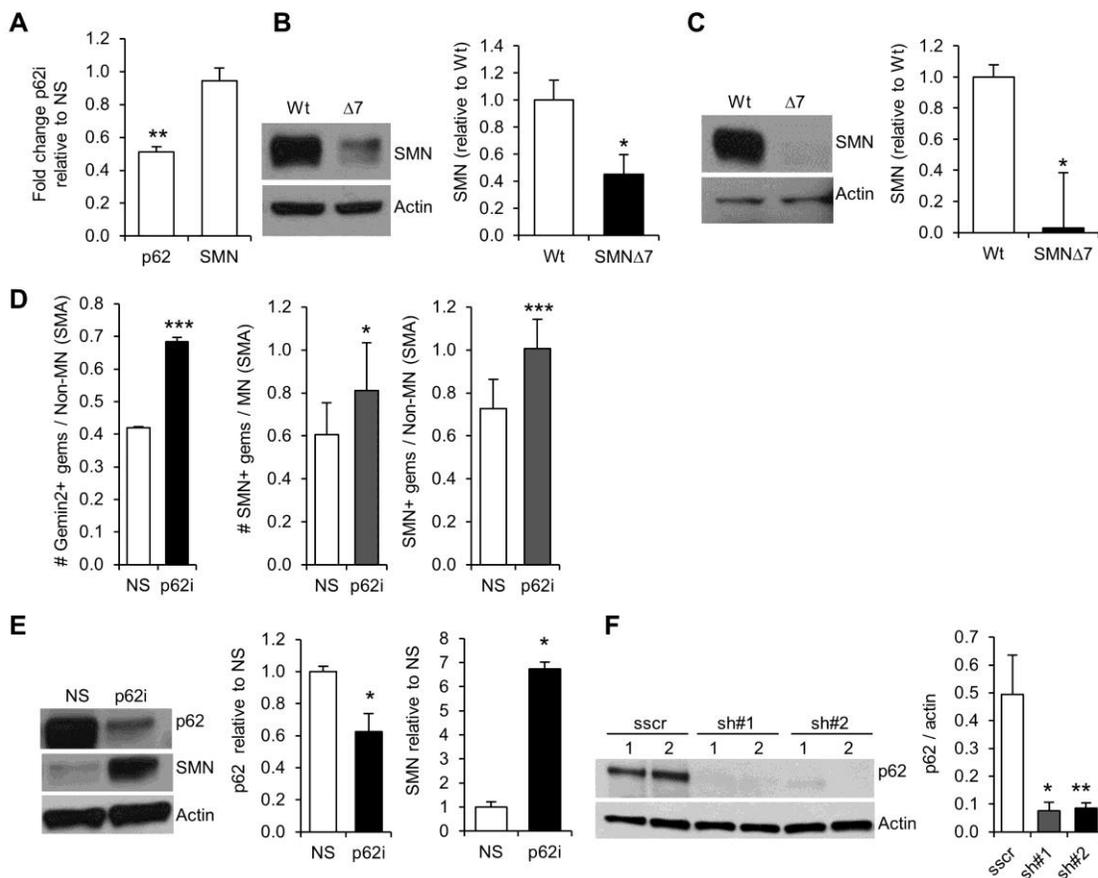
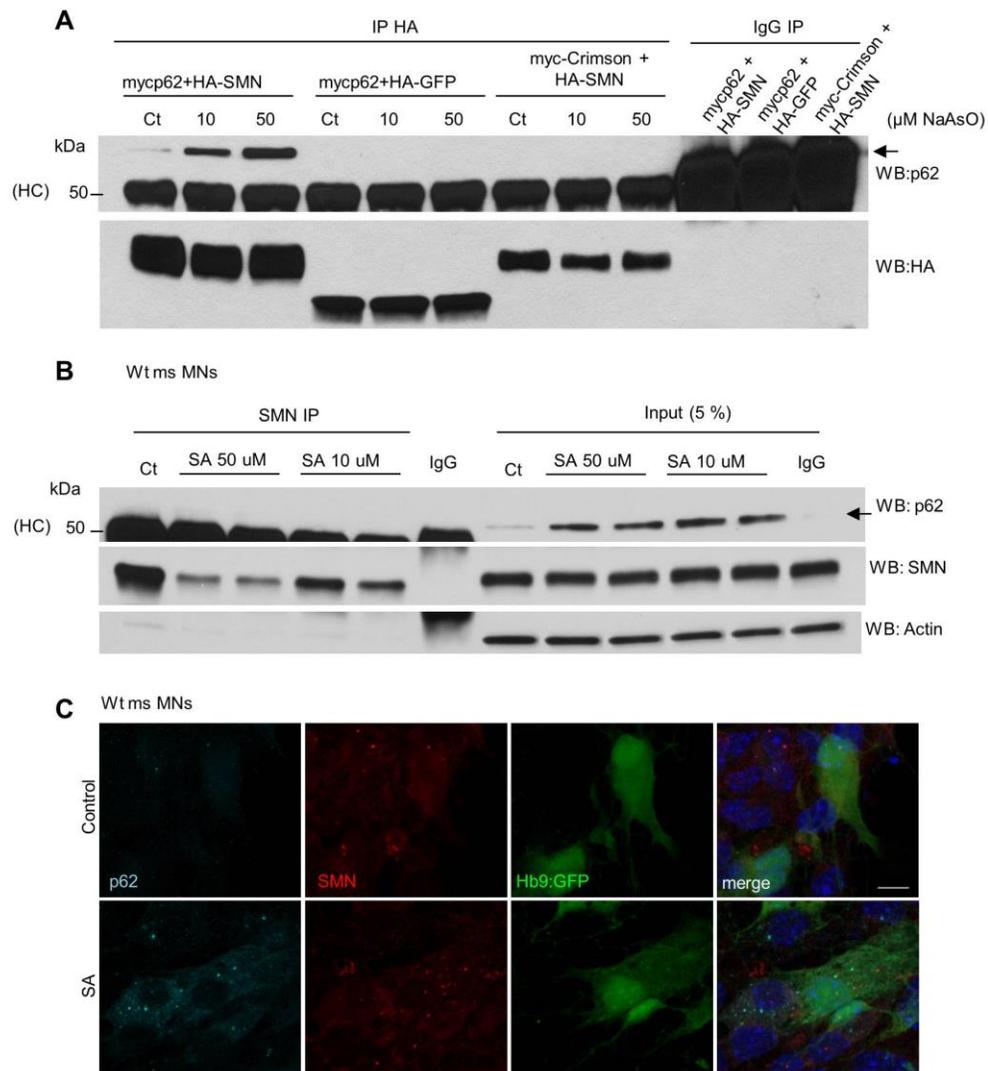


