Novel repertoire of tau biosensors to monitor pathological tau transformation and seeding activity in living cells

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ABSTRACT

Aggregates of the tau protein are a well-known hallmark of several neurodegenerative diseases, collectively referred as tauopathies, including Frontal Temporal Dementia and Alzheimer's disease (AD). Monitoring the transformation process of tau from physiological monomers into pathological oligomers or aggregates in a high-throughput, quantitative manner and in a cellular context is still a major challenge in the field. Identifying molecules able to interfere with those processes is of high therapeutic interest. Here, we developed a series of inter- and intramolecular tau biosensors based on the highly sensitive Nanoluciferase (Nluc) binary technology (NanoBiT) able to monitor the pathological conformational change and self-interaction of tau in living cells. Our repertoire of tau biosensors reliably report ***i.*** molecular proximity of physiological full-length tau at microtubules; ***ii.*** changes in tau conformation and self-interaction associated with tau phosphorylation, as well as ***iii.*** tau interaction induced by seeds of recombinant tau or from brain lysates of a mouse model of tau pathology. By comparing biosensors comprising different tau forms (*i.e.* full-length or short fragments, wild-type or the disease-associated tau(P301L) variant) further insights in the tau transformation process are obtained. Proof-of-concept data for the high-throughput suitability and identification of molecules interfering with the pathological tau transformation processes are presented. This novel repertoire of tau biosensors are aimed to boost the disclosure of molecular mechanisms underlying pathological tau transformation in living cells and to discover new drug candidates for tau-related neurodegenerative diseases.

Keywords: biosensor, tau, Alzheimer`s disease, Nanoluciferase complementation, seeding.

INTRODUCTION

Tauopathies are a group of neurodegenerative diseases that display abnormal neuronal aggregates of the tau protein, also called neurofibrillary tangles (NFT). Examples of tauopathies include Alzheimer's disease (AD), the most common dementia worldwide, Frontotemporal Dementia (FTD), Pick disease, and others. Under physiological conditions tau is found mainly associated to microtubules (MTs), participating in the regulation of MT assembly/disassembly, with impact on axonal transport and synapsis structural organization. In AD and other tauopathies, tau undergoes a pathological transformation which involves hyperphosphorylation, conformational changes, loss of MT-binding affinity, and oligomerization, ultimately resulting in tau mislocation and NFTs formation (1, 2). Progressive accumulation of NFTs in specific brain areas is the molecular marker that mostly correlates with AD cognitive dysfunction and is the basis of the Braak classification of AD into VI stages (3).

Tau is encoded by the *MAPT* gene and is expressed as six different isoforms due to alternative splicing, referred to as 0N3R, 1N3R, 2N3R, 0N4R, 1N4R, and 2N4R, depending on the presence or absence of two near-amino-terminal inserts (N) and of three (3R) or four (4R) repeats in its central repeat domain (4). Except for the 0N3R isoform, which is found only in newborns, all the other isoforms are expressed in human brain and all can be found in aggregates observed in AD brains. Tau phosphorylation/dephosphorylation determines its affinity to bind to MTs and this is a tightly controlled and dynamic process under physiological conditions. Disease-associated tau is hyperphosphorylated and shows lower capacity to bind MTs while its propensity to aggregate is increased. Once started, tau pathology is propagated and spread between neurons in a prion-like mechanism, transmitting the misfolded

pathological conformation to other native tau molecules through a prion-like seeding effect (5). Whilst tau conformational changes, self-interaction and aggregation are clearly linked to tauopathies, the underlying molecular mechanisms are still poorly understood, limiting thus the development of therapeutics targeting these processes.

Tau self-interaction has been extensively studied using *in vitro* aggregation reporter systems like the fluorescent reporters thioflavin T or congo red. Although those experiments provided important information on the kinetics of fibrils formation and on the *in vitro* conditions to induce tau aggregation, such as the requirement of heparin or other polyanions (6), these assays rely on high concentrations (μM to mM) of tau and, thus, are not suitable to probe the initial oligomeric state and are unlikely to fully mimic the transformation process that occurs in a cellular context. The development of cellular-based sensors overcame part of these obstacles. Initial assays were mainly based on visualization of intracellular tau aggregates by western blot or fluorescent microscopy using antibodies or recombinant tau molecules fused to fluorescent proteins (GFP, CFP, YFP), or complementation assays based on the reconstitution of fluorescent proteins (BiFC) (7-13). A Fluorescence Resonance Energy Transfer (FRET)-based biosensor, combined with microscopy imaging or fluorescence assisted cell sorting (FACS), has been widely used for the detection of tau seeding activity and propagation (14, 15). More recently, FRET-based biosensors combined with fluorescence lifetime (FLT) detection allowed improved quantification of the signal for screening applications (16). Despite significant improvements, the existing biosensors still have limitations either in terms of sensitivity (*i.e.* microscopy or western blot for visualization of small tau oligomers), of laborious data analysis (FRET-based assays), or of high-throughput screening compatibility (17). In order to develop improved tau

biosensors for research and drug discovery application we used the recently developed highly sensitive Nanoluciferase (Nluc) binary technology (NanoBiT) (18, 19).

Nluc-based luminescence assays are highly sensitive and quantitative, have a large dynamic range, minimize the impact of the fusion protein due to its small seizer (19kDa) compared to fluorescent proteins, and require minimal post hoc analysis compared to fluorescence-based methods. This technology has been previously applied to investigate the effect of kinase inhibitors on spontaneous tau self-aggregation (20). Here, we have developed a series of tau biosensors using the NanoBiT complementation system to monitor spontaneous and phosphorylation- and seeding-induced *intra*molecular conformational changes, as well as *inter*molecular tau self-interaction of a variety of tau forms, in living cells and in a quantitative and high-throughput-compatible manner.

RESULTS

Development of Nanoluciferase enzyme complementation-based tau biosensors

In order to monitor changes in tau conformation and aggregation we developed intra- and inter-molecular biosensors, respectively, using the NanoBiT technology. In this system, the Nluc enzyme is split into two fragments, a large 18kDa fragment (LgBit) and a smaller 1.3kDa fragment (SmBit), that are fused to the proteins of interest (18). To design tau biosensors using this system, the LgBit was fused to the N-terminus of tau and the SmBit either at the C-terminus of the double-fused tau (*intra*molecular sensors) or to the N-terminus of single-fused tau (*inter*molecular sensor) (**Figure 1A**). Different forms of tau exist, depending to the presence or absence of two domains at the N-terminal part (0N-2N) and to the number of repeats (R1 to R4) at its central repeat

domain. In this study we used full-length wild-type tau (WT-Tau), full-length tau carrying the pro-aggregating P301L mutation (Tau(P301L)), and the pro-aggregating K18 fragment of tau (21-23) comprising only the four-repeat domains (R1-R4) and carrying the P301L mutation (K18(P301L)) (**Figure 1B**). Among the advantages of the NanoBiT system used for these tau biosensors is that it allows monitoring the behavior of tau molecules in real time, in living cells, in a straightforward pipeline. As shown in **Figure 1C**, cells can be treated with test compounds and Nluc activity is measured immediately after addition of its substrate, reflecting the fast and high-throughput properties of the assay.

In order to validate the approach of using the NanoBiT system to develop tau biosensors, we first developed an intermolecular tau interaction biosensor comprising the aggregation-prone K18 fragment displaying the P301L mutation (K18(P301L)), as the aggregation properties of this tau fragment has been extensively reported (21-23). HEK293T cells expressing a fixed amount of LgBit-K18(P301L) and increasing amounts of SmBit-K18(P301L), showed the expected saturation profile of the complementation signal, reflecting specific interaction and progressive saturation of LgBit-K18(P301L) with SmBit-K18(P301L) (**Figure 1D**). Expression levels of the sensor were monitored in parallel by western-blot using anti-HA antibodies recognizing both constructs (**Figure 1E**). The cellular distribution of the K18(P301L) biosensor was visualized by immunofluorescence microscopy using antibody against the HA tag of the biosensor. The images revealed a punctuated pattern of the K18(P301L) biosensor, the absence of artefactual large aggregates, few co-localization with tubulin and some nuclear localization as well (**Figure 1F**). Next, we challenged biosensor cells with recombinant K18 protein, a treatment that has been previously reported to efficiently induce ("seed") tau aggregation (14, 24). Heparin-induced aggregates of recombinant

K18 (aggK18) and of full-length tau (aggTau) were obtained as previously reported (25). A robust increase in signal was observed in K18(P301L) sensor-expressing cells treated with either monomeric K18 (mK18) or aggK18 (**Figure 1G**). Seeds of aggTau also led to a significant increase of the sensor signal, while monomeric tau (mTau) and oligomers of the amyloid-beta peptide (oAß) had no effect (**Figure 1G**), confirming thus the specificity of the sensor response towards tau seeds and the lack of cross-seeding activity to Aß. No aggK18-induced increase in the complementation signal was observed when either LgBit-K18(P301L) or SmBit-K18(P301L) were expressed alone (**Figure 1 – figure supplement 1A-B**). Of note, the kinetics of the Nluc enzyme:substrate reaction reaches a plateau in less than 10min after substrate addition (**Figure 1 – figure supplement 1C**), and all results are taken at this time-point to avoid any interference of the enzymatic activity kinetics on the comparison between different treatments. These results demonstrate the suitability of the NanoBiT technology to monitor the pathologically relevant seeding response and self-interaction of tau.

