**Reviewer 1**

**Comment**: S4-6, the units for the y-axis should be provided. Counts or FPKM? Are they transformed with log2 or log10?

**Response**: Normalised values are in counts, and are not log transformed.

**Comment**: The brief description should be provided for the table S16. For example, In "Figure\_S16\_Unmapped-reads\_assignment\_charts", what are the boxes and what do different colors represent? (add legend in the figure as to what the degree of green color represent) Why are there multiple boxes? Also, relationships between labels and boxes are not so clear (for example, which boxes are associated with Eukaryota and with Bilateria)? In short, tell readers how to parse this diagram.

**Response**: A short description has been added to Figure S16 to elaborate the diagram.

**Comment**: L228, L237: Clearly indicate whether normalised fold change is log2 or log10

**Response**: To my knowledge, the CLCGW normalisation suite does not log2 or log10 transform the count data during normalisation. Normalised values used for subsequent analyses were untransformed and directly used for further analyses.

**Comment**: Table\_S3\_Differentially\_expressed\_genes:

According to the authors, PRB3 is thymus-specific, but in the fold change is reported to be 2201.25 in "Spleen vs. Thymus" sheet whereas -2114.04 in "Lymph vs. Thymus". It seems that the third sheet should have been labeled as "Thymus vs. Spleen" instead of "Spleen vs. Thymus". Furthermore, I suggest that "Normalised fold change" column be clearly labeled so as to indicate the direction of the fold change.

**Response**: Labelling of tissue comparisons were amended in the main text and Table S3 to reflect fold change directionality, which involves “Spleen vs. Lymph”, and “Thymus vs. Lymph” tissue comparisons. Labelling of “Normalised fold change” tabs in Table S3 were also amended to reflect fold change directionality.

**Comment**: RNA-Seq Normalised Means (as in Table\_S5\_Comparison\_with\_Peng\_et\_al) should be reported for all genes as an additional supplementary table.

**Response**: While I am aware that it is pertinent to report a comparison for all genes, it is unfortunately presently beyond our technical knowledge and capabilities to perform a transcriptome-wide comparison of our data with that of Peng et al. (and to a certain degree, time-consuming to tabulate manually). Hence we made a preliminary comparison of the gene expression of tissue-specific genes. In the future, we hope to perform a more robust comparison.

**Comment**: CLEC4G, SHISA3, and CCL20 are reported to be lymph node-specific. Question is, why aren't CLEC4G and SHISA3 reported in "Lymph vs. Thymus" sheet in Table S3? and CCL20 in "Lymph vs. Spleen" sheet? Similarly, NKX2-3 is reported to be spleen-specific but isn't reported in "Spleen vs. Thymus" sheet in Table S3. Indeed, those genes have a mean expression level equal to 0 in thymus tissues (as reported in Table\_S5) and I speculate that those genes were filtered out because their log fold change values are infinity. However, authors should process their data so that those genes are included in their differential gene expression analysis list so that it is consistent with results of the tissue-specific analysis.

**Response**: Indeed, while performing the DEG analysis, genes with normalised fold change values infinite and negative infinite were filtered out. They have now been added back to Table S3.

**Comments for the Author**:

- L613: completeness -> incompleteness

- L622: ofnine -> of nine

- Table S3: what is the unit of Normalised fold change? log2 or log10? Indicate it on the column label.

- L190: normalized -> normalised (for consistency)

- L242, 245: ver. -> version (to be consistent with the usage of version in L238)

- L88: Insert a space before "High throughput..."

- L189: Insert a space before "The final ..."

- L244: Insert a space before "KEGG pathway ..."

- L318: Insert a space before "In Lymph vs. Spleen ..."

- L322: Insert a space before "Table S3 ..."

- L389: Insert a space before "The TNF ..."

- L623: Insert a space before "An alternative ..."

- L625: Insert a space before "Peng et al ..."

**Response**: Corrections for the above comments have been performed as suggested.

**Reviewer 2**

**Comment**: L160-165 - As indicated in the rebuttal add a comment relating to the animals being free of disease upon visual examination and members appeared to be of the same family group.

**Response**: The suggested statements were added into the text.

**Comment**: Thymus M2 and M5 are particularly weak with 1/3 to 1/6 of the reads mapped as compared to other samples. While I do not consider this a risk to the observations made during the aggregate analysis it does lower the resolution as observed in both the MCA and various gene expression data. Adding more sequence data would significantly raise the value of the data set to the research community. While I strongly recommend adding sequence data to these samples I do not consider it a bar to publishing this paper.

**Response**: We whole-heartedly agree with the additional sequencing suggestion, however at this stage of our research, the RNA-seq library was constructed and sequenced nearly 2.5 years ago, and to perform additional sequencing of the same library perhaps may not provide additional data of value, due to the age of the library samples. In addition, budgetary concerns also hinders us from performing any additional sequencing runs on the samples.