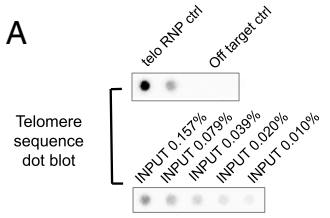
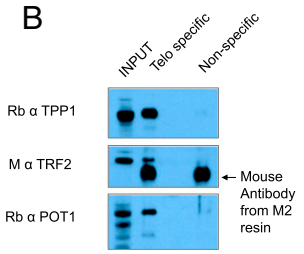
Supporting Information

Tsui et al. 10.1073/pnas.1718844115

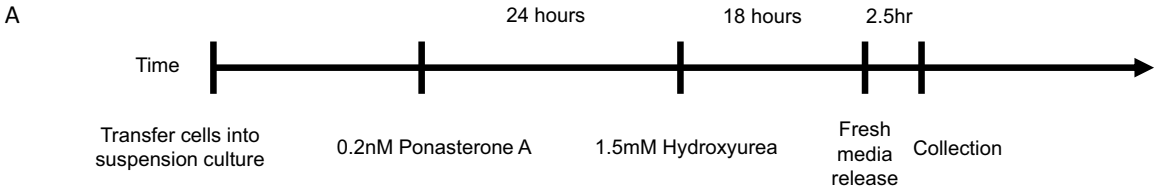




C

Protein Name	Telomere/ Non-specific dNSAF ratio
Tankyrase 1 Binding Protein	2.40
TRF2	>999
RAP1	>999
PURβ	>999
TELO2	>999
TPP1	>999
PRKDC	0.93
RIF1	>999

Fig. S1. (*A*) dCas9-3×FLAG is bound to either telomere-specific or nontargeting sgRNA and incubated with formaldehyde-crosslinked and sheared HeLa chromatin. dCas9 and associated chromatin is eluted off beads with 3×FLAG peptide. Specific and nontargeting DNA enrichment is assessed with telomere DNA-specific and Alu DNA-specific radioactive probes, respectively. dCas9-3×FLAG RNP loaded with telomere guide RNA specifically pulls down telomere-sequence DNA, while off-target dCas9-3×FLAG RNP does not. Both pulldowns have similar amounts of nonspecific Alu DNA contamination. (*B*) Western blot analysis of other shelterin subunits using protein samples from telomere-specific and nontargeting dCas9-3×FLAG pulldown. Telomere-specific dCas9-3×FLAG pulldown can specifically enrich for shelterin components such as TPP1, TRF2, and POT1, while nontargeting guide RNA-loaded dCas9-3×FLAG has no discernible enrichment. (*C*) Table of proteins associated with telomeres identified via MudPIT mass spectrometry. TPP1, RAP1, and RIF1 were identified when samples were digested with AspN and GluC enzymes; the rest were found with a trypsin and LysC combination.



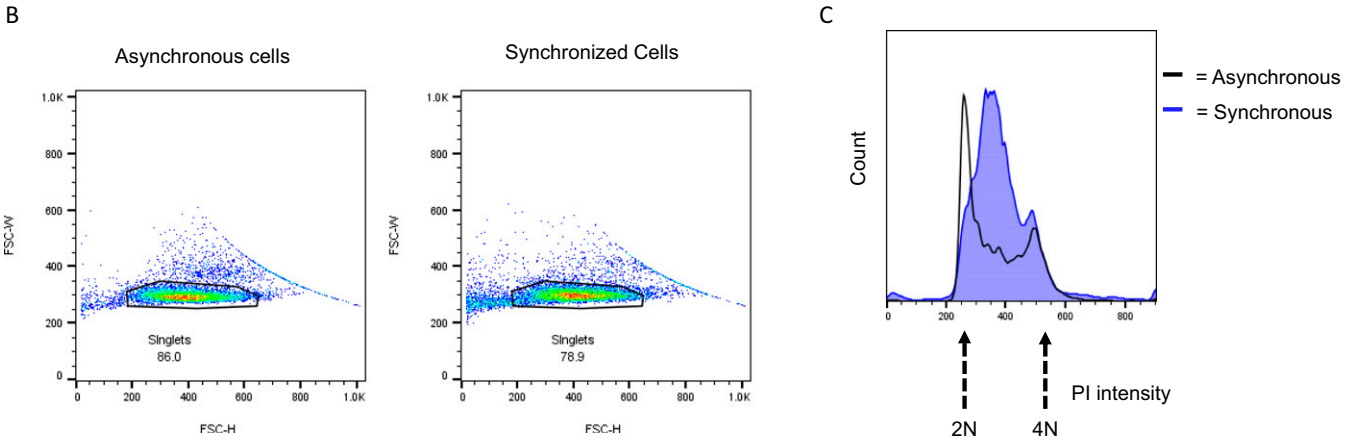


Fig. S2. S2 cells can be properly synchronized in suspension cultures without greatly affecting cell viability. (*A*) Synchronization scheme for S2 cells in suspension culture. (*B*) Comparison of the forward scatter width (FSC-W) and forward scatter height (FSC-H) of sorted S2 cells to distinguish the proportion of viable singlet cells. (*C*) Synchronized and asynchronous S2 cells are stained with PI, and the intensity is measured by the flow cytometer.

