

Figure S1 Knockout strategy of target genes. (A) Strategy for single knockout. *hph*: hygromycin-resistance cassette. (B) Strategy for double knockout. *nat*: nourseothricin-resistance cassette.

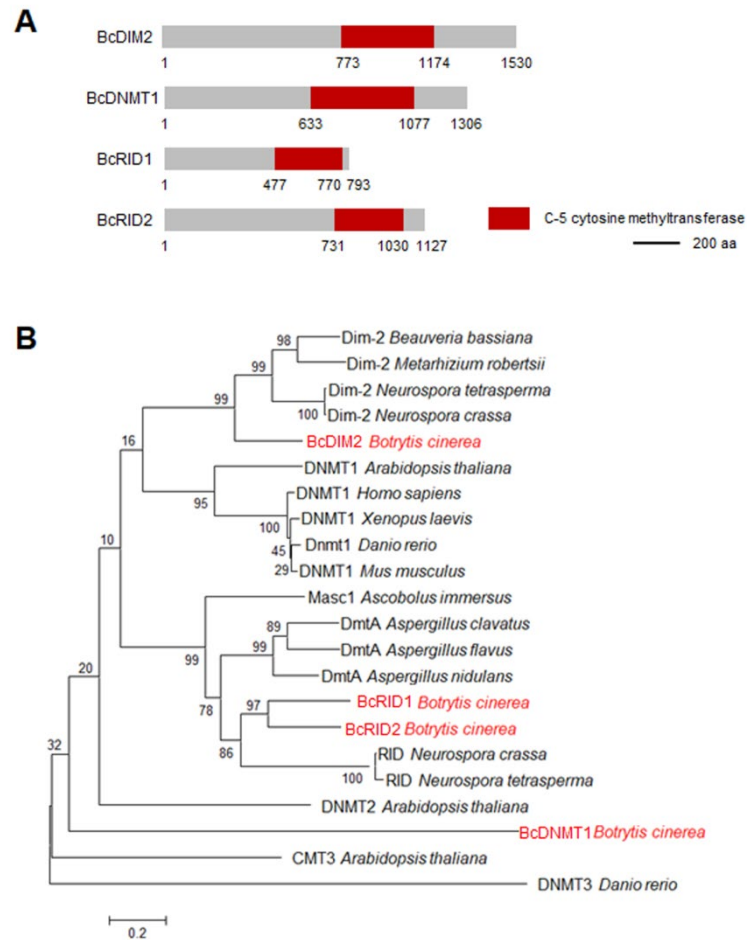


Figure S2 Analysis of DNA methyltransferases of *B. cinerea*. (A) Schematic diagram of conserved domain of C5 cytosine methyltransferase. (B) Phylogenetic tree of the of 5mC MTase proteins in *B. cinerea*.

