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Liver X Receptor signaling in the striatum and neuroprotection in Huntington's Disease

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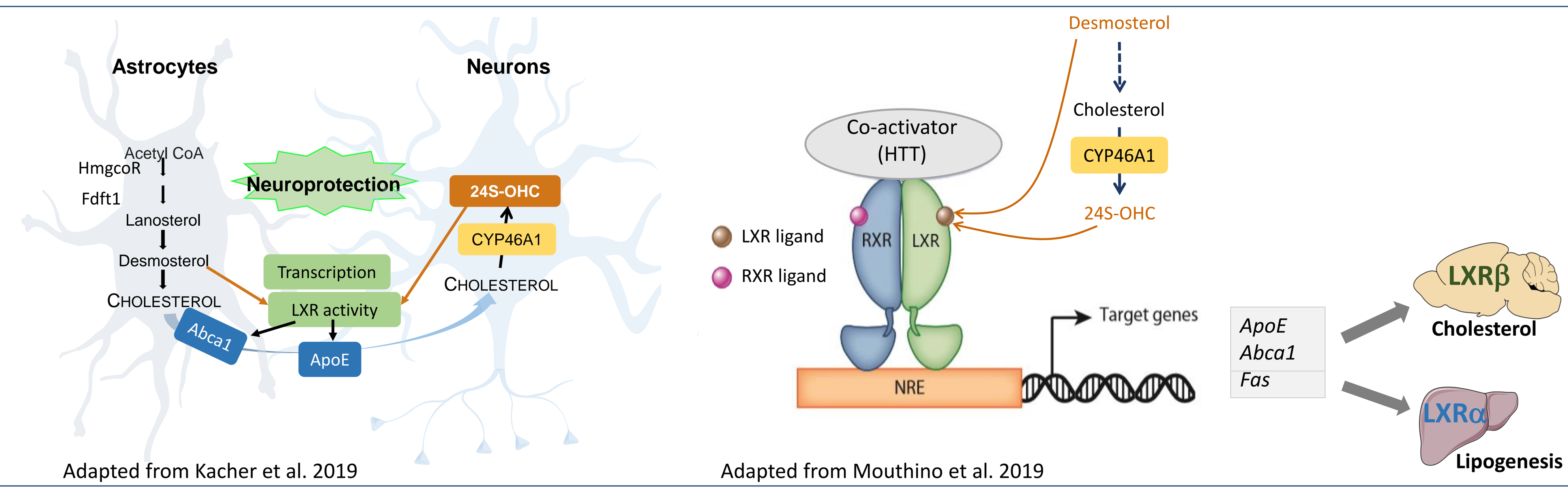
Introduction

Huntington's disease (HD) is associated with cholesterol metabolism deregulation[1]. The main pathway of cholesterol elimination is its catabolization by the neuronal enzyme CYP46A1 into 24S-OHC, a ligand of LXR. CYP46A1 level is decreased in HD and its restoration induces a neuroprotection with an upregulation of LXR target genes[2,3]. A therapeutic interest was raised for the LXR in several neurodegenerative diseases[4], and here we hypothesized the involvment of LXR in CYP46A1 neuroprotection. There are two LXR isoforms, LXRα mainly expressed in the liver and LXRβ enriched in the brain, involved in cholesterol metabolism. Commercialized LXR agonists suffer from side effects on lipogenesis due to the activation of LXRα in the liver. Here we propose to activate specifically the LXRβ in the brain, with specific agonists[5], to investigate the role of LXR activation in cellular and mice model of Huntington's disease.



Transcriptional activity of LXR agonists in primary striatal neurons and astrocytes

