**Supplementary Figure 1. CO and LCO response in barley.**  

**a** Responses of barley atrichoblasts to COs and LCOs at early developmental stages. Representative traces in atrichoblasts of 5-day-old lateral roots under nutrient limited conditions, responding to treatment of $10^{-8}$ M CO4, $10^{-8}$ M CO8, $10^{-7}$ M SmLCO and $10^{-7}$ M MlLCO. Numbers indicate cells responding compared to total cells analyzed.  

**b** A simplified signaling pathway of arbuscular mycorrhizal (AM) symbiosis. The Myc factors (Myc-LCOs and Myc-COs) secreted by AMF are perceived by the Myc-factor receptors (LysM receptor kinases) and the receptor-like kinase SYMRK in the plasma membrane of the plant cells. Then an unknown secondary messenger transduces the signal into the nucleus, causing nuclear calcium oscillations which are supported by the channels POLLUX/DMI, CNGC15 and MCA8 in the nuclear membrane. The nuclear calcium signal is decoded by CCaMK via association with calmodulin (CaM) and calcium, inducing the phosphorylation of CYCLOPS. The CCaMK-CYCLOPS complex promotes the expression of transcription factor RAM1, which is a major regulator of AM symbiosis.  

**c** Related to Fig. 1d, showing AMF colonization measured at 5- and 7-weeks post inoculation of barley wild type (WT), symrk and cyclops mutants.  

**d** Images of barley plants grown at indicated time points under nutrient limited conditions. Note in 16- and 30-day old plants the leaves begin to senesce (arrows) and anthocyanin accumulates in the stem, timepoints when LCO recognition is observed in the roots. Scale bars = 2 cm.
Supplementary Figure 2. Nutrient starvation and pretreatment of strigolactones and karrikins enhance calcium oscillations. **a** Related to Fig. 3a, the representative calcium traces in atrichoblasts of barley lateral roots grown under different nutrient regimes for 16 days, responding to $10^{-7}$ M SmLCO. **b** Related to Fig. 3b, the calcium traces in atrichoblasts of *M. truncatula* lateral roots grown under nutrient depleted and replete conditions, responding to $10^{-11}$ M SmLCO. **c** and **d** The calcium traces of atrichoblasts of *M. truncatula* responding to NS-LCO (**c**) and wheat lateral roots responding to $10^{-7}$M SmLCO (**d**) after pretreatment of control buffer (mock), 1 µM 5DS and a mixture of 1 µM KARs for 12 hrs. Plants were grown on +P-N medium for 10 days (*M. truncatula*) or 5 days (wheat) before pretreatment. Numbers indicate cells responding compared to total cells analyzed. **e** The percentage of atrichoblasts of barley lateral roots with calcium oscillations after pretreatment of mock, 1 µM 5DS, 1µM karrikin 1, 1µM karrikin 2, a mixture of 1 µM karrikin 1 and karrikin 2 (KARs) and 1 µM GR24. The plants were grown on +P-N medium with 100nM AVG for three days.
Supplementary Figure 3. Phylogenetic trees of gene families, related to Figure 4, 6 and 7.

