Figure S1. PCR amplicons of 3′RACE (**A**), 5′RACE (**B**), and in gDNA and cDNA (**C**) of *TaABCG36* from wheat TcLr19. M_1: DL2000 marker, M_λ: λDNA/HindIII Marker.

```
1      atggacgcgacggcggaaatccacaatgtcgccagcatgcggcgcgacagcgggtccatatggcggccggggacgacgtgttctcgcgatcgtcc
1      M  D  A  T  A  E  I  H  N  V  A  S  M  R  R  D  S  G  S  I  W  R  R  G  D  D  V  F  S  R  S  S

97     aggggacgaggacgacgaggaggcgctgcgctgggccgcgctcgagaaactgcccacctacgaccgcgtccgccgtgccatcctcccgcctctcgacggcggcgagggcgcggcccccggaggcggcggcggggaag
33     R  D  E  D  D  E  E  A  L  R  W  A  A  L  E  K  L  P  T  Y  D  R  V  R  R  A  I  L  P  P  L  D  G  G  E  G  A  A  P  G  A  A  A  G  K

232    ggggtcgttgacgtgcacggcctcgggccgcgagcgccgggctcttcctcgagcgcctcgtgcgcgtcgccgacgaggacaacgagcgcttcctgctcaagctcaaggaccgcctcgagcgggttgggattgaa
78     G  V  V  D  V  H  G  L  G  P  R  E  R  R  A  L  L  E  R  L  V  R  V  A  D  E  D  N  E  R  F  L  L  K  L  K  D  R  L  E  R  V  G  I  E

367    atggccgacgatcgaggtgccggttcgagcacctggtggcggaggcggaggtccgggtcggcaacagcggcctcccaccgtcctcaactccatcaccaacacgctcgaggaagcggcgaacgcgctcgcgcatactc
123    M  P  T  I  E  V  R  F  E  H  L  V  A  E  A  E  V  R  V  G  N  S  G  L  P  T  V  L  N  S  I  T  N  T  L  E  E  A  A  N  A  L  R  I  L

502    cccaacaggaagcggaccatgcccatcctccacgacgtcagtggcatcatcaagcccgcaggatgacactcctgttgggcccacctgggtcaggcaagaccaccttgctgctcgcctttggccggaggctcgac
168    P  N  R  K  R  T  M  P  I  L  H  D  V  S  G  I  I  K [P  R  R  M  T  L  L  L |G  P  P  G  S  G  K  T  T| L  L  L  A  L  A  G  R  L  D]
                                                                                 Walker A

637    aaagacctcaaggtctcaggcaatgttacctacaacgggcacggatggaggagtttgtgccggagaggacggcggcgtacataagccagcagcgacctccacataggggagatgactgtgagggagacactgcc
213    K  D  L  K  V  S  G  N  V  T  Y  N  G  H  G  M  E  E  F  V  P  E  R  T  A  A  Y  I  S  Q  H  D  L  H  I  G  E  M  T  V  R  E  T  L  A

772    ttctccgcacgctgccagggtgttggcactcgctttgatatgctgactgagttgtcgaggcgagagaaggccgcaaatatcaagccagatgctgatattgcatttatgaaggcttcttcaatggggaggtctg
258    F  S  A  R  C  Q  G  V  G  T  R  F  D  M  L  T  E  L  S  R  R  E  K  A  A  N  I  K  P  D  A  D  I  D  A  F  M  K  A  S  S  M  G  G  L

907    gaggccaatgtgaacacagactatatactgaagatattaggactagaagatgtgtgcagacacgatggttggggacgagatgctcagggggcatttctggtggacaacggaagcgtgttacaactggtgagatgctg
303    E  A  N  V  N  T  D  Y  I  L  K  I  L  G  L  E  M  C  A  D  T  M  V  G  D  E  M  L  R [G  I  S  G  G  Q  R  K  R  V  T  T  G  E  M  L]
                                                                                         Walker C (ABC transporter signature)

1042   gttgggccggccaaggccgctcttcatggacgagatctcaactggctcgacagctcaactacatttcagattgtaaactcactcaggcaatctgtgcacatccttggtggcacagccgtcatctccctgctgcag
