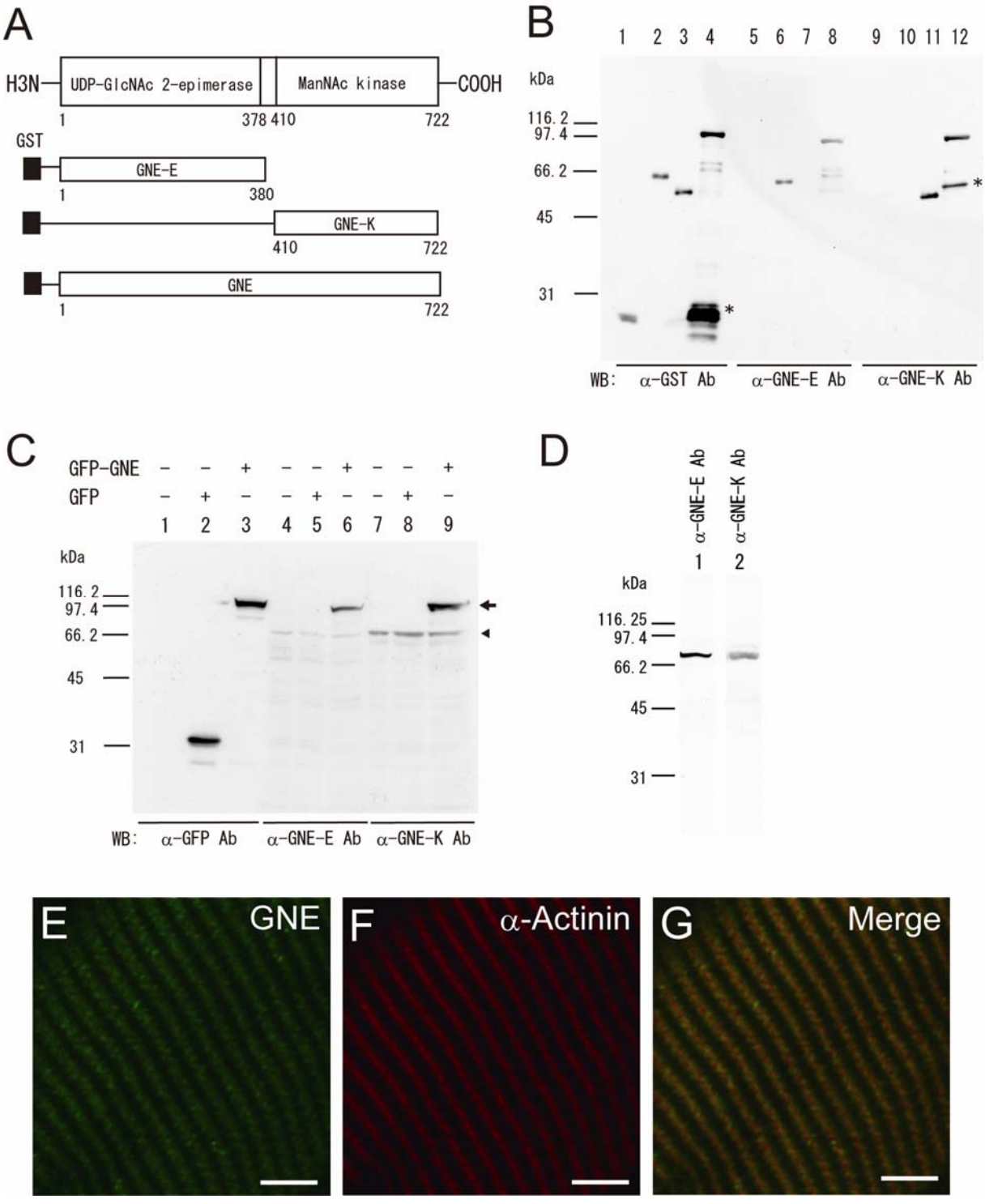
A
H3N
UDP-GlcNAc 2-epimerase
ManNAc kinase
COOH
1
378 410
722
GST
GNE-E
1
380
GNE-K
410
722
GNE
1
722
B
1 2 3 4 5 6 7 8 9 10 11 12
kDa
116.2
97.4
66.2
45
31
*
WB: α-GST Ab
α-GNE-E Ab
α-GNE-K Ab
C
GFP-GNE
GFP
1 2 3 4 5 6 7 8 9
kDa
116.2
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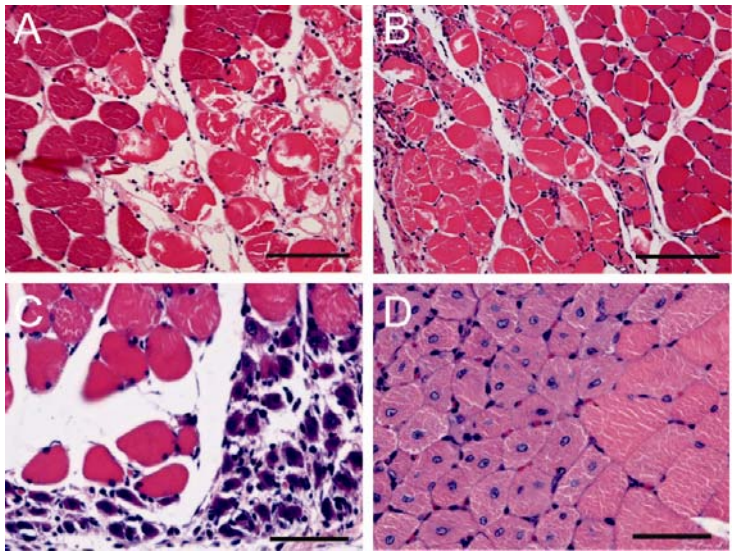
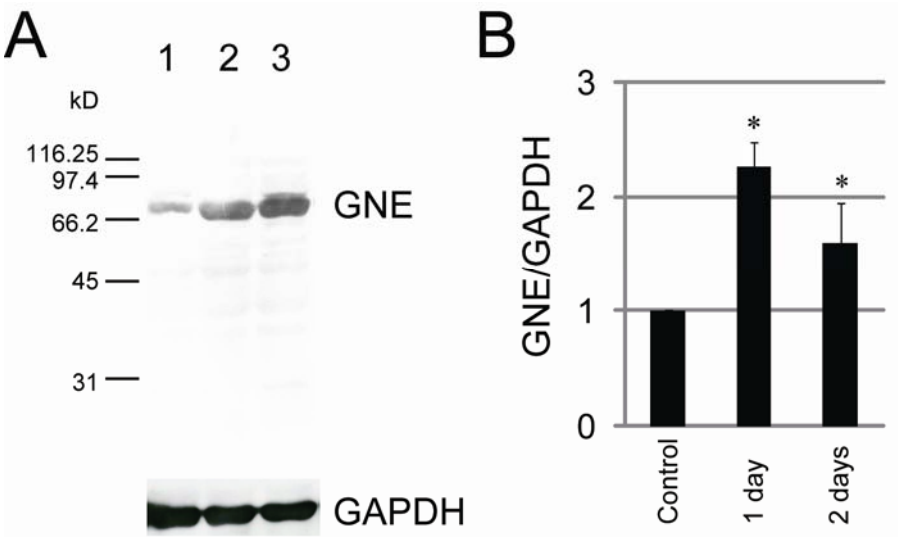
45
31
WB: α-GFP Ab
α-GNE-E Ab
α-GNE-K Ab
D
α-GNE-E Ab
α-GNE-K Ab
1 2
kDa
116.25
97.4
66.2
45
31
E GNE
F α-Actinin
G Merge

Supplementary Figure 1. Identification of GNE with specific polyclonal antibodies. (A) Overview of recombinant GST-fused GNE proteins. (B) Western blotting of recombinant GST-fused GNE proteins. Purified recombinant GST-fused GNE proteins (lanes 1, 5, 9, GST; lanes 2, 6, 10, GST-GNE-E; lanes 3, 7, 11, GST-GNE-K; lanes 4, 8, 12, GST-GNE) were separated on a 10% SDS-polyacrylamide gel followed by blotting onto nitrocellulose membrane. α-GST Ab was immunoreactive with all of GST, GST-GNE-E, GST-GNE-K, and GST-GNE (lanes 1--4), α-GNE-E Ab was immunoreactive only with GST-GNE-E and GST-GNE (lanes 6 and 8), and α-GNE-K Ab was immunoreactive only with GST-GNE-K and GST-GNE (lanes 11 and 12). Cleaved GST-GNE was also detected in lanes 4 and 12 (asterisks). (C) Western blotting of GFP-tagged GNE protein. HEK293 (human embryonal kidney) cells transiently transfected with pEGFP-C2 (Clontech, Mountain