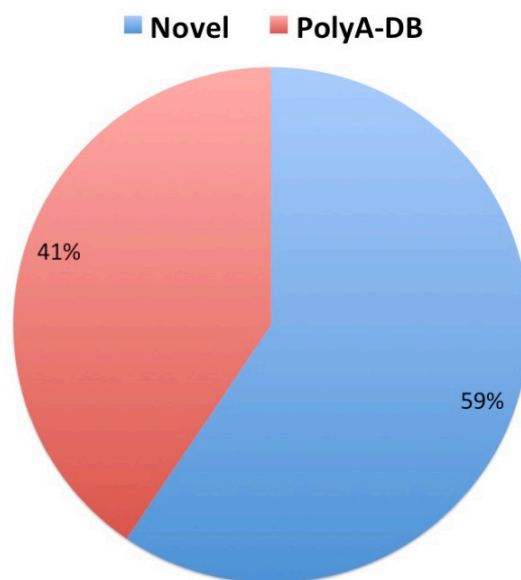


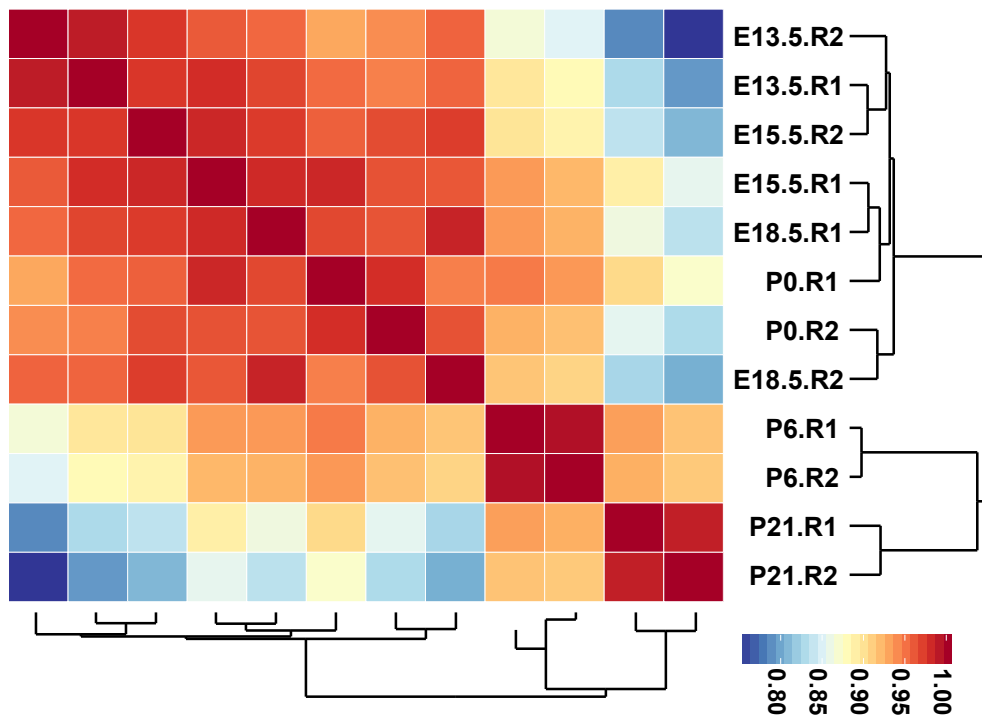
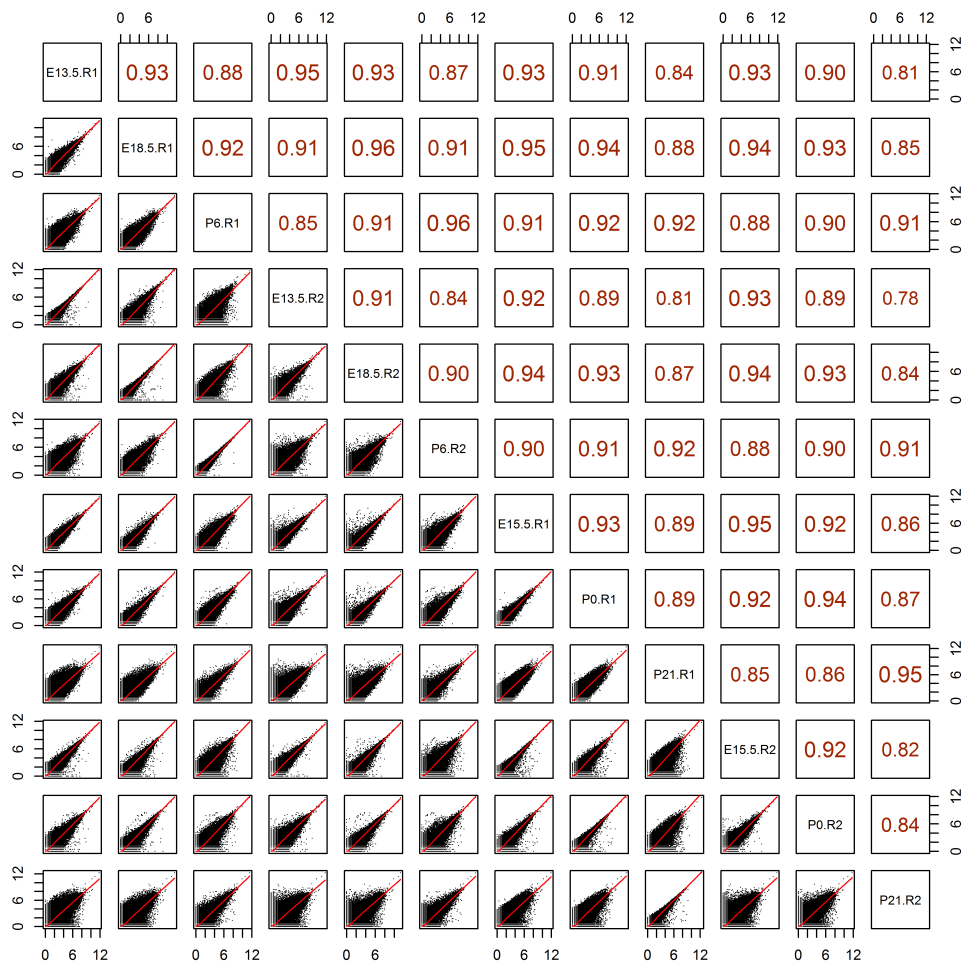
Dynamic Landscape of Alternative Polyadenylation during Retinal Development

