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Supplemental information

C9orf72 poly(PR) mediated neurodegeneration is associated with nucleolar stress

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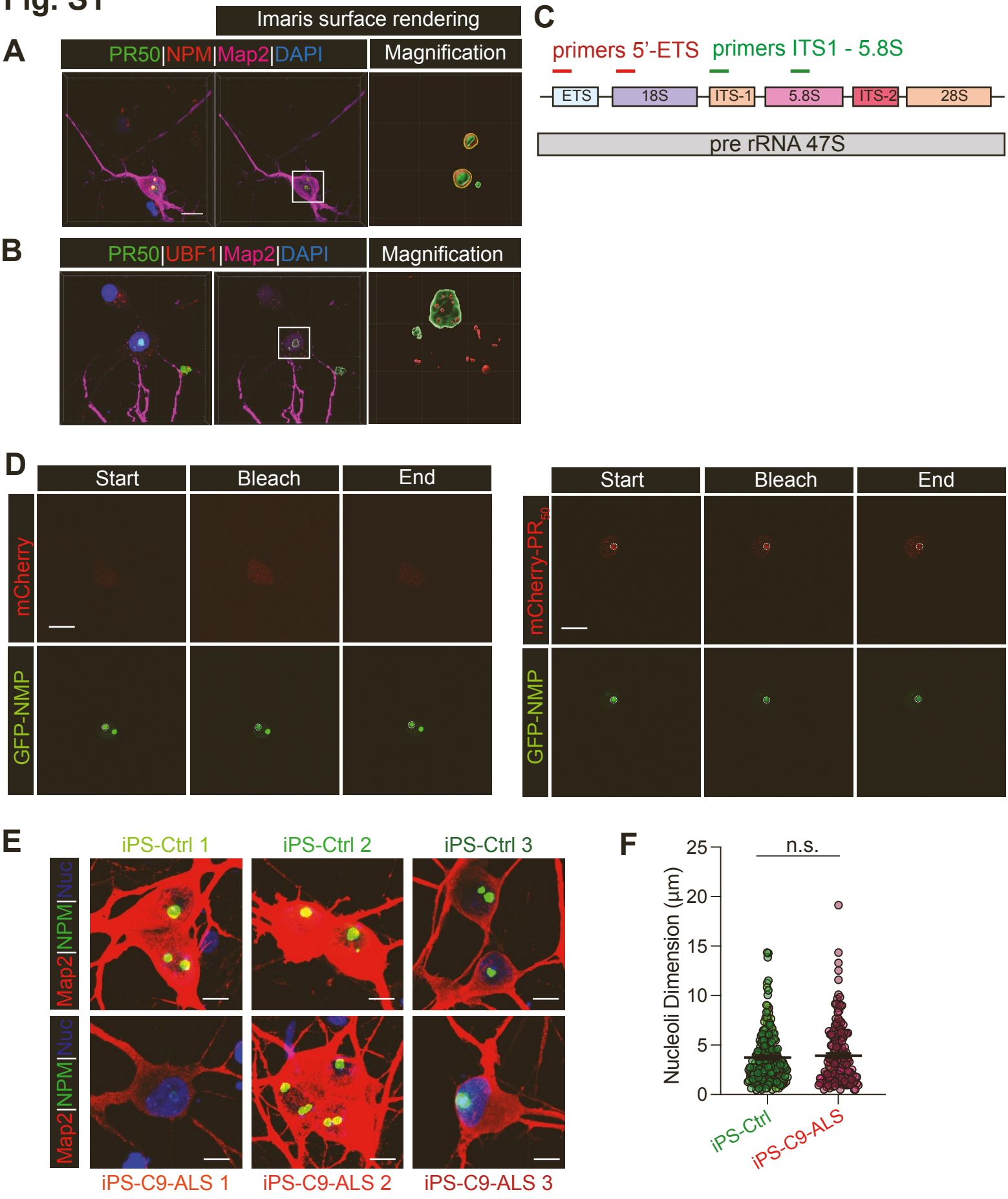
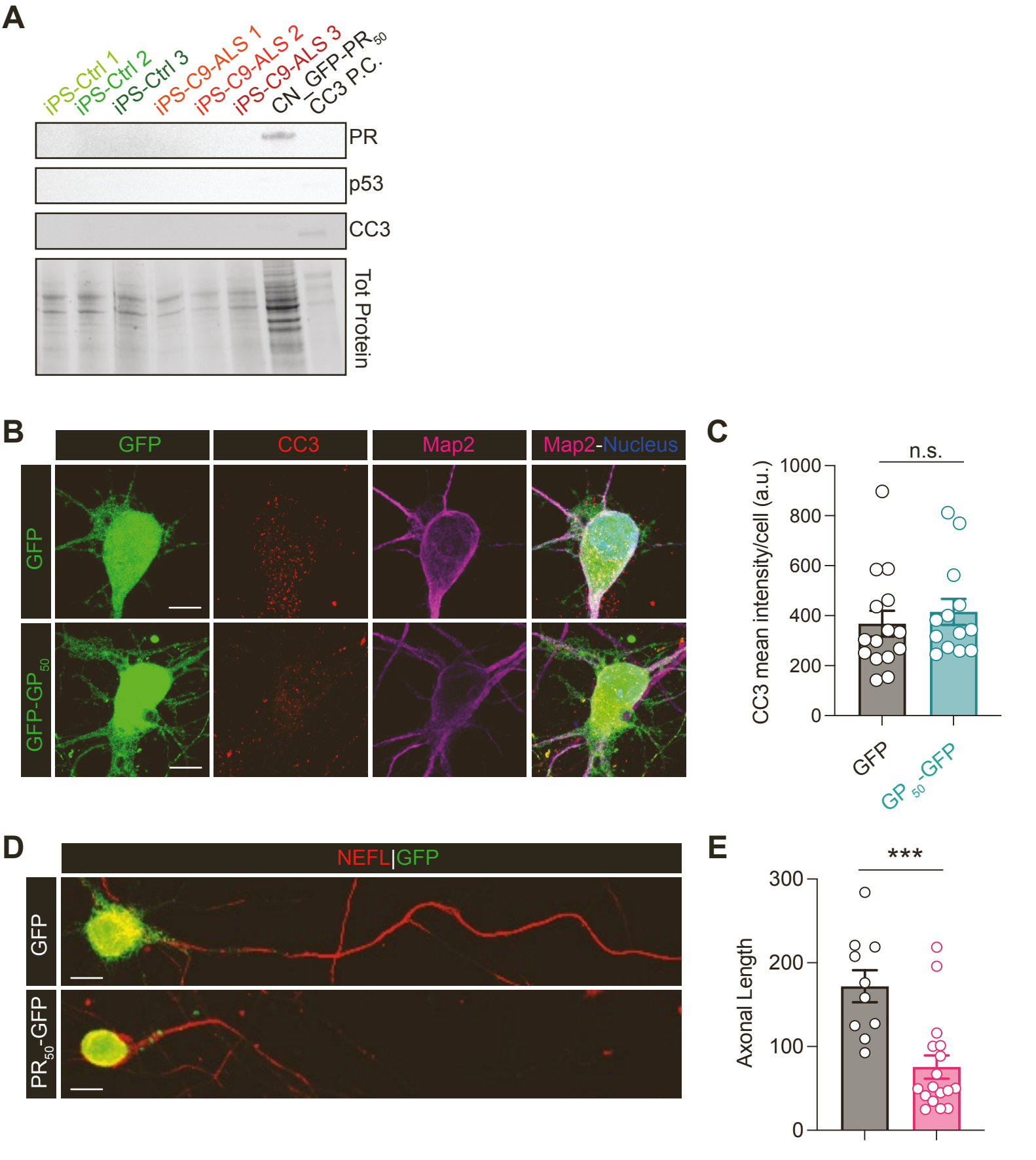
Fig. S1


