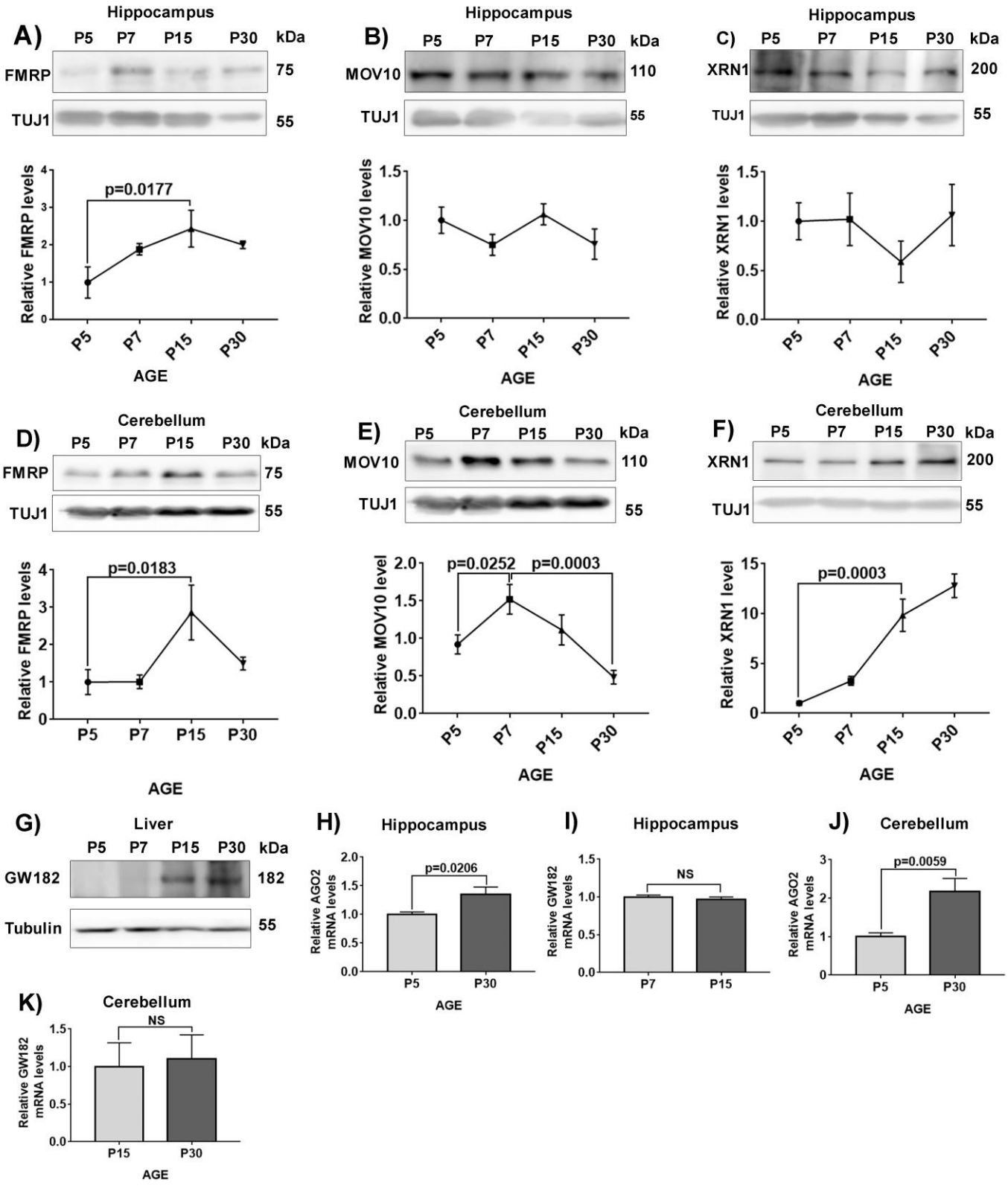

Fig. S1. A) Representative immunoblots (top) and line graph (bottom) depicting FMRP expression profile during hippocampal development. Data represent relative FMRP levels normalized to TUJ1, Data: mean +/- SEM, n=3-5 animals per group. One Way ANOVA followed by Bonferroni's multiple comparisons test.

B) Representative immunoblots (top) and line graph (bottom) depicting MOV10 expression profile during hippocampal development. Data represent relative MOV10 levels normalized to TUJ1, Data: mean +/- SEM, n=3-5 animals per group. One Way ANOVA followed by Bonferroni's multiple comparisons test.

C) Representative immunoblots (top) and line graph (bottom) depicting XRN1 expression profile during hippocampal development. Data represent relative XRN1 levels normalized to TUJ1, Data: mean +/- SEM, n=3-5 animals per group. One Way ANOVA followed by Bonferroni's multiple comparisons test.

