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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Table S1** A summary of read retention after trimming, mapping, and counting. On the left column sample replicates are listed where C = normoxia, 3.5 = mild hypoxia, 0.5 = *P*crit, A = anoxia, and R = recovery time points. | | | | | | | |
| **Sample** | **Reads before trim** | **After trimming** | **Percent retained after trim** | **After mapping** | **Percent mapped** | **After counting** | **Percent counted and mapped** |
| **Average** | 14721690 | 13377440 | 91.10% | 12768055 | 95.43% | 10377819 | 77.66% |
| **Min** | 9296007 | 9047410 | 88.92% | 8519329 | 94.16% | 7217475 | 74.84% |
| **Max** | 17740487 | 15882022 | 97.48% | 15139948 | 96.38% | 11886378 | 80.96% |
| **0.5-1** | 15080682 | 13631979 | 90.39% | 13042446 | 95.68% | 10559447 | 77.46% |
| **0.5-2** | 15066384 | 13604844 | 90.30% | 12866570 | 94.57% | 10488187 | 77.09% |
| **0.5-3** | 16352714 | 14582883 | 89.18% | 13980632 | 95.87% | 11322965 | 77.65% |
| **0.5-4** | 14173108 | 12798392 | 90.30% | 12213780 | 95.43% | 9937041 | 77.64% |
| **0.5-5** | 15420160 | 13914306 | 90.23% | 13246498 | 95.20% | 10667103 | 76.66% |
| **0.5-6** | 11043075 | 10620558 | 96.17% | 10102560 | 95.12% | 8364220 | 78.75% |
| **3.5-1** | 14476481 | 13263476 | 91.62% | 12782737 | 96.38% | 10377008 | 78.24% |
| **3.5-2** | 9296007 | 9047410 | 97.33% | 8519329 | 94.16% | 7217475 | 79.77% |
| **3.5-3** | 15640161 | 14041008 | 89.78% | 13465718 | 95.90% | 10967343 | 78.11% |
| **3.5-4** | 14354239 | 12804950 | 89.21% | 12184688 | 95.16% | 9904052 | 77.35% |
| **3.5-5** | 13265079 | 11929472 | 89.93% | 11363570 | 95.26% | 9193371 | 77.06% |
| **3.5-6** | 16113538 | 14769787 | 91.66% | 14143855 | 95.76% | 11586855 | 78.45% |
| **A-1** | 17740487 | 15882022 | 89.52% | 15139948 | 95.33% | 11886378 | 74.84% |
| **A-2** | 14881716 | 14066504 | 94.52% | 13364222 | 95.01% | 10858084 | 77.19% |
| **A-3** | 12735602 | 11649936 | 91.48% | 11180066 | 95.97% | 8965773 | 76.96% |
| **A-4** | 15890635 | 14152874 | 89.06% | 13539623 | 95.67% | 10896969 | 76.99% |
| **A-5** | 14137237 | 12771722 | 90.34% | 12256955 | 95.97% | 9998329 | 78.28% |
| **A-6** | 15499781 | 14750710 | 95.17% | 14079068 | 95.45% | 11551500 | 78.31% |
| **C-1** | 14184895 | 12905095 | 90.98% | 12417484 | 96.22% | 10210518 | 79.12% |
| **C-2** | 13418024 | 12079876 | 90.03% | 11557566 | 95.68% | 9480287 | 78.48% |
| **C-3** | 15671108 | 14302458 | 91.27% | 13694425 | 95.75% | 11002387 | 76.93% |
| **C-4** | 15725830 | 14100184 | 89.66% | 13476971 | 95.58% | 10876383 | 77.14% |
| **C-5** | 15201451 | 13717258 | 90.24% | 13123455 | 95.67% | 10722031 | 78.16% |
| **C-6** | 16714325 | 15143352 | 90.60% | 14443593 | 95.38% | 11774587 | 77.75% |
| **R-1** | 9764254 | 9518329 | 97.48% | 9076983 | 95.36% | 7705652 | 80.96% |
| **R-2** | 13633312 | 12122216 | 88.92% | 11495975 | 94.83% | 9287748 | 76.62% |
| **R-3** | 17051950 | 15183092 | 89.04% | 14552705 | 95.85% | 11863513 | 78.14% |
| **R-4** | 16607656 | 14836614 | 89.34% | 14060646 | 94.77% | 11263233 | 75.92% |
| **R-5** | 16630154 | 14802169 | 89.01% | 14130335 | 95.46% | 11245707 | 75.97% |
| **R-6** | 15880651 | 14329728 | 90.23% | 13539256 | 94.48% | 11160431 | 77.88% |

