Meiosis and Kinetochore genes are used by cancer cells as genome destabilizers and transformation catalysts

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Abstract:

Cancer cells have an altered transcriptome which contributes to their altered behaviors compared to normal cells. Indeed, many tumors express high levels of genes participating in meiosis or kinetochore biology, but the role of this high expression has not been fully elucidated. In this study we explore the relationship between this overexpression and genome instability and transformation capabilities of cancer cells. For this, we obtained expression data from 5 different cancer types which were analyzed using computational information-theoretic analysis. We were able to show that highly expressed meiotic/kinetochore genes were enriched in the altered gene networks characterizing unstable cancer types with high chromosome instability (CIN). However, altered networks characterizing the cancers with low CIN did not include meiotic and kinetochore genes. Representative gene candidates, found by the analysis to be correlated with a CIN phenotype, were further explored by transfecting genomically-stable (HCT116) and unstable (MCF7) cancer cell lines with vectors overexpressing those genes. This overexpression resulted in abnormal cell divisions, defective spindle formation and increased transformation properties in stable cancer HCT116 cells. Interestingly the genome stability properties of the unstable MCF7 cancer cells were less affected by gene overexpression. Our results indicate that overexpression of both meiosis and kinetochore genes drives genomic instability and cancer progression.

64

65 **Introduction:**

66 Cancer is a complex disease, characterized by numerous genomic aberrations and by dysregulation
67 of gene expression. Along with overexpression of oncogenes and repression of tumor suppressors,
68 tumors often express various tissue specific genes, not necessarily related to their primary tissue
69 of origin (1-3). In particular, cancer cells have been found to frequently express genes that are
70 normally restricted to the testis. These genes can be referred to as cancer/testis antigens (CTA) (4).
71 Many CTA have been found to be involved in meiotic divisions, which occur in the testis and
72 include processes with inherent genome instability (see below). This property of the meiotic genes
73 has led to the intriguing idea that the expression of CTA in tumors drives genome and chromosome
74 instability in those tumors (5, 6).

75 Chromosome instability (CIN) is considered to be one of the most important cancer hallmarks.
76 CIN tumors, which have the propensity to constantly change their genome, have worse prognosis
77 than non-CIN tumors (7, 8). Previous work shows that CIN tumors use several molecular
78 mechanisms to achieve their instability, such as replication stress and modulation of the spindle
79 assembly checkpoint (9-11). Due to inherent functions of CTA genes involved in meiosis, that
80 include mono-orientation of sister kinetochores and DNA double-strand break formation and
81 repair, CTA have become prime candidates initiating an additional mechanism to achieve CIN
82 (12-15).

83 Several small scale studies have already shown that a cohort of meiotic genes is expressed in
84 different tumors (6, 16-19). Of special note is the fact that overexpression of meiotic cohesin Rec8
85 in fission yeast cells causes uniparental disomy of chromosomes in mitotic cells (20).

86 Another emerging player in the generation of CIN in cancer is the kinetochore (21). Kinetochores
87 are protein complexes built on centromeres, the specialized loci on eukaryotic chromosomes,
88 playing a key role in mediating chromosome segregation (21). This is mainly achieved through
89 the physical connection between microtubules and the centromeric DNA (22). The balance
90 between all the different kinetochore components is crucial for maintaining genome stability and
91 correct ploidy. Under- or overexpression of different kinetochore components may lead to the
92 formation of chromosomes with very little microtubule attachment, or on the contrary, too many
93 microtubules binding to a chromosome (22-25). Eventually this may lead to non-disjunction and
94 aneuploidy (23-25). Overexpression of specific kinetochore components such as the inner-

centromere protein CENP-A (centromere specific ortholog of histone H3 serving as the structural basis of the kinetochore) leads to deposition of kinetochore components on additional loci in a chromosome already containing a centromere, and the formation of di-centric chromosomes, resulting in a breakage-fusion-bridge cycle of chromosomes and CIN (26). On the other hand, insufficient CENP-A can result in senescence of cells and apoptosis (27, 28). Misregulation of kinetochore components has been observed in many tumors (29). Alterations in the expression levels of kinetochore genes may also cause CIN in tumors, as well as affect the prognosis of specific patients and their response to therapy (29). Despite all these studies the role that the kinetochores play as drivers of CIN during tumorigenesis is not fully understood.

To explore further the relationship between meiosis and kinetochore genes and genome instability we performed a large scale computational analysis of normal and cancer tissues which were obtained from breast, bladder, stomach, colorectal and cervical cancer and normal tissues, all from TCGA data (https:\\xena.ucsc.edu\). We have demonstrated that tumors with high CIN overexpressed meiosis and kinetochore genes in cancer specific gene correlation networks. Although tumors were heterogeneous and could be characterized by different altered gene expression networks, meiosis and kinetochore altered transcripts could be found in different compositions in high CIN tumors but not in low CIN patients within the same type of cancer (see Fig. 1).

To further validate our hypothesis that meiosis and kinetochore genes drive CIN we performed experimental studies in genomically stable and unstable cancer cell lines (CIN+ and CIN-,(8)). We have demonstrated that overexpression of meiosis and kinetochore genes in cancer cell-lines increases genome instability in this setting. Moreover, we show that this over-expression elevates significantly genome instability in genomically stable cancer cell lines, but less in unstable cell lines. Overexpression of these genes also led to the enhanced transformation and invasiveness properties of the cancer cell lines, providing an experimental evidence for the involvement of meiosis and kinetochore genes in genome instability and cellular transformation. An overview of the study is summarized in Figure 1.

126

127 **Materials and Methods:**

128 **1. Data analysis**

129 **1.1 Thermodynamic-based information theoretical approach (Surprisal Analysis)**

130 Matrix of gene expression data was obtained from TCGA database for each cancer type. Every
131 dataset is profiled for thousands of transcripts (total 20,530). The matrix was used as an input file
132 for the information-theoretic surprisal analysis using MATLAB software (30). The analysis was
133 used in several studies, for example (30-32). Briefly, we identify in normal and tumor subsets, the
134 expected gene expression levels at the steady state (a state in which the biological processes are
135 balanced) and deviations thereof for each transcript *i*. The deviations occur due to
136 environmental/genomic constraints. Any biochemical/genetic perturbation can be considered as a
137 constraint and elicit a coordinated change in a group of transcripts. These groups of transcripts are
138 named **unbalanced processes** and identified through calculations of $G_{i\alpha}$ values (=weights of
139 participation) for each transcript *i* in each process α (α=1,2..3). Each transcript can participate in
140 more than one unbalanced process due to non-linearity of biological networks. Only the transcripts
141 located on the tails of the distributions of $G_{i\alpha}$ values (See plots in the Tables S3-S7) are analyzed
142 further for biological meaning. Additionally, the analysis identifies an amplitude, $\lambda_\alpha(k)$, or an
143 importance of each process α in each tissue *k* (**Fig. S1**). Sign of $G_{i\alpha}$ and, $\lambda_\alpha(k)$ means correlation
144 or anti-correlation between the transcripts in the same process α (in case of $G_{i\alpha}$) or α between the
145 same processes in different tumors (in case of $\lambda_\alpha(k)$). For example, if the process α is assigned the
146 values: $\lambda_\alpha(1) = 37$, $\lambda_\alpha(20) = 0$, $\lambda_\alpha(33) = -39$, it means that this process influences the tumors of
147 the patients indexed 1 and 33 in the opposite directions, while it is inactive in patient 20. In order
148 to calculate whether a particular transcript was induced or reduced due to a process α, the product
149 $G_{i\alpha}*\lambda_\alpha(k)$ is calculated for each transcript.

150 In summary we identify for each transcript a set of unbalanced processes in which it is involved
151 and quantify how important each process is in each normal/cancer sample. Thus, a comprehensive
152 map of unbalanced processes is obtained for each cancer or normal sample that allows to
153 characterize each tissue in heterogeneous datasets in detail. Detailed description on how surprisal

154 analysis is implemented in biology and how $G_{i\alpha}$ and, $\lambda_{\alpha}(k)$ are computed is provided in detail in
155 (30-32)
156
157 **1.2 Computation of copy number variation**
158 We obtained data of copy number variation (CNVs) in cancer population from the TCGA genomic
159 database. Copy number variation (CNVs) are a type of structural variant involving alterations in
160 the number of copies of specific regions of DNA, which can be either deleted or duplicated. These
161 chromosomal deletions and duplications involve large stretches of DNA (that is, thousands of
162 nucleotides, which may span many different genes) but can range considerably in size as well as
163 prevalence. Only CNVs larger than 1 Mb (large CNVs usually correlate with the genomic stability)
164 were considered for further analysis and are thus termed lCNVs (large chromosome number
165 variations). The number of lCNVs for each sample were summed and determined using R tools.
166 The distribution for each cancer type is shown in **Fig. 2**.
167
168 **2. Experimental methods**
169
170 **2.1 Cell lines and culture**
171 HCT116 human colon adenocarcinoma cell lines were maintained in Dulbecco's Modified Eagle's
172 medium (DMEM, Sigma), supplemented with 10% fetal bovine serum (FBS), 1% PenStrep (100
173 U/mL Penicillin and 100 µg/mL Streptomycin) and 4 mM L-glutamine in a 37 °C incubator (5%
174 CO2). MCF-7 breast cancer cell lines were maintained in RPM-1640, (Sigma), supplemented with
175 10% fetal bovine serum (FBS), 1% PenStrep (100 U/mL Penicillin and 100 µg/mL Streptomycin)
176 and 4 mM L-glutamine in a 37 °C incubator (5% CO2).
177
178 **2.2 DNA preparation and Transfection.**
179 Meiotic and kinetochore genes tagged with Enhanced Green Fluorescent Protein (EGFP) (from
180 BD Biosciences) were used. The GenElute, HP Plasmid Midiprep Kits (Bio Basic Inc, Canada) .
181 was used to isolate the plasmid. All cells were transfected 24h after initial plating. Transfections
182 were performed using the Mirus transfection reagent (cat-81094967, Zotal, USA) with a 4:1
183 (transfection reagent: DNA) ratio. Transfection efficiency for HCT116 and MCF-7 cells was