D) Representative immunoblots (top) and line graph (bottom) depicting FMRP expression profile during Cerebellar development. Data represent relative FMRP levels normalized to TUJ1, Data: mean +/- SEM, n=3-5 animals per group. One Way ANOVA followed by Bonferroni's multiple comparisons test.

E) Representative immunoblots (top) and line graph (bottom) depicting MOV10 expression profile during Cerebellum development. Data represent relative MOV10 levels normalized to TUJ1, Data: mean +/- SEM, n=3-5 animals per group. One Way ANOVA followed by Bonferroni's multiple comparisons test.

F) Representative immunoblots (top) and line graph (bottom) depicting XRN1 expression profile during cerebellum development. Data represent relative XRN1 levels normalized to TUJ1, Data: mean +/- SEM, n=3-5 animals per group. One Way ANOVA followed by Bonferroni's multiple comparisons test.

G) Representative immunoblots depicting GW182 expression profile during liver development.

H) qPCR analysis of AGO2 mRNA expression in P5 and P30 hippocampus. The levels of AGO2 mRNA were normalized using the expression of ß-actin mRNA, Data: mean +/- SEM, n=3-5 independent experiments, Unpaired t-test.

I) qPCR analysis of GW182 mRNA expression in P7 and P15 hippocampus. The levels of GW182 mRNA were normalized using the expression of ß-actin mRNA, Data: mean +/- SEM, n=3-5 independent experiments, Unpaired t-test.

J) qPCR analysis of AGO2 mRNA expression in P5 versus P30 Cerebellum. The levels of AGO2 mRNA were normalized using the expression of ß-actin mRNA, Data: mean +/- SEM, n=3-5 independent experiments, Unpaired t-test.

K) qPCR analysis of GW182 mRNA expression in P15 versus P30 Cerebellum. The levels of GW182 mRNA were normalized using the expression of ß-actin mRNA, Data: mean +/- SEM, n=3-5 independent experiments, Unpaired t-test.

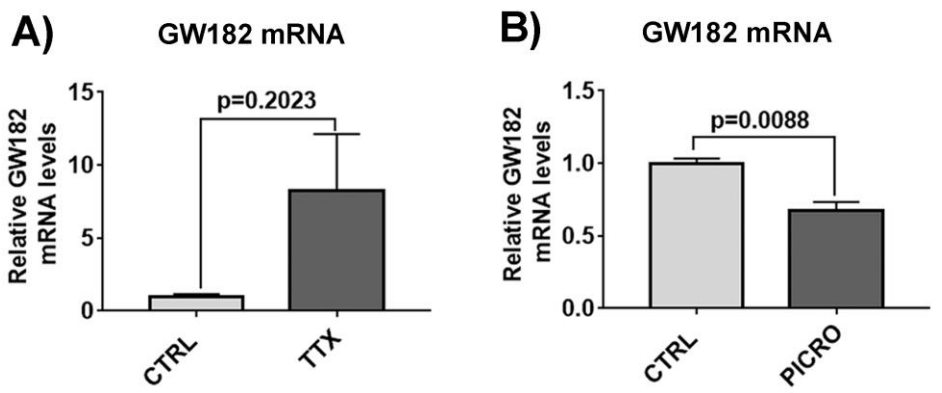

Fig. S2. A) qPCR analysis of GW182 mRNA expression from DIV7 hippocampal neurons treated with mock or tetradotoxin for 48hrs. The levels of GW182 mRNA were normalized using the expression of GAPDH mRNA, Data: mean +/- SEM, n=3 independent experiments, Unpaired t-test.

B) qPCR analysis of GW182 mRNA expression from DIV7 hippocampal neurons treated with mock or picrotoxin for 48hr. The levels of GW182 mRNA were normalized using the expression of GAPDH mRNA, Data: mean +/- SEM, n=3 independent experiments, Unpaired t-test

