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Original research

Multiomics of the intestine-liver-adipose axis in multiple studies unveils a consistent link of the gut microbiota and the antiviral response with systemic glucose metabolism

Anna Castells-Nobau,[1,2,3,4] José Maria Moreno-Navarrete ,[1,2,4,5] Lisset de la Vega-Correa,[1,2,4] Irene Puig,[1,2,4] Massimo Federici ,[6] Jiuwen Sun,[3,7,8,9] Remy Burcelin ,[7,8,9] Laurence Guzylack-Piriou,[10] Pierre Gourdy,[11,12] Laurent Cazals,[11,12] María Arnoriaga-Rodríguez,[1,2,4,5] Gema Frühbeck,[4,13] Luisa Maria Seoane ,[4,14] José López-Miranda,[4,15] Francisco J Tinahones,[4,16] Carlos Dieguez,[4,17] Marc-Emmanuel Dumas ,[18,19,20,21] Vicente Pérez-Brocal,[22,23] Andrés Moya ,[22,23,24] Nikolaos Perakakis,[25] Geltrude Mingrone,[26] Stefan Bornstein,[25] Jose Ignacio Rodriguez Hermosa,[27] Ernesto Castro,[27] Jose Manuel Fernández-Real ,[1,2,4,5] Jordi Mayneris-Perxachs [1,3,4]

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For numbered affiliations see end of article.

Correspondence to
Dr Jose Manuel Fernández-Real; jmfreal@idibgi.orgDr Jordi Mayneris-Perxachs; jmayneris@idibgi.org and Dr José Maria Moreno-Navarrete; jmoreno@idibgi.org

AC-N and JMM-N contributed equally.

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ABSTRACT

Background The microbiota is emerging as a key factor in the predisposition to insulin resistance and obesity.

Objective To understand the interplay among gut microbiota and insulin sensitivity in multiple tissues.

Design Integrative multiomics and multitissue approach across six studies, combining euglycaemic clamp measurements (used in four of the six studies) with other measurements of glucose metabolism and insulin resistance (glycated haemoglobin (HbA1c) and fasting glucose).

Results Several genera and species from the Proteobacteria phylum were consistently negatively associated with insulin sensitivity in four studies (ADIPOINST, n=15; IRONMET, n=121, FLORINASH, n=67 and FLOROMIDIA, n=24). Transcriptomic analysis of the jejunum, ileum and colon revealed T cell-related signatures positively linked to insulin sensitivity. Proteobacteria in the ileum and colon were positively associated with HbA1c but negatively with the number of T cells. Jejunal deoxycholic acid was negatively associated with insulin sensitivity. Transcriptomics of subcutaneous adipose tissue (ADIPOMIT, n=740) and visceral adipose tissue (VAT) (ADIPOINST, n=29) revealed T cell-related signatures linked to HbA1c and insulin sensitivity, respectively. VAT Proteobacteria were negatively associated with insulin sensitivity. Multiomics and multitissue integration in the ADIPOINST and FLORINASH studies linked faecal Proteobacteria with jejunal and liver deoxycholic acid, as well as jejunal, VAT and liver transcriptomic signatures involved in the actin cytoskeleton, insulin and T cell signalling. Fasting glucose was consistently linked to interferon-induced genes and antiviral responses in the intestine and VAT. Studies in *Drosophila melanogaster* validated these human insulin sensitivity-associated changes.

Conclusion These data provide comprehensive insights into the microbiome-gut-adipose-liver axis and its impact on systemic insulin action, suggesting potential therapeutic targets.Cite Now

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ In recent years, there has been growing recognition of the significant systemic effects of microbiota diversity and composition on the predisposition to obesity, type 2 diabetes and metabolic syndrome. However, most studies investigating the connections between gut microbiota and insulin sensitivity have used single-omics approaches focused on specific tissues and limited to individual human studies. Consequently, our understanding remains fragmented. The limitations of previous studies highlight the necessity for a more integrative approach to comprehend the interplay among gut microbiota, intestine, adipose tissue and liver in influencing systemic insulin action.

INTRODUCTION

Obesity is associated with multiple metabolic alterations, specifically insulin resistance and type 2 diabetes (T2D). The last years witnessed the recognition of strong systemic effects of microbiota in determining propensity to obesity, T2D and metabolic syndrome.[1 2] In experimental models, the gut microbiota has been shown to increase intestinal permeability, favouring the translocation of microbiome-derived lipopolysaccharide (LPS) to the bloodstream. This leads to metabolic endotoxaemia, which initiates obesity and insulin

WHAT THIS STUDY ADDS

⇒ It is the first study to offer comprehensive insights into the microbiome-gut(jejunum, ileum and colon)-adipose-liver axis and its impact on systemic insulin action in humans, underscoring: (1) the pivotal role of Proteobacteria, bile acids, and T-cell related genes in influencing insulin sensitivity, (2) the involvement of Enterobacteria through several of the identified genes, and (3) associations between antiviral response genes across different tissues and fasting glucose. These findings significantly contribute to our understanding of the complex interplay among tissues and the gut microbiota within the context of obesity and insulin resistance.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The novel insights gained from our findings may pave the way for the development of innovative therapeutic strategies targeting the gut microbiota and the intestine-adiposeliver axis. Such strategies and targets hold the potential to enhance insulin sensitivity and improve metabolic health, representing promising avenues for advancement in clinical practice.

resistance.[3 4] While some research has explored the links between gut microbiota and insulin sensitivity in humans, most studies have used single-omic approaches focussing on individual tissues and are often limited to a singular human cohort. Consequently, our understanding remains fragmented, lacking a holistic understanding of the intricate interplay among the gut microbiota, intestine, adipose tissue and liver influencing systemic insulin action.[5] The limitations of prior studies underscore the need for a more comprehensive and integrative approach to grasp the complexities of these interactions.

In this work, we conducted an unparalleled investigation of the gut-adipose-liver axis in humans by applying a pioneering comprehensive multiomics, multitissue, cross-cohort integrative approach encompassing transcriptomics in the intestine (jejunum, ileum and colon), liver and adipose tissue (both visceral and subcutaneous). In addition, we incorporated plasma metabolomics, lipidomics of the intestine and plasma, and faecal, ileal and colon metagenomics, coupled with the gold standard assessment of insulin sensitivity using the hyperinsulinaemic-euglycaemic clamp, complemented by indirect measurements of insulin sensitivity such as fasting glucose and glycated haemoglobin (Hb1Ac) levels. This investigation was performed across six independent studies (ADIPOINST, n=29; IRONMET, n=121; SIMMUNIDIA, n=55; FLOROMIDIA, n=37; FLORINASH, n=80 and ADIPOMIT, n=740, online supplemental table 1) and validations in *Drosophila melanogaster*. Our unique approach highlighted an intricate crosstalk between the intestine, liver and adipose tissue and revealed a robust and reproducible microbial-metabolic-transcriptomics signature involving Proteobacteria, bile acids and T cell-related genes linked to insulin sensitivity.

RESULTS

Proteobacteria and Erysipelotrichaceae species are negatively associated with insulin sensitivity

Few studies have investigated the relationship between gut microbiota and insulin sensitivity using the hyperinsulinaemic-euglycaemic clamp.[6–11] Importantly, all these studies assessed microbial composition using 16S rRNA sequencing, which did not provide information on microbial composition at the species levels or microbial function and did not take into account the compositional nature of the metagenomics datasets. To overcome these limitations, we analysed the faecal microbiota of three independent studies (ADIPOINST, n=15; IRONMET, n=121 and FLORINASH, n=67) by shotgun metagenomics. We applied linear regression models in the log-scale taking into account the underlying compositional structure of the metagenomics data using the analysis of compositions of microbiome with bias correction (ANCOM-BC)[12] adjusting for age, sex and body mass index (BMI). We found a consistent negative association of insulin sensitivity with several genera and species (figure 1A-E and online supplemental tables 2–7) from the *Erysipelotrichaceae* family and the Proteobacteria phylum, specifically from the *Enterobacteriaceae* family, across three independent studies. We further validated these results on a subset of patients from the FLOROMIDIA cohort with available hyperinsulinaemic-euglycaemic clamps (n=24) using faecal 16S rRNA sequencing. Once again, the genus *Desulfovibrio* from the Proteobacteria phylum was strongly negatively associated with insulin sensitivity (online supplemental figure 1 and online supplemental table 8).

