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Sampling of colon specimens

After CO₂ asphyxiation of each mouse the entire colon was quickly removed, placed into ice-cold PBS and 1 cm colonic specimens were taken just distal to the cecum (proximal colon) and above the rectum (distal colon). The remaining colon was cut in half longitudinally. One half was snap frozen and stored in liquid nitrogen (LN2) for and the other half immersed in RNA later™ (Ambion), kept at 4C° for 24h and stored in LN2 for RNA isolation. Proximal and distal colon specimens were embedded in Optimal Cutting Temperature medium, frozen in LN2 pre-cooled isopentane, and stored at -80°C until cryosectioning. Eight µm sections were cut onto Poly-L-lysine coated superfrost/plus slides for F4/80 immunostaining.

Analyses and functional interpretation of microarray data

Functional network and pathway analyses -Networks, canonical pathways and functional analyses were generated through the use of Ingenuity Pathways Analysis (IPA) (Ingenuity® Systems) (1). IPA Knowledge Base comprises manually curated molecular interactions. Three and 6 mo differentially expressed data sets (P -value < 0.05, no FDR applied) were used for the analysis. Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathway Knowledge Base. These so called “focus genes” were then used as a starting point for generating networks and identifying pathways or biological functions. The functional analysis and canonical pathways analyses identified biological functions and pathways that were most significant to the data set. Fischer’s exact test was used to calculate p -values determining the probability that the association between the genes in the dataset and either canonical pathway or biological function could be explained by chance alone. Significances for biological functions or canonical pathways were assigned to each network by determining a P -value for the enrichment of the genes in the network for such functions or pathways compared with the whole Ingenuity pathway knowledge base as a reference set. A score of greater than 2 indicates that there is a less than 1 in 100 chance that the Focus Genes were assembled into a network due to random chance alone.

Gene Ontology- EASE program (NIH, NIAID) (2) was used to determine which Gene Ontology (GO) categories were statistically over-represented among differentially expressed genes. The enrichment P -values were calculated using modified Fisher’s exact test with Bonferroni adjustment for multiple testing. P -values < 0.05 were considered significant. 3 and 6 month differentially expressed gene sets (P -values < 0.05, no FDR applied) were used for the analysis

Gene Set Enrichment Analysis (GSEA) was used to identify statistically significant coordinated changes in a defined gene set between the WD and control groups at 3 and 6 mo. A custom gene set was generated based on thoroughly researched panels of oxidative stress related genes (Superarray Bioscience, Mouse Pathway Focused PCR Array Lists). GSEA analysis was performed using a desktop application available at Broad Institute (3). Normalized expression values for the whole microarray gene set were extracted from GeneSifter and the data were converted from probe sets to gene symbols.

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The input gene set was comprised of C2 pathway and a manually curated literature collection of the Molecular Signature Database Version 1 (MSigDB)(4). *P*-values were calculated by permuting genes 1000 times. Permutation type was set to 'gene set'. False positive rate was controlled by the proportion of false positives by calculating the false discovery rate (FDR) corresponding to each normalized enrichment score (NES). The FDR was computed by comparing the tails of the observed and null distributions for the NES. Gene sets with $FDR < 0.25$ and nominal *P*-values < 0.05 were considered significant.

Validation of Micoarray data by qPCR

Quantitative RT-PCR (qPCR) was used to verify the microarray results. The same batch of total RNA samples used for microarray analysis from 4 control and 4 experimental mice at both time points were reverse transcribed with random nonamers using Eurogentec (San Diego, CA) RT core kit (RT-RTCK-05) to yield first-strand cDNA. 11 Taqman Gene Expression Assays (ABI, Applied Biosystems; Supplemental Table 3) were designed across exon boundaries to avoid genomic DNA detection. Each assay contains pre-formated primers and Taqman probe in a 20X concentration. Taqman assay reactions were performed in 384-well optical plate and run in the ABI 7900HT Sequence Detection System. Efficiency of all assays was equal as determined from ΔC_t vs. log input in validation experiment; therefore the comparative $\Delta\Delta C_t$ method was used for quantification. All amplification were done in triplicate and real-time thermal profile was set as 50°C/2min, 95°C/10min and 40 cycles of 95°C/15sec; 60°C/1min. The threshold cycle (*C_t*) scores were averaged for subsequent calculations of relative expression values by using Applied Biosystems Sequence Detection System 2.3 (SDS) software. The *C_t* scores represent the cycle number at which fluorescence signal crosses the threshold value. The *C_t* scores for genes of interest for each sample were normalized against *C_t* scores for the corresponding endogenous control gene, which was GAPDH. The comparative *C_t* method was used to calculate relative expression between the WD group and controls, the formula is expressed as: relative expression = $2^{-\Delta\Delta C_t}$.
2 = The fold change between *C_t* values (doubling of PCR amplicon).
 $\Delta\Delta C_t = (C_t \text{ experiment} - C_t \text{ GAPDH}) - (C_t \text{ control} - C_t \text{ GAPDH})$.
This equation means how many doublings or fold changes of amplicon occur between cycles of your control (calibrator) vs. experiment samples.

Digital image acquisition for computerized histomorphometry analysis

Digital images of microscopic fields of cross sections (x20 objective and x10 ocular lenses) were acquired with a Zeiss Axioplan2 microscope equipped with QImaging Retiga Camera system and integrated LCD filter. Camera settings were at maximal sensitivity and 1/400 sec exposure time. White balancing was performed before image acquisition. Digital images were saved in 24-bit color TIFF format. For each mouse, 5-7 images of the colonic cross section were taken to represent the entire section.

Pro- and anti-inflammatory protein expression profiling

Clarified tissue extracts of mid colon from 10 mice/group, 40 samples in total, were analyzed by a Rodent Multi-Analyte Profile testing (Rules-Based Medicine, Rodent MAP version 1.6 antigen panel;) (5), a high-density, quantitative immunoassay of pro-and anti-inflammatory protein expression in small samples. Tissues were homogenized in 9

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volumes of lysis buffer (weight/volume; 0.05 mol/L, Tris-HCL with 2 mmol/L EDTA, pH 7.4) containing protease and phosphatase inhibitor cocktails (P 8340 and P 5726, Sigma, MI, U.S.A) and centrifuged for 2 min at 13, 000xg. Supernatant fractions were collected and frozen at -80°C until analysis by the Rodent MAP testing. The Least Detectable Dose (LDD) for each analyte is defined as the mean background value for 20 determinations plus three standard deviations. Values above the LDD possess excellent precision with coefficients of variation (CV) usually below 10%. The Lower Assay Limit (LAL) is defined as each assay's working sensitivity in plasma as defined by the lowest concentration calibrator used for quantitation. Values between the LDD and LAL are less precise and were set to the LAL for data mining. Two-tailed student's *t*-test was used to compare diet-dependent protein expression levels and values of $P < 0.05$ were considered to be statistically significant.

Quantitative analysis of redox active compounds in colon tissues

The concentrations of selected redox active compounds were measured using a Perkin-Elmer HPLC equipped with an 8 channel coulometric array detector (ESA) (6). Frozen colonic segments from 6 animals fed the WD or the control diet for 6 mo were homogenized in 5 volumes of cold PBS (weight/volume) and a 50 μ L aliquot was mixed with 15 μ L of 25% solution of metaphosphoric acid. Samples were allowed to stand for 15 minutes at 4 °C and were centrifuged at 10,000-x g for 10 minutes to sediment protein. Cell pellet precipitates were dissolved in 0.1 mol/L NaOH and saved for protein determinations by a spectrophotometric quantitation method using BCA reagent (Pierce Chemical). The concentrations of redox sensitive compounds in the supernatant fractions were determined by injecting 5 μ L aliquots onto an Ultrasphere 5 u, 4.6 x 250 mm, C18 column and eluting with a mobile phase of 50 mmol/L NaH_2PO_4 , 0.05 mmol/L octane sulfonic acid, 2% acetonitrile (pH 2.62) at a flow rate of 1 mL/min. The 8-channel CoulArray detectors were set at 200, 350, 400, 450, 500, 550, 600 and 700 mV, respectively. Peak areas were analyzed using ESA, Inc. software. The concentrations of redox active compounds in the samples were compared against standards for each derivative (cysteine, cystine, ascorbate, methionine, glutathione, oxidized glutathione, and uric acid) and were reported as nmol/mg protein. The range of concentrations of each standard curve was 10 to 200 pmol/L.

