

Transcriptome analysis of the anhydrobiotic cell line Pv11 infers the mechanism of desiccation tolerance and recovery

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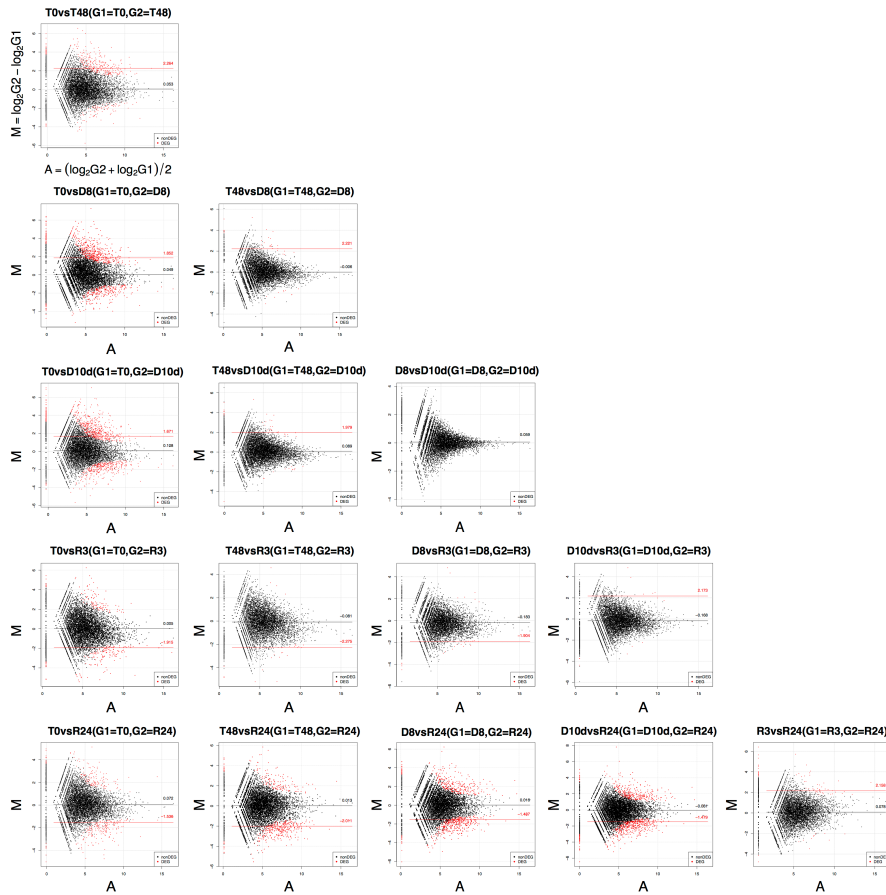
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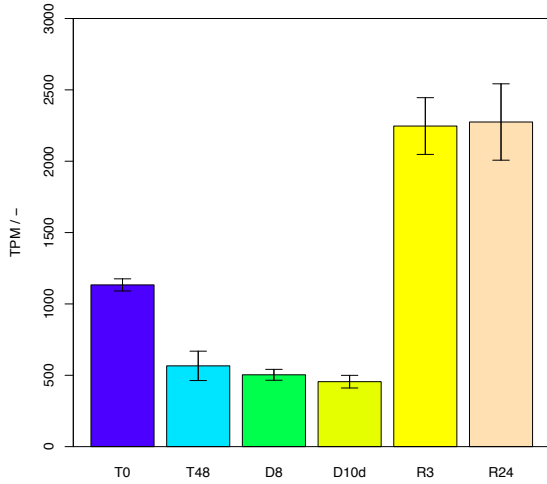
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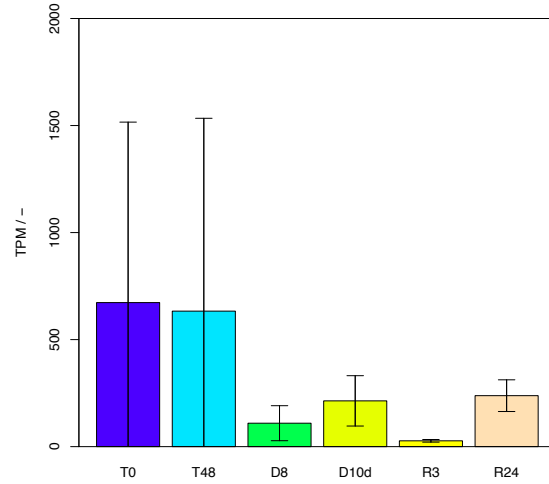
†Deceased



Supplementary Figure S1. M-A plots of differentially expressed genes (DEGs) for each pair of samples. The M value indicates the log ratio (sample group 2 (G2) relative to sample group 1 (G1), $\log_2 G_2 - \log_2 G_1$). The A value indicates the average expression of a gene in samples G1 and G2 ($(\log_2 G_2 + \log_2 G_1)/2$). The median M values are indicated by black lines for non-DEGs and red lines for DEGs.

