

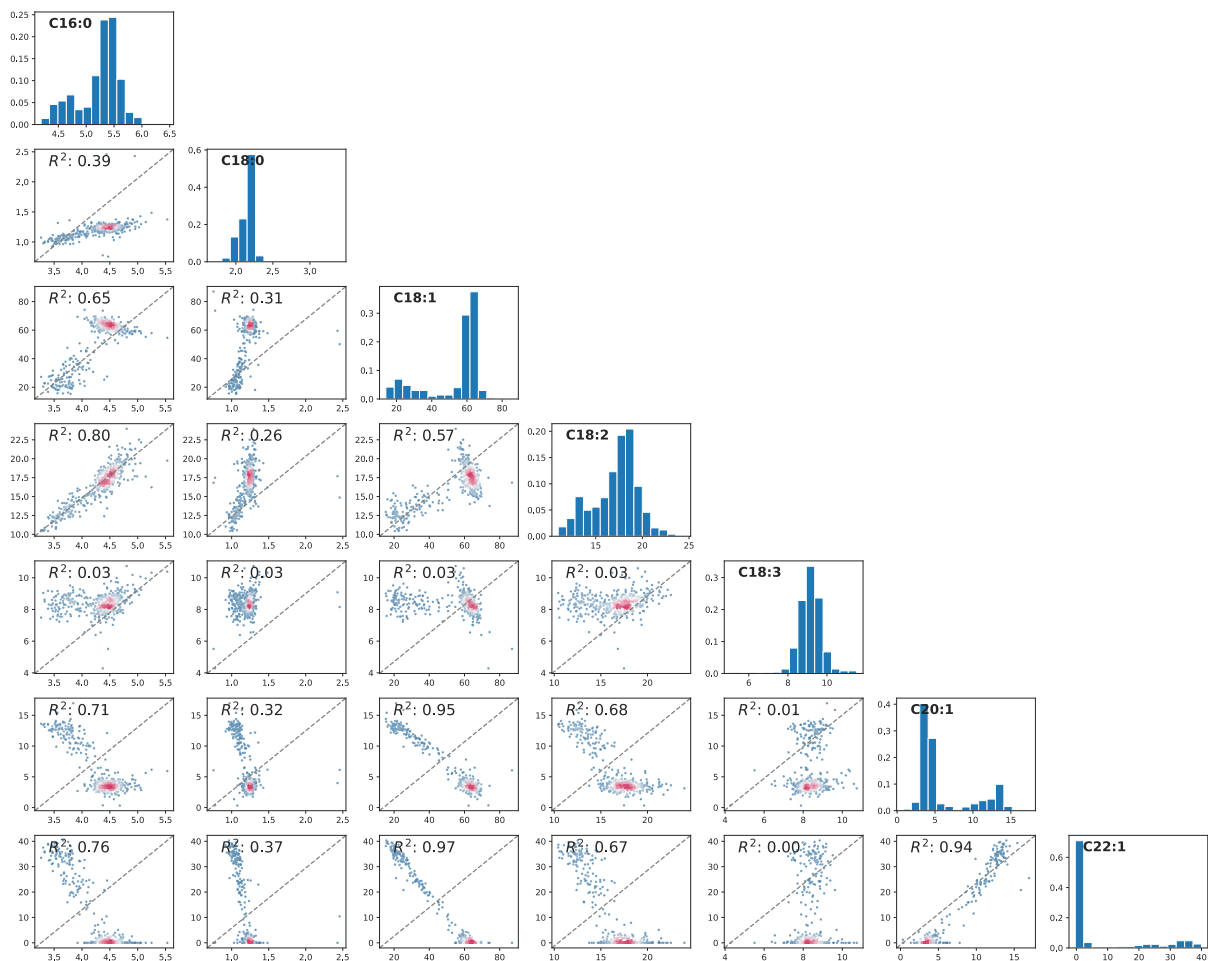


Fig. S1 FAs composition and phenotypic correlation. The histogram shows the phenotype distribution of each FAs in rapeseed following BLUP analysis. Scatter plots represent the correlation analysis among different FAs. R^2 represents the square of Pearson's correlation coefficient.

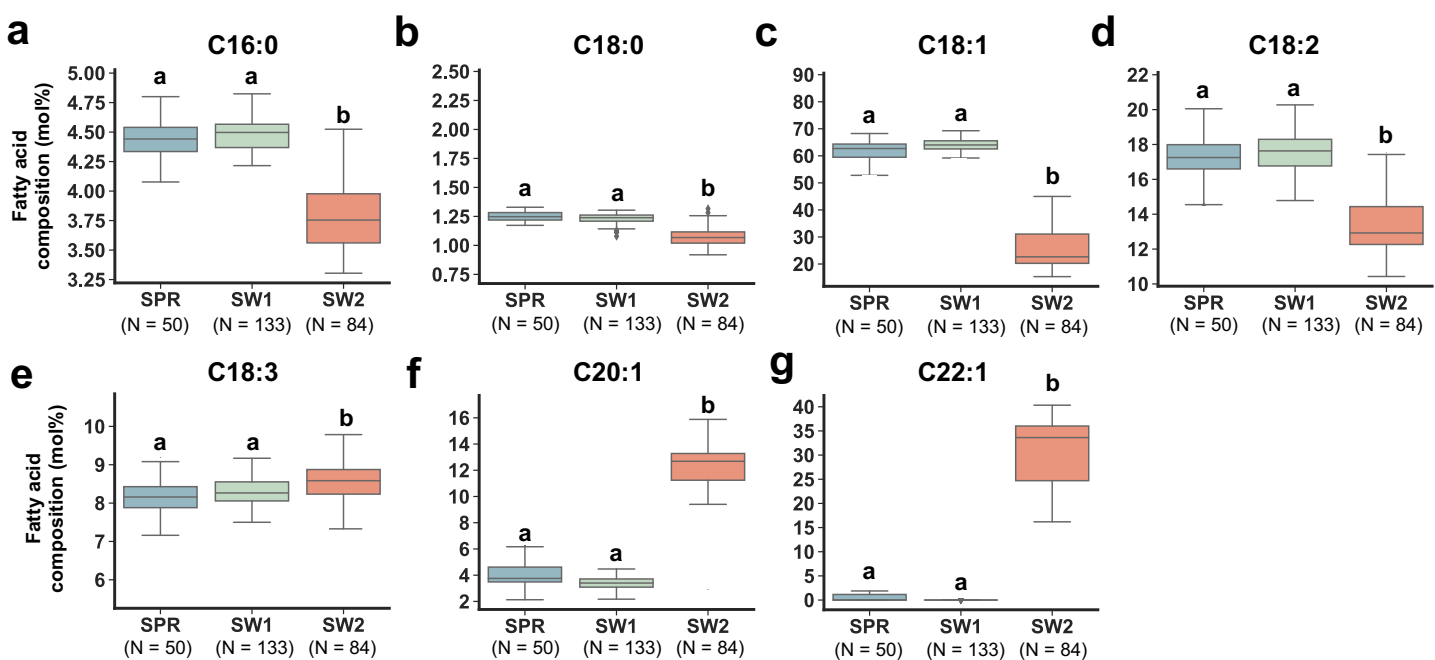


Fig. S2 Distribution of different FAs across three subgroups. a-g Distribution of palmitic acid (C16:0, **a**), Stearic acid (C18:0, **b**), Oleic acid (C18:1, **c**), Linoleic acid (C18:2, **d**), Linolenic acid (C18:3, **e**), 11-Eicosenoic acid (C20:1, **f**) and Erucic acid (C22:1, **g**). Different letters indicate significant differences among subgroups (one-way ANOVA for multiple comparisons, $P < 0.05$).

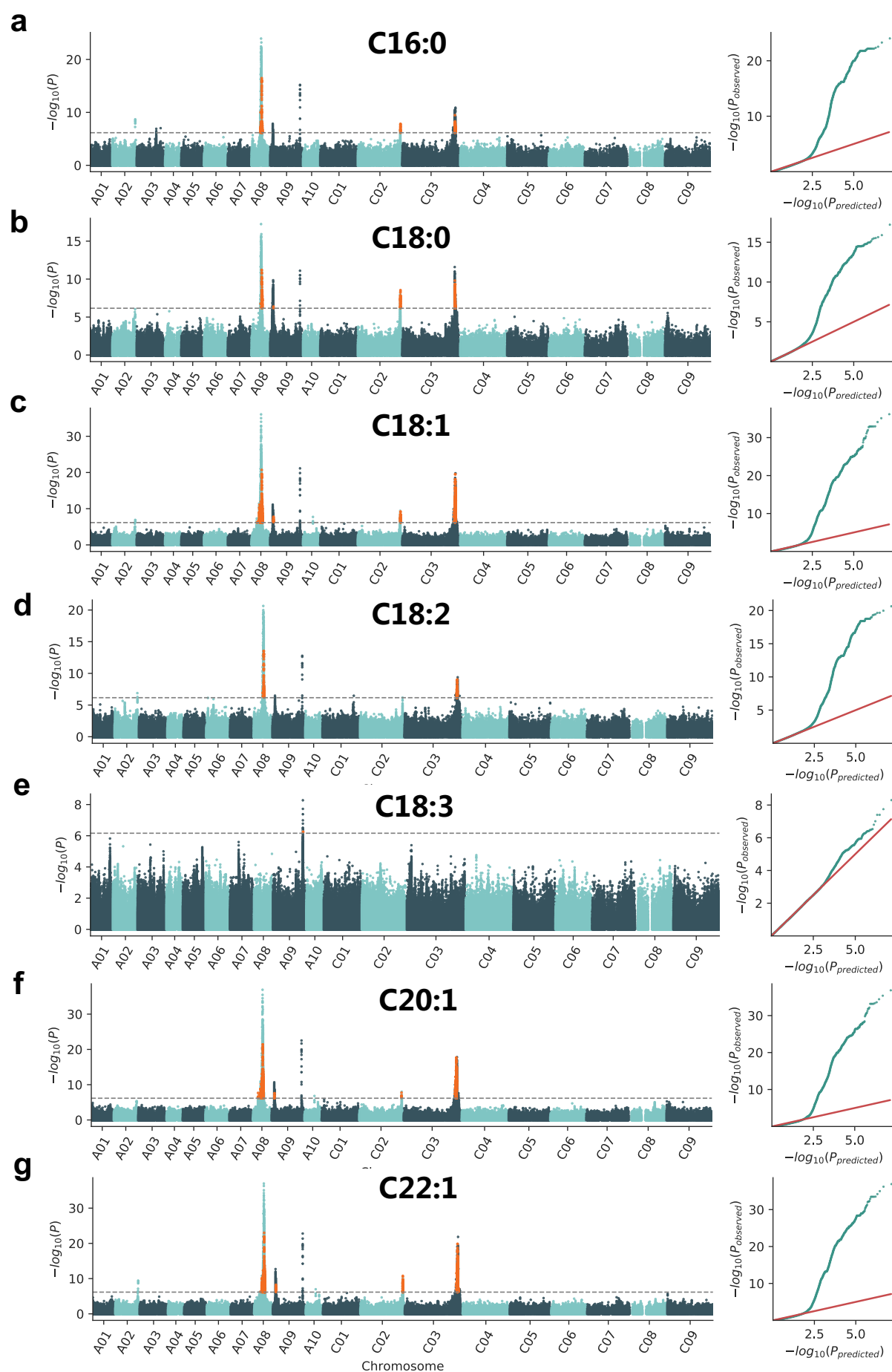


Fig. S3 (a-g) Manhattan and QQ plot of genome-wide association analysis of seven fatty acid compositions in rapeseed.

