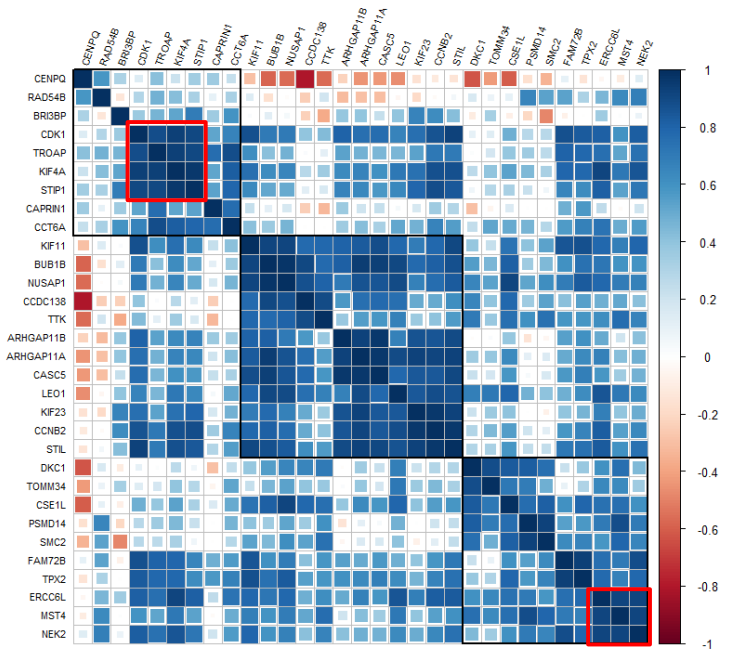
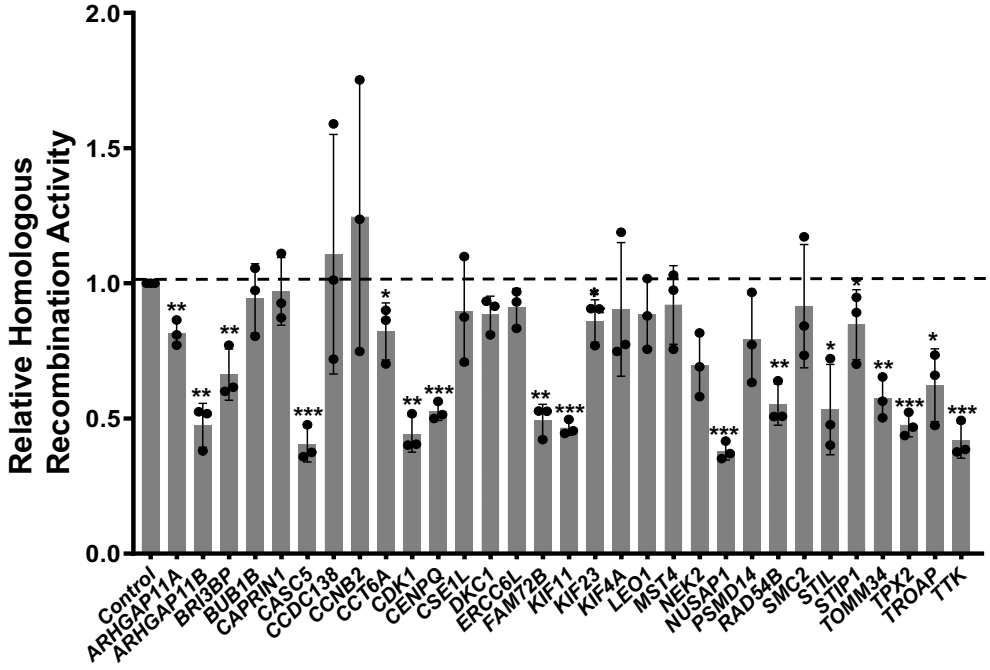
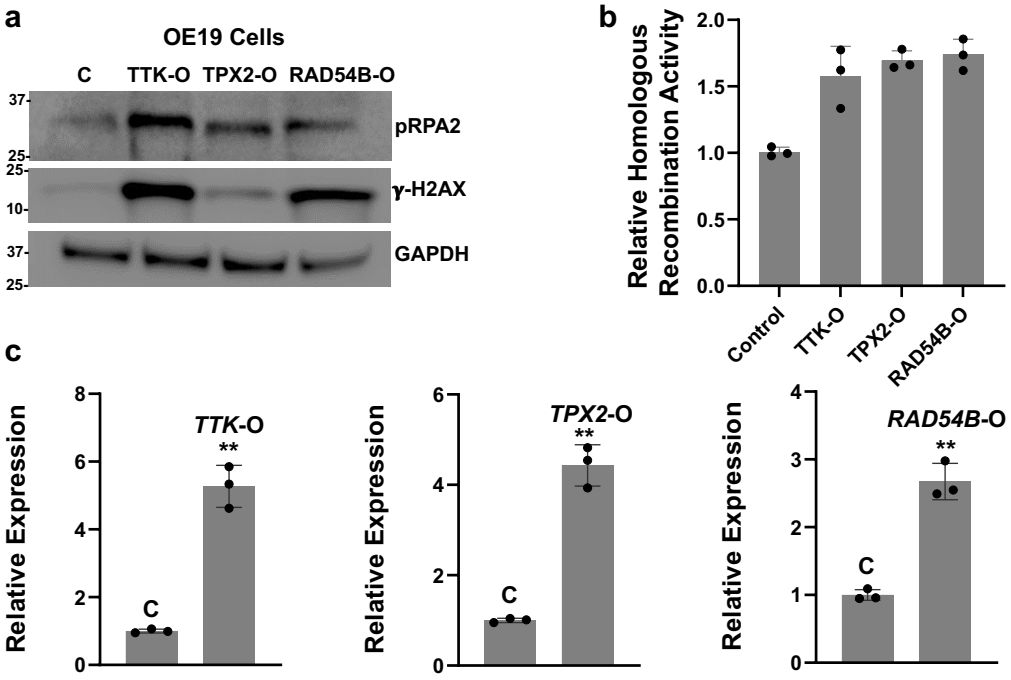
SUPPLEMENTARY MATERIAL:



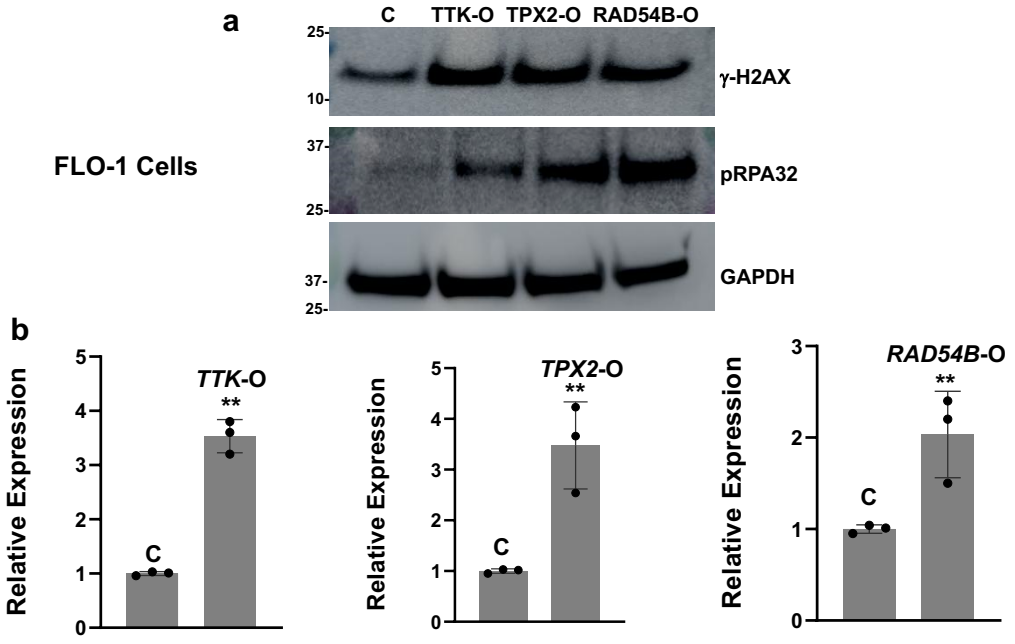
Supplementary Figure 1. Co-upregulated GIS31 genes in EAC. GIS31 genes in EAC patient dataset (from TCGA) were analyzed by a pairwise correlation of their expression, using Pearson method.



