

Figure S1: Biological function gene ontology of differentially expressed transcripts after seven days of low temperature stress.

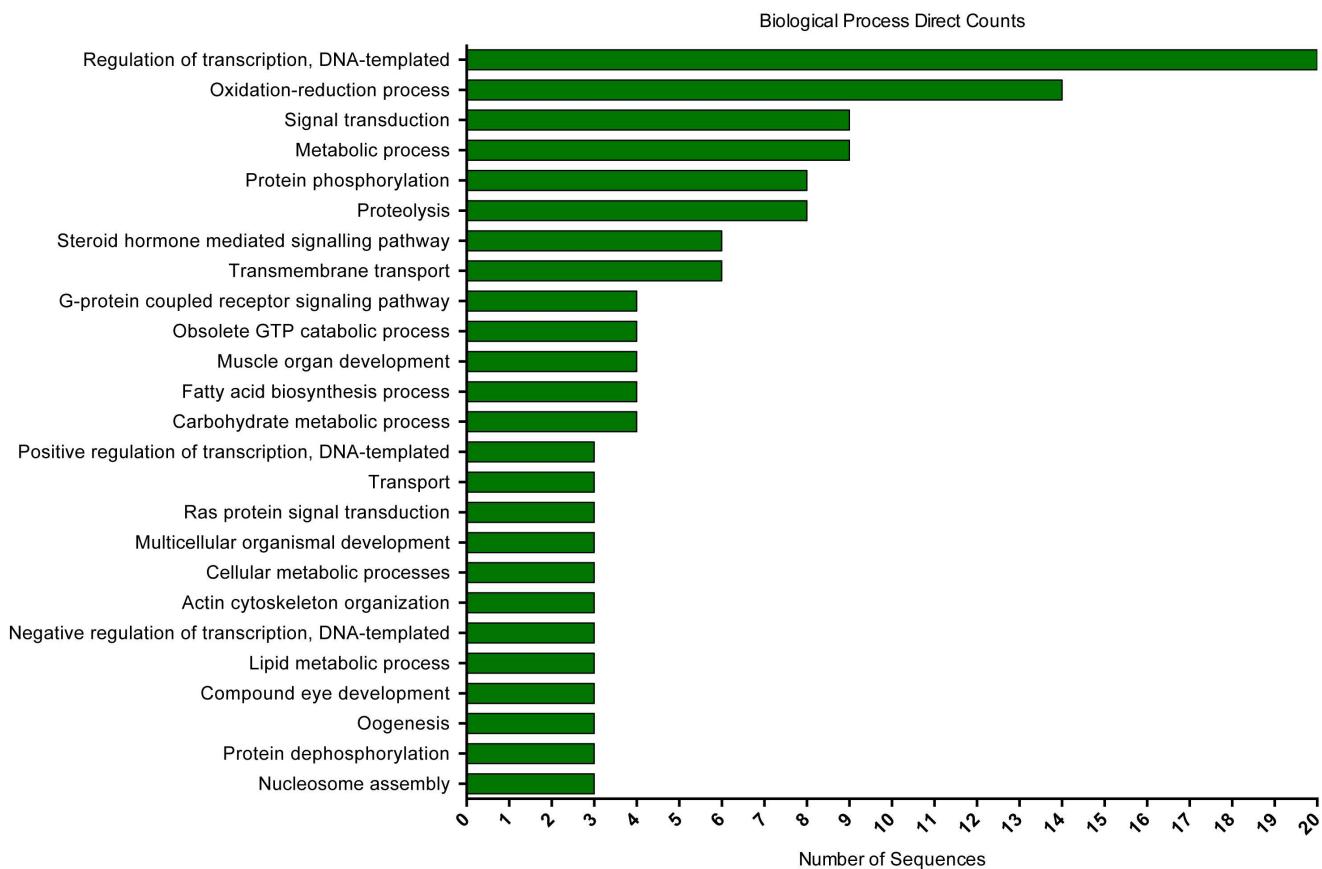


Figure S2: Expression profiles of candidate genes during temperature stress. The expression of candidate genes identified in the RNA-seq analysis was assessed throughout the entire duration of low temperature stress after extended overwintering ($n=6$). The data are presented as mean \pm s.e.m. Asterisks indicate a significant difference in expression between STR and FTR determined by ANOVA.

Table S1. qPCR validation primers. Primer sets highlighted in grey were reference gene candidates.

