SUPPLEMENT–FIGURE LEGENDS

Fig. 1S. The mouse *Cabp4* gene and the targeting construct. (**A**) **Organization of *CABP4* gene.** The coding regions are shown in black boxes and the untranslated regions in white boxes. The numbers represent the size of the introns. (**B**) **Generation of CaBP4 knockout mice using gene targeting.** In the targeting vector, the Neo cassette replaces exon 1 and part of exon 2. H3: *HindIII*, X: *XhoI*. The primers used for genotyping the mice are represented by arrows. Specifically, the targeting vector was constructed by using 1.5 kb DNA fragment as short arm, which was a PCR fragment from *CABSA1* to *CABSA2*. CABSA1 is located 1.5 kb upstream of exon 1, with a sequence of 5'–*GTACTGGGGAGGTGGAAGCAGG*–3'. *CABSA2* is partially located inside of exon 1, 13 bp upstream of start ATG codon, with a sequence of 5'–*GGACTCAGTGATGTAGCTCCCTG*–3'. The short arm was inserted into the 5'–end of the *Neo* gene cassette using the *MluI* site. The long arm was from a 7.3 kb *PspOMI* genomic fragment to the end of the clone. In this strategy, exon 1 and part of exon 2 were replaced by the *Neo* gene cassette. Ten micrograms of targeting vector were linearized by *NotI* and then transfected by electroporation into 129 SvEv iTL1 embryonic stem cells. After selection in G418, surviving colonies were expanded, and PCR analysis was performed to identify clones that had undergone homologous recombination. The correctly targeted embryonic stem cell lines were then microinjected into C57BL/6J blastocysts. The chimeric mice were generated and they gave germline transmission of the disrupted *Cabp4* gene. CaBP4–deficient mice were generated by Ingenious Targeting Laboratory (Stony Brook, NY). (**C**) **PCR–based genotyping of mice.** *Cabp4* and the targeted loci give 730 bp and 800 bp PCR products, respectively. To identify the wild–type allele, the primer pair K181 (5'–*CCAGGGTCCCAGAAAGCC*–3', located 130 bp downstream the ATG) and K184 (5'–*TCGGGGTTAGCAGCGGTCA*–3', hybridizing at the end of intron 2) was used and gives a PCR product of 730 bp. The targeted *Cabp4* allele was identified with primers K183 (5'–*CTTGGCGAATATCATGGTGG*–3', located in the neo cassette) and K184 (~800 bp) and gives a PCR product of ~ 800 bp. The PCR were cycled at 94°C for 5 min followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, 68°C for 1.5 min, followed by a final extension at 68°C for 7 min.

Fig. 2S. (A) and (B) Cross-section through the outer plexiform layer of *Cabp4*$^{+/+}$ and *Cabp4*$^{-/-}$ mice analyzed by transmission EM. Many inflated photoreceptor terminals are visible in the section of C*abp4*$^{+/+}$ mice compared with only a few flattened and

disorganized terminals in the *Cabp4*$^{-/-}$ mice (1 photoreceptor terminal is delimited by a white line). The OPL of the *Cabp4*$^{-/-}$ mice is very thin. The outer and inner nuclear layer are closer to each other and both visible in the *Cabp4*$^{-/-}$ mouse while only the outer nuclear layer is seen in *Cabp4*$^{+/+}$ at the same magnification. Many synaptic ribbons are evident in the *Cabp4*$^{+/+}$ section and indicated by arrows compared to only one in the *Cabp4*$^{-/-}$ section. (**C**) **Montage of cross–sections through the retina of two–month–old and eight–month–old mice.** The outer nuclear layer is thinner in eight–month–old than in two–month–old *Cabp4*$^{-/-}$ mice.

