

**Table S1:** Primer pairs used for candidate genes in qRT-PCR validation. Unless otherwise stated, primers were designed *de novo* (as described in the methods).

Primer	Direction	Sequence	Source
<i>Actin79B</i>	F	5'-TTGGAGATCCACATCTGCTG-3'	Sinclair et al. 2007
	R	5'-CCAGGTATCGCTGACCGTAT-3'	
<i>TotA</i>	F	5'-GCACCCAGGAACTACTTGACATCT-3'	Ekengren et al.2001
	R	5'-GACCTCCCTGAATCGGAACTC-3'	
<i>TotM</i>	F	5'-CGGACTTGAACACACGCGCC-3'	
	R	5'-CCAGAATCCGCCTTGTGCGGG-3'	
<i>Cp16</i>	F	5'-GAAAGTGACTGGTCATCGAG-3'	
	R	5'-AATTAACCAGAGCGTGTGTC-3'	
<i>Upheld</i>	F	5'-GAGACAAAACCACCTCAGAC-3'	
	R	5'-GGACGAGAGTCCCAACAC-3'	
<i>CG6300</i>	F	5'-TCTCGGGCTGCCTTGGAGGT-3'	
	R	5'-TCCGATCTCATCGGGTCCCAGG-3'	
<i>CG33669</i>	F	5'-ACTCCGAAGCGAGAATGAGC-3'	
	R	5'-AACGTCGCCGTGGAGCATGT-3'	
<i>Tm2</i>	F	5'-AGTGTGCGTGTGCGTTGGATCT-3'	
	R	5'-CGTTTCAAACACACTATACGCTGTCTG-3'	
<i>Lsp1 beta</i>	F	5'-AGCGCCTGTGCGACGGATTTC-3'	
	R	5'-TCGATGGCCTCGGGTTTGCG-3'	
<i>CG14995</i>	F	5'-ATTACGCGCCCTGCCGAACC-3'	
	R	5'-GGCAACTGGGGCGGCTCTTT-3'	
<i>CG6188</i>	F	5'-TGACCAGTGAGTTCCGTCTG-3'	
	R	5'-ATGTAGAAGGCGGGGTTCTT-3'	
<i>CG13921</i>	F	5'-TGGCTCCATAGCCCATCGCCA-3'	
	R	5'-AGGGTGGCGGGCACAAAGGT-3'	
<i>CG16711</i>	F	5'-GGCAGTGGACCACGGTGACG-3'	
	R	5'-GCAGCGGCCGCCATCTGATT-3'	

**Table S2:** Genes differentially expressed in adult female virgin *D. melanogaster* after repeated cold exposures (five daily 2h exposures to -0.5 °C) but not after prolonged (one 10h exposure to -0.5 °C) or a single short cold exposure (one 2h exposure to -0.5 °C), with a description of associated biological processes or molecular functions, as well as their locations on *D. melanogaster* chromosomes (X, 2L, 2R, 3L, 2R, 4). Genes were classified based on their GO terms that were identified by DAVID. \* indicates differentially expressed genes among the three cold treatments after F-test (ANOVA, p-value < 0.05).

Functional category	Gene Name	Fold change	Biological process and molecular function	Location
<b>Muscle protein/ Actin binding</b>	<i>Upheld</i>	2.6 up	Calcium ion binding; sarcomere organization; myofibril assembly	X
	<i>Tropomyosin 2*</i>	2.4 up	Actin binding; muscle thin filament tropomyosin	3R
	<i>Paramyosin*</i>	2.4 up	Myofibril assembly; structural constituent of muscle; motor activity	3L
	<i>Fhos</i>	2.3 up	Actin binding	3L
	<i>Myosin light chain 2</i>	2.1 up	ATPase activity; calcium ion binding; microfilament motor activity	3R
<b>Chorion</b>	<i>Chorion protein 16*</i>	2.7 up	Structural constituent of chorion; multicellular organismal development	3L
	<i>Chorion protein 19*</i>	2.6 up	Structural constituent of chorion; multicellular organismal development	3L
	<i>Chorion protein 38*</i>	2.1 up	Eggshell chorion assembly	X
<b>Metabolic</b>	<i>CG6300*</i>	2.5 up	Catalytic activity; long-chain fatty acid transporter activity	3R

