Strigolactones (SLs), a newly discovered class of carotenoid–derived phytohormones, are essential for developmental processes that shape plant architecture and interactions with parasitic weeds and symbiotic arbuscular mycorrhizal fungi. Despite the rapid progress in elucidating the SL biosynthetic pathway, the perception and signalling mechanisms of SL remain poorly understood. Here we show that DWFAR 3 (D53) acts as a repressor of SL signalling and that SLs induce its degradation. We find that the rice (Oryza sativa) d53 mutant, which produces an exaggerated number of tillers compared to wild-type plants, is caused by a gain–of–function mutation and is insensitive to exogenous SL treatment. The D53 gene product shares predicted features with the class I Clp ATPase proteins and can form a complex with the α/β hydrolase protein DWFAR 14 (D14) and the F-box protein DWFAR 3 (D3), two previously identified signalling components potentially responsible for SL perception. We demonstrate that, in a D14– and D3– dependent manner, SLs induce D53 degradation by the proteasome and abrogate its activity in promoting axillary bud outgrowth. Our combined genetic and biochemical data reveal that D53 acts as a repressor of the SL signalling pathway, whose hormone–induced degradation represents a key molecular link between SL perception and responses.

Shoot branching (tillering in crops) is a major determinant of plant architecture and crop yield, which is under the integrated control of hormonal, developmental and environmental factors1–5. Although the existence of a root-derived transmissible shoot-repressing signal was proposed more than 70 years ago6, the identity of this signal(s) has remained elusive. Recent studies with branching mutants in several plant species have demonstrated that, in a specific group of terpenoid lactones, are the long-sought branching-repressing hormones, whose function are highly conserved in both monocots and dicots6–8. In addition to repressing shoot branching, these lactones also have a role in regulating root growth, leaf senescence and flower development. SLs also act as exogenous signals to promote the symbiosis between land plants and arbuscular mycorrhizal fungi9 and stimulate the germination of the parasitic weeds Striga and Orobanche, which are serious agricultural pests in many parts of the world6.

Previous studies have shown that DWFAR 3 (D3), D10, D14 (also known as HIGH-TILLERING DWFAR 2, HTD2 and D88), D17 (also known as HTD1) and D27 in rice10–14, MORE AXILLARY GROWTH 1 (MAX1), MAX2, MAX3 and MAX4 in Arabidopsis15–17, RAMOUSUS 1 (RMS1), RMS4 and RMS5 in pea18 and DECREASED APICAL DOMINANCE 1 (DAD1), DAD2 and DAD3 in petunia19,20 are involved in either the biosynthesis or signalling of SLs. Among these genes, MAX3/RMS5/D17/DAD3, MAX4/RMS1/D10/DAD1, MAX1 in Arabidopsis and D27 in rice encode the carotenoid cleavage dioxygenase 7 (CCD7), CCD8, CYP711A1 (a cytochrome P450) and a novel β-carotene isomerase, respectively, and are involved in the sequential cleavage of β-carotene and synthesis of SLs20. By contrast, MAX2/RMS4/D14 and D14/DAD2, which encode an F-box protein and a protein of the α/β-hydrolase superfamily, respectively, probably have a role in SL signalling12,19,21.

The structural similarity between MAX2/RMS4/D3 proteins with the auxin receptor TRANSPORT INHIBITOR RESPONSE 1 (TIR1)22,23 and jasmonate receptor CORONATINE INSENSITIVE 1 (COI1)24, and D14/DAD2 proteins with the gibberellin receptor GIBBERELLIN INSENSITIVE DWFAR 1 (GID1)25, has sparked the speculation that both MAX2/RMS4/D3 and D14/DAD2 could be candidates for the SL receptors26 and that binding and hydrolysis of SLs by D14/DAD2 (refs 19, 27, 28) might be required for triggering proteasome-mediated degradation of an unknown repressor by the Skp1–Cullin–F-box-containing (SCF)MAX2 complex29,30. However, the identity of such a repressor and its regulatory mechanisms in SL signalling have remained unknown.

In this study, we identified a gain–of–function rice mutant, d53, which displays a SL-insensitive and increased-tillering phenotype. Map-based cloning revealed that D53 encodes a protein sharing predicted features with the class I Clp ATPase proteins and that it can form a complex with the α/β hydrolase protein D14 and the F-box protein D3. We show that SLs induce D53 degradation by the proteasome–ubiquitin pathway in a D14– and D3– dependent manner. Our studies establish D53 as a repressor of the SL signalling pathway, whose hormone–induced degradation is essential for SL signalling.

**d53 is a rice SL–insensitive mutant**

Previous studies have identified several rice mutants defective in SL biosynthesis or signalling10–14. Because of their highly branched and dwarf phenotype, these mutants were termed ‘d mutants’, such as d3, d10, d14, d17 and d27. The rice d53 mutant10 also displayed reduced height and increased tillering, as well as thinner stem and shorter crown root, compared to the wild-type strain (Fig. 1a, b and Extended Data Fig. 1a, b).

