

Supplemental Data:

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Figure S1.

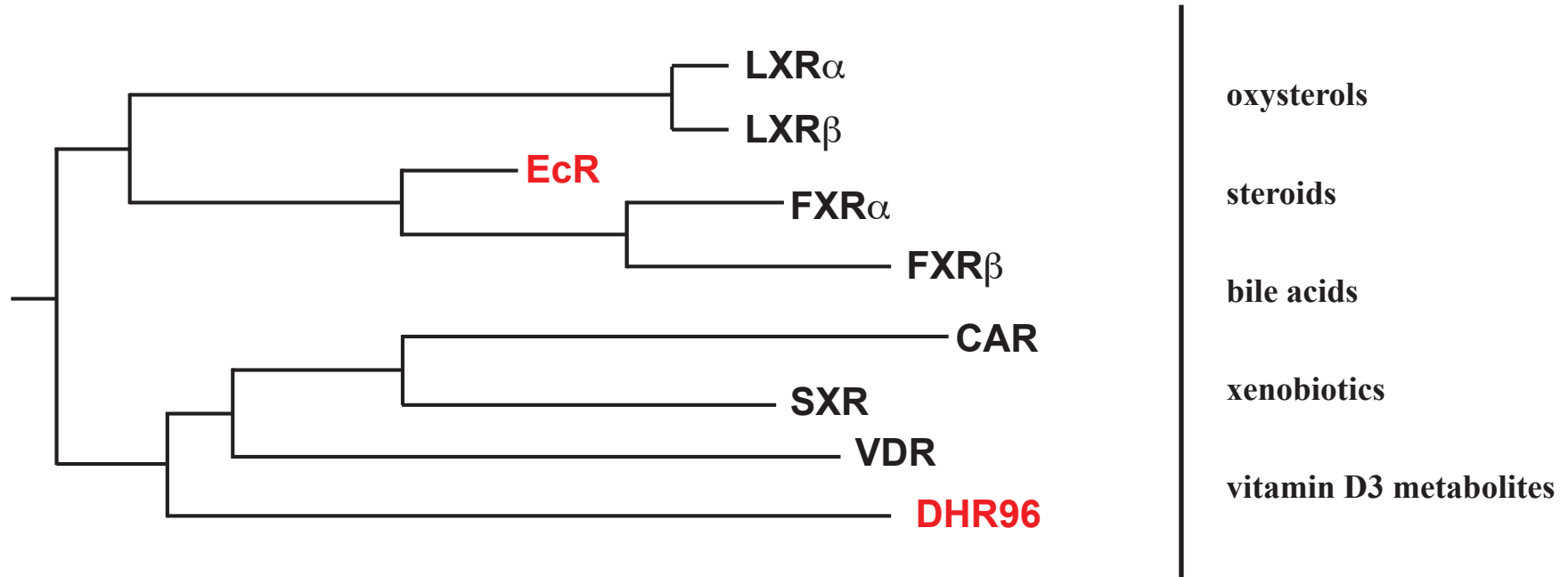


Figure S1. Phylogenetic tree comparing LXR-like nuclear receptors in humans and *Drosophila*. Modified after Laudet and Bonneton (Laudet et al., 2005). Human nuclear receptors are shown in black, fly receptors in red. On the right, classes of ligands that were identified for members of this subgroup are listed. LXR: Liver X Receptor. FXR: Farnesoid X Receptor. EcR: Ecdysone Receptor. CAR: Constitutive Androstane Receptor. SXR: Steroid and Xenobiotic Receptor. VDR: Vitamin D Receptor. DHR96: *Drosophila* Hormone Receptor 96.

