

## **Supplemental Information**

### **TPX2 Amplification-Driven aberrant mitosis in Long-Term Cultured Human Embryonic Stem Cells**

Ho-Chang Jeong, Young-Hyun Go, Joong-Gon Shin, Yun-Jeong Kim, Min-Guk Cho, Dasom Gwon, Hyun Sub Cheong, Haeseung Lee, Jae-Ho Lee, Chang-Young Jang, Hyoung Doo Shin, and Hyuk-Jin Cha

This PDF file includes:

Supplemental Material and Methods

Supplemental Figure Legends

## **Supplemental Material and Methods**

### **Live cell imaging**

Cells were placed on 4-well Chambered Coverglass (Thermo Scientific) coated with matrigel and then incubated with 1 µg/ml of Hoechst 33342 (Thermo, H1399) to visualize chromosomes. Fluorescent images were acquired every 5 min for 24 hrs using a Nikon eclipse Ti with a 40x dry Plan-Apochromat objective, captured with an iXonEM +897 Electron Multiplying charge-coupled device camera, and analyzed using a NIS elements Ar microscope imaging software.

### **Immunoblotting and Immunofluorescence**

Cells were lysed with tissue lysis buffer (TLB, 20mM Tris-HCl, pH 7.4, 137mM NaCl, 2mM EDTA, 1% Triton X-100, and 10% glycerol) supplemented with 0.2mM sodium vanadate and 1mM protease inhibitor cocktail (Roche), and Immunoblotting assay was performed as described previously [65]. Primary antibodies used in this study were as followed: TPX2 (Cell signaling, #12245), pAurora A (Cell signaling, #3079), Aurora A (Cell signaling, #4718), LIN28A (Cell signaling, #3979), GAPDH (AbFrontier), Cyclin B1 (Santa cruz, sc-245), and  $\alpha$ -tubulin (Santa cruz, sc-8035). For Immunofluorescence, cells were fixed with 4% paraformaldehyde, permeabilized with 0.25% Triton X-100, and then blocked with TBS-T containing 3% BSA. The coverslips were incubated with primary antibodies:  $\alpha$ -tubulin (1:400),  $\beta$ -tubulin (1:400), and Aurora A (1:200) at 4°C overnight and stained with either Cy2- (Jackson ImmunoResearch, 1:200) or Alexa 594- (Life Technologies, 1:200) conjugated secondary antibody with DAPI (1:200) at room temperature for 1 hr. Cell images were captured and analyzed by a BX53 research microscope.

### **Flow cytometry analysis**

1 x 10<sup>6</sup> cells were fixed/permeabilized with BD Cytofix/Cytoperm (BD Biosciences, 554722) and stained with following primary antibodies: Oct4 (Abcam, ab19857, 1:100), Nanog (Cell signaling, #4903, 1:100), and TPX2 (Cell signaling, 1:200), followed by incubation with Alexa 488- (Life Technologies, 1:200) or PerCP- (R&D, F0114, 10µl per each sample) conjugated secondary antibodies. The cells were washed, suspended in BD Perm/Wash (BD Biosciences, 554723), and measured by flow cytometry on a FACSCalibur (BD Biosciences). For SSEA4 staining, fluorescence-labeled SSEA4-FITC antibodies (BD Pharmingen, 560126) were used. To detect DNA contents, cells were fixed with chilled 70% ethanol at 4°C overnight, incubated with RNase A (Sigma, 100µg/ml) and propidium iodide (Sigma, 50µg/ml) in dark room, and analyzed by the flow cytometer. The acquired data were analyzed by FlowJo software.

### Fluorescence-based competitive proliferation assay

GFP-expressing hESC (EGFP-P1) and P3 hESC are cultured together. Cells are detached with Accutase (BD Bioscience) and rinsed with DPBS three times before flow cytometry. GFP+ cells in the total population are measured using flow cytometry.

