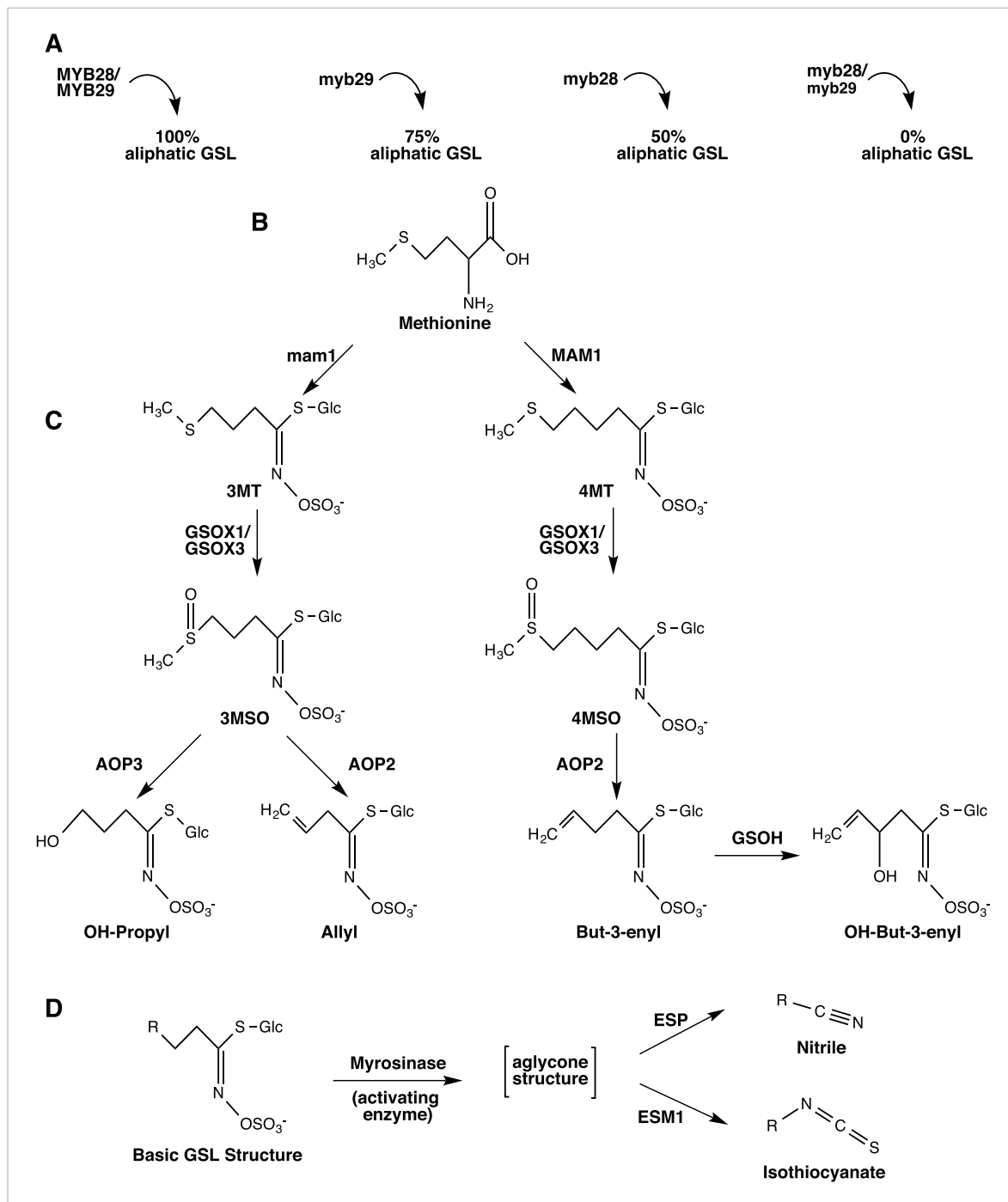


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## Figures and figure supplements

Natural genetic variation in *Arabidopsis thaliana* defense metabolism genes modulates field fitness

**Rachel Kerwin, et al.**

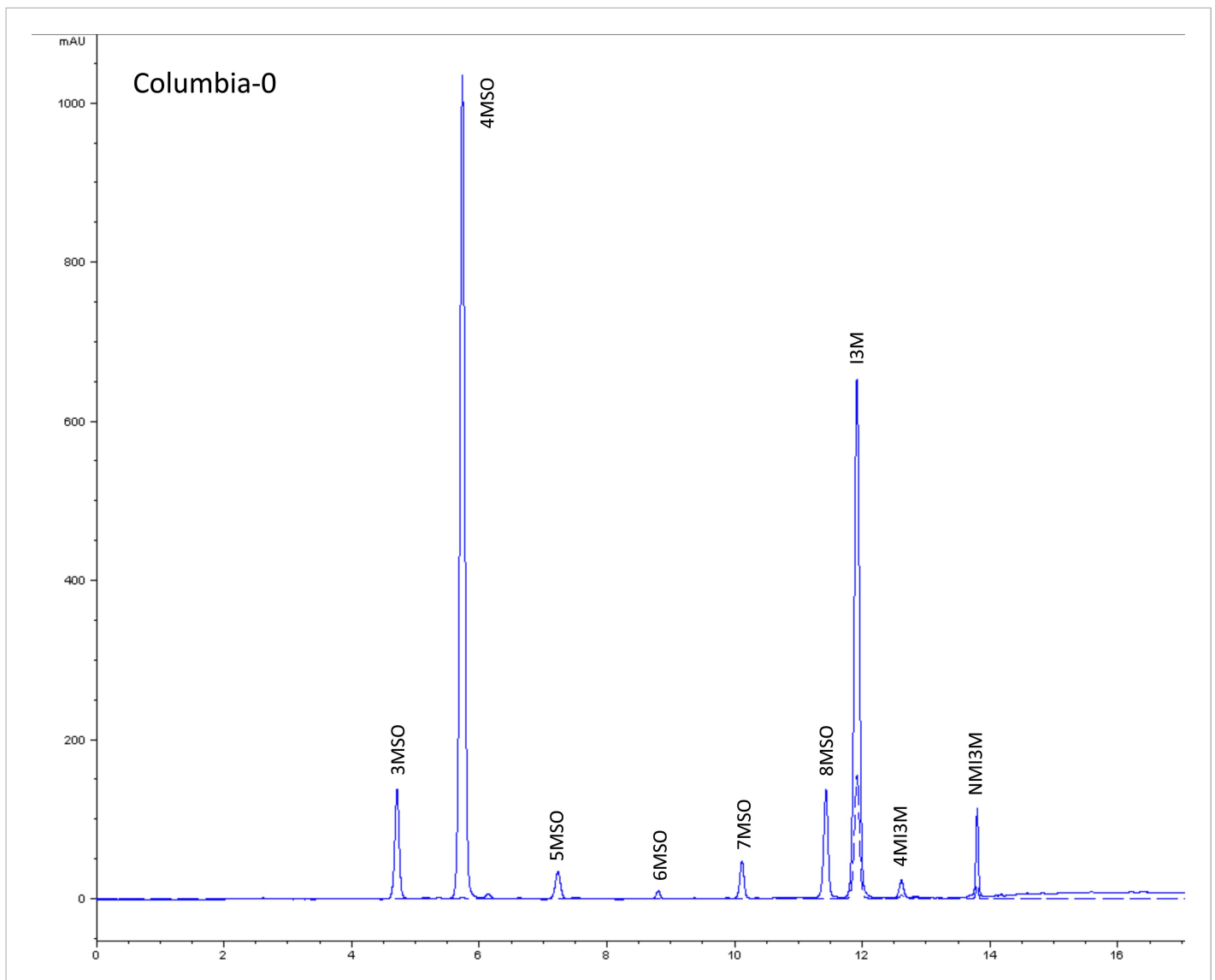


**Figure 1.** Overview of aliphatic GSL biosynthesis and activation in *Arabidopsis thaliana*. Arrows represent the steps involved in aliphatic glucosinolate (GSL) biosynthesis that have been validated through laboratory experiments and are naturally variable within *A. thaliana*. Gene names are listed next to or above the arrows. **(A) Regulation of aliphatic GSL biosynthesis.** The transcription factors MYB28 and MYB29 control accumulation of aliphatic GLS. A double knockout in these genes results in no aliphatic GLS accumulation, while a single knockout in these genes leads to a 50% reduction in aliphatic GSL (myb28) or a 25% reduction in aliphatic GSL (myb29), compared to WT Col-0. The biosynthetic enzymes MAM1 and AOP2 also influence aliphatic GLS accumulation and a non-functional allele at either locus leads to decreased GLS accumulation. **(B) Amino acid chain elongation.** During chain elongation, carbons are added to a methionine precursor through a series of reactions producing an elongated amino acid. Variation at the Elong locus controls the number of carbons added to the amino acid precursor and therefore, the length of the GSL side chain, R. A functional allele at this locus, MAM1, leads primarily to accumulation of GSL with four carbon (4C) length side chains, whereas a non-functional allele, gsm1 leads to accumulation of GSL with three carbon (3C) length side chains. The elongated amino acid is subsequently converted into a GLS via the core pathway (not shown). **(C) Side chain modification.** The GSL compounds produced can then undergo a series of side chain modifications that lead to the suite of diverse GSL compounds *Figure 1. continued on next page*

Figure 1. Continued

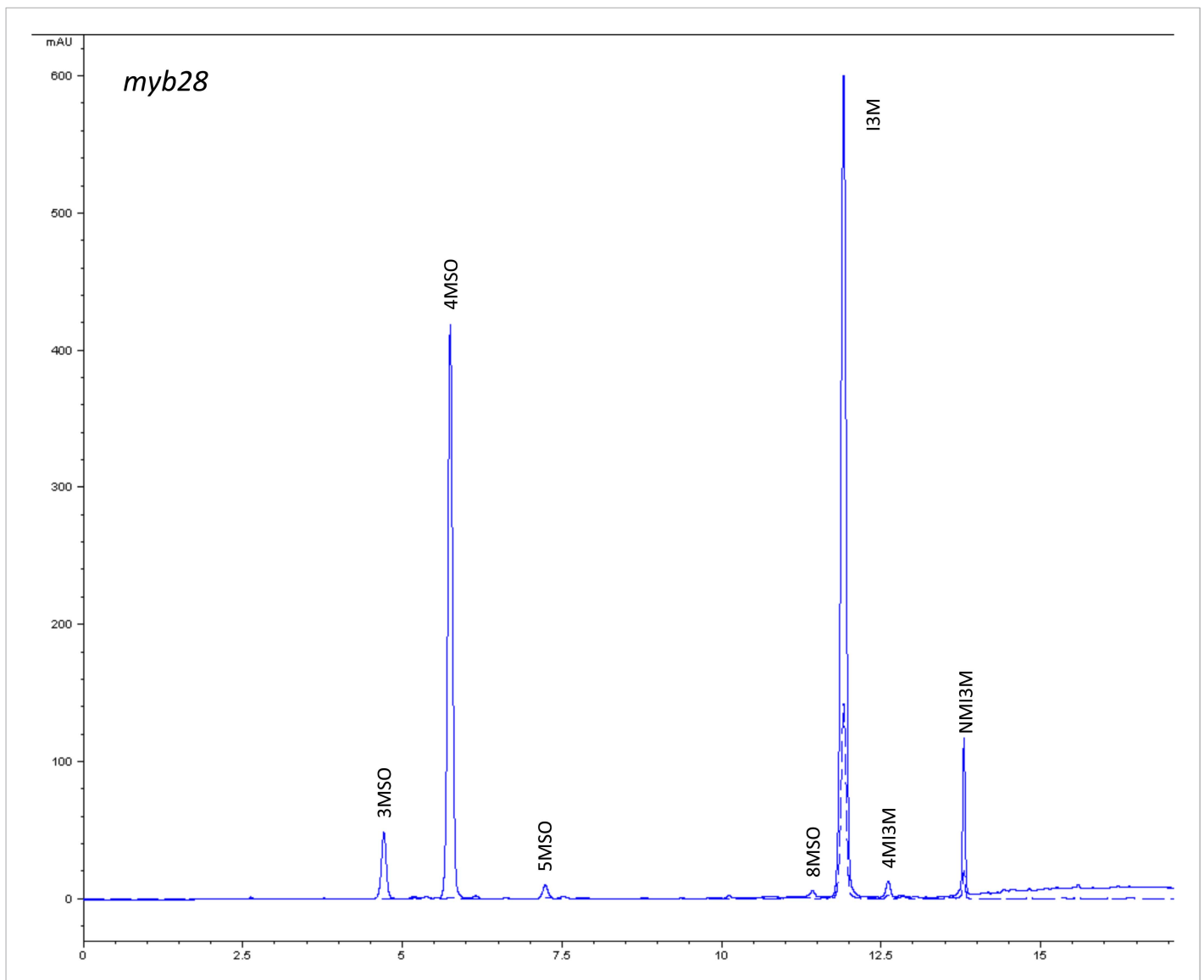
found in *Arabidopsis*. Side chain modification is controlled by variation of GSOX1, GSOX3, AOP2, AOP3 and GSOH. GSOX1 & GSOX3 oxygenate a methylthio (MT) to methylsulfinyl (MSO) GSL. AOP2 converts MSO to alkenyl, such as allyl and but-3-enyl. AOP3, on the other hand converts only 3C length MSO to OH-propyl GSL and cannot act on the 4MSO GSL. GSOH oxygenates the 4C but-3-enyl to the OH-alkenyl GSL, OH-but-3-enyl. Since GSOH acts on but-3-enyl GSL, which is a product of AOP2, a functional AOP2 is necessary for GSOH to function and AOP2 is said to be epistatic to GSOH. Col-0 is functional for MAM1 and the GSOX's, null for both AOP2 and AOP3, and functional for GSOH, resulting in accumulation of primarily 4MSO GLS. See **Figure 1—figure supplements 1–17** for images of GSL traces for each GSL genotypes in our mutant laboratory population. **(D) GSL Activation.** Once produced, GLS are stored in the vacuole in their stable, unreactive form until activation occurs. Upon cellular disruption, such as occurs during pathogen attack, insect herbivory or even wind damage, GLS come into contact with their own plant-made activating enzyme, myrosinase. After production, myrosinase is stored in vacuoles of idioblastic cells called myrosin bodies. Myrosinase activates the GSL compound by cleaving the glucose moiety, yielding an unstable aglycone structure that non-enzymatically rearranges to either nitriles or isothiocyanates, depending on the presence of the co-activators ESM1 and ESP.

