Supplemental material

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HeLa YFP-H2B + siARP1; Chromosome scattering phenotype

t=0 min	t=12 min	t=30 min	t=102 min	t=168 min	t=228 min	t=288 min	t=348 min	t=408 min
t=468 min	t=528 min	t=612 min	t=672 min	t=732 min	t=792 min	t=852 min	t=912 min	t=948 min

Figure S1. **Mitotic progression in U2OS cells and chromosome scattering phenotype.** (A) U2OS cells were transfected with the indicated siRNAs and the mitotic index was determined 72 h after transfection, as described in the Materials and methods section. The dotted line indicates the siGAPDH average + 2x standard deviation. Bars represent an average of three individual experiments and error bars display SEM. (B) A representative example the chromosome scattering phenotype. HeLa cells stably expressing H2B-YFP transfected with ARP1 siRNA normally aligns all its chromosomes. After a prolonged metaphase arrest, single chromosomes start to fall out of the metaphase plate. The amount of chromosomes leaving the metaphase plate increases over time. Bar, 10 µm.



Figure S2. Validation of results in different cell lines. (A) Quantification of centrosome-nuclear distance in prophase HeLa cells 72 h after transfection with indicated siRNAs. Bars represent average of three experiments (n = 20 centrosomes/experiment). Error bars represent SEM. (B) Quantification of the percentage of bipolar spindles in U2OS cells treated with 1.5 μ M STLC. Remaining spindles are all monopolar. Bars are an average of three independent experiments (n = 50 cells/experiment). Error bars represent SEM. (C) Quantification of spindle pole focusing defects in HeLa cells 72 h after transfection with indicated siRNAs. n = 75 cells/condition in three independent experiments. Error bars represent SD.



Figure S3. **Dynactin-depleted cells do not retain checkpoint proteins at their kinetochores.** (A) HeLa cells were transfected with indicated siRNAs for 72 h. 5 h before fixation, cells were treated with the CDK1 inhibitor RO3306. After 3 h, cells were released from the RO3306 block by washing four times with PBS to allow cells to go in mitosis. One sample was treated with nocodazole to create unattached KTs. After 2 h, cells were fixed and stained with BUBR1 antibody and with CREST antibody to visualize the centromeres. (B) HeLa cells were treated as in A. Cells were stained for MAD1 and CREST. (C) HeLa cells were treated as in A. Cells were stained for Spindly and CREST. Insets are enlargements of individual KT pairs. Bars, 5 µm (insets, 0.5 µm).

		Prometaphase				Metaphase				
		DAPI	DHC-GFP	CREST	Merge	DAPI	DHC-GFP	CREST	Merge	
dynein subunits siTCTEX1 siRoadbl-1 siDLIC1/2 siDIC2 siGAPDH	siGAPDH	Refer				and the second s	0	and the state of t	2	
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Figure S4. **Dynein kinetochore localization in the presence of microtubules.** DHC-GFP–expressing HeLa cells were transfected with indicated siRNAs. 72 h after transfection, cells were fixed and stained with anti-GFP antibody and CREST antibody to visualize the centromeres. DAPI was used to visualize the DNA. Based on chromosome and spindle morphology, prometaphase and metaphase cells were imaged.



Figure S5. **Dynactin depletion leads to reduced dynein at the spindle poles.** HeLa Kyoto cells stably expressing mouse DHC-GFP were transfected with either GAPDH siRNA, p150glued siRNA, or DIC2 siRNA. 72 h after transfection, cells were fixed and stained with anti-GFP antibody. Line scans were performed from the spindle pole toward the spindle midzone and the average amount of DHC-GFP was determined (n = 10 cells/condition from 2 individual experiments).

Table S1. List of selected proteins

Gene name	Aliases	Accession_number	Gene ID	Size (kD)
DYNC1H1	DHC	NM_001376	1778	532
DYNC2H1	DHC2	NM_001080463	79659	493
DYNC111	DIC1	NM_004411	1780	73
DYNC112	DIC2	NM_001378	1781	71
DYNC1LI1	DLIC1	NM_016141	51143	57
DYNC1LI2	DLIC2	NM_006141	1783	54
DYNC2LI1	D2LIC	NM_016008	51626	40
DYNLL1	DLC 1	NM_003746	8655	8–10
DYNLL2	DLC2	NM_080677	140735	10
DYNLRB1	Roadblock-1	NM_014183	83658	11
DYNLRB2	Roadblock-2	NM_130897	83657	11
DYNLT1	tctex 1	NM_006519	6993	12.5
DYNLT3	tctex1L	NM_006520	6990	13
DCTN1	p150glued	NM_004082	1639	127
DCTN2	Dynamitin/p50	NM_006400	10540	50
ACTR1A	Arp1/centractin α	NM_005736	10121	43
ACTR1B	Arp1/centractin β	NM_005735	10120	43
ACTR3B	Arp11	NM_020445	57180	48
DCTN4	p62	NM_016221	51164	52
DCTN5	p25	NM_032486	84516	20
DCTN6	p27	NM_006571	10671	21
DCTN3	p22/P24	NM_007234	11258	21
CAPZA1	F-actin–capping protein subunit α-1	NM_006135	829	33
CAPZB	F-actin–capping protein subunit β	NM_004930	832	31
PAFAH1B1	LIS1	NM_000430	5048	45
NDE1	nudE nuclear distribution gene E homologue 1	NM_017668	54820	39
NDEL1	Nudel/NudE like protein	NM_001025579	81565	38
BICD2	bicaudal D homolog 2 (Drosophila)	NM_015250	23299	94
CCDC99	Spindly	NM_017785	54908	70
ZW10	ZW10, zeste white 10 homologue (Drosophila)	NM_004724	9183	89

