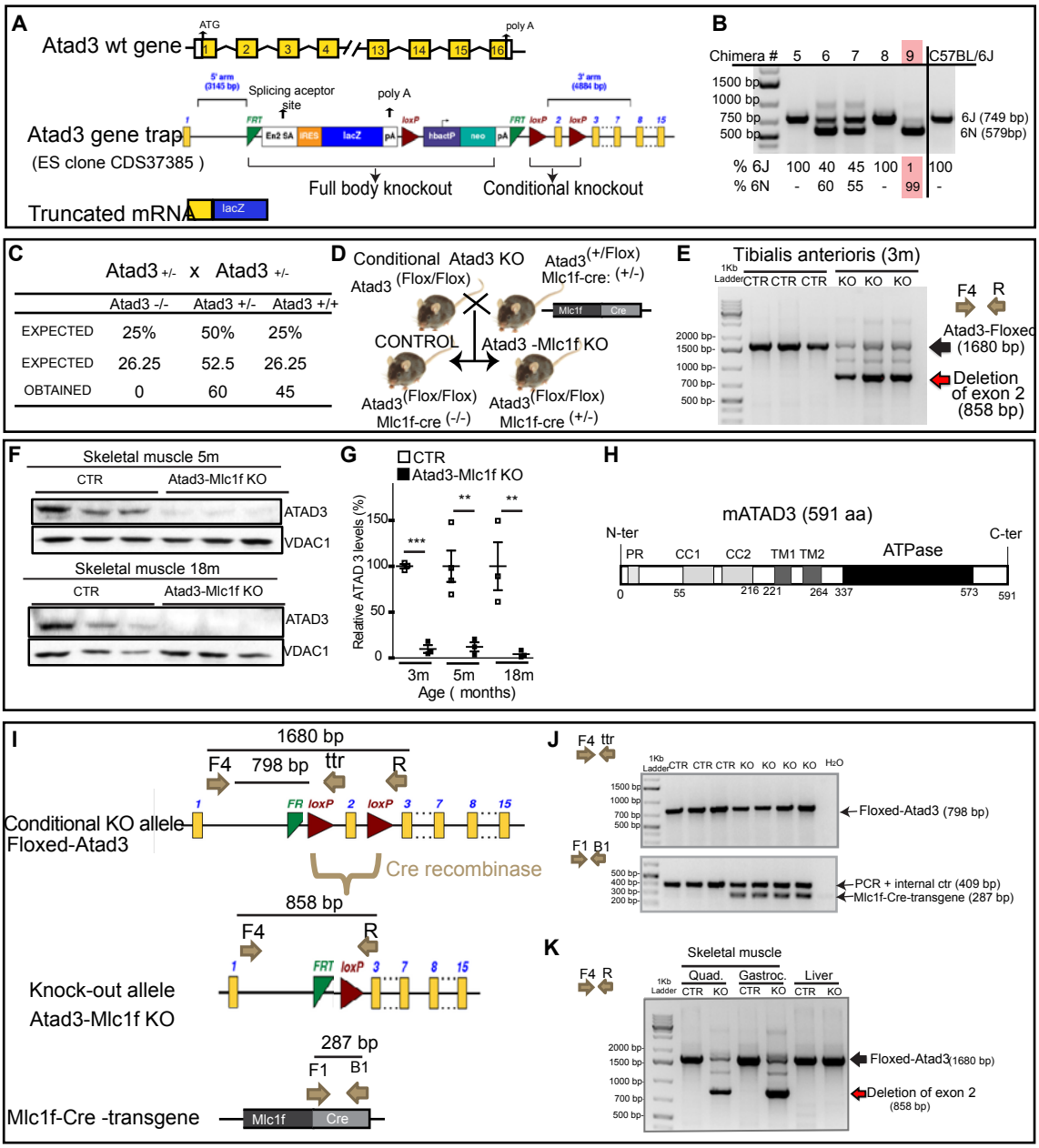
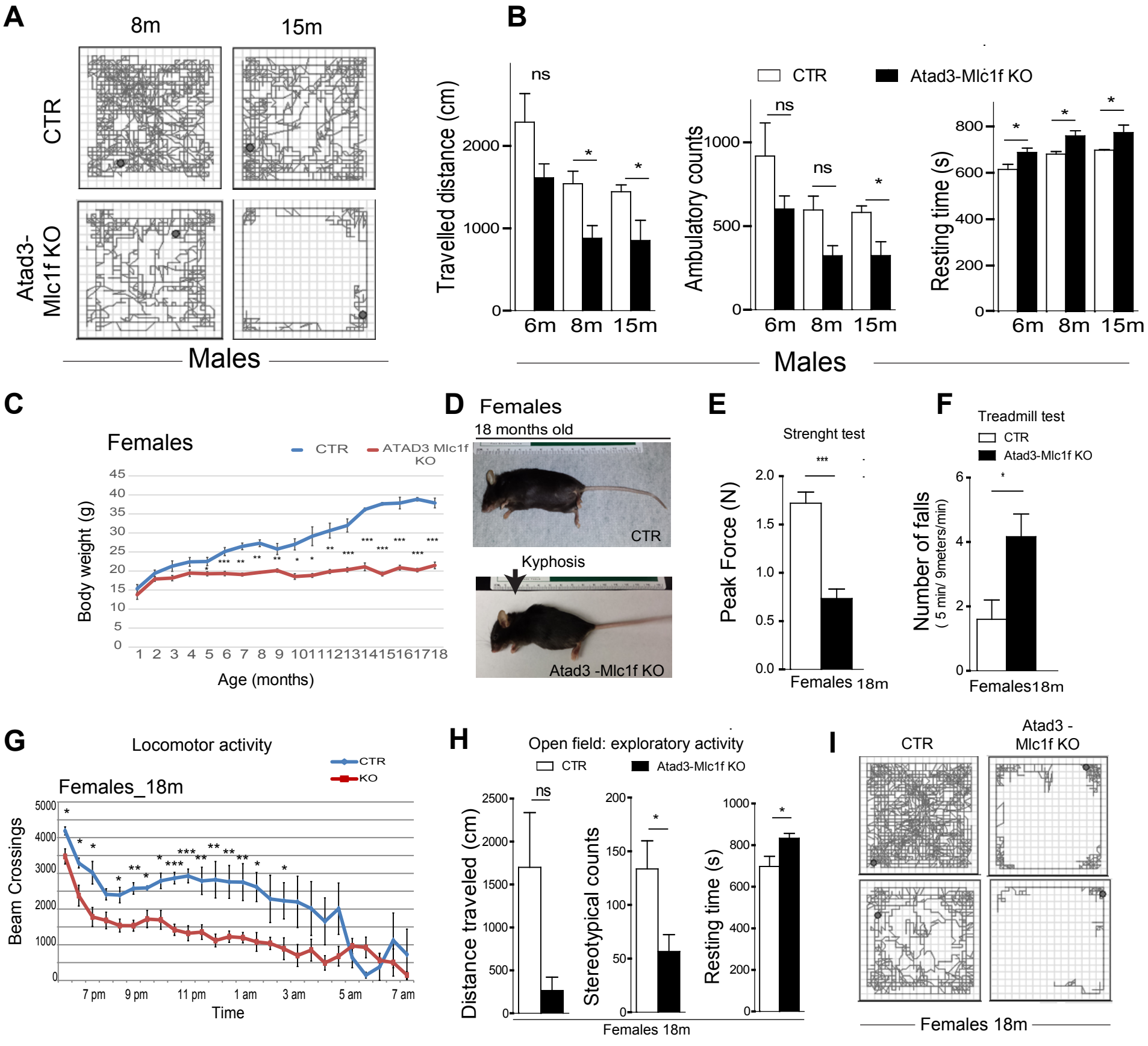
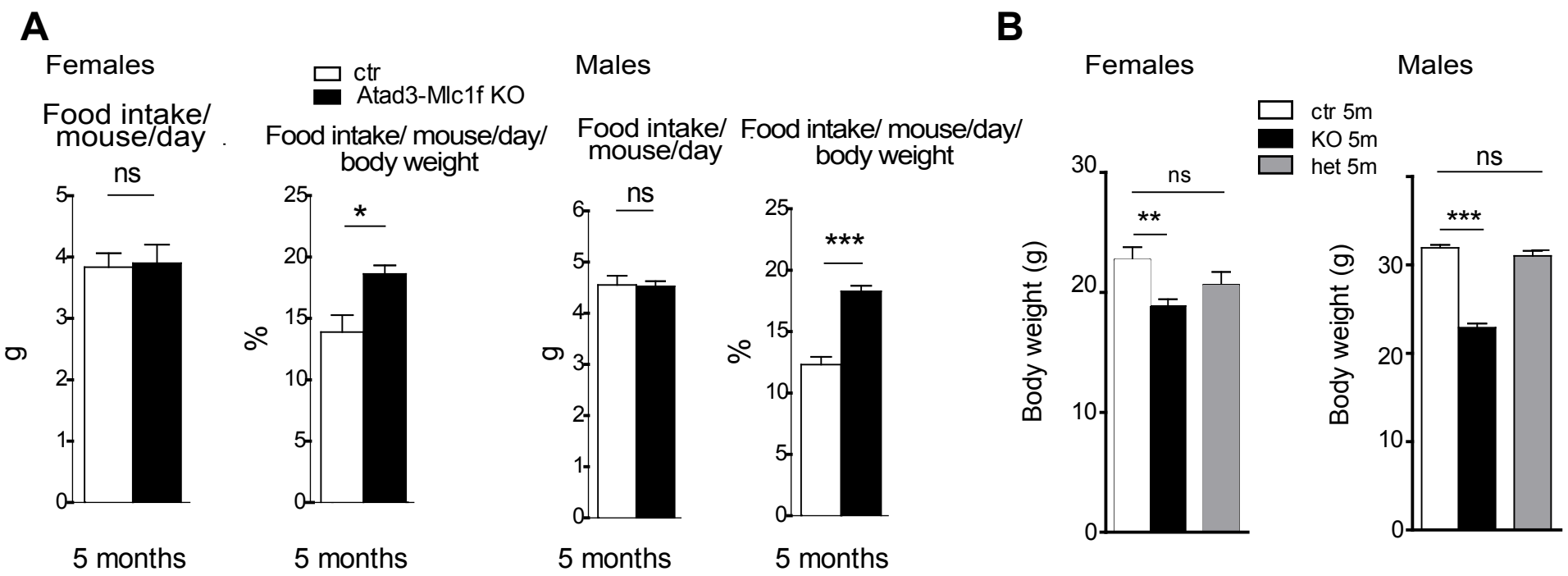


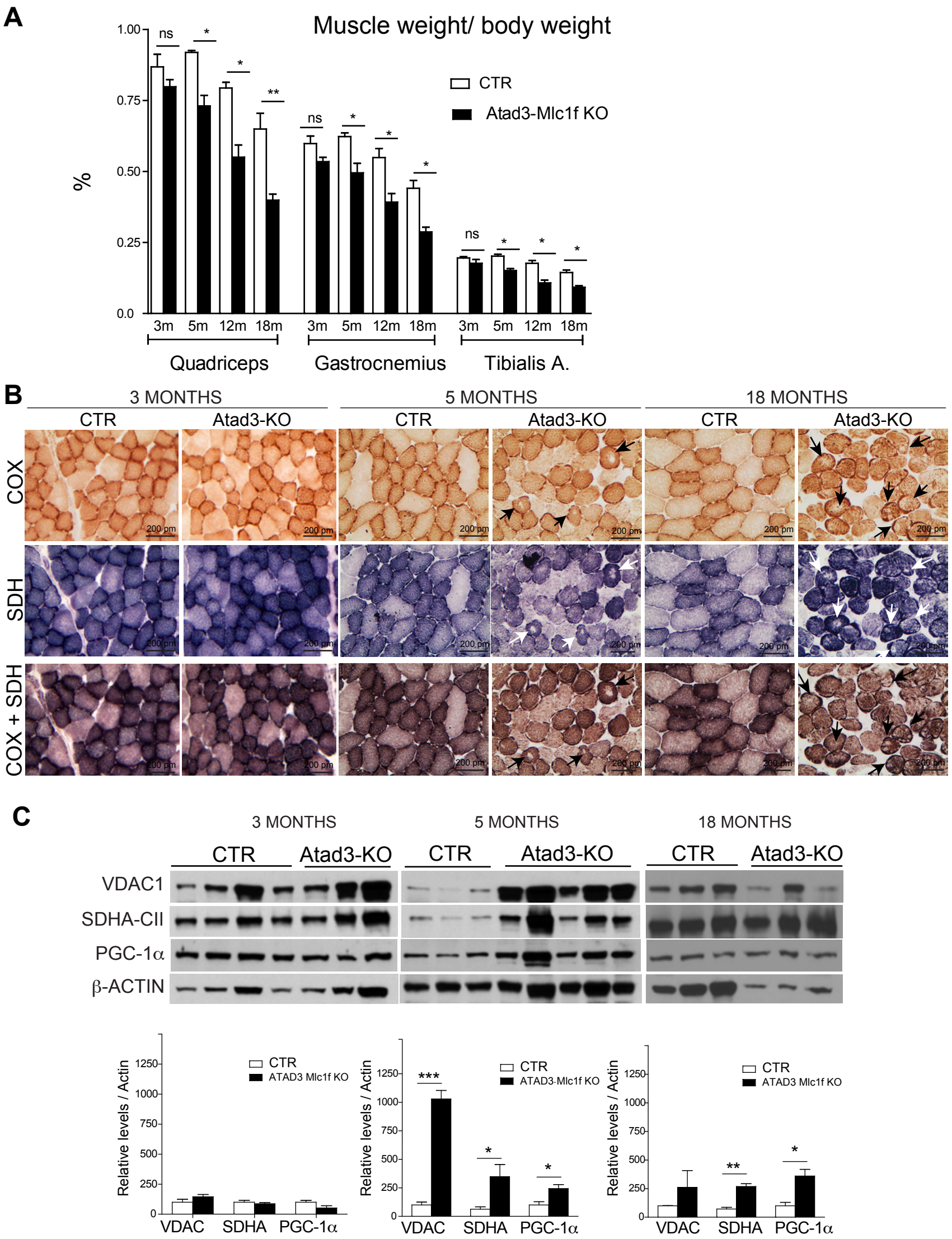
Figure Supplementary S1. Conditional Disruption of the Atad3 gene and creation of skeletal muscle-specific Atad3 knockout mice. (A) Schematic representation of Mus musculus Atad3 gene (NCBI gene ID: 108888) and construct used to generate transgenic mice with conditional potential into intron 1 (KOMP, clone CSD37385). The construct contains a neomycin resistance cassette flanked by flipase recognition target (FRT) sites inserted in the intronic region between exons 1 and 2. Exons are indicated by numbered boxes and introns by lines. En2SA, splicing acceptor; IRES, an internal ribosome entry site; pA, polyadelination sequences. (B) Genotyping analysis of Atad3 chimeras by amplification of Nnt locus, 579pb, in background 6N and 749pb, in