<b>process/ Regulation of biological process</b>	<i>ia2*</i>	2.2 up	Protein tyrosine phosphatase activity; protein amino acid dephosphorylation	2L
	<i>Rab GTPase 9E</i>	2.1 up	GTPase activity; protein transport; small GTPase mediated signal transduction	X
	<i>CG3635</i>	2.1 up	Triglyceride lipase activity	2L
	<i>NADH-ubiquinone oxidoreductase chain 4</i>	2.1 up	NADH dehydrogenase (ubiquinone) activity; mitochondrial respiratory chain complex I	Mitochondrion genome
	<i>CG3894</i>	2.1 up	Intracellular signaling pathway	2R
	<i>Rab GTPase 9Db</i>	2.0 up	GTP binding; protein transport; small GTPase mediated signal transduction	X
	<i>Phosphatidylinositol 3 kinase 59F</i>	2.0 down	Phosphoinositide-mediated signaling; dsRNA transport; cellular response to starvation	2R
	<i>CG32174*</i>	2.1 down	Ubiquinone biosynthetic process	3L
	<i>Heat shock protein 22*</i>	2.1 down	Response to heat; determination of adult lifespan; response to oxidative stress; protein refolding	3L
	<i>CG8303*</i>	2.1 down	Catalytic activity	2R
<b>Metal ion binding</b>	<i>CG6188</i>	2.3 down	Folic acid binding; methionine metabolic process	3R
	<i>CG13830</i>	2.0 up	Calcium ion binding	3R
	<i>CG14306</i>	2.1 up	Protein binding; zinc ion binding	3R

	<i>CG32850</i>	2.2 up	Zinc ion binding; protein binding	4
	<i>Sirt4</i>	2.1 down	Zinc ion binding; chromatin silencing; NAD binding; protein amino acid deacetylation	X
	<i>Neu3*</i>	2.1 down	zinc ion binding; proteolysis	3R
<b>Other</b>	<i>Turandot M*</i>	4.9 up	Encodes a humoral factor	2L
	<i>CG33669</i>	2.4 up	Unknown	X
	<i>Histone H3*</i>	2.4 up	DNA binding; chromatin assembly or disassembly; nucleosome assembly	2L
	<i>CG41495*</i>	2.4 up	Unknown	
	<i>Stellate protein CG33245*</i>	2.4 up	Protein kinase regulator activity; regulation of protein kinase activity	X
	<i>CG42287</i>	2.4 up	Unknown	2R
	<i>CG32821</i>	2.3 up	Unknown	X
	<i>CG11585</i>	2.3 up	Unknown	X
	<i>CG41124*</i>	2.2 up	Unknown	
	<i>CG33223</i>	2.2 up	Unknown	X
	<i>CG33664*</i>	2.2 up	Unknown	X
	<i>CG32074</i>	2.2 up	Unknown	3L
	<i>CG1894*</i>	2.2 up	Histone acetyltransferase activity; regulation of transcription	3R
	<i>Met75C</i>	2.2 up	Unknown	3L

	<i>Z band alternatively spliced PDZ-motif protein 66*</i>	2.2 up	Mesoderm development; Z disc	3L
	<i>Lcp65Ag1</i>	2.2 up	Structural constituent of chitin-based cuticle; larval chitin-based cuticle development	3L
	<i>CG13055</i>	2.2 up	Unknown	3L
	<i>Sperm-specific dynein intermediate chain 3*</i>	2.1 up	Unknown	X
	<i>CG12587*</i>	2.1 up	Unknown	3R
	<i>CG13622</i>	2.1 up	Unknown	3R
	<i>CG33667</i>	2.1 up	Unknown	X
	<i>CG18437</i>	2.1 up	Unknown	3L
	<i>Chorion protein a at 7F</i>	2.0 up	Unknown	X
	<i>CG3984</i>	2.0 up	Unknown	3R
	<i>CG13704*</i>	2.0 down	Unknown	3L
	<i>CG9396</i>	2.0 down	Unknown	3R
	<i>CG4025</i>	2.0 down	Unknown	X
	<i>CG4749</i>	2.1 down	Unknown	2L
	<i>CG5428</i>	2.1 down	Sulfotransferase activity	2R
	<i>CG14104</i>	2.2 down	Unknown	3L

