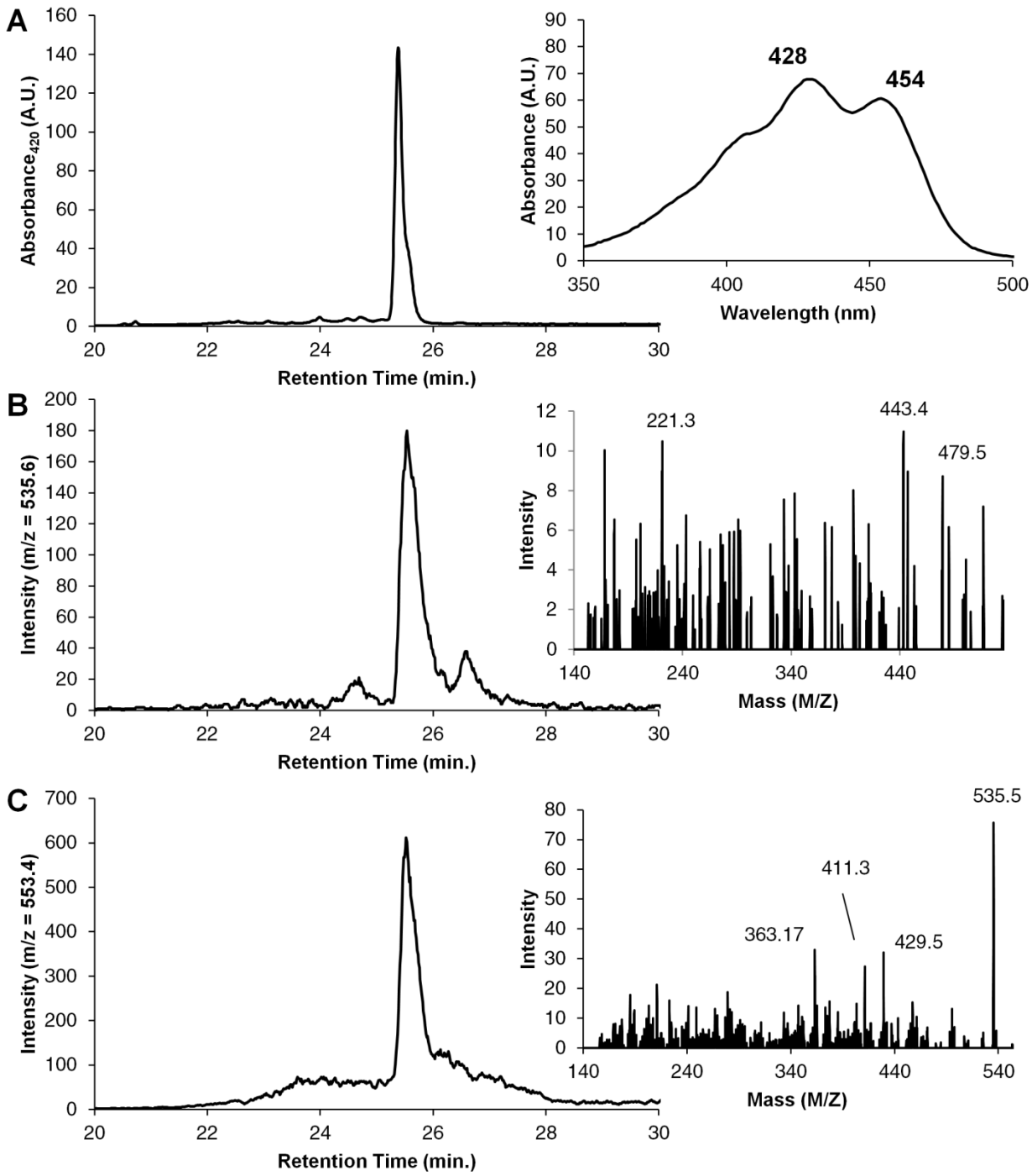


Supplemental Figure S1. LC-MS and LC-MS/MS characterization of a β-carotene metabolite. The previously observed β-carotene metabolite was separated by normal-phase HPLC and collected. Following this, the metabolite was subjected to reverse-phase HPLC, MS, and MS/MS analysis. **(A)** HPLC trace at 420 nm of β-carotene metabolite isolated from β-carotene fed mice. LC-MS trace at **(B)** m

/ z = 535.6 and **(C)** 553.4, the most abundant peaks that match the retention time of the unknown β-carotene metabolite. Insets on the right show MS/MS fragmentation patterns of these peaks.

