Transcriptional dynamics during lens fiber cell differentiation and novel insights into the denucleation process

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Materials Included:
Figures S1 to S7 and Tables S1-S2

Fig. S1. Chromosomal localization, probe design, and stages of lens fiber cell differentiation. A) Chromosomal localization of genes on three mouse chromosomes: 1 (γA-Crystallin), 5 (βB1-Crystallin and β-actin), and 17 (αA-Crystallin). Genes examined and their neighbors are shown in red and black boxes, respectively. B) A summary of probes and their dyes used in this study.

Fig. S1A

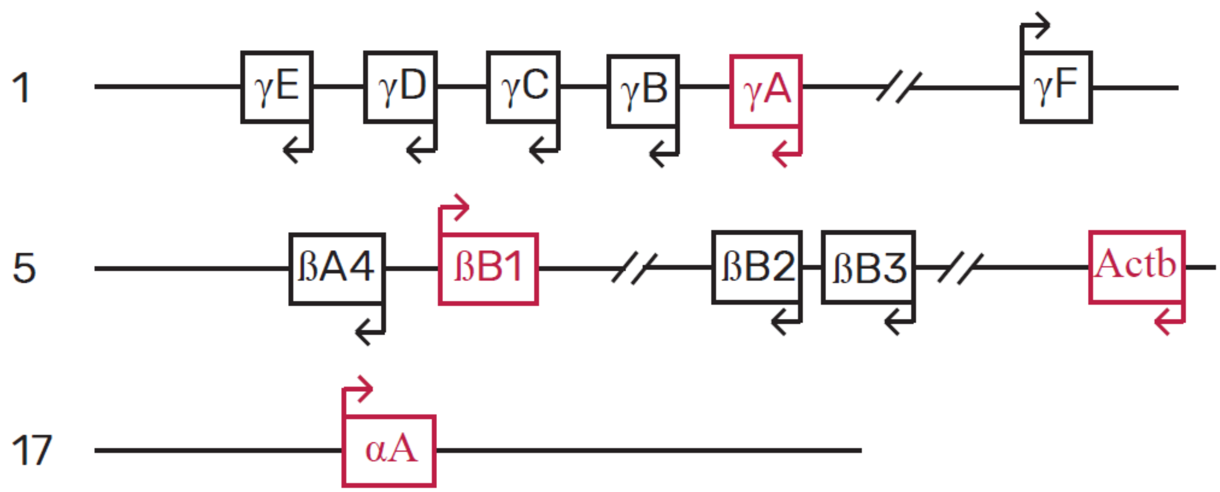

Fig. S1B

Gene	Probe length	Exon or Intron	Dye	# of probes
αA-Crystallin	20	Exon	Quasar 570	25
αA-Crystallin	20	Intron	Quasar 670	21
γA-Crystallin	20	Exon	Quasar 670	16
βB1-Crystallin	20	Exon	CAL Fluore Red 610	12
β-actin	20	Exon	Quasar 670	35

Fig. S2. RNA FISH of αA-crystallin introns and exons to identify and confirm nascent transcription sites in cell nuclei of newborn mouse lens tissue. Exons and introns were detected by Quasar 570- and Quasar 670-labeled probes (see Fig. S1B), respectively.