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| **Table S2** Top 10 genes in each maSigPro cluster, arranged by statistical significance, along with possible roles that may relate to hypoxia response and reoxygenation stress during recovery. | | | |
| **Cluster** | **Gene ID** | **Gene Annotation** | **Known Functional Role(s)** |
| **Cluster 1** |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | 01682  12432  00370  04867  12088  06799  09070  14535  03517  11503 | ninaB  hmp Flavohemoprotein  B3GALT1  SLC13A5  tim (timeless)  CHAC2  aos (giant-lens)  per  Mgst3  At5g337910 | Carotenoid dual-oxygenase and isomerase  Nitric oxide detoxification and redox homeostasis  Biosynthesis of glycoproteins and glycolipids  Citrate transport within neurons  Circadian clock and DNA damage checkpoint  Glutathione degradation for antioxidant homeostasis  Photorecepter axon pathfinding and cell determination  Regulation of circadian rhythm  Cell detoxification via glutathione reactions  Mitochondrial chaperone |
| **Cluster 2** |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | 01551  05776  04965  07926  06511  02126  03439  10671  05534  09693 | cuticle-like  DDO  Siah1  Tmem9  Pyx (pyrexia)  Gbs-76A  Hsp68Bb  Dipetalogastin homolog  arrd-17  SPCC1494.01 | Exoskeleton modification  Deamination of D-aspartate for cell detoxification  Positive inducer of HIF-1α through PHD degradation  Regulation (+) of Wnt pathway and intracellular pH reduction  Heat stress response and circadian temperature synchronization  Regulation (-) of glycogen binding and (+) glycogen biosynthesis  Heat shock protein  Thrombin inhibitor to protect fibrinogen-related proteins  Responds to osmotic stress and stimulation after starvation  2-oxoglutarate-dependent dioxygenase |
| **Cluster 3** |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | 08675  06802  07801  08592  17390  12220  03482  08955  07840  08629 | norpA  Venom carboxylesterase-6  CYP6J1  Nrf-6 Nose  Slc12a3 homolog  Cyp49a1  Glutathione reductase (Gsr)  AHCYL2  UGP2  Tret1 | Phototransduction in photoreceptors  Lipolytic activity to generate free fatty acids  Oxidoreductase related to cell detoxification (GSH)  Lipid transport  Sodium and chloride homeostasis and receptor for cytokines  Estrogen hormone biosynthesis  Mitochondrial oxidative stress resistance and cell detoxification  Regulation of sodium/bicarb transport and magnesium sensitivity  Generates precursors to glycogen  Trehalose sugar transporter for chitin synthesis |
| **Cluster 4** |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | 02557  08167  15032  11065  14798  04178  05313  13736  00225  05545 | Ets98B  Ptchd3  colt  SIK2  Gmcl1  Atxn1l  CPPED1  Chitin deacetylase 5  mucin-5AC  METTL9 | Transcription factor binding during cell differentiation  Sperm development or function  Gas filling of trachea and epithelial morphogenesis  Fatty acid oxidation and glucose metabolism  Nuclear envelope protein involved in spermatogenesis  Repressor of Notch signaling  Apoptosis promoter and inhibits glucose uptake in adipocytes  Conversion of chitin to chitosan  Extracellular matrix structural constituent of internal epithelia  Methylation in mitochondria including electron transport subunits |
