Supplemental Table 1A Primer sequences

Primer name	**Sequence**	**Notes**
pTDH3_SacI_F	TACGACTCACTATAGGGCGAATTGGAGCTCTCAGTTCGAGTTTATCATTATCAATACTGC	Forward and reverse primers to amplify the TDH3 promoter from the yeast genome for Gibson Assembly into pRS306
pTDH3_R	GATGCATGAATTCTTTGTTTGTTTATGTGTGTTTATTCGAAACT	
Rvar_Dsup_F	TTTCGAATAAACACACATAAACAAACAAAGAATTCATGCATCATCATCATCATCACATGG	Forward and reverse primers to amplify the Dsup gene from pET21b-nHis6-Rvar-DSUP-CFLAG for Gibson Assembly into pRS306
Rvar_Dsup_R	GGCGCGCCTTATTTGTCATCGTCGTCTTTGTAGTC	
tADH1_F	GACGACGATGACAAATAAGGCGCGCCACTTCTAAATAAGCGAATTTCTTATGATTTATGA	Forward and reverse primers to amplify the ADH1 terminator from the yeast genome for Gibson Assembly into pRS306
tADH1_BglII_R	CCCCTCGAGCGGGGACGAGGCAAGCTAAACAGATCTATATTACCCTGTTATCCCTAGCGG	
roGFP_BamHI_F	ACTAGTGGATCCACCATGGCTAGCGAGTT	Forward and reverse primers to amplify the roGFP2-Grx1 sequence from p415TEF cyto roGFP2-Grx1
Grx_2xNLS_HindIII_R	ATCGATAAGCTTTTAAACCTTTCTCTTCTTCTTTGGAACCTTTCTCTTCTTCTTTGGATTTGCAAGAATAGGTTC	
sod1_F	CGTACGCTGCAGGTCGACAAATGTTTAGCTGTAACTATGTTGCGGAAAAACAGGCAAG	Forward and reverse primers to amplify the KANMX sequence from pFA6a_KANMX6 to delete *SOD1*
sod1_R	ATCGATGAATTCGAGCTCGGAGGGAAATGAAAATGAATGAATTGATGCGCTTACTACTT	
Dsup_M1M2_gRNA_F	CTTTATACGTCCGCTGAGCCGCCT	Complementary oligos to ligate a guide RNA sequence to target the Dsup HMGN-like region into pCASB
Dsup_M1M2_gRNA_R	AAACAGGCGGCTCAGCGGACGTAT	
Dsup_EV_gRNA_F	CTTTTTCTGTGGATGATTGGTGTG	Complementary oligos to ligate a guide RNA to target the N-terminus of Dsup into pCASB
Dsup_EV_gRNA_F	CTTTTTCTGTGGATGATTGGTGTG	Complementary oligos to ligate a guide RNA to target the N-terminus of Dsup into pCASB
Dsup_EV_gRNA_R	AAACCACACCAATCATCCACAGAA	
Dsup_M1NLS_gRNA_F	CTTTGCGGGGCGCTGGTAAGAAAG	Complementary oligos to ligate a guide RNA to target the C-terminus of Dsup ΔC into pCASB
Dsup_M1NLS_gRNA_F	AAACCTTTCTTACCAGCGCCCCGC	
Dsup_M1_HDR_F	ATCAGGCAGTGAGGGTGGAGCTAAGAAAGGCAGAGGGCGGGGCGCTGGTAAGAAAGCGGA	Forward, middle, and reverse primers used to amplify an HDR template for generation of Dsup ΔC
Dsup_M1_HDR_M	AGAAAGCGGATGCGGGTGATACGTCCGCTGACTACAAAGACGACGATGACAAATAA	
Dsup_M1_HDR_R	AATAAAAATCATAAATCATAAGAAATTCGCTTATTTAGAAGTGGCGCGCCTTATTTGTCA	
Dsup_M2_HDR_F	CAGGCAGTGAGGGTGGAGCTAAGAAAGGCAGAGGGCGGGGCGCTGGTAAGAAAGCGGATG	Forward, middle, and reverse primers used to amplify an HDR template for generation of Dsup 3R/3E
Dsup_M2_HDR_M	AAAGCGGATGCGGGTGATACGTCCGCTGAGCCGCCTGAAGAATCGTCCGAACTGACGTCTTCAGGTACAGGGGCGGGTTCCGCTCCAGCT	
Dsup_M2_HDR_R	GAAGGTGTACTGGAGGAAGAAGCAGCACGCTTCGCTCCGCCTTTCGCTGCAGCTGGAGCG	
Dsup_EV_HDR_F	ATAGTTAGTCTTTTTTTTAGTTTTAAAACACCAAGAACTTAGTTTCGAATAAACACACAT	Forward, middle, and reverse primers used to amplify an HDR template for generation of Empty Vector cells
Dsup_EV_HDR_M	AAACACACATAAACAAACAAAGAATTCATGTAACATCATCATCATCACATGGCATCTACA	
Dsup_EV_HDR_R	TTCGTTTCCTCAGATTTACCTGTGGAAGAGGGTTCTGTGGATGATTGGTGTGTAGATGCC	
Dsup_M1NLS_HDR_F	GAAGGTGTACTGGAGGAAGAAGCAGCACGCTTCGCTCCGCCTTTCGCTGCAGCTGGAGCG	Forward, middle, and reverse primers used to amplify an HDR template for generation of Dsup ΔC-NLS
Dsup_M1NLS_HDR_M	AAGAAGAAGAGAAAGGTTCCAAAGAAGAAGAGAAAGGTTGACTACAAAGACGACGATGAC	
Dsup_M1NLS_HDR_R	AATCATAAATCATAAGAAATTCGCTTATTTAGAAGTGGCGCGCCTTATTTGTCATCGTCG	
Dsup_M1_HMGN_HDR_F	TGGAGCTAAGAAAGGCAGAGGGCGTGGCGCTGGTAAGAAAGCAGATGCGGGTGATACGTC	Forward, middle, and reverse primers used to amplify an HDR template for generation of Dsup HMGN ΔC+NLS from Dsup ΔC+NLS
Dsup_M1_HMGN_HDR_M	GTGATACGTCCGCTGAGCCGCCTCGGCGGTCGTCCCGCCTGACGTCTCCAAAGAAGAAGA	
Dsup_M1_HMGN_HDR_R	GTCATCGTCGTCTTTGTAGTCAACCTTTCTCTTCTTCTTTGGAACCTTTCTCTTCTTCTT	

