

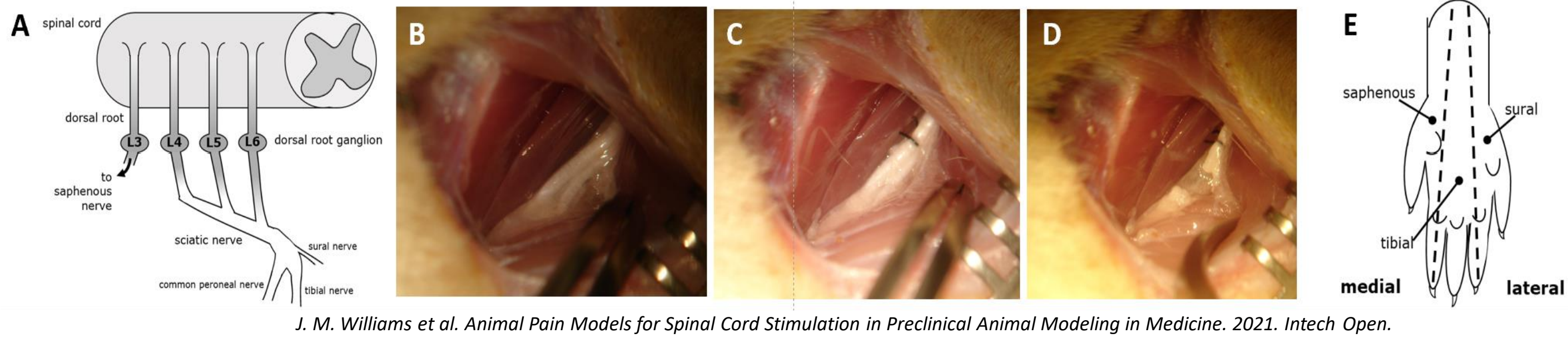
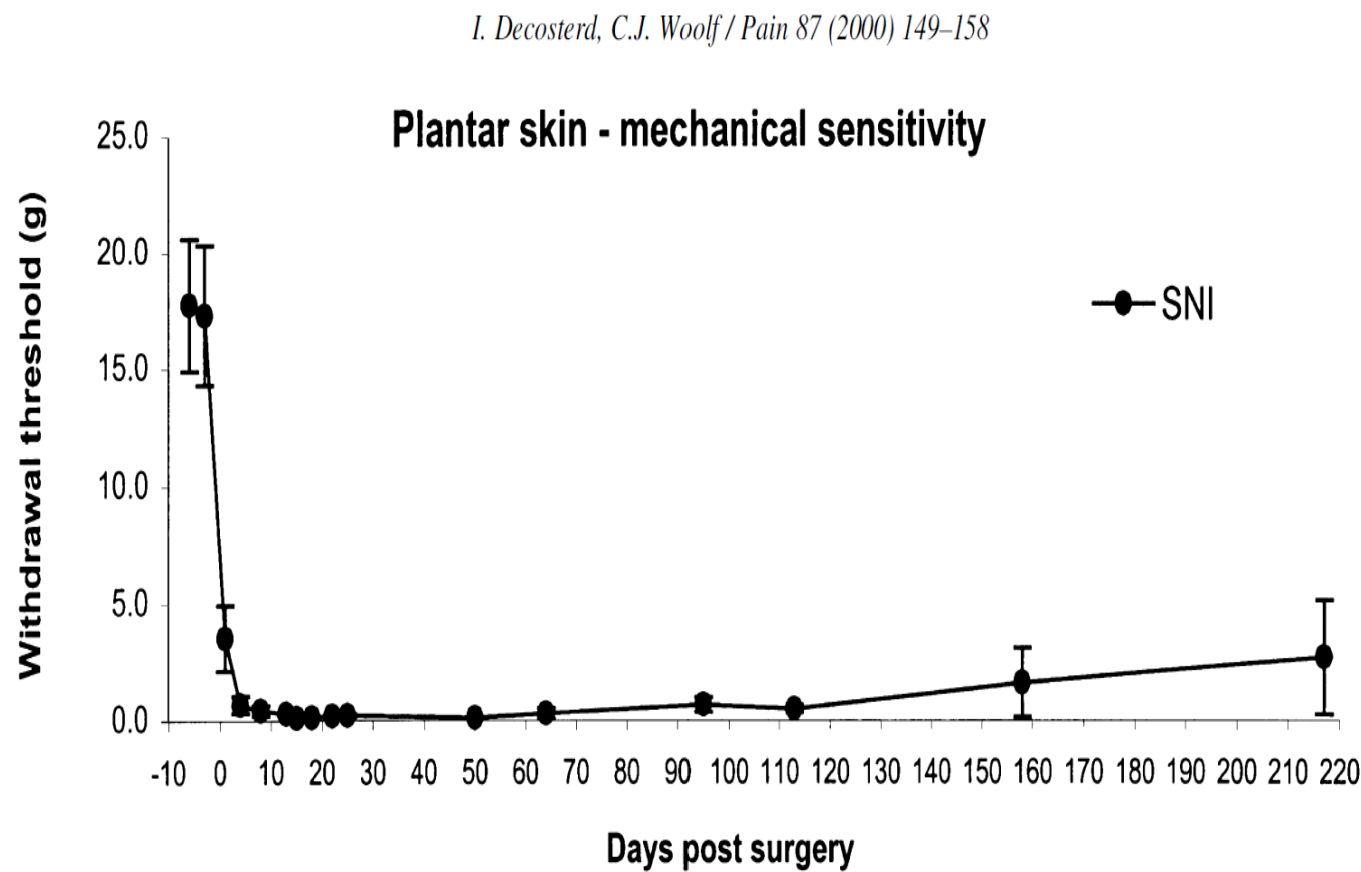
A Transcriptomic Study of the L5-DRG in the Spared Nerve Injury Model of Neuropathic Pain

