

**The histone H3 variant H3.3 regulates gene body DNA methylation in
*Arabidopsis thaliana***

Additional file 1: Supplemental Tables

Supplemental Table 1: Summary of H3.3 T-DNA insertion lines.

Gene	ID	T-DNA line	Name	Leaf phenotype
<i>HTR4</i>	At4g40030	N582765 ^{a,b}	<i>htr4-1</i>	wildtype-like
		N807939 ^b	<i>htr4-2</i>	wildtype-like
<i>HTR5</i>	At4g40040	N510583 ^a	<i>htr5-1</i>	wildtype-like
		N808947	<i>htr5-2</i>	wildtype-like
		N846395 ^b	<i>htr5-3</i>	wildtype-like
<i>HTR8</i>	At5g10980	N587850 ^a	<i>htr8-1</i>	wildtype-like
		N641101 ^b	<i>htr8-2</i>	wildtype-like

^a[32], ^b this work

Supplemental Table 2: Plant genetic backgrounds.

Name	T-DNA / CRISPR/Cas9	Transgene	Description
<i>h3.3KO</i>	<i>htr4;htr5;htr8-2</i>	none	<i>H3.3</i> knockout
<i>h3.3kd-1</i>	<i>htr4-2;htr8-2</i>	<i>amiR-HTR5-I</i>	<i>H3.3</i> knockdown
<i>h3.3kd-2, -3</i>	<i>htr4-2;htr8-2</i>	<i>amiR-HTR5-II</i>	<i>H3.3</i> knockdown
<i>h3.3kd/+</i>	<i>htr4-2;htr8-2</i>	<i>amiR-HTR5-II/+</i>	F1 cross of <i>h3.3kd-3</i> , hemizygous transgene
<i>rH3.3</i>	<i>htr4-2;htr8-2</i>	<i>rHTR5</i>	<i>amiR-HTR5-II</i> resistant <i>rHTR5</i>
<i>h3.3kd/+;rH3.3/+</i>	<i>htr4-2;htr8-2</i>	<i>amiR-HTR5-II/+</i> , <i>rHTR5/+</i>	F1 cross of <i>h3.3kd-3</i> and <i>rH3.3</i> , both transgenes hemizygous

Supplemental Table 3: Expression of selected chromatin factors in WT and *h3.3kd*.

Category	Gene	Gene ID	WT	<i>h3.3kd</i>	log2(FC)	p_value	q_value
Potential H3 chaperone	<i>HIRA</i>	AT3G44530	3.83	4.86	0.34	0.18	0.68
	<i>ATRXL</i>	AT1G08600	11.56	12.11	0.07	0.74	0.97
	<i>ASF1A</i>	AT1G66740	16.49	14.52	-0.18	0.49	0.91
	<i>ASF1B</i>	AT5G38110	10.29	13.26	0.37	0.23	0.74
	<i>MSI1</i>	AT5G58230	20.06	24.37	0.28	0.20	0.70
	<i>FAS1</i>	AT1G65470	6.79	6.58	-0.05	0.86	0.98

