

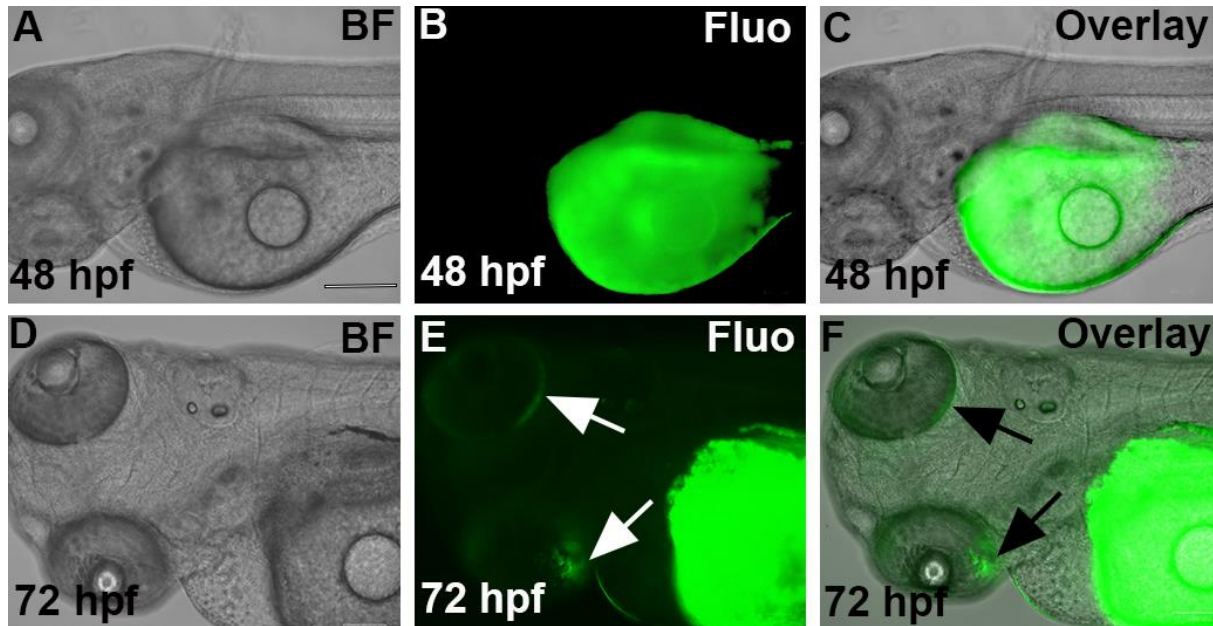
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Supplemental information

**Excess glucose or fat differentially affects
metabolism and appetite-related gene expression
during zebrafish embryogenesis**

