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Supplemental Information

Antisense Oligonucleotides Reduce RNA Foci in Spinocerebellar Ataxia 36 Patient iPSCs

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Supplemental Figures

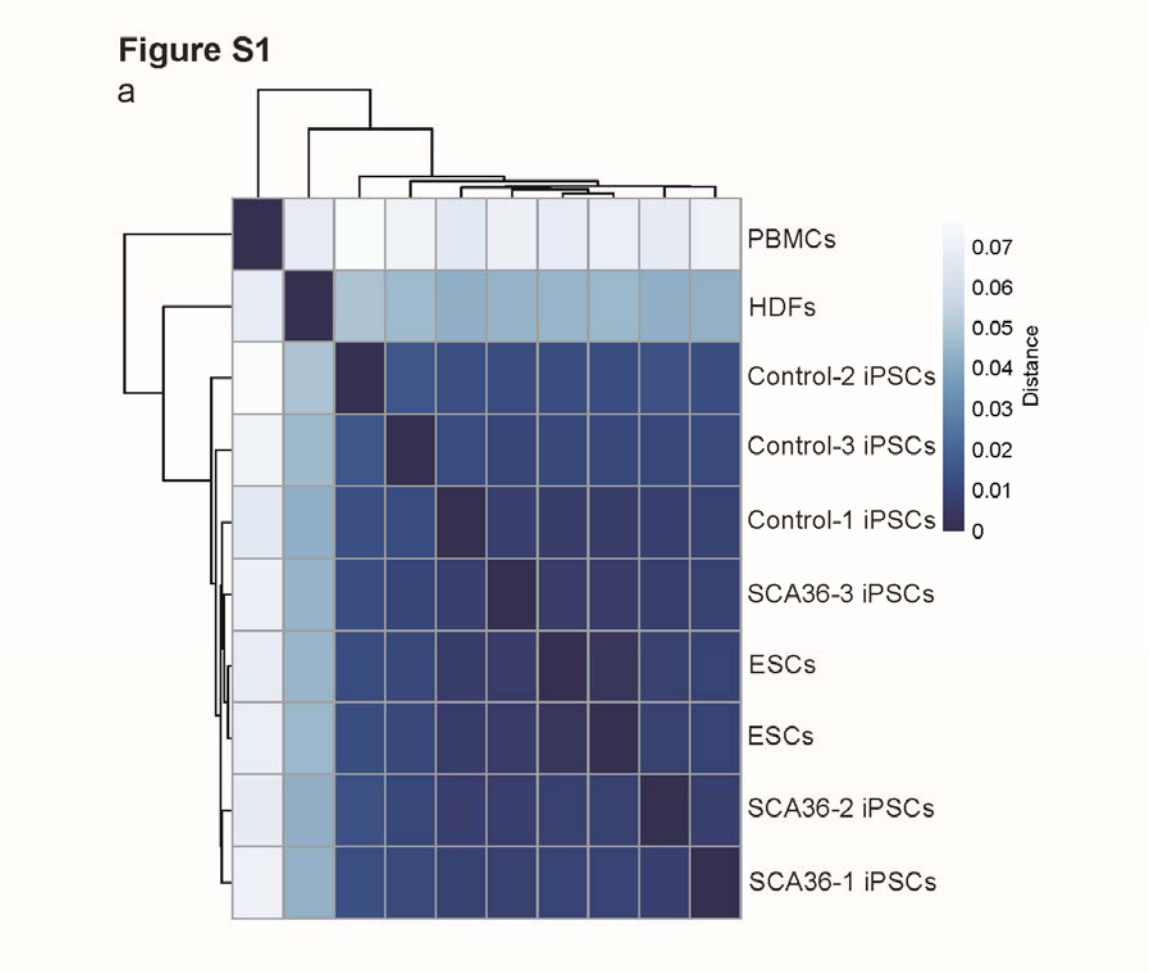

Figure S1: RNA-Seq analysis

All six iPSCs and embryonic stem cells (ESCs) for positive control were categorized in the same cluster, which was differentiated from HDF or PBMC clusters.

