

# RNA-Seq Analysis of Perturbation on the Anterior and Posterior Axis in the Developing Allotetraploid Species *Xenopus laevis*

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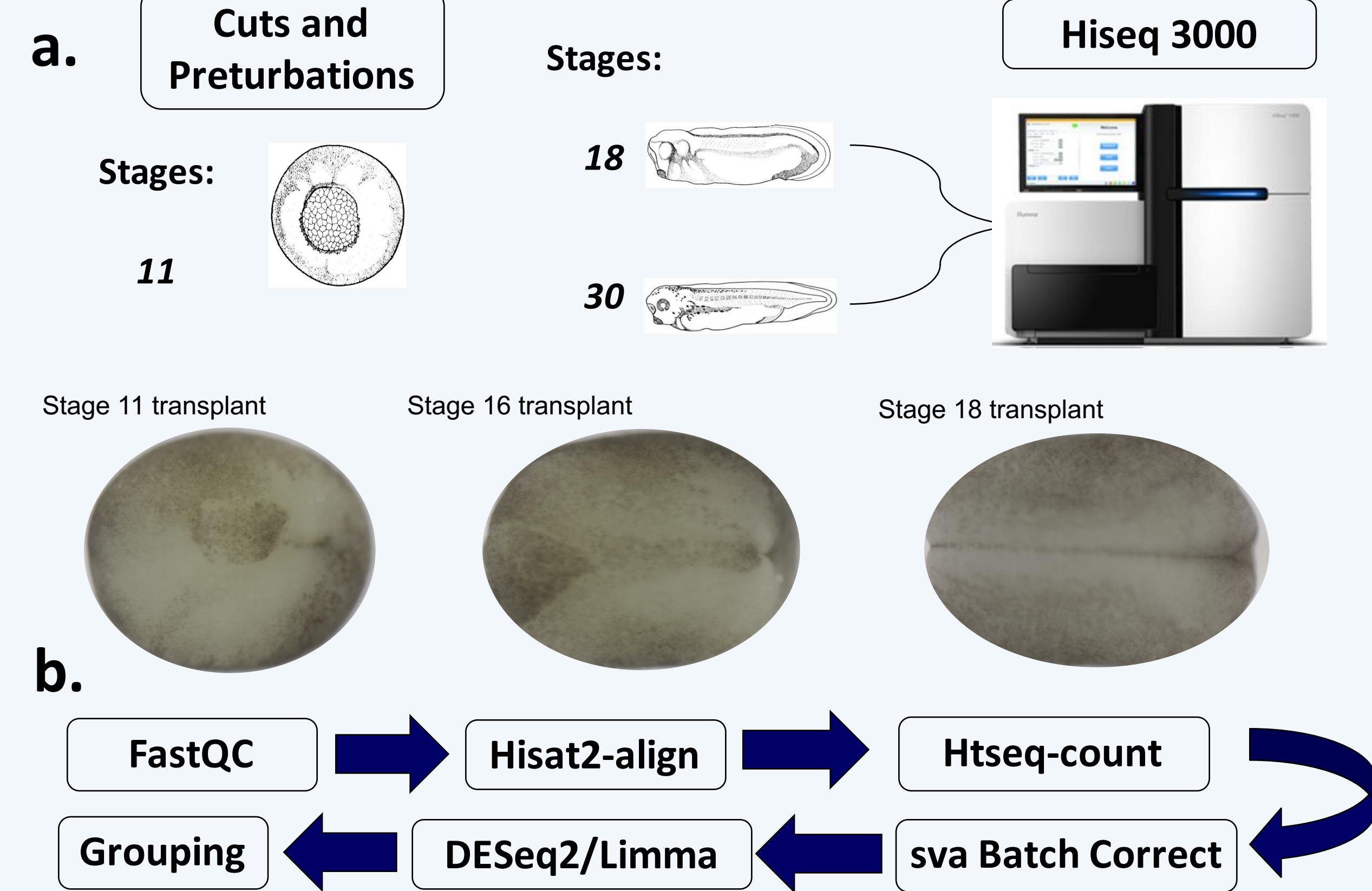
## I. Introduction

The Anterior-Posterior axis directs the orientation of embryonic development during the formation of the neural tube. Early embryonic stages allow for a heightened ability of plasticity in the event of injury. The repatterning of the A-P axis is crucial in early embryonic stages, specifically in healing and continuing healthy development of the neural tube. The results of this RNA-Seq analysis has suggested several candidate genes that contribute to the embryonic plasticity of the A-P axis in various crucial development stages. Three categories: healing, transplant, and repatterning, were used to group differentially expressed genes based on their type of perturbation. These inferential assumptions, investigated the effects of transplanting foreign tissue to the embryo, rotating the transplanted tissue, and injuring the original tissue, resulting in several candidate neurogenesis related genes that are conserved as well as some unique to each perturbation.