Phylogenetic trees represent the evolutionary relationship among NSP1 (a), NSP2 (b), CCD8 (c), D14/D14L (d), SMAX-LIKE (e) and LysM-RLK (f) sequences from Medicago truncatula, Hordeum vulgare, Lotus japonicus, Arabidopsis thaliana, Oryza sativa, Zea mays and Fragaria vesca. The genes in M. truncatula and/or barley (Hordeum vulgare) are highlighted in bold. Bootstrap information is displayed as circles.
Supplementary Figure 4. Mycorrhizal colonization and nodulation of *M. truncatula* mutants. **a** The position of *Tnt1* insertion is annotated in the structure of each gene. **b** Semi-quantitative RT-PCR showing the transcript of each gene in the corresponding mutants, using *MtHistone* as a loading control. The assay was repeated three times with similar results. **c** Fungal colonization of wild type (A17) and *nsp2-2* mutants under P-deficient conditions, measured at 3 weeks post inoculation. **d** Related to Fig. 5c, a repeat of *R. irregularis* colonization in the critical mutants. n=5 biologically independent samples. Total: total colonization; A: arbuscules. **e** Nodulation of *M. truncatula* in strigolactone biosynthesis and strigolactone/karrikin signaling mutants under N-limited conditions. Number of nodules formed per plant was measured at 2 weeks post inoculation. n=10 biologically independent plants. For statistical analysis a one-sided Wilcoxon test was performed. **: p<0.01.
Supplementary Figure 5. Mycorrhizal colonization of barley mutants. a A repeat assay of Fig. 3f, showing root-length colonization of barley wild type, *nsp1a* and *nsp2* mutants at 7 weeks post inoculation. n=5 biologically independent samples. b Related to Fig. 5d, Root-length colonization at 9 weeks post inoculation in barley wild type and *d14l* mutant roots under P deficient conditions. n=8 biologically independent samples. Total: total colonization; A: arbuscules. *p*-values for colonization levels were determined by a one-sided Wilcoxon test. **: p<0.01; *: 0.01<p<0.05.
Supplementary Figure 6. Overexpression of NSP2 in barley restored mycorrhization suppressed by high P. **a** Mycorrhizal colonization levels of barley wild-type roots grown under P gradients, assessed at 7 weeks post inoculation. n=3 biologically independent plants. Total: total colonization; A: arbuscules. For statistical analysis a one-sided Wilcoxon test was performed. **: p<0.01. **b** and **c** Levels of MtNSP1 (b) and MtNSP2 (c) transcripts in barley roots transformed with overexpression of respective genes. The plants were grown under +P+N for three weeks. n=3 biologically independent samples. ±s.e.m. **: p<0.001, measured using a Student’s t-test (one-tailed, two-sample equal variance). **d** A repeat experiment showing root-length colonization of barley roots overexpressing MtNSP1 and MtNSP2. The plants were inoculated with R. irregularis and grown under different P levels for 5 weeks. n=3-5 biologically independent plants. Total: total colonization; A: arbuscules. p-values were determined by a one-sided Wilcoxon test. **: p<0.01.
Supplementary Figure 7. N and/or P starvation induces a subset of genes regulated by NSP1 and NSP2.

Heatmaps showing the *M. truncatula* (a) and barley (b) genes upregulated by either N and/or P starvation, repressed in *nsp1* or *nsp2* mutants in at least one nutrient condition and activated by overexpression of *miRR-MtNSP2* in *M. truncatula* (a) and *MtNSP2* in barley (b). +P-N, -P+N and -P-N represent the expression of these starvation-induced genes in wild-type plants by comparing -N or/and -P conditions to +P+N conditions. *nsp* show gene expression in *nsp* mutants compared to wild-type plants grown in the same nutrient conditions (-N or/and -P), while *NSPox* show *NSP* overexpression roots compared to wild type under nutrient replete conditions. Genes are clustered by expression pattern. A 1.5-fold cutoff was used for log2 fold changes with all the heatmaps.
Supplementary Figure 8. NSP1 and NSP2-regulated genes involve the apocarotenoid biosynthetic pathway. **a** A subset of the biosynthetic pathway of apocarotenoids in plants. The enzymes in each step are highlighted in bold and their full names are listed in the inset. The genes regulated by nutrient starvation in an NSP-dependent manner (Fig. 5a, b) are highlighted on the pathway, with blue stars representing *M. truncatula* genes and red circles representing barley genes. **b** Root expression *pMtD27::GUS* in *M. truncatula* wild type and *nsp1-1* mutants. The plants were grown on modFP plates for 4 weeks after hairy-root transformation. Bars = 200 µm. The assay was repeated three times with similar results. **c** Expression levels of strigolactone biosynthetic genes in *M. truncatula* roots with overexpression of GFP, NSP1, NSP2 and a combination of NSP1 and NSP2, using PT4 as a negative control. The plants were grown on modFP plates for 4 weeks after hairy-root transformation of the respective construct. Bars represent means of 4 biological replicates ± s.e.m. Different letters indicate different statistical groups (ANOVA, post hoc Tukey, *P* < 0.05).
Supplementary Figure 9. Nutrient regulation of barley RLK genes via 5DS and KARs. a Relative expression of barley RLK genes in wild-type roots grown under different nutrient conditions. The expression values were obtained by conducting TMM normalization from transcriptome data. n=2-3 biologically independent samples. ±s.e.m. b qPCR showing regulation of symbiosis genes by 5DS and KARs. Wild type plants were grown under repressive P-conditions and pretreated for 2 days on solid media containing 1 µM 5-deoxystrigol (5DS) or a mixture of 1 µM karrikin 1 and karrikin 2 (KARs). D53 acts as a marker gene for strigolactone treatment, while DLK2 responding to both strigolactone and karrikin treatment. n=4 biologically independent samples, ±s.e.m.; ** indicates p<0.01, * indicates 0.01<p<0.05, measured using a Student’s t-test (one-tailed, two-sample equal variance). c Root-length colonization of barley wild type and rlk2-2 mutant grown under low P conditions. The colonization levels were measured at 5 weeks post inoculation. n=3 biologically independent samples. d A repeat of Fig. 6e, showing root-length colonization of barley rlk2, rlk10 and rlk2/rlk10 double mutants grown under P-limited conditions, measured at 7 weeks post inoculation. The RLK10 mutation in the rlk2/rlk10 double mutant is equivalent to rlk10-1. n=14-15 biologically independent samples. Total: total colonization; A: arbuscules. p-values for colonization levels were determined by a one-sided Wilcoxon test. **: p<0.01.
Supplementary Table 1. Time course analysis of barley root cells responding to $10^{-7}$ M *SmLCO* after incubation with 5DS and KARs.