	<i>FAS2</i>	AT5G64630	3.81	4.58	0.27	0.28	0.79
Potential H2A.Z chaperone	<i>ARP6</i>	AT3G33520	23.94	22.39	-0.10	0.66	0.95
	<i>PIE1</i>	AT3G12810	6.67	7.81	0.23	0.27	0.77
	<i>SEF</i>	AT5G37055	17.34	16.53	-0.07	0.83	0.98
	<i>MET1</i>	AT5G49160	12.32	11.62	-0.08	0.68	0.96
DNA methylation related	<i>DRM2</i>	AT5G14620	10.33	9.38	-0.14	0.56	0.93
	<i>DRM3</i>	AT3G17310	10.02	10.13	0.02	0.92	0.99
	<i>CMT2</i>	AT4G19020	7.59	7.34	-0.05	0.83	0.98
	<i>CMT3</i>	AT1G69770	11.69	12.46	0.09	0.67	0.96
	<i>SUVH4</i>	AT5G13960	6.02	5.58	-0.11	0.69	0.96
	<i>SUVH5</i>	AT2G35160	2.17	3.34	0.62	0.06	0.44
	<i>SUVH6</i>	AT2G22740	7.37	7.16	-0.04	0.87	0.98
	<i>DDM1</i>	AT5G66750	5.20	5.76	0.15	0.58	0.94
	<i>ROS1/DML1</i>	AT2G36490	17.79	12.40	-0.52	0.01	0.14
	<i>RPA32A</i>	AT2G24490	8.13	11.80	0.54	0.08	0.48
	<i>DME</i>	AT5G04560	22.02	19.30	-0.19	0.33	0.83
	<i>DML2</i>	AT3G10010	5.81	5.44	-0.10	0.68	0.96
	<i>DML3</i>	AT4G34060	0.82	0.49	-0.74	0.14	0.63
	<i>IBM1</i>	AT3G07610	8.84	7.25	-0.29	0.16	0.65
	<i>SHH1</i>	AT1G15215	10.75	9.96	-0.11	0.68	0.96
	<i>SDC</i>	AT2G17690	0.21	0.97	2.18	0.00	0.09
	Histone H1	<i>HON1</i>	AT1G06760	226.36	299.03	0.40	0.05
<i>HON2</i>		AT2G30620	167.00	272.96	0.71	0.00	0.02
<i>HON3</i>		AT2G18050	25.09	231.05	3.20	0.00	0.00
Histone H2A	<i>HTA1</i>	AT5G54640	37.31	41.34	0.15	0.54	0.93
	<i>HTA2</i>	AT4G27230	40.64	49.73	0.29	0.21	0.72
	<i>HTA10</i>	AT1G51060	96.15	119.95	0.32	0.12	0.58
	<i>HTA13</i>	AT3G20670	27.52	35.67	0.37	0.16	0.64
	<i>HTA3</i>	AT1G54690	45.62	61.73	0.44	0.05	0.36
	<i>HTA5</i>	AT1G08880	118.90	130.04	0.13	0.52	0.92
	<i>HTA6</i>	AT5G59870	58.41	81.57	0.48	0.02	0.24
	<i>HTA7</i>	AT5G27670	54.10	90.96	0.75	0.00	0.01
	<i>HTA12</i>	AT5G02560	13.34	15.32	0.20	0.56	0.93
	<i>HTA8</i>	AT2G38810	13.11	14.18	0.11	0.69	0.96
	<i>HTA9</i>	AT1G52740	133.18	110.22	-0.27	0.17	0.66
	<i>HTA11</i>	AT3G54560	38.77	41.66	0.10	0.67	0.96
<i>HTA4</i>	AT4G13570	5.94	5.22	-0.18	0.77	0.97	
Histone H2B	<i>HTB1</i>	AT1G07790	40.66	54.21	0.41	0.07	0.45
	<i>HTB2</i>	AT5G22880	151.69	189.51	0.32	0.10	0.55
	<i>HTB3</i>	AT2G28720	137.08	174.74	0.35	0.08	0.48
	<i>HTB4</i>	AT5G59910	181.31	196.89	0.12	0.54	0.93
	<i>HTB5</i>	AT2G37470	51.99	68.18	0.39	0.09	0.51
	<i>HTB6</i>	AT3G53650	8.31	33.70	2.02	0.00	0.00
	<i>HTB7</i>	AT3G09480	5.40	8.94	0.73	0.11	0.55
	<i>HTB8</i>	AT1G08170	0.00	0.00	0.00	1.00	1.00
	<i>HTB9</i>	AT3G45980	183.58	268.07	0.55	0.01	0.10
	<i>HTB10</i>	AT5G02570	1.63	3.20	0.97	0.22	0.73
	<i>HTB11</i>	AT3G46030	158.87	201.37	0.34	0.08	0.49
Histone H3	<i>HTR1</i>	AT5G65360	119.02	205.75	0.79	0.00	0.00
	<i>HTR2</i>	AT1G09200	154.17	272.70	0.82	0.00	0.00
	<i>HTR3</i>	AT3G27360	20.81	48.81	1.23	0.00	0.00
	<i>HTR9</i>	AT5G10400	48.68	58.86	0.27	0.23	0.74
	<i>HTR13</i>	AT5G10390	18.08	29.26	0.69	0.01	0.18
	<i>HTR4</i>	AT4G40030	336.33	79.26	-2.09	0.00	0.00
	<i>HTR5</i>	AT4G40040	426.33	197.87	-1.11	0.00	0.00

