# Expanded View Figures

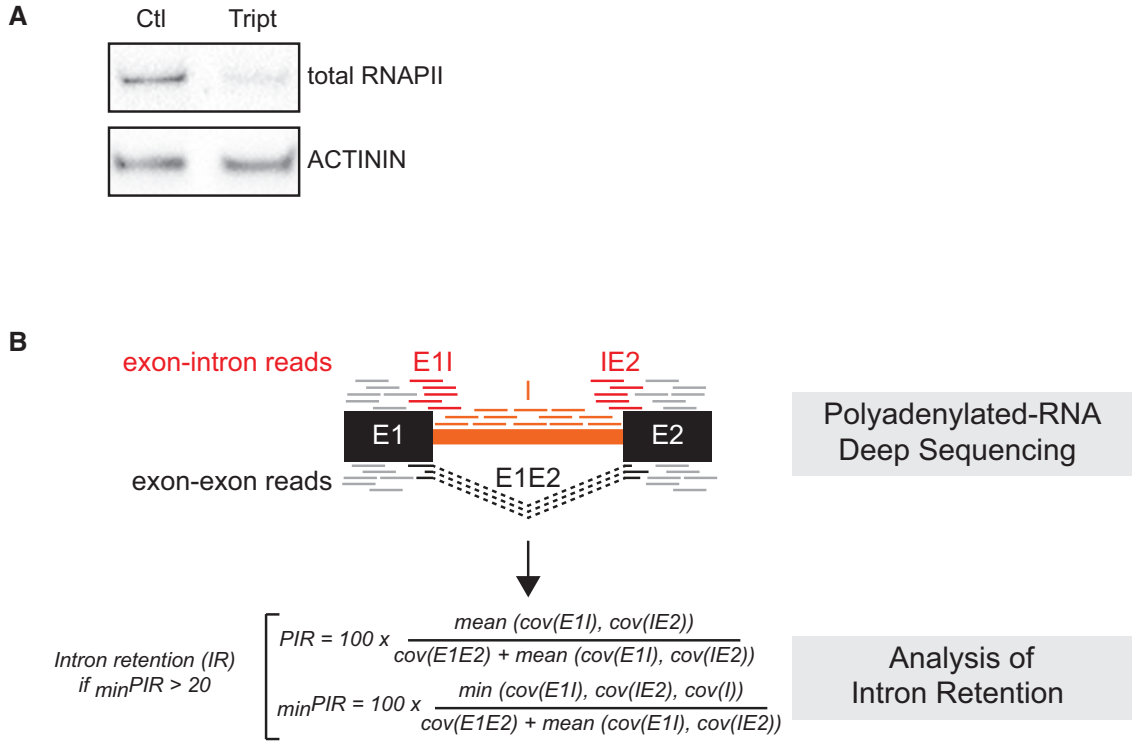
**Figure EV1. related to Fig 1.**

A Activity of the RNA polymerase II inhibitor triptolide (tript) treatment was probed by assessing global expression of RNA polymerase II. Triptolide results in transcription block as it induces a fast proteaosome-dependent degradation of the RNA-polymerase II. Mouse primary neocortical cultures were treated for 2 h with triptolide (1 μM). ACTININ was used as a loading control (four independent cultures).

B Polyadenylated RNAs were isolated and sequenced. For each segment comprised of two consecutive exons and the intervening intron, coverages (cov) of reads spanning the 5′ and 3′ exon-intron junctions (E1I and IE2, respectively), the exon-exon junction (E1E2) and mapping the intron sequence (I) were calculated. Then, the percentage of intron retention (PIR) and the minimal PIR (minPIR) was evaluated as described in the formulas. Introns were considered as retained if PIR exceeded 20 and if there was a minimum (20%) sequence coverage across the entire intron.

C Bar graph displaying proportion of genes containing or not IRs.

D Bar graph displaying—among genes that contain at least one IR—the proportion of genes containing 1, 2 or more IRs.



