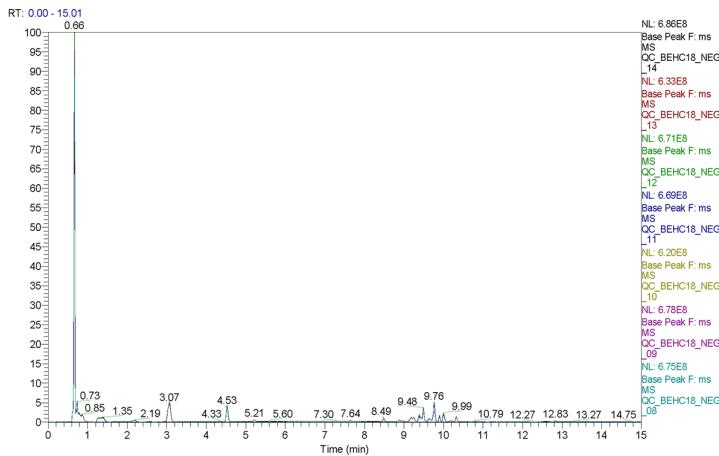


## Supplemental

### Supplemental Figures

Figure S1. Proteins identified in malpighian tubule samples in the (A) positive ion mode and (B) negative ion mode. y-axis, intensity.

(A)



(B)

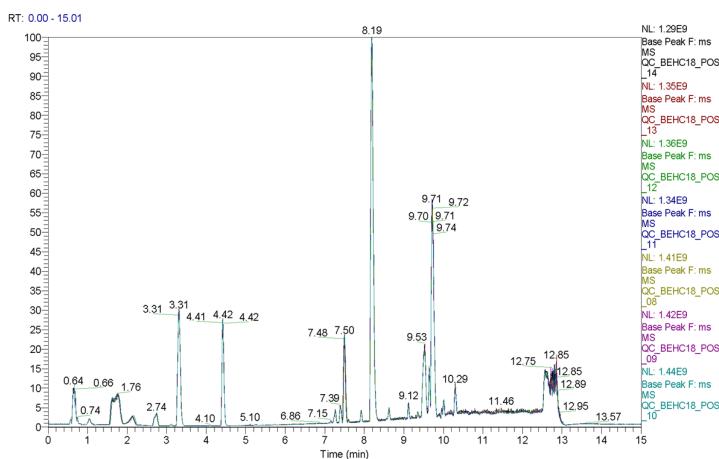
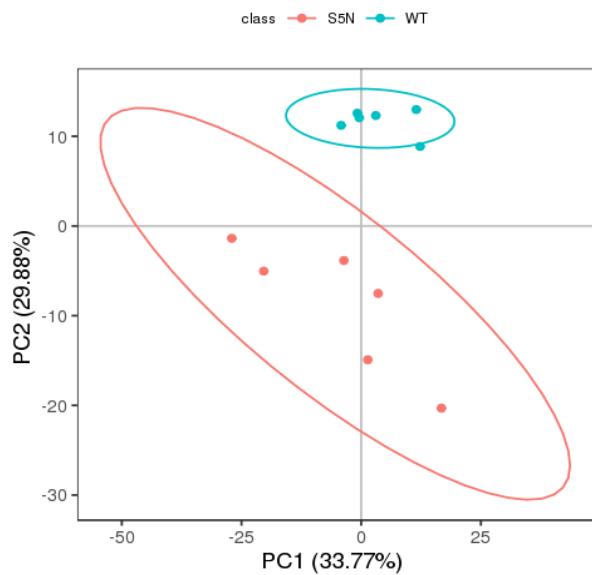


Figure S2. Principal component analysis of proteins differentially expressed in malpighian tubule samples identified in (A) positive ion mode and (B) negative ion mode.

(A)



(B)