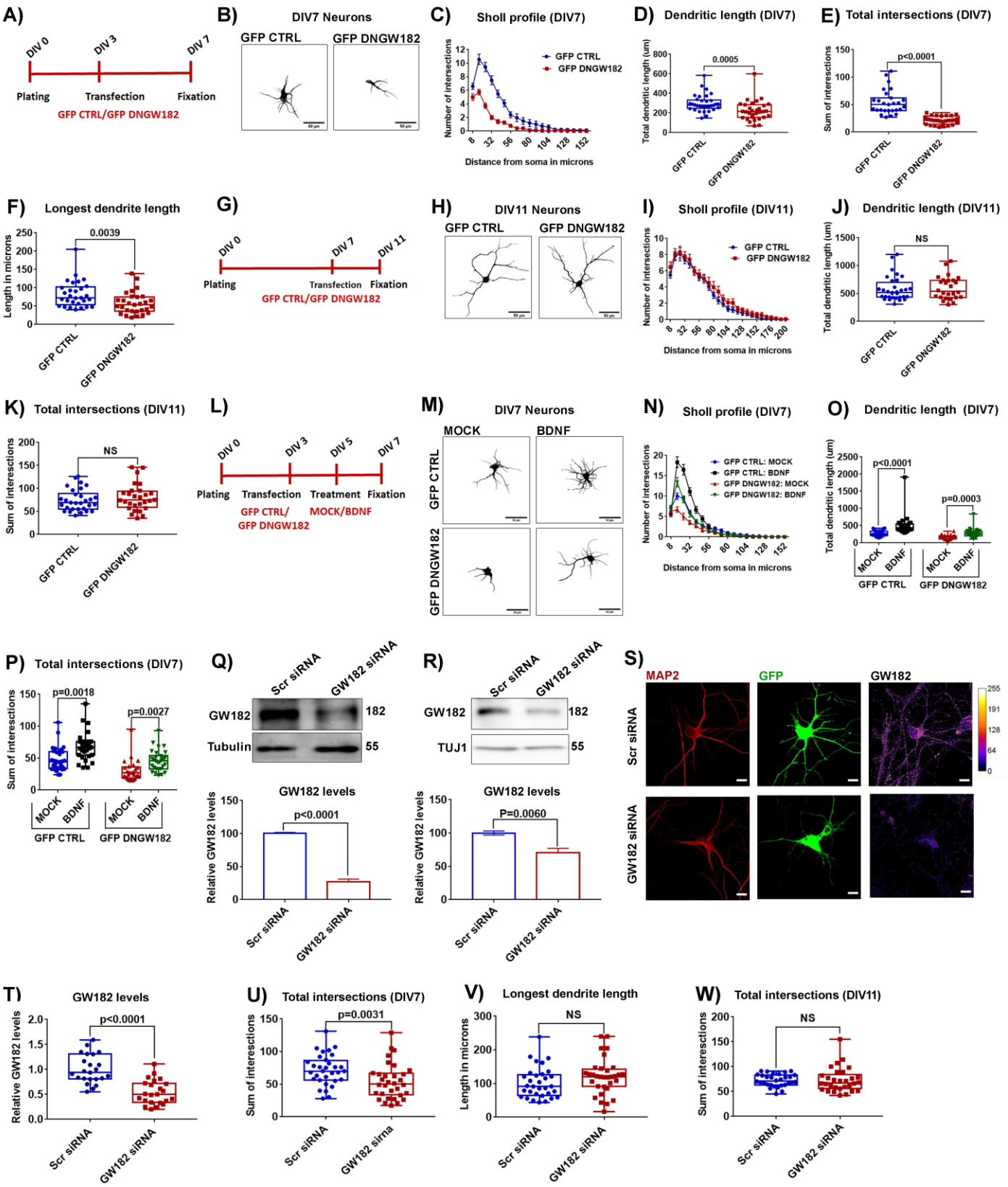

Fig. S3. A) Schematic showing the experimental procedure and timeline: Cultured hippocampal neurons were transfected with either control GFP or GFP DNGW182 on DIV3 and fixed on DIV7, followed by immunostaining.

B) Representative micrographs of DIV7 cultured hippocampal neurons transfected with either control GFP or GFP DNGW182 on DIV3. The images are derived from the threshold MAP2 intensity of transfected neurons. Scale bar represents 50 microns.

C) Sholl curve of DIV7 cultured hippocampal neurons transfected with either control GFP or GFP DNGW182 on DIV3. Data: mean +/- SEM, n=32 neurons from 4 independent experiments, GFP DNGW182 overexpressing neurons had significantly more dendrites from GFP overexpressing neurons at 16-48 microns from the soma, Two way ANOVA followed by Bonferroni's multiple comparisons test.

D) Quantification of the total dendritic length of cultured hippocampal neurons transfected with either control GFP vector or DN GW182 on DIV3, n=31-32 neurons from 4 independent cultures, Mann-Whitney test.

E) Quantification of total intersections of DIV7 hippocampal neurons transfected with either GFP or GFP DNGW182 on DIV 3, n=27 neurons from 3 independent cultures, Mann-Whitney test.

F) Quantification of the length of longest dendrite of DIV7 cultured hippocampal neurons transfected with either control GFP vector or DN GW182 on DIV3, n=29 neurons from 4 independent cultures, Mann-Whitney test.

G) Schematic showing the experimental procedure and timeline: Cultured hippocampal neurons were transfected with either control GFP or GFP DNGW182 on DIV7 and fixed on DIV11, followed by immunostaining.

H) Representative micrographs of DIV11 cultured hippocampal neurons transfected with either control GFP vector or GFP DNGW182 mutant on DIV7. The images are derived from the threshold MAP2 intensity of transfected neurons. Scale bar represents 50 microns.

