nature plants

LETTERS

https://doi.org/10.1038/s41477-021-00852-x

Check for updates

Suppression of rice miR168 improves yield, flowering time and immunity

He Wang[1,2,5], Yan Li[1,2,5], Mawsheng Chern[3,5], Yong Zhu[1,2,5], Ling-Li Zhang[2], Jun-Hua Lu[2], Xu-Pu Li[2], Wen-Qiang Dang[2], Xiao-Chun Ma[2], Zhi-Rui Yang[4], Sheng-Ze Yao[4], Zhi-Xue Zhao[1,2], Jing Fan[1,2], Yan-Yan Huang[1,2], Ji-Wei Zhang[1,2], Mei Pu[1,2], Jing Wang[1,2], Min He[1,2], Wei-Tao Li[1,2], Xue-Wei Chen[1,2], Xian-Jun Wu[1,2], Shi-Gui Li[1,2], Ping Li[1,2], Yi Li[4], Pamela C. Ronald[3] and Wen-Ming Wang[1,2] ✉

MicroRNA168 (miR168) is a key miRNA that targets *Argonaute1* (*AGO1*), a major component of the RNA-induced silencing complex[1,2]. Previously, we reported that miR168 expression was responsive to infection by *Magnaporthe oryzae*, the causal agent of rice blast disease[3]. However, how miR168 regulates immunity to rice blast and whether it affects rice development remains unclear. Here, we report our discovery that the suppression of miR168 by a target mimic (MIM168) not only improves grain yield and shortens flowering time in rice but also enhances immunity to *M. oryzae*. These results were validated through repeated tests in rice fields in the absence and presence of rice blast pressure. We found that the miR168–*AGO1* module regulates miR535 to improve yield by increasing panicle number, miR164 to reduce flowering time, and miR1320 and miR164 to enhance immunity. Our discovery demonstrates that changes in a single miRNA enhance the expression of multiple agronomically important traits.

Rice is a staple food for half of the world's population. Yield, flowering time and disease resistance are key factors in rice production. However, the presence of disease-resistance genes can penalize crop yield[4]. For example, a trade-off between biomass and resistance has been documented in 56% of disease-resistance studies[5]. There is also a correlation between growth duration and yield. Crops with higher yields usually have longer vegetative growth[6,7]. Despite these challenges, recent reports indicate that certain immune regulators can promote immunity without yield penalties; in particular, *ideal plant architecture 1* (*ipa1*) *-1D* promotes both yield and immunity[8,9]. In addition, the presence of a long noncoding RNA has been demonstrated to reduce flowering time without yield penalty[10]. To date, no regulators have been reported to promote yield, early maturity and immunity together.

MicroRNAs (miRNAs) are global gene regulators controlling plant growth, development and immunity[11,12]. We therefore examined miRNAs that have the potential to affect rice growth, yield and immunity. miR168 is responsive to *Magnaporthe oryzae* infection[3] and targets *Argonaute1* (*AGO1*), which encodes the key component of the RNA-induced silencing complex[1,13]. For these reasons, miR168 serves as a good candidate for such a study. Here, we created miR168 target mimic (MIM168) transgenic lines and overexpression (OX168) lines (Supplementary Fig. 1a,b). OX168 plants showed significantly higher miR168 expression, leading to lower *AGO1* expression, whereas MIM168 displayed significantly lower miR168 expression, resulting in higher expression of *AGO1* compared with the Nipponbare (NPB) control plants (Supplementary Fig. 1b–d).

We observed pleiotropic phenotypes in OX168 and MIM168 plants. Compared with the NPB control, OX168 plants displayed increased height, significantly fewer panicles, slightly higher 1,000-grain weight and similar grain number per panicle (Fig. 1a–c and Supplementary Table 1). Conversely, MIM168 plants were shorter and displayed significantly more panicles, with similar grain number per panicle but slightly lower 1,000-grain weight than the control (Fig. 1a–c and Supplementary Table 1). In addition, OX168 lines exhibited an approximately ten-day delay in flowering time and developed 17 leaves on average, whereas MIM168 flowered approximately three days earlier and developed 14 leaves, with the control developing 15 leaves (Fig. 1d,e and Supplementary Fig. 2a,b). These results clearly show that the alteration of miR168 amounts affects plant architecture and flowering time and may influence yield.

To test the capacity of MIM168 and OX168 lines in grain yield, we grew them along with the NPB control in rice fields from 2017 to 2019. Three independent lines were included for each of MIM168 and OX168. MIM168 lines yielded significantly more grain (up to 30–40% higher), calculated both per plant and per area (m^2) in the rice paddy fields; in contrast, OX168 lines yielded significantly less grain (approximately 20–40% lower) (Fig. 1f and Supplementary Table 1). These results demonstrate that the suppression of miR168 changes plant architecture, resulting in higher grain yields.

AGO1, the target of miR168, was previously shown to be required for pathogen-associated molecular-pattern-triggered immunity in *Arabidopsis*[14]. We therefore tested two lines for each of MIM168 and OX168 in a rice field with high rice blast pressure to assess their potential effects on resistance to *M. oryzae*. We found that, while the NPB control yield was 13% less under blast disease pressure, MIM168 lines had only a 1–4% reduction in yield. In contrast, OX168 lines displayed a 20–40% reduction in yield (Fig. 2a,b and Supplementary Fig. 2c). These results suggest that lower miR168 levels enhance resistance, whereas higher miR168 levels decrease resistance to *M. oryzae*. Under blast nursery conditions, MIM168 lines yielded up to 75% higher per m^2 than the NPB control (Fig. 2a,b and Supplementary Fig. 2c). Consistent with the field results, OX168

[1]State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Sichuan Agricultural University, Chengdu, China. [2]Rice Research Institute and Key Lab for Major Crop Diseases, Sichuan Agricultural University, Chengdu, China. [3]Department of Plant Pathology, University of California Davis, and the Joint BioEnergy Institute, Davis, CA, USA. [4]The State Key Laboratory of Protein and Plant Gene Research, College of Life Sciences, Peking University, Beijing, China. [5]These authors contributed equally: He Wang, Yan Li, Mawsheng Chern, Yong Zhu. ✉e-mail: j316wenmingwang@sicau.edu.cn

