Formation, release, and internalization of stable tau oligomers in cells

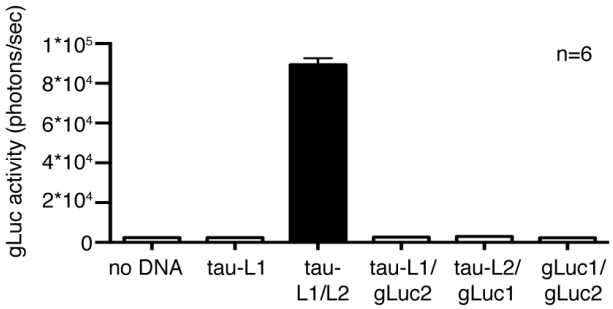
Susanne Wegmann[1,#], Samantha Nicholls[1,#], Shuko Takeda[1], Zhanyun Fan[1], Bradley T. Hyman[1,]

[1]Massachusetts General Hospital, Dept. of Neurology, Charlestown, MA and Harvard Medical School, Boston, MA, Massachusetts Institute of Neurodegenerative Diseases (MIND)

[#] authors contributed equally
corresponding authors:

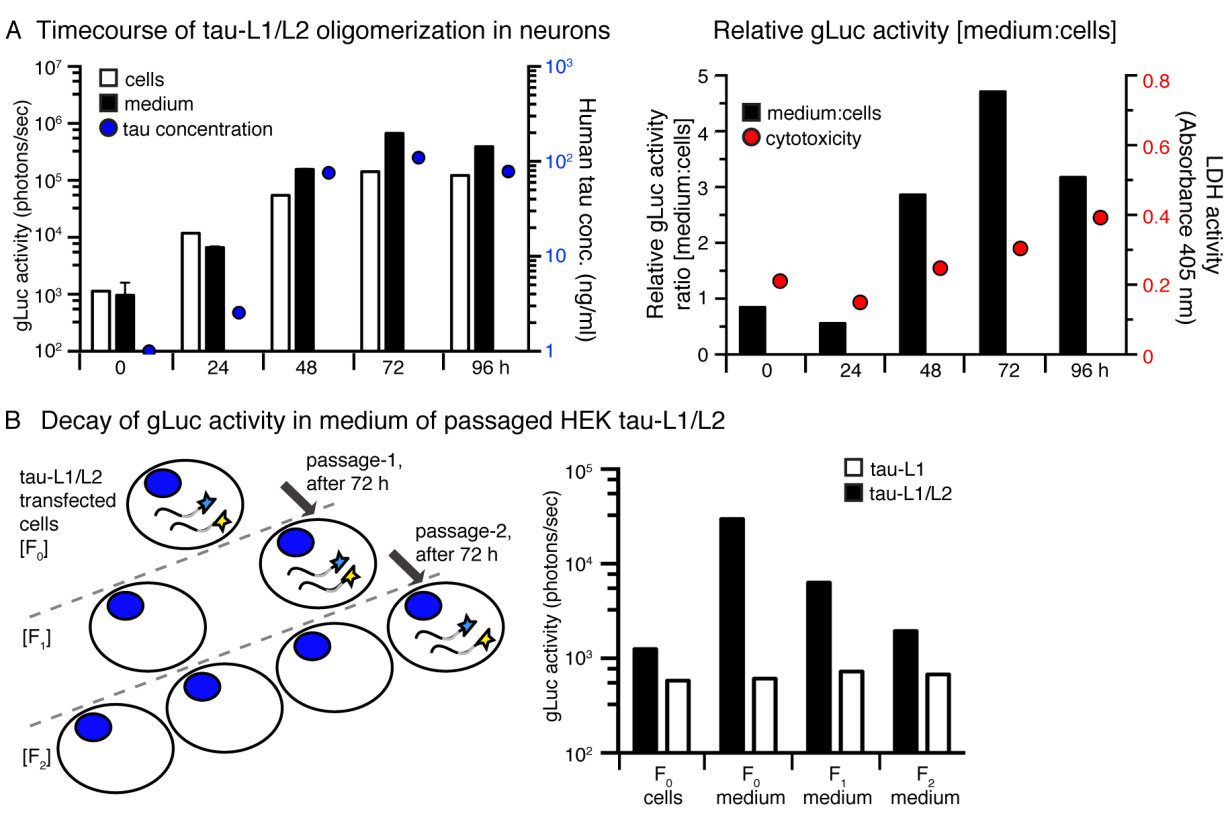
Bradley T. Hyman (bhyman@mgh.harvard.edu) and Susanne Wegmann (swegmann@mgh.harvard.edu)

gLuc complemenation depends on tau oligomerization



Supplementary Figure 1. Tau oligomerization drives gLuc complementation.

