Supporting Information

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SI Materials and Methods

The *ebs3-1 bri1-9* (Col-0) mutant was crossed with *bri1-9* (Ws-2) and the resulting F1 plants were self-fertilized to generate F2 mapping populations. Genomic DNAs isolated from 480 F2 *ebs3-1 bri1-9* plants and molecular markers listed in Table S1 were used for PCR-based genetic mapping to locate the *EBS3* locus to a 200-kb region on the top of chromosome I. The DNA fragment of *At1g16900* was independently amplified from four individual *ebs3-1 bri1-9* seedlings, sequenced, and compared with the published WT Col-0 sequence to identify a nucleotide change.
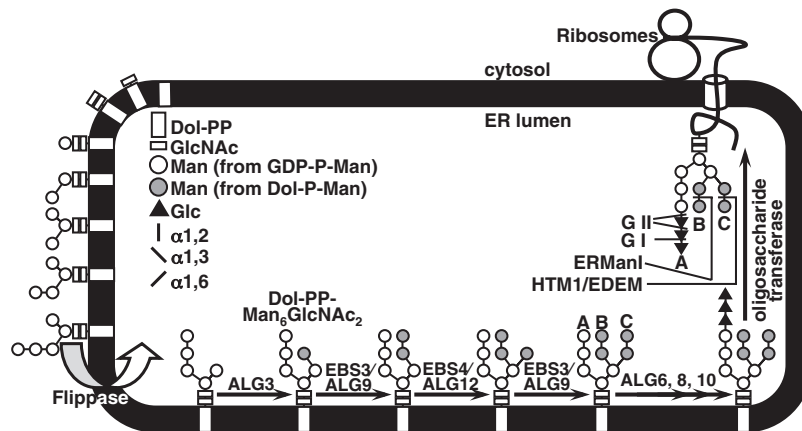

Fig. S1. Hypothetical assembly pathway of N-glycan precursor in *Arabidopsis*. The Dol-PP-Man$_5$GlcNAc$_2$ is assembled at the cytosolic face of the ER membrane. After flipping into the ER lumen, four Man and three Glc residues are added sequentially from Dol-P-Man and Dol-P-Glc donor substrates, respectively, to form Glc$_3$Man$_9$GlcNAc$_2$, which is then transferred to nascent polypeptide by the multiprotein oligosaccharyltransferase complex. Names of yeast luminal enzymes and their corresponding *Arabidopsis* homologs involved in each glycosyltransfer reaction are indicated above the dark arrows. The white bar indicates the dolichol linker, the white rectangle represents GlcNAc, the circle denotes Man (shaded circle indicating the ER luminal addition), and the triangle designates Glc. A, B, and C denote the three dimannose arms. Also shown are the sugar donors, the three types of sugar linkage, three deglycosylation enzymes, and their target bonds.