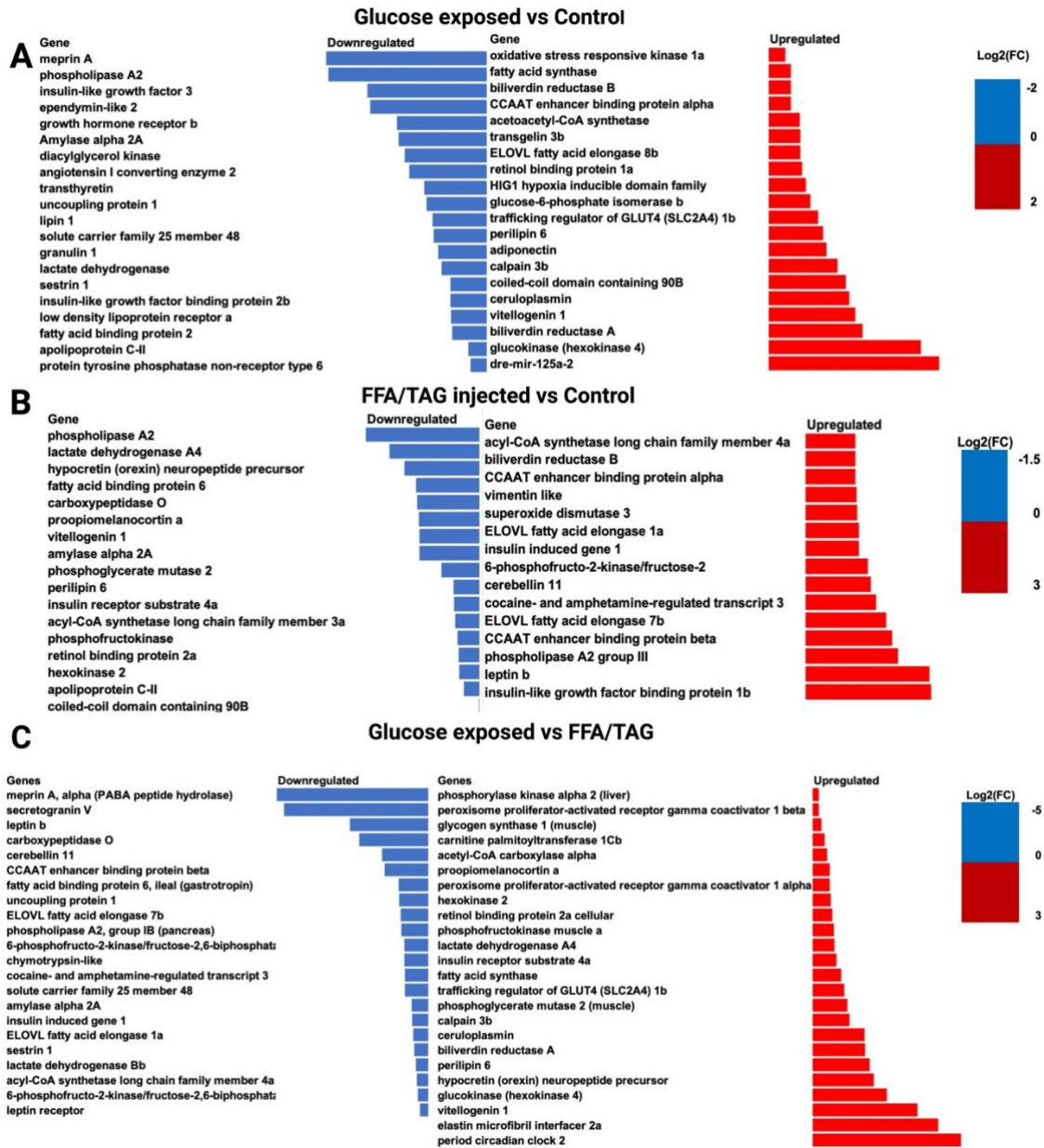
Bridget Konadu, Carol K. Cox, Michael R. Garrett, and Yann Gibert

