

Supplemental information

**Central synaptopathy is the most conserved
feature of motor circuit pathology across spinal
muscular atrophy mouse models**

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Supplemental Information

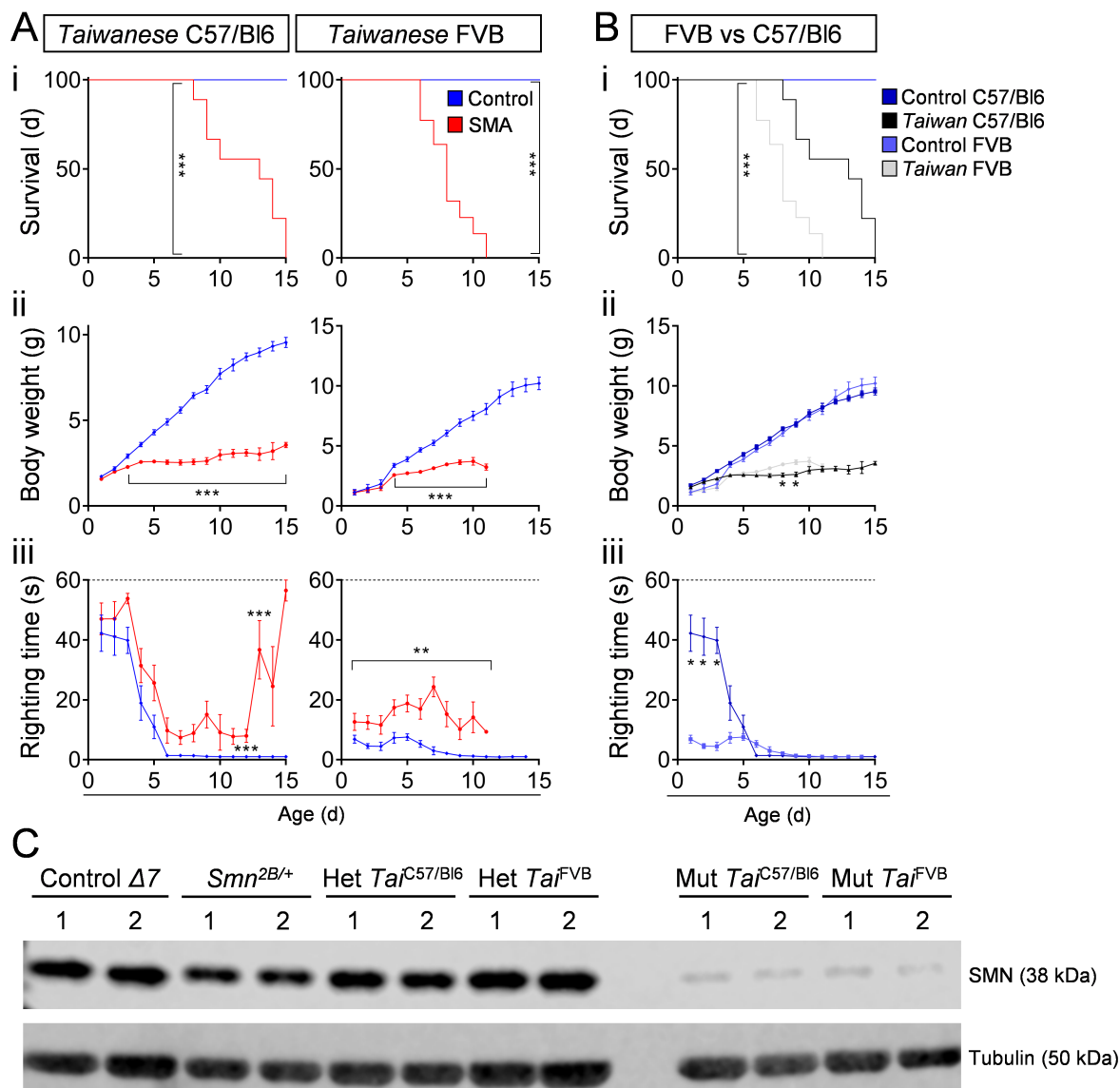
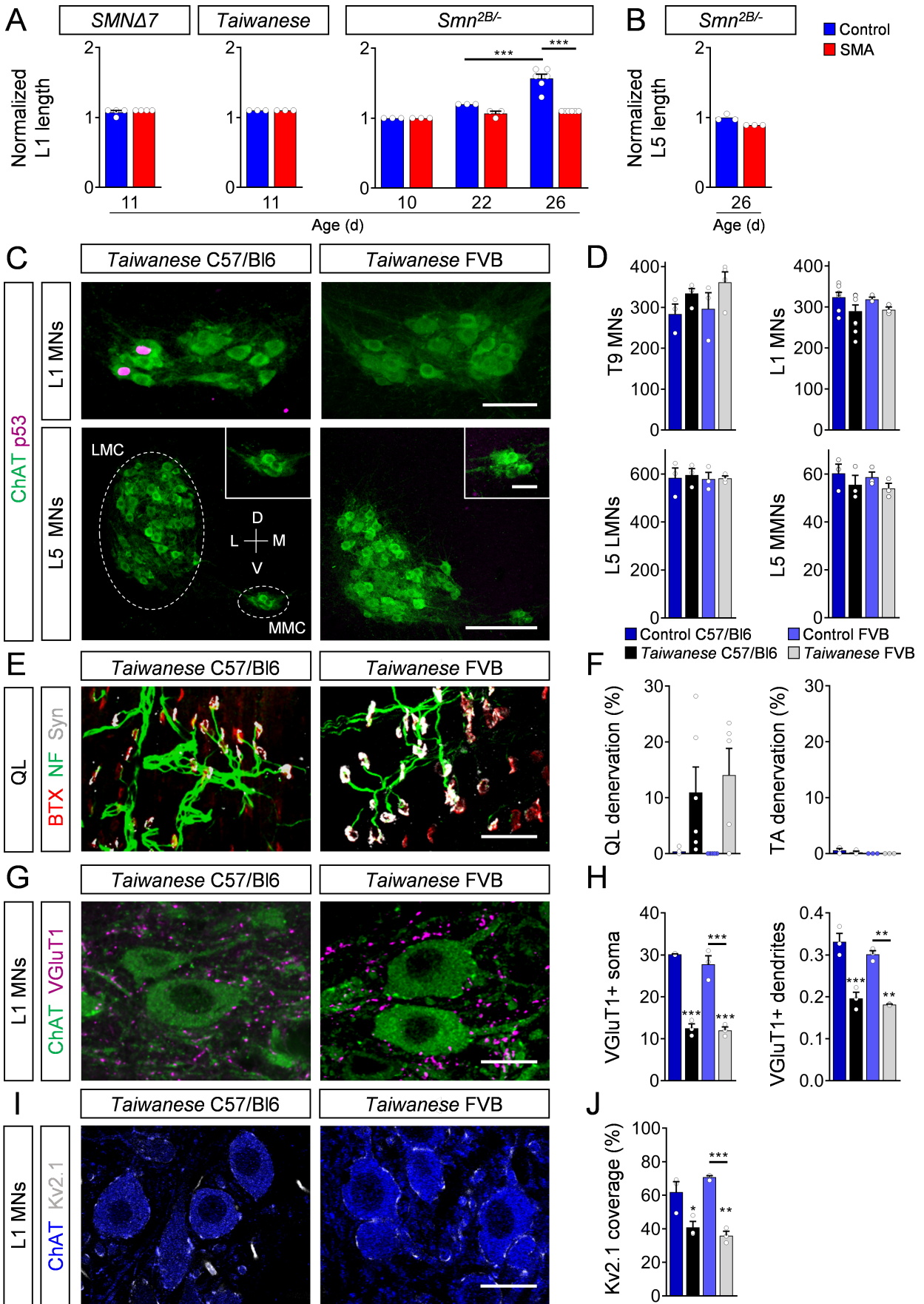


