Supporting Information

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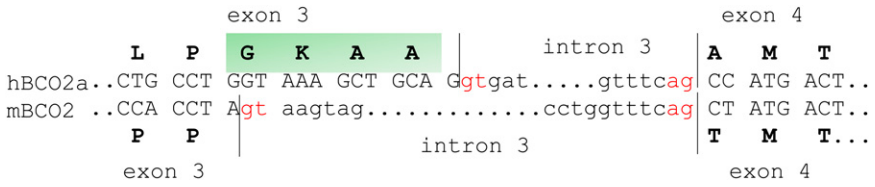

Fig. S1. Splice junctions of exon 3/intron 3 and intron 3/exon 4 of the human and mouse β,β-carotene-9′,10′-dioxygenase (BCO2) genes. The insertion of GKAA in the human gene is caused by a change in the donor splice site in the human gene. Intron sequences are in lowercase, 5′ and 3′-ends are printed in red.

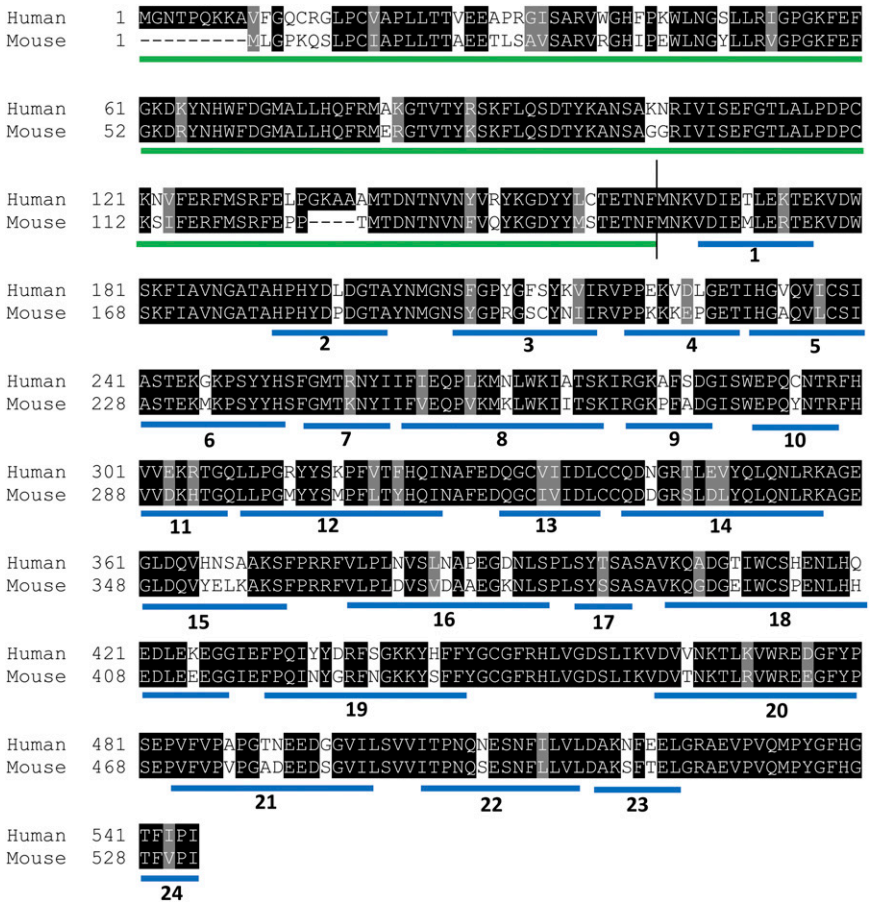

Fig. S2. Protein sequences of human BCO2 and mouse BCO2 used to design primers of mutagenesis experiments. Green underline shows the fragments switched between human BCO2 and mouse BCO2 and the vertical line shows the connection position. Blue underline shows the substituted amino acid positions between human BCO2 and mouse BCO2. These were numbered and the corresponding numbers are given to the primers in Table S1.

