Supplementary Information

Material and Methods

DNA constructs

The firefly reporter constructs UBE3A-luc, APT1-luc, LIN41-luc WT, LIN41-luc mutant and LIMK1-luc were generated by inserting the corresponding 3'UTRs downstream of the firefly gene into the XbaI restriction site of pGL3-promoter or pGL4.13 (Promega). UBE3A-luc contains a 606 bp fragment of the rat UBE3A 3'UTR (Valluy J. et al., 2015). APT1-luc is derived from pGL3-Apt1 (Siegel et al., 2009). The wildtype and mutant 3'UTRs of *C.elegans* LIN41 were kindly provided by D. Bartel. LIN41 WT 3'UTR was excised by NotI and MssI and LIN41 mutant 3'UTR was PCR amplified. The LIMK1 3'UTR was PCR amplified from pGL3-Limk1 (Schratt et al., 2006). The 138-sponge-luc reporter was generated by inserting six binding sites for miR-138 carrying a bulge at position 9-12 (Ebert et al., 2007) into the XbaI site of pGL4.13. The ctrl sponge was created by inserting the reverse sequence of 138 sponge in pGL4.13. The renilla gene from phRL-TK was first cloned into pGL3-promoter using unique NcoI and XbaI sites and subsequently excised with HindIII and XbaI to generate pGL4-RL. For GFP-fusion proteins, open reading frames from a mouse brain cDNA library (C57BL/6) were PCR amplified and inserted into pEGFP_C1 (Clonetech) using the indicated restriction sites. The open reading frame (ORF) of Ncoa3 was amplified by PCR from a mouse cDNA clone (Clone ID: 30104508, Openbiosystems) and inserted to KpnI and AgeI restriction sites of pEGFP_N1 (Clontech). Silent mutations were introduced by site directed mutagenesis to generate shRNA-resistant Nova1 ($Nova1^R$) and Ncoa3 ($Ncoa3^R$). A predicted nuclear receptor binding site in the mouse Ago2 promotor (GRCm38/mm10, chr. 15 pos. 73185409-73185468) was cloned into the BglII and HindIII sites of pGL4.

For improved expression, a minimal promoter was further inserted to the HindIII site by oligo annealing and ligation. The Flag/HA-Ago2 expression vector was kindly provided by Thomas Tuschl. For both types of shRNA expression vectors, pSuper basic (oligoengine) and AAV (Christensen et al., 2009), the same DNA oligos were annealed and ligated into the BglII and HindIII restriction sites.

Plasmids containing wild-type and mutant 3'UTRs of mouse IQGAP1 and HMGA2 were kindly provided by D. Bartel. The 3'UTRs were excised using the SacI/XbaI and XbaI/NotI restriction sites respectively and ligated to the XbaI site of pGL4 after blunt end generation.

Antibodies

Immunohistochemistry (ICC) or Western Blot analysis (WB) at the given dilution: rabbit anti-Ncoa3 (SRC-3 clone 5E11 from Cell Signaling, WB 1:1000, ChIP), mouse anti-Ncoa3 (AIB-1 clone34 from BD Biosciences, ICC: 1:200), rabbit anti-MAP2 (Cell Signaling cat.# 4542 , ICC: 1:200), mouse anti-MAP2 (clone HM-2 from Sigma-Aldrich, ICC: 1:2000), rabbit anti-α-Tubulin (Cell Signaling cat.# 2144, WB: 1:5000), mouse anti-β-Actin (clone AC-15 from Sigma-Aldrich, WB: 1:10,000), rabbit anti-HDAC2 (ab32117 from Abcam, WB: 1:5000), mouse anti-Limk1 (clone 42 from BD Biosciences, WB: 1:750), mouse anti-Ago2 (clone 2D4 from Wako, WB: 1:1000), rabbit anti-Ago2 (ab32381 from Abcam, ICC: 1:200), chicken anti-GFP (ab13970 from Abcam, WB: 1:10,000), rabbit anti-Nova1 (Merck Millipore cat.# 07-637, WB: 1:1500; ICC: 1:250), rabbit anti Nova-pan (obtained from R. Darnell; WB: 1:5000), rabbit anti-HA (ab9110 from Abcam, ICC: 1:500), mouse anti-Flag (clone M2 from Sigma-Aldrich, WB: 1:1000), rabbit anti-Drosha (sc-33778 from SCBT, WB: 1:500).

Species specific secondary antibodies for WB were HRP-conjugated (Calbiochem) and applied at 1:20,000 dilutions.

Secondary antibodies for ICC were anti-rabbit conjugated to Alexa 488, anti-mouse conjugated to Alexa 647 and anti-rabbit conjugated to Alexa 647 (Life technologies) at a dilution of 1:1000.

RNA extraction and Reverse Transcription-quantitative PCR (RT-qPCR)

For RNA extraction, hippocampal neurons were cultured in the presence of FUDR and infected on 4DIV. Total RNA was extracted on 14DIV using mirVana miRNA isolation Kit (Ambion). RNA was further treated with Turbo DNase (Ambion) according to the manufacturer's protocol. After denaturation of the DNase for 10 min. at 75°C in the presence of 15 mM EDTA we reverse transcribed 1 μg total RNA to cDNA using the iScript cDNA synthesis Kit (BioRad) supplemented with Mg^{2+} (final concentration 6 mM $MgCl_2$ and 12 mM EDTA). Quantitative PCR was processed in triplicates with 10μl total volume (0.67 μl cDNA and 0.25 μM primer concentration) using the iTaq SYBR Green Supermix with ROX (BioRad) on a Step One Plus instrument (Applied Biosystems).

Luciferase assay

Hippocampal neurons (11DIV) or cortical neurons (5DIV, 12DIV) were transfected as above in duplicates with 50-100 ng of the indicated pGL4 construct, equal amount of pGL4-RL and either 10 ng of the given pSuper vector or 7,5 pmol of siRNA duplex, or co-transfected with 5 pmol of miRNA duplex RNA (miR-134 or miR-138) (amount for one well of 24 well plate). Lysis was carried out 2-3 days later and the activity of both firefly and renilla was measured using the Dual Luciferase Reporter System (Promega) on the GloMax R96 Microplate Luminometer (Promega). Firefly activity was normalized to

renilla activity and a basal condition which did not contain a shRNA expression vector or siRNA was set as 1. Tethering experiments were performed by co-transfecting cortical neurons at 5DIV with 150 ng pCI-Neo-NHA or 250-400 ng NHA-fusion constructs, 50 ng of pRL-5boxB (obtained from R. Pillai) and 50 ng of pGL4.13 (Promega). Luciferase assay was performed 2 days after transfection and renilla activity was normalized to firefly activity.

Western blot analysis

The concentration of extracts was determined by BCA assay (Pierce) and equal protein amounts were separated on a SDS-PAGE. After wet transfer (BioRad) to a PVDF membrane (Millipore) unspecific binding was blocked by incubation with 5% milk powder in Tris-buffered saline containing 0.1% Tween 20 (TBS-T). The same milk solution was used to dilute primary and secondary antibodies which were incubated at 4°C overnight or for 1 h at RT respectively, each followed by 3 wash steps with (TBS-T) for 10 min. Secondary HRP-conjugated antibodies were detected using the ECL Prime Western Blotting Detection Reagent (GE Healthcare). Band intensities of scanned films were measured with Image J.

Immunocytochemistry and confocal microscopy

For microscopic analysis hippocampal neurons were fixed by 4% paraformaldehyde/sucrose in PBS for 15 min. followed by three PBS washes. For analysis of neuronal morphology (see below), the GFP expressing cells were mounted directly in Aquapoly (Polysciences). For immunocytochemistry the cells were pre-incubated with GDB (20 mM $NaPO_4$ pH 7.4, 450 mM NaCl, 0.3% Triton X-100, 0.1% Gelatine) for 10 min. followed by incubation with the primary antibody for 1 h at RT

diluted in GDB. After the cells were washed four times with PBS, the secondary antibody was applied for 1 h at RT diluted in GDB. After three further PBS washes, Hoechst was incubated at 1:5000 in PBS for 5 min. and the cells were mounted as above.

