Supporting Information

Hartwig et al. 10.1073/pnas.1108359108

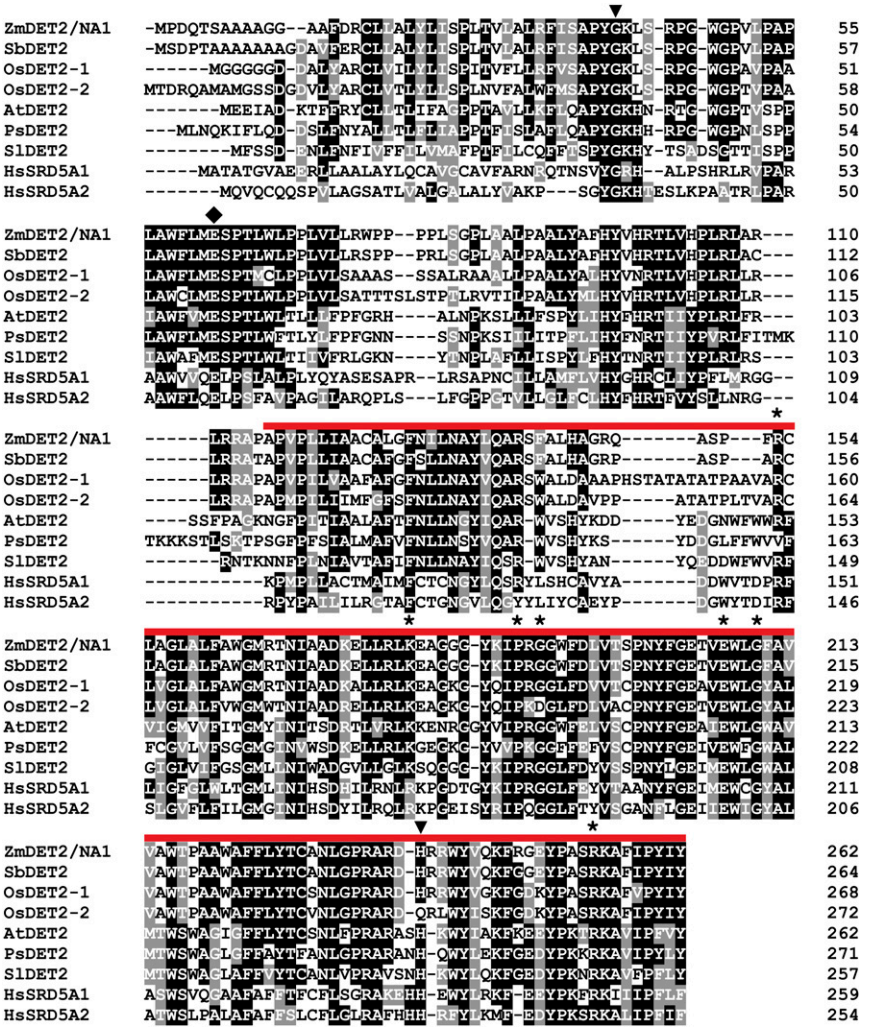

Fig. S1. Amino acid alignment of NA1 to selected 5α-steroid reductases. Sequences were aligned with ClustalW2 (version 2.0.12; http://www.ebi.ac.uk/Tools/clustalw2/), and similarity shading was applied using Boxshade (version 3.21). Dashes represent gaps introduced to maximize the alignment. A red bar above the residues indicates the predicted 3-oxo-5α-steroid 4-dehydrogenase domains (pfam02544). Conserved glycine and histidine residues, important for sterol binding, are indicated by inverted triangles; asterisks indicate residues important for cofactor binding originally identified in a human DET2 ortholog (1, 2); black diamond marks a glutamic acid residue shown to be important for DET2 function in Japanese morning glory (*Ipomoea nil*) (3). Zm, *Zea mays*; Sb, *Sorghum bicolor*; Os, *Oryza sativa*; At, *Arabidopsis thaliana*; Ps, *Pisum sativum*; Sl, *Solanum lycopersicum*; Hs, *Homo sapiens*.

1. Russell DW, Wilson JD (1994) Steroid 5 α-reductase: Two genes/two enzymes. *Annu Rev Biochem* 63:25–61.
2. Luo M, et al. (2007) GhDET2, a steroid 5α-reductase, plays an important role in cotton fiber cell initiation and elongation. *Plant J* 51:419–430.
3. Suzuki Y, et al. (2003) A dwarf mutant strain of *Pharbitis nil*, Uzukobito (*kobito*), has defective brassinosteroid biosynthesis. *Plant J* 36:401–410.

