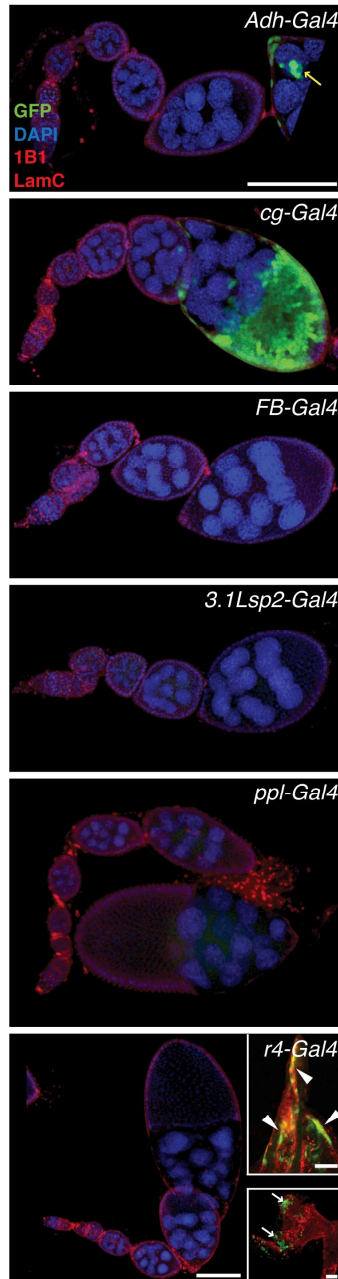


Supplemental Figure 1
Armstrong, Laws and Drummond-Barbosa

Fig. S1. In adult females, *3.1Lsp2-Gal4* is exclusively expressed in adipocytes. Expression of *UAS-GFP* (green) induced by several larval and/or adult fat body Gal4 drivers in adult female tissues shows that only *3.1Lsp2-Gal4* is exclusively expressed in adipocytes. DAPI (blue) labels nuclei in brains, guts and oenocytes; α -spectrin (red) labels cell membranes in

oenocytes (except in *ppl-Gal4*). Arrowheads indicate GFP-positive nuclei in the gut, for *ppl-Gal4*. Scale bars: 50 μm (brains), 50 μm (guts, for all except *ppl-Gal4*), 20 μm (gut, for *ppl-Gal4*), 10 μm (oenocytes), or 20 μm (adipocytes).