View, CA, USA) alone (lanes 2, 5, 8) or with pEGFP-C2 into which the cDNA of GNE inserted into the GNE-pGEM-T easy vector was subcloned at the *EcoRI* site (lanes 3, 6, 9), or not transfected (lanes 1, 4, 7), by use of FuGENE6 (Roche) in accordance with the manufacturer's instructions. Cells were harvested after incubation for 48 h and then total proteins were extracted, as described previously [20]. Twenty micrograms of total protein was separated on 10% SDS-polyacrylamide gel followed by blotting onto a nitrocellulose membrane. While α-GFP Ab (lanes 1--3) was immunoreactive with GFP protein and GFP-fused GNE protein, both α-GNE-E Ab (lanes 4--6) and α-GNE-K Ab (lanes 7--9) were immunoreactive only with GFP-fused GNE protein (arrow). Endogenous GNE was also detected (arrowhead). (D) Western blotting of endogenous GNE protein in the murine gastrocnemius muscle. Fifty micrograms of total protein was separated on 10% SDS-polyacrylamide gels, followed by blotting onto nitrocellulose membrane. GNE was detected with α-GNE-E Ab (lane 1) or α-GNE-K Ab (lane 2). Molecular mass markers (Bio-Rad SDS-PAGE Molecular weight standards, Broad range) are indicated on the left (B-D). (E-G) Double-labeled immunofluorescence of GNE and α-actinin in normal muscle.-Tissue sections from murine quadriceps muscle were analyzed by double-labeled immunostaining with α-GNE-K Ab (green) together with α-actinin Ab (red). Scale bars: 5 μm.



