

1 **Supplementary information to:**

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3 Growth Cone Localization of the mRNA Encoding the Chromatin Regulator HMGN5
4 Modulates Neurite Outgrowth

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17 Running Head: *Hmgn5* mRNA localization controls neurite outgrowth

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1 **Figure S1. Validation of the effectiveness of the EHNA treatment.** The anterograde
2 and retrograde movement of acidic organelles in N1E-115 cells was imaged using 20 nM
3 LysoTracker green dye 20 minutes before imaging. Representative micrographs of cells
4 treated with either vehicle (A) or 1mM EHNA 1 hour before imaging (B). White
5 arrowheads point at retrograde movement while magenta arrowheads point at anterograde
6 movement. The neurite runs distally off bottom of the frame in panel (A) and off top of
7 the frame in panel (B). Scale bars: 20 μ m. Time scale is in minutes:seconds.

8

9 **Figure S2. Transcriptomic analysis of *Hmgn5* KD N1E-115 cells.** Total RNA (n=3
10 preparations) was extracted from control (non-differentiated and differentiated) and
11 *Hmgn5* KD cells and subjected to microarray analysis. (A) List of all the genes whose
12 expression is significantly affected by *Hmgn5* KD. “kd vs ctrl” indicates the difference in
13 expression levels between *Hmgn5* KD and differentiated control cells while “4h vs 24h”
14 indicates the difference in expression levels between non-differentiated and differentiated
15 control cells. Up-regulated genes are presented in shades of green, while down-regulated
16 genes are presented in shades of red. All genes with a positive B-statistics (posterior
17 probability of being differentially expressed) were considered to be differentially
18 expressed. (B) Validation of part of the microarray data by RT-qPCR (on two
19 independent RNA preparations, mean \pm s.e.m). Microarray data are presented as mean \pm
20 s.e.m (n=3).

21

22 **Figure S3. mRNA localization and protein expression levels of HMGN5-GFP**
23 **constructs in N1E-115 cells.** (A) Confocal fluorescence micrographs of FISH with

1 riboprobes anti-sense and sense (negative control) to *GFP* mRNA. N1E-115 cells were
2 transfected with the different rescue/overexpression constructs and then subjected to
3 FISH analysis. FISH signal is represented in ibw contrast. Black arrowheads indicate
4 punctate structures. Scale bars: 20 μ m. (B) Western blot analysis with anti-GFP and anti-
5 α tubulin antibodies and quantification to show that the different rescue/overexpression
6 constructs are expressed to approximately the same level in N1E-115 cells (n=3
7 experiments, mean \pm s.e.m.).

8

9 **Figure S4. Chromatin analysis of HMGN5-GFP expression constructs.** (A)

10 Representative micrographs of DAPI stained N1E-115 cells transfected with GFP,
11 HMGN5-GFP, HMGN5-GFP-3'UTR, HMGN5S17,21E-GFP or HMGN5S17,21E-GFP-
12 3'UTR. GFP signal is shown in green while DAPI staining is shown in ibw contrast.
13 Scale bar: 10 μ m. (B) Measurement of the number of heterochromatic foci in the DAPI
14 staining of N1E-115 cells transfected with GFP, HMGN5-GFP, HMGN5-GFP-3'UTR,
15 HMGN5S17,21E-GFP or HMGN5S17,21E-GFP-3'UTR (n= 20 cells).

16

17 **Figure S5. mRNA localization of HMGN5-GFP constructs in hippocampal neurons.**

18 Confocal fluorescence micrographs of FISH with riboprobes anti-sense and sense
19 (negative control) to *GFP* mRNA. Hippocampal neurons were transfected with the
20 different rescue/overexpression constructs and then subjected to FISH analysis. FISH
21 signal is represented in ibw contrast while F-actin staining is in cyan. Black arrowheads
22 indicate punctate structures. Scale bar: 20 μ m for whole cell micrographs, 5 μ m for
23 growth cone micrographs.

1

2 **Figure S6. Analysis of axonal specification in *Hmgn5* KD hippocampal neurons.**

3 Confocal fluorescence micrographs of hippocampal neurons transfected with control or
4 *Hmgn5* siRNA and GFP and stained with anti-MAP2 and anti-SMI312 antibodies.
5 Neurons were fixed either at 3 or 7 DIV. Scale bar: 20 μ m.

6

7 **Table S1.** List of all the primers used in this study.

8

9 **Movie S1. Visualization of growth cone mRNA translation using PalX2-Dendra2**

10 **reporters.** Time-lapse imaging of growth cones of PalX2-Dendra2 and PalX2-
11 Dendra2/*Hmgn5* 3'UTR transfected N1E-115 cells, treated or non-treated with 40 μ m
12 anisomycin 30 minutes before bleaching. Pre-bleaching images and fluorescence
13 recovery after bleaching time-lapses are shown. The images are color-coded so that warm
14 and cold colors represent high and low fluorescence intensity. Note that all growth cones
15 are in the protrusive phase. Timescale is in minutes:seconds. Scale bars: 20 μ m.

16

17 **Movie S2. Visualization of anterograde and retrograde transport using LysoTracker**

18 **in N1E-115 cells treated with vehicle.** Time-lapse green fluorescence imaging of N1E-
19 115 cells treated with vehicle and 20 nM LysoTracker dye 20 minutes before imaging.
20 The arrowheads (white for retrograde movement and magenta for anterograde movement)
21 refer to the micrographs presented in Fig. S1A. The neurite runs distally off bottom of the
22 frame. Timescale is in minutes:seconds. Scale bars: 20 μ m.

