uORF–mediated translation allows engineered plant disease resistance without fitness costs

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Controlling plant disease has been a struggle for humankind since the advent of agriculture. Studies of plant immune mechanisms have led to strategies of engineering resistant crops through ectopic transcription of plants’ own defence genes, such as the master immune regulatory gene NPR1 (ref. 1). However, enhanced resistance obtained through such strategies is often associated with substantial penalties to fitness2, making the resulting products undesirable for agricultural applications. To remedy this problem, we sought more stringent mechanisms of expressing defence proteins. On the basis of our latest findings that translation of key immune regulators, such as TBF1 (ref. 3), is rapidly and transiently induced upon pathogen challenge (see accompanying paper3), we developed a ‘TBF1-cassette’ consisting of not only the immune-inducible promoter but also two pathogen-responsive upstream open reading frames (uORFsTBF1) of the TBF1 gene. Here we demonstrate that inclusion of uORFsTBF1-mediated translational control over the production of snc1-1 (an autoimmune receptor) in Arabidopsis thaliana and AtNPR1 in rice enables us to engineer broad-spectrum disease resistance without compromising plant fitness in the laboratory or in the field. This broadly applicable strategy may lead to decreased pesticide use and reduce the selective pressure for resistant pathogens.

To meet the demand for food production caused by the rapid expansion of world population while limiting the use of pesticides, which are potential pollutants, new strategies must be developed to control crop diseases. As an alternative to traditional chemical and breeding methods, studies of plant immune mechanisms have made it possible to engineer resistance through ectopic expression of plants’ own resistance-conferring genes5. The first line of active defence in plants involves recognition of microbial/damage-associated molecular patterns (M/DAMPs) by host pattern-recognizing receptors (PRRs) in pattern-triggered immunity (PTI)6. Ectopic expression of PRRs for MAMPs7,8 and the DAMP signal eATP9, as well as in vivo release of the DAMP molecules, oligogalacturonides10, have all been shown to enhance resistance in transgenic plants. Besides PRR-mediated basal resistance, plant genomes encode hundreds of intracellular nucleotide-binding and leucine-rich repeat immune receptors (also known as ‘R proteins’) to detect the presence of pathogen effectors delivered inside plant cells11. Individual or stacked R genes have been transformed into plants to confer effector-triggered immunity12,13. In addition to PRR and R genes, NPR1 is another favourite gene used in engineering plant resistance5. Unlike immune receptors that are activated by specific MAMPs and pathogen effectors, NPR1 is a positive regulator of broad-spectrum resistance induced by a general plant immune signal, salicylic acid1. Overexpression of Arabidopsis NPR1 (AtNPR1) could enhance resistance against a variety of pathogens in diverse plant families such as rice14–16.

A major challenge in engineering disease resistance, however, is to overcome the associated fitness costs2. In the absence of specialized immune cells, immune induction in plants involves switching from growth-related activities to defence1,17. Plants normally avoid auto-immunity by tightly controlling transcription, messenger RNA (mRNA) nuclear export, and degradation of defence proteins18. However, only transcriptional control has been used prevalently so far in engineering disease resistance2. On the basis of our global translatome analysis4, we discovered translation to be a fundamental layer of regulation during immune induction, which can be explored to allow more stringent pathogen-inducible expression of defence proteins.

To test our hypothesis that tighter control of defence protein translation can minimize the fitness penalties associated with enhanced disease resistance, we used the TBF1 promoter (TBF1p) and the 5’ leader sequence (before the start codon for TBF1), which we designated as the ‘TBF1-cassette’. TBF1 is an important transcription factor for the growth-to-defence switch upon immune induction. Translation of TBF1 is normally suppressed by two uORFs within the 5’ leader sequence2. BLAST analysis showed that uORF2TBF1, the major mRNA feature conferring the translational suppression3,4, is conserved across plant species (>50% identity) (Extended Data Fig. 1), suggesting an evolutionarily conserved control mechanism and a potential use of TBF1-cassette to regulate defence protein production in plant species other than Arabidopsis.