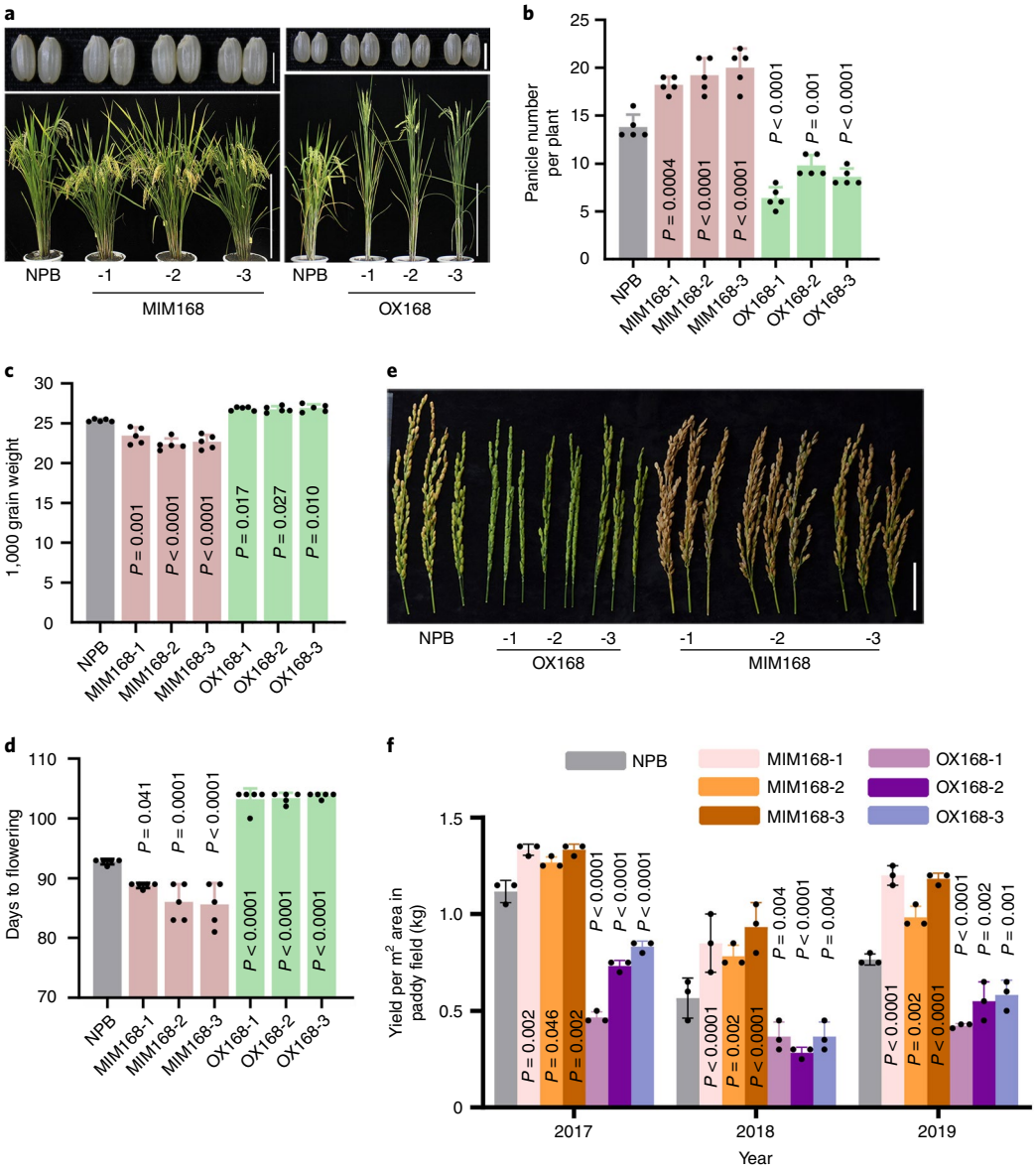

Fig. 1 | miR168 regulates rice yield and flowering time. a, Gross morphology and husked grains of the NPB control, OX168 and MIM168. Scale bars, 50 cm for gross morphology and 5 mm for grains. **b**–**d**, Quantification of panicle number (**b**), 1,000 grain weight (**c**) and flowering time (**d**). The data are shown as mean ± s.d. ($n = 5$ independent plants). **e**, Panicle morphology. Scale bar, 5 cm. **f**, Yields of the NPB control, OX168 and MIM168 per 1 m^2 area in the paddy fields in 2017, 2018 and 2019. Each dataset contains three plots. The data are shown as mean ± s.d. ($n = 3$ biologically independent plots). For **b**,**c**,**e**, and **f**, the *P* values were determined by one-way analysis of variance (ANOVA).

lines were more susceptible to *M. oryzae* strain Guy11, as well as 97-27-2 and NC-34 (two strains isolated from rice fields in north China and south China, respectively). The OX168 lines displayed significantly larger disease lesions and 2–4.5-fold higher fungal biomass, whereas MIM168 plants were more resistant, displaying smaller lesions and less than 50% fungal biomass compared with the NPB control (Fig. 2c,d and Supplementary Fig. 2d,e). Moreover, OX168 showed significant increased invasive hyphae progression at 18–48 hours post-inoculation (hpi) with Guy11 in sheath cells and reduced H_2O_2 accumulation at 48 hpi. In contrast, MIM168 showed delayed invasive hyphae progression and increased H_2O_2 accumulation at the infected sites (Fig. 2e,f), a marker of defence responses. Consistently, MIM168 lines accumulated higher mRNA levels of the defence-related genes, including *OsPR1*, *OsPR10b*, *Os04g10010* and the H_2O_2 production genes *respiratory burst oxidase homologues* *RbohB* and *RbohE*, but lower levels of a *catalase* gene encoding H_2O_2 degradation enzyme (Supplementary Fig. 2f–k). OX168 lines, however, displayed an opposite or unchanged expression pattern of these genes in response to *M. oryzae* infection. These results demonstrate that miR168 suppresses rice immunity against *M. oryzae*, and the suppression of miR168 enhances rice immunity to *M. oryzae*.

We next created *AGO1*-silencing transgenic lines (AGO1i) to explore whether miR168 regulates rice growth and immunity via AGO1. AGO1i lines showed significantly lower *AGO1* (*AGO1a-d*) amounts compared with the NPB control (Supplementary Fig. 3a,b). Consistent with the yield traits of OX168, AGO1i lines showed taller plants, slightly larger seeds, fewer tillers, lower 1,000-grain weights and significantly reduced yields than the NPB control (Supplementary Fig. 3c–e and Supplementary Table 1). Moreover, as observed for the OX168 lines, the AGO1i lines displayed enhanced