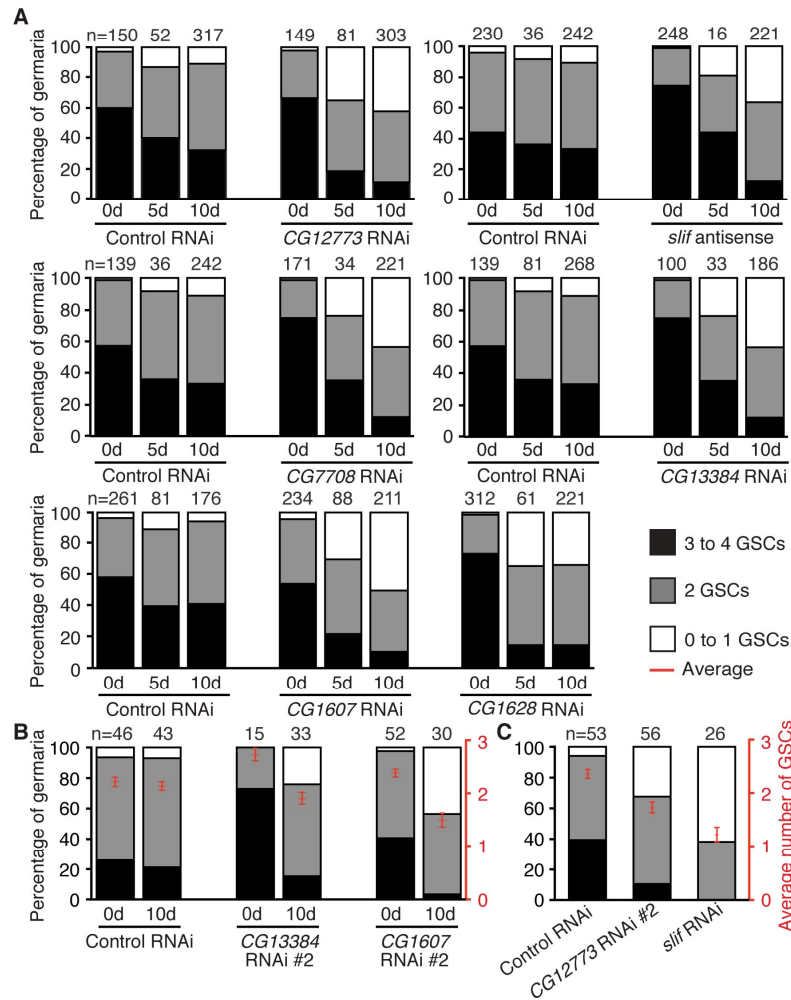


Supplemental Figure 2
Armstrong, Laws and Drummond-Barbosa

Fig. S2. *3.1Lsp2-Gal4* is not expressed in ovaries.

Analysis of *UAS-GFP* (green) induced by fat body Gal4 drivers shown in Figure 1 in adult ovaries shows that *3.1Lsp2-Gal4* has no ovarian expression. *Adh-Gal4* is expressed late follicle cells, including border cells (yellow arrow), *cg-Gal4* is expressed in stage 10 and later

follicle cells, and *r4-Gal4* is expressed in late dorsal-anterior follicle cells (arrowheads) and oviduct (white arrows). DAPI (blue) labels nuclei; 1B1 (red) labels cell membranes; LamC (red) labels nuclear envelopes of a subset of terminally differentiated cells. Scale bars: 100 μm (main panels), 50 μm (top inset), 50 μm (bottom inset).