Figure S1. FVB background shortens life span of *Taiwanese* mice independent of SMN levels. Related to Fig. 1. (A-B) Survival (i), body weight (ii) and righting time (iii) of (A) *Taiwanese* shown separately for C57/Bl6 (left panel), FVB background (middle panel) and (B) on both backgrounds for side-by-side comparison. Statistics: Mantel-Cox test for survival (i), multiple t-test with Holm-Sidak method for body weight (ii) and righting time (iii). animal numbers (n) = *Taiwanese C57/Bl6*: control = 19, SMA = 18; *Taiwanese FVB*: control = 23, SMA = 26. (C) Western blot analysis of SMN and tubulin (loading control) protein levels in the spinal cord of control animals from *SMN $\Delta 7$* , *Smn*^{2B/+}, heterozygous *Taiwanese C57/Bl6* and *Taiwanese FVB* mice together with mutant *Taiwanese* on C57/Bl6 and FVB background mice at postnatal day 10. Data are presented as mean \pm SEM. Asterisks on top of bars without horizontal line indicate the significance compared to the control group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



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Figure S2. Genetic background does not impact motor circuit pathology. Related to Fig. 2. (A) Quantification of normalized L1 spinal segment length of *SMNΔ7*, *Taiwanese* at P11 and *Smn^{2B/-}* mice at P10, P22 and P26 including age-matched control littermates. Statistics: two-tailed t-test for *SMNΔ7*, *Taiwanese* and two-way ANOVA with Tukey's correction for *Smn^{2B/-}*. n values for L1 segment length = *SMNΔ7*: control = 4, SMA = 4; *Taiwanese*: control = 3, SMA = 4; *Smn^{2B/-}*: control P10 and P22 = 3, SMA = 3; control P26 = 6, SMA = 6. n values for L5 segment length P26 *Smn^{2B/-}*: control = 3, SMA = 3. (B) Quantification of normalized L5 spinal segment length of *Smn^{2B/-}* mice with age-matched control littermates at P26. Statistics: two-tailed t-test. (C) Immunostaining of ChAT+ motor neurons (green) and p53 (magenta) of L1 (upper panel) and L5 (lower panel) spinal cord sections from P11 mutant *Taiwanese* on *C57/Bl6* and FVB background P11. Scale bar: upper panel = 100μm, lower panel = 200μm and insets = 20μm. (D) Number of T9, L1, L5 lateral and L5 medial motor neurons of *Taiwanese C57/Bl6* and *Taiwanese FVB* mice at P11 with including age-matched control littermates. Statistics: one-way ANOVA with Tukey's correction. n values T9 MNs = *Taiwanese C57/Bl6*: control = 3, SMA = 3; *Taiwanese FVB*: control = 3; SMA = 4. n values L1 MNs = *Taiwanese C57/Bl6*: control = 7, SMA = 9; *Taiwanese FVB*: control = 3; SMA = 3. n values L5 LMNs and MMNs = 3 per group. (E) NMJ staining with bungarotoxin (BTX, red), synaptophysin (Syn, gray), and neurofilament (NF, green) in quadratus lumborum (QL) muscles from the same groups as in (C) at P11. Scale bar = 50μm. (F) Percentage of denervation of QL and tibialis anterior (TA) from the same groups as in (D) at P11. Statistics: one-way ANOVA with Tukey's correction. n values QL denervation = *Taiwanese C57/Bl6*: control = 3, SMA = 6; *Taiwanese FVB*: control = 5; SMA = 5. n values TA denervation = 3 per group. (G) Immunostaining of VGluT1+ synapses (magenta) and ChAT+ motor neuron (green) somata of the same groups as in (C) at P11. Scale bar = 20μm. (H) Number of VGluT1+ synapses on L1 motor neuron somata and dendrites of the same groups as in (D) at P11. Statistics: one-way ANOVA with Tukey's correction. (I) Single optical plane confocal images of L1 ChAT+ motor neurons (blue) expressing Kv2.1 channels (gray) from the same groups as in (C) at P11. Scale bar = 20μm. (J) Percentage of somatic coverage of Kv2.1 expression in L1 motor neurons for the same groups as in (D) at P11. Statistics: one-way ANOVA with Tukey's correction. n values for VGluT1 soma, dendrites and Kv2.1 = 3 animals per group. Data are presented as mean ± SEM. Asterisks on top of bars without horizontal line indicate the significance compared to the control group. *p<0.05; **p<0.01; ***p<0.001.

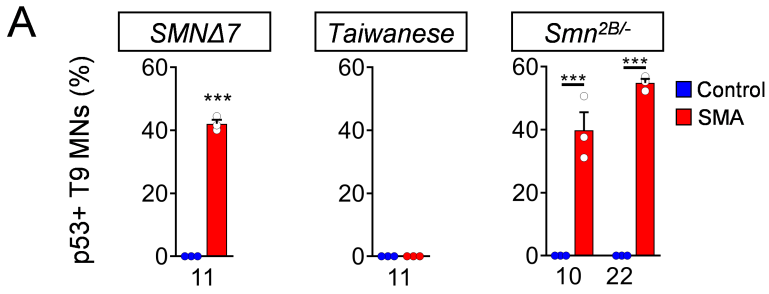


Figure S3: p53 expression in T9 motor neurons. Related to Fig. 3. (A) Percentage of p53+ T9 motor neurons of *SMNΔ7*, *Taiwanese* C57/Bl6 and *Smn^{2B/-}* mice at end-stage with age-matched controls. Statistics: two-tailed t-test for *SMNΔ7*, *Taiwanese* and two-way ANOVA with Tukey's correction for *Smn^{2B/-}*. n values p53+ T9 = 3 animals per group. Data are presented as mean ± SEM. Asterisks on top of bars without horizontal line indicate the significance compared to the control group. *p<0.05; **p<0.01; ***p<0.001.

