A multi-omics longitudinal study of the murine retinal response to chronic low-dose irradiation and/or simulated microgravity

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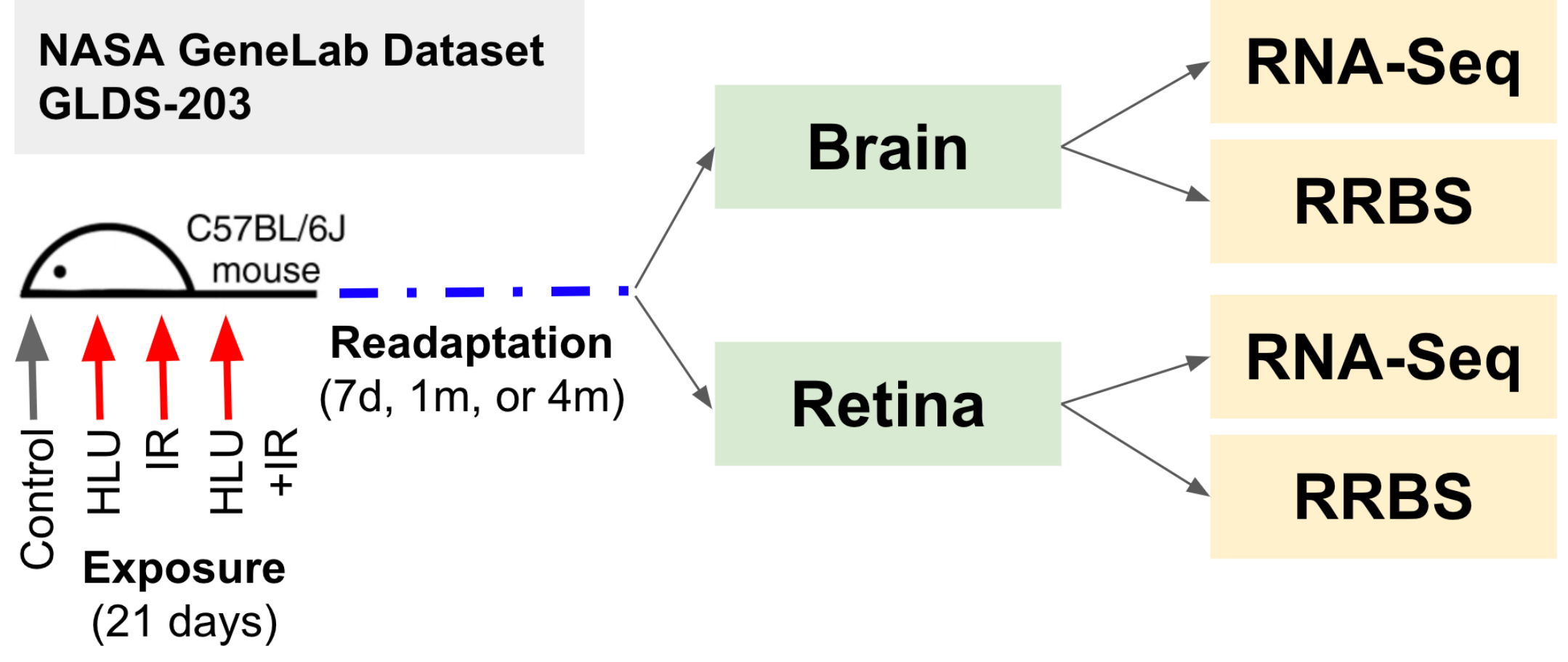
BACKGROUND

Spaceflight has been reported to have detrimental impact on ocular structure and function.

Objective: Characterize expression and methylation changes in mouse retina due to chronic low-dose radiation (IR) and/or hindlimb unloading (HLU).



Exposure	7d	1m	4m
Control	6	5	4
HLU	5	5	5
IR	4	6	5
HLU+IR	6	4	4

Table 1: Sample counts.

DIFFERENTIAL GENE EXPRESSION

- RNA-seq counts were processed and analyzed for differential gene expression with DESeq2.
- Exposure groups were compared against controls matched by timepoint. Differential expression (DE) defined as $|\log_2(\text{fold change})|>0.263$ and adjusted p-value<0.05.
- DEG in 7d IR and 1m HLU+IR enriched in ocular disease terms and in the *somatodendritic compartment*, respectively.