348    V  G  P  A  K [A  L  F  M  D  E] I  S  T  G  L  D  S  S  T  T  F  Q  I  V  N  S  L  R  Q  S  V  H  I  L  G  G  T  A  V  I  S  L  L  Q
                     Walker B

1177   ccagccccctgaaacgtacaacttat[tcgatgacatcatcctcctctctgatggccaagtcgtgtaccaggcgcctcgtgacgacgtgcttgaattcttcgagtctgtgggttcaagtgtcctgaaaggaagggc]
393    P  A  P  E  T  Y  N  L  F  D  D  I  I  L  L  S  D  G  Q  V  V  Y  Q  G  P  R  E  D  V  L  E  F  F  E  S  V  G  F  K  C  P  E  R  K  G

1312   [gtcgccgacttcttgcaagaggtgacttcgagaaagatcaaaagcaatactgggtgc]ggggtgatgagctcctaccggttcgtgccggttaaggactttgtgcgtgcattcagtgcattatgaaggctcttcatgaaggtctg
438    V  A  D  F  L  Q  E  V  T  S  K  K  D  Q  K  Q  Y  W  V  R  G  G  D  E  S  Y  R  F  V  P  V  K  D  F  V  R  A  F  Q  S  F  H  T  G  R  A

1447   ataagaaaggaggctcgctgtgccattcgacagagcaagagccatccagcagcattgacaactacgaggtatggtgtgagtggcacggagctgctaaaggcaaacattggcaggagagatcctcctcatgaaaagg
483    I  R  K  E  L  A  V  P  F  D  K  S  K  S  H  P  A  A  L  T  T  T  R  Y  G  V  S  G  T  E  L  L  K  A  N  I  G  R  E  I  L  L  M  K  R

1582   aactcttttcgtctgcatgtttagtaacttccagctgatcctcatgtcattcattattgcaatgacgctcttcttccgtacaaagatgaagccggactcggtgacaaatggggggcatctacatggggggcactcttcttt
528    N  S  F  V  C  M  F  R  T  F  Q  L  I  L  M  S  F  I  A  M  T  L  F  F  R  T  K  M  K  R  D  S  V  T  N  G  G  I  Y  M  G  A  L  F  F

1717   ggtgtgctcatgatcatgttcaatggcttctctgagcttgcactcaccgtcttttaagctacccgtgttctctacccagcgatggataccctcatggatcctcaag
573    G  V  L  M  I  M  F  N  G  F  S  E  L  A  L  T  V  F  K  L  P  V  F  F  K  Q  R  D  L  L  F  Y  P  A  W  A  Y  T  I  P  S  W  I  L  K

1852   atcccaataacatttgttgaggttggcggttatgttttcataacatactatgtcatggggttcgacccaaatgtgaggcaggttcttcaagcaatatcttcttatgttagcaatcaatcagatgcggcatcactc
618    I  P  I  T  F  V  E  V  G  G  Y  V  F  I  T  Y  Y  V  M  G  F  D  P  N  V  G  R  F  F  K  Q  Y  L  L  M  L  A  I  N  Q  M  A  A  S  L

1987   ttccgattcattggtggagcaggcaaagaacatgattgttgcgaatgtcttgcatcattcatgctgtcattttatggtattgggcggattcattctagtaagagaaaaagtgaagaaatggtggattggggc
663    F  R  F  I  G  G  A  A  R  N  M  I  V  A  N  V  F  A  S  F  M  L  L  I  F  M  V  L  G  G  F  I  L  V  R  E  K  V  K  K  W  W  I  W  G

2122   tactggatctcccactaatgtatgcacagaatgccatctcagtgaatgagttcttcggtcacagctgggataaaatatgaatagcacggcctcaaacgagaccctggagtgcaagtcctgaagtatcgcgga
708    Y  W  I  S  P  L  M  Y  A  Q  N  A  I  S  V  N  E  F  F  G  H  S  W  D  K  I  L  N  S  T  A  S  N  E  T  L  G  V  Q  V  L  K  Y  R  G

2257   gtgttccagaagcaaagtggtactggatcggtttgggtgcaatgctcgggtacaccttgttgttcaatgctctcttcactcttgccctttacatacctgaaggcatacgtgaactcccgttcgtcggtatctgaa
753    V  F  P  E  A  K  W  Y  W  I  G  L  G  A  M  L  G  Y  T  L  L  F  N  A  L  F  T  L  A  L  T  Y  L  K  A  Y  G  N  S  R  S  S  V  S  E

