```
OsHYD1 : MATGLSGGAMTSFAVKKPLLAAAVRRRSWPPPSGRALPFSPLTRTPRSRG..LGTVTCFVPQGTESQQAPAPSPPPTVPV
ZmHYD3 : .....MAAAMTSFVAKNPLLAAAARRR.APPLAGRALPFSPLASTRAPRR....TVTCFVPQDTAAPAAPVPA.......
ZmHYD4 : MAAGLSGAAMTSFVAKNPLLAAAARRRALPPLAGRALPFSPLTTARAPRRRGLGTVTCFVPQDTEHPAAAAPAP..VAPV
OsHYD2 : .....MAVARLVVITPAVLLGRTARVSPSAVP...........RLRPIVAGRRAV...AAPTRAVLGDGAGVGG......
OsHYD3 : .....MAVARLVAARAPLLSPAAVAAAHRSPP...........ALLRLAFAPLPARRLAVPLRVAVGEP...........
ZmHYD5 : .....MAVARLVSAPFPLAPLRVRAPRPALPPGAHAGP..RPPVLALAPPAASAAPRRAVPARAAPEDG...........
ZmHYD6 : .....MAVARLVAAPFPLATCRLRRPRPALPP.AHAGPGPRPQVLVLAPPAATAA.RRAVPVRAAPEDEAVVAG......

                                              TMH1                                  TMH2
OsHYD1 : PVPSLEEEAAAAAARRIAERKARKLSERRTYLVAAVMSSLGFTSMAVAAVYYRFHWQLEGGD..VPMTEMFGTFALSVGA
ZmHYD3 : ....LDEEARAAAARRVAEKEARKRSERRTYLVAAVMSSLGVTSMAVAAVYYRFSWQMEGGE..VPVIETLGTFALSVGA
ZmHYD4 : PETALDEEARAAAARRVAERKARKRSERRTYLVAAVMSSLGVTSMAVAAVYYRFSWQMEGGA..VPVSEMFGTFALSVGA
OsHYD2 : ...EEDAVVAVVEEDAVARRAARKRSERRTYLVAAVMSSLGFTSMAAAAVYYRFAWQMEAGGGDVPATEMVGTFALSVGA
OsHYD3 : ...E....PEEDARRAVAERAARKQSERRTYLVAAVMSSLGITSMAAAAVYYRFAWQMEVGG.EIPVTEMFGTFALSVGA
ZmHYD5 : .........GRGDAAAAAARAARKQSERRTYLVAAVMSSLGITSMAAAAVYYRFAWQMEGGG.AIPVTEMVGTFALSVGA
ZmHYD6 : ...D....GGGGDAEAVAARAARKQSERRTYLVAAVMSSLGITSMAAAAVYYRFAWQMEGGG.EIPVTEMVGTFALSVGA

                                                       TMH3                   TMH4
OsHYD1 : AVGMEFWAQWAHRSLWHASLWHMHESHHRAR.EGPFELNDVFAITNAVPAISLLAYGFFHRGIVPGLCFGAGLGITLFGM
ZmHYD3 : AVGMEFWARWAHRALWHASLWHMHESHHRPR.EGPFELNDVFAIVNAAPAISLLAYGFFHRGIVPGLCFGAGLGITLFGM
ZmHYD4 : AVGMEFWARWAHRALWHASLWHMHESHHRPR.EGPFELNDVFAIVNAVPAISLLAYGFFHRGLVPGLCFGAGLGITLFGM
OsHYD2 : AVGMEFWARWAHRALWHASLWHMHESHHRPR.DGPFELNDVFAIANAAPAISLLAYGLLNRGLLPGLCFGAGLGITLFGM
OsHYD3 : AVGMEFWARWAHRALWHASLWHMHESHHRPR.DGPFELNDVFAITNAVPAMSLLAYGFFTRGLVPGLCFGAGLGITLFGM
ZmHYD5 : AVGMEFWARWAPRALWHASLWHMHESHHRARDDGPFELNDVFAIVNAVPAMSLLAYGFFNRGLVPGLCFGAGLGITLFGM
ZmHYD6 : AVGMEFWARWAHRALWHASLWHMHQSHHRPR.DGPFELNDVFAIVNAVPAMSLLAYGFFNRGLVPGLCFGAGLGITLFGM
                             HXXXXH HXXHH

OsHYD1 : AYMFVHDGLVHRRFPVGPIANVPYFRRVAAAHKIHHTDKFEGVPYGLFLGPKELEEVGGLEELEKELARINRSL......