Imaris surface rendering
A
PR50|NPM|Map2|DAPI
Magnification
B
PR50|UBF1|Map2|DAPI
Magnification
C
primers 5'-ETS
primers ITS1 - 5.8S
ETS
18S
ITS-1
5.8S
ITS-2
28S
pre rRNA 47S
D
Start
Bleach
End
mCherry
GFP-NMP
mCherry-PR50
E
iPS-Ctrl 1
iPS-Ctrl 2
iPS-Ctrl 3
Map2|NPM|Nuc
iPS-C9-ALS 1
iPS-C9-ALS 2
iPS-C9-ALS 3
F
n.s.
Nucleoli Dimension (µm)
25
20
15
10
5
0
iPS-Ctrl
iPS-C9-ALS

Supplementary Fig. S1 - C9-ALS iPS-CN do not show nucleolar stress - Related to Figure 1

A. Confocal imaging and 3D Imaris rendering of rat primary cortical neurons transfected with PR_{50}-GFP. GFP is shown in green, NPM is shown in red, Map2 is shown in magenta, and nuclei (Hoechst) are shown in blue. Scale bar = 10 μm
B. Confocal imaging and Imaris rendering of rat primary cortical neurons transduced with PR_{50}-GFP. GFP is shown in green, Ubf1 is shown in red, Map2 is shown in magenta, and nuclei (Hoechst) are shown in blue. Scale bar = 10 μm
C. Schematic of primers used for qPCR for pre-processed rRNA.
D. Representative images of nucleoli bleached for FRAP experiment (white ROI). Rat primary cortical neurons are transfected with GFP-NPM and mCherry or PR_{50}-mCherry. Time points are shown: start (0 sec), bleach (5 sec), and end (180 sec). Scale bar = 10μm.
E. Representative images of iPS-induced cortical neurons, DIV 25. NPM is shown in green, Map2 is shown in red, and nuclei are shown in blue. Scale bar = 5 μm
F. Dot plot showing quantification of nucleoli dimension. p=n.s. (mean ± s.e.m., n=1, Student's t-test, non-parametric)

