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**Supplementary information**

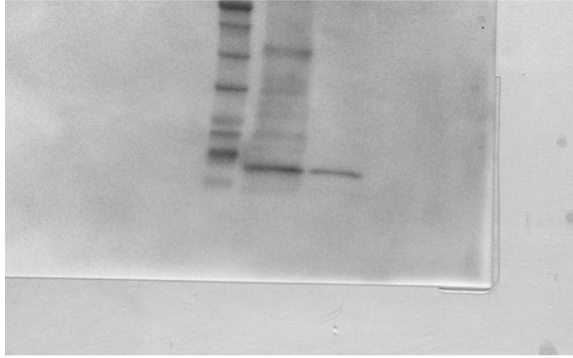
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**Histone H2B.8 compacts flowering plant sperm through chromatin phase separation**

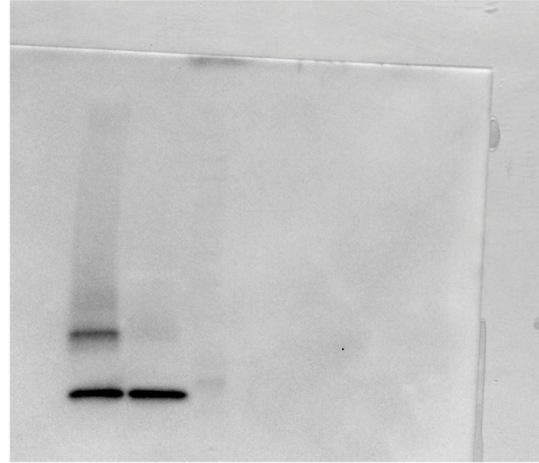
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In the format provided by the  
authors and unedited

