

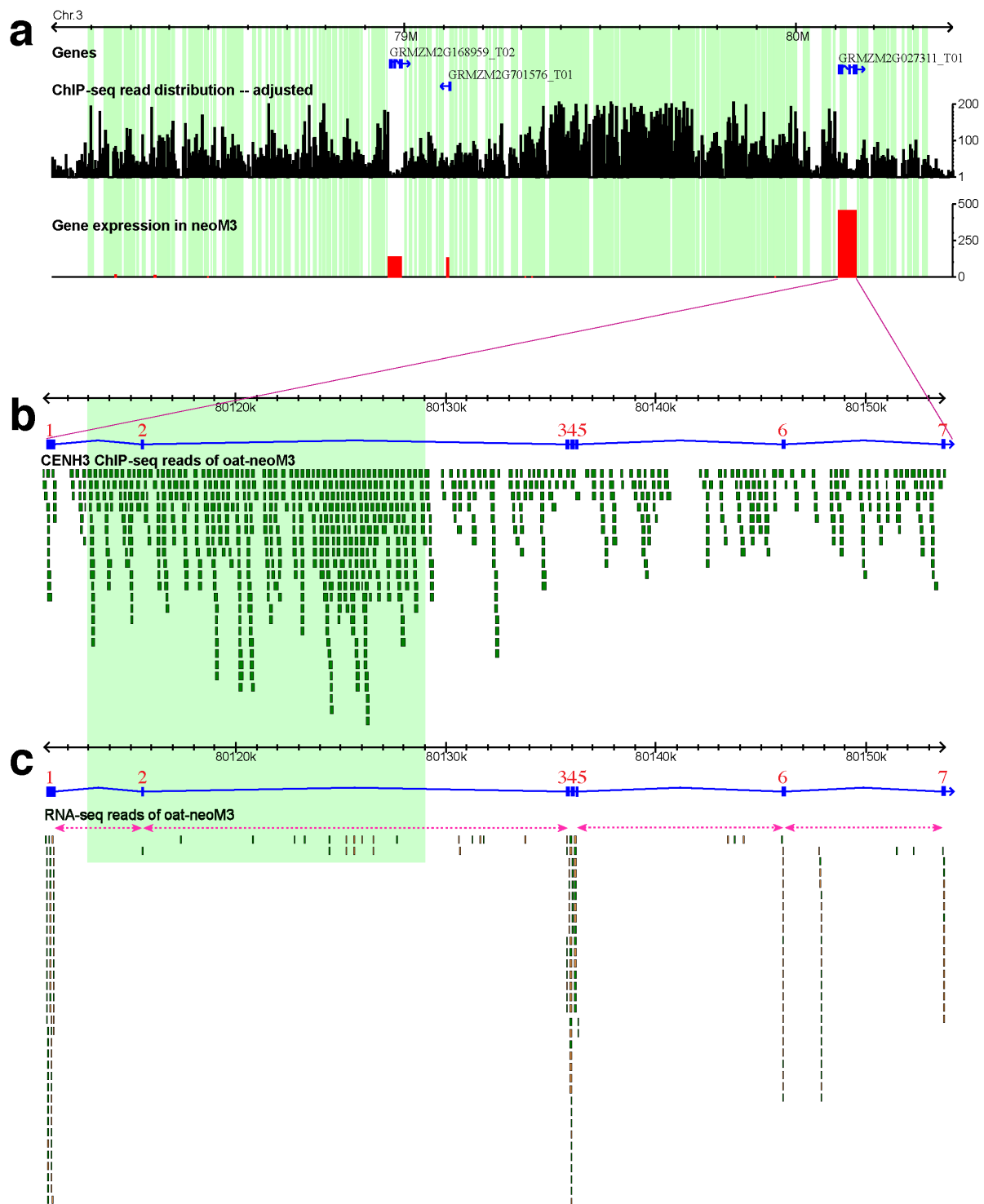


Figure S1. Mapping of ChIP-seq and RNA-seq sequence reads within gene *G311*. **(a)** Mapping ChIP-seq reads and three transcribed genes within the neocentromere *nCenM3*. This diagram was extracted from Figure 5. **(b)** Mapping of ChIP-seq reads (small green blocks) within gene *G311*. The seven small exons of gene *G311* are numbered in red color. The large green box covers a region significantly enriched with CENH3. This region spans parts of intron #1, intron #2, and exon #2. **(c)** Mapping of RNA-seq reads within gene *G311*. The dashed purple lines with arrows at both ends indicate multiple reads that connect the two consecutive exons. Small brown blocks represent reads from the positive strand, small green blocks represent reads from the negative strand.

