

Fig. S1. Generation of mutant alleles at the zebrafish *alx3* locus. *alx3* mutations were induced by CRISPR/Cas9 mutagenesis targeted to the second exon of the *alx3* locus. *alx3^{uw2113}* allele contains a net insertion of 13 nt (magenta letters) which is predicted to cause a frameshift and a premature stop (red letters).

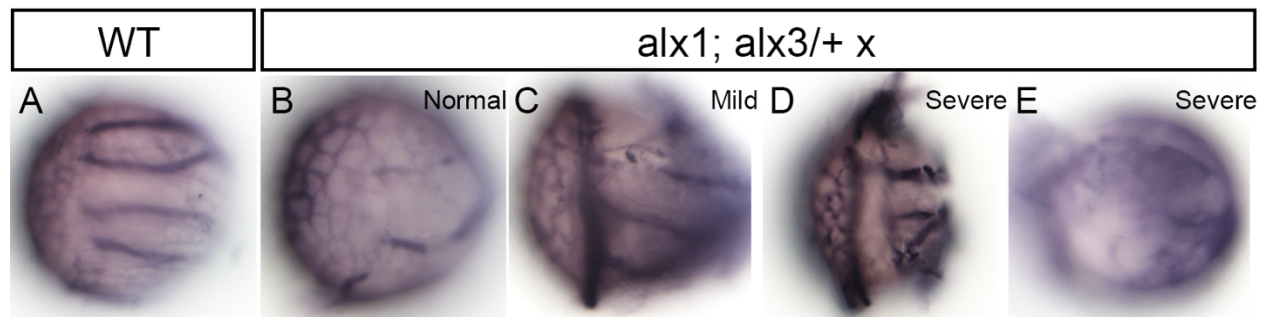


Fig. S2. *alx1* and *alx3* are required for hyaloid vascular patterning. Embryos derived from WT (A) or *alx1; alx3/+* (B – E) parents were first scored for the presence of misshapen eyes and stained for alkaline phosphatase to visualize hyaloid vasculature that surrounds the lens at 5 dpf. A: Normal hyaloid pattern in WT (6/6), B: Reduced hyaloid network formation in embryo with normally shaped eyes (5/6), C: Embryos with mildly misshapen eyes and disorganized hyaloid pattern (5/6). D-E: Embryos with severely misshapen eyes and disorganized (D; 2/5) or no hyaloid vasculature (E; 2/5 lens).

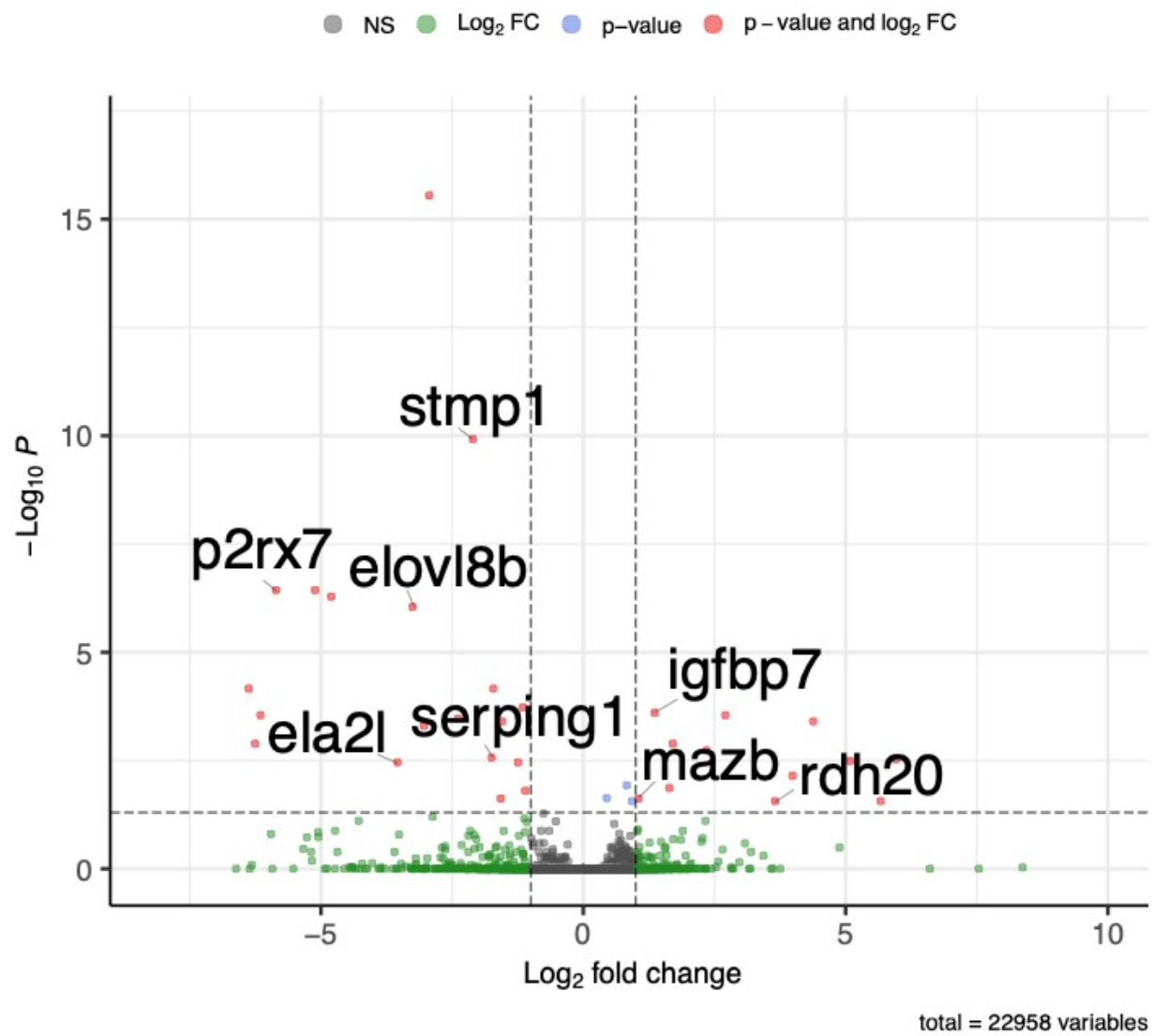
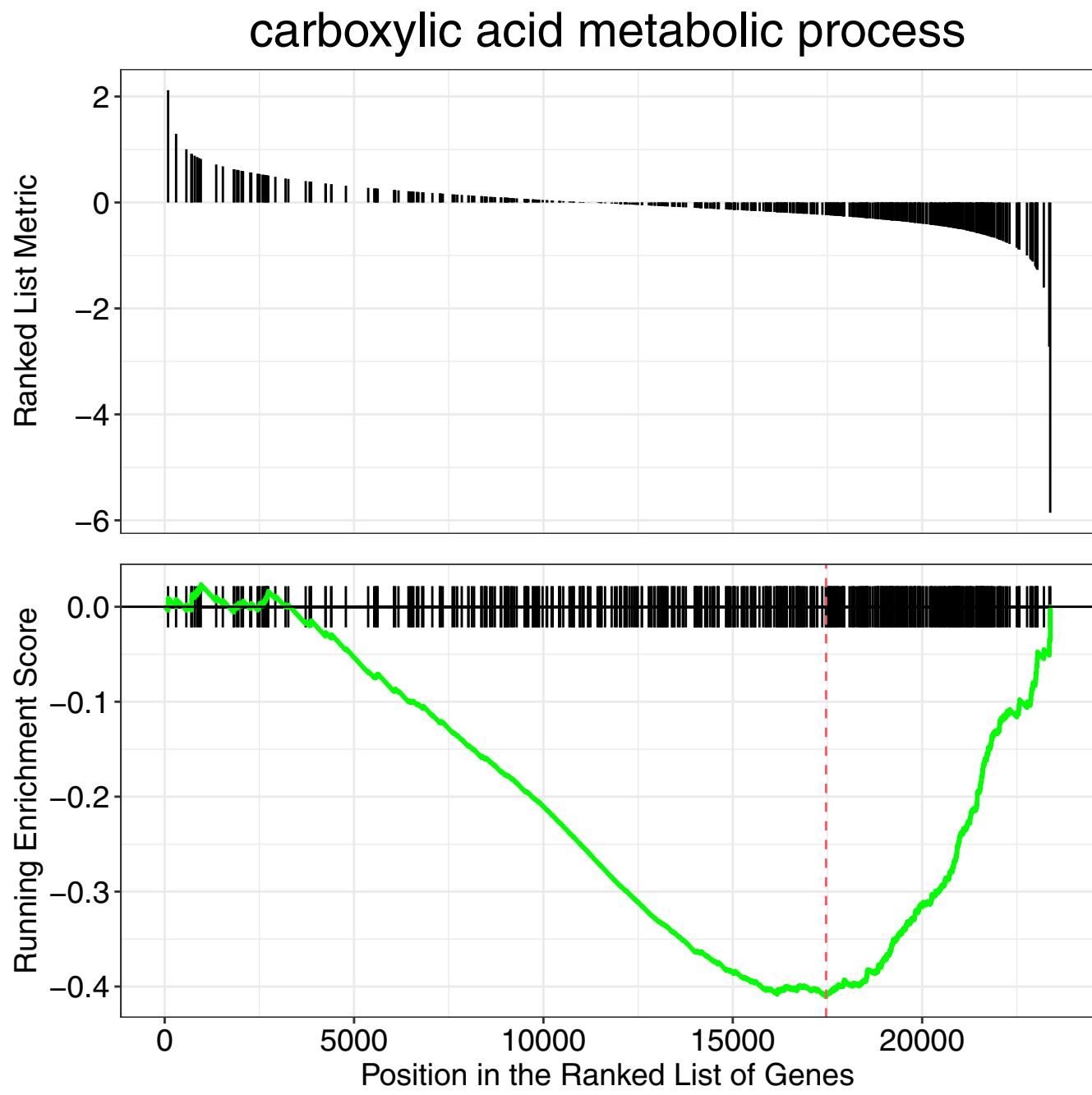
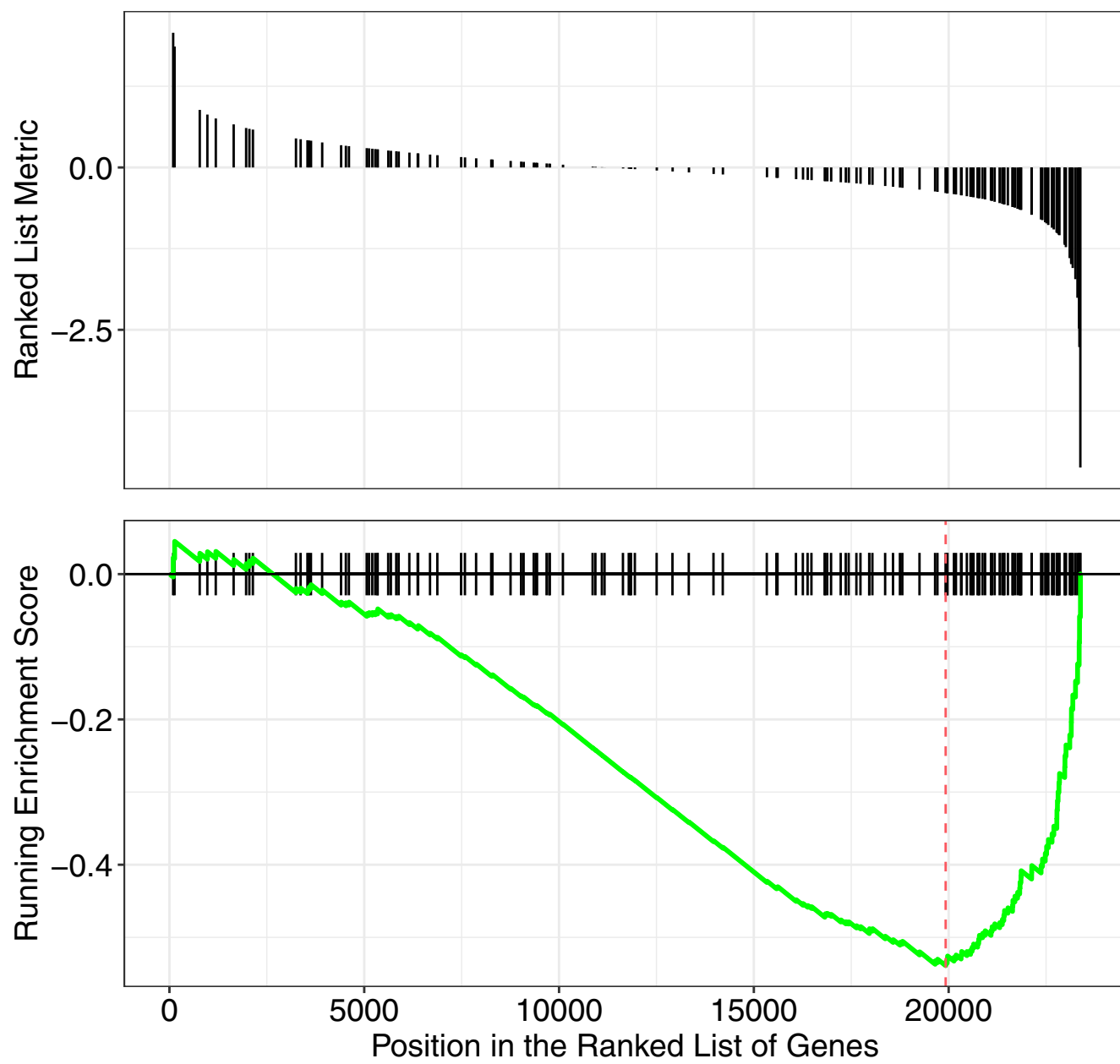


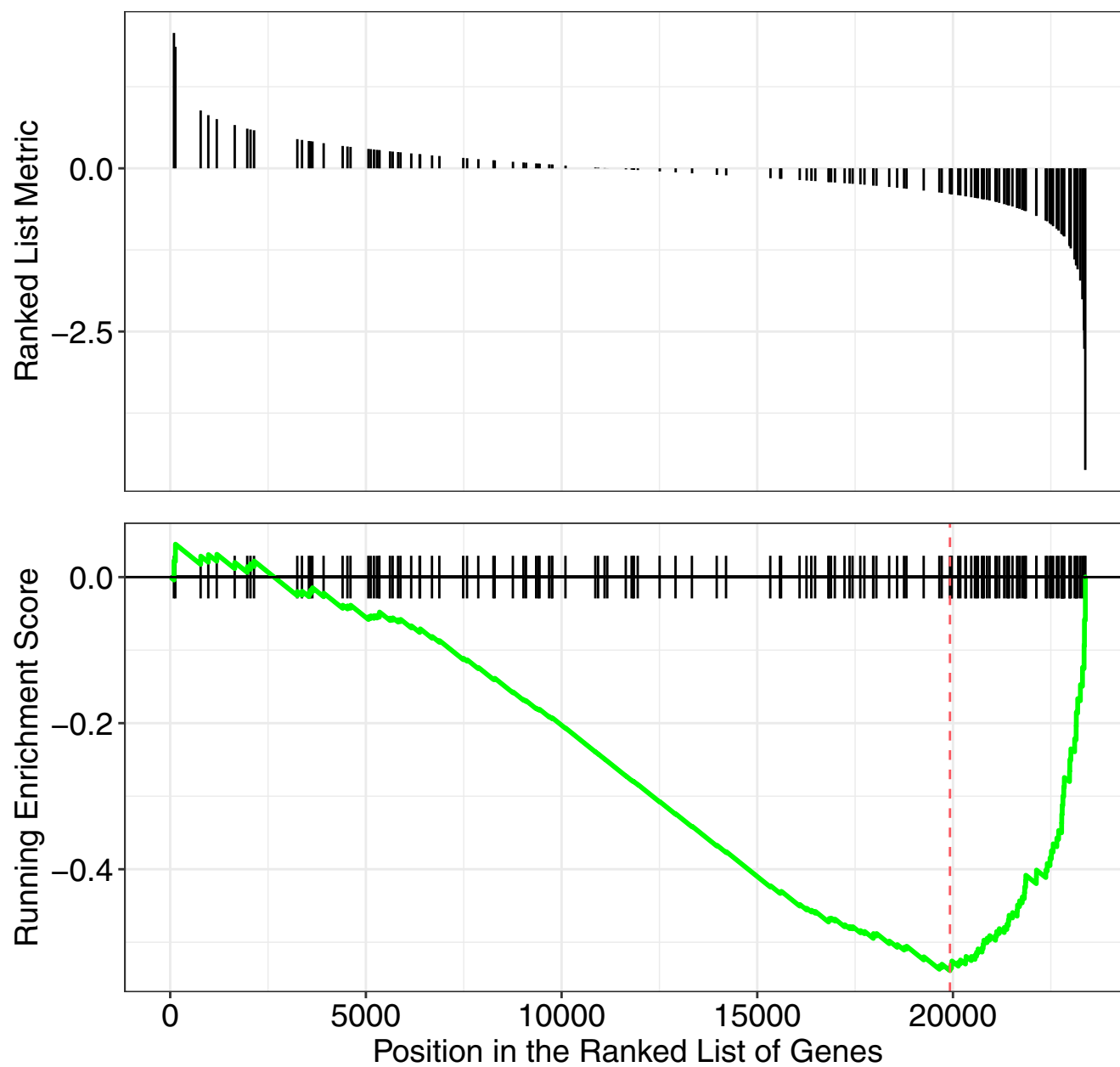
Fig. S3. Differentially expressed genes in *alx1;alx3/+* mutants relative to wildtype siblings. A heat map (A) and volcano plot diagram (B) of differentially expressed genes in *alx1;alx3/+* mutants relative to wildtype siblings (see Figure 6 for details).



