**Editorial comments:**

1. Formatting:

-Please change the title to reflect the method rather than an experimental result.

Done.

-Please re-write the short abstract to address the methods presented rather than the results.

Done.

-Please use italics for Latin phrases such as “in vivo” and “in vitro”.

Done.

-Paraformaldehyde is toxic and requires a caution statement.

Done.

-Please reformat bold protocol section headings to reflect the method rather than the result.

Done.

2. Grammar:

-Line 47 – The following what? Manuscript? Article?

The text has been changed (new manuscript, line 46).

-2.10 – “Analyze the stainings”

The text has been changed (new manuscript, line 199).

-Discussion - Please correct the punctuation in the third paragraph. A list should not be separated by complete sentences.

The text has been changed (new manuscript, lines 312-318).

-Line 306 – “for receptors which, when unstimulated, exhibits”

The text has been changed (new manuscript, lines 330-332).

3. Additional detail is required:

-1.2, 2.2, 3.2 – About how long does this take?

As long as it takes (it all depends on the initial cell-seeding density).

-2.7, 2.8 – Is this 5% saponin?

No it is 0.5% Saponin. 0.5% has been added to the text (new manuscript, lines 186, 189, 192, 196).

-2.8 – What antibody dilution?

Antibody dilutions have been added throughout the text.

-3.8 – What are the settings? Is this performed on ice?

The proper settings and conditions are now given (new manuscript, lines 230-232).

4. Branding:

-1.4, 3.5 – cOmplete

Done

-3.5 – PhosSTOP

Done

5. Results:

-Please describe the results (ie what the data show) in more detail in there results section. One sentence for figures 4, 5 and 6 is insufficient.

The results have been rewritten in accordance with the requests

-Please indicate any statistical tests used.

Done.

-Figure 5 requires a scale bar.

Done

**Reviewer #1:**

*Manuscript Summary:*

Comments for author

Overall an interesting paper but given that it is a methods paper one would expect at least as much methods detail as a standard paper. I would suggest a general increase in such detail. Specific points below.

Since it is a methods paper it would be best to not refer to the authors' standard (not methods) paper for details of the methods e.g., (lines 128, 197). It would be better to include the details in this current paper. For example, what are the specifics of the vectors. What procedure was used for transfection?

Information regarding constructs and reagents used for transfection are now included (new manuscript, lines 126-130).

Line 129 indicates myc tag not required but myc antibody is specified on line 157.

The line (line 129 in the original manus) has been omitted as it is somewhat confusing – instead, the use of a Myc-tag and anti-Myc Ab’s has been clarified in the discussion section (new manuscript, lines 341-347).

Shouldn't the text on protocol points 1., 2., etc be the questions addressed not the conclusions drawn from the experiments.

The protocol points 1, 2, and 3 has been rewritten in accordance with the requests.

Why different cell confluence used in 1.2 vs 2.2 steps?

High confluency was used simply to ensure enough protein for Western blotting, whereas a lower confluency was preferred for immunocytochemistry to allow a clear view of single cells by avoiding overlap and clustering.

Specify all primary and secondary antibody concentrations used? This is generally included even in non-methods papers because it is so critical.

Antibody dilutions have been added throughout the text.

Saponin concentration used in antibody solution?

The Saponin concentration has been added to the text (new manuscript, lines 186, 189, 192, 196).

How do you know it is the downregulation of CNTFR that lowers the response to subsequent CLC:CLF-1 stimulation. All that is shown is that CLC/CLF-1 treatment lowers the response to subsequent treatment with the same ligand, a very common pharmacological effect that could be mediated by many different things independent of CNTFR. The authors need to discuss and qualify their conclusions and/or explain the logic.

The low response to CLC:CLF-1 in pre-stimulated cells is shown because it agrees with (and is to be expected upon) a downregulation of CNTFRα. But we fully agree with the reviewer and acknowledge that other factors may contribute - this has now been emphasized in the discussion section (new manuscript, lines 319-325).

Is there a spin step between steps 3.5 and 3.6?

No.

Typo line 282 ..figur

Corrected.

Typo line 309 in stead

Corrected

Line 306 exhibit not exhibits

corrected

*Major Concerns:* N/A

*Minor Concerns:* N/A

*Additional Comments to Authors:* N/A

**Reviewer #2:**

*Manuscript Summary:*

This manuscript summarises the techniques used by this group to investigate the role of CRLF1 in the functions of the cytokine CLCF1. It definitively complementary to the previous publications of this group and deserves to be published.

*Major Concerns:* N/A

*Minor Concerns:* Figure 6 is unclear. Are the cells used HEK293-CNTFRa-Myc/sorLA or HEK293-sorLA?

The cells used are HEK293-sorLA. As mentioned (new manuscript, line 203) they express endogenous CNTFRα (without a Myc-tag).

*Additional Comments to Authors:* N/A