**Table S4:** Putative high-impact SNPs that increased significantly in frequency between the mass-bred and generation 13 in the desiccation replicate lines. These SNPs caused the loss or gain of a stop codon and hence are predicted to impact protein function. Gene function is presented where this is known, using information from Flybase [1] and references cited.

| **line** | **pos** | **effect** | **effect type** | **codon substi-tution** | **amino acid substitution / base substitution** | **gene name / FBgn name** | **gene description** | **CDS length (bp)** | **protein length (amino acids)** | **GO Biological Process** | **GO Cellular Component** | **GO Molecular Function** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| D5 | 2L:3506557 | splice\_donor \_variant |  |  | c.1G>T | tim / FBgn0014396 | produces circadian rhythms through its binding with period, subsequent nuclear localization of the heterodimer and indirect inhibition of transcription. Degradation is induced by light. | 3136-5404 (numerous isoforms) | 890-1398 (numerous isoforms) | cellular response to light stimulus GO0071482, circadian behavior GO0048512, circadian regulation of heart rate GO0003053, circadian temperature homeostasis GO0060086, copulation GO0007620, entrainment of circadian clock GO0009649, locomotor rhythm GO0045475, mating behaviour GO0007617, negative phototaxis GO0046957, negative regulation of transcription from RNA polymerase II promoter GO0000122, negative regulation of transcription regulatory region DNA binding GO2000678, photoperiodism GO0009648, regulation of circadian sleep/wake cycle GO0042749, regulation of circadian sleep/wake cycle sleep GO0045187, circadian rhythm GO0007623, eclosion rhythm GO008062, protein import into nucleus GO0006606, regulation of protein import into nucleus GO0042306, rhythmic behaviour GO0007622, sleep GO0030431 | cytoplasm GO0005737, nucleus GO0005634, protein complex GO0043234 | protein binding GO0005515, protein heterodimerization activity GO0046982, transcription factor binding GO0008134 |
| D3 | 2L:3722019 | splice\_donor \_variant |  |  | c.568T>C | Shaw / FBgn0003386 | encodes a voltage-gated potassium channel | 2428-3086 (5 isoforms) | 498-619 | sleep GO0030431, potassium ion transport GO0006813, protein homooligomerization GO0051260, transmembrane transport GO0055085 | integral component of membrane GO0016021, plasma membrane GO0005886, voltage-gated potassium channel complex GO0008076 | delayed rectifier potassium channel activity GO0005251, voltage-gated cation channel activity GO0022843, voltage-gated potassium channel activity GO0005249 |
| D3 | 2L:3779318 | splice\_donor \_variant |  |  | c.1G>C | bowl / FBgn0004893 | putative transcription factor functioning in leg joint formation, terminal pathway of embryogenesis, foregut and hindgut patterning. | 3394-3630 (7 isoforms) | 744 | embryonic foregut morphogenesis GO0048617, embryonic hindgut morphogenesis GO00048619, hindgut morphogenesis GO0007442, imaginal disc-derved leg joint morphogenesis GO0016348, imaginal disc-derived leg morphogenesis GO0007480, lateral inhibition GO0046331, terminal region GO0007362, wing disc development GO0035220, negative regulation of transcription from RNA polymerase II promter GO0000122, negative regulation of transcription, DNA-templated GO0045892, positive regulation of transcription from RNA polymerase II promoter GO0045944, positive regulation of transcription, DNA-templated GO0045893 | nucleus GO0005634 | metal ion binding GO0046872, nucleic acid binding GO0003676, sequence-specific DNA binding RNA polymerase II transcription factor activity GO0000981 |
| D2 | 2L:5808472 | splice\_accept or \_variant |  |  | c.1931G>T | CG11034 / FBgn0031741 | unknown | 2409-2545 (3 isoforms) | 85-751 (3 isoforms) | proteolysis GO0006508 | membrane GO0016020 | dipeptidyl-peptidase activity GO0008239, serine-type peptidase activity GO0008236 |
