Supplemental Information

The Circular RNome of

Developmental Retina in Mice

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Supplementary Figure 1. circRNA expression analysis at E18.5 vs. P1, P1 vs. P7, P7 vs. P14, P14 vs. P30.
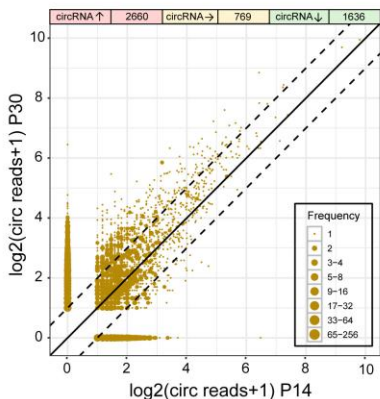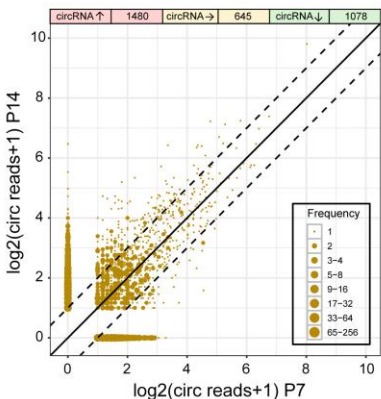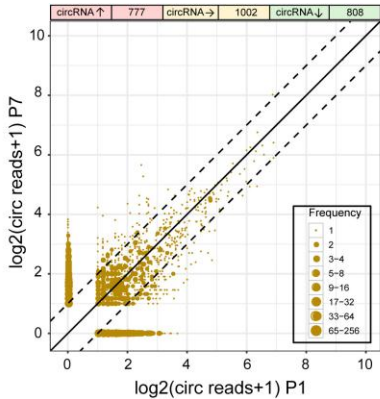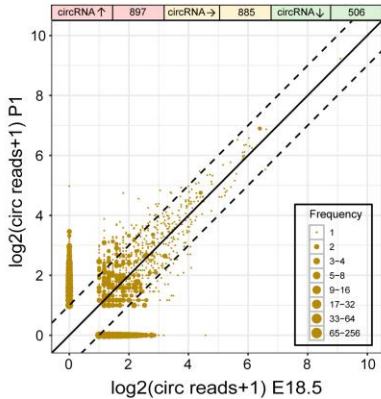
Supplementary Figure 2. Head to tail junction sequences of circRNAs.

mmu_circ_0001878 |Cdr1as

mmu_circ_0001757|Bbs9

mmu_circ_0001468|Hipk2

mmu_circ_0001692|Arhgap10
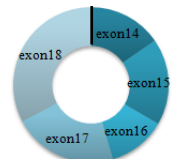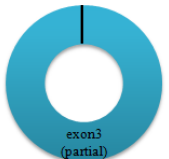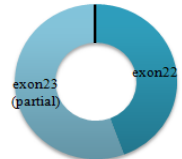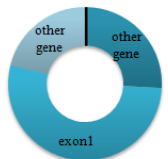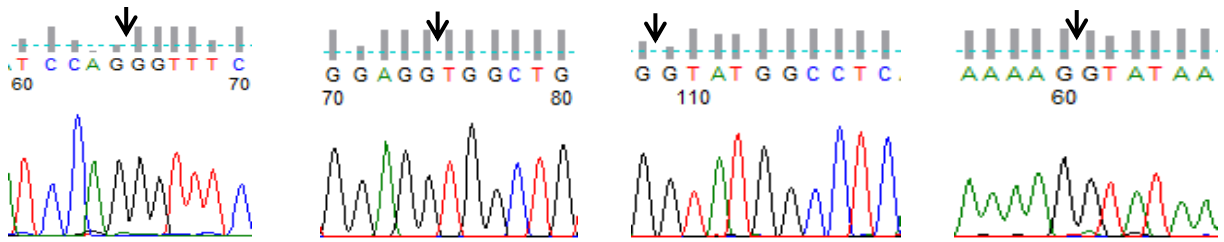
mmu_circ_0000377|Arhgap5