### Primer information

**Table 1.** Primer Sequences for quantitative real-time PCR analysis

| Gene         | Forward Sequence (5' to 3') | Reverse Sequence (5' to 3') |
|--------------|-----------------------------|-----------------------------|
| <i>TPX2</i>  | GCTCAACCTGTGCCACATTA        | CGAGAAAGGGCATATTTCCA        |
| <i>AURKA</i> | TCCTGAGGAGGAACTGGCATCAAA    | TACCCAGAGGGCGACCAATTTCAA    |

## Supplemental Figure Legends

**Figure S1.** (A) Short tandem repeat profile of P1, P3, or P4 hESCs compared to that of WiCell H9 hESC line (B) Competitive cell growth assay for P3 hESCs mixed with P1 hESCs expressing EGFP (C) Flow cytometry analysis for Annexin V and 7-AAD, Cells were treated with etoposide for 24 hrs and the percentages of live cells (dual negative for Annexin V and 7-AAD) were presented. (D) Flow cytometry analysis for pluripotency markers in P1, P3, or P4 hESCs (E) BrdU incorporation assay was performed in P1, P3, or P4 hESCs. The percentages of active proliferating cells (BrdU+) were graphically represented in lower right panel.

**Figure S2.** (A) Log R ratio (LRR) plots for chromosome 17 region assessed by SNP array, Individual intensity of genetic probes is represented as a blue-filled circle. (B) The LRR plots for 17q24 (about 3 Mb sub-chromosomal region) were magnified and the y-axis ranges from -2 to 2.

**Figure S3.** (A) Overall gene expression patterns of the four hESC lines (P1-P4) in duplicate. TPM was taken as gene expression level and a total of 11,203 genes with standard deviation > 0.1 were used. For visualization purposes, gene-wise z-score was shown. The hierarchical clustering results by Pearson correlation of samples and genes were displayed as dendrograms in the left and top panels of the heatmap, respectively. (B) Volcano plots showing DEGs in P2, P3, and P4 compared to P1 hESCs, and DEGs in LP-hESCs compared to EP-hESCs. DEGs were selected using DESeq2 with  $|\log_2\text{fold-change}| > 1$  and a false discovery rate (FDR) < 0.01. (C) Heatmap showing gene set similarity of significantly enriched GO Biological Process (BP) terms in LP-hESCs compared to EP-hESCs. Similarity score was driven by the hypergeometric P-value. Representative GO terms of each cluster were marked as underline. (D) GSEA results

for microtubule-related GO terms. The top 5 leading genes were selected from the leading-edge subsets, defined as the genes that contributed the most to the enrichment signal of a given set.

**Figure S4.** (A) CNV intensity for *AURKA* was assessed by SNP array. (B) Cell cycle profiles in P1, P3, or P4 hESCs, DNA contents of cells were analyzed by PI staining (C) Relative expression level of *TPX2* and *BCL2L1* of hPSCs from indicated datasets (D) The information of GSE dataset used in (C) (E) Relative expression level of indicated gene in hESCs and human dermal fibroblasts (hDFs)

**Figure S5** Relative expression level of TPX2 in iTPX2 hESCs at 24 hours after indicated dose of Dox treatment

**Movie S1** Time lapse images of P1 (A), P2 (B), P3 (C) and P4 (D) hESCs at mitosis

**Table S1** List of differentiated expressed genes between EP and LP-hESCs

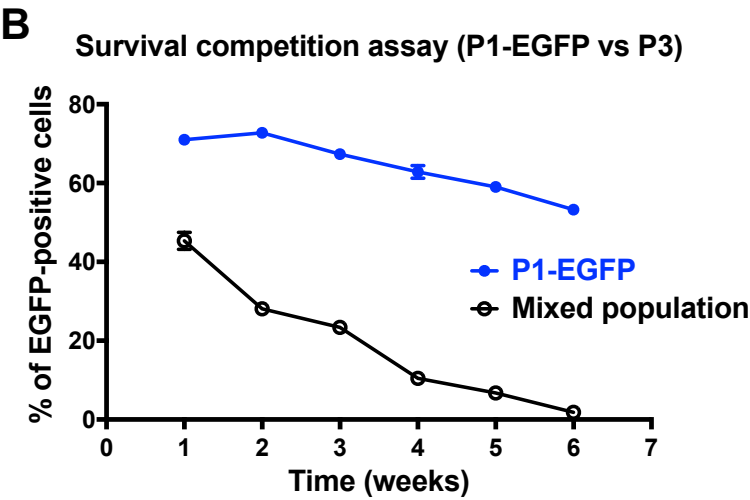
**Table S2** List of upregulated functions in LP-hESCs