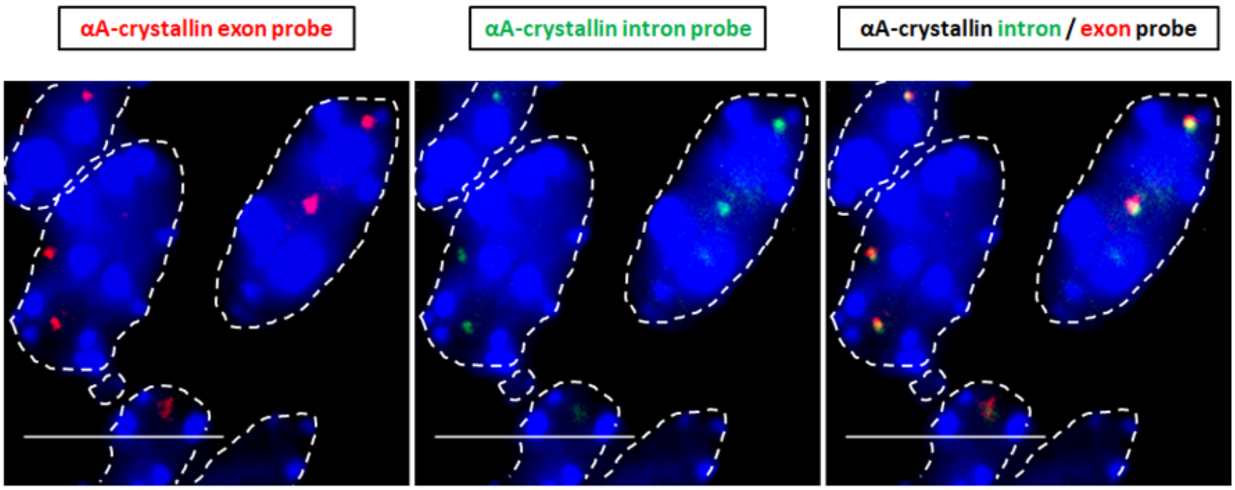

Fig. S3. Co-localization of active RNA PolII and the transcription sites of β-actin gene to confirm that RNA FISH technique detects nascent transcription sites. The co-localization shows β-actin by RNA FISH (red) and RNA polymerase II (green) by immunofluorescence in newborn mouse lens fiber cell nuclei. RNA FISH signal were detected by various Quasar or CAL Fluor dyes indicated in Fig. S1B and RNA polymerase II signal was detected by Alexa-Fluor 488 conjugated secondary antibody against an anti-Pol II primary antibody.

