

**Supplemental Figure 1. In situ hybridization of *Pk1* expression during development.** **A**, Weak and uniform expression of *Pk1* in developing cortex (CCX), anlage of hippocampus (Hip, between short yellow lines) and lateral ganglion eminence (LGE) at E13.5. LV, lateral ventricle. **B**, *Pk1* is weakly expressed in retina (Re), and decently expressed in lens (Le) and a subset of mesenchymal cells in eye region. **C**, Similar to brain cortex, weak and uniform expression of *Pk1* in both ventricular zone (VZ) and differentiating area of the developing spinal cord. *Pk1* expression is also detected in dorsal root ganglion (DRG). **D**, An example of *Pk1* expression in non-neural tissue of tongue muscle (TM) and Meckel's cartilage (MC). **E**, An overview of *Pk1* expression in E15.5 brain coronal section. 3V, third ventricle, LGE, lateral ganglionic eminence. **F**, Enlarged boxed area in **E** showing *Pk1* expression in ventricular, subventricular and intermediate zones, and lateral ganglion eminence (LGE). **G**, *Pk1* expression in P1 hippocampus. DG, dentate gyrus; CA, *Cornu Ammonis*. **H**, *Pk1* expression in P5 hippocampus. **I**, *Pk1* expression in olfactory bulb (OB) and hair follicles (HF) at P5. **J**, *Pk1* expression in P5 retina (Re), lens (Le) and hair follicles (HF). **K-O**, *Pk1* sense probe control in situ hybridization. **K**, E16.5 retina. **L**, P0 retina. **M**, P5 retina. **N**, P21 retina. **O**, P0 hippocampus.

**Supplemental Figure 2. *Pk1* expression in striatum and thalamus revealed by the endogenous EYFP reporter.** **A**. Overlapping of *Pk1* (green) and NeuN (red) labeling in the striatum of the forebrain (note merged red and green channels). **B**. Overlapping of *Pk1* (green) and NeuN (red) labeling in the posterior

thalamus (note merged red and green channels). **C-D**. Section levels of the images: C, for panels in A, striatum (ST); D, for panels in B, thalamus (TH). CCX, cerebral cortex; SC, superior collicullus; HT, hypothalamus; MM, medial mammillary nucleus. Short lines indicate cells that are NeuN positive only.

**Supplemental Figure 3. Validation of *Pk1* shRNAi in HEK293 cells and primary neuronal culture.**

**A.** shRNA efficiently knocks down *Pk1* expression in HEK 293 cells. **a)** Overexpression of *Pk1* in HEK 293 cells detected by anti-Pk1 antibody (red). **b)** shRNA and *Pk1* cotransfected cells greatly reduce Pk1 expression (red). **c)** Cotransfected GFP (green) with Pk1 (red). **d)** Cotransfected GFP, shRNA (green), and Pk1 (IHC red). *Pk1* but not GFP expression is knocked down by shRNA (compared **c)** with **d)**). **B,** GFAP-positive feeder astrocytes in neuronal culture. **C,** Map2/GFAP staining of the same field showing healthy hippocampal neurons on top of the astrocytes. **D,** Zoom-in of hippocampal neurons stained with anti-MAP2 at day 7 (DIV7) culture.