Figure S2

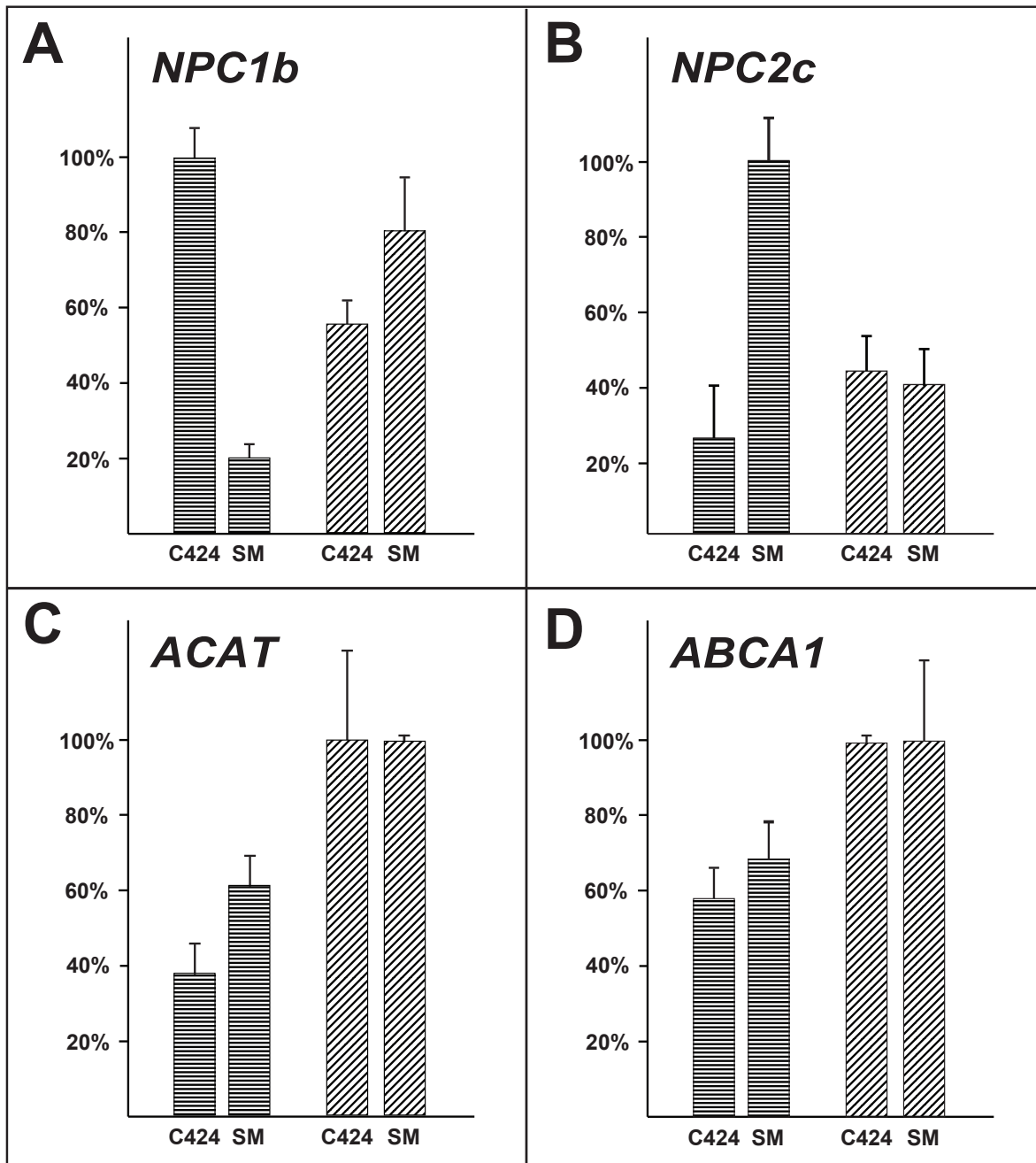


Figure S2. Microarray results (Affymetrix) for the *NPC1b*, *NPC2c*, *ACAT* and *ABCA1* genes. Compare this figure to data shown in Figure 6A-D; the latter is a repeat experiment using qPCR and independent samples for validation of microarray data shown here. Second instar larvae of comparable size were collected from Carolina 424 (C424) or standard medium (SM). Correct staging of larvae to the first half of the second instar was accomplished by collecting animals the appropriate time after egg deposition. In addition, samples were analyzed with qPCR for low expression of molecular markers that are turned on in the second half of the second instar. Samples with high expression of these markers (*DHR4* and *β FTZ-F1*) were discarded. For each gene, the condition with the highest level of expression was set to 100%. Parallel stripes: *CanS* controls, diagonal stripes: *DHR96* mutants.

Figure S3

High cholesterol

FC	SE	induced	repressed	neutral
0.00	0.00		NPC2d	
0.13	0.01		CG5932	
0.31	0.05		Nol	
0.43	0.08		CG2065	
0.47	0.09		CG11314	
0.53	0.16		Prat2	
0.57	0.20		CG10300	
0.64	0.23		CG16708	
0.69	0.16		CG11315	
0.71	0.23			CG10514
0.71	0.32			Mmp1
0.77	0.36			Brat
0.77	0.36			FancL
0.80	0.51			CG31148
0.84	0.46			ACAT
0.86	0.34			Thor
0.90	0.33			Egh
0.93	0.47			CG11781
0.94	0.42			Nubbin
0.98	0.28			LpR2
1.11	0.40			CG1819
1.16	0.51			Cyp12d1
1.23	0.78			Brummer
1.28	0.59			NPC2a
1.37	0.55			Net-RA
1.42	0.39			Peste
1.52	0.47	NPC1b		
2.35	0.71	TotC		
2.92	0.50	Atet		
3.13	1.17	CG15533		
3.30	1.20	LpR1		
4.13	0.75	Lip3		
5.01	0.53	CG4783		
32.09	2.21	NPC2e		

CanS (0%) controls vs. *CanS* (1% cholesterol)