Effect of FFA/TAG injection on embryos at 48- and 72-hours post fertilization



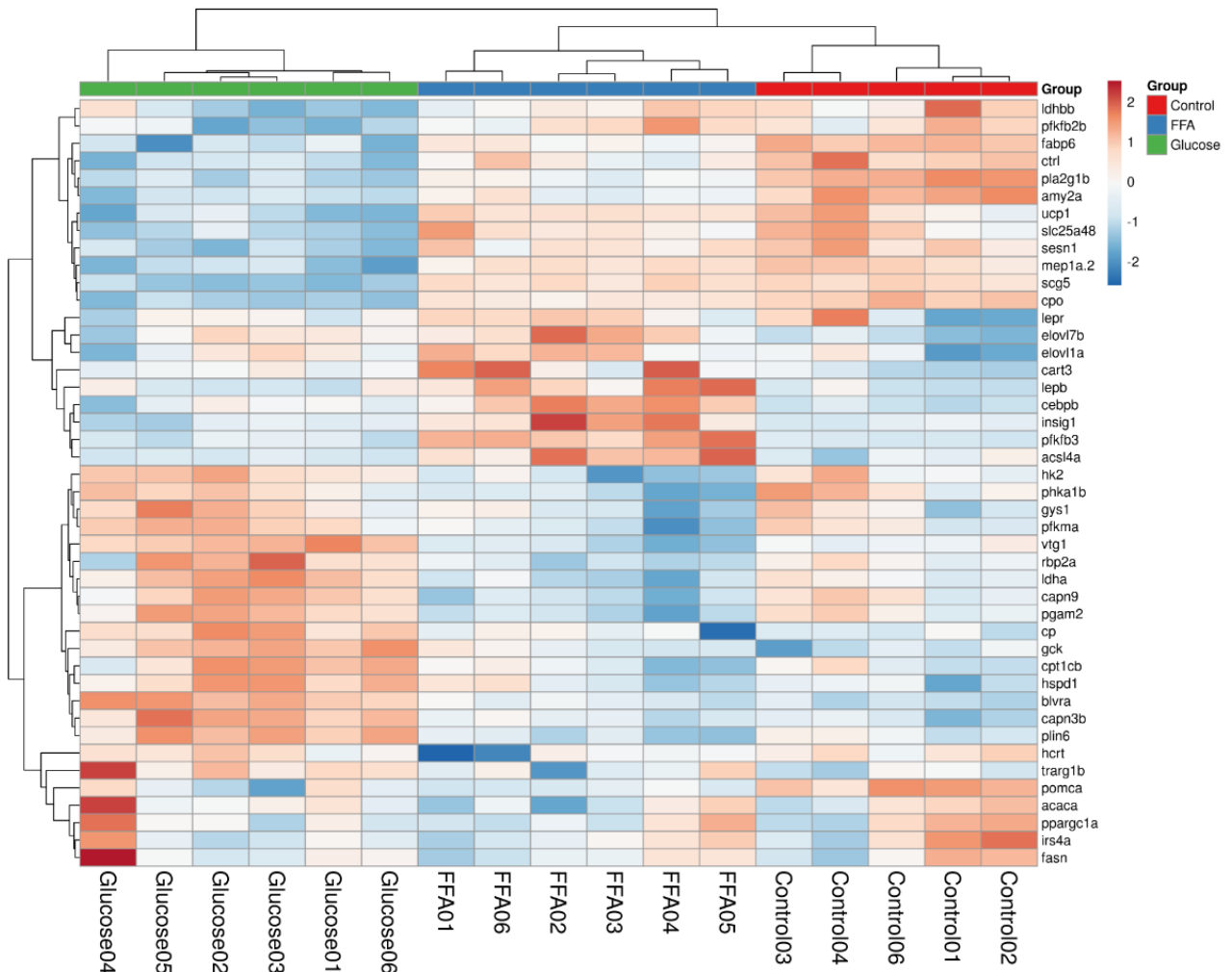
Supplementary Fig. 2. Comparison of the effect BODIPY-labeled fluorescent FLC12 FFA/TAG injected on zebrafish embryos at 48 and 72 hpf. A. Brightfield image showing site of injection in a 48 hpf FFA/TAG injected embryo. B. Fluorescent image of the embryo shown in A. C. Merged image of A and B. D. Brightfield image showing site of injection in a 72 hpf FFA/TAG injected embryo. E. Fluorescent image of the embryo shown in D with fluorescence detected in the head (white arrows). F. Merged image of D and E, the black arrows depict fluorescent diffusion in the head. Scale bar: 100 μ m

Comparison of upregulated and upregulated DEGs between Glucose treated embryos and FFA/TAG injected embryos