David L. Cedeno PhD^{1,2}, Sandra L. Rodriguez-Zas PhD³, David C. Platt MS^{1,2}, Joseph M. Williams PhD², Bruce R. Southey PhD³, Elena V. Romanova PhD³, Jonathan V. Sweedler PhD³, Ricardo Vallejo MD PhD^{1,2}.

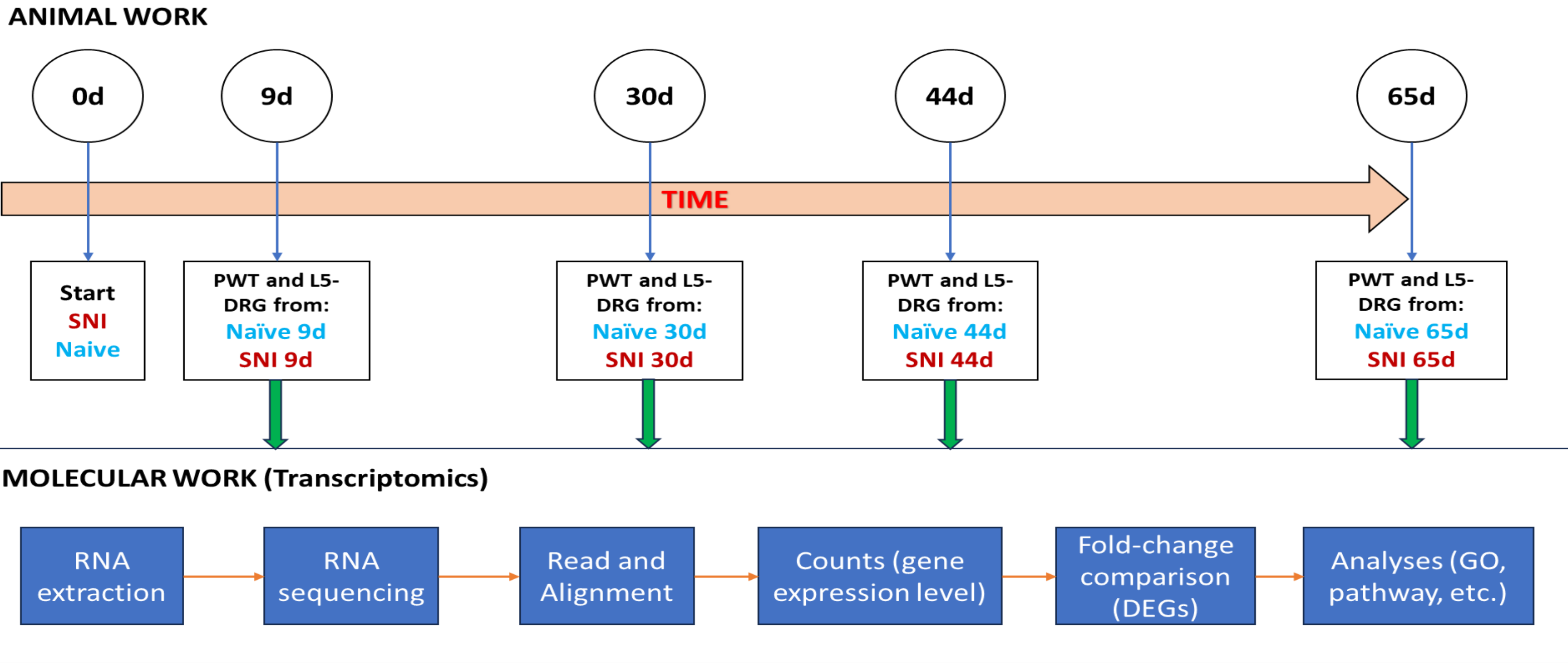
1. SGX Medical, Bloomington, IL; 2. Illinois Wesleyan University, Bloomington, IL; 3. University of Illinois at Urbana-Champaign, Champaign, IL.

