

Supplementary Data

Molecular mechanisms underpinning transgenerational plasticity in the green sea urchin *Psammechinus miliaris*

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Supplementary Information S1: Assignment of the HSP annotations: HSP90 and HSP70. Alignments of the seven HSP70 gene family members identified from the transcriptome data annotated with domain motifs, with the information also tabulated below.

ClustalW alignments of the seven putative transcripts with sequence similarity to HSP70 gene family members. These are annotated with the following characteristic signature motifs as described in Rensing & Maier (1994): I: [IVL]-D-[LF]-G-T-T-x-S' II: D-[LF]-G(3)-T-F-D, III: [TS]-[VC]-P-A-[YN]-[FY]-N, IV: [NP]-[EG]-P-[TS]-A-A and V: R-A-[RK]-F-E-[ED]-[LM]. Also annotated: A = the hydrophobic linker site between the nuclear binding domain (NBD) and the substrate binding domain (SBD) (Zuiderweg *et al.*, 2013) and two N-linked glycosylation sites (NKSI and NVSA (consensus of N-X-S)) (Munro & Pelham, 1986; Laursen *et al.*, 1997). Although the region where the putative nuclear localisation signal domain is marked (NLS), none of the sequences have sufficient positively charged amino acids in this region to satisfy this motif. Also see Table below for identification of motifs associated with each transcript.

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Contig13435      1 -----
Contig37720     1 -----
Contig62830     1 -----MAKAPA
Contig62842     1 -----AKAPA
Contig62178     1 -----MRKLLMCMVMALSASLTLADDDDDSKKEVGTV
Contig44027     1 SKNSKNCLRGTSGCVRQISQQRHFSSVLKSTCSALNSDSRHHQRHPCRYASDTVRCVAV
Contig46238     1 -----
consensus      1 -----

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I

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Contig13435      1 -----
Contig37720     1 -----
Contig62830     7 VGIDLGTTYS CVGV FQHGKVEI IANDQGNRTTPSYVAFT-DTERLI GDAAKNQ TAMNPYN
Contig62842     6 VGIDLGTTYS CVGV FQHGKVEI IANDQGNRTTPSYVAFT-DTERLI GDAAKNQ TAMNPSN
Contig62178     34 VGIDLGTTYS CVGV FKNRVEI IANDQGNRTTPSYVAFTSDNERLI GDAAKNQ LTSNPEN
Contig44027     61 VGIDLGTTINS CVVA IMEGKTPKVI ENAEGARTTPSVVAFTKDDERLV GMPAKRQAVTNAKN
Contig46238     1 -----
consensus      61 .....

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Contig13435      1 -----
Contig37720     1 -----
Contig62830     66 TIE DAKRL IGRNFS DAAVQSDMEHWPF EIVINDNKKPKIQVEFKGEKKQFYAEEI SSMVLL
Contig62842     65 TIE DAKRL MGRNFT DSAVQSDMKHWPF EKVVDSSKKPKIEVEFKGEKKQFFAEEI SSMVLL
Contig62178     94 TVE DAKRL IGRNFD DSSVQHD I KYFPFKVMNKNSKPHVKVQVEGQDRIFAAEEI SAMVLT
Contig44027     121 TLYATKRL IGRRED DKETQKDI GTVSYETTKASSG---DAWVKANDKVYSPSQVGFVLT
Contig46238     1 -----
consensus      121 ...

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III

IV

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Contig13435      1 -----
Contig37720     1 -----PAYFNDAQRQETKDAGVIAGLNVLRIINEPTAAALAYGL
Contig62830     126 KMKETAENYLGKTVTD AVVTVPAYFNDSQRHATKDAGVISGLN I LRIINEPTAAALAYGL
Contig62842     125 KMKETAENYLGKTTIT DAVVTVPAYFNDSQRHATKDAGIISGLNVLRIINEPTAAALAYGL
Contig62178     154 KMKETAENYLGKVT HAVVTVPAYFNDAQRQATKDAGVIAGLNMRIINEPTAAALAYGM
Contig44027     178 KMKETAENYLGTPAKNAV VTVPAYFNDSQRQATKDAGQIAGLNVLRVINEPTAAALAYGM
Contig46238     1 --KETAEHOCGKETNS TV I TVPVDFSDNQRVLIKKAAEAAGFKILRLISDPSAALLAYQI
consensus      181 .....

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Identification of signature motifs in the seven putative transcripts with sequence similarity to HSP70 gene family members. Annotation notes: NBD = nuclear binding domain, SBD = substrate binding domain. None of the sequences contain a putative nuclear localisation signal, which comprises two positively charged amino acids separated by 10-11 amino acids from another block of five, which contains at least three positively charged amino acids.

Motif	Note on motif	Contigs							Reference
		13435	37720	62830	62842	62178	44027	46238	
[IVL]-D-[LF]-G-T-T-x-S	Signature I	-	-	√	√	√	√	-	Rensing & Maier (1994)
D-[LF]-G(3)-T-F-D	Signature II	X	√	√	√	√	√	X	Rensing & Maier (1994)
[TS]-[VC]-P-A-[YN]-[FY]-N	Signature III	-	-	√	√	√	√	X	Rensing & Maier (1994)
[NP]-[EG]-P-[TS]-A-A	Signature IV	-	√	√	√	√	√	X	Rensing & Maier (1994)
R-A-[RK]-F-E-[ED]-[LM]	Signature V: Characteristic of HSP70, HSC70 and GRP78	X	√	√	√	√	X	X	Rensing & Maier (1994)
KRK*.KDL**.RAL*R	Putative nuclear localisation signal	X	X	X	X	X	X	X	Rensing & Maier (1994)
[DE]-[VIL]-L-LD-V	Hydrophobic linker between NBD & SBD	X	X	√	√	√	√	-	Zuiderweg <i>et al.</i> (2013)
NKSI (N-X-S)	N-linked glycosylation site	√	√	√	√	X	X	-	Munro & Pelham (1986)
NVSA (N-X-S)	N-linked glycosylation site	X	X	√	-	X	√	-	Munro & Pelham (1986)

References:

- Laursen JR, di Liu H, Wu X-J, Yoshino TP (1997) Heat-shock response in a molluscan cell line: Characterisation of the response and cloning of an inducible HSP70 cDNA. *Journal of Invertebrate Pathology* **70**, 226-33.
- Munro S & Pelham HRB (1986) An Hsp70-like protein in the ER: Identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. *Cell* **46**, 291-300.
- Rensing SA & Maier U-G (1994) Phylogenetic analysis of the stress-70 protein family. *Journal of Molecular Evolution*. **38**, 80-86.
- Zuiderweg ERP, Bertelsen EB, Rousaki A, Mayer MP, Gestwicki JE, Ahmad A (2013) Allostery in the Hsp70 chaperone proteins. *Topics in Current Chemistry* **328**, 99-153.

Supplementary Information S2: Morphological measurements and abnormality rates in *P. miliaris* larvae cultured under normal and low pH conditions.

These data are taken from a study which was conducted alongside the experiments described in this paper. The data formed part of a Masters report produced by Clara L. Mackenzie entitled “Life in a high CO₂ world: The impacts of near-future ocean acidification to the growth, reproductive success and larval recruitment of *Psammechinus miliaris* (Echinodermata: Echinoidea) (2010) University of Bangor. Supervisors A.J. Davies and C.C. Suckling.

The same broodstock and aquarium systems were used as described in this paper, although only control acclimated adults (ambient pH) or two month low pH acclimated adults (End-Century pH) were used to produce larvae, which were then cultured to recruitment. These experiments provided comparative data on larval morphometrics and abnormality rates between the two conditions.

Larval morphometrics

Larval morphometrics were conducted at six time points, with measurements taken at each stage as defined below.

Table 1: Overview of egg and larval measurements used for comparing effects of pH on larval development.

DAY	DEVELOPMENTAL STAGE	n	MEASUREMENT
1	Egg	100	egg diameter
1	Egg	50-100	egg density (ml ⁻¹) fertilisation success
2	Blastula/Prism	30	hatching success length and width of early larvae
7	Echinopluteus	30	larval length and width, body length and width, stomach length and width, mouth width, post-oral arm length
14, 23, 29	Echinopluteus	30	larval length and width, body length and width, stomach length and width, mouth width, post-oral arm length, rudiment length

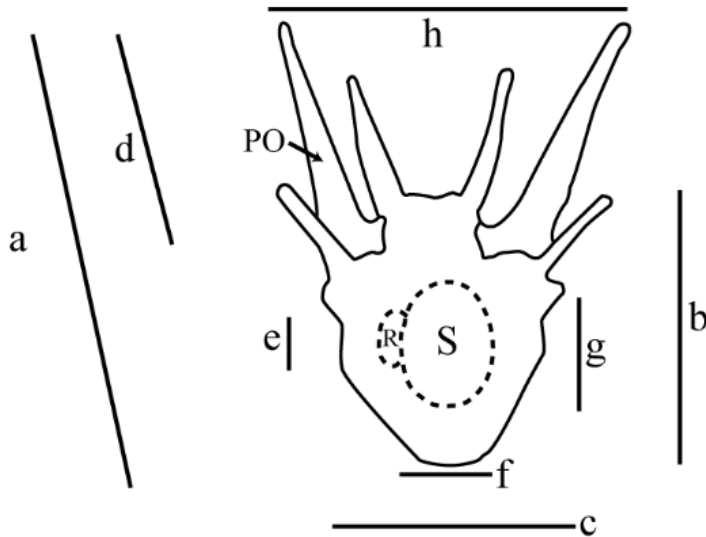


Figure 1: Morphometrics of *P. miliaris* larvae: a = total larval length, b = body length, c = body width, d = postoral arm length, e = rudiment length, f = stomach width, g = stomach length, h = total larval width. PO = postoral arm, R = rudiment, S = stomach (adapted from Kelly *et al.*, 2000).

