



Peer Community In Evolutionary Biology

Supporting Information

Transcriptional differences between the two host strains of Spodoptera frugiperda (Lepidoptera: Noctuidae)

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Fig. S1 - A. Fertility represented by the number of egg-masses divided by the number of females present in mating cages. Values represent the mean of fertility with the standard error for sf-C (green) and sf-R (red) according to different experimental trials: choice (in presence of corn and rice plants), no-choice (either in presence of corn only or in presence of rice only). The letters above the bars means indicated the significant differences in the mean fertility ($P < 0.05$). For sf-C (**B**) and sf-R (**C**), we counted the percentage of eggs (y-axis) that gave rise to a live larva for sf-C and sf-R in each trial. Error bars represent the variations between egg-masses. No statistical differences were observed between trials.

Fig. S2 - Fitness traits in sf-C and sf-R according to the diet: corn plant (red), rice plant (blue). Bars represent the developmental time until adult emergence for sf-C (**A**) and sf-R (**B**). The variation between replicates is represented by the standard error (except for the developmental time which are exactly the same for both strain on corn plant) and the different letters above bars indicate significant differences between plant diets for each strain ($P < 0.05$).

Fig. S3 - Survival from egg hatching for 50 individuals reared on artificial diet with low (exp. #1) or high (exp. #2) hygrometry.

Fig. S4 - Example of manual gene annotation

A. In the *S. frugiperda* genome (Gouin et al., 2017) the gene GSSPFG00032711001 is differentially expressed between sf-C and sf-R, however its function is unknown. In this WebApollo browser screenshot, the predicted gene of the official gene set (OGS2.0) is shown in green. The alignment of RNAseq reads in this region, shown in gray, reveals an intron darker gray. We used this support to correct the structure of this gene in the yellow track. **B.** The corrected sequence is now used to perform blastp annotations and reveal that this gene has in fact been identified as *polycalin* in other Lepidoptera (Mauchamp et al. 2006).

Fig. S5 - 50 most expressed genes in laboratory sf-R strain

This heatmap displays the relative gene expression of the top 50 most differentially expressed gene in sf-R across the MORT2 experimental datasets, where red is overexpressed and blue underexpressed (z-scores). The columns on the right indicate the gene identification name and its manual reannotation. Genes are ordered from most overexpressed (top) to less.

Fig. S6 - 50 most expressed genes in laboratory sf-C strain

This heatmap displays the relative gene expression of the top 50 most differentially expressed gene in sf-C across the MORT2 experimental datasets, where red is overexpressed and blue underexpressed (z-scores). The columns on the right indicate the gene identification name and its manual reannotation. Genes are ordered from most overexpressed (top) to less.

Fig. S7 - qPCR validation of RT RNAseq experiments

This figure shows two examples of strain associated gene expressions. The first one (top left: slack-LINE1) is a series of 3 LINE-type transposable elements expressed in sf-R. The IGV browser screenshot shows the RNA-Seq coverage across this region. On the right are the qPCR measurements ($\Delta\Delta Ct$ values on the y-axis) of expression associated to slack-LINE1 in three independent individual larvae of each strain, confirming its overexpression in sf-R.

At the bottom, another example is shown for the Fatty Acid Binding protein 10 (FABP-10), a member of a cluster of similar genes involved in fatty acid transport in the midgut, whose expression is associated to sf-R.

Fig. S8 - Genotyping of individual larvae using the COI diagnostic gene

A diagnostic locus of 550 bp in the mitochondrial gene Cytochrome Oxidase I (COI) (Meagher Jr. and Gallo-Meagher 2003) has been amplified by PCR. **A.** Digestion by the Mspl restriction enzyme is possible only in the sf-C strain and liberates one 500 bp fragment and a 50bp fragment. This PCR_RFLP is tested on individual L4 larvae from our laboratory colonies. All sf-C are digested, none of the sf-R. **B.** Test on 32 L4 individual larvae from the Citra sweet corn field. **C.** Test on 18 larvae from the Tifton corn field and 6 larvae from the Jacksonville pasture grass field. **D.** Proportion of diagnosed sf-C and sf-R individuals in each field.

Fig. S9 - Genotyping of individual larvae using the *tpi* gene SNP

A diagnostic locus of 800 bp in the Z-linked gene *Triose Phosphate Isomerase (Tpi)* (Nagoshi 2010) has been amplified by PCR. The PCR fragment encompasses introns 2 and 3 of the *tpi* gene. **A.** Digestion by the Avall restriction enzyme is possible only in the sf-R strain and liberates one 500 bp fragment and one 300bp fragment. This PCR-RFLP method is tested on individual L4 larvae from our laboratory colonies. All sf-R are digested, none of the sf-C. **B.** Test of the marker in select individuals from each field. The names in red indicate the putative sf-R larvae according to COI genotype. An R is noted when individuals show a proper restriction. Only one individual from Tifton (B25) is tested as sf-R with this marker. Individuals A11 and B20 show two amplified bands, indicating that they may be heterozygous for the intron length. It has been shown that intron length polymorphism exists at this gene (Nagoshi and Meagher 2016). All tested larvae from Jacksonville show the expected sf-R digestion pattern.

Fig. S10 - Genotyping of individual larvae using the FR1 repeat

The FR1 repeat is a sex-linked repeat element associated with the sf-R strain. It is present in sf-C but with less copies (Nagoshi and Meagher 2003b; Nagoshi and Meagher 2003a). **A.** In the laboratory population, some sf-R individuals show a strong multiband amplification, indicative of the presence of this repeat. These copies are supposedly on the W chromosome and as such can only be detected in males. In natural populations, only two individuals from the Tifton field show this amplification. The B25 individual, that was genotyped as sf-R with COI and *Tpi* markers, doesn't show the FR1 amplification, probably because it is a male. **B.** Low copy numbers are detected in the Jacksonville individuals, except for the B5 individual, which might be the only female.

Fig. S11 - qPCR measurement of DE genes in natural populations

Examples shown here are qPCR expression measurements ($\Delta\Delta Ct$ values on the y-axis) examples for two sf-R strain associated gene expressions: slack-LINE1 and ngf1a, a nervous system associated transcription factor. We tested the expression of these 2 genes in individual larvae from our laboratory colonies (Lab) and from the Florida collections of sf-C or sf-R genotypes. The overexpression is observed only in laboratory sf-R larvae.

Fig. S12 - A. Principal component analysis (PCA) of normalized RNA-seq reads of sf-R and sf-C individual larvae sampled in Tifton (blue) or Jacksonville fields (red). The samples cluster by collection groups. **B.** Correlogram of the FL15 RNAseq experiments showing no clear overall correlation per genotype. **C.** PCA of all RNA-seq samples from the laboratory and field conditions. The laboratory sf-R experiments cluster with field individuals while laboratory sf-C samples cluster away.

Fig. S13 - Heatmap of 50 most DE genes overexpressed in laboratory sf-R strain (same as **Fig. S5**) across all RNAseq experiments.

Each raw represents z-score normalized expression for one gene across all RT and field samples. Genes are ordered from top to bottom, from the most significant to the 50th most significant and the blue-white-red color scale indicates lower, no and higher variation of gene expression for each gene. These genes are clearly overexpressed in laboratory sf-R and underexpressed in laboratory sf-C. But no clear pattern is observable in other RNAseq experiments or from field collections.

Fig. S14 - Heatmap of 50 most DE genes overexpressed in laboratory sf-C strain (same as **Fig. S6**) across all RNAseq experiments.

Each raw represents z-score normalized expression for one gene across all RT and field samples. Genes are ordered from top to bottom, from the most significant to the 50th most significant and the blue-white-red color scale indicates lower, no and higher variation of gene expression for each gene. These genes are clearly overexpressed in laboratory sf-C but are mostly underexpressed in all other experiments.

Fig. S15 – Constitutive Sf-C associated gene expression across all RNAseq experiments.

These genes have a sf-C specific expression in laboratory experiments as well as in field collection samples. This heatmap shows the relative expression of each of these genes across all RNAseq samples analyzed (z-scores).

Fig. S16 - Annotation of COI-numt in the *S. frugiperda* genome

A. Webapollo screenshot showing the GSSPFG00006578001-RA predicted gene on scaffold-722 and RNAseq coverage underneath. In the yellow track, the part that has a sequence

homology with mitochondrial COI gene is shown in magenta. **B.** log₂ fold changes of expression of the COI-numt in all RNAseq samples showing their sf-R associated expression.

Fig. S17 - Staging of L4 larvae

- A.** Actual size chart that was used after calibration in laboratory conditions to stage *S. frugiperda* larvae. The width of the lines should correspond to the width of cephalic capsule.
B. In field collections, larvae were placed on the chart printouts so that their body follows a line. To be considered an L4 larva, the width of the head should be the same size or slightly bigger than the width of the line.

Table S1 - Sequencing and alignment statistics of RNAseq experiments

This table is presenting the number of reads processed per sample and their different alignment statistics with bowtie2 (Langmead and Salzberg 2012).

Table S2 - Comparison of RNAseq data and qPCR

This table is a list of 30 genes that are found overexpressed in sf-R compared to sf-C in the RT experiment. Last two columns on the right indicate the log₂ Fold Change observed in RNAseq experiments and the $\Delta\Delta Ct$ values obtained by qPCR. Except for peroxidase, all genes tested show a confirmed overexpression of these genes in sf-R.

Table S3 - Candidate genes primers sequences for qPCR used in **Table S2**

Table S4 – Manual annotation of the 50 genes with the most constitutive sf-R associated expression

Table S5 – Manual annotation of the 50 genes with the most constitutive sf-C associated expression

FIGURE S1

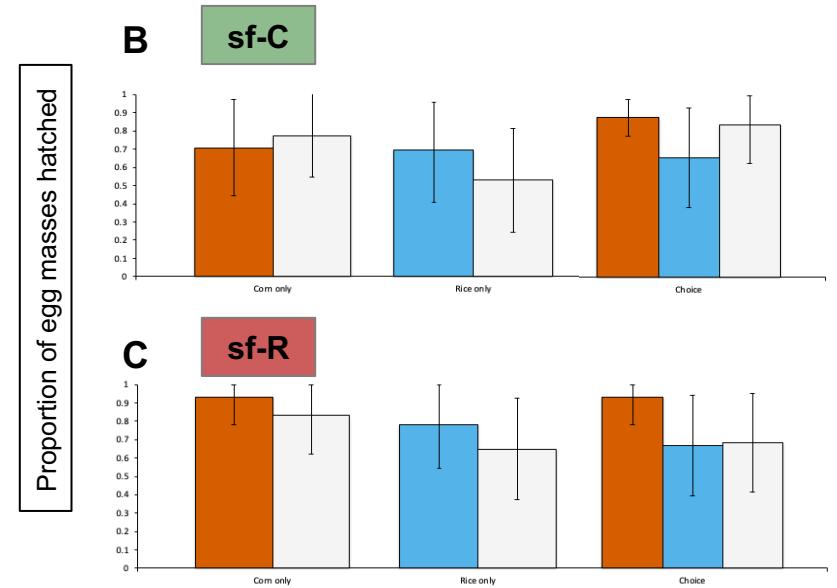
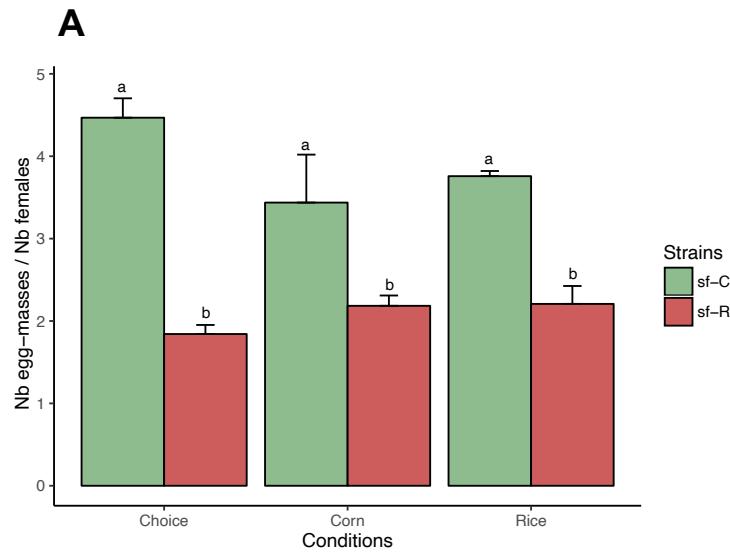