background 6J. (C) Frequencies expected and obtained from breeding of heterozygous Atad3 full body knockouts. (D) Crossing scheme used to obtain skeletal muscle-specific knockout mice. (E) PCR product amplified from skeletal muscle showing the deletion of exon 2 in the Atad3-skeletal muscle knockouts mice. (F) Western blot analysis of ATAD3 protein levels in mitochondria from hind limbs skeletal muscles of 5 and 18 months old animals. (G) Quantification of western blot analysis of ATAD3 protein showed in F. (H) Schematic representation of the Mus musculus ATAD3 protein with its domains. PR, Proline rich domain; CC1-2, coiled-coil domain; TM, transmembrane domain; and ATPase domain. (I) Strategy for the conditional disruption of Atad3 gene in skeletal muscle. Schematic representation of the Atad3 targeted allele (conditional knockout-Atad3LoxP), and the knocked-out allele after Cre-recombinase-mediated excision of the exon 2. The Cre-enzyme is expressed under the muscle myosin light chain promoter 1 (Mlc1f). The position of forward (F4, F1) and reversed (ttr) primers used for genotyping, and forward (F4) and reversed (R) primers used for detection of the deletion of exon 2 are indicated by arrows. (J) Genotyping analysis of Atad3 conditional knock-out allele, and Mlc1f-Cre transgene in DNA extracted from tails of control (CTR) and knockout mice (KO). (K) Genotyping analysis of Atad3 conditional allele and Atad3 deleted allele in skeletal muscle and liver of control (CTR) and knockout mice (KO). DNA fragments corresponding to the Atad3 conditional allele (Atad3LoxP, 1680 bp) and Atad3 deleted allele (KO, 858 bp) are indicated. Bars represent means ± standard error (SE). Student's t-test was used to determine the level of statistical significance. Where *** indicates $p<0.0001$, ** indicates $p<0.001$.