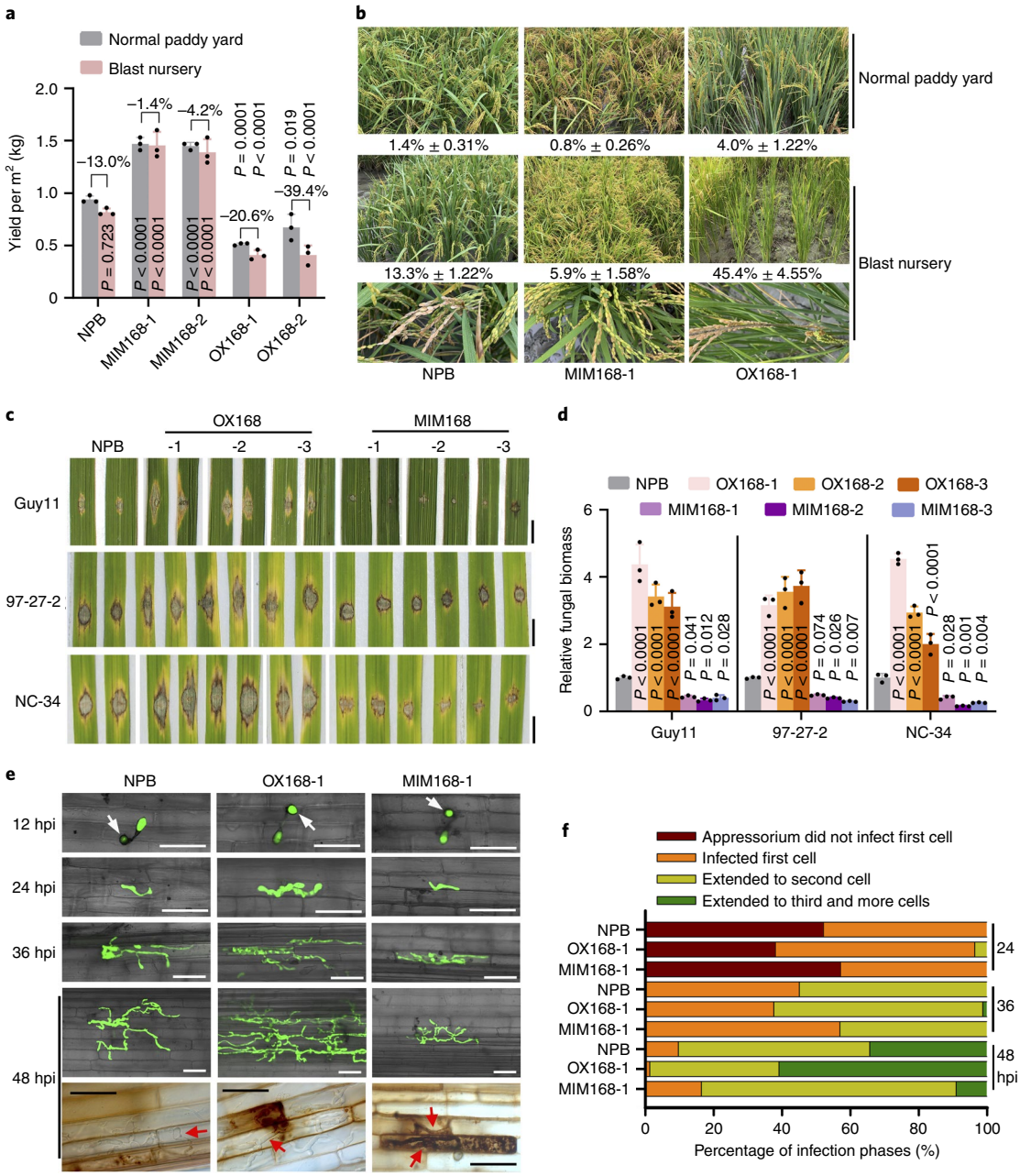

Fig. 2 | miR168 regulates rice immunity against *M. oryzae*. **a**, Yields of the NPB control, OX168 and MIM168 with or without blast disease pressure (blast nursery) in 2019. The bars indicate mean ± s.d. ($n = 3$ biologically independent plots). **b**, Blast disease severities of the NPB control, OX168 and MIM168 in fields with or without blast disease pressure in Wenjiang, Sichuan Province, China. The percentages indicate *M. oryzae*-infected panicles to total counted panicles. **c**, Disease lesions of detached leaves at five days post-inoculation (dpi) by punch inoculation with three blast strains. Scale bars, 5 mm. **d**, qPCR of fungal DNA of the samples in **c**. The data are shown as mean ± s.d. ($n = 3$ independent samples). **e**, Microscopic images showing the infection status of GFP-tagged Guy11 at 12, 24, 36 and 48 hpi. Scale bars, 20 μm. The white arrows indicate appressoria, and the red arrows indicate invasive hypha. H_2O_2 in cells was stained by DAB, and the brownness intensity indicates the H_2O_2 amount in the cells. **f**, Quantification of pathogenesis of Guy11. Over 150 conidia in each line were analysed in three independent experiments. For **a** and **d**, the *P* values were determined by one-way ANOVA.

susceptibility to and accelerated invasive progression of *M. oryzae*, and less H_2O_2 accumulation upon inoculation with three strains (Supplementary Fig. 3f–i). These results indicate that AGO1 mediates miR168 function.

Because the miR168–*AGO1* module regulates the accumulation of miRNAs globally, we performed small RNA sequencing to examine changes of miRNAs in leaves at the seedling and tillering stages and in panicles at the booting stage. Many miRNAs altered expression in OX168 and MIM168 lines (Supplementary Table 2). We found 162 miRNAs at the seedling stage, 156 at the tillering stage and 197 at the booting stage that were regulated by miR168 (Supplementary Fig. 4a and Supplementary Table 3). Among them, we found 15 miRNAs[11,15–17] that were previously identified as regulators of rice developmental processes (Supplementary Fig. 4b). To identify the miR168–*AGO1* module-regulated miRNAs that are probably involved in immunity, we compared those miRNAs affected in MIM168 and OX168 (Supplementary Table 3) with those responsive to *M. oryzae* (Supplementary Table 2 in ref. [3]) and