| Incubation time (hr) | $10^{-7}$ M 5DS | $10^{-7}$ M KARs |
|----------------------|----------------|-----------------|
| 1                    | 0 / 21         | 0 / 29          |
| 2                    | 0 / 31         | 0 / 27          |
| 3                    | 0 / 28         | 0 / 26          |
| 4                    | 0 / 29         | 0 / 27          |
| 8                    | 0 / 30         | 3 / 25          |
| 16                   | 25 / 37        | 22 / 31         |

Cells spiking / total cells
Supplementary Table 2. *NSP1* and *NSP2* percentage dependencies of nutrient upregulated DEGs in *M. truncatula* and barley

### Medicago

| Condition                                      | -N+P 15D | +N-P 15D | -N-P 15D |
|------------------------------------------------|----------|----------|----------|
| nutrient upregulated and dependent on MtNSP1  | 332      | 173      | 181      |
| nutrient upregulated and dependent on MtNSP2  | 442      | 366      | 395      |
| nutrient upregulated                           | 3635     | 1041     | 4047     |
| *MtNSP1*-dependent genes of those nutrient upregulated (%) | 9.1      | 16.6     | 4.5      |
| *MtNSP2*-dependent genes of those nutrient upregulated (%) | 12.2     | 35.2     | 9.8      |
| nutrient upregulated and dependent on MtNSP1 in at least one starvation condition | 588      |          |          |
| nutrient upregulated and dependent on MtNSP2 in at least one starvation condition |          |          |          |
| nutrient upregulated in at least one starvation condition |          |          |          |
| *MtNSP1*-dependent genes of those nutrient upregulated in at least one starvation condition (%) |          |          | 11.5     |
| *MtNSP2*-dependent genes of those nutrient upregulated in at least one starvation condition (%) |          |          | 17.6     |

### Barley

| Condition                                      | -N+P 21D | +N-P 21D | -N-P 21D |
|------------------------------------------------|----------|----------|----------|
| nutrient upregulated and dependent on HvNSP1  | 440      | 536      | 999      |
| nutrient upregulated and dependent on HvNSP2  | 437      | 1046     | 1404     |
| nutrient upregulated                           | 5932     | 5648     | 7009     |
| *HvNSP1*-dependent genes of those nutrient upregulated (%) | 7.4      | 9.5      | 14.3     |
| *HvNSP2*-dependent genes of those nutrient upregulated (%) | 7.4      | 18.5     | 20.0     |
| nutrient upregulated and dependent on HvNSP1 in at least one starvation condition |          |          | 1576     |
| nutrient upregulated and dependent on HvNSP2 in at least one starvation condition |          |          | 2436     |
| nutrient upregulated in at least one starvation condition |          |          | 10412    |
| *HvNSP1*-dependent genes of those nutrient upregulated in at least one starvation condition (%) |          |          | 15.1     |
| *HvNSP2*-dependent genes of those nutrient upregulated in at least one starvation condition (%) |          |          | 23.4     |
### Supplementary Table 3. List of constructs

