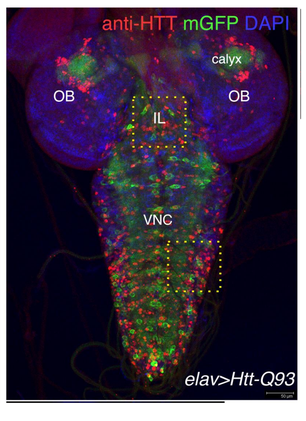


UAS-Gal4 system to express in neurons using Elav=Gal4

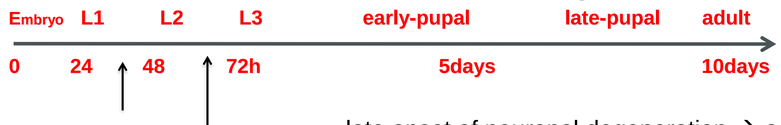


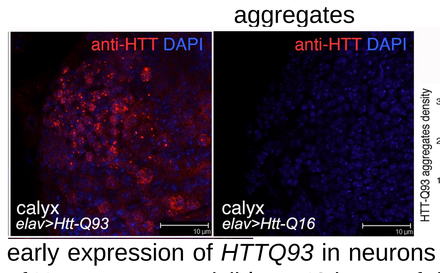


mHTTQ93 aggregates
in the brain of larvae, stained with antibody anti human HTT (IF)



Time line of HD in Drosophila:





early expression of *HTTQ93* in neurons results in the formation of Htt aggregates visible at 48 hours of development

mHTTQ93 aggregates in adult brain (IF)

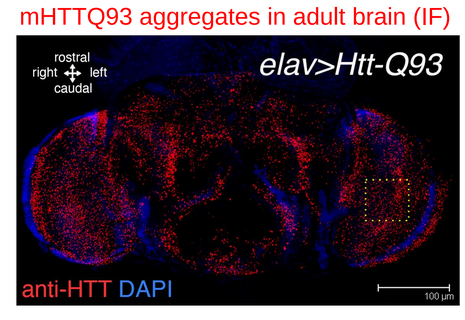
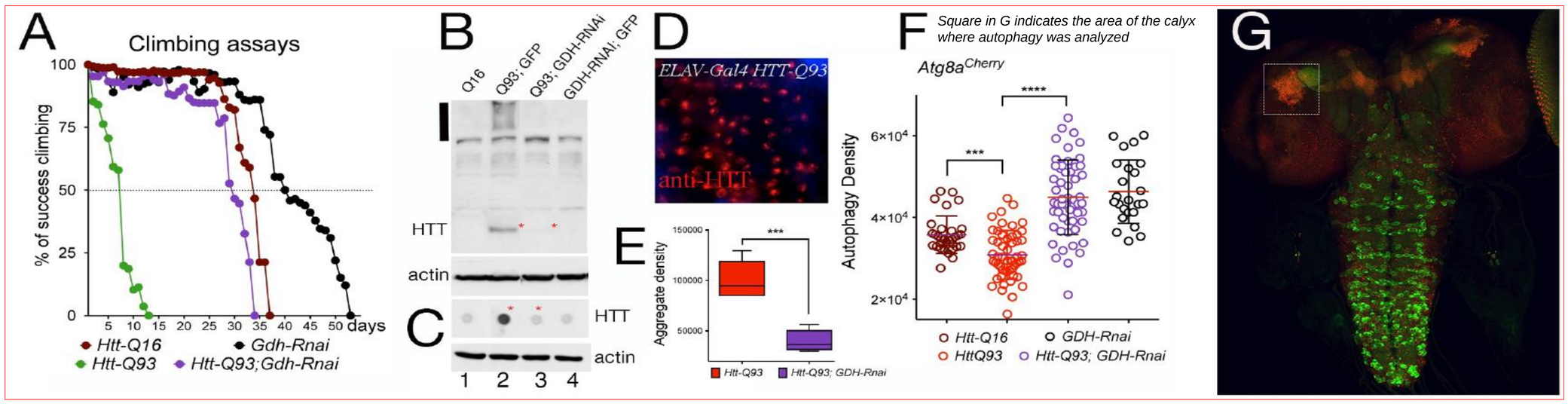


FIGURE 1 - Using a *Drosophila* model that expresses the first *Exon* of the human *Htt* gene with 93-CAG repetitions (*HTTQ93*) (Steffen at al, 2001) we performed a genetic interaction analysis to identify the function of member of the GGC in the toxicity phenotypes of HD. We identify that reduction of GDH ameliorates animal motility performing climbing assays (1A*). GDH-RNAi decreases the levels of HTT protein analyzed in the heads of adults (1B/C). GDH reduction reduces also the size of HTT aggregates in brains of animals expressing HTTQ93 (1D/E), due to activation of the autophagic flux (Fig 2A-C). Indeed, GDH-RNAi induces the formation of autophagosome measured with mCherry-Atg8 (1F/E).