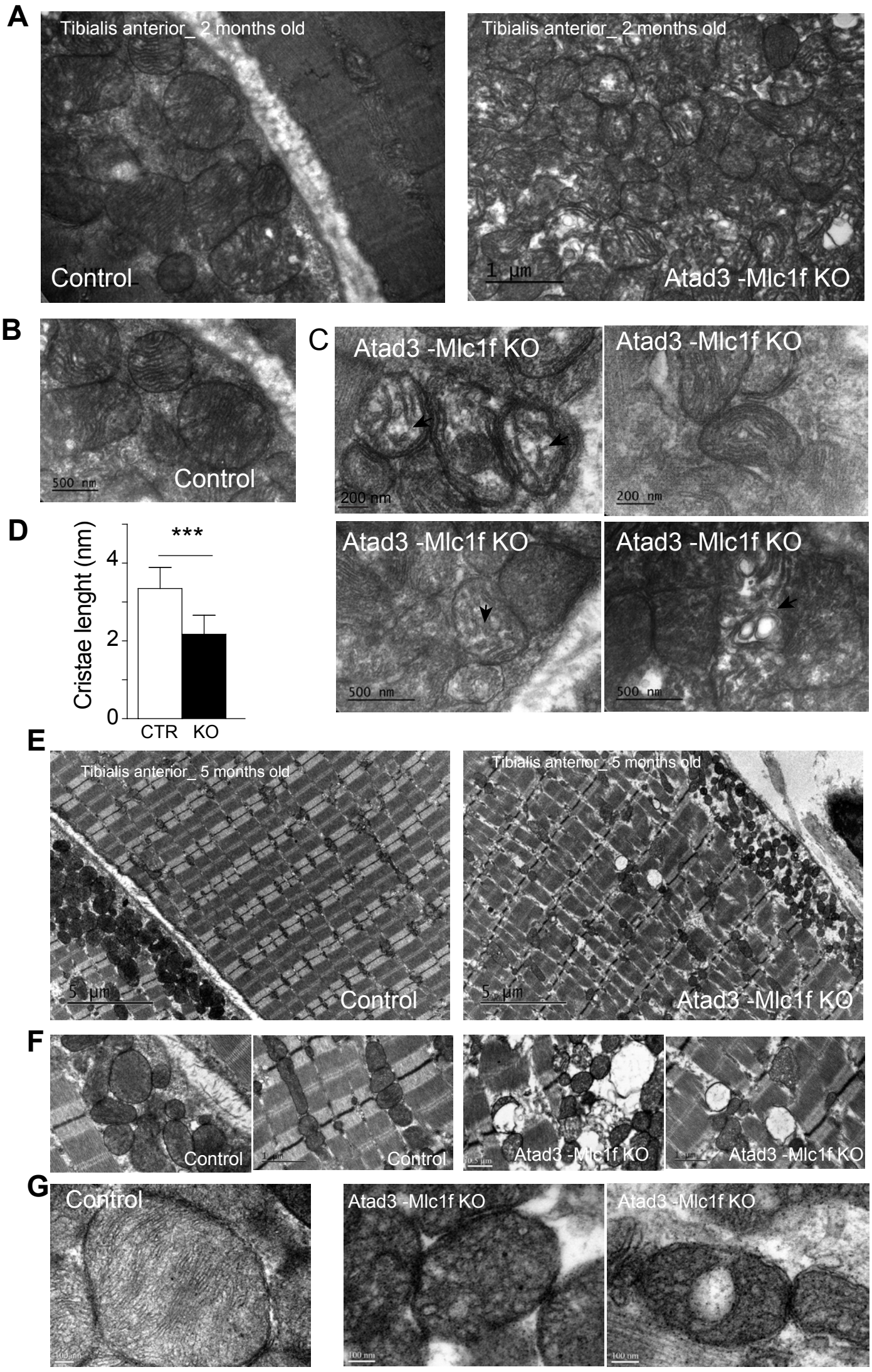
Supplementary Figure S2. Phenotype of Atad3 knockout mice in skeletal muscle. (A) Representative images of route travelled by Atad3-Mlc1f KO and control mice of 8 and 15 months age. (B) Open field test: measurements of exploratory activity, travelled distance, ambulatory activity and resting (n=6). (C) Atad3-Mlc1f female knockout mice (red) showed a significant reduction in weight (n=8-10). (D) Representative images of control and Atad3-Mlc1f KO mice, showing decreased body weight and kyphosis. (E)18-month-old Atad3-Mlc1f KO mice showed reduced muscle strength (n=5). (F) Treadmill performed by Atad3-Mlc1f KO and control mice, showing increased number of falls in the KO group (n=6). (G) Activity cage: 18-month-old Atad3-Mlc1f female knockout mice (red) showed a significant reduction in locomotor activity compared to age matched control mice (blue) (n=6). (H) Open field test: measurements of travelled distance, stereotypical counts, and resting time (n=6). (I) Representative images of route travelled by Atad3-Mlc1f KO and control mice. Bars represent means ± standard error (SE). P values were calculated by Student's t test to determine the level of statistical difference. Where n.s. indicates not statistically significant; * $p<0.05$, ** $p<0.01$ and *** $p<0.0001$.