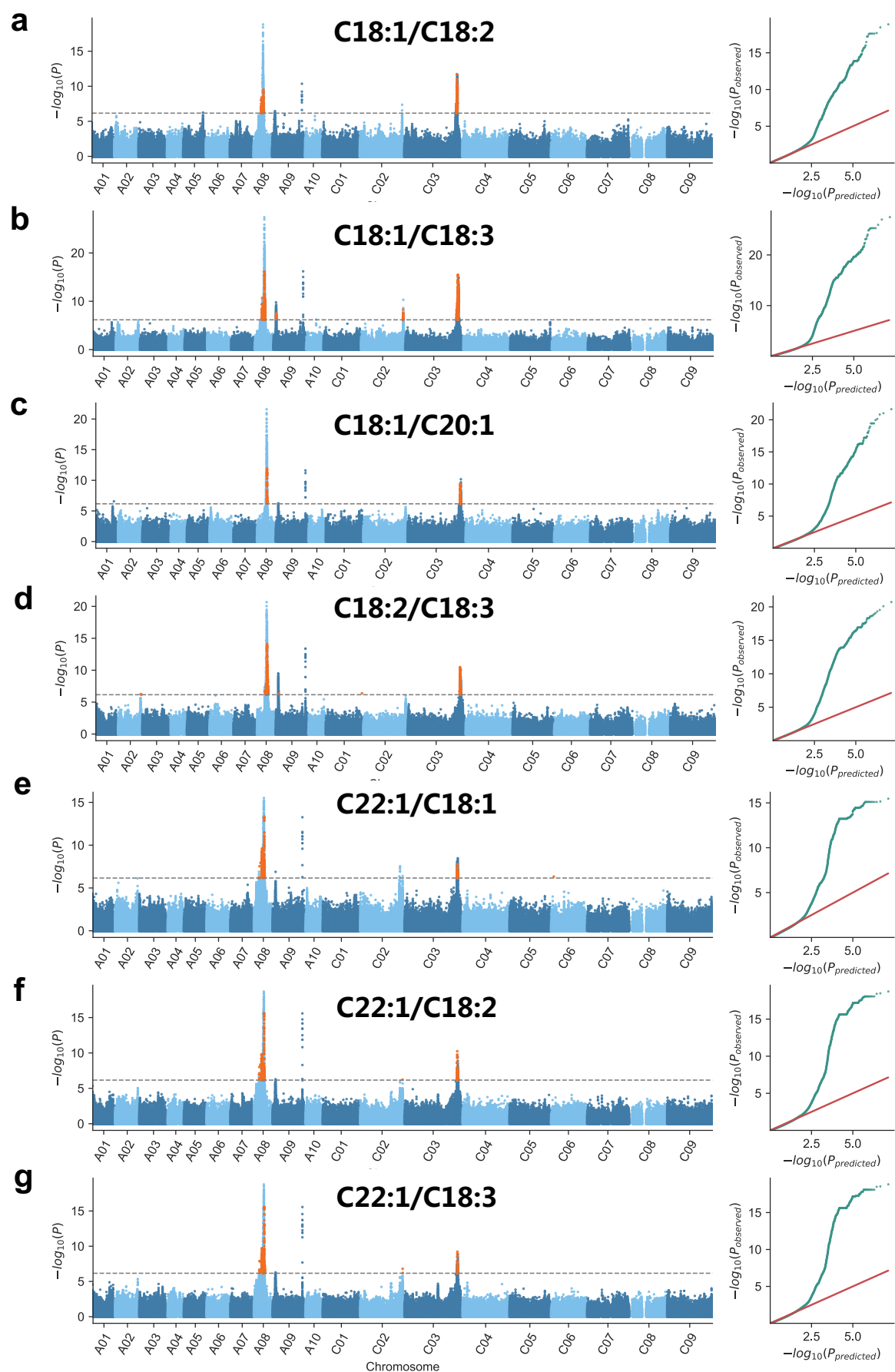


Fig. S4 (a-g) Manhattan and QQ plot of genome-wide association analysis of FAs ratio in rapeseed.

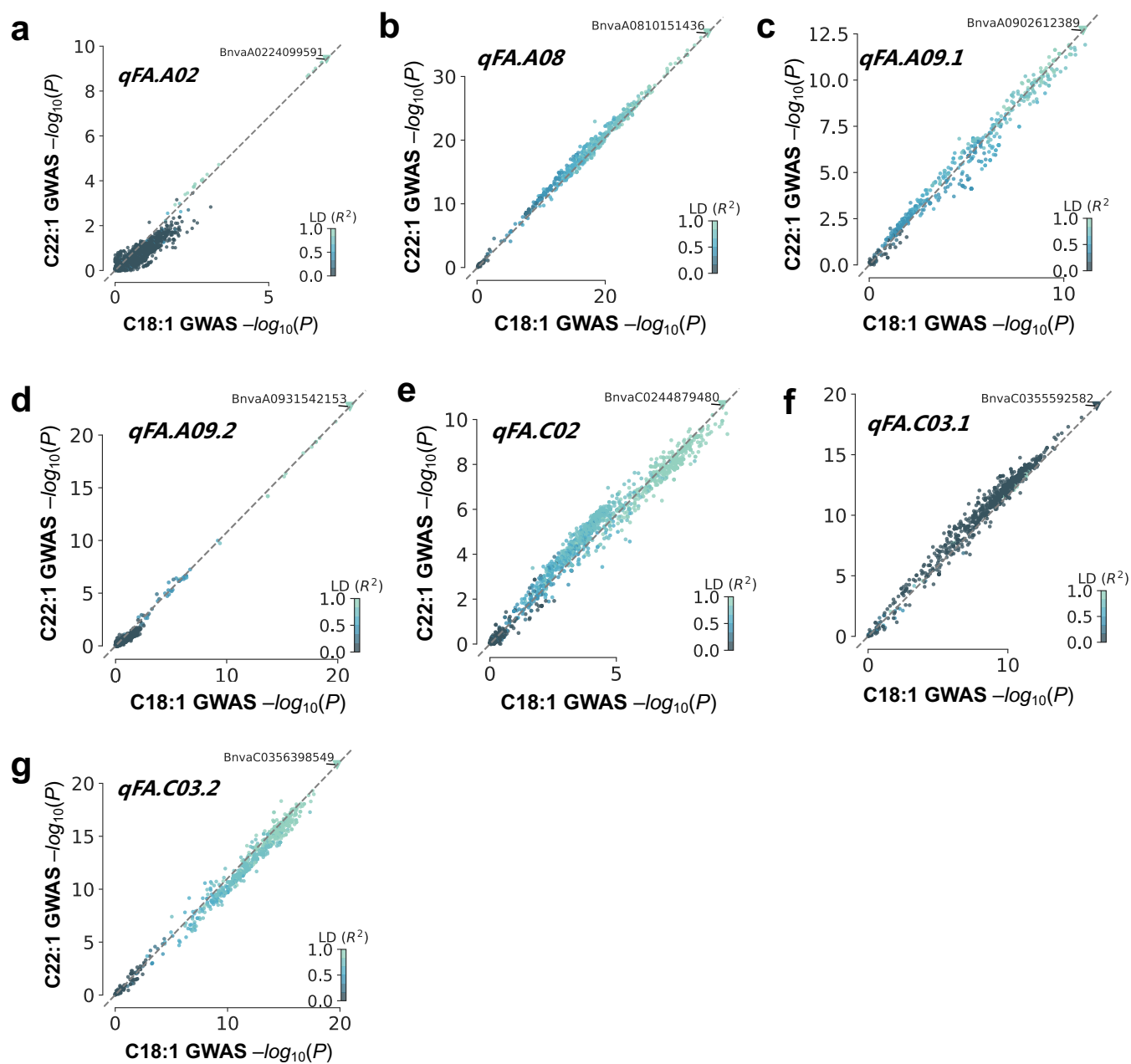


Fig. S5 (a-g) Colocalization analysis of fatty acid compositions GWAS data.

