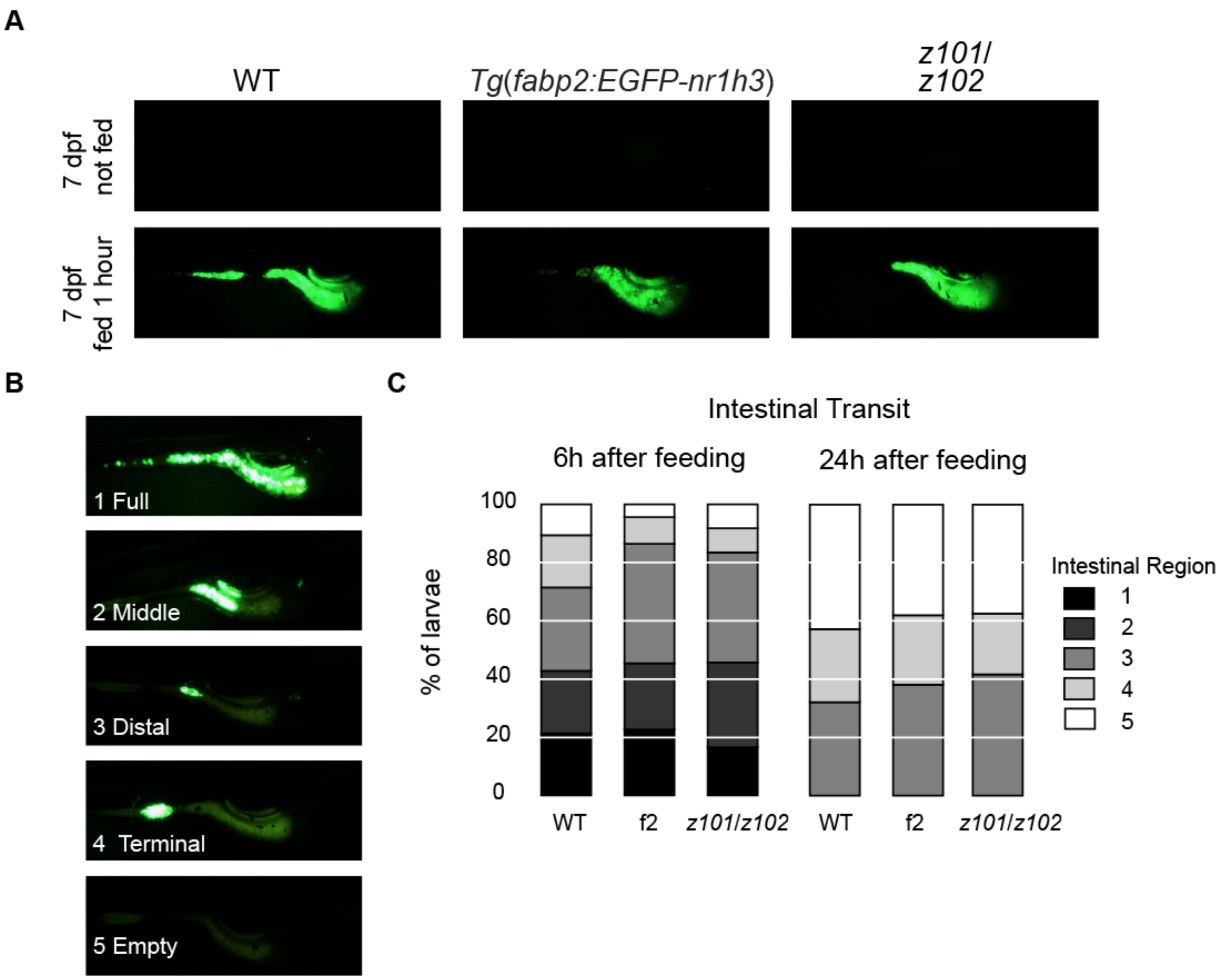
Lxr-driven enterocyte lipid droplet formation delays transport of ingested lipids