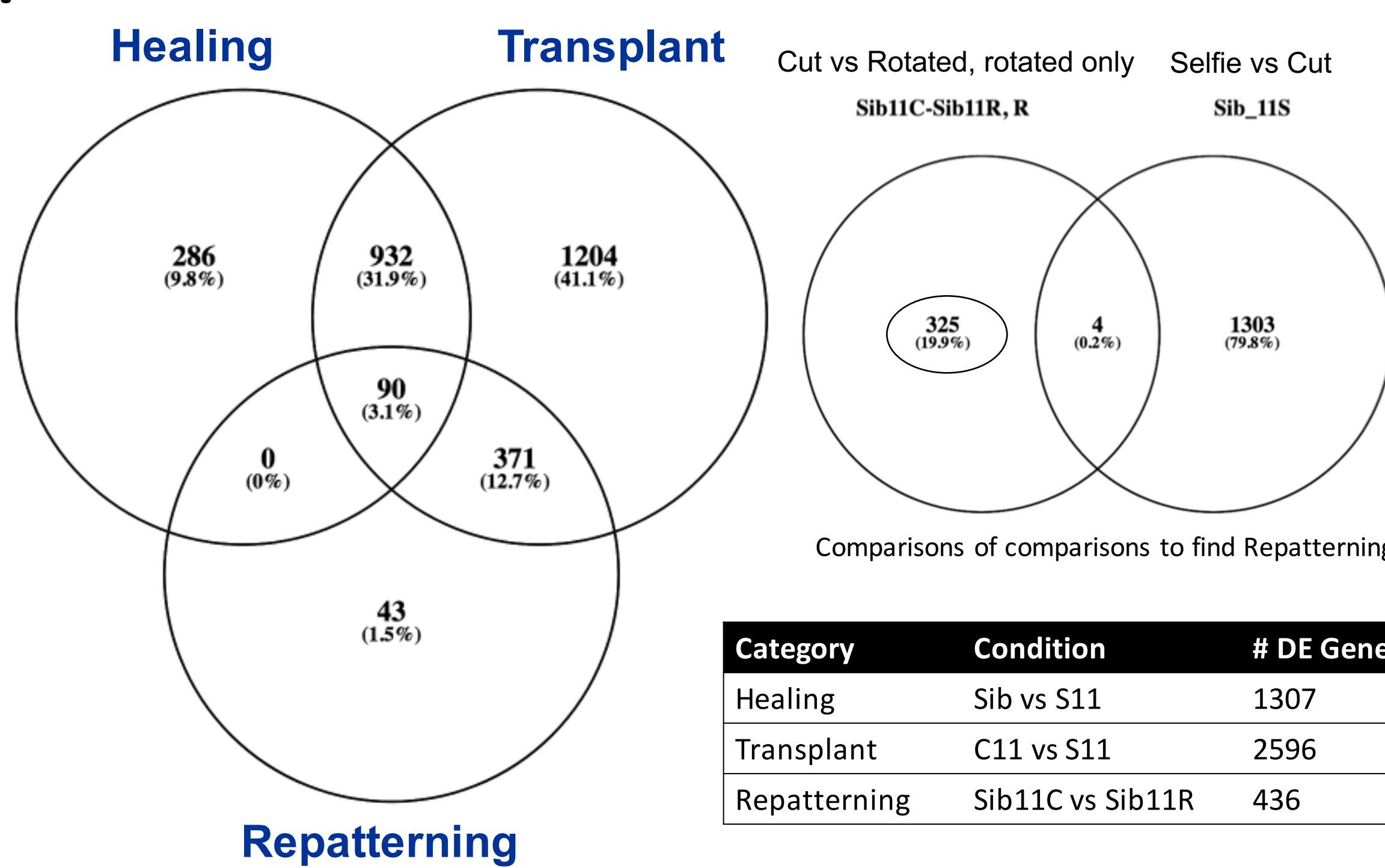
## II. Methods

RNA-Seq data was gathered from three different experiments, with three perturbations and controls. The data was prepared with polyA selection, paired-end layout, and the Illumina HiSeq 3000. The cuts and transplants were done at stage 11, and the embryos were imaged and sequenced at stages 18 and 30. Stage 10/11 is the beginning of the gastrula stage where the embryo has begun differentiation and begins the development of the anterior-posterior axis [3]. Stage 18 is in the middle of the neurula stage where neurulation occurs, the folding of the neural plate into the neural tube. Stage 30 is the late tailbud stage where neurulation is complete and tail formation begins. The cut (transplant) condition is where the tissue was removed and replaced with foreign tissue from a new embryo. The Selfie condition was when the tissue was cut out but replaced on the same embryo. The Rotated condition was foreign tissue transplanted but rotated to change the axis. All raw reads are processed through the pipeline in 1b. Read data was aligned to a reference genome and quantified into read counts with RNA-Seq programs. All read counts were clustered to determine unwanted variation, later removed through batch effect removal and filtered to have a mean of 5 counts for each gene. Differential expression between controls and the conditions was done by comparing expression in the various conditions and stages. Batch correction with sva to remove effects between Rotations and Selfies data as they are from two separate conducted experiments.