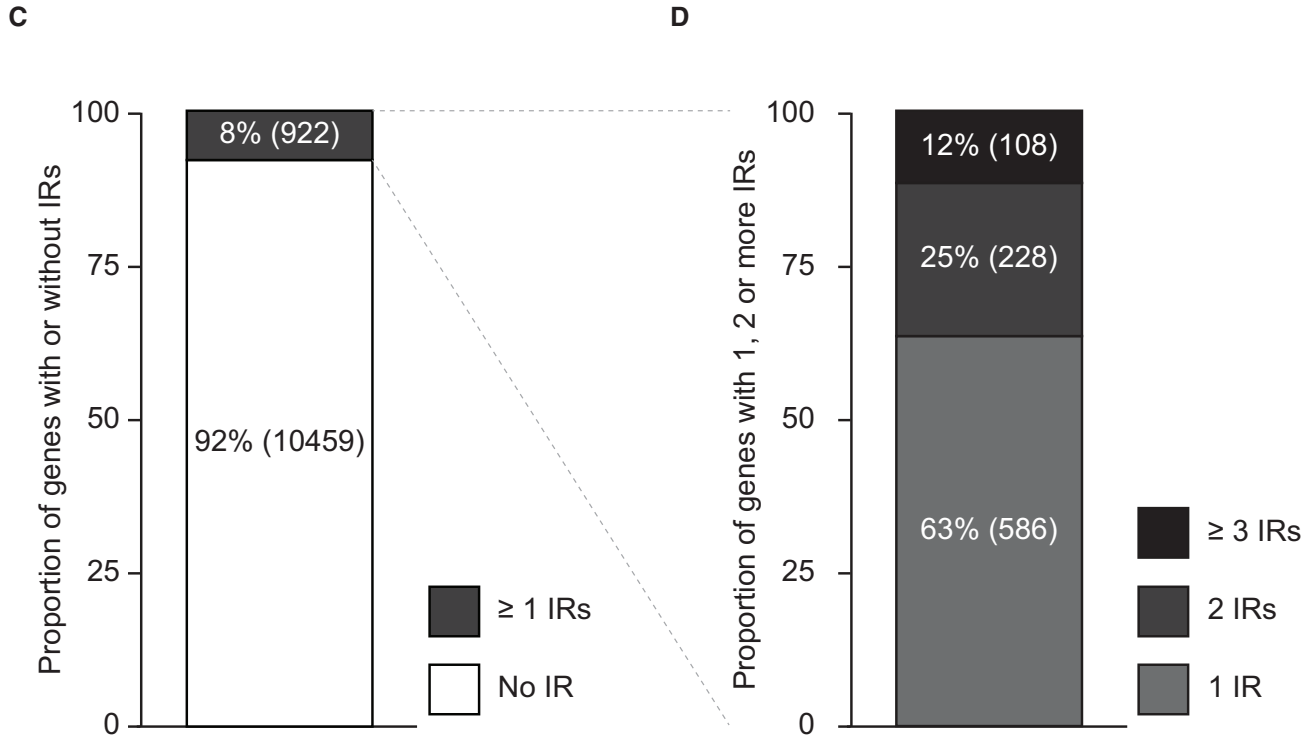




**Figure EV1.**

**Figure EV2. related to Fig 2.**

A Activity of RNA polymerase II inhibitor DRB treatment was probed by assessing C- terminal domain (CTD) phosphorylation and global expression of RNA polymerase II. DRB inhibits the CDK9 kinase and consequently prevents phosphorylation of the RNA polymerase II CTD (C-terminal domain). ACTININ was used as a loading control.

B Efficiencies of bicuculline (A) and BDNF (B) stimulation were assessed by controlling the induction of ERK, AKT and CREB phosphorylation by western blot assays. ACTININ and COFILIN were used as a loading control. Mouse primary neocortical cells (14 days in culture) were stimulated with bicuculline (20 μM) or BDNF (50 ng/ml) for 5 to 60 min.

C Cumulative frequency plot displaying percentage of phospho-ERK-positive (pERK) neurons control (black), bicuculline-stimulated (blue) and BDNF-stimulated (orange) neocortical cultures.

D Violin plots displaying quantification of phospho-ERK signal in control (gray), bicuculline-stimulated (blue) and BDNF-stimulated mouse primary neocortical cells (orange). For the inner boxplot, the central line represents the median; the upper and lower bounds of the box represent, respectively, the $75^{th}$ (Q3) and $25^{th}$ (Q1) percentiles; the upper whisker corresponds to the highest observed value within [Q3, 1.5*Q3] and the lower whisker corresponds to the lowest observed value within [0.5*Q1, Q1]. The *P*-values calculated with a two-tailed Mann–Whitney test are indicated (four independent cultures; $N = 200$ neurons (control), 201 neurons (bicuculline), 165 neurons (BDNF)).