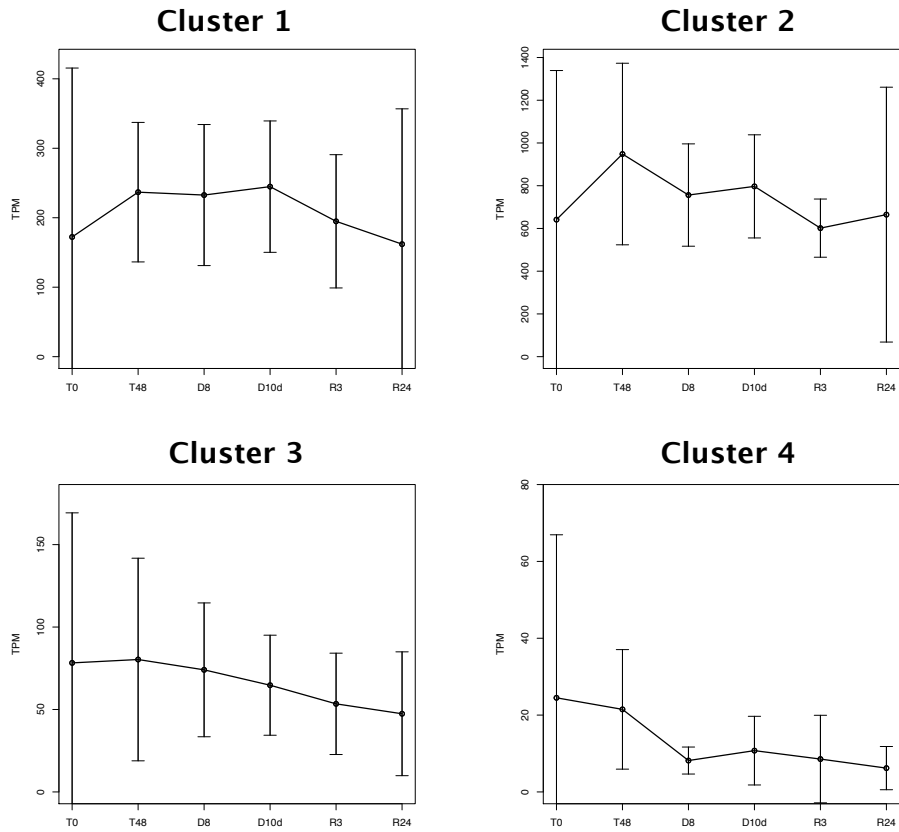


Pv.01867

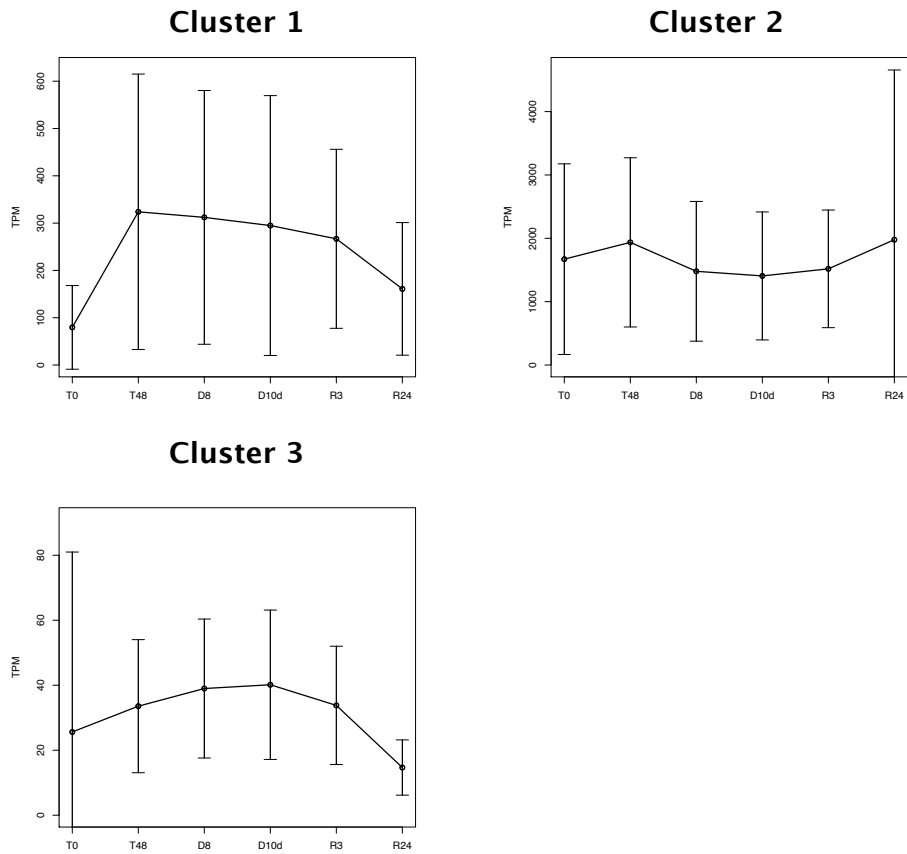


Pv.04558

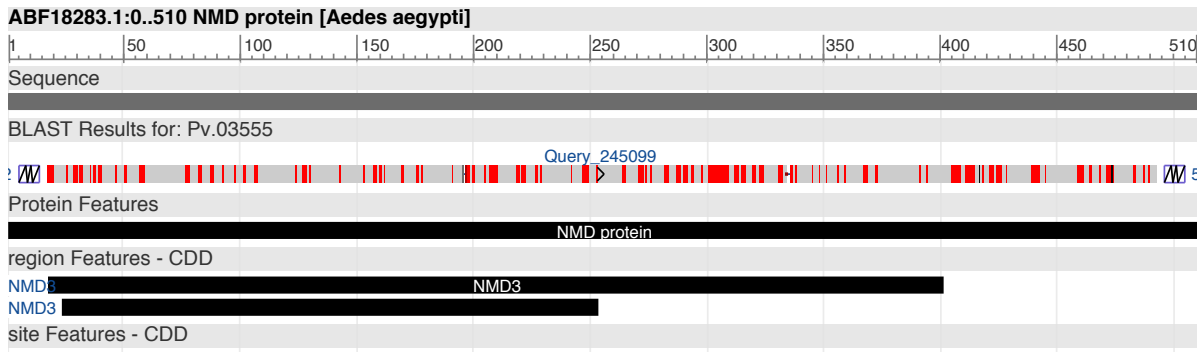
Supplementary Figure S2. Expression of Pv.01867 and Pv.04558. Horizontal axis shows sample names. TPM, tags per million. T0, control Pv11 cells. T48, trehalose treatment for 48 h. D8, desiccation for 8 h. D10d, desiccation for 10 days. R3, rehydration of D10d cells for 