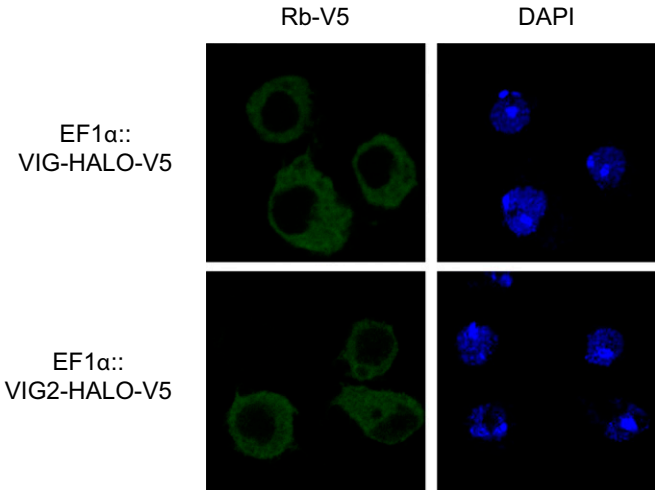


Fig. S3. Localization of Vig and Vig2 expression in S2 cells. Vig and Vig2 cDNA is overexpressed as a HALO-V5–fused protein driven by an EF1α promoter in S2 cells. Cells are then fixed, and the overexpressed protein is visualized by Rabbit anti-V5 (Rb-V5) antibody.

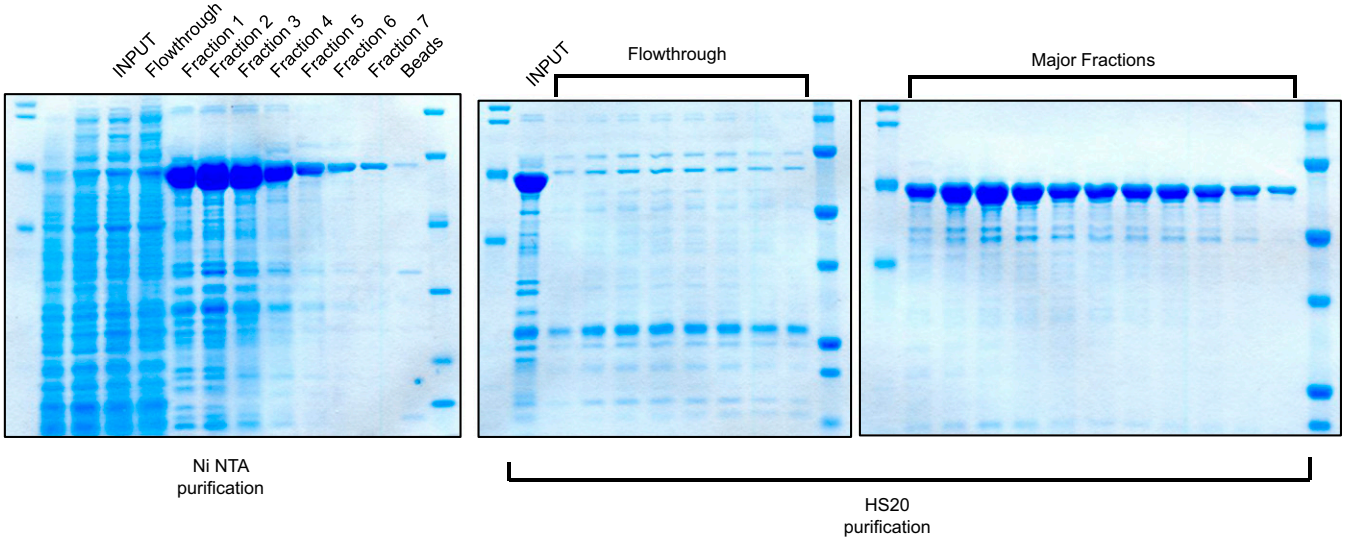


Fig. S4. Purification of 6×His-VIG recombinant protein. 6×His-VIG was driven in a pET302 vector and transformed into BL21-Codon Plus RIPL-competent cells (Agilent). Cultures are induced at 18 °C overnight at 0.3 mM IPTG, lysed, and purified with Ni-NTA., Select fractions are further purified with a POROS HS20 column. The 6×His-VIG–enriched fractions are collected, dialyzed, and flash frozen.