Mutation in *DHR96*

FC	SE	induced	repressed	neutral
0.04	0.00		NPC2d	
0.14	0.02		Nol	
0.15	0.01		CG5932	
0.15	0.01		CG10514	
0.18	0.05		CG31148	
0.32	0.08		FancL	
0.33	0.05		ACAT	
0.39	0.05		CG2065	
0.44	0.07		CG11314	
0.49	0.13		CG10300	
0.64	0.16		CG11315	
0.70	0.25			Brat
0.77	0.28			CG16708
0.79	0.33			Mmp1
0.84	0.22			CG1819
0.93	0.42			Thor
0.97	0.50			Prat2
0.99	0.51			Egh
1.01	0.39			LpR2
1.05	0.43			Net-RA
1.39	0.53			NPC2a
1.54	0.54	Brummer		
1.59	0.46	Nubbin		
1.67	0.42	Peste		
1.73	0.61	Atet		
1.80	0.63	TotC		
1.81	0.60	Lip3		
2.09	0.66	Cyp12d1		
2.55	0.27	CG11781		
3.67	1.21	CG15533		
6.31	0.54	CG4783		
7.41	0.69	NPC1b		
13.21	2.09	LpR1		
97.34	4.62	NPC2e		

CanS vs. *DHR96*¹ mutants on standard medium

Figure S3. The transcriptional changes triggered by high cholesterol phenocopy the *DHR96* mutation. Both tables represent qPCR analysis of 34 genes in samples that were isolated from larvae reared on standard medium plus/minus 1% cholesterol. qPCR is based on the Fluidigm Biomark instrument using 5 internal control genes per sample. Fold changes are averages of eight independent samples, standard error is indicated as “SE”. To facilitate the comparison between the two tables, genes are grouped in different columns, depending on whether the gene was found to be induced (>1.5fold change, shown in blue), repressed (<-1.5fold change, shown in red) or considered to not change significantly (<1.5 and >-1.5fold changes). Genes are sorted top to bottom starting with the most strongly downregulated gene.