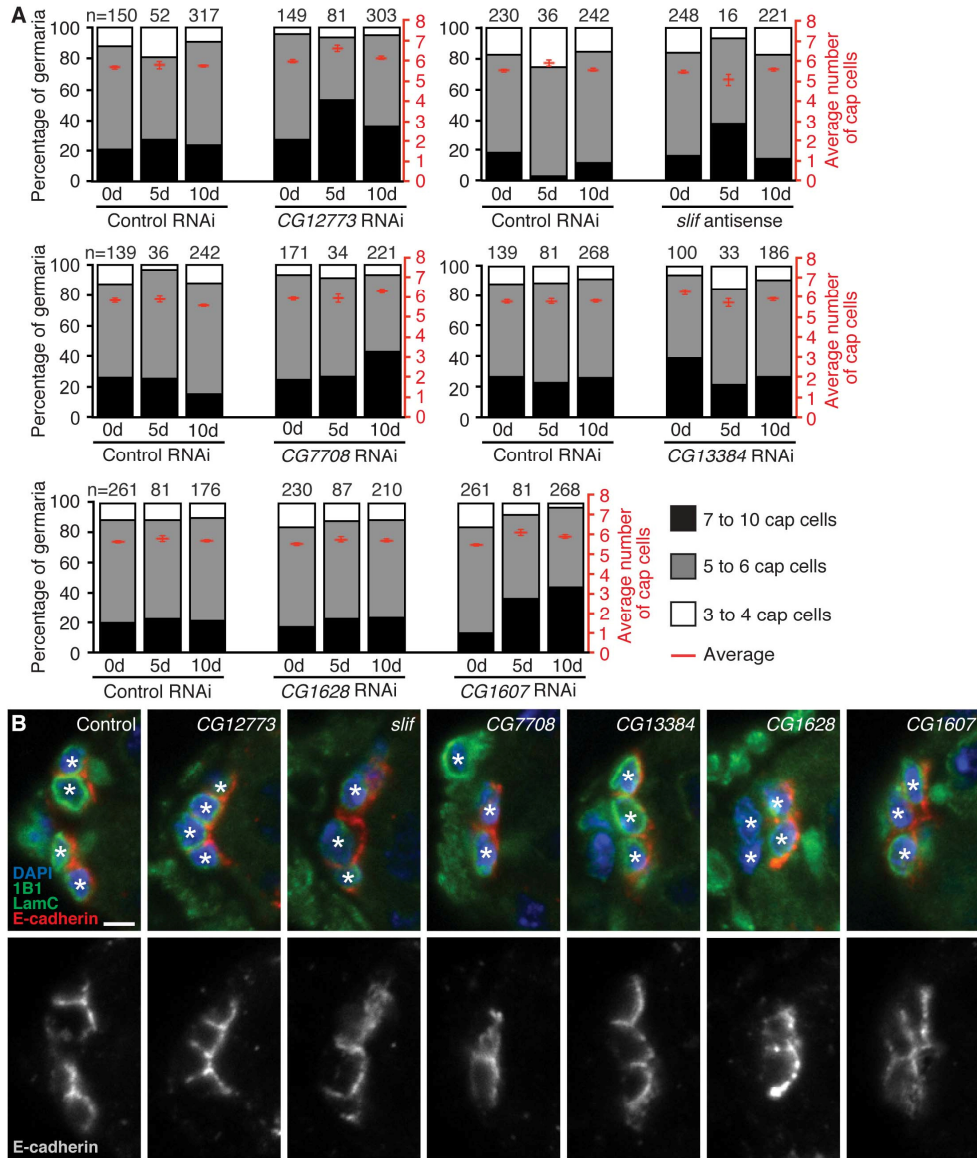


Supplemental Figure 3
Armstrong, Laws, and Drummond-Barbosa

Fig. S3. Reduced amino acid transport in adipocytes leads to higher rates of GSC loss in the ovary.

(A-C) Frequencies of germaria containing zero-or-one, two, or three-or-four GSCs at different days after switch to 29°C for *Gal80^{ts}*; *Lsp2*-mediated induction of a *UAS-slif*

antisense or *UAS-RNAi* transgenes against amino acid transporters *CG12773*, *CG7708*, *CG13384*, *CG1607*, *CG1628*, *CG12943* or *white* control. The same data used to calculate GSC number averages in Fig. 4 are plotted in (A). In (C), data at 10 days after switch to 29°C are shown. The reduction in average GSC numbers upon adipocyte inhibition of amino acid transport (Fig. 4) reflects an increased percentage of germaria showing zero-or-one GSC and decreased fraction retaining two or three-or-four GSCs. The right y-axis in (B,C) shows the average number of cap cells per germarium. Number of germaria analyzed is shown above each bar.



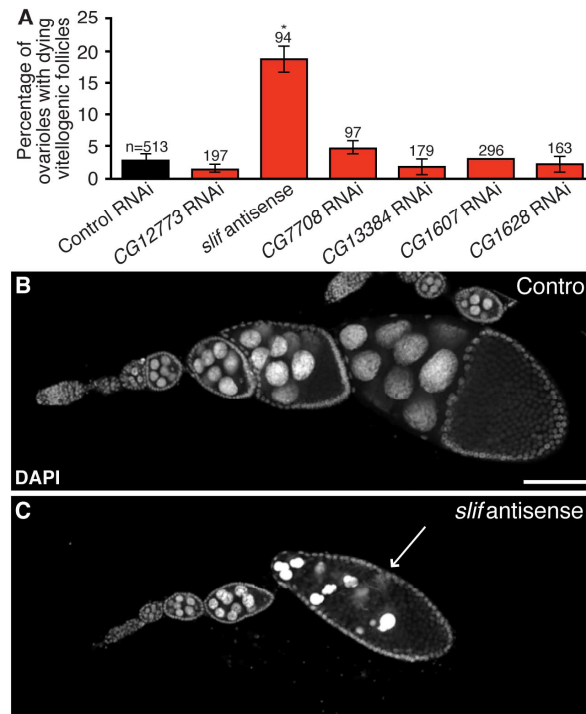
Supplemental Figure 4
Armstrong, Laws, and Drummond-Barbosa

Fig. S4. Reduced amino acid transport in adipocytes does not affect cap cell number or E-cadherin levels.

(A) Frequencies of germaria containing three-or-four, five-or-six, or seven-to-10 cap cells (left y-axis), and average number of cap cells per germarium (right y-axis) at different days

after switch to 29°C for *Gal80^{ts}*; *Lsp2*-mediated induction of a *UAS-slif antisense* or *UAS-RNAi* transgenes against amino acid transporters *CG12773*, *CG7708*, *CG13384*, *CG1607*, *CG1628*, *CG12943* or *white* control. Number of germaria analyzed is shown above each bar.

