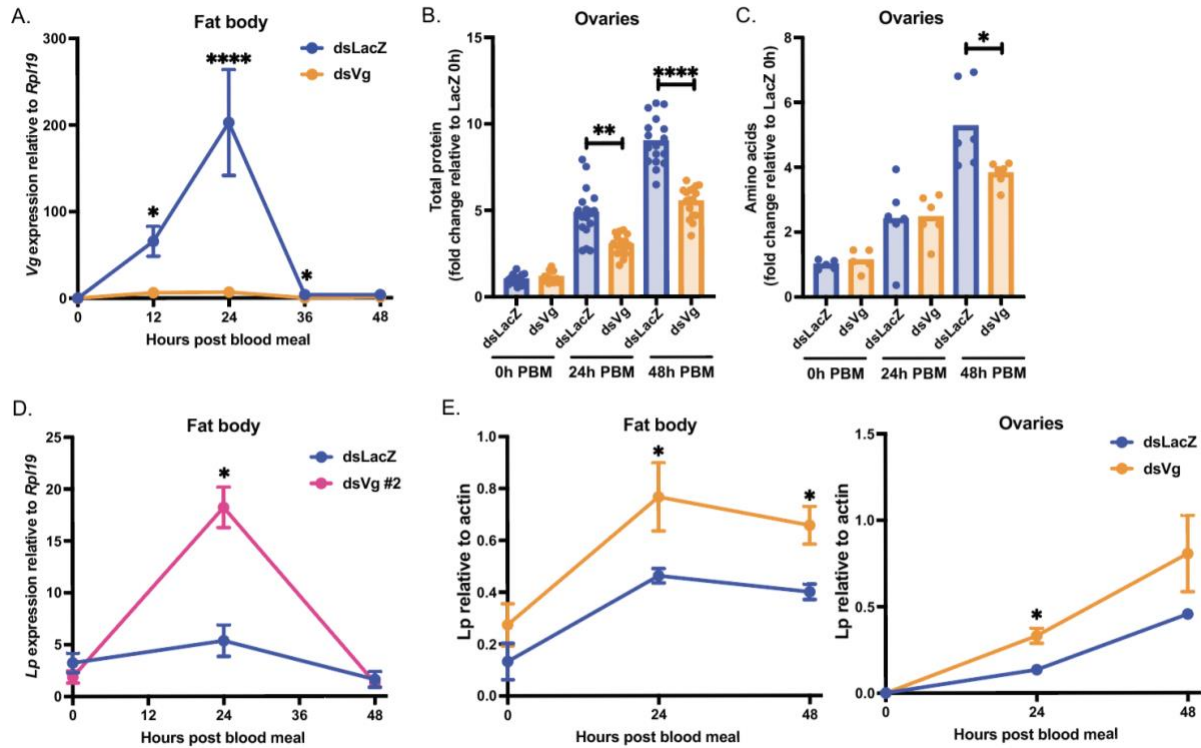
