molecular systems biology

Model-driven multi-omic data analysis elucidates metabolic immunomodulators of macrophage activation

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 06 February 2012

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the two referees who agreed to evaluate your manuscript. As you will see from the reports below, the referees find the topic of your study of potential interest. However, they raise several concerns on your work, which should be addressed in a revision of the present work.

Reviewer #1 feels that experimental follow up of some of the network-based predictions would be very important. We have circulated the reports among the present referees to give them the chance to comment on each other's report. As an outcome of this process, Reviewer #2 agreed that experimental validation of novel immune modulators would indeed dramatically increase the impact of the study, but felt that it would not be mandatory in the context of this particular study. We do recognize that the study already combines computational predictions with multiple omics data and we thus tend to share the opinion of reviewer #2.

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If you feel you can satisfactorily deal with these points and those listed by the referees, you may

wish to submit a revised version of your manuscript. Please attach a covering letter giving details of the way in which you have handled each of the points raised by the referees. A revised manuscript will be once again subject to review and you probably understand that we can give you no guarantee at this stage that the eventual outcome will be favourable.

Best wishes,

Editor
Molecular Systems Biology

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Referee reports:

Reviewer #1 (Remarks to the Author):

The authors have reconstructed the complete metabolic network of RAW 264.7 macrophages and have used this reconstruction to predict the impact of gene deletions and restrictions in the exchange of external metabolites on the production of several metabolites that are known to be of importance for the immune response (e.g. generation of NO) of the cell. Their analysis identified a couple of nutrients that upon restriction should significantly modulate the immune response. Moreover, the authors generated omic's data to validate the theoretically predicted changes of pathway fluxes which occur during macrophage activation.

In summary, this is a well-done study combining large-scale network analysis with high-throughput data.

Main criticism:
1.) The presented study is basically confirmative. All external metabolites identified to be relevant for the efficient production of those internal metabolites required for the anti-pathogenic action of the macrophage have already been described and extensively studied in the literature. In some cases, the predicted effects are even discordant with experimental findings (see below). To demonstrate the real benefit and of their large-scale systems biology approach it is imperative that the study is extended with the aim to generate experimentally testable hypothesis on novel metabolic immune modulators and to present at least one successful experimental verification of such network-based prediction.

2. Another critical point pertains the physiological relevance of this study as all results were generated for macrophages cultivated under in vitro conditions. Speaking of metabolic modulators of the macrophage's immune response one gets the impression that changes in the availability of certain nutrients (glucose, glutamine, oxygen etc.) will somehow modulate (i.e. either enhance or attenuate) the activation of the macrophages. Are there examples for a limited supply of these metabolic modulators in vivo? Are changes in the plasma concentration of glucose, arginine or glutamine in a range that fits to the degree of inhibition of the exchange fluxes that according to the model simulations are required to compromise the immune response? To make the simulations more compatible with the in vivo situation the rate of the exchange fluxes should be modelled by the (known) transport kinetics and in the presence of an external environment that resembles the nutrient composition of the interstitial fluid.

Other critical points:

3. "To characterize the metabolic functionality of the three algorithm-derived networks as well as the reconciled model, the networks were put through stringent testing of 288 metabolic functions validated in Recon 1"
Recon1 is an omnipotent network that doesn't exist in any human cell. Is it, therefore, feasible to demand the macrophage network to satisfy all metabolic objectives tested with Recon1? Please provide a list of metabolic objectives reported for macrophages including a reference to the source of this information.

4. "Oxygen, glucose, and glutamine have all been previously shown to be of great importance in macrophage metabolism and activation as they are key for cellular respiration, energy production, and respiratory burst".
From this statement one might conclude that high glucose concentrations are beneficial for macrophages to fulfil their anti-pathogenic action. However, the opposite is true. Hyperglycemic levels of glucose (8-20mM) have been shown to inhibit the release of IL-1 by lipopolysaccharide-stimulated RAW 264.7 murine macrophage cells and thus to attenuate their capacity to fight bacterial infections (Hill, J.R., Kwon, G., Marshall, C.A. and McDaniel, M.L. (1998). Hyperglycemic levels of glucose inhibit interleukin 1 release from RAW 264.7 murine macrophages by activation of protein kinase C. J Biol Chem 273, 3308-13) . This dissent also demonstrates the limitations of the presented study which considers the immune-modulating role of exchangeable metabolites (like glucose) only as being necessary precursors for the production of pro-inflammatory target metabolites but disregards their role as regulators of signalling pathways (see also 5.)

