

## Effect of *phyB* and *phyC* loss-of-function mutations on the wheat transcriptome under short and long day photoperiods

Additional file 1. Tables and figures in this additional file:

**Figure S1:** Heading date of *phyB* and *phyC* mutants in SD photoperiods. For each phytochrome gene, data is presented for plants carrying non-functional copies of only the A homeolog (-A), only the B homoeologue (-B) or both A and B alleles combined (null). Experiments were performed in the tetraploid variety 'Kronos' (Genomes AABB).

**Figure S2:** Representative plants of WT, *phyB*-null and *phyC*-null genotypes at eight-weeks of age. SD RNA-seq data was generated from leaf tissue harvested at this stage.

**Figure S3:** Transcript levels (TPM) from RNA-seq data of selected flowering time genes in wild-type, *phyB*-null and *phyC*-null plants grown in SD conditions. *FT-A3* transcripts were not detected in the RNA-seq experiment and are not displayed here. *VRN1* genes are displayed in a separate panel. Error bars represent standard error.

**Figure S4:** MDS plot for all SD and LD RNA-seq samples. LD data consists of two experimental replicates each with four biological replicates of each genotype. SD data consists of one experimental replicate.

**Figure S5:** Number of DE genes between SD and LD samples of each genotype. SD samples were harvested at 8 weeks of age and consisted of 4 biological replicates. LD samples were harvested at 4 weeks of age and consisted of 8 biological replicates.

**Table S1:** Summary of RNA-seq reads and mapping rates from SD samples.

**Table S2 –** Top 10 significant enriched GO terms among genes differentially regulated by *PHYB*, by *PHYC*, and by both *PHYB* and *PHYC* in SD conditions.

**Table S3:** Summary of RNA-seq reads and mapping rates from LD samples.

**Table S4 –** Top 10 significant enriched GO terms among genes differentially regulated by both *PHYB* and *PHYC* under different photoperiod conditions.

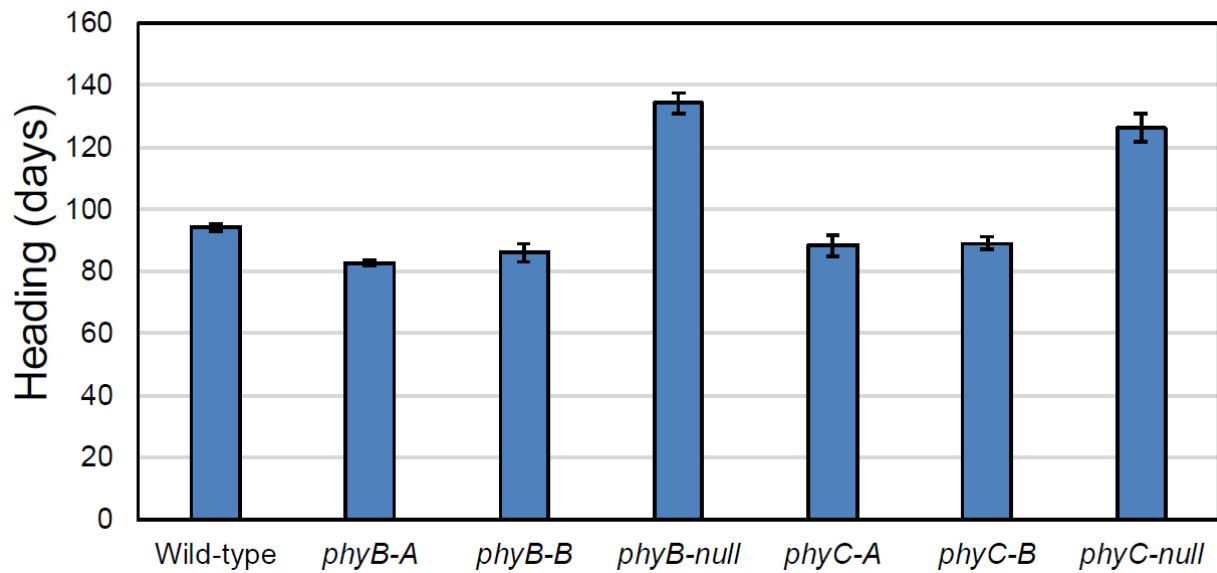
**Table S5:** Selected genes regulated by both *PHYB* and *PHYC* in both SD and LD photoperiods. Fold change between WT and respective *phy*-null mutants are presented. The values for LD are the mean fold-change in expression between both experimental replicates. a - *FT-A1* and *FT-B1* expression was zero in the *phy*-null mutants in these comparisons.

**Table S6:** Selected genes regulated by both *PHYB* and *PHYC* in SD photoperiods but not LD photoperiods. Fold change between WT and respective *phy*-null mutants are presented as the mean fold-change between both experimental replicates. a - *FT-A4* expression was zero in the *phy*-null mutants in these comparisons.

**Table S7:** Selected genes regulated by both *PHYB* and *PHYC* in LD photoperiods but not SD photoperiods. Fold change between WT and respective *phy*-null mutants are presented as the mean fold-change between both experimental replicates.

**Table S8:** *PHYB*- and *PHYC*-regulated splicing-related genes. Mean TPM and fold-change for each genotype was calculated from four biological replicates in SD samples, and eight biological replicates for LD samples.