184 evaluated by counting the number of GFP positive cells by immunoflorescent microscope and
185 calculating the percentage based on the total number of cells.
186
187 **2.3 Cell synchronization and immunostaining.**
188 HCT116 and MCF-7 cells were synchronized by double thymidine block (33). Cells were treated
189 with 2mM thymidine for 18 h in medium supplemented with 10% FBS. After washing twice
190 with PBS, cells were cultured in fresh medium for 9h and again treated for 15h with media
191 containing 2mM thymidine (10% FBS). After washing cells with PBS, the block was released by
192 the incubation of cells in fresh medium and cells were harvested at 9h (HCT116) and 11h (MCF-
193 7) and fixed with methanol. After that immunostaining was performed. Cells were washed 3
194 times with PBS and blocked with 5% BSA diluted with PBS. After that, cells were incubated
195 with first Ab (α-tubulin antibodies, T5168, Sigma, USA, 1:400 dilution) for 2 hr at room temp.
196 After washing twice with PBS, cells were incubated with a secondary Ab (donkey anti-mouse
197 IgG antibodies, life science,USA, 1:200 dilution) for 1 hr at room temp followed by washing the
198 cells twice with PBS. Again, the cells were incubated with Hoechst 33342 (cat: PIR-62249
199 Thermo scientific, Germany) diluted 1:10,000 for 5-30min for DNA visualization.
200
201 **2.4 Soft agar assay**
202 Colony formation on soft agar was assayed in triplicate by plating 5000 cells in a layer of 0.3%
203 (w/v) agar in assay DMEM (HCT116) and RPMI medium (MCF-7) medium, on top of a 0.6%
204 (w/v) agar layer. Plates were incubated at 37 °C and 5% (v/v) CO2 for 3 weeks, and the medium
205 was replaced every 4 d. Colonies were stained using 0.005% (w/v) Crystal Violet solution, and an
206 image of the whole well was acquired using an Olympus SZ61 stereomicroscope. Colonies were
207 counted using ImageJ software (http://imagej.nih.gov/ ij/). The area and number of colonies was
208 calculated.
209
210 **3. Statistics**
211 Significance was determined using a two-tailed Student's *t* test. The $p < .001$ was considered as
212 extremely significant (***), $p < .01$ as highly significant (**), and $p < .05$ as statistically
213 significant (*).
214

215
216
217
218 **Results:**
219 **Degree of genome instability varies between different cancer types**
220 We hypothesized that the extent of alterations in gene expression levels of meiotic and kinetochore
221 genes may be related to the degree of genome instability. To explore this, we determined the
222 distribution of large DNA tracts which exhibited copy number variations in five different cancer
223 types, namely breast (n=1104), bladder (n=407), stomach (n=415), colorectal (n=383) and cervical
224 (n=305) cancer patients and compared them to normal tissues from breast (n=114), bladder (n=19)
225 stomach (n=20) colorectal (n=51) and cervix (n=3), all from TCGA database. Copy number
226 variation (**CNV**) is a type of chromosomal structural variation that involves alterations in DNA
227 copy number of specific genome regions. Those regions can be either deleted or duplicated. The
228 chromosomal deletions and duplications can involve large stretches of DNA, e.g. thousands of
229 nucleotides, which may span many different genes. In our analysis we included only CNVs that
230 were larger than 1 Mb (lCNV= large CNVs of more than $1*10^6$ Base pairs, 1 Mb). We found that
231 Bladder tumors had large number of DNA regions with lCNVs (high lCNV value) with a
232 maximum value of 850 1CNVs and an average value of 112 1CNVs. Breast and stomach cancers
233 also show high values of lCNVs (with a maximum of 821 1CNVs, and average of 109 1CNVs for
234 breast and a maximum of 650 lCNVs and average of 108 1CNVs for stomach cancer) (**Fig 2,B**).
235 On the other hand, colorectal and cervical cancer types have less DNA regions with lCNV (max
236 402, avg lCNVs=79 and max 278, avg 1CNVs= 80 respectively) **(Fig. 2A, B)**. **($p<0.001$)**
237
238 **Altered gene expression networks, characterizing cancer samples with genome instability,**
239 **are enriched with overexpressed kinetochore and meiosis transcripts**
240 To explore the relationship between the altered expression of meiosis and kinetochore genes and
241 genome instability we performed a large scale unbiased computational analysis of over 2800
242 normal and cancer tissues from 5 cancer types mentioned above. We utilized a computational
243 information-theoretic surprisal analysis (SA) (31, 32) in order to identify altered gene co-expressed
244 networks in cancer tissues, named *unbalanced processes.* Several different unbalanced processes
245 (=networks) may occur in a particular cancer type due to inter-patient heterogeneity. SA deciphers

246 a number of unbalanced processes in each cancer type, by decoding the expected expression levels
247 of the tested molecules at the steady state (i.e. the balanced, unconstrained state), and the deviations
248 thereof due to environmental or genomic constraints. These constraints elicit unbalanced processes
249 in the system (see (31, 32) for more details). Co-varying altered transcripts that deviate from the
250 steady state significantly and in a coordinated manner are grouped and each group represents an
251 unbalanced process α. The analysis determines those transcripts through calculation of a "weight
252 of participation", $G_{i\alpha}$, of each transcript i in a process α (**Table S1**). Transcripts with significant
253 $G_{i\alpha}$ values are analyzed further using the David database (https://david.ncifcrf.gov/) in order to
254 assign a biological meaning for each process α. Every altered transcript can be involved in several
255 unbalanced processes.
256 Next the analysis assigns an amplitude, $\lambda_\alpha(k)$, an importance of each process α in each tissue k.
257 **Table S2** lists amplitudes of all processes in every cancer type and in every tissue. Several distinct
258 unbalanced processes can be active in each cancer type/cancer tissue(32, 34). Detailed description
259 of the analysis can be found in (32).
260 Using SA we identified 16 distinct unbalanced processes in breast cancer **(Fig. 3A**, rigorous error
261 analysis, as described in (32, 34, 35) and Methods, is applied in order to determine a number of
262 unbalanced processes beyond the noise in each dataset). Processes with lower indices, such as
263 processes 1 and 2, were the most dominant and appeared in high a percentage of the patients **(Fig.
264 S1)**. Each process included different biological categories. For example, the most dominant
265 process, process 1, which was found in 21% of breast cancer patients (239 of 1095 breast cancer
266 samples, **Fig. S1**). This process included induced transcripts involved in biological categories such
267 as cell division, sister chromatid cohesion and chromosome segregation **(Table S3)**. Process 2
268 appeared in 20% of breast cancer patients **(Table S3)** and included induced transcripts involved in
269 cell division, mitotic nuclear division and sister chromatid cohesion **(Table S3)**. Interestingly, less
270 common unbalanced processes (with higher indices) included less kinetochore and meiosis genes
271 and consequently less kinetochore/meiosis related enriched biological categories **(Fig. 3A**).
272 Similar results were found for the two other types of cancer with high values of lCNVs: bladder
273 cancer (**Fig. 3B)** and stomach cancer **(Fig. 3C) (Tables S4, S5)**.
274 In contrast, the most dominant unbalanced processes in cancers with lower values of lCNVs
275 (colorectal and cervical cancers) were not particularly enriched for meiosis and/or kinetochore

genes (**Fig. 3D, E, Table S6-S7).** These results show a correlation between high lCNV values and overexpression of meiosis/ kinetochore genes **(Fig. 3A-C)** and evince a possibility that highly expressed meiosis and kinetochore genes in cancer might be involved in genome instability of those cancers.

To further investigate the correlation between the altered expression levels of meiosis/kinetochore genes and genome instability, we examined every cancer type individually. We compared cancer samples with highly unstable genomes (10% of the samples with highest lCNVs values) to the cancer samples with relatively stable genomes (10% of the samples with lowest lCNVs values) within the same cancer type. The results demonstrate that in breast cancer, the most dominant processes harboring meiosis/kinetochore genes appeared mainly in cancer tissues with highly unstable genomes (high lCNVs, **Fig. 4A**). Patients having more stable genomes (lower lCNVs) did not harbor those networks (**Fig. 4B**). Similar results were found in bladder and stomach cancers (**Fig. 4C, D and Fig. 4E, F** respectively**).** In contrast, the correlation between gene expression levels of meiosis/kinetochore genes and genome instability was not found in the cancer types with relatively stable genomes, (colorectal and cervical cancers, **Fig. S2)**.

These results point to a high correlation between participation of meiosis/kinetochore in dominant unbalanced cancer-specific processes and genome instability, showing that meiosis and kinetochore genes may have an active role in driving CIN. This strong correlation prompted us to examine our hypothesis experimentally.