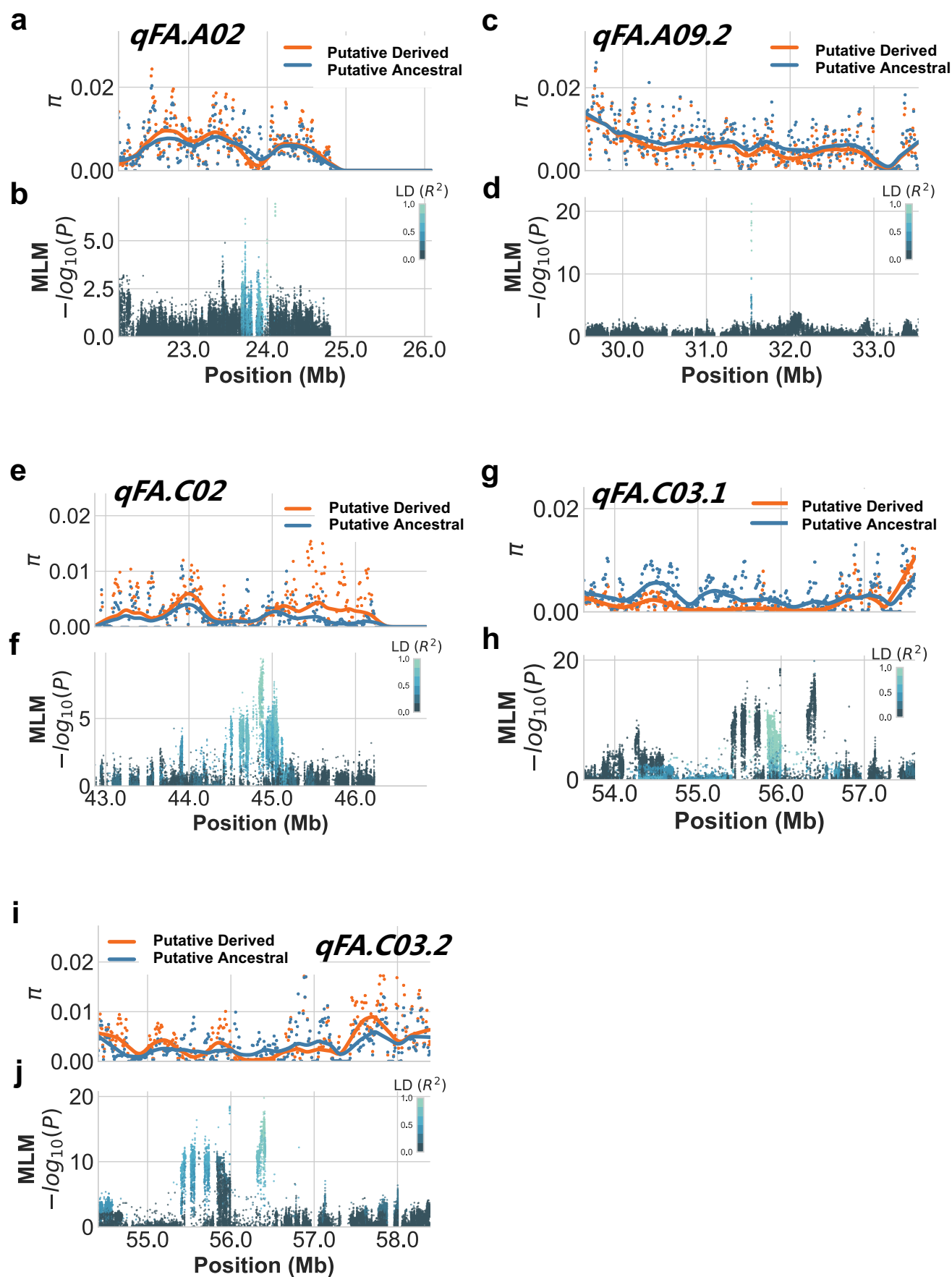
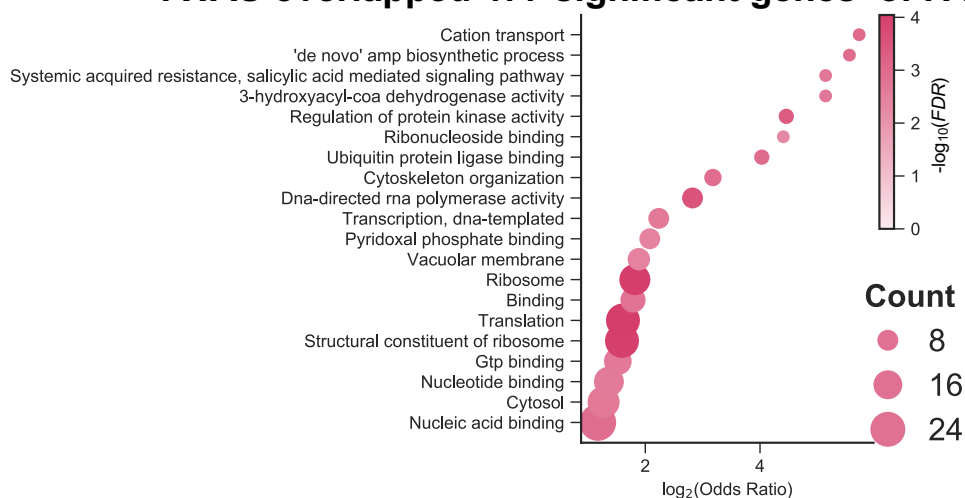
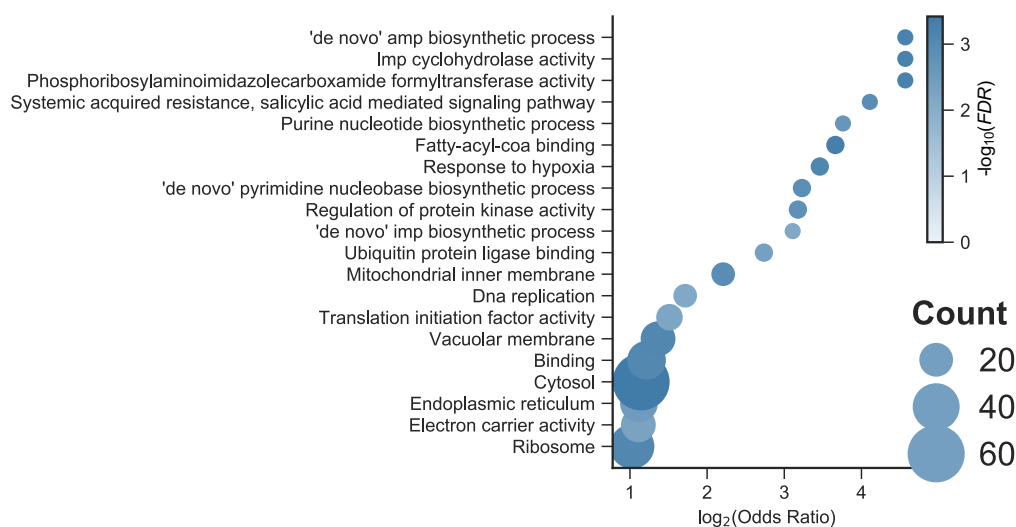


Fig. S6 FAs association significant loci selected analysis. Nucleotide diversity (π) comparison between accessions with ancestral and derived alleles at the QTLs *qFA.A02* (a), *qFA.A09.2* (c), *qFA.C02* (e), *qFA.C03.1* (g) and *qFA.C03.2* (i), and Manhattan plot of GWAS results of the 2 Mb region upstream and downstream of the QTLs *qFA.A02* (b), *qFA.A09.2* (d), *qFA.C02* (f), *qFA.C03.1* (h) and *qFA.C03.2* (j).

a TWAS overlapped 477 significant genes of FAs



b TWAS significant genes of FAs at 20 DAF



c TWAS significant genes of FAs at 40 DAF

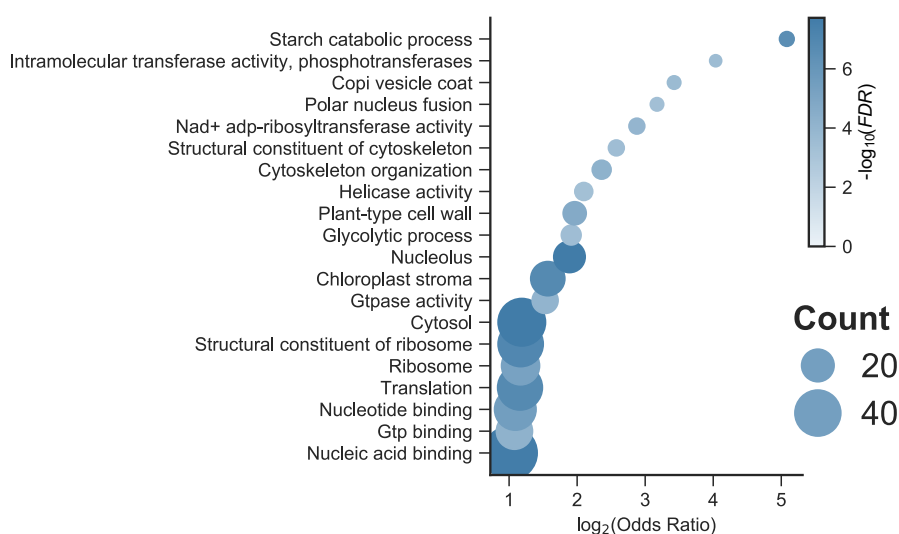


Fig. S7 GO enrichment analysis of FA TWAS significant genes. **a** GO enrichment analysis of significant FA-associated genes at 20 DAF. **b** GO enrichment analysis of significant FA-associated genes at 40 DAF. **c** GO enrichment analysis of the overlapped significant FA-associated genes at 20 DAF and 40 DAF. The y-axis represents the name of the enriched pathways, the count value represents the number of overlapped genes in each pathway, and FDR represents the significance of enrichment.

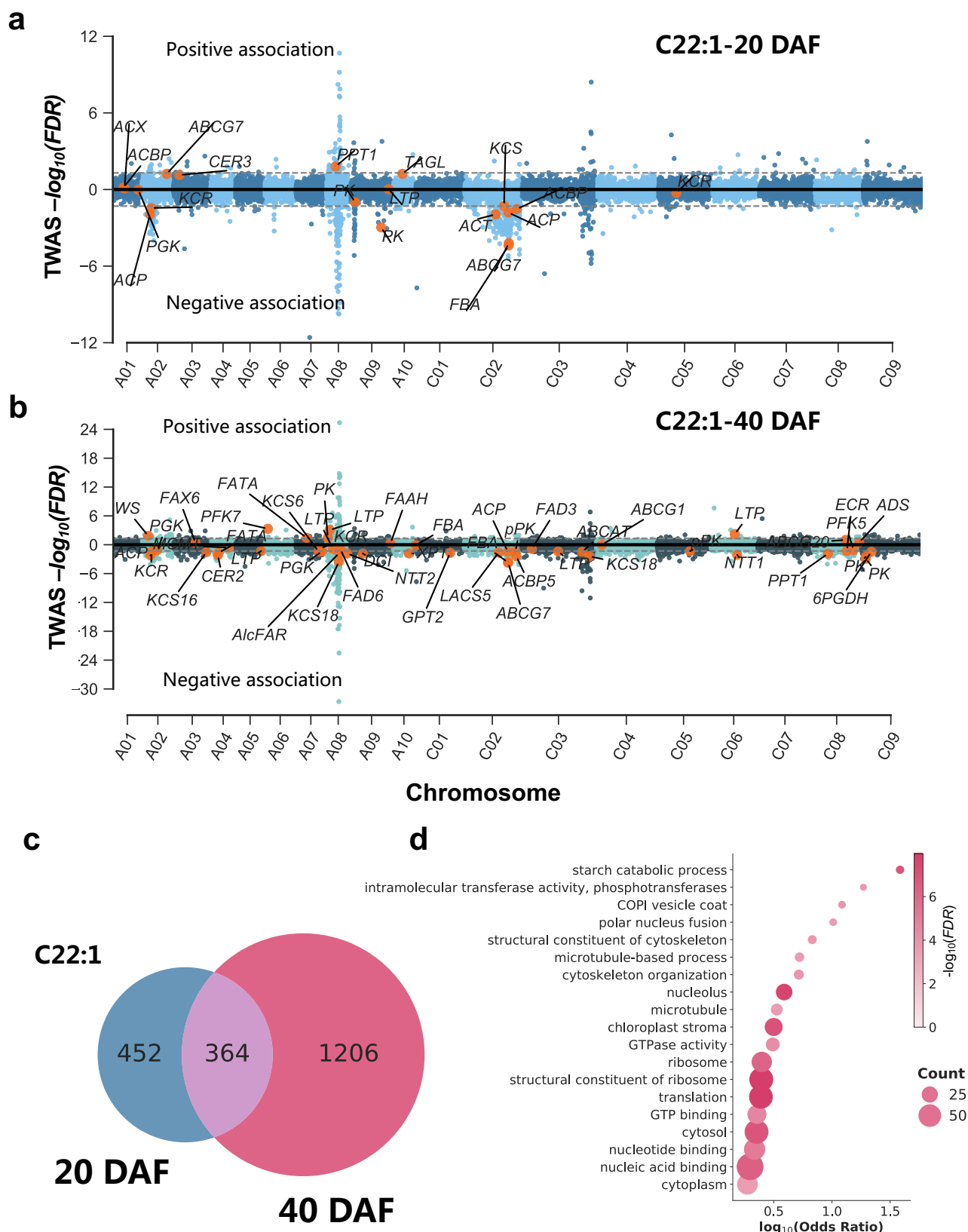


Fig. S8 Transcriptome wide association studies of C22:1. **a** Manhattan plot of C22:1 at 20 DAF. **b** Manhattan plot of C22:1 at 40 DAF. Each point represents a gene, with those positively or negatively associated with C22:1 depicted above or below the thick black line, respectively. Gold dots indicate significant lipid metabolism genes previously identified in Arabidopsis, showing notable performance in one of the TWAS results. **c** Venn diagram shows the significant C22:1 associated genes from TWAS at 20 and 40 DAF. **d** GO enrichment analysis of significant overlapping C22:1 associated genes at 20 and 40 DAF. The y-axis represents the names of the enriched pathways. The count value indicates the number of overlapped genes in each pathway, while the FDR value reflects the significance of enrichment.

