

	1	11	21	31	41	51	60
sp Q9UHM6-1 hOPN4	MNPPSGPRVP	PSPTQEPSCM	ATPAPPSWWD	SSQSSISLGL	RLPSISPTAP	GTWAAAWVPL	60
sp Q9UHM6-2 hOPN4 (v2)	MNPPSGPRVP	PSPTQEPSCM	ATPAPPSWWD	SSQSSISLGL	RLPSISPTAP	GTWAAAWVPL	60
sp Q9QXZ9-1 musOpn4L	MDSPSGPRVL	SSLTQDPSFT	TSPALQGIWN	GTQ-NVSVRA	QLLSVSPPTS	AHQAAAWVPF	59
sp Q9QXZ9-2 musOpn4S	MDSPSGPRVL	SSLTQDPSFT	TSPALQGIWN	GTQ-NVSVRA	QLLSVSPPTS	AHQAAAWVPF	59
h/mOpn4L Chimera	MNPPSGPRVP	PSPTQEPSCM	ATPAPPSWWD	SSQSSISLGL	RLPSISPTAP	GTWAAAWVPL	60
	*:*****	. * **:*	::** . *	::* .:* .	.* *:*:.	. *****:	
TM1							
sp Q9UHM6-1 hOPN4	PTVDVPDHAH	YTLGTVILLV	GLTGMLGNLT	VIYTFPCR---	-----SR	SLRTPANMFI	109
sp Q9UHM6-2 hOPN4 (v2)	PTVDVPDHAH	YTLGTVILLV	GLTGMLGNLT	VIYTFCRAVL	RGVTVMMSQR	SLRTPANMFI	120
sp Q9QXZ9-1 musOpn4L	PTVDVPDHAH	YTLGTVILLV	GLTGMLGNLT	VIYTFPCR---	-----NR	GLRTPANMFI	108
sp Q9QXZ9-2 musOpn4S	PTVDVPDHAH	YTLGTVILLV	GLTGMLGNLT	VIYTFPCR---	-----NR	GLRTPANMFI	108
h/mOpn4L Chimera	PTVDVPDHAH	YTLGTVILLV	GLTGMLGNLT	VIYTFPCR---	-----SR	SLRTPANMFI	109
	*****	*****	*****	*****		. * *****	
TM2							
sp Q9UHM6-1 hOPN4	INLAVSDFLM	SFTQAPVFFT	SSLYKQWLFGL	ETGCEFYAFC	GALFGISSMI	TLTAIALDRY	169
sp Q9UHM6-2 hOPN4 (v2)	INLAVSDFLM	SFTQAPVFFT	SSLYKQWLFGL	ETGCEFYAFC	GALFGISSMI	TLTAIALDRY	180
sp Q9QXZ9-1 musOpn4L	INLAVSDFLM	SVTQAPVFFA	SSLYKKWLFGL	ETGCEFYAFC	GAVFGITSMI	TLTAIAMDRY	168
sp Q9QXZ9-2 musOpn4S	INLAVSDFLM	SVTQAPVFFA	SSLYKKWLFGL	ETGCEFYAFC	GAVFGITSMI	TLTAIAMDRY	168
h/mOpn4L Chimera	INLAVSDFLM	SFTQAPVFFT	SSLYKQWLFGL	ETGCEFYAFC	GALFGISSMI	TLTAIALDRY	169
	*****	*.*****:	*****:****	*****	**:*:*:*	*****:***	
TM3							
sp Q9UHM6-1 hOPN4	INLAVSDFLM	SFTQAPVFFT	SSLYKQWLFGL	ETGCEFYAFC	GALFGISSMI	TLTAIALDRY	169
sp Q9UHM6-2 hOPN4 (v2)	INLAVSDFLM	SFTQAPVFFT	SSLYKQWLFGL	ETGCEFYAFC	GALFGISSMI	TLTAIALDRY	180
sp Q9QXZ9-1 musOpn4L	INLAVSDFLM	SVTQAPVFFA	SSLYKKWLFGL	ETGCEFYAFC	GAVFGITSMI	TLTAIAMDRY	168