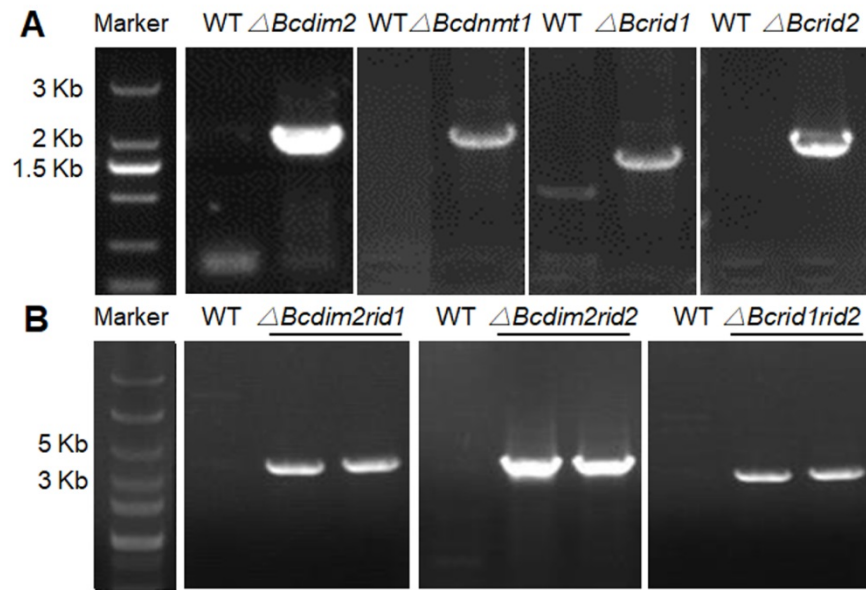


Figure S3 PCR diagnosis of knockout mutants. Flank-spanning PCR for single knockout mutants (A) and double knockout mutants (B).

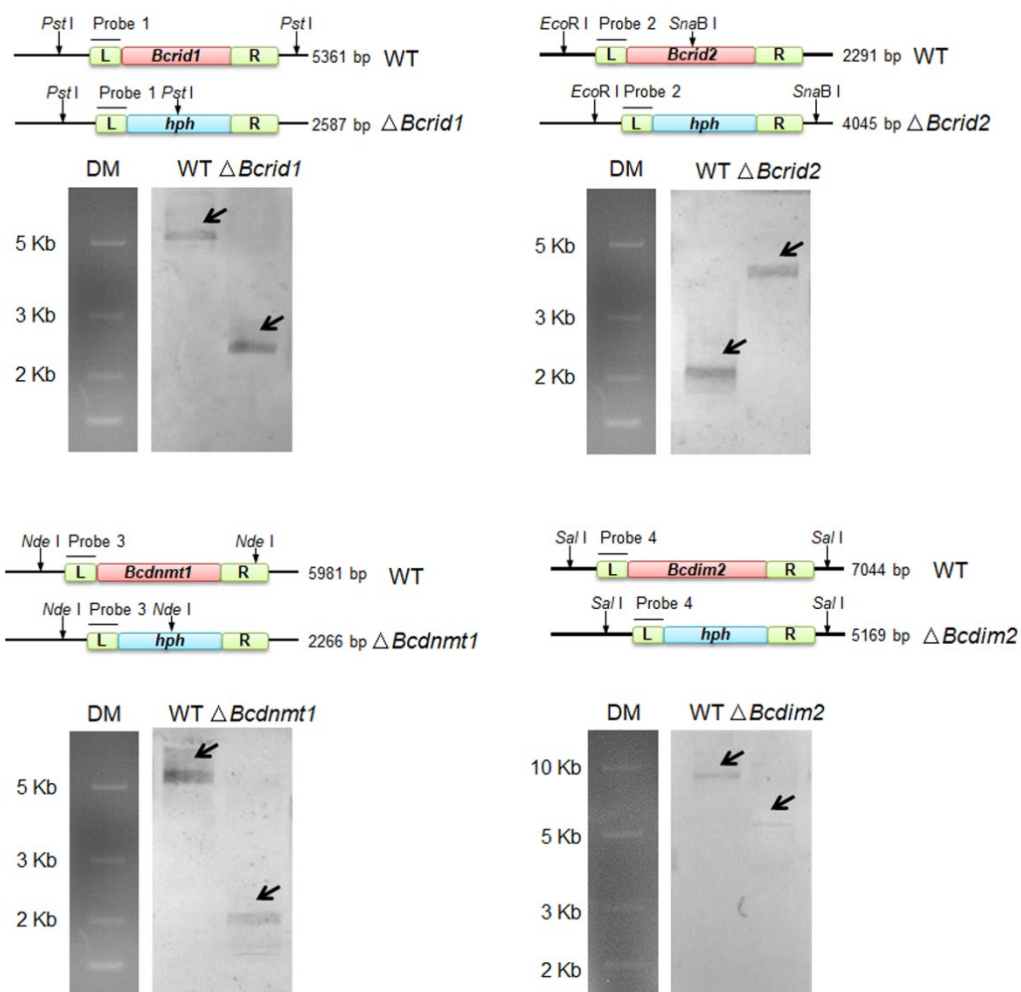


Figure S4 Southern blot analysis of DNA MTase mutants.

