

Supplementary material

SFig. 1. Analysis of autophagy induction by different stress conditions in *Chlamydomonas*. (A), Log phase cells growing in TAP medium were treated with 5 µg/ml tunicamycin (tun), 1 mM H₂O₂, 1 µM methyl viologen (MV) or 20 µM norflurazon (NF) and samples were taken at the indicated times and processed for expression analysis of *ATG8*, *ATG3* and *CAL2* genes by qPCR. Values are means of three independent experiments. (B), Thirty micrograms of total extracts from cells treated as indicated in panel A were resolved by 15% SDS-PAGE followed by western blot analysis with anti-ATG8 and anti-FKBP12 antibodies.

SFig. 2. Expression analysis of the *FKBP12* gene by qPCR. Log phase cells grown in TAP medium were treated with 5 µg/ml tunicamycin (tun) and samples were collected and processed for RNA isolation at the indicated times. Expression of the *FKBP12* gene was analyzed by qPCR. Values are means of two independent experiments.

SFig. 3. (A) Domain structure of CAL2, ERO1 and PDI6 proteins from *Chlamydomonas*. Evolutionarily conserved domains were identified with the Conserved Domain search tool at NCBI (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). (B) Amino acid sequence alignment of CAL2 protein homologues from *Chlamydomonas reinhardtii* (CrCAL2; Cre01.g038400.t1.2), *Arabidopsis thaliana* (AtCAL2; NP_172392.1), human (HsCAL2; AAB51176.1) and *Saccharomyces cerevisiae* (CNE1). ER retention motifs are underlined. (C) Amino acid sequence alignment of ERO1 protein homologues from *Chlamydomonas reinhardtii* (CrERO1; Cre17.g723150.t1.3), *Arabidopsis thaliana* (AtERO1; AEE35298.1), human (HsERO1; AAH08674.1) and *Saccharomyces cerevisiae* (ScERO1). (D) Amino acid sequence alignment of PDI6 protein homologues from

Chlamydomonas reinhardtii (CrPDI6; Cre12.g518200.t1.3), *Volvox carteri* (VcPDI6; XP_002948079.1), *Chlorella variabilis* (CvPDI6; XP_005852011.1) and *Arabidopsis thaliana* (AtATERDJ3A; NP_187509.1). The thioredoxin-like motif is underlined.