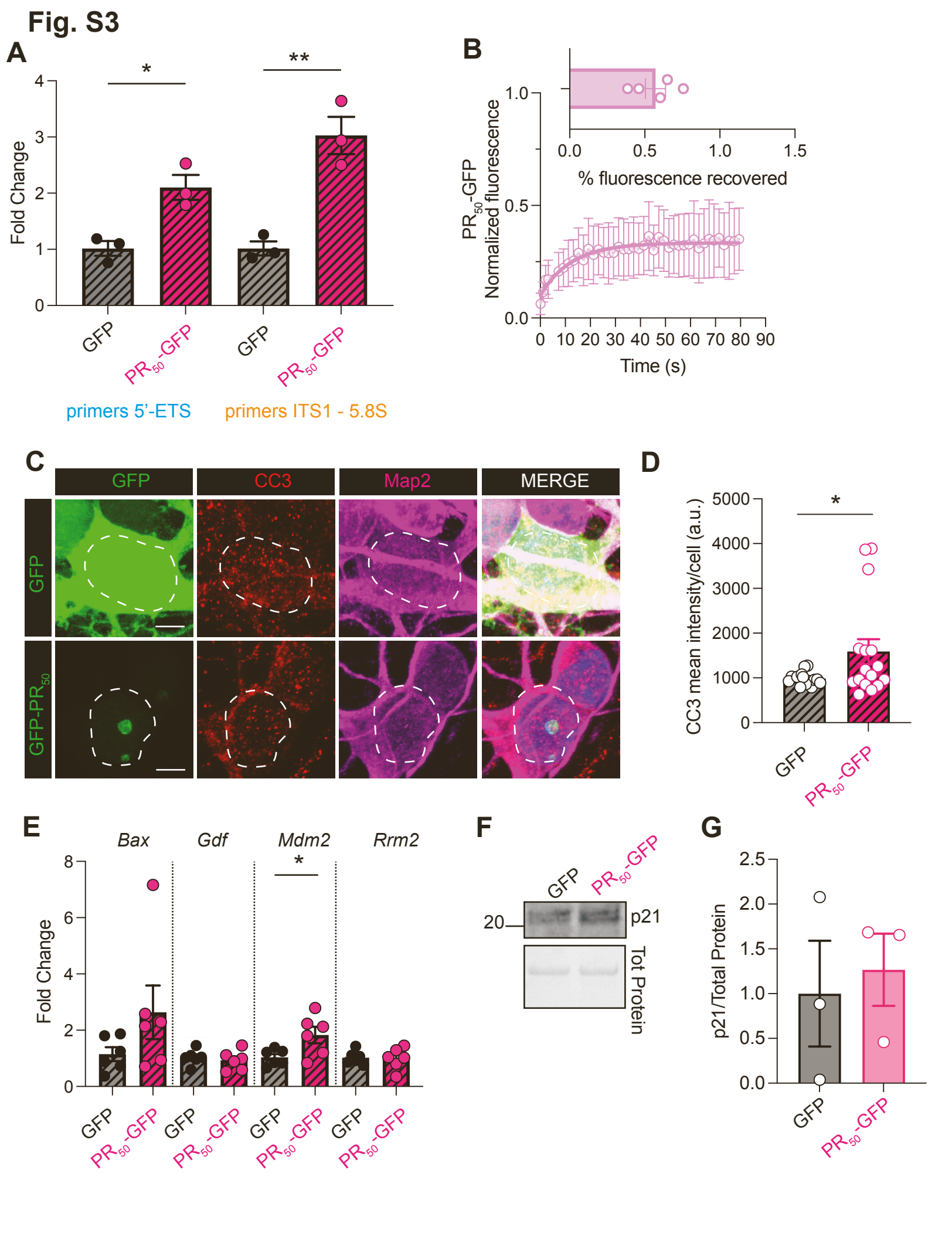
Fig. S2


A
iPS-Ctrl 1
iPS-Ctrl 2
iPS-Ctrl 3
iPS-C9-ALS 1
iPS-C9-ALS 2
iPS-C9-ALS 3
CN_GFP-PR50
CC3 P.C.
PR
p53
CC3
Tot Protein
B
GFP
CC3
Map2
Map2-Nucleus
GFP
GFP-GP50
C
n.s.
CC3 mean intensity/cell (a.u.)
1000
800
600
400
200
0
GFP
GP50-GFP
D
NEFL|GFP
GFP
PR50-GFP
E
***
Axonal Length
300
200
100
0