All images were acquired with a LSM 5 Pascal (Zeiss) microscope. Presented immunocytochemistry images are the maximum projections of 2-4 confocal stacks with a *z*-distance of 0.5-1 μm. Measurements of average signal intensity and *x*-*y*-distance as well as further image cropping/magnification steps were carried out with Image J software.

Primers

All primer sequences are given 5' to 3'. The following primers were used for qPCR:

Ncoa3 FW: GCTTCGTCTCGACCCACTTC

Ncoa3 REV: GGTCGGATGCCTTGTCCTAC

Ago1 FW: TCATTATCGTCATCCTGCCCG

Ago1 REV: GGCAGAGGTTGGACAGAGTC

Ago2 FW: TCACATTCATCGTGGTGCAGA

Ago2 REV: TCTTTGTGTCGACGGTTGTG

Ago3 FW: GCTGTAGGCGACGCAAGTTC

Ago3 REV: TGAAAGCTCCAGGCAGAAGAG

Ago4 FW: GTTCAGTCAGGAGGTCGTGC

Ago4 REV: TGGCCAAGCTACCTGTTTCA

Tnrc6a FW: GCTCAAGTGCCTCCTCCATT

Tnrc6a REV: GGTTCAGCATGGCTACCTGT

Tnrc6b FW: CCCATAGGACACAACCCCAC

Tnrc6b REV: CTCCAAGGAGATGCCGGTTT

Tnrc6c FW: CACTTTTGCCAGAACTTACTAAGAC

Tnrc6c REV: ACAGGCAGATGCACGTTTG

Dicer FW: CAGTTGTCCATCATGCCCTC

Dicer REV: CTCAGCGCCACTCTTGAGAA

Trbp FW: CAGTCTGAGTGCAACCCTGT

Trbp REV: TGGTGAACTCTTTGCGGTGA

Arhgdib FW: TACAGAGACACACAGGGCGG

Arhgdib REV: CGGCTTGTAGTTGAGCTTGC

Htra4 FW: GGCTCCGGAACTCCGATATG

Htra4 REV: ATCCCAATCACCTCGCCATC

Gpr3 FW: AACCCGGTCATTTACGCCTT

Gpr3 REV: CTGGCGGACCTAACCTTCTG

Spast FW: GCGCATCGACGAGGAAGAG

Spast REV: TGTTCACCTTGGCCCGTAAC

Cyb5r3 FW: TTCAAGGACACGCATCCCAA

Cyb5r3 REV: GGACTTCTTGTCTGCACGGA

Map9 FW: GGCAGCGGTTTATCAGGAGT

Map9 REV: CCAGGCCTCAAATGACGCTA

U6 snRNA FW: CTCGCTTCGGCAGCACA

U6 snRNA REV: AACGCTTCACGAATTTGCGT

GAPDH FW: GCCTTCTCTTGTGACAAAGTGGA

GAPDH REV: CCGTGGGTAGAGTCATACTGGAA

Limk1 FW: CCTCCGAGTGGTTTGTCGA

Limk1 REV: CAACACCTCCCCATGGATG

Rgs4 FW: ACAAGCCGGAACATGTTAGAG

Rgs4 REV: AGACTTGAGGAAACGACGGT

The following oligos were used to create shRNA expression constructs:

Control shRNA	FW1: GATCCCCAAACCTTGTGGTCCTTAGGTTCAAGAGA
	FW2: CCTAAGGACCACAAGGTTTTTTTTA
	REV1: AGCTTAAAAAAAACCTTGTGGTCCTTAGGTCTCTTGAA
	REV2: CCTAAGGACCACAAGGTTTGGG
Ncoa3 shRNA#1	FW1: GATCCCCGCAGCAGTAATGATGGATCTTCAAGAGA
	FW2: GATCCATCATTACTGCTGCTTTTTA
	REV1: AGCTTAAAAAGCAGCAGTAATGATGGATCTCTCTTGAA
	REV2: GATCCATCATTACTGCTGCGGG
Ncoa3 shRNA#2	FW1: GATCCCCGGAGACAGTGAGACAGATATTCAAGAGA
(used in all major	FW2: TATCTGTCTCACTGTCTCCTTTTTA
experiments)	REV1: AGCTTAAAAAGGAGACAGTGAGACAGATATCTCTTGAA
	REV2: TATCTGTCTCACTGTCTCCGGG
Nova1 shRNA	FW1: GATCCCCGGTACTACTGAGAGGGTTTTCAAGAGA
	FW2: AAACCCTCTCAGTAGTACCTTTTTA
	REV1: AGCTTAAAAAGGTACTACTGAGAGGGTTTTCTCTTGAA
	REV2: AAACCCTCTCAGTAGTACCGGG
Drosha shRNA	FW1: GATCCCCCAACATAGACTACACGATTTTCAAGAGA
	FW2: AATCGTGTAGTCTATGTTGTTTTTGGAAA
	REV1: AGCTTTTCCAAAAACAACATAGACTACACGATTTCTCTTGAA

REV2: AATCGTGTAGTCTATGTTGGGG

DGCR8 shRNA FW1: GATCCCCAACAATTTGGAGACTAGATGAATTCAAGAGA

FW2: TTCATCTAGCTCCAAATTGTTTTTTTGGAAA

REV1: AGCTTTTCCAAAAAAACAATTTGGAGCTAGATGAATCTCTTGAA

REV2: TTCATCTAGCTCCAAATTGTTGGG

The following primers were used to clone the mouse Ncoa3 ORF to pEGFP_N1:

Ncoa3-KpnI-FW: GACTGGTACCATGAGTGGACTAGGCGAAAG

Ncoa3-AgeI-REV: GACTACCGGTGTGCAGTATTTCTGATCGG

The following primers were used to clone the mouse Nova1 ORF to pEGFP_C1:

Nova1-XhoI-FW: GACTCTCGAGGCATGGCGGCAGCTCCCATTC

Nova1-SmaI-REV: GACTCCCGGGTCAACCCACTTTCTGAGGATTGGC

The following primers were used to clone the mouse Ewsr1 ORF to pEGFP_C1:

Ewsr1-XhoI-fw: GACTCTCGAGGCGCGTCCACGGATTACAGT

Ewsr1-SmaI-rev: GACTCCCGGGCTAGTAGGGCCGGTCTCTG

The following primers were used for site directed mutagenesis:

Ncoa3^{R}-FW: GTGCCATCCTAAAGGAGACCGTTCGCCAGATACGGCAAATAAAAG

Ncoa3^{R}-REV: CTTTTATTTGCCGTATCTGGCGAACGGTCTCCTTTAGGATGGCAC

Nova1^{R}-FW: GATTTTTATCCAGGTACCACCGAGCGGGTTTGCTTGATCCAGG

Nova1^{R}-REV: CCTGGATCAAGCAAACCCGCTCGGTGGTACCTGGATAAAAATC

The following primers were used to clone 3'UTRs to pGL4:

LIN41-FW: GATCTCTAGACACTTTCTTCTTGCTCTTTAC

LIN41-REV: GATCGCTAGCTTTATTCCAATTATGTTATCAG

LIMK1-FW: CTTCTAGAGATACTTGGAGGATAGACCCTCACC

LIMK1-REV: GCCCCGACTCTAGCTAGCGGGAGCACAGAATTGAT

The following primers were used for cloning of NHA-fusion constructs:

NHA-Nova1-Xba1-FW: TCTCTAGAATGGCGGCAGCTCCCATTCAGCAGAACG

NHA-Nova1-Not1-REV:

CCGGTGGCGGCCGCGTCAACCCACTTTCTGAGGATTGGCAG

NHA-Nova1-del1-Not1-REV:

CTGTGGATCCTCTGCGGCCGCCTGCTAGATAAGTTCAACAG

NHA-Nova1-del2-Xba1-FW:

TGAATCTAGAATCCAGAAGATACAAGAGGATCCACAGAGTG

NHA-EGFP-FW: CCACCGGTCGACACCATGGTGAGCAAGGGCG

NHA-EGFP-REV: ATCTAGAGTCGCGGCCGCTTTACTTGTAC

NHA-Ncoa3-MluI-FW: GACTACGCGTATGAGTGGACTAGGCGAAAG

NHA-Ncoa3-XbaI-REV: GATCTCTAGATCAGCAGTATTTCTGATCGGGG

The following primers were used for cloning of the mouse Ago2 nuclear receptor element and the minimal promotor to pGL4.13:

Ago2-RE-FW1: GATCTGCCGGGAGGTGGCGGTGTGGTCACG

Ago2-RE-FW2: CGCACGGGTCTGGCCGCGTCCAAGTTCAAGCTCTGA

Ago2-RE-REV1: ACCCGTGCGCGTGACCACACCGCCACCTCCCGGCA

Ago2-RE-REV2: AGCTTCAGAGCTTGAACTTGGACGCGGCCAG

Min-prom-FW1: AGCTTAGACACTAGAGGG

Min-prom-FW2: TATATAATGGAAGCTCGACTTCC

Min-prom-FW1: AGCTGGAAGTCGAGCTTCC

Min-prom-REV2: ATTATATACCCTCTAGTGTCTA

The following primers were used to amplify rat genomic regions proximal to the transcriptional start site in ChIP experiments:

Ago2-genomic-FW: ATGGTGCACCCTAAGCTTCC

Ago2-genomic-REV: TGTGATGAAGTGGATCTGCACG

Cyb5r3-genomic-FW: AGAATGGCAGTGAAGCACCA

Cyb5r3-genomic-REV: ATCTGCCCTGGAAGATTGCC

Map9-genomic-FW: ATAAGCTGATAGGGCACTGCG

Map9-genomic-REV: AACCGACTGGTCTCTTGGGT

Spast-genomic-FW: CCAGTCAGACTCCTGCGAAC

Spast-genomic-REV: AGATCTGGGGTTTCGATGGC

beta-globin-genomic-FW: TCTAGAAGGTACCCTCATGGCTGAA

beta-globin-genomic-REV: GGATATGCCCTGTGGAGTGTTGAC

Figure legends

Figure S1: Validation of siRNA specificity in luciferase reporter assays.

A) Primary rat cortical neurons were transfected with the unmodified pGL4 (empty-luc) and luciferase activity was quantified as in Fig. 1 C and D. B) Western blot analysis with lysates obtained from HEK293 cells that had been co-transfected with the indicated GFP-tagged protein expression constructs and different siRNAs. Upper panels: anti-GFP Western. Lower panel: anti-ß-Actin Western (loading control). For GFP-Ewsr1, a non-specific band (*) serves as internal loading control.

Figure S2: Nova1 expression in cortical neurons and validation of the anti-Ncoa3 antibody for ICC.

A) Western blot analysis of Nova1 protein using whole cell extracts harvested from rat cortical neurons at the indicated days of *in vitro* (DIV) culture. ß-actin was used as a loading control. B) Western blot analysis of Nova1 protein using brain extracts from Nova1 knockout mice. C) Western blot analysis of Ncoa3 protein using whole cell extracts harvested from FUDR-treated rat hippocampal neurons at the indicated days of *in vitro* (DIV) culture. α-Tubulin was used as a loading control. D) Immunocytochemistry analysis of Ncoa3 expression (red) in hippocampal neuron cultures at 7DIV. MAP2 staining (green) was used to visualize neurons, Hoechst (blue) to visualize nuclei. Right: Magnification of insert depicted on the left. For simplicity, only the Ncoa3 signal is shown in greyscale. Cell and nuclear outlines derived from MAP2 and Hoechst staining, respectively, are shown in yellow. Scale bar = 10 μm. E) Immunocytochemistry for Ncoa3 (white in left panel, red in right panel) in hippocampal neurons (14DIV) that were transfected with the indicated shRNA expression vectors and GFP. F) Quantification of

the average Ncoa3 signal intensity in the soma of transfected neurons (right panel). Presented values are mean ± standard deviation from three independent experiments (10 cells per condition) with normalization to a basal condition without shRNA. **p<0.01 (student's t-test). Scale bar = 50 μm.

Figure S3: Validation of Ncoa3/Nova1 knockdown and target regulation.

A, B) Western blot analysis of Nova1 (A) and Ncoa3 (B) protein using whole cell extracts from cortical neurons (4-6DIV) that have been nucleofected with the indicated shRNA expression vectors. Migration of the Nova1-specific band is marked by an arrowhead. ß-Actin was used as a loading control. C-E) Reporter gene assay performed in either rat hippocampal neurons (14DIV, C) or cortical neurons (14DIV, D, E) that have been transfected with pGL4 vectors carrying the indicated 3'UTR together with either control, Nova1 or Ncoa3 shRNA expression vectors 3 days before. Relative luciferase activity (RLA) represents the ratio of firefly reporter activity to renilla control reporter activity which is normalized to a basal condition without shRNA expression vector. Presented are averages ± standard deviations from three independent experiments. One-way ANOVA: p<0.0001 (E), p<0.001 (D), p<0.05 (C). Tukey HSD test (C) and unpaired student's t-test (D, E): *p<0.05, **p<0.01.(student's t-test to control shRNA). F, G) Reporter gene assays in cortical neurons (14DIV) were performed in principle as described in C-E), instead that GFP expression vectors were used. Presented are averages ± standard deviations from three independent experiments. unpaired student's t-test: *p<0.05.

Figure S4: Validation of Nova1 and Ncoa3 dependent regulation of Limk1.

A) Technical replicate of the experiment in Fig. 4A) and the corresponding quantification of n=2 in B). C, D) Technical replicates of the experiments in Fig. 4B) and the quantification of n=3 in E). *p<0.05 (student's t-test).

Figure S5: Validation of Nova1-Ago interaction by co-IP.

A) This experiment is a biological replicate of Fig. 5A. B) Nova1 band intensities from figures 5A and S5A were quantified using ImageJ software. Values are given as percent intensity compared to the input signal and represent the average of two independent experiments. C, D) Equal experimental setup as in Fig. 5A) and S5A) except that RNAse A was added to the lysate before IP. E) Validation of anti-pan-Nova antibody. Western blot analysis using brain lysate from Nova knockout mice. F) Western blot analysis in lysates from HEK293 cells transfected with the indicated NHA-fusion constructs. Upper panel: anti-HA Western Blot; lower panel: anti-ß-Actin Western Blot as loading control. * non-identified cross-reactive protein. G) Immunocytochemistry in neurons transfected with the indicated NHA-Nova1 constructs. Upper panel: anti-HA. Middle panel: Hoechst (nuclei); lower panel: MAP2 (dendrites). H) Tethering assay in HEK293 cells with the indicated expression constructs as in Fig. 5D-F. Presented are averages ± standard deviations from three independent experiments. One-way ANOVA: p<0.001. Tukey HSD test: *p<0.05, **p<0.01, ns= not significant.

Figure S6: Dendritic outgrowth after Nova1 knock-down and validation of Ncoa3 and Drosha shRNA efficacy.

A) Dendritic outgrowth of cultured hippocampal neurons was analyzed by Sholl's method after transfection with GFP and Nova1 shRNA, control shRNA expression vector, or no

additional vector as in Fig. 6. B) Summation of the intersection number over all analyzed distances from the data in A). C) Western blot analysis of GFP-Ncoa3 in cell extracts from HEK293 cells that had been transfected with indicated shRNA constructs together with GFP-Ncoa3 expression vectors. Expression of co-transfected GFP was used as a loading control. Note that GFP-Ncoa3^{R} is not resistant to Ncoa3 shRNA#1, but to shRNA#2 which is used for all experiments in the main body. D) Western blot analysis of Drosha in hippocampal neurons (19DIV) that had been infected with rAAV expressing the indicated shRNAs after 12DIV. ß-Actin was used as a loading control. E) qPCR analysis of indicated mRNAs in rAAV-Drosha or control shRNA infected neurons. Values are normalized to the rAAV control shRNA condition for each target mRNA. N=3. F) Taqman qPCR analysis of indicated mature miRNAs in rAAV-Drosha or control shRNA infected neurons. Values are normalized to the rAAV control shRNA condition for each target mRNA. N=2.