**Supplemental Figure 4. Expression and subcellular localization of Pk1 and its mutant proteins in HEK 293 cells.**

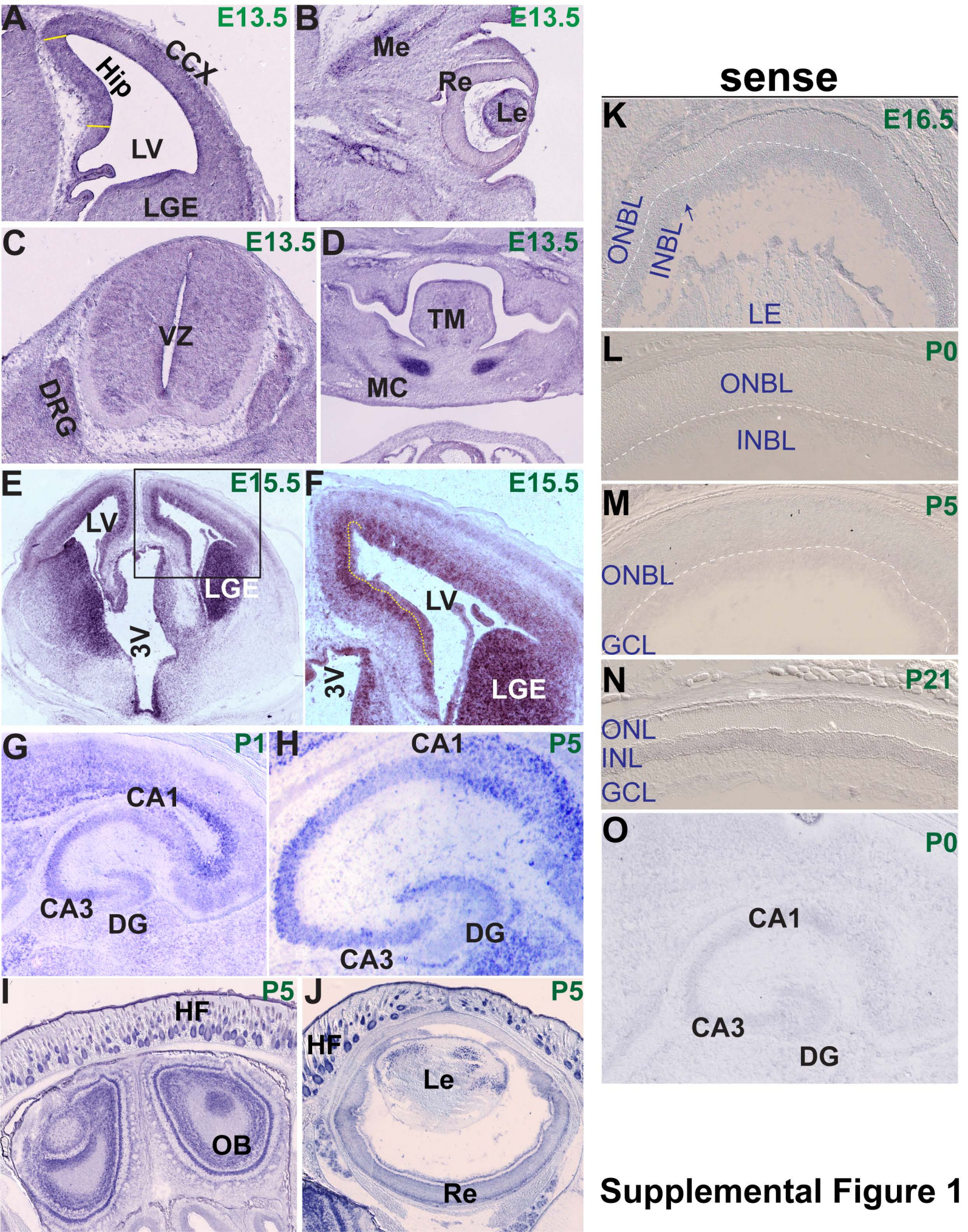
**A,** Graphical illustration of GFP- and myc-tagged full-length (FL) Pk1, and various deletion constructs used in this study. Different deletion constructs are named by an affixed “ $\Delta$ ” to the deleted domains. Color codes indicate domains and tags in each construct. **B-C.** Referring to panel order from top to bottom: localization of Pk1 full-length (Pk1-

FL) in the nuclear envelope and membrane protrusions in the nucleus (white arrows); deletion of PET (DeltaPET), LIM (DeltaLIM), CIIS (DeltaCIIS), and both LIM and CIIS (DeltaLIM/CIIS) cause vesicle-like localization of Pk1 on nuclear membrane (yellow arrows); deletion of PET and CIIS (DeltaPET/CIIS) causes nuclear localization of Pk1 (green arrows); while deletion of PET and LIM (DeltaPET/LIM) causes complete cytoplasmic localization. Deletion of PET, LIM and CIIS (DeltaPET/ LIM/CIIS) altogether cause similar phenotypes as deletion of PET and LIM. **D**, Differential localization of Pk1 deletion construct compared with GFP-tagged full-length Pk1 within the same cell. Myc-tagged DeltaPET/CIIS localizes solely in the nucleus (red and yellow), while GFP-tagged full-length Pk1 localizes on the both nuclear envelope and nucleus (green and yellow). **E**, Differential localization of DeltaPET/LIM/CIIS compared with GFP-tagged full-length Pk1. With PET, LIM and CIIS deletion, Pk1 protein mostly localizes in the cytoplasm (red, encircled by dashed lines), while GFP-tagged Pk1 remains in the nucleus (green, arrow).