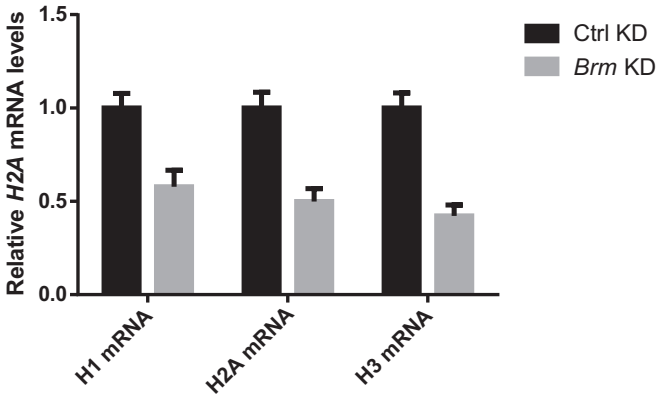


Fig. S5. Knockdown (KD) of Brahma (brm) leads to decreased mRNA expression for *H1*, *H2A*, and *H3*. S2 cells are treated with dsRNA against *Brm* for 3 d, and the level of H2A mRNA is assessed by qPCR. Total RNA is isolated by TRIzol extraction, and cDNA is synthesized with the iScript reverse transcriptase kit. The knockdown of brm leads to a decrease in mRNA levels for both linker histone genes and core histone genes, suggesting that brm regulates the entire HisC.

Table S1. RT-qPCR primer sequences

Targets	Forward (5′ → 3′)	Reverse (5′ → 3′)
dmH1	TCCCCAGTGGCTGCCCCACC	GTGACGGCGTCGCAGAGGCT
dmH2A	AGCGTGTTGGTGCAGGCGCT	TGGCCAGTTGCAGATGACGCGG
dmH3	TGGTGGAAAGGCGCCACGCA	AGGCCACGGTTCCAGGGCGA
dmH4	CAGAGCGTACACAACATCCA	GTGTGAAGCGCATATCTGGA
dmTUB84b	CGTGAATGTATCTCTATCCATGT	TTGCCAGCTCCAGTCTCGCT
dmACTIN	GCGTCGGTCAATTCAATCTT	AAGCTGCAACCTCTTCGTCA
dmRPL32	CCAGTCGGATCGATATGCTAA	GTTCGATCCGTAACCGATGT

Table S2. qPCR primer sequences

Targets	Forward (5′ → 3′)	Reverse (5′ → 3′)
Telomere TARGET PLASMID CONTROL	GGGAATTGTGAGCGGATAAC	AGCGAATTCACGTGATGATG
H2A gene 3′ end	AATTATTCCGCGTCATCTGC	GGCCTTCTTCTCGGTCTTCT
H2B gene 5′ end	GTGTCAGGATGGACCTGCTT	CTAGTGGAAAGGCAGCCAAG
H2A/H2B promoter	CCTTCACTTTGCCACCTTTT	GTCACCCACCCCTAACTGAA
H1 promoter	CACTTCAAGCAAACTTCGACA	CTGCCTACCAACCTCCTTTG
H2A gene 5′ end	ACTACGCAGAGCGTGTTGGT	CTTGTTGTCACGAGCAGCAT

Table S3. sgRNA DNA targets

Target	Sequence (5′ → 3′)
dm sgRNA #1	AGAACCTTGTAAATGTAGA
dm sgRNA #2	TTCTTTTTCTTGTCGGTCT
dm sgRNA #3	GAAACTACGCAGAGCGTGT
dm sgRNA #4	TTACGGCAGCTAGGTAAAC
dm sgRNA #5	CTGCCTTTCCACTAGTTTT
dm sgRNA #6	GGTGGCAAAGTGAAGGGAA
dm sgRNA #7	GCCGGTCTTCAATTCCCTG
dm sgRNA #8	CTGAGGTTCTCGAGTTGGC
Nontargeting sgRNA	ACATGTTGATTTCCTGAAA