mmu_circ_0001311|Ankib1

mmu_circ_0000723|Tulp4

mmu_circ_0001624|Mettl9
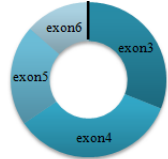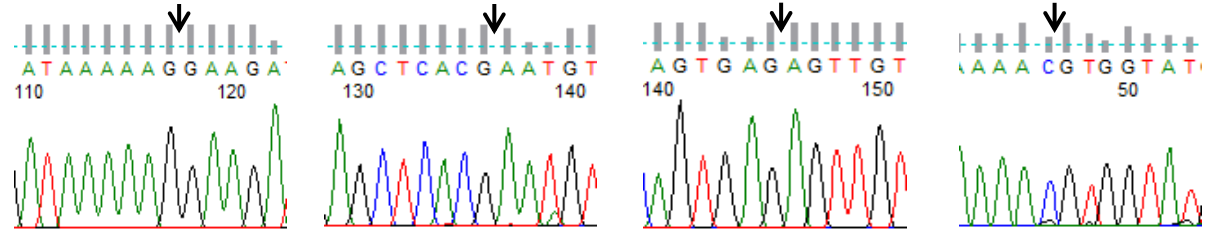
Supplementary Figure 3. Comparsion the results between RNA-seq and qRT-PCR.
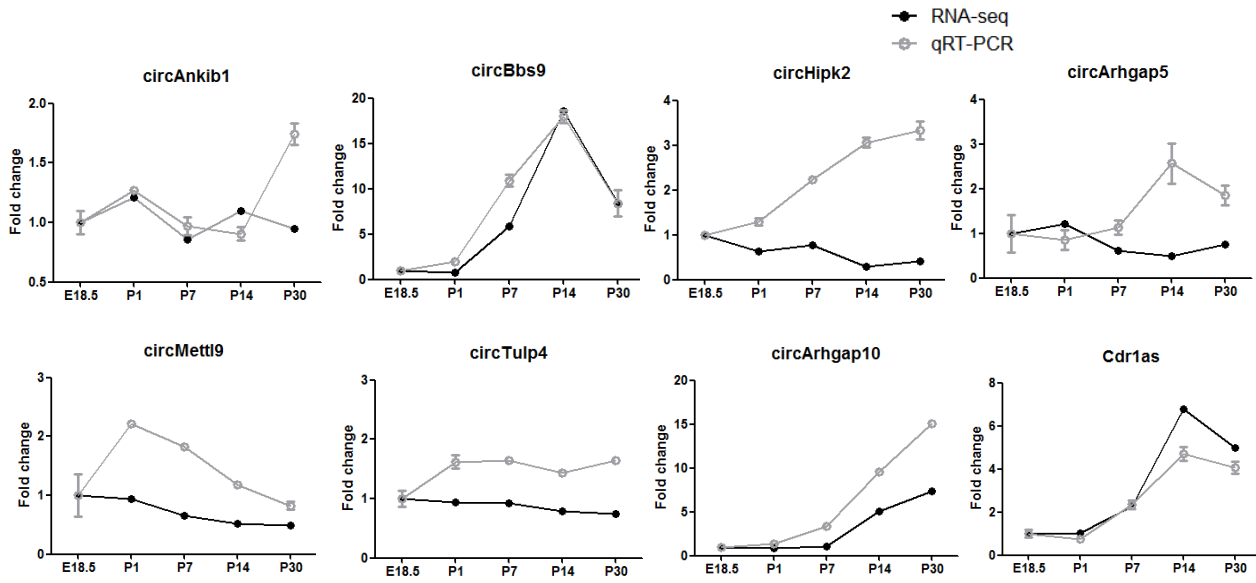
Supplementary Figure 4. (A) circRNA expression change between P7 and P1 are positively correlated with host genes expression changes. (B) Summary of expression changes between circRNA and host gene at P7 vs. P1. (C) circRNA expression change between P14 and P1 are positively correlated with host genes expression changes. (D) Summary of expression changes between circRNA and host gene at P14 vs. P1.
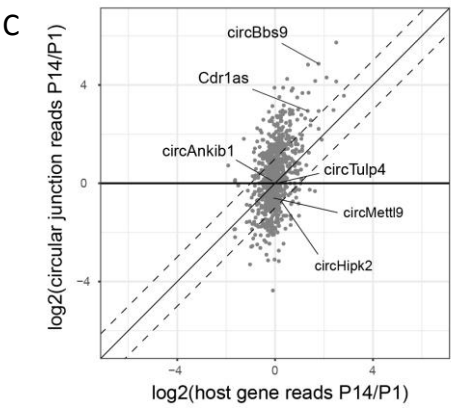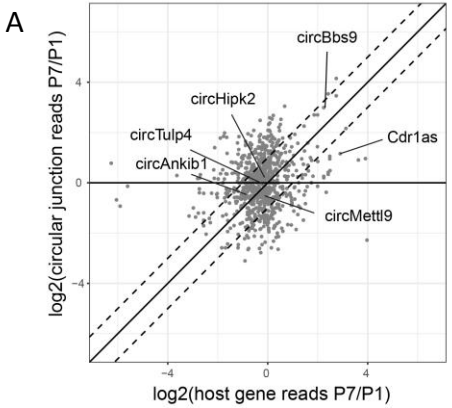
B P7 vs. P1

	mRNA ↑	mRNA →	mRNA ↓
circRNA ↑	235	733	61
circRNA →	67	414	37
circRNA ↓	113	705	153