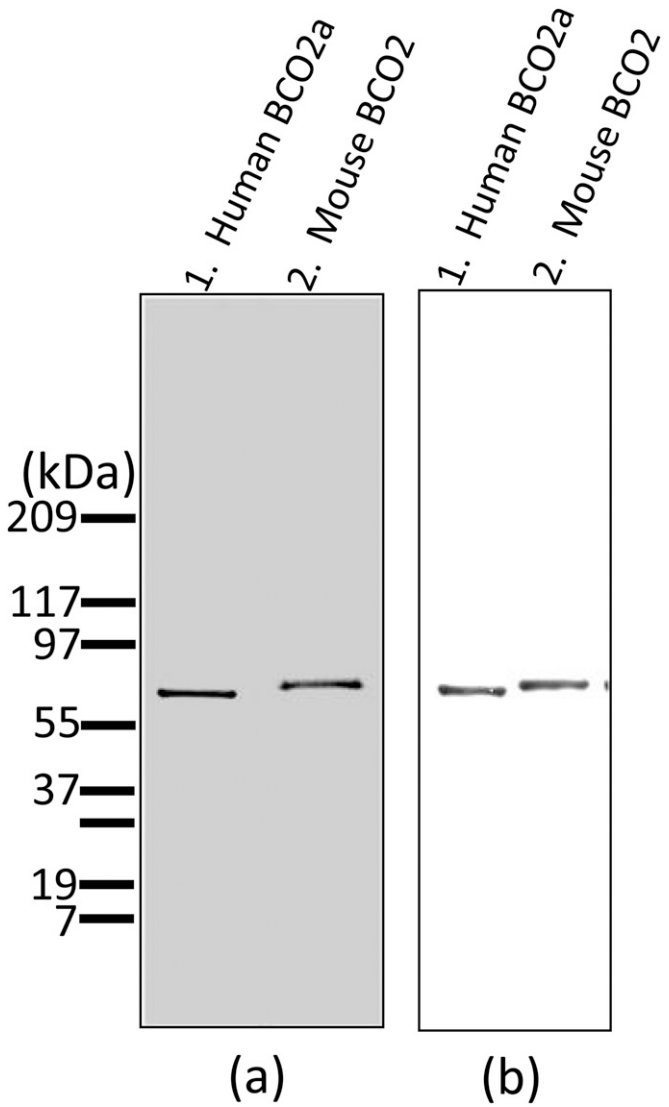

Fig. S3. SDS/PAGE (*A*) and Western blot (*B*) of recombinant human BCO2a (1), and mouse BCO2 (2) used for surface plasmon resonance (SPR).

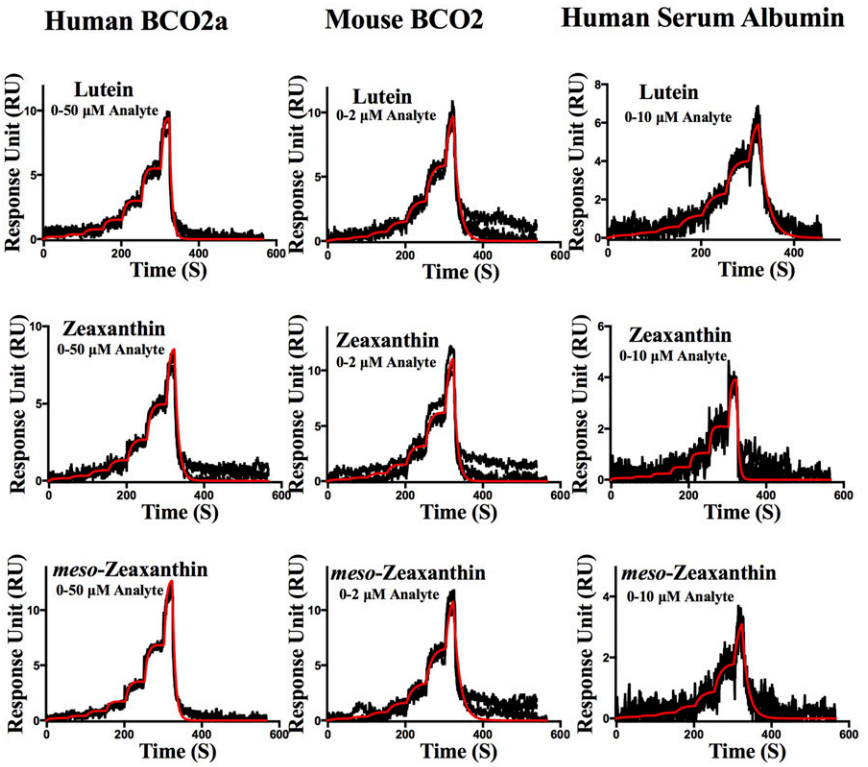

Fig. S4. SPR sensorgrams of carotenoids with human BCO2a, mouse BCO2, and human serum albumin.

