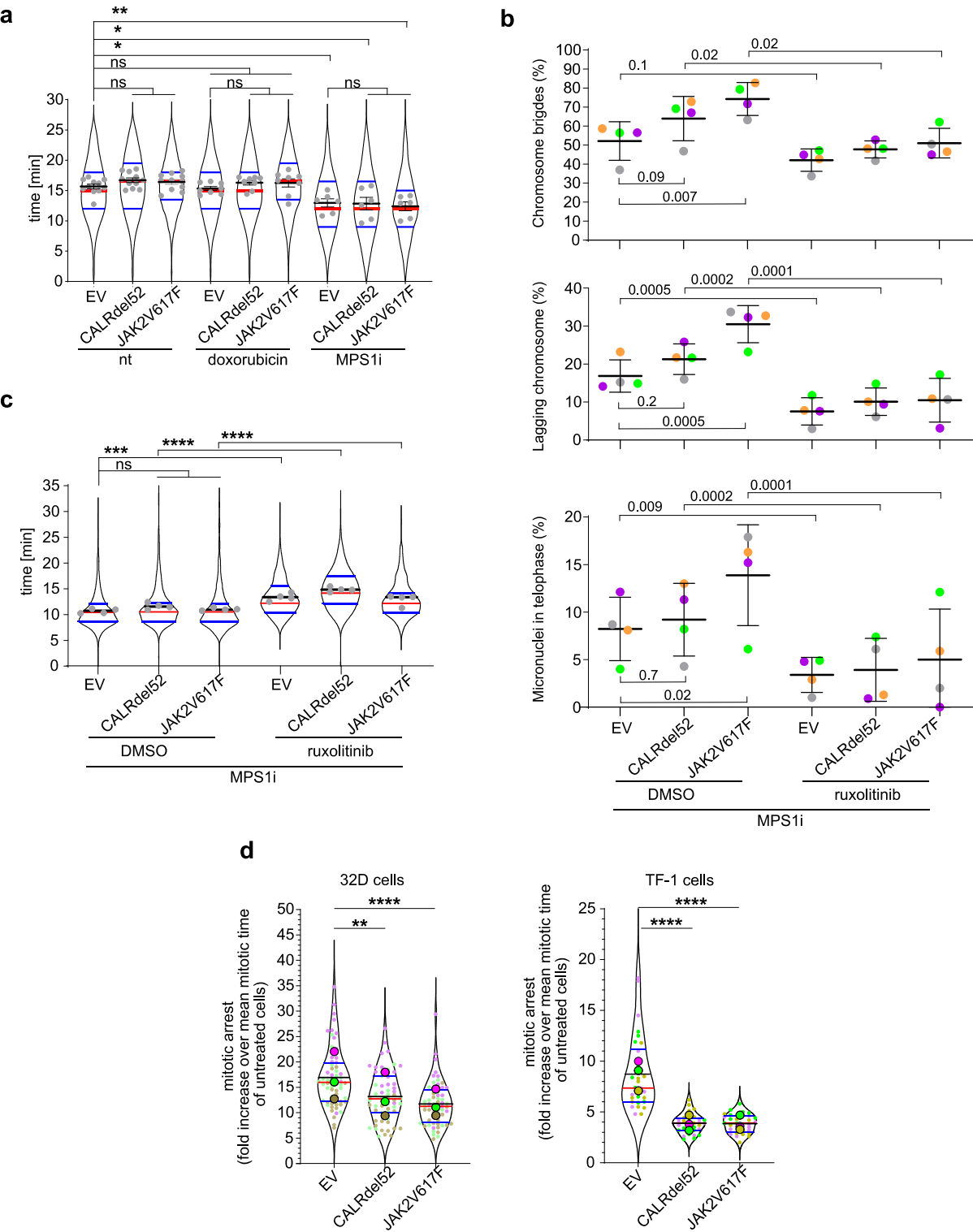


## **Calreticulin and JAK2V617F driver mutations induce distinct mitotic defects in myeloproliferative neoplasms**

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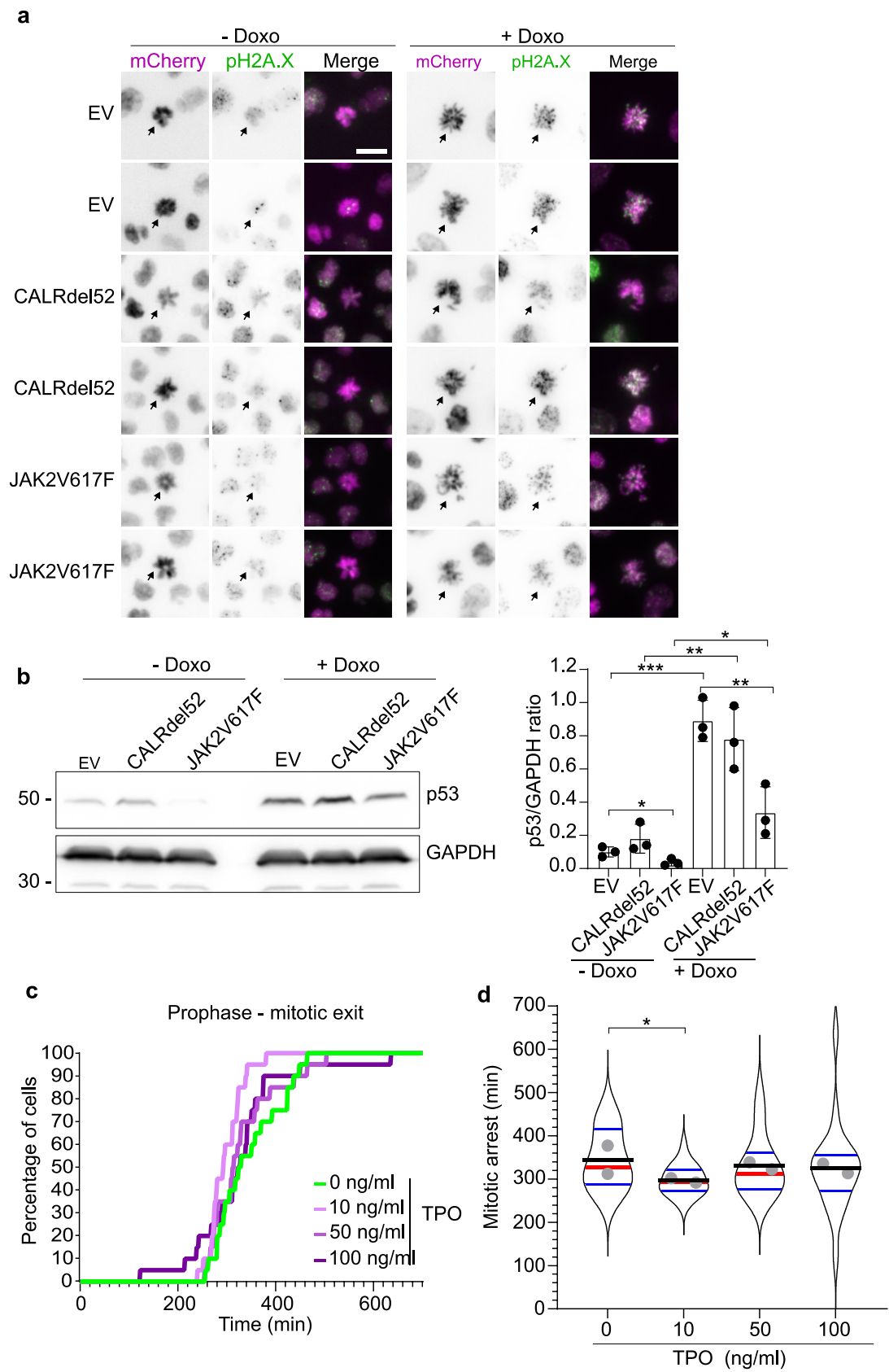
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Supplementary Figure S1