Figure. S1

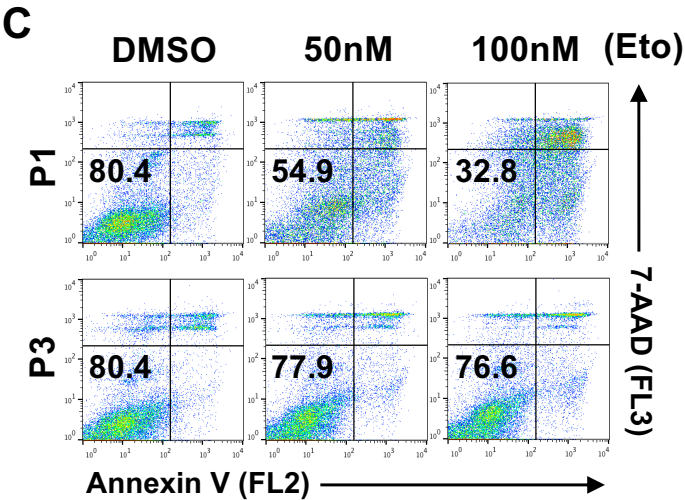
A

| Samples     | D16S539 | D7S820 | D13S317 | D5S818 | CSF1PO | TPOX   | Amelogenin | TH01     | vWA    |
|-------------|---------|--------|---------|--------|--------|--------|------------|----------|--------|
| H9 (WiCell) | 12, 13  | 9, 11  | 9, 9    | 11, 12 | 11, 11 | 10, 11 | X, X       | 9.3, 9.3 | 17, 17 |
| P1 (40s)    | 12, 13  | 9, 11  | 9, 9    | 11, 12 | 11, 11 | 10, 11 | X, X       | 9.3, 9.3 | 17, 17 |
| P3 (200s)   | 12, 13  | 9, 11  | 9, 9    | 11, 12 | 11, 11 | 10, 11 | X, X       | 9.3, 9.3 | 17, 17 |
| P4 (300s)   | 12, 13  | 9, 11  | 9, 9    | 11, 12 | 11, 11 | 10, 11 | X, X       | 9.3, 9.3 | 17, 17 |

