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SUPPLEMENTARY MATERIAL

corresponding to:

Pitx3 directly regulates *Foxe3* during early lens development

NAFEES AHMAD, MUHAMMAD ASLAM, DORIS MUENSTER, MARION HORSCH,
MUHAMMAD A. KHAN, PETER CARLSSON, JOHANNES BECKERS and JOCHEN GRAW

***Address correspondence to:** Jochen Graw. Helmholtz Centre Munich – German Research Center for Environmental Health, Institute of Developmental Genetics, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany. Tel: +49-89/3187-2610. E-mail: graw@helmholtz-muenchen.de

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TABLE S1

PROBES FOR EMSA

Gene	Probe	Sequence*
Foxe3	Fox3-1-EMSA	5'-Biotin-AATCCCTGGCCATT <u>TAATCCCT</u> CCTGCCAGCCC-3'
	Fox3-2-EMSA	5'-Biotin-ACGCTGAAAACGCGGATTAGCCCTTGGGCCG-3'
Prox1	Prox1-EMSA	5'-Biotin-AGGGGGGGCAGTT <u>TAATCCCT</u> GTAAATGTGGT-3'
Tube1	Tube1-3-1-EMSA	5'-Biotin-GACAAGCTGCTAATAAGCTGTTTCTGCCATCT-3'
	Tube1-3-2-EMSA	5'-Biotin-TGTAATAACAAACTAAGCTGTATCCTGGCCG-3'

*Pitx3 putative binding sites are underlined.

TABLE S2

PRIMERS FOR GENOTYPING OF *APHAKIA* MICE

Primer	Sequence	Annealing (°C)	Product size (bp)	
			wt	ak
Pitx3-1/2NF	5'-ATTTCGGTGCAGAGTAAGG-3'	63	1,165	399
Pitx3-2R	5'-ATTGGATTGGCTCTGATGGT-3'			

TABLE S3

PRIMERS FOR RT-QPCR

Gene	Primer	Sequence	Annealing (°C)	Product size (bp)
E4f1	E4FqF	5'-AGTACATTATTGAGGCCACTGC-3'	60	219
	E4FqR	5'-CAATGGTGATCGTGCTGTC-3'		
Foxe3	Foxe3-lt	5'-GCCGCCCTACTCATAATC-3'	60	172
	Foxe3-rt	5'-ACAGTCGTTGAGGGTGAGG-3'		
Prox1	Prox1qF	5'-ATGCTGTGTCCTGTTTCTCT-3'	60	101
	Prox1qR	5'-GCTTATCAGGCTCAAATCAAAC-3'		
Tuba*	TubeaF	5'-CCAGATGCCAAGTGACAAGA-3'	60	117
	TubeaR	5'-GTGGGTTCCAGGTCTACGA-3'		
Tube1	Tube1-mqF	5'-CAGTGCTTCTTCATCATCCA-3'	60	126
	Tube1-mqR	5'-GGAAGGATAAACCGCTGTC-3'		

*: Primers from qprimerDepot (<http://mouseprimerdepot.nci.nih.gov/>)

TABLE S4

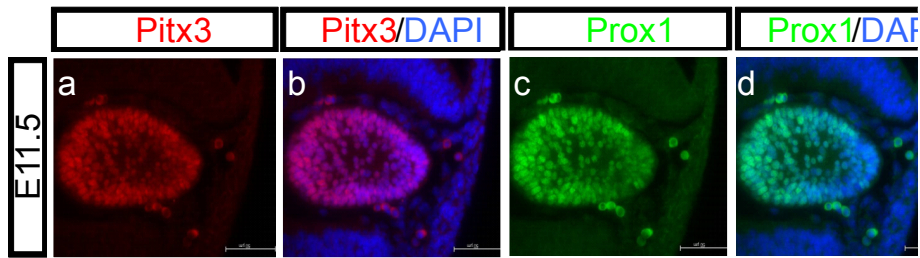
PRIMERS FOR CLONING OF *FOXE3* PROMOTER AND CHIP-PCR

Primer	Sequence	Annealing temperature (°C)	Product size (bp)
Foxe3ch-1F	5'-CAGAGTGGAGCAAGCTGGTG-3'	58	162
Foxe3ch-1R	5'-TAAGACGGCCAGTGAAGGTG-3'		
Foxe3ch-2F	5'-TAAGACGGCCAGTGAAGGTG-3'	58	283
Foxe3ch-2R	5'-CTTTGGACAAGGGTGGGAAT-3'		
Foxe3ch-1F	5'-CAGAGTGGAGCAAGCTGGTG-3'	58	401
Foxe3ch-2R	5'-CTTTGGACAAGGGTGGGAAT-3'		

