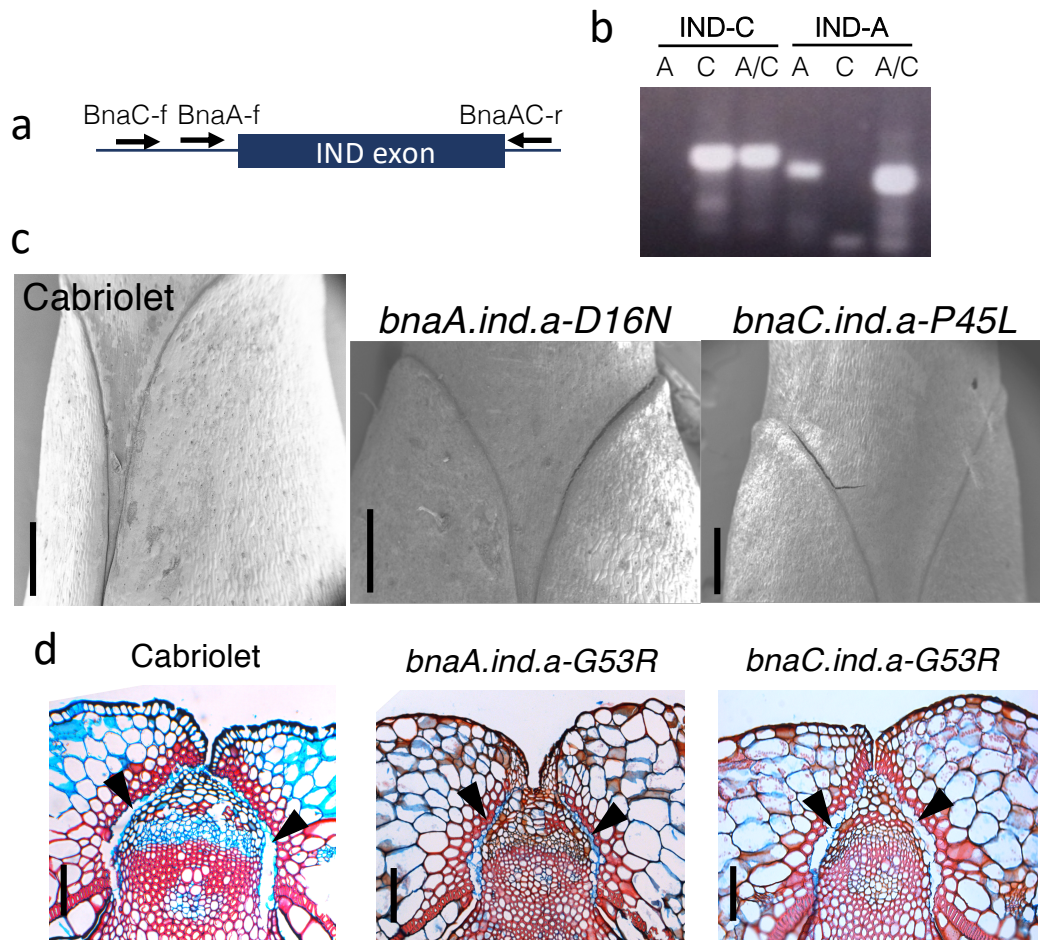Online Resource 1

```
HEC         ------MNNYNMNPSLFQNYTWNNIINSSNNNNK--NDDHHHQHNNDPIGMAMDQYTQLHI   53
IND         MMEPQPHHLLMD---------WNKANDLLTQEHAAFLNDPHHLMLDPP-------------   39
BraA.IND.a  MME--HHHLLMN---------WNKPIDLITEENS--FNHNPHFIVDPP-------------   35
BnaA.IND.a  MME--HHHLLMN---------WNKPIDLITEENS--FNHNPHFIVDPP-------------   35
BolC.IND.a  MME--PHHLLMN---------WNKPIDLITQENS--FNHNPHFMVDPP-------------   35
BnaC.IND.a  MME--PHHLLMN---------WNKPIDLITQENS--FNHNPHFMVDPP-------------   35
                   ::   *:         **:  :   .::::    :   *    : *

HEC         FNPFSSSHFPPLSSSLTTTTLLSGDQEDDEDEEEPLEELGAMKEMMYKIAAMQSVDIDPA  113
IND         --PETLIHLD------------------EDEEYDEDMDAMKEMQYMIAVMQPVDIDPA    77
BraA.IND.a  --SETLSHFQPPPTIFSDHG--GGEEAEEEEEEEGEEEMDPMKKMQYAIAAMQPVDLDPA   91
BnaA.IND.a  --SETLSHFQPPPTIFSDHG--GGEEAEEEEEEEGEEEMDPMKKMQYAIAAMQPVDLDPA   91
BolC.IND.a  --SETLSHFQPPPTVFSDHG--GGEEA---EDEEGEEEMDEKEMQYAIAAMQPVDIDPA   88
BnaC.IND.a  --SETLSHFQPPTVFSDEG--GGEEA---EDEEGEEEIDEMKEMQYAIAAMQPVDIDPA   88