Tau biosensors report conformational changes and tau-tau interaction in response to microtubule destabilization and phosphorylation

The conditions triggering the transformation processes of full-length wild-type tau in a cellular context are largely unknown. We therefore aimed to apply the NanoBiT approach to full-length wild-type tau. The WT-Tau intermolecular interaction sensor (LgBit-Tau/SmBit-Tau) behaved similar to the K18(P301L) interaction sensor in that it showed a robust saturation curve in HEK293T cells expressing a fixed amount of LgBit-Tau and increasing amounts of SmBit-Tau (**Figure 2A**). Replacement of the SmBit-Tau by the non-related Halo protein fused to SmBit generated no signal confirming the specificity of the tau intermolecular self-interaction assay (**Figure 2A**).

Successful expression of sensors at the protein level were monitored by western-blot using the HA tag common to all constructs (**Figure 2A, bottom**). Immunofluorescence microscopy revealed an extensive colocalization of the tau sensor with MTs (**Figure 2B**), which correspond to the reported localization of endogenous full-length tau and is distinct from the cytoplasmic localization of K18 (24).

The central repeat domain of tau is known to be involved in MT binding and in the pathological intermolecular interaction of tau (26), meaning that both events are mutually exclusive. To determine whether the basal signal observed with the WT-Tau interaction sensor originates from MT-bound tau or cytosolic tau, we treated biosensor cells with colchicine to destabilize MTs. Under this condition, the sensor signal was decreased by 75% (**Figure 2C**) indicating that the basal signal most likely reflects molecular proximity between MT-bound tau molecules rather than self-interaction of cytoplasmic tau. Consistently, colchicine had no effect on the basal signal of the K18(P301L) interaction sensor, confirming that the signal of this sensor is MT-independent and reflects K18(P301L) self-interaction in the cytosol (**Figure 2D**). Collectively, these data indicate that the intermolecular tau sensor, like endogenous tau, is located at the MT and generates a complementation signal reflecting molecular proximity between tau molecules that are attached to MTs.

We then probed the conformational state of full-length tau with the *intra*molecular tau conformational biosensor where LgBit and SmBit are fused to the same tau molecule (LgBit-Tau-SmBit). Expression of increasing quantities of the sensor resulted in a linear increase of the luminescence signal as would be expected for an intramolecular complementation signal (**Figure 2E**). Immunofluorescence microscopy confirmed the localization of the LgBit-Tau-SmBit sensor at MTs (**Figure 2F**). Treatment of WT tau sensor cells with colchicine reduced the signal of the tau

conformation sensor by 60% indicating a change in WT tau conformation when unbound to MTs (**Figure 2G**), whereas the signal of a LgBit-K18(P301)-SmBit conformation sensor was unaffected as expected (**Figure 2H**). Taken together, these data indicate that the tau conformation sensor faithfully monitors different conformational states of tau depending on its MTs-bound or unbound status. This observation is consistent with previous *in vitro* observations and molecular models showing that MTs stabilizes tau into a "paperclip fold" structure, in contrast to the natively unfolded structure of soluble tau (27-29).

In the course of its pathological transformation, tau is known to become hyperphosphorylated and to dissociate from MTs (30, 31). However, the link of these events to tau transformation (conformational changes, seeding, aggregation) is not clear. To address this question, we induced tau phosphorylation by treating biosensor cells expressing either the conformation or interaction WT-Tau sensors with forskolin for 24 hours. In both cases a 2-3-fold increase in signal was observed, indicative of the induction of a change in conformation (**Figure 2I**) and the induction of tau self-interaction (**Figure 2J**). Successful phosphorylation of tau biosensors by forskolin was confirmed by the increased immmunoreactivity of the anti-phospho-tau specific AT8 antibody (Ser202/Thr205), a phosphorylation site that correlates well to pathological tau species observed in AD (32) (**Figure 2I-J, right panels**). In order to assess whether phosphorylation-induced self-interaction of WT tau biosensor can be favorized by increasing the MT-free population of tau, we treated sensor cells with colchicine followed by forskolin. Surprisingly, the co-treatment condition completely abrogated the forskolin effect (**Figure 2K**), suggesting that WT-tau phosphorylation occurs mainly at MT-bound tau. In summary, our tau biosensors have proven to be useful tools to monitor the behavior of tau in a physiological cellular context, while enabling the

investigation of different factors suspected to promote changes in its conformation and self-interaction properties.

Seeding response of tau biosensors

The mechanisms underlying the aggregation of physiological forms of tau remains poorly known. To investigate whether the high sensitivity of the NanoBiT system is able to report on the seeding response of physiological tau we assessed the response of our WT-Tau interaction sensor towards different seeds. Differently to the K18(P301L) interaction sensor (see **Figure 1G**), none of the recombinant K18 or tau seeds induced self-interaction of the WT-Tau biosensor when run in parallel (**Figure 3A**). The WT-Tau interaction biosensor also did not respond to seeds obtained from brain lysates of the transgenic (Tg) tauopathy mouse model carrying the P301S mutation **(Figure 3B**), while the K18(P301L) interaction biosensor cells showed a robust 107-fold increase in signal in the presence of brain lysates from the Tg mice compared to brain lysates from WT mice (**Figure 3B**). Such an increase was not observed in biosensor cells expressing LgBit-K18(P301L) and the non-related SmBit-Halo protein (**Figure 3 – figure supplement 1A**), confirming the specificity of the biosensor response. The K18(P301L) seeding response to Tg brain lysates was concentration-dependent, reaching saturation at around 1 μg of protein lysate (**Figure 3C**). This sensor showed a large dynamic range spanning 4 logs of brain lysate concentrations (**Figure 3C**), with a minimum detection limit at 300 pg of total protein (**Figure 3C insert**). The robustness and reproducibility of this biosensor response was further confirmed using brain lysates from four independent WT and Tg mice to account for interindividual variability on tauopathy development (**Figure 3 – figure supplement 1B**). In contrast to the pronounced seeding response of the K18(P301L)

interaction sensor, no change in the K18(P301L) conformation sensor signal was observed in response to most of the recombinant seeds, with the exception of aggK18, nor to brain lysates (**Figure 3 – figure supplement 2**). These results suggest that the seeds do not induce any detectable conformational changes in the K18(P301L) sensor containing the P301L mutation and that the P301L mutation is fully compatible with seeds-induced aggregation.

Next, we raised the question whether our WT-Tau sensor molecules, despite being resistant to the seeds from Tg brain lysates, would show increased interaction with seeding-responding forms of tau (*i.e*. K18) in the presence of pathological seeds from Tg brain lysates. For this, we assessed the complementation between LgBit-Tau and Smbit-K18(P301L) in the presence of brain lysates from WT and Tg mice. Interestingly, we observed a significant increase in the complementation signal upon treatment with brain lysates from Tg mice and not from WT mice (**Figure 3D**). This result indicates that full-length WT tau can be recruited to and interact with seeding-responsive K18 species.

The pro-aggregating P301L mutation renders full-length tau responsive to seeding

In order to investigate whether the conformational-impacting P301L mutation would be sufficient to render full-length tau susceptible to seeding and consequent increased self-interaction, we developed full-length Tau(P301L) biosensors. The corresponding Tau(P301L) interaction biosensor was responsive to forskolin-induced interaction (2.5-fold increase) (**Figure 4A**), similarly to the WT-Tau sensor (**Figure 2J**). The Tau(P301L) conformation biosensor showed a larger (5-fold) forskolin-induced fold-change in signal (**Figure 4B**) compared to the corresponding WT-Tau biosensor (2.5-fold) (**Figure 2I**), suggesting a higher overall phosphorylation or a

higher impact of phosphorylation on the conformation of the Tau(P301L) sensor. Efficient phosphorylation was confirmed by AT8 antibody immunoreactivity (**Figure 4A-B, right panels**). Fluorescence microscopy analysis of the cellular distribution of the Tau(P301L) sensor revealed a partial co-localization with tubulin, similar to WT-Tau biosensor, and, to lesser extent, some punctuated pattern (**Figure 4C**) similar to that observed for the K18(P301L) sensor. Colchicine treatment led to an intermediate phenotype, with modest decrease in the complementation signal of both conformation and interaction Tau(P301L) biosensors (**Figure 4D-E**) compared to the robust decrease of the MT-bound WT-Tau sensors and the colchicine-insensitivity of the cytosolic K18(P301L) sensors. Collectively, these observations indicate that Tau(P301L) sensors exist as a mixture of MT-bound and cytosolic tau. In respect to the seeding response of the Tau(P301L) interaction sensor, recombinant aggK18 (**Figure 4F**) and brain lysates from Tg mice (**Figure 4G**) induced a robust response. Similar to the K18(P301L), no seeding-induced conformational change was observed for the Tau(P301L) biosensor (**Figure 4 – figure supplement 1**). The effect of Tg brain lysate seeds correlated well with the positive immunoreactivity of the tau phosphorylation-specific AT8 antibody and the MC-1 antibody recognizing pathological tau conformation (33) (**Figure 4H, left panel**). Consistently, immunoreactivity for both antibodies were undetectable in cells treated with brain lysates from WT mice and in cells expressing the WT-Tau interaction biosensor that is not responsive to either WT or Tg brain lysates (**Figure 4H**). Taken together, these data indicate that Tau(P301L) biosensors are located at MTs and in the cytoplasm and show an intermediate phenotype between the WT-Tau and the K18(P301L) biosensors, with overall intermediate sensitivity to seeding most likely because of the pro-aggregating conformational effect of the P301L mutation.

Altogether, these results indicate that our tau biosensors efficiently report changes in tau conformational, self-interaction or seeding responses, enabling direct comparison between the behavior of different tau forms in living cells and under different challenging contexts.