#### L1 plasmids for barley transformation

| ENSA ID | ENSA Standard name | Backbone | PU       | S                  | C   | SC | T       |
|---------|-------------------|----------|----------|--------------------|-----|----|---------|
| EC15567 | pL1M-R5-pZmUBI-   | EC47841  | pL1V-R5  | EC15455 pZmUBI-   |     |    |         |
|         | MtNSP1-FLAG-tAg7- |          |          | MtNSP1            |     |    |         |
|         | 15567             |          |          |                    |     |    |         |
| EC15568 | pL1M-R5-pOsUBI3-  | EC47841  | pL1V-R5  | EC15328 pOsUBI3-  |     |    |         |
|         | MtNSP2-FLAG-tRbeS- |          |          | MtNSP2            |     |    |         |
|         | 15568             |          |          |                    |     |    |         |
| EC15031 | pL1M-R2-pNFBx4-NLS- | EC47811  | pL1V-R2  | EC15059 pNFBx4    |     |    |         |
|         | cyPET-t35S-       |          |          | NLS               |     |    |         |
|         | 15031             |          |          |                    |     |    |         |
| EC15571 | pL1M-R4-pINF-NLS- | EC47831  | pL1V-R4  | EC15249 pINF       |     |    |         |
|         | yPET-tActin2-15571 |          |          | NLS               |     |    |         |

#### L2 plasmids for barley transformation

| ENSA ID | ENSA Standard name | 2i-1 Backbone | position 1 | position 2 | position 3 | position 4 | position 5 | End linker |
|---------|-------------------|---------------|------------|------------|------------|------------|------------|------------|
| EC15027 | pL2V-HYG-15027    | EC50505 p2S-HYG |            |            |            |            |            | EC49255 pELB-1 |
|         |                   | pL2V-1        |            |            |            |            |            |            |
| EC15551 | pL2B-HYG-MtNSP1-  | EC15027 pL2V-HYG |            |            |            |            |            | EC41800 pELE-5 |
|         | FLAG-cyPyP-15551  |               |            |            |            |            |            |            |
| EC15552 | pL2B-HYG-MtNSP2-  | EC15027 pL2V-HYG |            |            |            |            |            | EC41800 pELE-5 |
|         | FLAG-cyPyP-15552  |               |            |            |            |            |            |            |

#### L1 plasmids for Medicago hairy-root transformation

| ENSA ID | ENSA Standard name | Backbone | PU       | S                  | C   | T       |
|---------|-------------------|----------|----------|--------------------|-----|---------|
| EC22522 | pL1M-R2-pLjUBI1-  | EC47811  | pL1V-R2  | EC15251 pLjUBI1   |     | EC41432 |
|         | 3xFLAG-MtNSP1-IOCS- |          |          |                    |     | tocs    |
|         | 22522              |          |          |                    |     |         |
| EC22523 | pL1M-R2-pBdEF1a-  | EC47811  | pL1V-R2  | EC15336 pBdEF1a   |     |         |
|         | 3xMyc-MtNSP2-t35S- |          |          |                    |     |         |
|         | 22523              |          |          |                    |     |         |
| EC43038 | pL1M-R2-pBdEF1a-  | EC47811  | pL1V-R2  | EC15336 pBdEF1a   |     |         |
|         | 3xMyc-MtNSP2-miRR-t35S-43038 |          |          |                    |     |         |
| EC59216 | pL1M-R1-pAtUBI10- | EC47802  | pL1V-R1  | EC15062 pAtUBI10  |     |         |
|         | RUBY-tNOS-59216    |          |          |                    |     |         |