FIGURE S2

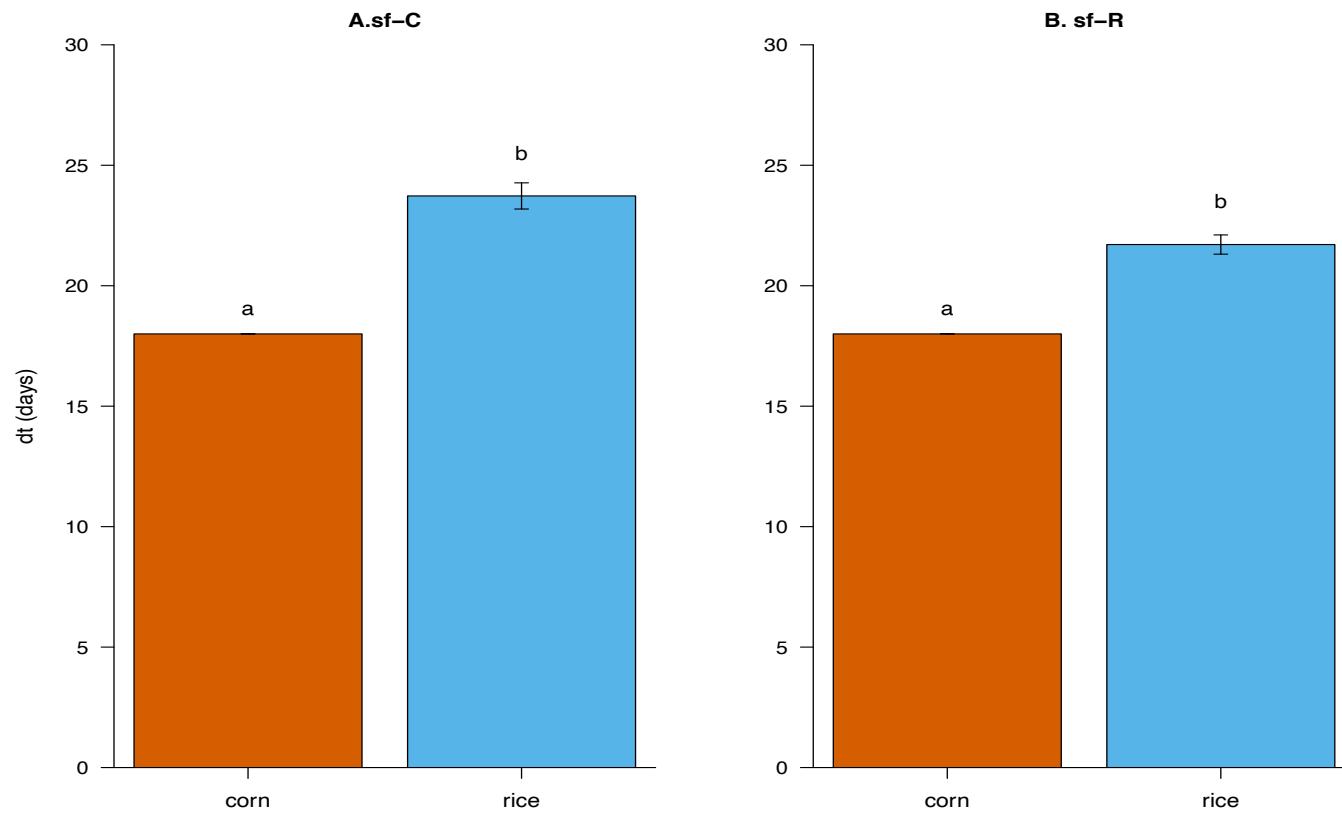


FIGURE S3

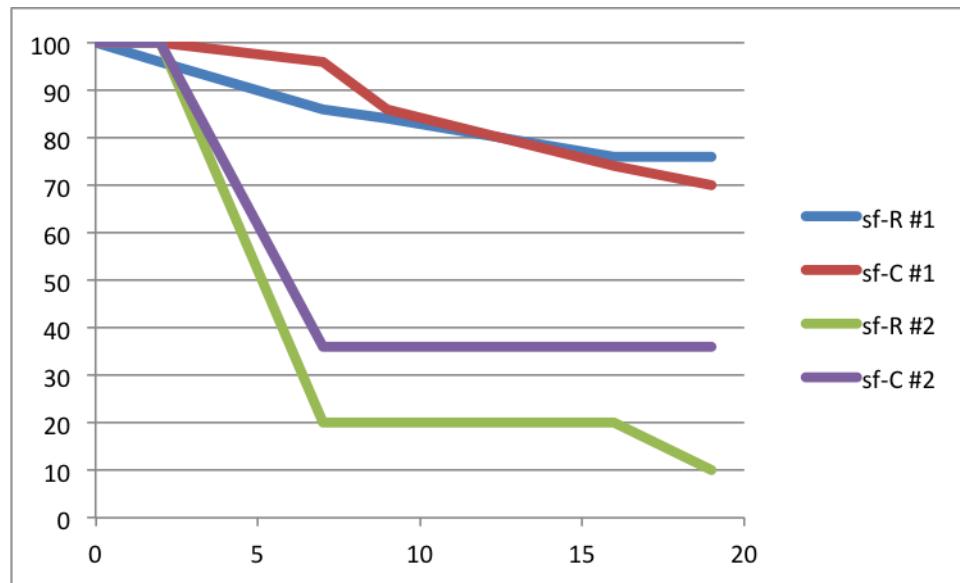
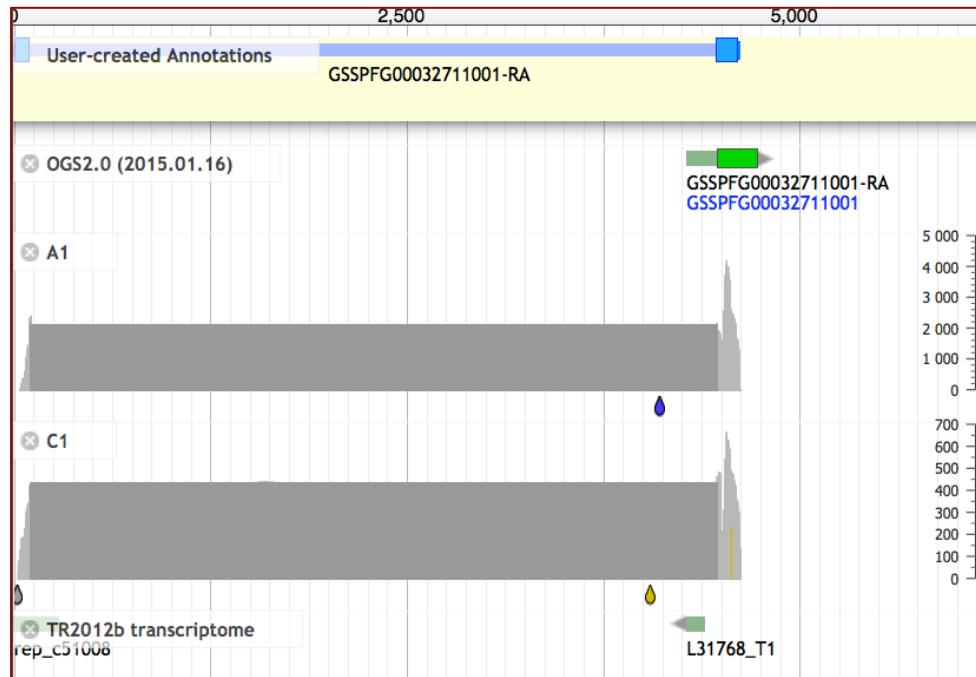


FIGURE S4

A.



B.

Sequences producing significant alignments:	(Bits)	Value
gb AEA76321.1 polycalin [Mamestra configurata]	62.8	4e-09
gb AJQ81210.1 polycalin [Helicoverpa armigera]	56.6	4e-07
ref XP_012553082.1 PREDICTED: chlorophyllide A binding prote...	57.0	4e-07
ref NP_001037071.1 chlorophyllide A binding protein precursor...	57.0	4e-07
gb AGM34046.1 chlorophyllide A binding protein precursor [Bo...	57.0	4e-07
ref XP_012553081.1 PREDICTED: chlorophyllide A binding prote...	57.0	4e-07
gb ACB54957.2 polycalin [Helicoverpa armigera]	56.2	5e-07
gb ACB54956.1 polycalin [Helicoverpa armigera]	56.2	6e-07
gb ACB54951.1 polycalin [Helicoverpa armigera]	56.2	6e-07
gb ABU98612.1 multi-domain lipocalin [Helicoverpa armigera]	55.8	7e-07