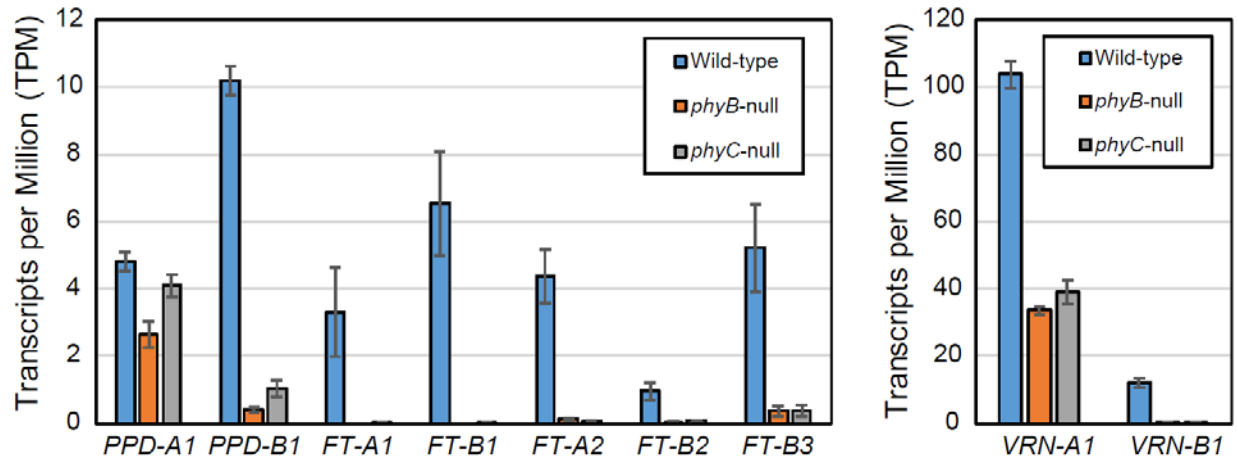
**Figure S1:** Heading date of *phyB* and *phyC* mutants in SD photoperiods. For each phytochrome gene, data is presented for plants carrying non-functional copies of only the A homeolog (-A), only the B homoeologue (-B) or both A and B alleles combined (null). Experiments were performed in the tetraploid variety ‘Kronos’ (Genomes AABB).



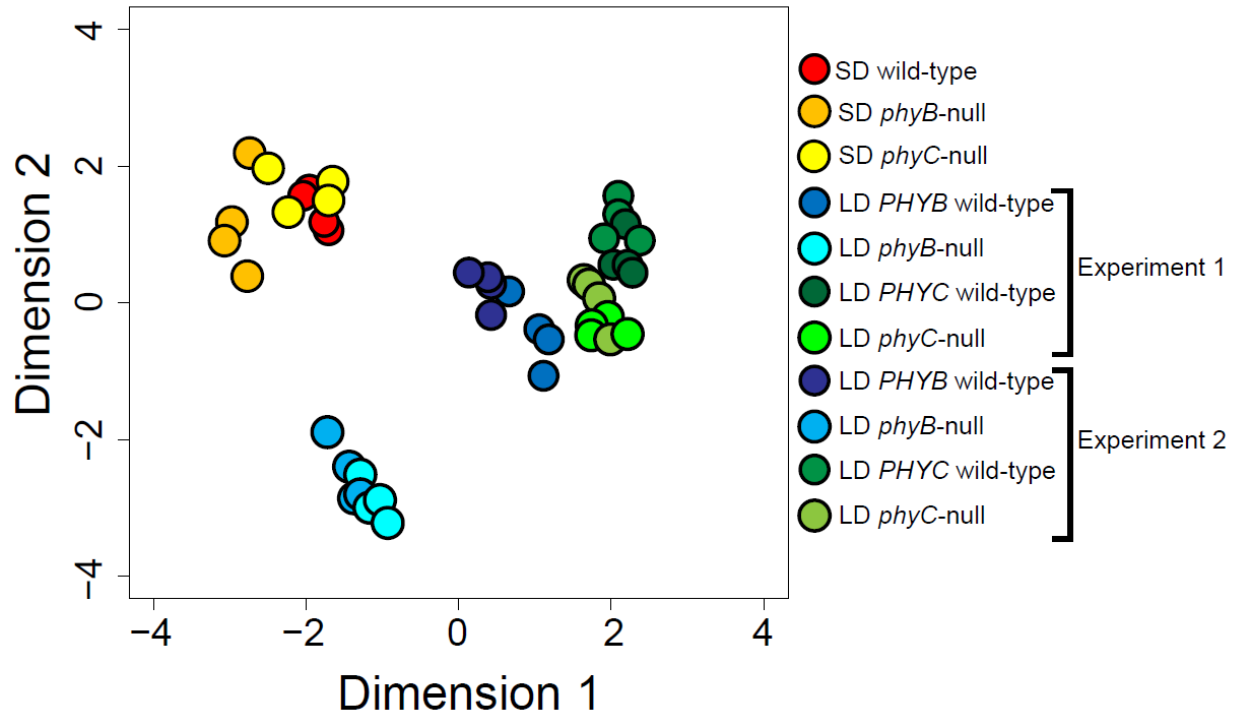
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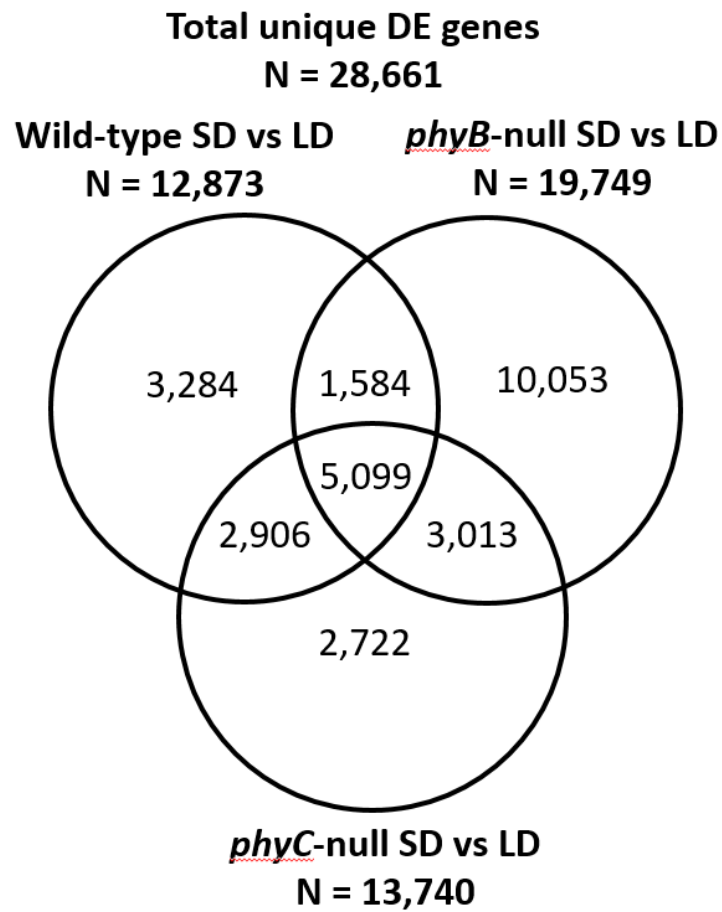
**Figure S3:** Transcript levels (TPM) from RNA-seq data of selected flowering time genes in wild-type, *phyB*-null and *phyC*-null plants grown in SD conditions. *FT-A3* transcripts were not detected in the RNA-seq experiment and are not displayed here. *VRN1* genes are displayed in a separate panel. Error bars represent standard error.