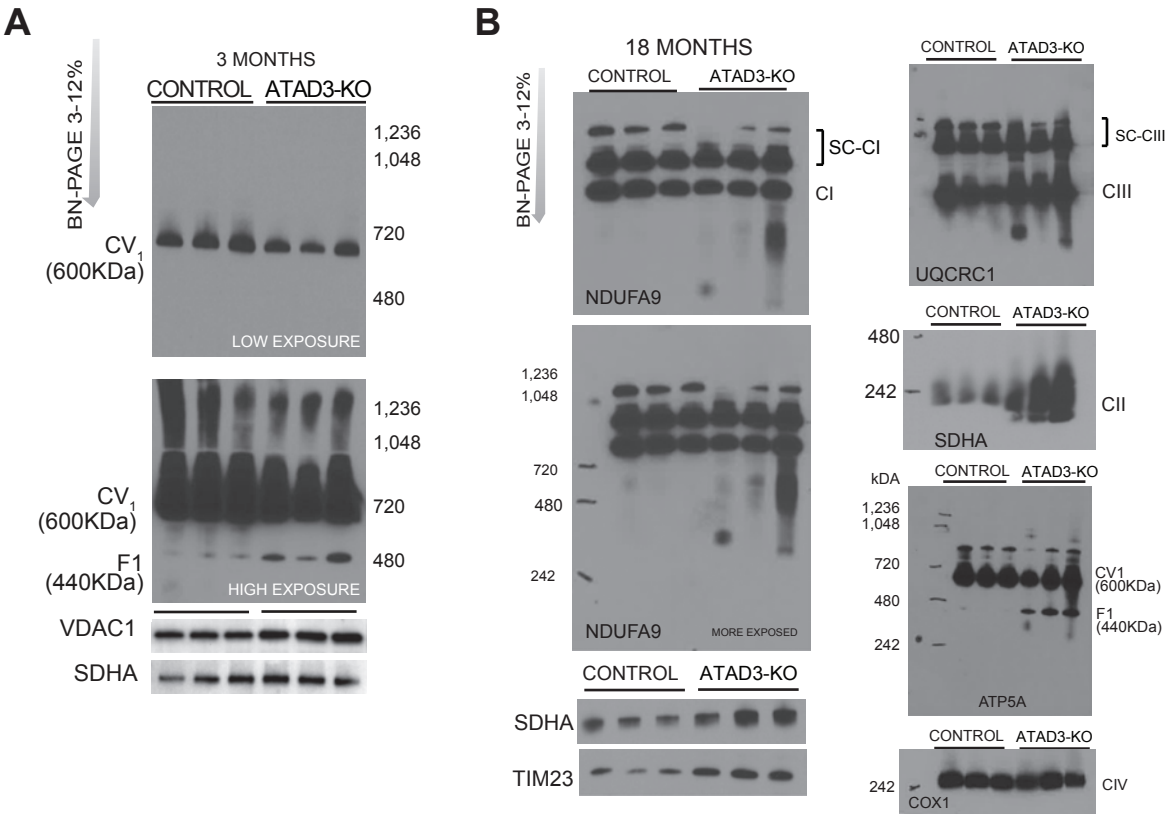
Supplementary figure S3. Atad3 Mlc1f knockout mice showed similar absolute food intake to control animals. (A) Quantification of daily food intake in females and males Atad3 Mlc1f knockout mice compared to control littermates. Absolute food intake was expressed as grams of food pellet/mouse/day/. Relative food intake was expressed as grams of food/mouse/day/body weight and expressed in percentage. (B) Body weight of control, heterozygous and Atad3 Mlc1 KO females at 5 months of age (C) Absolute muscle weight of Grastrocnemius and Tibialis anterior from 18 months old females (n=5). (D) Heart weight versus body weight of 5-12 months old Atad3-Mlc1f knockout and control male mice (n=6). (E) Western blot of muscle homogenates of control and Atad3-Mlc1f KO animals at different ages using antibodies against MF-20 (myosin heavy chain); and 12/101 (used as a marker for regenerating skeletal muscle). Actin was used as loading control. (F) Hematoxylin and eosin staining of skeletal muscle (Tibialis anterior) showing progressive degeneration of muscle fibers (asterisks) in the Atad3 Mlc1f knockout mice. Fibers from Atad3 Mlc1f KO mice had reduced size and appeared detached from each other. Bars represent means ± standard error (SE). P values were calculated by Student's t test to determine the level of statistical difference. Where n.s. indicates not statistically significant; **p<0.01 and ***p<0.0001.



