

**Dr Groppa\_14 June 2016**

**Subject: FR52 Summary project**

**Aim:** to profile the different cell subsets contributing to skeletal muscle regeneration in order to identify novel paracrine signaling pathways that might be harnessed to rescue muscle related disorders, such as muscle dystrophies.

**Short explanation of the experimental plan:**

1) *cell subsets analyzed:* endothelial cells, fibre/adipogenic mesenchymal stem/progenitor cells (FAP), muscle progenitors and inflammatory cells were sorted from skeletal muscle after acute damage induced with notexin, a myotoxin that acts on terminally differentiated myofibers;

2) *time points:* cells were collected from skeletal muscle at 1,2,3,4,5,6,7,10, and 14 days after notexin intramuscular injection or from undamaged tissue. At each time point, the four populations were purified with Influx sorter taking advantage of specific staining and reporter transgenic mouse. Most of the time, no replicates have been performed.

The gating strategy was based on the following staining:

CD31 APC

VCAM PE

CD45 Alexa700

Alpha7 APC Cy7

PDGFRa EGFP (reporter mouse)

The file “EXAMPLE” shows the gating strategy performed at the sorter facility (LSC) with Andy and Justin.

3) *mouse models:* wild type mouse was used to study muscle regeneration, and CCR2KO to model acute fibrosis after injection of notexin

4) *sequencing:* after cell isolation, RNA is extracted and cDNA libraries are generated from mRNA. Sequencing is performed with Illumina NextSeq500. Using TopHat and Cufflinks, gene and transcript lists in FPKM are generated.

5) *contribution to this project:* Dr Groppa defined the details of the project with the supervision of Dr Rossi, did the experiments, collected, stored, and analyzed the data; Lin Yi helped Dr Groppa for the sample sorting and RNA extraction. Ryan Vancer Werff did the sequencing. Dr Paolo Martini (from University of Padua, Italy) has helped and is currently helping with the data analysis taking advantage of cutting edge tools for time course data analysis.