Fig. 3S. **Retina histology of six to eight–month–old *Cabp4*$^{-/-}$ and *Cabp4*$^{+/+}$ mice.** (**A**, **B** and **C).** ROS, ONL, and OPL thickness (in micrometers) plotted as a function of the retinal location (in millimeters) from the optic nerve head. The mice were 6–8 months old (mean ± SD from 3 animals). Open circles: *Cabp4*$^{+/+}$, closed circles: *Cabp4*$^{-/-}$. See Materials and Methods for details. The datasets of ROS, ONL and OPL thickness at 250–2000 μm from the ONH were evaluated by one–way ANOVA, which showed significant differences between *Cabp4*$^{+/+}$ and *Cabp4*$^{-/-}$ ($P < 0.001$). (**D**) Comparison of outer nuclear layer thickness in *Cabp4*$^{-/-}$ and *Cabp4*$^{+/+}$ mice. Layers of nuclei were counted at 750–1750 μm from the optic nerve heads (mean ± SD, $n = 60$ from 3 animals). Significant differences between *Cabp4*$^{-/-}$ and *Cabp4*$^{+/+}$ were observed (**, $P < 0.001$). (**E**) High magnification image of the outer plexiform layer reveals the spaces in cone pedicle and rod spherule of *Cabp4*$^{+/+}$ mice (left image, circled by white line) surrounded by plasma membrane (PSD95 immunostaining, green). Such spaces are not observed in *Cabp4*$^{-/-}$ mice (right image). The mice used in this experiment were 8–10 wks old. Red color represents anti–mGluR6 immunoreactivity to show the invaginations of on–bipolar cells. Bar indicates 5μm.

Fig. 4S. ERG responses from *Cabp4*$^{-/-}$ and C*abp4*$^{+/+}$ mice. (**A**) Leading edge (initial –5 to 10 ms) of maximal photoresponse (filled circles, evoked at 2.8 log cd·s·m^{-2}) in dark–adapted condition is represented and fitted with a model of

phototransduction (smooth lines) from *Cabp4*$^{-/-}$ and C*abp4*$^{+/+}$ mice at 10 weeks and 6 months of age. Units of amplitude and time indicate 200 µV and 5 ms respectively. (**B**) The maximum amplitude and sensitivity of the *Cabp4*$^{-/-}$ mouse photoresponses in ERG measurements are reduced from maximal responses in dark–adapted condition. Both parameters in *Cabp4*$^{-/-}$ mice are compared to the results in *Cabp4*$^{+/+}$ mice. *Cabp4*$^{-/-}$ mice showed significant differences in maximum amplitudes at 10 weeks and 6 months of age (*, $P < 0.01$), but not in sensitivities at both ages.

