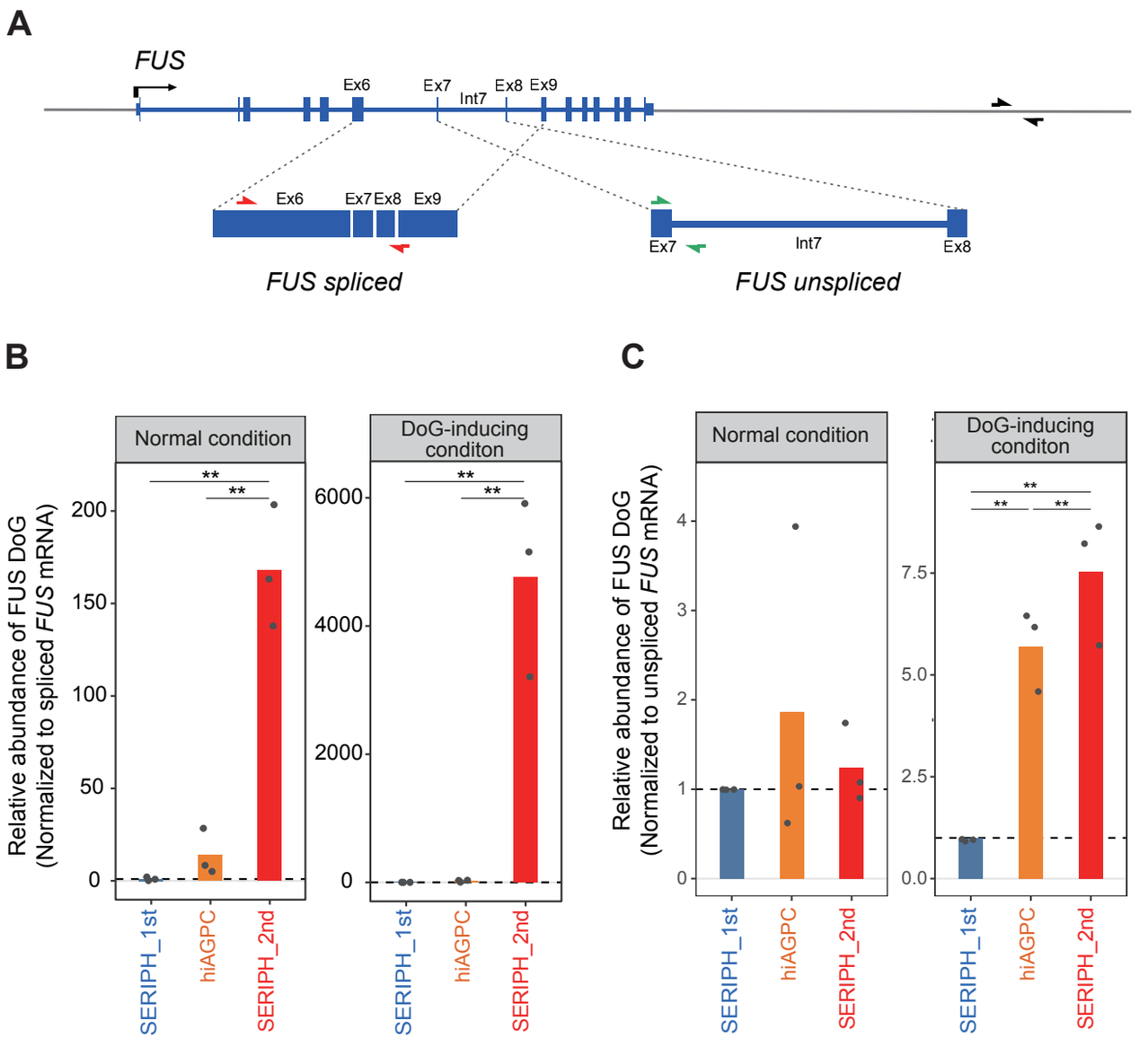

Supplemental Fig. 5

Supplemental Fig S5: Relative abundance of DoG transcripts normalized to spliced *FUS* mRNA.

(A) Schematic representation of qPCR primer sets used for analysis. DoG transcripts were detected ~7 kb downstream of the annotated 3′ end of the *FUS* gene (primers shown in black). Spliced *FUS* mRNA was quantified using primers spanning exon junctions (Exons 6–9; primers shown in red). Unspliced *FUS* transcripts were detected using primers spanning Exon 7 and Intron 7, targeting an intron that tends to be retained in transcripts associated with DoG transcription (primers shown in green).
(B) DoG transcript levels normalized to spliced *FUS* mRNA (Ex6–Ex9) within each biological replicate and further scaled to the mean value of the SERIPH 1st-step fraction. Dots represent individual biological replicates, and bars indicate mean values (n = 3 biological replicates). Statistical significance was evaluated using repeated-measures one-way ANOVA followed by Holm-adjusted post hoc tests. ** indicates adjusted $P < 0.01$.
(C) DoG transcript levels normalized to unspliced *FUS* mRNA (Ex7–Int7) within each biological replicate and further scaled to the mean value of the SERIPH 1st-step fraction. Dots represent individual biological replicates, and bars indicate mean values (n = 3 biological replicates). Statistical significance was evaluated using repeated-measures one-way ANOVA followed by Holm-adjusted post hoc tests. ** indicates adjusted $P < 0.01$. Because unspliced transcripts containing this intron tend to be detected in conjunction with DoG transcription and are present at relatively low abundance, the relative difference between DoG and upstream unspliced transcripts remained comparatively small even in RNA prepared using the improved method, similar to the SERIPH 2nd-step fraction.