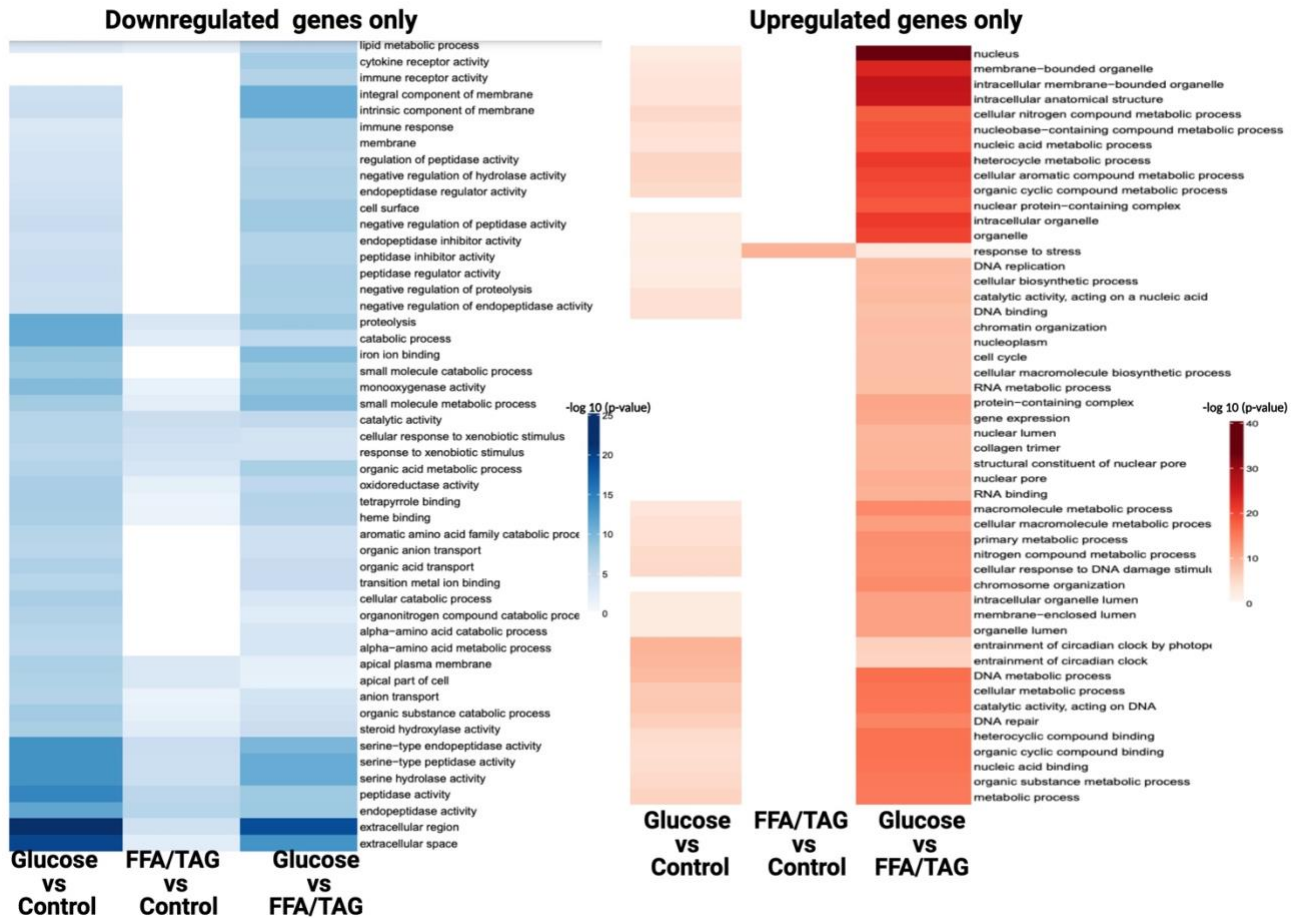
Supplementary Fig. 3. Analysis of differentially expressed genes. The first 50 upregulated or downregulated genes represented within a range of (-2 to 2) of log 2 of the fold change were chosen. A. Shows downregulated (blue) and upregulated (red) genes of treatment glucose compared to controls. B. Shows downregulated (blue) and upregulated (red) genes of FFA/TAG injected samples compared to controls. C. FFA/TAG injected samples compared to glucose-exposed samples show significant upregulated genes (blue) and downregulated genes (red).

Heatmap comparing DEGs in glucose-exposed embryos, FFA/TAG-injected embryos, and controls