Literature cited

1. Ingenuity Pathway Analysis Software- Complete Pathway Database (April, 2009), <http://www.ingenuity.com/>.
2. DAVID 2008 Functional Annotation Bioinformatics Microarray Analysis (December, 2008), <http://david.abcc.ncifcrf.gov/>.
3. Gene Set Enrichment Analysis (December, 2008), <http://www.broad.mit.edu/gsea/>.
4. Gene Set Enrichment Analysis/ Molecular Signatures Database/ Browse Collections (December, 2008), <http://www.broad.mit.edu/gsea/msigdb/collections.jsp>.
5. Rules-Based Medicine, Inc: The Biomarker Testing Laboratory (June, 2008) <http://www.rulesbasedmedicine.com>.

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6. Pinto JT, Van Raamsdonk JM, Leavitt BR, Hayden MR, Jeitner TM., Thaler HT, Krasnikov BF, Cooper AJ. Treatment of YAC128 mice and their wild-type littermates with cystamine does not lead to its accumulation in plasma or brain: implications for the treatment of Huntington disease. *J Neurochem.* 2005;94:1087-101.

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Supplemental Table 1. Diet Compositions^{1,2}

Ingredients	AIN-76A	WD
Fat (corn oil), %	5 (13)	20 (40)
Calcium, mg/g	5 (2700)	0.5 (220)
Cholecalciferol $\mu\text{g/g}$	0.025 (600)	0.0125 (50)
Phosphorus, mg/g	4 (2200)	3.6 (1600)
Fiber (cellulose), %	5 (25)	5 (25)
Folic acid, g/g	2 (1100)	2 (1100)
kJ/g (approximate)	3.6	4.5

¹Numbers in parenthesis are the calculated equivalents, based on nutrient density, in a daily human 2000 kJ diet. ²Modified from reference (18).

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Supplemental Table 2. Custom Gene Lists related to oxidative stress used in GSEA

Gene Symbols

SCD1, GPX2, GPX3, APOE, NCF2, NOS2, ALS2, DUOX1, RAG2, PRDX4, MPP4, SLC41A3, EPX, AASS, TPO, IL19, KIF9, GPX5, UCP3, TMOD1, NUDT15, ZMYND17, CYBA, EHD2, MPO, SOD3, GPX7, LPO, RECQL4, PRDX2, PARK7, IL22, XPA, PRDX6-RS1, CTSB, SERPINB1B, APC, TXNRD2, PRDX1, FMO2, GPX6, NOX1, GPX1, NOX4, NXN, PRDX5, ERCC2, CCS, GPX4, PRNP, NOXO1, TXNRD3, GSR, PRDX6, GAB1, SOD1, PSMB5, PRDX3, PTGS2, TXNRD1, NOXA1, PTGS1, CAT, IDH1, PPP1R15B, NQO1

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Supplemental Table 3. Expression of 11 genes in the colon of WD fed mice compared to controls at 3 and 6 months identified by microarray and validated by qPCR¹

Gene symbol	Time points				TaqMan® Gene Expression Assays
	3 months		6 months		
	Microarray	qPCR	Microarray	qPCR	
Fold					
Upregulated					
Mt2	3.44	3.48			Mm00809556_s1
Per2	1.89	2.06	1.98	2.61	Mm00478113_m1
Gsta2	1.49	1.32	1.45	1.13	Mm00833353_mH
Cyp4b1	1.72	1.79	1.72	2.05	Mm00484138_m1
Sqle	1.59	1.89	1.40	1.41	Mm00436772_m1
Tef	1.94	2.06	1.79	2.12	Mm00457513_m1
Downregulated					
Scd1	-4.42	-10.20	-1.72	-1.59	Mm00772290_m1
Lox	-1.48	-2.00	-1.52	-2.16	Mm00495386_m1
Arntl	-1.59	-2.27	-1.65	-2.06	Mm00500226_m1
Hsp110	-1.74	-2.11		-1.36	Mm00442864_m1
Spon2	-2.15	-2.10		-1.68	Mm00513596_m1

¹Data are expressed as fold change and are means; n=8 for microarray and n=4 for qPCR analyzes.

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Supplemental Table 4. Differentially expressed genes in the colon of WD fed C57Bl/6 mice at both the 3 mo (n=8) and 6 mo (n=8) time points¹

Gene Symbol	RefSeq	3 months Fold	6 months Fold	Description	Gene Ontology
Entpd5	NM_007647	1.29	1.23	Mus musculus ectonucleoside triphosphate diphosphohydrolase 5 (Entpd5) mRNA.	
Gsta2	NM_008181	1.43	1.37	Mus musculus glutathione S-transferase alpha 1 (Ya) (Gsta1) mRNA.	GO:6749(glutathione metabolism)
Gsta2	NM_008182	1.49	1.45	Mus musculus glutathione S-transferase alpha 2 (Yc2) (Gsta2) mRNA.	
Gstp1	NM_013541	1.29	1.25	Mus musculus glutathione S-transferase pi 1 (Gstp1) mRNA.	GO:6749(glutathione metabolism); GO:8152(metabolism)
Gsta2	NM_008182	1.41	1.33	Mus musculus glutathione S-transferase alpha 2 (Yc2) (Gsta2) mRNA.	
Fgfbp1	NM_008009	1.24	1.24	Mus musculus fibroblast growth factor binding protein 1 (Fgfbp1) mRNA.	GO:6355(regulation of transcription, DNA-dependent)
Gstp1	NM_013541	1.32	1.27	Mus musculus glutathione S-transferase pi 1 (Gstp1) mRNA.	GO:6749(glutathione metabolism); GO:8152(metabolism) GO:6695(cholesterol biosynthesis); GO:8203(cholesterol metabolism); GO:8610(lipid biosynthesis); GO:6694(steroid biosynthesis); GO:16126(sterol biosynthesis)
Nsdhl	NM_010941	1.2	1.26	Mus musculus NAD(P) dependent steroid dehydrogenase-like (Nsdhl) mRNA.	
Aard	NM_175503	1.23	1.24	Mus musculus alanine and arginine rich domain containing protein (Aard) mRNA.	GO:4(biological process unknown)
AI842788	NM_198616	1.22	1.25	Expressed sequence AI842788 (AI842788), mRNA	
Cndp2	NM_023149	1.23	1.22	Mus musculus CNDP dipeptidase 2 (metallopeptidase M20 family) (Cndp2) mRNA.	GO:6508(proteolysis)
1500041J02Rik	NM_026424	1.3	1.42	Mus musculus RIKEN cDNA 1500041J02 gene (1500041J02Rik) mRNA.	
Cyp4f16	NM_024442	1.22	1.47	Mus musculus cytochrome P450 family 4 subfamily f polypeptide 16 (Cyp4f16) mRNA.	GO:6118(electron transport) GO:6695(cholesterol biosynthesis); GO:8610(lipid biosynthesis); GO:8152(metabolism); GO:6694(steroid biosynthesis)
Hsd17b7	NM_010476	1.37	1.21	Mus musculus hydroxysteroid (17-beta) dehydrogenase 7 (Hsd17b7) mRNA.	
Per2	NM_011066	1.89	1.98	Mus musculus period homolog 2 (Drosophila)	GO:7623(circadian rhythm);