23

1 **Movie S3. Visualization of anterograde and retrograde transport using LysoTracker**
2 **in N1E-115 cells treated with EHNA.** Time-lapse green fluorescence imaging of N1E-
3 115 cells treated with 1mM EHNA 1 hour before imaging and 20 nM LysoTracker dye
4 20 minutes before imaging. The arrowhead (magenta for anterograde movement) refers to
5 the micrographs presented in Fig. S1B. The neurite runs distally off top of the frame.
6 Timescale is in minutes:seconds. Scale bars: 20 μ m. While anterograde movement is not
7 perturbed, treatment with EHNA causes inhibition of retrograde movement.

8

9 **Movie S4. Neurite outgrowth dynamics of control and *Hmgn5* KD N1E-115 cells.**
10 Phase-contrast time-lapse imaging of control and *Hmgn5* KD N1E-115 cells. Timescale
11 is in hours:minutes. Scale bars: 50 μ m. Note that control cells establish long neurites over
12 time, while *Hmgn5* KD cells fail to do so.

13

14 **Movie S5. Representative movie of the histone H1 FRAP analysis.** Time-lapse green
15 fluorescence imaging of an N1E-115 cell nucleus transfected with histone H1-GFP and
16 mRuby2. Pre-bleaching image, bleaching and fluorescence recovery after bleaching are
17 shown. The images are presented in black and white contrast. Timescale is in
18 seconds:milliseconds. Scale bar: 3 μ m.

19

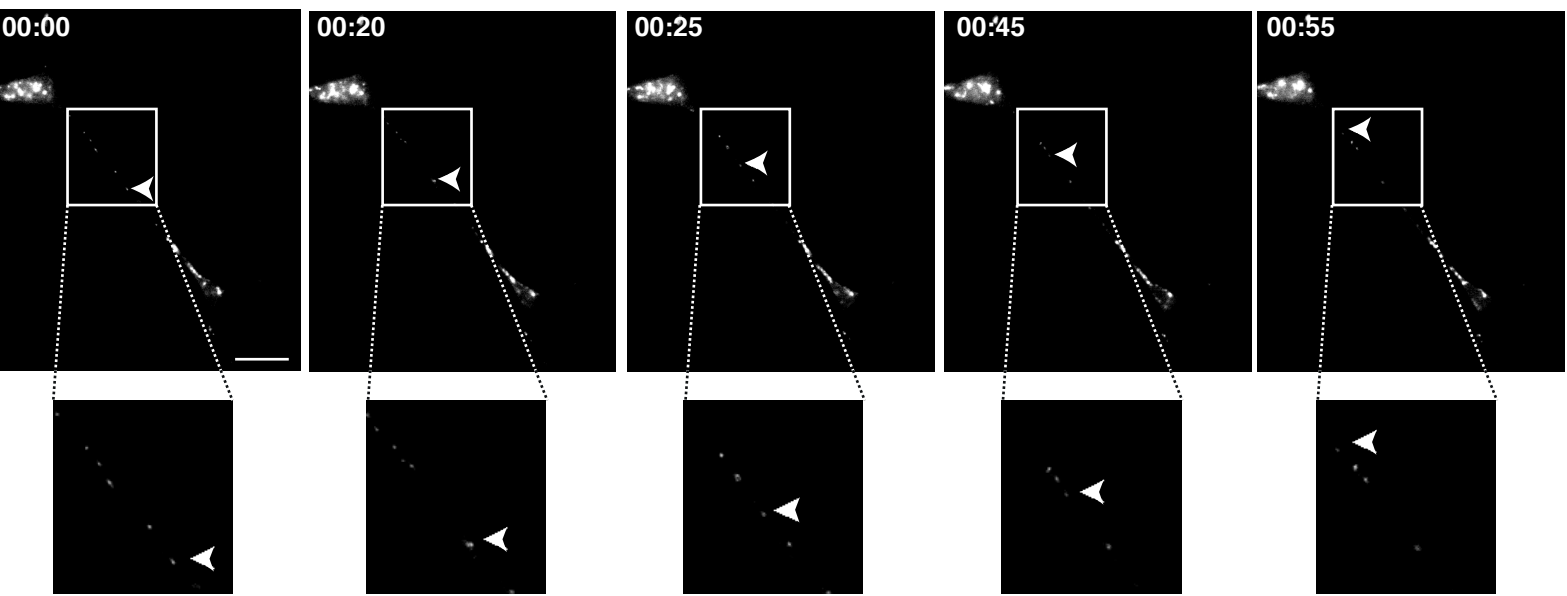
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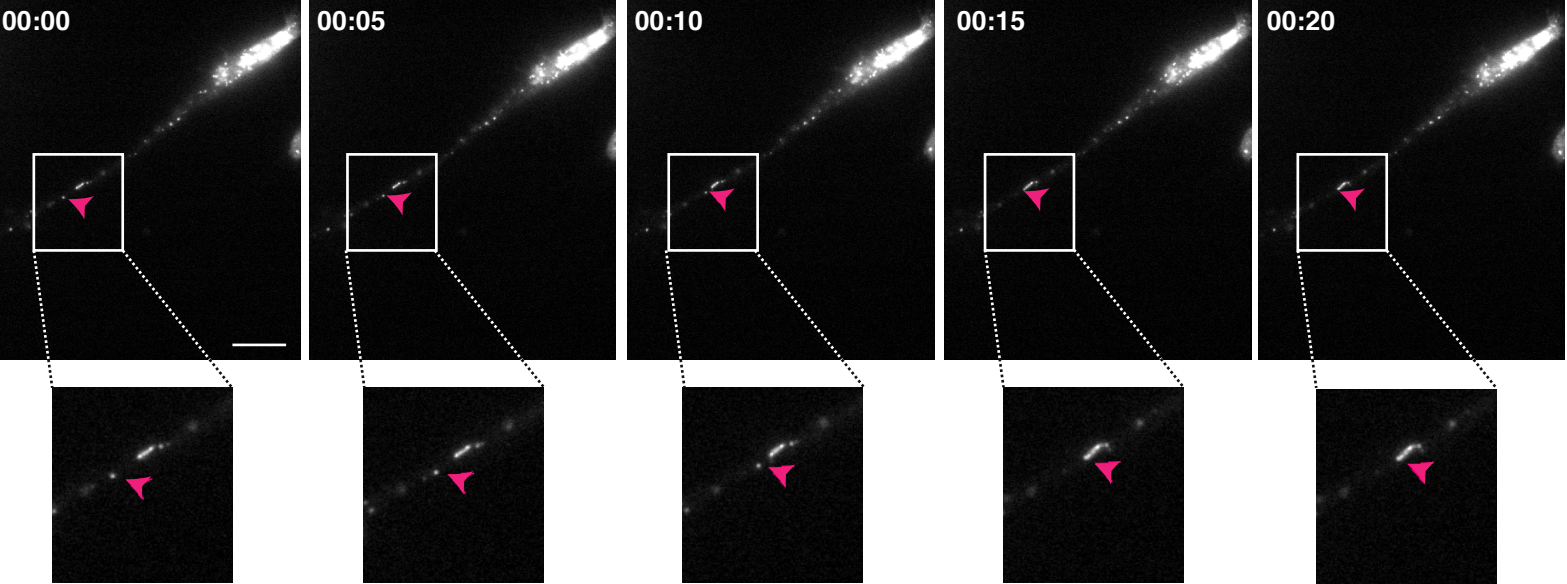
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A Vehicle