	<i>CG16711</i>	2.2 down	Unknown	3L
	<i>CG13921</i>	2.3 down	Unknown	3L
	<i>CG14995</i>	2.6 down	Autophagic cell death; salivary gland cell autophagic cell death	3L
	<i>Larval serum protein 1 <math>\beta^*</math></i>	2.9 down	Larval serum protein complex; nutrient reservoir activity; oxygen transporter activity	2L

**Table S3:** Gene differentially expressed in adult female virgin *D. melanogaster* after a prolonged (10h) cold exposure but not after repeated (five daily 2h exposures) or a single short (2h) cold exposure, with a description of associated biological processes or molecular functions, as well as their locations on *D. melanogaster* chromosomes (X, 2L, 2R, 3L, 2R, 4). Genes were classified based on their GO terms that were identified by DAVID. \* indicates differentially expressed genes among the three cold treatments after F-test (ANOVA, p-value < 0.05).

GO terms	Gene Name	Fold change	Biological process and molecular function	Location
<b>Immune response</b>	<i>Immune induced molecule 23*</i>	2.7 up	Toll signaling pathway; antibacterial humoral response; defense response	2R
	<i>Chorion protein b at 7F*</i>	2.5 up	Unknown	X
	<i>Immune iduced molecule 1*</i>	2.3 up	Defense response	2R
	<i>Metchnikowin*</i>	2.3 up	Defense response; antibacterial humoral response; antifungal humoral response	2R
	<i>Peptidoglycan-recognition protein SC1a/b*</i>	2.2 up	Peptidoglycan binding; protein binding; defense response; innate immune response	2R
<b>Metabolic process</b>	<i>NADH-ubiquinone oxidoreductase chain 3</i>	2.5 up	NADH dehydrogenase (ubiquinone) activity; mitochondrial electron transport, NADH to ubiquinone	Mitochondrial genome
	<i>CG9747*</i>	2.0 up	acyl-CoA delta11-desaturase activity; lipid metabolic process; oxidation reduction	3R
	<i>CG4099</i>	2.0 up	Heme binding; oxidation reduction	3R
	<i>CG13309*</i>	2.2 down	Unknown	3L
	<i>CG31661*</i>	2.4 down	Unknown	2L
	<i>Target of brain insulin*</i>	2.4 down	Carbohydrate metabolic process	3R

	<i>CG9466</i>	2.4 down	Zinc ion binding; carbohydrate binding;	2L
	<i>Larval visceral protein L*</i>	2.9 down	Cation binding; carbohydrate metabolic process	2R
	<i>Neither inactivation nor afterpotential D*</i>	3.6 down	scavenger receptor activity; phototransduction	2L
<b>Reproduction</b>	<i>Odorant-binding protein 19c*</i>	4.4 up	Odorant binding; sensory perception of chemical stimulus; transport	X
	<i>Vitelline membrane 32E*</i>	5.1 up	Vitelline membrane formation in chorion-containing eggshell	2L
	<i>Defective chorion 1*</i>	3.6 up	Chorion-containing eggshell formation	X
	<i>Lipid storage droplet-1</i>	2.4 up	Lipid storage	3R
	<i>CG13887*</i>	2.4 up	Intracellular protein transport;	3L
	<i>Chorion protein 15*</i>	2.7 down	Structural constituent of chorion; eggshell chorion assembly	3L
<b>Transport</b>	<i>CG14309*</i>	5.9 up	Unknown	3R
	<i>Follicle cell protein 3C</i>	5.7 up	Unknown	X
	<i>CG10407*</i>	4.4 up	Unknown	3R
<b>Others</b>	<i>Glutathione S transferase D2*</i>	3.9 up	Glutathione peroxidase activity	3R
	<i>Chorion protein b at 7F*</i>	3.9 up	Oxidation reduction	X
	<i>Heat shock protein 23*</i>	3.3 up	Response to hypoxia; response to heat	3L
	<i>CG15784*</i>	3.2 up	Unknown	X
	<i>Vitelline membrane-like*</i>	3.1 up	Dorsal/ventral axis specification	X
	<i>CG15829*</i>	3.0 up	acyl-CoA binding; enzyme inhibitor activity	3L