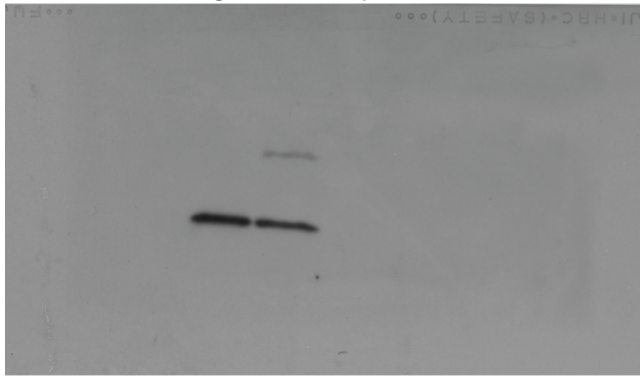
Extended Data Figure 1a - Rep1 H3K9me2



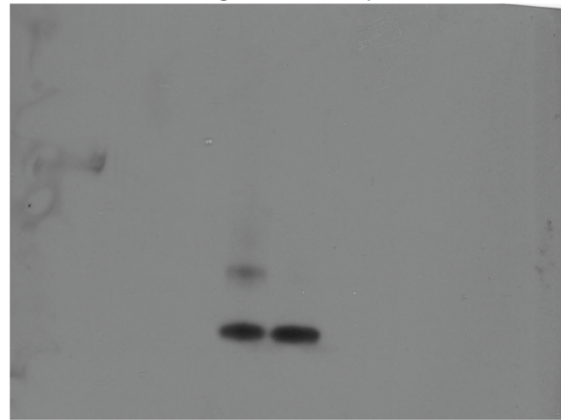
Extended Data Figure 1a - Rep1 H3



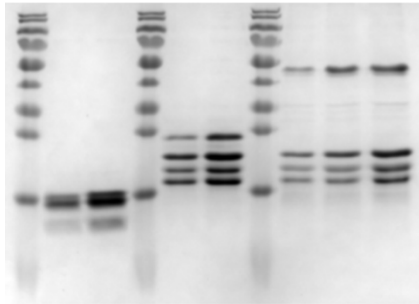
Extended Data Figure 1a - Rep2 H3K9me2



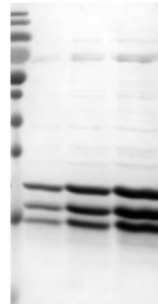
Extended Data Figure 1a - Rep2 H3



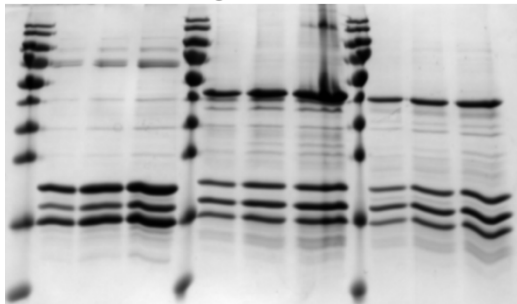
Extended Data Figure 3c - H2B.2 and H2B.8



