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Supporting information for article:

A novel inhibitor stabilizes the inactive conformation of MAPK-interacting kinase 1

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Kinase enzyme assay

1. Protein preparation

Human Mnk1 (full length) was expressed with an N-terminal histidine tag in *E.coli* BL21(DE3) strain. The cells were grown in LB media at 37 °C up to OD600nm 1.0, and then induced with 1 mM isopropyl-β-D- thiogalactopyranoside (IPTG). After 4 hours of incubation at 25 °C, the cells were harvested. After sonication and centrifugation, the supernatant was loaded on a HisTrap HP column (GE Healthcare), and the protein was eluted with a gradient of 20–400 mM imidazole. Subsequent gel filtration was performed on a Superdex 200 10/300 GL column (GE Healthcare) using buffer consisting of 50 mM Tris-HCl (pH 7.5), 200 mM NaCl, and 1 mM DTT. Peptide mapping analysis by mass spectrometry confirmed that two Thr residues (residues 209 and 214) of Mnk1 targeted by Erk2 or p38 kinase were not phosphorylated. The GST-tagged phosphorylated Mnk1 (full length) and the GST-tagged phosphorylated Erk2 were purchased from Carna Biosciences.

2. Kinase enzyme assay

Unphosphorylated Mnk1 was pre-incubated with the compounds in an assay buffer (50 mM HEPES pH 7.5, 10 mM $MgCl_2$, 0.01% Brij-58, and 2.5 mM DTT) for 30 min. Then Erk2 was added to the mixture to activate Mnk, in addition with a substrate peptide and ATP, which was then incubated for 120 min. Next, anti-phosphorylated-eIF4E antibody and EDTA were mixed and the reaction was stopped. After the incubation with AlphaScreen beads (PerkinElmer) for 60 min, the plate was read at 570 nm using an EnVision multilabel plate reader (PerkinElmer).

The assay using phosphorylated Mnk1 was performed in a similar manner as described above, except that Erk2 was not included in the assay system.