Application of tau interaction biosensors for the identification of drug candidates

Given the established role of the seeding activity in spreading tau pathology in different brain areas, identification of compounds able to interfere with this process holds great therapeutic potential. In view of the robust responses of our K18(P301L) and Tau(P301L) interaction biosensors towards seeds from Tg mouse brain lysates, we aimed to characterize their suitability to be used in screening for compounds interfering with their seeding/interaction response. We determined the Z' to estimate the high-throughput suitability and robustness of the K18(P301L) and Tau(P301L) interaction biosensor assays. Z' values of 0.7 and 0.5 were obtained, respectively, when comparing brain lysates from WT and Tg mice (**Figure 5A-B**). Z' values between 0.7 to 1 are considered as excellent performance for high-throughput screenings (34).

As a proof-of-concept, we evaluated the seeding response of the K18(P301L) and Tau(P301L) interaction sensors in the presence of compounds previously reported in the literature to interfere with protein aggregation. Compounds ID220249 (35), CINQ-trp (36), ID220255 (35) and spermine (37) had no significant effect on the P301S seeds-induced complementation whereas compounds ANLE138b (38), LMTMeSO4 (39) and bb14 (40) showed a significant inhibition for both sensors (**Figure 5C-D**). Interestingly, higher inhibition efficacy was observed for the K18(P301L) sensor, suggesting that the effect of compounds was mainly dependent on the 4RD tau domain. Concentration-response curves performed with the K18(P301L) biosensor for these 3

compounds showed a bi-phasic inhibition curve, with a partial inhibition at nanomolar concentration and up to 80% inhibition at micromolar concentrations (**Figure 5E-G**). Decrease of the biosensor signal due to cell toxicity of the compounds was excluded (**Figure 5 – figure supplement 1A**), as well as a non-specific effect due to interference with Nluc activity (**Figure 5 – figure supplement 1B**).

Taken together, these results suggest that the NanoBiT-based tau biosensors are useful tools to monitor the behavior of the different forms of tau in a living cell context over time, and to identify or characterize candidate drugs able to interfere with the tau seeding/self-interaction processes.

DISCUSSION

Tau conformational changes and self-interaction are known to comprise the very first molecular changes linked to AD development, preceding in decades the appearance of cognitive symptoms. Understanding, monitoring and preventing those processes are still among the main challenges and goals in AD field, as it holds great therapeutic and clinical value to the development of new drugs and to monitor AD progression at an early stage. Here, we developed a series of original tau biosensors based on the NanoBiT complementation technique and demonstrate the application of these biosensors as innovative research tools able to monitor tau behavior in terms of conformational changes, self-interaction and seeding of different tau forms, in living cells and under chemical and biological challenges. These are high-throughput-compatible tau conformation and seeding/interaction assays, able to identify molecular mechanisms driving tau pathological transformation processes, as well as new drug candidates displaying potential inhibitory effects on these early tau conformation/oligomerization/spreading steps.

The molecular mechanisms initiating the transformation of tau into pathological species, especially in the case of full-length WT tau as in sporadic AD, is still poorly known. Tau aggregates in AD brains are found in a microtubule-free and hyperphosphorylated state, implying that hyperphosphorylation and microtubule dissociation are key steps preceding tau aggregation (3, 30). Phosphorylation is indeed a key post-translational modification of tau, regulating its dynamics of association and dissociation from MTs under physiological conditions (41), and probably related to the early conformational changes in tau pathology (31, 42, 43, 44). In agreement with previous reports (45), our tau conformational sensors evidenced that mutations like P301L impose a specific conformation that is spontaneously aggregation-prone, probably by increased exposition of the amyloidogenic domain of tau compared to WT tau.

In spite of the evident key role of changes of tau phosphorylation and conformational status, assays monitoring these processes are still scarce (17). The conformational biosensors we developed here faithfully reported the conformational change of tau induced by phosphorylation or by MTs destabilization. Our tau biosensors based on full-length WT tau behaves exactly like endogenous WT tau, inasmuch as they are found highly associated to MTs, do not easily self-aggregate, and do not adopt a MC-1-reactive pathological conformation even in the presence of potent tau seeds. In agreement with previous reports using FRET-based intramolecular biosensors (29, 46), we observed that the conformation of full-length WT-Tau is highly impacted by destruction of MTs. The main advantage of our intramolecular biosensor compared with those previously developed is the high signal-to-noise ratio, sensitivity, reproducibility and straight forward analysis, while the FRET-based sensors require several steps of analysis. A similar impact of MTs destruction was also observed for

the *inter*molecular interaction tau biosensor. This indicates that the WT-Tau biosensor signal reports on the proximity of tau molecules within MTs rather than on tau-tau self-interaction properties. This information is highly relevant, especially in view of recent approaches aiming to identify inhibitors of spontaneous WT-tau interaction (16, 20). It is therefore highly likely that molecules identified by those studies act by detaching tau from microtubules and would be therefore rather detrimental for tau physiological functions. From our extensive characterization of the different sensors, we observed that sensors comprising mutated tau, in both full-length and K18 forms, are the most adequate and very sensitive tools to screen for compounds inhibiting the pathological oligomerization of tau, while the WT-tau sensor can be used as control, to assure that tau-MTs interaction is not destabilized. Our WT-tau full-length sensors provided further important insights on tau behavior by demonstrating, for example, that phosphorylation of WT tau takes place when it is still bound to MTs, as colchicine prevented its phosphorylation. Both intra- and intermolecular tau biosensors are, thus, useful tools to elucidate the mechanisms underlying the pathological transformation of native non-mutated tau by identifying cellular stressors and/or signaling pathways linked to age-related tau conformation change and/or to dissociation from MTs.

Propagation of tau pathology from cell-to-cell through a prion-like mechanism has been now extensively described and it implies on the ability of pathological tau forms to transmit its conformation and self-interaction behavior to other tau molecules from neighboring cells, spreading the pathology in a cell-to-cell mechanism to several brain areas (24). While the seeding response and aggregation of tau have been extensively shown using high micromolar concentrations of purified tau or overexpression of tau or K18 fragment displaying the pro-aggregating P301L/S mutations, the mechanisms underlying the formation of WT tau oligomers in a

physiological context compatible to the very initial phases of tauopathies are still to be elucidated. Accordingly, our WT-tau biosensor revealed to be highly resistant to oligomerize, even in the presence of potent tau seeds present in brain lysates of P301S mouse model, further confirming that this sensor does not monitor pathological tau interaction. Interestingly, when combining LgBit-Tau with SmBit-K18(P301L) we observed low basal interaction between these molecules, which was however significantly increased in the presence of seeds. This result suggests that WT tau molecules do not self-interact easily, but they can interact with and be sequestered into oligomers formed by seeding-responsive tau species. This new biosensor comprising K18 and WT-tau full-length might represent an interesting new target for the identification of therapeutic molecules preventing the seeding of physiological tau molecules. These data provide new insights on the mechanisms of tau seeding by demonstrating that physiological tau molecules can be sequestered by aggregation-prone tau seeds, highlighting also the importance of evaluating the dynamic responses of tau in a cell context rather than in *in vitro* assays.

Previously developed cell-based tau oligomerization biosensors were mainly based on tau conjugated to fluorescent proteins or on bi-molecular fluorescence complementation (BiFC) technique for visualization of tau aggregates by fluorescent microscopy (11, 12), or on FRET (14, 16, 29). The first assays on the luciferase complementation technology applied to tau aggregation were based on *Renilla* or *Gaussia luciferase* complementation assays (25, 47-49). Such quantitative property was essential to determine the minimum functional unit of tau fibrils required to induce self-interaction of K18-based complementation sensor (25), as well as to characterize the seeding activity of distinct tau strains (47) and tau-containing exosomes (49). To the best of our knowledge, our K18(P301L) and tau(P301L) full-length sensors are the first

NanoBiT-based tau seeding assays. As previously mentioned, one of the main advantages of NanoBit-based sensors is their quantitative properties, fast application and straightforward data analysis. In accordance with previous reports (25, 47-49), we also observed that sensors comprising the K18 fragment show the best signal amplitude in response to extracellular seeds, including those obtained from brain lysates of Tg mouse model of tauopathy. The study from Wegmann and collaborators (48) was the only one to use full-length WT tau and, similarly to our results, no significant self-interaction of the sensor was observed in the presence of brain homogenates from the transgenic P301S mouse model, confirming the resistance of WT-Tau to be seeded compared to K18 or mutated tau. The sensibility of our K18(P301L) interaction sensor is outstanding, spanning 4 logs of protein concentration of brain lysates and being able to discriminate between brain lysates from control and transgenic mice in as low as 300 pg of total protein after only 24h of incubation. Compared to existing FRET-based K18 aggregation sensors (14, 17, 50), the Nluc complementation-based Tau(P301L) and K18(P301L) interaction assays presented here are high-throughput compatible and require no further data integration following plate reading of the bioluminescence signal. We provide "proof-of-principle" evidence of the robustness of both assays and its application in the identification of small molecules able to interfere with tau seeding / self-interaction response, opening novel possibilities for the discovery of new drug candidates for tau-related neurodegenerative diseases.

In summary, we developed a series of highly sensitive NanoBiT tau biosensors able to monitor tau conformational changes and self-interaction/seeding responses in living cells exposed to different challenges related to diseases development, including over activity of kinases, microtubule disruption or presence of pathological tau seeds. Our study is the first one that generated and systematically compared the response of

different tau forms in terms of conformational changes and self-interaction towards different challenges in a cellular context. These sensors comprise a full platform for the investigation of tau physiological and pathological behaviors, as they proved to be useful tools to better understand the diversity of response of different tau forms, the cellular conditions that might favor tau pathological transformation and seeding, as well as to be applied in high-throughput screening programs to the identification of potential drug candidates disrupting tau seeding and self-interaction responses.