### 1.

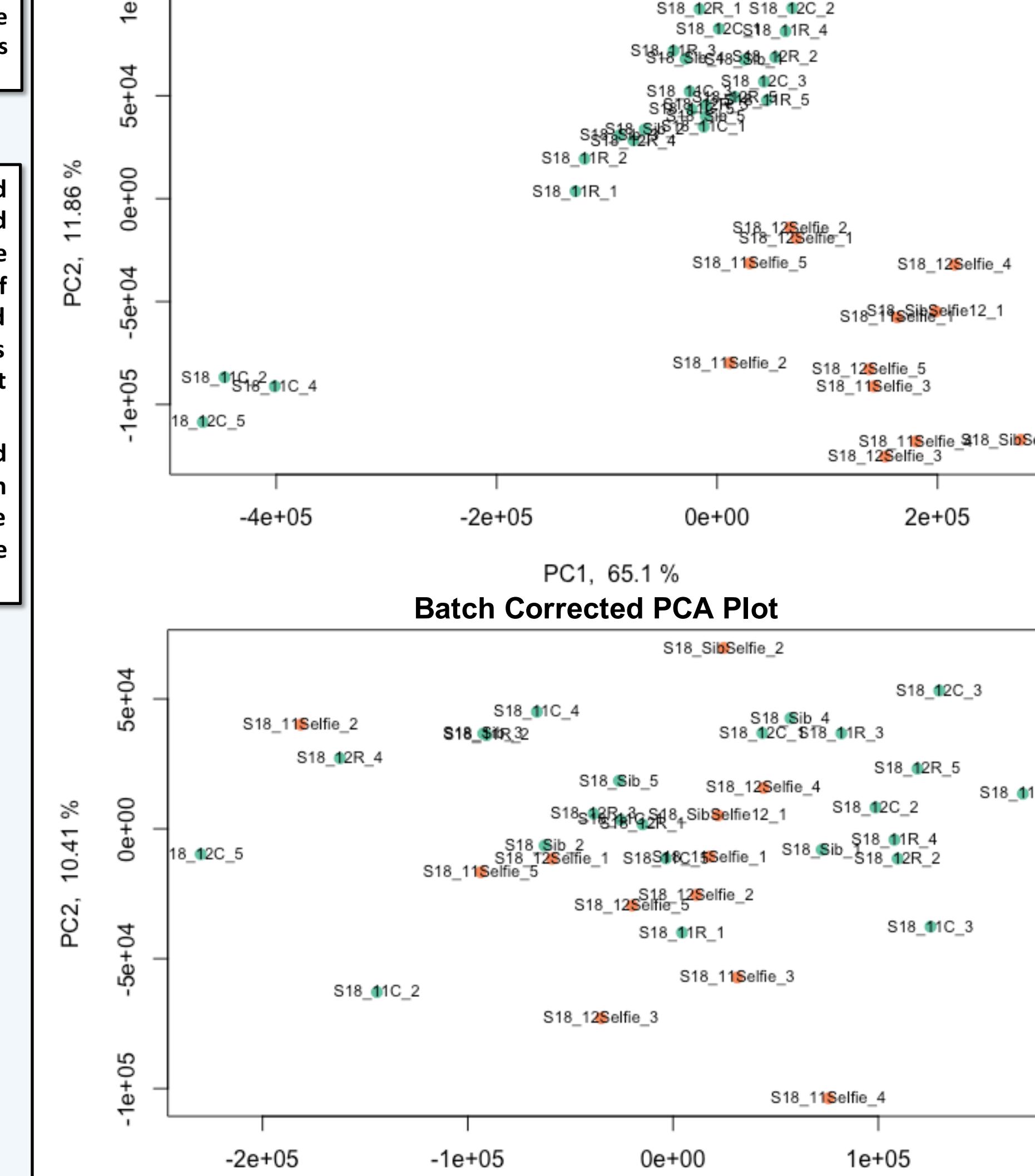


## 2. Differentially Expressed Genes in 3 Experiments Conservation



## III. Results

### 3. Clustering of Batch Corrected Data with Siblings and Conditions



### 4. Unique Genes to the Gene Groups

Healing Specific	GO Enrichment	Log2fold change
yeats4	Cellular/developmental pro.	0.307400912
akna	Cell projection organization	0.85762617
odf3	Forebrain/head developm.	0.647935759

Transplant Specific	GO Enrichment	Log2fold change
yap1	cellular resp. to DNA damage stim.	0.208805378
Xelaev18039104m	Reg. of establishment of cell polarity	0.187524981
id3	somitogenesis	0.149684125

Repatterning Specific	GO Enrichment	Log2 fold change
