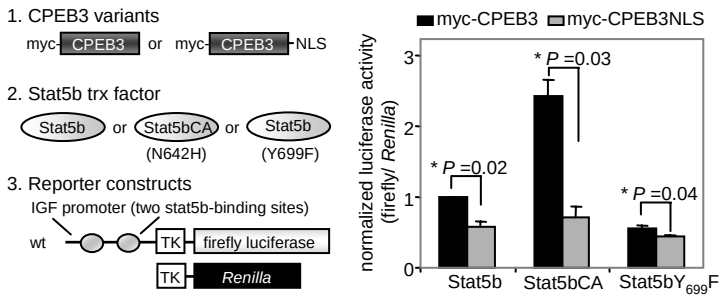
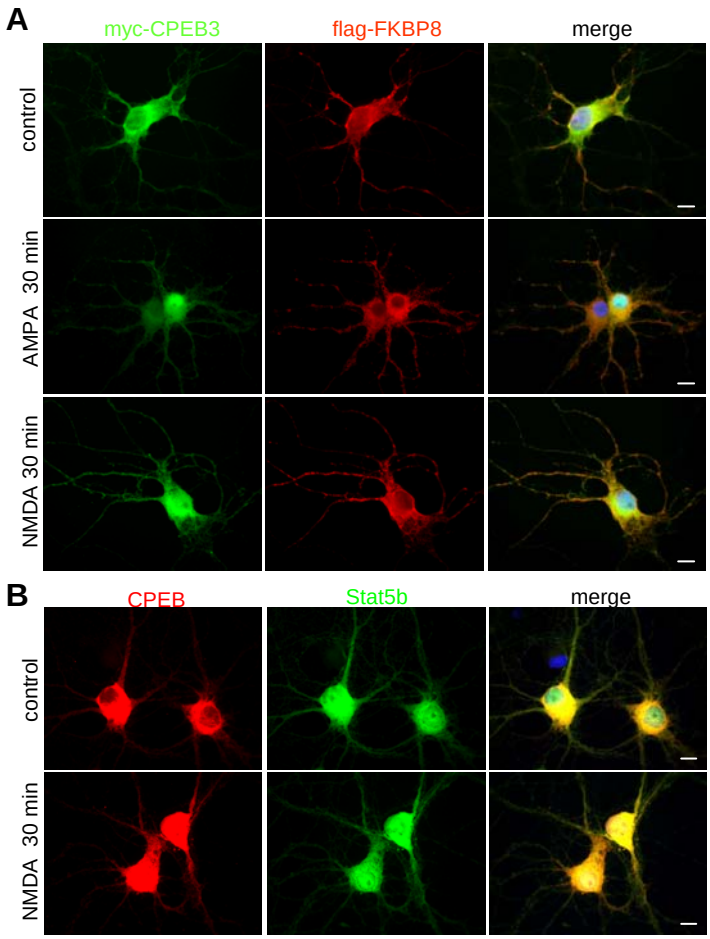
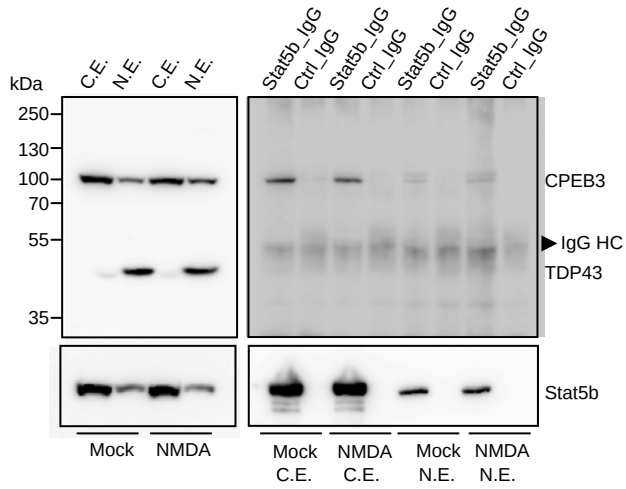


Supplementary Figure S1. CPEB3 inhibits Stat5b's transcriptional activity regardless of the phosphorylation status of Y699. The 293T cells were transfected with plasmids encoding 1) myc-CPEB3 or myc-CPEB3 fused with the nuclear localization sequence (myc-CPEB3NLS) and 2) wild type (WT), constitutively active (CA) Stat5b with N642H mutation or phosphorylation-defective Stat5b with Y699F mutation and 3) reporters expressing firefly luciferase driven by the IGF1 promoter containing two Stat5b-binding sites along with *Renilla* luciferase. TK: thymidine kinase promoter. Stat5b-mediated transactivation is expressed as normalized luciferase activity (firefly/*Renilla*). Three independent experiments were analyzed and expressed as mean ± s.e.m. and the asterisks denote significant difference (Student's *t* test).