Fig. S2. Effect of *EBS1* and *EBS2* overexpression on *ebs3-1 bri1-9*. Images of 1-mo-old soil-grown transgenic *ebs3 bri1-9* plants containing a *gEBS3*, an empty vector (*pPZP212*), a *p35S-EBS1*, or a *gEBS2* transgene.
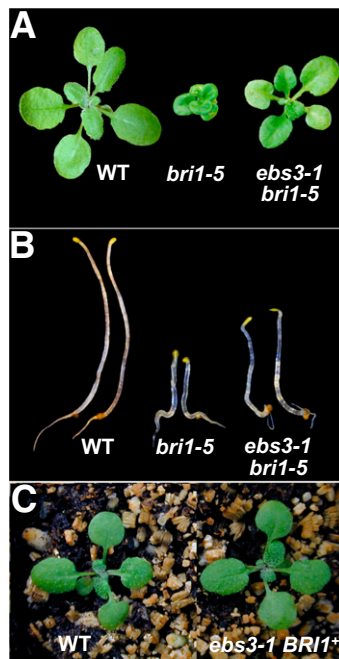
Fig. S3. *ebs3-1* mutation also suppresses the *bri1-5* mutation but has no effect on plant growth in a *BRI1+* background. (*A* and *B*) Images of 3-wk-old soil grown plants (*A*) and 5-d-old etiolated seedlings (*B*) of WT, *bri1-5*, and *ebs3-1 bri1-5*. (*C*) Images of 3-wk-old soil-grown plants of an *ebs3-1 BRI1+* mutant and its WT control.
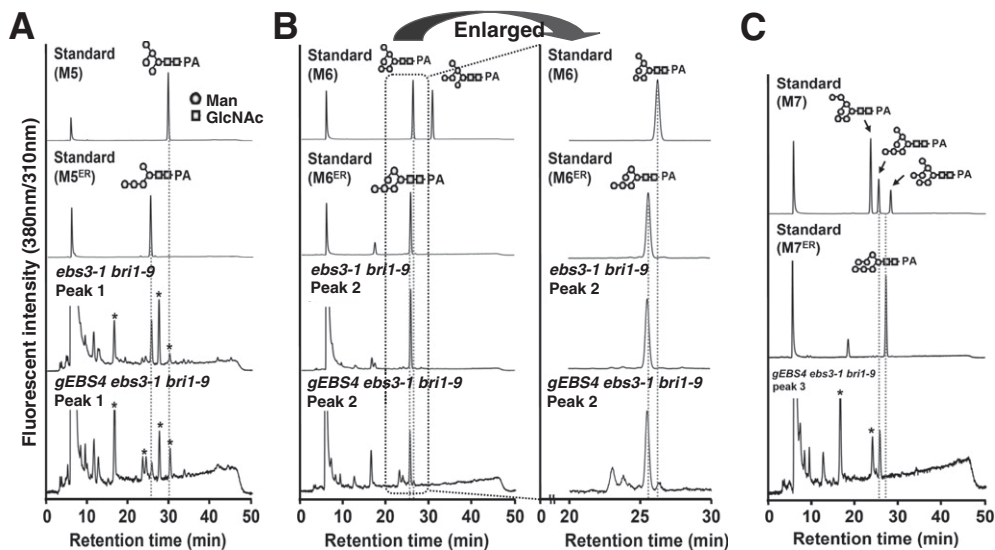