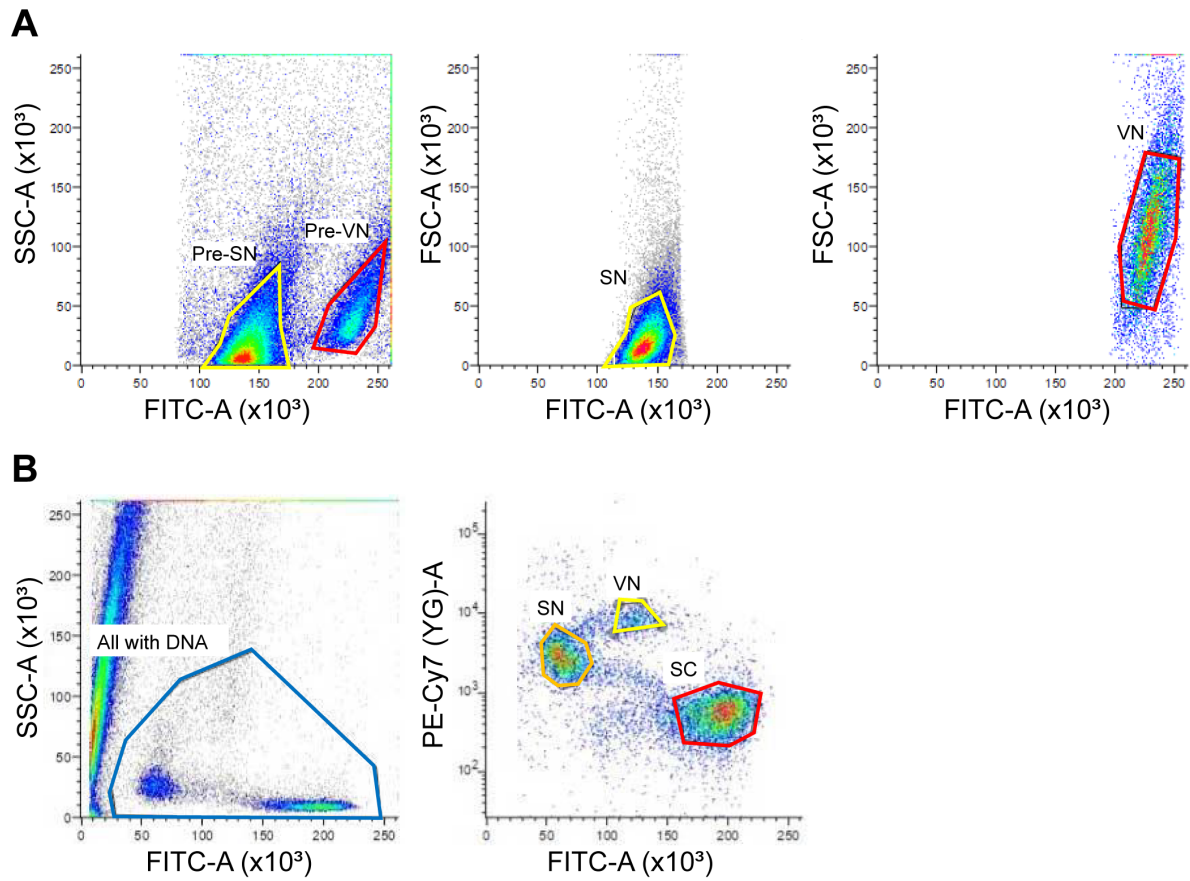
Extended Data Figure 3c - H2B.8ΔIDR



Extended Data Figure 3c - H2B.8-scrambledIDR



**Supplementary Figure 1.** Source data for blots or gels in Extended Data Figure 1a and Extended Data Figure 3c.



**Supplementary Figure 2.** Example FACS scatterplots showing (A) the gating strategy to isolate sperm (SN) and vegetative (VN) nuclei by SYBR Green intensity (FITC-A) and side scatter area (SSC-A) and then by forward scatter area (FSC-A) and (B) the gating used to purify sperm cells (SC) from pollen by first gating for all events with DNA by SYBR Green intensity (FITC-A) and side-scatter (SSC-A) and then against SYTOX Orange staining (PE-Cy7 (YG)-A).

**Supplementary Table 1.** List of H2B.8 homologs identified in flowering plant species.

File attached.

**Supplementary Table 2.** Multivariate linear regression modelling of H2B.8. The table lists the regression weight for each variable in the multivariate linear regression models (MLR1 and MLR2) of H2B.8. The formula and adjusted R<sup>2</sup> for both models are shown (P-value < 0.001, F-statistic, for all variables in both models). Additionally, for each variable, a separate model was made to predict H2B.8 and the adjusted R<sup>2</sup> value is listed.

<b>MLR1</b>	<b>H3K4me3</b>	<b>Log<sub>10</sub> (RPKM+1)</b>	<b>GC</b>	<b>H3K9me2</b>	<b>H1.1</b>	<b>H1.2</b>	<b>H2A.W</b>
Regression weight	-0.276	-0.334	-1.654	0.017	-0.176	-0.129	0.017
Adjusted R <sup>2</sup>	0.360	0.342	0.205	0.021	0.110	0.124	0.013
<b>MLR1</b>	<b>H2A.Z</b>	<b>H3.1</b>	<b>H3.3</b>	<b>H3K27ac</b>	<b>H3K27me1</b>	<b>H3K27me3</b>	<b>mCG</b>
Regression weight	0.204	-0.154	0.240	0.079	0.122	0.088	0.231
Adjusted R <sup>2</sup>	0.001	0.016	0.012	0.077	0.033	0.022	0.002
MLR1: H2B.8 = 1 - 0.276×H3K4me3 - 0.334×Log10(RPKM+1) - 1.654×GC + 0.017×H3K9me2 - 0.176×H1.1 - 0.129×H1.2 + 0.017×H2A.W + 0.204×H2A.Z - 0.154×H3.1 + 0.240×H3.3 + 0.079×H3K27ac + 0.122×H3K27me1 + 0.088×H3K27me3 + 0.231×mCG (Adjusted R <sup>2</sup> = 0.567)							
<b>MLR2</b>	<b>gene TPM&gt;5</b>	<b>gene TPM 1-5</b>	<b>gene TPM 0-1</b>	<b>euTE</b>	<b>hetTE</b>	<b>intergenic</b>	
Regression weight	-0.933	-0.791	-0.097	0.613	-0.190	0.666	
Adjusted R <sup>2</sup>	0.183	0.091	0.0001	0.061	0.0008	0.209	
MLR2: H2B.8 = - 0.074 - 0.933×gene (TPM>5) - 0.791×gene (TPM 1-5) - 0.097×gene (TPM 0-1) + 0.613×euTE - 0.190×hetTE + 0.666×intergenic (Adjusted R <sup>2</sup> = 0.422)							

**Supplementary Table 3.** *h2b.8* transmission data. *h2b.8*<sup>+/-</sup>, heterozygous *h2b.8* mutant; *P* value, Fisher's exact test. Results shown in the first two rows were obtained from one reciprocal crossing experiment performed by one researcher, and those from the third row were obtained from a separate experiment performed by an independent researcher.