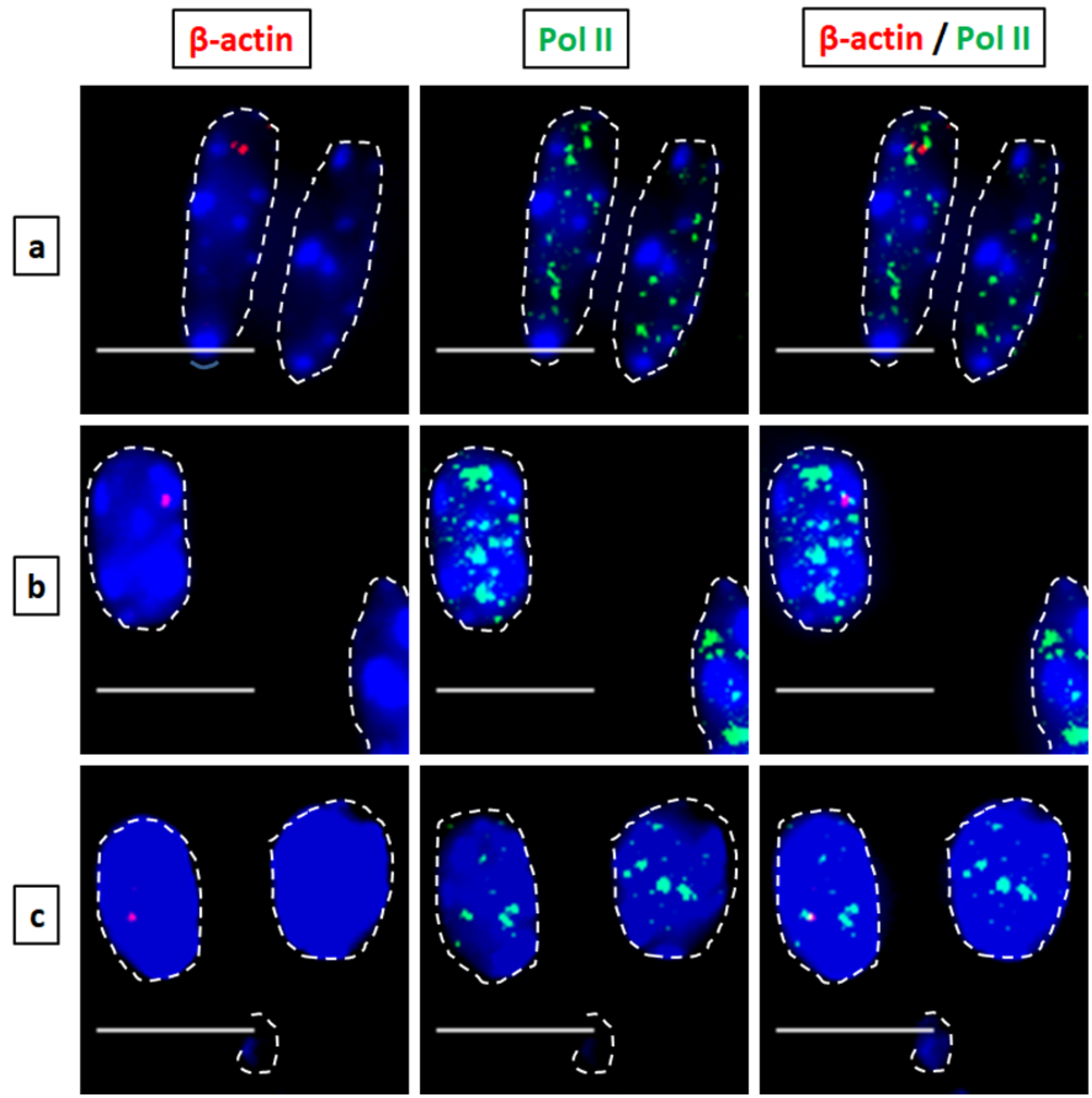

Fig. S4. Co-localization of active RNA PolII and the transcription sites of αA-crystallin gene to confirm that RNA FISH technique detects nascent transcription sites. The co-localization shows αA-crystallin by RNA FISH (red) and RNA polymerase II (green) by immunofluorescence in newborn mouse lens fiber cell nuclei. RNA FISH signal were detected by various Quasar or CAL Fluor dyes indicated in Fig. S1B and RNA polymerase II signal was detected by Alexa-Fluor 488 conjugated secondary antibody against an anti-Pol II primary antibody.