Figure S2

a

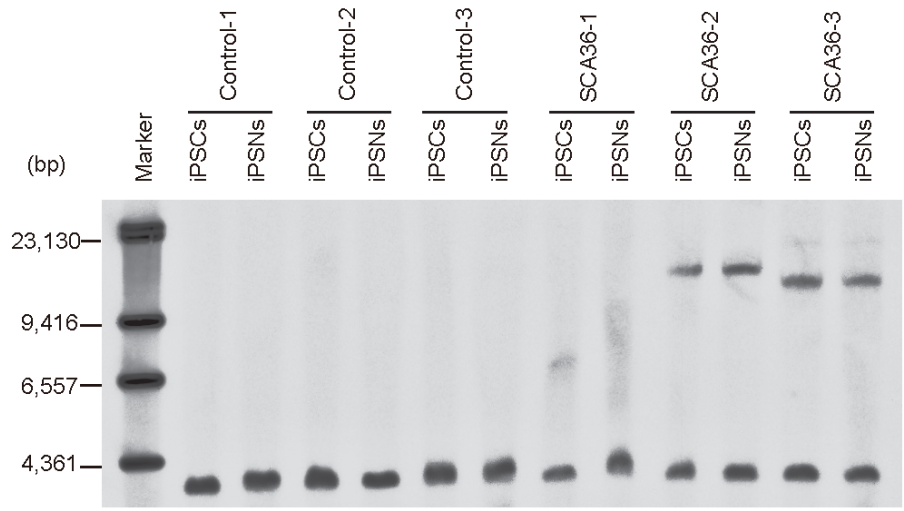

Figure S2: Southern blot analysis of iPSCs and iPSNs

An expanded GGCCTG repeat allele was shown in the SCA36 patient iPSCs and the SCA36 patient iPSNs.