D P14 vs. P1

	mRNA ↑	mRNA →	mRNA ↓
circRNA ↑	115	833	17
circRNA →	7	451	8
circRNA ↓	22	1442	295

Supplementary Figure 5. luciferase assay detection the binding between miRNAs and circAnkib1 (A), circBbs9 (B)and circMettl9 (C) in 293D cell.
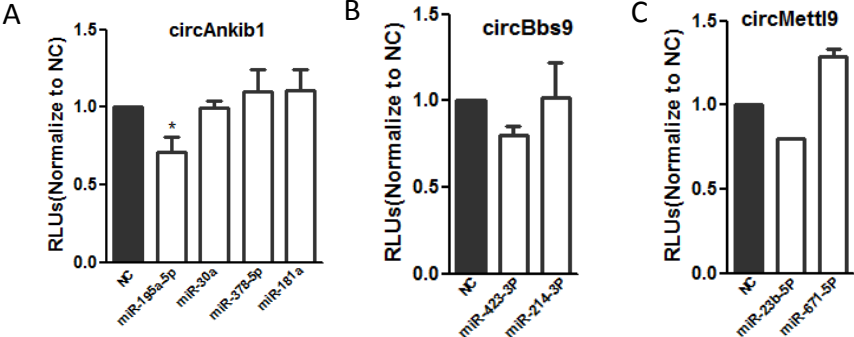
Supplementary Figure 6. The target sites of miRNAs in corresponding circRNAs

miR-204-5p UCCGUAUCCUACUG**UUUCCCUU**
......GGGTGGCTTCAGAGGGAA.......

UCGGAUAGGACCUAA**UGAACUU**   miR-26a-5p
......TCTCTGCACTTGAACTATTG......
......TTAAGTTACTTGAAATCTAT......
.....GCTAAGAACTTGAAAATGAG......

miR-671-3p CCACCUCGGGACU**CUUGGCCU**
......AACTCTGAACCGGAGCTCA......

UCCGUAUCCUACUG**UUUCCCUU** miR-204-5p
......GGCGCTACTATGAAGAGGGAT......

circTulp4

1162
1213
1266
1378
362
1 1946
1806

circHipk2

miR-124-3p CCGTAAGTGGCG**CACGG**AAT
......ACCCTTCAATCAAGTGCCTTC......

36
1 1084

Cdr1as

954
820
2142
2505
1 2927

mmu-miR-204-3p:UGCAGGGAAACGG**AAGGGUC**G
820-2505:   43 binding site: ......TTCCCAGA......

mmu-miR-378-3p: AGAAGACUGAG**GUUCAGGU**C
954-2142:11   binding site: ......AAGTCCA......

Ankib1

miR-195a-5p CGGUUAUAAAGA**CACGACGAU**
......TTACACTATGCTGCTAGACA......

178
1 873

Supplementary Figure 7. qRT-PCR identify the interference efficiency of si-circTulp4 in N2a cell.