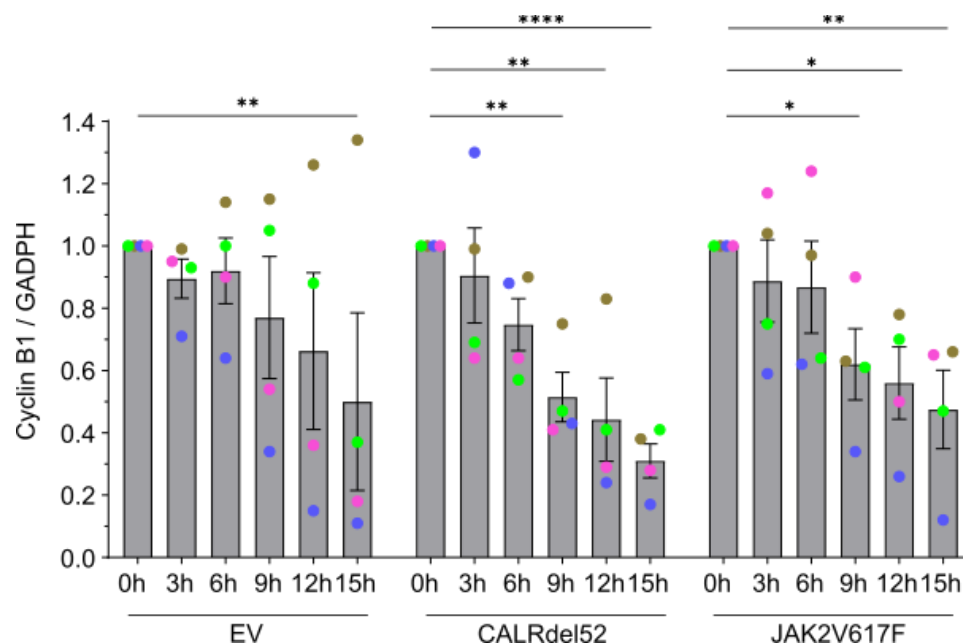
**Supplementary Figure S1. CALRdel52 and JAK2V617F mutations do not affect mitotic duration in murine 32D<sup>MPL</sup>.** a) Superplots of the mitotic duration in control (EV), CALRdel52, and JAK2V617F transduced 32D<sup>MPL</sup> cells expressing H2B-mCherry, untreated (nt) or treated with 200 nM doxorubicin or 3  $\mu$ M MPSi. The violin superplots show pooled data from 6 to 11 independent experiments with 100 cells per experiment and condition. The thick grey dots indicate independent experiment means. Horizontal red lines indicate medians, black lines mean, and blue lines are quartiles of the pool. One-Way ANOVA \* $p < 0.05$  \*\* $p < 0.01$ . b) Plots showing the percentage of chromatin bridges, lagging chromosomes and micronuclei in control (EV), CALRdel52 or JAK2V617F 32D cells in the presence of 3  $\mu$ M MPS1 inhibitor NMS-P715 after treatment with DMSO or 1  $\mu$ M ruxolitinib for 4 h (Four independent, color-coded experiments with >90 cells per experiment and condition, the horizontal black lines, and dispersion bars indicate means and SD of the means). The significance p-values were obtained with the exact Fisher test. c) Superplots of mitotic duration in control (EV), CALRdel52 or JAK2V617F 32D cells in presence of 3  $\mu$ M MPS1 inhibitor NMS-P715 after treatment with DMSO or 1  $\mu$ M ruxolitinib for 4h. The violin superplots show pooled data from 4 independent experiments with >90 cells per experiment and condition. The thick grey dots indicate independent experiment means. Horizontal red lines indicate medians, black line mean, and blue lines are quartiles of the pool. One-Way ANOVA \* $p < 0.05$  \*\* $p < 0.01$ . d) Fold increase of mitotic time in arrest in 100 ng/ml nocodazole treated control (EV), CALRdel52 and JAK2V617F 32D<sup>MPL</sup> or TF-1 cells over mean mitotic time of untreated cells. The violin superplots show three independent (color-coded) experiments with 20 cells per experiment and cell line. The thick colored dots indicate independent experiment means. Horizontal red lines indicate medians, black lines mean, and blue lines are quartiles of the pools. Kruskal-Wallis with Dunn's post-test; \*\* $p < 0.01$  \*\*\*\* $p < 0.001$ .

Supplementary Figure S2



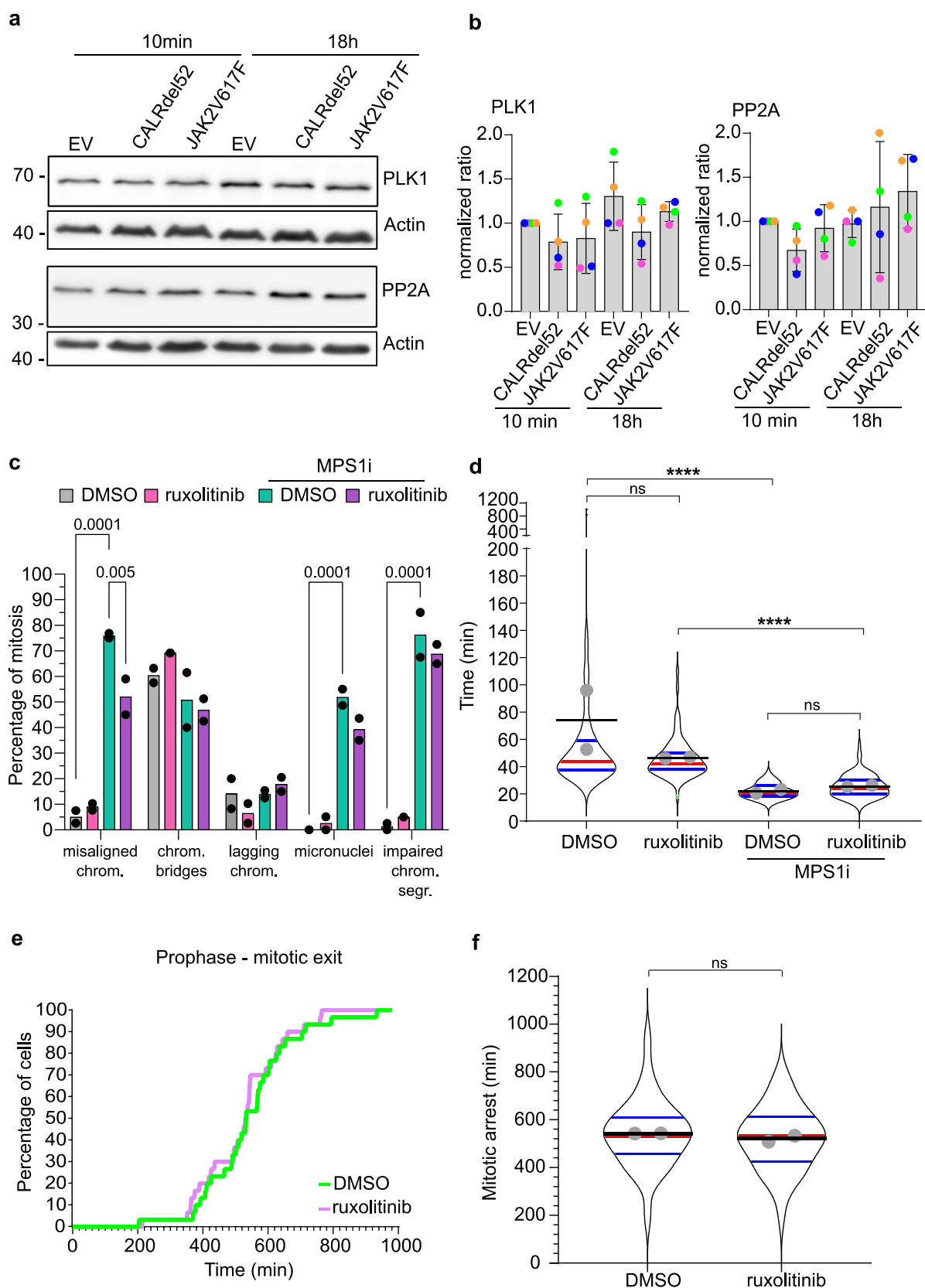
**Supplementary Figure S2. DNA damage and p53 levels are unchanged in mitotic control (EV), CALRdel52, or JAK2V617F transduced 32D<sup>MPL</sup> cells.** a) Representative immunofluorescence images of untreated or doxorubicin-treated (30h, 200nM) prometaphase cells stained for DNA double-strand breaks with the  $\gamma$ -H2AX (H2AX S139ph). Scale bar 10  $\mu$ m. b) Representative cropped western blots for p53 and GAPDH as loading control of whole cell extracts after being treated or not with doxorubicin (30h, 200nM). Quantitation of p53 protein expression normalized to GAPDH. The columns indicate the means, error bars SDs, and black dots individual data points of three independent experiments. Unpaired t-test; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns  $p > 0.05$ . For uncropped WB see Supplementary Figure S8. c) Plots show the fraction of control (EV) 32D<sup>MPL</sup> cells cultured with the indicated concentrations of TPO for more than 4h that exit mitosis at the given time after mitotic entry in the presence of 100 ng/ml nocodazole. d) Superplots of mitotic duration in control (EV) 32D<sup>MPL</sup> from (c). The violin superplots show pooled data from 2 independent experiments with 10 cells per experiment and condition. The thick grey dots indicate independent experiment means. Horizontal red lines indicate medians, black lines mean, and blue lines quartiles of the pool. Kruskal-Wallis test with uncorrected Dunn's post-test; \*  $p < 0.05$ .