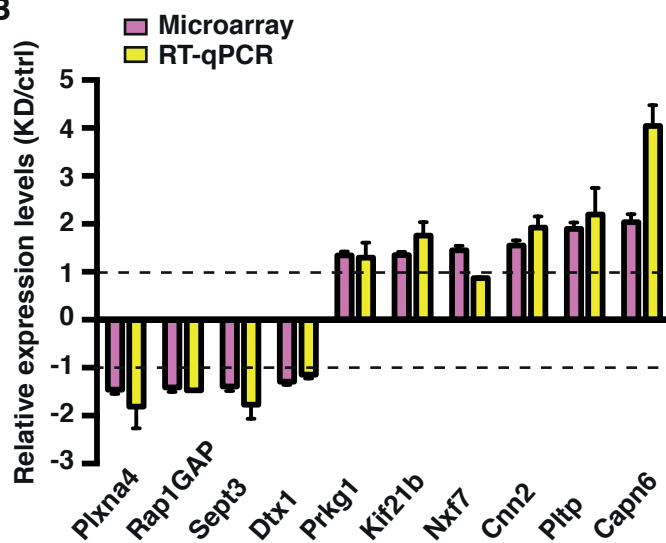
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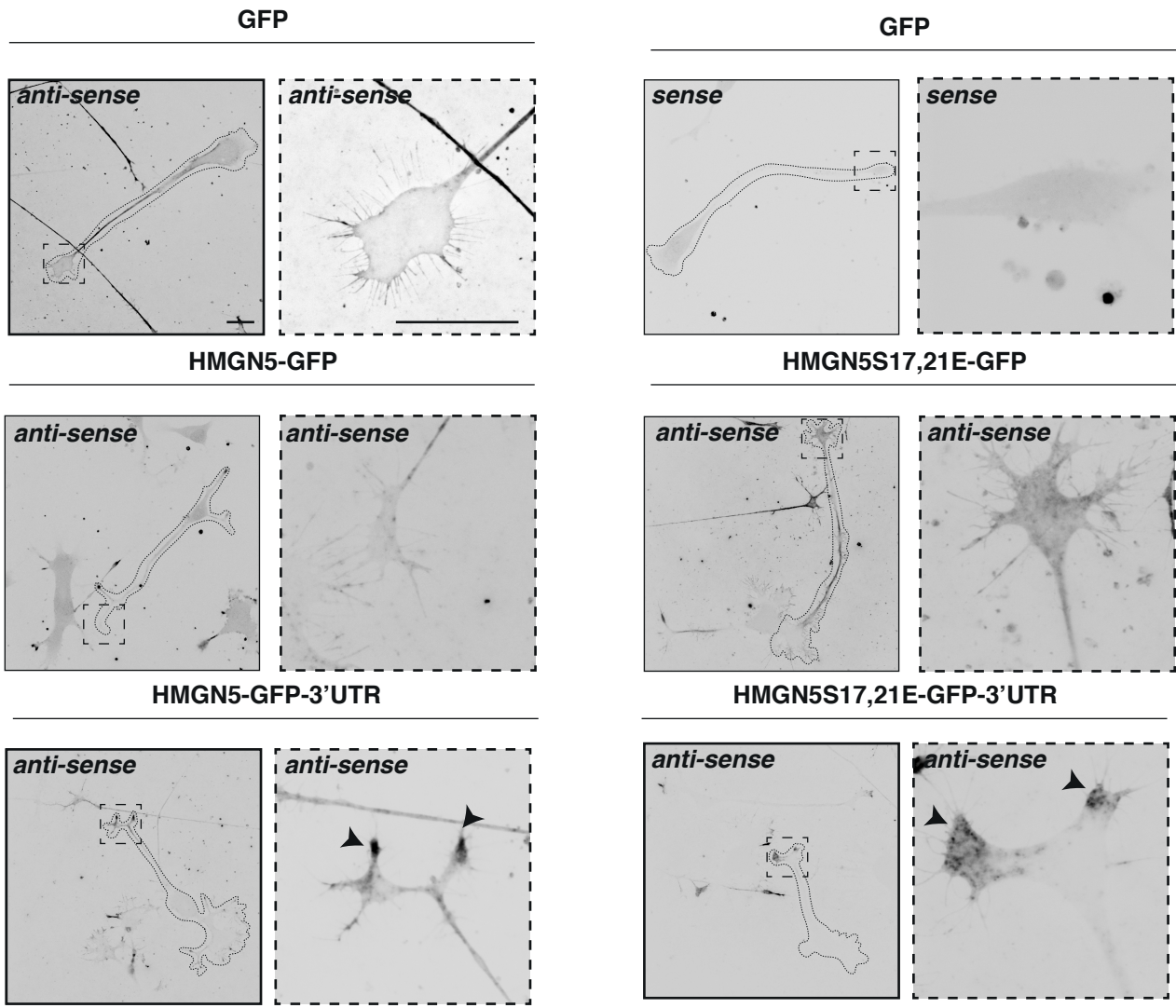
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| Symbol | GeneName | kd vs ctrl | B statistics | 4h vs 24h | B statistics |
|----------|--|------------|--------------|-----------|--------------|
| Igfbp5 | insulin-like growth factor binding protein 5 | 2.09 | 4.39 | | |
| Capn6 | calpain 6 | 2.03 | 3.89 | 2.28 | 6.05 |
| Pltp | phospholipid transfer protein | 1.89 | 4.01 | 1.81 | 2.57 |
| Gm885 | predicted gene 885 | 1.68 | 0.06 | | |
| Cnn2 | calponin 2 | 1.55 | 2.28 | 2.33 | 6.29 |
| Olfir763 | olfactory receptor 763 | 1.52 | 0.49 | | |
| Gm5424 | argininosuccinate synthase pseudogene | 1.49 | 0.97 | 1.64 | 4.43 |
| Necab1 | N-terminal EF-hand calcium binding protein 1 | 1.48 | 0.78 | | |
| Nxf7 | nuclear RNA export factor 7 | 1.45 | 1.64 | 2.08 | 5.63 |
| Nrp2 | neuropilin 2 | 1.41 | 2.29 | | |
| Nupr1 | nuclear protein 1 | 1.39 | 0.79 | 1.75 | 3.34 |
| Adam12 | a disintegrin and metallopeptidase domain 12 | 1.39 | 1.12 | | |
| Radil | Ras association and DIL domains | 1.37 | 1.69 | | |
| Sparc | secreted acidic cysteine rich glycoprotein | 1.36 | 0.46 | | |