Results of morphometric measurements

Only the results of certain time-points are shown to illustrate the points raised in the main discussion, namely the change in larval morphology with treatment over time and abnormality rates.

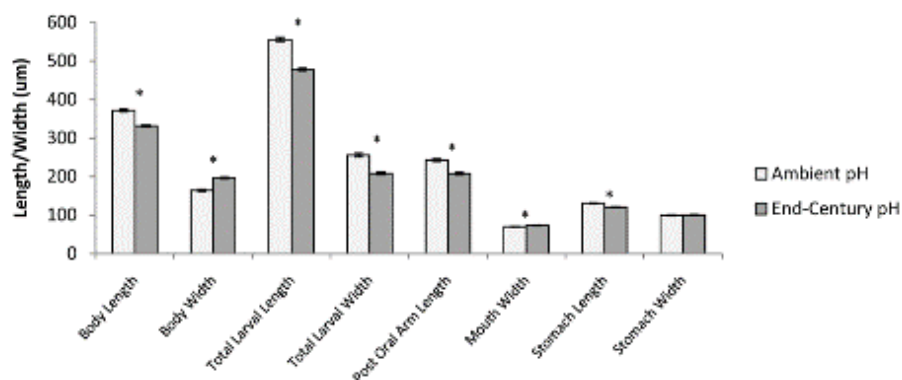


Figure 2: Day 7 larval morphometrics of *P. miliaris* larvae under ambient and elevated CO₂. * indicates a significant difference between larvae reared under ambient pH and End-century pH. Error bars indicate one standard error of the mean.

Day 7 ambient larvae had significantly greater body length-width ratios ($F_{1,174} = 194.300$, $p < 0.001$) and significantly lower larval length-width ratios ($F_{1,174} = 4.375$, $p = 0.038$) than those reared in high CO₂. Stomach length-width ratios did not vary significantly between the two treatment groups.

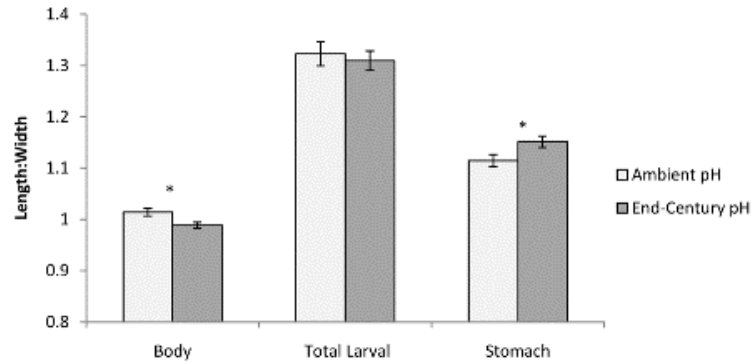


Figure 3: Day 23 gross larval morphometrics of *P. miliaris* larvae under ambient and elevated CO₂. * indicates a significant difference between larvae reared under ambient pH and End-century pH. Error bars indicate one standard error of the mean.

Day 23 high CO₂ larvae had significantly smaller body length-width ratios ($F_{1,174} = 8.035$, $p = 0.005$) but significantly larger stomach length-width ratios ($F_{1,174} = 5.465$, $p = 0.021$) than counterparts reared under ambient conditions. No significant difference was identified in total larval length-width ratios.

Abnormality rates in culture

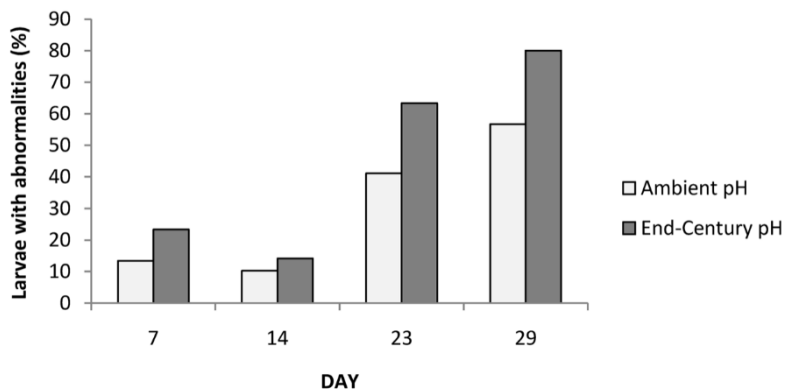


Figure 4: Percentage of larvae with abnormalities over larval development (Day) under ambient and End-Century pH conditions.

Larvae raised under End-Century pH conditions had an increased percentage of abnormalities (25-40%) than those raised under ambient conditions. The severity of abnormalities in End-Century conditions was more pronounced with missing arms, substantial length differences in arm length, asymmetric body shape etc. compared to ambient pH raised larvae, which had only minor length differences in arm pairs and minor imbalance to body shape.

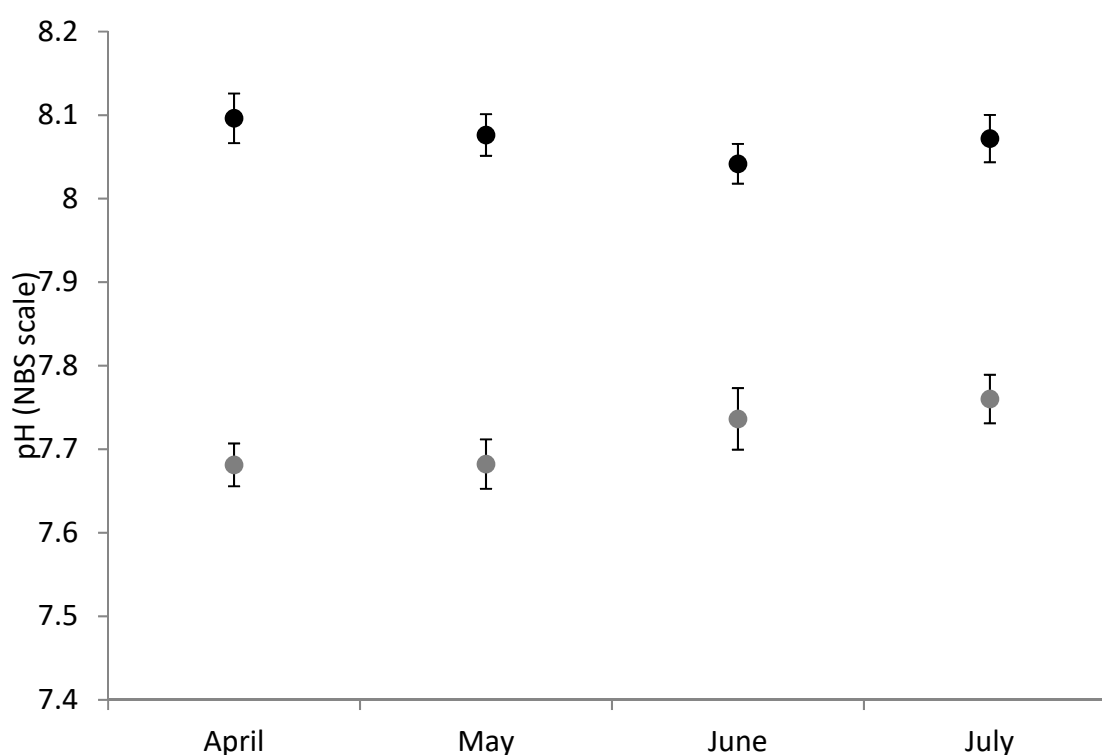
Reference

Kelly MS, Hunter AJ, Scholfield CL, McKenzie JD (2000) Morphology and survivorship of larval *Psammechinus miliaris* (Gmelin) (Echinodermata: Echinoidea) in response to varying food quantity and quality. *Aquaculture*, 183, 223-240.

Supplementary Information S3

Urchin culture

OA: Seawater temperature increased over the course of the adult acclimation period (from 10°C at the beginning of April to 16°C by the beginning of July) as expected with seasonal variability, but temperature compensated pH_{NIST} remained relatively constant for both treatments. Larvae were cultured in July at 16°C. In the End-Century pH trial, temperature and salinity values of seawater were not significantly different, while control pH was significantly higher than the End-Century pH treatment (Kruskal Wallis: pH: $H_{(599)} = 448.50$, $P < 0.001$; One way ANOVA: temperature: $F_{(1,592)} = 0.21$, $P = 0.648$; salinity: $F_{(1,485)} = 1.59$, $P = 0.208$) (Table 1; Supplementary Figure S1 (below)).



Supplementary Figure S1: Mean monthly pH values (\pm SE) in control (black circles) and low seawater pH (grey circles) trial.

There was no significant difference in the test diameters (TD) of the adult broodstock used in this trial between the treatments (Control (mean TD mm \pm SD) 31.03 ± 1.19 and End-Century 32.08 ± 2.06) and there were no mortalities of adults during the two month acclimation period.

Heat shock trial: High temperature treatments were significantly higher than control temperatures (One way ANOVA: $F_{(1,17)} = 2148.70$, $P < 0.001$) and salinity values remained the same throughout the experimental period. Adults which were heat shocked were not significantly different in size and mass (Control: TD (\pm SE) = 25.46 ± 1.58 mm, whole animal wet mass = 8.51 ± 1.54 g; High temperature: TD = 26.14 ± 2.24 mm, whole animal wet mass = 8.80 ± 2.28 g) (One Way ANOVA: TD: $F_{(1,9)} = 0.06$, $P = 0.810$; Wet mass: $F_{(1,9)} = 0.01$, $P = 0.919$).