foxn4	Cell fate commitment	0.67753328
sox8	epithelium development	-0.388583069
spdef	embryonic morphogenesis	0.643610531

Figure 4: Several unique genes for each of the three gene groups, with their specific GO enrichment related term. Log2 fold is the regulation of the gene within the cell, this is an average of all of the replicates, a positive value signifies upregulation while a negative value means downregulation.

### Shared Genes between Gene Groups

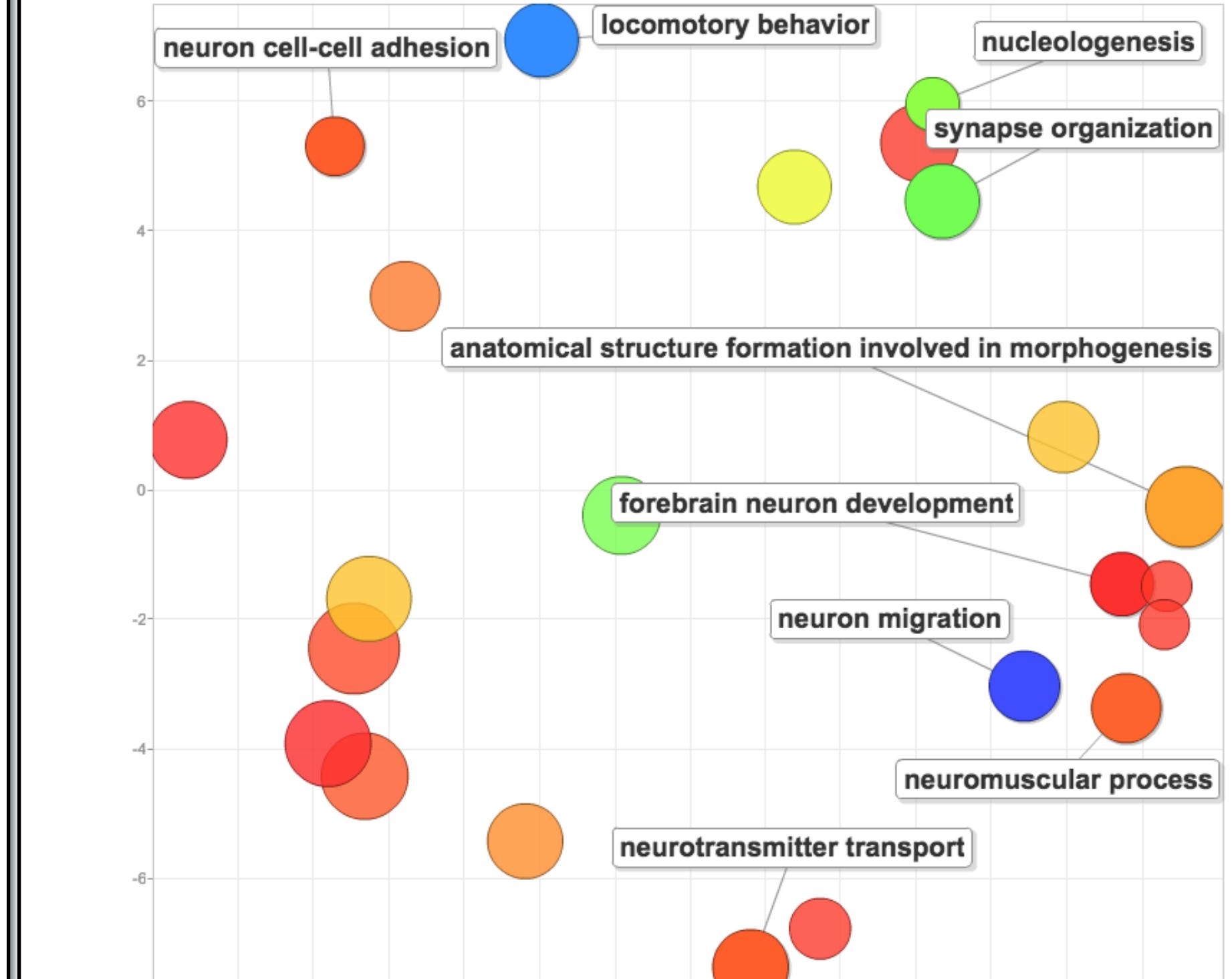
Shared Healing & Transplant	GO Enrichment	Log2 fold change
fdps	Biosynthetic membrane pro.	3.115791112
sod1	Neuron cellular homeostasis	-1.407961629
rarres1	Neg. reg. of cell population	-1.636185581