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Kinetic analysis showed that at the heading stage, the total tiller number of \( d_3 \) was almost three times that of the wild type, resulting from an increase in both higher-order and high-node tillers (Fig. 1c and Extended Data Fig. 1c, d). Histological analysis revealed that the sizes of vascular bundles and parenchyma cells in internodes were largely comparable between \( d_3 \) and wild-type plants, implying that the shortening and thinning of the \( d_3 \) stem were mainly caused by a reduction in cell number (Extended Data Fig. 1e–h). The phenotypes of \( F_1 \) heterozygotes was also similarly downregulated in the \( F_2 \) generation (Extended Data Fig. 2a–g). Genetic analyses of an \( F_2 \) population derived from a cross of \( d_3 \) and a wild-type parent (Norin 8) showed that the normal, intermediate and dwarf plants segregated as 1:2:1 (33:58:28, \( \chi^2 = 0.09, P > 0.05 \)), indicating that the \( d_3 \) mutation behaved in a semi-dominant manner (Extended Data Fig. 2b).

The phenotypic similarity between \( d_3 \) and the previously reported rice \( d \) mutants prompted us to examine whether \( d_3 \) is defective in SL-mediated inhibition of axillary bud outgrowth. Quantitative PCR with reverse transcription (qRT–PCR) analysis showed that expression of \( D10 \) (encoding CCDC8) was similarly upregulated in \( d_3 \) (Fig. 1d) as in other \( d \) mutants, owing to feedback regulation in the SL pathway. In addition, expression of an inhibitor of axillary bud outgrowth, \( FINE CULM 1 \) (FC1), which is orthologous to the maize TEOSINTE BRANCHED1 (TB1) and the Arabidopsis BRANCHED 1 (BRC1), was also similarly downregulated in the \( d_3 \), \( d14 \) and \( d27 \) mutants (Fig. 1d), suggesting that \( D3 \) is probably involved in SL biosynthesis or signalling. Moreover, exogenous application of a SL analogue, GR24, effectively inhibited the outgrowth axillary buds of \( d_27 \), but not \( d14 \) or \( d53 \) (Fig. 1e and Extended Data Fig. 3a–c). Further, measurement of SLs produced in the root exudates showed that \( d_3 \) accumulated markedly higher levels of 2’-epi-5-deoxystrigol (epi-5DS), a native SL of rice, than the wild-type cultivar Norin 8 (Fig. 1f). These results indicate that \( d_3 \) is a SL-insensitive mutant.

**D3 acts as a repressor of SL signalling**

\( D3 \) was previously mapped to the terminal region of the short arm of rice chromosome 11 (ref. 35). To decipher the molecular defect in \( d_3 \), we isolated \( D3 \) by a map-based cloning approach. Using an \( F_2 \) population of ~12,000 plants generated from the cross between Ketan Nangka and the mutant, we further delimited the \( D3 \) locus to a 34-kilobase DNA region on the bacterial artificial chromosome (BAC) clone OSJNBA0032J07, which contains three putative genes (Fig. 2a). Sequence analysis revealed a single-nucleotide substitution and 15-nucleotide deletion in the third exon of LOC_Os11g01330 in \( d_3 \), which resulted in an amino acid substitution (R812T) and deletion of five amino acids (E813GKTGI817) (Fig. 2b). To verify that this mutation caused the tillering dwarf phenotype, we generated transgenic plants expressing the wild-type or mutant \( D3 \) gene under the control of the promoter of the cauliflower mosaic virus large subunit (CaMV) 35S. Transgenic plants, like the wild type, produced no tillers, whereas the mutant displayed increased tillering (Fig. 2c), implicating that the decreased expression of SLs results in an increase in the number of tillers in the mutant. A transgenic line expressing an enhanced SL pathway (E35S:GR24) showed no phenotypic difference from the wild type, whereas another line showing a reduced SL pathway (D35S:GR24) displayed an increase in tiller number (Fig. 2d).

**Figure 1** | Phenotype of \( d_3 \) mutant. a, Phenotype of wild-type (WT) and \( d_3 \) mutant at 4-week-old seedling stage (a) or heading stage (b). White arrows indicate the first tillers in \( d_3 \), which is usually absent in wild type, and red arrows show the second tillers. c, Comparison of tillering kinetics at different developmental stages. d, qRT–PCR assay showing altered expression of \( D10 \) and FC1 in \( d \) mutants. e, Responses of rice seedlings to GR24 treatment. Red and white arrowheads indicate the first and second tillers, respectively. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) measurement of epi-5DS levels in root exudates, gfw, per gram fresh weight; ND, not detected. Scale bars, 5 cm (a), 30 cm (b), 2 cm (c). Values are means ± s.d. (n = 30 plants; d, f, n = 3 replicates). The Student’s t-test analysis indicates a significant difference (compared with wild type, *P < 0.05, **P < 0.01).