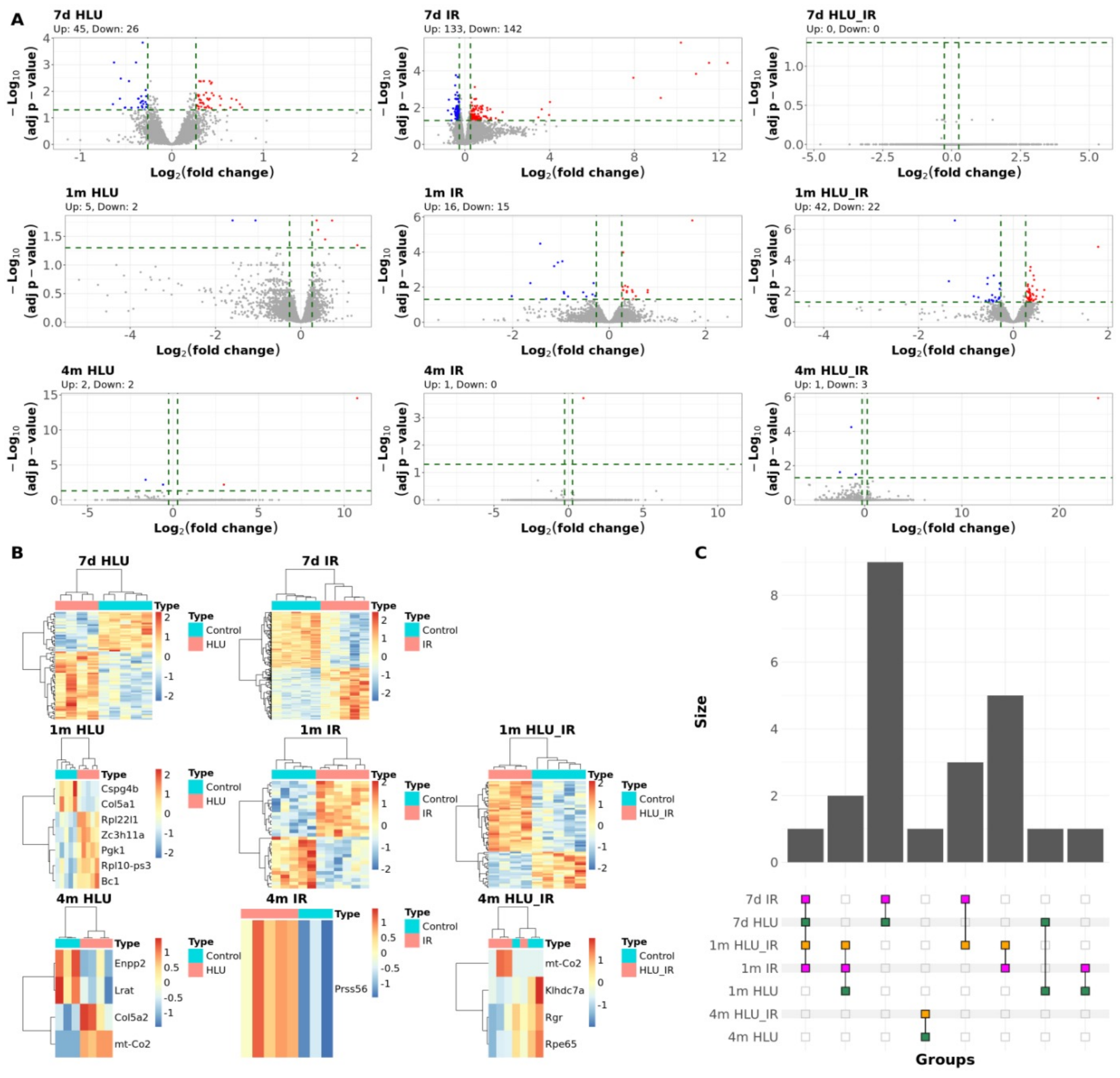


Figure 1: Differential gene expression. (A) Volcano plots showing DEG across in each exposure group at different time points. **(B)** Heatmaps with hierarchical clustering analysis of DEG in each group. **(C)** Upset plot showing DEG shared between at least two of the nine groups.