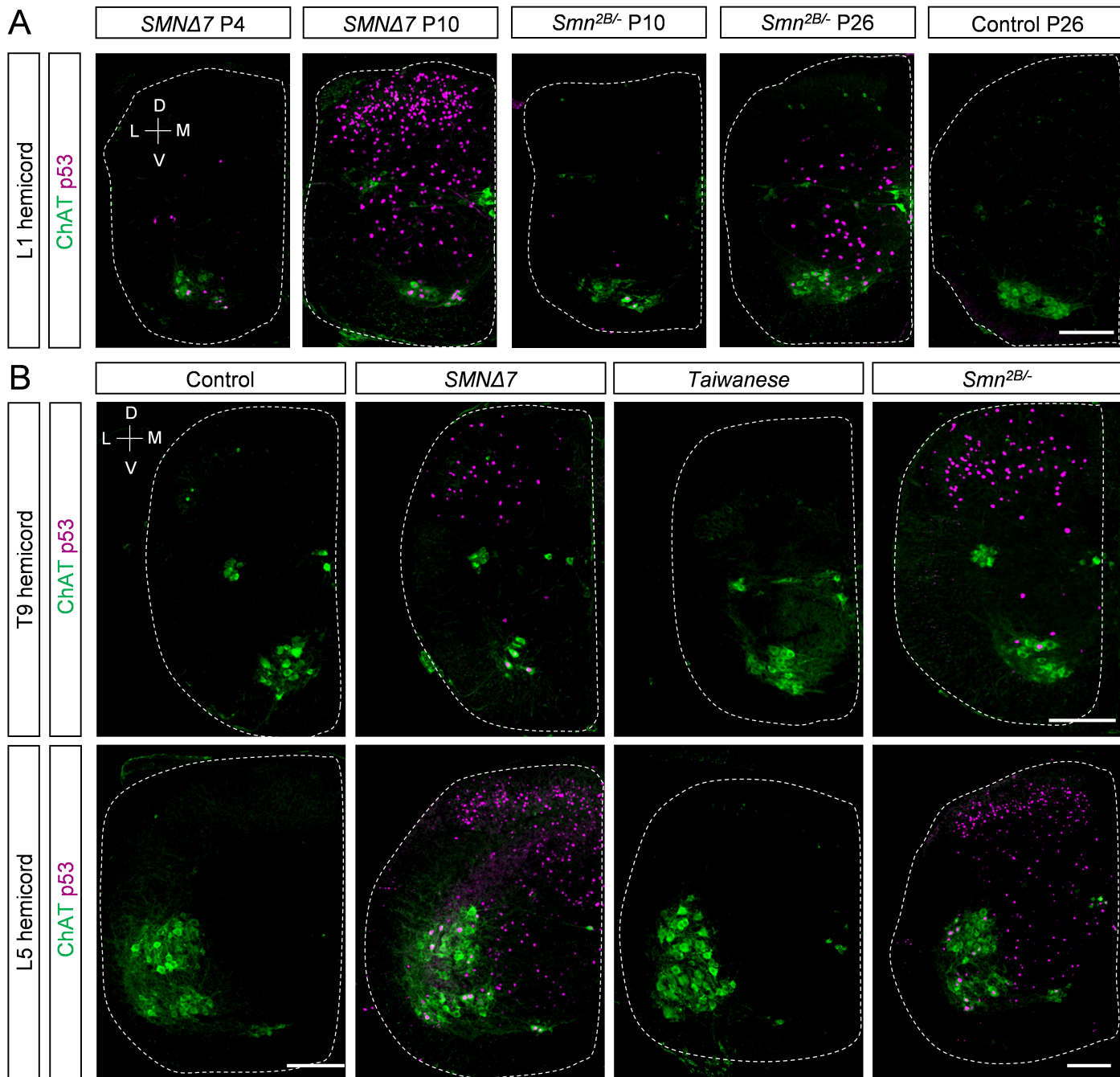
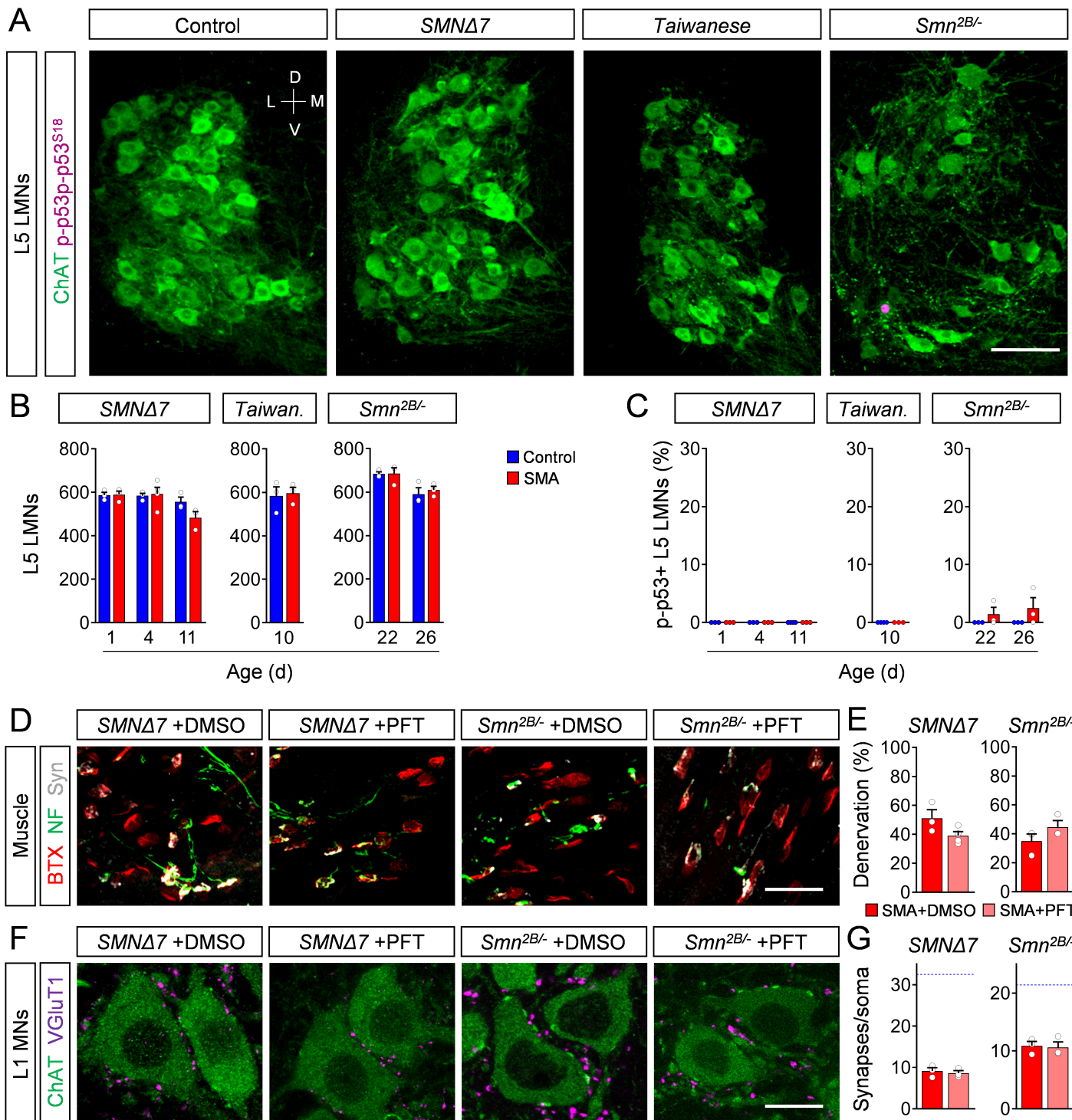