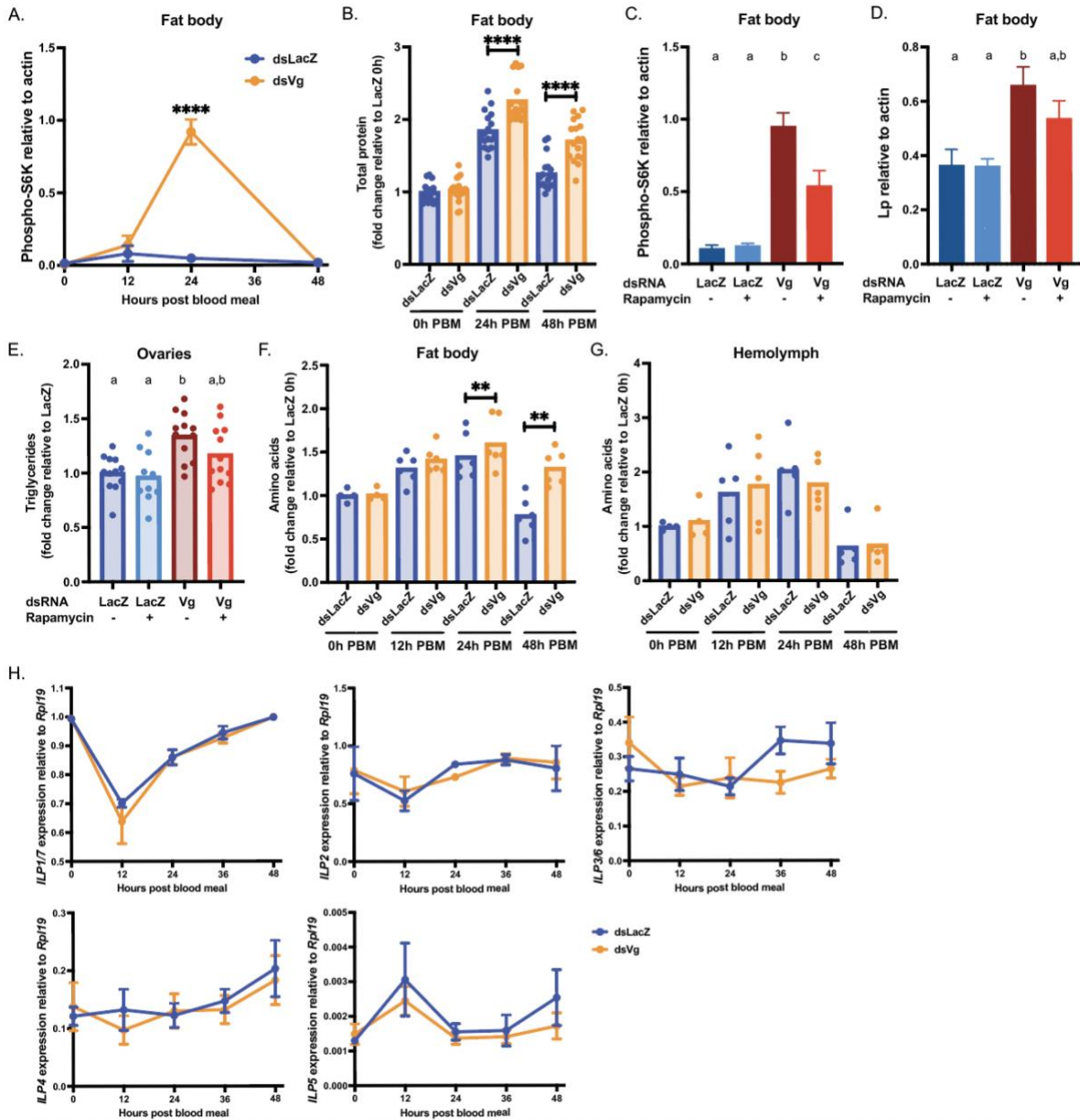


