

## SUPPLEMENTARY INFORMATION

**Table 1.** Protein alignment of B73 RefGen\_v3 [15] with RWS. Sequenced region is shaded in grey, and remaining gaps were assumed to match reference sequence.

Highlighted sequences indicate polymorphisms.

|       |                                                               |     |
|-------|---------------------------------------------------------------|-----|
| B73   | MGTKVNIIVGSHVWAEDPDTCWVDGEVVKINGEEAEIQATNGKKIVANLSKLYPKDMEAA  | 60  |
| RWS   | MGTKVNIIVGSHVWAEDPDTCWVDGEVVKINGEEAEIQATNGKKIVANLSKLYPKDMEAA  | 60  |
| ***** |                                                               |     |
| B73   | AGGVDDMTKLSYLPHEPGVLQNLAIRYELNEIYTYTGNILIAVNPQRLPHLYDPHMMHQY  | 120 |
| RWS   | AGGVDDMTKLSYLPHEPGVLQNLAIRYELNEIYTYTGNILIAVNPQRLPHLYDPHMMHQY  | 120 |
| ***** |                                                               |     |
| B73   | KGAPFGELSPHVFAVADVAYRAMVNENKSNAILVSGESGAGKTETTKMLMRYLAYLGGRA  | 180 |
| RWS   | KGAPFGELSPHVFAVADVAYRAMVNENKSNAILVSGESGAGKTETTKMLMRYLAYLGGRA  | 180 |
| ***** |                                                               |     |
| B73   | ATEGRTVEQQVLESNPVLEAFGNAKTVRNNNSSRFGKFVEIQFDKHGRISGAAIRTYLLE  | 240 |
| RWS   | ATEGRTVEQQVLESNPVLEAFGNAKTVRNNNSSRFGKFVEIQFDKHGRISGAAIRTYLLE  | 240 |
| ***** |                                                               |     |
| B73   | RSRVCQVSDPERNYHCFYLLCAAPQEDVDKYKLGPNKTFHYLNQSNCYELVGVSDAHEYL  | 300 |
| RWS   | RSRVCQVSDPERNYHCFYLLCAAPQEDVDKYKLGPNKTFHYLNQSNCYELVGVSDAHEYL  | 300 |
| ***** |                                                               |     |
| B73   | ATTRAMDIVGISTQEQDAIFRVVAAILHIGNIEFSKGKEADSSVLKDEKSKFHLETTAEL  | 360 |
| RWS   | ATTRAMDIVGISTQEQDAIFRVVAAILHIGNIEFSKGKEADSSVLKDEKSKFHLETTAEL  | 360 |
| ***** |                                                               |     |
| B73   | LMCNPGALEDALCKRVMVTPEEVIKRSOLDPYNATISRDGLAKTIYSRLFDWLVDKINSSI | 420 |
| RWS   | LMCNPGALEDALCKRVMVTPEEVIKRSOLDPYNATISRDGLAKTIYSRLFDWLVDKINSSI | 420 |
| ***** |                                                               |     |
| B73   | GQDASSKCLIGVLDIYGFESFKANSFEQFCINYTNEKLQQHFNQHVFKMEQEEYTKQID   | 480 |
| RWS   | GQDASSKCLIGVLDIYGFESFKANSFEQFCINYTNEKLQQHFNQHVFKMEQEEYTKQID   | 480 |
| ***** |                                                               |     |
| B73   | WSYIEFVDNQDVLDLIEKKPGGVIALLDACMFPKSTHETFAQKLYQTFQKHKRFVVKPKL  | 540 |
| RWS   | WSYIEFVDNQDVLDLIEKKPGGVIALLDACMFPKSTHETFAQKLYQTFQKHKRFVVKPKL  | 540 |
| ***** |                                                               |     |
| B73   | SRTDFTICHYAGEVLYQSDQFLDKNKDYVVAEHQELLSASKCSFISGLFPPPEETSKSS   | 600 |
| RWS   | SRTDFTICHYAGEVLYQSDQFLDKNKDYVVAEHQELLSASKCSFISGLFPPPEETSKSS   | 600 |
| ***** |                                                               |     |
| B73   | KFSSIGARFKQQLQALMDTLNSTEPHYIRCVKPNNVLKPAIFENVNVMQQLRCCGVLEAI  | 660 |
| RWS   | KFSSIGARFKQQLQALMDTLNSTEPHYIRCVKPNNVLKPAIFENVNVMQQLRCCGVLEAI  | 660 |

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B73 RISCAGYPTRRTFYEFLHRFGILAPEALEGNSDEKAACKRILEKKGLLGFQIGKTKVFLR 720  
RWS RISCAGYPTRRTFYEFLHRFGILAPEALEGNSDEKAAACKRILEKKGLLGFQIGKTKVFLR 720

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B73 AGQMAELDARRTEVLSAAAKTIQGKMRTHIMRKKFLSLRKASVCVQAIWRGRLACKLYD 779  
RWS AGQMAELDARRTEVLSAAAKTIQGKMRTHIMRKKFLSLRKASVCVQAIWRGGRLACKLYD 780

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B73 NMRREAAAIKVQKNQRRHQARRSYKLRYASVLVVQTALRAMAARNEFRFKKQSTGAVTIQ 839  
RWS NMRREAAAIKVQKNQRRHQARRSYKLRYASVLVVQTALRAMAARNEFRFKKQSTGAVTIQ 840

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B73 ARYRCYRAHKYHKKLKCAAIVAQCRWRGRIARKELKLLKMEARETGALKEAKDKLEKKVE 899  
RWS ARYRCYRAHKYHKKLKCAAIVAQCRWRGRIERTNSKRKGR----- 881