Supplementary Table S1: Putative annotation of transcripts upregulated in CE2 experiment compared to Control samples. Lines annotated in yellow are those involved in the one main STRING network identified in these transcripts

SwissProt ID	Gene Symbol	Gene Name
Q9NRK6	ABCB10	ATP-binding cassette, sub-family B (MDR/TAP), member 10
P00813	ada	adenosine deaminase
Q9BV57	ADI1	acireductone dioxygenase 1
Q8NEM8	Agb13	ATP/GTP binding protein-like 3
Q7Z5J8	ankar	ankyrin and armadillo repeat containing
Q96B67	arrdc3	arrestin domain containing 3
Q9H2C2	ARV1	ARV1 homolog (S. cerevisiae)
Q9Y5Z6	B3galt1	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 1
O00555	Cacna1a	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
P62158	CALM1	calmodulin 3 (phosphorylase kinase, delta); calmodulin 2 (phosphorylase kinase, delta); calmodulin 1 (phosphorylase kinase, delta)
Q96PB1	casd1	CAS1 domain containing 1
P17787	CHRN2	cholinergic receptor, nicotinic, beta 2 (neuronal)
Q86X52	CHSY1	chondroitin sulfate synthase 1
Q14008	ckap5	cytoskeleton associated protein 5
Q95406	CNIH	cornichon homolog (Drosophila)
Q86VX2	commd7	COMM domain containing 7
Q9UJA2	CRLS1	cardiolipin synthase 1
Q9H467	CUEDC2	CUE domain containing 2
Q9NXE8	Cwc25	coiled-coil domain containing 49
Q9C098	DCLK3	doublecortin-like kinase 3
Q8NEL9	DDHD1	DDHD domain containing 1
Q8N7U6	EFHB	EF-hand domain family, member B
P11161	Egr2	early growth response 2
Q2NXX8	ERCC6L	excision repair cross-complementing rodent repair deficiency, complementation group 6-like
Q99502	Eya1	eyes absent homolog 1 (Drosophila)
Q9H5I5	Fam38b	family with sequence similarity 38, member B
Q14320	Fam50a	family with sequence similarity 50, member A
Q9UJT9	FBXL7	F-box and leucine-rich repeat protein 7
Q9UHY8	fez2	fasciculation and elongation protein zeta 2 (zygin II)
P49326	FM05	flavin containing monooxygenase 5
P15408	Fosl2	FOS-like antigen 2
Q9BQS8	Fyco1	FYVE and coiled-coil domain containing 1
Q9NXP7	GIN1	gypsy retrotransposon integrase 1
Q4V328	Gripap1	GRIP1 associated protein 1
P63132	HERVK113	Endogenous retrovirus group k member 113 polypolyprotein
Q8IY31	IFT20	intraflagellar transport 20 homolog (Chlamydomonas)
Q94829	IPO13	importin 13
Q6DN90	Iqsec1	IQ motif and Sec7 domain 1
Q8NAB2	KBTBD3	kelch repeat and BTB (POZ) domain containing 3
Q96J84	KIRREL	kin of IRRE like (Drosophila)
Q6ZMI0	KLRAQ1	KLRAQ motif containing 1
Q9BRS8	LARP6	La ribonucleoprotein domain family, member 6
O00370	LORF2	LINE-1 retrotransposable element
A6PVS8	Lrriq3	leucine-rich repeats and IQ motif containing 3
Q16706	MAN2A1	mannosidase, alpha, class 2A, member 1
P10636	mapt	microtubule-associated protein tau
Q16820	mep1b	mepirin A, beta
Q9UHC1	MLH3	mutL homolog 3 (E. coli)
Q8N119	MMP21	matrix metalloproteinase 21
Q9Y5R2	mmp24	matrix metalloproteinase 24 (membrane-inserted)
Q9UMX5	NENF	neuron derived neurotrophic factor
Q60500	NPHS1	nephrosis 1, congenital, Finnish type (nephrin)
Q9H668	OBFC1	oligonucleotide/oligosaccharide-binding fold containing 1
Q9H4Z3	PCIF1	PDX1 C-terminal inhibiting factor 1
Q13563	Pkd2	polycystic kidney disease 2 (autosomal dominant)
O00444	PLK4	polo-like kinase 4 (Drosophila)
O15162	plscr1	phospholipid scramblase 1
Q9ULJ8	ppp1r9a	protein phosphatase 1, regulatory (inhibitor) subunit 9A
Q9UJF2	RASAL2	RAS protein activator like 2
Q9NYR8	Rdh8	retinol dehydrogenase 8 (all-trans)
Q6NUQ1	rint1	RAD50 interactor 1
Q92753	RORB	RAR-related orphan receptor B
A6NKG5	RTL1	retrotransposon-like
Q96F10	SAT2	spermidine/spermine N1-acetyltransferase family member 2
Q9H792	SGK269	NKF3 kinase family member
Q8WUT9	Slc25a43	solute carrier family 25, member 43
Q8N370	Slc43a2	solute carrier family 43, member 2
Q8NBI5	Slc43a3	solute carrier family 43, member 3
Q43246	slc7a4	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4
Q6X4U4	SOSTDC1	sclerostin domain containing 1
Q86XZ4	Spats2	spermatogenesis associated, serine-rich 2
P18405	srd5a1	steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)
Q8WXE9	STON2	stonin 2
Q9UGT4	Susd2	sushi domain containing 2
O75478	Tada2a	transcriptional adaptor 2 (ADA2 homolog, yeast)-like
Q5VWG9	taf3	TAF3 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 140kDa
Q9Y6L7	TLL2	tolloid-like 2
P68371	TUBB2C	tubulin, beta 2C
P18206	vcl	vinculin
Q9NQW7	XPNPEP1	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble
Q8N883	ZNF614	zinc finger protein 614
Q6ZMW2	znf782	zinc finger protein 782

Supplementary Table S2: Putative annotation of transcripts upregulated in E2 experiment compared to control samples. Lines annotated with green and yellow represent those involved in the two main STRING networks identified in these transcripts

SwissProt ID	Gene Symbol	Gene Name
Q96QU6	ACCS	1-aminocyclopropane-1-carboxylate synthase homolog (Arabidopsis)(non-functional)
P22303	ache	acetylcholinesterase (Yt blood group)
Q9H981	actr8	ARP8 actin-related protein 8 homolog (yeast)
Q86UN6	Akap14	A kinase (PRKA) anchor protein 14
Q7Z5J8	ankar	ankyrin and armadillo repeat containing
P15144	ANPEP	alanyl (membrane) aminopeptidase
P55087	AQP4	aquaporin 4
P18848	ATF4C	activating transcription factor 4 (tax-responsive enhancer element B67); activat
P06276	bchE	butyrylcholinesterase
Q9BSD7	C10orf57	chromosome 10 open reading frame 58; chromosome 10 open reading frame 57
Q9BRX8	C10orf58	chromosome 10 open reading frame 58; chromosome 10 open reading frame 57
Q9BTY2	C1orf57	chromosome 1 open reading frame 57
Q8NHS4	C2orf63	chromosome 2 open reading frame 63
P62158	CALM3	calmodulin 3 (phosphorylase kinase, delta); calmodulin 2 (phosphorylase kinase,
Q15822	CHRNA2	cholinergic receptor, nicotinic, alpha 2 (neuronal)
Q9NPF2	CHST11	carbohydrate (chondroitin 4) sulfotransferase 11
Q9ULY5	Clec4e	C-type lectin domain family 4, member E
Q9Y6N1	COX11	COX11 homolog, cytochrome c oxidase assembly protein (yeast)
Q9H467	CUEDC2	CUE domain containing 2
Q86UP6	CUZD1	CUB and zona pellucida-like domains 1
Q9NXE8	Cwc25	coiled-coil domain containing 49
P08684	CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4
Q5H9S7	DCAF17	chromosome 2 open reading frame 37
Q9C098	DCLK3	doublecortin-like kinase 3
Q99489	DDO	D-aspartate oxidase
Q9NUL7	Ddx28	DEAD (Asp-Glu-Ala-Asp) box polypeptide 28
Q14147	DHX34	DEAH (Asp-Glu-Ala-His) box polypeptide 34
Q9UGM3	DMBT1	deleted in malignant brain tumors 1
O43854	edil3	EGF-like repeats and discoidin I-like domains 3
P11161	Egr2	early growth response 2
Q9BY44	Eif2a	eukaryotic translation initiation factor 2A, 65kDa
P21128	Endou	26 serine protease
Q6UWV6	ENPP7	ectonucleotide pyrophosphatase/phosphodiesterase 7
Q2NKX8	ERCC6L	excision repair cross-complementing rodent repair deficiency, complementation gr
O60427	Fads1	fatty acid desaturase 1
Q9UBU6	FAM8A1	family with sequence similarity 8, member A1
Q9NW38	fancl	Fanconi anemia, complementation group L
P57775	FBXW4	F-box and WD repeat domain containing 4
Q8N8Q3	flj35220	hypothetical protein FLJ35220
P49326	FMO5	flavin containing monooxygenase 5
P58012	Foxl2	forkhead box L2
Q9BX68	FUCA2	fucosidase, alpha-L- 2, plasma
Q11128	FUT5	fucosyltransferase 5 (alpha (1,3) fucosyltransferase)
P51993	FUT6	fucosyltransferase 6 (alpha (1,3) fucosyltransferase)
P50440	gatm	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
P04062	GBA	glucosidase, beta; acid (includes glucosylceramidase)
P19440	ggt1	gamma-glutamyltransferase light chain 3; gamma-glutamyltransferase 4 pseudogene;
Q8N2G8	GHDC	GH3 domain containing
Q9NXP7	GIN1	gypsy retrotransposon integrase 1
Q96SL4	GPX7	glutathione peroxidase 7
P42262	GRIA2	glutamate receptor, ionotropic, AMPA 2
Q13889	gtf2h3	general transcription factor IIH, polypeptide 3, 34kDa
P84243	h3f3a	H3 histone, family 3B (H3.3B); H3 histone, family 3A pseudogene; H3 histone, fam
Q9UJM8	HAO1	hydroxyacid oxidase (glycolate oxidase) 1
Q96MB7	HARBI1	harbinger transposase derived 1
Q9BY42	HINT2	histidine triad nucleotide binding protein 2
Q7LGA3	Hs2st1	heparan sulfate 2-O-sulfotransferase 1
P22304	ids	iduronate 2-sulfatase
Q8WV93	LACE1	lactation elevated 1