*HttQ16 vs HttQ93: p-value<0.0001; HttQ93 vs HttQ93;GDH-RNAi: p-value<0.0001

Climbing assay: adult are tested for their ability to climb above a fix line in a tube within 15 sec. Their ability is monitored over time.



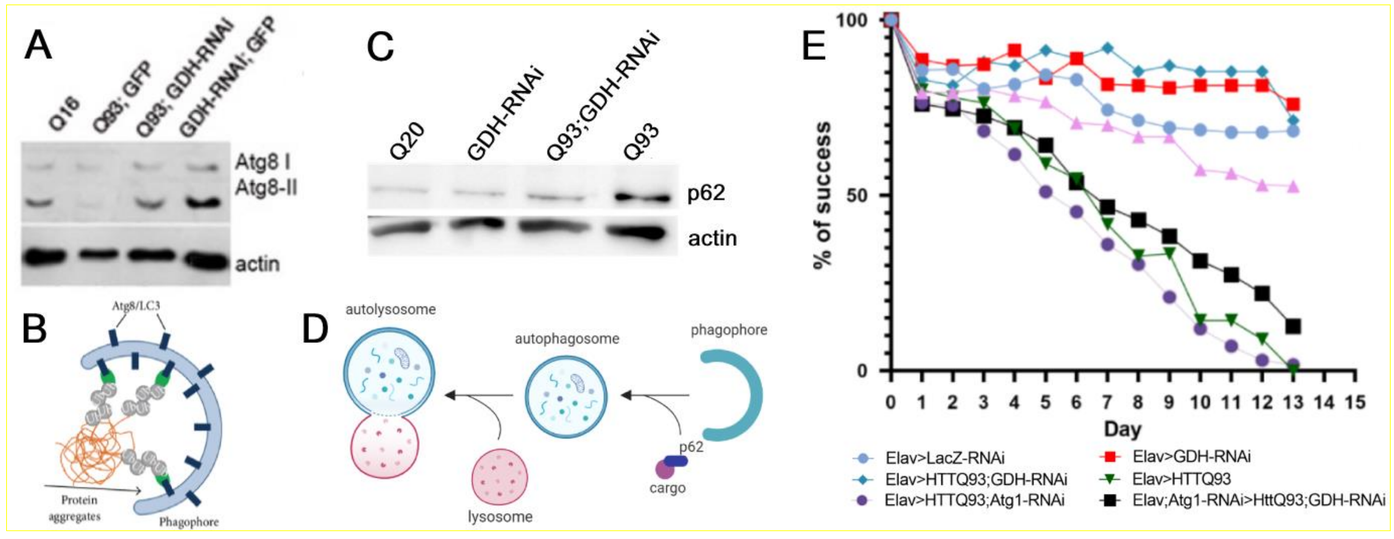


FIGURE 2 - GDH reduction is responsible for the activation of the autophagic flux, measured by the cleavage of Atg8 (2A) and the decrease in p62 levels (2C). The reduction of Atg1 eliminates the beneficial effect played by GDH downregulation on the amelioration of the motility of adult flies expressing HTTQ93 (2E*).

*Elav>HttQ93;GDH-RNAi vs Elav>HttQ93;Atg1-RNAi,GDH-RNAi: p-value<0.0001)

