The transcription factor CaRF limits NMDAR-dependent transcription in the developing brain

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Figure S1

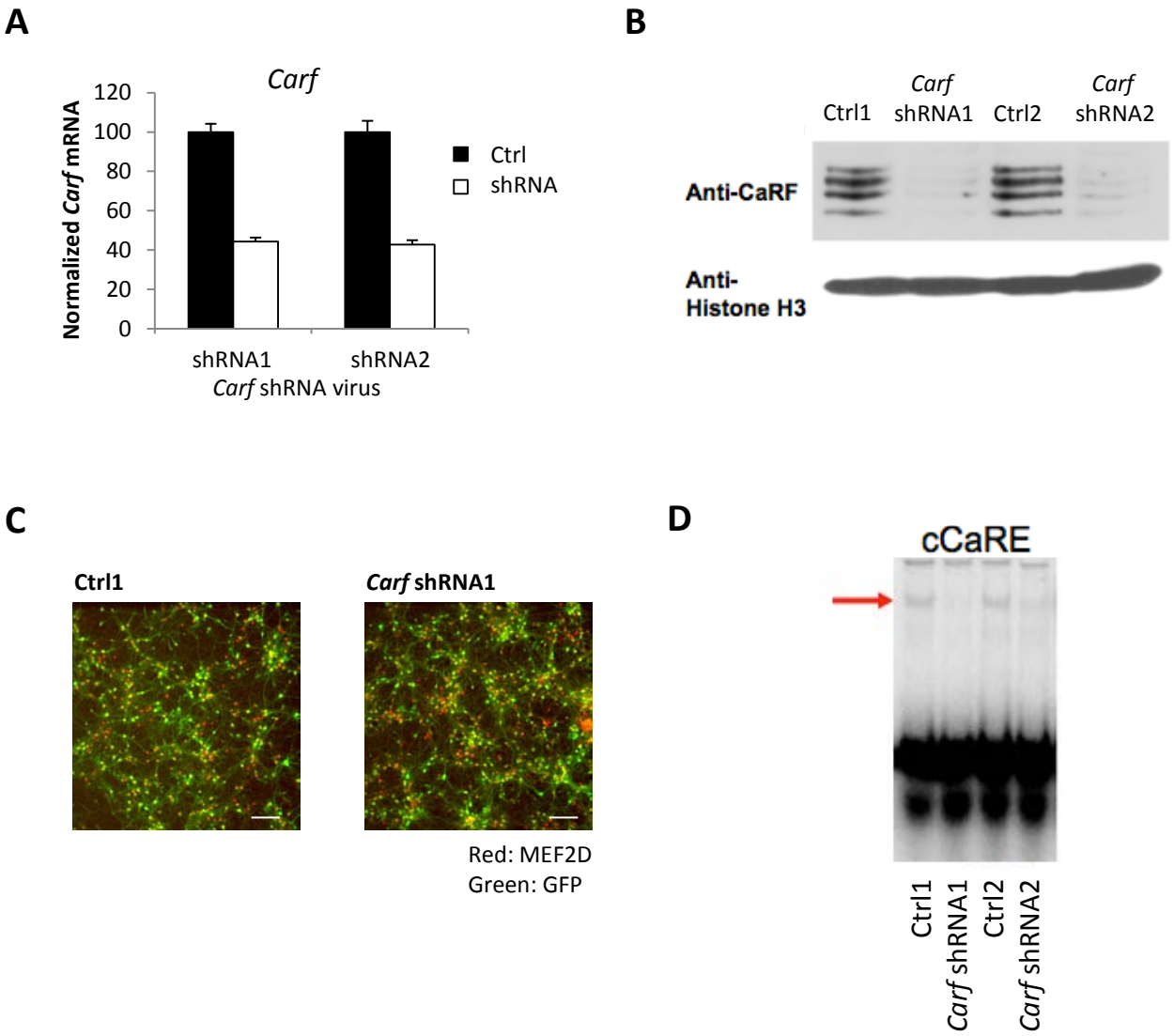

Figure S1: Validation of lentiviral *Carf* knockdown.
(A) Levels of *Carf* mRNA in mouse cortical neurons infected with lentiviruses encoding shRNAs targeting two independent sequences in *Carf*. *Carf* mRNA levels are shown normalized to expression in cells infected with the paired control viruses. n=3. **(B)** Nuclear extracts from cultured neurons infected with the indicated lentiviruses were analyzed by Western blot using an antibody raised against the CaRF protein. Histone H3 is shown as a nuclear loading control. **(C)** Fluorescence image