

Supplementary Table S2. Total number of Amps (frequency > 0.3%) detected for each T0 line and their T1 and T2 offspring lines from the hexaploid THA53 genotype. We also represented the number of CRISPR Amps, non-targeted WT Amps, and putative targeted WT Amps per line. The number of Amps for the WT has been calculated as the mean of all WT lines.

| T0 | T1 | T2 | Cas9 +/- | Total Amps/line ^a | CRISPR Amps ^b | Non-targeted WT Amps ^c | Putative targeted WT Amps ^d |
|-----|------|------|----------|------------------------------|--------------------------|-----------------------------------|--|
| | WT | | NA | 47 | NA | NA | NA |
| P06 | - | - | Cas9 + | 47 | 0 | 47 | 0 |
| | T450 | - | Cas9 + | 49 | 2 | 47 | 0 |
| | | V791 | Cas9 + | 49 | 2 | 47 | 0 |
| P20 | - | - | Cas9 + | 47 | 2 | 45 | 2 |
| | V464 | - | Cas9 - | 50 | 3 | 47 | 0 |
| | V465 | - | Cas9 + | 50 | 3 | 47 | 0 |
| | V467 | - | Cas9 + | 46 | 1 | 45 | 2 |
| | V468 | - | Cas9 - | 37 | 1 | 36 | 11 |

T0: generation 0; T1: generation 1; T2: generation 2; WT: wild type; Cas +/-: presence or absence of Cas9 coding sequence; NA: not applicable; Amps: unique denoised amplicons.

^a Amps with frequencies > 0.3%. A more restrictive threshold was applied for considering putative targeted WT Amps in lines with no CRISPR Amps present.

^b Number of new generation Amps present in CRISPR lines and absent in WT lines.

^c WT Amps detected in CRISPR lines.

^d Number of WT Amps not detected in CRISPR lines and considered as putative targeted Amps.