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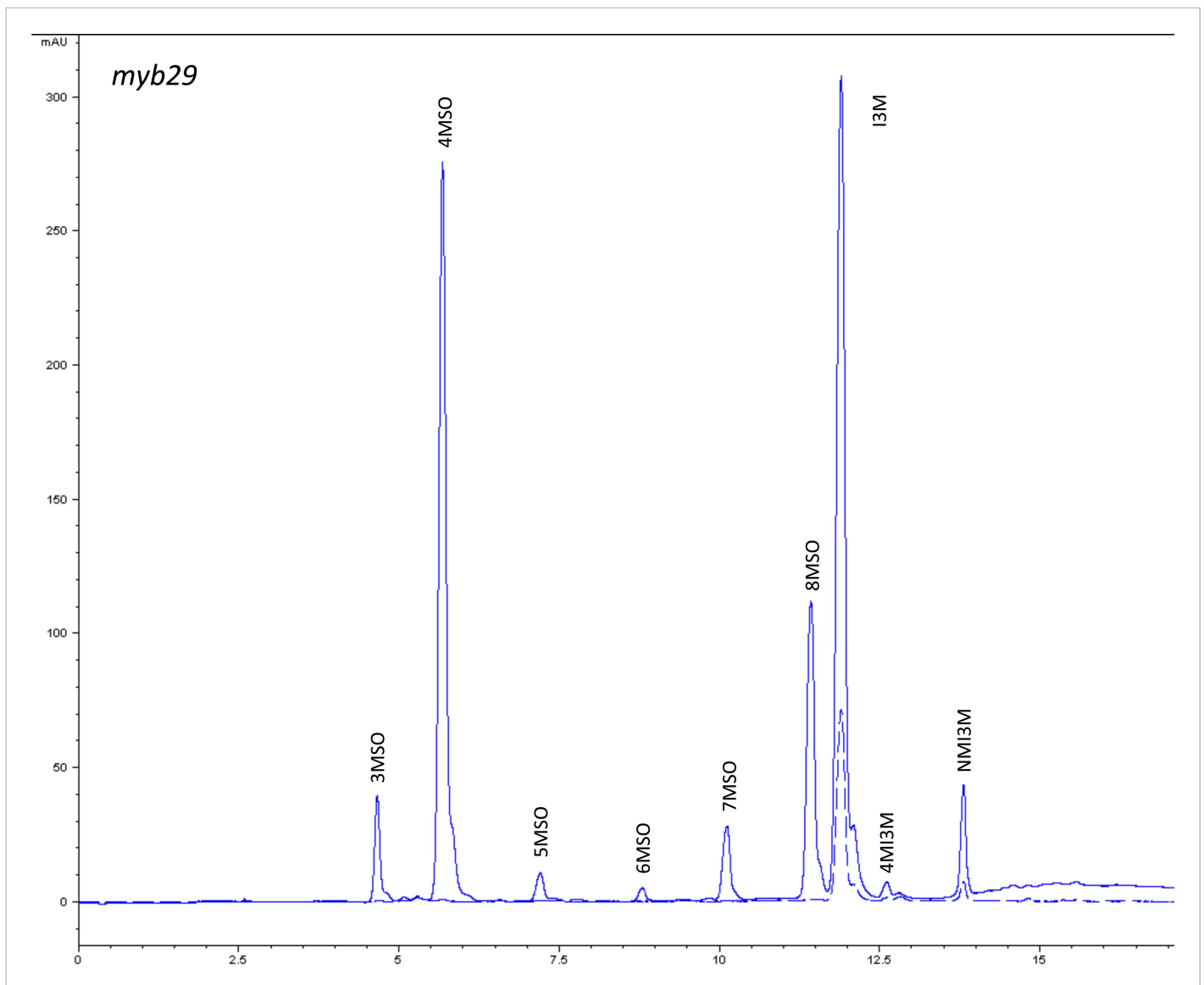
**Figure 1—figure supplement 1.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 wild-type genotype. Shown is an HPLC trace from a representative individual of field-grown Col-0 that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 5MSO = 5-Methylsulfinylpentyl GSL, 6MSO = 6-Methylsulfinylhexyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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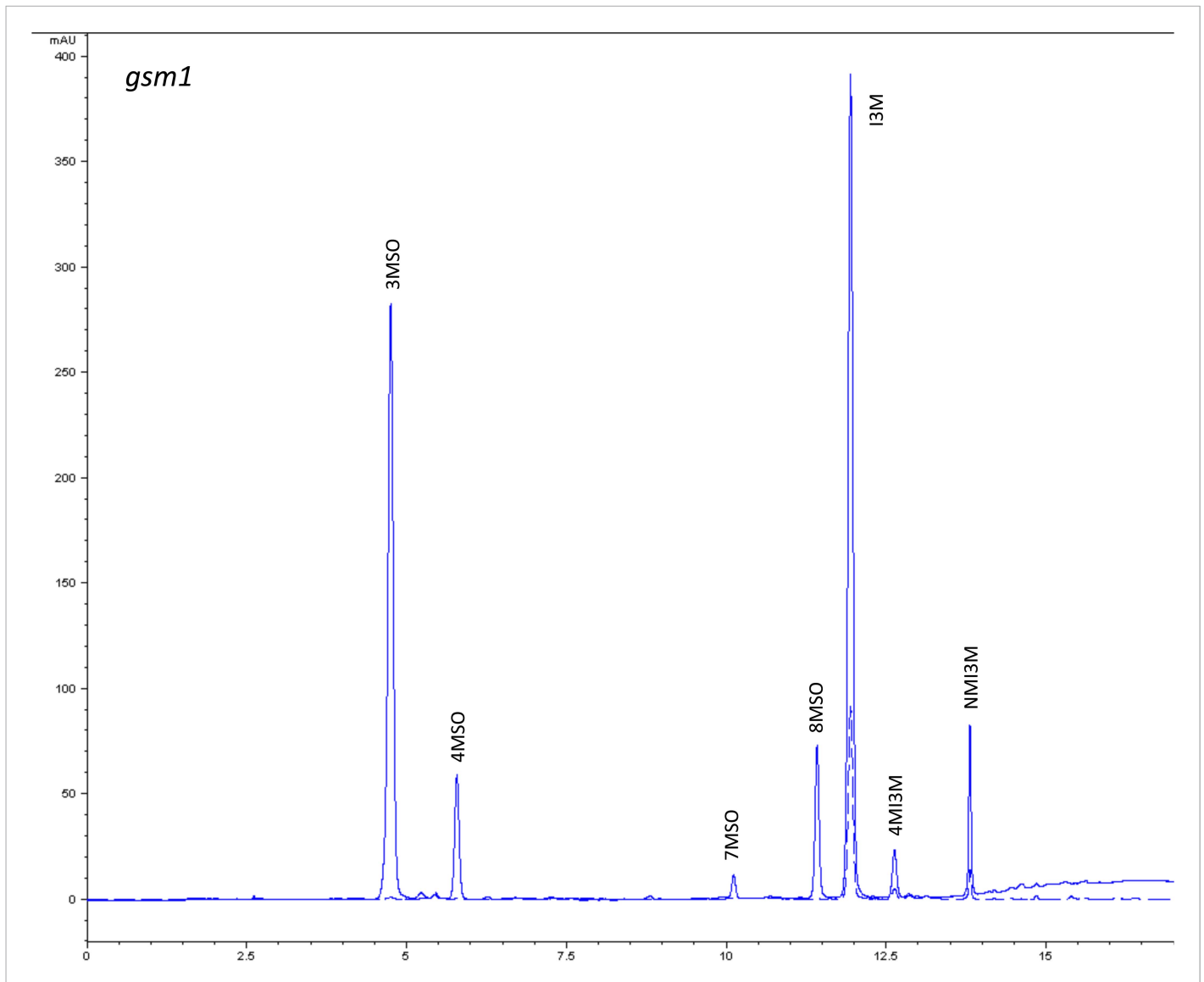
**Figure 1—figure supplement 2.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 *myb28* genotype. Shown is an HPLC trace from a representative individual of field-grown *myb28* genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 5MSO = 5-Methylsulfinylpentyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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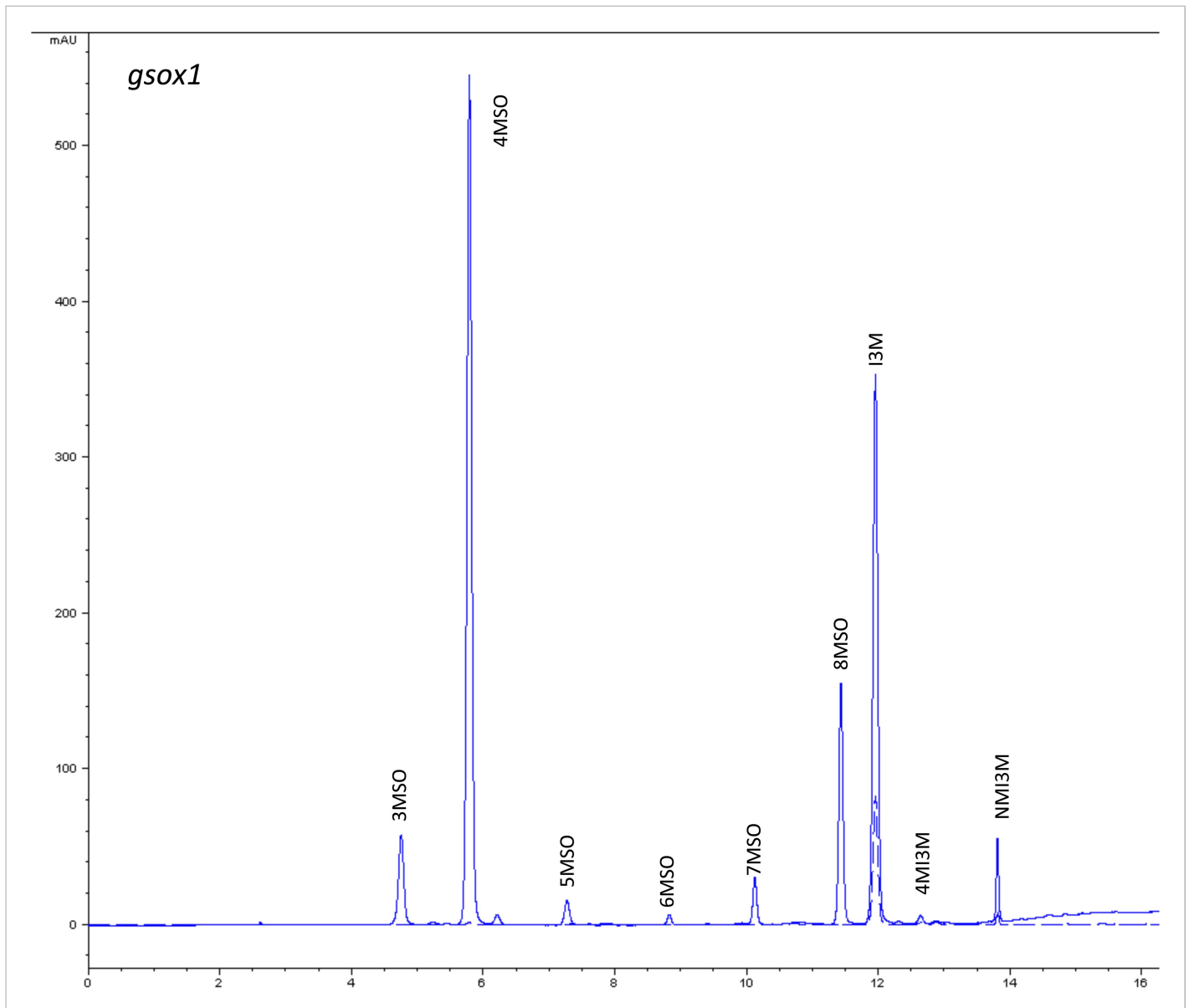
**Figure 1—figure supplement 3.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 *myb29* genotype. Shown is an HPLC trace from a representative individual of field-grown *myb29* genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 5MSO = 5-Methylsulfinylpentyl GSL, 6MSO = 6-Methylsulfinylhexyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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**Figure 1—figure supplement 4.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 *gsm1* genotype. Shown is an HPLC trace from a representative individual of field-grown *gsm1* genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

