SUPPLEMENTARY MATERIAL

SYPPLEMENTARY FIGURE LEGENDS

Figure S1. *Phylogenic studies of the miR-183/96/182 cluster and 3'-UTR of Casp2*. **(A)** Genomic arrangement of the miR-183/96/182 cluster in vertebrates. Numbers under the x-axis represent relative locations on the chromosome in base pairs. The y-axis shows different species. (+) means transcription from centromere to telomere; (-) means transcription from telomere to centromere. **(B)** 3'-UTRs of *Casp2* are highly conserved in mammals. Letters in purple represent the seed regions for miR-183; letters in red indicate the seed regions for miR-96 and miR-182. Letters in the red box denote the conserved region for the miR-183/96/182 cluster.

Figure S2. *Creation of the miR-183/96/182 cluster sponge construct.* **(A) a.** Schematic representation of the sponge construct: Ten copies of binding sites for each miR-183/96/182 cluster miR were inserted into the 3'-UTR of EGFP. The construct under control of a mouse opsin promoter ended with a SV40 polyadenylation signal. The whole fragment containing 6.8 kb was cut out by NotI/MluI. The SpongeFor/SpongeRev primer pair was designed for genotyping analysis and the BindingFor/BindingRev primer pair was included to check expression of the sponge element. Binding sequences of miR-183, miR-96 and miR-182 are shown in **b**. **(B)** Testing for the mouse *Opsin* promoter. Only Y79 cells transfected with the sponge construct showed a bright EGFP signal. Phase contrast images are shown on the right. Scale bars: 20 μm.

Figure S3. *Test of cluster probe specificities.* There were no cross-reactions between different probes.

Figure S4. *Creation of miR-183/96/182 stable cell lines.* **(A)** Inserted partial pri-miR sequences of miR-183/96/182 cluster miRs. Letters in purple indicate mature miR sequences; letters in red represent the remaining regions of pre-miR sequences; letters in blue denote cloning and detecting primer sequences. **(B)** Confirmation of miR expression levels in stable cell lines. Note that **a** demonstrates the specific pri-miR expression of target miRs detected by RT-PCR in different stable cell lines, whereas **b** shows specific mature miR expression in different cell lines by splinted ligation.

Figure S5. *Creation of target luciferase reporters.* **(A)** Schematic representation of luciferase reporter constructs with four copies of the targeted regions from each target gene's 3'-UTR. The targeted regions in target genes' 3-UTRs are listed; letters in green represent the seed regions, letters in red denote the complementary nucleotides. **(B)** Testing the linear range of genetically engineered luciferase reporter constructs. From 100-200 ng was found to be in the linear range for all plasmid constructs and the empty pGL3P plasmid whereas 20-50 ng was in the linear range for the *Renilla* plasmid.

Figure S6. *Supplementary data for the establishment of miR-183/96/182 sponge transgenic mouse cell lines.* **(A)** A standard curve of sponge element DNA is shown with Ct-values derived from quantitative real-time PCR. The slope (efficiency), R^2 (coefficients) and y-intercept (sensitivity) are listed. A transgene size of 1 kb was estimated as 0.17 picograms of transgene per microgram of mouse genomic DNA. **(B)** Tissue specificity of sponge component expression. The opsin promoter mRNA of sponge component constructs was detected only in retina and not in brain, heart, liver or intestine by semiquantitative RT-PCR. RT-minus [RT (-)] and *Gapdh* reactions were used as negative and positive controls. Amplification cycle numbers are indicated on the right. **(C)** Statistical analysis of mature miRs in different transgenic mouse lines. Error bars represent the standard errors of the means (n=3), * $p<0.05$. **(D)** Immunoblotting results show that *Opsin*

expression levels were not affected by the exogenous mouse *Opsin* promoter in all three transgenic mouse lines. β-Tubulin was used as the internal control.

Figure S7. *Casp2 expression levels in 4-month-old wt mouse retina under different light intensity exposure.* Immunoblotting data show that both pro-Casp2 and cleaved-Casp2 were increased after 5,000 and 10,000 lux light intensity exposure for 30 min. β-Tubulin was used as the internal control.