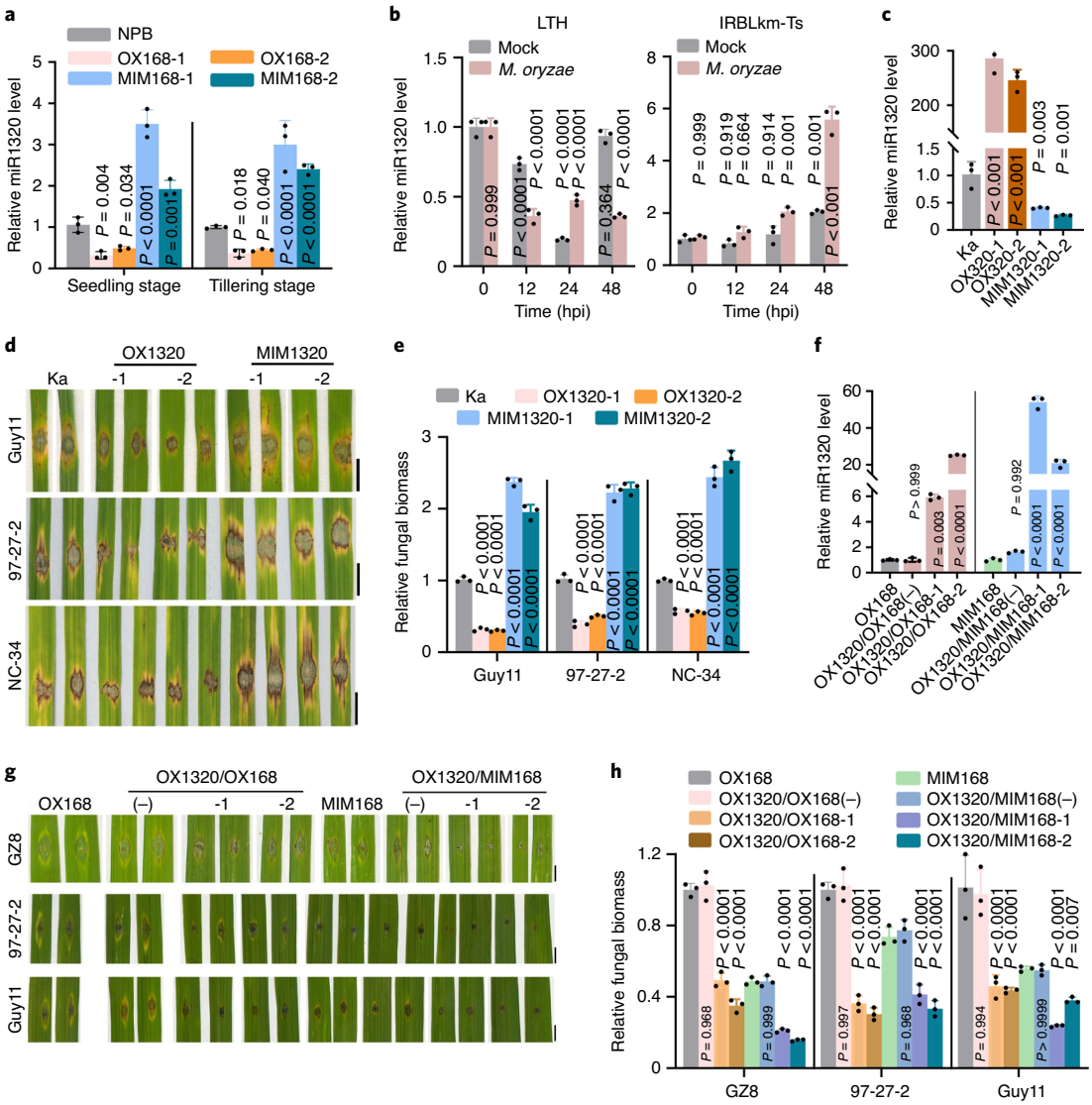

Fig. 3 | miR1320 contributes to miR168-regulated immunity. a, RT–qPCR of miR1320 in the NPB control, OX168 and MIM168. **b**, RT–qPCR of miR1320 in a susceptible variety (LTH) and a resistant variety (IRBLkm-Ts) upon *M. oryzae* infection or mock treatment. The data were normalized so that the level at 0 hpi was arbitrarily set as 1. **c**, RT–qPCR of miR1320 in the Kasalath (Ka) control, OX1320 and MIM1320. **d**, Blast lesions of detached leaves at 5 dpi with three blast strains. Scale bars, 5 mm. **e**, qPCR of fungal DNA of the samples in **d**. Kasalath is used as the control. **f**, RT–qPCR of miR1320 in OX168, OX1320/OX168, OX1320/OX168 null segregant (–), MIM168, OX1320/MIM168 and OX1320/MIM168 null segregant (–). OX168 and MIM168 are used as controls, respectively. **g**, Blast lesions of detached leaves at 5 dpi with three blast strains. Scale bars, 5 mm. **h**, qPCR of fungal DNA of the samples in **g**. For the *P* value analysis, the data were compared with those of OX168 and MIM168, respectively. For **a**–**c**,**e**,**f** and **h**, the data are shown as mean ± s.d. ($n = 3$ independent samples). All the *P* values were determined by one-way ANOVA.

identified 54 miRNAs from 39 families. Among these, we found nine miRNAs[3,12,18–23] that were previously identified to be involved in immunity (Supplementary Fig. 4b,c and Supplementary Table 4). Consistently, the transcript levels of many target genes of these nine miRNAs were changed in OX168 and MIM168 (Supplementary Fig. 4d). These data indicate that many miRNAs mediate miR168 function, and some of these are critical to immunity.

First, the previously uncharacterized miR1320 was upregulated in MIM168 and downregulated in OX168 at the seedling and tillering stages. Its expression was validated by quantitative PCR with reverse transcription (RT–qPCR) and northern blotting analysis, although northern blot seems not sensitive enough to detect the alteration of low-abundance miR1320 (Fig. 3a, Supplementary Fig. 4e and Supplementary Table 3). Importantly, miR1320 was differentially upregulated in a blast-resistant rice variety (International Rice Blast Line Pyricularia-Kanto51-m-Tsuyuake (IRBLkm-Ts)), consistent with a previous report[24], whereas it was downregulated in the susceptible variety Lijiang xin Tuan Heigu (LTH) upon *M. oryzae* infection (Fig. 3b). These results suggest that miR1320 may mediate miR168 function in immunity to *M. oryzae*. We therefore tested the effects of miR1320 directly by generating overexpression (OX1320) and target mimic (MIM1320) lines (Fig. 3c) in Kasalath, and overexpression lines in OX168 (OX1320/OX168) and MIM168 (OX1320/MIM168) (Fig. 3f). We found that OX1320 lines showed enhanced resistance, with smaller lesions and lower fungal biomass (by ~50%), whereas MIM1320 displayed the opposite phenotypes (Fig. 3d,e and Supplementary Fig. 5a,b). Moreover, OX1320/OX168 lines were more resistant than OX168 and null segregants, and OX1320/MIM168 lines were more resistant than MIM168 and null segregants (Fig. 3g,h). Consistently, OX1320 lines showed delayed