E Boxplots displaying the fold change (FC) of read coverage along the whole retained intron of regulated transcripts. The central line represents the median; the upper and lower bounds of the box represent, respectively, the $75^{th}$ (Q3) and $25^{th}$ (Q1) percentiles; the upper whisker corresponds to the highest observed value within [Q3, 1.5*Q3] and the lower whisker corresponds to the lowest observed value within [0.5*Q1, Q1]. For bicuculline-IR down (light blue): $N = 382$; for bicuculline-IR up (dark blue): $N = 48$; for BDNF-IR down (orange): $N = 243$; for BDNF-IR up (red): $N = 142$ (three independent cultures).

F Density plot (based on kernel density estimates) displaying the relative localization of IR along their host transcripts. Data are plotted for all IRs (gray) or for regulated IRs (purple, either by bicuculline or BDNF).

G Violin plots displaying the IR-transcript expression associated with stable IRs (gray, $N = 2{,}210$), bicuculline-regulated IRs (blue, $N = 430$) and BDNF-regulated IRs (orange, $N = 385$). For the inner boxplot, the central line represents the median; the upper and lower bounds of the box represent, respectively, the $75^{th}$ (Q3) and $25^{th}$ (Q1) percentiles; the upper whisker corresponds to the highest observed value within [Q3, 1.5*Q3] and the lower whisker corresponds to the lowest observed value within [0.5*Q1, Q1]. The *P*-values calculated with a two-tailed Mann–Whitney test are indicated on the top of the panel.

H Violin plots displaying the nucleus-to-cytosol expression ratio of IR-transcripts. All stable IR-transcripts (gray, $N = 2{,}210$), bicuculline-regulated (blue, $N = 430$) and BDNF-regulated (orange, $N = 385$) transcripts are displayed. Percentages of nuclear-enriched ($N/C > 2$) and cytosolic-enriched ($N/C < 0.5$) transcripts are indicated on the panel. For the inner boxplot, the central line represents the median; the upper and lower bounds of the box represent, respectively, the $75^{th}$ (Q3) and $25^{th}$ (Q1) percentiles; the upper whisker corresponds to the highest observed value within [Q3, 1.5*Q3] and the lower whisker corresponds to the lowest observed value within [0.5*Q1, Q1] (three independent cultures).

