Supplementary material; Figures, Legends

Structural and molecular distinctions of primary and secondary spines in the sea urchin *Lytechinus variegatus*

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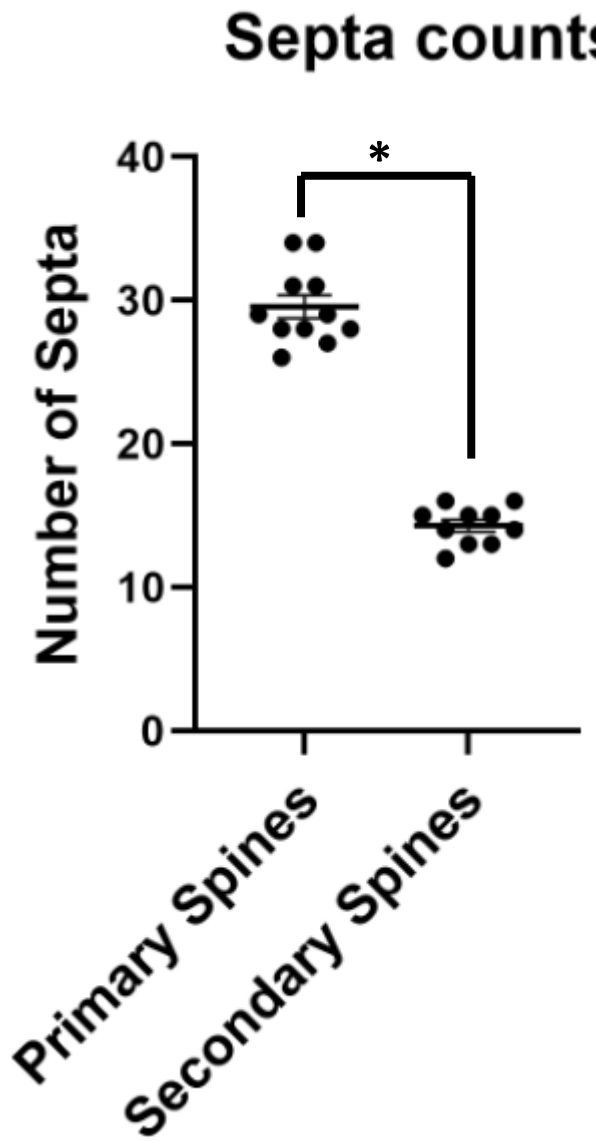
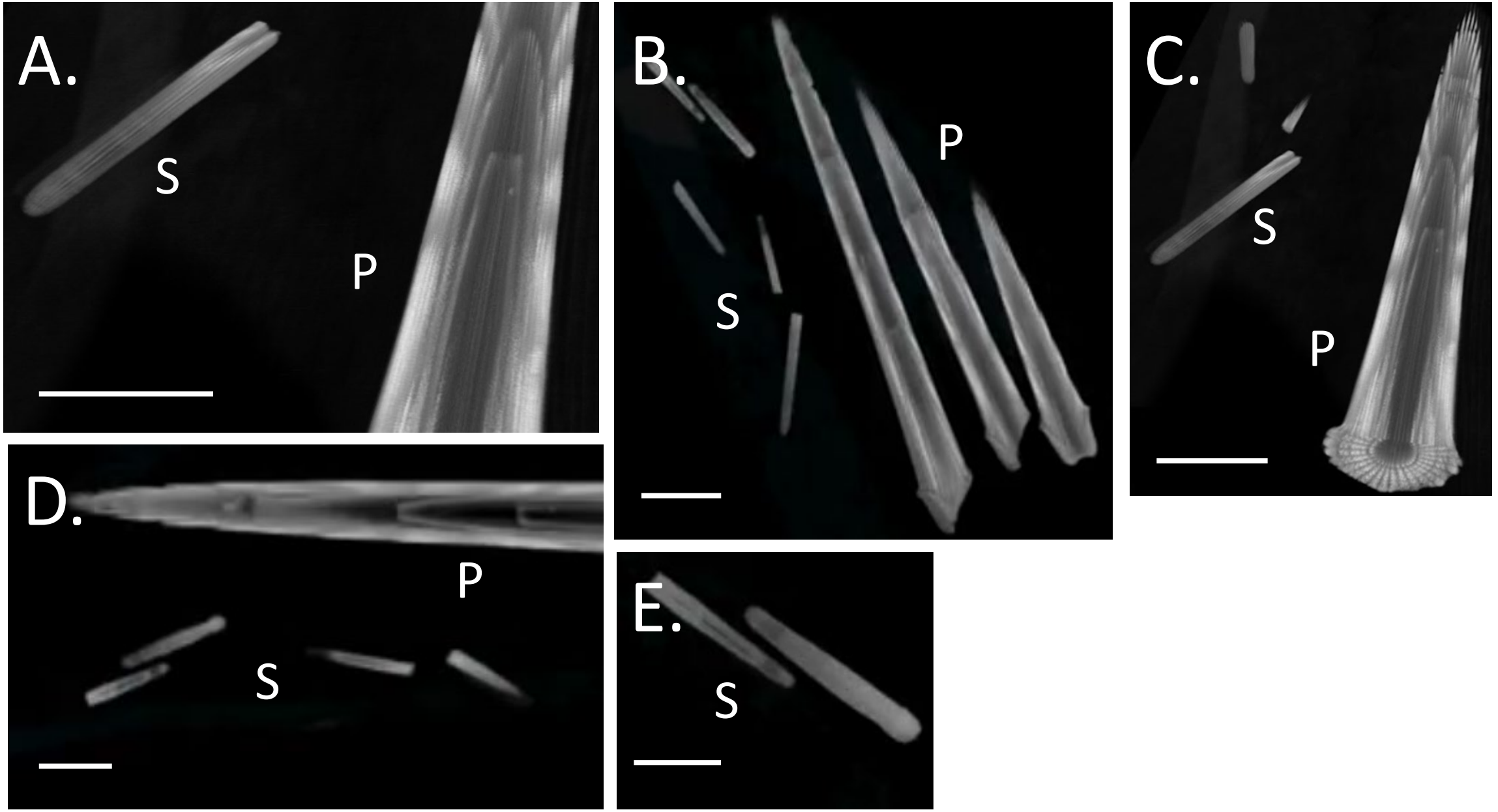
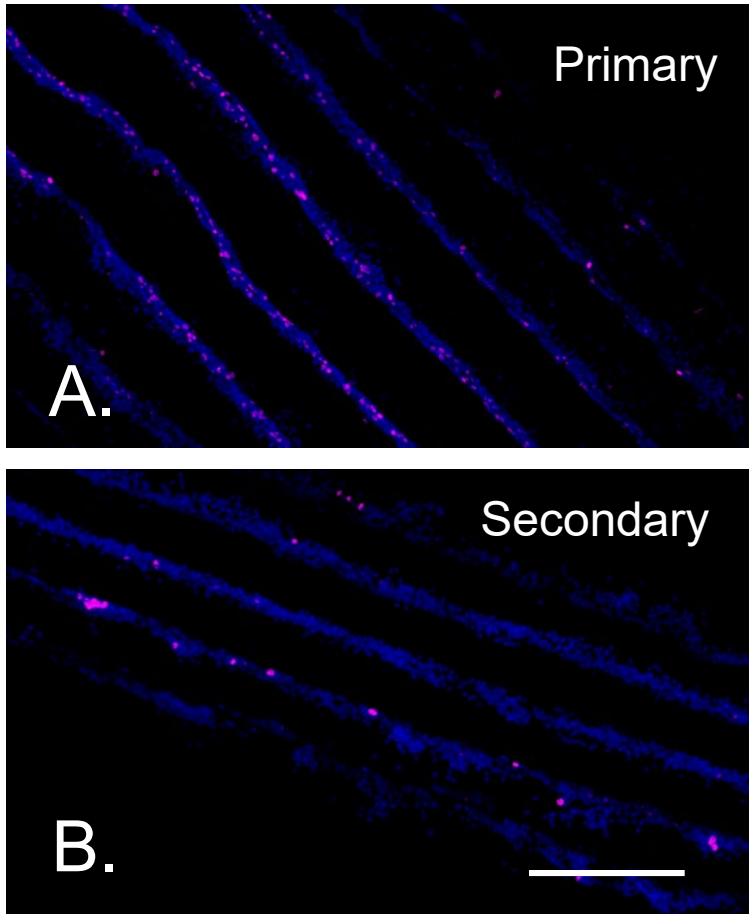
Hebert et al., Supplemental Figure 1

