MiR-33a is a therapeutic target in SPG4-related hereditary spastic paraplegia human neurons

Fumiko Nakazeki[1†], Itaru Tsuge[2,3†], Takahiro Horie[1], Keiko Imamura[2,4,5], Kayoko Tsukita[2,4], Akitsu Hotta[2], Osamu Baba[1], Yasuhide Kuwabara[1], Tomohiro Nishino[1], Tetsushi Nakao[1], Masataka Nishiga[1], Hitoo Nishi[1], Yasuhiro Nakashima[1], Yuya Ide[1], Satoshi Koyama[1], Masahiro Kimura[1], Shuhei Tsuji[1], Motoko Naitoh[3], Shigehiko Suzuki[3], Yuishin Izumi[6], Toshitaka Kawarai[6], Ryuji Kaji[6], Takeshi Kimura[1], Haruhisa Inoue[2,4,5]*, and Koh Ono[1]*

1 Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, Japan.

2 Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan.

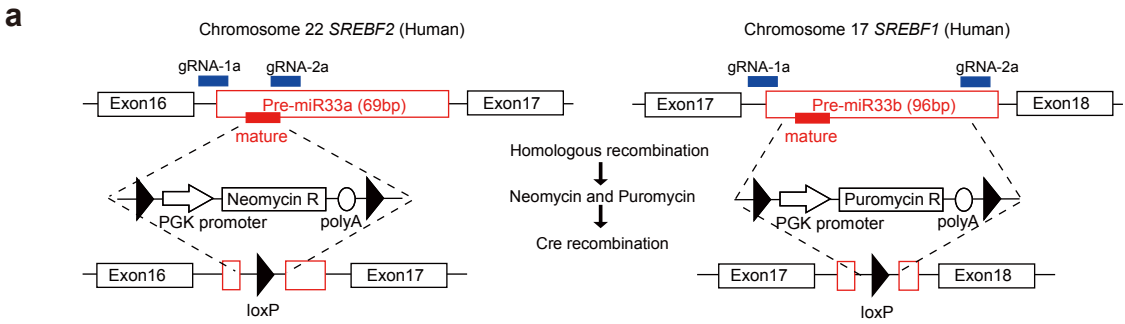
3 Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan

4 iPSC-based Drug Discovery and Development Team, RIKEN BioResource Center (RIKEN BRC), Kyoto, Japan

5 Medical-risk Avoidance based on iPS Cells Team, RIKEN Center for Advanced Intelligence Project (RIKEN AIP), Kyoto, Japan

6 Department of Clinical Neuroscience, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima 770-8503, Japan

Supplementary Figure 1



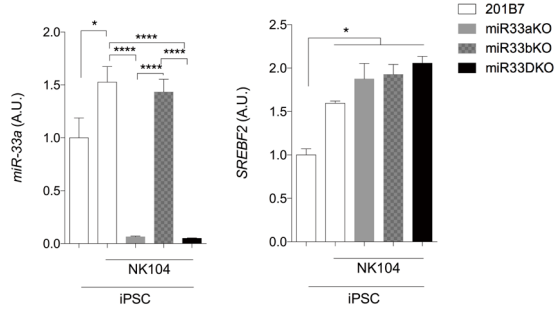
b

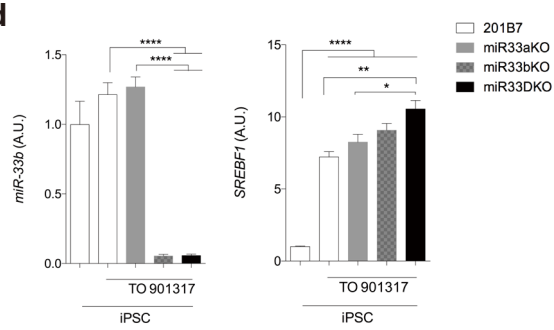
premiR-33a without seed sequence

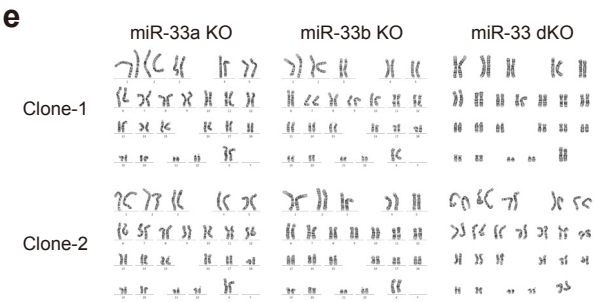
CTGTGGCGCAACGCAATTAGT**GATAACTTCGTATAGCATACATTATACGAAGTTAT**GTTTGTTACCATTTG
TGTTCTAATGGTACCCATGCAATGTTTCCACAGTGCATCACAG