**Supplemental Figure 5. Expression of Pk1 during retinal development and its downregulation in *Nrl*<sup>-/-</sup> retina. A.** qPCR detection of *Pk1* expression at different developmental stages in both wild-type (green bars) and *Nrl*<sup>-/-</sup> (blue bars) retinas. All stages of *Pk1* expression are normalized to embryonic day 14 (E14). Dashed line indicates basal level expression at E14 (arbitrarily defined as 1). A decreased expression of *Pk1* in *Nrl*<sup>-/-</sup> retina is consistently observed (blue

bars). **B.** Western blot using Pk1 specific antibody shows reduced protein expression in 4-week old *Nrl*<sup>-/-</sup> retina. **C.** eYFP reporter revealed widely-expressing *Pk1* by retinal neurons including ONL cells at P21. **D-F,** Expression of Pk1 (green) in cones (red) identified by co-labeling of GFP with cone arrestin (CAR) (above short lines).

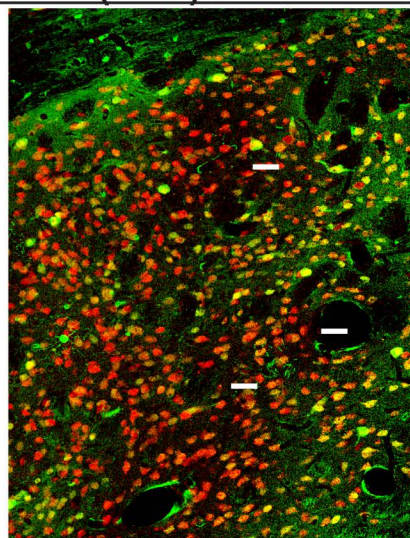
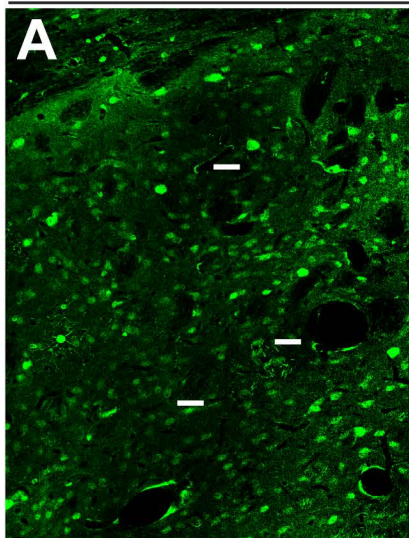
**Supplemental Figure 6. Normal rhodopsin trafficking in shRNA-transfected photoreceptors. A-C.** Relative normal rhodopsin expression and localization in shRNA-transfected photoreceptors at both P8 and P21. Arrowheads point to rhodopsin. **D-E,** Some shRNA photoreceptors develop outer segment (arrowhead in E). Red is Rds immunostaining.



Supplemental Figure 1

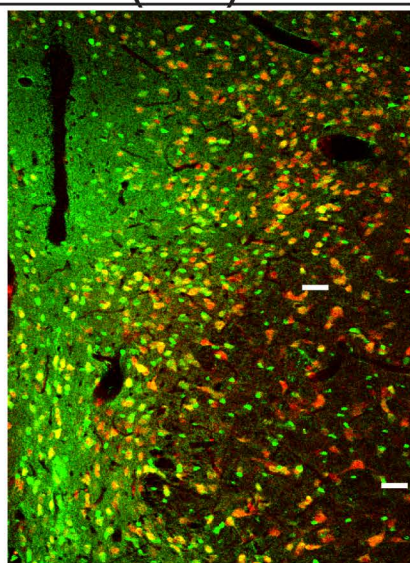
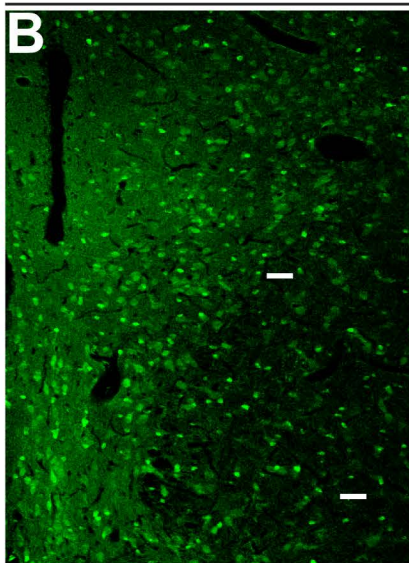
# Striatum (ST)

