Supplemental Figure Legends

Fig. S1. Immunostaining of cultured neurons and astrocytes. *A*, DIV12 neurons were stained with neuron-specific Tuj1 antibody (Millipore, Billerica, MA). ***B*,** DIV12 neurons were stained with glia-specific GFAP antibody (Millipore, Billerica, MA). Most cells were neurons. ***C*,** Cultured astrocytes were stained with GFAP antibody, which labeled most cells.

Fig. S2. Information of primers. *A*, Primers used for RT-qPCR. Shown were sequences of forward and reverse primers, length of anticipated PCR products, and annealing temperatures. From "RT-I" to "BDNF" are primers for rat. From "RT-H-I" to "RT-H-VI" are primers for human. ***B*,** Primers used for luciferase reporters.

Fig. S3. Negative and positive controls of dual FISH. *A*, Negative control. Dual FISH experiment was done with probes against E. coli DapB, a protein not expressed in mammalian cells. No signal was detected. ***B*,** Positive control. Dual FISH experiment was done with probes against UBC (ubiquitin C), a protein expressed in mammalian cell. Strong signals were detected.

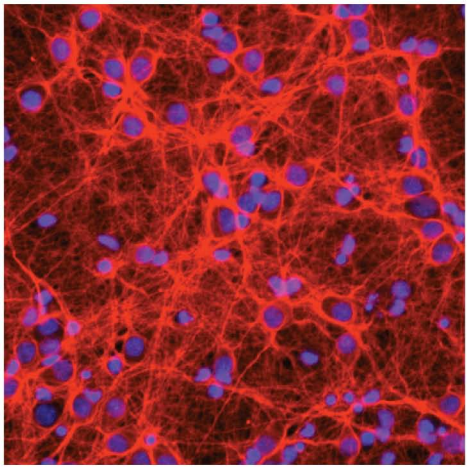
Fig. S4. KA stimulation of NRG1 type expression in cultured neurons. Cortical neurons (DIV9) were treated for 6 hr without or with 100 μM KA. RT-qPCR was conducted as in Fig. 6. **$p<0.01$.

Fig. S5. Neuronal activity regulation of type I NRG1 expression. *A*, Identification of minimal promoter of type I NRG1. Luciferase constructs containing different length of type I promoter (from 96 bp to 7 kb) were used in dural luciferase assay. Luciferase activity was measured 24 hr after transfection. Values were normalized to internal control, *Renilla* activity and corrected by the size of luciferase constructs. Data were presented as folds above the values of the 7-kb promoter construct. ***B*,** Diagram of luciferase constructs. NFAT-1 or Egr-1 was deleted in the 2.9-kb 5'UTR. ***C*,** Luciferase activities of indicated luciferase constructs in SH-SY5Y cells. Experiments were performed as in Fig. 6. Values were normalized to corresponding controls. ED, Egr-1 deletion; ND, NFAT-1 deletion. *$p<0.05$; **$p<0.01$; NS, no significant difference.

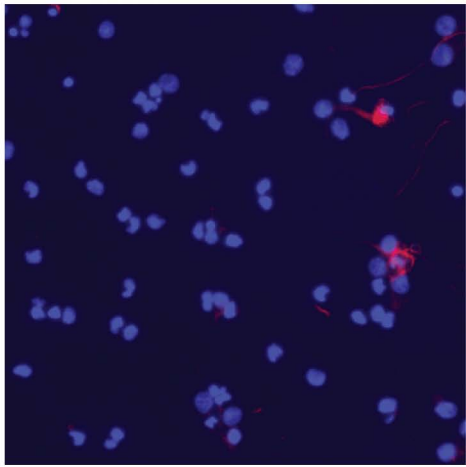
Fig. S6. NRG1 isoform expression patterns of cultured neurons at different ages. DIV-dependent expression of NRG1 isoforms in cultured neurons.

Fig. S1.