A6PVS8	Lrriq3	leucine-rich repeats and IQ motif containing 3
Q16820	mep1b	mepirin A, beta
Q6ZSS7	MFSD6	major facilitator superfamily domain containing 6
Q96AX9	mib2	mindbomb homolog 2 (Drosophila)
O75431	MTX2	metaxin 2
Q8WXR4	MYO3B	myosin IIIB
Q9Y3Q0	NAALAD2	N-acetylated alpha-linked acidic dipeptidase 2
Q8NBP7	pcsk9	proprotein convertase subtilisin/kexin type 9
Q13608	PEX6	peroxisomal biogenesis factor 6
O75928	PIAS2	protein inhibitor of activated STAT, 2
O00625	PIR	pirin (iron-binding nuclear protein)
Q13563	Pkd2	polycystic kidney disease 2 (autosomal dominant)
Q63HM9	PLCXD3	phosphatidylinositol-specific phospholipase C, X domain containing 3
Q9P215	Pogk	pogo transposable element with KRAB domain
Q9H1D9	Polr3f	polymerase (RNA) III (DNA directed) polypeptide F, 39 kDa
P15918	RAG1	recombination activating gene 1
Q8NHQ8	rassf8	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8
P78509	RELN	reelin
O00370	RNASSET2	ribonuclease T2
O00584	RNASSET2	ribonuclease T2
Q92753	RORB	RAR-related orphan receptor B
P46777	rpl5	ribosomal protein L5 pseudogene 34; ribosomal protein L5 pseudogene 1; ribosomal
P04843	RPN1	ribophorin I
A6NKG5	RTL1	retrotransposon-like
Q9HBX9	RXFP1	relaxin/insulin-like family peptide receptor 1
Q8WTV0	SCARB1	scavenger receptor class B, member 1
Q9HD40	sepsecs	Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase
Q9H173	SIL1	SIL1 homolog, endoplasmic reticulum chaperone (S. cerevisiae)
Q12908	SLC10A2	solute carrier family 10 (sodium/bile acid cotransporter family), member 2
Q9NY64	SLC2A8	solute carrier family 2 (facilitated glucose transporter), member 8
Q07837	Slc3a1	solute carrier family 3 (cystine, dibasic and neutral amino acid transporters, a
Q8NBI5	Slc43a3	solute carrier family 43, member 3
Q9GZV3	SLC5A7	solute carrier family 5 (choline transporter), member 7
O95238	SPDEF	SAM pointed domain containing ets transcription factor
P30874	Sstr2	somatostatin receptor 2
O75478	Tada2a	transcriptional adaptor 2 (ADA2 homolog, yeast)-like
Q5VWG9	taf3	TAF3 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 140kDa
Q8WUH2	TGFBRAP1	transforming growth factor, beta receptor associated protein 1
Q3ZCQ8	tim50	translocase of inner mitochondrial membrane 50 homolog (S. cerevisiae)
O43897	TLL1	tolloid-like 1
Q9Y6L7	TLL2	tolloid-like 2
Q53FA7	TP53I3	tumor protein p53 inducible protein 3
Q13748	TUBA3D	tubulin, alpha 3d; tubulin, alpha 3c
P68371	TUBB2C	tubulin, beta 2C
Q8TDH9	TXNDC5	thioredoxin domain containing 5 (endoplasmic reticulum); muted homolog (mouse)
P19971	Tymp	thymidine phosphorylase
Q8N6F8	wbscr27	Williams Beuren syndrome chromosome region 27
P47989	XDH	xanthine dehydrogenase
Q9NQW7	XPNPEP1	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble
Q8TBF4	ZCRB1	zinc finger CCHC-type and RNA binding motif 1
Q6ZMW2	znf782	zinc finger protein 782

Supplementary Table S3: Putative annotation of transcripts upregulated in CE3 experiment compared to control samples. Lines annotated with annotated with green and yellow represent those involved in the two main STRING networks identified in these transcripts

SwissProt ID	Gene Symbol	Gene Name
P12821	ACE	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1
Q09666	ahnak	AHNAK nucleoprotein
Q9NRL2	BAZ1A	bromodomain adjacent to zinc finger domain, 1A
Q6W2J9	bcor	BCL6 co-repressor
Q5VT06	CEP350	centrosomal protein 350kDa
Q12996	CSTF3	cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kDa
Q7Z449	CYP2U1	cytochrome P450, family 2, subfamily U, polypeptide 1
Q9Y2E4	dip2c	DIP2 disco-interacting protein 2 homolog C (Drosophila)
Q8WXX0	DNAH7	dynein, axonemal, heavy chain 7
Q92874	DNASE1L2	deoxyribonuclease I-like 2
Q9BQC3	DPH2	DPH2 homolog (S. cerevisiae)
Q9C0D9	Ept1	selenoprotein I
Q8IUD2	ERC1	ELKS/RAB6-interacting/CAST family member 1
O60447	EVI5	ecotropic viral integration site 5
Q6ZV73	Fgd6	FYVE, RhoGEF and PH domain containing 6
Q8N3X1	FNBP4	formin binding protein 4
P85037	Foxk1	forkhead box K1
P58012	Foxl2	forkhead box L2
Q9UQL6	HDAC5	histone deacetylase 5
P37235	hpcal1	hippocalcin-like 1
P35475	Idua	iduronidase, alpha-L-
O00370	LORF2	LINE-1 retrotransposable element
Q5SY16	NOL9	nucleolar protein 9
P46531	notch1	Notch homolog 1, translocation-associated (Drosophila)
Q15738	nsdhl	NAD(P) dependent steroid dehydrogenase-like
Q7Z3B4	NUP54	nucleoporin 54kDa
O15381	NVL	nuclear VCP-like
Q16825	PTPN21	protein tyrosine phosphatase, non-receptor type 21
Q8IZ41	Rasf	RAS and EF-hand domain containing
Q9UHR5	Sap30bp	SAP30 binding protein
Q9HD40	sepsecs	Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase
Q96BD0	SLCO4A1	solute carrier organic anion transporter family, member 4A1
Q6ZQN7	SLCO4C1	solute carrier organic anion transporter family, member 4C1
Q6Q759	SPAG17	sperm associated antigen 17
O95238	SPDEF	SAM pointed domain containing ets transcription factor
Q9H5L6	THAP9	THAP domain containing 9
Q86W42	THOC6	THO complex 6 homolog (Drosophila)
Q53HI1	UNC50	unc-50 homolog (C. elegans)
Q9Y5J1	utp18	UTP18, small subunit (SSU) processome component, homolog (yeast)
Q6RFH5	WDR74	WD repeat domain 74
Q8N5D0	WDTC1	WD and tetratricopeptide repeats 1
Q8N184	ZNF567	zinc finger protein 567
Q8IYH5	ZZZ3	zinc finger, ZZ-type containing 3

Supplementary Table S4: Putative annotation of transcripts upregulated in E3 experiment compared to control samples. Lines annotated with yellow, blue and green are those involved in the three main STRING networks identified in these transcripts

