## Supplementary Table legends

### Table S1

**Xenopus and human proteins entries.** Entries from all the *Xenopus laevis* proteins detected by mass spectrometry and the corresponding human protein entries.

### Table S2

**Ran Spindle Assembly Factors in the RanMT proteome.** RanGTP regulated SAFs present in the RanMT proteome.

### Table S3

**The RanMT proteome.** Complete human entries for the RanMT proteome after filtering and overlaps with previously published proteomes of interest. The following data are shown: spindle/MT related GOs; presence in MiCroKITS; or interaction with these; presence in the general RanMT network; number of connections in this network; presence in the functional networks; classification as a dynamic or non-dynamic protein and presence in any of the clusters. Ran SAFs, kinases and phosphatases are also specified.

### Table S4

**Protein candidates.** Features of the selected protein candidates.

### Table S5

**Protein dynamic profiles.** Clusters classification of the 286 dynamic proteins and their relative abundance values for each time-point.

## Supplementary figure legends

## Figure S1

### Gene Ontology analysis of the RanMT proteome

Clusters of GO terms represented in the RanMT proteome obtained with the online CleverGO tool analysis. Three of the major clusters are shown in red with the corresponding Wordclouds on the left.

## Figure S2

### Candidate analysis

**A**- Western blot of control (CTRL) and CBX3 silenced cells using specific against anti-CBX3 and anti-tubulin antibodies. 30µg of cell protein lysate were loaded per lane. CBX3 was efficiently silenced.

**B**- Western blot of control (CTRL) and DnaJB6 silenced cells using specific against anti-DnaJB6 and anti-tubulin antibodies. 30µg of cell protein lysate were loaded per lane. DnaJB6 was efficiently silenced.

**C-** Schematic representation of the MT regrowth experiment in control and CBX3 or DnaJB6 silenced cells.

**D-** Schematic representation of the MT regrowth experiment in cells incubated with DMSO (control) or the CK2 inhibitor Quinalizarin.

## Figure S3

**Functional modules in the RanMT proteome**

**A-** Venn diagram showing the overlap between the proteins (including the selected key factors and their interactors) present in each of the four functional modules. The table shows the number of proteins (selected key proteins and their interactors) in each module. Note that the total number of nodes and connections is smaller than the sum of all the individual categories due to overlaps.

**B**- Protein-protein interactome network of the proteins included in the MT nucleation module.

**C**- Protein-protein interactome network of the proteins included in the MT dynamics module including both MT stabilization and destabilization factors (green dots are proteins specific for MT-stabilization and blue dots are proteins specific for MT destabilization).

**D**- Protein-protein interactome network of the proteins included in the MT organization module.

In all cases only experimentally validated interactions were considered. The selected key proteins are in orange.

## Figure S4

**Intensity areas of the dynamic and non-dynamic proteins in the RanMT proteome**

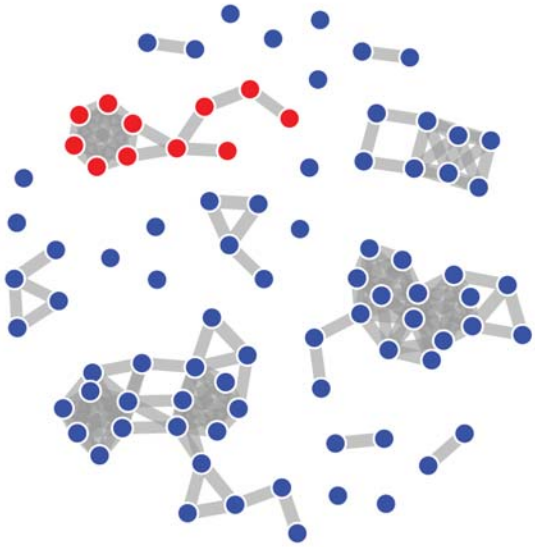
The average of the intensity areas for all the dynamic (white boxes) and non-dynamic (grey boxes) proteins in the RanMT proteome were calculated at each time point. Box and whiskers plot showing protein intensity area averages comparison between dynamic (white) and non-dynamic proteins (grey). Boxes show values between the 25th and the 75th percentiles, with a line at the median and a + at the mean, whiskers extend from the 10th to the 90th percentile and dots correspond to outliers values. No significant differences were observed between the two groups at any of the time-points (ANOVA test).