Shared Transplant & Repatterning	GO Enrichment	Log2 fold change
heatr1	rRNA from transcript	-0.357205867
znf703	Hindbrain development	-0.370436093
alpl	Resp. to mechanical stimul.	-2.416010771

Shared Amongst All 3	GO Enrichment	Log2 fold change
vsx1	Negative reg. of transcription	2.578479196
cdh20	Calcium ion binding	0.739433067
six6	Ectoderm differentiation	1.741598997

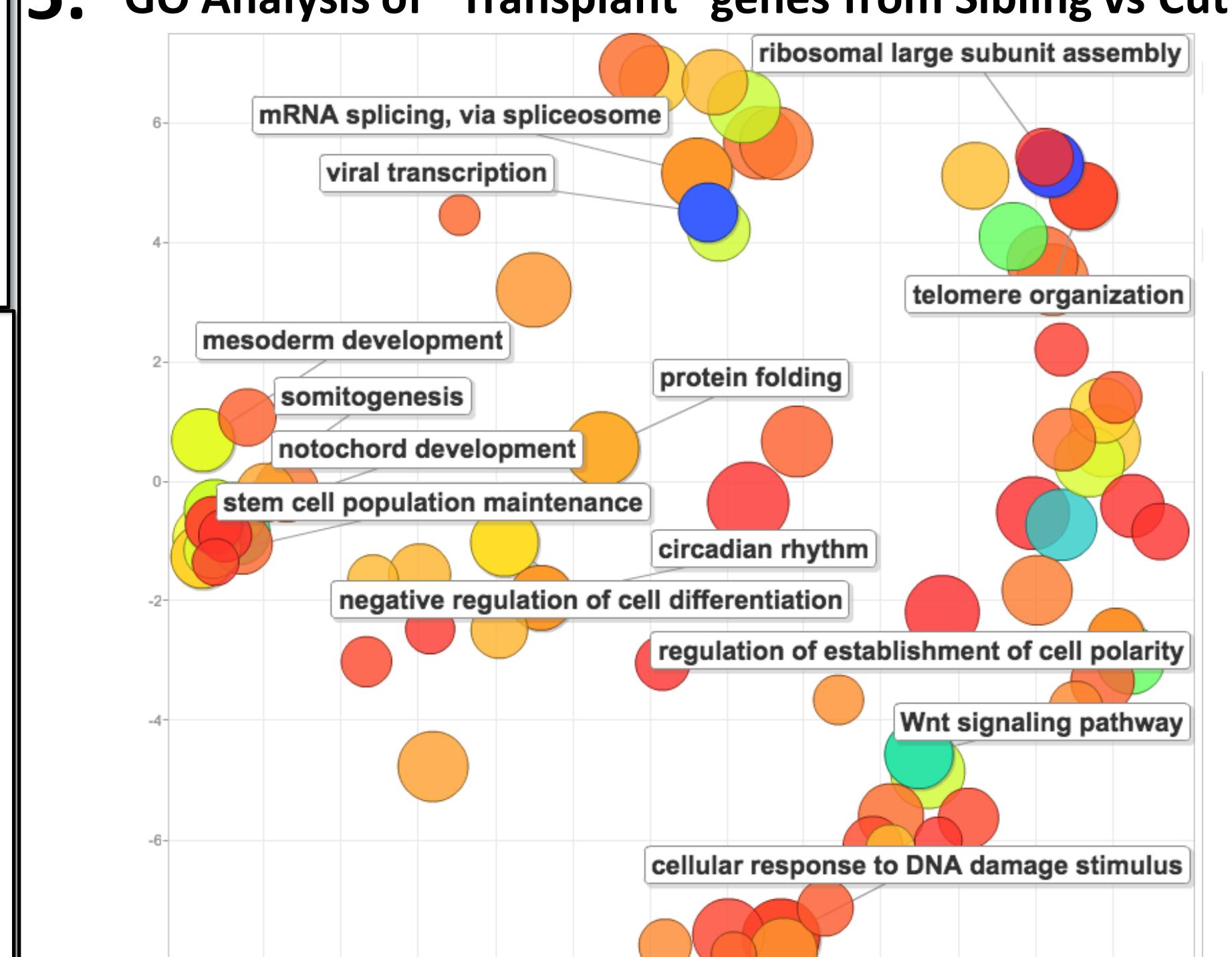
## III. Results

### 6. GO Analysis of "Healing" genes from Sibling vs Selfie



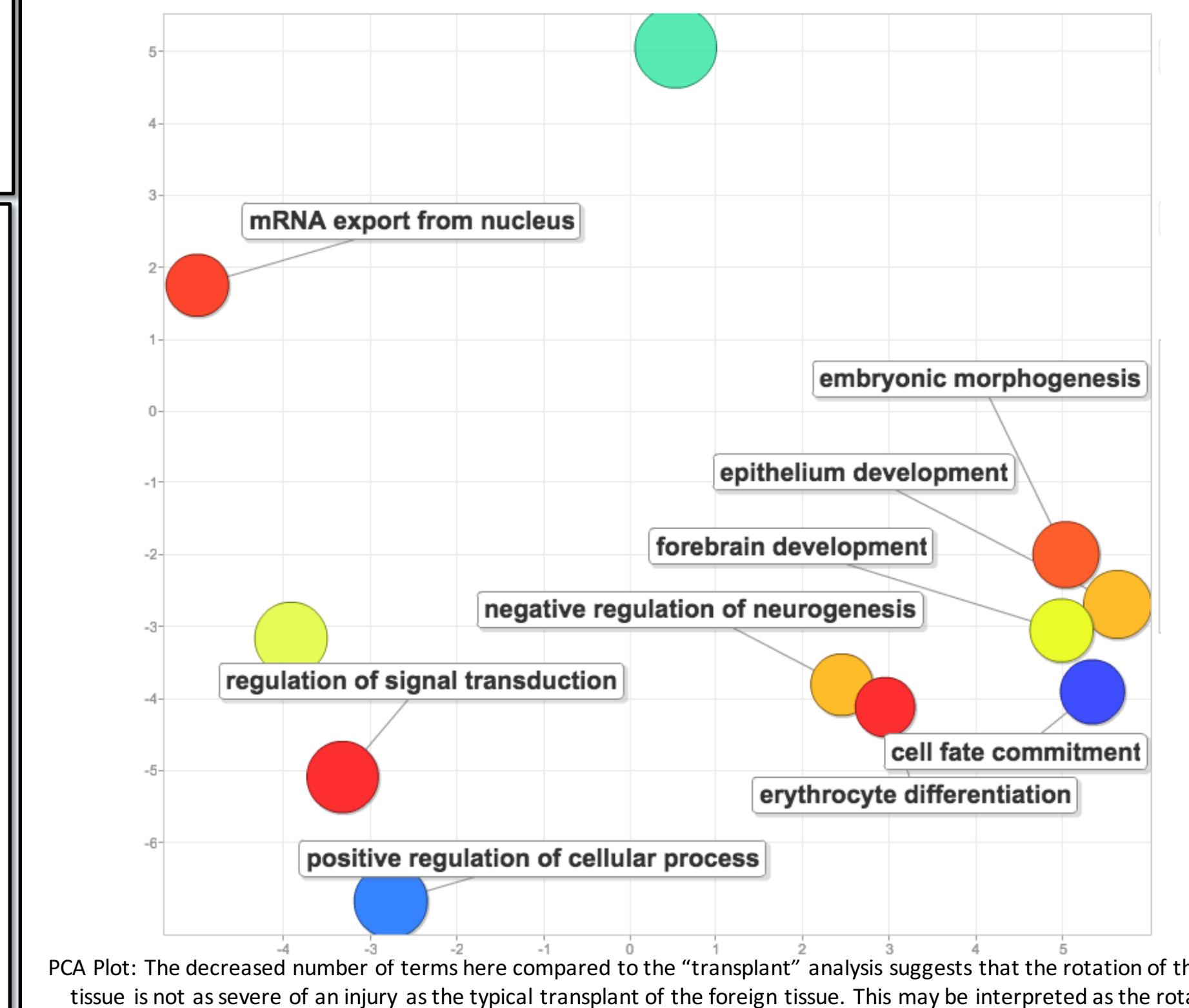
PCA: The majority of the functions are related to basic embryonic development such as neuron cell-cell adhesion or migration. Healing follows the typical pattern of growth and utilizes cell processes that undergo during healthy development without perturbation. Morphogenesis is interesting as it suggests the construction of the neural tube. At this early stage (18), an injury is likely simple to repair as other structures are building and the presence of continual checks and repairs.