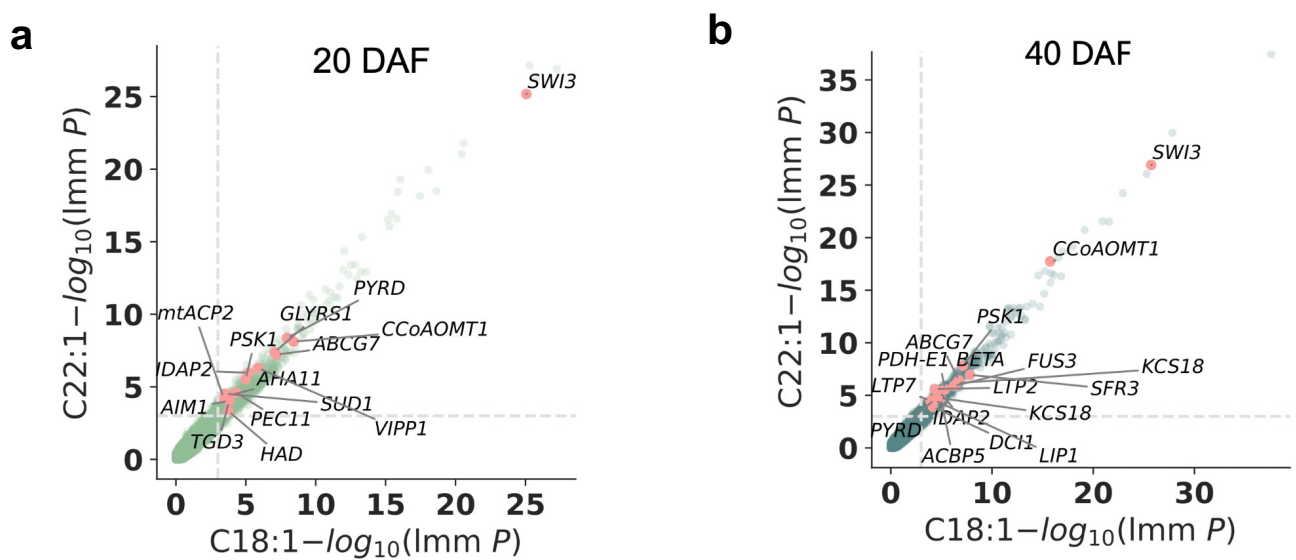


Fig. S9 Significant degree correlation analysis of significant C18:1 associated genes and C22:1 associated genes from TWAS. a Significant degree correlation analysis of C18:1 and C22:1 TWAS significant genes at 20 DAF. **b** Correlation analysis of the significance of C18:1 and C22:1 TWAS significant genes at 40 DAF. lmm P represents the significant level of genes in the TWAS of C18:1 and C22:1 content.

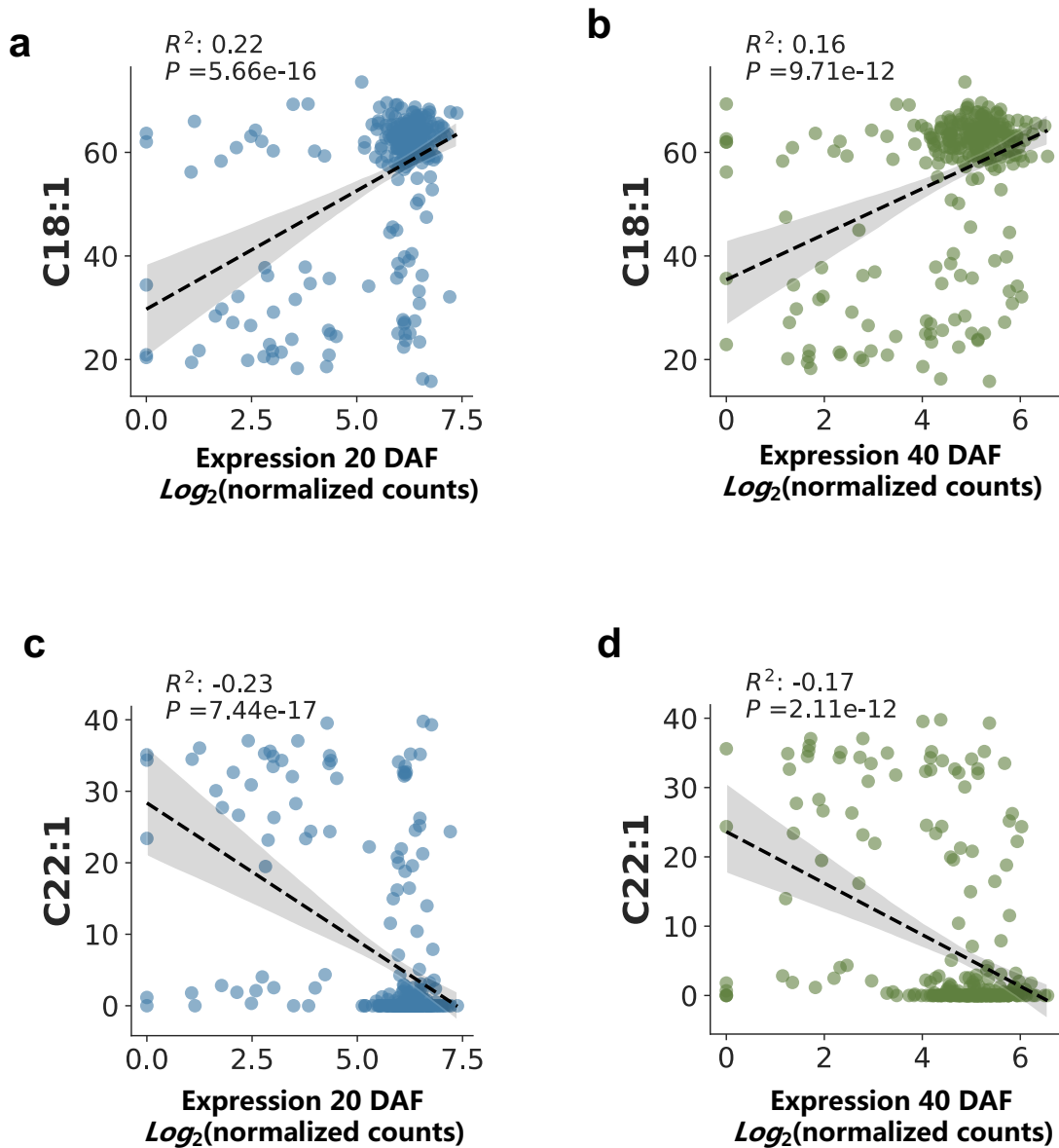


Fig. S10 (a-d) Correlation analysis of *BnaA09.PYRD* gene expression values at 20 and 40 DAF with C18:1 and C22:1 content. The x-axis indicates the normalized expression value, while the y-axis represents phenotype value.

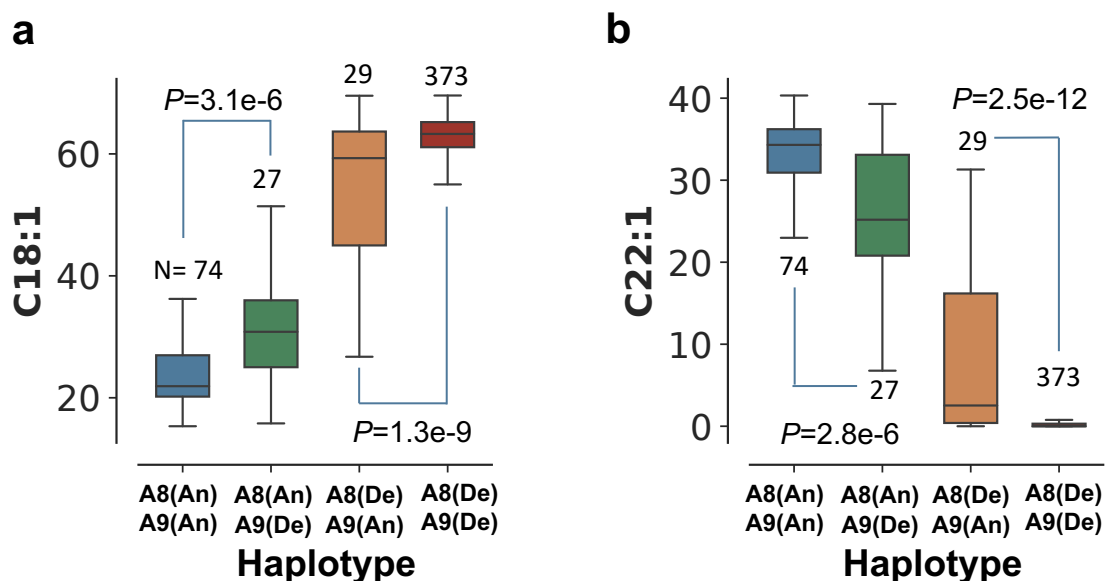


Fig. S11 Box plots of the effects of haplotypes on C18:1 and C22:1 constructed from the variants within the *qFA.A08* and the *qFA.A09.1* locus. (An) indicates the presence of ancestral allele, (De) indicates the presence of derived allele. The center line of the box plot is the median C18:1 or C22:1 in each haplotype, with the upper and lower edges of the box showing the respective quartiles. Dots represent the values of C18:1 or C22:1 content for each variety. The MLM module from EMMAX software was applied to calculate the effect of haplotypes.

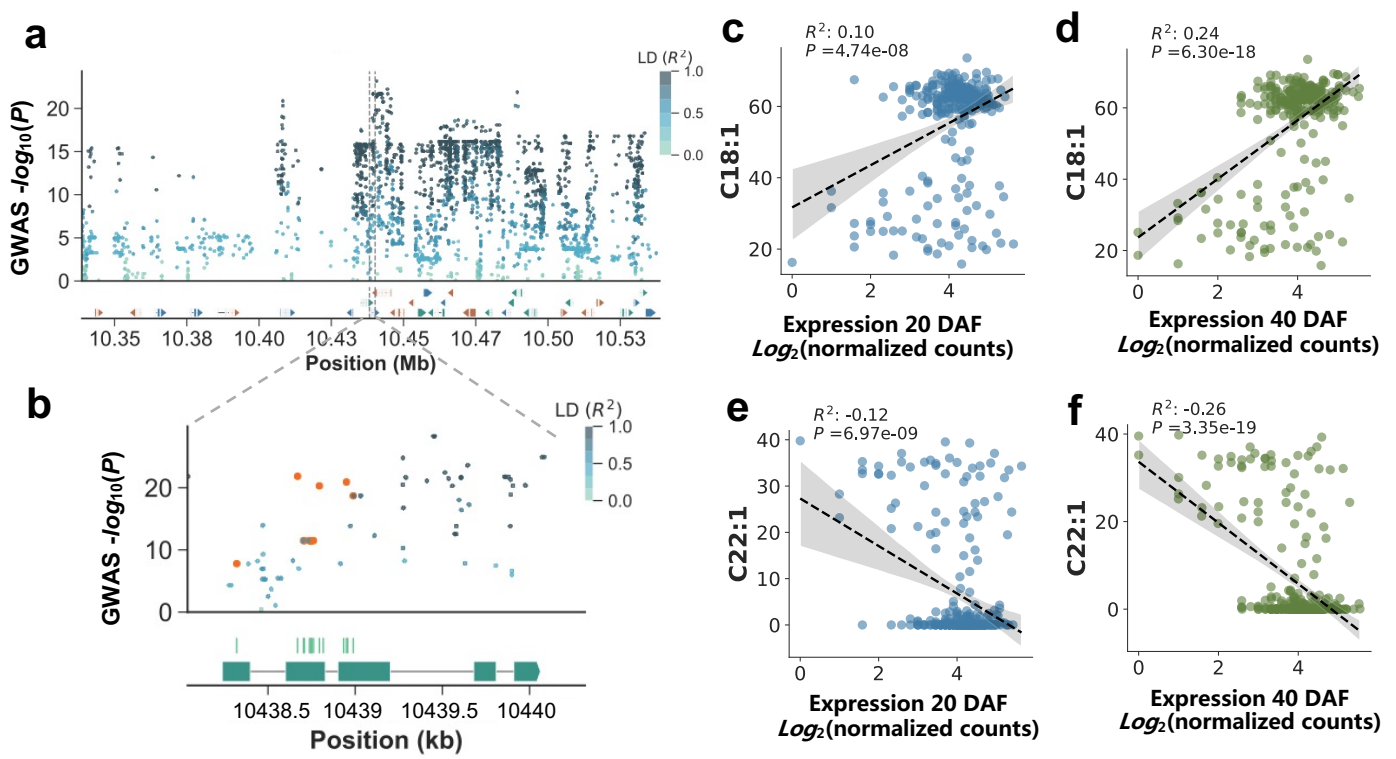


Fig. S12 Characterization of *BnaA08.PSK1* at the population level. **a** Manhattan plot and gene information for the 100 kb genomic region upstream and downstream of the *BnaA08.PSK1* gene. The dots indicate variants within the region, with the colour of each dot indicating the LD distance (R^2) between the variants and the lead SNP. The grey dashed line marks the position of the candidate gene's position, and the gene structure in the locus is displayed at the bottom. **b** GWAS results around *BnaA08.PSK1*, with nonsynonymous variants marked by gold dots. **(c-f)** Correlation analysis of *BnaA08.PSK1* gene expression values at 20 and 40 DAF with C18:1 and C22:1 content. The x-axis indicates the normalized expression value, while the y-axis represents phenotype value.