Conversely, insulin sensitivity was positively associated with microbial species from the *Bifidobacterium* and *Prevotella* genera. In the ADIPOINST and FLORINASH cohort, we also found a positive association between short-chain fatty acid (SCFA)-producing species from the *Blautia* and *Faecalibacterium* genus and insulin sensitivity (figure 1D-E). Recent studies also demonstrate the causal role of gut microbiota in insulin sensitivity regulation. Faecal microbiota transplantation (FMT) from lean donors has been shown to enhance peripheral insulin sensitivity,[7 9] whereas FMT from metabolic syndrome donors has had the opposite effect.[6] Consistent with our results, FMT from lean male donors to male subjects with obesity increased SCFA-producing bacteria like *Bifidobacterium pseudolongum*, while reducing *Escherichia coli* levels compared with the autologous group.[9] Additionally, increases in *Desulfovibrio* spp. predicted a ≥10% decrease in insulin sensitivity, whereas *Prevotellaceae* spp predicted non-deterioration.[6] Similarly, treatment with vancomycin in male patients with obesity decreased peripheral insulin sensitivity and increased gram-negative bacteria, mainly *Proteobacteria*.[8] Elevated Proteobacteria levels have also been observed in individuals with T2D,[13 14] although these studies often did not account for medication use. Conversely, other studies found no clear link between increased Proteobacteria and insulin sensitivity after vancomycin treatment in obese individuals,[15] and under certain conditions, a decrease in Proteobacteria has been associated with insulin sensitivity or with improved metabolic outcomes, such as after gastric bypass[16 17] or in toll-like receptor 2 knockout mice.[18]

Medication is known to impact the gut microbiota.[19 20] Therefore, to rule out the confounding effects of medication on our current associations, we conducted additional analyses controlling for hypertension and dyslipidaemia medication, as well as the consumption of proton pump inhibitors, which were the primary medications in our studies. Additionally, although the ADIPOINST and IRONMET studies did not include patients with T2D (HbA1c <6.5% and fasting glucose <126 mg/dL), nine patients (13%) in the FLORINASH cohort had T2D. To further eliminate the confounding effect of diabetes, we controlled the analyses for the presence of T2D in the FLORINASH cohort. Remarkably, after controlling for these additional covariates, not only did most of the associations remain significant, but genera from the Proteobacteria phylum also exhibited the strongest

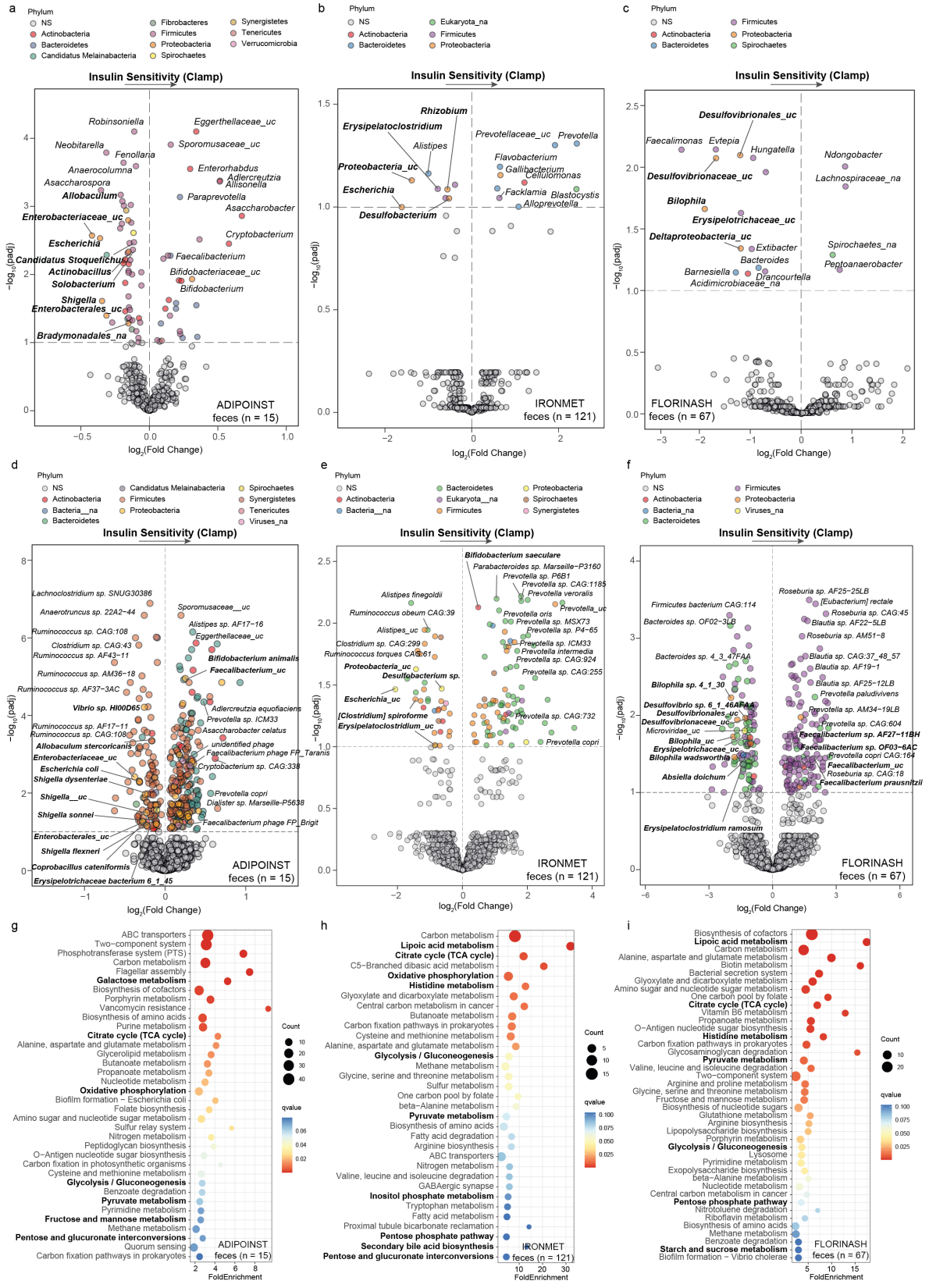

Figure 1 Associations of the faecal microbiota composition and functionality with the hyperinsulinaemic-euglycaemic clamp across studies. (A–F) Volcano plots of differential microbial (a-c) genera and (d-f) species associated with insulin sensitivity (hyperinsulinaemic-euglycaemic clamp) in the ADIPOINST (n=15), IRONMET (n=121) and FLORINASH (n=67) studies, respectively; identified using the analysis of microbiomes with bias correction compared. The $\log_2$ (Fold Change) and the $-\log_{10}$ (p values) adjusted for multiple testing are plotted for each taxon. Significantly different taxa are coloured according to phylum. (g–i) Dot plots of the KEGG pathway over-representation analyses (q value <0.1) mapping the KEGG orthologues significantly associated with insulin sensitivity in the ADIPOINST, IRONMET and FLORINASH studies, respectively. Dots are coloured according to the q value. KEGG, Kyoto Encyclopaedia of Genes and Genomes.

negative fold changes and most significant (p_{adj}) associations with insulin sensitivity (online supplemental figure 2 and online supplemental tables 9–11).

Previous studies have overlooked microbial functionality analyses, which are essential to accurately capture the host-microbiome interactions due to microbial functional redundancy.[21] Hence, we next performed functional analyses based on Kyoto Encyclopaedia of Genes and Genomes (KEGG) orthologs in the three studies (online supplemental tables 12–14) and a pathway enrichment analysis using those microbial genes associated with insulin sensitivity ($p_{adj}<0.05$). In the ADIPOINST, IRONMET and FLORINASH studies, we found a consistent over-representation of pathways involved in energy metabolism such as the tricarboxylic acid cycle, oxidative phosphorylation, glycolysis/gluconeogenesis, pyruvate metabolism and the pentose phosphate pathway (figure 1G-I and online supplemental tables 15–17). Notably, both the IRONMET and FLORINASH studies showed a strong association with lipoic acid and histidine metabolism. Alpha-lipoic acid has been demonstrated to increase insulin sensitivity measured with hyperinsulinaemic-euglycaemic clamp in patients with T2D.[22–24] A functional analysis based on KEGG modules identified histidine degradation as one of the most significant pathways (online supplemental figure 3 and online supplemental tables 18–20). Notably, imidazole propionate, a microbially produced histidine metabolite, is elevated in subjects with prediabetes and diabetes and has been shown to impair insulin signalling.[25 26]

A T cell-related jejunal transcriptomic signature is positively associated with systemic insulin sensitivity

Recent evidence suggests the gut is a key tissue in obesity-related insulin resistance through the gut microbiota-induced inflammation[27] by disrupting intestinal permeability and enhancing absorption of microbial products such as LPS.[3 4] Most of the digestion of lipids and sugars takes place in the jejunum and is regulated by insulin.[28] However, little is known about the jejunal transcriptome in relation to insulin sensitivity. Considering this, we performed an RNAseq of jejunal samples from the ADIPOINST cohort (n=26) and applied robust linear regression models adjusted for age, BMI and sex, to identify jejunal transcriptome signatures associated with the hyperinsulinaemic-euglycaemic clamp (figure 2A and online supplemental table 21). A pathway over-representation analysis highlighted that most of the upregulated genes played a key role in both the CD4 and CD8 helper T cell receptor signalling (TCR) and differentiation (Th1 and Th2), innate immunity, modulation of the inflammatory response, the RHO GTPase cycle and maintenance of the intestinal epithelium integrity (figure 2B,C and online supplemental table 22). *CR2*, involved in the complement cascade, and *CCL11*, encoding for an eosinophil-specific chemokine, were the two jejunal genes that had the highest effect size. These findings hint at the preservation of intestinal immunity and integrity with increased insulin sensitivity.