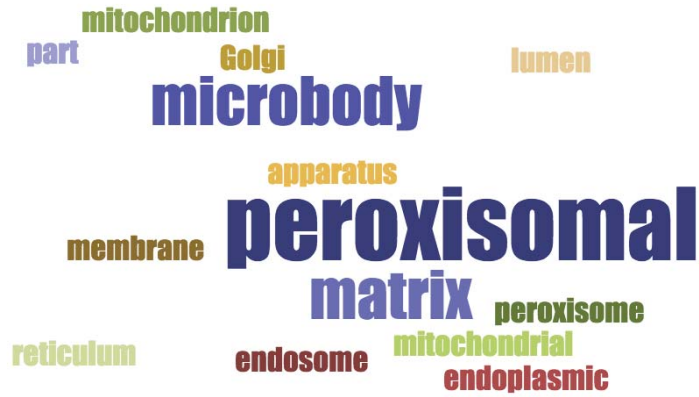
## Figure S5

**Dynamic abundance profiles for selected proteins and protein complexes**

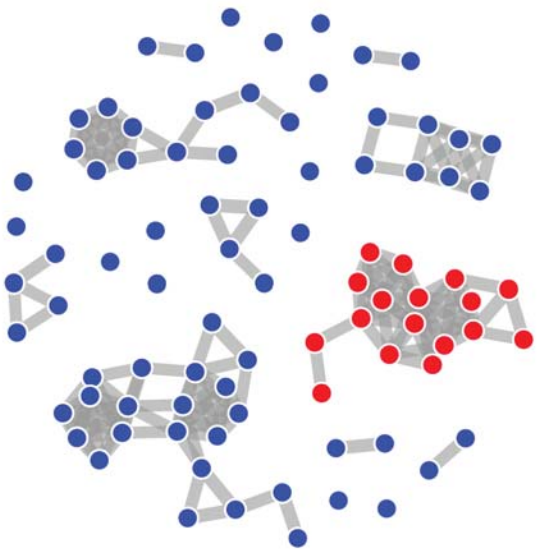
Graphical representation of the changes in relative protein abundance for some key proteins (as indicated) involved in (A) MT nucleation (cluster 6), (B) MT dynamics (clusters 5 and 6) and (C) MT organization (clusters 6 and 7).