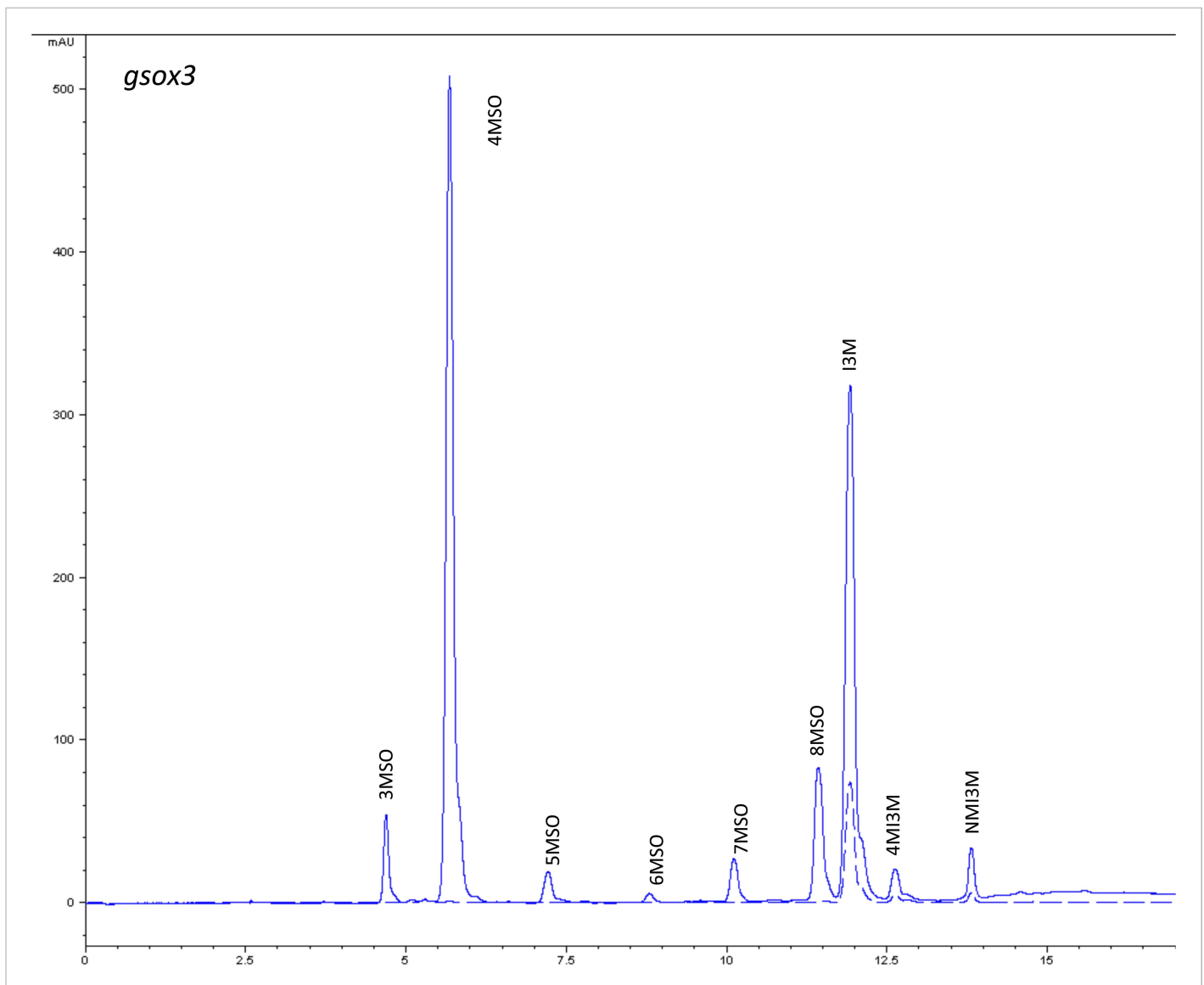
DOI: [10.7554/eLife.05604.007](https://doi.org/10.7554/eLife.05604.007)



**Figure 1—figure supplement 5.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 *gsox1* genotype. Shown is an HPLC trace from a representative individual of field-grown *gsox1* genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 5MSO = 5-Methylsulfinylpentyl GSL, 6MSO = 6-Methylsulfinylhexyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, 13M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

