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Gravitational and Space Research

Supplement: The Impact of Hindlimb Suspension on the Rat Eye: A Molecular and Histological Analysis of the Retina

Supplementary Table 5. Number of eyes received and used as part of this tissue sharing study.

Time (days)	YM				YF				OM			
	Right Eye (RNA)		Left Eye (Histo)		Right Eye (RNA)		Left Eye (Histo)		Right Eye (RNA)		Left Eye (Histo)	
	Control	HS	Control	HS	Control	HS	Control	HS	Control	HS	Control	HS
0	4		4		4		3		4			
14	4	4	3	3	4	4	3	3	4	4	3	3
90	4	4	3	3	4	4						
104	4	4	3	2	4	4	3	3	4	4	3	3
180	2	2	3	3	4	6	3	3	4	4	3	3

Sample size is denoted at each time point for the three different cohorts indicating the right eye used for gene expression and the left eye used for histological analysis. Sample size for both control animals and hindlimb suspended animals are included. HS, hindlimb suspended; OM, old males; YF, young females; YM, young males.

Supplementary Table 6. Sample size by cohort for collection of IOP measures.

Time (days)	YM	YF	OM
0	76	96	72
7	12	18	6
14	14	16	0
28	6	8	0
45	28	34	44
90	50	60	38
97	6	10	0
104	12	26	14
118	6	0	0
135	10	6	8
180	10	8	8

Sample size (n) are provided for each cohort to summarize the repeated collection of IOP measures at baseline (day = 0) throughout the HS time course (Day 1–90) and recovery periods (Day 91–180) of the study. IOP was collected longitudinally on each eye (left, right). The sample size per timepoint varied due to the sacrifice time of the animals, as well as due to reasons undisclosed to us from the experimentation site. HS, hindlimb suspended; IOP, intraocular pressure; OM, old males; YF, young females; YM, young males.