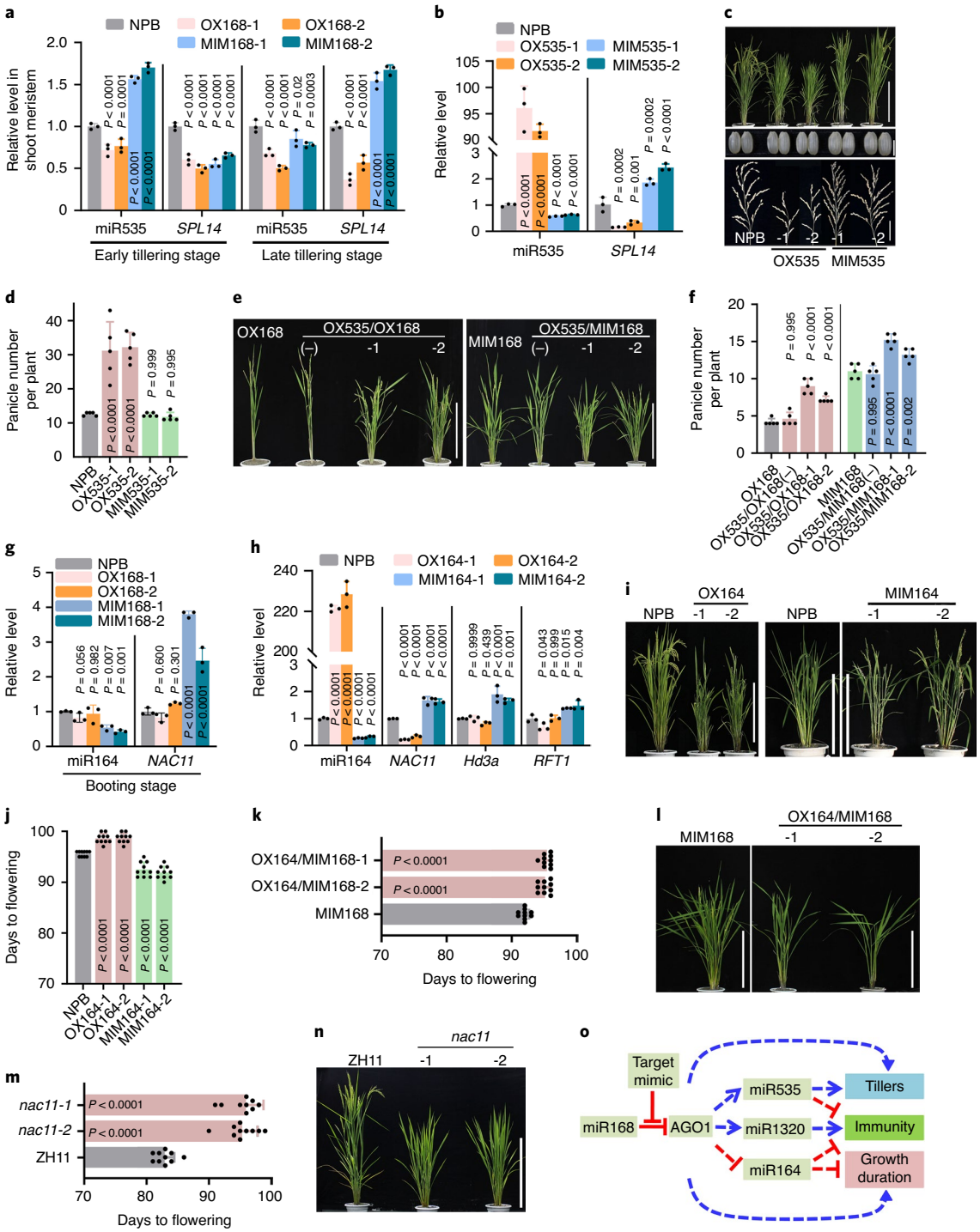

Fig. 4 | miR535 and miR164 contribute to miR168-regulated development. a, RT–qPCR of miR535 and *SPL14* mRNA in the shoot meristem of the NPB control, OX168 and MIM168. **b**, RT–qPCR of miR535 and *SPL14* mRNA in the NPB control, OX535 and MIM535. **c**, Gross morphology (top; scale bar, 50 cm), husked grains (middle; scale bar, 5 mm) and panicle morphology (bottom; scale bar, 5 cm) of the indicated lines. **d**, Panicle numbers of the NPB control, OX535 and MIM535. **e**, Gross morphology of OX168, OX535/OX168, OX535/OX168 null segregant (−), MIM168, OX535/MIM168 and OX535/MIM168 null segregant (−). **f**, Panicle numbers of the indicated lines. OX168 and MIM168 are used as independent controls. **g**, RT–qPCR of miR164 and *NAC11* mRNA in the NPB control, OX168 and MIM168 at the booting stage. **h**, RT–qPCR of miR164 and mRNAs of *NAC11*, *Hd3a* and *RFT1* in the NPB control, OX164 and MIM164. **i**,**j**, Gross morphology (**i**; scale bars, 50 cm) and flowering time (**j**) of the NPB control, OX164 and MIM164. **k**,**l**, Flowering time (**k**) and gross morphology (**l**; scale bars, 50 cm) of the MIM168 control and OX164/MIM168 lines. **m**,**n**, Flowering time (**m**) and gross morphology (**n**; scale bar, 50 cm) of the Zhonghua 11 (ZH11) control and *nac11* mutants. **o**, Model for miR168–*AGO1* regulating rice immunity and growth through three miRNAs. For **a**,**b**,**g** and **h**, the data are shown as mean ± s.d. ($n = 3$ independent samples). For **d** and **f**, the data are shown as mean ± s.d. ($n = 5$ independent plants). For **j**,**k** and **m**, the data are shown as mean ± s.d. ($n = 10$ independent plants). All the *P* values were determined by one-way ANOVA.

invasive hyphae progression, more H_2O_2 and higher mRNA levels of H_2O_2-generation and defence-related genes than the Kasalath control upon *M. oryzae* infection (Supplementary Fig. 5c–f). MIM1320 showed the opposite (Fig. 3g,h and Supplementary Fig. 5c–f). In addition, OX1320 showed similar yields as the Kasalath control (Supplementary Table 1). These results suggest that miR1320 contributes to MIM168-conferred resistance to *M. oryzae*.

Second, miR156, miR529 and miR535 target *SQUAMOSA promoter binding protein-like (SPL) 14* (also known as *IPA1*), which positively regulates panicle development[15,25,26]. We examined their accumulation in the shoot apical meristem. miR156 was decreased or unchanged, and miR529 was not detected in OX168 and MIM168 lines (Supplementary Fig. 6). In contrast, miR535 was repressed in OX168 and elevated in MIM168 at the early tillering stage, whereas it was repressed in both OX168 and MIM168 at the late tillering stage (Fig. 4a and Supplementary Fig. 4e). Because miR535 was not previously demonstrated to regulate *SPL14*, we tested whether miR535 could repress *SPL14*. We fused the putative miR535 target site of *SPL14* to yellow fluorescence protein (YFP) at the 5′ terminus (*35S-SPL14*$_{ts}$–YFP) and transfected *Nicotiana benthamiana* with or without miR535. The YFP signal was clearly decreased in the presence of miR535 (Supplementary Fig. 7a–c), indicating that miR535 represses *SPL14*. Consistently, *SPL14* was suppressed at the early tillering stage but elevated at the late tillering stage in MIM168 in the shoot meristem (Fig. 4a).

To test the role of miR535, we generated miR535 overexpression (OX535) and target mimic (MIM535) lines (Fig. 4b). We found that OX535 greatly reduced *SPL14* levels and MIM535 significantly increased them (Fig. 4b), indicating that miR535 suppresses *SPL14* expression in rice. More importantly, OX535 lines developed approximately threefold more tillers, although they carried smaller panicles with less filled grains, leading to lower yield; conversely, MIM535 lines displayed unchanged tiller numbers, but carrying bigger panicles with more grains per panicle, leading to higher yield per plant than the control (Fig. 4c,d and Supplementary Table 1). Consistently, both OX535/OX168 and OX535/MIM168 lines developed more tillers than OX168 and MIM168, respectively (Fig. 4e,f). Moreover, MIM535 displayed enhanced resistance with more H_2O_2 accumulation compared with the NPB control, while OX535 showed the opposite (Supplementary Fig. 8). These results suggest that higher miR535 levels in MIM168 at the seedling to early tillering stages favour the development of more tillers/panicles via the suppression of *SPL14* but disfavour immunity (Fig. 4a–d), whereas lower miR535 levels at the late tillering to booting stages shift the balance from more tillers/panicles to larger panicle size, and improve immunity via *SPL14* (ref. [9]).

We next assessed the miRNAs that contribute to the alteration of flowering time in OX168 and MIM168. miR164a was significantly repressed in MIM168 and remained unchanged in OX168 at the booting stage (Fig. 4g and Supplementary Table 3). Conversely, miR164-targeted *NAC11*, which contributes to shortening flowering time[27], was significantly increased in MIM168 (Fig. 4g and Supplementary Fig. 9b), suggesting that the decrease in miR164 at the booting stage contributes to the increase in *NAC11*, resulting in earlier flowering in MIM168. To validate this hypothesis, we generated miR164a overexpression (OX164) and target mimic (MIM164) lines in NPB, OX168 (OX164/M168) and MIM168 (OX164/M168) lines (Supplementary Fig. 9g). OX164 displayed a shorter stature and an approximately four-day delay in flowering associated with significantly decreased *NAC11* levels than the control, and OX164/MIM168 showed an approximately three-day delay in flowering in comparison with MIM168; conversely, MIM164 showed a similar plant height and flowered approximately five days earlier, accompanied with increased levels of *NAC11* and the flowering genes *Hd3a* and *RFT1* (Fig. 4h–l). Furthermore, we generated two homozygous knockout lines, *nac11-1* and *nac11-2*, through CRISPR–Cas9 technology (Supplementary Fig. 10a–d) and found that the resulting lines displayed significantly delayed flowering (Fig. 4m,n), indicating that *NAC11* contributes to the early flowering of MIM168 lines. In addition, miR164 was enhanced in OX168 but repressed in MIM168 at the seedling and booting stages (Supplementary Figs. 5e and 9a). Consistently, OX164 showed reduced resistance to *M. oryzae*, whereas MIM164 displayed enhanced resistance[24] (Supplementary Fig. 9c–f). Moreover, OX164/OX168 showed lower resistance than OX168, and OX164/MIM168 showed lower resistance than MIM168 and the null segregants (Supplementary Fig. 9g–i).