As the TCR signalling pathway had the strongest association with insulin sensitivity in the jejunum, we sought to measure the content of T cells in the ileum of another independent cohort (SIMMUNIDIA, n=42). We found that both the numbers and proportion of ileal T cells were negatively associated with fasting glucose and HbA1c (figure 2D-G). While interpreting these results, it is important to consider that the ileum has a slightly distinct immune microenvironment than the jejunum.[29] Overall, these findings indicate that the presence of T cells seems to exercise a protective function in the small intestine. Similarly, the frequency of Th2 cells has also been negatively correlated with insulin resistance in mice and humans in other tissues like VAT.[30 31]

Ileal Proteobacteria and deoxycholic acid (DCA) exhibit a negative association with insulin sensitivity

The gut microbiota and derived metabolites are key in intestinal barrier disruption and local immune responses that contribute to inflammation and lead to metabolic disease.[32] However, only a limited number of studies have described the composition of the small intestine microbiota, and there is even little evidence about its relationship with obesity and insulin resistance.[33] Thus, we analysed the microbiota of the ileum mucosa in the SIMMUNIDIA cohort (n=42) using 16S rRNA gene sequencing and identified bacterial genera associated with HbA1c as an indirect marker of insulin resistance (figure 2H). In agreement with our findings in faeces, bacterial genera from the Proteobacteria phylum (*Escherichia, Shigella* and *Enterobacter*) were associated with insulin resistance (figure 2H and online supplemental table 23) and negatively with the total number of T cells (figure 2I and online supplemental table 24), while the *Bifidobacterium* genus and SCFA-producing genera such as *Faecalibacterium* were negatively associated. In line with our results, a high-fat diet (HFD) resulted in alterations in the ileum microbiota that impaired the immune system of the small intestine by decreasing the number of interleukin (IL)-17-producing CD4 T cells (Th17) and induced T2D in mice.[34]

We next profiled the lipidome and metabolome of the jejunum in the ADIPOINST cohort (n=26) to elucidate the small intestine biochemical pathways linked to insulin sensitivity. We applied a random forest-based variable selection machine learning algorithm to identify lipids and metabolites predictive of the hyperinsulinaemic-euglycaemic clamp. DCA, a microbial-derived secondary bile acid, was identified as the most important metabolite negatively associated with insulin sensitivity (figure 2J and online supplemental table 25). Valine and isoleucine were also strongly negatively associated with insulin sensitivity, whereas taurine had a positive association.

Consistently, jejunal levels of DCA were positively associated with the presence of proinflammatory bacterial species in faeces such as *Gammaproteobacteria* (*E. cloacae, E. coli* and *Citrobacter*) and negatively associated with species from the *Bifidobacterium* and *Faecalibacterium* genera (online supplemental figure 4 and online supplemental table 26). In line with our results, FMT from donors with metabolic syndrome resulted in an increase in faecal levels of DCA and other secondary bile acids while decreasing the insulin sensitivity of recipient patients.[6] DCA is known to induce intestinal inflammation, disrupting the epithelial barrier and increasing gut permeability.[35–37] Elevated levels of intestinal DCA have been linked to HFDs and western diets[38] and increased abundance of the genus *Clostridium*, the main producer of DCA, promoting colonic inflammation.[38] Plasma levels of DCA were associated with insulin resistance and significantly elevated in patients with T2D.[39]

Proteobacteria and a T cell-related transcriptomic signature in the colon are also associated with insulin sensitivity

We also performed a colon transcriptomics analysis in the FLOROMIDIA cohort to identify genes associated with insulin sensitivity (figure 3A and online supplemental table 27). Consistent with our findings in the jejunum, pathways involved in the CD4/CD8 helper T cell lineage commitment and the Th17 type immune response were enriched (figure 3B, online supplemental