**Figure 2** | Map-based cloning and characterization of \( D3 \). a, \( D3 \) was fine-mapped on chromosome 11. The numbers of recombinants are shown in brackets. b, Molecular lesions in \( d_3 \) mutant. c, Phenotypic comparison of \( pAct1::D3-GFP \) and \( pAct1::d3-GFP \) transgenic plants. Vector (Vec.), \( pAct1::D3-GFP \) control. L, independent transgenic line. Scale bar, 10 cm. d, e, Tiller number (d) and relative expression of \( D3 \) (e) of transgenic plants in c. OE, overexpression. f, \( D3 \) expression in various organs, including young panicles (YP), young roots (YR), shoots (S), leaf blades (LB), leaf sheaths (LS), culms (C) and nodes (N). g, GR24 treatment induces \( D3 \) expression. h, Relative expression levels of \( D3 \) in two wild-type varieties Norin 8 (N) and Shikori (S), and six rice \( d \) mutants. Each value in d–h represents the mean ± s.d. (d and f–h, n = 3 replicates; e, n = 20 plants). The Student’s t-test analysis indicates a significant difference (compared with control, *P < 0.05, **P < 0.01).
of rice ACTIN 1 promoter (pAct1), in a wild-type background. Notably, all transgenic plants expressing the mutant d53 gene showed a more exaggerated tillering phenotype than those expressing the wild-type D53 gene. The severity of tillering phenotype in these transgenic plants was correlated with the expression level of the transgene. Notably, over-expression of the wild-type D53 gene also caused a moderate increase in tillering, compared to the vector control plants (Fig. 2c–e). These observations suggested that the D53 protein acts as a repressor in the SL-mediated branching-inhibition pathway and that the dominant tillering phenotype of the d53 mutant was most likely caused by a gain-of-function mutation in d53. To further confirm this, we generated D53 knockdown transgenic plants using an RNA interference (RNAi) approach. As expected, reducing D53 expression in a d53 background markedly reduced the tiller number (Extended Data Fig. 3d, e). Taken together, these data support the proposition that the d53 mutation enhances D53 activity in repressing SL signalling.

D53 is predicted to encode a protein of 1,131 amino acids. A BLAST search identified a closely related homologue of D53 (designated D53-like, LOC_Os12g01360) with 96.6% amino acid sequence identity in the rice genome. Further, D53-like proteins were found in other monocots and dicots, but not in lower plants, animals or microbes, indicating that the D53-like clade is specific to higher plants (Extended Data Fig. 4). Sequence analysis by the HHpred structure prediction server revealed that D53 shares a similar secondary structure composition, despite low primary sequence homology, to proteins of the class I Clp ATPase family, which are characterized by an N-terminal domain, a D1 ATPase domain, an M domain and a D2 ATPase domain. Notably, the D2 domain of D53 contains a highly conserved linear sequence, Phe-Asp-Leu-Asn-Leu, which closely matches the ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motif (Extended Data Fig. 5), which is known to interact with the TOPLESS family of proteins and is involved in transcriptional repression.

Figure 3 GR24 promotes the D53–D14 and D14–D3 interaction. a, Y2H assay showing that D53 and D14 interact with D14 in the presence of GR24. Yeast transformants were spotted on the control medium (SD – Leu/–Trp (–LT)) and selective medium (SD – Leu/–Trp/–His/–Ade (–LTHA) plus X-α-gal). AD, activating domain; BD, binding domain; SD, synthetic dropout. b, BiFC analysis of D53 and D14. The positions of nuclei are indicated by DAPI (4,6-diamidino-2-phenylindole) staining. eYFP, enhanced yellow fluorescent protein. NY and CY stand for the N terminus and C terminus of eYFP, respectively. BF, bright-field image. Scale bars, 10 μm. c, In vitro pull-down assay of recombinant maltose binding protein (MBP)–D3 or MBP using resins containing GST–D14. Asterisks indicate the full-length MBP–D3 protein. d, Pull-down assay showing co-immunoprecipitation of D14 from the d3 mutant plant extracts, using GST–D3–OSK1 as the bait. 'Input' shows that roughly equal amounts of total plant proteins were used.

Figure 4 GR24 promotes D14- and D3-dependent proteasomal degradation of D53. a, Western blot analysis showing that GR24 promotes D53 protein degradation in wild type (a), but not in d mutants (b). 10 μg of total protein was applied in each lane. c, Confocal scanning images showing different degradation patterns of D53–GFP and D53–GFP fusion proteins in wild-type, d3 or d14 backgrounds. d, Relative luciferase activity of D53–GFP and D53–GFP in wild-type, d3 or d14 backgrounds. Values are means ± s.d. of three independent experiments. The double asterisks represent significant difference compared with control (LUC) determined by the Student’s t-test at P < 0.01. NS, not significant. e, f, Phenotype of d14 d53 (e) and d3 d53 (f) double mutants. g, Phenotypes of D53–RNAi transgenic plants in d3 and d14 backgrounds. Scale bars, 100 μm (c), 20 cm (e–g).
SLs promote D53–D14 and D14–D3 interaction

Previous studies have identified the F-box protein D3 and the α/β-hydrolase D14 as two key components of SL signalling in rice,
which have been proposed to directly participate in SL perception. A domain deletion analysis indicated that the D1 domain of D53 was essential for the GR24-dependent D53–D14 interaction. Interestingly, its binding activity was inhibited by the M and D2 domains, although their negative effect can be overcome by the N domain (Extended Data Fig. 7).