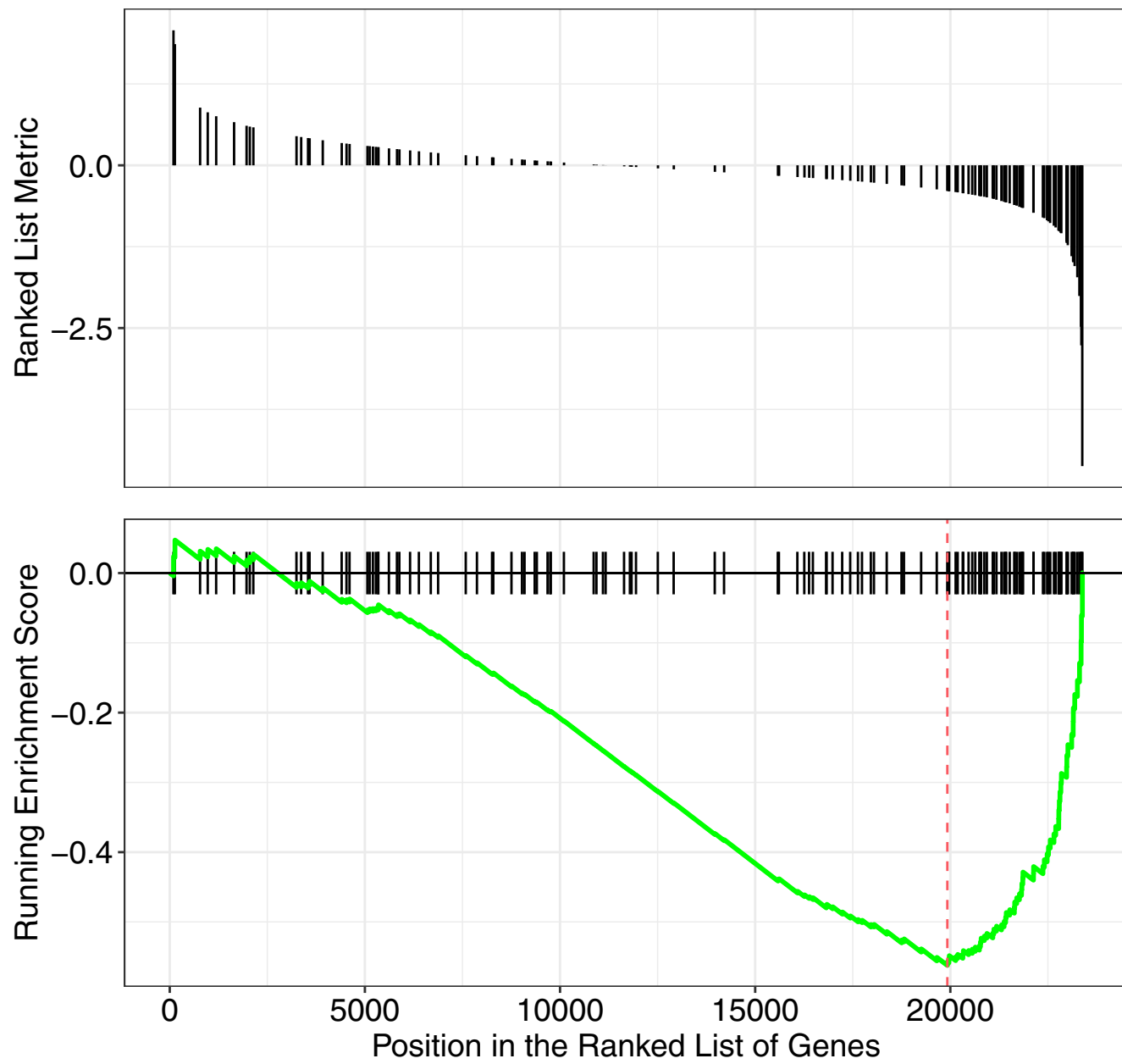
serine-type peptidase activity



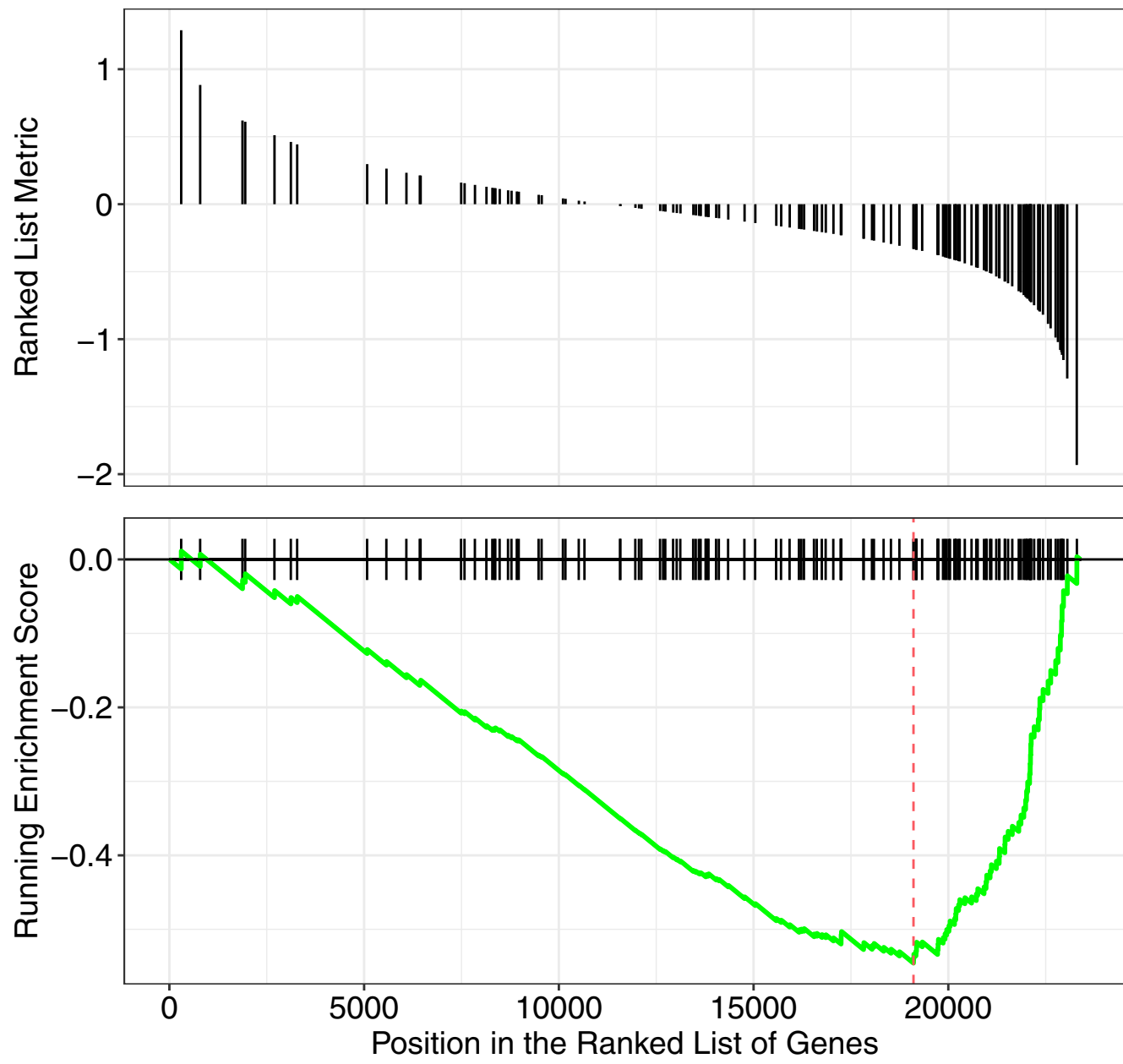
serine hydrolase activity



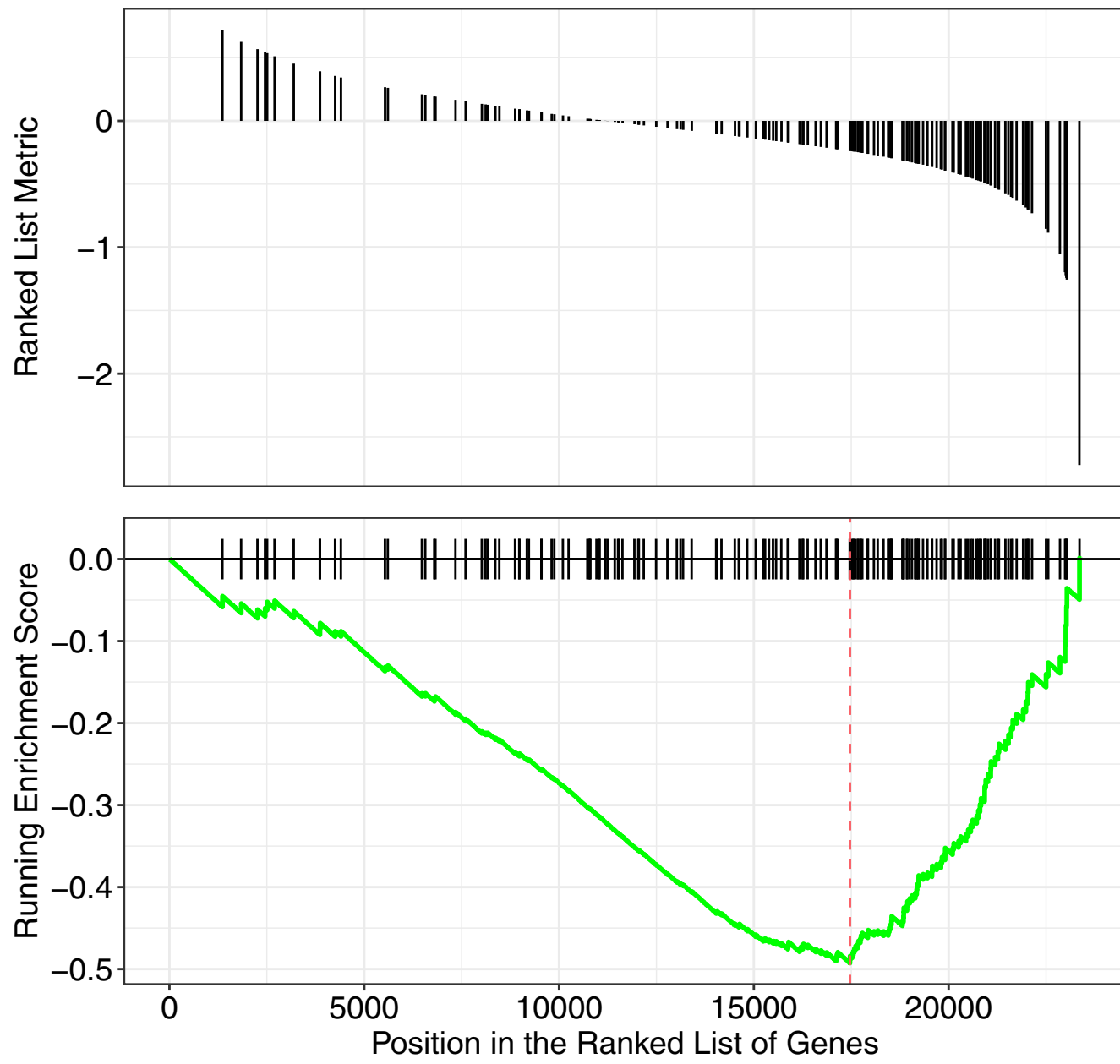
serine-type endopeptidase activity



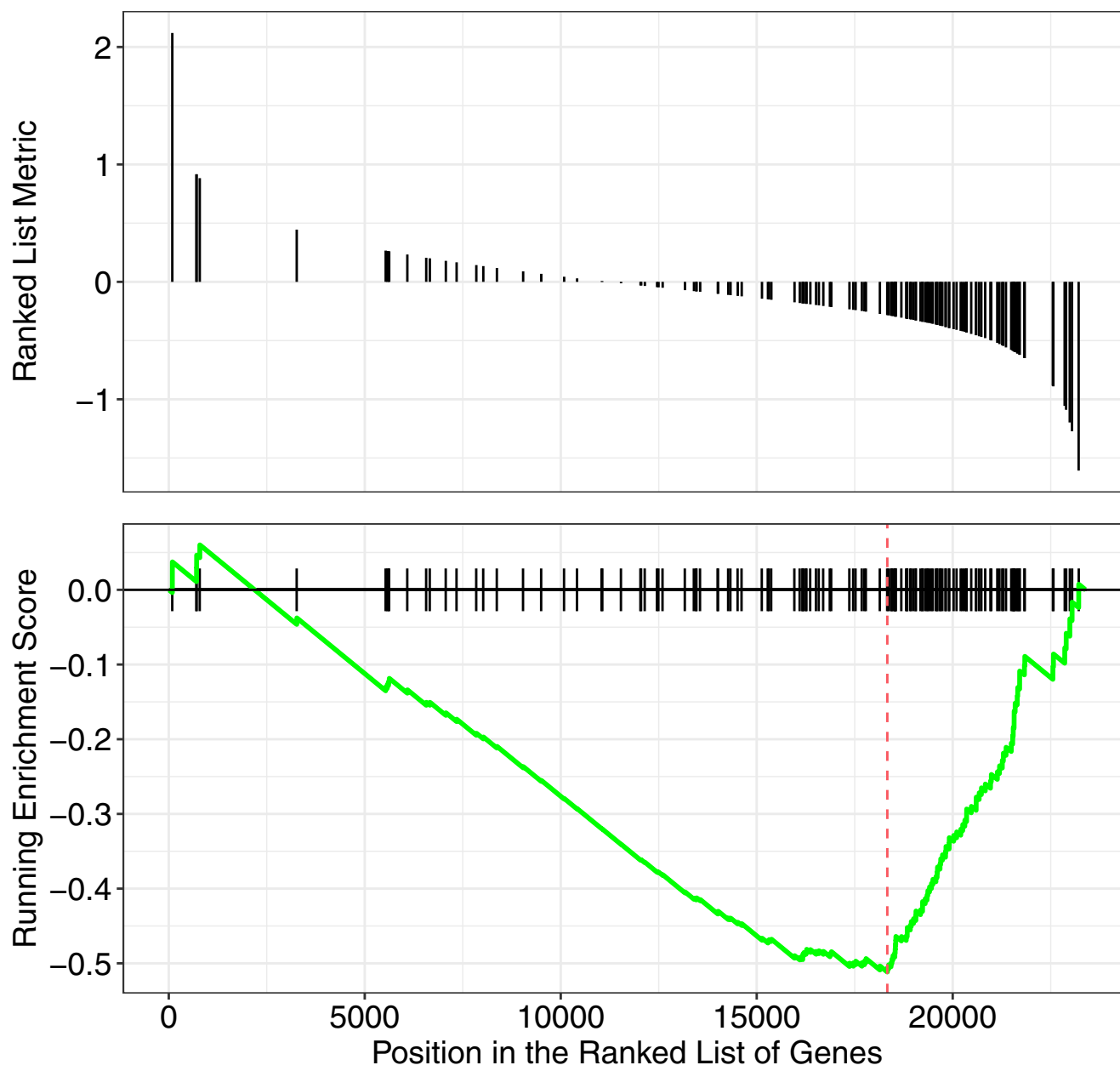
monooxygenase activity



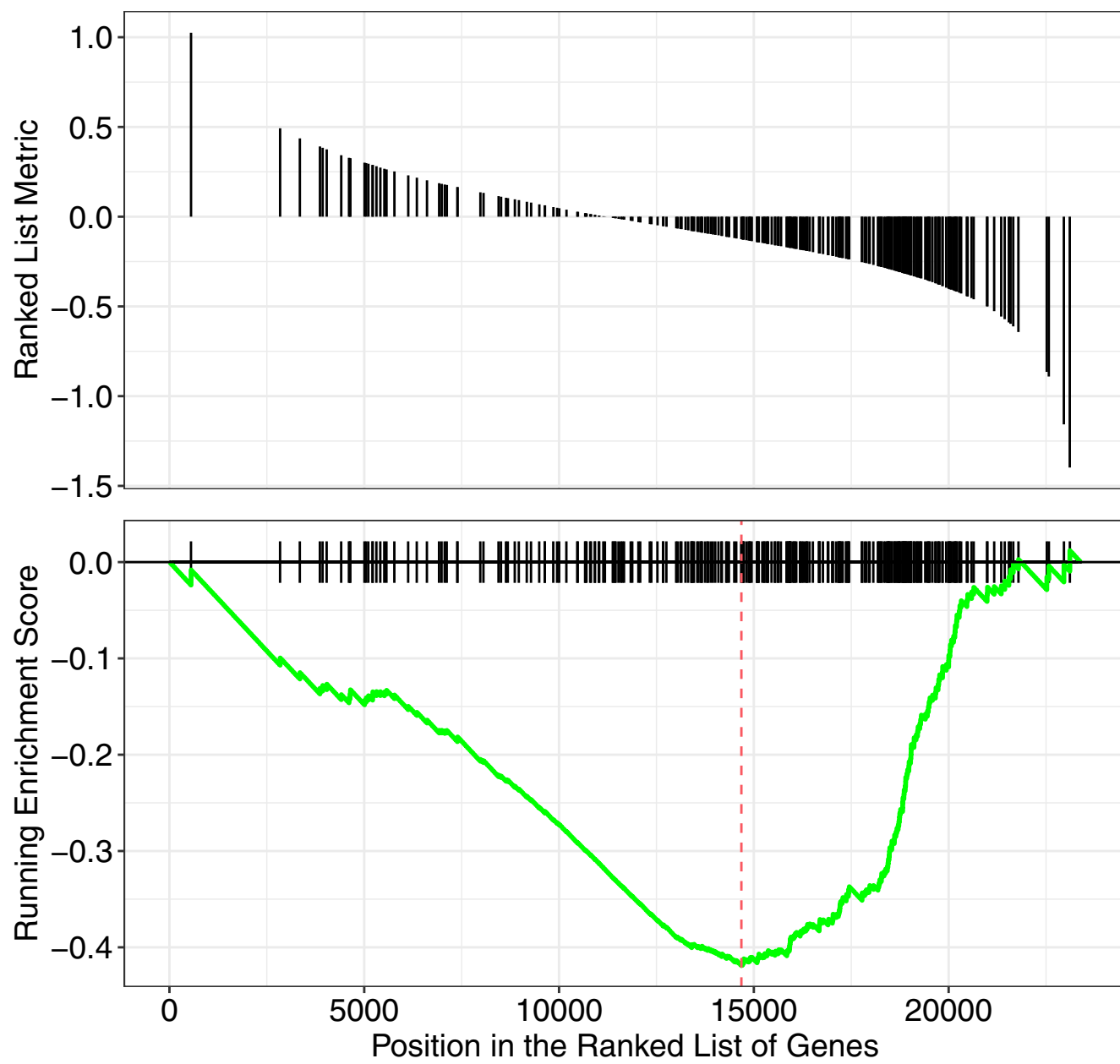
fatty acid metabolic process



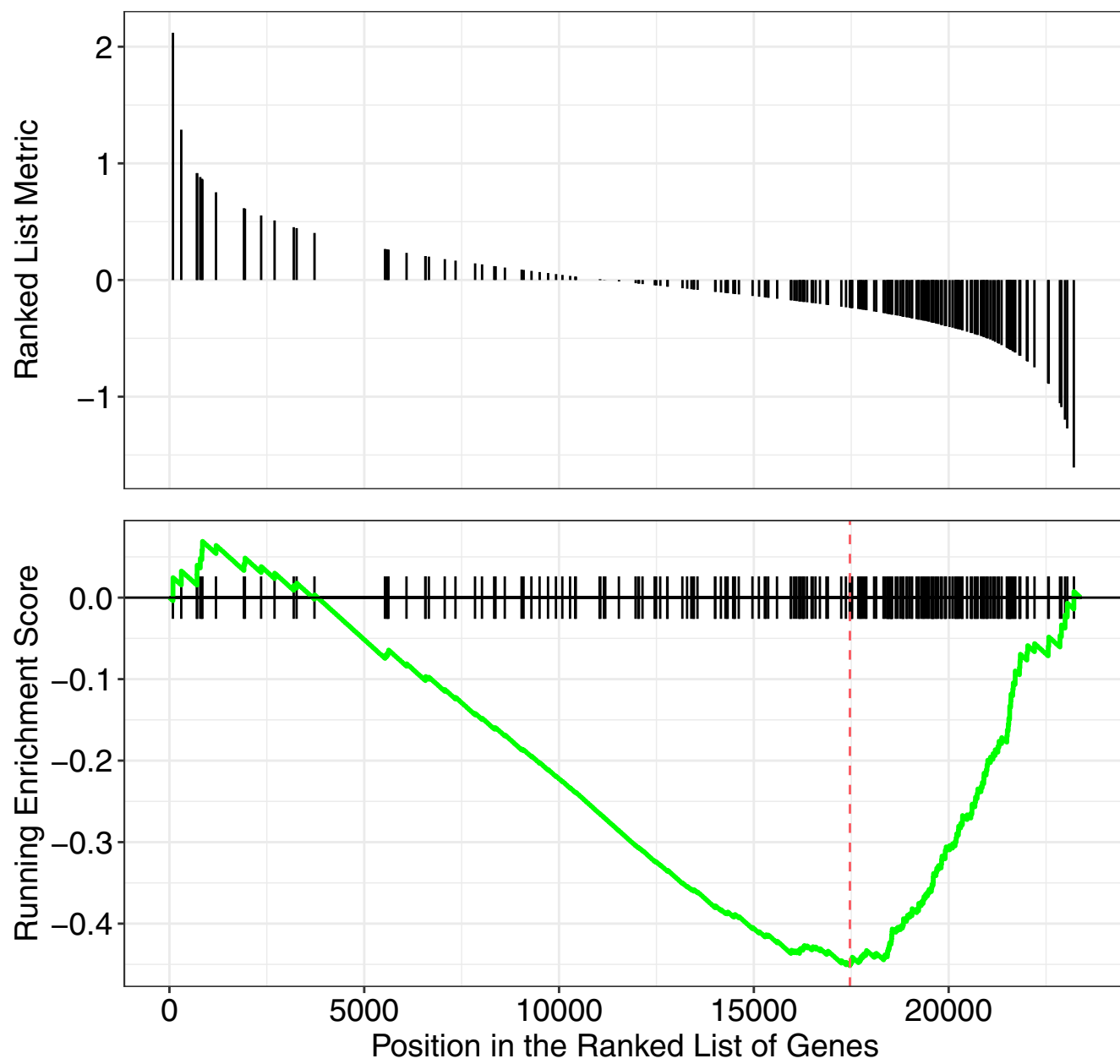
carboxylic acid catabolic process



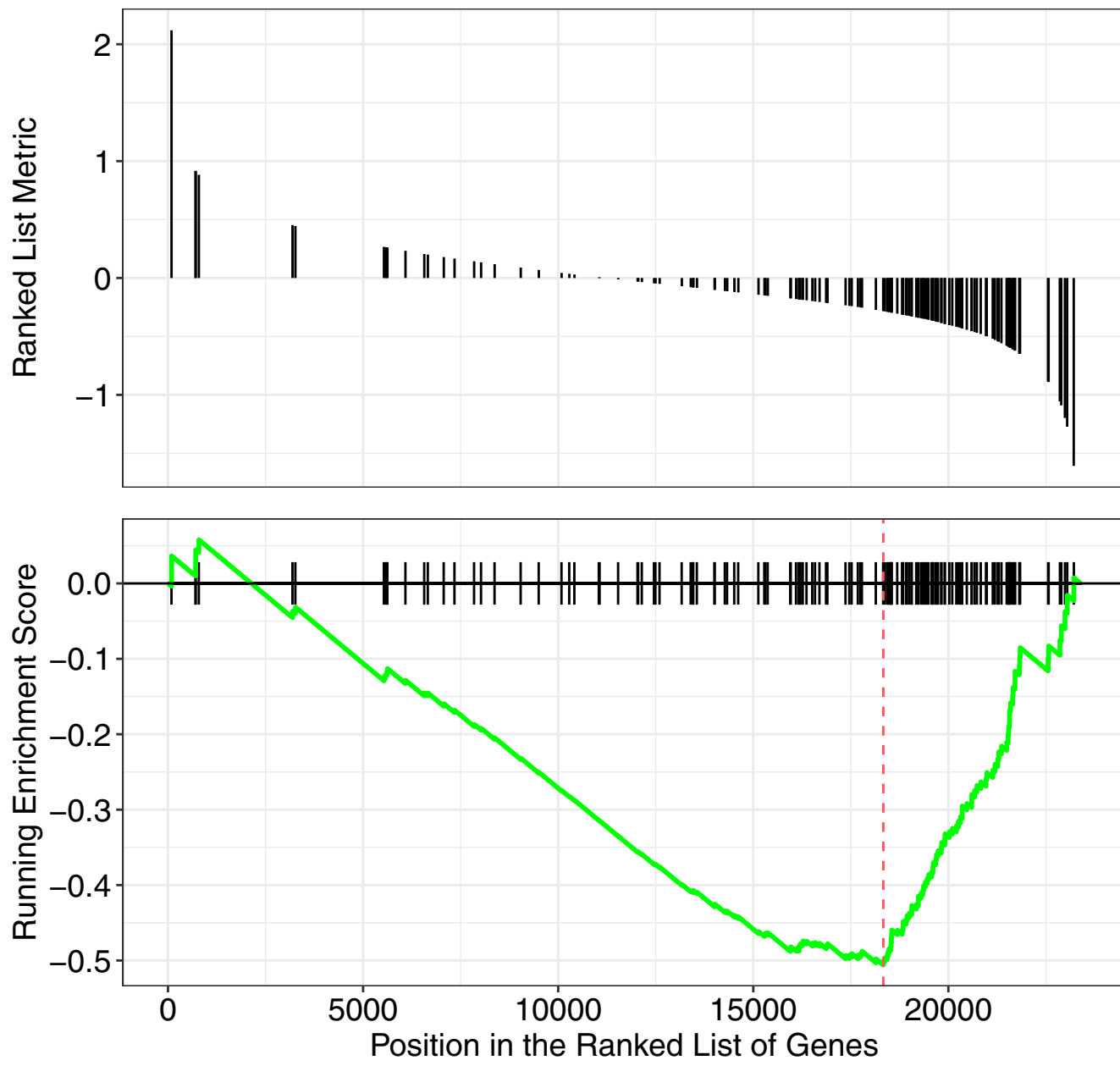
ncRNA processing



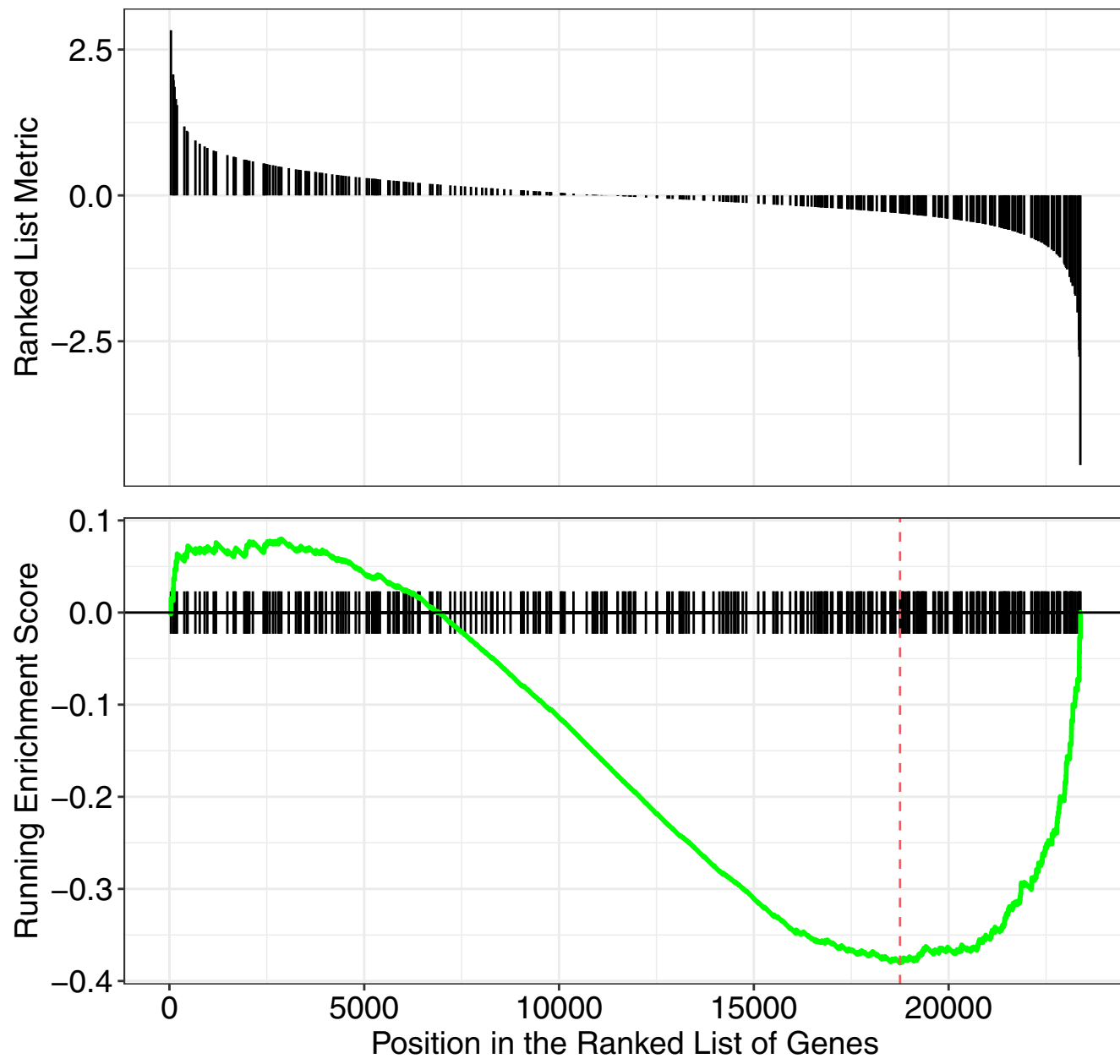
small molecule catabolic process



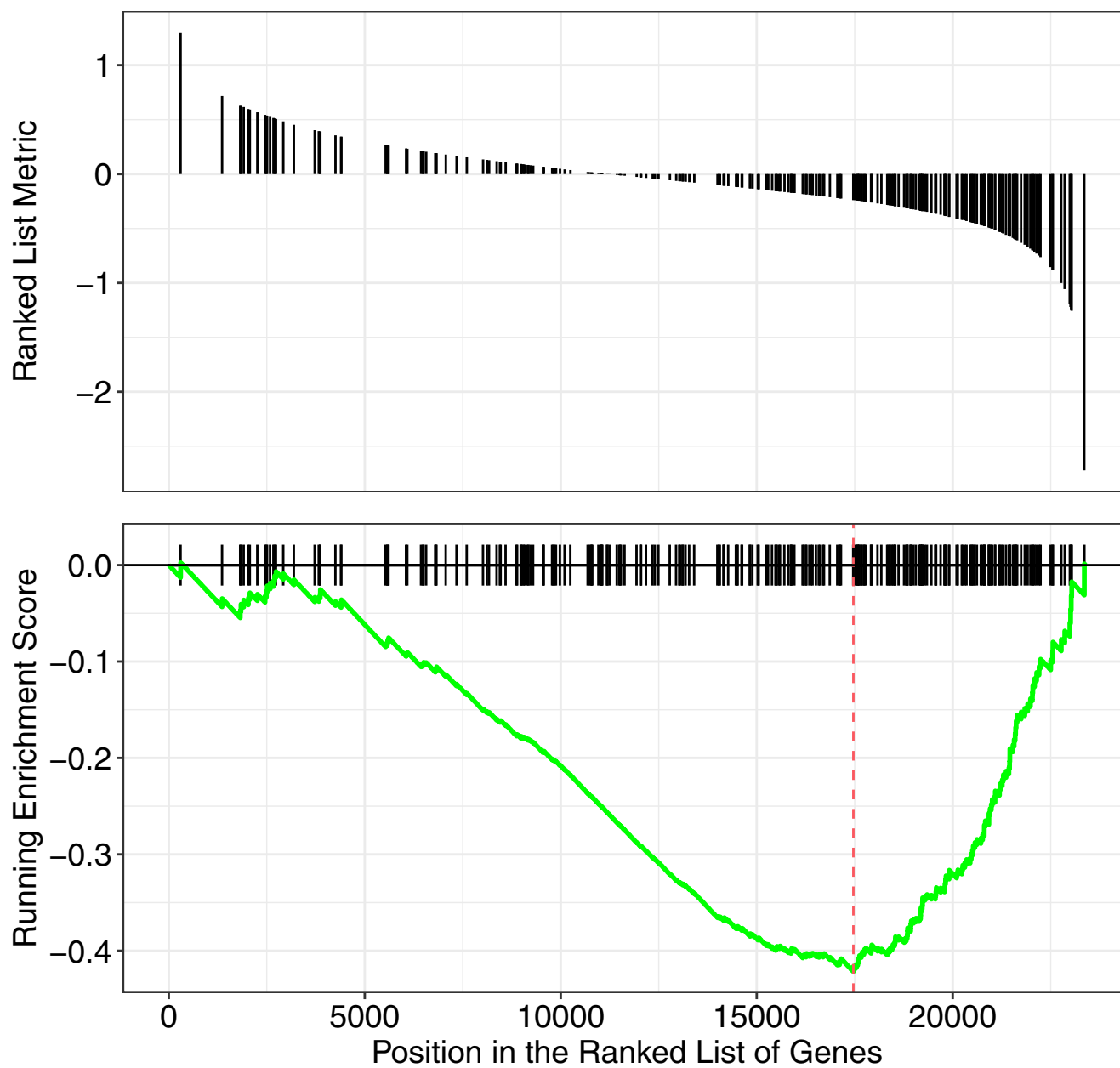
organic acid catabolic process



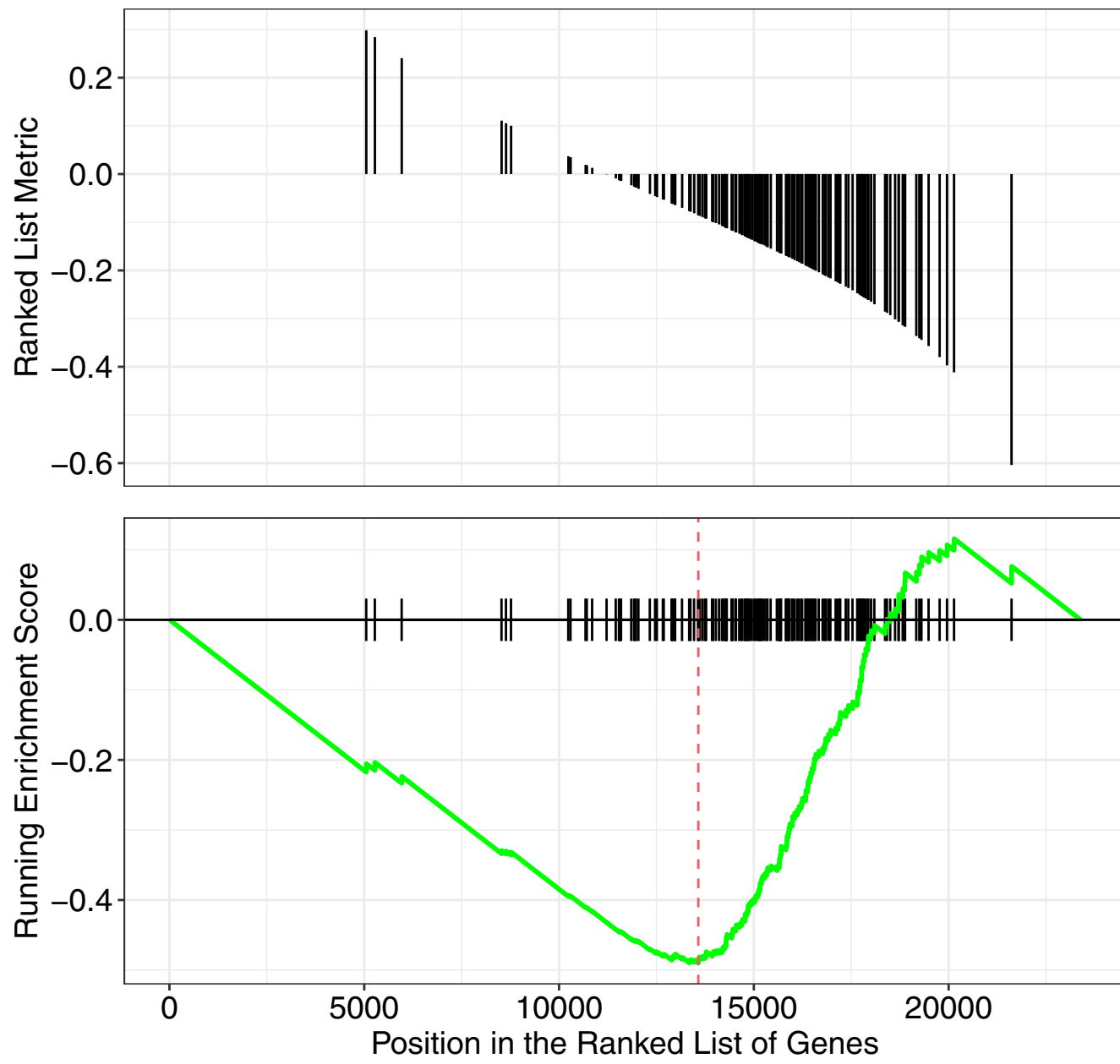
endopeptidase activity



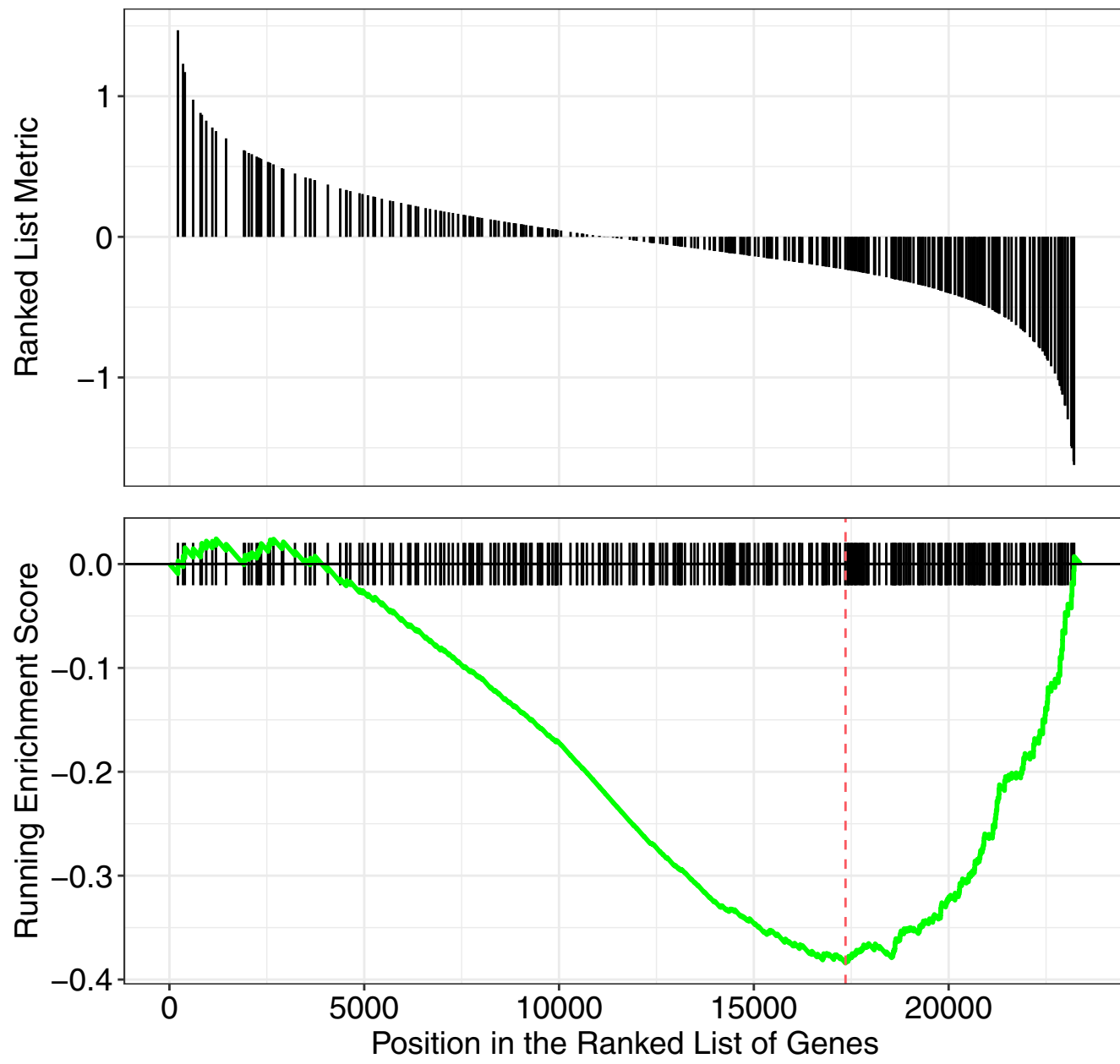
monocarboxylic acid metabolic process



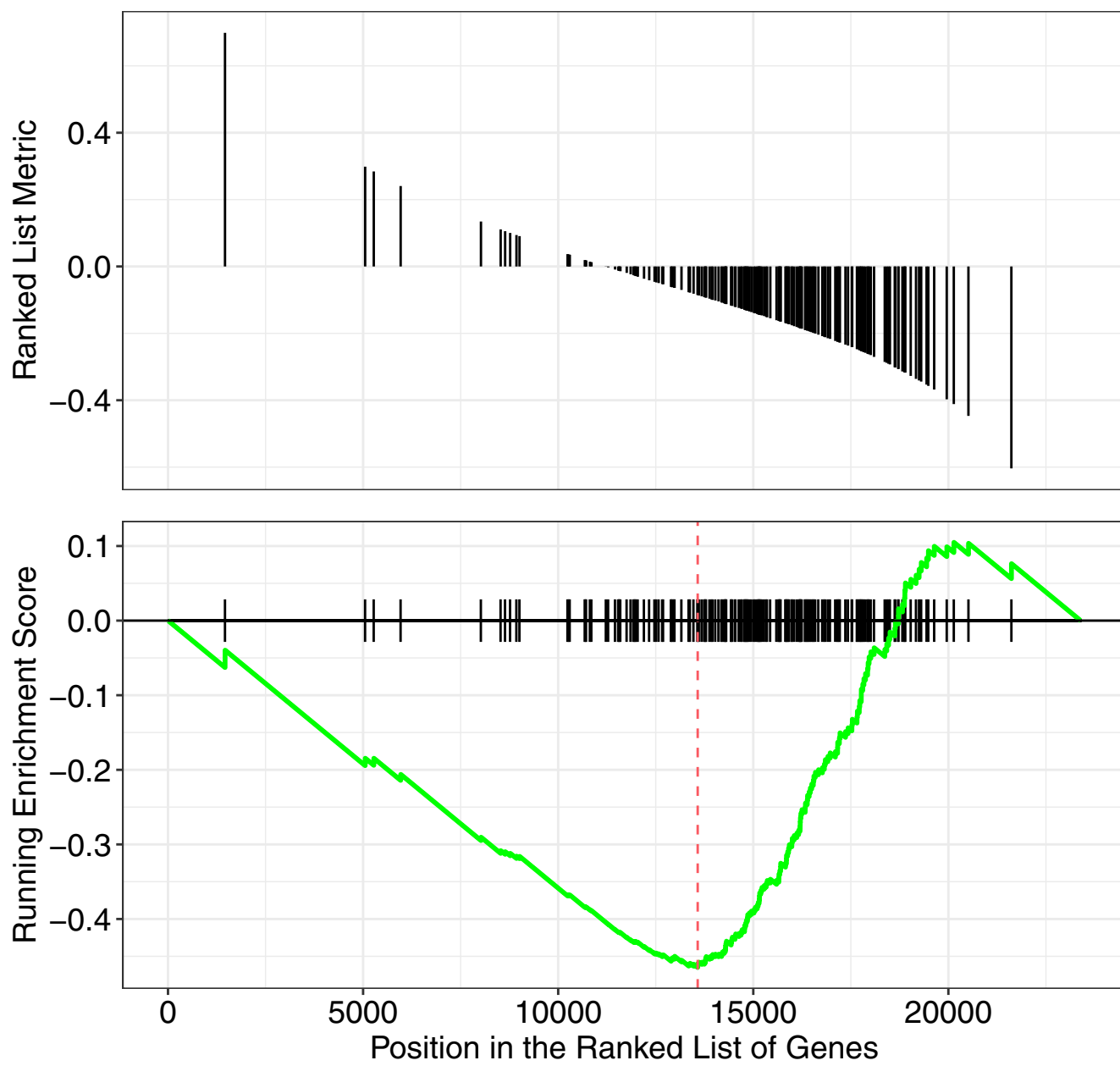
structural constituent of ribosome



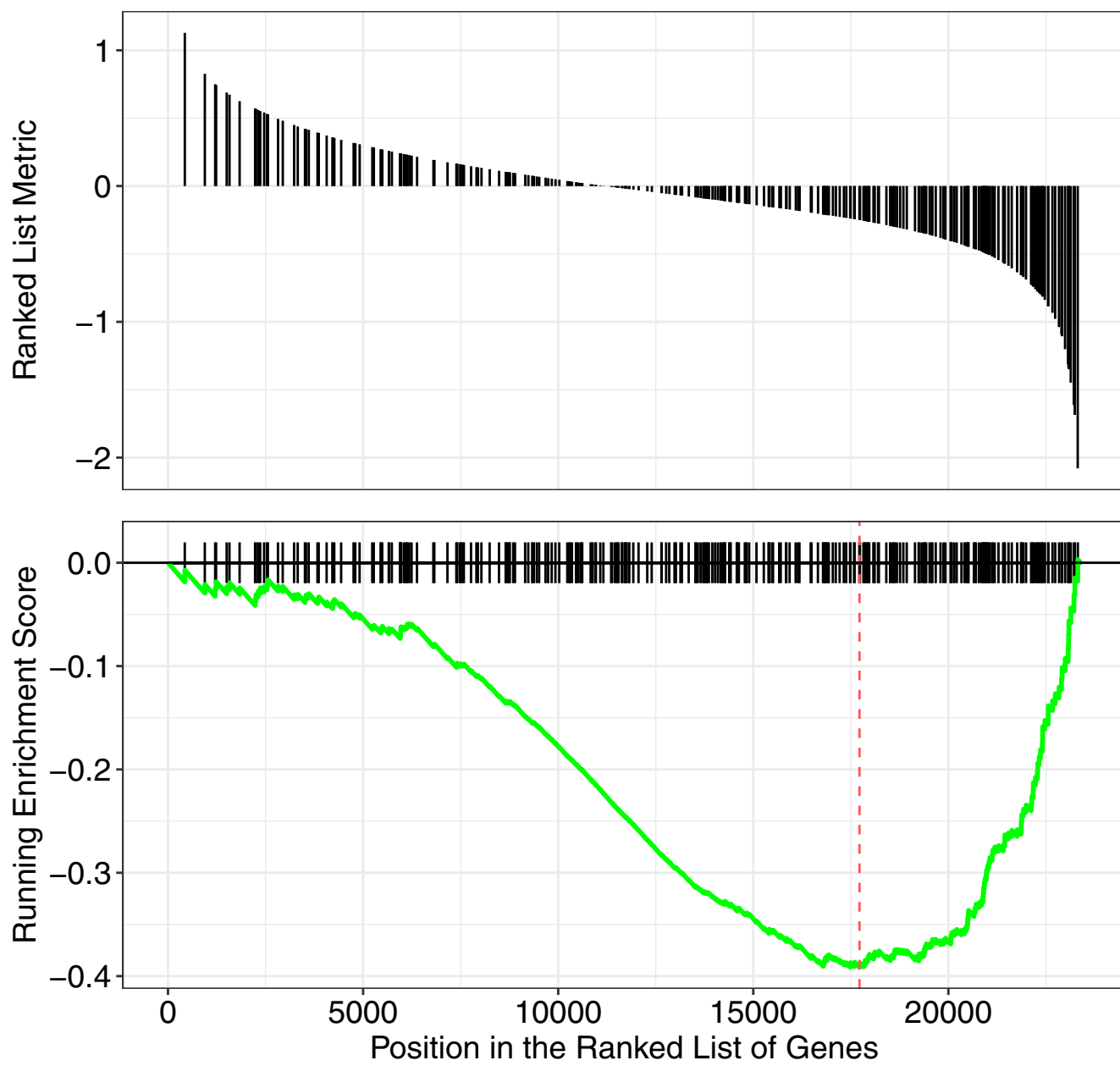
carbohydrate metabolic process

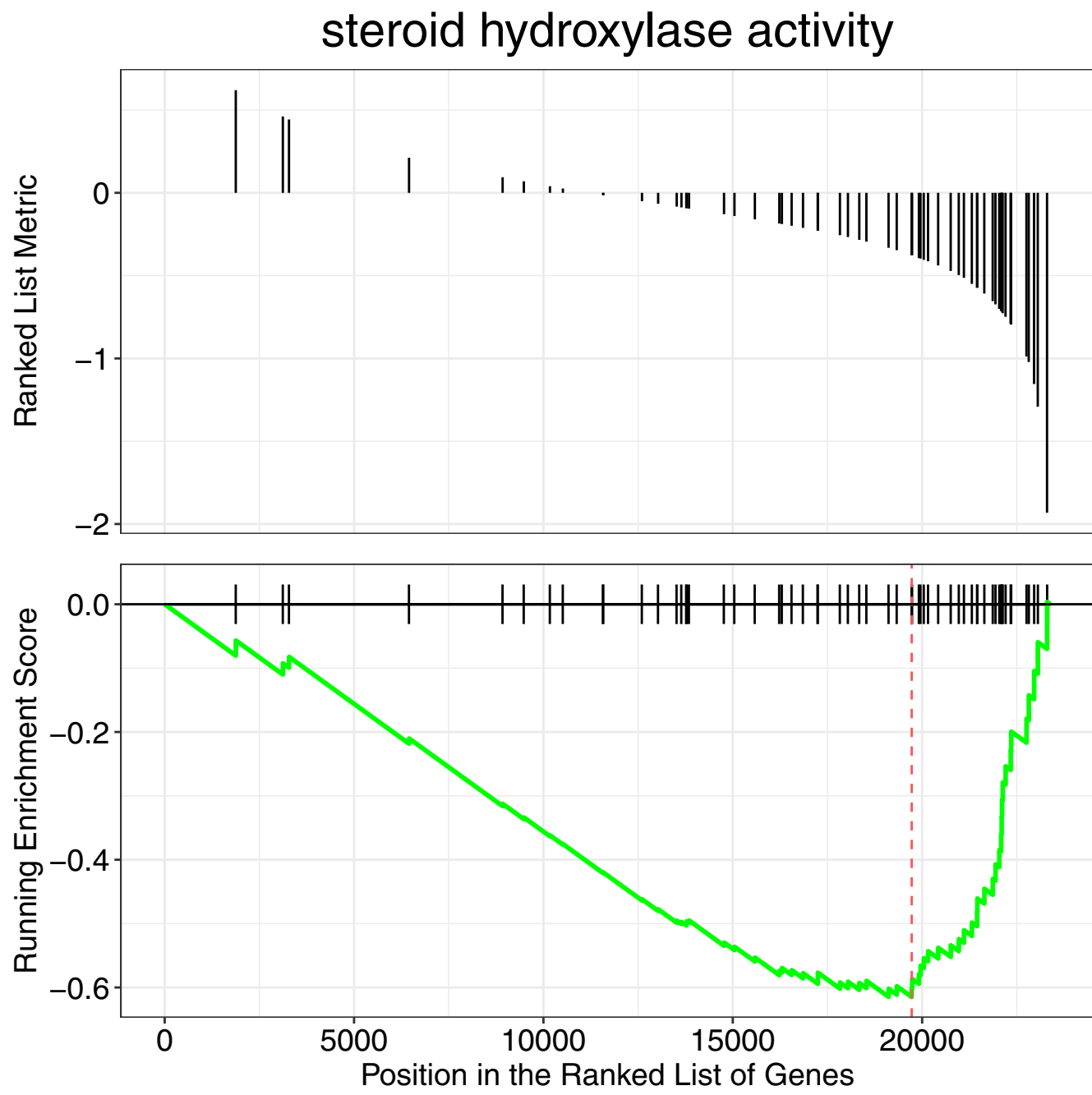


ribosome

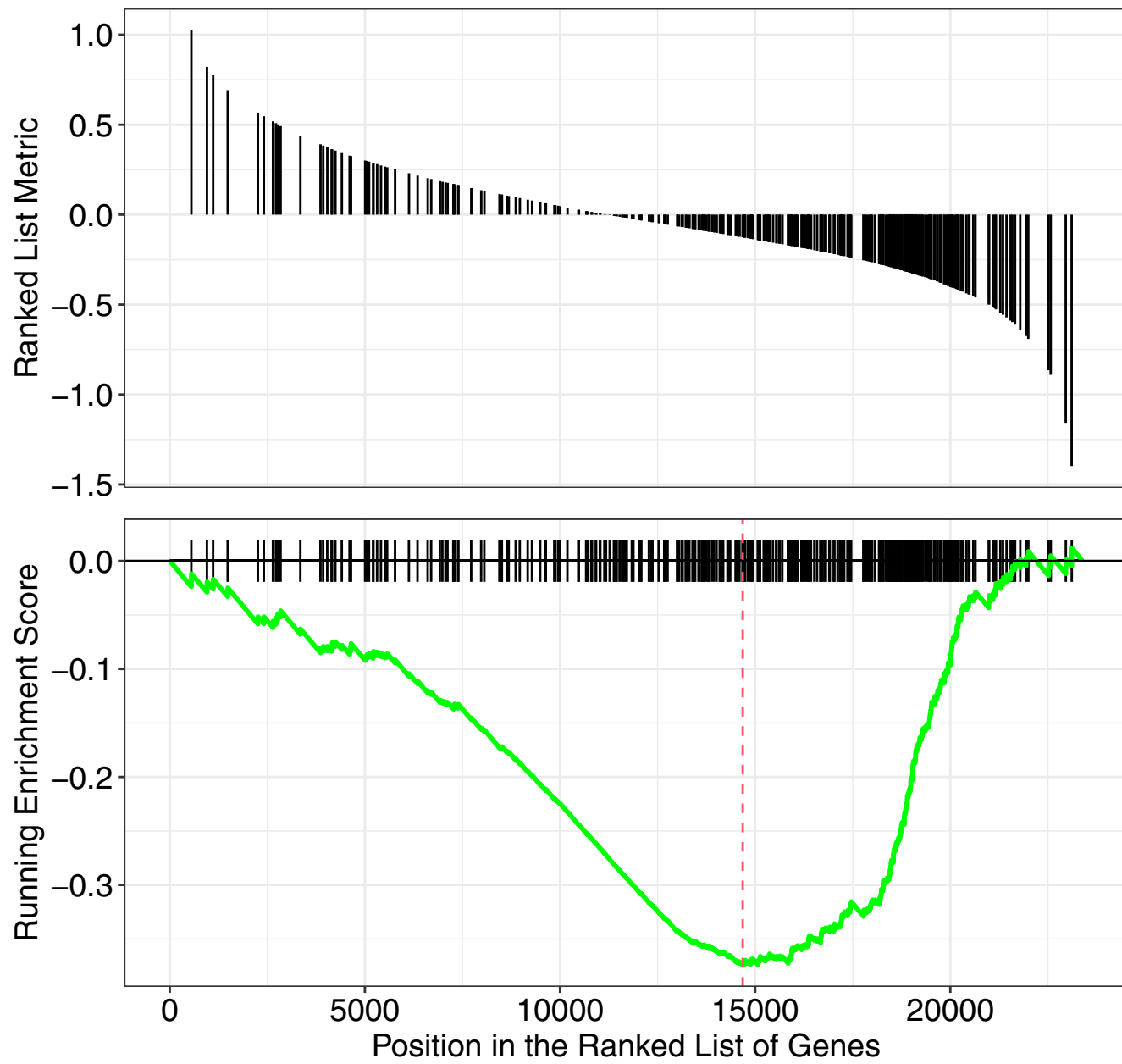


transferase activity, transferring glycosyl groups

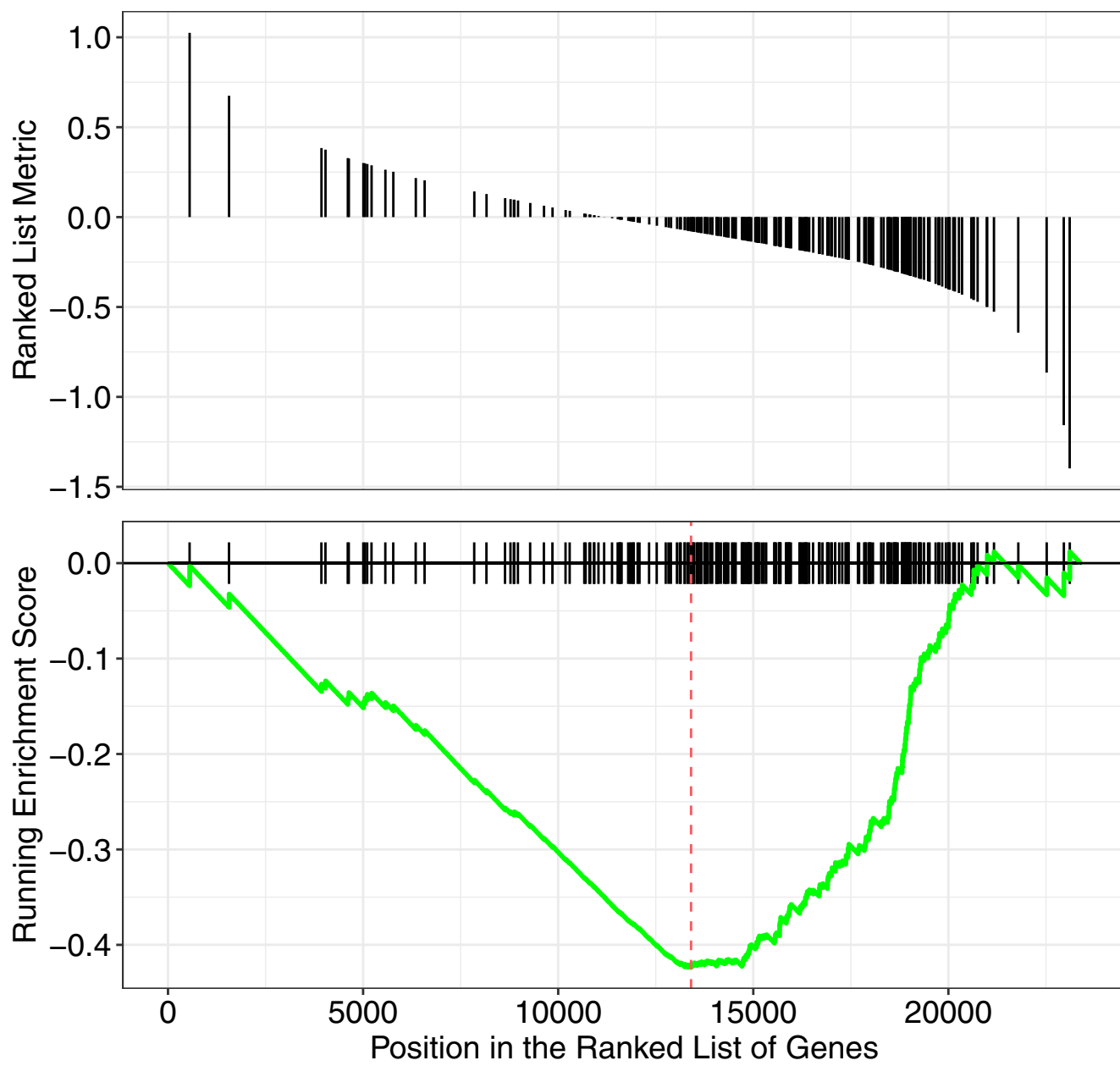




ncRNA metabolic process



ribosome biogenesis



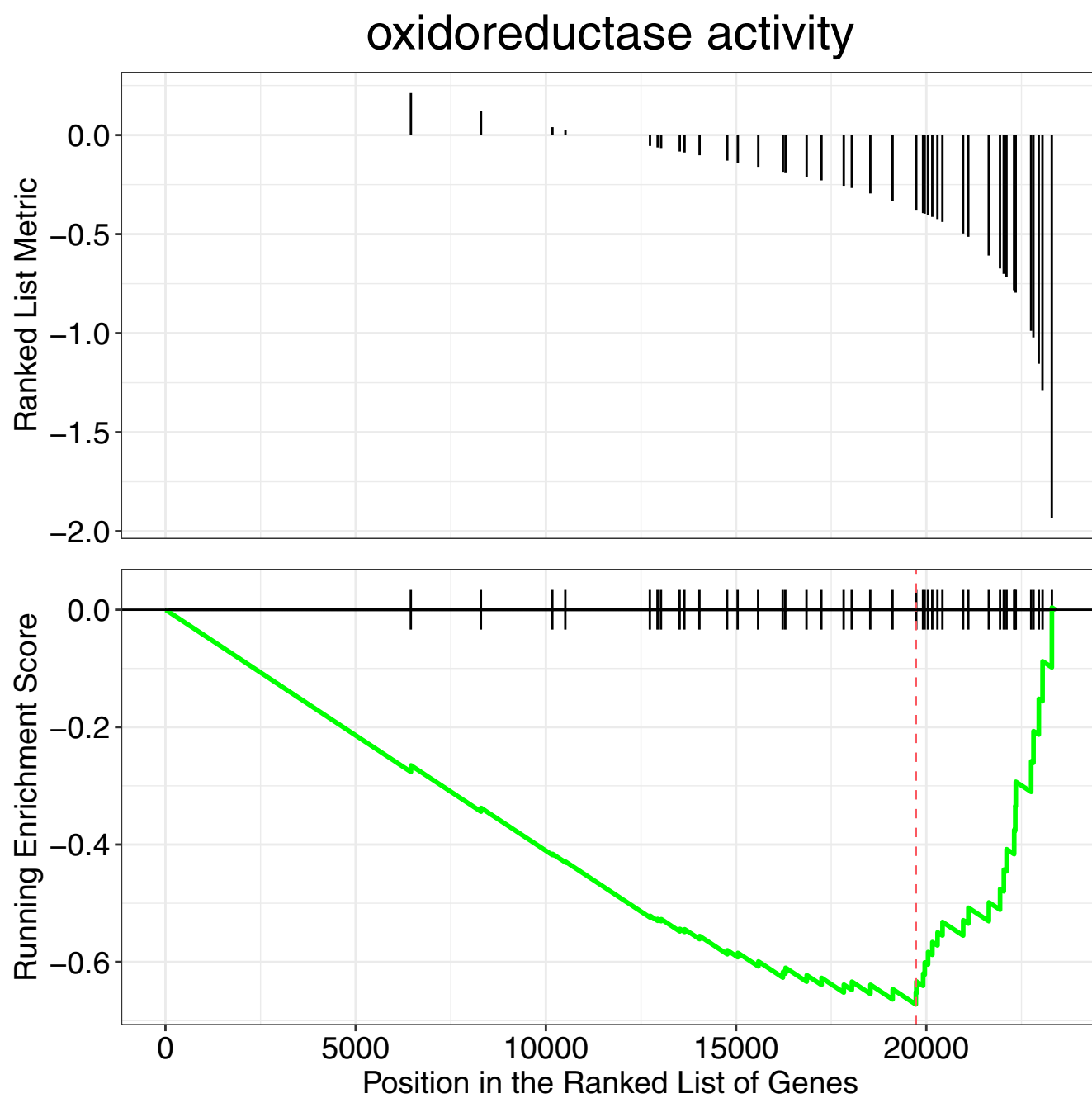
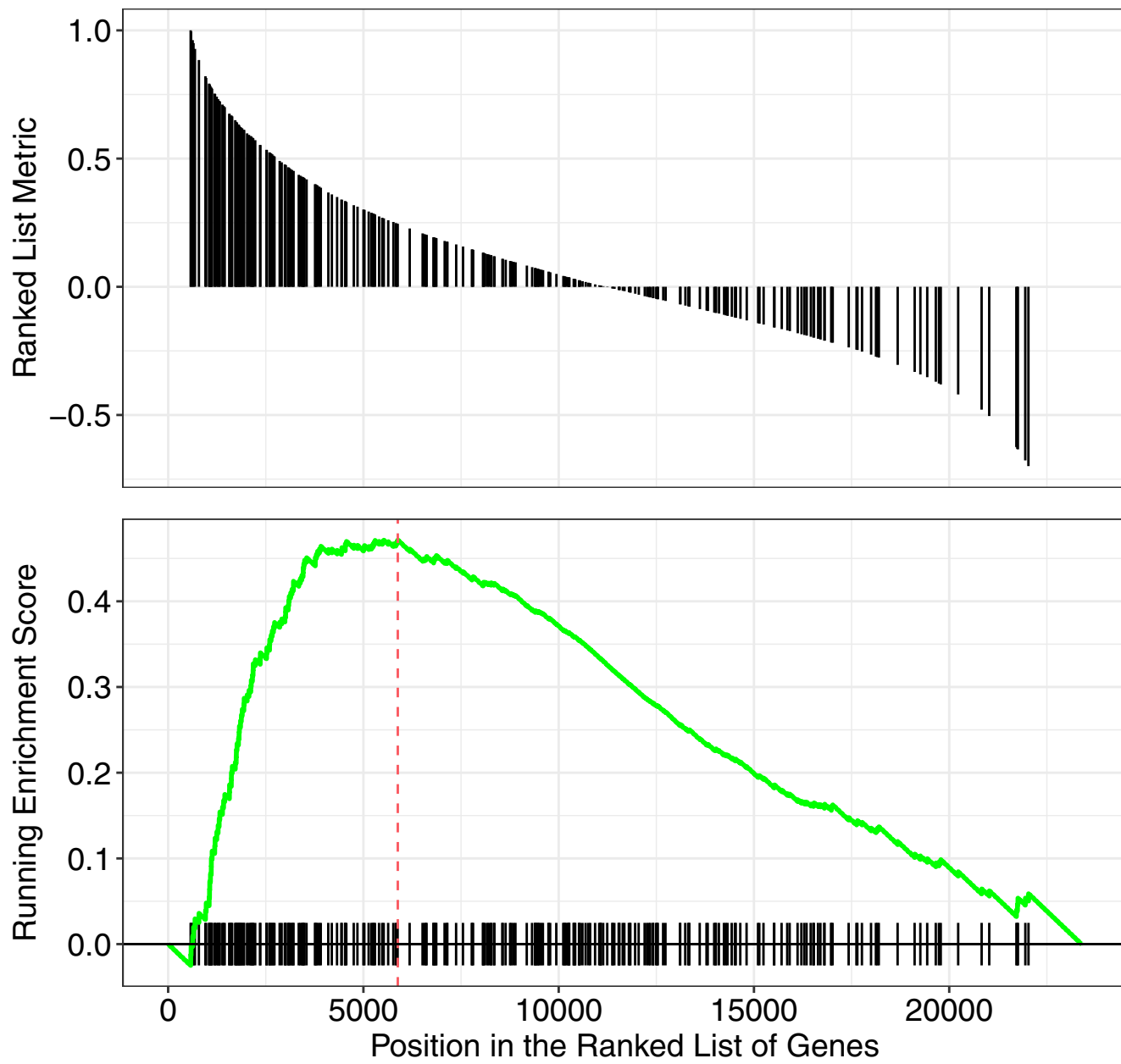
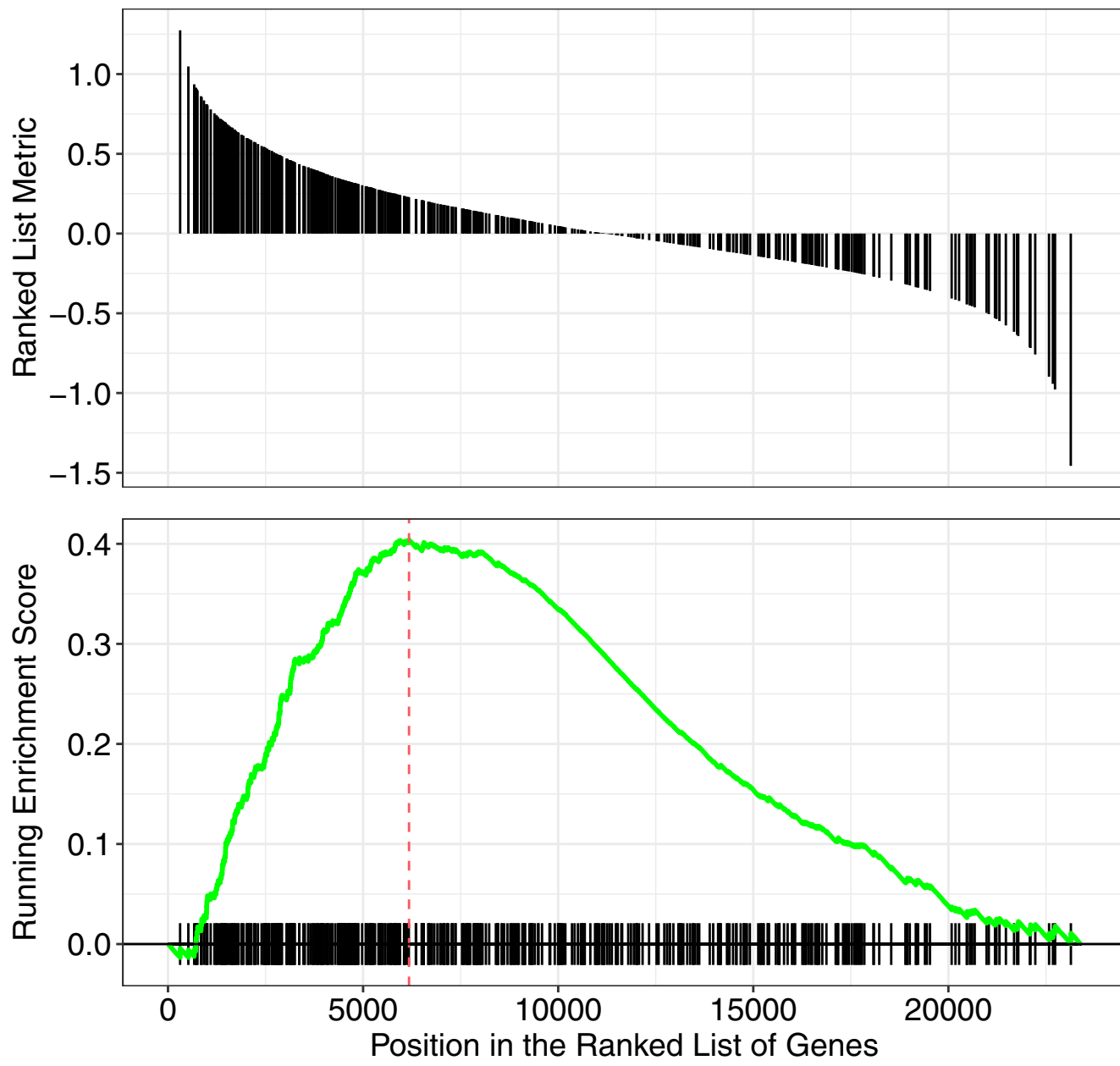


Fig. S4. Enrichment plots of Gene set enrichment analysis (GSEA) of differentially expressed genes in *alx1;alx3* mutants. Running score plot and pre-ranked list of top 25 suppressed GSEA terms shown in Figure 5C.