### 5. GO Analysis of "Transplant" genes from Sibling vs Cut



PCA Plot: There are significantly larger amount of specific gene functions compared to typical development and healing. DNA damage stimulus is notable as a large cluster and likely due to the more severe nature of the injury, regulation of establishment of cell polarity signals A-P axis related repair and basic repatterning of the new foreign tissue.

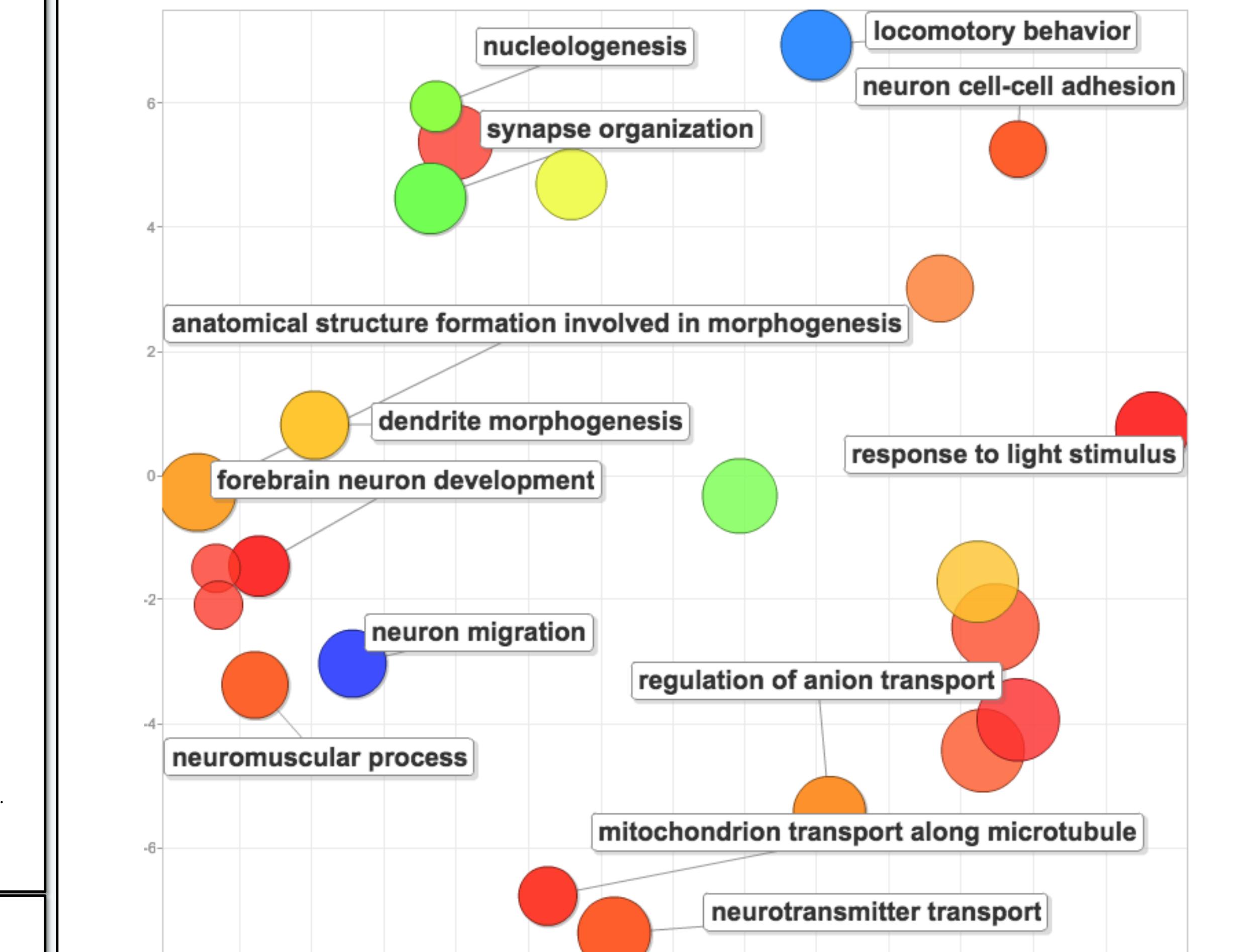
### GO Analysis of "Repatterning" genes from Sibling vs Rotated