### Supplementary Figure S3



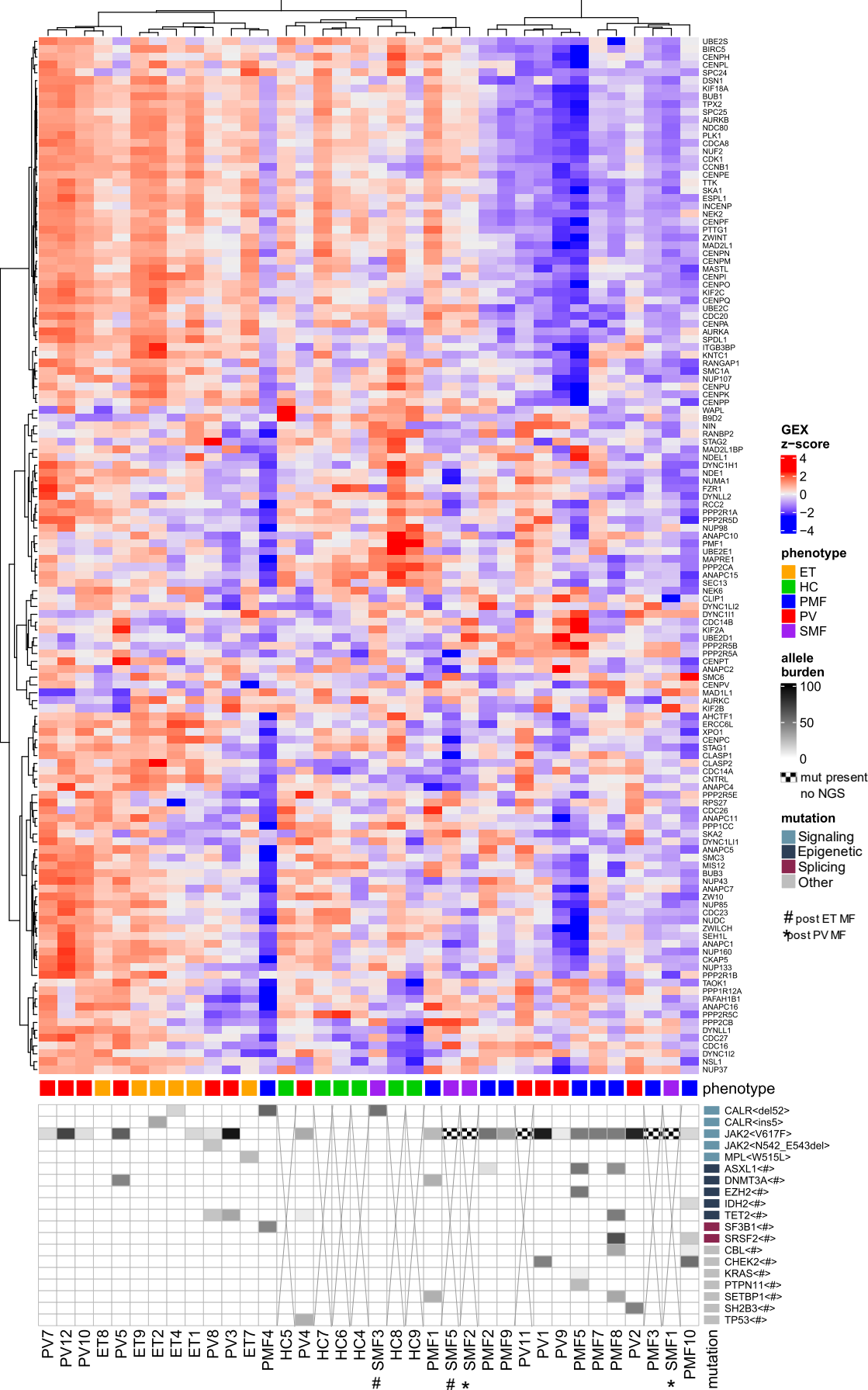
**Supplementary Figure S3. Cyclin B1 degradation is faster in cells expressing CALRdel52 and JAK2V617F mutations.** Cyclin B1 levels in cells, treated for 3 h with 200ng/ml nocodazole. Time points were taken after nocodazole wash out at t=0. Samples are, normalized to GAPDH and t=0. Columns indicate the means, error bars, SDs, and colored dots the values of four independent experiments. Two-way ANOVA with Dunnett post-test; \* p<0.05; \*\*p<0.01; \*\*\*p<0.001.

## Supplementary Figure S4



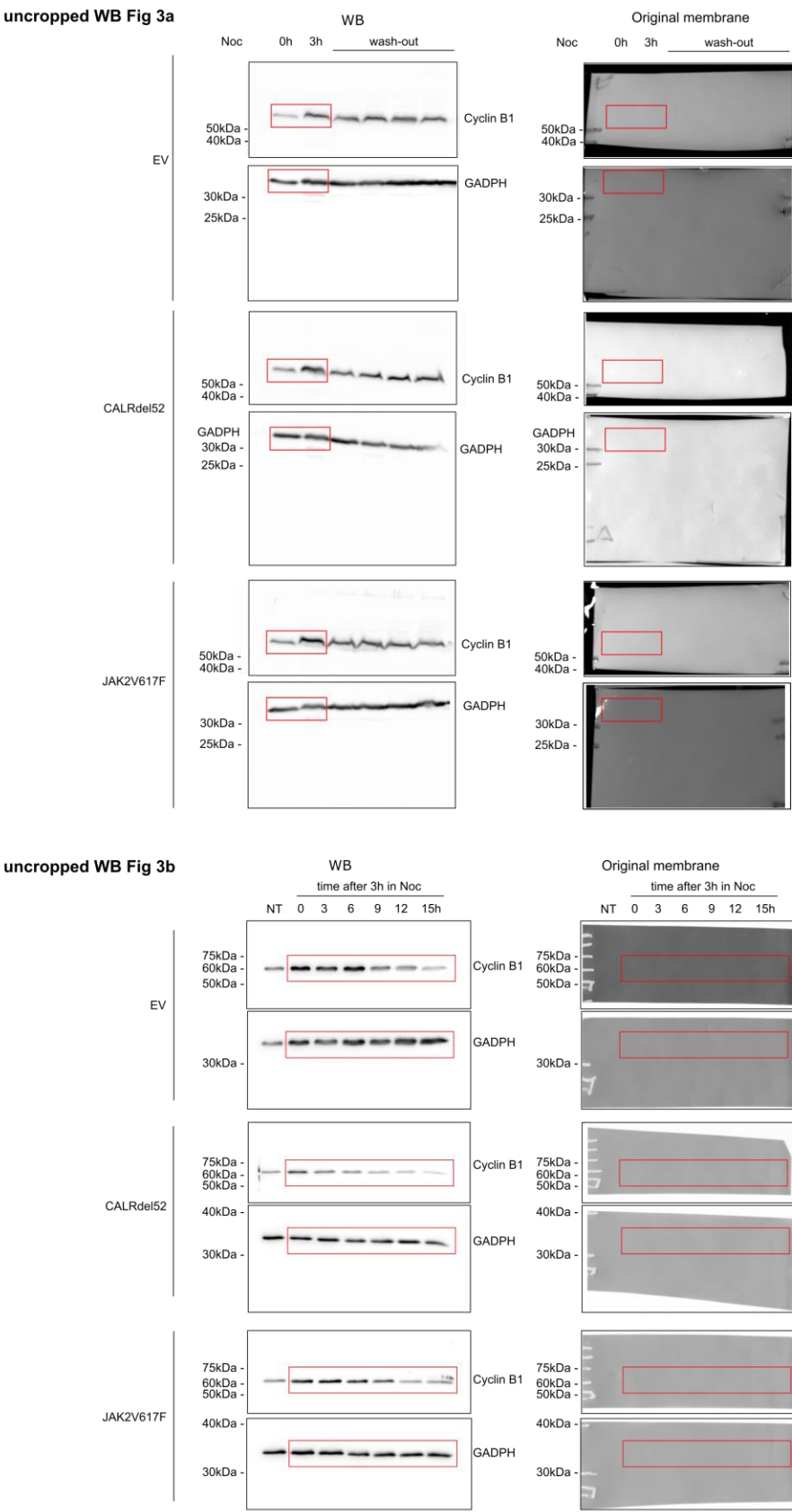
**Supplementary Figure S4. CALRdel52 and JAK2V617F mutations do not affect protein expression of the mitotic regulators PLK1 and PP2A.** a) Cropped western blots for PLK1, PP2A, and actin as loading control of whole cell extracts from cells at 10 min and 18 h after mitotic arrest induction with 200ng/ml nocodazole. b) Quantitation of the expression of PLK1 or PP2A normalized to actin and EV control. Grey columns indicate the means, the error bars the SDs, and the colored dots the values of four independent experiments. One-way ANOVA (Kruskal-Wallis test). For uncropped WB see Supplementary Fig. S8. c) Plots showing the percentage of chromatin bridges, lagging chromosomes and micronuclei in HEL cells in the presence of 3  $\mu$ M MPS1 inhibitor NMS-P715 after treatment with DMSO or 1  $\mu$ M ruxolitinib for 4 h (two independent experiments with >30 cells per condition, the bars indicate means and the dots the percentages of independent experiments). The significance p-values were obtained with Two-Way ANOVA. d) Superplots of mitotic duration in HEL cells from (c). The violin superplots show pooled data from 2 independent experiments with >30 cells per experiment and condition. The thick grey dots indicate independent experiment means. Horizontal red lines indicate medians, black lines mean, and blue lines are quartiles of the pool. Kruskal-Wallis with Dunn's post-test; ns  $p>0.05$  \*\*\*\* $p<0.001$ . e) Fraction of HEL cells treated with DMSO or 1  $\mu$ M ruxolitinib for 4 h that exit mitosis at the given time after mitotic entry in the presence of 100 ng/ml nocodazole. f) Mitotic duration in HEL cells from (c). The violin superplots show pooled data from 2 independent experiments with 15 cells per experiment and condition. The thick grey dots indicate independent experiment means. Horizontal red lines indicate medians, black lines mean, and blue lines are quartiles of the pool. Two-tailed t-test; \*  $p<0.05$ .

