Supplementary Material

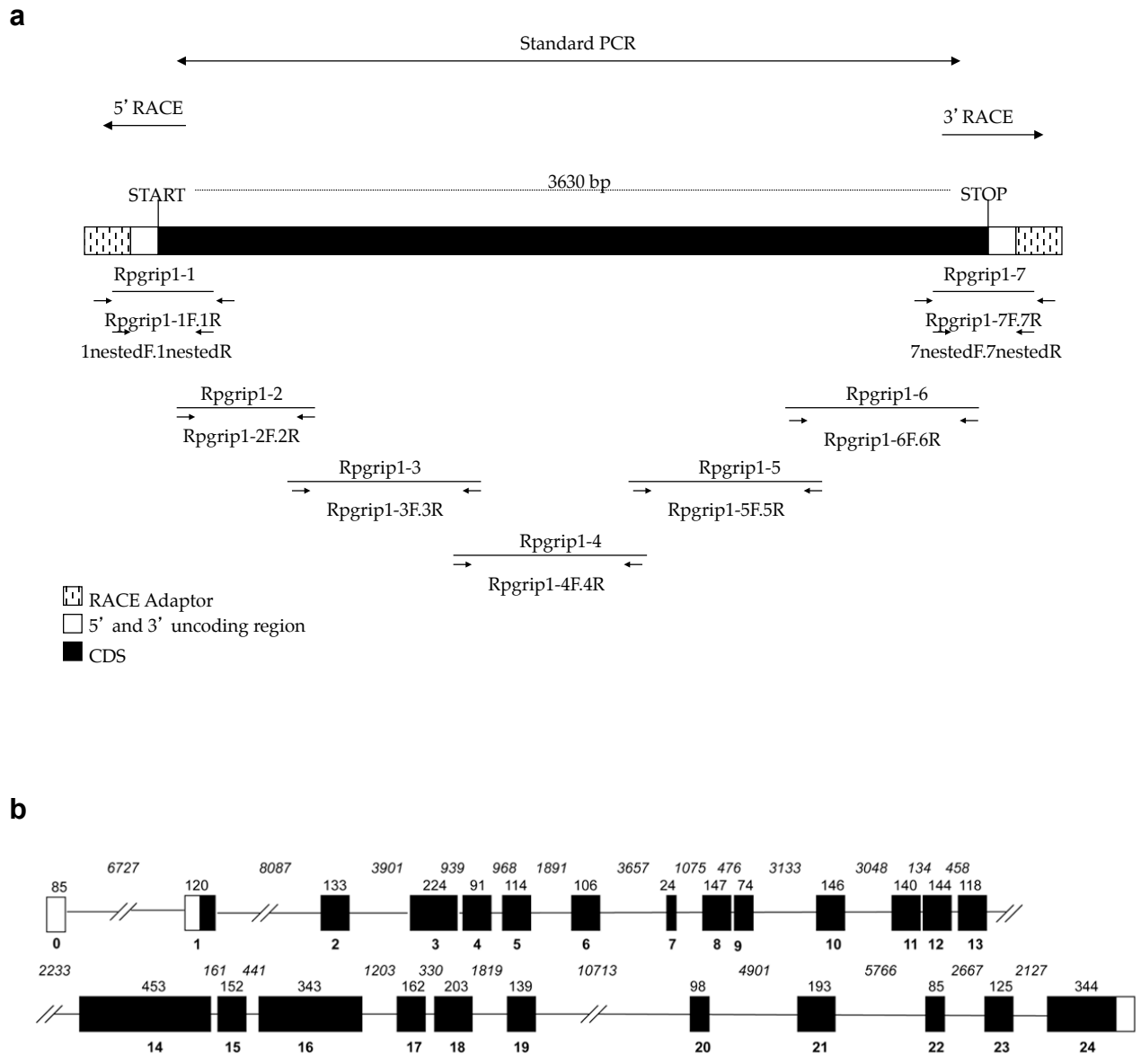

Figure S1. Canine *Rpgrip1* amplification strategy. (**a**) Standard PCR and RLM-RACE PCR amplification strategy. Seven overlapping fragments covering the entire coding region were amplified (Rpgrip1-1 to Rpgrip1-7). (**b**) Canine *Rpgrip1* gene organization. Exons are represented as boxes and introns as lines. White and black boxes represent non-coding and coding exons, respectively. Exon numbers are indicated behind exon boxes. Exon sizes are indicated above exon boxes in base pairs (bp). Intron sizes are indicated in italics above intron lines in base pairs.