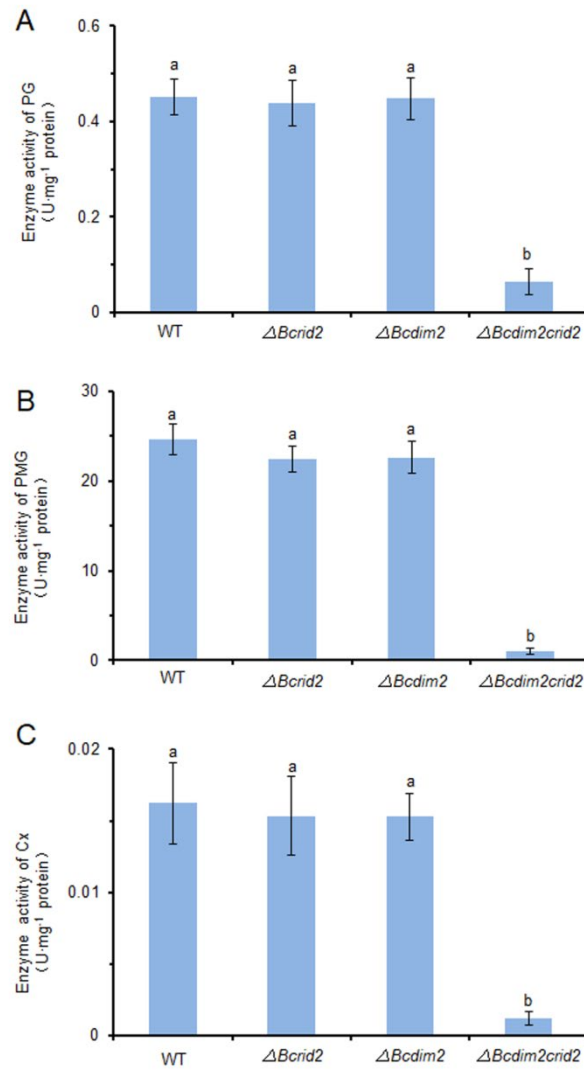


Figure S5 Activities of extracellular pathogenic proteins are down-regulated in double knockout mutant $\Delta Bcdim2rid2$. (A) Activity of polygalacturonase (PG). (B) Activity of poly-methyl galacturonate (PMG). (C) Activity of carboxymethyl cellulose (Cx).