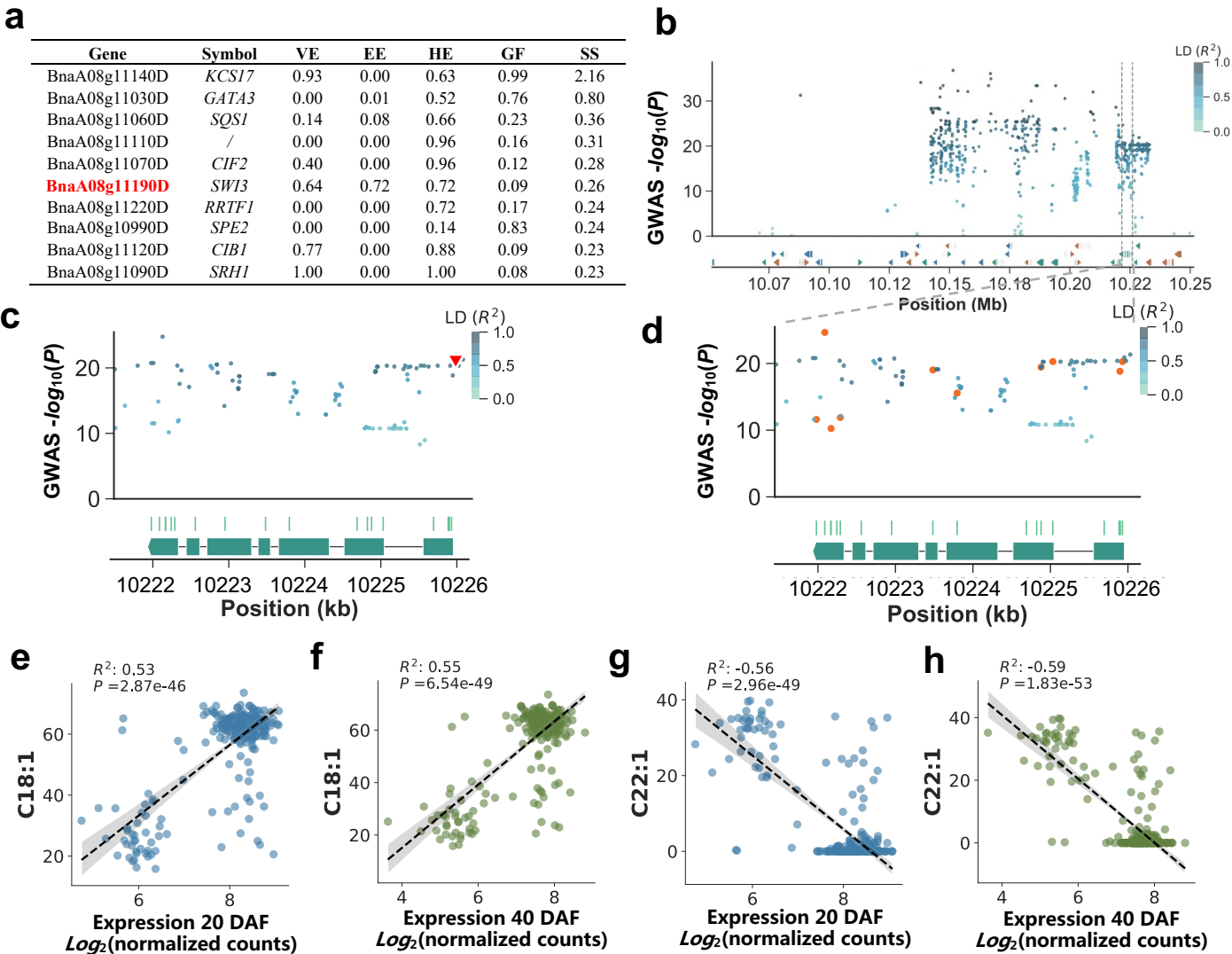


Fig. S13 Characterization of *BnaA08.SWI3* at the population level. **a** List of top 10 genes ranked within the QTL *qFA.A08*. **b** Manhattan plot and gene information for the 100 kb genomic region upstream and downstream of the *qFA.A08* locus. The dots indicate variants within the region, with the colour of each dot indicating the LD distance (R^2) between the variants and the lead SNP. The grey dashed line marks the position of the candidate gene's position, and the gene structure in the locus is displayed at the bottom. **(c-d)** GWAS results around *BnaA08.SWI3*, with code-shifting mutation marked by red inverted triangles (**c**), nonsynonymous variants marked by gold dot (**d**). **(e-h)** Correlation analysis of *BnaA08.SWI3* gene expression values at 20 and 40 DAF with C18:1 and C22:1 content. The x-axis indicates the normalized expression value, while the y-axis represents phenotype value.

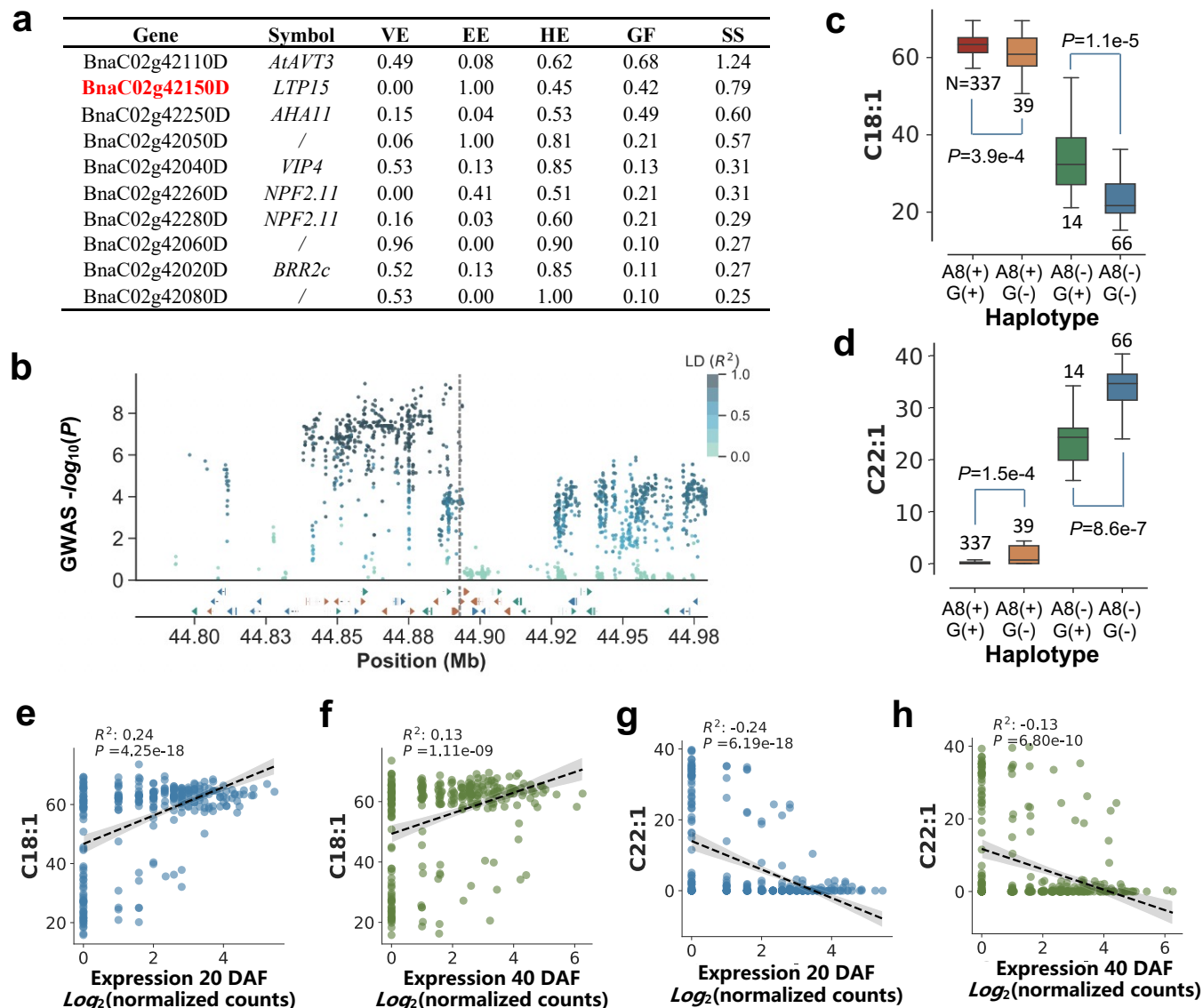


Fig. S14 Characterization of *BnaC02.LTP15* at the population level. **a** List of top 10 genes ranked within the QTL *qFA.C02*. **b** Manhattan plot and gene information for the 100 kb genomic region upstream and downstream of the *qFA.C02* locus. The dots indicate variants within the region, with the colour of each dot indicating the LD distance (R^2) between the variants and the lead SNP. The grey dashed line marks the position of the candidate gene's position, and the gene structure in the locus is displayed at the bottom. **(c-d)** Box plots of the effects of haplotypes on C18:1 and C22:1 constructed from the variants within the *BnaC02.LTP15* and the upstream 2 kb region. (+) indicates the presence of effects at the *qFA.A08* locus or gene, (-) indicates the absence of effects at the *qFA.A08* locus or gene. The center line of the box plot is the median C18:1 or C22:1 content in each haplotype, with the upper and lower edges of the box showing the respective quartiles. Dots represent the values of C18:1 or C22:1 content for each variety. The MLM module from EMMAX software was applied to calculate the effect of haplotypes. **(e-h)** Correlation analysis of *BnaC02.LTP15* gene expression values at 20 and 40 DAF with C18:1 and C22:1 content. The x-axis indicates the normalized expression value, while the y-axis represents phenotype value

