

Supplemental Information

Melanopsin modulates refractive development and myopia

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Abbreviations: mRGC = melanopsin-expressing retinal ganglion cell; DA = dopamine; DOPAC = L-3,4-dihydroxyphenylalanine; FD = form-deprivation; L-DOPA = L-3,4-dihydroxyphenylalanine; CT = corneal thickness; ACD = anterior chamber depth; LT = lens thickness; VCD = vitreous chamber depth; RT = retinal thickness; AL = axial length; CC = corneal curvature; MEA = two-way repeated-measures mixed-effects analysis; HSK = Holm-Sidak multiple comparisons posthoc test

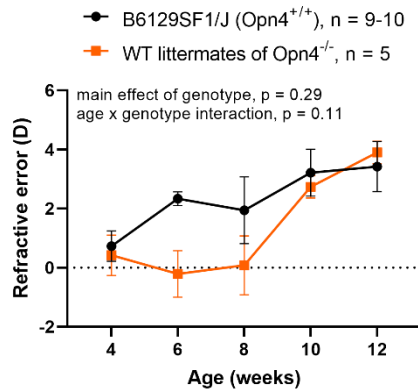


Figure S1. Comparison of refractive development in wildtype controls. *Opn4*^{+/+} (B6129SF1/J, black) and wildtype littermates of *Opn4*^{-/-} mice (orange) had similar refractive error at 4 weeks of age and developed to similar hyperopic refractions after 10 weeks of age. Despite more negative refractions at 6 and 8 weeks in wildtype littermates of *Opn4*^{-/-} than B6129SF *Opn4*^{+/+} mice, there were no significant age by genotype interactions. Data are presented as mean \pm SEM. Comparisons were performed with RM two-way mixed-effects analysis (MEA) with Holm-Sidak multiple comparisons (HSK) tests.