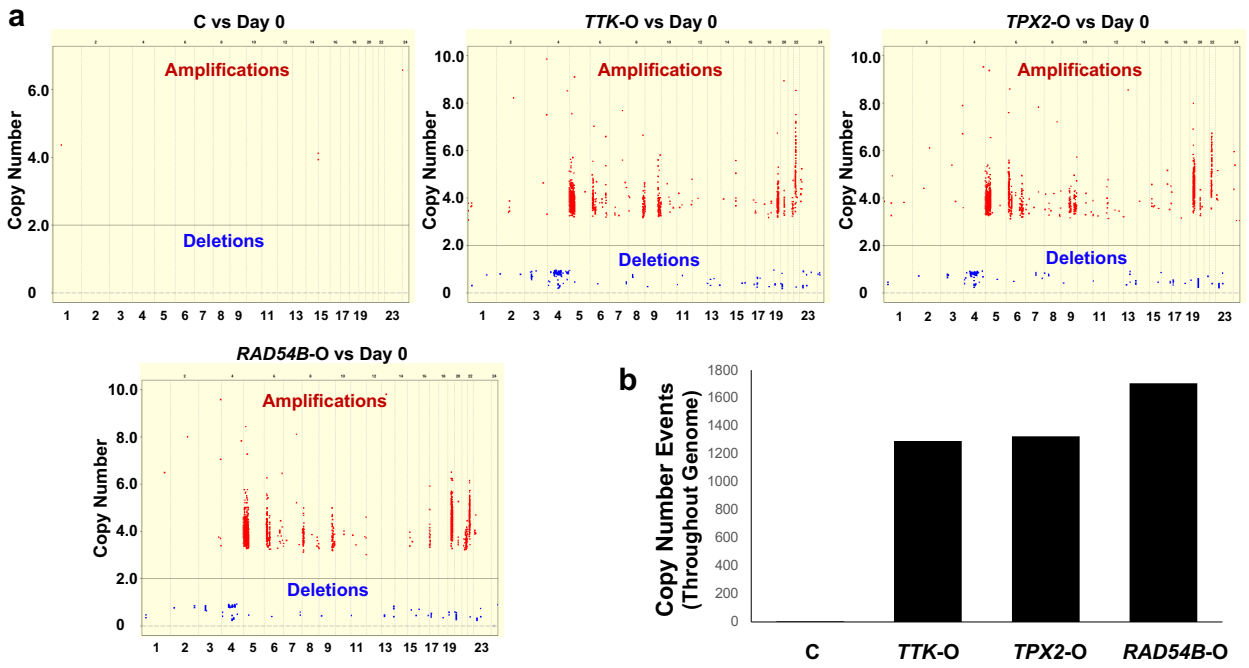
Supplementary Figure 2. Functional siRNA screen evaluating GIS31 genes for impact on homologous recombination (HR) activity in EAC cells. EAC (FLO1) cells were transfected with siRNAs, either control (non-targeting) or those targeting 31 potential genomic instability (GIS31) genes, and impact on homologous recombination assessed using strand exchange assay described in Methods section. Bar graphs show percent inhibition of homologous recombination activity; error bars represent SDs of three independent experiments. Two-tailed p-values, indicating significance of difference relative to control siRNA-transfected cells, are shown as: * < 0.05 – > 0.005; ** < 0.005 – > 0.0001; *** < 0.0001 – < 0.000005.



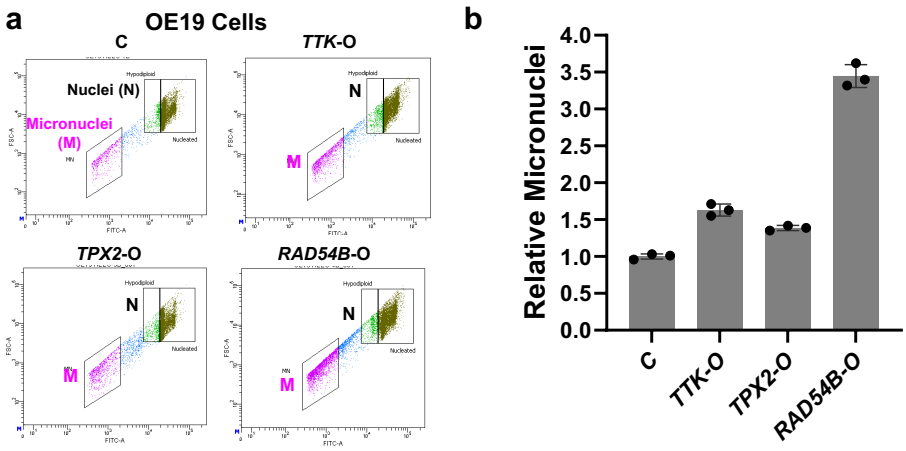
Supplementary Figure 3. Overexpression of *TTK*, *TPX2* and *RAD54B* increases DNA breaks and homologous recombination activity in EAC (OE19) cells. (a-b) OE19 cells were transfected with control plasmid (C) or those overexpressing *TTK* (*TTK*-O), *TPX2* (*TPX2*-O) or *RAD54B* (*RAD54B*-O), selected in puromycin and evaluated for γ-H2AX and phosphorylated-RPA32, using Western blotting (a), and homologous recombination activity, using a plasmid-based assay (b). Error bars indicate SDs of experiments conducted in triplicate; Two-tailed p values: = p < 0.5; (**c)** The transgene overexpression confirmed by Q-PCR.