To investigate how SL regulates D53, we performed a set of additional experiments. Both western blot analysis and fluorescence microscopy pull-down assay (BiFC) showed that D53–D14 interaction was rapid and degradation of D53 protein via the ubiquitin-proteasome pathway. Degradation of D53 protein via the ubiquitin-proteasome pathway leads to expression of SL-responsive genes (such as FCI) and SL responses.

Discussion

It has been speculated that perception of SLs triggers the degradation of putative repressors by the SCFMAX2 ubiquitin ligase complex to suppress shoot branching. In this study, we established that D3 acts as a repressor of SL signalling in rice. Consistent with the previous observation of GR24-dependent interaction between DAD2 and PhMAX2A (ref. 19), we found that GR24 also promotes the interaction between D14 with D3 and D3 (Fig. 3). Further, we showed that D53 is targeted for degradation by the proteasome in a D14- and D3-dependent manner (Fig. 4a–d and Extended data Fig. 8). Together, these data collectively support the notion that SL perception by D14 acts to promote ubiquitination of D53 by the D14–SCFD3 ubiquitin ligase, and subsequent degradation of D53 by the proteasome, leading to the propagation of SL signal and downstream physiological responses (Fig. 5). Our findings reveal a remarkable similarity between the hormonal perception and signalling mechanism of SL and several other classes of plant hormones, including auxin, jasmonate and gibberellins.

Interestingly, a recent study reported that a D53 homologue in Arabidopsis, SMX1, acts downstream of MAX2 (orthologue of rice D3) in repressing the seed germination and seedling morphogenesis phenotypes of max2, but not the lateral root formation, axillary shoot growth, or senescence phenotypes of max2 (ref. 41). Further, as observed for D53, three closest homologues of D53 in Arabidopsis (designated SMXL6, SMXL7 and SMXL8) were also induced by GR24 treatment, suggesting that D53 and its homologues have a broad role in regulating different developmental processes and that the D3/D53 functional module is conserved between monocots and dicots. Consistent with this notion, the SL-analogous compounds known as karrikins also use this mechanism. D3/D53 homologues have a broad role in regulating different developmental processes and that the D3/D53 functional module is conserved between monocots and dicots. Consistent with this notion, the SL-analogous compounds known as karrikins also use this mechanism.
and LC/MS–MS measurement of epi-SDS, qRT–PCR, histological analysis and GUS staining. Y2H assay, in vivo degradation assay, BiFC assays, recombinant protein preparation, in vitro pull-down assays and transient expression assays.

**Online Content** Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Plant materials and growth conditions. The wild-type rice (Oryza sativa L. subspecies japonica) varieties used in this study were Norin 8 (for d3, d4, and d27), Akumuro (for d3), Shikohari (for d10 and d17) and Kitakea (for transformation analyses). The d35-carrying line, KL908, was initially identified from a mutant library of Norin 8 mutagenized by \(^{60}\)Co-\(\gamma\) irradiation\(^1\). The d27 and d14 mutants were described previously\(^2\)\(^-\)\(^4\). The d3 mutant used in this work carries a premature stop codon (unpublished data). The \(d4\) and \(d33\) double mutants were generated by crossing their respective parental lines and identified by genotyping from their respective \(F_2\) or \(F_3\) populations.

Rice plants were cultivated in the experimental field at the Nanjing Agricultural University in Nanjing in the natural growing seasons. For qRT–PCR, GR24 treatment, SL analyses and transient expression assay, the seedlings of wild-type and mutants were grown in climate chambers (HP1500GS, Ruihua) at 70% humidity, under long-day conditions with daily cycles of 14 h of light at 30 °C and 10 h of dark at 25 °C. Light was provided by fluorescent white-light tubes (400–700 nm, 250 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)).