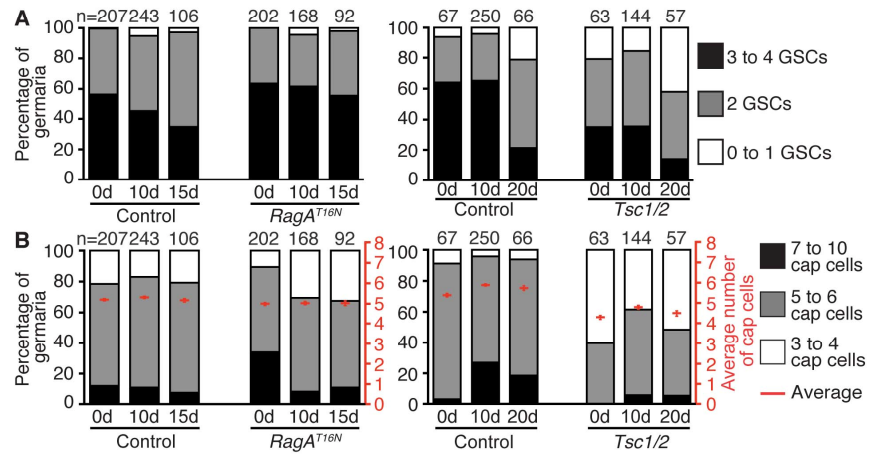
(B) Germaria from females at 10 days of adult adipocyte-specific knockdown of amino acid transporters or *white* control gene showing no obvious difference in levels of E-cadherin (red) at GSC-cap cell junctions. DAPI (blue) labels nuclei; 1B1 (green) labels fusomes; LamC (green) labels cap cell nuclear envelopes. Asterisks indicate cap cells. Scale bar, 2.5 μm.



Supplemental Figure 5
Armstrong, Laws and Drummond-Barbosa

Fig. S5. Adult adipocyte-specific knockdown of amino acid transporters does not disrupt vitellogenesis, except in the case of *slif*.

(A) Percentage of ovarioles containing dying vitellogenic follicles at 10 days of adipocyte knockdown of amino acid transporters. Number of ovarioles analyzed is shown above each bar. * $P < 0.05$, Student's t test. Error bars indicate mean \pm s.e.m. (B,C) DAPI-stained ovarioles from control (B) or *slif* (C) RNAi genotypes shown in (A). Arrow indicates degenerating follicle, recognized by the presence of pyknotic nuclei. Scale bar, 100 μm .