| Kif21b | kinesin family member 21B | 1.35 | 1.12 | 1.71 | 3.80 |
| Prkg1 | protein kinase, cGMP-dependent, type I | 1.34 | 0.20 | 1.66 | 4.14 |
| Fam129a | family with sequence similarity 129, member A | 1.34 | 1.34 | 1.42 | 1.00 |
| Tmem173 | transmembrane protein 173 | 1.32 | 0.07 | 1.46 | 1.51 |
| Dtx1 | deltex 1 homolog (Drosophila) | -1.29 | 0.40 | -1.41 | 1.22 |
| Csdc2 | cold shock domain containing C2, RNA binding | -1.30 | 0.00 | -1.44 | 0.71 |
| BC005764 | cDNA sequence BC005764 | -1.31 | 0.06 | -1.30 | 1.46 |
| Pdzk1ip1 | PDZK1 interacting protein 1 | -1.37 | 0.11 | -1.90 | 5.11 |
| septin3 | septin 3 | -1.39 | 0.32 | | |
| Tle1 | transducin-like enhancer of split 1 | -1.40 | 0.11 | -1.86 | 4.70 |
| Rsph4a | radial spoke head 4 homolog A (Chlamydomonas) | -1.40 | 0.07 | 1.39 | 0.06 |
| Rap1gap | Rap1 GTPase-activating protein | -1.40 | 0.30 | -1.53 | 5.48 |
| Apol11b | apolipoprotein L 11b | -1.41 | 0.34 | | |
| Plxna4 | plexin A4 | -1.45 | 1.72 | | |
| Sctr | secretin receptor | -1.48 | 0.68 | | |
| Cacna1i | calcium channel, voltage-dependent, alpha 1I subunit | -1.87 | 3.52 | -1.95 | 5.33 |
| Hmgn5 | high-mobility group nucleosome binding domain 5 | -2.30 | 3.51 | | |
| Mettl7a3 | methyltransferase like 7A3 | -2.35 | 1.65 | | |