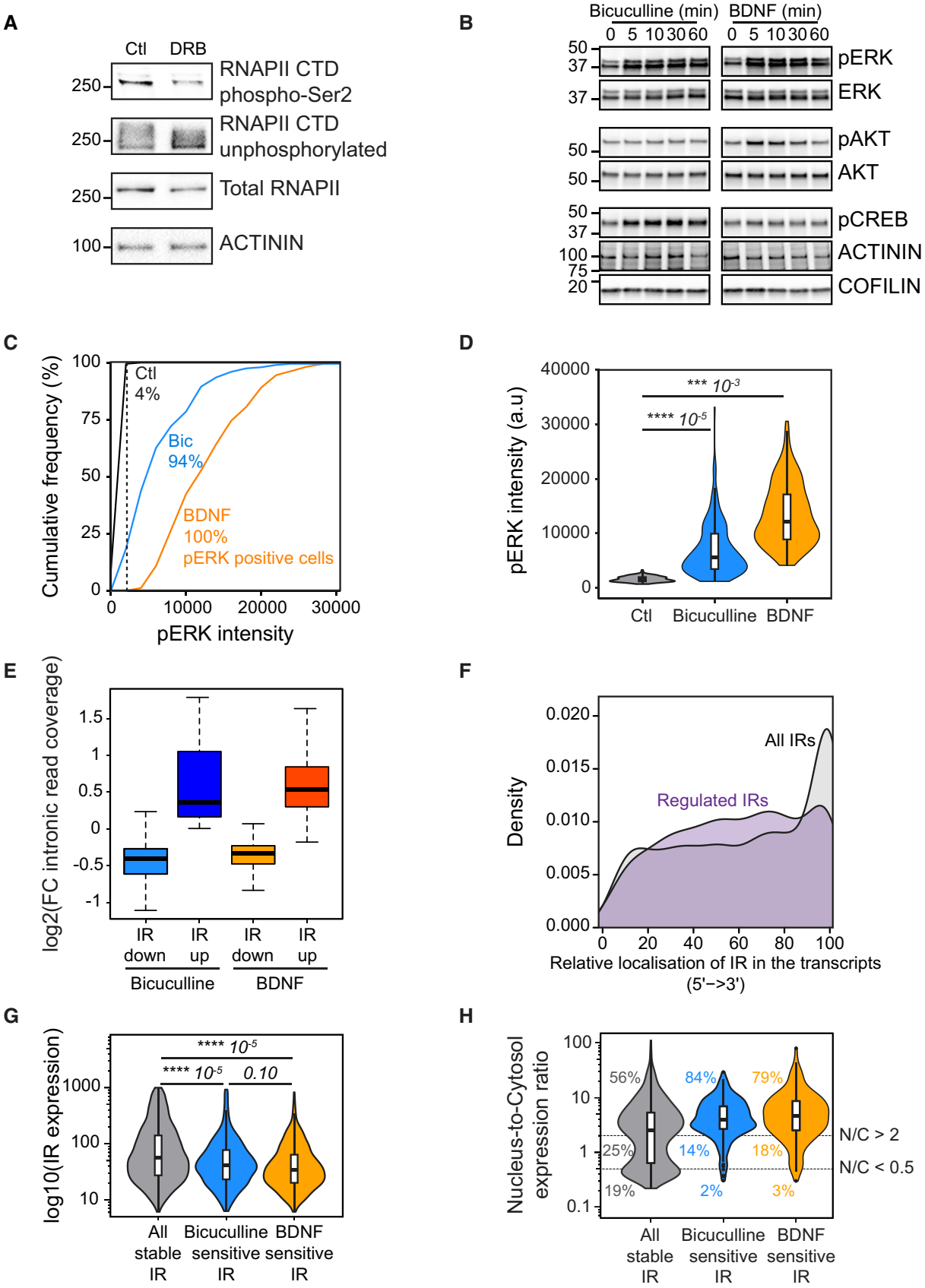

A
Ctl
DRB
250
RNAPII CTD phospho-Ser2
250
RNAPII CTD unphosphorylated
250
Total RNAPII
100
ACTININ
B
Bicuculline (min)
BDNF (min)
0 5 10 30 60
0 5 10 30 60
50
37
pERK
37
ERK
50
pAKT
50
AKT
50
37
pCREB
100
75
ACTININ
20
COFILIN
C
Cumulative frequency (%)
100
75
50
25
0
Ctl
4%
Bic
94%
BDNF
100%
pERK positive cells
0
10000
20000
30000
pERK intensity
D
pERK intensity (a.u)
40000
30000
20000
10000
0
*** 10-3
**** 10-5
Ctl
Bicuculline
BDNF
E
log2(FC intronic read coverage)
1.5
1
0.5
0
-0.5
-1
IR down
IR up
IR down
IR up
Bicuculline
BDNF
F
Density
0.020
0.015
0.010
0.005
0.000
All IRs
Regulated IRs
0
20
40
60
80
100
Relative localisation of IR in the transcripts (5'->3')
G
log10(IR expression)
1000
100
10
**** 10-5
**** 10-5
0.10
All stable IR
Bicuculline sensitive IR
BDNF sensitive IR
H
Nucleus-to-Cytosol expression ratio
100
10
1
0.1
56%
84%
79%
25%
14%
18%
19%
2%
3%
N/C > 2
N/C < 0.5
All stable IR
Bicuculline sensitive IR
BDNF sensitive IR