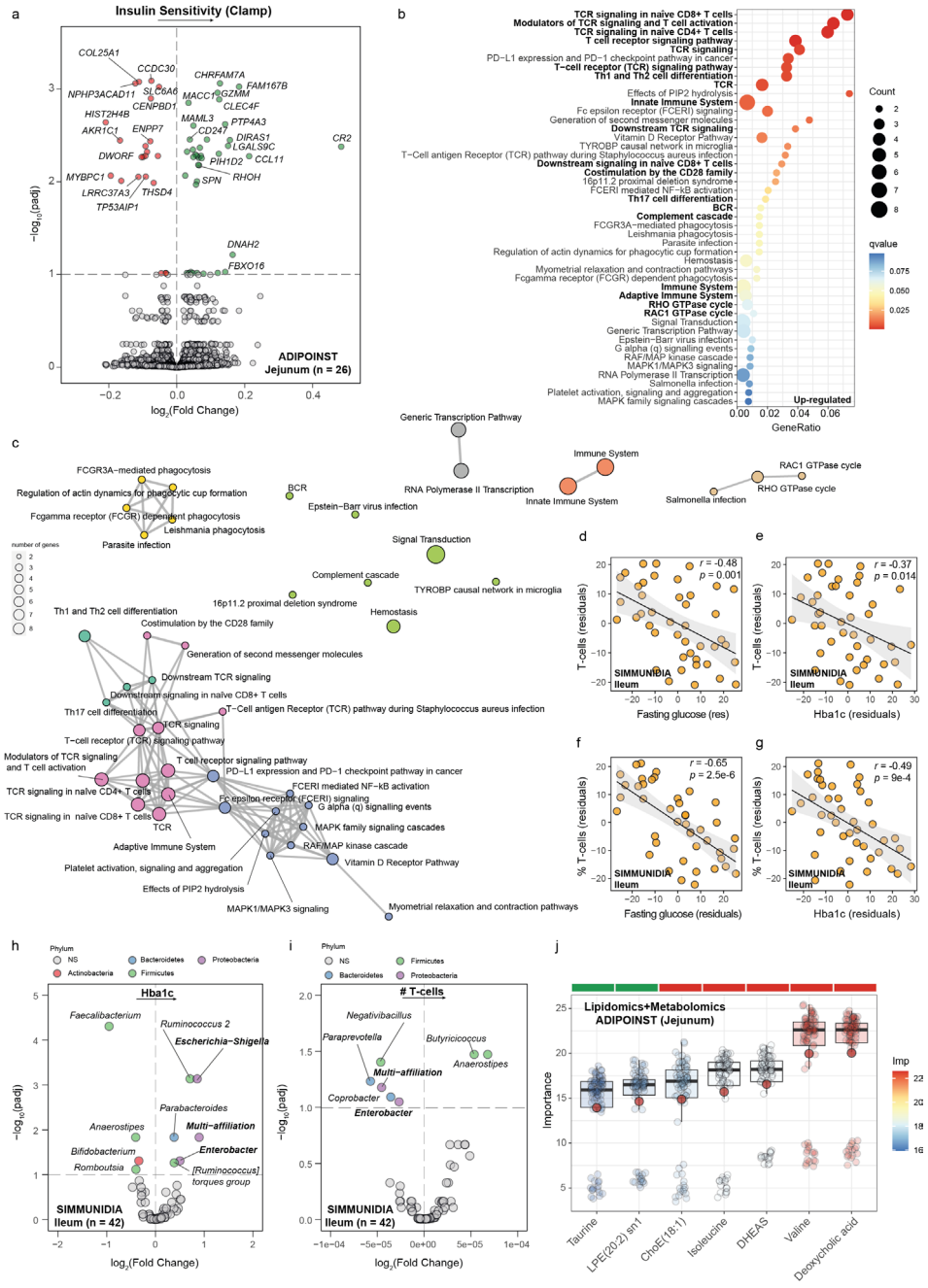

Figure 2 Cross-cohort and cross-omics associations in the small intestine with insulin sensitivity and resistance. (a) Volcano plot of differentially expressed genes associated with insulin sensitivity (hyperinsulinaemic-euglycaemic clamp) in the jejunum of patients from the ADIPOINST cohort (n=26) identified by limma-voom analysis controlling for age, sex and BMI. The $\log_2$ fold change associated with a unit change in the clamp and the $\log_{10}$ p values adjusted for multiple testing are plotted for each gene. (b) Dot plot of pathways significantly associated (q value <0.1) with insulin sensitivity in the jejunum identified from a pathway over-representation analysis mapping significantly upregulated genes to the Reactome, Kyoto Encyclopaedia of Genes and Genomes, Wikipathways, PID and NetPpath databases. Dots are coloured by the q value. (c) Over-representation analysis results were mapped as a function network of pathways using an enrichment map. Edges connect overlapping gene sets, while node size reflects the total number of genes in each pathway. Overlapping gene sets tend to cluster together, making it easy to identify functional modules. Functionally related pathways are clustered based on the Markov Cluster Algorithm and coloured with the same colour. (d–g) Scatter plot of the partial Spearman's rank correlations (adjusted for age, sex and BMI) between the fasting glucose or HbA1c levels and the number of T cells or the percentage of T cells in the ileum of patients from the SIMMUNIDIA cohort (n=43). The ranked residuals are plotted. (h) Volcano plots of differential microbial genera associated with HbA1c and (i) the number (#) of T cells in the ileum of the SIMMUNDIA cohort (n=42) identified using ANCOM-BC controlling for age, sex and BMI. The $\log_2$ (Fold Change) and the $-\log_{10}$ (p values) adjusted for multiple testing are plotted for each taxon. Significantly different taxa are coloured according to phylum. (j) Boxplots of the normalised variable importance measure for the metabolites/lipids associated with insulin sensitivity in the jejunum of the ADIPOINST cohort. The red dot represents the mean and the colour bar above each plot indicates the sign of the association between the metabolites/lipids and insulin sensitivity, with red indicating negative correlation and green positive correlation. Significant metabolites were identified using the Boruta algorithm with 5000 trees and 500 iterations. ANCOM-BC, analysis of microbiomes with bias correction; BMI, body mass index; HbA1c, glycated haemoglobin.

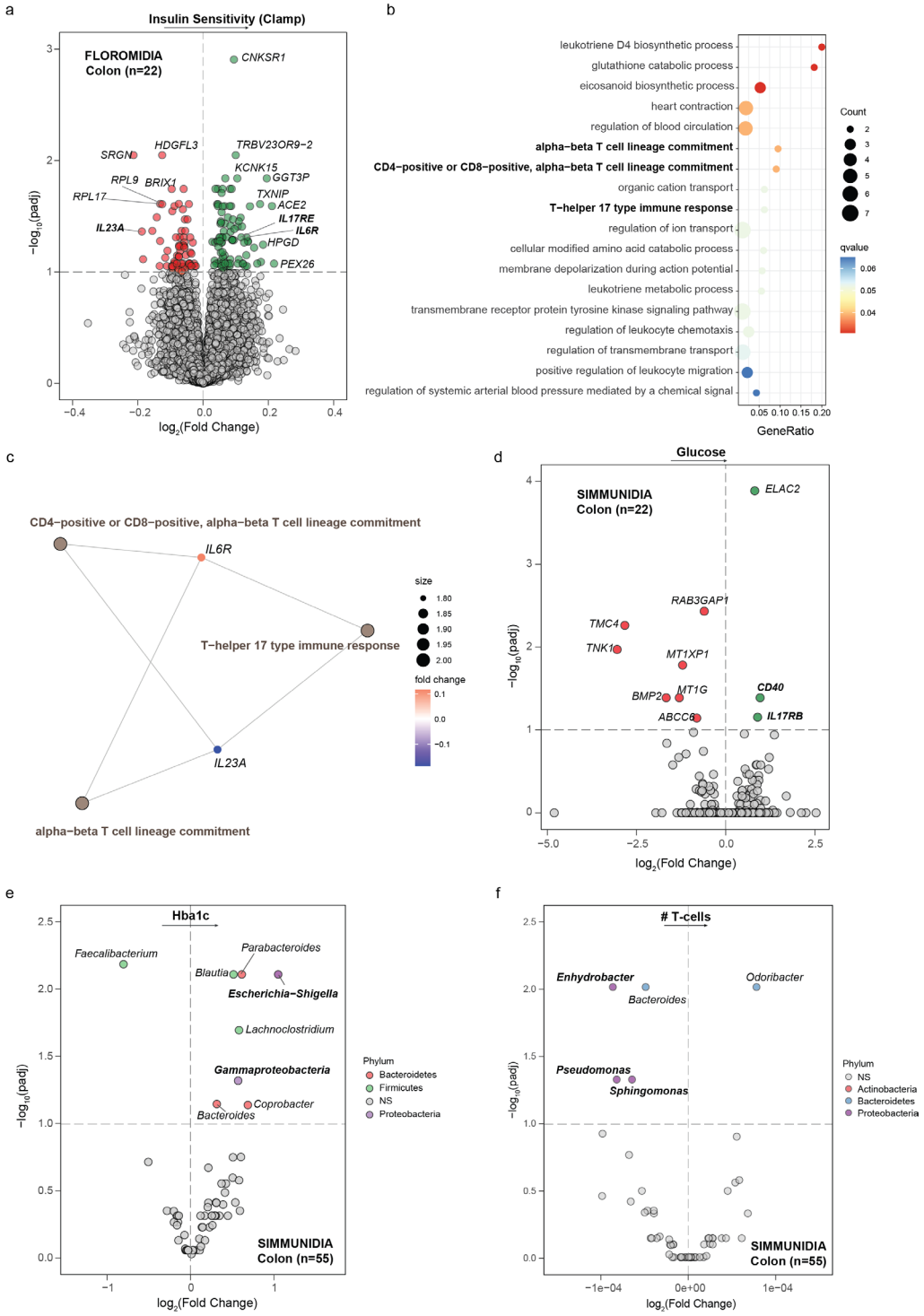

Figure 3 Cross-cohort and cross-omics associations in the colon with insulin sensitivity and resistance. (a) Volcano plot of differentially expressed genes associated with insulin sensitivity (hyperinsulinaemic-euglycaemic clamp) in the colon of patients from the FLOROMIDIA cohort (n=22), identified by limma-voom analysis controlling for age, sex and BMI. The $\log_2$ fold change associated with a unit change in the clamp and the $\log_{10}$ p values adjusted for multiple testing are plotted for each gene. (b) Dot plot of gene ontology-biological processes significantly associated (q value <0.1) with insulin sensitivity in the colon identified from a gene ontology over-representation analysis using significant genes associated with insulin sensitivity. Dots are coloured by the q value. (c) Gene-concept network depicting the linkage of significant genes associated with insulin sensitivity participating in Th17 immune response and CD4+ or CD8+, alpha-beta T cell lineage commitment. (d) Volcano plot of differentially expressed genes associated with the fasting glucose levels in the colon of patients from the SIMMUNIDIA cohort (n=22), identified by limma-voom analysis controlling for age, sex and BMI. The $\log_2$ fold change and the $\log_{10}$ p values adjusted for multiple testing are plotted for each gene. (e) Volcano plots of differential microbial genera associated with HbA1c and (F) the number (#) of T cells in the colon of the SIMMUNDIA cohort (n=55) identified using ANCOM-BC controlling for age, sex and BMI. The $\log_2$ (Fold Change) and the $-\log_{10}$ (p values) adjusted for multiple testing are plotted for each taxon. Significantly different taxa are coloured according to phylum. ANCOM-BC, analysis of microbiomes with bias correction; BMI. body mass index; HbA1c, glycated haemoglobin.

table 28). We found that insulin sensitivity was positively associated with interleukin 17 receptor E (*IL17RE)* and interleukin 6 receptor *(IL6R*), but negatively with IL-23 subunit alpha (*IL23A*) (figure 3A,C). Similarly, the fasting glucose levels in the SIMMUNIDIA cohort were associated with the expression of IL-17 receptor B (*IL17RB*) and tumour necrosis factor receptor (*CD40*) (figure 3D and online supplemental table 29). We then analysed the microbiota of the colon in the SIMMUNIDIA cohort (n=55) using 16S rRNA gene sequencing. In agreement with our results at the ileum, we found that genera from the Proteobacteria phylum (*Escherichia*, *Shigella* and *Gammaproteobacteria*) were positively associated with insulin resistance assessed with the HbA1c and negatively associated with the number of T cells in the colon, whereas *Faecalibacterium* were again negatively associated (figure 3E,F and online supplemental tables 30,31).

