

## Supplemental information – Table 1: Primers, Plasmids and gBlocks

### Primers and sgRNAs

	Cloning primers
dSpt6_F1	TCCGACTAGTATGGCCGAATTTCTGGAATCCGA
dSpt6_R2	GCATGATATCGTTCTCGTCGTAGAGGGGCGT
1kup_F_BstZ17I	ATAGTATACCAACAACAGGAGCACCGGACAC
2kup_R_NheI	CGAGCTAGCAATATAATGGAATAAAGCTTAACCTAATAATAAAGTGCG
dSpt6_R-Ascl	ATATGGCGCGCCTAGATGTTCTCGTCGTAGAGGGGCGT
T2A_CD4_E1_F	CCGGAATTCGAGGGCAGAGGAAGTCTTCTAACATGCG
T2A_CD4_B1_R	AAAGGATCCCTAGCCGGTGTGGCGGCACCTGA
	RNAi primers
White fw (RNAi)	TTAATACGACTCACTATAGGGACTGCTCAATGGCCAACCTGTGGAC
White rev (RNAi)	TTAATACGACTCACTATAGGGCCTCGGCCATCAGAAGGATCTTGTC
Spt6_F(RNAi)	TTAATACGACTCACTATAGGGATGCCGGGCAAGTTCCTGCTGTCCTA
Spt6_R(RNAi)	TTAATACGACTCACTATAGGGGGCGTCATGAACGGAGTCTGTCCAC
CID fw (RNAi)	TTAATACGACTCACTATAGGGAGACCGTCGGCGAACAACCTCAAAGT
CID rev (RNAi)	TTAATACGACTCACTATAGGGAGAAGCCGCTGCGTCAAGTACATCTC
Neg9	UACGACCGGUCUAUCGUAGTT
	sgRNA primers
dU6_2_sgRNA_F	GTTCTGACTTGCAGCCTGAAATACGGCACG
dU6_2_3_sgRNA_R	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAA
Spt6_sgRNA_5_fw	TTCGTGACGACTTCTCAAAGTACG
Spt6_sgRNA_5_rev	AACCGTACTTTGAGAAGTCGTCC
Spt6_sgRNA_9_fw	TTCGGTTACGATTCCGATGGCGT
Spt6_sgRNA_9_rev	AACACGCCATCGGAATCGTAACC
	Primers for Site-Directed Mutagenesis
S20A_F	CAACTCAAAGgCGCCGAACGACG
S20A_R	TTCGCCGACGGCCTGGGT
S75A_F	GAGAGACAGCgCCACCACTGGCG
S75A_R	GCTGCCGGCTGCCTCCGT
S77A_F	CAGCTCCACCgcTGGCGAGGAGGAGGACC
S77A_R	TCTCTCGCTGCCGGCTGC
B75AM77A_F	CAGCgCCACCgcTGGCGAGGAGGAGGAC
S20D_F	CAACTCAAAGgacCCGAACGACGACGACACGGCC

S20D_R	TTCGCCGACGGCCTGGGT
S75A_F	GAGAGACAGCgaCACCAGTGGCGAGGAGGAGG
S75A_R	GCTGCCGGCTGCCTCCGT
S77D_F	CAGCTCCACCgacGGCGAGGAGGAGGACC
S77D_R	TCTCTCGCTGCCGGCTGC
B75DM77D_F	CGAGAGACAGCgacACCgacGGCGAGGAGGAGGACC
B75DM77D_R	GGTCCTCCTCCTCGCCgtcGGTGTCTGTCTCTCG

### Plasmids:

pMT-CID-GFP-hygro	Heun et al., 2006 <sup>1</sup>
pMT-Spt6-GFP-hygro	This study
pIB-resSpt6-stop	This study
pMT_FKBP_L106P_Fbox_GFPbinder_hygro_opt	This study
pMT-CID-HA-ERT2_kana_opt	This study
pRITE_MT_CID_V5_puro_3myc_CD4	This study
1kup_resGFPSpt6_stop	This study
pIB_Cas9_CD4_Blast	
pET-His6-Sumo_Spt6199-338	This study
pET22b_H3	gift from K. Luger
pET22b_H4	gift from K. Luger
pMT-SNAP-CENP-A	Boltengagen et al., 2016 <sup>2</sup>
pMT-SNAP-CENP-A_S77D	This study
pMT-SNAP-CENP-A_S20/75/77D	This study