To explore the application of uORFsTBF1, we first demonstrated its capacity to control both cytosol- and endoplasmic reticulum (ER)-synthesized proteins (‘Target’) using firefly luciferase (LUC; Extended Data Fig. 2a) and green fluorescent protein (GFPER; Extended Data Fig. 2b), respectively, as proxies through transient expression in Nicotiana benthamiana (Fig. 1a–c and Extended Data Fig. 2c, d). This uORFsTBF1-mediated translational suppression was strong enough to prevent cell death induced by overexpression of TBF1 (TBF1–YFP, TBF1 fused with yellow fluorescent protein) observed in 35S::uORFsTBF1–TBF1–YFP (Fig. 1d and Extended Data Fig. 2e). Similar repression activity was observed for uORF2bZIP11 of the sucrose-responsive bZIP11 gene (Extended Data Fig. 2f–l). However, unlike uORFsTBF1, the uORF2bZIP11-mediated repression could not be alleviated by the MAMP signal elf18 (Extended Data Fig. 2m, n). These results support the potential utility of uORFsTBF1 in providing stringent control of cytosol- and ER-synthesized defence proteins specifically for engineering disease resistance.

To monitor the effect of uORFsTBF1 on translational efficiency, a dual-luciferase system was constructed to calculate the ratio of LUC activity to the control renilla luciferase (RLUC) activity (Fig. 1e). The resulting transgenic plants were tested for responsiveness to bacterial pathogens Pseudomonas syringae pathovar (pv.) maculicola ES4326 (Psm ES4326), Pseudomonas pv. tomato (Pst) DC3000, and the corresponding mutant of the type III secretion system Pst DC3000 hrcC-, as well as to MAMP signals, elf18, and flg22. The equally rapid induction in the reporter translational efficiency by all treatments suggests that it is probably a part of pattern-triggered immunity, which does not
involve bacterial type III effectors (Fig. 1f). The transient increases in translation were not correlated with significant changes in mRNA levels (Fig. 1d). The transient increases in translation were not correlated with significant changes in mRNA levels (Fig. 1d). The transient increases in translation were not correlated with significant changes in mRNA levels (Fig. 1d). These data provide a proof of concept that adding pathogen-inducible either the 35S promoter or its native promoter21,22, limiting the utility of SNC1, and perhaps other R genes, in engineering resistant plants. To overcome the fitness penalty associated with the snc1 mutant, we put it under the control of uORFsTBF1 driven by either the 35S promoter or TBF1p to create 35S:uORFsTBF1-snc1 and TBF1p:uORFsTBF1-snc1, respectively. As controls, we also generated 35S:uORFsTBF1-snc1 and TBF1p:uORFsTBF1-snc1, in which the start codons of the uORFs were mutated. The first generation of transgenic Arabidopsis (T1) with these four constructs displayed three distinct developmental phenotypes: type I plants were small in rosette diameter, dwarf, and exhibited chlorosis; type II plants were healthier but still dwarf; and type III plants were indistinguishable from WT (Extended Data Fig. 3). We found that regulating either transcription or translation of snc1 markedly improved plant growth, as judged by the increased percentage of type III plants. The highest percentage of type III plants was found in TBF1p:uORFsTBF1-snc1 transformants, in which snc1 was regulated by TBF1-cassette at both transcriptional and translational levels. The absence of type I plants in these transformants clearly demonstrated the stringency of TBF1-cassette (Extended Data Fig. 3).

We propagated the transformants to obtain homozygotes for the transgene. For the TBF1p:uORFsTBF1-snc1 and 35S:uORFsTBF1-snc1 lines, homozygosity caused most of the type III plants in T1 to show the type II phenotype in T2. But for TBF1p:uORFsTBF1-snc1 transformants, they maintained their normal growth phenotype as homozygotes. We then picked four independent TBF1p:uORFsTBF1-snc1 lines for further disease resistance and fitness tests (Fig. 2a, b). We first showed that these transgenic lines indeed had elevated resistance to Psm ES4326 by either spray inoculation or infiltration (Fig. 2c, d and Extended Data Fig. 4a, b). They also displayed enhanced resistance to Hyaloperonospora arabidopsidis Noco2 (Hpa Noco2), an oomycete pathogen which causes downy mildew in Arabidopsis (Fig. 2e, f and Extended Data Fig. 4c). However, in contrast to snc1, these transgenic lines showed almost the same fitness as WT, including total seed weight per plant (Fig. 2g–i and Extended Data Fig. 4d–g). Upon Psm ES4326 challenge, we detected significant increases in the snc1 protein within 2 hours post infection (h.p.i.) in all four TBF1p:uORFsTBF1-snc1 transgenic lines, but not in WT or snc1 (Extended Data Fig. 4h, i). These data provide a proof of concept that adding pathogen-inducible

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translational control is an effective way to enhance plant resistance without fitness costs.