An adipose tissue signature involved in T cell and Rho GTPase signalling is associated with systemic insulin sensitivity and resistance

Chronic inflammation in the adipose tissue has become a hallmark of obesity-related insulin resistance. Hence, we performed an RNA-seq analysis of the subcutaneous adipose tissue (SAT) of patients with and without obesity from the ADIPOMIT cohort (n=740). After fitting robust linear regression models controlling for age, BMI and sex, we identified several genes associated with HbA1c (figure 4A and online supplemental

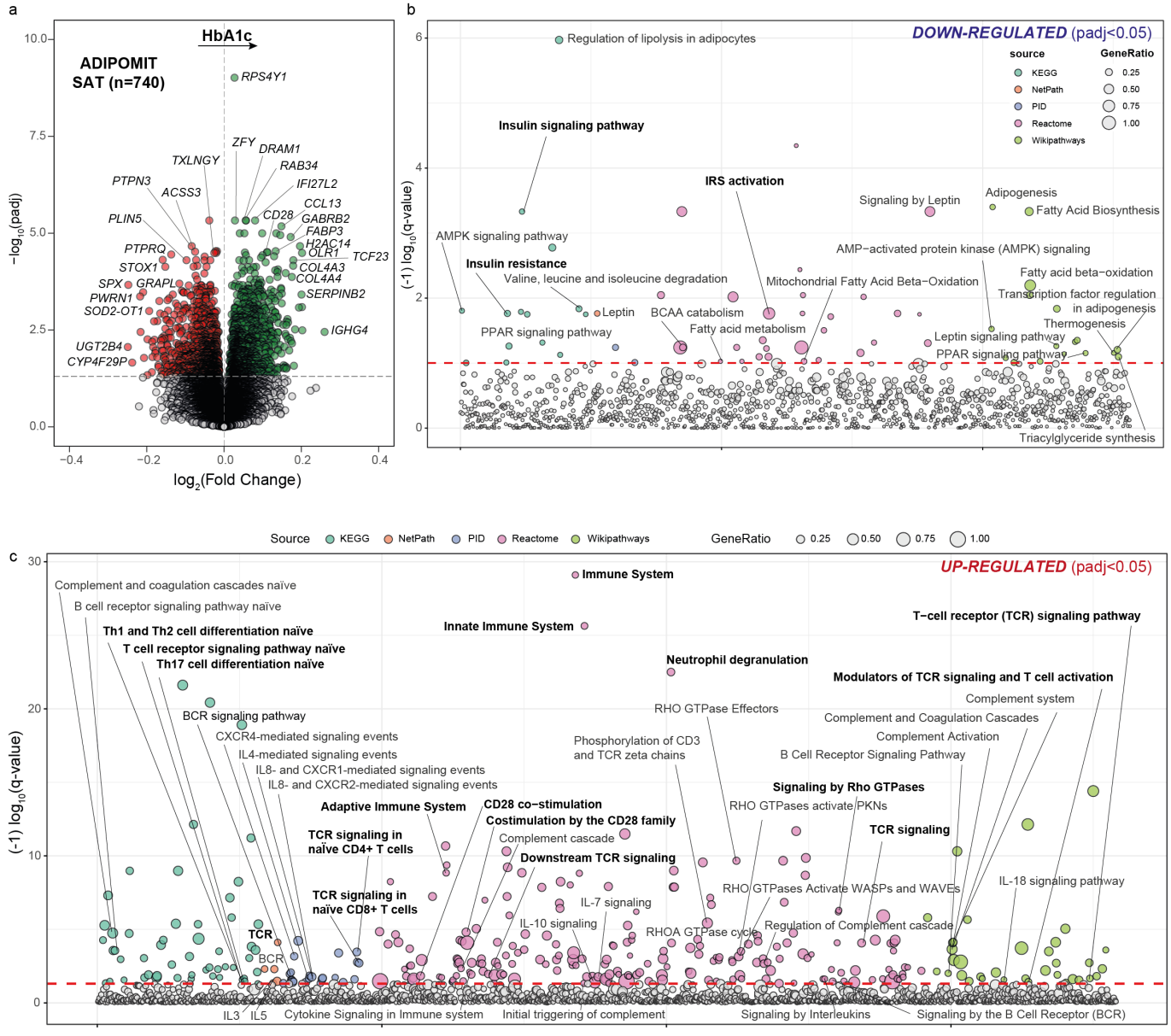

Figure 4 SAT transcriptomic signatures associated with HbA1c. (a) Volcano plot of differentially expressed genes associated with HbA1c in the SAT of patients from the ADIPOMIT cohort (n=740), identified by limma-voom analysis controlling for age, sex and BMI. The $\log_2$ fold change and the $\log_{10}$ p values adjusted for multiple testing are plotted for each gene. (b) Manhattan-like plot of pathways significantly associated (q value <0.1) with insulin sensitivity in the jejunum identified from a pathway over-representation analysis mapping significantly downregulated genes and (c) significantly upregulated genes to the Reactome, Kyoto Encyclopaedia of Genes and Genomes, Wikipathways, PID and NetPath databases. HbA1C, glycosylated haemoglobin; SAT, subcutaneous adipose tissue.

table 32). An enrichment analysis highlighted that genes negatively associated with HbA1c were involved in pathways that included insulin signalling, insulin resistance, IRS activation or leptin signalling, whereas genes positively associated with HbA1c were mainly involved in the TCR signalling, the immune system and the signalling by Rho GTPases (figure 4B,C and online supplemental tables 33,34), consistent with our findings in the jejunum and colon. However, while higher expression of the genes associated with these pathways in the intestine appears to be linked to greater insulin sensitivity, in adipose tissue, it seems to be associated with increased insulin resistance. This disparity might indicate different stages of development and/or asynchronous responses to environmental stimuli. In the intestine, higher insulin sensitivity could correspond to a stronger immune response, suggesting that an adequate intestinal immune response might prevent gut dysbiosis-induced insulin resistance. Conversely, in adipose tissue, an increased immune response could indicate insulin resistance-associated adipose tissue dysfunction. Chronic activation of this response in adipose tissue can become detrimental. However, it should be noted that these interpretations remain speculative.

We next performed an RNA-seq analysis of the VAT of patients from the ADIPOINST cohort (n=29). After applying robust linear regression models adjusted for age, BMI and sex, we identified a large number of VAT genes associated with insulin sensitivity (figure 5A and online supplemental table 35). Remarkably, a pathway enrichment analysis based on the genes negatively associated with insulin sensitivity revealed associations with innate immunity, specifically an over-representation of the TCR signalling pathway; the signalling by Rho GTPases; the IL4-, IL8-, IL3-, IL-18-, IL2-mediated signalling events and anticipated metabolic pathways such as leptin, adiponectin, glucagon and phosphatidylinositol 3-kinase (PI3K)/protein kinase B signalling (figure 5B and online supplemental table 36).

A multiomics and multitissue integration links Proteobacteria with jejunal DCA, jejunal and VAT genes involved in actin cytoskeleton, insulin and T cell signalling

Single-omics analyses only provide information about one layer of biological regulation. Therefore, we integrated the metagenomic, metabolomic and transcriptomic (jejunal and VAT) profiles linked to insulin sensitivity in the ADIPOINST cohort to obtain a holistic picture of the mechanisms underlying the gut microbiome-host interactions related to insulin sensitivity. Due to the large number of VAT genes associated with insulin sensitivity, we applied a multivariate method based on the multiblock-sparse projection to latent structures (MB-sPLS) using an L1 regularisation to select the 50 most relevant VAT genes and improve interpretation.

Cluster 1 depicted a strong negative association with insulin sensitivity and jejunal metabolites and lipids (DCA, valine, isoleucine, dehydroepiandrosterone sulfate (DHEAS)), microbial genera from the Proteobacteria phylum (*Escherichia, Shigella* and *Legionella*) and the *Erysipelotrichaceae* family (*Allobaculum* and *Solobacterium*) and transcriptomic signatures in the jejunum and VAT (figure 5C). Notably, this cluster was strongly negatively associated with cluster 2, with a strong correlation among metabolites (taurine), microbial genera (*Faecalibacterium*), jejunal and VAT genes strongly positively associated with insulin sensitivity.

DHEAS), microbial genera from the Proteobacteria phylum (*Escherichia, Shigella* and *Legionella*) and the *Erysipelotrichaceae* family (*Allobaculum* and *Solobacterium*) and transcriptomic signatures in the jejunum and VAT (figure 5C). Notably, this cluster was strongly negatively associated with cluster 2, with a strong correlation among metabolites (taurine), microbial genera (*Faecalibacterium*), jejunal and VAT genes strongly positively associated with insulin sensitivity.