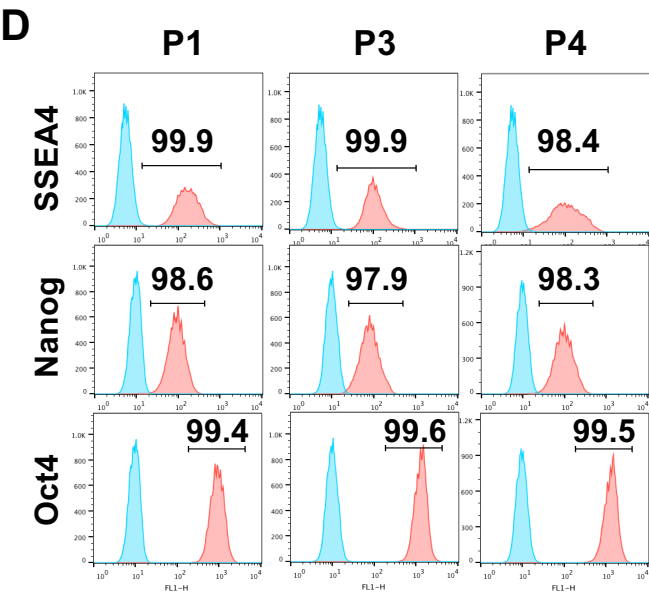
B



C



D



E

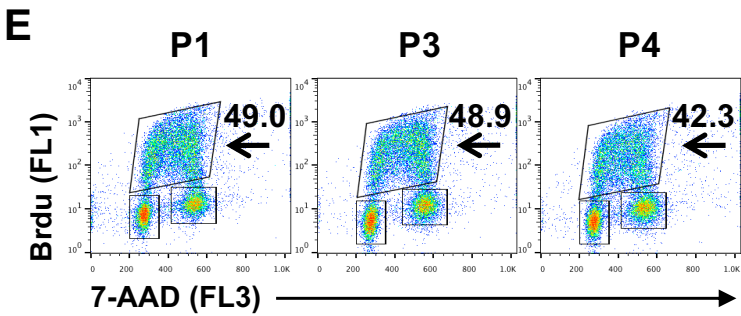