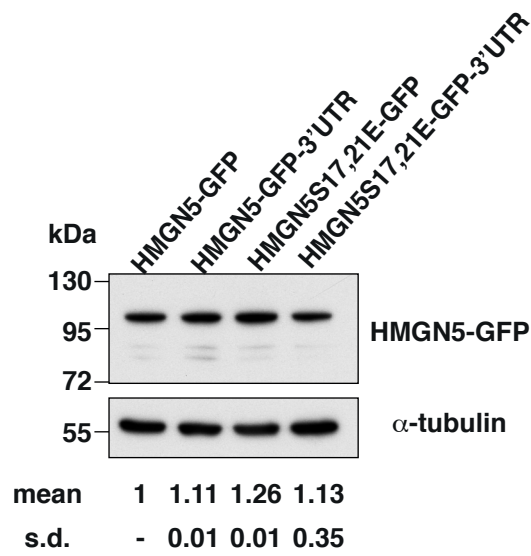
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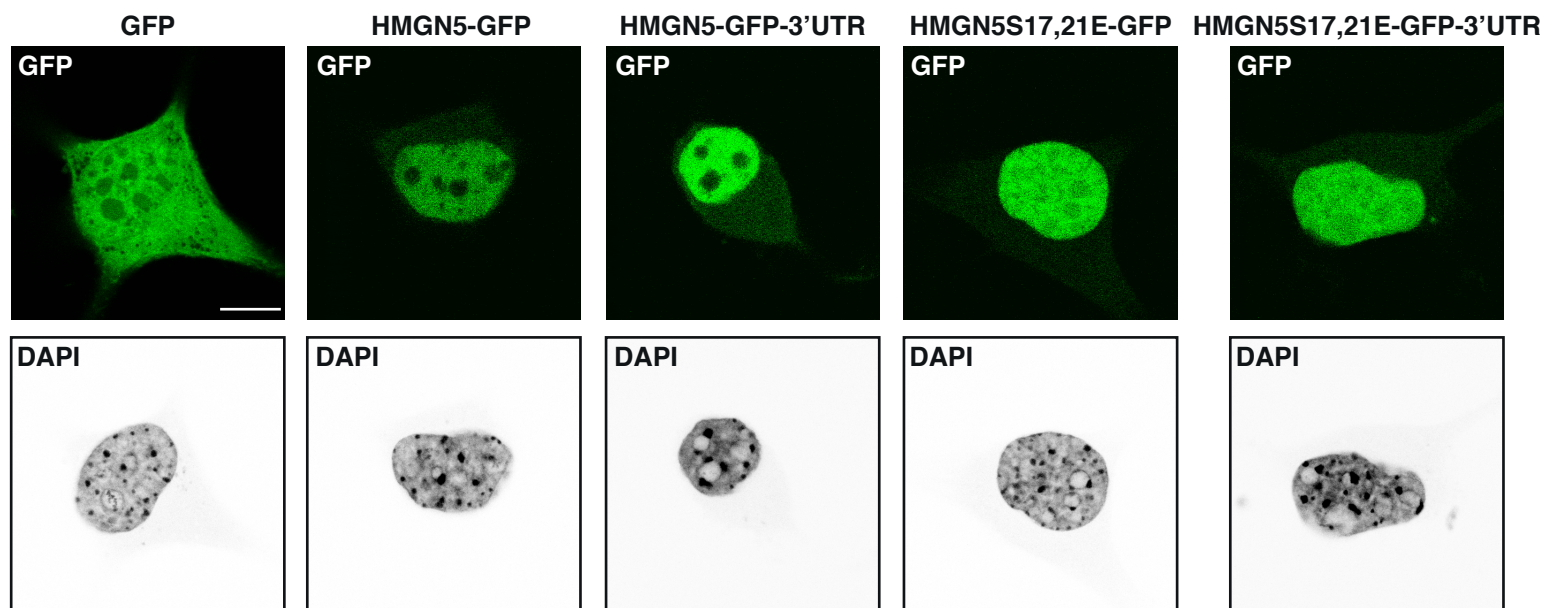
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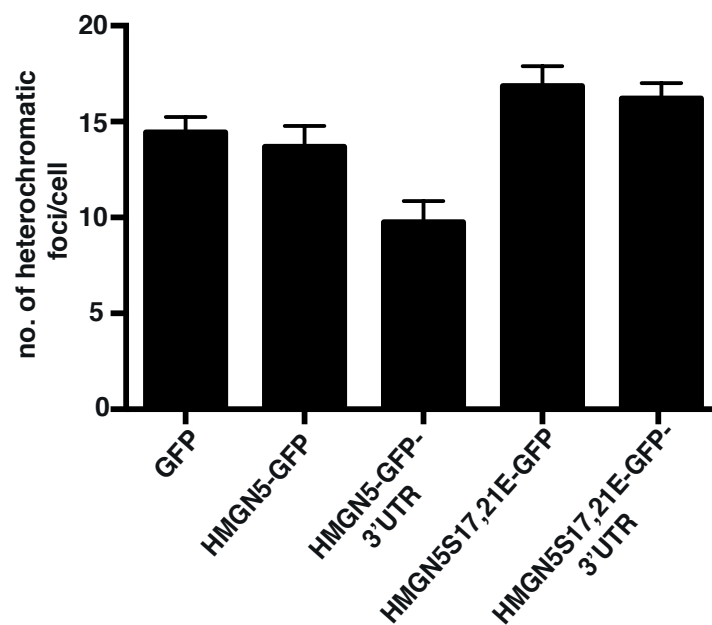
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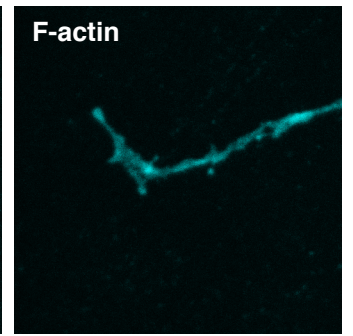
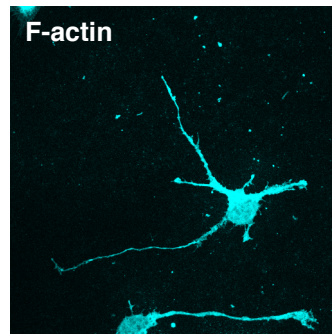
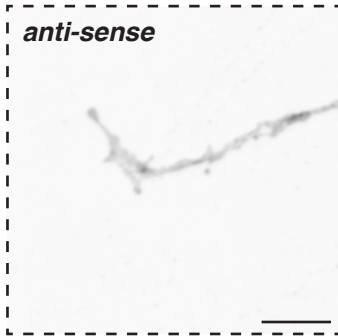
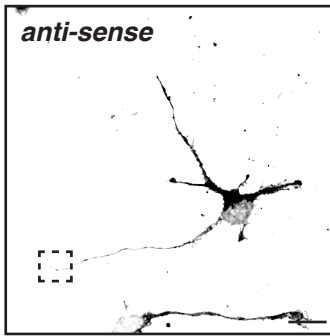
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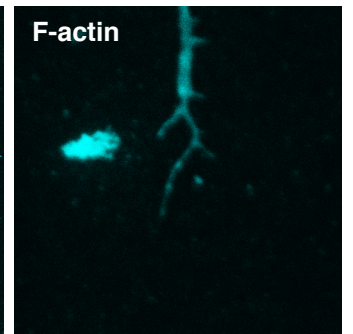
