**A**

Chst14 (chr2)

ATG

Exon1

100 3p

Coding region

Non-coding region

5' ATTTTCCCCGCTCAGCTAACCCTTAGCTGCCCGAGGGGAAAGCAGCGAGACCTCTTGC6GACGCACCGGACAGCCGATTTGAGCCGCGGTGCTGCGGCGGGGGG

3' TACTAAGGGGAAAGGTACCTGGGATCCAGACAGCAGCGAGACCTCTTGC6GACGCACCGGACAGCCGATTTGAGCCGCGGTGCTGCGGCGGGGGG

Start

sgRNA #6

sgRNA #7

sgRNA #1

sgRNA #5

sgRNA #3

**B**

pX330-Cent1 (Positive control)

pX330 (Negative control)

pX330-sgRNA#1

pX330-sgRNA#3

pX330-sgRNA#5

pX330-sgRNA#6

pX330-sgRNA#7

pX330-sgRNA#8

Chst14 (chr2)

Exon1

100 3p
Fig. S1. *In vitro* evaluation of the sgRNAs’ DNA cleavage activities.

(A) Six sgRNAs (#1, 3, 5, 6, 7, and 8) to target the downstream region from the *Chst14* translation start site ATG are indicated by arrows within the sequences. (B) Screening of candidate sgRNAs using a method that explores homologous recombination and reconstituting EGFP expression. The pCAG-EGxxFP target plasmid contained 5 and 3 EGFP fragments, and a *Chst14* genomic fragment or *Cent1* as a control between EGFP fragments (pCAG-EG *Chst14* FP, or pCAG-EG *Cent1* FP). The target plasmid was co-transfected with pX330 plasmids expressing sgRNA and hCas9 into HEK293T cells. The efficiency of homology-dependent repair was validated by observing EGFP fluorescence 2 days after transfection (pX330 with *Cen1*/sgRNA1, positive control; pX330 without sgRNA, negative control). Bars, 200 µm.
Fig. S2. CRISPR/Cas9-mediated Chst14 mutant alleles used in this study and their off-target analyses

(A) The detailed sequences for two independent nonsense mutant alleles. (B) Sequencing results from five off-target candidate loci for each sgRNAs (#6 and #7) in founder mice. The PAM sequences are underlined. No sign of off-target cleavages was detected within the genomic regions evaluated.
Fig. S3. Reduction in growth curve and grip strength in Chst14 mutant mice

(A) Growth curve of 1- to 24-week-old male and female Chst14+/+ (n = 3–4), and +6/−10-bp mutant homozygous mice (Chst14−/−, n = 3–4). (B) Growth curve of 1- to 12-month-old Chst14+/+ (n = 5–8), and −1-bp mutant heterozygous (Chst14+/−, n = 6–9) and homozygous mice (Chst14−/−, n = 3–4). (C) Grip strength data (g) from 2- to 12-month-old male and female Chst14+/+ (n = 3), and Chst14−/− mutant mice (+6/−10-bp mutant, n = 3). All data are presented as mean ± SD. Significant differences, Chst14+/+ vs. Chst14−/− (**p < 0.005; ****p < 0.0001), Chst14+/+ vs. Chst14−/− (####p < 0.0001), were evaluated using two-way ANOVA, and t-test.
Fig. S4. Early-life growth rate and deficiency of dermatan sulfate biosynthesis in Chst14 gene trap-knockout (KO) mice

(A) Early-life growth rate of male and female Chst14+/+ (n = 5), gene trap-KO Chst14+/- (n = 9), and Chst14−/− mice (n = 3). (B) Total amounts of chondroitin sulfate (CS) and dermatan sulfate (DS) disaccharides derived from urine of 5–6-week-old Chst14+/+ (n = 6), Chst14+/- (n = 5), and Chst14−/− mice (n = 3) were analyzed by anion-exchange HPLC after enzymatic digestion. N.D., not detected (< 0.1 pmol/mg creatinine). All data are presented as mean ± SD. Significant differences, Chst14+/+ vs. Chst14−/− (*p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001), Chst14+/− vs. Chst14−/− (##p < 0.01, ###p < 0.001, ####p < 0.0001), were evaluated using two-way or one-way ANOVA.
A