Figure. S2

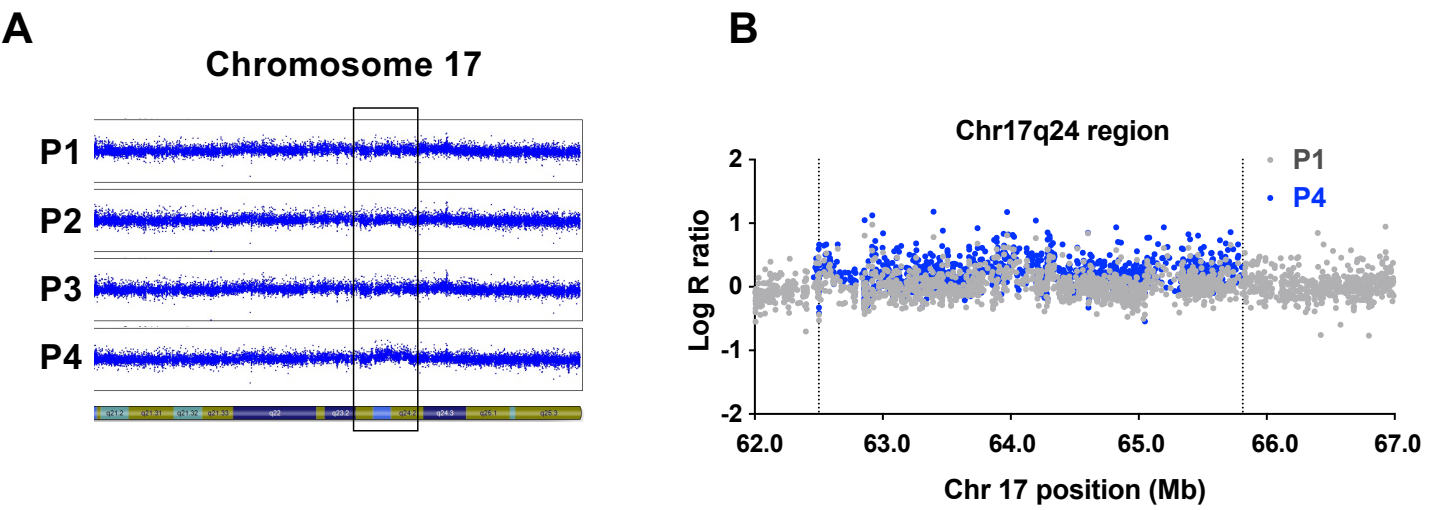
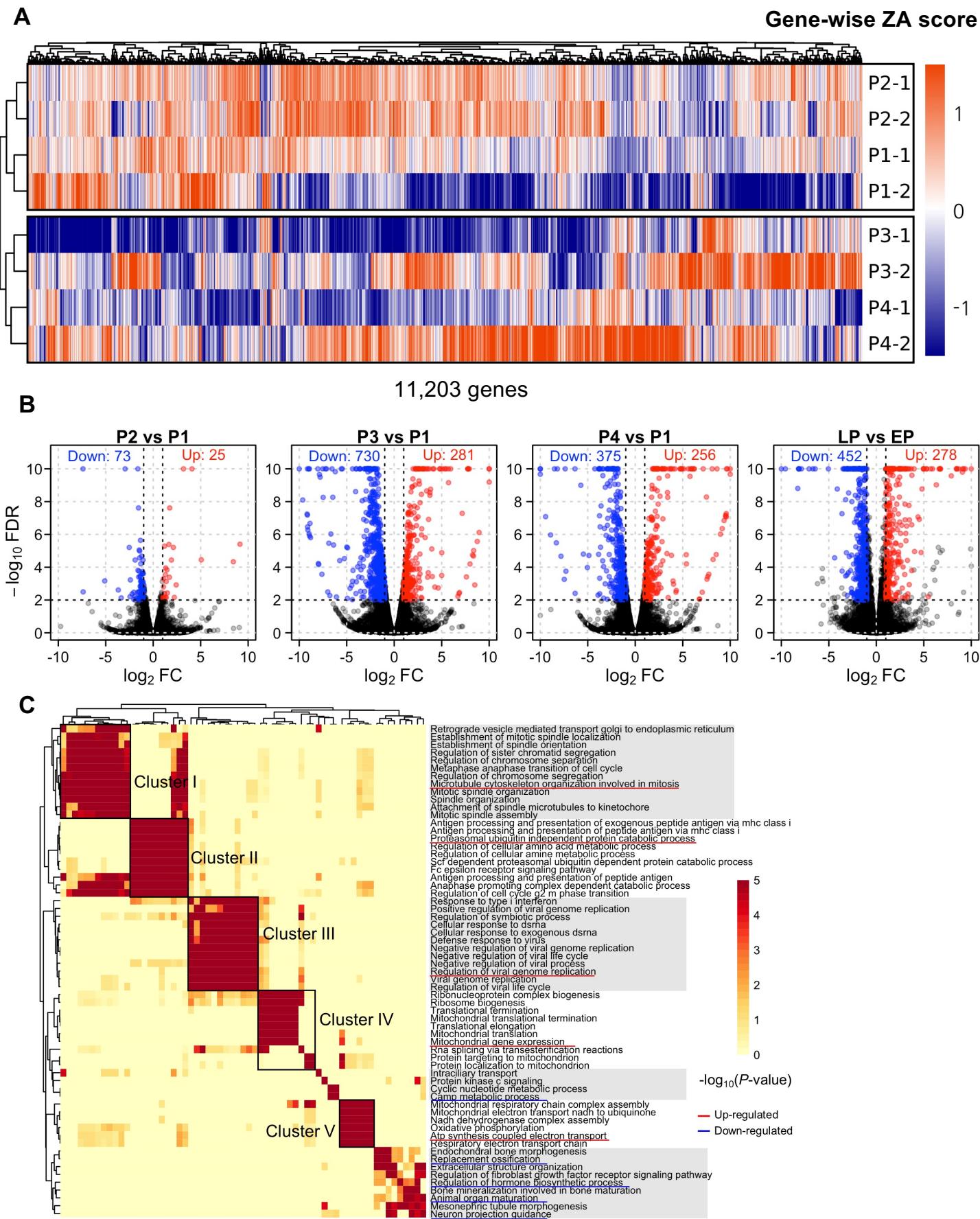