Figure S4. p53 expression in the spinal cord over time. Related to Fig. 4. (A) Immunostaining of ChAT+ motor neurons (green) and p53 (magenta) of L1 spinal cord hemicord sections from mutant *SMNΔ7* at P4 and P10 and *Smn*^{2B/-} mice at P10, P26 and a control littermate at P26. Scale bar = 200µm. (B) Immunostaining of ChAT+ motor neurons (green) and p53 (magenta) of T9 (upper panel) and L5 (lower panel) spinal cord hemicord sections from control, *SMNΔ7*, *Taiwanese* C57/Bl6 and *Smn*^{2B/-} mice at end-stage. Scale bars = 200µm.



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Figure S5. p-p53^{S18} expression is absent in resistant motor neuron pools. Related to Fig. 5. (A) Immunostaining of ChAT+ motor neurons (green) and phosphorylated p53 (magenta) of lateral L5 spinal cord segments from control, *SMNΔ7*, *Taiwanese* and *Smn^{2B/-}* mice at end-stage. Scale bar = 100μm. (B) Number of L5 lateral motor neurons of *SMNΔ7* at P1, P4 and P11, *Taiwanese* at P10 and *Smn^{2B/-}* mice at P22 and P26 with age-matched control littermates. Statistics: two-tailed t-test for *Taiwanese* and two-way ANOVA with Tukey's correction for *SMNΔ7* and *Smn^{2B/-}*. n values L5 LMNs = 3 for all genotypes and ages, except *SMNΔ7* P4 = 4. (C) Percentage of phosphorylated p53^{S18}+ L5 lateral motor neurons of the same groups and ages as in (B). Statistics: two-tailed t-test for *Taiwanese* and two-way ANOVA with Tukey's correction for *SMNΔ7* and *Smn^{2B/-}*. n values for p-p53^{S18}+ L5 LMNs = 3 for each group and ages, except *SMNΔ7* control P11 = 4 and *Taiwanese* control = 4. (D) NMJ staining with bungarotoxin (BTX, red), synaptophysin (Syn, gray), and neurofilament (NF, green) in quadratus lumborum (QL) muscles from *SMNΔ7* + DMSO, *SMNΔ7* + PFT, and tibialis anterior (TA) from *Smn^{2B/-}* + DMSO and *Smn^{2B/-}* + PFT mice at end-stage. Scale bar = 50μm. (E) Percentage of denervation in the QL muscle of *SMNΔ7* mice treated with DMSO or PFT and in TA muscle of *Smn^{2B/-}* mice treated with DMSO or PFT at end-stage. Statistics: two-tailed t-test. n values for denervation = 3 per group, except *SMNΔ7* SMA = 4. (F) Immunostaining of VGluT1+ synapses (magenta) and ChAT+ motor neuron (green) somata of the same groups as in (D) at end-stage. Scale bar = 20μm. (G) Number of VGluT1+ synapses on L1 motor neuron somata of the same groups as in (D) at end-stage. Blue dotted lines indicate synaptic numbers of control littermates. Statistics: two-tailed t-test. n values for denervation = 3 per group. Data are presented as mean ± SEM. Asterisks on top of bars without horizontal line indicate the significance compared to the control group. *p<0.05; **p<0.01; ***p<0.001.