A
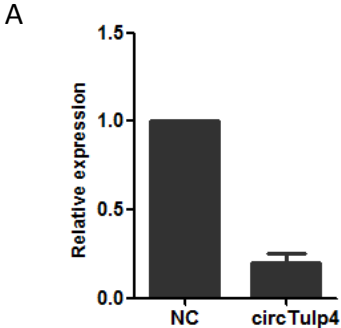
Supplementary Figure 8. Representative ERG response recording of retina after AAV-circTulp4 shRNA and AAV-EGFP subretinal injection. Representative ERG curve that consisted of a negative a-wave and a positive b-wave; the distance between dashed lines labeled by a or b is the amplitude of the a and b wave, respectively.
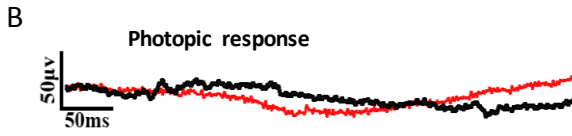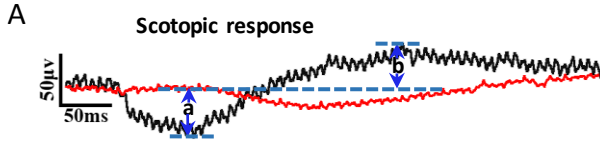
Supplementary Figure 9. qRT-PCR detection of miR-204-5p, miR-26a-5p (a), miR-204-5p and miR-26a-5p target genes (b) and target s of Meis2 (C) in mouse retina after AAV-circTulp4 shRNA and AAV-EGFP subretinal injection.
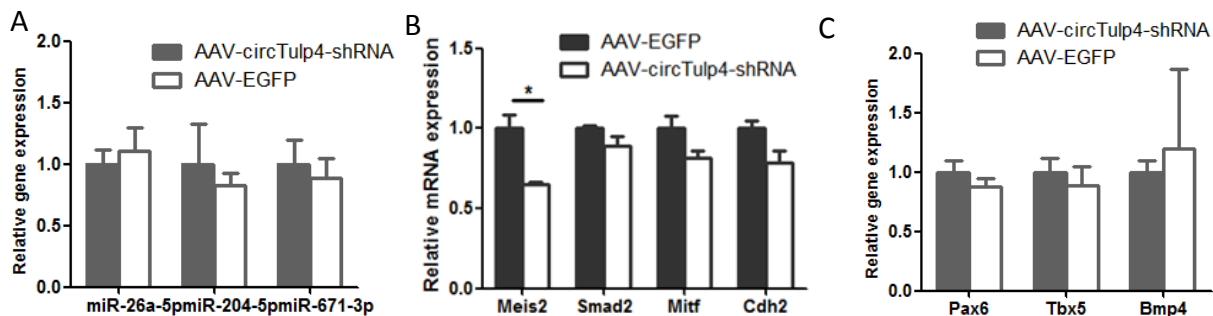
Supplementary Figure 10. Negative control DAPB of ISH in mouse retina.
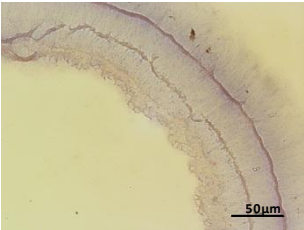
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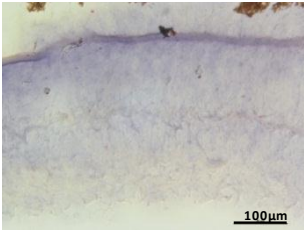
40X

Supplementary Table 1. The replicates overlap of circRNAs across the five stages.

	E18.5	P1	P7	P14	P30
E18.5	301	736	647	705	802
P1	736	452	813	862	983
P7	647	813	384	929	1078
P14	705	862	929	697	1543
P30	802	983	1078	1543	2147

Supplementary Table 2. Primers used in QPCR.

	Forward	Reverse
mmu_circ_0001624	GGTCATCCTGGCATTGGT	GCCAGCCAGATTTCTCAATG
mmu_circ_0001468	TATCCATGCTGACCTCAAACC	CACACTACAGAAGGCACTTGA
mmu_circ_0000723	CCCAAGAGTGAGAAGGAGAAAC	CGGTTAATTCAGGAGCCATCA
mmu_circ_0001311	CCGAAGAAATAGAAGCGGAGTAT	GATGAGTGCTTTGCGGAATTT
mmu_circ_0001757	TGGAGTAATGCTAATGAGTTGAGG	GCTGAGACTTCAGGCATGG
mmu_circ_0000629	GGGTCCATGAGGGTTGTATT	CGAGGGCTGAACTCTTCTATG
mmu_circ_0000377	AGACATCGTGGAAGTGAAGAAG	GGGTCGAGGCTCTTTGTTT
mmu_circ_0001878	CGCAGTGTCTCCAGTGTATC	TGCAGTTGCTGGAAGACC
lin-Mettl9	GAAAACGTAGGTGGCAAGTGG	AGCTTCGACGACAAAGCCGGC
lin-Bbs9	ACCTGCAGTGCTCGTATC	GGGTACCTTGCAACTTTACATC
lin-Tulp4	TCGTCTCTGTTCCTACCTCTC	CAAAGTCTCGCATGTTGTTGG
lin-Ankib1	AGCACTTTCGATCTCCCAGAG	TGCAGAGCGACTGGCAGGGCT
lin-Arhgap5	TGACAGCAGACAACTTATCCATC	CCACAGTGTTCGCAGTTTCTA
lin-Hipk2	GGTCATTGACTTTGGTTC	CTTCACACTACAGAAGGC
Q-m-Mitf	CCTCTGAAGAGCAGCAGTTCT	CTCCCTGCGCTCCTGCTCCTG
Q-m-Meis2	GCAATCTATGGGCACCCGTTG	GGAACAGACGTCTCCGCCGGC
Q-m-Stat3	CAGTTTCTGCAGAGCAGGTAT	CTGGGCTGCCGTGGCTGCCGT
Q-m-Smad2	ACCATACCAAGGTCTCTTGAT	ATGATGACTGTGAAGGTCCGG
Q-m-Ulk2	GGTTCTCCAAGATCTGCAGTA	TCTTCGTCCTCCTGTCTGCAC
Q-m-Rd3	GCCATCCTCAGGTTCAGGCAG	GGTTCGGATGTCACTGGCAAA
Q-m-Pde4d	CGGAGCAAAAGTGCCTCTGAG	ACTTGAGTCTGCAGATGTGAC
Q-m-Bbs9	GGGAACGGCCACGATAAAATC	ATCTTCAGCCTGAGGCCCACC
Q-m-Rnf217	AGCTCTCAGGTACAACTTGGC	CTTGATAGAGTCCTCATGGGT
Q-m-Slc6a6	AATGGTGGAGGTGCGTTCCTC	ACAGATCTTCTCCCAGCAGGT
Q-m-GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