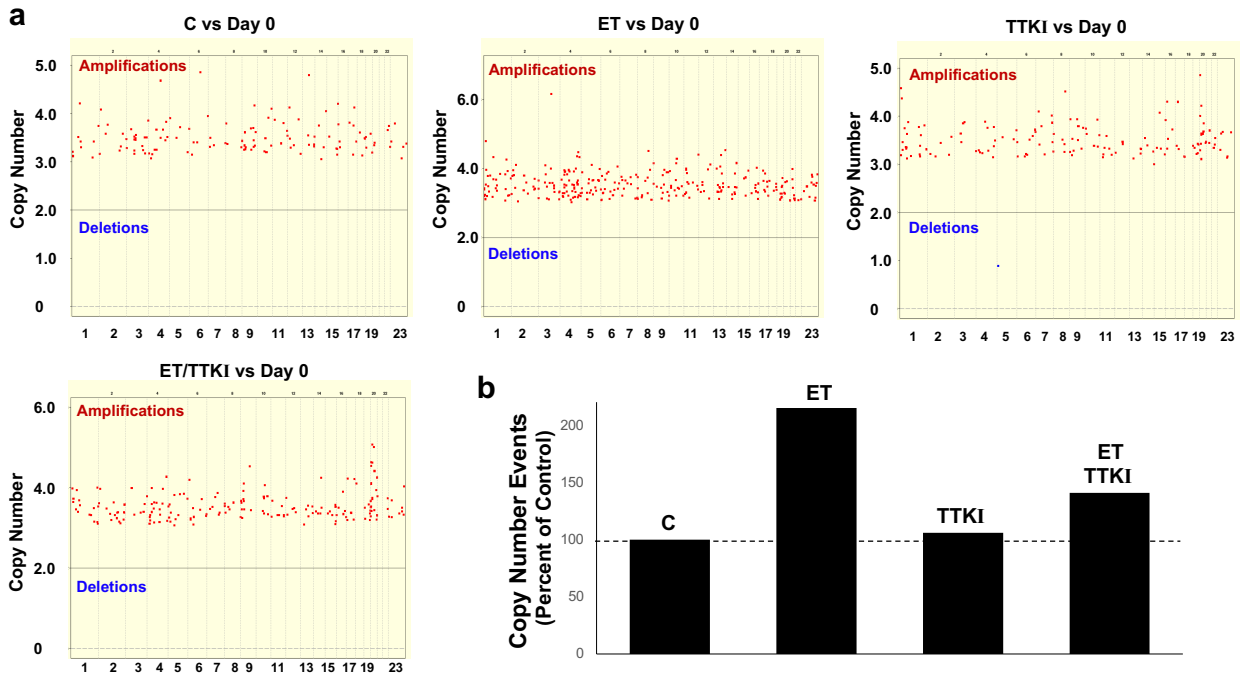
Supplementary Figure 4. Overexpression of *TTK*, *TPX2* and *RAD54B* increases DNA breaks and DNA end resection in EAC (FLO-1) cells. a) FLO-1 cells were transfected with control plasmid (C) or those overexpressing *TTK* (*TTK*-O), *TPX2* (*TPX2*-O) or *RAD54B* (*RAD54B*-O), selected in puromycin and evaluated for γ-H2AX and phosphorylated-RPA32, using Western blotting; **b)** The transgene overexpression confirmed by Q-PCR.