FIGURE S5

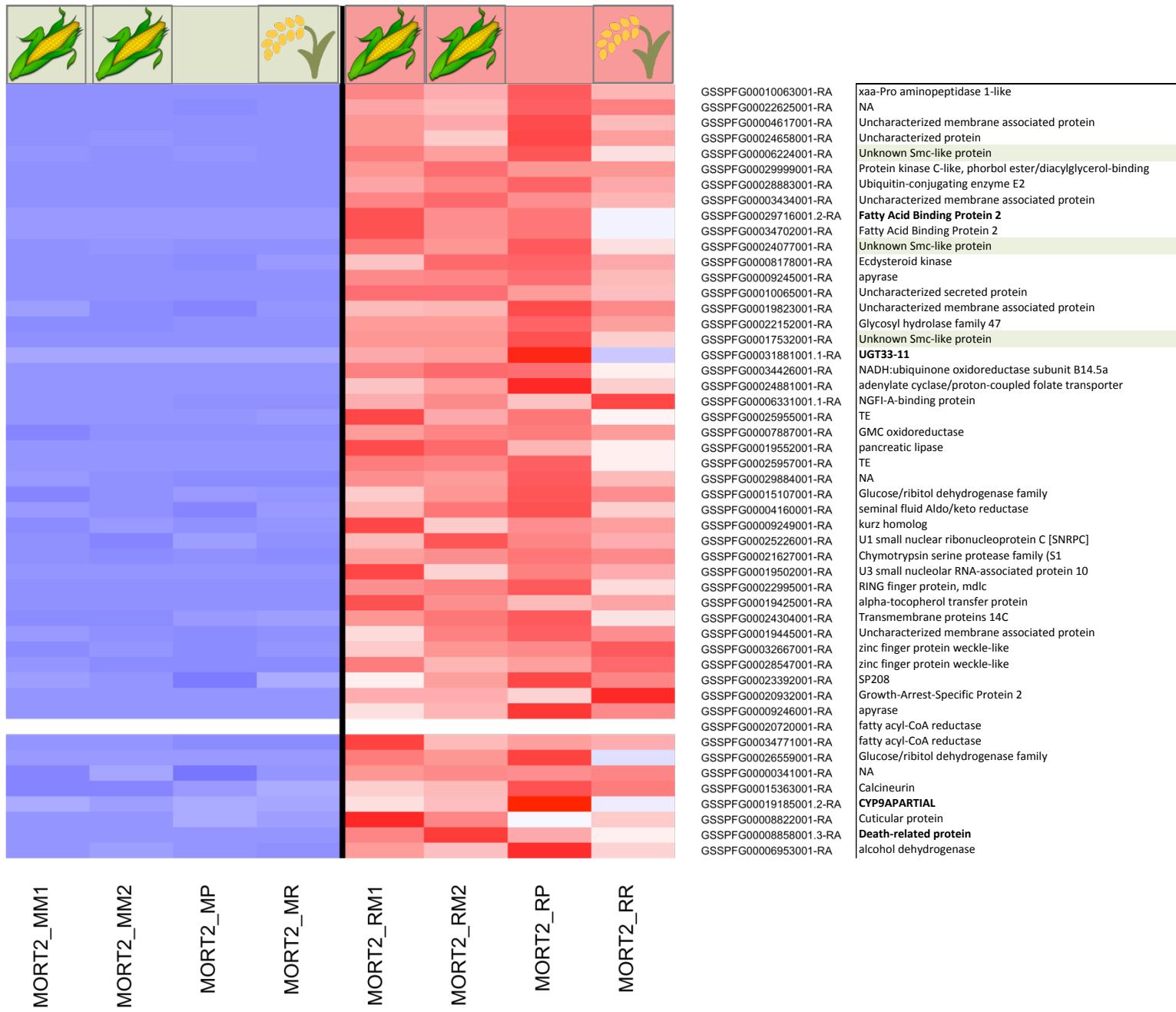


FIGURE S6

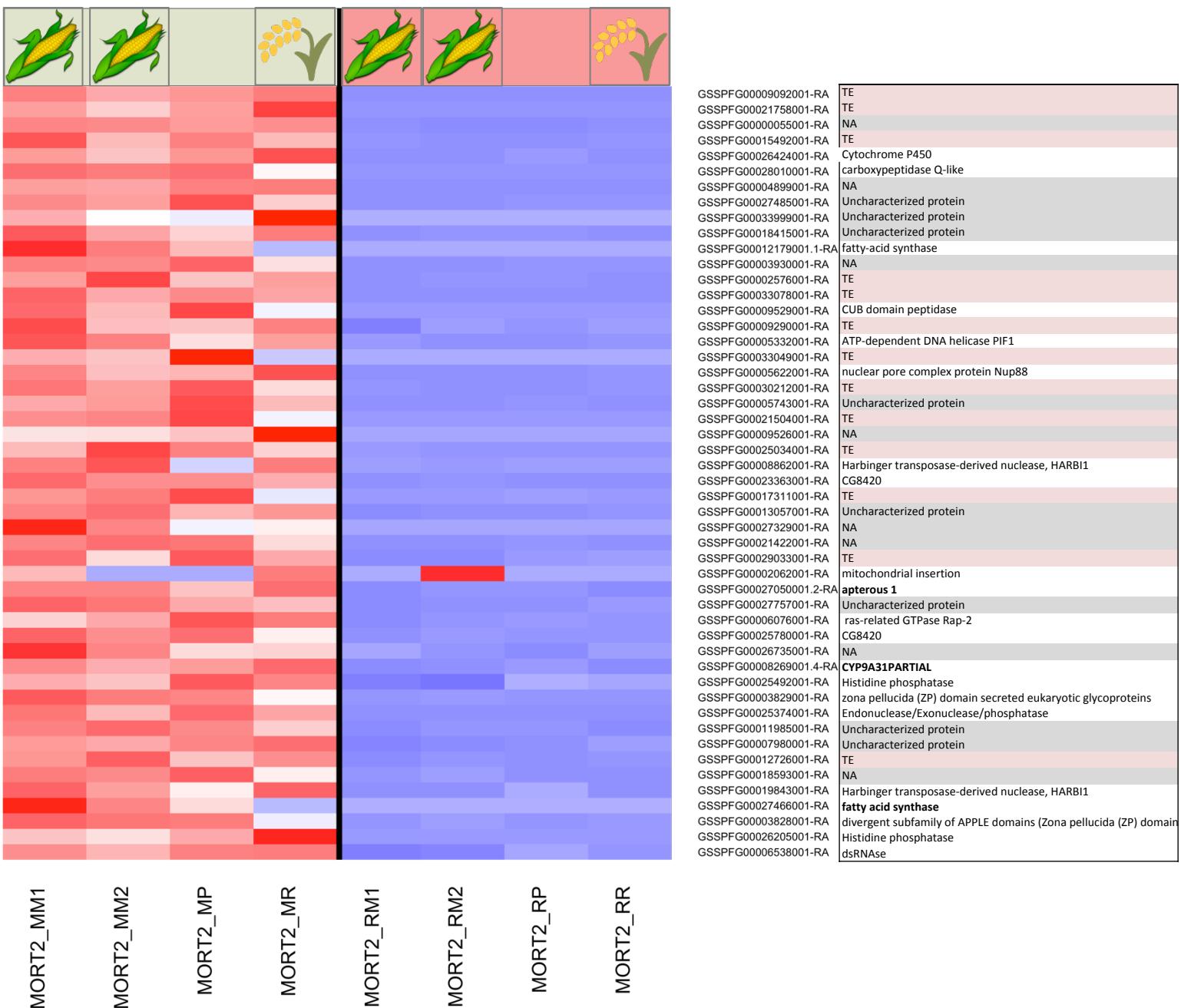


FIGURE S7

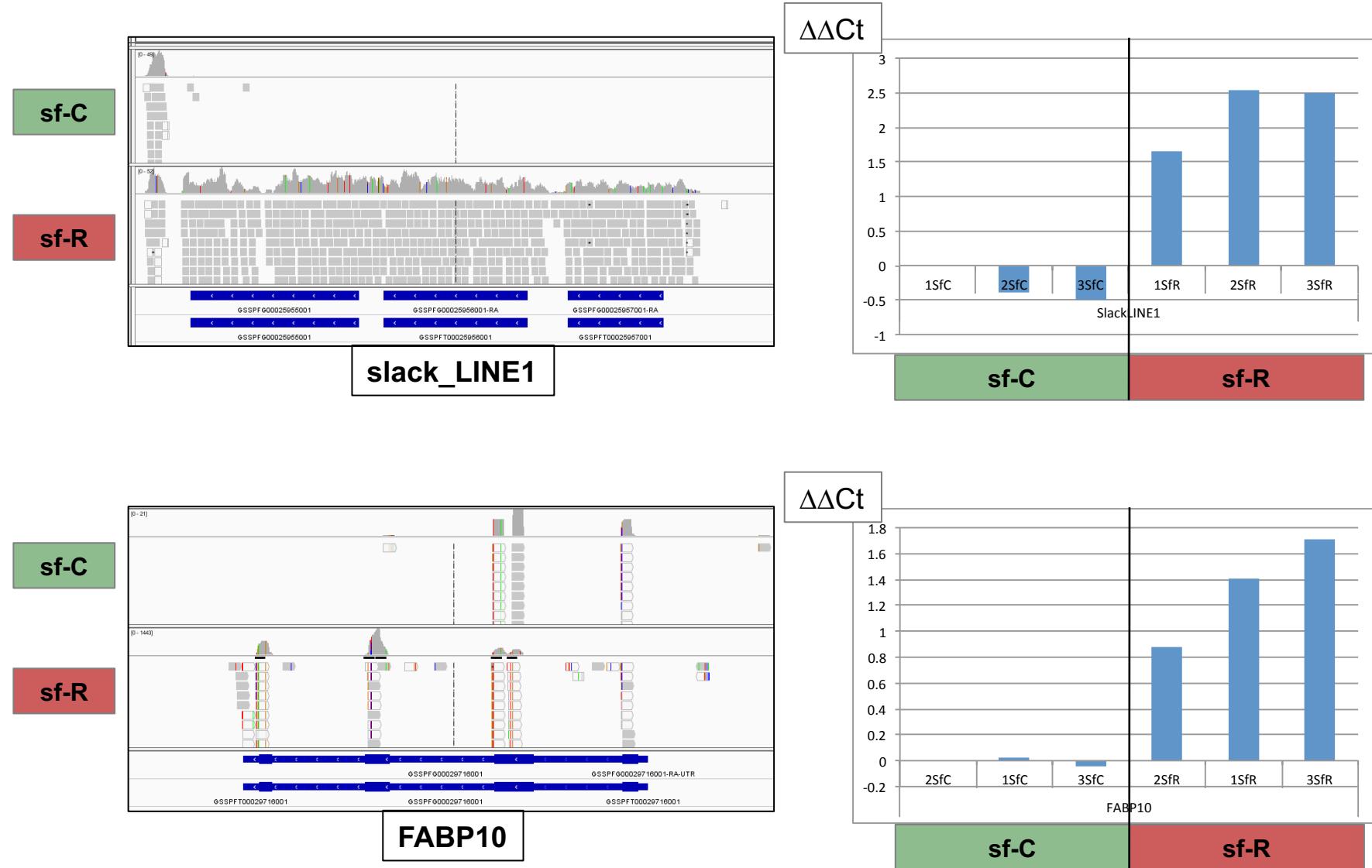
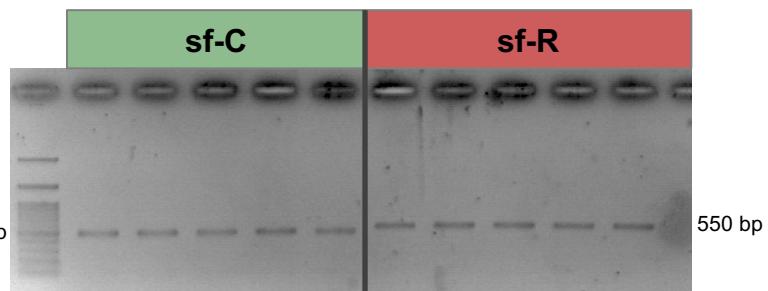


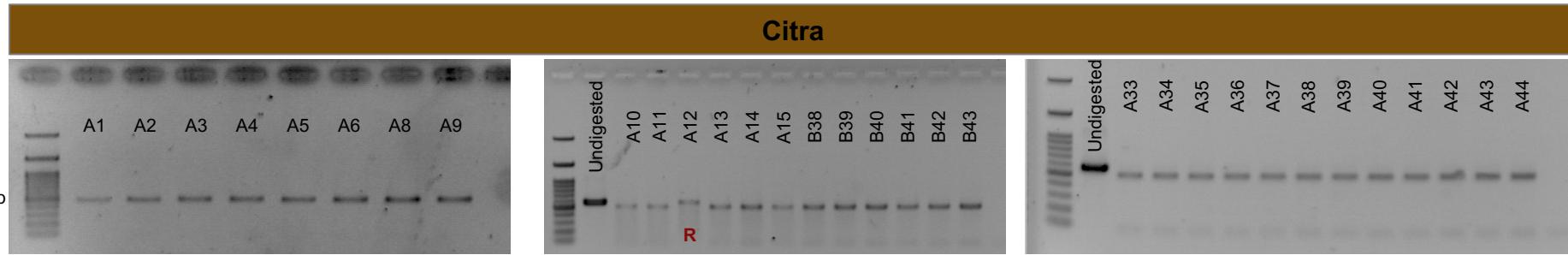
FIGURE S8

A



550 bp

B



Undigested

A10

A11

A12

A13

A14

A15

B38

B39

B40

B41

B42

B43

Undigested

A33

A34

A35

A36

A37

A38

A39

A40

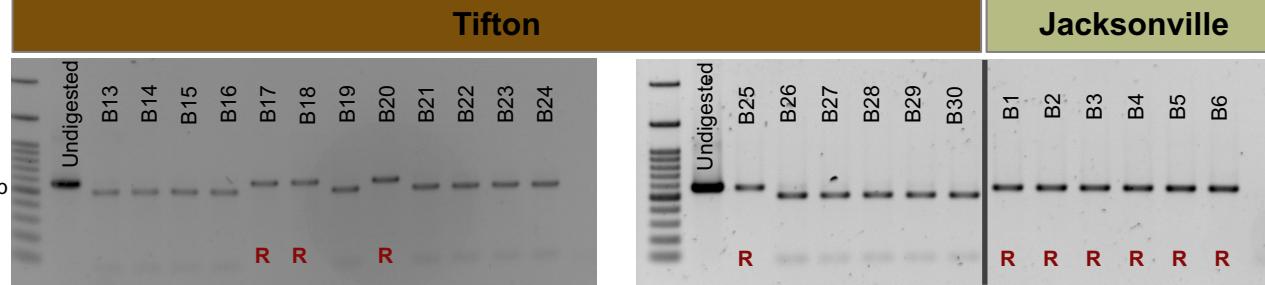
A41

A42

A43

A44

C



Undigested

B25

B26

B27

B28

B29

B30

Undigested

B1

B2

B3

B4

B5

B6

R

R

R

R

R

R

R

R

Undigested

A33

A34

A35

A36

A37

A38

A39

A40

A41

A42

A43

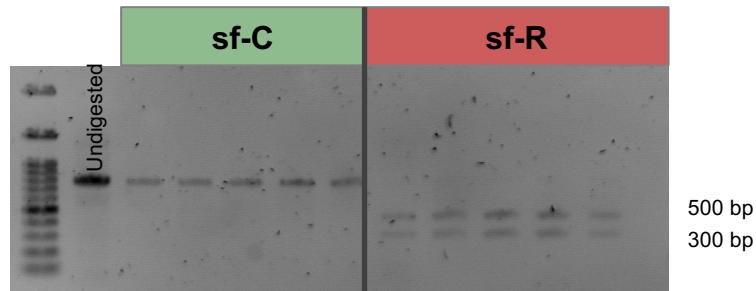
A44

D

	Citra		Tifton		Jacksonville	
	SfC	SfR	SfC	SfR	SfC	SfR
Col	31	1	14	4	0	6

FIGURE S9

A



B

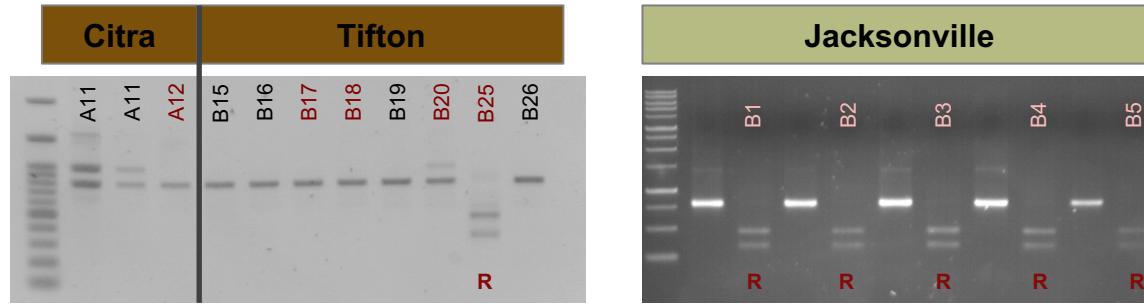
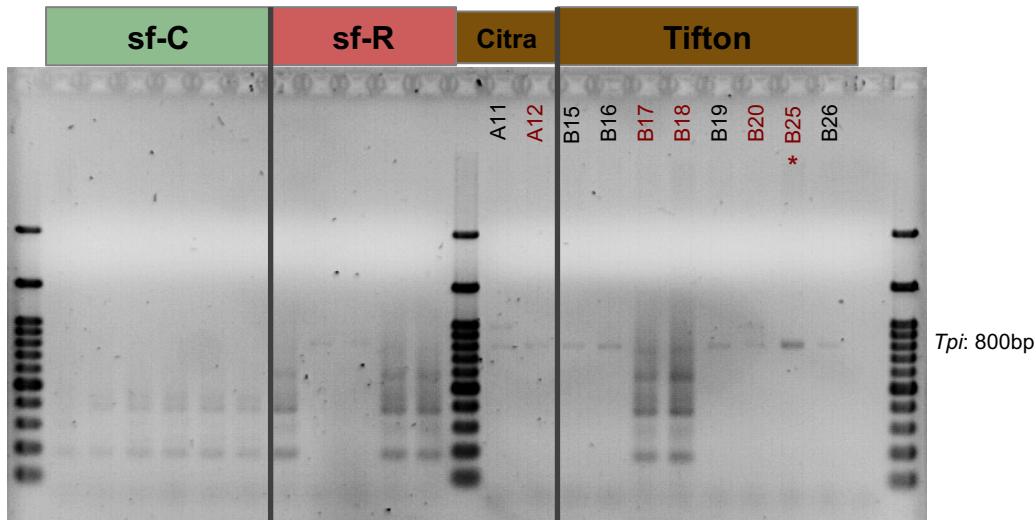