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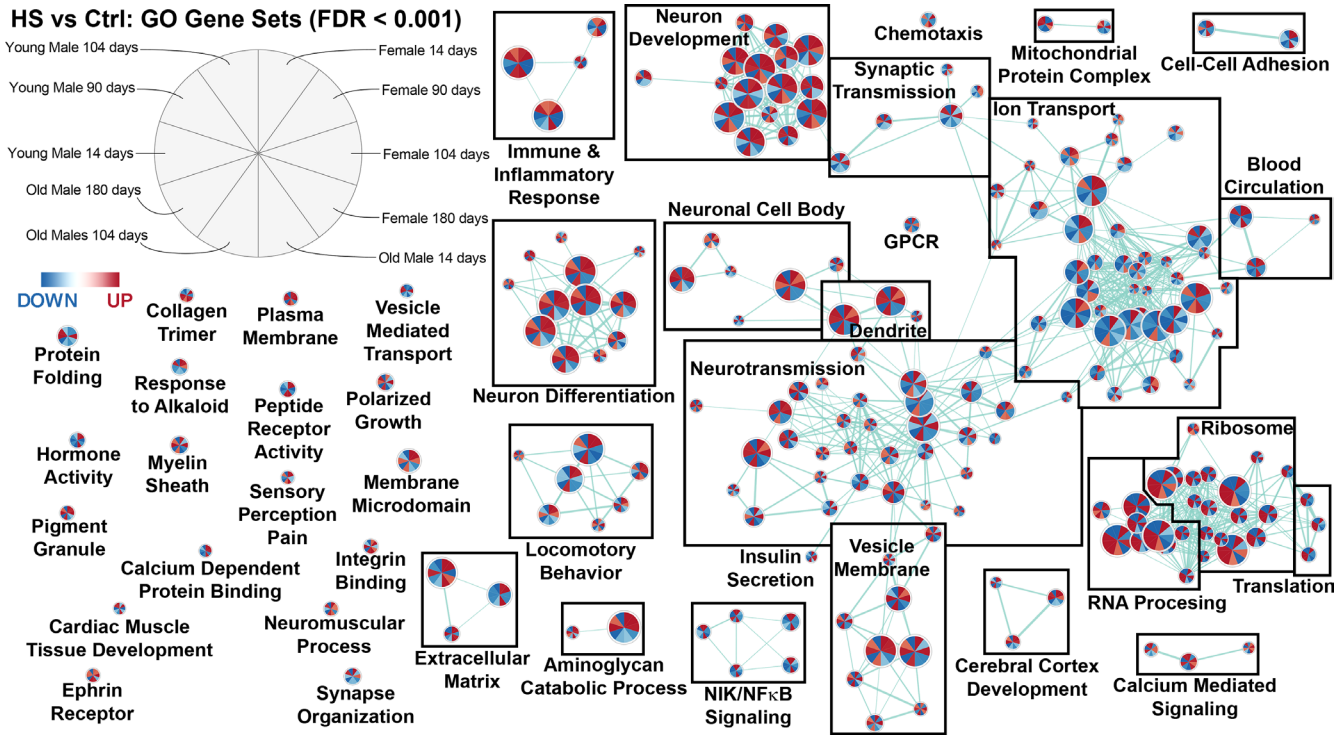