Fig. S4. Structural determination of the Man$_5$GlcNAc$_2$ and Man$_6$GlcNAc$_2$ glycans. The peaks 1 (*A*), 2 (*B*), and 3 (*C*) (at a retention time of 49, 52.5, and 55 min, respectively, in Fig. 3*B*) of the PA-labeled LLOs of the *ebs3-1 bri1-9* mutant and the *gEBS4 ebs3-1 bri1-9* transgenic line were analyzed by RP-HPLC. Their elution profiles were compared with authentic PA-oligosaccharide standards (Man$_5$GlcNAc$_2$-PA and Man$_6$GlcNAc$_2$-PA) of known isomeric configurations. (Circle indicates a Man residue, whereas square denotes a GlcNAc residue.) *Contaminants with unknown glycan structure. (*B*, *Right*) Magnified view of the RP-HPLC elution profiles between retention time of 20 and 30 min at left.
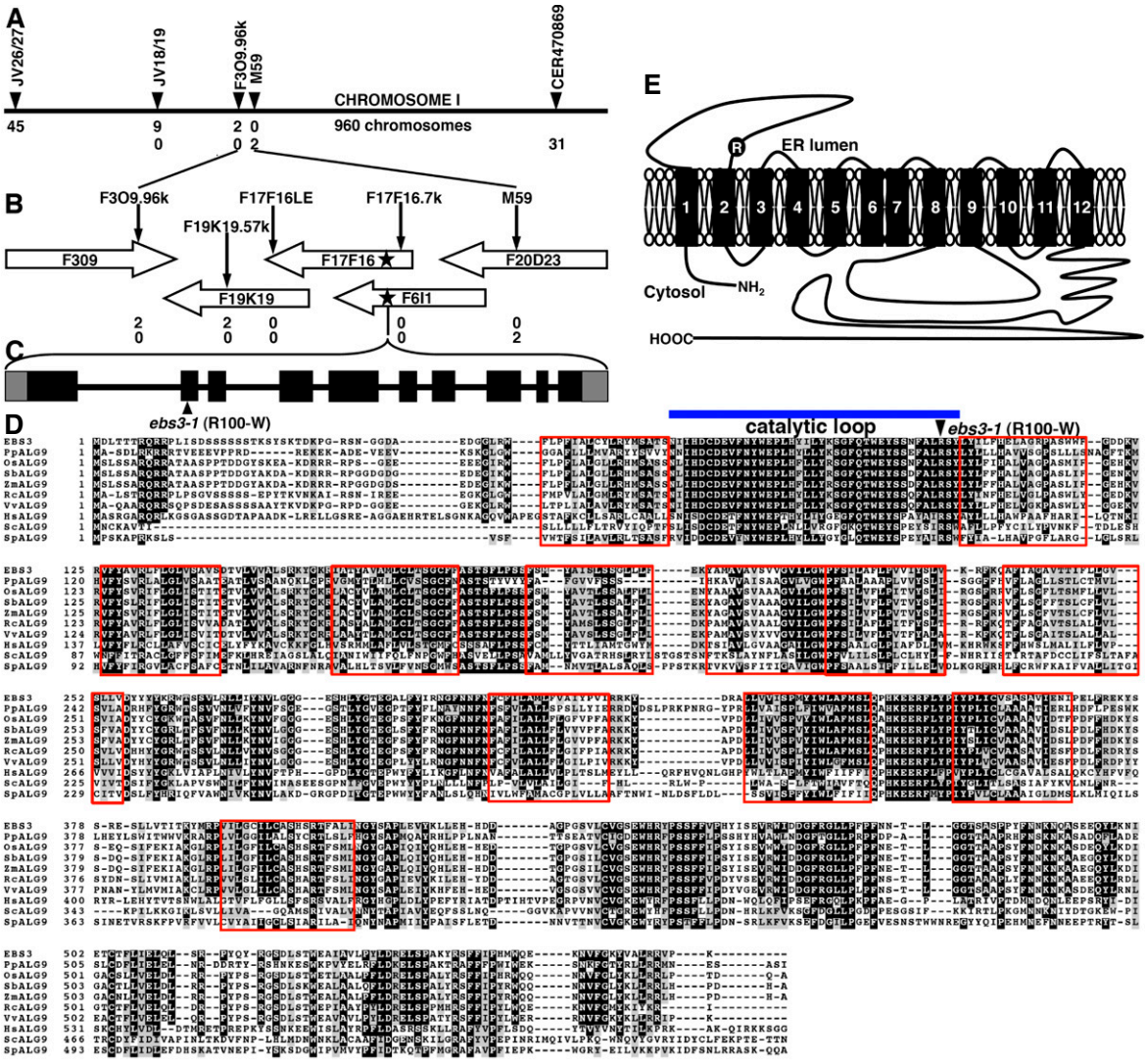
Fig. S5. *EBS3* is mapped to a 200-kb region on the top of chromosome I and encodes a multipass membrane protein highly similar to the yeast/human ALG9. (*A*) The line represents genomic DNA, and PCR-based markers (see Table S1 for details), and numbers of chromosome recombinants are shown above and below the line, respectively. (*B*) A BAC contig of five overlapping BACs between markers F3O9.96k and M59 was also shown. Additional PCR markers (with arrows indicating their positions) and corresponding numbers of recombinant chromosomes are indicated above and below the contig, respectively. Star indicates the location of the *EBS3* gene. (*C*) Schematic presentation of the gene structure of *EBS3*. Dark bar indicates exon, thick line represents intron, and arrow indicates the position of the *ebs3-1* mutation. (*D*) Sequence comparison between EBS3 and known/predicted ALG9 proteins from other eukaryotes. Alignment of EBS3 (GenBank accession no. NP_173134) with known and predicted ALG9s from *Physcomitrella patens* (PpALG9; GenBank accession no. XP_001785514), sorghum (SbALG9; GenBank accession no. XP_002457152), maize (ZmALG9; GenBank accession no. NP_001148995), rice (OsALG9; GenBank accession no. NP_001042360), caster bean (RcALG9; GenBank accession no. XP_002523375), grapevine (VvALG9; GenBank accession no. CAN72375), human (HsALG9; GenBank accession no. NP_079016), budding yeast (ScALG9; GenBank accession no. NP_014180), and fission yeast (SpALG9; GenBank accession no. NP_594684). Sequence alignment was performed at the T-Coffee server (http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee_cgi/index.cgi) and shaded using the BoxShade server (http://www.ch.embnet.org/software/BOX_form.html). Identical amino acids in >7 sequences are shaded in black, whereas similar ones are in gray. The triangle indicates the R residue mutated in *ebs3-1*. Red boxes define predicted transmembrane segments, and the blue bar indicates the predicted ER luminal catalytic loop located between the first two predicted transmembrane domains. (*E*) Predicted topology of the EBS3 protein. Prediction of 12 potential transmembrane domains was first performed at the dense alignment surface server (http://www.sbc.su.se/~miklos/DAS) and the hidden Markov model for topology prediction server (http://www.enzim.hu/hmmtop) and was later adjusted based on the predicted topology of other ALG9 homologs (1). The dark-circled R residue in the first cytoplasmic loop is mutated in *ebs3-1*.