of neurons infected with lentiviruses containing either the Ctrl1 or *Carf* shRNA1. Green, GFP indicates pLLx3.8-infected cells; red MEF2D antibody staining indicates neurons. Scale bar= 100µm. **(D)** Electrophoretic mobility shift assay. Nuclear extracts from neurons infected with the indicated viruses were incubated with radiolabeled DNA probes containing a high-affinity CaRF binding sequence (cCaRE). The red arrows indicate sequence specific nuclear protein-DNA complexes.

Figure S2

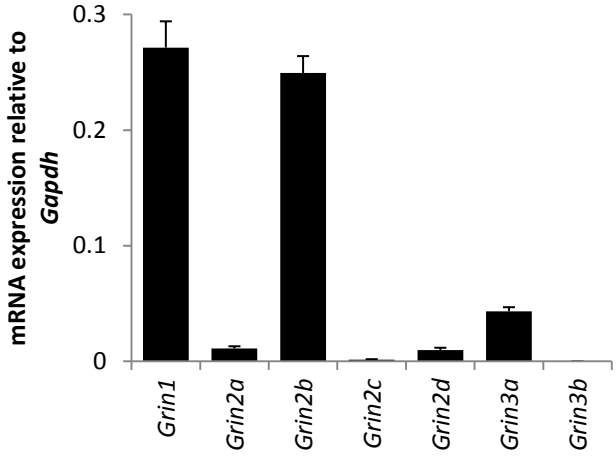

Figure S2: mRNA levels of NMDAR subunits in cultured mouse cortical neurons.
Levels of the indicated mRNAs in cultured mouse cortical neurons (DIV 7). mRNA levels are shown relative level to *Gapdh* expression. n=4.