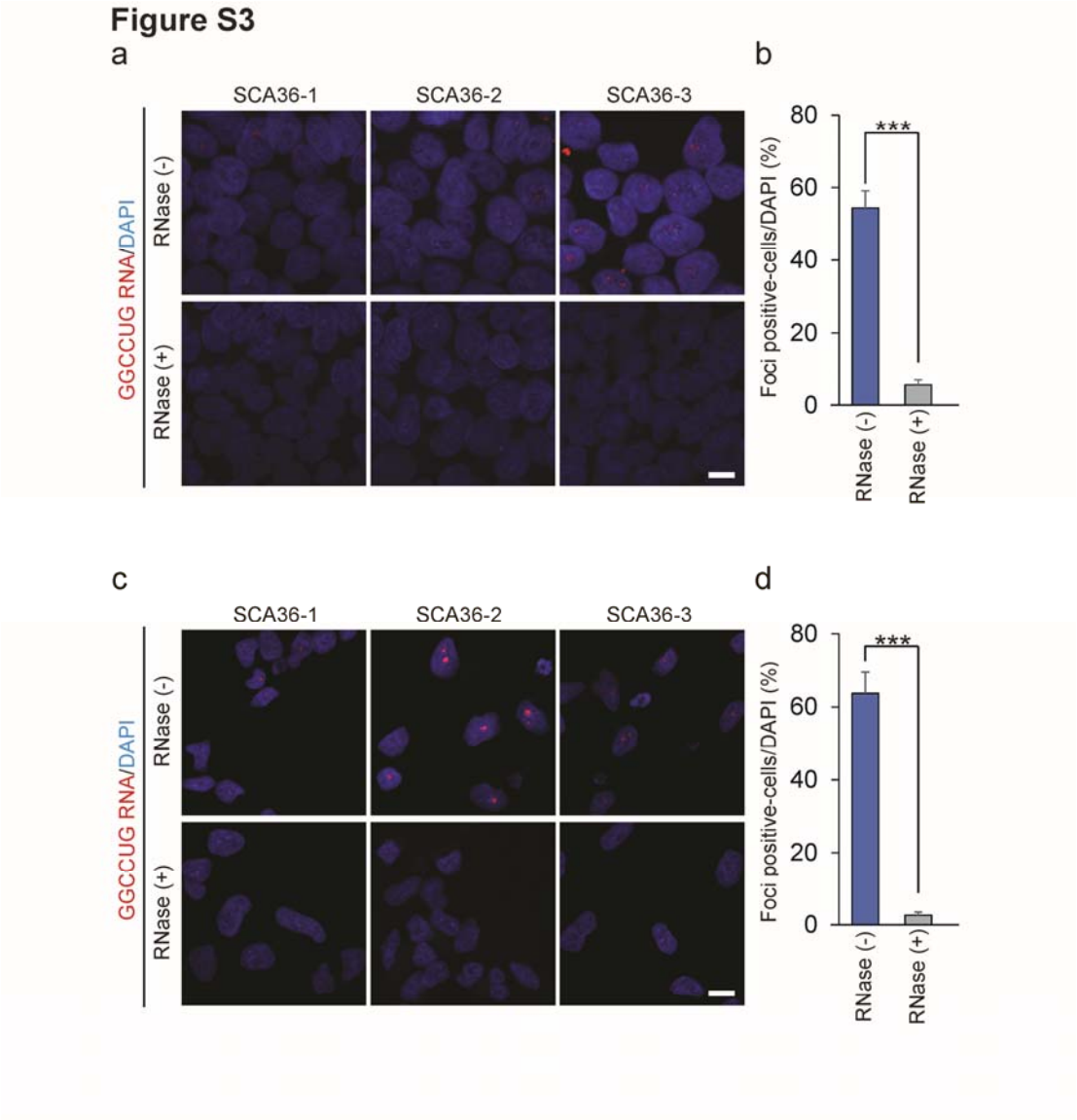

Figure S3: RNA foci in SCA36 patient iPSCs and iPSNs disappeared by RNase A treatment

(a) Sense RNA foci in SCA36 patient iPSCs disappeared by RNase A treatment. Scale bar: 10μm. (b) The graph shows that almost no RNA foci were detected by RNase A treatment. ***$p < 0.001$. Data presented as mean ± SEM from n = 3 clones. (c) Sense RNA foci in

SCA36 patient iPSNs disappeared by RNase A treatment. Scale bar: 10μm. (d) The graph shows that almost no RNA foci were detected by RNase A treatment. ***$p < 0.001$. Data presented as mean ± SEM from n = 3 clones.