EYFP/NeuN

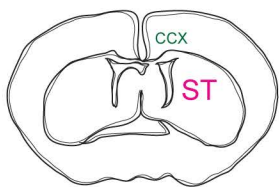


# Thalamus (TH)

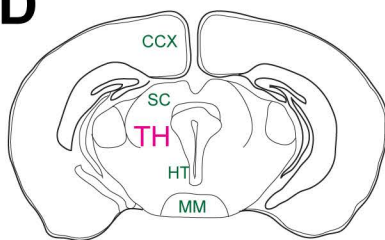
EYFP/NeuN



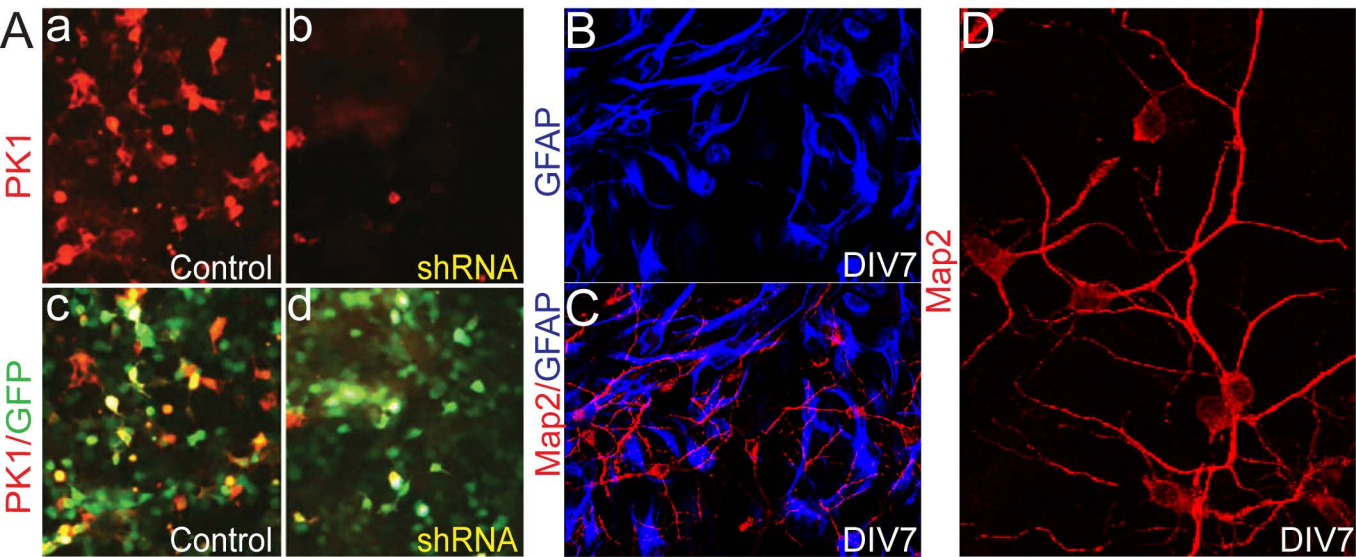
