

Table S1. Mutation frequency of *BrPRR1a*, *BrPRR1b* and *BrPRR1ab* genes using CRISPR/Cas9 system.

| Target region | Sample | Total count | Insertion | Deletion | Indel | Indel frequency | Genotype | Mutation analysis | | | | | | | |
|----------------|--------------------|-------------|-----------|----------|-------|-----------------|--------------|-------------------|--------|--------|--------|-------|--------|-------|--------|
| BrPRR1a-sgRNA3 | <i>prr1a-g3-1</i> | 8436 | 0 | 8428 | 8428 | 99.91% | bi-allelic | 1 del | 65.20% | 2 del | 33.30% | | | | |
| | <i>prr1a-g3-2</i> | 7464 | 568 | 6896 | 7464 | 100.00% | hetero | 1 del | 49.30% | mosaic | | | | | |
| | <i>prr1a-g3-3</i> | 7035 | 0 | 7035 | 7035 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-4</i> | 6794 | 0 | 6794 | 6794 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-5</i> | 7661 | 0 | 7661 | 7661 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-6</i> | 7594 | 1043 | 6551 | 7594 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-7</i> | 6344 | 0 | 6344 | 6344 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-8</i> | 6976 | 0 | 6976 | 6976 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-9</i> | 5856 | 0 | 5856 | 5856 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-10</i> | 7159 | 0 | 7159 | 7159 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-11</i> | 7626 | 0 | 7626 | 7626 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-12</i> | 7436 | 539 | 6897 | 7436 | 100.00% | mosaic | | | | | | | | |
| | <i>prr1a-g3-13</i> | 7062 | 558 | 6504 | 7062 | 100.00% | mosaic | | | | | | | | |
| BrPRR1a-sgRNA4 | <i>prr1a-g4-1</i> | 8972 | 2959 | 6013 | 8972 | 100.00% | bi-allelic | 1 del | 63.50% | 24 ins | 34.20% | | | | |
| | <i>prr1a-g4-2</i> | 10321 | 0 | 10321 | 10321 | 100.00% | bi-allelic | 8 del | 53.00% | 1 del | 44.80% | | | | |
| | <i>prr1a-g4-3</i> | 11652 | 0 | 11652 | 11652 | 100.00% | bi-allelic | 8 del | 52.20% | 1del | 45.90% | | | | |
| | <i>prr1a-g4-4</i> | 10359 | 0 | 10353 | 10353 | 99.94% | bi-allelic | 8 del | 51.90% | 1 del | 45.90% | | | | |
| | <i>prr1a-g4-5</i> | 8369 | 2844 | 5525 | 8369 | 100.00% | bi-allelic | 1 del | 62.60% | 24 ins | 35.50% | | | | |
| | <i>prr1a-g4-6</i> | 9061 | 3138 | 5923 | 9061 | 100.00% | bi-allelic | 1 del | 61.80% | 24 ins | 35.90% | | | | |
| | <i>prr1a-g4-7</i> | 10651 | 2 | 10649 | 10651 | 100.00% | bi-allelic | 8 del | 50.90% | 1 del | 46.90% | | | | |
| | <i>prr1a-g4-8</i> | 10917 | 2001 | 8916 | 10917 | 100.00% | tri-allelic | 1 del | 52.80% | 12 del | 27.00% | 1 ins | 18.00% | | |
| | <i>prr1a-g4-9</i> | 10564 | 0 | 10564 | 10564 | 100.00% | bi-allelic | 26 del | 58.10% | 9 del | 39.60% | | | | |
| | <i>prr1a-g4-10</i> | 10303 | 0 | 10303 | 10303 | 100.00% | bi-allelic | 26 del | 55.70% | 9 del | 42.00% | | | | |
| | <i>prr1a-g4-11</i> | 9916 | 0 | 9916 | 9916 | 100.00% | bi-allelic | 26 del | 57.90% | 9 del | 39.50% | | | | |
| | <i>prr1a-g4-12</i> | 7381 | 3952 | 3424 | 7376 | 99.93% | Quad-allelic | 1 ins | 35.30% | 7 del | 26.40% | 1 del | 19.20% | 1 ins | 17.60% |
| | <i>prr1a-g4-13</i> | 10180 | 0 | 10180 | 10180 | 100.00% | bi-allelic | 26 del | 59.10% | 9 del | 38.60% | | | | |
| | <i>prr1a-g4-14</i> | 9433 | 2 | 9431 | 9433 | 100.00% | bi-allelic | 26 del | 67.80% | 9 del | 30.10% | | | | |
| | <i>prr1a-g4-15</i> | 7528 | 3589 | 3935 | 7524 | 99.95% | bi-allelic | 2 del | 51.30% | 1 ins | 46.70% | | | | |
| | <i>prr1a-g4-16</i> | 6734 | 3221 | 3511 | 6732 | 99.97% | bi-allelic | 2 del | 51.40% | 1 ins | 47.10% | | | | |