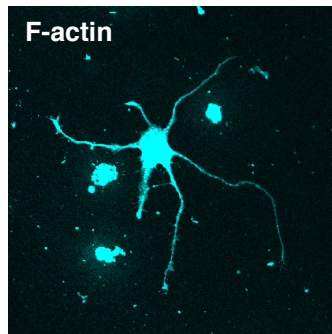
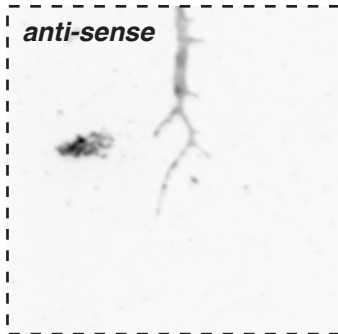
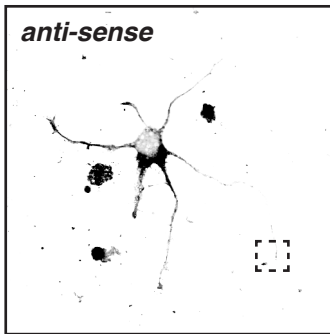
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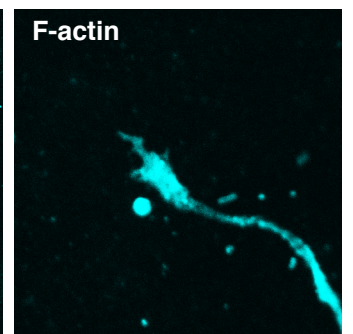
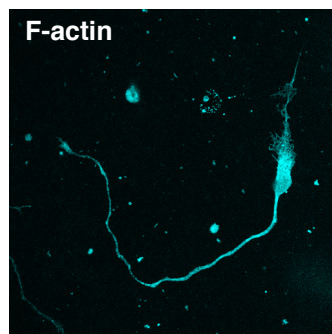
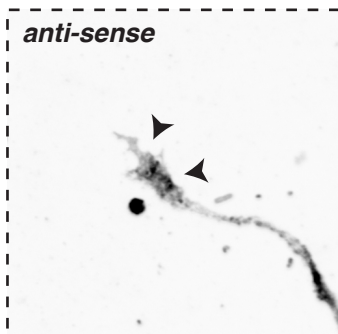
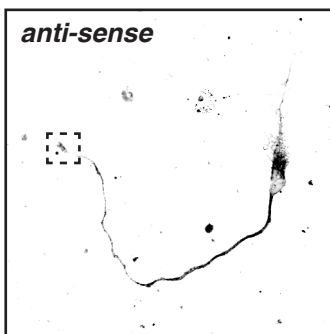
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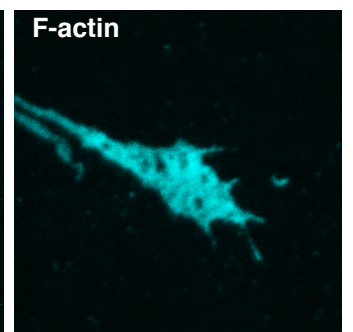
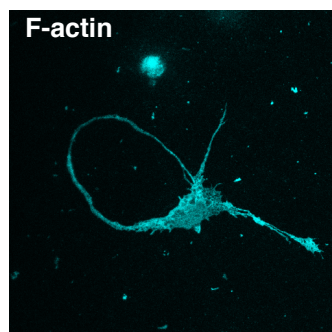
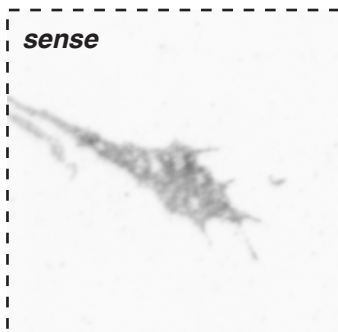
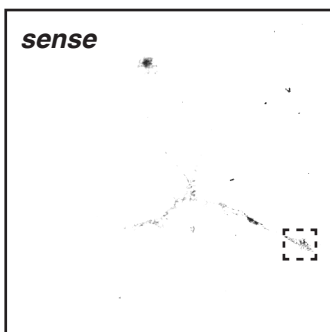
HMGN5-GFP