Supplemental Figure 1.



Supplemental Figure 1. Increased amounts of SMN and number of gems upon p62 depletion. (A) qPCR from wildtype mouse ESC-derived MNs after p62 knock-down showing mRNA expression levels of p62 and SMN (Mean \pm s.e.m. $**p < 0.01$, Two-way *t*-test, $n=3$ independent experiments). (B) Representative immunoblot and quantification from protein lysates from wildtype and SMN Δ 7 mouse ESCs and (C) ESC-derived MNs (Mean \pm s.e.m. $*p < 0.05$, Two-way *t*-test, $n=5$ independent experiments). (D) Quantification of the number of gemin2⁺ gems per cell in the non-MN population of the culture and the number of SMN⁺ gems per cell in MNs and non-MNs in SMA mouse cultures 7 days after infection with non-silencing control (NS) or shRNA against p62 (p62i) (Mean \pm s.e.m. $***p < 0.001$, $*p < 0.05$, Two-way *t*-test, $n=3, 7, 7$ independent experiments from left to right, 600 MNs and 40,000 non-MNs per experiment were counted). (E) Representative immunoblot from protein lysates from 3T3 cells 5 days after being infected with non-silencing control (NS) or shRNA against p62 (p62i), and quantification of p62 and SMN protein levels (Mean \pm s.e.m. $*p < 0.05$, Two-way *t*-test, $n=3$ independent experiments). (F) Representative immunoblot and quantification from human healthy control BJ iPSC-derived MNs 7 days after infection with lentivirus carrying scramble (sscr)-control shRNA or two different shRNA against p62 (Mean \pm s.e.m. $**p < 0.01$, $*p < 0.05$, Two-way *t*-test, $n=4$ independent experiments).