METHODS

Expression vectors

Plasmids encoding for NanoBiT system (SmBit and LgBit) were purchased from Promega (Madison, USA) and cloned in front of the tau protein in pcDNA3.1 vectors by restriction enzymes. The tau constructs used in this study comprises: **i.** tau full-length (aa 1-441 of 2N4R tau); **ii.** tau(P301L) (tau full-length displaying the P301L mutation); **iii.** K18(P301L) (aa 244-39 comprising the 4 repeat domains of tau with the P301L mutation). The tau P301L mutants were generated by PCR using QuickChange Lightning Site-Direct mutagenesis kit (Agilent Technologies, Santa Clara, USA). For all intermolecular sensors, we generated N- and C-terminal LgBit / SmBit conjugated tau constructs. All the combination pairs were tested and the best configuration for each biosensor was chosen based on the criteria of displaying a measurable basal signal. Similarly, LgBit and SmBit at either N- or C-terminal positions was also tested for all intramolecular sensors. All constructs include a HA-tag and their sequences were verified by DNA sequencing.

Cell Culture and cell transfection

HEK293T (Sigma-Aldrich) were maintained in Dulbecco's Modified Eagle's Medium (DMEM) Glutamax (Invitrogen) supplemented with 4.5 g/L glucose, 10% fetal bovine serum, 100 U/mL penicillin and 0.1 mg/mL streptomycin, at 37°C (95% O_2, 5% CO_2). Cells were transfected with specific amounts of each construct (as indicated) in 6-well or 12-well plates using jetPEI reagent according to the supplier's instructions (Polyplus-transfection, New York, NY, USA). One day after transfection, cells were plated into white 96-well plates (Perkin Elmer Life Sciences, Waltham, MA) precoated with 10 µg/ml poly-L-lysine (Sigma-Aldrich), and the Nluc complementation assay was performed 48h post-transfection.

Preparation of recombinant proteins and mouse brain lysates

His6-K18 (244-372) and -His6-WT 2N4R tau were produced in E. coli BL21 (DE3) bacteria and purified using IMAC capture followed by ion exchange chromatography (GTP biotechnology). K18 protein was eluted in 20mM sodium phosphate, 500mM NaCl, pH 7, 1 mM EDTA and 2 mM DTT, The 2N4R tau protein was eluted in 25mM Phosphate de sodium, 25mM NaCl, 2,5mM EDTA pH 6,6 and lyophilized in 50mM acétate d'ammonium pH 8,2. Aggregated species of recombinant tau full-length and K18 fragment were prepared as previous reported (25). Briefly, monomeric species were preincubated in 10 mM dithiothreitol for 60 min at room temperature, followed by incubation in oligomerization buffer (10 mM HEPES, 100 mM NaCl, and 2 to 6.8 µM heparin (1:1 ratio of monomeric tau to heparin, 37°C, 24h). Oligomers of amyloid beta peptide were obtained from lyophilized $A\beta_{1-42}$ (Anaspec), which was firstly dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol, evaporated, aliquoted and reconstituted in anhydrous DMSO and PBS, followed by incubation for 24h at 4°C prior to use (51).

Brain-derived tau seeds were obtained from the P301S transgenic mice model of tauopathy, as their brain lysate has been shown to facilitate the formation of tau-tau interaction in HEK cells (14). Heterozygous mice expressing human tau 1N4R with the P301S mutation (52), and WT littermates (referred to as Tg and WT mice, respectively) were obtained from Jackson Laboratories (Stock No: 008169) and bread for SERVIER at Charles River (Lyon, France). Nine-month-old male mice were sacrificed, and the cortex rapidly removed and flash-frozen. Frozen tissue was lysed in 100 mg/mL of PBS (-Ca^{2+}, -Mg^{2+}), using a cooled Precellys® homogeniser. Lysates were sonicated (Q800R2, Sonica) at 4°C for 1 min at 90% power, and centrifuged for 15 min at 21.000g, 4°C. The supernatant was collected, aliquoted and stored at -80 °C. Protein concentration was determined using a Pierce™ BCA Protein Assay Kit (ThermoFisher). Prior to use, lysates were sonicated again in a probe sonicator (Branson SLPe, 90%, 6x10sec, with 10 sec interval). Sample sizes were designed to give statistical power while minimizing animal use.

Nluc complementation Assay

Transfected cells expressing tau sensors were incubated with forskolin (10 µM, 24h) and/or colchicine (10 µM, 1h), in the 96-well plate. For tau seeding experiments, recombinant tau proteins (100 nM, 24h) or brain lysates from Tg and WT mice (up to 3 ug of total protein / well, 24h) were mixed with Lipofectamine 2000 reagent (1 uL / well) in Opti-MEM (Invitrogen) and incubated for 20 min before adding to HEK293T cells, as previously reported (14). Immediately before the assay, medium was removed, washed once with PBS, and replaced by PBS. The nanoluciferase substrate Nano-Glo Live Cell (Promega) was added to the wells at a final dilution of 1:100 and

luminescence signal was read immediately and for several cycles in order to assure the detection of the plateau of the enzyme : substrate reaction.

Immunofluorescence analysis

HEK293T cells transiently expressing each sensor were seeded onto sterile poly-L-lysine coated 24-well format cover glass one day after transfection with the indicated tau biosensors, and treated with the indicated compounds. At the end of the incubation time, cells were washed, fixed with paraformaldehyde 4% or methanol:acetone (1:1) solution (15 min), permeabilized in 0.2% Triton X-100, and blocked for 1h with 5% horse serum. Cells were then incubated (overnight, 4°C) with monoclonal anti-HA antibody (rabbit, dilution 1:500, cat. 3724S; Cell Signaling), or anti-tubulin (rat, MAB1864; Millipore; 1:2000), or with anti-phospho tau antibody AT8 (MN1020; Thermofisher; 1:200), or with the tau conformation specific MC-1 antibody (dilution 1:500, (33)). MC-1 antibody was generously provided by Dr. Peter Davies. After several washes, cells were incubated with anti-mouse or anti-rat or anti-rabbit fluorescein-conjugated secondary antibodies (dilution 1:500, Invitrogen) and cell nuclei were stained with DAPI (Sigma-Aldrich). After mounting the cover glass into glass slides, the slides were analyzed under Zeiss Observer Z1 microscope (Zeiss, Germany). Images obtained were further analyzed using ImageJ software (53).

SDS-PAGE/Western Blot

Cells expressing tau biosensors were lysed in Laemli buffer (62.5 mmol/L Tris/HCl pH 6.8, 5% SDS, 10% glycerol, 0.005% bromophenol blue) and resolved in SDS-PAGE gel (10% or 12%), followed by protein transfer to nitrocellulose membranes. Membranes were blocked in 5% non-fat dried milk in TBS (10 mM Tris-HCl, pH 8,

150 mM NaCl), and immunoblotted with primary antibodies against the HA tag (rabbit, dilution 1:1000, cat. 3724S; Cell Signaling), or anti-phospho tau antibody AT8 (MN1020; Thermofisher; 1:200), diluted in 0.3% BSA in TBS (overnight, 4°C), followed by incubation with secondary antibodies coupled to 680 or 800 nm fluorophores (1:15,000 dilution in 0.3% non-fat dried milk in TBST, 1h at room temperature; LI-COR Biosciences, Lincoln, NE, USA). Membranes were read using the Odyssey LI-COR infrared fluorescent scanner (LI-COR Biosciences).

Data analysis

Data are presented as means ± SEM of the indicated *n* (number of independent experiments). A minimum of 3 independent experiments, each performed in triplicates (technical replicates), was performed to ensure accurate estimation of experiment variability. Nluc complementation experiments were each performed in triplicates to ensure the reliability of single values, and luminescence data are expressed as the raw data or normalized to % of basal signal or maximal signal, as indicated. Non-specific complementation signal was determined for inter-molecular sensors by expressing only one of the constructs or co-transfecting with a complementation construct comprising a protein (halo) non-related to tau. For the experiments on concentration-response curves of inhibitory compounds, data were fitted to the non-linear regression log (inhibitor) versus response (three parameters) fitting equation (GraphPad Prism software version 6). Statistical analysis was performed using GraphPad Prism software version 6, applying Student's *t* test to compare two groups or ordinary one-way or two-way analysis of variance (ANOVA) when comparing multiple groups, followed by Tukey's or Dunnett's multiple comparison post hoc test when appropriate. Values of $p < 0.05$ were considered statistically significant. Z' values (34) of the Nluc

complementation assay for the K18(P301L) and Tau(P301L) interaction biosensors were determined using the following equation, with cells treated with brain lysates from WT mice used as negative control and cells treated with Tg brain lysates as positive control:

$$\text{Z-factor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}$$

where σp= standard deviation of positive control; σn= standard deviation of negative control; μp=mean of positive control; μn=mean of negative control.

Ethics Committee Approval

All animal experimental procedures were performed in agreement with the European Communities Council Directive 2010/63/EU and were approved by the Ethical Committee for Animal Experimentation of the Institut de Recherches Servier.

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AUTHOR CONTRIBUTIONS:

Conceptualization, E.C., A.O., F.P., J.D., P.M. and R.J.; Biochemical investigations, E.C., A.O. M.L., D.N.L, and A.F.; Writing – Original Draft, Review and Editing, E.C., J.D., P.M. and R.J.; Funding Acquisition, R.J.

CONFLICT OF INTEREST:

The authors have nothing to declare.