A



B



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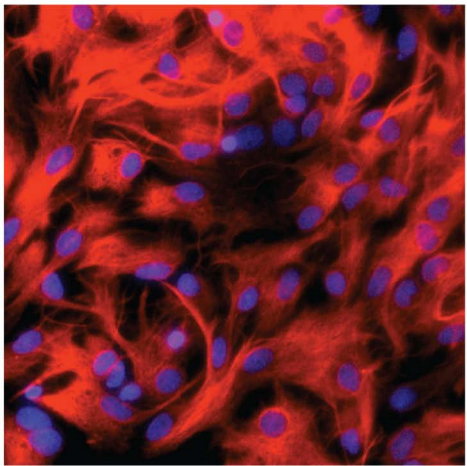


Fig. S2.

A

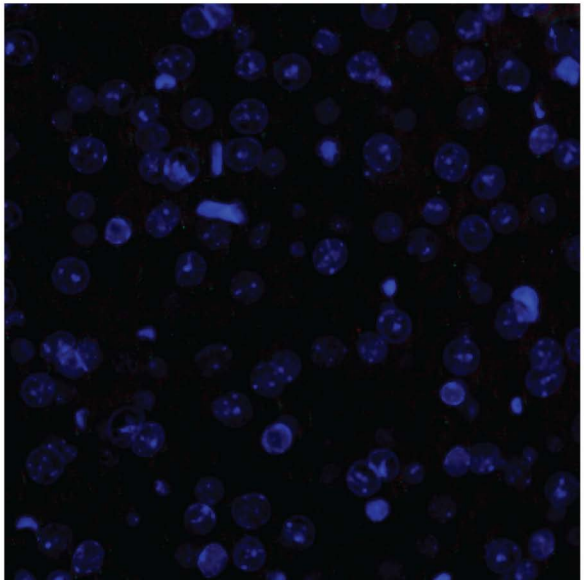
Name	Forward-primer	Reverse-primer	Length (bp)	Annealing temp (°C)
RT-I	TCATCTTCGGCGAGATGTCTG	CTCCTGGCTCTTCATTTCTTTCA	152	60
RT-II	GAGACTGGCCGCAACCTCA	TGACTCCTGGCTCTTCATTTCTTT	97	60
RT-III	GGACCCCTGAGGTGAGAACA	CAGTCGTGGATGTCGATGTGG	102	60
RT-IV	GGATCAGCACGGGGAAGG	CACCGGAGCACTAGCTTGGA	121	70
RT-V	GCAAACCTCCTACGGAGTTTTAA	TCCTGGCTCTTCATTTCTTTCA	127	60
RT-VI	ACAGGGTATGGAAGAGCAAGAAA	GGTCCCAGTCGTGGATGTCG	88	60
RT-EGF	ACCAGCCATCTCATAAAGTGCG	TTGACGGGTTTGACAGGTCC	94	60
RT-b-1	CAGATGTGGATCAGCAAGCAGG	TTGTCAAAGAAAGGGTGTAAAACG	112	60
RT-b-2	GCGTCCACCCGCGAGTACAACC	TGCACATGCCGGAGCCGTTGT	121	70
BDNF	TGTGGTTTGTTGCCGTTGC	TTTGTCTGTTTTCTGAAAGAGGGA	117	60
RT-H-I	AGGGAAGGGCAAGAAGAAGGAGC	TTCAATCGGGGAGGCAAGG	90	60
RT-H-II	GGCTCAAGGAGGACAGCAGGTAC	TTTCAATCGGGGAGGCAAGG	173	60
RT-H-III	CGCCATCCTTCCCTTCACCC	GTTTTCTCCTTCTCCGCACATTTTAC	166	60
RT-H-IV	GCATGGGGAAAGGACGCG	CAATTCATTCCCATTCTTGAACCACT	170	60
RT-H-V	AATTCTTCTACGGAGTTTTAACCTACAC	GCCGATTCCTGGCTTTTCAT	131	60
RT-H-VI	CTGGACTTCAAAGAGCAGGAAAGTATG	GATGGCTTGTCCCAGTGGTGG	97	60