### Notes
- For plasmids marked with "nt", the corresponding KNAB plasmid was used.
- For plasmids marked with "nt2", the corresponding pSOS plasmid was used.
- For plasmids marked with "nt3", the corresponding pCOS plasmid was used.
- For plasmids marked with "nt4", the corresponding pSC plasmid was used.
- For plasmids marked with "nt5", the corresponding pTOS plasmid was used.
- For plasmids marked with "nt6", the corresponding pUBI plasmid was used.
- For plasmids marked with "nt7", the corresponding pActin2 plasmid was used.
- For plasmids marked with "nt8", the corresponding p35S plasmid was used.
- For plasmids marked with "nt9", the corresponding pL0M-S-3xFlag plasmid was used.
- For plasmids marked with "nt10", the corresponding pL0M-C-MtNSP1 plasmid was used.
- For plasmids marked with "nt11", the corresponding pL0M-C-MtNSP2 plasmid was used.
- For plasmids marked with "nt12", the corresponding pL0M-C-MtNSP2-miRR plasmid was used.
- For plasmids marked with "nt13", the corresponding pL0M-C-MtNSP2-miRR plasmid was used.
- For plasmids marked with "nt14", the corresponding pL0M-C-MtNSP2-miRR plasmid was used.
- For plasmids marked with "nt15", the corresponding pL0M-C-MtNSP2-miRR plasmid was used.
- For plasmids marked with "nt16", the corresponding pL0M-C-MtNSP2-miRR plasmid was used.
- For plasmids marked with "nt17", the corresponding pL0M-C-MtNSP2-miRR plasmid was used.
| ENSA ID | ENSA Standard name | 2i-1 Backbone | position 1 | position 2 | End linker |
|---------|--------------------|---------------|------------|------------|------------|
| EC59234 | pL2B-RUBY-EV-59234 | EC50507-L2    | EC59216-R1-pAtUBI10-RUBY-tNOS | -          | EC41722 pELE-1 |
| EC59217 | pL2B-RUBY-pLjUBI-3xFLAG-MtNSP1-59217 | EC50507-L2 | EC59216-R1-pAtUBI10-RUBY-tNOS | EC22522-R2-pLjUBI1-3xFLAG-MtNSP1-tOCS | EC41744 pELE-2 |
| EC59218 | pL2B-RUBY-pBdEF1a-3xMyc-MtNSP2-59218 | EC50507-L2 | EC59216-R1-pAtUBI10-RUBY-tNOS | EC22523-R2-pBdEF1a-3xMyc-MtNSP2-t35S | EC41744 pELE-2 |
| EC59220 | pL2B-RUBY-pBdEF1a-3xMyc-MtNSP2-miRR-59220 | EC50507-L2 | EC59216-R1-pAtUBI10-RUBY-tNOS | EC43038-R2-pBdEF1a-3xMyc-MtNSP2-miRR-t35S | EC41744 pELE-2 |
Supplementary Table 4. Primer sequences

**Primer sequences for quantitative PCR**

| Gene          | Primer Forward                      | Primer Reverse                      |
|---------------|------------------------------------|-------------------------------------|
| MtHistone     | ATTCCAAAGCGGCTGCTCA                | CTGCGCTTTTGTGTTTGGAGATG             |
| MtHistone     | CTGCCTCGCTCTCCATTT                 | CACGCTGCTCTCTCCATTT                |
| MtNSP1-qPCR   | GGGATTTGCACCTGGATTC                | GAACTCTTTTATGAGCCTGTT              |
| MtNSP2-qPCR   | CAGCCTGCTCTCCATTT                 | CACGCTGCTCTCTCCATTT                |
| MtUbiquitin   | GCAGATAGACACGCTGGGA                | AAACGTTCCTGGTGTTTGGAGATG            |
| MtD27         | CAGCCTGCTCTCCATTT                 | CACGCTGCTCTCTCCATTT                |
| MtCCD7        | GATGTGGGGGAAGAAGCTATTG             | TCTCAACTTACCAGCAGGAGAGAAAT         |
| MtCCD8        | CAGCCTGCTCTCCATTT                 | CACGCTGCTCTCTCCATTT                |
| MtGGPS        | TGTCCGTCTTCCATCTGTTTTG             | AGTCCAGCTCTAATATGAGGAGAAAT         |
| MtMAX1        | AGGTTCTTTTATGAGCCTGTT             | TCTCAACCTTACCAGCAGGAGAGAAAT        |
| MtMAX1        | CACGCTGCTCTCTCCATTT                | CACGCTGCTCTCTCCATTT                |
| MtPT4         | GACACGAGGCGCTTTCATAGC              | CTCAACTTACCAGCAGGAGAGAAAT         |
| HvADP         | GAGACATCCAGCATCGATCTCC             | CACGCTGCTCTCTCCATTT                |
| HvRSK2        | GACGCTGCTCTCCATTT                 | CACGCTGCTCTCTCCATTT                |
| HvD14         | GTCTCTTTTCTTTCCATTTTTG             | AGTCCAGCTCTAATATGAGGAGAAAT         |
| HvD14La       | GACAGGAGTGGTGTTTGTGAGGAGG          | CACGCTGCTCTCTCCATTT                |
| HvD14Lb       | GACGCTGCTCTCTCCATTT                | CACGCTGCTCTCTCCATTT                |
| HvCYCLOPS     | CACGCTGCTCTCTCCATTT                | CACGCTGCTCTCTCCATTT                |
| HvCYCLOPS     | TCTTGTTCTGGTTTATGAGGAGGAGAGA      | CACGCTGCTCTCTCCATTT                |

