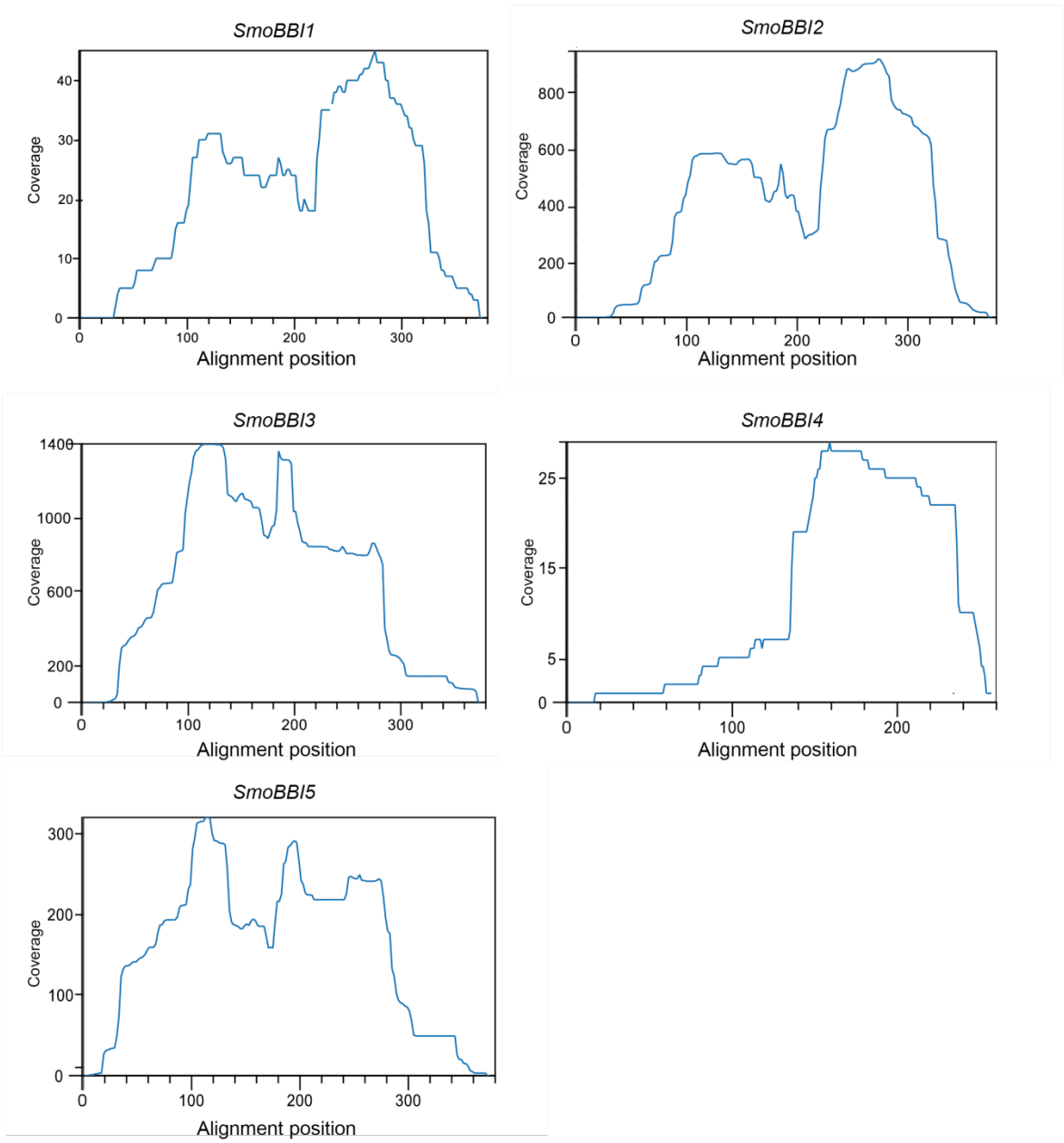
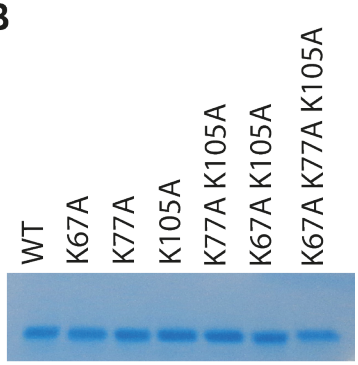


Supplemental Figure 1. Relative *BBI* transcript abundance in *S. moellendorffii* tissue shown as the number of clean RNAseq reads that mapped to each open reading frame (Supports Figure 2). Coverage (Y axis) is the number of reads contributing to a given position in the mapping.