FIGURE S10

A



B

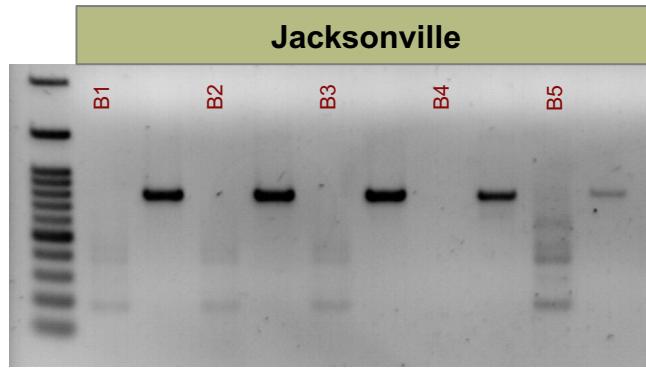


FIGURE S11

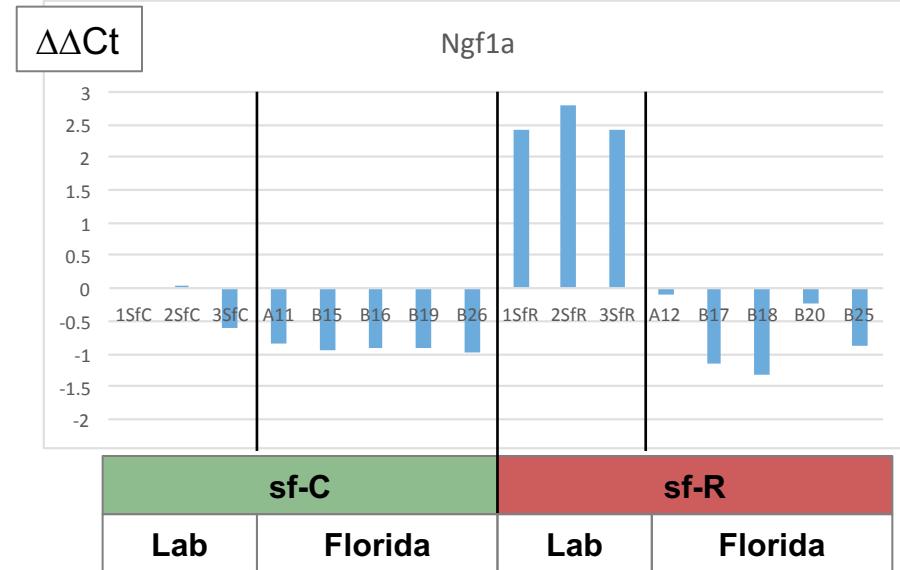
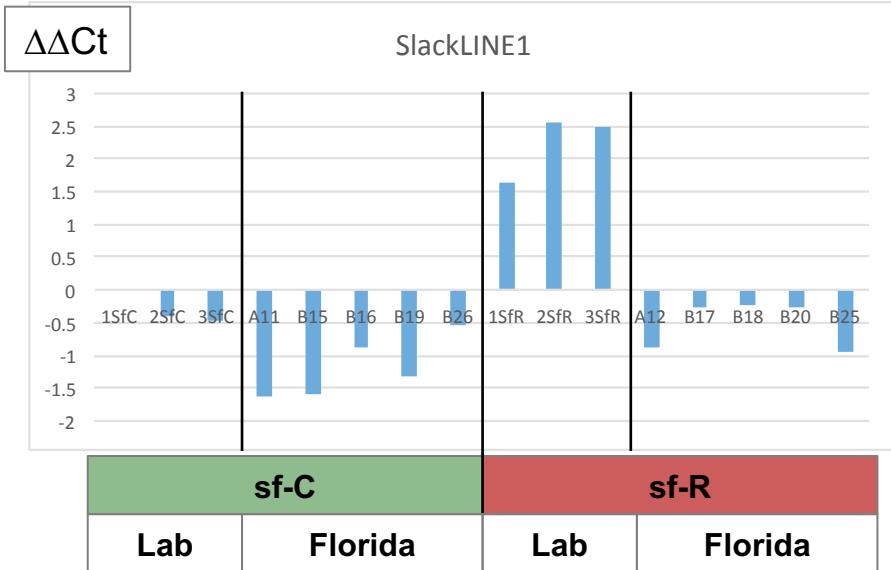
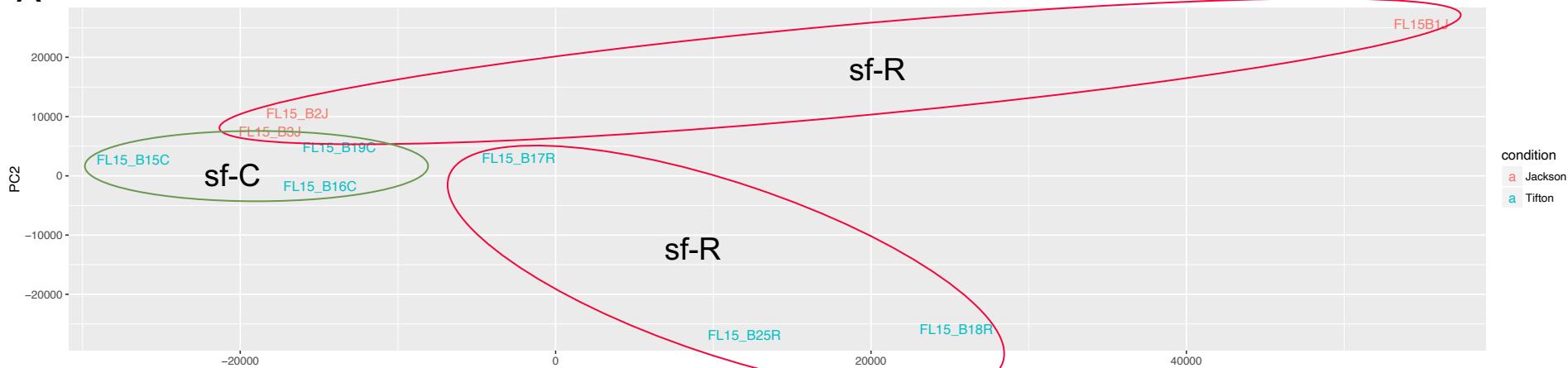
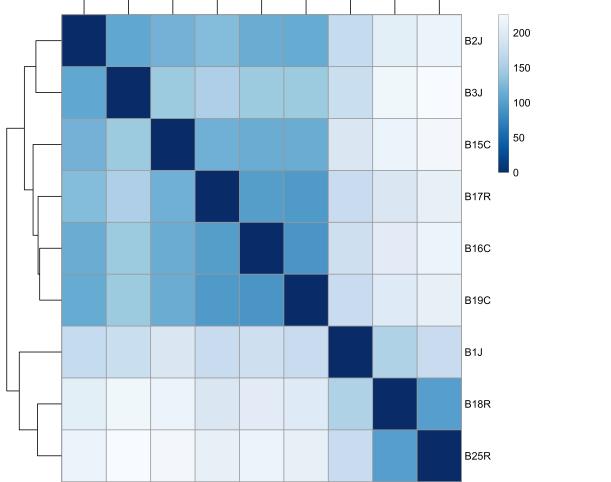