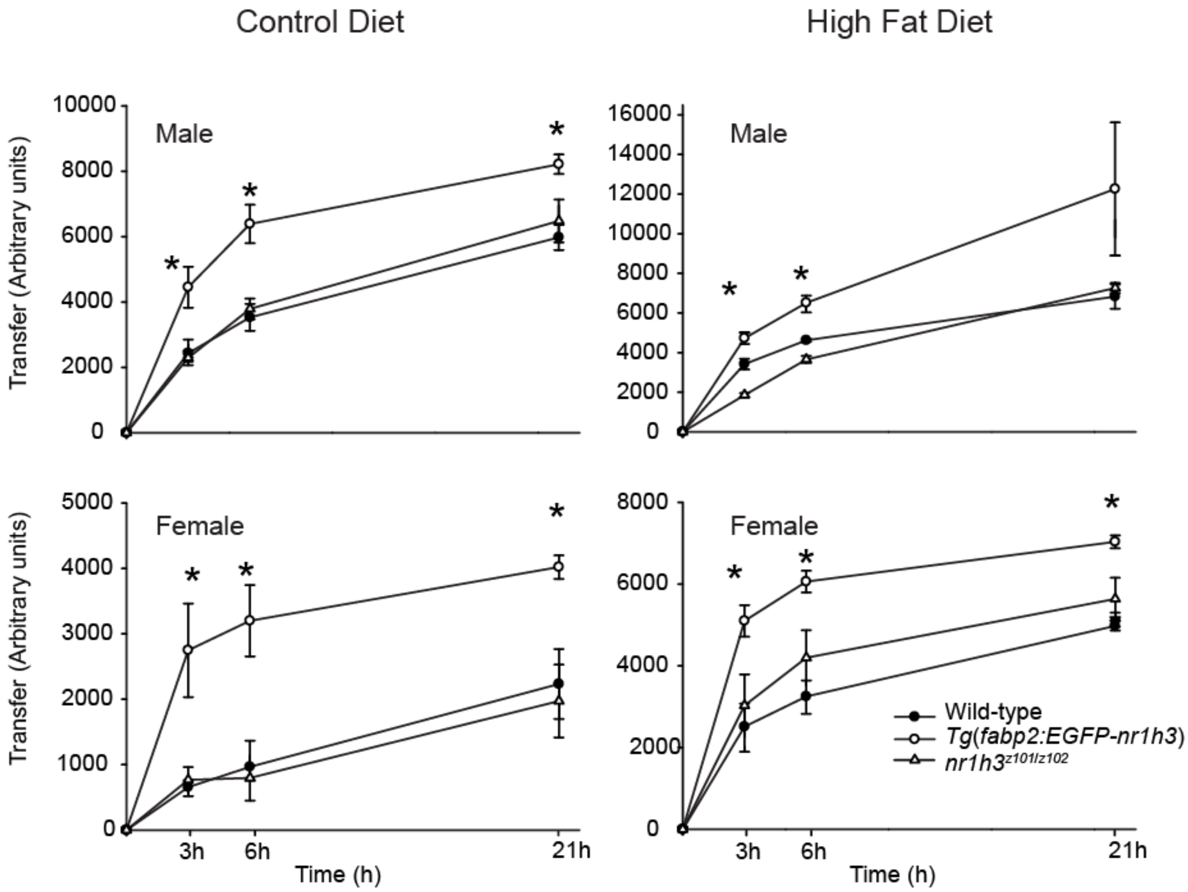
Lourdes Cruz-Garcia, Amnon Schlegel

Supplementary Materials

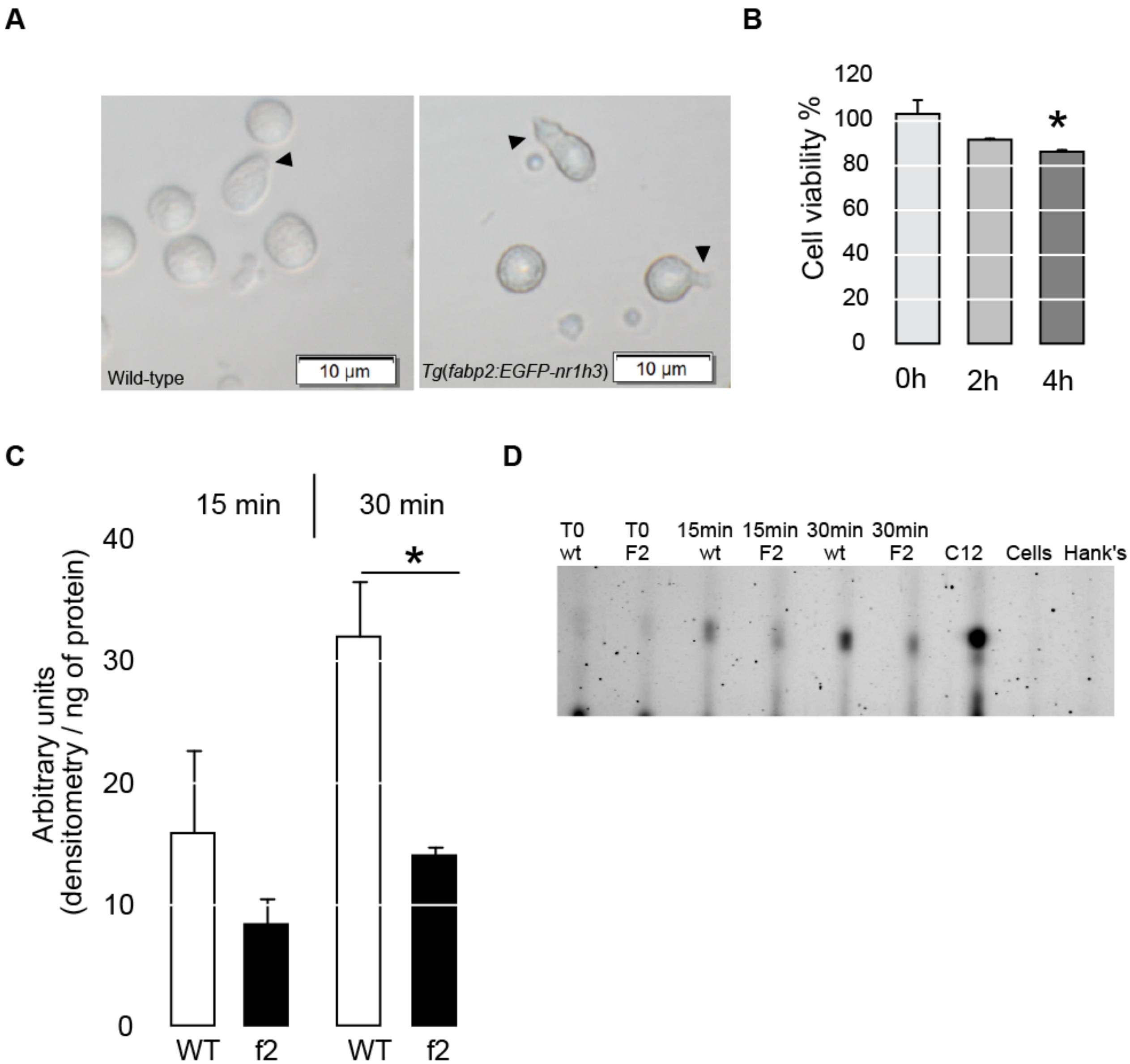
Supplementary Figures



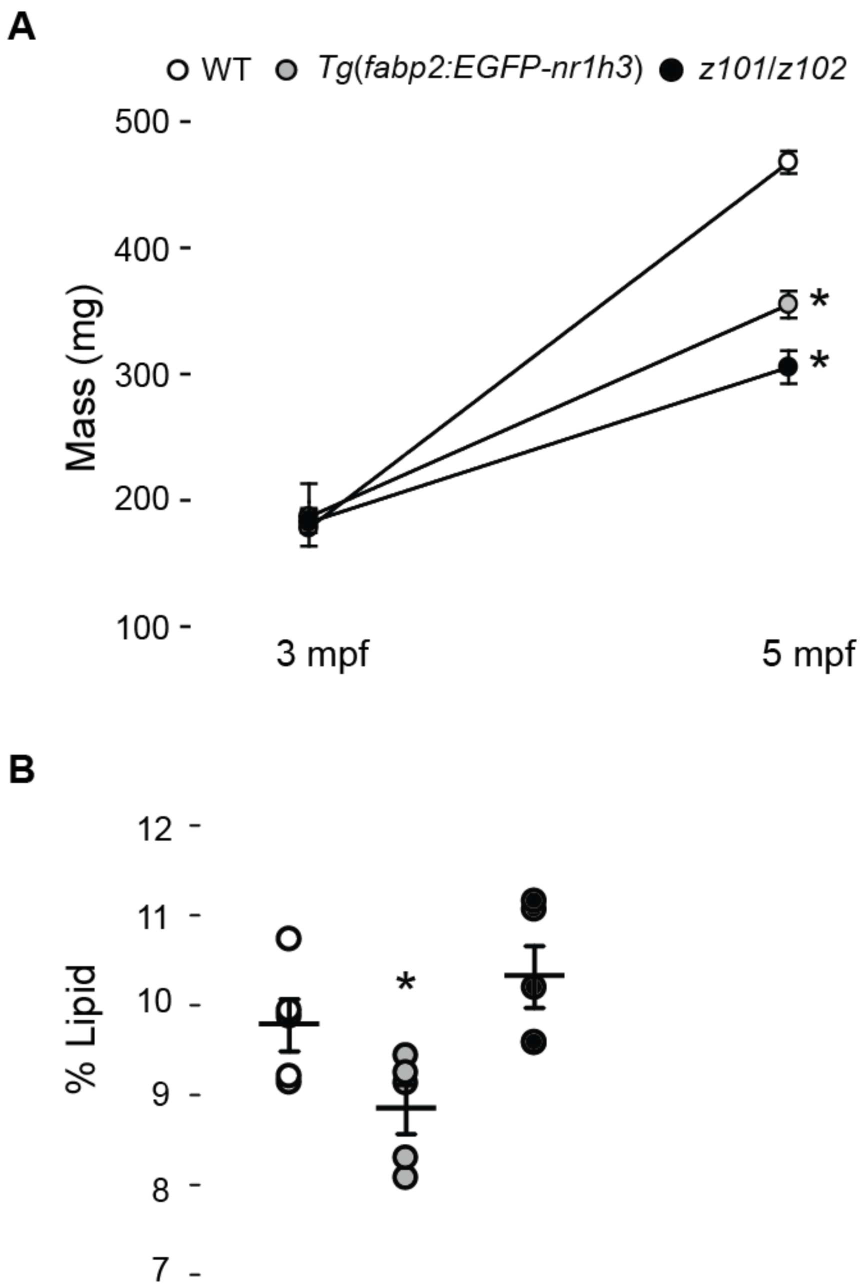
Supplementary Figure 1. Intestinal transit is not affected by Lxr. (A) 7 dpf larvae were fed a meal spiked with non-absorbable fluorescent beads for 1h. (B and C) The location of the beads was monitored 6 and 24 h after feeding (n= 25-28). No differences in intestinal transit were found among the 3 genotypes. WT, wild-type; *z101/z102*, *nr1h3*$^{z101/z102}$; f2, *Tg*(*fabp2:EGFP-nr1h3*).



Supplementary Figure 2. Intestinal Mtp activity is greater in *Tg*(*fabp2:nr1h3*) animals. Mtp activity was measured in anterior intestines from animals fed a control or high fat diet (*) P < 0.05 for each genotype compared to wild-type (n=4).