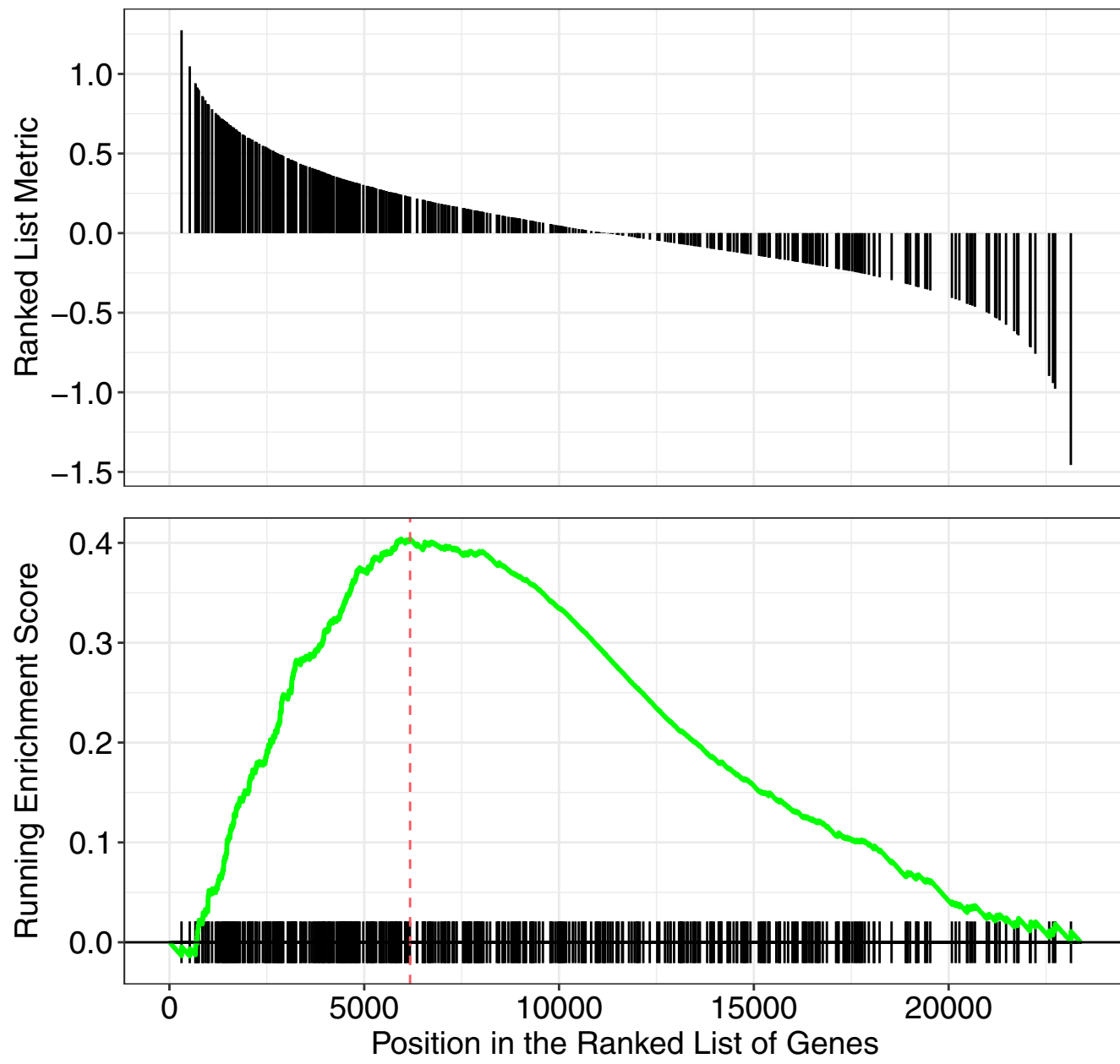
transcription coregulator activity



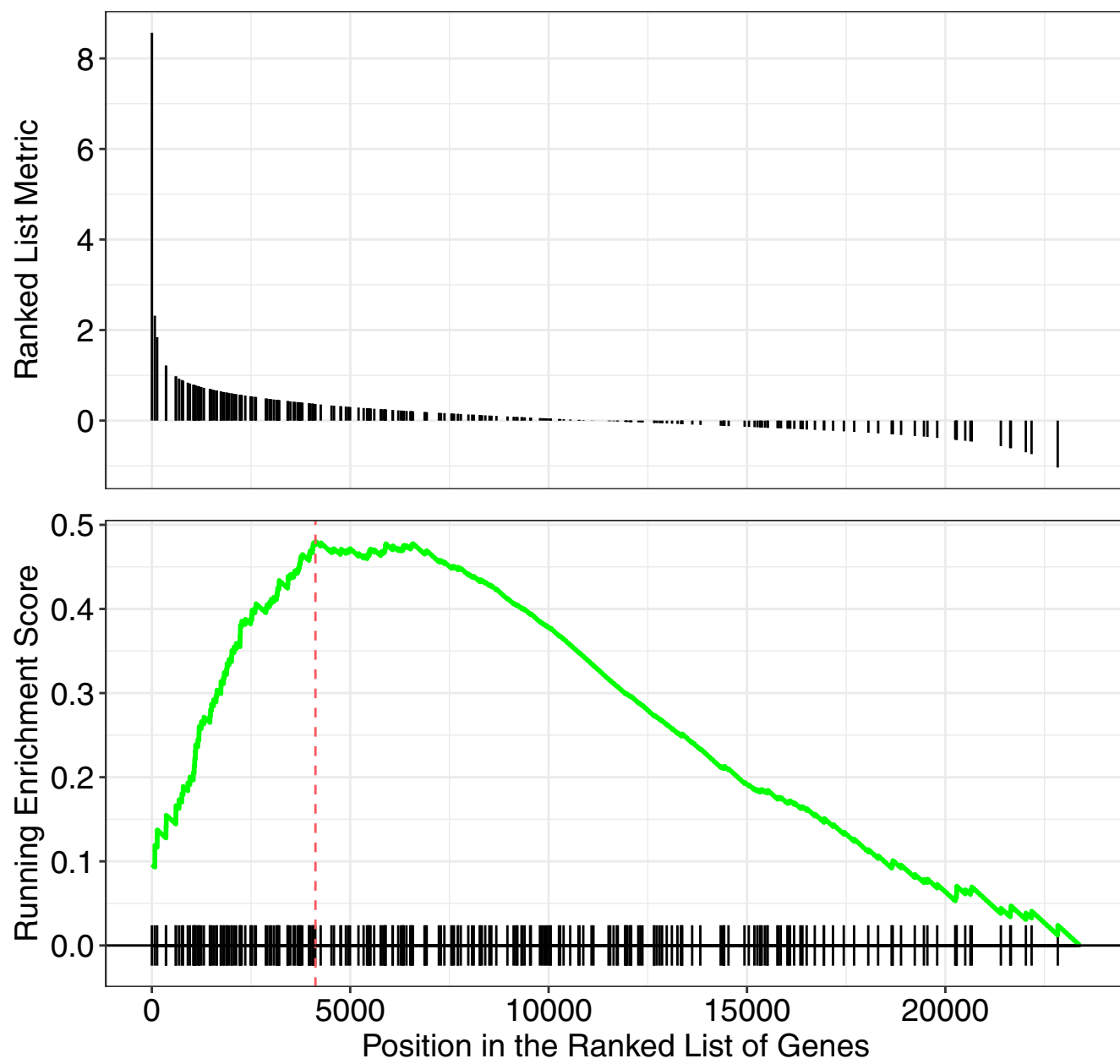
brain development



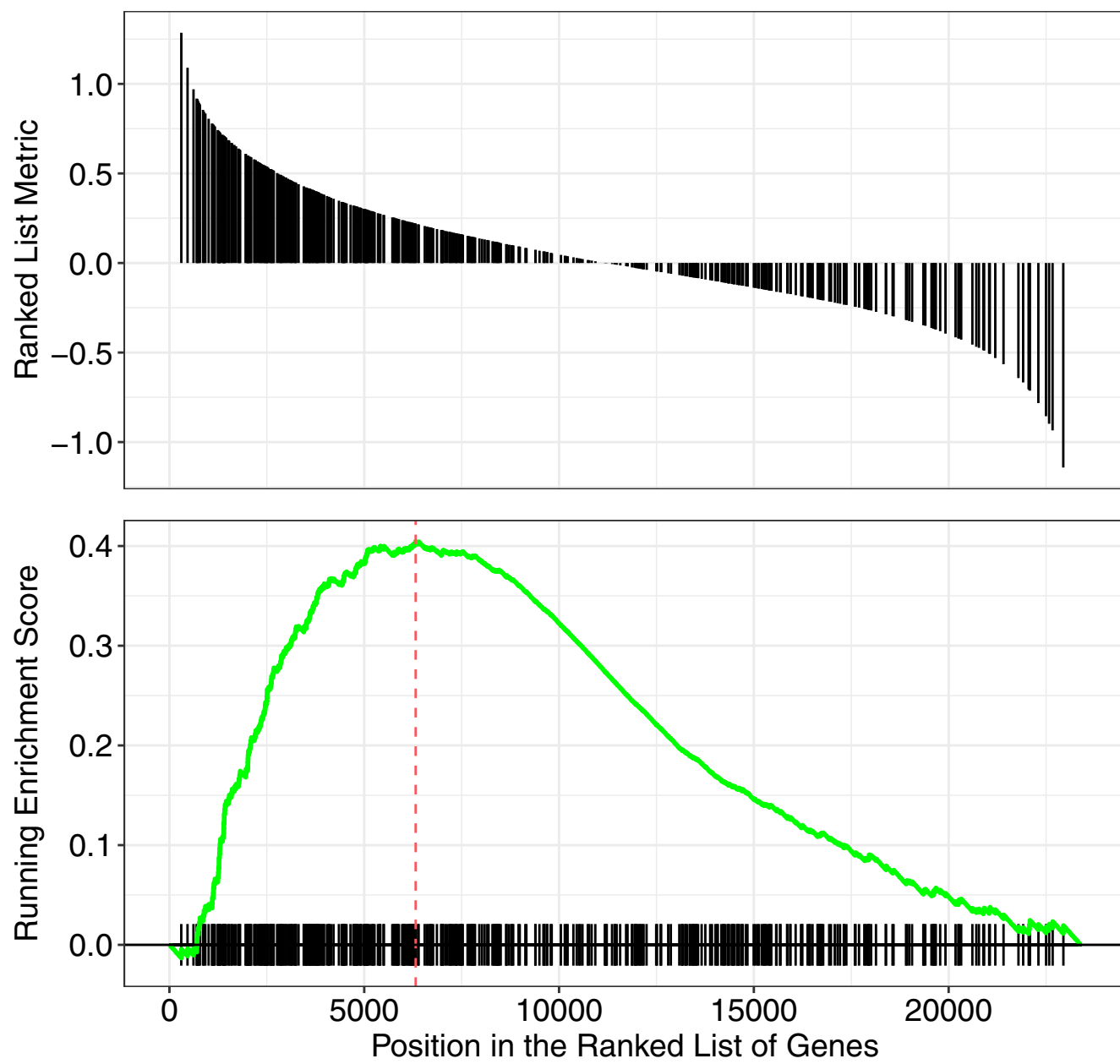
head development



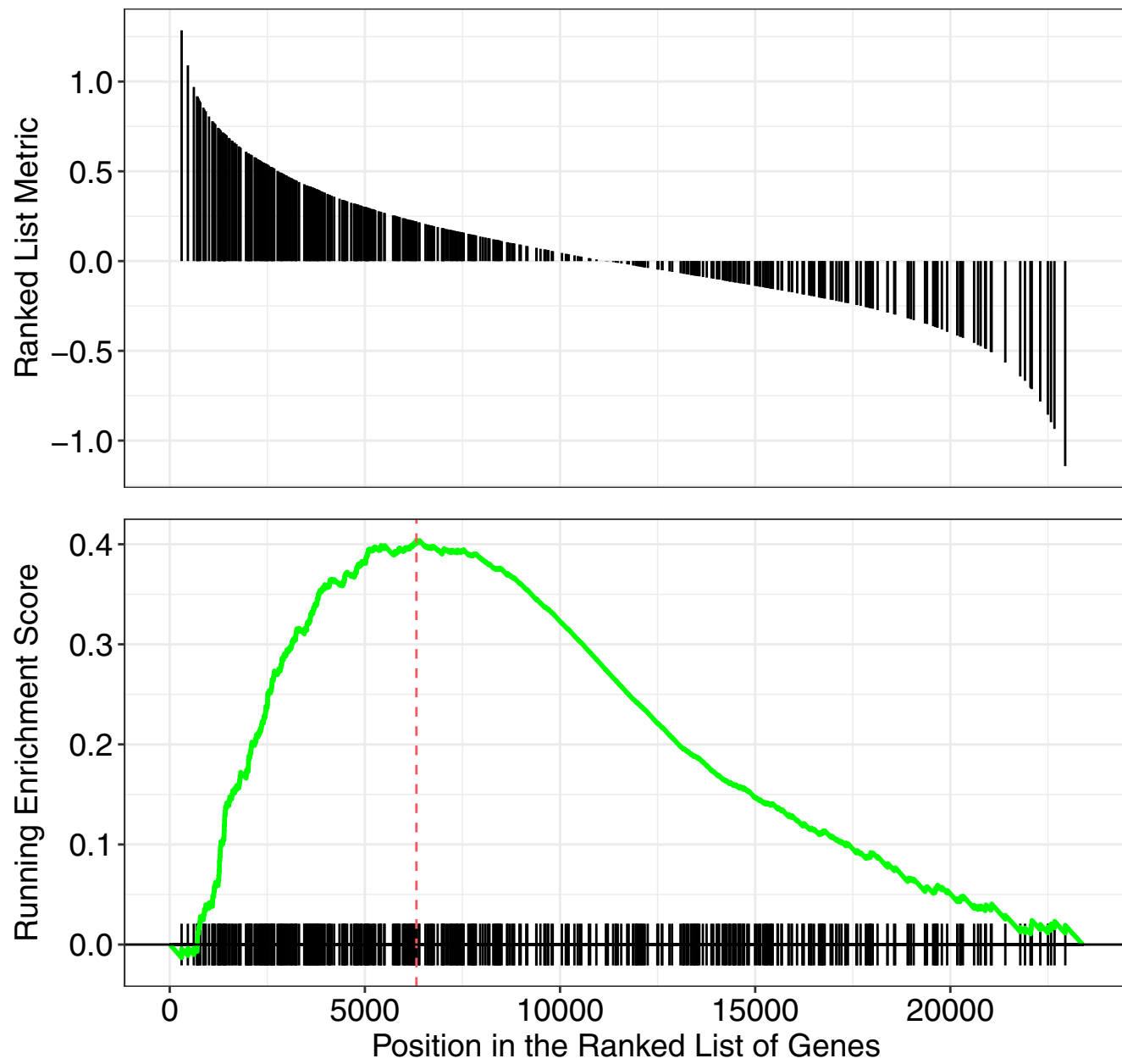
transcription regulator complex



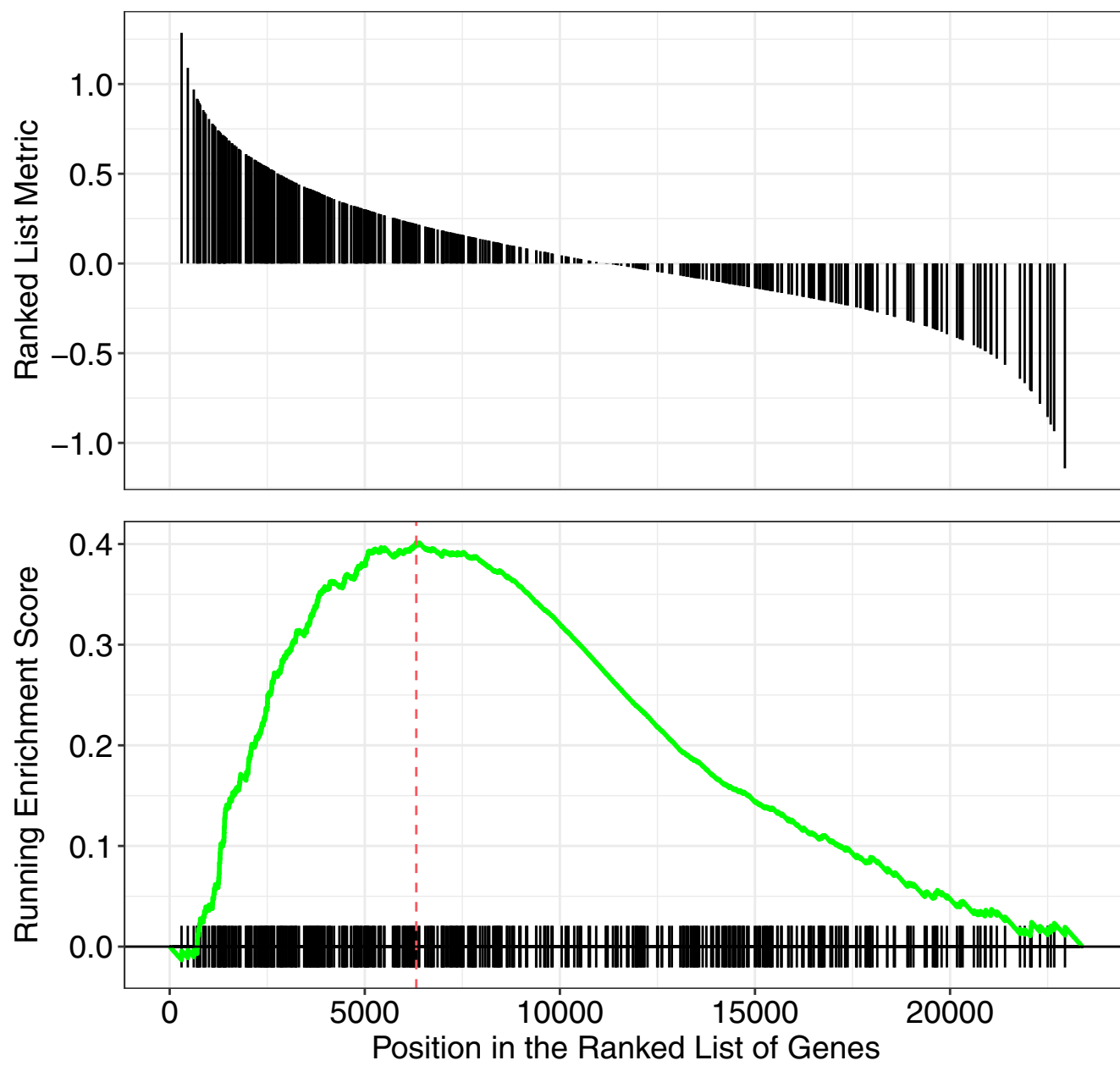
cell projection morphogenesis



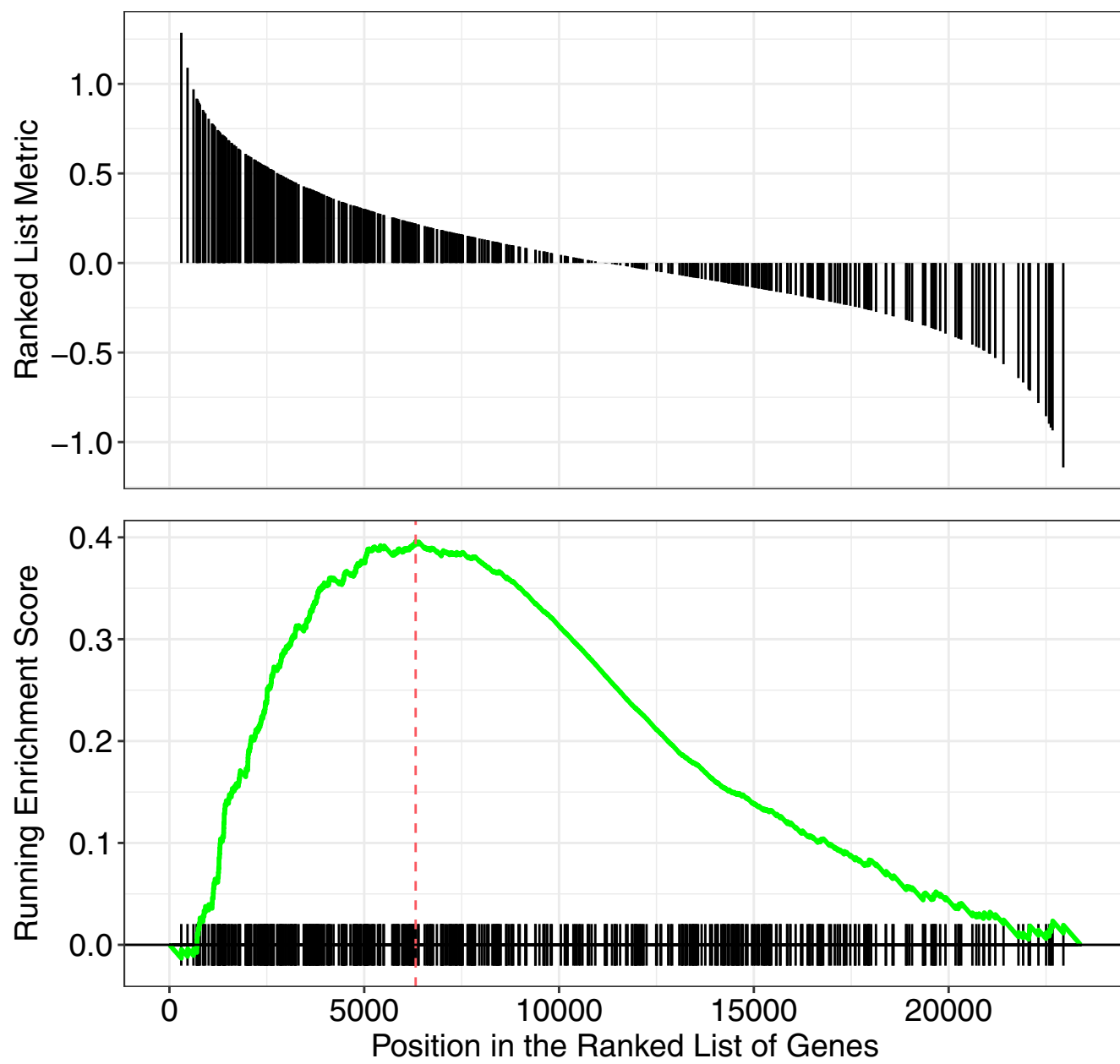
neuron projection morphogenesis



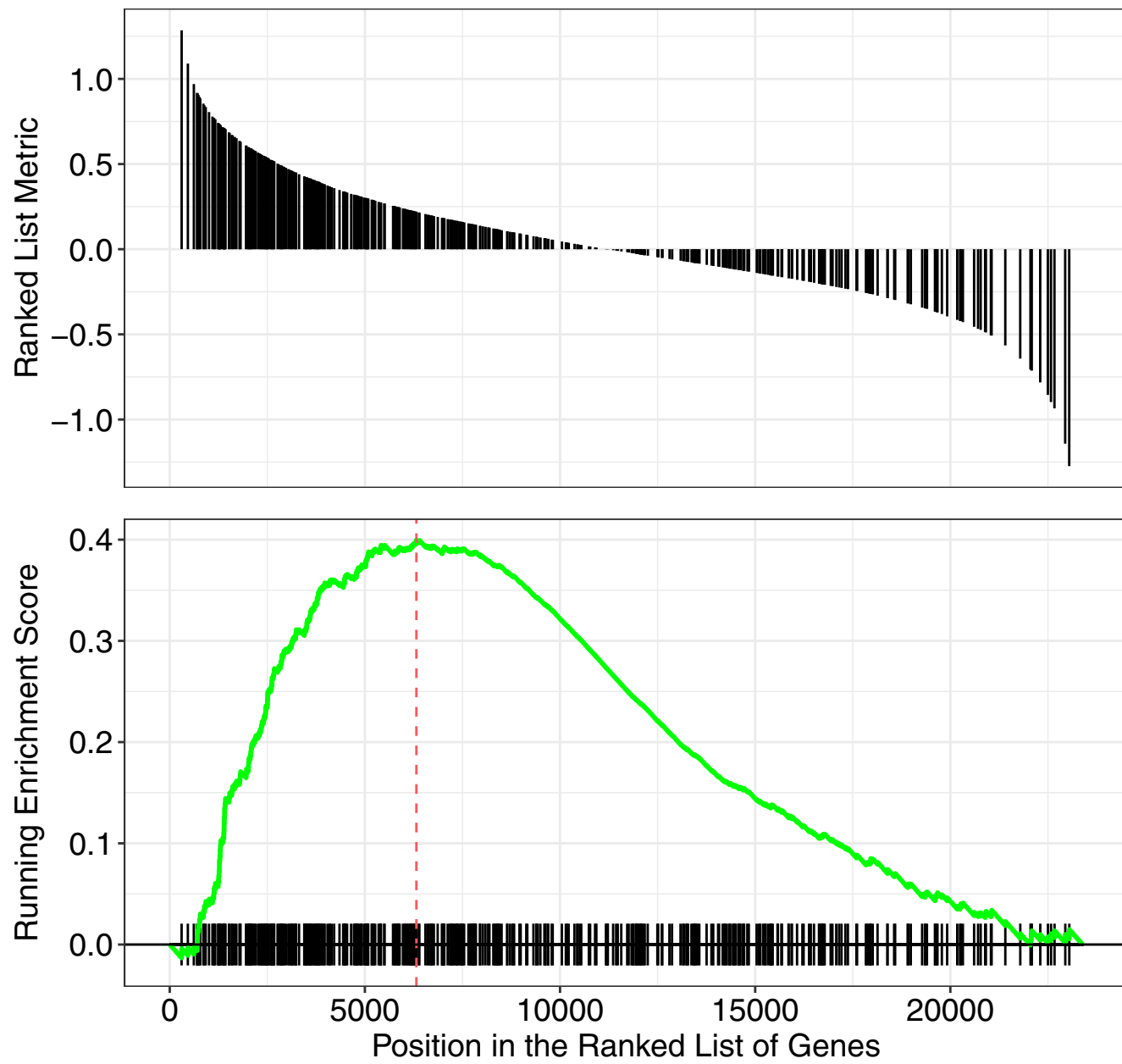
plasma membrane bounded cell projection morphogenesis



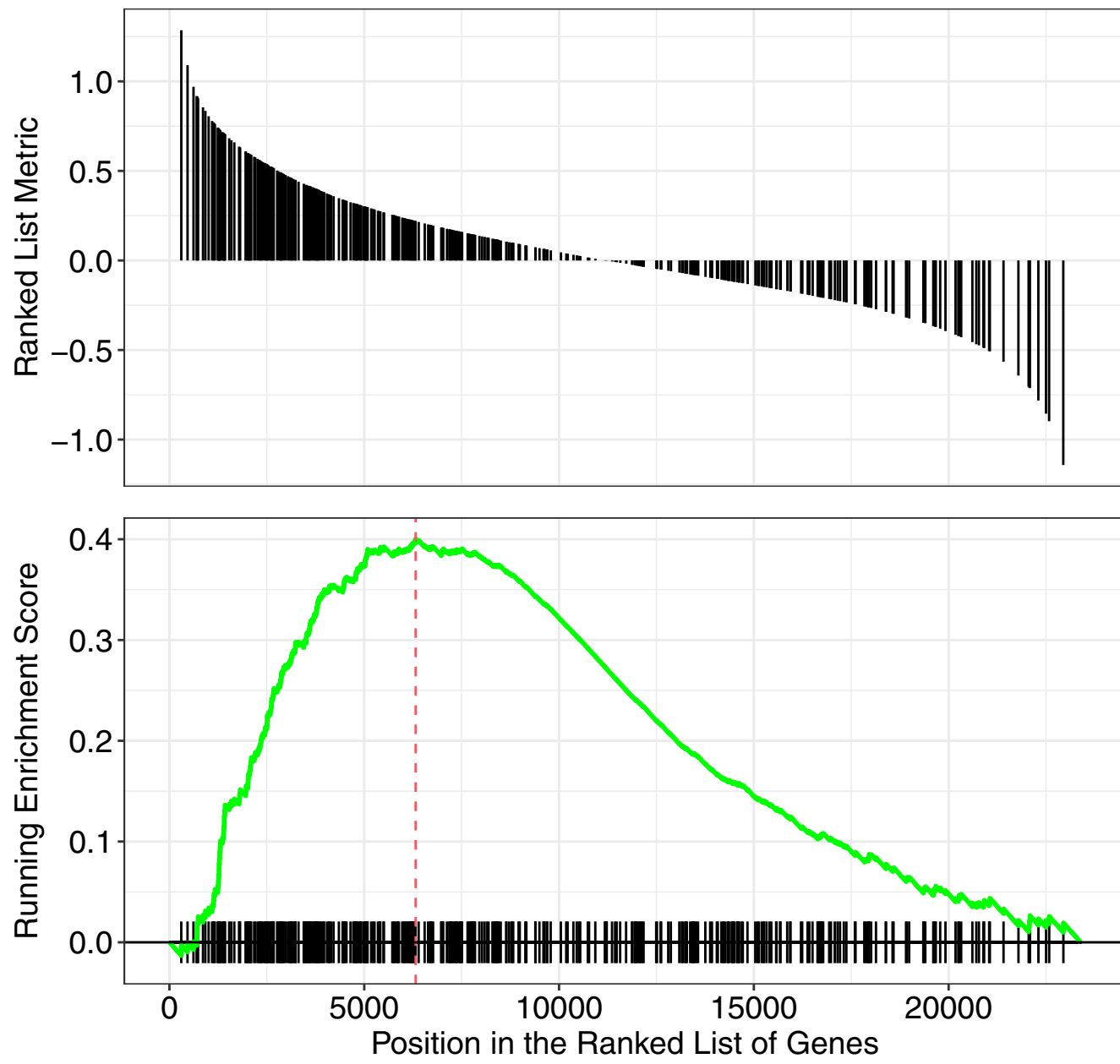
cell part morphogenesis



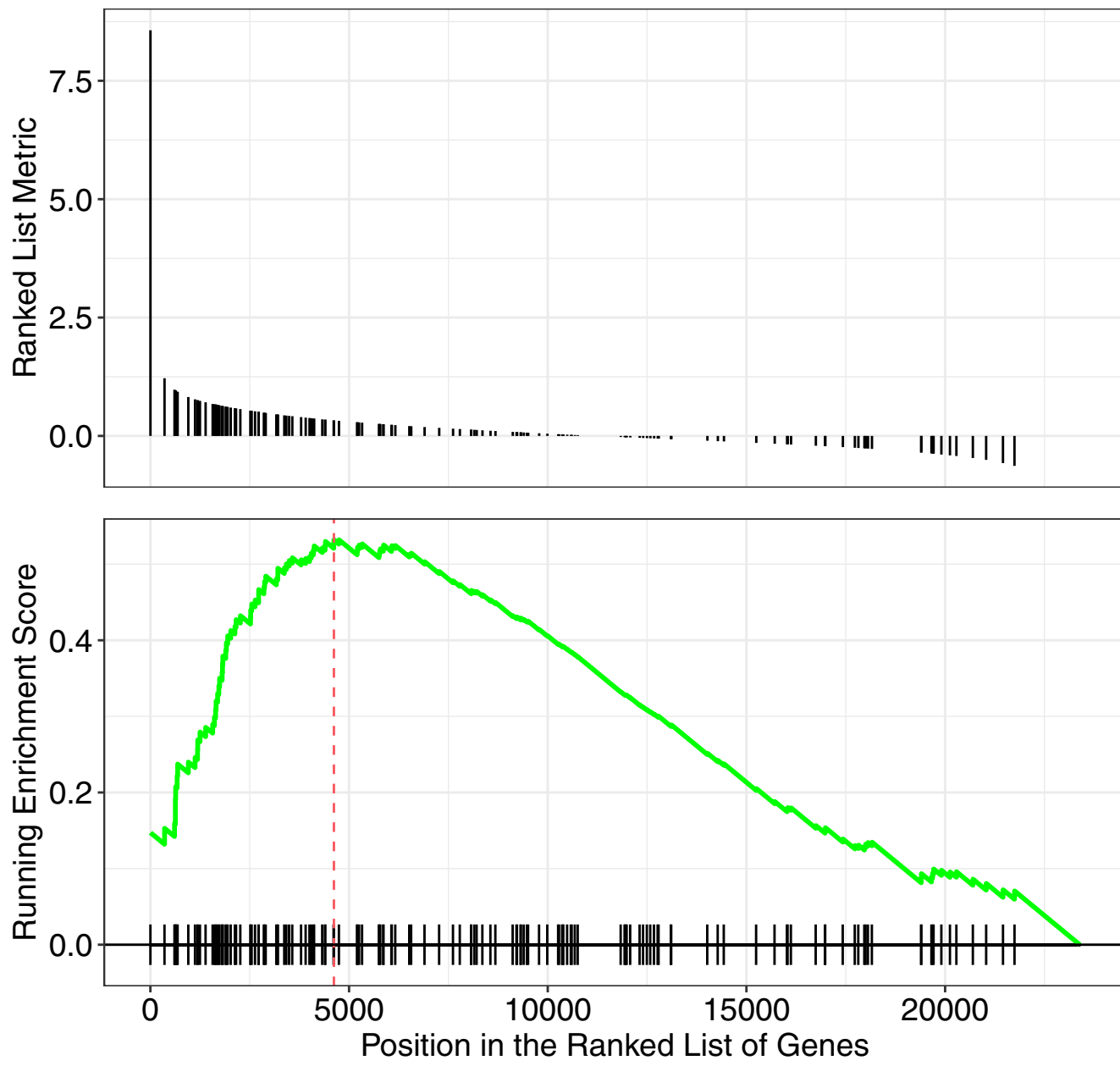
cell morphogenesis involved in neuron differentiation