B

Name	Froward-primer	Reverse-primer
IVwt-Luc	CACACGCGTGCAGAGCCATCAATGAGGTC	CACGCTAGCGCTGCTGCCGGAGCG
IVdmt-Luc	GCATGGAGTAGAGGAGGCATCTGAAGTGCACACTG	CAGTGTGCACTTCAGATGCCTCCTCTACTCCATGC
IVpmt-Luc	CCTTGCTCACTGAAGCCTCTCACTCCCCAG	CTGGGGAGTGAGAGGCTTCAGTGAGCAAGG
7k	CACGAGCTCAGCCCTGTTATCATGATGACATTATGAAG	CACACGCGTCTCAGTCCCCTGGCAATGCA
4.8k	CACACGCGTTGCATTGCCAGGGGACTGAG	CACGCTAGCCTCGCCGGAGACGGAGCGCT
2.9k	CACACGCGTTAATGTGAGATCTAAATTTT	CACGCTAGCCTCGCCGGAGACGGAGCGCT
861	CACACGCGTCTACCCCTCCCTGCCTGCTT	CACGCTAGCCTCGCCGGAGACGGAGCGCT
441	CACACGCGTCCCCGAACTCTTCTGG	CACGCTAGCCTCGCCGGAGACGGAGCGCT
341	GGTACCGAGCTCTTACGCGTAACTTTTCTCTGCGCCG	CGGCGCAGAGAAAAGTTACGCGTAAGAGCTCGGTACC
241	GGTACCGAGCTCTTACGCGTTCTCCGCCTGGGTTC	GAACCCAGGCGGAGAACGCGTAAGAGCTCGGTACC
96	GGTACCGAGCTCTTACGCGTCTCGGGGTGGGGG	CCCCCACCCCGAGACGCGTAAGAGCTCGGTACC
Egr-1-D	GTCCGCGCCTCGGGTGGTGGGGAAGAGGGAGGG	CCCTCCCTCTTCCCCACCACCCGAGGCGCGGAC
NFAT-1-D	CCCGGCCAGCGCGGGGGGCTGCGCCCG	CGGGCGCAGCCCCCCGCGCTGGCCGGG

Fig. S3.

A



B

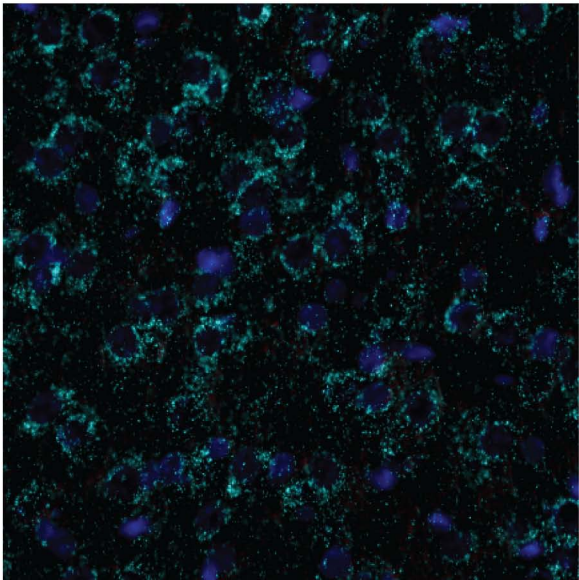


Fig. S4.