Figure S7: rAAV-infection efficacy, microarray validation and additional Ncoa3-ChIP experiments.

A) Immunocytochemistry in hippocampal neurons (14DIV) that had been infected with rAAV expressing the indicated shRNAs after 4DIV. MAP2 staining (red) was used to visualize neurons, Hoechst (blue) to visualize cell nuclei. Co-expression of GFP from rAAV vectors in MAP2-positive cells was used to determine transfection efficiency with approx. 80% from 3 independent infections (approx. 80%). Scale bar = 100 µm. B) Matrix of Pearson's correlation coefficients of signals obtained from 6 independent microarray hybridizations. C) qPCR analysis of indicated mRNAs in hippocampal

neurons (14DIV) that had been infected with rAAV for the expression of indicated shRNAs at 4DIV. mRNA levels were normalized to GAPDH and a non-infected basal condition was set to 1. Presented are averages ± standard deviations from three independent infections. **p<0.01 (unpaired student's t-test). D) Quantification of Ago2 protein levels in hippocampal neurons from three independent Western Blots performed as in Fig. 8D. Band densities were normalized to α-Tubulin and a non-infected condition (basal) was set as 1. Presented are averages ± standard deviations. *p<0.05 (unpaired student's t-test). E, F) qPCR analysis of indicated Ncoa3-regulated transcripts from two additional Ncoa3-ChIP experiments performed in either control shRNA (black) or Ncoa3 shRNA (grey) infected neurons. Values are presented as fraction of the respective input DNA used for ChIP. As a specificity control, ChIP with an unrelated IgG was performed in control shRNA transfected neurons (white). G) Alignment of mouse, rat and human Ago2 promoter sequences for the construction of the Ago2 luciferase promoter reporter used in Fig. 8F. The putative RXRA binding site is depicted in bold. Non-conserved nucleotides are shown in italics.

Figure S8: Validation of Flag/HA-Ago2 expression in hippocampal neurons and HEK293 cells.

A) Left panel: Immunocytochemistry using anti-HA antibody (red) in hippocampal neurons (14DIV) that had been transfected with either Flag/HA-Ago2 (top) or an empty Flag/HA plasmid (control vector, bottom) three days before. Co-transfection of GFP (green) was used to outline neuronal morphology. Hoechst counterstain (blue) was used to visualize nuclei. Scale bar = 40 μm. Right panel: Higher magnification of the neurons shown on the left, illustrating the predominant cytoplasmic localization of Flag/HA-Ago2.

Scale bar = 15 μm. B) Western blot analysis of Flag/HA-Ago2 using anti-Flag antibody in cell extracts from HEK293 cells that had been transfected with Flag/HA-Ago2 or empty Flag/HA plasmid (control vector). α-Tubulin was used as a loading control. C) Immunocytochemistry using anti-Ago2 antibody (upper panel) in hippocampal neurons (12DIV) treated as described in Fig. 9A. Co-transfection of GFP (green, lower panel) served to outline the neuronal soma and was used for quantification of the signal. Scale bar = 10 μm.

Supplementary Dataset S1: List of all siRNAs used for in initial screen.

Supplementary Dataset S2: Results of the initial screen.

Supplementary Dataset S3: Complete list of genes altered by rAAV-Ncoa3 shRNA (Fc>1.3, p<0.05) including non-annotated transcripts.