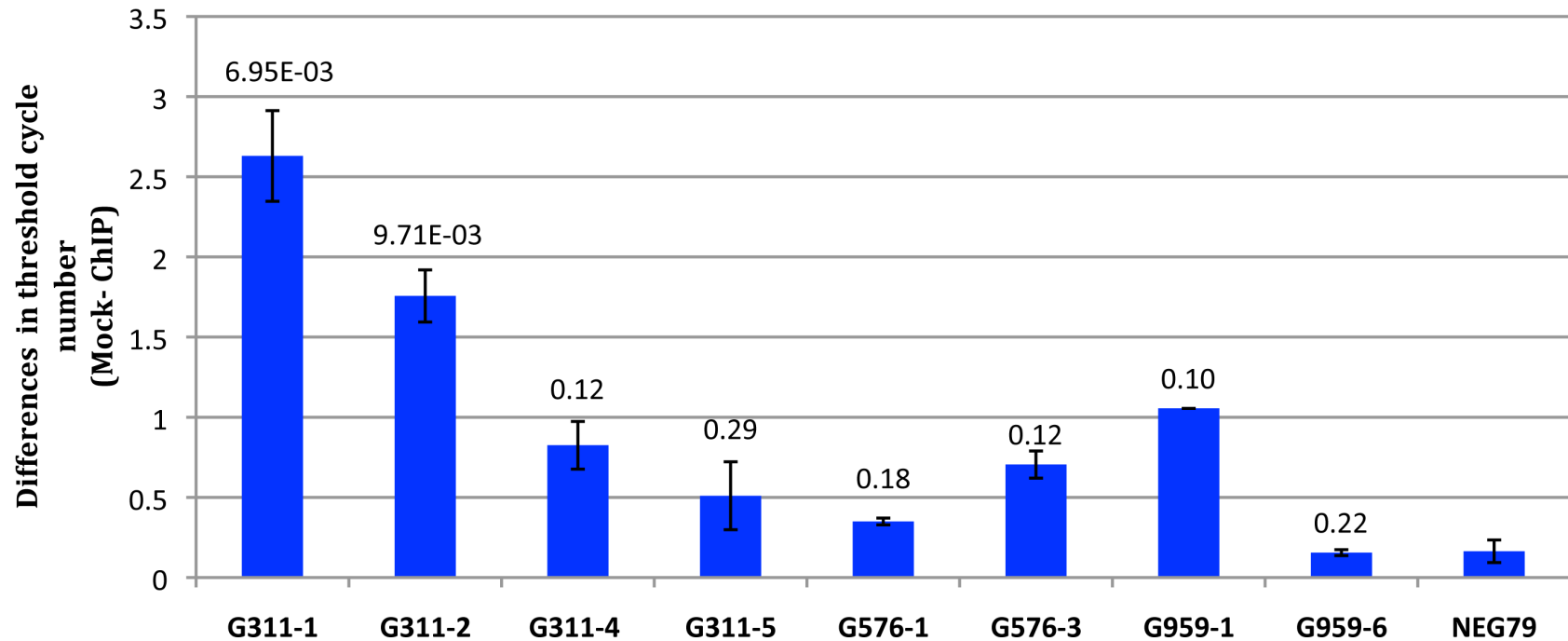


Figure S2. Confirmation of ChIP-seq data for three genes by quantitative ChIP-PCR. Multiple primers were designed from each of the three genes (*G959*, *G576*, and *G311*). Primers G311-1 and G311-2 were designed from regions of *G311* enriched with CENH3; primers G311-4 and G311-5 were designed from regions of *G311* that were not enriched with CENH3 according to ChIP-seq data. Primer (NEG79) was used as a negative control and was designed from a region at 79 Mb on maize chromosome 3. Difference in threshold cycle number (Mock-ChIP) is normalized with the negative control and is indicated by the height of columns with standard error. For each primer, significance of enrichment from antibody-binding fraction over mock treatment was tested using one-tailed Student's t-test at a significance level of $\alpha=0.05$; the corresponding *p*-values are shown above the primer numbers. The ChIP-qPCR results confirmed the association of CENH3 with *G311*, but not with *G959* and *G576*.