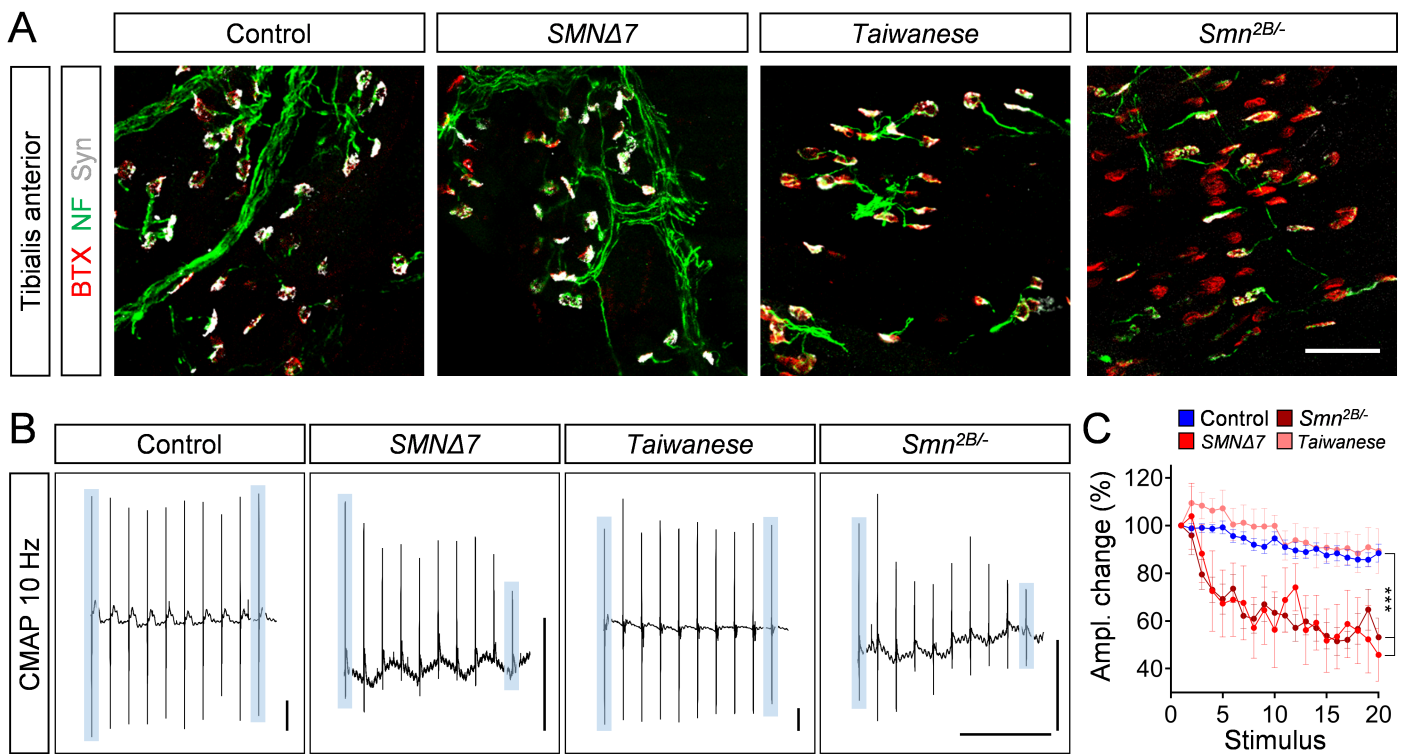


Figure S6. Distal muscles are more resistant in SMA mouse models. Related to Fig. 6. (A) NMJ staining with bungarotoxin (BTX, red), synaptophysin (Syn, gray), and neurofilament (NF, green) in tibialis anterior (TA) muscles from control, *SMNΔ7*, *Taiwanese* and *Smn^{2B/-}* mice at end-stage. Scale bar = 50μm. (B) Example trains of CMAP recordings at 10Hz stimulation from P11 TA muscles of the same groups as in (A). Blue transparent boxes indicate first and tenth muscle response of each train. Scale bars = 0.5mV, 50ms. (C) Percentage of CMAP amplitude change at 10Hz stimulation from P11 TA muscles of the same groups as in (A). Control group is pooled from controls of each individual line. n values for 10 Hz stimulation: control = 24; *SMNΔ7* = 5; *Taiwanese* = 8; *Smn^{2B/-}* = 7. Statistics: multiple t-test with Holm-Sidak method. Data are presented as mean ± SEM. Asterisks on top of bars without horizontal line indicate the significance compared to the control group. *p<0.05; **p<0.01; ***p<0.001.