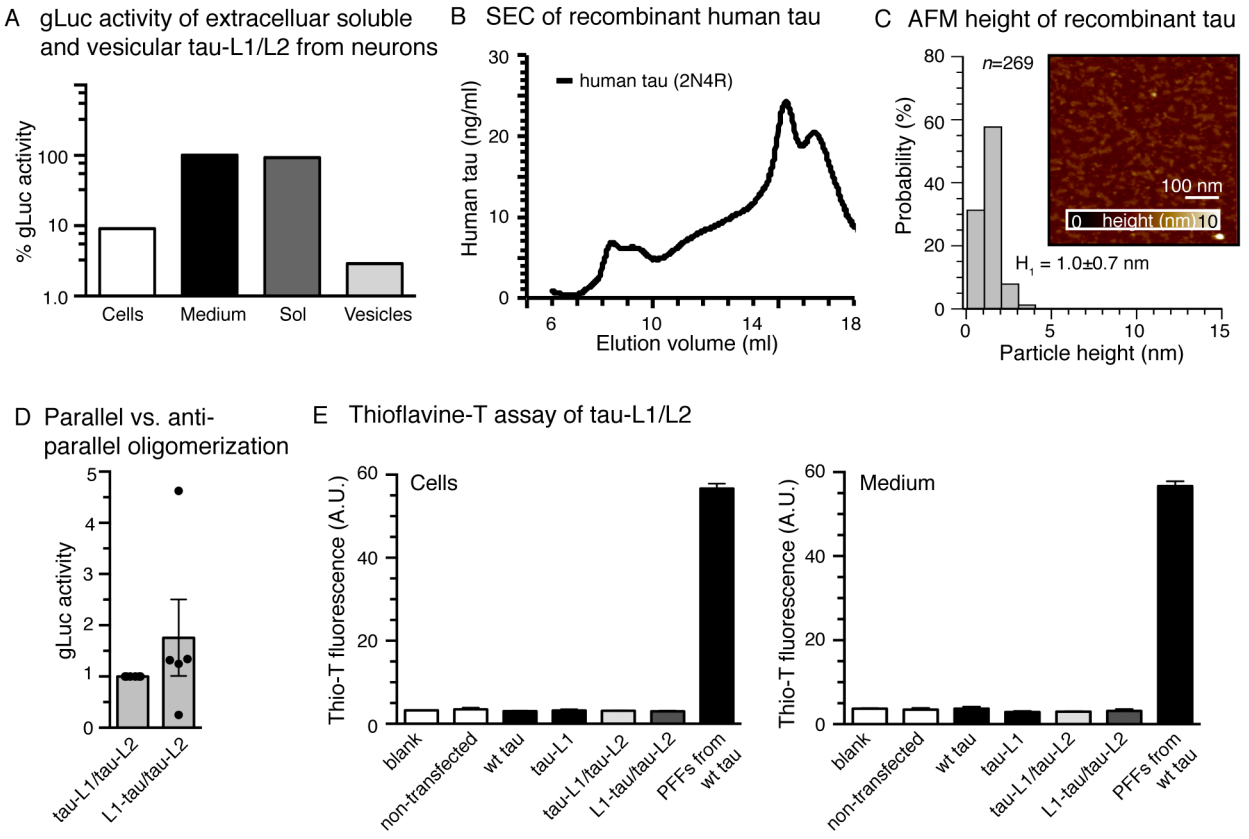
Luciferase activity in conditioned medium (40 hours) could be detected only in cells expressing tau-L1 and tau-L2, but not in cells expressing tau-L1 and the complement gLuc fragment (gLuc2), in cells expressing tau-L2 and the complement gLuc fragment (gLuc1), or in cells expressing gLuc1 and gLuc2. Mean±SEM, n=2 experiments, 6 repeats.



Supplementary Figure 2. Tau-L1/L2 is release over multiple generations of HEK and by primary neurons.

A) The timecourse of tau-L1/L2 expression and release in primary cortical mouse neurons is similar to HEK tauL1/L2 cells. The release of tau-L1/L2 into the medium (blue dots) increases largely between 24 and 48 hours post lipotransfection. Cytoxicity determined by LDH assay (red dots) increases only slightly at 48 hours and then after. Mean±SEM, n=4 repeats.