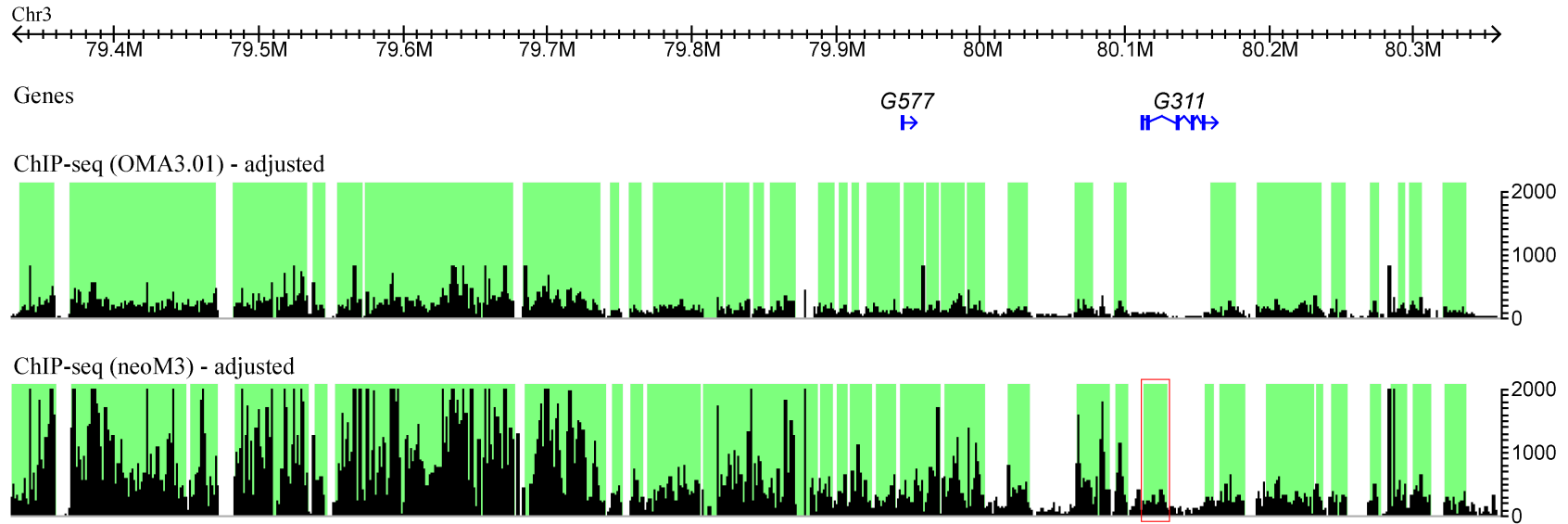


Figure S3. High-resolution mapping of CENH3 ChIP-seq reads in the 79.3-80.3 Mb region that is shared by *nCenM3* and *nCen3*. Each black bar represents the number of ChIP-seq reads (normalized in read number per 1 kb mappable region per million reads, y-axis) in a 1 kb windows. The green blocks indicate subdomains significantly enriched with CENH3, which are interspersed by subdomains that were not enriched with CENH3 (white blocks). Note: Gene *G311* spans a CENH3 subdomain (red box) in *nCenM3* but not in *nCen3*.