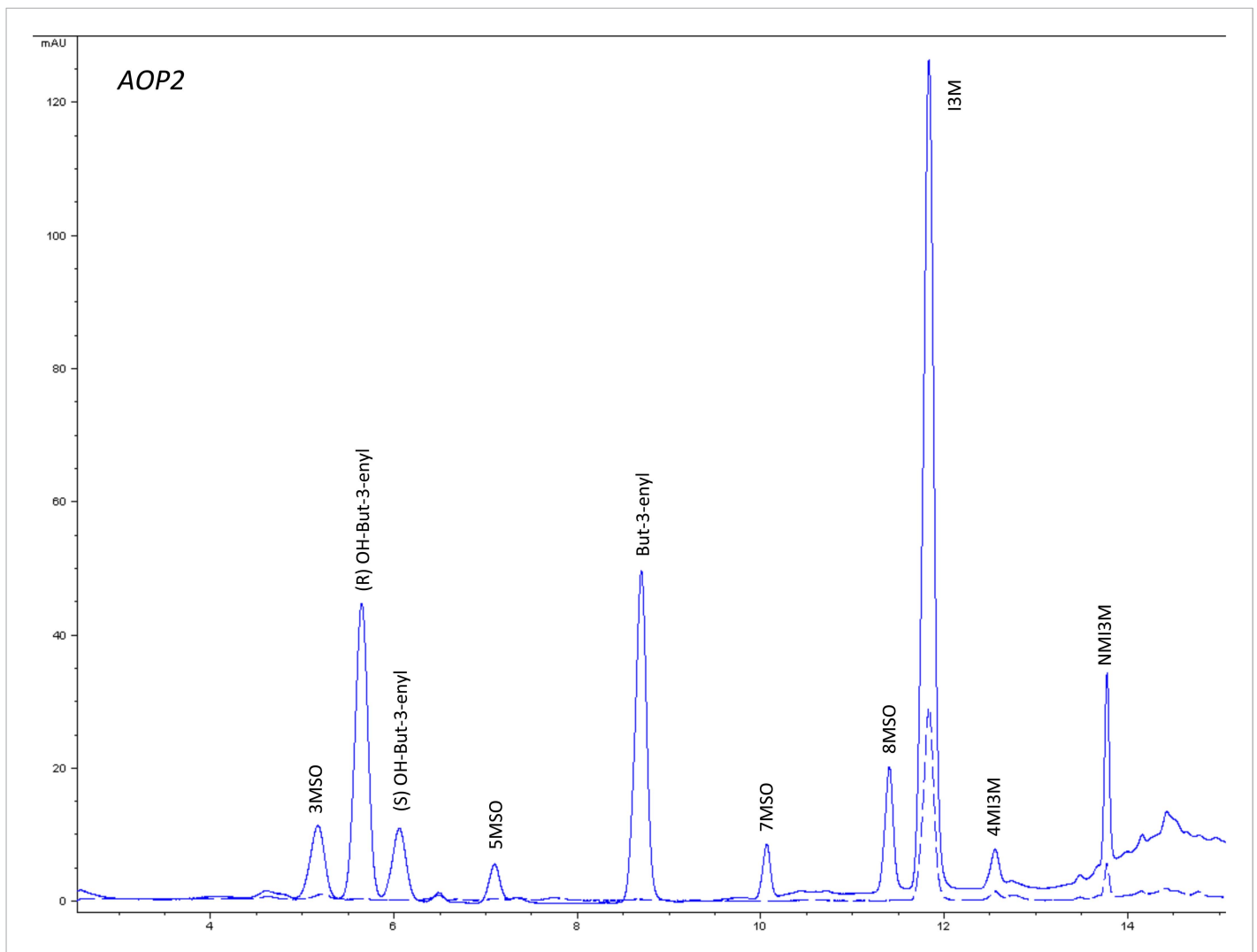
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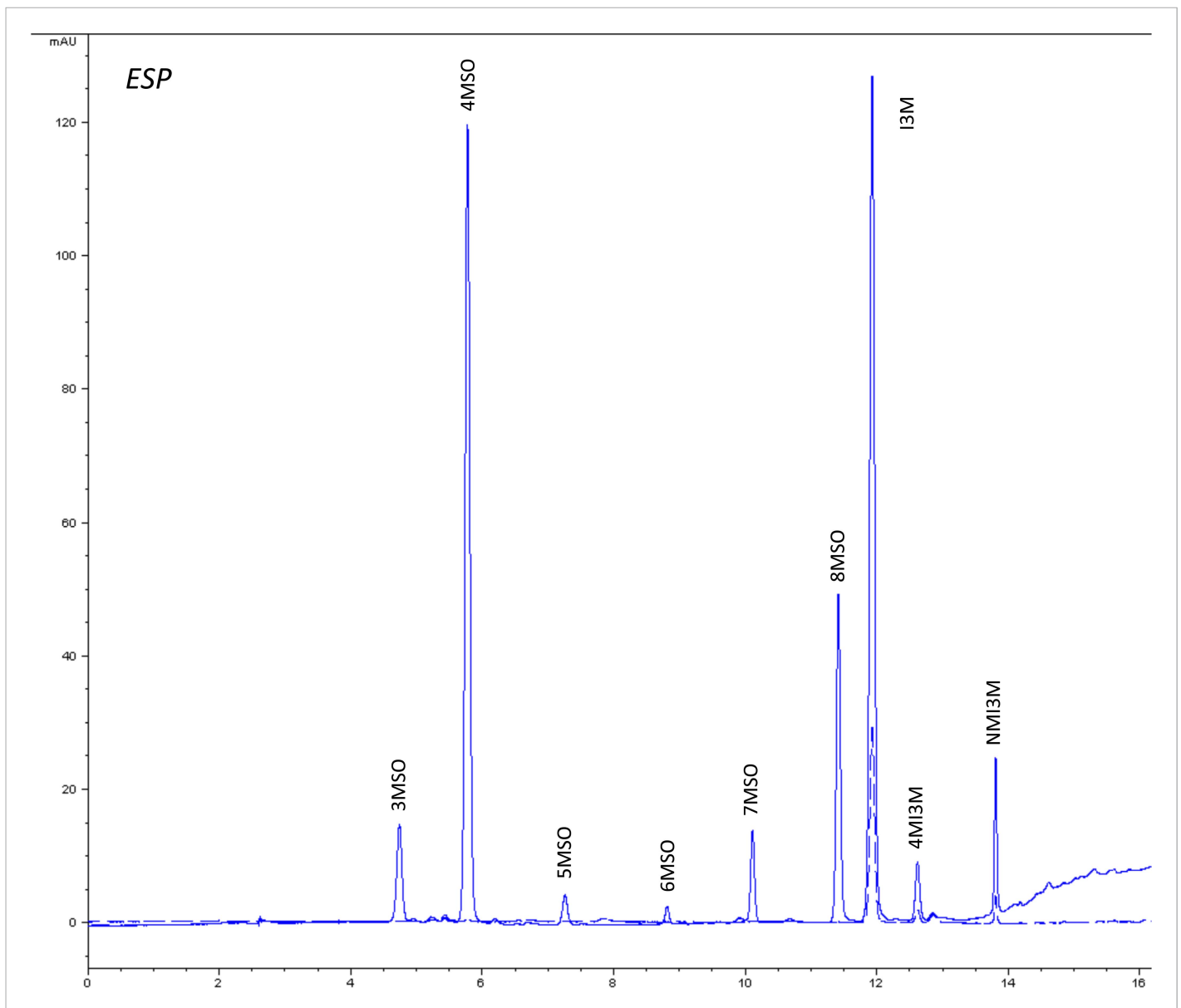
**Figure 1—figure supplement 6.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 gsox3 genotype. Shown is an HPLC trace from a representative individual of field-grown gsox3 genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 5MSO = 5-Methylsulfinylpentyl GSL, 6MSO = 6-Methylsulfinylhexyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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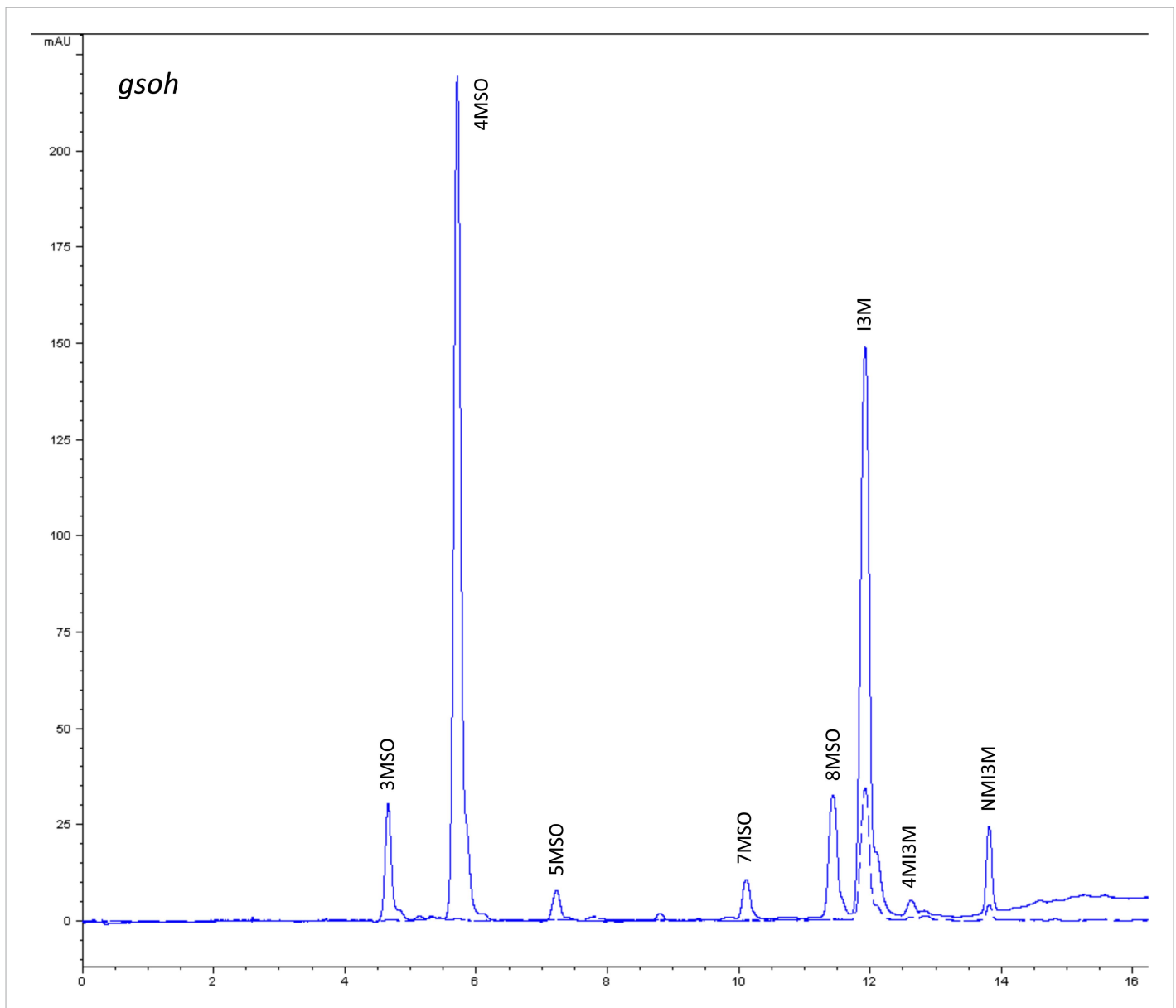
**Figure 1—figure supplement 7.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 AOP2 genotype. Shown is an HPLC trace from a representative individual of field-grown AOP2 genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), (R) OH-But-3-enyl = (R) OH-But-3-enyl GSL, (S) OH-But-3-enyl = (S) OH-But-3-enyl GSL, 5MSO = 5-Methylsulfinylpentyl GSL, But-3-enyl = But-3-enyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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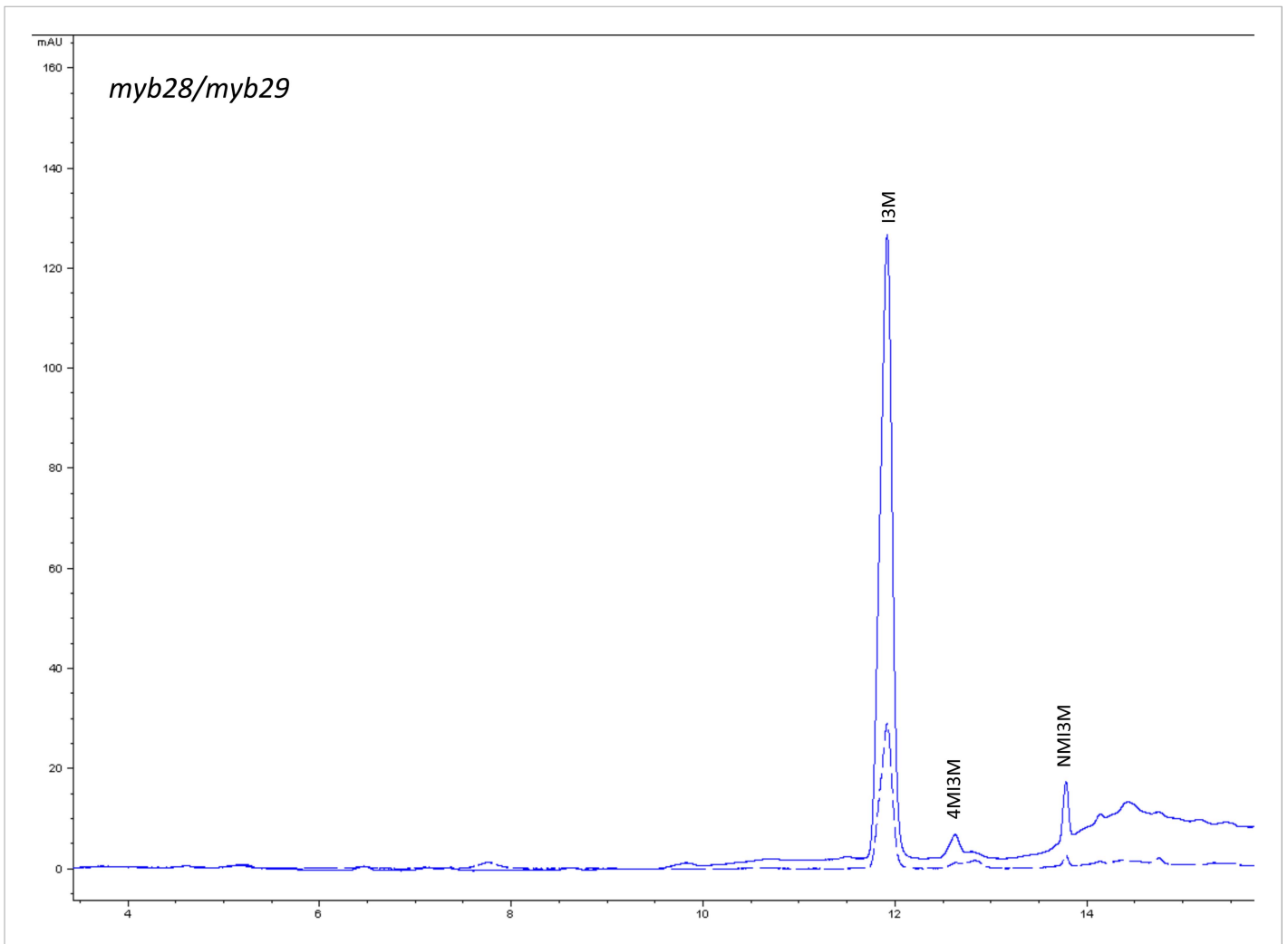
**Figure 1—figure supplement 8.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 ESP genotype. Shown is an HPLC trace from a representative individual of field-grown ESP genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 5MSO = 5-Methylsulfinylpentyl GSL, 6MSO = 6-Methylsulfinylhexyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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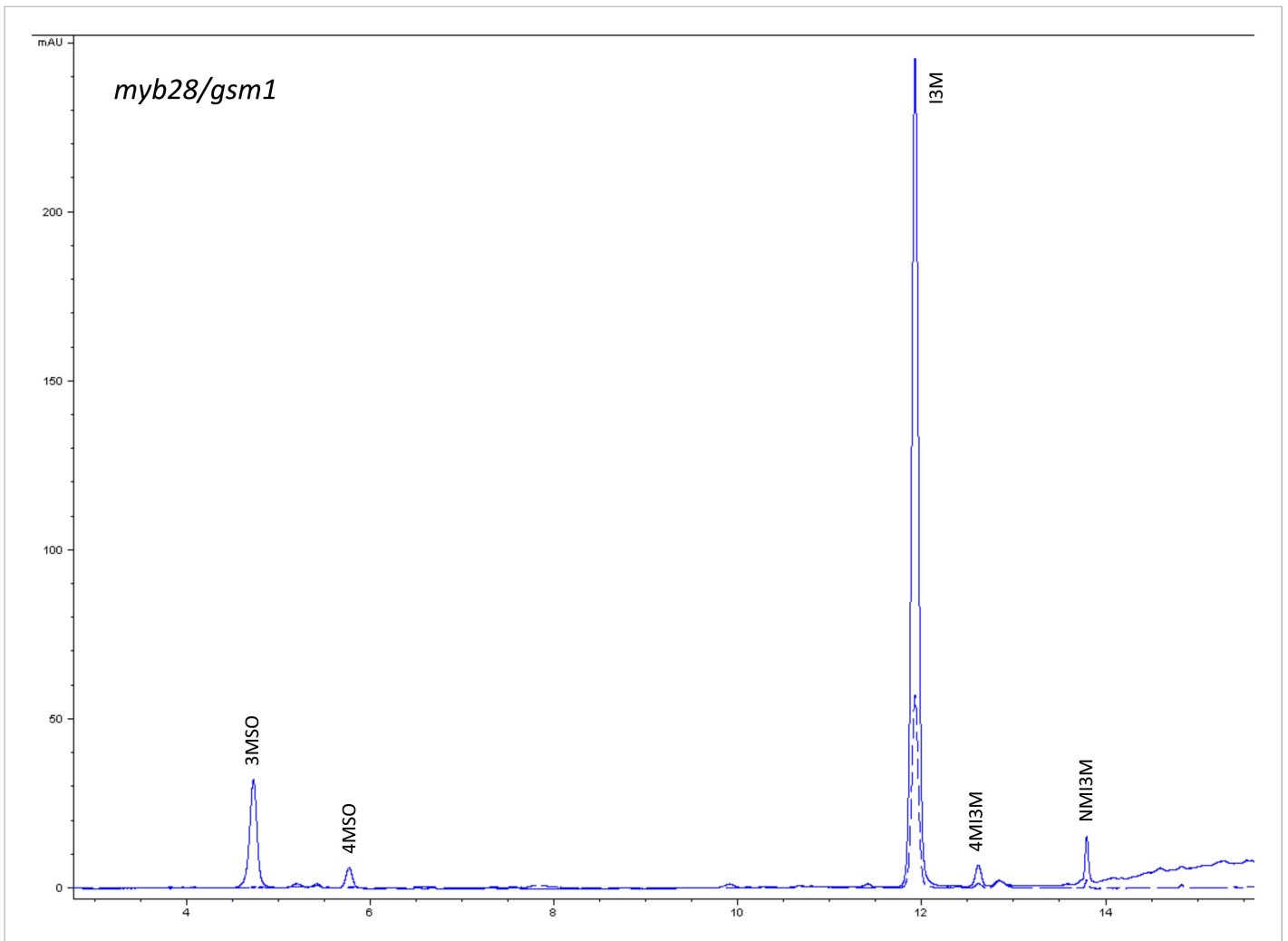
**Figure 1—figure supplement 9.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 *gsoh* genotype. Shown is an HPLC trace from a representative individual of field-grown *gsoh* genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 5MSO = 5-Methylsulfinylpentyl GSL, But-3-enyl = But-3-enyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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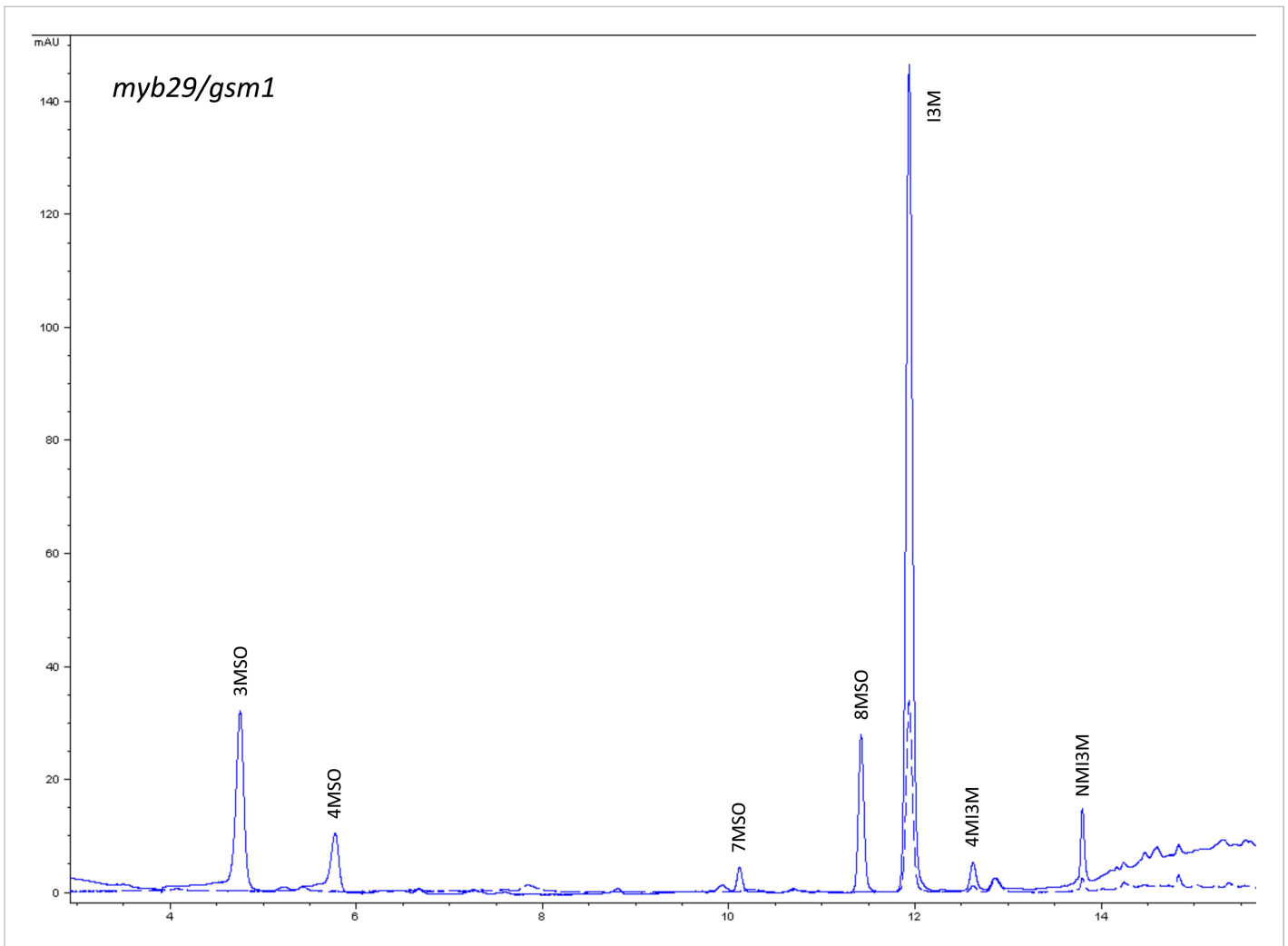
**Figure 1—figure supplement 10.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 myb28/myb29 genotype. Shown is an HPLC trace from a representative individual of field-grown myb28/myb29 genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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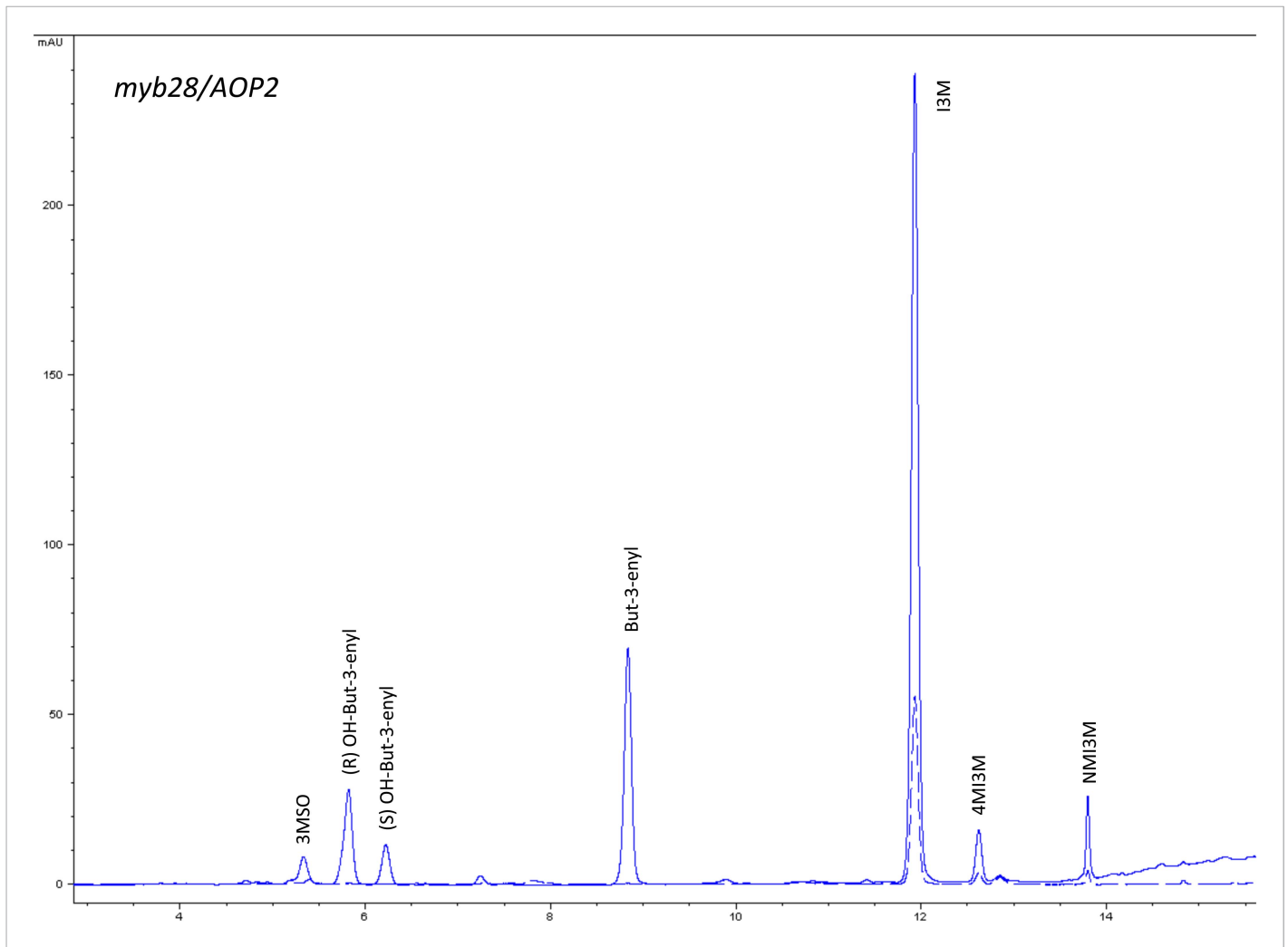
**Figure 1—figure supplement 11.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 *myb28/gsm1* genotype. Shown is an HPLC trace from a representative individual of field-grown *myb28/gsm1* genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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**Figure 1—figure supplement 12.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 *myb29/gsm1* genotype. Shown is an HPLC trace from a representative individual of field-grown *myb29/gsm1* genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