SwissProt ID	Gene Symbol	Gene Name
P33527	ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1
O00370	Abcc3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3
Q8TB40	abhd4	abhydrolase domain containing 4
Q9NPJ3	ACOT13	acyl-CoA thioesterase 13
Q09666	ahnak	AHNAK nucleoprotein
Q96NU7	amdhd1	amidohydrolase domain containing 1
Q99856	arid3a	AT rich interactive domain 3A (BRIGHT-like)
Q9H6L4	ARMC7	armadillo repeat containing 7
Q5FYB0	ARSJ	arylsulfatase family, member J
P08243	asnS	asparagine synthetase
P18848	ATF4	activating transcription factor 4 (tax-responsive enhancer element
O43505	b3gnt1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1; UDP-
Q99933	bag1	BCL2-associated athanogene
Q9NRL2	BAZ1A	bromodomain adjacent to zinc finger domain, 1A
Q6W2J9	bcor	BCL6 co-repressor
P35226	BMI1	BMI1 polycomb ring finger oncogene
P12643	bmp2	bone morphogenetic protein 2
Q08AG7	c13orf37	chromosome 13 open reading frame 37
Q63HM2	C14orf13	chromosome 14 open reading frame 135
Q9UJK0	c16orf42	chromosome 16 open reading frame 42
Q9BSD7	C1orf57	chromosome 1 open reading frame 57; NTPCR
Q9NVP4	C20orf12	chromosome 20 open reading frame 12
Q9NWX4	C4orf27	chromosome 4 open reading frame 27
O43598	C6orf108	chromosome 6 open reading frame 108
Q13951	Cbfb	core-binding factor, beta subunit
O75828	Cbr3	carbonyl reductase 3
Q9BW85	CCDC94	coiled-coil domain containing 94
Q9UEE9	cfdp1	craniofacial development protein 1
Q9H2X0	CHRD	chordin
Q9NPF2	CHST11	carbohydrate (chondroitin 4) sulfotransferase 11
Q9UJA2	CRLS1	cardiolipin synthase 1
Q12996	CSTF3	cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kDa
Q9UBR2	CTSZ	cathepsin Z
Q9NXE8	Cwc25	coiled-coil domain containing 49
Q16678	CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide 1
P10635	cyp2d6	cytochrome P450, family 2, subfamily D, polypeptide 6
P08684	CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4
Q99489	DDO	D-aspartate oxidase
Q7L014	ddx46	DEAD (Asp-Glu-Ala-Asp) box polypeptide 46
Q9H2U1	DHX36	DEAH (Asp-Glu-Ala-His) box polypeptide 36
Q8WXX0	DNAH7	dynein, axonemal, heavy chain 7
Q9NZQ0	DNAJC27	DnaJ (Hsp40) homolog, subfamily C, member 27
Q9ULA0	DNPEP	aspartyl aminopeptidase
Q9BQC3	DPH2	DPH2 homolog (<i>S. cerevisiae</i>)
O43854	edil3	EGF-like repeats and discoidin I-like domains 3
Q8N9N8	Eif1ad	eukaryotic translation initiation factor 1A domain containing
Q8IUD2	ERC1	ELKS/RAB6-interacting/CAST family member 1
Q03828	EVS2	even-skipped homeobox 2
Q96CS3	FAF2	Fas associated factor family member 2
Q6ZV73	Fgd6	FYVE, RhoGEF and PH domain containing 6

P07954	FH	fumarate hydratase
Q8N8Q3	flj35220	hypothetical protein FLJ35220
Q8N3X1	FNBP4	formin binding protein 4
P58012	Foxl2	forkhead box L2
Q14331	frg1	FSDH region gene 1
Q9NXP7	GIN1	gypsy retrotransposon integrase 1
P23378	Gldc	glycine dehydrogenase (decarboxylating)
Q13439	golga4	golgi autoantigen, golgin subfamily a, 4
Q9UHW5	Gpn3	GPN-loop GTPase 3
Q96SL4	GPX7	glutathione peroxidase 7
Q16772	GSTA3	glutathione S-transferase alpha 3
P78417	GSTO1	glutathione S-transferase omega 1
P30711	GSTT1	glutathione S-transferase theta 1
Q8N3Z3	Gtpbp8	GTP-binding protein 8 (putative)
Q96MB7	HARB11	harbinger transposase derived 1
Q7Z4H3	hddc2	HD domain containing 2
Q7Z4V5	Hdgrfp2	hepatoma-derived growth factor-related protein 2
Q9H0R4	hdhd2	haloacid dehalogenase-like hydrolase domain containing 2
P54198	HIRA	HIR histone cell cycle regulation defective homolog A (<i>S. cerevisia</i>)
Q99880	Hist1h2b	histone cluster 1, H2bl
P61978	Hnrnpk	heterogeneous nuclear ribonucleoprotein K; similar to heterogeneous
P08107	HSPA1A	heat shock 70kDa protein 1A; heat shock 70kDa protein 1B
O75874	IDH1	isocitrate dehydrogenase 1 (NADP+), soluble
P35475	Idua	iduronidase, alpha-L-
Q13123	Ik	similar to CG18005; IK cytokine, down-regulator of HLA II
Q96LU5	IMMP1L	IMP1 inner mitochondrial membrane peptidase-like (<i>S. cerevisiae</i>)
P10914	Irf1	interferon regulatory factor 1
Q7LBC6	KDM3B	lysine (K)-specific demethylase 3B
Q12767	KIAA0195	KIAA0195
Q99538	LGMN	legumain
Q9C099	LRRCC1	leucine rich repeat and coiled-coil domain containing 1
O95232	LUC7L3	cisplatin resistance-associated overexpressed protein
O60502	mgea5	meningioma expressed antigen 5 (hyaluronidase)
Q5JRA6	MIA3	melanoma inhibitory activity family, member 3
P22894	MMP8	matrix metalloproteinase 8 (neutrophil collagenase)
Q7Z2W9	MRPL21	mitochondrial ribosomal protein L21
Q8WY64	MYLIP	myosin regulatory light chain interacting protein
O95251	Myst2	MYST histone acetyltransferase 2; KAT7
Q96S42	NODAL	nodal homolog (mouse)
P46531	notch1	Notch homolog 1, translocation-associated (<i>Drosophila</i>)
Q96CB9	nsun4	NOL1/NOP2/Sun domain family, member 4
Q13608	PEX6	peroxisomal biogenesis factor 6
Q9P215	Pogk	pogo transposable element with KRAB domain
Q9H9Y6	Polr1b	polymerase (RNA) I polypeptide B, 128kDa
Q9BT43	Polr3gl	polymerase (RNA) III (DNA directed) polypeptide G (32kD)-like
O43172	PRP4	U4/U6 small ribonuclear protein PRP4
O15438	PRPF4	PRP4 pre-mRNA processing factor 4 homolog (yeast)
O94906	PRPF6	similar to U5 snRNP-associated 102 kDa protein (U5-102 kDa protein)
P23467	PTPRB	protein tyrosine phosphatase, receptor type, B
P15918	RAG1	recombination activating gene 1
Q86X10	RALGAPB	KIAA1219
Q8NHQ8	rassf8	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8
P98175	RBM10	RNA binding motif protein 10
Q8NDT2	RBM15B	RNA binding motif protein 15B
Q86U06	RBM23	RNA binding motif protein 23

P49756	RBM25	RNA binding motif protein 25
Q8NBN7	RDH13	retinol dehydrogenase 13 (all-trans/9-cis)
Q99496	rnf2	ring finger protein 2
A6NKG5	RTL1	retrotransposon-like
Q14151	SAFB2	scaffold attachment factor B2
Q9HD40	sepsecs	Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase
Q9NVD3	SETD4	SET domain containing 4
Q12908	SLC10A2	solute carrier family 10 (sodium/bile acid cotransporter family), m
Q8TF71	SLC16A10	solute carrier family 16, member 10 (aromatic amino acid tr
Q8IZD6	SLC22A15	solute carrier family 22, member 15
Q9Y345	SLC6A5	solute carrier family 6 (neurotransmitter transporter, glycine), me
Q96BD0	SLCO4A1	solute carrier organic anion transporter family, member 4A1
Q9H2Y9	slco5a1	solute carrier organic anion transporter family, member 5A1
P18583	SON	SON DNA binding protein
Q06945	Sox4	SRY (sex determining region Y)-box 4
O95238	SPDEF	SAM pointed domain containing ets transcription factor
Q96T58	spen	spen homolog, transcriptional regulator (<i>Drosophila</i>)
Q9GZT4	srr	serine racemase
Q9NP77	ssu72	SSU72 RNA polymerase II CTD phosphatase homolog (<i>S. cerevisiae</i>)
O00267	supt5h	suppressor of Ty 5 homolog (<i>S. cerevisiae</i>)
Q4LDE5	SVEP1	sushi, von Willebrand factor type A, EGF and pentraxin domain conta
O75528	TADA3	transcriptional adaptor 3 (NGG1 homolog, yeast)-like
Q7Z7C8	TAF8	TAF8 RNA polymerase II, TATA box binding protein (TBP)-associated f
Q9NXG2	THUMPD1	THUMP domain containing 1
Q9Y584	TIMM22	translocase of inner mitochondrial membrane 22 homolog (yeast)
Q53FA7	TP53I3	tumor protein p53 inducible protein 3
Q6PGP7	TTC37	tetratricopeptide repeat domain 37
P07437	tubB	tubulin, beta; similar to tubulin, beta 5; tubulin, beta pseudogene
P55851	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
Q8IWX7	UNC45B	unc-45 homolog B (<i>C. elegans</i>)
Q53HI1	UNC50	unc-50 homolog (<i>C. elegans</i>)
Q5SQQ9	VAX1	ventral anterior homeobox 1
P23381	wars	tryptophanyl-tRNA synthetase
Q5VUA4	ZNF318	zinc finger protein 318
O94892	ZNF432	zinc finger protein 432
Q8N184	ZNF567	zinc finger protein 567
Q5TYW1	ZNF658	zinc finger protein 658

Supplementary Table S5: Putative annotation of transcripts upregulated in CE4 experiment compared to control samples. Most of the transcripts cluster in one big network so no STRING networks are annotated for this gene listing

