Supporting Information

Chuck et al. 10.1073/pnas.1407401112

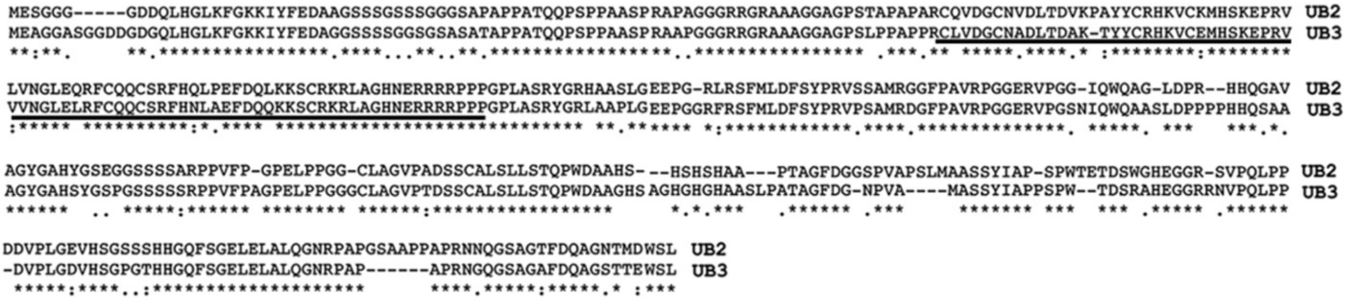
SI Materials and Methods

Association of *ub3* SNPs with ERN and TBN in the NAM Population. Linkage and joint linkage analyses of ERN and TBN in the NAM population have been described previously (1). Briefly, in the joint linkage analysis of ERN across all 26 NAM families, the closest marker to *ub3* (m388) was the first QTL to enter the model, explained 12.0% of the variation in ERN across the entire NAM population, and was the largest-effect ERN QTL across all of the NAM (Fig. S6*A*) and in 13 of 26 individual families (Fig. S6*B*). In the joint linkage analysis of TBN across all 26 NAM families, the closest marker to *ub3* (m388) was the sixth QTL to enter the model, explained 1.4% of the variation in TBN across the entire NAM population, and was not remarkable in terms of effect size (Fig. S7).

Here, we used the newly available set of HapMap2 SNPs (www.panzea.org) to redo a genome-wide association study scan across an ~18-Mb interval encompassing the 95% confidence intervals of the *ub3*-linked joint linkage QTL for ERN and TBN. The phenotypes used for the genome-wide association study consisted of the residuals from the complete joint linkage models excluding QTL on chromosome 4, and confidence intervals were estimated as described previously for leaf architecture traits (2). A set of 575,668 SNPs across this region was filtered to include only those that were polymorphic and contained no missing or heterozygous genotypes across 27 founder lines (8,851). Interval genotypes were imputed as the mean of two flanking markers weighted by physical distance, and phenotypes were regressed on each SNP genotype in turn using family as a covariate.

***ub3* Genotyping and Phenotypic Analysis of IBM Lines.** In total, 167 IBM families were scored for either the B73 or Mo17 alleles using a CAPS marker to a fragment amplified using the *ub3* 7F1 primer (TGCTGGATTTCTCATACCCAAGG) and the 7R2 primer (TGTGCGGCTACGACGATGTGC) and then digested with HpaII, distinguishing the two alleles. These results were corroborated using published *ub3* SNP data (3). These genotype data were correlated with raw measurements for TBN, BZ length, and ERN for these same families across multiple field environments as described previously (1). Best linear unbiased predictors for branch number, BZ, and ERN were compared between 89 RILs that received the B73 *ub3* allele and 79 RILs that received the Mo17 *ub3* allele, and *P* values were calculated by two-sided *t* test.

1. Brown PJ, et al. (2011) Distinct genetic architectures for male and female inflorescence traits of maize. *PLoS Genet* 7(11):e1002383.
2. Tian F, et al. (2011) Genome-wide association study of leaf architecture in the maize nested association mapping population. *Nat Genet* 43(2):159–162.
3. Ganal MW, et al. (2011) A large maize (Zea mays L.) SNP genotyping array: Development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. *PLoS ONE* 6(12):e28334.