Over expression of representative meiosis and kinetochore genes in genomically stable and unstable cancer cell lines

In order to test our hypothesis, we have overexpressed representative kinetochore and meiosis related genes, which were found in the analysis (see for example Table S2), in cancer cell lines. The two kinetochore genes we overexpressed, CENP-A and HJURP, represent the structural basis of the kinetochore structure (19, 36). CENP-A is the histone H3 homolog that forms a platform upon which all other kinetochore components assemble (37). HJURP is the CENP-A chaperone, responsible for recruiting it to the centromere (38). We also overexpressed four meiosis-related genes: DMC1, SPO11, SMC1B and REC8. DMC1 and SPO11 are involved in homologous recombination and DNA double strand break repair during meiosis (39, 40). SMC1B and REC8 are meiosis specific components of cohesin, and participate in homologous chromosome pairing

307 and in sister chromatid mono-orientation (41-43). We have overexpressed these genes in two
308 cancer cell-lines- HCT116, a colon cancer cell line which is CIN negative and has a relatively
309 stable genome, and MCF7, a breast cancer cell line which shows high chromosome instability (8,
310 44). The genes were fused to GFP to monitor their expression (**Fig. S3**).
311
312 **Over expression of meiosis and kinetochore genes promotes genome instability in cancer cell**
313 **lines**
314 In order to check whether the overexpressed meiosis and kinetochore genes promote genome
315 instability we evaluated the number of cells with lagging chromosomes, anaphase bridges, uneven
316 segregation of chromosomes and deviation from a bipolar spindle configuration as a means to
317 estimate genome stability (25, 45-47). **Figure 5 (B, C and G)** shows that overexpression of both
318 kinetochore genes, CENP-A and HJURP, and one of the meiosis genes, DMC1 in HCT116, caused
319 a significant elevation in anaphase bridges and lagging chromosomes (more examples in **Fig. S4)**.
320 Other meiosis-related genes did not affect the chromosome segregation phenotype when over-
321 expressed. **Figure 5 (E, F and I)** also shows that overexpression of all meiosis and kinetochore
322 genes in HCT116 cells caused a significant elevation in mono-polar and multi-polar spindle
323 formation compared to an empty plasmid (G, H, I and J) (more examples in **Fig. S5**).
324 In contrast, overexpression of meiosis and kinetochore genes in the genomically unstable MCF7
325 cells caused a significantly less severe phenotype **Figure 5** (**H and J**). Only CENP-A
326 overexpression caused a small elevation in the occurrence of mono-polar and multi-polar spindles
327 (**Fig. 5J**). However, all other phenotypes, related to the spindle (**Fig. 5J**) and chromosome
328 segregation (**Fig. 5H**), were not affected in MCF7 cells in response to induced expression of
329 meiosis/kinetochore genes.
330 These results demonstrate that overexpression of kinetochore and meiosis genes in genomically
331 stable cells has the ability to promote genome instability. The same overexpression has a
332 significantly smaller effect in a cell line that has already acquired a high degree of genome
333 instability before the gene transfection.
334
335 **Over expression of meiosis and kinetochore genes promotes invasiveness of cancer cells**
336 Cancers with unstable genomes are often more invasive than cancers with stable genomes (8, 48,
337 49). Therefore, we hypothesized that overexpression of meiosis and kinetochore genes and

338 promotion of genome instability could induce invasiveness and transformation properties of cancer
339 cells.
340 To examine a change in the transformation properties of the cells we tested an ability of HCT116
341 and MCF7 cells to generate colonies in soft agar following overexpression of the
342 meiosis/kinetochore genes (50, 51). **Figure 6 and S6** show that overexpression of all meiosis and
343 kinetochore genes in HCT116 cells enhanced both the number of colonies generated and the size
344 of the colonies, demonstrating enhanced cancer transformation properties. Surprisingly, although
345 genome instability parameters were not increased in the MCF7 cells upon the gene overexpression,
346 an elevation in the number and size of the colonies in soft agar was detected in this cell line (**Fig.**
347 **S7**). These results show that induced invasiveness and cellular transformation of the tested cell
348 lines may correspond to the induced expression of meiosis and kinetochore genes, although not
349 necessarily linked to the ability of those genes to induce genome instability parameters.
350
351
352 **Discussion**
353 Genome instability and the mechanisms behind it are among central questions in cancer biology
354 in recent decades (52). Here we investigate a new possible route to achieve genome instability in
355 cancer by overexpression of genes which participate in meiosis or in kinetochore formation. The
356 process of meiosis includes inherent genome instability which occurs through meiotic homologous
357 recombination and sister chromatid mono-orientation. The perturbed expression of kinetochore
358 proteins has also potential to affect the processes involved in proper chromosome segregation and
359 genome stability.
360 We have taken a multi-pronged approach to support our hypothesis and have used computational
361 analysis of large cancer datasets and an experimental approach utilizing cancer cell line models.
362 Using information-theoretic computational analysis of five different cancer types obtained from
363 TCGA database, we have shown that the most abundant altered gene expression networks,
364 characterizing unstable cancers, were enriched with meiosis and kinetochore genes. Altered gene
365 expression networks characterizing cancers with low lCNVs were not enriched with those genes.
366 Moreover, in unstable cancers patients with the highest lCNVs were characterized by the
367 unbalanced processes enriched with meiosis and kinetochore genes in contrast to the patients with
368 low lCNVs within the same cancer type which did harbor those processes.

369 Although these analyses were merely restricted to a correlation, the finding of this correlation in
370 five major cancer types, and the extension of the correlation to the specific patient groups within
371 each cancer hints to a strong link between the overexpression of meiosis and kinetochore genes
372 and genomic instability in tumors.
373 In order to go beyond this correlation and demonstrate a causative effect, we performed
374 experiments in which several representative meiosis and kinetochore genes, as identified by the
375 computational analysis, were expressed in two cancer cell lines. The overexpression caused an
376 elevation of genome instability parameters in the stable HCT116 cell lines, but not in the less stable
377 MCF7 cell line. However, overexpression caused both cell lines to increase their invasiveness and
378 transformation properties as measured by colony formation ability in soft agar.
379 These results could suggest that induced invasiveness (observed in both cell lines) is not directly
380 related to the induced genome instability (observed in HCT116) although they both result from the
381 overexpression of the same genes. Another possibility is that even a slight and undetectable
382 increase in genome instability (as in MCF-7), can cause a large effect on invasiveness of the cells.
383 Further experiments are needed to distinguish between these possibilities.
384 Our results also demonstrate that meiosis and kinetochore genes can serve as markers for genome
385 instability. Future work should assess the accuracy and sensitivity of those markers and whether
386 downregulating meiosis and kinetochore genes could be used as a therapeutic approach.
387 In conclusion, we have shown that genome instability in tumors could be driven by overexpression
388 of specific classes of genes, namely meiosis and kinetochore genes, which are involved in genome
389 organization and maintenance of undifferentiated cells. This finding may have medical
390 implications regarding the identification of genome instability in tumors and the treatment of
391 unstable tumors through manipulation of these gene networks.
392
393 **Figure Legends:**
394
395 **Figure 1. Schematic overview of how mis-expressed meiosis and kinetochore genes induce**
396 **chromosome instability.** 2821 tumors tissues of five cancer types obtained from TCGA datasets
397 were analyzed. Cancer types were categorized into two subtypes: cancer with unstable genomes
398 (unstable cancers) and cancer with a more stable genome (stable cancers). Information-theoretic
399 analysis is utilized to study the altered gene expression networks in the entire population. We find

400 that the most dominant cancer-specific altered networks in unstable cancers were enriched with
401 meiotic and kinetochore but not in stable cancers. The experimental overexpression of meiosis and
402 kinetochore genes in cancer cell lines induced genomic instability phenotypes: anaphase bridges
403 (right) and spindle defects (multipolar spindle, left).
404
405 **Figure 2. Distribution of Large Copy number variation (lCNVs) values in different cancer**
406 **types.** Bladder cancer has a high number of DNA regions with lCNVs (max 850, avg lCNVs=
407 112) followed by breast cancer and stomach cancers (max 821, avg lCNVs =109 and max 650 avg
408 CNVs = 108 respectively). These three cancer types are categorized as unstable cancer types
409 ($p<0.001$). On the other hand, colorectal, and cervical cancer types have a low number of DNA
410 regions with lCNVs (max 402, avg lCNVs=79 and max 278, avg 1CNVs= 80 respectively), and
411 are categorized as more stable cancer types. Fig. 2A shows the full distribution of lCNVs values
412 in all samples, while Fig. 2B shows a zoom–in on cases having up to 200 DNA regions with lCNVs
413 (low CNV values).
414
415 **Figure 3. Involvement of kinetochore and meiosis genes in unbalanced processes as**
416 **identified using surprisal analysis in both unstable and stable cancers**. Every dataset is
417 profiled for thousands of transcripts (total 20,530), which are resolved into altered networks
418 (unbalanced processes) characterizing each tumor and normal tissue. All transcripts, which
419 deviate from the balance state in the same (coordinated) way, are organized in groups,
420 unbalanced processes. Meiosis and kinetochore genes were found to participate in the most
421 dominant processes in unstable cancers: processes 1 and 2 in breast cancer (Bc, **A)**, processes 2,
422 3 and 4 in bladder cancer (Bl, **B)** and process 1and 3 in stomach cancer (Sc, **C)**. There is a
423 significantly lower percentage of meiosis and kinetochore genes in the dominant processes
424 characterizing colorectal (Co, **D**) and cervical cancers (Ce, **E).**
425
426 **Figure 4. The correlation between over-expression of meiosis/kinetochore genes and genomic**
427 **instability within each cancer type.** Unstable breast, bladder and stomach cancers were analyzed
428 in this section. Each cancer type was analyzed separately as following: Distribution of lCNVs
429 values was generated for each cancer type. 10% of the samples with highest lCNVs values
430 (unstable group) and 10% of the samples with lowest lCNVs values (stable group) were selected