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				(Per2) mRNA.	GO:6355(regulation of transcription, DNA-dependent); GO:48511(rhythmic process); GO:7165(signal transduction); GO:6350(transcription); GO:160(two-component signal transduction system (phosphorelay))
Ngef	NM_019867	1.25	1.23	Mus musculus neuronal guanine nucleotide exchange factor (Ngef) mRNA.	GO:50875(cellular physiological process)
Pcsk9	NM_153565	1.52	1.4	Mus musculus proprotein convertase subtilisin/kexin type 9 (Pcsk9) mRNA.	GO:8203(cholesterol metabolism); GO:6629(lipid metabolism); GO:43086(negative regulation of enzyme activity); GO:30182(neuron differentiation); GO:6508(proteolysis); GO:8202(steroid metabolism)
Akr1b8	NM_008012	1.26	1.34	Mus musculus aldo-keto reductase family 1 member B8 (Akr1b8) mRNA.	
Dhcr7	NM_007856	1.21	1.29	Mus musculus 7-dehydrocholesterol reductase (Dhcr7) mRNA.	GO:1568(blood vessel development); GO:30154(cell differentiation); GO:6695(cholesterol biosynthesis); GO:8610(lipid biosynthesis); GO:30324(lung development); GO:42127(regulation of cell proliferation); GO:6694(steroid biosynthesis); GO:16126(sterol biosynthesis)
Tm4sf7	NM_053082	1.25	1.54	Mus musculus transmembrane 4 superfamily member 7 (Tm4sf7) mRNA.	
Dbp	NM_016974	1.59	1.66	Mus musculus D site albumin promoter binding protein (Dbp) mRNA.	GO:7623(circadian rhythm); GO:6355(regulation of transcription, DNA-dependent); GO:48511(rhythmic process); GO:6350(transcription)
Sc4mol	NM_025436	1.51	1.35	Mus musculus sterol-C4-methyl oxidase-like (Sc4mol) mRNA.	GO:8610(lipid biosynthesis); GO:8152(metabolism); GO:6694(steroid biosynthesis); GO:16126(sterol biosynthesis)
4931406C07Rik	NM_133732	1.49	1.51	Mus musculus RIKEN cDNA 4931406C07 gene (4931406C07Rik) mRNA.	
Creb3l3	NM_145365	1.49	1.42	Mus musculus cAMP responsive element binding protein 3-like 3 (Creb3l3) mRNA.	
Dp11l	NM_139292	1.24	1.25	Mus musculus deleted in polyposis 1-like 1	

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Tef	NM_017376	1.94	1.79	(Dp111) mRNA. Mus musculus thyrotroph embryonic factor (Tef) transcript variant 2 mRNA.	GO:45944(positive regulation of transcription from RNA polymerase II promoter); GO:6355(regulation of transcription, DNA-dependent); GO:48511(rhythmic process); GO:6350(transcription) GO:6118(electron transport); GO:8152(metabolism)
Sqle	NM_009270	1.59	1.4	Mus musculus squalene epoxidase (Sqle) mRNA.	GO:8152(metabolism)
Cbr3	NM_173047	1.4	1.38	Mus musculus carbonyl reductase 3 (Cbr3) mRNA.	GO:8152(metabolism)
Aldh1a7	NM_011921	1.26	1.48	Mus musculus aldehyde dehydrogenase family 1 subfamily A7 (Aldh1a7) mRNA.	GO:8152(metabolism); GO:42574(retinal metabolism)
Mid1ip1	NM_026524	1.22	1.31	Mid1 interacting protein 1 (gastrulation specific G12-like (zebrafish)), mRNA (cDNA clone MGC:18710 IMAGE:4215464)	GO:7026(negative regulation of microtubule depolymerization)
AI987692	NM_177912	-1.32	-2.54	Mus musculus expressed sequence AI987692 (AI987692) mRNA.	
Eln	NM_007925	-1.4	-1.34	Mus musculus elastin (Eln) mRNA.	GO:30833(regulation of actin filament polymerization); GO:43149(stress fiber formation); GO:7519(striated muscle development)
Ifi202b	NM_172648	-1.23	-1.31	Mus musculus interferon activated gene 205 (Ifi205) mRNA.	
9030623N16Rik	NM_025777	-1.31	-1.84	Mus musculus RIKEN cDNA 9030623N16 gene (9030623N16Rik) mRNA.	
Arntl	NM_007489	-1.59	-1.65	Mus musculus aryl hydrocarbon receptor nuclear translocator-like (Arntl) mRNA.	GO:7623(circadian rhythm); GO:60(protein import into nucleus, translocation); GO:42176(regulation of protein catabolism); GO:45449(regulation of transcription); GO:6355(regulation of transcription, DNA-dependent); GO:7165(signal transduction); GO:6350(transcription); GO:160(two-component signal transduction system (phosphorelay))
Col4a1	NM_009931	-1.26	-1.34	Mus musculus procollagen type IV alpha 1 (Col4a1) mRNA.	GO:7155(cell adhesion); GO:6817(phosphate transport)

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Nfil3	NM_017373	-1.34	-2.09	Mus musculus nuclear factor interleukin 3 regulated (Nfil3) mRNA.	GO:6355(regulation of transcription, DNA-dependent)
Slc39a4	NM_028064	-1.27	-1.36	Mus musculus solute carrier family 39 (zinc transporter) member 4 (Slc39a4) mRNA.	GO:6811(ion transport); GO:30001(metal ion transport); GO:6810(transport); GO:6882(zinc ion homeostasis); GO:6829(zinc ion transport)
Tfrc	NM_011638	-1.33	-1.5	Mus musculus transferrin receptor (Tfrc) mRNA.	GO:6897(endocytosis); GO:6879(iron ion homeostasis); GO:6508(proteolysis)
Lox	NM_010728	-1.48	-1.52	Mus musculus lysyl oxidase (Lox) mRNA.	
Scd1	NM_009127	-4.42	-1.72	Mus musculus stearyl-Coenzyme A desaturase 1 (Scd1) mRNA.	GO:6633(fatty acid biosynthesis); GO:8610(lipid biosynthesis); GO:6801(superoxide metabolism)
Ctgf	NM_010217	-1.37	-1.2	Mus musculus connective tissue growth factor (Ctgf) mRNA.	GO:6260(DNA replication); GO:1525(angiogenesis); GO:1502(cartilage condensation); GO:7155(cell adhesion); GO:30154(cell differentiation); GO:16477(cell migration); GO:7160(cell-matrix adhesion); GO:8543(fibroblast growth factor receptor signaling pathway); GO:7229(integrin-mediated signaling pathway); GO:1503(ossification); GO:1558(regulation of cell growth)
2900041A09Rik	NM_182839	-1.3	-1.6	Mus musculus RIKEN cDNA 2900041A09 gene (2900041A09Rik) mRNA.	GO:6118(electron transport)

¹Data were generated by GeneSifter Microarray analysis restricting analysis to genes with signal intensities greater than 0.95. 1.2 fold cutoff was applied and pairwise statistical analysis was performed using Student's t-test (P -value < 0.05).