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Figure Legends

Figure 1 with 1 supplement

NanoBiT-based biosensors development and application to monitor the tau seeding response in living cells. A) Scheme of NanoBiT-based intramolecular (conformational) and intermolecular (interaction) biosensors. **B)** Biosensors were developed for three tau forms: full-length wild-type tau (WT-Tau) corresponding to the 2N4R isoform displaying both of the two domains at the N-terminal part (N1,N2), the proline-rich (PR) region, and four-repeat domains (R1-R4); full-length tau carrying the P301L mutation (Tau(P301L)); and the tau fragment comprising only the 4 repeat domains (R1-R4) and carrying the P301L mutation (K18(P301L)). **C)** Workflow of NanoBiT assay (scheme created with Biorender). **D)** Saturation curve of the intermolecular interaction sensor K18(P301L) in HEK293T cells expressing a fixed amount of LgBit-K18(P301L) (250 ng of transfected vector) and increasing amounts of SmBit-K18(P301L) (0 to 500 ng of transfected vector); data are expressed as mean ± SEM of 4 independent experiments. **E)** Representative western blot analysis of the expression of K18(P301L) biosensor pairs used in panel D. **F)** Cellular distribution of the K18(P301L) biosensor monitored by immunofluorescence microscopy (tubulin: in green; biosensor: anti-HA antibody, in red; nuclei: DAPI staining, in blue). Scale bar: 10 µm. **G)** K18(P301S) interaction biosensor signal in the presence of recombinant monomeric K18 (mK18), aggregated K18 (aggK18) or tau (aggTau), or oligomeric amyloid beta peptide (oAβ); data are expressed as mean ± SEM of 5 independent experiments; ** $p<0.01$, **** $p<0.001$ by one-ANOVA, followed by Dunnett's multiple comparisons test to basal condition.

Figure 1 – Figure Supplement 1

Validation of tau sensors based on the NanoBiT system by the K18(P301S) interaction sensor. K18(P301S) interaction signal in biosensor cells treated with recombinant aggregated K18 (aggK18) and in cells expressing only LgBiT-K18(P301L) **(A)** or only SmBiT-K18(P301L) **(B)**; data are expressed as mean ± SEM of 3 independent experiments; ** $p<0.01$, by Student t test. **C)** Kinetics of the Nluc enzyme:substrate activity following addition of Nluc substrate in K18(P301L) expressing cells treated with the indicated concentrations of recombinant aggK18. Data shown as mean ± SD of triplicates.

Figure 2

Tau biosensors monitor conformational change and self-interaction of WT full-length tau. A) Saturation curve of the intermolecular WT-Tau interaction sensor in HEK293T cells expressing a fixed amount of LgBit-Tau (250 ng of transfected vector) and increasing amounts of SmBit-Tau or SmBit-Halo (0 to 500 ng of transfected vector); data are expressed as mean ± SEM of 5 independent experiments. Bottom: Representative western blot (WB) analysis of the expression of the tau biosensor pairs. **B)** Cellular distribution of the WT-Tau interaction biosensor monitored by immunofluorescence microscopy (biosensor: anti-HA antibody, in red; tubulin: in green; nuclei: DAPI staining, in blue); Scale bar: 10 µm. **C)** Signals of WT-Tau and K18(P301L) **(D)** interaction biosensors in cells treated or not with colchicine (10 µM, 1h); data are expressed as mean ± SEM of 7 independent experiments; ** $p<0.005$ by two-tailed paired Student *t* test; n.s. = not-significant. **E)** WT-Tau conformational sensor signal in HEK293T cells expressing increasing amounts of LgBit-Tau-SmBit vector (0 to 500 ng of transfected vector); data are expressed as mean ± SEM of 7 independent experiments. Bottom: Representative WB analysis of the expression of the Tau conformational biosensor. **F)** Cellular distribution of the WT-Tau conformational biosensor monitored by immunofluorescence microscopy (biosensor: anti-HA antibody, in red; tubulin: in green; nuclei: DAPI staining, in blue); Scale bar: 10 µm. **G)** WT-Tau and K18(P301L) conformational **(H)** biosensors signal in cells treated or not with colchicine; data are expressed as mean ± SEM of 7 independent experiments; *** $p<0.001$ by two-tailed paired Student *t* test; n.s. = not-significant. **I)** Signals of WT-Tau conformational and interaction **(J)** biosensors in cells treated or not with forskolin (10 µM, 24h); data are expressed as mean ± SEM of 7 independent experiments; **$p<0.01$, *** $p<0.005$ by two-tailed paired Student *t* test; right panel: WB analysis of phosphorylated tau (P-Tau) and Total tau (T-tau) using AT8 and anti-HA antibodies, respectively. L: Lgbit-Tau; S: SmBit-Tau. **K)** WT-Tau interaction biosensor response in cells treated with colchicine (10 µM, 1h), forskolin (10 µM, 24h), or both (colchicine followed by forskolin); data are expressed as mean ± SEM of 5 independent experiments; **$p<0.01$, *** $p<0.005$, **** $p<0.001$ by one-way ANOVA followed by Sidak's multiple comparison test.

Figure 3 with 2 supplements

Tau seeding response monitored by NanoBiT tau biosensors. A) WT-Tau interaction biosensor signal in cells treated with monomeric (m) or aggregated (agg) forms of recombinant K18 or tau; data are

expressed as mean ± SEM of 5 independent experiments. **B)** K18(P301L) and WT-Tau interaction biosensors signal in cells treated with brain lysates (3 µg total protein, 24h) obtained from WT or Tg mice; data are expressed as mean ± SEM of 7 independent experiments; **** $p<0.001$ by one-ANOVA, followed by Sidak's multiple comparison tests. **C)** K18(P301L) interaction biosensor signal in the presence of increasing concentrations of brain lysates (in µg of total protein) obtained from WT or Tg mice; representative curve of 4 independent experiments expressed as mean ± SD of triplicates. Insert: zoom of data points indicated by the square. **D)** LgBit-Tau + SmBit-K18 interaction biosensor signal in the presence of brain lysates obtained from WT or Tg mice (3 µg); data are expressed as mean ± SEM of 5 independent experiments; *** $p<0.005$ by one-ANOVA, followed by Sidak's multiple comparison tests.

Figure 3 – Figure Supplement 1

Specificity of the K18(P301S) interaction sensor response. A) Biosensor signal in cells expressing K18(P301S) interaction biosensor or the non-related LgBiT-K18(P301L) + SmBiT-Halo biosensor interaction pair following treatment with brain lysates obtained from WT or Tg mice; data are expressed as mean ± SEM of 3 independent experiments; *$p<0.05$, by Student t test compared to WT group. **B)** Reproducibility of the K18(P301S) interaction biosensor response towards seeds from brain lysates obtained from four independent WT (WT 1-4) or Tg (Tg 1-4) mice; data are expressed as mean ± SEM of 3 independent experiments; ****$p<0.001$, by one-way ANOVA followed by Sidak's multiple comparison tests.

Figure 3 – Figure Supplement 2

Effect of recombinant seeds or brain lysates on K18(P301L) conformation monitored by NanoBiT conformation biosensors. A) K18(P301L) conformation biosensors signal in cells treated with monomeric (m) or aggregated (agg) forms of recombinant K18 or tau, or with brain lysates obtained from WT or Tg mice **(B)**. Data are expressed as mean ± SEM of 3-6 independent experiments. ** $p<0.01$ by one-ANOVA, followed by Sidak's multiple comparison tests.

Figure 4 with 1 supplement

Tau(P301L) self-interaction and seeding responses monitored by Nluc biosensors. A) Tau(P301L) interaction and conformation **(B)** biosensor signal in cells treated or not with forskolin (10 µM, 24h); data are expressed as mean ± SEM of 4 independent experiments; *p<0.05, ** p<0.01 by Student *t* test. Right panel: western blot analysis of phosphorylated tau (P-Tau) and total tau (T-tau) using AT8 and anti-HA antibodies, respectively; L: Lgbit-Tau; S: SmBit-Tau. **C)** Cellular distribution of the Tau(P301L) biosensor monitored by immunofluorescence microscopy (biosensor: anti-HA antibody, in red; tubulin: in green; nuclei: DAPI staining, in blue); Scale bar: 10 µm. **D)** Tau(P301L) and WT-Tau conformation and interaction **(E)** biosensors signal in cells treated or not with colchicine (10 µM, 1h); data are expressed as mean ± SEM of 3 independent experiments; * p<0.05, ** p<0.01, *** p<0.005 by Student *t* test. **F)** Tau(P301L) interaction biosensor signal in cells treated for 24h with recombinant proteins or brain lysates obtained from WT or Tg mice **(G)**; data are expressed as mean ± SEM of 3-5 independent experiments; ** p<0.01 by one-ANOVA, followed by Sidak's multiple comparison tests to the indicated group. **H)** Immunofluorescence microscopy detection of tau pathological conformation (MC-1 antibody, green, upper panel) or phospho-tau (AT8 antibody, green, bottom panel) in cells expressing Tau(P301L) or WT-Tau interaction biosensors (HA antibody, red) and treated with brain lysates obtained from WT or Tg mice for 24h; nuclei are stained with DAPI (blue); Scale bar: 10 µm.

Figure 4 – Figure Supplement 1

Effect of recombinant seeds or brain lysates on Tau(P301L) conformation monitored by NanoBiT conformation biosensors. A) Tau(P301L) conformation biosensors signal in cells treated with monomeric (m) or aggregated (agg) forms of recombinant K18 or tau, or with brain lysates obtained from WT or Tg mice **(B)**. Data are expressed as mean ± SEM of 3-6 independent experiments. ** p<0.01 by one-ANOVA, followed by Sidak's multiple comparison tests.