**Figure S4:** MDS plot for all SD and LD RNA-seq samples. SD data consists of four biological replicates of each genotype and one wild-type sample was used as a control for both mutants. LD data consists of two experimental replicates each including four biological replicates of *phyB*-null, *phyC*-null and wild-type sister lines for each respective mutant.



**Figure S5:** Number of DE genes between SD and LD samples of each genotype. SD samples were harvested at 8 weeks of age and consisted of 4 biological replicates. LD samples were harvested at 4 weeks of age and consisted of 8 biological replicates.



**Table S1:** Summary of RNA-seq reads and mapping rates from SD samples.

| Genotype          | Biological replicate | Total raw reads | Reads discarded |     | Total trimmed reads | Uniquely mapped reads |      |
|-------------------|----------------------|-----------------|-----------------|-----|---------------------|-----------------------|------|
|                   |                      |                 | N               | %   |                     | N                     | %    |
| Wild type         | 1                    | 58,502,128      | 1,013,691       | 1.7 | 57,488,437          | 39,607,623            | 68.9 |
|                   | 2                    | 58,830,113      | 1,108,319       | 1.9 | 57,721,794          | 40,219,742            | 69.7 |
|                   | 3                    | 67,577,803      | 1,139,114       | 1.7 | 66,438,689          | 45,702,198            | 68.8 |
|                   | 4                    | 65,430,265      | 1,191,428       | 1.8 | 64,238,837          | 44,160,635            | 68.7 |
| <i>phyB</i> -null | 1                    | 54,007,718      | 837,760         | 1.6 | 53,169,958          | 37,368,473            | 70.3 |
|                   | 2                    | 63,069,027      | 1,079,413       | 1.7 | 61,989,614          | 43,188,697            | 69.7 |
|                   | 3                    | 64,820,390      | 1,047,399       | 1.6 | 63,772,991          | 44,409,244            | 69.6 |
|                   | 4                    | 66,273,908      | 1,174,901       | 1.8 | 65,099,007          | 45,971,041            | 70.6 |
| <i>phyC</i> -null | 1                    | 66,985,324      | 1,317,576       | 2.0 | 65,667,748          | 45,956,376            | 70.0 |
|                   | 2                    | 66,376,725      | 1,289,943       | 1.9 | 65,086,782          | 45,148,294            | 69.4 |
|                   | 3                    | 86,487,404      | 1,503,903       | 1.7 | 84,983,501          | 58,948,262            | 69.4 |
|                   | 4                    | 72,794,612      | 1,384,225       | 1.9 | 71,410,387          | 49,720,482            | 69.6 |
| Mean              |                      | 65,929,618.08   | 1,173,972.67    | 1.8 | 64,755,645          | 45,033,422            | 69.7 |