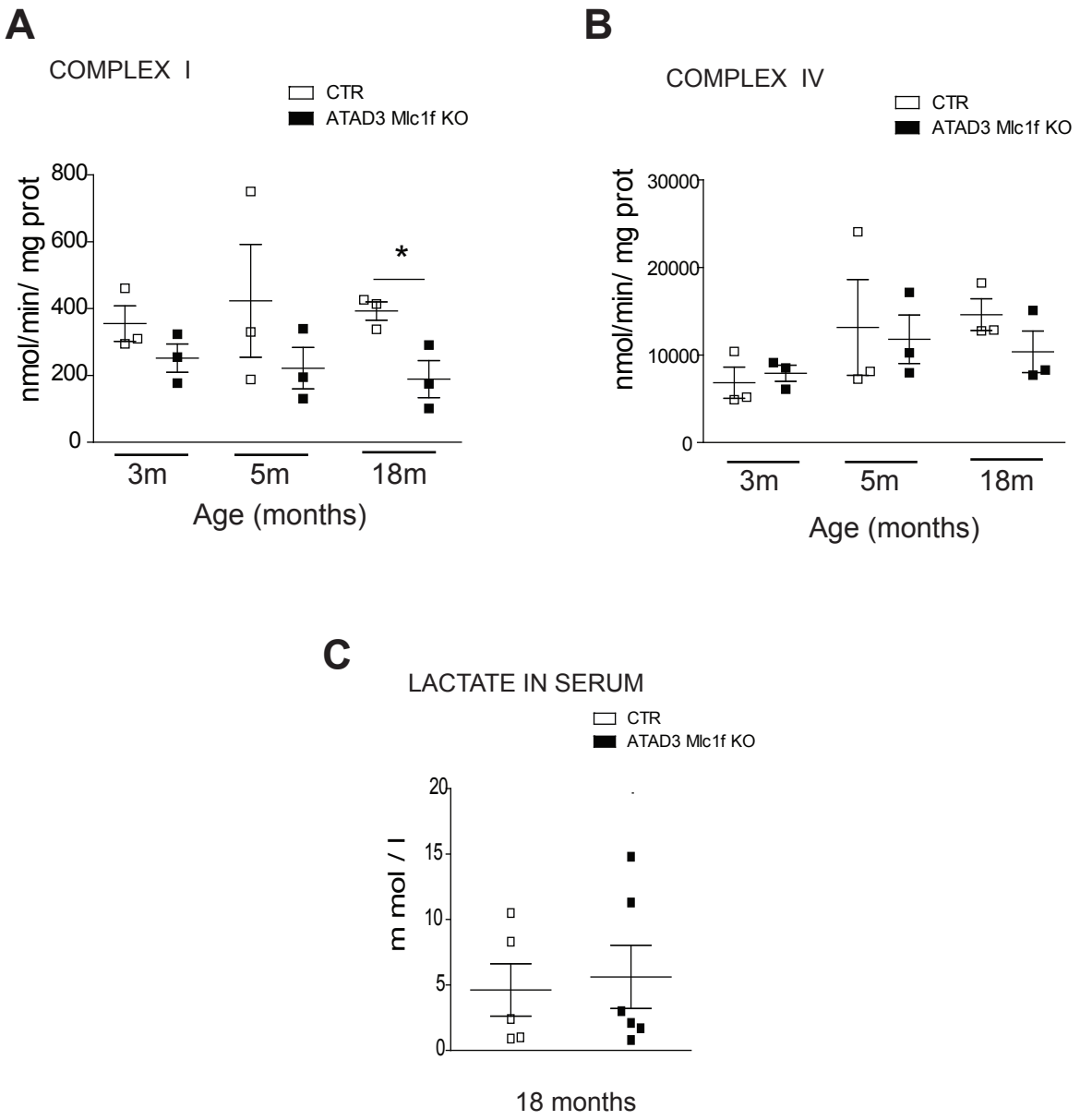
Supplementary figure S4. Lack of ATAD3 in the skeletal muscle induces progressive muscle wasting and central cores devoid of mitochondrial stain. (A) Relative muscle weight to body weight from control and Atad3-Mlc1f KO animals of 3, 5, 12, and 18 months expressed in percentage (n=6-8). (B) Representative COX, SDH, and COX/SDH staining of skeletal muscle (Tibialis anterior) from control and Atad3-Mlc1f KO of 3, 5 and 18 months old (n=3-4). COX activity is showed in brown and SDH in blue. Arrows indicate central cores lacking COX and SDH activity. Scale bar represents 200 pm. (C) Western of muscle homogenates (20 µg protein) of control Atad3-Mlc1f KO animals at different ages using antibodies against VDAC1, SDHA (complex II subunit) and PGC-6|. Actin was used as loading control. Bars represent means ± standard error of the mean (SEM). P values were calculated by Student's t test to determine the level of statistical difference - n.s. indicates not statistically significant; * $p<0.05$, ** $p<0.01$ and *** $p<0.0001$.