<b>Maternal parent</b>	<b>Paternal parent</b>	<b>Total F1 progeny</b>	<b>WT F1 progeny</b>	<b><i>h2b.8</i><sup>+/-</sup> F1 progeny</b>	<b>Ratio (WT: <i>h2b.8</i><sup>+/-</sup>)</b>	<b><i>h2b.8</i> transmission (%)</b>	<b><i>P</i> value</b>
Wild type	<i>h2b.8</i> <sup>+/-</sup>	575	333	242	1:0.73	42.09	0.0078
<i>h2b.8</i> <sup>+/-</sup>	Wild type	574	278	296	1:1.06	51.57	0.6367
Wild type	<i>h2b.8</i> <sup>+/-</sup>	743	436	307	1:0.70	41.32	0.0009

**Supplementary Table 4.** *In vitro* germination assay of wild-type and *h2b.8* mutant pollen.

<b>Rep</b>	<b>Genotype</b>	<b>Elongated (%)</b>	<b>Germinated (%)</b>	<b>Not germinated (%)</b>
1	WT	85.37	5.11	9.52
2	WT	80.9	4.33	14.77
3	WT	81.74	6.27	11.99
4	WT	80.49	7.38	12.13
5	WT	85.51	3.25	11.24
6	WT	85.73	2.07	12.2
Average	WT	83.29	4.74	11.97
1	<i>h2b.8</i>	85.4	3.32	11.28
2	<i>h2b.8</i>	86.98	2.22	10.79
3	<i>h2b.8</i>	86.36	2.91	10.73
4	<i>h2b.8</i>	77.08	6.25	16.67
5	<i>h2b.8</i>	85.81	1.92	12.28
6	<i>h2b.8</i>	82.87	3.69	13.44
Average	<i>h2b.8</i>	84.08	3.39	12.53

**Supplementary Table 5.** Segregation among progeny from self-pollinated *h2b.8* heterozygous mutant plants.

<b>Selfed F1 plant</b>	<b>F2 # WT</b>	<b>F2 # <i>h2b.8</i> +/-</b>	<b>F2 # <i>h2b.8</i> -/-</b>	<b>Total F2 #</b>
1	97	174	85	356
2	103	190	88	381
3	93	193	97	383
4	64	192	86	342
total	357	749	356	1462

**Supplementary Table 6.** List of primers used in this study.

<b>Primer name</b>	<b>Sequence (5' - 3')</b>	<b>Purpose</b>	<b>Construct</b>
<b>H2B.8 CRISPR sgRNA</b>			
TB201	TGTGGTCTCAATTGACAATCA AAGTAACCGTCAGTTTAAGAG CTATGCTGGAA	H2B.8 sgRNA 1	pICSL4723 with 4 H2B.8 sgRNA via pICH47751
TB202	TGTGGTCTCAATTGAAGTAAC CGTCACGGAAGAGTTTAAGAG CTATGCTGGAA	H2B.8 sgRNA 2	pICSL4723 with 4 H2B.8 sgRNA via pICH47761
TB203	TGTGGTCTCAATTGGGATCTCC TTCTTCCGTGAGTTTAAGAGCT ATGCTGGAA	H2B.8 sgRNA 3	pICSL4723 with 4 H2B.8 sgRNA via pICH47772
TB204	TGTGGTCTCAATTGCTGTCTCG GTGATCACACAGTTTAAGAGC TATGCTGGAA	H2B.8 sgRNA 4	pICSL4723 with 4 H2B.8 sgRNA via pICH47781
<b>H2B.8 CRISPR screening</b>			
SH335	CCACCGCGTAGTAGACAG	H2B.8 forward primer	N/A
SH336	AAGCGGGAGTTTCCGGTG	H2B.8 reverse primer	N/A
SH337	GCTAAGGTATTTCGAACGAC	H2B.8 CRISPR sequencing primer	N/A
TB296	AGGTCGTCGTCGTAGGTGTC	Cas9 forward primer	N/A
TB297	GTGCAGACCTACAACCAGCT	Cas9 reverse primer	N/A
<b><i>h2b.8-1</i> and <i>h2b.8-2</i> genotyping</b>			