Supplemental Figure 1. *Lp* knockdown significantly impairs oogenesis and affects Vg expression and localization. (A) Successful *Lp* knockdown as determined by RT-qPCR of *Lp* expression levels relative to *Rpl19* in the fat body of dsLacZ and dsLp females (REML variance component analysis: * = $p < 0.05$; **** = $p < 0.0001$; three biological replicates). (B) RT-qPCR of *Vg* expression levels relative to *Rpl19* in the fat body of dsLacZ and dsLp females (REML variance component analysis: ** = $p < 0.01$; **** = $p < 0.0001$; four biological replicates). (C) Western blot quantification from Figure 1C showing an accumulation of Vg in the fat body and a decrease of Vg in the ovaries upon *Lp* knockdown (REML variance component analysis: ** = $p < 0.01$; **** = $p < 0.0001$; three biological replicates). (D) Images of ovaries at 24 and 48h post blood meal showing that *Lp* depleted ovaries develop normally at first before degenerating by 48h; scale bar = 2mm.



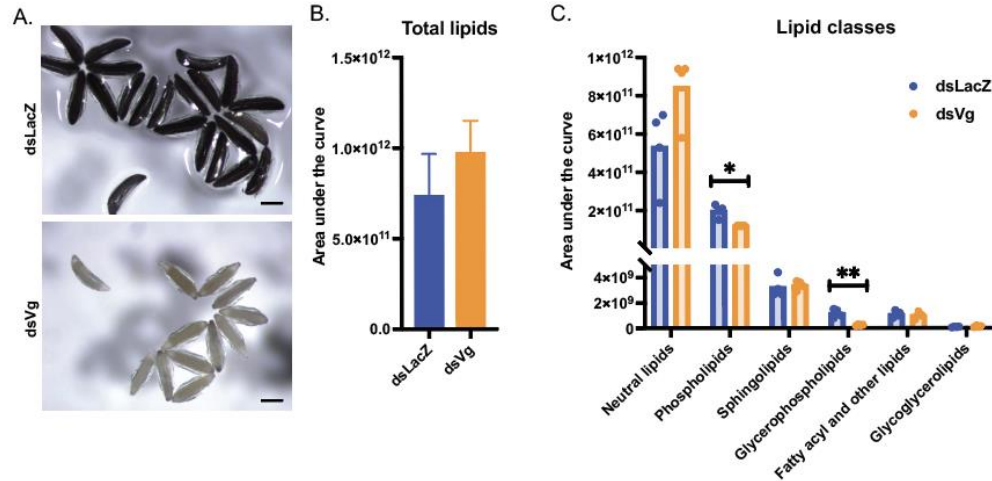
Supplemental Figure 2. *Vg* knockdown effects in fat body and ovaries. (A) Successful *Vg* knockdown as determined by RT-qPCR of *Vg* expression levels relative to *Rpl19* in the fat body (REML variance component analysis: * = $p < 0.05$; **** = $p < 0.0001$; three biological replicates). (B) Fold change in protein levels measured by Bradford assay in the ovaries of dsLacZ and dsVg females before blood meal and at 24h and 48h post blood meal (PBM); each dot is representative of three ovaries (REML variance component analysis by timepoint: ** = $p < 0.01$; **** = $p < 0.0001$; three biological replicates). (C) Fold change in free amino acid levels in the ovaries of dsLacZ and dsVg females before blood meal and at 24h and 48h PBM; each dot is representative of five ovaries (REML variance component analysis by timepoint: * = $p < 0.05$; three biological replicates). (D) *Vg* knockdown by second target also results in an increase in *Lp* levels as determined by RT-qPCR (REML variance component analysis: * = $p < 0.05$; three biological replicates). (E) Western blot quantification from Figure 2G showing an accumulation of *Lp* in the fat body and ovaries upon *Vg* knockdown (REML variance component analysis: fat body – dsRNA: $p < 0.01$; * = $p < 0.05$; ovaries – dsRNA: $p < 0.05$; * = $p < 0.05$; three biological replicates).



Supplemental Figure 3. *Vg* expression regulates Lp-mediated accumulation of lipids via TOR signaling. (A) Western blot quantification from Figure 3B showing an increase in phospho-S6K levels in the fat body upon *Vg* knockdown (REML variance component analysis: **** = $p < 0.0001$; three biological replicates). (B) Fold change in protein levels, measured by Bradford assay of the fat body are increased in ds*Vg* females; each dot is representative of three fat bodies (REML variance component analysis by timepoint: **** = $p < 0.0001$; three biological replicates). (C) Western blot quantification from Figure 3D showing a decrease in phospho-S6K levels in the fat body upon rapamycin treatment (ANOVA; three biological replicates). (D) Western blot quantification from Figure 3E showing Lp protein levels upon *Vg* knockdown and rapamycin treatment (ANOVA; three biological replicates). (E) Triglyceride levels measured in dsLacZ and ds*Vg* ovaries upon 0.5 μ l of 40 μ M rapamycin treatment at 72h post blood meal and normalized to

Supplemental Table 1: Vg amino acids and their decrease in embryos upon Vg depletion.

