Well	Sample	Target	Conc(copies/µL)	Status	Experiment	SampleType	TargetType	Supermix	DyeName(s)	Copies/20µL Well	PoissonConfMax	PoissonConfMin	Accepted Droplets	Positives	Negatives	Ch1+Ch2+	Ch1+Ch2-	Ch1-Ch2+	Ch1-Ch2-	Linkage	CNV	PoissonCNVMax	PoissonCNVMin	Reference Copies	Reference Used	Ratio	PoissonRatioMax	PoissonRatioMin	Fractional Abundance	PoissonFractional AbundanceMax	PoissonFractional AbundanceMin	MeanAmplitude Of Positives	MeanAmplitude Of Negatives	MeanAmplitude Total	PoissonConfidence eMax68	PoissonConfidence Min68	PoissonCNV Max68	PoissonCNV Min68	PoissonRatio Max68	PoissonRatio Min68	PoissonFractional AbundanceMax68	PoissonFractional AbundanceMin68
A01	CENH2_Aldine_Canola	FAM	22.2867120	Manual	CNV	Treatment 1, Biological Replicate 1, Technical Replicate 1	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	447.9142615	24.60475159	20.19249015	21000	396	20604	396	0	1227	19377	22.3967	0.9467	1.050711727	0.842658128	4	HEX	0.2367	0.262677553	0.210674582	19.13809158	20.83825136	17.4279354	10745.42127	8655525.988	10651.75649	23.52274513	21.27170222	0.989760587	0.893644667	0.238942297	0.221410117	26.00251957	18.27660739
A01	CENH2_Aldine_Canola	HEX	94.6301951	Manual	CNV	Treatment 1, Biological Replicate 1, Technical Replicate 1	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1892.603271	99.24435425	90.03406421	21000	1623	19377	396	0	1227	19377	22.3967												5600.647931	1270.164885	1604.81471	96.98267855	92.28294373						
B01	CENH2_Aldine_Canola	FAM	19.3853350	Manual	CNV	Treatment 1, Biological Replicate 2, Technical Replicate 2	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	387.7907264	21.4417838	17.33225768	20927	342	20585	342	0	978	19607	19.3853	1.0116	1.131952372	0.891393317	4	HEX	0.2529	0.282908693	0.222825329	20.18536978	22.10143851	18.26503016	10938.59648	824.5713379	989.8714396	20.40465342	18.33755112	1.072995727	0.958229031	0.268248932	0.237527328	21.16295494	19.20778661
B01	CENH2_Aldine_Canola	HEX	76.6512986	Manual	CNV	Treatment 1, Biological Replicate 2, Technical Replicate 2	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1533.024658	80.79436493	72.52262878	20927	1320	19607	342	0	978	19607	19.3853												5673.714135	1282.754581	1529.722261	78.76325526	74.54299164						
C01	CENH2_Aldine_Canola	FAM	16.3829119	Manual	CNV	Treatment 1, Biological Replicate 3, Technical Replicate 3	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	327.6258631	18.26264517	14.59154153	21115	292	20823	292	0	1011	19812	16.383	0.8745	0.985483637	0.763520568	4	HEX	0.2186	0.241570909	0.190880142	17.94013696	19.80862236	16.07282157	10998.28615	8391892158	9791317616	17.3421357	15.45463207	0.931125255	0.81787895	0.232789314	0.204409757	18.89355777	16.9871163
C01	CENH2_Aldine_Canola	HEX	74.9361744	Manual	CNV	Treatment 1, Biological Replicate 3, Technical Replicate 3	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1498.726318	79.01296234	70.87376404	21115	1303	19812	292	0	1011	19812	16.383												5717.49406	1291.665742	1564.794556	77.01466533	72.84181617						
D01	CENH2_Aldine_Canola	FAM	19.1736927	Manual	CNV	Treatment 1, Biological Replicate 4, Technical Replicate 4	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	383.4738464	21.16436386	17.18638839	22084	357	21727	357	0	987	20740	19.1737	1.0382	1.159414957	0.917075631	4	HEX	0.2596	0.289853739	0.229268908	20.60727959	22.51667237	18.6978868	10946.42734	825.4565015	989.4508849	20.18891987	18.159388	1.180966486	0.976424141	0.275016612	0.244106035	21.58145793	19.63310124