Overview of all selected proteins that were included in the siRNA library. Listed are: gene name, known aliases, accession numbers, Gene ID, and protein size.

Table S2. **qRT-PCR primers**

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)		
DYNC1H1	CGCCCAACATACCCATTATC	TGCGTCTCCACTTCAATCTG		
DYNC2H1	GCAAGTATTCGCACCAATGTT	CCCAACCCAGCTTCTAGTTC		
DYNC111	TCCTGCCAAGGGAAGTAGTG	TCTGAGTCCTGGCCAACTTT		
DYNC112	TCACTTCATCGTTTGACTGGAC	ATCCACAGGGCAAACAGG		
DYNC1LI1	CAGAAATGGGCAAGTGTTGTT	GGGAAGTCTTCTCCTGGCTCT		
DYNC1LI2	GGCTGGGACAATGAAAAGAA	CACCTGCTCATCTTCTGCTG		
DYNC2LI1	AGAAGAGCAAAAGGGCACAA	CACCTGTGATGGGTATGCTG		
DYNLL1	TGTCGGAAGAGATGCAACAG	TGCCAGGTGGGATTGTACTT		
DYNLL2	TCTGAGGACATGCAACAGGA	CGATACAATGCCAGGTAGGG		
DYNLRB1	GGTGGAGGAGACACTGAAGC	GTTGTCCATGGTGCTCTTGA		
DYNLRB2	AAGCCAAAAGCACAGTTCGT	CCATCGCAGGTCTATTCACA		
DYNLT1	AACTCACCAAGCTGGGAAAA	GTCCCAGAAGCAGGAACTTG		
DYNLT3	TGGGGTTTTAGGTGGTGAAG	CTCTTCTGGACCACTGCACA		
DCTN1	GCTCATGCCTCGTCTCATTT	GTACACCAGTCCAGCAGCAA		
DCTN2	TTGCCAAGCATAAAGCCTCT	GCTGCTTGATGGTGACAAGT		
ACTR1A	AGCATCCTGTGCTCCTGACT	GTGGTCCTGCCTGTAGCGTA		
ACTR1B	CTCTGGAGACGGAGAAGGTG	ACTCTCATCCCCGACAAGGT		
ARP3B	CCTCCACTCAATACACCAGAAA	ACCCACTTGTCGAGATGTCC		
DCTN4	CCCAACGTCAATCAAATTCA	GGGTGAGGTTCTCAACTGGA		
DCTN5	GGTCAACGCAGCACAGATT	GGTGGAACCACAGTTTCTGG		
DCTN6	ATTGGCGAAGGGAACCTAAT	GCTTGGGAATAACAGCCAAC		
DCTN3	GGACCAGTGTGTGGAAATCA	GCTGGCAAAGTAGCTCATCC		
CAPZA1	CCATTATTCCAACGGCTTCT	ATCTCCAACGACCATTCCAG		
CAPZB	GGCTGGAGATGGATCAAAGA	CCAGAGCCAGATTTGTTGGT		
BICD1	GTGTTGAAAGCCAACAAGCA	GGTTGCAGCATCTTCTTTCA		
BICD2	TGGAGGAGATCCTCAAGCTG	CAAGCTG ACCATGGCCTTCTCATTCTC		
NDE1	CGGAGCAGCAAGAACAGAG	AAGAACAGGCTTCAGCAGGA		
NDEL1	TGCAGGCTGATAACCAAAGA	AATGGCCCGAGTCTGACTTA		
LIS 1	ATGGGTCGTAGCAACAAGG	GGCCCAGGTTTACCACTTT		
hSpindly	TGAAATGATGACCATGACTGA	TTCACATTCGCAGCTCAAAC		
ZW10	CATTGACCTGCTGAAATCCA	GCAGTGGAAAACTCCTGCAA		
β-Actin	AGAAAATCTGGCACCACACC	AGAGGCGTACAGGGATAGCA		

Primer sequences used for qRT-PCR analysis. Primers were designed with a Tm close to 60 degrees to generate 90–120 bp amplicons, mostly spanning exon–exon junctions.