To explore the role of the genes of both clusters in relation to insulin sensitivity, we conducted gene ontology-biological process (GO-BP) and pathway enrichment analyses. The most significant BP from jejunal genes in cluster 2 (positively associated with insulin sensitivity) involved lymphocyte migration and the regulation of the immune system (figure 5D). Consistently, the most significant jejunal pathways from this cluster included modulators of TCR signalling and T cell activation, the complement cascade, the innate immune system and the Rho GTPase cycle (figure 5E), whereas the most significant VAT pathway was precisely involved in the regulation of insulin-like growth factor (IGF) transport and uptake by IGFBPs (q value=0.0008).

Additionally, supramolecular fibre organisation (*CCL1*, *CHRFAM7A, CYFIP2* and *RHOH*) and actin cytoskeleton organisation (*CCL11* and *RHOH*) were among the GO-BP over-represented in cluster 2. Actin disassembly in both adipose and muscle cells has been shown to inhibit insulin-induced events such as glucose transporter recruitment to the cell surface and enhanced glucose transport[40]; actin is indispensable for the insulin-stimulated translocation of the glucose transporter GLUT4.[41] Plasma *CCL11*, has been positively associated with steatosis severity[42] and *CCL11* knockout mice fed a high-fat, high-calorie diet improved insulin sensitivity.[43] Additionally, *RHOH* is a key factor for the development and proper function of T cells.[44 45]

Of note, the GO-BP and pathways from the VAT most strongly associated with cluster 1 (negatively associated with insulin sensitivity) comprised again the regulation of the actin cytoskeleton organisation and regulation but also the insulin signalling (figure 5F,G) Similarly, the over-represented BP from these cluster in the jejunum also included the musculoskeletal movement and the supramolecular fibre organisation (figure 5H).

Finally, to assess whether the negative associations between faecal Proteobacteria and insulin sensitivity translated to the adipose tissue, we performed 16S rRNA sequencing of the VAT in a subset of patients from the IRONMET cohort (n=12). In line with our results, we found that genera from the Proteobacteria phylum (*Bradyrhizobium* and *Moraxellaceae* families) in the VAT were negatively associated with insulin sensitivity (figure 5I and online supplemental table 37).

A multiomics and multitissue integration links proteobacteria, liver DCA and liver genes involved in actin cytoskeleton, insulin secretion and the immune system

The liver plays a central role in modulating glucose and insulin. Therefore, we next profiled the liver transcriptome of obese patients from the FLORINASH cohort (n=80). After fitting robust linear regression models adjusted for age, BMI and sex, we identified 1356 genes associated with the hyperinsulinaemic-euglycaemic clamp (figure 6A and online supplemental table 38). Consistent with our findings in the jejunum and the VAT, these genes were involved in the TCR signalling, the complement system, the Ras and Rap1 GTPase signalling and the cytokine–cytokine receptor interaction (figure 6B and online supplemental table 39). We also profiled the lipidome of the liver of a subset of these patients. We found several lysophosphatidylcholines and lysophosphatidylethanolamines associated with insulin sensitivity

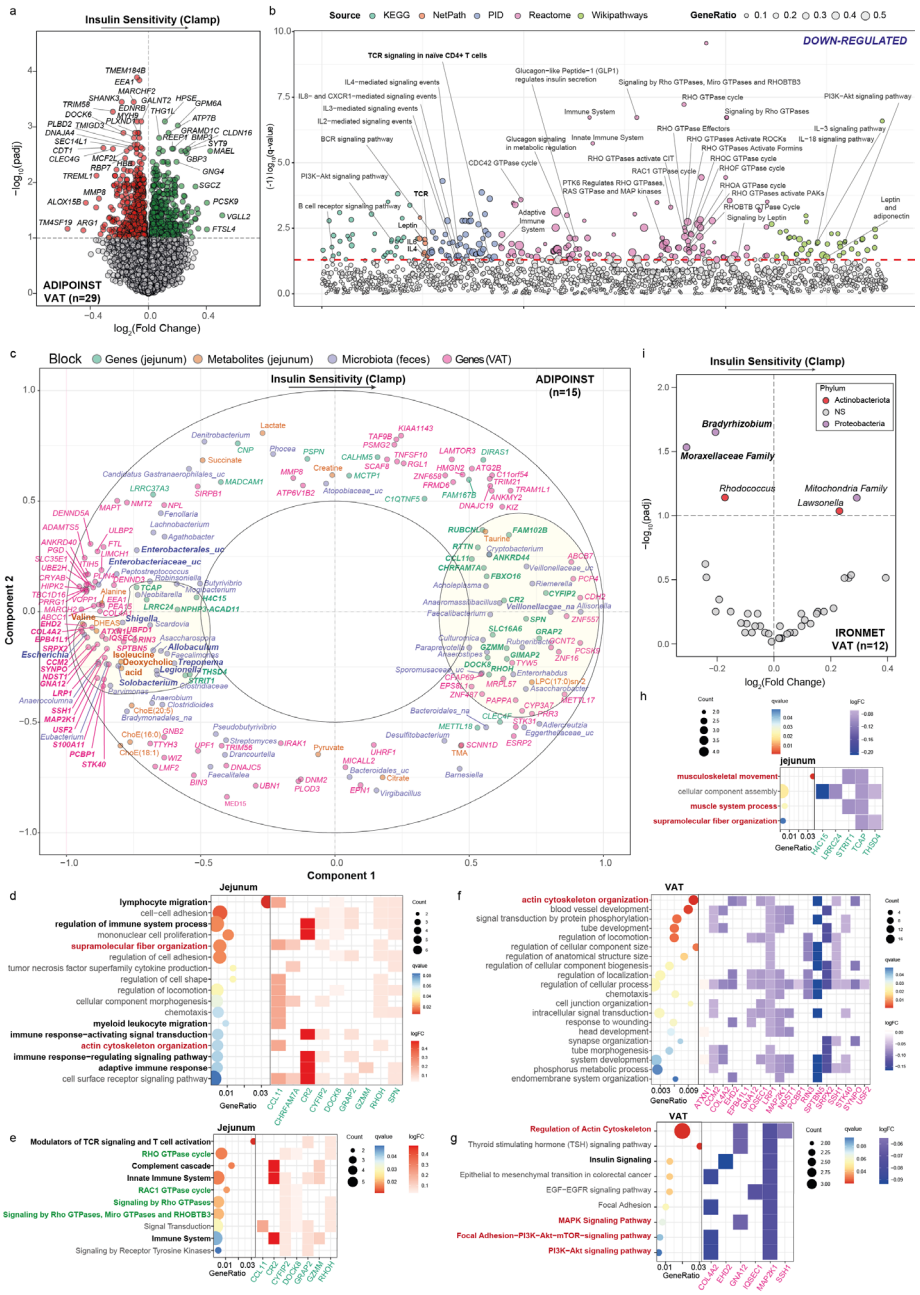

Figure 5 VAT transcriptomic signatures associated with insulin sensitivity and cross-omics and cross-tissue integration in the ADIPOINST cohort. (a) Volcano plot of differentially expressed genes associated with insulin sensitivity (hyperinsulinaemic-euglycaemic clamp) in the VAT of patients from the ADIPOINST cohort (n=29) identified by limma-voom analysis controlling for age, sex and BMI. The $\log_2$ fold change associated with a unit change in the clamp and the $\log_{10}$ p values adjusted for multiple testing are plotted for each gene. (b) Manhattan-like pathways significantly associated (q value <0.1) with insulin sensitivity in the VAT identified from a pathway over-representation analysis mapping significantly downregulated genes to the Reactome, Kyoto Encyclopaedia of Genes and Genomes, Wikipathways, PID and NetPath databases. (c) Correlation circle plot for the integration of the jejunum and VAT genes, jejunum metabolites and faecal microbial species associated with insulin sensitivity in the ADIPOINST cohort using a multiblock sparse PLS model. Strongly positively associated variables or groups of variables are projected close to one another on the correlation circle (~0° angle). The variables or groups of variables strongly negatively associated are projected diametrically opposite (~180° angle) on the correlation circle. Variables not correlated are situated ~90° from one another. (d) Dot plot of significantly enriched (q value <0.1) gene ontology-biological processes and (e) pathways from jejunal genes strongly positively associated with insulin sensitivity included in cluster 2 with a heatmap displaying the gene participating in each biological term. Dots are coloured by the q value and genes in the heatmap are coloured by the $\log_2$ Fold Change of the association with insulin sensitivity. (f) Dot plot of significantly enriched (q value <0.1) gene ontology-biological processes and (g) pathways from VAT and (h) jejunal genes strongly negatively associated with insulin sensitivity included in cluster 1 with a heatmap displaying the gene participating in each biological term. Dots are coloured by the q value and genes in the heatmap are coloured by the $\log_2$ Fold Change of the association with insulin sensitivity. (i) Volcano plots of differential microbial genera associated with insulin sensitivity in the VAT of the IRONMET (n=12) identified using ANCOM-BC controlling for age, sex and BMI. The $\log_2$ (Fold Change) and the $-\log_{10}$ (p values) adjusted for multiple testing are plotted for each taxon. Significantly different taxa are coloured according to phylum. ANCOM-BC, analysis of microbiomes with bias correction; BMI, body mass index; VAT, visceral adipose tissue