Figure 1S

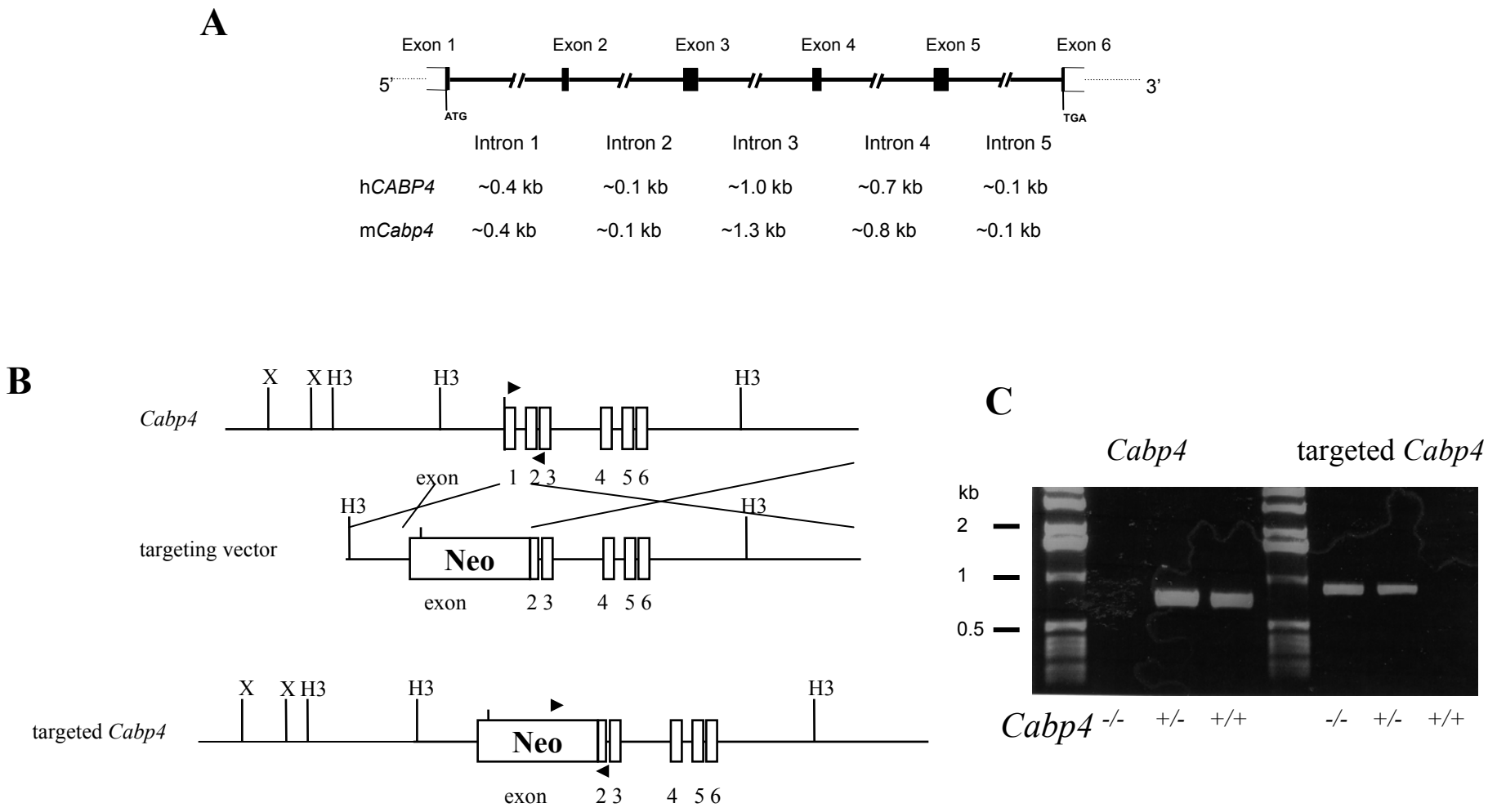

A
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Exon 6
5'
3'
ATG
TGA
Intron 1
Intron 2
Intron 3
Intron 4
Intron 5
hCABP4
~0.4 kb
~0.1 kb
~1.0 kb
~0.7 kb
~0.1 kb
mCabp4
~0.4 kb
~0.1 kb
~1.3 kb
~0.8 kb
~0.1 kb
B
Cabp4
X
X H3
H3
H3
exon
1 2 3
4 5 6
H3
H3
targeting vector
Neo
exon
2 3
4 5 6
targeted Cabp4
X
X H3
H3
H3
Neo
exon
2 3
4 5 6
C
Cabp4
targeted Cabp4
kb
2
1
0.5
Cabp4 -/- +/- +/+ -/- +/- +/+