TB298	AGTAGACAGTTAATCACCAAT GCTAAGGTA	<i>h2b.8-1</i> dCAPS genotyping forward nested PCR	N/A
TB299	TGATCACACACGGATCTCCTTC CTCCGTGA	<i>h2b.8-1</i> dCAPS genotyping reverse nested PCR	N/A
TB293	AGTAGACAGTTAATCACCAAT GCTAAGGTA	<i>h2b.8-1</i> dCAPS genotyping forward	N/A
TB294	TGATCACACACGGATCTCCTTC CTCCGTGA	<i>h2b.8-1</i> dCAPS genotyping reverse	N/A
TB335	GTGAAAGGGTGATCGTGGTG	<i>h2b.8-2</i> genotyping forward	N/A
TB336	TGGCGGGAGATGAGTATAGG	<i>h2b.8-2</i> genotyping reverse	N/A
<b>Reporter and ectopic H2B.8 cloning (Multisite Gateway cloning system)</b>			
TB137	GGGGACAACCTTTGTATAGAAA AGTTGATCAGTGGAGGATGAC ATGGC	H2B.8 promoter cloning forward with attB4 site	<i>pH2B.8::H2B.8-eGFP</i> / <i>pH2B.8::H2B.8-Myc</i>
TB138	GGGGACTGCTTTTTTGTACAAA CTTGATTCTTCGTTAGAAATAA CCG	H2B.8 promoter cloning reverse with attB1r site	<i>pH2B.8::H2B.8-eGFP</i> / <i>pH2B.8::H2B.8-Myc</i>
TB139	GGGGACAAGTTTGTACAAAAA AGCAGGCTATGGCGCCGAGAA AACCAAAGGT	H2B.8 cloning forward with attB1 site	<i>pH2B.8::H2B.8-eGFP</i> / <i>pH2B.8::H2B.8-Myc</i> / <i>p35S::H2B.8-eGFP</i>
TB140	GGGGACCACTTTGTACAAGAA AGCTGGGTAACGTTTCCTACTA TCATAACCA	H2B.8 cloning reverse with attB2 site	<i>pH2B.8::H2B.8-eGFP</i> / <i>pH2B.8::H2B.8-Myc</i> / <i>p35S::H2B.8-eGFP</i>

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**H2B.8ΔIDR and H2B.2 cloning (In-Fusion cloning system)**

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SH469	TATGACCATGATTACGAATTAT	H2B.8ΔIDR	<i>p35S::H2B.8ΔIDR-YFP /</i>
	CAGTGGAGGATGACATGGCG	overlapping forward	<i>pH2B.8::H2B.8ΔIDR-</i>
		N-terminus	<i>eGFP</i>
SH475	TCAAAGTGCCGACGGTTACTTT	H2B.8ΔIDR	<i>p35S::H2B.8ΔIDR-YFP /</i>
	GATTGTCTCTTCC	overlapping reverse	<i>pH2B.8::H2B.8ΔIDR-</i>
		N-terminus	<i>eGFP</i>
SH476	AGTAACCGTCGGCACTTTGAA	H2B.8ΔIDR	<i>p35S::H2B.8ΔIDR-YFP /</i>
	GAAAACAGATAAGG	forward C-terminus	<i>pH2B.8::H2B.8ΔIDR-</i>
			<i>eGFP</i>
SH474	CTCGCCCTTGCTCACGGATCCC	H2B.8ΔIDR reverse	<i>p35S::H2B.8ΔIDR-YFP /</i>
	AGATCTTCCTCAGAGATGAGC	C-terminus	<i>pH2B.8::H2B.8ΔIDR-</i>
			<i>eGFP</i>
TB333	TCTATCTCTCTCGAGGTACCAT	H2B.2 cloning	<i>p35S::H2B.2-YFP</i>
	GGCGAAGGCAGATAAGAAA	forward	
TB335	TGTCGACTCCGAATTCAGAAC	H2B.2 cloning	<i>p35S::H2B.2-YFP</i>
	TCGTAAACTTCGTAACCGC	reverse	

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**Chimeric H2B.8 construct cloning (Multisite Gateway cloning system)**

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PSH295	GGGGACCACTTTGTACAAGAA	<i>H2B.8 with N234K</i>	<i>pH2B.8::H2B.8-N234K-</i>
	AGCTGGGTAAGAACTCGTAAA	substitution	<i>Myc</i>
	CTTCGTAACAGCTTTAGAGCCT		
	TC		

