Supplemental Table S1: Oligonucleotide primers[1] for site-directed mutagenesis of canine RPE65

Mutant	Forward primer[2]	Reverse primer[2]
F61L	CTGAACCATTTTACCACCTGTTAGACGGACAAGCC	GGCTTGTCCGTCTAACAGGTGGTAAAATGGTTCAG
F61Y	ATCTGAACCATTTTACCACCTGTATGACGGACAAGC	GCTTGTCCGTCATACAGGTGGTAAAATGGTTCAGAT
F61W	GAACCATTTTACCACCTGTGGGACGGACAAGCCCTTCTGC	GCAGAAGGGCTTGTCCGTCCCACAGGTGGTAAAATGGTTC
Y275F	GTCTTTGGGGAGCCAACTTCATGGATTGTTTTGAGTC	GACTCAAAACAATCCATGAAGTTGGCTCCCCAAAGAC
Y275W	AGTCTTTGGGGAGCCAACTGGATGGATTGTTTTGAGTCCA	TGGACTCAAAACAATCCATCCAGTTGGCTCCCCAAAGACT
Y275I	GAGTCTTTGGGGAGCCAACATCATGGATTGTTTTGAGTCC	GGACTCAAAACAATCCATGATGTTGGCTCCCCAAAGACTC
Y239W	GACCGATTCAAGCCATCGTGGGTCCATAGTTTTGGTTTGA	TCAAACCAAAACTATGGACCCACGATGGCTTGAATCGGTC
Y239F	CGATTCAAGCCATCGTTCGTCCATAGTTTTGGTTTG	CAAACCAAAACTATGGACGAACGATGGCTTGAATCG
Y239L	CGATTCAAGCCATCGCTCGTCCATAGTTTTGGTTTG	CAAACCAAAACTATGGACGAGCGATGGCTTGAATCG
Y239S	CGATTCAAGCCATCGTCCGTCCATAGTTTTGGTTTG	CAAACCAAAACTATGGACGGACGATGGCTTGAATCG
Y239T	CGATTCAAGCCATCGACCGTCCATAGTTTTGGTTTG	CAAACCAAAACTATGGACGGTCGATGGCTTGAATCG
Y239C	CGATTCAAGCCATCGTGCGTCCATAGTTTTGGTTTG	CAAACCAAAACTATGGACGCACGATGGCTTGAATCG
Y239D	CGATTCAAGCCATCGGACGTCCATAGTTTTGGTTTG	CAAACCAAAACTATGGACGTCCGATGGCTTGAATCG
W331Y	GTGGATCTCTGCTGCTACAAAGGATTTGAATTC	GAATTCAAATCCTTTGTCGCAGCAGAGATCCAC
W331F	GTGGATCTCTGCTGCTTCAAAGGATTTGAATTC	GAATTCAAATCCTTTGAAGCAGCAGAGATCCAC
W331L	GTGGATCTCTGCTGCTTGAAAGGATTTGAATTC	GAATTCAAATCCTTTCAAGCAGCAGAGATCCAC
W331Q	GTGGATCTCTGCTGCCAGAAAGGATTTGAATTC	GAATTCAAATCCTTTCTGGCAGCAGAGATCCAC
T147W	GATTACTATGCCTGCTGGGAGACCAACTTCATTAC	GTAATGAAGTTGGTCTCCCAGCAGGCATAGTAATC
T147Y	GATTACTATGCCTGCTACGAGACCAACTTCATTAC	GTAATGAAGTTGGTCTCGTAGCAGGCATAGTAATC
T147V	GGGAAGATTACTATGCCTGCGTGGAGACCAACTTCATTACAA	TTGTAATGAAGTTGGTCTCCACGCAGGCATAGTAATCTTCCC
T147A	GAAGATTACTATGCCTGCGCGGAGACCAACTTCATTA	TAATGAAGTTGGTCTCCGCGCAGGCATAGTAATCTTC
T147C	GGGGAAGATTACTATGCCTGCTGCGAGACCAACTTCATTACAAAG	CTTTGTAATGAAGTTGGTCTCGCAGCAGGCATAGTAATCTTCCCC
T147G	GGAAGATTACTATGCCTGCGGGGAGACCAACTTCATTACA	TGTAATGAAGTTGGTCTCCCCGCAGGCATAGTAATCTTCC
T147S	GATTACTATGCCTGCAGCGAGACCAACTTCATTAC	GTAATGAAGTTGGTCTCGCTGCAGGCATAGTAATC
F103W	GAGAAAAGGATCGTCATAACGGAATGGGGCACCTGTGCGT	ACGCACAGGTGCCCCATTCCGTTATGACGATCCTTTTCTC
F103Y	GAAAAGGATCGTCATAACGGAATATGGCACCTGTGC	GCACAGGTGCCATATTCCGTTATGACGATCCTTTTC
F103I	GAAAAGGATCGTCATAACGGAAATTGGCACCTGTG	CACAGGTGCCAATTTCCGTTATGACGATCCTTTTC
F103L	GAAAAGGATCGTCATAACGGAACTTGGCACCTGTG	CACAGGTGCCAAGTTCCGTTATGACGATCCTTTTC