**Primer sequences for genotyping of *Medicago Tnt1* mutants**

| Gene          | Primer Forward                      | Primer Reverse                      |
|---------------|------------------------------------|-------------------------------------|
| Tnt-F1        | TCTTTTGTTGATTGATGCAACTTTTGTGG      | TGATACACCCAGATGCGGTAATTAACAAAGA     |
| Tnt-R1        | TGATACACCCAGATGCGGTAATTAACAAAGA   | TCTTTTGTTGATTGATGCAACTTTTGTGG      |
| MtNSP1-NF9220 | GTCTCTTTTCTTTCCATCTTATTGT        | ATGCCATCAATGACCTTCACCT              |
| MtNSP2-NF10950| ATGCCATCAATGACCTTCACCT            | ATGCCATCAATGACCTTCACCT              |
| MtNSP2-LIKE-NF17492 | ATGCCATCAATGACCTTCACCT           | ATGCCATCAATGACCTTCACCT              |
| MtNSP2-LIKE-NF17492-R | ATGCCATCAATGACCTTCACCT           | ATGCCATCAATGACCTTCACCT              |
| CCD7-NF1485   | CGACGAGTATGAGCAGAAGGAGG          | TGATACACCCAGATGCGGTAATTAACAAAGA     |
| CCD7-NF1485-R | TGATACACCCAGATGCGGTAATTAACAAAGA | CGACGAGTATGAGCAGAAGGAGG          |
| CCD8-NF18323 & 11036-F | TGATACACCCAGATGCGGTAATTAACAAAGA | TGATACACCCAGATGCGGTAATTAACAAAGA   |
| CCD8-NF18323 & 11036-R | TGATACACCCAGATGCGGTAATTAACAAAGA | TGATACACCCAGATGCGGTAATTAACAAAGA   |
| D14-NF18262-F | AATAGGGTTTTTATGAGCAGGAGG          | TGATACACCCAGATGCGGTAATTAACAAAGA     |
| D14-NF18262-R | AATAGGGTTTTTATGAGCAGGAGG          | TGATACACCCAGATGCGGTAATTAACAAAGA     |
| D14La-NF13623-F | GACGAGGAGTGGTGTTTGTGAGGAGG        | GACGAGGAGTGGTGTTTGTGAGGAGG        |
| D14La-NF13623-R | GACGAGGAGTGGTGTTTGTGAGGAGG        | GACGAGGAGTGGTGTTTGTGAGGAGG        |
| D14Lb-NF5873-F | TGATACACCCAGATGCGGTAATTAACAAAGA   | TGATACACCCAGATGCGGTAATTAACAAAGA   |
| D14Lb-NF5873-R | TGATACACCCAGATGCGGTAATTAACAAAGA   | TGATACACCCAGATGCGGTAATTAACAAAGA   |

**Primers for genotyping of *Medicago Tnt1* mutants**

| Gene          | Primer Forward                      | Primer Reverse                      |
|---------------|------------------------------------|-------------------------------------|
| D14-NF18262-F | AATAGGGTTTTTATGAGCAGGAGG          | TGATACACCCAGATGCGGTAATTAACAAAGA     |
| D14-NF18262-R | AATAGGGTTTTTATGAGCAGGAGG          | TGATACACCCAGATGCGGTAATTAACAAAGA     |
| D14La-NF13623-F | GACGAGGAGTGGTGTTTGTGAGGAGG        | GACGAGGAGTGGTGTTTGTGAGGAGG        |
| D14La-NF13623-R | GACGAGGAGTGGTGTTTGTGAGGAGG        | GACGAGGAGTGGTGTTTGTGAGGAGG        |
| D14Lb-NF5873-F | TGATACACCCAGATGCGGTAATTAACAAAGA   | TGATACACCCAGATGCGGTAATTAACAAAGA   |
| D14Lb-NF5873-R | TGATACACCCAGATGCGGTAATTAACAAAGA   | TGATACACCCAGATGCGGTAATTAACAAAGA   |
## Primers for semi-quantitative RT-PCR