Amino acid	Percent content in Vg	Decrease upon Vg KD in mothers (fold change)	Significance	
Ser (S)	8.50%	2.2	0.09205	
Tyr (Y)	8.00%	52.6	0.00010	***
Phe (F)	7.70%	63.2	0.00000	****
Glu (E)	6.50%	1.2	0.33025	
Ala (A)	6.30%	2.0	0.02859	*
Asp (D)	6.30%	2.1	0.15012	
Lys (K)	6.20%	10.0	0.00000	****
Gln (Q)	6.10%	1.1	0.88614	
Val (V)	5.80%	3.1	0.00005	****
Asn (N)	5.60%	5.5	0.00006	****
Leu (L)	5.60%	3.7	0.00024	***
Gly (G)	4.40%	2.2	0.02060	*
Pro (P)	4.40%	1.4	0.01066	*
Thr (T)	4.40%	3.9	0.00015	***
Arg (R)	3.90%	2.9	0.00012	***
Ile (I)	3.20%	8.7	0.00001	****
His (H)	2.90%	1.0	0.69815	
Met (M)	2.20%	4.9	0.00010	***
Cys (C)	1.10%	Not detected		
Trp (W)	0.80%	13.2	0.00002	****



Supplemental Figure 4. *Vg* knockdown in females prevents embryo melanization and causes early embryonic arrest. (A) Light microscopy of embryos from dsLacZ- and dsVg-derived females at 3-5h post oviposition; scale bar = 200 μ m. (B-C) Total lipids (B) and lipid classes (C) in dsLacZ- and dsVg-derived embryos 3-5h post oviposition as determined by mass spectrometry (unpaired t tests, followed by FDR correction: * = $p < 0.05$; ** = $p < 0.01$; four biological replicates).

Supplemental Table 4

Gene	Primer	Citation, if previously published
Rpl19	F CCAACTCGCGACAAAACATTC R ACCGGCTTCTTGATGATCAGA	Werling, Shaw, Itoe et al., 2019
Lp	F CAGCCAGGATGGTGAGCTTAA R CACCAGCACCTTGCGTT	Werling, Shaw, Itoe et al., 2019
Vg	F CCGACTACGACCAGGACTTC R CTTCCGGCGTAGTAGACGAA	Werling, Shaw, Itoe et al., 2019
ILP1/7	F GCAAAAAGTCCGAGAATCTACTGATGA R CGAACGATCGTTCAATGTGTGGA	
ILP2	F CTACCTCTACGCCAACAGC R CGTGTACATAATCTGTGCGATAGTG	
ILP3/6	F GGTAAAGGTACTGTCCTTCCTG R AGTATCTGCTGCGTGTGTC	Arsic and Guerin, 2008
ILP4	F TCTCCGAAAGAACACAGTTGA R GGTTTCTGCCTGAACCACAT	Arsic and Guerin, 2008
ILP5	F GTGGCACCAGGAGAGTCATT R GCCCAGTACAGATGGCGTAT	

Supplemental Table 5. Details of statistical tests and outputs are summarized for each figure. For qRT-PCR, at least three independent biological replicates of a gene expression timecourse were analyzed, except for ILP1, where one replicate was excluded as an outlier. Effect test outputs are reported here. Multiple comparisons were calculated using pairwise Student's t tests at each timepoint followed by FDR correction (see **Table S6**). KD = knock down; rand = random effect; FDR = false discovery rate.