FIGURE S12

A



B



C

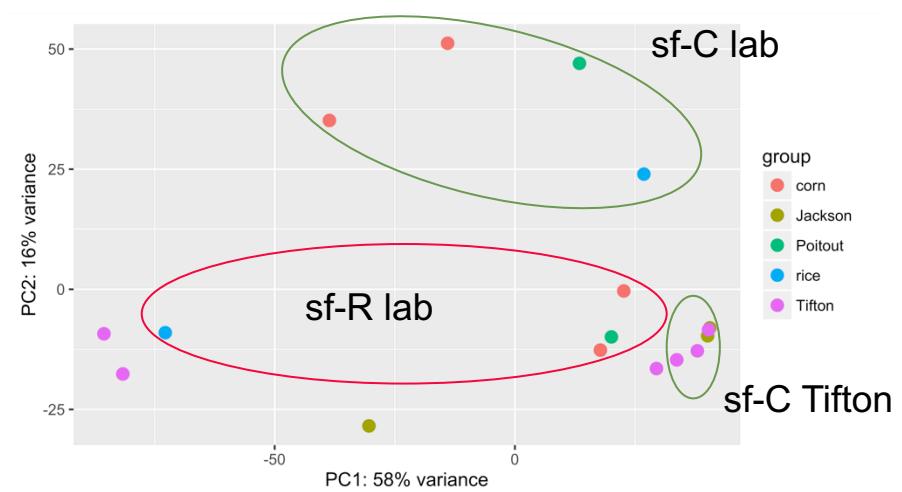


FIGURE S13

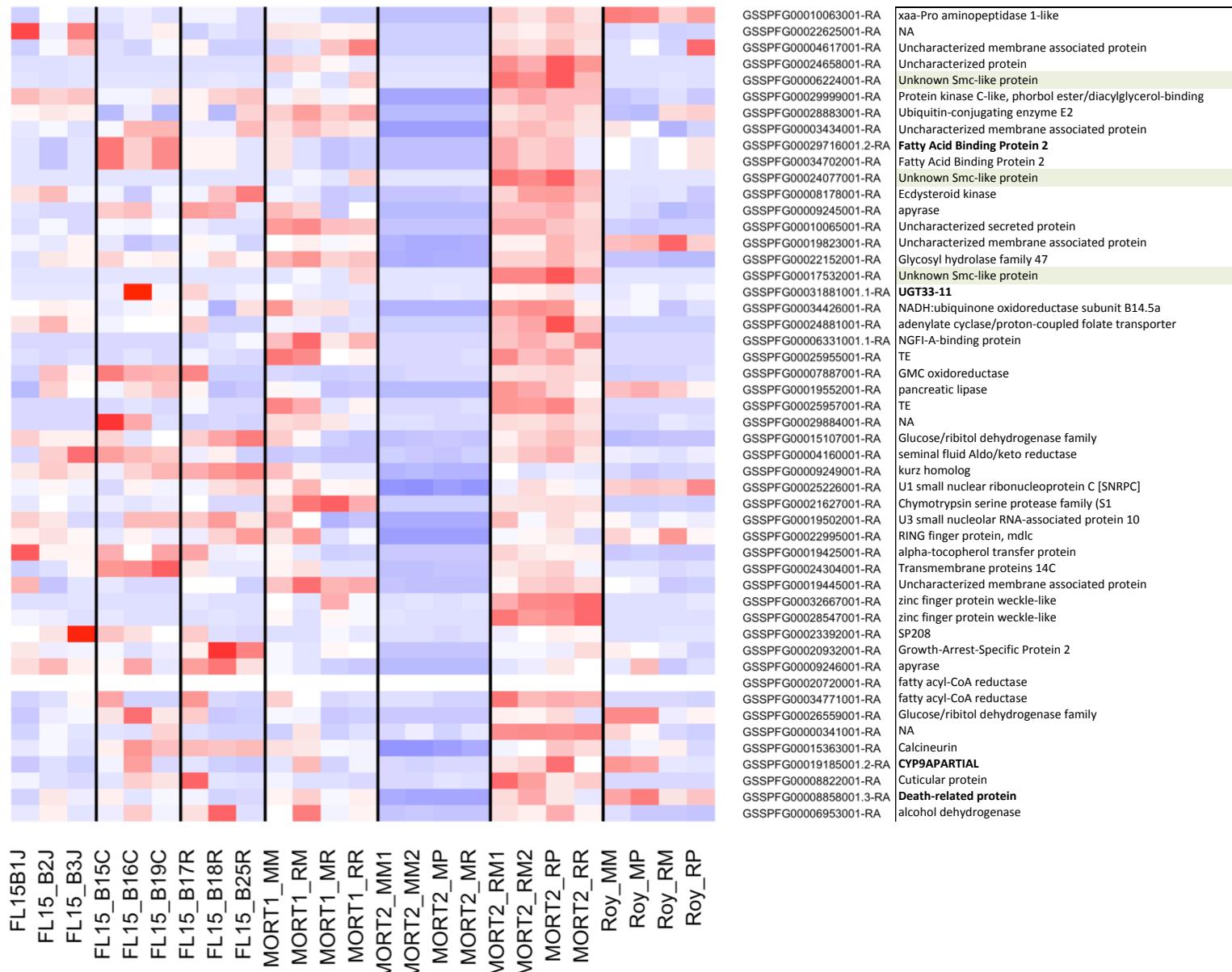


FIGURE S14

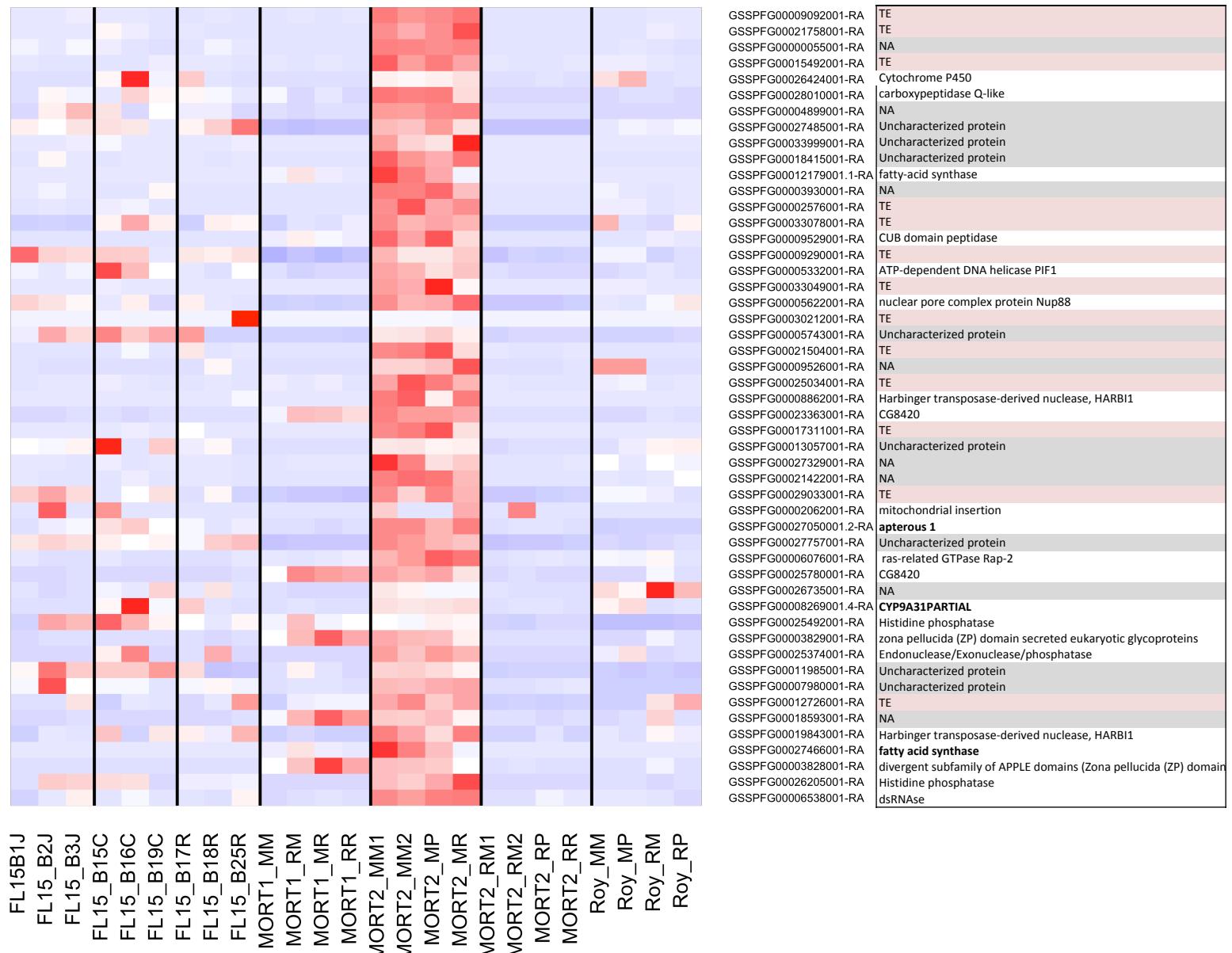


FIGURE S15

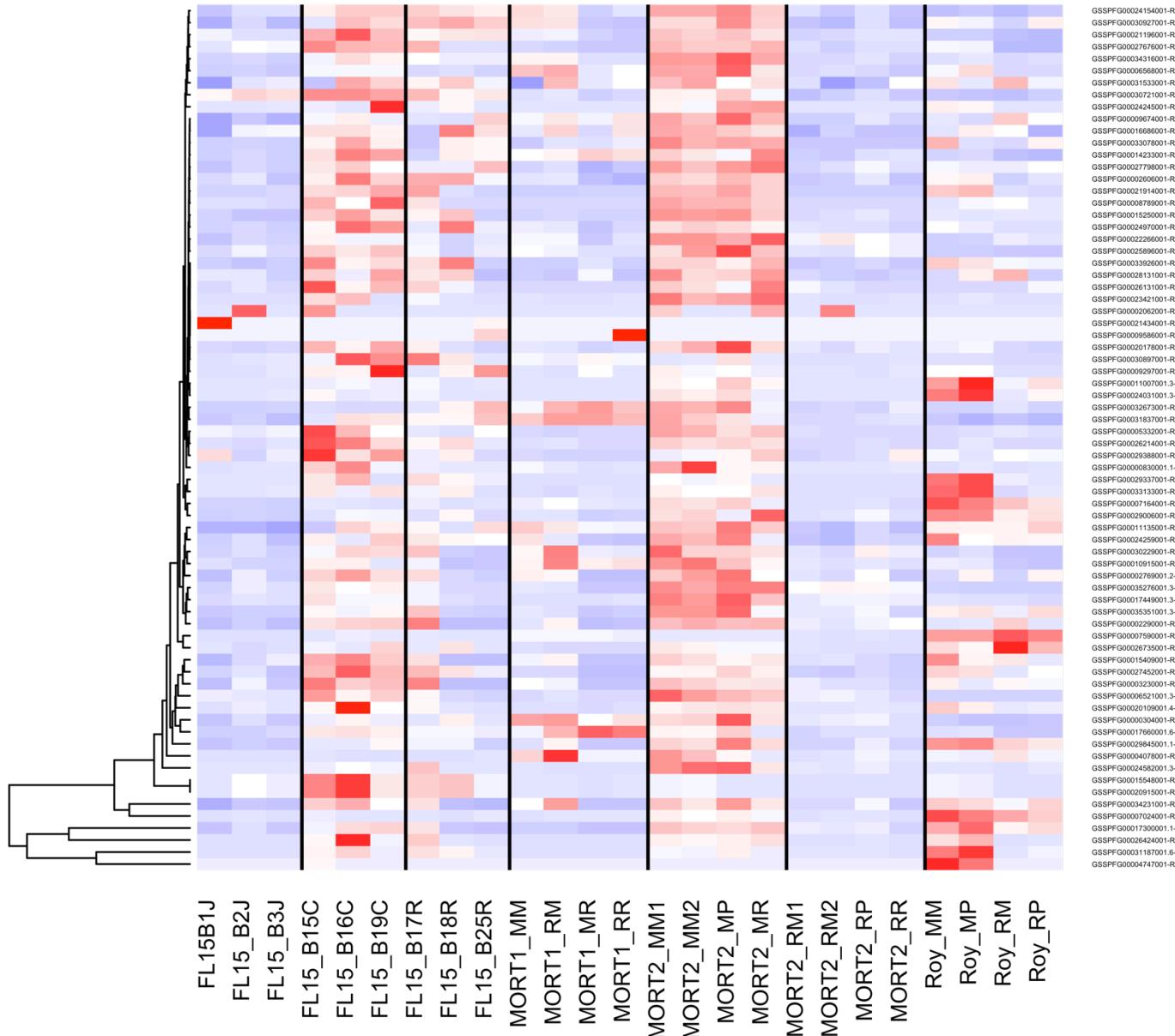
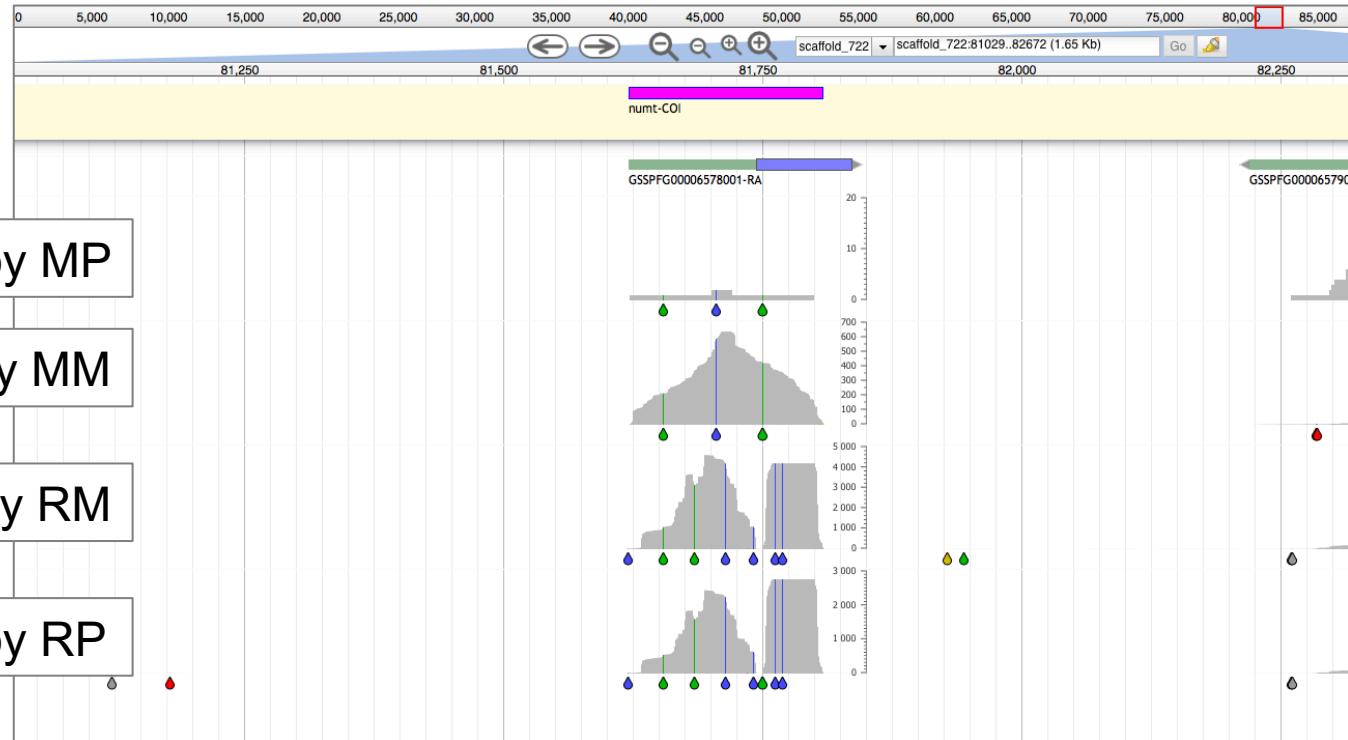


FIGURE S16

A.



B.

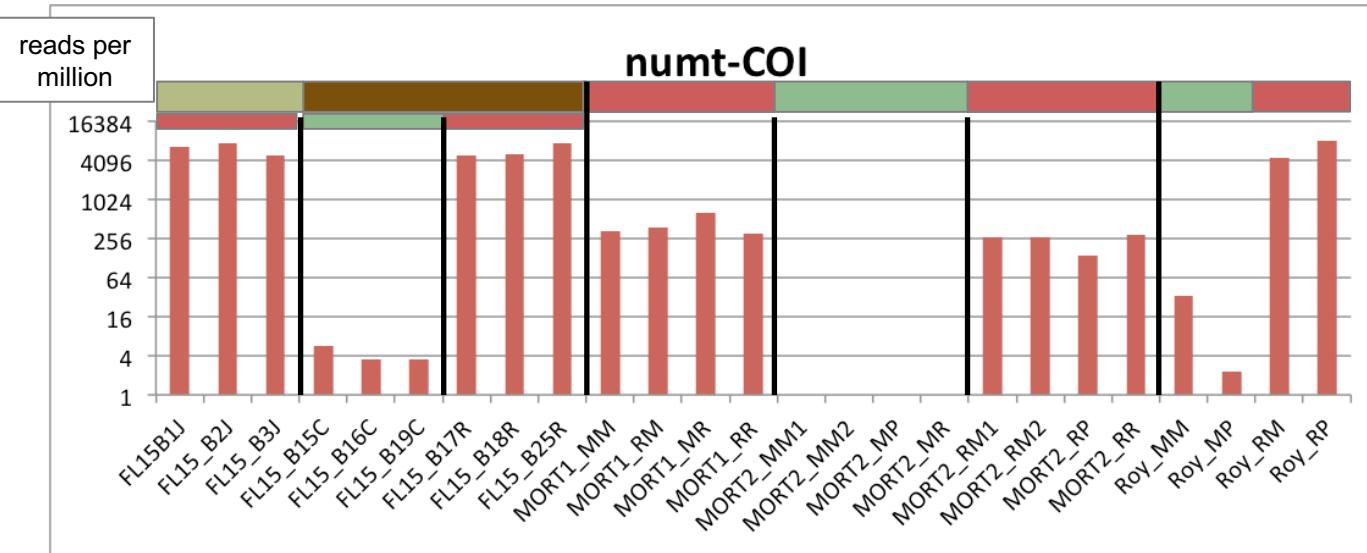
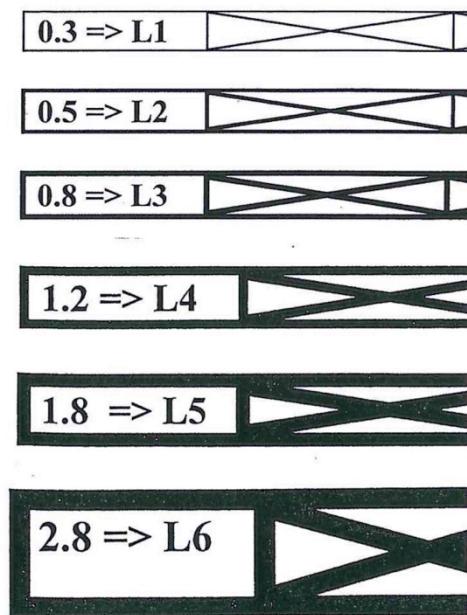


FIGURE S17

A.