**C**



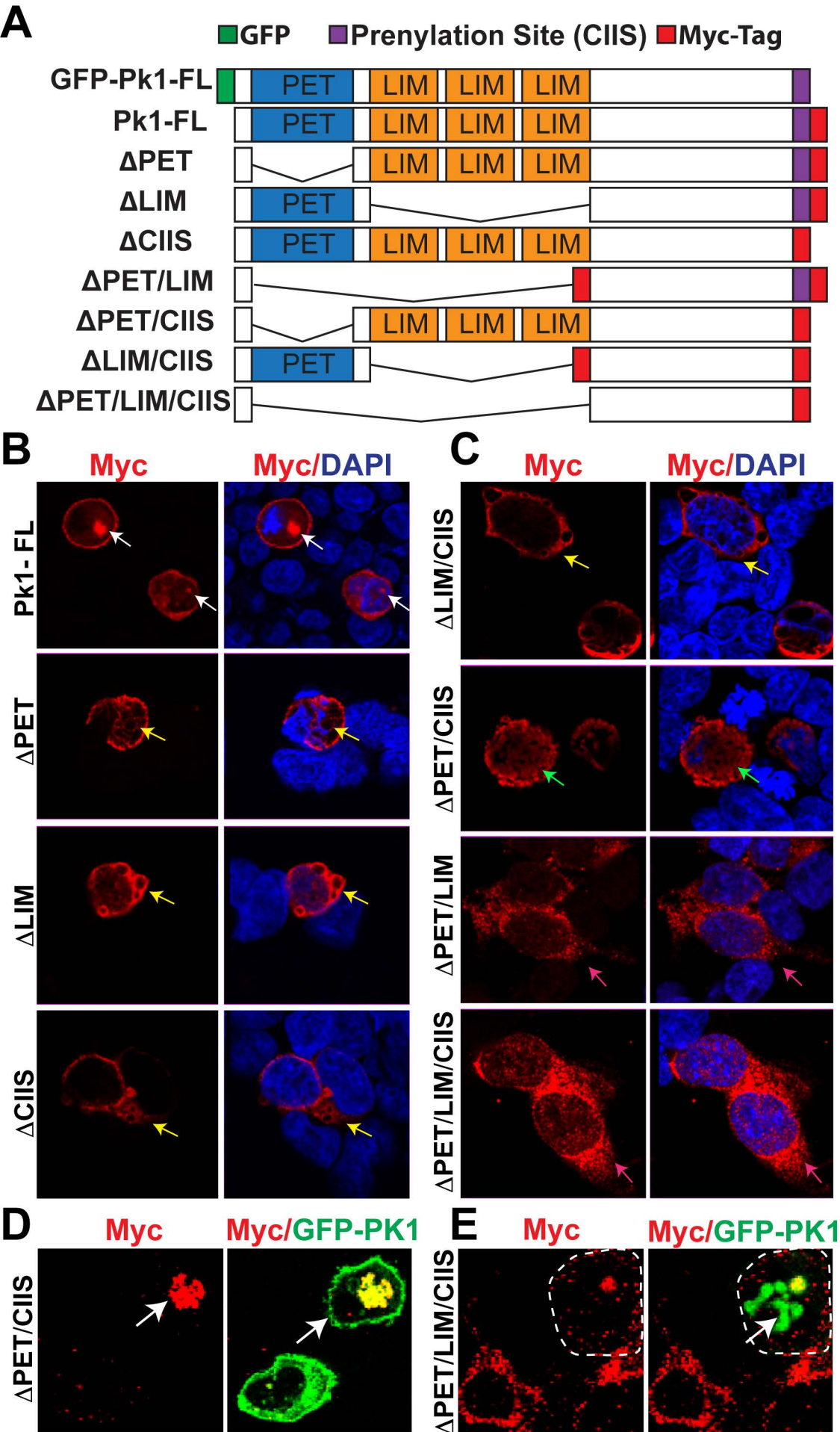
**D**



Supplemental Figure 2

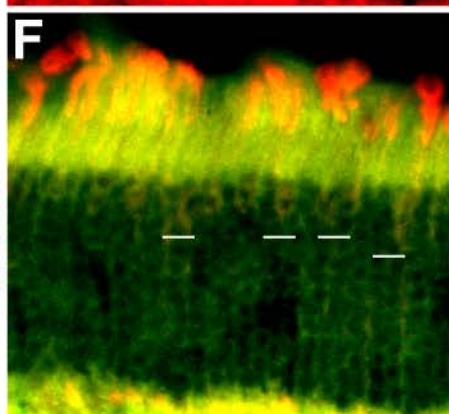
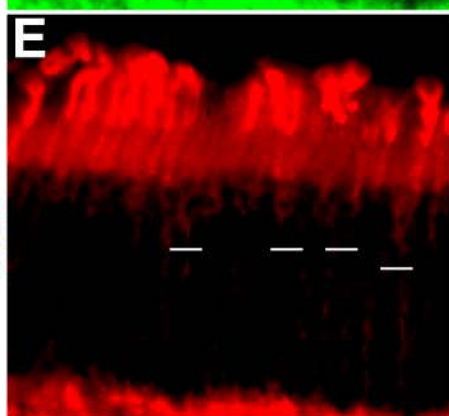