Continued

| Target region | Sample | Total count | Insertion | Deletion | Indel | Indel frequency | Genotype | Mutation pattern | | | | | | | |
|----------------|--------------------|-------------|-----------|----------|-------|-----------------|--------------|------------------|--------|--------|--------|-------|--------|--|--|
| BrPRR1b-sgRNA3 | <i>prrlb-g3-1</i> | 9903 | 3 | 9898 | 9901 | 99.98% | homo | 1 del | 99.10% | | | | | | |
| | <i>prrlb-g3-2</i> | 10586 | 0 | 10584 | 10584 | 99.98% | homo | 1 del | 98.10% | | | | | | |
| | <i>prrlb-g3-3</i> | 10074 | 0 | 10072 | 10072 | 99.98% | bi-allelic | 5 del | 49.50% | 7 del | 48.40% | | | | |
| | <i>prrlb-g3-4</i> | 10069 | 0 | 10069 | 10069 | 100.00% | bi-allelic | 5 del | 48.90% | 7 del | 48.70% | | | | |
| | <i>prrlb-g3-5</i> | 8573 | 0 | 8569 | 8569 | 99.95% | bi-allelic | 7 del | 49.70% | 5 del | 48.80% | | | | |
| | <i>prrlb-g3-6</i> | 9467 | 0 | 9467 | 9467 | 100.00% | bi-allelic | 5 del | 49.80% | 7 del | 48.40% | | | | |
| | <i>prrlb-g3-7</i> | 9447 | 4071 | 5368 | 9439 | 99.92% | hexa-allelic | 13 del | 22.40% | 5 del | 17.80% | 1 del | 15.60% | | |
| | | | | | | | | 1 ins | 15.10% | 1 ins | 14.30% | 1 ins | 13.40% | | |
| | <i>prrlb-g3-8</i> | 7780 | 3176 | 4573 | 7749 | 99.60% | mosaic | | | | | | | | |
| | <i>prrlb-g3-9</i> | 8373 | 3200 | 5170 | 8370 | 99.96% | hexa-allelic | 1 ins | 25.20% | 13 del | 19.50% | 3 del | 14.30% | | |
| | | | | | | | | 1 del | 14.20% | 1 del | 13.00% | 1 ins | 12.60% | | |
| BrPRR1b-sgRNA4 | <i>prrlb-g3-10</i> | 6337 | 2530 | 3733 | 6263 | 98.83% | mosaic | | | | | | | | |
| | <i>prrlb-g3-11</i> | 9467 | 0 | 9467 | 9467 | 100.00% | bi-allelic | 5 del | 49.80% | 7 del | 48.40% | | | | |
| | <i>prrlb-g4-1</i> | 6633 | 9 | 3432 | 3441 | 51.88% | hetero | 1 del | 50.80% | | | | | | |
| | <i>prrlb-g4-2</i> | 6289 | 6268 | 2 | 6270 | 99.70% | bi-allelic | 1 ins | 50.70% | 1 ins | 47.60% | | | | |
| | <i>prrlb-g4-3</i> | 6733 | 4415 | 2310 | 6725 | 99.88% | tri-allelic | 1 ins | 43.20% | 1 del | 33.70% | 1 ins | 21.45% | | |
| | <i>prrlb-g4-4</i> | 6504 | 0 | 6502 | 6502 | 99.97% | bi-allelic | 21 del | 49.30% | 9 del | 48.70% | | | | |
| | <i>prrlb-g4-5</i> | 10358 | 10346 | 2 | 10348 | 99.90% | bi-allelic | 1 ins | 49.20% | 1 ins | 49.20% | | | | |
| | <i>prrlb-g4-6</i> | 12878 | 6238 | 6636 | 12874 | 99.97% | bi-allelic | 1 del | 50.50% | 2 ins | 47.80% | | | | |
| | <i>prrlb-g4-7</i> | 11300 | 5545 | 4 | 5549 | 49.11% | hetero | 1 ins | 48.50% | | | | | | |
| | <i>prrlb-g4-8</i> | 8103 | 0 | 8093 | 8093 | 99.88% | bi-allelic | 21 del | 54.20% | 7 del | 44.00% | | | | |
| | <i>prrlb-g4-9</i> | 8283 | 0 | 8283 | 8283 | 100.00% | bi-allelic | 21 del | 56.20% | 7 del | 42.10% | | | | |
| | <i>prrlb-g4-10</i> | 7291 | 623 | 6668 | 7291 | 100.00% | mosaic | | | | | | | | |
| | <i>prrlb-g4-11</i> | 4732 | 1967 | 2765 | 4732 | 100.00% | mosaic | | | | | | | | |
| | <i>prrlb-g4-12</i> | 4341 | 431 | 3910 | 4341 | 100.00% | mosaic | | | | | | | | |
| | <i>prrlb-g4-13</i> | 6504 | 0 | 6502 | 6502 | 99.97% | bi-allelic | 21 del | 49.30% | 9 del | 48.70% | | | | |