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MESGGG-----GDDQLHGLKFGKKIYFEDAAGSSSGSSSGGGSAPAPPATQQPSPPAASPRAPAGGGRRGRAAAGGAGPSTAPAPARCQVDGCNVDLTDVKPAYYCRHKVCKMHSKEPRV UB2
MEAGGASGGDDGDGQLHGLKFGKKIYFEDAGGSSSSGGSGSASATAPPATQQPSPPAASPRAAPGGGRRGRAAAGGAGPSLPPAPPRCLVDGCNADLTDAK-TYYCRHKVCEMHSKEPRV UB3
**:**.      **.****************.****...**..**.*****************..*****************  .***.** *****.****.* :********:********

LVNGLEQRFCQQCSRFHQLPEFDQLKKSCRKRLAGHNERRRRPPPGPLASRYGRHAASLGEEPG-RLRSFMLDFSYPRVSSAMRGGFPAVRPGGERVPGG-IQWQAG-LDPR--HHQGAV UB2
VVNGLELRFCQQCSRFHNLAEFDQQKKSCRKRLAGHNERRRRPPPGPLASRYGRLAAPLGEEPGGRFRSFMLDFSYPRVPSAMRDGFPAVRPGGERVPGSNIQWQAASLDPPPPHHQSAA UB3
:***** **********:*.**** ****************************** **.***** *:*************.****.***************. *****. ***    ***.*.

AGYGAHYGSEGGSSSSARPPVFP-GPELPPGG-CLAGVPADSSCALSLLSTQPWDAAHS---HSHSHAA---PTAGFDGGSPVAPSLMAASSYIAP-SPWTETDSWGHEGGR-SVPQLPP UB2
AGYGAHSYGSPGSSSSSRPPVFPAGPELPPGGGCLAGVPTDSSCALSLLSTQPWDAAGHSAGHGHGHAASLPATAGFDG-NPVA----MASSYIAPPSPW--TDSRAHEGGRRNVPQLPP UB3
******  .. *****:****** ******** ******:*****************     *.*.***   .****** .***     ******* ***  *** .***** .******

DDVPLGEVHSGSSSHHGQFSGELELALQGNRPAPGSAAPPAPRNNQGSAGTFDQAGNTMDWSL UB2
-DVPLGDVHSGPGTHHGQFSGELELALQGNRPAP------APRNGQGSAGAFDQAGSTTEWSL UB3
 *****:****..:********************       ****.*****:*****.* :***
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Fig. S1. Amino acid alignment of UB2 and UB3. Amino acid alignment of (upper rows) UB2 and (lower rows) UB3 full-length proteins. Underlined sequence represents the SBP-box.