5 "Tryptophan and phenylalanine were highly ranked amongst substrates that had a suppressive effect on M1 and M2 metabolic activation phenotypes".
The authors cite the wrong papers to verify the validity of their finding. The contrary is true: In tryptophan-starved macrophages the induction of NO synthase induction was found to be significantly reduced (Chiarugi, A., Rovida, E., Dello Sbarba, P. and Moroni, F. (2003). Tryptophan availability selectively limits NO-synthase induction in macrophages. J Leukoc Biol 73, 172-7). Again (see 4.), the discrepancy between predicted and observed effect points to the limitation of the approach die to the neglect of the gene-regulatory role of metabolites.

6. The authors should explain in more detail why the measured fold-changes of transcripts and reporter metabolites are consistent with their theoretical predictions. Does the FBA calculationd allow to make inferences about changes of metabolite levels (except exchangeable end products)?

.

Reviewer #2 (Remarks to the Author):

Summary:
Bordbar et al. constructed a genome-scale model of the RAW 264.7 (Mouse Leukaemic Monocyte Macrophage) cell line to study the metabolic alterations that accompany macrophage activation. By applying the model the authors examined the affects of the different metabolites and metabolic pathways on the immune response. Glucose and arginine were identified as immune-activators, while tryptophan and vitamin D3 were identified as immune-suppressors. The regulation of glutamine and glutamate towards nucleotide synthesis and malate aspartate shuttle was suggested to suppress macrophage activation. The model predictions were shown to be supported by the literature and by experimental measurements across three levels of gene expression, protein abundance and metabolites. The analysis demonstrates the relationship between metabolism and immunity, indicating that the immune response is dictated to some extent by the metabolic capabilities of the macrophages.
In general, the paper makes an important systems wide contribution to the study of macrophage activation and the relations between metabolism and the immune system. However, some aspects of the study need to be elucidated and strengthened to further increase the potential impact of the paper. Below we give specific suggestions for improving the paper, and assuming those can be adequately addressed, this paper is bound to be of considerable interest to the wide readership of MSB.

Major Comments:
1. The model reconstruction is performed by utilizing three alternative methods (iMAT,GimmeBM, and GimmeP), resulting in three different models. Then, based on the latter a unified model is manually obtained.

a. It is unclear why three methods are used instead of one improved version of a construction algorithm. Please explain.

b. The final stage of manually integrating the models is not clearly explained - please revise the write-up and extend.

c. One of the methods that were applied is iMAT. As iMAT is not a model constructing algorithm (having potentially many solutions at optima), it is unclear how a model was obtained via this method, and should be further explained.

2. As the cell-line model is a subset of the published homolog-based mouse reconstruction (Sigurdsson et al. 2010), it is important to compare between the performances of the original and macrophage specific model. To convince the readers that the RAW 264.7 model is more suitable to study macrophages' metabolism than the original model, the authors should provide functional analysis demonstrating that the predictive ability of the new model outperforms that of original.

3. Regarding the sensitivity and sampling analysis of metabolite exchanges, are the relations between the exchange reactions and the objectives completely monotonic? If not, how were fluctuations accounted for?

4. The authors show, by referring to specific examples, that the in-silico model-based predictions are consistent with gene expression, protein abundance and metabolite measurements. The significance of this consistency should be statistically quantified and supported by p-values - these now are only partially provided in a non-systematic manner.

5. It could be interesting to constraint the model according to the gene expression that was measured in the times of macrophage stimulation (using iMAT or Gimme) and examine whether the predicted metabolic phenotypes are consistent with the previous predictions already given.

