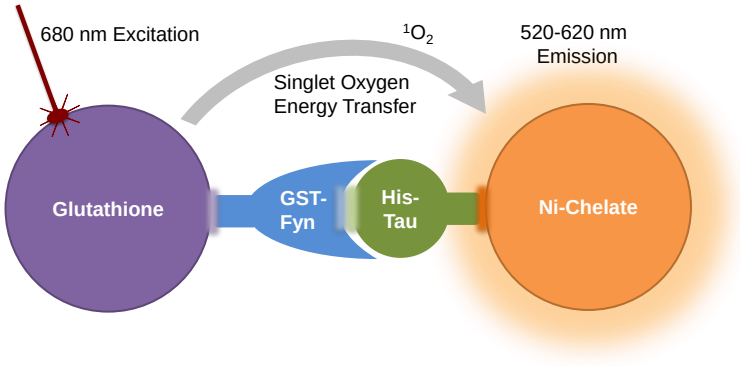
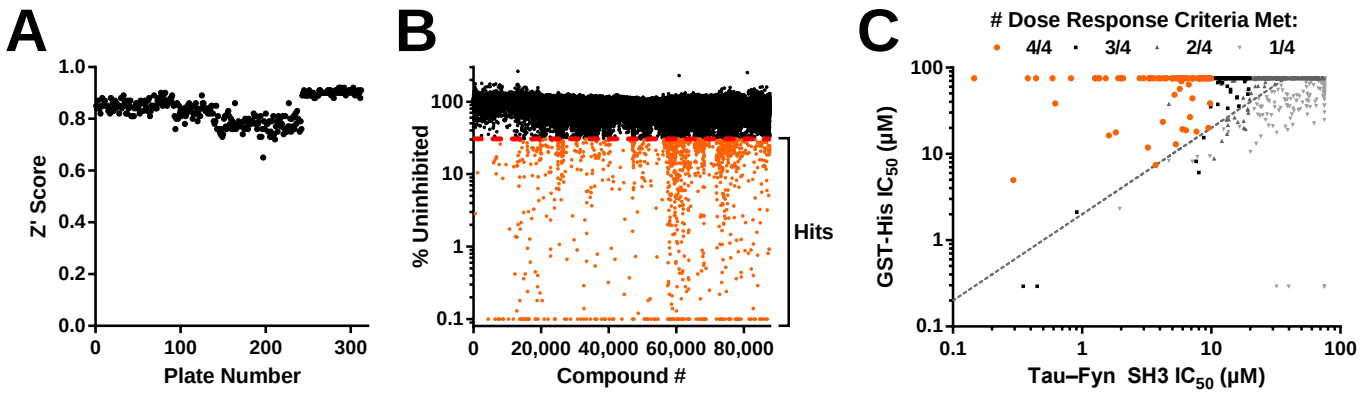
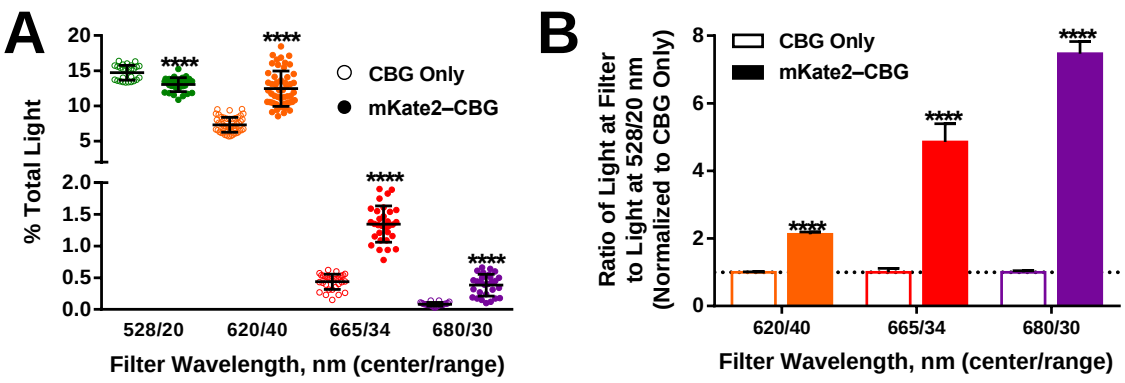


Supplementary Figure S1. (Related to Figure 1) The AlphaScreen assay uses recombinant Tau and Fyn SH3 coupled to fluorescent beads. GST-tagged Fyn binds the glutathione donor beads and His-tagged Tau binds the nickel chelate acceptor beads.



Supplementary Figure S2. HTS with the AlphaScreen Tau–Fyn SH3 assay identifies potential Tau–Fyn SH3 interaction inhibitors. (**A**) Z' score was consistently high throughout HTS. (**B**) Hits were defined as compounds that reduced the Tau–Fyn interaction by more than 3 standard deviations (i.e., below 30.68% of the control signal). Points with <0.1% of control signal are plotted at 0.1% for display on the log plot shown. (**C**) Activity in the primary screen plotted vs. activity in the GST-His counterscreen. Dotted line represents a selectivity index of 2, i.e. an IC_{50} for GST-His two-fold greater than the IC_{50} for the Tau–Fyn SH3 interaction. The 64 hits meeting all four criteria described in the text (activity in the main Tau–Fyn SH3 AlphaScreen assay and selectivity indices of >2 with a GST-His control AlphaScreen assay and LL47 & THP-1 cell-based toxicity assays) are indicated in orange. Compounds with no detectable activity at or above 75 μM in the GST-His counterscreen are shown here at the 75 μM line for display purposes.



Supplementary Figure S3. (Related to Figure 5) A BRET assay allows measuring the Tau–Fyn interaction in living cells. (**A**) Light emitted by an mKate2–CBG fusion protein was red-shifted compared to CBG only, consistent with BRET. Signal was read 30 minutes after substrate addition, **** indicates $p < 0.0001$ by t-test for each filter set (n = 24–53 independent transfections per group). Error bars indicate SD. (**B**) One way to measure BRET signal is to evaluate the ratio of red light to green light. The mKate2–CBG fusion signal was easily measured by the ratio of red light (at any of several different red wavelength filters) to the amount of green light at 528/20 nm (double ratio). Signal was read 30 minutes after substrate addition, **** indicates $p <$ 0.0001 mKate2 effect by two-way ANOVA (n = 9–31 independent transfections per group). Error bars indicate SEM.