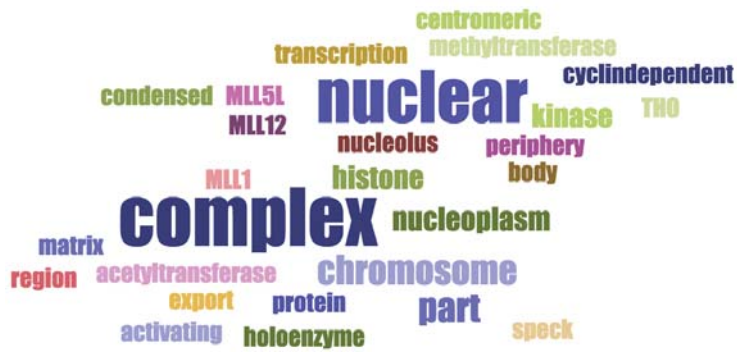
A






B






C

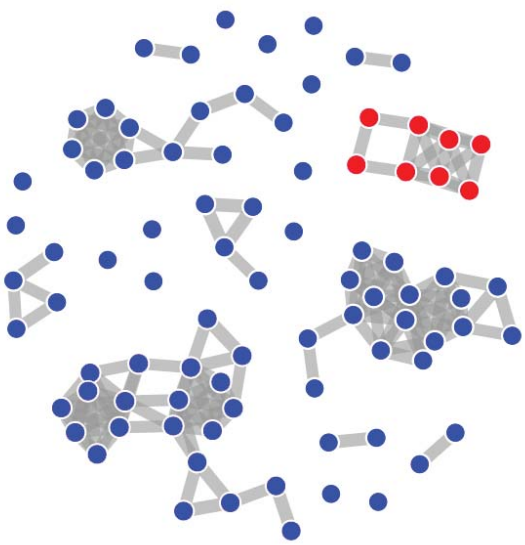



**Figure S1**

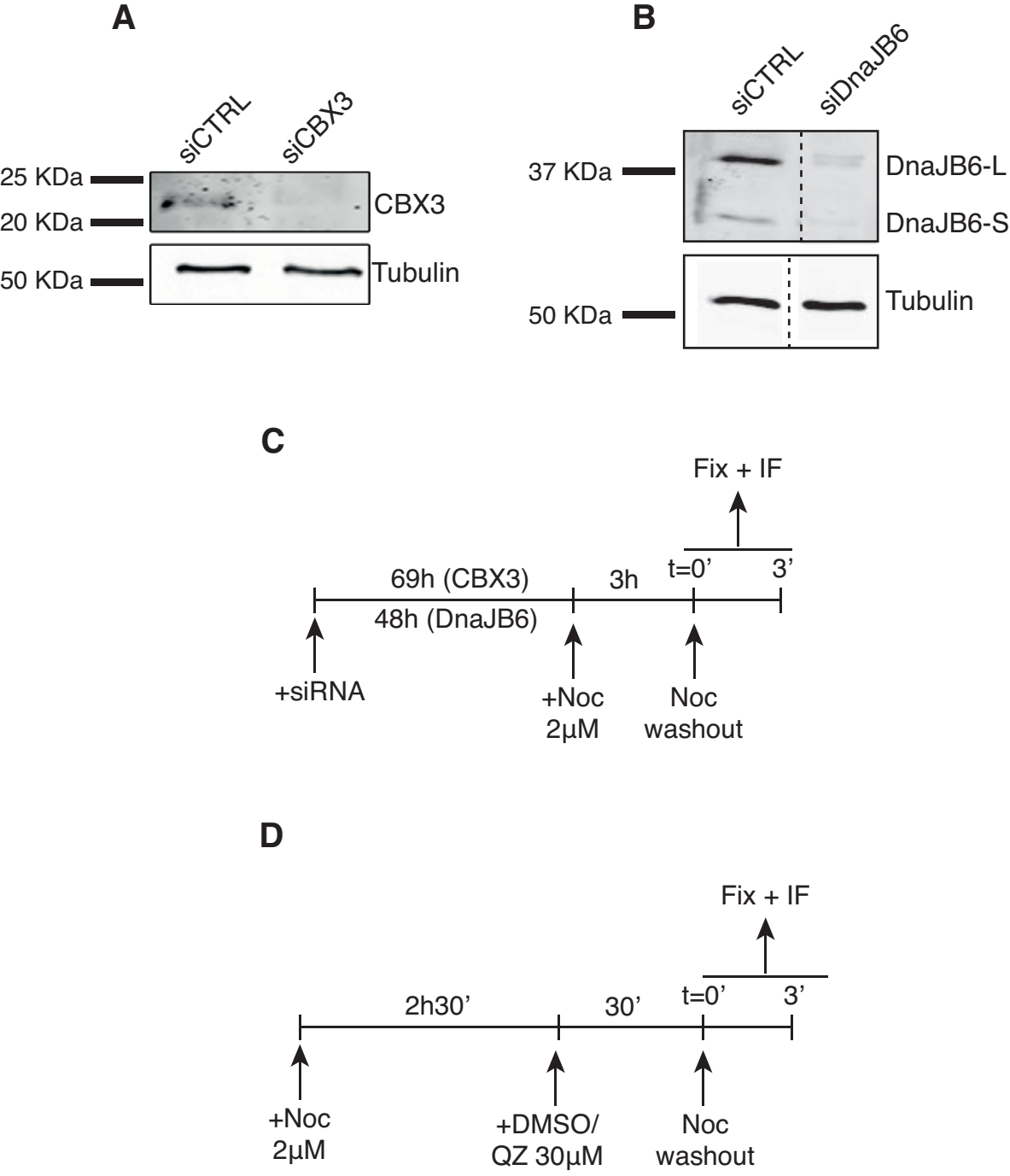
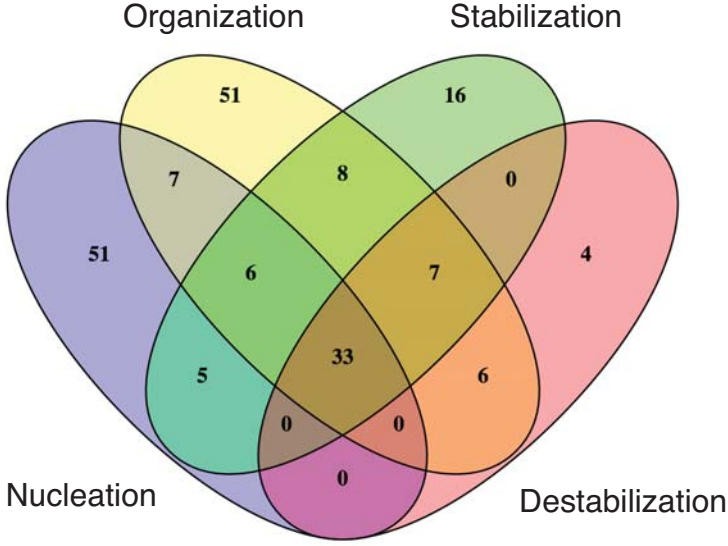