431 in each cancer type. Cancer specific unbalanced processes of 10% of the samples with highest
432 lCNVs values **(A, C, E)** were compared to the processes appeared in 10% of the samples with
433 lowest lCNVs values (**B, D, F**). The most dominant processes harboring meiosis/kinetochore genes
434 appeared mainly in unstable group **(A, C, E))** in all three cancer types. Patients having more stable
435 genomes (stable group) harbored the processes which did not include meiosis and kinetochore
436 genes (**B, D, F**). Sign "-" means anticorrelation: -1 in **B, D, F** means that all induced transcripts,
437 including meiosis and kinetochore genes, in process 1 are reduced in process -1 and vice versa.
438 Tables S3-S7include the information regarding biological categories induced in processes -1, -2
439 and -3 (**B, D, F**), which characterize stable groups within breast, bladder and stomach cancers.
440
441 **Figure 5**. **Overexpression of meiosis (DMC1, REC8, SPO11 and SMC1B) and kinetochore**
442 **genes (CENP-A and HJURP) causes a genomic instability phenotype**. The effect of
443 overexpressed meiosis and kinetochore genes in stable (HCT116) and unstable (MCF-7) cancer
444 cell lines was measured by staining cells with DAPI and anti-tubulin IF (see Methods). Scoring
445 the cells for genome instability was performed by counting defects during anaphase (anaphase
446 bridges, lagging chromosomes) and apparent spindle formation defects (multipolar and unipolar).
447 Presented are immunofluorescence images showing the effects of overexpression of meiosis
448 (DMC1) and kinetochore (CENP-A) genes. Showing abnormal cell divisions i.e. Anaphase Bridge
449 (**B**) and lagging chromosomes (**C**) and apparent spindle formation defects (unipolar **E** and tripolar
450 **F**). Quantification of chromosome segregation and spindle defects in meiosis and kinetochore
451 transfected stable cell line HCT116 shows more significant defects compared to the MCF 7 cell
452 line (**G-J**). Statistical significance is shown by asterisks (*P < 0.05; **P < 0.01; ***P < 0.001).
453 Scale bar equals 10μm.
454
455 **Figure 6. Overexpression of meiosis (DMC1, REC8, SPO11 and SMC1B) and kinetochore**
456 **genes (CENP-A and HJURP) causes elevated invasiveness** Overexpressed meiotic and
457 kinetochore genes in a stable cell line (HCT 116) have the ability to promote invasiveness in a soft
458 agar assay. A soft agar colony formation assay was applied for the detection of transformed cells
459 when overexpressed with meiotic and kinetochore genes in a stable cell line. The number of
460 colonies **(A)** and size of the colonies **(B** for full scale or **C** for a zoom in on colonies up to 3000
461 mm^2) were compared (see Methods). Statistically significant differences compared with empty

vector (No genes) were determined using a Student's 2-tailed t test (*P < 0.05; **P < 0.01; ***P < 0.001)

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Availability of data and reagents:

The authors affirm that all data necessary for confirming the conclusions of this article are represented fully within the article and its tables and figures. All the necessary reagents from this study can be shared.