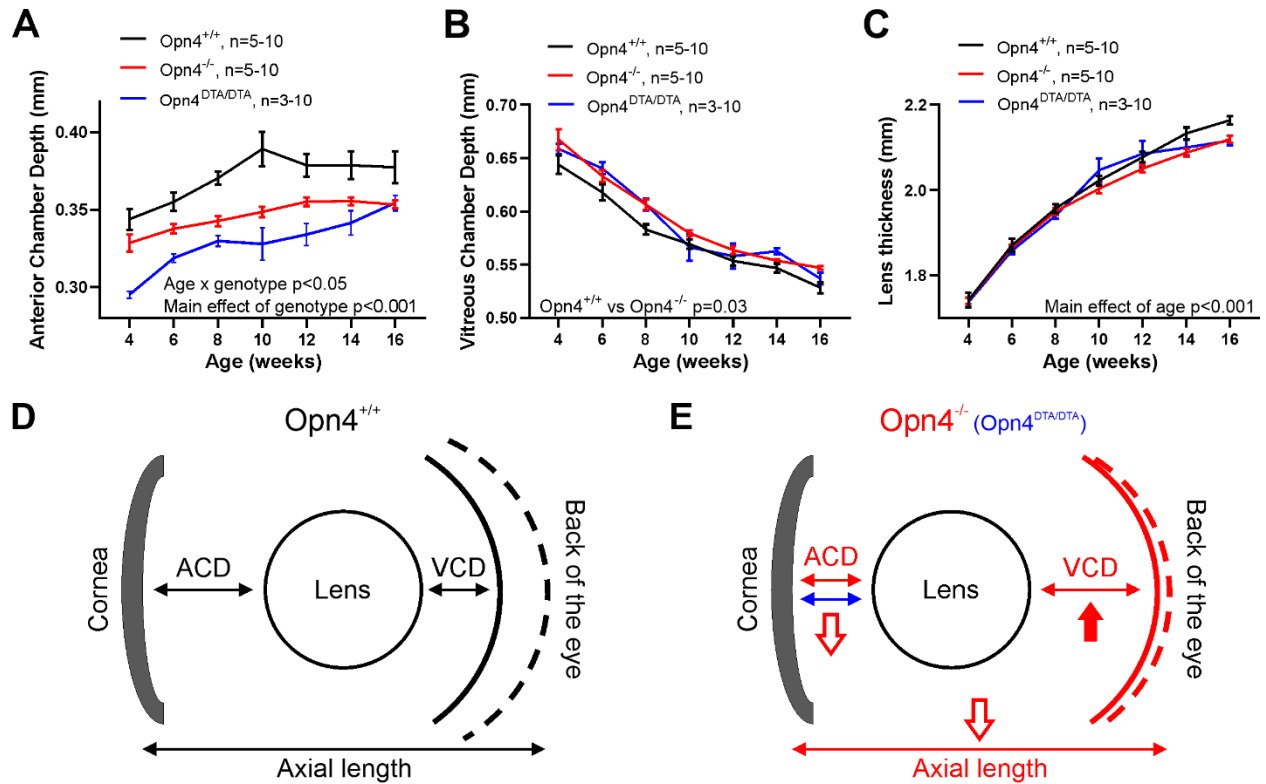


Figure S2. Loss of melanopsin alters ocular dimensions. A) *Opn4*^{-/-} (red) and *Opn4*^{DTA/DTA} mice (blue) had significantly shorter anterior chamber depths (ACD) than *Opn4*^{+/+} mice (black) across almost all ages. B) *Opn4*^{-/-} but not *Opn4*^{DTA/DTA} mice had a slight but significantly longer vitreous chamber depth (VCD) than *Opn4*^{+/+} mice across all ages. C) There was no difference in lens thickness (LT) between *Opn4*^{+/+}, *Opn4*^{-/-}, and *Opn4*^{DTA/DTA} mice. D-E) Cartoon showing major changes in ocular structure after 16 weeks of eye development in *Opn4*^{+/+} (D) and *Opn4*^{-/-} / *Opn4*^{DTA/DTA} mice (E) where a lack of melanopsin may result in a slight anterior shift of the crystalline lens causing a change in ocular chamber dimensions. Red arrows indicate an increase (solid) or decrease (open) in the measured value compared to *Opn4*^{+/+} mice. Data are presented as mean \pm SEM. Comparisons were performed with MEA with HSK.