Figure 2S

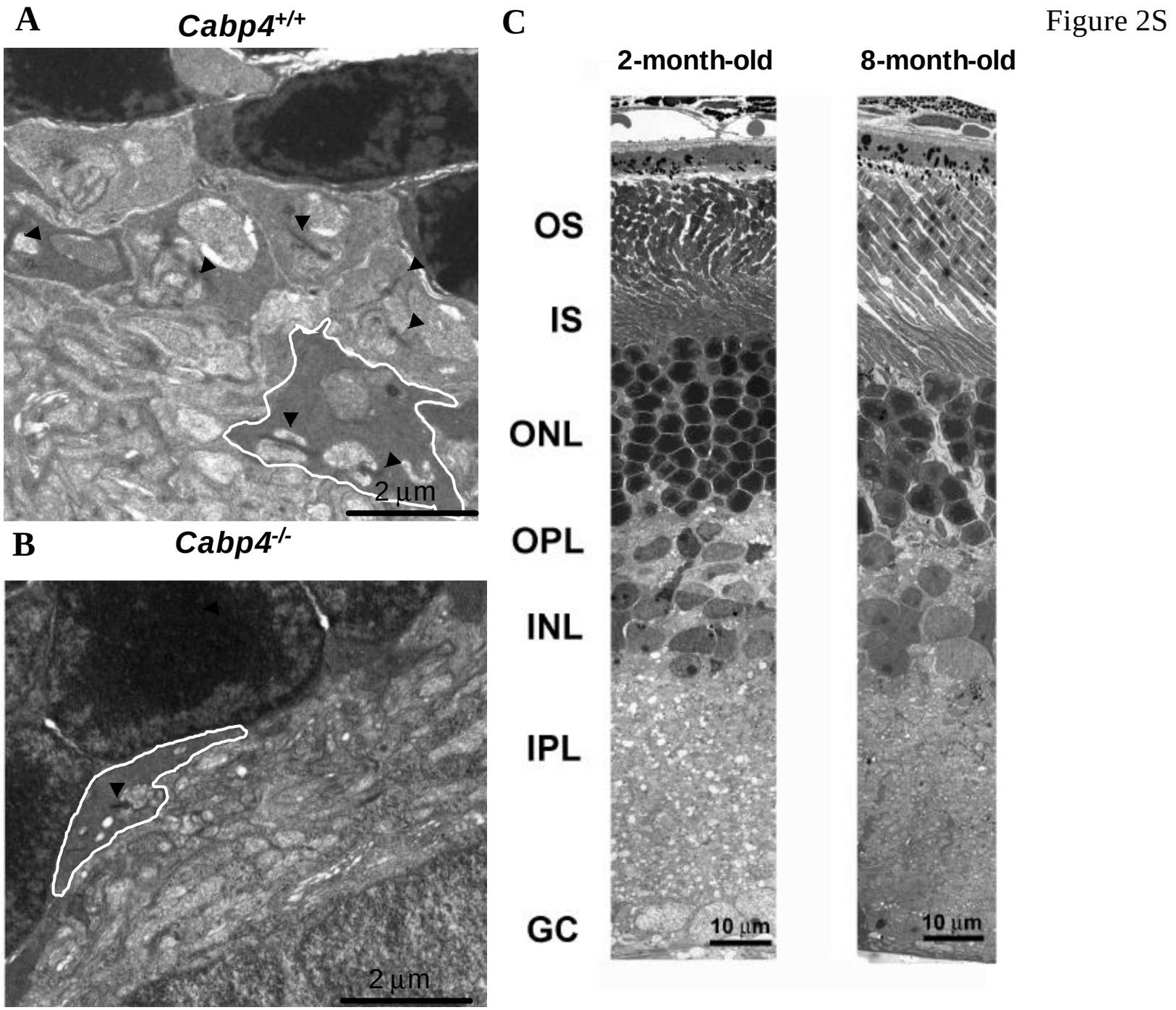

A
Cabp4+/+
2 µm
B
Cabp4-/-
2 µm
C
2-month-old
8-month-old
OS
IS
ONL
OPL
INL
IPL
GC
10 µm
10 µm