Figure. S3



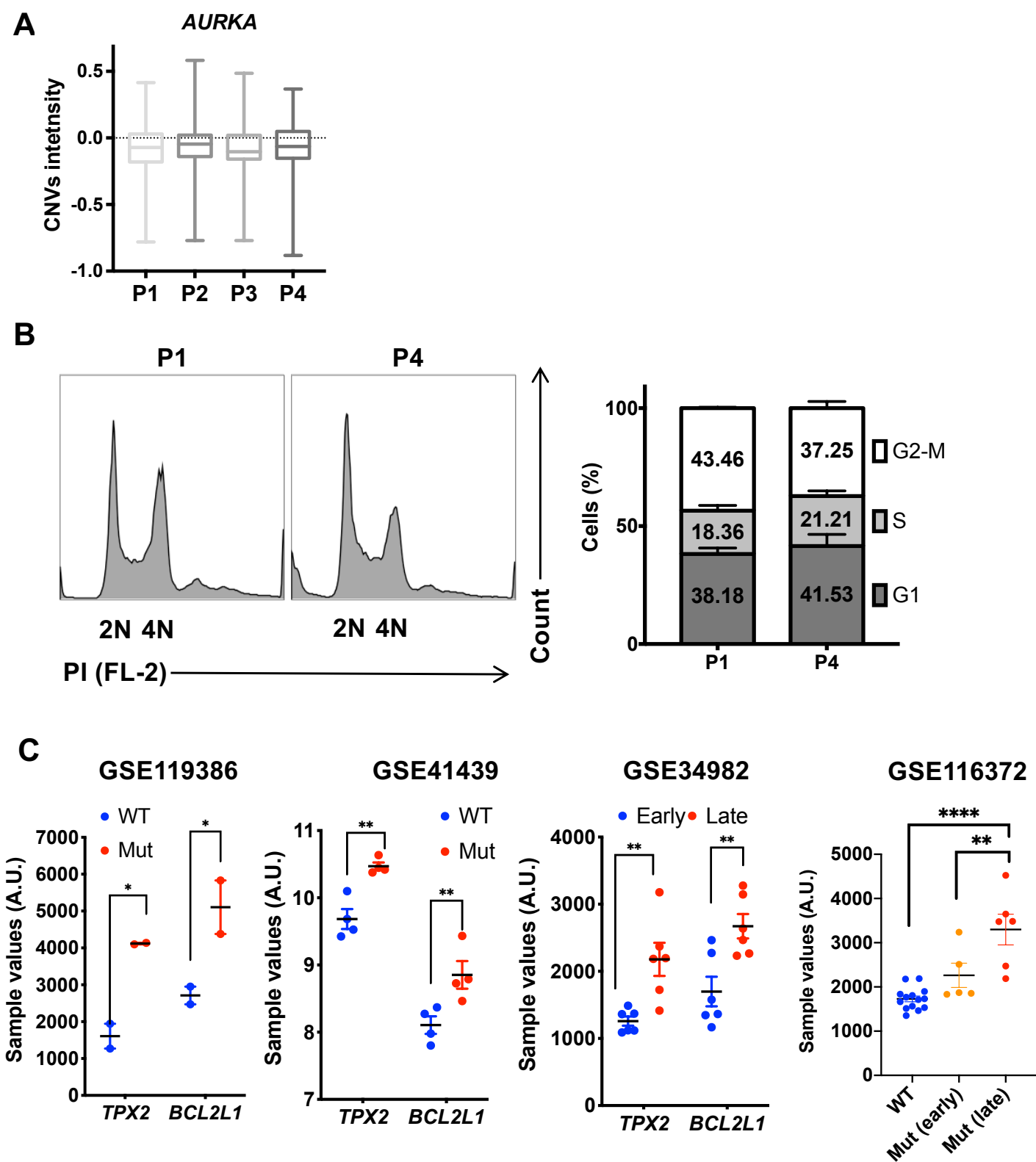


D

## Microtubule, spindle, mitosis related GO terms

| GO term                                                   | NES  | P-value | Top5 leading genes                          |
|-----------------------------------------------------------|------|---------|---------------------------------------------|
| Microtubule cytoskeleton organization involved in mitosis | 2.35 | 0.00022 | <b>KIF3B, TPX2</b> , AURKC, INSC, NDC80     |
| Mitotic spindle organization                              | 2.20 | 0.00022 | <b>KIF3B, TPX2</b> , AURKC, NDC80, CENPE    |
| Establishment of mitotic spindle localization             | 2.17 | 0.00021 | INSC, NDC80, ITGB1, MCPH1, ZW10             |
| Attachment of spindle microtubules to kinetochore         | 2.08 | 0.00021 | AURKC, NDC80, CENPE, KNSTRN, FAM175B        |
| Spindle microtubule                                       | 2.08 | 0.00022 | <b>KIF3B</b> , AURKC, CENPE, KIFAP3, KIF2A  |
| Establishment of spindle orientation                      | 2.07 | 0.00021 | INSC, NDC80, ITGB1, MCPH1, ZW10             |
| Mitotic spindle assembly                                  | 2.04 | 0.00021 | <b>KIF3B, TPX2</b> , AURKC, FAM175B, KIF2A  |
| Spindle organization                                      | 2.02 | 0.00022 | <b>KIF3B, TPX2</b> , AURKC, UVRAG, NDC80    |
| Regulation of cell cycle g2 m phase transition            | 2.01 | 0.00022 | <b>TPX2</b> , OFD1, DYNC1I2, KCNH5, PSMD1   |
| Cell cycle g2 m phase transition                          | 1.99 | 0.00022 | <b>TPX2</b> , OFD1, DYNC1I2, KCNH5, CCNA2   |
| Negative regulation of cell cycle g2 m phase transition   | 1.98 | 0.00022 | PSMD1, PSMB1, PSMD12, PSMC6, PSMA1          |
| Spindle assembly                                          | 1.90 | 0.00022 | <b>KIF3B, TPX2</b> , AURKC, FAM175B, KIF2A  |
| Spindle                                                   | 1.85 | 0.00023 | <b>KIF3B, TPX2</b> , AURKC, UXT, SNCG       |