We next applied TBF1-cassette to engineering resistance in rice, which is one of the most important staple crops in the world. Using 35S:uORFsTBF1-LUC and 35S:uORFsTBF1-LUC (Fig. 1b), we first showed that the Arabidopsis uORFsTBF1 could suppress translocation without significantly influencing mRNA levels in the rice (Oryza sativa) cultivar ZH11 (Extended Data Fig. 5a, b). We then chose AtNPR1 (ref. 1), which has been shown to confer broad-spectrum disease resistance in a variety of plants, as the transgene. However, it is known that overexpressing AtNPR1 in rice by the maize ubiquitin promoter causes growth retardation, seed size reduction, and development of the so-called lesion mimic disease phenotype under certain environmental conditions15,23. To remedy the fitness problem, we expressed the AtNPR1–EGFP fusion gene under the following four regulatory systems: 35S:uORFsTBF1–AtNPR1–EGFP, 35S:uORFsTBF1–AtNPR1–EGFP, TBF1p:uORFsTBF1–AtNPR1–EGFP, and TBF1p:uORFsTBF1–AtNPR1–EGFP. These four constructs were assigned different codes for blind testing of resistance and fitness phenotypes. Under growth chamber conditions, either the TBF1p-mediated transcriptional or the uORFsTBF1-mediated translational control largely decreased the ratio and the severity of rice plants with lesion mimic disease (Extended Data Fig. 5c). However, the best results were obtained using TBF1-cassette with both transcriptional and translational control. Next, we tested plant resistance to the bacterial pathogen Xanthomonas oryzae pv. oryzicola (Xoo), the causal agent for rice blight, in the first (T0 in rice research) and the second (T1) generations of transformants under the greenhouse conditions where lesion mimic disease was not observed even for 35S:uORFsTBF1–AtNPR1. Unsurprisingly, the 35S:uORFsTBF1–AtNPR1 plants displayed the highest level of resistance to Xoo, owing to the constitutive transcription and translation of AtNPR1 (Extended Data Figs 6 and 7a, b). However, similar levels of resistance were also observed in plants with either transcriptional or translational control or both. Notably, these resistance results were faithfully reproduced in the field (Fig. 3a, b and Extended Data Fig. 7c). In response to Xoo challenge, transgenic lines with functional uORFsTBF1 displayed transient AtNPR1 protein increases, which peaked around 2 h.p.i., even in the absence of significant changes in mRNA levels (for example, 35S:uORFsTBF1–AtNPR1 in Extended Data Fig. 7d, e).

To determine the spectrum of AtNPR1-mediated resistance, we inoculated the third generation of transgenic rice plants (T2) with X. oryzae pv. oryzicola (Xoc) and Magnaporthe oryzae (M. oryzae), the causal pathogens for rice bacterial leaf streak and fungal blast, respectively. We observed similar patterns of enhanced resistance against Xoc and M. oryzae in growth chambers designated for these controlled pathogens (Fig. 3c–f) as for Xoo, confirming the broad spectrum of AtNPR1-mediated resistance. The lack of significant variation among the different transgenic lines suggests that they all had saturating levels of AtNPR1 in conferring resistance.

We then performed detailed fitness tests on these transgenic plants in the field and found that constitutive transcription and translation of AtNPR1 in 35S:uORFsTBF1–AtNPR1 plants clearly had fitness penalties (Fig. 3g–i and Extended Data Fig. 8). Addition of transcriptional or and translational control of AtNPR1 significantly reduced costs to agronomically important traits, with a combination of both transcriptional and translational control performing the best in eliminating cost on yield on the basis of the number of grains per plant and 1,000-grain weight (Fig. 3h, i).

Using TBF1-cassette, we established a new strategy of enhancing broad-spectrum disease resistance with minimal adverse effects on plant growth and development. The ubiquitous presence of uORFs in mRNAs of organisms ranging from yeast (13% of all mRNA)24 to humans (49% of all mRNA)25 suggests the potentially broad utility of these mRNA features for the precise control of transgene expression.