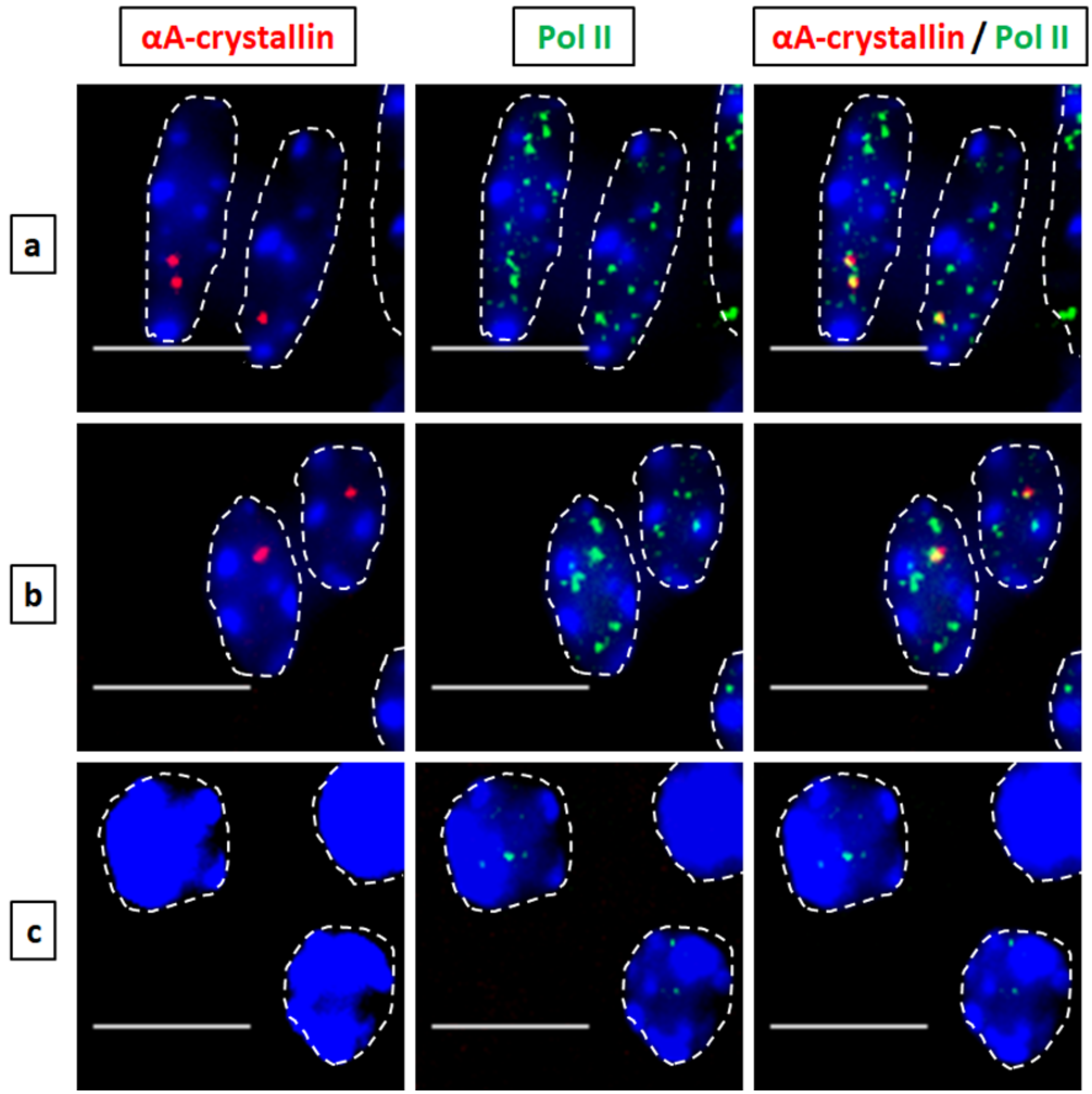

Fig. S5. Co-localization of active RNA PolII and the transcription sites of βB1-crystallin gene to confirm that RNA FISH technique detects nascent transcription sites. The co-localization shows βB1-crystallin by RNA FISH (red) and RNA polymerase II (green) by immunofluorescence in newborn mouse lens fiber cell nuclei. RNA FISH signal were detected by various Quasar or CAL Fluor dyes indicated in Fig. S1B and RNA polymerase II signal was detected by Alexa-Fluor 488 conjugated secondary antibody against an anti-Pol II primary antibody.