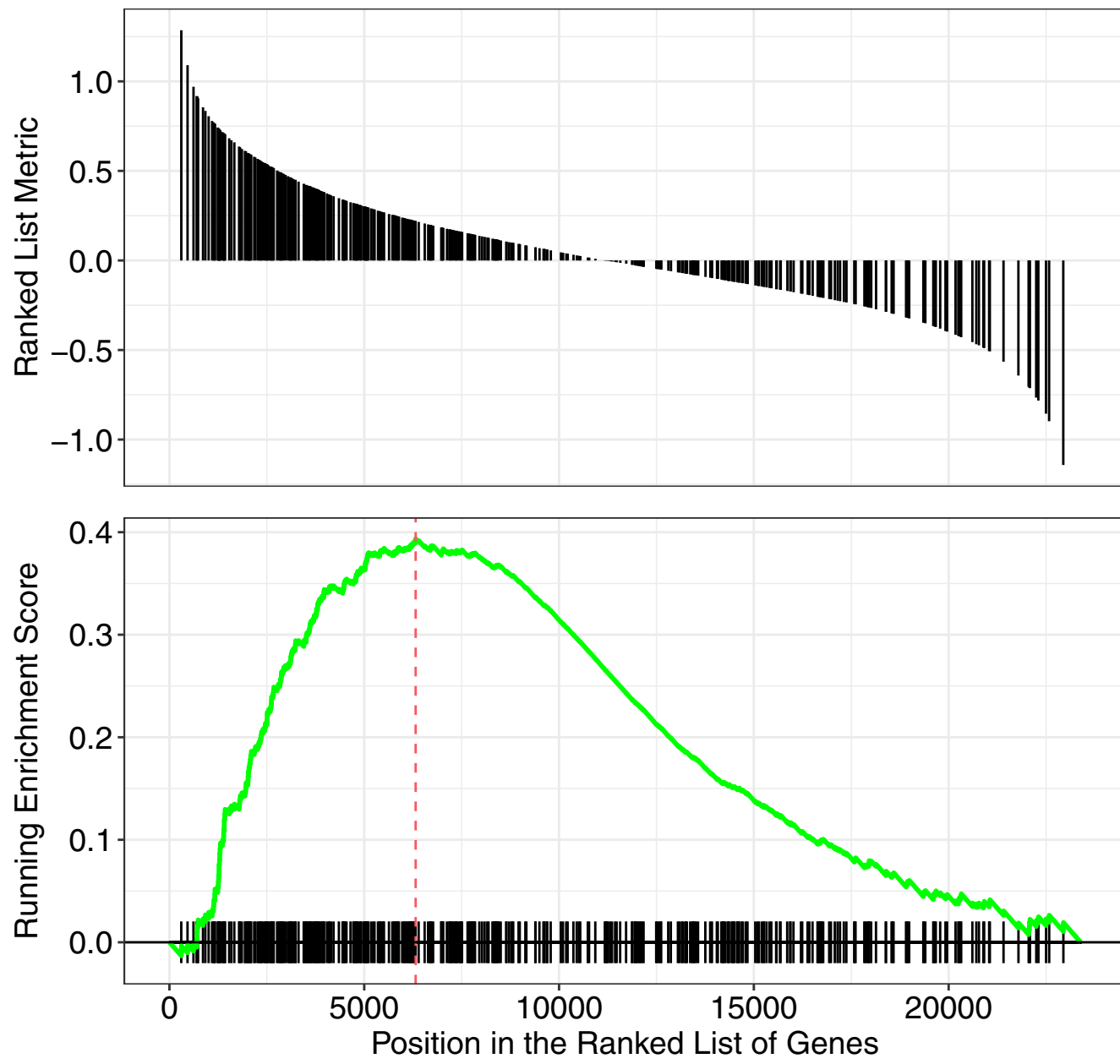
axonogenesis



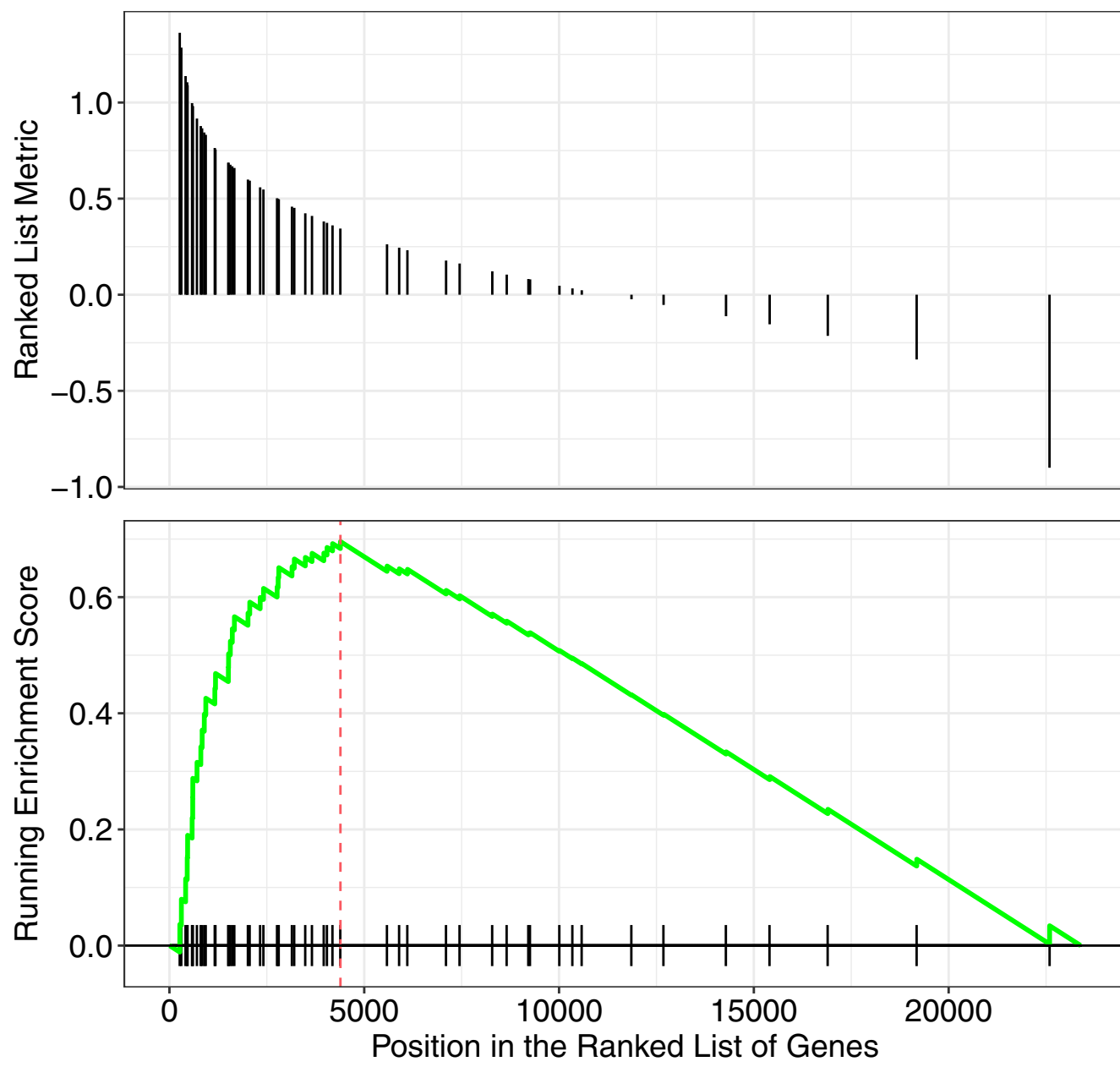
transcription factor binding



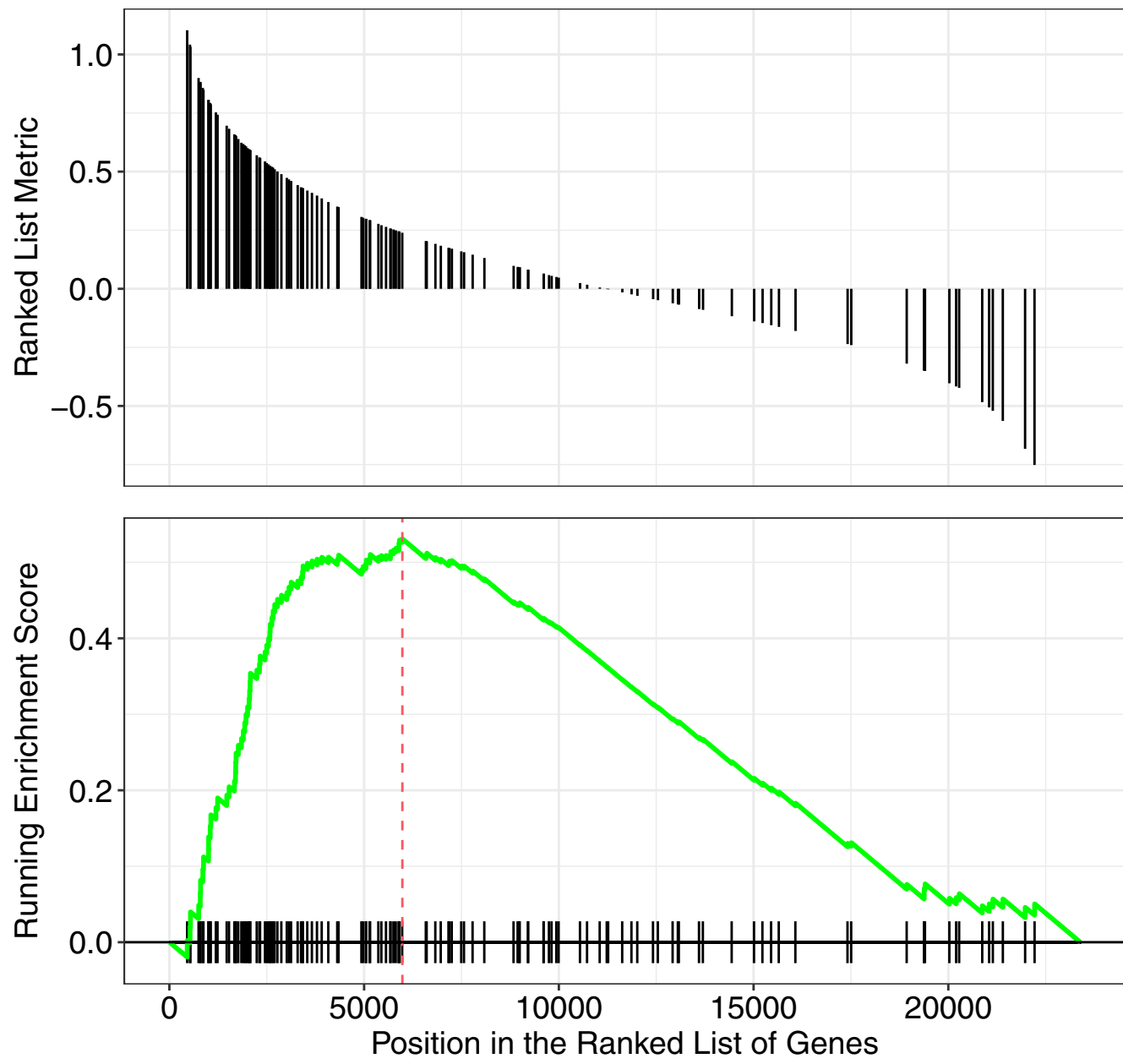
axon development



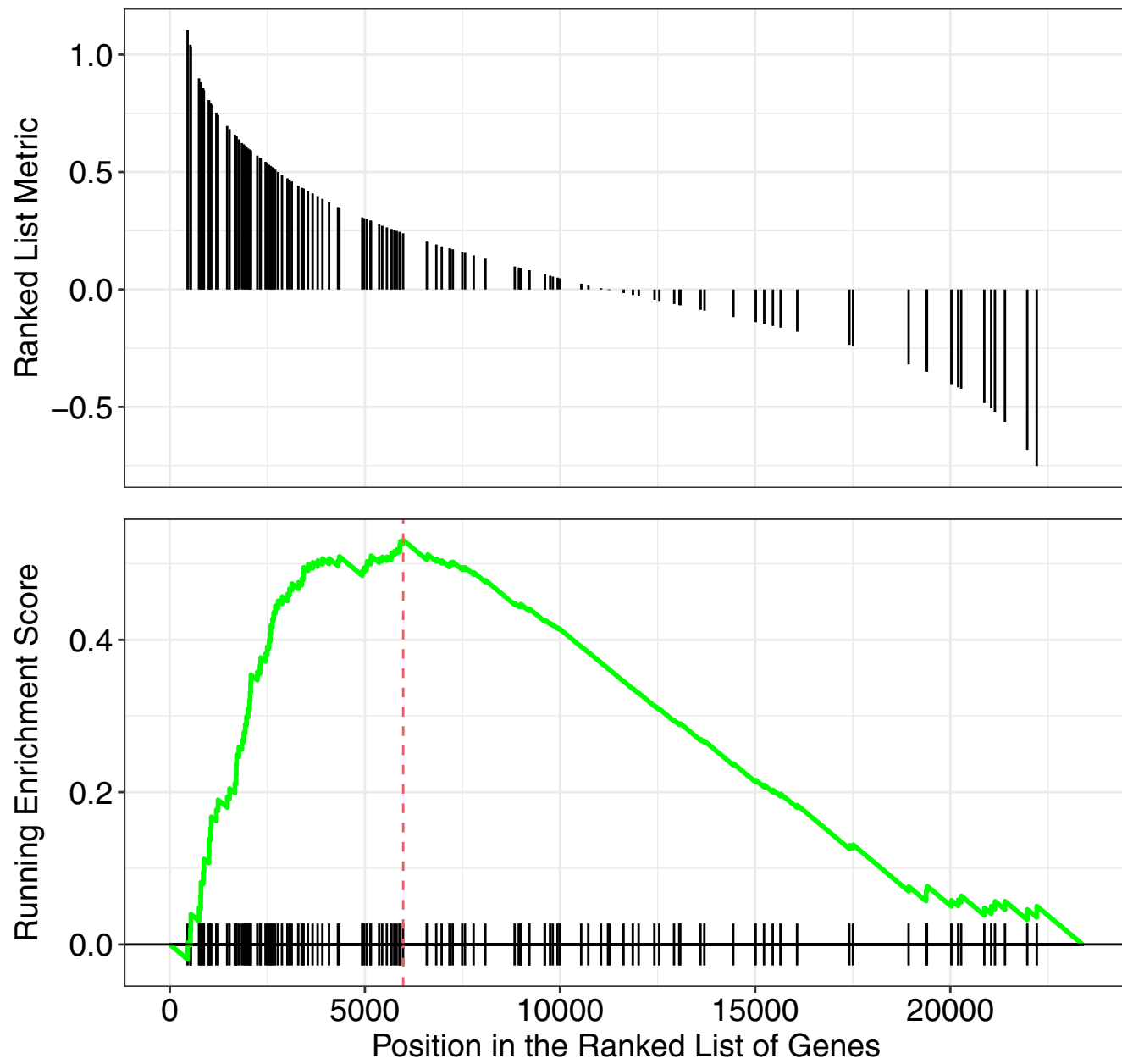
collagen trimer



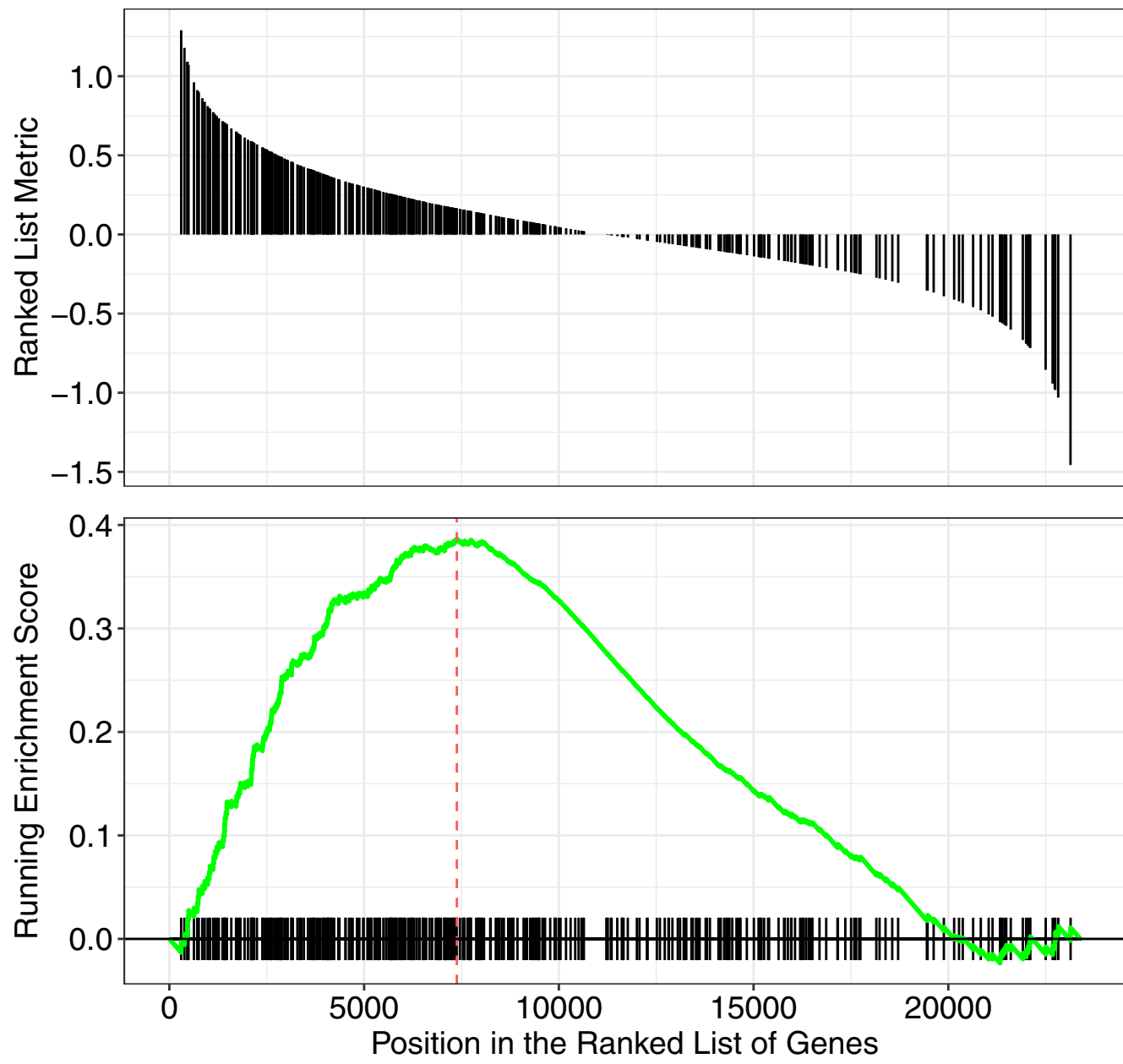
modulation of chemical synaptic transmission



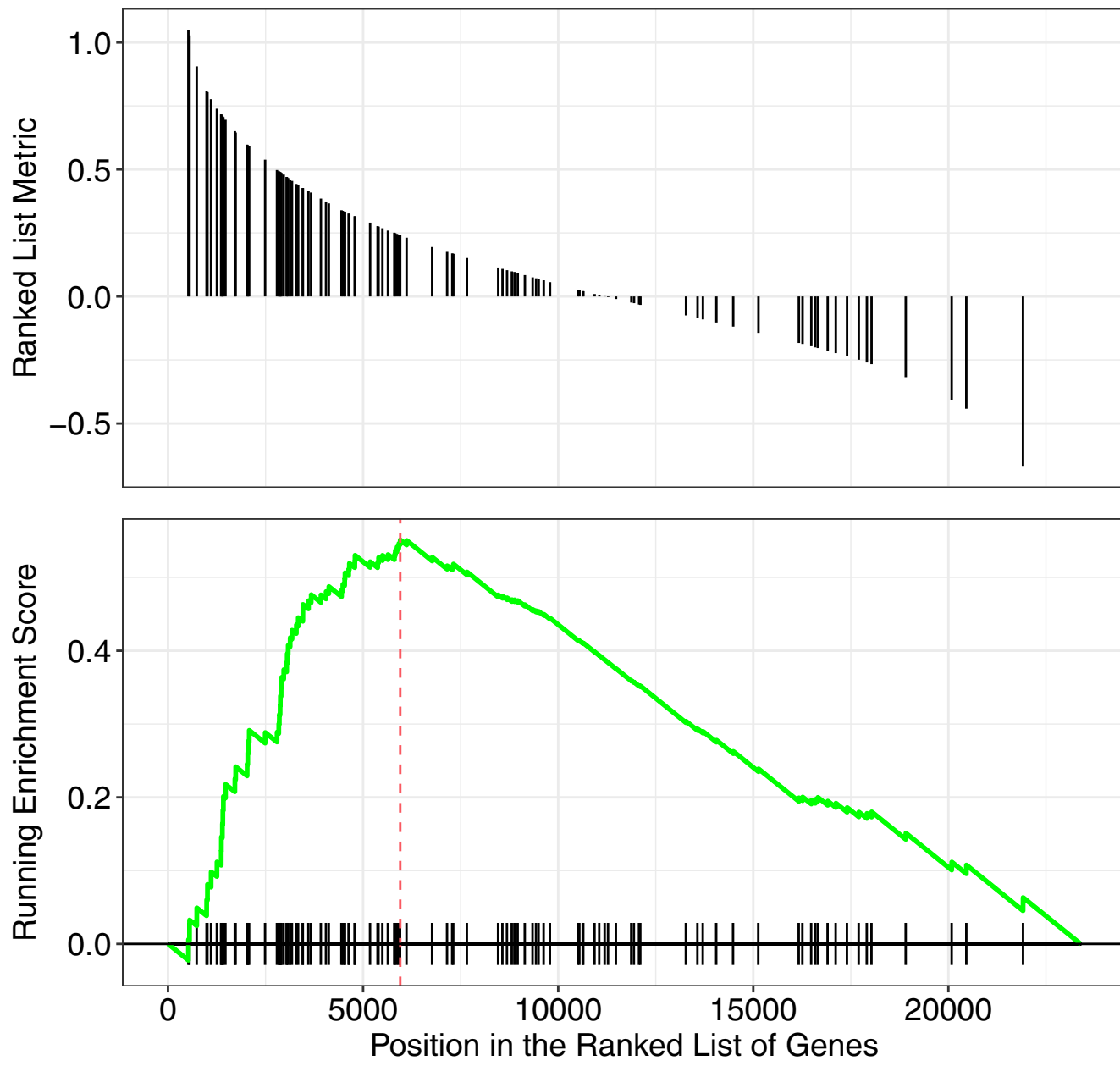
regulation of trans-synaptic signaling



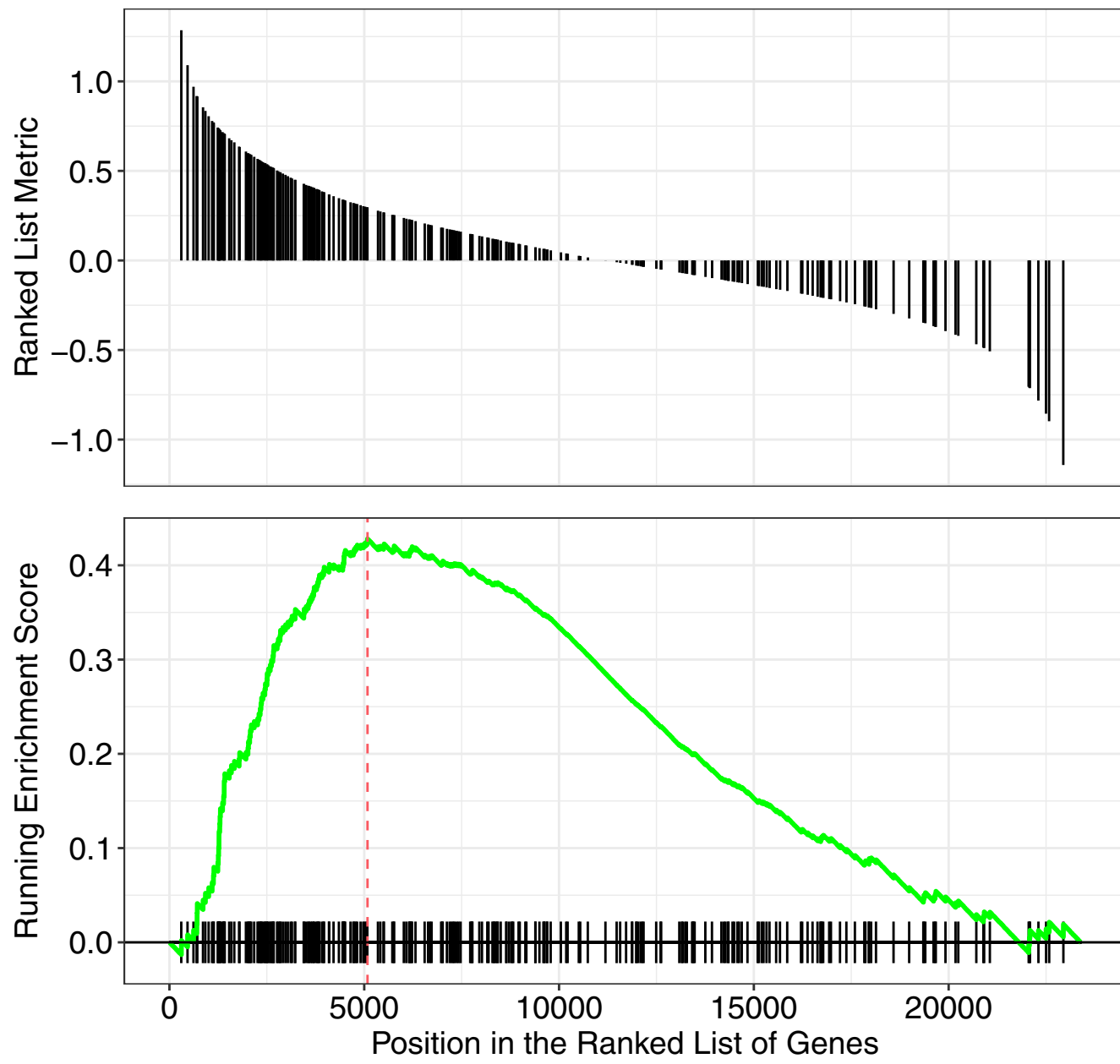
regionalization



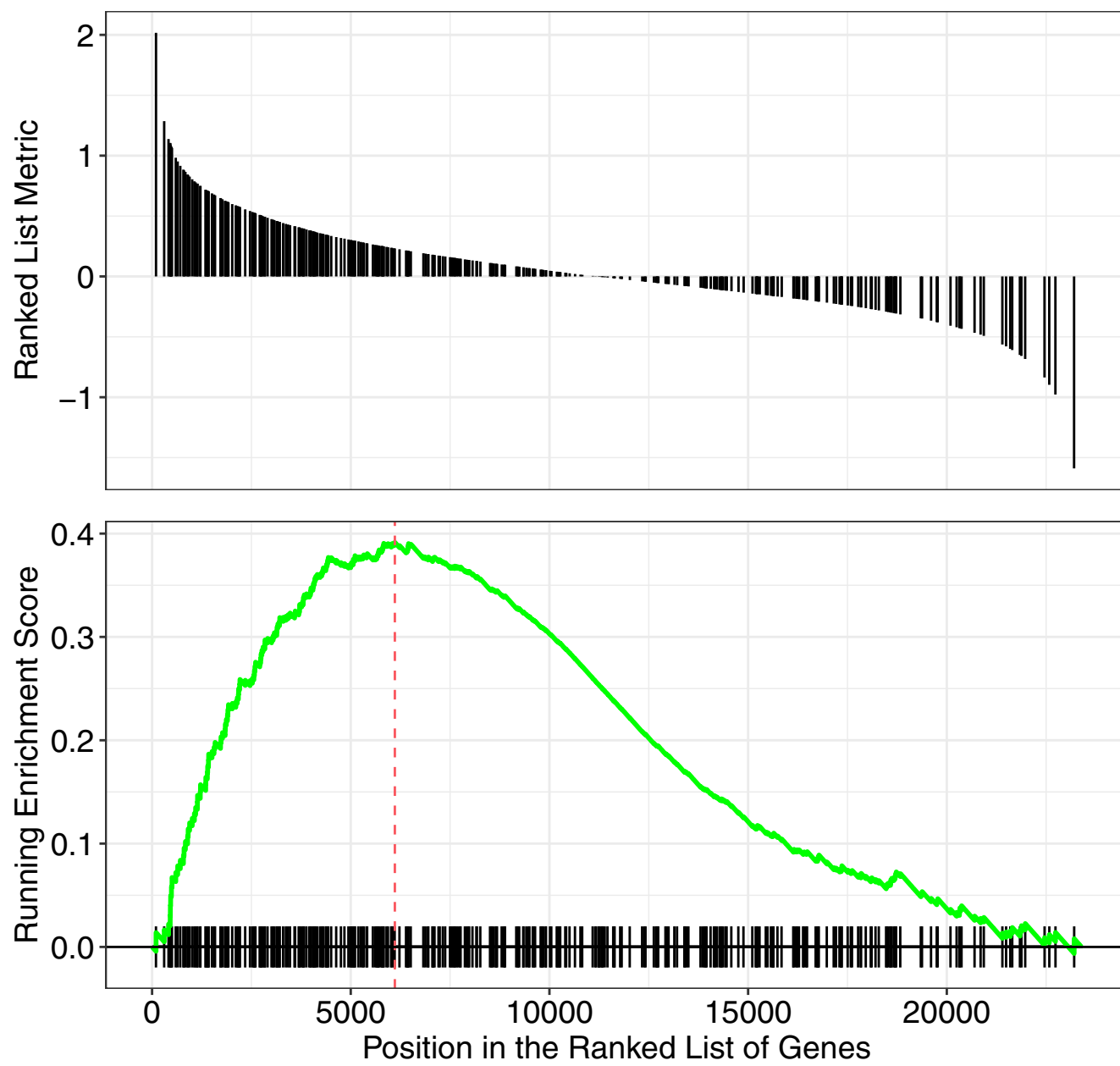
central nervous system neuron differentiation



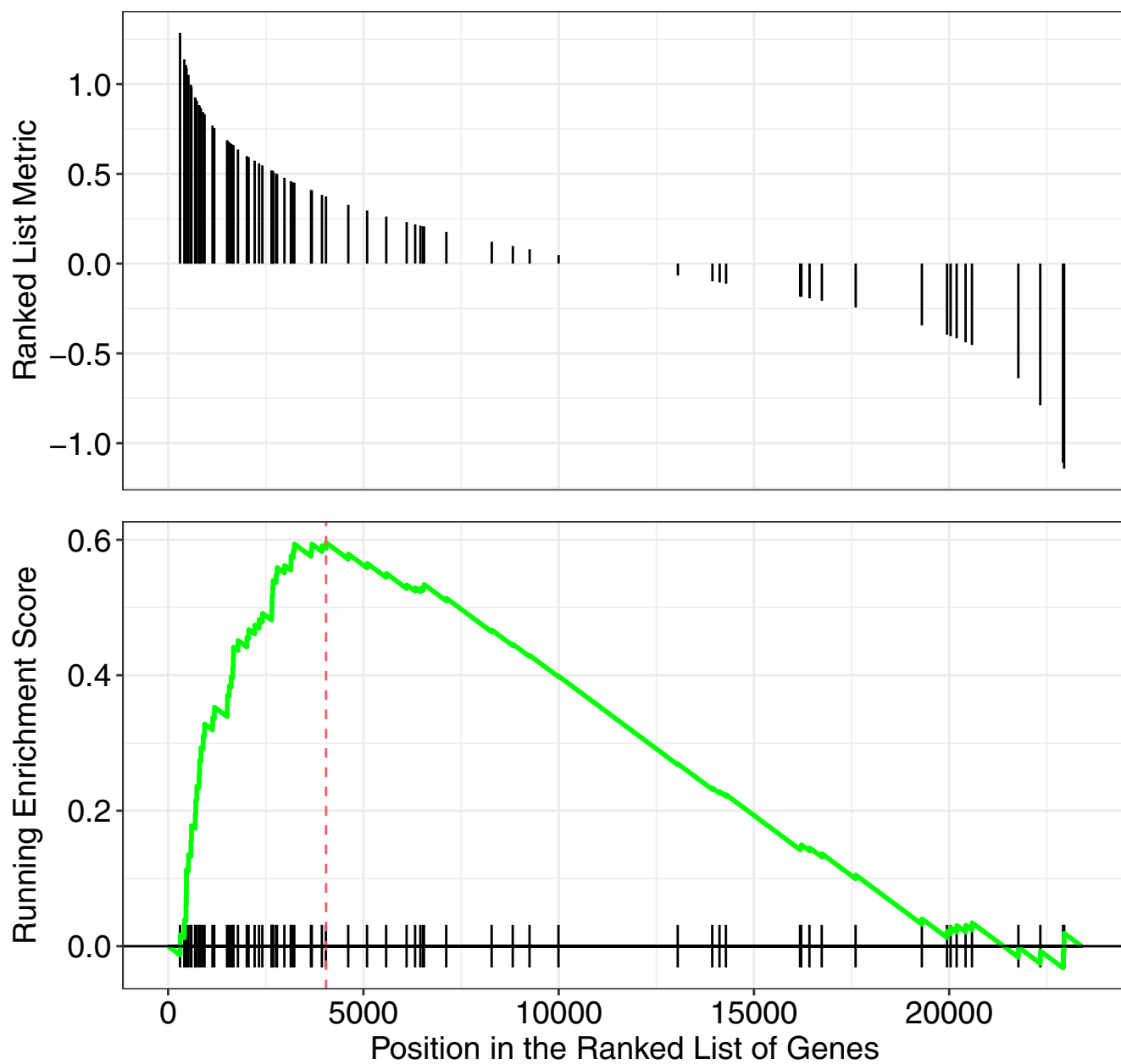
axon guidance



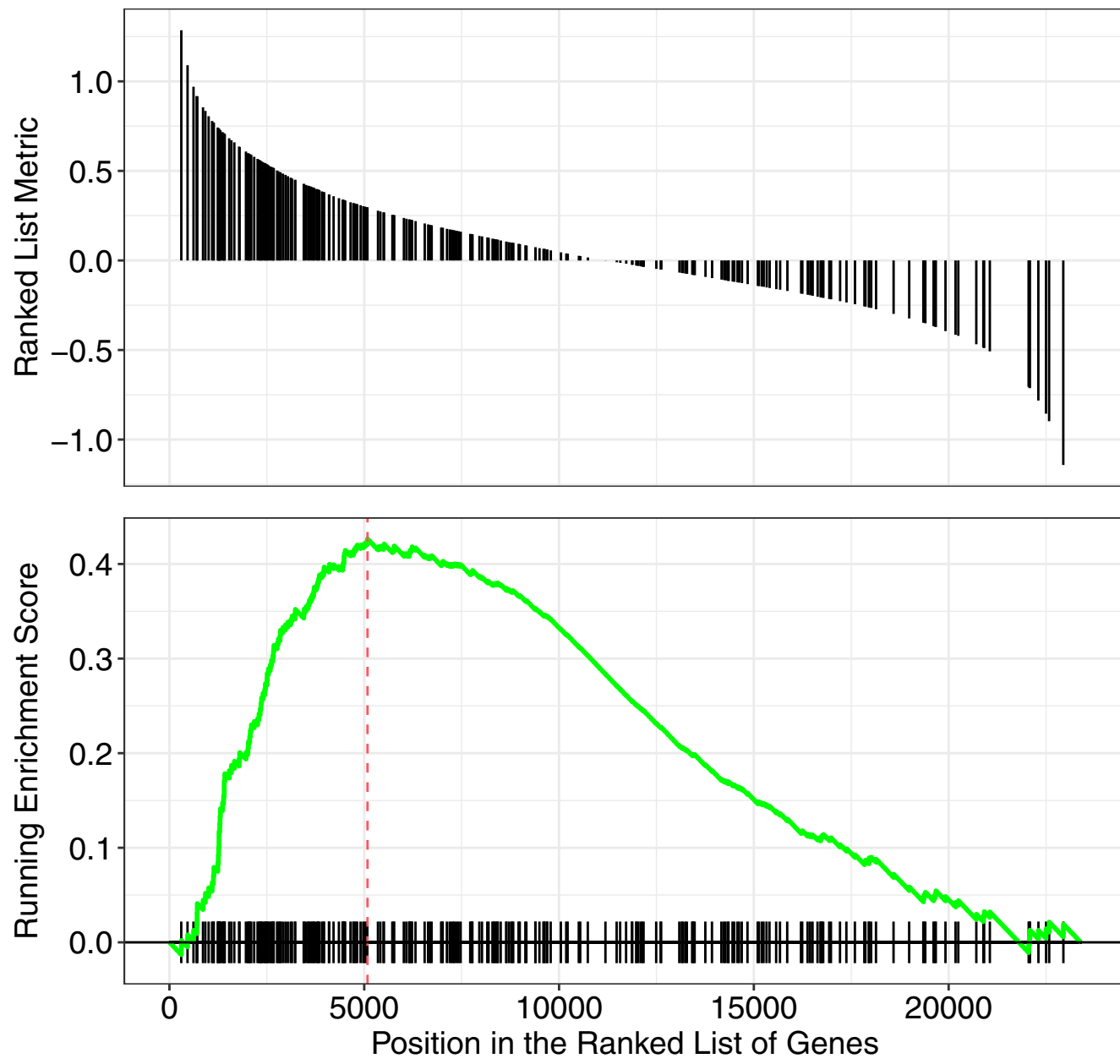
skeletal system development



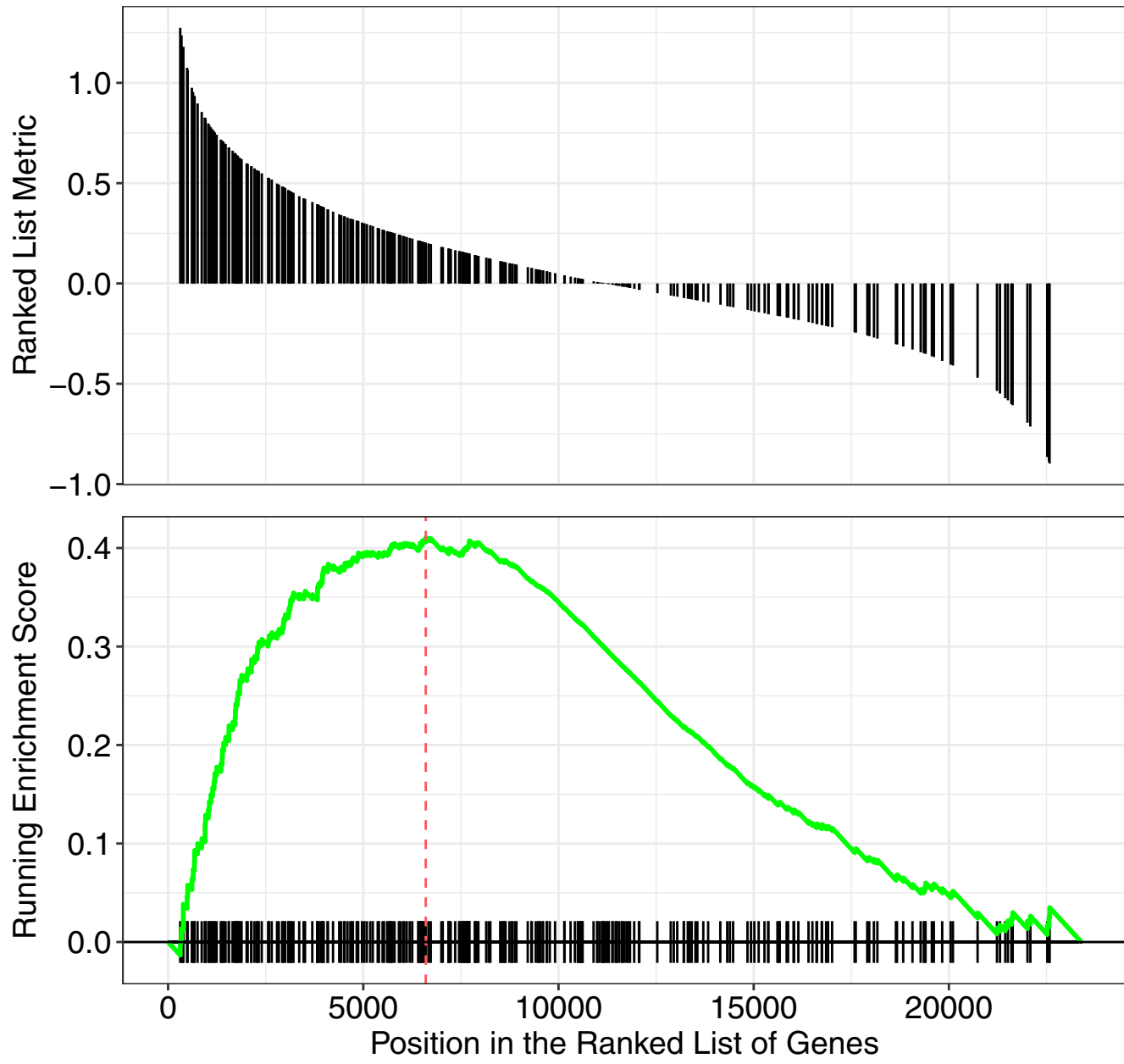
extracellular matrix structural constituent



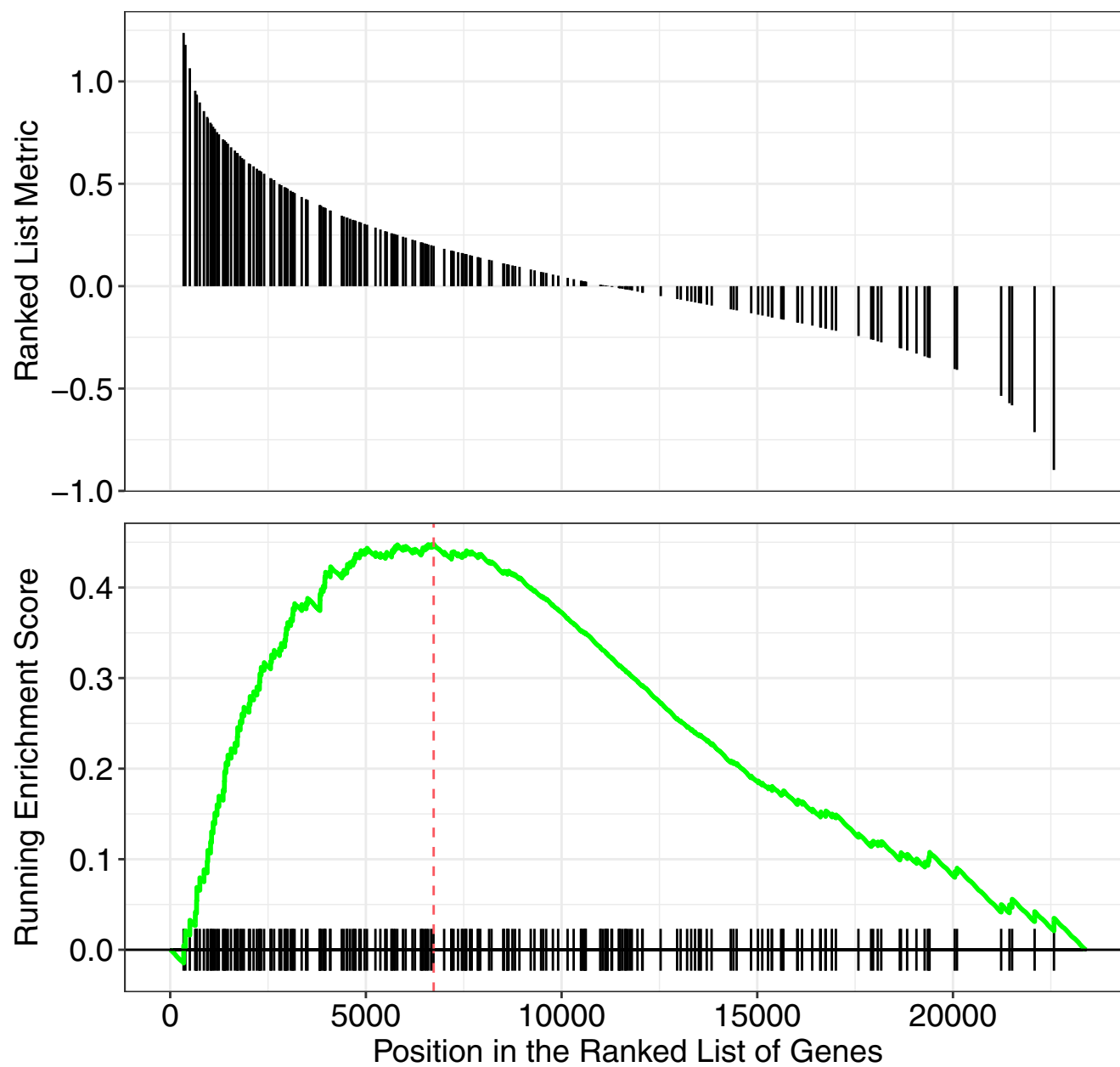
neuron projection guidance



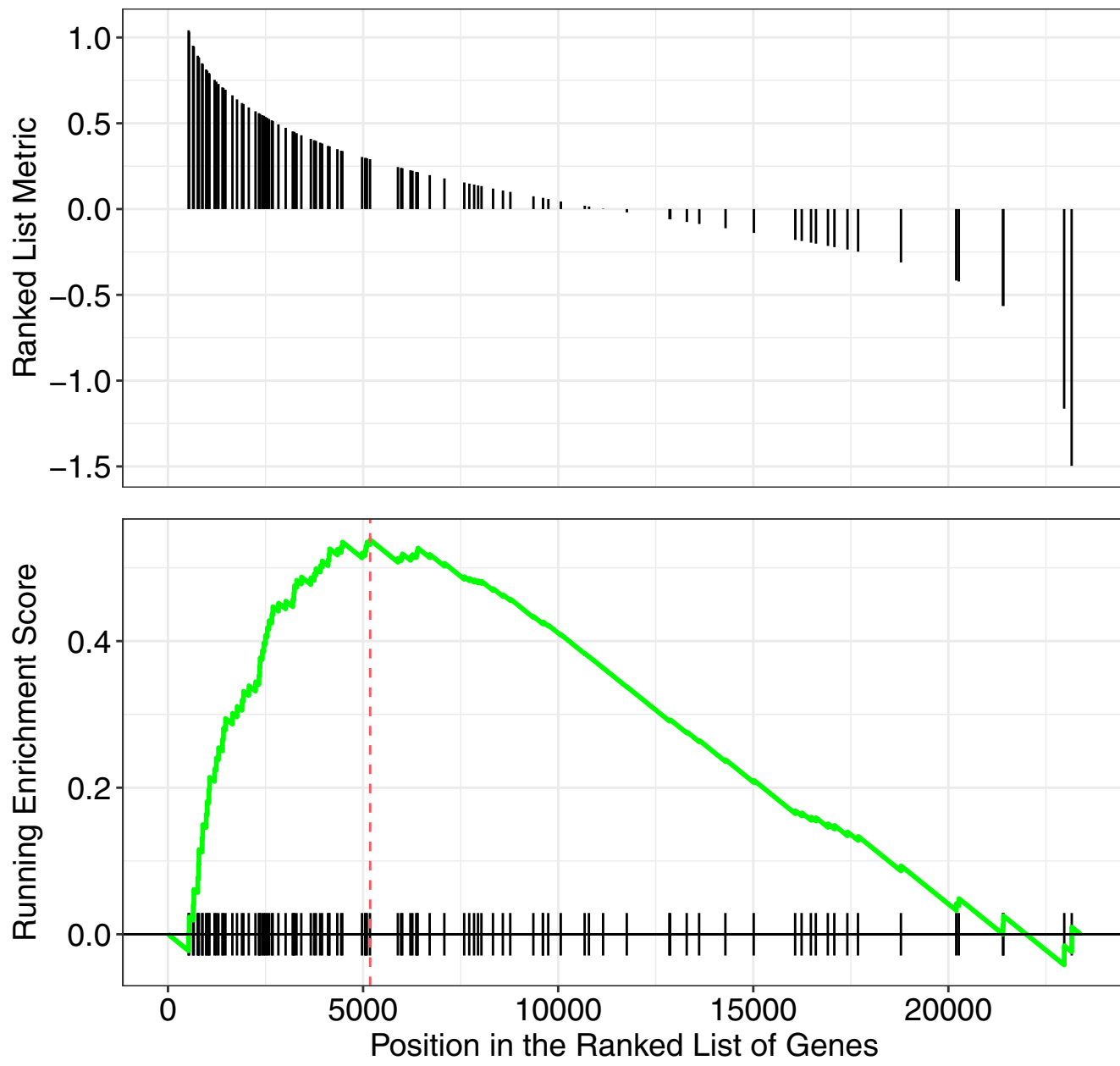
sensory organ morphogenesis



eye morphogenesis



postsynaptic specialization



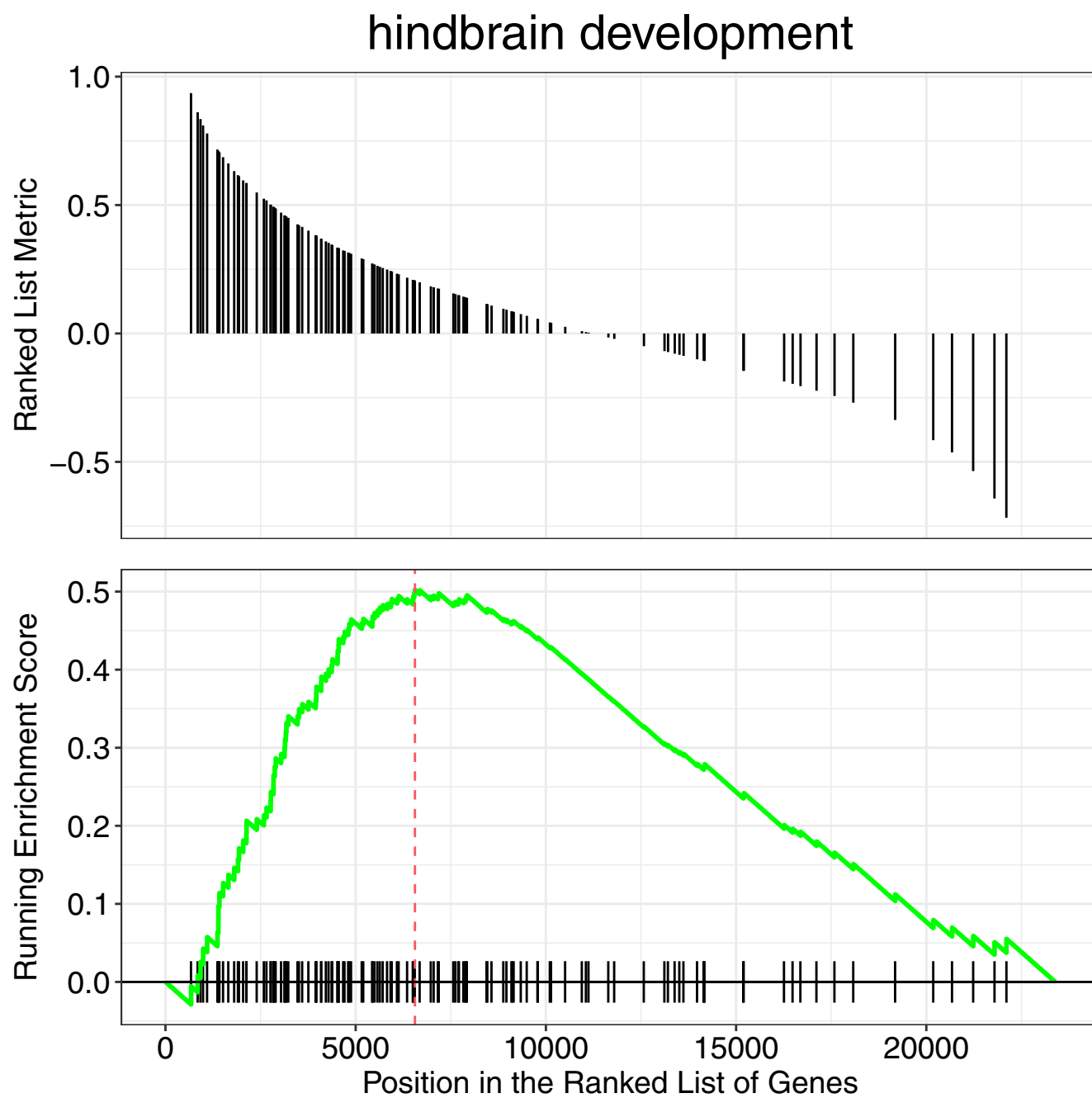


Fig. S5. Enrichment plots of Gene set enrichment analysis (GSEA) of differentially expressed genes in *alx1;alx3* mutants. Running score plot and pre-ranked list of top 25 activated GSEA terms shown in Figure 5D.