              .  :   *:                  ::**   *::.  **:* *  **.**.**:***

HEC         TVKKPKRRNVRISDDPQSVAARHRRERISERIRILQRLVPGGTKMDTASMLDEAIRYVKF  173
IND         TVPKPNRRNVRISDDPQTVVARRRRERISEKIRILKRIVPGGAKMDTASMLDEAIRYTKF  137
BraA.IND.a  TVPKPNRRNVRVSDDPQTVVARRRRERISEKIRILKRMVPGGAKMDTASMLDEAIRYTKF  151
BnaA.IND.a  TVPKPNRRNVRVSDDPQTVVARRRRERISEKIRILKRMVPGGAKMDTASMLDEAIRYTKF  151
BolC.IND.a  TVPKPNRRNVRVSEDPQTVVARRRRERISEKIRILKRMVPGGAKMDTASMLDEAIRYTKF  148
BnaC.IND.a  TVPKPNRRNVRVSEDPQTVVARRRRERISEKIRILKRMVPGGAKMDTASMLDEAIRYTKF  148
            **  **:*****:*:***:*.**.*******.****:*:****:**************.**

HEC         LKRQIR-LLNNNTGYTPPPPQDQASQAVTTSWVSPPPPPSFGRGGRGVGELI          224
IND         LKRQVR-ILQPHSQIGAPMANPSYL-----CYYHNSQP-------------           169
BraA.IND.a  LKRQVR--------LASSASHSAWS-----SYV------------------           171
BnaA.IND.a  LKRQVRLLLQPHTQLGAPMSDPSCL-----CYYHNSDT-------------           184
BolC.IND.a  LKRQVR-LLQPHTQLGAPMSDPSRL-----CYYHNSDT-------------           180
BnaC.IND.a  LKRQVR-LLQPHTQLGAPMSDPSRL-----CYYHNSDT-------------           180
            ****:*            .. .            .:
```