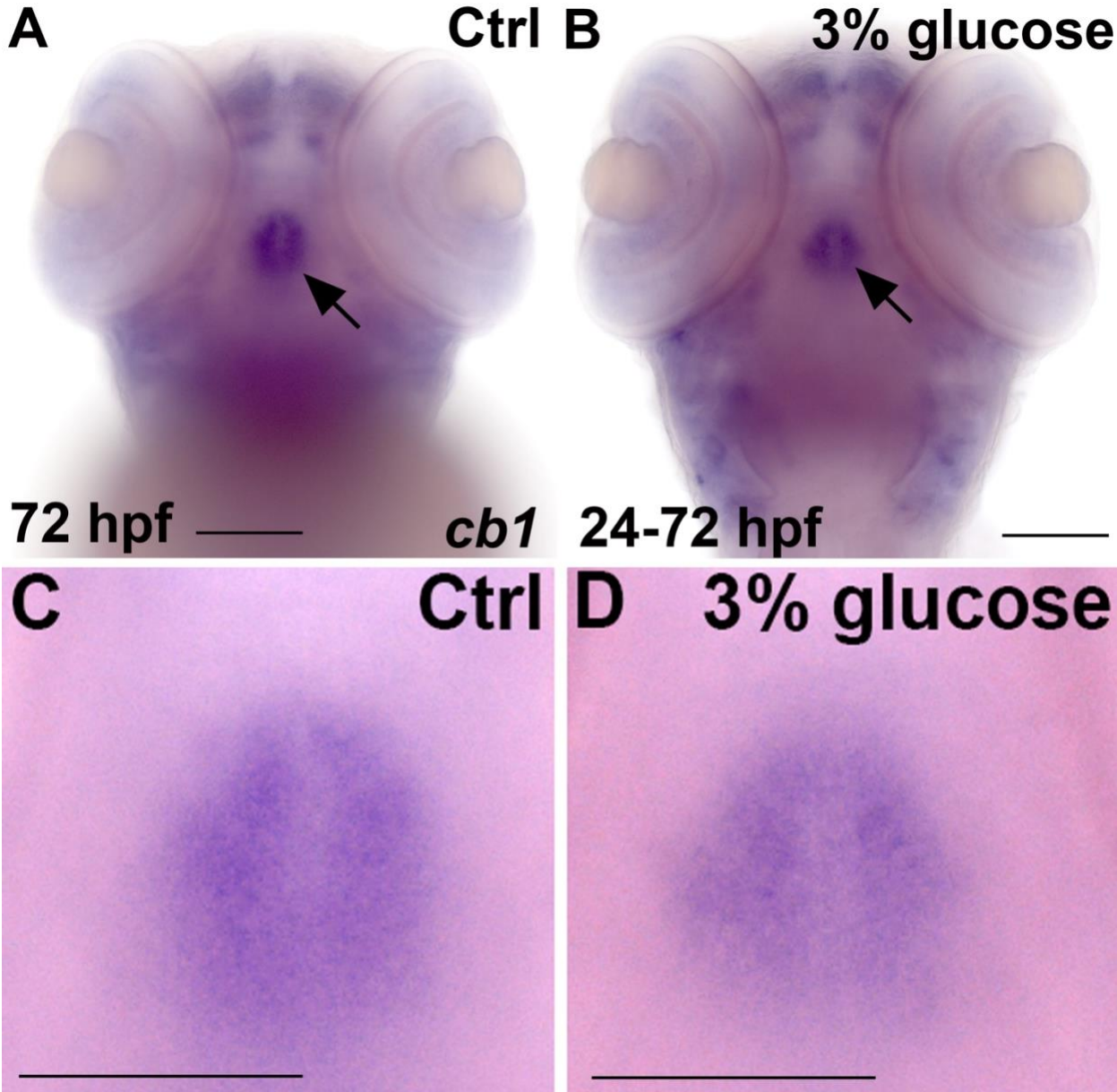
Supplementary Fig. 4. Heatmap showing gene intensity per sample relative to the average level across all samples. Individual genes are shown on the Y axis while samples are shown along the X axis. Red and blue cells correspond to higher and lower RNAseq levels, respectively. FFA/TAG injected samples compared to glucose-exposed samples show significantly upregulated genes (blue) and downregulated genes (red).

Heatmap comparison of Gene ontology terms between glucose exposed embryos, FFA/TAG injected embryos and controls



Supplementary Fig. 5. Heatmaps of significantly enriched GO terms. Comparisons are shown on the X axis with GO terms on the Y axis between Glucose, FFA/TAG and controls. Enrichment terms were restricted to include only those with 2 or more genes. Only GO terms with an enrichment p-value less than 0.05 and the top 50 terms were included. Results are shown separately for up-regulated and down-regulated genes, Colour (red for enriched among up-regulated genes and blue for those enriched among down-regulated genes) is assigned based on the $-\log_{10}$ (enrichment p-value), with lighter colours implying less significant enrichment.

Close up view of CB1 transcript by Whole Mount In- situ hybridization



Supplementary Fig. 6. Display mRNA expression of CB1 gene (arrows) by WISH in embryos at 72 hpf. A. control B. Glucose-treated embryos (3% w/v). Close-up view of CB1 expression in C. control and D. Glucose. Scale bars 100 μm (A,B); Scale bars 50 μm (C,D).