ZmHYD3 : AYMFVHDGLVHRRFPVGPIADVPYFRRVAASHKIHHMDKFGGVPYGLFLGPKELEEVGGLDELVSSPVSEATDTEDAGEE
ZmHYD4 : AYMFVHDGLVHRRFPVGPIANVPYFRRVAAAHKIHHMDKFEGVPYGLFLGPKELEEVGGLDELEKELARIGRTI......
OsHYD2 : AYMFVHDGLVHRRFPVGPIENVPYFRRVAAAHQIHHTDKFEGVPYGLFLGPKELEEVGGTEELDKEIKKRIKRKEAMDAI
OsHYD3 : AYMFVHDGLVHRRFPVGPIANVPYFRRVAAAHQIHHMDKFEGVPYGLFLGPKELEEVGGIEELEKEIKRRIKRKETLDAI
ZmHYD5 : AYMFVHDGLVHRRFPVGPIENVPYFRRVAAAHQIHHMDKFQGVPYGLFLGPKELKEVGGTEELEKEIKRRIRRREALDAT
ZmHYD6 : AYMFVHDGLVHRRFPVGPIENVPYFRRVAAAHQIHHMDKFQGVPYGLFLGPKELKEVGGTEELEKEIKKRIRRREALDAI
              HXXXXH                   HXXHH

OsHYD1 : .......................
ZmHYD3 : KTRPVVCVVRTSVFMGQSVPNEF
ZmHYD4 : .......................
OsHYD2 : R......................
OsHYD3 : Q......................
ZmHYD5 : Q......................
ZmHYD6 : Q......................
```