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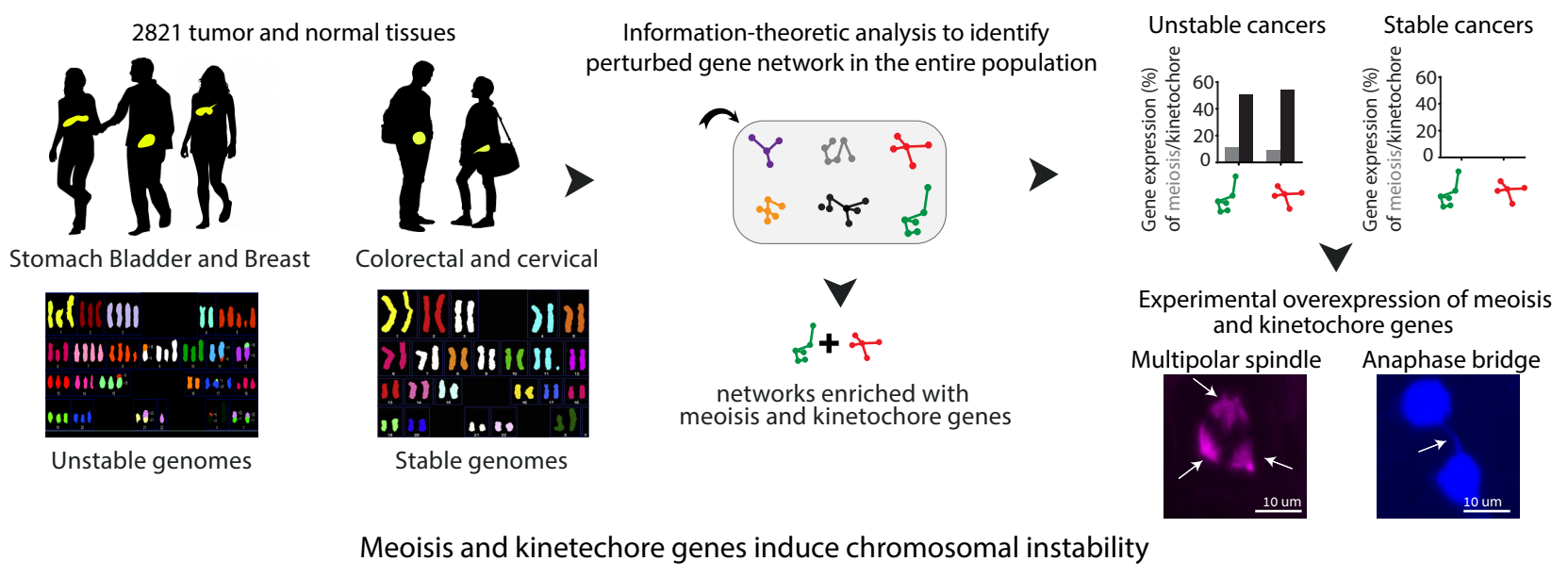
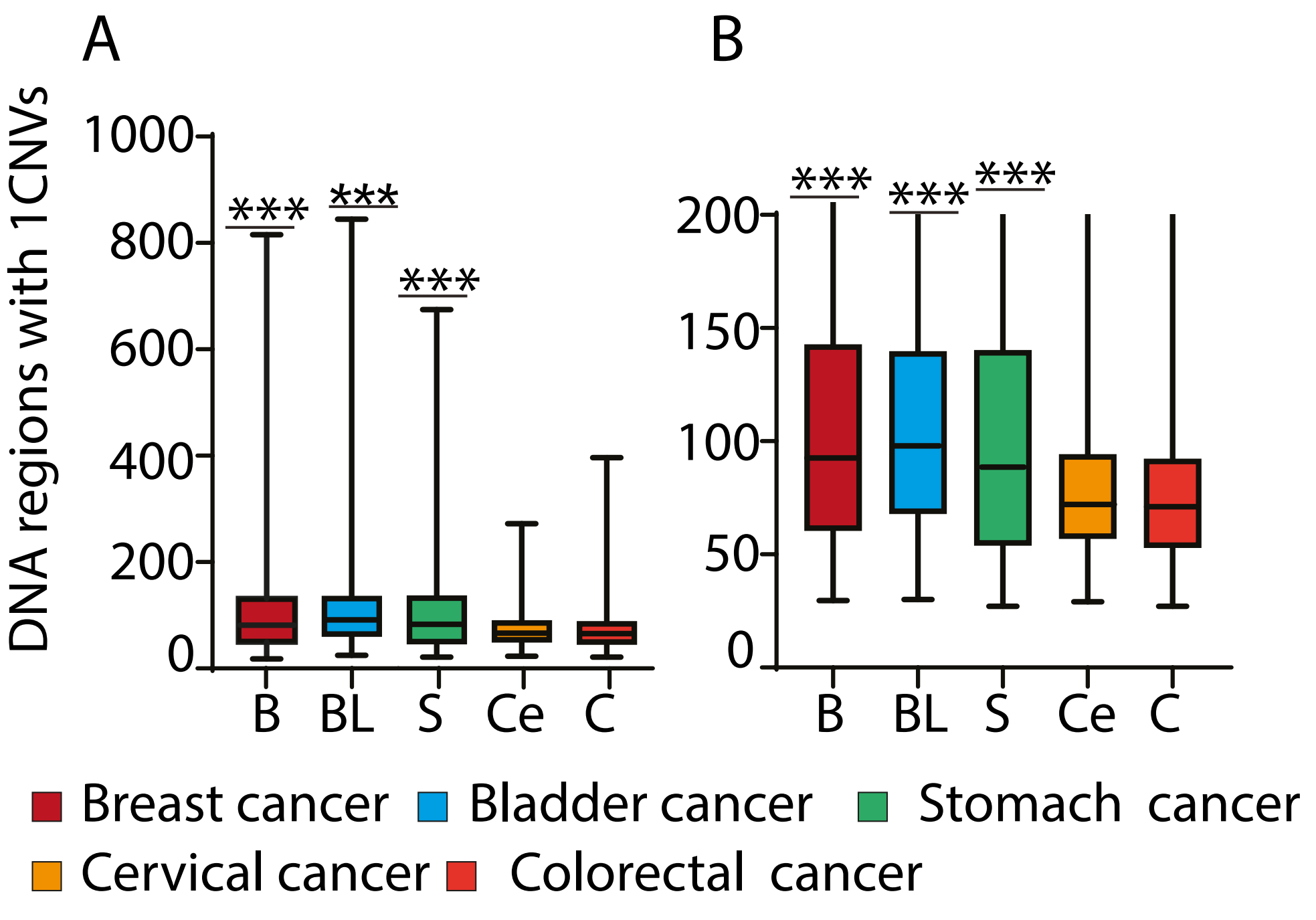
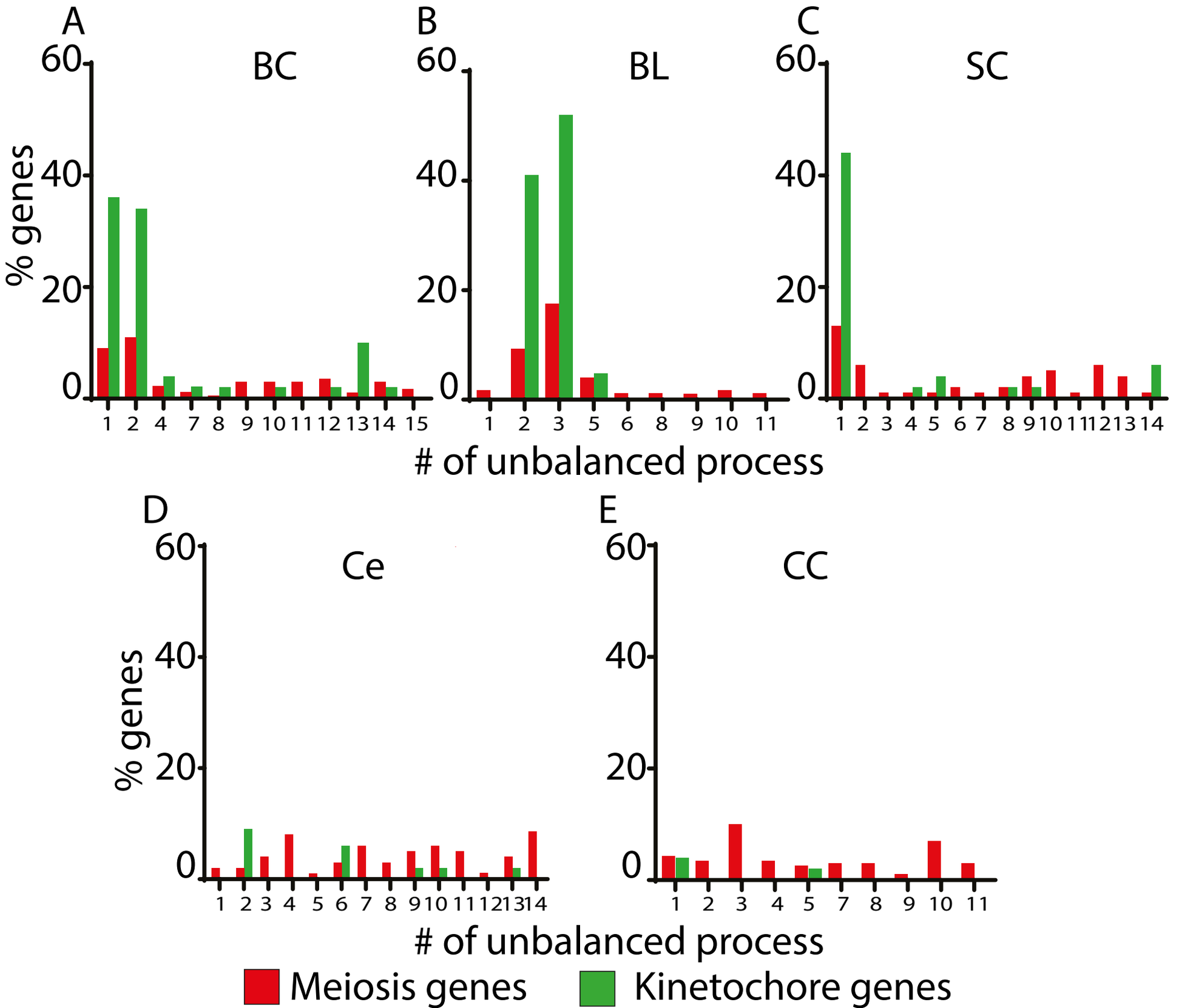
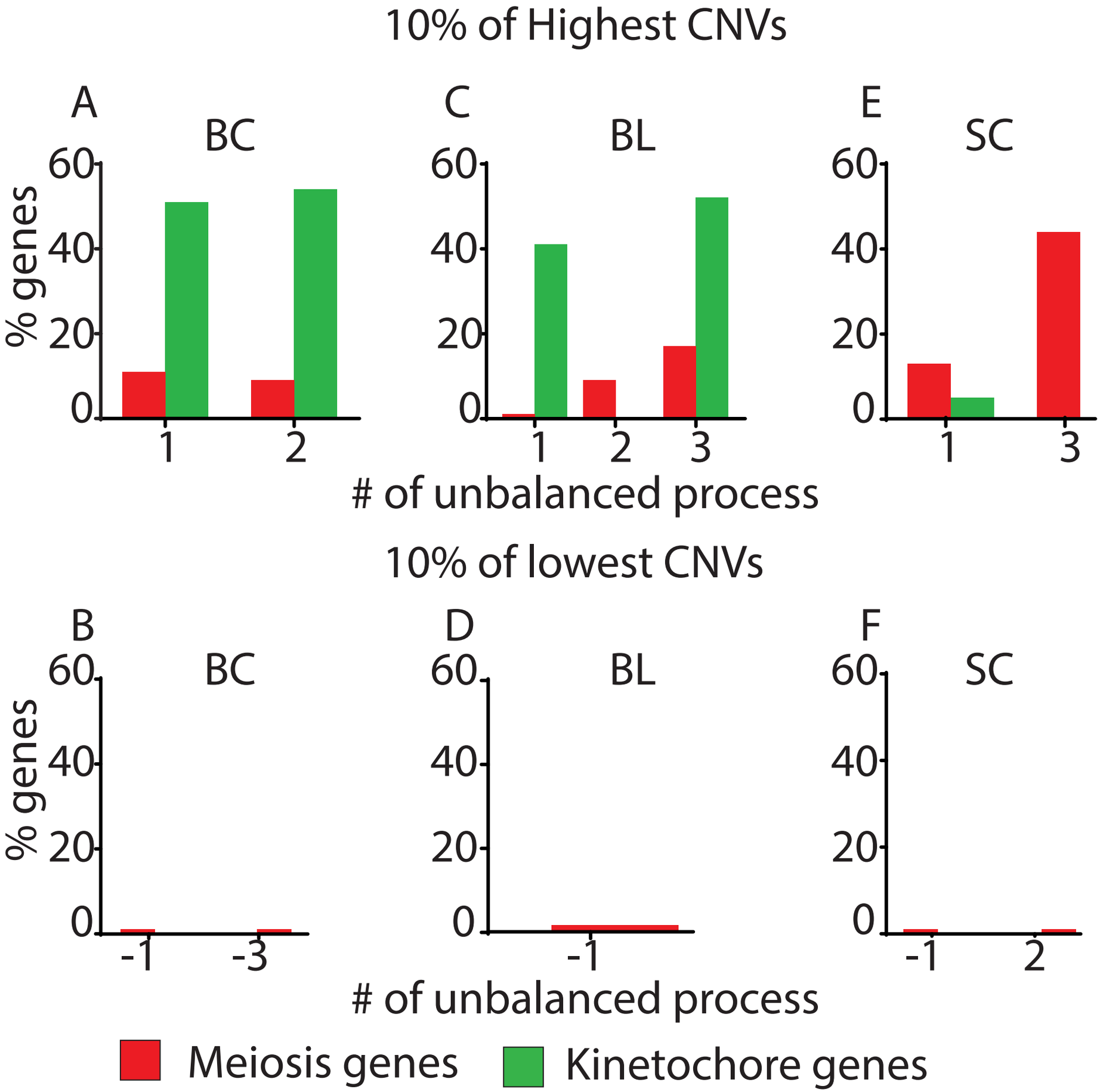
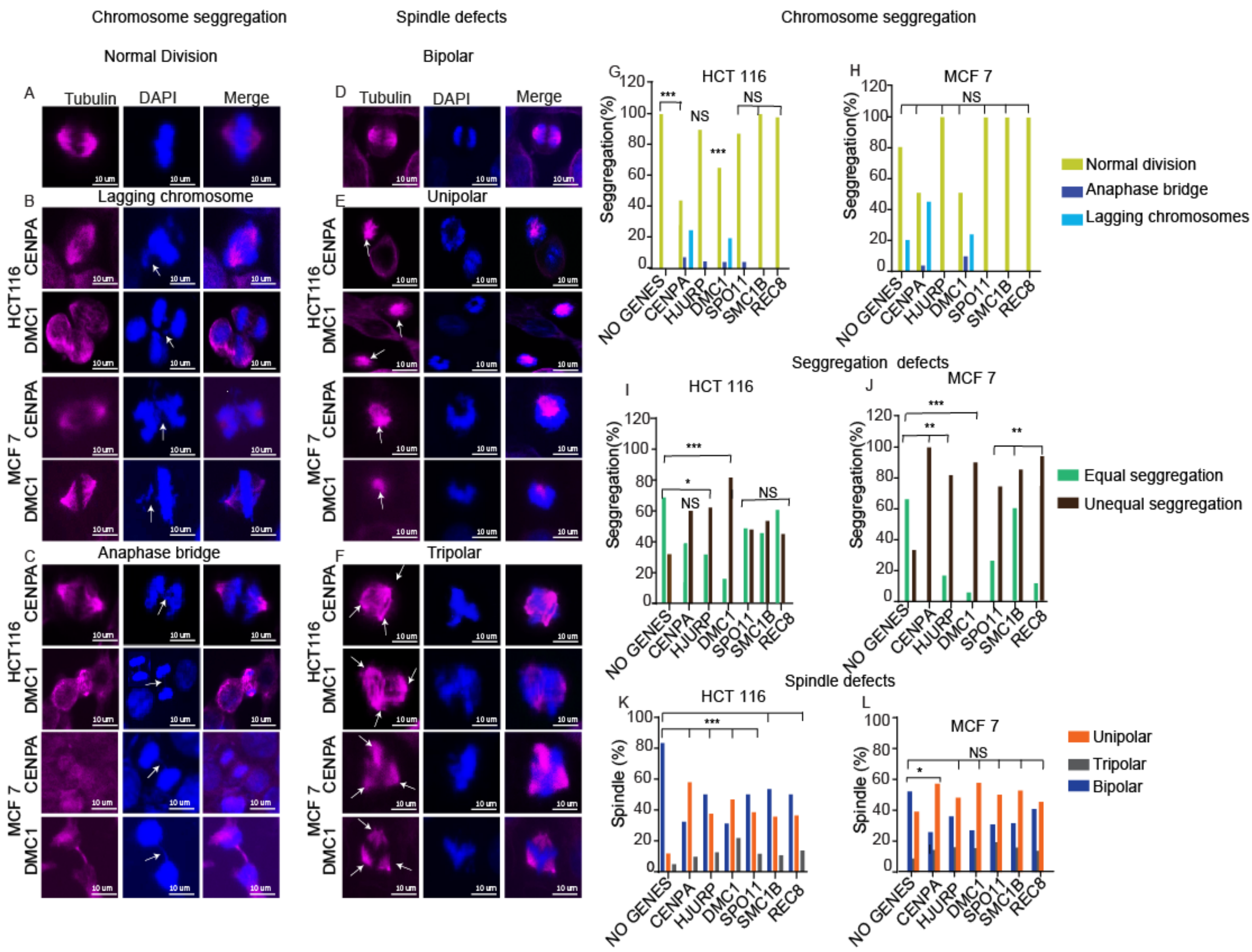
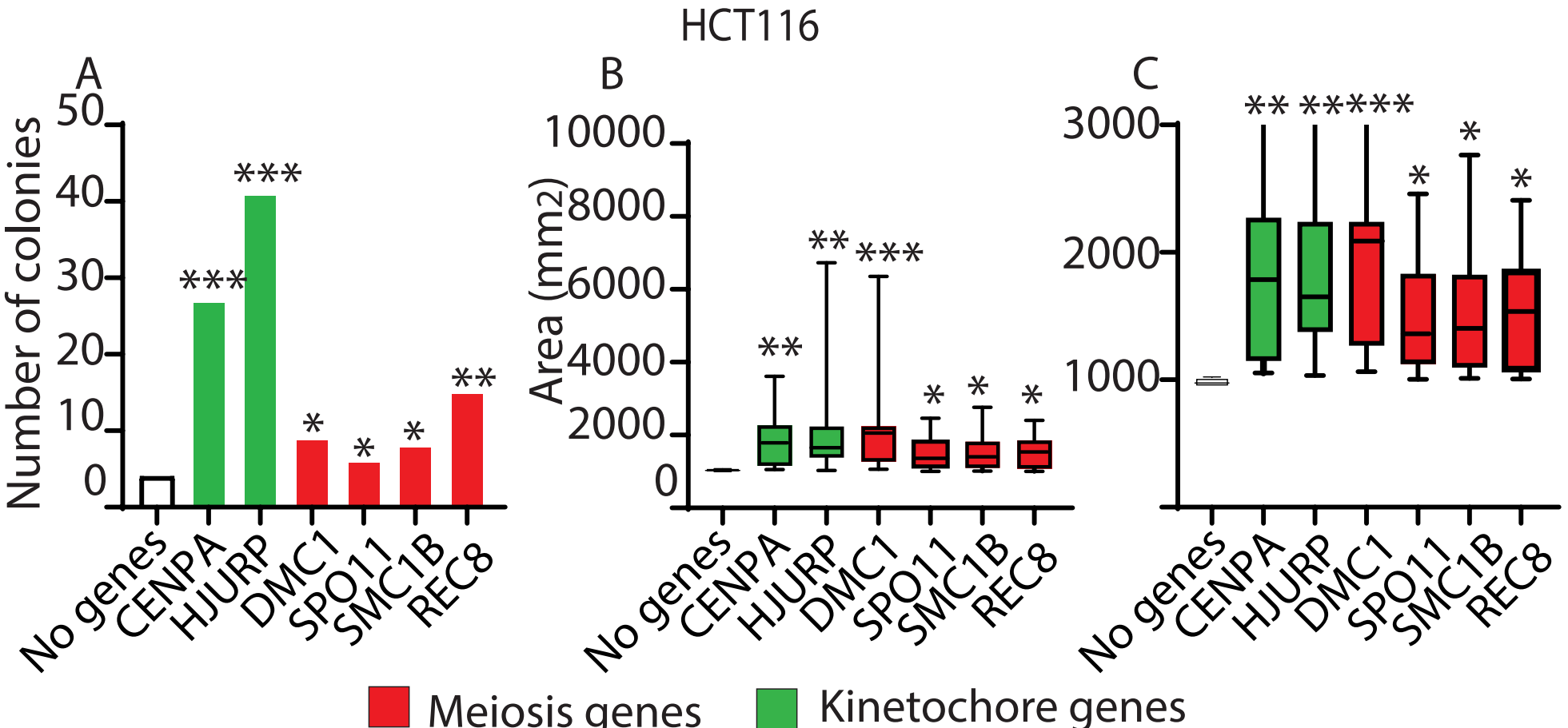

