nature portfolio

Peer Review File

Size-dependent plastic exposure disrupts macrophage function and tissue-specific metabolism

Corresponding Author: Professor Elvira Mass

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Metabolism.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In the manuscript entitled “Size-dependent plastic exposure disrupts macrophage function and tissue-specific metabolism”, Nikola Makdissi and colleagues investigate the impact of chronic micro- and nano-plastic exposure on liver biology and systemic metabolism. Using a combination of in vivo models, metabolic phenotyping, omics (lipidomics, single-nucleus RNA sequencing) and functional macrophage assays, the authors identify KCs as the major hepatic reservoir for MP/NP, and show that chronic exposure induces KC reprogramming. Importantly, the study suggests that MC and NP exert distinct metabolic effects: particularly in the context of HFD feeding, nano-plastics predominantly promote energy expenditure, provide resistance to HFD (despite systemic glucose handling worsens); on the contrary, microplastics mainly promote hepatic lipid accumulation. The authors further propose that altered KC-hepatocyte crosstalk contributes to the observed hepatic metabolic phenotype.

The observation that chronic plastic exposure may reprogram tissue-resident macrophages and thereby alter hepatic and systemic metabolism is conceptually intriguing, and potentially relevant for the readership of Nature Metabolism. Importantly, the work also moves beyond the simplistic inflammatory narrative that dominates much of the current literature in the field, and instead proposes a more nuanced model involving tissue-specific metabolic dysregulation and macrophage functional adaptation.

The study is also timely, given the increasing evidence that micro- and nano-plastics accumulate in human tissues and may have clinically relevant biological consequences in metabolic disease and its complications (e.g. in atherosclerosis). The identification of KC as key cellular mediators linking plastic exposure to metabolic dysfunction provides an interesting conceptual framework that could open important future directions in environmental metabolism and immunometabolism research.
The amount of work performed is substantial and many datasets are of high quality. Overall, this is an ambitious and potentially impactful study. The manuscript is generally well written, the English language is excellent, and the work is supported by many experimental approaches and in vivo models. In particular, the combination of metabolic phenotyping with advanced single-cell approaches, KC lineage tracing and depletion/repopulation models represents a major strength of the study.

However my main concern relates to how the data are presented and interpreted, and to the fact that the mechanisms driving: A) The changes in hepatic lipidome (LPC/LPE/SM) in chow conditions; B) the changes in BW, EE, BAT function, hepatic fat accumulation in HFD and in the in vitro experiments; are currently not clear from a mechanistic metabolic perspective.

In specific:

1)Despite I might agree with the authors that data point to “metabolic alteration” (widely claimed in the manuscript – but not clear in substance) very little metabolic details are shown in this manuscript:

a.Please systematically provide for all the mouse experiments BW, body composition (Fat/Lean Mass %), tissue weights, Fasting Glucose, Insulin, NEFA/FFA, TG, Cholesterol; also calculate HOMA-IR (with glucose/insulin) and At-IR (with

NEFA/insulin) as those will help to understand the systemic metabolic context in each experiment (and why in some comparisons there are differences among groups that are absent in others) and the reasons of the divergent phenotypes among NP/MP: this is particularly important in the experiments where changes in AT/liver (weights, lipids and/or glycogen) are observed or when BW is different among groups. Without this information it is difficult to interpret the results from a systemic metabolic perspective. This is very important especially in the experiments depicted in figure 2 (and for negative results in extended data figure
7) where BW, AT weight and hepatic fat content differ among the treatments (thus changes in AT lipolysis are expected among the groups as result of improved AT function).
b. It is currently not clear how NP might promote the divergent phenotype between increased EE (and likely improved AT function and peripheral insulin sensitivity: circulating NEFA and AT-IR index would help …) and reduced glucose tolerance (in presence of unchanged ITT) in HFD: these findings, although intriguing, need to be better characterised. For example, it might be useful (if blood volumes allow) to quantify circulating glucagon or FGF21 that might be implicated in the phenotype: is circulating FGF21 (or any other hepatokines able to modulate EE) increased (at least provide gene expression information)? Or, on the contrary, might reduced glucagon explain the impaired glucose tolerance in GTT (given ITT are not significant)? I agree with the authors that the findings are intriguing but they are counterintuitive (as result or improved AT function and reduced hepatic fat, anyone would expect an improved glucose tolerance) and without a clear explanation, I am hesitant to consider this manuscript a good fit for the readership of Nat Met;
c. Please characterise main changes (e.g. UCP1, FAO, Lipolysis, etc) in WAT/BAT that might explain the systemic/in vivo phenotype and the resistance to HFD: explaining it with reduced AT weight is somehow oversimplistic since reduced it can either be associated to reduced expandability (thus worsening systemic metabolism – e.g. lipodystrophy) or improved AT insulin sensitivity (that seems to be at play in nanoparticle data): this is a "standard" (and very basic) requirement for AT function profiling; check any paper focussing on AT function/flexibility in obesity (e.g. from Arner, Vidal-Puig, Spiegelman labs);
d. Thee mechanism by which MP might promote hepatic fat accumulation is currently completely uncharacterised mechanistically, and the claims on APO sound anectodical: changes in hepatic fat result from an imbalance between lipid influx (FFA, DNL, dietary lipids, HDL/LDL coming back to the liver) and output (FAO, VLDL). It is therefore not clear to this reviewer how the transcriptional upregulation of APOs might contribute to the phenotype (e.g. Apoc3 might exert negative effects on systemic metabolism by inhibiting LPL thus impacting VLDL metabolism: however, I am doubtful this mechanism would be at play here and no proof is provided). Moreover, the snSEQ data show a consistent suppression in most cell types of Cyp7b1: are oxysterols increased? Could an activation of the LXR>SREBP>DNL axis explain the hepatic fat accumulation observed here? Are canonical targets of LXR and SREBP1c modulated by NP/MP? Conceptually speaking this would be a much easier explanation (also for in vitro mechanistic confirmation, see below) seq data point in this direction.
e. The discussion of the metabolic consequences of the observed phenotypes and metabolic changes is quite superficial throughout the manuscript: it looks like the authors show difficulty in interpreting their metabolic results.

I am afraid that, from a metabolic perspective, currently the manuscript opens more questions rater than providing answers … which is a pity given the intriguing phenotype.