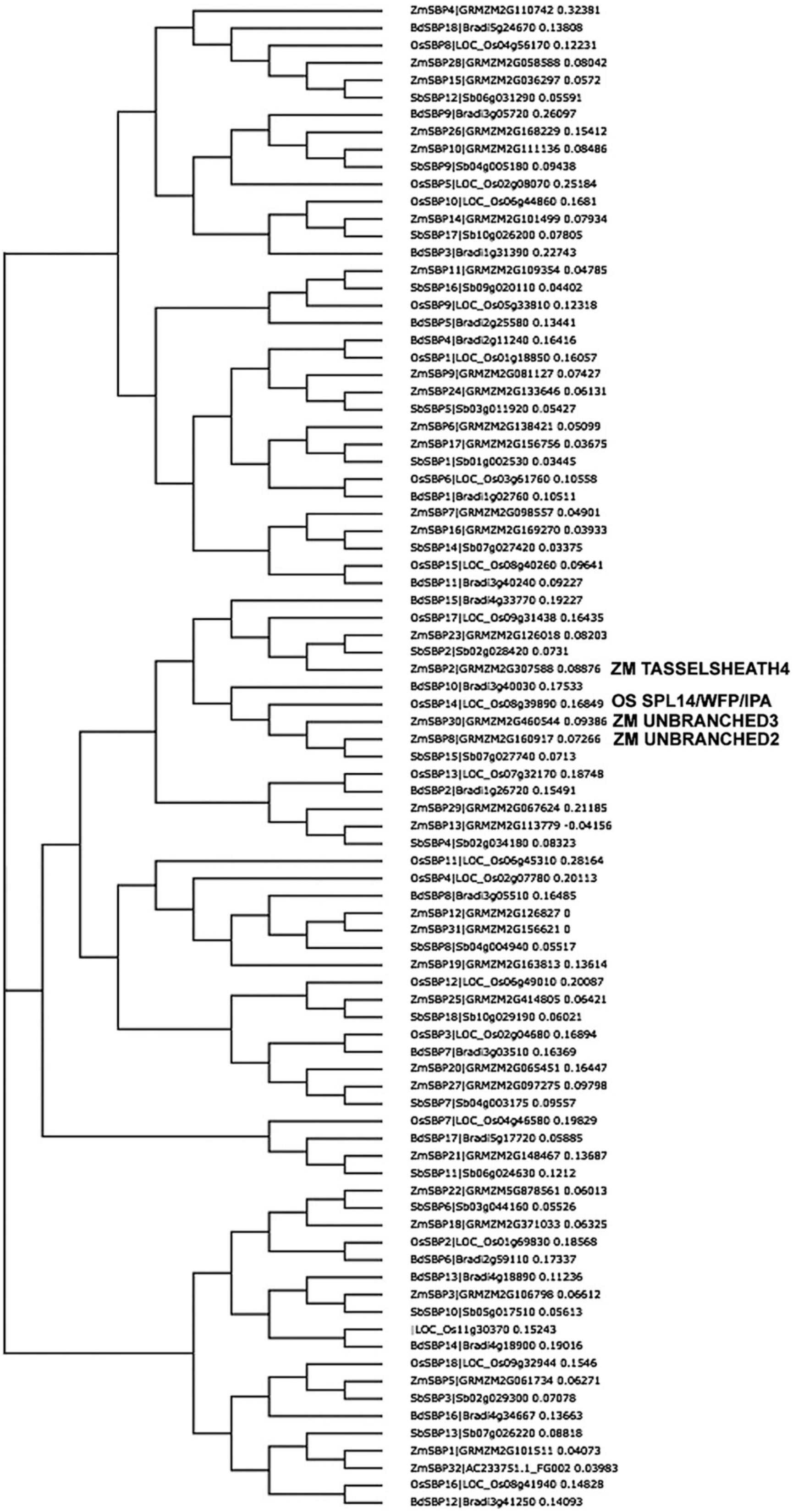

Fig. S2. Phylogenetic tree of SBP-box transcription factors from grasses. Neighbor-joining tree of all annotated SBP-box proteins from maize, sorghum, *Brachypodium*, and rice.