premiR-33b without seed sequence

GCGGGCGGGCGCAACGCAATTAGT**GATAACTTCGTATAGCATACATTATACGAAGTTAT**GTTTGTTACCATTTG
CCCCTGGCACCAC







Supplementary Figure. 1

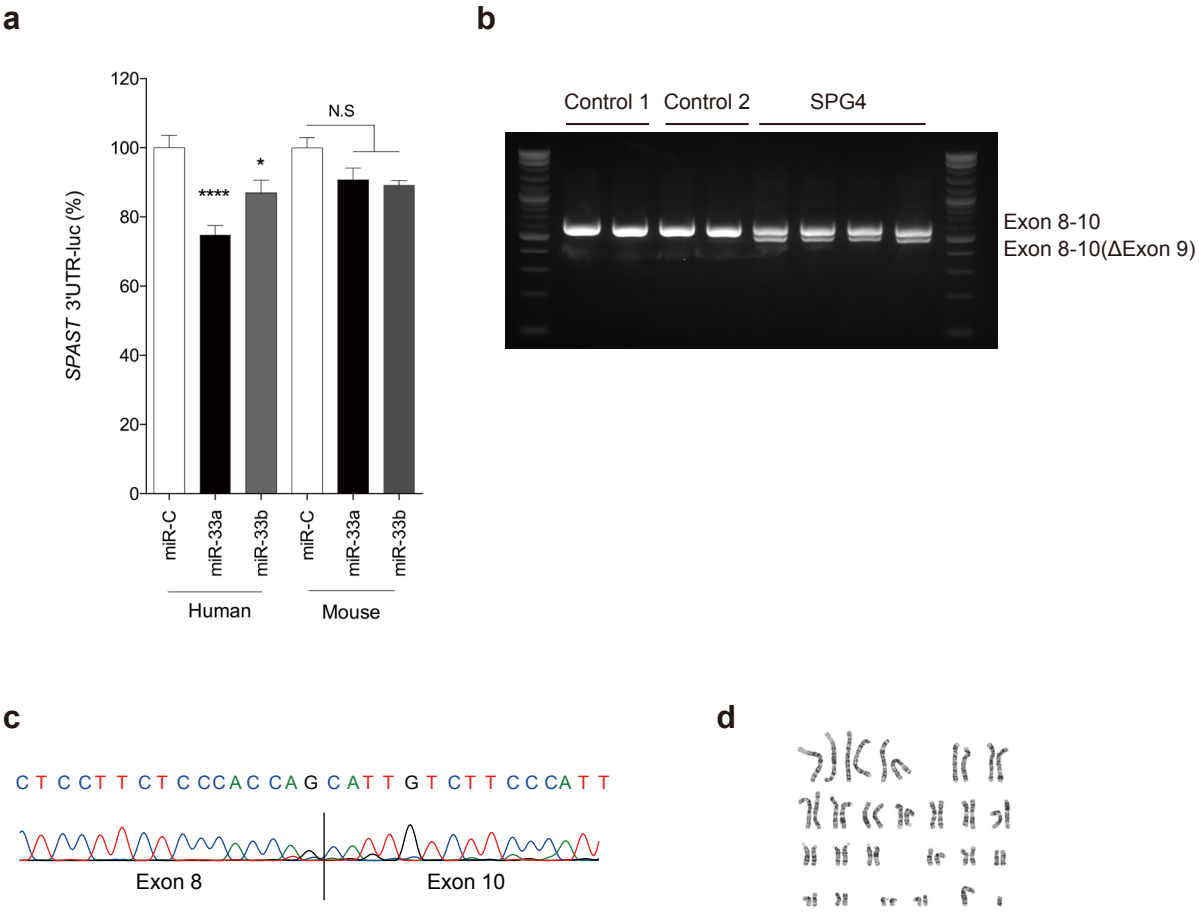
(**a**) Schematic overview of human *SREBF2* and *SREBF1* locus with targeting strategy. The donor template was designed to have PGK-Neomycin and/or Puromycin selection cassette flanked by two loxP sites and homology arms. PGK promoter: phosphoglycerol kinase promoter, Neomycin R: neomycin resistance gene, Puromycin R: puromycin resistance gene, polyA: polyadenylation sequence.

(**b**) DNA sequencing confirmed the deletions and insertions generated by CRISPR-Cas9 technology. loxP sequenced are highlighted by blue.