**Figure EV2.**

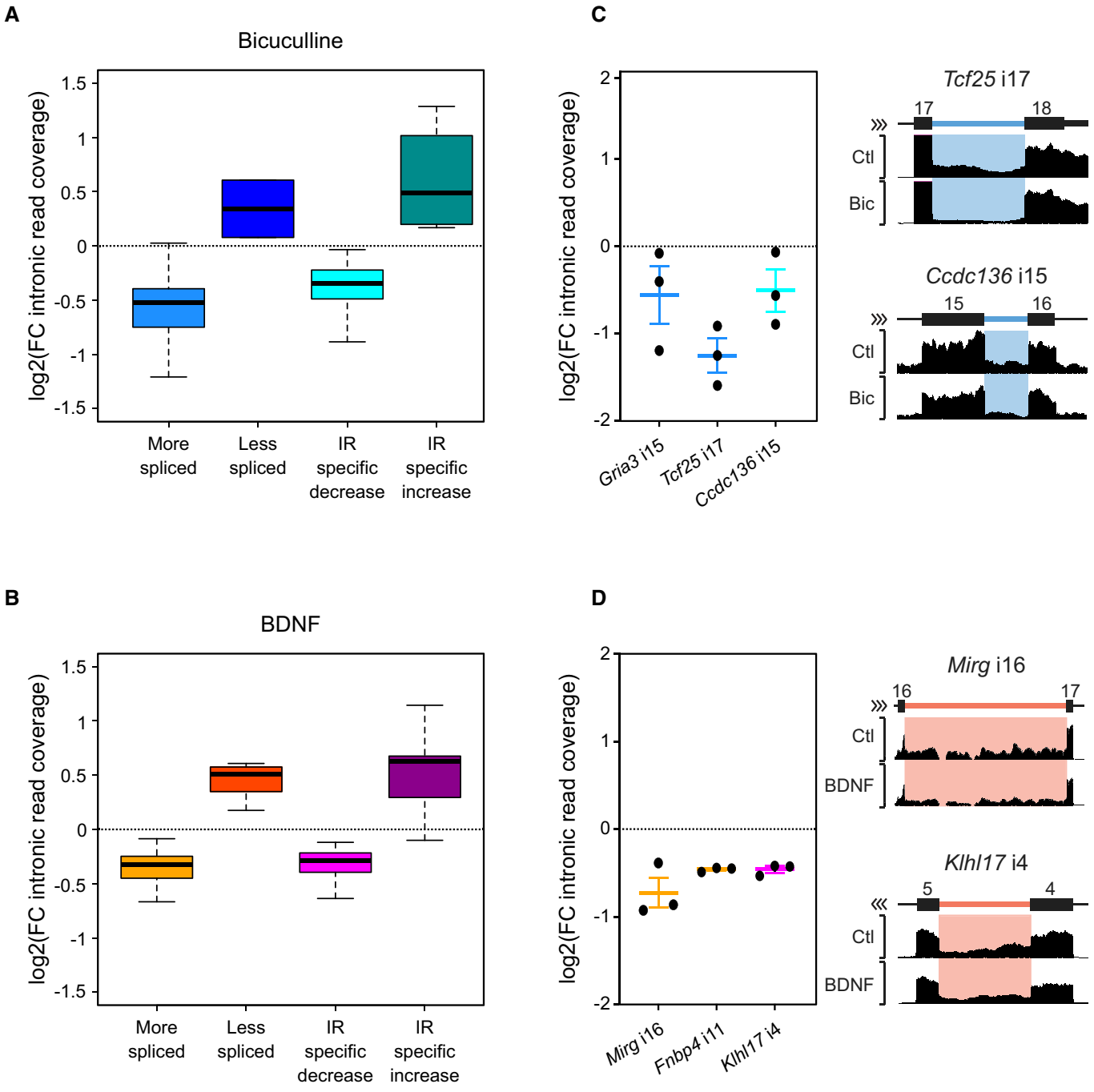



**Figure EV3. related to Fig 3.**

A, B Boxplots displaying the fold change (FC) of read coverage along the whole retained intron in the different categories of regulated IR-transcripts upon stimulation with bicuculline (A) or BDNF (B). The central line represents the median; the upper and lower bounds of the box represent, respectively, the 75$^{th}$ (Q3) and 25$^{th}$ (Q1) percentiles; the upper whisker corresponds to the highest observed value within [Q3, 1.5*Q3] and the lower whisker corresponds to the lowest observed value within [0.5*Q1, Q1].