sp Q9QXZ9-2 musOpn4S	INLAVSDFLM	SVTQAPVFFA	SSLYKKWLFGL	ETGCEFYAFC	GAVFGITSMI	TLTAIAMDRY	168
h/mOpn4L Chimera	INLAVSDFLM	SFTQAPVFFT	SSLYKQWLFGL	ETGCEFYAFC	GALFGISSMI	TLTAIALDRY	169
	*****	*.*****:	*****:****	*****	**:*:*:*	*****:***	
TM4							
sp Q9UHM6-1 hOPN4	LVITRPLATF	GVASKRRAAF	VLLGVWLYAL	AWSLPPFFGW	SAYVPEGLLT	SCSWDYSMFT	229
sp Q9UHM6-2 hOPN4 (v2)	LVITRPLATF	GVASKRRAAF	VLLGVWLYAL	AWSLPPFFGW	SAYVPEGLLT	SCSWDYSMFT	240
sp Q9QXZ9-1 musOpn4L	LVITRPLATI	GRGSKRRAL	VLLGVWLYAL	AWSLPPFFGW	SAYVPEGLLT	SCSWDYMTFT	228
sp Q9QXZ9-2 musOpn4S	LVITRPLATI	GRGSKRRAL	VLLGVWLYAL	AWSLPPFFGW	SAYVPEGLLT	SCSWDYMTFT	228
h/mOpn4L Chimera	LVITRPLATF	GVASKRRAAF	VLLGVWLYAL	AWSLPPFFGW	SAYVPEGLLT	SCSWDYSMFT	229
	*****:	*.*****:	*****	*****	*****	*****:	**
TM5							
sp Q9UHM6-1 hOPN4	PAVRAYTMLL	CCFVFFLPLL	IIICYIFIF	RAIRETGRAL	QTFGACKGNG	ES-LWQR---	285
sp Q9UHM6-2 hOPN4 (v2)	PAVRAYTMLL	CCFVFFLPLL	IIICYIFIF	RAIRETGRAL	QTFGACKGNG	ES-LWQR---	296
sp Q9QXZ9-1 musOpn4L	PQVRAYTMLL	FCFVFFLPLL	IIIFCYIFIF	RAIRETGR--	----ACEGCG	ESPLRQRQW	282
sp Q9QXZ9-2 musOpn4S	PQVRAYTMLL	FCFVFFLPLL	IIIFCYIFIF	RAIRETGR--	----ACEGCG	ESPLRQRQW	282
h/mOpn4L Chimera	PAVRAYTMLL	CCFVFFLPLL	IIICYIFIF	RAIRETGRAL	QTFGACKGNG	ES-LWQR---	285
	*****	*****	***:*****	*****	**:* * * * *		
TM6							
sp Q9UHM6-1 hOPN4	QRLQSECKMA	KIMLLVILLF	VLSWAPYSAV	ALVAFAGYAH	VLTPYMSSVP	AVIAKASAIH	345
sp Q9UHM6-2 hOPN4 (v2)	QRLQSECKMA	KIMLLVILLF	VLSWAPYSAV	ALVAFAGYAH	VLTPYMSSVP	AVIAKASAIH	356
sp Q9QXZ9-1 musOpn4L	QRLQSEWKMA	KVALIVILLF	VLSWAPYSTV	ALVAFAGYSH	ILTPYMSSVP	AVIAKASAIH	342
sp Q9QXZ9-2 musOpn4S	QRLQSEWKMA	KVALIVILLF	VLSWAPYSTV	ALVAFAGYSH	ILTPYMSSVP	AVIAKASAIH	342
h/mOpn4L Chimera	QRLQSECKMA	KIMLLVILLF	VLSWAPYSAV	ALVAFAGYAH	VLTPYMSSVP	AVIAKASAIH	345
	*****	***	*:*****	*****:	*****:	*****	*****
H8							
sp Q9UHM6-1 hOPN4	NPIIYAIHP	KYRVAIAQHL	PCLGVLLGVS	RRHSRPYPSY	RSTHRSTLTS	HTSNLSWISI	405