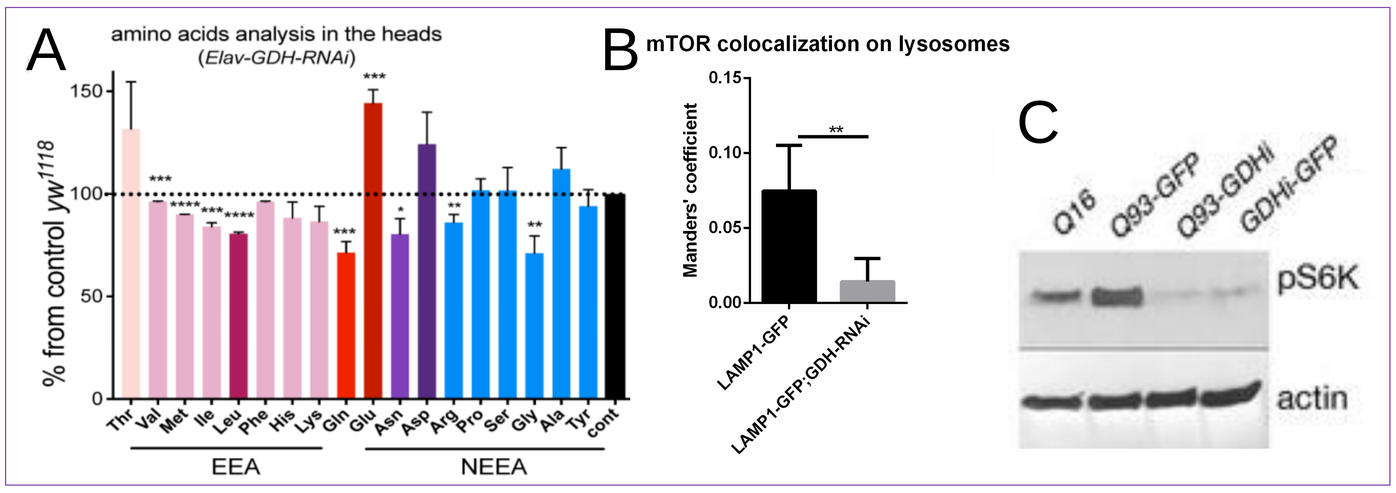
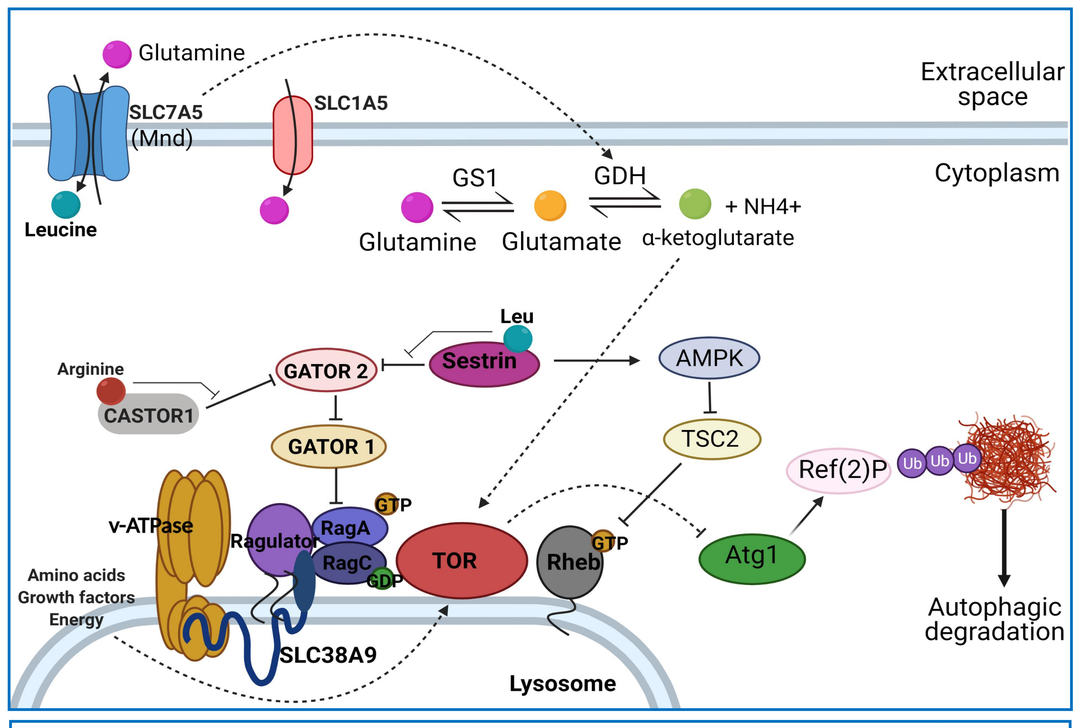


FIGURE 3 - Reduction of GDH in neurons decreases the levels of essential aminoacids (3A) resulting in a reduced localization of mTOR (mammalian target or Rapamycin) on the lysosomal membrane (3B) and to a reduced phosphorylation of one of its substrates, S6K, on Tre398 (3C).



MODEL: since leucin was shown to regulate GDH ability in controlling autophagy (Lorin et al, 2013) we are currently analyzing if modulation of Sestrin and Minidics (SLC7A5/Mnd) may be involved in the downregulation of TOR by GDH-RNAi

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