

Fig. S1. Sequence alignment. Alignment of BRX family proteins from *Arabidopsis thaliana* (AtBRX), *Amborella trichopoda* (AmbBRXL1 and AmbBRXL2), *Selaginella moellendorffii* (SmBRX), *Physcomitrium patens* (PpBRXL1 and PpBRXL2), and *Marchantia polymorpha* (MpBRXL1). The AGC kinase target phosphosite of AtBRX and AmbBRXL2 in the linker between the BRX-domains is highlighted in red.

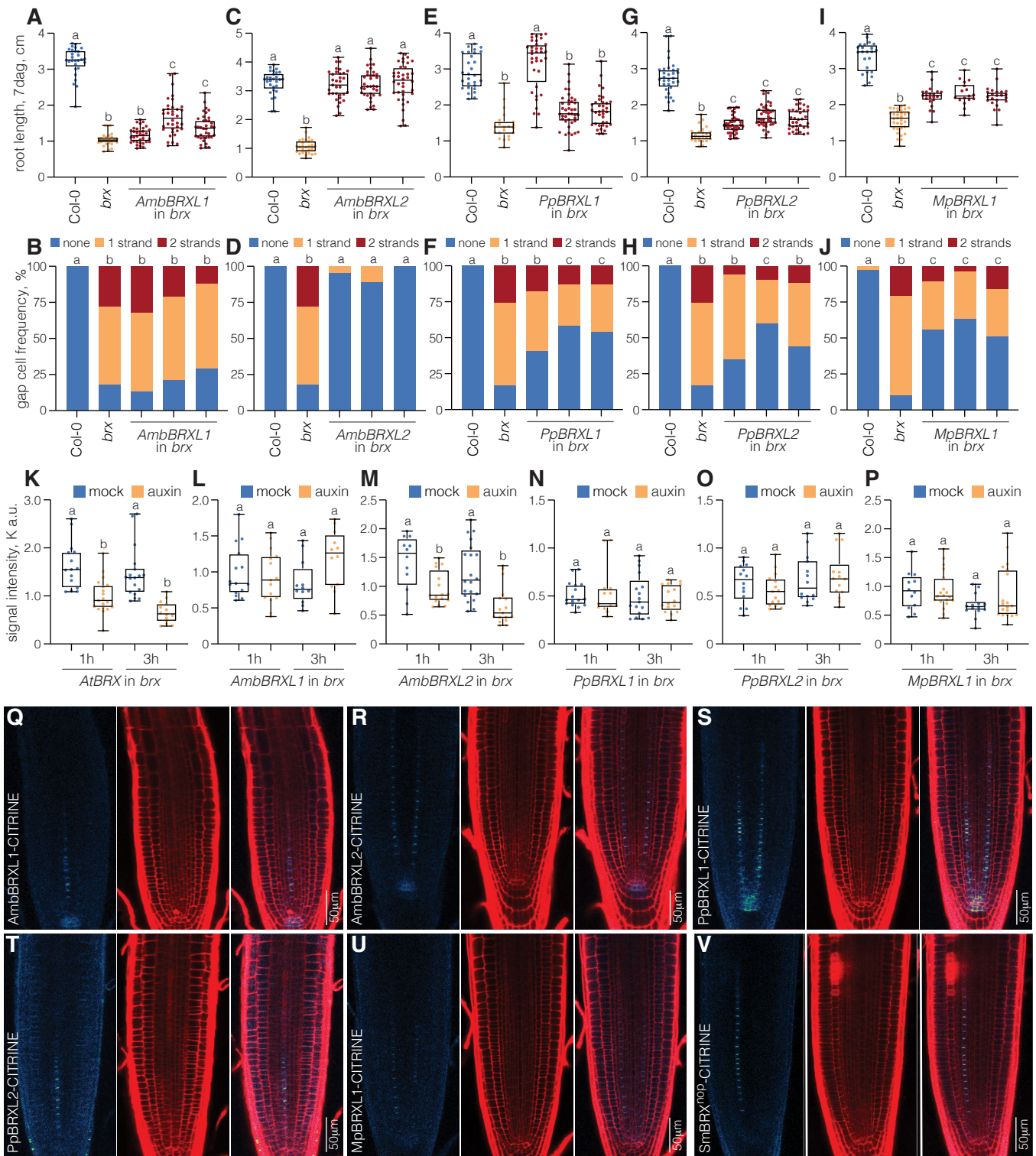


Fig. S2. Functional assays of BRX family proteins across the green lineage. (A-J) Root length of 7-day-old seedlings and corresponding quantification of protophloem sieve element differentiation defects (“gap cells”) in 7-day-old roots from Col-0 wildtype, *brx* mutant, and three representative independent transgenic lines each expressing the indicated BRX family proteins under control of the *A. thaliana* *BRX* promoter in *brx* background. (A) n=26-38 roots; (B) n=17-31 roots; (C) n=28-38 roots; (D) n=26-41 roots; (E) n=21-40 roots; (F) n=22-31 roots; (G) n=25-40 roots; (H) n=17-25 roots; (I) n=17-33 roots; (J) n=29-45 roots. (K-P) Signal intensity quantification of indicated CITRINE fusion proteins (expressed under control of the *A. thaliana* *BRX* promoter) at the rootward plasma membrane of developing protophloem sieve elements, 1h or 3h after mock (DMSO) or auxin (10 μ M 1-naphthalene-acetic acid) treatment. (Q-V) Confocal microscopy images of indicated CITRINE fusion proteins (expressed under control of the *A. thaliana* *BRX* promoter), showing their protophloem-specific expression and polar localization (green fluorescence, left panels). Central panels show propidium iodide-stained cell wall outline (red fluorescence), right panels show the overlay. Box plots display 2nd and 3rd quartiles and the median, bars indicate maximum and minimum. Statistically significant different samples (lower case letters) were determined by Fisher’s exact test (B, D, F, H, J) or ordinary one-way ANOVA (A, C, E, G, I, K-P).