Figure S3

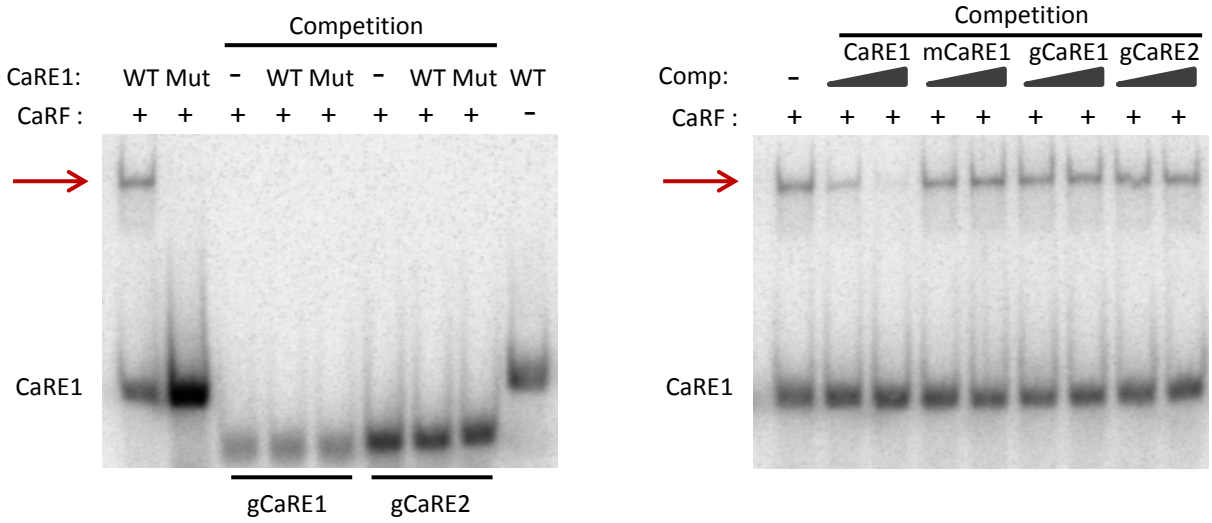

Figure S3: CaRF does not bind to *Grin3a* promoter directly.
Electrophoretic mobility shift assays. Left, in vitro transcribed and translated CaRF protein was incubated with either with a radiolabeled CaRF binding sequence (CaRE1) or one of the two sequences with partial homology to the CaRF binding sequence identified in the proximal *Grin3a* promoter (gCaRE1 and gCARE2). The red arrows indicate nuclear protein-DNA complexes. Specificity of the interaction is shown by competition of the interaction with incubation of an excess of unlabeled WT CaRE1 probe but not an unlabeled mutant probe (MUT) that lacksCaRF binding. Right, CaRF is first bound to the CaRE1 probe and association with the gCaRE sequences is tested by asking if the gCaREs can compete binding. The ramps indicate increasing concentration of unlabeled competitor probe. Competition of labeled CaRE1 binding by unlabeled CaRE1 is shown as a positive control. The red arrows indicate nuclear protein-DNA complexes.

Figure S4

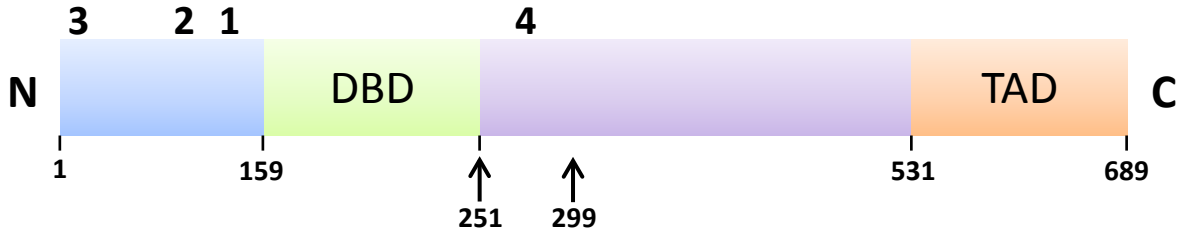

Figure S4: Schematic diagram showing the protein domain organization of CaRF and the targeting sites of *Carf* shRNAs.

The numbers above the diagram indicate the target site of *Carf* shRNAs. The numbers below the diagram indicate the protein residues corresponding to mouse CaRF (NP_631889.1). The arrows mark the C-terminal end of truncated forms of CaRF reported previously (Tao et al. 2002). The rCaRF(1-268) construct corresponds to the truncated mouse CaRF terminating at amino acid 251. DBD, DNA binding domain; TAD, transcriptional activation domain.