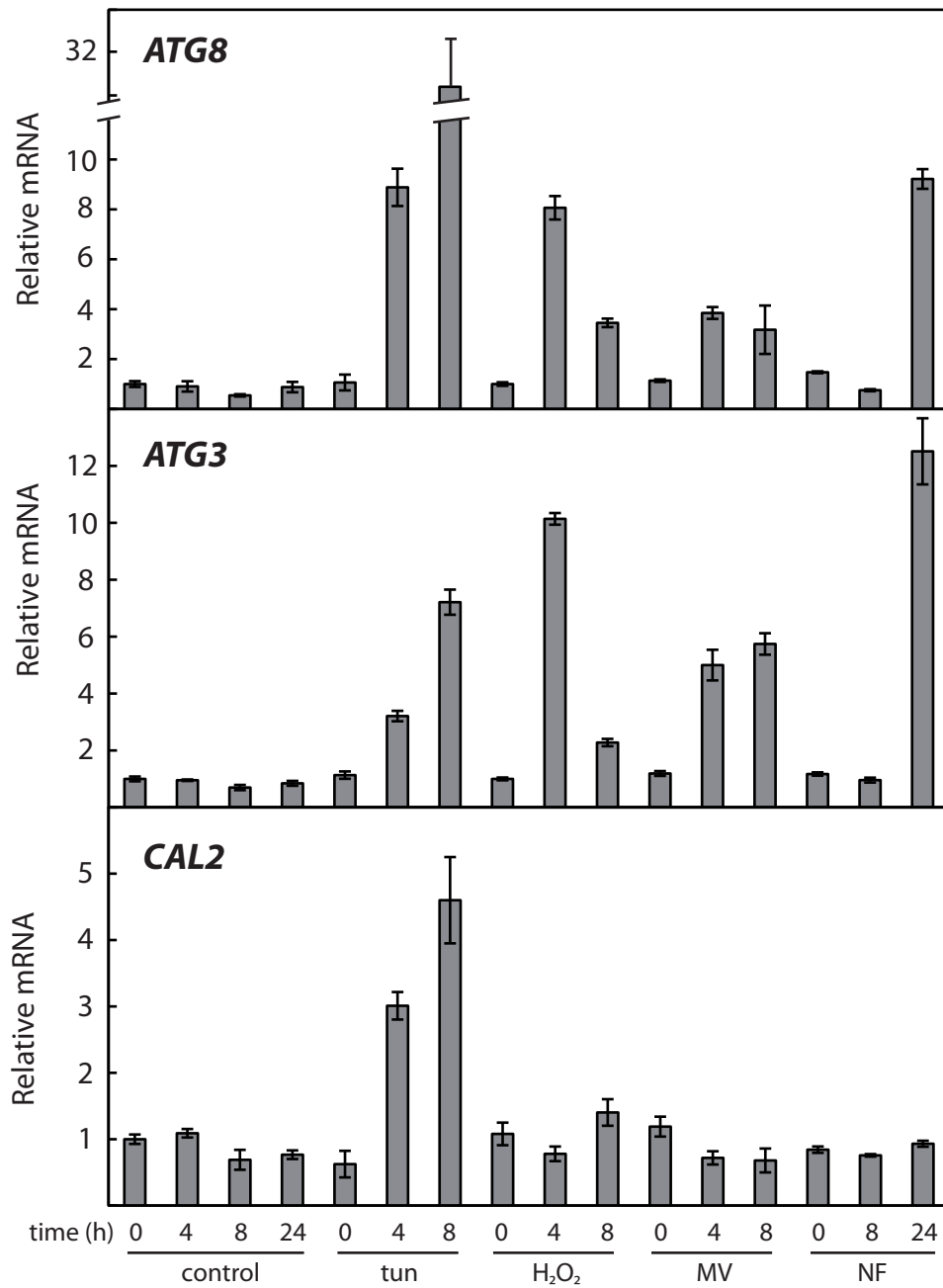
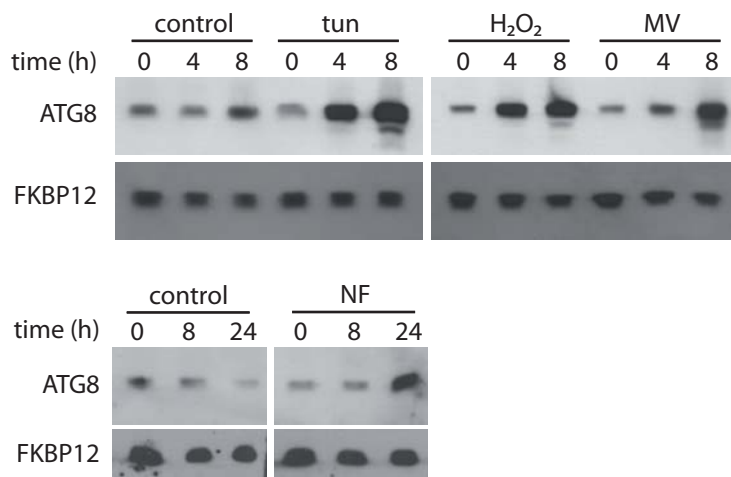
SFig. 4. Effect of thapsigargin on autophagy and ER stress. Log phase cells grown in TAP medium were treated with 1 µg/ml thapsigargin (thap) or 5 µg/ml tunicamycin (tun) for 8 hours and then processed for western blot analysis of ATG8 and FKBP12 proteins (A) or qPCR analysis of *ATG8*, *ERO1* and *PDI6* genes (B). Values are means of two independent experiments.

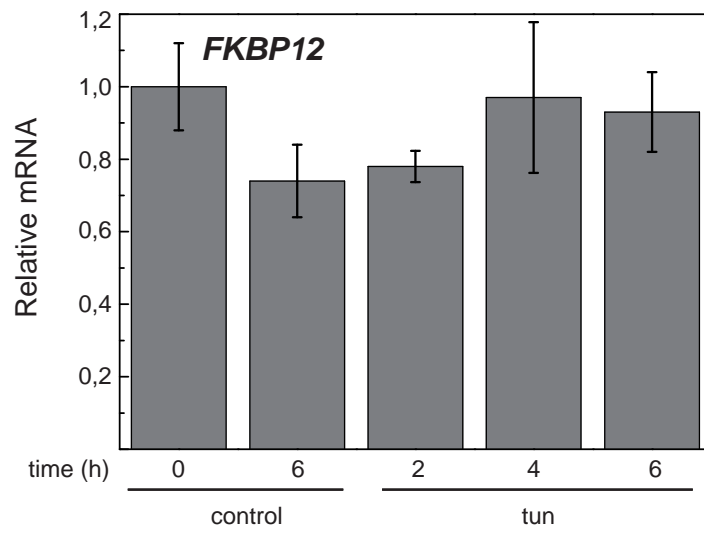
SFig. 5. Expression analysis of *CAL2*, *ERO1* and *Rb60* genes by qPCR. Log phase cells grown in TAP medium were treated for 8 hours with 2.5 mM DTT (A) or 5 µg/ml tunicamycin (B) alone or in combination with 10 mM GSH. Samples were collected and processed for RNA isolation. Expression of the indicated genes was analyzed by qPCR. Values are means of three independent experiments. “*”, Differences were significant at $P < 0.05$ according to Student’s t-test between DTT and DTT/GSH.

SFig. 6. Expression analysis of the *GSTS1* gene by qPCR. Log phase cells grown in TAP medium were treated for 30 minutes or 8 hours with 5 mM ascorbate or left untreated (control). Samples were collected and processed for RNA isolation and qPCR analysis. Values are means of two independent experiments.