| **Cluster 5** |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | 11456  10176  02167  13139  04517  15195  16261  11277  01071  04316 | AVT3B  TACR2  nhr-14  Slc18b1  Heat shock 70 kDa cognate 4  setd7  Jph3  Dat  HMCN1  Gls | Neutral amino acid transport  Receptor for tachykinins (neural, muscular)  Immune regulator and enabler of steroid hormone binding activity  Translocation of polyamines from cytosol to secretory vesicles  Used in protein degradation for healthy embryogenesis  DNA damage response and heterochromatin organization  Forms junctional complexes between plasma membrane and ER  Dopamine and noradrenaline transport  Influences components of epidermal or epithelial structure  Glutaminase used in energy production and acid-base homeostasis |
| **Cluster 6** |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | 11302  12235  13288  07499  00248  08165  01675  07652  12109  04108 | Spns2 (spinster homolog)  D4Des  slc46a1  Luciferin 4-monooxyganase  CTDSPL2  gst-6 glutathione transferase  Tret1-2 homolog  GstD1  NDRG3  ATP13A3 | Lipid transporter required for immune response  Biosynthesis of polyunsaturated fatty acid docosahexaenoic acid  Transport of folate across cell membranes in acidic conditions  Fatty-acyl-CoA biosynthesis for downstream ATP production  Regulator of transcription by RNA polymerase II  Cell detoxification against oxidative stress  Trehalose sugar transporter for chitin synthesis  Insect glutathione transferase for cell detoxification  Lactate signaling during hypoxia  Transport of polyamines to the cytosol |
| **Cluster 7** |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | 14591  08242  12804  00766  05569  05663  11917  01737  08982  08241 | cwo transcription factor  Leech-der. tryptase inh. c  lcc2 Laccase-2  Slc22a1  lhpp  fabG  SQOR  Slc22a7  Soma ferritin  Dipetalogastin homolog | Circadian pacemaker and regulator of Clock gene  Tryptase inhibitor  Used to make exoskeleton insoluble and hard  Transporter critical for uptake of cations and toxins  Metabolizes phosphate compounds and inhibits aerobic glycolysis  Fatty acid synthesis  Detoxifies mitochondria from sulfides for hypoxia resistance  Hub gene for excretion of toxic anions during hypoxic stress  Storage of iron ions during hypoxia  Thrombin inhibitor to protect fibrinogen-related proteins |
| **Cluster 8** |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | 13810  09890  05274  11475  01161  09874  07649  09466  09716  12742 | urad  clpX  EHD4  xsc  mmlH  agmo  Similar to oxlT  nad Epmerase dehydratase  Similar to oxlT  TMEM68 | Purine catabolic processes in the peroxisome  Unfolded protein response in mitochondria  Membrane reorganization and endosomal transport  Conversion of taurine to sulfite and acetate  4-methylmuconolactone transporter in aromatics metabolism  Alkylglycerol monooxygenase that be involved in cuticle stability  Uptakes oxalate from the gut  Helps regulate cell surface properties  Uptakes oxalate from the gut  Alternative triglyceride synthesis and mitochondrial stability |
| **Cluster 9** |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | 02439  11523  01542  12977  03519  05698  12659  112107  05177  05281 | CD63  Venom toxin 11-like  mdh  CG9705  CG13624  PNKP  rost (rolling stone)  NA+/H+ exchanger beta  Slc12a3 homolog  XK-related 6-like | Promotes cell survival, cell adhesion, and sequestration of pigment  Secretes OcyC11 like compound affecting cell adhesion  Needed for citric acid cycle and NADH supply to mitochondria  Cold shock protein needed for mRNA 3’-UTR binding activity  Positive regulator of transcription involved with TORC1 signaling  DNA damage repair following oxidative damage  Needed for myoblast fusion during muscle growth and repair  Important for insulin secretion during homeostasis  Sodium and chloride homeostasis and receptor for cytokines  Sodium and chloride homeostasis and receptor for cytokines |