Table S1. Primers used in the mutagenesis experiments to restore the carotenoid cleavage function of human BCO2

Primer	Sequence
1. T171M + k174R	5′-AAATTTGCTCCAATCTACCTTTTCTGTTCTTTCCAGCATTTCAATGTCCACTTTATTCATAAAGTTG-3′
	5′-CAACTTTATGAATAAAGTGGACATTGAAATGCTGGAAAGAACAGAAAAGGTAGATTGGAGCAAATTT-3′
2. L197P	5′-CACATCCTCATTATGACCCGGATGGAACAGCATACAA-3′
	5′-TTGTATGCTGTTCCATCCGGGTCATAATGAGGATGTG-3′
3. F208Y, Y211R, F213S, S214C, K216N, V217I	5′-CCTTCTCTGGAGGAACCCGAATAATATTATAGCAGCTACCACGTGGCCCATAGGAGTTCCCCATATTGTATGCTG-3′
	5′-CAGCATACAATATGGGGAACTCCTATGGGCCACGTGGTAGCTGCTATAATATTATTCGGGTTCCTCCAGAGAAGG-3′
4. E223K, V225K, D226E, L227P	5′-GGATTGTCTCCCCAGGCTCCTTCTTCTTTGGAGGAACCCGAATAACCTTATAGGA-3′
	5′-TCCTATAAGGTTATTCGGGTTCCTCCAAAGAAGAAGGAGCCTGGGGAGACAATCC-3′
5. V234, I237L	5′-AGAAGCAATAGAACATAACACCTGGGCTCCATGGATTGTCTCC-3′
	5′-GGAGACAATCCATGGAGCCCAGGTGTTATGTTCTATTGCTTCT-3′
6. G246M	5′-AAAGCTATGGTAGTAAGAAGGTTTCATTTTCTCTGTAGAAGCAATAGAACAT-3′
	5′-ATGTTCTATTGCTTCTACAGAGAAAATGAAACCTTCTTACTACCATAGCTTT-3′
7. R258K	5′-GTTGTTCAATGAAAATTATATAGTTCTTTGTCATTCCAAAGCTATGGTAGTAA-3′
	5′-TTACTACCATAGCTTTGGAATGACAAAGAACTATATAATTTTCATTGAACAAC-3′
8. I264V, L268V, N271K, A276I	5′-CTTTCCCCGAATTTTAGAAGTGATAATTTTCCACAGGTTCATCTTTAGAG-3′
	5′-GTTCATCTTTAGAGGTTGTTCAACGAAAATTATATAGTTCCTTGTCATTC-3′
	5′-TAGAAGTGGCAATTTTCCACAGCTTCATCTTTACAGGTTGTTCAATGAAAATTATA-3′
	5′-TATAATTTTCATTGAACAACCTGTAAAGATGAAGCTGTGGAAAATTGCCACTTCTA-3′
	5′-GAATGACAAGGAACTATATAATTTTCGTTGAACAACCTCTAAAGATGAAC-3′
	5′-CTCTAAAGATGAACCTGTGGAAAATTATCACTTCTAAAATTCGGGGAAAG-3′
9. A284P, S286A	5′-CCAGCTTATCCCATCTGCAAAGGGCTTTCCCCGAATTTTAG-3′
	5′-CTAAAATTCGGGGAAAGCCCTTTGCAGATGGGATAAGCTGG-3′
10. C295Y	5′-CATGAAACCGCGTATTATACTGGGGTTCCCAGCTT-3′
	5′-AAGCTGGGAACCCCAGTATAATACGCGGTTTCATG-3′
11. E303D, R305H	5′-GAAGGAGCTGTCCAGTGTGTTTATCCACCACATGAAACCG-3′
	5′-CGGTTTCATGTGGTGGATAAACACACTGGACAGCTCCTTC-3′
12. R313M, K317M, V320L, F322Y	5′-AAAGGCATTGATTTGATGATATGTAAGAAAAGGCATGCTGTAGTACATCCCTGGAAGGAGCTGTCCAGT-3′
	5′-ACTGGACAGCTCCTTCCAGGGATGTACTACAGCATGCCTTTTCTTACATATCATCAAATCAATGCCTTT-3′
13. V334I, I335V	5′-TTGACAGCACAAATCAATTACAATACAGCCCTGGTCCTCAAAGG-3′