C, D Left: Means and SEMs of the fold change (FC) of read coverage along the whole retained intron of transcript candidates regulated upon stimulation with bicuculline (C) or BDNF (D). Displayed transcripts correspond to those assessed by PCRs in the main Fig 3 (three independent cultures). Right: genome browser screenshots showing the read coverage on regulated intron retentions in response to bicuculline (C) or BDNF (D) stimulation.

**Figure EV4. related to Fig 4.**

A Violin plots displaying the nucleus-to-cytosol expression ratio of IR-transcripts unregulated (brown) or regulated through splicing (blue). Percentages of nuclear-enriched (N/C > 2) and cytosolic-enriched (N/C < 0.5) transcripts are indicated on the panel. The central dots represent the median.

B, C Pairwise comparison displaying the fold change (FC) regulation of IR-transcripts in the nucleus in function to spliced isoforms in the cytosol. All IR-transcripts are displayed and those corresponding to regulated IRs through splicing are colored in blue (B) and those associated with unregulated IRs are colored in brown (C). Transcripts were considered regulated through splicing (B, blue) if the followings are applied: (i) IR-transcript expression fold change ≥ 20% and |z-score| ≥ 1.5 in the WCE, (ii) spliced isoform expression fold change ≥ 20% and |z-score| ≥ 1.5 in the WCE and (iii) expression of the IR- and the spliced isoforms evolved in opposite directions in the WCE. Transcripts were considered unregulated (C, brown) if the followings are applied: (i) fold change in the expression of the intron retaining transcripts ≤ 5% and |z-score| ≤ 1 in the WCE.

D Box plot displaying the fold change in the overall gene expression of gene containing a regulated retained intron upon bicuculline stimulation. The central line represents the median; the upper and lower bounds of the box represent, respectively, the $75^{th}$ (Q3) and $25^{th}$ (Q1) percentiles; the upper whisker corresponds to the highest observed value within [Q3, 1.5*Q3] and the lower whisker corresponds to the lowest observed value within [0.5*Q1, Q1].