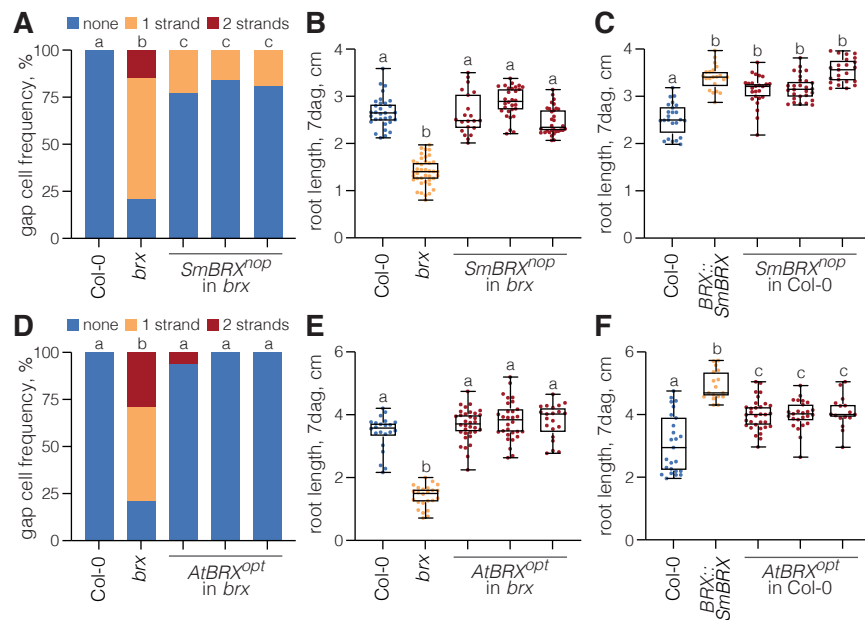


Fig. S3. Functional assays of codon-(non)-optimized SmBRX and AtBRX variants. (A) Quantification of protoxylem sieve element differentiation defects (“gap cells”) in 5-day-old roots from Col-0 wildtype, *brx* mutant, and three independent transgenic lines expressing a non-codon-optimized SmBRX variant (SmBRX^{nop}) under control of the *A. thaliana* BRX promoter in the *brx* background. n=27-48 roots. (B) Root length of 7-day-old seedlings corresponding to the genotypes assayed in (A). n=21-40 roots. (C) Root length of 7-day-old seedlings from Col-0, *brx*, and three independent transgenic lines expressing SmBRX^{nop} under control of the *A. thaliana* BRX^{opt} promoter in Col-0 background. n=21-28 roots. (D-F) Similar to (A-C), for an *A. thaliana* codon-optimized AtBRX variant (AtBRX^{opt}). (D) n=14-27 roots; (E) n=21-34 roots; (F) n=16-32 roots. Box plots display 2nd and 3rd quartiles and the median, bars indicate maximum and minimum. Statistically significant different samples (lower case letters) were determined by Fisher’s exact test (A, D) or ordinary one-way ANOVA (B, C, E, F).

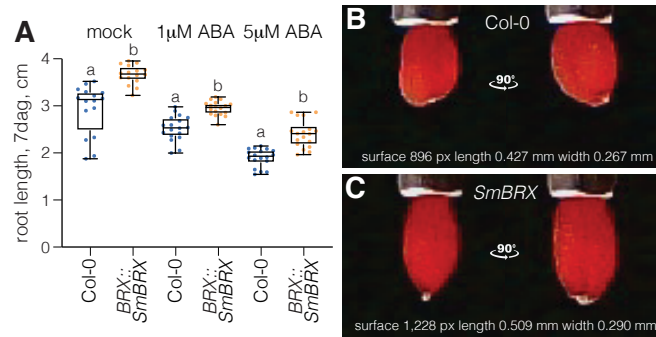


Fig. S4. Illustration of increased seed and embryo size upon heterologous SmBRX expression.

(A) Root length of 7-day-old seedlings from Col-0 and *BRX::SmBRX* plants, grown on media supplemented with increasing amounts of abscisic acid. n=16-18 roots; (B-C) Illustration of high resolution seed imaging with the *Boxeed* platform, for a Col-0 wildtype (B) and an *SmBRX* transgenic seed (C). Measured parameter values for the seeds shown are indicated.

Dataset 1. Protein sequence alignment of 302 BRX family proteins retrieved from a cross the green lineage with scaffold IDs (One Thousand Plant Transcriptomes, 2019) and species names, html file.

[Click here to download Dataset 1](#)

Dataset 2. Open reading frame coding sequences of BRX family genes and variants used for the creation of transgenes, pdf file.