	5′-CCTTTGAGGACCAGGGCTGTATTGTAATTGATTTGTGCTGTCAA-3′
14. N343D, T346S, E348D, V349L	5′-CTGAGATTCTGTAACTGGTAAAGATCTAGGCTTCTTCCATCATCTTGACAGCACAAATCAATT-3′
	5′-AATTGATTTGTGCTGTCAAGATGATGGAAGAAGCCTAGATCTTTACCAGTTACAGAATCTCAG-3′
15. H366Y, N367E, S368L, A369K	5′-CAAACCTTCGAGGGAAAGATTTGGCTTTTAACTCATAGACCTGATCAAGCCCTTCCCCAGCCT-3′
	5′-AGGCTGGGGAAGGGCTTGATCAGGTCTATGAGTTAAAAGCCAAATCTTTCCCTCGAAGGTTTG-3′
16. N382D, L385V, N386D, P388A, D391K	5′-TCGAAGGTTTGTTTTGCCTTTAGATGTCAGTGTGGATGCCGCTGAGGGAAAGAACCTGAGTCCA-3′
	5′-TGGACTCAGGTTCTTTCCCTCAGCGGCATCCACACTGACATCTAAAGGCAAAACAAACCTTCGA-3′
17. T399S	5′-CAGCACTGGCTGAAGAATAGGACAATGGACTCAGG-3′
	5′-CCTGAGTCCATTGTCCTATTCTTCAGCCAGTGCTG-3′
18. A407G, T410E, H415P, Q420H, K425E	5′-CAATGCCTCCTTCCTCTTCTAGGTCCTCCTGATGT-3′
	5′-ACATCAGGAGGACCTAGAAGAGGAAGGAGGCATTG-3′
	5′-TCTCATGAAAATCTACATCATGAGGACCTAGAAAAGGAAGG-3′
	5′-GAACGATCTGGTGCTCTCCTGAAAATCTACATCAGGA-3′
	5′-CAGTGCTGTGAAACAGGGTGATGGAGAGATCTGGTGCTCTCATG-3′
	5′-CATGAGAGCACCAGATCTCTCCATCACCCTGTTTCACAGCACTG-3′
	5′-CCTTCCTTTTCTAGGTCCTCATGATGTAGATTTTCATGAGA-3′
	5′-TCCTGATGTAGATTTTCAGGAGAGCACCAGATCGTTC-3′
19. Y435N, D437G, S440N, H445S	5′-CCACAGCCATAAAAGAAACTATACTTTTTGCCACTGAATCGATCATAGTAGAT-3′
	5′-ATCTACTATGATCGATTCAGTGGCAAAAAGTATAGTTTCTTTTATGGCTGTGG-3′
	5′-AAAAGAAATGATACTTTTTGCCATTGAATCGACCATAGTTGATCTGAGGAAATTCAATGCCTC-3′
	5′-GAGGCATTGAATTTCCTCAGATCAACTATGGTCGATTCAATGGCAAAAAGTATCATTTCTTTT-3′
20. V466T, K471R, D476E	5′-GAGGGATAAAAGCCCTCTTCTCTCCAAACCTTCAGTG-3′
	5′-CCAAACCTTCAGTGTCTTATTCGTCACATCAACCTTGATCAGAGAA-3′
	5′-TTCTCTCCAAACCCTCAGTGTCTTATTCACCACATCAAC-3′
	5′-TTCTCTGATCAAGGTTGATGTGACGAATAAGACACTGAAGGTTTGG-3′
	5′-CACTGAAGGTTTGGAGAGAAGAGGGCTTTTATCCCTC-3′
	5′-GTTGATGTGGTGAATAAGACACTGAGGGTTTGGAGAGAA-3′
21. A488V, T491A, N492D, G496S	5′-AGAATAACCCCACTATCTTCTTCATTGGTTCCTGGTGC-3′
	5′-CCCACCATCTTCTTCATCGGCTCCTGGTACTGGAACAAAAACAGGT-3′
	5′-GCACCAGGAACCAATGAAGAAGATAGTGGGGTTATTCT-3′
	5′-ACCTGTTTTTGTTCCAGTACCAGGAGCCGATGAAGAAGATGGTGGG-3′
22. N509S, I514L	5′-CATCCAAAACTAGGAGAAAATTGCTTTCATTCTGGTTGGGAGTGA-3′
	5′-GGATAAAATTGCTTTCACTCTGGTTGGGAGTGATCACCA-3′

Table S1. Cont.

