Hi there,

Thanks for optimising the lentivirus infection conditions! It's clear that we should be using 5 MOI going forward.

With NT3 treatment that also gave improvement in your analysis, I still had some questions though. As you know, MAP2 that was checked in the optimisation experiment is a marker of mature neurons that is not specific to sensory neurons, let alone to their nociceptor subset that we are trying to generate. So, I thought we needed to dig a bit deeper.

Using 5 MOI of the NGN2 virus with and without NT3, I've now repeated the protocol but additionally used a lentivirus that expresses the GFP reporter gene driven by the MAP2 promoter. I've got smaller proportions of GFP+ cells than the proportions of MAP2-expressing cells you observed in your optimisation experiment (~8% and ~5% with and without NT3, respectively). This is expected, however, because I had to use an additional lentivirus to generate the MAP2-GFP label, so it may not have worked in every MAP2-positive cell.

Using these cells, I then checked the expression of TRPA that specifically marks nociceptor neurons. I used immunostaining with APC-conjugated anti-TRPA antibodies for this. Please see the plot below. (Note that 100% corresponds to all MAP2-GFP-positive cells, not all cells overall.)

I also used the Fura2-AM dye to profile the Ca2+ response of MAP2-GFP+ cells to mustard oil, which is a feature of nociceptor neurons. This is our first functional test in these cells! It turns out that we have quite a bit of responding cells, but more without NT3 than with – see second plot below. (Again, 100% corresponds to all MAP2-GFP-positive cells.)

We should discuss this properly soon (we'll have a good chance for this at your presentation next week), but in the meantime – any thoughts on whether using NT3 is a good idea going forward?

Kind regards,
Ela