Continued

| Target region | Sample | Total count | Insertion | Deletion | Indel | Indel frequency | Genotype | Mutation pattern | | | | | |
|-----------------|---------------------|-------------|-----------|----------|-------|-----------------|-------------|------------------|--------|--------|--------|-------|--------|
| BrPRR1ab-sgRNA2 | <i>prrlab-g2-1</i> | 5908 | 3702 | 2201 | 5903 | 99.92% | bi-allelic | 1 ins | 61.30% | 5 del | 36.90% | | |
| | <i>prrlab-g2-2</i> | 6429 | 6417 | 5 | 6422 | 99.89% | homo | 1 ins | 98.50% | | | | |
| | <i>prrlab-g2-3</i> | 7552 | 4424 | 3124 | 7548 | 99.95% | bi-allelic | 1 ins | 58.10% | 5 del | 41.00% | | |
| | <i>prrlab-g2-4</i> | 7363 | 4341 | 3015 | 7356 | 99.90% | bi-allelic | 1 ins | 58.10% | 5 del | 40.50% | | |
| | <i>prrlab-g2-5</i> | 7542 | 4340 | 3188 | 7528 | 99.81% | bi-allelic | 1 ins | 56.80% | 5 del | 41.60% | | |
| | <i>prrlab-g2-6</i> | 6818 | 3864 | 2949 | 6813 | 99.93% | bi-allelic | 1 ins | 56.10% | 5 del | 42.80% | | |
| | <i>prrlab-g2-7</i> | 1206 | 804 | 303 | 1107 | 91.79% | hetero | 1 ins | 52.30% | | | | |
| | <i>prrlab-g2-8</i> | 2915 | 1464 | 24 | 1488 | 51.05% | hetero | 1 ins | 49.50% | | | | |
| | <i>prrlab-g2-9</i> | 11319 | 3397 | 7908 | 11305 | 99.88% | tri-allelic | 2 del | 36.70% | 1 del | 32.10% | 1 ins | 29.60% |
| BrPRR1ab-sgRNA3 | <i>prrlab-g3-1</i> | 10773 | 26 | 6546 | 6572 | 61.00% | hetero | 14 del | 35.70% | | | | |
| | <i>prrlab-g3-2</i> | 10969 | 4507 | 4052 | 8559 | 78.03% | hetero | 1 ins | 40.10% | | | | |
| | <i>prrlab-g3-3</i> | 11992 | 6186 | 0 | 6186 | 51.58% | hetero | 1 ins | 50.40% | | | | |
| | <i>prrlab-g3-4</i> | 9181 | 4354 | 4710 | 9064 | 98.73% | mosaic | 10 del | 37.00% | | | | |