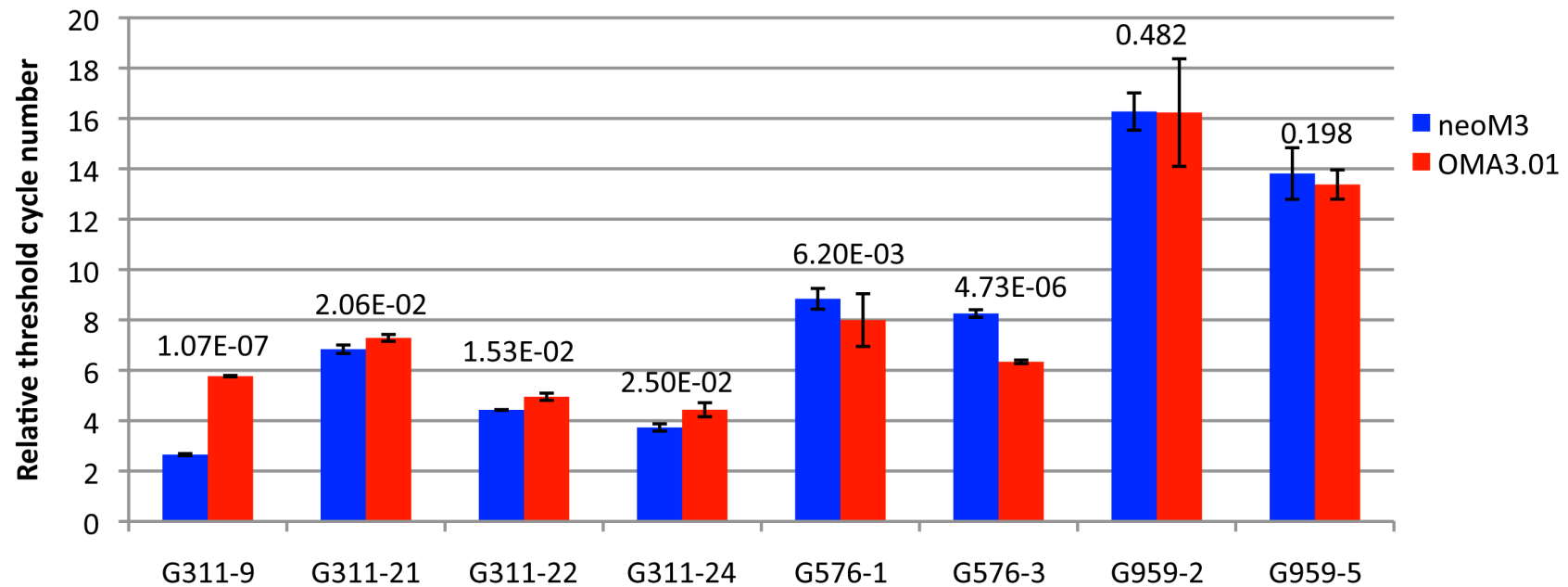


Figure S4. Quantitative transcription analysis of three active genes associated with *nCenM3* and *nCen3*. Multiple primers were designed from each of the three genes. The *glyceraldehyde-3-phosphate dehydrogenase* gene of oat was used as an internal control. For each gene, relative threshold cycle number was calculated and normalized over the internal control. Significance of differences in threshold cycle number between *nCenM3* and *nCen3* was tested using Student's t-test at significant level of $\alpha=0.05$; the corresponding *p*-values are shown above the primer numbers. The qPCR confirmed that expression level of gene *G311* is significantly increased in *nCenM3* compared to *nCen3*. By contrast, expression level of gene *G576* was significantly decreased in *nCenM3*. For gene *G959*, there was no significant difference in expression levels between the two neocentromeres.