| D3 | 2L:6338646 | splice\_donor \_variant |  |  | c.1T>C | Pez / FBgn0031799 | regulates intestinal stem cell proliferation. Required for hippo pathway activity in midgut epithelium and involved in maintenance of homeostasis in gut [2] | 4455, 4546 (2 isoforms) | 1252 | negative regulation of hippo signaling GO0035331, negative regulation of stem cell proliferation GO2000647, protein dephosphorylation GO0006470 | apical part of cell GO0045177, cell cortex GO0005938, cytoskeleton GO0005856 | cytoskeletal protein binding GO0008092, protein tyrosine phosphatase activity GO0004725 |
| D3 | 2L:7718976 | splice\_donor \_variant |  |  | c.272T>C | CG13793 / FBgn0031935 | probable neurotransmitter transporter | 1927 | 583 | neurotransmitter transport GO0006836 | integral component of membrane GO0016021 | neurotransmitter transporter activity GO0005326, neurotransmitter:sodium symporter activity GO0005328 |
| D4 | 2L:11171549 | stop\_gained | NONSENSE | tCa/tAa | p.Ser29\*/c.86C>A | Vm32E / FBgn0014076 | Major early eggshell protein secreted by follicle cells. Loss is supposed to be associated with defects in eggshell membrane integrity [3] | isoform a: 437 bp; isoform b: 499 bp | both isoforms 116 bp | [vitelline membrane formation involved in chorion-containing eggshell formation GO0007305](http://flybase.org/cgi-bin/cvreport.html?id=GO:0007305) | [extracellular space GO0005615](http://flybase.org/cgi-bin/cvreport.html?id=GO:0005615) | [structural constituent of vitelline membrane GO0008316](http://flybase.org/cgi-bin/cvreport.html?id=GO:0008316) |
| D3 | 2L:11937500 | stop\_lost | MISSENSE | tAa/tCa | p.Ter380Serext\*?/c.1139A>C | Or33b / FBgn0026391 | Forms an odorant receptor complex with Orco that appears to detect pyrazines, including 2,5-dimethylpyrazine. This particular receptor reaches saturation at very low doses and shows a long-lasting response. Larvae are attracted to 2,5-dimethylparazine [4] | 1140 | 379 | [detection of chemical stimulus involved in sensory perception of smell GO0050911, sensory perception of smell GO0007608](http://flybase.org/cgi-bin/cvreport.html?id=GO:0050911) | [integral component of membrane GO0016021, dendrite membrane GO0032590](http://flybase.org/cgi-bin/cvreport.html?id=GO:0016021) | [olfactory receptor activity GO0004984, odorant binding GO0005549, transmembrane signaling receptor activity GO0004888](http://flybase.org/cgi-bin/cvreport.html?id=GO:0004984) |
| D4 | 2R:9041933 | splice\_acceptor \_variant |  |  | c.1G>A | Ance-4 / FBgn0033366 | unknown | 1948-2008 (2 isoforms) | 514-609 (2 isoforms) | proteolysis GO0006508 | membrane GO0016020 |  |
| D4 | 2R:10378394 | stop\_gained | NONSENSE | Cag/Tag | p.Gln449\*/c.1345C>T | Ir47a / FBgn0033515 | A taste receptor gene expressed in probably non-neuronal cells of the larval terminal organ. In adults, expressed in labellar bristles (taste sensillae) and certain leg sensillae, likely in neurons [5,6] | 2982 | 593 | [detection of chemical stimulus GO0009593](http://flybase.org/cgi-bin/cvreport.html?id=GO:0009593) | [integral component of membrane GO0016021](http://flybase.org/cgi-bin/cvreport.html?id=GO:0016021) | [ligand-gated ion channel activity GO0015276](http://flybase.org/cgi-bin/cvreport.html?id=GO:0015276) |
| D4 | 2R:11294829 | stop\_gained | NONSENSE | taT/taA | p.Tyr114\*/c.342T>A | CG42336 / FBgn0259238 | unknown | 5 isoforms ranging between 509-901 bp | 5 isoforms ranging between 65-162 aa |  |  |  |