SUPPLEMENTARY TABLES

Table S1. Primer Sets for Real Time PCR.[1]

Name	mRNA	Forward	Reverse
Neurod4	NM_007501	AGACCGGGCTCTTATGGAAT	GAATCTTTCAAGGCGAGCTT
Hes1	NM_008235	GGCCTCTGAGCACAGAAAGT	GCCGGGAGCTATCTTTCTTA
Dcx	NM_001110222	CAGAAGCGATCAAACTGGAA	GGACCACAAGCAATGAACAC
Ncald	NM_001170866	TGGGACTGCACACATTGAA	ACTCCTGGATCTCGTGCTCT
Rarg	NM_001042727	TCATCTGTGGAGACCGAATG	CCTTGGGAACATGTAGGGTT
Gnai3	NM_010306	TTGATGTAGGTGGCCAAAGA	ATGCATTCGGTTCATTTCCT
Clock	NM_007715	GGCACCACCAATAATAGGCT	TGCATTAAGTGCTCGTGACA
Bhlhe22	NM_021560	GGTTTAGTGCGGATGAAGTG	TTTCGCCTCCAAAGAAGACT
Casp2	NM_007610	GCACAGGAAATGCAAGAGAA	CTTGGAGCTGAAGCAGTTTG
Bcl2	NM_009741	GAGTACCTGAACCGGCATCT	GAAATCAAACAGAGGTCGCA
Arrdc3	NM_001042591	TCTGGGAAGACAGAGACGTG	ATCCACGTACACCATTAGCG
Binding		TCCTGCTGGAGTTCGTGAC	CGTCCCCGGGTTTAAACTA
Pri-miR96		GGGCCATAAACAGAGCAGAG	GGCAGTGAAAGGTGATCTGG
Pri-miR182		TACAGGCCGAAGGACCATAG	GGCGCAGGGAAACATTAAG
Pri-miR183		CTGGTGAGGAGGGTTGCTAA	GTACTGGAACAGGCCCTCTG
Gapdh	NM_008084	GTGTTCCTACCCCCAATGTG	GGAGACAACCTGGTCCTCAG

[1]Primer sets used in this research. The first column shows the identities of the primer sets; the second column indicates the corresponding accession number; and the third and fourth columns present the sequences of the forward and reverse primers, respectively.

Table S2. Oligonucleotides used for Splinted Ligation.[1]

Name	Sequence
Bridge-mmu-miR-96	GAATGTCATAAGCGAGCAAAAATGTGCTAGTGCCAAA
Bridge-mmu-miR-182	GAATGTCATAAGCGCGGTGTGAGTTCTACCATTGCCAAA
Bridge-mmu-miR-183	GAATGTCATAAGCGAGTGAATTCTACCAGTGCCATA
Bridge-mmu-miR-125a	GAATGTCATAAGCGTCACAGGTTAAAGGGTCTCAGGGA
Bridge-mmu-Let-7	GAATGTCATAAGCGAACTATACAACCTACTACCTCA
Bridge-mmu-miR-107	GAATGTCATAAGCGTGATAGCCCTGTACAATGCTGCT
Mimic-mmu-miR-96	TTTGGCACTAGCACATTTTTGCT
Mimic-mmu-miR-182	TTTGGCAATGGTAGAACTCACACCG
Mimic-mmu-miR-183	TATGGCACTGGTAGAATTCACT

[1]Oligonucleotides used for splinted ligation. Bridge oligonucleotides were used to hybridize the detection probe and target miRs; mimic oligonucleotides simulating the endogenous miR sequences were used as positive controls.

Table S3. Statistical analysis of Figure 3Cb.[1]

	wt			**transgene**		
	0 min	**10 min**	**30 min**	**0 min**	**10 min**	**30 min**
rod *Opsin*	1.0±0.1	0.9±0.1	0.9±0.1	1.0±0.1	0.7±0.1*	0.4±0.0*
Mw-opsin	1.0±0.1	0.9±0.1	0.8±0.1	1.0±0.1	0.8±0.1	0.7±0.1*

[1]Immunoblotting data were quantified by Image J. Both *Opsin* and *Mw-opsin* levels were normalized by *Tubulin*. Data represent means±S.D. (n=3), * $p<0.05$.