Supplementary Figure S2. NMDAR signaling changes the nucleocytoplasmic distribution of CPEB3 but not that of Stat5b.(**A**) Immunostaining of exogenously expressed myc-CPEB3 and flag-FKBP8 in cultured hippocampal neurons treated with AMPA and NMDA for 30 min. (**B**) Immunostaining of CPEB3 and Stat5b in control and NMDA-stimulated hippocampal neurons. Scale: 10 μm.



Supplementary Figure S3. CPEB3 interacts with Stat5b in nuclear and cytoplasmic extracts. The cortical neurons treated with or without 30 min of NMDA were used for subcellular fractionation. The nuclear and cytoplasmic lysates were precipitated with Stat5b and control IgGs and immunoblotted with CPEB3 Ab. The nucleus-enriched RNA-binding protein, TDP43, was used as a fractionation control. N.E.: nuclear extract, C.E.: cytoplasmic extract.

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A Rat EGFR promoter sequence (-3000 to +300)

TGAGGGATGGGGGGAGGTGCCATCCCACAGTCAAAAACTCTGACCCAGAATTGTTCCTGTCTGAAAGAACTGCAGGAATGAAGATGGAGAAGGGCCTGAG
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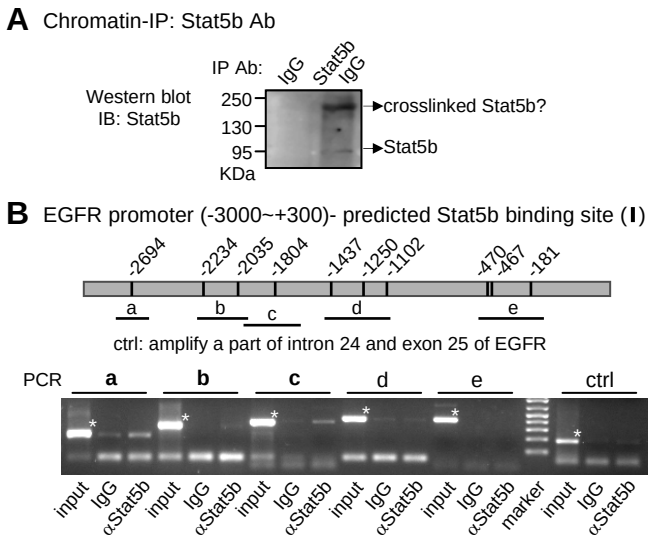
B Consensus Stat5b-binding sequence

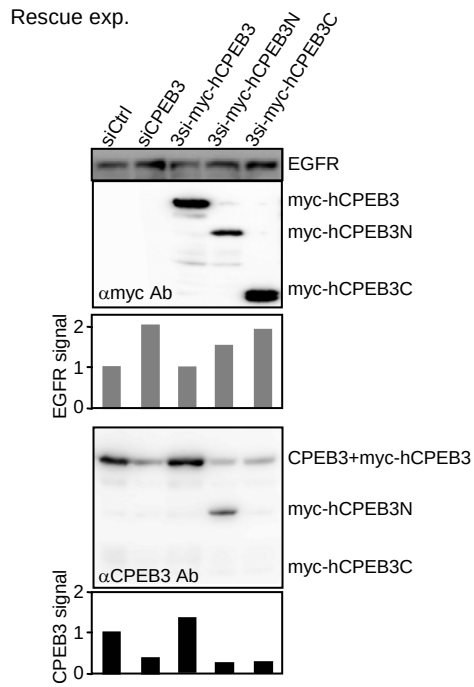
C Predicted Stat5b-binding sites in the EGFR promoter

Location (strand)	Core Score	Matrix Score	Binding sequence
-2694 (-)	1.00	0.80	gaatttggGGAAAac
-2234 (-)	0.95	0.77	tgtctctgGGAAGgg
-2035 (-)	0.97	0.82	gggtacctAGAAAtg
-1804 (+)	1.00	0.78	tgTTTCCtttaaaaa
-1437 (-)	0.92	0.77	cactcctgTGAAAac
-1250 (+)	0.87	0.77	ggCTTCGtgaaaagg
-1102 (+)	0.97	0.78	caTTTCTcagatggc
-470 (+)	0.97	0.95	ttTTTCTtggaagaa
-470 (-)	0.95	0.91	tttttcttGGAAGaa
-467 (-)	0.97	0.76	ttcttggaAGAAAga
-181 (+)	0.97	0.77	agTTTCTctggatcc