2821 tumor and normal tissues
Stomach Bladder and Breast
Colorectal and cervical
Unstable genomes
Stable genomes
Information-theoretic analysis to identify
perturbed gene network in the entire population
networks enriched with
meoisis and kinetochore genes
Unstable cancers
Stable cancers
Gene expression (%)
of meiosis/kinetochore
60
40
20
0
Experimental overexpression of meoisis
and kinetochore genes
Multipolar spindle
Anaphase bridge
10 um
10 um
Meoisis and kinetechore genes induce chromosomal instability


A
B
DNA regions with 1CNVs
1000
800
600
400
200
0
***
***
***
B
BL
S
Ce
C
200
150
100
50
0
***
***
***
B
BL
S
Ce
C
Breast cancer
Bladder cancer
Stomach cancer
Cervical cancer
Colorectal cancer


A
BC
B
BL
C
SC
D
Ce
E
CC
% genes
0
20
40
60
1 2 4 7 8 9 10 11 12 13 14 15
1 2 3 5 6 8 9 10 11
1 2 3 4 5 6 7 8 9 10 11 12 13 14
1 2 3 4 5 6 7 8 9 10 11 12 13 14
1 2 3 4 5 7 8 9 10 11
# of unbalanced process
Meiosis genes
Kinetochore genes


10% of Highest CNVs
A
BC
C
BL
E
SC
% genes
60
40
20
0
1
2
3
# of unbalanced process
10% of lowest CNVs
B
BC
D
BL
F
SC
-1
-3
2
# of unbalanced process
Meiosis genes
Kinetochore genes


Chromosome seggregation
Normal Division
A
Tubulin
DAPI
Merge
B
Lagging chromosome
C
Anaphase bridge
HCT116
MCF 7
CENPA
DMC1
Spindle defects
Bipolar
D
E
Unipolar
F
Tripolar
Chromosome seggregation
G
HCT 116
H
MCF 7
Seggregation(%)
NO GENES
CENPA
HJURP
DMC1
SPO11
SMC1B
REC8
Normal division
Anaphase bridge
Lagging chromosomes
Seggregation defects
I
HCT 116
J
MCF 7
Equal seggregation
Unequal seggregation
Spindle defects
K
HCT 116
L
MCF 7
Spindle (%)
Unipolar
Tripolar
Bipolar


HCT116
A
B
C
Number of colonies
Area (mm2)
50
40
30
20
10
0
10000
8000
6000
4000
2000
0
3000
2000
1000
***
***
*
*
*
**
**
**
***
*
*
*
** *****
*
*
*
No genes
CENPA
HJURP
DMC1
SPO11
SMC1B
REC8
Meiosis genes
Kinetochore genes

Misexpression of genes with roles in kinetochore structure or in meiosis contributes to genome instability in cancer

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Supplementary files include:

Fig. S1. Amplitudes (λ_1 (k)) of unbalanced process 1 in each tumor and normal tissues.

