Response to Reviewer 3 Comments

Point 1: In the work with the title “Reciprocal regulation of TRPS1 and miR-221 in intervertebral disc cells” the authors show, that decreased TRPS1 expression goes along with higher IVD degeneration. Concerning stress response, SOD2 protective for IVD, since it acts against oxidative stress by clearing mitochondrial reactive oxygen species, is lower expressed with increased IVD degeneration. Overexpression of TRPS1 in IVD primary cell cultures, used at P2 in a de-differentiated but not senescent state, shows increased level of chondrogenic marker expression, therefore a restorage of a chondrogenic like phenotype, which is lost in the de-differentiating process. Moreover, the authors could show, that TRPS1 and miR-221 expression are linked together, overexpression of TRPS1 in these primary cell cultures led to significantly reduced expression of miR-221. Furthermore, the authors of this manuscript could demonstrate that TRPS1 regulates miR-221 expression by binding to different promotor regions. The more IVD degeneration exists, the less binding sites for TRPS1 are accessible on the miR-221 promotor region. This group could also show, that vice versa miR-221 binds to the 3’UTR of TRPS1, regulating its expression. This work provides new promising targets in the therapy of IVD degeneration. Overall the authors provided a very nice work, nevertheless I have some minor comments mainly concerning the language which in some parts is very good but appear in other parts with mistakes, therefore the authors should carefully edit the language in this manuscript.

Response 1: We thank the Reviewer for her/his positive comments

Point 2:
Row ”46”: occur is without an s in that case and spina should be spine
Row “54”: citation 4 is in a different style than the rest
Row “56”: Consequently, detecting potential specific target therapeutic molecules should be
Consequently, detecting potential specific therapeutic target molecules

Response 2: All errors have been corrected

Point 3:
Row “77”: What is meant with loop involving cells? This should be written more specific.

Response 3: We have referred to a concept of the degenerative circle of IDD described in the reference 15

Point 4:
Row “81”: molecule instead of molecule
Row “231”: It is Vectastain ABC solution not Vecstain
Row “243-245”: Formatting needs revision
Row “286”: There is too much space before “All”

Response 4: All errors have been corrected

Point 5: Figure1 A+B: The bar does not show a size, at least not in the version I got, if it is there it should be bigger.
**Response 5:** We checked the Figure 1

**Point 6:** Row “305”: The authors state that SOD2 plays an important role in cellular stress response, this should be written more precisely, like antiapoptotic and against oxidative stress, although it is discussed later, so it is easier for the reader to understand.

**Response 6:** The sentence: “… and implicated in antiapoptotic action and defense against oxidative stress” has been added (Row 314-315)

**Point 7:** Row “324”: Before “In these” is too much space

**Response 7:** OK, thanks.

**Point 8:** Figure 2: Labelling of the white, black and grey bar is missing, which one is control, hTRPS1 and EMPTY

**Response 8:** A small legend indicating the color code for the bar graphs has been included.

**Point 9:** Figure 2 C: The authors show that on the one hand chondrogenic markers are higher expressed in TRPS1 transfected cells, but on the other hand that these cells also express higher level of SOX2 a stem cells marker, which is somehow confusing. Control staining of totally differentiated chondrocytes would be needed to compare expression intensity and in which range TRPS1 transfection leads to chondrogenic differentiation. Moreover, the authors state in the discussion that it was previously shown that there is a potential stem cell niche detectable in IVD cells, this should be mentioned in this part of the manuscript shortly, not only in the discussion otherwise it is misleading for the reader.

**Response 9:** The aim of the experiments reported in Figure 2 was to demonstrate that through the modulation of a transcription factor such as TRPS1 it is possible both to improve the phenotype of de-differentiated/degenerated cells that regain the ability to express the chondrogenic markers, and to awaken the activity of those progenitors (stem cells) that are essential for tissue regeneration. In the hypothesis of target therapy for IDD, these are the ideal characteristics for reverting degeneration through an injectable molecule.

Regarding the effect of TRPS1 overexpression on the stemness, this result is preliminary and has to be confirmed by analyzing other stemness markers, however, SOX2 increase encourages us to continue on this hypothesis.

As suggested by the Reviewer we modified the text and added a sentence on the Results to better explain the data (Row 359-362).

For what concerns control staining of totally differentiated chondrocytes we referred to previous data as data not shown, by adding the sentence: “The expression level of these chondrogenic markers in TRPS1 overexpressing IVD cells were comparable with those by us detected in human primary chondrocytes (data not shown) [17]” (Row 347-349). Below we report the Figure with the data to which we refer:
Immunocytochemical analysis performed on freshly isolated human nasal septum chondrocytes.

**Point 10:**
Row “340”: immunohystochemical should be immunohistochemical
Row “362”: potential TRPS1 binding sites, here the s was missing

**Response 10:** OK, thanks.

**Point 11:** Figure 3: The authors used only one patient for the respective degradation stage, to confirm the conclusions the authors made, it should be done at least in 3 patients of the same stage each.

**Response 11:** In order to perform Chromatin immunoprecipitation assay high cells number is mandatory: from a human disc surgical sample the number of cells obtainable is very low and it is difficult to have a large number of IVD samples which are suitable for carrying out the ChIP experiment. We are trying to collect them, but for now we are not able to satisfy the Reviewer requests.

One possibility to overcome this limitation would be to use cadaveric samples, but unfortunately the Italian legislation does not allow it. For this reason, we are looking for collaborations with foreign research centers interested in our research and able to recruit cadaver samples through specific organ donation program.

**Point 12:**
Row “389”: Citation 16 is in a different style than the rest
Row “477-478”: Please change the sentence to: this extends our previous finding

**Response 12:** OK, thanks.