INTRODUCTION

The rat spared nerve injury (SNI) model has been widely used for studying neuropathic pain. Animals develop thermal and mechanical hypersensitivity in the hind paw ipsilateral to injury, which is sustained for more than 70 days. Behavioral assessment of hypersensitivity is an objective measurement of the degree of pain. This is the first transcriptome-based study of the long-term time-evolution of the model.



MATERIALS AND METHODS



Animal procedures were approved by the Institutional Animal Care and Utilization Committee at Illinois Wesleyan University. Male rats (Sprague-Dawley, Envigo) weighing 300 ± 20 g were used. SNI done as described in the literature¹. Mechanical hypersensitivity (PWT) was measured before SNI and weekly using an electronic von Frey anesthesiometer. Fold changes (FC = SNI/naïve expressed as $\log_2FC$) were obtained from mean RNA counts of 3 to 4 L5-DRG specimens per group. ANOVAs were run for $\log_2FC$ in every endpoint using a false discovery rate (FDR) method to correct for multiple comparisons.

1. Decosterd and Woolf. Pain. 2000; 87:149-154

RESULTS

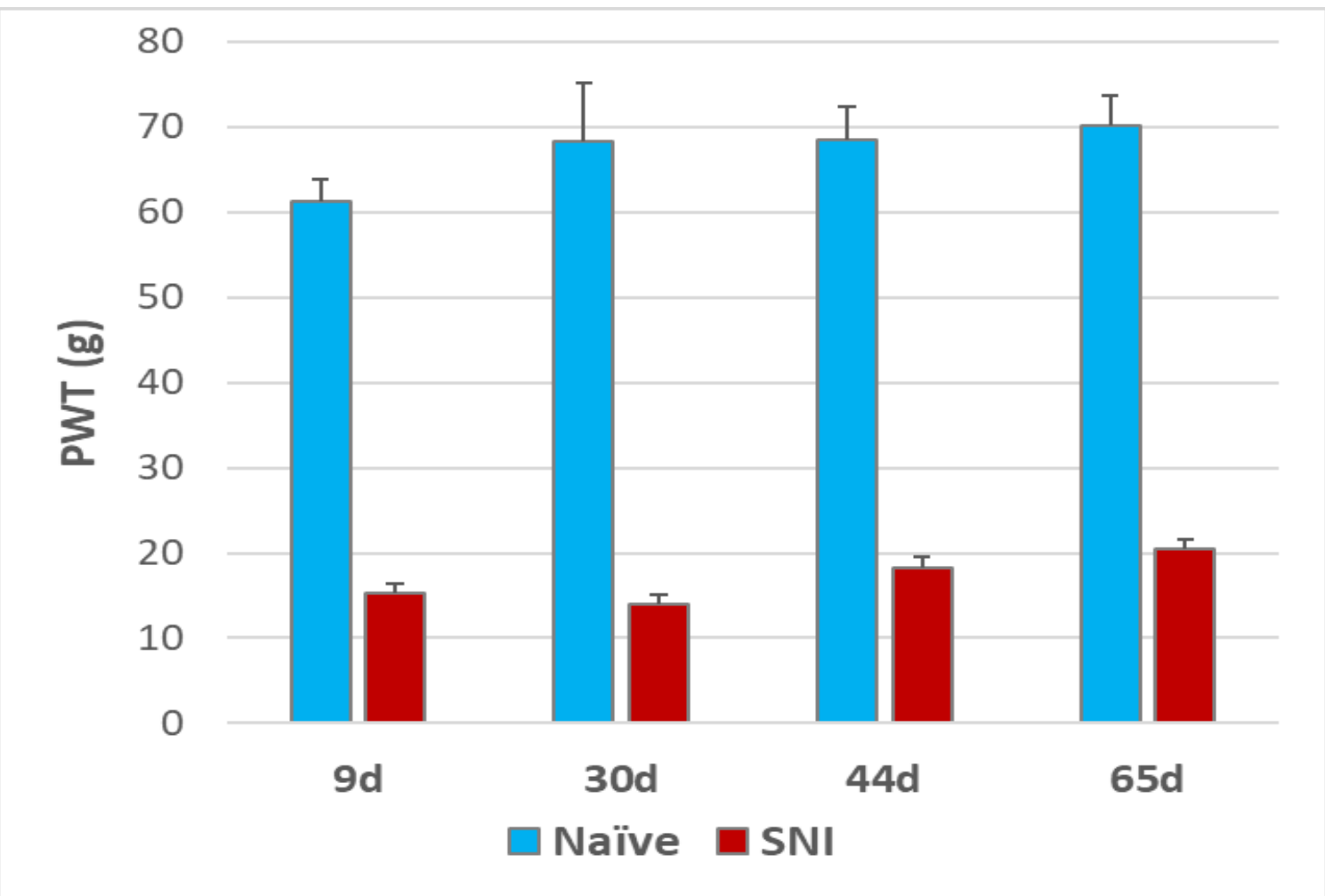


Figure 1. Mean PWTs ($\pm$ standard error, SE) for Naïve and SNI groups at every experimental endpoint. Differences between SNI and Naïve PWTs are significant (Holm-Sidak adjusted $P < 0.001$) at every time point. There were no significant differences among Naïve PWTs or SNI PWTs as a function of time.