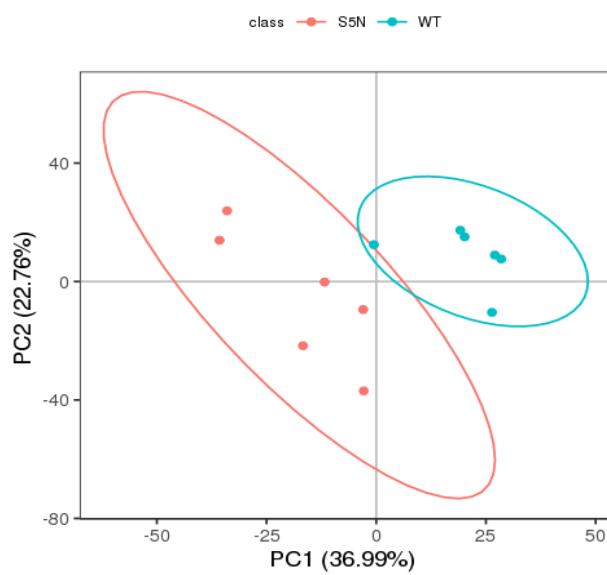
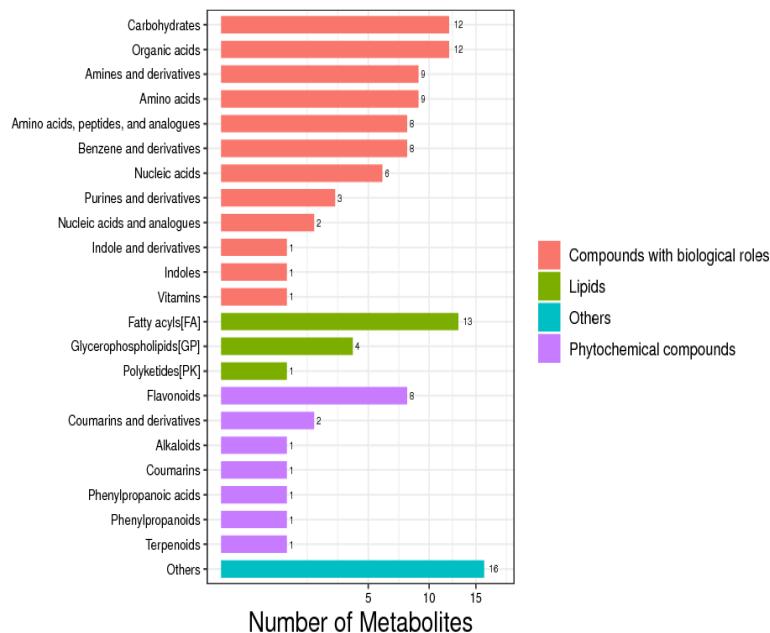


Figure S3. Classification of metabolites. (A) Classification of metabolites in positive ion mode. (B) Classification of metabolites in negative ion mode. x-axis, number of metabolites; y-axis, KEGG pathway annotations.

(A)



(B)

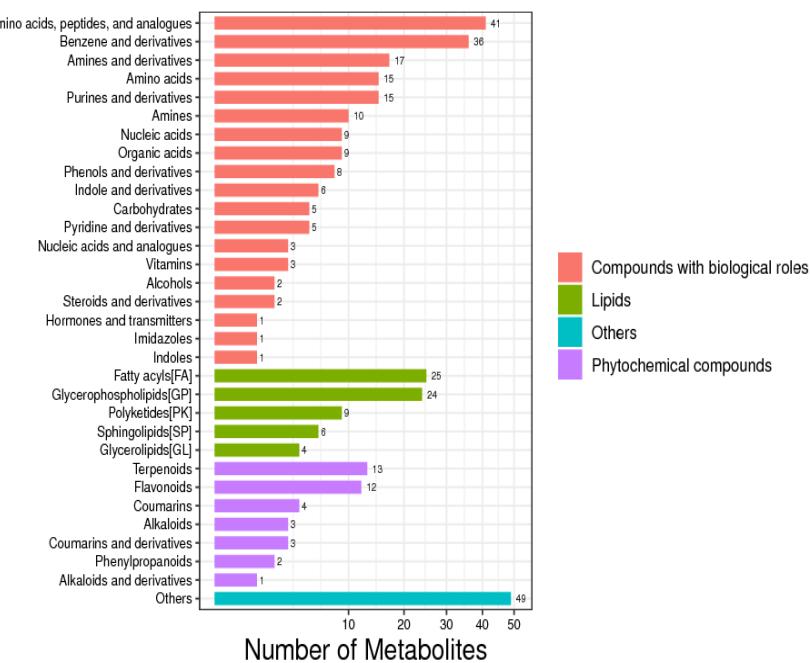
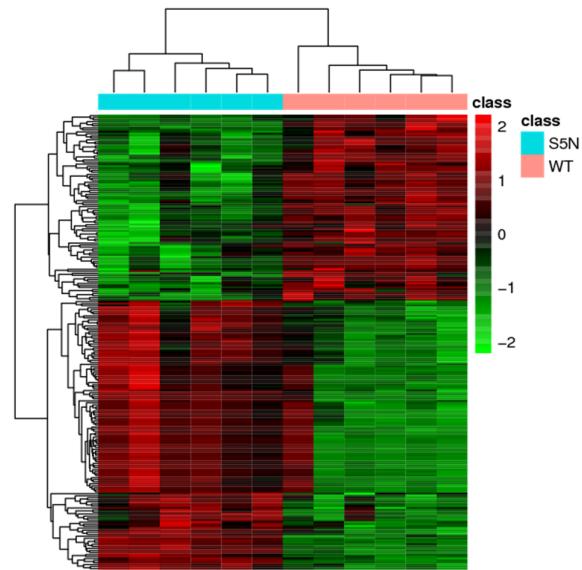