Supplementary Figure S5



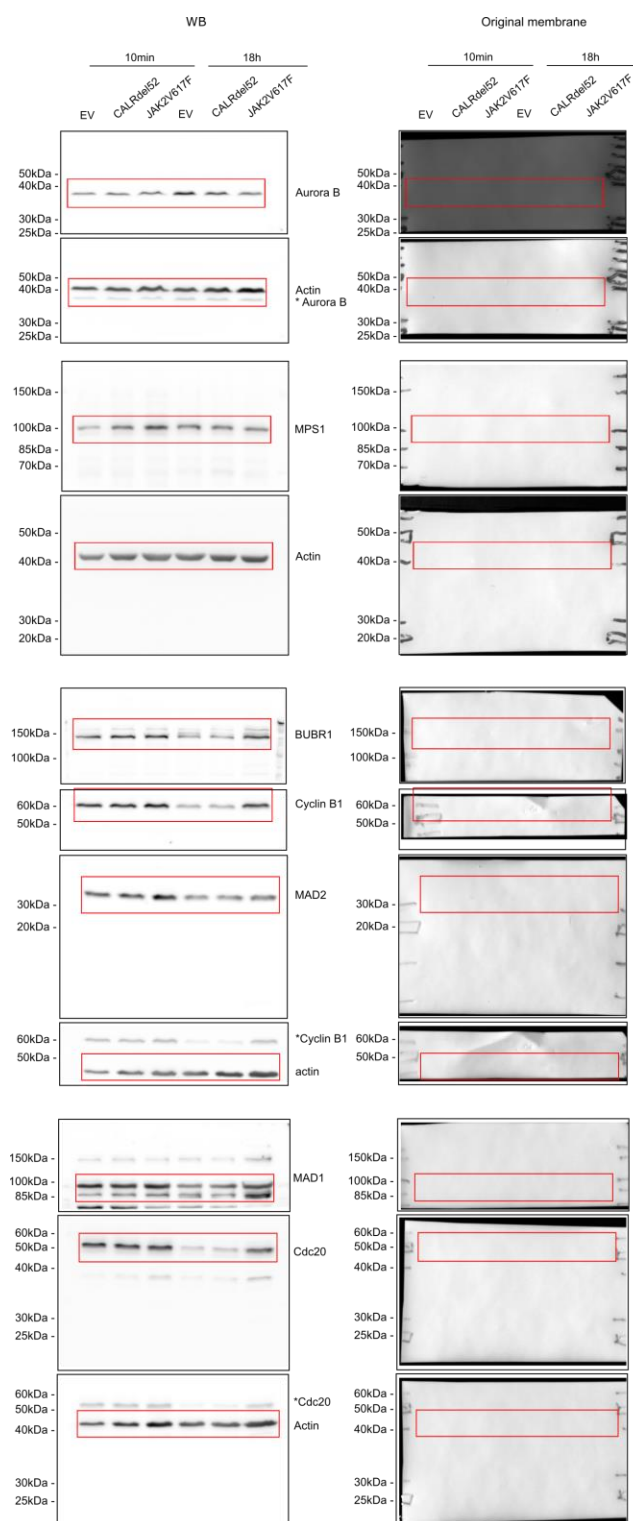
**Supplementary Figure S5. Hierarchical clustering of differentially expressed mitotic genes in CD34+ enriched mononuclear cells separates healthy controls (HC) from ET, PV, PMF, and SMF patients.** Heatmap of unsupervised clustering using the expression profile of 132 mitotic genes (see Supplementary Table 1) in each Ph-neg. MPN subtype and HC samples. Allele burden of MPN-associated driver and bystander mutations are indicated by shades of gray. The process affected by the mutations are indicated by a color code: signaling (light blue), epigenetic (dark blue), splicing (garnet), and others (grey). Samples lacking genotype by NGS are marked with x. Instances of JAK2V617F detection through alternative methods are indicated by hatching. The disease phenotype is indicated by a color code: ET (orange), healthy controls (HC; green), PMF (Blue), PV (red) and SMF (purple). Patients with secondary myelofibrosis post ET are indicated with (#) and post PV are indicated with (\*).

Supplementary Figure S6



**Supplementary Figure S6.** Uncropped WB and original membranes from Figure 3a and b. The red rectangle indicates the cropped area displayed in the main and supplementary figures.