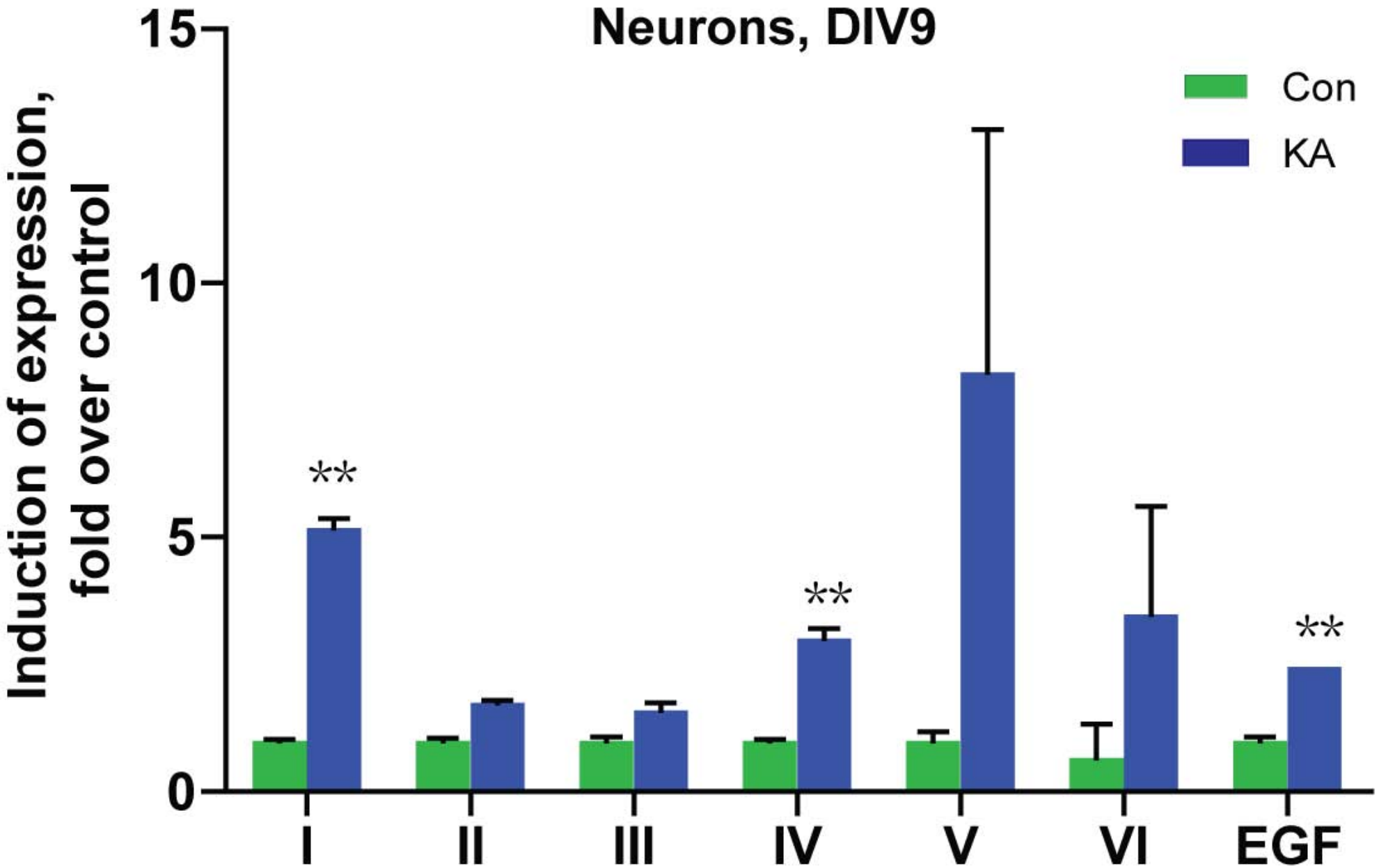