**Table S2** – Top 10 significant enriched GO terms among genes differentially regulated by *PHYB*, by *PHYC*, and by both *PHYB* and *PHYC* in SD conditions.

| <b><i>PHYB</i>-regulated</b>      |                                       |                  |                    |                 |          |
|-----------------------------------|---------------------------------------|------------------|--------------------|-----------------|----------|
| <b>GO ID</b>                      | <b>Term</b>                           | <b>Annotated</b> | <b>Significant</b> | <b>Expected</b> | <b>P</b> |
| GO:0055114                        | Oxidation-reduction process           | 4,000            | 540                | 396.1           | 5.30E-15 |
| GO:0006468                        | Protein phosphorylation               | 3,908            | 529                | 387.0           | 6.60E-15 |
| GO:0044710                        | Single-organism metabolic process     | 9,737            | 1,161              | 964.1           | 8.50E-15 |
| GO:1903825                        | Organic acid transmembrane trans.     | 244              | 64                 | 24.2            | 2.30E-13 |
| GO:1905039                        | Carboxylic acid transmembrane trans.  | 237              | 61                 | 23.5            | 2.00E-12 |
| GO:0015849                        | Organic acid transport                | 324              | 75                 | 32.1            | 2.10E-12 |
| GO:0009812                        | Flavonoid metabolic process           | 489              | 98                 | 48.4            | 9.80E-12 |
| GO:0046942                        | Carboxylic acid transport             | 317              | 72                 | 31.4            | 1.50E-11 |
| GO:0016310                        | Phosphorylation                       | 5,363            | 669                | 531.0           | 1.70E-11 |
| GO:0044699                        | Single-organism process               | 17,160           | 1,886              | 1699.1          | 2.30E-11 |
| <b><i>PHYC</i>-regulated</b>      |                                       |                  |                    |                 |          |
| GO:0006952                        | Defense response                      | 1,209            | 43                 | 19.0            | 7.70E-07 |
| GO:0042592                        | Homeostatic process                   | 1,321            | 43                 | 20.8            | 7.20E-06 |
| GO:0006468                        | Protein phosphorylation               | 3,908            | 94                 | 61.6            | 2.10E-05 |
| GO:0016310                        | Phosphorylation                       | 5,363            | 121                | 84.5            | 2.20E-05 |
| GO:0016045                        | Detection of bacterium                | 5                | 3                  | 0.1             | 3.80E-05 |
| GO:0098543                        | Detection of other organism           | 5                | 3                  | 0.1             | 3.80E-05 |
| GO:0098581                        | Detection of external biotic stimulus | 8                | 3                  | 0.1             | 0.00021  |
| GO:0065008                        | Regulation of biological quality      | 2,033            | 53                 | 32.0            | 0.00025  |
| GO:0048878                        | Chemical homeostasis                  | 621              | 22                 | 9.8             | 0.00041  |
| GO:0006879                        | Cellular iron ion homeostasis         | 42               | 5                  | 0.7             | 0.0005   |
| <b><i>PHYB/PHYC</i>-regulated</b> |                                       |                  |                    |                 |          |
| GO:0006952                        | Defense response                      | 1,209            | 30                 | 11.1            | 9.10E-07 |
| GO:0006468                        | Protein phosphorylation               | 3,908            | 62                 | 35.7            | 1.10E-05 |
| GO:0006879                        | Cellular iron ion homeostasis         | 42               | 5                  | 0.4             | 4.00E-05 |
| GO:0016310                        | Phosphorylation                       | 5,363            | 76                 | 49.0            | 5.10E-05 |
| GO:0055072                        | Iron ion homeostasis                  | 92               | 6                  | 0.8             | 0.00021  |
| GO:0050896                        | Response to stimulus                  | 7,059            | 90                 | 64.5            | 0.0004   |
| GO:0042592                        | Homeostatic process                   | 1,321            | 25                 | 12.1            | 0.00054  |
| GO:0006950                        | Response to stress                    | 3,927            | 54                 | 35.9            | 0.00153  |
| GO:0048872                        | Homeostasis of number of cells        | 7                | 2                  | 0.1             | 0.0017   |
| GO:0009812                        | Flavonoid metabolic process           | 489              | 12                 | 4.5             | 0.00199  |