Figure S4. Hierarchical cluster analyses of the metabolite distribution in  $\Delta$ Bm5'N mutant and WT of metabolites detected in (A) positive ion mode and (B) negative ion mode. Each column represents an individual, and each row represents a metabolite. The color (from green to red) represents metabolite expression intensity from low to high.

(A)



(B)

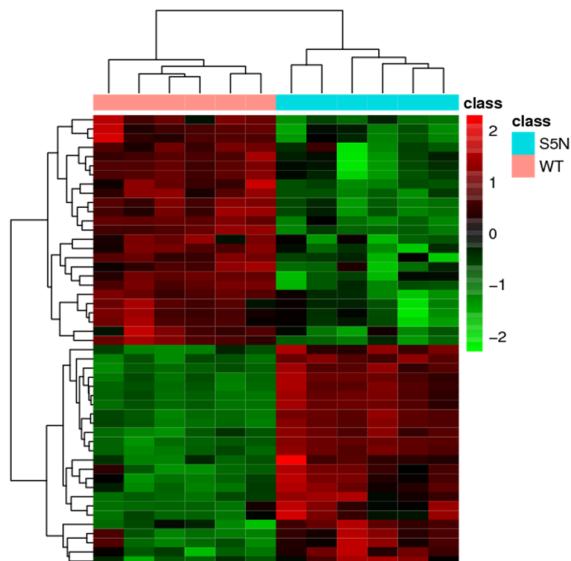


Figure S5. CRISPR/Cas9 induced mutagenesis of Bm5'N mutant. Target sites are shown as red squares. The deletion mutations were detected in the five mutant samples.

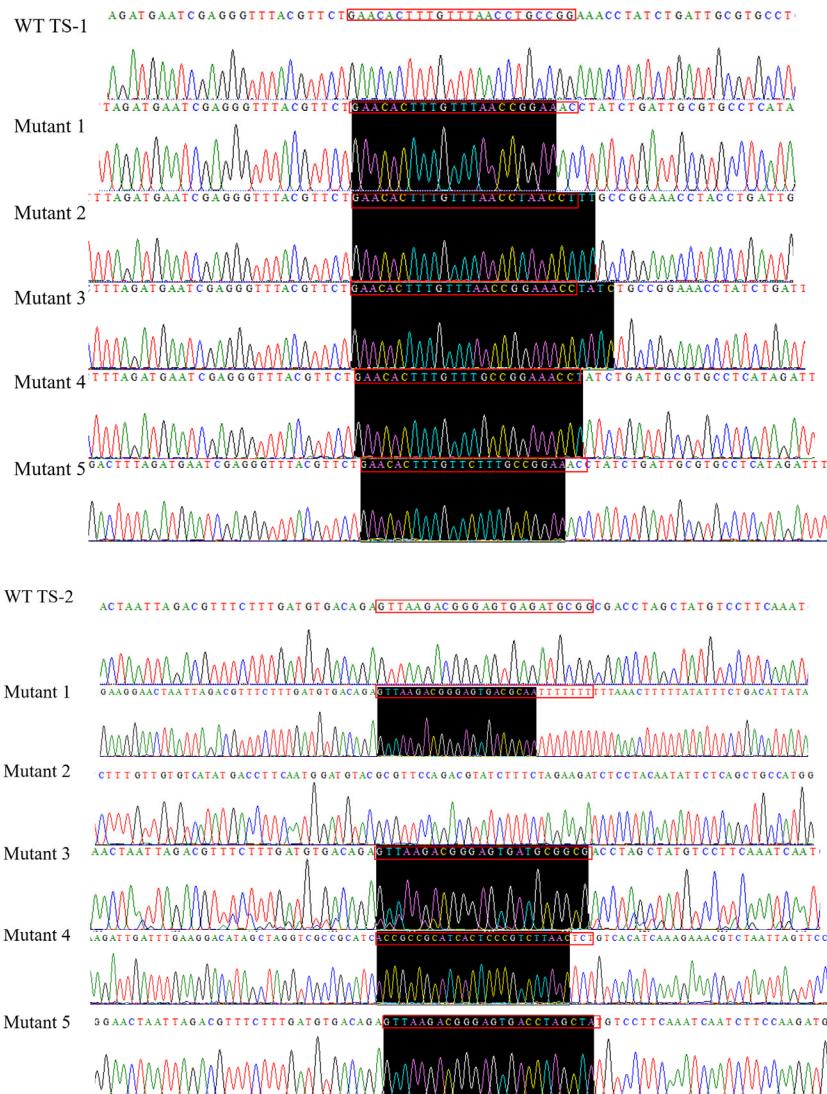
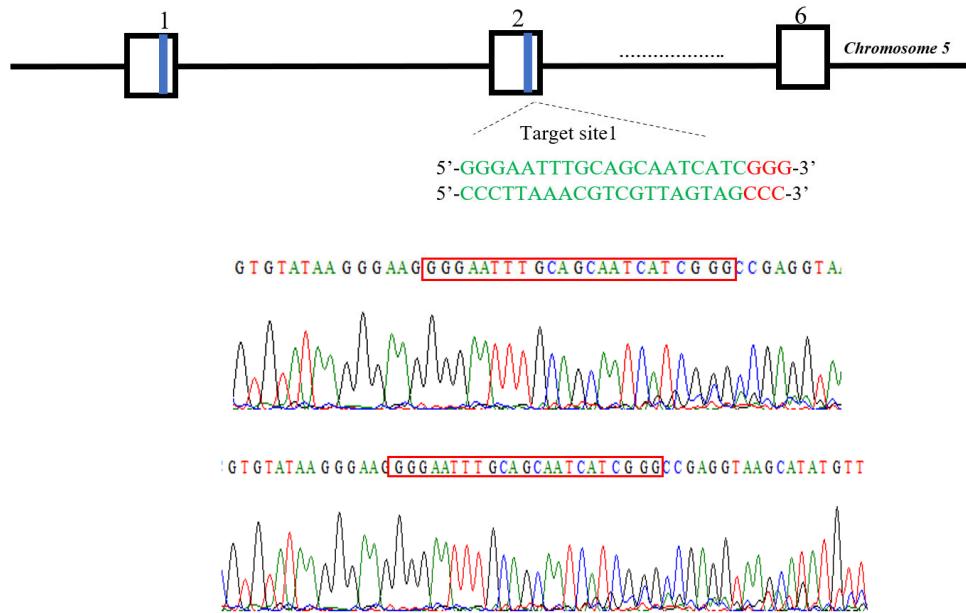