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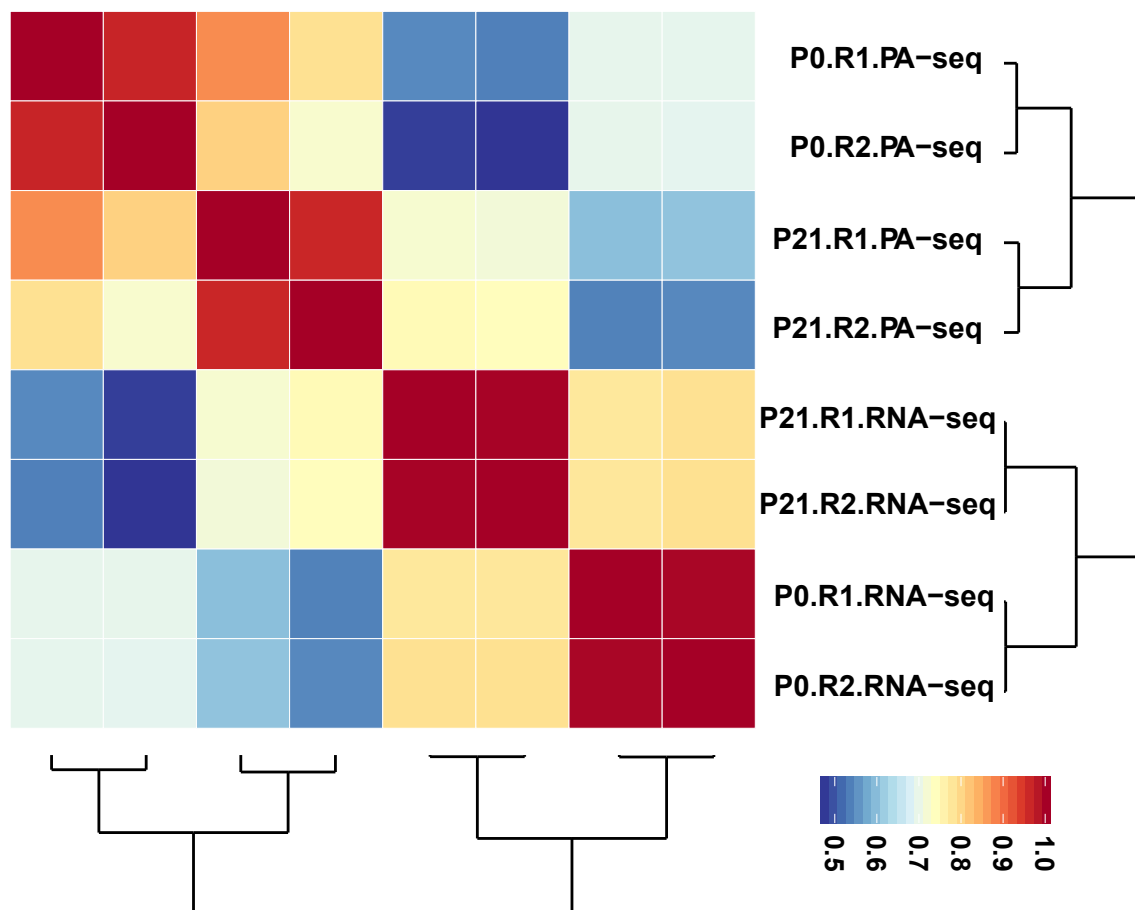
SUPPLEMENTARY FIGURES AND TABLES