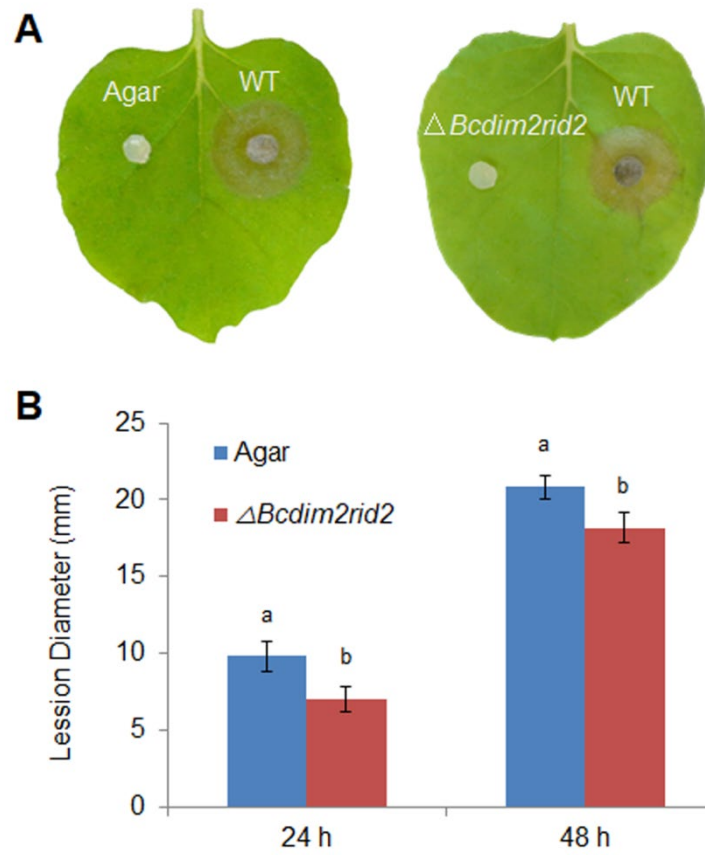


Figure S6 Pre-infection of $\Delta Bcdim2rid2$ induced the resistance of host. (A) Disease symptoms on detached tobacco leaves (48 hpi). (B) Lesion diameters on detached tobacco leaves.

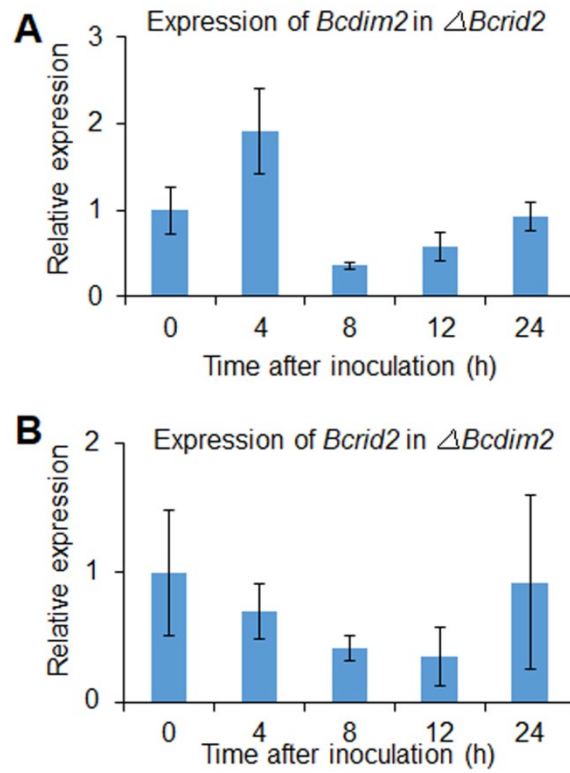


Figure S7 The expression pattern of *Bcdim2* in $\Delta Bcrd2$ (A) and the expression pattern of *Bcrd2* in $\Delta Bcdim2$ during conidial germination.

Table S1 The primers used for generation of knockout mutants.

