

Supplemental Table S 1. Oligonucleotides used for PCR amplification and site-specific mutation of GLYK genes from maize (*ZmGLYK*), rice (*OsGLYK*) and Arabidopsis (*AtGLYK*). Restriction sites used for cloning are underlined.

| Enzyme variant | Direction | Sequence |
|----------------------------------|-----------|---|
| ZmGLYK | Forward | 5'- <u>AGATCTT</u> CCTCGTCGTCGTCGTCGACC-3' |
| | Reverse | 5'- <u>GGTACCT</u> CACAAACATGAGATGTC-3' |
| ZmGLYK ^{C428S} | Forward | 5'- <u>AGATCTT</u> CCTCGTCGTCGTCGTCGACC-3' |
| | Reverse | 5'- <u>GGTACCT</u> CACAA AGATGAGATGTC-3' |
| ZmGLYK ^{Δ423-429I} | Forward | 5'- <u>AGATCTT</u> CCTCGTCGTCGTCGTCGACC-3' |
| | Reverse | 5'- <u>GGTACCT</u> CATCCACCACAGATAGGGTT-3' |
| ZmGLYK ^{C420S} | Forward | 5'- <u>AGATCTT</u> CCTCGTCGTCGTCGTCGACC-3' |
| | Reverse | 5'- <u>GGTACCT</u> CACAAACATGAGATGTCCCATGATCCACCAGAGATAG-3' |
| ZmGLYK ^{C420S,428S} | Forward | 5'- <u>AGATCT</u> CCTCGTCGTCGTCGTCGACC-3' |
| | Reverse | 5'- <u>GGTACCT</u> CACAAAGATGAGATGTCCCATGATCCACCAGAGATAG-3' |
| ZmGLYK ^{C420S,Δ423-429} | Forward | 5'- <u>AGATCTT</u> CCTCGTCGTCGTCGTCGACC-3' |
| | Reverse | 5'- <u>GGTACCT</u> CATCCACCAGAGATAGGGTTCTATTTTC-3' |
| ZmGLYK ^{C96S} | Forward | 5'-CTGGTGGACAGGATCGGCTAC-3' |
| | Reverse | 5'-CGGGCCGCAGGAGATGAAGTC-3' |

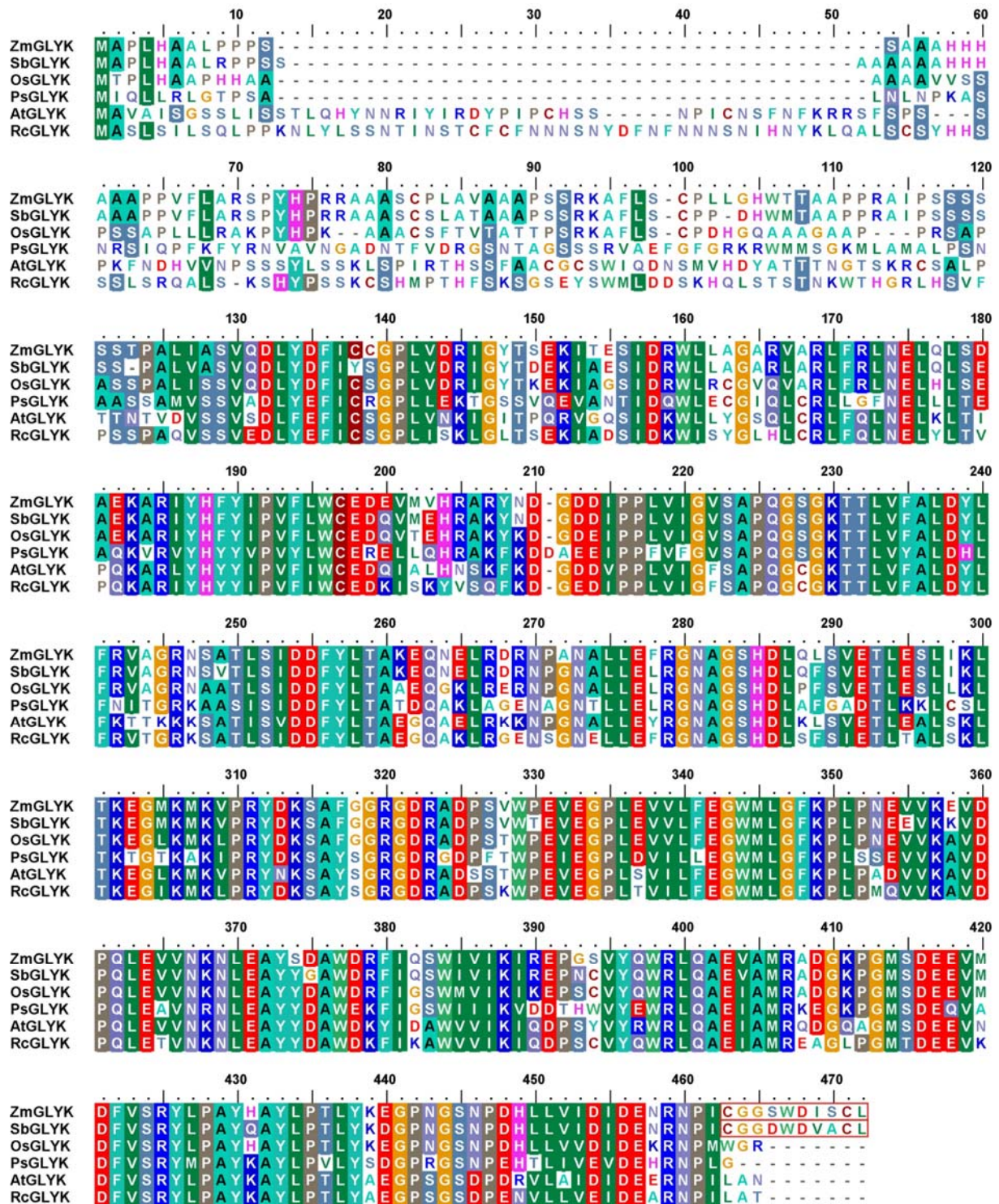
Supplemental Table S1 (continued).