CSase AC digests of GAGs

Chst14<sup>+/+</sup>  Chst14<sup>+/−</sup>  Chst14<sup>−/−</sup>

−1 bp mutants

B

CSase B digests of GAGs

Chst14<sup>+/+</sup>  Chst14<sup>+/−</sup>  Chst14<sup>−/−</sup>

−1 bp mutants
Fig. S5. Chromatograms of the chondroitinase AC and B digests of glycosaminoglycans derived from muscles

Chromatograms show chondroitinase AC and chondroitinase B digests of glycosaminoglycans derived from Chst14+/−, Chst14−/+1-bp mutant Chst14+/−, and Chst14−/− mice. The elution positions of authentic 2-aminobenzamide-labeled CS/DS disaccharides are indicated by the following numbers: 1, ΔHexUA-GalNAc; 2, ΔHexUA-GalNAc(6S); 3, ΔHexUA-GalNAc(4S); 4, ΔHexUA(2S)-GalNAc(6S); 5, ΔHexUA(2S)-GalNAc(4S); 6, ΔHexUA GalNAc(4S,6S); 7, ΔHexUA(2S)-GalNAc(4S,6S). ΔHexUA, GalNAc, 2S, 4S, and 6S represent 4,5-unsaturated hexuronic acid, N-acetylgalactosamine, 2-O-sulfate, 4-O-sulfate, and 6-O-sulfate, respectively.
Fig. S6. Total amounts of CS and DS disaccharides derived from urine

The total amounts of CS and DS disaccharides derived from urine of 1-year-old Chst14+/+, +6/-10-bp mutants Chst14+/-, and Chst14−/− mutant female mice were analyzed by anion-exchange HPLC after enzymatic digestion. Data are presented as mean ± SD. Statistical differences, compared to Chst14+/+ mice and Chst14−/− mutant mice (n = 6; **p < 0.01, ***p < 0.001), were evaluated using t-test; N.D., not detected (< 0.1 pmol/mg creatinine).
Fig. S7. Skin fragility in Chst14 mutant mice

(A) Hematoxylin and eosin (H&E) staining (upper and middle panels) and immunohistochemical analysis by horseradish peroxidase (HRP)-diaminobenzidine (DAB)-labeled observation of decorin (bottom panels) in skin derived from Chst14+/+, +6/-10 bp mutant Chst14+/−, and Chst14−/− mice. Bars, 100 µm. (B) Tensile stress (N/mm²) were derived from Chst14+/+, +6/-10 bp mutants Chst14+/−, and Chst14−/− mice. Data are presented as mean ± SD. Statistical differences, compared to Chst14+/+ (***p < 0.001) were evaluated using one-way ANOVA.
Fig. S8. Tensile stress and stress-strain curves from the skin of Chst14 mutant mice. The tensile stress and tensile strain on the skin were derived from the linear slopes of the force-displacement and stress-strain curves for Chst14+/+ and 1-bp mutant Chst14−/− mice, respectively. Red dots indicate the points at the maximum stress.
Fig. S9. Radiographs of Chst14 mutant mice

(A, B) Forelimb radiographs for 1-year-old female Chst14+/+, +6/-10-bp mutants Chst14+/−, and Chst14−/− mice (A), and Chst14 gene trap-KO mouse (B). (C) Representative lateral radiographs of 1-year-old female Chst14+/+ and Chst14−/− gene trap-KO mice. (D, E) Hindlimb radiographs of 1-year-old female Chst14+/+, +6/-10-bp mutants Chst14+/−, and Chst14−/− (D), and Chst14 gene trap-female KO mice (E).
Fig. S10. The frequency distribution of myofiber area in Chst14 mutant mice and gene trap-KO-mouse

Frequency distribution of myofiber area (µm²) counted from H&E staining photographs of 1-year-old Chst14+/+, +6/-10-bp mutant Chst14−/− mice (A) and Chst14 gene trap-KO mice (B) (n = 3, each). Area values show frequency (% of total fibers) and distribution comparisons. Data are represented as mean ± SD. Significant differences compared to Chst14+/+ (*p < 0.05) were evaluated using the multiple t-test.
Fig. S11. Reduced voluntary activity in Chst14 mutant mice and gene trap-KO mice. The mice were analyzed for voluntary running activity in the wheel cage, yielding voluntary running activity and maximum running speed (A, C), and the daily running distance (B, D) for 1-year-old Chst14+/+ (n = 3–4), Chst14+- (n = 4), and Chst14-/- gene trap-KO mice (n = 3) (A, B), and -1-bp mutant mice (n = 3–4) (C, D). All data are presented as the mean ± SD. Significant differences compared to Chst14+/+ (p < 0.05, and ****p < 0.0001) were evaluated using one-way ANOVA.
Table S1. Substitution rate of congenic marker