Fig	Comparison	Statistical test	Effect Test Outputs
1A	Egg number after Lp KD	Mann-Whitney	p<0.0001
1B	TAG levels after Lp KD (Ovaries)	Ln(x+1.1) transformation; Linear Mixed Model at each timepoint	(0h) dsRNA p=0.7964 replicate p=0.3755 (24h) dsRNA p<0.0001 replicate p=0.3400 (48h) dsRNA p<0.0001 replicate p=0.0109
1B	TAG levels after Lp KD (Midgut)	Ln(x+1.1) transformation; Linear Mixed Model at each timepoint	(0h) dsRNA p=0.2984 replicate p=0.3317 (24h) dsRNA p<0.0001 replicate p=0.3573 (48h) dsRNA p<0.0001 replicate p=0.3228
2A	Egg number after Vg KD	Mann-Whitney test	p<0.0001
2B	Fertility after Vg KD	Kruskal-Wallis test	dsLacZ vs dsVg: p<0.0001 dsLacZ vs dsVg#2: p<0.0001 dsVg vs dsVg#2: p>0.05
2E	Triglycerides after Vg KD (ovaries 48h)	Unpaired t test on transformed data	p=0.0222
2F	Lp expression after Vg KD	4 th root transformation; Linear Mixed Model followed by 5 post-hoc t-tests (Table S6)	timepoint p<0.0001 dsRNA p<0.0001 dsRNA x timepoint p=0.0032 replicate[rand] p=0.3090
3A	Vg upon rapamycin treatment	Unpaired t test	p<0.0001

3C	Lp mRNA levels upon rapamycin treatment	ANOVA	LacZ Control vs. LacZ Rapamycin p=0.9956 LacZ Control vs. Vg Control p=0.0002 LacZ Control vs. Vg Rapamycin p=0.2500 LacZ Rapamycin vs. Vg Control p=0.0003 LacZ Rapamycin vs. Vg Rapamycin p=0.3469 Vg Control vs. Vg Rapamycin p=0.0226
4D	Embryo triglycerides	Unpaired t test	p=0.0496
S1A	Lp expression after Lp KD	4 th root transformation; Linear Mixed Model followed by 5 post-hoc t-tests (Table S6)	timepoint p<0.0001 dsRNA p<0.0001 dsRNA x timepoint p=0.0004 replicate[rand] p=0.478
S1B	Vg expression after Lp KD	4 th root transformation; Linear Mixed Model followed by 5 post-hoc t-tests (Table S6)	timepoint p<0.0001 dsRNA p=0.044 dsRNA x timepoint p=0.0001 replicate[rand] p=0.536 replicate x timepoint[rand] p=0.033
S1C	Lp expression after Vg KD (Ovaries)	Ln(x+1) transformation; Linear Mixed Model followed by 3 post-hoc t-tests (Table S6)	timepoint p<0.0001 dsRNA p=0.0103 dsRNA x timepoint p=0.0192 replicate p=0.3836
S1C	Lp expression after Vg KD (Fatbody)	No transformation; Generalized Linear Model followed by 3 post-hoc t-tests (Table S6)	timepoint p<0.0001 dsRNA p=0.0148 dsRNA x timepoint p<0.0001 replicate p=0.0012
S2A	Vg expression after Vg KD	8 th root transformation; Linear Mixed Model followed by 5 post-hoc t-tests (Table S6)	timepoint p<0.0001 dsRNA p<0.0001 dsRNA x timepoint p=0.0068 replicate[rand] p=0.6864
S2B	Protein levels after Vg KD (Ovaries)	No suitable transformation; Generalized Linear Mixed Model at each timepoint	(0h) dsRNA p=0.8072 replicate p=0.4399 (24h) dsRNA p=0.0044 replicate p=0.0002 (48h) dsRNA p<0.0001 replicate p=0.0092