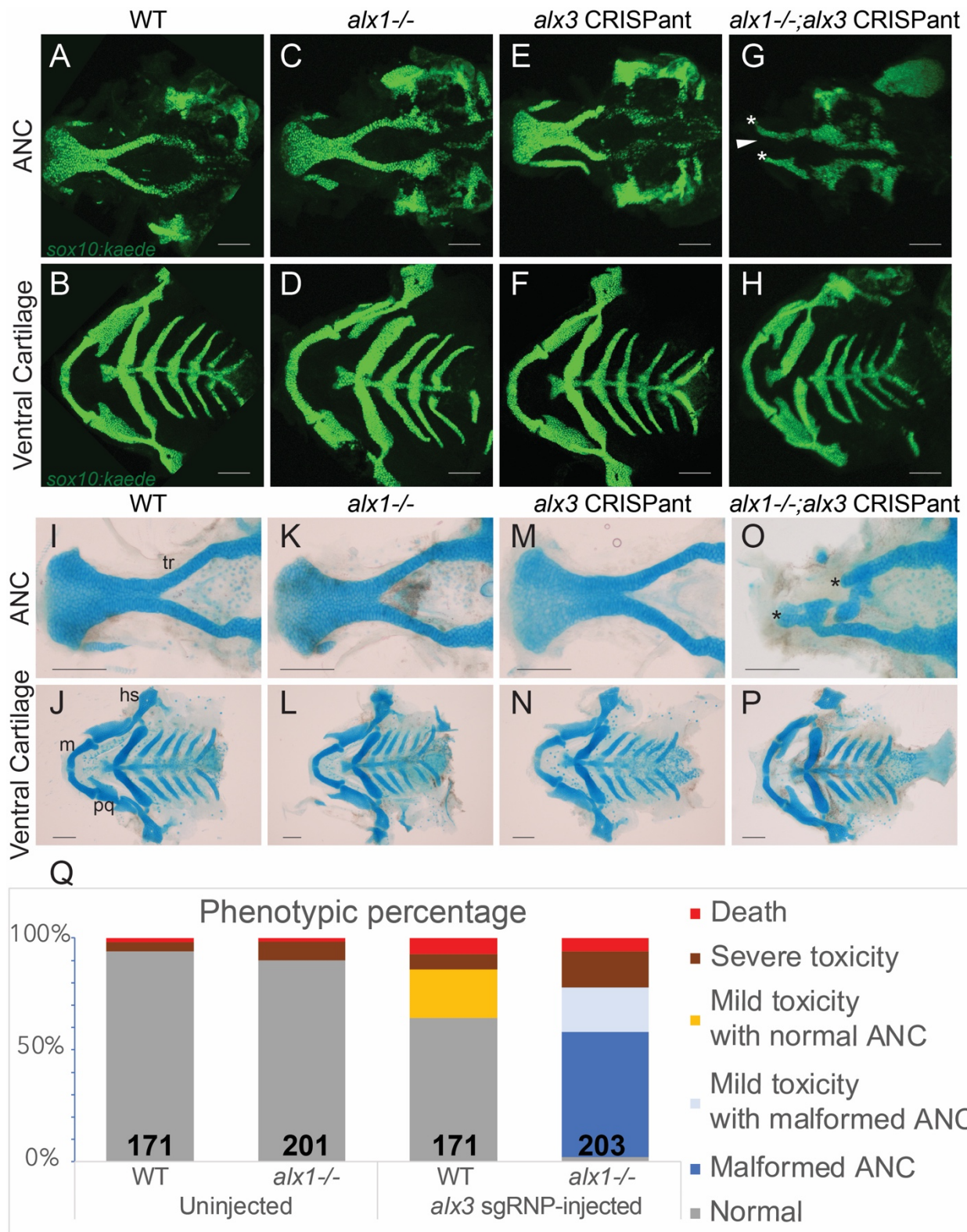
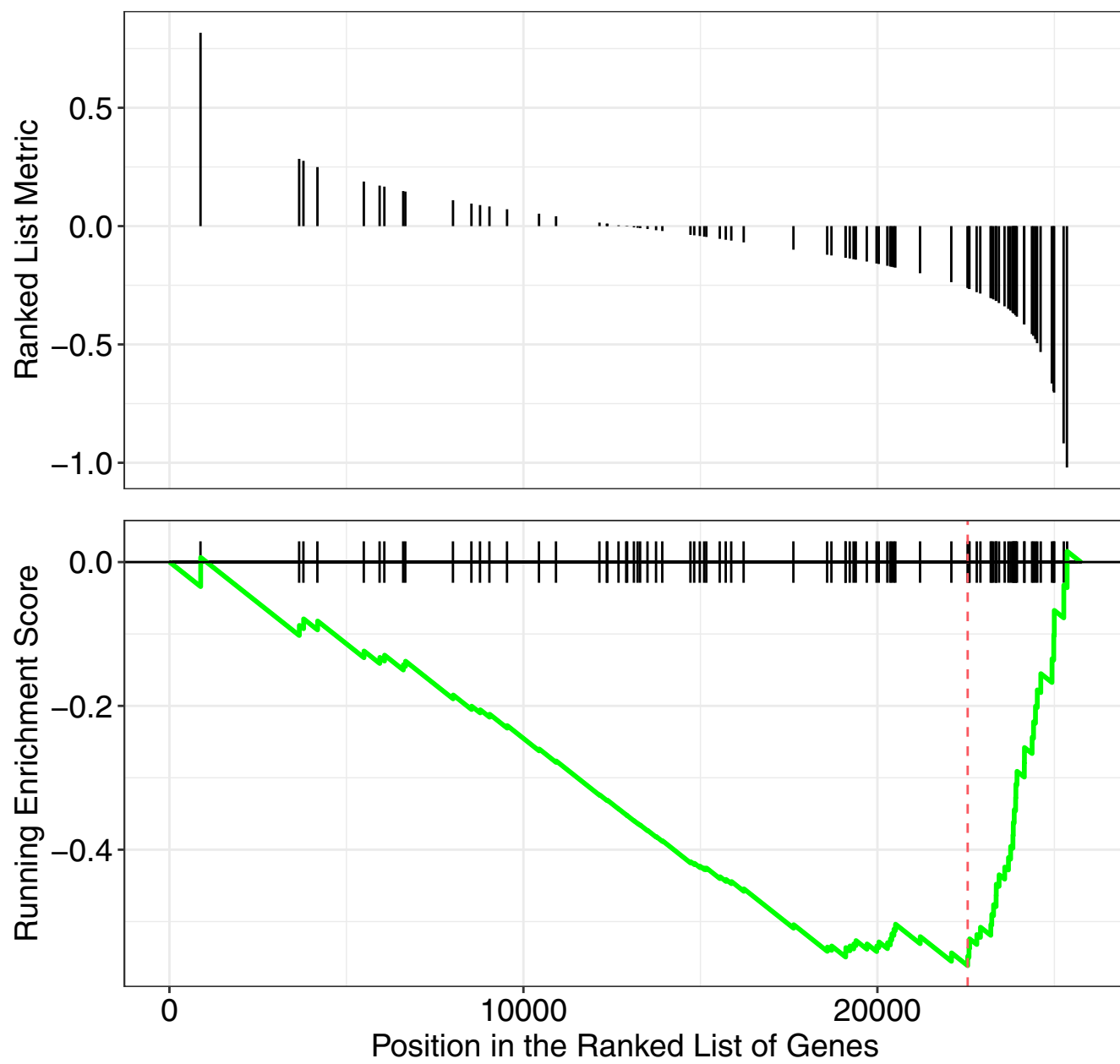
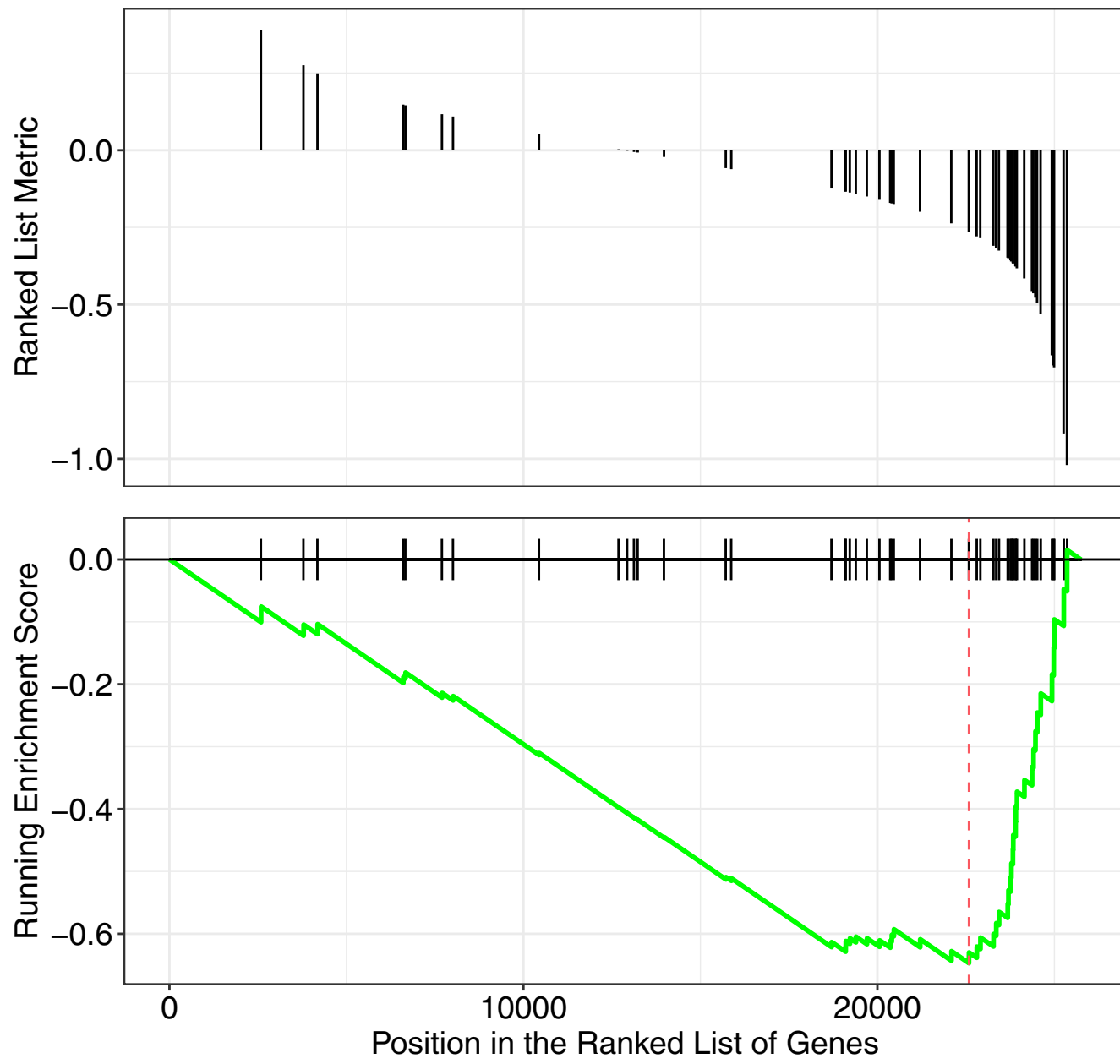


Fig. S6. *alx1*; *alx3CR* phenocopy *alx1*; *alx3* double mutants. Embryos derived from Tg(sox10:kaede) embryos or *alx1*^{uw2016}; Tg(sox10:kaede) were injected with *alx3* CRISPR/Cas9 and fixed at 4 dpf. Embryos were dissected, and flat-mounted in order to image the anterior neurocranium (ANC) and ventral pharyngeal cartilage. *alx1*; *alx3CR* developed with midline cleft (arrowhead in G), with the lateral elements (asterisks in G) separated due to the missing median element. I – P: Dissected flat-mount Alcian Blue-stained cartilage in larvae of different genotypes, anterior to the left. I: WT embryo with normal paired trabeculae (tr) and a normal ANC. O: *alx1*; *alx3CR* with defective trabeculae fusion (asterisks) and depleted ethmoid plate. Q: Percentage of embryos displaying malformed anterior neurocranium (ANC) phenotype in larvae of different genotypes. All embryos are shown with anterior to the left. Scale bar: 100µm.