3 h. R24, rehydration of D10d cells for 24 h. Data are mean \pm SD, n=3.



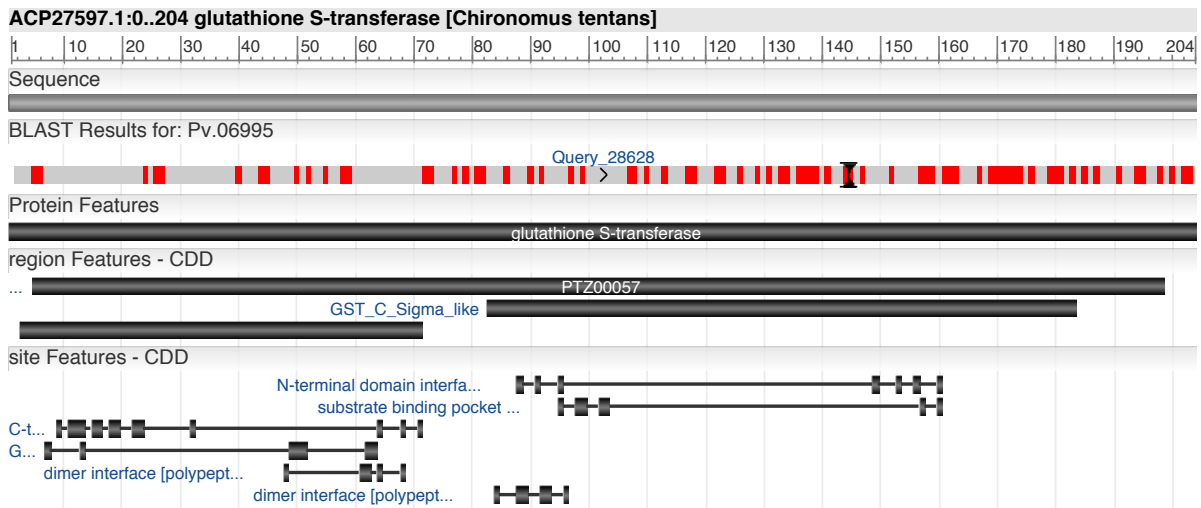
Supplementary Figure S3. Time course of the expression of DEGs annotated by GO:0008152 (metabolic process) (mean \pm SD).



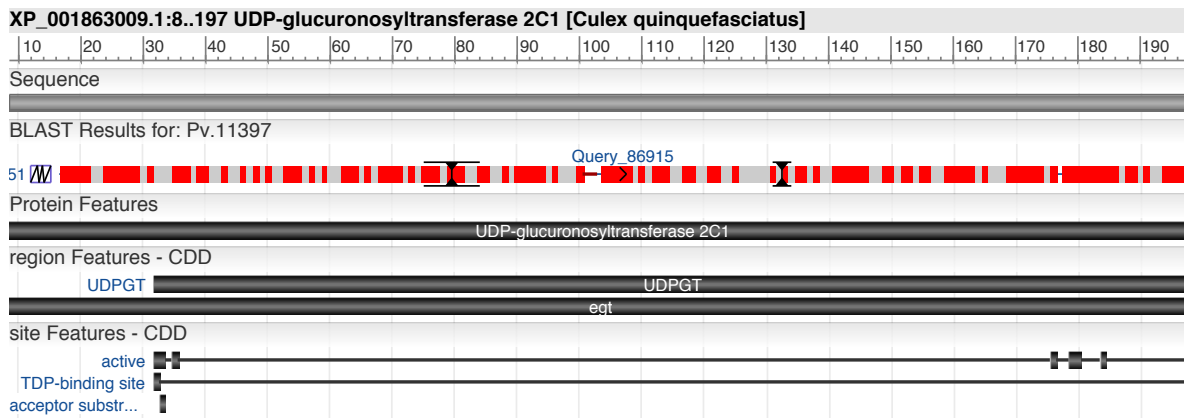
Supplementary Figure S4. Time course of the expression of DEGs annotated by GO:0055114 (oxidation-reduction process) (mean \pm SD).