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**H2B.8 IDR swap construct cloning (In-Fusion cloning system)**

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PSZ644	ACAAATCTATCTCTCTCGAGGT	H2B.8-EWSR1	<i>p35S::H2B.8-EWSR1-</i>
	ACCATGGCGCCGAGAAAACCA	overlapping forward	<i>IDR-eGFP</i>
	AAG	N-terminus	

PSZ650	CCGTGGACGCGACGGTTACTT TGATTGTCTCTTCC	H2B.8-EWSR1 overlapping reverse N-terminus	<i>p35S::H2B.8-EWSR1-IDR -eGFP</i>
PSZ651	AGTAACCGTCGCGTCCACGGA TTACAGTACC	H2B.8-EWSR1 overlapping forward EWSR1-IDR	<i>p35S::H2B.8-EWSR1-IDR -eGFP</i>
PSZ652	TCAAAGTGCCCTGCCATAAA CACCCATGC	H2B.8-EWSR1 overlapping reverse EWSR1-IDR	<i>p35S::H2B.8-EWSR1-IDR -eGFP</i>
PSZ653	TTATGGGCAGGGCACTTTGAA GAAAACAGATAAGG	H2B.8-EWSR1 overlapping forward C-terminus	<i>p35S::H2B.8-EWSR1-IDR -eGFP</i>
PSZ645	ACTAGTGTCGACTCCGAATTC ACGTTTCCTACTATCATAACCA ACG	H2B.8-EWSR1 overlapping reverse C-terminus	<i>p35S::H2B.8-EWSR1-eGFP</i>
PSZ644	ACAAATCTATCTCTCTCGAGGT ACCATGGCGCCGAGAAAACCA AAG	H2B.8-TAF15 overlapping forward N-terminus	<i>p35S::H2B.8-TAF15-IDR -eGFP</i>
PSZ666	CAGAATCCGAGACGGTTACTT TGATTGTCTCTTCC	H2B.8-TAF15 overlapping reverse N-terminus	<i>p35S::H2B.8-TAF15-IDR -eGFP</i>
PSZ667	AGTAACCGTCTCGGATTCTGG AAGTTACGG	H2B.8-TAF15 overlapping forward TAF15-IDR	<i>p35S::H2B.8-TAF15-IDR -eGFP</i>
PSZ668	TCAAAGTGCCTTGTGAATGAT AGGACTGCTGG	H2B.8-TAF15 overlapping reverse TAF15-IDR	<i>p35S::H2B.8-TAF15-IDR -eGFP</i>

PSZ669	TCATTCACAAGGCACTTTGAA GAAAACAGATAAGG	H2B.8-TAF15 overlapping forward C-terminus	<i>p35S::H2B.8-TAF15-IDR</i> <i>-eGFP</i>
PSZ645	ACTAGTGTCGACTCCGAATTC ACGTTTCCTACTATCATAACCA ACG	H2B.8-TAF15 overlapping reverse C-terminus	<i>p35S::H2B.8-TAF15-IDR</i> <i>-eGFP</i>
PSZ690	TCTATCTCTCTCGAGGTACCAT GGCTCCTAGAAAGCCTAAGGT	H2B.8- scrambledIDR forward	<i>p35S::H2B.8-</i> <i>scrambledIDR-eGFP</i>
PSZ691	TGTCGACTCCGAATTCTCTCTT TCTAGAATCATATCCAACA	H2B.8- scrambledIDR reverse	<i>p35S::H2B.8-</i> <i>scrambledIDR-eGFP</i>

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**Supplementary Table 7.** List of *P*-values associated with statistical tests in this study.