| | <i>prrlab-g3-5</i> | 8555 | 3567 | 2798 | 6365 | 74.40% | hetero | 1 ins | 39.80% | | | | |
| | <i>prrlab-g3-6</i> | 9095 | 3723 | 5363 | 9086 | 99.90% | tri-allelic | 1 ins | 39.90% | 14 del | 33.40% | 6 del | 24.60% |
| | <i>prrlab-g3-7</i> | 9642 | 4194 | 5446 | 9640 | 99.98% | tri-allelic | 1 ins | 42.20% | 14 del | 31.20% | 6 del | 24.30% |
| | <i>prrlab-g3-8</i> | 10980 | 41 | 4240 | 4281 | 39.0% | mixed | | | | | | |
| | <i>prrlab-g3-9</i> | 9379 | 4292 | 1797 | 6089 | 64.92% | hetero | 1 ins | 42.90% | | | | |
| | <i>prrlab-g3-10</i> | 1468 | 31 | 542 | 573 | 39.03% | mixed | | | | | | |
| | <i>prrlab-g3-11</i> | 1572 | 238 | 875 | 1113 | 70.80% | mixed | 12 del | 32.70% | | | | |
| | <i>prrlab-g3-12</i> | 1107 | 546 | 465 | 1011 | 91.33% | mixed | 1 ins | 49.80% | 14 del | 24.60% | | |
| | <i>prrlab-g3-13</i> | 716 | 640 | 71 | 711 | 99.30% | mixed | 1 ins | 41.30% | 1 ins | 33.40% | | |

Table S2. Predicted Off-target sites of each guide RNA in *B. rapa* genome using Cas-OFFinder (<http://www.rgenome.net/cas-offfinder/>) used in this study.

| Label | Sequences | Predicted off-target sites | | | | |
|------------------------|-------------------------|----------------------------|---|---|----|----------|
| | | 1 | 2 | 3 | 4 | mismatch |
| BrPRR1a-sgRNA3 | GCCTGACATCGATATAATACTGG | 0 | 0 | 0 | 0 | |
| BrPRR1a-sgRNA4 | GAGGCAAGACGAGGTCCCTGTGG | 0 | 0 | 0 | 3 | |
| BrPRR1b-sgRNA3 | CATCTTCATACCCTTGGCCATGG | 0 | 0 | 1 | 12 | |
| BrPRR1b-sgRNA4 | AGGTCCTCTCAGCGGGTTGGTGG | 0 | 0 | 0 | 4 | |
| BrPRR1ab-sgRNA2 | ATAAGAACACTGTGAAAGGAGGG | 0 | 0 | 2 | 22 | |
| BrPRR1ab-sgRNA3 | CTTGATAAGAACACTGTGAAAGG | 0 | 0 | 2 | 21 | |

Table S3. Sequences and position of predicted off-target sites with 3 bp mismatch.