To discriminate 129S2/SvImJ and C57BL/6J mice strain-specific 58 SSLP markers, DNA from mouse tails was amplified by PCR using SSLP-specific primers. Heterozygous mice with the highest replacement rate for markers of 129 mice in B6 mice were selected for mating to obtain the next generation. The tables show the numbers of C57BL/6J marker and heterozygous 129S2/SvImJ and C57BL/6J markers and their substitution rate for C57BL/6J (%) in F9 and F11 generation gene trap mice (total 8 or 7 mice, respectively).

| F9 generation gene trap mouse # | Marker (No of sites) | Substitution rate (%) |
|-------------------------------|---------------------|----------------------|
|                               | C57BL/6J x 129S2/SvImJ |                      |
| 1                             | 33                  | 11                   | 75.0 |
| 2                             | 40                  | 18                   | 69.0 |
| 3                             | 42                  | 16                   | 72.4 |
| 4                             | 48                  | 10                   | 82.8 |
| 5                             | 42                  | 16                   | 72.4 |
| 6                             | 42                  | 15                   | 73.7 |
| 7                             | 42                  | 14                   | 75.0 |
| 8                             | 39                  | 18                   | 68.4 |

| F11 generation gene trap mouse # | Marker (No of sites) | Substitution rate (%) |
|-------------------------------|---------------------|----------------------|
|                               | C57BL/6J x 129S2/SvImJ |                      |
| 1                             | 55                  | 3                    | 94.8 |
| 2                             | 53                  | 5                    | 91.4 |
| 3                             | 55                  | 3                    | 94.8 |
| 4                             | 55                  | 3                    | 94.8 |
| 5                             | 56                  | 2                    | 96.6 |
| 6                             | 55                  | 3                    | 94.8 |
| 7                             | 57                  | 1                    | 98.3 |
Table S2. Birth rate of gene trap Chst14 KO mice

| Genotype       | Birth (Number, Rate %) |
|----------------|------------------------|
| Chst14^{+/+}   | 78 (25.7 %)            |
| Chst14^{+/−}   | 220 (72.6 %)           |
| Chst14^{−/−}   | 5 (1.7 %)              |
| Total          | 303 (100%)             |

Table S3. Offspring number of CRISPR/Cas9 genomic-engineered mice

| Genotype                          | sgRNA #6 | sgRNA #7 |
|-----------------------------------|----------|----------|
| Homozygous (Frameshift mutation*) | 1        | 1        |
| Heterozygous                      | 1        | 0        |
| Compound heterozygous             | 18       | 19       |
| Wild type                         | 0        | 1        |
| Total                             | 20       | 21       |

* The mutation was introduced a premature termination codon.
Table S4. A. The potential off-target candidate loci (OT1-OT5) for Chst14 sgRNA #6 and #7 predicted by CRISPOR web-tool.

| sgRNA #6 | Off-target sequence | Mismatch position | Mismatch count | MIT Off-target score | CFD Off-target score | Chromosome | Start | End | Strand | Locus description |
|----------|---------------------|-------------------|----------------|---------------------|---------------------|-------------|-------|-----|--------|-----------------|
| OT1      | GCTGCC TGGACA GAAAGG CAGGG | ......* | 4 | 0.316 | 0.580 | chr1 | 4656 | 2060 | + | intergenic:Srf-Ptk7 |
| OT2      | CCGGCC TATCCCG AAAAGG TAGG | ......** | 4 | 0.353 | 0.576 | chr7 | 7996 | 8057 | + | intergenic:Gm2 4541-Zfp710 |
| OT3      | ACTGGG TACCCA GAAAGG CATGG | ......* | 4 | 0.187 | 0.335 | chr1 | 7610 | 4879 | + | intron:Vpe53 |
| OT4      | GCTGCC TGGACC GGGAGG CAGGG | ......* | 4 | 0.028 | 0.284 | chr1 | 8738 | 2553 | - | intergenic:Tx7-1768011E4Rk |
| OT5      | GCCGCG TGCCCC GGGAGC AGGGG | ......* | 4 | 0.317 | 0.262 | chr1 | 1763 | 1786 | + | intergenic:PK85-Gm26104 |