| Enzyme variant | Direction | Sequence |
|--------------------------------|-----------|--|
| ZmGLYK ^{C96,97S} | Forward | 5'-CTGGTGGACAGGATCGGCTAC-3' |
| | Reverse | 5'-CGGGCCGGAGGAGATGAAGTC-3' |
| ZmGLYK ^{C155S} | Forward | 5'-GTCATGGTGCACAGAGCCAGG-3' |
| | Reverse | 5'-CTCGTCCTCGGACCAGAGGAAG-3' |
| OsGLYK | Forward | 5'- <u>CTCGAGATCTCCTCCGTCCAGGAC</u> -3' |
| | Reverse | 5'- <u>GGTACCTCATCTACCCACATAGGATTCCTC</u> -3' |
| OsGLYK ^{ZmGLYK-Cterm} | Forward | 5'- <u>CTCGAGATCTCCTCCGTCCAGGAC</u> -3' |
| | Reverse | 5'- <u>GGTACCTCACAAACATGAGATGTCCCATGATCCACCACAGATAGGATTCCTCTTTTCGTCTAT</u> -3' |
| AtGLYK | Forward | 5'- <u>CTCGAGACAGTGGATGTCTCTTCGGTGTCAG</u> -3' |
| | Reverse | 5'- <u>CTCGAGTTAGTTTGCGAGTATCGGGTTCCT</u> -3' |
| AtGLYK ^{ZmGLYK-Cterm} | Forward | 5'- <u>CTCGAGACAGTGGATGTCTCTTCGGTGTCAG</u> -3' |
| | Reverse | 5'- <u>CTCGAGTCACAAACATGAGATGTCCCATGATCCACCACATATCGGGTTCCTTTCTTCATCGAT</u> -3' |
| ZmTrx <i>f1</i> | Forward | 5'- <u>CATATGTCCAGCGGGATCGAGACGAGC</u> -3' |
| | Reverse | 5'- <u>GGATCCTCAGTCTGACTTGACTGTGTTCGATGGC</u> -3' |
| ZmTrx <i>f2</i> | Forward | 5'- <u>CATATGTCGAGCGGGATCGAGACGAGC</u> -3' |
| | Reverse | 5'- <u>GGATCCTCAGCCTGACTTCTTGTTGGCTATCTCG</u> -3' |