Figure S4

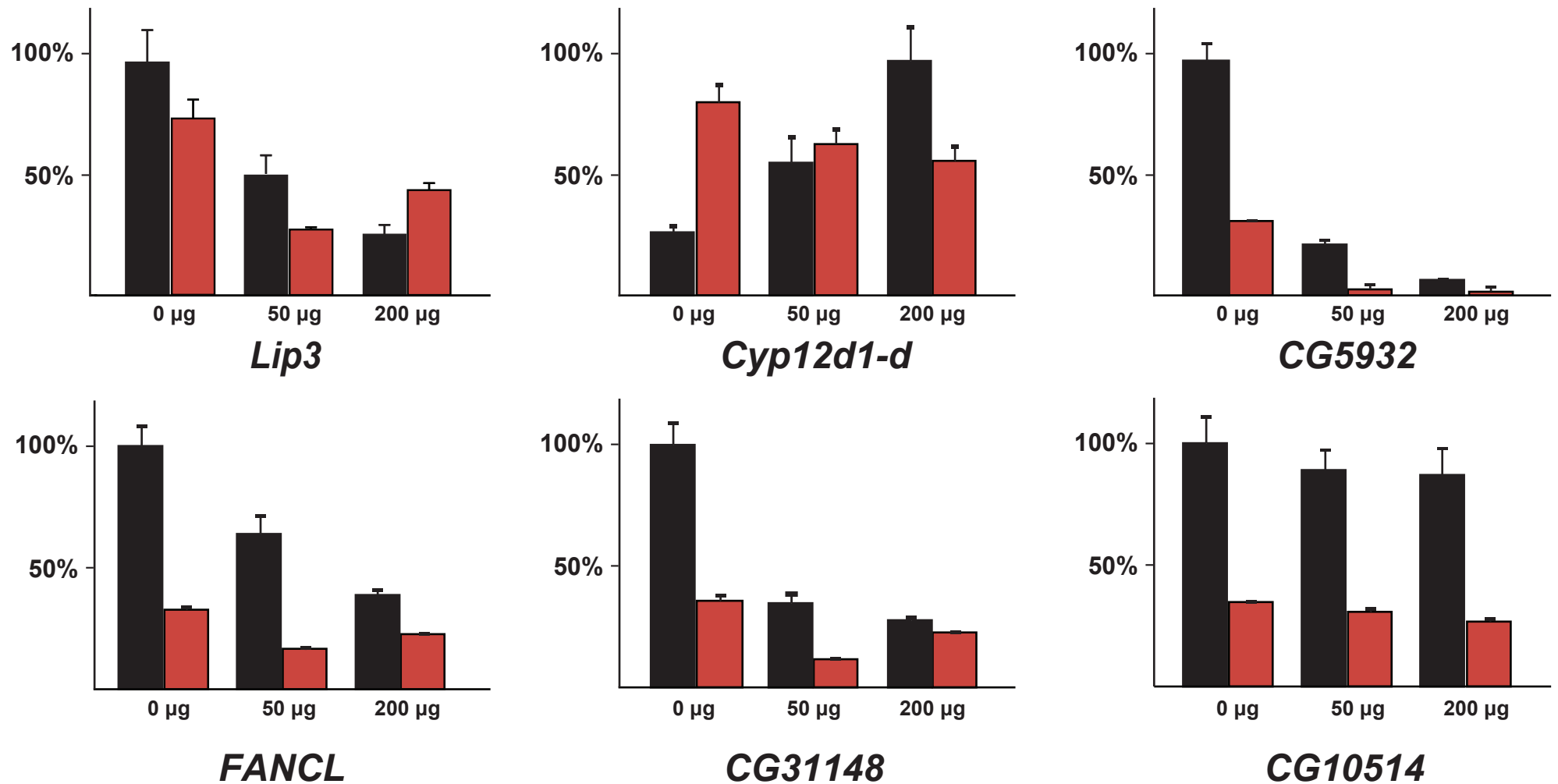


Figure S4. Dietary cholesterol regulates genes in a concentration- and *DHR96*-dependent manner. Staged second instar larvae were collected from lipid-depleted C424 medium that was supplemented with either 0 µg, 50 µg or 200 µg cholesterol per gram (dry weight, 1 g per vial). Total RNA was reverse-transcribed and subjected to qPCR analysis whereby every data point is based on four biological samples each tested in triplicate. For each gene, the highest expression level was normalized to 100%. Black: wild type (*CanS*), red: *DHR96* mutants. Error bars indicate standard error. *FANCL*: *Fanconi anemia complementation group L*.

Gene ID	Molecular Function/Activity	Human Homolog	WT (<i>CanS</i>)			<i>DHR96</i> ¹		
			C	CA	TS	C	CA	TS
Atet	ATPase Coupled Transport	ABCG4	↑					
ABCA1**	ATPase Coupled Transport	ABCA1/ABCA3	↑					
CG1819	ATPase Coupled Transport	ABCA12	↑		↓			↑
CG11781	Membrane Transport	Transmembrane Protein 93						
NPC1b	Intestinal Cholesterol absorption/transport	NPC1L1	↑		↓	↑		
NPC2a	Sterol Transport	NPC2						
NPC2d	Sterol Transport	NPC2	↓			↓	↑	↑
NPC2e	Sterol Transport	NPC2	↑	↑				
NPC2g	Sterol Transport	NPC2	↓	↓				
NPC2h	Sterol Transport	NPC2	↓	↑	↑			
peste	Fatty Acid Transport	Scavenger Receptor Class B, member 2	↑	↓	↓	↑		
LPR1	Lipid (Lipoprotein) Transport	VLDL-b	↑	↑	↑			
LPR2	Lipid (Lipoprotein) Transport	VLDL-a						
ACAT**	Sterol O-Acyltransferase	ACAT1/2						↑
CG5932	Triacylglycerol Lipase	Gastric Lipase A (LIPA)	↓		↓	↓	↑	
brummer	Triacylglycerol Lipase	Patatin-Like Phospholipase Domain						
Lip3	Cholesteryl ester hydrolase	Acid Lipase	↓				↑	
CG31148	Glucosylceramidase, Sphingolipid metabolism	Beta-Glucocerebrosidase		↑		↑	↑	↑
CG15533	Sphingomyelin Phosphodiesterase	Sphingomyelin Phosphodiesterase 1	↑		↓	↑		
egghead	Sphingolipid metabolism	-						
CG10514	Choline Kinases	-						
CG16708	Ceramide Kinase, Sphingolipid metabolism	CERKL	↓	↑	↑	↓	↑	↑
Prat2	Amidophosphoribosyl Transferase	GPAT	↓		↑			
FANCL	E3 Ubiquitin Ligase	FANCL						
CG2065	Short-Chain Dehydrogenases	3-Hydroxyacyl-CoA Dehydrogenase II	↓	↓				