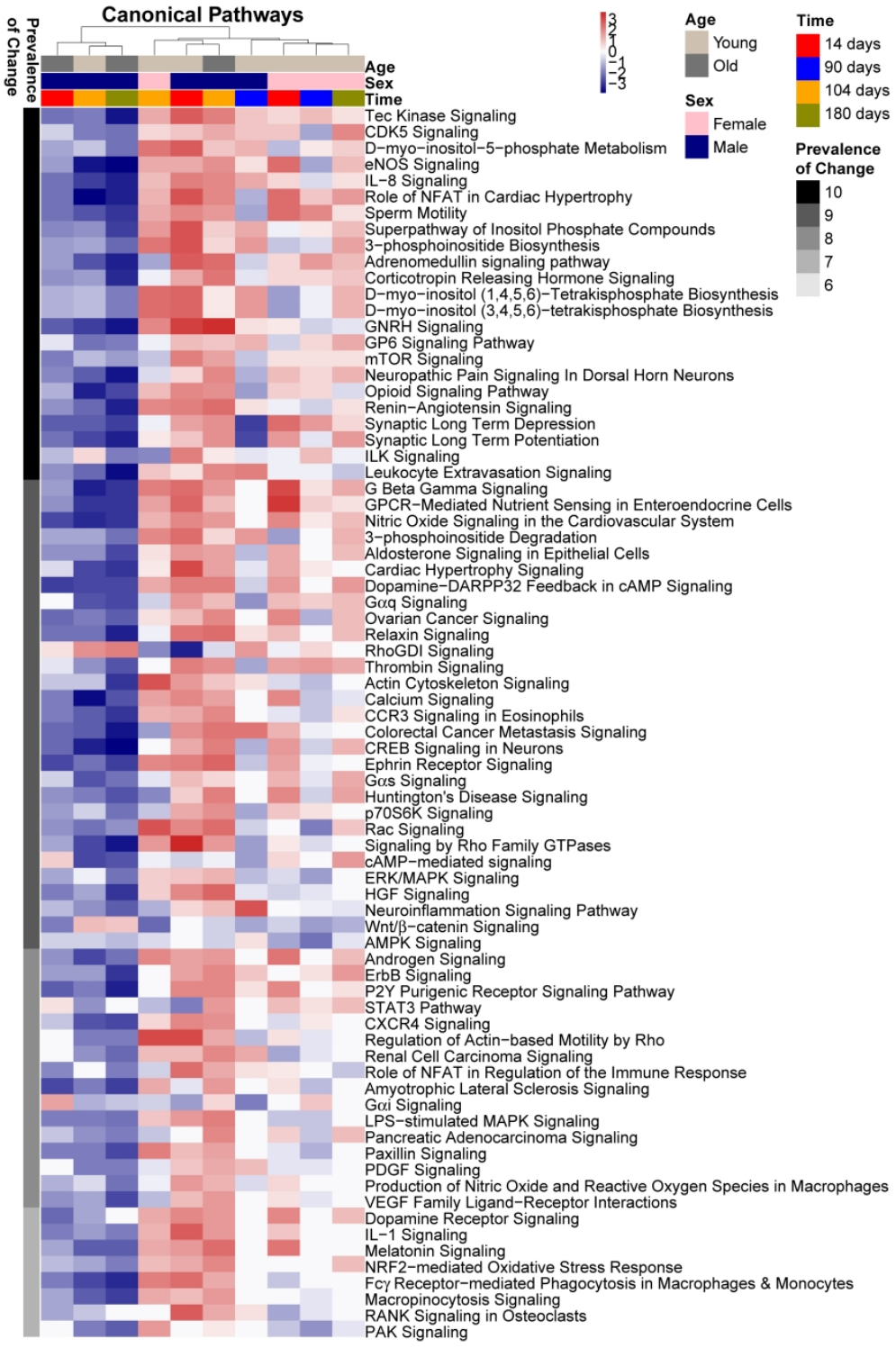
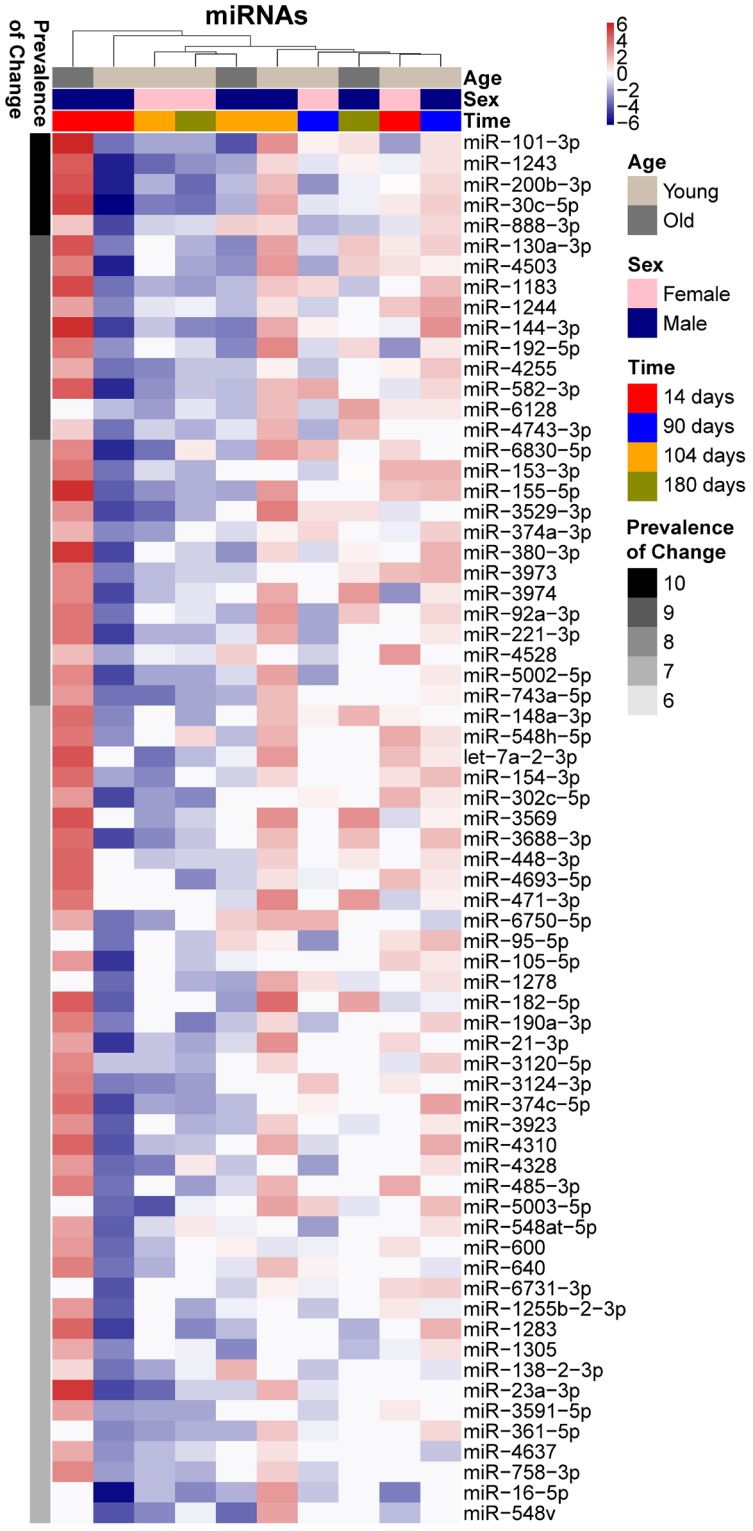