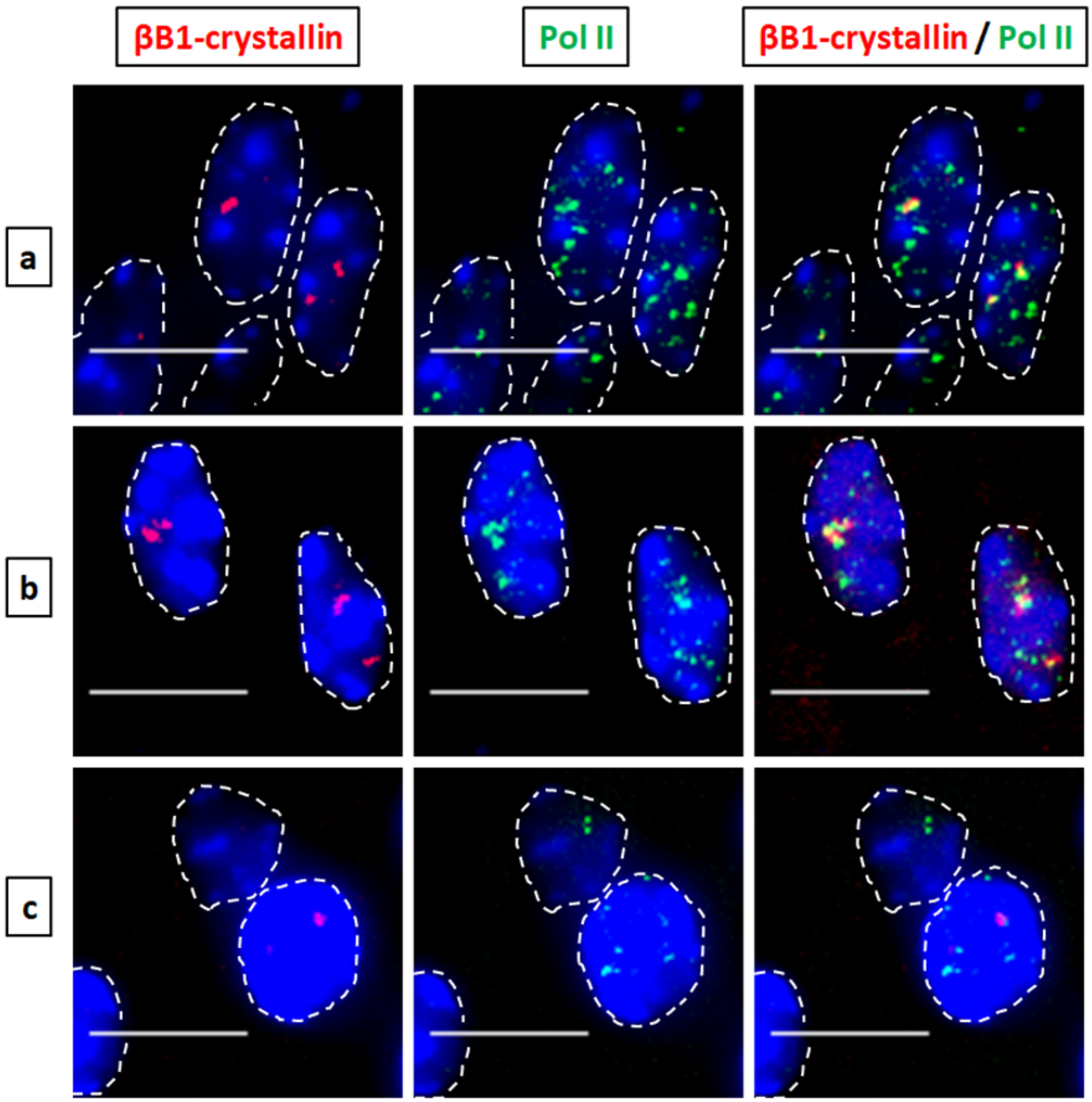

Fig. S6. Co-localization of active RNA PolII and the transcription sites of γA-crystallin gene to confirm that RNA FISH technique detects nascent transcription sites. The co-localization shows γA-crystallin by RNA FISH (red) and RNA polymerase II (green) by immunofluorescence in newborn mouse lens fiber cell nuclei. RNA FISH signal were detected by various Quasar or CAL Fluor dyes indicated in Fig. S1B and RNA polymerase II signal was detected by Alexa-Fluor 488 conjugated secondary antibody against an anti-Pol II primary antibody.