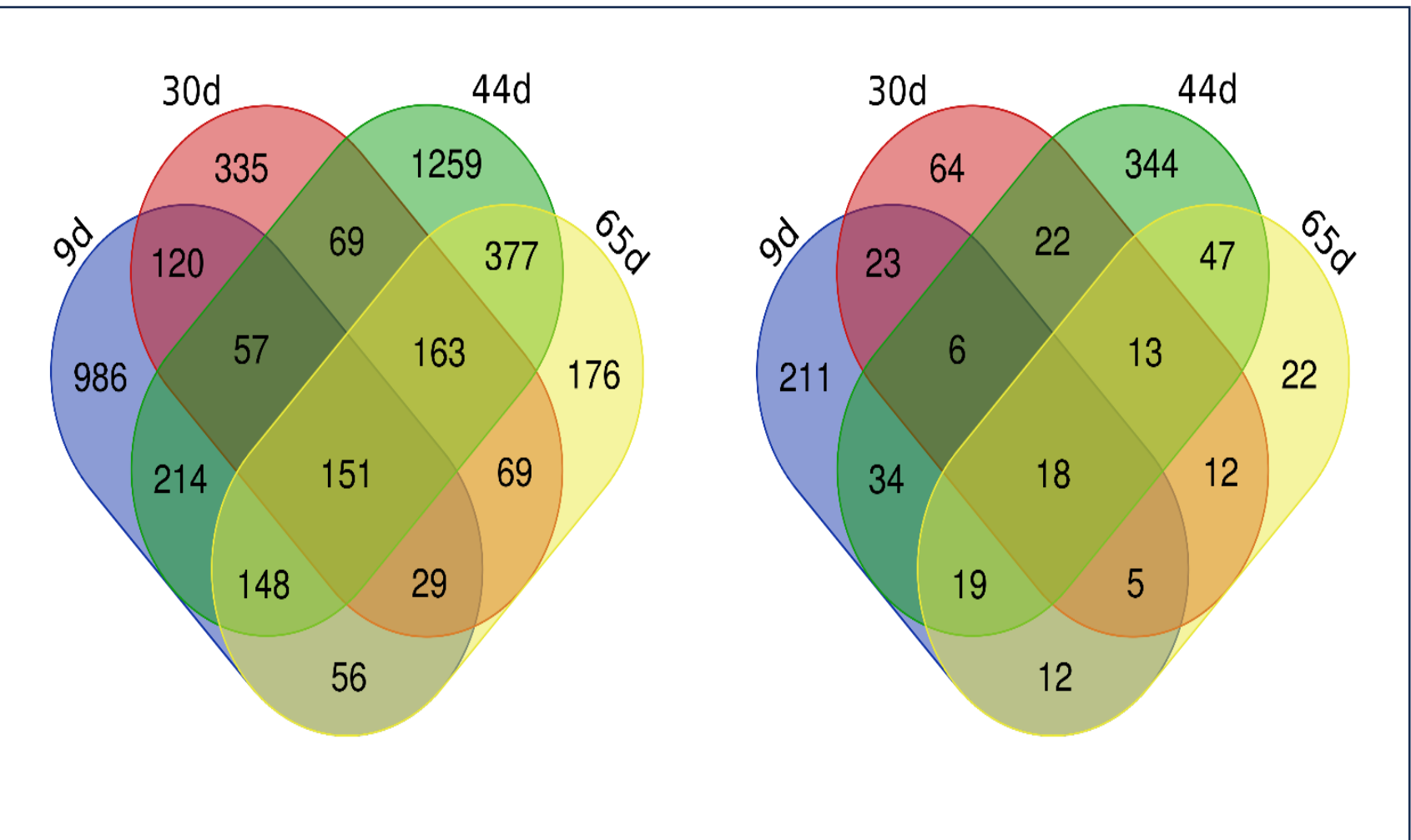


Figure 2. Venn diagrams showing the distribution of unique SNI/Naïve differentially expressed genes (DEGs) at each study endpoint for all significant (FDR-P < 0.1) DEGs (Left diagram) and for those with $|\log_2FC| \geq 1$ (FDR-P < 0.01) (Right diagram). Venn diagrams were built courtesy of the Van de Peer Lab for Bioinformatics and Evolutionary Genomics at Gent University.