Online Resource 1. Multiple alignment of IND protein sequences from Arabidopsis and Brassica species including HEC3 from Arabidopsis as an outgroup. Alignment was done using ClustalOmega. Mutated residues used in the analysis of the manuscript are indicated by a black circle.

Online Resource 2

a

BnaC-f BnaA-f   BnaAC-r

→ → [IND exon] ←

b

IND-C			IND-A		
A	C	A/C	A	C	A/C

c

Cabriolet   *bnaA.ind.a-D16N*   *bnaC.ind.a-P45L*

**d** Cabriolet   *bnaA.ind.a-G53R*   *bnaC.ind.a-G53R*

Online Resource 2. a Schematic outline of *IND* gene showing position of paralogue-specific primers (BnaC-f and BnaA-f) and BnaAC-r, which will amplify both. **b** Agarose gel showing paralogue-specific PCR amplification using primers BnaC-f/BnaAC-r to amplify *BnaC.IND.a* (IND-C) or BnaA-f/BnaAC-r to amplify *BnaA.IND.a* (IND-A) on gDNA from *B. rapa* (A), *B. oleracea* (C) and *B. napus* (A/C). **c** Effect of single mutations in *bna.ind* genes. SEM images of mature (stage 17) fruits from Cabriolet (wild type) and indicated *bna.ind* single mutants. **d** Tissue sections of mature (stage 17c) fruits from Cabriolet (wild type) and indicated *bna.ind* single mutants. *Scale bars* correspond to 1 mm in c and 100 μm in d.
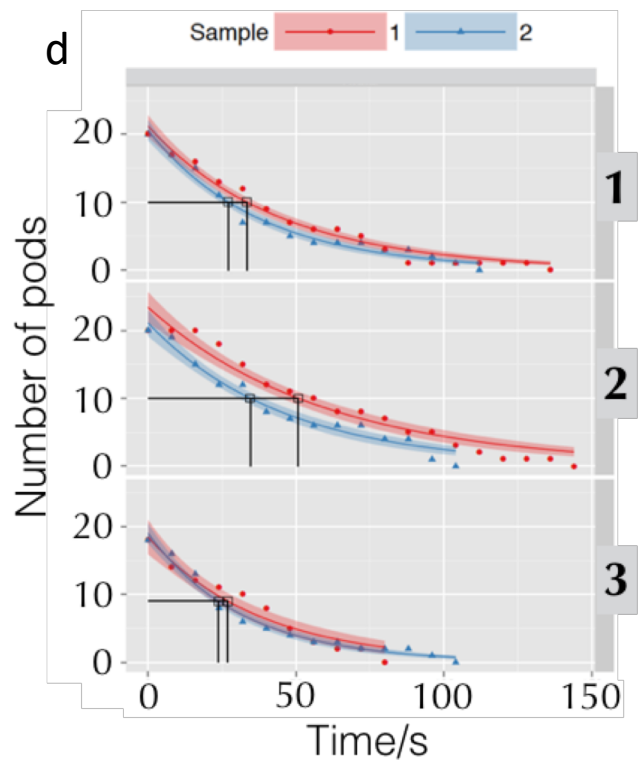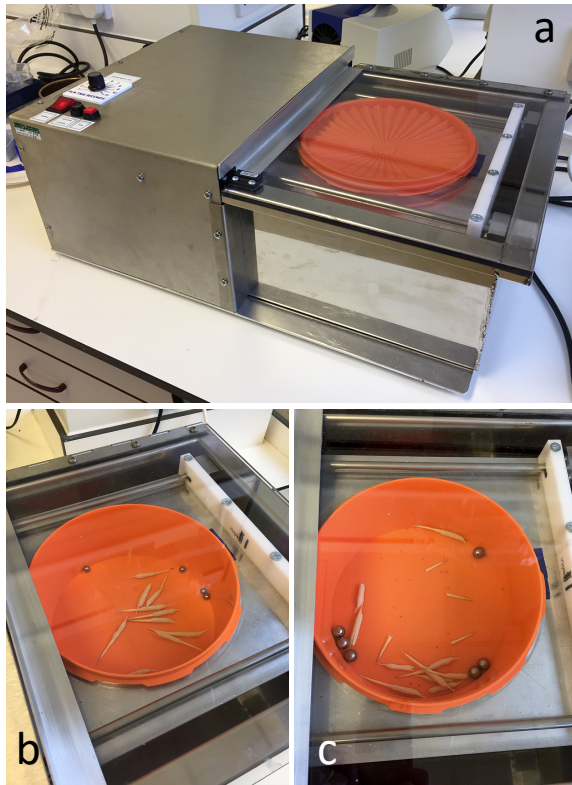
Online Resource 3

a

	bnaC.ind.a			
bnaA.ind.a	P45L	G53R	R95H	T146I
D16N	X	X	X	X
P45L	-	X	X	-
G53R	X	X	X	X
M70I	X	X	-	X