Supplementary Figure S1

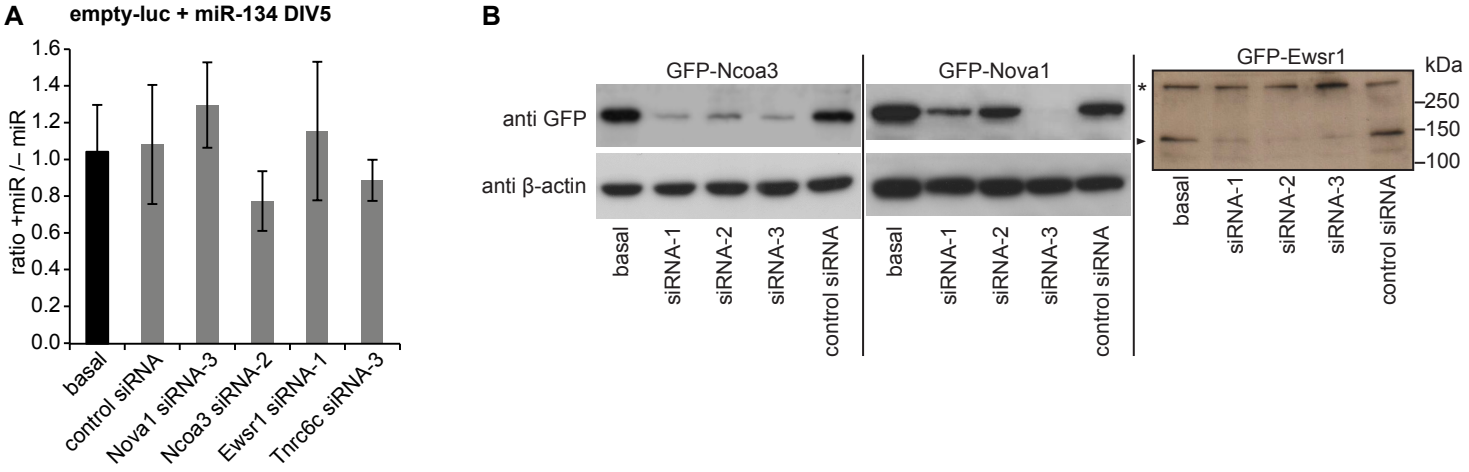


Figure S2

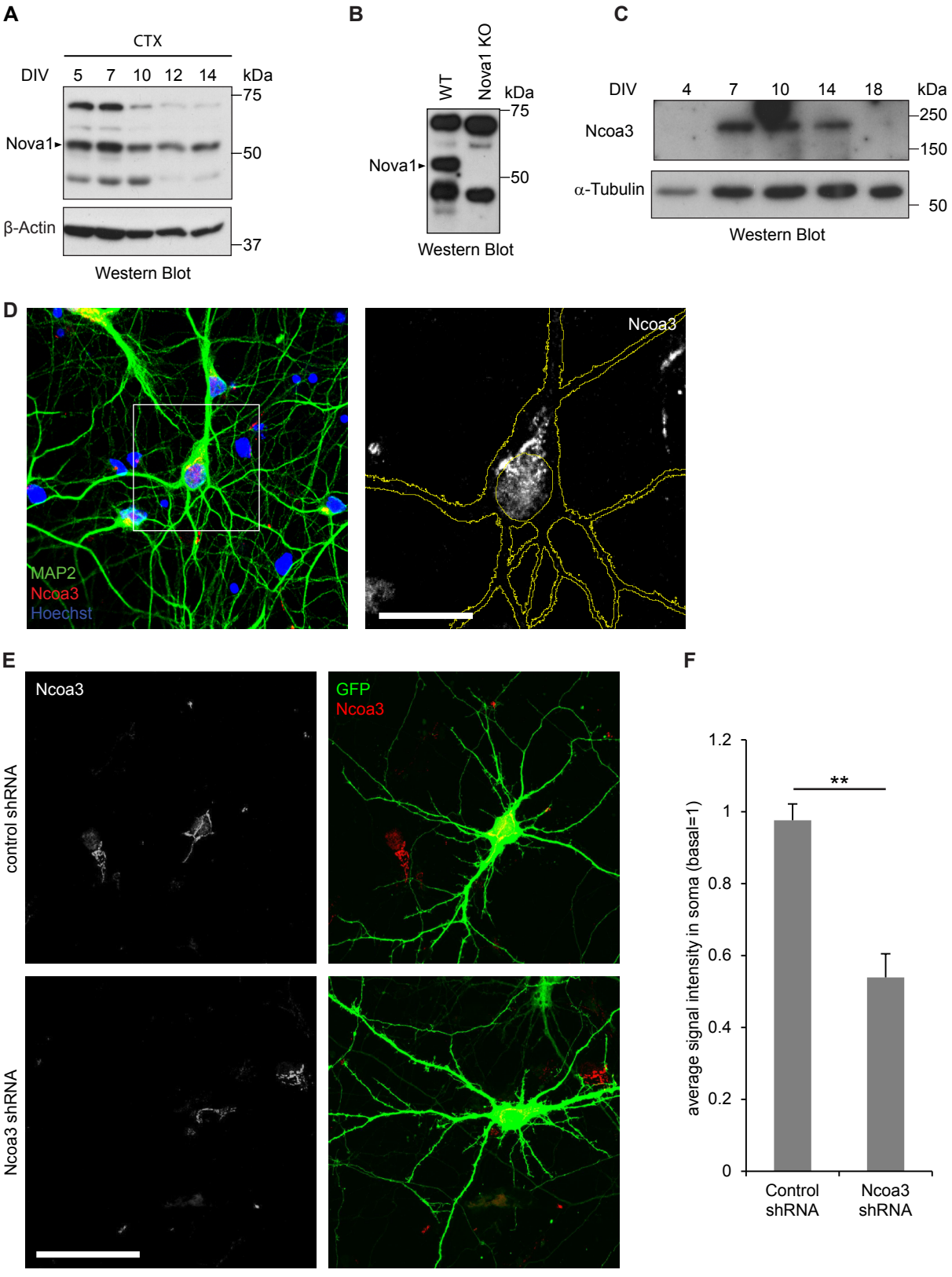

A
CTX
DIV 5 7 10 12 14 kDa
Nova1
β-Actin
75
50
37
Western Blot
B
WT
Nova1 KO
kDa
75
Nova1
50
Western Blot
C
DIV 4 7 10 14 18 kDa
Ncoa3
250
150
α-Tubulin
50
Western Blot
D
MAP2
Ncoa3
Hoechst
Ncoa3
E
Ncoa3
GFP
Ncoa3
control shRNA
Ncoa3 shRNA
F
average signal intensity in soma (basal=1)
1.2
1
0.8
0.6
0.4
0.2
0
**
Control shRNA
Ncoa3 shRNA

Figure S3

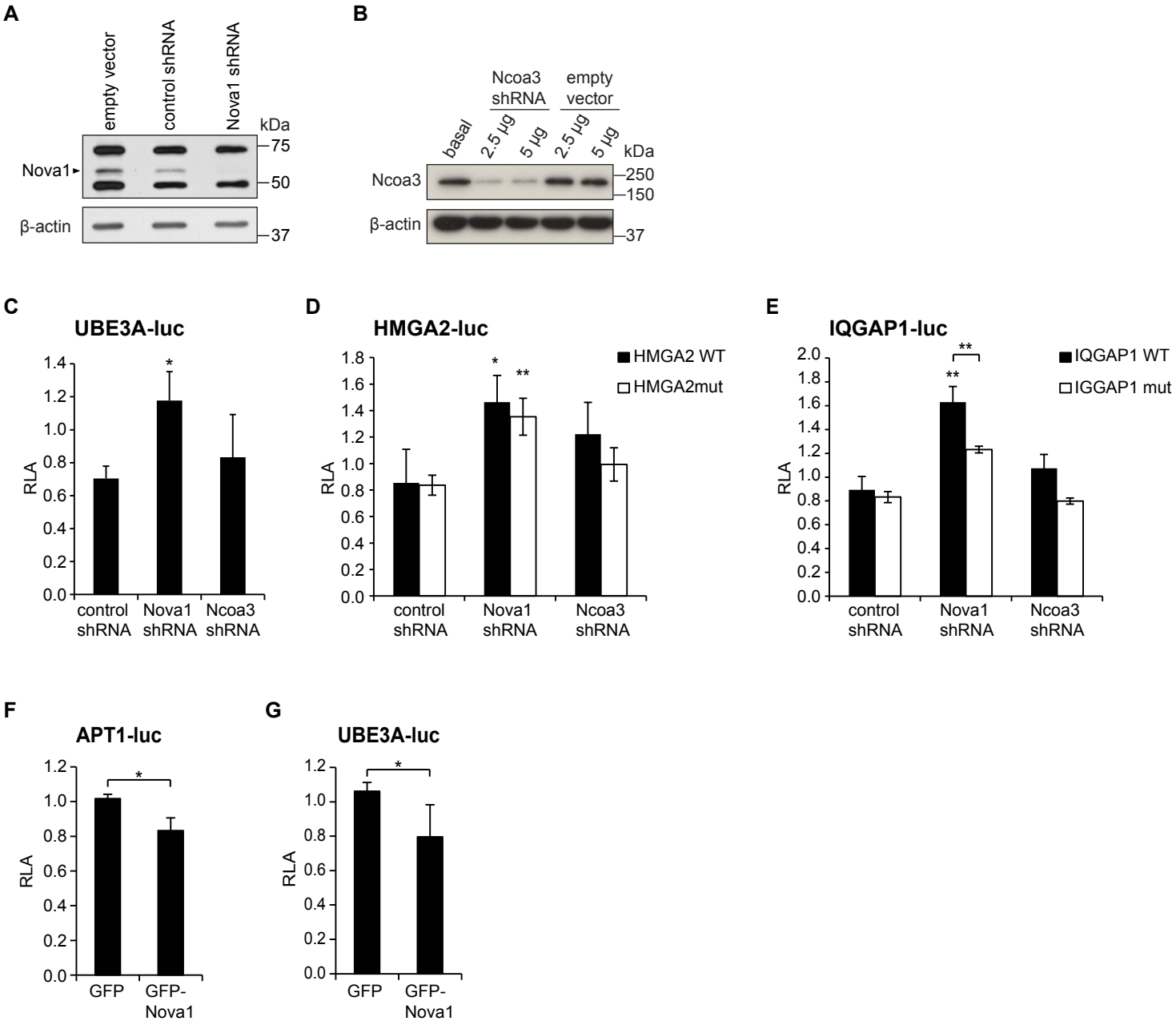

A
empty vector
control shRNA
Nova1 shRNA
kDa
Nova1
75
50
β-actin
37
B
Ncoa3 shRNA
empty vector
basal
2.5 μg
5 μg
2.5 μg
5 μg
kDa
Ncoa3
250
150
β-actin
37
C
UBE3A-luc
RLA
control shRNA
Nova1 shRNA
Ncoa3 shRNA
D
HMGA2-luc
HMGA2 WT
HMGA2mut
RLA
control shRNA
Nova1 shRNA
Ncoa3 shRNA
E
IQGAP1-luc
IQGAP1 WT
IGGAP1 mut
RLA
control shRNA
Nova1 shRNA
Ncoa3 shRNA
F
APT1-luc
RLA
GFP
GFP-Nova1
G
UBE3A-luc
RLA
GFP
GFP-Nova1