| D4 | 2R:11981758 | splice\_acceptor \_variant |  |  | c.838A>C | CG33145/GalT1 / FBgn0053145 | encodes a beta-1,3-galactosyltransferase involved in synthesising terminal N-linked glycans [7] | 2249, 2316 (2 isoforms) | 287, 466 (2 isoforms) | protein N-linked glycosylation GO0006487 | membrane GO0016020 | beta-1,3-galactosyltransferase activity GO0048531, UDP-galactose:beta-N-actylglucosamine beta-1,3-galactosyltransferase activity GO0008499 |
| D4 | 2R:13545307 | splice\_donor \_variant |  |  | c.1G>C | CG6145 / FBgn0033853 | unknown | 1826-2260 (5 isoforms) | 420-548 (5 isoforms) | neuron projection morphogenesis GO0048812, NAD metabolic process GO0019674, NADP biosynthetic process GO0006741 | cytoplasm GO0005737, nucleus GO0005634 | NAD+ kinase activity GO0003951 |
| D4 | 2R:13757850 | stop\_gained | NONSENSE | Gag/Tag | p.Glu759\*/c.2275G>T | CG6280 / FBgn0033866 | unknown | 3706 | 1093 |  |  |  |
| D3 | 2R:22764807 | splice\_donor \_variant |  |  | c.870C>G | CG13527 / FBgn0034776 | unknown | 1017-1519 (2 isoforms) | 290-292 (2 isoforms) |  |  |  |
| D4 | 3L:3810968 | start\_lost | MISSENSE | atG/atA | p.Met1?/c.3G>A | CG32263 / FBgn0052263 | unknown | 803 | 206 |  | integral component of membrane GO0016021 |  |
| D4 | 3L:16327391 | stop\_gained | NONSENSE | Aag/Tag | p.Lys71\*/c.211A>T | CG13038 / FBgn0040795 | unknown | 509 | 94 |  |  |  |
| D3 | 3L:18890344 | splice\_donor \_variant |  |  | c.1T>A | CG3961 / FBgn0036821 | unknown | 2649-3373 (4 isoforms) | 654-704 (4 isoforms) | long-chain fatty acid metabolic process GO0001676 |  | long-chain fatty acid-CoA ligase activity GO0004467 |
| D3 | 3L:18998608 | stop\_gained | NONSENSE | tCa/tGa | p.Ser588\*/c.1763C>G | CG3808 / FBgn0036838 | unknown | 2522 | 615 | [RNA processing GO0006396](http://flybase.org/cgi-bin/cvreport.html?id=GO:0006396) |  | [mRNA binding GO0003729, nucleic acid binding GO0003676, nucleotide binding GO00000166, RNA methyltransferase activity GO0008173](http://flybase.org/cgi-bin/cvreport.html?id=GO:0003729) |
| D3 | 3R:4319820 | stop\_gained | NONSENSE | Gaa/Taa | p.Glu12\*/c.34G>T | CG9795 / FBgn0037234 | unknown | 2390-2825 (4 isoforms) | 501-736 (4 isoforms) |  |  |  |
| D3 | 3R:6373845 | start\_lost | MISSENSE | Atg/Gtg | p.Met1?/c.1A>G | CG1041/CRAT / FBgn0037440 | catalyses final step of peroxisomal beta-oxidation of fatty acids [8] | 2267-2380 (4 isoforms) | 638-662 (4 isoforms) | fatty acid beta-oxidation GO0006635 | nucleoplasm GO0005654, peroxisome GO0005777, mitchondrion GO0005739 | carnitine O-acetyltransferase activity GO0004092 |
| D3 | 3R:14328211 | start\_lost | MISSENSE | Atg/Gtg | p.Met1?/c.1A>G | spn-B / FBgn0003480 | involved in double-strand DNA break repair in meiosis; regulating grk-Efgr signalling and thereby affecting dorso-ventral patterning in the embryo [9] | 1384 | 341 | germarium-derived oocyte fate determination GO0007294, intracellular mRNA localization GO0008298, karyosome formation GO0030717, oogenesis GO0048477, polarity specification of anterior/posterior axis GO0009949, polarity specification of dorsal/ventral axis GO0009951, chromosome condensation GO0030261, DNA recombination GO006310, DNA repair GO0006281, double-strand break repair GO0006302, meiotic nuclear division GO0007126, oocyte differentiation GO0009994, reciprocal meiotic recombination GO0007131, regulation of translation GO0006417 |  | ATP binding GO0005524, DNA binding GO0003677, DNA-dependent ATPase activity GO0008094, recombinase activity GO0000150 |
| D3 | 3R:14567153 | splice\_donor \_variant |  |  | c.188T>G | CG31313 / FBgn0051313 | unknown | 470-782 (3 isoforms) | 59-124 (3 isoforms) |  |  | cysteine-type endopeptidase inhibitor activity GO0004869 |