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Supplemental Table 5. Biological function GO categories over-represented in the colon of mice after 3 mo (n=8) or 6 mo (n=8) feeding of WD¹

3 month GO	Fold	6 month GO	Fold
angiogenesis	3.69*	angiogenesis	2.80
carboxylic acid metabolism	2.26*	carboxylic acid metabolism	1.94*
cellular lipid metabolism	3.56***	cellular lipid metabolism	2.40***
lipid biosynthesis	5.80***	lipid biosynthesis	3.20***
steroid biosynthesis	13.89***	steroid biosynthesis	6.30***
steroid metabolism	7.57***	steroid metabolism	3.27**
sterol metabolism	12.71***	sterol metabolism	7.32***
blood vessel morphogenesis	3.68*	anion transport	2.47*
cation homeostasis	5.38**	antigen presentation, exogenous antigen	6.00*
cell growth	4.95**	chemotaxis	5.29***
coenzyme metabolism	3.15*	complement activation	5.02*
electron transport	2.19*	cytokine and chemokine mediated signaling pathway	6.00*
fluid secretion	55.50*	cytokine biosynthesis	4.35*
glutathione metabolism	16.00**	cytokine metabolism	4.28*
isoprenoid biosynthesis	19.20**	cytokine production	4.15*
isoprenoid metabolism	14.50**	humoral defense mechanism	5.22**
nitric oxide mediated signal transduction	41.70*	immune cell activation	2.87*
positive regulation of cell proliferation	3.70*	immune cell chemotaxis	9.00*
regulation of cell size	4.80**	inflammatory response	4.10***
		innate immune response	6.23**
		lymphocyte differentiation	3.75*
		mast cell activation	17.99**
		myeloid cell differentiation	4.32*
		negative regulation of myeloid cell differentiation	15.87***
		negative regulation of protein kinase activity	6.75*

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negative regulation of protein metabolism	4.09*
positive regulation of biosynthesis	4.69*
positive regulation of immune response	4.55**
positive regulation of protein kinase activity	4.91*
positive regulation of protein metabolism	4.09*
positive regulation of signal transduction	5.14*
regulation of endocytosis	6.17*
regulation of immune response	3.30**
regulation of protein kinase activity	4.15**
regulation of protein metabolism	3.23***
thymic T cell selection	11.57*

¹The enrichment *P*-values were calculated using modified Fisher's exact test with Bonferroni adjustment for multiple testing. * Different from control, $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$.

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Supplemental Table 6. Canonical pathways significantly altered by the WD (n=8) in the colon of C57Bl/6 mice compared to controls (n=8) at 3 months or 6 months^{1,2}

3 month					
Signaling Pathways	Ratio ³	Gene Symbol	Description	RefSeq	Fold
LPS/IL-1 Mediated Inhibition of RXR Function	10/170***	ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	XM_358306	1.219**
		ALDH1B1	aldehyde dehydrogenase 1 family, member B1	NM_028270	1.369***
		FABP6	fatty acid binding protein 6, ileal (gastrotropin)	NM_008375	1.226*
		FMO5	flavin containing monooxygenase 5	NM_010232	1.307*
		GSTA4	glutathione S-transferase A4	NM_010357	1.406***
		GSTA5	glutathione S-transferase A5	NM_008182	1.486***
		GSTO1	glutathione S-transferase omega 1	NM_010362	1.201***
		GSTP1	glutathione S-transferase pi	NM_013541	1.317***
		HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	NM_145942	1.574***
		HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	NM_008256	1.478**
Nrf-2 Mediated Oxidative Stress Response	8/142***	ACTA2	actin, alpha 2, smooth muscle, aorta	NM_007392	1.219**

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		GCLM	glutamate-cysteine ligase, modifier subunit	NM_008129	1.369***
		GSTA4	glutathione S-transferase A4	NM_010357	1.226*
		GSTA5	glutathione S-transferase A5	NM_008182	1.307*
		GSTO1	glutathione S-transferase omega 1	NM_010362	1.406***
		GSTP1	glutathione S-transferase pi	NM_013541	1.486***
		HMOX1	heme oxygenase (decycling) 1	NM_010442	1.201***
		STIP1	stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein)	NM_016737	1.317***
Circadian rhythm signaling	4/31***	ARNTL	aryl hydrocarbon receptor nuclear translocator-like	NM_007489	-1.589***
		CLOCK	clock homolog (mouse)	NM_007715	-1.207**
		NR1D1	nuclear receptor subfamily 1, group D, member 1	NM_145434	-1.234*
		PER2	period homolog 2 (Drosophila)	NM_011066	1.895***
Xenobiotic Metabolism Signaling	8/228**	ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	XM_358306	1.219**
		ALDH1B1	aldehyde dehydrogenase 1 family, member B1	NM_028270	1.369***
		FMO5	flavin containing monooxygenase 5	NM_010232	1.307*
		GSTA4	glutathione S-transferase A4	NM_010357	1.406***
		GSTA5	glutathione S-transferase A5	NM_008182	1.486***
		GSTO1	glutathione S-transferase omega 1	NM_010362	1.201***
		GSTP1	glutathione S-transferase pi	NM_013541	1.317***

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Aryl Hydrocarbon receptor signaling	6/147**	HMOX1	heme oxygenase (decycling) 1	NM_010442	1.684*
		ALDH1B1	aldehyde dehydrogenase 1 family, member B1	NM_028270	1.369***
		CCND2	cyclin D2	NM_009829	-1.221*
		GSTA4	glutathione S-transferase A4	NM_010357	1.406***
		GSTA5	glutathione S-transferase A5	NM_008182	1.486***
		GSTO1	glutathione S-transferase omega 1	NM_010362	1.201***
IGF1-Signaling	4/90*	GSTP1	glutathione S-transferase pi	NM_013541	1.317***
		CTGF	connective tissue growth factor	NM_010217	-1.37**
		IGFBP2	insulin-like growth factor binding protein 2, 36kDa	NM_008342	-1.203*
		IGFBP4	insulin-like growth factor binding protein 4	NM_010517	-1.233***
		SFN	stratifin	NM_018754	-1.227**
Metabolic Pathways	Ratio	Gene Symbol	Description		Fold
Glutathione Metabolism	6/102***	GCLM	glutamate-cysteine ligase, modifier subunit	NM_008129	1.231*
		GSTA4	glutathione S-transferase A4	NM_010357	1.406***
		GSTA5	glutathione S-transferase A5	NM_008182	1.486***
		GSTO1	glutathione S-transferase omega 1	NM_010362	1.201***
		GSTP1	glutathione S-transferase pi	NM_013541	1.317***
		IDH1	isocitrate dehydrogenase 1 (NADP+), soluble	NM_010497	1.278**

Online Supporting Material

Butanoate Metabolism	6/127***	AACS	acetoacetyl-CoA synthetase	NM_030210	1.481***
		ALDH1A7	aldehyde dehydrogenase family 1, subfamily A7	NM_011921	1.257***
		ALDH1B1	aldehyde dehydrogenase 1 family, member B1	NM_028270	1.369***
		HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	NM_145942	1.574***
		HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	NM_008256	1.478*
		ILVBL	ilvB (bacterial acetolactate synthase)-like	NM_173751	1.204***
Biosynthesis of Steroids	6/127**	DHCR7	7-dehydrocholesterol reductase	NM_007856	1.206***
		FDPS	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransferase, geranyltranstransferase)	NM_134469	1.295*
		HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	XM_127496	1.249*
		LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	NM_146006	1.484***
		MVD	mevalonate (diphospho) decarboxylase	NM_138656	1.232*
		SQLE	squalene epoxidase	NM_009270	1.587***
Metabolism of Xenobiotics	8/213**	CYP2D10	cytochrome P450, family 2, subfamily	NM_010005	1.281*

Online Supporting Material

by Cytochrome P450

	d, polypeptide 10		
CYP2D26	cytochrome P450, family 2, subfamily d, polypeptide 26	NM_029562	1.567*
CYP4B1	cytochrome P450, family 4, subfamily B, polypeptide 1	NM_007823	1.724)
CYP4F12	cytochrome P450, family 4, subfamily F, polypeptide 12	NM_022434	1.409**
GSTA4	glutathione S-transferase A4	NM_010357	1.406***
GSTA5	glutathione S-transferase A5	NM_008182	1.486***
GSTO1	glutathione S-transferase omega 1	NM_010362	1.201***
GSTP1	glutathione S-transferase pi	NM_013541	1.317***