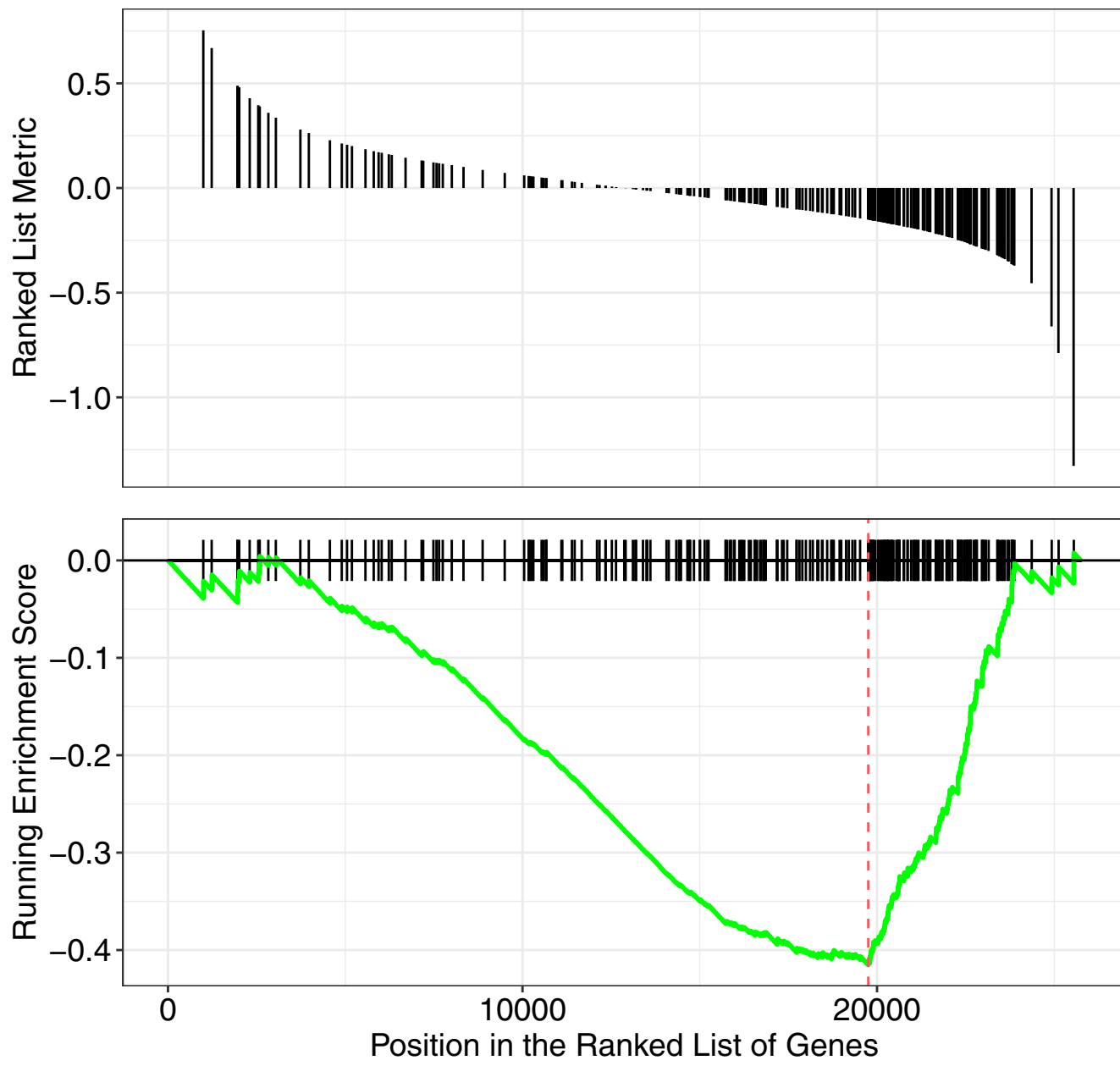
lens development in camera-type eye

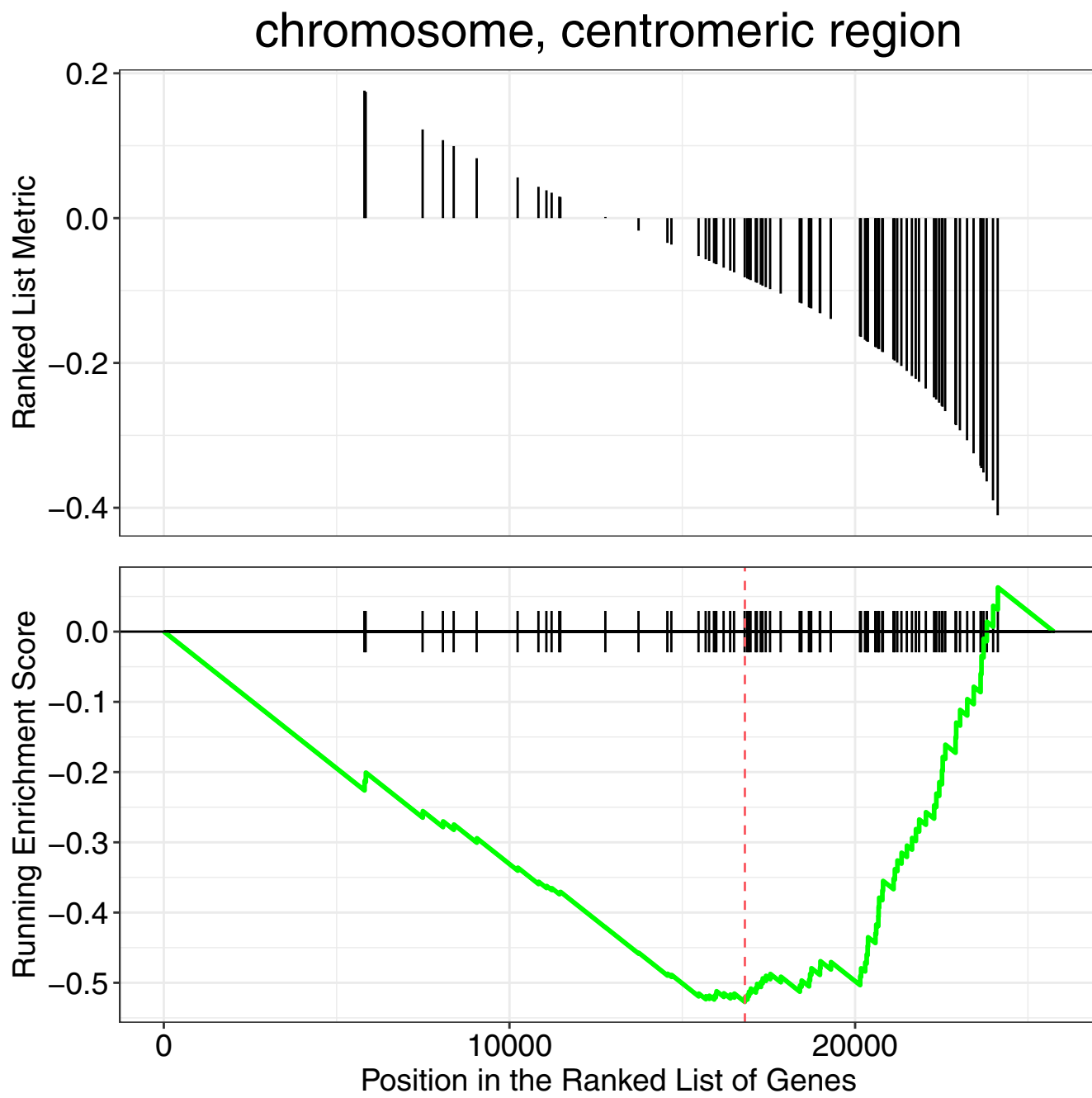


structural constituent of eye lens

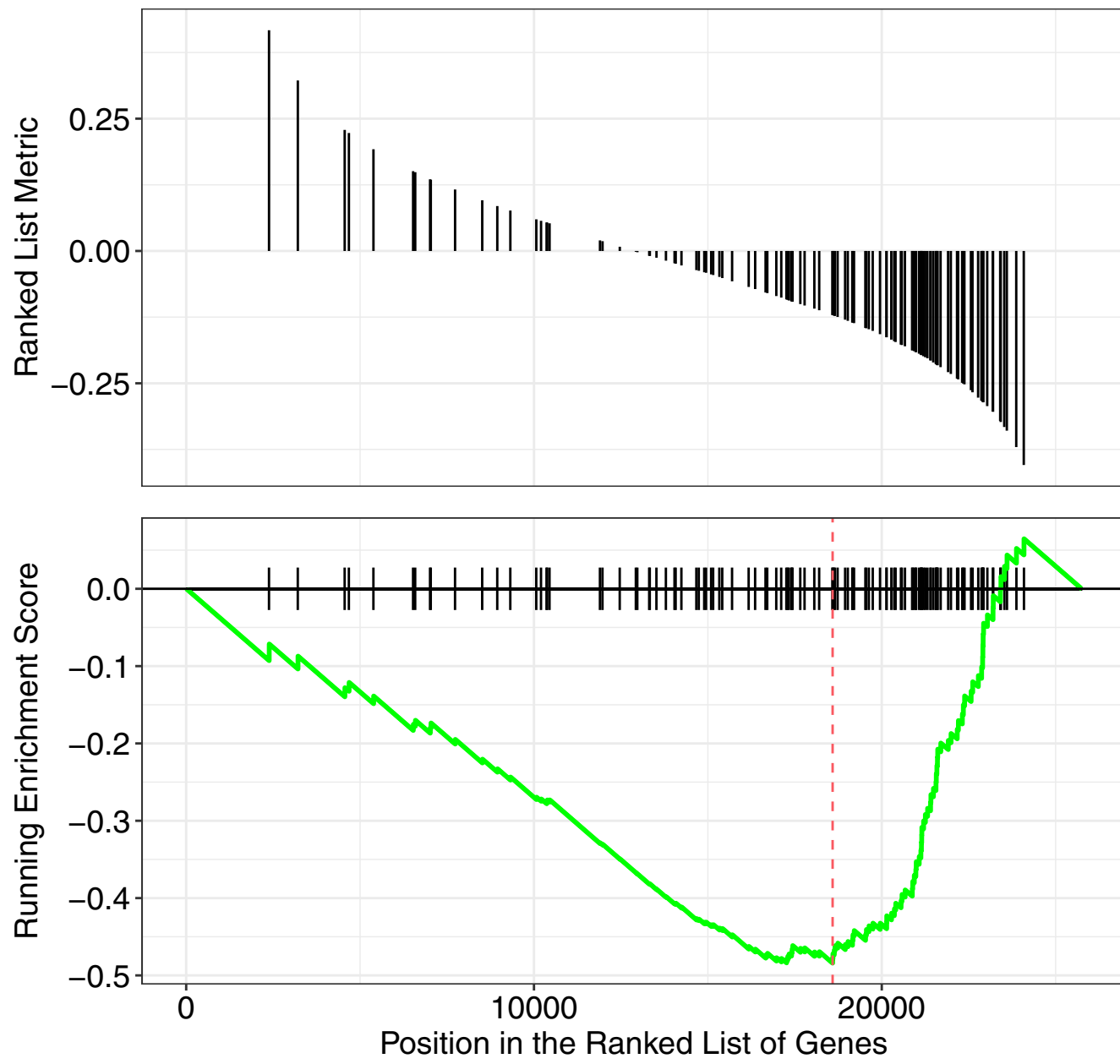


tubulin binding

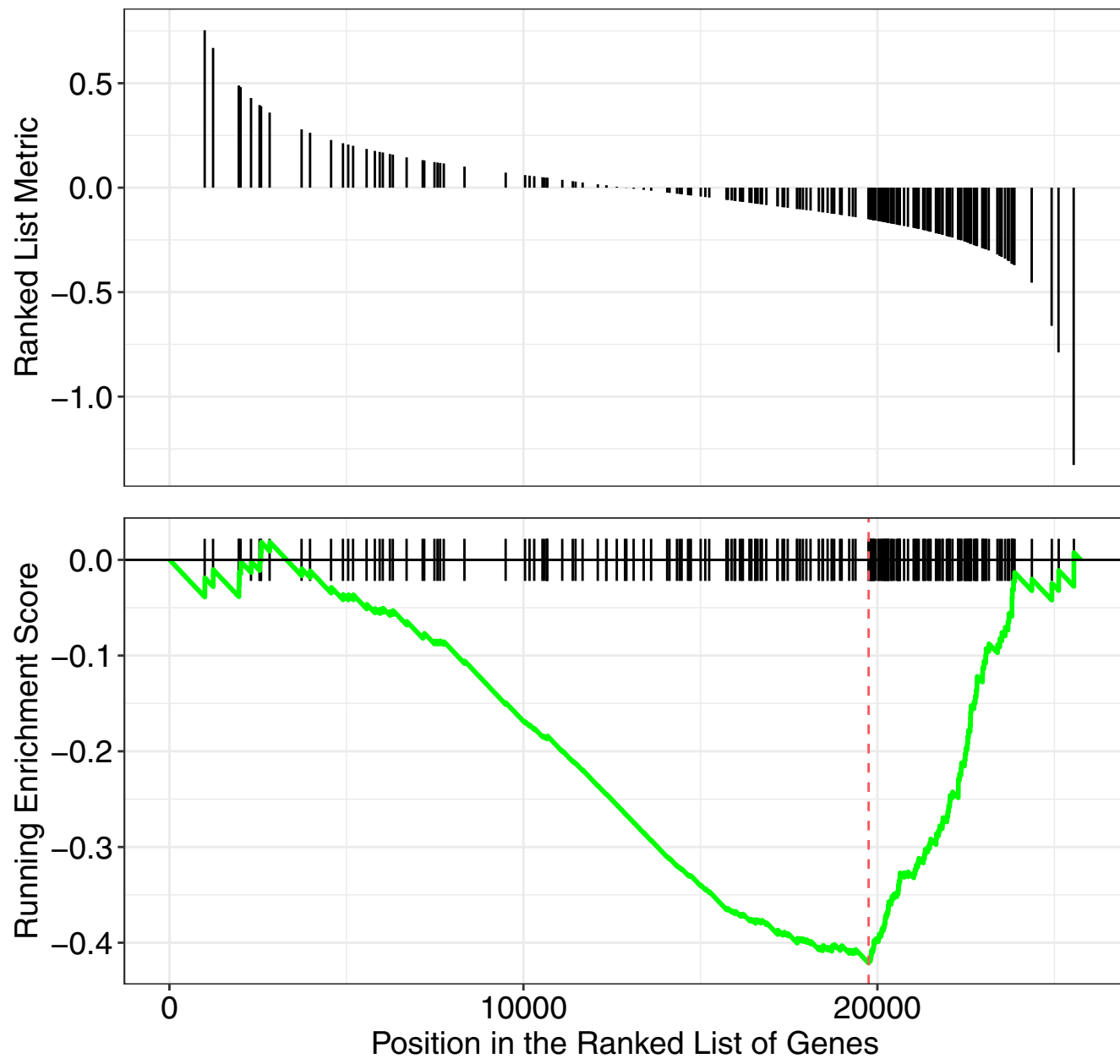


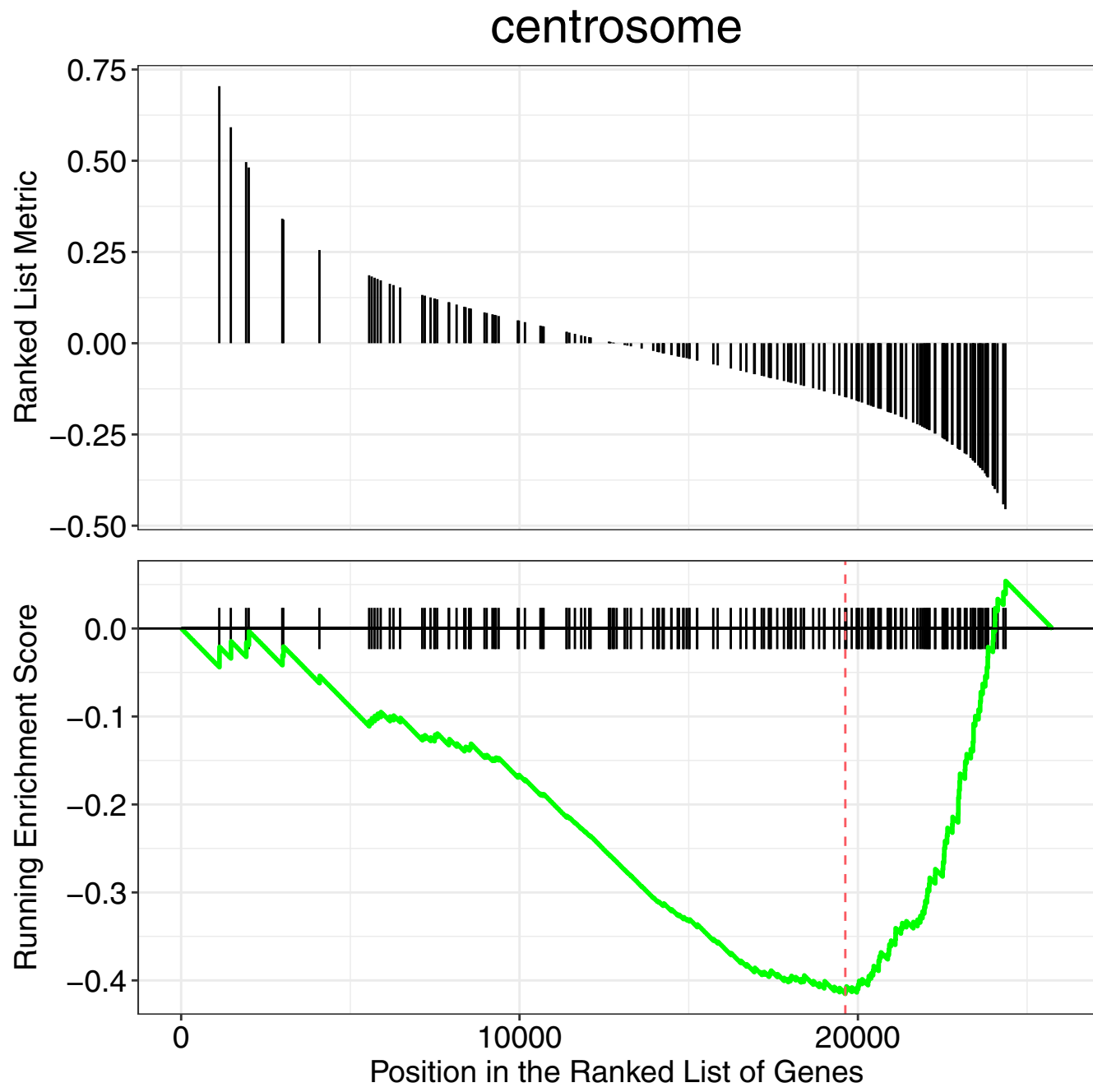


histone binding

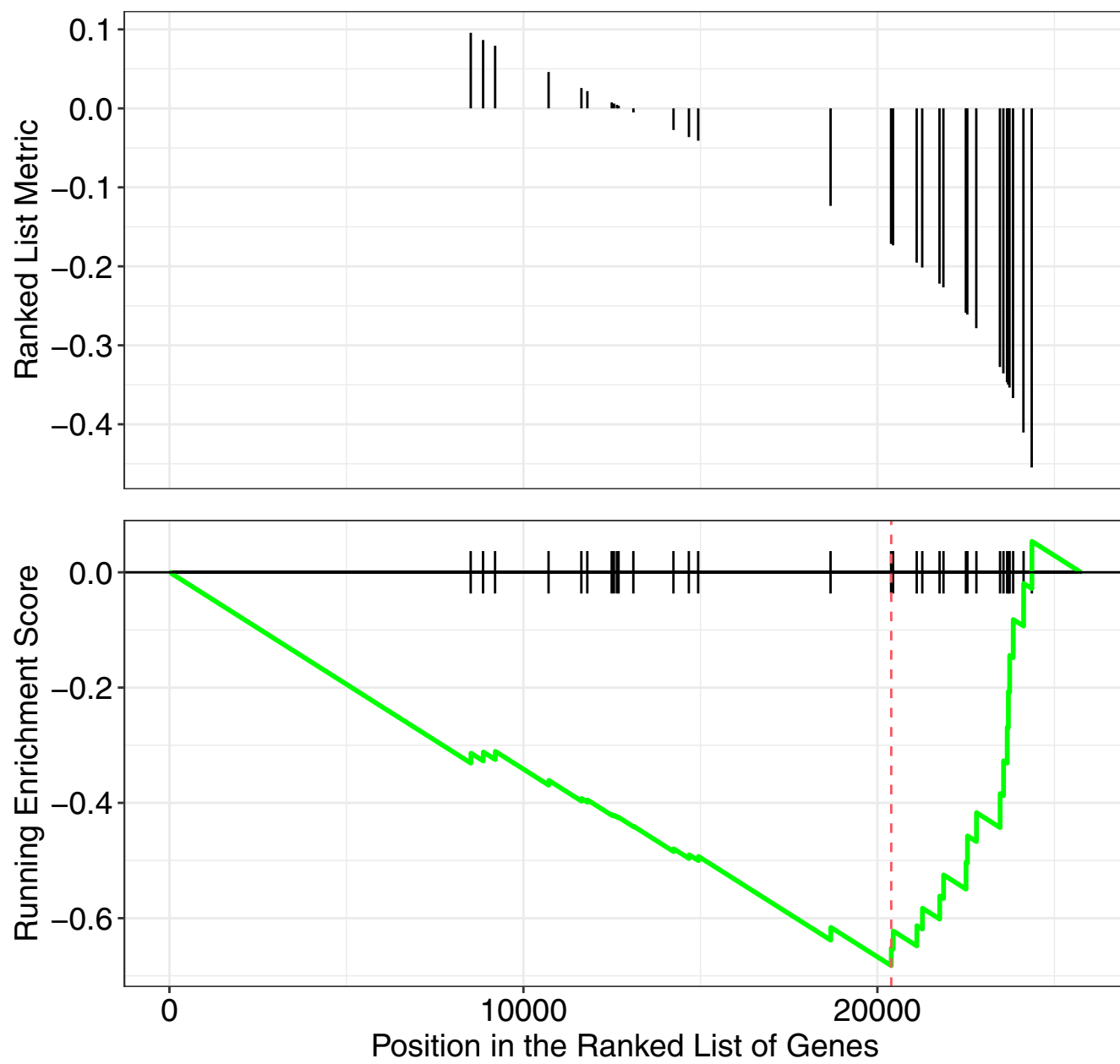


microtubule binding

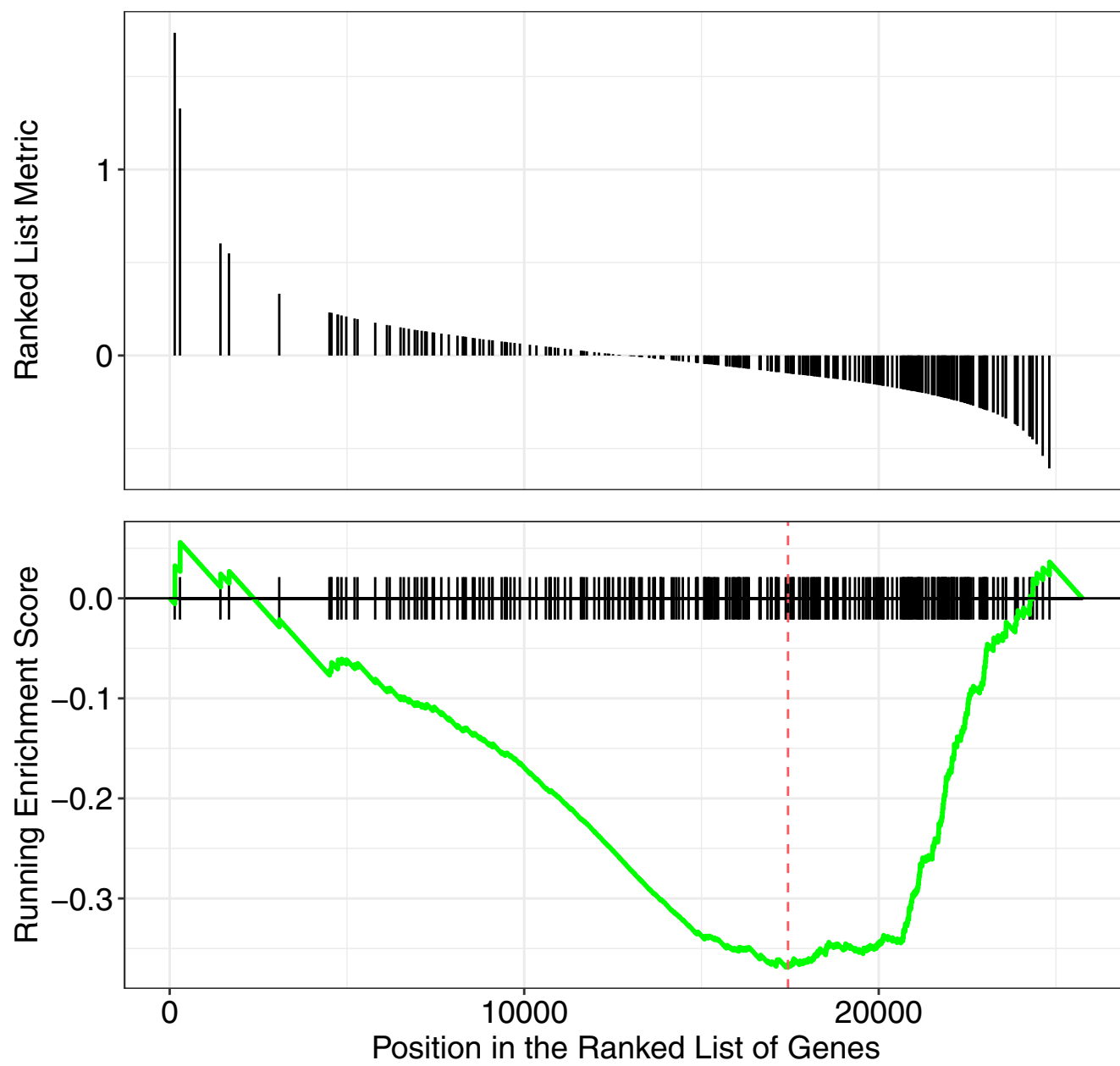


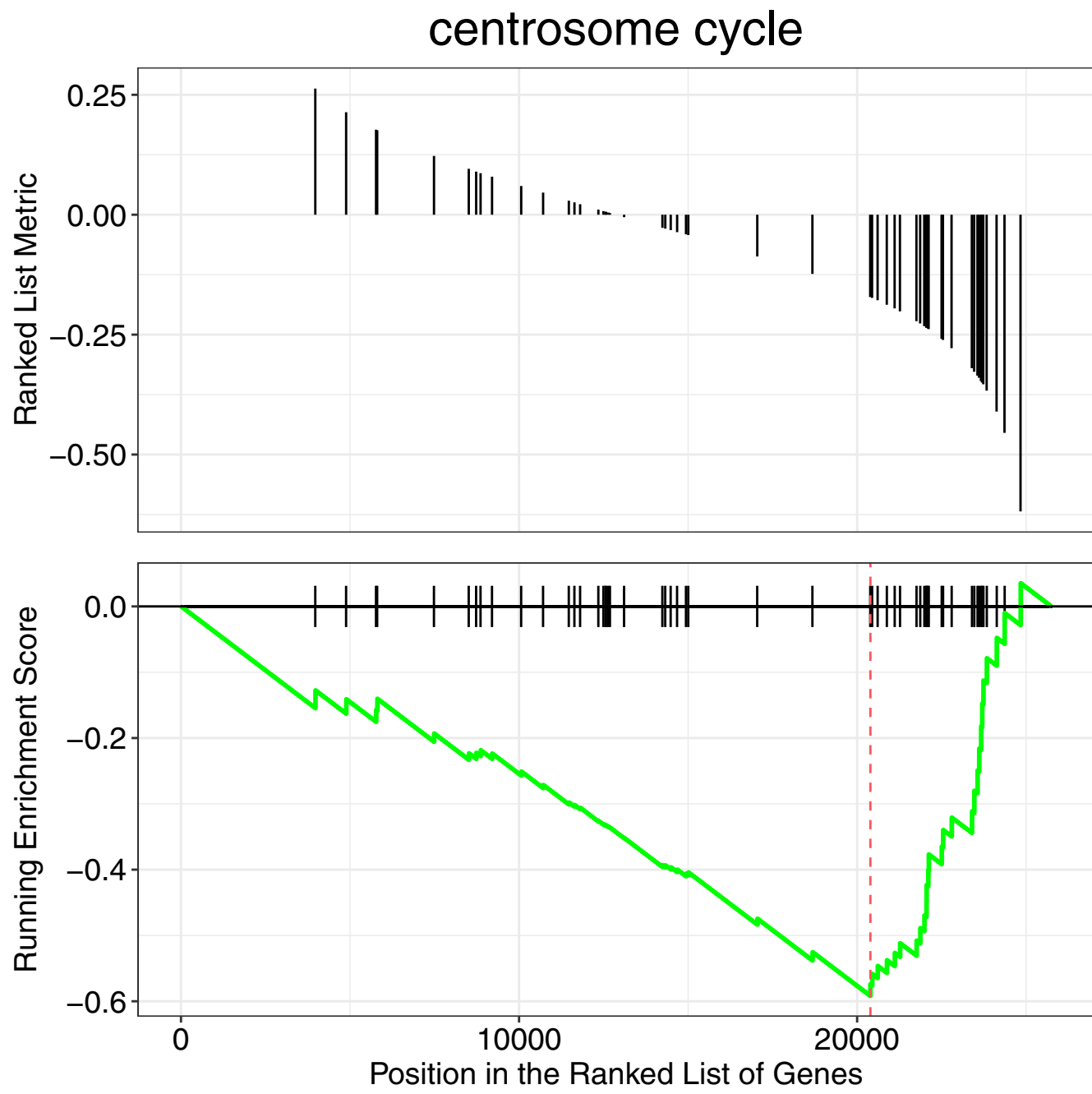


centrosome duplication

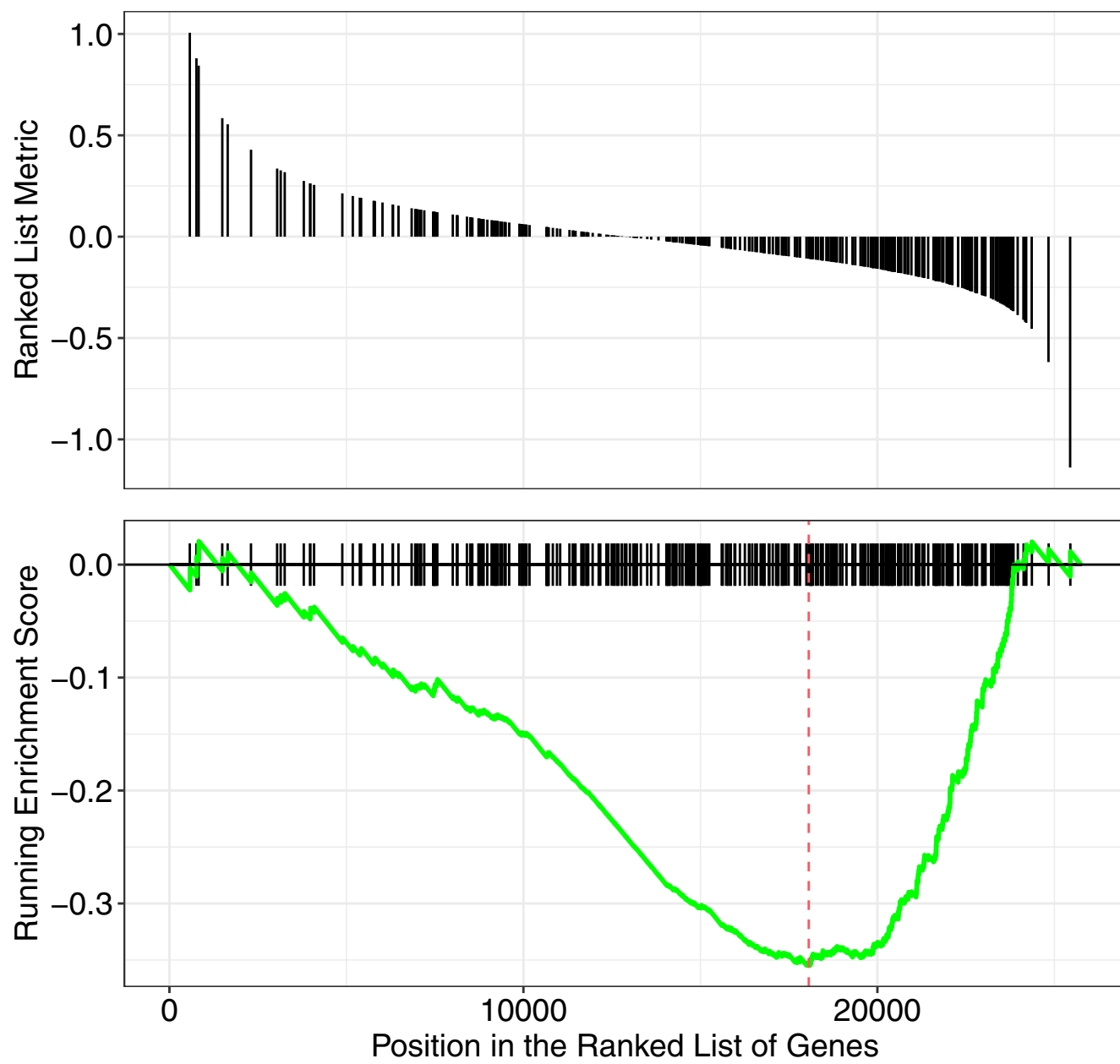


RNA splicing

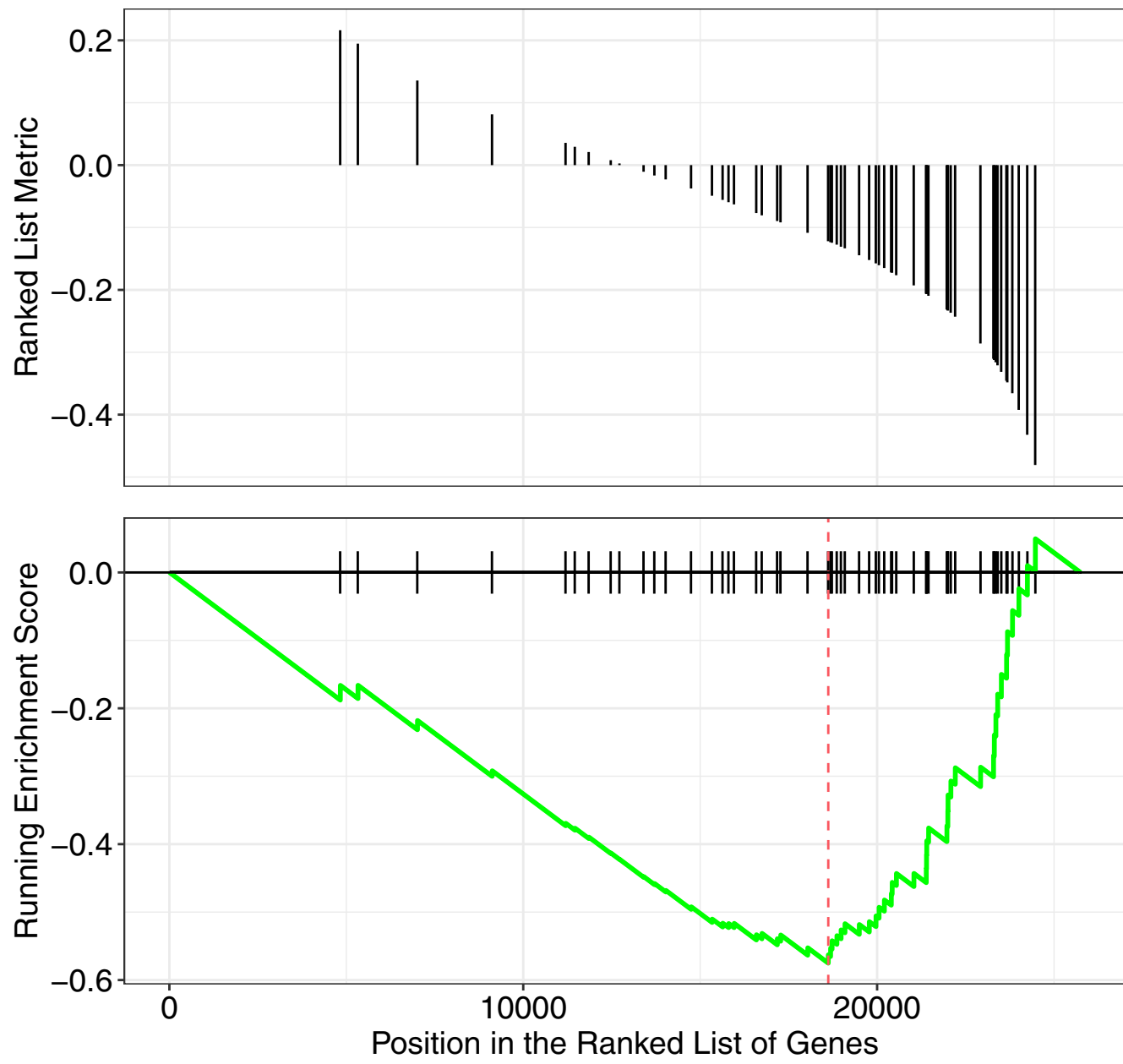


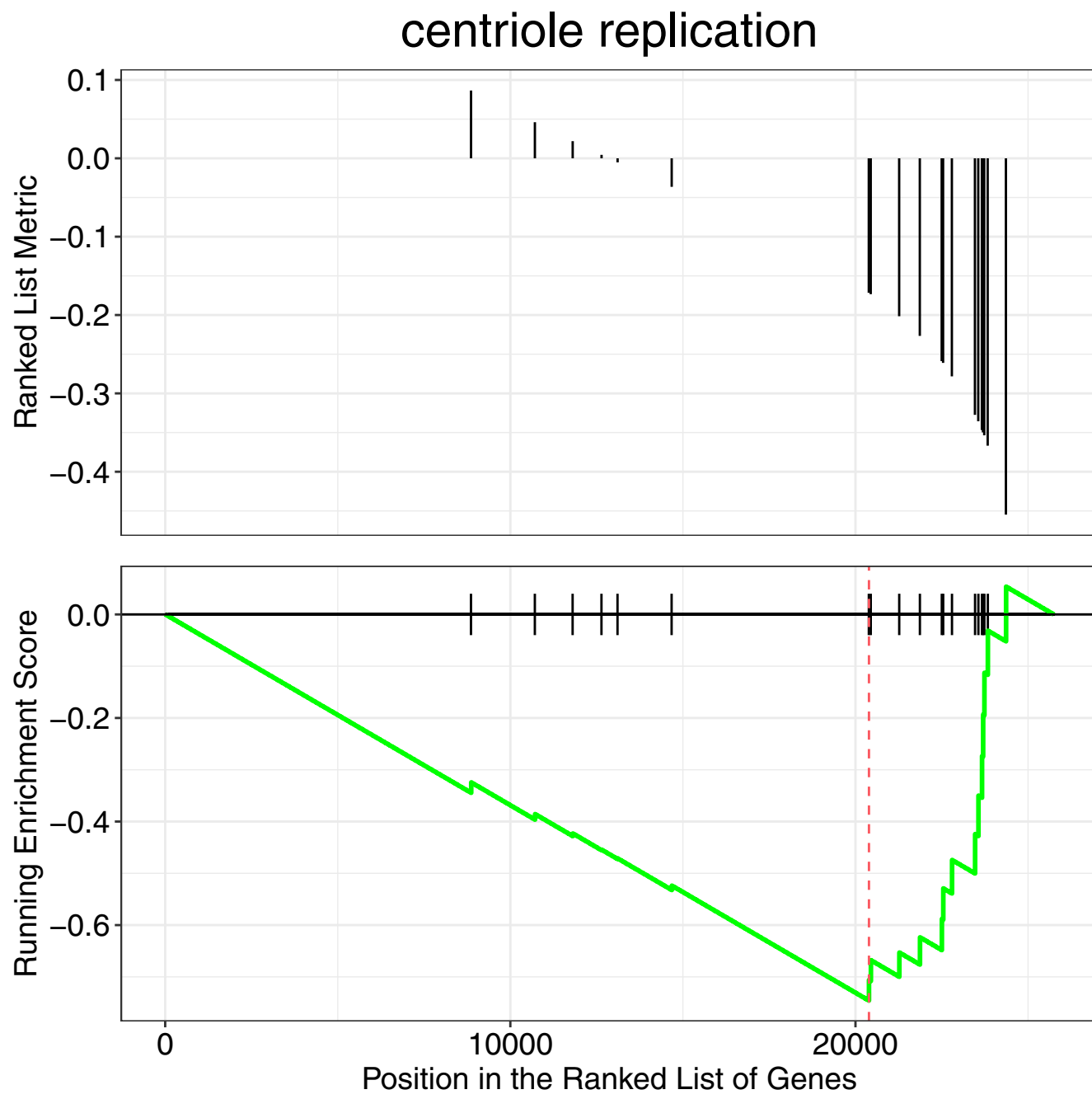


microtubule cytoskeleton organization

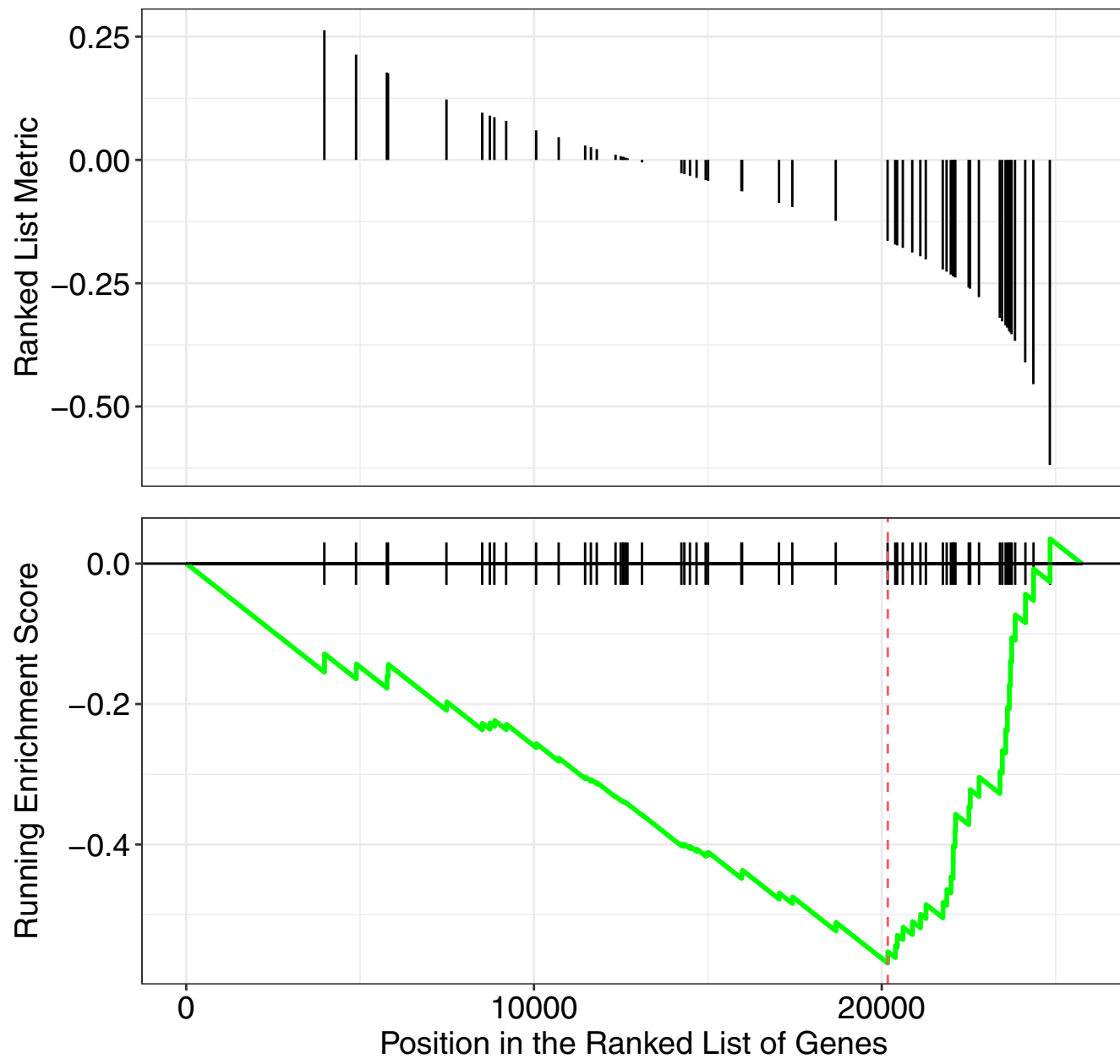


DNA helicase activity

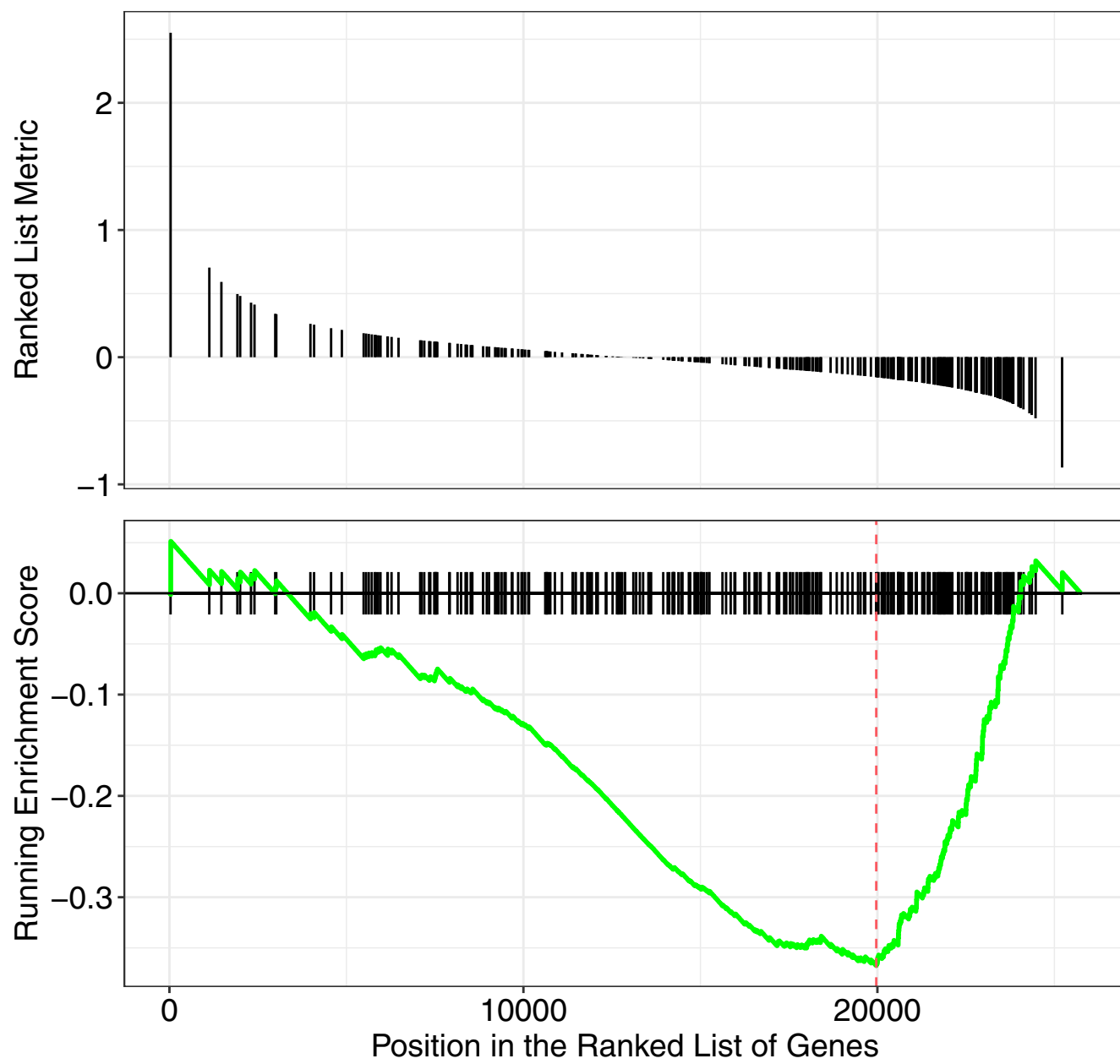




microtubule organizing center organization



microtubule organizing center



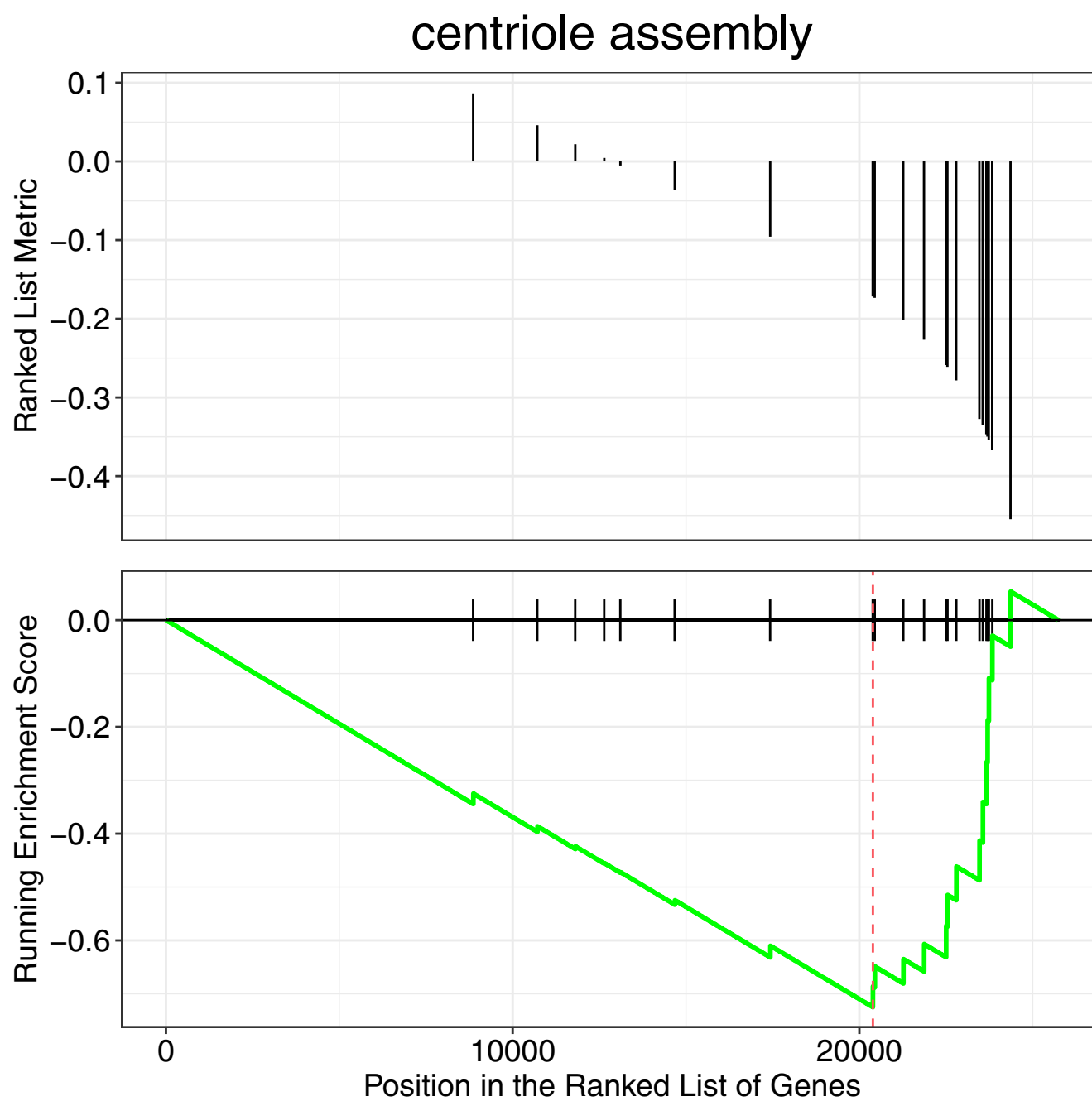
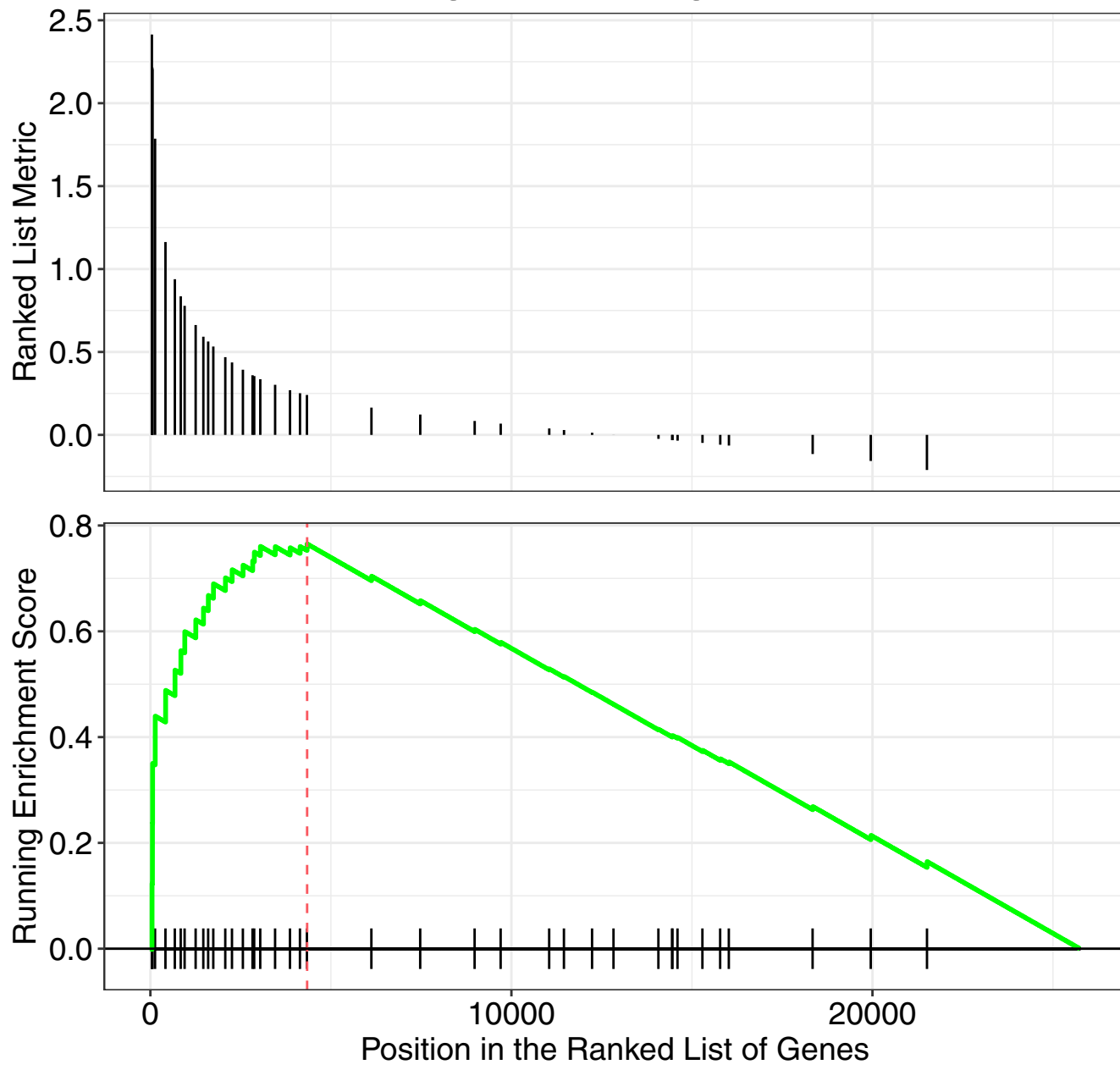
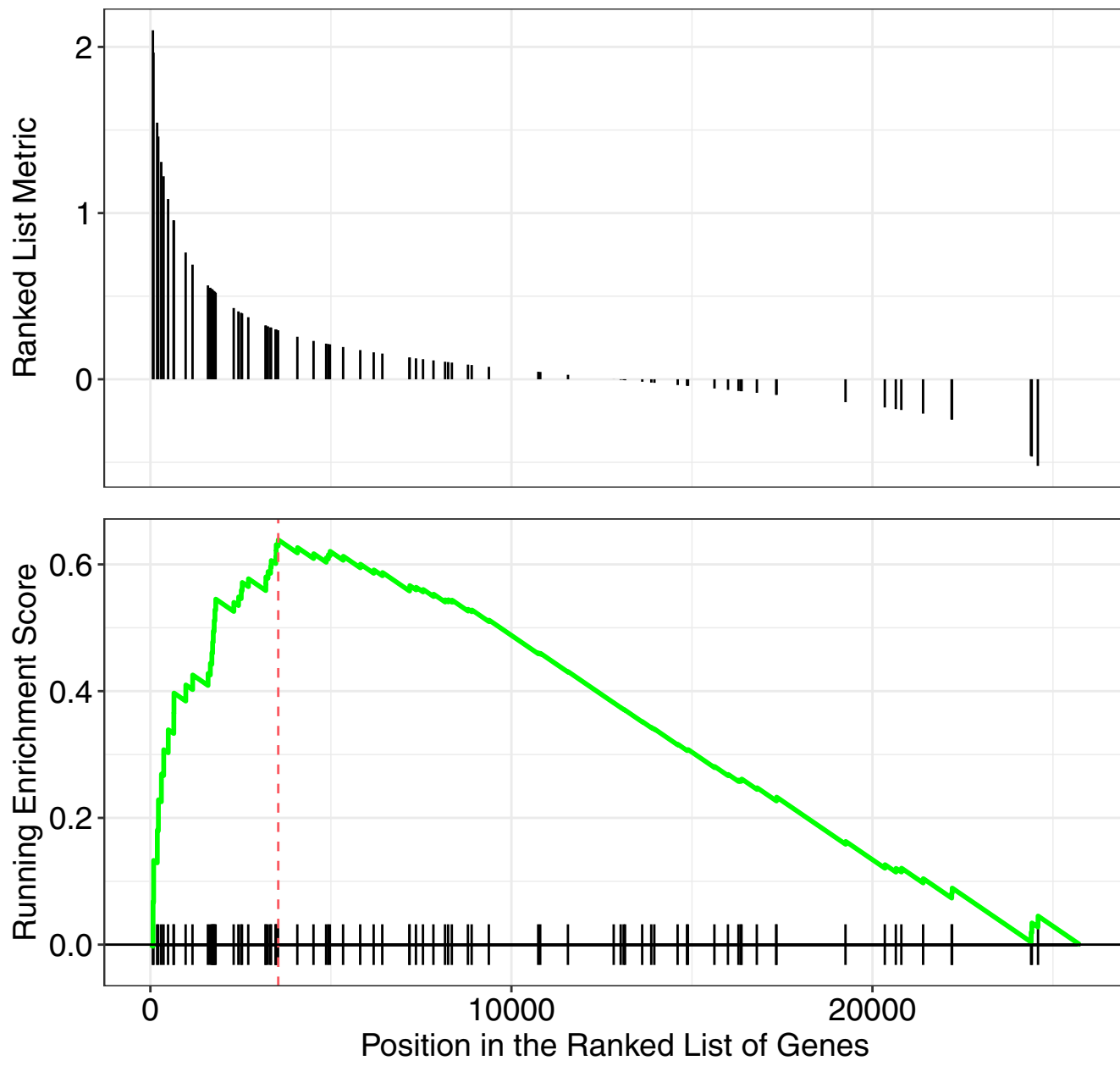


Fig. S7. Enrichment plots of Gene set enrichment analysis (GSEA) of differentially expressed genes in *alx1;alx3* crispants. Running score plot and pre-ranked list of all suppressed GSEA terms shown in *alx1;alx3* crispants.