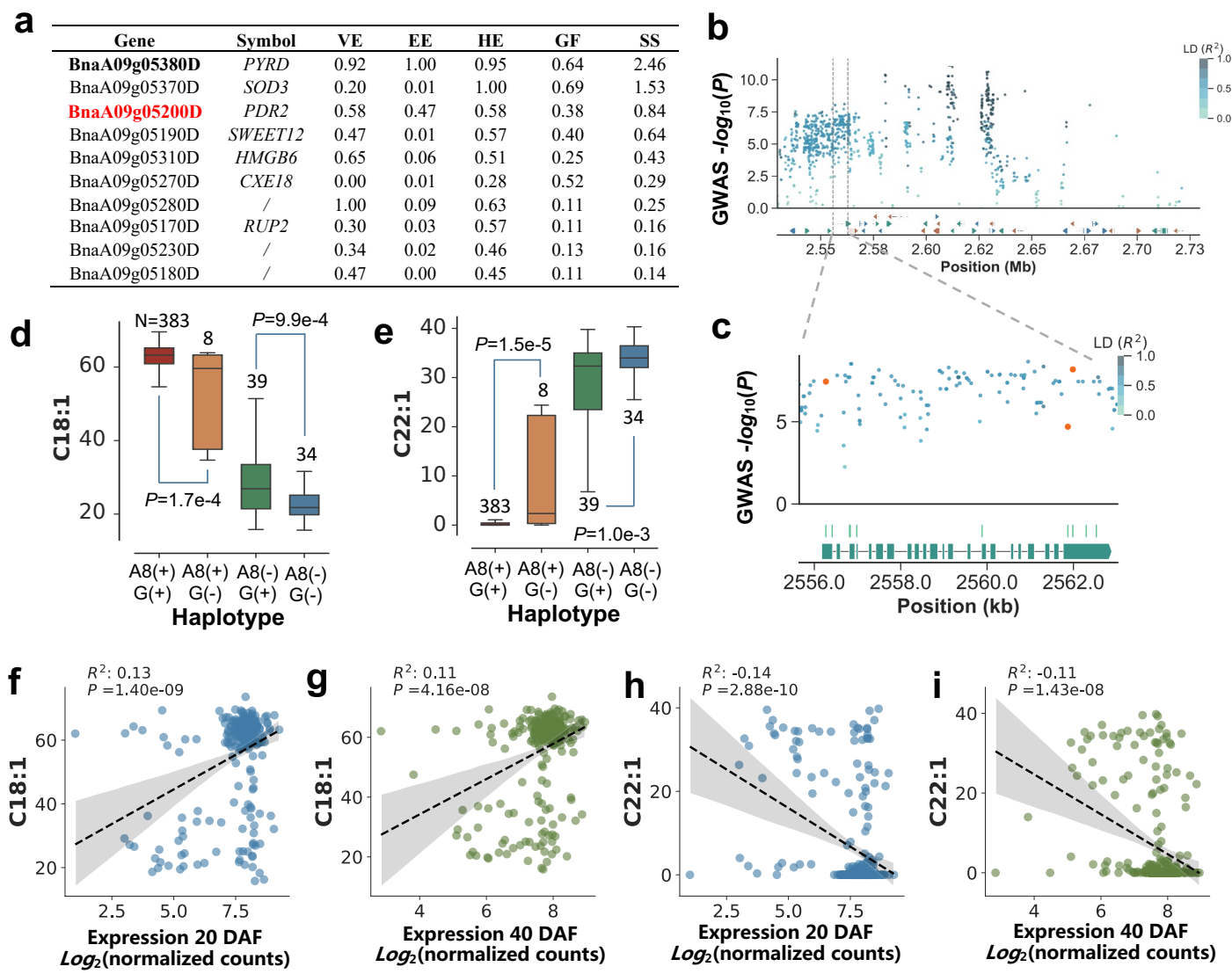


Fig. S15 Characterization of *BnaA09.PDR2* at the population level. **a** List of top 10 genes ranked within the QTL *qFA.A09.1*. **b** Manhattan plot and gene information for the 100 kb genomic region upstream and downstream of the *qFA.A09.1* locus. The dots indicate variants within the region, with the colour of each dot indicating the LD distance (R^2) between the variants and the lead SNP. The grey dashed line marks the position of the candidate gene's position, and the gene structure in the locus is displayed at the bottom. **c** GWAS results around *BnaA09.PDR2*, with nonsynonymous variants marked by gold dots. **(d-e)** Box plots of the effects of haplotypes on C18:1 and C22:1 constructed from the variants within the *BnaA09.PDR2* and the upstream 2 kb region. (+) indicates the presence of effects at the *qFA.A08* locus or gene, (-) indicates the absence of effects at the *qFA.A08* locus or gene. The center line of the box plot is the median C18:1 or C22:1 content in each haplotype, with the upper and lower edges of the box showing the respective quartiles. Dots represent the values of C18:1 or C22:1 content for each variety. The MLM module from EMMAX software was applied to calculate the effect of haplotypes. **(f-i)** Correlation analysis of *BnaA09.PDR2* gene expression values at 20 and 40 DAF with C18:1 and C22:1 content. The x-axis indicates the normalized expression value, while the y-axis represents phenotype value.

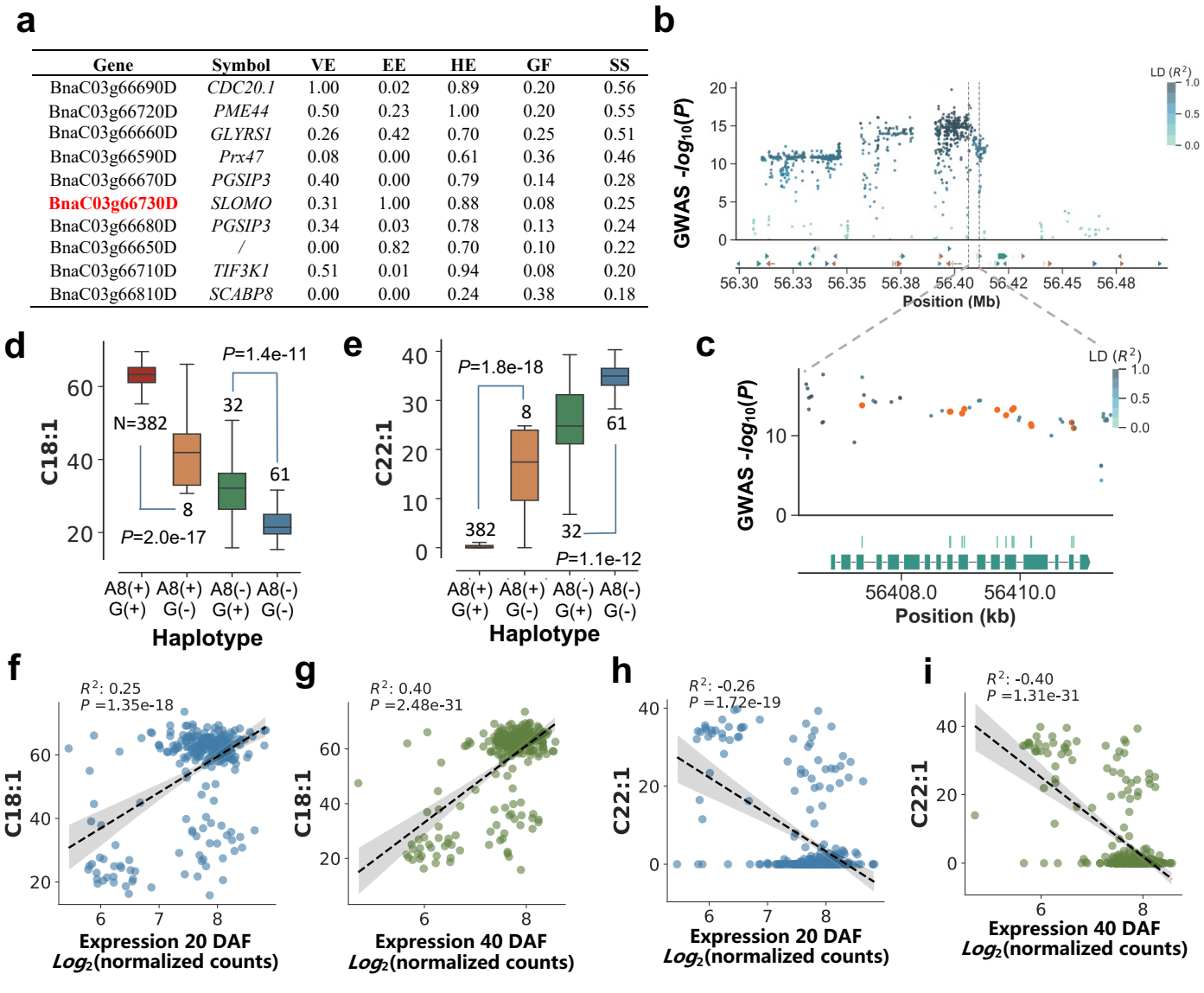
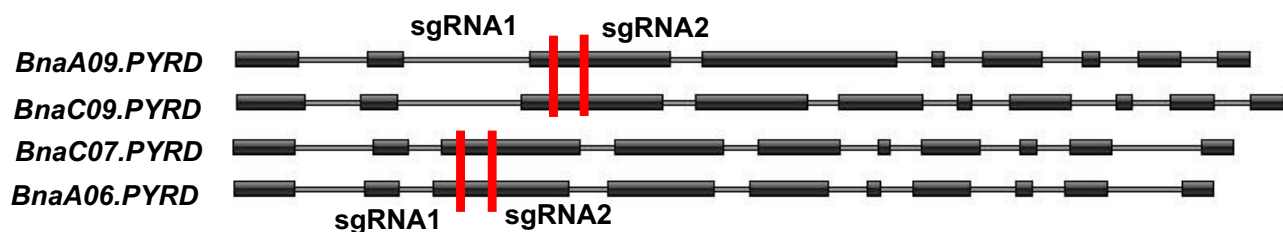


Fig. S16 Characterization of *BnaC03.SLOMO* at the population level. **a** List of top 10 genes ranked within the QTL *qFA.C03.2*. **b** Manhattan plot and gene information for the 100 kb genomic region upstream and downstream of the *qFA.C03.2* locus. The dots indicate variants within the region, with the colour of each dot indicating the LD distance (R^2) between the variants and the lead SNP. The grey dashed line marks the position of the candidate gene's position, and the gene structure in the locus is displayed at the bottom. **c** GWAS results around *BnaC03.SLOMO*, with nonsynonymous variants marked by gold dots. **(d-e)** Box plots of the effects of haplotypes on C18:1 and C22:1 constructed from the variants within the *BnaC03.SLOMO* and the upstream 2 kb region. (+) indicates the presence of effects at the *qFA.A08* locus or gene, (-) indicates the absence of effects at the *qFA.A08* locus or gene. The center line of the box plot is the median C18:1 or C22:1 content in each haplotype, with the upper and lower edges of the box showing the respective quartiles. Dots represent the values of C18:1 or C22:1 content for each variety. The MLM module from EMMAX software was applied to calculate the effect of haplotypes. **(f-i)** Correlation analysis of *BnaC03.SLOMO* gene expression values at 20 and 40 DAF with C18:1 and C22:1 content. The x-axis indicates the normalized expression value, while the y-axis represents phenotype value.