Table S4. Statistical analysis of Figure 5Cb.[1]

	NIH3T3	miR-96	miR-182	miR-183
pro-*Casp2*	1.0±0.0	0.4±0.1*	0.5±0.1*	1.1±0.1
cleaved-*Casp2*	1.0±0.0	0.6±0.2*	0.5±0.2*	1.0±0.2

[1]Immunoblotting data were quantified by Image J. Both pro-*Casp2* and cleaved-*Casp2* levels were normalized by *Tubulin*. Data represent mean±S.D. (n=3), * $p<0.05$.

Table S5. Statistical analysis of Figure 6Ab.[1]

	0 h	1 h	3 h	6 h	12 h	24 h	72 h
miR-96	1.0±0.0	0.9±0.3	1.2±0.2	1.5±0.3	1.7±0.2*	2.5±0.4*	1.1±0.2
miR-182	1.0±0.0	1.0±0.2	1.1±0.2	1.3±0.2	1.2±0.4	1.3±0.4	1.1±0.1
miR-183	1.0±0.0	1.2±0.1	1.3±0.2	1.6±0.2	1.6±0.1*	2.1±0.1*	1.0±0.0

[1]Splinted ligation data were quantified by Image J. miR-96, miR-182 and miR-183 levels were normalized by Let-7. Data represent means±S.D. (n=3), * $p<0.05$.

Table S6. Statistical analysis of Figure 6Bb.[1]

	wt		**transgene**	
	not treated	**light-induced**	**not treated**	**light-induced**
pro-*Casp2*	1.0±0.0	1.2±0.2	1.0±0.4	1.3±0.4
cleaved-*Casp2*	1.0±0.0	1.4±0.4	1.1±0.4	3.0±0.3*
Bid	1.0±0.0	1.0±0.2	1.0±0.4	0.9±0.3
tBid	1.0±0.0	1.8±0.5*	1.6±0.2*	3.3±0.2*

[1]Immunoblotting data were quantified by Image J. All protein levels were normalized by *Tubulin*. Data represent means±S.D. (n=3), * $p<0.05$.

Table S7. Statistical analysis of Figure S7.[1]

	0 lux	500 lux	1,000 lux	2,500 lux	5,000 lux	10,000 lux
pro-*Casp2*	1.0±0.2	1.2±0.3	1.3±0.3	1.2±0.3	1.8±0.3*	1.6±0.4
cleaved-*Casp2*	1.0±0.2	1.1±0.2	1.5±0.3	1.4±0.2	2.4±0.3*	2.0±0.3*

[1]Immunoblotting data were quantified by Image J. Both pro-*Casp2* and cleaved-*Casp2* levels were normalized by *Tubulin*. Data represent means±S.D. (n=3), * $p<0.05$.

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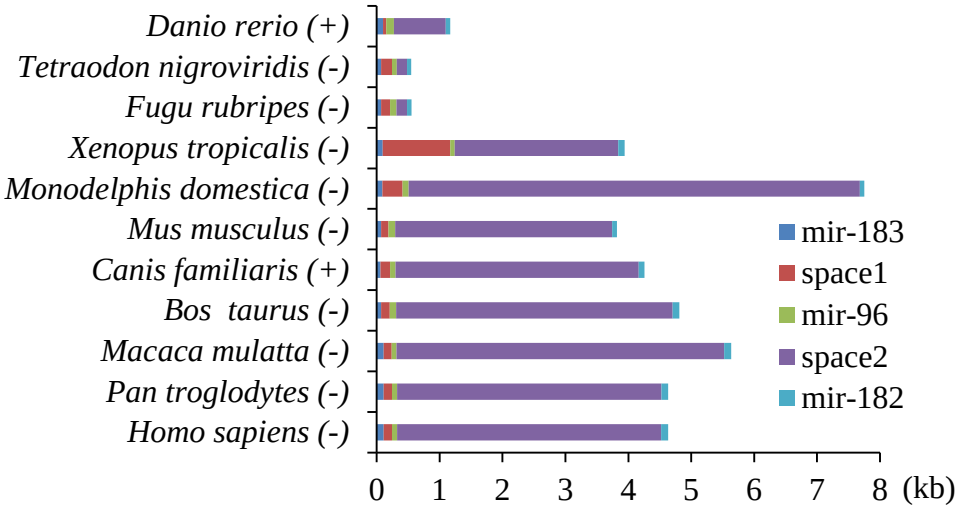