Supplementary Table 1.**Table 1. List of primers used for quantitative PCR**

GENE	5'FORWARD PRIMER3'	5'REVERSE PRIMER3'
Glut2	GCAGAAGAACCCTCACTC	TCTCCGCCACAATAAACC
Insulin	GGTCCTGTTGGTCGTGTC	GTTGTAGAAGAAGCCTGTTGG
Insulin receptor a	GATCTGCATCCCTCGTTTCA	CTCCAGGTCCATGTCAAACCTC
Insulin receptor a	GATCTGCATCCCTCGTTTCA	CTCCAGGTCCATGTCAAACCTC
Insulin receptor b	AAGGTATGAAAGTGCCGTCC	GCATTTTCGGTTTTGGGACAG
atgl	CCTGCAAGGAGTGAGGTATG	CTGTAGAGGTTGGCGAGTGT
hsla	CAGGACACCGTAAAGATGCT	TCCACCAGACAACCTAGACCG
mgll	CGAGAGGCCGCGAGGATTTTA	TGAGTTTAGGAGCCAAGCG
Cpt1a	TCTACCTGAGAGGTCGTGGG	TAGCCGTTCCCATGAGCAG
Cpt2	AATGGATTGGGTGCAACGTG	TGAGTTCTAACCTTCAGGCTC
POMC	CACTGCTCACACTCTCA	GCCACCTTCGTTTCTAT
npy	GCGTTTCTCTTGTTTCGTCTGCTT	TGACCTTTTCCCATACCTCTGCCT
agrp	TTGAAGCCTATGATGAGGATCTGG	CTTAAAGAAGCGGCAGTAGCAGGT
PACAP 1a	CTGAAGGCGGTGGCAGTAAT	TTGCAGCCCACAGATCCATAG

PACAP 1b	TGCACTCGCAACTACATCCA	AGCCAACAGAACCAGTGGAG
CART1	CGAGCAGGACGAGCAGTTCATC	GCTCCCCTACGTCACACCTG
crh	TCGTCCATGATCTTGCGGTT	GAATCTGCACGTGGTTGTCG
Oxytocin	AAAGGCCTGCGGTTATGAGG	TTGGCCGGTTGATTGACAGC
Orexin(hcrt)	CTACGAGATGCTGTGCCGAG	CCAAGAGTGAGAATCCCGAC
CB1	AGCGGAGCAGGACCAGTTCA	GGGAACAGCATGGTCAGAGATGC
Sf-1	CATCCTCTTCAACCCCAACG	GCAGGGCACTTCTCCACACA
pfk (liver)	GTAACACGCATGGGCATTTTTG	TCGCCAGTTTGATGTGATCTCCT
pfk (muscle)	ATCACATCCGTCCTGCTACATGG	TGGTCTGGAAATCCTTACAGCG
lpl	CGCAGTTAACAAAATTCGCA	TCCTTCTCGCCATGTATTCC
cd36	CTCTGCCTCACTTCCTCT	GCCGTCTCATTCAACCAT
fabp2	TCAGCACTTTCGCACACTG	CTTGGCCTCGACTCCATCAT
Ifabp	ACGTGGCAGGTTTACGCTCAG	TTGGAGGTGATGGTGAAGTCG

Supplementary Table 3.**Table 3. Gene table of RNAseq Data (FFA/TAG injected vs Control)**