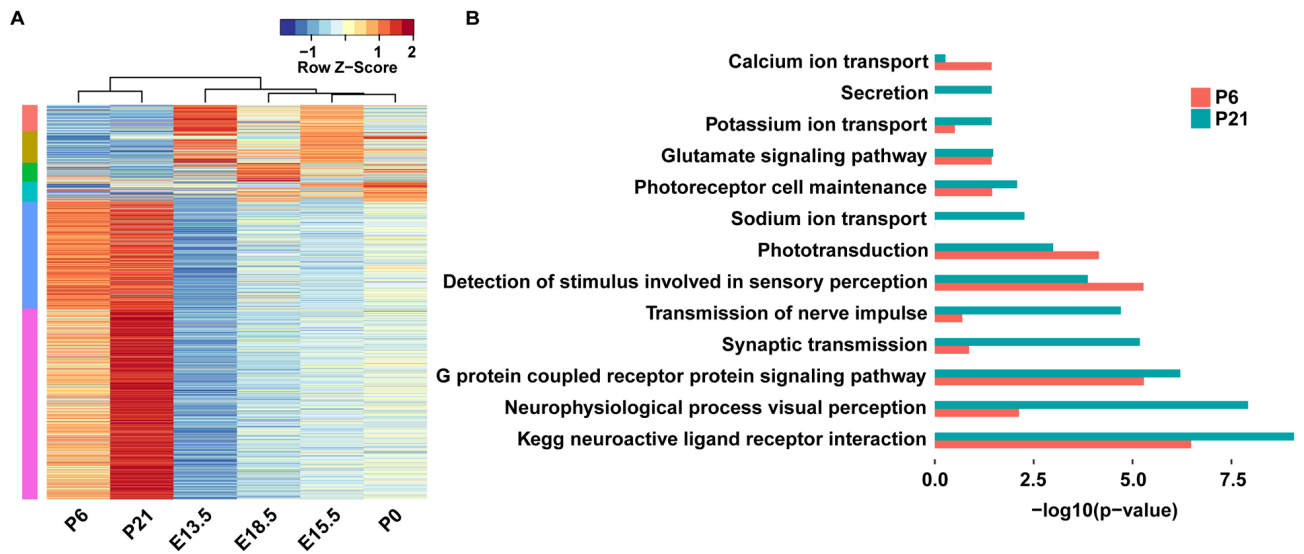
Supplementary Figure S1. Pie chart of fractions of distinct novel pA sites and known pA sites present in the PolyA-DB database.