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B73 ELTWVRVQLEKRLRTDLEEAK--AQEVSKLQNSMEALQAKLDETNTKLAKEREAAKTIEE 956  
RWS TPWRAVR-KRLRDRPGRSKSSRGVETAELYGSITG----- 915

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B73 APPVVQETQVLVQDTEKIDSLTAEVQDLKTSLQSEKERAGDLEKKHSEEQQANEEKQKKL 1016  
RWS ----- 915

B73 DETEIKMRQFQDYLRRLEEKLANVESENKVLRQQAVSMAPSKILSGRSKSNLQRNSENVO 1076  
RWS ----- 915

B73 VSSNDPKTAPESNSTSSPKKEYDIDDKPQKSLNEKQQENQDLLIRCIQHLGYAGNRPVA 1136  
RWS ----- 915

B73 ACIIYKCLLHWRSEFEVERTSVFDRIIQTIGHAIETQDNNEVLAYWLSNASTLLLLLQRTL 1196  
RWS ----- 915

B73 KASGSTGMAPQRRRSSATLFGRMTQSFRGAPQGVNLSLINGSMTGVTGVLTRQVEAKYPA 1256  
RWS ----- 915

B73 LLFKQQLTAYVEKIYGMIRDNLKKEISPLLGLCIQAPRTSRASLMKGSSRSNTNTAAQQA 1316  
RWS ----- 915

B73 LIAHWQGIVKSLGNFLNILKVNNVPPFLVRKVFTQIFSFINVQLFNSLLLRRECCSFSNG 1376  
RWS ----- 915

B73 EYVKAGLAELEHWCYRATDEYAGSAWDELKHIRQAIGFLVIHQPKKTLDEISHDLCPVL 1436  
RWS ----- 915

B73 SIQQLYRISTMYWDDKYGTHSVSPEVISNMRVLMTEDSNNPISNSFLLDDDDSSIPFSVDD 1496  
RWS ----- 915

B73 ISKSMQQIDISDIEPPPLIRENSGFVFLPPPE 1529  
RWS ----- 915

**Table S2.** Sequence information for RNAi targets and primers

| Name         | Sequence                                                                                                     | Product Size (nt) | Purpose     |
|--------------|--------------------------------------------------------------------------------------------------------------|-------------------|-------------|
| MEP_F        | TGTACTCGGCAATGCTCTTG                                                                                         | 203               | qPCR primer |
| MEP_R        | TTTGATGCTCCAGGCTTACC                                                                                         |                   |             |
| MYO_F        | TGACCCAGAGCGCAATTAC                                                                                          | 145               | qPCR primer |
| MYO_R        | ATCACTTACACCGACCAACTC                                                                                        |                   |             |
| MYO18_F      | TTTCCTAGGACTAGTGTGTGCTTGAGGC                                                                                 | 96                | RNAi primer |
| MYO18_R      | CATCAG<br>TTTGTATACCCCGGGCCTCAGGCGCAAG<br>TATTCC                                                             |                   |             |
| MYO16_F      | TTTCCTAGGACTAGTCAAAGACTACGTT                                                                                 | 126               | RNAi primer |
| MYO16_R      | GTGGCGG<br>TTTGTATACCCCGGGGCTCCAATGGAAG<br>AGAACTTG                                                          |                   |             |
| MYO18 Target | GTGTGCTTGAGGCCATCAGGATCAGTTG<br>TGCCGGGTACCCGACGCGTCGCACATTC<br>TACGAGTTTCTGCATCGTTTCGGAATAC<br>TTGCGCCTGAGG |                   |             |
| MYO16 Target | CAAAGACTACGTTGTGGCGGAGCACCA<br>GGAAGTCTGAGCGCTTCTAAATGCTCG<br>TTTATCTCCGGATTGTTTCCGCCTCCGCC                  |                   |             |

AGAGGAGACGTCCAAATCGTCCAAGTT  
CTCTCCATTGGAGC

**Table 3.** Primers used for SSR marker analysis and the presence or absence of QTL regions. Relevant information provided here is based on B73 RefGen\_v4 [30].

| Marker Name | QTL          | Bin  | Product Length (bp) | Anneal Temp (°C) | Sequence                                                        |      | Starting Coordinates | Relevant    | Gene          |
|-------------|--------------|------|---------------------|------------------|-----------------------------------------------------------------|------|----------------------|-------------|---------------|
| 1.04_682414 | <i>qhir1</i> | 1.04 | 150-200             | 57               | Fwd: TTGTTCTTGCATCCATCCAG<br>CATGCACTTGCCGTTGTACT               | Rev: | 69429443             | within      |               |
| GSS_44      | <i>qhir1</i> | 1.04 | 150-200             | 57               | Fwd: AATGTGGCATGTACGAGGTG<br>AGGGGTTAGGCGGATCAAT                | Rev: | 69445286             | downstream  | GRMZM2G471240 |
| umc1040     | <i>qhir8</i> | 9.01 | 100-200             | 57               | Fwd: CATTCACTCTCTTGCCAACTTGA<br>Rev: AGTAAGAGTGGGATATTCTGGGAGTT |      | 4446555              | overlapping | GRMZM2G124276 |
| bnlg1272    | <i>qhir8</i> | 9.01 | 250                 | 57               | Fwd: ACCGAAGATGAGGTGTGACA<br>TCAGTGCAAGGGCAATTTAG               | Rev: | 85302                | downstream  | GRMZM2G310569 |

**Figure 1.** Correlations between relative expression (fold change) of *MYO* transgenic events and a) survival rates after spraying a herbicidal treatment for transgene selection (data for B73 x <sup>*MYO*</sup>F<sub>1</sub> crosses), or b) haploid induction rates (% haploids found for *qhir1,MYO*BC<sub>2</sub>F<sub>1</sub>). .