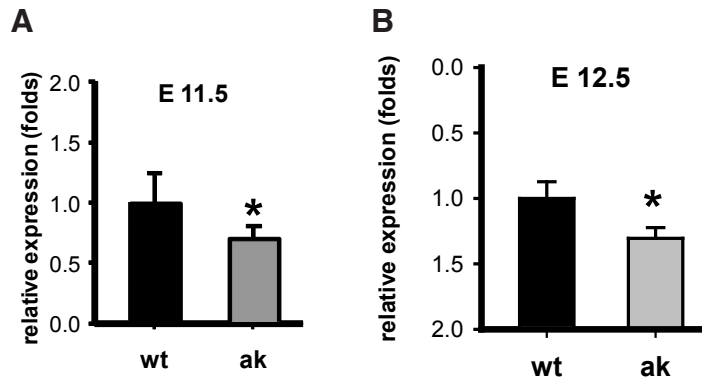
TABLE S5

PRIMERS FOR SITE DIRECTED MUTAGENESIS

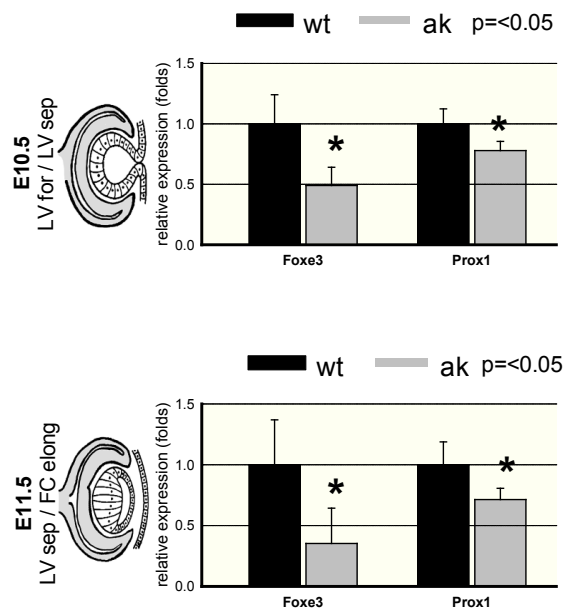
Primer	Sequence
Prox1-mut	GTAAAAATAAGGGGGGGCAGTTTGTAAATGTGGTGCG
Foxe3-mut1	CAATCCCTGGCCATCTCCTGCCAGCC
Foxe3-mut2	CGCTGAAAACGCGCCCTTGGGCCG



Supplementary Fig. S1. Analysis of Pitx3 and Prox1 expression in *Foxe3* mutant. Immunofluorescence staining for Pitx3 (a) and Prox1 (b) was performed on sections from *Foxe3* mutant embryos at E11.5. Co-staining for both of these genes revealed that their expression completely overlap in this mutant (c); however, the expression of Prox1 is observed more anterior compared to the wild-type lens (Fig. 2o) at this stage, indicating that *Foxe3* inhibits Prox1. Immunofluorescence staining was performed on 8 μm thick, PFA fixed paraffin sections. Scale bars, 50 μm .



Supplementary Fig. S2. RT-qPCR was performed at E11.5 for *E4f1* (A) and at E12.5 for *Tube1* (B) using RNA from the head of littermate embryos. Expression is shown as fold changes of values normalized to *Tuba* and calculated using $2^{-\Delta\Delta CT}$ method. Values from wild-type samples are represented as one. Data represents mean \pm standard deviations from three samples run in duplicate. Statistical analysis was done using student's t test. $p \leq 0.05$.



Supplementary Fig. S3. Quantification of *Foxe3* and *Prox1* transcripts at different developmental stages (adopted from Lang, 2004 and <http://www.mc.vanderbilt.edu/>) using RNA from the head of littermate embryos. Expression is represented as fold changes normalized to *Tuba* and calculated using $2^{-\Delta\Delta CT}$ method. Values from wild-type samples are represented as one. Data represents means \pm standard deviations from five samples run in duplicate. Statistical analysis was done using student's t test. $p < 0.05$. Abbreviations: LV for, lens vesicle formation; LV sep, lens vesicle separation; FC elong, fiber cell elongation.