2392   gacgaactgaaggagaagcatgccaacctgaacggtgaagttctggacaatgatcgacagtcctgccggattcgatggaacactgataacgatcaggaatgactcagcaatagtcagaagaaattctagc
798    D  E  L  K  E  K  H  A  N  L  N  G  E  V  L  D  N  D  R  M  L  S  P  S  N  D  G  P  I  R  M  N  T  G  N  D  S  A  I  V  E  E  N  S  S

2527   cccatgcaaagggggatggtggtgcttccatttctcccgcttttcactcacctttgacaacatcagatactctgttgacatgcacactgga[atgaagcacaaggcgtagttgaagaccgattggagctcctcaaaggt]
843    P  M  Q  R  G  M  V  L  P  F  L  P  L  S  L  T  F  D  N  I  R  Y  S  V  D  M  P  L  E  M  K  A  Q  G  V  V  E  D  R  L  E  L  L  K  G

2662   [gttagtggatctttcaggccaggggtactgacctgccctgatggggtgtcagttggttgctggcaagactactctgatggatgttgtgcagggagaaagacaggtgggtacattcaaggaaacatcagcattt]cagga
888    V  S  G  S  F  R  P  G  V  L  T  A  L  M [G  V  S  G  A  G  K  T  T] L  M  D  V  L  A  G  R  K  T  G  G  Y  I  Q  G  N  I  S  I  S  G
                                               Walker A

2797   [tacccgaagaaacaagagacctttgcacg]cgtatcaggatcaggacgaatgatatcctccgcaggtgacagtctatgagtcgctgctttttctcagcatggctccgtctccccgaggatgttggattca
933    Y  P  K  K  Q  E  T  F  A  R  V  S  G  Y  C  E  Q  N  D  I  H  S  P  Q  V  T  V  Y  E  S  L  L  F  S  A  W  L  R  L  P  E  D  V  D  S

2932   aacaaaagaaggatgtcatcgagaggtgatggacttgtggagctcaagcctct[caaagatgcattggttggtctaccggagtgaatggtctgtcaactgaacagaggagagaggctgaccattgctgtggag]
978    N  K  R  R  R  M  F  I  E  E  V  M  E  L  V  E  L  K  P  L  K  D  A  L  V  G  L  P  G  V  N [G  L  S  T  E  Q  R  K  R  L  T  I  A  V  E]
                                                                                               Walker C (ABC transporter signature)

3067   [ctcgttgctaatccttctatcatttttcatggatgagccaacctccggacttgatgcccgagcagccgcaatcgtgatgaggacagtgaggaacactgttgatactggtagaacgtgtggtttgcacga]ttcatcaa
1023   L  V  A  N  P  S [I  I  F  M  D  E] P  T  S  G  L  D  A  R  A  A  A  I  V  M  R  T  V  R  N  T  V  D  T  G  R  T  V  V  C  T  I  H  Q
                       Walker B

3202   cctagcattgacatatttgaagcatttgatgagctattcctgatgaagcgaggtgagaagagatctatgctggtccattgggccatcattctgcagacttaatcaattactatgagggcattcatggggtcagc
1068   P  S  I  D  I  F  E  A  F  D  E  L  F  L  M  K  R  G  G  E  E  I  Y  A  G  P  L  G  H  H  S  A  D  L  I  N  Y  Y  E  G  I  H  G  V  S

3337   aaaattaaggatggctacaatccggcaacatggatgttggaagtgacaacgatcggtcaagaacagatgctaggcattgattcagtgacatatacaagaaatctgaactctaccagagaaacaaggcattgata
1113   K  I  K  D  G  Y  N  P  A  T  W  M  L  E  V  T  T  I  G  Q  E  Q  M  L  G  I  D  F  S  D  I  Y  K  K  S  E  L  Y  Q  R  N  K  A  L  I

3472   aaggaacagagccaaccccgcgccgggttcaactgatctgtatttccctacccagtactcgcagtcttctcgatcacacaatgcatggctttgctgtggaagcagaaccctgcttacaat
1158   K  E  Q  S  Q  P  A  P  G  S  T  D  L  Y  F  P  T  Q  Y  S  Q  S  S  I  T  Q  C  M  A  C  L  W  K  Q  N  L  S  Y  W  R  N  P  Y  N