	<i>CG14834*</i>	2.9 up	Unknown	3L
	<i>Follicle cell protein 26Ac*</i>	2.8 up	Unknown	2L
	<i>CG13997*</i>	2.8 up	Unknown	2L
	<i>CG31086*</i>	2.7 up	Unknown	3R
	<i>Frost*</i>	2.6 up	Response to cold	3R
	<i>Cuticular protein 47Eb</i>	2.5 up	Structural constituent of chitin-based cuticle; structural constituent of chitin-based larval cuticle	2R
	<i>CG13324*</i>	2.5 up	Unknown	2R
	<i>CG13905</i>	2.4 up	Unknown	3L
	<i>CG15065*</i>	2.3 up	Unknown	2R
	<i>CG14187*</i>	2.3 up	Unknown	3L
	<i>HMG protein Z</i>	2.2 up	DNA binding	2R
	<i>CG13114*</i>	2.1 up	Unknown	2L
	<i>CG16775</i>	2.1 up	Unknown	3L
	<i>CG13323*</i>	2.1 up	Unknown	2R
	<i>Niemann-Pick type C-2e</i>	2.1 up	Proteolysis	3R
	<i>CG10993</i>	2.1 up	Nucleic acid binding; nucleotide binding	X
	<i>Yellow-g*</i>	2.0 up	Unknown	3L
	<i>CG12378*</i>	2.0 up	Unknown	3R

	<i>Mucin 12Ea</i>	2.0 down	Unknown	X
	<i>CG10513</i>	2.4 down	Unknown	3R
	<i>Metallothionein C*</i>	3.1 down	Metal ion binding	3R

**Table S4:** Genes differentially expressed after single short cold exposure, but not in the flies experienced multiple and sustained cold exposures, with a description of associated biological processes or molecular functions, as well as their locations on *D. melanogaster* chromosomes (X, 2L, 2R, 3L, 2R, 4). Genes were classified based on their GO terms that were identified by DAVID. \* indicates differentially expressed genes among the three cold treatments after F-test (ANOVA, p-value < 0.05).

Gene Name	Fold change	Biological process and molecular function	Location
<i>Raptor</i>	2.1 down	Positive regulation of protein amino acid phosphorylation; response to DNA damage stimulus	X
<i>CG42574*</i>	2.1 down	Acid-amino acid ligase activity zinc ion binding; protein modification process	3R
<i>RhoGEF3</i>	2.2 down	Signal transducer activity; regulation of Rho protein signal transduction	3L
<i>CG11505*</i>	2.5 down	Nucleic acid binding	3L
<i>CG34415</i>	2.6 down	Regulation of transcription; DNA-dependent	2R

**Table S5:** Pathways identified by KEGG\_PATHWAY from genes differentially expressed six hours after repeated (five daily 2h exposures to -0.5 °C), prolonged (one 10h exposure to -0.5 °C), and single short (one 2h exposure to -0.5 °C) cold exposures by female adult *Drosophila melanogaster*.

Database	Pathway terms	Genes associated with pathway		
		Repeated	Prolonged	Single short
KEGG	Lysine degradation		<i>CG10814</i>	<i>CG10814</i>
	Lysosome	<i>CG9463</i>	<i>CG9463</i> <i>CG9466</i>	
	Purine metabolism		<i>Urate oxidase</i>	<i>Urate oxidase</i>
	Glycine	<i>CG6188</i>		
	Oxidative phosphorylation	<i>NADH-ubiquinone oxidoreductase chain 4</i>	<i>NADH-ubiquinone oxidoreductase chain 3</i>	
	p53 pathway	<i>CG3187</i> <i>Phosphatidylinositol 3 kinase 59F</i>		
	Ras pathway	<i>Phosphatidylinositol 3 kinase 59F</i>		<i>Raptor</i>
	Galactose metabolism		<i>CG11909</i> <i>Larval visceral protein L</i>	
	Biosynthesis of unsaturated fatty acids		<i>CG9747</i>	
	Glutathione metabolism		<i>Glutathione S transferase D2</i>	