sp Q9UHM6-2 hOPN4 (v2)	NPIIYAIHP	KYRVAIAQHL	PCLGVLLGVS	RRHSRPYPSY	RSTHRSTLTS	HTSNLSWISI	416
sp Q9QXZ9-1 musOpn4L	NPIIYAIHP	KYRVAIAQHL	PCLGVLLGVS	GQRSHPSLSY	RSTHRSTLSS	QSSDLSWISG	402
sp Q9QXZ9-2 musOpn4S	NPIIYAIHP	KYRVAIAQHL	PCLGVLLGVS	GQRSHPSLSY	RSTHRSTLSS	QSSDLSWISG	402
h/mOpn4L Chimera	NPIIYAIHP	KYRVAIAQHL	PCLGVLLGVS	GQRSHPSLSY	RSTHRSTLSS	QSSDLSWISG	405
	*****	*****	*****	::*:* *	*****:	::*:*:***	
C-TERM							
sp Q9UHM6-1 hOPN4	RRRQESLGSE	SEVGWTHMEA	AAVWGAAQQA	NGRSLYGQGL	EDLEAKAPPR	PQGHEAETPG	465
sp Q9UHM6-2 hOPN4 (v2)	RRRQESLGSE	SEVGWTHMEA	AAVWGAAQQA	NGRSLYGQGL	EDLEAKAPPR	PQGHEAETPG	466
sp Q9QXZ9-1 musOpn4L	RKRQESLGSE	SEVGWTDDET	TAAWGAAQQA	SGQSFCSQNL	EDGELKASSS	PQVQRSKTPK	462
sp Q9QXZ9-2 musOpn4S	RKRQESLGSE	SEVGWTDDET	TAAWGAAQQA	SGQSFCSQNL	EDGELKASSS	PQTK-----	456
h/mOpn4L Chimera	RKRQESLGSE	SEVGWTDDET	TAAWGAAQQA	SGQSFCSQNL	EDGELKASSS	PQVQRSKTPK	465
	*:*****	*****	*.*****	.*:* .:*	** * * * .	** :	
TM7							
sp Q9UHM6-1 hOPN4	-----	KTKGLIPSQD	PR-----	-----	-----	-----M	478
sp Q9UHM6-2 hOPN4 (v2)	-----	KTKGLIPSQD	PR-----	-----	-----	-----M	489
sp Q9QXZ9-1 musOpn4L	VPGFSTCRPM	KGQGARPSSL	RGDQKGRILAV	CTGLSECPHP	HTSQFPLAFL	EDDVTLRHLIM	522
sp Q9QXZ9-2 musOpn4S	-----	---GHLPSLD	LG-----	-----	-----	-----M	466
h/mOpn4L Chimera	VPGFSTCRPM	KGQGARPSSL	RGDQKGRILAV	CTGLSECPHP	HTSQFPLAFL	EDDVTLRHLIM	525
		* **				*	

Fig. S1. Aligned amino acid sequences of hOPN4 and musOpn4 isoforms

Amino acid sequences of hOPN4 (Q9UHM6-1), hOPN4(v2)(Q9UHM6-2), musOpn4L (Q9QXZ9-1), musOpn4S (Q9QXZ9-2) and h/musOpn4L chimera. Sequences aligned using L-INS-I in MAFFT (v7.475) (Kato and Standley, 2013). Transmembrane helices indicated in grey. Helix 8 indicated in dark grey. C-terminal tail indicated in blue. "*" indicates positions with fully conserved residue, ":" indicates strongly similar properties between residues, "." Indicates weakly similar properties between residues.