Supplementary Figure 3. Transgenic over-expression of Lxra in enterocytes decreases uptake of fatty acids. (A) Enterocytes were isolated from wild-type and *Tg*(*fabp2a:EGFP:nr1h3*) ('F2') animals. Arrowheads point to the apical brush border. (B) Viability of the isolated cells. (C and D) The isolated enterocytes were incubated with a BODIPY FL C_{12} for 15 and 30 min, and the lipids were extracted and run on a TLC plate, and quantified. (*) $P < 0.05$ compared to wild-type (n=3 pools of three wells each).



Supplementary Figure 4. Mass and body composition (% lipid) analysis of adults fed the control diet for two months. (A) Masses of animals in the indicated cohorts were measured at the start and conclusion of a two month protocol of feeding the control diet shown in Table S1 (B) Body composition was measured at the conclusion of the feeding period. Note *Tg*(*fabp2:EGFP-nr1h3*) adults had lower masses at the end of the study. (*) $P < 0.05$.

Supplementary Tables

Supplementary Table 1. Composition of define diets used in this study.

Ingredient	Control Diet	High Fat Diet	Vendor
Corn Dextrin	7	2.67	Sigma-Aldrich
Fish Meal	30	23.56	The Scoular Company
Casein	18.75	13.5	Sigma-Aldrich
Wheat Gluten	12	9	Red Mill
Cod Liver	0.4	7	Gateway Products
Flaxseed Oil	2	12.5	Now Foods
Sunflower Oil	0.4	7	Bizim Sunflower Oil
Dried Egg Yolk	6	10	Sigma-Aldrich
Gelatin	4	4	Sigma-Aldrich
Carboxymethylcellulose	2	2	Acros Organics
Alpha cellulose	8.68	0	Sigma-Aldrich
Vitamin Mix[a]	3.81	3.81	Sigma-Aldrich
Mineral Mix[b]	2.5	2.5	
Canthaxanthin	2.3	2.3	LTK Laboratories
Betaine[a]	0.15	0.15	Sigma-Aldrich
Butylated Hydroxytoluene	0.01	0.10	Alfa Aesar
Energy (cal/ 100 g)	318.4	425.7	
Crude Protein (%)	51.2	39.9	
Crude Lipid (%)	10.2	31.1	
Total Cholesterol (%)	0.34	0.38	
Ash (%)	10.4	8.8	