2) For the lipidomics analyses:

a. on top of clustering the results on lipid classes, would be nice to check if there are changes in the degree of saturation within the significantly different lipid classes: I am asking since some plastics can contain (and might potentially release in KC?) huge amounts of palmitate (saturated FA, notoriously lipotoxic) – this is a very well known "issue" in users of GC-MS (analyses are usually performed using glassware to avoid huge palmitate contamination from plastics): are plastics directly releasing palmitate in KC (and, secondarily, to other cells?) that is then incorporated in complex lipids? Why FFAs are not shown in the heatmap? They would have been extremely instructive here …
b. it is not clear how the authors explain LPC/LPE/SM changes in Fig 1: if plastics do not induce huge changes in inflammatory cells infiltrates (that contain a lot of these lipid classes – this would have been an easy explaination), how the authors justify the observed changes in lipid composition? No explanation is provided. Do the snSEQ provide any information regarding a reshaping of metabolic pathways involved in LPC/LPE/SM metabolism? Please show at least the gene expression (e.g. by extracting data from Seq, or performing qPCR) of key rate limiting enzymes of these pathways that can justify the observed phenotype;
c. The sentence "… all of which are associated with hepatic metabolic disorders" is very general … authors should clarify what do they mean: the fact that changes in a specific lipid class might have been characterised in a specific disease does not necessarily mean that same concepts apply to this specific context. This superficial discussion of metabolic data is repeatedly used throughout the manuscript and very confusing.

3) I disagree with the sentence at page 7 line 232 "consistent with preserved insulin sensitivity, quantification of insulin-producing B-cells in the pancreas revealed no difference among conditions". This is conceptually wrong: insulin production and sensitivity are two completely different concepts (moreover in an ITT insulin is provided from outside the body … so insulin production does not really matter during an ITT …). I instead wonder if changes in insulin/glucagon/GLP1 occur at 60-120 min of GTT (assuming blood is still available – I understand it is very little and not be feasible retrospectively) as they might justify the worsening of glucose tolerance (GTT) despite the body weight loss. Or focussing on intrahepatic mechanisms (e.g. AKT phosphorylation to prove hepatic insulin resistance?).

4) The pathway analysis in snRNA-seq should be improved to extract more biological value:

a. By showing DEGs that enrich the different significantly regulated pathways;

b. By focussing on LPC/LPE/SM metabolism;
c. By focussing on aspects of liver metabolism involved in hepatic lipid input and output (see above);
d. By performing a transcription factor analysis (e.g. VIPER or IPA Upstream Regulator Analysis) in hepatocytes and KC to provide a better understanding of which processes might be occurring there. In light of the changes in BW/EE/hepatic fat and GTT it is likely, for example, that there will be a prediction of modulation of glucose/lipid transcriptional regulators such as PPARs, ChREBP, SREBPs, and/or other lipid sensing nuclear receptors (that might enlighten the mechanism behind the phenotype); again, can the suppression of Cyp7b1 can be associated to increased LXR activity (as discussed above)?
e. The above mentioned results can be better discussed in light of (and correlated to) systemic changes and histological findings; for example the changes in APOA1 might reflect changes in HDL metabolism? Does APOCIII influence VLDL metabolism (toward inhibition of LPL increased TGs are expected: however, as discussed above, the value of these results cannot be appreciated here since circulating HDL/LDL cholesterol and TGs have not been shown …)?
As it sits, the snSEQ data do not allow to fully capture the degree of metabolic dysregulation occurring in the liver, and how much these changes are due to intra-hepatic mechanism or rather reflecting systemic metabolic changes that need to be taken in deep consideration. As it currently reads, these data look like a pure bioinformatic exercise with very little biological value (which is a pity given the nice phenotype and the huge amount of work).

5) The in vitro co-culture experiments should be better described and expanded: why MP-KCs induce hepatocyte lipid droplet formation? It is a matter of increased hepatocyte input (uptake? DNL?) or reduced hepatic output (FAO? VLDL export?)? Plastics releasing palmitate in KC? Ideas will come from the results of points above while in vitro experiments should validate these predicted mechanisms mechanistically with a single well designed “killer experiment” (better if using appropriate advanced MASLD models and avoiding 2D cultures – see PMID: 41582251). This is a crucial point for Nat Met readership.

6) KC phagocytic capacity: are these results any relevant in this manuscript? I wonder if these data can be used for a follow-up story? They look substantially unrelated to the main trajectory of the paper that in my opinion should be more tailored to systemic and intra-hepatic metabolic unbalances (that are the main results). Moreover, these data sound a bit confusing considering that no mayor changes in the immune system of the liver is observed in the specific obesity settings explored (and the role of microbiota/KC in MASLD is pretty much established). These data can be a good basis for a follow-up story focussing on wound healing, liver regeneration and advanced disease stages (using appropriate models of disease progression).

7) Following up from the previous point, in the discussion, please stress the fact that the observations provided in this manuscript relate to “lean” (chow) and very mild (short term HFD) Obesity/MASLD-inducing conditions, and that it cannot be excluded that in the context of longer challenges and progressive MASH models (e.g. WDs), effects on inflammation and fibrosis might arise. No need to provide additional experiments, but this is a crucial point to discussed to shape future directions in the field (see – see PMID: 41582251; PMID: 38867022). In line with this, it is also relevant to discuss for Extended Data Figure 5 the fact that short term HFD is not a good model to study hepatic fibrosis in the context of MASLD, and more studies are needed with appropriate models to exclude an impact of plastics on disease progression.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have carefully addressed the major concerns raised during the previous round of review, and the manuscript has substantially improved in both quality and readability. I appreciate the considerable effort invested in the revision. I only have a few minor comments that, in my opinion, would further strengthen the manuscript:

1. Plasma lipid profile. I respectfully disagree with the decision not to report the circulating lipid profile because the differences were not statistically significant. In this case, the absence of significant changes is informative, as it supports the authors' interpretation that the observed phenotype is predominantly driven by intrahepatic mechanisms, particularly given that fasting insulin and NEFA measurements were not available. Along the same lines, I believe the lack of information regarding systemic insulin resistance and adipose tissue function should be more explicitly acknowledged in the limitations. Since adipose tissue function is central to the interpretation of systemic and hepatic metabolism, these missing data somewhat limit the mechanistic interpretation of the findings.