D01	CENH2_Aldine_Canola	HEX	71.8696902	Manual	CNV	Treatment 1, Biological Replicate 4, Technical Replicate 4	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1477.39209	77.82621002	69.92624664	22084	1344	20740	357	0	987	20740	19.1737												5685.239983	1283.75116	1551.619344	75.88661967	71.85604095						
E01	CENH2_Aldine_Canola	FAM	16.6947470	Manual	CNV	Treatment 1, Biological Replicate 5, Technical Replicate 5	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	333.8949585	18.55377411	14.82684671	21708	307	21401	307	0	650	20831	16.6947	1.2637	1.42617546	1.10124251	4	HEX	0.3159	0.356543865	0.275310629	24.00795696	26.35349916	21.66243232	11129.32891	784.4374447	938.280327	17.64756557	15.76259671	1.346599974	1.180818003	0.336649993	0.295204501	25.29465217	22.81112675
E01	CENH2_Aldine_Canola	HEX	52.843647	Manual	CNV	Treatment 1, Biological Replicate 5, Technical Replicate 5	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1056.872925	56.19675827	49.50006104	21708	957	20831	307	0	650	20831	16.6947												5794.345708	1303.285873	1509.547864	54.55322266	51.13654799						
F01	CENH2_Aldine_Canola	FAM	No Call	CHECK	CNV	Treatment 1, Biological Replicate 2, Technical Replicate 2	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM				0	0	0	0	0	0	0	0															0							
F01	CENH2_Aldine_Canola	HEX	No Call	CHECK	CNV	Treatment 1, Biological Replicate 2, Technical Replicate 2	Reference	ddPCR Supermix for Probes (no dUTP)	HEX				0	0	0	0	0	0	0	0															0							
G01	CENH2_aldine_R26	FAM	17.2759410	Manual	CNV	Treatment 2, Biological Replicate 3, Technical Replicate 3	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	345.5188293	19.12743996	15.45736634	22981	335	22646	335	0	753	21893	17.2759	1.2111	1.359368412	1.062753745	4	HEX	0.3028	0.339849606	0.265683436	23.24945222	25.42723242	21.05582201	10266.73962	793.4648058	931.5587966	18.22021675	16.33242798	1.286726069	1.135401478	0.321687672	0.28385057	24.35211536	22.1357286
G01	CENH2_aldine_R26	HEX	57.0589082	Manual	CNV	Treatment 2, Biological Replicate 3, Technical Replicate 3	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1141.196167	60.45560874	53.67378998	22981	1088	21893	335	0	753	21893	17.2759												6939.189939	1438.119549	1690.932795	58.79113087	55.3310118						
H01	CENH2_aldine_R26	FAM	15.7874154	Manual	CNV	Treatment 2, Biological Replicate 4, Technical Replicate 4	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	315.7483046	17.55050641	14.08519168	22342	298	22044	298	0	671	21373	15.7875	1.2114	1.360620199	1.054903075	4	HEX	0.3028	0.342157998	0.263529769	23.24456776	25.56968318	20.92812233	10678.9134	796.5718893	925.4494486	16.79286883	14.88278664	1.291599544	1.131115232	0.323998686	0.28277888	24.43641149	22.0627240
H01	CENH2_aldine_R26	HEX	52.1645546	Manual	CNV	Treatment 2, Biological Replicate 4, Technical Replicate 4	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1043.291138	55.45392227	48.88435745	22342	969	21373	298	0	671	21373	15.7875												7657.679953	1456.967819	1699.794168	53.84165573	50.48984526						