DIFFERENTIAL GENE METHYLATION

- RRBS reads were processed with a Nextflow pipeline and *MethylKit* was used for detecting diff. methylated (DM) loci, regions and genes.
- Hypermethylation persisted at 4m after IR in genes involved in eye morphogenesis, patterning and pathogenesis (*Wnt* signaling regulator *Maz, Adamts5*, *Fscn2*).
- Highest correlation in methylation difference was seen across different exposure groups at the same time point.

Figure 2: Differential methylation in exposure groups. (A) Total number of differentially methylated loci. **(B)** Pairwise correlation plots for common differentially methylated sites. **(C)** Upset plot showing overlapping differentially methylated genes.

GENES WITH DIFF. EXPRESSION AND METHYLATION

- 23 shared genes; 14 had DM in the promoter and 9 in the body.
- *Eef1a1* (involved in peptide chain elongation) was down-regulated and hypomethylated in IR-only and HLU-only at 7d.

Group	Genes with diff. expression and methylation (sign of expression $\log_2$fold-change, sign of methylation change)
7d HLU	*Cdk14* (-,+), *Eef1a1* (-,-), *Fam222a* (+,-), *Pitpnm3* (+,+), *Sipa1l3* (+,+), *Sox9* (+,-)
7d IR	*BC031181* (-,+), *Crybb3* (+,+), *Dcp1b* (+,-), *Eef1a1* (-,-), *Fgfr1* (+,-), *Flnb* (+,+), *Mbd6* (+,+), *Ncor2* (+,+), *Plec* (+,+), *Ppm1a* (-,+)
1m HLU+IR	*B4galt6* (+,-), *Kcnip3* (+,-), *Lamp5* (+,+), *Nacad* (+,+), *Rundc3a* (+,+), *Sphkap* (+,+), *Tle3* (+,+)

Table 2: Genes with statistically significant expression and methylation changes. Genes that are differentially expressed in a given group compared to timepoint-matched controls (adjusted p-val < 0.05) and contain at least one differentially methylated locus or region (adjusted p-value < 0.05) are listed.

GENE SET ENRICHMENT ANALYSIS

- *Nucleotide metabolism* enriched in all exposures (↑ at 1m, ↓ at 7d,4m); *Pdk1* (↑ at 1m, ↓ at 7d,4m; involved in metabolism of glucose & fatty acid and hypoxia response) in all gene sets
- At 4m, all shared BPs were suppressed; included *lens development, wound healing, angiogenesis.*
- *Wnt signaling, actin filament-based process* and *epithelium morphogenesis* were among 52 BP shared between DE and DM in 4m HLU+IR.