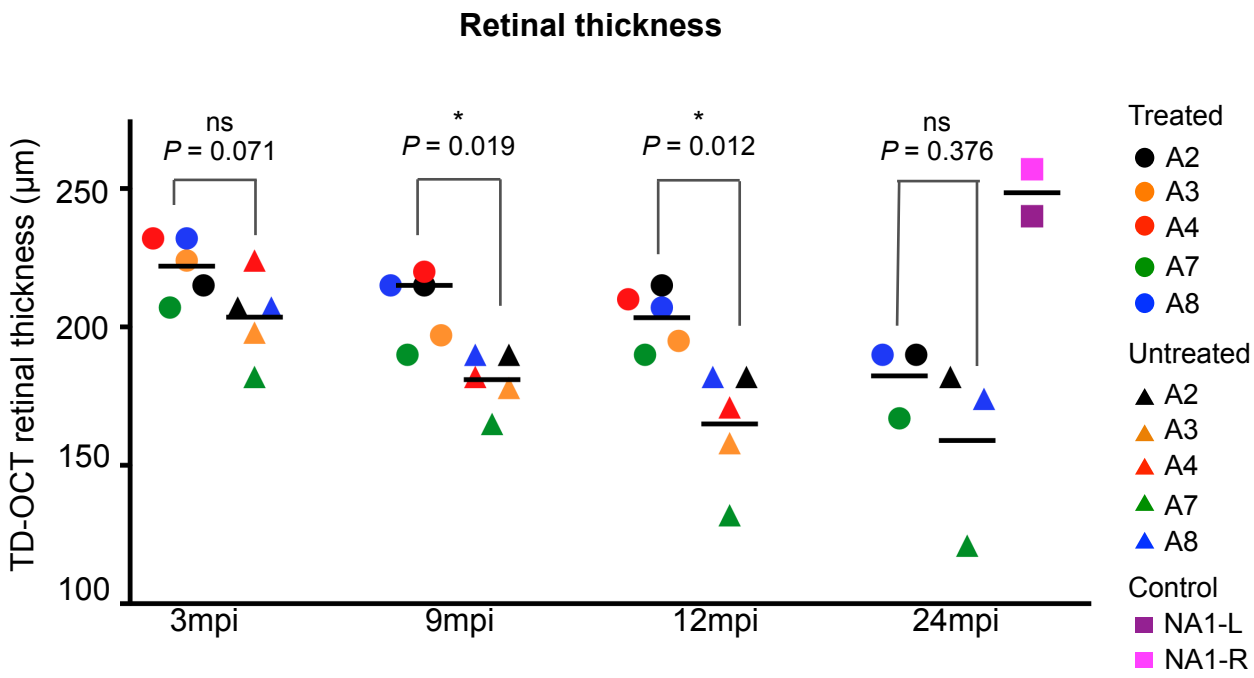

Figure S2. Gene therapy did not halt retinal degeneration in treated MLHD-*Cord1* eyes. Central retinal thickness were measured in treated and untreated eyes of dogs A2, A3, A4, A7 and A8 at 3, 9, 12 and 24 months postinjection (mpi) from TD-OCT scans obtained 13 mm above the optic nerve head. Results obtained in treated and untreated eyes were compared statistically at each time-point using unpaired Mann-Whitney t-test. One and two asterisk(s) indicate(s) significant difference between treated and untreated eyes with *P*-value <0.05 or <0.005, respectively. We note a significant higher retinal thickness in treated eyes compared to untreated eyes at 9 and 12 mpi. Dark bar represents the mean. Ns, non significant. NA1, non-affected *Rpgrip1*$^{+/-}$ dog. L, left eye; R, right eye.