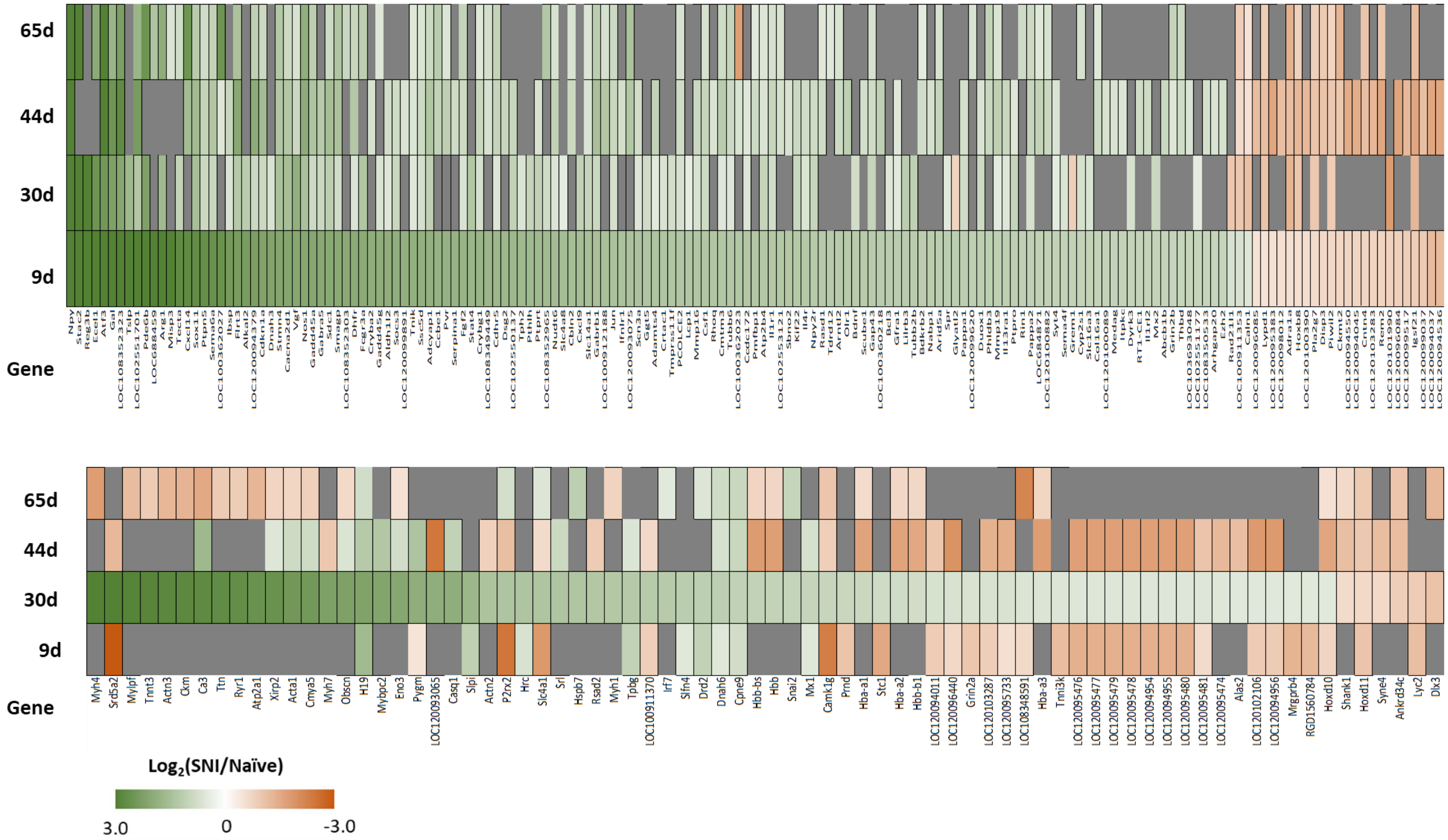


Figure 3. Heat maps of significant (FDR-P < 0.01) DEGs (N = 644) arranged in 4 sets according to whether the SNI/Naïve is the largest at a given study endpoint. Top row: 9d (N = 165), Second top row: 30d (N = 76).