BnaA09.PYRD-sgRNA

Reference:

TGGCTGTTCTCAGCAA-CAAAGG

GGGACTTGAAGTTTGGGGAAGG

L1:

TGGCTGTTCTCAGCAATCAAAGG

GGGACTTGAAGTTTGGGGAAGG

L35:

TGGCTGTTCTCAGCAA - AAAGG

GGGACTTGAAGTTTGGGGAAGG

BnaC09.PYRD-sgRNA

Reference:

TGGCTGTTCTCAGCAA-CAAAGG

GGGACTTGAAGTTTGGGGAAGG

L1:

TGGCTGTTCTCAGCAATCAAAGG

GGGACTTGAAGTTTGGGGAAGG

L35:

TGGCTGTTCTCAGCAAACAAAGG

GGGACTTGAAGTTTGGGGAAGG

BnaC07.PYRD-sgRNA

Reference:

TGGCTGTTCTCAGCAA-CAAAGG

GGGACTTGAAGTTTGGGGAAGG

L1:

TGGCTGTTCTCAGCAAACAAAGG

GGGACTTGAAGTTTGGGGAAGG

L35:

TGGCTGTTCTCAGCATCAAAGG

GGGACTTGAAGTTTGGGGAAGG

BnaA06.PYRD-sgRNA

Reference:

TGGCTGTTCTCAGCAA-CAAAGG

GGGACTTGAAGTTTGG-GGAAGG

L1:

TGGCTGTTCTCAGCAA-CAAAGG

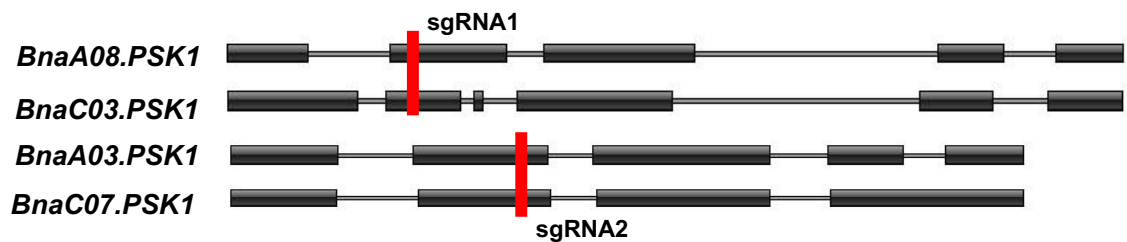
GGGACTTGAAGTTTGG-GGAAGG

L35:

TGGCTGTTCTCAGCAA-CAAAGG

GGGACTTGAAGTTTGGTGGAAGG

Fig. S17 Genotype and the location of *BnaPYRD-sgRNA* mutant lines. Black sequences are sgRNA sequences, green indicate PAM sequences, red -/bases indicate base deletions or insertions.



***BnaA08.PSK1*-sgRNA1**

Reference TCTGAGAAAGTGCTT-GGTAAGG

L5 TCTGAGAAAGTGCTT - - - AAGG

L26 TCTGAGAAAGTGCTTGGTAAGG

***BnaC03.PSK1*-sgRNA1**

Reference TCTGAGAAAGTGCTT-G-GTAAGG

L5 TCTGAGAAAGTGCTT-G-TAAGG

L26 TCTGAGAAAGTGCTT-GAGTAAGG

***BnaA03.PSK1*-sgRNA2**

Reference TTGCCACGACATTGAG-TTCGGG

L5 TTGCCACGACATTGA- -TTCGGG

L26 TTGCCACGACATTGAGTTTCGGG

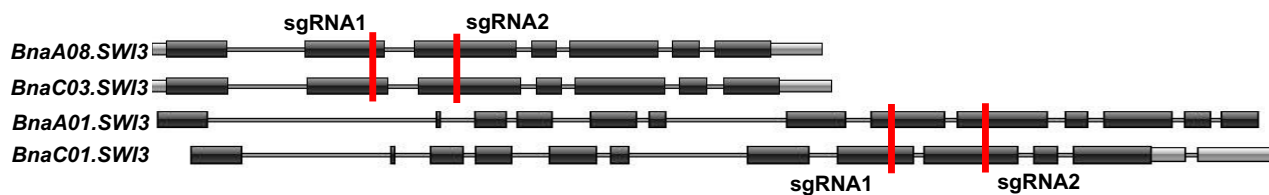
***BnaC07.PSK1*-sgRNA2**

Reference TTGCCACGACATTGAG-TTCGGG

L5 TTGCCACGACATTGAGTTTCGGG

L26 TTGCCACGACATTGAG-TTCGGG

Fig. S18 Genotype and the location of *BnaPSK1*-sgRNA mutant lines. Black sequences are sgRNA sequences, green indicate PAM sequences, red -/bases indicate base deletions or insertions.



BnaA08.SWI3

Reference:	TGGAGTTCTTGGACTA-CTG	GGG	GGCGTTGGTAGTGGGAAGT	
L3:	TGGAGTTCTTGGAG	GTA-CTG	GGG	GGCGTTGGTAGTGGGAAGT
L10:	TGGAGTTCTTGGACTA	ACT	GGG	GGCGTTGGTAGTGGGAAGT
L27:	TGGAGTTCTTGG- -	A-CTG	GGG	GGCGTTGGTAGTGGGAAGT

BnaC03.SWI3

Reference:	TGGAGTTCTTGGACTACTG	GGG	GGCG-TTGGTAGTGGGAAGT	
L3:	TGGAGTTCTTGGACT-	CTG	GGG	GGCG-TTGGTAGTGGGAAGT
L10:	TGGAGTTCTTGGAG	TACTG	GGG	GGCGTTTGGTAGTGGGAAGT
L27:	TGGAGTTCTTGGACTACTG	GGG	GGC--TTGGTAGTGGGAAGT	

BnaA01.SWI3

Reference:	TGGAGTTCTTGGACTA-CTG	GGG	GGCGTTGGTAGTGGGAAGT	
L3:	TGGAGTTCTTGGACT-	CTG	GGG	GGCGTTGGTAGTGGGAAGT
L10:	TGGAGTTCTTGGACTA-CTG	GGG	GGCGTTGGTAGTGGGAAGT	
L27:	TGGAGTTCTTGGACTACTG	GGG	GGCGTTGGTAGTGGGAAGT	

BnaC01.SWI3

Reference:	TGGAGTTCTTGGACTACTG	GGG	GGCGTTGGTAGTGGGAAGT
L3:	TGGAGTTCTTGGACTACTG	GGG	GGC-TTGGTAGTGGGAAGT
L10:	TGGAGTTCTTGGACTA-CTG	GGG	GGCGTTGGTAGTGGGAAGT
L27:	TGGAGTTCTTGGACTACTG	GGG	GGCGTTGGTAGTGGGAAGT

Fig. S19 Genotype and the location of *BnaSWI3*-sgRNA mutant lines. Black sequences are sgRNA sequences, green indicate PAM sequences, red -/bases indicate base deletions or insertions.



BnaLTP15.C02

Reference:	CTTGTTTGGTCTTGGCACC AGG	CCAGG -T-CAGGTGCCAACAAGAC
L3:	CTTGTTTGGTCTTGGCACC AGG	CCAGG -T A CAGGTGCCAACAAGAC
L11:	CTTGTTTGGTCTTGGCACC AGG	CCAGG -T- -AGGTGCCAACAAGAC
L20:	CTTGTTTGGTCTTGGCACC AGG	CCAGG -T-CAGGTGCCAACAAGAC

BnaLTP15.A02

Reference:	CTTGTTTGGTCTTGGC-ACC AGG	CCAGG -TCAGGTGCCAACAAGAC
L3:	CTTGTTTGGTCTTGGC T ACC AGG	CCAGG -TCAGGTGCCAACAAGAC
L11:	CTTGTTTGGTCTTGGC- ACC AGG	CCAGG T TCAGGTGCCAACAAGAC
L20:	CTTGTTTGGTCTTGGC A ACC AGG	CCAGG T TCAGGTGCCAACAAGAC

Fig. S20 Genotype and the location of *BnaLTP15*-sgRNA mutant lines. Black sequences are sgRNA sequences, green indicate PAM sequences, red -/bases indicate base deletions or insertions.

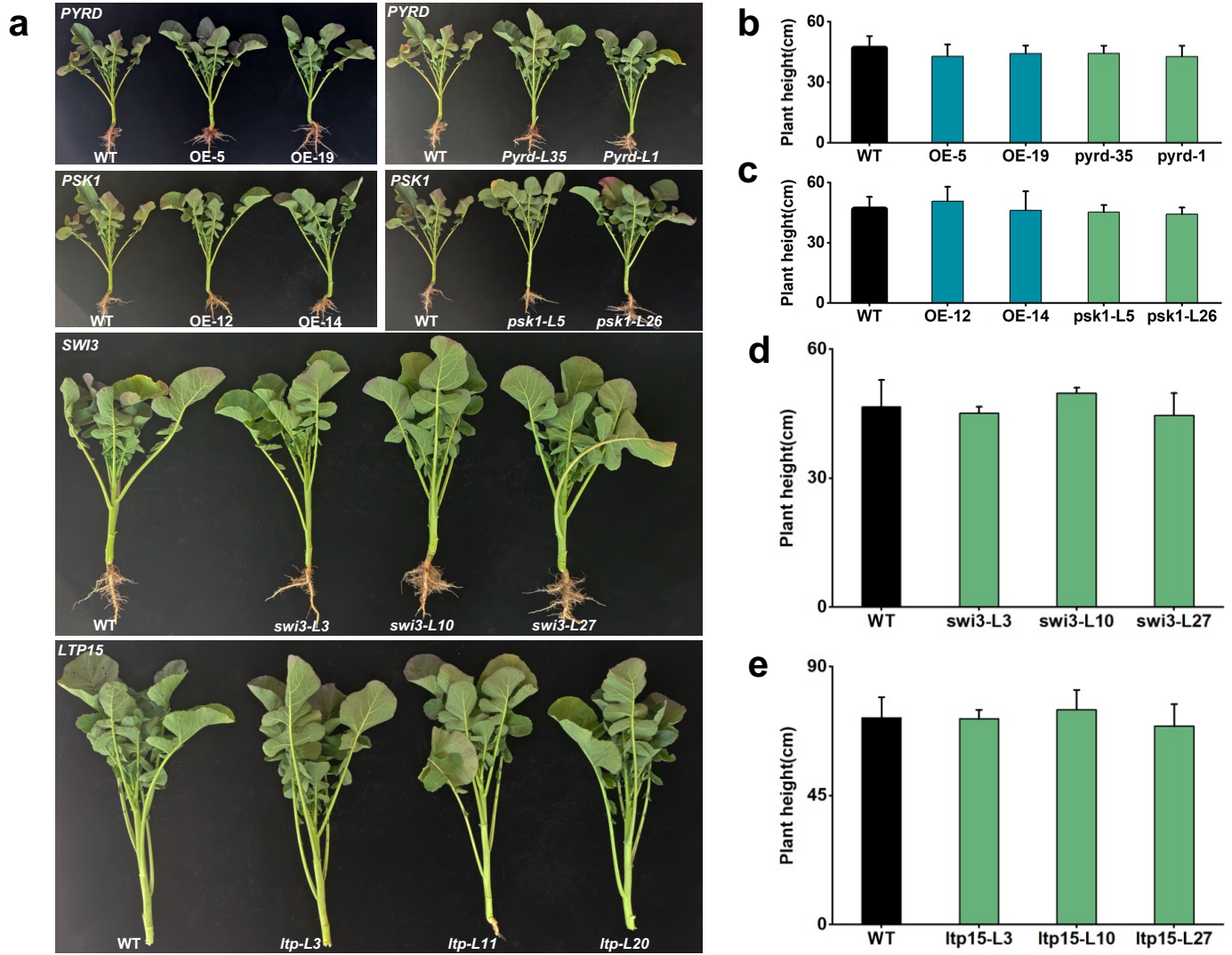


Fig. S21 Phenotypic evaluation of transgenic and wild-type rapeseed lines. (a) Growth performance of wild-type (WT) plants and transgenic lines overexpressing or carrying mutations in four candidate genes. (b–e) Comparative analysis of plant height (cm) for *BnaPYRD* (b), *BnaPSK1* (c), *BnaSWI3* (d), and *BnaLTP15* (e) shows no significant differences between WT and mutant lines (n = 3–6).