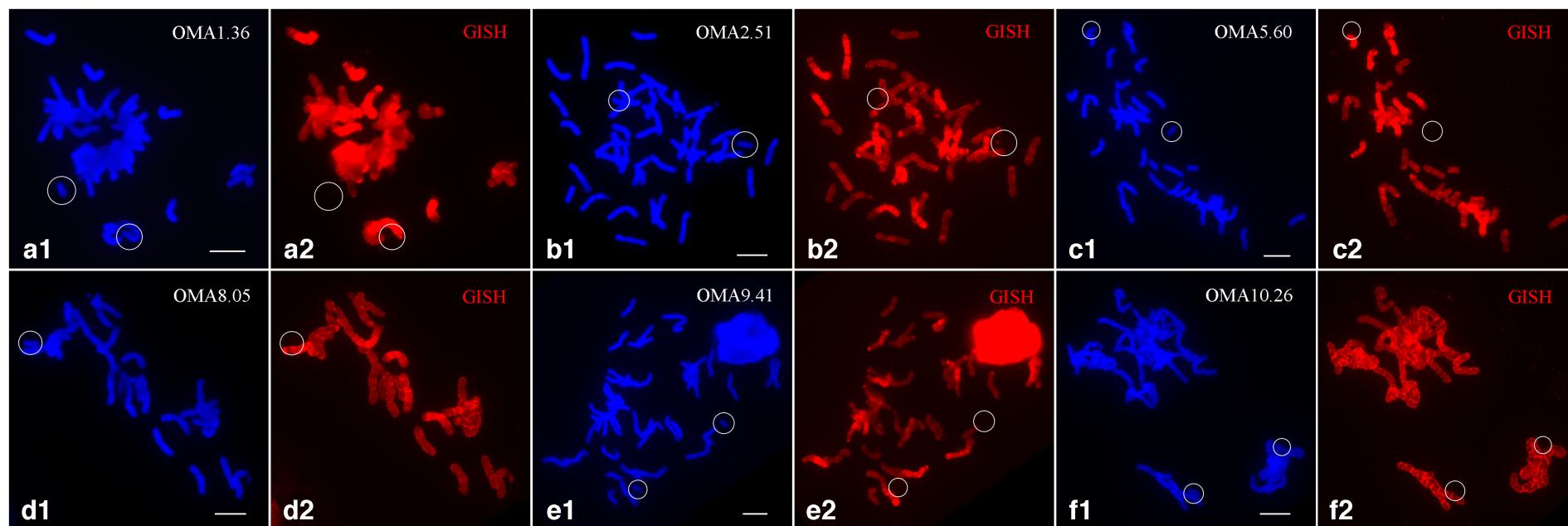


Figure S5. Genomic in situ hybridization analysis of oat-maize chromosome addition lines. **(a1-f1)** A somatic metaphase cell from OMA1.36 (oat-maize chromosome addition 1, a1), OMA2.51 (oat-maize chromosome addition 2, b1), OMA5.60 (oat-maize chromosome addition 5, c1), OMA8.05 (oat-maize chromosome addition 8, d1), OMA9.41 (oat-maize chromosome addition 9, e1), and OMA10.26 (oat-maize chromosome addition 10, f1), respectively. Open circles indicate the maize chromosome(s) in each line. **(b2-f2)** GISH signals associated with each cell. Unambiguous hybridization signals were only observed in the telomeric region of maize chromosome 2 (b2). Bars = 10 μ m.