Primer	Sequence
	5′-TGGTGATCACTCCCAACCAGAGTGAAAGCAATTTTATCC-3′
	5′-TCACTCCCAACCAGAATGAAAGCAATTTTCTCCTAGTTTTGGATG-3′
23. N521S, E523T	5′-ACCTCTGCTCGGCCCAGCTCCGTAAAGCTCTTGGCATCCAAAACTAGG-3′
	5′-CCTAGTTTTGGATGCCAAGAGCTTTACGGAGCTGGGCCGAGCAGAGGT-3′
24. I543V	5′-CTCGAGCTAGATGGGTACGAAGGTACCATGGAACC-3′
	5′-GGTTCCATGGTACCTTCGTACCCATCTAGCTCGAG-3′
25. Mouse BCO2+GKAA	5′-ATGTCAAGGTTTGAGCCACCTGGTAAAGCTGCAACTATGACTGACAACAC-3′
	5′-GTGTTGTCAGTCATAGTTGCAGCTTTACCAGGTGGCTCAAACCTTGACAT-3′
26. Human BCO2-GKAA+A→T	5′-GTCCAGGTTTGAGCTGCCTATGACTGACAATACTAATG-3′
	5′-CATTAGTATTGTCAGTCATAGGCAGCTCAAACCTGGAC-3′
	5′-TCCAGGTTTGAGCTGCCTACCATGACTGACAATA-3′
	5′-TATTGTCAGTCATGGTAGGCAGCTCAAACCTGGA-3′
27. N-terminal switch	5′-ATGAATAAGGTGGACATTGAGATGC-3′
	5′-CCCCGGGCTCTTTCTTTTTTG-3′
	5′-TTCGAAGGAATTCGGTACCATGGGAAATACTCCTCAGAAAAAAGC-3′
	5′-AGCATCTCAATGTCCACCTTATTCATAAAGTTGGTCTCAGTGCAG-3′
	5′-AAAACCTGTATTTTCAGGGCGAAGGAGTTCGAACCATGTTGGGACCGAAGCAAAG-3′
	5′-TCTGTTTTTTCCAGAGTTTCAATGTCCACTTTATTCATAAAATTAGTCTCTGTGCTC-3′
28. Human BCO2-GKAA with N122S	5′-TCTCCCGGATCCATGCAAGAGTATTTTTGAACGTTTCATGTCC-3′
	5′-GGACATGAAACGTTCAAAAATACTCTTGCATGGATCCGGGAGA-3′
29. Human BCO2-GKAA with L133P	5′-ATGTCCAGGTTTGAGCCGCCTACCATGACTGAC-3′
	5′-GTCAGTCATGGTAGGCGGCTCAAACCTGGACAT-3′
30. Human BCO2-GKAA with Y435N, D437G	5′-GGCATTGAATTTCCTCAGATCAACTATGGTCGATTCAGTGGCAAAAAGTA-3′
	5′-TACTTTTTGCCACTGAATCGACCATAGTTGATCTGAGGAAATTCAATGCC-3′
31. Human BCO2-GKAA with S440N	5′-TTCCTCAGATCAACTATGGTCGATTCAATGGCAAAAAGTATC-3′
	5′-GATACTTTTTGCCATTGAATCGACCATAGTTGATCTGAGGAA-3′
32. Human BCO2-GKAA with L133P and S440N	5′-ATGTCCAGGTTTGAGCCGCCTACCATGACTGAC-3′
	5′-GTCAGTCATGGTAGGCGGCTCAAACCTGGACAT-3′
	5′-TTCCTCAGATCAACTATGGTCGATTCAATGGCAAAAAGTATC-3′
	5′-GATACTTTTTGCCATTGAATCGACCATAGTTGATCTGAGGAA-3′