

Supplemental Figure 1: Over expression of NCS-1 I35A rescues NCS-1 levels and the calcium signal in SHSY-5Y cells. A: Representative western blot showing NCS-1 I35A-HA levels are affected by taxol treatment. Lane 1: NCS-1 I35A-HA, Lane 2: NCS-1 I35A-HA + taxol, Lane 3: NCS-1 I35A-HA + CAST + taxol. B: Western blot analysis of HA levels was used to show that NCS-1 I35A-HA was significantly decreased by taxol treatment and was only partially rescued in those cells also transfected with CAST prior to taxol treatment (n = 3) * $p \leq 0.05$. All treatments were normalized to cells transfected with HA tagged NCS-1 I35A. C: The intracellular calcium signal was reduced after a 6 hr treatment of Taxol. The over expression of NCS-1 WT prior to taxol treatment was unable to fully rescue the calcium signal to that of control cells. However, those cells expressing NCS-1 I35A prior to treatment with taxol had calcium signals similar to that of control cells (15-20 cells, n = 3). (A = 1uM ATP; T = 10uM thapsigargin) D: Comparison of normalized response durations to 1 μ M ATP in control cells, cells treated with taxol, and cells overexpressing NCS-1 WT or I35H. All treatments are normalized to control cells. All values are mean \pm SEM.

Supplemental Figure 2: WT and mutant versions of NCS-1 bind to InsP₃R. InsP₃R was immunoprecipitated with anti-InsP₃R1(TN1H) from SHSY-5Y lysates and then the samples were probed with anti-NCS-1. Lane 1: SHSY-5Y lysate of control cells, lane 2: SHSY-5Y lysate of cells transfected with NCS-1 WT-HA, lane 3: SHSY-5Y lysate of cells transfected with NCS-1 I35H-HA, lane 4: SHSY-5Y lysate of cells transfected with NCS-1 I35A-HA.

Supplemental Figure 3: Cleavage reactions of NCS-1 WT, I35H and I35A by μ -calpain. Digestion reactions resolved by SDS-PAGE gel were performed using a 1:4 molar ratios of μ -calpain to NCS-1 WT, I35H, and I35A (n = 4). Changes in NCS-1 levels after calpain cleavage were determined using UnSCAN-It. Lane 1: NCS-1 WT, lane 2: NCS-1 I35H, lane 3: NCS-1 I35A, lane 4: μ -calpain, lane 5: NCS-1 WT + μ -calpain, lane 6: NCS-1 I35H + μ -calpain, lane 7: NCS-1 I35A + μ -calpain.