Map-based cloning of \(D35\). To map the \(D35\) locus, the \(d3\) mutant was crossed with a polymorphic javanica variety, Keter Nanng. Rough mapping was performed with simple sequence repeat (SSR) markers W1 (\(5'-GGATGATGAGA\) TCTTGATGAGA-AAGACAGGA-3\') and W2 (\(5'-GGCCCTCTTTGCTGAGCAC-3'\) and \(5'\)-CCGGGATCAAACAGAATCTC-3\') using 242 normal plants obtained in the F\(_2\) family. Using 2,893 normal plants, \(D35\) was finally mapped to a region between the SSR marker Z7 (\(5'\)-AGAGACCGCAAACAGGAG-3\') and \(5'\)-CCGACACGTTGGATGCTACAC-3\') and a derived cleaved amplified polymorphic sequence (dCAPS) marker Z3 (\(5'\)-GGTCTGCCTCTCTGGACATC-3\') and \(5'\)-AGTAAAAATGTGGAGGGCA-3\').

Vector construction and plant transformation. To generate the pAct1:GFP and pAct1:d35-GFP constructs, full-length complementary DNAs (cDNAs) of \(D35\) and \(d35\) were amplified, and the PCR products were then cloned into the binary vector AHLG44 using the In-Fusion Advantage PCR Cloning Kit (Clontech) and vector construction and plant transformation.

Rice plants were cultivated in the experimental field at the Nanjing Agricultural University, Jiangsu, China. The wild-type rice cultivar Norin 8. All transgenic rice plants were generated using the Agrobacterium-mediated transformation of rice calli, as described previously\(^6\). In brief, surface-stereilized rice seeds were pre-equilibrated Oasis HLB 3cc cartridges (Waters) after adding 1 ng d6-5DS as the internal control. The tissues were sliced into 8–12-mm sections with a microtome (Leica RM2265), and affixed to microscope slides. Paraffin was removed from the sections using xylene, and then the sections were dehydrated through a gradient ethanol series, and stained with toluidine blue. Sections were viewed under a light microscope (Leica DM5000B) and photographed using a Micro Colour charge-coupled device camera (Leica DFC350; Diのでn). The coding region of \(D14\) was cloned into the Y2H bait vector pGBK7 (Clontech). The yeast strains 535 and \(d3\) and various domain deletion variants of \(D35\) were cloned into the Y2H prey vector pADT (Clontech). The \(Y2H\) assay. Coding regions of the rice \(d3\), \(d3\) and various domain deletion variants of \(D35\) were cloned into the \(Y2H\) bait vector pGBK7 (Clontech). The \(Y2H\) assay was performed as described previously\(^6\). Various tissues or hand-cut sections of \(D35\)-GUST1 generation transgenic plants were incubated in a staining solution containing 100 mM NaPO\(_4\) buffer, pH 7.0, 2 mM X-Gluc, 0.5 mM K\(_3\)Fe(CN)\(_6\), 0.5 mM K\(_4\)Fe(CN)\(_6\), 0.1% Triton X-100 and 10 mM Na\(_2\)-EDTA at 37 °C. Samples were vacuum-infiltrated briefly at the initiation of staining with X-Gluc solution. After staining, the staining solution was removed and the sample was washed with several changes of 50% ethanol until the tissue became clear. Images were taken directly or under the stereomicroscope.

Histological analysis and GUS staining. Segments of the rice \(d3\), \(d3\) and various domain deletion variants of \(D35\) were cloned into the \(Y2H\) prey vector pADT (Clontech). The \(Y2H\) assay was performed as described previously\(^6\). Various tissues or hand-cut sections of \(D35\)-GUST1 generation transgenic plants were incubated in a staining solution containing 100 mM NaPO\(_4\) buffer, pH 7.0, 2 mM X-Gluc, 0.5 mM K\(_3\)Fe(CN)\(_6\), 0.5 mM K\(_4\)Fe(CN)\(_6\), 0.1% Triton X-100 and 10 mM Na\(_2\)-EDTA at 37 °C. Samples were vacuum-infiltrated briefly at the initiation of staining with X-Gluc solution. After staining, the staining solution was removed and the sample was washed with several changes of 50% ethanol until the tissue became clear. Images were taken directly or under the stereomicroscope.

GUS histochemical staining was performed according to a method described previously\(^6\). Various tissues or hand-cut sections of \(D35\)-GUST1 generation transgenic plants were incubated in a staining solution containing 100 mM NaPO\(_4\) buffer, pH 7.0, 2 mM X-Gluc, 0.5 mM K\(_3\)Fe(CN)\(_6\), 0.5 mM K\(_4\)Fe(CN)\(_6\), 0.1% Triton X-100 and 10 mM Na\(_2\)-EDTA at 37 °C. Samples were vacuum-infiltrated briefly at the initiation of staining with X-Gluc solution. After staining, the staining solution was removed and the sample was washed with several changes of 50% ethanol until the tissue became clear. Images were taken directly or under the stereomicroscope.

Antibody preparation. For detection of \(D35\), a His-fused protein–specific polyclonal antibody (Leica DFC35; D35 protein), was expressed in E. coli (DE3) cells (Biomed), and then affinity purified. Subsequently, the recombination protein was injected into rabbits to produce polyclonal antibodies against \(D35\) (Abmart). The loading control used is anti-HSP82 antibody (Beijing Protein Innovation).