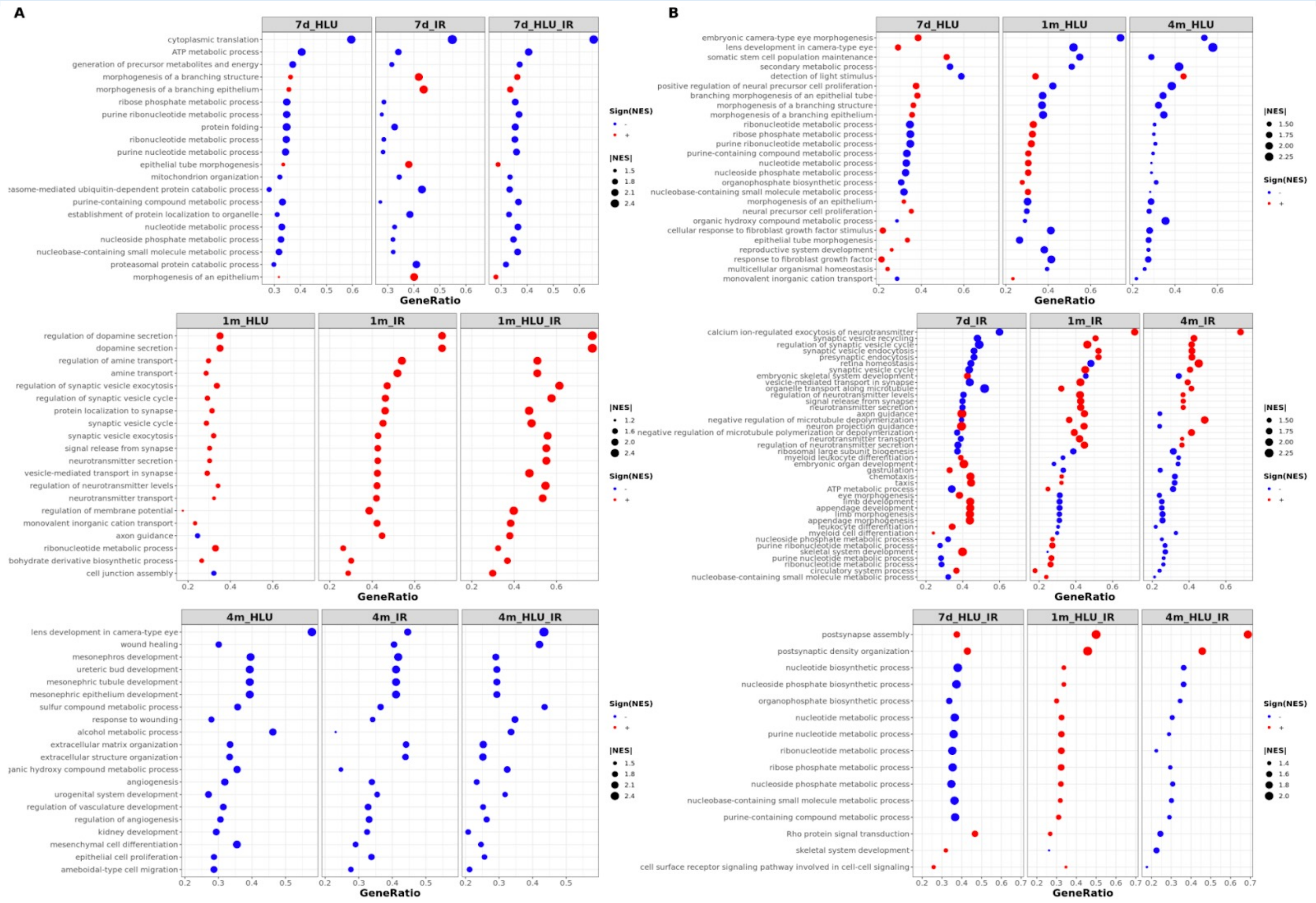


Figure 3: Common biological processes. (A) Top 20 BP common across exposure for each time point. **(B)** Top BP across all time points for each exposure.

NOTCH AND WNT SIGNALING

Gene or BP	Changes in expression or methylation; function or significance of gene
Wnt signaling	↑ in 7d IR, HLU+IR, ↓ in 1m HLU & 4m all groups
Bclaf3	methylation ↑ in 4m IR, HLU+IR; regulates proliferation/apoptosis by suppressing Wnt signaling in mouse epithelium
Tle3	expression and methylation ↑ in 1m HLU+IR; co-repressor involved in Wnt/Notch signaling
Notch3	expression ↑ in 7d IR; NASA Twins Study reports epigenetic changes in spaceflight blood samples
Rbx1, Ncor2	methylation ↑ in 4m HLU+IR, expression and methylation ↑ in 7d IR; genes in the Notch signaling pathway
Wnt3, Wnt11, Wnt7b, Lrp6, Tnik	gene set members in majority of the BP enriched in 4m HLU+IR based on diff. methylation

Table 3: Genes or BP related to *Notch* and *Wnt* signaling pathways with significant expression and/or methylation changes.

SIGNIFICANT FINDINGS AND FUTURE WORK

- Genes and BP related to *Notch/Wnt* signaling altered at 4m.
- *Nucleotide metabolism* enriched in all groups.
- Ongoing work interrogates epigenomic and transcriptomic changes in matched retina and brain samples at 4m.