Minor comments:

1. Page 6 line 223 - "It is important to note that that", redundant that.

2. Page 13, line 538- it is unclear why Figure 1A is referred to.

3. It should be clearly stated at an early stage that the RAW 264.7 cell line is a mouse leukaemic monocyte macrophage cell line.

1st Revision - authors' response 20 March 2012

We would like to thank both reviewers for their constructive criticism of the manuscript. Their suggestions have guided our efforts in improving and clarifying the content of our original manuscript, and we believe that the reviewers will now endorse the work for publication in MSB. In particular for Reviewer #1, we have further clarified our systems analysis that points to a highly complementary role for metabolism during activation/suppression signaling and highlight with additional experimental literature that support the immunomodulatory roles of glucose metabolism and tryptophan catabolism. For Reviewer #2, we have performed additional computational analyses to confirm the need for a cell-specific network, systemically shown the accordance of the omics data to the predictions, and updated the sensitivity analysis results. A line-by-line account of revisions is provided below.

Reviewer #1 (Remarks to the Author):

The authors have reconstructed the complete metabolic network of RAW 264.7 macrophages and have used this reconstruction to predict the impact of gene deletions and restrictions in the exchange of external metabolites on the production of several metabolites that are known to be of importance for the immune response (e.g. generation of NO) of the cell. Their analysis identified a couple of nutrients that upon restriction should significantly modulate the immune response. Moreover, the authors generated omic's data to validate the theoretically predicted changes of pathway fluxes which occur during macrophage activation.

In summary, this is a well-done study combining large-scale network analysis with high-throughput data.

Main criticism:
1.) The presented study is basically confirmative. All external metabolites identified to be relevant for the efficient production of those internal metabolites required for the anti-pathogenic action of the macrophage have already been described and extensively studied in the literature. In some cases, the predicted effects are even discordant with experimental findings (see below). To demonstrate the real benefit and of their large-scale systems biology approach it is imperative that the study is extended with the aim to generate experimentally testable hypothesis on novel metabolic immune modulators and to present at least one successful experimental verification of such network-based prediction.

The reviewer is correct in stating that the putative immunomodulatory metabolites predicted by our model are known to influence macrophage activation. However, the goal of our work was to provide a systems level framework for analyzing multi-omics data sets for this commonly used macrophage cell line. We found it rather interesting that metabolites that are known to primarily influence activation phenotypes through signaling and transcriptional processes also appeared to possess the ability to influence activation phenotypes solely by their metabolism. We have written more text around this point (Lines ~480) , as well as emphasizing publications that address the interface between metabolism and immunity that has recently emerged to be more important than previously thought.

2. Another critical point pertains the physiological relevance of this study as all results were generated for macrophages cultivated under in vitro conditions. Speaking of metabolic modulators of the macrophage's immune response one gets the impression that changes in the availability of certain nutrients (glucose, glutamine, oxygen etc.) will somehow modulate (i.e. either enhance or attenuate) the activation of the macrophages. Are there examples for a limited supply of these metabolic modulators in vivo? Are changes in the plasma concentration of glucose, arginine or glutamine in a range that fits to the degree of inhibition of the exchange fluxes that according to the model simulations are required to compromise the immune response? To make the simulations more compatible with the in vivo situation the rate of the exchange fluxes should be modelled by the (known) transport kinetics and in the presence of an external environment that resembles the nutrient composition of the interstitial fluid.

This is an interesting comment and would be highly appropriate if we were presenting a model derived from primary cells. The work that we've presented here was designed to provide a systems perspective for analyzing the immortalized RAW 264.7 cell line and it would likely be too great of a leap to try to interpret this model in an *in vivo* fashion. We would like to re-emphasize that our sensitivity analysis was not implying how nutrient concentrations would directly drive the immune response, per se. Rather, our analysis identifies the substrate nutrients that may be taken up more (or 'recruited') in order to more effectively produce the target activation metabolites (e.g. NO). This has been demonstrated in previous experiments on activated macrophages, in which the uptake of arginine and glucose were increased based on necessity to promote NO production during its activated form. These references are noted in Line 217.