HMGN5-GFP-3'UTR



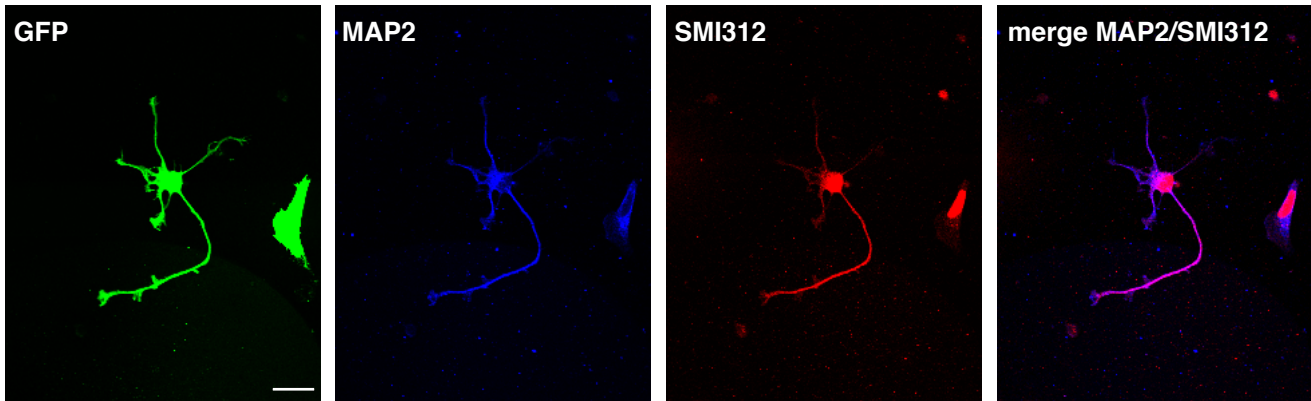
GFP



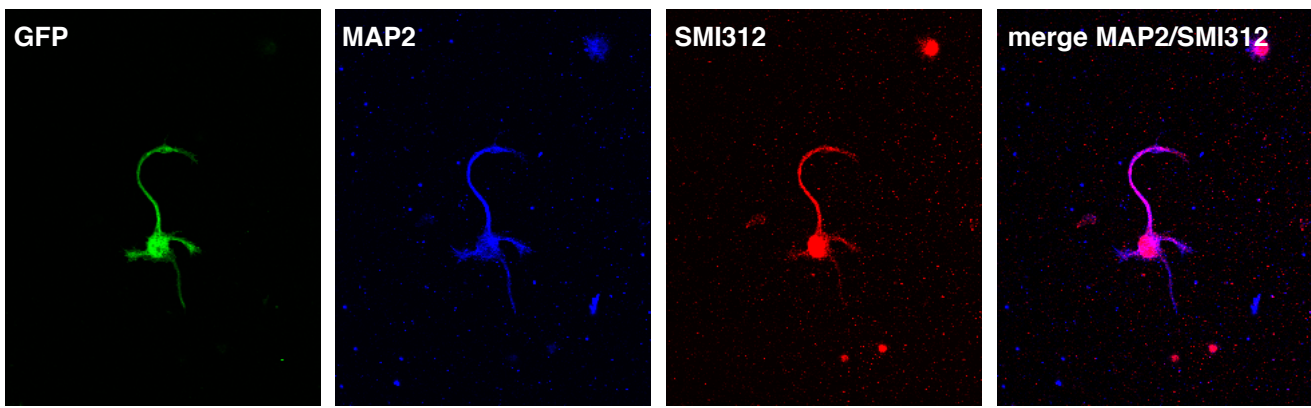
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DIV 3

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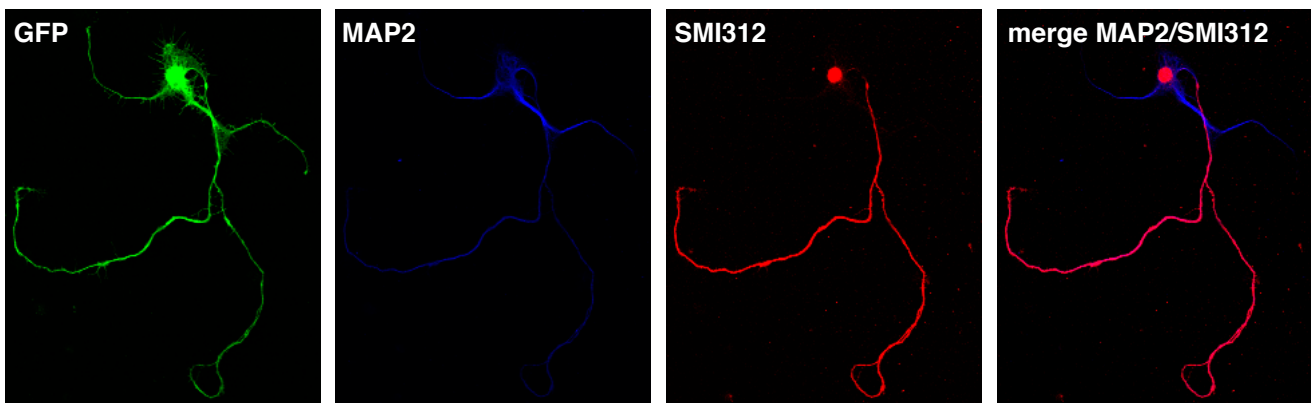


