SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell counting-For counting adherent, viable cells, media were aspirated and cultures briefly rinsed with warmed PBS. Adherent cells were briefly trypsinized in Hanks buffered saline solution and triturated in PBS. Equal volumes of cells and 37^{o}C Trypan Blue (0.04% w/v) were combined for 8 min. The number of viable cells was determined by hemacytometer counting of unstained cells. For determination of the percent viability, both the culture media and first rinse fractions were combined with adherent cells and the pool was centrifuged at 500 x g for 5 min at 37^{o}C. The supernatant was decanted and the pellet was re-suspended in PBS, Trypan Blue -stained and counted as above. All counting was performed by an observer blind as to conditions.

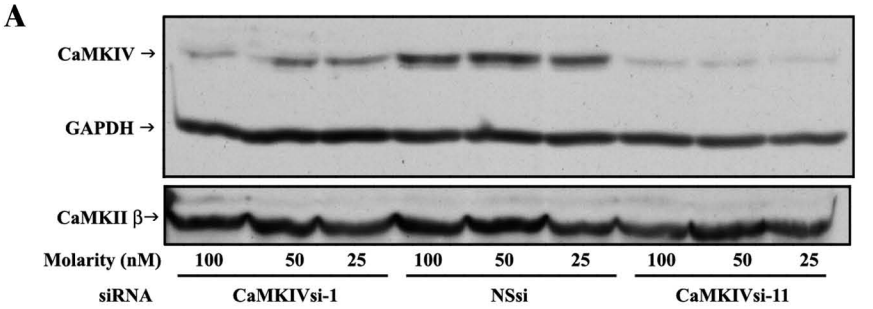
SUPPLEMENTAL FIGURE LEGENDS

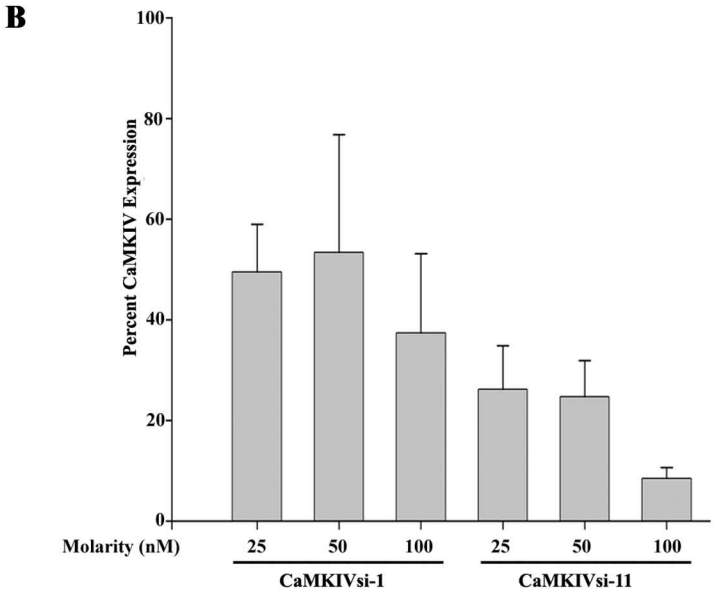
Supplemental Figure 1 **RNAi of CaMKIV. A**, BE(2)C cells were transfected for 48 h with control (NSsi) or CaMKIV exon 1 (CaMKIVsi-1) or 11 (CaMKIVsi-11)- directed siRNAs at various molarities. Lysates were subjected to immunoblotting for CaMKIV, and as a loading control, GAPDH. As an additional specificity control, blots were stripped and probed for a related kinase, CaMKIIβ. **B**, CaMKIV protein expression as detected by immunoblotting was quantified by scanning densitometry and normalized to GAPDH expression. CaMKIV expression in cells transfected with the indicated siRNAs is calculated as a percentage of expression in the concentration-matched NSsi controls. CaMKIV levels were reduced by 25, 50, and 100 nM CaMKIVsi-11 to 26.2 ± 15.06%, 24.7 ± 12.5%, and 8.45 ± 3.8%, respectively. CaMKIVsi-1 at 25, 50, and 100 nM decreased CaMKIV levels to 49.5 ± 16.5%, 53.4 ± 40.6%, and 37.3 ± 27.3% of control, respectively. **C,** CaMKIV mRNA was quantified by qRT-PCR 48 h after transfection with 50 nM CaMKIVsi-11. Expression was normalized to GAPDH, and calculated relative to NSsi control. 50nM CaMKIVsi-11 produced a 76 ± 11.51% reduction in CaMKIV mRNA virtually identical to the percentage reduction (75.3%) in protein at this CaMKIVsi-11 concentration.