Online Content Methods, along with any additional 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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Supplementary Information is available in the online version of the paper.

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Author Contributions G.X. and X.D. designed the research. G.X. performed the Arabidopsis-related experiments with help from E.Z. for fitness tests. L.L. isolated the snc1 mutant leads to constitutive, NPR1-independent pathogen resistance. Mol. Plant Microbe Interact. 14, 1131–1139 (2001).

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Plasmid construction. The 35S promoter with duplicated enhancers was amplified from pRNAi-LIC28 and flanked with Psfl and XbaI sites using primers P1/P2. The nopaline synthase (NOS) terminator was amplified from pRNAi-LIC and flanked with KpnI and EcoRI sites using primers P3/P4. Gateway cassette with LIC addresses was amplified and flanked with KpnI and AIII sites using primers P5/P6/P7 (the PCR fragment by P5/P6 was used as template for P5/P7) from pDEST375 (GenBank accession number KC614689.1). The NOS terminator, the 35S promoter, and the Gateway cassette were sequentially ligated into pCAMBIA1300 (GenBank accession number AF234296.1) via KpnI/EcoRI, Psfl, XbaI, and KpnI/AIII, respectively. The resultant plasmid was used as an intermediate plasmid. The S′ leader sequences of TBF1 (upstream of the ATG start codon of TBF1) with WT uORF1 mutants and uorf1 mutants were amplified with P8/P9 and P8/P10 from the previously published plasmids29 carrying uORF1–uORF2–GUS–uros1–uros2–GUS, and uorf1–uros1–uros2–GUS, respectively, and cloned into the intermediate plasmid via XbaI/KpnI. The resultant plasmids were designated as pGX179 (35S::uORF2aTBF1-Gateway-NOS) and pGX180 (35S::uros2f::Gus–Gateway-NOS). TBF1p was amplified from the Arabidopsis genomic DNA and flanked with HindIII/AscI using primers P11/P1, and the fied from the Gateway-NOS and pGX1 (used as template for PCR and introduction of HindIII/KpnI using primer P11/P8). The 35S promoter in pGX179 was replaced by the TBF1 promoter to produce pGX1 (TBF1:p::uORF2aTBF1-Gateway-NOS). The NOS promoter was amplified from the Arabidopsis genomic DNA and flanked with HindIII/SpeI using primers P4/P15 and ligated into pGX179, which was cut with HindIII/XbaI, to generate pGX181 (TBF1:p::uORFsTBF1-Gateway-NOS). LUC, GFPgfp, and sncl were amplified from pGWB235 (ref. 27), GFP–HDEL30, and the sncl mutant genomic DNA, respectively. TBF1–YFP and NPR1–EGFP were fused together through PCR, cloned via ligation independent cloning32. EFR was amplified from the Arabidopsis Information Resource, fused with EGFp and controlled by the 35S promoter. The S′ leader sequence of IZP1 (containing uORF3p·ZPI) was amplified from the Arabidopsis genomic DNA with G904/G905. The start codons (ATG) for uORF2a and uORF2b in the S′ leader sequence were mutated to CTG and TAG, respectively, to generate uorf2aZPI and uorf2bZPI, by PCR using primers containing point mutations. Primer and plasmid information can be found in Supplementary Table 1.

Arabidopsis growth, transformation, and pathogen infection. The Arabidopsis Col-0 accession was used for all experiments. Plants were grown on soil (Metro Mix 360) at 22°C with 55% relative humidity and under 12/12-h light/dark cycles for bacterial growth assay and measurements of plant radius and fresh weight, or 16/8-h light/dark cycles for seed weight and silique number measurements. Arabidopsis for uORF2a and uORF2b in the 5′ promoter. The 5′ cloned via ligation independent cloning26. EFR was amplified from U21686 (The Gateway-NOS) and pGX180 (35S::uros2f::Gus–Gateway-NOS) and used as template for PCR and introduction of HindIII/KpnI using primer P11/P8. The 35S promoter in pGX179 was replaced by the TBF1 promoter to produce pGX1 (TBF1:p::uORF2aTBF1-Gateway-NOS). The NOS promoter was amplified from the Arabidopsis genomic DNA and flanked with HindIII/SpeI using primers P4/P15 and ligated into pGX179, which was cut with HindIII/XbaI, to generate pGX181 (TBF1:p::uORFsTBF1-Gateway-NOS). LUC, GFPgfp, and sncl were amplified from pGWB235 (ref. 27), GFP–HDEL30, and the sncl mutant genomic DNA, respectively. TBF1–YFP and NPR1–EGFP were fused together through PCR, cloned via ligation independent cloning32. EFR was amplified from the Arabidopsis Information Resource, fused with EGFp and controlled by the 35S promoter. The S′ leader sequence of IZP1 (containing uORF3p·ZPI) was amplified from the Arabidopsis genomic DNA with G904/G905. The start codons (ATG) for uORF2a and uORF2b in the S′ leader sequence were mutated to CTG and TAG, respectively, to generate uorf2aZPI and uorf2bZPI, by PCR using primers containing point mutations. Primer and plasmid information can be found in Supplementary Table 1.