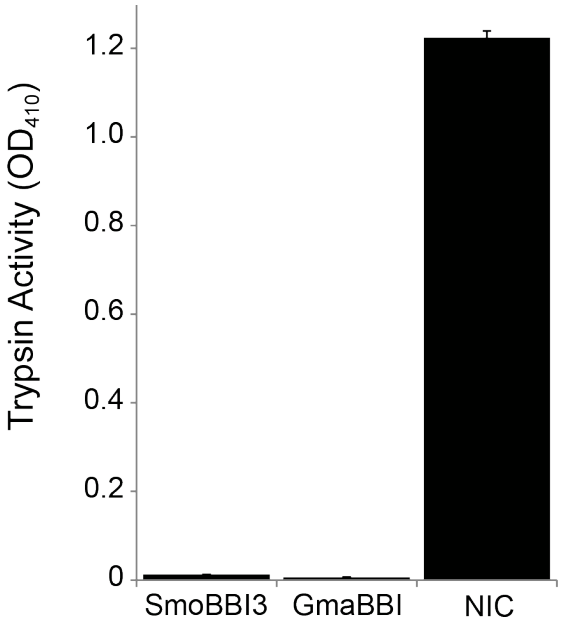
A

```
atgagaggatcgcatcaccatcaccatcacggatccgaaaacctgtattttcagggtgaa
 M  R  G  S  H  H  H  H  H  H  G  S  E  N  L  Y  F  Q  G  E
gttctggatctgcctggcgaatttttctttgttgatggtgcagcaagccgtccgagcgca
 V  L  D  L  P  G  E  F  F  F  V  D  G  A  A  S  R  P  S  A
tgtcgtccgaatagccagtgttgcccgaatagcaaattttgtccgaaaggtaaattttgc
 C  R  P  N  S  Q  C  C  P  N  S  K  F  C  P  K  G  K  F  C
accatctgcaccaaaagctatccgcctagctgttttgtaccaaagcaccgcctaattgt
 T  I  C  T  K  S  Y  P  P  S  C  F  C  T  K  A  P  P  N  C
ggtgttggtagcagctgttgtagtgatggtgattgcccgaaaggcaaagaatgtagcgtt
 G  V  G  S  S  C  C  S  D  G  D  C  P  K  G  K  E  C  S  V
tgcaccaaatcatttccgcctatttgtcgttgtggcaccaaaccgaaactggttctggtt
 C  T  K  S  F  P  P  I  C  R  C  G  T  K  P  K  L  V  L  V
agcgaataatga
 S  E  *  *
```