(**c**) Expression levels of mature miR-33a and the host gene (*SERBF2*) with treatment. n=3 in each clone, two clones per knockout line. *P<0.05, ****P<0.0001 by one-way ANOVA.

(**d**) Expression levels of mature miR-33b and the host gene (*SERBF1*) with treatment. n=3 in each clone, two clones per knockout line.

(**e**) Keryotype analysis in miR-33 KO iPSCs.



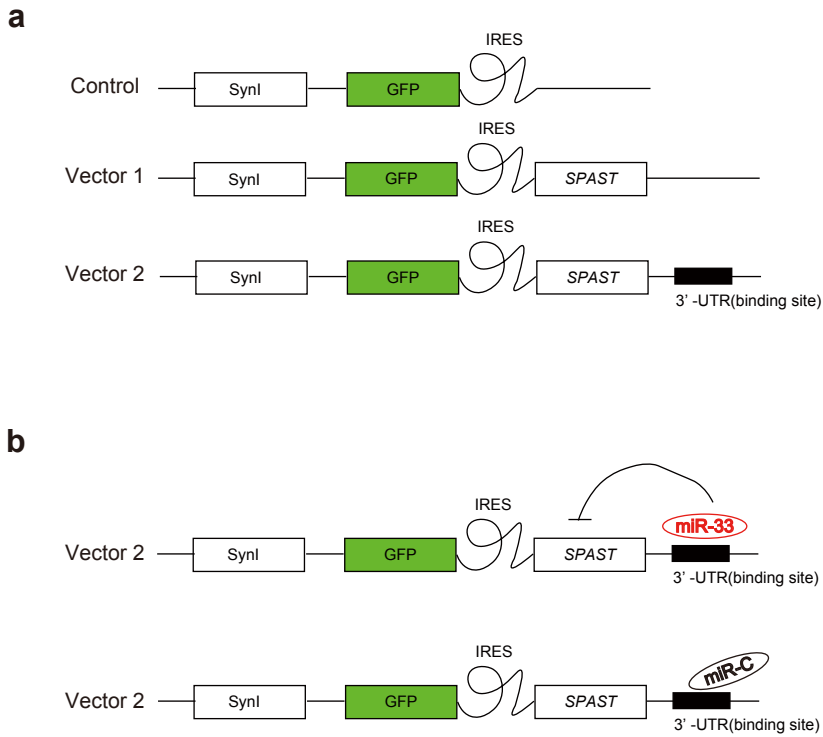
Supplementary Figure. 2

(**a**) Luciferase reporter activity of human and mouse *SPAST* gene 3' -UTR constructs in HEK293T cells overexpressing miR-control (miR-C) and miR-33. n=4 each, *P<0.05, ****P<0.0001 by unpaired t-test.

(**b**) RT-PCR analysis of SPAST in SPG4-iPSCs. Sense primer was designed in exon 8, and antisense primer was designed in exon 10 of SPAST.

(**c**) Sequencing of abnormal band (bottom band) in SPG4-iPSCs, indicating the skipped exon 9.