Supplementary Fig. S2 - PR but not GP causes axonal defects - Related to Figure 2

A. Western blot for PR, p53, CC3, and total protein was performed on iPS-induced cortical neurons DIV 25.
B. Representative images showed rat primary cortical neurons transfected with GFP or GP_{50}-GFP. GFP or GP_{50}-GFP are shown in green, CC3 is in red, Map2 is in magenta, and nuclei (Hoechst) are in blue. Scale bar = 10 μm
C. Graph bar showing quantification of CC3 nuclear intensity in rat primary cortical neurons transfected with GFP or PR_{50}-GFP. *** $p< 0.001$ (mean ± s.e.m., n=3, Student's t-test, non-parametric).
D. Representative images of rat cortical primary neurons transfected with GFP or PR_{50}-GFP. GFP is shown in green, and NFL is shown in red. Scale bar = 10 μm
E. Graph bar showing the quantification of the axonal length of neurons transfected with GFP or PR_{50}-GFP. $p<0.001$ (mean ± s.e.m., n=2, Student's t-test, non-parametric)

Fig. S3


A
Fold Change
GFP
PR50-GFP
primers 5'-ETS
primers ITS1 - 5.8S
B
PR50-GFP Normalized fluorescence
% fluorescence recovered
Time (s)
C
GFP
CC3
Map2
MERGE
GFP-PR50
D
CC3 mean intensity/cell (a.u.)
E
Bax
Gdf
Mdm2
Rrm2
Fold Change
F
p21
Tot Protein
G
p21/Total Protein

Supplementary Fig. S3 - Viral transduction of PR causes nucleolar stress - Related to Figure 3

A. qPCR of rat primary cortical neurons transduced with GFP or PR_{50}-GFP for pre-processed rRNA. ** $p< 0.01$, *** $p< 0.001$ (mean ± s.e.m., n=4, Student's t-test, non-parametric).

B. Graph showing the percentage of recovered fluorescence of PR_{50}-GFP over time in the presence (mean ± s.e.m., n=3).

   Inset: graph bar showing quantification of FRAP percentage of recovered fluorescence of PR_{50}-GFP after photobleaching (mean ± s.e.m., n=3)

   Graph showing the percentage of recovered fluorescence of GFP-NPM over time in the presence of mCherry or PR_{50}-mCherry *** $p< 0.001$ (mean ± s.e.m., n=3, two-way ANOVA).

C. Representative images showed rat primary cortical neurons transduced with GFP or PR_{50}-GFP. GFP or PR_{50}-GFP are shown in green, CC3 is in red, Map2 is in magenta, and nuclei (Hoechst) are in blue. Scale bar = 10 μm

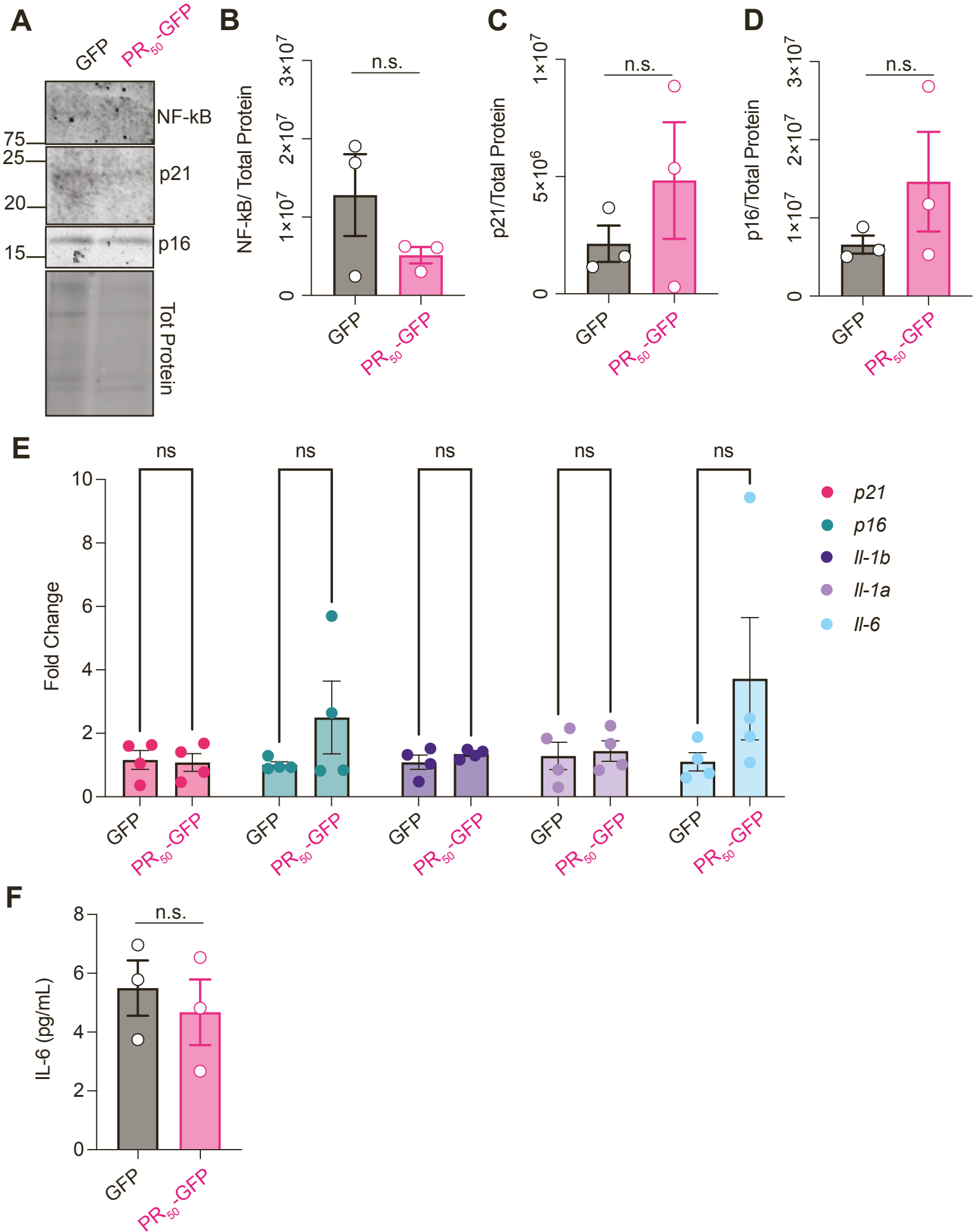
D. Graph bar showing quantification of CC3 nuclear intensity in rat primary cortical neurons transduced with GFP or PR_{50}-GFP. * $p< 0.05$ (mean ± s.e.m., n=2, Student's t-test, non-parametric).

