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Supplemental Information

Effective and Accurate Gene Silencing by a Recombinant AAV-Compatible MicroRNA Scaffold

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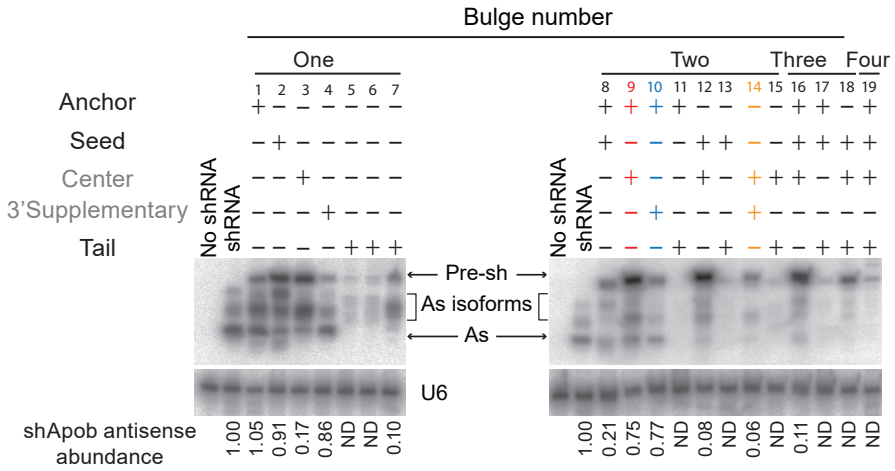
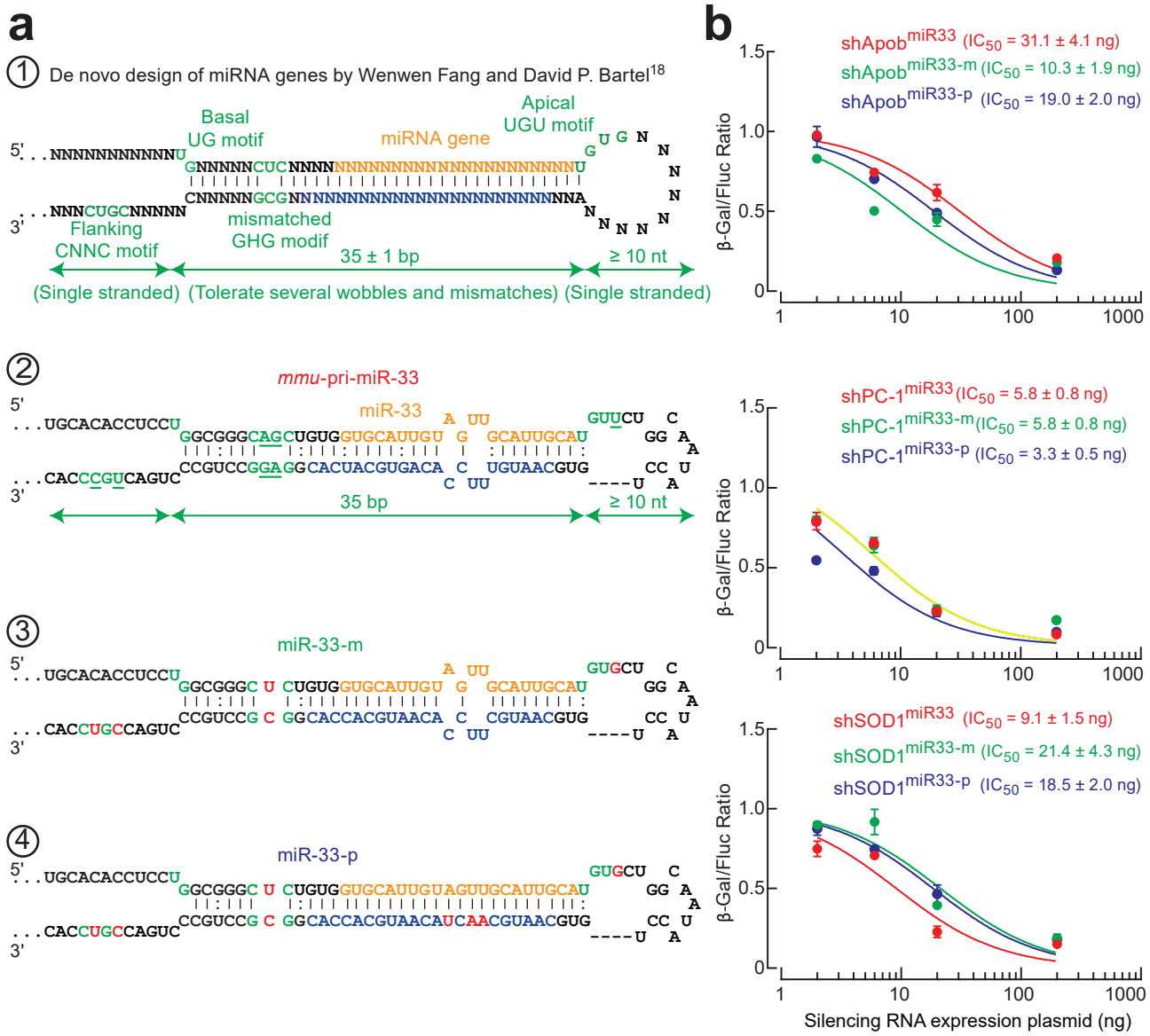
Supplementary Figure 1