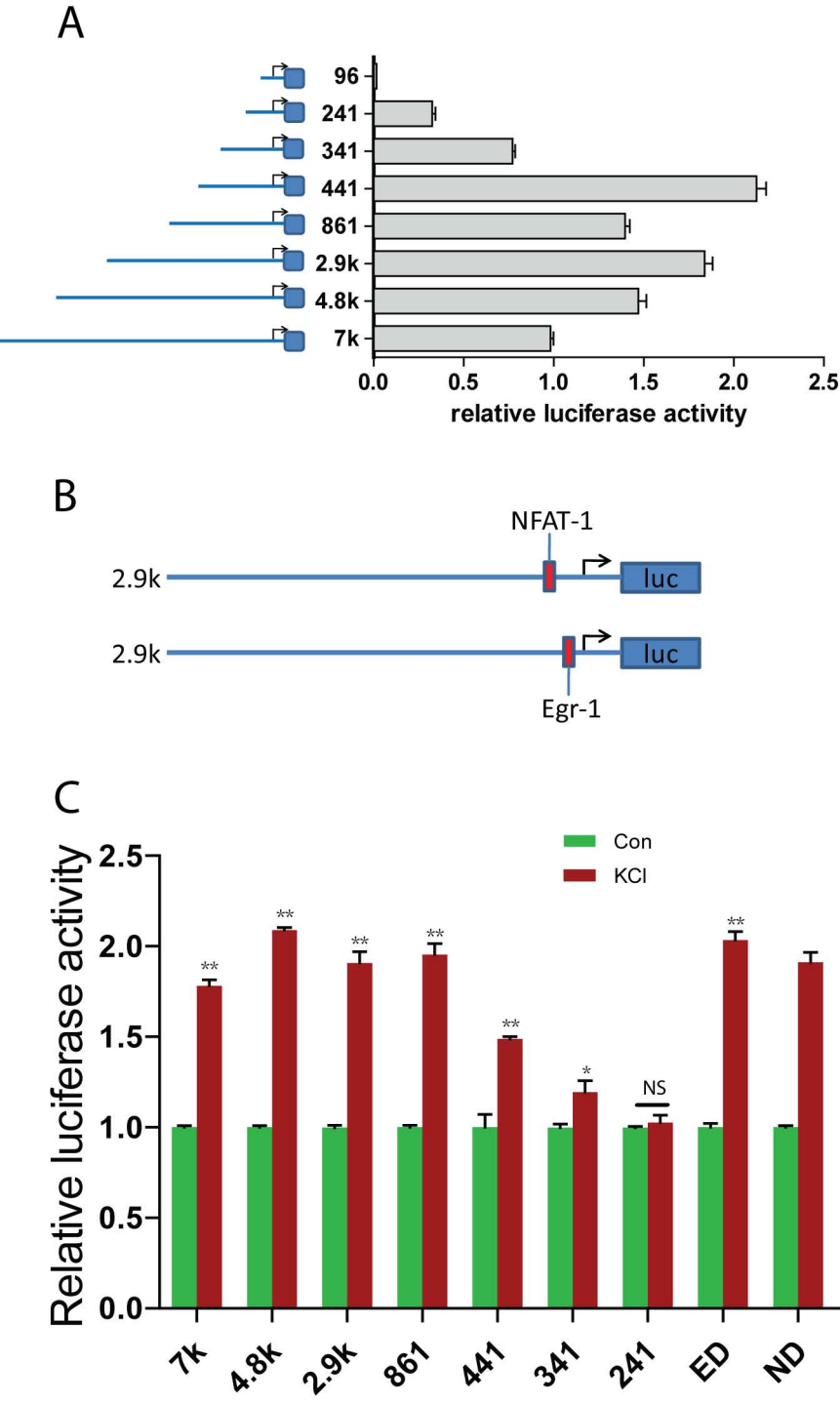