Figure S6. CRISPR/Cas9 induced mutagenesis of BmABCG5 mutant. Target sites are shown as red squares. The deletion mutations were detected in the five mutant samples.



**Table S1 Primers used in this work**

Primer name	Primer sequence(5' to 3')	The purpose
5N-CDS-F	ATGGACACCACTAGAATAATGC	CDS Clone
5N-CDS-R	TTATTGTGTTGCGCCGCCTT	CDS Clone
5N-qPCR-F	CGATGCCTACGGAAATATTTAGTG	qRT-PCR analysis
5N-qPCR-R	ATGCCGTGTATGTGAAACGT	qRT-PCR analysis
5N-TS1-F	AGTCAACTTAACAAAGTGAC	Identification of mutations
5N-TS1-R	ACGAGTACCATCCAGAGACT	Identification of mutations
5N-TS2-F	CTTGTGTCATATGACCTT	Identification of mutations
5N-TS2-R	AGAGTGGTCCCTCGCCGAAG	Identification of mutations
BmRP49-F	TCAATCGGATCGCTATGACA	qRT-PCR analysis
BmRP49-R	ATGACGGGTCTTCTTGTGG	qRT-PCR analysis
wh3-qPCR-F	AAATCGCAACTACGGAGCCA	qRT-PCR analysis
wh3-qPCR-R	CATGATGCCAGCAGTTCAC	qRT-PCR analysis
AB2-qPCR-F	TGTCGCCGCTAAACTAAAAT	qRT-PCR analysis
AB2-qPCR-R	ACTGTATCTAGTCCGCTAGTTGG	qRT-PCR analysis
AB3-qPCR-F	CACTGGGTCTGTACGAGCAT	qRT-PCR analysis
AB3-qPCR-R	AGTTCTTGTGGTGCCTGG	qRT-PCR analysis
AB4-qPCR-F	ACGAAGCAATGTCTCGCA	qRT-PCR analysis
AB4-qPCR-R	CCAGGAACATGAGGGATGGA	qRT-PCR analysis
AB5-qPCR-F	TCTAGTGTGTTAGCCGGGA	qRT-PCR analysis
AB5-qPCR-R	ATTGATTGTTCGGTTGCTTCGT	qRT-PCR analysis