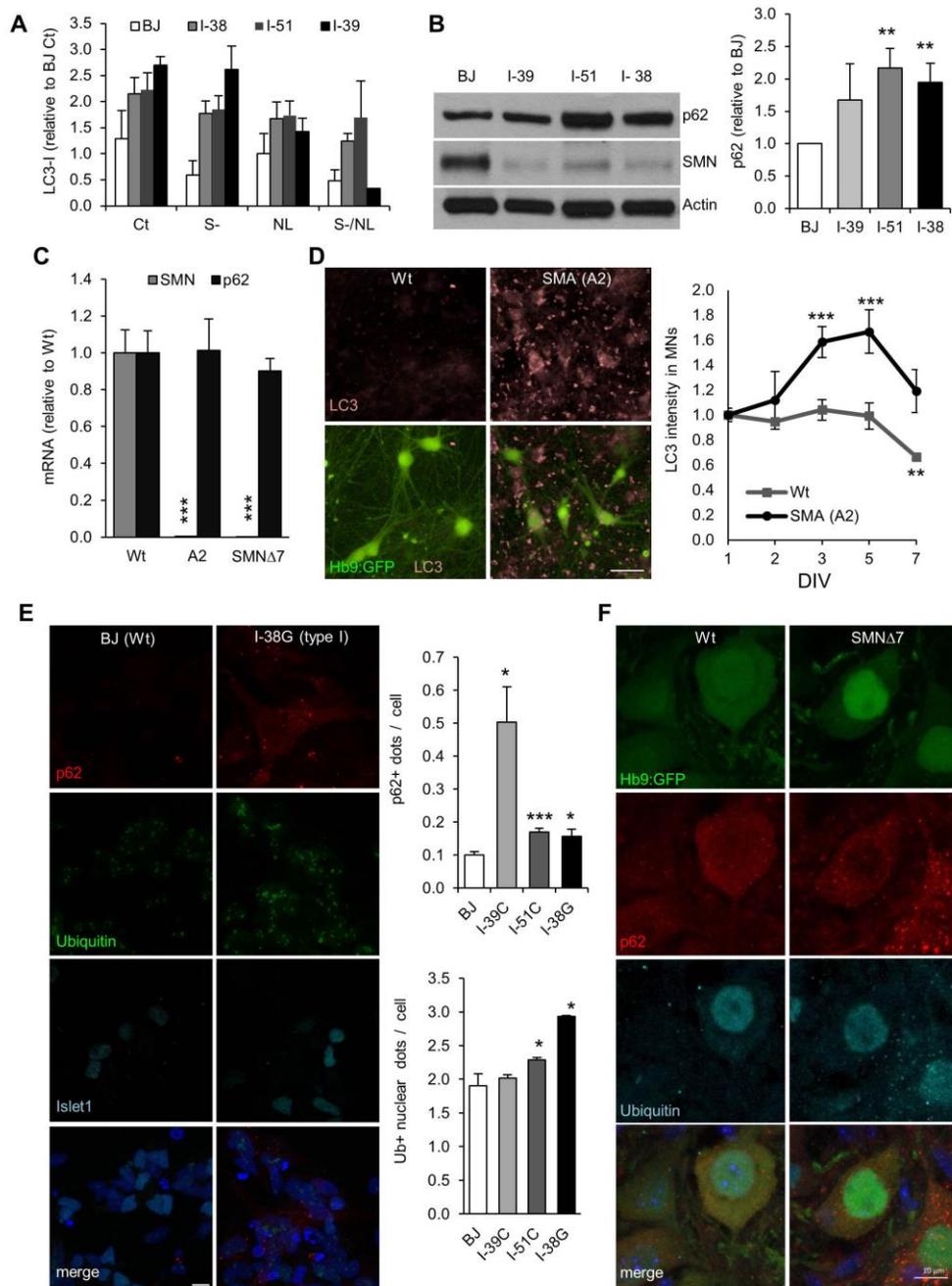
Supplemental Figure 2.



Supplemental Figure 2. Binding between SMN and p62 is seen in SG-induced conditions.