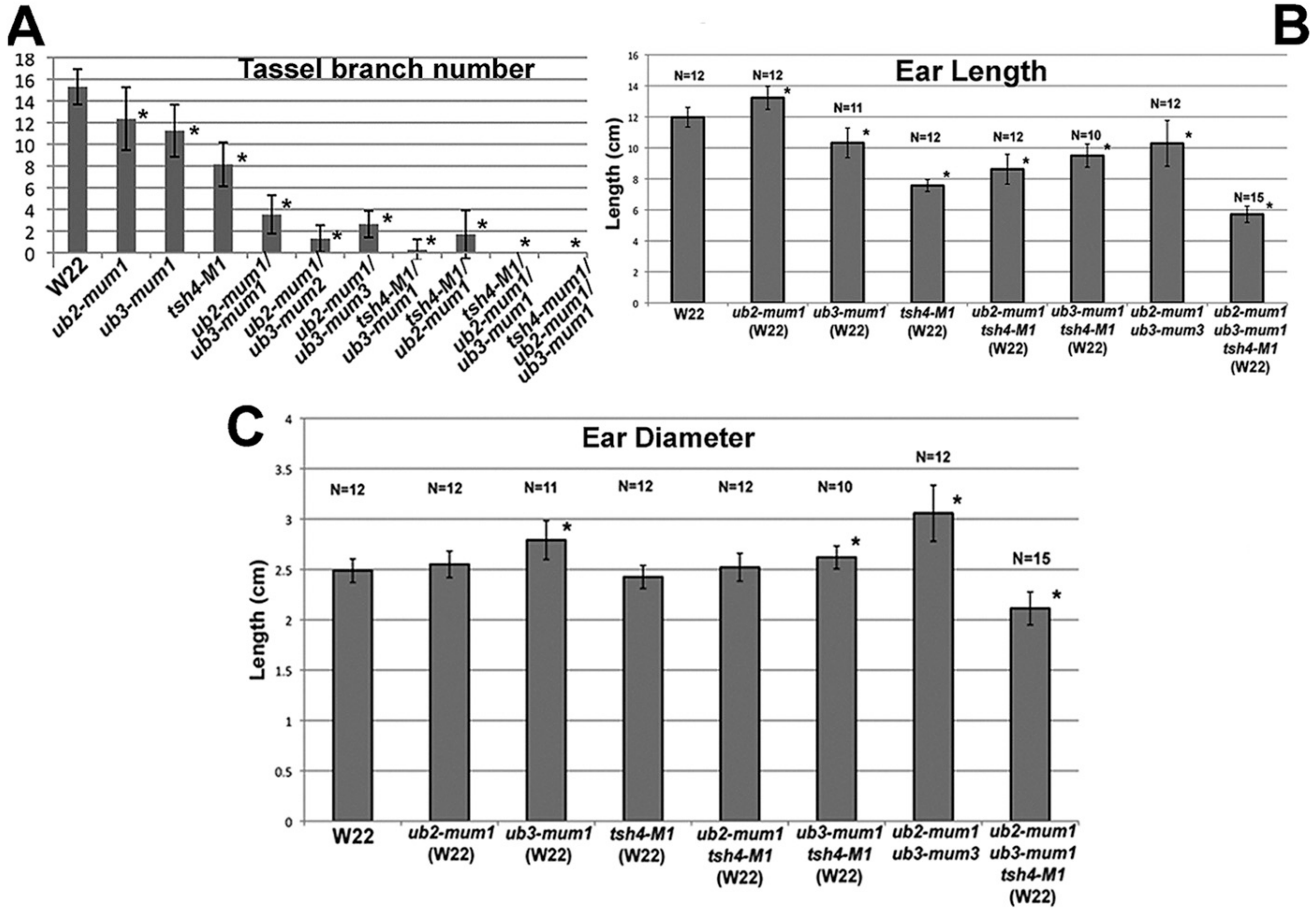

Fig. S3. Tassel and ear measurements of *SBP-box* mutants. (*A*) TBN in W22, *ub2*, *ub3*, and *tsh4* double- and triple-mutant combinations. *Significant differences with W22 (P value < 0.0003 was calculated by t test). (*B*) Average lengths of inbred W22 and *SBP-box* mutant ears. Error bar = SD. All mutants are significantly different from W22 based on two-tailed t tests. *P value < 0.0024. (*C*) Average diameters of W22 and *SBP-box* mutant ears at midpoint. Only single-, double-, and triple-mutant combinations with *ub3* are significantly different from the inbred based on two-tailed t tests. Error bar = SD. *P value < 0.0143.