[1] Predicted by QuikChange Primer Design Program (Stratagene division of Agilent Technologies)
[2] Primer sequences depicted in 5’-NNNN-3’ orientation

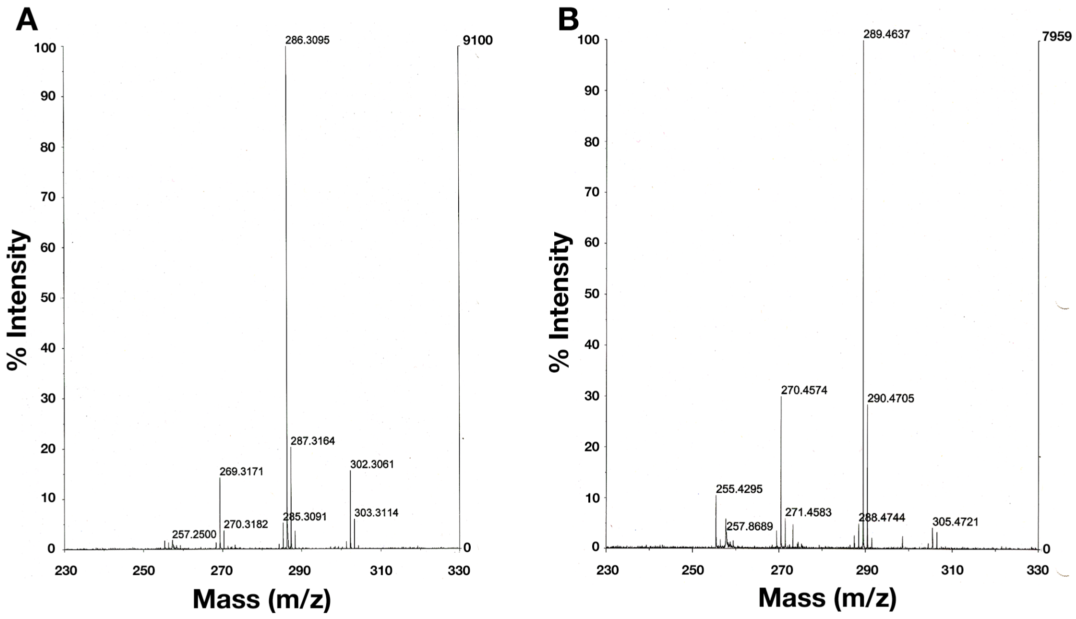
Supplemental Figure Legends

Fig. S1: MALDI-TOF analysis of retinol standards. A: All-*trans* retinol. The major mass of ~286 corresponds to intact all-*trans* retinol while the mass at ~287 is due to stochastic occurrence of ^{13}C. The masses at ~269 and ~270 are due to loss of hydroxyl from the intact ~286 and ~287 masses. The masses at ~302 and ~303 are ascribed to epoxidation of the intact ~286 and ~287 masses. B: [15-^{2}H,^{18}O]-all-*trans* retinol. The major mass of ~289 corresponds to intact labeled [15-^{2}H,^{18}O]-all-*trans* retinol while the mass at ~290 is due to stochastic occurrence of ^{13}C. The masses at ~270 and ~271 are due to loss of ^{18}O-labeled hydroxyl (^{18}OH) from the intact ~289 and ~290 masses. The extra 1 atomic mass unit, compared to masses ~269 and ~270 in A, is due to presence of ^{2}H label, which is not lost from the retinol. The mass at ~305 is ascribed to epoxidation of the intact ~289 species. Method as described in Experimental Procedures.