A**B**

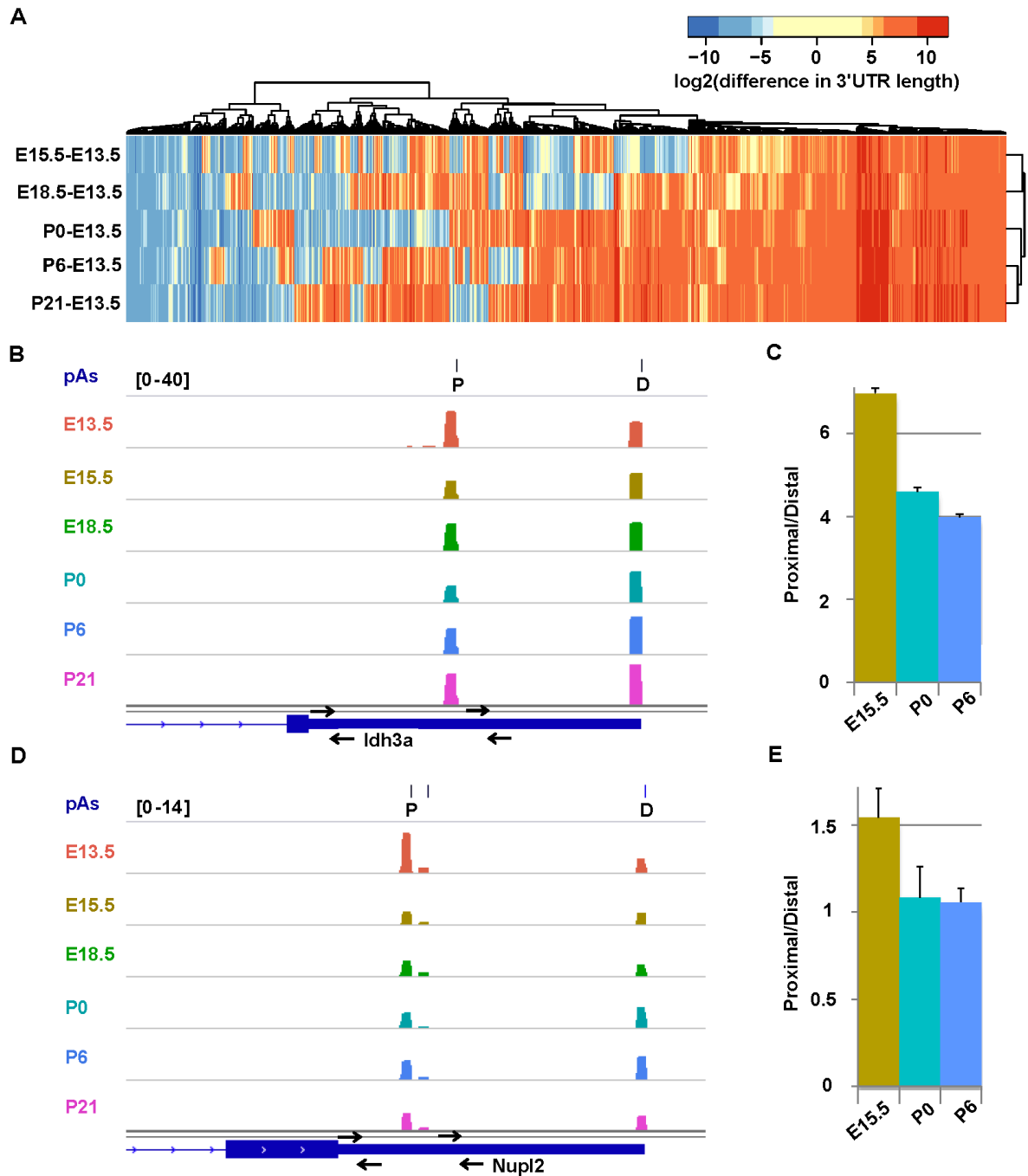
Supplementary Figure S2. Correlations between PA-seq replicates and samples at different developmental stages. **(A)** Heat map of the Pearson correlation between polyadenylation profiles of replicates and samples. The embryonic stages and P0 were highly correlated with each other and showed a low similarity with P6 and P21. **(B)** Correlation matrix with pair-wise scatter plots between samples and replicates in the lower diagonal and the corresponding correlation coefficients in the upper diagonal, scaling the font size to reflect the absolute value of the correlation. In the scatter plots, each point represents a single gene, such that the relative expression ($\log_2\text{RPM}$) of the identified pA sites between the samples (indicated in the diagonal) is plotted.