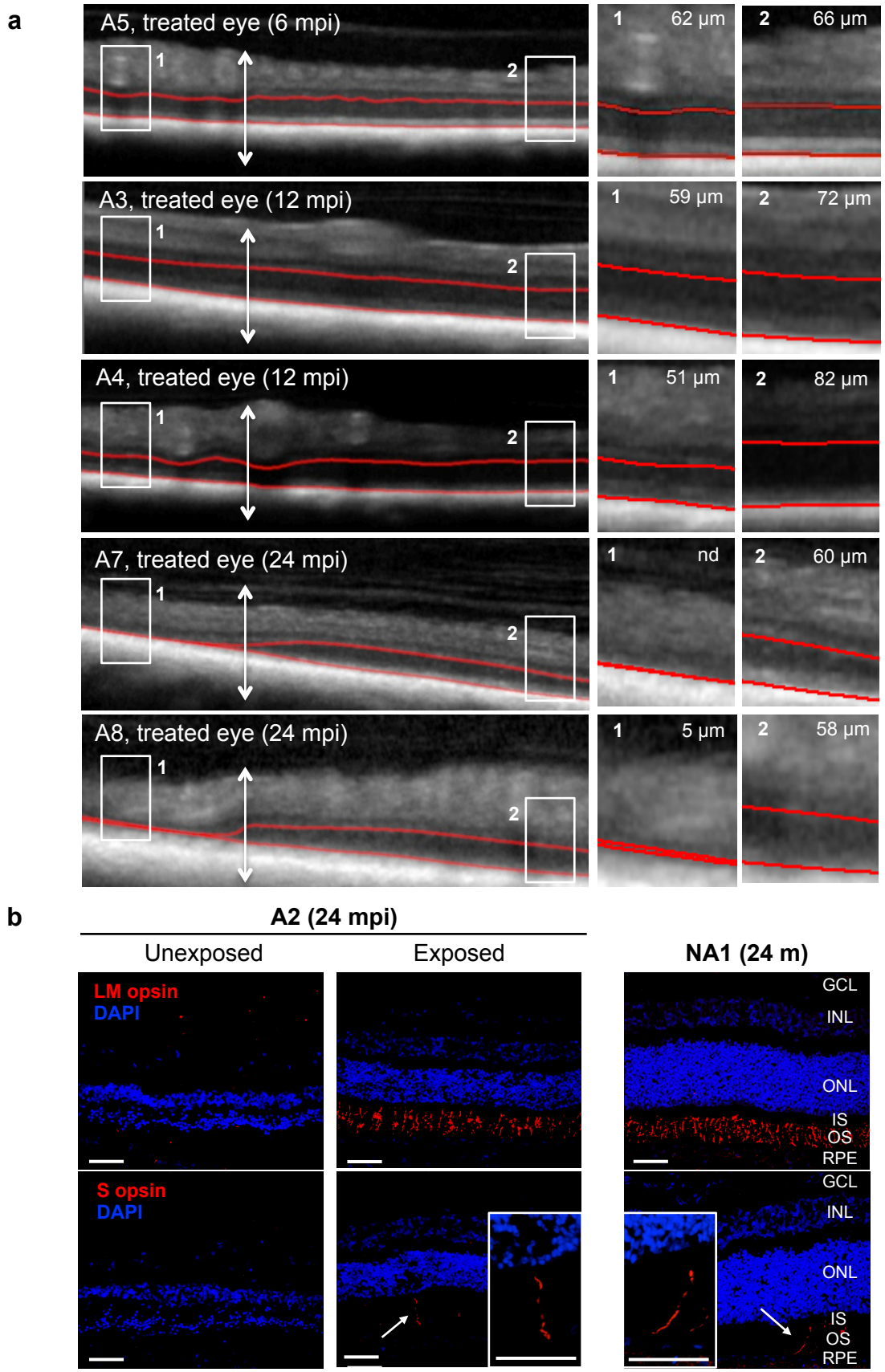

Figure S3. Gene therapy prevented long-term photoreceptor degeneration in all treated MLHD-*Cord1* eyes. (**a**) SD-OCT scans obtained 13 mm above the optic nerve head from the treated eye of dog A5, A3, A4, A7 and A8 at latest timepoint. Photoreceptor layer (ONL + outer and inner segments) was