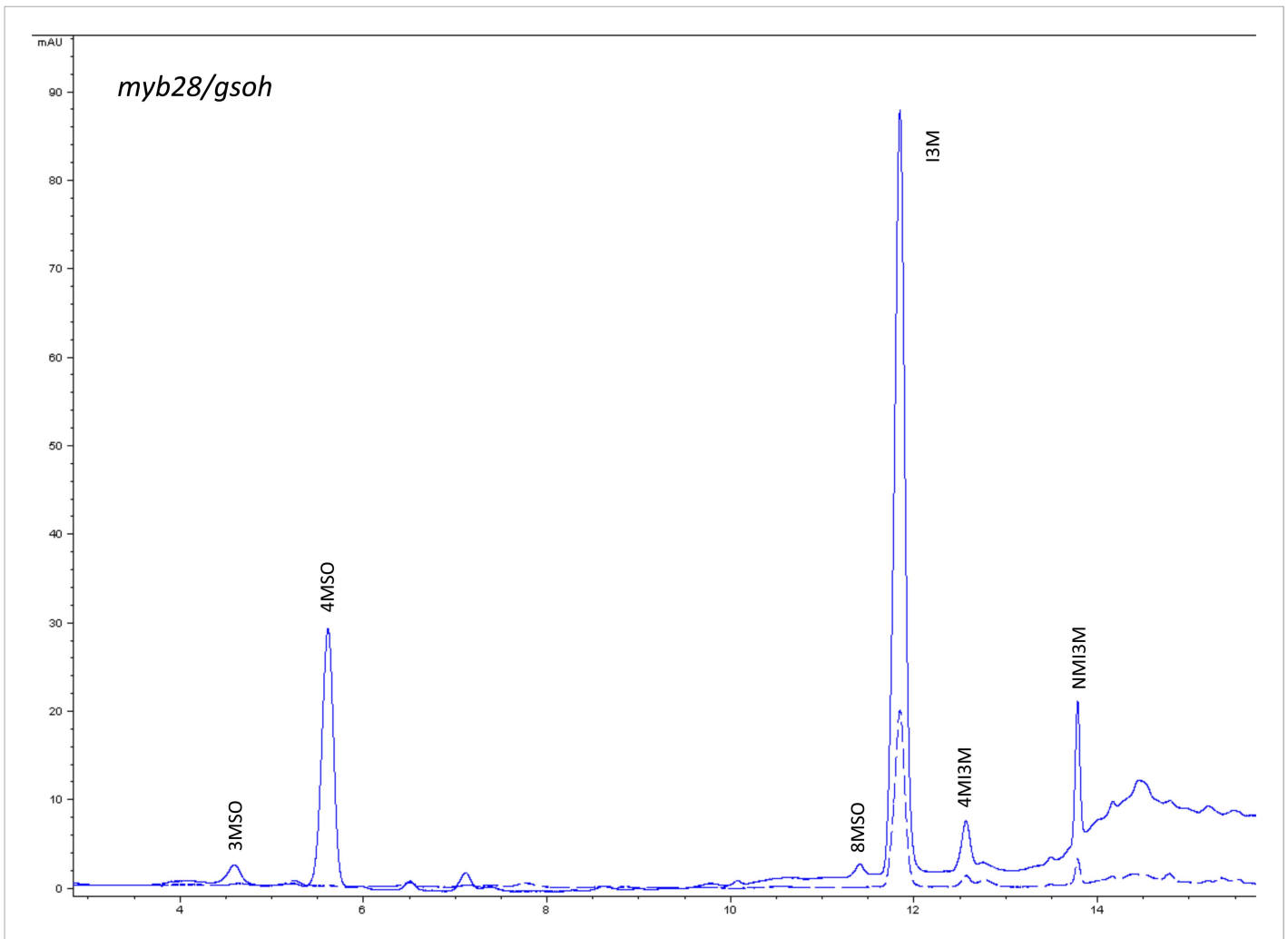
DOI: [10.7554/eLife.05604.015](https://doi.org/10.7554/eLife.05604.015)



**Figure 1—figure supplement 13.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 myb28/AOP2 genotype. Shown is an HPLC trace from a representative individual of field-grown myb28/AOP2 genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), (R) OH-But-3-enyl = (R) OH-But-3-enyl GSL, (S) OH-But-3-enyl = (S) OH-But-3-enyl GSL, But-3-enyl = But-3-enyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