Figure 5 with 1 supplement

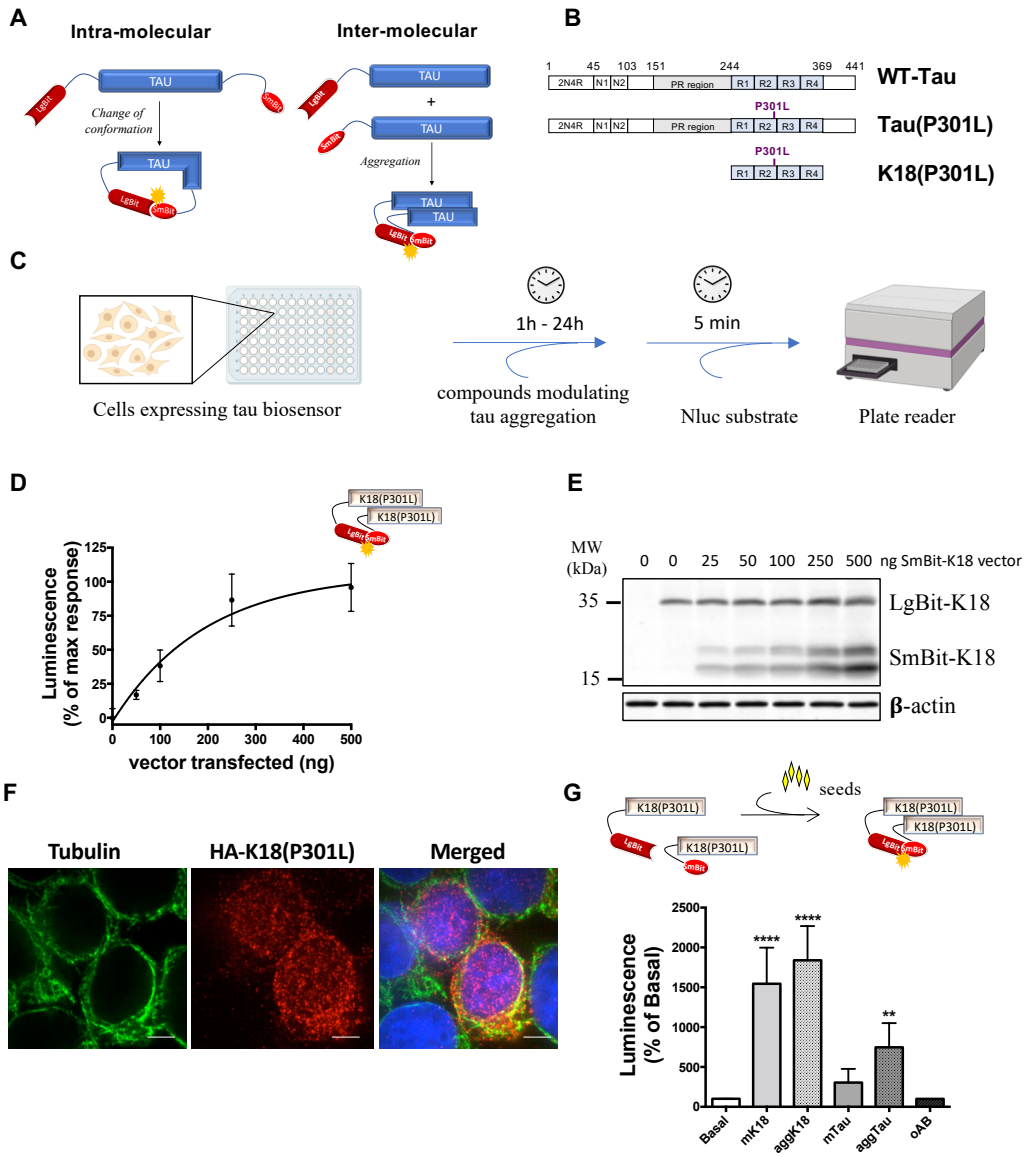
Tau NanoBiT biosensors application in the identification of inhibitors of tau seeding and self-interaction. A) Determination of Z' by collecting biosensor signals for K18(P301L) or Tau(P301L) **(B)** interaction biosensor cells in the presence of brain lysates from WT (red) or Tg (blue) mice. **C)** K18(P301L) or Tau(P301L) **(D)** interaction biosensor signal in the presence of candidate inhibitors of tau aggregation; data are expressed as mean ± SEM of 3-4 independent experiments; *p<0.05,

***p<0.005, ****p<0.001 by one-ANOVA, followed by Sidak's multiple comparison tests to the vehicle group. **E-G)** Concentration-response curves of the effect of compounds ANLE138b (**E**), LMTMeSO4 (**F**) and bb14 (**G**) on the K18(P301L) biosensor signal; data are expressed as mean ± SEM of 4 independent experiments.

Figure 5 – Figure Supplement 1

Effect of tested compounds on Nluc activity and cell viability. A) Cell viability assessed by MTT test in HEK293T cells incubated with vehicle (DMSO 0.1%) or the corresponding compounds (10 μM, overnight); Triton 20% was used as positive control of the assay; data are expressed as mean ± SD of triplicates. **B)** Nluc activity in HEK293T cells expressing Nluc construct and incubated with vehicle (DMSO 0.1%) or the corresponding compounds (10 μM, overnight); data are expressed as mean ± SD of triplicates.

1 **Figure 1**



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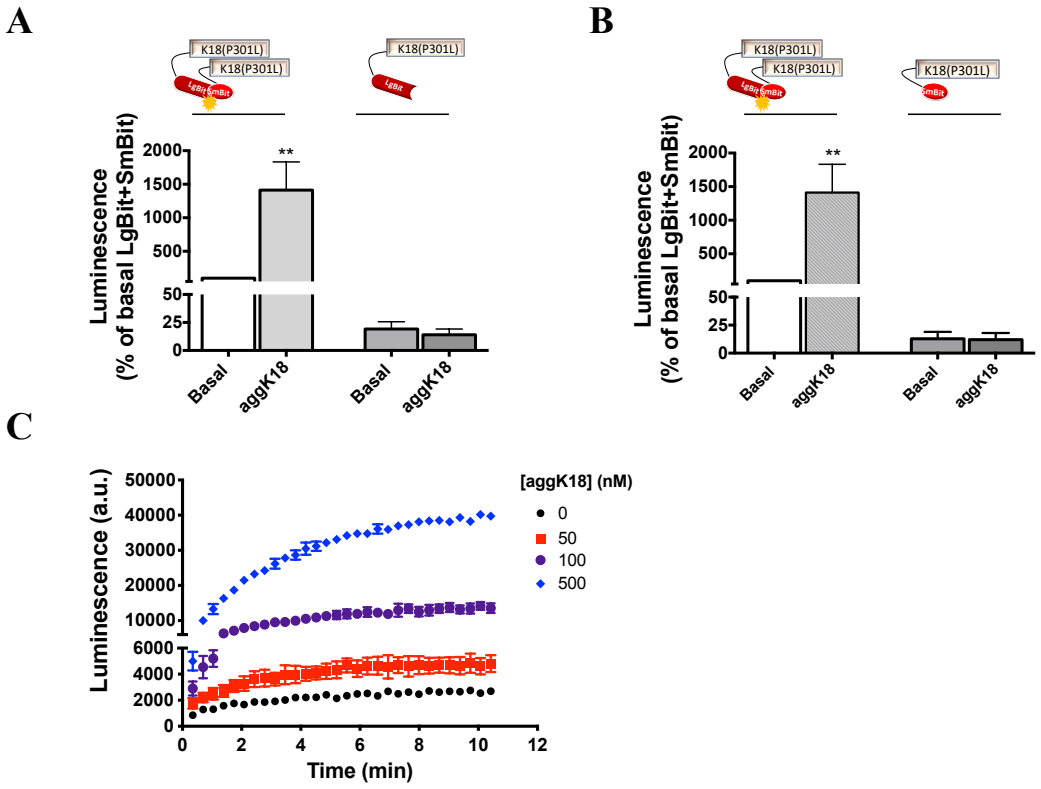
1 **Figure 1 – Figure Supplement 1**