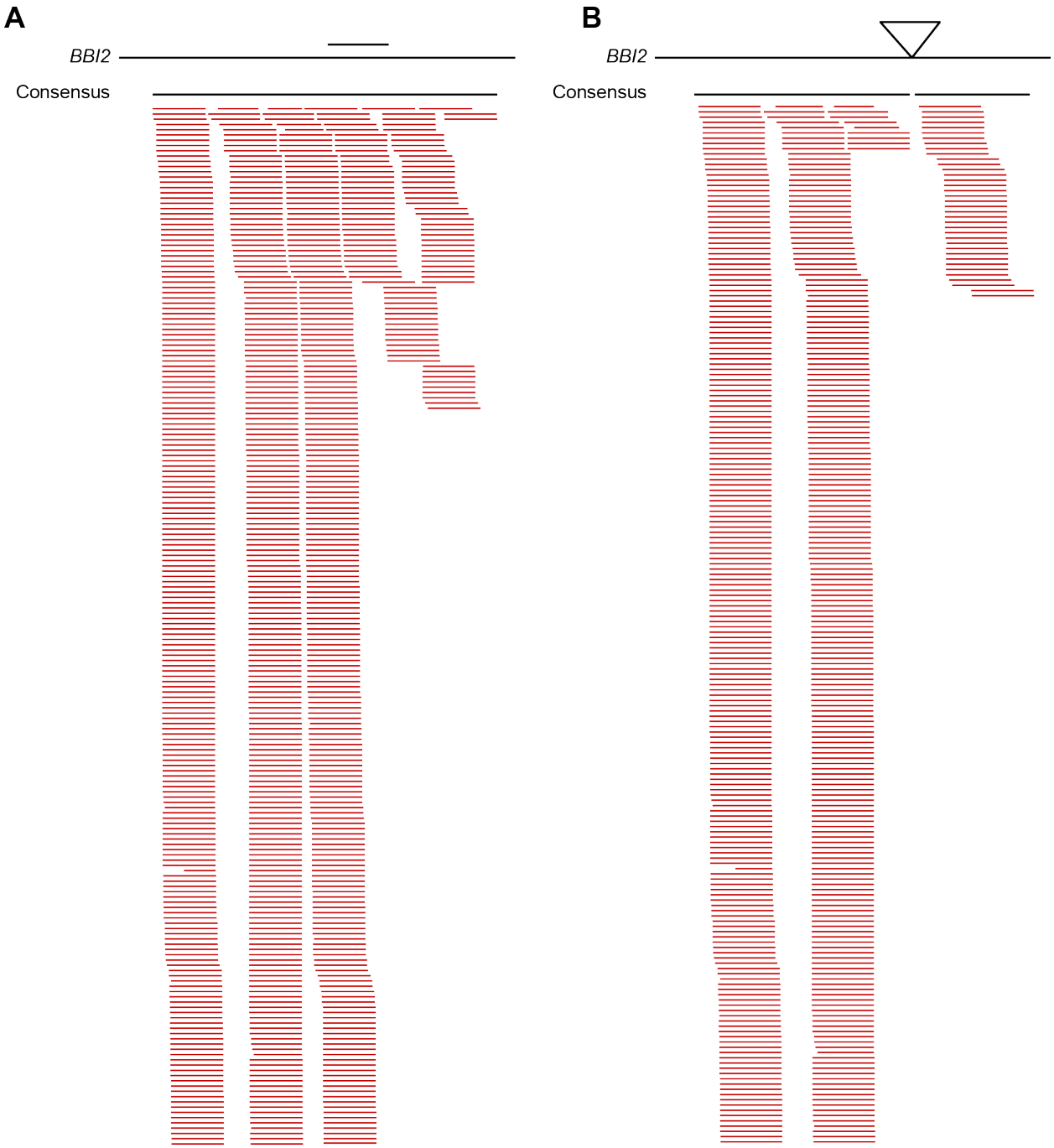
B



Supplemental Figure 2. Recombinant *S. moellendorffii* $BBI3_{22\text{-}124}$ (Supports Figure 3)
(A) *E. coli* codon-optimised ORF encoding BBI3 aligned with the translated sequence. The six-His tag is indicated in red and TEV recognition sequence is indicated in purple. Both conserved BBI-like inhibitory motifs are indicated in green.
(B) SDS-PAGE gel of recombinant BBI3 and BBI3 mutants indicating purity following immobilized metal affinity chromatography and gel-filtration chromatography.



Supplemental Figure 3. Trypsin inhibition by *S. moellendorffii* BBI3 (SmoBBI3) and *Glycine max* BBI (GmaBBI) (Supports Figure 3). Inhibitors were incubated with trypsin and the substrate of trypsin, BAPNA. Trypsin activity is reported as absorbance measurements at OD_{410} which is indicative of BAPNA conversion to p-nitroaniline by trypsin and compared to the no inhibitor control (NIC). Error bars represent standard deviation for three technical replicates in a single microtiter plate.