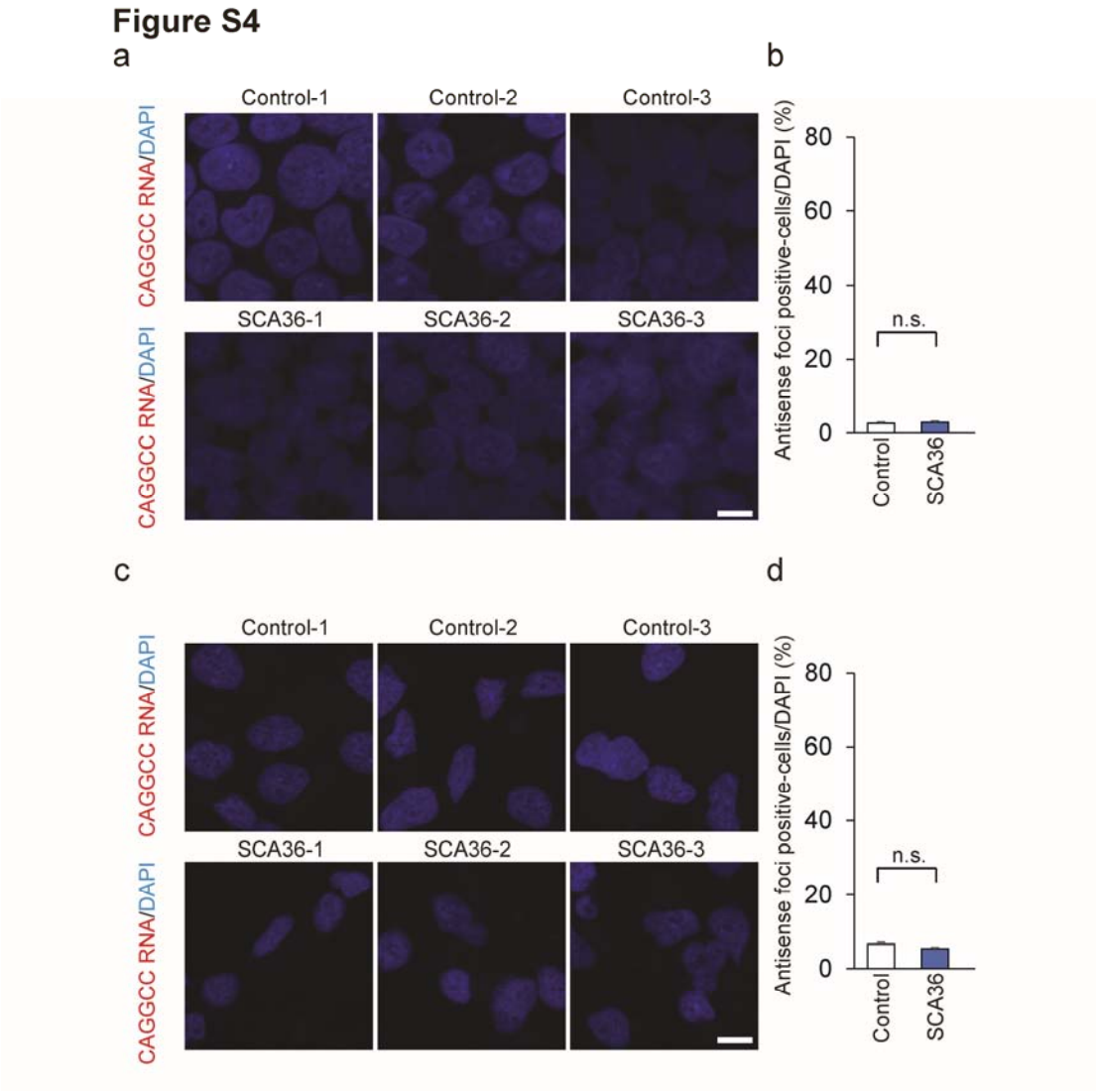

Figure S4: Antisense RNA foci were not detected in SCA36 patient iPSCs or iPSNs.

Fluorescence in situ hybridization (FISH) analysis was performed for each iPSC clone using a cy3-conjugated CUG(GGCCUG)$_2$G locked nucleic acid oligonucleotide probe. (a) No antisense RNA foci were detected in either healthy control or SCA36 patient iPSCs. Scale bar: 10μm. (b) The graph shows that there were no significant CAGGCC RNA foci-positive cells in iPSCs. Data presented as mean ± SEM from n = 3 clones. (c) No antisense RNA foci

were detected in either healthy control or SCA36 patient iPSNs. Scale bar: 10μm. (d) The graph shows that there were no significant CAGGCC RNA foci-positive cells in iPSNs. Data presented as mean ± SEM from n = 3 clones.

Figure S5

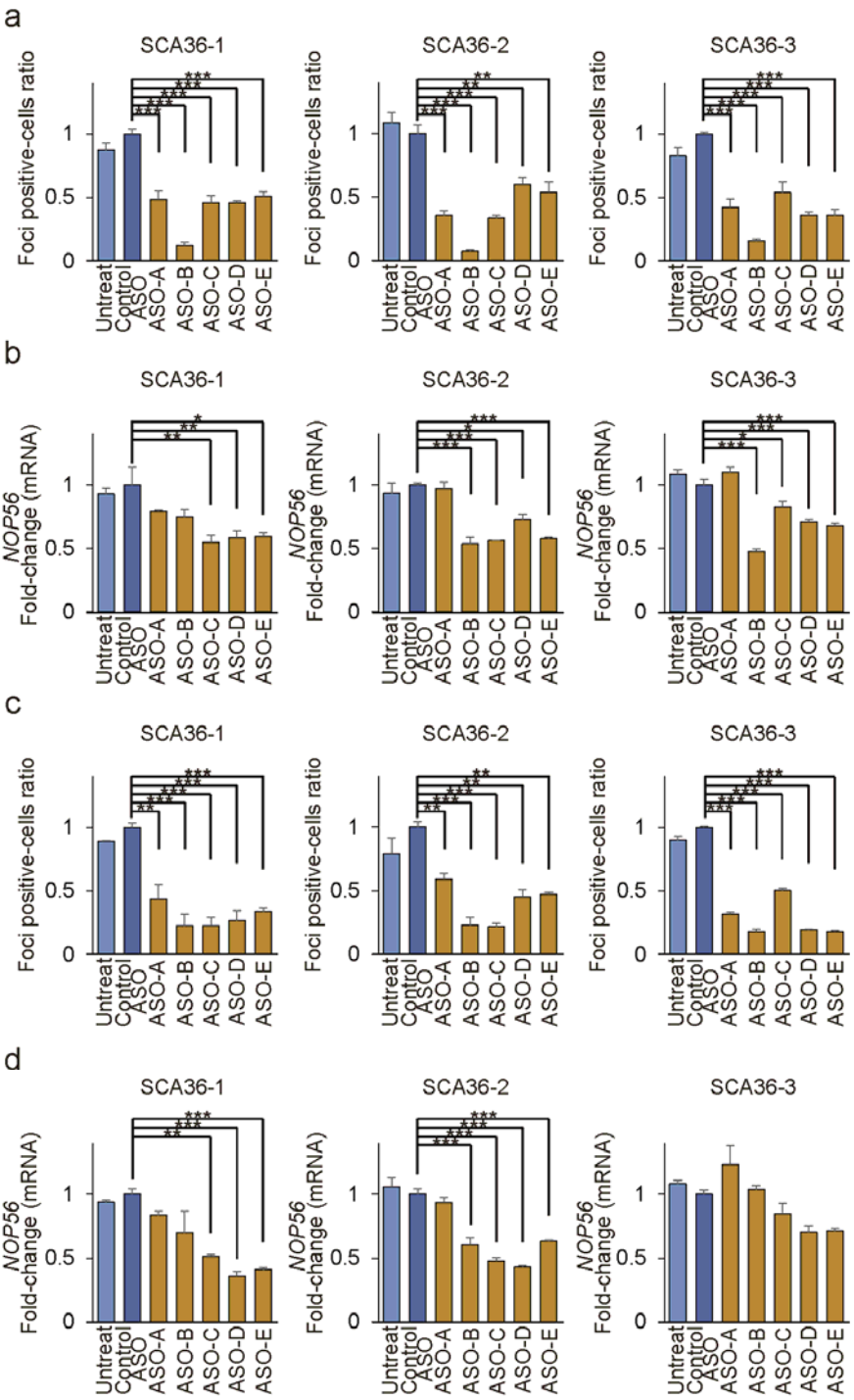
a
SCA36-1
SCA36-2
SCA36-3
Foci positive-cells ratio
b
c
d
NOP56
Fold-change (mRNA)
Untreat
Control
ASO
ASO-A
ASO-B
ASO-C
ASO-D
ASO-E