Table S4. *Drosophila* RNAi primers

Target	Forward (5′ → 3′)	Reverse (5′ → 3′)
BIN1	TTAATACGACTCACTATAGGGAGA ATGGCCAACGTGGAATCTAT	TTAATACGACTCACTATAGGGAGA GTACGGACGCTGGCGCC
BUBR1	TTAATACGACTCACTATAGGGAGA GGCCTGGAATAAGGCAAATG	TTAATACGACTCACTATAGGGAGA CCAACAACTCCTCTGGCT
CP190	TTAATACGACTCACTATAGGGAGA AAGCCTGCTATCGCC	TTAATACGACTCACTATAGGGAGA CGCCTTCTGTTGTGCT
CG3262	TTAATACGACTCACTATAGGGAGA GGAAAGGGTGGTGTTGGAA	TTAATACGACTCACTATAGGGAGA TTGCGCGGTAGAGAACTT
CG8771	TTAATACGACTCACTATAGGGAGA CAACCGGGTGTTACCGAG	TTAATACGACTCACTATAGGGAGA CACGAACTTTGGCTGTTTC
VIG2	TTAATACGACTCACTATAGGGAGA CAATCGTGACAACAGGGGA	TTAATACGACTCACTATAGGGAGA TCCGTCATTGCGGAAGCC
CG12608	TTAATACGACTCACTATAGGGAGA CGCGGAAACAGTCGCAG	TTAATACGACTCACTATAGGGAGA AGGCGATGGCCTTGAC
SYNCRIP Isoform C	TTAATACGACTCACTATAGGGAGA GGTCAGCGTAAATACGGC	TTAATACGACTCACTATAGGGAGA TTGTTTGCTCATCCGGCTC
DBP21E2	TTAATACGACTCACTATAGGGAGA GGTGAGGAACTCCAGCAGG	TTAATACGACTCACTATAGGGAGA CAGGATCATCTGGGTGCC
BAP60	TTAATACGACTCACTATAGGGAGA CATCGCTACTGCAGCGC	TTAATACGACTCACTATAGGGAGA GCTTGAACTGCAGCGGC
ROX8	TTAATACGACTCACTATAGGGAGA CCAGTCCCGGCAATCAG	TTAATACGACTCACTATAGGGAGA ACCTCGCTGTTGTGCG
CDC5	TTAATACGACTCACTATAGGGAGA CTCGCAAGTTGAAGCCCG	TTAATACGACTCACTATAGGGAGA GCTAGCAAAGCATCCGTCG
IRBP	TTAATACGACTCACTATAGGGAGA TGTCACGGACGTCAGGGA	TTAATACGACTCACTATAGGGAGA ACAAGCGCTTCGATCCG
NAP1	TTAATACGACTCACTATAGGGAGA AGGACGTCTACAAGCTGGA	TTAATACGACTCACTATAGGGAGA TCTGCTGGGAGTCGTCG
BRM	TTAATACGACTCACTATAGGGAGA GGGACAGCCATTGCCA	TTAATACGACTCACTATAGGGAGA TCGTTCAGCCTCTAGCTTC
CKIIalpha	TTAATACGACTCACTATAGGGAGA ACGACCACGGAAAAGTGC	TTAATACGACTCACTATAGGGAGA ATCGCTTTCGTGAGTGACG
ROW	TTAATACGACTCACTATAGGGAGA AACGGCGACTCCTTCG	TTAATACGACTCACTATAGGGAGA GGCCGCTTGTAGGTGG
VIG	TTAATACGACTCACTATAGGGAGA AGGAAGCGCGAGTTCG	TTAATACGACTCACTATAGGGAGA TGGGCCACGGTTTCCAC
CG31357	TAATACGACTCACTATAG G TGCGCATGGGATACTATGAA	TAATACGACTCACTATAG G TGCGAGTATGGCAACTTGAG
CG3295	TAATACGACTCACTATAG G AAGTCCTTGGCATGTGGAAC	TAATACGACTCACTATAG G ACCAACACCAGTCCTCGAAC
EBI	TAATACGACTCACTATAG G GTTCTCTTCAGATCAGCGGG	TAATACGACTCACTATAG G GTTCTTGTGTTTCCGCCAAT
CG7611	TAATACGACTCACTATAG G GCAAGTGCTATCAACTGCCA	TAATACGACTCACTATAG G AATTGAGTGTGGAACCCTCG
RanBPM	TAATACGACTCACTATAG G CCTTTGATTTGGCGTGATTT	TAATACGACTCACTATAG G TTCATGTTTGCCGAATGTGT
Smu1	TAATACGACTCACTATAG G GGTTTCCTTGAGGTGTGGAA	TAATACGACTCACTATAG G CCAAACCTTTACTGTGCCGT
Mahj	TAATACGACTCACTATAG G TTGTGGTCTTGCTATCGCTG	TAATACGACTCACTATAG G TACATTGGCCTTGTGCATGT
Mxc	TAATACGACTCACTATAG G CCGCAAACACCGCTTATTAT	TAATACGACTCACTATAG G TACCCACTCGTTGTTCACCA
Control	TAATACGACTCACTATAG GTTAAAATTCGCGTTAAATTT	TAATACGACTCACTATAG GTGTGGTGGTTACGCGCAGCG

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)