

Supplementary Table 1

Gene name	Tagging method	Cell line	Source	Cells analyzed after quality control	Number of independent experiments
KIF11	BAC	HK cDNA H2B-mCherry BAC mKIF11-GFP #2354	A. Hyman, MPI-CBG Dresden, Germany <sup>26</sup>	14	2
MIS12	BAC	HK BAC mMIS12-LAP cDNA H2B-mCherry cDNA #2341	A. Hyman, MPI-CBG Dresden, Germany <sup>53</sup>	35	5
TUBB4B	BAC	HK cDNA H2B-mCherry BAC mTUBB4B-LAP #2637	A. Hyman, MPI-CBG Dresden, Germany <sup>53</sup>	28	5
RACGAP1	BAC	HK cDNA H2B-mCherry BAC LAP-mRACGAP1 #2362	A. Hyman, MPI-CBG Dresden, Germany <sup>53</sup>	18	4
CDCA8	BAC	HK cDNA H2B-mCherry BAC mCDCA8-LAP #2607	A. Hyman, MPI-CBG Dresden, Germany <sup>53</sup>	13	2
NEDD1	BAC	HK cDNA H2B-mCherry BAC mNEDD1-LAP #311	A. Hyman, MPI-CBG Dresden, Germany <sup>53</sup>	28	4
CENPA	cDNA	HK cDNA EGFP-CENPA cDNA H2B-mCherry pool	T. Hirota, Cancer Institute Tokyo, Japan <sup>28</sup>	21	4
NES	cDNA	HK cDNA H2B-mCherry cDNA NES-mEGFP2 pool	J. Ellenberg/EMBL, this work	17	4
PLK1	ZFN	HK ZFN PLK1-mEGFP #24 cDNA H2B-mCherry pool	J. Ellenberg/EMBL, this work	16	3
AURKB	ZFN	HK ZFN AURKB-mEGFP #H24 cDNA H2B-mCherry pool	J. Ellenberg/EMBL <sup>29</sup>	14	4
BUB1	CRISPR	HK CRISPR mEGFP-BUB1 #63 cDNA H2B-mCherry pool	J. Ellenberg/EMBL, this work	12	2
NUP107	ZFN	HK 2xZFN mEGFP-NUP107 #26, 31	J. Ellenberg/EMBL <sup>16</sup>	16	4
RANBP2	CRISPR	HK CRISPR mEGFP-NUP358/RANBP2 #97	J. Ellenberg/EMBL this work	22	3
NUP214	CRISPR	HK CRISPR mEGFP-NUP214 #2-12	J. Ellenberg/EMBL, this work	14	5
TPR	CRISPR	HK CRISPR TPR-mEGFP #171	J. Ellenberg/EMBL, this work	15	3
CEP192	ZFN	HK ZFN CEP192-mEGFP #15	J. Ellenberg/EMBL, this work	13	2
CEP250	CRISPR	HK CRISPR CEP250-mEGFP #1A-142	J. Ellenberg/EMBL, this work	19	3
NCAPH2	CRISPR	HK CRISPR mEGFP-NCAPH2 #1	J. Ellenberg/EMBL <sup>30</sup>	20	3
TOP2A	CRISPR	HK CRISPR mEGFP-TOP2A #102	J. Ellenberg/EMBL, this work	16	3
KIF4A	CRISPR	HK CRISPR mEGFP-KIF4A #173	J. Ellenberg/EMBL, this work	21	4
WAPL	CRISPR	HK CRISPR WAPL-EGFP	J.M. Peters/IMP <sup>31</sup>	12	3
STAG1	CRISPR	HK CRISPR STAG1-EGFP #H8	J.M. Peters/IMP, this work	22	3
STAG2	CRISPR	HK CRISPR STAG2-EGFP #F2	J.M. Peters/IMP, this work	13	3
RAD21	CRISPR	HK CRISPR RAD21/SCC1-EGFP	J.M. Peters/IMP <sup>32</sup>	18	3
CTCF	CRISPR	HK CRISPR CTCF-EGFP #F2	J.M. Peters/IMP, this work	16	3
BUB1B	CRISPR	HK CRISPR EGFP-BUB1B #M04-A03	J.M. Peters/IMP, this work	20	3
ANAPC2	CRISPR	HK CRISPR mEGFP-ANAPC2 #M21-P1-A11	J.M. Peters/IMP, this work	14	3
MAD2L1	CRISPR	HK CRISPR MAD2L1-EGFP #M11-B11	J.M. Peters/IMP, this work	10	2

The LAP tag has EGFP as fluorescent protein. The reference number refer to the references in the Methods of Cai, Hossain, et al. 2018

**Supplementary Table 2**

<b>Gene</b>	<b>Genome editing tool</b>	<b>Sequences</b>
AURKB	ZFN	CGCCTGATGGTCCCTgtcattCACTCGGGTGCCTGTGTT
PLK1	ZFN	TGGGCCAGCAACCGTCTaaggccTCCTAATAGCTGCC
CEP192	ZFN	CTTGTCATTCAAACAGATGaaggcaAGAGTATTGCTATTG
NUP107	ZFN	TCAGTACTGATGgtggcaGCTGAGCCCAGTC
BUB1	CRISPR/Cas9D10A	ACCAGACGGACACTTACTGA GGCGCCTGGGGTCGGGCC
RANBP2	CRISPR/Cas9D10A	GGCGCGTGAGACCAGCGCTC GAGGCGCAGCAAGGCTGACG
NUP214	CRISPR/Cas9D10A	GCAGCCAACGCTGCCTCCCA CGGCGCGATGGGAGACGAGA
TPR	CRISPR/Cas9D10A	CTCCTCTCCCTCCCATTGCA CAGAGGAAATATTAATTAAA
CEP250	CRISPR/Cas9D10A	CTGCTACCTGGAGGGCGGCTT ACAGACAGAACGACTGTGTCA
MAD2L1	CRISPR/Cas9D10A	GTCATCCTCAGTCATTGAC TAATTGTAATTTGAAATG
BUB1B	CRISPR/Cas9D10A	CGCCGCCATCCTGCATTCC GGTGCTCTGAGGTAGGTAC
ANAPC2	CRISPR/Cas9D10A	CGCAGCCATGACGCGCACA CGCCGCGCCGAGCGAATCT
RAD21	CRISPR/Cas9D10A	CTTATATAATATGGAACCT CAAATTGCCCCCATGTGTA
STAG1	CRISPR/Cas9D10A	TCTTCAGACTTCAGAACAT GTTTCTCATCATTCTCTA
STAG2	CRISPR/Cas9D10A	CACAGATTAAATTGTGTAC CTCTCTCTCATTAGGTCT
CTCF	CRISPR/Cas9D10A	GAGGATCATCTCGGGCGTG CAGCATGATGGACCGGTGA
WAPL	CRISPR/Cas9D10A	CTAAGGGTAGTCCGTTGT TGGGGAGAGACCACATTAA
NCAPH2	CRISPR/Cas9D10A	ACCGCAGGGCTGCCTCCGA CCGTTCCCTCCGGACATGG

ZFN cut sites are indicated in lower case. For CRISPR/Cas9 editing, the first sequence is the antisense gRNA binding site, the second one is the sense gRNA binding site (double nicking approach).

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