(A) Representative HA immunoprecipitation from HEK293T lysates transfected with the indicated plasmids and treated with control media or sodium arsenite (SA) (10 or 50 μM) the last 6 hours of the culture. Membranes were immunoblotted against p62 and HA (HC, heavy IgG chain). (B) Representative immunoprecipitation of endogenous SMN protein from mouse wildtype MN lysates treated with control or SA for the last 6 hours of the culture and immunoblotted against p62, SMN, LC3 and actin (HC, heavy IgG chain). (C) Representative image from mouse wildtype ESC-derived MNs cultured in control conditions or with SA and immunostained against p62 (cyan) and SMN (red) (the MN promoter Hb9 is endogenously labeled with the reported GFP and nuclei are stained with DAPI, blue). Scale bar, 10 μm .

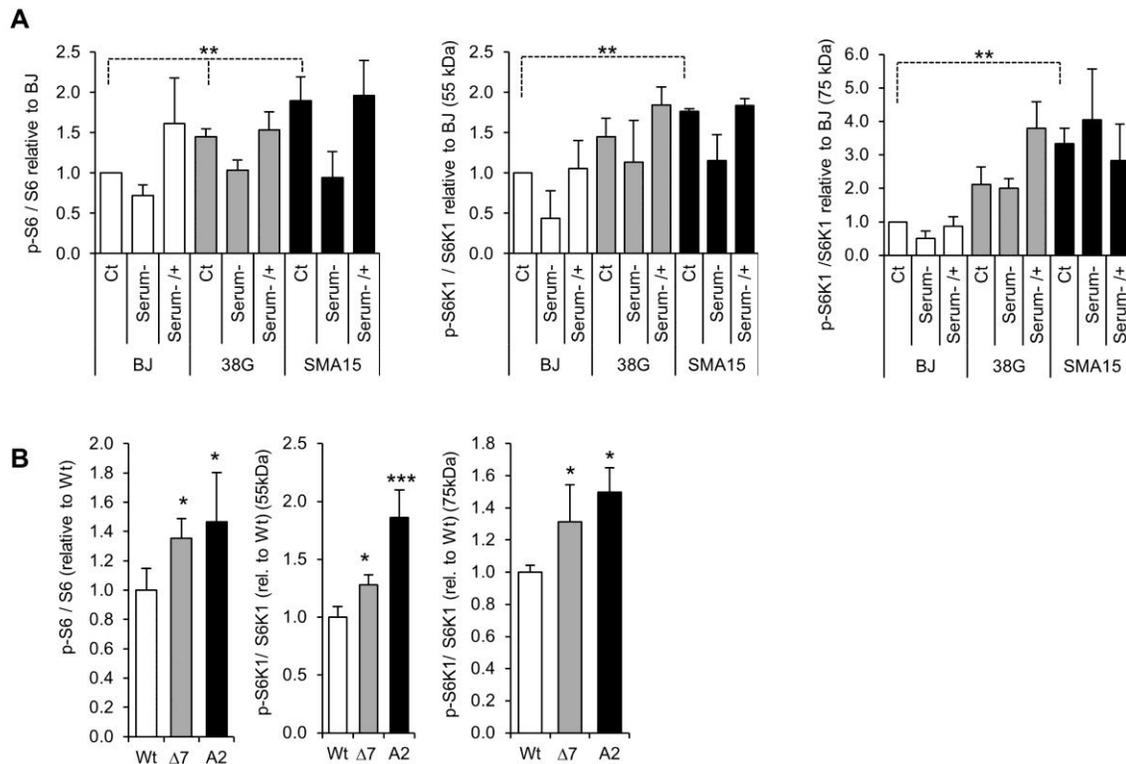
Supplemental Figure 3.



Supplemental Figure 3. Lack of SMN results in autophagy activity dysfunction. (A) Quantification of LC3-I protein levels from western blot from healthy control and SMA human fibroblasts exposed to autophagy modulators. (B) Representative immunoblot from protein lysates from healthy control and SMA human fibroblasts showing p62 and quantification (Mean \pm s.e.m. $^{**}p < 0.01$, Two-way *t*-test, $n=4$ independent experiments). (C) Quantification of SMN and p62 mRNA expression levels in wildtype and SMA mouse MNs (A2 and SMN Δ 7) (Mean \pm s.e.m. $^{***}p < 0.001$, Two-way *t*-test, $n=7, 7, 4$ independent experiments for Wt, A2 and SMN Δ 7