Figure 3S

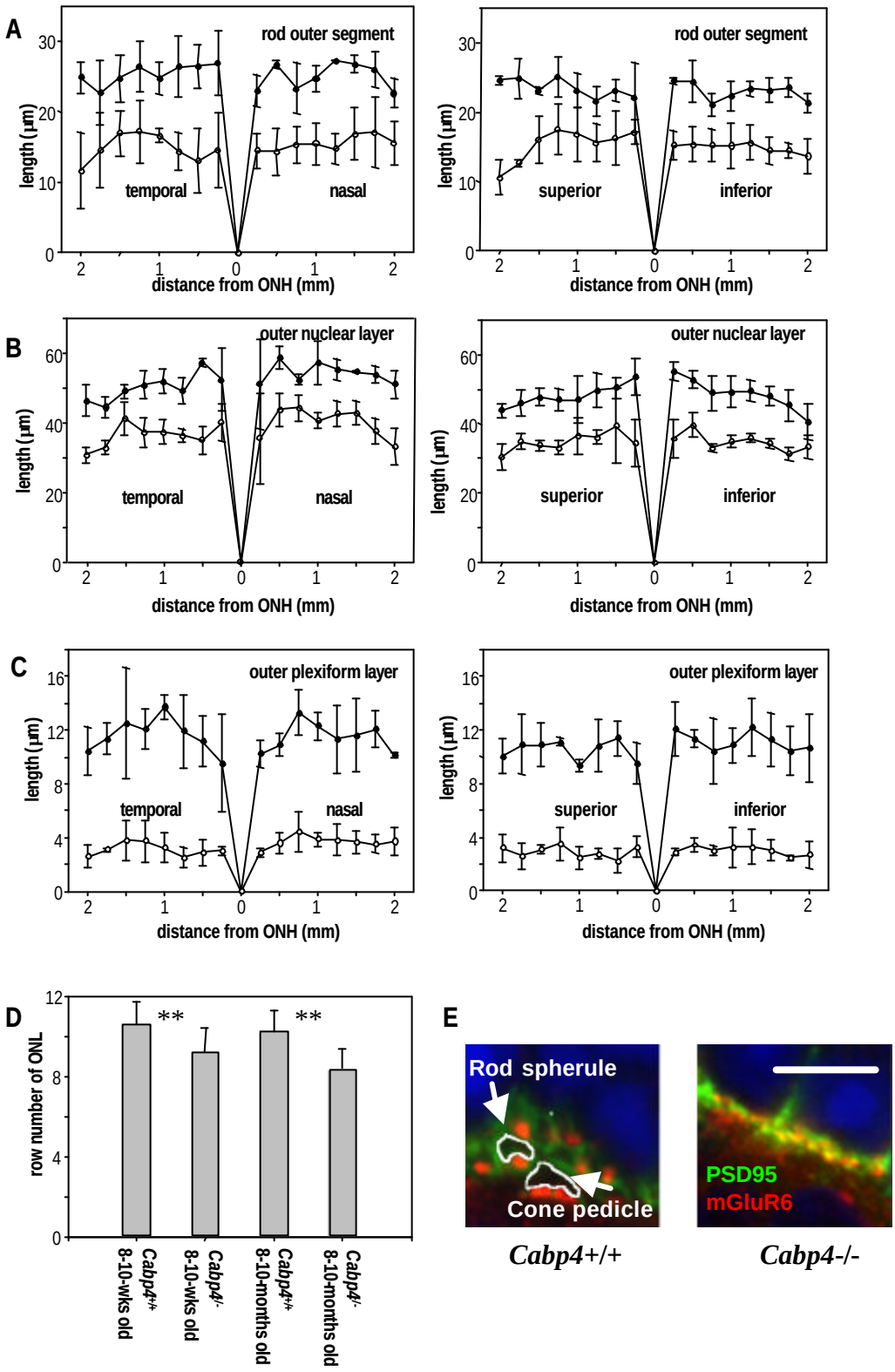
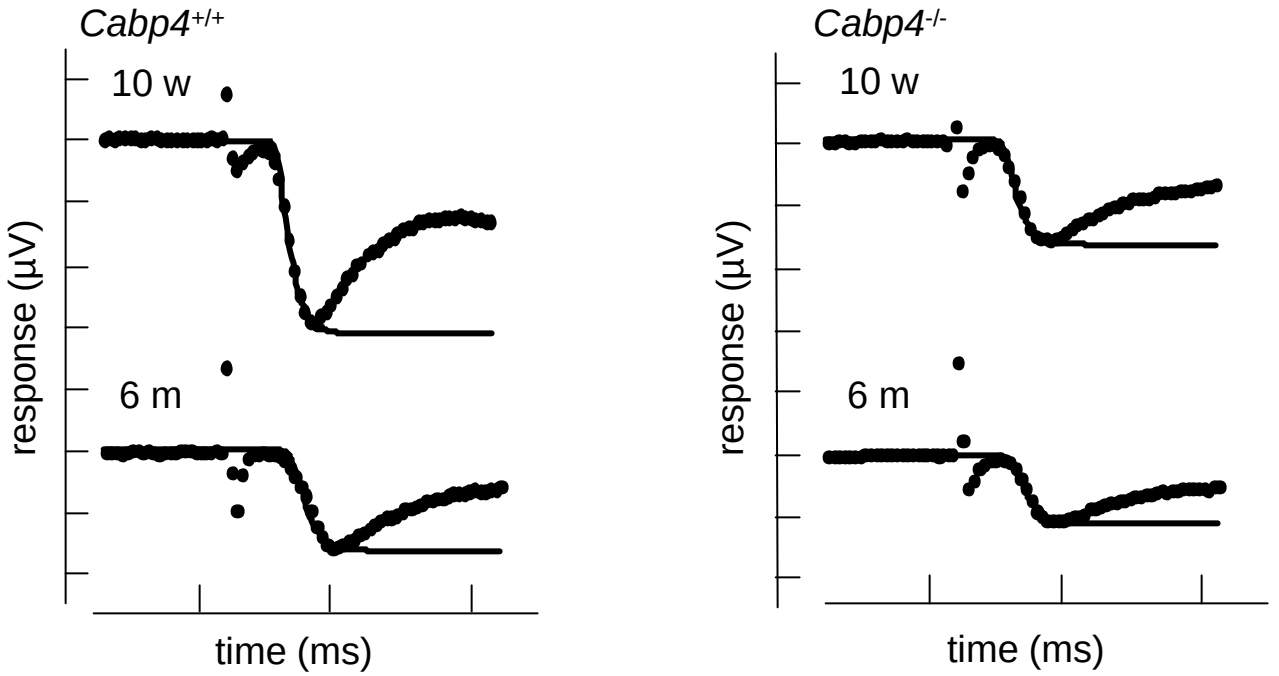