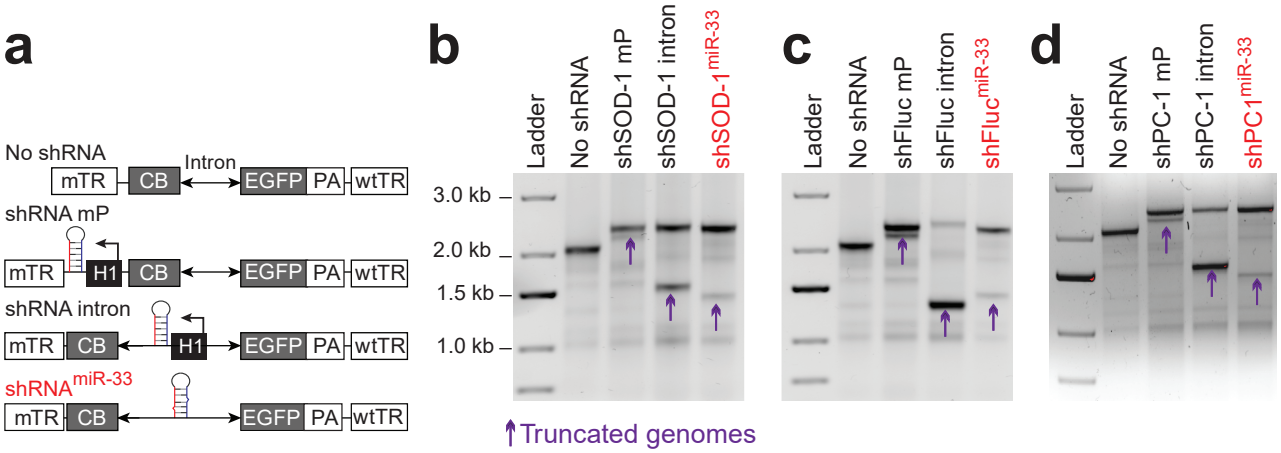
Supplementary Figure 1. Small RNA Northern blot analysis of shApob antisense levels in HEK293 cells transfected with the shApob constructs with/without bulge. As, shApob antisense; Pre-sh, shApob precursors.

Supplementary Figure 2



Supplementary Figure 2. Engineering the motif features of miRNA genes into the pri-mmu-miR-33 scaffold.