| D3 | 3R:15281600 | splice\_donor \_variant |  |  | c.1T>A | Tm1 / FBgn0003721 | tropomyosin; associates with the troponin complex to participate in calcium-dependent regulation of muscle contraction | 752-2651 (17 isoforms) | 161-711 (17 isoforms) | dendrite morphogenesis GO0048813, pole plasm oskar mRNA localization GO0045451, regulation of lamellipodium assembly GO0010591, muscle contraction GO0006936, oogenesis GO0048477, pole plasm assembly GO0007315 | cytosol GO0005829, colocalizes with filamentous actin GO0031941, investment cone GO70865, sarcomere GO0030017, colocalizes with sarcoplasmic reticulum GO0016529, muscle thin filament tropomyosin GO0005862 | actin filament binding GO0051015, actin binding GO0003779 |
| D3 | 3R:15844524 | stop\_lost | MISSENSE | taA/taT | p.Ter1471Tyrext\*?/c.4413A>T | cv-d / FBgn0265048 | Binds to and 'transmits' BMP ('bone morphogenic protein') signals in the wing - probably contributing to tissue organisation [10]. Likely to act in other tissues as well. Produced in the fat body and may be transported in the hemolymph | 4744 | 1470 | [imaginal disc-derived wing vein specification GO0007474, positive regulation of BMP signaling pathway GO0030513, lipid transport GO0006869](http://flybase.org/cgi-bin/cvreport.html?id=GO:0007474) | [extracellular space GO0005615](http://flybase.org/cgi-bin/cvreport.html?id=GO:0005615) | [BMP binding GO0036122, heparan sulfate proteoglycan binding GO0043395, lipid transporter activity GO0005319](http://flybase.org/cgi-bin/cvreport.html?id=GO:0036122) |
| D3 | 3R:15844525 | stop\_lost | MISSENSE | tAa/tTa | p.Ter1471Leuext\*?/c.4412A>T | cv-d / |  |  |  |  |  |  |
| D3 | 3R:17482661 | stop\_lost | MISSENSE | Taa/Caa | p.Ter1007Glnext\*?/c.3019T>C | Dscam3 / FBgn0261046 | a cell adhesion molecule that displays homomeric binding | 4 isoforms ranging from 3712 to 8231 bp | 4 isoforms ranging from 1006 to 2087 aa | [cell adhesion GO0007155, homophilic cell adhesion via plasma membrane adhesion molecules GO0007516](http://flybase.org/cgi-bin/cvreport.html?id=GO:0007155) | [integral component of plasma membrane GO0005887, plasma membrane GO0005886](http://flybase.org/cgi-bin/cvreport.html?id=GO:0005887) | [identical protein binding GO0042802](http://flybase.org/cgi-bin/cvreport.html?id=GO:0042802) |
| D3 | 3R:19608368 | stop\_gained | NONSENSE | Cga/Tga | p.Arg268\*/c.802C>T | CG17751 / FBgn0038717 | unknown | 1666 | 533 | [transmembrane transport GO0055085](http://flybase.org/cgi-bin/cvreport.html?id=GO:0055085) | [integral component of membrane GO0016021](http://flybase.org/cgi-bin/cvreport.html?id=GO:0016021) | [secondary active organic cation transmembrane transporter activity GO0008513](http://flybase.org/cgi-bin/cvreport.html?id=GO:0008513) |
| D3 | 3R:22769405 | stop\_lost | MISSENSE | tgA/tgG | p.Ter65Trpext\*?/c.195A>G | CG43092 / FBgn0262538 | unknown | 722 | 225 |  |  |  |
| D3 | 3R:23594457 | splice\_donor \_variant |  |  | c.1380G>A | CG16723 / FBgn0039092 | unknown | 2088 | 695 |  |  | transferase activity transferring acyl groups other than amino-acyl groups GO0016747 |
| D3 | 3R:24560524 | stop\_gained | NONSENSE | tCa/tAa | p.Ser751\*/c.2252C>A | CG13624/REPTOR / FBgn0039209 | forms half of a transcriptional activator complex repressed by TORC1: activity increases when nutrients are low or under rapamycin treatment. REPTOR appears to be responsible for mediating about 90% of transcriptional repression downstream of TORC1 in S2 cells [11] | 7 isoforms ranging from 3680 to 5651 bp | 7 isoforms ranging from 755 to 814 aa | [regulation of transcription, DNA-templated GO0006355](http://flybase.org/cgi-bin/cvreport.html?id=GO:0006355) |  | [protein homodimerization activity GO0042803, sequence-specific DNA binding GO0043565, sequence-specific DNA binding transcription factor activity GO0003700](http://flybase.org/cgi-bin/cvreport.html?id=GO:0042803) |
| D3 | 3R:24840987 | stop\_gained | NONSENSE | taC/taA | p.Tyr59\*/c.177C>A | CG33658 / FBgn0053658 | unknown | 546 | 181 |  |  |  |
| D3 | 3R:26261115 | splice\_donor \_variant |  |  | n.213T>G | CR44321 / FBgn0265380 | may be non-coding gene but appears to be transcribed |  |  |  |  |  |