I) Sholl curve of DIV11 cultured hippocampal neurons transfected with either control GFP or GFP DNGW182 mutant on DIV7. Data: mean +/- SEM, n=22-25 neurons from 4 independent experiments.

J) Quantification of the total dendritic length of DIV11 cultured hippocampal neurons transfected with either Control GFP or GFP DNGW182 on DIV7, n=26-32 neurons from 4 independent cultures, Mann-Whitney test.

K) Quantification of total intersections of DIV11 hippocampal neurons transfected with either GFP or GFP DNGW182 on DIV7, n=28-30 neurons from 4 independent cultures, Unpaired t-test with Welch's correction.

L) Schematic showing the experimental procedure and timeline: Cultured hippocampal neurons were transfected on DIV3 followed by BDNF treatment for 48hrs starting from DIV5 onwards, and fixed on DIV7.

M) Representative images of cultured hippocampal neurons transfected at DIV3 with either control GFP or GFP DNGW182. After transfection, the neurons were treated with BDNF (50ng/ml) on DIV 5 and were fixed on DIV7. Scale bar represents 50 microns.

N) Sholl Quantification of the effects of GFP DNGW182 on BDNF-induced dendrite arborization formation of hippocampal neurons. Data: mean +/- SEM, n=25-30 neurons from 4 independent experiments, BDNF treatment resulted in increased dendritic intersections in GFP transfected neurons at 16-40 microns from the soma and resulted in increased intersections in GFP DNGW182 transfected neurons as well at 16-32 microns from the soma, Two way ANOVA followed by Bonferroni's multiple comparisons test.

O) Quantification of the total dendritic length of neurons described in Figure S3R, n=25-30 neurons from 3 independent experiments, Kruskal- Wallis followed by Dunn's multiple comparisons test.

P) Quantification of total intersections of MOCK/BDNF treated (48 hrs) DIV7 hippocampal neurons transfected with either GFP or GFP DNGW182, n=25-30 neurons from 3 independent cultures, Kruskal- Wallis followed by Dunn's multiple comparisons test.

Q) Immunoblot validation of GW182 knockdown in Neuro2A cells: Representative immunoblots (top) and quantification (bottom) of GW182 levels from N2A cells treated with either scrambled or GW182 siRNA. Data: mean +/- SEM, n=3 independent experiments, Unpaired t-test.

R) Immunoblot validation of GW182 knockdown in cultured hippocampal neurons: Representative immunoblots (top) and quantification (bottom) of GW182 levels from cultured hippocampal neurons cells treated with either scrambled or GW182 siRNA. Data: mean +/- SEM, n=4 independent cultures, Unpaired t-test.

S) Immunostaining validation of GW182 knockdown in hippocampal neuronal culture: Representative images showing GW182 staining in DIV5 hippocampal cultured neurons transfected with GFP along with either scrambled siRNA or GW182 siRNA on DIV3.

T) Immunostaining quantification representing GW182 levels in neurons upon scrambled siRNA or GW182 siRNA transfections. Data: mean +/- SEM, n=23-24 neurons from 3 independent cultures, Unpaired t-test.

U) Quantification of total intersections of DIV7 hippocampal neurons transfected with either scrambled siRNA or GW182 siRNA on DIV3, n=32 neurons from 4 independent cultures, Unpaired t-test.

V) Quantification of length of longest dendrite of DIV7 hippocampal neurons transfected with either scrambled siRNA or GW182 siRNA on DIV3, n=32 neurons from 4 independent cultures, Unpaired t-test.

W) Quantification of total intersections of DIV11 hippocampal neurons transfected with either scrambled siRNA or GW182 siRNA on DIV7, n>25 neurons from 4 independent cultures, Unpaired t-test with Welch's correction.