Supplemental Table 1B Yeast Strains + Phenotypes

Strain name / description	**Genotype**	**Reference**
BY4741	*Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachmann et al (1998) Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115-132, doi:10.1002/(SICI)1097-0061(19980130)14:2<115::AID-YEA204>3.0.CO;2-2.
yap1Δ (from yeast deletion collection)	*Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 yap1::KANMX*	Giaever *et al.* (2002) Functional profiling of the Saccharomyces cerevisiae genome. *Nature* **418**, 387-391, doi:10.1038/nature00935.
RGY002, Dsup	*Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* P_{TDH3} *::P* $_{TDH3}$ *D* *FLAG URA3*	This study
RGY008, Dsup *sod1Δ*	RGY002 *sod1::KANMX*	This study
RAY149, Empty Vector	RGY002 *DsupΔ*	This study
RAY136, Dsup ΔC	RGY002 *DsupΔ360-446*	This study
RAY153, Dsup 3R/3E	RGY002 *Dsup R363E/R364E/R367E*	This study
RAY228, Dsup ΔC+NLS	RGY002 *DsupΔ360-446-NLS* (PKKKRKVPKKKRKV)	This study
RAY170, Dsup + [roGFP]	RGY002 (*p415TEF cyto roGFP2-Grx1)*	This study
RAY168, Empty Vector + [roGFP]	RAY149 (*p415TEF cyto roGFP2-Grx1*)	This study
RAY174, Dsup 3R/3E + [roGFP**]**	RAY153 *(p415TEF cyto roGFP2-Grx1)*	This study
RAY236, Dsup ΔC+NLS + [roGFP**]**	RAY228 *(p415TEF cyto roGFP2-Grx1)*	This study
RAY157, Dsup + [roGFP_NLS]	RGY002 (*p415TEF roGFP2-Grx1-NLS*)	This study
RAY158 Empty Vector + [roGFP_NLS]	RAY149 (*p415TEF roGFP2-Grx1-NLS*)	This study
RAY163 Dsup 3R/3E + [roGFP_NLS]	RAY153 (*p415TEF roGFP2-Grx1-NLS*)	This study
RAY237, Dsup ΔC+NLS + [roGFP_NLS]	RAY228 (*p415TEF roGFP2-Grx1-NLS*)	This study
RAY189, rtt106Δcac1Δ	*Mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hmr::P* $_{URA3}$ *GFP/URA3* *TRP::BrdU-Inc(TRP) bar1::LEU2 rtt106::HIS3 cac1::KANMX*	Rosas, Aguilar, et al (2023) A novel single alpha-helix DNA-binding domain in CAF-1 promotes gene silencing and DNA damage survival through tetrasome-length DNA selectivity and spacer function. Elife 12, e83538, doi:10.7554/eLife.83538.
YZS276, H2B-FLAG	*Mat* a, *ura3-1, trp1-1, leu2-3,-112, his3-11, ade2-1, can1-100* *hta1-htb1Δ::LEU2 hta2-htb2Δ (pZS145 HTA1-Flag-HTB1,CEN HIS3)*	Sun & Allis (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature 418, 104-108.
RAY274, Dsup HMGN ΔC +NLS	RGY002 *Dsup Δ374-460-NLS* (PKKKRKVPKKKRKV)	This study