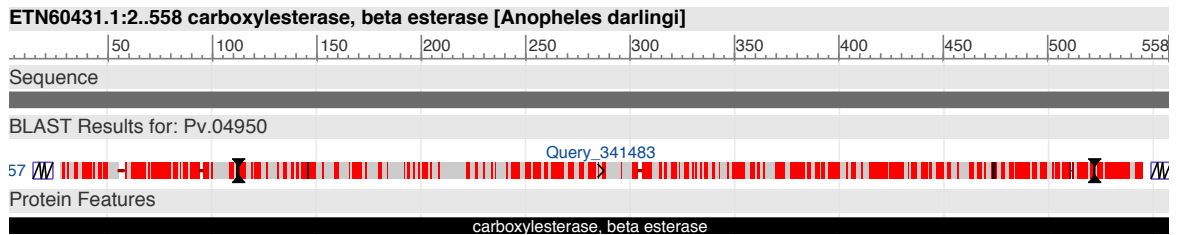
Supplementary Figure S5. Alignment of Pv.03555 with nonsense-mediated mRNA decay (NMD) protein. The visualization was created by using MSASviewer (<https://www.ncbi.nlm.nih.gov/projects/msaviewer/>). Sequence: sequence of the subject gene; Blast Results: alignment results between the *P. vanderplanki* gene and the subject gene by BLASTX; Protein Features, specific regions of the proteins; region Features, specific regions of the subject gene; site Features, specific sites of the subject gene. Under BLAST Results, gaps are represented as thick, dark-red, horizontal lines; insertions as black vertical bars and black arrowheads; and aligned regions as grey bars (match regions) or red bars (mismatch regions). The regions and sites of the subject gene that overlapped with those of the aligned *P. vanderplanki* gene were considered present.



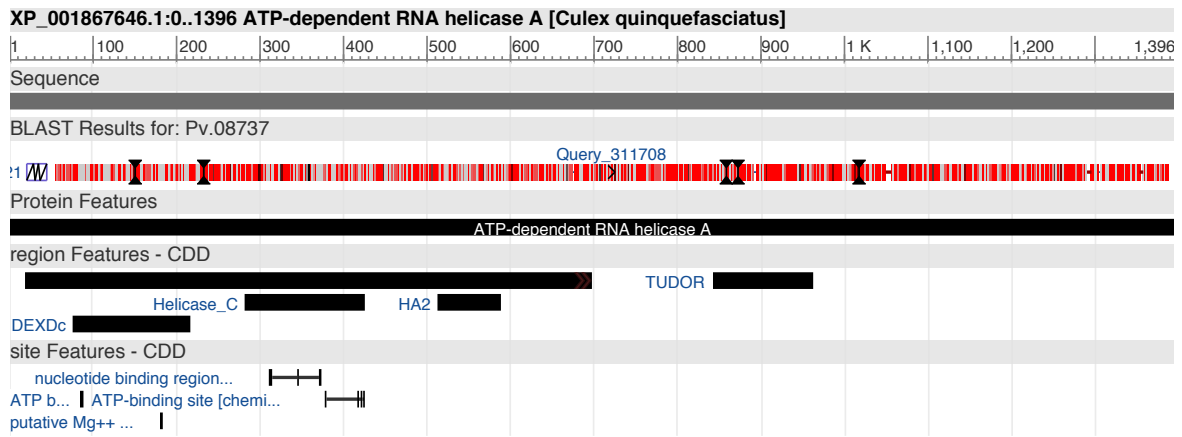
Supplementary Figure S6. Alignment of Pv.06995 with glutathione S-transferase.



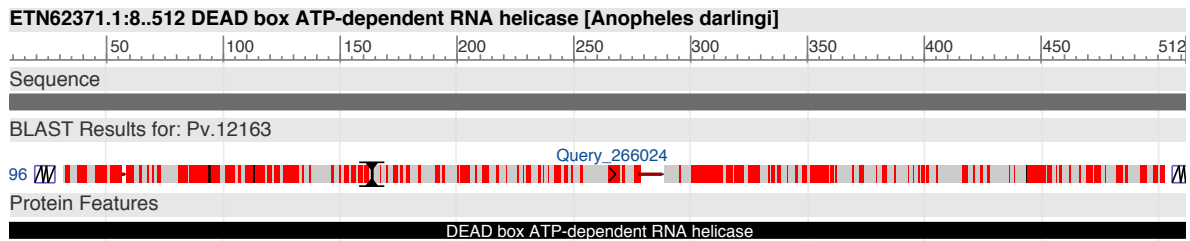
Supplementary Figure S7. Alignment of Pv.11397 with UDP-glucuronosyltransferase 2C1.