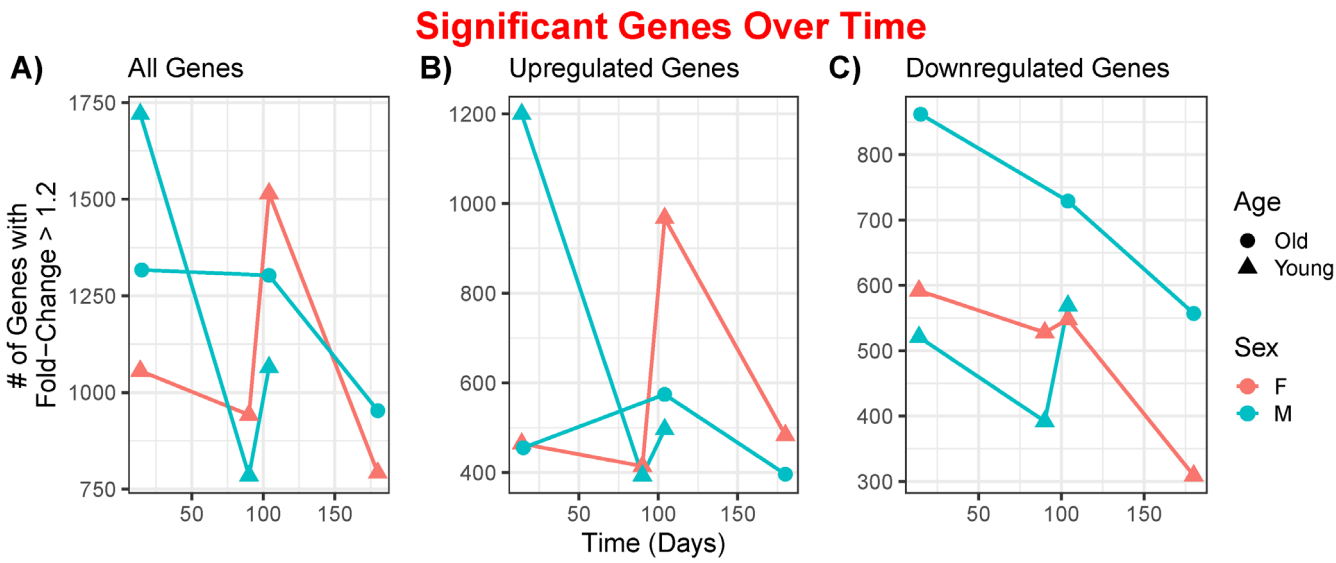
Supplementary Figure 1. Gene set enrichment analysis (GSEA) for the Gene Ontology (GO) gene sets commonly enriched in retina of rats comparing hindlimb suspension (HS) vs controls. All samples were comparing experimental conditions with the respective controls. The significant gene sets were determined with false discovery rate (FDR) ≤ 0.001. The legend in the upper left of the figure displays what each component/wedge of the nodes represent. Each node contains 10 wedges for each condition and the color of each wedge indicates if the gene set is downregulated (blue) or upregulated (red). The shade of the color indicates the degree of regulation. Each node represents one gene set and the size of the node indicates the number of genes involved with the predictions. Pathways with more than one node are grouped together in the black boxes under each major category.



