Supplemental figure legends

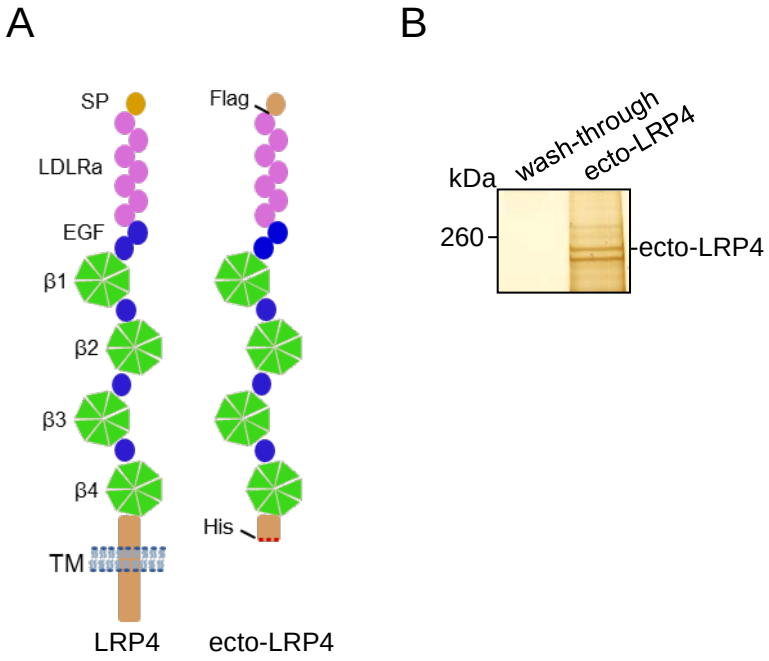
Suppl Figure 1. Expression and purification of ecto-LRP4. (A) Schematic diagrams of full length LRP4 and ecto-LRP4. (B) Sliver staining of wash-through and purified ecto-LRP4.

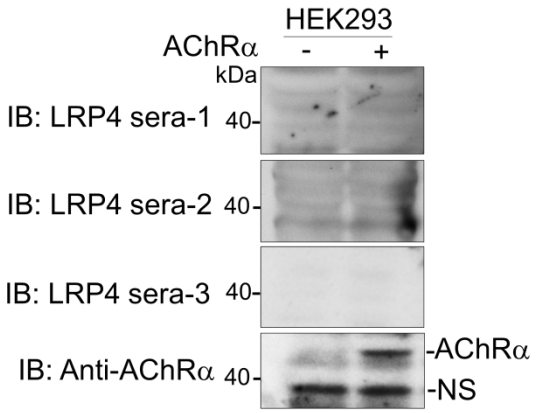
Suppl Figure 2. LRP4 sera did not recognize AChRα protein. Lysates of HEK293 cells expressing AChRα were subjected to western blot by anti-AChR antibody mAb35 and by anti-LRP4 sera.