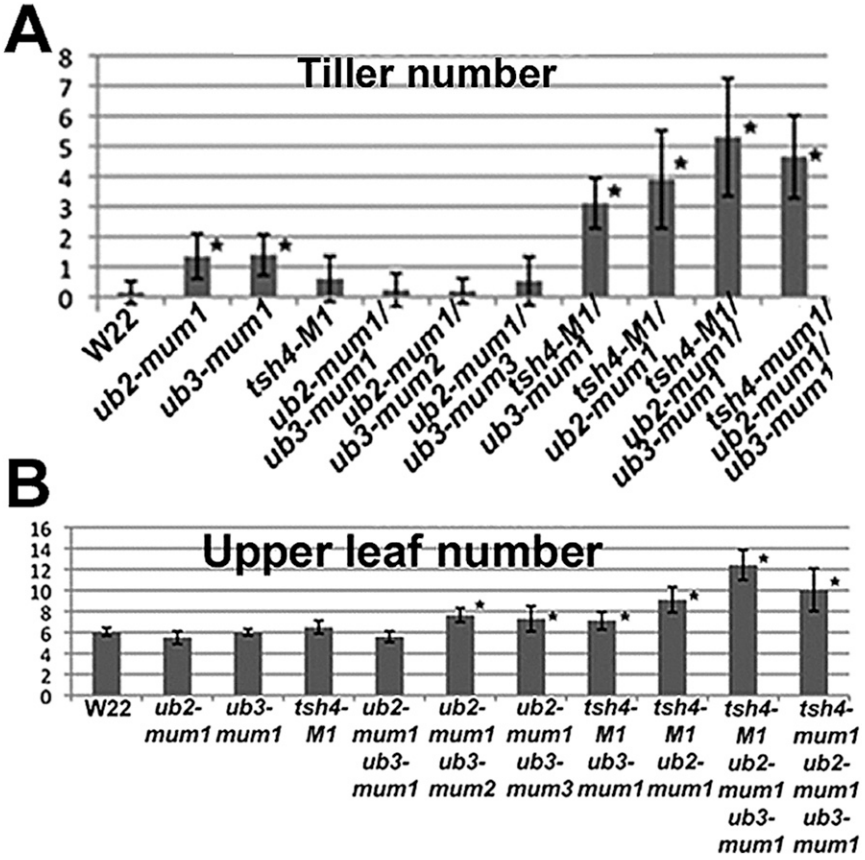

Fig. S4. Tiller and leaf counts of *SBP-box* mutants and expression of tillering markers. (*A*) Tiller counts in field-grown plants of single-, double-, and triple-mutant combinations. *Significant differences from W22 (*P* value < 1.34E-06 was calculated by *t* test). (*B*) Upper leaf number counts above ear nodes of single, double, and triple mutants. *Significant differences from WT W22 (*P* value < 5.09E-05 was calculated by *t* test).

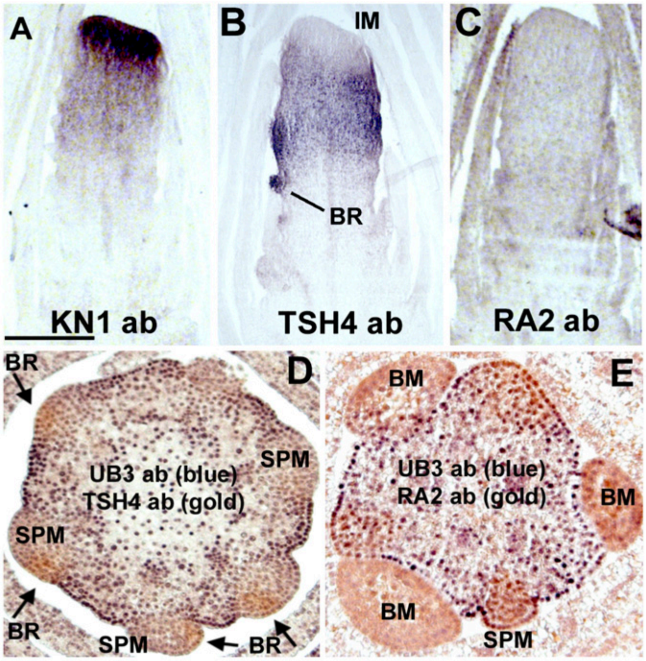

Fig. S5. Immunolocalization of early meristem markers in mutant and WT tassels. (*A*–*C*) Immunolocalization in adjacent sections of *ub2-mum1/ub3-mum3* tassel. (*A*) KN1 immunolocalization showing expression only in the IM. (*B*) TSH4 immunolocalization showing expression in stem and early bract primorida (BR). (*C*) RA2 immunolocalization showing lack of expression. (Scale bars: *A*–*C*, 500 μm.) (*D*) Double labeling of WT tassel with UB3 (blue) and TSH4 (gold) antibodies. The proteins occupy separate domains in the SPM; UB3 is absent from the SPM, whereas TSH4 is present in the BR of the SPM (arrows). (*E*) Double labeling of the base of WT tassel with UB3 (blue) and RA2 (gold). UB3 is absent from the BM and SPM, whereas RA2 is present in both.