Supplemental Table 1C C&R Abs

CUT&RUN Format	Target	Antibody
Native	IgG	*EpiCypher* #13-0042 (Lot 20036001-52)
Native	H3K4me3	*EpiCypher # 13-0041* (Lot SG2419844A)
Native	FLAG	*ThermoFisher* #MA1-91878 (Lot 19091001)

Supplemental Table 1D dCypher Targets

EpiCypher Catalog #	dNuc Identifier
16-0006	147x601 H3.1 (rNucleosome)
16-2044	199x601 H3.1
16-0013	H2AX
16-0366	H2AXS139ph
16-0014	H2AZ.1
16-0015	H2AZ.2
16-0011	H3.3 WT
16-0349	H3.3K4M
16-0350	H3.3K9M
16-1323	H3.3K27M
16-0346	H3.3G34R
16-0347	H3.3G34V
16-0348	H3.3G34W
16-0344	H3.3K36M
16-0029	H2AE61A
16-0030	H2AE92K
16-0031	H2BE105A,E113A
16-0027	Tailless
16-0023	H3.1NΔ2
16-0016	H3.1NΔ32
16-0017	H3.3NΔ32
16-0018	H4NΔ15
16-0321	H3K4me1
16-0334	H3K4me2
16-0316	H3K4me3
16-0325	H3K9me1
16-0324	H3K9me2
16-0315	H3K9me3
16-0338	H3K27me1
16-0339	H3K27me2
16-0317	H3K27me3
16-0322	H3K36me1
16-0319	H3K36me2
16-0320	H3K36me3
16-0390	H3.3K36me3
16-0367	H3K79me1
16-0368	H3K79me2
16-0369	H3K79me3
16-0393	H4K12me1
16-0331	H4K20me1
16-0332	H4K20me2
16-0333	H4K20me3
16-0342	H3K4ac
16-0314	H3K9ac
16-0371	H3K9bu
16-0351	H3K9cr
16-0343	H3K14ac
16-0372	H3K18ac
16-0373	H3K18bu
16-0337	H3K18cr
16-0336	H3K4,9,14,18ac (H3 Tetra Ac)
16-0335	H3K4me3,K9,14,18ac
16-0364	H3K23Ac
16-0365	H3K27ac
16-0384	H3K27bu
16-0383	H3K27cr
16-0385	H3K27ac, S28ph
16-0397	H3K27me3, S28ph
16-0345	H3S10ph
16-0388	H3S28ph
16-0389	H3.3S31ph
16-0378	H3K36ac
16-0352	H4K5ac
16-0353	H4K8ac
16-0312	H4K12ac
16-0354	H4K16ac
16-0377	H4K20ac
16-0313	H4K5,8,12,16ac (H4 Tetra Ac)
16-0374	H3K4,9,14,18ac/H4K5,8,12,16ac (H3/H4 Tetra Ac)
16-0376	H2AK5,8,13,15ac (H2A Tetra Ac)
16-0386	H2BK12ac
16-0387	H2BK20ac
16-0359	H2AR3me1
16-0360	H2AR3me2a
16-0361	H2AR3me2s
16-0359	H3R2me1
16-0360	H3R2me2a
16-0361	H3R2me2s
16-0379	H3R8me1
16-0380	H3R8me2a
16-0381	H3R8me2s
16-0382	H3R17me1
16-0375	H3R17me2a
16-0356	H4R3me1
16-0357	H4R3me2a
16-0358	H4R3me2s
16-0362	H3R2,8,17cit
16-0398	H3K14ub
16-0395	H2AK119ub
16-0400	H2AK129ub
16-0396	H2BK120ub
16-0399	H2AK15ub