Online Supporting Material

Fatty Acid Metabolism	7/186**	ACAA2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	NM_177470	1.242***
		ALDH1A7	aldehyde dehydrogenase family 1, subfamily A7	NM_011921	1.257***
		ALDH1B1	aldehyde dehydrogenase 1 family, member B1	NM_028270	1.369***
		CYP2D10	cytochrome P450, family 2, subfamily d, polypeptide 10	NM_010005	1.281*
		CYP2D26	cytochrome P450, family 2, subfamily d, polypeptide 26	NM_029562	1.567*
		CYP4B1	cytochrome P450, family 4, subfamily B, polypeptide 1	NM_007823	1.724*
		CYP4F12	cytochrome P450, family 4, subfamily F, polypeptide 12	NM_022434	1.409**
Valine, Leucine, Isoleucine Degradation	5/107**	ACAA2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	NM_177470	1.242***
		ALDH1A7	aldehyde dehydrogenase family 1, subfamily A7	NM_011921	1.257***
		ALDH1B1	aldehyde dehydrogenase 1 family, member B1	NM_028270	1.369***
		HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	NM_145942	1.574***
		HMGSC2	3-hydroxy-3-methylglutaryl-	NM_008256	1.478*

Online Supporting Material

Tryptophan Metabolism	7/237**	ALDH1A7	Coenzyme A synthase 2 (mitochondrial) aldehyde dehydrogenase family 1, subfamily A7	NM_011921	1.257***
		ALDH1B1	aldehyde dehydrogenase 1 family, member B1	NM_028270	1.369***
		CCBL1	cysteine conjugate-beta lyase; cytoplasmic (glutamine transaminase K, kynurenine aminotransferase)	NM_172404	1.467*
		CYP2D10	cytochrome P450, family 2, subfamily d, polypeptide 10	NM_010005	1.281*
		CYP2D26	cytochrome P450, family 2, subfamily d, polypeptide 26	NM_029562	1.567*
		CYP4B1	cytochrome P450, family 4, subfamily B, polypeptide 1	NM_007823	1.724*
		CYP4F12	cytochrome P450, family 4, subfamily F, polypeptide 12	NM_022434	1.409**
		Synthesis and degradation of ketone bodies	2/19*	HMGCS1	3-hydroxy-3-methylglutaryl- Coenzyme A synthase 1 (soluble)
HMGSC2	3-hydroxy-3-methylglutaryl- Coenzyme A synthase 2 (mitochondrial)			NM_008256	1.478*

Online Supporting Material

Pyruvate Metabolism	4/145*	AKR1B8	--	NM_008012	1.26*
		(includes EG:14187)			
		ALDH1A7	aldehyde dehydrogenase family 1, subfamily A7	NM_011921	1.257***
		ALDH1B1	aldehyde dehydrogenase 1 family, member B1	NM_028270	1.369***
Glycosphingolipid biosynthesis - Lactoseries	2/28*	ME1	malic enzyme 1, NADP(+)-dependent, cytosolic	NM_008615	-1.548*
		B3GNT5	beta3Gn-T5, Lc3 Synthase	NM_054052	-1.271*
		ST3GAL4	2,3-Alpha Sialyltransferase, Alpha-2,3-sialyltransferase, Beta-galactosidase alpha-2,3-sialyltransferase, CGS23, FLJ11867, NANTA3, SAT3, SIAT4, SIAT4C, ST3GAL IV, STZ	NM_009178	1.267*
Linoleic Acid Metabolism	4/119*	CYP2D10	cytochrome P450, family 2, subfamily d, polypeptide 10	NM_010005	1.281*
		CYP2D26	cytochrome P450, family 2, subfamily d, polypeptide 26	NM_029562	1.567*
		CYP4B1	cytochrome P450, family 4, subfamily B, polypeptide 1	NM_007823	1.724*

Online Supporting Material

CYP4F12 cytochrome P450, family 4, subfamily
F, polypeptide 12 NM_022434 1.409**

6 month

Signaling Pathways	Ratio	Gene Symbol	Description	RefSeq	Fold
Nrf-2 Mediated Oxidative Stress Response	10/142***	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	NM_010234	1.794**
		JUNB	jun B proto-oncogene	NM_008416	1.235*
		CBR1	carbonyl reductase 1	NM_007620	1.265**
		GSTA3	glutathione S-transferase A3	NM_010356	1.35*
		GSTA5	glutathione S-transferase A5	NM_008182	1.451***
		GSTP1	glutathione S-transferase pi	NM_013541	1.274***
		MGST3	microsomal glutathione S-transferase 3	NM_025569	1.26***
		NQO1	NAD(P)H dehydrogenase, quinone 1	NM_008706	1.33**
		CREBBP	CREB binding protein (Rubinstein- Taybi syndrome)	XM_148699	-1.305**
Circadian rhythm signaling	4/31**	GPX2	glutathione peroxidase 2 (gastrointestinal)	NM_030677	-1.379*
		PER1	period homolog 1 (Drosophila)	NM_011065	1.37*
		PER2	period homolog 2 (Drosophila)	NM_011066	1.982**
		CRY2	cryptochrome 2 (photolyase-like)	NM_009963	1.495**
T cell receptor signaling	6/104**	ARNTL	aryl hydrocarbon receptor nuclear translocator-like	NM_007489	-1.654**
		FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	NM_010234	1.794**

Online Supporting Material

IGF1-Signaling	5/90**	PTPRH	protein tyrosine phosphatase, receptor type, H	NM_207270	1.218*
		LCP2	lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)	NM_010696	-1.286**
		PPP33C	protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform	NM_008915	-1.202***
		CD3D	CD3d molecule, delta (CD3-TCR complex)	NM_013487	-1.435**
		CD3E	CD3e molecule, epsilon (CD3-TCR complex)	NM_007648	-1.391*
		CTGF	connective tissue growth factor	NM_010217	1.204*
		FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	NM_010234	1.794**
		NEDD4	neural precursor cell expressed, developmentally down-regulated 4	NM_010890	1.24**
LPS/IL-1 Mediated Inhibition of RXR Function	7/170**	NOV	nephroblastoma overexpressed gene	NM_010930	1.84**
		IGF1	insulin-like growth factor 1 (somatomedin C)	NM_010512	-1.254**
		SLC27A2	solute carrier family 27 (fatty acid transporter), member 2	NM_011978	1.417**
		GSTA3	glutathione S-transferase A3	NM_010356	1.35*
		GSTA5	glutathione S-transferase A5	NM_008182	1.451***
		GSTP1	glutathione S-transferase pi	NM_013541	1.274***
		MGST3	microsomal glutathione S-transferase 3	NM_025569	1.26***
		TLR4	toll-like receptor 4	NM_021297	-1.368***
Complement Coagulation Cascade	4/70**	ABCG1	ATP-binding cassette, sub-family G (WHITE), member 1	NM_009593	-1.342**
		C2	complement component 2	NM_013484	-1.608*
		C3	complement component 3	NM_009778	-1.553*

Online Supporting Material

Aryl Hydrocarbon receptor signaling	6/147**	C1QB	complement component 1, q subcomponent, B chain	NM_009777	-1.228*
		CD55	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	NM_010016	-1.364*
		FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	NM_010234	1.794**
		GSTA3	glutathione S-transferase A3	NM_010356	1.35*
		GSTA5	glutathione S-transferase A5	NM_008182	1.451***
		GSTP1	glutathione S-transferase pi	NM_013541	1.274***
B cell Receptor Signaling	6/149**	MGST3	microsomal glutathione S-transferase 3	NM_025569	1.26***
		NQO1	NAD(P)H dehydrogenase, quinone 1	NM_008706	1.33**
		BCL2A1	BCL2-related protein A1	NM_007534	-1.475*
		FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)	NM_010188	-1.216*
		FCGR2B	Fc fragment of IgG, low affinity IIb, receptor (CD32)	NM_010187	-1.234**
		MAP3K6	mitogen-activated protein kinase kinase kinase 6	NM_016693	-1.381**
		PPP3CC	protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform	NM_008915	-1.202***
RAC2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	NM_009008	-1.206*		
Metabolic Pathways	Ratio	Gene Symbol	Description	RefSeq	Fold
Glutathione Metabolism	7/102***	GPX2	glutathione peroxidase 2 (gastrointestinal)	NM_030677	-1.379*
		GSTA3	glutathione S-transferase A3	NM_010356	1.35*
		GSTA5	glutathione S-transferase A5	NM_008182	1.451***