Cyp12d1	Detoxification, Steroid metabolism	Cytochrome P450, 27A1		↑	↑			
TotC	Stress Response	-	↑	↑	↑			
CG10300	Carrier Vitamin E Binding	TTPA	↓	↑		↑		
DHR96	Transcriptional regulation, Xenobiotic response	VITAMIN D Receptor (VDR)	↓	↓	↓	n/a	n/a	n/a
Net	Transcriptional regulation	Neurogenic Differentiation 1						
CG4783	Insect development?	-	↑	↑	↑			
Thor	Translational regulation (developmental events)	eIF4e-Binding Protein 2				↓		
Brat	Translation repressor activity	-						
Nubbin	Transcriptional regulation	POU Class 2 Homeobox 1						
Mmp1	Metallo-endopeptidase	Matrix Metallopeptidase 1			↓			
Nol	Larval neurogenesis (Secreted glycoprotein?)	-	↓	↓				

Table S1. Summary of genes used for microfluidic qPCR and the effects of dietary lipids. We selected these genes based on loss- and gain-of function *DHR96* microarray studies, predicted gene functions as well as published work. The table summarizes the molecular functions and expression profiles of affected genes in wild type animals (*CanS*) and *DHR96*^l mutants reared on standard cornmeal medium containing 1% (wet weight) of three different fats: cholesterol (C), canola oil (CA) or tristearin (TS). The gene expression values represent fold changes based on data generated with the Biomark Fluidigm arrays. The arrows denote up- or downregulation higher or lower than 1.5fold. ** name not official yet – only used in this publication. *Atet*: ABC transporter expressed in trachea, *ABCG4*: ATP-binding cassette sub-family G member 4, *ABCA1*: ATP-binding cassette sub-family C member 1, *NPC1*: Niemann Pick type C-1, *NPC2*: Niemann-Pick type C-2, *VLDL*: very low density lipoprotein receptor, *ACAT*: Acyl coenzyme A:cholesterol acyltransferase, *CERKL*: Ceramide Kinase-like, *GPAT*: Glutamine phosphoribosylpyrophosphate amidotransferase, *TTPA*: Tocopherol Transfer Protein Alpha, *FANCL*: Fanconi anemia- complementation group L, *RNAPII*: RNA Polymerase II). Note: *ABCA1* was tested separately on a 96 well qPCR instrument (StepOnePlus, ABI) using SYBR Green as a fluorescent dye.