EpiCypher Catalog #	DNA Identifier
18-0005	Biotinylated-DNA (Widom 601, 147bp)
18-2044	Biotinylated-DNA (Widom 601, 199bp)

Supplemental Table 1E dCypher Queries (+ Misc)

Construct Number	Name	Description	Uniprot accession number (Region covered)
	Dsup protein info (HMGN underlined, red deleted in CΔ allele) For recombinant purified Dsup protein QC see **Suppl. Fig. 4**	Tardigrade Dsup (aa1-445) [Ramazzottius varieornatus (taxid:947166)] MASTHQSSTE PSSTGKSEET KKDASQGSGQ DSKNVTVTKG TGSSATSAAI VKTGGSQGKD SSTTAGSSST QGQKFSTTPT DPKTFSSDQK EKSKSPAKEV PSGGDSKSQG DTKSQSDAKS SGQSQGQSKD SGKSSSDSSK SHSVIGAVKD VVAGAKDVAG KAVEDAPSIM HTAVDAVKNA ATTVKDVASS AASTVAEKVV DAYHSVVGDK TDDKKEGEHS GDKKDDSKAG SGSGQGGDNK KSEGETSGQA ESSSGNEGAA PAKGRGRGRP PAAAKGVAKG AAKGAAASKG AKSGAESSKG GEQSSGDIEM ADASSKGGSD QRDSAATVGE GGASGSEGGA KKGRGRGAGK KADAGDTSAE PP**RR**SS**R**LTS SGTGAGSAPA AAKGGAKRAA SSSSTPSNAK KQATGGAGKA AATKATAAKS AASKAPQNGA GAKKKGGKAG GRKRK	P0DOW4
pCM048	H2A.1	human H2A (MSGRGKQGGK ARAKAKTRSS RAGLQFPVGR VHRLLRKGNY AERVGAGAPV YLAAVLEYLT AEILELAGNA ARDNKKTRII PRHLQLAIRN DEELNKLLGK VTIAQGGVLP NIQAVLLPKK TESHHKAKGK)	P0C0S8
pCM122	H2B.1C	human H2B (MPEPAKSAPA PKKGSKKAVT KAQKKDGKKR KRSRKESYSV YVYKVLKQVH PDTGISSKAM GIMNSFVNDI FERIAGEASR LAHYNKRSTI TSREIQTAVR LLLPGELAKH AVSEGTKAVT KYTSSK)	P62807
pCM112	H3.2	human H3 (MARTKQTARK STGGKAPRKQ LATKAARKSA PATGGVKKPH RYRPGTVALR EIRRYQKSTE LLIRKLPFQR LVREIAQDFK TDLRFQSSAV MALQEACEAY LVGLFEDTNL CAIHAKRVTI MPKDIQLARR IRGERA)	Q71DI3
pCM148	H4	human H4 (MSGRGKGGKG LGKGGAKRHR KVLRDNIQGI TKPAIRRLAR RGGVKRISGL IYEETRGVLK VFLENVIRDA VTYTEHAKRK TVTAMDVVYA LKRQGRTLYG FGG)	P62805
pCM001	601 DNA	147 bp Widom 601 nucleosome-positioning DNA sequence (ATCGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCGAT)	-

Blast Human histones to [*Ramazzottius varieornatus* (taxid:947166)]
Human H2A vs. hypothetical protein RvY_15450 [R. varieornatus] **(91% : aa 24-121)**
Human H2B vs. hypothetical protein RvY_05959 [R. varieornatus] **(>90% : aa 36-125)**
Human H3.1 vs. hypothetical protein RvY_05961 [R. varieornatus] **(100% : aa 1-136)**
Human H4 vs. hypothetical protein RvY_05962 [R. varieornatus] **(100% : aa 22-102)**