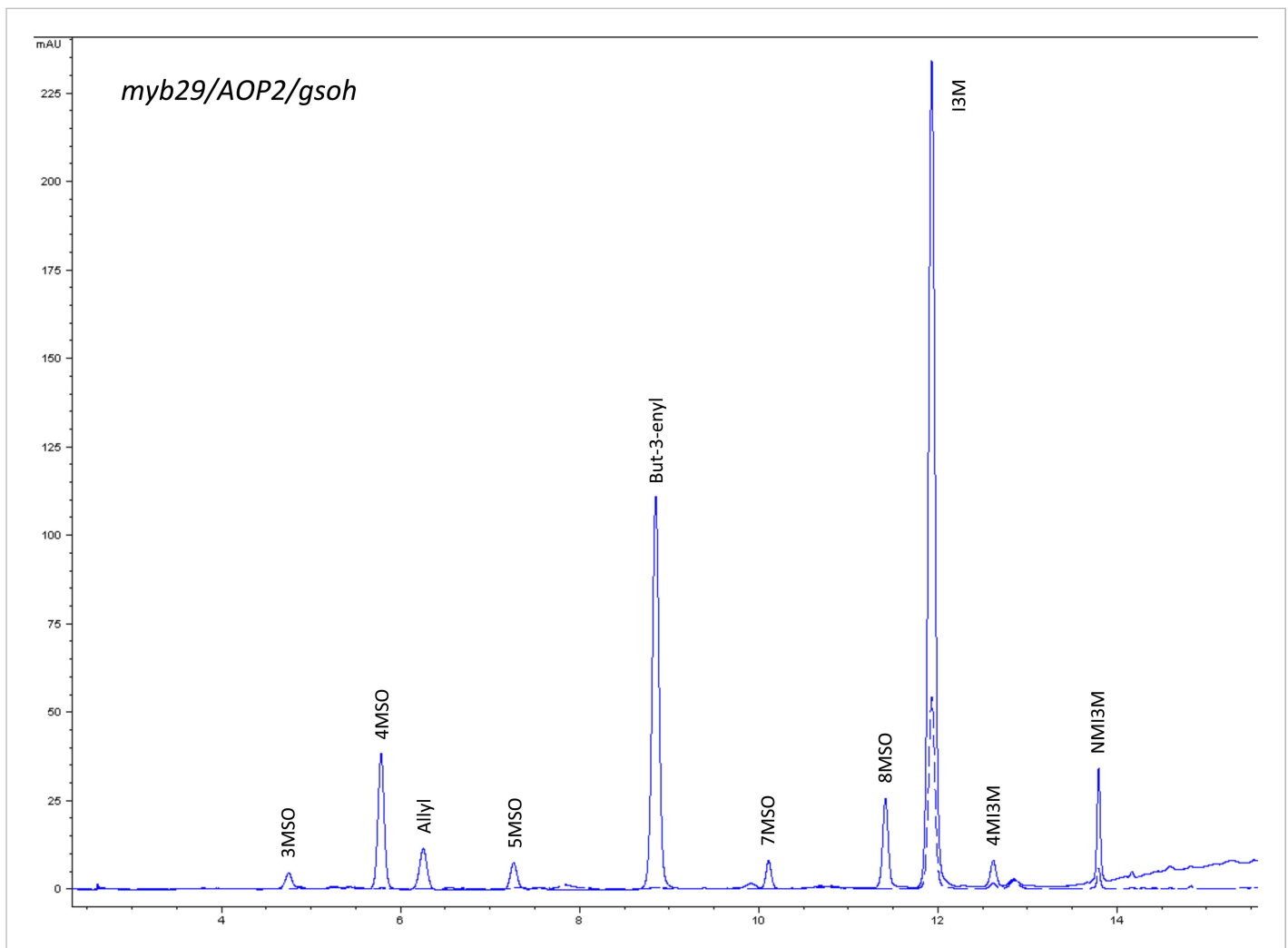
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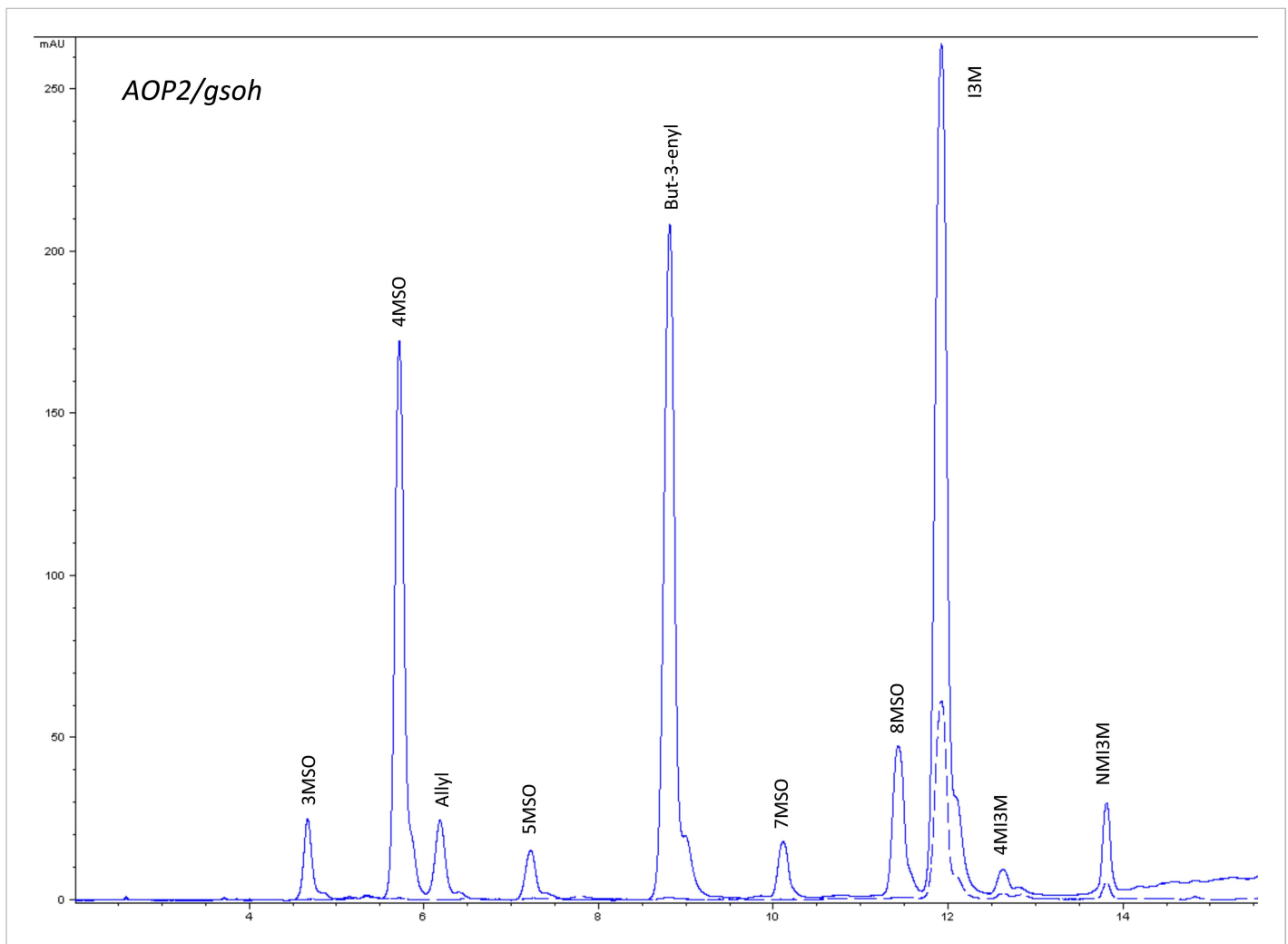
**Figure 1—figure supplement 14.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 *myb28/gsoh* genotype. Shown is an HPLC trace from a representative individual of field-grown *myb28/gsoh* genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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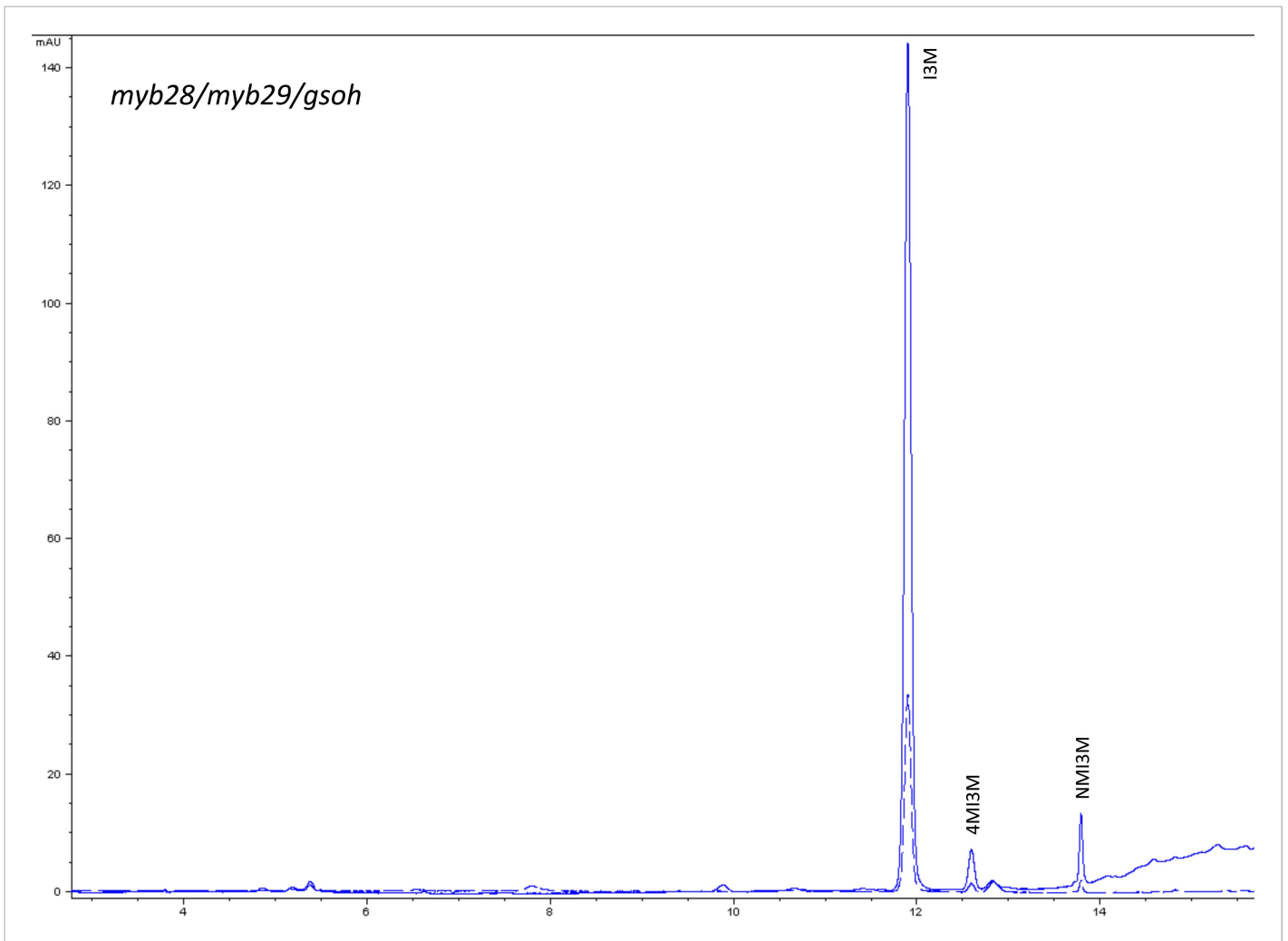
**Figure 1—figure supplement 15.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 *myb29/AOP2/gsoh* genotype. Shown is an HPLC trace from a representative individual of field-grown *myb29/AOP2/gsoh* genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, Allyl = Allyl (Propenyl) GSL, 5MSO = 5-Methylsulfinylpentyl GSL, But-3-enyl = But-3-enyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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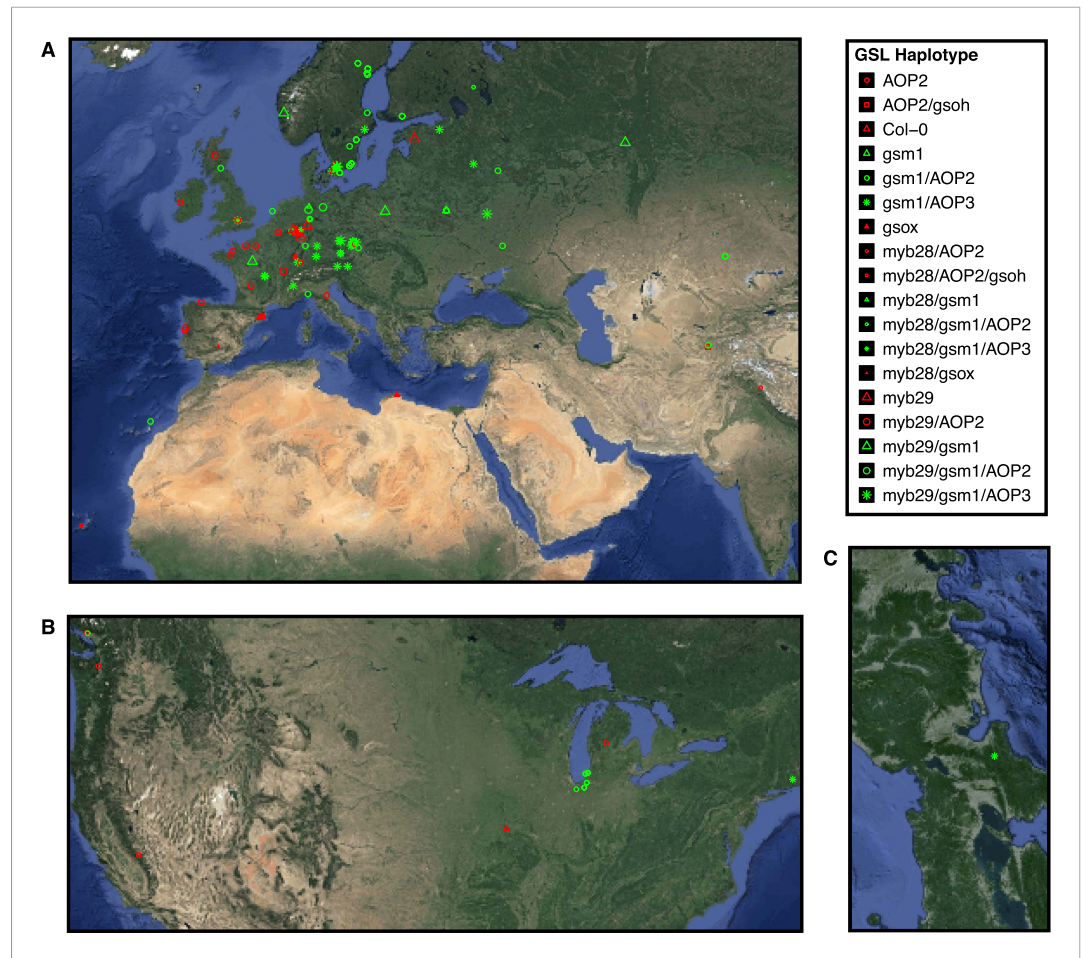
**Figure 1—figure supplement 16.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 AOP2/gsoh genotype. Shown is an HPLC trace from a representative individual of field-grown AOP2/gsoh genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. 3MSO = 3-Methylsulfinylpropyl glucosinolate (GSL), 4MSO = 4-Methylsulfinylbutyl GSL, Allyl = Allyl (Propenyl) GSL, 5MSO = 5-Methylsulfinylpentyl GSL, But-3-enyl = But-3-enyl GSL, 7MSO = 7-Methylsulfinylheptyl GSL, 8MSO = 8-Methylsulfinyloctyl GSL, I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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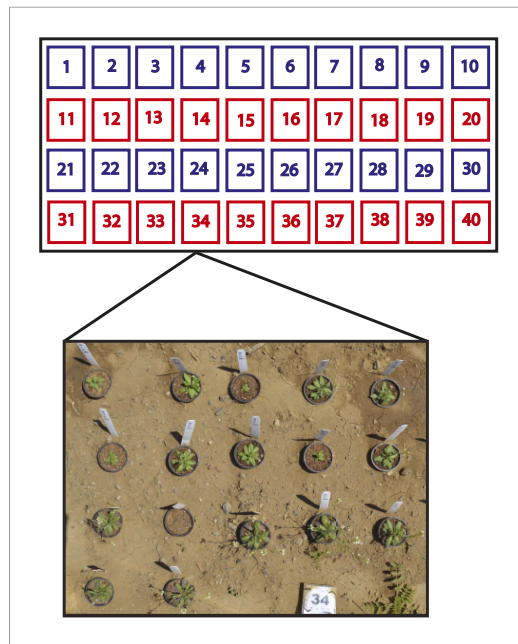
**Figure 1—figure supplement 17.** HPLC trace of *Arabidopsis thaliana* accession Columbia-0 myb28/myb29/gsoh genotype. Shown is an HPLC trace from a representative individual of field-grown myb28/myb29/gsoh genotype that depicts the GSL profile found within the leaves of this genotype. The mixture of various GSL structures present within the leaf and the relative amounts of each constitute the GSL profile in an individual. I3M = Indol-3-ylmethyl GSL, 4MI3M = 4-Methoxyindol-3-ylmethyl GSL, NMI3M = 1-Methoxyindol-3-ylmethyl GSL.