B

```
Homo_sapiens        AGAGAACCTTTCCACT-CCCACTGCCAGGATTTTGTATTGCCATCGGGTGCCAAATAAAT
Pan_troglodytes     AGAGAACCTTTCCACT-CCCACTGCCAGGATTTTGTATTGCCATCGGGTGCCAAATAAAT
Pongo_pygmaeus      CGAGAATCTTTCCACT-CCCACTGCCAGGATTTTGTATTGCCATCAGGTGCCAAATAAAT
Rattus_norvegicu    GTAGACTCTTCACACTTCCCACTGCCAAGATTTTGTATTGCCATCAGGTGCCAAATAAAT
Mus_musculus        GTAGAATCTTCACACTTCCCACTGCCAAGATTTTGTATTGCCATCAGGTGCCAAATAAAT
Monodelphis_dome    TGAGCCTCTCCCCACTCCCCACTGCCAGGGTTTTGTATTGTTTTCAGGTGCCAAATAAAT
Bos_taurus          ATAGAACCTTTCCAGTTCCCACTGCCAGGATTTTGTATTGCCATCTGGTGCCAAATAAAT
Canis_familiaris    ATAGAACCTTTCCACTTCCCACTGCCAGGATTTTACATTGCCTTCAGGTGCCAAATAAAT
Equus_caballus      ATAGAACCTTTCCACTTCCCACTGCCAGGATTTTGTATTGCCATCAGGTGCCAAATAAAT
                      **   **   ** * ********** * ****  ****   ** **************
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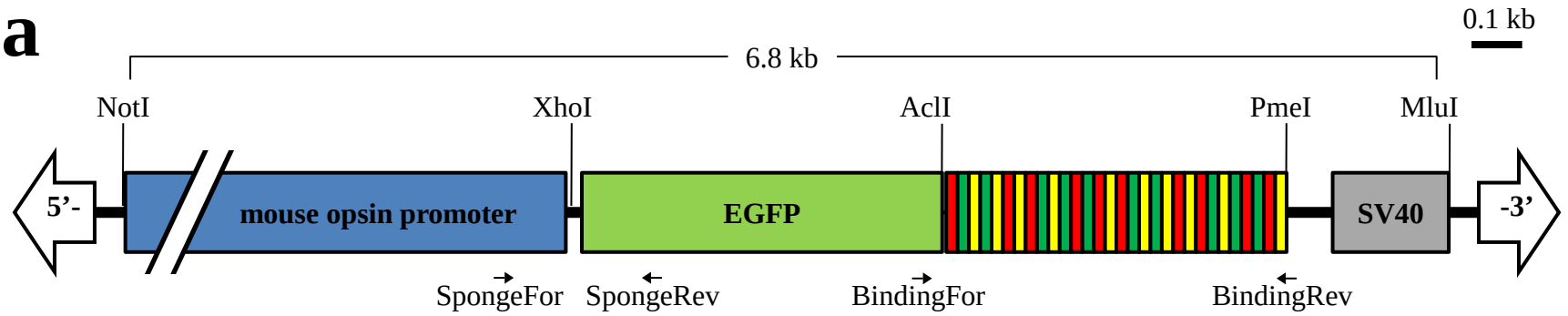
Figure S1

A

a



b

miR-183 binding sequence:

```
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UTR  5'-AGTGAATTCT      AGTGCCATA
miR     UCACUUAAGA      UCACGGUAU-5'
                UGG
```

miR-96 binding sequence:

```
                ACG
UTR  5'-AGCAAAAATG      AGTGCCAAA
miR     UCGUUUUUAC      UCACGGUUU-5'
                ACGA
```

miR-182 binding sequence:

```
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UTR  5'-CGGTGTGAGTT      ATTGCCAAA
miR     GCCACACUCAA      UAACGGUUU-5'
                GAUGG
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B