Arabidopsis growth, transformation, and pathogen infection. The Arabidopsis Col-0 accession was used for all experiments. Plants were grown on soil (Metro Mix 360) at 22°C with 55% relative humidity and under 12/12-h light/dark cycles for bacterial growth assay and measurements of plant radius and fresh weight, or 16/8-h light/dark cycles for seed weight and silique number measurements. The floral dip method29 was used to generate transgenic plants. The BGL2-GUS reporter line30 was used for sncl related transformation. For infection, bacteria were first grown on a King's B medium plate at 28°C for 2 days before being resuspended in 10 mM MgCl2 solution for infiltration. The antibiotic selection for transgenic plants was grown on nutrient agar medium (0.1% yeast extract, 0.3% beef extract, 0.5% polypeptone, and 1% sucrose) at 28°C for 2 days before being resuspended in sterile water and dilution to OD600nm = 0.5 for inoculation. For Xoo infection in the greenhouse (performed in 2016), rice was grown for 3 weeks from 2 February and inoculated on 23 February with data collection on 8 March. For Xoo infection in the field (performed in 2016), rice was grown on 10 May in the experimental stations of Huazhong Agricultural University, Wuhan, China (31°N latitude) and inoculated on 20 July with data collection on 4 August. Xoo strains PXO347 and PXO99 were grown on nutrient agar medium (0.1% yeast extract, 0.3% beef extract, 0.5% polypeptone, and 1% sucrose) at 28°C for 2 days before resuspension in sterile water and dilution to OD600nm = 0.5 for inoculation. For Xoo infection in the field (performed in 2016), rice was grown for 3 weeks from 2 February and inoculated on 23 February with data collection on 8 March. For Xoo infection in the field (performed in 2016), rice was grown on 10 May in the experimental stations of Huazhong Agricultural University, Wuhan, China (31°N latitude) and inoculated on 20 July with data collection on 4 August. Xoo strains PXO347 and PXO99 were grown on nutrient agar medium (0.1% yeast extract, 0.3% beef extract, 0.5% polypeptone, and 1% sucrose) at 28°C for 2 days before resuspension in sterile water and dilution to OD600nm = 0.5 for inoculation. Five to ten leaves of each plant were inoculated by the leaf-clipping method at the booting (panicle development) stage31,32. Disease was scored by measuring the lesion length at 14 days post inoculation (d.p.i.). PCR was performed using primer rice-F and rice-R (Supplementary Table 1) for identification of AtNPR1 transgenic plants. Both PCR-positive and -negative T1 plants were scored. For Xoc infection in the growth chamber (performed in 2016), rice was grown on 20 October and inoculated on 15 November, with data collection on 29 November. Xoc strain RH3 was grown on nutrient agar medium (0.1% yeast extract, 0.3% beef extract, 0.5% polypeptone, and 1% sucrose) at 28°C for 2 days before resuspension in sterile water and dilution to OD600nm = 0.5 for inoculation. For Xoo infection, disease was scored by measuring the lesion length at 7 d.p.i. For Xoc and M. oryzae, three independent transgenic lines for each construct were tested, with data from two lines shown in Fig. 3 and from the third line in the Source Data of Fig. 3. For Xoo, M. oryzae, and Xoc, four independent transgenic lines for each construct were tested, with data from two lines shown in Fig. 3 and from all four lines in Extended Data Figs 7, 8 and 9.

