**Supplemental Figure S1. Overview of the bioinformatics workflow.**

**Supplemental Figure S2. General experimental design.** Two different heat assays were conducted. In 40 ºC assay (upper part), plants were stressed and sampled at intervals up to 3 days to characterize short and medium responses: T1, T3. Then, RNA extracted from treated plants was sequenced in order to perform splicing analysis. 45 ºC assay (lower part) was divided in two phases. In Phase I, plants were stressed and sampled at intervals up to 5 days to characterize long responses: T1, T3, T5. At 6 months after the end of Phase I, the plants were subjected to another round of treatment (Phase II) to evaluate the potential acquisition of long-term splicing memory. C = Control; T1, T3, T5: stressed plants samples after 1,3 or 5 days of stress.

**Supplemental Figure S3. Annotation pipeline and non-supervised behavior clusters.** (a) KisSplice isoform sequences were mapped to different transcriptome assemblies using bowtie2. Then, full-transcript sequences were identified and annotated to achieve functional information using Mercator4-Mapmen and custom R-SQL scripts integrated with 12 gene ontology databases. Finally, all the annotation were completed with complementary assembly/splicing. (b) K-means clustering of all isoforms reported. The solid line shows the mean for each cluster. The sampling times correspond to the 40 ºC assay shown in **Figure 1**.

**Supplemental Figure S4. Splicing variation effect proportions across non-supervised behavior clusters.** (a) Pie-charts showing proportions corresponding to regions where the splicing variation was produced for all the isoforms of each cluster. NoRes = neither full model nor full-transcript sequences were predicted/mapped so events could not be classified. Different colors reflect transcript regions where the splicing variation was produced. (b) Pie-charts illustrating proportions corresponding to splicing variation effect for all the CDS isoforms of each cluster. Change = lead to protein sequence changes (gray). PTC = introduce premature termination codons (orange).

**Supplemental Figure S5. Heat stress-responsive intron retention hypothesis in gymnosperms.** Under control conditions, the spliceosome (represented only by U1 and U2 pre-spliceosome complex for a easier interpretation) binds co-transcriptionally to the unique splicing sites available, leading to constitutive splicing (Control). During heat stress, several factors, such as nucleus hypomethylation and faster kinetics, result in transcription loss of control which leads to simultaneous availability of multiple splice sites, thus, splice sites compete for spliceosome/splicing factors recruitment depending on their strength (Heat stress). As a product of this, a general increase of retained introns, specially those ones flanked by weak splice sites, would be induced (angiosperms). However, intron length divergence between seed plants (angiosperms and gymnosperms) may have crucial implications in this type of RNA processing. Despite being flanked by weak splice sites, gymnosperms long introns (indicated by a dashed line) might hinder intron recognition as a single unit to be retained because of their length or the potential appearance of strong cryptic splice sites (gymnosperms). All of this could ultimately cause the retention of fragmented instead of complete introns and/or a lower prevalence of IR in gymnosperms.

**Supplemental Figure S6. Stress-specific contrast functional description and isoform-protein relationships.** (a) Volcano analysis of DD event isoforms. Not significant = FDR-adjusted P > 0.05 and absolute log fold change < 1.8; Significant = FDR-adjusted P < 0.05 and absolute log fold change < 1.8; Fold Change = FDR-adjusted P > 0.05 and absolute log fold change > 1.8; Significant & Fold Change = FDR-adjusted P <0.05 and absolute log fold change > 1.8. (b) Gene set enrichment analysis of DD event isoforms using Mercator4 functional categories. Blue and gold indicate significative and non-significative enriched terms, respectively. (c) Volcano analysis of isoform-protein relationships. NoRelation = absolute isoform and protein log fold change < 0.5; Medium = absolute isoform and protein log fold change > 0.5; High = absolute isoform and protein log fold change > 1; VeryHigh = absolute isoform and protein log fold change > 1.8. (d) Scatter-plot of UMAP1 (x axis) and UMAP2 (y axis) illustrating the proteins projection over isoforms. Samples are coloured/shaped according to treatment (C, T1, T3) and molecular level (isoforms, proteins), respectively. The sampling times correspond to the 40 ºC assay shown in **Figure 1**.

**Supplemental Figure S7. Functional characterization of proteins and all regulatory layers biomarkers for each MOFA2 latent factor.** (a) Enriched Mercator4 functional categories and top weight proteins associated to latent factor 1 (top) and latent factor 2 (bottom). Positive = functional terms linked to samples with > 0 scores; Negative = functional terms linked to samples with < 0 scores. (b) For each regulatory layer (rows) and each biologically relevant latent factor (columns), weights distribution (right panel) and scatterplot of factor values (x-axis) versus expression/abundance values (y-axis) for the top features with largest weight (left panel) are displayed. Samples are coloured according to treatment (C, T1, T3). The sampling times correspond to the 40 ºC assay shown in **Figure 1**.

**Supplemental Table S1** All genome data sources for the species used in intron comparative analysis.

**Supplemental Table S2** List of primers used for RT-PCR.

**Supplemental Table S3** Sanger sequencing data validating RT-PCR results.

**Supplemental Table S4** K-means clusters data.

**Supplemental Table S5** Isoform sequences, expression, annotation, stats and potential event type data.

**Supplemental Table S6** Multi-omics integration data.