Online Supporting Material

		GSTM6	glutathione S-transferase, mu 6	NM_008184	1.374***
		GSTP1	glutathione S-transferase pi	NM_013541	1.274***
		H6PD	hexose-6-phosphate dehydrogenase (glucose 1- dehydrogenase)	NM_173371	-1.256**
		MGST3	microsomal glutathione S-transferase 3	NM_025569	1.26***
Valine, Leucine, Isoleucine Degradation	5/107*	ACAA1B	acetyl-Coenzyme A acyltransferase 1B	NM_146230	3.519**
		ACADS	acyl-Coenzyme A dehydrogenase, C- 2 to C-3 short chain	NM_007383	1.246*
		ALDH1A7	aldehyde dehydrogenase family 1, subfamily A7	NM_011921	1.483***
		BCAT2	branched chain aminotransferase 2, mitochondrial	NM_009737	-1.209*
		HMGCL	3-hydroxymethyl-3-methylglutaryl- Coenzyme A lyase (hydroxymethylglutaricaciduria)	NM_008254	1.266*
Biosynthesis of Steroids	5/127*	DHCR7	7-dehydrocholesterol reductase	NM_007856	1.289*
		MVK	Mevalonate kinase (mevalonic aciduria)	NM_023556	1.229*
		NQO1	NAD(P)H dehydrogenase, quinone 1	NM_008706	1.330**
		PMVK	Phosphomevalonate kinase	NM_026784	1.280*
		SQLE	Squalene epoxidase	NM_09270	1.395**

¹Ingenuity canonical pathway analyses of 3 mo and 6 mo differentially expressed total data sets (P -value < 0.05, no FDR applied). ²Fischer's exact test was used to calculate P -values. * Different from control, $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$. ³The ratio is calculated as the number of differentially expressed genes divided by the total number of genes that make up that pathway.

Online Supporting Material

Supplemental Table 7. Biological networks in the colon of WD fed C57Bl/6 mice (n=8) compared to controls (n=8) at 3 and 6 mo¹

A. Integrated dataset at 3 months

Genes in Network differentially express	Score ²	Focus molecules	Functions
ACTA2↓ ³ , ARNTL↓, CAV1↓, CCND2↓, CLOCK↓, COL1A1↓, DBP↑, EGFR↓, EIF2S2↑, ENAH↓, EPB41L3↑, GSTA5↑, GSTP1↑, HMGCS2↑, IGFBP2↓, IGFBP4↓, MT2A↑, MYOCD↓, NR1D1↓, NR1D2↑, PER2↑, PER3↑, PLAT↓, SERPINH1↓, SFRP1↓, TEF↑, TES↓, TSPAN4↑, USP2↑	54	29	Behavior, Nervous System Development and Function, Cancer
AREG↓, ARG2↑, DUSP6↓, ELN↓, FBLN2↓, FBN1↓, FGF10↓, FGFBP1↑, GHR↓, GREM1↓, HMGCR↑, HMGCS1↑, HMOX1↑, KCNQ1↑, LOX↓, LOXL1↓, LSS↑, NDRG1↑, NFIL3↓, P2RY2↑, PLEKHA1↓, PPP1R1A↓, PTN↓, RGS1↓, SCD↓, TNFRSF12A↓	46	26	Dermatological Diseases and Conditions, Genetic Disorder, Tissue Development
ANXA5↓, COL18A1↓, COL4A1↓, COL5A1↓, CTGF↓, E2F2↓, EDN1↓, GNAI1↓, LGALS1↓, LIG1↑, MCM4↑, MCM5↑, MCM6↑, MMP14↓, RGS4↓, SERPINF1↓, SLC9A3R1↑, TNFRSF17↑, TNFRSF11A↓, UHRF1↑, WEE1↑, YAP1↓	36	22	Cardiovascular System Development and Function, Cancer, Cellular Growth and Proliferation
ACAA2↑, AQP4↓, CDR2↓, COL5A1↓, CPD↓, EMP3↓, GHR↓, ↑GPT, LOC63928↓, PDLIM2↑, PDLIM4↓, PLEKHB1↓, RFX2↓, SFRP1↓, ST3GAL4↑, STIP1↓, TFRC↓, TLE1↓, TLE4↓, TSPO↓	32	20	Cancer, Cell Cycle, Reproductive System Disease
ABCC3↑, AKR1B8 (includes EG:14187) ↑, CNDP2↑, CYP2D10↑, CYP2D26↑, CYP4B1↑, CYP4F12↑, FGFBP1↑, FMO5↑, GSTA4↑, GSTO1↑, HSD17B7↑, KRT7↑, ME1↓, PLEKHA6↓, PPP1R3C↓, PTGER3↓, RERG↓, SLC7A8↓, SPSB1↓	32	20	Cell Morphology, Vitamin and Mineral Metabolism, Gene Expression

Online Supporting Material

ALDH1A7↑, CXCL13↑, CYB5B↑, CYP2D10↑, ELOVL6↑, ENTPD5↑, FMO5↑, GCLM↑, LAMB3↑, MT1E↑, PDE8B↑, PPP1R3C↓, RETSAT↑, SC4MOL↑, SCD↓, SFRP1↓, SLC13A2↑, SQLE↑, STARD5↑	30	19	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry
C13ORF15↓, CGI-38↓, CHAF1B↑, COL4A1↓, DMN↓, E2F2↓, FAH↓, HIST1H1C↓, HSPH1↓, IGFBP2↓, IGFBP4↓, MATN4↓, MCM4↑, PHLDA3↓, RORC↑, SFN↓, STOM↓, TMEM43↓, TPPP↓	30	19	Cell Cycle, Connective Tissue Development and Function, Cancer
C10ORF58↓, CENPA↓, COL4A1↓, CRYAB↓, CSRP1↓, DUSP6↓, HSPA4L↓, HTRA1↓, IGFBP4↓, KRT7↑, MTHFD1L↑, NNM1↓, PARP3↓, PDLIM4↓, SDF2↓, SRXN1↑	23	16	Amino Acid Metabolism, Post-Translational Modification, Small Molecule Biochemistry
AACS↑, DHCR7↑, FA2H↑, FABP6↑, FDPS↑, IDH1↑, IGK↑, MVD↑, NSDHL↑, PCSK9↑, SC4MOL↑, SLC7A9↑, SQLE↑, UCP2↑	19	14	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry
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B. Integrated dataset at 6 months			
ALDH1A7↑, DGAT2↑, DHCR7↑, FRZB↑, ID3↑, JUNB↑, KLF4↑, MVK↑, NSDHL↑, PCSK9↑, PF4↑, PMVK↑, PTPRH↑, SC4MOL↑, SREBF2↑, CCL8↓, CDC7↓, EXPI↓, FHL2↓, FST↓, GFPT2↓, IFI16↓, IGH-2↓, MSC↓, NFIL3↓, PDLIM5↓, SCD↓, TFRC↓, ZBTB16↓, POU2AF1↓, TCF12↓	54	31	Lipid metabolism, Small Molecule Biochemistry, Gene Expression
ADD1↑, APLP1↑ (includes EG:333), CENTA1↑, CRY2↑, CTGF↑, DBP↑, DES↑, ES22↑, HOXA7↑, NOV↑, PDLIM2↑, PER1↑, PER2↑, SGK2↑, NPAS2↓, ARNTL↓, GF11↓, IDE↓,	49	29	Behavior, Nervous System Development and Function, Cell Signaling