Table S2: 73 genes upregulated by 1% cholesterol in wild type.

row	probe set	Gene Symbol	FC CanS +- 1% C	p-value	FC DHR96 vs CanS	p-value
1	1623732_at	CG31410 (NPC2e)	11.59	1.21E-07	21.82	1.85E-08
2	1627895_at	CG18404	5.63	5.62E-03	12.96	5.05E-04
3	1627236_s_at	---	4.50	6.11E-04	1.18	5.67E-01
4	1629559_s_at	Atet	4.03	1.33E-06	3.12	6.56E-06
5	1634075_at	dp	3.65	8.14E-03	1.65	2.20E-01
6	1636132_at	---	3.35	3.41E-03	1.16	6.42E-01
7	1637253_s_at	CG17570	3.03	7.68E-03	1.90	7.75E-02
8	1635766_at	Fs(2)Ket	2.83	7.53E-04	1.29	2.45E-01
9	1626028_at	CG4783	2.71	2.70E-03	4.89	1.26E-04
10	1632177_at	hth	2.51	9.85E-04	1.36	1.34E-01
11	1631621_s_at	egh	2.47	4.53E-03	2.08	1.36E-02
12	1634980_s_at	CG18490	2.37	5.35E-03	1.58	8.39E-02
13	1635909_at	cic	2.37	9.61E-04	1.27	2.03E-01
14	1623605_a_at	cbt	2.35	4.25E-03	1.78	2.94E-02
15	1633512_at	toy	2.35	4.40E-03	1.37	1.92E-01
16	1639306_s_at	CG17090	2.24	9.37E-04	1.59	2.03E-02
17	1639785_s_at	dpld	2.23	1.81E-03	1.41	9.16E-02
18	1628489_at	dally	2.19	6.98E-03	1.46	1.23E-01
19	1624067_at	CG6704	2.15	5.52E-03	1.02	9.15E-01
20	1638568_s_at	H	2.10	2.16E-04	1.22	1.37E-01
21	1627446_at	net	2.08	1.50E-05	2.21	8.11E-06
22	1637144_a_at	Map205	2.06	6.31E-05	1.47	4.06E-03
23	1625471_s_at	CG4928	2.01	9.22E-03	1.31	2.33E-01
24	1629944_at	CG12814	2.00	8.53E-03	1.30	2.35E-01
25	1633801_s_at	CG9171	2.00	1.06E-03	1.52	1.73E-02
26	1631303_s_at	NK7.1	1.98	1.07E-03	1.68	5.64E-03
27	1627101_at	scyl	1.95	7.92E-05	1.74	3.04E-04
28	1638432_a_at	CG10082	1.94	1.24E-03	1.41	3.68E-02
29	1631280_at	CG9095	1.90	7.03E-03	1.64	2.43E-02
30	1624839_at	h	1.89	5.42E-04	1.48	9.69E-03
31	1627784_at	---	1.89	5.22E-03	1.54	3.37E-02
32	1633794_a_at	Pino	1.89	6.20E-03	2.11	2.47E-03
33	1639330_s_at	Tao-1	1.88	3.95E-03	1.14	4.39E-01
34	1628435_at	---	1.84	2.04E-03	1.46	2.45E-02
35	1641685_at	Edc3	1.81	3.66E-04	1.59	1.80E-03
36	1631534_at	sfl	1.81	8.16E-05	1.52	9.63E-04
37	1634573_a_at	---	1.80	3.51E-03	1.29	1.22E-01
38	1626899_at	GATAd	1.79	6.34E-03	1.21	2.62E-01
39	1640457_s_at	Bsg	1.78	4.93E-04	1.20	1.13E-01
40	1639414_at	Snoo	1.76	9.91E-03	1.56	2.94E-02
41	1624059_at	CG32223	1.75	8.38E-03	1.21	2.67E-01
42	1624021_a_at	dlg1	1.72	6.60E-04	1.14	2.47E-01
43	1634579_at	CG4747	1.70	2.93E-03	1.27	9.44E-02
44	1623812_at	CG10943	1.68	6.47E-03	2.46	2.10E-04
45	1628729_at	---	1.68	9.79E-03	1.24	2.06E-01
46	1634146_at	CG42258	1.68	7.94E-03	1.33	9.03E-02
47	1629479_a_at	fok	1.67	9.60E-03	1.30	1.21E-01
48	1640280_at	lola	1.66	5.20E-03	1.31	8.26E-02
49	1624321_at	Rac2	1.66	8.79E-03	1.49	2.65E-02
50	1624780_at	DereCG2995	1.66	6.06E-03	1.49	2.03E-02
51	1634771_a_at	CG8184	1.64	3.46E-03	1.30	6.76E-02
52	1629396_a_at	CG8486	1.64	2.17E-03	1.21	1.34E-01
53	1640817_at	zornin	1.63	7.61E-03	1.53	1.57E-02
54	1625594_s_at	Pka-R2	1.63	1.28E-03	1.33	2.41E-02
55	1633253_s_at	CG2991	1.63	6.67E-04	1.30	2.05E-02
56	1630396_at	CG4360	1.63	6.76E-04	1.16	1.41E-01
57	1627191_a_at	ena	1.62	2.48E-03	1.29	5.56E-02
58	1628778_at	itp	1.62	7.98E-04	1.32	1.78E-02
59	1634509_s_at	fwd	1.61	3.05E-03	1.11	4.12E-01
60	1636363_s_at	Mapmodulin	1.61	2.74E-03	-1.08	5.11E-01
61	1631321_s_at	His1:CG31617	1.59	6.41E-03	1.03	8.20E-01
62	1638741_at	---	1.59	7.78E-03	1.28	1.03E-01
63	1637487_at	Dcp-1	1.58	3.07E-04	1.96	1.80E-05
64	1640547_at	mew	1.57	3.68E-04	1.28	1.30E-02
65	1626766_s_at	daw	1.56	1.07E-04	1.32	2.32E-03
66	1628784_at	CG16817	1.56	1.90E-03	1.03	8.04E-01
67	1639054_s_at	---	1.54	8.51E-03	1.47	1.48E-02
68	1641104_s_at	Eaat1	1.53	5.67E-03	1.20	1.44E-01
69	1633443_s_at	CG2082	1.53	3.50E-03	1.26	5.92E-02
70	1631524_a_at	hth	1.51	1.46E-04	1.50	1.63E-04
71	1636099_s_at	mbl	1.51	4.28E-03	1.37	1.70E-02
72	1639064_s_at	Akt1	1.51	2.80E-03	1.29	3.07E-02
73	1632485_a_at	Nak	1.51	1.47E-03	1.29	1.80E-02