b

	Stage 16	Stage 17c
Cabriolet		
M70I G53R		
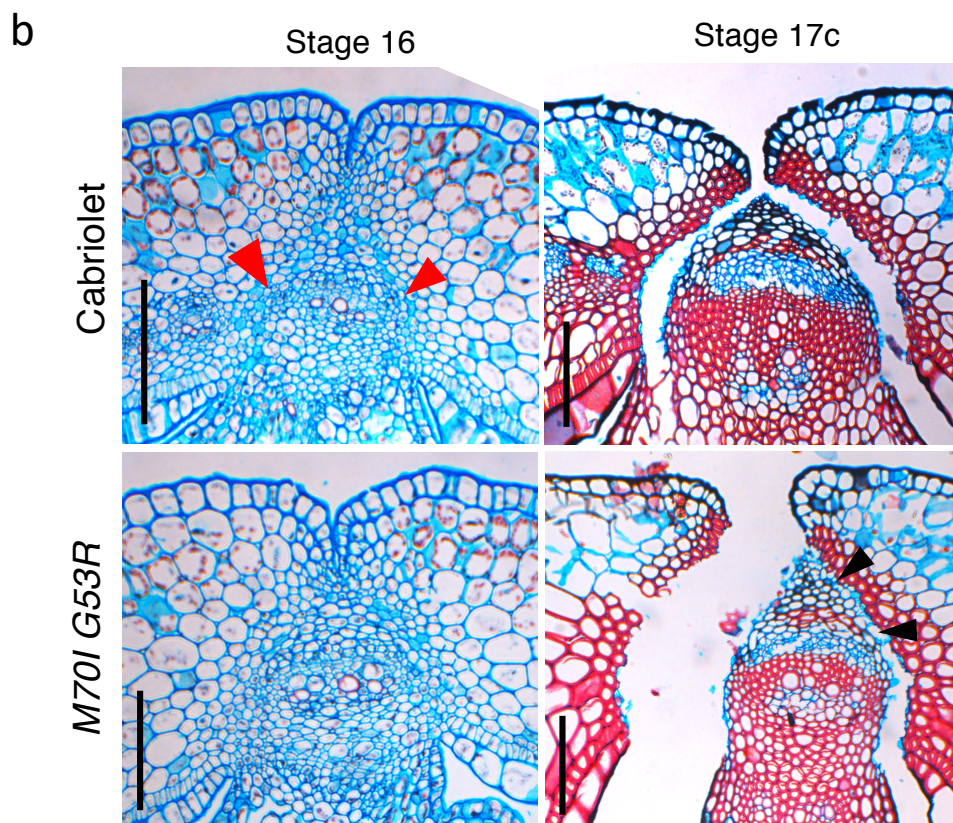
Online Resource 3. a Table of double-mutant combinations. 'X' indicates fruits were obtained for RIT while '-' indicates no RIT was performed on those lines due to infertile pods. **b** Tissue sections of fruits from Cabriolet (wild type) and the *bnaA.ind.a-M70I bnaC.ind.a-G53R* (*M70I/G53R*) double mutant at the indicated developmental stages. Red arrowheads point to separation layer primordium cells in Cabriolet. Black arrowheads indicate lack of complete separation in the *M70I/G53R* double mutant. *Scale bars* correspond to 100 μm.
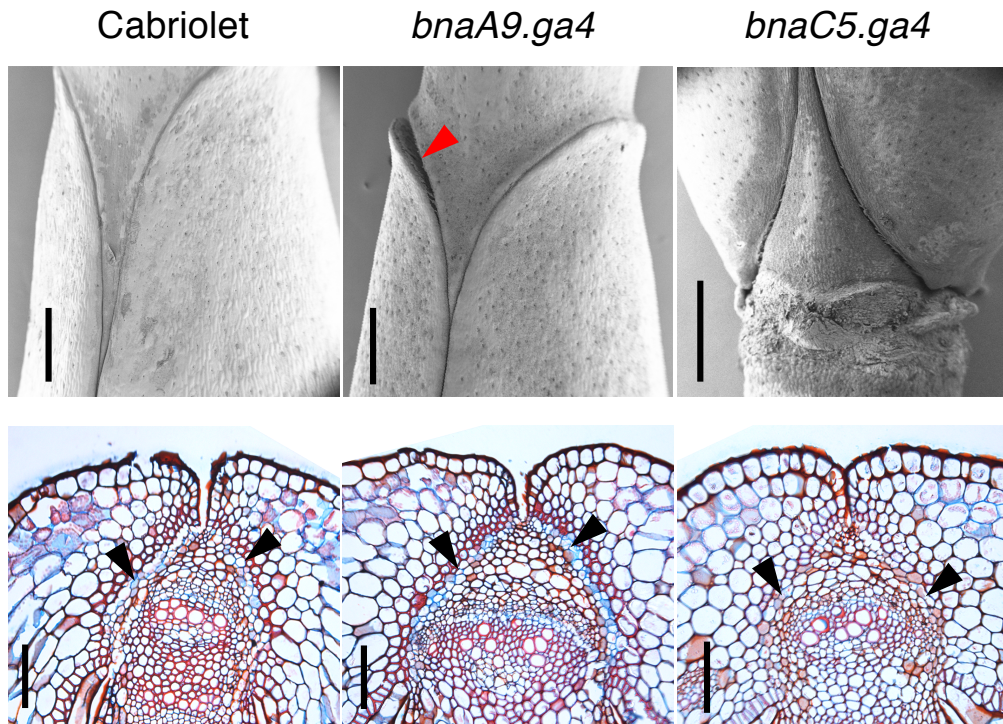
Online Resource 4