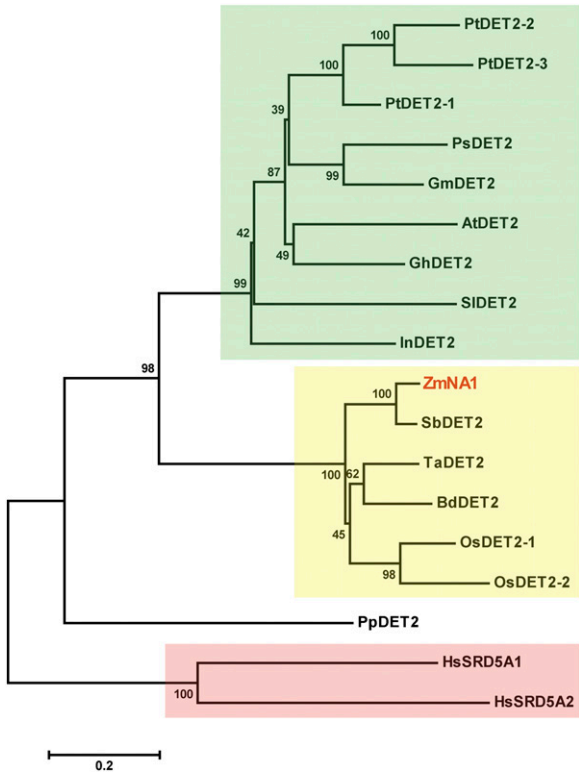

Fig. S2. Phylogenetic analysis of NA1 and selected 5α-steroid reductases. MEGA5 (1) was used to construct the phylogenetic tree based on the neighbor-joining method (2). Alignment was performed using the ClustalW function in Mega5 with default settings. The optimal tree with the sum of branch length = 5.50177703 is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the internal nodes (3). The evolutionary distances were computed using the JTT matrix-based method (4) and are in the units of the number of amino acid substitutions per site. All ambiguous positions were removed for each sequence pair. NA1 is highlighted in red. Monocotyledon cluster (yellow box), dicotyledon cluster (green box), and human 5α-steroid reductases (red box) are color-coded. Pt, *Populus tremuloides*; At, *Arabidopsis thaliana*; Gh, *Gossypium hirsutum*; Ps, *Pisum sativum*; Gm, *Glycine max*; Sl, *Solanum lycopersicon*; In, *Ipomoea nil*; Zm, *Zea mays*; Sb, *Sorghum bicolor*; Ta, *Triticum aestivum*; Bd, *Brachypodium distachyon*; Os, *Oryza sativa*; Pp, *Physcomitrella patens*; Hs, *Homo sapiens*.

1. Tamura K, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731–2739.
2. Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425.
3. Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791.
4. Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. *Comput Appl Biosci* 8:275–282.

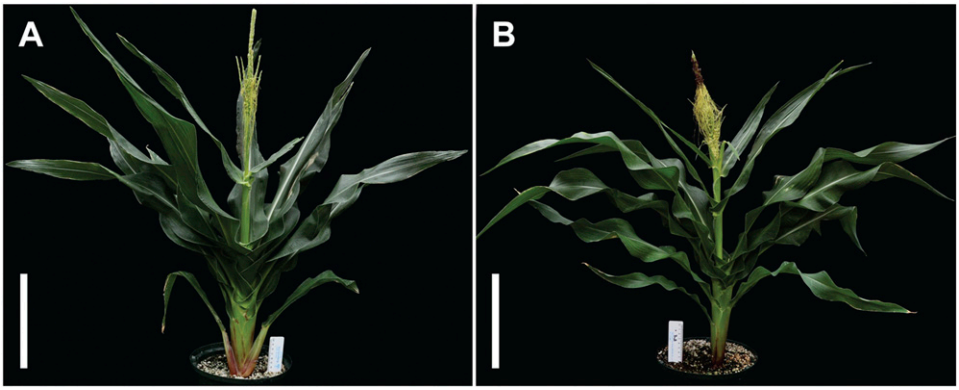

Fig. S3. Genetic complementation test of *na1-1* and *na1-2*. Pollen from homozygous *na1-1* plants was used to fertilize homozygous *na1-2* ears. (*A*) *na1-1* homozygous plant. (*B*) F1 plant of the cross between *na1-1* and *na1-2*. (Scale bars, 20 cm.)