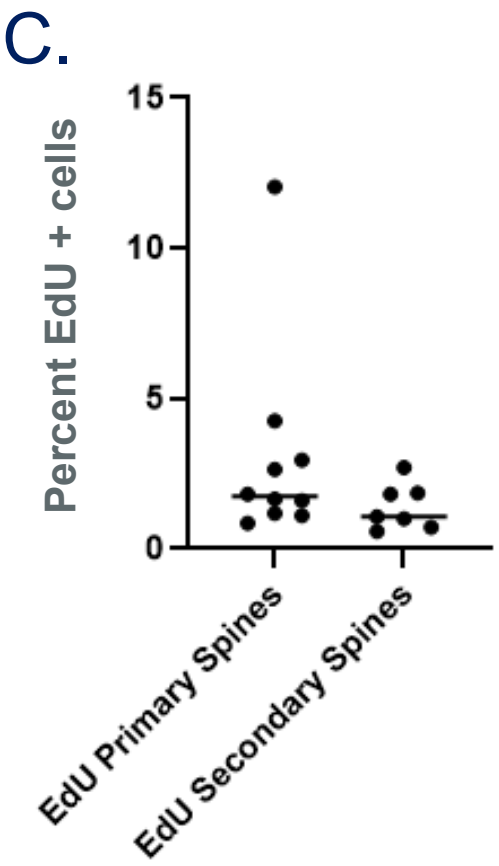


Hebert et al., Supplemental Figure 2

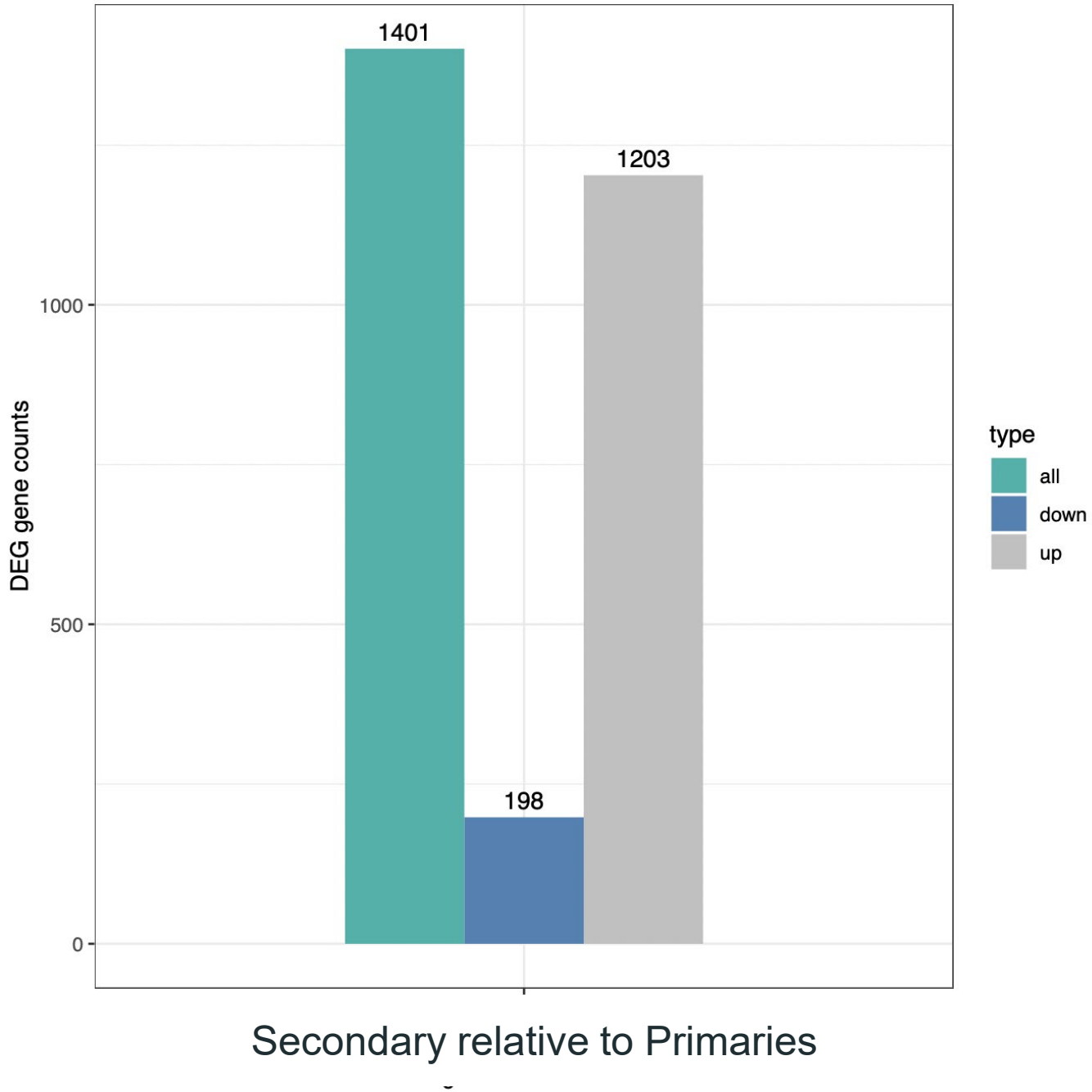


Hebert et al., Supplemental Figure 3

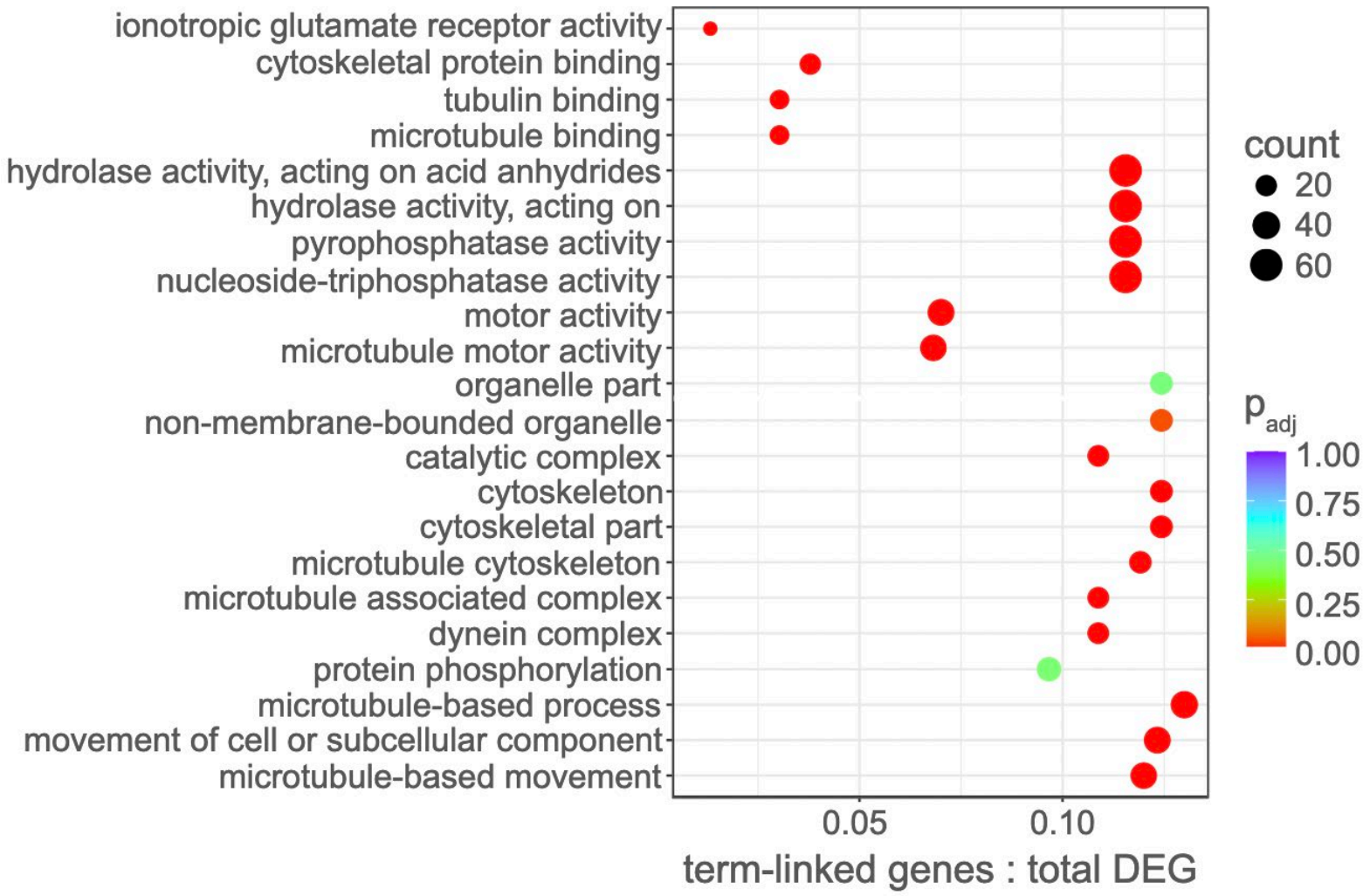




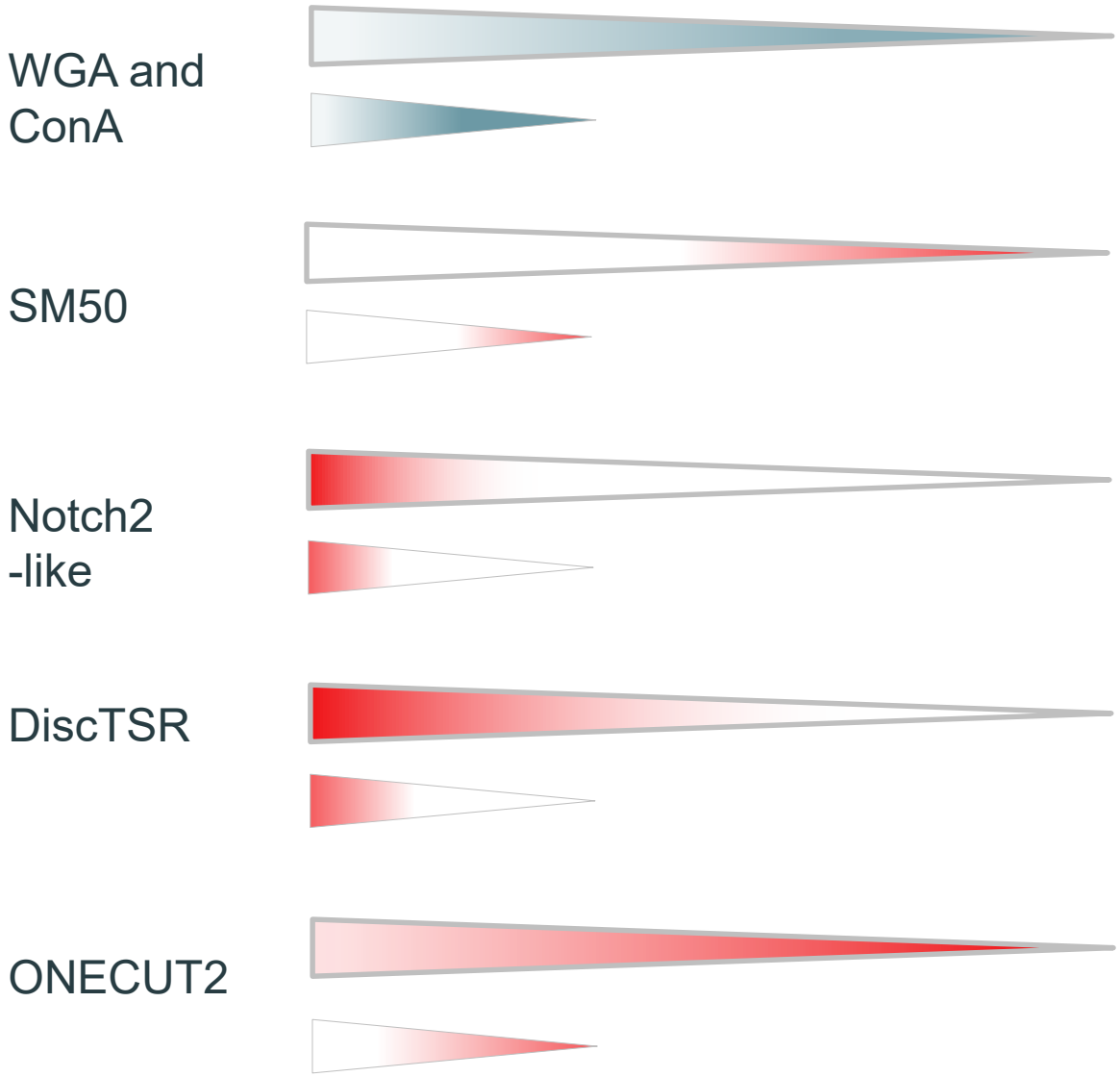
Hebert et al.,
Supplemental Figure 4



Hebert et al., Supplemental Figure 5



Hebert et al.,
Supplemental Figure 6



Hebert et al.,
Supplementary Figure Legends

SFig 1. Septa were counted from 11 primary spines and 10 secondary spines. The data were analyzed by an unpaired, two-tailed t-test and plotted as a box plot of individuals with the mean given as a line in the cell clusters. The mean of the primary septa was 29.55, and of the secondary septa was 14.30. The asterisk in the graph is indicative of a P value of <0.0001.