Figure S1. Conserved motifs in HYD proteins of maize and rice. Multiple sequence alignment of HYD protein sequences from maize (Zm; *Zea mays*) and rice (Os; *Oryza sativa*) indicates variable N-termini harboring the chloroplast transit peptide (light blue shaded box), four transmembrane helices (TMH1-4) and four histidine motifs necessary for enzymatic function (consensus sequences indicated by a thin overline).

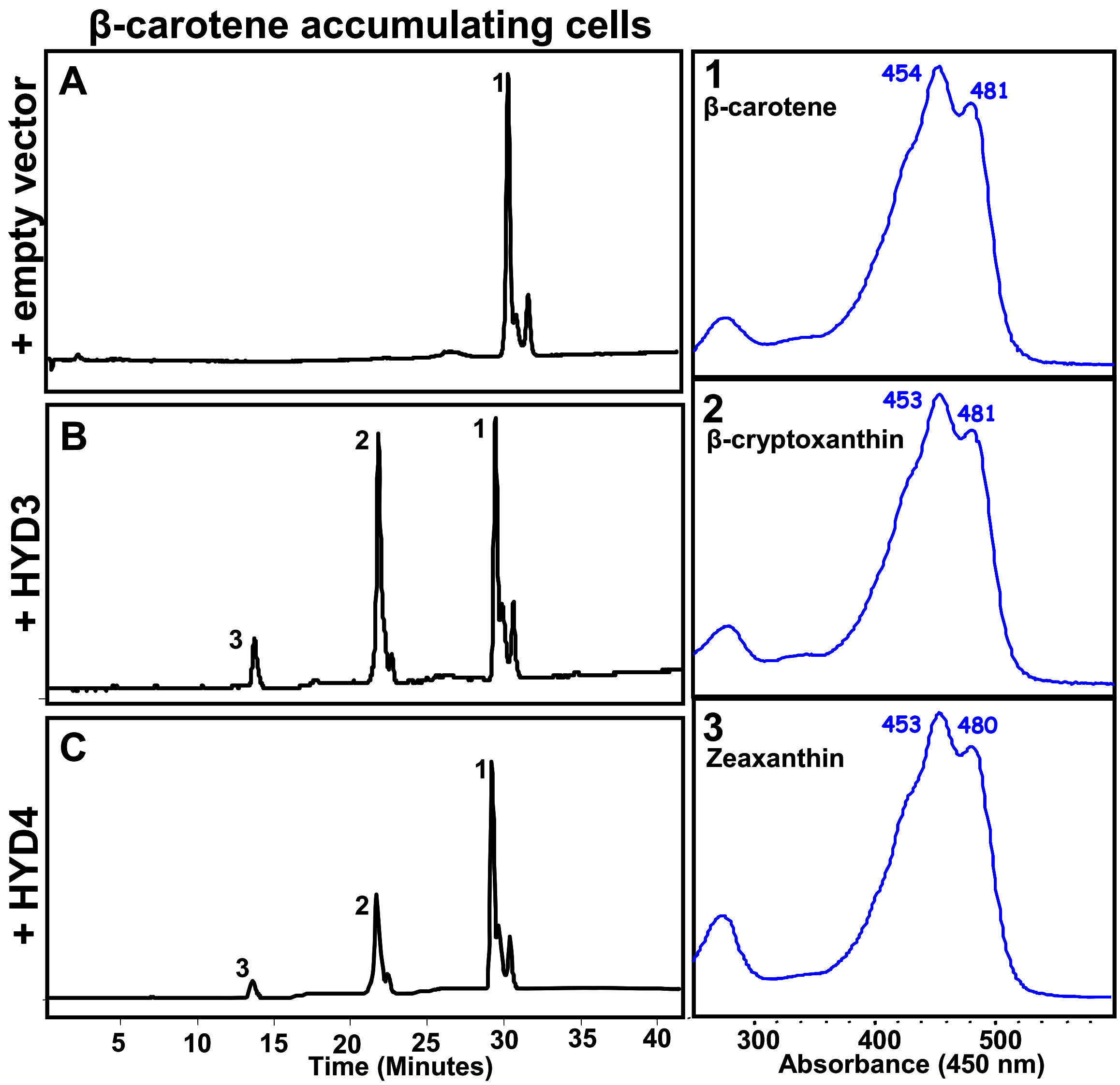
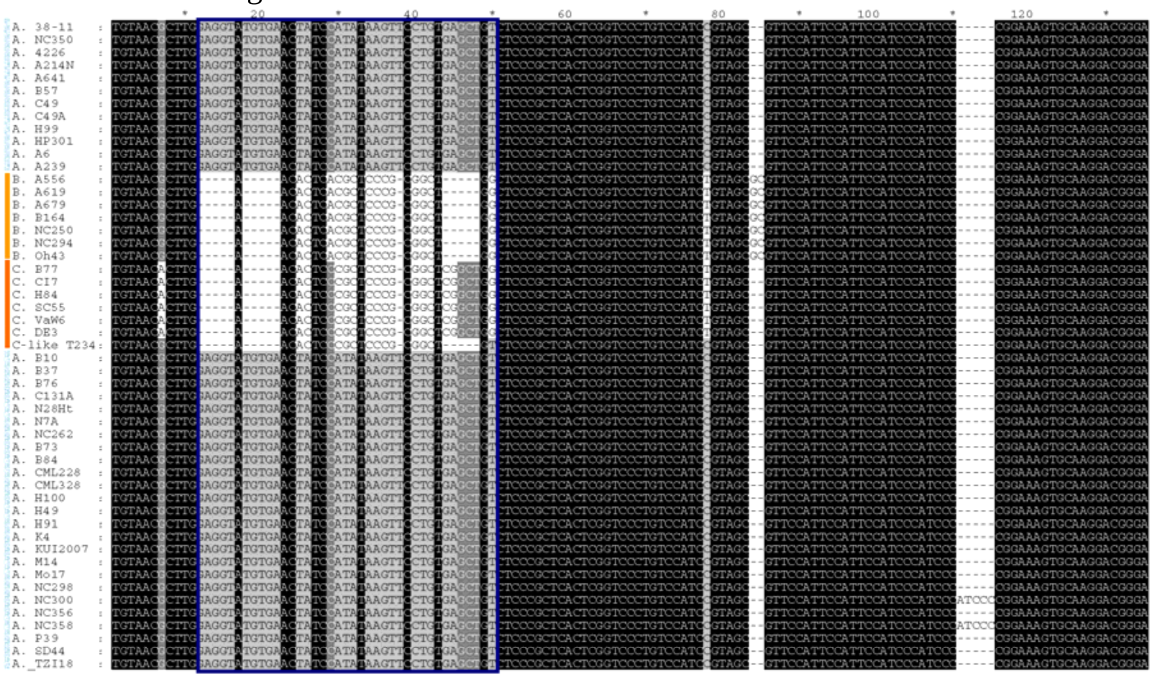


Figure S2. Functional complementation of *HYD* genes. Control cells carrying pAC-BETA-04 which contains genes encoding enzymes needed for β-carotene production (Sun et al., 1996) and an empty vector (pET23c) accumulated the β-carotene substrate (Fig. S2A). Alternatively, when β-carotene – accumulating cells were transformed with the *HYD3* (Fig. S2B) or *HYD4* (Fig. S2C) expression