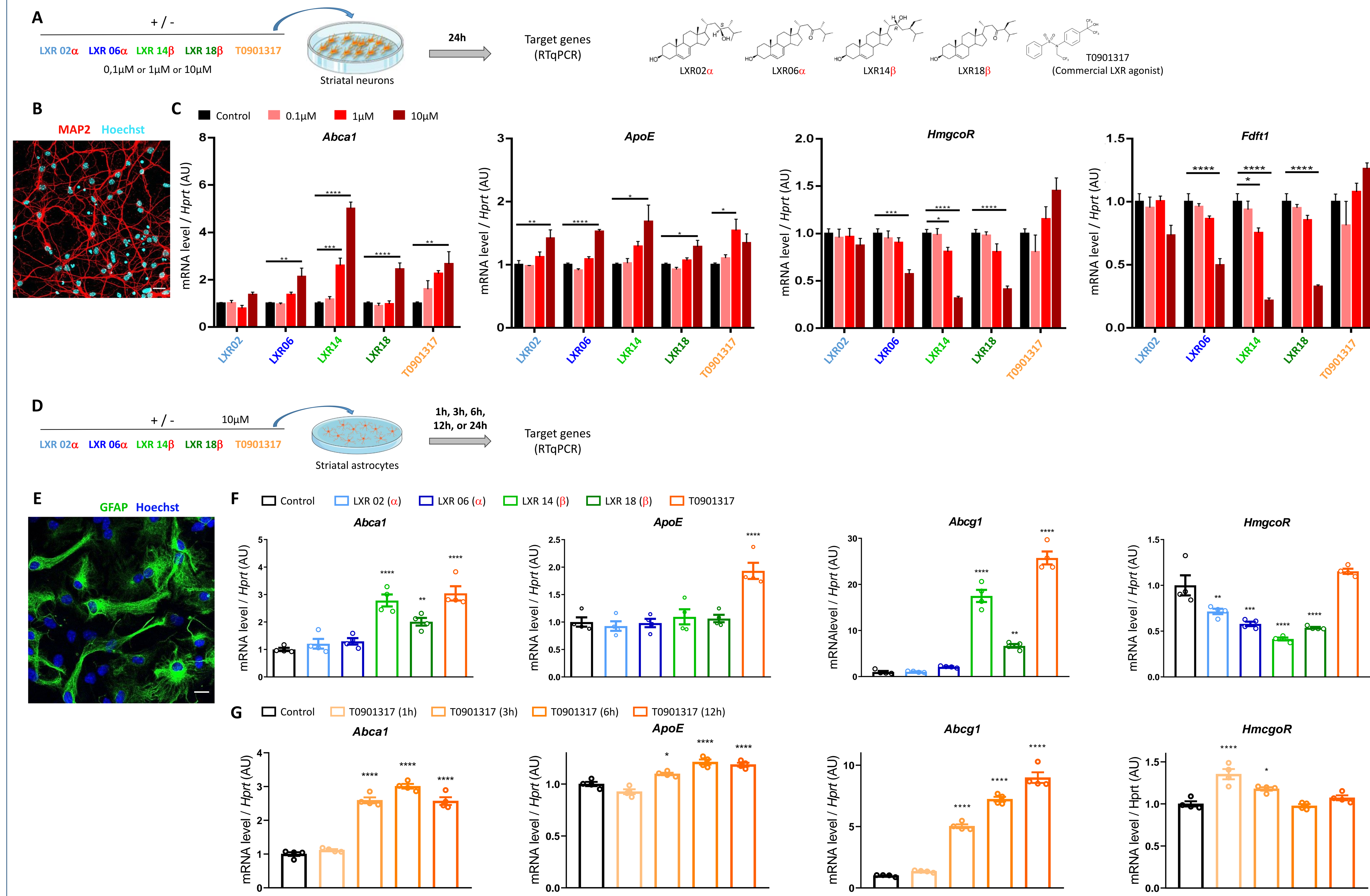


Figure 1. LXR agonists are transcriptionally active in primary cultures of neurons and astrocytes.
(**A**) Wild Type primary striatal cultures of neurons (**B**) were treated with increasing doses of LXR agonists for 24 hours. Structure of the four LXR agonists (Marinozzi et al. 2017) compared with T0901317. (**C**) mRNA levels of LXR target genes involved in cholesterol transport (*Abca1*, *ApoE*) or synthesis (*HmgcoR*, *Fdft1*) were measured after RTqPCR relative to *Hprt* house keeping gene.
(**D**) Wild Type primary striatal cultures of astrocytes (**E**) were treated with LXR agonists (10µM) for 24h (**F**) or with T0901317 (10µM) for increasing time (**G**).
(**F, G**) mRNA levels of LXR target genes involved in cholesterol transport (*Abca1*, *ApoE*, *Abcg1*) or synthesis (*HmgcoR*, *Fdft1*) were measured after RTqPCR relative to *Hprt* house keeping gene.
Data are represented as mean±SEM (n=4-5). Statistical analysis: one-way ANOVA followed by Tukey's post-hoc test. Treatment effect: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. (**B, E**) Scale bar 20µm.