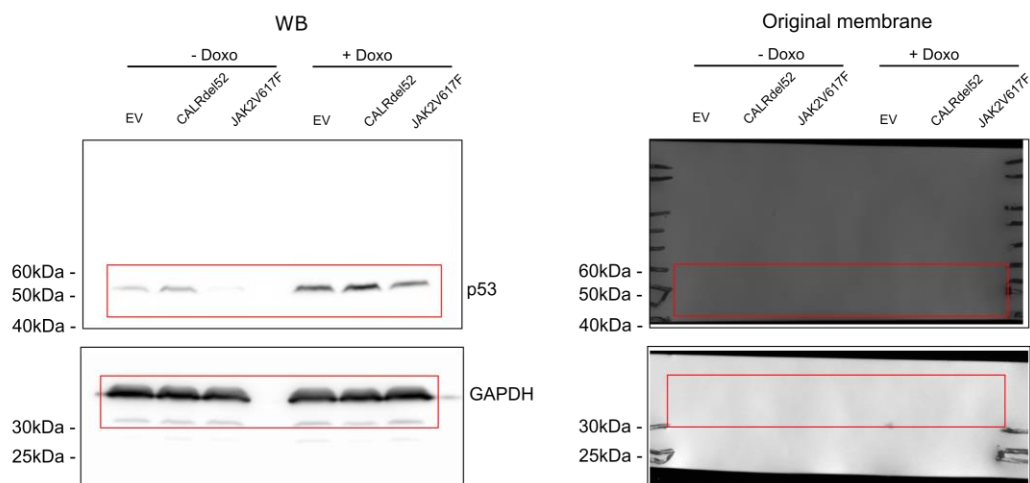
## Supplementary Figure S7



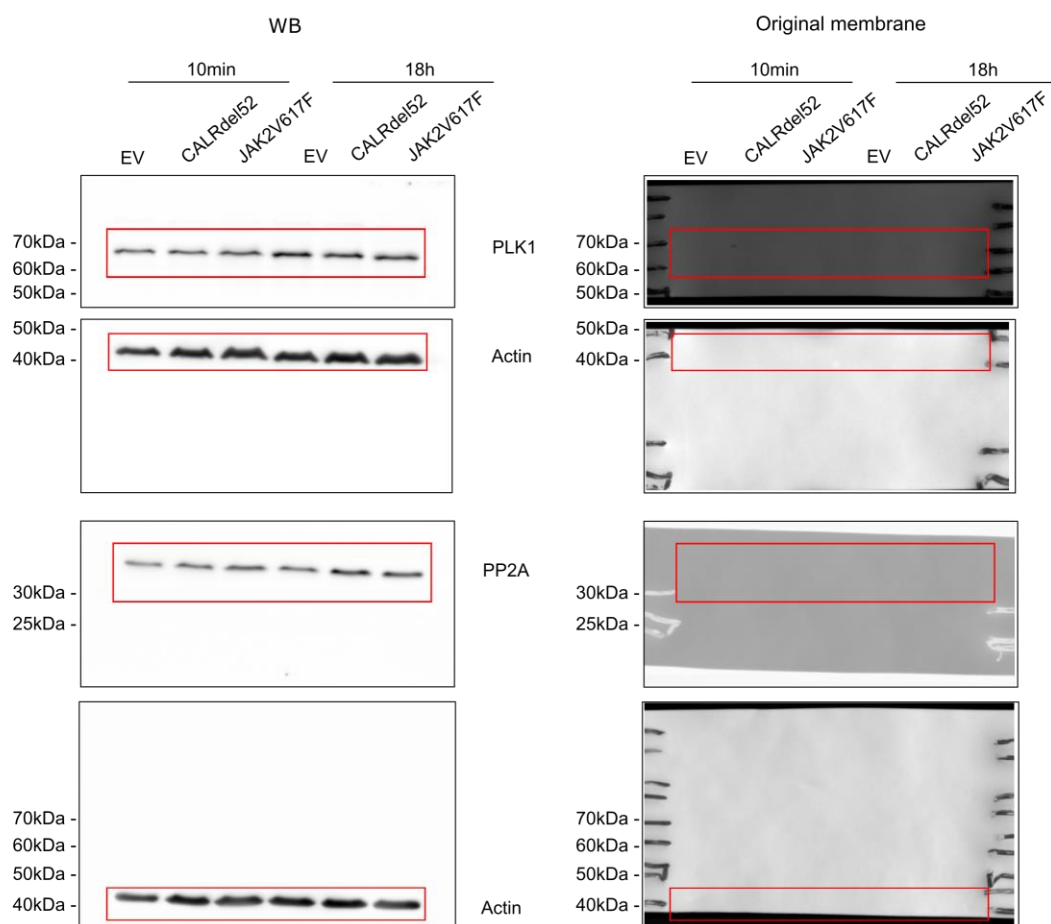
**Supplementary Figure S7.** Uncropped WB and original membranes from Figure 4a. The asterisks (\*) indicate that the band at that height comes from the WB performed on the same membrane crop prior to the detection of the actin loading control. The red rectangle indicates the cropped area displayed in the main and supplementary figures.

## Supplementary Figure S8

UNCROPPED WB Supplementary Figure S2b

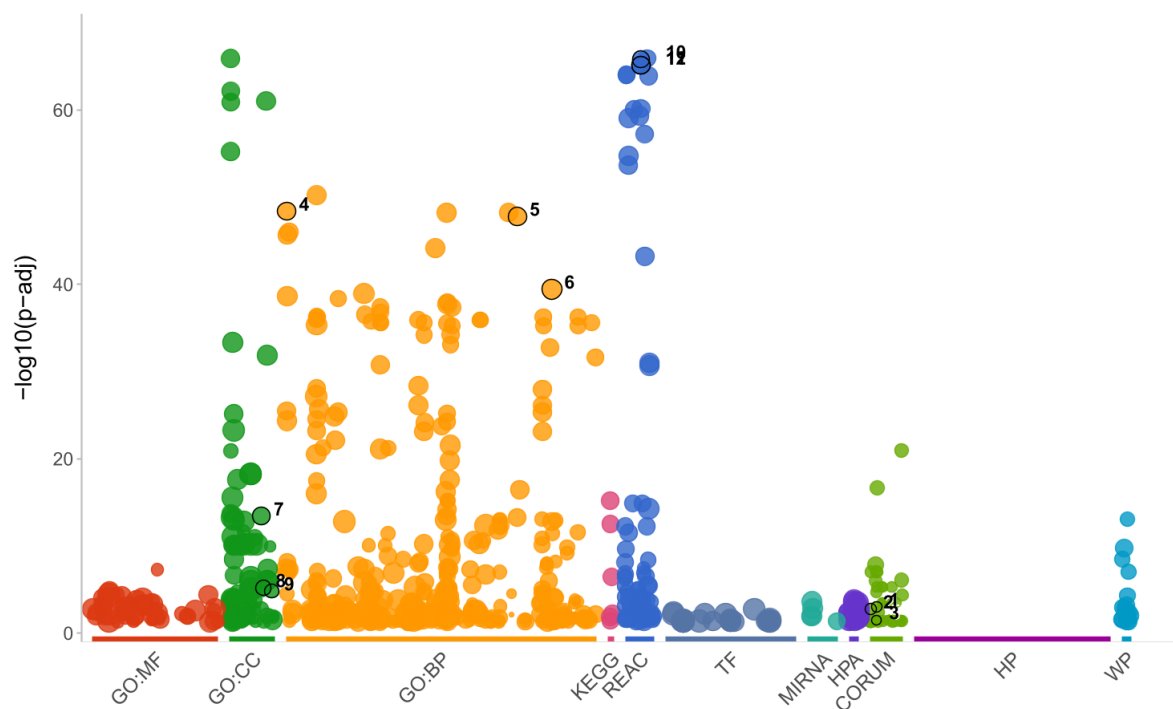


UNCROPPED WB Supplementary Figure S4a



**Supplementary Figure S8.** Uncropped WB and original membranes from Supplementary Figure S2a and Supplementary Figure S4a. The red rectangle indicates the cropped area displayed in the main and supplementary figures.

## Supplementary Figure S9



| id | source | term_id            | term_name                             | term_size | p_value |
|----|--------|--------------------|---------------------------------------|-----------|---------|
| 1  | CORUM  | CORUM:1116         | CRM1-Survivin-AuroraB mitotic complex | 3         | 9.4e-04 |
| 2  | CORUM  | CORUM:190          | Mitotic checkpoint complex (MCC)      | 4         | 1.8e-03 |
| 3  | CORUM  | CORUM:1117         | CRM1-Survivin mitotic complex         | 2         | 3.0e-02 |
| 4  | GO:BP  | GO:0000070         | mitotic sister chromatid segregation  | 187       | 4.1e-49 |
| 5  | GO:BP  | GO:0140014         | mitotic nuclear division              | 278       | 1.6e-48 |
| 6  | GO:BP  | GO:1903047         | mitotic cell cycle process            | 757       | 3.6e-40 |
| 7  | GO:CC  | GO:0072686         | mitotic spindle                       | 184       | 3.8e-14 |
| 8  | GO:CC  | GO:0097431         | mitotic spindle pole                  | 38        | 6.2e-06 |
| 9  | GO:CC  | GO:1990023         | mitotic spindle midzone               | 14        | 1.5e-05 |
| 10 | REAC   | REAC:R-HSA-69618   | Mitotic Spindle Checkpoint            | 109       | 1.5e-66 |
| 11 | REAC   | REAC:R-HSA-2555396 | Mitotic Metaphase and Anaphase        | 229       | 7.3e-66 |
| 12 | REAC   | REAC:R-HSA-68882   | Mitotic Anaphase                      | 228       | 7.3e-66 |

[g:Profiler \(biit.cs.ut.ee/gprofiler\)](http://g:Profiler.biit.cs.ut.ee/gprofiler)

**Supplementary Figure S9.** Enrichment analysis for mitotic processes of the negative GEX z-score genes of PMF/SMF/PV1/11/9 cohort (Supplementary Figure S5 and Supplementary Table 3). Top: Manhattan-like-plot showing terms, color-coded by data source (see annotation below). The y-axis shows the adjusted p-values in the negative log10 scale. Each circle is a term, whereas the size of the circle denotes the term size. Bottom: Table highlighting the top 3 terms by adjusted p-value for their respective data source, related to the search term "Mitotic" or "mitotic". Annotation: GO - Gene Ontology, MF - Molecular Function, CC - Cellular Component, BP - Biological Process; REAC - Reactome, WP - WikiPathways; TF - TRANSFAC; MIRNA - miRTarBase; HPA - Human Protein Atlas; HP - Human phenotype ontology.

