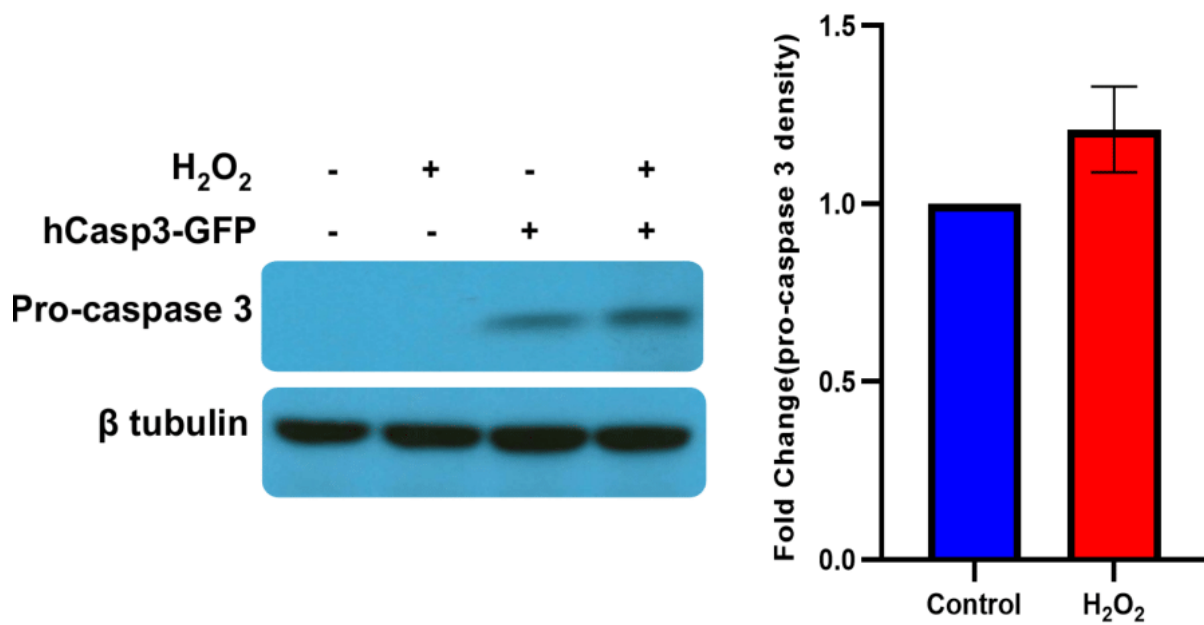
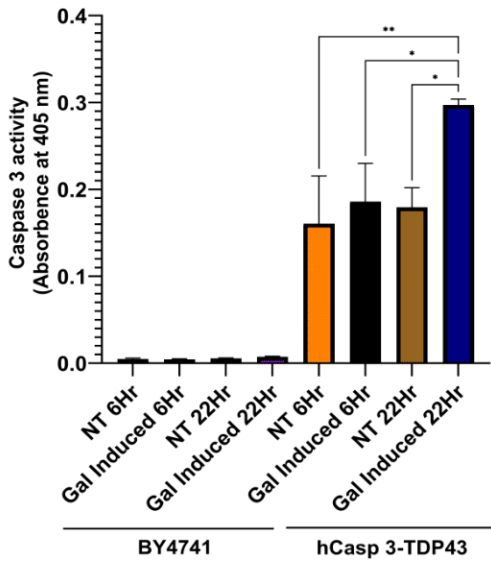


Supplemental Information - Caspase 3 exhibits a yeast metacaspase proteostasis function that protects mitochondria from toxic TDP43 aggregates