E. qPCR for *Bax*, *Mdm2*, *RRm2b*, *GDF15* was performed on primary cortical neurons transduced with GFP or PR_{50}-GFP 3 days post-transduction. * $p<0.05$ (mean ± s.e.m., n=3, Student's t-test, One-way ANOVA).

F. Western blot for p21 total protein performed on primary cortical neurons transduced with GFP or PR_{50}-GFP 36 hours post-transduction.

G. Graph bar showing p21 quantification of Western blot performed on primary cortical neurons transduced with GFP or PR_{50}-GFP 36 hours post-transduction. p=n.s. (mean ± s.e.m., n=3, Student's t-test, non-parametric).

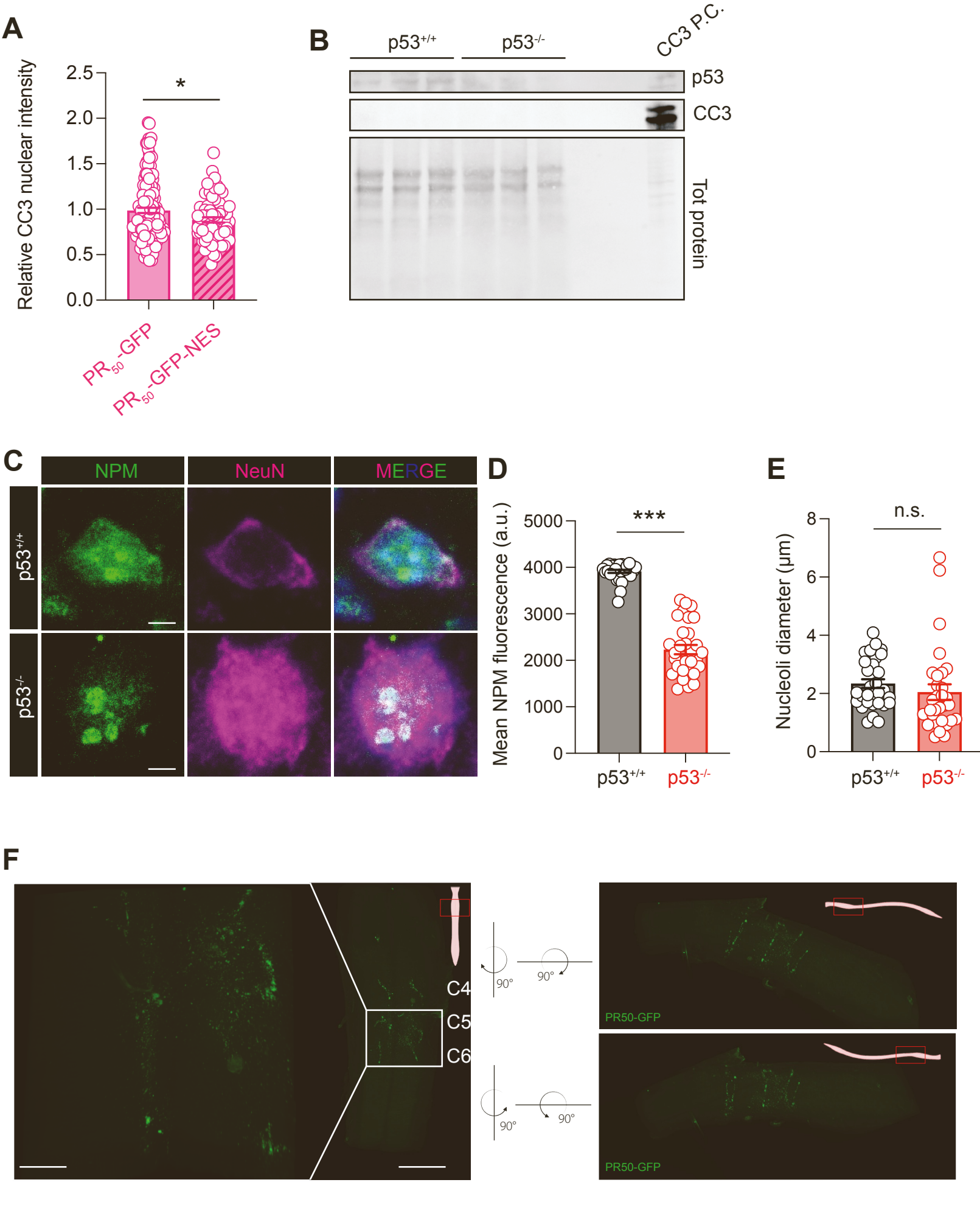
Fig. S4


A
GFP
PR50-GFP
NF-kB
p21
p16
Tot Protein
75
25
20
15
B
NF-kB/ Total Protein
n.s.
C
p21/Total Protein
n.s.
D
p16/Total Protein
n.s.
E
Fold Change
ns
p21
p16
Il-1b
Il-1a
Il-6
F
IL-6 (pg/mL)
n.s.

Supplementary Fig. S4 - PR does not induce senescence at 7 days post transduction - Related to Figure 3

A. Western blot for p21, p16, and NF-kB and total protein performed on mouse primary cortical neurons transduced with GFP or PR_{50}-GFP 7 days post-transduction.
B. Graph bar showing NF-kB quantification of Western blot performed on mouse primary cortical neurons transduced with GFP or PR_{50}-GFP. p=n.s. (mean ± s.e.m., n=3, Student's t-test, non-parametric).
C. Graph bar showing p21 quantification of Western blot performed on mouse primary cortical neurons transduced with GFP or PR_{50}-GFP. p=n.s. (mean ± s.e.m., n=3, Student's t-test, non-parametric).
D. Graph bar showing p16 quantification of Western blot performed on mouse primary cortical neurons transduced with GFP or PR_{50}-GFP. p=n.s. (mean ± s.e.m., n=3, Student's t-test, non-parametric).
E. qPCR for *p21*, *p16*, *Il6*, *Il1a,* and *Il1b* was performed on mouse primary cortical neurons transduced with GFP or PR_{50}-GFP 7 days post-transduction. p=n.s. (mean ± s.e.m., n=3, Student's t-test, One-way ANOVA).
F. ELISA to assay Il-6 concentration in cell culture media from mouse primary cortical neurons transduced with GFP or PR_{50}-GFP, 7 days post-transduction. p=n.s. (mean ± s.e.m., n=3, Student's t-test, Student's t-test, non-parametric).

