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Supplementary information

LRP1 is a master regulator of tau uptake and spread

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Supplementary Information

Supplemental methods

Internalization controls

Before flow cytometry analysis cells were washed and treated with trypsin (0.05%), proteinase K (0.5mg/ml), or washed again in a low pH buffer (0.1M Glycine pH 2.5, 150mM NaCl) according to previously published protocols[1]. Additionally, the identical uptake experiment was performed at 4°C (to prevent endocytosis) and no uptake was observed.

qPCR

Purelink RNA Extraction Kit (Invitrogen) was used to isolate RNA from samples. RNA (1 µg) was then converted to cDNA using SuperScript Reverse Transcriptase III (Invitrogen) according to the supplier's instructions. Real-time quantitative PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) according to QuantStudioTM 12K Flex Real-Time PCR System protocol. GAPDH mRNA level was used to normalize samples.

Surface quantification of mLRP

Cells expressing various HA-mLRP constructs were briefly fixed in 4% PFA (5min at RT) then blocked in PBS, 5% Donkey Serum (Sigma) for 1hr at RT. Primary HA antibody (Sigma H3663, 1:200) was added o/n at 4°C, and as a negative control for internalization GAPDH antibody (Abcam 181602, 1:200). Samples were washed and secondary antibodies (Invitrogen) were added for 1hr at RT. Cells were washed again, mounted and imaged on a Leica SP8 Confocal microscope. Corrected Integrated density of the fluorescent signal was quantified in FIJI for individual cells across three images (n=60).

Co-immunoprecipitation

H4i LRP1 sgRNA cells expressing either mLRP4 or empty vector (TetO) were lysed open in RIPA buffer (Sigma). Myc-tagged 2N4R was added to lysate (1ug) and lysates were incubated with either Anti-HA magnetic beads (Thermo Fisher) or IgG labeled magnetic beads (Thermo Fisher) for 2h at RT. After 2h, beads were washed with PBS, 0.05% Tween and eluted with 0.1M Glycine pH 2.8. Samples were loaded on a Tris-acetate gel (Invitrogen) and blotted for the presence of HA-mLRP4 (Sigma H3663, 1:1000) and myc-2N4R (Cell signaling 2272, 1:1000).

Mouse primary culture

Primary mouse hippocampal cultures were used to confirm neuronal specific expression of shRNAs. Mouse pups were decapitated at postnatal day 1 (P1), brains were removed and hippocampi were dissected. Hippocampi were enzymatically dissociated with papain for 40 minutes at 37°C and then triturated. Cells were plated on PLL coated dishes.

Cryo-sections

To produce cryosections for LRP1 immunohistochemistry brains were perfused transcardially with PBS for 10 mins, carefully dissected and then immersion fixed in 4% paraformaldehyde overnight at 4ºC. Next, samples were rinsed in cold PBS 5 x 10 mins and 1 x 1hr. Samples were then cryoprotected in a sucrose gradient; 10% sucrose for 2 hrs, 20% sucrose for 2 hrs, and 30% sucrose overnight. Brains were then embedded in Optimal Cutting Temperature medium (Electron Microscopy Sciences, Washington, PA) and frozen in LN_2. Subsequently, 25µm sections were cut using a cryostat (Leica, Buffalo Grove, IL) and collected on a poly-lysine coated glass slide. Sections were blocked for 30 mins in normal donkey serum diluted 1:20 in PBTA at room temperature. Next, primary antibodies were incubated for 1hr (anti-LRP1; Abcam, 92544; 1:200). Then, sections were rinsed 5 x 5 mins and 1 x 1hr after which

corresponding secondary antibodies were incubated for 1 hr (Jackson ImmunoResearch Laboratories; 1:200). Sections were once again rinsed in PBTA, 5 x 5 mins and 1 x 1hr. Lastly, sections were sealed as described above.

Supplemental References

1 Yu, A. *et al.* Protein aggregation can inhibit clathrin-mediated endocytosis by chaperone competition. *Proceedings of the National Academy of Sciences of the United States of America* **111**, E1481-1490, doi:10.1073/pnas.1321811111 (2014).