A02	CENH2_aldine_R26	FAM	14.6095875	Manual	CNV	Treatment 2, Biological Replicate 1, Technical Replicate 1	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	292.1917114	16.43299666	12.78899574	20914	247	19767	247	0	710	19057	14.6096	1.0138	1.152550128	0.871962218	4	HEX	0.2534	0.28889782	0.217900804	20.21983025	22.47640188	17.96325862	10603.74547	838.4837336	949.8623652	15.53954586	13.68693575	1.086131238	0.94143226	0.271532809	0.235353815	21.37144958	19.06851993
A02	CENH2_aldine_R26	HEX	57.6441626	Manual	CNV	Treatment 2, Biological Replicate 1, Technical Replicate 1	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1152.883301	61.30243517	53.9572496	20914	957	19957	247	0	710	19057	14.6096												7146.147148	1462.362919	1734.141749	59.50919724	55.7920816						
B02	CENH2_aldine_R26	FAM	15.5738586	Manual	CNV	Treatment 2, Biological Replicate 2, Technical Replicate 2	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	311.4773728	17.47213029	13.67865562	19666	259	19406	259	0	757	18670	15.5738	0.9997	1.136139998	0.863339635	4	HEX	0.2499	0.284035	0.215834909	19.99583685	22.1784669	17.81120679	10774.49023	843.2737345	973.8736653	16.54597603	14.60663687	1.060331674	0.930147959	0.267332918	0.23353699	21.10942245	18.8021523
B02	CENH2_aldine_R26	HEX	62.3164551	Manual	CNV	Treatment 2, Biological Replicate 2, Technical Replicate 2	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1246.22291	66.14959286	58.48583984	19695	1016	18679	259	0	757	18679	15.5738												7196.988893	1471.674555	1767.824663	64.26838684	60.35814667						
C02	CENH2_aldine_R26	FAM	13.3694771	Manual	CNV	Treatment 2, Biological Replicate 3, Technical Replicate 3	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	267.3889462	15.07238303	11.66852856	20974	237	20737	237	0	716	20021	13.3694	0.9775	1.116584577	0.838439588	4	HEX	0.2444	0.279146144	0.209609897	19.63850774	21.88387919	17.3922563	10873.84426	833.4543418	946.9677786	14.23831559	12.50132465	1.048467373	0.906556791	0.262116843	0.226429198	20.7841337	18.4930017
C02	CENH2_aldine_R26	HEX	54.7889122	Manual	CNV	Treatment 2, Biological Replicate 3, Technical Replicate 3	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1094.161255	58.18696213	51.23941803	20974	953	20021	237	0	716	20021	13.3694												7241.622865	1480.59837	1742.333992	56.48172379	52.93768894						
D02	CENH2_aldine_R26	FAM	15.4946529	Manual	CNV	Treatment 2, Biological Replicate 4, Technical Replicate 4	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	309.8932486	17.35812678	13.6246456	20330	266	20064	266	0	685	19380	15.4947	1.0879	1.235679908	0.949196155	4	HEX	0.272	0.308917727	0.235049039	21.38262676	23.60541893	19.09682259	10714.63365	839.4148867	957.7592945	16.46569315	14.54589888	1.163309717	1.012557346	0.290827429	0.253139357	22.54733329	20.21792823
D02	CENH2_aldine_R26	HEX	56.9691956	Manual	CNV	Treatment 2, Biological Replicate 4, Technical Replicate 4	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1139.383057	60.57695807	53.37238112	20330	961	19369	266	0	685	19369	15.4947												7199.963827	1470.562873	1741.391148	58.80847501	55.11326943						
E02	CENH2_aldine_R26	FAM	40.5739088	Manual	CNV	Treatment 2, Biological Replicate 5, Technical Replicate 5	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	811.4393311	43.62108994	37.53875582	20119	682	19437	682	0	1286	18151	40.5739	1.3401	1.456783	1.221303097	4	HEX	0.335	0.364197075	0.305834249	25.09451174	26.73183184	23.45719164	10807.74106	948.6518947	1283.857447	42.12665258	39.01914052	1.286528924	1.126928924	0.349904993	0.320127231	25.92987641	24.2591467