Immunoblot. Arabidopsis tissue (100 mg) infected by Psm ES4326 (OD600nm = 0.02) was collected and lysed in 200 μl lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 0.2% Nonidet P-40, protease inhibitor cocktail (Roche, one tablet for 10 ml)) before centrifugation at 12,000 × g for the supernatant. The same protocol was used to extract proteins from rice infected by Psm PX099, at OD600nm = 0.5) using a slightly different lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM DTT, 1 mM PMSF, 2 mM EDTA, 0.1% Triton X-100, protease inhibitor cocktail (Roche, one tablet for 10 ml)). Antibody information and the experimental conditions can be found in Supplementary Table 1. Immunoblot detection was performed using a membrane (Millipore) and a chemiluminescence detection system (ECL, Amersham). Two-sided one-way analysis of variance together with Tukey’s test was used for multiple comparisons. Sample size can be found in the Source Data. Unless specifically stated, sample size n means biological replicates. Experiments were done three times with similar results for all the Arabidopsis experiments. GraphPad Prism 6 was used for all the statistical analyses.

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Data availability. The authors declare that the main data supporting the findings of this study are available within the article and its Source Data files. Extra data are available from the corresponding author upon request.

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Extended Data Figure 1 | Conservation of uORF2 TBF1 nucleotide and peptide sequences in plant species. a, Schematic of TBF1 mRNA structure. The 5′ leader sequence contains two uORFs, uORF1 and uORF2. CDS, coding sequence. b–d, Alignment of uORF2 nucleotide sequences (b) and alignment (c) and phylogeny (d) of uORF2 peptide sequences in different plant species. The corresponding triplets encoding the conserved amino acids among these species are underlined. Identical residues (black background), similar residues (grey background), and missing residues (dashes) were identified using Clustlw2. At (Arabidopsis thaliana; AT4G36998), Pv (Phaseolus vulgaris; XP_007155927), Gm (Glycine max; XP_006600987), Gr (Gossypium raimondii; CO115325), Nb (Nicotiana benthamiana; CK286574), Ca (Cicer arietinum; XP_004509145), Pd (Phoenix dactylifera; XP_008797266), Ma (Musa acuminata subsp. Malaccensis; XP_009410098), Os (O. sativa; Os09g28354).
Extended Data Figure 2 | Characterization of uORFs TBF1 and uORFs bZIP11 in translational control. Related to Fig. 1. 

a, Subcellular localization of the LUC–YFP fusion (a) and GFPER (b). SP, signal peptide from Arabidopsis basic chitinase; HDEL, ER retention signal. Representative of eight images. Scale bar, 10 μm. 

c–e, mRNA levels of LUC in (Fig. 1b; n = 3), GFPER in (Fig. 1c; n = 4), and TBF1–YFP in (Fig. 1d; n = 3) 2 d.p.i. before cell death was observed in plants expressing TBF1.

f, Schematics of the 5′ leader sequences used in studying the translational activities of WT uORFs bZIP11, mutant uorf2abZIP11 (ATG to CTG), or uorf2bbZIP11 (ATG to TAG). 

g–i, uORFs bZIP11-mediated translational control of cytosol-synthesized LUC (g; chemiluminescence with pseudo-colour); ER-synthesized GFP ER (h; fluorescence under ultraviolet light); and cell death induced by overexpression of TBF1–YFP fusion (i; cleared using ethanol) after transient expression in N. benthamiana for 2 days (g, h) and 3 days (i), respectively. Representative of four images.

j–l, mRNA levels of LUC in (g; n = 2 experiments with three technical replicates), GFPER in (h; n = 3 experiments with three technical replicates), and TBF1–YFP in (i; n = 3 experiments with three technical replicates).

m, Translational efficiency changes in LUC controlled by the 5′ leader sequence containing WT uORFs bZIP11, mutant uorf2abZIP11, or uorf2bbZIP11 in response to elf18 in N. benthamiana. Mean of the LUC/RLUC activity ratios (n = 12).

n, LUC/RLUC mRNA changes in m. Mean of LUC/RLUC mRNA normalized to Mock from two experiments with three technical replicates. Bar with solid circles, mean with individual biological replicates.