3. "To characterize the metabolic functionality of the three algorithm-derived networks as well as the reconciled model, the networks were put through stringent testing of 288 metabolic functions validated in Recon 1"

Recon1 is an omnipotent network that doesn't exist in any human cell. Is it, therefore, feasible to demand the macrophage network to satisfy all metabolic objectives tested with Recon1? Please provide a list of metabolic objectives reported for macrophages including a reference to the source of this information.

The reviewer is correct in asserting that using these 288 metabolic functions as a requirement for a cell-line specific model is not a viable criterion. In our work, we did not require that the final RAW 264.7 meet these objectives; we used these objectives as a guide for identifying the differences amongst the 3 draft reconstructions that we further investigated by manual curation of biochemical literature. There is no perfect method for using omics data to make a cell-specific model thus we combined the results of two published methods and one that we developed here into a reconciled model; the reconciliation was guided in part by determining when there was discordance amongst the draft models regarding these functions (*i.e.,* there was not complete concordance among the models constructed with these automated methods). We have clarified this point in both the main (Lines ~534-538) and supplementary texts (Page 3, first paragraph). In addition, we have appended the differential metabolic functions and the citations for reconciliation to the Supplementary Material.

4. "Oxygen, glucose, and glutamine have all been previously shown to be of great importance in macrophage metabolism and activation as they are key for cellular respiration, energy production, and respiratory burst".

From this statement one might conclude that high glucose concentrations are beneficial for macrophages to fulfil their anti-pathogenic action. However, the opposite is true. Hyperglycemic levels of glucose (8-20mM) have been shown to inhibit the release of IL-1 by lipopolysaccharide-stimulated RAW 264.7 murine macrophage cells and thus to attenuate their capacity to fight bacterial infections (Hill, J.R., Kwon, G., Marshall, C.A. and McDaniel, M.L. (1998). Hyperglycemic levels of glucose inhibit interleukin 1 release from RAW 264.7 murine macrophages by activation of protein kinase C. J Biol Chem 273, 3308-13) . This dissent also demonstrates the limitations of the presented study which considers the immune-modulating role of exchangeable metabolites (like glucose) only as being necessary precursors for the production of pro-inflammatory target metabolites but disregards their role as regulators of signalling pathways (see also 5.)

Please see response to point #2 above regarding glucose's role during activation. The De Souza 2008 paper cited in the main text shows that LTA stimulated RAW 264.7 cells under hyperglycemia have higher metabolic activation properties such as producing more nitric oxide. Other non-metabolic increases include production of tumor necrosis factor alpha and secretion of matrix metalloproteinase 9. Plus chronic inflammation is a common symptom of type 2 diabetics.

We also state in Lines 294-296 that our approach is limited in scope (i.e. does not include regulation and signaling) but has the potential to evaluate the metabolic pathways that may be affected to support macrophage activation.

5. "Tryptophan and phenylalanine were highly ranked amongst substrates that had a suppressive effect on M1 and M2 metabolic activation phenotypes".

The authors cite the wrong papers to verify the validity of their finding. The contrary is true: In tryptophan-starved macrophages the induction of NO synthase induction was found to be significantly reduced (Chiarugi, A., Rovida, E., Dello Sbarba, P. and Moroni, F. (2003). Tryptophan availability selectively limits NO-synthase induction in macrophages. J Leukoc Biol 73, 172-7). Again (see 4.), the discrepancy between predicted and observed effect points to the limitation of the approach die to the neglect of the gene-regulatory role of metabolites.

In the case of the suppressive metabolites, such as tryptophan, increased flux through its catabolic pathway may be a potential mechanism by which it would inversely affect production of the target activation metabolites. Notably, it is widely known that tryptophan catabolites have an immunosuppressive role, and the tryptophan catabolite 3-hydroxyanthranilic acid has been shown to suppress NO production in activated murine macrophages. We have modified the text in Lines 227-234 which supports the immunosuppressive role of tryptophan catabolism. Lines 294-296 discuss the limitations of our approach regarding regulation and signaling, but has the potential to evaluate the metabolic pathways that are changed as a result of macrophage suppression.