2. FGF21 and glucagon measurements. I could not locate the newly generated FGF21 and glucagon data in the revised manuscript. While I understand that elucidating the upstream mechanisms regulating these hormones is beyond the scope of the present study and may represent future work, these measurements are important to provide a plausible framework explaining the apparent divergence between energy expenditure, hepatic steatosis and glucose tolerance in HFD-fed mice. I therefore encourage the authors to include these data in the manuscript and integrate them into the discussion.

To clarify, my intention is not to challenge the authors' decision to approach the study from a macrophage-centric perspective. Rather, I believe that providing this metabolic context would make the findings more accessible and interpretable for the broad readership of Nature Metabolism.

3. Discussion of lipidomics and transcriptomics. The expanded discussion of the lipidomic and sequencing datasets

considerably improves the mechanistic interpretation of the study. However, I find this section somewhat lengthy and, at times, repetitive. The interpretation of the different layers of evidence (DEGs, lipidomics, pathway analysis and VIPER) could likely be better integrated to avoid reiterating similar concepts. In addition, I would recommend avoiding extensive discussion of individual genes or lipid species, or mechanistic interpretations that are not directly supported by experimental evidence, as some conclusions (for example regarding SCD1 regulation and its downstream effects) appear somewhat speculative. As a minor point of curiosity, was FXR predicted to be inhibited, given the apparent changes in bile acid metabolism?

4. Pathway enrichment analysis. I am not entirely clear on how the pathway enrichment results are presented. Typically, only significantly enriched pathways are discussed, whereas several non-significant pathways also appear to be displayed and interpreted here. In addition, activation or inhibition is usually represented using the normalized enrichment score (NES). Finally, could the authors clarify whether VIPER analysis was performed on differentially expressed genes rather than on the complete expression matrix? A DEG-based analysis might more directly identify transcription factors whose activity is altered by plastic exposure and could further strengthen the mechanistic interpretation.

5. The authors may also wish to cite the recent review on plastics and liver disease published in Nature Reviews Gastroenterology & Hepatology (PMID: 41946921), which provides a timely overview of the field and would help place the present findings in a broader context.

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The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.
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Response to Editors' Comments

Dear Christoph,
We thank you for the detailed guidance. Below we address each editorial point, in case it has not already been part of the previously submitted manuscript:

Metabolic characterisation and mechanistic framework. In response to the referee's and your central request, we have strengthened the metabolic characterisation of the mice and, using our snRNA-seq data in combination with the lipidomics data, formulated a plausible and internally consistent mechanistic framework for how size-dependent plastic exposure alters hepatic and systemic lipid metabolism. We explicitly do not claim a fully validated molecular mechanism, and we have formulated the corresponding limitations and alternative interpretations in the discussion. We note that this has led to a substantial expansion of the manuscript; we would be willing to shorten this material and defer to the editor and referee on the appropriate final length.

Glucagon. As specifically requested, we have now measured circulating glucagon by ELISA in the available plasma from both chow- and HFD-fed cohorts. These data are presented and discussed in the point-by-point response (Figure R1, Reviewer point 1b).

Sex and gender reporting (SAGER). Almost all experiments in this study were performed exclusively in young male mice. This is now stated explicitly in the abstract and at the relevant points in the results and methods. The rationale for using male mice and the resulting limitation to generalisability are addressed in the discussion, and whether sex was considered in the study design is stated in the Reporting Summary.

Abstract. The abstract has been rewritten to 146 words.

Figure legends. Following the revision and the expansion in the number of sub-panels, main figure legends 1 and 2 exceed the 350-word guideline. Should we be condensing the hepatocyte phenotype, we would be able to move part of these data to the Supplementary Information, where they would not need to be presented as prominently, allowing the corresponding legends to be shortened accordingly.

Reporting Summary. A completed Reporting Summary form is uploaded with the revised submission.

Reviewer #7:

In the manuscript entitled “Size-dependent plastic exposure disrupts macrophage function and tissue-specific metabolism”, Nikola Makdissi and colleagues investigate the impact of chronic micro- and nano-plastic exposure on liver biology and systemic metabolism. Using a combination of in vivo models, metabolic phenotyping, omics (lipidomics, single-nucleus RNA sequencing) and functional macrophage assays, the authors identify KCs as the major hepatic reservoir for MP/NP, and show that chronic exposure induces KC reprogramming. Importantly, the study suggests that MC and NP exert distinct metabolic effects: particularly in the context of HFD feeding, nano-plastics predominantly promote energy expenditure, provide resistance to HFD (despite systemic glucose handling worsens); on the contrary, microplastics mainly promote hepatic lipid accumulation. The authors further propose that altered KC-hepatocyte crosstalk contributes to the observed hepatic metabolic phenotype.

The observation that chronic plastic exposure may reprogram tissue-resident macrophages and thereby alter hepatic and systemic metabolism is conceptually intriguing, and potentially relevant for the readership of Nature Metabolism. Importantly, the work also moves beyond the simplistic inflammatory narrative that dominates much of the current literature in the field, and instead proposes a more nuanced model involving tissue-specific metabolic dysregulation and macrophage functional adaptation.

The study is also timely, given the increasing evidence that micro- and nano-plastics accumulate in human tissues and may have clinically relevant biological consequences in metabolic disease and its complications (e.g. in atherosclerosis). The identification of KC as key cellular mediators linking plastic exposure to metabolic dysfunction provides an interesting conceptual framework that could open important future directions in environmental metabolism and immunometabolism research.
The amount of work performed is substantial and many datasets are of high quality. Overall, this is an ambitious and potentially impactful study. The manuscript is generally well written, the English language is excellent, and the work is supported by many experimental approaches and in vivo models. In particular, the combination of metabolic phenotyping with advanced single-cell approaches, KC lineage tracing and depletion/repopulation models represents a major strength of the study.

However my main concern relates to how the data are presented and interpreted, and to the fact that the mechanisms driving: A) The changes in hepatic lipidome (LPC/LPE/SM) in chow conditions; B) the changes in BW, EE, BAT function, hepatic fat accumulation in HFD and in the in vitro experiments; are currently not clear from a mechanistic metabolic perspective.

We appreciate the highly positive and constructive assessment of the conceptual framework and the recognition that the study moves beyond a simplistic inflammatory interpretation of plastic exposure. We agree that the metabolic characterization can be strengthened. At the same time, we would like to emphasize that the **central conceptual advance of the manuscript is the identification of Kupffer cells as a major cellular reservoir and sensor of chronic plastic exposure,** and the demonstration that chronic exposure induces long-term macrophage functional reprogramming that **secondarily** alters hepatic and systemic metabolism in a size-dependent manner.