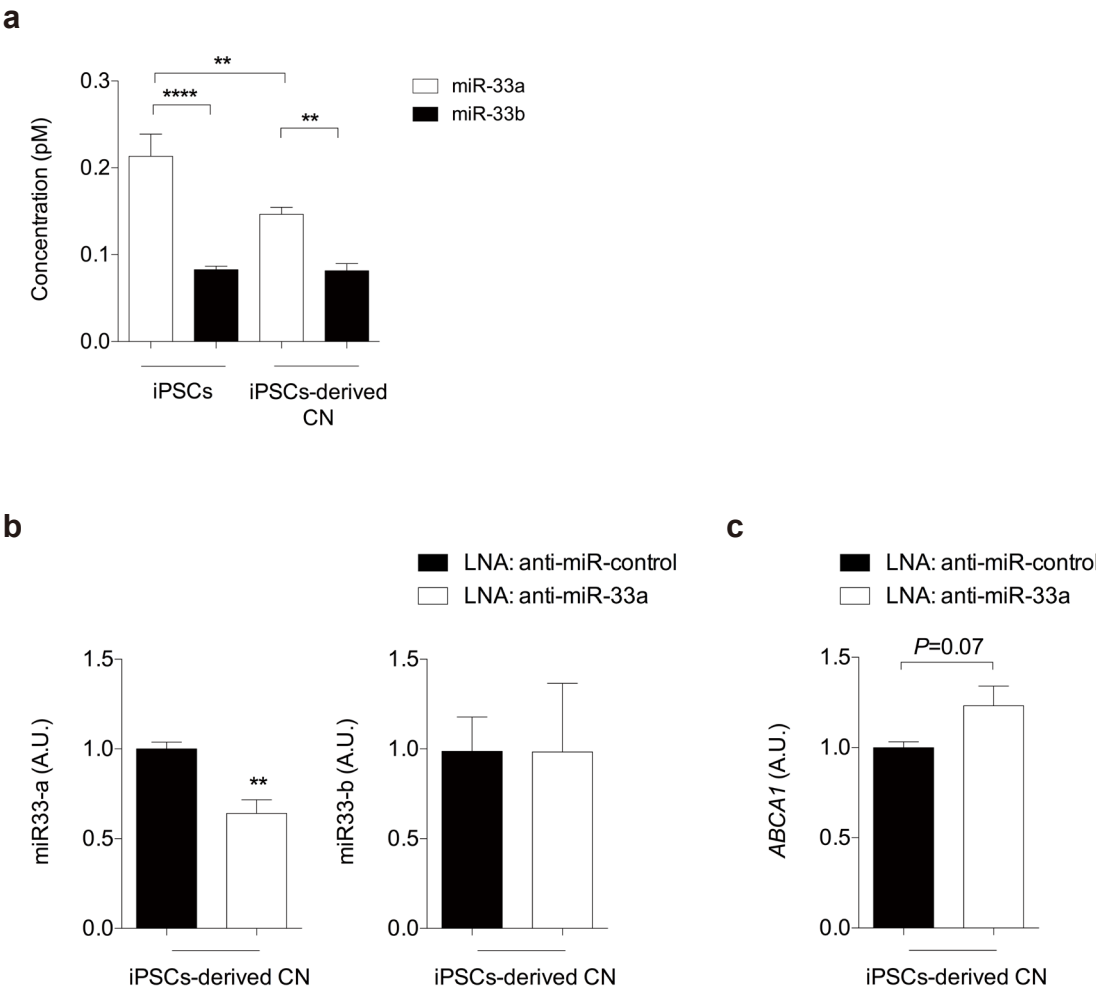
(**d**) Karyotype analysis in SPG4-iPSCs.



Supplementary Figure. 3

(**a**) Schematic map showing the lentivirus vector.

(**b**) Schematic overview of miR-33 mediated translational repression.