Online Supporting Material

IGF1↓, IL7R↓, LCN2↓, LY86↓, LY6A↓, GPD2↓, PAPP A↓,
PTK6↓, SERPINA3↓, SLPI↓, XDH↓

COL8A1↑, FOS↑, GSTA3↑, GSTA5↑, GSTM6↑, GSTP1↑, LMNA↑, MGST3↑, NQO1↑, PCYT2↑, CD86↓, ELN↓, GPR65↓, HSP90B1↓, IL1RL1↓, KNG1 (includes EG:16644)↓, LCP2↓, LOX↓, MSLN↓, OSMR↓, RAC2↓, SCIN↓, TLR4↓	35	23	Cellular Compromise, Cell Signaling, Amino Acid Metabolism
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DUSP8↑, DUSP16↑, GADD45A↑, MUC1↑, PIB5PA↑, TSPAN4↑, RHOU↑, CD74↓ CD3D↓, CD3E↓, CD3EAP↓, COL4A1↓, CSF2RB↓, FCGR2A↓, FCGR2B↓, GADD45G↓, HYOU1↓, POLR1A↓, POLR1E↓, SH2D2A↓, TLR3↓, TRAT1↓, TRIB3↓	35	23	Cellular Development, Inflammatory Disease, Skeletal and Muscular Disorders
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ACAA1B↑, EMID1↑, KLF4↑, NGEF↑, LOX↓, LY6C↓, NAPSA↓, RNF144↓, SLPI↓, SOCS1↓, STX11↓, SYNJ2↓, TNFAIP2↓, WARS↓, ANXA13↓, BCAT2↓, BLM↓, H2AFX↓. NUP210↓	26	19	Cancer, Gene Expression, Cellular Development
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AK1↑, ARPC1A↑, CBR1↑, CRIP1↑, LMNA↑, PPM1G↑, RASD2↑, TRIP10↑, CEP55↓, CSN3↓, DLG7↓, IFI16↓, IL7R↓, MPHOSPH1↓, NFIL3↓, RPRM↓, SESN1↓	22	17	Cell Cycle, Cellular Assembly and Organization, Cellular Development
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C. Upregulated genes at 3 months

ARG2, C10ORF119, CRY2, Cyclin A, DBP, E2f, EIF2S2, Glutathione transferase, GST, GSTA2, GSTA4, GSTA5, GSTM2, GSTM3 (includes EG:14864), GSTO1, GSTP1, Histone h3, HMGCR, HMGCS1, HMOX1, KCNQ1, LIG1, MCM4, MCM5, MCM6, MT1E, MT2A, MTF1, NDRG1, PDGF BB, PER2, PER3, Pka, Pkc(s), UHRF1	42	21	Amino Acid Metabolism, Cellular Compromise, Vitamin and Mineral Metabolism
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Online Supporting Material

<p>AACS, CFD, cholic acid, CYP2E1, CYP7A1, DHCR7, FA2H, FABP4, FABP6, fatty acid, FDPS, FGF19 (includes EG:9965), GH1, ID3, IDH1, IGK, INSIG1, LDLR, LSS, malonyl-coenzyme A, mevalonic acid, MVD, NSDHL, PCSK9, PPARGC1B, RETN, RORC, SC4MOL, SLC7A9, SQLE, SREBF1, SREBF2, sterol, TCF3, UCP2</p>	29	16	Lipid Metabolism, Small Molecule Biochemistry, Molecular Transport
<p>APOC3, ATP6V1B1, beta-estradiol, CBX5, CCDC85B, CDKN1A, CHAF1B, CLCN3, CTSB, CYP2D10, CYP2D26, CYP2E1, CYP4B1, CYP4F12, ELOVL6, EPB41L3, FGF19, HKDC1, HSD17B7, INS1, MCM3, MCM6, MT1E, NCOA5, NCOR1, NR1D1, NR1D2, OPRK1, ORC1L, PRLR, PRMT5, RETSAT, SLC9A3R1, SMARCA4, Unspecific monooxygenase</p>	29	16	Cell Morphology, Cellular Development, Connective Tissue Development and Function
<p>ACAA2, ADIPOR2, ADRB3, ALDH1A7, ALDH1B1, BIRC3, CA1 (includes EG:759), CBR3, CHST4, CTSD, CXCL13, CYP17A1, ENTPD5, ERBB2, F2, FOSB, GPT, IGF1, KRT7, L-triiodothyronine, LAMA3, LAMB3, LAMC2, LTA, LTB, MYLK, PDE8B, PDLIM2, PRDX2, PRKD1, PTHR1, SQLE, ST3GAL4, TNF, UCP2</p>	27	15	Connective Tissue Development and Function, Tissue Morphology, Cellular Development
<p>ABCC3, AKR1B8 (includes EG:14187), ATF2, ATF3, ATP, CFD, cholesterol, CNDP2, CYB5B, CYP2E1, DBP, FGF2, FMO5, FOSL1, GCLC, GCLM, GDF15, HMGCR, hydrogen peroxide, IL1B, LAMA3, MT2A, MTHFD1L, P2RY2, P38 MAPK, PLA2G4A, PRKAA2, PRKD1, progesterone, SLC3A1, STARD5, TERT, TNFRSF17, TSPAN4, ursodeoxycholic acid</p>	27	15	Cellular Growth and Proliferation, Cancer, Free Radical Scavenging
<p>ABCC3, AMPK, Ap1, ARNTL, carbon monoxide, CLOCK, Cyclin B, CYP2E1, EZH2, glycerol, HMGCS2, IL6, KITLG (includes EG:4254), KLF2, LDLR, LEP, MAP3K7, MID1IP1,</p>	22	13	Cancer, Cell Death, Nutritional Disease

Online Supporting Material

NFKB2, NGEF, PER2, PLK1, PRDX1, prostaglandin D2, RAC1, SC4MOL, SLC13A2, SRXN1, ST3GAL4, TEF, TNFRSF1B, TNFSF11, UCP2, USP2, WEE1

D. Upregulated genes at 6 months

APLP1 (includes EG:333), CENTA1, COL8A1, CPEB1, CRY2, CTGF, CYP51A1, DBP, ES22, FOS, FRZB, GSTA3, GSTA5, GSTM6, GSTP1, HOXA7, ID3, JUNB, LMNA, MGST3, NQO1, PCYT2, PER1, PER2, SSTR2	49	25	Behavior, Nervous System Development and Function, Cellular Compromise
ARL2BP, CBR1, DGAT2, DHCR7, GNA11, GNB5, MVK, NGEF, NSDHL, PCSK9, PMVK, RASD2, SC4MOL, SQLE, SREBF2, TRIP10	27	16	Cardiovascular System Development and Function, Cell Signaling, Nervous System Development and Function
CCDC85B, CDK10, CYP2C18, DCXR, DECR2, DIO1, EMID1, KLF4, MPST, MUC1, PIB5PA, TM7SF3, TSPAN4, TST	22	14	Free Radical Scavenging, Molecular Transport, Cellular Growth and Proliferation
CBR3, CLDN4, CNDP2, FGFBP1, HMGCL, HSD17B7, IHH, KHK, LEFTY1, MEP1A, MGLL, MIR16, TSC22D1, VDAC3	22	14	Cell Death, Reproductive System Disease, Organismal Development
AFM, AK1, AKR1B8 (includes EG:14187), CRIP1, DDAH2, LMNA, MID1IP1, NKX2-3, NOV, PPM1G, PTGS1, SLC27A2, TDO2	20	13	Cell Death, DNA Replication, Recombination, and Repair, Cell Cycle
ACAA1B, ACADS, ALDH1A7, ARPC1A, B4GALT3, CLDN4, ENTPD5, PF4, PLS3, SQLE, TCAP, TPPP	18	12	Cancer, Cellular Movement, Cell-To-Cell Signaling and Interaction
ADD1, DES, DUSP8, DUSP16, GADD45A, KLF4, MUC1, NEDD4, PTPRH, RHOD, RHOU	16	11	Cell Cycle, Cellular Movement, Cellular Assembly and Organization
AP1S1, CNN2, EIF2AK1, KRT17, PACSIN3, PDLIM2, RIPK4,	16	11	Amino Acid Metabolism, Post-