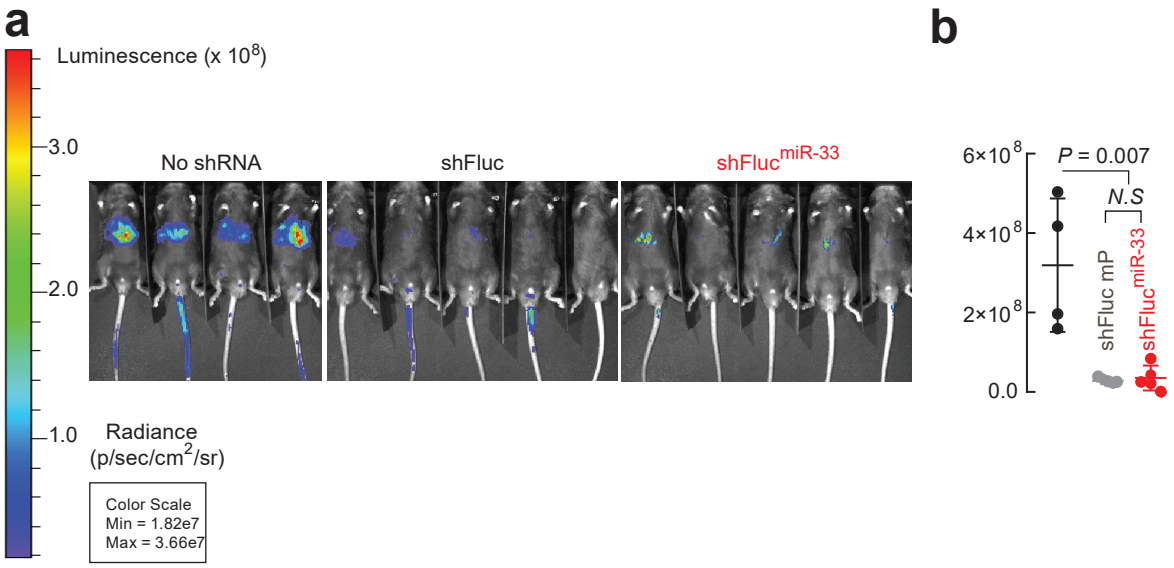
(a) Motifs (nucleotides in green) in miRNA genes described by Weiwei Fu and David P Bartel[29] (structure 1) and in *mmu*-pri-miR-33 (structure 2). The underlined variable nucleotides in *mmu*-pri-miR-33 were converted into the motifs of miRNA genes (red nucleotides in structures 3 and 4) to generate two modified miR-33 scaffolds. (b) Dose response curves for knockdown efficacy in the *Apob*, *PC1*, and SOD1 genes in HEK293 cells transfected with the shRNA$^{miR\text{-}33}$ and modified shRNA$^{miR\text{-}33}$ plasmids from 2 to 200 ng/well, together with their sensor plasmids (200 ng/well). Values are mean ± SD. IC50 values are listed for each construct. Note that the shPC-1$^{miR\text{-}33}$ and shPC-1$^{miR33\text{-}m}$ datapoints are overlapping (yellow curve).