constructs, additional peaks were observed that corresponded to enzyme intermediate, β-cryptoxanthin, and enzyme product, zeaxanthin. This observed hydroxylase activity was similar to that seen for the other class of β-ring hydroxylase, the P450 CYP97A enzyme, which also hydroxylates β-rings, in contrast to CYP97C which was shown to hydroxylate ε-rings (Quinlan et al., 2007). **Left,** HPLC chromatograms, at 450 nm of extracted pigments from *E. coli* transformed with pAC-BETA-04 and (**A)** pET23C (empty vector), showing accumulated β-carotene (peak 1); or (**B)** pTHYD3 encoding HYD3, showing β-carotene plus β-cryptoxanthin and zeaxanthin (peaks 1-3, respectively); or (**C**) pTHYD4 encoding HYD4, showing β-carotene plus β-cryptoxanthin and zeaxanthin (peaks 1-3, respectively). **Right,** UV spectra of peaks 1-3 shown in the chromatograms on the left.

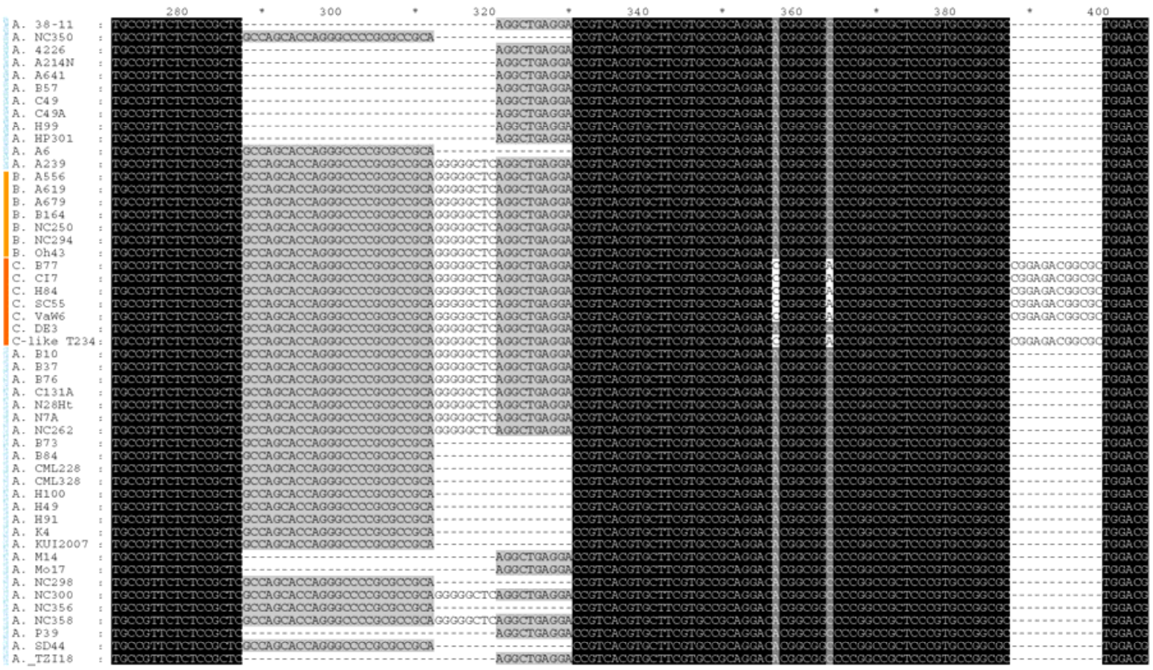
Figure S3. Alignment of maize *HYD3* in 51 lines. Variant 5' region was amplified by PCR primers P1/P2 (Fig. 7). Allele and corresponding inbred are denoted on left of each sequence. Colors are as in Fig 6.

①



②

③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A. C49A
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A. HP301
A. A6
A. A239
B. A556
B. A619
B. A679
B. B164
B. NC250
B. NC294
B. Oh43
C. B77
C. CI7
C. H84
C. SC55
C. VaW6
C. DE3
C-like T234
A. B10
A. B37
A. B76
A. C131A
A. N28Ht
A. N7A
A. NC262
A. B73
A. B84
A. CML228
A. CML328
A. H100
A. H49
A. H91
A. K4
A. KUI2007
A. M14
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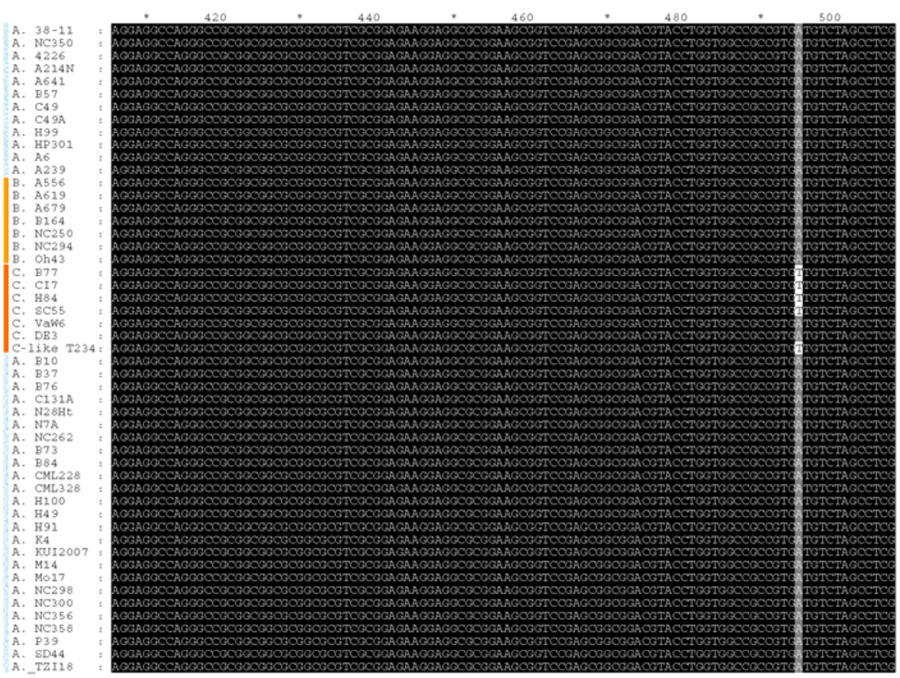
④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460
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500