**Table S3:** Summary of RNA-seq reads and mapping rates from LD samples.

| Genotype  | Exp. Rep. | Bio. Rep. | Raw reads  | Reads discarded |      | Trimmed reads | Uniquely mapped reads |      |
|-----------|-----------|-----------|------------|-----------------|------|---------------|-----------------------|------|
|           |           |           |            | N               | %    |               | N                     | %    |
| PHYB-WT   | 1         | 1         | 38,193,836 | 2,291,251       | 6.0  | 35,902,585    | 18,977,319            | 52.9 |
|           |           | 2         | 37,228,077 | 2,236,373       | 6.0  | 34,991,704    | 18,615,718            | 53.2 |
|           |           | 3         | 50,793,083 | 2,966,158       | 5.8  | 47,826,925    | 25,410,013            | 53.1 |
|           |           | 4         | 44,301,543 | 2,715,483       | 6.1  | 41,586,060    | 21,258,178            | 51.1 |
|           | 2         | 1         | 53,475,278 | 3,983,069       | 7.5  | 49,492,209    | 26,092,448            | 52.7 |
|           |           | 2         | 54,275,607 | 3,972,114       | 7.3  | 50,303,493    | 26,657,660            | 53.0 |
|           |           | 3         | 57,095,077 | 4,313,269       | 7.6  | 52,781,808    | 27,415,963            | 51.9 |
|           |           | 4         | 59,581,947 | 4,279,495       | 7.1  | 55,302,452    | 29,303,043            | 53.0 |
| phyB-null | 1         | 1         | 43,374,525 | 2,540,401       | 5.9  | 40,834,124    | 21,739,116            | 53.2 |
|           |           | 2         | 47,201,620 | 2,731,653       | 5.8  | 44,469,967    | 23,537,045            | 52.9 |
|           |           | 3         | 81,109,422 | 4,903,617       | 6.0  | 76,205,805    | 40,552,227            | 53.2 |
|           |           | 4         | 47,250,183 | 2,769,089       | 5.9  | 44,481,094    | 23,788,301            | 53.5 |
|           | 2         | 1         | 47,550,092 | 3,517,065       | 7.4  | 44,033,027    | 23,533,579            | 53.5 |
|           |           | 2         | 50,880,434 | 3,783,852       | 7.4  | 47,096,582    | 25,126,305            | 53.4 |
|           |           | 3         | 56,371,576 | 4,144,097       | 7.4  | 52,227,479    | 27,938,705            | 53.5 |
|           |           | 4         | 51,113,770 | 3,679,790       | 7.2  | 47,433,980    | 25,437,698            | 53.6 |
| PHYC-WT   | 1         | 1         | 40,263,724 | 4,609,655       | 11.5 | 35,654,069    | 18,918,318            | 53.1 |
|           |           | 2         | 40,409,065 | 4,363,202       | 10.8 | 36,045,863    | 20,341,146            | 56.4 |
|           |           | 3         | 42,605,342 | 4,775,194       | 11.2 | 37,830,148    | 20,222,651            | 53.5 |
|           |           | 4         | 39,796,668 | 4,597,431       | 11.6 | 35,199,237    | 18,893,499            | 53.7 |
|           | 2         | 1         | 37,994,979 | 3,339,800       | 8.8  | 34,655,179    | 18,184,337            | 52.5 |
|           |           | 2         | 43,262,009 | 3,678,319       | 8.5  | 39,583,690    | 21,115,165            | 53.3 |
|           |           | 3         | 41,619,486 | 3,613,203       | 8.7  | 38,006,283    | 20,202,760            | 53.2 |
|           |           | 4         | 31,887,538 | 2,770,104       | 8.7  | 29,117,434    | 15,386,865            | 52.8 |
| phyC-null | 1         | 1         | 44,904,496 | 4,989,736       | 11.1 | 39,914,760    | 21,705,064            | 54.4 |
|           |           | 2         | 46,776,974 | 5,163,917       | 11.0 | 41,613,057    | 22,393,932            | 53.8 |
|           |           | 3         | 43,960,996 | 4,795,476       | 10.9 | 39,165,520    | 21,053,322            | 53.8 |
|           |           | 4         | 42,873,364 | 4,595,730       | 10.7 | 38,277,634    | 20,718,521            | 54.1 |
|           | 2         | 1         | 35,291,647 | 2,998,278       | 8.5  | 32,293,369    | 17,279,830            | 53.5 |
|           |           | 2         | 36,712,107 | 3,016,466       | 8.2  | 33,695,641    | 17,747,367            | 52.7 |
|           |           | 3         | 45,656,274 | 3,815,739       | 8.4  | 41,840,535    | 22,551,769            | 53.9 |
|           |           | 4         | 38,922,327 | 3,391,092       | 8.7  | 35,531,235    | 13,034,303            | 36.7 |
| Mean      |           |           | 46,022,908 | 3,729,379       | 8.2  | 42,293,530    | 22,347,880            | 52.8 |

**Table S4** – Top 10 significant enriched GO terms among genes differentially regulated by both *PHYB* and *PHYC* under different photoperiod conditions.

