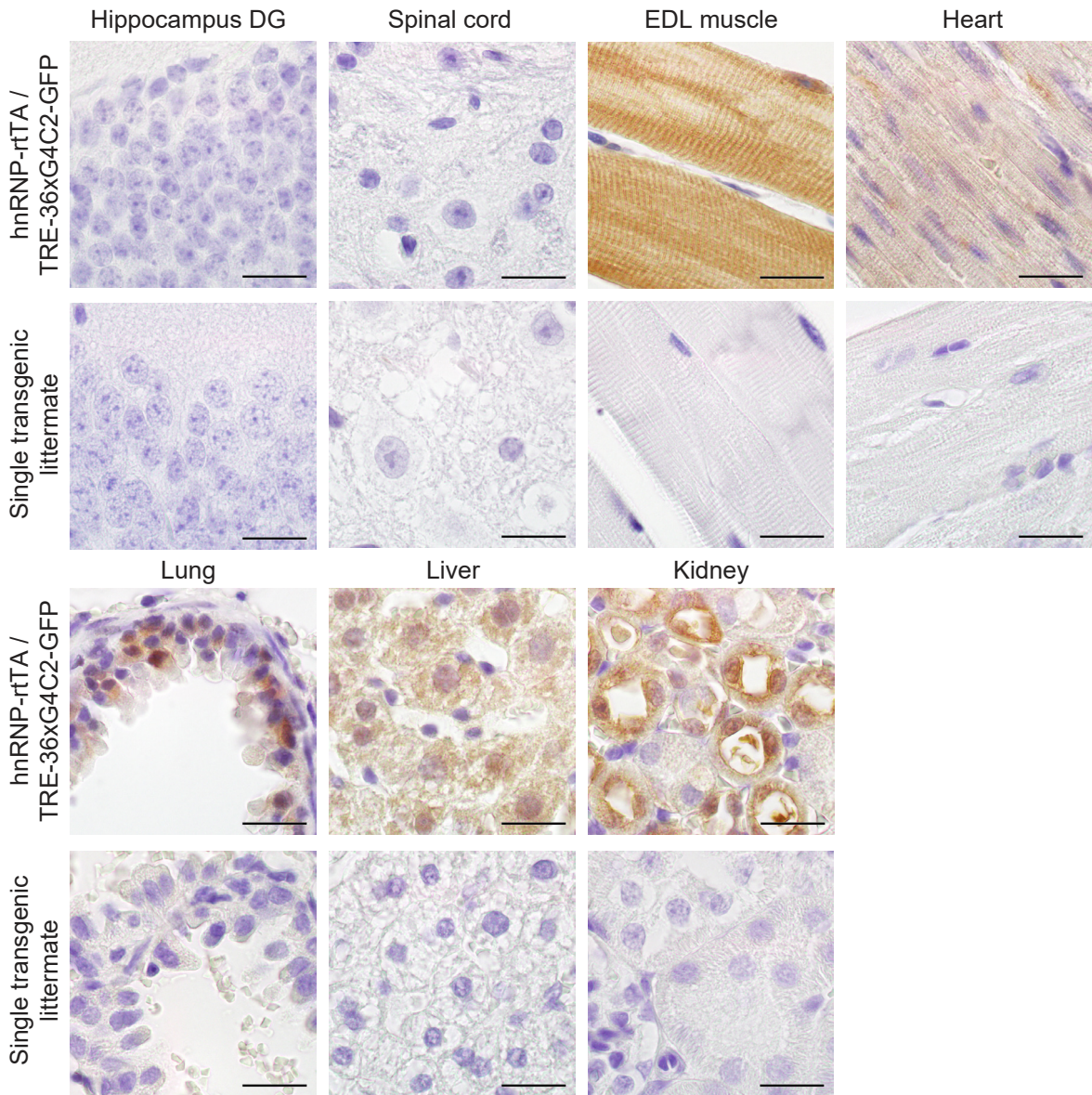
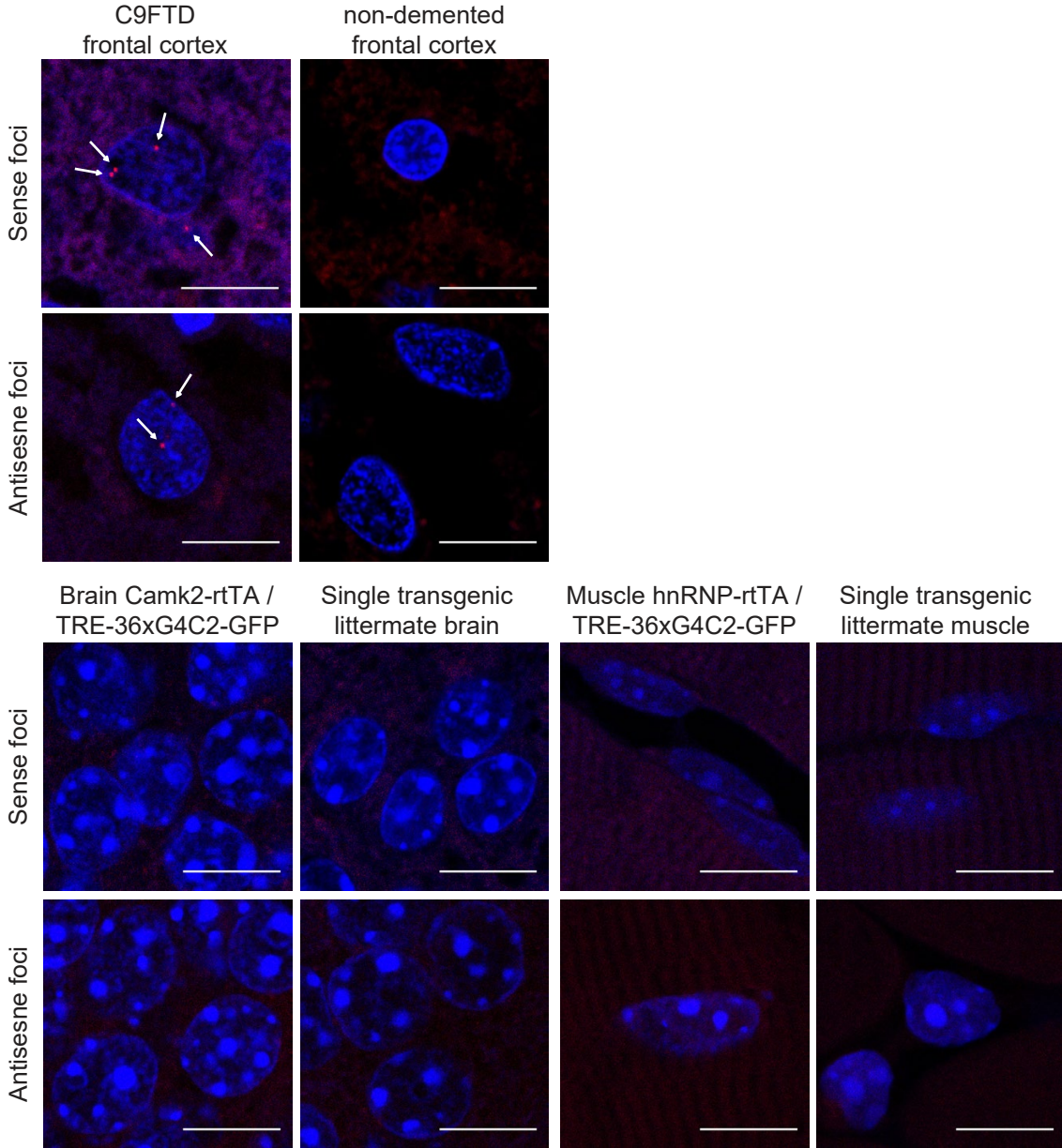


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Mouse    17  ----GGA-----ATTCGCCCTTGtttttcccacccctctctCTcccCa
        |||        .||        |||||
Human    151  TTTAGGAGGTGTGTGTTT-----TTGTTTTTCCCACC--CTCTCTCCCA
        55  ctacttgctcCCTcacagtactcgctgaggggtgaacaagaaaagacctga
        |||||
        194  CTACTTGCT--CTCACGTA TACTCGCTGAGGGTGAACAAGAAAAGACTGA
        105  taaagattaaccagaagaaaaacaaggaggggaaacaaccgcagcctgtagc
        |||||
        242  TAAAGATTAACCAGAAGAAAACAAGGAGGGAAACAACCGCAGCCTGTAGC
        155  aagctctggaactcaggagtgcgcgctaggggccggggccggggccggg
        |||||
        292  AAGCTCTGGAAC T CAGGAGT CGCGCGCTA-----
        205  gccggggccggggccggggccggggccggggccggggccggggccggggc
        321  -----
        255  cggggccggggccggggccggggccggggccggggccggggccggggccg
        321  -----
        305  gggccggggccggggccggggccggggccggggccggggccggggccggg
        321  -----
        355  gccggggccggggccggggccggggccggggccggggccggggccggggc
        |||||
        321  -----GGGGCCGGGGCCGGGGCCGGGGC
        405  gtggtcggggcccggggccggggcccggggccggggccggggctgcggttcgg
        |||||
        344  GTGGTCGGGGCGGGCCCGGGGCCGGGCCGGGGCTGCGGTTGCGG
        455  tgcctgcgcccgggcgggagggcgcaggcgggtggcgaagtgggtgagtg
        |||||
        394  TGCTGCGCCCGCGGGCGGAGGCAGGCGGTTGGCGAGTGGGTGAGTG
        505  aggaggc-----ggcggg-----cccggga--
        |||||          |||||          .|||
        444  AGGAGGCGGCATCCTGGCGGGTGGCTGTTTGGGGTTCGGCTGCCGGGAAG
```

**Supplementary figure 1: Alignment of DNA sequence of mouse transgene (lower case) and human C9ORF72 sequence (upper case/ capital letters) surrounding the repeat expansion.** NCBI Reference Sequence: NG\_031977.1. Our mouse model contains 118 bp upstream and 115 bp downstream human flanking region around the G4C2 repeat expansion.