| sgRNA #7 | Off-target sequence | Mismatch position | Mismatch count | MIT Off-target score | CFD Off-target score | Chromosome | Start | End | Strand | Locus description |
|----------|---------------------|-------------------|----------------|---------------------|---------------------|-------------|-------|-----|--------|-----------------|
| OT1      | CTGGCTT TACGTG CGGACC GCCGG | ......* | 4 | 0.426 | 0.279 | chr1 | 9033 | 6045 | - | exon:Arhgap27-Gm11647 |
| OT2      | TTGATCG GCCCTG CAGGCC TAGG | ......* | 4 | 0.104 | 0.242 | chr1 | 6918 | 4554 | - | exon:Mga2 |
| OT3      | CCGGAT TGGCCG GGGAGG GTGCCA | ......* | 4 | 0.085 | 0.227 | chr1 | 4302 | 8047 | - | intergenic:Hpse2-Gm22135 |
| OT4      | TTGGCTT CGGGAG CAGGCC AGGGG | ......* | 4 | 0.639 | 0.221 | chr1 | 2535 | 4009 | + | intergenic:Gm1 699-Gm26600 |
| OT5      | CGGAGG TCGGTG CCGGCC CGAGG | ......* | 4 | 0.697 | 0.215 | chr1 | 5688 | 6933 | + | intergenic:Gm2218-Znmy2 |

On target sequence for sgRNA #6; ACTGGCTGCCCGAAAAGCG CGG (chr14) On target sequence for sgRNA #7; CCGCCTTGCGCTCGGCGCCA GGG (chr14)
B. Primers used for Off-target analyses

| Name         | Sequence (5’ to 3’)                          |
|--------------|----------------------------------------------|
| sgRNA #6 OT1 F | AGAAGCTTCTTAGCAGTGATGGCAG                    |
| sgRNA #6 OT1 R | GTCTTGAACTCGCTACAGAGCTAAGG                   |
| sgRNA #6 OT2 F | CCTGACCTAGTGAAGTAGTGAATTCC                  |
| sgRNA #6 OT2 R | ATCTGCAAGGTTGTATACTCCCTAGC                  |
| sgRNA #6 OT3 F | CAGAGAGTGTCTCTGGAGTCTAGTGG                  |
| sgRNA #6 OT3 R | TTGCATGCAAGTGAGCTGCTGG                     |
| sgRNA #6 OT4 F | GATGACTGGAGCACATACAGGTCG                   |
| sgRNA #6 OT4 R | GTGGTTAAAGGAGACATTCTAGCCATCC               |
| sgRNA #6 OT5 F | GAAGTCATCAGGACACAGCAATC                     |
| sgRNA #6 OT5 R | GCTCTGCAAGTCCAGAACACCCAC                   |
| sgRNA #7 OT1 F | TAGACCTCTGGAGCCTGAGACCTCT                  |
| sgRNA #7 OT1 R | TACGACTCCGCCCTCGTGGACAC                   |
| sgRNA #7 OT2 F | CGCAGGTAAGGGAATCACGGTGTIC                 |
| sgRNA #7 OT2 R | CATTGCTACTCCAGAGGACAGAGC                 |
| sgRNA #7 OT3 F | CATCGGCTGGAAGTGAAAGTTACAGTG             |
| sgRNA #7 OT3 R | CATACGTAACCTGAGTGCTCTTCCTG               |
| sgRNA #7 OT4 F | CTAGTCACCTTGAGAAGGTGAACCTGAG          |
| sgRNA #7 OT4 R | AGTCTAGCCAGCAGCATGAGATTCCCT             |
| sgRNA #7 OT5 F | CAGAACCAGTGGAGCTTCTC                    |
| sgRNA #7 OT5 R | TCTCGGTTCAGACAGGCTGTG                   |