Gene ID	Symbol	Entrez	Gene name	Fold change	Log2 (fold change)	P-value
ENSDARG00000009153	pla2g1b	571738	phospholipase A2, group IB (pancreas)	-2.743	-1.456	5.37E-08
ENSDARG00000101251	ldha	30496	lactate dehydrogenase A4	-1.153	-0.205	0.00132
ENSDARG00000070932	hcrtr	613239	hypocretin (orexin) neuropeptide precursor	-1.943	-0.959	0.0167
ENSDARG00000044566	fabp6	415166	fatty acid binding protein 6, ileal (gastrotropin)	-1.758	-0.814	0.000428
ENSDARG00000077688	cpo	100005630	carboxypeptidase O	-1.734	-0.794	0.0000205
ENSDARG00000092233	vtg1	559475	vitellogenin 1	-1.705	-0.77	0.00054
ENSDARG00000013856	amy2a	406539	amylase alpha 2A	-1.698	-0.764	0.0000013
ENSDARG00000057571	pgam2	572733	phosphoglycerate mutase 2 (muscle)	-1.399	-0.485	1.31E-07
ENSDARG00000076844	plin6	794783	perilipin 6	-1.259	-0.333	0.00523
ENSDARG00000086551	irs4a	100329897	insulin receptor substrate 4a	-1.249	-0.321	0.0198
ENSDARG00000032079	acsl3a	567157	acyl-CoA synthetase long-chain family member 3a	-1.241	-0.311	0.000142
ENSDARG00000060797	pfkmb	568001	phosphofructokinase, muscle b	-1.225	-0.293	0.00108
ENSDARG00000070038	rbp2a	568032	Retinol-binding protein 2a, cellular	-1.205	-0.269	0.00707
ENSDARG00000101482	hk2	406339	hexokinase 2	-1.195	-0.257	0.00113

ENSDARG00000025375	idh1	100006589	isocitrate dehydrogenase (NADP (+)) 1	-1.183	-0.242	0.0000858
ENSDARG00000092155	apoc2	568972	apolipoprotein C-II	-1.149	-0.201	0.01
ENSDARG00000044626	ccdc90b	100149897	coiled-coil domain containing 90B	-1.008	-0.011	0.09
ENSDARG00000004078	acsl4a	393622	acyl-CoA synthetase long-chain family member 4a	1.281	0.357	0.0000484
ENSDARG00000096829	blvrb	436959	biliverdin reductase B	1.283	0.359	0.000116
ENSDARG00000036074	cebpa	140815	CCAAT enhancer binding protein alpha	1.287	0.364	0.000131
ENSDARG00000044501	viml	393746	vimentin like	1.313	0.393	0.00472
ENSDARG00000070168	sod3a	504078	superoxide dismutase 3, extracellular a	1.345	0.427	0.00196
ENSDARG00000099960	elov11a	449816	ELOVL fatty acid elongase 1a	1.39	0.475	0.0000234
ENSDARG00000010658	insig1	334189	insulin induced gene 1	1.396	0.481	0.000063
ENSDARG00000001953	pfkfb3	554477	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	1.745	0.803	4.35E-09
ENSDARG00000086654	cbln11	100149559	cerebellin 11	1.899	0.925	0.00000918
ENSDARG00000035852	cart3	557301	cocaine- and amphetamine-regulated transcript 3	2.161	1.112	0.000495
ENSDARG000000100185	elov17b	-	ELOVL fatty acid elongase 7b	2.768	1.469	0.0000054
ENSDARG00000042725	cebpb	140814	CCAAT enhancer binding protein beta	3.221	1.688	8.47E-07

ENSDARG00000008948	pla2g3	641423	phospholipase A2 group III	3.768	1.914	0.000296
ENSDARG00000045548	lepb	564348	leptin b	8.288	3.051	0.0000708

Supplementary Table 4.

Table 4. Gene table of RNAseq Data (Glucose exposed vs FFA/TAG injected)

Gene ID	Symbol	Entrez	Gene name	Fold change	Log2 (fold change)	P-value
ENSDARG00000008029	mep1a.2	565535	meprin A, alpha (PABA peptide hydrolase), tandem duplicate 2	-28.929	-4.854	1.76E-09
ENSDARG00000077045	scg5	393699	secretogranin V	-24.464	-4.613	5.93E-12
ENSDARG00000045548	lepb	564348	leptin b	-5.702	-2.512	0.000722
ENSDARG00000077688	cpo	100005630	carboxypeptidase O	-4.643	-2.215	1.43E-11
ENSDARG00000042725	cebpb	140814	CCAAT enhancer binding protein beta	-2.63	-1.395	0.0000158
ENSDARG00000044566	fabp6	415166	fatty acid binding protein 6, ileal (gastrotropin)	-1.937	-0.954	0.000131
ENSDARG00000023151	ucp1	83908	uncoupling protein 1	-1.905	-0.929	1.26E-08
ENSDARG00000100185	elovl7b	-	ELOVL fatty acid elongase 7b	-1.833	-0.875	0.00171
ENSDARG00000009153	pla2g1b	571738	phospholipase A2, group IB (pancreas)	-1.821	-0.865	0.0000649
ENSDARG00000001953	pfkfb3	554477	6-phosphofructo-2- kinase/fructose-2,6- biphosphatase 3	-1.698	-0.764	1.65E-08
ENSDARG00000068680	ctrl	447843	chymotrypsin-like	-1.695	-0.762	5.03E-07