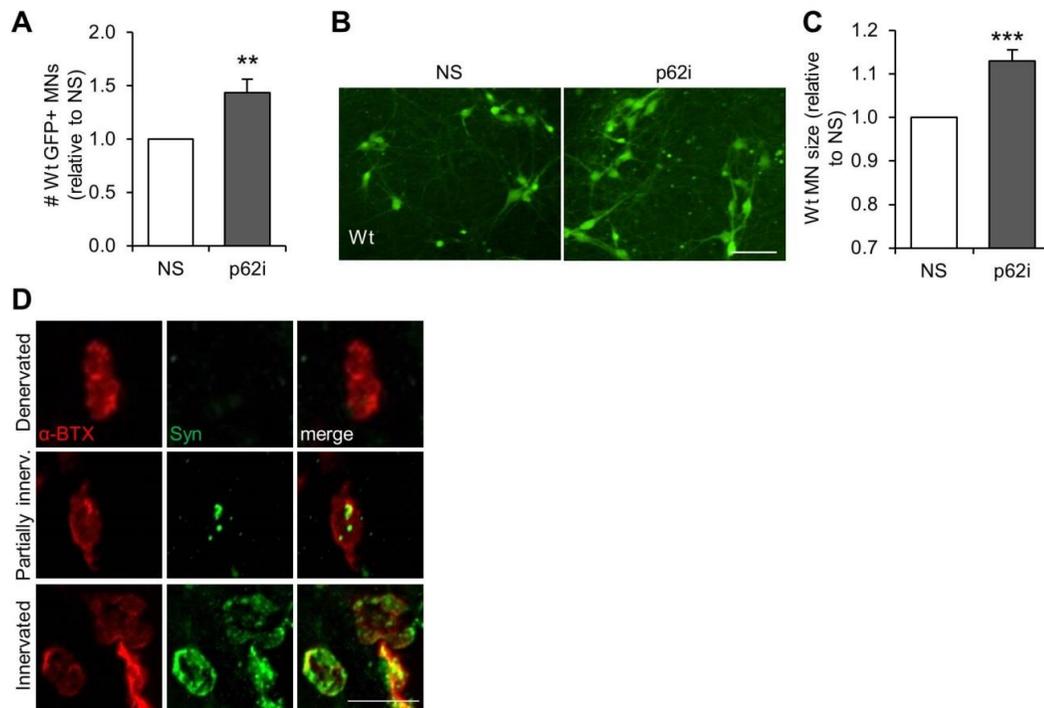
MNs respectively). **(D)** Representative image of immunostaining against LC3 (purple) on mouse Hb9:GFP⁺ wildtype and SMA MNs 7 days after dissociation. The quantification of LC3-immunostaining signal levels over the time course of the culture is shown on the right. Scale bar, 50 μ m (Mean \pm s.e.m. $**p < 0.01$, $***p < 0.001$, Two-way ANOVA followed by Bonferroni post-test, $n=3$ independent experiments. Average number of MNs studied throughout the time course, wildtype, 500; A2, 50). **(E)** Representative confocal images from human control and SMA type I MNs. Cells were fixed 5 days after dissociation and immunostained with p62 (red), ubiquitin (green) and Islet1 (cyan) antibodies (nuclei were stained with DAPI, blue). Quantifications of the number of p62 and ubiquitin dots per MN are shown. Scale bar, 10 μ m (Mean \pm s.e.m. $*p < 0.05$, $***p < 0.001$, Two-way t -test, $n= 2$ independent experiments. Number of cells analyzed for BJ, I-39C I-51C and I-38C, 1800, 1200, 4500 and 1500 respectively). **(F)** Representative confocal images of spinal cords sections from P10 wildtype and SMN Δ 7 littermates immunostained against p62 (red) and ubiquitin (cyan). Hb9 MNs endogenously labeled with GFP and nuclei stained with DAPI (blue) are shown. Scale bar, 10 μ m.

Supplemental Figure 4.