| Spindle pole                                              | 1.79 | 0.00022 | <b>TPX2</b> , AURKC, UXT, BEX4, CKAP2L      |
| Microtubule binding                                       | 1.59 | 0.00022 | <b>KIF3B, TPX2</b> , JAKMIP2, UXT, MID1     |
| Transport along microtubule                               | 1.58 | 0.00022 | <b>KIF3B</b> , UXT, TRIM58, DYNC1I2, HSPA8  |
| Microtubule                                               | 1.55 | 0.00023 | <b>KIF3B, TPX2</b> , AURKC, MID1, BEX4      |
| Spindle localization                                      | 1.85 | 0.00042 | INSC, NDC80, ITGB1, MCPH1, ZW10             |
| Regulation of microtubule cytoskeleton organization       | 1.56 | 0.00043 | <b>TPX2</b> , MID1, PAK1, FGF13, PHLDB2     |
| Microtubule associated complex                            | 1.58 | 0.00065 | <b>KIF3B</b> , AURKC, MID1, DYNC1I2, PEA15  |
| Microtubule polymerization or depolymerization            | 1.68 | 0.00066 | <b>TPX2</b> , MID1, PAK1, FGF13, KIF2A      |
| Microtubule based transport                               | 1.52 | 0.00087 | <b>KIF3B</b> , UXT, OFD1, TRIM58, DYNC1I2   |
| Microtubule based movement                                | 1.41 | 0.00092 | <b>KIF3B</b> , UXT, OFD1, TRIM58, DYNC1I2   |
| Spindle midzone                                           | 1.88 | 0.00105 | AURKC, CENPE, CDCA8, MAP10, KIF18A          |
| Mitotic spindle                                           | 1.59 | 0.00152 | <b>TPX2</b> , CENPE, CKAP2L, KNSTRN, MAP10  |
| Spindle midzone assembly                                  | 1.89 | 0.00163 | AURKC, MAP10, RACGAP1, KIF4A, AURKB         |
| Regulation of spindle organization                        | 1.79 | 0.00210 | <b>TPX2</b> , DRG1, GNAI1, CLTC, PSRC1      |
| Microtubule depolymerization                              | 1.73 | 0.00295 | <b>TPX2</b> , MID1, FGF13, KIF2A, MID1IP1   |
| Protein transport along microtubule                       | 1.65 | 0.00300 | <b>KIF3B</b> , KIFAP3, RPGR, IFT22, DYNC2H1 |