In vivo degradation assay of \(D35\). One-week-old rice seedlings were treated with or without 5 \(\mu\)M GR24 and collected at different time points. To block proteasomal protein degradation, seedlings were pre-treated with 40 \(\mu\)M MG132 for 2 h. The rice plants were then extracted in the SDS sample buffer containing 5% \(\beta\)-mercaptoethanol (BM) at 95 °C for 10 min. Western blots were performed using the antiserum against D35 and visualized by an enhanced horseradish peroxidase-diaminobenzidine (HRP-DAB) substrate kit (Tiangen).

The pAct1::D35-GFP and pAct1::d35-GFP transgenic plants were treated with 5 \(\mu\)M GR24 with or without the following inhibitors: MG132 (40 \(\mu\)M), AEBSF (500 \(\mu\)M), pepstatin A (1 \(\mu\)M) or leupeptin (20 \(\mu\)M). GFP fluorescence in nuclei of transgenic root cells was observed and imaged with a Zeiss LSM 710 laser scanning confocal microscope.

BiFC assay. The full-length \(D14\) and \(D35\) cDNAs were cloned into the binary vectors pSoup (Eichhorn et al., submitted) and pMYCE (M) to create the D14-NY and D35-CY vectors, respectively. For transient expression, A. tumefaciens strain EHA105 carrying the gene of interest (at an OD\(_{600}\) of 0.1) was co-infiltrated with p19 strain into a 5-week-old \(N.\) benthamiana leaf as described previously\(^6\). After 2 days, infiltrated leaves were sprayed with (+) or without (−) 5 \(\mu\)M GR24 24 h before leaf excision. The
eYFP and DAPI fluorescent signals of the infiltrated leaves were monitored sequentially using a laser confocal scanning microscope. The wavelengths for eYFP and DAPI were 514 and 405 nm for excitation, and 527 and 488 nm for detection, respectively.

**Recombinant protein preparation.** The full-length open reading frames of D3–OSK1 and D14 were cloned into the pGEX4T-1 vector, generating a fusion with MBP. Expression of GST, GST–D3–OSK1, GST–D14, MBP and MBP–D3 in BL21 Rosetta cells was induced with 0.1 mM isopropyl-β-D-thiogalactoside at 20 °C for 16 h. Fusion proteins were purified using GST Bind Resin (Novagen) or amylose-affinity chromatography (New England Biolabs) according to the manufacturer’s protocols and quantified by the Bio-Rad protein assay reagent.

**Pull-down assays.** One-week-old rice seedlings were pre-treated with 40 μM MG132 for 1 h and then treated with or without 5 μM GR24 for 30 min. Total proteins were subsequently extracted in a radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, pH 7.2, 150 mM NaCl, 10 mM MgCl2, 1× complete protease inhibitor cocktail, Roche) containing 40 μM MG132. After centrifugation (16,000 g at 4 °C), the supernatant was collected. Total protein concentration was determined by the Bradford protein assay kit (Bio-Rad). Roughly equal amounts of purified GST and GST–D3–OSK1 fusion proteins (about 50 μg) were affixed to GST Bind Resin and then mixed with 300 μl of plant extract (containing 1.5 mg total proteins). After being gently shaken at 25 °C for 15 min, the resin was washed five times with RIPA buffer. During all these procedures, 5 μM GR24 was added to the assay mixture for GR24-treated seedlings and was not added to the protein samples from seedlings not treated with GR24. Proteins were detected with anti-D14 antibodies at 1:1,000 dilution and visualized with enhanced chemiluminescence reagent (GE Healthcare).

For in vitro pull-down, GST–D14 (5 μg) was incubated with MBP–D3 (6 μg) or MBP (2 μg) at 25 °C for 15 min in 300 μl of binding buffer (20 mM Tris-HCl, pH 7.6, 2.5 mM β-ME and 0.1 M NaCl) with or without 10 μM GR24. After incubation, 20 μl of GST resin was added. After further incubation at 25 °C for 15 min, the resin was washed five times with the washing buffer (20 mM Tris-HCl, pH 7.5, 500 mM NaCl and 0.5% Triton X-100). The washing buffer also contains 10 μM GR24 where appropriate. After washing, 40 μl of 1× SDS–PAGE sample buffer were added, the mixture was denatured, and the sample was loaded on a 8% SDS–PAGE gel, and proteins were detected by the HRP-conjugated anti-MBP antibody (New England Biolabs) and visualized with enhanced chemiluminescence reagent (GE Healthcare).