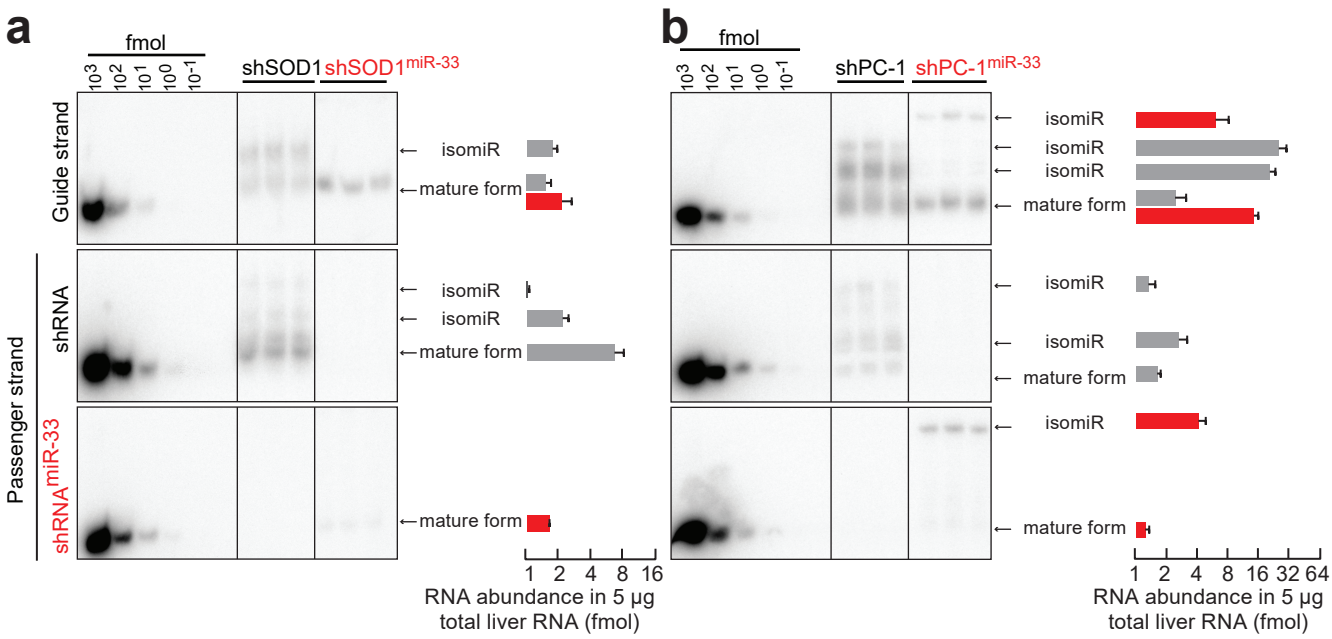
Supplementary Figure 3. Vector genome populations in purified rAAV gene silencing vectors

(a) Schematic of gene silencing constructs used for AAV packaging. (b-d) Agarose gel analysis of viral DNA extracted from purified vectors harboring shRNA or shRNA^{miR-33} against SOD-1, Fluc, and *PC*-1. The shFluc intron vector in panel (c) harbors the shRNA cassette in close proximity to the EGFP transgene, while the intronic shSOD-1 and shPC-1 vectors harbor the shRNA cassette in close proximity to the CB promoter as shown in panel (a). Purple arrows indicate truncated genomes caused by shRNA-encoding sequences or the miR-33 scaffold. The small truncated genomes are caused by obligate palindromic sequences residing in vector genomes as we previously reported[16].