A
siCTRL
siCBX3
25 KDa
20 KDa
CBX3
50 KDa
Tubulin
B
siCTRL
siDnaJB6
37 KDa
DnaJB6-L
DnaJB6-S
50 KDa
Tubulin
C
Fix + IF
69h (CBX3)
48h (DnaJB6)
3h
t=0'
3'
+siRNA
+Noc
2µM
Noc
washout
D
Fix + IF
2h30'
30'
t=0'
3'
+Noc
2µM
+DMSO/
QZ 30µM
Noc
washout

**Figure S2**

A




| | Nodes | Connections |
|---|---|---|
| Nucleation | 15 | 85 |
| Stabilization | 7 | 67 |
| Destabilization | 5 | 44 |
| Organization | 22 | 95 |
| Total | 47 | 142 |

B

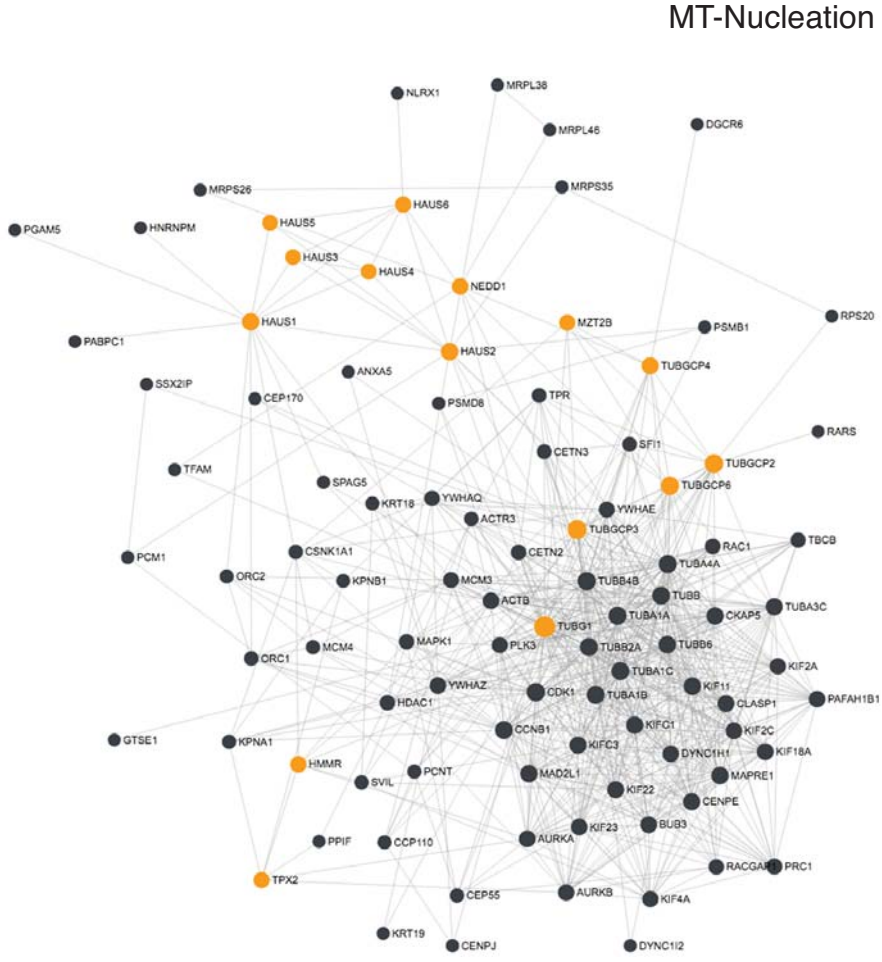



**Figure S3**

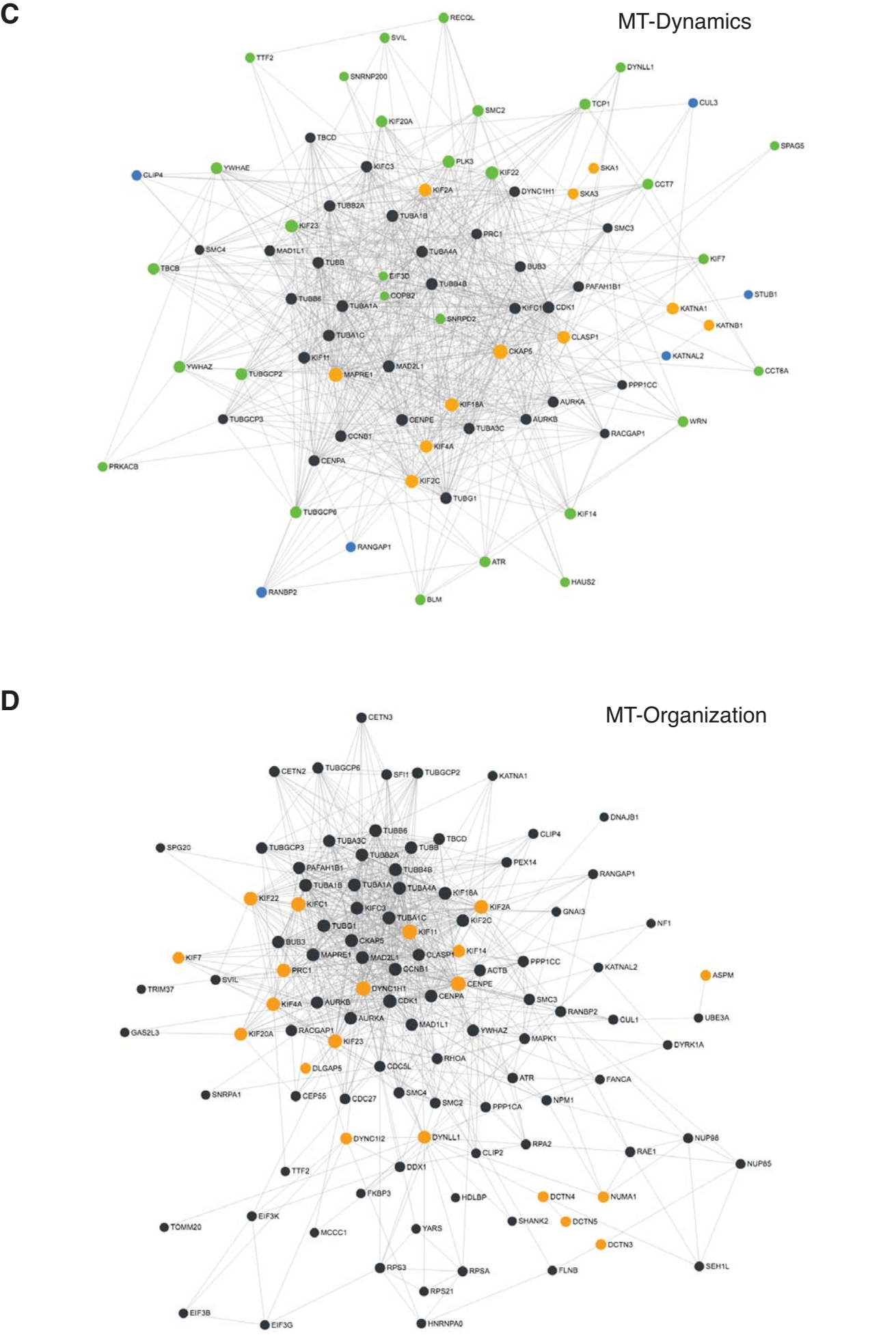

C
MT-Dynamics
RECQL
SVIL
TTF2
SNRNP200
DYNLL1
SMC2
TCP1
CUL3
KIF20A
TBCD
SPAG5
CLIP4