| D3 | 3R:27545222 | stop\_gained | NONSENSE | tgG/tgA | p.Trp260\*/c.780G>A | Gr98c / FBgn0046886 | likely to be a taste receptor but exact function unknown | 1227 | 408 | [sensory perception of taste GO0050909](http://flybase.org/cgi-bin/cvreport.html?id=GO:0050909) | [integral component of membrane GO0016021](http://flybase.org/cgi-bin/cvreport.html?id=GO:0016021) | [taste receptor activity GO0008527](http://flybase.org/cgi-bin/cvreport.html?id=GO:0008527) |
| D3 | 3R:28283392 | stop\_gained | NONSENSE | Cag/Tag | p.Gln231\*/c.691C>T | CG34436 / FBgn0085465 | unknown | 853 | 270 | [proteolysis GO0006508](http://flybase.org/cgi-bin/cvreport.html?id=GO:0006508) |  | [serine-type endopeptidase activity GO0004252](http://flybase.org/cgi-bin/cvreport.html?id=GO:0004252) |
| D3 | 3R:29587371 | stop\_gained | NONSENSE | Cag/Tag | p.Gln200\*/c.598C>T | CG7567 / FBgn0039670 | unknown | 770 | 211 |  |  |  |
| D2, D3, D4, D5 | dmel\_mitochondrion\_genome:7997 | stop\_lost | MISSENSE | aGa/aTa | p.Ter43Metext\*?/c.128G>T | mt:ND5 / FBgn0013684 | encodes a subunit of Complex I of the mitochondrial membrane NADH dehydrogenase that transfers electrons from NADH to the respiratory chain | 1717 | 572 | ATP synthesis coupled electron transport GO0042773 | mitochondrial respiratory chain complex I GO0005747, mitochondrion GO005739 | NADH dehydrogenase (ubiquinone) activity GO0008137 |
| D4 | X:2094941 | splice\_donor \_variant |  |  | c.174G>A | csw / FBgn0000382 | required component of all receptor tyrosine kinase signalling pathways, acting downstream of torso, Egfr and btl. May mediate heteromeric protein interactions. Required for cell specification of various embryo tissues and during imaginal development. | 4106-7664 (4 isoforms) | 682-945 (4 isoforms) | dephosphorylation GO0016311, dorsal/ventral axis specification ovarian follicular epithelium GO0008069, epidermal growth factor receptor signaling pathway GO0007173, epithelial cell migration open tracheal system GO0007427, fibroblast growth factor receptor signaling pathway GO0008543, imaginal disc development GO0007444, mesoderm development GO0007498, mitotic cell cycle GO0000278, negative regulation of apoptotic process GO0043066, open tracheal system development GO0007424, phagocytosis engulfment GO0006911, protein dephosphorylation GO0006470, R7 cell fate commitment GO0007465, regulation of compound eye photoreceptor development GO0045314, terminal region development GO0007362, torso signaling pathway GO0008293, ventral midline development GO0007418, anterior/posterior axis specificaiton embryo GO0008595, primary branching open tracheal system GO007428, sevenless signaling pathway GO0045500 | cytoplasm GO0005737 | protein binding GO0005515, protein tyrosine phosphatase activity GO0004725, non-membrane spanning protein tyrosine phosphatase activity GO0004726, receptor signalingprotein tyrosine phosphatase activity GO0004728 |
| D5 | X:11791714 | stop\_gained | NONSENSE | Caa/Taa | p.Gln262\*/c.784C>T | Karl / FBgn0030334 | unknown | 4 transcripts ranging from 1075 to 2447 bp | all 266 bp | [imaginal disc-derived wing morphogenesis GO0007476](http://flybase.org/cgi-bin/cvreport.html?id=GO:0007476) |  |  |
| D3 | X:11887054 | splice\_acceptor \_variant |  |  | c.1A>T | CG10353 / FBgn0030349 | unknown | 4203-4771 (7 isoforms) | 984-1029 (7 isoforms) | chloride transport GO0006821 | extracellular exosome GO0070062 | intracellular calcium activated chloride channel activity GO0005229 |
| D4 | X:14314821 | stop\_lost | MISSENSE | Tag/Gag | p.Ter198Gluext\*?/c.592T>G | betaNACtes2 / FBgn0030563 | suggested to be involved in reproduction; expressed in the testis [12] | 907 | 197 |  |  |  |

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