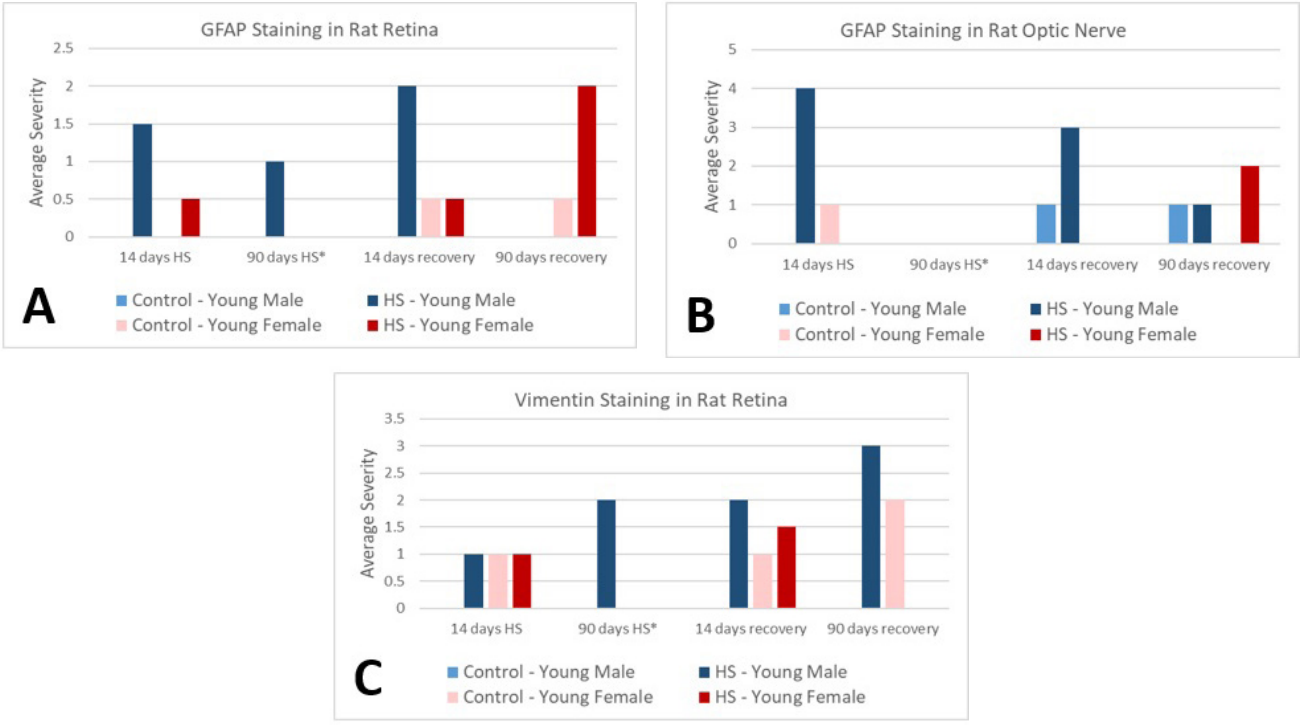
Supplementary Figure 2. Canonical pathway analysis utilizing Ingenuity Pathway Analysis (IPA) on the significantly regulated genes (FDR < 0.05). The results are shown only for the common canonical pathways for all conditions as a heatmap of the activated z-scores.



Supplementary Figure 3. miRNA predictions utilizing Ingenuity Pathway Analysis (IPA) on the significantly regulated genes (FDR < 0.05). The results are shown only for the common predicted miRNAs for all conditions as a heatmap of the activated z-scores.



Supplementary Figure 4. Significantly regulated genes in the rat retina as a function of time after hindlimb suspension. The scatter/line plots show the number of genes with a fold-change > 1.2 for **(A)** all genes, **(B)** only upregulated genes and (C) only downregulated genes. Age of the rats are indicated by different shapes and sex of the rats are indicated by different colors.



Supplementary Figure 5. Severity score of histologic effects of HS in the rat eye. Qualitative assessment was performed as described in methods. A GFAP immunostaining in retinas of both young male and female rates including HS and controls **(B)** GFAP immunostaining in the optic nerve of both young male and female rates including HS and controls. (**C)** Vimentin immunostaining in retinas of both young male and female rates including HS and controls. * Denotes that no data was available for female cohort at that timepoint.