E02	CENH2_aldine_R26	HEX	121.1046906	Manual	CNV	Treatment 2, Biological Replicate 5, Technical Replicate 5	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	2422.69575	126.4608868	115.7638397	20119	1968	18151	682	0	1286	18151	40.5739												7294.529421	1480.855126	2049.537019	123.8389016	118.3767119						
F02	CENH2_aldine_R26	FAM	40.9894631	Manual	CNV	Treatment 2, Biological Replicate 6, Technical Replicate 6	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	818.9892773	43.90143913	37.96613174	20926	715	20211	715	0	1341	18870	40.9895	1.4171	1.538460692	1.295662212	4	HEX	0.3543	0.384615173	0.323165253	26.15825519	27.81403784	24.50447254	10702.41835	930.3789074	1266.339263	42.41115564	39.37178184	1.479000616	1.355156299	0.369750154	0.338781572	27.00352927	25.2145981
F02	CENH2_aldine_R26	HEX	115.4513245	Manual	CNV	Treatment 2, Biological Replicate 6, Technical Replicate 6	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	2309.026367	120.5810089	110.341895	20926	1956	18970	715	0	1341	18970	40.9895												7271.686651	1475.732222	2021.111112	118.06571	112.8427277						
G02	CENH2_aldine_R26	FAM	39.4990018	Manual	CNV	Treatment 2, Biological Replicate 7, Technical Replicate 7	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	789.9001636	42.47613525	36.52959656	20535	678	19857	678	0	1284	18651	39.499	1.2871	1.519794693	1.274649855	4	HEX	0.3493	0.379849623	0.318612664	25.88643953	27.57987139	24.20918566	10685.49849	930.6166201	1252.674851	41.01709974	37.98296256	1.459709944	1.334534694	0.364927486	0.332423601	26.7458715	25.0350691
G02	CENH2_aldine_R26	HEX	113.0867691	Manual	CNV	Treatment 2, Biological Replicate 7, Technical Replicate 7	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	2261.735352	118.2091293	107.9866104	20535	1882	18653	678	0	1284	18653	39.499												7273.129435	1483.453549	2014.684128	115.6974335	110.4818878						
H02	CENH2_aldine_R26	FAM	39.1527238	Manual	CNV	Treatment 2, Biological Replicate 8, Technical Replicate 8	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	783.0145263	42.06438435	36.25435587	21333	698	20635	698	0	1191	19434	39.1527	1.4352	1.559784849	1.310554[illegible]	4	HEX	0.3588	0.389846212	0.327638525	26.49523872	28.09258973	24.7178877	10899.8438	932.3753848	1255.415811	40.63879776	37.67452621	1.498748623	1.371390326	0.374687126	0.342897281	27.29413841	25.5483470[illegible]
H02	CENH2_aldine_R26	HEX	109.1319962	Manual	CNV	Treatment 2, Biological Replicate 8, Technical Replicate 8	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	2182.638893	114.0652212	104.2199628	21333	1889	19434	698	0	1191	19434	39.1527												7351.660774	1497.218869	2015.863534	111.646149	106.6228133						
A03	CENH2_aldine_R26	FAM	61.2339146	Manual	CNV	Treatment 3, Biological Replicate 1, Technical Replicate 1	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	1224.339234	65.43678833	57.03914923	16074	815	15259	815	0	4	15255	61.236	3.9799	4.362448992	3.593945678	4	HEX	0.995	1.091487226	0.898492569	49.87435274	52.29900585	47.44970163	10203.22532	694.8007613	1180.59612	63.36264649	59.07350807	4.176889255	3.783007725	1.044221564	0.945751931	51.11141869	48.6372893[illegible]