Supplementary Figure 4



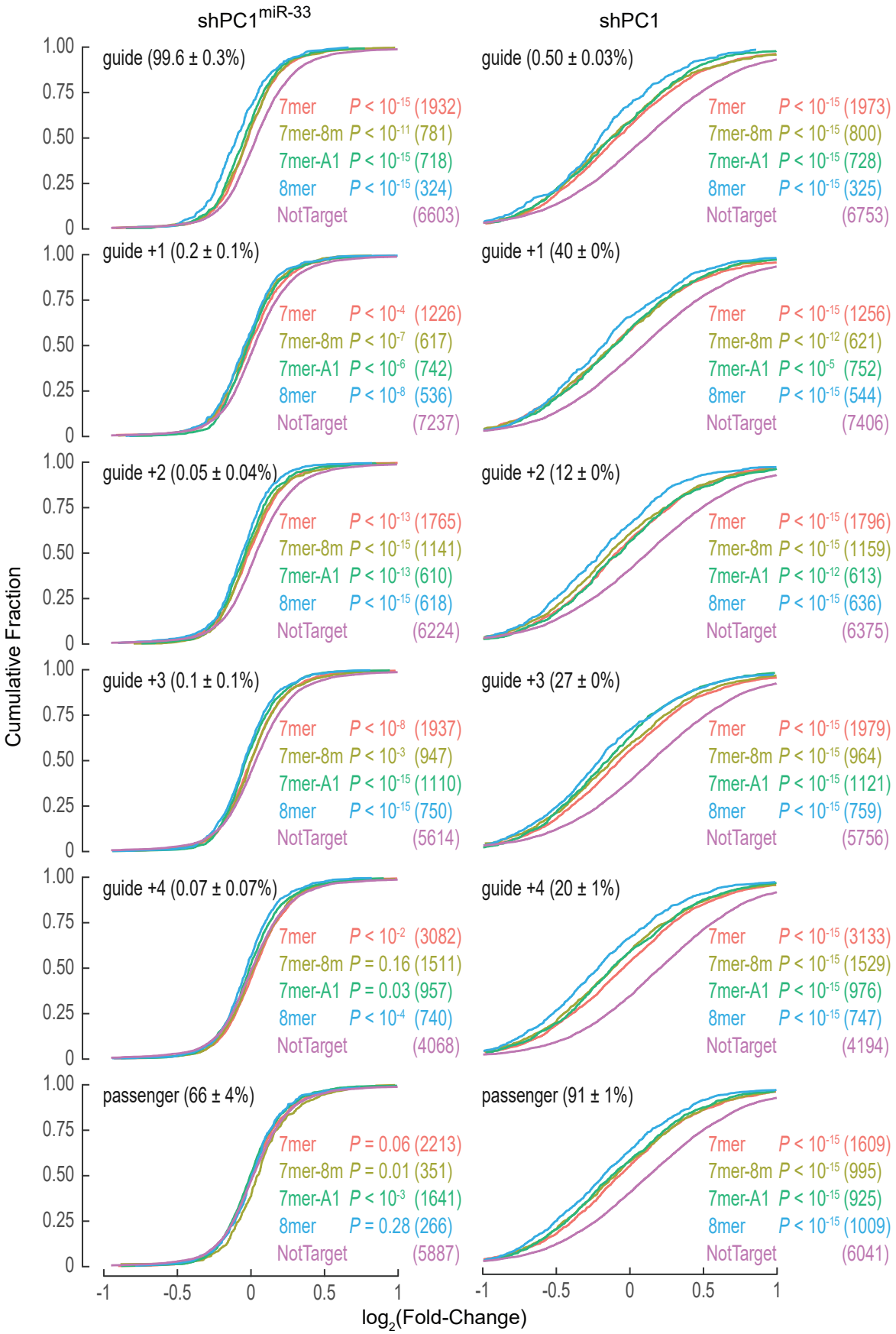
Supplementary Figure 4. Bioluminescence levels from mice co-injected with rAAV9-Fluc at 1.0 × 10^{11} GCs/mouse and rAAV9-no shRNA , shFluc, or shFluc^{miR-33} at 5.0 × 10^{11} GCs/mouse. (a) Live luminescence maging of mice treated with AAV vectors for three weeks. (b) Photo flux of liver region.



Supplementary Figure 5. Northern blot analysis of transcribed RNA from shSOD1 or shSOD1^{miR-33} (a), shPC-1 or shPC-1^{miR-33} (b) in HEK 293 cells.

Total RNA was extracted from the HEK 293 cells 48 hours after transfection. Five micrograms of total RNA were used for Northern blot analysis. Synthetic oligonucleotides at indicated amount were used as reference for quantification. The transcribed RNAs from shRNA and shRNA^{miR-33} are in grey and red, respectively.

Supplementary Figure 6



Supplementary Figure 6. Imprecise process of shRNA causes off-target knockdown.

Protein-coding genes are categorized based on pairing of their 3' UTR sequences to difference guide isoforms. The acumulative distribution of log $_2$ fold-change compared to control (NotTarget) is plotted. Guide +1, +2, +3, or +4: guide isoform whose 5' ends shift 1, 2, 3, or 4 nucleotides towards the 3' end.