SFig 2. Additional images of secondary spines to test for growth rings – see primary spines (P). None are seen in secondary spines. Note that spines in B. and D. are from the same set of spines, but at different Z-planes. Bar = 1 mM

SFig 3. EdU incorporation was used to test for replicating cells in the primary (A) and secondary (B) spines. Pulsing spines for 3 hours cultured in vitro resulted in significant EdU labeling averaging 1 - 4% of the cells per hour (C). Most of the EdU label appears to be within the spine stereom but was also found within the epithelium of the spines. The data were analyzed by an unpaired, two-tailed t-test and plotted as a box plot of individuals with the mean given as a line in the cell clusters. The mean of the primary spines was 3.01, and of the secondary septa was 1.39. The P-value of confidence distinguishing between these groups was 0.23, and thus we conclude that there is no significant difference in the percent of cycling cells in primary and secondary spines. Bar is B = 100 microns.

SFig.4. Most of the differentially expressed genes (1401) are enriched in secondaries (1203) and depleted in primaries (198).

SFig 5. Top 22 significant GO terms displayed by gene ratio, absolute gene count, and significance in functional analysis.

SFig 6. Summary of gene expression in spines. Proximal is to the left and distal is to the right. Primary spines are larger than secondary spines. Green signal resulted from lectin staining, whereas red signal is from FISH.

Primary Spines (Group 1) vs Secondary Spines (Group2)

See attached deg.xls “SSvsPS_deg_all”

Column 1	gene id
Column 2	sample normalized readcounts of all samples
Column 3	group average normalized readcount of group
Column 4	log2FoldChange the result performed by DESeq2 R/EdgeR R package.
Column 5	pvalue, p-value in statistical tests.
Column 6	padj adjusted p-value. The smaller the p-adjusted is, the more significance to the differentially expressed genes
Column 7	gene name
Column 8	name of chromosome in which the gene is located
Column 9	the start position of the gene at the chromosome
Column 10	the end position of the gene at the chromosome
Column 11	gene strand, the chain information of the gene
Column 12	gene length
Column 13	gene type
Column 14	gene function description
Column 15	the family name of the gene
Column 16*	the raw readcount of samples
Column 17*	the fpkm of each samples

*indicates groups of collumns