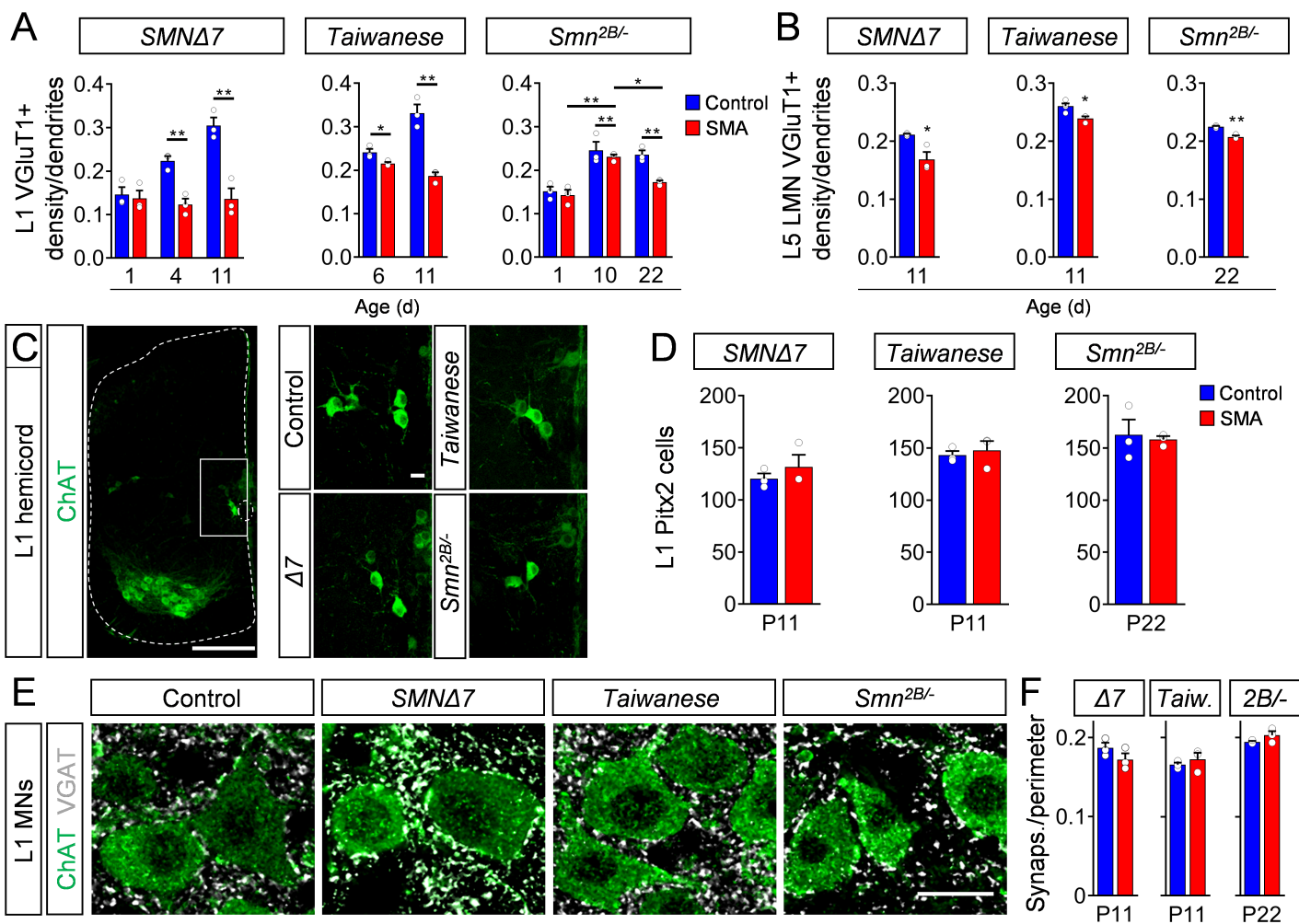


Figure S7. Selective loss of excitatory synapses. Related to Fig. 7. (A-B) Number of VGLuT1+ synapses on proximal dendrites of (A) L1 motor neurons and (B) L5 lateral motor neurons of *SMNΔ7*, *Taiwanese* and *Smn^{2B/-}* mice with age-matched control littermates. Statistics: two-way ANOVA with Tukey's correction for (A) and two-tailed t-test for (B). n values for L1 and L5 LMN VGLuT1+ = 3 animals per group. (C) Immunostaining of ChAT+ neurons (green) in a P11 control spinal cord hemicord. White box indicates location of cholinergic Pitx2 cells surrounding the central canal. Scale bar = 200μm. Magnified images show cholinergic Pitx2 from the same groups (A) at P11 and P22, respectively. Scale bar = 20μm (D) Number of cholinergic Pitx2 cells in L1 spinal segments of the same groups and ages as in (A). Statistics: two-tailed t-test. n values for Pitx2 = 3 animals per group (E) Immunostaining of VGAT+ synapses (gray) on ChAT+ motor neuron somata (green) of L1 spinal cord segments from the same groups and ages as in (A). Scale bar = 20μm. (F) Quantification of VGAT+ synapses per perimeter of L1 motor neurons from the same groups and ages as in (A). n value for VGAT = 3 animals per group. Statistics: two-tailed t-test. Data are presented as mean ± SEM. Asterisks on top of bars without horizontal line indicate the significance compared to the control group. *p<0.05; **p<0.01; ***p<0.001.