Supplementary Figure 5. Overexpression of *TTK*, *TPX2* and *RAD54B* in normal primary esophageal cells induces the acquisition of new copy number changes over time. Normal primary human esophageal epithelial cells (HEsEpi; ScienCell) were transfected with control plasmid (C) or those overexpressing *TTK* (*TTK*-O), *TPX2* (*TPX2*-O) or *RAD54B* (*RAD54B*-O), selected in puromycin and cultured for thirty days. DNA from these and baseline control (day 0) cells was purified and acquisition of copy number events during growth of cells in culture vs. day 0 cells (representing baseline genome) monitored, using SNP6.0 arrays (Affymetrix); a copy event was defined as a change in ≥ 5 consecutive CNV probes by 1 copy. **(a)** Images showing copy number events, as red (amplification) and blue (deletion) dots on all chromosomes, in cultured relative to baseline (day 0) cells; **(b)** Bar graph showing total copy-number change events, throughout genome.



Supplementary Figure 6. Overexpression of *TTK*, *TPX2* and *RAD54B* increases genomic instability in EAC (OE19) cells. OE19 cells were transfected with control plasmid (C) or those overexpressing *TTK* (*TTK*-O), *TPX2* (*TPX2*-O) or *RAD54B* (*RAD54B*-O), selected in puromycin and evaluated for micronuclei, a marker of genomic instability. Flow cytometry images of micronuclei (a) and bar graphs showing percentage of micronuclei (b) are shown.



Supplementary Figure 7. TTK inhibitor reduces etoposide-induced acquisition of copy number events in EAC cells. FLO-1 cells, control (C; DMSO only) or those treated with TTK inhibitor (TTK-I; 10 nM) and etoposide (ET; 1 μM), alone as well as in combination with each other for 3 weeks. DNA from these and baseline control (day 0) cells was purified and acquisition of copy number events during growth of cells in culture vs. day 0 cells (representing baseline genome) monitored, using Axiom™ Precision Medicine Diversity Arrays; a copy event was defined as a change in ≥ 3 consecutive CNV probes by 1 copy. **(a)** Apmlifications (red dots) and deletions (blue dots) on different chromosomes; **(b)** Bar graph showing copy number events throughout genome.

Supplementary Table 1. Known functions of GIS31 genes.

Gene Name	Family	Function/Pathway	Ref. #
ARHGAP11A	Rho GTPase	Cell cycle, DNA damage response	1
ARHGAP11B	Rho GTPase	Brain Development	2
BRI3BP	BRI3 binding protein	Involved in tumorigenesis	3
BUB1B	Serine/Threonine Kinase B	Mitotic checkpoint kinase	4
CAPRIN1	Cell Cycle Associated Protein	Cell proliferation	5
CASC5	Kinetochore protein	Cell cycle regulation	6
CCDC138	Coiled-coil domain-containing	Unknown	
CCNB2	B-type cyclins	G2/M Cell cycle regulation	7
CCT6A	Chaperonin protein	Protein folding	8
CDK1	Cyclin dependent kinase	G2/M Cell cycle regulation	9
CENPQ	Centromere protein	Regulation of mitosis	10
CSE1L	Nuclear export factor	Cell cycle and genomic instability	11
DKC1	Small nucleolar ribonucleoprotein	Cell cycle	12
ERCC6L	Mitotic helicase	Mitosis checkpoint regulation	13
FAM72B	Family with sequence similarity 72	Cell cycle regulation	14
KIF11	Kinetochore associated protein	Mitosis checkpoint regulation	15
KIF23	Kinetochore associated protein	Mitosis checkpoint regulation	15
KIF4A	Kinetochore associated protein	Mitosis checkpoint regulation	15
LEO1	RNA polymerase II associated factor Paf1	Oncogene	16
MST4	Serine/threonine protein kinase	Promote cell growth and transformation	17
NEK2	Mitotic kinase	Cancer progression	18
NUSAP1	Nucleolar & spindle associated	Promote cancer progression	19
PSMD14	Deubiquitinating enzyme	Promote tumor metastasis	20
RAD54B	DNA repair and recombination protein	Promotes homologous recombination	21
SMC2	Structural maintenance of chromosomes protein	Involved in DNA repair pathway and genomic instability	22
STIL	Centriolar replication factor	Involved in DNA damage response	23
STIP1	Stress induced phosphoprotein	Tumor growth, metastasis	24
TOMM34	Mitochondrial import receptor	Promotes cancer growth	25
TPX2	Microtubule-associated protein	Genomic instability, cancer	26
TROAP	Cytoskeleton, spindle assembly	Cancer and metastasis	27
TTK	Mitotic kinase	Homologous recombination and cancer growth	28

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