a. Composition: thiamine hydrochloride, 9 mg/kg; riboflavin, 9 mg/kg; pyridoxine hydrochloride, 10.5 mg/kg; nicotinic acid, 45 mg/kg; D-calcium pantothenate, 24 mg/kg; folic acid, 3 mg/kg; D-biotin, 0.3 mg/kg; cyanocobalamin, 0.015 mg/kg; retinyl palmitate, 24 mg/kg; DL-tocopherol acetate ,300 mg/kg; cholecalciferol, 3.75 mg/kg; menaquinone, 0.075 mg/kg; sucrose, 14.55 g/kg; phosphatidyl choline, 19 g/kg; choline chloride, 1.8 g/kg; sodium ascorbyl phosphate, 0.3 g/kg; inositol, 2 g/kg;

b. Calcium phosphate dibasic, 7.5 g/kg; sodium chloride, 1.11 g/kg; potassium citrate, 3.3 g/kg; potassium sulphate, 0.78 g/kg; magnesium oxide, 0.36 g/kg; manganese carbonate 52.5 mg/kg; ferric citrate, 90 mg/kg; zinc carbonate, 24 mg/kg; copper carbonate, 4.5 mg/kg; sucrose, 1.77 g/kg; potassium phosphate 10 g/kg.

Supplementary Table 2. qPCR primer sequences.

Gene	*Protein*	Accesion number	Primers 5' to 3'
abcg5	*ATP-binding cassette, sub-family G, member 5*	NM_001128690.1	TCCATGAAGGATGTTGTCCA AGATCCCACCAGGGTCCTAC
abcg8	*ATP-binding cassette, sub-family G, member 8*	XM_005156538.1	CAGCAATAAGCAGACGGTCA TTCCCGTTGATCAGGATCTC
acsl3a	*acyl-CoA synthetase long-chain family member 3a*	XM_005159236.1	CAAGCTCGTTGAGGATGACA TCCTCTGCTTCCCTTCTTGA
acsl3b	*acyl-CoA synthetase long-chain family member 3b*	XM_005163548.1	CTGGCTCAGAGGTTTGGAAG CAGGTCTTTGCTTGTGACGA
acsl5	*acyl-CoA synthetase long-chain family member 5*	NM_001004599.1	GCGTCAAAGGGACAAATGTT CCTGGGCCAGTTTGAAGATA
apoa1a	*apolipoprotein A-Ia*	NM_131128.1	CTAAGCTGACCGAGCGTCTT ATGTGGTCCTCACGTTCTCC
apoa1b	*apolipoprotein A-Ib*	NM_001100144.2	GCCCTACGTCCAGGAGTACA TTACTCCTTGCTGGCGAACT
apoa4	*apolipoprotein A-IV*	NM_001079861.1	TGCCAGGCCAACCTATTCTA TTCACTGGCCATATCAGCAC
apoba	*apolipoprotein Ba*	XM_689735.6	TGACCTCAAGCACGTCACTC GGGGAAAACCAGCACTTGTA
apobb	*apolipoprotein Bb*	XM_005160629.1	GCTTGAAGGAACCAGCAGTC AGTTGGTGGTTGGCATTAGC
cd36	*CD36 antigen*	NM_001002363.1	ATGAGACGGCCAAAATGTTC ATTCGTTTTTGACGGTTTCG
cyp7a1a	cytochrome P450, family 7, subfamily A, polypeptide 1a	NM_201173.1	GCAGGCGTGCCAATGTG CAGCTCGTTGAAGGTAGATAGTGTGT
dagt2	*diacylglycerol O-acyltransferase 2*	XM_005172674.1	CATGGCATCTTGTGTTTTGG TCGGTTTACTGGGCAGATTC
mtp	*microsomal triglyceride transfer protein*	NM_212970.1	CTCAGCTGGTGGATGCAGTA CAGGGATTGAAGCATGGACT
slc27a4	*solute carrier family 27 (fatty acid transporter), member 4*	NM_001017737.1	GCCCAGGATGTCTTCAAAAA TCTTTCATGTCCAGCAGTCG
rplp0	*ribosomal protein large P0*	XM_005165011.1	CTGAACATCTCGCCCTTCTC TAGCCGATCTGCAGACACAC

.