We therefore focused the revision on strengthening the mechanistic link between Kupffer cell dysfunction and hepatic lipid handling, particularly addressing whether altered hepatocyte lipid accumulation is associated with increased lipid uptake versus de novo lipogenesis via an extended analysis of our single-nucleus RNA-seq data. We further expanded the data on cohorts that underwent metabolic phenotyping. Some of the reviewer’s suggestion have led to new experiments, which we show in our response (energy expenditure, glucagon and FGF21 measurements). However, we have decided against their inclusion in the manuscript

as the new data is opening up new avenues for future studies, rather than improving the interpretation of the phenotypes we observe. We would like to emphasize that plastic is not a physiological trigger, therefore local and system metabolic responses are likely not corresponding to those observed under plastic-free feeding condition.

In specific:

1)Despite I might agree with the authors that data point to “metabolic alteration” (widely claimed in the manuscript – but not clear in substance) very little metabolic details are shown in this manuscript:

a.Please systematically provide for all the mouse experiments BW, body composition (Fat/Lean Mass %), tissue weights, Fasting Glucose, Insulin, NEFA/FFA, TG, Cholesterol; also calculate HOMA-IR (with glucose/insulin) and At-IR (with NEFA/insulin) as those will help to understand the systemic metabolic context in each experiment (and why in some comparisons there are differences among groups that are absent in others) and the reasons of the divergent phenotypes among NP/MP: this is particularly important in the experiments where changes in AT/liver (weights, lipids and/or glycogen) are observed or when BW is different among groups. Without this information it is difficult to interpret the results from a systemic metabolic perspective. This is very important especially in the experiments depicted in figure 2 (and for negative results in extended data figure 7) where BW, AT weight and hepatic fat content differ among the treatments (thus changes in AT lipolysis are expected among the groups as result of improved AT function).

We agree that additional systemic metabolic characterization would strengthen interpretation of the phenotype. In the previous manuscript we have already provided body weight trajectories and tissue weights. In the revised manuscript we now provide body composition analyses as well as body and organ weights from the metabolic cage experiments (see new **Extended Data Figure 8**). We have previously measured total cholesterol, HDL cholesterol, and triglycerides in the serum of HFD cohorts, however, we did not observe any significant differences, which is why we did not include the data in the previous manuscript. Due to the extensive serum analyses already performed on the HFD cohorts without major alterations, we unfortunately do not have sufficient samples remaining to additionally test parameters such as NEFA in a meaningful manner. The little serum we had left, we used to measure FGF21 and glucagon as this required very low volumes (see below, Figure R1).

We would respectfully argue that performing entirely new large-scale metabolic phenotyping cohorts, including repeated insulin stimulation studies (including HOMA-IR/AT-IR), endocrine profiling, and complete systemic metabolic re-characterization across all cohorts, would extend substantially beyond the scope of the current study and revision timeline. However, this is certainly something that we aim to address in future follow-up studies.

b. It is currently not clear how NP might promote the divergent phenotype between increased EE (and likely improved AT function and peripheral insulin sensitivity: circulating NEFA and AT-IR index would help …) and reduced glucose tolerance (in presence of unchanged ITT) in HFD: these findings, although intriguing, need to be better characterised. For example, it might be useful (if blood volumes allow) to quantify circulating glucagon or FGF21 that might be implicated in the phenotype: is circulating FGF21 (or any other hepatokines able to modulate EE) increased (at least provide gene expression information)? Or, on the contrary, might reduced glucagon explain the impaired glucose tolerance in GTT (given ITT are not significant)? I agree with the authors that the findings are intriguing but they are counterintuitive (as result or improved AT function and reduced hepatic fat, anyone would expect an improved glucose tolerance) and without a clear explanation, I am hesitant to consider this manuscript a good fit for the readership of Nat Met;

We thank the reviewer for this suggestion, which we have now addressed experimentally. We measured circulating glucagon and FGF21 by ELISA in the available plasma samples from both the chow-diet (freshly collected) and the HFD cohorts (serum stored for 2 years) (**Figure R1**). Under chow-diet conditions, glucagon was significantly increased in both NP- and MP-exposed mice relative to Ctrl, whereas FGF21 did not differ significantly between groups. After HFD, neither glucagon nor FGF21 reached significance, although FGF21 showed a non-significant trend towards an increase in NP-exposed mice.

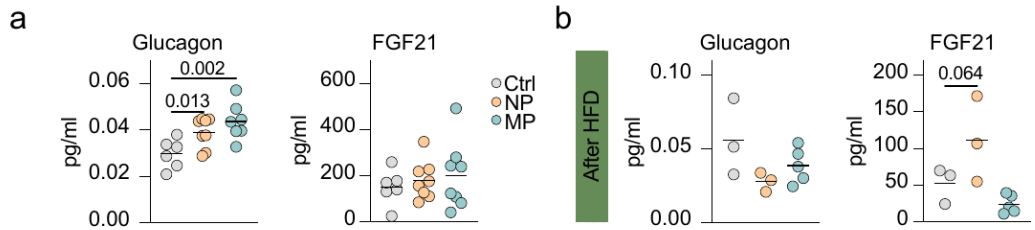

Figure R1: *ELISA-based quantification of glucagon and FGF21 in chow diet. (a) and HFD-fed mice (b). One-Way ANOVA and multiple testing corrections with a false discovery rate.*

The glucagon and FGF21 data reveal a context-dependent pattern. Under chow conditions, circulating glucagon was significantly elevated in both NP- and MP-exposed mice, raising the possibility that chronic MNP exposure affects pancreatic α-cell function or islet endocrine output even under homeostatic conditions. Dissecting whether the pancreas is a direct target of MNPs would require dedicated islet and α-cell characterisation that constitutes a separate study. After HFD, neither analyte reached significance, but glucagon showed a trend towards reduction and FGF21 a trend towards increase in NP-exposed mice. Together, a tendency for lower glucagon combined with higher FGF21 could at least partially explain the divergent NP phenotype under metabolic challenge, as reduced glucagon may impair hepatic glucose output contributing to the observed glucose intolerance, while increased FGF21 could drive the elevated energy expenditure and reduced adiposity. We plan to address these observations in dedicated follow-up studies.