SFig. 7. *DAD1* expression is upregulated upon prolonged ER stress. Log phase cells grown in TAP medium were treated with 5 µg/ml tunicamycin (tun) and samples were

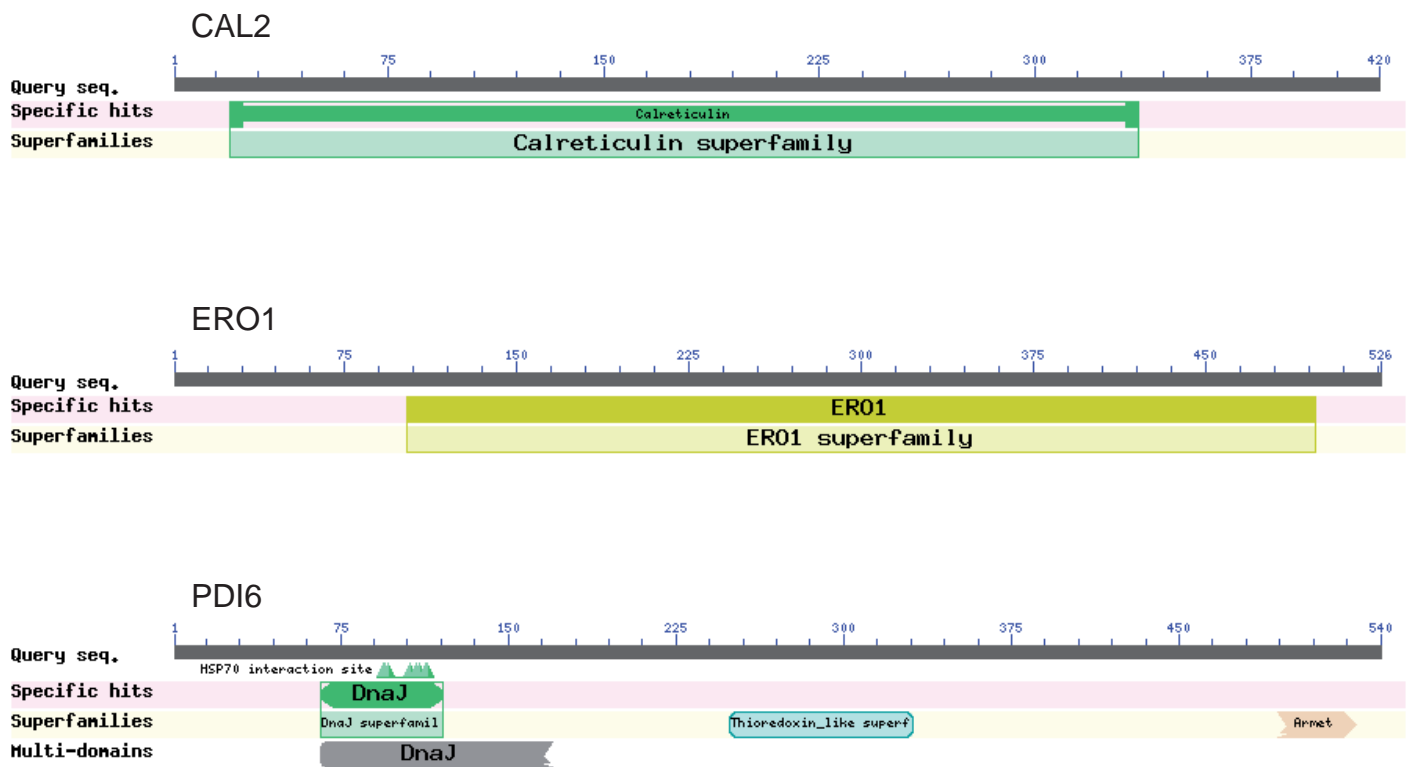
collected and processed for RNA isolation at the indicated times. Expression of the *DAD1* gene was analyzed by qPCR. Values are means of two independent experiments.

A**B****SFigure 1**



SFigure 2

A



SFigure 3

B

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CrCAL2      -----MKWGVVAVLLATL-----VVAASAKDYFKETFDGS-----WADRWTKSSWKVSDG
AtCAL2      -MAKMIPSLVSLILIGL-----VAIASAAVIFEERFDDG-----WENRWKSEWKKDDN
HsCAL2      -----MLLSVPLLLLGLL-----LAVAEPAVYFKEQFLDGD---GWTSRWIESKHKSD--
CNE1        MKFSAYLWWLFLNLALVKGTSLLSNVTLAEDSFWEHFQAYTNTKHLNQEWITSEAVNNEG
              : * : : . * * * . . * * . .