RESULTS

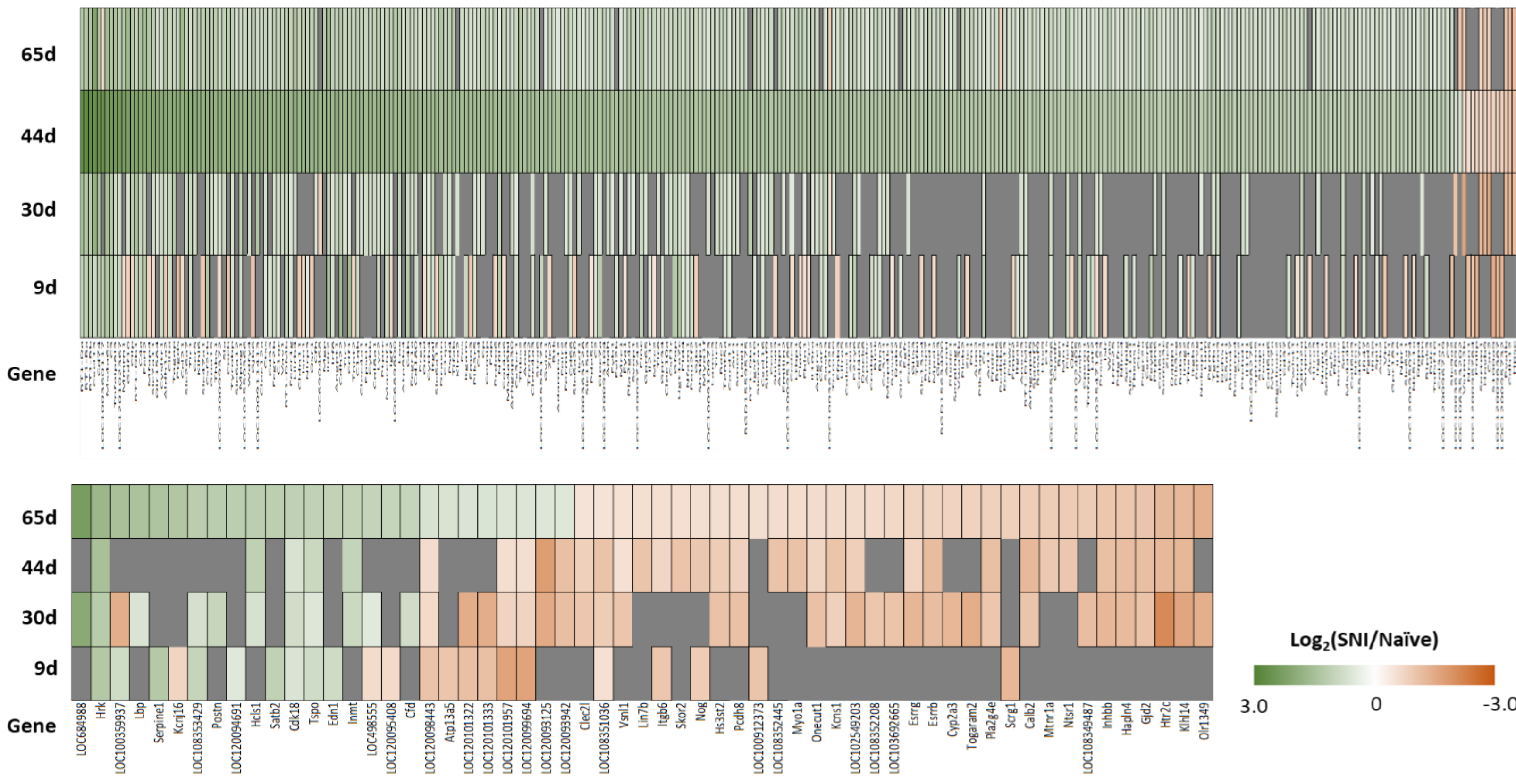


Figure 3 (Cont.). Heat maps of significant (FDR-P < 0.01) DEGs (N = 644) arranged in 4 sets according to whether the SNI/Naïve is the largest at a given study endpoint. Third row: 44d (N = 344), Bottom row: 65d (N = 59).

DISCUSSION

Enrichment analysis shared across all endpoints include gene ontology biological processes (BPs) of response to wounding, regulation of immune response, inflammatory response, regulation of cell activation, protein activation cascade, regulation of transmembrane transport, and regulation of membrane potential. Significant $\log_2FC$ in two or more endpoints were found for 644 DEGs. Most of these DEGs (>70%) are upregulated by the SNI model at every timepoint. The timepoints at which DEGs have the largest FCs are the 9d (acute stage) and 44d (chronic stage).

This study confirms the effect of the SNI pain model 65 days post-surgery and indicates that the transcriptome of the L5-DRG is greatly affected by the SNI pain model in the male rat. Results show the transition from an acute state (early time) to a chronic state. The chronic state seems to be prominent at the 44d day of implementation of the model as implied by the large number of DEGs with large FC at that time. Our results indicate that neuropathic pain due to peripheral injury will affect gene expression in sensory neural structures away from the site of injury. DEGs in the L5-DRG are related to immune response, inflammation, and signaling, which are important to central sensitization and chronification of pain.