S2C	Amino acid levels after Vg KD (Ovaries)	Square root transformation; Linear Mixed Model at each timepoint	(0h) dsRNA p=0.5067 replicate p=0.4232 (24h) dsRNA p=0.8833 replicate p=0.4843 (48h) dsRNA p=0.0163 replicate p=0.3562
S2D	Lp expression after Vg KD#2	4 th root transformation; Linear Mixed Model followed by 3 post-hoc t-tests (Table S6)	timepoint p=0.0004 dsRNA p=0.1804 dsRNA x timepoint p=0.0409 replicate[rand] p=0.9751
S2E	Vg expression after Lp KD (Ovaries)	Square root (x+0.03) transformation; Linear Mixed Model followed by 3 post-hoc t-tests (Table S6)	timepoint p<0.0001 dsRNA p=0.0100 dsRNA x timepoint p=0.1130 replicate p=0.8073
S2E	Vg expression after Lp KD (Fatbody)	No transformation; Linear Mixed Model followed by 3 post-hoc t-tests (Table S6)	timepoint p=0.0004 dsRNA p=0.0025 dsRNA x timepoint p=0.5277 replicate p=0.6241
S3A	Protein levels after Vg KD (Fat body)	Cube root transformation; Linear Mixed Model at each timepoint	(0h) dsRNA p=0.4585 replicate p=0.3221 (24h) dsRNA p<0.0001 replicate p=0.3232 (48h) dsRNA p<0.0001 replicate p=0.3260
S3B	pS6K expression after Vg KD (Fatbody)	No transformation; Generalized Linear Model followed by 4 post-hoc t-tests (Table S6)	timepoint p<0.0001 dsRNA p=0.1251 dsRNA x timepoint p<0.0001 replicate p=0.0084
S3C	Phospho S6K protein levels upon rapamycin treatment	ANOVA	LacZ Control vs. LacZ Rapamycin p=0.9976 LacZ Control vs. Vg Control p<0.0001 LacZ Control vs. Vg Rapamycin p=0.0072 LacZ Rapamycin vs. Vg Control p<0.0001 LacZ Rapamycin vs. Vg Rapamycin p=0.0100 Vg Control vs. Vg Rapamycin p=0.0102
S3D	Lp protein levels upon	ANOVA	LacZ Control vs. LacZ Rapamycin

	rapamycin treatment		<p>p>0.9999 LacZ Control vs. Vg Control p=0.0185 LacZ Control vs. Vg Rapamycin p=0.2184 LacZ Rapamycin vs. Vg Control p=0.0172 LacZ Rapamycin vs. Vg Rapamycin p=0.2044 Vg Control vs. Vg Rapamycin p=0.4834</p>
S3E	Triglycerides upon rapamycin treatment	ANOVA	<p>LacZ Control vs. LacZ Rapamycin P=0.9860 LacZ Control vs. Vg Control p=0.0030 LacZ Control vs. Vg Rapamycin p=0.2565 LacZ Rapamycin vs. Vg Control p=0.0018 LacZ Rapamycin vs. Vg Rapamycin p=0.1618 Vg Control vs. Vg Rapamycin p=0.2566</p>
S3F	Amino acid levels after Vg KD (Fat body)	No transformation Linear Mixed Model at each timepoint	<p>(0h) dsRNA p=0.8672 replicate p=0.3832 (12h) dsRNA p=0.1087 replicate p=0.3257 (24h) dsRNA p=0.0064 replicate p=0.3201 (48h) dsRNA p=0.0027 replicate p=0.4067</p>
S3G	Amino acid levels after Vg KD (Hemolymph)	No transformation Linear Mixed Model at each timepoint	<p>(0h) dsRNA p=0.7177 replicate p=0.7615 (12h) dsRNA p=0.6672 replicate p=0.4125 (24h) dsRNA p=0.2457 replicate p=0.3536 (48h) dsRNA p=0.8222 replicate p=0.7415</p>
S3H	ILP1 expression after Vg KD (2 replicates)	arcsine transformation; Linear Mixed Model No post-hoc t-testing	<p>timepoint p<0.0001 dsRNA p=0.426</p>

	ILP2 expression after Vg KD	arcsine transformation; Linear Mixed Model No post-hoc t-testing	timepoint p=0.0089 dsRNA p=0.920
	ILP3 expression after Vg KD	No transformation; Linear Mixed Model No post-hoc t-testing	timepoint p=0.248 dsRNA p=0.367
	ILP4 expression after Vg KD	No transformation; Linear Mixed Model No post-hoc t-testing	timepoint p=0.002 dsRNA p=0.408
	ILP5 expression after Vg KD	5 th root transformation; Linear Mixed Model No post-hoc t-testing	timepoint p=0.002 dsRNA p=0.241
S4B	Embryo total lipids	Unpaired t test	p=0.1606

Supplemental Table 6. Post-hoc testing for significant differences using an FDR of 0.05. See

Supplemental Table 5.