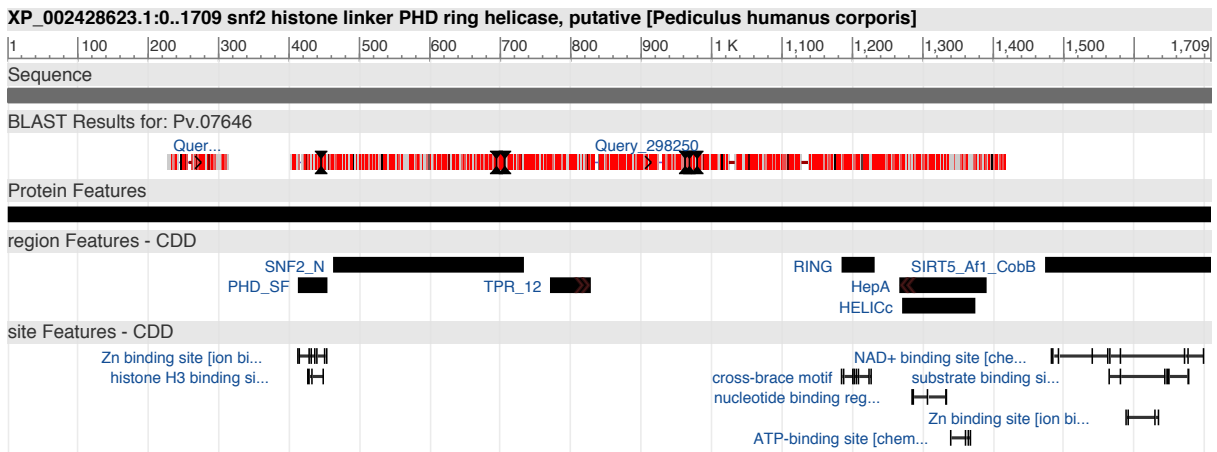
Supplementary Figure S8. Alignment of Pv.04950 with carboxylesterase, beta esterase.



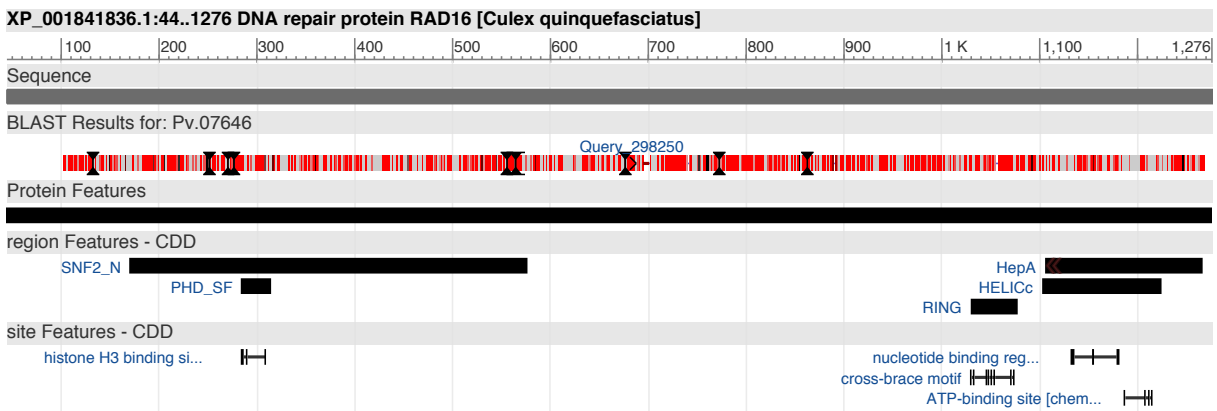
Supplementary Figure S9. Alignment of Pv.08737 with ATP-dependent RNA helicase A.