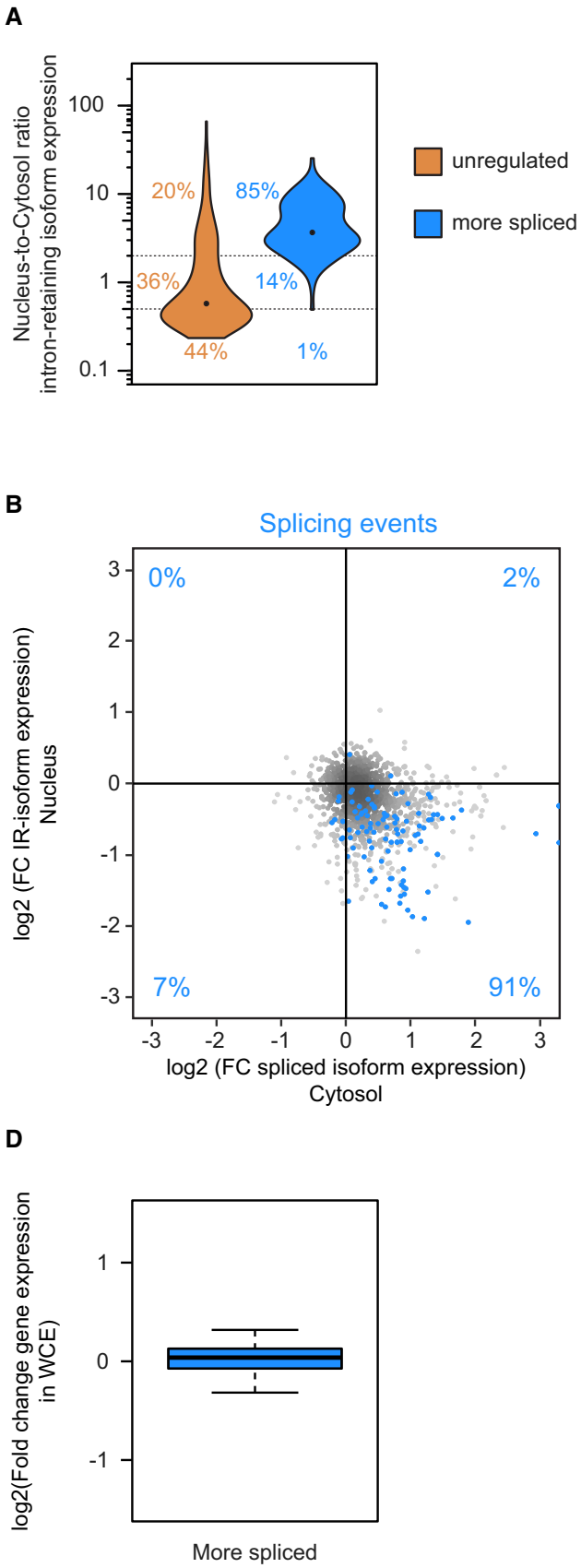



**Figure EV4.**

**Figure EV5. related to Fig 5.**

A RT-PCR validations of IR-transcripts specifically regulated upon bicuculline stimulation (*Smg1*), BDNF stimulation (*Gria2*), commonly regulated (*Cdr1os*). Fold changes (FC) in the expression of the IR- (red and orange) and spliced (dark gray) isoforms were assessed with real-time qPCR using three different primer sets, as represented in the right scheme. Means and SDs are displayed; the *P*-values calculated with a one-tailed *t*-test are indicated (as * when $P < 0.05$, as ** when $P < 0.01$, as *** when $P < 0.001$ and as numerical values when *P*-value is close to the 0.05-significance threshold) (three independent cultures).

B Means and SEMs of the fold change (FC) of read coverage along the whole retained intron of transcript candidates regulated upon stimulation with bicuculline or BDNF. Displayed transcripts correspond to those assessed by PCR in the main Figs 5 and EV5A (three independent cultures).