Online Resource 4. Random Impact Test analysis and visualisation of the podshatteR software output. **a** RIT machine on lab bench. **b** photo of sample with ball bearings prior to shaking. **c** photo of sample after one round of 8-sec shaking. **d** example of two samples tested in triplicates with number of remaining intact pods plotted against time of shaking. Values are fitted with decay curves calculated by software generated here. RIT_{50} values are calculated as the time it takes to break 10 pods out of 20.

```
AtGA4      MPAMLTDVFRGHPIHLPHSHIPDFTSLRELPDSYKWTPKDDLLFSAAPSPPATGENIPLI    60
BnaC5.GA4  MPTMLTDVFRGHPIHLPHSHQPDFTSLSELPDSYTWTPKDDPLLDAAPSPPAASENIPLI    60
BnaA6.GA4  MPTMLTDVFRGHPIHLPHSHQPDFTSLSELPDSYTWTPKDDPLLDAAPSPPAASENIPLI    60
BnaC8.GA4  MPTVLTDVFRGHPIHLPHTHQPDFTSLSELPDSYTWTSKDDPLFTAPPSPPGAGESIPLI    60
BnaA9.GA4  MPTVLTDVFRGHPIHLPHTHQPDFTSLSELPDSYWSKDDPLFTAPPSPPDAGESIPLI      60
           **:::***************:* ****** ******.** *** *:  * **** :.*.****

AtGA4      DLDHPDATNQIGHACRTWGAFQISNHGVPLGLLQDIEFLTGSLFGLPVQRKLKSARSETG   120
BnaC5.GA4  DLNHPDAANQIGSACRTWGAFQIANHGVPLELLQGIEFLTGSLFQLPVHRKLKAARSETG   120
BnaA6.GA4  DLNHPDAANQIGSACRTWGAFQIANHGVPLELLQGIEFLTGSLFQLPVQRKLKAARSETG   120
BnaC8.GA4  DLNHPDAANQIGRACRTWGAFQIANHGVPLELLQDIEFLTGSLFQLPVQHKLEAARSDAG   120
BnaA9.GA4  DLNHPDAANQIGRACRTWGAFQIANHGVPLELLQGIEFLTGSLFQLPVQRKLKAARSDTG   120
           **:****:**** ***********:****** ***.********* ***::**:::***::*

AtGA4      VSGYGVARIASFFNKQMWSEGFTITGSPLNDFRKLWPQHHL-NYCDIVEEYEEHMKKLAS   179
BnaC5.GA4  FSGYGVARISSFFNLQMWSEGFTITGSPLNDFRKLWPQHHLNNYCDIVEEYEEQMQKLAS   180
BnaA6.GA4  FSGYGVARISSFFNKQMWSEGFTITGSPLNDFRKLWPQHHLNNYCDIVEEYEEQMQKLAS   180
BnaC8.GA4  FSGYGVARISSFFNKKMWSEGFTITGSPLNDFRKLWPQLHL-NYCDIVEQYEEQMQKLAS   179
BnaA9.GA4  FSGYGVARISSFFNKKMWSEGFTITGSPLNDFRKLWPQLHL-NYCDIVEQYEEQMQKLAS   179
           .********:*****:*********************** ** *******:***:*:****

AtGA4      KLMWLALNSLGVSEEDIEWASLSSDLNWAQAALQLNHYPVCPEPDRAMGLAAHTDSTLLT   239
BnaC5.GA4  KLMWLSLTSLGVSEEDIKWASANSDSDWAQSALQLNHYPVCPEPDRAMGLAAHTDSTLLT   240
BnaA6.GA4  KLMWLSLTSLGVSEEDIKWASANPDLNWAQSALQLNHYPVCPEPDRAMGLAAHTDSTLLT   240
BnaC8.GA4  KLMWLSLNSLGVSEEDIKWARVSSDLNWAQSALQLNHYPVCPEPDRAMGLAPHTDSTLLT   239
BnaA9.GA4  KLMWLSLNSLGVTEEDIKWATVSSDLNWAQSALQLNHYPVCPEPDRAMGLAPHTDSTLLT   239
           *****:*.****:****:***  . * :***:****************** ********

AtGA4      ILYQNNTAGLQVFRDDLGWVTVPPFPGSLVVNVGDLFHILSNGLFKSVLHRARVNQTRAR   300
BnaC5.GA4  ILHQNNTAGLQVFRDDLGWVTVPPVPGSLVVNVGDLFHILSNGLFKSVLHRARVNQTRSR   300
BnaA6.GA4  ILHQNNTAGLQVFRDDLGWVTVPPVPDSLVVNVGDLFHILSNGLFKSVLHRARVNQTRSR   300
BnaC8.GA4  ILYQNNTAGLQVFRDDLGWVTVPPVPGSLVVNVGDLFHILSNGLFKSVIHRVRVNQTRPR   300
BnaA9.GA4  ILYQNNTAGLQVFRDDFGWVTVPPVPGSLVVNVGDLFHILSNGLFKSVIHRARVNQTRPR   300
           **:***********:.*.*******.*.**************************:**.****** *

AtGA4      LSVAFLWGPQSDIKISPVPKLVSPVESPLYQSVTWKEYLRTKATHFNKALSMIRNHREE   358
BnaC5.GA4  LSVAFLWGPQSDIKISPVPKLVSPVGSPLYRSVTWTEYLRTKATHFNEALSMIRNHIDE   359
BnaA6.GA4  LSVAFLWGPQSDIKISPVPKLVSPVGSPLYRSVTWTEYLRTKATHFNEALSMIKNDIDE   359
BnaC8.GA4  LSVAFLWGPRSDTKISPVPKLVSPDESPLYRSVTWTEYLRTKATHFNKALSMIRNHREK   358
BnaA9.GA4  LSVAFLWGPRSDTKISPVPKLVSPDESPLYRSVTWTEYLRTKATHFNKALSMIRNHREK   358
           *********:** ***********   ****:****.*************:*****:*. ::
```