| <b><i>PHYB-PHYC</i> regulated – SD specific</b> |                                                                |                  |                    |                 |          |
|-------------------------------------------------|----------------------------------------------------------------|------------------|--------------------|-----------------|----------|
| <b>GO ID</b>                                    | <b>Term</b>                                                    | <b>Annotated</b> | <b>Significant</b> | <b>Expected</b> | <b>P</b> |
| GO:0006468                                      | Protein phosphorylation                                        | 5,201            | 88                 | 41.65           | 5.10E-12 |
| GO:0016310                                      | Phosphorylation                                                | 7,036            | 105                | 56.35           | 4.60E-11 |
| GO:0019725                                      | Cellular homeostasis                                           | 845              | 23                 | 6.77            | 4.50E-07 |
| GO:0006952                                      | Defense response                                               | 1,563            | 33                 | 12.52           | 5.10E-07 |
| GO:0042592                                      | Homeostatic process                                            | 1,597            | 33                 | 12.79           | 8.10E-07 |
| GO:0006796                                      | Phosphate-containing compound metabolic process                | 9,005            | 109                | 72.12           | 2.00E-06 |
| GO:0006793                                      | Phosphorus metabolic process                                   | 9,117            | 109                | 73.02           | 3.60E-06 |
| GO:0045454                                      | Cell redox homeostasis                                         | 356              | 13                 | 2.85            | 7.20E-06 |
| GO:0006464                                      | Cellular protein modification process                          | 8,493            | 99                 | 68.02           | 3.60E-05 |
| GO:0036211                                      | Protein modification process                                   | 8,493            | 99                 | 68.02           | 3.60E-05 |
| <b><i>PHYB-PHYC</i> regulated – LD specific</b> |                                                                |                  |                    |                 |          |
| GO:0048586                                      | Regulation of LD photoperiodism, flowering                     | 30               | 2                  | 0.02            | 0.00015  |
| GO:0042752                                      | Regulation of circadian rhythm                                 | 31               | 2                  | 0.02            | 0.00016  |
| GO:2000028                                      | Regulation of photoperiodism, flowering                        | 41               | 2                  | 0.02            | 0.00029  |
| GO:2000243                                      | Positive regulation of reproductive process                    | 42               | 2                  | 0.03            | 0.0003   |
| GO:0048574                                      | LD photoperiodism, flowering                                   | 43               | 2                  | 0.03            | 0.00032  |
| GO:0005982                                      | Starch metabolic process                                       | 224              | 3                  | 0.14            | 0.00034  |
| GO:0048571                                      | LD photoperiodism                                              | 45               | 2                  | 0.03            | 0.00035  |
| GO:0010218                                      | Response to far red light                                      | 52               | 2                  | 0.03            | 0.00046  |
| GO:0007623                                      | Circadian rhythm                                               | 53               | 2                  | 0.03            | 0.00048  |
| GO:0048511                                      | Rhythmic process                                               | 57               | 2                  | 0.03            | 0.00056  |
| <b><i>PHYB-PHYC</i> regulated – SD and LD</b>   |                                                                |                  |                    |                 |          |
| GO:0050789                                      | Regulation of biological process                               | 10,086           | 17                 | 5.9             | 3.20E-06 |
| GO:0009889                                      | Regulation of biosynthetic process                             | 5,573            | 12                 | 3.26            | 2.40E-05 |
| GO:0065007                                      | Biological regulation                                          | 11,745           | 17                 | 6.88            | 2.90E-05 |
| GO:0006355                                      | Regulation of transcription, DNA-templated                     | 5,066            | 11                 | 2.97            | 5.80E-05 |
| GO:0080090                                      | Regulation of primary metabolic process                        | 6,135            | 12                 | 3.59            | 6.40E-05 |
| GO:1903506                                      | Regulation of nucleic acid-templated transcription             | 5,113            | 11                 | 2.99            | 6.40E-05 |
| GO:2001141                                      | Regulation of RNA biosynthetic process                         | 5,113            | 11                 | 2.99            | 6.40E-05 |
| GO:0051252                                      | Regulation of RNA metabolic process                            | 5,162            | 11                 | 3.02            | 7.00E-05 |
| GO:0019219                                      | Regulation of nucleobase-containing compound metabolic process | 5,317            | 11                 | 3.11            | 9.10E-05 |
| GO:0048573                                      | Photoperiodism, flowering                                      | 157              | 3                  | 0.09            | 0.00011  |

**Table S5:** Selected genes regulated by both *PHYB* and *PHYC* in both SD and LD photoperiods. Fold change between WT and respective *phy*-null mutants are presented. The values for LD are the mean fold-change in expression between both experimental replicates. a - *FT-A1* and *FT-B1* expression was zero in the *phy*-null mutants in these comparisons.

| Gene ID            | Annotation        | Fold change in expression |                       |                       |                       |
|--------------------|-------------------|---------------------------|-----------------------|-----------------------|-----------------------|
|                    |                   | Short days                |                       | Long days             |                       |
|                    |                   | WT/ <i>phyB</i> -null     | WT/ <i>phyC</i> -null | WT/ <i>phyB</i> -null | WT/ <i>phyC</i> -null |
| TraesCS7A01G115400 | <i>FT-A1</i>      | - <sup>a</sup>            | 168.88                | - <sup>a</sup>        | - <sup>a</sup>        |
| TraesCS7B01G013100 | <i>FT-B1</i>      | - <sup>a</sup>            | 286.72                | 927.23                | 481.46                |
| TraesCS3A01G143100 | <i>FT-A2</i>      | 38.02                     | 78.31                 | 119.44                | 16.64                 |
| TraesCS1B01G351100 | <i>FT-B3</i>      | 14.44                     | 14.18                 | 3.56                  | 7.52                  |
| TraesCS5A01G391700 | <i>VRN-A1</i>     | 3.09                      | 2.66                  | 15.64                 | 7.96                  |
| TraesCS5B01G396600 | <i>VRN-B1</i>     | 70.61                     | 52.50                 | 113.15                | 73.89                 |
| TraesCS2A01G261200 | <i>FUL-A2</i>     | 92.75                     | 644.50                | 800.56                | 127.00                |
| TraesCS2A01G174300 | <i>FUL-A3</i>     | 351.75                    | 369.78                | 740.74                | -                     |
| TraesCSU01G196100  | <i>PPD-B1</i>     | 25.37                     | 9.95                  | 322.38                | 35.17                 |
| TraesCS6A01G273200 | MYB               | 21.12                     | 8.30                  | 43.09                 | 2.89                  |
| TraesCS6B01G300600 | MYB               | 7.99                      | 5.11                  | 4.05                  | 3.63                  |
| TraesCS1A01G220300 | CONSTANS-like     | 3.36                      | 2.90                  | 3.82                  | 2.48                  |
| TraesCS3B01G365300 | VQ-motif protein  | 0.19                      | 0.31                  | 0.01                  | 0.08                  |
| TraesCS5A01G520200 | <i>TaFLC-A2</i>   | 0.35                      | 0.23                  | 0.12                  | 0.18                  |
| TraesCS4B01G351500 | <i>TaFLC-B2</i>   | 0.18                      | 0.11                  | 0.01                  | 0.02                  |
| TraesCS3A01G435000 | <i>TaFLC-A4-1</i> | 0.22                      | 0.06                  | 0.14                  | 0.12                  |
| TraesCS2A01G427200 | <i>WCOR15</i>     | 0.03                      | 0.11                  | 0.01                  | 0.05                  |