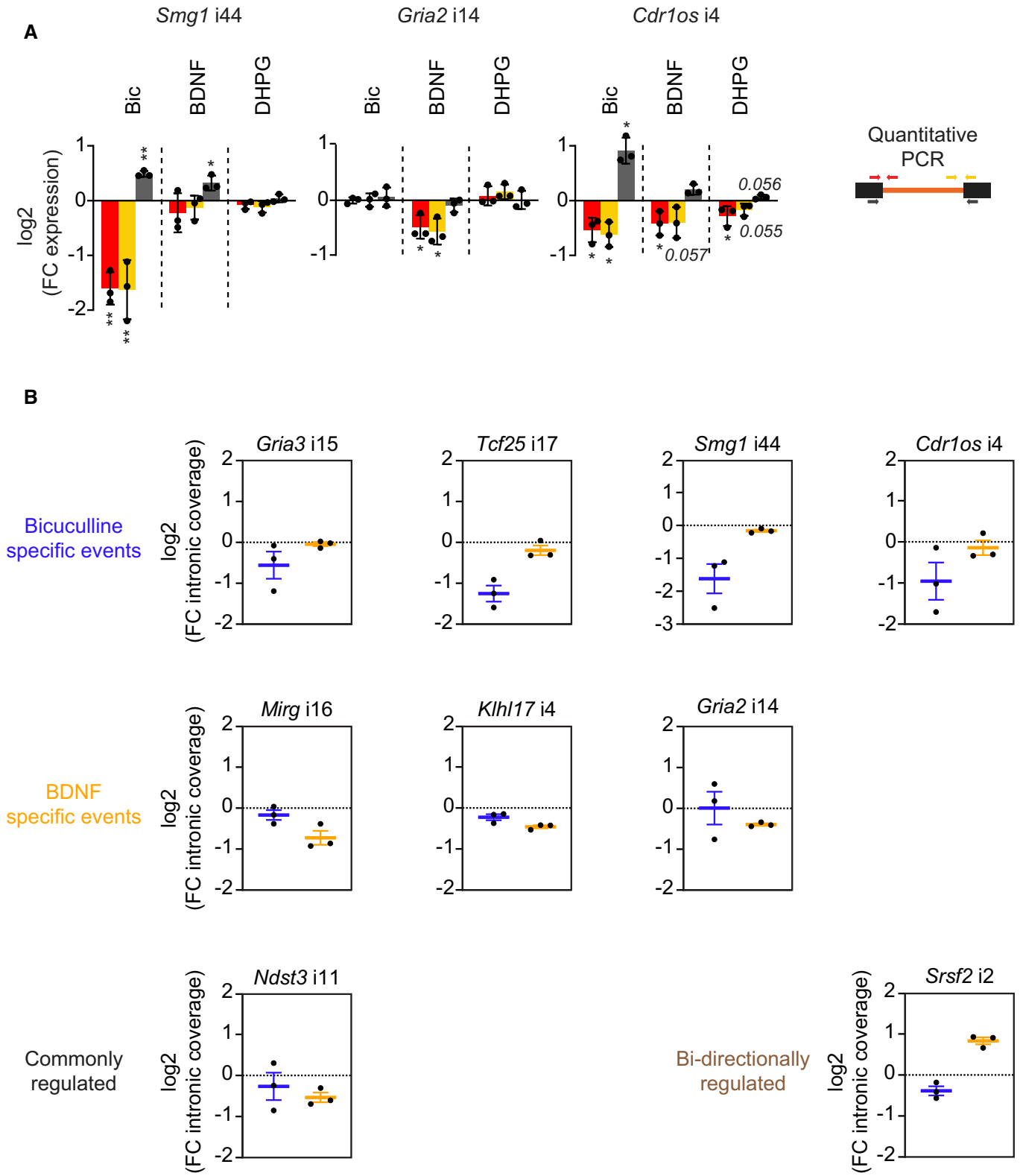



**Figure EV5.**

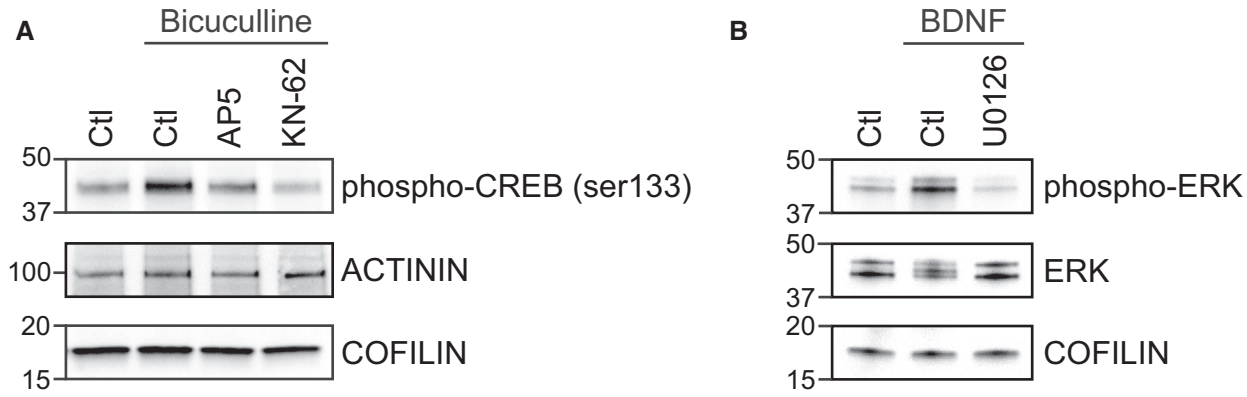



**Figure EV6. related to Fig 6.**

A Efficiency of the pharmacological compounds AP5 blocking NMDA receptors (NMDAR) and KN-62 targeting the calcium/calmodulin-dependent protein kinases (CaMK) was controlled by assessing the level of CREB serine 133 phosphorylation by western blot; ACTININ and COFILIN were used as a loading control. In all conditions, DRB (50 μM) was applied to mouse primary neocortical cells (14 days in culture) for 2 h; 1 h before cell collection, bicuculline (20 μM) was added to the cultures. 15 min before bicuculline stimulation, the NMDAR antagonist AP5 (50 μM) or CaMK blocker KN-62 (10 μM) were applied (three independent cultures).

B Efficiency of the pharmacological blocker U0126 of the mitogen-activated protein kinases (MAPK) was controlled by assessing the level of ERK phosphorylation by western blot; COFILIN was used as a loading control. In all conditions, DRB (50 μM) was applied to mouse primary neocortical cells (14 days in culture) for 2 h; 1 h before cell collection, BDNF (50 ng/ml) was added to the cultures. 15 min before BDNF stimulation, the MAPK inhibitor U0126 (10 μM) was applied (three independent cultures).