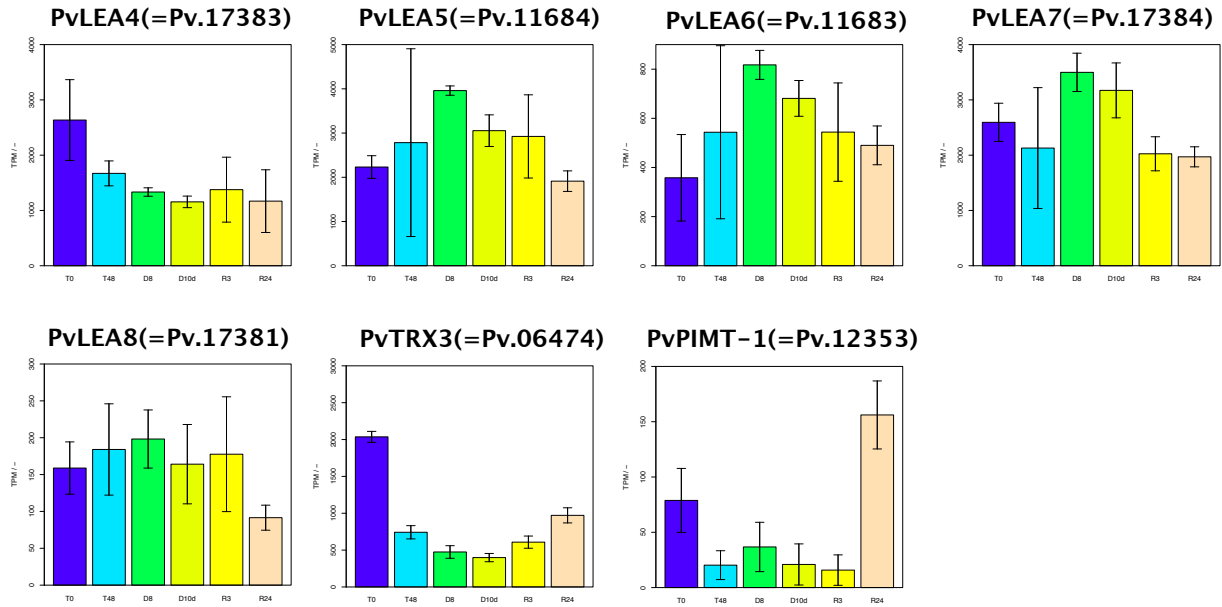
Supplementary Figure S10. Alignment of Pv.12163 with DEAD-box ATP-dependent RNA helicase.



Supplementary Figure S11. Alignment of Pv.07646 with SNF2 histone linker PHD-ring helicase, annotated as putatively involved in desuccinylation.



Supplementary Figure S12. Alignment Pv.07646 with the DNA-repair protein RAD16.



Supplementary Figure S13. Time course expression of the genes encoding late embryogenesis abundant proteins (LEA), thioredoxin (TRX), and protein L-isoaspartyl methyltransferase (PIMT) genes.

Supplementary Table S1. DEGs between D10d and R3.

	PvGene ID	Gene Identifier	BLAST first hit gene	E.value
Upregulated in R3	Pv.04891	194753680	GF12734	1.71E-9
	Pv.07646	170027903	DNA repair protein RAD16	0.0
	Pv.01911	765338102	AAEL005023-PA	1.17E-56
	Pv.00327	170050025	conserved hypothetical protein	2.20E-61
	Pv.08602	668455465	AGAP001139-PA-like protein	3.67E-102
	Pv.01867	157136676	AAEL013609-PA, partial	0.0
	Pv.03099		No Hits	
Downregulated in R3	Pv.04558	170036404	conserved hypothetical protein	2.62E-107
	Pv.08152		No Hits	
	Pv.11244	157128927	AAEL011272-PA, partial	2.15E-126
	Pv.13360	170052229	FAM96B	7.93E-77
	Pv.05172	157104834	AAEL004151-PB	4.96E-25
	Pv.13909	751792306	PREDICTED: Y+L amino acid transporter 2	0.0
	Pv.10501	170050626	cyclin b	1.49E-109

Supplementary Table S2. Annotated gene ontology to the DEGs identified between D10d and R3.