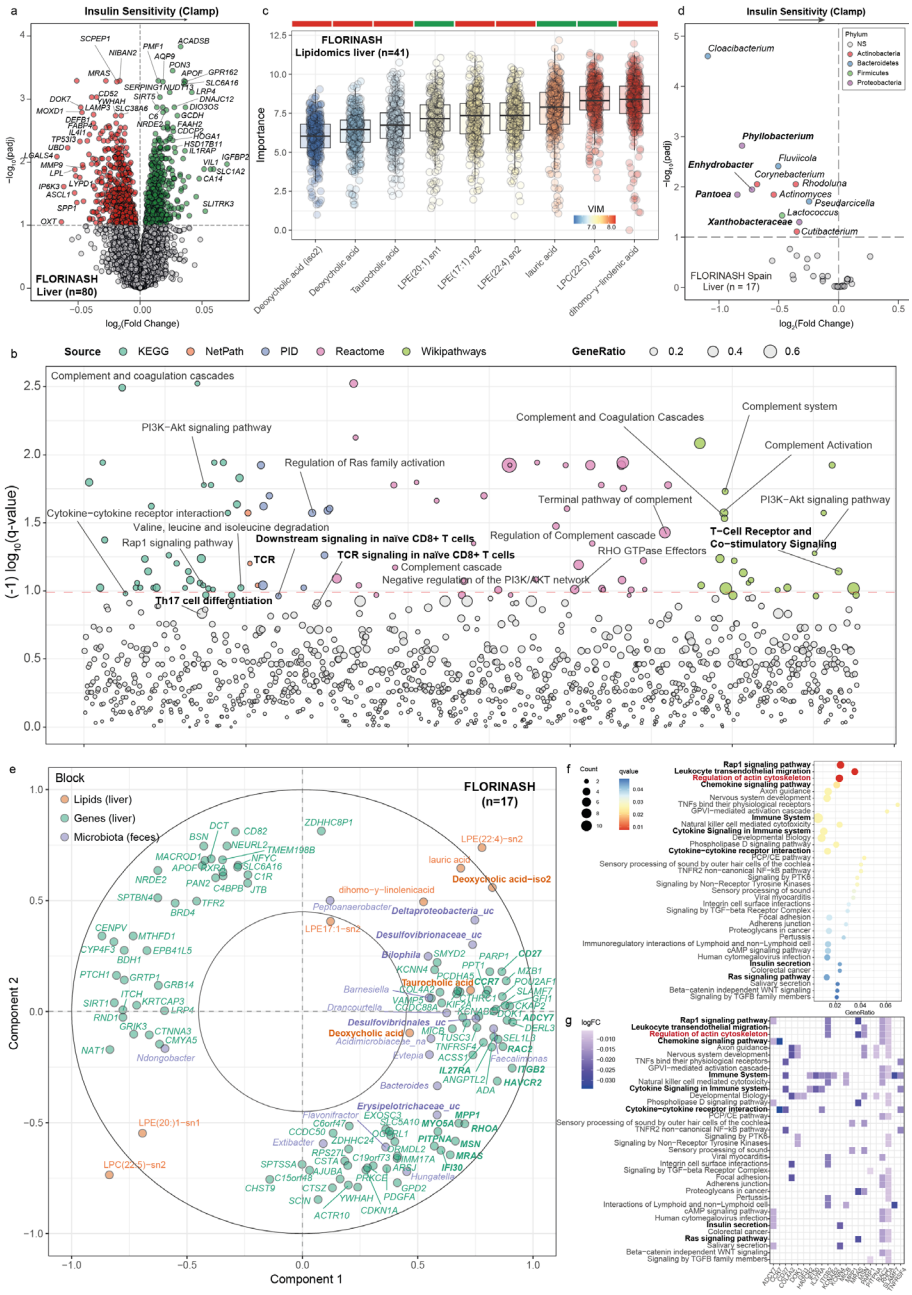

Figure 6 Liver transcriptomic signatures associated with insulin sensitivity and cross-omics and cross-tissue integration in the FLORINASH cohort. (a) Volcano plot of differentially expressed genes associated with insulin sensitivity (hyperinsulinaemic-euglycaemic clamp) in the liver of patients from the FLORINASH cohort (n=80) identified by limma-voom analysis controlling for age, sex and BMI. The $\log_2$ fold change associated with a unit change in the clamp and the $\log_{10}$ p values adjusted for multiple testing are plotted for each gene. (b) Manhattan-like plot of pathways significantly associated (q value <0.1) with insulin sensitivity in the liver identified from a pathway over-representation analysis mapping significant genes to the Reactome, KEGG, Wikipathways, PID and NetPpath databases. (c) Boxplots of the normalised variable importance measure for the lipids associated with insulin sensitivity in the liver of the FLORINASH cohort (n=41). The bar above each plot indicates the sign of the association between the lipids and insulin sensitivity, with red indicating a negative correlation and green a positive correlation. Significant metabolites were identified using the Boruta algorithm with 5000 trees and 500 iterations. (d) Volcano plots of differential microbial genera associated with insulin sensitivity (clamp) in the liver of a subset of patients from Spain of the FLORINASH cohort (n=17) identified using ANCOM-BC controlling for age, sex and BMI. The log2 (Fold Change) and the −log10 (p values) adjusted for multiple testing are plotted for each taxon. Significantly different taxa are coloured according to phylum. (e) Correlation circle plot for the integration of the liver lipids, liver genes and faecal microbial species associated with insulin sensitivity in the FLORINASH cohort using a multiblock sparse partial least squares model. Strongly positively associated variables or groups of variables are projected close to one another on the correlation circle (~0° angle). The variables or groups of variables strongly negatively associated are projected diametrically opposite (~180° angle) on the correlation circle. Variables not correlated are situated ~90° from one another. (f) Dot plot of significantly enriched (q value <0.1) pathways (based on Reactome, KEGG, Wikipathways, PID and NetPath) and (g) heatmap of genes participating in these pathways, identified from liver genes strongly negatively associated with insulin sensitivity from the transcriptome signature clustering with Proteobacteria and deoxycholic acid and negatively associated with insulin sensitivity. Dots are coloured by the q value and genes in the heatmap are coloured by the $\log_2$ Fold Change of the association with insulin sensitivity. KEGG, Kyoto Encyclopaedia of Genes and Genomes.