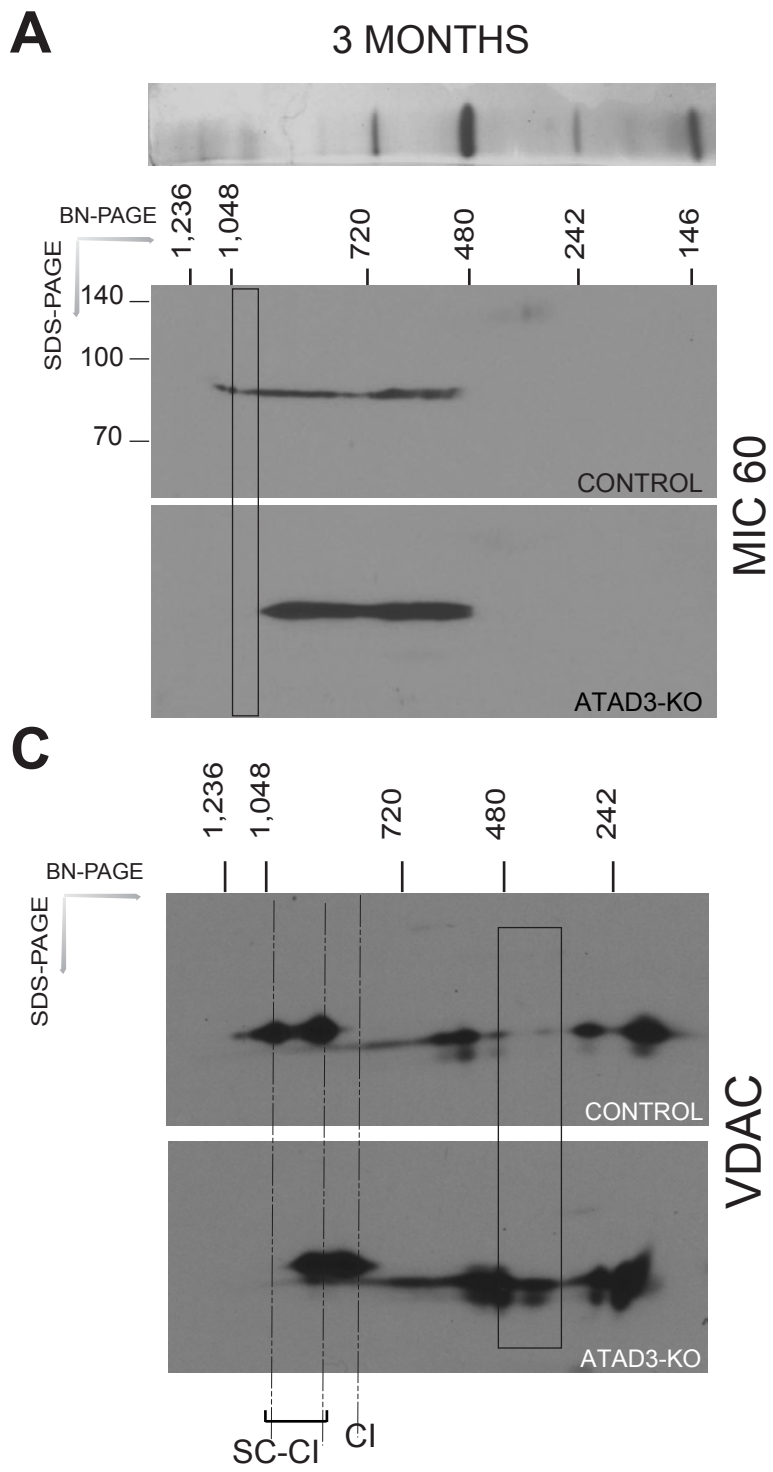
Supplementary figure S5. Lack of protein ATAD3 in muscle disrupts mitochondrial cristae structure. (A) Representative electron micrographs from 2 m control and Atad3-Mlc1f KO tibialis anterior. Mitochondria from KO showed disrupted cristae. (B) Electron micrographs of higher magnification from control (B) and Atad3-Mlc1f KO muscle (C) are shown. Arrows indicate disrupted cristae. (D) Quantification of the cristae length expressed from > 400 mitochondria from ctr and Atad3 knockout mice, expressed in nm. Bars represent means ± standard error (SE). (E) Representative electron micrographs from 5-month-old control and Atad3-Mlc1f KO muscle (tibialis anterior). (F) Higher magnification of mitochondria from KO showing disrupted cristae and possibly mitophagic vesicles. (G) Micrograph from control mitochondria showing lamellar cristae, and from Atad3- Mlc1f KO mitochondria showing disrupted cristae. Quantification of the cristae perimeter (H), width (I) and length (J) of mitochondria from > 400 mitochondria from ctrl and Atad3 knockout mice. Values indicated average of n=3 per group. Error bars indicate SEM. P values were calculated by Student's t test. Where, n.s. indicates not statistically significant; * $p<0.05$, ** $p<0.01$ and *** $p<0.0001$.

