Reviewer's report

Title: Androgen receptor and chemokine receptors 4 and 7 form a signaling axis to regulate CXCL12-dependent cellular motility

Version: 4 Date: 7 January 2015

Reviewer: Daniel Frigo

Reviewer's report:

The revised manuscript by Hsiao et al presents some new data that strengthens the study. This includes additional loading controls, revised text to better convey certain points and fantastic new data to demonstrate a physical interaction between CXCR7 and AR (new Figure 5D). As such many of this reviewer’s concerns have been addressed. However there are still three major points that continue to be a concern:

Major Revisions:

1) While this reviewer appreciates the difficulty of working with membrane proteins, the specificity of the CXCR7 antibody is still questionable. The knockdown of CXCR7 continues to be minor (never more than 30-40% at even the RNA level; most companies, including Qiagen—the company the authors used here, guarantee their chemical siRNAs will knock down mRNA levels at least 75% or 1) they will send you additional siRNAs or 2) they will refund you). Further, data from only one siRNA is shown (see below for additional comments on this topic). Also the different band sizes presented continually throughout the study inevitably leave the reader to wonder which bands are real and which are artifacts. Sometimes there are smears, sometimes there are different sized, distinct bands. This reviewer is willing to concede this point given the variety of possible explanations, but suspects many future readers will also question the validity of this antibody. As such, I do hope the authors, as biochemists, are sincere in that they will try to identify the different CXCR7 species as stated. Alternatively, as described below, the use of multiple siRNAs would also help verify the specificity of this antibody.

2) The authors response to earlier concerns about the purity of fractionations not being clean (response point 2) was that they have presented better western blot images. However, this was not the case. It appears in the revised manuscript the authors again used the exact same blots, but in Figure 2 they happened to move around the image layout. This fractionation issue continues to be a concern especially given that confocal microscopy was still not performed.

3) Validation of findings using multiple siRNAs or an add-back approach is now a standard approach that is a must for any molecular work. The off-target effects of chemical siRNAs are significant, even despite claims by the manufacturer than they validated the siRNAs. This only means the company validated that the
siRNA can knockdown their intended target in the one cell line they tested it in. They do not test for off-target effects and certainly do not test for off-target effects in multiple cell lines. The use of the control siRNA is helpful, but that is more just a control to make sure the process of siRNA transfection in general doesn't have an effect. The authors stated they used multiple siRNAs and they all yielded the same effect. If that is the case, they should show this data because it would greatly strengthen the manuscript.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.