<b>Figure</b>	<b>Group 1</b>	<b>Group 2</b>	<b>P value</b>
Fig. 1b	GN	Leaf	0
	Sperm	Leaf	0
	Sperm	GN	0
Fig. 2a	<i>h2b.8-1</i>	WT	0
	<i>h2b.8-2</i>	WT	0
	<i>pH2B.8::H2B.8-eGFP h2b.8-1</i>	WT	0.8040364
	<i>pH2B.8::H2B.8-Myc h2b.8-1</i>	WT	0.7102341
	<i>pH2B.8::H2B.8ΔIDR-eGFP h2b.8-1</i>	WT	0
	<i>pH2B.8::H2B.8-N234K-Myc h2b.8-1</i>	WT	0.5549251
	<i>h2b.8-2</i>	<i>h2b.8-1</i>	0.4003808
	<i>pH2B.8::H2B.8-eGFP h2b.8-1</i>	<i>h2b.8-1</i>	0
	<i>pH2B.8::H2B.8-Myc h2b.8-1</i>	<i>h2b.8-1</i>	0
	<i>pH2B.8::H2B.8ΔIDR-eGFP h2b.8-1</i>	<i>h2b.8-1</i>	0.9980652
	<i>pH2B.8::H2B.8-N234K-Myc h2b.8-1</i>	<i>h2b.8-1</i>	0
	<i>pH2B.8::H2B.8-eGFP h2b.8-1</i>	<i>h2b.8-2</i>	0
	<i>pH2B.8::H2B.8-Myc h2b.8-1</i>	<i>h2b.8-2</i>	0
	<i>pH2B.8::H2B.8ΔIDR-eGFP h2b.8-1</i>	<i>h2b.8-2</i>	0.9233628
	<i>pH2B.8::H2B.8-N234K-Myc h2b.8-1</i>	<i>h2b.8-2</i>	0
	<i>pH2B.8::H2B.8-Myc h2b.8-1</i>	<i>pH2B.8::H2B.8-eGFP h2b.8-1</i>	0.9999862
	<i>pH2B.8::H2B.8ΔIDR-eGFP h2b.8-1</i>	<i>pH2B.8::H2B.8-eGFP h2b.8-1</i>	0
	<i>pH2B.8::H2B.8-N234K-Myc h2b.8-1</i>	<i>pH2B.8::H2B.8-eGFP h2b.8-1</i>	0.9993517
	<i>pH2B.8::H2B.8ΔIDR-eGFP h2b.8-1</i>	<i>pH2B.8::H2B.8-Myc h2b.8-1</i>	0
	<i>pH2B.8::H2B.8-N234K-Myc h2b.8-1</i>	<i>pH2B.8::H2B.8-Myc h2b.8-1</i>	0.9999006
	<i>pH2B.8::H2B.8ΔIDR-eGFP</i>		
	<i>pH2B.8::H2B.8-N234K-Myc h2b.8-1</i>	<i>h2b.8-1</i>	0.92866346

Fig. 2d	<i>p35S::H2B.8-eGFP</i> #3	WT	0.002157
	<i>p35S::H2B.8-eGFP</i> #5	WT	0.0003166
	<i>p35S::H2B.8ΔIDR-YFP</i> #2	WT	0.9870122
	<i>p35S::H2B.8ΔIDR-YFP</i> #5	WT	0.9987245
	<i>p35S::H2B.2-YFP</i> #8	WT	0.9890218
	<i>p35S::H2B.2-YFP</i> #9	WT	0.9999989
	<i>p35S::H2B.8-eGFP</i> #5	<i>p35S::H2B.8-eGFP</i> #3	0.9993084
	<i>p35S::H2B.8ΔIDR-YFP</i> #2	<i>p35S::H2B.8-eGFP</i> #3	0.0268405
	<i>p35S::H2B.8ΔIDR-YFP</i> #5	<i>p35S::H2B.8-eGFP</i> #3	0.012598
	<i>p35S::H2B.2-YFP</i> #8	<i>p35S::H2B.8-eGFP</i> #3	0.0235015
	<i>p35S::H2B.2-YFP</i> #9	<i>p35S::H2B.8-eGFP</i> #3	0.0011189
	<i>p35S::H2B.8ΔIDR-YFP</i> #2	<i>p35S::H2B.8-eGFP</i> #5	0.0054939
	<i>p35S::H2B.8ΔIDR-YFP</i> #5	<i>p35S::H2B.8-eGFP</i> #5	0.0023178
	<i>p35S::H2B.2-YFP</i> #8	<i>p35S::H2B.8-eGFP</i> #5	0.0046637
	<i>p35S::H2B.2-YFP</i> #9	<i>p35S::H2B.8-eGFP</i> #5	0.0001511
	<i>p35S::H2B.8ΔIDR-YFP</i> #5	<i>p35S::H2B.8ΔIDR-YFP</i> #2	0.9999712
	<i>p35S::H2B.2-YFP</i> #8	<i>p35S::H2B.8ΔIDR-YFP</i> #2	1
	<i>p35S::H2B.2-YFP</i> #9	<i>p35S::H2B.8ΔIDR-YFP</i> #2	0.9666131
	<i>p35S::H2B.2-YFP</i> #8	<i>p35S::H2B.8ΔIDR-YFP</i> #5	0.9999847
	<i>p35S::H2B.2-YFP</i> #9	<i>p35S::H2B.8ΔIDR-YFP</i> #5	0.9943575
<i>p35S::H2B.2-YFP</i> #9	<i>p35S::H2B.2-YFP</i> #8	0.970609	
Fig. 5c	No change	Down	0
	Up	Down	0
	Up	No change	0
Fig. 5e	WT sperm	Generative nucleus	0
	<i>h2b.8</i> sperm	Generative nucleus	0.1425682
	<i>pH2B.8::H2B.8ΔIDR-eGFP h2b.8-1</i>	Generative nucleus	0.1395767