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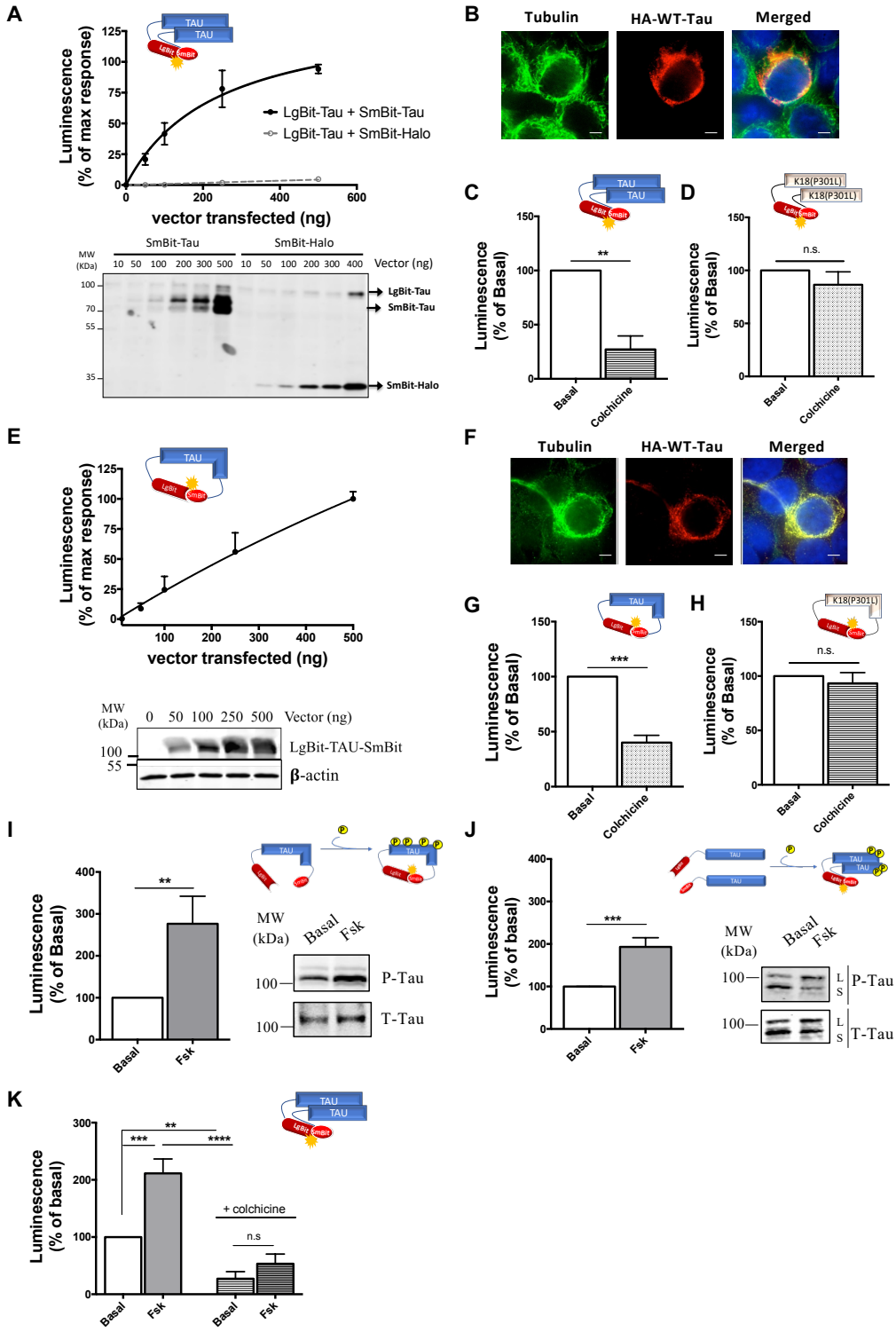

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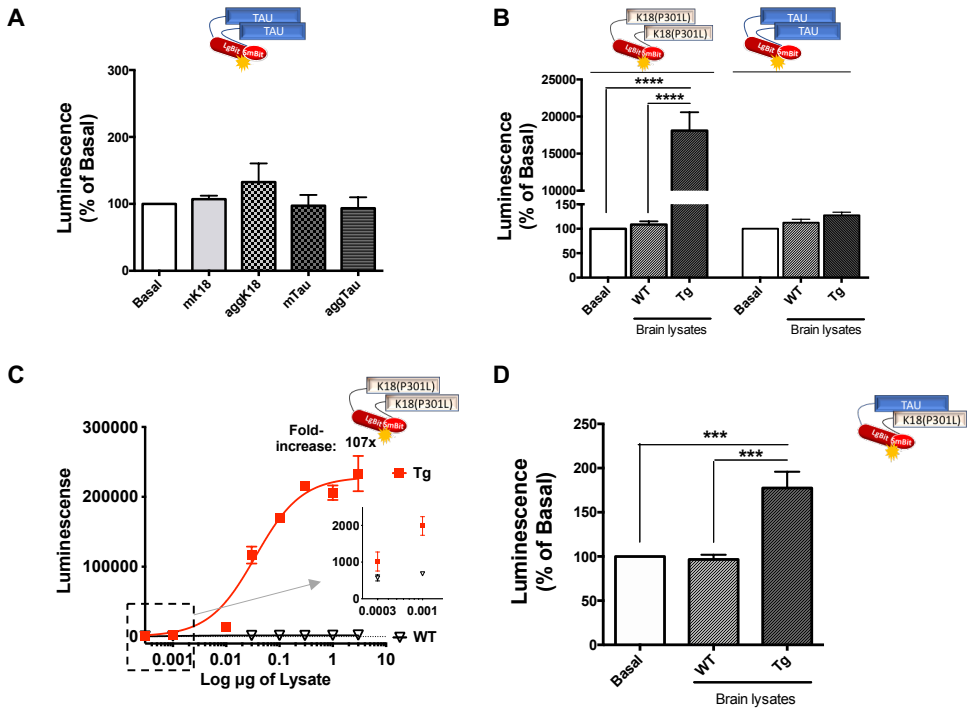
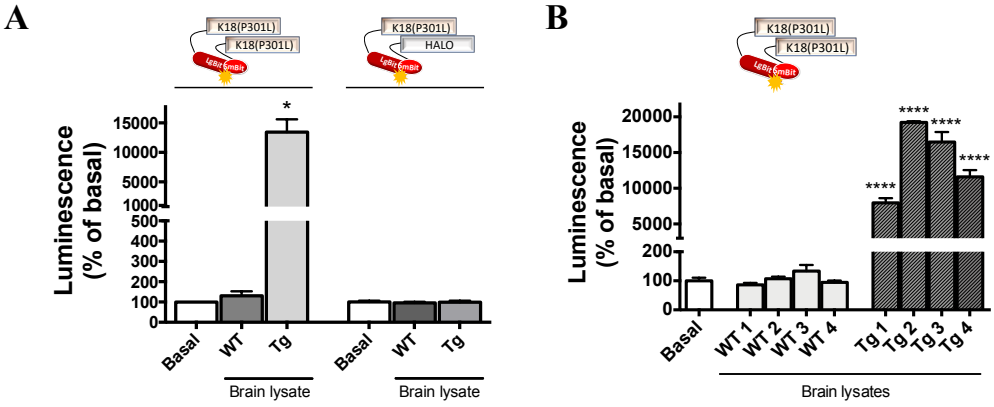
1 **Figure 3**


A
TAU
TAU
LgBit SmBit
Luminescence (% of Basal)
300
200
100
0
Basal
mK18
aggK18
mTau
aggTau
B
K18(P301L)
K18(P301L)
TAU
TAU
Luminescence (% of Basal)
25000
20000
15000
10000
150
100
50
0
****
****
Basal
WT
Tg
Brain lysates
Basal
WT
Tg
Brain lysates
C
K18(P301L)
K18(P301L)
Luminescense
300000
200000
100000
0
Fold-increase: 107x
Tg
2000
1000
0
0.0003
0.001
WT
0.001
0.01
0.1
1
10
Log µg of Lysate
D
TAU
K18(P301L)
Luminescence (% of Basal)
250
200
150
100
50
0
***
***
Basal
WT
Tg
Brain lysates

2

1 **Figure 3 – Figure Supplement 1**



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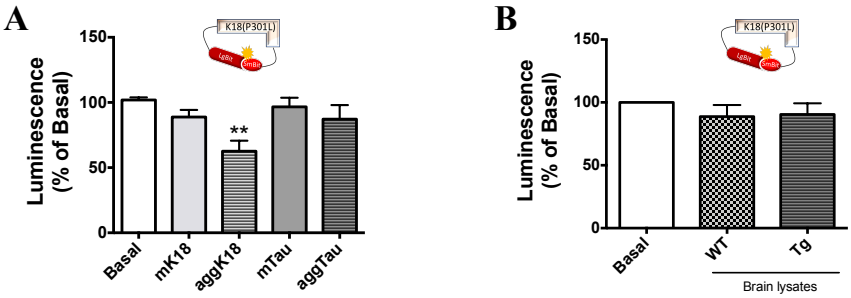
1 **Figure 3 – Figure Supplement 2**



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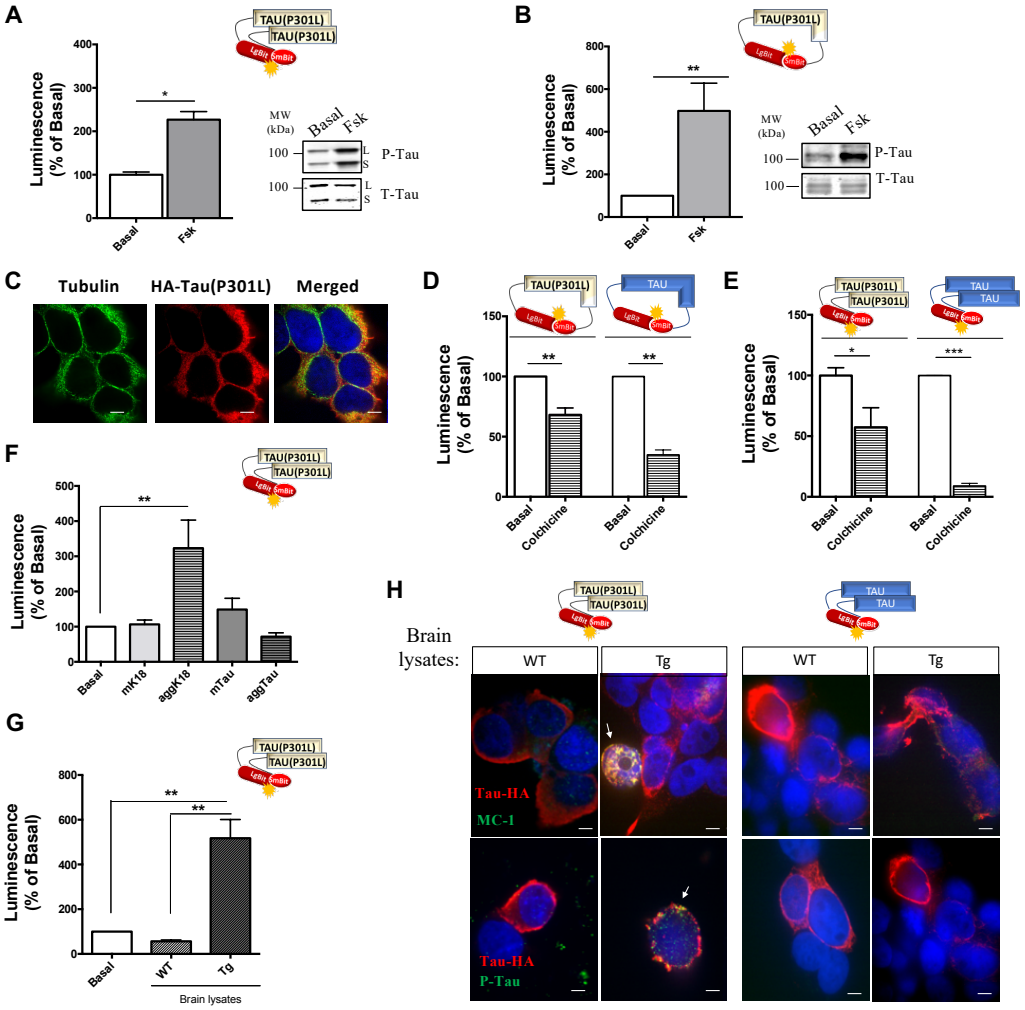
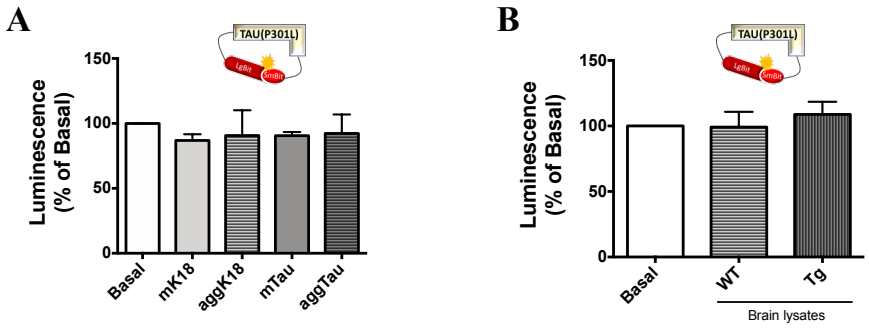
1 **Figure 4**