1. Oriol R, Martinez-Duncker I, Chantret I, Mollicone R, Codogno P (2002) Common origin and evolution of glycosyltransferases using Dol-P-monosaccharides as donor substrate. *Mol Biol Evol* 19:1451–1463.

Fig. S6. Phenotypic rescue of the *ebs3-1 bri1-9* mutant by a genomic *gEBS3* transgene. Images of 4-wk-old soil-grown T2 transgenic *ebs3-1 bri1-9* mutants carrying an empty vector or a genomic *gEBS3* transgene.
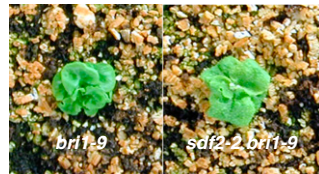

Fig. S7. A T-DNA insertional mutation in *Arabidopsis* SDF2 fails to enhance the *bri1-9* dwarfism. Images of 1-mo-old soil-grown plants of *bri1-9* and a double mutant between *bri1-9* and the *Arabidopsis sdf2-2* mutant known to be hypersensitive to ER stresses (1).

1. Schott A, et al. (2010) *Arabidopsis* stromal-derived Factor2 (SDF2) is a crucial target of the unfolded protein response in the endoplasmic reticulum. *J Biol Chem* 285:18113–18121.

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ScALG9       1  MN----------------------------------------CKAVTISLLLLLFLTRVYIQPTFSLISDCDETFNYWEPLN
EBS3         1  MDLTTTRQRRPLISDSSSSSSTKSYSKTDKPGRSNGGDAEDGGLRWFLPFIALCYLRYMSATSNIIHDCDEVFNYWEPLH
At5g14850    1  MDIRK---RKNAGGDGDGGADGASVNVGDEP-DSFGG---RIGSPRRIFLFCLAFRVVNALLIQTYFNPDEHWQSLEVAH
EBS4         1  MPTDS---KM-------AKFLQSY--------------GYDLILGSVAAIYVV-----MAPYTKVEESFNVQSMHD

ScALG9      43  LLVRGFGKQTWEYS--PEYSIRSWAFLLPFYCI---LYPVNKFTDLESHWNF-FITRACLGFFSFIMEFKLHREIAGSLA
EBS3        81  YILYKSGFQTWEYS--SNFALRSYLYILFHELA---GRPASWWFGDDKVRVF-YAVRLFLGLVSAVSDTVLVVALSRKYG
At5g14850   74  RTIFGYGYMTWEWK--R--GIRSYLHPMLFAF----LYKLLQVTGLDTPYIMIKAPRLMQSIFSAIGDLYLYKLSDALYG
EBS4        48  ILYHRHHLDSYDHLEFPGVVPRTFIGAFIVSVFASPVVSIISCLGFPKVYSL-VAARLVLGCIILSTLRFFRIQIKKKFG

ScALG9     117  LQIANIWIIFQLFNPGWFHAS-VELLPSAVAMLLYVGATRH--SLRYLSTGS--TSNFTKSLAYNFLASILGWPFVLILS
EBS3       155  KRIATYAVAMLCLTSGCFFAS-TSFLPSSFSMYAISLSSGL--LLF---------EKYAMAVAVSVVGVILGWPFSILAF
At5g14850  146  GNVATWS-LFCQMANWFIFFCLNRTFSNCLETVLTIMGLYYWPCIR--DSSIDYPVNRKWGLVIAALACAIRPTSAVIWL
EBS4       127  NQVETFFVLFTSLQFHFLFYC-TRPLPNILALGLVNLAYGN--WLK---------GNFYPALSFLIFATVIFRCDTMLLL