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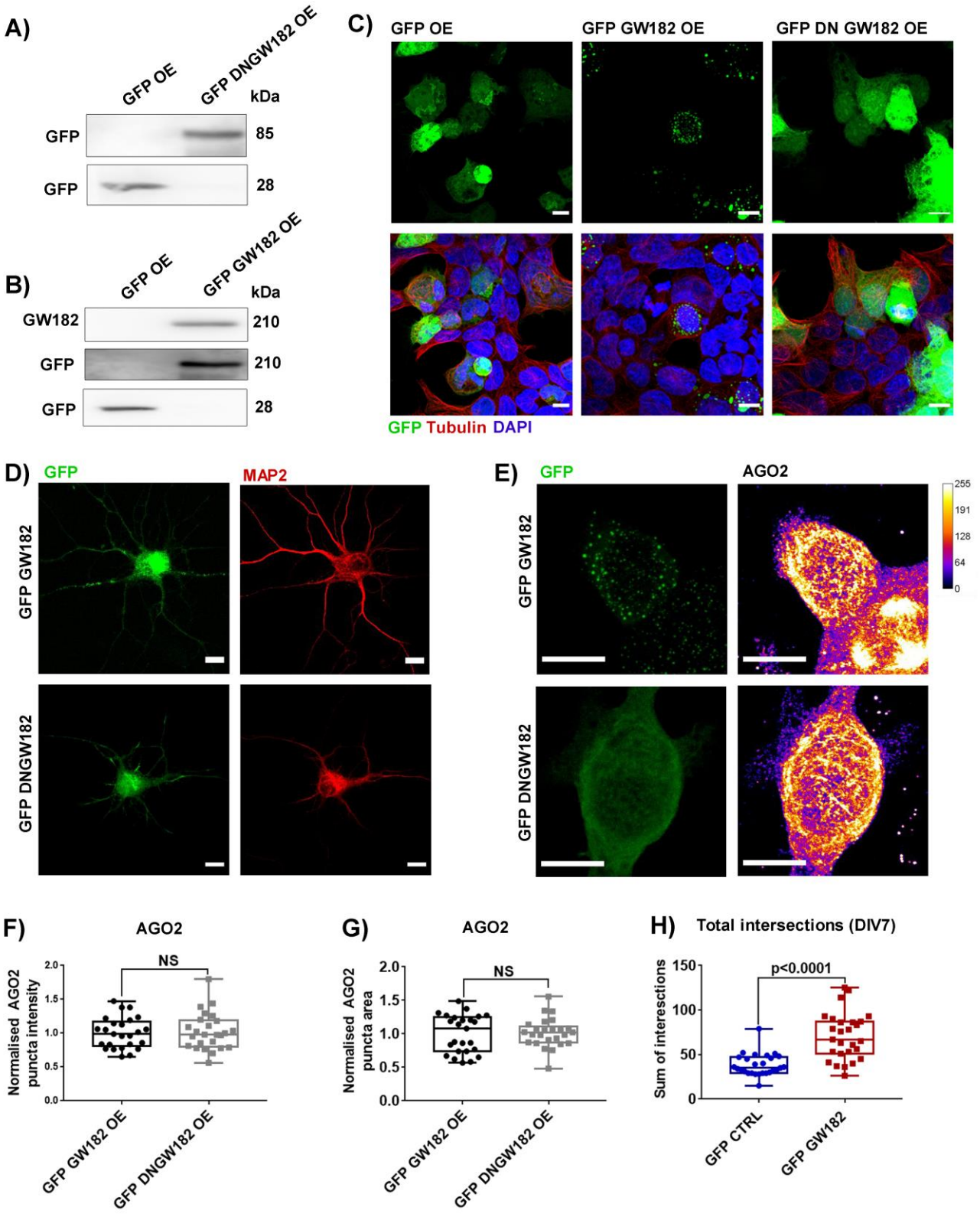
A)
GFP OE
GFP DNGW182 OE
kDa
GFP
85
GFP
28
B)
GFP OE
GFP GW182 OE
kDa
GW182
210
GFP
210
GFP
28
C)
GFP OE
GFP GW182 OE
GFP DN GW182 OE
GFP Tubulin DAPI
D)
GFP
MAP2
GFP GW182
GFP DNGW182
E)
GFP
AGO2
GFP GW182
GFP DNGW182
255
191
128
64
0
F)
AGO2
NS
Normalised AGO2 puncta intensity
2.0
1.5
1.0
0.5
0.0
GFP GW182 OE
GFP DNGW182 OE
G)
AGO2
NS
Normalised AGO2 puncta area
2.0
1.5
1.0
0.5
0.0
GFP GW182 OE
GFP DNGW182 OE
H)
Total intersections (DIV7)
p<0.0001
Sum of intersections
150
100
50
0
GFP CTRL
GFP GW182

Fig. S4.

A) Immunoblot validation of GFP DNGW182 overexpression in HEK293T cells using GFP antibody.

B) Immunoblot validation of GFP GW182 overexpression in HEK293T cells using GFP and GW182 antibody.

C) Immunostaining characterization of GFP GW182 and GFP DNGW182 overexpression in HEK293T cells. Cells were stained with DAPI and tubulin to identify nuclear and cytosolic compartments. Scale bar represents 10 microns.

D) Immunostaining Validation of GFP GW182 and GFP DNGW182 overexpression in cultured hippocampal neurons. Neurons were identified using MAP2 staining. Scale bar represents 10 microns.

E) Representative images showing AGO2 staining in GFP GW182 or GFP DN GW182 transfected HEK293T cells.

F) Quantification of AGO2 puncta intensity in GFP GW182 or GFP DN GW182 transfected HEK293T cells, n=23 cells from two independent experiments.