Figure S4

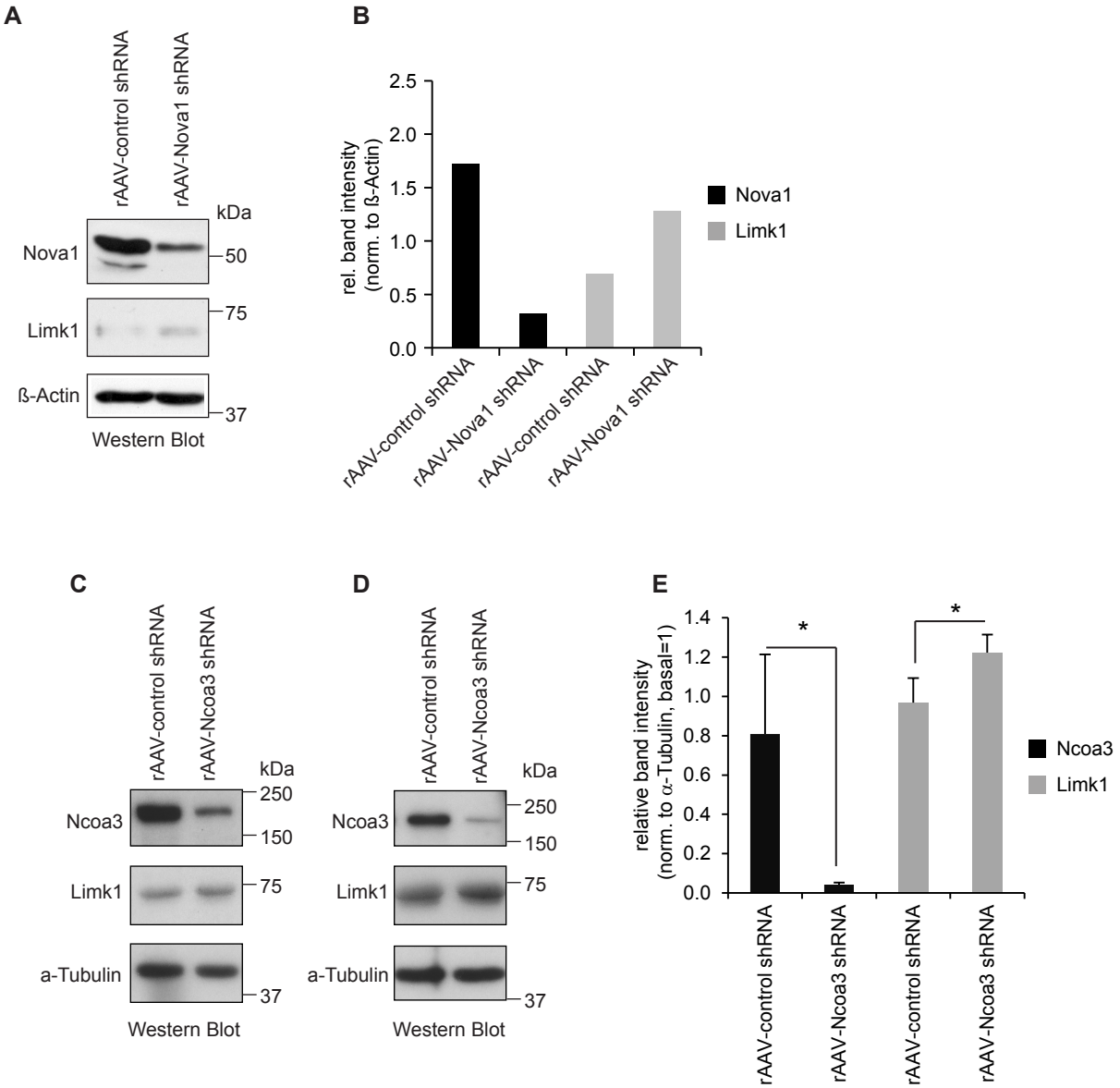

A
rAAV-control shRNA
rAAV-Nova1 shRNA
kDa
Nova1
50
Limk1
75
ß-Actin
37
Western Blot
B
rel. band intensity (norm. to ß-Actin)
2.5
2.0
1.5
1.0
0.5
0.0
Nova1
Limk1
rAAV-control shRNA
rAAV-Nova1 shRNA
rAAV-control shRNA
rAAV-Nova1 shRNA
C
rAAV-control shRNA
rAAV-Ncoa3 shRNA
kDa
Ncoa3
250
150
Limk1
75
a-Tubulin
37
Western Blot
D
rAAV-control shRNA
rAAV-Ncoa3 shRNA
kDa
Ncoa3
250
150
Limk1
75
a-Tubulin
37
Western Blot
E
relative band intensity (norm. to α-Tubulin, basal=1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Ncoa3
Limk1
rAAV-control shRNA
rAAV-Ncoa3 shRNA
rAAV-control shRNA
rAAV-Ncoa3 shRNA

Figure S5

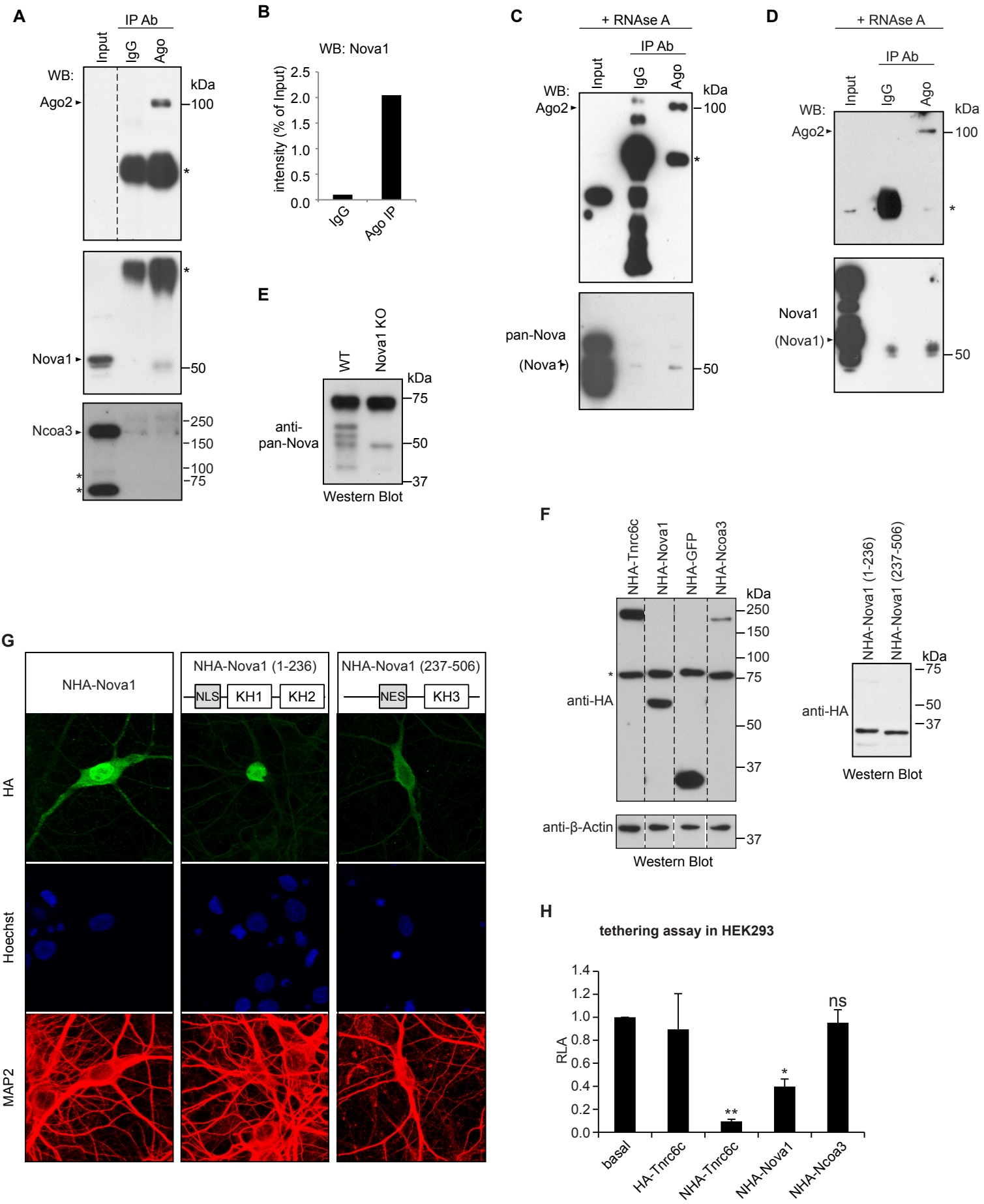

A
IP Ab
Input
IgG
Ago
WB:
Ago2
kDa
100
Nova1
50
Ncoa3
250
150
100
75
B
WB: Nova1
intensity (% of Input)
2.5
2.0
1.5
1.0
0.5
0.0
IgG
Ago IP
C
+ RNAse A
IP Ab
Input
IgG
Ago
WB:
Ago2
kDa
100
pan-Nova
(Nova1)
50
D
+ RNAse A
IP Ab
Input
IgG
Ago
WB:
Ago2
kDa
100
Nova1
(Nova1)
50
E
WT
Nova1 KO
kDa
75
anti-
pan-Nova
50
37
Western Blot
F
NHA-Tnrc6c
NHA-Nova1
NHA-GFP
NHA-Ncoa3
kDa
250
150
100
75
anti-HA
50
37
anti-β-Actin
37
Western Blot
NHA-Nova1 (1-236)
NHA-Nova1 (237-506)
kDa
75
anti-HA
50
37
Western Blot
G
NHA-Nova1
NHA-Nova1 (1-236)
NLS
KH1
KH2
NHA-Nova1 (237-506)
NES
KH3
HA
Hoechst
MAP2
H
tethering assay in HEK293
RLA
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ns
*
**
basal
HA-Tnrc6c
NHA-Tnrc6c
NHA-Nova1
NHA-Ncoa3