Neuroprotective role of LXR agonists in HD cellular model

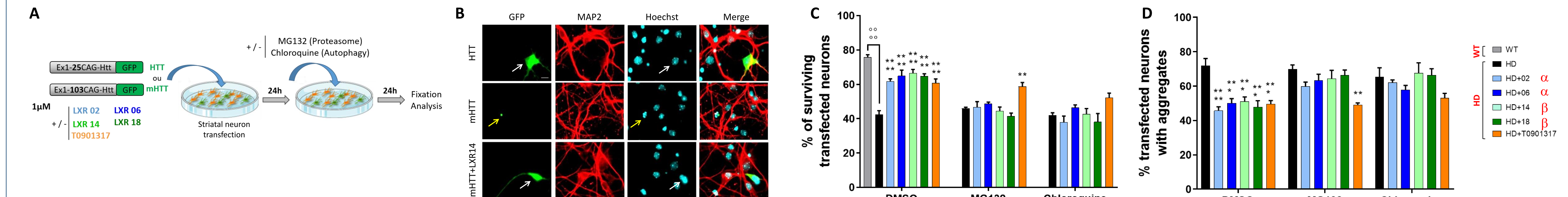


Figure 2. LXR agonists are neuroprotective in a cellular model of HD. (**A**) Striatal neurons were transfected with plasmids coding for either Exon1-25CAG-HTT (WT) or Exon1-103CAG (mHTT) and treated with LXR agonists (1µM). After 24h, cells were treated with proteasome inhibitor (MG132, 10µM) or autophagy inhibitor (chloroquine, 30µM) for 24h.
(**B**) GFP staining of HTT or mHTT (green) co-labeled with nuclei (hoechst, blue) and MAP2 (red). Scale bare 10µm. White arrows indicate a neuron with diffuse HTT and yellow arrows indicate a neuron with mHTT aggregate.
mHTT aggregates (**C**) and neuronal survival (**D**) were quantified. LXR agonists have beneficial effects on these two parameters. Data are represented as mean±SEM (n=200-250 tranfected neurons). Statistical analysis: two-way ANOVA followed by Bonferroni's post-hoc. Genotype effect: : °°°°p < 0.0001. Agonists LXR effect compared to HD cells **p < 0.01, *** p < 0.001, ****p < 0.0001