### gBlocks

resGFPSpt6\_HindIII\_Eco47III

GTAAAGCTTTATTCCATTATATTGCTAGTTATGGCCGAATTTCTGGACTCCGA  
GGCGGAAGAATCGGAGGAGGAGGAAGAATTGGACGTCAACGAGCGCAAGA  
GACTCAAGAACTGAAAGCAGCCGTCTCCGATAGTAGCGAAGAAGAAGAAG  
GTGAGTTTTTCCACTGGAGCCGCTGAAGGCGCGTACAAATACATACATACAT  
ACATACGTATGTGGCGATCTCTGGCGGGTTTTTCACCCCTCTCACCAGGCG  
CGCCAGATCCACACATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTG  
GTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAG  
CGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTG

AAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGT  
GACCACCCTGACCTACGGCGTGCAAGTGCCTTCAGCCGCTACCCCGACCACA  
TGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGG  
AGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAG  
GTGAAGTTCGAGGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCAT  
CGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAAC  
ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCA  
AGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTC  
GCCGACCCTACCAGCAGAACACCCCATCGGGCAGCGCCCCGTGCTGCT  
GCCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCA  
ACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCTCG  
ACTCGAGGTACCTGGTAACAGTTTTTCAGTTGCATATGTATATCTGCGGCTAC  
AATGAAGGTAAAAGGTAAACCGATATTCTTATTGCCCTATTCCGTAGACGAC  
GAGGAACGGCTGCGCGAAGAGCTCAAAGATCTGATCGACGACAATCCCATC  
GAGGAGGACGATGGCAGTG**GTTACGATTCCGACGGAGT**GGGCAGCGGCAA  
GAAGCGCAAAAAACATGAAGACGACGATCTGGACGATCGACTGGAGGACG  
ACGACTACGATCTTATCGAGGAGAATCTGGGCGTCAAAGTCGAGAGACGGA  
AGCGGTTCAAGCGTCTGCGTCGCATCCACGACAACGAAAGCGATGGCGAG  
GAGCAGCACGTGGACGAGGGTCTGGTGCCTGAGCAGATTGCTGAACAATT  
GTTTCGACGAGAACGATGAGAGCATTGGTCATCGCAGCGAGCGCAGTCACA  
GGGAGGCGGACGACTACGACGACGTGGATACGGAGTCGGACGCAGATGA  
CTTCATTGTGGACGATAATGGGCGTCCCATAGCCGAGAAGAAGAAGAAACG  
GCGCCCCATCTTTACAGACGCATCACTGCAGGAAGGTCAGGACATCTTTGG  
CGTAGACTTCGACTAT**GACGACTTCTCAAATATG**AAGAAGACGACTACGAA  
GATGACTCGGAAGGAGATGAGTACGATGAGGATCTGGGCGTCGGCGATGA  
TACGCGCGTCAAGAAGAAGAAAGCGCTCAA

GFP, Mutated sgRNA9 and sgRNA5 target regions (respectively)

dSpt6\_199-338\_Ecoli\_codopt\_biobrick\_IDT

**TACTTCCAATCCAATGCA**ATGACCGATGCGTCTCTGCAAGAAGGTCAAGATA  
TTTTTGGCGTGGATTTTGATTATGATGATTTAGCAAATATGAAGAAGACGAT  
TATGAAGATGACAGCGAAGGCGATGAATATGATGAAGATCTGGGCGTGGGC  
GACGATACCCGCGTGAAAAAGAAGAAAGCGCTGAAAAAGAAAGTGGTGAAG  
AAAACCATTTTTGATATTTATGAACCGAGCGAACTGAAACGCGGCCATTTTA  
CCGATATGGATAACGAAATTCGCAAAACCGATATTCCGGAACGCATGCAGC  
TGCGCGAAGTGCCGGTGACCCCGGTGCCGGAAGGCAGCGATGAACTGGAT  
CTGGAAGCGGAATGGATTTATAAATATGCGTTTTGCAAACATACCGTGAGCG  
AACAGGAAAAACCGGAAAGCCGCGAAAAAT**TAATAACATTGGAAGTGGATAA**

Biobrick

FKBP\_L106P degron

AAAGGTACCAAC**ATGGGTGTGCAGGTTGAGACGATCTCGCCCGGTGATGGT**  
**CGCACCTTCCCAAGCGTGGACAGACCTGCGTGGTCCATTACACTGGCATG**  
**CTGGAGGATGGTAAGAAGGTGGACTCCTCGCGTGACCGTAACAAGCCATTC**