A

Gene	Fold Change	p-value
Arrdc3	2.40	0.003
Hba-a1	-1.68	0.029
Hbb-b1	-1.84	0.019
LOC100047427	2.37	0.000
AI850995	-2.03	0.016
Nr1d1	2.02	0.005

B

Gene	Fold Change	p-value
Rnf186	2.19	0.018
Sntg2	1.61	0.022
Lzts2	1.56	0.003
0610008F07Rik	1.70	0.024
2900006A08Rik	-1.80	0.003
LOC380707	1.73	0.006
Rhpn2	-1.66	0.010
LOC100043257	1.60	0.003
Prkaa2	-1.55	0.002
Htatip2	2.50	0.028
Skil	-1.64	0.000
Dlat	-1.54	0.032
Decr1	-1.67	0.038
Sbk	1.65	0.033
1500011K16Rik	1.50	0.019
Sult1c2	2.00	0.049
Rnf125	-1.52	0.004
Slc25a30	-2.35	0.049
Elovl2	-1.60	0.011
Plk3	-1.87	0.040
Hhex	-2.10	0.019
Spon2	-1.66	0.019

Supplemental Figure S2. Effect of carotenoid accumulation on the liver expressome. Five-week old *Bco1*$^{-/-}$ *Bco2*$^{-/-}$ (DKO) female mice were supplemented on either a zeaxanthin (n = 5, control = 4) carotene (n = 5, control = 6) diet for 10 weeks. Livor βer -expressome was assessed by Illumina bead chip. Several genes were identified to have a significant change in expression in response to **(A)** β-carotene or **(B)** zeaxanthin supplementation

Pathway	Genes with changes in expression in pathway	Genes in Pathway	p-value
Trehalose Degradation II	1	1	0.0058
Aldosterone Signaling in Epithelial Cells	3	78	0.0102
Type II Diabetes Mellitus	3	80	0.0109
Spermidine Biosynthesis	1	2	0.0116
GDP Glucose Biosynthesis	1	2	0.0116
CREB Signaling in Neurons	3	87	0.0137
Glucose and Glucose 1 phosphate	1	3	0.0173
Melatonin Signaling	2	39	0.0212
Pyridoxal 5' phosphate salvage pathway	2	40	0.0222
GPCR Mediated Nutrient Sensing in Enteroendocrine cells	2	40	0.0222
Methylmalonyl Pathway	1	4	0.0230
2-oxobutanote Degradation I	1	5	0.0286
UDP-N-acetyl-D-galactosamine Biosynthesis	1	5	0.0286
Neuropathic Pain Signaling in Dorsal Horn Neurons	2	47	0.0301
Salvage Pathways of Pyrimidine Ribonucleotides	2	51	0.0349
Sperm Motility	2	52	0.0362
Huntington's Disease Signaling	3	130	0.0390
Prostanoid Biosynthesis	1	7	0.0399
Neuregulin Signaling	2	58	0.0442
Synaptic Long Term Potentiation	2	59	0.0455

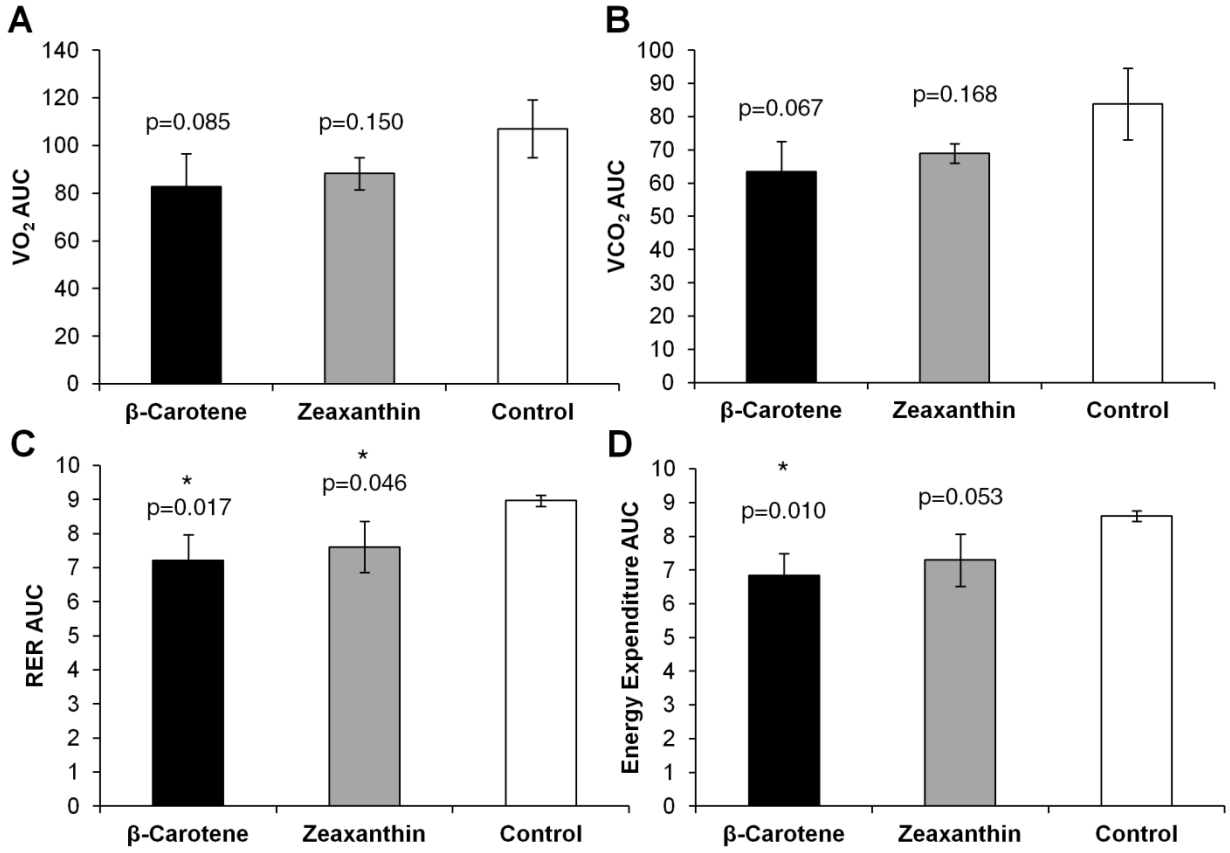
Supplemental Figure S3. Pathways with a significant change due to β-carotene supplementation.