G) Quantification of AGO2 puncta area in GFP GW182 or GFP DN GW182 transfected HEK293T cells, n=23 cells from two independent experiments

H) Quantification of total intersections of DIV7 hippocampal neurons transfected with either GFP or GFP GW182 at DIV 3, n=27 neurons from 3 independent cultures, Mann-Whitney test.

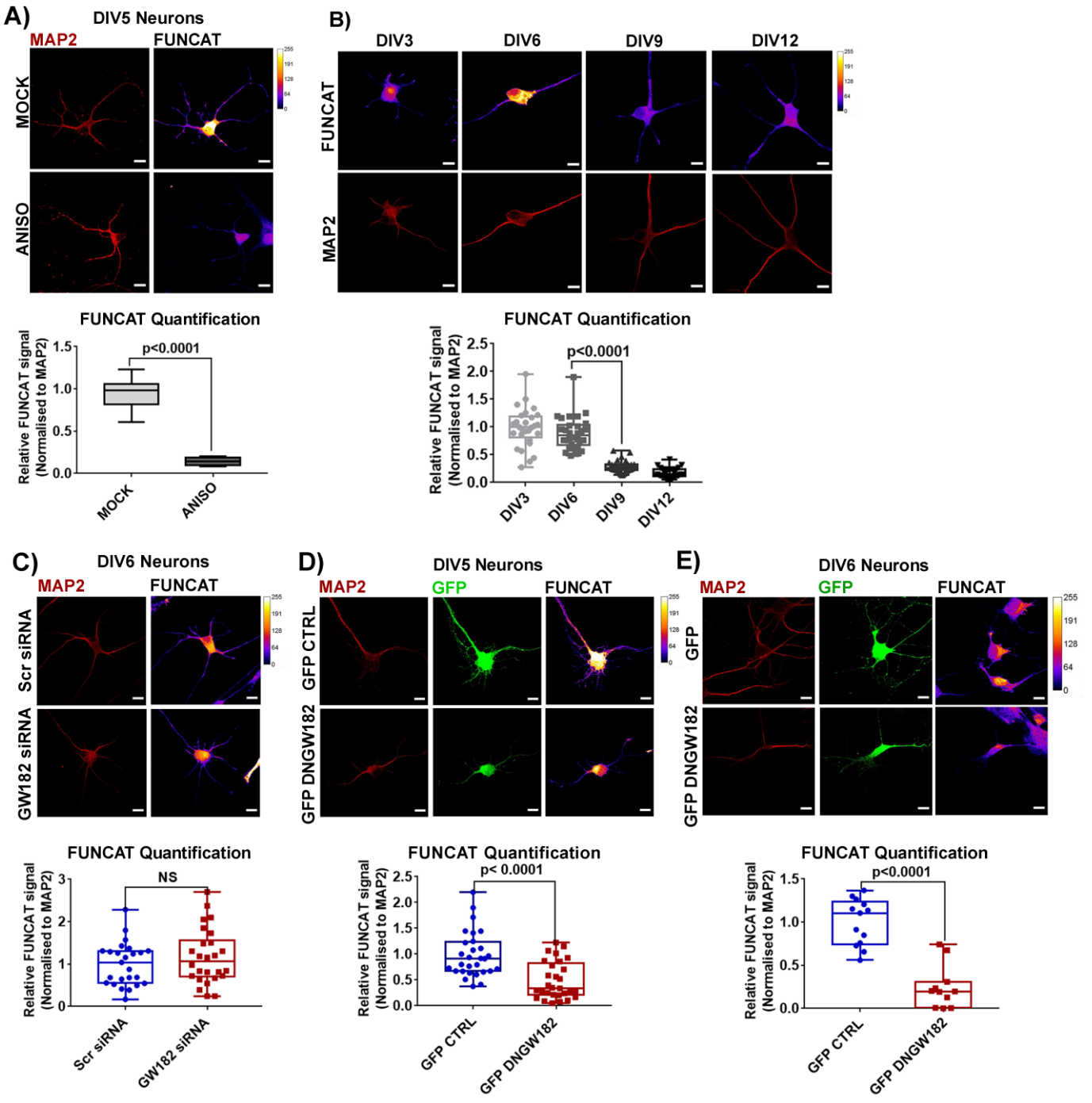
A)
DIV5 Neurons
MAP2
FUNCAT
MOCK
ANISO
FUNCAT Quantification
Relative FUNCAT signal (Normalised to MAP2)
p<0.0001
MOCK
ANISO
B)
DIV3
DIV6
DIV9
DIV12
FUNCAT
MAP2
FUNCAT Quantification
Relative FUNCAT signal (Normalised to MAP2)
p<0.0001
DIV3
DIV6
DIV9
DIV12
C)
DIV6 Neurons
MAP2
FUNCAT
Scr siRNA
GW182 siRNA
FUNCAT Quantification
Relative FUNCAT signal (Normalised to MAP2)
NS
Scr siRNA
GW182 siRNA
D)
DIV5 Neurons
MAP2
GFP
FUNCAT
GFP CTRL
GFP DNGW182
FUNCAT Quantification
Relative FUNCAT signal (Normalised to MAP2)
p< 0.0001
GFP CTRL
GFP DNGW182
E)
DIV6 Neurons
MAP2
GFP
FUNCAT
GFP
GFP DNGW182
FUNCAT Quantification
Relative FUNCAT signal (Normalised to MAP2)
p<0.0001
GFP CTRL
GFP DNGW182