Supplementary Table 1: Oligonucleotides used in this study.

Oligonucleotides	Sequence (5′ to 3′)	Aims
pri-miR-122 F	ATCGGGCCCGACTGCAGTTTCAGCGTTTG	Cloning of mouse *pri-miR-122*
pri-miR-122 R	CGCGGGCCCGACTTTACATTACACACAAT	
pri-miR-33 F	AGGGCTCTGCGTTTGCTCCAGG	Cloning of mouse *pri-miR-33*
pri-miR-33 R	AGGGTGACACTGTCCTTATT	
pri-miR-26a F	GCCCCTTCTCTTTGGCAG	Cloning of mouse *pri-miR-26a*
pri-miR-26a R	TTGGCCAGCAAGCTTGG	
pri-miR-126 F	GGAAGGCATTGTGGGGCGTAA	Cloning of mouse *pri-miR-126*
pri-miR-126 R	TGCAAAGTCTCTGGCTGTC	
pri-miR-22 F	ATTTCAGGTCGTCCCATATGTC	Cloning of mouse *pri-miR-22*
pri-miR-22 R	GTCCCTCACCTTCCGGATGATAG	
pri-miR-199 F	CTCAGTCCTGGGCCTACTTTTTCCA	Cloning of mouse *pri-miR-199*
pri-miR-199 R	TGCCACGTCAGAAGAGTTCAG	
pri-miR-99 F	GGATTCCCAGCCTTTAAAATATTTAC	Cloning of mouse *pri-miR-99*
pri-miR-99 R	GGATTAAAAGATCCATGAAG	
pri-miR-21 F	GATATCGACTGTTGGCATTAAGCCCC	Cloning of mouse *pri-miR-21*
pri-miR-21 R	GACTTTCCAAGTCTCACAAG	
pri-miR-375 F	ACCGCGGTGCTCAGGTGAGAG	Cloning of mouse *pri-miR-375*
pri-miR-375 R	CAGAGACTGAGCACGGT	
pri-miR-101 F	TTTTGCCTCCATCCAGAAGTGC	Cloning of mouse *pri-miR-101*
pri-miR-101 R	GGAAGAGTGGTGAACACAGGA	
pri-miR-451 F	AGTCTGGGTACCCCACCTCCAGAG	Cloning of mouse *pri-miR-451*
pri-miR-451 R	GCACAGTGAAGAGGAAAATGTACCC	
pri-miR-194 F	AGGTACAGGCTAGGTCTTGTCC	Cloning of mouse *pri-miR-194*
pri-miR-194 R	AGCTCCGTGCTCCGTAGTCT	
pri-miR-30a F	GTGTTTGACACTTAGTAGATGA	Cloning of mouse *pri-miR-30a*
pri-miR-30a R	AATATATTTCTTTGCTTAGC	
pri-miR-155 F	TTTCTCTTTGCAGGTGCTGC	Cloning of mouse *pri-miR-155*