Supplementary Figure 2. Histological changes in the gastrocnemius muscle after CTX injection. Paraffin-embedded tissue sections from murine gastrocnemius muscles at 1 (A), 2 (B), 4 (C), and 7 (D) days after CTX injection were stained with H&E. Scale bars: 100 μm (A and B), 50 μm (C and D).



Supplementary Figure 3. Upregulation of GNE protein in CTX-injured muscles. (A) Representative Western blots of CTX-injured muscles. Gastrocnemius muscles non-injected (control) (lane 1) and at 1 day (lane 2) and 2 days (lane 3) after CTX injection (treated) were excised surgically. Fifty micrograms of total protein was separated on 10% SDS-polyacrylamide gels followed by blotting onto nitrocellulose membrane. GNE was detected with α-GNE-K Ab (upper panel). The same membranes were reprobed with a mouse monoclonal α-GAPDH Ab (lower panel). Molecular mass markers are indicated on the left. (B) The signal intensities of GNE determined by densitometry using NIH ImageJ software were compared with those of GAPDH, and are shown as fold induction: (GNE in treated muscles / GAPDH in treated muscles) / (GNE in control muscles / GAPDH in control muscles). The results from three mice are expressed as means±SD, and were 2.3 ± 0.21 and 1.6 ± 0.35 fold at 1 day and 2 days after CTX injection, respectively. *Significantly different ($p < 0.05$) from controls (Mann-Whitney U-test) (C). Each immunoblot was repeated at least twice using independent samples.