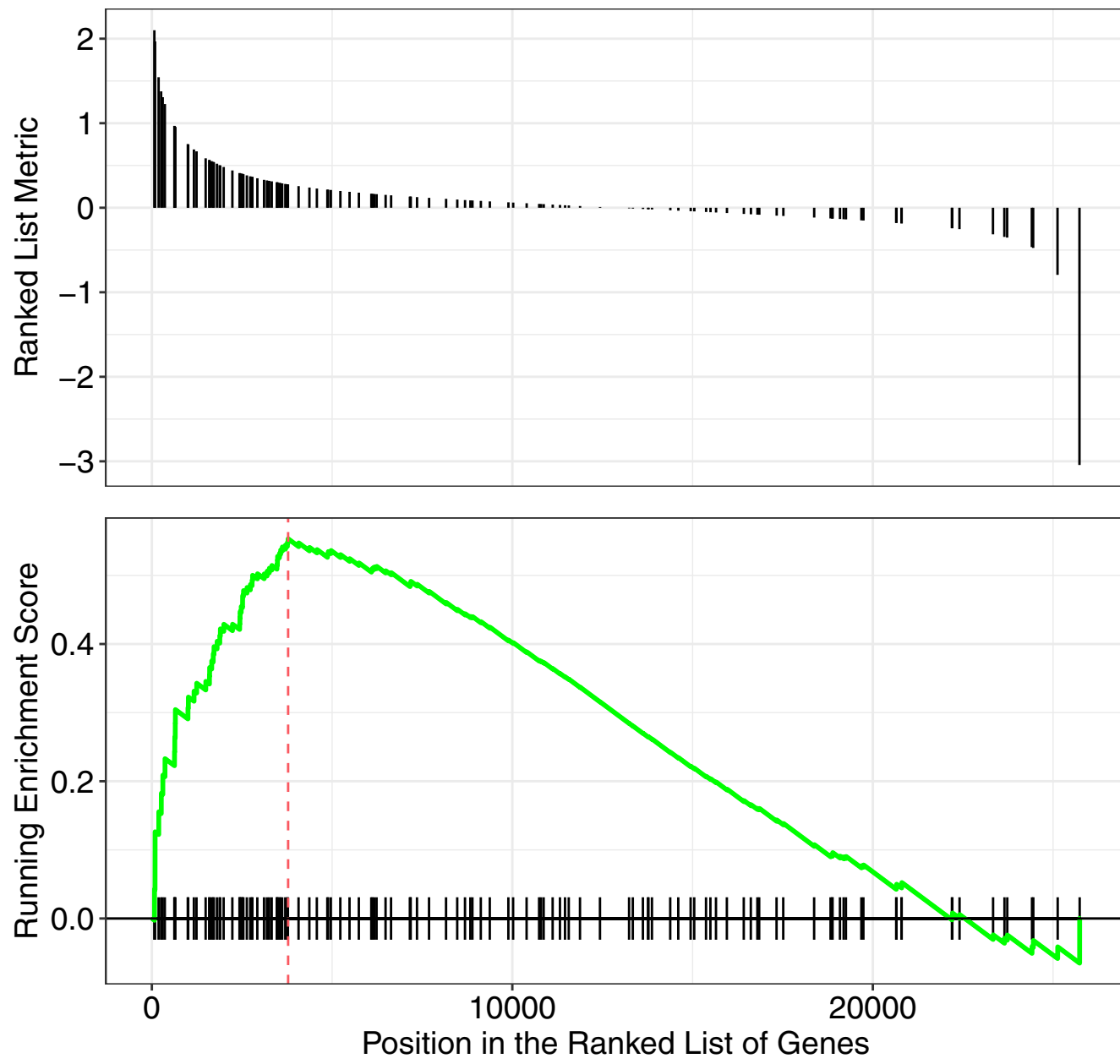
circadian regulation of gene expression



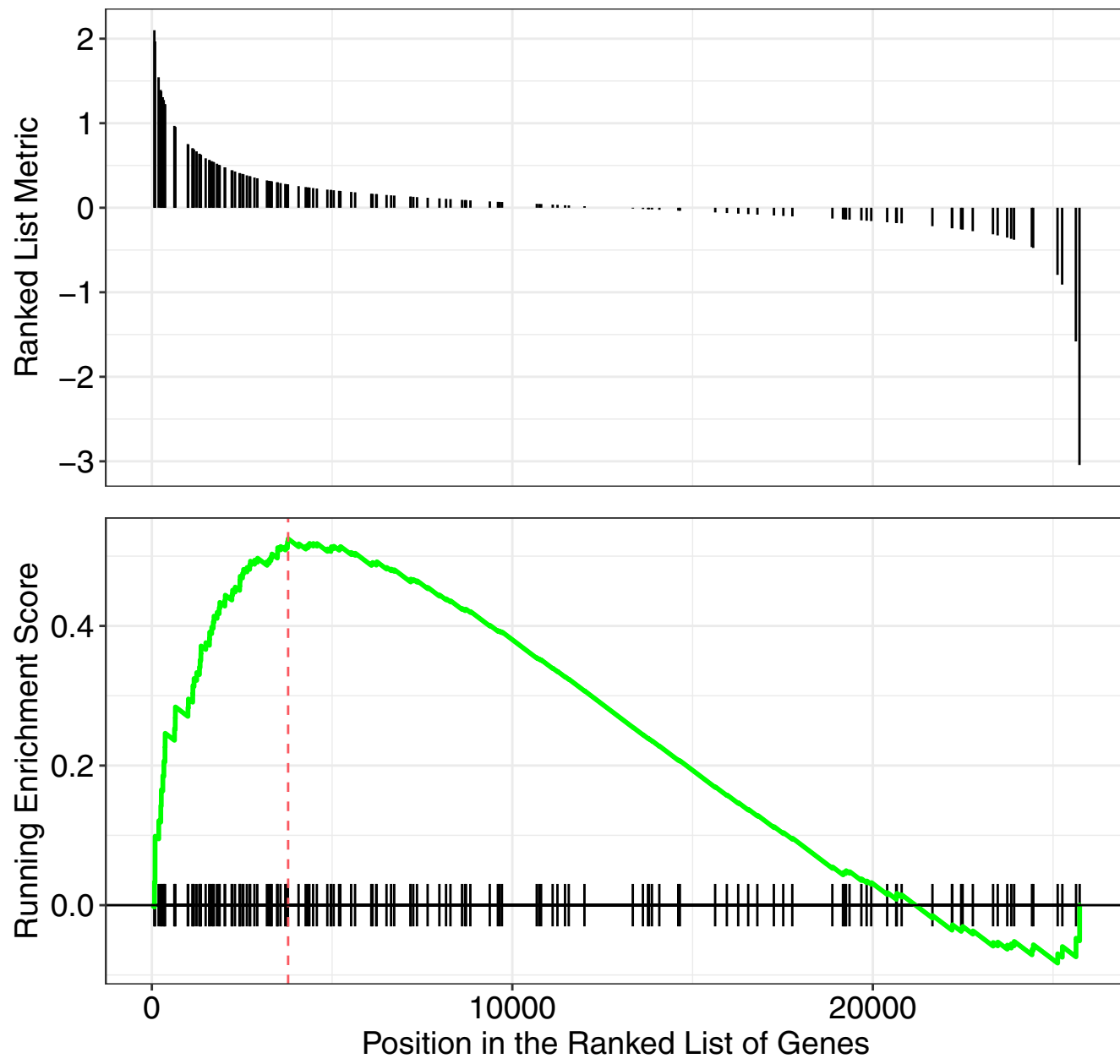
response to xenobiotic stimulus



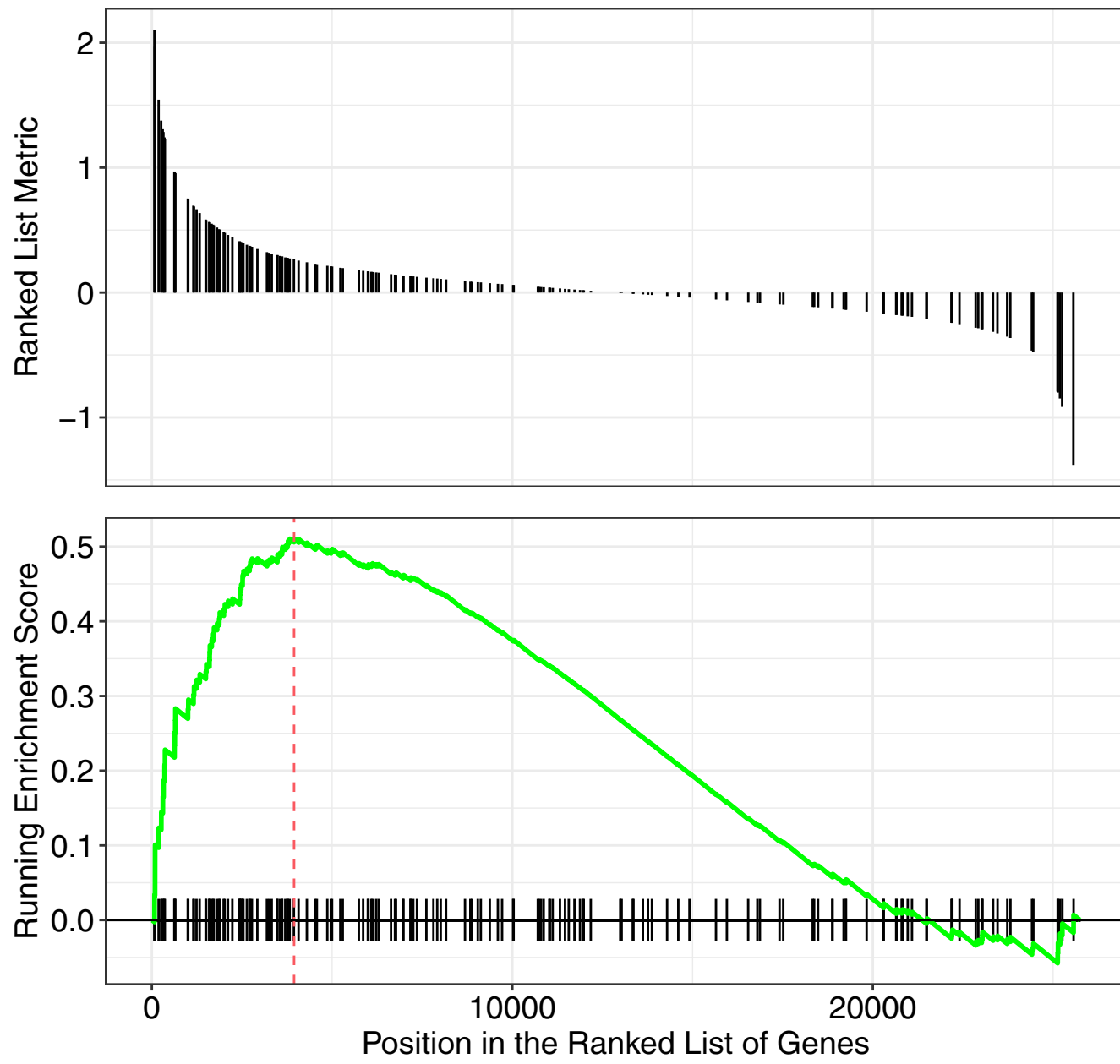
monooxygenase activity



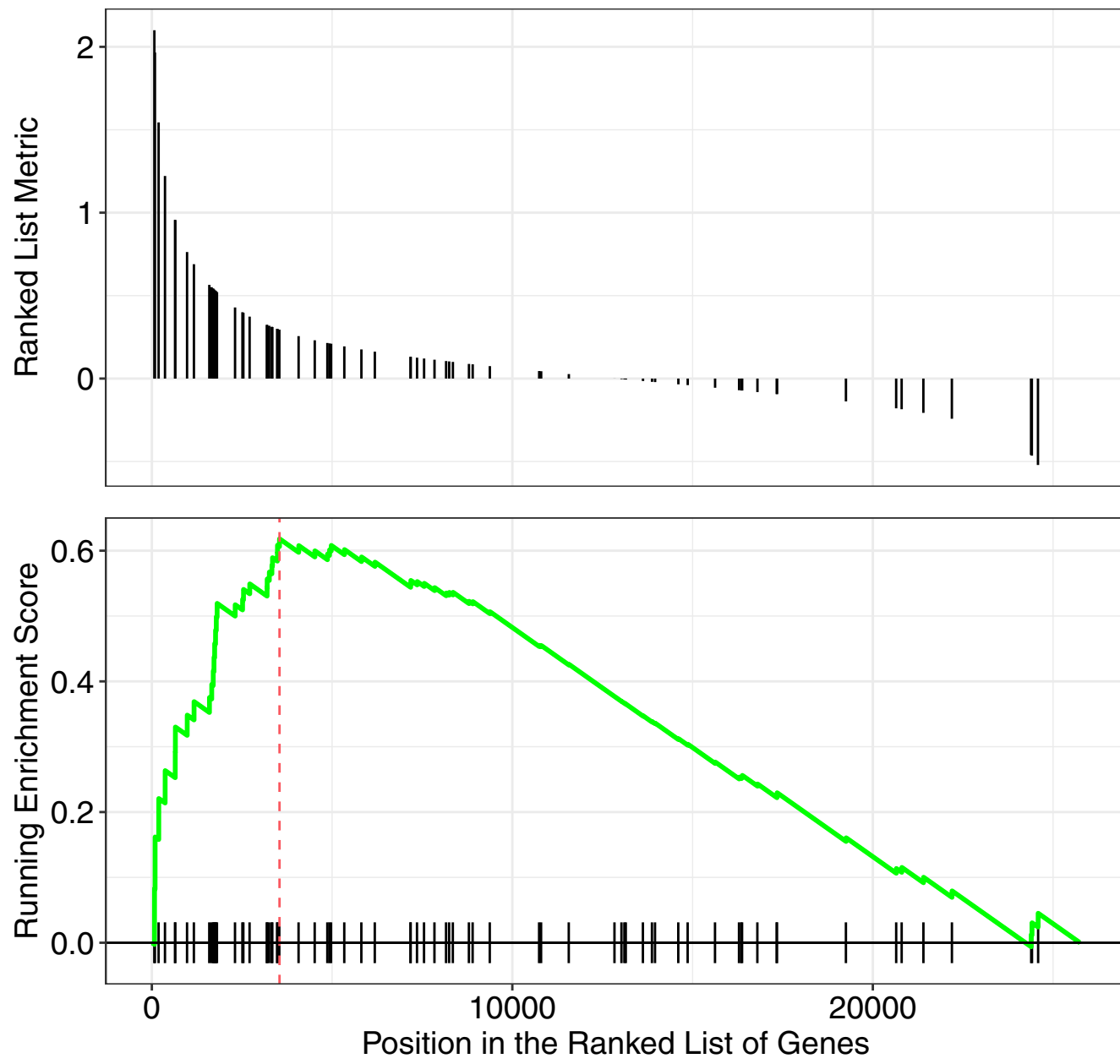
tetrapyrrole binding



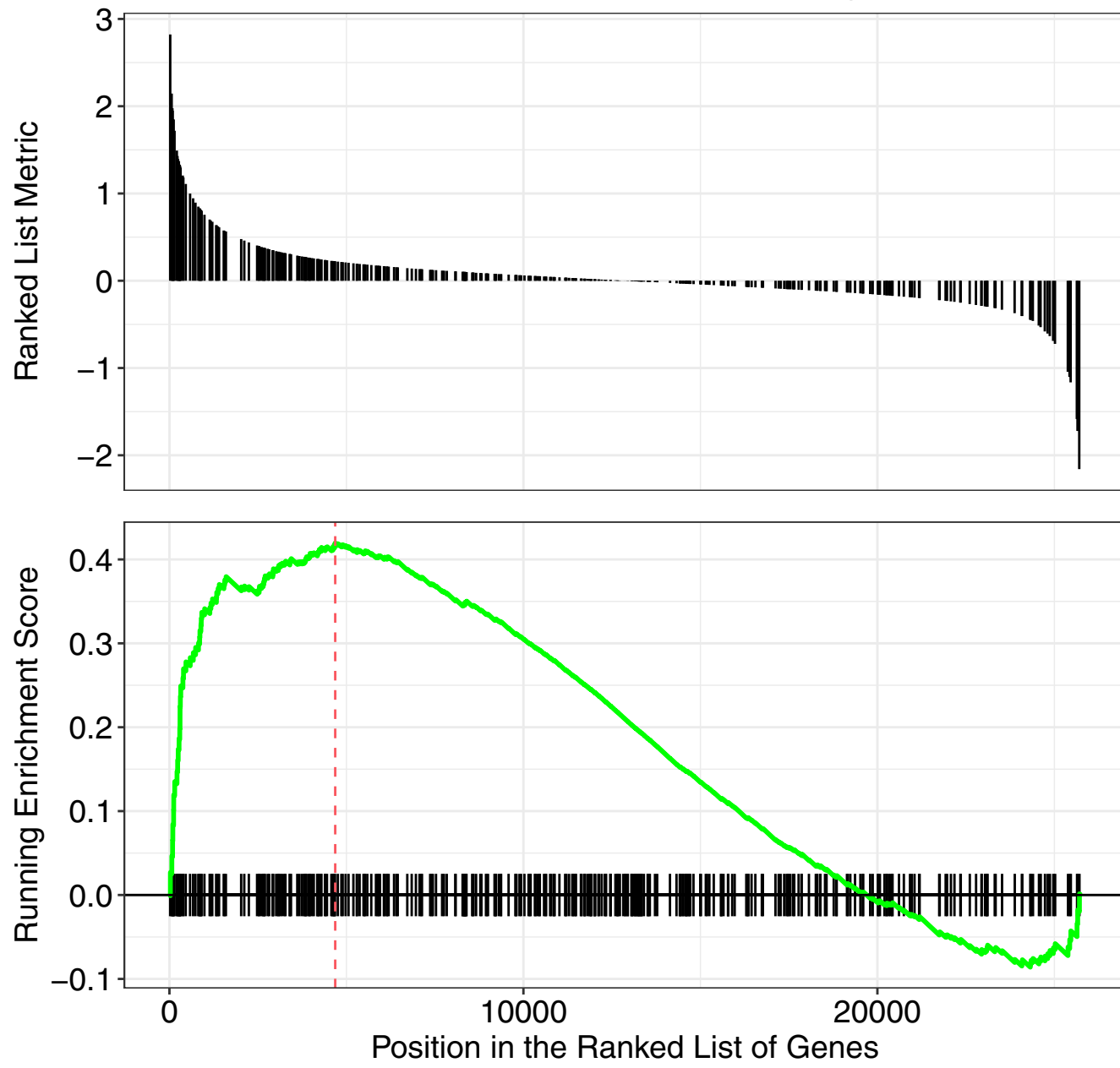
iron ion binding



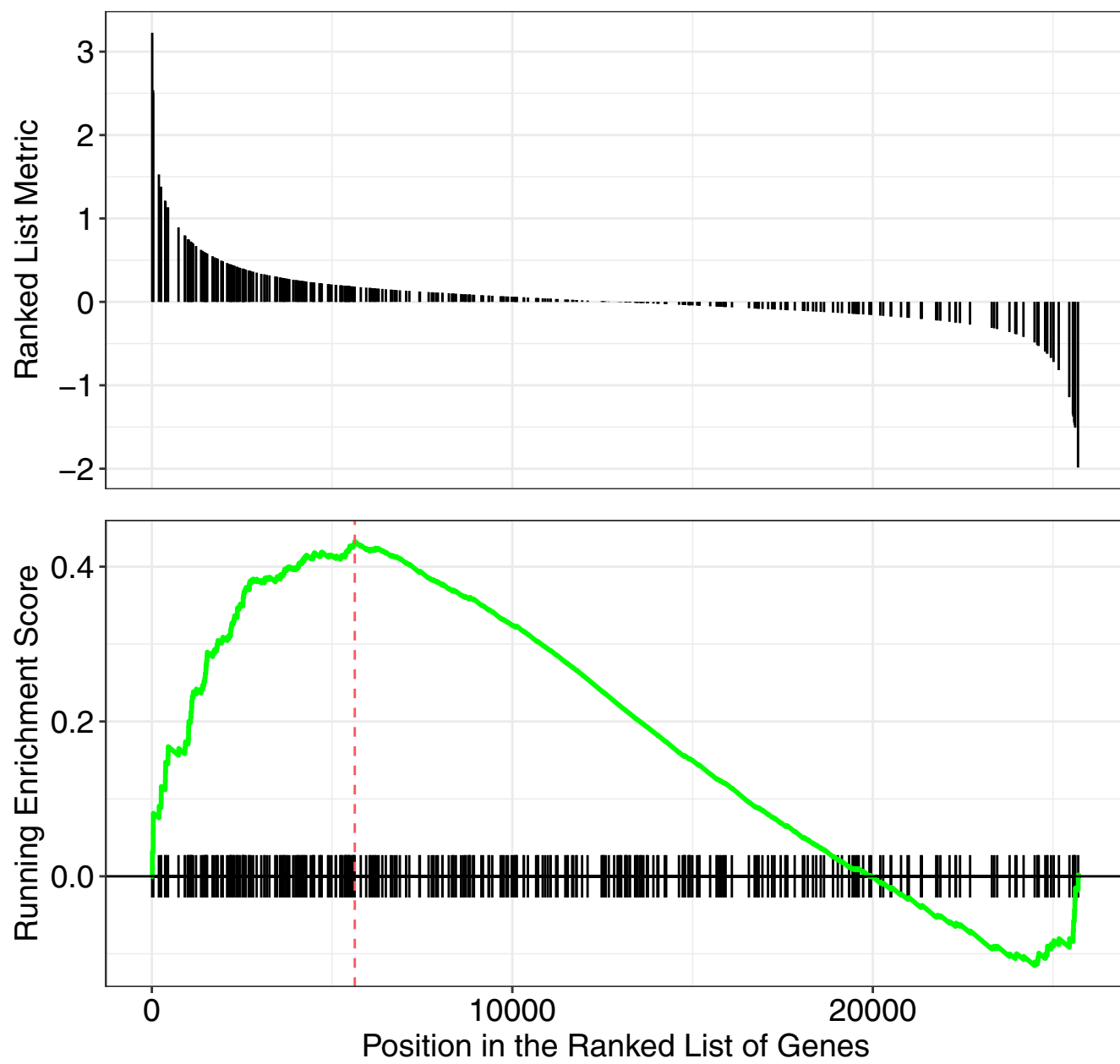
cellular response to xenobiotic stimulus



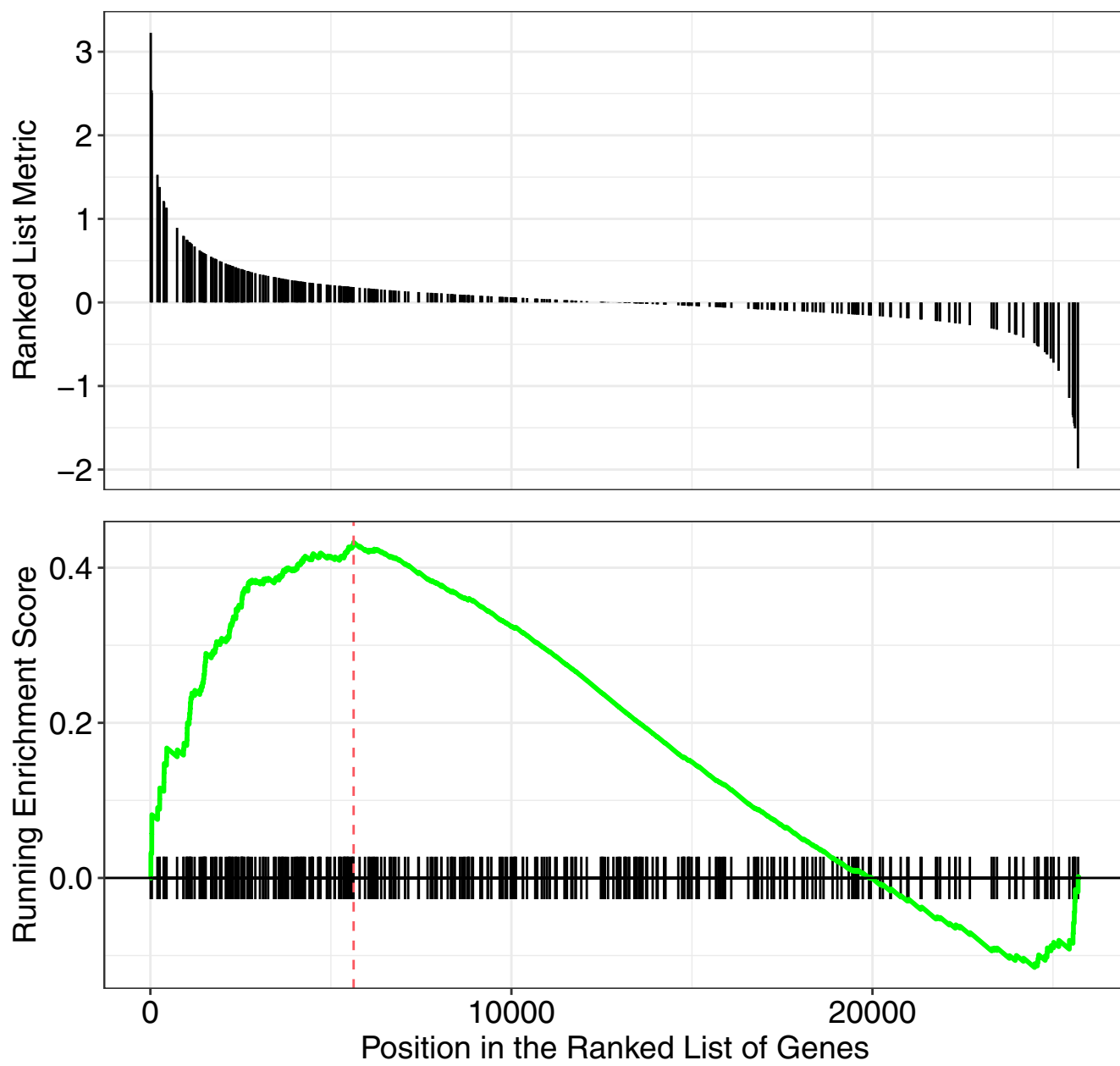
endopeptidase activity



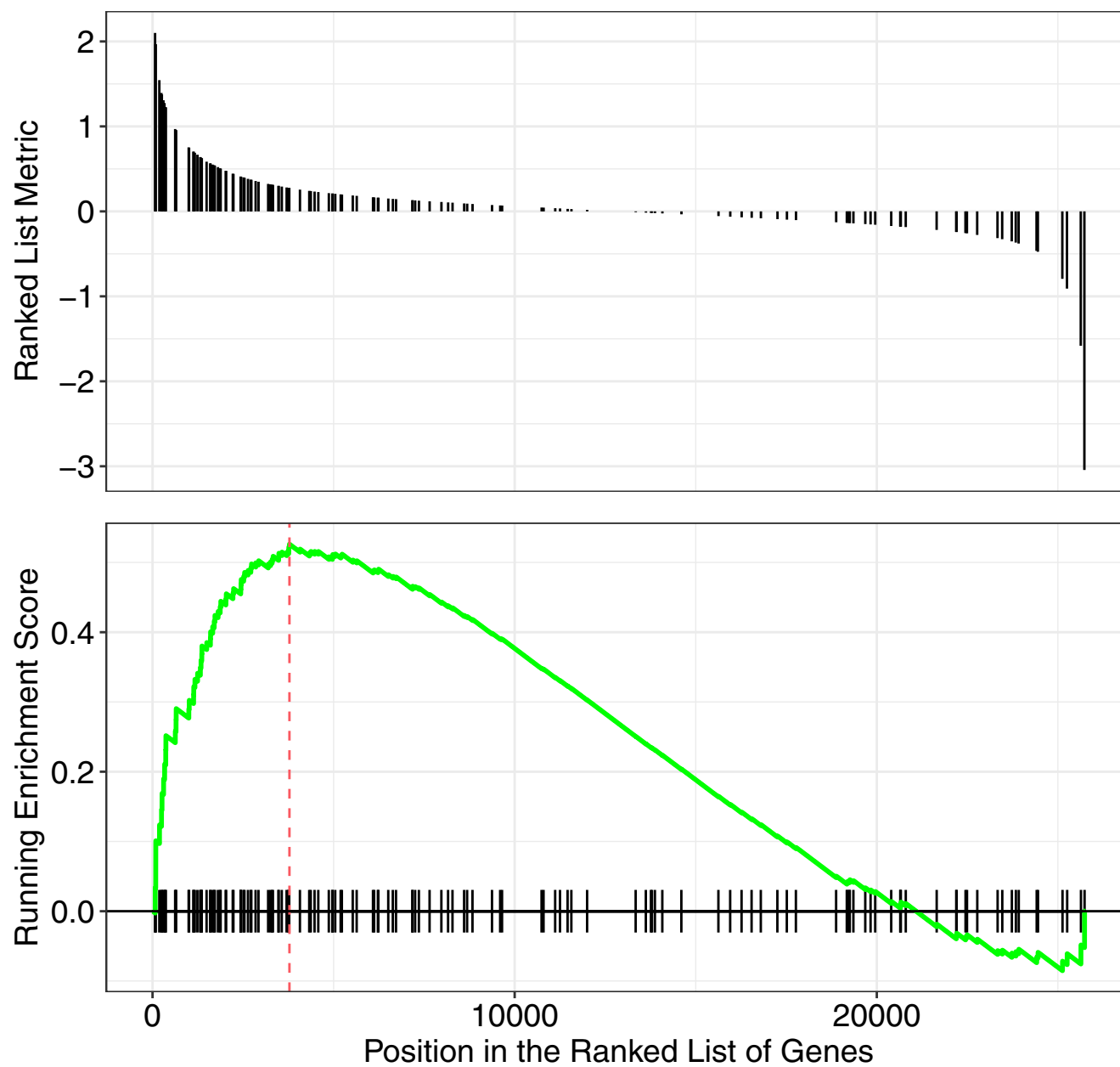
signaling receptor activator activity



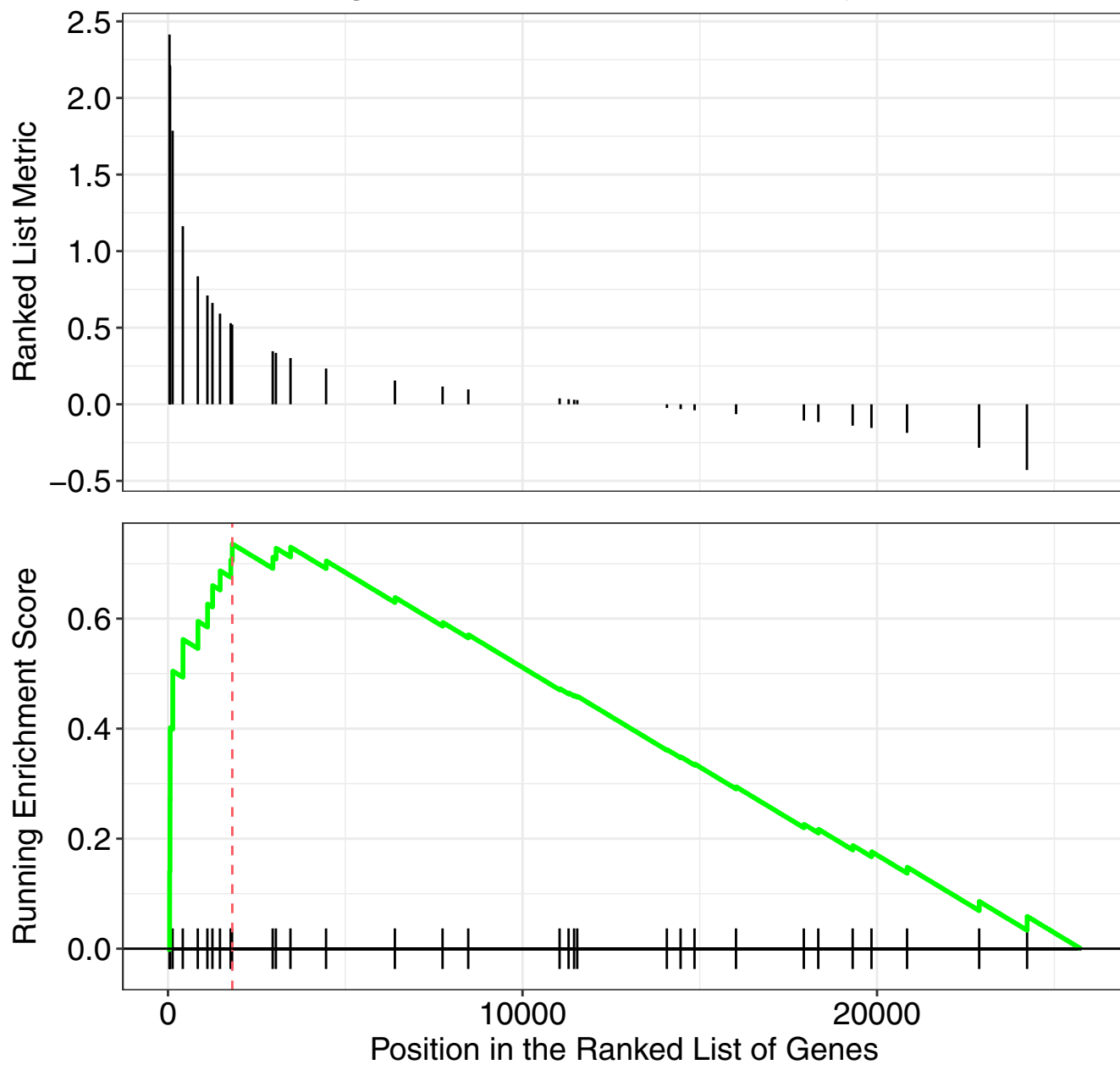
receptor ligand activity



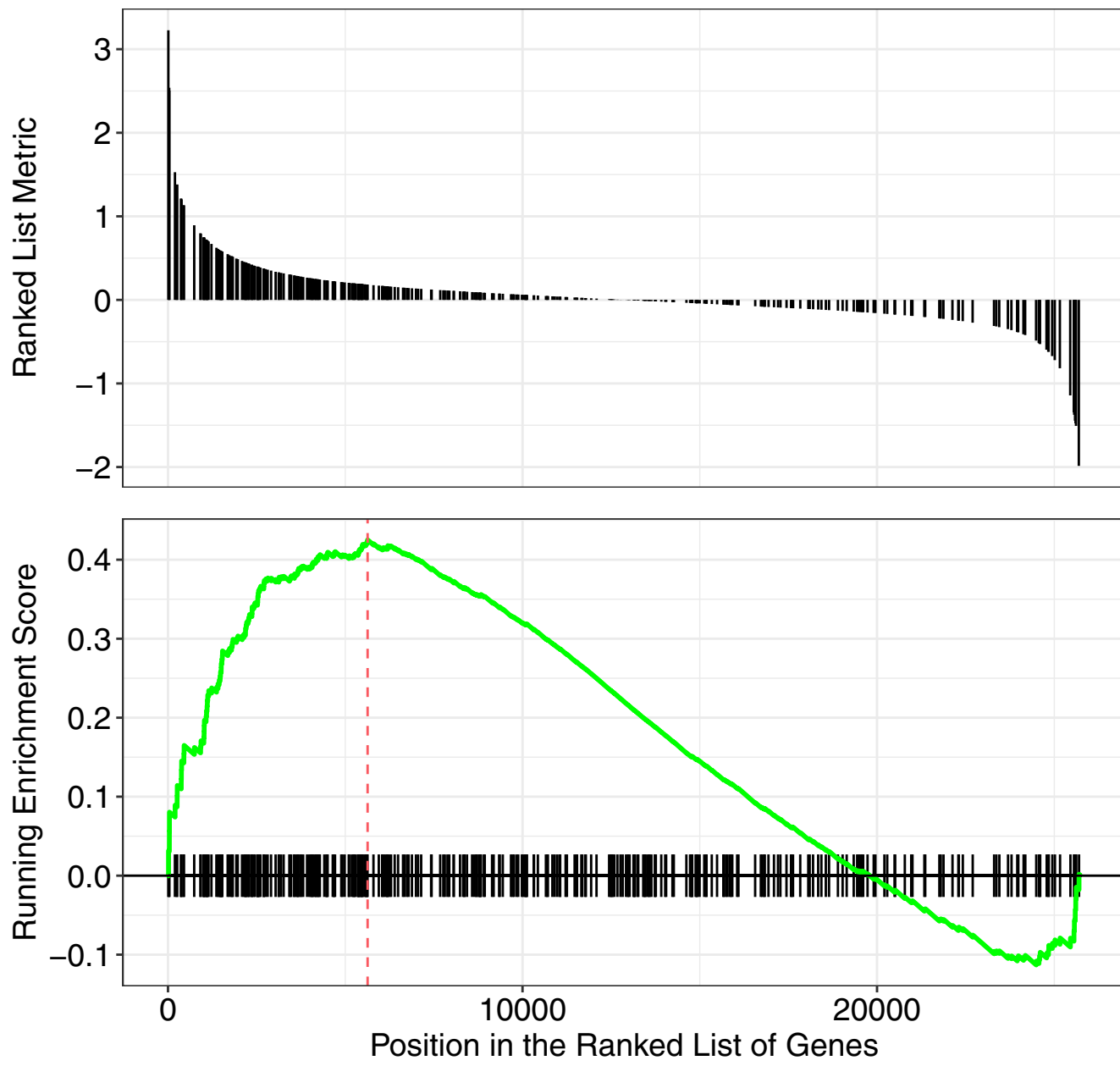
heme binding



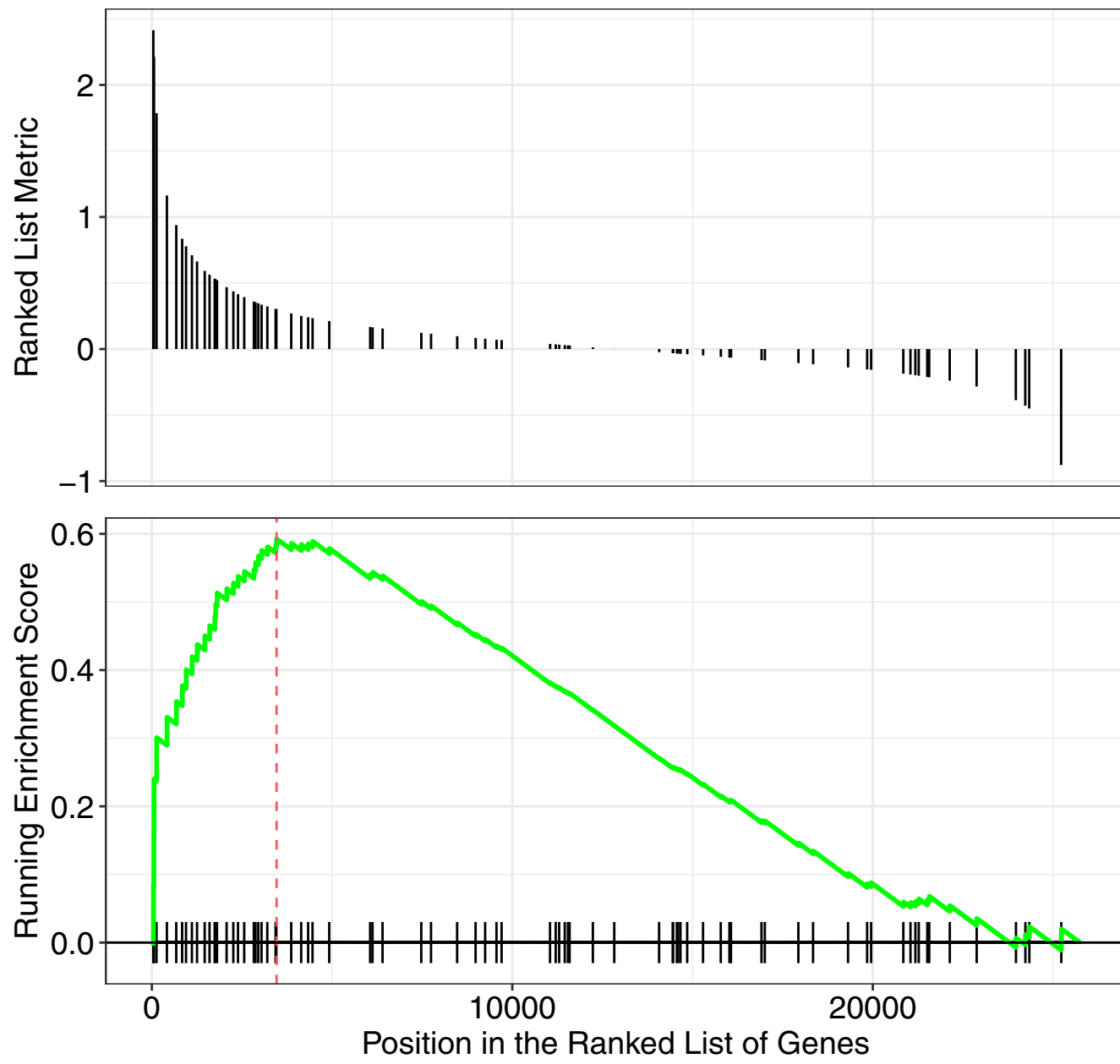
regulation of circadian rhythm



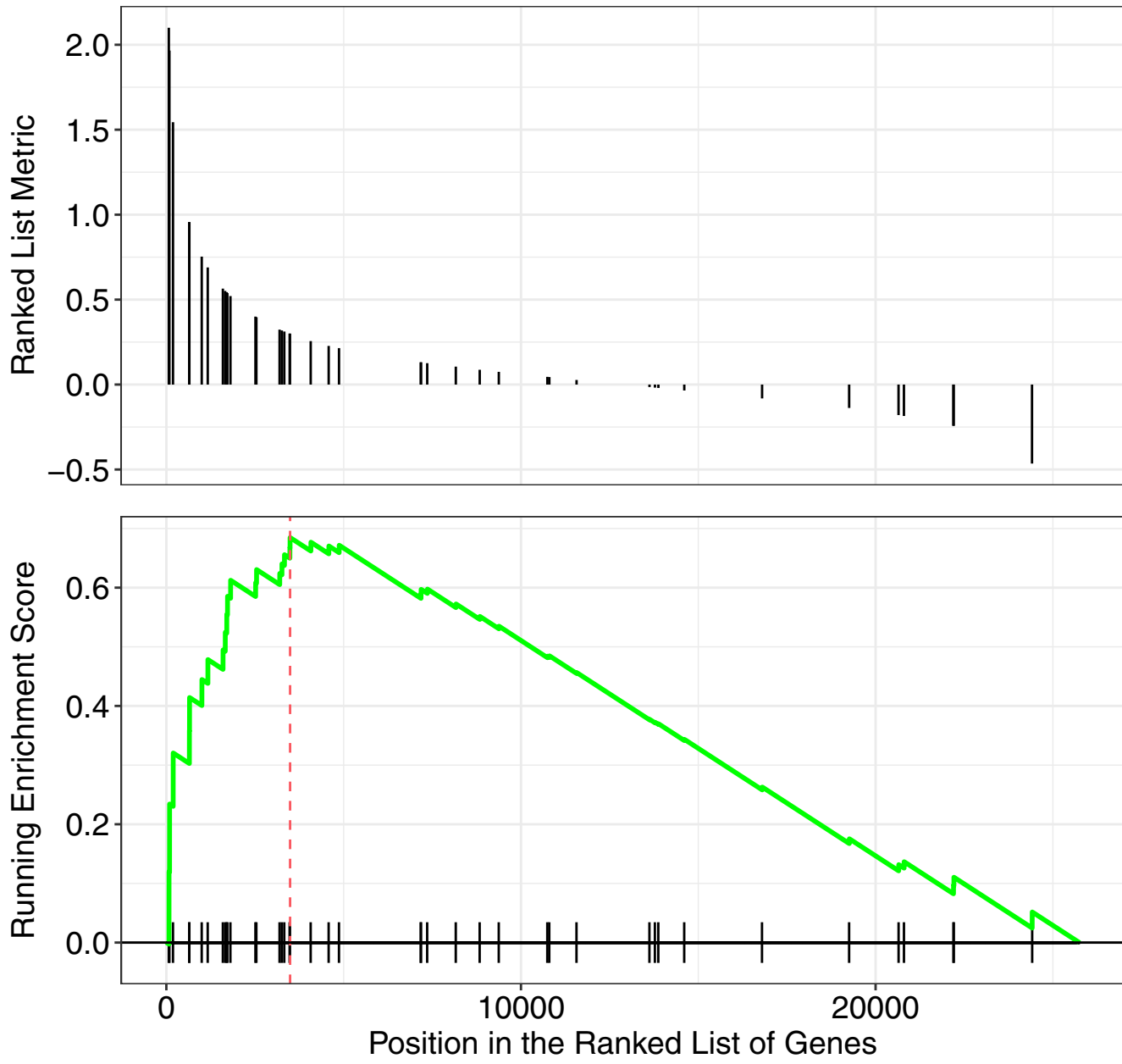
receptor regulator activity



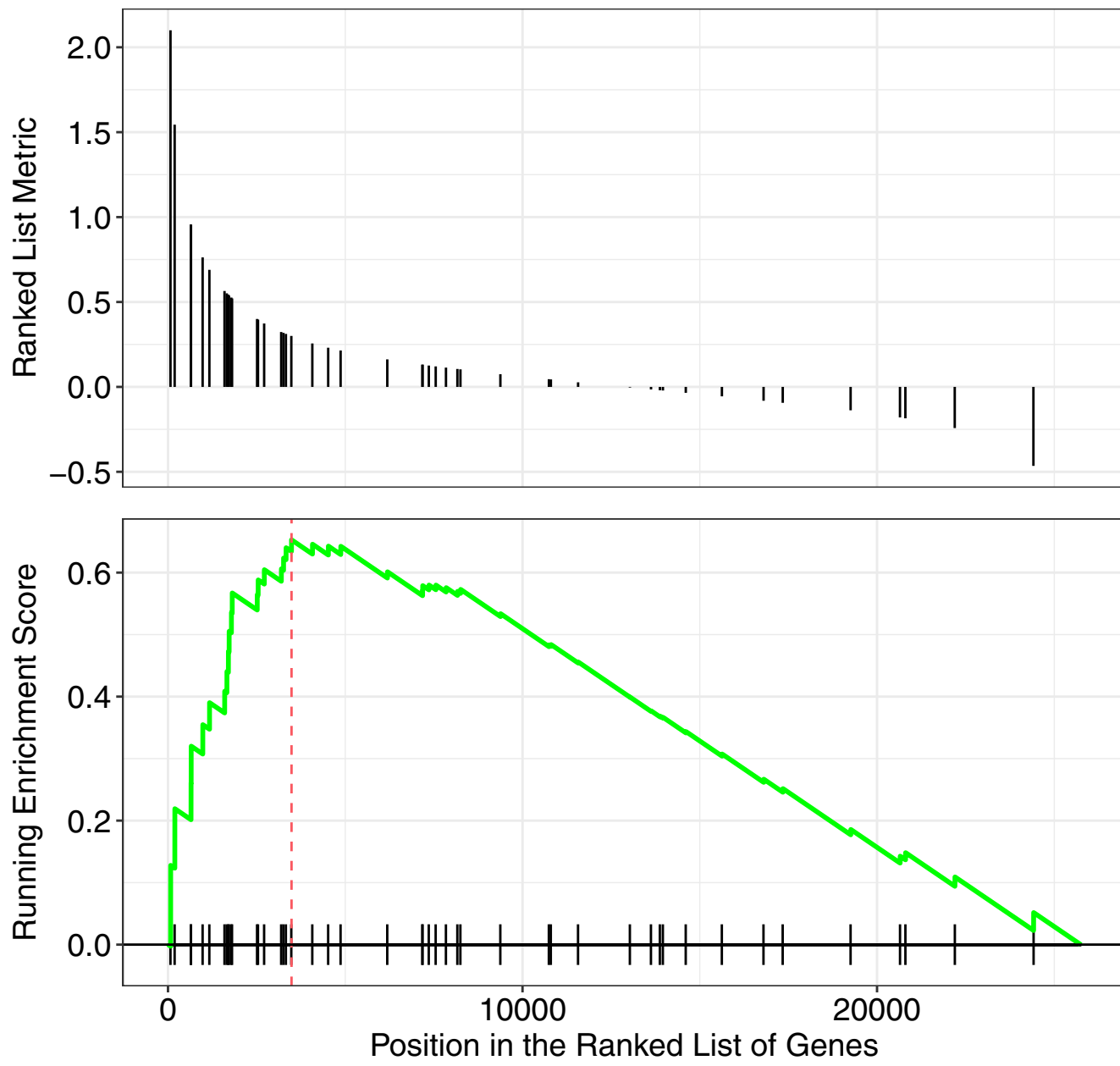
circadian rhythm



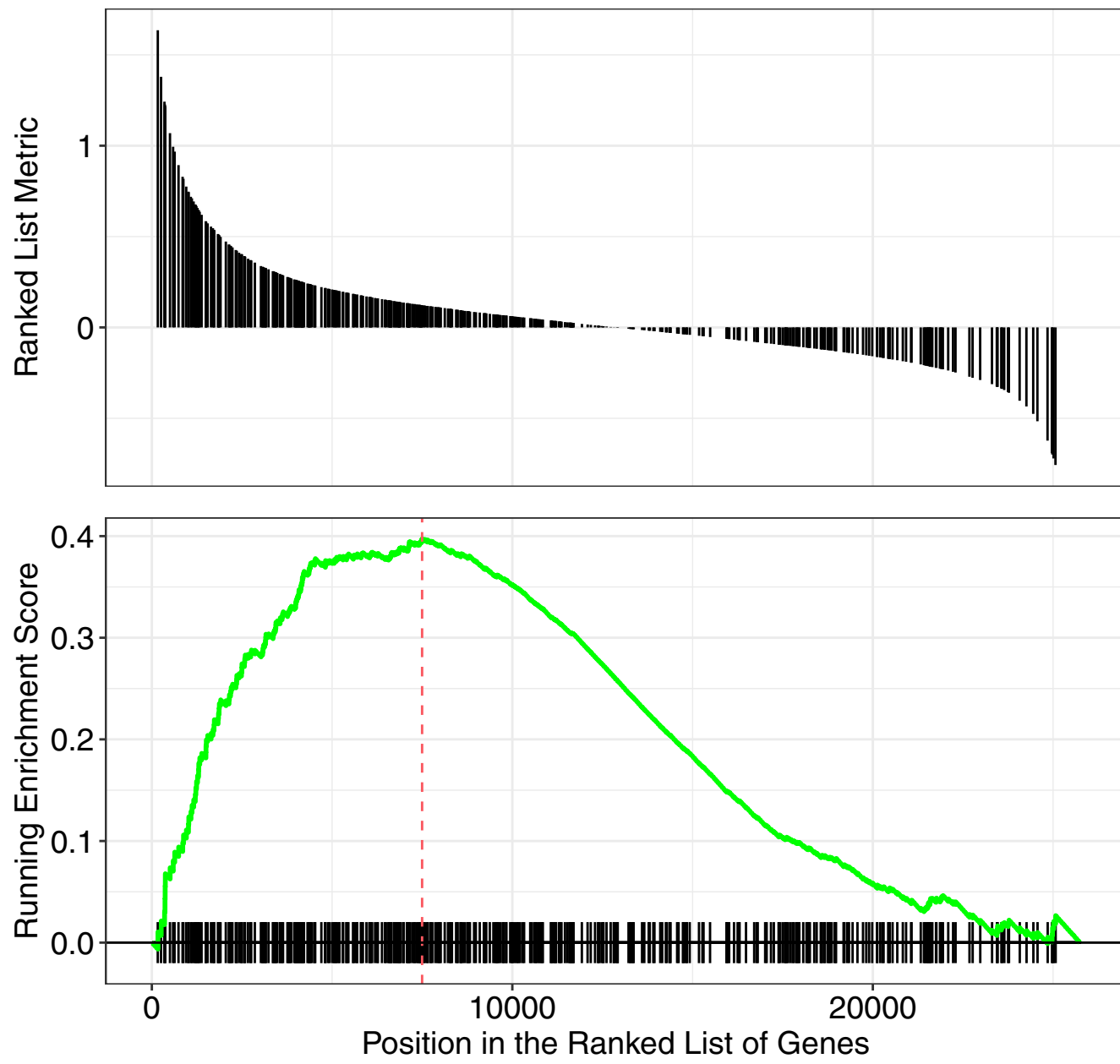
incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein



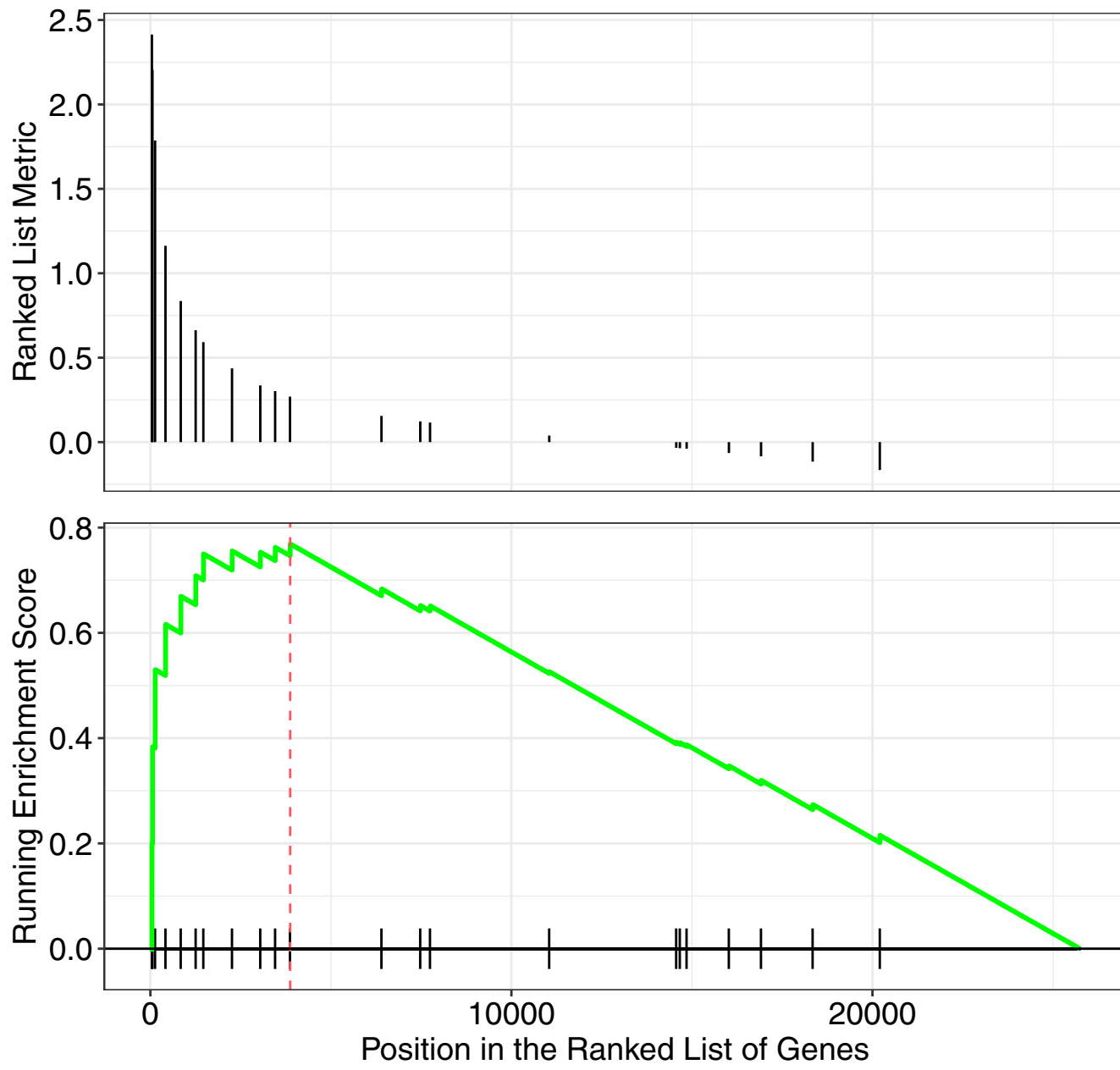
xenobiotic metabolic process



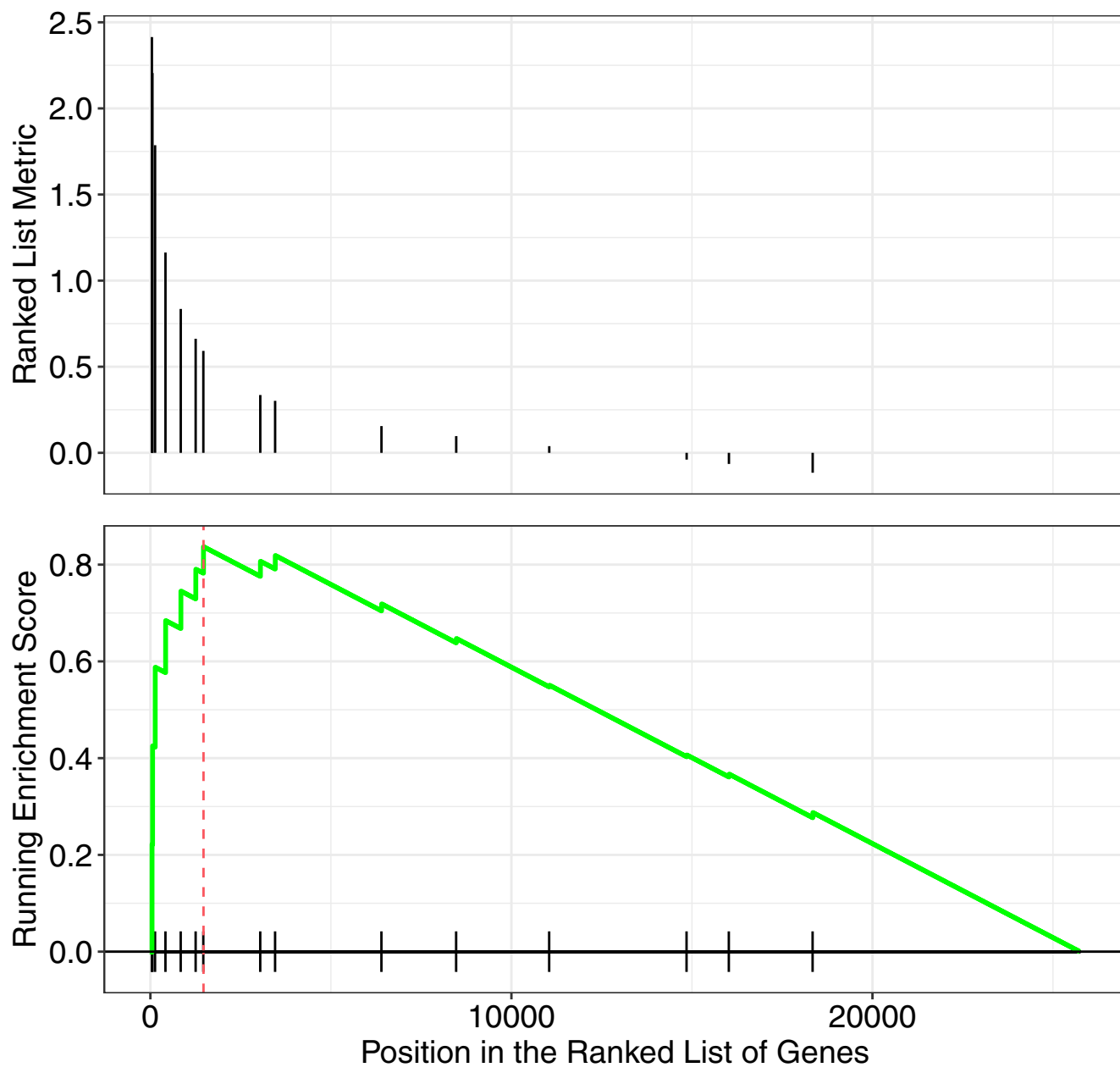
chemical homeostasis

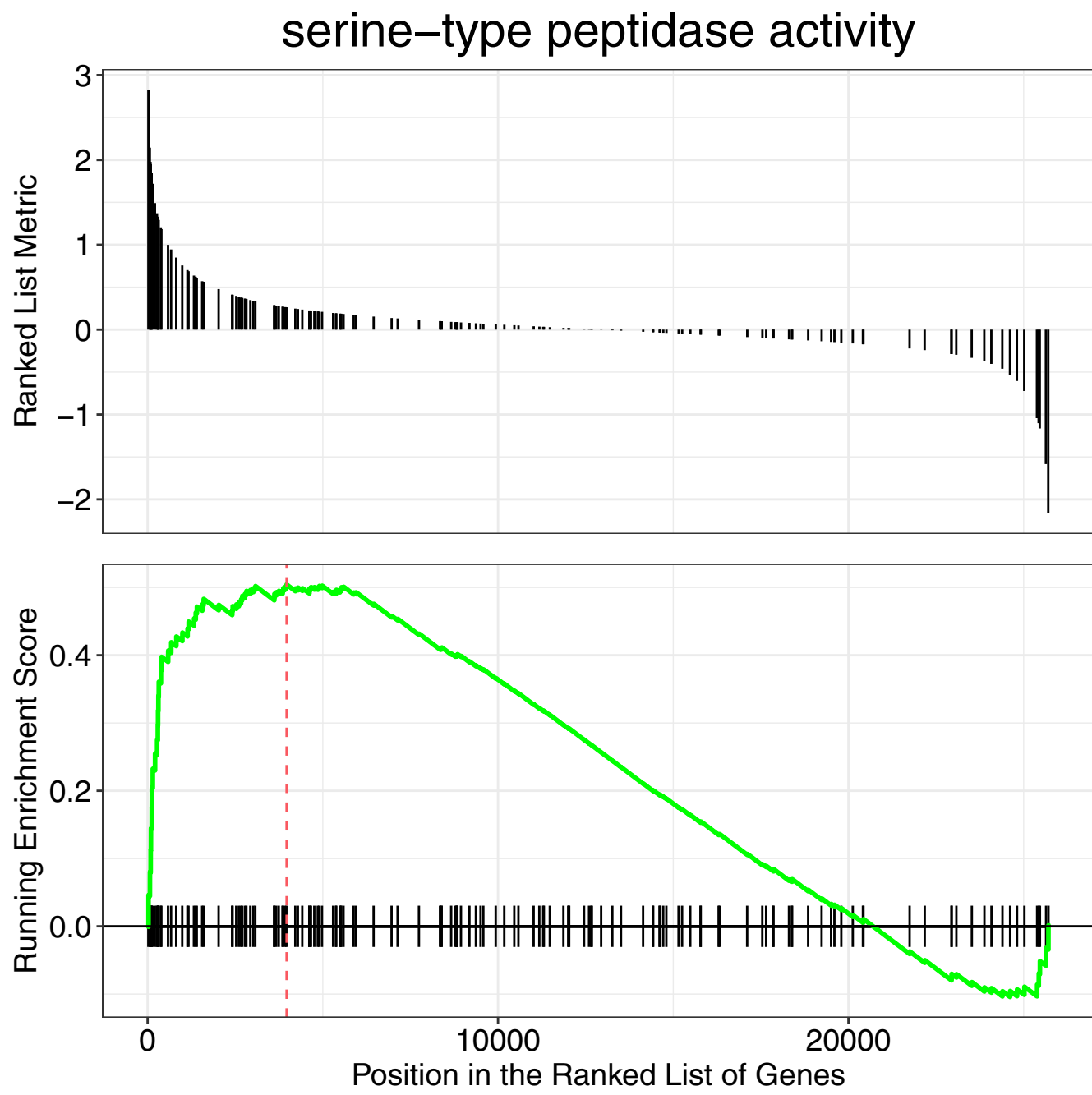


photoperiodism

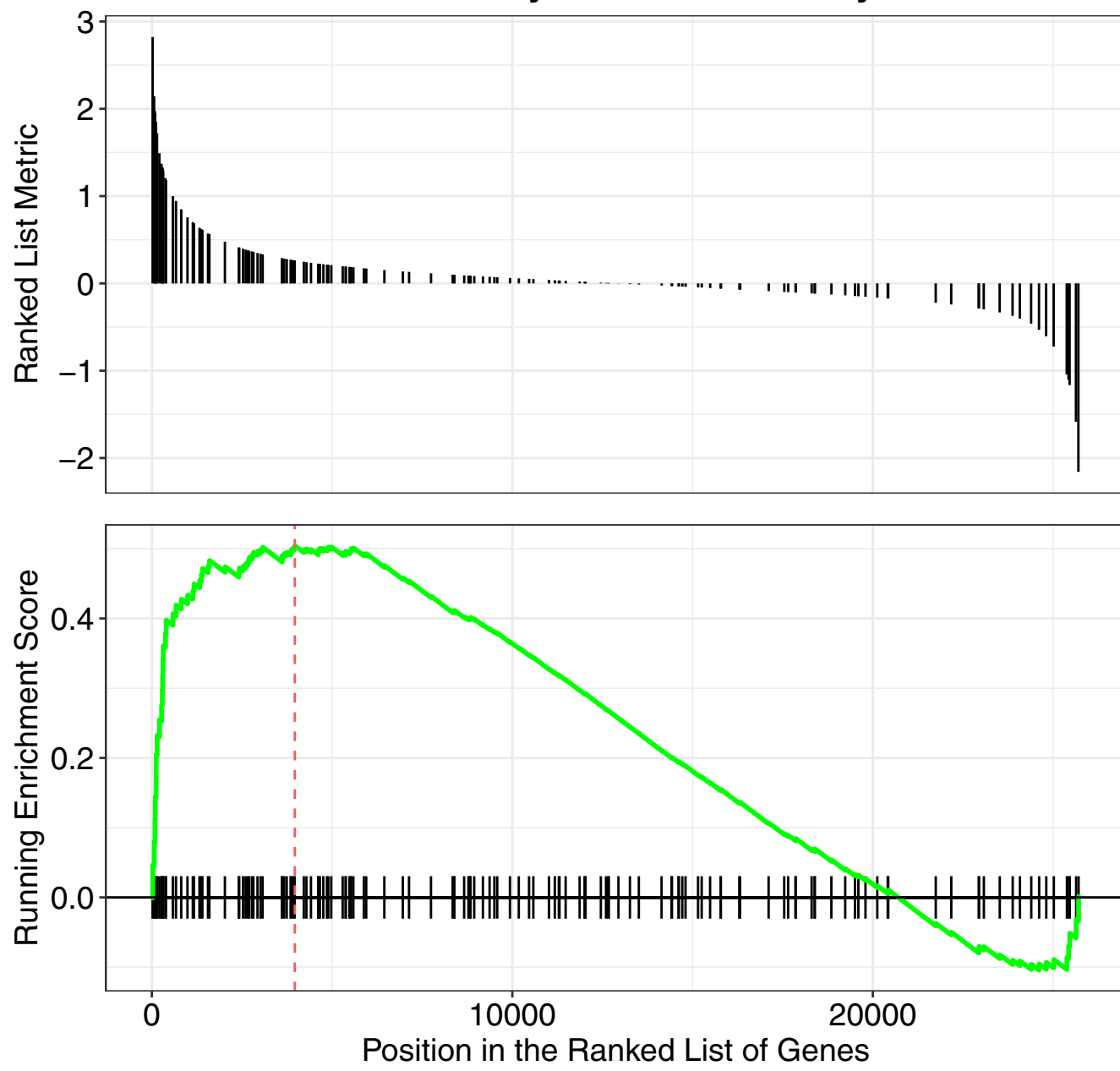


entrainment of circadian clock

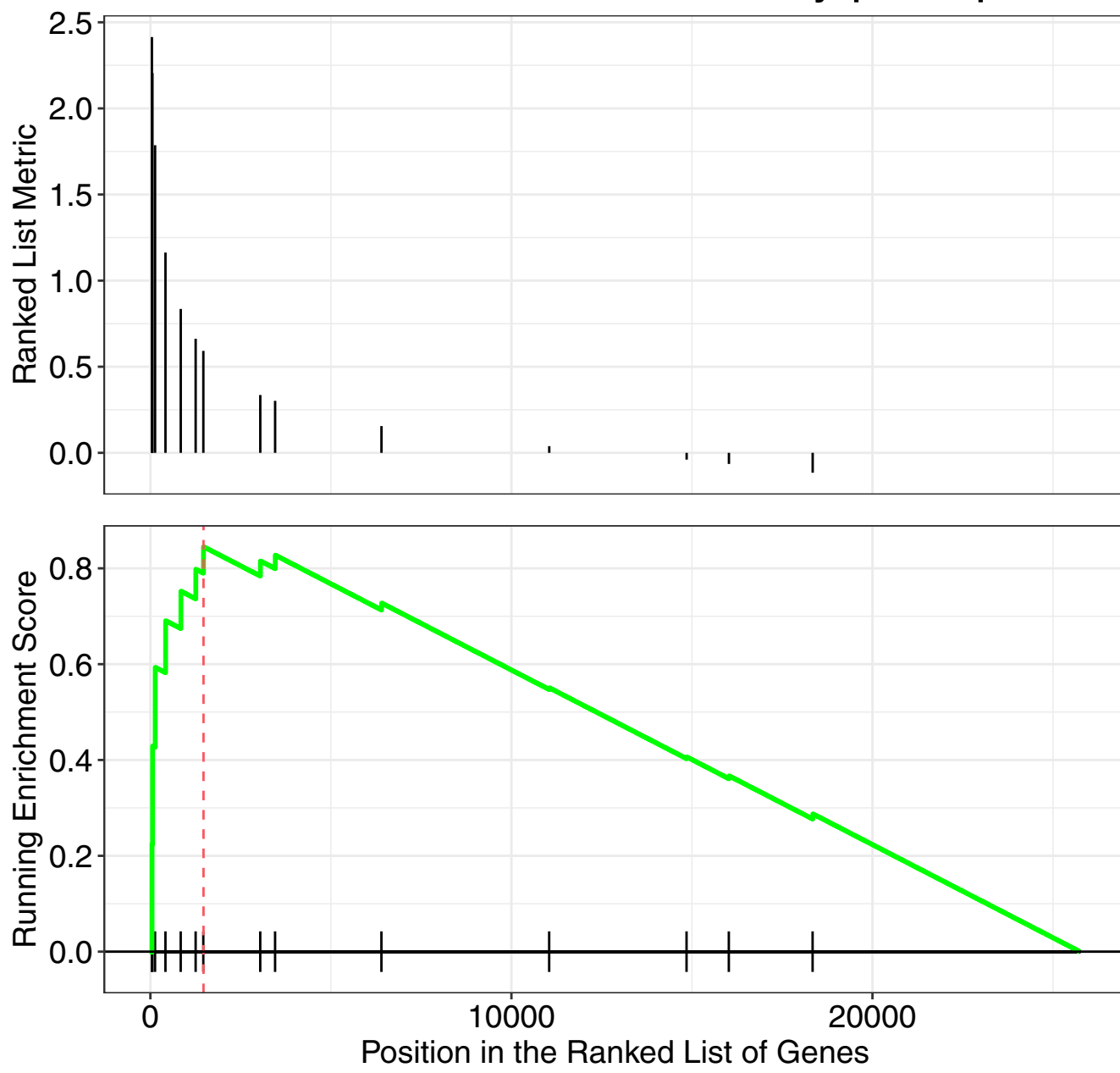




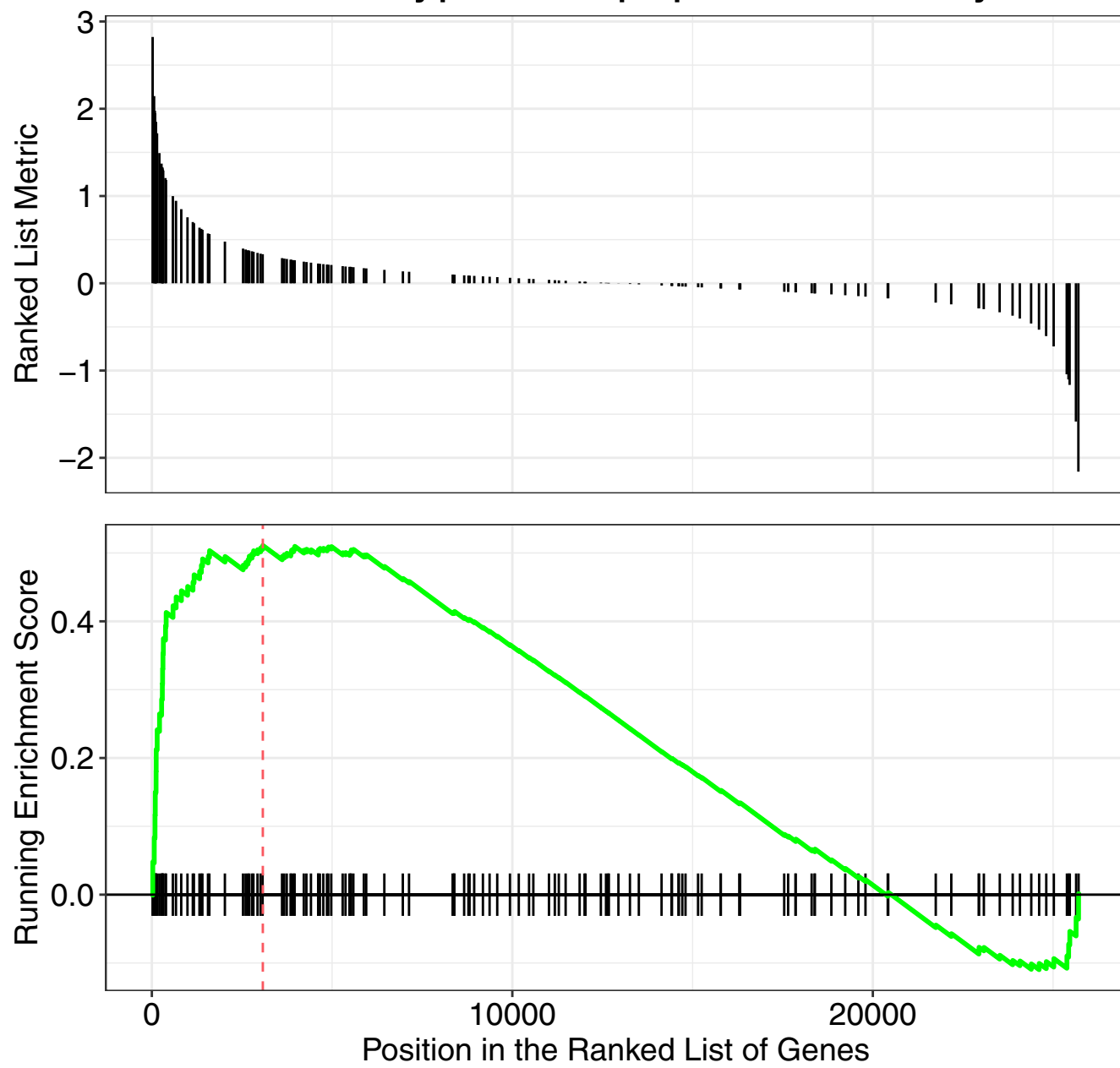
serine hydrolase activity



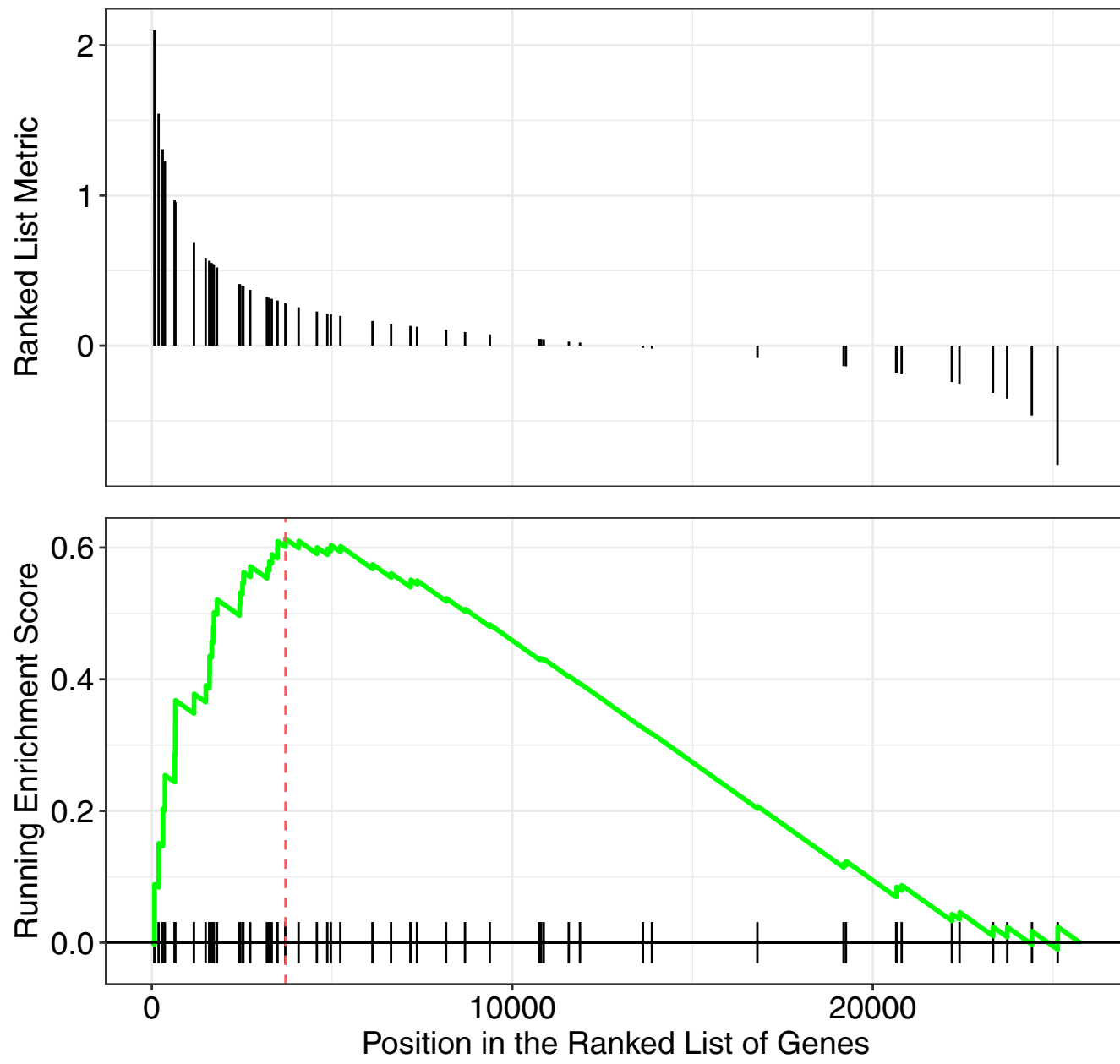
entrainment of circadian clock by photoperiod



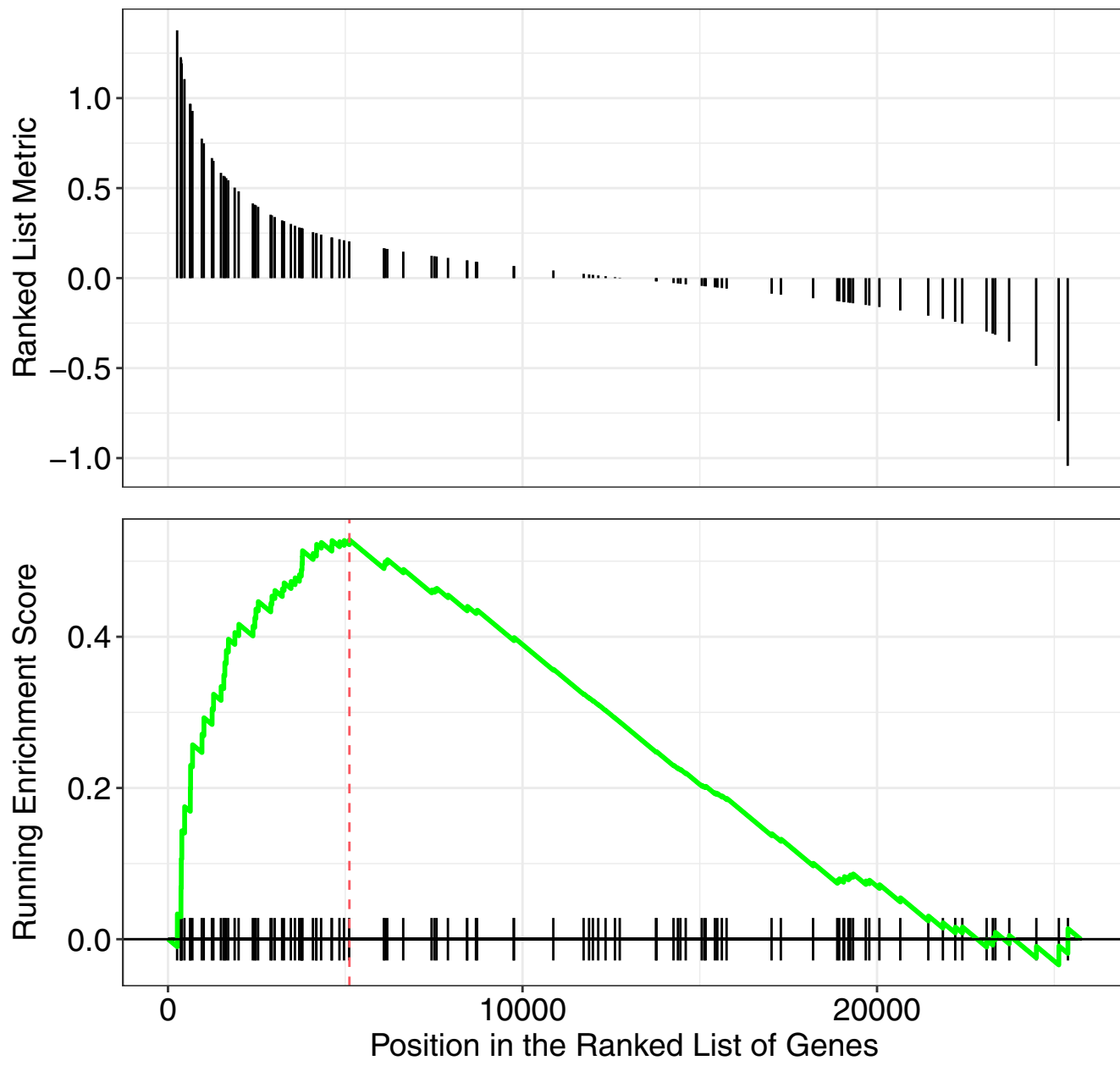
serine-type endopeptidase activity



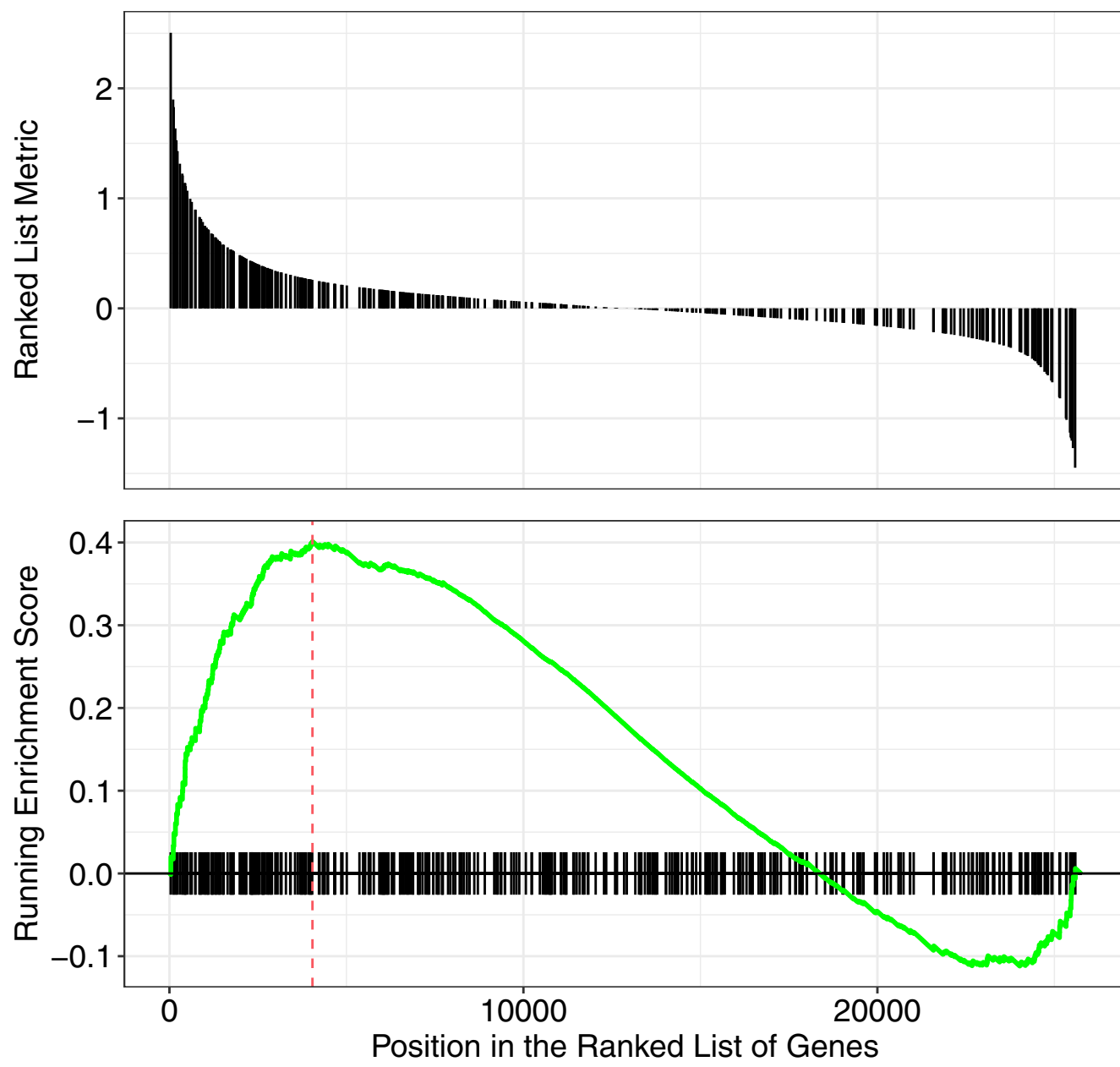
steroid hydroxylase activity

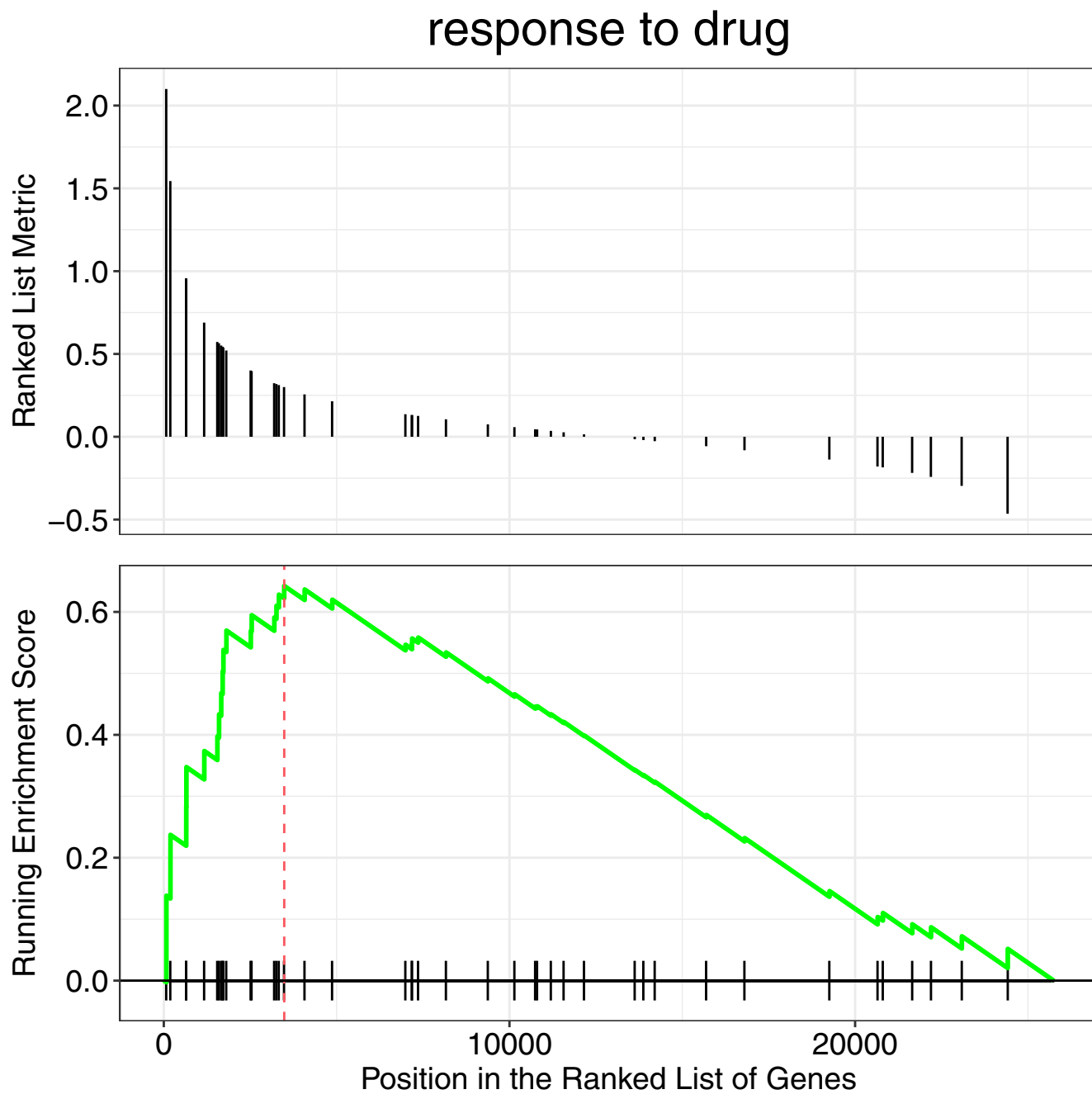


steroid metabolic process

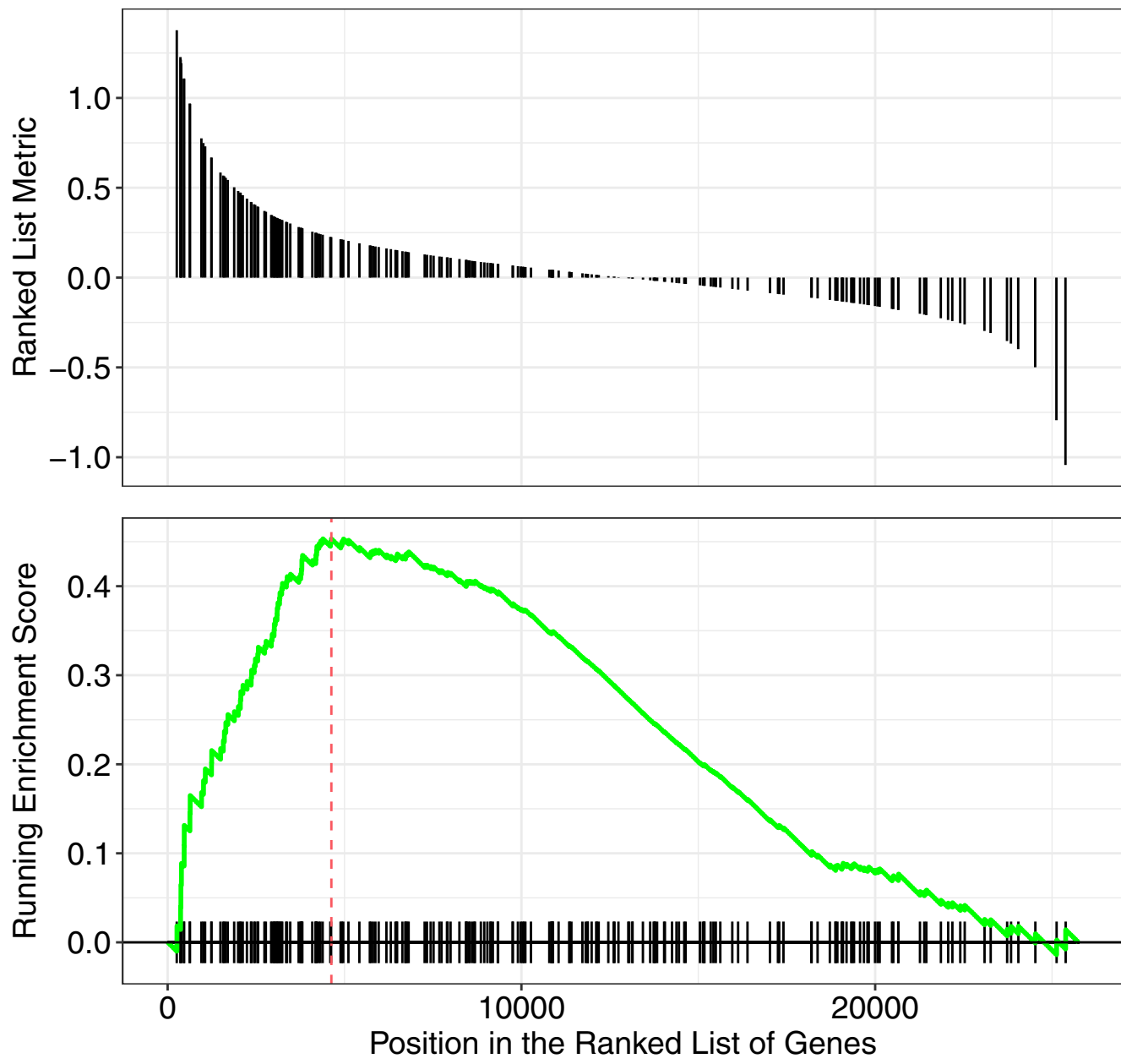


immune response

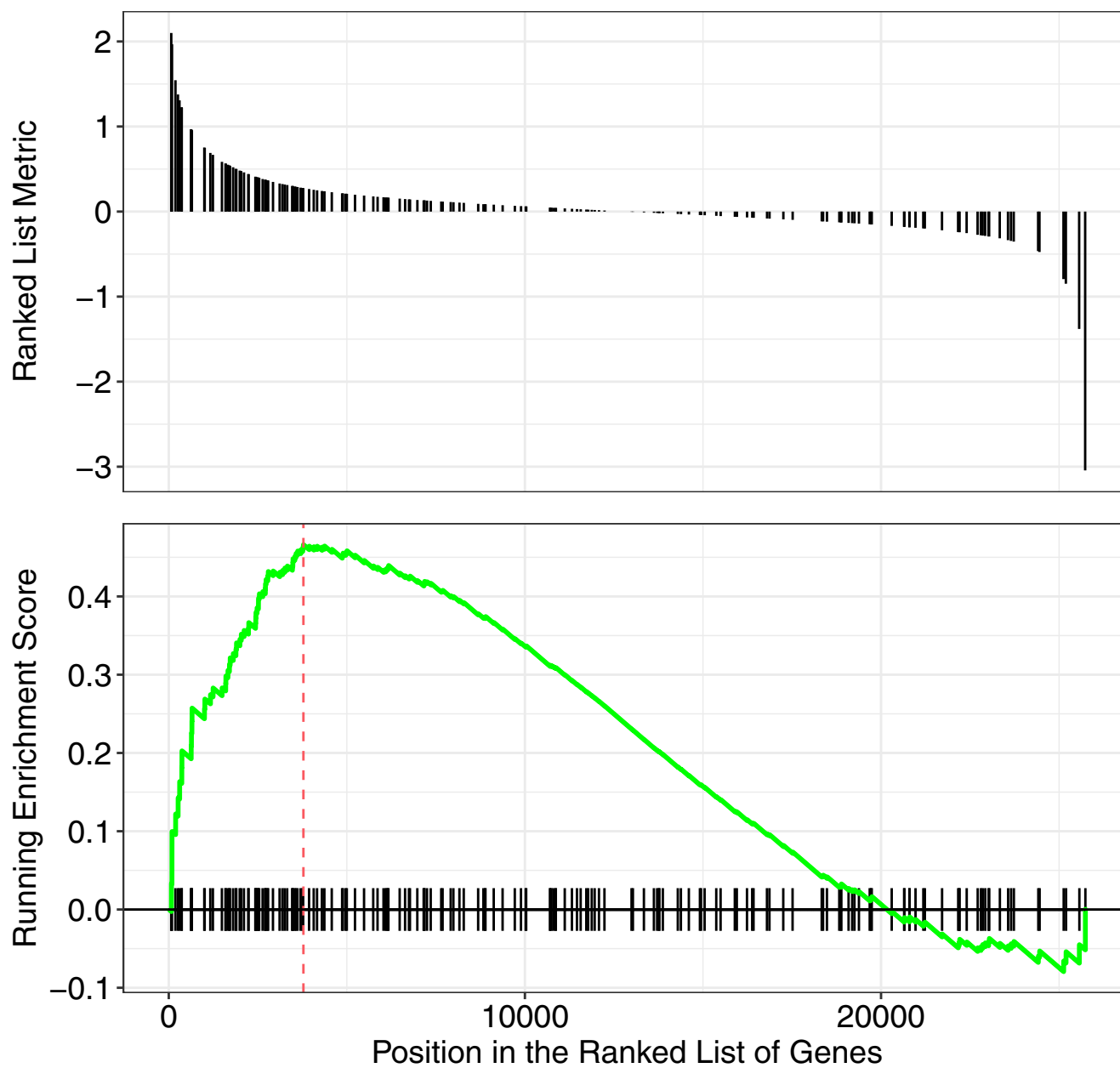




organic hydroxy compound metabolic process



oxidoreductase activity



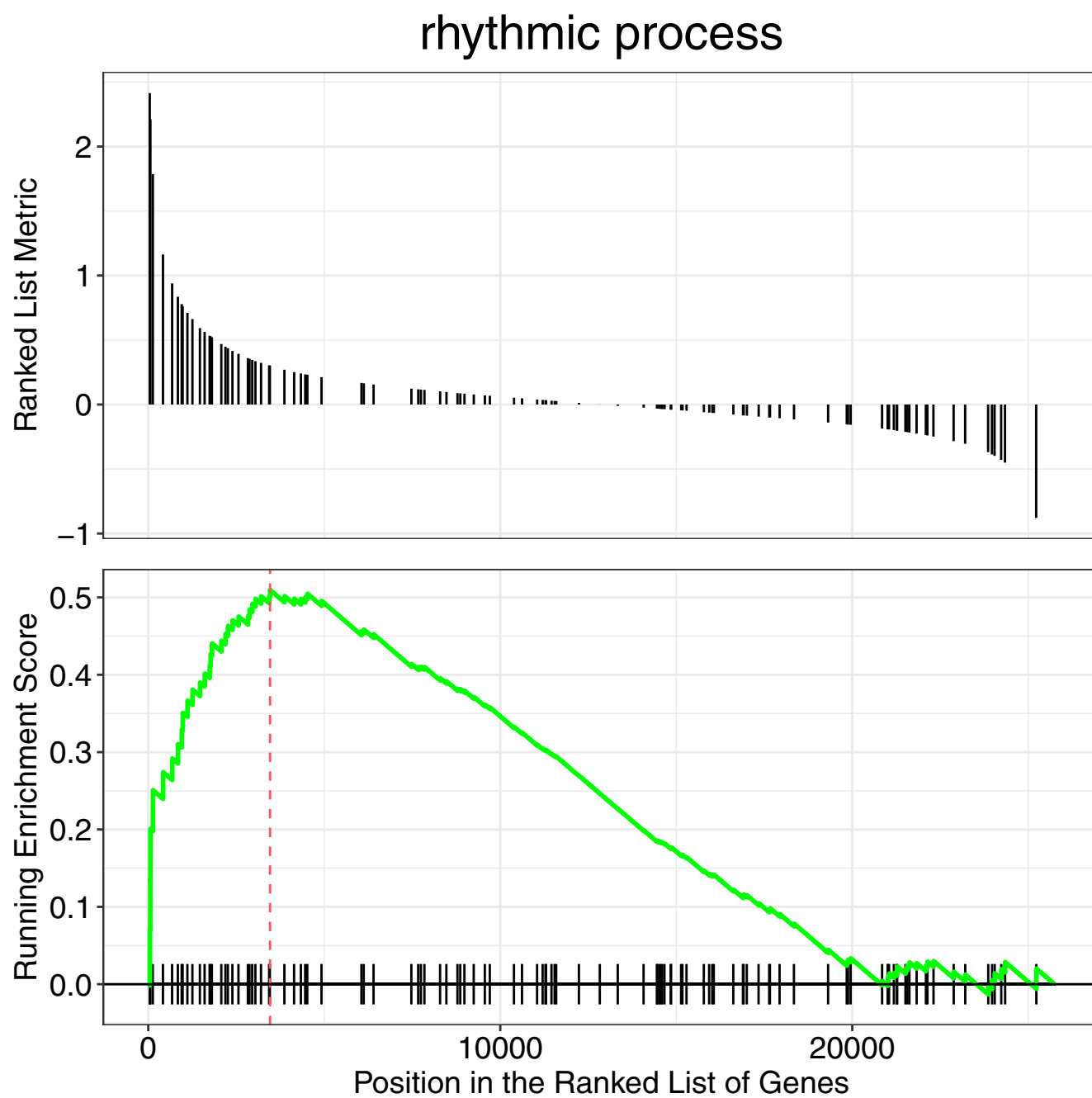


Fig. S8. Enrichment plots of Gene set enrichment analysis (GSEA) of differentially expressed genes in *alx1;alx3* crispants. Running score plot and pre-ranked list of all activated GSEA terms.

Table S1. Differentially expressed genes in alx1;alx3 mutants.

All differentially expressed genes with multiple-testing adjusted p-value smaller than or equal to 0.05 in 5dpf alx1;alx3 mutants when compared to age-matched alx^{uw2016} siblings.

[Click here to download Table S1](#)

Table S2. Gene set enrichment analysis (GSEA) of differentially expressed genes in alx1;alx3 mutants.

All enriched GO terms with multiple-testing adjusted p-value smaller than or equal to 0.05 from all differentially expressed genes in 5dpf alx1;alx3 mutants when compared to age-matched alx^{uw2016} siblings.

[Click here to download Table S2](#)

Table S3. Differentially expressed genes in alx1;alx3 crispants. All differentially expressed genes with multiple-testing adjusted p-value smaller than or equal to 0.05 in 3dpf **alx1;alx3 crispants** when compared to age-matched wildtype embryos.

[Click here to download Table S3](#)

Table S4. Gene set enrichment analysis (GSEA) of differentially expressed genes in alx1;alx3 crispants.

All enriched GO terms with multiple-testing adjusted p-value smaller than or equal to 0.05 from all differentially expressed genes in alx1;alx3 crispants when compared to age-matched wildtype.

[Click here to download Table S4](#)