Table S2. Genes upregulated by 1% cholesterol. This table summarizes genes upregulated in wild type (*CanS*) when treated with 1% dietary cholesterol using Affymetrix *Drosophila* 2.0 microarrays. Data was analyzed with *germa* and *limma*. Included are fold changes for *CanS* (1%) vs. *CanS* (0%) as well as *DHR96* (0%) vs. *CanS* (0%). Selection criteria for this list are a p value <0.01 and a fold change >1.5. Genes are ranked by fold change in the column representing the response to cholesterol in wild type, with the highest value at position 1.

Table S3: 55 genes downregulated by 1% cholesterol in wild type.

row	probe set	Gene Symbol	FC CanS +/- 1% C	p-value	FC DHR96 vs CanS	p-value
1	1633050_at	CG12813 (NPC2d)	-556.05	3.54E-08	-233.32	1.18E-07
2	1631446_at	Cht9	-43.04	3.74E-07	-27.14	1.07E-06
3	1631558_at	Cpr65Ax2	-13.04	4.18E-03	-3.61	8.56E-02
4	1636402_at	CG10081	-11.79	8.87E-03	-20.63	2.87E-03
5	1636583_at	CG5932	-10.80	7.81E-03	-5.04	4.44E-02
6	1641622_at	Lcp65Ae	-10.09	9.60E-03	-3.75	9.09E-02
7	1634815_at	CG31104	-6.98	9.71E-03	-7.35	8.50E-03
8	1641136_at	Cpr78Cc	-6.40	4.76E-03	-4.92	1.07E-02
9	1636387_at	CG10300	-4.65	4.31E-05	-8.59	3.29E-06
10	1625235_at	CG13325	-4.60	3.05E-03	-4.60	3.05E-03
11	1635467_a_at	CG7381	-4.44	3.92E-04	2.14	1.91E-02
12	1638132_at	CG10184	-3.58	1.58E-03	-2.04	3.16E-02
13	1639268_at	CG13324	-3.18	6.43E-03	5.21	7.64E-04
14	1638182_at	CG5999	-3.07	6.78E-04	-3.32	4.29E-04
15	1638903_at	CG5724	-3.04	5.53E-07	-2.88	8.17E-07
16	1631072_at	CG9512	-2.89	4.56E-04	-1.61	3.59E-02
17	1640065_at	GstE7	-2.85	4.03E-05	-1.76	2.66E-03
18	1622946_at	CG6908	-2.70	8.65E-04	-1.49	7.42E-02
19	1633492_at	hgo	-2.55	6.80E-03	-1.50	1.57E-01
20	1625042_at	CG31288	-2.52	7.40E-03	-2.54	7.09E-03
21	1637319_at	Fancl	-2.49	3.41E-06	-6.43	1.07E-08
22	1623769_at	CG7322	-2.45	4.56E-03	-1.50	1.19E-01
23	1628474_at	CG16712	-2.39	7.10E-03	-1.55	1.11E-01
24	1634697_at	CG32667	-2.32	6.81E-03	-1.85	3.00E-02
25	1627180_at	Cyp4d14	-2.31	1.03E-03	-1.21	2.97E-01
26	1634152_at	GstD5	-2.29	7.21E-03	-1.36	2.22E-01
27	1628657_at	GstE9	-2.26	6.29E-03	-1.95	1.74E-02
28	1627869_at	Jon25Bi	-2.19	5.19E-03	-2.77	1.09E-03
29	1633684_at	CG32444	-2.17	1.31E-05	-1.79	1.12E-04
30	1633471_at	Prx2540-2	-2.14	2.79E-03	-1.22	3.01E-01
31	1623840_at	CG18607	-2.11	7.03E-03	-1.34	1.99E-01
32	1630212_at	CG2065	-2.08	8.40E-03	-2.04	9.47E-03
33	1638246_at	CG5804	-2.04	6.72E-03	-1.33	1.85E-01
34	1630109_at	Hsc70-4	-1.86	3.94E-03	-1.04	8.26E-01
35	1641052_at	Jon66Cii	-1.77	8.89E-03	-1.42	7.16E-02
36	1629853_at	CG3699	-1.77	8.86E-04	-1.42	1.33E-02
37	1638038_at	CG4335	-1.76	9.42E-04	-1.67	1.71E-03
38	1633503_at	CG12171	-1.76	6.94E-03	-1.68	1.03E-02
39	1640466_s_at	CG6543	-1.74	3.13E-03	-1.96	9.83E-04
40	1625265_at	CG9119	-1.71	2.30E-03	-1.37	3.31E-02
41	1629009_at	Cyp28a5	-1.71	1.63E-04	-2.08	1.61E-05
42	1635800_at	CG5431	-1.69	6.47E-04	-1.69	6.83E-04
43	1630624_s_at	CG10151	-1.69	2.65E-03	-1.93	6.29E-04
44	1630505_a_at	Cyp311a1	-1.65	8.42E-03	-1.65	8.39E-03
45	1626664_at	CG3285	-1.64	4.12E-03	-1.13	3.58E-01
46	1631533_at	Cyp6a22	-1.64	6.93E-03	-1.24	1.63E-01
47	1630429_s_at	CG11889	-1.62	8.17E-04	-1.01	9.28E-01
48	1630802_at	Cyp6d4	-1.60	3.33E-03	-1.97	3.20E-04
49	1627854_at	CG9914	-1.58	5.80E-04	-1.54	8.77E-04
50	1626248_at	CG18547	-1.53	7.69E-03	-1.19	1.89E-01
51	1639553_at	CG9987	-1.53	4.54E-03	-1.14	2.75E-01
52	1641650_at	alpha-Est5	-1.53	1.09E-03	-1.18	9.39E-02
53	1633036_s_at	CG32495	-1.51	6.50E-03	-1.31	4.42E-02
54	1632342_a_at	CG33080	-1.51	2.82E-03	-1.41	7.82E-03
55	1624185_at	CG1041	-1.50	7.66E-03	-1.26	8.17E-02

Table S3. Genes downregulated by 1% cholesterol. This table summarizes genes downregulated in wild type (*CanS*) when treated with 1% dietary cholesterol using Affymetrix *Drosophila* 2.0 microarrays. Data was analyzed with gcrma and limma. Included are fold changes for *CanS* (1%) vs. *CanS* (0%) as well as *DHR96* (0%) vs. *CanS* (0%). Selection criteria for this list are a p value <0.01 and a fold change <-1.5. Genes are ranked by fold change in the column representing the response to cholesterol in wild type, with the most strongly downregulated gene in row 1.