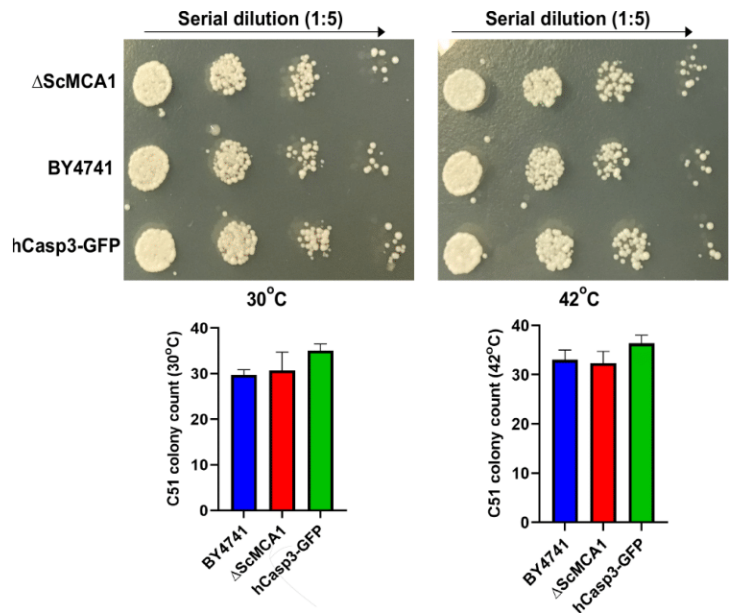


**FIGURE S1:** Western blot analysis of protein extract from BY4741 and hCasp3-GFP showing robust procaspase 3 protein expression following hydrogen peroxide treatment. The graph shows the quantification of the band intensity normalized to the control group. Values are mean  $\pm$  SEM of three independent experiments with p-value < 0.05.

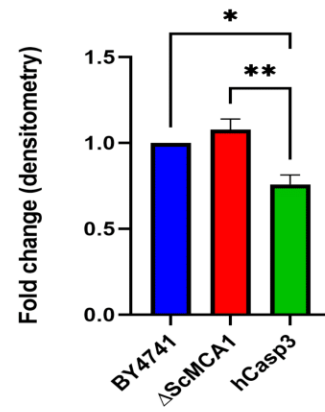
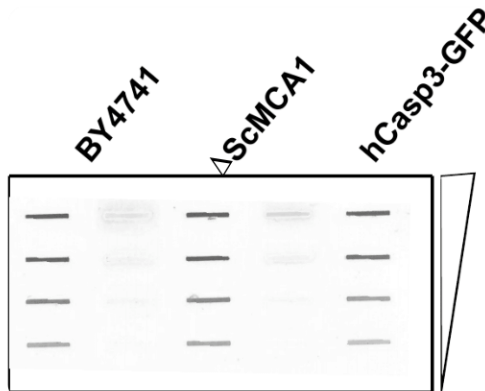
### S2A