6. The authors should explain in more detail why the measured fold-changes of transcripts and reporter metabolites are consistent with their theoretical predictions. Does the FBA calculationd allow to make inferences about changes of metabolite levels (except exchangeable end products)?

From our sensitivity and sampling analysis, we determined key reactions that were either characteristic of activation or inhibition of macrophage phenotypes (see Figure 4B). We compared our *in silico* derived areas key to activation to transcriptional changes. To do so, we determined where the major transcript changes were occurring using reporter metabolites analysis (Patil 2005 PNAS). This analysis does not predict changes in metabolite levels; instead the method determines the areas of significant statistical enrichment of transcription changes. We found that the major areas of enrichment were the areas we classified as characteristic for activation/inhibition (Figure 4). We have further clarified the reporter metabolite analysis in the text (Lines 361-375).

We also verified whether our model predictions on flux changes were consistent with the generated omics data. We hypothesized that areas important for activation would be up-regulated and areas important for suppression would be down-regulated in LPS stimulated RAW macrophages. The transcriptomic and proteomic data were in high accordance with model predictions.

Though metabolomics does not provide direct evidence of reaction changes, if there is a flux split (i.e. the split between activation and nucleotide metabolism from glutamine) and metabolites in one pathway are significantly higher, and whereas those in the other pathway are significantly lower, we can infer that there is an inverse pathway shift. This point has been clarified in the main text (Lines 411-414).

Reviewer #2 (Remarks to the Author):

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Bordbar et al. constructed a genome-scale model of the RAW 264.7 (Mouse Leukaemic Monocyte Macrophage) cell line to study the metabolic alterations that accompany macrophage activation. By applying the model the authors examined the affects of the different metabolites and metabolic pathways on the immune response. Glucose and arginine were identified as immune-activators, while tryptophan and vitamin D3 were identified as immune-suppressors. The regulation of glutamine and glutamate towards nucleotide synthesis and malate aspartate shuttle was suggested to suppress macrophage activation. The model predictions were shown to be supported by the literature and by experimental measurements across three levels of gene expression, protein abundance and metabolites. The analysis demonstrates the relationship between metabolism and immunity, indicating that the immune response is dictated to some extent by the metabolic capabilities of the macrophages.

In general, the paper makes an important systems wide contribution to the study of macrophage activation and the relations between metabolism and the immune system. However, some aspects of the study need to be elucidated and strengthened to further increase the potential impact of the

paper. Below we give specific suggestions for improving the paper, and assuming those can be adequately addressed, this paper is bound to be of considerable interest to the wide readership of MSB.

Major Comments:
1. The model reconstruction is performed by utilizing three alternative methods (iMAT,GimmeBM, and GimmeP), resulting in three different models. Then, based on the latter a unified model is manually obtained.
a. It is unclear why three methods are used instead of one improved version of a construction algorithm. Please explain.

At the time when the models were built, there were two methods to integrate omics data with a global metabolic network (GIMME and iMAT). Both methods have their deficiencies. Thus we decided to improve GIMME. Instead of the traditional version of GIMME that utilizes one objective (noted as GimmeBM for a singular biomass objective), we defined a set of core reactions that were determined from the proteomic data. We then ran GIMME for each objective separately utilizing the transcription data as the input. We were able to build sub-models, which we then combined for the final GIMMEp model. This approach outperformed all other approaches (see Supplementary Material). The GIMMEp approach is similar to the MBA method (originally Jerby MSB 2010 and updated in Folger MSB 2011) in the use of a core reaction set. However, the MBA approach (as per Folger MSB 2011) builds a parsimonious model by including as few additional reactions outside of the core reaction set. Instead, GIMMEp adds additional reactions based on the transcriptomic data on an as-needed basis to satisfy flux through the proteome-based objective functions.