Transcriptional activity of LXR agonist in WT mice

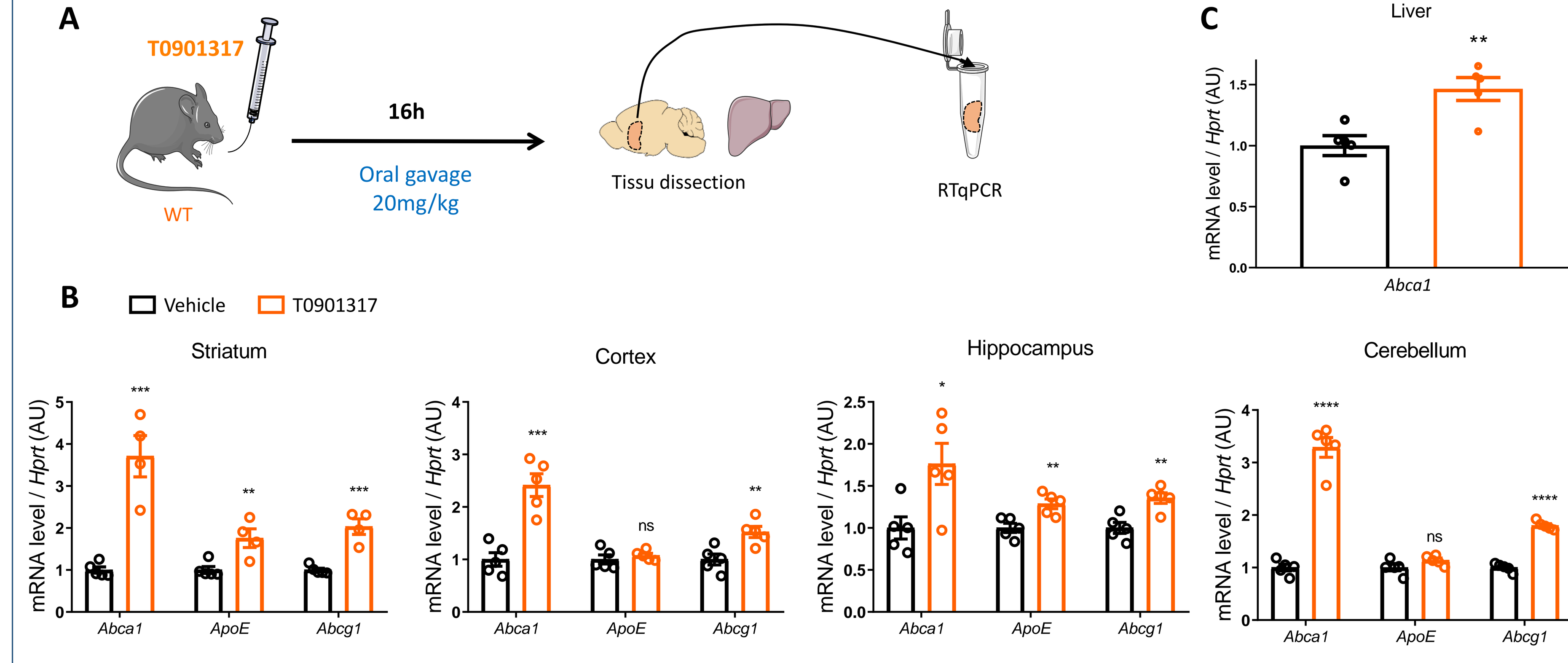


Figure 3. Validation of a treatment protocol with commercialized agonist in Wild Type mice
(**A**) Wild Type mice were treated with vehicle or T0901317 (20mg/kg) by oral gavage. Mice were sacrified 16h after treatment.
(**B-C**) mRNA level of LXR target genes (*Abca1*, *ApoE*, *Abcg1*) were measured in different brain regions (**B**) (Striatum, Cortex, Hippocampus, Cerebellum) and in the Liver (**C**) after RTqPCR relative to *Hprt* house keeping gene. Data are represented as mean±SEM (n=5). Statistical analysis: T-Test. Treatment effect : *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns=not significant.

Conclusions/Perspectives

- The bioactivity of LXRα and LXRβ agonists is validated in primary cultures of striatal neurons and astrocytes with an transcriptional regulation of target genes of LXR, involved in cholesterol metabolism and known to be decreased in HD.
- The LXR agonists induce a neuroprotection in a cellular model of HD, with a decrease of mHTT aggregates and an increase of cell survival.
  The neuroprotective role of LXR agonists is dependant of proteasome and autophagy mechanisms.
- A treatment protocole has been validated with a commercial agonist in WT mice.

➢ **The results support the interest to study the role of LXR activation in HD and the next step will be to better understand the mechanisms involved in LXR induced neuroprotection *in vitro* and to explore their effect in HD mice.**

References

1. Karasinska JM. And Hayden MR. (2011) Cholesterol metabolism in Huntington diseae. Nat Rev Neurol. 7(10):561-72
2. Boussicault L. et al. (2016) CYP46A1, the rate-limiting enzyme for cholesterol degradation, is neuroprotective in Huntington's disease. Brain, 139:935-970
3. Kacher R. et al. (2019) CYP46A1 gene therapy deciphers the role of brain cholesterol metabolism in Huntington's disease. Brain, 142(8):2432-2450
4. Moutinho M., Codocedo J.F. (2019). Nuclear Receptors as Therapeutic Targets for Neurodegenerative Diseases: Lost in Translation. Annu Rev Pharmacol Toxicol. 59:237-261
5. Marinozzi M. et al. (2017). Side-Chain Modified Ergosterol and Stigmasterol Derivated as Liver X receptor Agonsists. J. Med. Chem. 60:6548-6562