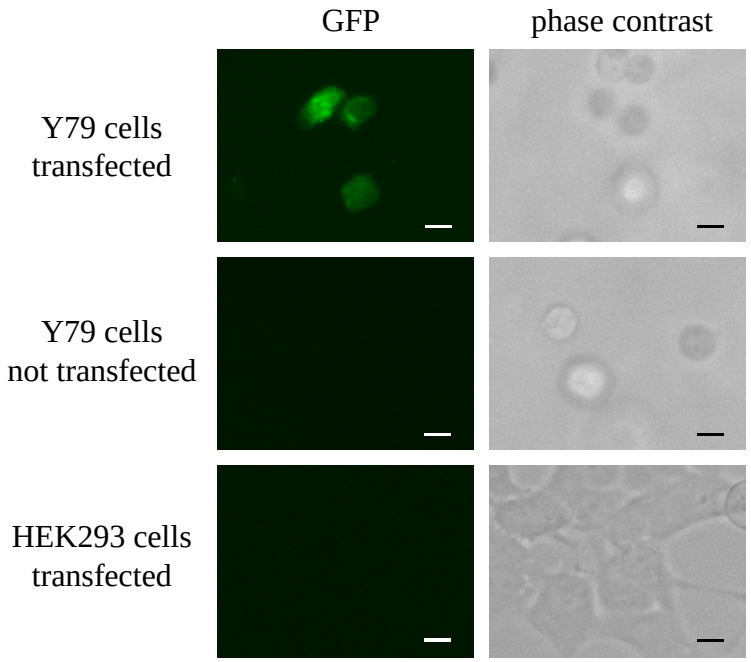

Figure S2

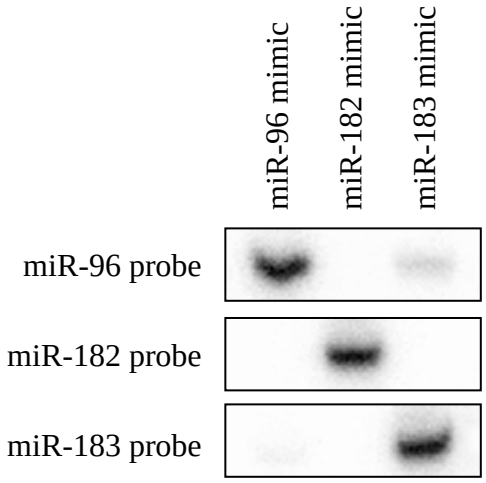
miR-96 mimic
miR-182 mimic
miR-183 mimic
miR-96 probe
miR-182 probe
miR-183 probe

Figure S3

A

Mmu-miR-96 pri-miR

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CCTTCCCCAGAGGGCCTGTTCCAGTACCATCTGCTTGGCCGATTTTGGCACTAGCACATTTTTGCTTGTGTCTCTCCGCTGTGAGCAATCATGTGTAGTGCCAATATGGGA
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ACCCTGGTGAACAGCCAGATCACCTTTCACTGCC

Mmu-miR-182 pri-miR

TACAGGCCGAAGGACCATAGTCTGGACCTTGTGTTAACTGTGGGAAGAGCGCCCTCCTAAAACCACCCTAACTGCTTCTTCTTCAGCATAGGCTTACTGGTCTGGCTGCT
GGAGGCCTCCCACCATTTTTGGCAATGGTAGAACTCACACCGGTAAGGTAATGGGACCCGGTGGTTCTAGACTTGCCAACTATGGTGTAAGTGCTGAGCTGCTGAAGGT
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TTCCCTGCGCC

Mmu-miR-183 pri-miR

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B

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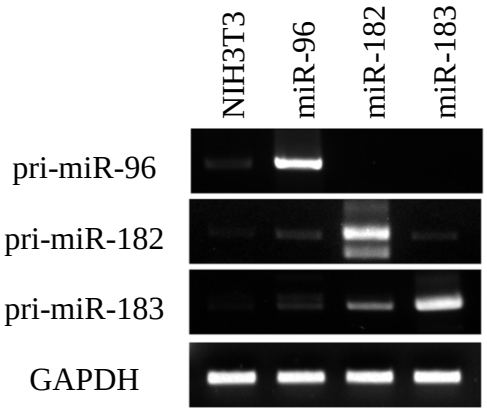