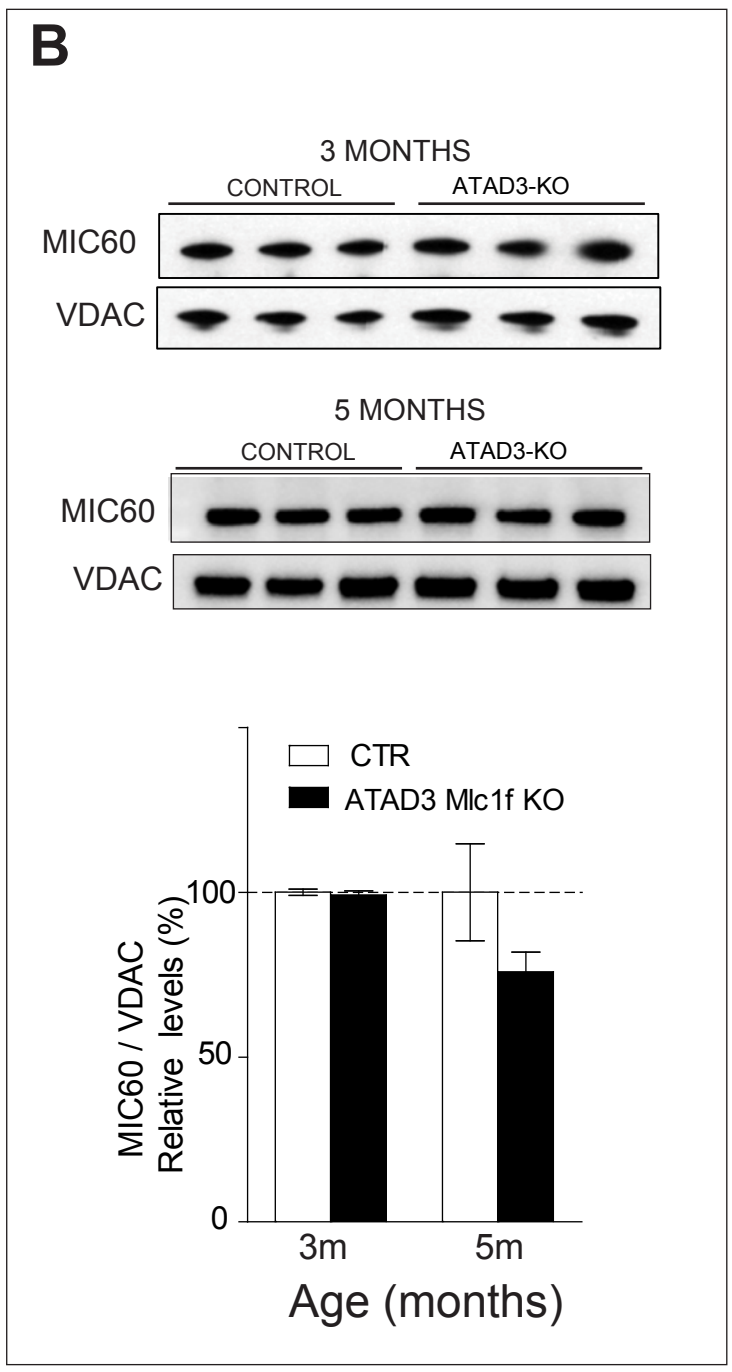


Supplementary figure S6. Impaired assembly of cristae macro complexes in Atad3-Mic1f KO muscles. (A-B) Blue Native PAGE (BN-PAGE) analysis of respiratory chain super-complexes followed by Western blot analysis using antibodies against C I (anti-NDUFA9), C II (anti-SDHA), C III (anti-UQCRC1), C IV (anti- COXI), and C V (anti- ATP5A) in mitochondrial extract from ctr and KO muscles of 3 and 18 months old mice. SDH-A (Complex II subunit), VDAC (voltage dependent anion channel-1) and TIM23 (Translocator of the inner membrane) were used as loading control.



Supplementary figure S7. Analysis of OXPHOS complexes in control and Atad3-Mlc1f KO skeletal muscle mitochondria. (A) Enzymatic activity of CI (NADH coenzyme Q reductase) and (B) CIV (Cytochrome c oxidoreductase) in mitochondria from skeletal muscle of 3, 5 and 18 months old mice. Each symbol corresponds to one mouse (n=3 per group). (C) Lactate levels in serum (n=5 in ctr group; and n =6 in KO group).





Supplementary Figure S8. Lack of ATAD3 affects MICOS complex assembly. (A) 2D-SDS PAGE of the Blue Native PAGE analysis of MICOS complex using antibody against MIC60 in mitochondria from ctr and Atad3-Mlc1f KO muscles of 3 months. (B) Western blot analysis using antibody against MIC60 in mitochondria from ctr and Atad3-Mlc1f KO muscles of 3 and 5 months old. VDAC1 (voltage dependent anion channel-1) was used as loadings control. (C) 2D-SDS-PAGE showing the impaired assembly of supercomplexes containing CI-subunit NDUFA9 and complexes of different molecular weight containing VDAC1 protein between control and KO.