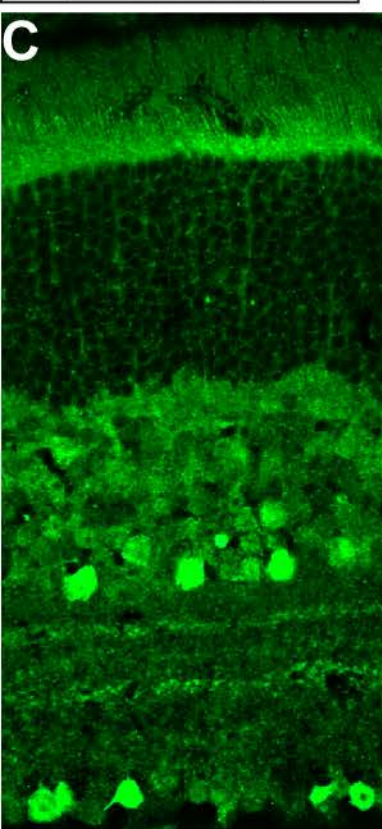
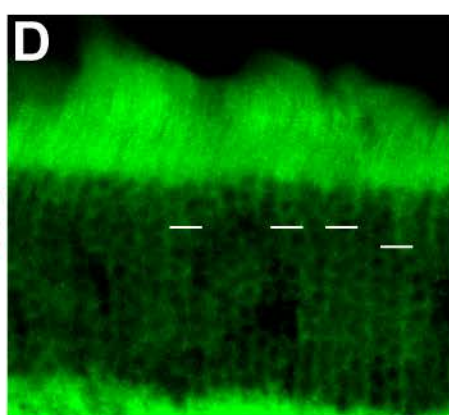
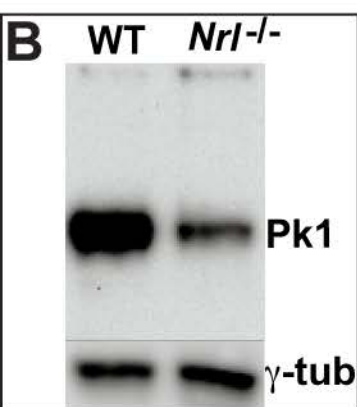
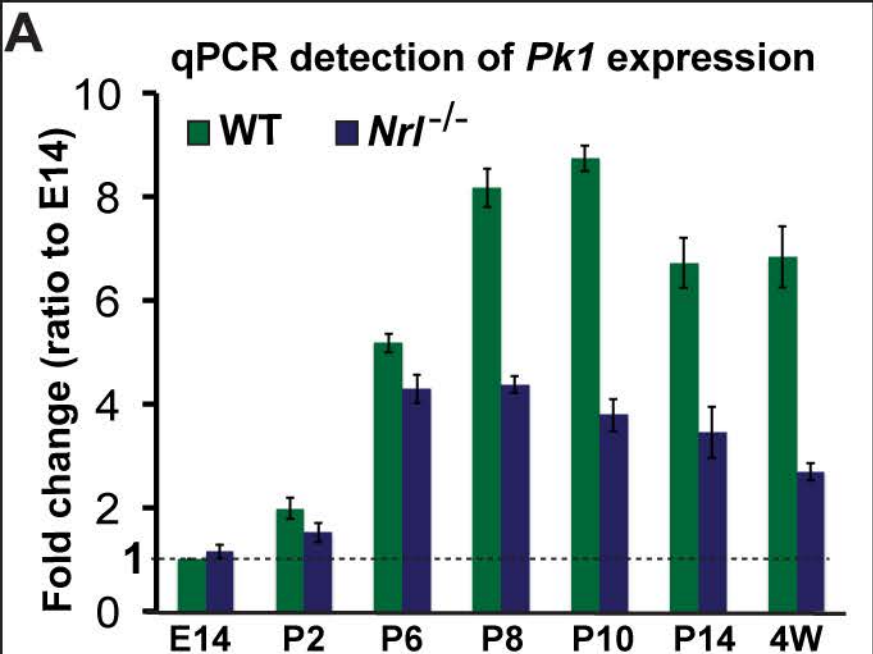