defined semi-automatically (red). Photoreceptor layer thickness was measured in two normalized temporal (1) and nasal (2) locations. (**b**) Microscopic images of retinal sections from the treated eye of dog A2 at 24 months postinjection (mpi) and the right eye of age-matched control dog NA1. Serial retinal cryosections encompassing the vector-exposed and the vector-unexposed areas of the treated retina were immunolabeled using L/M- (top) or S-opsin (bottom) antibodies. Primary antibodies were detected using Alexa 546-conjugated goat anti-rabbit IgG (red). Cell nuclei were counterstained with DAPI (blue). Scale bar = 30 μm. Abbreviations: m, months of age; GCL, ganglion cell layer; INL, inner nuclear layer; IS, inner segments; ONL, outer nuclear layer; OS, outer segments; RPE, retinal pigment epithelium; nd, not detectable.

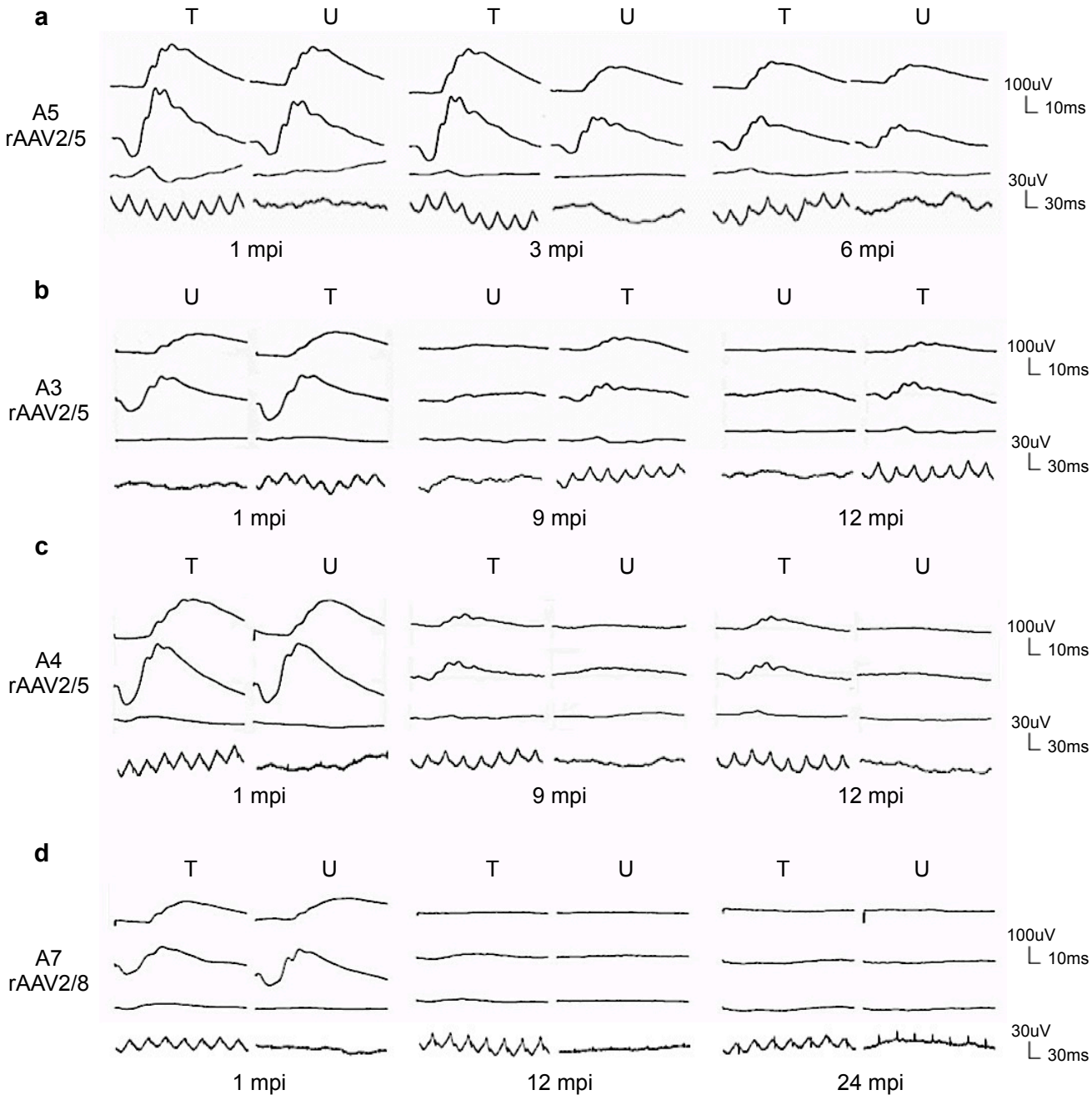

Figure S4. Bilateral full-field electroretinographic traces from dogs A7, A3, A4 and A5. (**a**) Electroretinographic trace from dog A5 treated with AAV2/5-RK-c*Rpgrip1* at 1, 3 and 6 months postinjection (mpi). (**b**) Electroretinographic traces from dog A3 treated with AAV2/5-RK-c*Rpgrip1* at 1, 9 and 12 mpi. (**c**) Electroretinographic traces from dog A4 treated with AAV2/5-RK-c*Rpgrip1* at 1, 9 and 12 mpi. (**d**) Electroretinographic traces from dog A7 treated with AAV2/8-RK-c*Rpgrip1* at 1, 12 and 24 mpi. The top two recordings are low- and high-intensity dark-adapted responses, whereas the bottom two recordings show light-adapted responses (responses to single flash and 30Hz flicker stimuli, respectively). Abbreviations: m, months of age; ms, milliseconds; μV, microvolts; T, treated eye; U, untreated eye.

