

Point-by-point reply

“Identification of the transcription factor ATF3 as a direct and indirect regulator of the LDLR”
(metabolites-1868994)

The authors want to thank the Editors and Reviewers for the evaluation of our manuscript and insightful comments (*in italic*). Please find our responses (Response (R) - blue) and revisions of the paper (yellow) below.

Reviewer 1

It's not clear to me what is declared by the authors in the section entitled “Author Contributions”. Indeed, it is stated that all authors have had a major role in the investigation, which means that all the listed authors including the so-called “DigiMed Bayern Consortium” have participated to the experimental work. This must be explained very carefully. As such, it doesn't seem realistic that about 100 authors have participated to the experimental work, and the Editorial Office must be aware of a potential issue of inappropriate authorship. Please explain in full details the role on this article of each expert included in the DigiMed Bayern Consortium.

R: We want to thank the reviewer for the evaluation of our manuscript.

We provided an accurate and detailed description of contribution. In detail, our work was presented at regular DigiMed Bayern Consortium meetings including fruitful discussion and advice how to proceed with ATF3. The DigiMed Bayern Consortium (fighting atherosclerosis) actively stimulated the recent investigations. Further, the manuscript was shared with all members of the consortium, and was reviewed and edited. Finally, the consortium agreed to publish the final version of the manuscript.

We agree, if ‘investigation’ should be considered as experimental work only by the editorial board, to remove the contribution of ‘all authors’ from the ‘investigation’ section.

Reviewer 2

The author provided significant evidence supporting that the transcription factor ATF3 has an impact on LDLR expression directly and indirectly. The findings in this manuscript depict a possible molecular mechanism that explains the role of ATF3 in the pathogenesis of cardiovascular diseases. However, a few discrepancies in the results remain to be further discussed. In the abstract (line 45 and 46), the author stated that the knockdown of ATF3 in Hep3B significantly reduced LDLR expression. However, the results presented in Figure 4 show an opposite trend.

Moreover, the correlation of ATF3 expression with LDLR and MAFF expression in Figure 6 suggests a positive correlation between them in the human liver. The author needs to explain the discrepancies between in vitro experiments and human data.

R: We want to thank the reviewer for the careful evaluation of our manuscript and the insightful comments.

The abstract incorrectly stated ‘downregulated’ by mistake. We corrected the section in the abstract in line with the reported results to ‘upregulated’.

R: We appreciate the comment to further elucidate potential confounding issues and changed the discussion/limitations section accordingly.

Comprehensive *in vivo* data from STARNET support the idea that high ATF3 levels are atheroprotective. However, extensive perturbation via knockdown of ATF3 in Hep3b cells led to immediate upregulation of LDLR *in vitro*. Interpretation of these results should take into account the following considerations. In general, ATF3 is a transcription factor with non-specific binding capacities to a range of interaction partners. As ATF3 binds the cAMP response element (CRE), a sequence present in many cellular promoters it has the capacity to up- or downregulate transcription from promoters with ATF sites. The regulatory impact might be influenced by different effect sizes and functions of interacting dimerization partners, differential splicing, presence of isoforms, environmental perturbation or intake of medication. In specific, *in vitro* models were limited to one human liver cell line, defined siRNA incubation times and a relatively high dose of LPS. Further, the molecular mechanisms affecting ATF3 expression and ATF3 downstream targets remain inadequately understood. In particular, molecular heterodimerisation partners of ATF3 influencing transcriptional activation or repression of MAFF and LDLR under different conditions remain unknown. In this regard, this study might be a good starting point and will stimulate further investigations.

Reviewer 3

The paper Metabolites-1868994 » Identification of the transcription factor ATF3 as a direct and indirect regulator of the LDLR« is the result of an extensive international collaboration with contributions from researchers involved in STARNET (Stockholm-Tartu Atherosclerosis Reverse Network Engineering Task), researchers working at the UCLA, the German Heart Centre Munich and several other prominent institutions. This work is an extension of the research on MAFF (MAF basic leucine zipper transcription factor F) as a regulator of atherosclerosis that has been published in Circulation 2021 (DOI: [10.1161/CIRCULATIONAHA.120.050186](https://doi.org/10.1161/CIRCULATIONAHA.120.050186)). Candidate genes associated with coronary artery disease were validated in mouse models. Gene regulatory networks, focusing on ATF3 and LDLR were studied by computer modeling /bioinformatics (Bayesian key driver analysis) and validated in cultures of human Hep3B cells, and finally validated in atherosclerotic phenotypes in humans and in mice.

The transcription factor ATF3 was found to be a regulator of a liver network linked to inflammation and cholesterol metabolism, directly and indirectly (via MAFF) regulating LDLR. Upregulation of ATF3 resulted in downregulation of LDLR. This work adds to the knowledge on links between inflammation and atherosclerosis and opens possibilities for testing ATF3 as a therapeutic target.

The paper covers a wide range of experiments and is necessarily at times only schematic, but is well written and balanced. The conclusions are supported by the results. I recommend accepting the paper for publication in Metabolites.

R: We want to thank the reviewer for the careful and positive evaluation of our manuscript.