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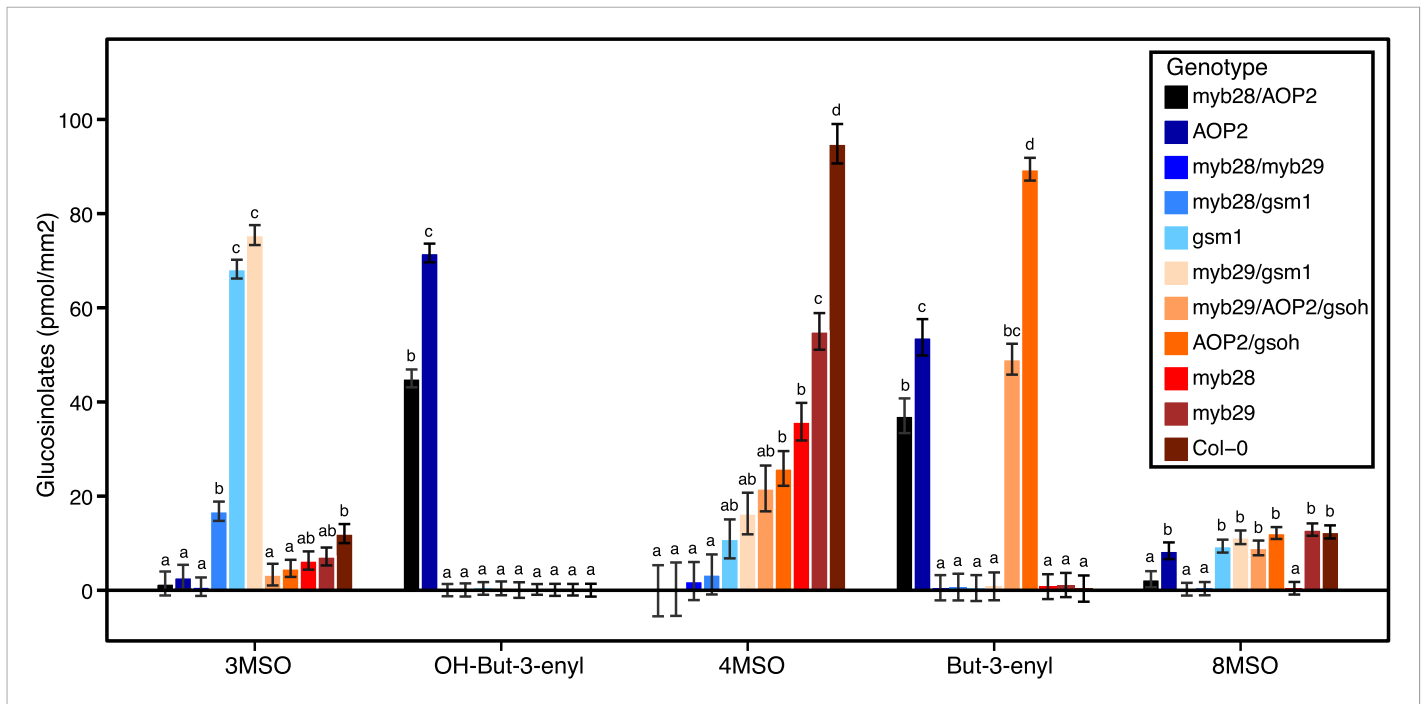
**Figure 2.** Globally distributed collection of *Arabidopsis thaliana* accessions that vary with respect to GSL haplotype. Shown are the geographic origins of 144 *Arabidopsis* accessions across (A) Europe and Northern Africa, (B) North America and (C) Japan, as well as their corresponding GSL haplotypes and chemotypes. GSL haplotype names correspond to allelic identity at six polymorphic loci involved in aliphatic GSL production, based on GSL profile data collected from each accession. Haplotype names use Col-0 as a reference, which is functional at four or the six loci. Symbol shape, color and size indicate GSL chemotype (i.e., phenotype based on GSL profile). Red = 3C (non-functional MAM1), green = 4C GSL (functional MAM1), triangle = MSO (non-functional AOP), square = alkenyl (functional AOP2), circle = OH-alkenyl (functional GSOH), star = OH-Propyl (functional AOP3), point size 1 = 100% accumulation of aliphatic GSL (compared to Col-0), point size 0.5 = 50% accumulation of aliphatic GSL (non-functional MYB28) and point size 1.5 = 75% accumulation of aliphatic GSL (non-functional MYB29). See **Figure 2—source data 1** for table of accession geographic information, **Figure 1** for schematic of biosynthetic pathway and **Figure 9** for more details on the allelic state at each locus for all 18 GSL haplotypes.

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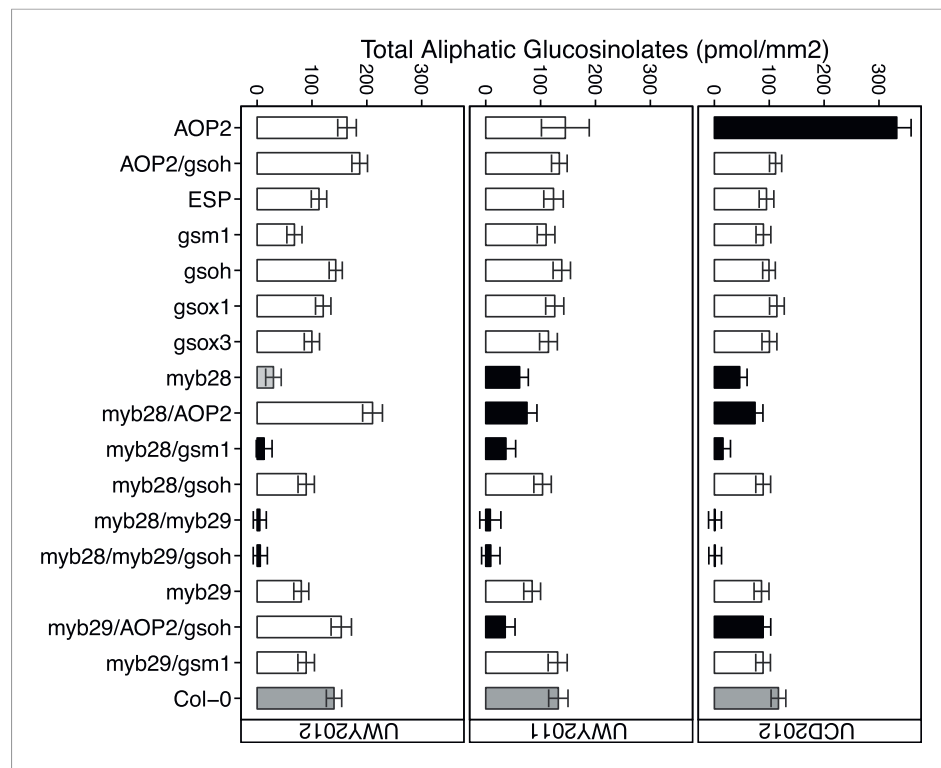
**Figure 3.** Split-plot field trial setup. Shown is the field trial setup used in all three environments. In each environment, 40 blocks were arranged into rows of 10 blocks and each row was called a plot. Within each block, the complete set of 17 genotypes was randomly organized, for a total of 40 genotype replicates per environment. Each plot (four per environment) was placed into one of two treatment groups. The ‘– Herbivory’ treatment group received pesticide application to prevent leaf damage (shown in blue). The ‘+ Herbivory’ or control treatment group did not receive pesticide application (shown in red). This setup was repeated in each of the three environments, for a total of 120 blocks/genotype replicates and 12 plots, split between the two treatment groups. Environment and treatment were nested within plot, making this field trial setup a split-plot design. Seedlings were transplanted from the greenhouse into the field at 2 weeks of age where they were allowed to flower and then subsequently harvested for further analysis in the laboratory.

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**Figure 4.** Average GSL profiles from select laboratory population genotypes grown in the field. Shown are the genotype averages of various aliphatic GSL chemical structures from GSL genotypes grown in all three environments in the field. The GSL structures present and the corresponding abundances make up the GSL profile of an individual. Results are based on single leaf analysis of 4 week old plants (see **Table 2** for full list of GSL genotypes used in this study). Each color represents a different aliphatic GSL genotype. Error bars represent standard error of the mean. Letters represent significantly different genotypes based on Tukey's HSD. See **Figure 4—source data 1** for full list of GSL genotypes used in this study and the corresponding LSM means and SE of GSL structures produced by all GSL genotypes used in study averaged across field trials.

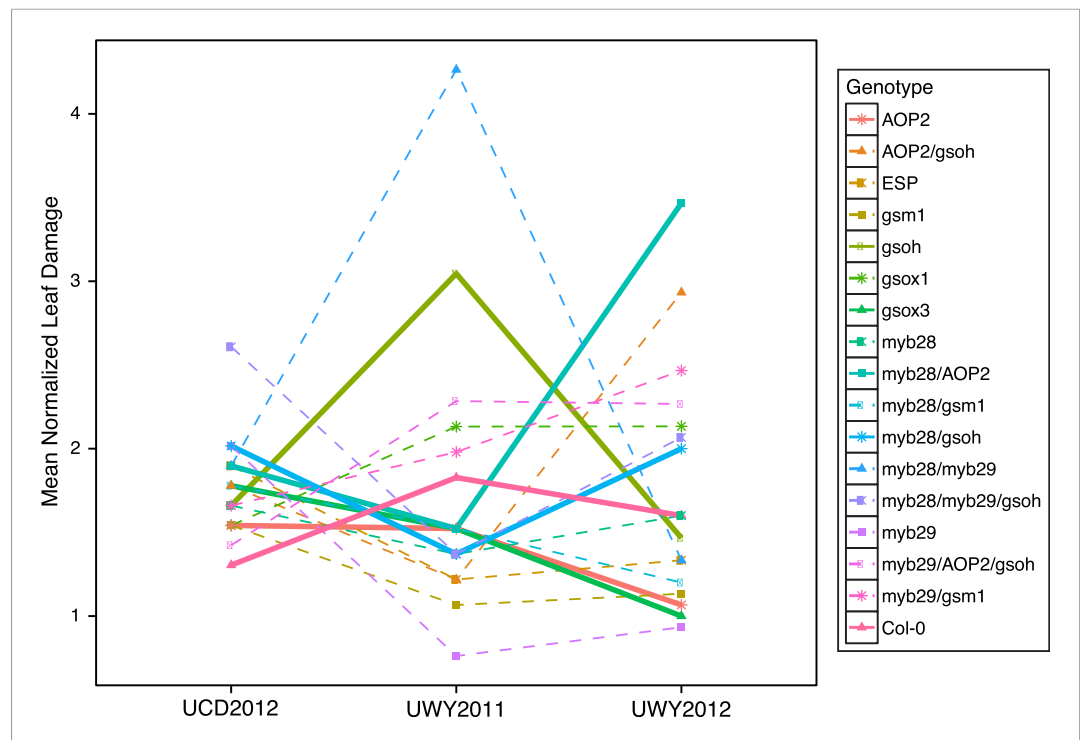
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**Figure 5.** Total aliphatic GSL accumulation of GSL genotypes from the laboratory population grown in the field. Shown are the genotype averages in all three environments of total aliphatic GSL from individuals grown in the field. Results are based on single leaf analysis of 4 week old plants. Bar color based on Dunnett's multiple comparison procedure. Within each environment, dark grey bars = Col-0 genotype, black bars = genotypes that accumulate significantly more or less total aliphatic GSL than Col-0 ( $p$  value  $\leq 0.05$ ), light grey bars = genotypes that accumulate suggestively more or less total aliphatic GSL than Col-0 ( $p$  value =  $0.05-0.1$ ) and white bars = genotypes that are not significantly different than Col-0 ( $p$  value  $>0.1$ ). Error bars represent standard error of the mean.