AAGTTCATGCTGGGCAAGCAGGAGGTGATTGCGGGTTGGGAGGAGGGTGT  
CGCCCAGATGTCCGTGGGCCAACGCGCCAAGCTGACCATCAGCCCAGACT  
ATGCTTACGGCGCCACTGGACACCCCGGTATCATCCACCCACGCCACCT  
TGGTGTTCGACGTGGAGCTGCTGAAGCCGGAGGGCGGAGGCAGCGGAGG  
TGGAAGCTCCGGCGGACTAGTAAC

FKBP\_L106P

F-box-GFP binder

TCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCAAATGATGAAAATGGA  
GACTGACAAAATAATGGACGAAACCAACTCCAATGCACAGGCCTTCACAAC  
CACTATGCTGTACGACCCGGTGCGCAAGAAAGACTCATCGCCACCTACCA  
AACGGAGCGGGAACCTCTGCTTTCAGTACTTCACCCAGTGGAGCGAGTCGG  
GCCAGGTGGACTTTGTGGAGCACCTGCTGTGCGCATGTGCCACTATCAAC  
ACGGACAGATCAATGCCTATCTCAAGCCGATGCTCCAGCGGGACTTTATCA  
CATTGCTGCCAATCAAGGGTCTGGATCACATCGCAGAAAACATTTTGTGTA  
CTTGGATGCCGAATCGCTCAAATCATCCGAGCTGGTCTGCAAGGAATGGCT  
GCGCGTCATTTCCGAGGGCATGCTCTGGAAGAAGCTCATCGAACGCAAGGT  
GCGCACAGATTCTTGTGGCGCGGACTGGCCGAGCGGCGTAATTGGATGC  
AGTACCTCTTCAAGCCAAGACCGGGCCAGACTCAACGGCCACACTCATTCC  
ATCGCGAGTTGTTCCCAAGATAATGAATGACATTGACAGCATAGAGAACAA  
CTGGCGGACTGGCCGCCACCTCGAGATGGATCAAGTCCAACCTGGTGGAGT  
CTGGTGGCGCTTTGGTGCAGCCAGGTGGCTCTCTGCGTTTGTCTGTGCCG  
CTTCTGGCTTCCCAGTGAACCGCTATTCCATGCGCTGGTATCGCCAGGCTC  
CAGGCAAAGAGCGTGAGTGGGTAGCCGGTATGTCCAGCGCGGGTGATCGT  
AGCTCCTATGAAGACTCCGTGAAGGGCCGTTTCACCATCAGCCGTGACGAT  
GCCCGTAACACGGTGTATCTGCAAATGAACAGCTTGAAACCTGAAGATACG  
GCCGTGTATTACTGTAATGTGAACGTGGGCTTCGAGTATTGGGGCCAAGGC  
ACCCAGGTCACCGTCTCCAGCTAATCTAGAGGG

NSImb (Fbox protein), GFP binder (GBP)

**DNA sequences assembled from multiple gBlocks and PCR products**

pRITE

AGTGGCGGCCGCTCGAGCGGTGGCTCCGGCGGAAGCATAACTTCGTATAA  
TGTATGCTATACGAAGTTATCACCGCGGTTCGAAAGTAAGCCTATCCCTAAC  
CCTCTCCTCGGTCTCGATTCTACCCGTACCGGTGAGGGCAGAGGAAGTCTT  
CTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTGCCAGCGGTAGCGG  
CAGCGGCAACATGACCGAGTACAAGCCCACCGTGCGTCTGGCCACCCGTG  
ACGATGTGCCACGTGCCGTTTCGACCCCTGGCCGCCGCCTTCGCCGACTAT  
CCAGCCACCCGTACACCGTGGACCCAGATCGCCACATCGAGCGTGTAC  
TGAGCTGCAGGAGCTGTTCTGACCCGCGTTGGTCTGGACATCGGCAAGG  
TGTGGGTGGCTGACGATGGCGCTGCCGTGGCTGTGTGGACTACCCAGAA  
TCCGTGGAGGCCGGAGCCGTGTTGCGCGAGATCGGCCACGTATGGCCGA  
GCTGTGCGGCTCGCGTCTCGCTGCCAGCAGCAGATGGAGGGACTCCTGG