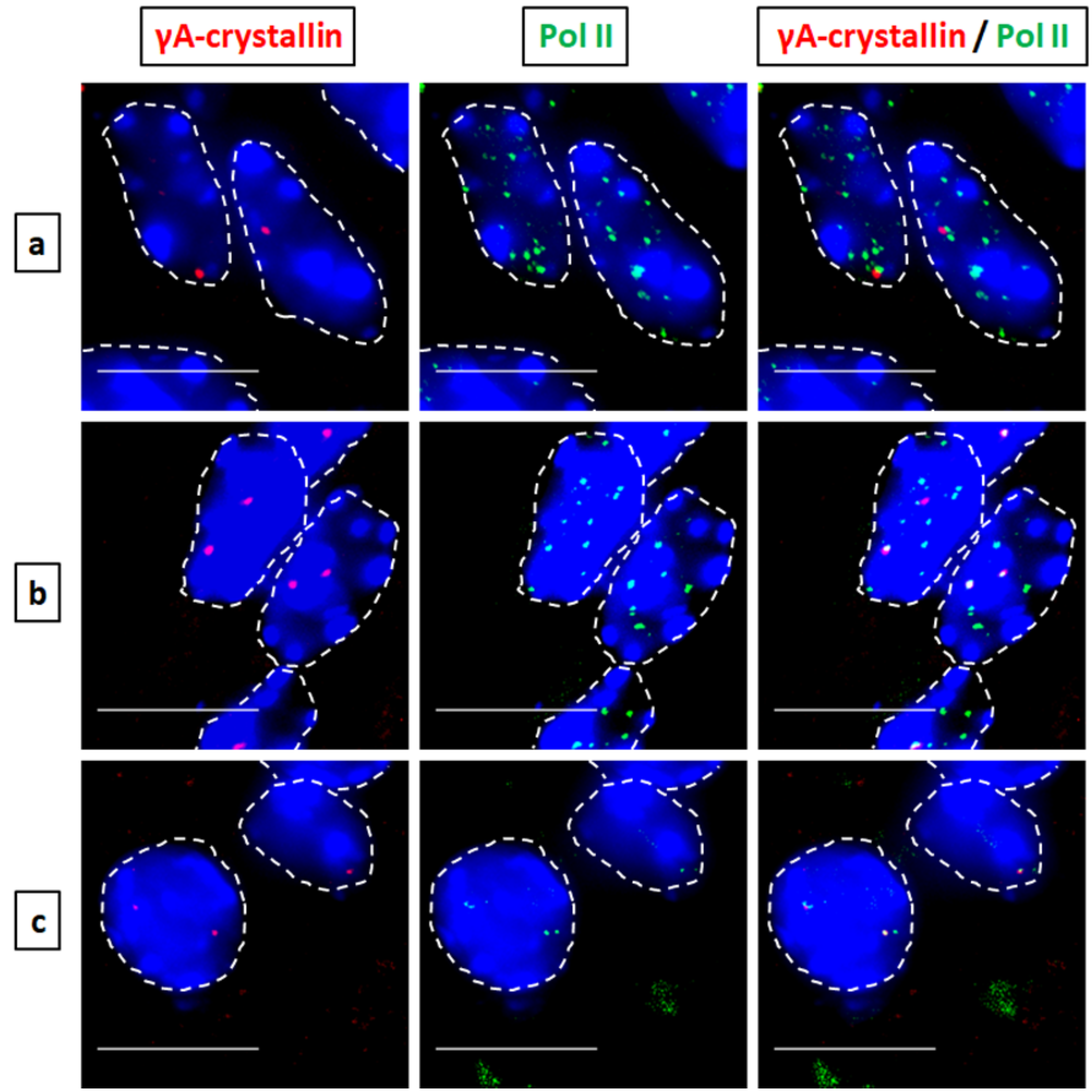

Fig. S7. RNA FISH in newborn mouse lens epithelium shows that βB1- and γA-crystallin shows no expression within the lens epithelium. The various indicated genes were detected by Quasar and CAL Fluor dyes indicated in figure S1B.

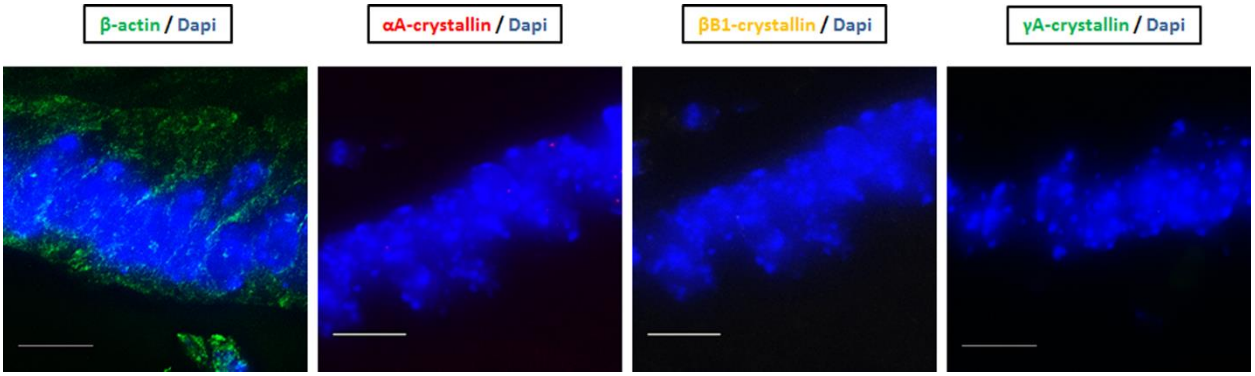

Table. S1. Number of cells counted at each of the four developmental stages, E12.5, E14.5, E16.5 and P1, from each of the four areas 'a', 'b', 'c', 'd', for each for the four genes studied, β-actin, αA-, βB1- and γA-crystallin.

E12.5	**a**	**b**	**c**	**d**
β-actin	145	132	152	138
αA-crystallin	293	267	301	276
βB1-crystallin	109	116	147	162
γA-crystallin	148	135	149	138

E14.5	**a**	**b**	**c**	**d**
β-actin	195	92	82	185
αA-crystallin	306	222	159	259
βB1-crystallin	194	113	91	70
γA-crystallin	112	109	68	189

E16.5	**a**	**b**	**c**	**d**
β-actin	229	140	107	111
αA-crystallin	340	219	177	108
βB1-crystallin	207	158	116	80
γA-crystallin	272	133	106	125

P1	**a**	**b**	**c**	**d**
β-actin	559	329	211	0
αA-crystallin	750	434	214	0
βB1-crystallin	381	211	114	0
γA-crystallin	326	246	162	0

	WT P1	**Snf2h null P1**
β-actin	901	540
αA-crystallin	1319	717
βB1-crystallin	794	534
γA-crystallin	805	504

Table. S2. Number of transcription sites counted at each of the four developmental stages, E12.5, E14.5, E16.5 and P1, from each of the four areas 'a', 'b', 'c', 'd', for each for the four genes studied, β-actin, αA-, βB1- and γA-crystallin.