Results from differential liver expressome were analyzed computationally to assess which cellular pathways may have changed as a result of carotenoid accumulation. Complete list of pathways affected by β-carotene accumulation.

Pathway	Genes with changes in expression in pathway	Genes in Pathway	p-value
Cholesterol Biosynthesis I	4	12	0.0003
Cholesterol Biosynthesis II (24,25 Dihydrolanosterol)	4	12	0.0003
Cholesterol Biosynthesis III (via Desmosterol)	4	12	0.0003
Zymosterol Biosynthesis	3	6	0.0005
Mitochondrial Dysfunction	7	111	0.0017
Prostate Cancer Signaling	7	59	0.0017
BMP Signaling	6	46	0.0022
Superpathway of Cholesterol Biosynthesis	4	22	0.0037
Regulation of IL-2 Expression in Lymphocytes	6	54	0.0051
ATM signaling	5	41	0.0070
Estrogen Dependent Breast Cancer Signaling	5	42	0.0078
Myc Mediated Apoptosis	5	45	0.0104
Rac Signaling	6	65	0.0124
Circadian Rhythm Signaling	3	17	0.0130
P2Y Purinergic Receptor Signaling Pathway	6	66	0.0134
PI3k/AKT Signaling	7	87	0.0147
ERK5 Signaling	5	49	0.0147
B Cell Recognition Signaling	8	110	0.0166
NGF Signaling	6	70	0.0175
Antiproliferative Role of TOB in T Cell Signaling	3	21	0.0233
Triacylglycerol Biosynthesis	3	21	0.0233
T Cell Receptor Signaling	5	57	0.0267
Epoxysqualene Biosynthesis	1	1	0.0298
L DOPA Degradation	1	1	0.0298
Sorbitol Degradation	1	1	0.0298
Synaptic Long Term Potentiation	5	59	0.0305
FcyRIIB Signaling in B Lymphocytes	3	24	0.0333
GNRH Signaling	6	82	0.0349
Neurotrophin/TRK Signaling	4	43	0.0381
PKC0 Signaling in T Lymphoma	5	64	0.0412
Chronic Myeloid Leukemia Signaling	5	65	0.0437
Oxidative Phosphorylation	5	65	0.0437
IL-17 Signaling	4	45	0.0441
ERK/MAPK Signaling	7	110	0.0454
Polyamine Regulation in Colon Cancer	2	12	0.0479

Supplemental Figure S4. Pathways with a significant change due to zeaxanthin supplementation.

Results from differential liver expressome were analyzed computationally to assess which cellular pathways may have changed as a result of carotenoid accumulation. Complete list of pathways affected by zeaxanthin accumulation.



Supplementary Figure S5. Carotenoid accumulation effects whole body respiration rates as area under the curve (AUC). Five-week old *Bco1*$^{-/-}$ *Bco2*$^{-/-}$ (DKO) female mice were supplemented on either β-carotene (n = 3), zeaxanthin (n = 3), or a control carotenoid-free diet (n = 3) for 10-weeks. Mice were then subjected to whole-body respiration analysis. (A) Oxygen consumption and carbon dioxide production were measured every 15 minutes for 6 hours. (B) Average values of oxygen consumption and (C) carbon dioxide production were lower in the carotenoid fed group.(D) Respiration quotient was calculated as the ratio of oxygen consumption to carbon dioxide production. (E) Total energy expenditure was then calculated from these values. Values are means, error bars represent standard error, p-values are given as compared to control mice.