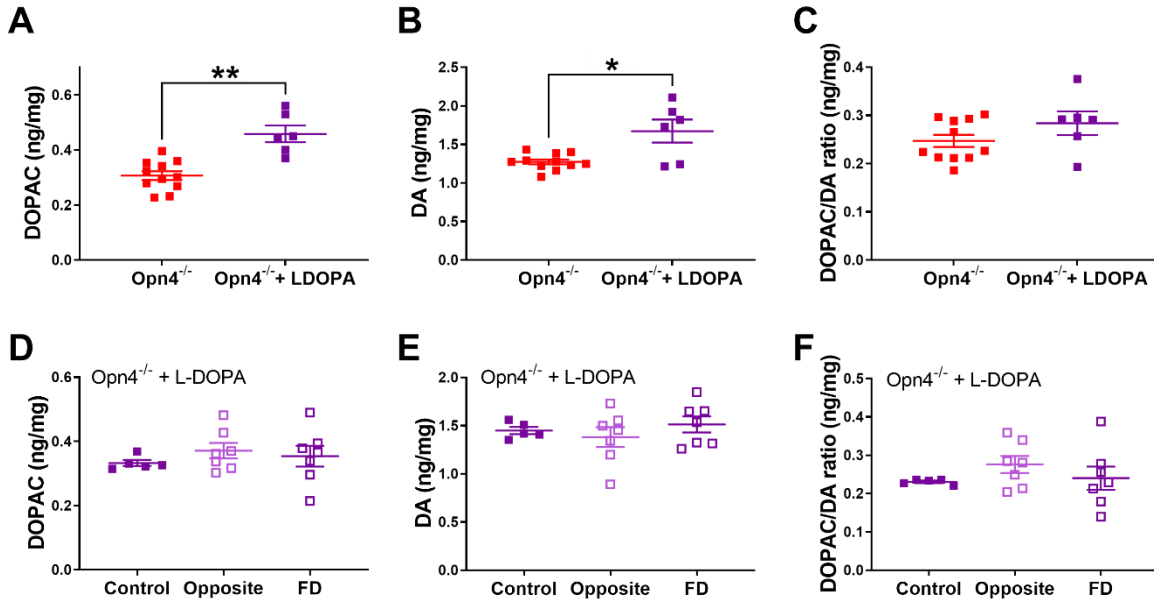


Figure S3. L-DOPA treatment increases DOPAC and DA levels in non-FD *Opn4*^{-/-} mice but does not affect levels after form-deprivation (FD). DA (A) and DOPAC (B) were significantly higher in *Opn4*^{-/-} mice after L-DOPA treatment (purple, n=11) than untreated *Opn4*^{-/-} mice (red, n=6). C) There was no change in the DOPAC/DA ratio with L-DOPA treatment. D-F) There was no significant difference between *Opn4*^{-/-} control (solid dark symbols, n=5), *Opn4*^{-/-} opposite (open light symbols, n=7), or *Opn4*^{-/-} FD (open dark symbols, n=7) retinas with L-DOPA treatment in levels of DOPAC, DA, or the DOPAC/DA ratio. However, DOPAC and DA levels in L-DOPA treated FD eyes were significantly higher than non-treated FD eyes ($p < 0.01$, data not shown). Data are presented as mean \pm SEM. Comparisons were performed in A-C with Welch's t-test and D-J with one-way ANOVA with HSK where * $p < 0.05$ and ** $p < 0.001$.

Table S1: Development of axial and corneal parameters in *Opn4^{+/+}* and *Opn4^{-/-}* mice under standard laboratory visual conditions (intact vision) including axial length (AL), anterior chamber depth (ACD), vitreous chamber depth (VCD), lens thickness (LT), retinal thickness (RT), corneal thickness (CT), and corneal curvature (CC). Data are presented as mean (top) and SEM (bottom). Comparisons between *Opn4^{+/+}* and *Opn4^{-/-}* mice were performed with RM two-way mixed-effects analysis (MEA) with Holm-Sidak multiple comparisons (HSK) where * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. NS = not significant.

Genotype	Age (wks)	AL (mm)	ACD (mm)	VCD (mm)	LT (mm)	RT (mm)	CT (mm)	CC (mm)
MEA (main effect of genotype)		(1,18) 6.468*	(1, 18) 25.18***	(1, 18) 5.86*	NS	(1,18) 5.118*	(1,18) 13.90**	NS
<i>Opn4^{+/+}</i> n=5-10	4	3.050 0.010	0.344 0.007	0.644 0.009	1.742 0.017	0.236 0.006	0.102 0.003	1.351 0.011
	6	3.178 0.013	0.355 0.006	0.618 0.007	1.871 0.015	0.228 0.003	0.108 0.003	1.429 0.008
	8	3.246 0.011	0.370 0.004	0.583 0.005	1.957 0.010	0.232 0.003	0.102 0.002	1.465 0.006
	10	3.304 0.009	0.389 0.011	0.569 0.004	2.022 0.011	0.230 0.004	0.108 0.004	1.503 0.006
	12	3.346 0.009	0.379 0.008	0.553 0.004	2.077 0.012	0.231 0.004	0.103 0.002	1.523 0.006
	14	3.399 0.009	0.379 0.009	0.547 0.004	2.133 0.014	0.233 0.003	0.106 0.003	1.536 0.005
	16	3.422 0.011	0.377 0.010	0.528 0.005	2.164 0.010	0.233 0.007	0.103 0.002	1.562 0.007
<i>Opn4^{-/-}</i> n=5-12	4	3.066 0.008	0.328 0.006	0.667 0.010	1.740 0.007	0.222 0.004	0.082 0.004*	1.365 0.004
	6	3.150 0.013	0.338 0.003	0.633 0.006	1.865 0.007	0.213 0.004	0.093 0.003*	1.435 0.004
	8	3.211 0.015	0.343 0.003***	0.606 0.005*	1.950 0.009	0.215 0.004*	0.099 0.001	1.471 0.006
	10	3.255 0.014*	0.349 0.003*	0.579 0.003	2.003 0.011	0.219 0.003	0.099 0.001	1.496 0.009
	12	3.289 0.012**	0.355 0.003	0.564 0.004	2.051 0.009	0.224 0.005	0.097 0.002	1.520 0.009
	14	3.320 0.014**	0.355 0.003	0.554 0.001	2.088 0.009	0.225 0.004	0.100 0.001	1.530 0.007
	16	3.344 0.014**	0.353 0.003	0.547 0.002	2.120 0.009	0.223 0.005	0.102 0.002	1.543 0.006

Table S2: Development of axial and corneal parameters in *Opn4^{DTA/DTA}* mice under standard laboratory visual conditions (intact vision) including axial length (AL), anterior chamber depth (ACD), vitreous chamber depth (VCD), lens thickness (LT), retinal thickness (RT), corneal thickness (CT), and corneal curvature (CC). Data are presented as mean (top) and SEM (bottom). Comparisons between *Opn4^{DTA/DTA}* and *Opn4^{+/+}* or *Opn4^{-/-}* mice were performed with MEA with HSK (*Opn4^{DTA/DTA}* vs *Opn4^{+/+}*: *p<0.05, **p<0.01, and ***p<0.001; *Opn4^{DTA/DTA}* vs *Opn4^{-/-}*: +p<0.05, ++p<0.01, and +++p<0.001). NS = not significant.