B) HEK tau-L1/L2 cells [F0] passaged over two generations [F1 and F2] and gLuc activity in the medium was measured in each generation after ~30h in culture. The release of tau-L1/L2 into the culture medium decayed exponentially with passage number, showing that HEK tau-L1/L2 are robust enough to be carried over to the next generation, and indicating that tau-L1/L2 cells diluted out through passaging rather than due to cell death (expected abrupt decay).



Supplementary Figure 3. Released tau-L1/L2 is mostly soluble and oligomeric.

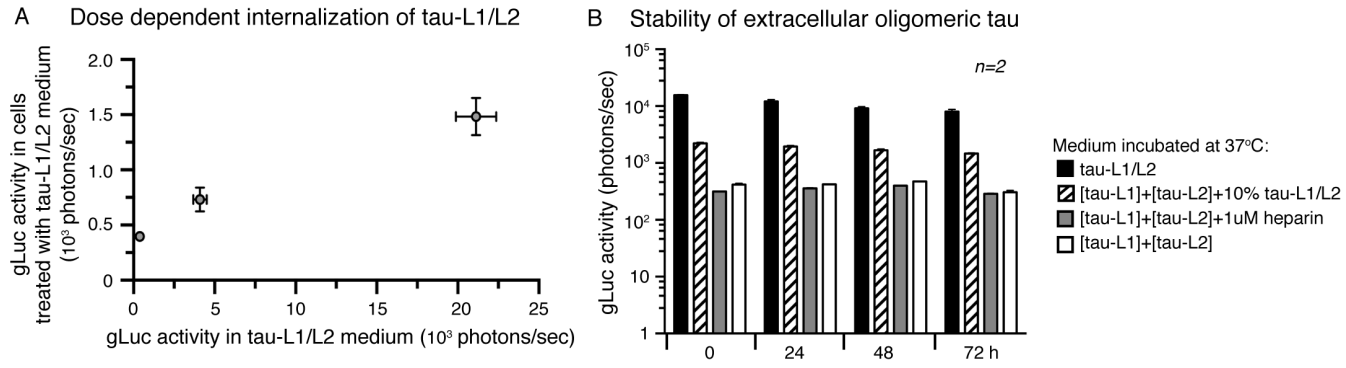
A) gLuc activity measured in cells and medium fractions of primary cortical neurons expressing tau-L1/L2 (48 h). Similar to HEK cells (Figure 3 A,B), tau-L1/L2 in the medium collected from primary neurons is mostly (~95%) soluble.

B) SEC of recombinant human full-length tau (2N4R) shows that the majority of tau exists as monomers (elution vol. 15-17 ml) with some oligomeric tau species (elution vol. 8-10 ml).

C) AFM images if recombinant tau showing mostly monomeric tau with most probable particle sizes (= AFM heights) of H_1=1.0±0.7 nm. Mean±SD, n=269 particle.

D) To compare between parallel (tau-L1/tau-L2) and anti-parallel (L1-tau/tau-L2) tau oligomer formation in the medium. HEK cells were co-transfected with either tau-L1 and tau-L2, or with L1-tau and tau-L2. gLuc activity in the medium (40 h) was only slightly higher (non-significant) for anti-parallel tau oligomer formation. Mean±SEM, n=4 experiments.

E) Thioflavine-T (Thio-T) assay to detect β-sheet aggregates of tau. No Thio-T activity was detected in either cells or medium of HEK transfected with wild-type (wt) tau, only tau-L1, tau-L1/tau-L2, or L1-tau/tau-L2. Pre-aggregated recombinant human tau (positive control) gave high readings of Thio-T fluorescence. Mean±SEM, n=3 repeats.



Supplementary Figure 4. Internalization and stability of extracellular tau-L1/L2.

A) The amount of tau-L1/L2 internalized by non-transfected HEK cells appears to correlate with the amount of tau-L1/L2 in the treatment medium. Mean±SEM, n=3 experiments, 3 repeats.

B) The stability of extracellular tau-L1/L2 oligomers was assessed by incubating pre-conditioned medium of HEK tau-L1/L2, after separation from the cells, at 37° C for 72 hours. gLuc activity from the same sample was measured in 24 hour intervals. To test the sensitivity gLuc activity to tau aggregation, pre-conditioned tau-L1 and tau-L2 medium (1:1 ratio) was incubated with either tau-L1/L2 (10% v/v) medium or with 1 μM heparin to trigger aggregation. In neither of these cases, tau-L1/L2 oligomerization could be detected, indicating that tau oligomerization rather than aggregation causes gLuc activity. Mean±SEM, n=3 repeats.