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Supplemental information

Identification of potent siRNA targeting complement C5 and its robust activity in pre-clinical models of myasthenia gravis and collagen-induced arthritis

Yoshikazu Kuboi, Yuta Suzuki, Sotaro Motoi, Chiyuki Matsui, Naoki Toritsuka, Tomoya Nakatani, Kazuhiro Tahara, Yoshinori Takahashi, Yoko Ida, Ayaka Tomimatsu, Motohiro Soejima, and Toshio Imai

Supporting Information

Western blotting

Serum C5 or C6 protein levels were analyzed using standard Western blotting with SDS-PAGE. Blood samples were collected under anesthesia with 2–2.5% vaporized isoflurane at week 11 (C6) and week 13 (C5) after the 1st immunization. For C5 detection, sera were diluted 1:200 into PBS and mixed with NuPAGE™ LDS Sample Buffer (#NP0007, Invitrogen), proteins were separated through electrophoresis on NuPAGE Novex Bis-Tris pre-cast 10% gels (# 567-1035, Bio-Rad Laboratories, Hercules, CA, USA). After electrophoretic transfer onto iBLOT2 PVDF Regular Stacks (IB24001, Thermo Fisher Scientific) using a iBlot2 Gel Transfer Device (IB21001, Thermo Fisher Scientific), membranes were blocked with blocking buffer (5% skim milk in Tris-buffered saline containing 0.1% Tween20 (TBS-T)) and incubated overnight at 4 °C with 1:1000 diluted Mouse Complement Component C5a Antibody (#AF2150, R&D Systems). Subsequently, 1:1000 diluted Rabbit anti-Goat IgG (H + L) Secondary Antibody-HRP (#811620, Invitrogen) was used. The immunoblotting signal was developed using the ECL Prime Western Blotting Detection Reagents (#RPN2232, cytiva), followed by imaging with the LAS3000 mini (FUJIFILM). For C6 detection, sera were diluted 1:50 into PBS and mixed with Laemmli Sample Buffer (#1610737, Bio-Rad), proteins were separated through electrophoresis on 4–20% Criterion TGX Precast Gels (#567-1095, Bio-Rad). After electrophoretic transfer onto iBLOT2 PVDF Regular Stacks (Invitrogen), membranes were blocked with the blocking buffer described above and incubated overnight at 4 °C with 1:2000 diluted C6 Polyclonal antibody (#17239-1-AP, Proteintech). Subsequently, 1:5000 diluted Peroxidase AffiniPure $F(ab')_2$ Fragment Donkey Anti-Rabbit IgG (H + L)(#711-036-152, Jackson ImmunoResearch) secondary antibody was used. The immunoblotting signal was developed and detected as described above.

Table S1. siRNA sequences

siRNA	Sense (5'-3')	Antisense (5'-3')
Control siRNA (Luciferase)	cuuAcGcuGAGuAcuucGAt^t	UCGAAGuACUcAGCGuAAGt^t
m/rC5-siRNA	cuGuGAAAGcAAGAuAuuuuu	AAAuAUCUUGCUUUcAcAGuu
m/rC6-siRNA	uGuucuAAGuccuGcAAuuuu	AAUUGcAGGACUuAGAAcAuu

N, RNA; n, 2'-OMe RNA; t, DNA(T); ^, phosphorothioate; m/r, mouse-rat.

Other C5-siRNA sequences can be identified using the indicated position of NM_001735.2.

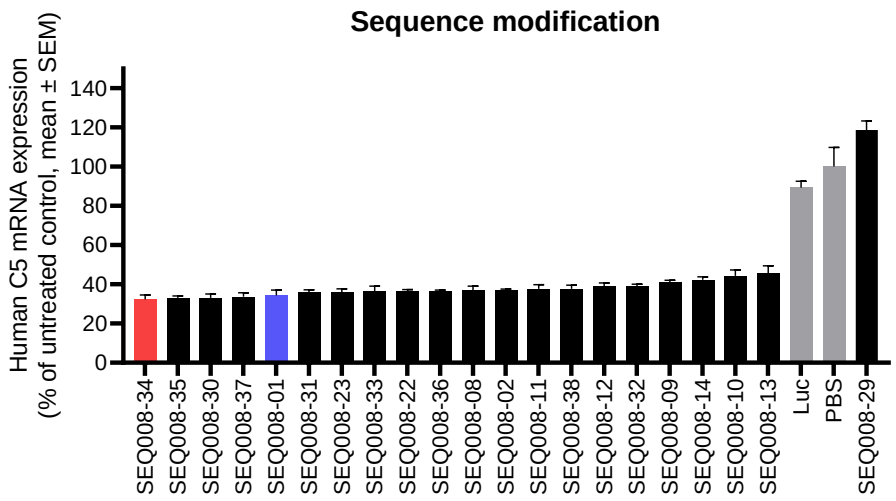

Figure S1. *In vitro* screening of chemically or structurally modified C5-siRNA. Human C5 mRNA levels in Hep3B cells transfected with a panel of siRNAs (1 nM) overnight were quantified using RT-qPCR and normalized to the housekeeping gene GAPDH. Data are presented as mean ± SEM.