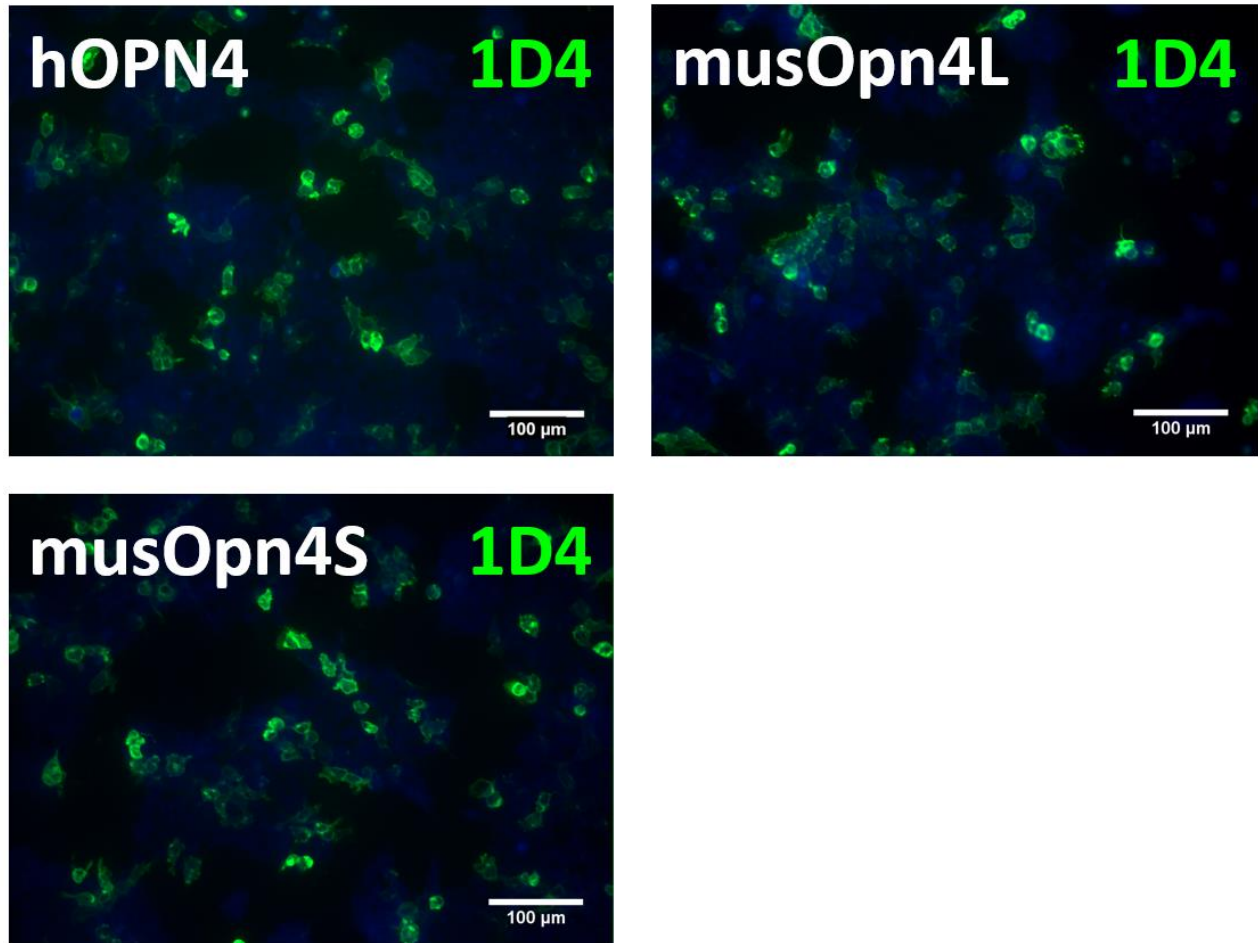


Fig. S2. Melanopsin expression in HEK293 cells Immunocytochemistry photomicrograph showing expression of hOPN4, musOpn4L, and musOpn4S via staining for 1D4 tag in HEK293 cells (in green). All opsins are expressed well and in cell membranes of HEK293 cells (nuclei stained with DAPI, in blue). Scale bar = 100μM.