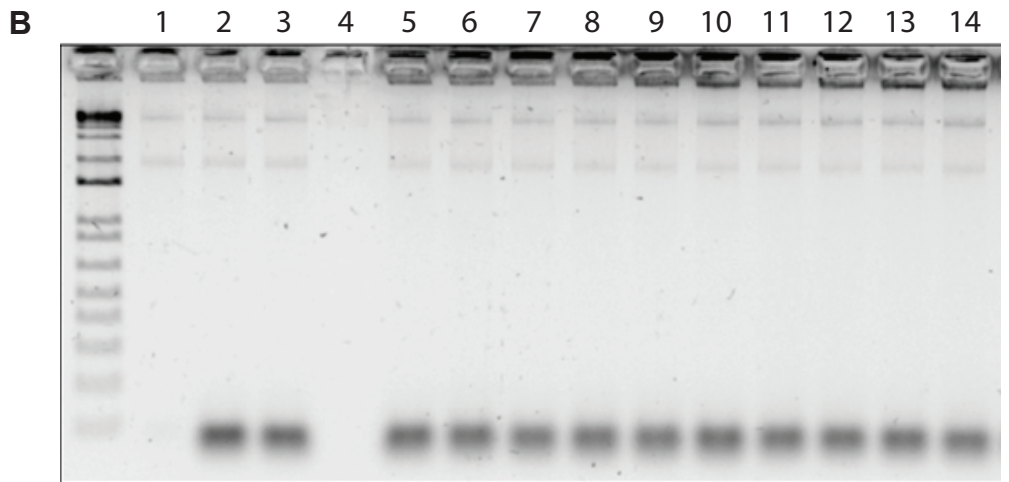
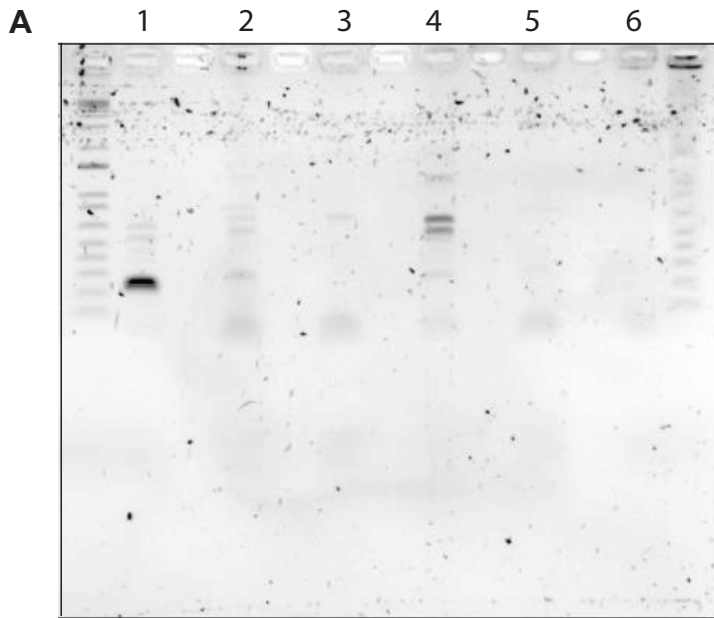


**Supplementary figure 2: GFP expression in EDL muscle, heart, lung, liver and kidney of TRE-36xG4C2-GFP/hnRNP-rtTA double transgenic mice.** No GFP staining was observed in the hippocampus dentate gyrus or in the spinal cord of DT mice. Single transgenic littermates, consisting of either TRE-only or rtTA-only, received the same dox treatment and are all negative for GFP staining. Scale bars are 20  $\mu$ m. All stainings were performed on all mice in this study. ST 4 weeks dox n=15, DT 4 weeks dox n=16

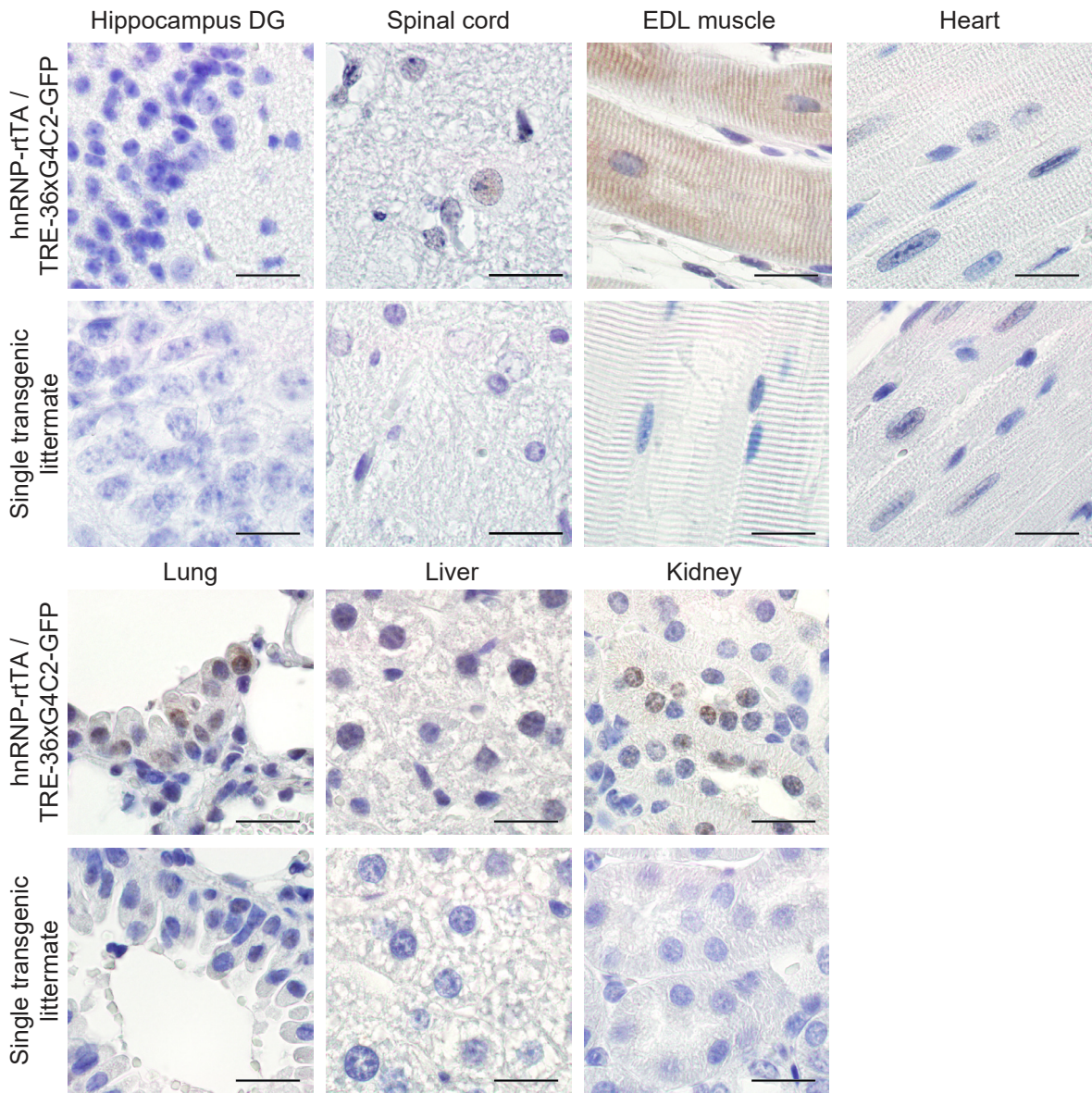