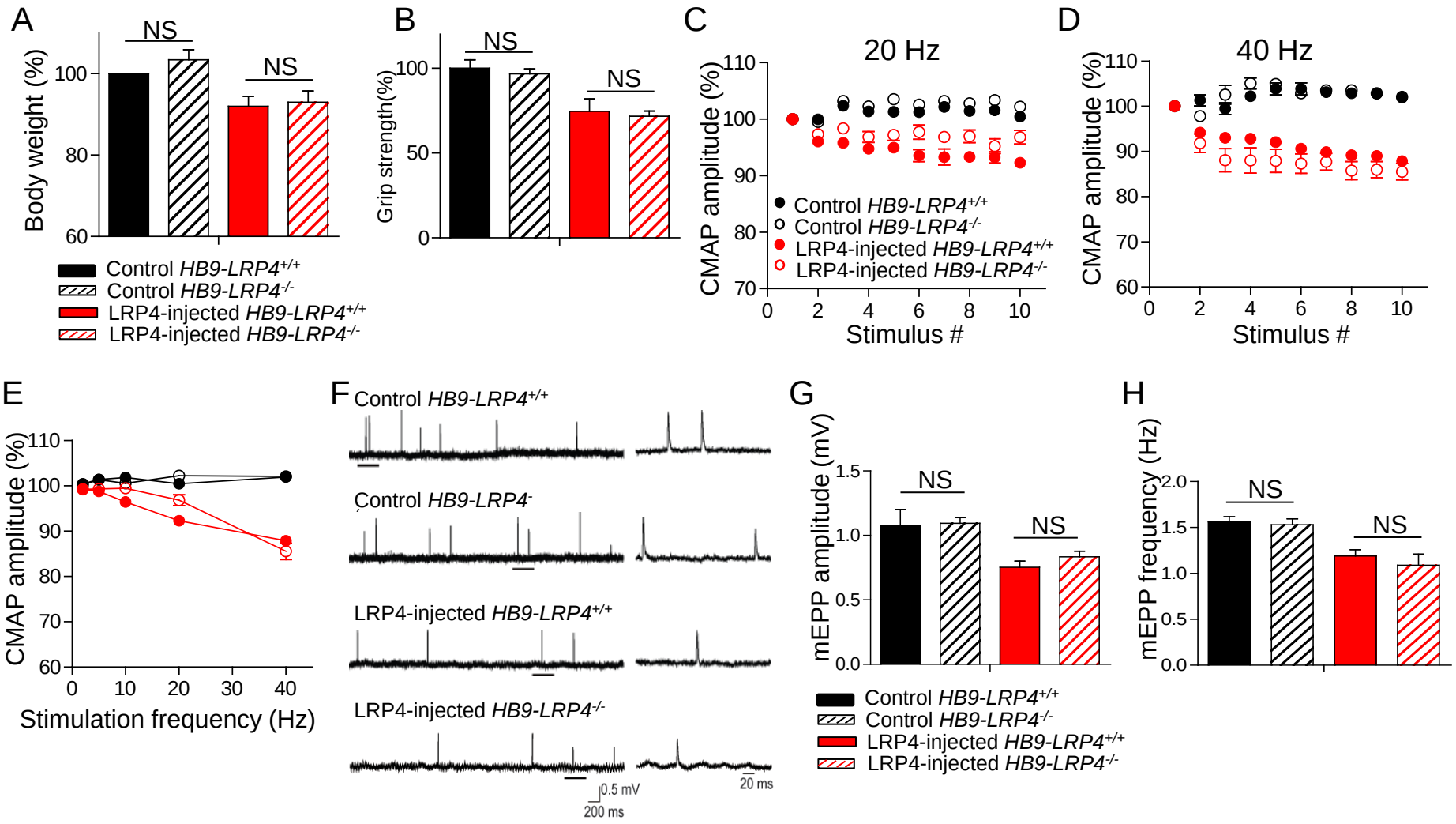
Suppl Figure 3. No effect of motor neuron-specific LRP4 mutation on muscle weakness induced by LRP4 immunization. (A, B) Similar reduction in body weight and muscle strength between LRP4-injected *HB9-LRP4*$^{+/+}$ mice (control) and *HB9-LRP4*$^{-/-}$ mice. (C, D) Reduced CMAP amplitudes at 20 Hz (C) or at 40 Hz (D) in LRP4-injected *HB9-LRP4*$^{+/+}$ mice (control) and *HB9-LRP4*$^{-/-}$ mice. (E) Reduced CMAP amplitudes of the 10th stimulation at different stimulation frequencies. (F) Representative mEPP traces. Traces on the right were enlarged from those underlined on the left. (G, H) Similar reduction of mEPP amplitude and frequency in the two genotypes after LRP4 immunization. Control, n = 3; *HB9-LRP4*$^{-/-}$ mice, n = 5; LRP4-injected control mice, n = 5; LRP4-injected *HB9-LRP4*$^{-/-}$ mice, n = 5. 3-4 muscle fibers were recorded from each mouse.

Suppl Figure 4. Immunization of rabbits with ecto-LRP4. (A) Detection of anti-LRP4 antibodies in sera of ecto-LRP4-injected rabbits. Sera were collected one week after first boost and subjected to ELISA. n = 3. (B) Images of representative control and LRP4-injected rabbits. (C) Reduced body weight of LRP4-injected rabbits.

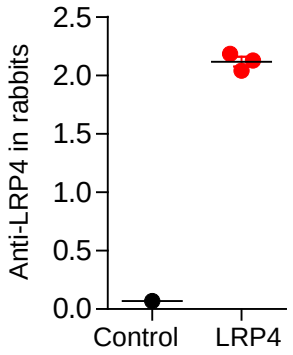
Suppl Figure 5. IgG purification from LRP4-injected rabbit sera. (A) Schematic diagram of IgG purification procedures. (B, C) Analysis of purified rabbit IgGs on 8% non-denaturing PAGE (B) and 12% denaturing SDS-PAGE (C). Fractions surrounding 25th fraction were pooled for injection.