Multiple methods were used because there are no perfect methods for using omics data to create a tissue-specific model with the existing methods having their own benefits and drawbacks. Combining multiple algorithms to improve performance is a relatively commonplace procedure. Because the methods employed are substantially different it was not possible to combine them into a super-algorithm, however, it is possible to compare the outcomes of each model to identify points that should be reconciled by comparison to existing literature.

b. The final stage of manually integrating the models is not clearly explained - please revise the write-up and extend.

Reconciliation was performed by identifying functional differences between the draft models and then resolving these differences by searching available biochemical literature. We have clarified this on lines 540-544. We have updated the Supplementary Data to include supporting literature citations.

c. One of the methods that were applied is iMAT. As iMAT is not a model constructing algorithm (having potentially many solutions at optima), it is unclear how a model was obtained via this method, and should be further explained.

c.) The reviewer is correct that iMAT is not a model building algorithm. It does however provide a flux distribution that is consistent with the omics data provided and is unbiased by objectives

(GIMME and GIMMEp). We used the omics data consistent flux distribution derived from iMAT as the third network as an unbiased assessment to further help with building the model. It is important to note that the final reconstructed model was most similar to the new GIMMEp model as the algorithm is a true model building algorithm.

We have updated the main (Lines 506-538) and Supplementary Text (Section 1) to address points a, b, and c.

2. As the cell-line model is a subset of the published homolog-based mouse reconstruction (Sigurdsson et al. 2010), it is important to compare between the performances of the original and macrophage specific model. To convince the readers that the RAW 264.7 model is more suitable to study macrophages' metabolism than the original model, the authors should provide functional analysis demonstrating that the predictive ability of the new model outperforms that of original.

Although the model of Sigurdsson *et al.* was constructed in a similar fashion as the global murine model used here, we did not use the model from Sigurdsson *et al.* to construct our model. We have updated our manuscript to include comparisons between our global murine model and the cell specific model. For the global murine model, the growth rate was higher (11.1 hour doubling time) but within the physiological range of 11-24 hours as mentioned in the cited references. Also, the ATP production and NO production were greatly inflated (i.e. 3X ATP and 3.3X NO production rates). These results strongly demonstrate that the smaller, RAW-specific model was quantitatively more accurate in predicting in vitro production rates than the Recon 1 network.

We also reran the sensitivity analysis and found the results to be significantly different than the results we previously determined for the cell specific network. For the activating substrates arginine and glutamine, they had little effect on activation properties and in particular had no effect on NO production. In addition, the most suppressive substrate was phosphate. As for produced metabolites, urea and glutamate were no longer activating as previously determined.

The comparison between the cell specific and generic metabolic networks is now mentioned in the text (Lines 132-138). A full analysis is also provided in the Supplementary Material (Supplemental Text, section 9; Figure S4).

3. Regarding the sensitivity and sampling analysis of metabolite exchanges, are the relations between the exchange reactions and the objectives completely monotonic? If not, how were fluctuations accounted for?

347 of the 355 sensitivity analyses yield monotonic relationships. The effect of L-cysteine production on ATP, L-glutamate production on NADH, L-cystine uptake on NOS, NH4 uptake on PTRC, and NH4 production on ATP, NADH, NOS, PRO functions was not monotonic. We re-ran our analyses for the 8 non-monotonic with a focus on consumption rates that are in physiologically measured ranges and updated the manuscript accordingly (Figure 2, Lines 223-225, 275, 573-579).

L-cysteine production, L-glutamate production, and ammonia uptake had little change with the new results. However we did find two differences. First, we find that ammonia production can be activating. This new result is more consistent with urea and glutamate production's result as both metabolites allow for homeostatic recycling of nitrogen. Second, we find that L-cystine uptake can be highly effective for activating NOS production, as high as L-histidine. It has been previously shown L-cystine uptake is induced during murine macrophage activation by LPS (PMID: 7654193).