pri-miR-155 R	GTCTGACATCTACGTTCATC	
shApob	CTGACTTTCATCTGTACTACATTCAAGAGATGTAGTACAG ATGAAAGTCAGTTTTT	Synthetic oligo for silencing *Apob* gene
shPC1	CGGGATTCTACCAGATATCTATTCAAGAGATAGATATCTG GTAGAATCCCGTTTTT	Synthetic oligo for silencing *PC1* gene
shSOD1	CATCATCAATTTCGAGCAGAATTCAAGAGATTCTGCTCGA AATTGATGATGTTTTT	Synthetic oligo for silencing SOD1 gene
shFluc	TTGACAAATACGATTTATCTATTCAAGAGATAGATAAATC GTATTTGTCAATTTTT	Synthetic oligo for silencing *Fluc* gene
Apob sensor-F	CGCCTCGAGAAATTGAAGAAGATCTGTTAAC	To generate partial *Apob* CDS as shApob sensor element
Apob sensor-R	CGCGCGGCCGCTCTTCTCTGGAGGGGACTGT	
PC1 sensor-F	CGCCTCGAGCCCAAAATGAATGCTTCTTTCTCG	To generate partial *PC1* CDS as shPC1 sensor element
PC1 sensor-R	CGCGCGGCCGCCCTGAAGAATCTGGTTCTTC	
hSOD1 sensor-F	ATAACTCGAGCGAAGGCCGTGTGCGTGCTGAAGGGC	To generate partial *hSOD1* CDS as shSOD1 sensor element
hSOD1 sensor-R	GCCAGCGGCCGCTTGGGCGATCCCAATTACACCACAAG	
shApob^{miR-33} gBlock	AGATCTAGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCT CCCTTGGGCCTGGGCCCACTGACAGCCCTGGTGCCTCTGG CCGGCTGCACACCTCCTGGCGGGCAGCTGTGTGTAGTAC AGATGAAAGTCAGTGTTCTGGCAATACCTGCTGACTTTAC TATGTACTACACACGGAGGCCTGCCCTGACTGCCCACGGT GCCGTGGCCAAAGAGGATCTAAGGGCACCGCTGAGGGCC TACCTAACCATCGTGGGGAATAAGGACAGTGTCACCCTTT TTCTGCAG	To generate *shApob^{miR-33}*
shPC1^{miR-33} gBlock	AGATCTAGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCT CCCTTGGGCCTGGGCCCACTGACAGCCCTGGTGCCTCTGG CCGGCTGCACACCTCCTGGCGGGCAGCTGTGTAGATATCT GGTAGAATCCCGTGTTCTGGCAATACCTGCGGGATTCGCC AAGATCTCTACACGGAGGCCTGCCCTGACTGCCCACGGT GCCGTGGCCAAAGAGGATCTAAGGGCACCGCTGAGGGCC TACCTAACCATCGTGGGGAATAAGGACAGTGTCACCCTTT TTCTGCAG	To generate *shPC1^{miR-33}*
shSOD1^{miR-33} gBlock	AGATCTAGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCT CCCTTGGGCCTGGGCCCACTGACAGCCCTGGTGCCTCTGG CCGGCTGCACACCTCCTGGCGGGCAGCTGTGTTCTGCTCG AAATTGATGATGTGTTCTGGCAATACCTGCATCATCATAT CCGAGCAGAACACGGAGGCCTGCCCTGACTGCCCACGGT GCCGTGGCCAAAGAGGATCTAAGGGCACCGCTGAGGGCC TACCTAACCATCGTGGGGAATAAGGACAGTGTCACCCTTT TTCTGCAG	To generate *shSOD1^{miR-33}*
shFluc^{miR-33} gBlock	AGATCTAGGGCTCTGCGTTTGCTCCAGGTAGTCCGCTGCT CCCTTGGGCCTGGGCCCACTGACAGCCCTGGTGCCTCTGG CCGGCTGCACACCTCCTGGCGGGCAGCTGTGTAGATAAA TCGTATTTGTCAATGTTCTGGCAATACCTGTTGACAAAAT CAATTTATCTACACGGAGGCCTGCCCTGACTGCCCACGGT GCCGTGGCCAAAGAGGATCTAAGGGCACCGCTGAGGGCC TACCTAACCATCGTGGGGAATAAGGACAGTGTCACCCTTT TTCTGCAG	To generate *shFluc^{miR-33}*
Apob-F	GTCCAGGTTGAATCACGGGT	qRT-PCR for *apob* mRNA
Apob-R	AGGATCCTGCAAGGTCAAGC	