Primers name	Sequence (5'→3')
Bcdim2-uF	CTCCACCAACTGCAATCACT
Bcdim2-uR	AATCGGAATGCGGCTCCACAATAAAGCCGAGCATACGAAA
Bcdim2-mF	TTTCGTATGCTCGGCTTTATTGTGGAGCCGCATTCCGATT
Bcdim2-mR	CTCTTCCCCTTTCTCTCCAAAGGCAACCATGCATGGTTAC
Bcdim2-dF	GTAACCATGCATGGTTGCCTTTGGAGAGAAAGGGGAAGAG
Bcdim2-dR	ATTAGTTTGGCGAGTGGATG
Berdnmt1-uF	CGATAACAAACCTCGGAATT
Bcdnmt1-uR	AATCGGAATGCGGCTCCACAATCTACGCAAACCTTGTCTCA
Bcdnmt1-mF	TGAGACAAGTTTGCGTAGATTGTGGAGCCGCATTCCGATT
Bcdnmt1-mR	CACATAGACGCTTGTCAATCAGGCAACCATGCATGGTTAC
Bcdnmt1-dF	GTAACCATGCATGGTTGCCTGATTGACAAGCGTCTATGTG
Bcdnmt1-dR	GAGCCAAGTGAGAAAGAACG
Berid1-uF	CTACCTTCTGCTCGGCTTCT
Berid1-uR	AATCGGAATGCGGCTCCACACCCTTACTGCCCTCTCTCTT
Berid1-mF	AAGAGAGAGGGCAGTAAGGGTGTGGAGCCGCATTCCGATT
Berid1-mR	TGTTTTCTGTATGCCAGTTTAGGCAACCATGCATGGTTAC
Berid1-dF	GTAACCATGCATGGTTGCCTAAACTGGCATAAGAAAACA
Berid1-dR	ATACCACAGAGGCTGAACAA
Berid2-uF	CAATCTTCCACTCCCTCATC
Berid2-uR	AATCGGAATGCGGCTCCACACAGTAGGTAGCAGGTCAAAC
Berid2-mF	GTTTGACCTGCTACCTACTGTGTGGAGCCGCATTCCGATT
Berid2-mR	CCATTCCCCTTCCACTTTTAGGCAACCATGCATGGTTAC
Berid2-dF	GTAACCATGCATGGTTGCCTAAAAGTGGAAGTGGAATGG
Berid2-dR	GTGAGTTAGTGGCTCGGATA
Bcdim2-uR2	ATGCGGCTCTAGAGGATCCCATAAAGCCGAGCATACGAAA
Bcdim2-mF2	TTTCGTATGCTCGGCTTTATGGGATCCTCTAGAGCCGCAT
Bcdim2-mR2	CTCTTCCCCTTTCTCTCCAACGCCGAGTTAGGCAACCATG
Bcdim2-dF2	CATGGTTGCCTAACTCGGCGTTGGAGAGAAAGGGGAAGAG
Berid1-uR2	ATGCGGCTCTAGAGGATCCCCCTTACTGCCCTCTCTCTT
Berid1-mF2	AAGAGAGAGGGCAGTAAGGGGGGATCCTCTAGAGCCGCAT
Berid1-mR2	TGTTTTCTGTATGCCAGTTTCGCCGAGTTAGGCAACCATG
Berid1-dF2	CATGGTTGCCTAACTCGGCGAAACTGGCATAAGAAAACA
Berid2-uR2	ATGCGGCTCTAGAGGATCCCCAGTAGGTAGCAGGTCAAAC
Berid2-mF2	GTTTGACCTGCTACCTACTGTGTGGAGCCGCATTCCGATT
Berid2-mR2	CCATTCCCCTTCCACTTTTAGGCAACCATGCATGGTTAC
Berid2-dF2	GTAACCATGCATGGTTGCCTAAAAGTGGAAGTGGAATGG
Bcdnmt5-uF	ATTGATAGGTGGACGAGACT
Bcdnmt5-uR	AATCGGAATGCGGCTCCACAGAGATGGAATGATTGAGAAA
Bcdnmt5-mF	TTTCTCAATCATTCCATCTCTGTGGAGCCGCATTCCGATT
Bcdnmt5-mR	ACAACATGAGCCTAACAGCTAGGCAACCATGCATGGTTAC

Bcdnmt5-dF	GTAACCATGCATGGTTGCCTAGCTGTTAGGCTCATGTTGT
Bcdnmt5-dR	CATATTTGAGTTCGCTGGAT
Bcdim2-detect-F	ACTGTATGGCCCCCTCTACC
Bcdnmt1-detect-F	CAAAGGTTATGAGGAAGGCC
Bcrd1-detect-F	TTGACAGCCAATTCCATACC
Bcrd2-detect-F	TTCCTCGTTCCTTGGGTGCT
Bcdnmt5-detect-F	TTTGCCGTTTCGTGTTATTGT
hph-detect-R	GTTTGCTGGTTACGCTTT
nat-detect-R	ATTCTATCCCTGGCTTTCCC

Table S2 The primer sequences for RT-qPCR.