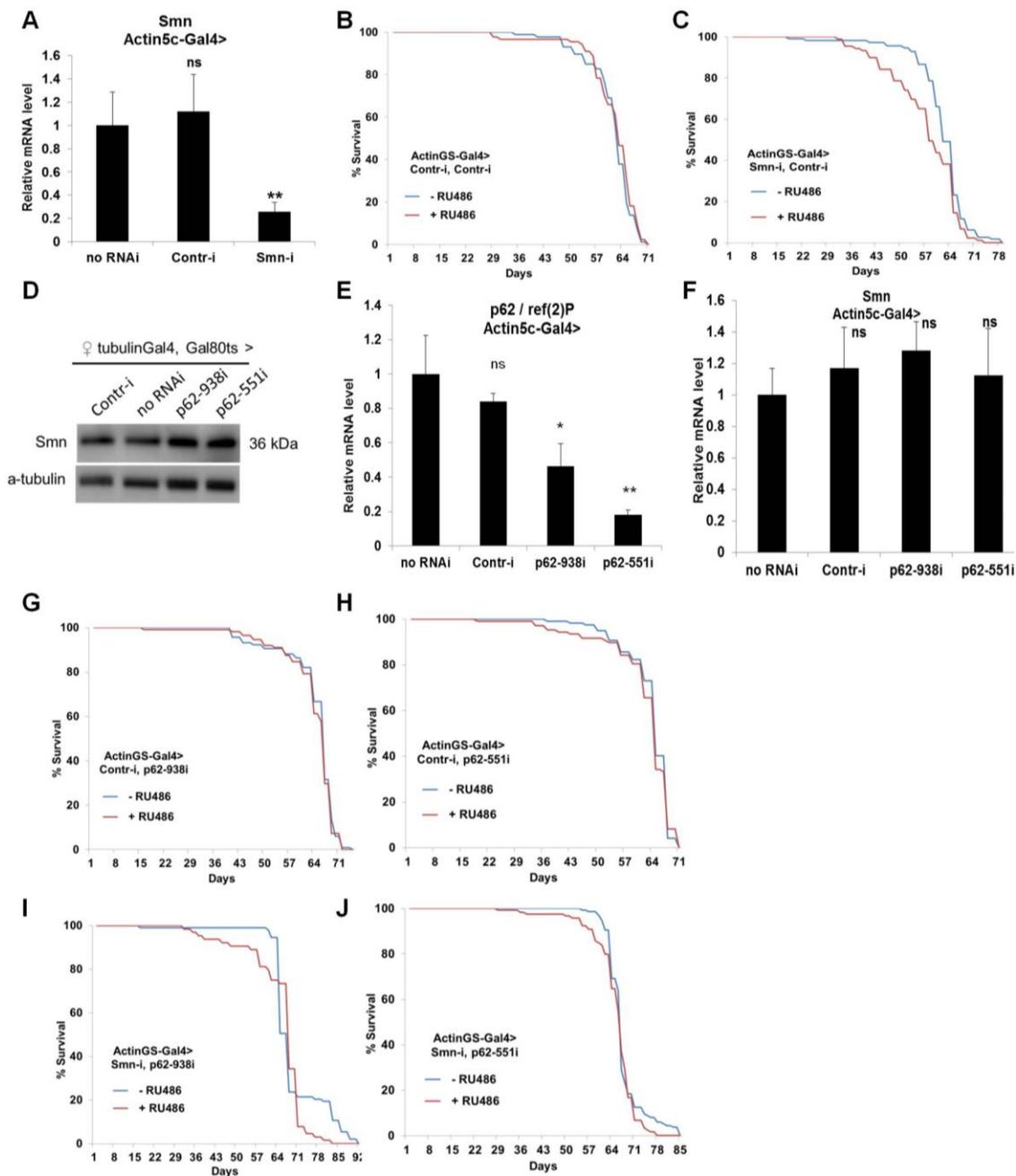
Supplemental Figure 4. Increased mTOR activity upon SMN deficiency. (A) Quantification of the levels of p-S6 (Ser235/236) over S6 total and p-S6K1 (Thr389) over p-S6K1 total in human type I and 0 SMA fibroblasts relative to healthy control BJ from western blot shown in Figure 5A (Mean \pm s.e.m. expressed relative to control-treated BJ healthy human fibroblasts. $**p < 0.01$, Two-way *t*-test, $n = 3$ independent experiments). (B) Quantification of the levels of p-S6 (Ser235/236) over S6 total and p-S6K1 (Thr389) over p-S6K1 total in mouse SMA ($\Delta 7$ and A2) MNs relative to wildtype from western blot shown in Figure 5B (Mean \pm s.e.m. $*p < 0.05$, $***p < 0.001$, Two-way *t*-test, $n = 6$ independent experiments).