Pv Gene ID	Annotated GO	GO Description	Pv Gene ID	Annotated GO	GO Description		
Pv.04891	GO:0016301 GO:0016310	kinase activity phosphorylation	Pv.01867	GO:0005886 GO:0012505 GO:0015279 GO:0048763 GO:0002115 GO:0007155 GO:0007476 GO:0022416 GO:0032237	plasma membrane endomembrane system store-operated calcium channel activity calcium-induced calcium release activity store-operated calcium entry cell adhesion imaginal disc-derived wing morphogenesis chaeta development activation of store-operated calcium channel activity		
Pv.00327	GO:0000166 GO:0003676 GO:0016779 GO:0046872 GO:0008152	nucleotide binding nucleic acid binding nucleotidyltransferase activity metal ion binding metabolic process		GO:0051533	positive regulation of NFAT protein import into nucleus		
Pv.08602	GO:0005634 GO:0005762	nucleus mitochondrial large ribosomal subunit		Pv.11244	GO:0017146 GO:0004972	NMDA selective glutamate receptor complex NMDA glutamate receptor activity	
	GO:0003676	nucleic acid binding		Pv.13360	GO:0005737 GO:0007059 GO:0016226	cytoplasm chromosome segregation iron-sulfur cluster assembly	
	GO:0003735 GO:0008270 GO:0006412	structural constituent of ribosome zinc ion binding translation		Pv.05172	GO:0022625 GO:0003682 GO:0003735 GO:0006412	cytosolic large ribosomal subunit chromatin binding structural constituent of ribosome translation	
Pv.07646	GO:0000786 GO:0005634 GO:0003677 GO:0004386 GO:0004842 GO:0005524 GO:0008270 GO:0016874 GO:0036054 GO:0036055 GO:0070403 GO:0000209	nucleosome nucleus DNA binding helicase activity ubiquitin-protein transferase activity ATP binding zinc ion binding ligase activity protein-malonyllysine demalonylase activity protein-succinyllysine desuccinylase activity NAD+ binding protein polyubiquitination		Pv.13909	GO:0016021 GO:0015171 GO:0003333 GO:0060356	integral component of membrane amino acid transmembrane transporter activity amino acid transmembrane transport leucine import	
	GO:0006334 GO:0022008 GO:0036047 GO:0036049	nucleosome assembly neurogenesis peptidyl-lysine demalonylation peptidyl-lysine desuccinylation		Pv.10501	GO:0000775 GO:0019908 GO:0045495 GO:0051233 GO:0072686 GO:0016538	chromosome, centromeric region nuclear cyclin-dependent protein kinase holoenzyme complex pole plasm spindle midzone mitotic spindle cyclin-dependent protein serine/threonine kinase regulator activity	
	Pv.01911	GO:0005794 GO:0016021 GO:0003676 GO:0008270 GO:0019706 GO:0006888 GO:0018345		Golgi apparatus integral component of membrane nucleic acid binding zinc ion binding protein-cysteine S-palmitoyltransferase activity ER to Golgi vesicle-mediated transport protein palmitoylation	GO:0000086 GO:0000281 GO:0000920 GO:0001700 GO:0006974 GO:0007079 GO:0008608 GO:0035186 GO:0035561	G2/M transition of mitotic cell cycle mitotic cytokinesis cell separation after cytokinesis embryonic development via the syncytial blastoderm cellular response to DNA damage stimulus mitotic chromosome movement towards spindle pole attachment of spindle microtubules to kinetochore syncytial blastoderm mitotic cell cycle regulation of chromatin binding	
	Pv.04558	GO:0005826 GO:0016324 GO:0016328 GO:0030478 GO:0045178 GO:0045179 GO:0005089 GO:0019992 GO:0046872 GO:0007015 GO:0007277 GO:0007349 GO:0007370 GO:0007374 GO:0007375 GO:0007377 GO:0016476 GO:0030589 GO:0031532 GO:0035025 GO:0035277 GO:0035556 GO:0038032 GO:0043547 GO:0050770 GO:0070252 GO:0090254		actomyosin contractile ring apical plasma membrane lateral plasma membrane actin cap basal part of cell apical cortex Rho guanyl-nucleotide exchange factor activity diacylglycerol binding metal ion binding actin filament organization pole cell development cellularization ventral furrow formation posterior midgut invagination anterior midgut invagination germ-band extension regulation of embryonic cell shape pseudocleavage involved in syncytial blastoderm formation actin cytoskeleton reorganization positive regulation of Rho protein signal transduction spiracle morphogenesis, open tracheal system intracellular signal transduction termination of G-protein coupled receptor signaling pathway positive regulation of GTPase activity regulation of axonogenesis actin-mediated cell contraction cell elongation involved in imaginal disc-derived wing morphogenesis			

Supplementary Table S3. Differentially expressed genes between T0 and T48 with GO:0008152 (metabolic process). PREDICTED, initial RefSeq record status meaning the annotation is only predicted by automated BLAST analysis and has not been validated experimentally.

Cluster Num.	Pv Gene ID	Gene Identifier	BLAST First Hit Gene	E-value
Cluster 1	Pv.00012	755889065	PREDICTED: uncharacterized protein LOC101899881 isoform X2	3.5155E-21
	Pv.00503	170031381	conserved hypothetical protein	0.0
	Pv.01170	195109112	GI23236	0.0
	Pv.01614	557775558	PREDICTED: tubulin beta-1 chain	0.0
	Pv.01749	158296880	AGAP008252-PA	0.0
	Pv.01933	755849759	PREDICTED: GTP-binding protein 1-like	0.0
	Pv.03555	94468868	NMD protein	0.0
	Pv.05750	347969792	AGAP003372-PB	8.73E-102
	Pv.06865	157120582	AAEL009068-PA	0.0
	Pv.07614	568250312	transport and golgi family organization	2.44E-75
	Pv.07843	668455678	hypothetical protein ZHAS_00011763	4.90E-162
	Pv.08737	170064729	ATP-dependent RNA helicase A	0.0
	Pv.11162	668458368	AGAP004610-PA-like protein	0.0
	Pv.12163	568253176	DEAD box ATP-dependent RNA helicase	3.32E-146
	Pv.13802	170049985	conserved hypothetical protein	2.60E-86
	Pv.15605	157107882	AAEL014906-PA, partial	2.81E-76
	Pv.17087	817062658	PREDICTED: E3 ubiquitin-protein ligase rnf146-like	1.19E-32
Cluster 2	Pv.02231	751789335	PREDICTED: 5-oxoprolinase	0.0
	Pv.06995	227343489	glutathione S-transferase	5.64E-87
	Pv.10576	347969074	AGAP003018-PA	0.0
	Pv.12167	157110933	AAEL000854-PA	9.32E-141
Cluster 3	Pv.02391	749772182	PREDICTED: abhydrolase domain-containing protein 4 isoform X1	9.38E-112
	Pv.06862	568250143	Elongator complex protein 3	0.0
	Pv.08047	332373216	unknown	8.53E-36
	Pv.11397	170054183	UDP-glucuronosyltransferase 2C1	8.03E-30
	Pv.12035	157117142	AAEL007889-PA	0.0
	Pv.13081	157136208	AAEL003433-PA	0.0
	Pv.13347	557763449	PREDICTED: E3 ubiquitin-protein ligase Topors isoform X2	3.02E-56
	Pv.16072	668446285	AGAP008326-PA-like protein	1.05E-145
Cluster 3	Pv.04950	568250792	carboxylesterase, beta esterase	1.67E-138
	Pv.06833	589278470	hypothetical protein TREMEDRAFT_39272	7.49E-26
	Pv.13234	668450732	hypothetical protein ZHAS_00007358	1.72E-28