SwissProt ID	Gene Symbol	Gene Name
O95477	ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1
O15439	abcc4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4
Q8IZP0	Abi1	abl-interactor 1
Q12979	ABR	active BCR-related gene
Q04771	acvr1	activin A receptor, type I
P55265	Adar	adenosine deaminase, RNA-specific
O60503	ADCY9	adenylate cyclase 9
Q9UPQ3	AGAP1	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1
O00468	AGRN	agrin
Q9UPW5	AGTPBP1	ATP/GTP binding protein 1
Q8N6S4	Ankrd13c	ankyrin repeat domain 13C
O15084	ANKRD28	ankyrin repeat domain 28
P09525	anxa4	annexin A4
Q13367	AP3B2	adaptor-related protein complex 3, beta 2 subunit
Q92572	ap3s1	adaptor-related protein complex 3, sigma 1 subunit
Q8IW19	aplf	aprataxin and PNKP like factor
P61204	ARF3	ADP-ribosylation factor 3
Q9P2F6	ARHGAP20	Rho GTPase activating protein 20
Q9UNA1	arhgap26	Rho GTPase activating protein 26
P36405	Arl3	ADP-ribosylation factor-like 3
Q96B67	arrdc3	arrestin domain containing 3
Q6PL18	ATAD2	ATPase family, AAA domain containing 2
Q2TAZ0	Atg2a	ATG2 autophagy related 2 homolog A (<i>S. cerevisiae</i>)
Q96BY7	ATG2B	ATG2 autophagy related 2 homolog B (<i>S. cerevisiae</i>)
Q9NT62	ATG3	ATG3 autophagy related 3 homolog (<i>S. cerevisiae</i>)
P38606	Atp6v1a	ATPase, H ⁺ transporting, lysosomal 70kDa, V1 subunit A
P21283	ATP6V1C1	ATPase, H ⁺ transporting, lysosomal 42kDa, V1 subunit
O43505	B3GNT2	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase
Q9UBX8	B4GALT6	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypep
O14874	BCKDK	branched chain ketoacid dehydrogenase kinase
P38398	BRCA1	breast cancer 1, early onset
Q9UID3	c11orf2	chromosome 11 open reading frame2
Q9Y4D8	c12orf51	chromosome 12 open reading frame 51
Q8IXR9	C12orf56	chromosome 12 open reading frame 56
Q96D70	c19orf22	chromosome 19 open reading frame 22
Q8WUQ7	C19orf29	chromosome 19 open reading frame 29
Q9NVP4	C20orf12	chromosome 20 open reading frame 12
Q5NDL2	c3orf64	chromosome 3 open reading frame 64
Q96LT7	C9orf72	chromosome 9 open reading frame 72
P00918	CA2	carbonic anhydrase II
Q08289	CACNB2	calcium channel, voltage-dependent, beta 2 subunit
Q96GE6	calml4	calmodulin-like 4
O15484	CAPN5	calpain 5
A2VCL2	CC162	coiled coil domain containing protein 162
Q9Y592	CCDC41	coiled-coil domain containing 41
Q8IWF9	CCDC83	coiled-coil domain containing 83
P30260	cdc27	cell division cycle 27 homolog (<i>S. cerevisiae</i>)

Q16543	cdc37	cell division cycle 37 homolog (<i>S. cerevisiae</i>)
P19022	CDH2	cadherin 2, type 1, N-cadherin (neuronal)
Q8N960	CEP120	centrosomal protein 120kDa
Q9UQN3	Chmp2b	chromatin modifying protein 2B
Q9BY43	CHMP4A	chromatin modifying protein 4A
Q9H444	CHMP4B	chromatin modifying protein 4B
Q9NZZ3	Chmp5	chromatin modifying protein 5
Q14008	ckap5	cytoskeleton associated protein 5
O94985	CLSTN1	calsyntenin 1
Q8N129	Cnpy4	canopy 4 homolog (zebrafish)
Q9Y215	COLQ	collagen-like tail subunit (single strand of homotrimer) of
Q9HCH3	CPNE5	copine V
P50416	Cpt1a	carnitine palmitoyltransferase 1A (liver)
Q5TZA2	Crocc	ciliary rootlet coiled-coil, rootletin
P41240	CSK	c-src tyrosine kinase
Q13948	CUX1	cut-like homeobox 1
Q9H720	CWH43	hypothetical protein FLJ21511
Q7L1T6	CYB5R4	cytochrome b5 reductase 4
Q9NQC7	CYLD	cyldromatosis (turban tumor syndrome)
P20813	CYP2B6	cytochrome P450, family 2, subfamily B, polypeptide 6
O95786	DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
Q8WYQ5	Dgcr8	DiGeorge syndrome critical region gene 8
Q8N5I4	DHRSX	dehydrogenase/reductase (SDR family) X-linked
Q96C10	dhx58	DEXH (Asp-Glu-X-His) box polypeptide 58
Q05193	DNM1	dynamamin 1
O00429	DNM1L	dynamamin 1-like
P50570	DNM2	dynamamin 2
Q9NRW4	dusp22	similar to mitogen-activated protein kinase phosphatase x;
Q16690	dusp5	dual specificity phosphatase 5
P42892	ECE1	endothelin converting enzyme 1
O14602	EIF1AY	eukaryotic translation initiation factor 1A, Y-linked
P26378	ELAVL4	ELAV (embryonic lethal, abnormal vision, <i>Drosophila</i>)-like 4
O94905	Erlin2	ER lipid raft associated 2
Q14674	ESPL1	extra spindle pole bodies homolog 1 (<i>S. cerevisiae</i>)
P14921	ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)
P50549	etv1	ets variant 1
Q96A65	EXOC4	exocyst complex component 4
Q9UNN5	FAF1	Fas (TNFRSF6) associated factor 1
Q9H5Z6	FAM124B	family with sequence similarity 124B
Q8N128	fam177a1	family with sequence similarity 177, member A1
Q9GZU8	fam192a	NEFA-interacting nuclear protein NIP30
Q8TBR7	FAM57A	family with sequence similarity 57, member A
Q5T7M9	fam69a	family with sequence similarity 69, member A
Q5HYJ3	fam76b	family with sequence similarity 76, member B
Q9UKA1	Fbx15	F-box and leucine-rich repeat protein 5
Q9UHY8	fez2	fasciculation and elongation protein zeta 2 (zygin II)
P21802	FGFR2	fibroblast growth factor receptor 2
Q8TF40	FNIP1	folliculin interacting protein 1
Q9P2Q2	Frmd4a	FERM domain containing 4A
Q8N475	Fstl5	follistatin-like 5
Q99999	GAL3ST1	galactose-3-O-sulfotransferase 1
Q96CN9	gcc1	GRIP and coiled-coil domain containing 1

O75603	GCM2	glial cells missing homolog 2 (Drosophila)
Q9NXN4	GDAP2	ganglioside induced differentiation associated protein 2
H0YKK7	GG6LS	putative golgin subfamily A member 6-like protein 19
Q5VSY0	Gkap1	G kinase anchoring protein 1
P60983	gmfb	glia maturation factor, beta
P09471	GNAO1	guanine nucleotide binding protein (G protein), alpha activ
Q9Y223	gne	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine
Q16774	GUK1	guanylate kinase 1
P42357	hal	histidine ammonia-lyase
P31268	HOXA7	homeobox A7
Q05084	Ica1	islet cell autoantigen 1, 69kDa
P46940	IQGAP1	IQ motif containing GTPase activating protein 1
Q6NYC1	JMJD6	jumonji domain containing 6
Q14678	KANK1	KN motif and ankyrin repeat domains 1; similar to ankyrin r
Q8IYT4	KATNAL2	katanin p60 subunit A-like 2
Q674X7	kaz	kazrin
Q9NS40	KCNH7	potassium voltage-gated channel, subfamily H (eag-related),
Q6PIL6	KCNIP4	Kv channel interacting protein 4
Q14500	KCNJ18	similar to hkir2.2x; similar to inward rectifying K+ channe
Q9UGI6	KCNN3	potassium intermediate/small conductance calcium-activated
O15550	Kdm6a	lysine (K)-specific demethylase 6A
O60303	kiaa0556	KIAA0556
Q9Y6X3	KIAA0892	KIAA0892
Q9HCE0	kiaa1632	KIAA1632
Q9BVG8	Kifc3	kinesin family member C3
Q9NSK0	KLC4	kinesin light chain 4
O00629	KPNA4	karyopherin alpha 4 (importin alpha 3)
Q9H9P8	L2HGDH	L-2-hydroxyglutarate dehydrogenase
P00338	LdhA	lactate dehydrogenase A
O43766	LIAS	lipoic acid synthetase
Q9Y561	LRP12	low density lipoprotein-related protein 12
O75581	LRP6	low density lipoprotein receptor-related protein 6
Q86X45	LRRC6	leucine rich repeat containing 6
Q9HD34	LYRM4	LYR motif containing 4
O15525	MAFG	v-maf musculoaponeurotic fibrosarcoma oncogene homolog G
Q9UNF1	MAGED2	melanoma antigen family D, 2
Q16706	MAN2A1	mannosidase, alpha, class 2A, member 1
P55145	manf	mesencephalic astrocyte-derived neurotrophic factor
Q99759	MAP3K3	mitogen-activated protein kinase kinase kinase 3
O43318	MAP3K7	mitogen-activated protein kinase kinase kinase 7
P28482	MAPK1	mitogen-activated protein kinase 1
Q8IW41	MAPKAPK5	mitogen-activated protein kinase-activated protein
P10636	mapt	microtubule-associated protein tau
P23508	MCC	mutated in colorectal cancers
Q7L590	Mcm10	minichromosome maintenance complex component 10
Q9HAF1	MEAF6	chromosome 1 open reading frame 149
Q9BV79	MECR	mitochondrial trans-2-enoyl-CoA reductase
Q14696	MESDC2	mesoderm development candidate 2
Q08431	MFGE8	milk fat globule-EGF factor 8 protein
Q09328	Mgat5	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucos
Q8N119	MMP21	matrix metalloproteinase 21
Q9Y5R2	mmp24	matrix metalloproteinase 24 (membrane-inserted)

