**Ligand receptor analysis**

We examined the expression of the ligands. We developed a tool to establish the strength of the expression. We compute the 30 percentile and the 80 percentile of all expression values at all time points. These percentiles were arbitrarily set as the thresholds for low/medium and medium/high respectively.

For any given gene, we assigned the high expression label if the expression of at least 20% of the time points is above the medium/high threshold. Similarly, if the expression of 80% of the time points were under the low/medium threshold we assigned to the gene the low class. All other cases were labeled as medium.

This approach is meant to establish the genes that in at least one time point have very high expression so likely they should be involved in regeneration.

We analyze manually the possible combination of expression level to create a database stored in the file “matrix-with-signal-analysis.xlsx”.

In the different tabs there are the most interesting signals (for me) and the cell involved.

The last tab is the complete list of ligands and receptors.

I made an attempt of analyzing the paracrine-fap signals (please see relative tab).

Basically I look if the pathways that contain the EC-expressed receptors are activated or perturbed. As you can see from the notes in the spreadsheet, this happens.

**Attempt to find downstram effector of the receptors.**

Using graphite (R package) we extracted all the edges per pathway. The edges are formatted as follows: pathway, source, destination, directed/undirected, process type.

From this database of edges, we kept those entries that had as source a receptor according to the database of Rezza et al. Thus, this database is made by the first neighbors of the receptors but only in the downstream direction.

In this shrinked database, ‘process type = binding’ correspond to “undirected” so we drop the information about directed/undirected edge building a new database with the following fields:

source, destination, process type, pathway.

Pathway annotations are redundant at this point: we eliminated these redundancies and obtained 3562 relations between receptor and putative downstream effectors.

Next we examined the process types. Process types of our putative downstream effectors are organized as follows:

|  |  |
| --- | --- |
| Process(activation) | 1653 |
| Process(indirect effect) | 516 |
| Process(indirect) | 368 |
| Process(binding/association) | 362 |
| Binding (undirected) | 325 |
| Process(phosphorylation) | 170 |
| Process(inhibition) | 120 |
| Process(expression) | 27 |
| Process(missing interaction) | 16 |
| Process | 3 |
| Process(dissociation) | 1 |
| Process(repression) | 1 |

To keep analysis as simple as possible we focus our attention only in process activation. We are going to examine this downstream effector to evaluate the activation of the receptor.

Here I partially stop. I tried many ways but nothing satisfied me.