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| **Table S3** Significantly enriched motifs in the promoter region (maximum 1000bp upstream) of genes in each cluster. in each maSigPro cluster, arranged by statistical significance, along with posible roles that may relate to hypoxia response and reoxygenation stress during recovery. | | | | | | |
| **Cluster** | **Motif\_ID** | **Name** | ***p*-adj** | **Consensus sequence** | **Class** | **Functional role** |
| **Cluster 1** |  |  |  |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | MA1700.1  MA0205.2  MA0255.1  MA0222.1  MA0015.1  MA0237.2  MA0193.1  MA0247.2 | Clamp  Trl  z  exd  Cf2  pan  schlank  tin | 4.43E-07  8.83E-04  4.88E-03  1.33E-02  2.77E-02  4.21E-02  4.23E-02  4.39E-02 | GWGMGAGCGAGAGR  RVAAGAGAGAGR  WTGAGTGRDW  NTTTGACR  RTATATRTAB  KCGSCKYBTTTGRT  CYACYAA  WBTCRAGTGS | C2H2 zinc finger  C2H2 zinc finger  Trithorax  Homeo domain  C2H2 zinc finger  High-mobility group  Homeo domain  Homeo domain | Sex specific alt splicing/regulates chromatin  Regulates chromatin structure  transvection-based gene expression  Regulation of neural development  Follicle cell fate and muscle gene regulation  Segment polarity during embryogenesis  Lipid metabolism  Development of heart and muscle |
| **Cluster 2** |  |  |  |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | MA1700.1  MA0205.2  MA0015.1  MA1457.1  MA0216.2  MA0193.1  MA0451.1 | Clamp  Trl  Cf2  grh  cad  schlank  kni | 2.03E-10  1.52E-07  9.42E-05  5.78E-03  1.50E-02  4.01E-02  5.07E-02 | GWGMGAGCGAGAGR  RVAAGAGAGAGR  RTATATRTAB  VAAACCAGTTTBN  RGYMATAAAAM  CYACYAA  AAWNTAGAGCAS | C2H2 zinc finger  C2H2 zinc finger  C2H2 zinc finger  Grainyhead  Homeo domain  Homeo domain  Nuc rec C4 zinc fingers | Sex specific alt splicing/regulates chromatin  Regulates chromatin structure  Follicle cell fate and muscle gene regulation  Epithelial cell and cuticular development  Regulation of gut AMP levels  Lipid metabolism  Regulates segmentation during development |
| **Cluster 3** |  |  |  |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | MA1700.1  MA0205.2  MA0015.1 | Clamp  Trl  Cf2 | 2.59E-08  4.69E-05  5.18E-02 | GWGMGAGCGAGAGR  RVAAGAGAGAGR  RTATATRTAB | C2H2 zinc finger  C2H2 zinc finger  C2H2 zinc finger | Sex specific alt splicing/regulates chromatin  Regulates chromatin structure  Follicle cell fate and muscle gene regulation |
| **Cluster 4** |  |  |  |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | MA1700.1  MA0205.2  MA0015.1  MA0255.1  MA0247.2 | Clamp  Trl  Cf2  z  tin | 7.79E-08  7.02E-07  2.35E-03  2.56E-03  4.72E-03 | GWGMGAGCGAGAGR  RVAAGAGAGAGR  RTATATRTAB  WTGAGTGRDW  WBTCRAGTGS | C2H2 zinc finger  C2H2 zinc finger  C2H2 zinc finger  Trithorax  Homeo domain | Sex specific alt splicing/regulates chromatin  Regulates chromatin structure  Follicle cell fate and muscle gene regulation  transvection-based gene expression  Development of heart and muscle |
| **Cluster 5** |  |  |  |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | MA0205.2  MA1700.1  MA0015.1 | Trl  Clamp  Cf2 | 1.62E-13  1.83E-13  1.53E-02 | RVAAGAGAGAGR  GWGMGAGCGAGAGR  RTATATRTAB | C2H2 zinc finger  C2H2 zinc finger  C2H2 zinc finger | Regulates chromatin structure  Sex specific alt splicing/regulates chromatin  Follicle cell fate and muscle gene regulation |
| **Cluster 6** |  |  |  |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | **MA1700.1**  **MA0205.2**  **MA0015.1**  **MA0255.1** | Clamp  Trl  Cf2  z | 3.10E-11  3.79E-11  9.35E-06  3.40E-02 | GWGMGAGCGAGAGR  RVAAGAGAGAGR  RTATATRTAB  WTGAGTGRDW | C2H2 zinc finger  C2H2 zinc finger  C2H2 zinc finger  trithorax | Sex specific alt splicing/regulates chromatin  Regulates chromatin structure  Follicle cell fate and muscle gene regulation  transvection-based gene expression |
| **Cluster 7** |  |  |  |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | **MA0015.1**  **MA1700.1**  **MA0255.1**  **MA0205.2**  **MA0531.1**  **MA0249.2** | Cf2  Clamp  z  Trl  CTCF  twi | 1.46E-05  1.87E-05  5.60E-04  5.62E-04  2.24E-02  3.27E-02 | RTATATRTAB  GWGMGAGCGAGAGR  WTGAGTGRDW  RVAAGAGAGAGR  CCRMYAGRTGGCGCY  RNACACATGTN | C2H2 zinc finger  C2H2 zinc finger  trithorax  C2H2 zinc finger  C2H2 zinc finger  bHLH | Follicle cell fate and muscle gene regulation  Sex specific alt splicing/regulates chromatin  transvection-based gene expression  Regulates chromatin structure  Short and long-range chromatin remodeling  Muscle development and Notch activation |
| **Cluster 8** |  |  |  |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | **MA1700.1**  **MA0205.2**  **MA0531.1**  **MA0015.1** | Clamp  Trl  CTCF  Cf2 | 1.47E-05  2.99E-05  1.18E-02  5.74E-02 | GWGMGAGCGAGAGR  RVAAGAGAGAGR  CCRMYAGRTGGCGCY  RTATATRTAB | C2H2 zinc finger  C2H2 zinc finger  C2H2 zinc finger  C2H2 zinc finger | Sex specific alt splicing/regulates chromatin  Regulates chromatin structure  Short and long-range chromatin remodeling  Follicle cell fate and muscle gene regulation |
| **Cluster 9** |  |  |  |  |  |  |
| A group of graphs showing the number of clusters  AI-generated content may be incorrect. | **MA1700.1** | Clamp | 4.22E-02 | GWGMGAGCGAGAGR | C2H2 zinc finger factors | Sex specific alt splicing/regulates chromatin |