A
SP
LDLRa
EGF
β1
β2
β3
β4
TM
LRP4
Flag
His
ecto-LRP4
B
kDa
wash-through
ecto-LRP4
260
ecto-LRP4

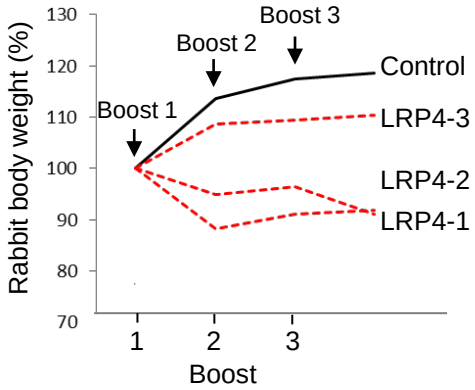

HEK293
AChRα
-
+
kDa
IB: LRP4 sera-1
40
IB: LRP4 sera-2
40
IB: LRP4 sera-3
40
IB: Anti-AChRα
40
-AChRα
-NS


A
Body weight (%)
NS
NS
Control HB9-LRP4+/+
Control HB9-LRP4-/-
LRP4-injected HB9-LRP4+/+
LRP4-injected HB9-LRP4-/-
B
Grip strength(%)
NS
NS
C
20 Hz
CMAP amplitude (%)
Stimulus #
Control HB9-LRP4+/+
Control HB9-LRP4-/-
LRP4-injected HB9-LRP4+/+
LRP4-injected HB9-LRP4-/-
D
40 Hz
CMAP amplitude (%)
Stimulus #
E
CMAP amplitude (%)
Stimulation frequency (Hz)
F
Control HB9-LRP4+/+
Control HB9-LRP4-
LRP4-injected HB9-LRP4+/+
LRP4-injected HB9-LRP4-/-
0.5 mV
200 ms
20 ms
G
mEPP amplitude (mV)
NS
NS
Control HB9-LRP4+/+
Control HB9-LRP4-/-
LRP4-injected HB9-LRP4+/+
LRP4-injected HB9-LRP4-/-
H
mEPP frequency (Hz)
NS
NS

A


Anti-LRP4 in rabbits
2.5
2.0
1.5
1.0
0.5
0.0
Control
LRP4

C


Rabbit body weight (%)
130
120
110
100
90
80
70
Boost 1
Boost 2
Boost 3
Control
LRP4-3
LRP4-2
LRP4-1
1
2
3
Boost

B

Control
LRP4

A

Size Exclusion Chromatography

Sera collection

↓

Saturated ammonium sulfate precipitation

↓

Dissolution in PBS

↓

Dialysis in PBS

↓

Concentration by centri-YM30 column

↓

Chromatography on sephacryl S-200 column

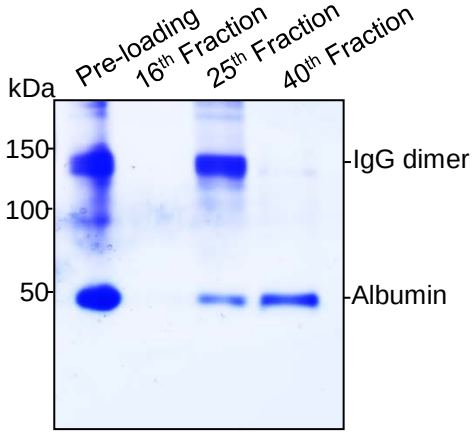
↓

OD280 quantification

↓

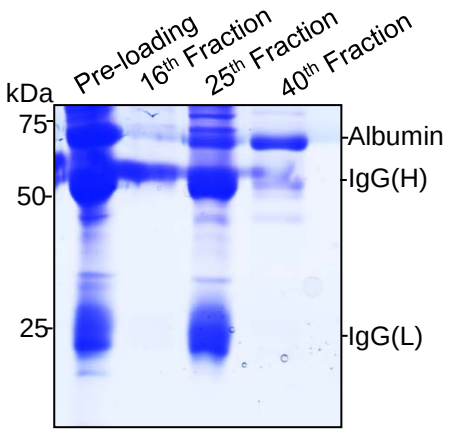
Analysis by PAGE

B



Non-denaturing gel

C



Denaturing gel