	<i>h2b.8</i> sperm	WT sperm	0.000001
	<i>pH2B.8::H2B.8ΔIDR-eGFP h2b.8-1</i>	WT sperm	0.0000003
	<i>pH2B.8::H2B.8ΔIDR-eGFP h2b.8-1</i>	<i>h2b.8</i> sperm	0.9998871
Fig. 5f	WT	<i>p35S::H2B.8-eGFP</i>	0
Extended			
Data Fig.			
2b	WT sperm	Generative nucleus	0
	<i>h2b.8-1</i> sperm	Generative nucleus	0
	<i>h2b.8-2</i> sperm	Generative nucleus	0
	<i>h2b.8-1</i> sperm	WT sperm	0
	<i>h2b.8-2</i> sperm	WT sperm	0
	<i>h2b.8-2</i> sperm	<i>h2b.8-1</i> sperm	0.4120998
Extended			
Data Fig.			
2d	WT	<i>h2b.8</i>	3.64E-07
Extended			
Data Fig.			
2e	WT	<i>h2b.8</i>	6.44E-14
Extended			
Data Fig.			
2h	Small (left plot)	Large (left plot)	0.00002294
	Small (middle plot)	Large (middle plot)	0.000052222
	Small (right plot)	Large (right plot)	0.6789
Extended			
Data Fig.			
3h	<i>p35S::H2B.8-eGFP</i>	WT	0.028352

<i>p35S::H2B.8-scrambledIDR-eGFP</i>	WT	0.0181214
<i>p35S::H2B.8-EWSR1-IDR-eGFP</i>	WT	0.0299781
<i>p35S::H2B.8-TAF15-IDR-eGFP</i>	WT	0.0306313
<i>p35S::H2B.8-scrambledIDR-eGFP</i>	<i>p35S::H2B.8-eGFP</i>	0.9999277
<i>p35S::H2B.8-EWSR1-IDR-eGFP</i>	<i>p35S::H2B.8-eGFP</i>	0.9967316
<i>p35S::H2B.8-TAF15-IDR-eGFP</i>	<i>p35S::H2B.8-eGFP</i>	0.9966074
	<i>p35S::H2B.8-scrambledIDR-</i>	
<i>p35S::H2B.8-EWSR1-IDR-eGFP</i>	<i>eGFP</i>	0.9886906
	<i>p35S::H2B.8-scrambledIDR-</i>	
<i>p35S::H2B.8-TAF15-IDR-eGFP</i>	<i>eGFP</i>	0.9883868
	<i>p35S::H2B.8-EWSR1-IDR-</i>	
<i>p35S::H2B.8-TAF15-IDR-eGFP</i>	<i>eGFP</i>	1

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