Hmgn5 siRNA

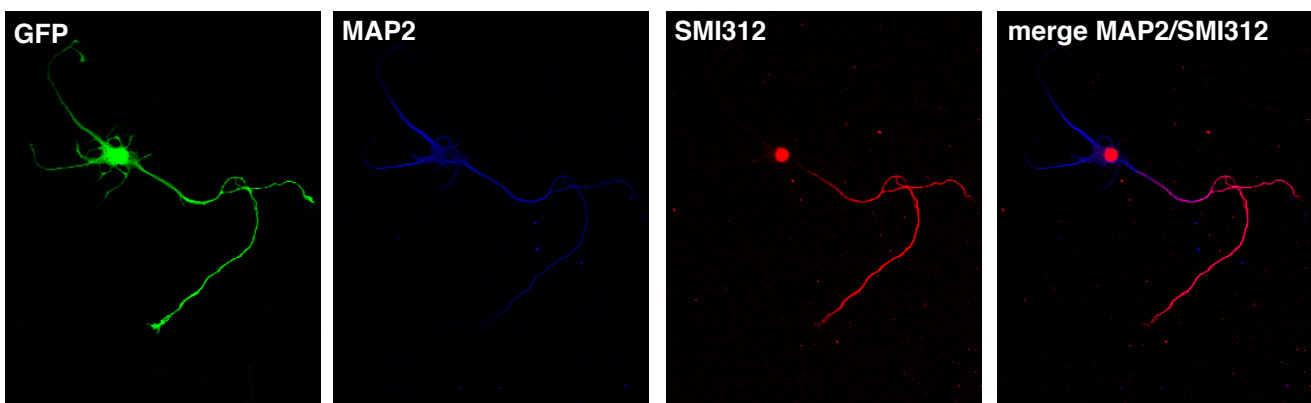


DIV 7

ctrl siRNA



Hmgn5 siRNA



Moretti_Table S1

| | |
|----------------------|---|
| Hmgn5 qPCR fw | AAAGAAAGGCTGCAGGTG |
| Hmgn5 qPCR rv | GTAAAGGGCACAGGCATAG |
| Snord15b qPCR fw | CAGAATGGCCACGTCTTGC |
| Snord15b qPCR rv | TCAATCAGTGCGCAGGACAC |
| Hmgn5 FISH fw | GGATCCGTTGCTGTAGATGAAGGGAG |
| Hmgn5 FISH rv | GGAATTCTCCTACTCTGC |
| Rpl19 qPCR fw | ACCCTGGCCCGACGG |
| Rpl19 qPCR rv | TACCCTTCCTCTCCCTATGCC |
| GFP FISH fw | GGATCCATCCTGGTCGAGCTGGAC |
| GFP FISH rv | GAATTCGTCCTCGATGTTGTGG |
| Hmgn5 3'UTR fw | GCGGCCGCGAGGAGCCTCTGAGTATTGTC |
| Hmgn5 3'UTR rv | GCGGCCGCTCATATTTGTGGAACTC |
| Hmgn5 CDS fw | GAATTCATGCCCAAAGAAAGGC |
| Hmgn5 CDS rv | GGATCCCGGACAATACTCAGAGG |
| Hmgn5 CDS SE mut fw | CCAAAGAGAAGAGAAGCCCGACTGGAGGCTATGCCTGTGC |
| Hmgn5 CDS SE mut rv | GCACAGGCATAGCCTCCAGTCGGGCTTCTCTTCTCTTTGG |
| Hmgn5 CDS res mut fw | CGGAAAAATAGAAGAGGAGGGACTCAATGAAAAACCAGGTACAGC |
| Hmgn5 CDS res mut rv | GCTGTACCTGGTTTTTCATTGAGTCCCTCCTCTTCTATTTTTCCG |
| Dendra no pal fw | GGATCCCATGAACACCCCGGAATTAACCTG |
| Plxna4 qPCR fw | CAGCAGTGCGCTCCTTAC |
| Plxna4 qPCR rv | AGACATAGGCGATGACAGAAG |
| Rap1GAP qPCR fw | AGAGTGTGTGGAGGAGTGATG |
| Rap1GAP qPCR rv | GTGTTCTTAGGGTAGGGTGAAG |
| Sept3 qPCR fw | ATGGTCGTTGGCCAGAGTG |
| Sept3 qPCR rv | AGAGGGTGTGACCAGTGTTG |
| Dtx1 qPCR fw | GCCTGATGAGGACTGTACC |
| Dtx1 qPCR rv | CAGGCAGAGCAGGTGATAC |
| Prkg1 qPCR fw | CCTCGAAGAGACCCACTATG |
| Prkg1 qPCR rv | TAACATTCACCTGCCCTTTAC |
| Kif21b qPCR fw | AGCCTATGGACAGACAGG |
| Kif21b qPCR rv | CTGCTCCTCTTCTGATGTC |
| Nxf7 qPCR fw | CCGTCAGTAAGACACACACC |
| Nxf7 qPCR rv | CTTCCTCCCTACTCCTATGTTG |
| Cnn2 qPCR fw | ATCCTATGCACACTCATGAAC |
| Cnn2 qPCR rv | CCTCAAACAGGTCCACAG |
| Pltp qPCR fw | CTCTGGATCTGGTGAAGC |
| Pltp qPCR rv | CTCACGTCCGAGATATTGTAG |
| Capn6 qPCR fw | ACTGGACAAAGGCAATTC |
| Capn6 qPCR rv | AAAGATTCCAGCGTATTTCTC |
| Hmgn5 CDS pCAG fw | GCTAGCATGCCCAAAGAAAGGC |
| Hmgn5 3'UTR pCAG rv | AGATCTTCATATTTGTGGAACTC |
| GFP 3'UTR pCAG rv | CTCGAGTACTTGTACAGCTCGTCC |