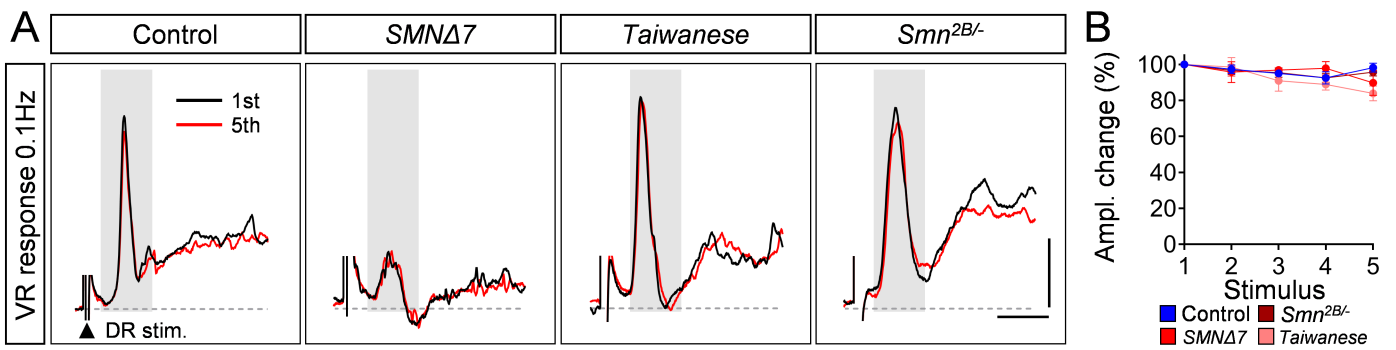


Figure S8: Proprioceptive synapses do not depress at low frequency stimulation. Related to Fig. 8. (A) Representative traces of the first (black) and fifth (red) VR responses recorded following stimulation of the homonymous L1 DR at 0.1Hz from P11 control, *SMNΔ7*, *Taiwanese* and *Smn^{2B/-}* mice. Scale bars = 0.5mV and 3ms. (B) Quantification of amplitude changes in percent of the monosynaptic ventral root response following 0.1Hz stimulation at P11 from the same groups as in (A). Statistics: multiple t-test with Holm-Sidak method. n values for 0.1Hz stimulation: control = 10; *SMNΔ7* = 7; *Taiwanese* = 5; *Smn^{2B/-}* = 5. Data are presented as mean ± SEM. Asterisks on top of bars without horizontal line indicate the significance compared to the control group. *p<0.05; **p<0.01; ***p<0.001.

Name	Company	Cat #	Host	Application	Dilution
SMN clone 8	BD Transd Lab	610646	Mouse	WB	1:10,000
Tubulin DM1A	Sigma	T9026	Mouse	WB	1:50,000
p53	Leica Novocastra	NCL-p53-CM5p	Rabbit	IF	1:1,000
p-p53 ^{S15}	Cell Signaling	9284 (Lots: 12,15)	Rabbit	IF	1:200
VGlut1	Synaptic Systems	135 304	Guinea pig	IF	1:5,000
Synaptophysin	Synaptic Systems	101-004	Guinea pig	IF	1:500
Neurofilament	Millipore	AB1987	Rabbit	IF	1:500
ChAT	Millipore	AB144P	Goat	IF	1:500
Bungarotoxin	Invitrogen	B35451	N/A	IF	1:500
Kv2.1	Neuromab	K89/34 concent.	Mouse	IF	1:500
Parvalbumin	Synaptic Systems	195 004	Guinea pig	IF	1:5,000
SmB	Santa Cruz	Sc-130670	Mouse	IF	1:100
VGAT	Synaptic Systems	131004	Guinea pig	IF	1:500

Table S1. List of antibodies. All first antibodies used in this study.

Gene Name	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
<i>p53</i>	GCCAAGTCTGTTATGTGCAC	GACTTCTTGTAGATGGCCATG
<i>Cdkn1a</i>	GACATTCAGAGCCACAGGCACC	GAGCGCATCGCAATCACGGCGC
<i>Perp1</i>	CAGAGCCTCATGGAGTACGC	GAGAATGAAGCAGATGCACAGG
<i>Gtse1</i>	TGACAAAGAGAACGTGGACTCAC	GAGGTGGGAGGCTTAGGTTC
<i>Stas Aber</i>	TGACGCCAAGGCTCTAGGAAAA	CCAAGTCCGGAGCATTGTACATAAAAAGG
<i>H1c pre</i>	GAGCCACCACTCCCACTTAAG	GGATCGAGTCCCTTGCAAC
<i>Chodl</i>	CCTACCTTTACCAGTGGAATGACG	TGGGTCTTTCAGGTTGGTTTG
<i>Gapdh</i>	AATGTGTCCGTCGTGGATCTGA	GATGCCTGCTTCACCACCTTCT
<i>Mdm2</i> E2-5	GATCACCGCGCTTCTCCTGC	CAATGTGCTGCTGCTTCTCG
<i>Mdm4</i> E5-8	TGTGGTGGAGATCTTTTGGG	TCAGTTCTTTTCTGGGATTGG

Table 2. List of primers for qPCR. Forward and reverse sequences for each primer set are listed.