**Supplementary figure 3: No sense nor antisense RNA foci were found in TRE-36xG4C2-GFP/Camk2- $\alpha$ -rtTA and TRE-36xG4C2-GFP/hnRNP-rtTA double transgenic mice and control single transgenic littermates.** Single and double transgenic mice received the same dox treatment. Only frontal cortex samples of C9FTD cases present with some nuclear sense and antisense foci. Scale bars are 10  $\mu$ m. The FISH was performed on all mice in this study. ST 4 weeks dox n=15, DT 4 weeks dox n=16



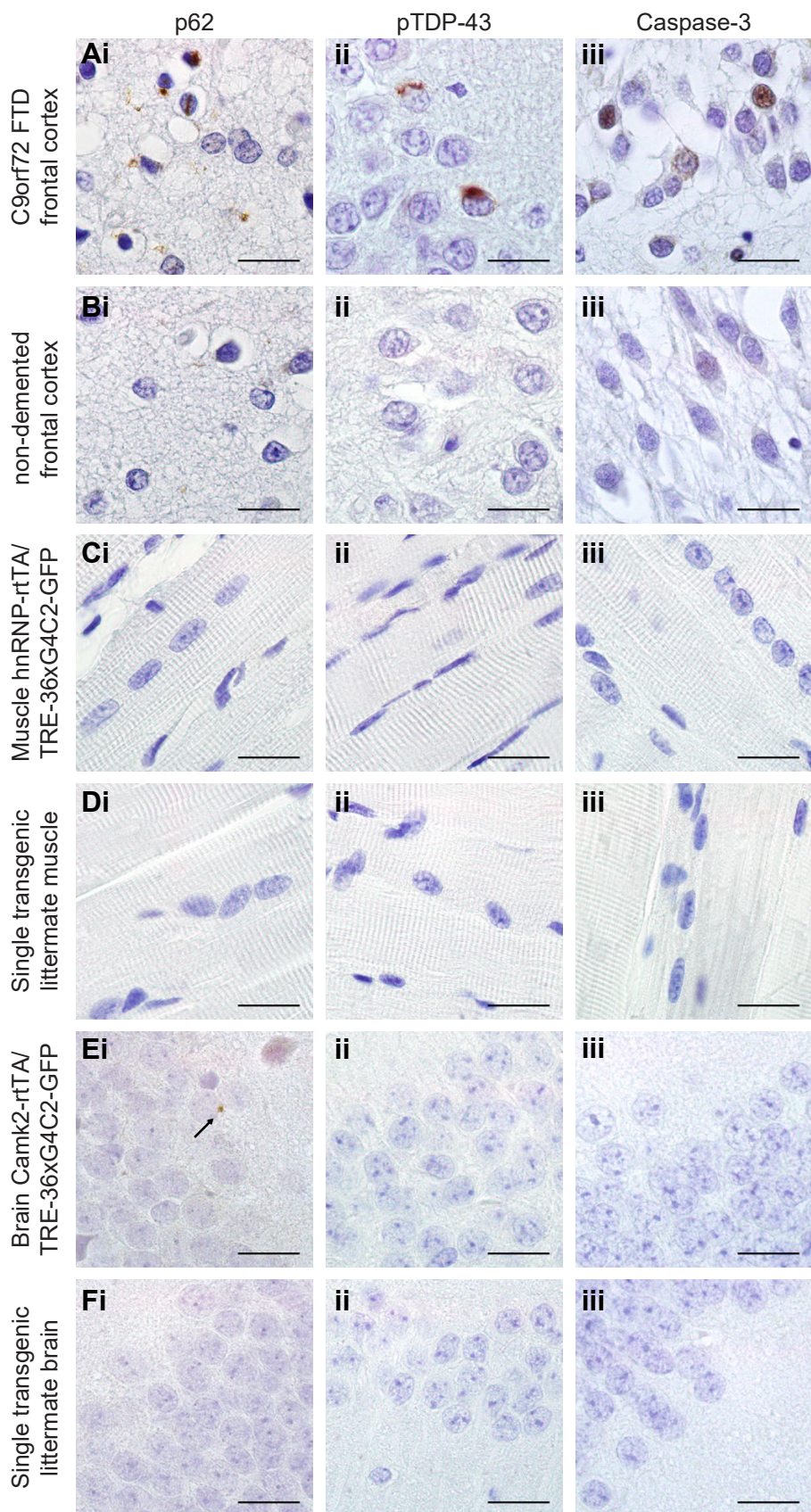


**Supplementary figure 4: reverse transcriptase PCR for C9orf72 antisense transcripts.** A) rt-PCR for C9orf72 antisense transcripts on frozen human prefrontal cortex samples with C9orf72 reverse specific primers. 1: NHB 05-123 (C9FTD) 2: NHB 09-044 (C9FTD) 3: NHB 08-150 (non-demented control) 4: NHB 07-089 (non-demented control) 5: NHB 05-123 (C9FTD) -rt control 6: H<sub>2</sub>O control. B) rt-PCR for C9orf72 antisense transcripts on TRE-36xG4C2-GFP mouse kidney samples with C9orf72 reverse specific primers. 1: H<sub>2</sub>O control 2: -rt mouse (15547-03 -rt) 3: -rt human (05-123 -rt) 4: empty cause of broken well 5: 15547-04 (DT) 6: 15547-03 (DT) 7: 12938-08 (DT) 8: 12938-06 (DT) 9: 18379-04 (DT) 10: 18379-03 (ST) 11: 18154-02 (DT) 12: 18154-01 (DT) 13: 18379-07 (DT) 14: 18379-01 (DT). Single and double transgenic mice received the same dox treatment.