CrCAL2      S---AGEFKLTAGKWYGDAEADKGIQTGPDSKFFAISAPLAT-VFDNTGKDTVVQFSVKH
AtCAL2      T---AGEWKHTAGNWSGDAN-DKGIQTSEDYRFYAISAEFP--EFSNKDKTLVFQFSVKH
HsCAL2      ----FGKFVLSSGKFYGDEEKDKGLQTSQDARFYALSASFE--PFSNKGQTLVVQFTVKH
CNE1        SKIYGAQWRLSQRLQGSAW-DKGIAVRTGNAAAMIGHLLETPINVSETDTLVVQYEIKL
              . : : * . * . * * : . . : : . . * . * : *

CrCAL2      EQDLDCGGGYIKVVV--ATSEKQMGEFGGDTPYSIMFGPDICG-----YSTRKVHVIL
AtCAL2      EQKLDCGGGYMKLLS--GDVDQKK--FGGDTPYSIMFGPDICG-----YSTKKVHAIL
HsCAL2      EQNIDCGGGYVKLFP--NSLDQTD--MHGDSEYNIMFGPDICG-----PGTKKVHVIF
CNE1        DNSLTCGGAFIKLMSGFMNVEALKHYAPDTEGVELVFGPDYCAPEINGVQFAINKVDKIT
              : : : * * : : * . . : : * * * * * . . * . * . *

CrCAL2      TYKGNYLIKKDIKAETDQ-LTHVYTLVIKP-DNTYQVLIDLKEVASGSLYEDWD-----
AtCAL2      TYNEANHLIKKDVPCETDQ-LTHVYTFILRP-DATYSILIDNVEKQTGSLYSDWD-----
HsCAL2      NYKGNVLINKDIRCKDDE-FTHLYTLIVRP-DNTYEVKIDNSQVESGSLEDDWD-----
CNE1        HESKLRYLQEMPLSKLTDTSQSHLYTLIIDESAQSFQILDGKTVMVREHIEDKKVNFE
              . . * : : * : * * * : : : * * . . * .

CrCAL2      --MLPPKTIKDPKASKPEDWDEREEIADPEDKKPEGWDD-----I
AtCAL2      --LLPPKIKDPSAKPEDWDEQEYISDPEDKKPDGYDD-----I
HsCAL2      --FLPPKIKDPASKPEDWDERAKIDDPTDSKPEDWD-----K
CNE1        PPITPPLMIPDVSVAKPHDWDDRIRIPDPEAVKLSDRDERDPLMIPHPDGTEPPEWNSSI
              : * * * * . . * . * * : : * * * * . . *

CrCAL2      PATIADKDAKKPEDWDEEDGTWEPPMIPNP-----EYKGEWKAKM
AtCAL2      PKEIPDTDSKKPEDWDEEDGEWTAPTIPNP-----EYMGEWKPKQ
HsCAL2      PEHIPDPDAKKPEDWDEEMDGEWEPPVIQNP-----EYKGEWKPRQ
CNE1        PEYILDPNAQKPSWWKELEHGEWIPPMIKNFLCTAERGCGQIPGLINNAKYKGPGELNE
              * * * : : * * . : * * * * * * * * * * * * * * * * * * * * *

CrCAL2      IKNPAYKIWVAPDIDNPDYVHDDK-LYNFKDLKFVGFELWQVKSGSIFDNILVTDDLEA
AtCAL2      IKNPNYKGKWEAPLIDNPDFKDDPE-LYVFPKLKYVGLELWQVKSGSLFDNVLICDDPDY
HsCAL2      IDNPDYKGTWIHPEIDNPEYSPDPS-IYAYDNFGVLGLDLWQVKSGTIFDNFLITNDEAY
CNE1        IINPNYMGEWHPEIENPLYEEQHPLRIENVISGVILEFWSGSPNMLISNIYVGKNVTE
              * * * * * * * * * * : : : : : : * . . . . * . : :

CrCAL2      AKKFAEDTWGKHKDEEKAMFDKVKEEDEKKADAPPPPVDAEAAEEDEDEYDKEEPSG
AtCAL2      AKKLADETWGKLKDAEKAAFDEAEKKNEEESKDAP-AESDAEDEPEDDEGDDSDSESK
HsCAL2      AEEFGNETWGVTKAAEKQMKDKQDEEQRLKEEEDK--KRKEEEEAEDKEDDEDKDEDE
CNE1        AQIIGNKTWLMRDRAFRGSDGPTERKFMNSRLGNLQTTFHNERESPNPFDRIDRILEQP
              * : : : * * . : . . . : . . : : * . .

CrCAL2      MGSIKIPKEEEES-----GHDEL
AtCAL2      AEETKSVDSEETSEKDATAHDEL
HsCAL2      DEEDKEEDEEEDVPG--QAKDEL
CNE1        LKFVLTAAVLLLTTSVLCCVVFT
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SFigure 3

C

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CrER01 MRRLCPSCALAAGACLLALLQHTALAGGAQANLTGISSTAALGAEAAADAFLTNRQLVGGQ
AtER01 -----MGKGA-----IKEESEKRRKTWRWPLATLVVVFLAVAVSSRTNSNVGFFF
HsER01 -----MGRGWGFLFGLLGAVVLLSSGHGEEQP
ScER01 -----MRLRTAIA TLCLTAFTSATSNNS--YIATDQT
          . : * :