P51948	Mnat1	menage a trois homolog 1, cyclin H assembly factor (Xenopus
Q9NZB8	Mocs1	molybdenum cofactor synthesis 1
O15442	MPPED1	metallophosphoesterase domain containing 1
Q5JR59	Mtus2	KIAA0774
Q15746	MYLK	myosin light chain kinase
O95544	nadK	NAD kinase
P54920	napA	N-ethylmaleimide-sensitive factor attachment protein, alpha
Q96PU5	Nedd4l	neural precursor cell expressed, developmentally down-regul
Q96PY6	nek1	NIMA (never in mitosis gene a)-related kinase 1
P21359	NF1	neurofibromin 1
Q15270	NKX1-1	NK1 homeobox 1
P43699	nkx2-1	NK2 homeobox 1
Q96PH1	nox5	NADPH oxidase, EF-hand calcium binding domain 5
P01111	NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
Q9BW91	nudt9	nudix (nucleoside diphosphate linked moiety X)-type motif 9
Q9NX40	ociad1	OCIA domain containing 1
O95897	olfm2	olfactomedin 2
O60313	Opa1	optic atrophy 1 (autosomal dominant)
Q92882	ostf1	osteoclast stimulating factor 1
Q6ZNF0	papL	iron/zinc purple acid phosphatase-like protein
Q9BYG4	PARD6G	par-6 partitioning defective 6 homolog gamma (C. elegans)
O14737	PDCD5	programmed cell death 5
Q9P0J1	Pdp1	pyruvate dehydrogenase phosphatase catalytic subunit 1
Q6ZMN7	Pdzrn4	PDZ domain containing ring finger 4
P08237	PFKM	phosphofructokinase, muscle
O15173	PGRMC2	progesterone receptor membrane component 2
Q96QT6	PHF12	PHD finger protein 12
Q9BUL5	Phf23	PHD finger protein 23
Q9UPP1	PHF8	PHD finger protein 8
P42338	Pik3cb	phosphoinositide-3-kinase, catalytic, beta polypeptide
P27986	Pik3r1	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)
Q9Y2I7	Pikfyve	phosphoinositide kinase, FYVE finger containing
P48426	PIP4K2A	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha
Q13393	PLD1	phospholipase D1, phosphatidylcholine-specific
Q9H8W4	plekhf2	pleckstrin homology domain containing, family F (with FYVE
A6NEE1	PLHD1	pleckstrin homology domain containing family D member 1
O15162	plscr1	phospholipid scramblase 1
O75051	Plxna2	plexin A2
P51805	Plxna3	plexin A3
P06746	polB	polymerase (DNA directed), beta
Q9HCU8	POLD4	polymerase (DNA-directed), delta 4
Q9NRF9	POLE3	polymerase (DNA directed), epsilon 3 (p17 subunit)
Q03181	Ppard	peroxisome proliferator-activated receptor delta
Q16537	PPP2R5E	protein phosphatase 2, regulatory subunit B', epsilon isofo
O43663	prc1	protein regulator of cytokinesis 1
Q05655	PRKCD	protein kinase C, delta
Q96LA8	PRMT6	protein arginine methyltransferase 6
Q14997	Psme4	proteasome (prosome, macropain) activator subunit 4
O43586	PSTPIP1	proline-serine-threonine phosphatase interacting protein 1
Q8N8N7	PTGR2	prostaglandin reductase 2
P43378	Ptpn9	protein tyrosine phosphatase, non-receptor type 9
Q16849	PTPRN	protein tyrosine phosphatase, receptor type, N

Q9UMZ3	ptprq	protein tyrosine phosphatase, receptor type, Q
P61026	RAB10	RAB10, member RAS oncogene family
P59190	RAB15	RAB15, member RAS oncogene family
Q9NP72	RAB18	RAB18, member RAS oncogene family
P62820	RAB1A	RAB1A, member RAS oncogene family
Q9UL25	rab21	RAB21, member RAS oncogene family
Q969Q5	RAB24	RAB24, member RAS oncogene family
P51148	rab5c	RAB5C, member RAS oncogene family
Q92930	RAB8B	RAB8B, member RAS oncogene family
P11233	RALA	v-ral simian leukemia viral oncogene homolog A (ras related
Q9UJF2	RASAL2	RAS protein activator like 2
Q0VAM2	RASGEF1B	RasGEF domain family, member 1B
P50749	RASSF2	Ras association (RalGDS/AF-6) domain family member 2
Q8WVW0	rassf5	Ras association (RalGDS/AF-6) domain family member 5
Q14206	RCAN2	regulator of calcineurin 2
P35241	rdx	radixin
P61586	Rhoa	ras homolog gene family, member A
Q9UQ26	RIMS2	regulating synaptic membrane exocytosis 2
Q96PX1	RNF157	ring finger protein 157
Q8IYW5	rnf168	ring finger protein 168
Q96GF1	RNF185	ring finger protein 185
Q99496	rnf2	ring finger protein 2
Q96LT9	RNPC3	RNA-binding region (RNP1, RRM) containing 3
O75582	RPS6KA5	ribosomal protein S6 kinase, 90kDa, polypeptide 5
Q8WVW3	RTN4IP1	reticulon 4 interacting protein 1
Q9HBX9	RXFP1	relaxin/insulin-like family peptide receptor 1
Q8WVM8	SCFD1	sec1 family domain containing 1
O76054	Sec14I2	SEC14-like 2 (<i>S. cerevisiae</i>)
O43236	SEPT4	septin 4
Q16181	SEPT7	septin 7
Q9NZV6	SEPX1	selenoprotein X, 1
Q96HU1	SGSM3	small G protein signaling modulator 3
P29353	SHC1	SHC (Src homology 2 domain containing) transforming protein
Q8IXJ6	Sirt2	sirtuin (silent mating type information regulation 2 homolo
O75563	skap2	src kinase associated phosphoprotein 2
Q16348	Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2
Q9Y226	SLC22A13	solute carrier family 22 (organic anion transporter
Q8NEW0	SLC30A7	solute carrier family 30 (zinc transporter), member 7
Q8IWA5	SLC44A2	solute carrier family 44, member 2
Q9Y2W3	Slc45a1	solute carrier family 45, member 1
Q96NT5	Slc46a1	solute carrier family 46 (folate transporter), member 1
Q2M3M2	Slc5a9	solute carrier family 5 (sodium/glucose cotransporter), mem
Q9UN76	Slc6a14	solute carrier family 6 (amino acid transporter), member 14
Q9H2G2	SLK	STE20-like kinase (yeast)
Q9UPR3	smg5	Smg-5 homolog, nonsense mediated mRNA decay factor (<i>C. elegans</i>)
Q86US8	SMG6	Smg-6 homolog, nonsense mediated mRNA decay factor (<i>C. eleg</i>
Q9H4F8	SMOC1	SPARC related modular calcium binding 1
P53814	smtn	smoothelin
P60880	SNAP25	synaptosomal-associated protein, 25kDa
Q9NRS6	snx15	sorting nexin 15
Q6X4U4	SOSTDC1	sclerostin domain containing 1
Q02446	SP4	Sp4 transcription factor

O43791	SPOP	speckle-type POZ protein
Q9GZT4	srr	serine racemase
Q8WVM7	STAG1	stromal antigen 1
Q92783	stam	signal transducing adaptor molecule (SH3 domain and ITAM mo
Q9Y2H1	stk38l	serine/threonine kinase 38 like
P61764	STXBP1	syntaxin binding protein 1
Q9H7L9	suds3	suppressor of defective silencing 3 homolog (<i>S. cerevisiae</i>)
Q9H5I1	suv39h2	suppressor of variegation 3-9 homolog 2 (<i>Drosophila</i>)
O95926	Syf2	SYF2 homolog, RNA splicing factor (<i>S. cerevisiae</i>)
P57105	synj2bp	synaptojanin 2 binding protein
Q86SS6	SYT9	synaptotagmin IX
Q96C24	SYTL4	synaptotagmin-like 4
Q5VWG9	taf3	TAF3 RNA polymerase II, TATA box binding protein (TBP)-asso
Q9Y6I9	Tex264	testis expressed 264
Q92481	TFAP2B	transcription factor AP-2 beta (activating enhancer binding
Q96J01	THOC3	similar to THO complex 3; THO complex 3
Q8NBT3	Tmem145	transmembrane protein 145
P82094	TMF1	TATA element modulatory factor 1
P28289	TMOD1	tropomodulin 1
Q8IUR5	TMTC1	transmembrane and tetratricopeptide repeat containing 1
P55327	TPD52	tumor protein D52
Q86SZ2	TRAPPC6B	trafficking protein particle complex 6B
P36406	trim23	tripartite motif-containing 23
Q13507	Trpc3	transient receptor potential cation channel, subfamily C, m
Q8TEL6	TRPC4AP	transient receptor potential cation channel, subfamily C, m
Q8NG11	TSPAN14	tetraspanin 14
Q9BWW7	TTL2	tubulin tyrosine ligase-like family, member 2
Q8WZ42	TTN	titin
O95551	Ttrap	TRAF and TNF receptor associated protein
Q8TDH9	TXNDC5	thioredoxin domain containing 5 (endoplasmic reticulum); mu
Q9Y2X8	ube2d4	ubiquitin-conjugating enzyme E2D 4 (putative)
P62256	Ube2h	ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)
P61086	UBE2K	ubiquitin-conjugating enzyme E2K (UBC1 homolog, yeast)
Q712K3	ube2r2	ubiquitin-conjugating enzyme E2R 2
Q13404	Ube2v1	ubiquitin-conjugating enzyme E2 variant 1; ubiquitin-conjug
Q8IWX7	UNC45B	unc-45 homolog B (<i>C. elegans</i>)
O75445	USH2A	Usher syndrome 2A (autosomal recessive, mild)
Q9Y6I4	usp3	ubiquitin specific peptidase 3
Q96K76	usp47	ubiquitin specific peptidase 47
Q93009	Usp7	ubiquitin specific peptidase 7 (herpes virus-associated)
Q9H269	VPS16	vacuolar protein sorting 16 homolog A (<i>S. cerevisiae</i>)
Q96AX1	vps33a	vacuolar protein sorting 33 homolog A (<i>S. cerevisiae</i>)
O75351	VPS4B	vacuolar protein sorting 4 homolog B (<i>S. cerevisiae</i>)
Q9BZH6	WDR11	bromodomain and WD repeat domain containing 2
Q8NEZ3	WDR19	WD repeat domain 19
Q9P2L0	WDR35	WD repeat domain 35
Q9Y2I8	WDR37	WD repeat domain 37
Q9Y4E6	WDR7	WD repeat domain 7
P41221	WNT5A	wingless-type MMTV integration site family, member 5A
P47989	XDH	xanthine dehydrogenase
Q9UBH6	XPR1	xenotropic and polytropic retrovirus receptor
Q9Y2Z4	Yars2	tyrosyl-tRNA synthetase 2, mitochondrial