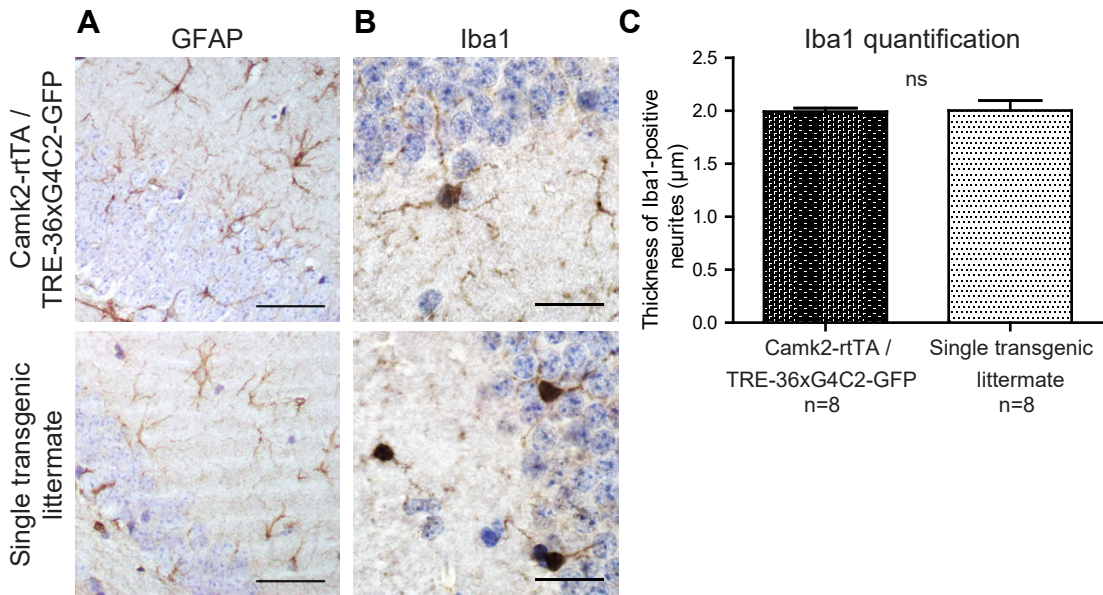


**Supplementary figure 5: Poly-GA expression in EDL muscle, lung, liver and kidney of TRE-36xG4C2-GFP/hnRNP-rtTA double transgenic mice.** No poly-GA staining was observed in the hippocampus dentate gyrus or in the spinal cord of DT mice. Single transgenic littermates, consisting of either TRE-only or rtTA-only, were treated similarly with dox and are all negative for poly-GA staining. Scale bars are 20  $\mu$ m. The poly-GA was performed on all mice in this study. ST 4 weeks dox n=15, DT 4 weeks dox n=16

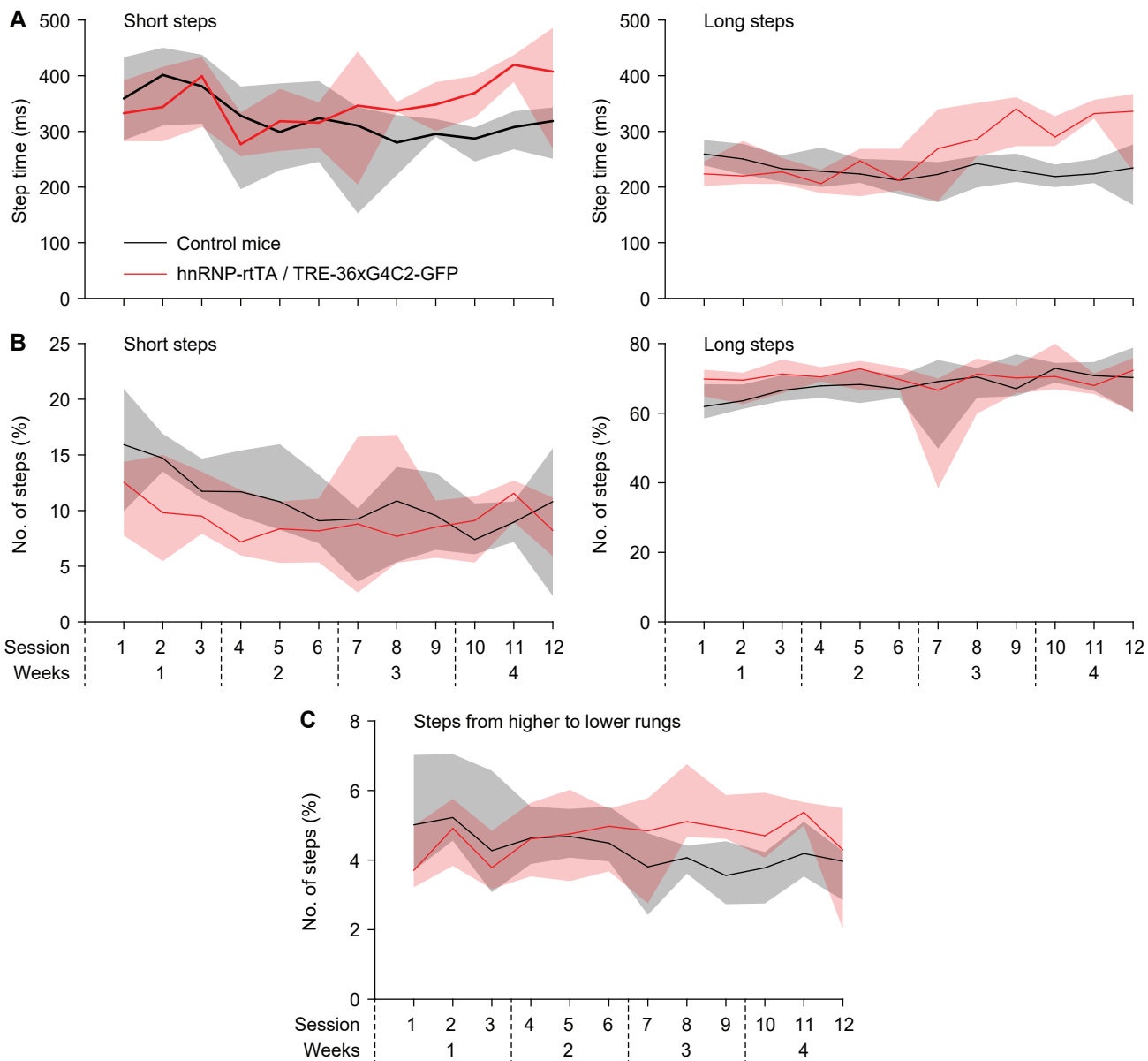




**Supplementary figure 6: Expression of 36x G4C2 human repeats does not cause abundant p62, pTDP-43 and cleaved-caspase 3 pathology.** Human prefrontal cortex of A) C9FTD patients or B) non-demented controls were used as positive and negative control for detection of pathology. C) TRE-36xG4C2-GFP/hnRNP-rtTA double transgenic mice do not present with any p62, pTDP-43 or cleaved-caspase-3 pathology in EDL muscle. E) TRE-36xG4C2-GFP/Camk2- $\alpha$ -rtTA double transgenic mice show some sparse perinuclear aggregates of p62 in the hippocampus dentate gyrus (arrow). D) and F) Single transgenic littermates, consisting of either TRE-only or rtTA-only, were treated similarly with dox and are negative for all pathology. All scale bars are 20  $\mu$ m. All stainings were performed on all mice in this study. ST 4 weeks dox n=15, DT 4 weeks dox n=16.

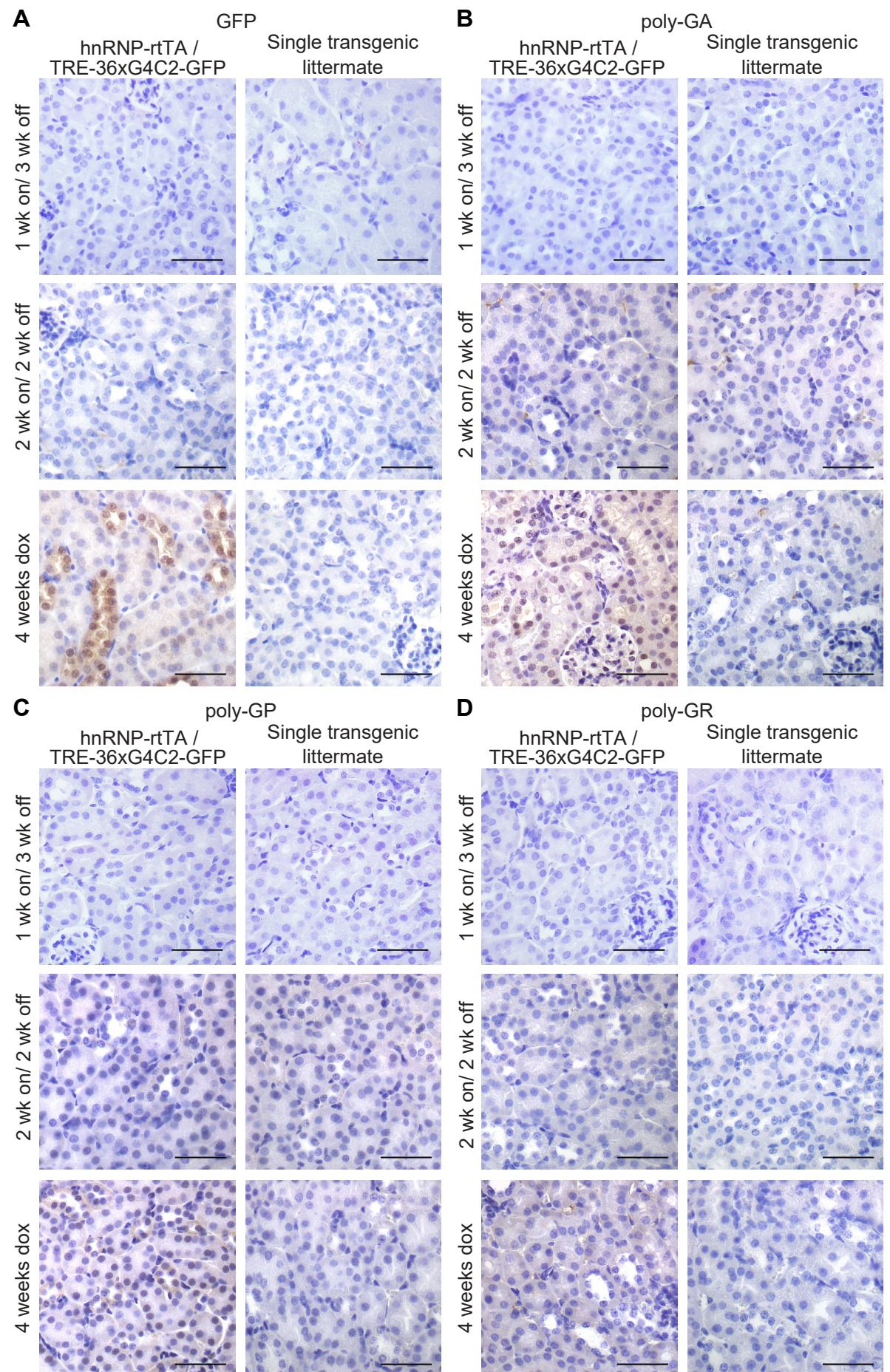