ScALG9     192  LPLCLHYLFNHRIISTIRTAFDCCLIFSLTAFAVIVTDSIFYGKLAPVSWNILFYNVINASEESGPNIFGVEPWYYYPLN
EBS3       223  LPVVIYSLVKRFKQAFIAGAV----TTIFLLGVSLLVDYYYYKRWTSSVLNLLIYNVLGGGES---HLYGTEGALFYIRN
At5g14850  223  YVGMLELFLTPNKVKFIILE-V-IPIGSLVLGFTCLLDRLMYGSWVIVPLNFLKFNFLSSGGD----YYGTHPWHWYFTQ
EBS4       195  GPIGLELLLTKS-ISFWKALKYCVGTALLAVGLTIFVDSIMWKKFVWPEFEVFWFNSILNRSS----DWGTHSIHWYFTS

ScALG9     272  LLLNFPLP----VLVLAIILGIFHLR-L--WP-LWASLFTWIAVFTQQPHKEERFLYPIYGLITLSASIAFYKVLNLFNRK
EBS3       296  GFNNFNFCFILAMLFVAIYPVIRRKYDRALLVVISPMYIWLAFMSLQPHKEERFLYPIYPLICVSASAVIENIPELFREK
At5g14850  297  GFLVMLFT----FTPFSIAGIIKSK-N---QKLSALILWVLAIYSILGHKEFRFVLPVLPIALIFSGYAFAQMEVSGGSS
EBS4       270  ALPRSLLV----AYPLSLLGTL---VDRRVPFFIVPVLSFVILYSKLPHKELRFIISSVPMFNLSAAVAASRIYN--NRK

ScALG9     344  P----------------ILKKGIKLSVLLIVAGQAMSRIVALVNNYTAPIAVYEQFSSLNQGGVKAPVVNVCTGREWYHF
EBS3       376  YS--------SRESLLVTITKYMRPVILGCILCASHSRTFALINGYSAPLEVYKLLEHHDDAG-PGSV--LCVGSEWHRY
At5g14850  369  SSSVTKKKQVPRQNHT---KWSPKLRLSVYFLLATNIPMALYMSLFHQRGTEDAMNYLSDEAY-KGRV-----------
EBS4       341  K----------------TIWKLVNMVMLAFFAISAGCTVVTFMASYNYPSGYALKRLHQIGH-PANV----AGEEWVHI

ScALG9     408  PSSFLLPDN-HRLKFVKSGFDGLLPGDFPESGSIFKKIRTLPKGMNNKNIYDTGKEWP-ITRCDYFIDIVAPINLTKDVF
EBS3       445  PSSFFVPHYISEVRWIDDGFRGLLP--FPFNNTL-GGTSASPPYFNNKNQASEEQYLKNIETCTFLIELQ----LSRPYQ
At5g14850  433  KSILFLMPCH-STPYYSTLHR-NIP--------MQFL-DCT-PSAEKGELDESDQFLVNPLGFASELAR----NWSEPPS
EBS4       400  -DTFGAMNGIS--RFCEDDF--------PWRYSKEEE-IVV-EELRNRNFTYLVNEHSSVD----------------GYK

ScALG9     486  NPLHLMDNWNKLACAAFIDGENSKILGRAFYVPEPINRIMQIVLPKQWNQ--VYGVRYIDYCLFEKPTETTN
EBS3       518  YRGSDLSTWEAIAVLPYLDRELSPAKYRSFFIPH-----------MWQEKNVFG-KYV---ALRRVP---K
At5g14850  497  HIVLFASE--ETKLRDFMIQHSFKE-VRRFFHAH----------FKVDRDLQSSVV---VYVVNHAF-------P
EBS4       451  CLFYEEGF-ERLELRRGFPPIVLVKKAKVYLHR----------EMKKEDPF---HKKW-----PG---C
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Fig. S8. Sequence comparison between the yeast ALG9 with EBS3, EBS4, and At5g18450. Alignment of the protein sequences of yeast ALG9 (GenBank accession no. NP_014180) with three *Arabidopsis* proteins [EBS3 (GenBank accession no. NP_173134); EBS4 (GenBank accession no. NP_001077448); At5g18450 (GenBank accession no. NP_568305)] was performed using the M-Coffee program at the T-Coffee server (http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee_cgi/index.cgi). The aligned sequences were shaded using the BoxShade server (http://www.ch.embnet.org/software/BOX_form.html) with identical amino acids in more than two proteins shaded in black and similar ones in gray. Arrow indicates the conserved Arg residue that is mutated in *ebs3-1* mutant.