Figure S5

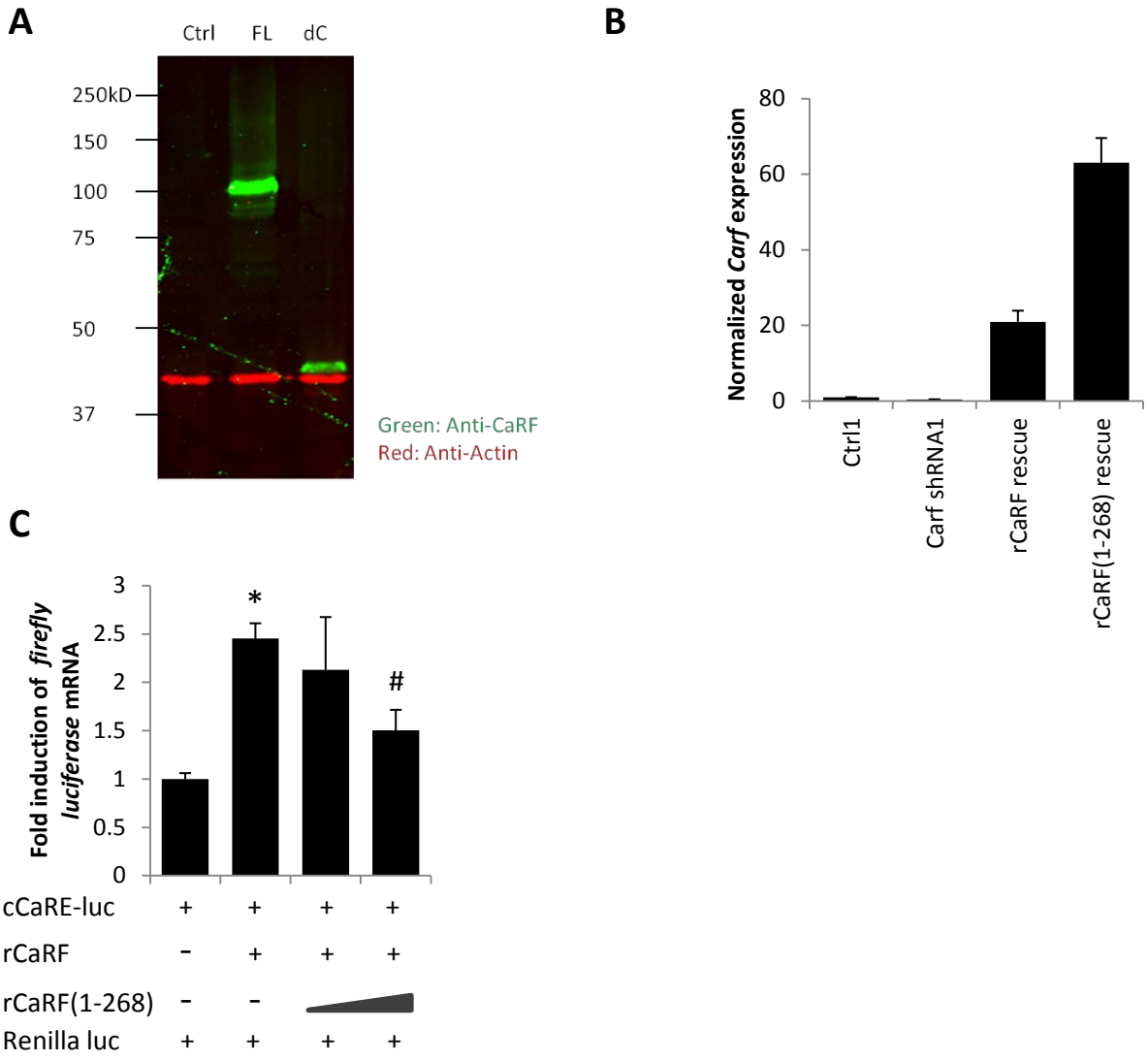

Figure S5: Validation of rat CaRF overexpression.
(A) Whole cell lysate from HEK293T cells transfected with control plasmid (Ctrl), rCaRF over-expression plasmid (FL) or rCaRF(1-268) overexpression plasmid (dC) was analyzed by Western blot using an antibody raised against CaRF. Actin is shown as a loading control. **(B)** Mouse/rat CaRF mRNA levels in mouse cortical neurons infected with the indicated lentiviruses. Mouse and rat *Carf* mRNA were measured by a primer pair against both mouse and rat *Carf* sequence. *n*=4-6. **(C)** HEK293T cells were transfected with indicated plasmids. At 2d after transfection, RNA was harvested . The *firefly luciferase* mRNA levels were normalized for each well to cotransfected *renilla luciferase* mRNA levels. The ramps indicate increasing amount of rCaRF(1-268) overexpression plasmid. *n*=3, *p<0.05 compared with no rCaRF. #p<0.05 compared with rCaRF only.