As for the sampling analysis, there were no changes as the solution space of a stoichiometric matrix is convex and thus all solutions are unimodal.

4. The authors show, by referring to specific examples, that the in-silico model-based predictions are consistent with gene expression, protein abundance and metabolite measurements. The significance of this consistency should be statistically quantified and supported by p-values - these now are only partially provided in a non-systematic manner.

To determine the significance of matching between model flux changes and the significantly changed genes and proteins, we did a permutation test 100000 times which was significant ($p < 1e-5$). We have now presented this test in a more clear manner in the supplement with the associated genes and proteins (fold change and p-value) and more information on the permutation test (Supplemental Text, part 10).

The metabolite measurements were compared qualitatively in terms of which pathways contained the significantly changed metabolites. There is no systematic method to compare the internal metabolomics data with the flux changes in the model.

5. It could be interesting to constraint the model according to the gene expression that was measured in the times of macrophage stimulation (using iMAT or Gimme) and examine whether the predicted metabolic phenotypes are consistent with the previous predictions already given.

This is an interesting observation, however, the omics data that we analyzed in this work were comparative (*i.e.,* two-channel) and used to provide an orthogonal view into portions of the network that were perturbed. To assess the consistency with the GIMME algorithm we would require a new one-channel quantitative data set. In addition, the portions of metabolism that we focus on would probably be active under both conditions, so no big change would occur in the inconsistency score as GIMME is dependent on transcripts being present or absent. Looking at the fold changes in the transcripts and proteins, as we have done, would provide a better sense of the actual induced change in those specific metabolic pathways

Minor comments:

1. Page 6 line 223 - "It is important to note that that", redundant that.

Has been removed

2. Page 13, line 538- it is unclear why Figure 1A is referred to.

Has been removed. Before the first submission, the text had been updated and the figure in question had been removed, but we missed updating that portion.

3. It should be clearly stated at an early stage that the RAW 264.7 cell line is a mouse leukaemic monocyte macrophage cell line.

Has been added (Line 58).

Accept letter 09 May 2012

Thank you again for sending us your revised manuscript. We have now heard back from the two reviewers how accepted to evaluate your revised study. Reviewer #2 is fully supportive of the work. Reviewer #1 still feels that the study should be extended by investigating experimentally one of the new hypotheses generated by your network analysis.

While we agree with reviewer #1 on the importance of generating novel biological insights, we also recognize the value of studies that find new ways to significantly advance the system-level interpretation of large-scale measurements in a biologically interesting context--in the case of this study, the impact of metabolic modulators of macrophage activation. In the case of this study, we feel therefore that the combination of a new human metabolic reconstruction with the integration of multi-omic data, including novel metabolomic profiling data, represents a sufficient advance to justify publication in Molecular Systems Biology. In view of the rather divergent views of both reviewers, we have also consulted with one of our Senior Editors who returned a postive and fully supportive recommendation. As such, I am pleased to inform you that your paper has been accepted for publication.

Thank you very much for submitting your work to Molecular Systems Biology.

Best wishes,

Editor
Molecular Systems Biology

http://www.nature.com/msb

Reviewer #1 (Remarks to the Author):

In my first report I criticized the paper as not being just very exciting for immunologists as all metabolic 'modulators' of the macrophage's immune response identified by the network-based approach are already known for a long while whereas experimentally testable hypothesis of novel modulators are missing. I think that the development of molecular systems biology has meanwhile reached the point where technical aspects should be more and more fade from the spotlight in favour of gaining new biological insights that other more traditional biochemical and cell biological methods will be able to provide only by chance and with much higher effort. Unfortunately, the authors declined to follow my strong advice to extend their study with the aim to generate experimentally testable hypothesis on novel metabolic immune modulators and to present at least one successful experimental verification of such network-based prediction. Therefore, I don't judge the paper as sufficiently innovative for publication in the leading scientific journal of molecular systems biology.

Reviewer #2 (Remarks to the Author):

The authors addressed our comments adequately, and modified the paper accordingly. Hence we find that the paper is suitable for publication in MSB.