Neurons, DIV9
Con
KA
Induction of expression, fold over control
15
10
5
0
**
**
**
I
II
III
IV
V
VI
EGF

Fig. S5.


A
96
241
341
441
861
2.9k
4.8k
7k
0.0
0.5
1.0
1.5
2.0
2.5
relative luciferase activity
B
NFAT-1
2.9k
luc
2.9k
luc
Egr-1
C
Con
KCl
Relative luciferase activity
2.5
2.0
1.5
1.0
0.5
0.0
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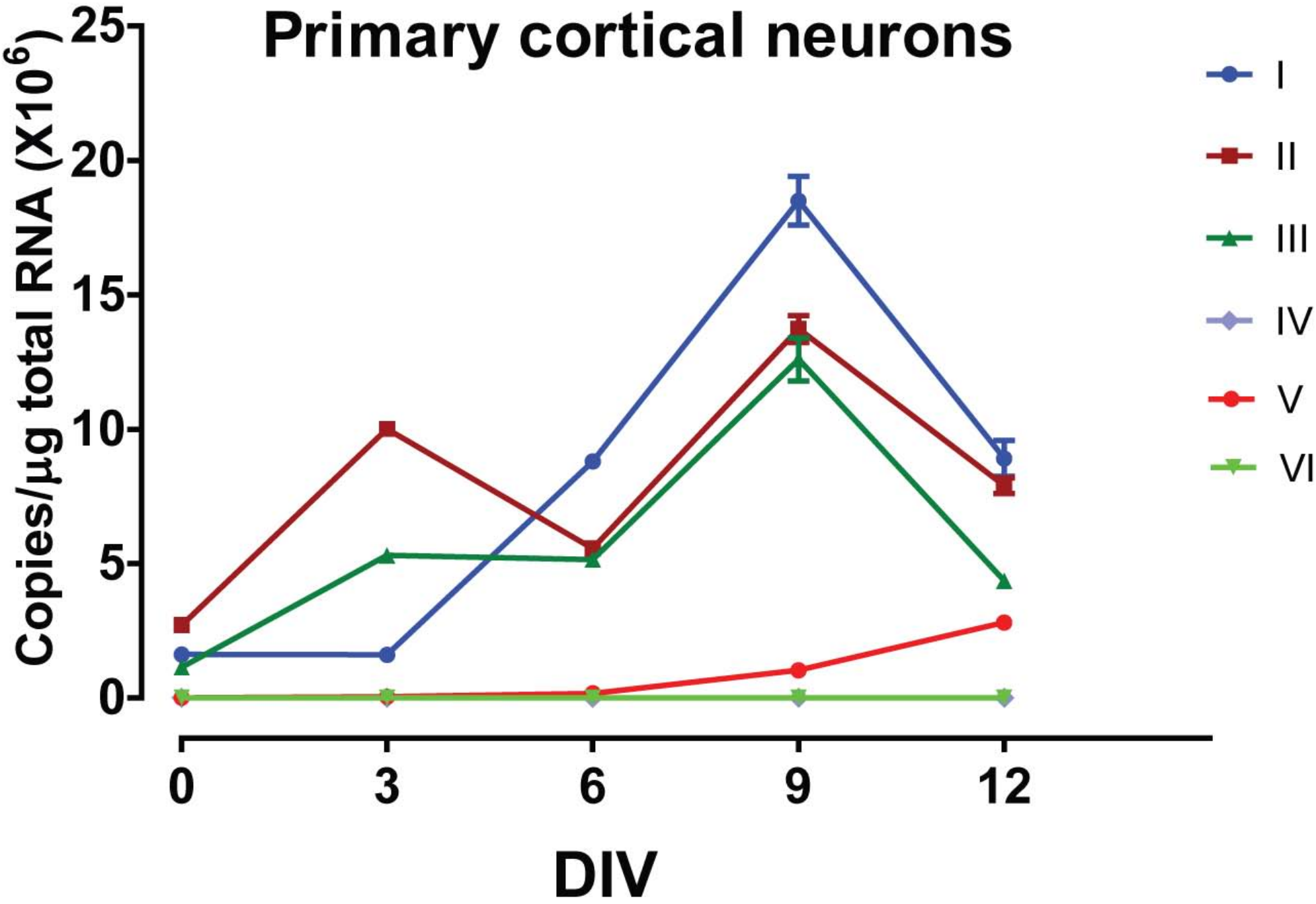
**
*
NS
**
7k
4.8k
2.9k
861
441
341
241
ED
ND

Fig. S6.


Primary cortical neurons
Copies/µg total RNA (X10^6)
25
20
15
10
5
0
0
3
6
9
12
DIV
I
II
III
IV
V
VI