**Table S6:** Selected genes regulated by both *PHYB* and *PHYC* in SD photoperiods but not LD photoperiods. Fold change between WT and respective *phy*-null mutants are presented as the mean fold-change between both experimental replicates. a - *FT-A4* expression was zero in the *phy*-null mutants in these comparisons.

| Gene ID            | Annotation      | Fold change in short days |                       |
|--------------------|-----------------|---------------------------|-----------------------|
|                    |                 | WT/ <i>phyB</i> -null     | WT/ <i>phyC</i> -null |
| TraesCS3B01G162000 | <i>FT-B2</i>    | 24.89                     | 12.53                 |
| TraesCS2A01G132300 | <i>FT-A4</i>    | - <sup>a</sup>            | 6.80                  |
| TraesCS7B01G158900 | <i>TaFLC-B1</i> | 12.18                     | 3.66                  |
| TraesCS2B01G378700 | NF-YB           | 2.70                      | 3.07                  |
| TraesCS7A01G233300 | MYB_related     | 4.26                      | 9.05                  |
| TraesCS7B01G131600 | MYB_related     | 14.02                     | 16.90                 |
| TraesCS5B01G054800 | bHLH            | 5.55                      | 2.59                  |
| TraesCS5B01G183700 | WRKY            | 7.80                      | 5.16                  |
| TraesCS3B01G129900 | WRKY            | 13.83                     | 6.20                  |
| TraesCS3B01G240200 | WRKY            | 17.62                     | 6.85                  |
| TraesCS7B01G249700 | WRKY            | 123.51                    | 205.05                |
| TraesCS3B01G199000 | WRKY            | 2.38                      | 2.94                  |
| TraesCS5B01G183800 | WRKY            | 4.22                      | 2.92                  |
| TraesCS6A01G146900 | WRKY            | 4.96                      | 2.58                  |
| TraesCS1B01G374900 | WRKY            | 5.07                      | 2.47                  |
| TraesCS3A01G347500 | WRKY            | 5.51                      | 2.87                  |
| TraesCS1A01G334400 | GA-2oxidase     | 0.05                      | 0.13                  |
| TraesCS6A01G143900 | BBox            | 0.06                      | 0.17                  |
| TraesCS2A01G100600 | G2-like         | 0.22                      | 0.40                  |
| TraesCS3A01G274900 | GATA            | 0.55                      | 0.46                  |
| TraesCS1B01G335100 | CBF             | 0.16                      | 0.17                  |
| TraesCS5A01G311100 | CBF             | 0.19                      | 0.37                  |
| TraesCS5A01G310800 | CBF             | 0.47                      | 0.18                  |
| TraesCS5A01G310700 | CBF             | 0.52                      | 0.33                  |

**Table S7:** Selected genes regulated by both *PHYB* and *PHYC* in LD photoperiods but not SD photoperiods. Fold change between WT and respective *phy*-null mutants are presented as the mean fold-change between both experimental replicates.

| Gene ID            | Annotation        | Fold change in long days |                       |
|--------------------|-------------------|--------------------------|-----------------------|
|                    |                   | WT/ <i>phyB</i> -null    | WT/ <i>phyC</i> -null |
| TraesCS3A01G116300 | <i>GIGANTEA-A</i> | 2.35                     | 3.21                  |
| TraesCS3B01G135400 | <i>GIGANTEA-B</i> | 2.59                     | 3.99                  |
| TraesCS6B01G315400 | CO-like           | 0.42                     | 0.23                  |
| TraesCS7B01G115200 | SPL               | 0.24                     | 0.25                  |
| TraesCS3A01G107200 | <i>TZF-A1</i>     | 0.09                     | 0.18                  |

**Table S8:** *PHYB*- and *PHYC*-regulated splicing-related genes. Mean TPM and fold-change for each genotype was calculated from four biological replicates in SD samples, and eight biological replicates for LD samples.