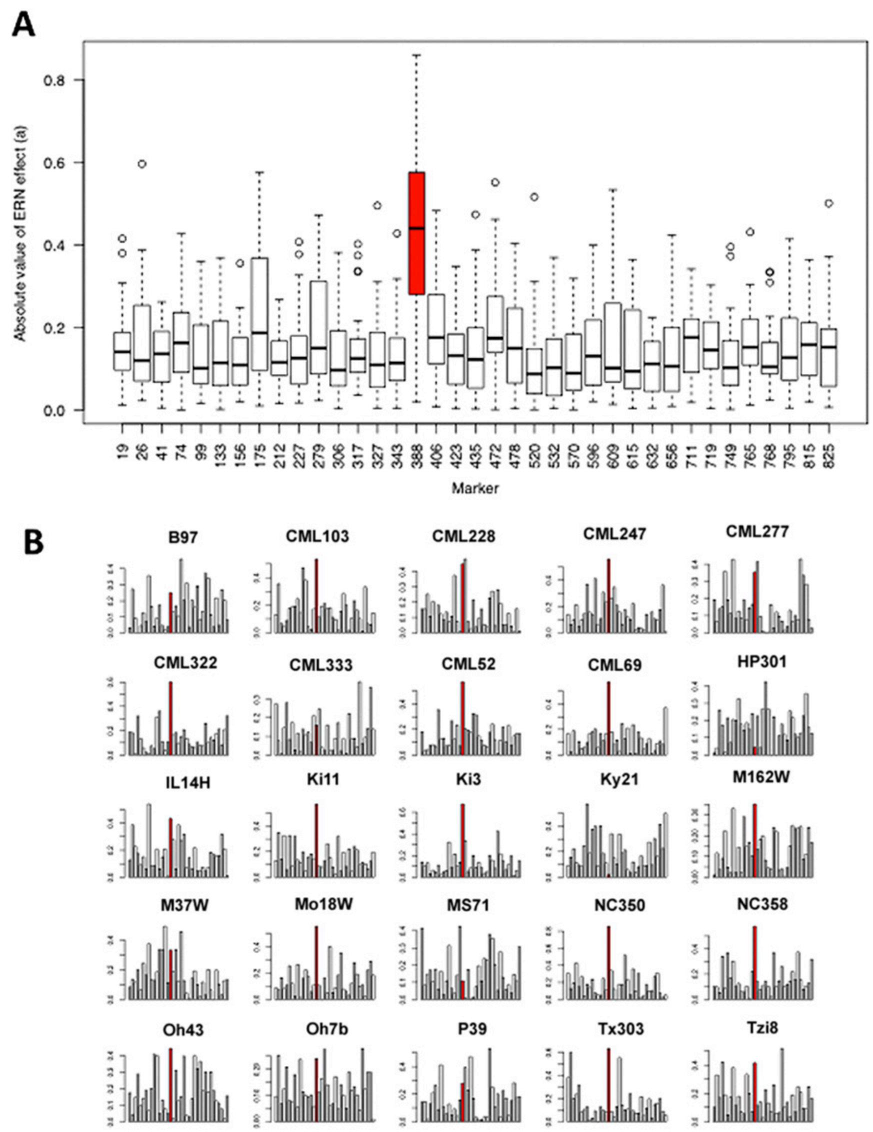

Fig. S6. QTL effects for ERN in the NAM population. (*A*) QTL effects from joint linkage analysis across the entire NAM population. (*B*) Joint linkage QTL effects separated by family. The *ub3*-linked ERN QTL is shown in red.