CCCCACACCGTCCCAAGGAGCCCGCCTGGTTCCTCGCCACCGTGGGTGTG  
TCCCAGATCACCAGGGCAAGGGCCTGGGCTCCGCCGTCGTTCTGCCGGG  
CGTCGAGGCCGCCGAGCGCGCTGGAGTCCCCGCCTTCCTGGAGACCTCG  
GCCCCCGTAACCTGCCCTTCTATGAGCGTCTGGGATTCACCGTCACCGCC  
GACGTTGAGGTTCCCGAGGGACCCCGCACCTGGTGCATGACCCGCAAGCC  
CGGTGCCTGAACGCGTCCGCTGATCAGCCTCGACTGTGCCTTCTAAGATCC  
AGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAG  
TGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACC  
ATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTT  
CAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTAC  
AAATGTGGTATGGCTGATTATGATCAATAACTTCGTATAATGTATGCTATACG  
AAGTTATCAAGATCTGCTAGCATGGCACCCGGGAGAGCAGAACTCATTAGT  
GAGGAGGACTTGGGCAGTGGCAGTGGAGGAGAACAAGCTCATCTCGGA  
GGAGGACCTGGGTTCCGGCTCCGGCGGAGAACAGAAAGTTGATCTCGGAGG  
AGGACCTGGAATTCGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTG  
GAGGAGAATCCCGGCCCTGCTAGTGGTAGCGGCAGCGGTAGCATGAACCG  
GGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCC  
AGCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAG  
TGGAAGTACCTGTACAGCTTCCCAGAAGAAGTCCATACAATTCCACTGGAA  
AACTCCAACCAGATAAAGATTCTGGGAAATCAGGGCTCCTTCTTAAGTAAA  
GGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAAGAAGCCTTTGGGAC  
CAAGGAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACTCAGATA  
CTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGT  
TCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGA  
CCCTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCCTCAGTGCAATGTAGGA  
GTCCAAGGGGTAAAAACATACAGGGGGGGGAAGACCCTCTCCGTGTCTCAG  
CTGGAGCTCCAGGATAGTGGCACCTGGACATGCACTGTCTTGCAGAACCAG  
AAGAAGGTGGAGTTCAAAATAGACATCGTGGTGCTAGCTTTCCAGAAGGCC  
TCCAGCATAGTCTATAAGAAAGAGGGGGGAACAGGTGGAGTTCTCCTTCCCA  
CTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGGCA  
GGCGGAGAGGGCTTCCTCCTCCAAGTCTTGATCACCTTTGACCTGAAGAA  
CAAGGAAGTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGG  
CAAGAAGCTCCCGCTCCACCTCACCTGCCCCAGGCCTTGCCTCAGTATGC  
TGGCTCTGGAAACCTCACCTGGCCCTTGAAGCGAAAACAGGAAAGTTGCA  
TCAGGAAGTGAACCTGGTGGTGTGATGAGAGCCACTCAGCTCCAGAAAAATT  
GACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTGAGCTTGAA  
ACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGG  
GTGCTGAACCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGAAGTGGG  
ACAGGTCCTGCTGGAATCCAACATCAAGGTTCTGCCACATGGTCGACCCC  
GGTGCAGCCAATGGCCCTGATTGTGCTGGGGGGCGTCGCCGGCCTCCTGC  
TTTTATTGGGCTAGGCATCTTCTTCTGTGTGAGGTGCCGCCACACCGGCTA  
GGGATCCGTTGTTTAAACCCG

loxP sites, V5, T2A, puromycin resistance, 3xMYC, CD4

1. Heun P, Erhardt S, Blower MD, Weiss S, Skora AD, Karpen GH. Mislocalization of the *Drosophila* Centromere-Specific Histone CID Promotes Formation of Functional Ectopic Kinetochores. *Dev Cell* **10**, 303-315 (2006).
2. Boltengagen M, *et al.* A novel role for the histone acetyltransferase Hat1 in the CENP-A/CID assembly pathway in *Drosophila melanogaster*. *Nucleic Acids Res* **44**, 2145-2159 (2016).
3. Foltz DR, Jansen LE, Black BE, Bailey AO, Yates JR, 3rd, Cleveland DW. The human CENP-A centromeric nucleosome-associated complex. *Nat Cell Biol* **8**, 458-469 (2006).