**Supplemental Figure 3**

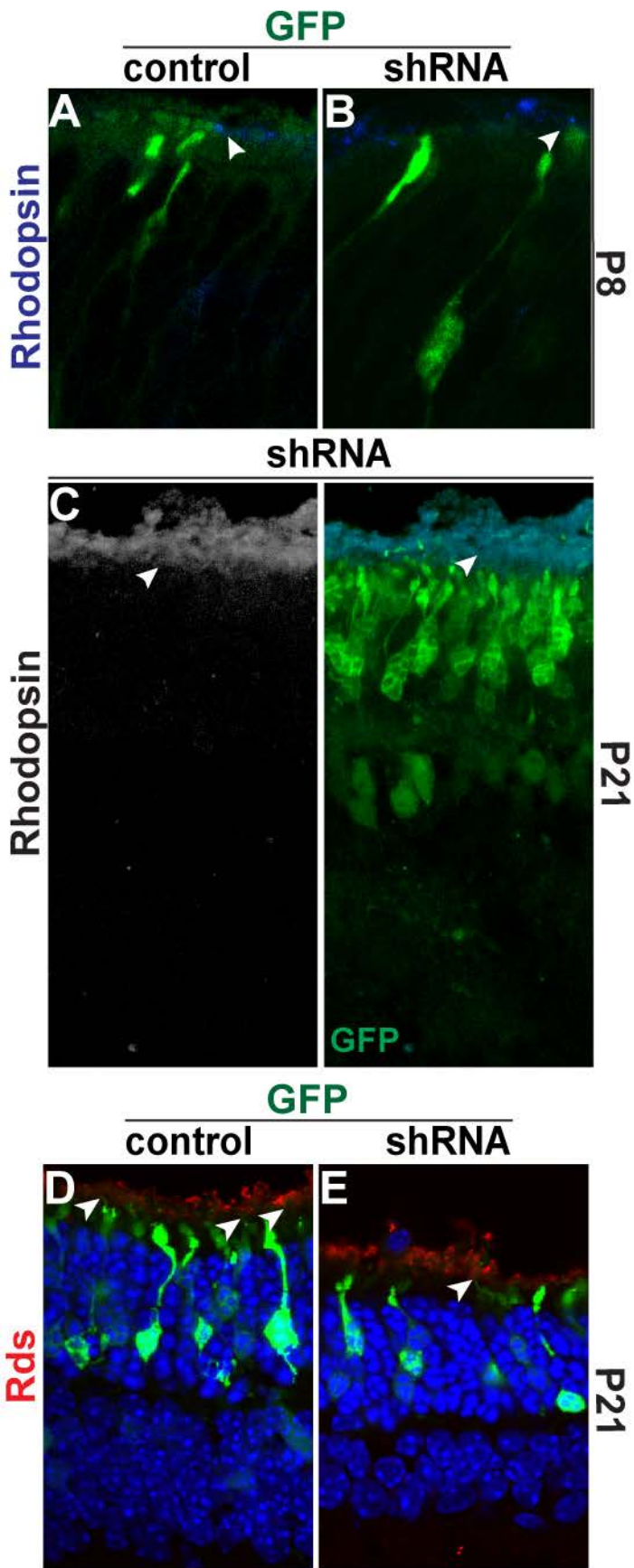


Supplemental Figure 4





Suppelemental Figure5



**Supplemental Figure 6**

**Supplemental table 1. Quantification of dendrites in cultured hippocampal neurons**

Constucts	Primary dendrites	average	P positive aberrant neuronal morpholog		Aberrant percentage
Control GFP	3+2+5+4+4+3+5+3+2+3+3+3 +5 +4	3.5	16	2	12.5
ShRNA	3+4+5+3+4+3+5+4+3	3.7	12	9	75
dPet	3+4+5+4+4+4+3+3	3.75	11	8	72.72727273
dPet/Lim	3+4+5+4+4+3+5+4+3	3.89	14	9	78.57142857
dLim/CIIS	3+4+5+5+4+3+5+3+3+3+3	3.72	14	11	85.71428571
dPet/lim/CIIS	3+5+5+4+5+3+5+4+3	4.11	11	9	81.81818182

Dendritic density: Crossing points/50um-diameter circle		average	STDEV	total neurons analysed
Control GFP	2+3+4+2+3+4+4	3.14285714	0.899735411	7
ShRNA	10 +12+9+7+6+7	8.5	2.258317958	6
dPet	6+8+6+5+7+5	6.16666667	1.169045194	6
dPet/Lim	5+5+6+4+5+7	5.33333333	1.032795559	6
dLim/CIIS	7+6+5+6+7+8	6.5	1.048808848	6
dPet/lim/CIIS	4+3+5+4+3+5+5	4.14285714	0.899735411	7

**Dendritic crossing points**

GFP construct	shRNA	dPet	dPet/Lim	dPet/CIIS	dPet/lim/ciis
2	10	6	5	7	4
3	12	8	5	6	3
4	9	6	6	5	5
2	7	7	4	6	4
3	6	5	5	7	3
4	7	5	7	8	5
4					5
<b>TTest P value</b>	5.99674E-05 gfp-shRNA	0.000263446	0.0017876 gfp-dpet gfp-dpet/lim	6.54162E-05 gfp/dpet/ciis	0.059695853 gpf-dpet/lim/CIIS

Supplemental table 2. Quantification of photoreceptor axon terminals and inner/outer segment length (IS+OS) using transrected GFP.