In addition, we characterized BAT activation by measuring UCP1 and TH protein abundance in animals that underwent metabolic phenotyping (**Figure R2**). Although we observed a tendency towards increased UCP1 abundance, expression was not significantly higher in the NP condition, indicating that the increased oxygen consumption in NP-exposed mice cannot fully be explained by classical UCP1-dependent thermogenesis alone. This raises the possibility that UCP1-independent mechanisms contribute to the elevated energy expenditure, for example futile substrate cycling (such as the creatine-driven substrate cycle or the lipid/triglyceride-fatty acid futile cycle) that dissipates energy without a proportional change in UCP1 protein.

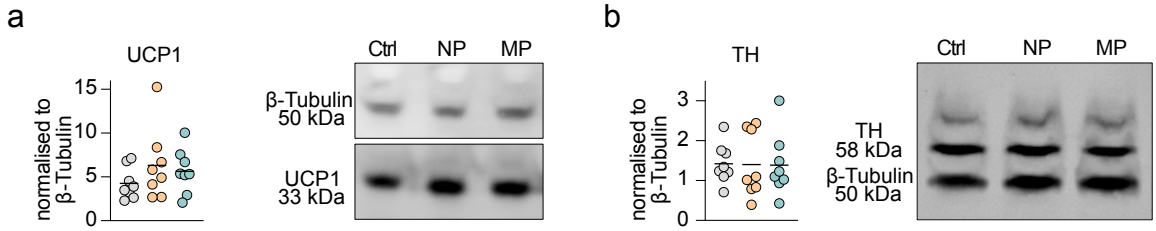

Figure R2: *Quantification of UCP1 (a) and TH (b) in BAT.*

Importantly, we would caution against interpreting BAT responses to MNP exposure through the lens of physiological cold- or diet-induced activation. Plastic is not a physiological stimulus, and chronic MNP accumulation may engage thermogenic and substrate-handling programmes that differ substantially from those triggered under normal conditions. The dissociation we observe between energy expenditure and UCP1 abundance is consistent with this view and suggests that the BAT phenotype reflects a non-canonical metabolic adaptation to plastic exposure rather than a conventional thermogenic response. Disentangling the relative contributions of UCP1-dependent and futile-cycle-based mechanisms will be an important direction for dedicated future studies.

We have repeatedly attempted to measure glucose uptake in tissues such as BAT, liver, and muscle to better understand how reduced glucose tolerance in the presence of unchanged ITT responses may occur. However, the enzymatic-based kit (Promega Glucose-Glo Assay, Cat #J6022) did not provide reliable readouts. Therefore, we did not pursue this line of experiments any further as this requires extensive mouse cohorts.

While we agree that the phenotypes are complex and somewhat counterintuitive after 12 weeks of plastic exposure, we respectfully note that this is the first study demonstrating that after only 4 weeks of plastic exposure, Kupffer cells are the first affected cells in the liver, whereas hepatocytes are not yet storing or producing excess lipids (Extended Data Figure 5g, h). Only subsequently does hepatic, and likely systemic, metabolism become altered. Thus, performing BAT characterization and hepatokine analyses in HFD cohorts represents a biologically meaningful and feasible strategy using a carefully designed longitudinal study. However, we believe that this is beyond the scope of the current manuscript.

Finally, we respectfully disagree that the study is not a good fit for the readership of Nature Metabolism, since many toxicology studies already point toward an impact of plastic exposure on liver metabolic perturbation (references 3, 12, 30-42). However, our study provides a cellular mechanism, as well as potential pathways, that may drive the phenotype through Kupffer cell-hepatocyte crosstalk. This is particularly important because the microplastics field is increasingly moving toward the interpretation that hepatocytes themselves directly take up microplastics, thereby causing the observed metabolic perturbations (PMID: 41946921 and related outreach article and posts from the related institute, e.g.: https://www.plymouth.ac.uk/news/scientists-explore-whether-plastic-particles-could-be-linked-to-significant-rises-in-liver-disease). While we cannot exclude that this may occur after even longer exposure periods, we do not observe plastic particles within hepatocytes, likely because Kupffer cells, as professional phagocytes residing within the sinusoids, efficiently filter particles directly from the blood, while endothelial cells provide an additional protective barrier.

Thus, we believe it is highly important that our study, approaching the problem from a macrophage-centric perspective, contributes to the field and helps future translational studies consider Kupffer cells as a causative and potentially druggable target.

c. Please characterise main changes (e.g. UCP1, FAO, Lipolysis, etc) in WAT/BAT that might explain the systemic/in vivo phenotype and the resistance to HFD: explaining it with reduced AT weight is somehow oversimplistic since reduced it can either be associated to reduced expandability (thus worsening systemic metabolism – e.g. lipodystrophy) or improved AT insulin sensitivity (that seems to be at play in nanoparticle data): this is a “standard” (and very basic) requirement for AT function profiling; check any paper focussing on AT function/flexibility in obesity (e.g. from Arner, Vidal-Puig, Spiegelman labs);

As outlined above, we characterised BAT activation by measuring UCP1 and TH protein abundance (point 1b, Figure R2). However, we would respectfully argue that comprehensive adipose tissue functional profiling comparable to dedicated obesity and adipose biology

studies would exceed the intended scope of the current manuscript. As pointed out above, a longitudinal study would ultimately be required to determine which tissues are initially affected beyond the liver and to disentangle systemic consequences from local NP accumulation within BAT.

d. Thee mechanism by which MP might promote hepatic fat accumulation is currently completely uncharacterised mechanistically, and the claims on APO sound anectodical: changes in hepatic fat result from an imbalance between lipid influx (FFA, DNL, dietary lipids, HDL/LDL coming back to the liver) and output (FAO, VLDL). It is therefore not clear to this reviewer how the transcriptional upregulation of APOs might contribute to the phenotype (e.g. Apoc3 might exert negative effects on systemic metabolism by inhibiting LPL thus impacting VLDL metabolism: however, I am doubtful this mechanism would be at play here and no proof is provided). Moreover, the snSEQ data show a consistent suppression in most cell types of Cyp7b1: are oxysterols increased? Could an activation of the LXR>SREBP>DNL axis explain the hepatic fat accumulation observed here? Are canonical targets of LXR and SREBP1c modulated by NP/MP? Conceptually speaking this would be a much easier explanation (also for in vitro mechanistic confirmation, see below) seq data point in this direction.