A
TAU(P301L)
TAU(P301L)
Luminescence (% of Basal)
Basal
Fsk
MW (kDa)
P-Tau
T-Tau
B
TAU(P301L)
C
Tubulin
HA-Tau(P301L)
Merged
D
TAU(P301L)
TAU
Colchicine
E
F
mK18
aggK18
mTau
aggTau
G
WT
Tg
Brain lysates
H
Brain lysates:
Tau-HA
MC-1
P-Tau

2

3

1 **Figure 4 – Figure Supplement 1**



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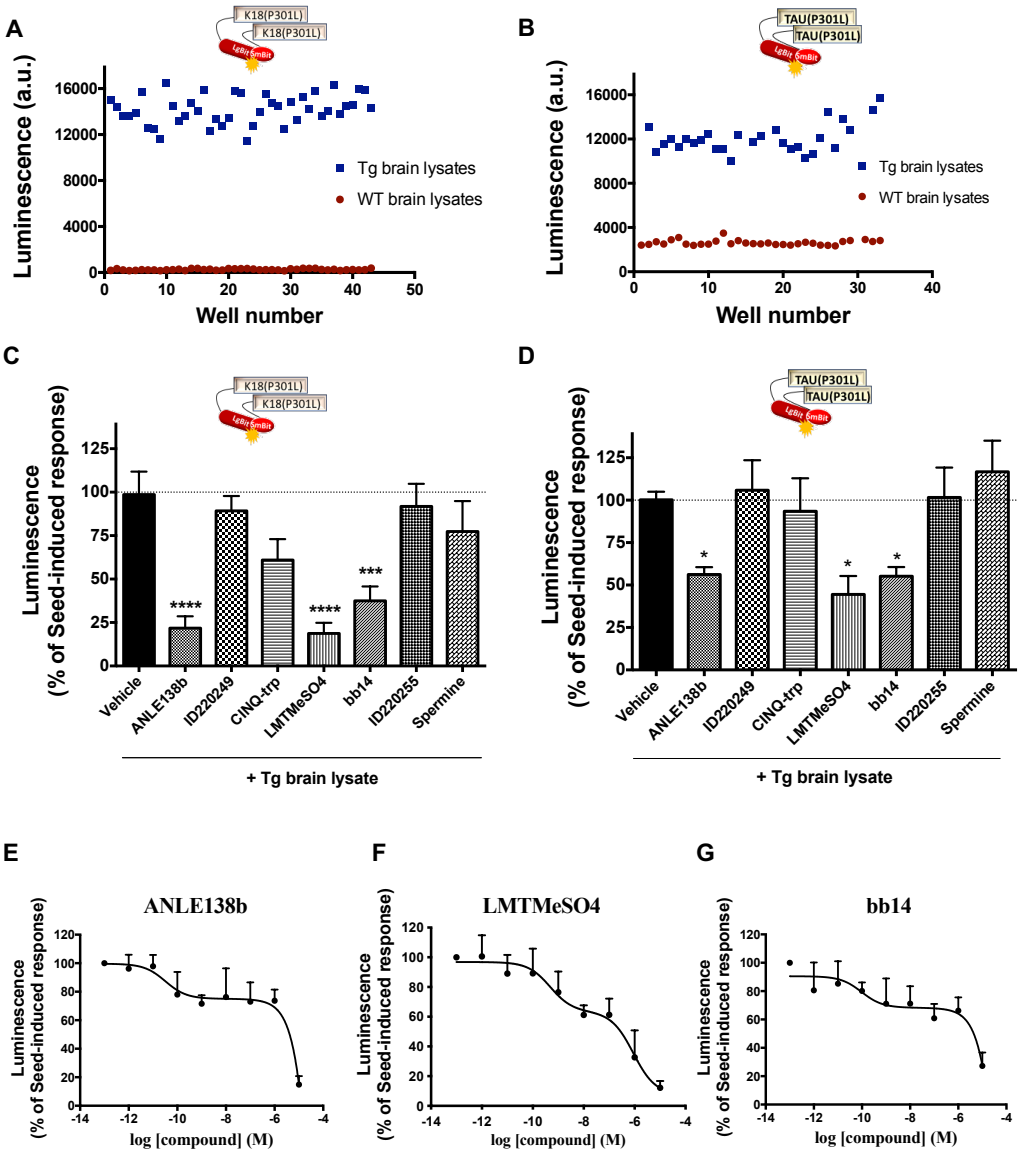
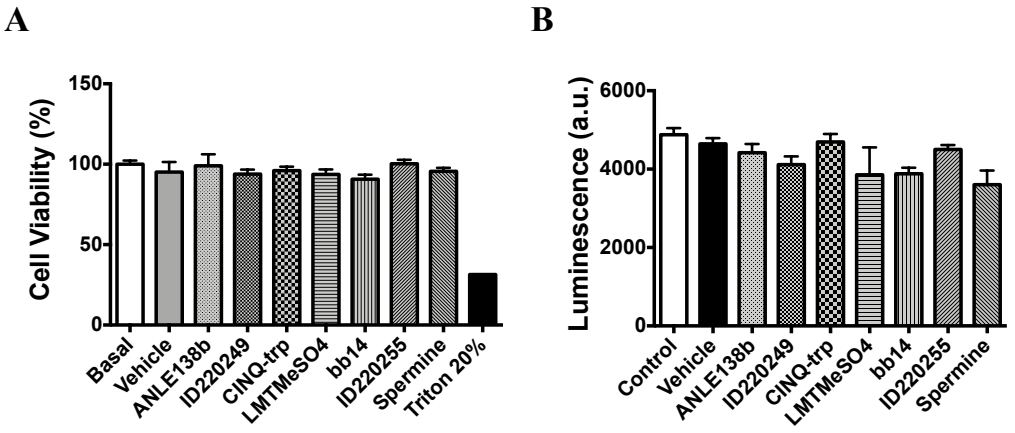
1 **Figure 5**


A
K18(P301L)
K18(P301L)
LgBit SmBit
Luminescence (a.u.)
16000
12000
8000
4000
0
0
10
20
30
40
50
Well number
Tg brain lysates
WT brain lysates
B
TAU(P301L)
TAU(P301L)
LgBit SmBit
Luminescence (a.u.)
16000
12000
8000
4000
0
0
10
20
30
40
Well number
Tg brain lysates
WT brain lysates
C
K18(P301L)
K18(P301L)
LgBit SmBit
Luminescence
(% of Seed-induced response)
125
100
75
50
25
0
****
****
***
Vehicle
ANLE138b
ID220249
ClNQ-trp
LMTMeSO4
bb14
ID220255
Spermine
+ Tg brain lysate
D
TAU(P301L)
TAU(P301L)
LgBit SmBit
Luminescence
(% of Seed-induced response)
125
100
75
50
25
0
*
*
*
Vehicle
ANLE138b
ID220249
ClNQ-trp
LMTMeSO4
bb14
ID220255
Spermine
+ Tg brain lysate
E
ANLE138b
Luminescence
(% of Seed-induced response)
120
100
80
60
40
20
0
-14
-12
-10
-8
-6
-4
log [compound] (M)
F
LMTMeSO4
Luminescence
(% of Seed-induced response)
120
100
80
60
40
20
0
-14
-12
-10
-8
-6
-4
log [compound] (M)
G
bb14
Luminescence
(% of Seed-induced response)
120
100
80
60
40
20
0
-14
-12
-10
-8
-6
-4
log [compound] (M)

2

3

1 **Figure 5 – Figure Supplement 1**



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