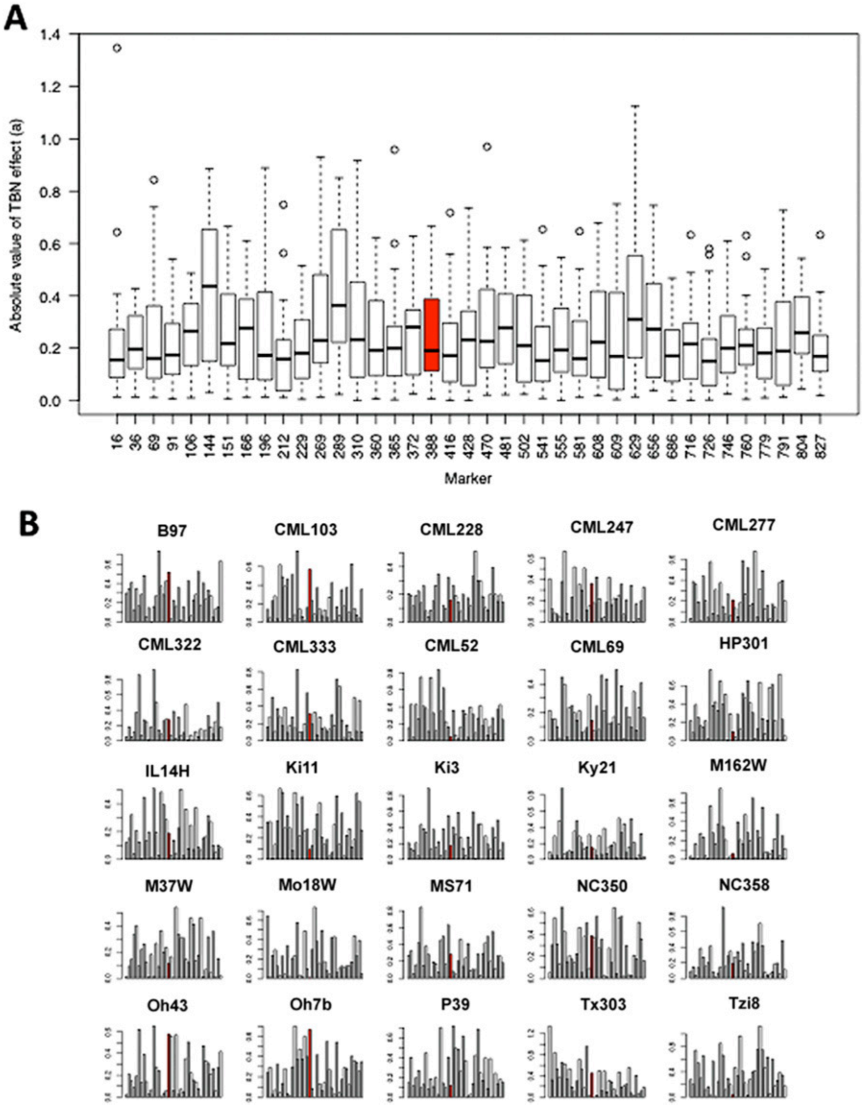

Fig. S7. QTL effects for TBN in the NAM population. (*A*) QTL effects from joint linkage analysis across the entire NAM population. (*B*) Joint linkage QTL effects separated by family. The *ub3*-linked TBN QTL is shown in red.

Table S1. Comparison of joint linkage and GWAS effects at *ub3*-linked QTL with genotypes underlying candidate polymorphisms

	ERN			TBN		
	Joint linkage*	GWAS†	Ser220Asn‡	Joint linkage*	GWAS†	Val260Met‡
B97	−0.24	1	1	0.52	1	0
CML103	−0.5	1	1	0.57	1	1
CML228	−0.48	1	1	NS	0	0
CML247	−0.52	1	1	0.36	1	0
CML277	−0.32	1	1	NS	0	0
CML322	−0.54	1	1	NS	1	0
CML333	−0.21	1	1	NS	1	0
CML52	−0.62	1	1	NS	0	0
CML69	−0.61	1	1	NS	0	0
HP301	NS	0	0	NS	0	0
IL14H	−0.42	1	1	NS	0	0
Ki11	−0.6	1	1	NS	0	0
Ki3	−0.66	1	1	NS	0	0
Ky21	NS	1	1	NS	0	0
M162W	−0.32	1	1	NS	0	0
M37W	−0.35	1	1	NS	0	0
Mo17	−0.44	1	1	0.56	1	1
Mo18W	−0.54	1	1	NS	0	0
MS71	NS	0	1	0.28	0	0
NC350	−0.83	1	1	−0.39	0	0
NC358	−0.57	1	1	NS	0	0
OH43	−0.37	1	1	−0.58	0	0
OH7B	−0.23	1	1	0.67	1	1
P39	−0.38	1	1	NS	0	0
Tx303	−0.65	1	1	0.45	1	1
TZI8	−0.36	1	1	NS	0	0

Effects/alleles that differ significantly from B73 are shaded.

*Joint linkage QTL effects. Effects not significant (NS) in an individual family ($P \geq 0.05$) are marked.

†Genotypes of the most significant SNP in each genome-wide association study (GWAS) analysis; 0 indicates the reference genome (B73) genotype.

‡Genotypes of the nonsynonymous SNPs in the third exon of ub3 at 199,457,549 and 199,457,430.