Figure S5: The data of antisense oligonucleotides effectiveness in each SCA36 patient iPSC or iPSN clone.

(a) The graph shows the number of RNA foci-positive cells in each SCA36 patient iPSC clone. $^{**}p < 0.01$, $^{***}p < 0.001$. Data presented as mean ± SEM from n = 3 wells.

(b) The graph shows the *NOP56* mRNA expression levels in each SCA36 patient iPSC clone after ASO treatment. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Data presented as mean ± SEM from n = 3 wells. (c) The graph shows the number of RNA foci-positive cells in each SCA36 patient iPSN clone. $^{**}p < 0.01$, $^{***}p < 0.001$. Data presented as mean ± SEM from n = 3 wells. (d) The graph shows the *NOP56* mRNA expression levels in each SCA36 patient iPSN clone after ASO treatment. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Data presented as mean ± SEM from n = 3 wells.

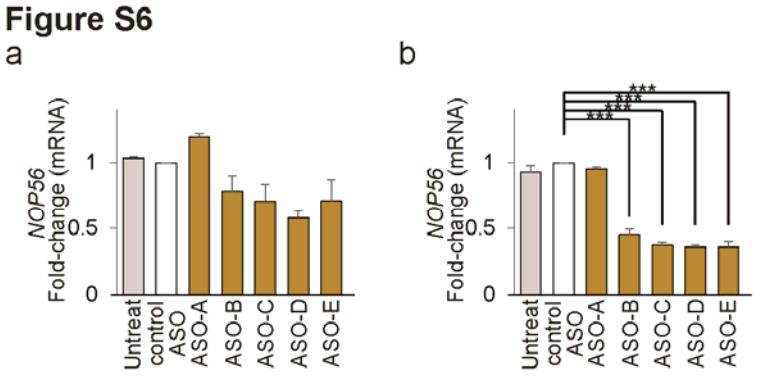

Figure S6: The *NOP56* mRNA expression levels in healthy control iPSCs and iPSNs after ASO treatment.

(a) The graph shows *NOP56* mRNA expression levels in healthy control iPSCs after ASO treatment. Data presented as mean ± SEM from n = 3 clones. (b) The graph shows *NOP56* mRNA expression levels in healthy control iPSNs after ASO treatment. ***$p < 0.001$. Data presented as mean ± SEM from n = 3 clones.