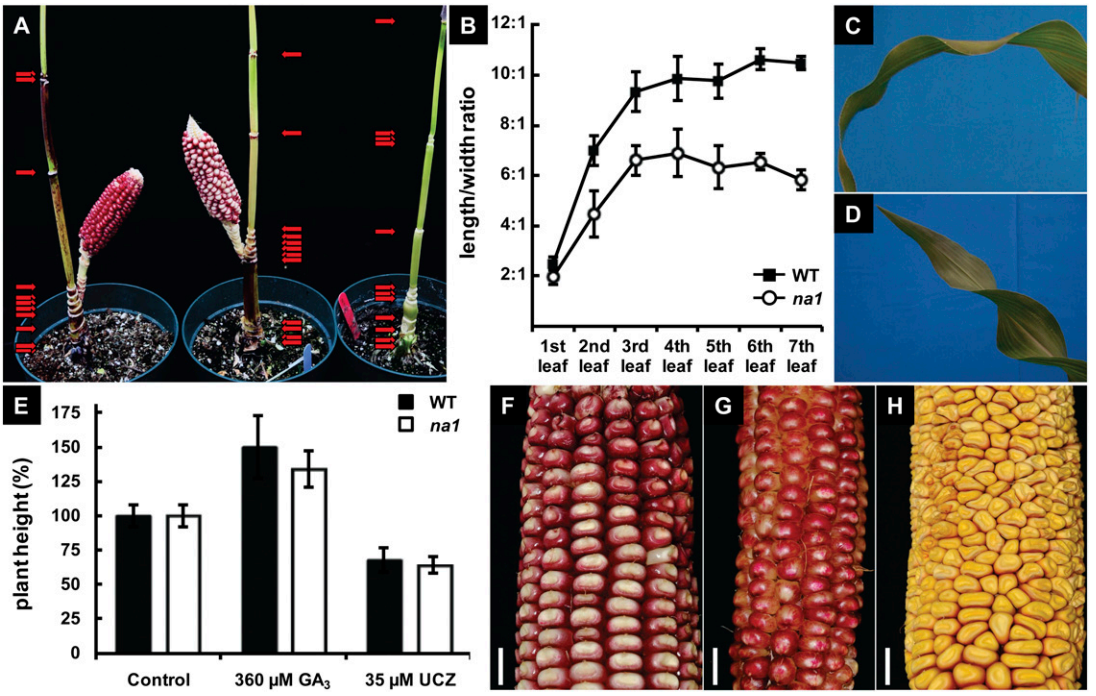

Fig. S4. Additional phenotypes of *na1* mutants. (*A*) Mature *na1-1* siblings with leaves removed for better visibility of internodes. Red arrows indicate stem nodes. Internode length varied considerably between *na1* plants. (*B*) Length to width ratio for the first seven fully expended leaves of *na1-1* and WT. (*C*) Leaf seven of wild type (WT). (*D*) Twisting leaf seven of *na1-1*. (*E*) Light-grown WT and *na1-1* plants both treated with 360 μM GA3 and 35 μM UCZ. The increase and reduction in plant height due to GA3 and UCZ treatment, respectively, is similar between WT and *na1* plants. (*F–H*) Sex determination in ears is not altered in *na1* mutants. Ears of WT (*F*), *na1-1* (*G*), and *ts2* (*H*). Stamen primordia and the gynoecium of the LF did not mature in WT and *na1* mutant ear spikelets, resulting in evenly rowed kernels. In contrast, *ts2* ears display irregular kernel placement due to the development of the normally suppressed gynoecium of the LF (1). (Scale bars, 1 cm.) (*B* and *E*) Data are means ± SD; $n = 10$ (*B*) and $n = 9$ (*E*).

1. DeLong A, Calderon-Urrea A, Dellaporta SL (1993) Sex determination gene *TASSELSEED2* of maize encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion. *Cell* 74:757–768.

Fig. S5. Light and dark development of wild-type, *d5*, and *na1* seedlings. (*A* and *B*) Wild-type seedlings grown in the light (*A*) or dark (*B*). (*C* and *D*) GA deficient *d5* (1) grown in the light (*C*) or dark (*D*). (*E* and *F*) *na1-1* grown in the light (*E*) or dark (*F*). Red arrows indicate the position of first (coleoptile) node. Plants were grown as described in *Chemical Treatments*. (Scale bar, 10 cm.)

1. Fujioka S, et al. (1988) Qualitative and quantitative analyses of gibberellins in vegetative shoots of normal, *dwarf-1*, *dwarf-2*, *dwarf-3*, and *dwarf-5* seedlings of *Zea mays* L. *Plant Physiol* 88:1367–1372.