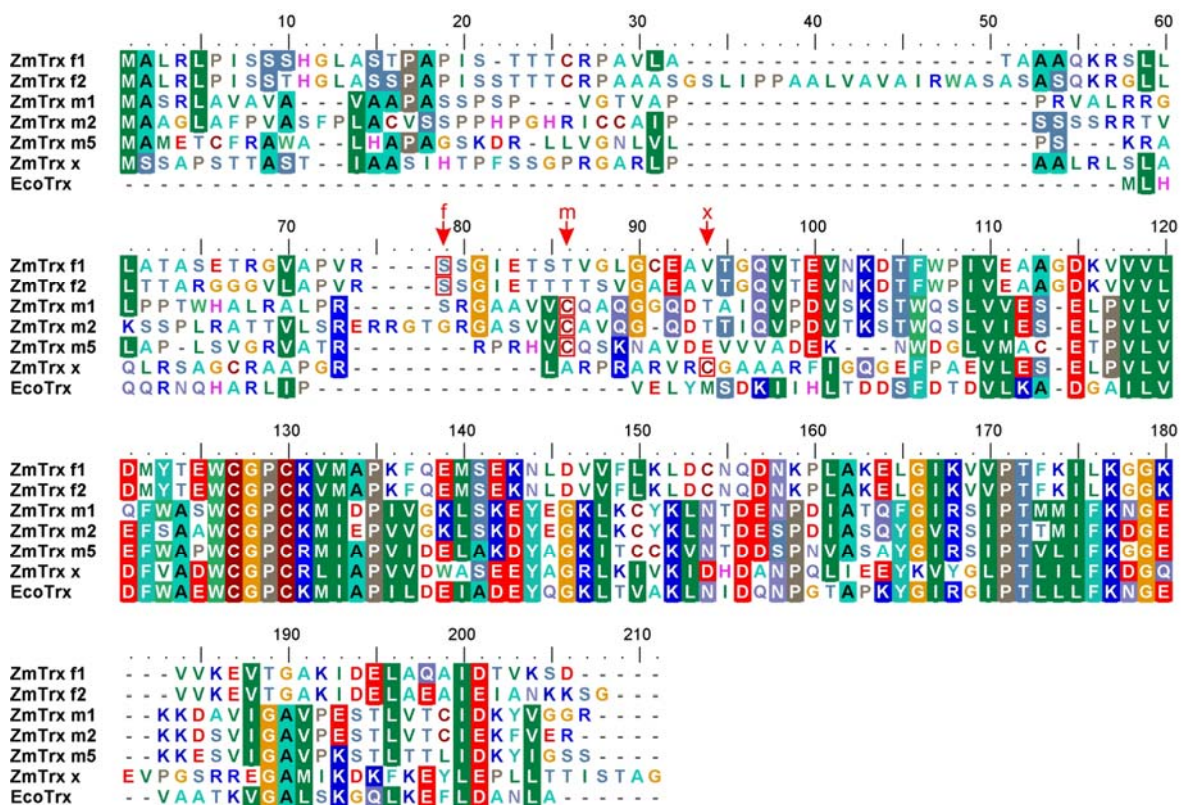
Supplemental Table S1 (continued).

| Enzyme variant | Direction | Sequence |
|-----------------|-----------|---|
| ZmTrx <i>m1</i> | Forward | 5'- <u>CATATGT</u> GCCAGGCCAGGGCGG-3' |
| | Reverse | 5'- <u>GGATCCT</u> CACCTCCCACCAACGTA CT TGT CG-3' |
| ZmTrx <i>m2</i> | Forward | 5'- <u>CATATGT</u> GCGCTGTCCAAGGCCAGG-3' |
| | Reverse | 5'- <u>GGATCC</u> CTACCTCTCGACAAATTTCTCGATGCATG-3' |
| ZmTrx <i>m5</i> | Forward | 5'- <u>CATATGC</u> AGTCCAAGAACGCAGTG-3' |
| | Reverse | 5'- <u>GGATCCT</u> CATGAACTACCGATGTACTTG-3' |
| ZmTrx <i>x</i> | Forward | 5'- <u>CATATGT</u> GCGGCGCGGCGGCCA-3' |
| | Reverse | 5'- <u>GGATCCT</u> TAAACCGGCTGTTGAAATTGTAGTCAACAGAGG-3' |

Supplemental Figure S 1. Alignment of GLYK protein sequences from *Zea mays* (ZmGLYK), *Sorghum bicolor* (SbGLYK), *Oryza sativa* (OsGLYK), *Picea sitchensis* (PsGLYK), *Arabidopsis thaliana* (AtGLYK) and *Ricinus communis* (RcGLYK). Accession numbers are: ZmGLYK, NP_001141904.1; SbGLYK, XP_002439104); OsGLYK, NP_001043886; PsGLYK, ABR16413; AtGLYK, NP_849912; RcGLYK, XP_002517532.



Supplemental Figure S 2. Amino acid alignment of the examined *Zea mays* chloroplast thioredoxins and of *E. coli* thioredoxin. Cleavage sites are indicated by arrows (first amino acid of native mature maize thioredoxins boxed). Nomenclature of maize thioredoxins follows the nomenclature of Chibani et al. (2009) for plant thioredoxins. Accession numbers are: ZmTrx *f1*, NP_001150158, LOC100283787; ZmTrx *f2*, ACF79373, LOC100273021; ZmTrx *m1*, NP_001150752, LOC100284385; ZmTrx *m2*, NP_001150752, LOC100284385; ZmTrx *m5*, NP_001105330, PCO147105; ZmTrx *x*, ACG33853, LOC100280852; EcoTrx, AAA67582.



Supplemental Figure S 3. Ribbon model of ZmGLYK (*Zea mays*) generated with the PHYRE server (Kelley and Sternberg, 2009) using the ScGLYK (*Saccharomyces cerevisiae*) primary and crystal structures (SCOP code: d1odfa). The model encompasses ZmGLYK amino acids 151 to 419, which are homologous to ScGLYK. The regulatory C-terminal amino acids 420-429 from ZmGLYK are missing but should be adjacent to the C-terminus at Ile⁴¹⁹. Note the close vicinity of the C-terminus to the ATP-binding P-loop.