O15498	YKT6	YKT6 v-SNARE homolog (<i>S. cerevisiae</i>)
P62699	YPEL5	yippee-like 5 (<i>Drosophila</i>)
Q9HBF4	ZFYVE1	zinc finger, FYVE domain containing 1
Q9C0D3	ZYG11B	zyg-11 homolog B (<i>C. elegans</i>)

Supplementary Table S6: Putative annotation of transcripts upregulated in E4 experiment compared to control samples. Most transcripts cluster in one big network so no STRING networks are annotated in this gene listing

SwissProt ID	Gene Symbol	Gene Name
O95477	ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1
O15439	abcc4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4
O60706	abcc9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
Q12979	ABR	active BCR-related gene
P28330	acadi	acyl-Coenzyme A dehydrogenase, long chain
Q9H3P7	ACBD3	acyl-Coenzyme A binding domain containing 3
O14734	ACOT8	acyl-CoA thioesterase 8
Q96GR2	Acsbg1	acyl-CoA synthetase bubblegum family member 1
Q9NUB1	acss1	acyl-CoA synthetase short-chain family member 1
P68133	ACTA1	actin, alpha 1, skeletal muscle
Q562R1	Actbl2	actin, beta-like 2
P00813	ada	adenosine deaminase
P55265	Adar	adenosine deaminase, RNA-specific
O60503	ADCY9	adenylate cyclase 9
Q96SZ5	Ado	2-aminoethanethiol (cysteamine) dioxygenase
Q9Y4W6	AFG3L2	AFG3 ATPase family gene 3-like 2 (yeast)
Q8NEM8	Agbl3	ATP/GTP binding protein-like 3
O00468	AGRN	agrin
Q9BRQ8	aifm2	apoptosis-inducing factor, mitochondrion-associated, 2
Q96NN9	aifm3	apoptosis-inducing factor, mitochondrion-associated, 3
P00568	ak1	adenylate kinase 1
P13716	Alad	aminolevulinate, delta-, dehydratase
P05091	ALDH2	aldehyde dehydrogenase 2 family (mitochondrial)
Q02252	Aldh6a1	aldehyde dehydrogenase 6 family, member A1
P49419	ALDH7A1	aldehyde dehydrogenase 7 family, member A1
P04075	ALDOA	aldolase A, fructose-bisphosphate
P09972	aldoc	aldolase C, fructose-bisphosphate
P18054	ALOX12	arachidonate 12-lipoxygenase
P09917	ALOX5	arachidonate 5-lipoxygenase
Q96NU7	amdhd1	amidohydrolase domain containing 1
Q9BS18	ANAPC13	anaphase promoting complex subunit 13
Q9UJX3	anapc7	anaphase promoting complex subunit 7
Q7Z5J8	ankar	ankyrin and armadillo repeat containing
Q9UPS8	ANKRD26	ankyrin repeat domain 26
O15084	ANKRD28	ankyrin repeat domain 28
Q5TZF3	ANKRD45	ankyrin repeat domain 45
Q9NQW6	Anln	anillin, actin binding protein
Q9UPM8	Ap4e1	adaptor-related protein complex 4, epsilon 1 subunit
Q8IW19	aplf	aprataxin and PNKP like factor
Q8WZ64	Arap2	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2
P84077	Arf1	ADP-ribosylation factor 1
P61204	ARF3	ADP-ribosylation factor 3
Q9P2F6	ARHGAP20	Rho GTPase activating protein 20
Q15052	ARHGEF6	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6
Q9Y4X5	ARIH1	ariadne homolog, ubiquitin-conjugating enzyme E2 binding protein, 1 (Drosophila)
Q8NEN0	ARMC2	armadillo repeat containing 2
Q96QS3	ARX	aristaless related homeobox
Q8IZT6	ASPM	asp (abnormal spindle) homolog, microcephaly associated (Drosophila)
O94817	Atg12	ATG12 autophagy related 12 homolog (S. cerevisiae)
Q2TAZ0	Atg2a	ATG2 autophagy related 2 homolog A (S. cerevisiae)
O75366	AVIL	advillin
Q9Y5Z6	B3galt1	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 1
Q43505	b3gnt1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1; UDP-GlcNAc:betaGal beta-1,3-N-acetylgluc
Q99933	bag1	BCL2-associated athanogene
Q8TBE0	BAHD1	bromo adjacent homology domain containing 1
O75531	BANF1	similar to barrier-to-autointegration factor; barrier to autointegration factor 1
Q07812	BAX	BCL2-associated X protein
Q9H6U6	BCAS3	breast carcinoma amplified sequence 3
O14874	BCKDK	branched chain ketoacid dehydrogenase kinase
Q07817	BCL2L1	BCL2-like 1
P12643	bmp2	bone morphogenetic protein 2
Q12981	BNIP1	BCL2/adenovirus E1B 19kDa interacting protein 1
P38398	BRCA1	breast cancer 1, early onset

Supplementary Table S7: Primer sequences used in Q-PCR analysis

Contig identifier	Primer sequences ^a	Amplicon size (bp)	Samples	Amplification efficiency	R ²
13435	F: 5'-ACCGACCTTGACATGACAGT-3' R: 5'-AGAAACCCCTGGAGAAGCTC-3'	191	Larvae ^b	Day 2: 98% Day 4: 128%	0.98
			Adults	125%	0.99
37720	F: 5'-TCGCACTGCGGCTGAACGA-3' R: 5'-CATCTTTGCATCAAGAATCGCC-3'	190	Larvae	101%	0.99
			Adults	112%	0.99
44027	F: 5'-CTTCAAGTGGAGATGCGTGG-3' R: 5'-CAATCCTGCAATCTGACCGG-3'	203	Larvae	102%	0.99
			Adults	80%	0.99
46238	F: 5'-GACCATCAACTCCTCCACCA-3' R: 5'-GGAATGCGACTTGATCCACC-3'	207	Larvae	99%	0.98
			Adults	119%	0.96
51464	F: 5'-GGGCTAGTAAGACGGAGACC-3' R: 5'-TCTGTGATGTCCTTGGGCAT-3'	217	Larvae	95%	0.99
			Adults	100%	0.97
52063	F: 5'-GACCTTATCGACACGGGAA-3' R: 5'-AGGCGGAGTAGAAACCAACA-3'	158	Larvae	91%	0.98
			Adults	109%	0.99
62178	F: 5'-TCCACAGCTGCAGATAACCA-3' R: 5'-CTTGTCTTCGGCGCTTACTC-3'	195	Larvae	105%	0.99
			Adults	99%	0.99
62830	F: 5'-CATCACTGCTAACAAAGAGCA-3' R: 5'-ATTTGGAGTCACTGAGAGCTT-3'	218	Larvae	104%	0.99
			Adults	111%	0.99
62842	F: 5'-ACAAGAAGGACATCTTACCA-3' R: 5'-TCCCTAGCTTGGAGTCGTTG-3'	236	Larvae	115%	0.99
			Adults	101%	0.99
18s rRNA	F: 5'-AGTTGGATCTTGGGTCCAGG-3' R: 5'-CCAACGCAATAGAACCGAGG-3'	209	Larvae	111%	0.99
			Adults	96%	0.99

^a F: forward primer; R: reverse primer.

^b A different Day 4 samples-enriched pool was used for Day 4 samples (hence the different efficiency) in order to include one outlier whose C_q were slightly outside the dynamic range of the standard curve prepared with the pool comprising all larval samples.

Supplementary Table S8: Raw Cqs of the reference gene (*18S*) used in the qPCR data analysis.

Developmental stage	Treatment	Sample	Raw Cq
Adults	Control	1	12.06673
		2	12.82645
		3	11.37784
		4	11.80275
		5	12.36306
	Heat shock	6	11.49351
		7	12.60066
		8	12.08099
		9	12.33766
		10	11.9397
2 dpf larvae	Control	1	19.19702
		2	18.51549
		3	19.74385
	Heat shock	4	17.96737
		5	17.41903
		6	18.91465
4 dpf larvae	Control	1	10.68156
		2	14.73164
		3	15.18901
	Heat shock	4	10.74604
		5	14.69566
		6	15.74854