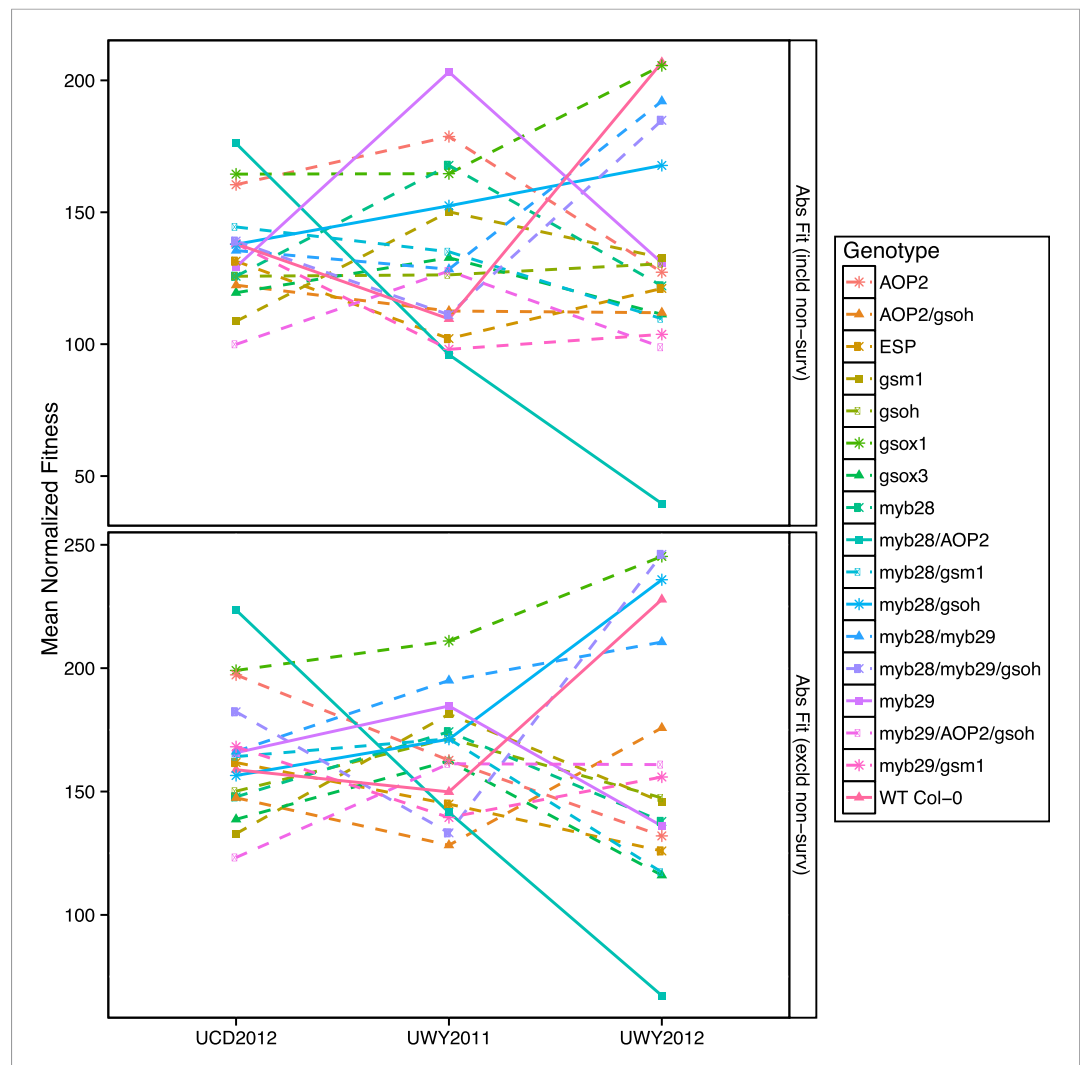
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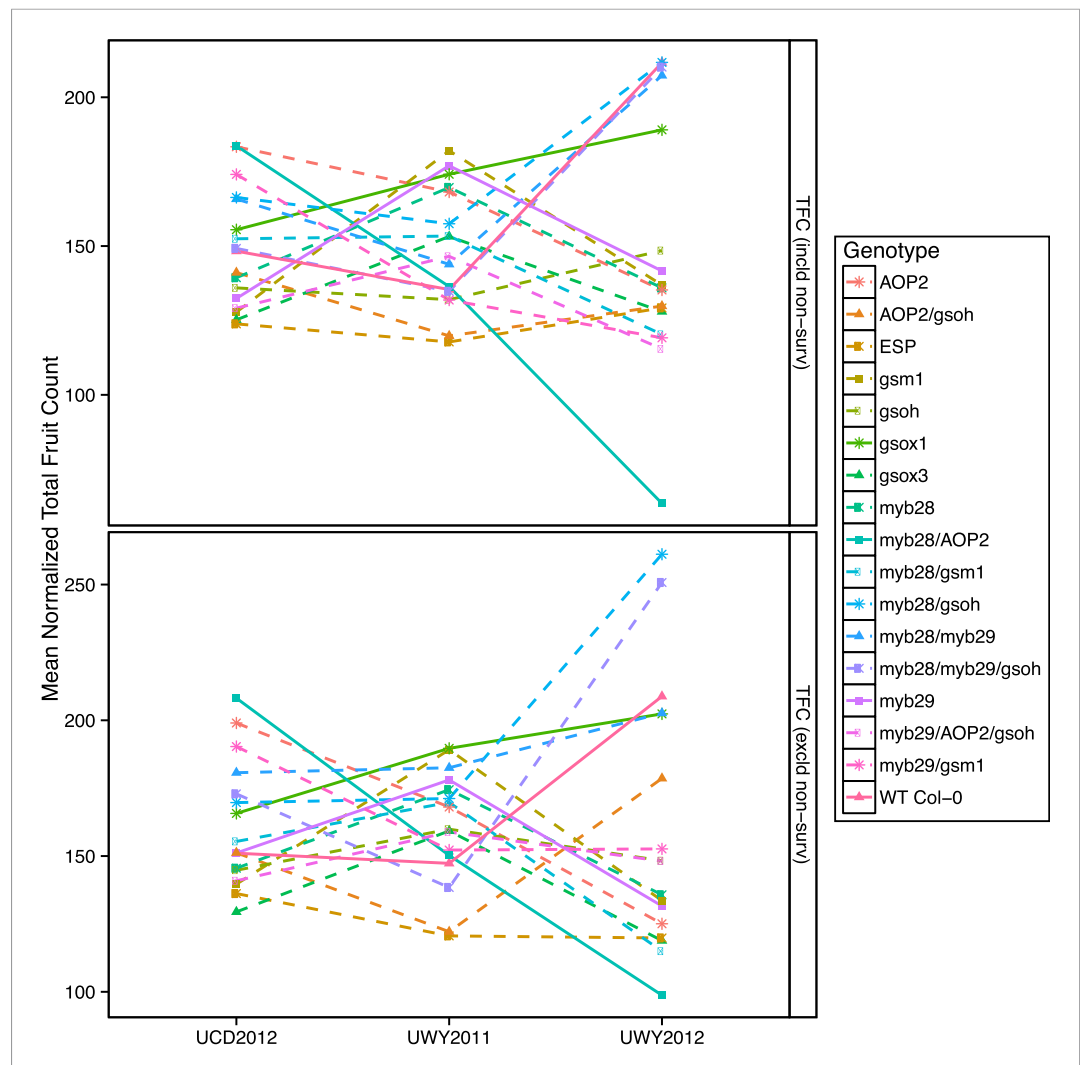
**Figure 6.** Mean normalized leaf damage of GSL genotypes from the laboratory population grown in the field. Shown are the mean normalized genotype averages in all three environments of leaf damage from GSL genotypes grown in the field. Mean normalization was conducted by first dividing the genotype average of each GSL genotype within an environment to the corresponding environment average. Then, each normalized value was multiplied by the grand mean across all three environments. This was done in order to put the leaf damage estimates in each environment on the same order of magnitude to ease visual comparisons of genotypes across environments and to highlight the fact that relative leaf damage of a given GSL genotype varies across environments.

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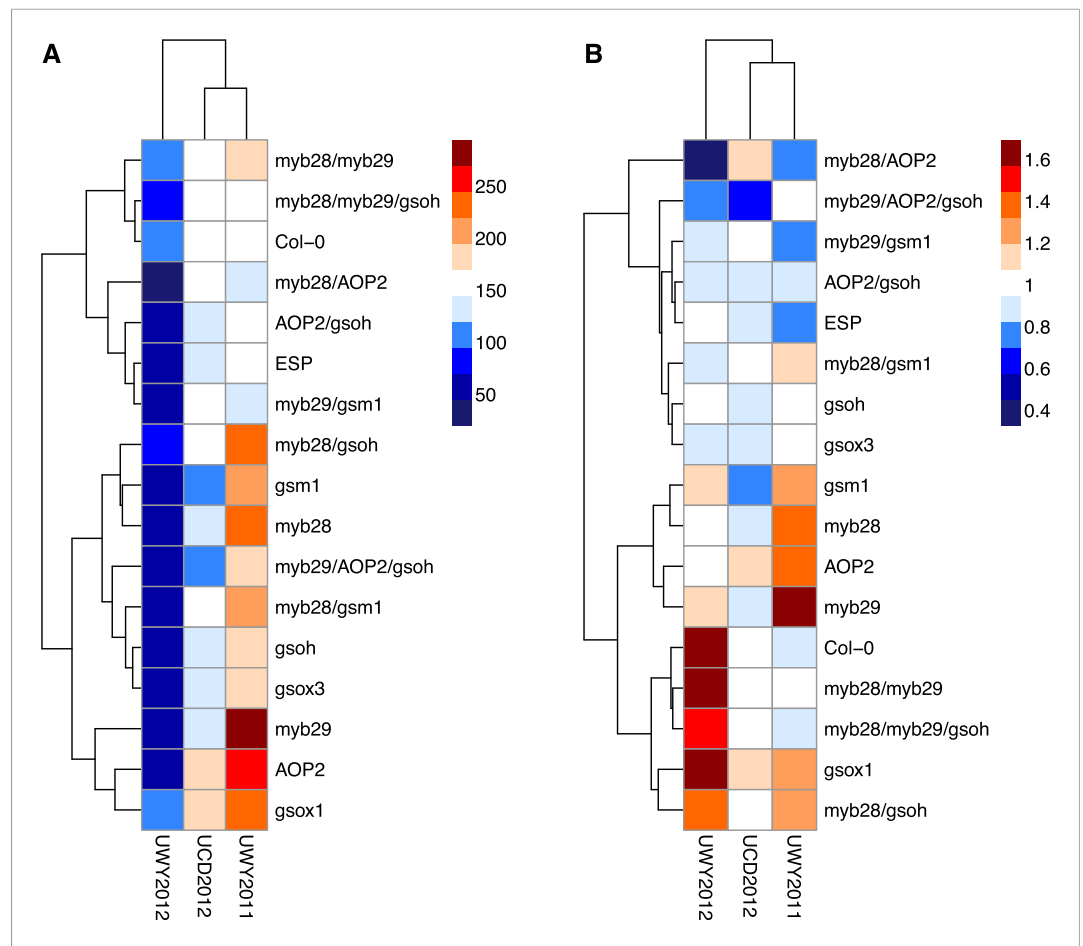
**Figure 7.** Mean normalized absolute fitness of GSL genotypes from the laboratory population grown in the field. Shown are the mean normalized genotype averages of absolute fitness from GSL genotypes grown in all three environments calculated either including or excluding survivorship, as indicated. Absolute fitness including survivorship was calculated as total fruit count (TFC)  $\times$  silique length  $\times$  survivorship, whereas absolute fitness excluding survivorship was calculated as TFC  $\times$  silique length for individuals that survived to harvest. Mean normalization was conducted for each phenotype by first dividing the average of each GSL genotype within an environment to the corresponding population mean for each environment. Then, each normalized value was multiplied by the grand mean across all three environments. This was done in order to put the phenotype estimates in each environment on the same order of magnitude to ease visual comparisons. Solid lines represent distinct patterns that GSL genotypes display across the environments and are meant as a visual aid.

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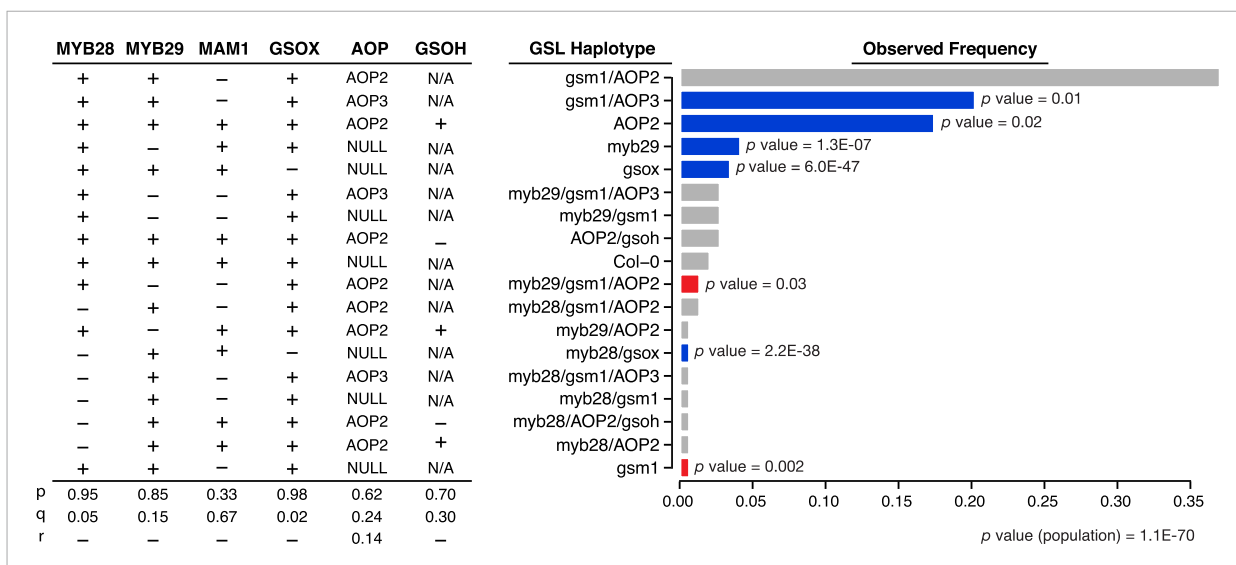
**Figure 7—figure supplement 1.** Mean normalized total fruit count of GSL genotypes from the laboratory population grown in the field. Shown are the mean normalized genotype averages of total fruit count (TFC) from GSL genotypes grown in all three environments calculated either including or excluding survivorship, as indicated. TFC was measured in the laboratory as the total number of fruits, flowers and buds per individual. Mean normalization was conducted for each phenotype by first dividing the average of each GSL genotype within an environment to the corresponding population mean for each environment. Then, each normalized value was multiplied by the grand mean across all three environments. This was done in order to put the phenotype estimates in each environment on the same order of magnitude to ease visual comparisons of genotypes across environments. Solid lines represent distinct patterns that GSL genotypes display across the environments and are meant as a visual aid.

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**Figure 8.** Relative and absolute of GSL genotypes from the laboratory population grown in the field. Heatmaps with hierarchical clustering of GSL genotypes representing the model corrected means of (A) absolute fitness including survivorship and (B) relative fitness of each genotype in each environment. Absolute fitness was calculated for each individual as the total fruit count  $\times$  silique length  $\times$  survivorship. Relative fitness was calculated by normalizing absolute fitness for each genotype against the population mean within an environment.

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**Figure 9.** GSL haplotype frequencies of *Arabidopsis thaliana* accessions based on GSL profile data from chamber-grown individuals. Shown are the GSL haplotypes observed among a population of 144 *Arabidopsis* accessions for which our lab had existing GSL data. Seven loci important in the aliphatic GSL pathway were called based on GSL profile data from the lab as '+' = functional, '-' = non-functional or 'NA' = unobservable due to epistasis (see **Figure 1** for an explanation of epistasis in the GSL biosynthetic pathway). Bar length represents the observed GSL genotype frequencies among 144 *Arabidopsis* accessions. Bar color represents the difference, for a given GSL haplotype, between expected and observed genotype frequencies, based on Chi Squared distribution (significant p values shown). Blue = GSL genotypes found more frequently than expected (p value  $\leq$  0.05), red = GSL genotypes found less frequently than expected (p value  $\leq$  0.05) and grey = GSL genotypes found as frequently as expected (p value  $>$  0.05).

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