We agree that the mechanism underlying MP-induced hepatic lipid accumulation requires improved mechanistic clarification. Based on discussions with Prof. Jörg Heeren, an expert in metabolism and now also co-author in this manuscript, we performed targeted transcriptional analyses of canonical de novo lipogenesis pathways and lipid uptake-associated genes, particularly focusing on LXR/SREBP-associated signaling pathways identified within the snRNA-seq dataset.

In addition, we interrogated the Lands cycle and lysophospholipid remodeling pathways within the snRNA-seq data, particularly LPC/LPE-associated reacylation pathways and related metabolic enzymes. We now provide a more coherent mechanistic framework linking altered phospholipid remodeling and hepatic lipid accumulation (see **new Fig. 1j-m, Extended Data Fig. 6i-k**).

Regarding APOs, we have previously shown that addition of ApoE and ApoA1 to hepatocytes induces increased lipid droplet formation in primary hepatocytes, and that these genes are upregulated in Kupffer cells following environmental perturbation (Huang et al. 2025 Nature PMID: 40533564). Thus, we believe that this represents a biologically plausible mechanism that could also contribute to increased lipid accumulation following plastic exposure. However, we would like to point out that proposing a definitive APO-driven mechanism was never our intention, as reduced Kupffer cell phagocytosis is likely the initial cellular process leading to accumulation of dead cells, molecular debris, and altered signaling within the parenchyma, which subsequently affects hepatocyte metabolism. This was also phrased carefully in the submitted manuscript.

We discussed several possibilities with Jörg Heeren regarding how to experimentally address the mechanisms promoting hepatic fat accumulation, and concluded that none of the currently feasible approaches alone would provide a timely and fully conclusive solution within the scope of a revision. While we are highly interested in understanding the effects of Kupffer cell-derived APOs on hepatocytes in more detail, this would require extensive ex vivo co-culture systems using Kupffer cells isolated from mice exposed to plastic for 12 weeks, as one mouse only provides sufficient Kupffer cells for approximately 2-3 hepatocyte wells. Such studies could additionally be combined with Ldlr- or Lrp1-deficient hepatocytes and labeled fatty acid tracing approaches. However, this would represent an entirely new project involving several hundred mice rather than a feasible extension of the current study.

At present, we also do believe that technically extensive VLDL production, turnover studies or LDL receptor-based genetic models would be helpful but are not mandatory for supporting the central conclusions of the manuscript.

e. The discussion of the metabolic consequences of the observed phenotypes and metabolic changes is quite superficial throughout the manuscript: it looks like the authors show difficulty in interpreting their metabolic results.

I am afraid that, from a metabolic perspective, currently the manuscript opens more questions rater than providing answers … which is a pity given the intriguing phenotype.

We appreciate this criticism and have substantially revised and deepened the discussion, providing candidate mechanisms for the observed metabolic phenotypes and integrating the transcriptional findings with the systemic and histological data.

2) For the lipidomics analyses:

a. on top of clustering the results on lipid classes, would be nice to check if there are changes in the degree of saturation within the significantly different lipid classes: I am asking since some plastics can contain (and might potentially release in KC?) huge amounts of palmitate (saturated FA, notoriously lipotoxic) – this is a very well known "issue" in users of GC-MS (analyses are usually performed using glassware to avoid huge palmitate contamination from plastics): are plastics directly releasing palmitate in KC (and, secondarily, to other cells?) that is then incorporated in complex lipids? Why FFAs are not shown in the heatmap? They would have been extremely instructive here …

We have re-assessed the lipidomics dataset and now provide a more detailed analysis of the significantly altered lipid classes, including their degree of saturation (**new Fig. 1j**). The heatmap in Fig. 1j shows the significantly altered lipid species, FFAs were not significantly altered in any condition and were therefore not included. As suggested, we calculated the double-bond index of the affected lipid classes **(new Extended Data Fig. 6i)**. Rather than the increase in saturation that direct palmitate release would predict, we observe a shift towards more unsaturated species: the double-bond index of triglycerides (TG) was increased in MP-treated mice, with additional changes in the double-bond indices of dihexosylceramide (DiHexCer), alkylphosphatidylcholine (PCO), and TG in NP-treated mice. This shift towards unsaturation, together with the accumulation of LPE and MG species in MP, is consistent with intracellular remodeling of endogenous lipid pools rather than with bulk incorporation of a saturated fatty acid such as palmitate released directly from the particles.

We are well aware that the plastic material used in the study can contaminate GC-MS analyses to some degree, as pointed out to us by Prof. Christoph Thiele, with whom these analyses were performed, and as our pyrolysis analyses also suggest (Extended Data Fig. 3c-e). Nevertheless, biologically speaking, the polystyrene particles used in this study pass through the gastrointestinal tract where they become coated with a protein corona. These aggregates are then likely opsonized by IgG antibodies in the circulation, resulting in their uptake by Kupffer cells. It is possible that prolonged residence within the phagolysosomal compartment of Kupffer cells may lead to partial degradation and release of plastic-associated compounds. However, previous attempts to isolate Kupffer cells for lipidomics analyses failed due to the limited cell numbers and the fragility of these cells during sorting, which is currently the only feasible approach to isolate Kupffer cells without hepatocyte contamination for immediate downstream applications.

Furthermore, our washout experiments do not indicate major degradation and release of plastic material, as NP and MP remain detectable in the liver even 6 months after cessation of exposure (Extended Data Fig. 7).

b. it is not clear how the authors explain LPC/LPE/SM changes in Fig 1: if plastics do not induce huge changes in inflammatory cells infiltrates (that contain a lot of these lipid classes – this would have been an easy explaination), how the authors justify the observed changes in lipid composition? No explanation is provided. Do the snSEQ provide any information regarding a reshaping of metabolic pathways involved in LPC/LPE/SM metabolism? Please show at least the gene expression (e.g. by extracting data from Seq, or performing qPCR) of key rate limiting enzymes of these pathways that can justify the observed phenotype;

We have extracted the rate-limiting enzymes of the LPC/LPE/SM pathways from the snRNA-seq data and now present them with a mechanistic interpretation (lines 246-365, **new Fig. 1k-m, Extended Data Fig. 6j, k**): downregulation of *Pcyt2* with upregulation of *Pemt* (PE depletion and diversion into PC), reduced *Mfsd2a* (restricted LPC-coupled import), and coordinated *Cers6*/*Degs1*/*Sgms2*/*Ugcg* changes (ceramide channeling toward sphingomyelin). This provides an enzyme-level explanation for the observed LPC/LPE/SM phenotype that does not require an inflammatory-infiltrate source.

c. The sentence "… all of which are associated with hepatic metabolic disorders" is very general … authors should clarify what do they mean: the fact that changes in a specific lipid class might have been characterised in a specific disease does not necessarily mean that same concepts apply to this specific context. This superficial discussion of metabolic data is repeatedly used throughout the manuscript and very confusing.

