Supplimental Material

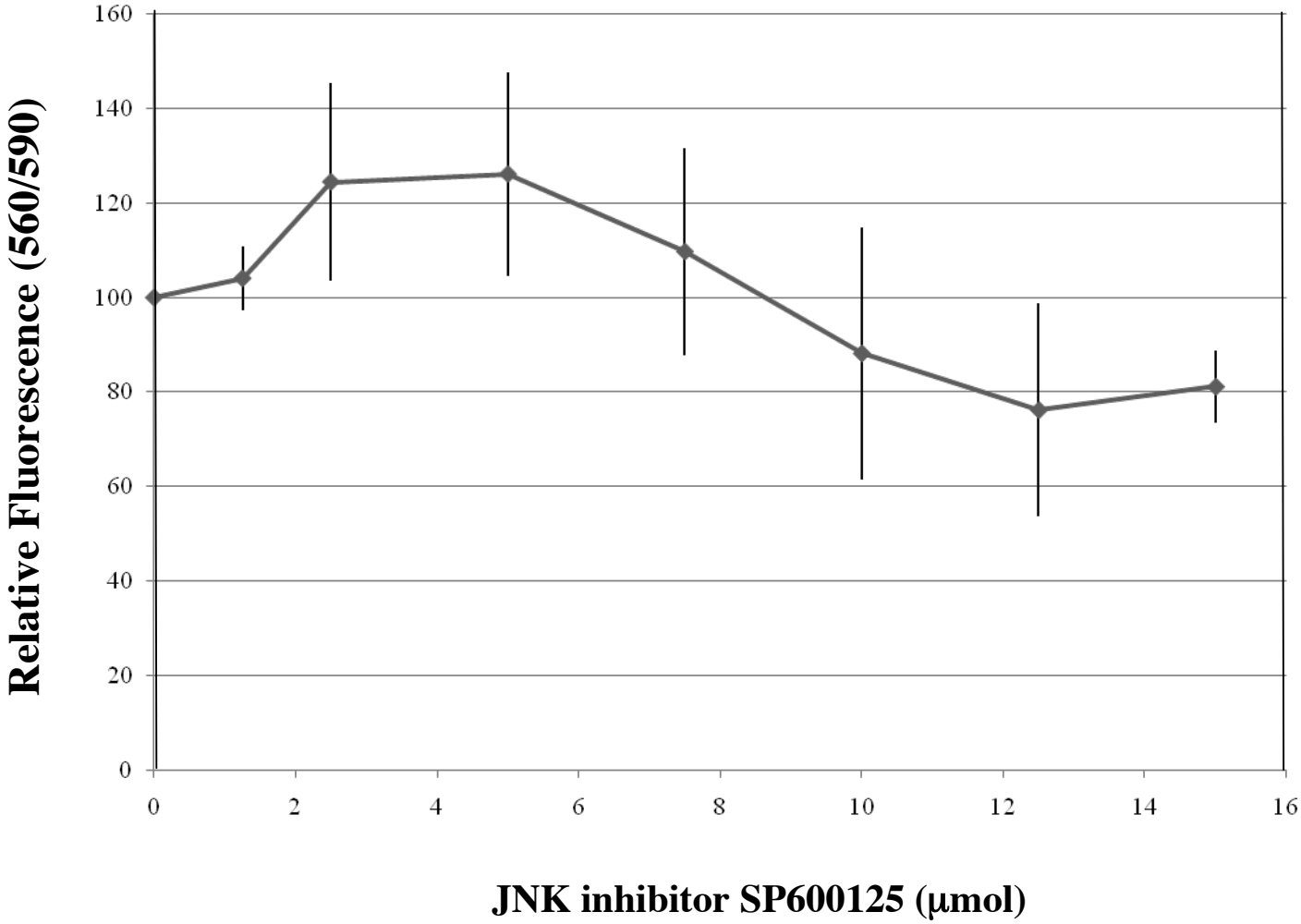
CellTiter-Blue Cell Viability Assay: PC12 cells were seeded in a 96 well plate. Next day, they were treated with dimethy sulphoxide (DMSO) or with various concentrations of the specific JNK inhibitor SP600125 (Invitrogen) for 2 hours before NGF treatment. Three days after the treatment, CellTiter-Blue Reagent (Promega)n containing resazurin was added to the cells to determine cell viability. Resazurin is known to be metabolized by viable cells to the fluorescent product, resorufin which can be measured. After three hours of incubation at 37 ºC, fluorescence with excitation 560nm and emission 590nm was measured using GloMax fluorometer (Promega).

Results in Supplemental Fig. 1 show that PC12 cells treated with various concentrations of the JNK inhibitor SP600125 showed high viability over a wide range of the inhibitor concentrations compared with the control cells. At the inhibitor concentration (10 μM) used in Fig. 5a, the cells showed of 88% viability. This suggests that lack of neurite outgrowth in the JNK inhibitor-treated cells is not due to the toxic side effects of the JNK inhibitor..

Supplemental Figure 1 Legend

PC12 cells were treated with DMSO or with various concentrations of the JNK inhibitor SP600125 two hours before being incubated with NGF. Three days after treatment, CellTiter Blue Reagent (Promega) was added. Three hours after incubation, fluorescence with excitation 560nm and emission 590nm [Fluorescence (560/590)] was measured.

Supp. Fig. 1


Relative Fluorescence (560/590)
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
JNK inhibitor SP600125 (μmol)