Supplemental material

Table S1. **MS-identified peptides, phosphorylation sites and kinase consensus motifs**

This table lists all peptide sequences and accession numbers of the identified *Drosophila* proteins and indicates the phosphorylated residues found in these peptides. Relevant parameter such as expected and calculated molecular weights and Mascot scores are also given. Distinct consensus recognition motives of the kinases most well-known to be involved in mitosis, Cdk1, Plk1, Aurora-A and the phosphopeptide binding motif of Plk1 (polo-box domain, PBD) as well as the recognition motif for CK2 are listed in the second sheet. Screening of the identified phosphorylation sites for these motifs resulted in the distribution shown in Table 1 (Fig 1).

Table S2. **RNAi phenotypes of phosphoproteins in SL2 cells assigned from single knockdowns and in a background of 4 simultaneously depleted kinases**

Significant phenotypes regarding centrosome duplication/segregation, centrosome morphology, cell cycle progression and chromosome segregation upon depletion of 25 MS-identified phosphoproteins and 4 kinases are listed in the left panel and highlighted in a dark shade. EGFP RNAi served as a control. Additionally, synergistic effects were analyzed by simultaneous knockdowns of phosphoproteins with each of the 4 kinases. Light and dark shades indicate a weaker and stronger phenotype, respectively compared to the single knockdown of polo (purple panel), aur (orange panel), cdc2 (blue panel) or CkIIβ (red panel). Panels shaded in light grey indicate that no significant phenotype was observed in the category. ● indicate that the

number of cells with abnormal chromosomes was significantly increased compared to control cells.

Table S3. **Oligos, plasmids, z-scores, p-values, GO terms**

Sequences of all RNAi and cloning primers as well as plasmids used in this study are listed (S3a). Furthermore, we show z-scores for FACS analysis and p-values for single knockdown analysis. Consistency of our results with relevant high throughput datasets and gene ontology annotation from Uniprot is also indicated (S3b). The third sheet (S3c) lists p-values obtained from double knockdown analysis with the 4 kinases polo, aur, cdc2 and CKIIβ. Both, deviation from the kinase single knockdown and from the egfp control were calculated. P-values below significance thresholds are highlighted in red.

Table S4. **Kinase profiling on peptide microarrays and MS-identification of Ote phosphosites after Aurora-A treatment**

Full length Ote (S4b) and 17 newly identified phosphorylation residues in 10 proteins from this study (S4a) were tested for *in vitro* phosphorylation by recombinant Aurora-A, PLK1, CDK1/Cyclin B and CK2 via peptide microarray analysis. Peptide sequences, kinase recognition motifs and corrected mean signal intensities for each kinase assay are listed. Peptides with signal intensities above 60% (AurA, Cdk1, CK2) or 40% (Plk1) of the control peptides were considered positive for *in vitro* phosphorylation by the respective kinase. To test if phosphorylation of Ote by Aurora-A can be confirmed by high resolution liquid chromatography MS (S4c), we incubated recombinant Ote with and without kinase under reaction conditions described in the Materials and Methods section. The resultant sample was analyzed on a nanoAcquity UPLC (Waters Corp) system coupled to an Orbitrap Velos

Instrument (ThermoScientific) running a water to acetonitrile gradient as described in the Materials and Methods section. In all injections performed (5x mock control, 5 x kinase assay), Ote was identified with a mascot score >2500 and with more than 70% sequence coverage. In the kinase-treated sample, one phosphorylated peptide (Table S4) was identified at high confidence in two separate runs by an MS/MS ion search using Mascot. Both peptides were not identified in five mock injections, indicating that phosphorylation was specifically due to the kinase treatment.