Figure S6

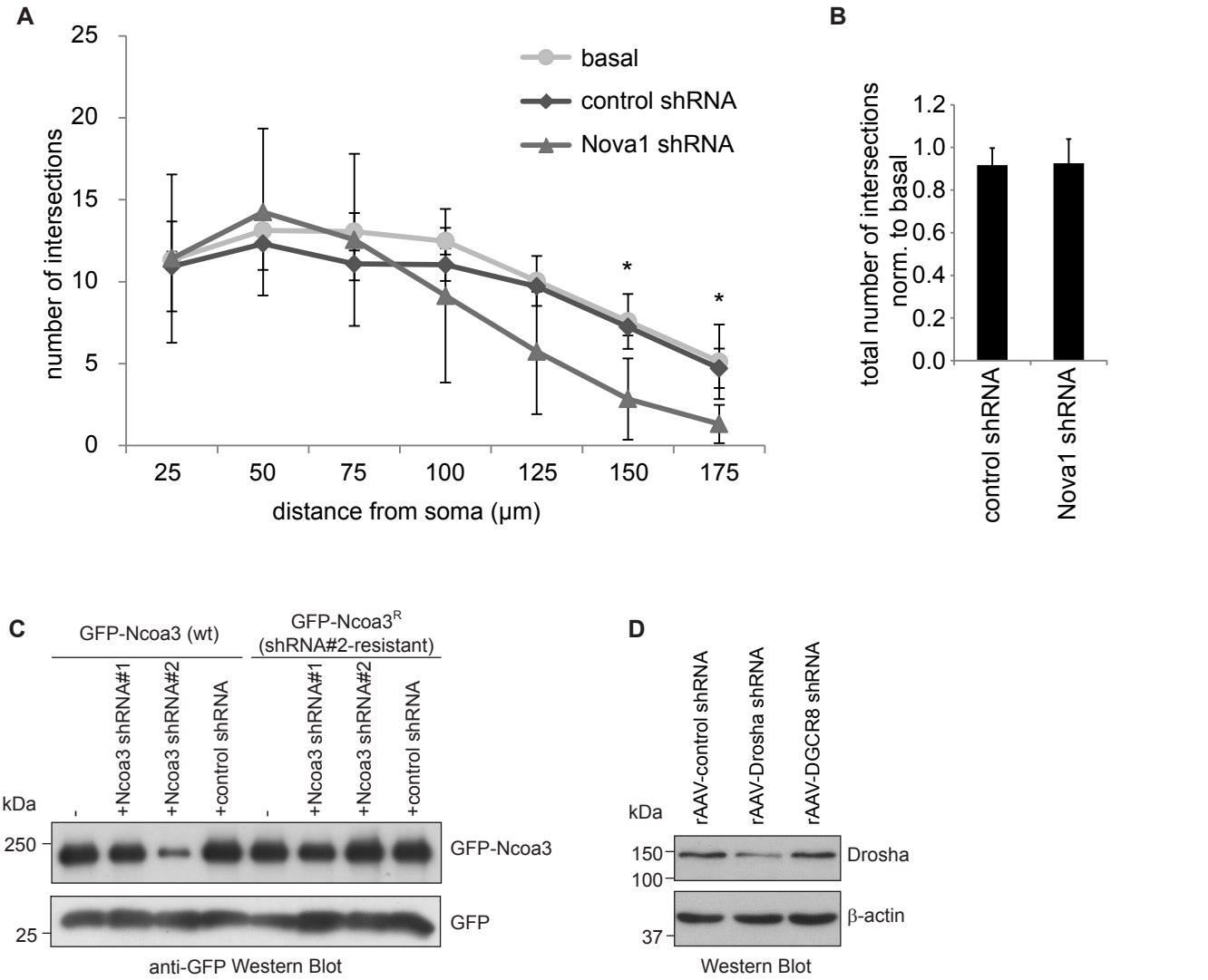
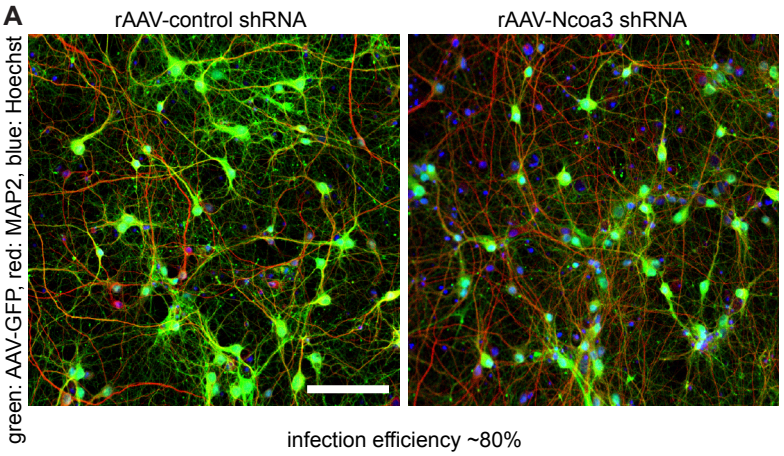

A
number of intersections
distance from soma (µm)
basal
control shRNA
Nova1 shRNA
B
total number of intersections norm. to basal
control shRNA
Nova1 shRNA
C
GFP-Ncoa3 (wt)
GFP-Ncoa3R (shRNA#2-resistant)
+Ncoa3 shRNA#1
+Ncoa3 shRNA#2
+control shRNA
kDa
250
GFP-Ncoa3
25
GFP
anti-GFP Western Blot
D
rAAV-control shRNA
rAAV-Drosha shRNA
rAAV-DGCR8 shRNA
kDa
150
100
Drosha
37
β-actin
Western Blot
E
rel. RNA levels (norm. to rAAV-control shRNA)
rAAV-control shRNA
rAAV-Drosha shRNA
drosha
pum2
F
rel. RNA amount (norm. to U6, rAAV-control shRNA=1)
AAV-control shRNA
AAV-Drosha shRNA
let-7c
mir-132
mir-134
mir-138