Fig. S5

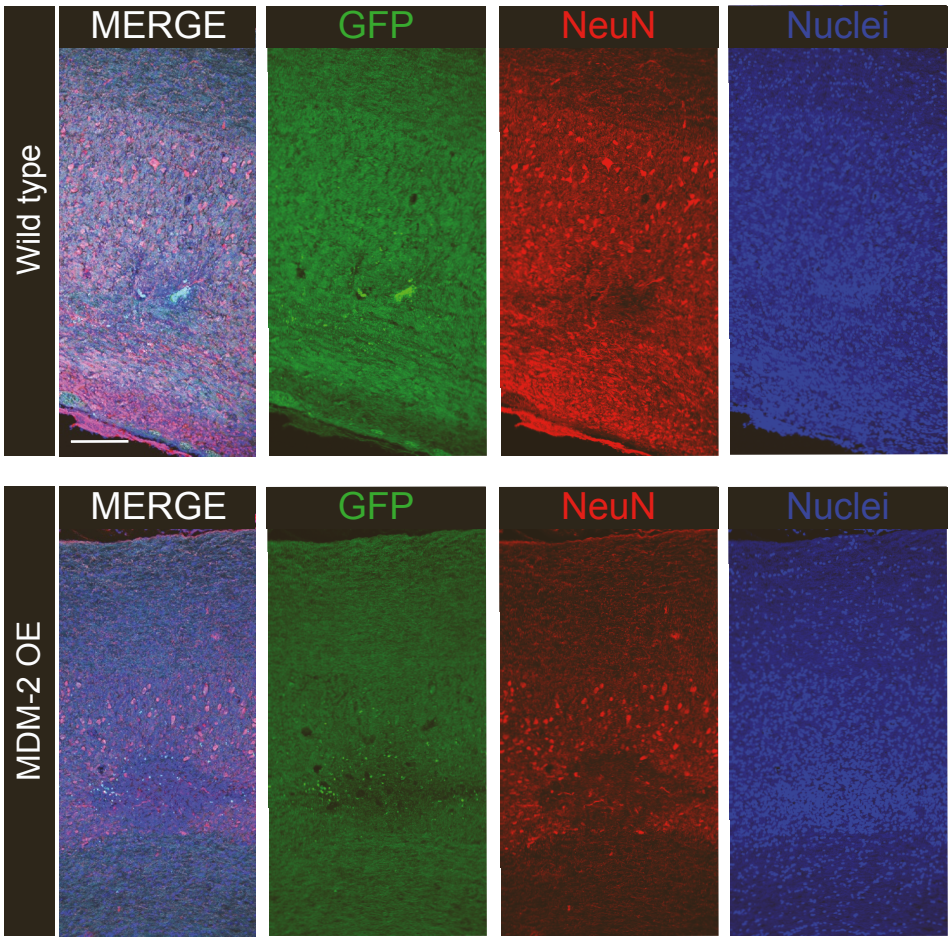

A
Relative CC3 nuclear intensity
2.5
2.0
1.5
1.0
0.5
0.0
*
PR50-GFP
PR50-GFP-NES
B
p53+/+
p53-/-
CC3 P.C.
p53
CC3
Tot protein
C
NPM
NeuN
MERGE
p53+/+
p53-/-
D
Mean NPM fluorescence (a.u.)
5000
4000
3000
2000
1000
0
***
p53+/+
p53-/-
E
Nucleoli diameter (µm)
8
6
4
2
0
n.s.
p53+/+
p53-/-
F
C4
C5
C6
90°
90°
90°
90°
PR50-GFP
PR50-GFP

Supplementary Fig. S5 - nucleolar stress characterization in p53 KO mice and injection representation - Related to Figure 6

A. Graph bar showing quantification of CC3 fluorescence in flies nuclei overexpressing the indicated constructs. * $p< 0.05$ (mean ± s.e.m., n=1, m>25, o>8. Student's t-test, non-parametric).
B. Western blot for p53, CC3, and total protein performed on $p53^{+/+}$ (WT) and $p53^{-/-}$ mice brain cortices extracts.
C. Representative images of $p53^{+/+}$ (WT) and $p53^{-/-}$ cortical neurons. NPM is shown in green, neuN is shown in magenta, and nuclei are shown in blue. Scale bar= 5 μm
D. Graph bar showing the quantification of nucleoli brightness. *** $p<0.001$ (mean ± s.e.m., n=1, m=60, Student's t-test, non-parametric).
E. Graph bar showing the quantification of nucleoli dimension. p=n.s. (mean ± s.e.m., n=1, m=60, Student's t-test, non-parametric).
F. Center: dorsal view of spinal cord tissue C4-C6 bilateral injected with PR_{50}-GFP acquired through light-sheet microscopy 4X magnification, 0.6X Zoom. Scale Bar = 0.5mm. Left: dorsal view of spinal cord tissue C4-C6 bilateral injected with PR_{50}-GFP acquired through light-sheet microscopy 4X magnification, 2.5X Zoom. Scale Bar = 2mm. Right: lateral views of spinal cord tissue C4-C6 bilateral injected with PR_{50}-GFP obtained through light-sheet microscopy 4X magnification, 2.5X Zoom. Scale bar = 2mm.