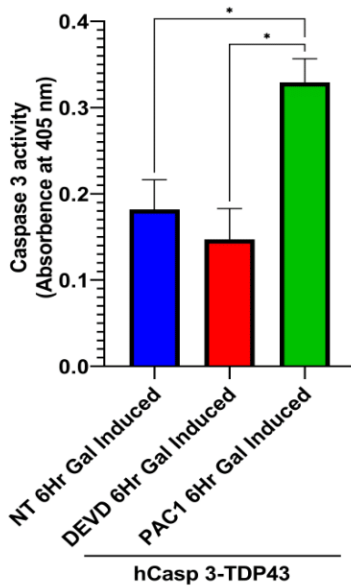
### S2B



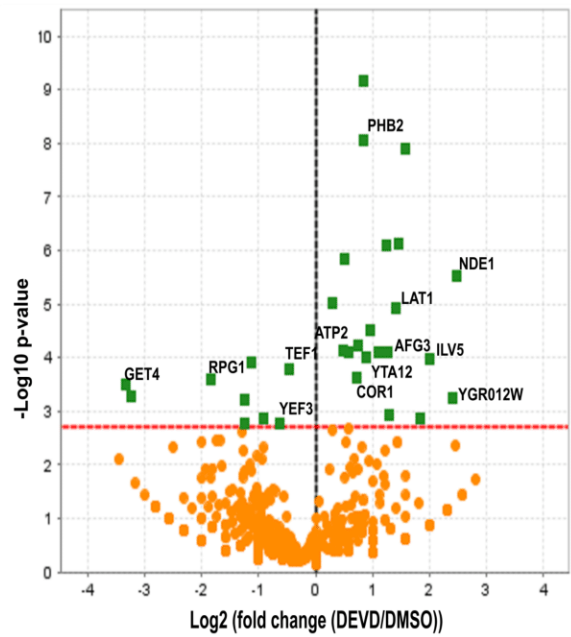
### S2C



### S2D



### S2E



## S2F

P40961 (100%), 31,427.9 Da

Prohibitin-1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=PHB1 PE=1 SV=2

7 exclusive unique peptides, 13 exclusive unique spectra, 14 total spectra, 120/287 amino acids (42% coverage)

```

MSNSAKLIDV ITKVALPIGI IASGIQYSMY DVKGGSRGVI FDRINGVKQ Q VVGEGETHFLV
PWLQKAIIDY VRTKPKSIAT NTGTKDLQMV SLTLRVLHRP EVLQLPAIQ NLGLDYDERV
LPSIGNEVLK SIVAQFDAAE LITQREISQ KIRKELSTRA NEFGIKLEDV SITHMTFGPE
FTKAVEQKQI AQQDAERAKF LVEKAEQERG ASVIRAEGEA ESAEFISKAL AKVGDGLLLI
RRL EASKDIA QTLANSSNVV YLPSQHSGGG NSESSGSPNS LLLNIGR
  
```

P50085 (100%), 34,407.2 Da

Prohibitin-2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=PHB2 PE=1 SV=2

2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 57/310 amino acids (18% coverage)

```

MNRSPGEFQR YAKAFQKQLS KVQQTGGRGQ VPSPRGAFAG LGGLLLLLGGG ALFINNALFN
VDGGRHAIIVY SRIHGVSSRI FNEGTHFIFP WLDTPIIYDV RAKPRNVASL TGTKDLQMVN
ITCRVLSRPD VVQLPTIYRT LGQDYDERVL PSIVNEVLKA VVAQFNASQL ITQREKVSRL
IRENLVRRAS KFNILLDDVS ITYMTFSPEF TNAVEAKQIA QQDAQRAAFV VDKARQEKQG
MVVRAQGEAK SAELIGEAIK KSRDYVELKR LDTARDIAKI LASSPNRVIL DNEALLLNTV
VDARIDGRGK
  
```

P39925 (100%), 84,547.4 Da

Mitochondrial respiratory chain complexes assembly protein AFG3 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=AFG3 PE=1 SV=1

1 exclusive unique peptides, 1 exclusive unique spectra, 2 total spectra, 31/761 amino acids (4% coverage)

```

MMMWRQYARG APRSLTSLSF GKASRISTVK PVLRSRMPVH QRLQTL SGLA TRNTIHRSTQ
IRSFHISWTR LNERNPKEG EGKNNGNKDN NSNKEDGKDK RNEFGSLSEY FRSKEFANTM
FLTIGFTIIF TLLTPSSNNS GDDSNRVLTF QDFKTKYLEK GLVSKIYVFN KFLVEAFALVN
TKQVVSFTIG SVDIFEEQMD QIQDNLNIPP RDRIPKIYIE RSSPFTYLFV FLPTIILLGG
LYFITRKINS SPPNANGGGG GGLGGMFNVG KSRAKLFNKE TDIKISFKNV AGCDEAKQEI
MEFVHFLKNP GKYTCLGAKI PRGAILSGPP GTGKTL LAKA TAGEANVPFL SVSGSEFVEM
FVGVGASRVRL DLFQTAR SMA PSII FIDEID AIGKER GKGG ALGGANDERE ATLNQLLVEM
DGFTTSDQVV VLAGTNRPDV LDNALMRPGR FDRHIQIDSP DVNGRQIYL VHLKRLNLDP
LLTDDMNNLS GKLATLTPGF TGADIANACN EAALIAARHN DPYITIHFHE QAIERVIAGL
EKKTRVLSKE EKRSVAYHEA GHAVCGWFLK YADPLLK VSI IPRGQ GALGY AQYLPDPQYL
ISEEQFRHRM IMA LAGGRVSE ELHFPSVTS G AHDDFKV TQ MANAMVTSLG MSPKIGYLSF
DQNDGNFKVN KPFNSKTART IDLEVKSIVD DAHRAC TELL TKNL D KVDLV AKELLRKEAI
TREDMIRLLG PRPFKERNEA FEKYLDPKSN TEPPEAP AAT N
  
```

P40341 (100%), 93,280.1 Da

Mitochondrial respiratory chain complexes assembly protein YTA12 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=YTA12 PE=1 SV=2