B.



Table S1

sample	Strain	Diet	Total_reads	Bowtie2 alignment			
				aligned 0 times	aligned exactly 1 time	aligned > 1 times	fraction_mapped
RT experiment							
MORT2_MM1	sf-C	Corn	67150390	18011798 (26.82%)	30746676 (45.79%)	18391916 (27.39%)	73.18%
MORT2_MM2	sf-C	Corn	43617452	11210535 (25.70%)	20344243 (46.64%)	12062674 (27.66%)	74.30%
MORT2_MP	sf-C	Poitout	31801441	8475906 (26.65%)	14912937 (46.89%)	8412598 (26.45%)	73.35%
MORT2_MR	sf-C	Rice	48323710	12899202 (26.69%)	22473995 (46.51%)	12950513 (26.80%)	73.31%
MORT2_RM1	sf-R	Corn	33742585	8997746 (26.67%)	15013813 (44.50%)	9731026 (28.84%)	73.33%
MORT2_RM2	sf-R	Corn	35347649	9334932 (26.41%)	15943900 (45.11%)	10068817 (28.49%)	73.59%
MORT2_RP	sf-R	Poitout	63139685	19832315 (31.41%)	27649786 (43.79%)	15657584 (24.80%)	68.59%
MORT2_RR	sf-R	Rice	70682628	21958766 (31.07%)	32163009 (45.50%)	16560853 (23.43%)	68.93%
Second generation RT experiment							
MORT1_MM	sf-R	Corn	36304954	9214731 (25.38%)	20459886 (56.36%)	6630337 (18.26%)	74.62%
MORT1_MR	sf-R	Rice	46719601	12461844 (26.67%)	27211195 (58.24%)	7046562 (15.08%)	73.33%
MORT1_RM	sf-R	Corn	41858774	9927539 (23.72%)	24195882 (57.80%)	7735353 (18.48%)	76.28%
MORT1_RR	sf-R	Rice	37354506	8593642 (23.01%)	22722574 (60.83%)	6038290 (16.16%)	76.99%
Natural populations							
FL15_B15C	sf-C	Corn	58940405	27961728 (47.44%)	23352881 (39.62%)	7625796 (12.94%)	52.56%
FL15_B16C	sf-C	Corn	74388159	28113552 (37.79%)	37128525 (49.91%)	9146082 (12.30%)	62.21%
FL15_B19C	sf-C	Corn	33627219	12941686 (38.49%)	16468020 (48.97%)	4217513 (12.54%)	61.51%
FL15_B17R	sf-R	Corn	39842098	15290479 (38.38%)	19573496 (49.13%)	4978123 (12.49%)	61.62%
FL15_B18R	sf-R	Corn	78623719	28419568 (36.15%)	41790045 (53.15%)	8414106 (10.70%)	63.85%
FL15_B25R	sf-R	Corn	23392758	8936331 (38.20%)	12240977 (52.33%)	2215450 (9.47%)	61.80%
FL15_B2J	sf-R	Grass	33537139	12307865 (36.70%)	17130166 (51.08%)	4099108 (12.22%)	63.30%
FL15_B3J	sf-R	Grass	42191185	16293397 (38.62%)	20834698 (49.38%)	5063090 (12.00%)	61.38%
FL15_B1J	sf-R	Grass	24904583	10145929 (40.74%)	11875708 (47.68%)	2882946 (11.58%)	59.26%

Table S2

OGS2.2	Annotation	Abbreviation	log2FC	ΔΔCt
GSSPFG00029721001-RA	S01.UNA + repeat motif	501VNA	4.841451941	3.362002334
GSSPFG00024881001-RA	adenylate cyclase	adenylate cyclase	4.636752333	1.816379812
GSSPFG00010063001-RA	xaa-Pro aminopeptidase 1-like	aminopeptidase	5.128397542	2.967979508
GSSPFG00035209001.5-RA	carboxylesterase 016c	carboxylesterase	6.776645953	2.548145672
GSSPFG00004817001.2-RA	Polycalin1_other-exons	cohesin4817	7.782372143	2.582019629
GSSPFG00031119001.2-RA	CYP340L	CYP	6.046285317	6.149297371
GSSPFG00017290001.2-RC	CYP340L1	CYP340L1	5.379910588	3.556942464
GSSPFG00002985001-RA	delta-24-sterol reductase	d245reductase	6.111188925	2.522842306
GSSPFG00029999001-RA	DEF8	Def8	4.771051161	2.347034643
GSSPFG00031106001.2-RA	DUF4602; C1orf131 homolog	DUF4601	6.932457522	1.390933337
GSSPFG00002727001-RA	Lipocalin - nitrobinding domain - DUF1794 protein	DVF1794	5.584081573	12.00773469
GSSPFG00029716001.2-RA	FABP	FABP10	6.361577058	1.334187653
GSSPFG00034702001-RA	FABP	FABP12	6.605986931	1.368224598
GSSPFG00020720001-RA	FAR	FAR-X	5.826344504	1.674146
GSSPFG00018006001-RA	Glycogen synthase	glyc synt	5.635375812	2.062591316
GSSPFG00024097001-RA	Hemicentin 2	hemicentin2	5.658948506	3.155072607
GSSPFG00006331001.1-RA	NGFI-A-binding protein	Ngf1a	5.296521038	2.729999007
GSSPFG00008932001-RA	intraflagellar transport protein 52 homolog isoform X2	p52	5.109329722	2.97790438
GSSPFG00022903001-RA	Peroxidase	peroxydase	5.70457573	-0.42053074
GSSPFG00020440001-RA	Polycalin1	polycalin	5.257370575	1.437914219
GSSPFG00035966001.2-RB	Polycalin1	polycalin1p3	7.373488645	1.753858172
GSSPFG00002897001-RA	putative inorganic phosphate cotransporter	Ptransporter	5.338207499	0.85946203
GSSPFG00014224001-RA	Rpb8	rpb8	5.334190603	1.498294088
GSSPFG00019426001-RA	phosphatidylinositol transfer protein (Sec14p)	Sec14P	5.052587086	0.438937448
GSSPFG00025955001-RA	Slack-LINE1	SlackLINE1	7.55220001	2.518786492
GSSPFG00025956001-RA	Slack-LINE2	SlackLINE2	8.408523346	3.316992707
GSSPFG00017532001-RA	putative cohesin	smc2	7.417473777	9.842471525
GSSPFG00004617001-RA	UGT33-11	UGT3311	8.168349428	4.136506264
GSSPFG00035441001.3-RA	UDP-glycosyltransferase-33-23	UGT3323	5.473349608	2.391054548
GSSPFG00031881001.1-RA	UDP-glycosyltransferase 33J2	UGT33J2	8.291597518	3.633597123

Table S3

OGS2.2	Abbreviation	Primer Orientation	Primer Seq	Primer Orientation	Primer Seq	Prod Size
GSSPFG00029721001-RA	501VNA	FORWARD	CCAAGGAAC TGATGGATTGG	REVERSE	GGGATCATGACAGAGGACACA	56
GSSPFG00024881001-RA	adenylate cyclase	FORWARD	CACGGTGGACACACTACCAG	REVERSE	TCATAACCCCTCCCAGCATA	50
GSSPFG00010063001-RA	aminopeptidase	FORWARD	ACTGGACGCAATTGAGGAG	REVERSE	GCTTCATCAGCTTCCAGAGG	54
GSSPFG00035209001.5-RA	carboxylesterase	FORWARD	TTGTGATA CCTGGCGATGAA	REVERSE	GGGGGTGTAGACATTGAGGA	50
GSSPFG00004817001.2-RA	cohesin4817	FORWARD	CGGGTGTCCCTGGAGAATT	REVERSE	TCGACTGTGCATCATTGGAT	51
GSSPFG00031119001.2-RA	CYP	FORWARD	GGGGTTTGATCGCTCATCTA	REVERSE	CGTCAAATGGCTTTACCC	51
GSSPFG00017290001.2-RC	CYP340L1	FORWARD	TTAACCGGAGCGATGGTTA	REVERSE	GCATTGGGTTTCTGGTAA	52
GSSPFG0002985001-RA	d245reductase	FORWARD	ATCATCGTATGGTGGCTCT	REVERSE	CCAGATCTTCAAACCAAGG	51
GSSPFG00029999001-RA	Def8	FORWARD	GTGCCAAACCGCATTAACTT	REVERSE	ATAATCGGGTTTCATTCCAC	50
GSSPFG00031106001.2-RA	DUF4601	FORWARD	GTGGGAATGTCGGGTTTG	REVERSE	CTATCCGCGCTTCTTCTTC	50
GSSPFG0002727001-RA	DVF1794	FORWARD	ATCAAACCTGGAACGAACGA	REVERSE	GCCCATGTTATGACTGACGA	51
GSSPFG00029716001.2-RA	FABP10	FORWARD	GTGTCCCCGATGACAAGATT	REVERSE	TCTGGTCTGGGTGTAGCTC	51
GSSPFG00034702001-RA	FABP12	FORWARD	GTGTCCCCGATGACAAGATT	REVERSE	TCTGGTCTGGGTGTAGCTC	51
GSSPFG00020720001-RA	FAR-X	FORWARD	CGGAGCTACCGTATTCTGA	REVERSE	TGAGCTGCTCCCAAGAAAT	53
GSSPFG00018006001-RA	glyc synt	FORWARD	GCTCCGACATGACAGTGGTA	REVERSE	TATTGCTTGGCAGGGAAG	51
GSSPFG00024097001-RA	hemicentin2	FORWARD	TGTGGTGCTGAAGAACACCT	REVERSE	TGGGCCATATTCCTATCA	50
GSSPFG00006331001.1-RA	Ngf1a	FORWARD	TTAATAACCCCGCCCTTTC	REVERSE	CAGTGGGCAGAGGTTAGGA	54
GSSPFG00008932001-RA	p52	FORWARD	ATCCAAAAGAATGCCACGTC	REVERSE	GGTGACGGCTGGTTAGTA	50
GSSPFG00022903001-RA	peroxydase	FORWARD	TAGCGCAATCTGGTATGAG	REVERSE	GGTTGAGACGGACGGTTCTA	51
GSSPFG00020440001-RA	polycalin	FORWARD	GGGCCAAACGATTGTTCTA	REVERSE	TATTGCCATGTCGGATCAAA	50
GSSPFG00035966001.2-RB	polycalin1p3	FORWARD	TGGTGGTGGCATCTCAGTAA	REVERSE	CGTTGCAAGTCTTGGTTCA	55
GSSPFG00002897001-RA	Ptransporter	FORWARD	TCCAATTCTACTGAAGCCAGAG	REVERSE	TTACATCCTCAGCTCTTCTACG	52
GSSPFG00014224001-RA	rbp8	FORWARD	AATGGCCGGTATTATTCTG	REVERSE	CCGGGTCAATATCTTCACG	53
GSSPFG00019426001-RA	Sec14P	FORWARD	ACCGCTTCCAAATTCTAT	REVERSE	TCCTAACGTCAAACAGCTGAA	51
GSSPFG00025955001-RA	SlackLINE1	FORWARD	GGAGAAGGGTGGCAAAGAT	REVERSE	GGCCTCCTCTAACGACTTCC	50
GSSPFG00025956001-RA	SlackLINE2	FORWARD	CCCCAACAGAGAAAGATCCA	REVERSE	TTGTGCATAGAATGGCCTG	50
GSSPFG00017532001-RA	smc2	FORWARD	CCATGGCCAATGGTATTAGG	REVERSE	CATCACCTGTTCTCGACA	53
GSSPFG00004617001-RA	UGT3311	FORWARD	GGTGGCAAAATGGGATT	REVERSE	CACGAGTCCAACCAAACAA	57
GSSPFG00035441001.3-RA	UGT3323	FORWARD	CAGTCCTTGGTGGAGCTT	REVERSE	CTGAAGCGCCAATATTCTCA	50
GSSPFG00031881001.1-RA	UGT33J2	FORWARD	CTCTGGAAGTGGACAAGGA	REVERSE	TCTGATGTTGCTGATTGTC	51