Supplemental Figure 5.



Supplemental Figure 5. Supplemental Figure 5. Effect of p62 knock-down in Wt mouse ESC-derived MNs and evaluation of FDB-2 denervation. (A) Quantification of number of Hb9:GFP⁺ wildtype mouse ESC-derived MNs treated as in Figure 6A. (Mean \pm s.e.m. expressed relative to NS-treated MNs. * p < 0.05, Two-way t -test, $n=8$ independent experiments). Representative image is shown in (B). Scale bar, 50 μ m. (C) Quantification of the wildtype MN soma size after p62 knock-down (Mean \pm s.e.m. expressed relative to NS-treated MNs. *** p < 0.001, Two-way t -test, 4700 and 6300 MNs were measured for NS and p62i, respectively). (D) High-magnification images of NMJs in FDB-2 muscles of SMN Δ 7 mice at P10 showing nerve terminals immunostained with anti-synaptophysin (green) and endplates with α -bungarotoxin (red). An example for evaluating endplates as fully denervated (top), partially (middle) and fully innervated (bottom) is shown. Scale bar = 20 μ m.

Supplemental Figure 6.



Supplemental Figure 6. p62 knock-down extends the lifespan of a SMA fly model. (A) Relative mRNA levels of Smn from wandering third instar larval stage expressing control RNAi or Smn RNAi (JF02057) under constitutive Actin5c-Gal4 driver (Mean \pm S.D. $**p < 0.01$, Two-way *t*-test). (B) Ubiquitous adult-onset expression of control RNAi does not affect lifespan in flies ($p = 0.3$, Log-Rank test, $n = 87$ (-RU486), $n = 88$ (+RU486)). (C) Ubiquitous adult-onset expression of Smn RNAi significantly suppresses lifespan in the presence of control RNAi ($**p < 0.01$, Log-Rank test, $n = 112$ (-RU486), $n = 89$ (+RU486)). (D) Representative immunoblot

of SMN in tubulinGal80ts, tubulinGal4 flies expressing either control RNAi, p62-551i RNAi (HMS00551) or p62-938i RNAi (HMS00938) for 2 weeks at 29.5°C. **(E)** Relative mRNA levels of p62 / ref(2)P from wandering third instar larval stage expressing either control RNAi, p62-551i RNAi (HMS00551) or p62-938i RNAi (HMS00938) under constitutive Actin5c-Gal4 driver (Mean \pm S.D. expressed relative to no RNAi-treated flies. * p < 0.05, ** p < 0.01. ns, not significant, Two-way t -test). **(F)** Relative mRNA levels of *Smn* from wandering third instar larval stage expressing either control RNAi, p62-551i RNAi (HMS00551) or p62-938i RNAi (HMS00938) under constitutive Actin5c-Gal4 driver (Mean \pm SD. expressed relative to no RNAi-treated flies. ns, not significant, Two-way t -test). **(G)** Ubiquitous adult-onset expression of p62-938i RNAi does not affect lifespan in the presence of control RNAi (p = 0.4, Log-Rank test, n = 117 (-RU486), n = 111 (+RU486)). **(H)** Ubiquitous adult-onset expression of p62-551i RNAi does not affect lifespan in the presence of control RNAi. **(I)** Ubiquitous adult-onset expression of *Smn* RNAi does not affect lifespan in the presence of p62-938i RNAi (p = 0.24, Log-Rank test, n = 93 (-RU486), n = 64 (+RU486)). **(J)** Ubiquitous adult-onset expression of *Smn* RNAi does not affect lifespan in the presence of p62-551i RNAi. Data are presented as mean + SEM.

Supplemental Experimental Procedures

Immunocytochemistry and Image Analysis.