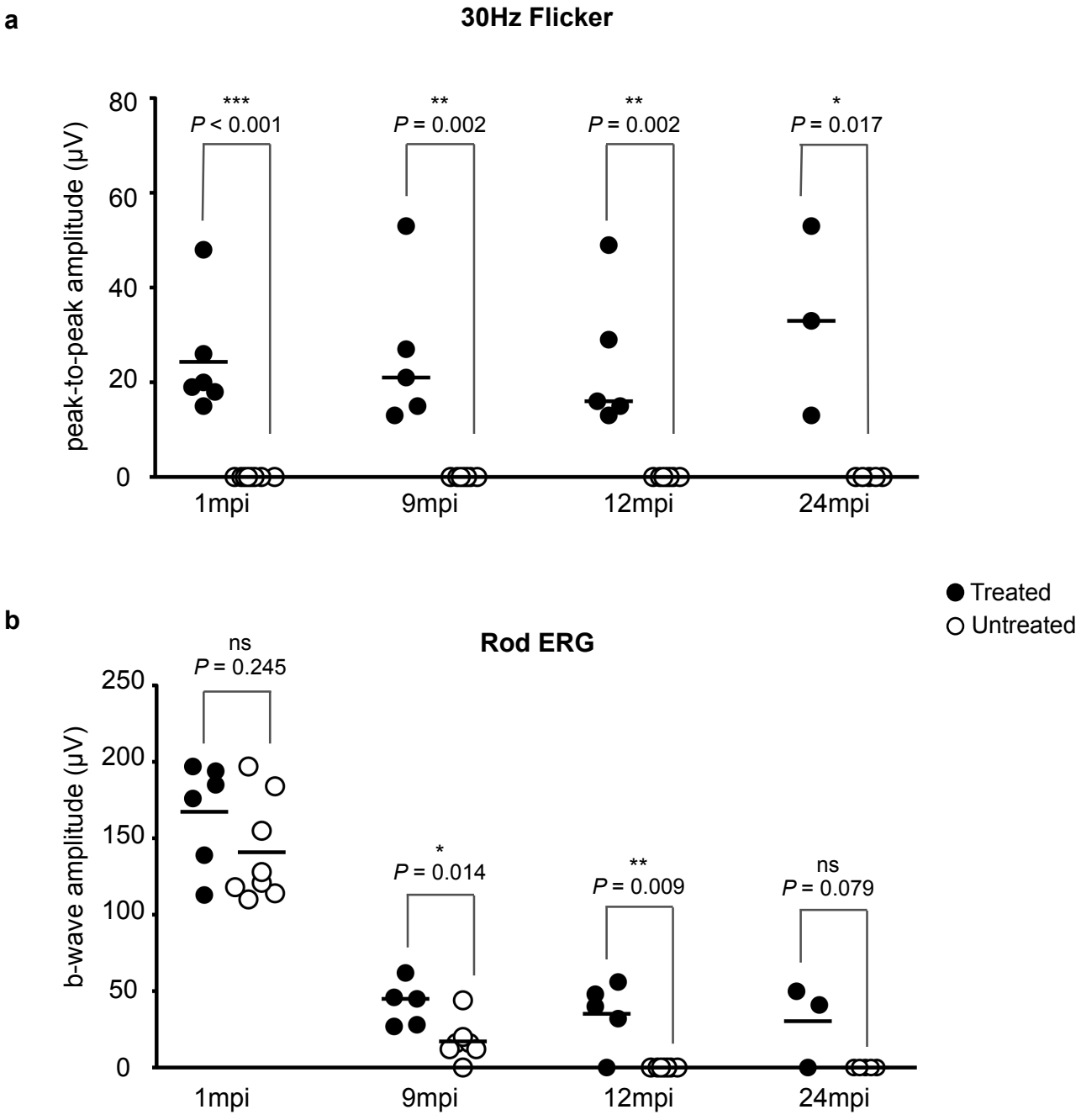

Figure S5. Statistical analysis of ERG responses in treated and untreated MLHD-*Cord1* eyes.

(**a**) Cone 30Hz Flicker- and (**b**) rod-mediated ERG responses amplitudes obtained in treated (dark circles) and untreated eyes (white circles) of dogs A1, A2, A3, A4, A7 and A8 were compared statistically at 1, 9, 12 and 24 mpi with unpaired Mann-Whitney t-test. The number of eyes analyzed per group was a follows: injected at 1 mpi, n=6, uninjected at 1 mpi, n=8, injected at 9 mpi and 12 mpi, n=5, uninjected at 9 mpi and 12 mpi, n=7, injected at 24 mpi, n=3, uninjected at 24 mpi, n=5. One, two and three asterisk(s) indicate(s) significant difference between treated and untreated eyes with *P*-value <0.05, <0.005 or <0.001, respectively. We clearly note a significant restoration of cone function in treated eyes

compared to untreated eyes at all time points. Regarding rod function, we note a significant better preserved function in treated eyes compared to untreated eyes at 9 and 12 mpi. At 24 mpi, no significant difference was found between treated and untreated eyes, probably due to the low number of examined eyes at this time point. Dark bars represent means. Abbreviations: ns, non significant.