Table S1. Expression of genes mapped within the neocentromeres

ID	Gene Name	Start	End	Centromere	FPKM
GRMZM2G111407*	<i>G407</i>	78261270	78263868	<i>nCenM3</i>	12.2718
GRMZM2G125800*	<i>G800</i>	78361435	78364039	<i>nCenM3</i>	7.35774
AC202959.3_FGT001*	<i>AC20201</i>	78496767	78497681	<i>nCenM3</i>	0
GRMZM2G168959	<i>G959</i>	78960843	78990086	<i>nCenM3</i>	107.417
GRMZM2G701576	<i>G576</i>	79113162	79114807	<i>nCenM3</i>	118.485
AC212709.3_FGT001*	<i>AC21201</i>	79307901	79308943	<i>nCenM3</i>	0
AC212709.3_FGT002*	<i>AC21202</i>	79325159	79325791	<i>nCenM3</i>	0
GRMZM2G701577*	<i>G577</i>	79945097	79945441	<i>nCenM3</i>	0
GRMZM2G701577	<i>G577</i>	79945097	79945441	<i>nCen3</i>	0
GRMZM2G027311*	<i>G311</i>	80110901	80153743	<i>nCenM3</i>	410.787
GRMZM2G027311	<i>G311</i>	80110901	80153743	<i>nCen3</i>	243.872
GRMZM2G100321	<i>G321</i>	80514284	80517540	<i>nCen3</i>	54.9448
GRMZM2G156033	<i>G033</i>	81036356	81074995	<i>nCen3</i>	64.7276
AC225179.3_FGT003	<i>A003</i>	81075088	81075801	<i>nCen3</i>	10.866
GRMZM5G839005	<i>G005</i>	81129015	81129609	<i>nCen3</i>	0
GRMZM5G856092	<i>G092</i>	81178553	81178945	<i>nCen3</i>	0
GRMZM2G701581	<i>G581</i>	81264296	81264718	<i>nCen3</i>	0
GRMZM2G085279	<i>G279</i>	81339451	81339973	<i>nCen3</i>	211.009
GRMZM2G317823	<i>G823</i>	81426471	81434279	<i>nCen3</i>	0
AC212769.3_FGT004	<i>A004</i>	81540784	81541471	<i>nCen3</i>	0
GRMZM2G154532	<i>G532</i>	82052898	82106476	<i>nCen3</i>	541.453
GRMZM2G154496	<i>G496</i>	82130646	82131652	<i>nCen3</i>	0
GRMZM5G826892	<i>G892</i>	82451382	82451604	<i>nCen3</i>	0
GRMZM2G081380	<i>G380</i>	83042235	83069098	<i>nCen3</i>	53.3467
AC198386.3_FGT001	<i>A001</i>	83259349	83259807	<i>nCen3</i>	12.8443
GRMZM2G135927*	<i>G927</i>	83533229	83533947	<i>nCen3</i>	0
GRMZM5G858754	<i>G754</i>	83582777	83583007	<i>nCen3</i>	0
GRMZM2G108527	<i>G527</i>	83689701	83690887	<i>nCen3</i>	475.409
GRMZM2G145886	<i>G886</i>	83717797	83740902	<i>nCen3</i>	0.361017
GRMZM5G865071	<i>G071</i>	83782336	83782827	<i>nCen3</i>	0

* Genes mapped partially or completely in a CENH3 subdomain.

Table S2. Primers used in isolation of the 8.7 kb DNA probe

Primer	Primer sequence	Start position (bp)	End position (bp)	Length (bp)	Tm (°C)	GC (%)	amplicon size (bp)
ISO30	5' AGGTTTCAGAATTTGATAGAGC 3'	3,103,550	3,103,570	21	50.8	38.1	899
	5' TACCAATGAGTGAAACCAAG 3'	3,104,448	3,104,467	20	50.6	40.0	
ISO31	5' TTGATAGACAAGCATTTACT 3'	3,104,506	3,104,525	20	44.9	30.0	716
	5' TACCCAGAGGCTATCTTACCC 3'	3,105,221	3,105,241	21	55.6	52.4	
ISO32	5' ATCAGCATTCTGGTCTCTGGTAG 3'	3,106,692	3,106,714	23	57.9	47.8	889
	5' AATGATTGACGATGGGTAGGC 3'	3,107,580	3,107,600	21	59.0	47.6	
ISO33	5' TTTCGTACAACCGTTCTCCAC 3'	3,107,626	3,107,646	21	57.7	47.6	1001
	5' ATTGCCTAGCATAGTAGCACTTC 3'	3,108,626	3,108,648	23	55.9	43.5	
ISO34	5' TATACTCTAAGAGTAGGGGTTCA 3'	3,109,187	3,109,209	23	50.7	39.1	702
	5' AATCTAATCATTGCCTTTGCT 3'	3,109,888	3,109,908	21	54.1	33.3	
ISO35	5' CTCCTACTGCTTCACCTCCAT 3'	3,110,412	3,110,432	21	56.0	52.4	1063
	5' TTACATTACAGCCACCACTACTTC 3'	3,111,474	3,111,497	24	56.3	41.7	
ISO36	5' ATGTAGGAAAAGAGAATGATGCT 3'	3,111,502	3,111,524	23	54.9	34.8	1151
	5' GTGGGAACGAGAATGAGTGTA 3'	3,112,652	3,112,672	21	54.8	47.6	
ISO37	5' AGCAGCACCTAACTAACCAC 3'	3,113,647	3,113,667	21	56.4	52.4	770
	5' AGAACGAAGAGCGAAGGAATA 3'	3,114,416	3,114,436	21	56.4	42.9	
ISO38	5' TTCCTTGAACCACCACCATTT 3'	3,114,416	3,114,436	21	59.3	42.9	734
	5' CGGTCCAACTTTTAGGTCTATGAG 3'	3,115,149	3,115,173	25	61.0	44.0	
ISO41	5' CCGTTGCTCTGGAGGTTGGTG 3'	3,118,531	3,118,551	21	65.0	61.9	847
	5' TCGGGTCATTCTGTGCTGGACT 3'	3,119,377	3,119,397	21	64.9	57.1	