3 exclusive unique peptides, 3 exclusive unique spectra, 3 total spectra, 36/825 amino acids (4% coverage)

```

MLLLSWSRIA TKVVRPVRVF RSY YGLTHIK SLHTQYRLLN RLQENKSGNK NEDNNEDAKL
NKEIPTDEEV EAIRKQVEKY IEQTK NNTIP ANWKEQKRKI DESIRRLLEDA VLKQESNRIO
EERKEKEEEN GPSKAKSNRT KEQGYFEGNN SRNIPPPPPP PPKPLNDP SNPVSKNVNL
FQIGLTFLL SFLLDLLNSL EEQSEITWQD FREKLLAKGY VAKLIVVNXS MVKVM LNDNG
KNQADN YGRN FYYFTIGSID SFEHLQKQAO DELDIDKDFR IPVLYVQEGN WAKAMFQILP
TVLMIAGI IW LTRRSQAQAG GSRGGIFGLS RSKAKKFNT T DVKIKFKDV AGCDEAKEEI
MEFVSFLKEP SRYEKMGAKI PRGAILSGPP GTGKTL LAKA TAGEAGVPFY FVSGSEFVEM
FVGVGAARVR DLFKTAARENA PSIVFIDEID AIGKARQKGN FSGANDEREN T LNQMLVEMD
GFTPADHV VV LAGTNRPDIL DKALLRPGRF DRHINIDKPE LEGRKAIFAV HLHHLK LAGE
IFDLK NRLAA LTPGFSGADI ANVCNEAALI AARSDEDAVK LNHFEOAIER VIGGVERKSK
LLSPEEKVVV AYHEAGHAVC GWYLYKADPL LKVSIIIPRG GALGYAQYLP GDIFLLTEQQ
LKDRMTPMSLG GRVSEELHFP SVTSGASDDF KKVTSMATAM VTELGM SDKI GWVNYQKRDD
SDLT KPFSD E TGD I IDSEVY RIVQECHDRR TKLLKEKAED VEKIAQVLLK KEVLTREDMI
DLLGKRPFPE RNDADF K YLN DYETEKIRKE EEKNEKRNEP KPSTN
  
```

P32795 (100%), 81,774.2 Da

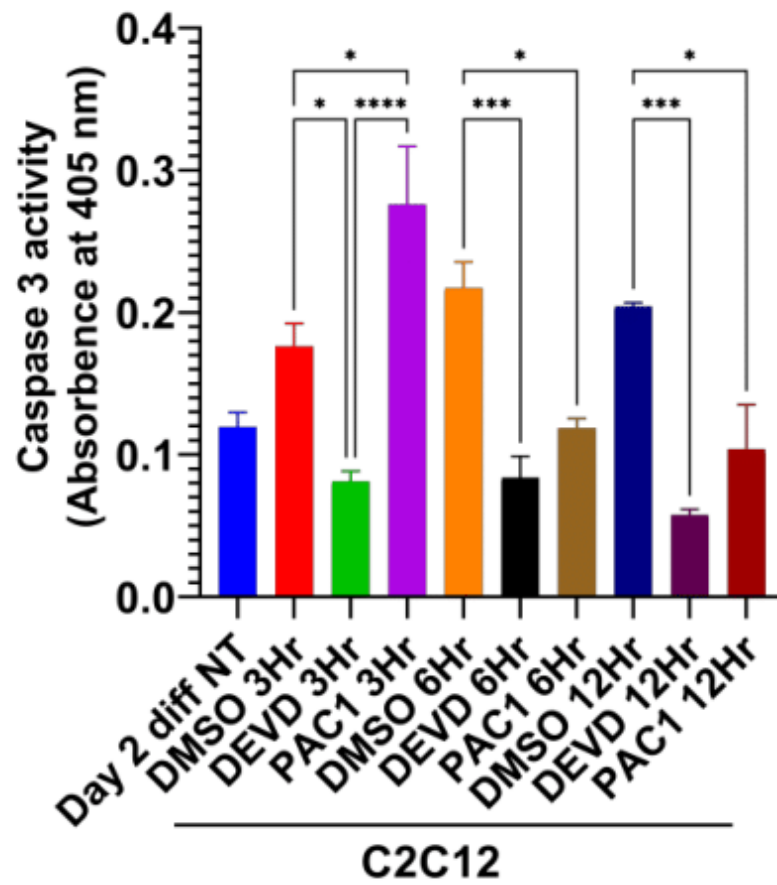
Mitochondrial inner membrane i-AAA protease supercomplex subunit YME1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=YME1 PE=1 SV=1

