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Supporting information for article:

The crystal structure of the catalytic domain of tau tubulin kinase 2 in complex with a small-molecule inhibitor

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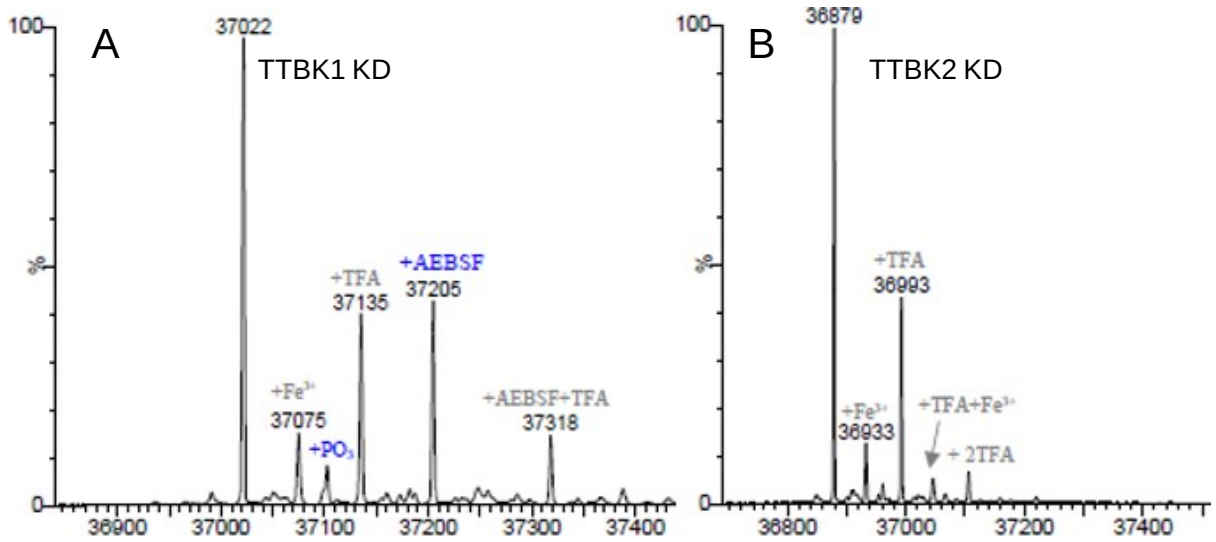
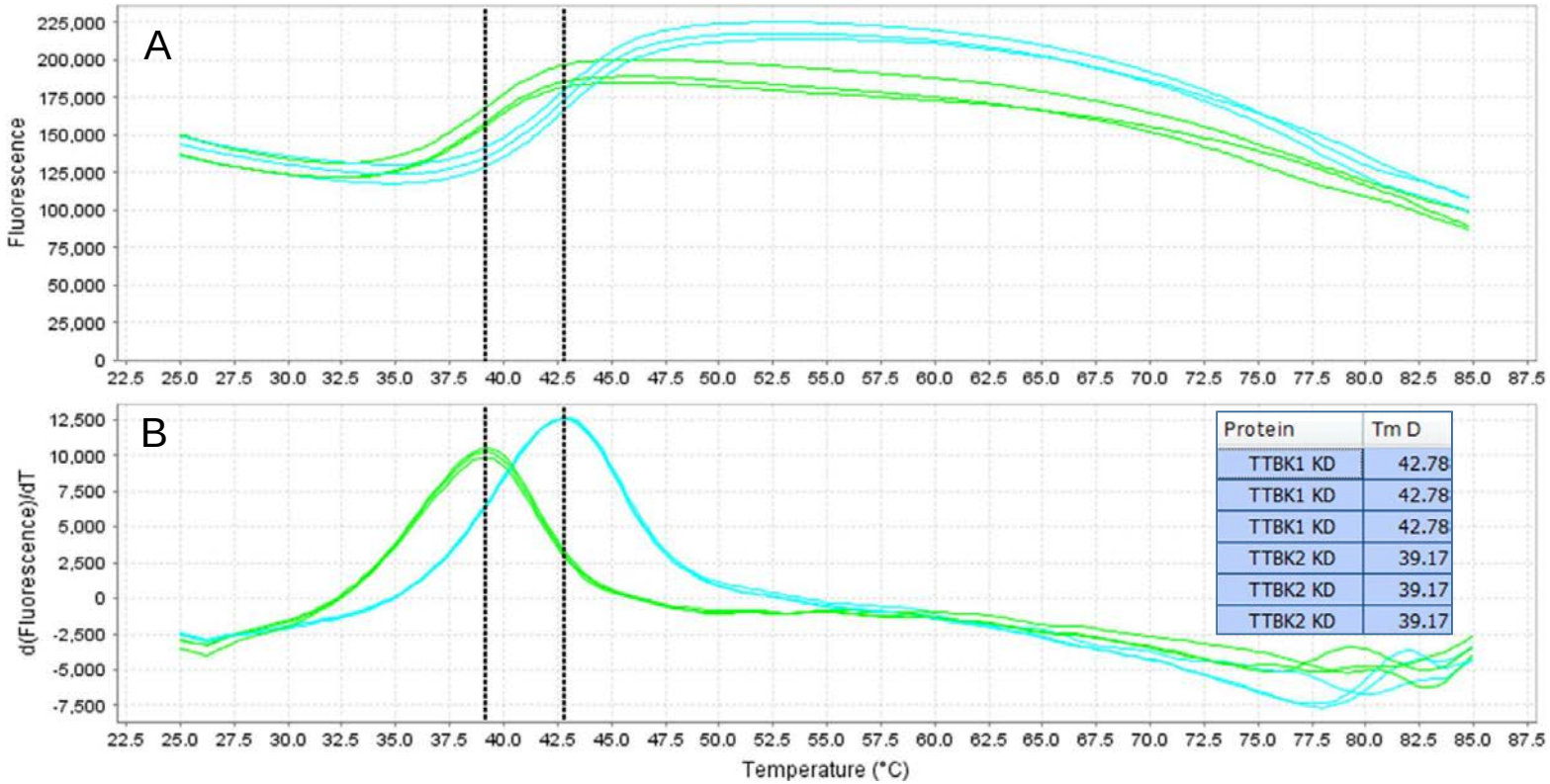
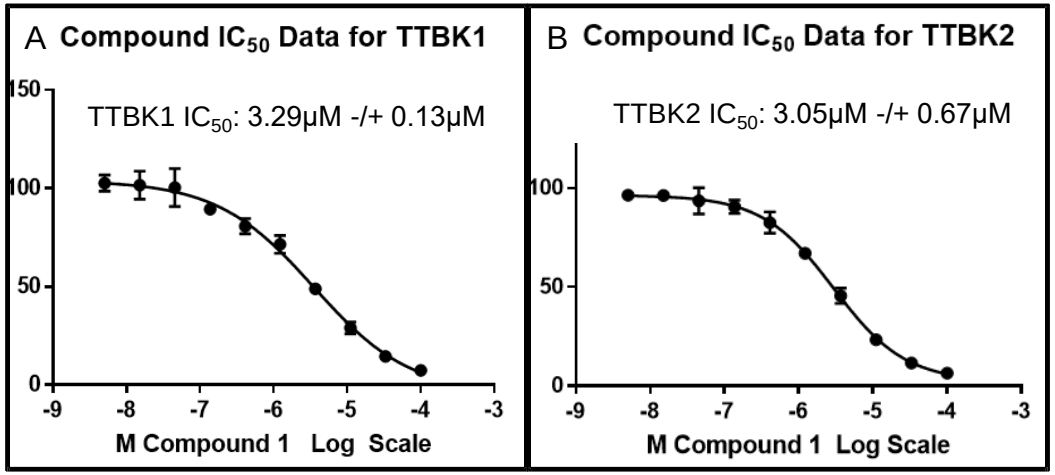