Table S4. Primer sequences and UPL probe codes for quantitative real-time PCR.		
Gene Symbol / Transcript ID	Primer Sequence (5' → 3')	Roche UPL Probe #
<i>ABCA1</i> (CG1718)	GACCAACACGCATTATGAACG TGAAGCAGTCGTCGATTCTC	86
<i>ACAT</i> (CG8112)	CACAAACTGAAACCGCACAG CGGACACGAAACAGAAGACCA	24
<i>Atet</i>	CCAGACAGGAGCCAGTGC GCCATTGCACAGGTTGTTC	102
<i>αTubulin 84B</i> (CG1913)	ACACTTCCAATAAAAACTCAATATGC CCGTGCTCCAAGCAGTAGA	3
<i>βFTZ-F1</i>	GCAGCTTCATTTGGTCGTC CCAGAATTTGTTCTCGCAAGT	104
<i>brat</i>	TGACAGAGGCCCTTCTCCT CCTGAATGCCCGTCTGTAGT	1
<i>brummer</i>	TGTCTCCTCTGCGATTTGC CGTTCACCACCCTGAAGAAG	44
<i>cabut</i>	GCCCCACTAAGGGAAAAC TCGTTACCTGTTGTTGTCGTC	141
CG10300	GAGAAGTGGGTGCAGTTGCT ACCACGAGTCCCATTCAAAA	149
CG10514	CAGATTCCCAGTTTGCATCA CACTACCGTAGAATGCAAAGCA	10
CG11781	TGGAATACTGTGCGACATCG GTACCACTCAGGCCAAAATAC	121
CG15533-RA	CAATTCCGAGCGAGAACG TGTGCTCTCCTCATTGTCCA	17
CG16708	CGTGCGTTTTCTGCTCAAC TGTGCGATATACCTCTACAAAAGG	29
CG1819 (CG34120)	CTTGTGATAATGCGGAATGGT CAAACGCTGCAGGGAGTC	61
CG2065	GCTGCTGGACGTATTGAAGA GTTTTAATGAAACCTTGGGTGTG	64
CG31148	TGCTTGGAGTGCTTCTGGTA CTGCATCGATGAGTTTGCAG	49
CG4783-RA	ATTGCAATTATCCTCCTGCTG GACCTCCACACTTTCGGATCT	20
CG5932	CAACGCCTTCATAATGTTTGC TCTCGATCAGGACGCTCAT	138
<i>Cyp12d1-p/d</i>	GGTCCCGTTCGATCTTCAA GGTCTTGTGCTCCTCCGTTA	75
<i>DHR4</i>	TGCTCTCCACATACCAGAGA CACGAAGGGCACATAGAACA	40
<i>DHR96</i>	ACCAGGTGGCCCTACTCA CATGTTCTCTTTGGTATGGGGTA	83
<i>egghead</i>	AAACCCAAACATTCGGACAC TCAGCTCTGTTTCTCGGTCA	143
<i>FANCL</i>	CGTCTGTTTGGAGGAGTGGT AAATGAGGTGGACAGCTTCG	158
<i>Lip3</i>	GTTCCGGCGATGATTGATTA CACATATTGCACCTGCTGCT	140
<i>LpR1</i>	CGGATTCCGGCTTTATTG AGCCAATGAGCAGGAGCA	131
<i>LpR2</i>	ATGGGACGCCACACATTTAC CAGATTTGAAGAGGGGGTTG	153
<i>Mmp-1</i>	GAAGGCTCGGACAACGAG GTCGTTGGACTGGTGATCG	150
<i>MtnA</i>	AACTCAATCAAGATGCCTTGC TTGCAGGATCCCTTGGTG	20

<i>Net-RA</i>	TCCGGGAGTTACGACTCTTC CGAGTGGTTCCAGCTGTTCT	44
<i>no optic lobe</i>	GCTACTGATCCAGCCACGA GGCGAAGTGGAAAGTGGTG	10
<i>NPC1b</i>	CGGATTTTGTTCAGCAACT GCCATTCTCAGTAAATCCCTCGT	112
<i>NPC2a (CG7291)</i>	ACAGTCGTCCACGGCAAG ACACAGGCATCGGGATTG	105
<i>NPC2c (CG3934)</i>	ACAGCACGCCAATTAGACAA CGTCGATTTGCACCATCA	30
<i>NPC2d (CG12813)</i>	GGATTATTACTATCGCTGGCTGA CCTCAAAGCGGAAAAGGTTTA	18
<i>NPC2e (CG31410)</i>	TTCTGGCCACAGTCAATGC CCAGTGGAAAACGGCTTATTT	120
<i>NPC2g (CG11314)</i>	GTCCGTACACCATCCGTTG GTCGATGGTGAAGCAGCAG	14
<i>NPC2h (CG11315)</i>	CAAGAGCGAGAACGTGGAG CCAACGGATGGTGTATGGAT	79
<i>nubbin (CG34395)</i>	CGGGATAAATCGAAGGAAGC AGTATTTGATGTGTTTGCAGCTTT	62
<i>peste</i>	CGCAACTGGATCGATATGTTT CTCGAATGATCGTGAATTGG	72
<i>Prat2</i>	ATTTGGTCAGCTCGGTTCC CCGACTCCCTGGCATAAC	44
<i>rp49 (CG7939)</i>	CGGATCGATATGCTAAGCTGT GCGCTTGTTCGATCCGTA	105
<i>Rpl1140 (CG3180)</i>	TGATGTACGACAACGAGGAAGA GCCACAGCTCGTGAGAGAT	63
<i>thor</i>	CCAGATGCCCGAGGTGTA AGCCCGCTCGTAGATAAGTTT	22
<i>TM1-RB</i>	GCGAGGAGTTCCACAAGC GTTTCAGAGCGGCAACCTC	10
<i>totC</i>	AATGAATGCCTCCATTTCTCTACTA GCAACCCTAAGGCTGTCAGA	117

Table S4. List of all primer-probe combinations. This table lists the primer sequences we used for our microfluidic-based qPCR analysis. In cases where gene expression was also analyzed on a 96 well qPCR instrument, the same primers were used either with the corresponding probes or with SYBR Green while omitting the UPL probe.

Supplemental Data – References:

Laudet V, Bonneton F (2005) Evolution of Nuclear Hormone Receptors in Insects. In *Molecular Insect Science*, Gilbert LI, Iatrou K, Gill SS (eds), Vol. 3, pp 287-318. Oxford: Elsevier.