Grain yield correlates with growth duration and competes with disease resistance. Here, we report that the suppression of miR168 results in enhanced yield, an earlier flowering time and increased resistance to *M. oryzae*. In the complex miRNA network modulated by the miR168–*AGO1* module, miR1320 plays a key role in resistance to *M. oryzae*, miR535 is important for balancing tiller development and immunity, and miR164 is critical for controlling flowering time and immunity. We propose a model in Fig. 4o to summarize our major finding that *AGO1* mediates miR168 function via multiple miRNAs. This model is not all inclusive, and other miRNAs and genes probably also contribute to tillering, flowering and resistance to *M. oryzae*.

Methods

Plant materials and growth conditions. The rice (*Oryza sativa* L.) accessions NPB (ssp. *japonica*), Kasalath (ssp. *indica*), LTH, ZH11 and IRBLkm-Ts were used in this study. LTH is a *japonica* accession highly susceptible to over 1,300 isolates of *M. oryzae* worldwide, and no major *R* gene is ever identified in it[28], whereas IRBLkm-Ts contains a single *R* gene, *Pikm*, that mediates ETI against *M. oryzae* strains expressing the avirulence gene alleles *AVR-PikA*/*D*/*E*[29,30]. For the yield traits assay and blast pressure assay, the control and transgenic plants were grown in paddy fields with high blast disease pressure (blast nursery) or no blast disease (normal field) in Chengdu, China (36° N, 103° E) during the normal rice-growing season from mid-April to mid-September. The rice seeds were immersed in water for two days at 37 °C in darkness for germination and then grown in a soil seed bed for four weeks before being transplanted into the paddies. For the blast disease assays, the control and transgenic plants were grown in a greenhouse with a 28/24 ± 1 °C day/night temperature, 70% relative humidity and a light/dark period of 14 h/10 h.

Construction of transgenic rice plants. To make transgenic lines overexpressing miRNAs, *MIR* gene-specific primers (Supplementary Table 5) were used to amplify the genomic sequences including 250–350 base pairs (bp) upstream and 200–300 bp downstream of the miRNAs from NPB total genomic DNA. The PCR products were then cloned into the binary vector 35S-pCAMBIA1300 at *Kpn*I and *Spe*I (or *Sal*I) sites and introduced into NPB (for miR168, miR164 and miR535) and Kasalath (for miR1320). To construct transgenic plants expressing the target mimic of the miRNAs, we utilized the gene *INDUCED BY PHOSPHATE STARVATION1* (*IPS1*) as a skeletal structure, which contains a mismatched loop at the *miR399* cleavage site[31]. The target mimic sequences of miR168, miR164, miR535 and miR1320 were inserted into *IPS1* to substitute the *miR399* target mimic site with specific primers (Supplementary Table 5). The mutated *IPS1* was then cloned into the binary vector 35S-pCAMBIA1300 at *Kpn*I and *Spe*I (or *Sal*I) sites and introduced into NPB (for MIM168, MIM164 and MIM535) or Kasalath (for MIM1320) via agrobacterium strain GV3101-mediated transformation. The transgenic plants were screened by a solution containing 0.1 mM 6-BA and 30 mg l^{-1} hygromycin (as previously described[3,20]) and confirmed by PCR to check the hygromycin-resistance genes. More than 20 independent transgenic lines were obtained for each construct, and two or three lines at T2 to T4 generation were used for the analyses of yield traits and the blast disease assays. To make transgenic lines overexpressing miRNAs in OX168 and MIM68, the PCR products of miR164, miR1320 and miR535 genes were cloned into the binary vector 35S-pCAMBIA2300 at *Kpn*I and *Pst*I sites, and the products were introduced into OX168 and MIM168, respectively. The transgenic plants were screened using a solution containing 0.1 mM 6-BA and 30 mg l^{-1} G418 and confirmed by PCR to check the G418-resistance gene. More than 15 independent transgenic lines were obtained for each construct, and two lines at T1 to T2 generation were used for the study.

Trait measurements. Plant height, panicle number per plant, grain number per panicle and 1,000-grain weight were measured at full maturity from five plants in the middle of three rows. Plant height was measured in the paddy fields. The 1,000-grain weight was weighted using an SC-A grain analysis system (Wanshen)

after the fully filled grains were dried in a 42 °C oven for one week. The data were analysed by a one-way ANOVA followed by post-hoc Tukey HSD analysis with significant differences ($P < 0.01$). These experiments were repeated two times using transgenic plants from two generations in two years.

Gene expression analyses. Total RNAs were extracted from rice leaves using TRIzol reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. The first-strand complementary DNA was synthesized from 1 μg of total RNA for transcriptional analysis using a PrimeScript RT Reagent Kit with gDNA Eraser (Takara Biotechnology) following the manufacturer's instructions. RT–qPCR was performed using specific primers and SYBR Green mix (QuantiNova SYBR Green PCR Kit, QIGEN) with a BIO-RAD C1000TM Thermal Cycler (Bio-Rad). The rice ubiquitin (*UBQ*) gene was selected as an internal reference for data normalization. To measure the accumulation of miRNAs, total RNA was reverse-transcribed using miRNA-specific stem-loop RT primers (Supplementary Table 4) with the PrimeScript RT Reagent Kit with gDNA Eraser (Takara Biotechnology), and the RT product was subsequently used as a template for qPCR by using miRNA-specific forward primers and the universal reverse primer (Supplementary Table 4). The snRNA U6 served as an internal reference for the detection of miRNAs. The qPCR analyses were performed with three technical replicates. The $2^{-\Delta\Delta CT}$ method was exploited to analyse the relative expression levels of miRNAs.

Pathogen infection and microscopy analysis. *M. oryzae* strains Guy11, 97-27-2, NC-34, GFP-tagged Guy11 and GFP-tagged strain Zhong8-10–14 (GZ8) were used for the blast disease assays. The *M. oryzae* strains were cultured in plates containing agar–oatmeal–tomato medium at 28 °C with 12-h/12-h light/dark cycles for two weeks. After the surface mycelia were washed with distilled water, the plates were further incubated for three days to promote sporulation. The spores were collected with distilled water, and the inoculum concentration was adjusted to 3×10^5 conidia per ml; the spores were then inoculated on 5-cm-long leaf sheaths from four-week-old plants as described previously[32]. The inoculated epidermal layer was excised, and conidia germination, appressorium development and invasive hyphae growth were recorded by a Nikon A1 Laser Scanning Confocal Microscope (Nikon Instruments) at 12, 24, 36 and 48 hpi. The quantitative analysis of infestation stage was conducted as described previously[3]. Wound inoculation was done following a previous report[33]. Briefly, dilution-drop conidia suspensions of *M. oryzae* strains Guy11, 97-27-2, GZ8 and NC-34 (3×10^5 conidia per ml) were placed against wounded sites or spray-inoculated on the three- to five-leaf-stage seedlings' leaves. Lesion formation was examined at four to six days after inoculation. The infection experiments were repeated twice. The relative fungal biomass was measured using the DNA amounts of *M. oryzae Mopot2* against rice ubiquitin DNA amounts by qPCR using specific primers and SYBR Green mix (QuantiNova SYBR Green PCR Kit, QIGEN) with a BIO-RAD C1000TM Thermal Cycler (Bio-Rad). The rice *UBQ* gene was selected as an internal reference for data normalization.