Online Resource 5. Multiple alignment of GA4 protein sequences from Arabidopsis and Brassica species. Alignment was done using ClustalOmega. Mutated residues used in the analysis of the manuscript are indicated by a black circle.

Online Resource 6

Cabriolet	*bnaA9.ga4*	*bnaC5.ga4*

Online Resource 6. Effect of mutations in *bna.ga4* genes. SEM (upper row) and tissue section (lower row) images of mature (stage 17) fruits from Cabriolet (wild type), *bnaA.ga4* and *bnaC.ga4* single mutants. Red arrowhead indicates valve that is opening, black arrowheads point to separation layer in cross sections. *Scale bars* correspond to 1 mm for SEMs and 100 μm for sections.

Online Resource 7

Oligonucleotides used in this study

Bna.GA4

```
A6-F    gtccaacacttccataatcttcc

A6-R    GAATGACATGGCGAAATCTCTGT


C5-F    gactctccgactcttccataatt

C5-R    ACATGGCAAATTTTCGGTTTCT


C8-F    gacacaaaacatctatcgaat

C8-R    gccgtctttagcatatgtaaattgagccg


A9-F    ccaaaacaagagcttaaacatatgc

A9-R    gcaaccaatatatatcatgacc
```

Bna.IND

```
IND A-F    aacattcatacacgcactac

IND A-R    atcaacatgaaacgcgtgat

IND C-F    ccgcaaatacaaacatatttagt

IND C-R    atcaacatgaaacgcgtgat
```