Supplemental Materials Molecular Biology of the Cell

Dhani et al.

Supplementary videos 0h_depletion 22h_depletion 25h_depletion

KT3711 h⁹⁰ ade6-M216 leu1+::P_{nmt81}-mzt1-S-GFP-2xFLAG P_{nda3}-mCherry-atb2-aur1^R mzt1::Hyg^R

Supplementary Figure S1. Examination of the Mzt1 depletion phenotypes.

Cells that are depleted for Mzt1 for 0 hour, 22 hours and 25 hours were filmed for 20 min at 1.5 min interval to record the Mzt1-GFP and mCherry-Atb2 signals. At each timepoint, 15 to 20 images along the Z-axis were taken at 0.4 μ m intervals to span the whole thickness of the cells and these are Z-projected to produce one flat image per timepoint. For a quantitative analysis, 130 cells (0 h depletion), 214 cells (22 h depletion) and 183 cells (25 h depletion) were observed and classified into categories depicted in the supplementary figure S2. Representative images of one timepoint of 0, 22 and 25 h depleted cells are presented. Corresponding movies for these images are provided as Supplementary movies.

Supplementary Figure S2.

Cartoon representations of categories of Mzt1 depletion phenotypes used in the quantitative analyses presented in Supplementary figure S1 and Figure 3G.

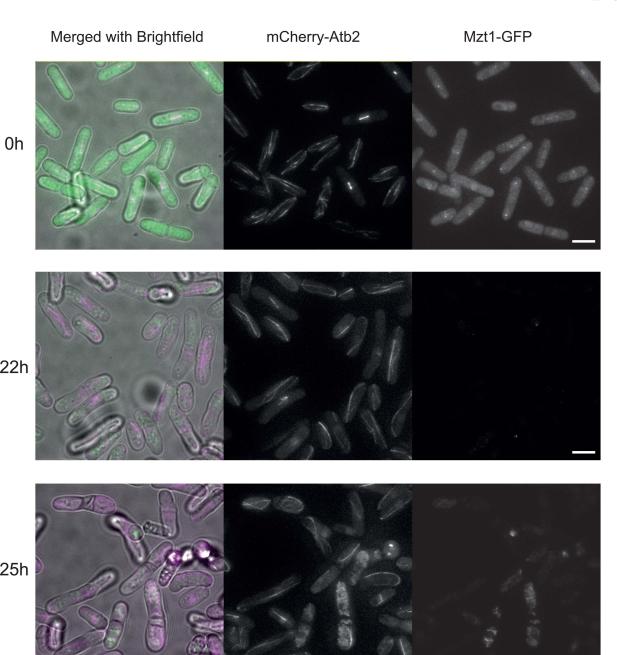
Supplementary Figure S3.

Sequence alignment of fission yeast GCP3^{Alp6} and human GCP3 by clustalW. Amino acids 118-186, which are predicted to be involved in Mzt1 binding, are indicated in yellow. The human GCP3 residues (551-555), proposed to be at the "hinge" region (Guillet *et al.*, 2011), and corresponding Alp6 residues (499-503) are indicated in red.

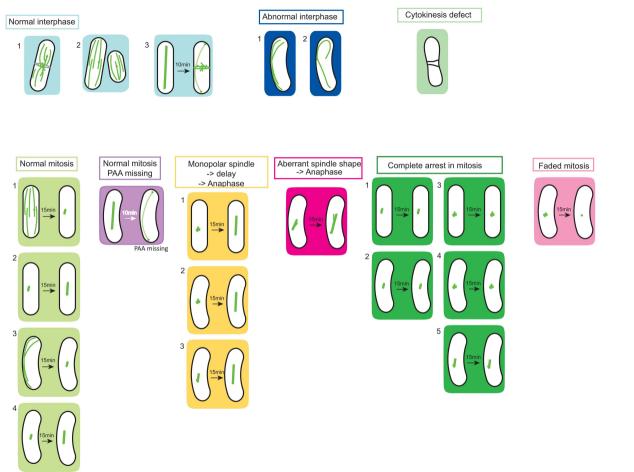
Supplementary videos

0h_depletion.mov, 22h_depletion.mov, 25h_depletion.mov

Live cell images of the cells presented in the Supplementary figure \$1. GFP tagged Mzt1 was depleted for 0 h, 22 h and 25 h respectively and signals of mCherry tagged α -tubulin Atb2 was observed at every 90 sec for 20min.