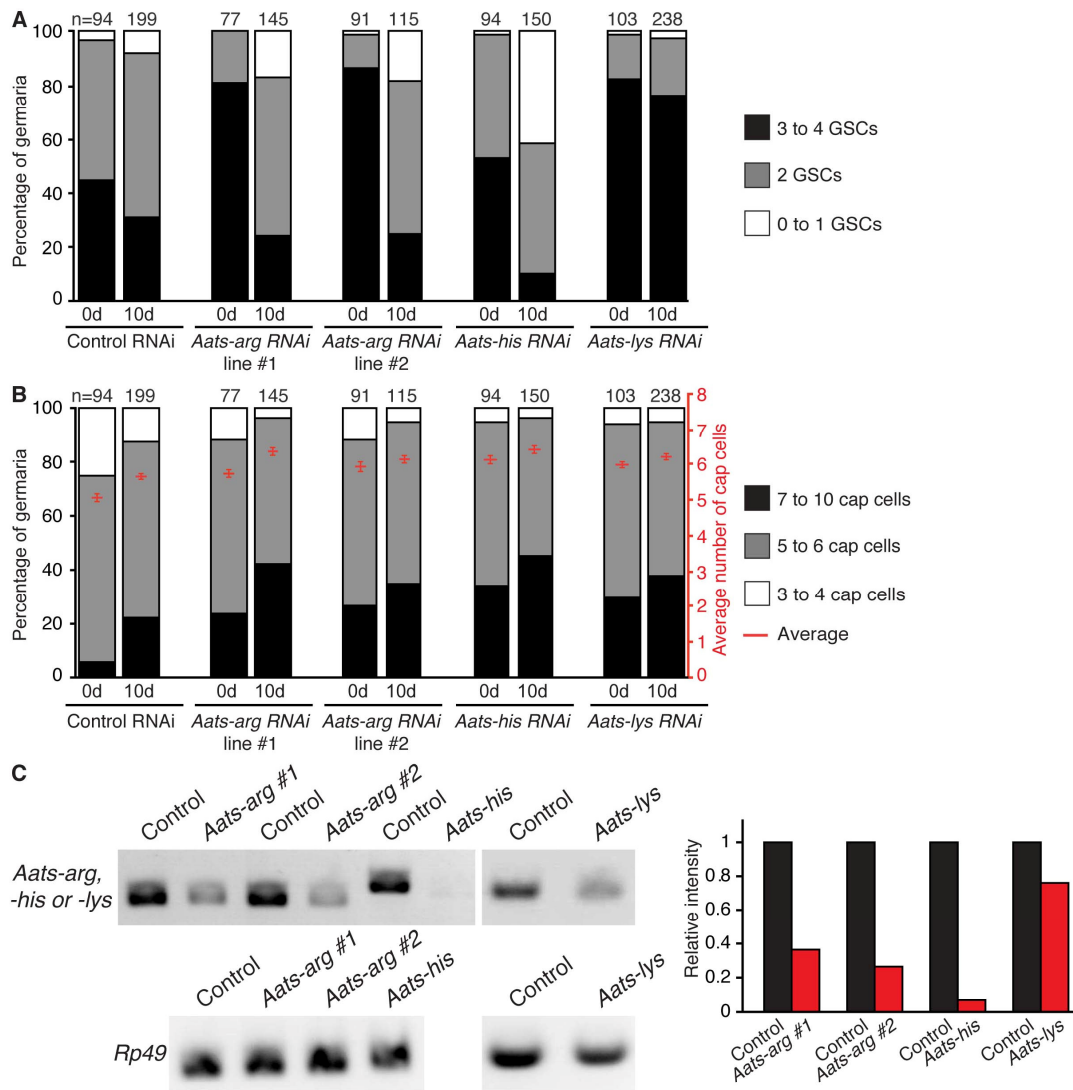


Supplemental Figure 6
Armstrong, Laws, and Drummond-Barbosa

Fig. S6. Reduced TOR signaling in adult adipocytes does not affect GSC or cap cell number.

(A,B) Frequencies of germaria containing zero-or-one, two, or three-or-four GSCs (A), or three-or-four, five-or-six, or seven-to-ten cap cells (B) at different days after switch to 29°C

for *Gal80^{ts}*; *Lsp2*-mediated induction of dominant negative *UAS-RagA^{T16N}* or *UAS-Tsc1* and *UAS-Tsc2* (*Tsc1/2*) transgenes. The same data used to calculate GSC number averages in Fig. 6A are plotted in (A). The right y-axis in (B) shows the average number of cap cells per germarium. Number of germaria analyzed is shown above each bar.



Supplemental Figure 7
Armstrong, Laws and Drummond-Barbosa

Fig. S7. Adult adipocyte-specific knockdown of aminoacyl-tRNA synthetases causes a reduction in GSC, but not cap cell, numbers.

(A,B) Frequencies of germaria containing zero-or-one, two, or three-or-four GSCs (A), or three-or-four, five-or-six, or seven-to-10 cap cells (B) at zero or 10 days after switch to 29°C

for *Gal80^{ts}*; *Lsp2*-mediated induction of *GFP* control, *Arginyl-tRNA synthetase* (*Aats-arg*), *Histidyl-tRNA synthetase* (*Aats-his*), or *Lysyl-tRNA synthetase* (*Aats-lys*) RNAi transgenes. The same data used to calculate GSC number averages in Fig. 7A are plotted in (A). The right y-axis in (B) shows the average number of cap cells per germarium. Number of germaria analyzed is shown above each bar. (C) RT-PCR analysis of hand-dissected fat bodies showing knockdown of amino acid transporters at 10 days of *Gal80^{ts}*; *Lsp2*-mediated induction of RNAi transgenes against *aminoacyl-tRNA synthetases* or *GFP* control. Note that *Aats-lys* knockdown was relatively inefficient and did not alter GSC number.