Table S3. Primers used in RT-PCR, ChIP-PCR and qRT-PCR

Target gene	Primer name	Primer pair sequence (5'-3')	Length (bp)	Tm	GC (%)	Amplicon size (bp)
G311	G311-92	CCTAGACCTAAACGGCTCCAT	21	58.0	52.4	840/831*
		GTTCAACATCCCTGTCTTTCC	21	56.0	47.6	
G311	G311-15	AAAGGCACAGAGAATATAGGC	21	53.6	42.9	707/707*
		ATACCTCCGGCACCACAAAAG	21	61.5	52.4	
G311	G311-1	AGCTCAGGAATGATTGCGTGTT	22	61.4	45.5	91
		CTAGCGATTGATGCACATAGAC	22	55.5	45.5	
G311	G311-2	TCTCACCACAGTCAAACCCAC	21	57.7	52.4	167
		CAAGAACCAAGTACAAACCTCC	22	56	45.5	
G311	G311-4	ACGATGTTGGTGTGCCCCGATTT	23	67.3	47.8	90
		CATAGCAACCATCCCTCAACA	21	64.7	57.1	
G311	G311-5	CTCCTTATCCTGCTTCTCCTACA	23	57.7	47.8	211
		CGAACCTAACGACCTATCTACAA	23	56.4	43.5	
G311	G311-9	CCTAGACCTAAACGGCTCCAT	21	58.0	52.4	233
		CTGTAGTCTTCCGGCTTGTG	20	55.7	55.0	
G311	G311-21	ACACATATTCCTGGTTGGGCTG	22	61.4	50.0	108
		ATACCTCCGGCACCACAAAAG	21	61.5	52.4	
G311	G311-22	ACACATATTCCTGGTTGGGCTG	22	61.4	50.0	103
		TCCGGCACCACAAAAGCAGAT	21	65.0	52.4	
G311	G311-24	ATATGCATCCTTCAGTGTCTTC	23	55.7	39.1	131
		AGTAGGCTGCAACATAGCAAA	21	55.8	42.9	
G576	G576-1	AAATCCCAAGCGTCGAGTGAA	21	62.1	47.6	272
		CCTTATGACATCCGACTTGAAACC	24	61.9	45.8	
G576	G576-3	CAGTTCGCTTGGTGTCA GTTCG	22	63.1	54.5	138
		GGGAGACGGCAGCATGGTTTT	21	65.9	57.1	
G959	G959-1	TGTTAGACCATGAAACTCAACAT	23	54.3	34.8	91
		CTCATTGACCAGTAGGTATAA	21	47.2	38.1	
G959	G959-2	GCTTATACGATGTCATCTAACTTT	24	53.1	33.3	76
		TTCAGGGAGTAATACCTCTAAAAC	24	54.5	37.5	
G959	G959-5	ACCAATCACACAAATAGTGATGAA	24	56.7	33.3	83
		TTGCCAGTAAAGAAGGAAATG	21	55.2	38.1	
G959	G959-6	AATCCAAACTGTTGTCAATAAGA	23	54.0	30.4	81
		CCATTGCCAGCTAAGACTTCA	21	58.2	47.6	
	NEG79	TCTGCACAGCCGAATTACATT	21	58.4	42.9	202
		ACCCTTCCACCAACAGCTCAA	21	61.8	52.4	

*The amplicon size in oats.