Hoechst 33342 (Life Technologies) was used for nuclear staining. Images of cells were captured using an automated Operetta wide field live-imaging (PerkinElmer) or Opera confocal microscope (PerkinElmer) as indicated in the corresponding figure legend. Subsequent image quantification was automatically performed using the Columbus Image Data Storage and Analysis System (PerkinElmer). MNs were identified either by GFP or Islet fluorescence in the nucleus. Nuclei were identified using Hoechst33342. A size and morphology threshold was used to eliminate apoptotic nuclei from quantification. After cell-identification a constant cytoplasmic region around the nucleus was defined. By using a fully automated imager and associated software, the intensities of all the pixels in that region of the cell for an indicated antibody immunostaining were added giving the “total intensity” in arbitrary units. That number was then divided by the number of pixels in that region, resulting in the “mean intensity” (of a pixel in the cell), in a way that is independent of the cell size. The average intensity of a given immunostaining per cell was averaged across at least 25 random fields per well and 3-5 wells per condition, for at least 3 biological replicates. For each experiment, 5 wells with no primary antibody were used to determine background fluorescence intensity.

Cell culture treatments.

For immunoprecipitation assays, cells were lysed by dounce homogenizer in 500 μ L of lysis buffer containing 50 mM Tris (pH 7.5), 150mM NaCl, 1% NP-40, 1 mM EDTA, and protease and phosphatase inhibitors (Thermo Fisher Scientific). Protein quantification was performed with BCA Protein Assay Kit (Thermo Scientific) following manufacturer’s instructions and 0.5 mg of lysates were pre-cleared by incubation with 10 μ L of magnetic dynabeads Protein G (Life Technologies) at 4°C for 30 min under rotation. Protein solution was collected by separating the beads with a magnet, final volume was brought to 800 μ L with lysis buffer, then 1 μ g of

antibody was added to the pre-cleared lysates and incubated overnight at 4°C in rotation. Bound proteins were eluted by boiling (95 °C for 10min) in 2x Laemmli Sample Buffer (Bio-Rad). Immunoprecipitated (IP) proteins and original lysates (input) were resolved on a SDS–PAGE gel. For cellular fractionation assays, the same number of cells per condition were lysed in ice-cold buffer containing 1M HEPES (pH 7.9), 1M MgCl₂, 2.5M KCl, 1M DTT and protease and phosphatase inhibitors, kept on ice for 5 min and lysed with a dounce homogenizer. Dounced cells were centrifuged at 228 g for 5 min at 4°C and the supernatant retained as the cytoplasmic fraction. 2%SDS, 50 mM Tris-HCl (pH 6.8), glycerol 10% buffer was added, mixed and centrifuged again to pellet any solids. The nuclear pellet was resuspended in sucrose buffer containing 0.25M sucrose, 10mM MgCl₂, protease and phosphatase inhibitors and laid over a 3 ml cushion of buffer containing 0.88M sucrose, 0.5 mM MgCl₂, protease and phosphatase inhibitors and then centrifuged at 2800 g for 10 min at 4°C. Pellet was resuspended in 4x Laemmli Sample Buffer (Bio-Rad). Cytoplasmic and nuclear fractions were resolved on a SDS–PAGE gel. Equivalent protein loading was verified using antibodies for β-actin and Lamin B1. For regular western blot assays, total protein was isolated from cells lysed in 50mM Tris-HCl (pH 6.8), glycerol 10%, 2% SDS, and protease and phosphatase inhibitors. For ubiquitination assays, 1,10-phenanthroline (VWR) was included in the lysis buffer to inhibit deubiquitinating enzymes. For mouse spinal cord or brain, tissues were mechanically homogenized, passed through a 21G syringe and lysates cleared by centrifugation at 12000 rpm for 10 min. Supernatants were collected and used for protein quantification. For fly immunoblot analyses, 10 flies were grinded in RIPA lysis buffer (Cell Signaling Technology). Lysates were resolved in precast SDS–PAGE gels (Bio-Rad), transferred to PVDF membranes (Trans-Blot Turbo Transfer Packs, Bio-Rad) for 10 min at 2.5V by using the Trans-Blot® Turbo™ Transfer System (Bio-Rad), blocked in 5% nonfat dry milk (Quality Biological, INC.) at RT for 1 hour and probed for the indicated antibodies. Densitometric analysis was performed on scanned autoradiographs using the Quantity One software (Bio-Rad).