CrER01 QEGAATIAADQLTAGESCRLSGHVDECCNCTYQNVDRLN-VALHPLLQQVVRTAFFRYFK
AtER01 SDRNSCSCSLQKTG----KYKGMIEDCC-CDYETVDNLNTEVLNPLLQDLVTPFFRYFK
HsER01 PETAAQRFCFCQVSG-----YLDDCT-CDVETIDRFNNYRLFPRLQKLLSDYFRYFK
ScER01 QNAFNDTHFCVKVDR-----NDHVSPSCNVTFNELNAIN-ENIRDDL SALLKSDFFKYFR
          : : : . : : : * : * . : : : * : : :

CrER01 VNIYCD CPLWPD-DSMCALRACSVCECEQSEVPPTWRRLEQGGQEEEEGSCNSNTCAA E
AtER01 VKLWCD CPFWD- DGMCR LDCSVCECPENEFPEPFKPFVPGLP SDD-LKQ E----GK
HsER01 VNLKRP CPFWD- ISQCGRDCAVKPCQSDEVPDGIKSASYKYSEAN--NLIEECEQAE
ScER01 LDLYKQCSFWDANDGLCLNRACSVDVVEDWDTLPEYWQPEILGSFNND--TMKEADSD
          : : * : * . * * * : . : . . .

CrER01 LDGKVNSTVDPDTALKLLNLKGWRGFNNPWMAEGEGDE-EYLYVNLLINPERYTG YAGEH
AtER01 PQGAVDR TIDNR-----AFRGWVETKNPWHDDDDTDSGEMSYVNLQLNPERYTG YTGPS
HsER01 RLGAVDESLS EETQKAVLQWTKHDDSSDNFCEADDIQSPEAEYVDLLL NPERYTG YKGPD
ScER01 ECKFLDQLCQTS-----KKPVDIEDTINYCDVNDFNGKNAVLIDL TANPERFTG YGGKQ
          : : . . : : : : : : * * * : * * *

CrER01 AHRIWRAIYSQSCFHSSSDSGSSSEDSSSSSSSGGGPPALSPASLPPDWCP EKR VFYRL
AtER01 ARRIWDSIYSENC PKYSSGE----T-----CPEKKVLYKL
HsER01 AWKIWNVIYEENC FKPQTIKR---PLNP--LASGQGTSEENTFYSWLEGLC VEKRAF YRL
ScER01 AGQIWS TIYQDNCF TIGETG-----ESLAKDAFYRL
          * : * * * : * . * . : * :

CrER01 ISGMHASISAHISANYLLSE--ERGLWGHNLTD FRMR LGRPE----LRDRIQNLYFAYLF
AtER01 ISGLHSSISMHIAADYLLDE--SRNQWQNIELMYDRILR-----HPDRVRNMYFTYLF
HsER01 ISGLHASINVHLSARYLLQETWLEKKWGHNIT EFQQRFDGILTEGEGPRRLKNLYFLYLI
ScER01 VSGFHASIGTHLSKEYLNTK---TGKWEPNLDLFMARIGN-----FPDRVTNMYFN YAV
          : * : * * * . * : * : * : * : * : * : * : * : * :

CrER01 VLRAVTKAAPLLARYDYVTGF GAEE DAATAQLMKSL L TSPELASSCPMPFDEGR LWKGG-
AtER01 VLRAVTKATAYLEQAEYDTGN-HAEDLKTQSLIKQLLYSPKLQ TACVPV FDEAKLWQQG-
HsER01 ELRALSKVLPFFERP DFLFT--GNKIQDENKMLLLEILHEIKSFPLHF DENSF FGDK
ScER01 VAKALWKIQPYLPEFS FCDLVNKEIKNRMDNVISQLDTK-----IFNEDLVFAND-
          : * : * . : . : . * * * * : : .

CrER01 -DGLALREELRAAFVNITRIMDCVGCEKCKLWGKQLT LGVATALKVLFSSDCNNTLPTS
AtER01 -SGPELKKQIQKQFRNISALMDCVGCCEKRLWGKLVQQLGTALKILF SVG--NQDIGD-
HsER01 -KEAHLKEDFR LHF RNISRIMDCVGCCEKRLWGKLVQQLGTALKILFSEK-LIANMPES
ScER01 -LSLT LKDEF RSRFKNVTKIMDCVQCDCRRLWGKIQT TGYATALKILFEINDADEFTKQH
          . * : : : : * * : : * * * * * : * : * * * : * . * * * * : * :

CrER01 PALSTLALERNEVIALVNLLERFSASIEYYRQLS-----AELQVAEG-----
AtER01 ---QTLQLQRNEVIALVNLLNRLSESVKMVHDMSPDVERLMEDQIAKVS-----
HsER01 GPSYEFHLTRQEIVSLFNAFGRISTSVKKLENFR-----
ScER01 ---IVGKLT KYELIALLQTFGR LSESVNMFEKMYGKRLNGSENRLSSFFQNNFFNII
          * : * : * : * : : * * * * : . :