Classification of MT status



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Alp6
      MSEIHVKTA---LSRLADKYLONSKNPSVPYTIETIVSFFOEIIHSISP---DTFOLDIDDILYKIYSKIP--PEENNDALFSKLSNLVS
      MATPDOKSPNVLLONLCCRILGRSE-----ADVAOOFOYAVRVIGSNFAPTVERDEFLVAEKIKKELIRORREADAALFSEL---HR
hGCP3
      *: . *:.
              * . * . * . * . * .
                                RLKSOTVIHNKSOILYFLYLLS--PISOSSRDVSSHLLDESISNPINIPSTEV------
Alp6
hGCP3
      KLHSOGVLKNKWSILYLLLSLSEDPRROPSK-VSSYATLFAOALPRDAHSTPYYYARPOTLPLSYODRSAOSAOSSGSVGSSGISSIGLC
      Alp6
      ----ESSNFGO-----TRYDOVPENPO-ITDWDEGLENESS---ISIAHDSSRLNRSTETSSVOHTLITEADLLSSISY
hGCP3
      ALSGPAPAPOSLLPGOSNOAPGVGDCLROOLGSRLAWTLTANOPSSOATTSKGVPSAVSRNMTRSRREGDTGGT--MEITEAALVRDILY
                            **** ** * *
Alp6
      VLQGISTEYVQFKNELALLSKR--IPVQYLLQ--MRALSETGLLYQELKVFSNYDPSVSQSIDGDNVSKAFINDQSLALQSLKSVISKEL
hGCP3
      VFOGIDGKNIKMNNTENCYKVEGKANLSRSLRDTAVRLSELGWLHNKIRRYTDQR-SLD-----RSFGLVGQSFCAALHQEL
      . .
                                                             .. .*. **: :.: :**
Alp6
      TNFLALIASLDSQIRAD-----ASLEKPMVTIRRCIAWTQVAKLKLRILSSVVNDNMNQENKKRLIQVVSKYNVHGDPLIQELSDKILT
hGCP3
      REYYRLLSVLHSOLOLEDDOGVNLGLES-SLTLRRLLVWTYDPKIRLKTLAALVDHCOG-RKGGELASAVHAYTKTGDPYMRSLVOHILS
                        .. *.. *.**
      EITGPLYEMIENWIYKGELVDPYOEFFVKEKNGSESHDHOGOGDVVWKGKYFLDKELIPSFLSEELVDKIFLIGKSLNFARYGCGDFDWA
Alp6
hGCP3
      LVSHPVLSFLYRWIYDGELEDTYHEFFVA-----SDPTVKTDRLWHDKYTLRKSMIPSFMTMDOSRKVLLIGKSINFLHOVCHDOTPT
       * • * • * • * * * * * * * • • * * • * * • * * • * * •
      QEHYQKLVKKLSYRDP-----HSLETVVDKAYTESINHLVYLMEEVFHLTDHLKAIKKYLLLGQGDFVDLLMESLGNSLDQPANTLF
Alp6
hGCP3
      TKMIAVTKSAESPODAADLFTDLENAFOGKIDAAYFETSKYLLDVLNKKYSLLDHMOAMRRYLLLGOGDFIRHLMDLLKPELVRPATTLY
                        RHNLTASLESAIRSSNASYEPEYVLKRLDARLLELSHGETGWDVFTLEYKVDSPINVIITPYCSROYLKIFNFLWRLKRIEFALAHSWRR
Alp6
hGCP3
      OHNLTGILETAVRATNAOFDSPEILRRLDVRLLEVSPGDTGWDVFSLDYHVDGPIATVFTRECMSHYLRVFNFLWRAKRMEYILT-DIRK
      VNLGERNVFRNLDYTKFEWHFVSCHL--AEMIHFVCQLQYYILFEVIEISWQELQLAMEKPNATLDTYIEAHEKYVTSITHKGLLGGGKS
Alp6
hGCP3
      GHMCNAKLLRNMP--EFSGVLHOCHILASEMVHFIHOMOYYITFEVLECSWDELWNKVOOA-ODLDHIIAAHEVFLDTIISRCLLDS---
       Alp6
      RNEDSFLHOLHDILKVILNFHDAIELLYNFSC-SLSNRIRINVPISTDALAAOYTP-----IKNELSNFTEEFO
hGCP3
      -DSRALLNQLRAVFDQIIELQNAQDAIYRAALEELQRRLQFEEKKKQREIEGQWGVTAAEEEEENKRIGEFKESIPKMCSQLRILTHFYQ
       : .:* :* :*
Alp6
      VRLOKLLHGLASHKDPEMRFLSVRLNYNEFYVSHRRRHD-----KDVTSO
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GIVQQFLVLLTTSSDESLRFLSFRLDFNEHYKAREPRLRVSLGTRGRRSSHT

hGCP3