Table S1. Characterization of *na1* alleles

Allele	Mutation	Position	Orig. genetic background	Seed source
na1-1	*Mu* insertion; 9 bp target site duplication (5′-CCGCACGCT-3′)	298–306	Mixed *Mu*-active	This work
na1-2	G to C transversion; results in nonsense substitution W163 > STOP	489	Unknown	G. Neuffer/Maize COOP
na1-3	*Mu* insertion; 9 bp target site duplication (5′-CGCGCAAGG-3′)	758–766	Mixed *Mu*-active	This work
na1-4	*Mu* insertion; 9 bp target site duplication (5′-AGATCCCGA-3′)	557–565	Mixed *Mu*-active	This work

To determine the position of a mutation in the *na1* gene, the A of the *na1* translational start (ATG) was counted as +1. Sequences of *na1* mutant alleles were deposited in GenBank (*na1-1*, JN020029; *na1-2*, JN020030; *na1-3*, JN020031; *na1-4*, JN020032).

Table S2. Induction of tassel floret feminization after exogenous application of PCZ

Line	PCZ, μM	No. of plants	Dwarf phenotype	Staminate tassel florets	Up to 50% of tassel florets feminized	>50% of tassel florets feminized
Wild type	0	13	0	13	0	0
na1-1	0	13	13	7	5	1
Wild type	500	13	13	0	11	2
na1-1	500	13	13	0	4	9

Plants were grown as described in *Chemical Treatments* in Turface and watered with fertilizer solution supplemented with 500 μM PCZ. A dwarf phenotype was scored if the total height did not exceed one-third of wild-type height and the secondary *na1* mutant phenotypic features (dark-green, short, twisting leaves) were present.

Table S3. Endogenous BR profiles of wild-type and *na1-1* plants for each independently grown biological replicate

BR intermediates	WT replica1, ng per g of fw	WT replica2, ng per g of fw	*na1* replica1, ng per g of fw	*na1* replica2, ng per g of fw
24-Methylenecholesterol	981	737	1,040	864
Campesterol	52,400	28,300	40,400	29,500
4-en-3-one	152	126	693	623
3-one	6.8	8.8	1.1	0.8
Campestanol	944	709	2.9	64.9
6-Deoxocathasterone	0.2	0.34	n.d.	0.05
6-Deoxoteasterone	0.01	0.05	n.d.	0.02
3-Dehydro-6-deoxoteasterone	0.35	0.21	n.d.	n.d.
6-Deoxotyphasterol	1.8	1.98	n.d.	0.23
6-Deoxocastasterone	5.18	6.26	n.d.	0.47
6-Oxocampestanol	8.1	19.9	4.9	14.4
Cathasterone	n.d.	n.d.	n.d.	n.d.
Teasterone	n.d.	n.d.	n.d.	0.09
Typhasterol	0.04	0.05	0.15	0.13
Castasterone	1.03	1.25	n.d.	0.13
Brassinolide	n.d.	n.d.	n.d.	n.d.

A pool of 20 g of fresh weight (WT replicate1, 20.71 g fw; WT replicate2, 20.05 g fw; *na1-1* replicate1, 20.57 g fw; and *na1-1* replicate2, 20.88 g fw) from 10 individuals was analyzed for each replicate. n.d., not detected.

Table S4. 5α-steroid reductases used in the phylogenetic analysis of ZmDET2/NA1

Sequence ID	Organism	GenBank accession (Gene ID)
AtDET2	*Arabidopsis thaliana*	NP_181340 (At2g38050)
BdDET2	*Brachypodium distachyon*	(Bradi2G55110)
GhDET2	*Gossypium hirsutum*	AAZ83346
GmDET2	*Glycine max*	AAG35638
HmSRD5A1	*Homo sapiens*	NP_001038
HmSRD5A2	*Homo sapiens*	NP_000339
InDET2	*Ipomoea nil*	BAC87862
OsDET2-1	*Oryza sativa*	NP_001044822 (Os01g0851600)
OsDET2-1	*Oryza sativa*	NP_001176391 (Os11g0184100)
PpDET2	*Physcomitrella patens*	XP_001769717
PsDET2	*Pisum sativum*	AAT76665
PtDET2-1	*Populus temuloides*	XP_002323554
PtDET2-2	*Populus temuloides*	XP_002319902
PtDET2-3	*Populus temuloides*	XP_002328371
SbDET2	*Sorghum bicolor*	JN020028 (Sb03g040050)
SlDET2	*Solanum lycopersicon*	CAH05260
TaDET2	*Triticum aestivum*	CBH32517
ZmDET2/NA1	*Zea mays*	NP_001149816