Supplementary Figure S3. Heat map showing the Pearson correlation of the transcript abundance of postnatal mouse retinas quantified by PA-seq and RNA-seq.



Supplementary Figure S4. Identification and characterization of the time-specific genes during retinal development. **(A)** Heat map of log-transformed RPM for all time-specific genes, row-wise normalization was applied. Each row of the heat map represents a gene. Red, high expression; blue, low expression using RPM Z-score values normalized for each gene individually, across all developmental stages. The color vector with group information of time-specific genes for each stage was added to the left side of the heat map corresponding to the color labels in Fig.2a. **(B)** Gene set enrichment analysis (GSEA) of the time-specific genes. The significance of enrichment is represented by the negative log₁₀-transformed FDR-corrected p-values. Functional annotation of the genes associated with P6- (red) and P21- (cyan) specific genes revealed an enrichment for those involved in neurophysiological process of visual perception, phototransduction, detection of stimulus involved in sensory perception, transmission of nerve impulse, and photoreceptor cell maintenance (p-value < 0.05).



Supplementary Figure S5. 3'UTR lengthening during retinal development and validation by qRT-PCR. **(A)** Heat map of difference in 3'UTR length when later stages are compared to E13.5. **(B-E)** Examples showing the validation of 3'UTR change by qRT-PCR. For the tested genes (lengthened: *Idh3a* and *Nupl2*), the height of each wiggle plot represents the number of PA-seq tags for the identified pA site (P, proximal; D, distal) at each developmental stage (B,D). The location of primer pairs used for PCR validation is indicated (B,D). The histograms represent the amount ratio of proximal/distal regions of the RNA transcript determined by qRT-PCR assays of retinal samples at the indicated developmental stages (C,E).


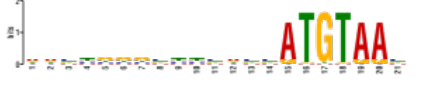



Supplementary Table S1. Mapping statistics of the PA-seq data for 6 stages of mouse retinal development including embryonic (E13.5, E15.5 and E18.5) and postnatal stages (P0, P6 and P21) with two replicates for each stage. The filtered, mapped, q20mapped, and q20mapped_rmdup represent the number of the reads after filtering low-quality reads, the filtered reads mapped to the mouse genome, the reads with a mapped quality ≥ 20 , and the q20mapped reads after the removal of duplicate reads, respectively

Sample	Replicate	Total	Filtered	Mapped	%mapped /filtered	q20mapped	%q20mapped /filtered	q20mapped_rmdup	%q20mapped_rmdup /filtered
E13.5	Replicate1	18,553,434	9,676,110	9,211,024	95	7,012,204	72	3,250,272	33.59
E15.5	Replicate1	19,036,590	9,149,472	8,698,124	95	6,882,125	75	3,342,449	36.53
E18.5	Replicate1	22,815,859	12,330,849	11,813,212	96	9,333,604	76	4,270,994	34.64
P0	Replicate1	14,644,173	7,159,956	6,801,492	95	5,477,785	77	2,901,426	40.52
P6	Replicate1	17,530,414	9,954,618	9,555,772	96	8,081,224	81	4,060,211	40.79
P21	Replicate1	17,426,868	8,637,847	8,274,514	96	7,200,882	83	3,597,354	41.65
E13.5	Replicate2	9,742,636	7,164,040	6,925,849	97	5,337,605	75	2,781,103	38.82
E15.5	Replicate2	10,865,060	8,134,808	7,896,920	97	6,302,515	77	3,210,178	39.46
E18.5	Replicate2	11,858,133	9,060,816	8,823,408	97	7,142,149	79	3,663,378	40.43
P0	Replicate2	8,227,698	6,400,298	6,205,677	97	5,053,858	79	2,869,142	44.83
P6	Replicate2	9,689,150	7,972,935	7,761,658	97	6,636,411	83	3,662,161	45.93
P21	Replicate2	10,340,964	7,998,325	7,808,614	98	6,899,091	86	3,691,264	46.15
