We have revised this statement to specify the particular lipid species and the directional change relevant to our context, and removed the broad disease association, contextualising the finding within hepatic phospholipid remodelling rather than a generic disorder reference.

3) I disagree with the sentence at page 7 line 232 "consistent with preserved insulin sensitivity, quantification of insulin-producing B-cells in the pancreas revealed no difference among conditions". This is conceptually wrong: insulin production and sensitivity are two completely different concepts (moreover in an ITT insulin is provided from outside the body … so insulin production does not really matter during an ITT …). I instead wonder if changes in insulin/glucagon/GLP1 occur at 60-120 min of GTT (assuming blood is still available – I understand it is very little and not be feasible retrospectively) as they might justify the worsening of glucose tolerance (GTT) despite the body weight loss. Or focussing on intrahepatic mechanisms (e.g. AKT phosphorylation to prove hepatic insulin resistance?).

We agree with the reviewer that the statement linking preserved insulin sensitivity to pancreatic beta-cell abundance was conceptually imprecise. We have removed this wording accordingly. Regarding dynamic endocrine responses during GTT experiments, we are unfortunately limited by remaining sample availability. Further, we do not believe that these extensive studies are necessary for the central conclusions of the study but we appreciate the reviewer's suggestions, which will be incorporated into the design of future experiments.

4) The pathway analysis in snRNA-seq should be improved to extract more biological value:

a. By showing DEGs that enrich the different significantly regulated pathways;
b. By focussing on LPC/LPE/SM metabolism;
c. By focussing on aspects of liver metabolism involved in hepatic lipid input and output (see above);
d. By performing a transcription factor analysis (e.g. VIPER or IPA Upstream Regulator Analysis) in hepatocytes and KC to provide a better understanding of which processes might

be occurring there. In light of the changes in BW/EE/hepatic fat and GTT it is likely, for example, that there will be a prediction of modulation of glucose/lipid transcriptional regulators such as PPARs, ChREBP, SREBPs, and/or other lipid sensing nuclear receptors (that might enlighten the mechanism behind the phenotype); again, can the suppression of Cyp7b1 can be associated to increased LXR activity (as discussed above)?

These analyses are now included (lines 246-365):
a) DEGs underlying each enriched module focusing on lipid metabolism are shown (**new Fig. 1l, Extended Data Fig. 6j**), including
b) the LPC/LPE/SM enzymes and
c) lipid input (uptake receptors *Cd36*, *Ldlr*, *Vldlr*, *Scarb1*) and output (VLDL: *Mttp*, *Sar1b*, *Creb3l3*; FAO components)
d) VIPER transcription-factor activity is now reported for both hepatocytes (**new Fig. 1m**) and KCs (**new Fig. 3i**), recovering PPARα/PPARγ and nuclear-receptor regulators as predicted by the reviewer. Regarding *Cyp7b1*/LXR: as *Nr1h3* (LXRα) is downregulated and DNL targets are suppressed, our data do not support increased LXR activity as the driver.

e. The above mentioned results can be better discussed in light of (and correlated to) systemic changes and histological findings; for example the changes in APOA1 might reflect changes in HDL metabolism? Does APOCIII influence VLDL metabolism (toward inhibition of LPL increased TGs are expected: however, as discussed above, the value of these results cannot be appreciated here since circulating HDL/LDL cholesterol and TGs have not been shown …)?
As it sits, the snSEQ data do not allow to fully capture the degree of metabolic dysregulation occurring in the liver, and how much these changes are due to intra-hepatic mechanism or rather reflecting systemic metabolic changes that need to be taken in deep consideration. As it currently reads, these data look like a pure bioinformatic exercise with very little biological value (which is a pity given the nice phenotype and the huge amount of work).

We have revised the discussion to integrate the transcriptional findings with the systemic and histological phenotypes, linking hepatocyte lipid-handling gene changes to the ORO and lipidomic data (see response to 4d).

5) The in vitro co-culture experiments should be better described and expanded: why MP-KCs induce hepatocyte lipid droplet formation? It is a matter of increased hepatocyte input (uptake? DNL?) or reduced hepatic output (FAO? VLDL export?)? Plastics releasing palmitate in KC? Ideas will come from the results of points above while in vitro experiments should validate these predicted mechanisms mechanistically with a single well designed “killer experiment” (better if using appropriate advanced MASLD models and avoiding 2D cultures – see PMID: 41582251). This is a crucial point for Nat Met readership.

We have expanded the description of the co-culture system, which is an overnight culture, and now interpret it explicitly in the context of the uptake-versus-disposal framework: because MP-KC-derived signals induce hepatocyte lipid droplets overnight in cells with intact export machinery, the phenotype reflects increased lipid input rather than a VLDL-export block, which we propose is a later consequence of sustained overload in vivo. We respectfully note that advanced 3D MASLD systems and lipid-flux validation would constitute a separate project (see Point 1d) and exceed the revision scope; we now state this as a future direction in the discussion.

6) KC phagocytic capacity: are these results any relevant in this manuscript? I wonder if these data can be used for a follow-up story? They look substantially unrelated to the main trajectory of the paper that in my opinion should be more tailored to systemic and intra-hepatic metabolic unbalances (that are the main results). Moreover, these data sound a bit confusing considering that no mayor changes in the immune system of the liver is observed

in the specific obesity settings explored (and the role of microbiota/KC in MASLD is pretty much established). These data can be a good basis for a follow-up story focussing on wound healing, liver regeneration and advanced disease stages (using appropriate models of disease progression).