Genotype	Age (wks)	AL (mm)	ACD (mm)	VCD (mm)	LT (mm)	RT (mm)	CT (mm)	CC (mm)	
<i>Opn4^{DTA/DTA}</i> n=3-12	4	2.996 0.012 ^{**} , ⁺⁺⁺	0.295 0.002 ^{***} , ⁺	0.659 0.005	1.737 0.011	0.207 0.004 ^{**}	0.098 0.002	1.366 0.004	
	6	3.117 0.008 ^{**}	0.319 0.003 ^{***} , ⁺⁺	0.640 0.006	1.858 0.008	0.204 0.003 ^{***}	0.096 0.002	1.436 0.006	
	8	3.177 0.009 ^{***}	0.330 0.003 ^{***}	0.606 0.006	1.940 0.008	0.203 0.003 ^{***}	0.098 0.002	1.471 0.006	
	10	3.218 0.010 ^{***} , ⁺	0.328 0.010 ^{**}	0.566 0.013	2.047 0.028	0.192 0.007 ^{**} , ⁺	0.105 0.005	1.495 0.010	
	12	3.250 0.012 ^{***} , ⁺	0.334 0.007 ^{**}	0.558 0.012	2.086 0.031	0.198 0.007 ^{**} , ⁺	0.102 0.007	1.522 0.007	
	14	3.310 0.014 [*]	0.341 0.008 [*]	0.563 0.003	2.100 0.019	0.208 0.004 ^{**}	0.098 0.002	1.529 0.006	
	16	3.266 0.017 ^{***} , ⁺⁺	0.354 0.005	0.537 0.005	2.116 0.012	0.160 0.011 ^{***} , ⁺⁺	0.096 0.004	1.553 0.007	
	MEA (vs <i>Opn4^{+/+}</i>)		(1,18) 37.33 ^{***}	(1,18) 52.55 ^{***}	NS	NS	(1,18) 50.00 ^{***}	NS	NS
	MEA (vs <i>Opn4^{-/-}</i>)		(1,18) 7.089 ⁺	(1,18) 15.29 ⁺⁺	NS	NS	(1,18) 23.15 ⁺⁺⁺	NS	NS

Table S3: Development of axial and corneal parameters in *Opn4^{+/+}* and *Opn4^{-/-}* mice after form-deprivation (FD) including axial length (AL), anterior chamber depth (ACD), vitreous chamber depth (VCD), lens thickness (LT), retinal thickness (RT), corneal thickness (CT), and corneal curvature (CC). Values are presented as the 'shift' (OD-OS) and normalized to the 4-week baseline measurement for each treatment group. Data are presented as mean (top) and SEM (bottom). Comparisons between control and FD mice and between genotypes were performed with MEA (or ANOVA) with HSK where *p<0.05. NS = not significant.