CrER01 -----AEGAPLPVAVGEPVA-----
AtER01 -----AKPARLRRIWDLAVSFW-----
HsER01 -----N--LLQNIH-----
ScER01 KEAGKSIRYTIENINSTKEGKKKTNNSQSHVFDDLKMPKAEIVPRPSNGTVNKWKKA WNT
          : :

CrER01 -----
AtER01 -----
HsER01 -----
ScER01 EVNNVLEAFRFIYRSYLDLPRNIWELSLMKVYKFWNKFIGVADYVSEETREPI SYKLDIQ
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SFigure 3

D

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CrPDI6      MGSHPISCLCCRAHVEDGAAAGVHAGRARS LIATVAVVLL LAAAQAPGAEAFRRGGGG
VcPDI6      -----MTLRLFLASIALLGLLLAQH AIAA-----R--G
CvPDI6      -----MGRSCLGRRLGSSLL LLA AAAAVAAVR-----
AtATERDJ3A  -----MVRTRLAISVVLVST LLLLNVKAK-----
                :   :   *   .

CrPDI6      GGGGRDFYSL LGVAK DAD EAT IKKAYRRQAL KWH PDR NTDKKEE AAARFRDIAAA YEVL S
VcPDI6      ---GRDFYSL LGVAR DAD DAT IKKAYRKQAL KWH PDR NPENKEKAEERFRDIAAA YEALS
CvPDI6      ---DTKY YDHLGVSP DAD ERT IQKAYRRAAL RYHPDR NPD-KPDAEERFREVA AAAYEVL S
AtATERDJ3A  ---SVDPYKVLGVSKDAK QRE IQKAFHKQSL KYHPDKNKD--KGAQEKFAEINNAYE ILS
                . . * . *** : ** : * : * : * : * : * : * : * : * : * : * : * : *

CrPDI6      DADKRRTYDQF GEEGLKHG--GGGGPGG-PGGAGGFNMNFQGGDPFEMFNMFFG-GGGGG
VcPDI6      DPQKR RVYDQF GEEGLKGG--GGGGPGGRPGGPGGFQAHFGGGDPFEMFNMFFGRGAAGG
CvPDI6      DSEKRQIYDRYGEQQLNQQQQGGGPGG-----NFHFQHGD AFNI FETVFG-----
AtATERDJ3A  DEEKRNKYDLYGDEKGP G-FDSGFP GG-----NG--GYSYSSSGGGFNFGGPGG
                * : * : * * : * : : : . . * * * * : * . . . * .

CrPDI6      GGMGGGGMGQRRAQFNMGGGG--MGGGF GGMG--GGMGGGGRGG-----HSGGGGEG
VcPDI6      GGMGGGGGAR-VHFNMGG-----MGGMGGMG--GGMGGG-----RGGGQP
CvPDI6      -GMGGGGQRMHFQFGGGGG--MGGGF GGMGGGHGGQHGGGR-----RHETAG
AtATERDJ3A  WQNMGGGGGSKSFSFSFGG PSESSFGFGMDDIFSMFSGGSSK GKEQFGFGSSSNAESKS
                ** * * * * * : * * : . * . *

CrPDI6      LYDDDPHVQTLTTDFPAG--DTAWVWLV EFYAPWCGHC KQLAPKWSA VAKSLKGVVKVG
VcPDI6      MYDSDPYVQQLDGS SLPP---DTNWWV LVEFYAPWCGHC KQLAPQWSKAAASLKG VVRVG
CvPDI6      LYDNDAFVQDLTEDTFPEGD-GDGWIVLIEFYAPWCGHC RQLAPKWRKVAEALHGVAKVA
AtATERDJ3A  KSSTVAAIKTINSQVYK KDVV DQGMTWLLLSY LPSQRGSQYHESIIEEVAESLQ GALKVG
                . . : : : . . * : * * . : . . * : * : * : *

CrPDI6      AINCEDAKELCGAHGVRGYPTIQAFVP---GSGAKP----YSGDRS AKAISDWALDLVP
VcPDI6      AVNCDERQQACQAQGLKGYPTIKAFVP---SGGAAAPSVREYNGERS AKAISDWALGLVP
CvPDI6      AVNCEQQQALCQEQQV RGYPTIKAF-----SGGHLH----EYKGDRSARHLKDWALGLLP
AtATERDJ3A  RLNCETESSLCKQLGIVPRRAPRMFVYSYTS SSKAT--LAEYTEELVAKKVK SFCQEHL P
                : * : . * * : : * . . * : * : * : * : * : *

CrPDI6      SR-----VSQIT TDSLRL-QLLARC SGGSSSGSSGSSSKGKDRASWSACV MLVSA
VcPDI6      SR-----VVRIS DEKSLDKLLLAQC SG-----KSKGKDAAAWNVCVLLISN
CvPDI6      KH-----IKTVNKQQHLA-DFLRQCTS-----GTGGSSK WGVCALLLSD
AtATERDJ3A  RFSKKIDLNTFDVSAVSSQKTPKVL LLS TKKDT PVIWR-VLSGLYNGRFV FYNTEVH DTS
                : : : : * . . . . : : :