Fig. S2. The correlation between over-expression of meiosis/kinetochore genes and genomic instability within stable cancer types

Fig. S3. Overexpression of meiosis and kinetochore genes in HCT116 and MCF-7.

Fig. S4. Immunofluorescent staining showing the cell division defects.

Fig. S5. Immunofluorescent staining showing the spindle defects.

Fig. S6. Meiosis/kinetochore genes induce number and size if the colonies in soft agar

Fig. S7. Soft agar assay analysis showing the number of colonies and area of MCF-7 cell line when overexpressed with meiotic and kinetochore genes.

Figure S1

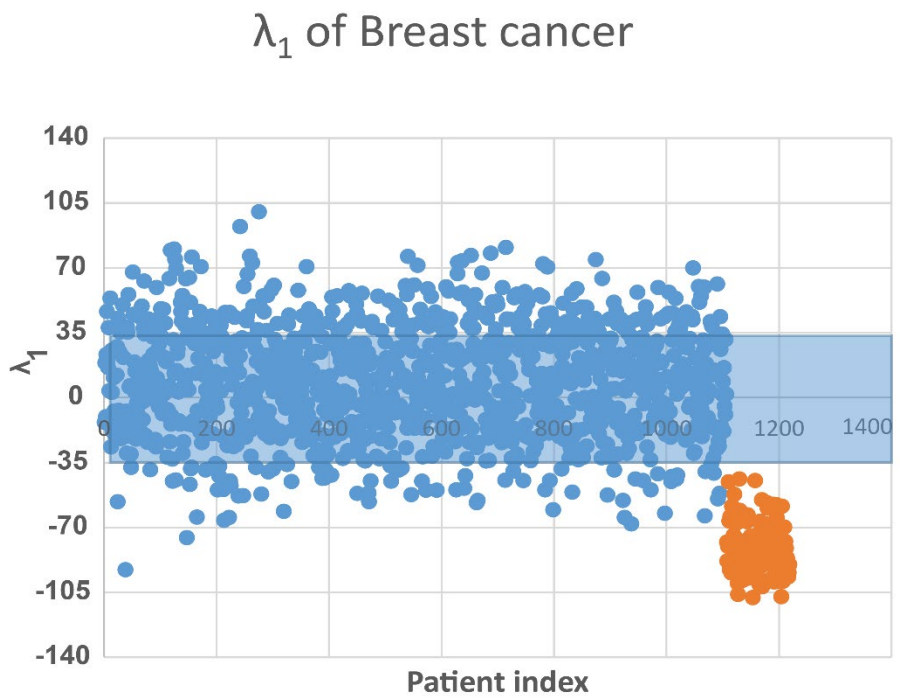


Figure S1. Amplitudes (λ_1 (k)) of unbalanced process 1 in each breast tumor (blue dots) and breast normal tissues (orange dots). The most dominant (characterized the biggest number of the patients in the dataset) process 1 distinguishes between cancer (blue dots) and non- cancer (orange dots) tissues. The blue box marks threshold limits. Plots of amplitudes for all unbalanced processes in breast and other cancer types can be found in **Table S2**.

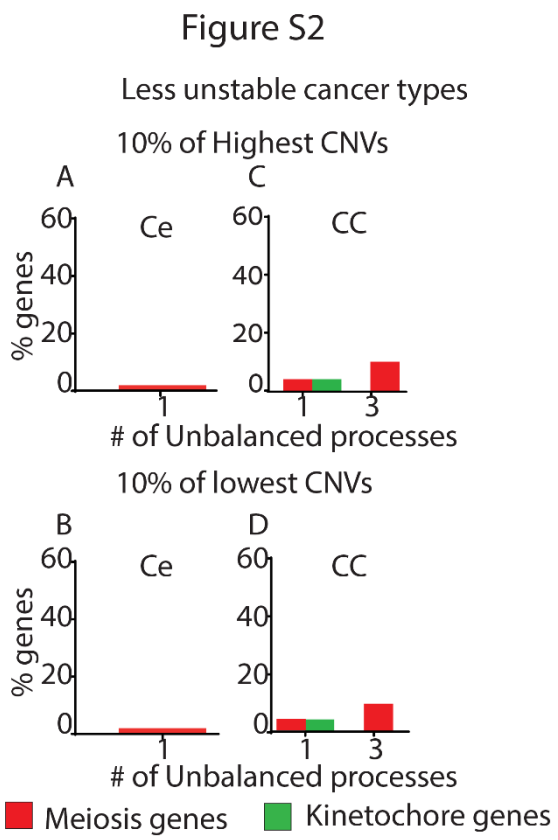
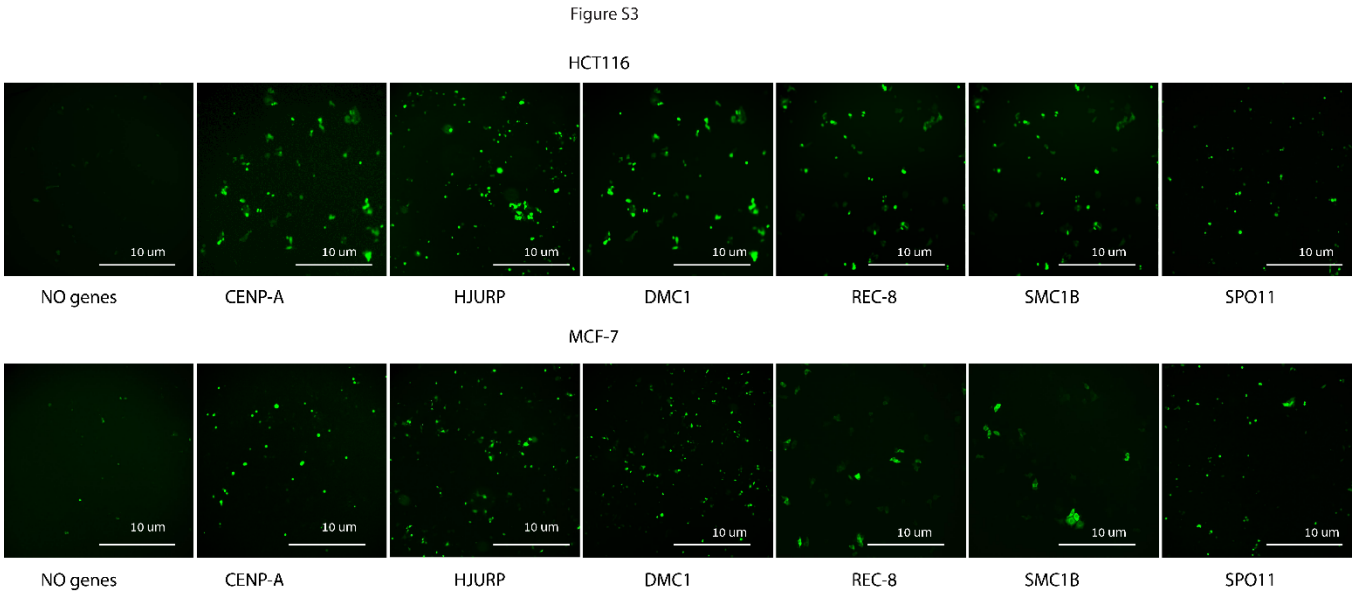
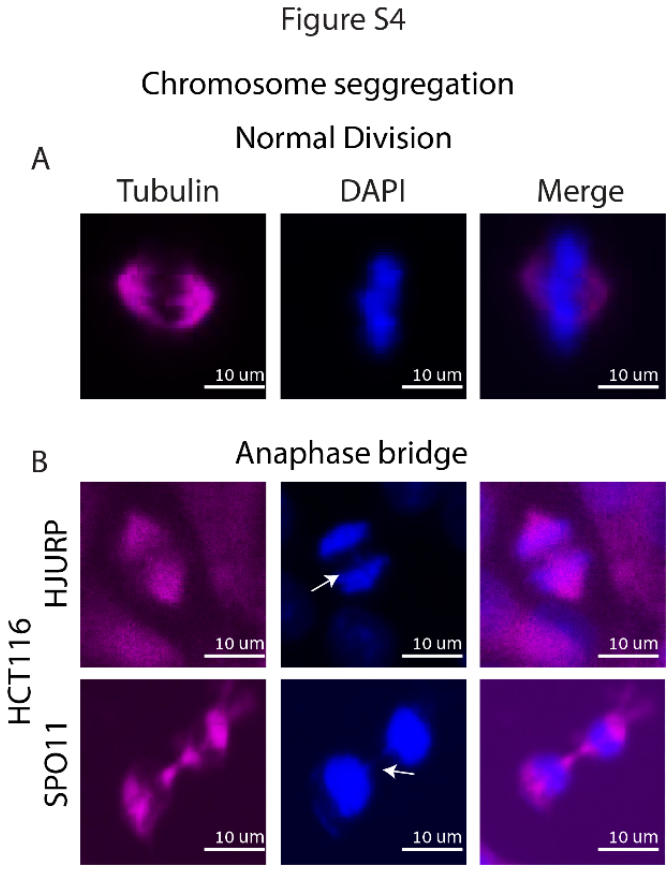


Figure S2. The correlation between over-expression of meiosis/kinetochore genes and genomic instability within stable cancer types. Stable cancer types, cervical cancer (Ce) and colorectal cancer (CC), were analyzed in this section. Each cancer type was analyzed separately as following: Distribution of lCNVs values was generated for each cancer type. 10% of the samples with highest lCNVs values (unstable group) and 10% of the samples with lowest lCNVs values (stable group) were selected in each cancer type. Cancer specific unbalanced processes of 10% of the samples with highest lCNVs values **(A, C)** were compared to the processes appeared in 10% of the samples with lowest lCNVs values (**B, D**). The most dominant processes characterizing two groups were not enriched with meiosis/kinetochore genes.