Fig. S2: Structural model and alignment of RPE65 and ACO. A: Stereo-pair model of RPE65 based on ACO template. Mouse RPE65 was modeled on *Synechocystis* ACO using the Swiss-Pdb viewer with apocarotenal (yellow) mounted in the ACO substrate-binding cleft. RPE65 structure is in green, ACO in silver. The propeller structure is well-maintained in RPE65, but the loops forming the dome above the catalytic site are more extensive in RPE65 and thus cannot be modeled on this template. B: Sequence alignment of RPE65 and ACO to show residues studied (F61, F103, T147, Y239, Y275 and W331) highlighted in red. Initial alignment obtained with DeepView/Swiss-PdbViewer (v3.7) was manually adjusted to maximize both gap suppression and alignment with predicted structural features (beta sheets and alpha helices). Residues 338-375 of RPE65 are restricted to metazoan carotenoid oxygenase family members. *=identity; .=similarity.

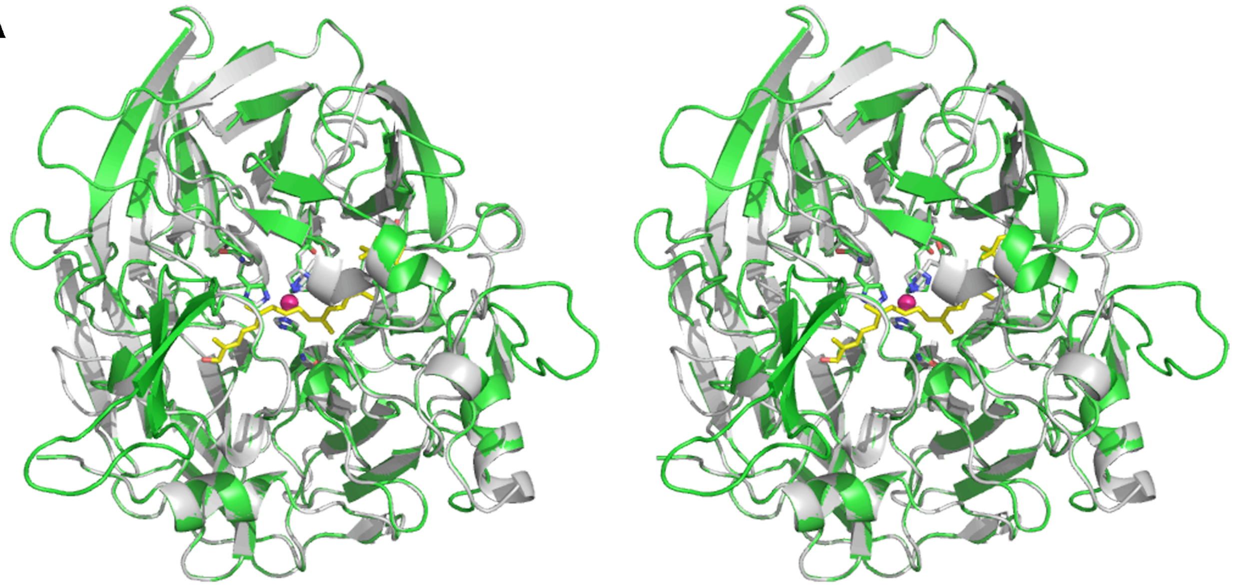
Fig. S3: Co-production of 11-*cis* and 13-*cis* retinols by RPE65. Data from Fig. 1B of Redmond et al. (8) were re-analyzed to extract 13-*cis* isomer information. Cultures were transfected with six different combinations: 1, pVitro2/RPE65+CRALBP; 2, pVitro2/RPE65 and pVitro3/LRAT+RDH5; 3, pVitro2/RPE65+CRALBP and pVitro3/LRAT+RDH5; 4, pVitro3/LRAT+RDH5; 5, pVitro2/CRALBP and pVitro3/LRAT+RDH5; and 6, no DNA. These were incubated with 2.5 μM all-*trans* retinol, as described in (8).