A03	CENH2_aldine_R26	HEX	61.5243988	Manual	CNV	Treatment 3, Biological Replicate 1, Technical Replicate 1	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1230.488037	65.74612427	57.31777191	16074	819	15255	815	0	4	15255	61.236												7197.72957	1414.690754	1709.355809	63.67644882	59.37428555						
B03	CENH2_aldine_R26	FAM	70.4853165	Manual	CNV	Treatment 3, Biological Replicate 2, Technical Replicate 2	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	1409.616608	76.58128122	66.41594866	19688	1145	18543	1145	0	1	18542	70.4985	3.9664	4.323750379	3.669954714	4	HEX	0.9991	1.088937595	0.917263678	49.97750658	52.02527102	47.92974017	10861.61607	721.1313239	1504.872221	72.57287433	68.40887451	4.163416141	3.829388683	1.048854102	0.957347171	51.02228187	48.9327297[illegible]
B03	CENH2_aldine_R26	HEX	76.5259856	Manual	CNV	Treatment 3, Biological Replicate 2, Technical Replicate 2	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1411.076712	74.64063036	66.47350964	19688	1146	18542	1145	0	1	18542	70.4985												7684.665522	1479.91583	1836.621871	72.64929694	68.47133925						
C03	CENH2_aldine_R26	FAM	64.9838152	Manual	CNV	Treatment 3, Biological Replicate 3, Technical Replicate 3	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	1299.63623	68.85530699	61.12102589	20191	1085	19106	1085	0	1	19105	64.9818	3.9662	4.33246259	3.539662777	4	HEX	0.9991	1.083115648	0.914990644	49.97632119	52.07987695	47.87277143	10873.39896	720.474874	1266.859856	66.95468956	63.01044883	4.167768549	3.824617778	1.041942147	0.954164445	51.04955827	48.9030841[illegible]
C03	CENH2_aldine_R26	HEX	65.0433817	Manual	CNV	Treatment 3, Biological Replicate 3, Technical Replicate 3	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1300.867798	68.91875084	61.18072891	20191	1086	19105	1085	0	1	19105	64.9818												7579.056146	1474.579014	1892.914134	67.81902771	63.87165617						
D03	CENH2_aldine_R26	FAM	72.8	Manual	CNV	Treatment 3, Biological Replicate 4, Technical Replicate 4	Unknown	ddPCR Supermix for Probes (no dUTP)	FAM	1456	76.9	68.7	20366	1222	19144	1222	0	5	19139	72.8	3.98	4.3	3.67	4	HEX	0.996	1.07	0.917	49.9	51.9	47.9	10914	729	1340	74.9	70.7	4.14	3.82	1.04	0.956	50.9	48.9
D03	CENH2_aldine_R26	HEX	73.1	Manual	CNV	Treatment 3, Biological Replicate 4, Technical Replicate 4	Reference	ddPCR Supermix for Probes (no dUTP)	HEX	1462	77.2	69	20366	1227	19139	1222	0	5	19139	72.8												7630	1486	1850	75.2	71						

Supplementary Table 2. Raw results of droplet digital PCR ablation assay for canola protoplast samples transfected with a CRISPR/CAS9 ribonucleoprotein (RNP) targeting the *CENH3* gene. In treatments 1 and 2, RNPs were delivered in Buffer 3.1 (New England Biolabs) and Modified Transfection Buffer respectively. Treatment 3 was a PEG-only negative control. Genomic DNA was extracted from protoplasts and analysed using a single-amplicon, dual probe droplet digital PCR ablation assay. The FAM probe matched the CRISPR ablation site within the canola *CENH3* gene. The HEX probe matched another site within the canola *CENH3* gene. Data was collected using a QX200 Droplet Reader (Bio-Rad Laboratories) and analysed using Quantasoft software (Bio-Rad Laboratories). CENH3: centromere-specific histone H3; CNV: copy number variation; ddPCR: droplet digital PCR.