AtBRX^{opt} – *Arabidopsis thaliana* BRX, codon-optimized for *A. thaliana*

ATGTTTCAGCTGTATCGCTTGCACCAAGGCTGATGGTGGTGAAGAGGTTGAACATGGTGTCTAGAGGTGGAACCTACCCCGAACACAAAAGAGGCTGTTAAGTCCTT
CACCATTCCAGATCAAGGACATGGCCCTTAAGTTTCAGCGGAGCTTACAAGCAGTGAAGCCTTGTACCGGGTCTCTTCTCACCTCTCAAGAAGGGACACCGTAG
CTTCCCTGATTACGACAATGCTTCTGAGGGTGTGCCGATCTCTTTTATGGGAGGATCTGCTGGATCTACCCCTGCTTGGGATTTACCAACTCTTCTCATCATCTG
CTGGCAGGCTCGAGTCTAAGTTCACTTCTATCTACGGGAACGACCGTGAGAGCATCTCTGCTCAGTCTTGTGATGTGGTGTCTGATGACGACGGACCGAAAGAAT
GGATGGCTCAAGTTGAACCTGGTGTGCATATTACCTTCGCTTCTCCCTACCGGTGGAAACGATCTCAAGAGGATCAGATTACGCCGTGAGATGTTGACAAGT
GGCAAGCTCAAGATGGTGGGGAGAGAAGTACGACAAGATCGTCGAGCTTTACAACGTGAGAGGTTCAACAGACAGGCTCTCAAACTCTGCTAGGTCTGAT
GATCAGTCTCAGAGGGACAGCCTACAGCAAGATGGATTCTGCTGTGAGTCTAAGGATTGGACCCCTAGACACAACCTCAGGCTCCAGGATCTGTGCCTCAT
CATTTCTACGGCGGCTCATCTAATTACGGACCGGATCTTATCATGTGGACCTCAATGGATGCTGTCTAGAAGTACCCCTCTTAGGGATGACCCCTCCGCTCAT
GTCTAACGCTTCTGAGATGCAAGCTGAGTGGATCGAAGAGGATGAGCCTGGTGTGTACATCAGATCAGGCAGCTTTCTGATGGAACAGAGAGCTTCGTCGAG
TCAGGTTCTCAAGAGAGGTTTCGGAGAAGTTCACGCTAAGACTTGGTGGGAGCAGAACAGAGAGAGAATCCAGACTCAGTACCTC

SmBRX^{nop} – *Selaginella moellendorffii* BRX, non-optimized original sequence

ATGCTGCCCTGTATTGCTTGAACGCGCATGCGATTGAGGACGATGACGAAAGCAGAGGACGAGGACGATTACACTGGAGGCCAAACACAATTACAAGG
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CGGAAGTGAATAAACAACACTACGAGTACGAACTGACCAATGGTGTGAAAGACGAGCCCGGGTTTATCACTGTGAAGCTCGGCCAAAATGGCAAC
CGAGAATAAAGCGTGAAGATTCAGTTCGCGAGAAGTTTACGAGAGGCAAGCAAAGCTATGGTGGGAGAGGAATCGCTTGAATACACGACAATTATTTG

AtBRX^{sm1} – “*S. moellendorffii*-like” *A. thaliana* BRX, in frame deletion of amino acids 219-266

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AAGCTCAACGGTGGTGGGGTGAAGACTATGACAAGATCGTTGAGCTTACAATGTACAGAGATTAACCGCAAGCTCTCAAACCGCTGCAAGATCCGACGATC
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AmbBRXL1 – *Amborella trichopoda* BRXL1, codon-optimized for *A. thaliana*

ATGCTCACCTGTATCGCTTGTCTAAGCACTTCGATGGATCTCTCAGGGATGCTGAAGAAGATGAGGCTGTACCCCTAACACCAAGAGGCTGTTAAGACTCTCA
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AGCTCAGTTCAAGATCATGGTATCGATCTTCAACCTTCACTCCTGAT

AmbBRXL2 – *Amborella trichopoda* BRXL2, codon-optimized for *A. thaliana*

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GAGGCTCATGTAAGCTTGGTGGGAGGAAAACAGAGAGAGAATCCAGGCTCAGTACCTC

S_mBRX – *S. moellendorffii* BRX, codon-optimized for *A. thaliana*

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P_pBRXL1 – *Physcomitrium patens* BRXL1

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P_pBRXL2 – *Physcomitrium patens* BRXL2

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TTCAGTCTGAAAAGTTCCCGGAGAACAGGCCAAAATATGGTGGGACGAGAACCAGCGGGGCAATTCACAACAGTATCTA

M_pBRXL1 – *Marchantia polymorpha* BRXL1, codon-optimized for *A. thaliana*

ATGCTTGTGCTCGCTCGTGTGCACTAGAACCCTGGATGATGTTGGAGGTGGTGGTGAAGGACCTCATGGATCTGAGACTAAGACCATCTCTACCAAGCAGGGGCTC
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GAGCAGGATGAACCTGGTGTACCTACCATCATCTACGTGCAAGGTGTGGGAAAGCAGCTCAAGAGAGTGAAGTCTCTAGGGACAAGTACTCTGAGGCTGA
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