

Table S5. Phenotypic segregation in BC₄S_{2:3} and BC₄S_{2:4} populations containing the 90-kb target genomic introgression.

| Population | Traits | <i>Pal9</i> -/- | <i>Pal9</i> +/- | <i>Pal9</i> +/+ | A | D | R ² | P value |
|----------------------------------|---------------|-----------------|-----------------|-----------------|-------|-------|----------------|----------|
| BC ₄ S _{2:3} | C16:0 (mg/g) | 5.37 ± 0.52 | 6.19 ± 0.69 | 6.52 ± 0.48 | 0.53 | 0.14 | 32.5 | 2.81E-10 |
| | C16:0/ALL (%) | 13.86 ± 0.51 | 15.34 ± 0.78 | 17.06 ± 0.79 | 1.66 | -0.06 | 69.3 | 1.85E-29 |
| | SFA/ALL (%) | 16.21 ± 0.56 | 17.72 ± 0.85 | 19.33 ± 0.93 | 1.59 | -0.03 | 63.6 | 2.52E-25 |
| | UFA/ALL (%) | 82.15 ± 0.63 | 80.65 ± 0.92 | 78.94 ± 1.06 | -1.66 | 0.05 | 60.3 | 3.51E-23 |
| | SFA/UFA (%) | 19.73 ± 0.14 | 21.97 ± 0.13 | 24.49 ± 1.01 | 2.03 | -0.06 | 62.6 | 1.24E-24 |
| BC ₄ S _{2:4} | C16:0 (mg/g) | 6.00 ± 0.62 | 6.70 ± 0.59 | 7.36 ± 0.54 | 0.61 | 0.02 | 41.3 | 7.08E-13 |
| | C16:0/ALL (%) | 13.52 ± 0.52 | 14.61 ± 0.61 | 16.27 ± 0.52 | 1.53 | -0.17 | 77.4 | 1.35E-34 |
| | SFA/ALL (%) | 15.65 ± 0.56 | 16.83 ± 0.55 | 18.36 ± 0.54 | 1.45 | -0.11 | 76.7 | 6.11E-34 |
| | UFA/ALL (%) | 82.76 ± 0.83 | 81.58 ± 1.23 | 80.17 ± 0.58 | -1.35 | 0.07 | 48.8 | 5.21E-16 |
| | SFA/UFA (%) | 18.91 ± 0.23 | 20.63 ± 0.16 | 22.91 ± 0.04 | 1.93 | -0.15 | 75.2 | 1.53E-32 |

One hundred and thirty two BC₄S₂ individuals were analyzed via ANOVA test in Excel combining with the genotypic segregation of QTL-*Pal9* and the phenotypic variation of C16:0, C16:0/ALL, SFA/ALL, UFA/ALL and SFA/UFA. One hundred and twenty one pooled kernels with the same genotype in a BC₄S_{2:4} family from 1,043 BC₄S_{2:3} populations were measured and analyzed for the phenotypic segregation according to the QTL-*Pal9* alleles. “A” represents the additive effect of By804 allele and “D” is the dominant effect. -/-, +/- and +/+ is the homozygous allele of B73, allele that are heterozygous for B73 and By804 and homozygous allele of By804 based on target genomic markers, respectively. The abbreviations of traits can be found in Table S2.