ENSDARG00000035852	cart3	557301	cocaine- and amphetamine-regulated transcript 3	-1.684	-0.752	0.0122
ENSDARG00000021250	slc25a48	436640	solute carrier family 25 member 48	-1.674	-0.743	1.21E-07
ENSDARG00000013856	amy2a	406539	amylase alpha 2A	-1.444	-0.53	0.000154
ENSDARG00000010658	insig1	334189	insulin induced gene 1	-1.435	-0.522	0.0000412
ENSDARG00000099960	elov1a	449816	ELOVL fatty acid elongase 1a	-1.399	-0.484	0.00003
ENSDARG00000020693	sesn1	436933	sestrin 1	-1.357	-0.441	0.00000931
ENSDARG00000071076	ldhbb	436747	lactate dehydrogenase Bb	-1.314	-0.394	0.0129
ENSDARG00000004078	acsl4a	393622	acyl-CoA synthetase long chain family member 4a	-1.292	-0.37	0.0000527
ENSDARG00000002037	pfkfb2b	564852	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2b	-1.255	-0.328	0.000989
ENSDARG00000070961	lepr	567241	leptin receptor	-1.204	-0.268	0.000455
ENSDARG00000037943	cpt1cb	550361	carnitine palmitoyltransferase 1Cb	1.156	0.209	0.00674
ENSDARG00000105039	phka1b	562402	phosphorylase kinase, alpha 1b (muscle)	1.382	0.467	0.000826
ENSDARG00000067829	ppargc1a	553418	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	1.232	0.301	0.0134
ENSDARG00000016875	gys1	394155	glycogen synthase 1 (muscle)	1.115	0.157	0.0394

ENSDARG00000056160	hspd1	282676	heat shock 60 protein 1	1.139	0.188	0.00493
ENSDARG00000078512	acaca	559403	acetyl-CoA carboxylase alpha	1.195	0.257	0.00179
ENSDARG00000043135	pomca	353221	proopiomelanocortin a	1.23	0.298	0.035
ENSDARG00000101482	hk2	406339	hexokinase 2	1.245	0.316	0.000241
ENSDARG00000070038	rbp2a	568032	retinol binding protein 2a, cellular	1.268	0.342	0.00166
ENSDARG00000014179	pfkma	447836	phosphofructokinase, muscle a	1.296	0.374	0.000509
ENSDARG00000101251	ldha	30496	lactate dehydrogenase A4	1.306	0.385	2.46E-06
ENSDARG00000086551	irs4a	100329897	insulin receptor substrate 4a	1.337	0.419	0.00479
ENSDARG00000087657	fasn	559001	fatty acid synthase	1.412	0.498	2.90E-05
ENSDARG00000094675	trarg1b	556978	trafficking regulator of GLUT4 (SLC2A4) 1b	1.469	0.555	0.000676
ENSDARG00000012341	capn9	445107	calpain 9	1.503	0.588	5.01E-07
ENSDARG00000057571	pgam2	572733	phosphoglycerate mutase 2 (muscle)	1.522	0.606	9.47E-09
ENSDARG00000043035	capn3b	100034405	calpain 3b	1.565	0.646	1.80E-08
ENSDARG00000010312	cp	84702	ceruloplasmin	1.878	0.909	0.00318
ENSDARG00000059857	blvra	767661	biliverdin reductase A	1.891	0.919	2.78E-07
ENSDARG00000076844	plin6	794783	perilipin 6	2.001	1.001	4.32E-08
ENSDARG00000070932	hert	613239	hypocretin (orexin) neuropeptide precursor	2.109	1.077	0.0108
ENSDARG00000068006	gck	751668	glucokinase (hexokinase 4)	2.467	1.303	0.000375
ENSDARG00000092233	vtg1	559475	vitellogenin 1	3.57	1.836	2.03E-08