Transcript ID	Forward	Reverse
MR0T_00009775 Set 1	CGA AAC GCT CGC TTA CAA ATA G	CAT TGA GGT GGC TCG TAT CTC
MR0T_00009775 Set 2	CCT CAG AGA CAT GAA GGA ACA C	GTG GGA AGA ACT GGG TGA TAT G
MR0T_0000590 Set 1	AAT GAG CCT ACG GAA AGA AGA G	GCT TTG GTG CTA GAG GAT GT
MR0T_0000590 Set 2	TCG TGC CCT TAC AAA GAC TG	CGA ACT TCT TCT CTC CGG TAT G
MR0T_00008799 Set 1	GAC CTG AAC CGA CGT CTT TAA T	CAG TTG ACT CAC AGA GGA GAA C
MR0T_00008799 Set 2	CTC CTG GGA ATC AGA TGG TTA G	GGC TTG AAC TCC GCT GAT AA
MR0T_00010646 Set 1	GCC GAT ACG ATT GTG AGA GAG	GTT CCA TGT TGG TTC CGT TTC
MR0T_00010646 Set 2	ACG CGA GAG GAA CAA CAT AG	GTC GTT GTC CTT CAC CAG AA
MR0T_00008869 Set 1	TGC CAA CCA ACA CAA GGA	AGC TAC CGG TTT GCC ATA TT
MR0T_00008869 Set 2	CAA AGC CAC AGC ACA CAT TC	ATC GCG ATG ATC TCT GGT TG
MR0T_00005226 Set 1	GGC AAA GGG AAT GAC ACT TG	GAG TAC ACA TTC ACA ATC CAT GAG
MR0T_00005226 Set 2	CCA ATG GAT GAA TGC CAG AAA T	GAG AAC AAG TGT CAT TCC CTT TG
MR0T_00008103 Set 1	GGT CGT GCC AAG ATC AAG AA	CTT AGG CTT CCG AGG ATG ATT G
MR0T_00008103 Set 2	CAA TCA TCC TCG GAA GCC TAA G	GCC GAA CAA CCT CTG GAA A
MR0T_00002094 Set 1	TGT GAG ATT GGA TGC GAA GAA	CAA CGA GCC TAG CAG AGA TTT A
MR0T_00002094 Set 2	GCA TCG GGC AAC AAA TCT TC	CAC GAC TCG TAC GGC TTA AA
MR0T_00008639 Set 1	GTG TGG TTA GCA GTT CAG TAG AG	GAA TTT GGC CTT CCT TGT GAT G
MR0T_00008639 Set 2	GTT ACG TCC GAG CCT TGA AA	GAG GAA TGC AGC TCA GGA TAA T
MR0T_00004919 Set 1	ACA ACC CAC AAC CAG AAG AG	TTC CAC TGT GCA CAT CGT ATT A
MR0T_00004919 Set 2	GAG GAG AAT GGC GAT CAC TAA A	CTT CCC TTC GTA GCG TTT CT
MR0T_00001385 Set 1	CCA GTT GGT GTT CGA GGA ATA G	TGC TCC AAG TCC AGT GTT ATG
MR0T_00002467 Set 1	TAC CTC CTC CGA CTT TAC	CTG GCC GTC CGC ATT ATT A
MR0T_00003376 Set 1	CTG GAC CAA AGC AAC AAA CG	CTG GGC CTG TAT CTT CTT CTT C
MR0T_00003926 Set 1	GGA GGC GAT CCT ACA AAT ACT G	GTC TAC CTC GAC TTT CTC AAT CG
MR0T_0000714 Set 1	CAG TAC ATG CTT TGG CGT TAT G	CCA GAA TCT CCT GTT TCT CCT G
MR0T_0000812 Set 1	AGC AGA GAA GAA GAC GAG AAA C	GTG CAG CAG TAC GAC CAA TA
MR0T_00008586 Set 1	TGA AAG CTT CTG CGG CTA TTA	CCA ACT TCT GTC TGG TCT CTT C
MR0T_00009058 Set 1	GAT GGC AAA CAA GCT AGA AGA AC	GGC TGA TAG AGC AAC GAA CA

Table S2. Ten most up- and down-regulated transcripts after treatment. Log₂ fold change relative to FTR. A negative fold change is an upregulation in STR.

Transcript ID	Sequence Description	Fold Change (log ₂)
MR0T_00006789	cuticle protein 6	-8.56374
MR0T_00008105	osiris 16	-8.42492
MR0T_00010933	PREDICTED: uncharacterized protein LOC100879602	-7.38722
MR0T_00010985	leucine-rich repeat-containing g-protein coupled receptor 4-like	-6.34828
MR0T_00007545	zinc carboxypeptidase a 1-like	-5.2878
MR0T_00002812	carboxypeptidase b	-4.91085
MR0T_00002813	trypsin-1	-4.91085
MR0T_00001862	heavy metal-translocating p-type atpase	-4.42962
MR0T_00002788	PREDICTED: hypothetical protein LOC100741758	-4.41335
MR0T_00008052	cuticle collagen 3a3	-4.35375
MR0T_00009775	tbc1 domain family member 4-like	1.71071
MR0T_00005906	cabut	1.72365
MR0T_00008799	zinc finger SCAN domain containing protein	1.78574
MR0T_00010646	ccaaat enhancer-binding protein	1.87841
MR0T_00008869	actin-binding rho-activating isoform 1	2.04768
MR0T_00005226	seminal fluid protein hacp027	2.07091
MR0T_00008103	osiris 14	2.07664
MR0T_00002094	zinc finger protein noc-like	2.26969
MR0T_00008639	myofilin isoform b	2.33654
MR0T_00004919	protein argonaute-2	3.67798

Table S3. qPCR validation. Yellow indicates transcripts that were validated with one primer set and green indicates those validated with two. Genes identified as non-differentially expressed were those used to determine stable reference genes.