A
rod outer segment
length (µm)
temporal
nasal
distance from ONH (mm)
rod outer segment
superior
inferior
distance from ONH (mm)
B
outer nuclear layer
length (µm)
temporal
nasal
distance from ONH (mm)
outer nuclear layer
superior
inferior
distance from ONH (mm)
C
outer plexiform layer
length (µm)
temporal
nasal
distance from ONH (mm)
outer plexiform layer
superior
inferior
distance from ONH (mm)
D
row number of ONL
**
**
8-10-wks old
Cabp4+/+
8-10-wks old
Cabp4-/-
8-10-months old
Cabp4+/+
8-10-months old
Cabp4-/-
E
Rod spherule
Cone pedicle
PSD95
mGluR6
Cabp4+/+
Cabp4-/-

Figure 4S

A



B

	Cabp4$^{+/+}$		*Cabp4*$^{-/-}$	
	10 w	6 m	10 w	6 m
maximum a-wave amplitude (µV)	535.0 ± 36.7	317 ± 9.8	366.0 ± 31.0*	221.0 ± 13.2*
sensitivity (log $cd^{-1} \cdot m^2 \cdot s^{-3}$)	6.5 ± 0.4	5.1 ± 0.7	5.4 ± 0.6	4.6 ± 0.2

*, P< 0.01