YWHAE
KIFC3
PLK3
KIF22
SKA1
KIF2A
DYNC1H1
SKA3
CCT7
TUBB2A
TUBA1B
KIF23
PRC1
SMC3
SMC4
MAD1L1
TUBA4A
KIF7
TBCB
TUBB
BUB3
EIF3D
TUBB4B
PAFAH1B1
STUB1
TUBB6
COPB2
TUBA1A
KIFC1
CDK1
KATNA1
SNRPD2
KATNB1
TUBA1C
CLASP1
CKAP5
KIF11
KATNAL2
YWHAZ
MAD2L1
CCT6A
TUBGCP2
MAPRE1
PPP1CC
KIF18A
AURKA
TUBGCP3
CENPE
AURKB
TUBA3C
WRN
CCNB1
RACGAP1
KIF4A
PRKACB
CENPA
KIF2C
TUBG1
TUBGCP6
KIF14
RANGAP1
ATR
HAUS2
RANBP2
BLM
D
MT-Organization
CETN3
CETN2
TUBGCP6
SFI1
TUBGCP2
KATNA1
DNAJB1
TUBB6
CLIP4
TBCD
SPG20
TUBGCP3
TUBA3C
TUBB
TUBB2A
PEX14
PAFAH1B1
TUBB4B
RANGAP1
TUBA1B
TUBA1A
TUBA4A
KIF18A
KIF22
KIFC1
KIFC3
KIF2A
GNAI3
TUBA1C
KIF2C
NF1
TUBG1