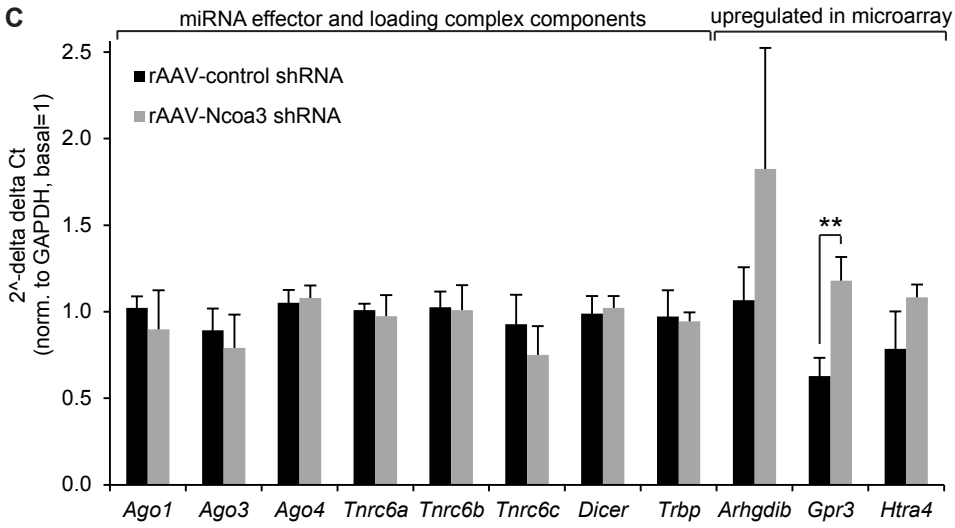
Figure S7

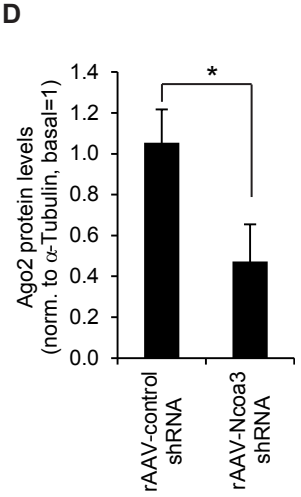


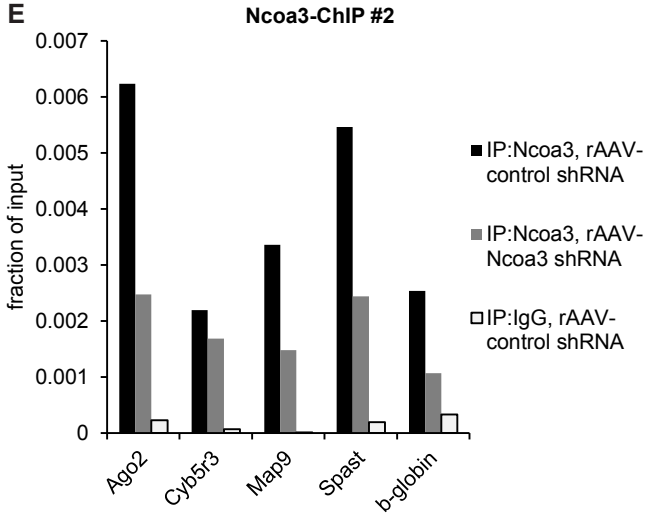
B

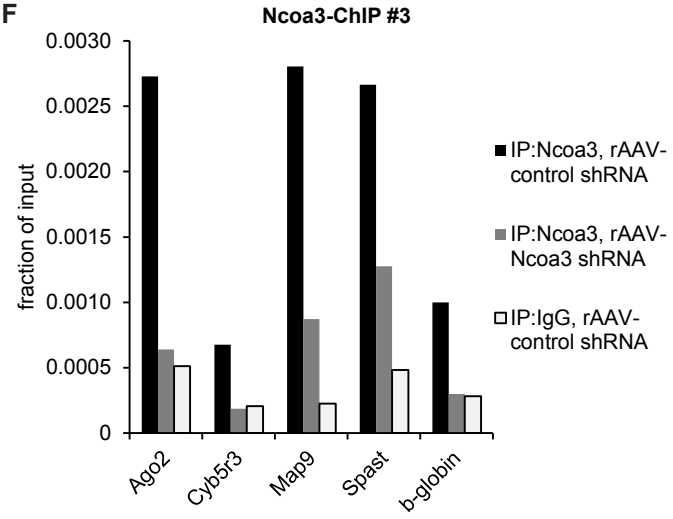
Pearson's correlation coefficients

		rAAV-control shRNA			rAAV-Ncoa3 shRNA		
		I	II	III	I	II	III
rAAV-control shRNA	I	1	0.994	0.976	0.995	0.992	0.975
	II	0.994	1	0.974	0.993	0.992	0.973
	III	0.976	0.974	1	0.974	0.975	0.994
rAAV-Ncoa3 shRNA	I	0.995	0.993	0.974	1	0.993	0.974
	II	0.992	0.992	0.975	0.993	1	0.975
	III	0.975	0.973	0.994	0.974	0.975	1





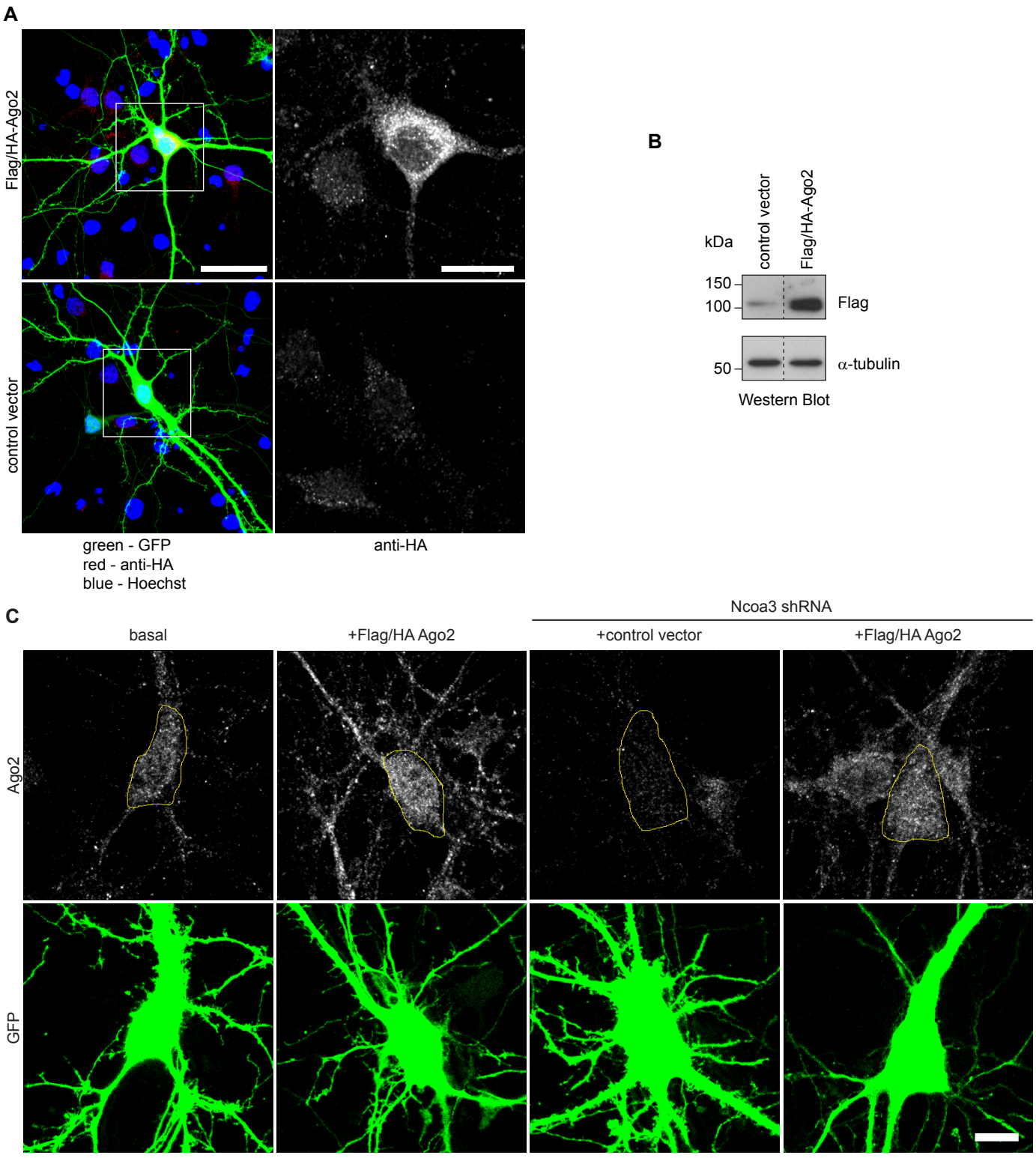




G

```
Mmu: GCCGGGAGGTGGCGGTGTGGTCACGCGCACGGGTCTGGCCGCGTCCAAGTTCAAGCTCTG
Rno: GCCGGGAGGTGGCGGTGTGGTCACGCGCACGGGTCTGGCCGCGTCCAAGTTCAAGNNNNN
Hsa: GCCGGGAGGTGGCGGTGTGGTCCTGCGCGAGCGGCCGGCCGCGTTCAAGTTCAAGCTCCG
```

Figure S8


A
Flag/HA-Ago2
control vector
green - GFP
red - anti-HA
blue - Hoechst
anti-HA
B
kDa
control vector
Flag/HA-Ago2
150
100
Flag
50
α-tubulin
Western Blot
C
Ncoa3 shRNA
basal
+Flag/HA Ago2
+control vector
+Flag/HA Ago2
Ago2
GFP