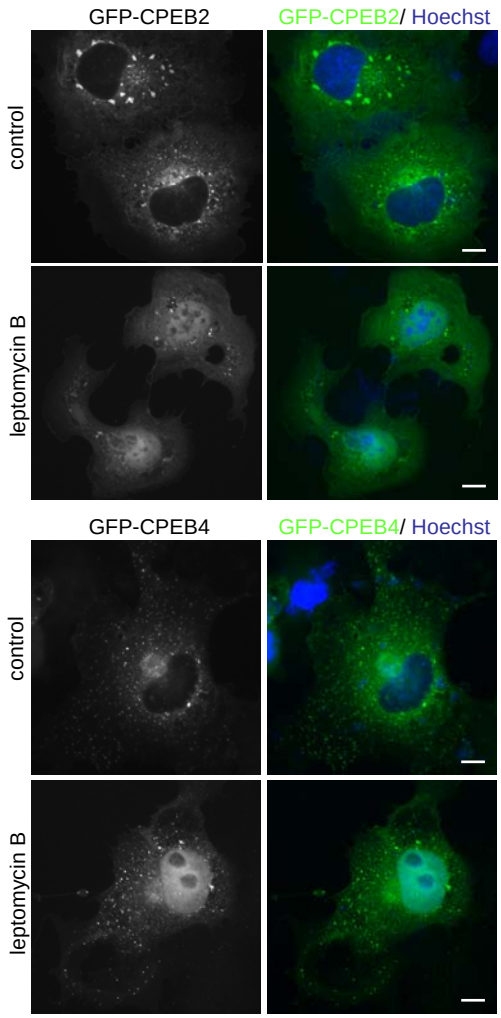
Supplementary Figure S4. Identification of Stat5b-binding sites in the EGFR promoter *in silico.* (**A**) The -3000 to +300 bps of EGFR promoter sequences are displayed. In particular, the designated PCR-amplified regions in the ChIP assay (Figure 5C) are shown in blue and the predicted Stat5b-binding elements are in bold and underlined. (**B**) Consensus Stat5b-binding sequence. (**C**) Information of the MATCH-analyzed Stat5b-regulatory elements including location, sense (+) and antisense (-) strand, core similarity score, matrix similarity score and binding sequences.



Supplementary Figure S5. Chromatin immunoprecipitation of cortical neuronal lysate with Stat5b or control IgG. The pulled-down substances were analyzed for proteins and DNA. (**A**) The Western blot shows precipitated Stat5b and the band of 190 kDa molecular weight is likely to be crosslinked dimeric Stat5b. (**B**) The predicted Stat5b-binding sites within the -3000 to +300 EGFR promoter and the PCR-amplified regions (a-e) are illustrated. The specific amplified bands are indicated with asterisks. The regions, a, b and c (in bold), were amplified more evidently in the Stat5b immunoprecipitate.



Supplementary Figure S6. The elevated EGFR level in CPEB3 knockdown neurons could be rescued with the expression of shRNA-resistant human CPEB3 (hCPEB3). The hippocampal neurons were infected with the lentivirus expressing the shRNA targeted to rat CPEB3 (3si) as well as the full-length, N- or C-terminal fragment of hCPEB3. The bar graphs are the quantified results of the immunoblotted signals of EGFR and CPEB3 on the Western blots. The CPEB3 antibody used here does not recognize the C-terminal RNA-binding domain.



Supplementary Figure S7. CRM1-mediated nuclear export of CPEB2 and CPEB4. The COS7 cells expressing GFP-CPEB2 and GFP-CPEB4 were treated for 30 min with leptomycin B, fixed and stained for nuclei with Hoechst 33342. Scale: 10 μm.

Supplemental Methods

Cytoplasmic and nuclear extract preparation

Approximated $6x10^7$ cortical neurons were homogenized in 2 ml of hypotonic IP buffer (20 mM Hepes, pH 7.4, 10 mM NaCl, 1mM $MgCl_2$, 10% glycerol, 0.5 mM DTT, 1X protease inhibitor cocktail) and centrifuged at 1000 xg for 5 min at 4ºC. The pellet was washed once with 2 ml of hypotonic buffer and centrifuged at 1000 xg for 5 min to obtain the nuclei. The supernatant was adjusted to 100 mM NaCl and centrifuged at 10K xg for 10 min to collect the supernatant as cytoplasmic extract. The nuclei were resuspended in 2 ml IP buffer containing 100 mM NaCl, sonicated on ice and centrifuged at 10K xg for 10 min to obtain the supernatant as nuclear extract. Equal volumes of lysate were incubated with Stat5b or control mouse IgG and the immunoprecipitates were probed with CPEB3 Ab.

Computational analysis of Stat5b-regulatory elements in the EGFR promoter

The sequence from -3000 to +300 bps of the transcription start site of rat EGFR gene was used for analysis. To identify the Stat5b-binding sites *in silico*, MATCH analysis (1) was applied to predict putative binding elements in the 3.3-kb EGFR promoter using the transcription factor matrices obtained from TRANSFAC (2). The search algorithm used two score values: the matrix similarity score was calculated using all positions of matrix and the core similarity score was calculated using the core positions. The sequences with both scores above 0.75 were tested in the ChIP assay.

Supplemental References

1. Kel, A.E., Gossling, E., Reuter, I., Cheremushkin, E., Kel-Margoulis, O.V. and Wingender, E. (2003) MATCH: A tool for searching transcription factor binding sites in DNA sequences. *Nucleic Acids Res*, **31**, 3576-3579.

2. Matys, V., Kel-Margoulis, O.V., Fricke, E., Liebich, I., Land, S., Barre-Dirrie, A., Reuter, I., Chekmenev, D., Krull, M., Hornischer, K. *et al.* (2006) TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. *Nucleic Acids Res,* **34**, D108-110.