To observe the immune responses of rice transgenic plants upon *M. oryzae* infection, three-leaf-stage seedlings were inoculated with *M. oryzae* strain Guy11 at a concentration of 5×10^5 conidia per ml. At 48 hpi, the leaves were collected and incubated in 1 mg ml^{-1} DAB (Sigma, Merck Life Science Co.) at 22 °C for 8 h at illumination. The DAB-stained leaves were double stained with trypan blue and observed under a microscope (Zeiss imager A2, Carl Zeiss). To detect the expression of defence-related genes and H_2O_2-related genes, the leaves were collected at indicated time points for RNA extraction and RT–qPCR. The experiments were repeated twice.

Small RNA-seq and data analysis. Total RNA was isolated with TRIzol (Thermo Fisher Scientific) from rice leaf samples collected at the three-leaf seedling and 28-day-old tillering stages and from young panicle samples collected at the booting stage. To obtain the young panicles at similar developmental stages, we collected samples from tillers with zero distance between the pulvinus of the flag leaf and that of the top-second leaf from nine-week-old plants. RNA integrity and concentration were checked with gel electrophoresis and the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies). Three micrograms of total RNA were subjected to the construction of a small RNA library with NEB Next Multiplex Small RNA Library Prep Set for Illumina (New England Biolabs), following the manufacturer's instructions. Index codes were added to attribute sequences to each sample. Library quality was checked on the Agilent Bioanalyzer 2100 system with High Sensitivity Chips. Clustering was carried out on a cBot Cluster Generation System with TruSeq SR Cluster Kit v3-cBot-HS (Illumina). The prepared libraries were loaded on an Illumina Hiseq 2500 platform for 50-bp single-end sequencing. The raw data were subjected to removing reads containing ploy-N, with 5′ adapter contaminants, without 3′ adapter or the insert tag, containing ploy A or T or G or C and low-quality reads. The obtained clean data were mapped to the rice reference genome (ftp://ftp.ensemblgenomes.org/pub/release-31/plants/fasta/oryza_sativa) by Bowtie[34]. The mapped small RNA reads were used to align with known miRNA in miRBase v.20.0 without mismatch using the modified software mirdeep2 (refs. [35]). The miRNA expression data were estimated by transcript per million. The fold change in the expression of miRNAs between OX168/NPB and MIM168/NPB was calculated. miRNAs regulated by miR168 were classified into two groups. Group one showed reverse expression patterns between OX168 and MIM168 ($\log_2 \geq 0.5$ or $\log_2 \leq -0.5$ at any stage, OX168 or MIM168 versus control). Group two showed synchronous changes in both OX168 and MIM168 ($\log_2 \geq 0.5$ or $\log_2 \leq -0.5$ in OX168 or MIM168 versus control at any stage).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data generated or analysed during this study are included in this Article and in its Supplementary Information files. The data are available upon request. Source data are provided with this paper.

Received: 10 December 2019; Accepted: 12 January 2021;
Published online: 15 February 2021

References

1. Li, Y. F. et al. Transcriptome-wide identification of microRNA targets in rice. *Plant J.* **62**, 742–759 (2010).
2. Peters, L. & Meister, G. Argonaute proteins: mediators of RNA silencing. *Mol. Cell* **26**, 611–623 (2007).
3. Li, Y. et al. Multiple rice microRNAs are involved in immunity against the blast fungus *Magnaporthe oryzae*. *Plant Physiol.* **164**, 1077–1092 (2014).
4. Nelson, R., Wiesner-Hanks, T., Wisser, R. & Balint-Kurti, P. Navigating complexity to breed disease-resistant crops. *Nat. Rev. Genet.* **19**, 21–33 (2018).
5. Bergelson, J. & Purrington, C. B. Surveying patterns in the cost of resistance in plants. *Am. Nat.* **148**, 536–558 (1996).
6. Evans, J. R. Improving photosynthesis. *Plant Physiol.* **162**, 1780–1793 (2013).
7. Rossi, M., Bermudez, L. & Carrari, F. Crop yield: challenges from a metabolic perspective. *Curr. Opin. Plant Biol.* **25**, 79–89 (2015).
8. Xu, G. et al. uORF-mediated translation allows engineered plant disease resistance without fitness costs. *Nature* **545**, 491–494 (2017).
9. Wang, J. et al. A single transcription factor promotes both yield and immunity in rice. *Science* **361**, 1026–1028 (2018).
10. Fang, J. et al. Ef-cd locus shortens rice maturity duration without yield penalty. *Proc. Natl Acad. Sci. USA* **116**, 18717–18722 (2019).
11. Tang, J. & Chu, C. MicroRNAs in crop improvement: fine-tuners for complex traits. *Nat. Plants* **3**, 17077 (2017).
12. Chandran, V. et al. *miR396-OsGRFs* module balances growth and rice blast disease-resistance. *Front. Plant Sci.* **9**, 1999 (2019).
13. Mallory, A. C., Elmayan, T. & Vaucheret, H. MicroRNA maturation and action—the expanding roles of ARGONAUTEs. *Curr. Opin. Plant Biol.* **11**, 560–566 (2008).
14. Li, Y. et al. Identification of microRNAs involved in pathogen-associated molecular pattern-triggered plant innate immunity. *Plant Physiol.* **152**, 2222–2231 (2010).
15. Sun, M. Z. et al. The multiple roles of *OsmiR535* in modulating plant height, panicle branching and grain shape. *Plant Sci.* **283**, 60–69 (2019).
16. Zhao, Y. F. et al. miR1432-*OsACOT* (acyl-CoA thioesterase) module determines grain yield via enhancing grain filling rate in rice. *Plant Biotechnol. J.* **17**, 712–723 (2019).
17. Yang, R. et al. Fine-tuning of MiR528 accumulation modulates flowering time in rice. *Mol Plant.* **12**, 1103–1113 (2019).
18. Salvador-Guirao, R., Hsing, Y. I. & San Segundo, B. The polycistronic miR166k-166h positively regulates rice immunity via post-transcriptional control of EIN2. *Front. Plant Sci.* **9**, 337 (2018).
19. Zhao, Z. X. et al. *Osa-miR167d* facilitates infection of *Magnaporthe oryzae* in rice. *J. Integr. Plant Biol.* **62**, 702–715 (2019).
20. Li, Y. et al. Osa-miR169 negatively regulates rice immunity against the blast fungus *Magnaporthe oryzae*. *Front. Plant Sci.* **8**, 2 (2017).
21. Zhang, X. et al. *Magnaporthe oryzae* induces the expression of a microRNA to suppress the immune response in rice. *Plant Physiol.* **177**, 352–368 (2018).
22. Li, Y. et al. Osa-miR398b boosts H_2O_2 production and rice blast disease-resistance via multiple superoxide dismutases. *N. Phytol.* **222**, 1507–1522 (2019).
23. Xiao, Z. Y. et al. MiR444b.2 regulates resistance to *Magnaporthe oryzae* and tillering in rice. *Acta Phytopathol. Sinica* **47**, 511–522 (2017).
24. Wang, Z. Y. et al. Osa-miR164a targets *OsNAC60* and negatively regulates rice immunity against the blast fungus *Magnaporthe oryzae*. *Plant J.* **95**, 584–597 (2018).
25. Jiao, Y. et al. Regulation of *OsSPL14* by OsmiR156 defines ideal plant architecture in rice. *Nat. Genet.* **42**, 541–544 (2010).
26. Wang, L. et al. Coordinated regulation of vegetative and reproductive branching in rice. *Proc. Natl Acad. Sci. USA* **112**, 15504–15509 (2015).
27. Mannai, Y. E., Akabane, K., Hiratsu, K., Satoh-Nagasawa, N. & Wabiko, W. The NAC transcription factor gene *OsY37* (ONAC011) promotes leaf