Table S1. Primers used for the amplification of c*Rpgrip1* cDNA.

Primers	Sequences	Location	Tm(°C)	Product name and size
Rpgrip1-1F	GCTGATGGCGATGAATGAACACTG	5'RACE Adaptor	72	942bp+adaptor
Rpgrip1-1R	ACTTCTGTTAACTGTGCTTTTG	Exon 6	60	
1nestedF	CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG	5'RACE Adaptor	110	Rpgrip1-1
1nestedR	CGGTCTTCTCACTTGTTGC	Exon 4	58	(707bp+adaptor)
Rpgrip1-2F	AGAGCACATGTTGGTGAAGGA	Exon 2	62	Rpgrip1-2
Rpgrip1-2R	ACTTCTGTTAACTGTGCTTTTG	Exon 6	60	(582bp)
Rpgrip1-3F	CAGAGAAGCTCTTTGGAGTG	Exon 5	60	Rpgrip1-3
Rpgrip1-3R	GTCTCTGTGTGATCCTTACTG	Exon 13	62	(884bp)
Rpgrip1-4F	ATCAACGTGTGTTACCAGGA	Exon 12-13	58	Rpgrip1-4
Rpgrip1-4R	TGGTCAGAAAAGGTGAAGAA	Exon 16	56	(875bp)
Rpgrip1-5F	GGTGTTAGAATACTGGATGA	Exon 15	56	Rpgrip1-5
Rpgrip1-5R	TGGCCAGCTTCGGTGGGA	Exon 18	60	(675bp)
Rpgrip1-6F	AAGACTCAGAGCCTGGCTC	Exon 16	60	Rpgrip1-6
Rpgrip1-6R	TTCAATGCACATCTTCTCCGA	Exon 21	60	(704bp)
Rpgrip1-7F	ATTCAGTAGTGATGCAGAAAGAAG	Exon 19	66	Rpgrip1-7
Rpgrip1-7R	GCGAGCACAGAATTAATACGACT	3'RACE Adaptor	66	(898bp+adaptor)
7nestedF	CTAAATCCTGTGAATGATAAAGAATC	Exon 19-20	68	872bp+adaptor
7nestedR	CGCGGATCCGAATTAATACGACTCACTATAGG	3'RACE Adaptor	94	