Fig. S5. A) Representative FUNCAT images (top) and corresponding quantification (bottom) of either Mock or Anisomycin treated DIV5 cultured hippocampal neurons, Scale bar represents 10 microns, n=6 neurons from 1 experiment, Unpaired t-test.

B) Representative FUNCAT images (top) and corresponding quantification (bottom) showing FUNCAT signal across different stages of hippocampal neuron development. Scale bar represents 10 microns, n=29-32 neurons from 3 independent cultures, Kruskal-allis test along with Dunn's multiple comparison test.

C) Representative FUNCAT images (top) and corresponding quantification (bottom) of FUNCAT signal (Normalized to corresponding MAP2 signal) in DIV6 cultured hippocampal neurons, transfected with either Scrambled siRNA or GW182 siRNA on DIV3, Scale bar represents 10 microns, n=26 neurons from 3 independent experiments, Unpaired t-test.

D) Representative FUNCAT images (top) and corresponding quantification (bottom) of FUNCAT signal (Normalized to corresponding MAP2 signal) in DIV5 cultured hippocampal neurons, transfected with either GFP control or GFP DNGW182 on DIV3, Scale bar represents 10 microns, n=29 neurons from 3 independent experiments, Mann-Whitney test.

E) Representative FUNCAT images (top) and corresponding quantification (bottom) of FUNCAT signal (Normalized to corresponding MAP2 signal) in DIV6 cultured hippocampal neurons, transfected with either GFP control or GFP DNGW182 on DIV3, Scale bar represents 10 microns, n=25-30 neurons from 3 independent experiments, Unpaired t-test.

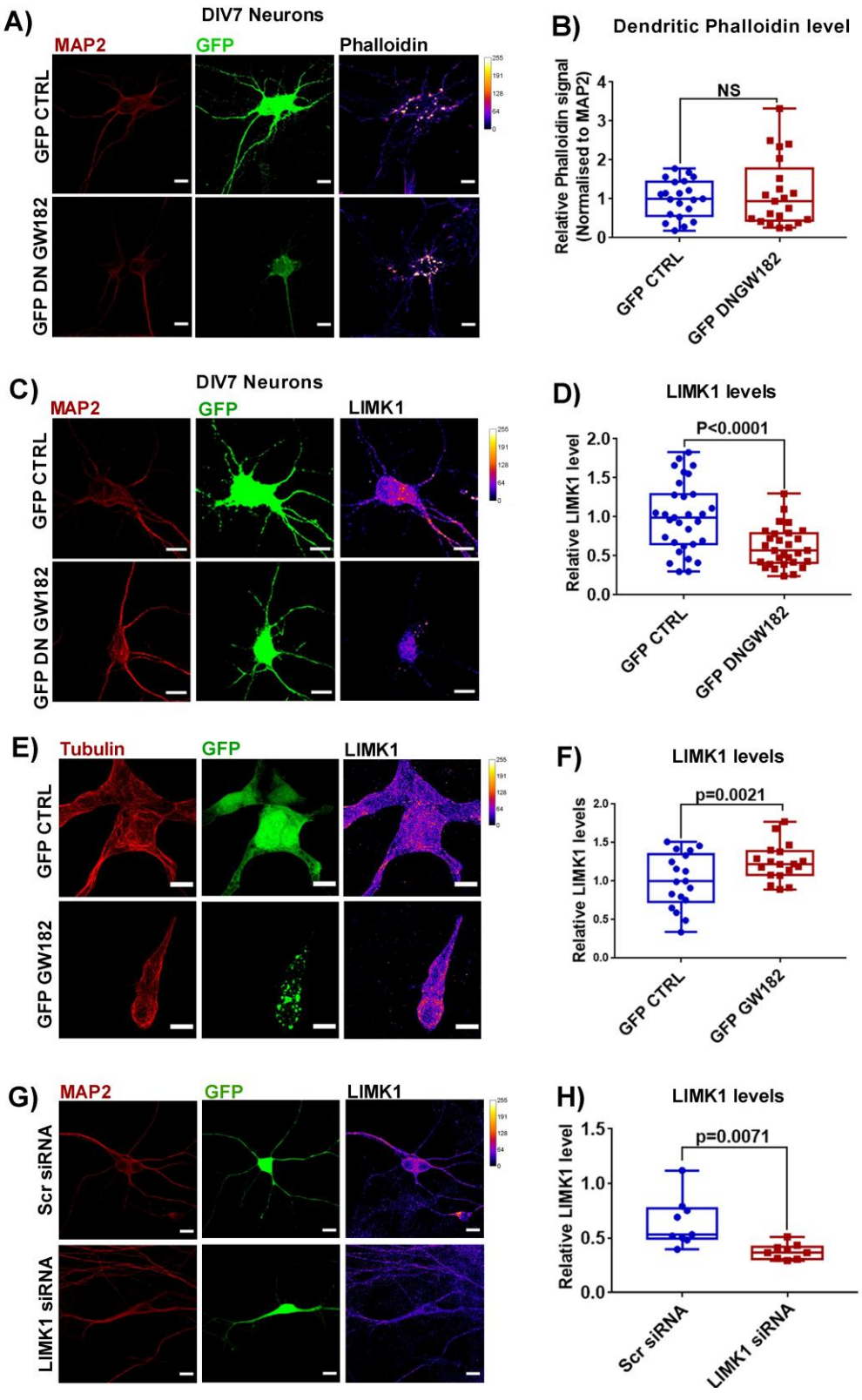
A)
DIV7 Neurons
MAP2
GFP
Phalloidin
GFP CTRL
GFP DN GW182
B)
Dendritic Phalloidin level
Relative Phalloidin signal (Normalised to MAP2)
NS
GFP CTRL
GFP DNGW182
C)
DIV7 Neurons
MAP2
GFP
LIMK1
GFP CTRL
GFP DN GW182
D)
LIMK1 levels
P<0.0001
Relative LIMK1 level
GFP CTRL
GFP DNGW182
E)
Tubulin
GFP
LIMK1
GFP CTRL
GFP GW182
F)
LIMK1 levels
p=0.0021
Relative LIMK1 levels
GFP CTRL
GFP GW182
G)
MAP2
GFP
LIMK1
Scr siRNA
LIMK1 siRNA
H)
LIMK1 levels
p=0.0071
Relative LIMK1 level
Scr siRNA
LIMK1 siRNA
I)
Total intersections
NS
p<0.0001
p=0.0262
Sum of intersections
Scr siRNA
LIMK1 siRNA
Scr siRNA
LIMK1 siRNA
GFP CTRL
GFP GW182