Transcript ID	Differentially Expressed in RNA-seq	qPCR Validated
MR0T_00009775	Yes	Yes
MR0T_00005906	Yes	Yes
MR0T_00008799	Yes	Yes
MR0T_00010646	Yes	No
MR0T_00008869	Yes	Yes
MR0T_00005226	Yes	Yes
MR0T_00008103	Yes	No
MR0T_00002094	Yes	Yes
MR0T_00008639	Yes	Yes
MR0T_00004919	Yes	Yes
MR0T_00001385	No	Yes
MR0T_00002467	No	Yes
MR0T_00003376	No	Yes
MR0T_00003926	No	Yes
MR0T_00007147	No	No
MR0T_00008127	No	Yes
MR0T_00008586	No	Yes
MR0T_00009058	No	No

Table S4: Gene ontology of non-cell-membrane transcripts. Log₂ fold change relative to FTR. A negative fold change (grey rows) is an upregulation in STR.

Transcript ID	Function	Transcript	Fold Change
MROT_00000137	Apoptotic Process	death associated protein	-1.04078
MROT_00007022	Apoptotic Process	intraflagellar transport protein 57 homolog	-1.06966
MROT_00004114	Developmental Process	homeobox protein	1.25495
MROT_00005807	Developmental Process	udp-glucose 6-dehydrogenase	1.17127
MROT_00002687	Developmental Process	ras association domain-containing protein 8-like	0.808743
MROT_00006453	Developmental Process	e3 ubiquitin-protein ligase su -like	0.799208
MROT_00010835	Developmental Process	paired box protein pax-2-b	-0.916377
MROT_00000481	Developmental Process	ecdysone-induced protein 78c	-1.30957
MROT_00008639	DNA repair	myofilin isoform b	2.33654
MROT_00004041	DNA repair	dna damage-binding protein 1	1.02919
MROT_00007044	DNA repair	p53 regulated pa26 nuclear protein	0.797203
MROT_00004541	DNA repair	meiotic recombination 11	-1.27712
MROT_00008401	Oxidation-reduction	acyl- delta desaturase-like	-1.17683
MROT_00010957	Oxidation-reduction	acyl- delta desaturase	-1.94342
MROT_00003853	Neurogenesis	isoform b	0.860505
MROT_00004354	Neurogenesis	storkhead-box protein 1	-0.726152
MROT_00002967	Neurogenesis	kn motif and ankyrin repeat domain-containing protein 1	-0.802562
MROT_00007176	Neurogenesis	peptidylglycine alpha-hydroxylating monooxygenase	0.996843
MROT_00007352	Oxidation/reduction	cytochrome b5-related protein	-0.85795
MROT_00000322	Oxidation/reduction	fatty acyl- reductase cg5065-like	-0.954789
MROT_00001392	Oxidation/reduction	probable cytochrome p450 305a1	-1.11015
MROT_00002750	Oxidation/reduction	1-cys peroxiredoxin	-1.35647
MROT_00005092	Oxidation/reduction	d-3-phosphoglycerate dehydrogenase	-1.57624
MROT_00007629	Oxidation/reduction	homogentisate -dioxygenase	-1.57882
MROT_00009098	Oxidation/reduction	pro-phenol oxidase subunit 2	-1.712
MROT_00008416	Oxidation/reduction	cytochrome p450 mitochondrial	-1.73091
MROT_00008044	Oxidation/reduction	oxidase peroxidase	-2.22979
MROT_00009438	Oxidation/reduction	10-formyltetrahydrofolate dehydrogenase	-2.33033
MROT_00003522	Oxidation/reduction	glyoxylate reductase hydroxypyruvate reductase	-2.36061
MROT_00008221	Oxidation/reduction	short-chain dehydrogenase	-2.41981
MROT_00010247	Oxidation/reduction	cytochrome p450 6b1-like	-2.44822

MROT_00000691	Oxidation/reduction	retinol dehydrogenase 14	-2.57532
MROT_00008095	Oxidation/reduction	osiris 2 cg1148-pb	-3.3388
MROT_00000580	Oxidation/reduction	fatty acid synthase	-4.00072
MROT_00000146	Oxidation/reduction	protein kintoun-like	1.41663
MROT_00008499	Oxidation/reduction	cytochrome p450 4g15	-2.2377
MROT_00002050	Stress response	myb domain-containing protein	-2.08356