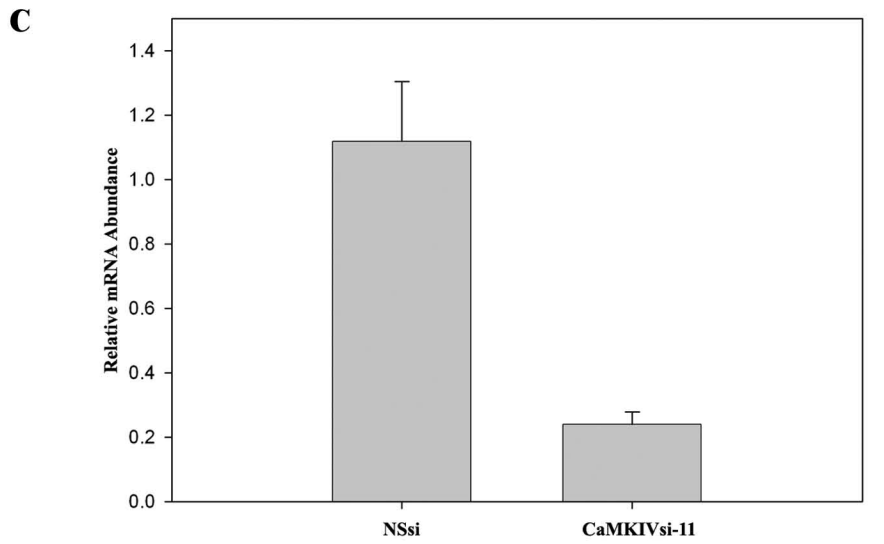
Supplemental Figure 2 **CREB phosphorylation as a function of time and NMDA concentration.** BE(2)C cells were serum-starved for 3 h, and then pre-incubated with HEPES-Tyrode's Buffer with glycine substituted for Mg^{2+} (119 mM NaCl, 2.5 mM $CaCl_2$, 2 mM glycine, 25 mM HEPES, 30 mM glucose) for 15 min. Cultures were then treated with NMDA or DMSO (vehicle) as indicated and analyzed by immunoblotting for CREB phosphorylation and total (pan) CREB expression.

Supplemental Figure 3 **CaMKK inhibition by STO-609 decreases proliferation of BE(2)C neuroblastoma cells.** Equal numbers of BE(2)C cells were plated in replicate plates and cultured for the indicated number of days in the absence (vehicle control, DMSO), or presence, of 10 μM STO-609. Viable (trypan blue-excluding) cells were counted by an observer blind as to conditions.

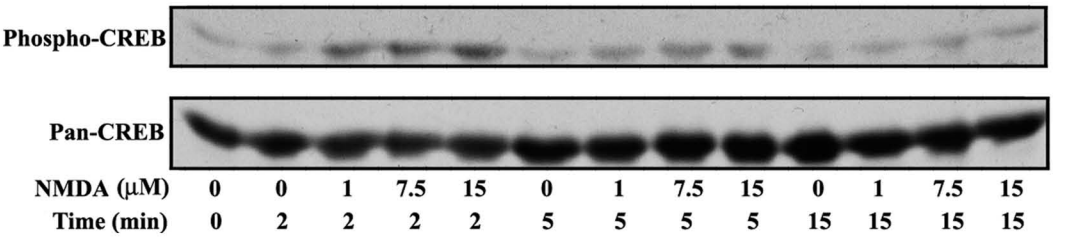
Supplemental Figure 4 **CaMKK inhibition by STO-609 reduces BE(2)C cell viability in a concentration-dependent manner.** The effect of incubating BE(2)C cells with various concentrations (0.01-50 nM) of STO-609 for 144 h on cell viability was assessed by counting the number of both viable (trypan-blue excluding) and non-viable (trypan blue-stained), cells. Percent normalized viable cells is calculated as: [number of viable cells divided by total cells in STO-609 treated cultures] divided by [number of viable cells divided by total cells in vehicle control treated cultures] x 100. Dotted line, IC50 = 10 μM