**Supplementary table 1. Human Gene Set of important mitotic genes.**

The table combines non redundant HALMARKS of the Molecular Signatures Database (MSigDB) for Mitotic spindle checkpoint ([https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/REACTOME\\_MITOTIC\\_SPINDLE\\_CHECKPOINT.html](https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/REACTOME_MITOTIC_SPINDLE_CHECKPOINT.html)) and the Mitosis (<https://www.gsea-msigdb.org/gsea/msigdb/cards/MITOSIS>).

| Human Gene Set Mitosis & SAC |                          |                                       |
|------------------------------|--------------------------|---------------------------------------|
| NCBI (Entrez)<br>Gene Id     | Gene<br>Symbol           | Gene Description                      |
| <a href="#">8379</a>         | <a href="#">MAD1L1</a>   | mitotic arrest deficient 1 like 1...  |
| <a href="#">996</a>          | <a href="#">CDC27</a>    | cell division cycle 27 [Source:HG...  |
| <a href="#">5048</a>         | <a href="#">PAFAH1B1</a> | platelet activating factor acetyl...  |
| <a href="#">23279</a>        | <a href="#">NUP160</a>   | nucleoporin 160 [Source:HGNC Symb...  |
| <a href="#">55166</a>        | <a href="#">CENPQ</a>    | centromere protein Q [Source:HGNC...  |
| <a href="#">54908</a>        | <a href="#">SPDL1</a>    | spindle apparatus coiled-coil pro...  |
| <a href="#">29945</a>        | <a href="#">ANAPC4</a>   | anaphase promoting complex subuni...  |
| <a href="#">5525</a>         | <a href="#">PPP2R5A</a>  | protein phosphatase 2 regulatory ...  |
| <a href="#">3796</a>         | <a href="#">KIF2A</a>    | kinesin family member 2A [Source:...  |
| <a href="#">5526</a>         | <a href="#">PPP2R5B</a>  | protein phosphatase 2 regulatory ...  |
| <a href="#">55746</a>        | <a href="#">NUP133</a>   | nucleoporin 133 [Source:HGNC Symb...  |
| <a href="#">7321</a>         | <a href="#">UBE2D1</a>   | ubiquitin conjugating enzyme E2 D...  |
| <a href="#">54820</a>        | <a href="#">NDE1</a>     | nudE neurodevelopment protein 1 [...  |
| <a href="#">23332</a>        | <a href="#">CLASP1</a>   | cytoplasmic linker associated pro...  |
| <a href="#">79023</a>        | <a href="#">NUP37</a>    | nucleoporin 37 [Source:HGNC Symbo...  |
| <a href="#">1781</a>         | <a href="#">DYNC1I2</a>  | dynein cytoplasmic 1 intermediate...  |
| <a href="#">5527</a>         | <a href="#">PPP2R5C</a>  | protein phosphatase 2 regulatory ...  |
| <a href="#">10403</a>        | <a href="#">NDC80</a>    | NDC80 kinetochore complex compone...  |
| <a href="#">7514</a>         | <a href="#">XPO1</a>     | exportin 1 [Source:HGNC Symbol;Ac...  |
| <a href="#">81929</a>        | <a href="#">SEH1L</a>    | SEH1 like nucleoporin [Source:HGNC... |
| <a href="#">9183</a>         | <a href="#">ZW10</a>     | zw10 kinetochore protein [Source:...  |
| <a href="#">8655</a>         | <a href="#">DYNLL1</a>   | dynein light chain LC8-type 1 [So...  |
| <a href="#">51433</a>        | <a href="#">ANAPC5</a>   | anaphase promoting complex subuni...  |
| <a href="#">332</a>          | <a href="#">BIRC5</a>    | baculoviral IAP repeat containing...  |
| <a href="#">10726</a>        | <a href="#">NUDC</a>     | nuclear distribution C, dynein co...  |
| <a href="#">8697</a>         | <a href="#">CDC23</a>    | cell division cycle 23 [Source:HG...  |
| <a href="#">79019</a>        | <a href="#">CENPM</a>    | centromere protein M [Source:HGNC...  |
| <a href="#">5905</a>         | <a href="#">RANGAP1</a>  | Ran GTPase activating protein 1 [...  |
| <a href="#">22919</a>        | <a href="#">MAPRE1</a>   | microtubule associated protein RP...  |
| <a href="#">2491</a>         | <a href="#">CENPI</a>    | centromere protein I [Source:HGNC...  |
| <a href="#">80152</a>        | <a href="#">CENPT</a>    | centromere protein T [Source:HGNC...  |
| <a href="#">5516</a>         | <a href="#">PPP2CB</a>   | protein phosphatase 2 catalytic s...  |
| <a href="#">5518</a>         | <a href="#">PPP2R1A</a>  | protein phosphatase 2 scaffold su...  |
| <a href="#">27338</a>        | <a href="#">UBE2S</a>    | ubiquitin conjugating enzyme E2 S...  |

|                        |                          |                                      |
|------------------------|--------------------------|--------------------------------------|
| <a href="#">25906</a>  | <a href="#">ANAPC15</a>  | anaphase promoting complex subuni... |
| <a href="#">4928</a>   | <a href="#">NUP98</a>    | nucleoporin 98 and 96 precursor [... |
| <a href="#">57122</a>  | <a href="#">NUP107</a>   | nucleoporin 107 [Source:HGNC Symb... |
| <a href="#">5528</a>   | <a href="#">PPP2R5D</a>  | protein phosphatase 2 regulatory ... |
| <a href="#">5515</a>   | <a href="#">PPP2CA</a>   | protein phosphatase 2 catalytic s... |
| <a href="#">1058</a>   | <a href="#">CENPA</a>    | centromere protein A [Source:HGNC... |
| <a href="#">991</a>    | <a href="#">CDC20</a>    | cell division cycle 20 [Source:HG... |
| <a href="#">25936</a>  | <a href="#">NSL1</a>     | NSL1 component of MIS12 kinetocho... |
| <a href="#">1063</a>   | <a href="#">CENPF</a>    | centromere protein F [Source:HGNC... |
| <a href="#">348995</a> | <a href="#">NUP43</a>    | nucleoporin 43 [Source:HGNC Symbo... |
| <a href="#">91687</a>  | <a href="#">CENPL</a>    | centromere protein L [Source:HGNC... |
| <a href="#">81930</a>  | <a href="#">KIF18A</a>   | kinesin family member 18A [Source... |
| <a href="#">11130</a>  | <a href="#">ZWINT</a>    | ZW10 interacting kinetochore prot... |
| <a href="#">64105</a>  | <a href="#">CENPK</a>    | centromere protein K [Source:HGNC... |
| <a href="#">80776</a>  | <a href="#">B9D2</a>     | B9 domain containing 2 [Source:HG... |
| <a href="#">79902</a>  | <a href="#">NUP85</a>    | nucleoporin 85 [Source:HGNC Symbo... |
| <a href="#">151648</a> | <a href="#">SGO1</a>     | shugoshin 1 [Source:HGNC Symbol;A... |
| <a href="#">8881</a>   | <a href="#">CDC16</a>    | cell division cycle 16 [Source:HG... |
| <a href="#">6249</a>   | <a href="#">CLIP1</a>    | CAP-Gly domain containing linker ... |
| <a href="#">55143</a>  | <a href="#">CDCA8</a>    | cell division cycle associated 8 ... |
| <a href="#">1783</a>   | <a href="#">DYNC1LI2</a> | dynein cytoplasmic 1 light interm... |
| <a href="#">5519</a>   | <a href="#">PPP2R1B</a>  | protein phosphatase 2 scaffold su... |
| <a href="#">57082</a>  | <a href="#">KNL1</a>     | kinetochore scaffold 1 [Source:HG... |
| <a href="#">79172</a>  | <a href="#">CENPO</a>    | centromere protein O [Source:HGNC... |
| <a href="#">1062</a>   | <a href="#">CENPE</a>    | centromere protein E [Source:HGNC... |
| <a href="#">84643</a>  | <a href="#">KIF2B</a>    | kinesin family member 2B [Source:... |
| <a href="#">51529</a>  | <a href="#">ANAPC11</a>  | anaphase promoting complex subuni... |
| <a href="#">23421</a>  | <a href="#">ITGB3BP</a>  | integrin subunit beta 3 binding p... |
| <a href="#">11004</a>  | <a href="#">KIF2C</a>    | kinesin family member 2C [Source:... |
| <a href="#">83540</a>  | <a href="#">NUF2</a>     | NUF2 component of NDC80 kinetocho... |
| <a href="#">51143</a>  | <a href="#">DYNC1LI1</a> | dynein cytoplasmic 1 light interm... |
| <a href="#">1060</a>   | <a href="#">CENPC</a>    | centromere protein C [Source:HGNC... |
| <a href="#">3619</a>   | <a href="#">INCENP</a>   | inner centromere protein [Source:... |
| <a href="#">79980</a>  | <a href="#">DSN1</a>     | DSN1 component of MIS12 kinetocho... |
| <a href="#">79682</a>  | <a href="#">CENPU</a>    | centromere protein U [Source:HGNC... |
| <a href="#">57405</a>  | <a href="#">SPC25</a>    | SPC25 component of NDC80 kinetoch... |
| <a href="#">64946</a>  | <a href="#">CENPH</a>    | centromere protein H [Source:HGNC... |
| <a href="#">64682</a>  | <a href="#">ANAPC1</a>   | anaphase promoting complex subuni... |
| <a href="#">5903</a>   | <a href="#">RANBP2</a>   | RAN binding protein 2 [Source:HGN... |
| <a href="#">25909</a>  | <a href="#">AHCTF1</a>   | AT-hook containing transcription ... |
| <a href="#">5529</a>   | <a href="#">PPP2R5E</a>  | protein phosphatase 2 regulatory ... |
| <a href="#">9184</a>   | <a href="#">BUB3</a>     | BUB3 mitotic checkpoint protein [... |
| <a href="#">220134</a> | <a href="#">SKA1</a>     | spindle and kinetochore associate... |