KIF11
BUB3
CKAP5
KIF7
MAPRE1
MAD2L1
CLASP1
KIF14
PPP1CC
PRC1
CCNB1
ACTB
KATNAL2
ASPM
SVIL
TRIM37
DYNC1H1
CENPE
KIF4A
AURKB
CDK1
CENPA
SMC3
RANBP2
CUL1
UBE3A
AURKA
MAD1L1
YWHAZ
GAS2L3
KIF20A
RACGAP1
MAPK1
KIF23
DYRK1A
RHOA
DLGAP5
CDC5L
ATR
FANCA
SNRPA1
CEP55
CDC27
SMC4
NPM1
SMC2
PPP1CA
DYNC1I2
DYNLL1
RPA2
NUP98
CLIP2
RAE1
NUP85
TTF2
DDX1
FKBP3
HDLBP
DCTN4
NUMA1
EIF3K
SHANK2
DCTN5
TOMM20
MCCC1
YARS
DCTN3
RPS3
RPSA
FLNB
SEH1L
RPS21
EIF3B
EIF3G
HNRNPA0

**Figure S3**

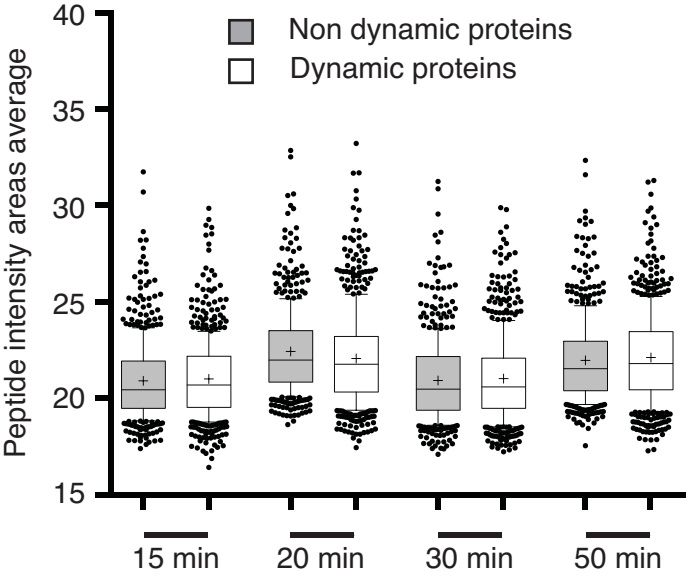

Non dynamic proteins
Dynamic proteins
Peptide intensity areas average
40
35
30
25
20
15
15 min
20 min
30 min
50 min