Supplementary Table 3. siRNA target sequence.

	sequence
si-circTulp4	ACAACAGTGAGAGTTGTAA
si-nc	CGCTGAGTACTTCGAAATGTC

Supplementary Table 4. The concentration and quality of RNA used for RNA-seq. RNAs were extracted with the TRIzol reagent and RNeasy mini kit (Qiagen).

Sample	Concentration (ng/g)	OD (260/280)	Quality (RIN)	Sample	Concentration (ng/g)	OD (260/280)	Quality (RIN)
E18.5-1	21.3	1.94	9.7	P7-2	3.6	1.94	10
E18.5-3	3.39	1.97	10	P14-1	4.48	1.88	9
P1-1	5.22	1.93	10	P14-3	3.41	1.97	8.4
P1-2	5.84	1.95	10	P30-2	3.3	1.87	8.6
P7-1	4.17	1.88	9.6	P30-3	3	1.91	8.7

Supplementary Table 5. Filtration and quality control of raw data. Raw reads/bases: reads/bases from original sequence, clean reads/bases: reads/bases from filtrated sequence, clean Q30: ratio of clean bases with quality score > 30 in total base.

Sample ID	raw reads	Clean reads	raw bases (Mb)	Clean bases (Mb)	clean Q30
E18-5-1	75997539	75243933	22798	20775	97.24%
E18-5-3	77546099	76967520	23262	21379	97.23%
P1-1	77543283	76978763	23262	21384	97.45%
P1-2	70357032	69759219	21106	19432	97.41%
P14-1	68346318	67787248	20502	18721	97.22%
P14-3	75127421	74552395	22538	20711	97.41%
P30-2	77687557	77030376	23306	21548	97.37%
P30-3	78367493	77531361	23510	21464	97.06%
P7-1	80589244	79991846	24176	22100	97.44%
P7-2	65589116	65124438	19676	18050	97.43%

Supplementary Table 6. Primers for luciferase assay.

	Forward	Reverse
Lucif-m-circ_0001878	CTCGAGGGTTTCCAGTGGTGCCAGTAC	GCGGCCGCCCAGAAAACCTGAATGTC
Lucif-m-circ_0001468	CTCGAGGTATGGCCTCACATGTGC	GCGGCCGCCGGTAGTATCTGGATTGC
Lucif-m-circ_0000723	GCGGCCGCAGTTGTAAGAGTCCATCCAGG	GTTTAAACCTCACTGTTGTGGCCTCGCAG
Lucif-m-circ_0001311	CTCGAGAATGTGAAACATGTTGTA	GCGGCCGCCGTGAGCTCGAAGCAGTG
Lucif-m-circ_0001757	CTCGAGGTGGCTGTACTCCAATCCCAG	GCGGCCGCCTCCAGAGATGAGCTTGG
Lucif-m-circ_0001624	CTCGAGTGGTATGTGTGCAACAGAGAG	GCGGCCGCCGTTTTCTACATAGGGAT