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Extended Data Figure 3 | Three developmental phenotypes observed in primary Arabidopsis transformants expressing snc1. The three developmental phenotypes observed in T$_1$ (that is, the first generation) Arabidopsis transgenic lines carrying 35S::uorf$_{TBF1}$::snc1, 35S::uORFs$_{TBF1}$::snc1, TBF1p::uorf$_{TBF1}$::snc1, TBF1p::uORFs$_{TBF1}$::snc1, and TBF1p::uORFs$_{TBF1}$::snc1 (above). Representative of five images. Fisher’s exact test was used for the pairwise statistical analysis (below). Different letters in ‘Total’ indicate significant differences between type III versus type I + type II ($P < 0.01$).
Extended Data Figure 4 | Effects of controlling transcription and translation of snc1 on defence and fitness in Arabidopsis. Related to Fig. 2. a, b, Psm ES4326 growth in WT, snc1, transgenic line numbers 1–4 after inoculation by spray (a) or infiltration (b). Mean ± s.e.m. c, Hpa Noco2 growth as measured by spore counts 7 d.p.i. Mean ± s.e.m. d–g, Analyses of plant radius (d), fresh weight (e), silique number (f), and total seed weight (g). Mean ± s.e.m. h, i, Relative levels of Psm ES4326-induced snc1 protein (h; numbers below immunoblots; see Supplementary Fig. 1 for gel source data) and mRNA (i; mean from two experiments with three technical replicates). Solid circles, individual biological replicates. Numbers 1–4, four independent transgenic lines carrying TBF1p:uORFsTBF1-snc1 with 1 and 2 shown in Fig. 2. h.p.i., hours after Psm ES4326 infection; CBB, Coomassie brilliant blue. See Source Data for sample size (n). Different letters above bars indicate significant differences (P < 0.05).
Extended Data Figure 5 | Functionality of uORFsTBF1 in rice.

a, b, LUC activity (a) and mRNA levels (b) in three independent primary transgenic rice lines (called ‘T0’ in rice research) carrying 35S:uorfsTBF1-LUC and 35S:uORFsTBF1-LUC. Mean of LUC activities (RLU, relative light unit) of three biological replicates. Solid circles, individual biological replicates; and mean of LUC mRNA levels of three technical replicates after normalization to the 35S:uorfsTBF1-LUC line 1.

c, Representative lesion mimic disease (LMD) phenotypes (above) and percentage of AtNPR1-transgenic rice plants showing lesion mimic disease in the second generation (T1) grown in the growth chamber (below).
Extended Data Figure 6 | Effects of controlling transcription and translation of AtNPR1 on defence in T0 rice. Related to Fig. 3.

a–d, Lesion length measurements after infection by Xoo strain PXO347 in primary transformants (T0) for 35S::uorfsTBF1-AtNPR1 (a), 35S::uORFsTBF1-AtNPR1 (b), TBF1p::uorfsTBF1-AtNPR1 (c), and TBF1p::uORFsTBF1-AtNPR1 (d).

Lines further analysed in T1 and T2 are circled. e, Average leaf lesion lengths. WT, recipient O. sativa cultivar ZH11. Mean ± s.e.m. Different letters above bars indicate significant differences (P < 0.05). See Source Data for sample size (n).
Extended Data Figure 7 | Effects of controlling transcription and translation of AtNPR1 on defence in T1 rice. Related to Fig. 3.

a, Representative symptoms observed in T1 AtNPR1-transgenic rice plants grown in the greenhouse (a) after Xoo inoculation and corresponding leaf lesion length measurements (b). PCR was performed to detect the presence (+) or the absence (−) of the transgene gene.

b, Quantification of leaf lesion length of four lines for Xoo inoculation in field-grown T1 AtNPR1-transgenic rice plants. Mean ± s.e.m. See Source Data for sample size (n). Different letters above bars indicate significant differences (P < 0.05).

c, Quantification of leaf lesion length of four lines for Xoo inoculation in field-grown T1 AtNPR1-transgenic rice plants. Mean ± s.e.m. See Source Data for sample size (n). Different letters above bars indicate significant differences (P < 0.05).

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Extended Data Figure 8 | Effects of controlling transcription and translation of AtNPR1 on fitness in $T_1$ rice under field conditions. Related to Fig. 3. Mean ± s.e.m. See Source Data for sample size ($n$). Different letters above bars indicate significant differences among constructs ($P < 0.05$).