senescence and accelerates heading time in rice. *Int. J. Mol. Sci.* **18**, 2165–2185 (2017).
28. Lin, Z. Z., Jiang, W. W., Wang, J. L. & Lei, C. L. Research and utilization of universally susceptible property of japonica rice variety Lijiangxintuanheigu. *Sci. Agricultura Sinica* **34**, 116–117 (2001).
29. Tsumematsu, H. et al. Development of monogenic lines of rice for blast resistance. *Breed. Sci.* **50**, 229–234 (2000).
30. Kanzaki, H. et al. Arms race co-evolution of *Magnaporthe oryzae AVR-Pik* and rice Pik genes driven by their physical interactions. *Plant J.* **72**, 894–907 (2012).
31. Franco-Zorrilla, J. M. et al. Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat. Genet.* **39**, 1033–1037 (2007).
32. Kankanala, P., Czymmek, K. & Valent, B. Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus. *Plant Cell* **19**, 706–724 (2007).
33. Kong, L. A. et al. Different chitin synthase genes are required for various developmental and plant infection processes in the rice blast fungus *Magnaporthe oryzae*. *PLoS Pathog.* **8**, e1002526 (2012).
34. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10**, R25 (2009).
35. Friedlander, M. R., Mackowiak, S. D., Li, N., Chen, W. & Rajewsky, N. miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic Acids Res.* **40**, 37–52 (2012).

Acknowledgements

We thank C.-L. Lei (Institute of Crop Science, Chinese Academy of Agricultural Sciences) for providing IRBLkm-Ts and J.-M. Zhou (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences) for suggestions on writing the manuscript. This work was supported by grants from the Sichuan Applied Fundamental Research Foundation (no. 2020YJ0332) and National Natural Science Foundation of China (nos. U19A2033, 31672090 and 31430072) to W.-M.W. and by grants from the National Institutes of Health (no. GM59962), USDA NIFA (no. 2017-67013-26590) and the Joint BioEnergy Institute funded by the US DOE (no. DE-AC02-05CH11231) to P.C.R. and M.C.

Author contributions

Yan Li and W.-M.W. conceived the project. H.W., Y.Z., L.-L.Z., J.-H.L., W.-Q.D., Z.-R.Y., S.-Z.Y. and Z.-X.Z. carried out the experiments. X.-P.L. and X.-C.M. performed the transgenic plant generation and analysis. J.-W.Z. and M.P. conducted the field trials. M.C., J.F. and X.-J.W. analysed the data. Yan Li, M.C., P.C.R. and W.-M.W. wrote the paper. X.-W.C., W.-T.L., J.W., M.H., Y.-Y.H., S.-G.L., P.L. and Yi Li discussed the results and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41477-021-00852-x.

Correspondence and requests for materials should be addressed to W.-M.W.

Peer review information *Nature Plants* thanks Yong-Hwan Lee, Shaoqing Li and Guo-Liang Wang for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

nature research

Corresponding author(s): Wen ming Wang

Last updated by author(s): Dec 20, 2020

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	BIO-RAD C1000TM Thermal Cycler (Bio-Rad Inc, Chengdu, China) was used to collect qRT-PCR raw data. Image Lab version 3.0 build 11 (Bio-Rad) was used for WB data collection. Nikon A1 Laser Scanning Confocal Microscopy was used for confocal imaging and data collection.
Data analysis	Clustal Omega program (http://www.ebi.ac.uk/Tools/msa/clustalo) was used for multiple sequence alignment. The Microsoft Excel 2003 and Graphpad Prism 8 (Graphpad Prism software, version 8.0.1)) were used for statistics and bar graphs overlaid with dot plots.ImageJ 1.52a plugin Colocalization Finder was used for determining the proteins colocalization ratio and confocal image (https://imagej.nih.gov/ij/).The obtained small RNA-seq data were mapped to rice reference genome (ftp://ftp.ensemblgenomes.org/pub/release-31/plants/fasta/ory).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data generated or analyzed during this study are included in this article and in its supplementary information files. Data are available upon requirement.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	When conducting phenotypic analyses, we used the sample size following standard procedures from previous publications. For resistance-related assay, we observed and measured rice blast resistance from at least ten infected leaves. For agronomic straits-related analysis, we measured from at least five independent plants. The sample size was included in the main text where possible. No statistical methods were used to predetermine sample sizes.
Data exclusions	No data were excluded from our analyses.
Replication	In a parallel experiment, we set up triple replications for determining the mean value and standard deviation. All the experiments were conducted at least two times to verify the reproducibility.
Randomization	The experimental groups were allocated based on the genotype as wild-type strain or deletion mutants, or based on control and different chemical treatments. Within each experiment group, the samples was randomly selected for statistic analysis.
Blinding	The data collection was not blinded. Blinding was not possible as the investigators were conducting the experiments and had to be aware of controls and treated groups.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*

Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☑ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access and import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	anti-OsAGO1，conducted by Zhejiang Hua'an Biology Company (http://www.huabio.cn/)，anti-Hsp and anti-GFP(Invitrogen, MA5-15256)
Validation	Anti-OsAGO1 and anti-Hsp mouse secondary antibody were used in our immunoblott analysis to detect the protein amounts of AGO1a and Hsp in rice. The results showed that the antibody allow detection of the specific corresponding protein with expected size.Anti-GFP were used in our immunoblott analysis in Nicotiana benthamiana expressing SPL_{ts}-GFP.The results showed that the antibody allow detection of the specific corresponding protein with expected size.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	*State the source of each cell line used.*
Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	*For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.*
Wild animals	*Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*

Outcomes — *Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.
For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission — *Provide a list of all files available in the database submission.*

Genome browser session
(e.g. UCSC)
Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates — *Describe the experimental replicates, specifying number, type and replicate agreement.*

Sequencing depth — *Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*

Antibodies — *Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*

Peak calling parameters — *Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*

Data quality — *Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*

Software — *Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation — *Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*

Instrument — *Identify the instrument used for data collection, specifying make and model number.*

Software — *Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*

Cell population abundance — *Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*

Gating strategy — *Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*
Volume censoring	*Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings	*Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*
Effect(s) tested	*Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	*Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*
Correction	*Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity	*Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*
Graph analysis	*Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.