AB8-qPCR-F	AGGTTGCGAACAAATGCGTC	qRT-PCR analysis
AB8-qPCR-R	TCCATTGTGAGTATGATAGCGGT	qRT-PCR analysis
KpnI-F	CGAGGTCGACGGTATCGATAAGGTTATGTAGTACAC ATTGTTGTA	Plasmid construction
U6-5NTS1-R	GCAGGGTAAACAAAGTGGTCACTTGTAGAGCACGAT ATTTTGTAT	Plasmid construction
U6-5NTS1-F	GAACACTTGTTAACCTGCCGTTTAGAGCTAGAAAT AGCAAGTT	Plasmid construction
overlap-R	CCGGGGAGTCAATGGCTAGAAAAAGCACCGACT CGGTG	Plasmid construction
overlap-F	GCTAGCCATTGACTCCCGGGAGGTTATGTAGTACAC ATTG	Plasmid construction
U6-5NTS2-R	CATCTCACTCCCGTCTAACACTTGTAGAGCACGATA TTTTGTAT	Plasmid construction
U6-5NTS2-F	GTAAAGACGGGAGTGAGATGGTTTAGAGCTAGAAA TAGCAAGTT	Plasmid construction
HindIII-R	TTTCCTGTTAGATATCAAAAAAGCACCGACTCG GTG	Plasmid construction
AD-CDS-F	ATGCTTCAGCTTCAAAGGTATTATGT	CDS Clone
AD-CDS-R	TCTTCCAATGATTAACCTCTAATAT	CDS Clone
AK-CDS-F	ATGGACGTTCTGATTCCATATGT	CDS Clone
AK-CDS-R	TCAGTCATTGATTGCTGGTC	CDS Clone
AMPD1b-CDS-F	ATGTCGAGGTCCGGCGACAG	CDS Clone
AMPD1b-CDS-R	TACGTACGCTAACAAAGTTGTCCAG	CDS Clone
ART1-CDS-F	ATGCCCCAACACGATTACATCAT	CDS Clone
ART1-CDS-R	CAGCCAAAGCTCTAATTTACAG	CDS Clone
PNP-CDS-F	ATGGCACCTATAAACCGCGAACGATAT	CDS Clone
PNP-CDS-R	TCATGGTCATCAGGGTCGGCCT	CDS Clone
PRPS1b-CDS-F	ATGCCAACATAAAAGTGTACTGG	CDS Clone
PRPS1b-CDS-R	TTAATATGGCACGTTAGTAAAAGATAC	CDS Clone
PRPS1-CDS-F	ATGTCTGTGCACAAGTCGAACCA	CDS Clone
PRPS1b-CDS-R	TTAATATGGCACGTTAGTAAAAGATAC	CDS Clone
Xan-CDS-F	ATGAAATCTAGTAATCAGATCAAC	CDS Clone
Xan-CDS-R	TTGCACCTATAATTGAGTTGACAT	CDS Clone
XDH-CDS-F	TAACGATAAACATTACACTGTGGAC	CDS Clone
XDH-CDS-R	CGGTGAATGGCAGATCGATATCT	CDS Clone
Xan2-CDS-F	ATGGTCATTTGTCTCGTTCACATC	CDS Clone
Xan2-CDS-R	GCTTCCTATCCTGGGTGAAATC	CDS Clone
ABCG5-sg1	TAATACGACTCACTATAGGGAATTGCAATCATC GTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCT AGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG GTGCTTT	Gene editing

AMPD1-F	ATGTGTTAATTGCAGGCAGTGAAAG	CDS Clone
AMPD1-R	CTAAGTGGTAGGTTGAGCTT	CDS Clone

**Table S2 Silkworm homologous gene ID**

Human gene	Silkworm homologous gene
phosphoribosyl pyrophosphate synthetase 1(PRPS1)	BGIBMGA001386
(PRPS1b)	BGIBMGA001570
adenosine monophosphate deaminase 1 (AMPD1)	BGIBMGA000806
(AMPD1b)	BGIBMGA000807
adenine phosphoribosyl transferase (ART1)	BGIBMGA011818
adenosine kinase (AK)	BGIBMGA007418
adenosine deaminase(AD)	BGIBMGA001278
purine nucleoside phosphorylase(PNP)	BGIBMGA011774
Xanthine Dehydrogenase(Xan)	BGIBMGA008244
(Xan2)	BGIBMGA008421
5'-nucleotidase (5N)	BGIBMGA011479
XDH xanthine dehydrogenase(XDH)	BGIBMGA008439