Supplementary Table S4. Differentially expressed genes between T0 and T48 with GO:0055114 (oxidation-reduction process).

Cluster Num.	Pv Gene ID	Gene Identifier	BLAST First Hit Gene	E-value
Cluster 1	Pv.00927	646723765	hypothetical protein L798_06909	2.76E-50
	Pv.02233	157115285	AAEL001183-PA	4.90E-121
	Pv.02556	568253142	augmenter of liver regeneration	1.03E-70
	Pv.03565	170049364	cytochrome P450	2.25E-162
	Pv.03626	157123150	AAEL009396-PA	6.56E-86
	Pv.03974	157124708	AAEL001860-PA	1.28E-16
	Pv.05128	568248619	hypothetical protein AND_009968	4.30E-154
	Pv.05617	262410959	ferritin heavy chain-like protein	6.71E-100
	Pv.05897	157112413	AAEL006129-PA	9.68E-144
	Pv.08902	304367635	catalase	0.0
	Pv.10292	751781624	PREDICTED: dehydrogenase/reductase SDR family member 11-like	1.70E-66
	Pv.12828	157132840	AAEL002901-PA	3.11E-87
	Pv.12949	766940976	PREDICTED: superoxide dismutase	5.00E-64
	Pv.15455	157132518	AAEL012457-PA	1.20E-142
Cluster 2	Pv.05587	584594454	cytochrome P450 6FV2	0.0
	Pv.05872	157111212	AAEL005790-PA	0.0
	Pv.06474	528744594	thioredoxin	5.87E-91
	Pv.07012	157137514	AAEL003645-PA	1.56E-101
Cluster 3	Pv.00404	528744570	thioredoxin	1.20E-90
	Pv.01417	584594454	cytochrome P450 6FV2	0.0
	Pv.01758	157130887	AAEL002658-PB	1.04E-92
	Pv.04458	347967826	AGAP002418-PA	5.58E-129
	Pv.07504	528744568	thioredoxin	7.07E-77
	Pv.07510	528744554	thioredoxin, partial	3.38E-95
	Pv.08589	157135753	AAEL013389-PA	0.0
	Pv.10571	157118100	AAEL008160-PA	0.0

Supplementary Table S5. Preprocessing and mapping. Samples were prepared in biological triplicate. totalSequences, number of sequenced reads; tooShort, number of reads shorter than 14 base pairs; tooManyN, number of reads containing more than 2 N bases; totalPassed, tooShort and tooManyN subtracted from totalSequences; mapped, number of reads mapped to the *P. vanderplanki* genome; unmapped, number of reads not mapped to the same genome; MappingRate, proportion of mapped reads among all passed reads.

	Preprocess Result				Mapping Result		
	totalSequences	tooShort	tooManyN	totalPassed	mapped	unmapped	MappingRate
T0-Sample1	9329152	1089685	33	8239434	1682490	6556944	20.4
T0-Sample2	8618767	1642930	23	6975814	2463740	4512074	35.3
T0-Sample3	5486398	1002516	12	4483870	1517136	2966734	33.8
T48-Sample1	8249645	907419	27	7342199	1282918	6059281	17.5
T48-Sample2	4527055	923602	9	3603444	1340121	2263323	37.2
T48-Sample3	5862177	1172255	20	4689902	1747773	2942129	37.3
D8-Sample1	7162127	867901	23	6294203	1263235	5030968	20.1
D8-Sample2	5497776	914899	15	4582862	1384844	3198018	30.2
D8-Sample3	4710977	789877	19	3921081	1214370	2706711	31.0
D10d-Sample1	9786341	972550	49	8813742	1410369	7403373	16.0
D10d-Sample2	5046272	887863	13	4158396	1358339	2800057	32.7
D10d-Sample3	6176969	1146552	21	5030396	1716458	3313938	34.1
R3-Sample1	2269038	431391	3	1837644	687580	1150064	37.4
R3-Sample2	1966504	340563	6	1625935	533487	1092448	32.8
R3-Sample3	4409262	614660	6	3794596	1188580	2606016	31.3
R24-Sample1	3580658	819575	8	2761075	1228917	1532158	44.5
R24-Sample2	5913507	1330397	16	4583094	2078353	2504741	45.3
R24-Sample3	5071881	1122334	14	3949533	1752922	2196611	44.4