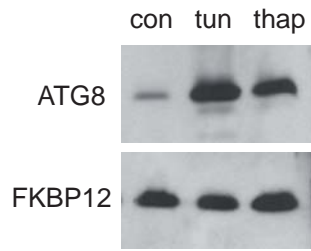
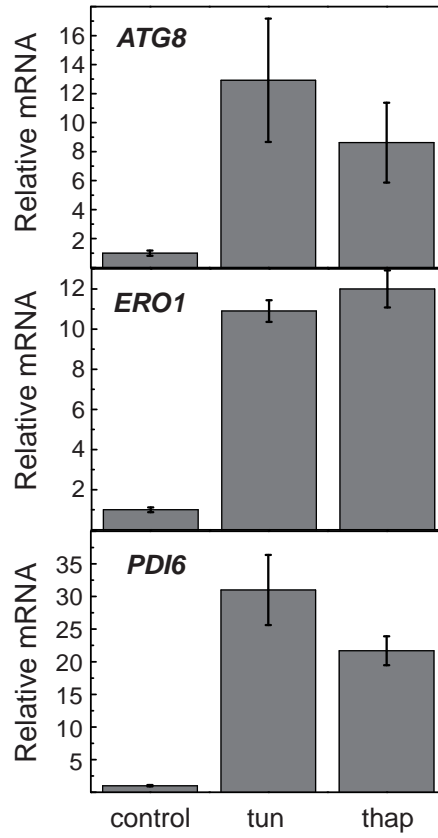
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VcPDI6      KSETPALYRALS VAYGK VAFGELNAAASSGK---AAAAAAVA AKLG-----
CvPDI6      KSETSALYKSLALRYKGI AFGEA-----LR SNAEISREF-----
AtATERDJ3A  DPKIQLGLGVDFPAIVGWLSN GEAQV LKTGITVKNLKS AVQELGK LLEGLEKKNK VSSK
                . . : * . . * : : * * . . . : :

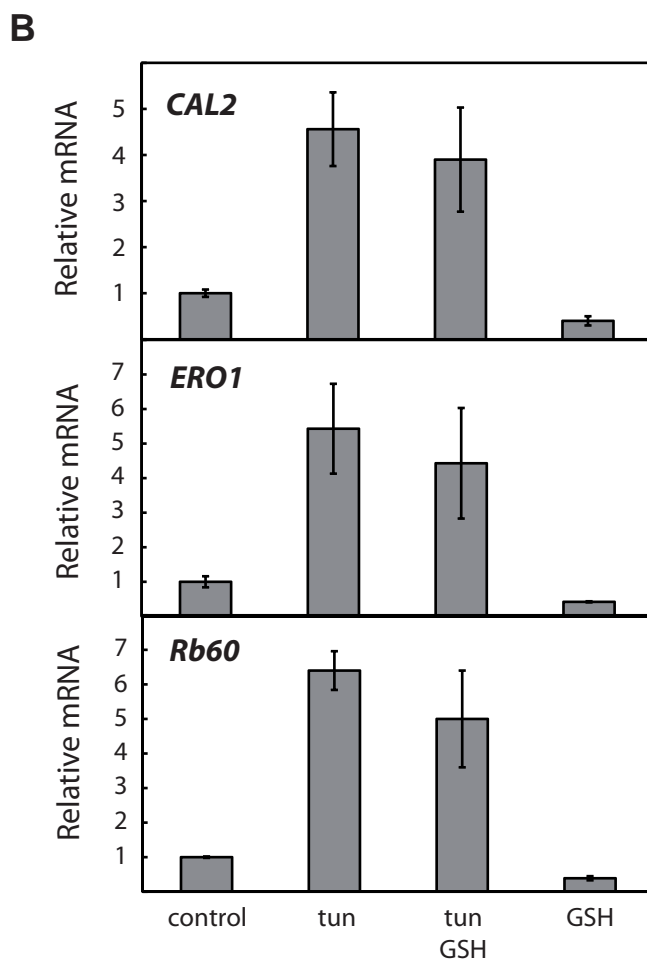
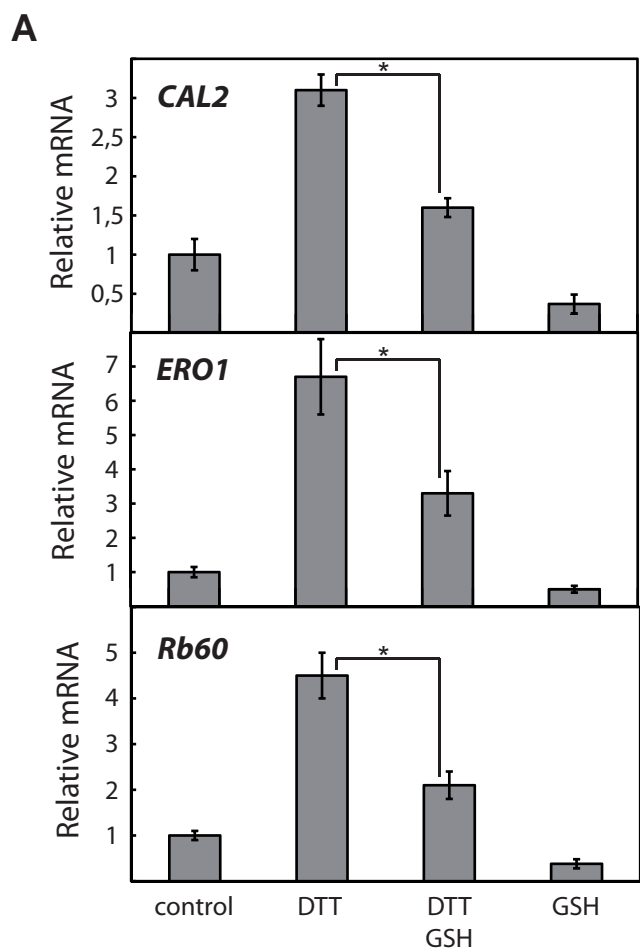
CrPDI6      -VDVEAER-AAGKLP LLIT-----ICNGDLALVDR-----YIGTLKSDSLA
VcPDI6      -VDLAAEREGSGKLP LLS-----ICNGDVAVAER-----YGGQLKSEPLG
CvPDI6      ------G--VSKYPTLLV-----ICGNKDVVVK-----YEGEMKSTKLS
AtATERDJ3A  SQAGQAPNESSEKI PLLSRPNFDS ICGENTPVCII GAFRSSNGKEKLQSIMS QSQSLS
                * * * * * : : : . . . * .