|                        |                         |                                                 |
|------------------------|-------------------------|-------------------------------------------------|
| <a href="#">701</a>    | <a href="#">BUB1B</a>   | BUB1 mitotic checkpoint serine/th...            |
| <a href="#">6396</a>   | <a href="#">SEC13</a>   | SEC13 homolog, nuclear pore and C...            |
| <a href="#">1780</a>   | <a href="#">DYNC1I1</a> | dynein cytoplasmic 1 intermediate...            |
| <a href="#">57551</a>  | <a href="#">TAOK1</a>   | TAO kinase 1 [Source:HGNC Symbol;...            |
| <a href="#">11243</a>  | <a href="#">PMF1</a>    | polyamine modulated factor 1 [Sou...            |
| <a href="#">147841</a> | <a href="#">SPC24</a>   | SPC24 component of NDC80 kinetoch...            |
| <a href="#">151246</a> | <a href="#">SGO2</a>    | shugoshin 2 [Source:HGNC Symbol;A...            |
| <a href="#">23122</a>  | <a href="#">CLASP2</a>  | cytoplasmic linker associated pro...            |
| <a href="#">4085</a>   | <a href="#">MAD2L1</a>  | mitotic arrest deficient 2 like 1...            |
| <a href="#">10393</a>  | <a href="#">ANAPC10</a> | anaphase promoting complex subuni...            |
| <a href="#">119504</a> | <a href="#">ANAPC16</a> | anaphase promoting complex subuni...            |
| <a href="#">55839</a>  | <a href="#">CENPN</a>   | centromere protein N [Source:HGNC...            |
| <a href="#">81565</a>  | <a href="#">NDEL1</a>   | nudE neurodevelopment protein 1 l...            |
| <a href="#">5347</a>   | <a href="#">PLK1</a>    | polo like kinase 1 [Source:HGNC S...            |
| <a href="#">79003</a>  | <a href="#">MIS12</a>   | MIS12 kinetochore complex compone...            |
| <a href="#">699</a>    | <a href="#">BUB1</a>    | BUB1 mitotic checkpoint serine/th...            |
| <a href="#">7324</a>   | <a href="#">UBE2E1</a>  | ubiquitin conjugating enzyme E2 E...            |
| <a href="#">55055</a>  | <a href="#">ZWILCH</a>  | zwilch kinetochore protein [Sourc...            |
| <a href="#">11065</a>  | <a href="#">UBE2C</a>   | ubiquitin conjugating enzyme E2 C...            |
| <a href="#">9793</a>   | <a href="#">CKAP5</a>   | cytoskeleton associated protein 5...            |
| <a href="#">378708</a> | <a href="#">CENPS</a>   | centromere protein S [Source:HGNC...            |
| <a href="#">29882</a>  | <a href="#">ANAPC2</a>  | anaphase promoting complex subuni...            |
| <a href="#">246184</a> | <a href="#">CDC26</a>   | cell division cycle 26 [Source:HG...            |
| <a href="#">6232</a>   | <a href="#">RPS27</a>   | ribosomal protein S27 [Source:HGN...            |
| <a href="#">9212</a>   | <a href="#">AURKB</a>   | aurora kinase B [Source:HGNC Symb...            |
| <a href="#">55920</a>  | <a href="#">RCC2</a>    | regulator of chromosome condensat...            |
| <a href="#">348235</a> | <a href="#">SKA2</a>    | spindle and kinetochore associate...            |
| <a href="#">9735</a>   | <a href="#">KNTC1</a>   | kinetochore associated 1 [Source:...            |
| <a href="#">5501</a>   | <a href="#">PPP1CC</a>  | protein phosphatase 1 catalytic s...            |
| <a href="#">54821</a>  | <a href="#">ERCC6L</a>  | ERCC excision repair 6 like, spin...            |
| <a href="#">401541</a> | <a href="#">CENPP</a>   | centromere protein P [Source:HGNC...            |
| <a href="#">51434</a>  | <a href="#">ANAPC7</a>  | anaphase promoting complex subuni...            |
| <a href="#">1778</a>   | <a href="#">DYNC1H1</a> | dynein cytoplasmic 1 heavy chain ...            |
| <a href="#">140735</a> | <a href="#">DYNLL2</a>  | dynein light chain LC8-type 2 [So...            |
| <a href="#">54820</a>  | <a href="#">NDE1</a>    | nudE neurodevelopment protein 1 [...            |
| <a href="#">57405</a>  | <a href="#">SPC25</a>   | SPC25 component of NDC80 kinetoch...            |
| <a href="#">55920</a>  | <a href="#">RCC2</a>    | regulator of chromosome condensat...            |
|                        |                         |                                                 |
| <a href="#">6790</a>   | <a href="#">AURKA</a>   | aurora kinase A [Source:HGNC Symbol;Acc:HG...   |
| <a href="#">6795</a>   | <a href="#">AURKC</a>   | aurora kinase C                                 |
| <a href="#">891</a>    | <a href="#">CCNB1</a>   | cyclin B1 [Source:HGNC<br>Symbol;Acc:HGNC:1579] |
| <a href="#">8555</a>   | <a href="#">CDC14B</a>  | cell division cycle 14B [Source:HGNC Symbol...  |
| <a href="#">8556</a>   | <a href="#">CDC14A</a>  | cell division cycle 14A [Source:HGNC Symbol...  |

|                        |                          |                                                     |
|------------------------|--------------------------|-----------------------------------------------------|
| <a href="#">983</a>    | <a href="#">CDK1</a>     | cyclin dependent kinase 1 [Source:HGNC Symb...      |
| <a href="#">201161</a> | <a href="#">CENPV</a>    | centromere protein V                                |
| <a href="#">9587</a>   | <a href="#">MAD2L1BP</a> | MAD2L1 binding protein                              |
| <a href="#">84930</a>  | <a href="#">MASTL</a>    | microtubule associated serine/threonine kinase like |
| <a href="#">7272</a>   | <a href="#">TTK</a>      | TTK protein kinase [Source:HGNC Symbol;Acc...       |
| <a href="#">9232</a>   | <a href="#">PTTG1</a>    | PTTG1 regulator of sister chromatid separat...      |
| <a href="#">4926</a>   | <a href="#">NUMA1</a>    | nuclear mitotic apparatus protein 1 [Sourc...       |
| <a href="#">51343</a>  | <a href="#">FZR1</a>     | fizzy and cell division cycle 20 related 1 ...      |
| <a href="#">4659</a>   | <a href="#">PPP1R12A</a> | protein phosphatase 1 regulatory subunit 12A        |
| <a href="#">9700</a>   | <a href="#">ESPL1</a>    | extra spindle pole bodies like 1, separase...       |
| <a href="#">10274</a>  | <a href="#">STAG1</a>    | stromal antigen 1 [Source:HGNC Symbol;Acc:H...      |
| <a href="#">10735</a>  | <a href="#">STAG2</a>    | stromal antigen 2 [Source:HGNC Symbol;Acc:H...      |
| <a href="#">8243</a>   | <a href="#">SMC1A</a>    | structural maintenance of chromosomes 1A [...       |
| <a href="#">9126</a>   | <a href="#">SMC3</a>     | structural maintenance of chromosomes 3 [S...       |
| <a href="#">79677</a>  | <a href="#">SMC6</a>     | structural maintenance of chromosomes 6             |
| <a href="#">22974</a>  | <a href="#">TPX2</a>     | TPX2 microtubule nucleation factor [Source...       |
| <a href="#">23063</a>  | <a href="#">WAPL</a>     | WAPL cohesin release factor                         |
| <a href="#">4751</a>   | <a href="#">NEK2</a>     | NIMA related kinase 2 [Source:HGNC Symbol;...       |
| <a href="#">10783</a>  | <a href="#">NEK6</a>     | NIMA related kinase 6 [Source:HGNC Symbol;...       |
| <a href="#">11064</a>  | <a href="#">CNTRL</a>    | centriolin                                          |
| <a href="#">51199</a>  | <a href="#">NIN</a>      | ninein                                              |