Figure. S4



| D | GSE Number | Array Platform | Cell Line    | Passage number | Class       | Karyotype                      | Ref (PMID) |  |
|---|------------|----------------|--------------|----------------|-------------|--------------------------------|------------|--|
|   | GSE119386  | RNA seq        | H9_P1        | Under 40       | WT          | 46, XX                         | 30293852   |  |
|   |            |                | H9_P2        | 101-200        |             | 46, XX                         |            |  |
|   |            |                | H9_P3        | 201-300        | Mut         | 46, XX, A                      |            |  |
|   |            |                | H9_P4        | Over 300       |             | 46, XX, Amplification 20q11.21 |            |  |
|   | GSE41439   | Microarray     | VUB01        |                | WT          | 46, XY                         |            |  |
|   |            |                | VUB01_20qdup |                | Mut         | 46, XX, dup (20)(q11.21)       |            |  |
|   |            |                | VUB02        |                | WT          | 46, XY                         |            |  |
|   |            |                | VUB02_20qdup |                | Mut         | 46, XX, dup (20)(q11.21)       |            |  |
|   |            |                | VUB03        |                | WT          | 46, XY                         |            |  |
|   |            |                | VUB03_20qdup |                | Mut         | 46, XX, dup (20)(q11.21)       |            |  |
|   |            |                | VUB07        |                | WT          | 46, XY                         |            |  |
|   |            |                | VUB07_20qdup |                | Mut         | 46, XX, dup (20)(q11.21)       |            |  |
|   | GSE34982   | Microarray     | WA09_Rep A   | 5              | Early       | -                              | 25714340   |  |
|   |            |                | WA09_Rep B   | 5              |             |                                |            |  |
|   |            |                | WA09_Rep C   | 5              |             |                                |            |  |
|   |            |                | WA09_Rep D   | 5              |             |                                |            |  |
|   |            |                | WA09_Rep E   | 5              |             |                                |            |  |
|   |            |                | WA09_Rep F   | 5              |             |                                |            |  |
|   |            |                | WA09_Rep A   | 161            | Late        |                                |            |  |
|   |            |                | WA09_Rep B   | 161            |             |                                |            |  |
|   |            |                | WA09_Rep C   | 161            |             |                                |            |  |
|   |            |                | WA09_Rep D   | 161            |             |                                |            |  |
|   |            |                | WA09_Rep E   | 161            |             |                                |            |  |
|   |            |                | WA09_Rep F   | 161            |             |                                |            |  |
|   | GSE116372  | RNA seq        | VUB01        | 75-89          | WT          | 46, XY                         | 31178415   |  |
|   |            |                | VUB01_mt     | 289-303        | Mut (late)  | 46, XY, dup (20)(q11.21)       |            |  |
|   |            |                | VUB02        | 15             | WT          | 46, XY                         |            |  |
|   |            |                | VUB02_mt1    | 377-383        | Mut (late)  | 46, XY, dup (20)(q11.21)       |            |  |
|   |            |                | VUB02_mt2    | 19-25          | Mut (early) | 46, XY, i(20)(p11.1)           |            |  |
|   |            |                | VUB03        | 23-29          | WT          | 46, XX                         |            |  |
|   |            |                | VUB03_mt     | 207-209        | Mut (late)  | 46, XX, dup (20)(q11.21)       |            |  |
|   |            |                | VUB14        | 22-25          | WT          | 46, XX                         |            |  |

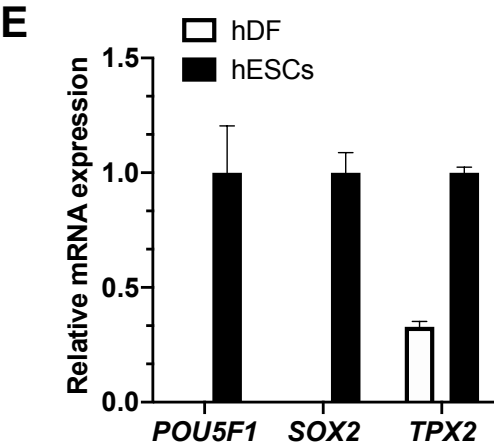


Figure. S5