PC1-F	AAAGGCCGCTGCTTTGAAAG	qRT-PCR for *pc1* mRNA
PC1-R	CCGCACCTGAATTTGTTGCA	
Actin-F	ATGCCAACACAGTGCTGTCTGG	qRT-PCR for *actin* mRNA
Actin-R	TGCTTGCTGATCCACATCTGCT	
Egfp-F	AGCAAAGACCCCAACGAGAA	Quantification of AAV genome copies in liver and AAV vector preparations
Egfp-R	GGCGGCGGTCACGAA	
Egfp-probe	6FAM-CGCGATCACATGGTCCTGCTGG-TAMRA	
shApob and shApob^{miR-33} AS probe; standard for *shApob sense*	GACTTTCATCTGTACTACA	Probe and standard for small RNA Northern blot
shPC-1 and shPC-1^{miR-33} AS probe; standard for *shPC-1 sense*	CGGGATTCTACCAGATATCTA	
shSOD-1 and shSOD-1^{miR-33} AS probe; standard for *shSOD-1 sense*	CATCATCAATTTCGAGCAGAA	
shApob^{miR-33} sense probe	GTGTGTAGTACATAGTAAAGTC	
shPC-1^{miR-33} sense probe	GTGTAGATATCTTGGCGAATCC	
shSOD-1^{miR-33} sense probe	GTGTTCTGCTCGGATATGATGA	
shApob^{miR-33} sense reference	GACTTTACTATGTACTACACAC	
shPC-1^{miR-33} sense reference	GGATTCGCCAAGATATCTACAC	
shSOD-1^{miR-33} sense reference	TCATCATATCCGAGCAGAACAC	
shApob sense probe; standard for *shApob and shApob^{miR-33} AS*	TGTAGTACAGATGAAAGTCAG	
shPC-1 sense probe; standard for *shPC-1 and shPC-1^{miR-33} AS*	TAGATATCTGGTAGAATCCCG	
shSOD-1 sense probe; standard for *shSOD-1 and shSOD-1^{miR-33} AS*	TTCTGCTCGAAATTGATGATG	
U6 probe	CTCTGTATCGTTCCAATTTTAGTATA	

Supplementary Table 2: Decreased mRNAs in AAV-*shPC1* treated mice

Seed type	Guide	Guide+1	Guide+2	Guide+3	Guide+4
7mer	283	204	250	269	413
7mer-8m	131	107	192	163	241
7mer-A1	124	122	82	175	146
8mer	62	103	134	164	181
No Target	615	679	557	444	234
Sum	1215	1215	1215	1215	1215

Supplementary Table 3: Decreased mRNAs in AAV-*shPC1*$^{miR\text{-}33}$-treated mice

Seed type	Guide	Guide+1	Guide+2	Guide+3
7mer	18	15	17	16
7mer-8m	10	6	7	12
7mer-A1	8	3	6	3
8mer	6	9	13	8
Not Target	51	60	50	54
Sum	93	93	93	93

Supplementary Table 4: Dysregulated genes in AAV-*shPC1*$^{miR\text{-}33}$-treated mice and PC-1 loss-of-function patients

	Increased genes			Decreased genes	
	PC-1 loss-of-function patients vs healthy controls (fold)	AAV-*shPC1*$^{miR\text{-}33}$ vs AAV-*Egfp* treated mice (fold)		PC-1 loss-of-function patients vs healthy controls (fold)	AAV-*shPC1*$^{miR\text{-}33}$ vs AAV-*Egfp* treated mice (fold)
Ak1	5.4	2.7	*Arid5b*	0.11	0.71
Anp32b	11.3	2.4	*Fasn*	0.07	0.58
Hist1h1c	29.3	1.5	*Hmgcr*	< 0.01	0.59
Lrrc42	9	1.7	*Lss*	< 0.01	0.70
Map7d1	3.1	1.5	*Mme*	0.03	0.64
Mgat4b	7.4	1.6	*Tfrc*	0.23	0.66
Pgp	19.3	1.8	*Cers6*	0.03	0.68
Arhgef1	8.1	1.6	*Myc*	0.19	0.53
Col1a1	54.8	1.7	*Nt5e*	0.04	0.55
Gigyf1	2.7	1.6			
Ncor2	4.7	1.8			
Wdfy1	3.4	1.8			