**Transient expression assays.** The D53, d53, D3 and D14 full-length cDNAs were cloned into the vector pA7-GFP or pGreenII 0800-LUC to generate D53–GFP or D53–LUC recombinant plasmids for transient expression assays, respectively. Plasmids DNA was prepared using the Plasmid Midi Kits (Qiagen) according to the manufacturer’s instructions. For preparation of rice protoplasts, the sterilized seeds were germinated and grown in climate chambers for 7–10 days. Green tissues from the stem and sheath of 40–60 rice seedlings were used. The protoplasts isolation and polyethyleneglycol (PEG)–mediated transfections procedure were carried out as described previously6. GFP fluorescence from protoplasts was observed and imaged as described above. For measurement of the relative luciferase activity, protoplasts were incubated overnight and then treated with or without 5 μM GR24 for 4 h. For MG132 treatment, 40 μM MG132 was added to the sample and incubated for 1 h before addition of GR24. Activities of firefly luciferase (fLUC) and Renilla luciferase (rLUC) were determined with the Dual-Glo Luciferase Assay System (Promega). The data were represented as the ratio of fLUC/rLUC activity.

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Extended Data Figure 1 | Phenotypes of d53 mutant. a, Comparison of crown root growth in wild-type (WT) and d53 mutant. DAG, days after germination. Each value represents the mean ± s.d. of 25 seedlings. b, Root phenotype of 7-week-old wild type and d53 at the tillering stage. Red dots indicate the main culms. c, Comparison of different types of tillers between wild type and d53 at the heading stage. Pt, primary tillers; St, secondary tillers; Tt, tertiary tillers; Qt, quaternary tillers. Each value represents the mean ± s.d. of 20 seedlings. d, Morphology comparison of tiller buds at the second node between wild type and d53. White arrows and arrowheads indicate the tiller buds and the second nodes, respectively. e, Transverse sections of the first internode of wild type and d53. f, Number of vascular bundles (VB) calculated from transverse sections of the first internode of wild type and d53. SVB, small vascular bundle; LVB, large vascular bundle. Data are means ± s.d. (n = 10). g, Longitudinal sections of the first internode of wild type and d53. h, Comparison of parenchyma (PC) cell length in first internode and root between wild type and d53. Data are means ± s.d. (n = 10). Differences with respect to the wild type that were found to be significant in a t-test are indicated with asterisks (*P < 0.05; **P < 0.01; NS, not significant). Scale bars, 10 cm (b), 2 cm (d), 100 μm (e, g).
Extended Data Figure 2 | $d53$ mutation behaves in a semi-dominant manner. a, b, Comparison of wild type, heterozygous (F1) and homozygous $d53$ plants at the heading stage (a), flag leaf (b), cross section of the first internode (c), panicle (d), plant height (e), tiller number (f) and diameter of the third internode (g). Scale bars, 20 cm (a), 5 cm (b, d) and 500 μm (c). For e–g, each value represents the mean ± s.d. (n = 25). h, Segregation of F2 progeny from a self-pollinated F1 plant ($d53 \times$ Norin 8).

|          | Total | Normal | Semi-dwarf | Dwarf | $\chi^2$ (1:2:1) |
|----------|-------|--------|------------|-------|------------------|
| $d53$/Norin 8 | 119   | 33     | 58         | 28    | 0.09 (p > 0.05)  |

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Extended Data Figure 3 | d53 is insensitive to GR24 treatment and confers enhanced tillering-promoting activity. a, Response of 5-week-old wild type, d53, d14 and d27 to the application of 1 μM GR24. b, Tiller bud length of 2-week-old wild type, d53, d14 and d27 seedling treated with (+) or without (−) 1 μM GR24. Data are means ± s.d. (n = 10). ND, not detected. c, Numbers of tillers showing outgrowth (>2 mm) for 5-week-old wild type, d53, d14 and d27 plants treated with (+) or without (−) 1 μM GR24. Data are means ± s.d. (n = 10). Asterisks in c denote significant differences between treated and untreated samples within the same genotype (two tailed Mann–Whitney U test, P < 0.01; NS, not significant). d, D53 RNAi transgenic plants exhibit reduced tillering in the d53 mutant background. d53Vec., d53 transformed with the pCUbi1390-3FAD2 control. e, Tiller number of RNAi transgenic lines in d at the tillering stage. Each value represents the mean ± s.d. of six plants (T1 generation). L4, L10 and L11 represent three independent lines. The t-test analysis indicated a significant difference (compared with vector control, **P < 0.01). Scale bars, 20 cm (a), 10 cm (d).
Extended Data Figure 4 | Phylogenetic analysis of D53 protein. Using the D53 protein sequence as the query in tBLASTn searches, homologues were identified from different organisms with a permissive cutoff E value of $1 \times 10^{-23}$. The sequences chosen from representative genomes were aligned and used to generate the neighbour-joining phylogenetic tree with 1,000 bootstrap replicates. The clade names were given on the basis of known sequences in each clade, which is supported by a bootstrap value $> 85$. 