Table S4 – Manual annotation of the 50 genes with the most constitutive sf-R associated expression

OGS2.2	baseMean	log2FoldChange	padj	scaffold	start	end	strand	Annotation	Best Homology	InterPro
GSSPFG00012499001-RA	41.438	5.356	9.1E-17	scaffold_24562	2044	2184	PLUS	Partial peptidase S1A, chymotrypsin family	>XP_022827099.1 uncharacterized protein LOC111356844 [Spodoptera litura]	IPR009003 Peptidase S1, PA clan
GSSPFG00017312001-RA	72.309	2.778	1.9E-11	scaffold_5799	9191	11743	PLUS TE		g 1573721284 ref XP_028042951.1 (piggyBac transposable element-derived protein 4-like isoform X2 [Bombbyx mori])	PiggyBac transposable element-derived protein
GSSPFG00006331001.1-RA	38.630	4.966	3.7E-09	superscaffold_207	307369	312517	PLUS	NGFI-A-binding protein	g 1199392082 ref XP_021191057.1 (NGFI-A-binding protein homolog [Helicoverpa armigera])	NGFI-A BINDING PROTEIN
GSSPFG00033823001-RA	102.169	1.554	2.5E-08	scaffold_665	62519	63360	PLUS NA		/	/
GSSPFG00033815001.4-RA	1204.896	2.556	3.6E-07	scaffold_665	1731	7761	PLUS	PGRP	g 1274144291 ref XP_022832520.1 (peptidoglycan recognition protein-like isoform X1 [Spodoptera litura])	Peptidoglycan recognition protein
GSSPFG00006224001-RA	277.127	3.794	4.2E-07	scaffold_8364	313	2849	PLUS	Unknown Smc-like protein	g 1274132455 ref XP_022830871.1 (coiled-coil domain-containing protein 40 isoform X2 [Spodoptera litura])	COILED-COIL DOMAIN-CONTAINING PROTEIN 40
GSSPFG00016090001-RA	179.703	3.750	2.3E-06	superscaffold_67	8782	9731	MINUS	alpha-tocopherol transfer protein	g 1274144492 ref XP_022823268.1 (alpha-tocopherol transfer protein-like [Spodoptera litura])	CRAL/TRIO N-terminal domain
GSSPFG00012333001-RA	202.416	4.183	2.6E-06	scaffold_419	24249	27320	MINUS	Fatty acyl-CoA reductase	g 1274118142 ref XP_022824237.1 (putative fatty acyl-CoA reductase CG5065 [Spodoptera litura])	Fatty acyl-CoA reductase
GSSPFG00004574001-RA	32.459	4.934	3.1E-06	scaffold_965	16341	25645	PLUS	Serine protease, S01.034: transmembrane peptidase, serine 4	g 1274124282 ref XP_022826384.1 (transmembrane protease serine 9-like [Spodoptera litura])	Peptidase S1A, chymotrypsin family
GSSPFG00018418001-RA	120.502	3.447	7.3E-06	scaffold_10763	1141	1281	PLUS	numt_COI_ND4	/	/
GSSPFG00008178001-RA	57.597	2.801	8.8E-06	scaffold_11019	4453	5244	PLUS	Ecdysteroid kinase	g 1274103665 ref XP_02283597.1 (uncharacterized protein LOC111364787 isoform X1 [Spodoptera litura])	Ecdysteroid kinase-like
GSSPFG00025164001-RA	249.863	4.622	8.9E-06	scaffold_9398	824	2510	PLUS NA		g 1274098509 ref XP_022834526.1 (uncharacterized protein LOC111362190 [Spodoptera litura])	/
GSSPFG000171532001-RA	49.167	4.954	1.1E-05	scaffold_42011	32	1151	PLUS	Unknown Smc-like protein	g 1274132453 ref XP_022830870.1 (coiled-coil domain-containing protein 40 isoform X1 [Spodoptera litura])	/
GSSPFG00011475001-RA	1468.212	4.561	1.1E-05	superscaffold_515	270856	300209	MINUS		g 1274125088 ref XP_022826287.1 (PAX-interacting protein 2-like [Spodoptera litura])	/
GSSPFG00030114001-RA	86.306	3.734	1.2E-05	scaffold_35751	459	1476	MINUS	alpha-tocopherol transfer protein	g 1274144492 ref XP_022832628.1 (alpha-tocopherol transfer protein-like [Spodoptera litura])	RETINALDEHYDE BINDING PROTEIN-RELATED
GSSPFG00015325001-RA	21.918	3.405	1.2E-05	scaffold_1961	38313	40593	PLUS TE		g 1486920932 ref XP_026493425.1 (piggyBac transposable element-derived protein 2-like [Vanessa tameamea])	PiggyBac transposable element-derived protein: DDE_Tnp_1_7
GSSPFG00019515001-RA	205.546	1.024	1.3E-05	scaffold_18956	70	1750	MINUS	Uncharacterized BTP/POZ transcription factor	g 1274098409 ref XP_022833473.1 (uncharacterized protein LOC111362155 [Spodoptera litura])	SKP1/BTB/POZ domain superfamily
GSSPFG00011838001-RA	2475.550	2.620	2.1E-05	scaffold_4541	7397	12803	PLUS	clavensin	g 1274145132 ref XP_022832976.1 (clavensin-like [Spodoptera litura])	CRAL-TRIO lipid binding domain superfamily
GSSPFG00025165001-RA	92.976	4.375	2.1E-05	scaffold_517	12864	14446	PLUS	Uncharacterized protein; s_517	g 1549086025 gb RVE41430.1 (hypothetical protein evm_0313924, partial [Chilo suppressalis])	/
GSSPFG00021956001-RA	202.072	0.898	3.7E-05	scaffold_15156	394	6160	PLUS	Tbkl kinase	g 1274100408 ref XP_022835755.1 (LOW QUALITY PROTEIN: serine/threonine protein kinase TBK1 [Spodoptera litura])	TANK binding kinase 1, ubiquitin-like domain
GSSPFG00014620001-RA	5.207	3.972	4.9E-05	superscaffold_813	83352	84278	PLUS	Zinc-finger protein	g 1496238990 ref XP_026745493.1 (uncharacterized protein LOC111360684 [Trichoplusia ni])	FVFE/PHD zinc finger + Baculovirus FP protein
GSSPFG00025955001-RA	122.996	4.151	5.2E-05	scaffold_183	169301	170656	MINUS	Stack LINE1	g 298204367 gb AD161832.1 (endonuclease-reverse transcriptase [Bombyx mori])	Reverse transcriptase domain
GSSPFG00007463001-RA	263.490	1.755	7.8E-05	scaffold_14772	1486	2878	MINUS	Ecdysteroid kinase	g 1274103669 ref XP_022837613.1 (uncharacterized protein LOC111364787 isoform X3 [Spodoptera litura])	Ecdysteroid kinase-like
GSSPFG00006526001-RA	90.653	1.572	1.1E-04	scaffold_19200	933	3186	PLUS	Protein artichoke	g 1274104572 ref XP_022816805.1 (protein artichoke [Spodoptera litura])	Leucine-rich repeat domain superfamily
GSSPFG00011681001.1-RA	264.791	3.249	1.1E-04	superscaffold_608	9468	12872	PLUS	Glucose dehydrogenase	g 1274137345 ref XP_022837607.1 (glucose dehydrogenase [FAD, quinone]-like isoform X2 [Spodoptera litura])	FAD/NAD(P)-binding domain superfamily - Glucose-methanol-choline oxidoreductase
GSSPFG00028883001-RA	307.920	2.094	1.1E-04	scaffold_2808	24600	28945	MINUS	Ubiquitin-conjugating enzyme E2	g 1274144092 ref XP_022832411.1 (ubiquitin-conjugating enzyme E2-22 kDa [Spodoptera litura])	Ubiquitin-conjugating enzyme E2
GSSPFG00017010001-RA	451.199	0.942	1.1E-04	scaffold_1685	43159	53355	MINUS	Rho guanine nucleotide exchange factor	g 1496285061 ref XP_026732580.1 (rho guanine nucleotide exchange factor 1-like isoform X1 [Trichoplusia ni])	DB homology (DH) domain
GSSPFG00013575001-RA	386.916	3.999	1.6E-04	scaffold_4472	2	8543	PLUS	takeout	g 1275386485 gb ATU07277.1 (takeout [Spodoptera litura])	Haemolymph juvenile hormone binding
GSSPFG00017882001.1-RA	916.677	3.863	2.4E-04	superscaffold_306	11517	13529	MINUS	yellow h2	g 1274099564 ref XP_022835105.1 (protein yellow-like, partial [Spodoptera litura])	Major royal jelly protein/protein yellow
GSSPFG00002468001-RA	328.535	3.930	2.4E-04	scaffold_190	16628	20217	MINUS	takeout	g 1275386485 gb ATU07277.1 (takeout [Spodoptera litura])	Haemolymph juvenile hormone binding
GSSPFG000262628001-RA	581.378	1.312	2.6E-04	superscaffold_106	38580	40189	PLUS	/	g 1274098298 ref XP_022834413.1 (uncharacterized protein LOC111362112 [Spodoptera litura])	/
GSSPFG00010450001-RA	52.899	2.135	2.6E-04	scaffold_1076	74513	75201	PLUS NA			TM domain
GSSPFG00001797001-RA	873.270	2.536	2.7E-04	superscaffold_345	13891	146770	MINUS	endocuticle structural glycoprotein	g 1274103967 ref XP_022814927.1 (endocuticle structural glycoprotein SbAbd-3-like [Spodoptera litura])	Insect cuticle protein - Chitin-binding type R&R consensus
GSSPFG00007187001-RA	57.501	1.086	3.1E-04	superscaffold_259	121981	124396	MINUS	islet cell autoantigen	g 1274131622 ref XP_022830425.1 (islet cell autoantigen 1 [Spodoptera litura])	Arfaptin homology (AH) domain
GSSPFG00034405001-RA	3934.962	4.139	3.2E-04	scaffold_899	32079	34798	PLUS	neurofilament heavy polypeptide	g 1274106589 ref XP_022820115.1 (neurofilament heavy polypeptide isoform X1 [Spodoptera litura])	TM domain
GSSPFG00028547001-RA	13.673	3.778	3.2E-04	scaffold_1264	68168	71148	PLUS	Sp3-like glucocorticoid receptor	g 1274113568 ref XP_022821723.1 (transcription factor Sp3-like [Spodoptera litura])	Glucocorticoid receptor-like (DNA-binding domain) + 3 Zinc finger C2H2-type
GSSPFG00031080001-RA	21.142	3.018	3.3E-04	scaffold_7275	3039	4697	PLUS NA			TM domain
GSSPFG00011415001-RA	1042.695	0.686	3.3E-04	scaffold_8188	30	3176	MINUS NA		g 1274113154 ref XP_022821497.1 (uncharacterized protein LOC111352977 [Spodoptera litura])	/
GSSPFG00008177001-RA	353.888	1.624	3.5E-04	scaffold_11019	1065	1859	PLUS	Ecdysteroid kinase	g 1274103667 ref XP_022837605.1 (uncharacterized ecdoreductase dhs-27-like isoform X2 [Spodoptera litura])	Ecdysteroid kinase-like
GSSPFG00030139001-RA	763.770	1.947	3.7E-04	scaffold_2510	37531	39530	PLUS	/	g 1274140375 ref XP_022814957.1 (uncharacterized protein LOC111348539 [Spodoptera litura])	/
GSSPFG00008472001-RA	190.464	3.679	3.9E-04	scaffold_7187	6435	7951	PLUS	/	g 1486899259 ref XP_026500633.1 (glycine-rich cell wall structural protein-like [Vanessa tameamea])	/
GSSPFG00034784001-RA	611.883	3.246	3.9E-04	scaffold_5	338897	350259	MINUS	Fatty acyl-CoA reductase	g 1274118142 ref XP_022824237.1 (putative fatty acyl-CoA reductase CG5065 [Spodoptera litura])	Male_sterile_NAD-bd
GSSPFG00012223001.1-RA	395.579	3.579	4.1E-04	superscaffold_596	31792	36073	MINUS	Reeler domain protein	g 1274122069 ref XP_022825151.1 (putative defense protein 3 [Spodoptera litura])	Reeler domain superfamily
GSSPFG00011683001.1-RA	56.502	3.550	4.5E-04	superscaffold_608	2521	6717	PLUS	glucose dehydrogenase	g 1274137343 ref XP_022837606.1 (glucose dehydrogenase [FAD, quinone]-like isoform X1 [Spodoptera litura])	Glucose-methanol-choline oxidoreductase
GSSPFG00027105001-RA	113.987	1.098	5.0E-04	superscaffold_658	48126	55291	PLUS	Broad complex core protein	g 1274137725 ref XP_022837815.1 (broad-complex core protein isoforms 1/2/3/4/5 isoform X3 [Spodoptera litra BTB/POZ domain - Zinc finger C2H2-type	
GSSPFG00004390001-RA	241.490	4.076	5.4E-04	scaffold_8617	4109	4848	MINUS	/	g 1274138702 ref XP_022814045.1 (uncharacterized protein LOC111347889 [Spodoptera litra])	/
GSSPFG00012336001-RA	884.031	3.154	6.2E-04	scaffold_419	85316	93664	PLUS	Fatty acyl-CoA reductase	g 1274117912 ref XP_022824155.1 (putative fatty acyl-CoA reductase CG5065 [Spodoptera litra])	Fatty acyl-CoA reductase
GSSPFG00028400001-RA	528.332	3.534	6.2E-04	scaffold_696	88259	89695	MINUS	/	g 1274125665 ref XP_022827146.1 (uncharacterized protein LOC111356881 [Spodoptera litra])	/
GSSPFG00010616001-RA	31.503	3.032	6.6E-04	scaffold_26453	749	2860	PLUS	/	g 1402415181 gb PZC74914.1 (hypothetical protein B5X24_HaOG207044 [Helicoverpa armigera])	/
GSSPFG00030439001-RA	49.406	3.287	6.6E-04	scaffold_4057	20799	25587	PLUS	Acytransferase	g 1274134691 ref XP_022831691.1 (nose resistant to fluoxetine protein 6-like isoform X1 [Spodoptera litra])	Acytransferase 3