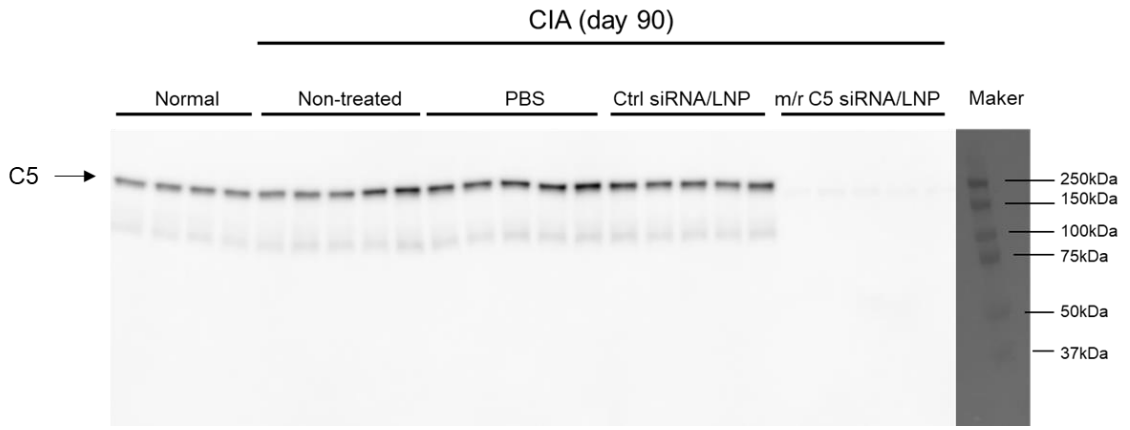

Figure S2. Western blot analysis for complement C5 protein expression levels in serum. Complement C5 levels in the serum of mice treated with either PBS, 1 mg/kg of Ctrl siRNA/LNP, 1 mg/kg of m/rC5-siRNA/LNP, or that of normal mice were evaluated. Full scan of western blots is shown.

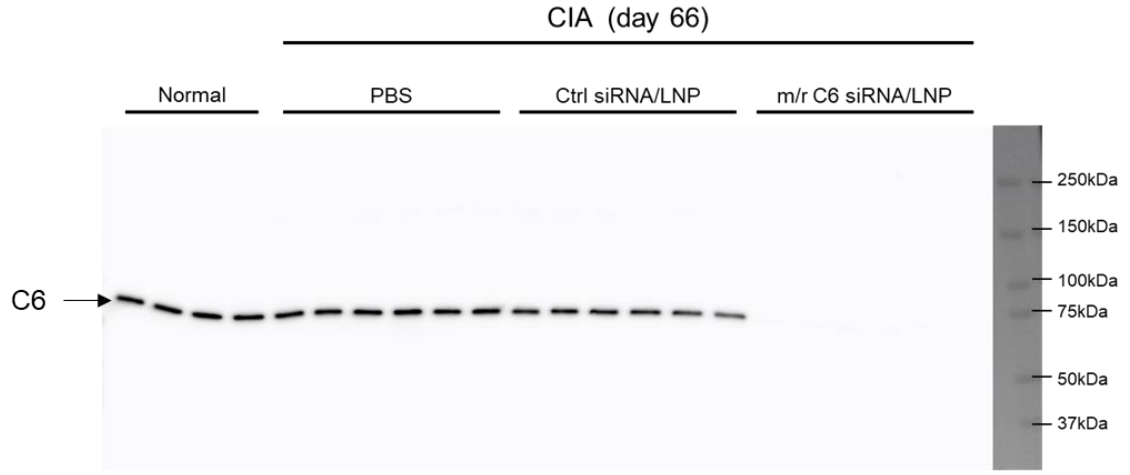

Figure S3. Western blot analysis for complement C6 protein expression levels in serum. Complement C6 levels in plasma of mice treated with either PBS, 1 mg/kg of Ctrl siRNA/LNP, 1 mg/kg of m/rC6-siRNA/LNP, or that of normal mice were evaluated. Full scan of western blots is shown.