Supplemental Figure S1



Supplemental Fig. S2

A



B

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RPE65     1        MSIQIEHPA GGYKKLFETV EELSSPLTAH VTGRIPLWLT GSLLRCGPGL
ACO       12          QRSYSP QDWLRGYQSQ PQEWDYWVED VEGSIPPDLQ GTLYRNGPGL
                      *   ..     . ...   .         * * **  *  *.* * ****
RPE65     50      FEVGSEPFYH LFDGQALLHK FDFK-EGHVT YHRRFIRTDA YVRAMTEKRI
ACO       58      LEIGDRPLKH PFDGDGMVTA FKFPGDGRVH FQSKFVRTQG YVEEQKAGKM
                  .*.*  *. *  ***....   * *  .*.*  .. .*.**.. **      ..
RPE65     99      VITE-FGTCA FPDPCKNIFS RFFSYFKGVE VTDNALVNIY PVGEDYYACT
ACO       108     IYRGVFGSQP AGGWLKTIFD LRLKNIAN-- -T-----NIT YWGDRLLALW
                  .    **.     .  * **. . .   .     *     **     *.   *
RPE65     148     ETNFITKINP ETLETIKQVD LCNYISVNG- ATAHPHIESD GTVYNIGNCF
ACO       150     EGGQPHRLEP SNLATIGLDD LGGILAEGQP LSAHPRIDPA STFDGGQPCY
                  *     ...*   * **   * *    ..     .***.*.. . *      *.
RPE65     197     GKNFTVAYNI IKIPPLKADK EDPINKSEVV VQFPCSDRFK PSYVHSFGLT
ACO       200     VTFSIKSSLS STLTLLELDP ------QGKL LRQKTETFPG FAFIHDFAIT
                        .      .  *  *           .. .          ...* *..*
RPE65     247     PNYIVFVETP VKINLFKFLS SWSLWGANYM DCFESNESMG VWLHVADKKR
ACO       244     PHYAIFLQNN VTLNGLPYL- -FGLRGAG-- ECVQFHPDKP AQIILVPRDG
                  *.* .*..   * .* . .*     .*.**    .* . .  .    . .  .
RPE65     297     RKYFNNKYRT SPFNLFHHIN TYEDNGFLIV DLCCWKGFEF VYNYLYLANL
ACO       290     GEIKRIPVQ- -AGFVFHHAN AFEENGKIIL DSICYNSLPQ V---------
                          .    .  .*** * ..*.** .*. *  * ...   *
RPE65     347     RENWEEVKRN AMKAPQPEVR RYVLPLTIDKV DTGRNLVTL PHTTATATLR
ACO       328     ---------- ---------- ---------DT DGDFRSTNF DNLDPGQLWR
                                                    *       .  .  .    *
RPE65     397     SDETIWLEPE VLFSGPRQAF EFPQINYQKF GGKPYTYAYG LGLNH----F
ACO       350     FTIDPAAATV EKQLMVSRCC EFPVVHPQQV G-RPYRYVYM GAAHHSTGNA
                                    .   *** .. *   * .** * *   . .*
RPE65     443     VPDKLCKMNV KT-KEIWMWQ EPDSYPSEPI FVSQPDALEE DDGVVLSVVV
ACO       399     PLQAILKVDL ESGTETLRSF APHGFAGEPI FVPRPGGVAE DDGWLLCLIY
                    . . *...  .  *       * ....*** **..* .. * *** .* ..
RPE65     492     SPGAGQKPAY LLVLNAKDLS EIARAEVETN IPVTFHGLFK RS
ACO       449     KADLHRSELV ILDAQDITAP AIATLKLKHH IPYPLHGSWA QT
                       .     .*  .       **  ... . **. .** .  ..
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Supplemental Figure S3