Seven overlapping fragments (Rpgrip1-1 to Rpgrip1-7) covering the entire *cRpgrip1* coding sequence were amplified with PCR and RLM-RACE PCR using primers designed from human, bovine and rodent *RPGRIP1* consensus sequences and putative canine sequences. Abbreviations: bp, base pair; F, forward primer; R, reverse primer; Tm, primer melting temperature calculated with the 2*(A+T)+4*(G+C) method.

Table S2. Characterization of the exon-intron junctions of the c*Rpgrip1* gene.

Exon	Exon size (bp)	3'-Splice intron/exon acceptor sequence	5'-Splice exon/intron donor sequence	Intron	Intron size (bp)
0	ND	ND	CAAAAG**gt**ttgt	1	6727
1	120	ttt**ag**TGCCCT	CGAAAG**gt**aaat	2	8087
2	133	ctt**ag**GTAAGA	CAAAAG**gt**acct	3	3901
3	224	tca**ag**GGCGAG	GGAGGG**gt**gagt	4	939
4	91	ttt**ag**AGCCCA	TGAGCC**gt**gagt	5	968
5	114	gac**ag**CAATAC	GCTGCG**gt**aaga	6	1891
6	106	ttc**ag**AGCTTC	CAAGAG**gt**gagg	7	3657
7	24	ttt**ag**GCATAT	CAGAAG**gt**actt	8	1075
8	147	ccc**ag**AATCAG	AAGGAG**gt**aaat	9	476
9	74	tct**ag**TTTCAG	AGAAAA**gt**gagt	10	3133
10	146	tcc**ag**CATGCT	AGAAAG**gt**aggt	11	3048
11	140	ccc**ag**CCCAGA	GCCGAG**gt**aaga	12	134
12	144	ccgc**ag**CCCCGG	TACCAG**gt**gtgg	13	458
13	118	tct**ag**GAGGAA	TGGAAG**gt**attt	14	2233
14	453	ctct**ag**AACAGC	TTACTG**gt**aagt	15	161
15	152	atcc**ag**GAGCTG	GATAAG**gt**gaga	16	441
16	343	ttc**ag**TCCAGA	TTAAAG**gt**ggga	17	1203
17	162	tatc**ag**GTGATT	AAAGAG**gt**aaag	18	330
18	203	attt**ag**GACCAG	TTACAA**gt**aagg	19	1819
19	139	ctaa**ag**CAGGTG	TGAATG**gt**attg	20	10713
20	98	ttgc**ag**ATAAAG	AAGGCA**gt**aagt	21	4901
21	193	tgtc**ag**GACTCG	GCAAAG**gt**gacg	22	5766
22	85	gccc**ag**TGATCC	AGGACG**gt**aagg	23	2667
23	125	ctt**ag**TTTAAA	TAGAGA**gt**gagt	24	2127
24	nd	ccgc**ag**TCTTGA	nd	nd	nd

Exons are numbered beginning at the 5' end of the gene. Intron sequences are in lower case letters and exon sequences in uppercase letters. Conserved 3' splice acceptor sites (**ag**) and 5' splice donors sites (**gt**) are in bold. Analysis of the exon-intro junctions demonstrated that boundaries followed the gt/ag consensus splicing rule. Abbreviations: bp, base pairs; nd, not determined.

Video S1. Assessment of dim- and bright-light vision of dog A7 at 12 months postinjection. Video shows ambulation of dog A7 through an obstacle avoidance course 12 months following the subretinal injection of AAV2/8-RK-c*Rpgrip1*. (**Part I**) Behavioral test was performed under bright-light conditions (260 ± 13 lux). An opaque lens was used to sequentially cover the untreated (left) and the treated (right) eye. (**Part II**) Behavioral test was performed under dim-light conditions (1,5 ± 0,8 lux). An opaque lens was used to sequentially cover the untreated (left) and the treated (right) eye.