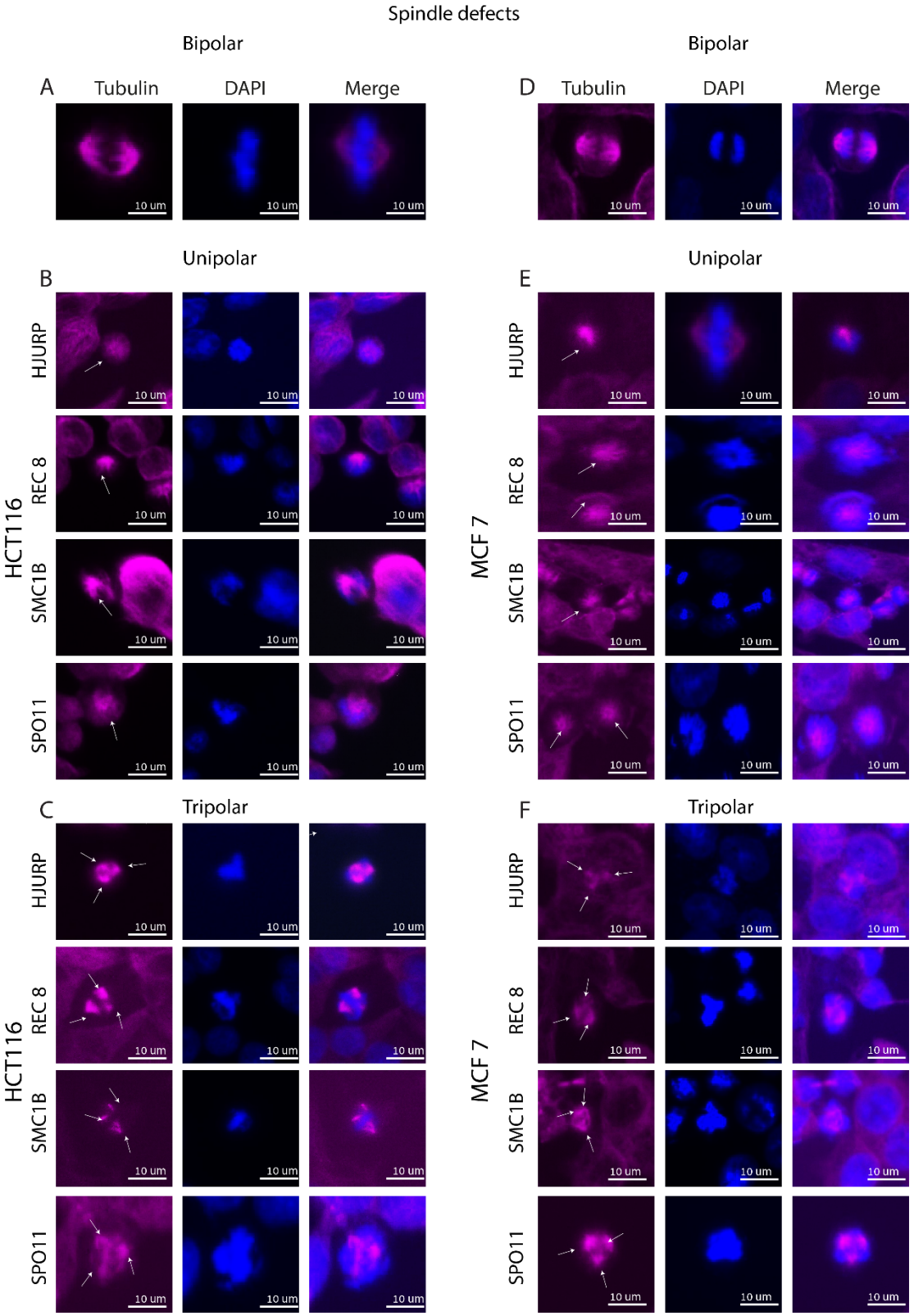


Supplementary Figure 3 (S3): Meiosis/kinetochore genes were fused to GFP and the over-expression pattern in the cell lines shows that the overexpression was successful. Overexpression of meiotic genes (DMC1, REC8, SMC1B, SPO11) and kinetochore genes (CENP-A and HJURP) in the HCT 116 cell line (**A**) and MCF-7 cell line (**B**) is presented.

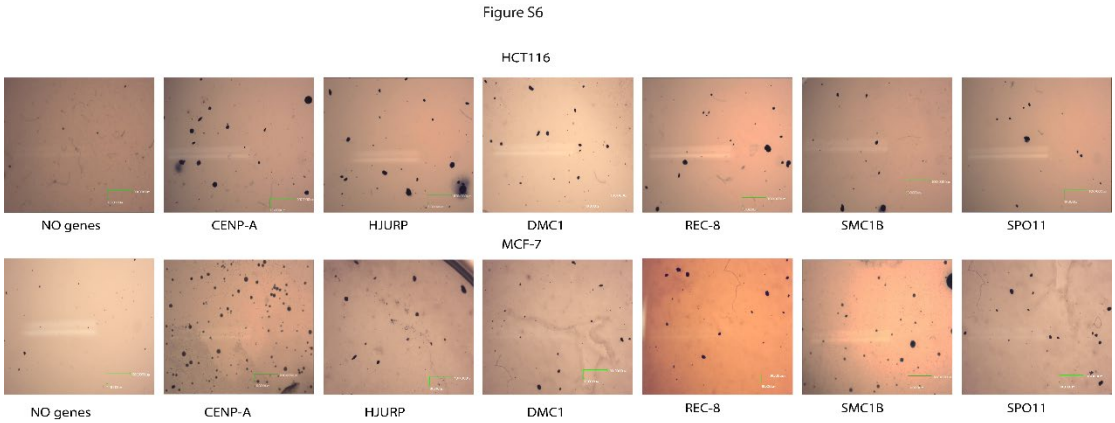


Supplementary Figure 4 (S4): Immunofluorescent staining showing the effect of overexpressed meiosis genes (DMC1, REC8, SMC1B and SPO11) and kinetochore genes (CENP-A and HJURP). The figure shows normal division (**A**) and anaphase bridges (white arrow) in HJURP and SPO11 overexpression (**B**). Scale bar is 10µm.

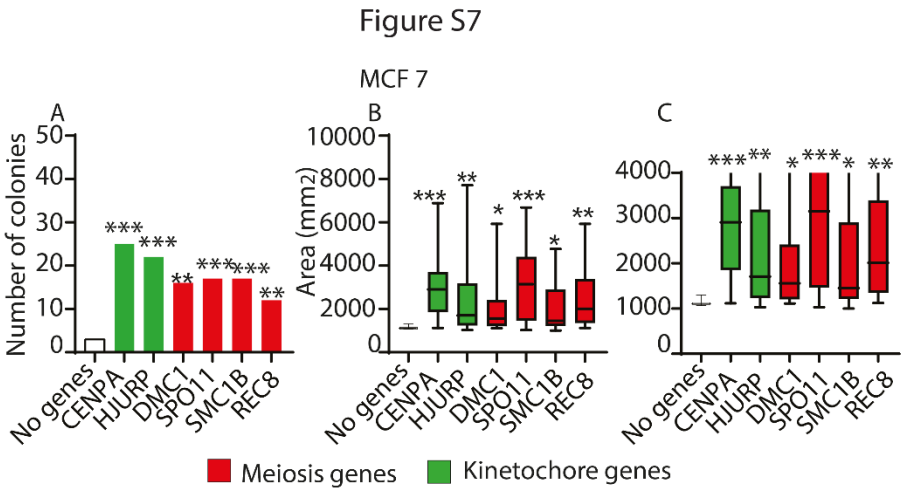
Figure S5



Supplementary Figure 5 (S5): Immunofluorescent staining showing effect of Overexpressed meiosis genes (DMC1, REC8, SMC1B and SPO11) and kinetochore genes (CENP-A and HJURP) in HCT116 and MCF-7 cell lines. **A&D** represent normal division. Overexpressed genes caused abnormal spindle defects: unipolar spindle formation (**B&E** in HCT116 and MCF 7 cells) and tripolar spindle formation (**C &F** in HCT116 and MCF-7 cells). Scale bar in B is 10 µm.



Supplementary Figure 6 (S6): Meiosis/kinetochore genes induce number and size of colonies in soft agar. HCT116 and MCF7 cells overexpressing the meiosis/kinetochore genes mentioned above were seeded in soft agar. Representative images of soft agar assay for HCT116 (**A**) and MCF-7 cell lines (**B**) are shown. Scale bar 10µm.



Supplementary Figure 7 (S7): A soft agar colony formation assay was applied for the detection of transformed cells when overexpressed with meiotic and kinetochore genes in an unstable cell line (MCF-7). The number of colonies **(Fig A)** and size of the colonies **(Fig B** for full scale or **C** for a zoom in on colonies up to 4000 mm^2**)** were measured using imageJ . Statistically significant differences compared with empty vector (No genes) were determined using a Student's 2-tailed t test (*P < 0.05; **P < 0.01; ***P < 0.001).