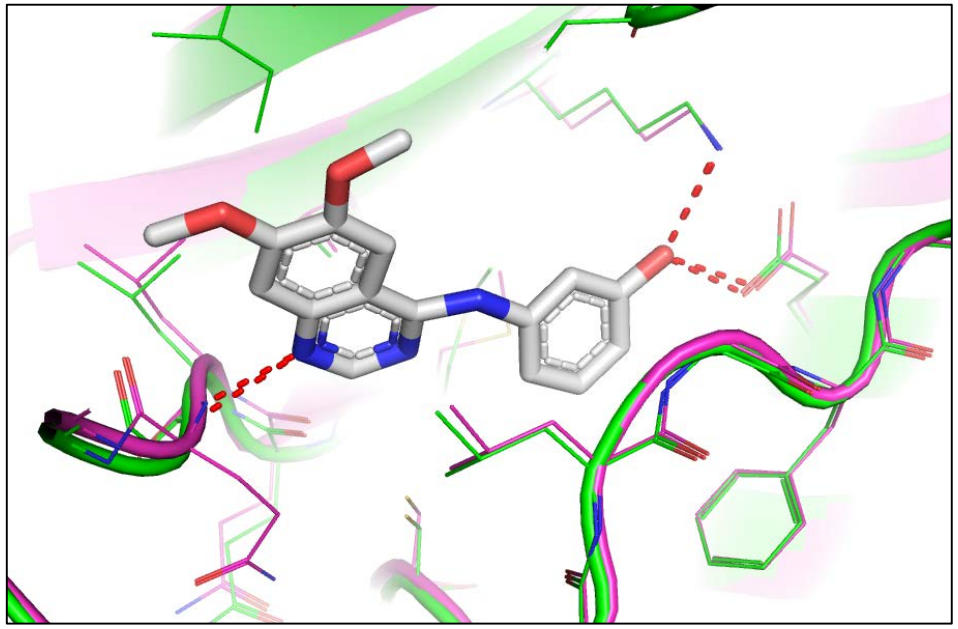
Supplementary Figure S1: ESI mass spectrometry (ESI-MS)characterization of **(A)** TTBK1 KD and **(B)** TTBK2 KD.



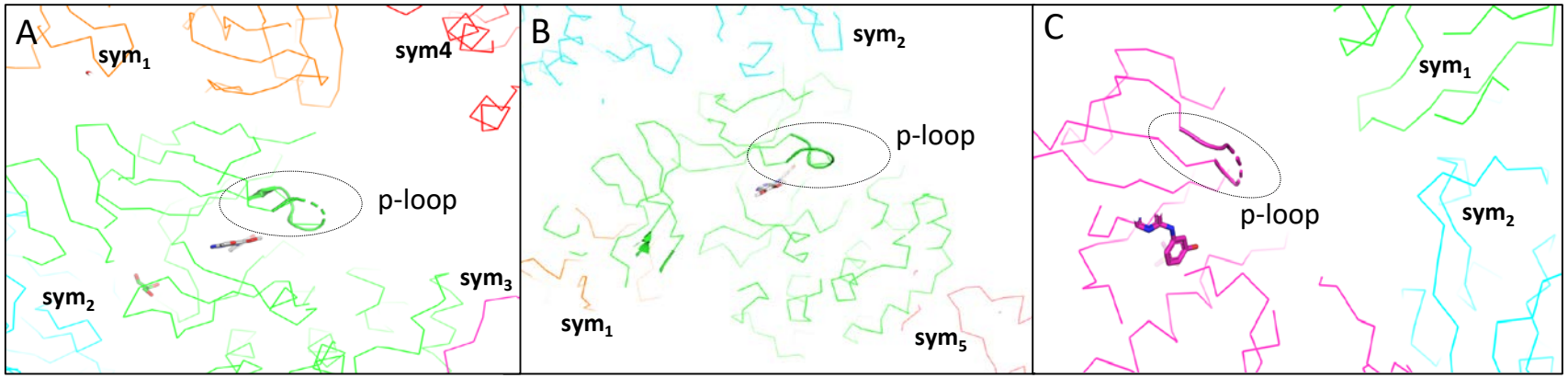
Supplementary Figure S2: TTBK1 KD (Cyan) and TTBK2 KD (Green) DSF results **(A)** Raw fluorescence data. **(B)** Fluorescence data derivative.