**Supplementary figure 7: TRE-36xG4C2-GFP/Camk2-alpha-rtTA double transgenic mice do not show astrogliosis or microgliosis.** A) Astrogliosis was assessed with GFAP labeling and B) microgliosis was tested with Iba1 staining. No differences in amount or morphology of GFAP-positive and Iba1-positive cells were seen in the hippocampus dentate gyrus of TRE-36xG4C2-GFP/Camk2-alpha-rtTA double transgenic mice and single transgenic control littermates. Single transgenic littermates, consisting of either TRE-only or rtTA-only, were treated similarly with dox. Scale bars are 20  $\mu\text{m}$ . C) To quantify the thickness of Iba1-positive neurites, averages were taken of 10 pictures per mouse. N=8 TRE-36xG4C2-GFP/Camk2-alpha-rtTA double transgenic mice and n=8 single transgenic controls. T-test  $p=0.9099$ .



**Supplementary figure 8: Erasmus ladder readouts.** A) Step times of short steps (from one higher rung to the next; left) and long steps (skipping one higher rung, right). B) Fraction of short and long steps of all steps. C) Fraction of steps that were made from a higher rung to a lower rung. Lines indicate medians and shaded areas the interquartile ranges.





**Supplementary figure 9: GFP and sense DPRs are cleared from the kidney after 2 weeks of dox withdrawal.** A) GFP staining on kidney of TRE-36xG4C2-GFP/hnRNP-rtTA double transgenic mice shows clearance of GFP staining when mice received 2 weeks of dox water followed by 2 weeks of normal drinking water compared to DT littermates that received 4 weeks of dox. B) Poly-GA staining of kidney