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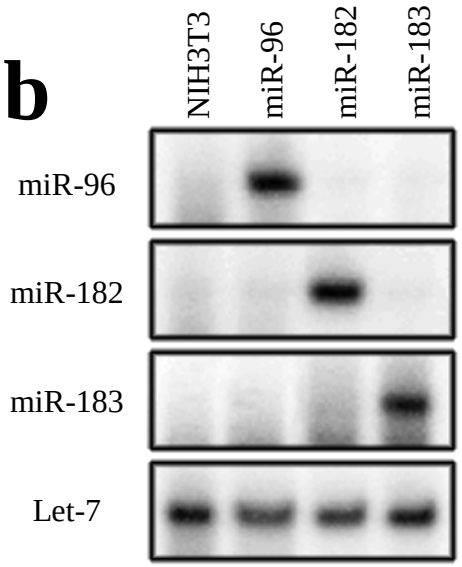

Figure S4

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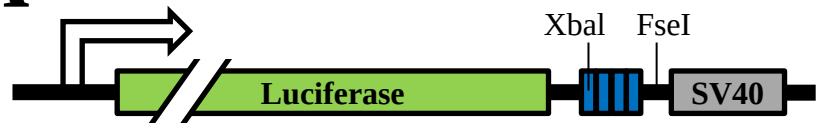


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miR-96          UCGUUUUUACACGAUC-ACGGUUU
miR-182       GCCACACUCAAGAUGGUAACGGUUU
miR-183         UCACUUAAGAUGGUCACGGUAU

Neurod4-3-UTR CAGGCCCTGTTCCAATGGTGCCAAA
miR-96          UCGUUUUUACACGAUCACGGUUU
miR-182       GCCACACUCAAGAUGGUAACGGUUU
miR-183         UCACUUAAGAUGGUCACGGUAU

Casp2-3-UTR   TTTTGTATTGCCATCAGGTGCCAAA
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B

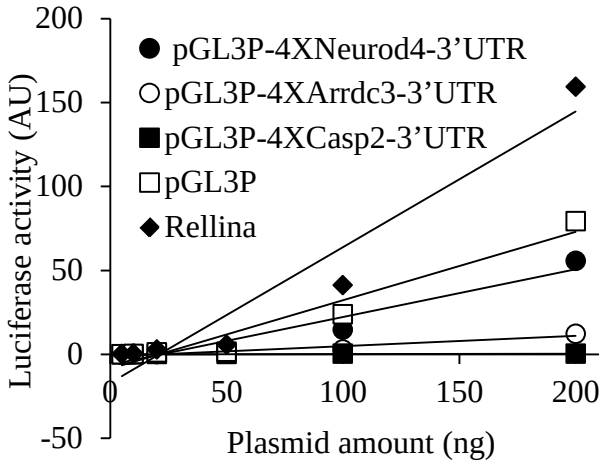
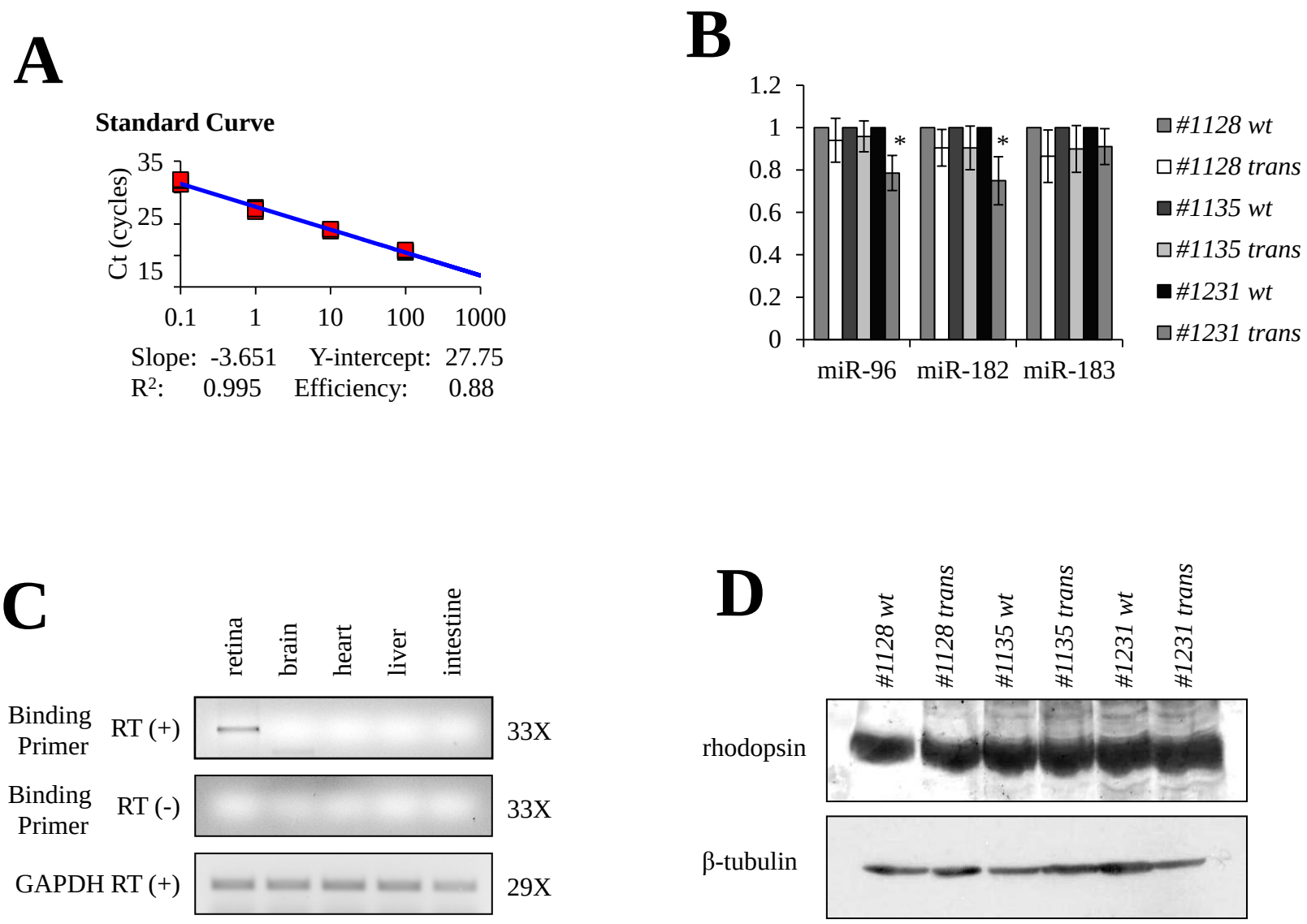