Table S1. Amino acid transporters tested in this study

AAT ^a	Type ^b	RNAi transgene #1	RNAi transgene #2 ^c	Fat body expression ^d
<i>CG1607</i> ^e	polyamine transporter	<i>P{GD4651}v14925</i>	<i>P{KK107364}VIE-260B</i>	larval/adult
<i>CG1628</i>	L-ornithine transporter	<i>P{KK108506}VIE-260B</i>	<i>P{GD8885}v47475</i>	adult
<i>CG4991</i>	n.s. ^f	<i>P{GD3406}v30263</i>	-	-
<i>CG5535</i>	cationic amino acid transporter	<i>P{KK100907}VIE-260B</i>	-	-
<i>CG7255</i>	cationic amino acid transporter	<i>P{KK110010}VIE-260B</i>	-	-
<i>CG7708</i>	proline:sodium symporter; choline transporter	<i>P{KK109385}VIE-260B</i>	<i>P{GD3648}v30302</i>	-
<i>CG7888</i>	n.s.	<i>P{GD2411}v37263</i>	-	-
<i>CG8785</i>	n.s.	<i>P{GD1961}v4650</i>	-	-
<i>CG9413</i>	polyamine transporter	<i>P{KK101306}VIE-260B</i>	-	-
<i>CG12531</i>	polyamine transporter; cationic amino acid transporter	<i>P{KK109373}VIE-260B</i>	-	-
<i>CG12773</i>	sodium:potassium:chloride symporter	<i>P{KK102472}VIE-260B</i>	<i>P{GD3189}v9899</i>	larval/adult
<i>CG12943</i>	n.s.	<i>P{KK112469}VIE-260B</i>	-	-
<i>CG13248</i>	polyamine transporter; cationic amino acid transporter	<i>P{KK103406}VIE-260B</i>	-	-
<i>CG13384</i>	n.s.	<i>P{KK102447}VIE-260B</i>	<i>P{GD1007}v44246</i>	adult
<i>CG13646</i>	n.s.	<i>P{GD257}v1571</i>	-	-
<i>CG13743</i>	n.s.	<i>P{GD3488}v40974</i>	-	-
<i>CG16700</i>	GABA:hydrogen symporter	<i>P{GD3405}v45188</i>	-	-
<i>CG17119</i>	L-cystine transporter	<i>P{GD3122}v51127</i>	-	-
<i>CG30394</i>	n.s.	<i>P{GD2127}v3470</i>	-	-
<i>CG32079</i>	n.s.	<i>P{KK107121}VIE-260B</i>	-	-
<i>dmGlut</i>	glutamate transporter	<i>P{TRiP.HMS01615}attP2</i>	-	larval
<i>kazachoc</i>	potassium:chloride symporter activity	<i>P{TRiP.HMS01058}attP2</i>	-	-
<i>minidiscs</i>	polyamine transporter; leucine import	<i>P{GD453}v42485</i>	-	adult ^g
<i>Ncc69</i>	sodium:potassium:chloride symporter	<i>P{KK108763}VIE-260B</i>	-	-
<i>pathetic</i>	n.s.	<i>P{KK104735}VIE-260B</i>	-	larval
<i>slimfast</i>	polyamine transporter; cationic amino acid transporter	<i>slif antisense</i> ^h	<i>P{GD12619}v45590</i>	larval ^h

^a AAT, amino acid transporter. The *Drosophila* genome encodes 40 predicted amino acid transporters; for 26 of them, RNAi lines were available (www.flybase.org).

^b Type of amino acid transporter according to FlyBase annotation (www.flybase.org).

^c The second set of RNAi lines target sequences that are different from those targeted by the first set (stockcenter.vdrc.at).

^d Fat body expression is listed as reported in FlyBase, except where indicated.

^e The red font indicates amino acid transporters followed up on in this study.

^f n.s., not specified.

^g Adult fat body expression of *minidiscs* reported in Martin et al., 2000.

^h Larval fat body expression of *slif* and *UAS-slif* antisense transgene described in Colombani et al., 2003.

Table S2. Primers used for RT-PCR analyses

Gene	Forward	Reverse
<i>CG1607</i>	DDB788 (5'-AGTATCGGTGTGGCTGTATTG-3')	DDB789 (5'-CTGGCAGAAGTTGTTGTGTATTT-3')
<i>CG12773</i>	DDB763 (5'-CATGTTAATGCCCGACAG-3')	DDB764 (5'-CATAGCTCTCGTCAGCGTC-3')
<i>CG13384</i>	DDB790 (5'-CTGGATCGGGAGATGATGAAAT-3')	DDB791 (5'-ACGCCACAAAGAGGAAGTAG-3')
<i>Aats-arg</i>	DDB796 (5'-CCGAACGATCTGCTATCCTAAA-3')	DDB797 (5'-TCTTAGCCAGCTCCATTCC-3')
<i>Aats-his</i>	DDB794 (5'-CCACATCGCCAAGGTCTATC-3')	DDB795 (5'-ATCGAAGCTAACTCGCTTATCC-3')
<i>Aats-lys</i>	DDB792 (5'-GGCTCCTACAAGGTCATCTATC-3')	DDB793 (5'-GGTATACGCGTTGCAAATCTC-3')
<i>Gcn2</i>	DDB811 (5'-ACACTGGCCCTAAGCCAATC-3')	DDB812 (5'-GCCTTGCTGGTGAATATGCG-3')
<i>Rp49</i>	DDB137 (5'-CAGTCGGATCGATATGCTAAGC-3')	DDB138 (5'-AATCTCCTTGCCTTCTTGG-3')