PCA Plot: The decreased number of terms here compared to the "transplant" analysis suggests that the rotation of the new tissue is not as severe of an injury as the typical transplant of the foreign tissue. This may be interpreted as the rotated tissue being more foreign and requiring mechanisms to essentially "start over" and rebuild with typical processes.

## III. Results

### 7. GO Analysis of "Transplant" genes in a later stage (30)



GO Enrichment: In later development, GO enrichment shows that there are a different amount of GO terms related with transplant genes in a later stage (30). Rather than the neurogenesis and cellular repair response categories, there are more typical neuronal development terms. This suggests that most repair and healing is complete by this stage and that most transplant significant genes are growth related rather than transplant specific, there are also fewer unique to transplant genes at this point.

## IV. Conclusions

- 1) Healing genes are more consistent with typical growth genes expressed during normal development, the injury is less severe and can be repaired easily
- 2) Transplant genes are more focused around repairing damage to DNA, neurogenesis, and fixing the A-P axis orientation, the introduction of new foreign tissue is a massive injury and requires more resources to repair.
- 3) Batch correcting using ~batch+condition accounts for both the batch effects and the independence of each variable (the three categories), using the Siblings or controls as the base experiment, the batch effects can be removed and result in more meaningful data.
- 4) The Repatterning group has a decreased number of DE genes and GO terms associated with the injury. This is likely due to the method of which this comparison was made, a comparison of comparisons, in order to get a list of genes strictly repatterning, without transplant. The purpose of this group is to see what genes are involved in realigning the A-P axis rather than just repairing the foreign tissue.
- 5) Combining Transplant and Repatterning genes results in a list that depicts what occurs in the rotated transplant situation, the focal point of the original A-P experiment. This also results in a larger list of candidate genes which could be further investigated.

## V. Future Direction

- 1) Gene enrichment analysis to determine function and common motifs in conserved and unique genes beyond GO analysis
- 2) Investigating specific neurogenesis genes conserved in all perturbations, they are likely crucial to neurogenesis development rather than neural axis plasticity. Including genes unique to each category,
- 3) Observing A-P related perturbations within *X. borealis* (tetraploid) RNA-Seq data, another species from the same subgenus as *X. laevis*, as well as *X. andrei*, an octoploid species
- 4) Homeologs in the conserved and unique genes to each category, L/S pairs presence, up or downregulation of specific homeologs in differentially expressed genes
- 5) Pooling data of other A-P axis related perturbations and comparing with our transplant perturbations.

## References

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