shows clearance of poly-GA after two weeks of dox withdrawal. C) Poly-GP staining and D) Poly-GR staining are also cleared from kidneys after 2 weeks of dox withdrawal. Single transgenic littermates received 2 or 4 weeks of dox and are all negative for GFP and DPRs. All scale bars are 50  $\mu$ m. All stainings were performed on all mice in this study. Numbers per group are: ST 1 week dox n=7, DT 1 week dox n=8, ST 1 week on/3 weeks off n=4, DT 1 week on/3 weeks off n=7, ST 2 weeks dox n=6, DT 2 weeks dox n=8, ST 2 weeks on/2 weeks off n=6, DT 2 weeks on/2 weeks off n=5, ST 4 weeks dox n=15, DT 4 weeks dox n=16

**Supplementary table 1: antibodies**

<b>Ab name</b>	<b>Host</b>	<b>Company</b>	<b>Cat.nr</b>	<b>Dilution</b>
GA	mouse	Millipore, clone 5E9	MABN889	1:500
GP	rabbit	Bio Connect Life Sciences	24494-1-AP	1:250
GR	mouse	LifeTein Services	n.a. (costum-made)	1:4000
PR	mouse	LifeTein Services	n.a. (costum-made)	1:500
PA	mouse	Gift from Petrucelli	n.a.	1:2500
pTDP-43	mouse	Cosmo bio	CAC-TIP-PTD-M01	1:1000
p62	mouse	BD Biosciences	610833	1:100
Neurofilament	chicken	2BScientific Ltd.	CPCA-NF-H-25ul	1:500
GFAP	Rabbit	Sigma	G-9269	1:100
Iba1	rabbit	Wako	019-19741	1:200
ChAT	goat	Chemicon	AB144P	1:500
poly-HRP anti Ms/Rb IgG	goat	Immunologic	DPV055HRP	undiluted
anti-mouse HRP	goat	DAKO	P0260	1:100
anti-rabbit HRP	goat	DAKO	P0217	1:100
anti-mouse Cy2	goat	Jackson	715-255-150	1:100
anti-rabbit Cy3	goat	Jackson	711-165-152	1:100
anti-chicken 488	goat	Jackson	303-545-006	1:100
anti-goat HRP	rabbit	DAKO	P0449	1:100