Supplementary Table S2. Gene-specific primers in proximal and distal regions of 3'UTRs used for qRT-PCR validation

Gene Symbol	Proximal	Distal
Aak1	5'GCTTCACCTCTGCTGGAATC	5'AGAGAGGCCCTAGGTTGAGC
	5'GCTGCCATGTCCTCATGATA	5'GAGCTTGCTAAGCTGCCTGT
Bcat1	5'AGCCCTTCCAGCACATAGAC	5'CCTGTGTCTGCCTCCTAAGC
	5'CTGTAGTCTCCCTCACCTGT	5'CAAAGGCCCAGGTAGAATCA
Idh3a	5'ACCATTGGTTTGCTTGCTTC	5'CCCGGATGCTCTCTGTGTAT
	5'AAGGTTTACCAGACGGACAA	5'AGGCAGTGACTGGTTGCTTT
Nupl2	5'GGTTTAAATACAGAAAGAATGCCTTAG	5'CGTACATGCCTGGTTCCTTT
	5'TCAAAGCCATTCAGAGCTTTCTC	5'TGTAAAAGGTGAGGGCTTGG
Gapdh	5'ACCACAGTCCATGCCATCAC	
(control)	5'TCCACCACCCTGTTGCTGTA	

Supplementary Table S3. Enriched microRNA binding sites in the lengthened/shortened part of 3'UTRs caused by APA

Logo	ID	Name	<i>p</i> -value	Adjusted <i>p</i> -value
	mmu-miR-5101	MIMAT0020608	4.27E-06	8.14E-03
	mmu-miR-5098	MIMAT0020605	4.58E-06	8.73E-03
	mmu-miR-669l-3p	MIMAT0017345	1.20E-05	2.28E-02
	mmu-miR-1192	MIMAT0005850	1.70E-05	3.21E-02
	mmu-miR-6951-5p	MIMAT0027802	2.25E-05	4.22E-02
	mmu-miR-590-3p	MIMAT0004896	2.35E-05	4.39E-02

Supplementary Table S4. Enriched RBP binding sites in the lengthened/shortened part of 3'UTRs caused by APA

Logo	ID	Name	<i>p</i> -value	Adjusted <i>p</i> -value
	M042_0.6	Gm10110	3.34E-12	3.11E-10
	M025_0.6	Hnrnpc	1.06E-11	9.89E-10
	M149_0.6	Cpeb4	2.71E-11	2.52E-09
	M075_0.6	Tial1	4.77E-10	4.44E-08
	M150_0.6	Raly	1.05E-08	9.75E-07
	M156_0.6	Tia1	1.05E-08	9.75E-07
	M077_0.6	U2af2	2.32E-07	2.16E-05
	M328_0.6	Elavl2	6.24E-07	5.81E-05
	M062_0.6	Sart3	1.07E-06	9.96E-05
	M146_0.6	Pabpc1	1.07E-06	9.96E-05
	M012_0.6	Cpeb2	1.15E-05	1.07E-03
	M169_0.6	Hnrpll	7.95E-05	7.36E-03
	M051_0.6	Rbm41	1.37E-04	1.27E-02
	M052_0.6	Rbm46	3.74E-04	3.42E-02
	M244_0.6	A1cf	4.31E-04	3.93E-02