2F Lp after Vg KD	p-value	FDR-adjusted p-value	Significant?
dsLacZ – dsVg at 0h	0.6414	0.6414	No
dsLacZ – dsVg at 12h	0.0731	0.0914	No
dsLacZ – dsVg at 24h	4.13×10^{-6}	2.07×10^{-5}	Yes
dsLacZ – dsVg at 36h	0.0020	0.0050	Yes
dsLacZ – dsVg at 48h	0.0450	0.0750	No
S1B Vg after Lp KD	p-value	FDR-adjusted p-value	Significant?
dsLacZ – dsLp at 0h	0.9198	0.9198	No
dsLacZ – dsLp at 12h	0.7704	0.9198	No
dsLacZ – dsLp at 24h	0.0029	0.0048	Yes
dsLacZ – dsLp at 36h	0.0028	0.0048	Yes
dsLacZ – dsLp at 48h	7.55×10^{-5}	3.78×10^{-4}	Yes
S1A Lp after Lp KD	p-value	FDR-adjusted p-value	Significant?
dsLacZ – dsLp at 0h	2.64×10^{-5}	6.60×10^{-5}	Yes
dsLacZ – dsLp at 12h	4.36×10^{-7}	2.18×10^{-6}	Yes
dsLacZ – dsLp at 24h	0.0138	0.0173	Yes
dsLacZ – dsLp at 36h	0.8884	0.8884	No
dsLacZ – dsLp at 48h	2.67×10^{-4}	4.45×10^{-4}	Yes
S1C (Ovaries)	p-value	FDR-adjusted p- value	Significant?
dsLacZ – dsVg at 0h	0.7766	0.7766	No
dsLacZ – dsVg at 24h	0.6119	0.7766	No
dsLacZ – dsVg at 48h	0.0009	0.0027	Yes
S1C (Fat body)	p-value	FDR-adjusted p- value	Significant?
dsLacZ – dsVg at 0h	0.7447	0.7447	No
dsLacZ – dsVg at 24h	0.1780	0.2670	No
dsLacZ – dsVg at 48h	2.86×10^{-10}	8.56×10^{-10}	Yes
S2A Vg after Vg KD	p-value	FDR-adjusted p-value	Significant?
dsLacZ – dsVg at 0h	0.9000	0.9000	No
dsLacZ – dsVg at 12h	0.0057	0.0115	Yes
dsLacZ – dsVg at 24h	2.94×10^{-5}	1.47×10^{-4}	Yes
dsLacZ – dsVg at 36h	0.0069	0.0115	Yes
dsLacZ – dsVg at 48h	0.5501	0.6877	No

S2D Lp after Vg KD#2	p-value	FDR-adjusted p-value	Significant?
dsLacZ – dsVg at 0h	0.3785	0.5678	No
dsLacZ – dsVg at 24h	0.0098	0.0294	Yes
dsLacZ – dsVg at 48h	0.8191	0.8191	No
S2E (Ovaries)	p-value	FDR-adjusted p- value	Significant?
dsLacZ – dsVg at 0h	0.9392	0.9392	No
dsLacZ – dsVg at 24h	0.0210	0.0315	Yes
dsLacZ – dsVg at 48h	0.0177	0.0315	No
S2E (Fat body)	p-value	FDR-adjusted p- value	Significant?
dsLacZ – dsVg at 0h	0.1927	0.1927	No
dsLacZ – dsVg at 24h	0.0133	0.0399	Yes
dsLacZ – dsVg at 48h	0.0298	0.0447	Yes
S3A (Fat body)	p-value	FDR-adjusted p- value	Significant?
dsLacZ – dsVg at 0h	0.9058	0.9058	No
dsLacZ – dsVg at 12h	0.1829	0.3658	No
dsLacZ – dsVg at 24h	8.06×10^{-25}	3.22×10^{-24}	Yes
dsLacZ – dsVg at 48h	0.6257	0.8343	No