We would like to emphasize that that impaired phagocytosis is central to the whole story, and we have clarified this in the text (lines 553-559). The phagocytic defect is detectable after only 4 weeks of MP exposure, **before** hepatic lipid accumulation is measurable (Fig. 3j, Extended Data Fig. 5g, h). Positioning macrophage dysfunction upstream of the metabolic phenotype is rather causal than as a parallel observation. We make this temporal, upstream relationship explicit in the discussion (lines 745-750).

7) Following up from the previous point, in the discussion, please stress the fact that the observations provided in this manuscript relate to “lean” (chow) and very mild (short term HFD) Obesity/MASLD-inducing conditions, and that it cannot be excluded that in the context of longer challenges and progressive MASH models (e.g. WDs), effects on inflammation and fibrosis might arise. No need to provide additional experiments, but this is a crucial point to discussed to shape future directions in the field (see – see PMID: 41582251; PMID: 38867022). In line with this, it is also relevant to discuss for Extended Data Figure 5 the fact that short term HFD is not a good model to study hepatic fibrosis in the context of MASLD, and more studies are needed with appropriate models to exclude an impact of plastics on disease progression.

We have added an explicit limitation to the discussion stating that our findings were obtained under lean and mild high-fat conditions, that short-term high-fat feeding is not an appropriate model for MASH or fibrosis, and that progressive models such as Western diets will be required to assess the inflammatory and fibrotic consequences of chronic plastic exposure on disease progression (lines 699-706).

Response to Editors' Comments

Dear Christoph, dear Yanina,
We thank you for the detailed guidance. Below we address each editorial point, in case it was not already part of the previously submitted manuscript:

Limitations of the work. We have now expanded the limitation in the discussion as suggested by the reviewer.

Addition of data. All data mentioned by the reviewer which he/she wanted to be included are now part of the manuscript (in Extended Data Fig 8 and SI Fig. 8).

Redundancy in the text. We have extensively shortened the part of the results that the reviewer requested during the last revision on gene expression and transcription factor activity. The reviewer now seems also to be fine with this shortening. As suggested by you, we did not implement the comments by the reviewer on pathway enrichment analysis.

We have already changed the manuscript to adhere to the provided guidelines during the last revision.

Reviewer #7:

The authors have carefully addressed the major concerns raised during the previous round of review, and the manuscript has substantially improved in both quality and readability. I appreciate the considerable effort invested in the revision. I only have a few minor comments that, in my opinion, would further strengthen the manuscript:

1. Plasma lipid profile. I respectfully disagree with the decision not to report the circulating lipid profile because the differences were not statistically significant. In this case, the absence of significant changes is informative, as it supports the authors' interpretation that the observed phenotype is predominantly driven by intrahepatic mechanisms, particularly given that fasting insulin and NEFA measurements were not available. Along the same lines, I believe the lack of information regarding systemic insulin resistance and adipose tissue function should be more explicitly acknowledged in the limitations. Since adipose tissue function is central to the interpretation of systemic and hepatic metabolism, these missing data somewhat limit the mechanistic interpretation of the findings.

We have now added the circulating lipid profile as SI Fig. 8 as well as a paragraph on the limitations (lines 694-697 in the manuscript version with track changes).

2. FGF21 and glucagon measurements. I could not locate the newly generated FGF21 and glucagon data in the revised manuscript. While I understand that elucidating the upstream mechanisms regulating these hormones is beyond the scope of the present study and may represent future work, these measurements are important to provide a plausible framework explaining the apparent divergence between energy expenditure, hepatic steatosis and glucose tolerance in HFD-fed mice. I therefore encourage the authors to include these data in the manuscript and integrate them into the discussion.

To clarify, my intention is not to challenge the authors' decision to approach the study from a macrophage-centric perspective. Rather, I believe that providing this metabolic context would make the findings more accessible and interpretable for the broad readership of Nature Metabolism.

We have included the FGF21 and glucagon data in Extended Data Figure 8c, d.

3. Discussion of lipidomics and transcriptomics. The expanded discussion of the lipidomic and sequencing datasets considerably improves the mechanistic interpretation of the study. However, I find this section somewhat lengthy and, at times, repetitive. The interpretation of the different layers of evidence (DEGs, lipidomics, pathway analysis and VIPER) could likely be better integrated to avoid reiterating similar concepts. In addition, I would recommend avoiding extensive discussion of individual genes or lipid species, or mechanistic interpretations that are not directly supported by experimental evidence, as some conclusions (for example regarding SCD1 regulation and its downstream effects) appear somewhat speculative. As a minor point of curiosity, was FXR predicted to be inhibited, given the apparent changes in bile acid metabolism?

We have adjusted and cut the manuscript to avoid repetitions and reiterations of concepts. Regarding FXR: Neither the gene *Nr1h4* encoding for nor the TF activity of FXR were altered upon MNP treatment.

4. Pathway enrichment analysis. I am not entirely clear on how the pathway enrichment results are presented. Typically, only significantly enriched pathways are discussed, whereas several non-significant pathways also appear to be displayed and interpreted here. In addition, activation or inhibition is usually represented using the normalized enrichment score (NES). Finally, could the authors clarify whether VIPER analysis was performed on

differentially expressed genes rather than on the complete expression matrix? A DEG-based analysis might more directly identify transcription factors whose activity is altered by plastic exposure and could further strengthen the mechanistic interpretation.

On presentation: our over-representation analysis (ORA) is run on the DEG sets, and the terms shown are the significantly enriched ones. Non-significant terms appear in the combined delta module scores for nine curated lipid metabolism gene sets. The way these were generated are outlined in the methods.
We note that the NES is a metric specific to GSEA. In this study, we deliberately employed ORA because, given the limited number of biological replicates, it provides a more conservative and robust approach for identifying biological processes associated with chronic plastic exposure than rank-based enrichment methods that rely on a stable genome-wide gene ranking.

On VIPER: the analysis was performed on pseudobulk expression profiles per cell type, sample and condition using the DoRothEA regulon, i.e. on the coordinated expression of each regulon rather than on a pre-filtered DEG list. We agree that a DEG-restricted analysis is a reasonable complementary approach and we note it as such. We retain the regulon-based approach here because it recovers coordinated activity changes that are not captured by thresholded DEGs alone.

5. The authors may also wish to cite the recent review on plastics and liver disease published in Nature Reviews Gastroenterology & Hepatology (PMID: 41946921), which provides a timely overview of the field and would help place the present findings in a broader context

We have added this review to the discussion (now Ref. 31).