Gene names	Primer names	Sequences (5'-3')
<i>Bcpgl</i>	Bcpgl-q-F	GATGTTGGTTCCTCCAGCGATA
	Bcpgl-q-R	CCGGAGTTGATAGCGAGACAGT
<i>Bcpg2</i>	Bcpg2-q-F	CGCCCCAGCAGATCTTGATA
	Bcpg2-q-R	AGCAGGTGGTCTTTGAGGCTAT
<i>Bcxyn11a</i>	Bcxyn11a-q-F	ACATACTCCAACGGAGCCAATG
	Bcxyn11a-q-R	CATCCTTTTCCACCGACGAA
<i>BcnoxA</i>	BcnoxA-q-F	TCGCGTCTCCGTTGTATGAA
	BcnoxA-q-R	TCCCTGTGGCTTGATTAACCA
<i>BcnoxB</i>	BcnoxB-q-F	GGTCCAAAAGTGTTGGGTTAGTCA
	BcnoxB-q-R	TTGCCCCAGCAGAAATTGA
<i>BcnoxD</i>	BcnoxD-q-F	AGTGATAGCAGGTGTTTGGGAAGCT
	BcnoxD-q-R	AGCACGCCATCATGTCCTTT
<i>BcnoxR</i>	BcnoxR-q-F	ACCCAATTCGTTCTGCTTCGT
	BcnoxR-q-R	ACCGAATGAAGATGCGGATAA
<i>Bcrho3</i>	Bcrho3-q-F	CTTCTTCCTCCTGCCAACCA
	Bcrho3-q-R	TGATTGCGAAGGAATGCCTAT
<i>Bcsod1</i>	Bcsod1-q-F	ATCTCCGGCACCGTCACTT
	Bcsod1-q-F	ATCTCCGGCACCGTCACTT
<i>Bcbmp1</i>	Bcbmp1-q-F	TCTTTCAATGTCAGCGAGCAA
	Bcbmp1-q-R	TGCAAAGCTGAGCAGACAACA
<i>BcG1</i>	BcG1-q-F	ACCACGGGTATCACCGAAAC
	BcG1-q-R	TCGGAACGTTGTCCTCCAA
<i>Bcbtp1</i>	Bcbtp1-q-F	GGCCGTGTTGTGATCGAAGT
	Bcbtp1-q-R	AGCTTTTCAGGGCGGATGT
<i>Bcplc1</i>	Bcplc1-q-F	TCCCGCAGGACTCGATAACT
	Bcplc1-q-R	TATGGCTTCCACTCGGGTTT
<i>Bcbos5</i>	Bcbos5-q-F	ACCCACTTCCATGGCAGAAA
	Bcbos5-q-R	CGGGTAACTGTGGTCGTTGTG
<i>Bcbac</i>	Bcbac-q-F	CCGTGTCGCTTACCAAGAAAC
	Bcbac-q-R	TTGCATCGCTGGTAGACCATT
<i>Bcras2</i>	Bcras2-q-F	TCTTCCCTCGACAGCGTTTG
	Bcras2-q-R	ACGCCGTATGCCACTCTTTT
<i>Bcmsb2</i>	Bcmsb2-q-F	CTCACTCCGACCTCACCTCTTC
	Bcmsb2-q-R	TTCGCTCTCGAGTTCGATTGT
<i>Bccrz1</i>	Bccrz1-q-F	CATGCAACAGCAGCCTATGG
	Bccrz1-q-R	GTGCAGCAGGTAAGCCGTAAT
<i>Bcste12</i>	Bcste12-q-F	CCCCACTCGATTTAGTTGACATC
	Bcste12-q-R	TCATATGCGCCATTCAATTGAG
<i>Bchox8</i>	Bchox8-q-F	ATGTCAATGATGCACCGAAGTG
	Bchox8-q-R	CCGGTACCATCATCGGAGAT
<i>Bcmads1</i>	Bcmads1-q-F	GAAGGCAAAGCTCCCTGGTA