A
CaMKIV →
GAPDH →
CaMKII β→
Molarity (nM) 100 50 25 100 50 25 100 50 25
siRNA CaMKIVsi-1 NSsi CaMKIVsi-11

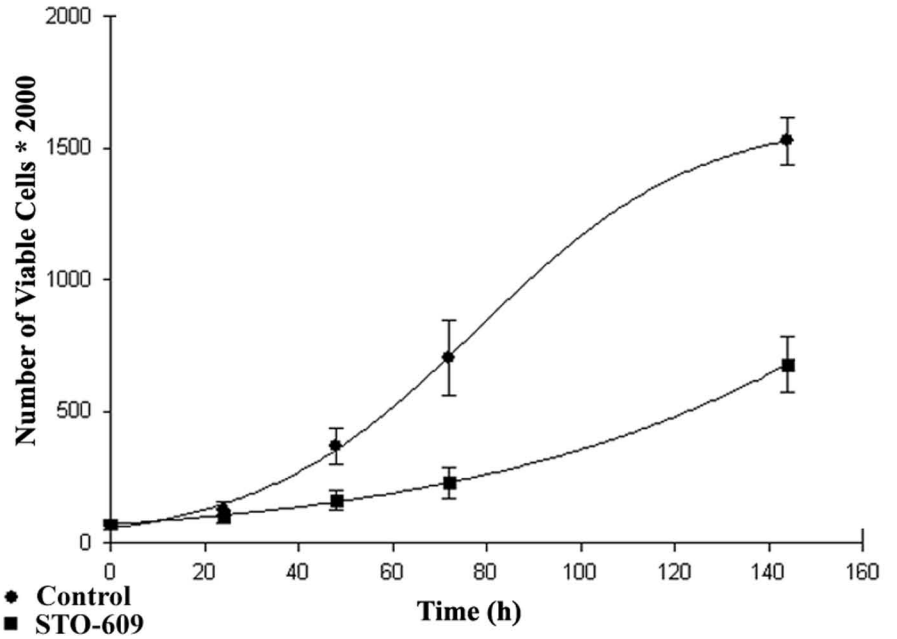

B
Percent CaMKIV Expression
100
80
60
40
20
0
Molarity (nM) 25 50 100 25 50 100
CaMKIVsi-1
CaMKIVsi-11


C
Relative mRNA Abundance
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
NSsi
CaMKIVsi-11

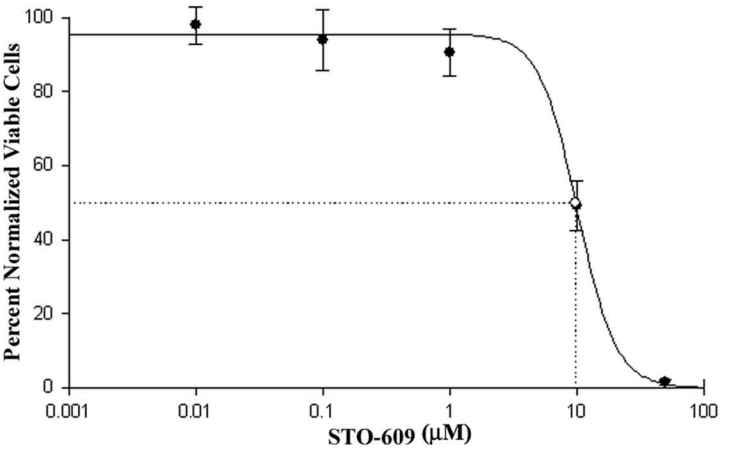
Suppl. Fig. 1


Phospho-CREB
Pan-CREB
NMDA (μM) 0 0 1 7.5 15 0 1 7.5 15 0 1 7.5 15
Time (min) 0 2 2 2 2 5 5 5 5 15 15 15 15

Suppl. Fig. 2


Number of Viable Cells * 2000
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
Time (h)
Control
STO-609

Suppl. Fig. 3


Percent Normalized Viable Cells
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
STO-609 (μM)

Suppl. Fig. 4