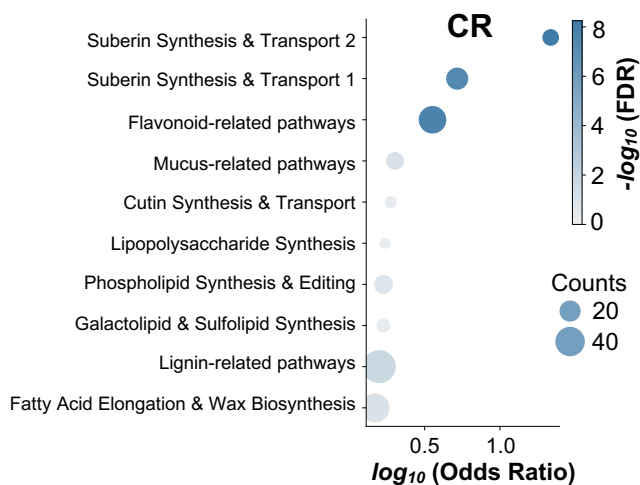


Fig. S22 GO enrichment analysis of DEGs in the comparison between *bna.pyrd* mutants and WT. The x-axis represents the name of the enriched pathway, the count value represents the number of overlapped genes in each pathway, and the FDR indicates the enrichment significance.

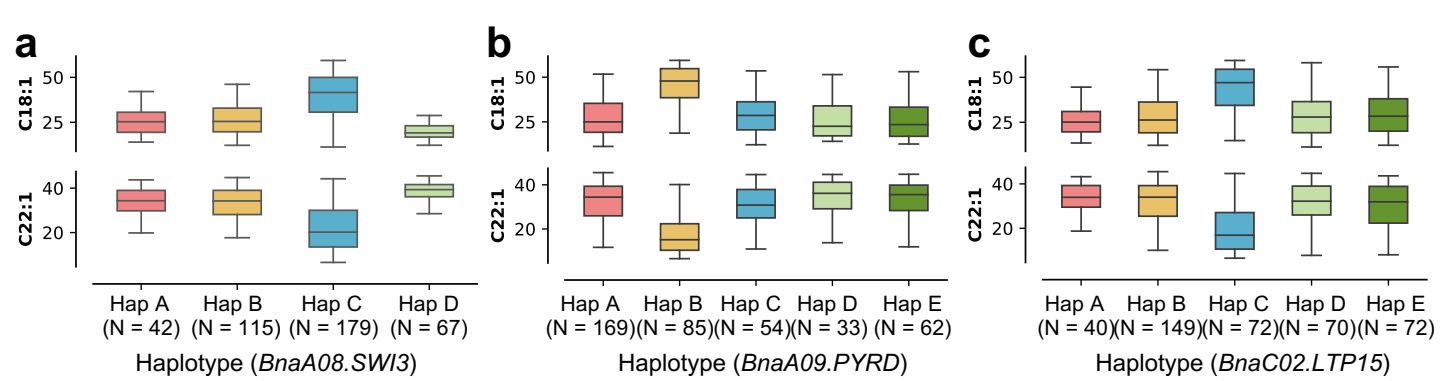


Fig. S23 Box plots of the effects of haplotypes in 418 accessions on C18:1 and C22:1. (a) is *BnaA08.SWI3*. (b) is *BnaA09.PYRD*. (c) is *BnaC02.LTP15*.

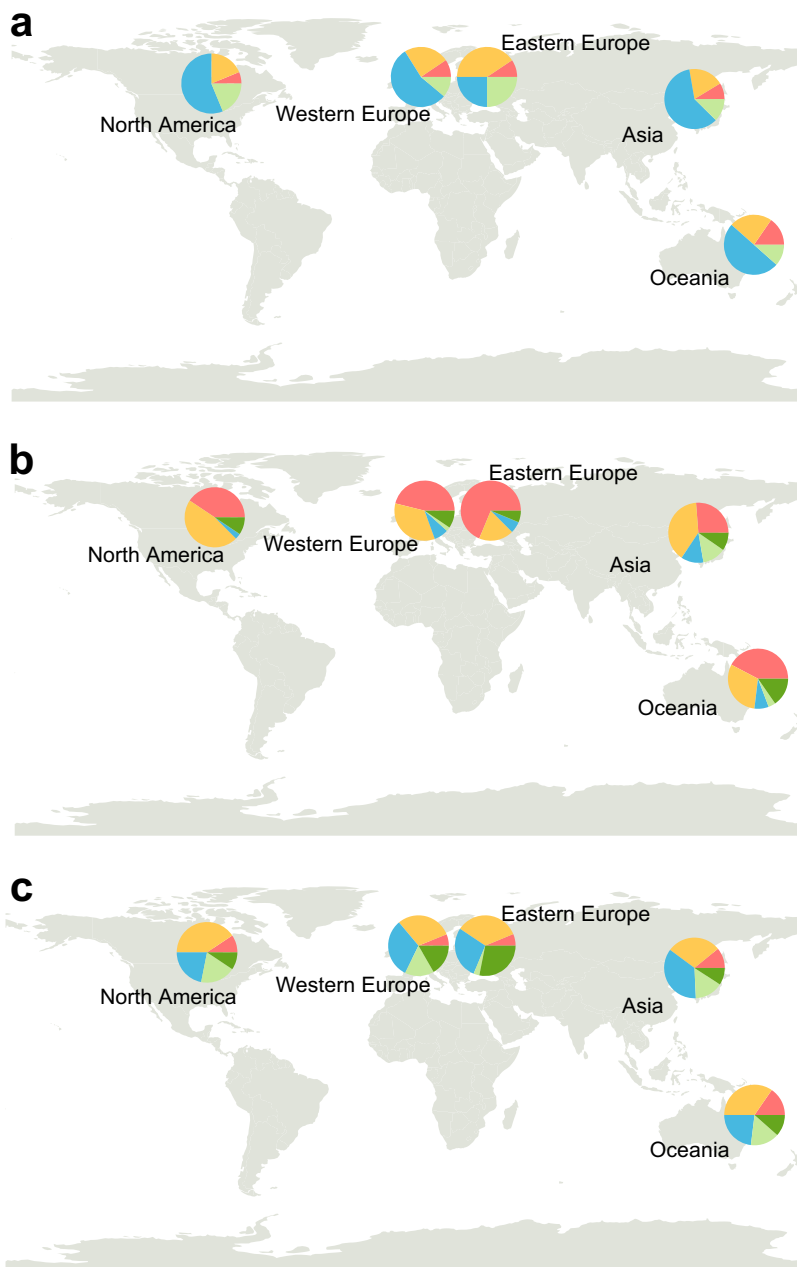


Fig. S24 Geographic distribution of different haplotypes. (a) is *BnaA08.SWI3*. **(b)** is *BnaA09.PYRD*. **(c)** is *BnaC02.LTP15*.

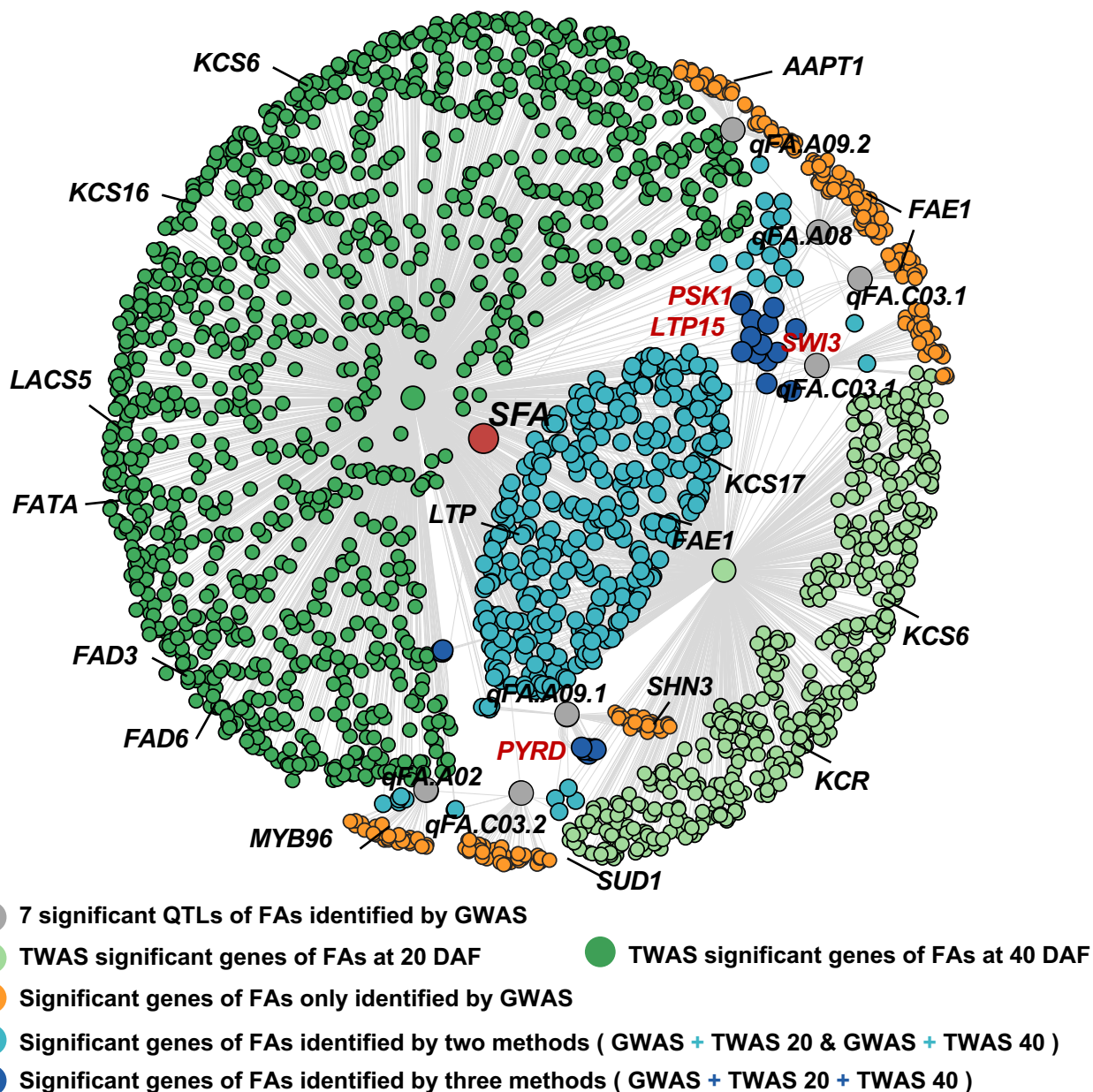


Fig. S25 Construction of a multi-omic gene network influencing fatty acid composition in rapeseed. The network contains seven QTLs, 1,576 TWAS significantly associated genes at 20 DAF and 2,196 TWAS significantly associated genes at 40 DAF. Identified QTLs and the previously reported genes known to affect FA composition are also labeled within the network.