- Plants
- Animals
- Microbes
Extended Data Figure 5 | Multiple sequence alignment of the deduced amino acid sequence of D53 with its homologues. D53 protein is predicted to contain an N-terminal domain, a D1 ATPase domain, an M domain and a D2 ATPase domain (http://toolkit.tuebingen.mpg.de/hhpred). The beginning and ending sites of each domain are indicated above the sequences. The predicted Walker A (P-loop) and Walker B motifs are shown in red boxes in the D1 domain and green boxes in the D2 domain, respectively. Note that the deletion of five amino acids in the D2 domain of d53 protein overlaps with the GYVG loop in ClpC. The conserved putative EAR motif in D53 and ClpP-binding loop in ClpC are also shown. The sequences used for alignment are D53 (Oryza sativa, LOC_Os11g01330), D53-like (Oryza sativa, LOC_Os12g01360), SMXL6 (Arabidopsis, At1g07200), SMXL7 (Arabidopsis, At2g29970) and ClpC (Bacillus subtilis, GI: 16077154).
Extended Data Figure 6 | Histochemical staining of the pD53::GUS reporter gene and subcellular localization of D53 protein.

a–h. Histochemical staining of young root (a), shoot (b), leaf (c), leaf sheath (d), panicle (e), transverse section of the leaf sheath (f), stem (g) and node (h). Scale bars, 1 mm (a, b, c, d, f, h), 1 cm (e), 100 μm (g). i–l. Subcellular localization of D53–GFP fusion protein in rice protoplast cells. A nuclear marker protein, OsMADS3, fused with mCherry, was used as a positive control. Scale bars, 5 μm. m–p. Confocal scanning images showing nuclear localization of the D53–GFP fusion protein in transgenic root cells. Scale bars, 100 μm.
Extended Data Figure 7 | Mapping of the D14-binding domain of D53.

a. Schematic structure of the D53 protein. Numbers indicate amino-acid (aa) residues. 
b. Y2H analysis showing interaction between full-length and various domain deletion variants of D53 with D14 in the presence or absence of 5 μM GR24. –LT, control medium (SD –Leu/–Trp); –LTHA, selective medium (SD –Leu/–Trp/–His/–Ade).
Extended Data Figure 8 | GR24 promotes D53 protein degradation.

a, Confocal scanning images showing that AEBSF, pepstatin A and leupeptin are not effective in blocking D53–GFP fusion protein degradation in transgenic seedlings treated with 5 μM GR24. Scale bars, 100 μm. b, Degradation of D53–GFP fusion protein but not D3–GFP or D14–GFP fusion proteins expressed in rice protoplasts, in the presence of 5 μM GR24. Pre-treatment with 40 μM MG132 for 1 h before addition of GR24 effectively blocks D53–GFP degradation. c, D53–GFP is degraded in the d53 mutant protoplasts in the presence of GR24, but not in d3 or d14 protoplasts. For b and c, each figure represents at least 50 cells observed. Scale bars, 10 μm.
Extended Data Figure 9 | D53-RNAi transgenic lines in d3 and d14 backgrounds. 

a, Comparison of plant height, diameter of the third internode and tiller number between wild type, d53, d14, d3 and their double mutants. Values are mean ± s.d. (n = 10). b, c, qRT–PCR assay (b) and western blot analysis (c) showing that the endogenous level of D53 messenger RNAs and proteins are downregulated in three representative D53-RNAi lines in d53, d14 and d3 mutant backgrounds, compared to wild-type control. Data are means ± s.d. (n = 3); significant difference determined by t-test (**P < 0.01). Anti-HSP82 was used as a loading control. d, Tiller number of representative D53-RNAi transgenic lines in d3 and d14 mutant backgrounds at the heading stage. Each value represents the mean ± s.d. of six plants. L2, L6 and L11 represent three independent lines in d3 background, and L1, L4, L6, L9 and L10 represent five independent lines in d14 background. Akumuro (A) and Norin 8 (N) are the wild-type varieties correspond to d3 and d14 mutants, respectively. d3Vec. and d14Vec. transgenic lines were used as the controls. Asterisks represent significant difference compared with vector control determined by the t-test at *P < 0.05 and **P < 0.01, respectively.
Corrigendum: D14–SCF<sup>D3</sup>–dependent degradation of D53 regulates strigolactone signalling

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In this Article, the bottom panel of Fig. 3c was assembled from processed digital images of separate western blot autoradiographs to show approximately equal amounts of MBP–D3 protein input in the presence or absence of GR24 treatment. During figure preparation, a single MBP–D3 lane was duplicated in error to create the third and fourth lanes. In addition, the last set of images in Extended Data Fig. 8b, right panel, was used in error for Extended Data Fig. 8c, bottom left panel. We have now repeated the relevant experiments, and Fig. 1 of this Corrigendum shows the amended Fig. 3c, and the Supplementary Information to this Corrigendum shows the corrected Extended Data Fig. 8c. None of these corrections alters the original design of the experiments, results, interpretation or conclusions of the paper. We apologize for any confusion that this may have caused.

Supplementary Information is available in the online version of the Corrigendum.

Figure 1 | This is the corrected Fig. 3c of the original Article.