CrPDI6      RHLDTYAAGKKCAKQVRLDAGT-----DLGKFSAGQLKQLVKDKGLDCRGCTEKDDF
VcPDI6      RHLESYAAGKKCAKAVRLDANT-----DLSGLSTRQLKDLLQAKGLECRGCAEKDEL
CvPDI6      RFLNQFYSGKACAAAIKID AAN-----DLSKMRVGLKQI LESRGEKCKE CVEKADY
AtATERDJ3A  RRQASTTGSQD TVSYSLLDATKQSAFLSSLDKSEFKTSSDKLLIAYKPRRGKFATFKGDM
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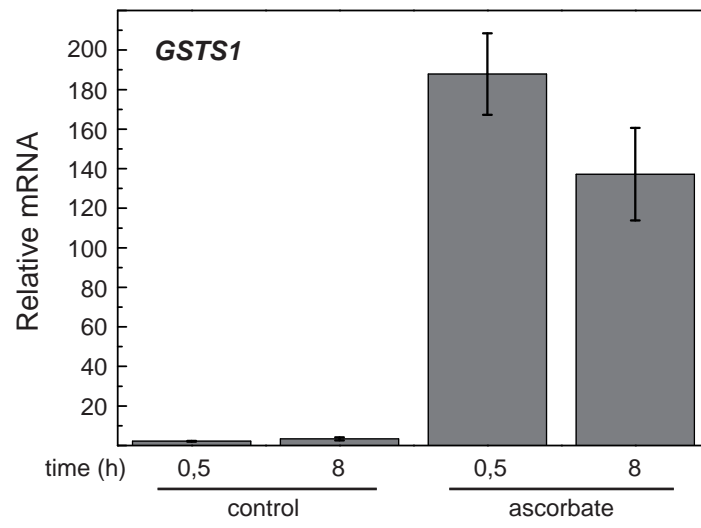
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VcPDI6      VRRLRDYVTS GV-----
CvPDI6      VAKIRAVFGIGDTAQQPPHSEL-----
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                . . . .
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SFigure 3

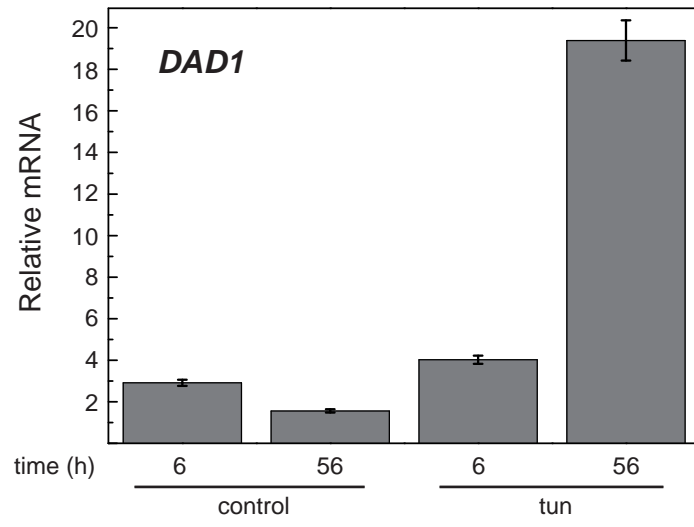
A**B****SFigure 4**



SFigure 5



SFigure 6



SFigure 7