Table S5. Manual annotation of the 50 genes with the most constitutive sf-C associated expression

OGS2.2	baseMean	log2FoldChange	padj	scaffold	start	end	strand	Annotation	Homology	IP
GSSPF00003930001-RA	65.426	-5.504	3.52E-15	superscaffold_328	16022	16834	PLUS	/	/	/
GSSPF00014445001-RA	53.167	-2.872	2.89E-14	scaffold_404	31734	33821	PLUS	DNA helicase	uncharacterized protein LOC110380119 [Helicoverpa ...	DNA helicase Pif1-like
GSSPF0000902001-RA	676.632	-6.477	6.66E-12	scaffold_1577	12569	14890	PLUS	TE	uncharacterized protein LOC113494593 [Trichoplusia...]	Reverse transcriptase domain
GSSPF00033049001-RA	287.649	-5.722	5.41E-11	superscaffold_601	170017	174234	PLUS	TE	hypothetical protein BSV51_5889 [Heliothis virescens]	Reverse transcriptase domain
GSSPF00033999001-RA	836.982	-5.546	1.81E-10	superscaffold_1180	158004	158412	PLUS	/	/	Transmembrane region
GSSPF00034206001-RA	925.406	-5.645	6.11E-09	superscaffold_816	59297	66958	PLUS	fatty-acyl-CoA reductase	fatty acyl-CoA reductase wnt-like [Spodoptera litura]	Fatty acyl-CoA reductase
GSSPF00009529001-RA	5513.420	-4.451	2.36E-08	scaffold_81	110099	118043	MINUS	Spermadhesin-like lectin	uncharacterized protein LOC111350041 [Spodoptera litura]	Spermadhesin, CUB domain superfamily
GSSPF00010240001-3-RA	1233.191	-2.321	2.41E-08	scaffold_13709	3294	5629	PLUS	Calcium-dependent lectin 4	hemolymph lipopolysaccharide-binding protein-like, partial [Spodoptera litura]	C-type lectin-like
GSSPF00015043001-RA	366.426	-2.175	3.03E-08	scaffold_29813	360	2044	MINUS	GNBP	beta-1,3-glucan-binding protein-like [Spodoptera litura]	GRAM-NEGATIVE BACTERIA-BINDING PROTEIN 1-RELATED
GSSPF00027050001-2-RA	128.368	-1.786	5.34E-08	scaffold_3897	4633	19471	PLUS	apterous 1	protein apterous-like isoform XI [Helicoverpa armigera]	Homeobox domain
GSSPF00024233001-RA	50.412	-2.765	7.24E-08	scaffold_1362	17970	20057	MINUS	DNA helicase	uncharacterized protein LOC110380119 [Helicoverpa armigera]	DNA helicase Pif1-like
GSSPF00013166001-RA	49.428	-2.753	1.98E-07	scaffold_882	51328	53415	MINUS	DNA helicase	uncharacterized protein LOC110380119 [Helicoverpa armigera]	DNA helicase Pif1-like
GSSPF00003295001-3-RA	1828.842	-2.837	2.37E-07	scaffold_22553	1941	3508	PLUS	odorant-binding protein 36	odorant binding protein 17 [Spodoptera exigua]	Insect pheromone/odorant-binding proteins
GSSPF00024351001-RA	451.875	-0.972	2.78E-07	scaffold_28552	59	2295	PLUS	TM protein	uncharacterized protein LOC111352652 [Spodoptera litura]	PMP-22/EMP/MP20/Claudin superfamily
GSSPF00015043001-RA	55.248	-4.696	3.61E-07	scaffold_22559	265	2480	MINUS	/	fibrinogen silencer-binding protein-like [Spodoptera litura]	/
GSSPF00027329001-RA	105.513	-4.262	4.07E-07	scaffold_6725	11537	12291	PLUS	/	/	/
GSSPF00005332001-RA	187.315	-2.921	4.16E-07	superscaffold_751	153518	156286	MINUS	DNA helicase	uncharacterized protein LOC110380119 [Helicoverpa armigera]	DNA helicase Pif1-like
GSSPF00021758001-RA	901.184	-4.987	7.32E-07	scaffold_1475	45647	49735	PLUS	TE	Retrovirus-related Pol polyprotein from transposon TNT 1-94 [Eumeta japonica]	Retrotransposon Ty1/copia-like
GSSPF00021650001-RA	1036.158	-2.601	1.30E-06	scaffold_29236	64	1943	MINUS	glucose dehydrogenase	glucose dehydrogenase [FAD, quinone]-like [Spodoptera litura]	Glucose-methanol-choline oxidoreductase / FAD/NAD(P)-binding domain superfamily
GSSPF00015431001-1-RA	3095.855	-5.096	1.62E-06	scaffold_25	365539	377676	PLUS	Fatty acid synthase	fatty acid synthase-like [Spodoptera litura]	Fatty acid synthase
GSSPF00008269001-4-RA	10079.204	-3.445	2.01E-06	scaffold_11622	5807	8216	MINUS	CYP9A31PARTIAL	cytochrome P450 SE-CYP9A21z2, partial [Spodoptera exigua]	Cytochrome P450, E-class, group I
GSSPF00029033001-RA	1946.912	-2.442	2.39E-06	superscaffold_334	42062	43502	PLUS	TE	hypothetical protein [Piscirickettsia salmonis]	/
GSSPF00015492001-RA	134.477	-4.271	3.77E-06	scaffold_5015	1654	3189	PLUS	TE	uncharacterized protein LOC111359856 [Spodoptera litura]	/
GSSPF00030456001-4-RA	371.255	-3.717	3.80E-06	superscaffold_667	32764	34262	PLUS	Cecropin D2	cecropin C [Spodoptera exigua]	Cecropin
GSSPF00025034001-RA	47.979	-3.169	3.94E-06	scaffold_119	8323	11097	MINUS	TE	piggyBac transposable element-derived protein 4-like [Bombyx mandarina]	PiggyBac transposable element-derived protein
GSSPF00011213001-RA	26.035	-1.501	4.45E-06	scaffold_1341	14383	19256	PLUS	Orc4	origin recognition complex subunit 4 [Spodoptera litura]	Origin recognition complex subunit 4
GSSPF00017887001-1-RA	689.116	-4.965	4.70E-06	scaffold_25	334090	363141	PLUS	Fatty acid synthase	fatty acid synthase-like [Spodoptera litura]	FAFTY ACID SYNTHASE 3
GSSPF00028982001-RA	237.134	-0.908	9.57E-06	scaffold_11342	3664	7637	PLUS	RNA methyltransferase	putative methyltransferase NSUN6 [Helicoverpa armigera]	RNA (C5-cytosine) methyltransferase
GSSPF00008611001-RA	182.154	-1.328	1.06E-05	scaffold_23545	1099	2682	PLUS	Mcm replication licensing factor Mcm3 [Spodoptera litura]	DNA replication licensing factor Mcm3 [Spodoptera litura]	/
GSSPF00023421001-RA	14.073	-3.500	1.06E-05	scaffold_1914	33353	35936	MINUS	Major facilitator, sugar transmembrane transporter	facilitated trehalose transporter Tret1-like [Spodoptera litura]	Major facilitator, sugar transporter-like
GSSPF00000830001-1-RA	394.040	-2.926	1.29E-05	superscaffold_636	59933	63312	PLUS	glucose dehydrogenase	glucose dehydrogenase [FAD, quinone]-like [Spodoptera litura]	Glucose-methanol-choline oxidoreductase
GSSPF000018074001-RA	512.151	-0.557	1.82E-05	scaffold_7139	5677	12546	MINUS	HMG box protein	HMG domain-containing protein 4 isoform X1 [Spodoptera litura]	High mobility group box domain
GSSPF00002576001-RA	73.215	-3.822	1.94E-05	scaffold_27664	441	2372	PLUS	TE	piggyBac transposable element-derived protein 4-like isoform X1 [Spodoptera litura]	PiggyBac transposable element-derived protein
GSSPF00018367001-RA	175.907	-4.389	2.06E-05	scaffold_9785	5077	9577	PLUS	DUF1676	uncharacterized protein LOC111357194 isoform X1 [Spodoptera litura]	Protein of unknown function DUF1676
GSSPF00011154001-1-RA	91.034	-1.228	2.38E-05	scaffold_924	9409	14940	PLUS	Claspin like	microtubule-associated protein futsch-like [Spodoptera litura]	Claspin
GSSPF00023769001-RA	200.369	-1.346	2.62E-05	scaffold_12811	5528	7142	MINUS	Nucleoporin NSP1/NUP62	nuclear pore glycoprotein p62-like [Helicoverpa armigera]	Nucleoporin NSP1/NUP62
GSSPF00003828001-RA	890.911	-3.717	2.65E-05	scaffold_1985	41560	42289	PLUS	/	/	/
GSSPF00002062001-RA	279.560	-3.354	3.14E-05	scaffold_459	105497	105630	PLUS	numt-ND2	/	/
GSSPF000025780001-RA	1050.775	-2.608	4.37E-05	scaffold_2063	30092	40049	PLUS	/	uncharacterized protein LOC111357139 [Spodoptera litura]	Signal Peptide
GSSPF00016432001-1-RA	250.152	-3.953	4.50E-05	scaffold_1877	52654	54924	MINUS	/	uncharacterized protein LOC111348319 [Spodoptera litura]	TRANSMEMBRANE
GSSPF00018669001-2-RB	162.057	-3.682	4.89E-05	scaffold_22469	1969	3330	MINUS	CYP338A1	cytochrome CYP338A2 [Spodoptera littoralis]	Cytochrome P450, E-class, group IV
GSSPF000223363001-RA	279.368	-4.025	5.07E-05	scaffold_5632	3032	5922	PLUS	/	uncharacterized protein LOC111357139 [Spodoptera litura]	signal peptide
GSSPF00002117001-RA	909.154	-3.597	5.21E-05	scaffold_9208	1816	4928	MINUS	/	uncharacterized protein LOC110384158 [Helicoverpa armigera]	Zona pellucida domain
GSSPF00027037001-RA	258.729	-0.823	6.42E-05	scaffold_15135	344	2637	PLUS	Leo1	another transcription unit protein [Spodoptera litura]	Leo1-like protein
GSSPF000024631001-RA	34.842	-1.601	7.74E-05	scaffold_13033	4861	5525	PLUS	/	/	TRANSMEMBRANE
GSSPF00004275001-RA	14.350	-3.334	7.77E-05	scaffold_14274	196	2776	PLUS	/	uncharacterized protein LOC111357540 [Spodoptera litura]	/
GSSPF000032900001-RA	17.524	-2.389	8.20E-05	scaffold_32	16629	17758	PLUS	Cog7	conserved oligomeric Golgi complex subunit 7-like [Hypomocoma kahamanoa]	Conserved oligomeric Golgi complex subunit 7
GSSPF000021626001-RA	305.017	-3.529	8.35E-05	scaffold_2114	4137	5142	MINUS	/	/	/
GSSPF000003829001-RA	3809.960	-3.627	8.35E-05	scaffold_1985	42432	51963	MINUS	/	uncharacterized protein LOC111356160 [Spodoptera litura]	Zona pellucida domain
GSSPF000024020001-RA	1179.432	-1.684	8.40E-05	scaffold_3326	660	6100	MINUS	Glyoxalase I	lactoylglutathione lyase [Spodoptera litura]	Glyoxalase/Bleomycin resistance protein/Dihydroxybiphenyl dioxygenase