Figure S5


A
Standard Curve
Ct (cycles)
35
25
15
0.1
1
10
100
1000
Slope: -3.651 Y-intercept: 27.75
R²: 0.995 Efficiency: 0.88
B
1.2
1
0.8
0.6
0.4
0.2
0
*
*
miR-96
miR-182
miR-183
#1128 wt
#1128 trans
#1135 wt
#1135 trans
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#1231 trans
C
retina
brain
heart
liver
intestine
Binding Primer
RT (+)
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Binding Primer
RT (-)
33X
GAPDH RT (+)
29X
GAPDH RT (-)
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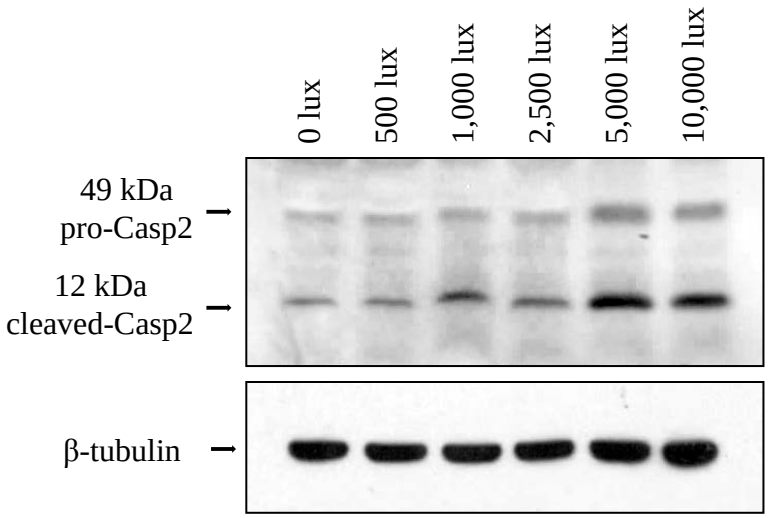
rhodopsin
β-tubulin

Figure S6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500 lux
1,000 lux
2,500 lux
5,000 lux
10,000 lux
49 kDa
pro-Casp2
12 kDa
cleaved-Casp2
β-tubulin

Figure S7