| Primer  | Sequence          |
|---------|-------------------|
| MtNSP1-RF | GCCACAAATAGCACAAACCAACA |
| MtNSP1-RR  | CGAAATGCACAAACTACTGC |
| MtNSP2-RF | ATGCCATCAATGACCTCCACT |
| MtNSP2-RR  | TATTAACCCACACCCACCTCCTT |
| MtNSP2L-RF | GGCCCAATCTTCGCACTCAG |
| MtNSP2L-RR  | TACAAGTCAAACAGAAGCAGAAA |
| CCD7-RF | CATTCACCAACCCCTCATCTTACA |
| CCD7-RR  | CCCACATACTGCCCTCCATTG |
| CCD8a-RF | GCGTGGGAAAGCTCGTGATG |
| CCD8a-RR  | GTTCTTAGCCCTTCTGATTGTAG |
| D14-RF | CGTAAAGGCTCCGGGCAAAATA |
| D14-RR  | ACTGCACCAAGTACTCCATCCCACAA |
| D14La-RF | GACAGGGACTACTTGGAGGATT |
| D14La-RR  | TATTCACTAGCCAAACAAGCAA |
| D14Lb-RF | TTGCATATTTGTTGGTCAATTTCGT |
| D14Lb-RR  | ATCTGCCTCCATTTCATCAAG |
| MAX2-RF | ATACTACCACAGCCGCCTCACTCC |
| MAX2-RR  | GACGCCTATTCAACGACATCTCTTA |
| SMAX1-RF | AAGGAAATTAGGGGAGGCTGTAT |
| SMAX1-RR  | TGGCGTAGGTGTCACAGGCTT |
### Supplementary Table 5. Generation of barley CRISPR mutants

| Mutants | Guide A                  | Guide B                  | Deletion sites                                      |
|---------|--------------------------|--------------------------|-----------------------------------------------------|
| symrk-1 | `cccatgtgccccgaaggttc`   | `gggccaattcegcgcccatcgg` | a 2 bp deletion at +94-95 bp and a 31 bp deletion from +719 bp to +749 bp |
| ecamk-1 | `ggttttcctatgtgagaaggg` | `gcgatgatggggatgacaggg` | a 4 bp deletion from +166 bp to +169 bp             |
| ecamk-2 | `gtcagatgtgtctggcccagg` | `gcgttgcctctgctatgatgg` | a 1 bp deletion at +58 bp                           |
| cyclops-1| `gagggagtcatgtgagatgg`   | `gcgccgagatggatgatgg`   | a 1 bp deletion at +8 bp and a 35 bp deletion from +113 bp to +147 bp |
| cyclops-2| `gagggagtcatgtgagatgg`   | `gcgccgagatggatgatgg`   | a 4 bp deletion from +8 bp to +11 bp and a 2bp deletion at +114-115 bp |
| ram1-1  | `ggaggagactccgccccctgagg`| `gccggagacacagggggcgg` | a 1 bp insertion at +961 bp                          |
| ram1-2  | `ccacacttacacacacagcacc`| `gtcagtaatacatggatccagg`| a 1 bp insertion at +1013 bp                         |
| nsp1-1  | `gtgctgctgtgctgctgca`    | `gacgccccatgtcctct`     | a 1 bp deletion at +164 bp                           |
| nsp1-2  | `gtcgccgctgctgctgctg`    | `gcgccccatgtcctctct`    | a 4 bp deletion from +286 bp to +289 bp              |
| nsp2-1  | `gtgctgctgctgctgctg`     | `gacgagctgctgctgctg`    | a 314 bp deletion from +48 bp to +361 bp             |
| nsp2-2  | `gtgctgctgctgctgctg`     | `gacgagctgctgctgctg`    | a 3 bp deletion from +38 bp to +40 bp and a 1 bp insertion at +356 bp |
| rlk2-1  | `gcagacactgacagctcag`    | `gtcagacactgacagctcag`  | a 1 bp insertion at +678 bp                          |
| rlk2-2  | `gcagacactgacagctcag`    | `gtcagacactgacagctcag`  | a 1 bp insertion at +628 bp and a 1 bp insertion at +678 bp |
| rlk10-1 | `cccgccgcttcctctgctgctgc`| `ggacctctgctggcgtgagccgg`| a 1 bp insertion at +14 bp                           |
| rlk10-2 | `cccgccgcttcctctgctgctgc`| `ggacctctgctggcgtgagccgg`| a 1 bp insertion at +273 bp                          |
| d14l-1  | `gacatagccctgagcgttgc`   | `tcgttgaggtcctcc`       | a 32 bp deletion from +88 bp to +119 bp              |
| d14l-2  | `aactctctaactcgcc`       | `tctacactgcteggtc`      | a 1 bp insertion at +169 bp                          |