2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 32/747 amino acids (4% coverage)

```

MNVSKILVSP TVTTNVLRIF APRLPQIGAS LLVQKKWALR SKKFYRFYSE KNSGEMPPKK
EADSSGKASN KSTISSIDNS QPPPPSNTND KTKQANVAVS HAMLATREQE ANKDLTSPDA
QAIFYKLLLO SNYPQYVVS R FETPGIASSP EGMELYMEAL QRIGRHSEAD AVRQNL L T AS
SAGAVNPSLA SSSSNQSGYH GNFPMSY SPL YGSRKEPLHV VVSESTFTVV SRWVKWLLVF
GILTYSFSEG FKYITENTTL LKSSSEVADKS VDVAK TNVKF DDVCGCDEAR AELEEVDFL
KDPTKYESLG GKLPKGVLLT GPPGTGKTL ARATAGEAGV DFFFGSGSEF DEYVGVGAK
RIRDLFAQAR SRAPAIFID ELDAI GGRN PKDQAYAKQT LNQLLVELDG FSQTSGII I
GATNFPEALD KALTRPGRFD KVVNVLDLPDV RGRADILKHH MKK I TLADNV DPT I IARGTP
GLSGAELANL VNQAAYVACQ KNAVSVDM SH FEWAKDKILM GAERKTMVLT DAARKATAFH
EAGHAIMAKY TNGATPLYKA TILPRGRALG ITFQLPEMDK VDI TKREACQA RLDVCMGGKI
AEELIYGKDN TTSGCCSDLQ SATGTARAMV TQYGM SDDVG PVNLS ENWES WSNKIRDIAD
NEVIELL KDS EERARRLLTK KNVELHRLAQ GLIEYETLDA HEIEQVCKGE KLDK LKTSTN
TVVEGPD SDE R KDIGDDKPK IPTMLNA
  
```

**FIGURE S2:** (A) Colorimetry assay was performed to determine the Caspase 3 activity in BY4741 and hCasp3-TDP43 expressing strain with Gal induced expression of TDP43-RFP for 6hr and 22hr. The value represents three independent experiments and are shown as mean  $\pm$  SEM with p-value  $< 0.05$ . (B) Caspase 3 activity assay in hCasp3-TDP43 expressing strain subjected to treatment with z-DEVD-FMK and PAC1. The values correspond to mean  $\pm$  SEM with p-value  $< 0.05$ . (C) Spot assay to assess the viability of three yeast strains BY4741,  $\Delta$ ScMCA1 and hCasp3-GFP subjected to heat stress (30°C & 42°C). The arrow above the panel indicates a 5-fold serial dilution of plated cells starting from left to right (1:1, 1:5, 1:25, 1:125). The colonies were counted and showed no difference in viability between the three yeast strains as indicated in the graph. The data represents three independent experiments and are shown as mean  $\pm$  SEM with p-value  $< 0.05$ . (D) Protein aggregation observed in BY4741,  $\Delta$ ScMCA1 and hCasp3-GFP strain using filter trap assay. The graph shows fold difference in protein density normalized to the control BY4741. The values are represented as mean  $\pm$  SEM with p-value  $< 0.05$ . (E) Volcano plot showing protein change in DMSO Vs. z-DEVD-FMK treated yeast cells. Protein differentially regulated with a p-value  $< 0.05$  are shown in green. Grey represents proteins that were not found to differ significantly between z-DEVD-FMK treatment and DMSO control. The horizontal axis indicates log 2-fold change of protein abundance while the vertical axis shows  $-\log_{10}$  of the p-value. (F) Coverage of five TDP43 interacting proteins PHB1, PHB2, AFG3, YTA12 and YME1 identified in the LC-MS/MS mass spectrometry analysis.



**FIGURE S3:** Substrate based colorimetry assay was performed to assess the Caspase 3 activity in C2C12 cells differentiated for 2 days and subjected to z-DEVD-FMK and PAC1 treatment for 3hr, 6hr & 12hr. The data is represented as mean  $\pm$  SEM with p-value < 0.05.