Table S1. Oligonucleotide primers used in this study

Name	Sequence	Comment
JV26/27*	CAAGAGATTGCAACATCCACA	SSLP
	AAGCTCCTTGGATCCGATTT	Ws-2 < Col-0
JV18/19*	TGTCGTATATCAATCGAAAAAGAGAT	SSLP
	AATTCAGTATCGAGATACCCCTCT	Ws-2 < Col-0
F3O9.96k[†]	CATTAAGGTAATCTAATGACGATT	dCAPS
	CATGCATCGTTACCCTATGG	HinfI cuts Col-0 DNA
F17F16.LE[†]	CTCTTGTTTCTCTGACACC	dCAPS
	GTTGCTGAGACTGAGGAACA	ScaI cuts Ws-2 DNA
F17F16.7k[†]	GAGGTGAACAAAGTCAAGAG	dCAPS
	TTGAGAGCTTCCCGAATCTAGACT	HinfI cuts Col-0
F19K19.57k[†]	CAGTCTTGTAATCAACCGTGA	dCAPs
	CACCCTTGCATGAACGTTTC	MspI cuts Col-0
M59*	GTGCATGATATTGATGTACGC	CAPS
	GAATGACATGAACACTTACACC	BstUI cuts Col-0 2 x, Ws-2 1 x
CER470869[‡]	GAAGAGACAAACGAGAATCTCG	SSLP
	CTTTGCAACAACGAACTAAATGC	Ws-2 < Col-0
ebs3-1F	GCAGTTCCAACTTTGCTCCT	dCAPs
ebs3-1R	CATGCAGTTATATCTATACCG	MnlI cuts Col-0
ebs3-1SMF	CAGTTCCAACTTTGCTCTTTGGTCGTACTTATAC	To create the *ebs3-1* mutation in a yeast expression
ebs3-1SMR	GTATAAGTACGACCAAAGAGCAAAGTTGGAACTG	plasmid
ebs4-1F	ATCGTTCGTTATACTAGACT	dCAPS
ebs4-2R	AGCAGACAAGTTGAACATTG	HinfI cuts Col-0 DNA
alg3t-For	TTTGCTTGCGTATCCCTCG	For screening T-DNA insertional *alg3-t2* mutation
alg3t-Rev	GTAGACTTCCCCTCCAGTTA	
LBa1	TGGTTCACGTAGTGGGCCATCG	
yALG9SwaIF	CACCCTGAAGCACATTTAAATGGGAACGATGAATTGC	Site-directed mutagenesis tocreate a SwaI site in *yALG9*
yALG9SwaIR	GCAATTCATCGTTCCCATTTAAATGTGCTTCAGGGTG	
yALG9SpeIF	CCAACTGAGACTACTAGTTGATATACAGGTTCTC	Site-directed mutagenesis tocreate a SpeI site in *yALG9*
yALG9SpeIR	GAGAACCTGTATATCAACTAGTAGTCTCAGTTGG	
EBS3SwaIF	AAATGGGAACGATGGATCTAACGACGACGC	To amplify an *EBS3* cDNA for cloning into a *yALG9*
EBS3SpeIR	CCACTAGTCACTGAGAAGAGTCCCAAAA	plasmid
EBS1cDNAF	GCGAGCTCGAGAAATGGGGACCACCACCAAT	To amplify the full-length *EBS1* cDNA
EBS1cDNAR	GCGGTACCTTACAGTTCTGCTTTAGAT	
F17F16SacI	CGGAGCTCGTAATGTCTCCGCAATCTC	To amplify a genomic fragment of the *EBS3* gene
F17F16KpnI	GCGGTACCTACCATGATCTCGATG	

*The primer sequences were obtained from the TAIR database (http://www.arabidopsis.org).
[†]These primers were designed using the dCAPS Finder program (see ref. 1) (http://helix.wustl.edu/dcaps/dcaps.html) based on the sequences of PCR products amplified from the *bri1-9* (Ws) mutant and the published genomic sequences of the WT Col-0.
[‡]This primer set was designed according to the Monsanto *Arabidopsis* SNP/INDEL database (see ref. 2).

1. Neff MM, Neff JD, Chory J, Pepper AE (1998) dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: Experimental applications in Arabidopsis thaliana genetics. *Plant J* 14:387–392.
2. Jander G, et al. (2002) Arabidopsis map-based cloning in the post-genome era. *Plant Physiol* 129:440–450.