Figure S6

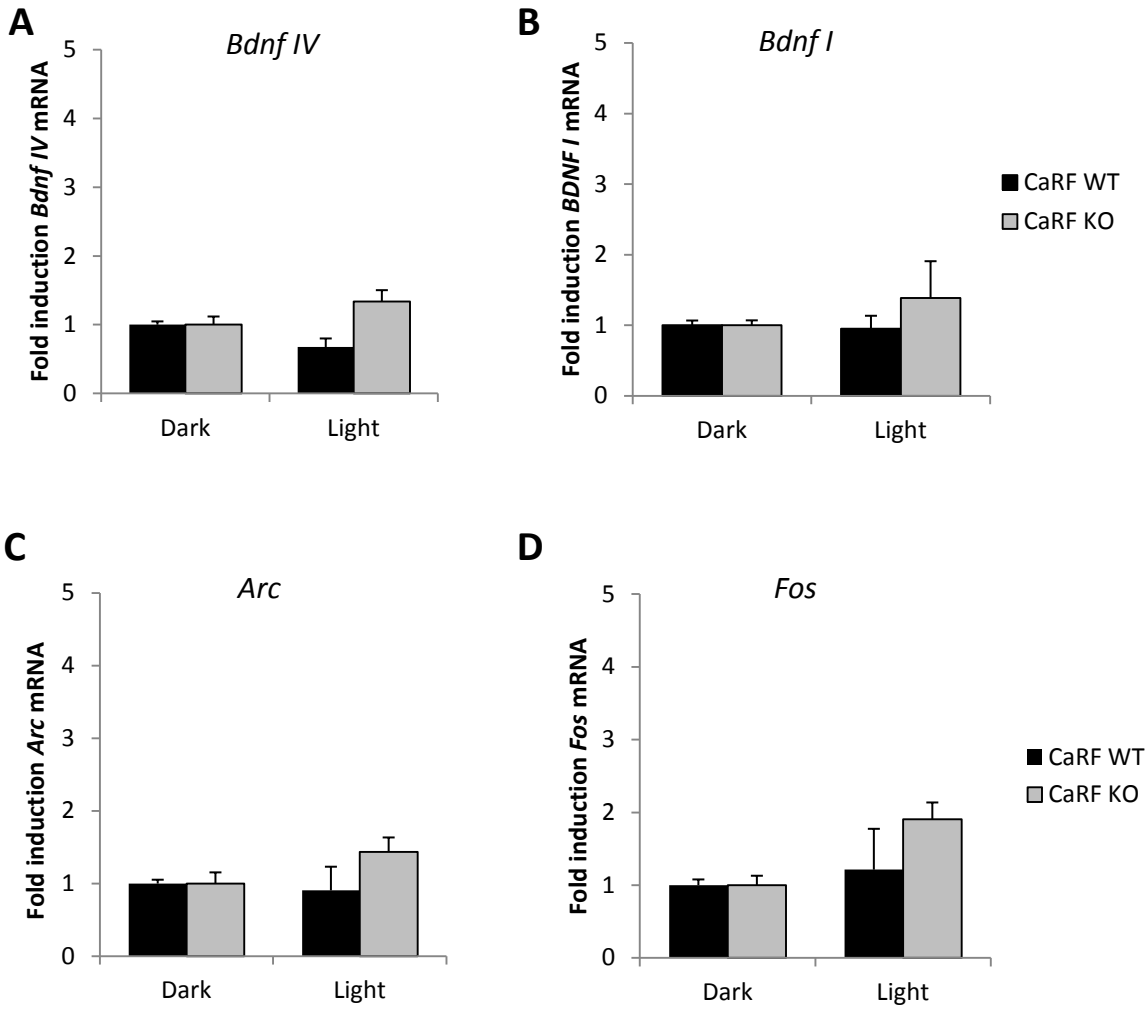

Figure S6: Light exposure does not induce gene transcription in somatosensory cortex.
Levels of **(A)** *Bdnf* exon IV, **(B)** *Bdnf* exon I, **(C)** *Arc*, and **(D)** *Fos* in the primary somatosenory cortex of P21 CaRF WT and KO mice after 7 days of constant darkness (Dark) or following exposure to light for 6hrs (Light) prior to tissue harvesting. *n*=9-11.

Figure S7

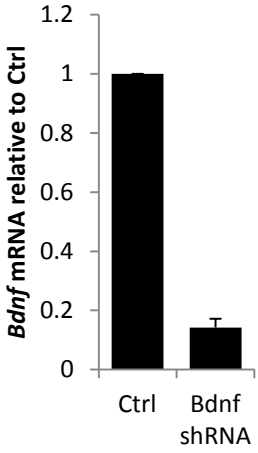

Figure S7: Validation of lentiviral *Bdnf* knockdown
Levels of *Bdnf* mRNA in mouse cortical neurons infected with lentiviruses encoding shRNAs targeting *Bdnf*. *Bdnf* mRNA levels are shown normalized to expression in cells infected with the paired control viruses (Ctrl). n=3.