**Figure S4**

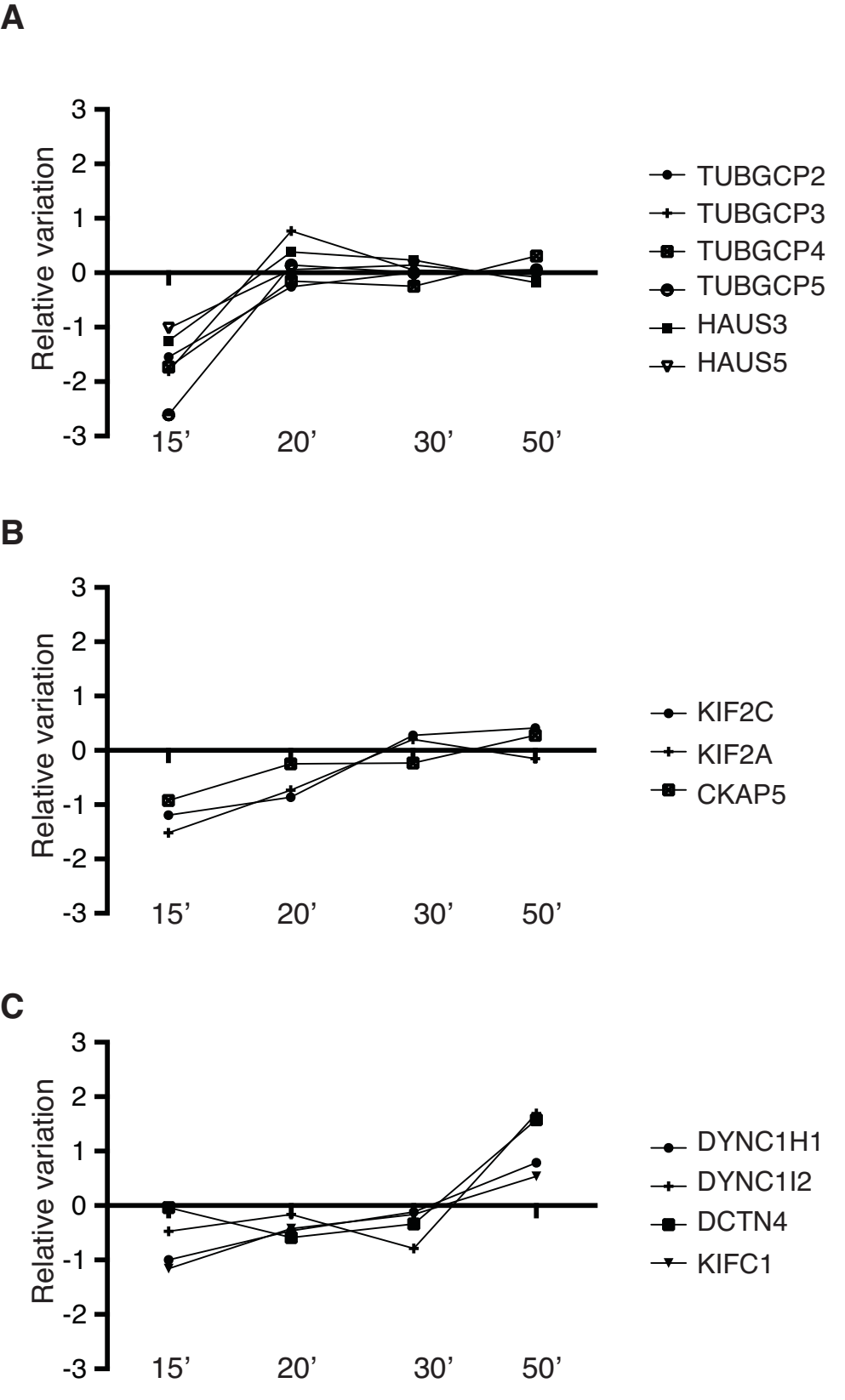

A
Relative variation
3
2
1
0
-1
-2
-3
15’
20’
30’
50’
TUBGCP2
TUBGCP3
TUBGCP4
TUBGCP5
HAUS3
HAUS5
B
Relative variation
3
2
1
0
-1
-2
-3
15’
20’
30’
50’
KIF2C
KIF2A
CKAP5
C
Relative variation
3
2
1
0
-1
-2
-3
15’
20’
30’
50’
DYNC1H1
DYNC1I2
DCTN4
KIFC1

**Figure S5**