Fig. S6

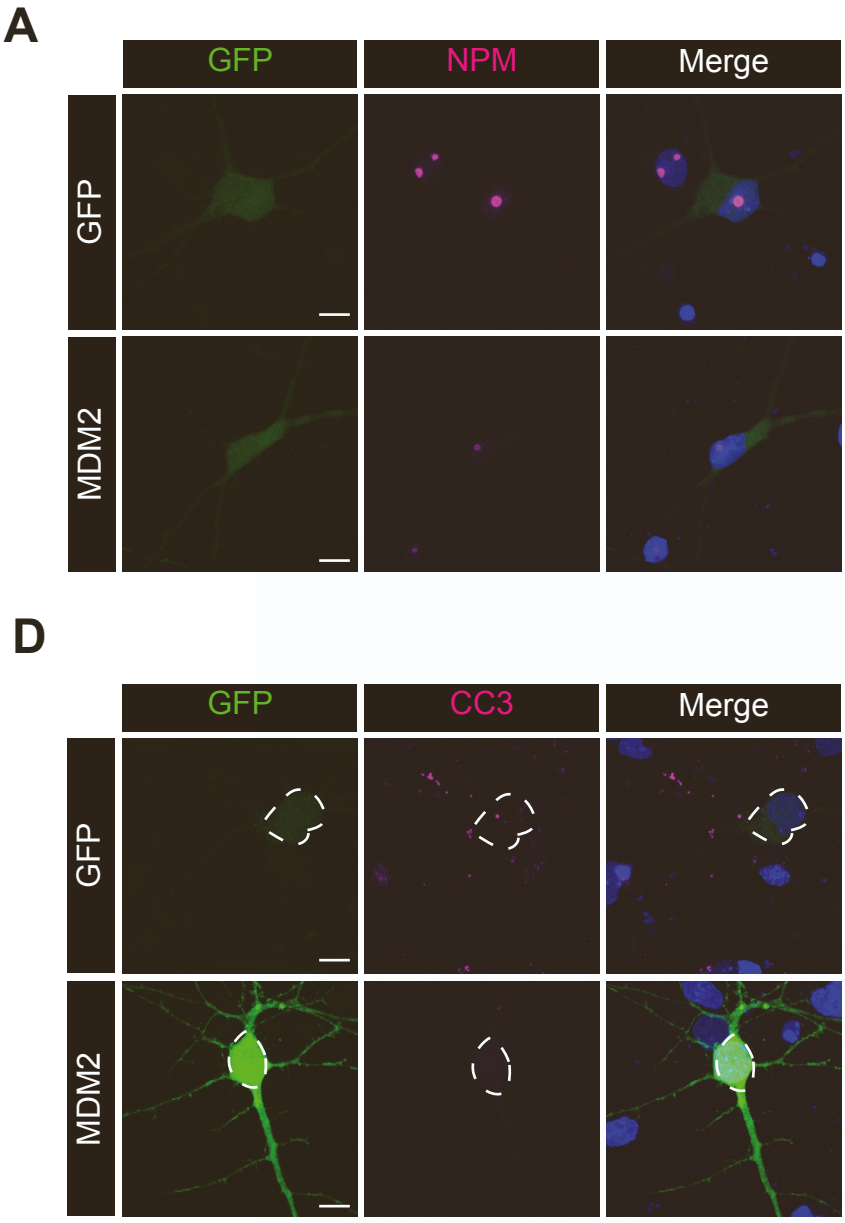
A


MERGE
GFP
NeuN
Nuclei
Wild type
MERGE
GFP
NeuN
Nuclei
MDM-2 OE

Supplementary Fig. S6 - PR injections in MDM2 OE mice - Related to Figure 7

A. Confocal imaging of PR_{50}-GFP injection site in WT animal. Scale bar = 100μm
B. Confocal imaging of PR_{50}-GFP injection site in Mdm2 OE animal. Scale bar = 100μm

Fig. S7


A
GFP
NPM
Merge
GFP
MDM2
B
Mean NPM fluorescence (a.u.)
5000
4000
3000
2000
1000
0
n.s.
GFP
MDM2
D
GFP
CC3
Merge
GFP
MDM2
C
Nucleoli diameter (μm)
10
8
6
4
2
0
n.s.
GFP
MDM2
E
CC3 mean intensity/cell (a.u.)
1500
1000
500
0
n.s.
GFP
MDM2
F
PR50 fluorescence (a.u.)
4000
3000
2000
1000
0
n.s.
GFP
MDM2

Supplementary Fig. S7 - Characterization of nucleolar defects in MDM2 OE mice - Related to Figure 7

A. Representative images of cortical neurons transfected with GFP and MDM2. GFP is shown in green, and NPM is shown in magenta. Scale Bar = 10μm
B. Graph bar showing the quantification of nucleoli brightness. P=n.s. (mean ± s.e.m., n=2, Student's t-test, non-parametric).
C. Graph bar showing the quantification of nucleoli dimension. P=n.s. (mean ± s.e.m., n=2, Student's t-test, non-parametric).
D. Representative images of cortical neurons transfected with GFP and MDM2. GFP is shown in green, and CC3 is shown in magenta. Scale Bar = 10μm
E. Graph bar showing the quantification of CC3 fluorescence intensity. P=n.s. (mean ± s.e.m., n=2, Student's t-test, non-parametric).
F. Graph bar showing the quantification of PR_{50} fluorescence intensity. P=n.s. (mean ± s.e.m., n=2, Student's t-test, non-parametric).