	Bcmads1-q-R	TGATGGATTTGGCGCATACA
<i>Bcboa2</i>	Bcboa2-q-F	GGCTTGTCGAGACTACCGATCT
	Bcboa2-q-R	TGCTCCTAAAGCGCCGATAA
<i>Bcboa6</i>	Bcboa6-q-F	CTCTTACGCTTACATGCGTCACTT
	Bcboa6-q-R	CCAGTTAAACAAAGGACGGTGAT

Table S3. The statistics of whole-genome bisulfite sequencing for 12 samples

Samples	Clean data		Mapping rate	Conversion rate	Coverage	Coverage (d \geq 5)
WT-1	2.2	G bp	63.4%	99.6%	99.99%	94.4%
WT-2	2.1	G bp	61.8%	99.5%	99.99%	94.0%
WT-3	1.9	G bp	62.3%	99.6%	99.99%	92.3%
<i>ΔBcdim2-1</i>	2.1	G bp	76.6%	99.6%	99.99%	97.3%
<i>ΔBcdim2-2</i>	2.5	G bp	77.0%	99.6%	99.99%	98.5%
<i>ΔBcdim2-3</i>	2.1	G bp	78.5%	99.6%	99.99%	97.8%
<i>ΔBcrid2-1</i>	2.2	G bp	64.1%	99.6%	99.99%	96.1%
<i>ΔBcrid2-2</i>	2.3	G bp	66.3%	99.6%	99.99%	96.6%
<i>ΔBcrid2-3</i>	2.2	G bp	86.5%	99.6%	99.99%	98.5%
<i>ΔBcdim2rid2-1</i>	2.3	G bp	86.2%	99.7%	99.99%	98.8%
<i>ΔBcdim2rid2-2</i>	2.0	G bp	87.0%	99.6%	99.99%	96.6%
<i>ΔBcdim2rid2-3</i>	2.0	G bp	85.9%	99.6%	99.99%	96.2%

Table S4. Global DNA methylation levels of different strains

	C			CG			CHG			CHH		
	Repeat1	Repeat2	Repeat3	Repeat1	Repeat2	Repeat3	Repeat1	Repeat2	Repeat3	Repeat1	Repeat2	Repeat3
WT	0.38%	0.44%	0.36%	0.34%	0.40%	0.32%	0.37%	0.43%	0.36%	0.39%	0.45%	0.37%
<i>ΔBcdim2</i>	0.37%	0.39%	0.43%	0.33%	0.36%	0.39%	0.37%	0.40%	0.44%	0.38%	0.40%	0.44%
<i>ΔBcri2</i>	0.39%	0.39%	0.43%	0.36%	0.36%	0.39%	0.39%	0.40%	0.43%	0.40%	0.40%	0.44%
<i>ΔBcdim2rid2</i>	0.28%	0.43%	0.37%	0.25%	0.39%	0.34%	0.28%	0.43%	0.37%	0.29%	0.44%	0.39%

Table S5 The numbers of methylated cytosines in each sample

Samples	Total C	mC	mC%
WT-1	16851479	37	0.0002%
WT-2	16779126	35	0.0002%
WT-3	16486434	42	0.0002%
<i>ΔBcdim2-1</i>	17406799	34	0.0002%
<i>ΔBcdim2-2</i>	17625101	33	0.0002%
<i>ΔBcdim2-3</i>	17487257	38	0.0002%
<i>ΔBcrid2-1</i>	17178586	32	0.0002%
<i>ΔBcrid2-2</i>	17278470	45	0.0002%
<i>ΔBcrid2-3</i>	17620206	76	0.0002%
<i>ΔBcdim2rid2-1</i>	17667930	13	0.0002%
<i>ΔBcdim2rid2-2</i>	17255226	91	0.0002%
<i>ΔBcdim2rid2-3</i>	17196563	73	0.0002%