| Label | Sequences | Position in genome | | Direction |
|----------------------|---|--------------------|----------|-----------|
| BrPRR1b-sgRNA3-off1 | CATCTTCATACC aa <u>TGGCC</u> t TGG | A06 | 2820332 | - |
| BrPRR1ab-sgRNA2-off1 | ATAA c AACAa <u>TGTGAAAGG</u> g TGG | A09 | 28541205 | + |
| BrPRR1ab-sgRNA2-off2 | A a AAGAACA t <u>TGTGAAAG</u> a AAGG | A09 | 35155954 | - |
| BrPRR1ab-sgRNA3-off1 | CTTG c TAAGAA <u>CACTGT</u> tt AAGG | A01 | 19835080 | + |
| BrPRR1ab-sgRNA3-off2 | a TTGA g AAGAA <u>CACTGT</u> t AAAGG | A08 | 10887388 | - |

Red low letters represented mismatch with gRNA and underbar showed seed region of gRNA.

Table S4. List of primers used in this study

| Primer name | DNA sequence (5'-3') | Purpose |
|-------------------|--|--------------------------|
| β-actin F | TGGCATCACACTTTTCTACAA | RT-qPCR |
| β-actin R | CAACGGAATCTCTCAGCTCC | |
| BrCCA1 F | TCTCTGTCACATGCTCCTCCTT | |
| BrCCA1 R | CGGCTAAGTTCCTTGTGG | |
| BrLHYa F | AGACATTCATGGAATCTTCTG | |
| BrLHYa R | GCGGTAATCATGCTGGAAAG | |
| BrLHYb F | CTGTATGCGCTCCTCTGCT | |
| BrLHYb R | TTGTCTTTGAGCCAGCGTTG | |
| 1a-sgRNA4 1st F | GTTTGAGCTGCTATCGATTGTT | Targeted deep-sequencing |
| 1a-sgRNA4 1st R | AAGCATTGAGCGCAAATAAG | |
| 1a-sgRNA4 2nd F | ACACTCTTTCCCTACACGAC GCTCTTCCGATCT CATTACTTTTCTCTACTTAGCAAAG | |
| 1a-sgRNA4 2nd R | GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT GCATAGAGAGAACTAAAGATAGA | |
| 1b-sgRNA3 1st F | AATGCATATGTAGTGTCTATTGCA | Targeted deep-sequencing |
| 1b-sgRNA3 1st R | TAGCATTCACAGCAACAAAGCT | |
| 1b-sgRNA3 2nd F | ACACTCTTTCCCTACACGAC GCTCTTCCGATCT CTTGAAACGCAGTGACTTCAG | |
| 1b-sgRNA3 2nd R | GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT CTAAGGAAATGATCATGCTGTC | |
| 1b-sgRNA4 1st F | AATGCATATGTAGTGTCTATTGCA | |
| 1b-sgRNA4 1st R | TAGCATTCAGCAACAAAGCT | |
| 1b-sgRNA4 2nd F | ACACTCTTTCCCTACACGAC GCTCTTCCGATCT TTTGATCTTGTGCCATCTGATC | |
| 1b-sgRNA4 2nd R | GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT CTGTTAGTGTCTGCTGCTA | |
| 1ab-sgRNA3 1st F1 | GTTTGAGCTGCTATCGATTGTT | Targeted deep-sequencing |
| 1ab-sgRNA3 1st F2 | GTTTGAGCTGCTATCGATTGTT | |
| 1ab-sgRNA3 1st R1 | AAGCATTGAGCGCAAATAAG | |
| 1ab-sgRNA3 1st R2 | AAGCATTGAGCGCAAATAAG | |
| 1ab-sgRNA3 2nd F1 | ACACTCTTTCCCTACACGAC GCTCTTCCGATCT TTTGATCTTGTGGGATCTGATC | |
| 1ab-sgRNA3 2nd F2 | GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT TTTGATCTTGTGGGATCTGATC | |
| 1ab-sgRNA3 2nd R1 | ACACTCTTTCCCTACACGAC GCTCTTCCGATCT GAGTATTGTGGATGGCTGTTT | |
| 1ab-sgRNA3 2nd R2 | GTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT GAGTATTGTGGATGGCTGTTT | |