**Supplementary Table 2. Antibodies.**

| <b>1. antibody</b>           | <b>Cat. No</b> | <b>company</b>              |
|------------------------------|----------------|-----------------------------|
| Anti-actin                   | 691001         | MP Biomedicals              |
| Anti-Aurora B                | ab 45145       | abcam                       |
| Anti-BubR1                   | ab172581       | abcam                       |
| Anti-Cdc20                   | ab183479       | abcam                       |
| Anti-Cyclin B1               | ab181593       | abcam                       |
| Anti-GAPDH (6C5)             | sc-32233       | Santa Cruz<br>Biotechnology |
| Anti-MAD1                    | ab175245       | abcam                       |
| Anti-MAD2xl                  | SA0503         | Ag Antonin                  |
| Anti-TTK/Mps1                | A01132-1       | Antibody Picoband           |
| Anti-p53 (1C12)              | #2524          | Cellsignalling              |
| Anti-phospho-BRCA1 (Ser1524) | #9009          | Cell signalling             |
| Anti-phospho-H2A.X (Ser193)  | 05-636         | Millipore                   |
| Anti-PLK1                    | 05-844         | Millipore                   |
| Anti-PP2A-Ca/b               | sc-56950       | Santa Cruz<br>Biotechnology |

| <b>2. antibody</b>                                              | <b>Cat. No</b> | <b>company</b> |
|-----------------------------------------------------------------|----------------|----------------|
| Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody     | #A-21244       | Invitrogen     |
| Goat Anti-Mouse IgG, H&L Chain Specific Peroxidase Conjugate    | 401215         | Sigma-Aldrich  |
| Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody     | #A-11001       | Invitrogen     |
| Goat Anti-Rabbit IgG, H & L Chain Specific Peroxidase Conjugate | 401353         | Sigma-Aldrich  |
| Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody    | #A-11008       | Invitrogen     |

**Supplementary table 3. Negative GEX z-score genes of PMF/SMF/PV1/11/9 cohort**

The table shows the genes negatively downregulated in the PMF/SMF/PV1/11/9 cohort from the upper part of the heatmap in Fig. 7 and Fig. S5.

| <b>NCBI (Entrez)<br/>Gene Id</b> | <b>Gene<br/>Symbol</b> | <b>Gene Description</b>                                |
|----------------------------------|------------------------|--------------------------------------------------------|
| <a href="#">27338</a>            | <a href="#">UBE2S</a>  | ubiquitin conjugating enzyme E2 S...                   |
| <a href="#">332</a>              | <a href="#">BIRC5</a>  | baculoviral IAP repeat containing...                   |
| <a href="#">64946</a>            | <a href="#">CENPH</a>  | centromere protein H [Source:HGNC...                   |
| <a href="#">91687</a>            | <a href="#">CENPL</a>  | centromere protein L [Source:HGNC...                   |
| <a href="#">147841</a>           | <a href="#">SPC24</a>  | SPC24 component of NDC80 kinetoch...                   |
| <a href="#">79980</a>            | <a href="#">DSN1</a>   | DSN1 component of MIS12 kinetoch...                    |
| <a href="#">81930</a>            | <a href="#">KIF18A</a> | kinesin family member 18A [Source...                   |
| <a href="#">699</a>              | <a href="#">BUB1</a>   | BUB1 mitotic checkpoint serine/th...                   |
| <a href="#">22974</a>            | <a href="#">TPX2</a>   | TPX2 microtubule nucleation factor [Source...          |
| <a href="#">57405</a>            | <a href="#">SPC25</a>  | SPC25 component of NDC80 kinetoch...                   |
| <a href="#">9212</a>             | <a href="#">AURKB</a>  | aurora kinase B [Source:HGNC Symb...                   |
| <a href="#">10403</a>            | <a href="#">NDC80</a>  | NDC80 kinetochore complex compone...                   |
| <a href="#">5347</a>             | <a href="#">PLK1</a>   | polo like kinase 1 [Source:HGNC S...                   |
| <a href="#">55143</a>            | <a href="#">CDCA8</a>  | cell division cycle associated 8 ...                   |
| <a href="#">83540</a>            | <a href="#">NUF2</a>   | NUF2 component of NDC80 kinetoch...                    |
| <a href="#">983</a>              | <a href="#">CDK1</a>   | cyclin dependent kinase 1 [Source:HGNC<br>Symb...      |
| <a href="#">891</a>              | <a href="#">CCNB1</a>  | cyclin B1 [Source:HGNC<br>Symbol;Acc:HGNC:1579]        |
| <a href="#">1062</a>             | <a href="#">CENPE</a>  | centromere protein E [Source:HGNC...                   |
| <a href="#">7272</a>             | <a href="#">TTK</a>    | TTK protein kinase [Source:HGNC<br>Symbol;Acc...       |
| <a href="#">220134</a>           | <a href="#">SKA1</a>   | spindle and kinetochore associate...                   |
| <a href="#">9700</a>             | <a href="#">ESPL1</a>  | extra spindle pole bodies like 1, separase...          |
| <a href="#">3619</a>             | <a href="#">INCENP</a> | inner centromere protein [Source:...                   |
| <a href="#">4751</a>             | <a href="#">NEK2</a>   | NIMA related kinase 2 [Source:HGNC<br>Symbol;...       |
| <a href="#">1063</a>             | <a href="#">CENPF</a>  | centromere protein F [Source:HGNC...                   |
| <a href="#">9232</a>             | <a href="#">PTTG1</a>  | PTTG1 regulator of sister chromatid separat...         |
| <a href="#">11130</a>            | <a href="#">ZWINT</a>  | ZW10 interacting kinetochore prot...                   |
| <a href="#">4085</a>             | <a href="#">MAD2L1</a> | mitotic arrest deficient 2 like 1...                   |
| <a href="#">55839</a>            | <a href="#">CENPN</a>  | centromere protein N [Source:HGNC...                   |
| <a href="#">79019</a>            | <a href="#">CENPM</a>  | centromere protein M [Source:HGNC...                   |
| <a href="#">84930</a>            | <a href="#">MASTL</a>  | microtubule associated serine/threonine kinase<br>like |
| <a href="#">2491</a>             | <a href="#">CENPI</a>  | centromere protein I [Source:HGNC...                   |
| <a href="#">79172</a>            | <a href="#">CENPO</a>  | centromere protein O [Source:HGNC...                   |

|                        |                         |                                                  |
|------------------------|-------------------------|--------------------------------------------------|
| <a href="#">3796</a>   | <a href="#">KIF2A</a>   | kinesin family member 2A [Source:...             |
| <a href="#">55166</a>  | <a href="#">CENPQ</a>   | centromere protein Q [Source:HGNC...             |
| <a href="#">11065</a>  | <a href="#">UBE2C</a>   | ubiquitin conjugating enzyme E2 C...             |
| <a href="#">991</a>    | <a href="#">CDC20</a>   | cell division cycle 20 [Source:HG...             |
| <a href="#">1058</a>   | <a href="#">CENPA</a>   | centromere protein A [Source:HGNC...             |
| <a href="#">6790</a>   | <a href="#">AURKA</a>   | aurora kinase A [Source:HGNC<br>Symbol;Acc:HG... |
| <a href="#">54908</a>  | <a href="#">SPDL1</a>   | spindle apparatus coiled-coil pro...             |
| <a href="#">23421</a>  | <a href="#">ITGB3BP</a> | integrin subunit beta 3 binding p...             |
| <a href="#">9735</a>   | <a href="#">KNTC1</a>   | kinetochore associated 1 [Source:...             |
| <a href="#">5905</a>   | <a href="#">RANGAP1</a> | Ran GTPase activating protein 1 [...             |
| <a href="#">8243</a>   | <a href="#">SMC1A</a>   | structural maintenance of chromosomes 1A [...    |
| <a href="#">57122</a>  | <a href="#">NUP107</a>  | nucleoporin 107 [Source:HGNC Symb...             |
| <a href="#">79682</a>  | <a href="#">CENPU</a>   | centromere protein U [Source:HGNC...             |
| <a href="#">64105</a>  | <a href="#">CENPK</a>   | centromere protein K [Source:HGNC...             |
| <a href="#">401541</a> | <a href="#">CENPP</a>   | centromere protein P [Source:HGNC...             |