| <i>PHYB</i> regulated under SD  |                                                            | TPM          |                      | Fold-change |
|---------------------------------|------------------------------------------------------------|--------------|----------------------|-------------|
| Gene ID                         | Annotation                                                 | Wild-type SD | <i>phyB</i> -null SD |             |
| TraesCS3B01G157400              | Pre-mRNA-splicing factor of RES complex protein            | 6.4          | 3.4                  | 1.9         |
| TraesCS1B01G125800              | Pre-mRNA-splicing factor <i>cwc26</i>                      | 10.0         | 2.3                  | 4.3         |
| TraesCS7B01G194600              | Pre-mRNA-splicing factor <i>SLU7</i>                       | 6.6          | 3.3                  | 2.0         |
| TraesCS5B01G017300              | Splicing factor 3B subunit 1                               | 0.2          | 0.9                  | 0.2         |
| TraesCS7B01G450300LC            | Splicing factor 3B subunit 4                               | 0.0          | 2.0                  | -           |
| TraesCS2B01G720700LC            | Splicing factor U2AF small subunit A                       | 0.1          | 3.2                  | 0.0         |
| TraesCS4B01G209600              | SR-rich pre-mRNA splicing activator                        | 4.4          | 1.7                  | 2.6         |
| TraesCS4A01G094900              | SR-rich pre-mRNA splicing activator                        | 25.2         | 4.9                  | 5.1         |
| TraesCS2A01G122400              | Splicing factor U2AF, large subunit                        | 3.0          | 1.3                  | 2.3         |
| TraesCS7A01G075100              | Splicing factor U2AF large subunit A                       | 0.2          | 0.0                  | 17.9        |
| <i>PHYC</i> -regulated under SD |                                                            | TPM          |                      | Fold-change |
| Gene ID                         | Annotation                                                 | Wild-type SD | <i>phyC</i> -null SD |             |
| TraesCS1B01G125800              | Pre-mRNA-splicing factor <i>cwc26</i>                      | 10.0         | 2.2                  | 4.6         |
| TraesCS2A01G646800LC            | Pre-mRNA-processing-splicing factor 8                      | 0.1          | 1.1                  | 0.1         |
| TraesCS1B01G416400              | Pre-mRNA-processing-splicing factor 8                      | 3.1          | 0.2                  | 15.6        |
| TraesCS1B01G130200              | Arginine/serine-rich splicing factor                       | 0.2          | 1.8                  | 0.1         |
| TraesCS5B01G017300              | Splicing factor 3B subunit 1                               | 0.2          | 0.6                  | 0.3         |
| TraesCS7B01G450300LC            | Splicing factor 3B subunit 4                               | 0.0          | 2.7                  | -           |
| TraesCS7B01G384700LC            | Splicing factor family-like                                | 0.5          | 0.0                  | -           |
| TraesCS5A01G366000              | Splicing factor U2AF large subunit                         | 0.0          | 0.2                  | 0.2         |
| TraesCS2B01G720700LC            | Splicing factor U2AF small subunit A                       | 0.1          | 2.1                  | 0.0         |
| <i>PHYB</i> -regulated under LD |                                                            | TPM          |                      | Fold-change |
| Gene ID                         | Annotation                                                 | Wild-type LD | <i>phyB</i> -null LD |             |
| TraesCS2A01G122400              | Splicing factor U2AF, large subunit                        | 3.5          | 1.5                  | 2.3         |
| TraesCS3A01G086900              | Splicing factor                                            | 5.8          | 9.8                  | 0.6         |
| TraesCS4A01G399500              | Pre-mRNA-splicing factor <i>CWC22</i> -like protein        | 3.0          | 9.1                  | 0.3         |
| TraesCS3A01G426500              | Lysine ketoglutarate reductase trans-splicing-like protein | 14.2         | 7.0                  | 2.0         |
| TraesCS5A01G189600              | Pre-mRNA-processing-splicing factor 8                      | 0.9          | 1.6                  | 0.6         |
| TraesCS3B01G293100              | Arginine/serine-rich splicing factor                       | 9.3          | 11.6                 | 0.8         |
| TraesCS3A01G260000              | Arginine/serine-rich splicing factor                       | 11.7         | 18.4                 | 0.6         |

|                    |                                      |      |      |     |
|--------------------|--------------------------------------|------|------|-----|
| TraesCS4A01G091300 | RNA-binding protein                  | 37.6 | 53.9 | 0.7 |
| TraesCS1B01G244200 | Splicing factor-like protein         | 4.3  | 6.5  | 0.7 |
| TraesCS5A01G267500 | Splicing factor U2AF small subunit A | 19.9 | 34.7 | 0.6 |

| <b><i>PHYC</i>-regulated under LD</b> |                                      | <b>TPM</b>          |                            | <b>Fold-change</b> |
|---------------------------------------|--------------------------------------|---------------------|----------------------------|--------------------|
| <b>Gene ID</b>                        | <b>Annotation</b>                    | <b>Wild-type LD</b> | <b><i>phyC</i>-null LD</b> |                    |
| TraesCS1B01G130200                    | Arginine/serine-rich splicing factor | 0.1                 | 0.5                        | 0.2                |
| TraesCS1B01G615200LC                  | Splicing factor 3B subunit 5         | 2.1                 | 11.7                       | 0.2                |