Genotype and treatment	Age (wks)	AL shift (mm)	ACD shift (mm)	VCD shift (mm)	LT shift (mm)	RT shift (mm)	CT shift (mm)	CC shift (mm)
MEA (main effect of treatment)		NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS
<i>Opn4^{+/+}</i> Control n=6-8	5	0.0031 0.0049	0.0065 0.0043	0.0005 0.0145	-0.0001 0.0028	-0.0048 0.0109	0.0009 0.0029	-0.0063 0.0129
	6	0.0013 0.0024	0.0043 0.0026	0.0118 0.0110	-0.0034 0.0034	-0.0089 0.0075	-0.0025 0.0018	0.0146 0.0090
	7	-0.0085 0.0046	0.0021 0.0019	-0.0104 0.0124	0.0007 0.0047	0.0018 0.0068	-0.0027 0.0033	0.0077 0.0081
<i>Opn4^{+/+}</i> FD n=3-7	5	-0.0051 0.0069	0.0012 0.0034	-0.0062 0.0095	-0.0005 0.0038	-0.0044 0.0072	0.0047 0.0041	0.0162 0.0153
	6	-0.0146 0.0057	-0.0018 0.0049	-0.0098 0.0154	0.0037 0.0038	-0.0045 0.0101	-0.0022 0.0032	0.0146 0.0054
	7	-0.0108 0.0060	-0.0002 0.0033	-0.0113 0.0135	-0.0012 0.0049	0.0044 0.0103	-0.0032 0.0013	0.0131 0.0100
MEA (main effect of treatment)		NS	NS	NS	NS	NS	NS	(1,14) 6.223*
<i>Opn4^{-/-}</i> Control n=4-7	5	-0.0088 0.0048	0.0026 0.0042	0.0052 0.0080	-0.0046 0.0026	-0.0054 0.0066	-0.0066 0.0067	0.0145 0.0094
	6	-0.0098 0.0060	0.0047 0.0046	0.0034 0.0093	-0.0043 0.0023	-0.0085 0.0115	-0.0058 0.0032	0.0069 0.0121
	7	-0.0077 0.0064	0.0017 0.0121	-0.0248 0.0181	0.0119 0.0088	0.0031 0.0138	0.0005 0.0025	0.0062 0.0062
<i>Opn4^{-/-}</i> FD n=7-9	5	0.0011 0.0109	-0.0070 0.0076	-0.0011 0.0072	-0.0080 0.0078	0.0087 0.0136	-0.0038 0.0291	-0.0081 0.0115
	6	0.0025 0.0078	0.0001 0.0032	0.0038 0.0065	0.0024 0.0037	-0.0023 0.0077	-0.0012 0.0022	-0.0064 0.0078
	7	0.0088 0.0075	0.0026 0.0053	0.0005 0.0100	0.0048 0.0047	0.0048 0.0116	-0.0029 0.0034	-0.0186 0.0106
Control MEA (<i>Opn4^{+/+}</i> vs <i>Opn4^{-/-}</i>)		NS	NS	NS	NS	NS	NS	NS
FD MEA (<i>Opn4^{+/+}</i> vs <i>Opn4^{-/-}</i>)		NS	NS	NS	NS	NS	NS	(1,12) 7.491*

Table S4: Development of axial and corneal parameters in *Opn4^{DTA/DTA}* mice after FD including axial length (AL), anterior chamber depth (ACD), vitreous chamber depth (VCD), lens thickness (LT), retinal thickness (RT), corneal thickness (CT), and corneal curvature (CC). Values are presented as the 'shift' (OD-OS) and normalized to the 4-week baseline measurement. Data are presented as mean (top) and SEM (bottom). Comparisons between *Opn4^{DTA/DTA}* and *Opn4^{+/+}*, *Opn4^{-/-}*, and naïve mice were performed with MEA (or ANOVA) with HSK (*Opn4^{DTA/DTA}* vs *Opn4^{+/+}*: *p<0.05). NS = not significant.

Genotype	Age (wks)	AL shift (mm)	ACD shift (mm)	VCD shift (mm)	LT shift (mm)	RT shift (mm)	CT shift (mm)	CC shift (mm)
MEA (main effect of treatment)		NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS
<i>Opn4^{DTA/DTA}</i> N=4-7	5	0.0054 0.0048	0.0026 0.0059	-0.0074 0.0152	0.0022 0.0021	0.0081 0.0133	-0.0001 0.0070	-0.0060 0.0148
	6	0.0038 0.0063	0.0055 0.0031	-0.0185 0.0149	0.0022 0.0044	0.0173 0.0127	-0.0027 0.0067	-0.0058 0.0118
	7	0.0081 0.0074	0.0007 0.0037	-0.0006 0.0075	0.0017 0.0026	0.0064 0.0069	-0.0002 0.0062	-0.0130 0.0186
<i>Opn4^{DTA/DTA}</i> FD n=3-6	5	-0.0005 0.0095	0.0063 0.0059	-0.0122 0.0099	-0.0034 0.0031	0.0110 0.0062	-0.0022 0.0031	-0.0028 0.0039
	6	0.0109 0.0089	0.0060 0.0055	-0.0007 0.0140	-0.0008 0.0019	0.0082 0.0091	-0.0019 0.0023	-0.0184 0.0080
	7	-0.0016 0.0090	0.0090 0.0072	-0.0145 0.0149	-0.0036 0.0027	0.0138 0.0099	-0.0062 0.0056	-0.0117 0.0170
MEA (<i>Opn4^{DTA/DTA}</i> FD vs <i>Opn4^{+/+}</i> FD)		NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	(1,9) 7.157*
MEA (<i>Opn4^{DTA/DTA}</i> FD vs <i>Opn4^{-/-}</i> FD)		NS	NS	NS	NS	NS	NS	NS
MEA (<i>Opn4^{DTA/DTA}</i> FD vs naïve)		NS	NS	NS	NS	NS	NS	NS