No. of PRs	P9 control		No. of PRs	P14 control		No. of PRs	P21 control	
	IS + OS	P9 shRNA IS +OS		IS +OS	P14 shRNA IS +OS		IS+OS	P21 shRNA IS+OS
1	107.935	103.325	1	102.821	102.325	1	64.195	31.953
2	106.283	75.18	2	101.213	77.19	2	111.288	66.038
3	120.167	100.319	3	88.906	89.319	3	90.449	73.027
4	113.719	132.85	4	93.42	121.85	4	136.015	49.497
5	102.181	67.268	5	85.321	77.264	5	128.81	48.508
6	93.68	106.231	6	93.683	98.233	6	83.726	50.16
7	63.953	110	7	62.923	80.06	7	105.043	70.859
8	69.462	85.147	8	70.461	85.142	8	104.01	61.685
9	109.11	98.082	9	105.12	88.08	9	106.892	76.792
10	96.84	89.538	10	93.84	77.438	10	128.876	69.065
11	117.047	84.172	11	112.044	64.162	11	132.197	70.859
12	98.351	75.472	12	88.353	75.481	12	109.005	41.195
13	95.336	73.11	13	95.345	76.15	13	114.018	76.007
14	118.106	75.213	14	98.762	75.211	14	102.489	62
15	107.154	32.202	15	107.131	33.205	15	94.048	47.802
16	65.299	36.139	16	80.23	38.138	16	68.029	44.911
17	89.961	55.082	17	89.554	56.082	17	82.152	57.079
18	106.452	81.056	18	103.451	79.056	18	109.659	67.03
19	98.433	38.601	19	98.435	68.601	19	121.202	42.059
20	122.381	67.03	20	112.361	57.031	20	89.56	32.14
21	119.549	73.763	21	119.538	73.773	21	95.352	37.108
22	104.547	63.6	22	101.547	63.63	22	91.022	44.553
23	87.92	94.202	23	87.927	84.202	23	93.086	51.039
24	71.176	77.026	24	70.177	56.026	24	92	52.612
25	102.528	59.54	25	98.528	79.55	25	94.133	75.107
26	123.033	70.711	26	100.035	77.712	26	87.207	65.97
27	136.125	38.079	27	126.135	58.089	27	98.858	49
28	108.301	94.366	28	109.443	64.213	28	94.048	41.11
29	113.146	56.569	29	103.156		29	56.515	39.294
30	103.817	67.119	30	105.673		30	64.07	
31	113.296	50.488	31	115.297		31	94.133	
<b>Average</b>	102.7512258	75.20903226		97.44612903	74.1861786	<b>Average</b>	98.13183871	54.9813448
<b>STDEV</b>	17.35026299	23.55851132		14.17992486	18.2086567	<b>STDEV</b>	19.7659235	13.911328
<b>P value</b>	1.0865E-06			9.24027E-07		<b>P value</b>	8.91575E-14	

No. of sections	P9 control		No. of sections	P9 shRNA	
	Axon terminals/cells x100			Axon terminals/cellsx100	
1	0.52	52	1	0.342857143	34.28571429
2	0.5111111111	51.11111111	2	0.375	37.5
3	0.588235294	58.82352941	3	0.3111111111	31.11111111
<b>Average</b>	0.342989418	34.2989418	<b>Average</b>	0.342989418	34.2989418
<b>STDEV</b>	0.042196381	4.21963808	<b>STDEV</b>	0.03194465	3.194464984
<b>P value</b>	0.001495206				

No. of sections	P14 control		No. of sections	P14 shRNA	
	Axon terminals/cells x100			Axon terminals/cellsx100	
1	0.46	46	1	0.416666667	41.6666667
2	0.511627907	51.1627907	2	0.333333333	33.3333333
3	0.55	55	3	0.369565217	36.95652174
<b>Average</b>	0.373188406	50.72093023	<b>Average</b>	0.373188406	37.31884058
<b>STDEV</b>	0.045162407	4.516240749	<b>STDEV</b>	0.041784647	4.178464708
<b>P value</b>	0.009778386				

No. of sections	P21 control		No. of sections	P21 shRNA	
	axon terminals/cell nox100			axon terminal/cell number	
1	0.416666667	41.6666667	1	0.25	25
2	0.470588235	47.0588235	2	0.26	26
3	0.518518519	51.8518519	3	0.24	24
<b>Average</b>	0.46859114	46.85911402	<b>Average</b>	0.25	25
<b>STDEV</b>	0.05095287	5.09528657	<b>STDEV</b>	0.01	1
<b>P value</b>	0.001880918				

shRNA		Control	
<b>P: P9 vs P14</b>	0.376279256	<b>P: P9 vs P14</b>	0.412988742
<b>P: P14 vs P21</b>	0.00767247	<b>P: P14 vs P21</b>	0.381535384
<b>P: P9 vs P14</b>	0.00857487	<b>P: P9 vs P14</b>	0.135802261