Supplementary Figure S3: Biochemical inhibition profile of compound 1 for TTBK1 **(A)** and TTBK2 **(B).**



Supplementary Figure S4: Binding mode of compound 1 in ATP site of TTBK2 (green) and TTBK1 (magenta).



Supplementary Figure S5: Symmetry mates around TTBK2 Cmp1 (green): A) molecule A and B) molecule B. C) Symmetry mates around molecule A of TTBK1 Cmp1 cocrystal structure (magenta (PDB 4BTK)).

Supplementary Methods

ESI mass spectrometry (ESI-MS) Mass Spectrometry

The TTBK kinase domains were reduced with 20 mM dithiothreitol in Tris-buffered saline, pH 8.0, containing 10 mM EDTA. Both TTBK kinase domains were then analyzed on an LC-MS system comprised of a UPLC (ACQUITY, Waters Corp.), a TUV dual-wavelength UV detector (Waters Corp.), and an LCT Premier mass spectrometer (Waters Corp.). A C4 Symmetry cartridge (2.1-mm x 10 mm, particle size 3.5 µm, Waters Corp.) was used for desalting. Molecular masses were obtained by deconvolution of raw mass spectra using the MaxEnt 1 program embedded in MaxLynx 4.1 software (Waters Corp.).

Differential Scanning Fluorimetry

Thermal denaturation (Tm) for the TTBK kinase domains were determined using a QuantStudio 12K Flex QPCR instrument. The assay was performed in 25mM TRIS pH8.0, 125mM NaCl, 5% glycerol, 5mM $MgCl_2$, 1mM TCEP and 1x Protein Thermal Shift dye (Life Technologies) with a protein concentration of 1µM. The TTBK kinase domains were thermally denatured using a temperature gradient from 20 to 85°C with a 1°C/minute gradient and fluorescence measured every 0.5 seconds.

Determination of TTBK1 and TTBK2 IC50 values for compound 1.

IC50 values for compound 1 were determined for TTBK1 and TTBK2 at Reaction Biology Corporation. The IC50 values of compounds were measured by incubating 1 nM TTBK1 or TTBK2,with 10uM ATP and 20 uM myelin basic protein, in 20 mM Hepes (pH 7.5), 10 mM MgCl2, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na3VO4, 2 mM DTT, 1% DMSO, for 2 hours at ambient temperature. Briefly, two independent 3 fold serial dilutions of each compound were prepared from 10 mM DMSO stocks for an assay 10 point compound titration from 5.1 nM through 100 uM. 1.5% DMSO (in uninhibited controls or assay matrix without enzyme), or 1.5% of 100x compound stocks in DMSO, were added to 1.5 nM of TTBK1 or TTBK2 with 30 uM myelin basic protein in assay buffer. After a 20 minute preincubation, kinase activity was initiated with 1/3 volume of 30 uM ATP (containing ~ 50 uCi [g33P]ATP/umole of ATP) in assay buffer. After 2 hours at ambient temperature, the activity was quenched with 1.5 volumes of 1% phosphoric acid. The quenched mixture was added to wells of a phosphocellulose filter plate. The filter wells were washed with 0.1% phosphoric acid, and dried before adding Liquid Scintillation Cocktail. The incorporated 33P was measured in a Microbeta Counter. The IC50 values was obtained from a 4 parameter fit of the net incorporated 33P cpm versus log [compound] using the Graphpad Prism Program.