3607   gccgttaggctcctttttcactactgtgattgctctcctctttcggccaccatcttctgggacctttggtggcaaaatgagccagtcacaagactgttcaacgccatgggtcaatgtatgctgcagtgctgttcatt
1203   A  V  R  F  L  F  T  T  V  I  A  L  L  F  G  T  I  F  W  D  L  G  G  K  M  S  Q  S  Q  D  L  F  N  A  M  G  S  M  Y  A  A  V  L  F  I

3742   ggtattatgaactgtacttcagttcaaccagtggtggctgtggaggacagtctttaccgtgaaagagcagccggacatgtactcagcattcccgtacgcattcgggcaggtggtcatcgaactcccatacaca
1248   G  I  M  N  C  T  S  V  Q  P  V  V  A  V  E  R  T  V  F  Y  R  E  R  A  A  G  M  Y  S  A  F  P  Y  A  F  G  Q  V  V  I  E  L  P  Y  T

3877   ctcgctcaggctaccgtatacggagtcatagtttactcgatgatttgggtttgagtggacagctcctaaattcttctggtacctcttcttcatgtacttcacgctgctctacttcacatctacggcatgatggct
1293   L  A  Q  A  T  V  Y  G  V  I  V  Y  S  M  F  W  Y  L  F  F  M  Y  F  T  L  L  Y  F  T  F  Y  G  M  M  A

4012   gttggcctgacacccaactaccacatcgcctcaatcgtctcgtcagcattctacgccatctggaatctcttctcaggattcatcatccccgcaccaaaagttccaatctggtggagatggtattgctgggtatgc
1338   V  G  L  T  P  N  Y  H  I  A  S  I  V  S  S  A  F  Y  A  I  W  N  L  F  S  G  F  I  I  P  R  P  K  V  P  I  W  W  R  W  Y  C  W  V  C

4147   ccgtagcgtggacactgaccgctggtgtctcacagtttggcgatgtccacaagtttcgactgaaagactttatagaggctgacttcgactcaagcacagctggttaggttatgtt
1383   P  V  A  W  T  L  Y  G  L  V  V  S  Q  F  G  D  V  T  T  P  M  E  D  G  T  P  V  K  D  F  I  E  G  Y  F  D  F  K  H  S  W  L  G  Y  V

4282   gctacggtggtcgttggcggttgcggtgctgtttgccttcttgttcggtttttgccatcatgaagctcaacttccagaagaggtag  4365
1428   A  T  V  V  V  A  F  A  V  L  F  A  F  L  F  G  F  A  I  M  K  L  N  F  Q  K  R  *  1454
```

Figure S2. Analysis of nucleotide and predicted amino acid sequences of TaABCG36. Nucleotide-binding domains are shown in grey; Walker A, Walker B and Walker C are boxed; Transmembrane segments are underlined; Silence site of V1 (yellow), V2 (blue), and V3 (green) are highlighted.

Table S1. Off-target prediction of the three BSMV fragments by si-Fi software.

Targets	BSMV-V1		BSMV-V2		BSMV-V3	
	Total siRNA Hits	Efficient siRNA Hits	Total siRNA Hits	Efficient siRNA Hits	Total siRNA Hits	Efficient siRNA Hits
TraesCS3D1G234400.1	149	72	185	89	177	82
TraesCS3B1G248400.1	146	72	165	77	156	70
TraesCS3A1G217900.1	59	29	186	89	99	49

Table S2. Statistically analysis of the VIGS TcLr19 plants.

	Replication 1		Replication 2		Replication 3		Silenced Ratio (%)
	Total Plants	Silenced Plants	Total Plants	Silenced Plants	Total Plants	Silenced Plants	
BSMV-V1	15	11	16	11	14	9	68.3[a]†
BSMV-V2	18	12	17	13	18	11	69.3[a]
BSMV-V3	17	11	16	10	15	11	66.0[a]

† Note: Different lowercase letters marked after the mean values identify significantly different means (Duncans' new multiple-range test ($p < 0.05$, $n = 3$).