Online Supporting Material

SGK2, SLC25A1, SRPK3, TEF

Translational Modification, Small Molecule Biochemistry

E. Downregulated genes at 3 months

ACTA2, Akt, AREG, CAV1, COL1A1, CTGF, EGFR, ELN, FBLN2, FBN1, GHR, Gpcr, GREM1, growth factor receptor, Igfbp, IGFBP2, IGFBP4, Integrin, Jnk, LOX, LOXL1, Mmp, MYOCD, Pdgf, PDGF BB, PLAT, PLEKHA1, PTN, Ras, RGS1, SERPINF1, Tgf beta, TNFRSF11A, TNFRSF12A, Vegf	45	23	Skeletal and Muscular System Development and Function, Cellular Movement, Cellular Growth and Proliferation
ADIG, CD2, CD274, CD1D, CD59A, CDR2, CPD, CXCL16, DBI, ENAH, HSPA1L, IFNG, LAMP1, LPIN1, ME1, MYC, NFIL3, NR1D1, PIGR, PPARG, progesterone, PSMC5, PSMD6, PSMD7, RFX1, RFX2, SCD, SDPR, SPSB1, TES, TFRC, TLE4, TSPO, TTC28, ZYX	40	21	Cell-To-Cell Signaling and Interaction, Cellular Growth and Proliferation, Hematological System Development and Function
ACLY, amino acids, B3GNT5, CENPA, COL5A1, COL7A1, CSRP1, DAD1, EMP3, ERBB2, F2, FBLN2, FGF10, ITGB4BP, LOC63928, LPIN1, MFAP2, NNMT, PARP3, PDLIM4, PLEKHB1, PPAP2A, PTPRK, retinoic acid, RHOJ, SCD, SDF2, SDPR, SERPINH1, SPOCK1, TGFB1, TLE1, TMOD3, TPM4, TSPAN8	33	18	Tissue Development, Cancer, Cellular Growth and Proliferation
Adenylate Cyclase, ANXA5, Ap1, ARNTL, Arntl-Clock, BAG2, BRD2, CCND2, CLOCK, COL18A1, COL4A1, COL5A1, CRY2, Cry-Period, Cyclin A, DUSP6, E2f, E2F2, EDN1, Gαi/o, ganglioside GD2, GNAI1, Histone h3, LGALS1, Mapk, Mek, MMP14, NR1D1, P38 MAPK, PI3K, Pka, Pkc(s), PPP1R1A, RGS4, SSTR3	28	16	Behavior, Cell Signaling, Nervous System Development and Function
BAG1, BIK, C10ORF58, CGI-38, COL4A1, CRYAB, DHFR, DNAJB1, HIST1H1C, HSPA1L, HSPA4L, HSPH1, HTRA1, ID1,	28	16	Cancer, Cellular Growth and Proliferation, Reproductive System

Online Supporting Material

IFI16, IGFBP4, KLF6, MATN4, MYOD1, PHLDA3, PTGES3, RRM2, RXRB, SERPINB2, SFRP1, SMN1, SNCA, STIP1, TEAD2, TERT, TMEM43, TOP1, TP53, TPPP, WNT4			Disease
alpha-estradiol, APBB1, AQP4, ARNT2, beta-estradiol, C13ORF15, CDC2, Cdc2-CyclinB-Sfn, COPZ2, CRABP2, DMN, EFNB2, FAH, FBLN2, GJB1, GPC1, IGFBP2, IGFBP4, KRAS, KRT5, NFE2, PDLIM4, PLEKHA6, PPP1R3C, PTGER3, RERG, RNA polymerase II, SFN, STOM, TEAD2, TEAD4, TGFB3, TRIM25, VEGFA, YAP1	28	16	Cellular Growth and Proliferation, Cancer, Endocrine System Development and Function
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F. Downregulated genes at 6 months			
CD74, COL4A1, CSF2RB, FCGR2A, FCGR2B, GFII, GPD2, GPR65, HYOU1, IDE, IGF1, IL7R, KNG1 (includes EG:16644), LCN2, LCP2, LOX, LY6A, OSMR, PAPP, PTK6, SERPINA3, SH2D2A, SLPI, SOCS1, TRIB3, ZBTB16	48	26	Cellular Growth and Proliferation, Hematological System Development and Function, Immune Response
ARNTL, CCL8, CD86, CD3D, CD3E, CD3EAP, CREBBP, EXPI, FHL2, GADD45G, GFPT2, HIRIP3, HSP90B1, IFI16, IGH-2, IL1RL1, NFIL3, NPAS2, POLR1A, POLR1E, TFRC, TLR3, TLR4, TRAT1	43	24	Cellular Development, Hematological System Development and Function, Immune and Lymphatic System Development and Function
ADAM17, ARRDC3, C10ORF10, C1QB, CCL21, IL1RL1, IL7R, KLRD1, LY86, PES1, RASD1, RIOK1, RNASE6, SELPLG, SERPINA3, SPTA1, XCL1	27	17	Cellular Movement, Hematological System Development and Function, Immune Response
ANXA13, AURKA, BLM, CEP55, CSN3, DLG7, ELN, H2AFX, IL7R, LOX, MPHOSPH1, MSLN, PPP1R10, RPRM, SLPI, SMPD3, TNFAIP2	27	17	Cell Cycle, Cancer, DNA Replication, Recombination, and Repair

Online Supporting Material

CYP4F3, HIST1H3F, IL13RA2, NCAPH, NFIL3, NMU, PACAP, POU2AF1, RETNLA, RETNLB (includes EG:57263), RIPK3, SAMSN1, SESN1, SH3KBP1, SLC16A6, SYNJ2, XDH	27	17	Cellular Compromise, Immune and Lymphatic System Development and Function, Cell-To-Cell Signaling and Interaction
BCAT2, CCL17, CD160, EGLN3, IRF4, IRF5, LOX, LY6C, MICAL1, NAPSA, NUP210, SLC39A4 (includes EG:55630), SLPI, STAT4, STX11, WARS	25	16	Cancer, Cell-To-Cell Signaling and Interaction, Reproductive System Disease
ABCG1, ADAMTS4, BHLHB8, C1ORF164, CALCB, CASC5, CDCA8, CLDN10, ELN, HIST1H3C, HLA-DQB2, LOX, SLC5A3, TNFAIP2	20	14	Tissue Development, Respiratory Disease, Cellular Growth and Proliferation
CITED4, COL4A1, FST, GPX2, KIF23, KLF15, MYRIP, PDIA6, PPP1R9A, PPP3CC, RAC2, SCIN, SEMA5A, UCK2 (includes EG:7371)	20	14	Immune Response, Cell-To-Cell Signaling and Interaction, Tissue Development
BCL2A1, beta-estradiol, C2, C3, CD55, CDC7, CRSP9 (includes EG:9443), H6PD, KCNJ8, MSC, PDLIM5, RNF144, SECTM1, TCF12	19	13	Cell Cycle, Infectious Disease, Cell Death
ADSL, CLDN1, GRPR, IL1RL1, MAP3K6, NUPL2, PTPN22, PTPRO, PTPRT, REFBP2, SCD, TRIB3, TSSK6	19	13	Amino Acid Metabolism, Post-Translational Modification, Small Molecule Biochemistry

¹Biological Networks in upregulated, downregulated, and integrated total differentially expressed datasets (P -value < 0.05 , no FDR applied) were generated through the use of IPA. ²The network Score is based on the hypergeometric distribution and is calculated with the right-tailed Fisher's exact test. The score is the negative log of this P -value. (A score of greater than 2 indicates that there is a less than 1 in 100 chance that the Focus Genes were assembled into a network due to random chance alone.) ³Upward-pointing arrows indicate increased expression and downward-pointing arrows indicate decreased expression in the WD group (n=8) compared to Controls (n=8).

Online Supporting Material