Supplemental Figure 4. Mapping of RNA-seq reads to genomic DNA sequence shows that *BBI2* is intronless (Supports Figure 2). No RNA-seq reads mapped across the currently annotated exon-exon junction in the gene model (GenBank: XP_002981001). **(A)** RNA-seq reads mapped onto genomic DNA including the sequence coding for *BBI2* (*S. moellendorffii* genome v1.0: scaffold_47:332896...333651). The position of the currently (erroneously) annotated intron is indicated above with a black bar. **(B)** RNA-seq reads mapped to same sequence as in panel **A**, but lacking the predicted intron from the current predicted gene model for *BBI2* (XM_002980955.1). The location of the removed sequence is indicated with a triangle.

Supplemental Table 1. All primers used in this study shown in the 5' to 3' direction. Primers include those designed for determining sequences of all *S. moellendorffii* BBI-like genes (SmoBBI) by RACE and subsequent full length transcripts (FL). The mutated codon in primers for site directed mutagenesis of *BBI3* are underlined. Primers for cloning full length BBI-like genes are labelled by species *Musa acuminata* (Mac) and *Isoetes drumondii* (Idr).

Use	Primer
SmoBBI1 5' RACE	TTGGGCTGTAGCCGGTTTCAC
SmoBBI1 5' RACEb	CAGGGTTGACCGCCACATGA
SmoBBI2 5' RACE	AGCGCCACAGCGACAAATAGGT
SmoBBI2 3' RACE	TTGCTCCTCTCATTGCGCTGCT
SmoBBI3 5' RACE	CACAGTCAACAGGGTTGACCAGCA
SmoBBI3 3' RACE	CCCTCCAAACTGCGGAGTAGGCAGT
SmoBBI4 5' RACE	TGCGGCGCTGCATCTTTTCG
SmoBBI4 3' RACE	TCCAGGCCATGGTGTGCAGG
SmoBBI5 5' RACE	CATAGCCACACATTATCATTATGA
SmoBBI5 3' RACE	CCCTCCAAACTGCGGAGTAGGCAGT
SmoBBI1 FL forward	ATTCTCTATTTCGCTATGGCTCAGTCTATC
SmoBBI1 FL reverse	TAGCCACATTATCATTATGAAAGATCTC
SmoBBI2/SmoBBI3/SmoBBI5 FL forward	ATCCTCTGTTTCGCTATGGCTCAG
SmoBBI2/SmoBBI3/SmoBBI5 FL reverse	GCATAGCCACACATTATCATTATGG
SmoBBI4 FL forward	TGATGAGCAACGCGATGATCTAGTCTC
SmoBBI4 FL reverse	AACCAAAAAAACTCTCTTTACTTTCTCGAAAC
SmoBBI3 K67A forward	TGCACCATCTGCACCGCAAGCTATCCG
SmoBBI3 K67A reverse	CGGATAGCTTGCGGTGCAGATGGTGCA
SmoBBI3 K77A forward	TGTTTTTGTACCGCAGCACCGCCTAATTG
SmoBBI3 K77A reverse	CAATTAGGCGGTGCTGCGGTACAAAAACA
SmoBBI3 K105A forward	TAGCGTTTGCACCGCATCATTTCCGCC
SmoBBI3 K105A reverse	GGCGGAAATGATGCGGTGCAAACGCTA
MacM0RX78 FL forward	TGGTGTGGAAGATGAGGAGC
MacM0RX78 FL reverse	AATGAACACCAAACCGCTGC
MacM0RX79 FL forward	TACAGCTGAGTGCGAAGTGG
MacM0RX79 FL reverse	ACCAAACCACTAGCTTGCGA
MacM0TRV2 FL forward	CGCGGATAGAAGACGCAGAT
MacM0TRV2 FL reverse	ACAATTTCCCGGAAGCGGAT
IdrBBI FL forward	ATCCGAGAACCGGGGCG
IdrBBI FL reverse	TCGTATAGGCTACATATGCACGC

Supplemental Data Set 1. Accession numbers and sequence information for sequences indicated in **Figure 4**. Sequences used in the phylogeny presented in **Figure 6** have a corresponding ID. Assembly version numbers are given for genomes accessed through the Phytozome BLAST portal. Assembly accession numbers are given for genomes accessed through the NCBI BLAST portal.

Supplemental File 1. Sequence alignment used to generate the BBI phylogeny shown in Figure 6. Sequences were aligned using ClustalW (2.0.12) followed by manual editing in BioEdit Sequence Alignment Editor (7.2.5).