Fig. S6. A) Representative images of DIV7 cultured hippocampal neurons showing Phalloidin staining in GFP control or GFP DNGW182 transfected neurons. Scale bar represents 10 microns.

B) Quantification of Phalloidin intensity (Normalized to corresponding MAP2 signal) in GFP control or GFP DNGW182 transfected neurons, n=21 neurons from 3 independent experiments, Unpaired t-test with Welch's correction.

C) Representative images of DIV7 cultured hippocampal neurons showing LIMK1 staining in GFP control or GFP DNGW182 transfected neurons. Scale bar represents 10 microns.

D) Quantification of normalized LIMK1 signal in GFP control or GFP DNGW182 transfected neurons. n=29-31 neurons from 4 independent experiments, Unpaired t-test with Welch's correction.

E) Representative images showing LIMK1 staining in HEK293T cells with either GFP or GFP GW182 overexpression. Scale bar represents 10 microns.

F) Quantification of LIMK1 intensity in GFP or GFP GW182 overexpressing HEK293T cells. n=18 cells, from 2 independent experiment, unpaired t-test.

G) Immunostaining validation of LIMK1 siRNA in cultured hippocampal neurons using LIMK1 antibody. Scale bar represents 10 microns.

H) Quantification of LIMK1 intensity (Normalized to MAP2 intensity of corresponding neurons) in neurons transfected with either scrambled siRNA or LIMK1 siRNA. n=9 neurons from 1 experiment, unpaired t-test with Welch's correction.

I) Quantification of total intersections of cultured hippocampal neurons transfected with either control GFP or GFP GW182 along with scrambled or LIMK1 siRNA. n=26-27 neurons from 3 independent experiments, Kruskal-Wallis followed by Dunn's multiple comparisons test.

Table S1. List of primers used in the study.

List of primers		
Transcript	**Forward primer (5'-3')**	**Reverse primer (5'-3')**
GAPDH	CAACTCCCTCAAGATTGTCAGCA	GGCATGGACTGTGGTCATGA
β-ACTIN	GGCTCCTAGCACCATGAAGAT	AAACGCAGCTCAGTAACAGTC
TNRC6A	ACACCTCAGATTGACGGCTC	AGCATTTCCATGTGGGAGGTT
AGO2	TGAGCGGGTTGGAAAGAGTG	TAAGCTGGCGCAGGAATTGA