E12.5	**a**	**b**	**c**	**d**
β-actin	50	50	80	73
αA-crystallin	100	198	285	203
βB1-crystallin	23	67	86	58
γA-crystallin	4	8	11	8

E14.5	**a**	**b**	**c**	**d**
β-actin	71	73	68	97
αA-crystallin	226	289	173	372
βB1-crystallin	112	108	84	58
γA-crystallin	26	110	95	241

E16.5	**a**	**b**	**c**	**d**
β-actin	203	110	68	51
αA-crystallin	448	272	139	13
βB1-crystallin	128	157	93	41
γA-crystallin	76	115	83	112

P1	**a**	**b**	**c**	**d**
β-actin	439	249	121	0
αA-crystallin	791	306	11	0
βB1-crystallin	275	151	41	0
γA-crystallin	146	272	182	0

	WT P1	**Snf2h null P1**
β-actin	651	240
αA-crystallin	909	315
βB1-crystallin	543	268
γA-crystallin	555	235

Table. S3. Table of standard deviations for transcription burst fraction measurements.

E12.5	**a**	**b**	**c**	**d**
β-actin	2.7882	2.9289	2.9236	3.2262
αA-crystallin	2.4722	2.5925	2.5358	2.4697
βB1-crystallin	2.6075	3.6279	3.0261	2.5343
γA-crystallin	1.121	2.2824	2.7867	2.736

E14.5	**a**	**b**	**c**	**d**
β-actin	2.1847	4.2984	4.629	2.7256
αA-crystallin	2.1323	2.1575	2.6829	1.8326
βB1-crystallin	2.6858	3.8106	4.5014	4.7104
γA-crystallin	1.3306	3.1414	3.7352	2.7256

E16.5	**a**	**b**	**c**	**d**
β-actin	2.7003	3.6358	3.8264	3.8217
αA-crystallin	2.0324	2.0283	2.2346	0.6192
βB1-crystallin	2.5718	3.2439	3.7435	4.0658
γA-crystallin	1.8998	3.5347	4.1766	3.7829

P1	**a**	**b**	**c**	**d**
β-actin	1.7641	2.1979	2.717	NA
αA-crystallin	1.4224	1.885	1.097	NA
βB1-crystallin	2.0262	2.713	2.8809	NA
γA-crystallin	1.8854	2.6379	3.1792	NA

	WT P1	**Snf2h null P1**
β-actin	1.3676	1.4698
αA-crystallin	1.7556	1.8734
βB1-crystallin	1.5129	1.8816
γA-crystallin	2.22	1.5469

Table. S4. Table of standard deviations for transcription burst intensity measurements.

E12.5	**a**	**b**	**c**	**d**
β-actin	7.3733	3.6022	3.2183	2.5498
αA-crystallin	2.4522	1.6984	1.3836	1.7059
βB1-crystallin	12.7818	6.5483	6.3446	8.2172
γA-crystallin	90.935	8.2466	15.6604	12.0328

E14.5	**a**	**b**	**c**	**d**
β-actin	12.9059	11.2358	10.152	7.4696
αA-crystallin	5.5745	7.8456	11.8615	4.0176
βB1-crystallin	10.0917	12.8424	9.2171	10.4935
γA-crystallin	21.931	4.1301	3.9967	2.2957

E16.5	**a**	**b**	**c**	**d**
β-actin	2.0217	2.5324	3.8396	3.9512
αA-crystallin	1.3673	1.9192	3.4025	7.7329
βB1-crystallin	3.8434	3.7089	2.9297	6.0089
γA-crystallin	6.8814	2.5694	2.4465	7.2049

P1	**a**	**b**	**c**	**d**
β-actin	1.2197	2.6165	5.7212	NA
αA-crystallin	1.9589	3.6046	20.3828	NA
βB1-crystallin	1.3478	2.283	6.8795	NA
γA-crystallin	4.0081	2.4814	3.5708	NA

	WT P1	**Snf2h null P1**
β-actin	1.4502	2.5658
αA-crystallin	1.0032	2.858
βB1-crystallin	1.8053	3.5122
γA-crystallin	1.5066	3.506