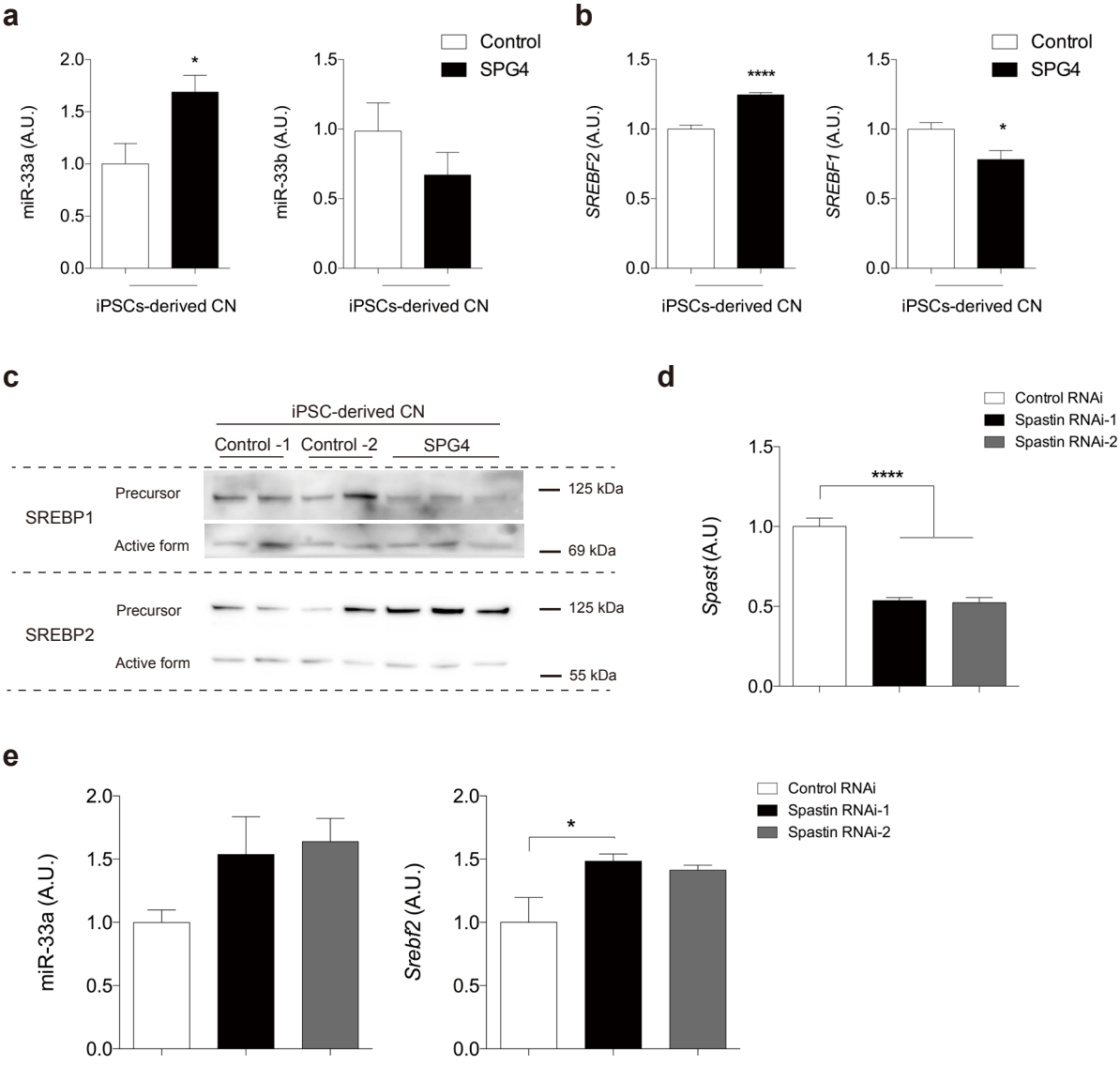


Supplementary Figure. 4

(**a**) Absolutely levels of miR-33a and miR-33b in both an undifferentiated state and neural differentiation. n=3~4 in each clone. **P<0.01, ****P<0.0001 by unpaired t-test.

(**b**) Expression levels of miR-33a/b with LNA treatment in iPSC-derived cortical neurons. n=4~5 each, **P<0.01 by unpaired t-test.

(**c**) Expression levels of *ABCA1* with LNA treatment in iPSC-derived cortical neurons. n=4~5 each.



Supplementary Figure. 5

(**a**) Expression levels of miR-33 in SPG4-derived cortical neurons. n=4~5 each, *P<0.05 by unpaired t-test.

(**b**) Expression levels of *SREBF2* and *SREBF1* in SPG4-derived cortical neurons. n=4~5 each, *P<0.05, ****P<0.0001 by unpaired t-test.

(**c**) Protein levels of SREBP1 and SREBP2 in SPG4-derived cortical neurons. Results obtained from the different part of the same membrane of Figure 3b.

(**d**) RT-PCR analysis conforming knockdown of Spastin in Neuro 2a cells. n=5 each, ****P<0.0001 by one-way ANOVA.

(**e**) Expression levels of miR-33a and *Srebf2* in two spastin RNAi Neuro 2a. n=5 each, *P<0.05 one-way ANOVA.

Table S1. Sequences for the gene targeting strategy

Selected sequences for gene targeting of CRISPR-Cas9n are shown.

Gene targeting sequence	
miR-33a KO gRNA-1a	GCTGCCCGCCAGGAGGTATGCGG
miR-33a KO gRNA-2a	TGTAGTTGCATTGCATGTTCTGG
miR-33b KO gRNA-1b	TGCAACAGCAATGCACCGCG
miR-33b KO gRNA-2b	TCGGCAGTGCAGCCCGGAGC

Table S2. Primer sequences

Gene-specific oligonucleotide primer sequences used in this study

Gene	Species	Forward	Reverse
SREBF1	Human	AACAGTCCCACTGGTCGTAGAT	TGTTGCAGAAAGCGAATGTAGT
SREBF2	Human	AGGAGAACATGGTGCTGA	TAAAGGAGAGGCACAGGA
SPAST	Human	AGCTGGTCAAGACTTGGCAA	AGGTTGCATTCGATTCTGCA
ABCA1	Human	GTCCTCTTTCCCGCATTATCTGG	AGTTCCTGGAAGGTCTTGTTCAC
18S	Human	AGAAACGGCTACCACATCCA	CCCTCCAATGGATCCTCGTT
Srebf2	Mouse	GTGGAGCAGTCTCAACGTCA	TGGTAGGTCTCACCCAGGAG
Spast	Mouse	CGGGCCAAGGTGAACAGTAT	GATGTCCATTGCGGCATGTC
18S	Mouse	CGCGGTTCTATTTTGTTGGT	AGTCGGCATCGTTTATGGTC