Place holder

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Figure S1 Venn diagram of the number of genes identified by DESeq2 as differentially expressed between select time points.

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Description automatically generated with medium confidenceFigure S2 Cluster 1 treemap from rrvgo reduction and grouping of TopGO enrichment analysis of genes. Genes in this cluster increased expression levels steadily through anoxia and then decreased to pre-stress levels during recovery.

A group of squares with different colors

Description automatically generated

Figure S3: Cluster 2 treemap from rrvgo reduction and grouping of TopGO enrichment analysis of genes. Genes in this cluster increased expression levels steadily through anoxia and then decreased to pre-stress levels during recovery.

A group of squares with text

Description automatically generatedFigure S4 Cluster 3 treemap from rrvgo reduction and grouping of TopGO enrichment analysis of genes. Genes in this cluster increased expression levels steadily through anoxia and then decreased to pre-stress levels during recovery.

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Figure S5 Cluster 5 treemap from rrvgo reduction and grouping of TopGO enrichment analysis of genes. Genes in this cluster increased expression levels steadily through anoxia and then decreased to pre-stress levels during recovery.

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Description automatically generated

Figure S6 Cluster 4 treemap from rrvgo reduction and grouping of TopGO enrichment analysis of genes. Genes in this cluster increased expression levels steadily through anoxia and then decreased to pre-stress levels during recovery.

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Description automatically generatedFigure S7 Cluster 6 treemap from rrvgo reduction and grouping of TopGO enrichment analysis of genes. Genes in this cluster increased expression levels steadily through anoxia and then decreased to pre-stress levels during recovery.

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Description automatically generated

Figure S8 Cluster 7 treemap from rrvgo reduction and grouping of TopGO enrichment analysis of genes. Genes in this cluster increased expression levels steadily through anoxia and then decreased to pre-stress levels during recovery.

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Description automatically generated with medium confidence

Figure S9 Cluster 8 treemap from rrvgo reduction and grouping of TopGO enrichment analysis of genes. Genes in this cluster increased expression levels steadily through anoxia and then decreased to pre-stress levels during recovery.

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Description automatically generated with medium confidence

Figure S10 Cluster 9 treemap from rrvgo reduction and grouping of TopGO enrichment analysis of genes. Genes in this cluster increased expression levels steadily through anoxia and then decreased to pre-stress levels during recovery.

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Figure S11 The expression pattern of significant genes identified by maSigPro or DESeq2 in the glycolysis, starch-sucrose, pyruvate, pentose-phosphate, fructose-mannose, TCA cycle, and amino sugar/chitin synthesis pathways. Significant genes are highlighted light blue on the pathways. Genes highlighted dark grey are genes listed by NCBI and KEGG (CITE) as being present in the *T. californicus* genome while genes with no highlighting are not. The heat map of each gene has five segments which correspond to the five time points of the hypoxia course: normoxia, mild hypoxia, hypoxia at *P*crit, anoxia, and recovery.