	<i>HTR8</i>	AT5G10980	208.31	9.62	-4.44	0.00	0.00
	<i>HTR6</i>	AT1G13370	0.10	0.50	2.30	0.15	0.63
	<i>HTR10</i>	AT1G19890	0.00	0.00	0.00	1.00	1.00
	<i>HTR14</i>	AT1G75600	0.14	0.27	0.97	0.58	1.00
	<i>HTR12</i>	AT1G01370	4.61	4.64	0.01	0.98	1.00
	<i>HTR11</i>	AT5G65350	1.22	2.50	1.04	0.12	0.59
	<i>HTR15</i>	AT5G12910	0.00	0.00	0.00	1.00	1.00
	<i>HTR7</i>	AT1G75610	0.00	0.47	1.79769E+308	0.02	0.24
Histone H4	<i>HF01</i>	AT3G46320	26.76	51.27	0.94	0.00	0.03
	<i>HF02</i>	AT5G59690	93.17	210.59	1.18	0.00	0.00
	<i>HF03</i>	AT2G28740	73.05	175.16	1.26	0.00	0.00
	<i>HF04</i>	AT1G07820	76.76	164.69	1.10	0.00	0.00
	<i>HF05</i>	AT3G53730	116.10	505.44	2.12	0.00	0.00
	<i>HF06</i>	AT5G59970	41.24	88.68	1.10	0.00	0.00
	<i>HF07</i>	AT3G45930	30.40	63.46	1.06	0.00	0.00
	<i>HF08</i>	AT1G07660	30.96	51.18	0.73	0.00	0.06

Supplemental Table 4: Limited overlap between H3.3 enrichment in WT and transcriptional changes in *h3.3kd*.

Location of H3.3 enrichment	Number of genes*	Overlap with misregulated genes	
		Downregulated (N=661)	Upregulated (N=269)
promoter-only (TSS +/-500 bp)	983	26 (2.6%)	16 (1.6%)
gene body-only (TSS +500bp to TTS)	9,035	175 (1.9%)	71 (0.8%)
promoter and gene body	9,860	283 (2.9%)	133 (1.3%)

*Overlap of all genes >500bp (N= 25,390) and defined H3.3 regions [18]

Supplemental Table 5: Primer sequences.

Purpose	Primer	Sequence
Cloning <i>amiR-HTR5-I</i>	I miR-s	gaTATCTATTTAGAAAACCGCCctctcttttgattcc
	II miR-as	gaGGGCGGTTTTCTAAATAGATAtcaagagaatcaatga
	III miR*-s	gaGGACGGTTTTCTATATAGATTtcacaggctgatgatg
	IV miR*-as	gaAATCTATATAGAAAACCGTCCctacatatattctct
Cloning <i>amiR-HTR5-II</i>	I miR-s	gaTTCCAGTAGACTTACGCGCTGtctcttttgattcc
	II miR-as	gaCAGCGGTAAGTCTACTGGAAtcaagagaatcaatga
	III miR*-s	gaCAACGCGTAAGTCAACTGGATtcacaggctgatgatg

	IV miR ⁺ -as	gaATCCAGTTGACTTACGCGTTGtctacatatattcct
amiR fusion with <i>attB</i>	<i>attB1-A</i>	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGATAAGCTTGATATCGAATTCCT
	<i>attB2-B</i>	GGGGACCACTTTGTACAAGAAAGCTGGGTGGCCGCTCTAGAAGCTAGTGGA
Cloning <i>HTR5</i>	<i>HTR5</i> -fwd	GGGGACAAGTTTGTACAAAAAAGCAGGCTGTATGGCTCGTACTAAGCAAACA
	<i>HTR5</i> -rev	GGGGACCACTTTGTACAAGAAAGCTGGGTAGCACGTTCTCCTCTGATCCTG
Mutagenesis <i>rHTR5</i>	<i>rHTR5</i> -fwd	CTAAGCAAACAGCTCGTAAAAGCACAGGAGGAAAGGCTCCTAGG
	<i>rHTR5</i> -rev	CCTAGGAGCCTTTCCTCCTGTGCTTTTACGAGCTGTTTGCTTAG
Mutagenesis <i>rHTR5-STOP</i>	<i>STOP</i> -fwd	GAGGAGAACGTGCTTAAAACCCAGCTTTCTTG
	<i>STOP</i> -rev	CAAGAAAGCTGGGTTTTAAGCACGTTCTCCTC
RT-PCR	<i>HTR4</i>	TCCCCTATTACTGCTTTTGTACG
		AAATCTCGGATTCTTGTTGG
	<i>HTR5</i>	TTTTCGAATTCGGAGCTCAA
		AGAAAACCACCCACATTCACA
	<i>HTR8</i>	CGAAAAGTCACATCTTTTGGAA
		GCGATAGGAAACAAGAATCCA