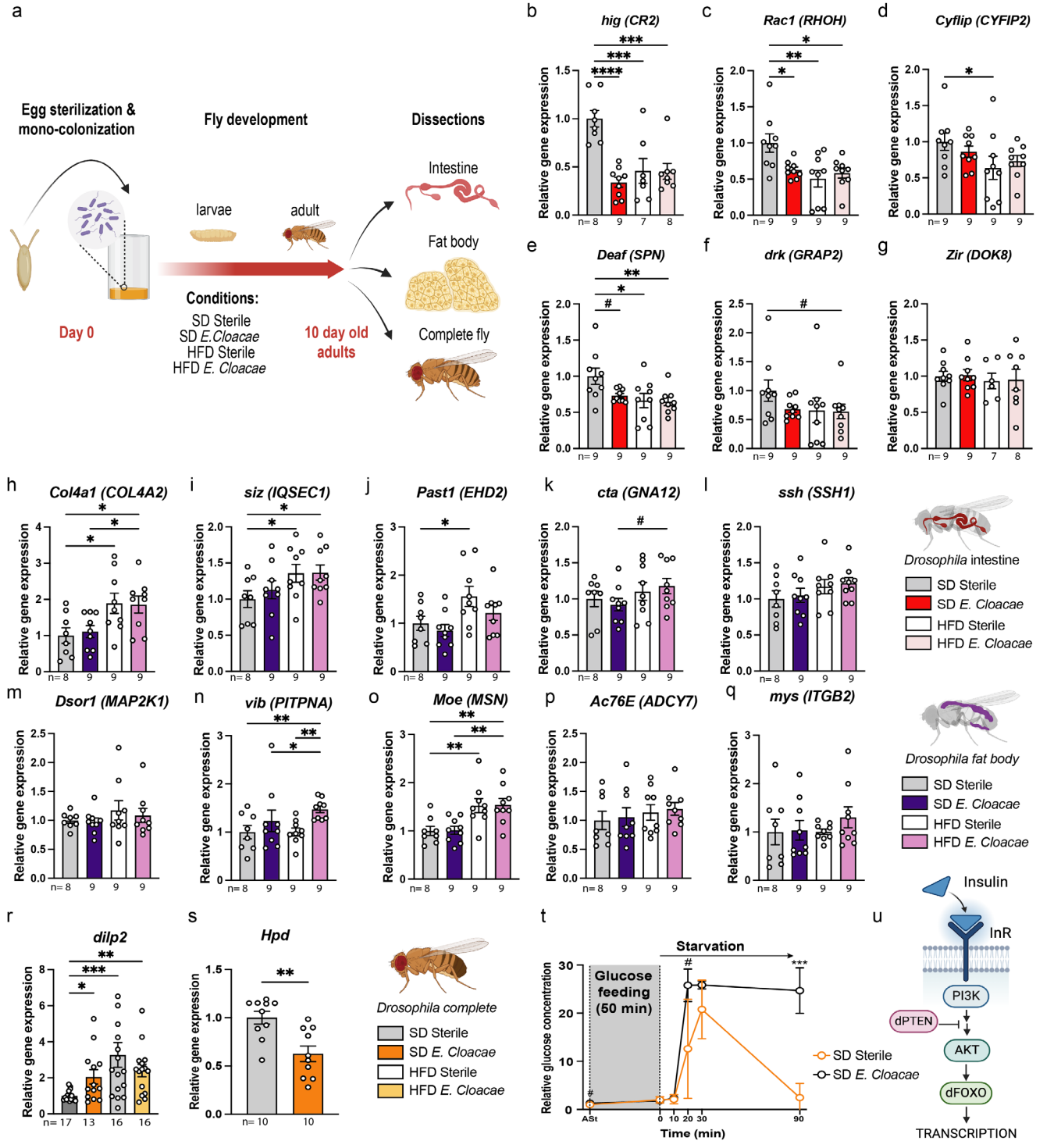

Figure 7 *Enterobacter cloacae* mono-association and HFD supplementation produce transcriptional changes in orthologues of insulin sensitivity-associated genes in the *Drosophila* intestine and fat body. (a) An experimental scheme was followed to generate *Drosophila* wild-type flies under sterile (germ-free) or monocolonisation conditions. Sterile flies can be easily generated by egg sterilisation. Subsequently, mono-associations were established by fly food supplementation with *E. cloacae* or the vehicle (sterile flies). On the 10th day of adulthood, dissections were conducted. 25–30 fly intestines or fat bodies were collected per sample. For complete flies, eight adults were collected per sample. Quantitative reverse transcription-PCR results; bars represent relative gene expression of b) *hig,* c) *Rac1,* (d) *Cyflip,* (e) *Deaf,* (f) *drk,* (g) *Zir,* (h) *Col4a1,* (i) *Moe,* (j) *siz,* (k) *Past1,* (l) *cta,* (m) *ssh,* (n) *vib,* (o) *Ac76E,* (p) *Dsor1,* (q) *mys,* (r) *dilp2* and (s) Hpd in flies fed with SD and HFD non-colonised with *E. cloacae* or left sterile for the. P values were determined using the one-way analysis of variance combined with Fisher's (least significant difference) multiple comparisons test when unequal variances Kruskal-Wallis non-parametric test with multiple comparisons was conducted. (t) Larval haemolymph glucose clearance after glucose feeding at 0, 10, 20, 30 and 90 min. Statistical significance at each time point was tested using an unpaired, two-sided t-test. n=3 independent replicates pulling eight larvae each. Error bars represent SE of the mean (#p<0.1, *p<0.05, *p<0.01 and *p<0.001). (u) Schematic representation of FOXO activation through insulin signalling in *Drosophila*. HFD, high-fat diet; SD, standard diet

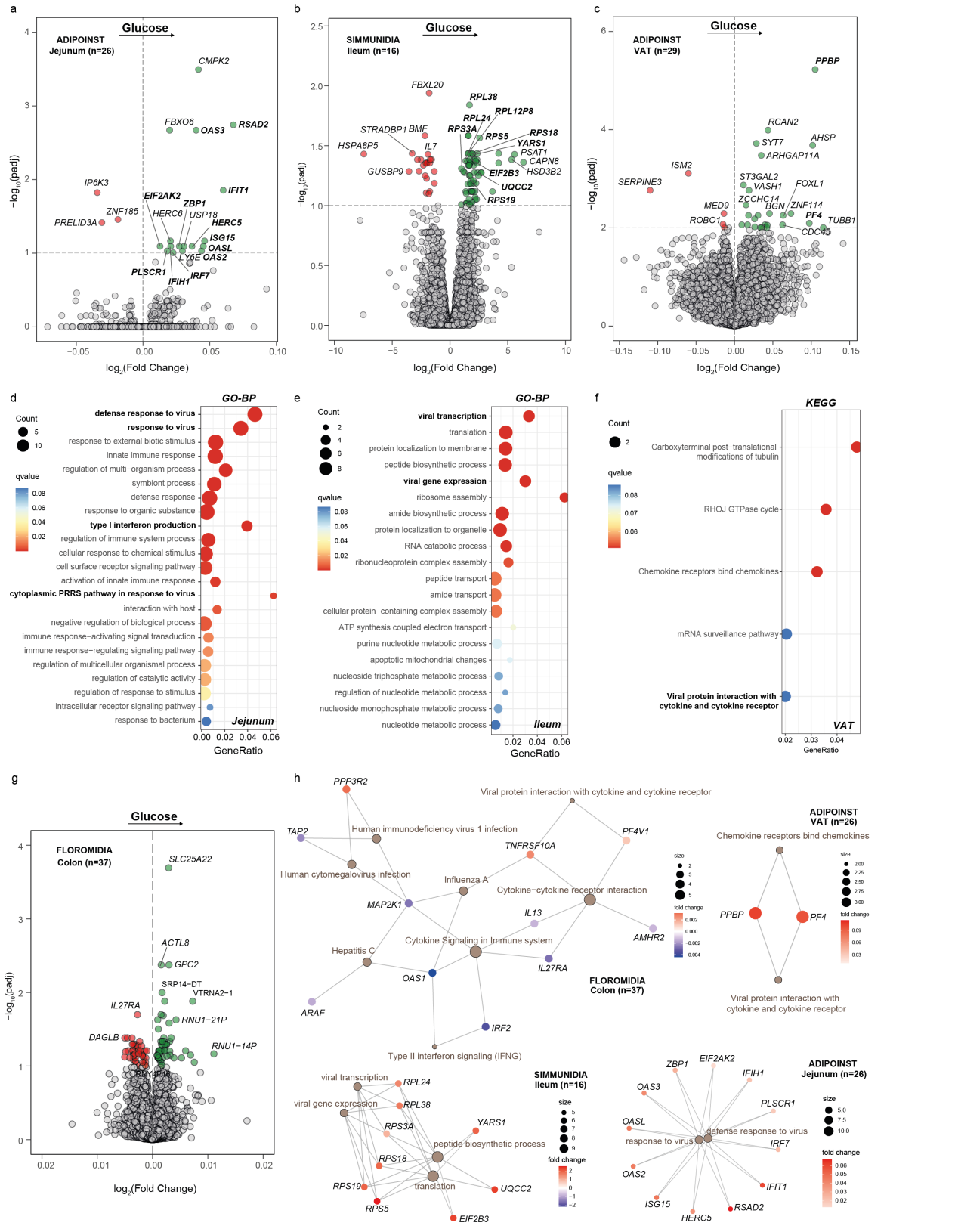

Figure 8 Cross-cohort and cross-tissue transcriptomic associations with fasting glucose levels. Volcano plot of differentially expressed genes associated with the fasting glucose levels in the (a) jejunum, (b) ileum, (c) VAT and (g) colon from the ADIPOINST (n=26), SIMMUNIDIA (n=16), ADIPOINST (n=29) and FLOROMIDIA (n=37) studies, respectively. The $\log_2$ fold change associated with a unit change in the fasting glucose levels and the $\log_{10}$ p values adjusted for multiple testing are plotted for each gene. (d–f) Dot plot of gene ontology biological processes (d,e) and Kyoto Encyclopaedia of Genes and Genomes pathways (F) significantly associated (q value <0.1) with the fasting glucose levels in the jejunum, ileum and VAT, respectively. (h) Gene-concept network depicting the linkage of significant genes associated with fasting glucose levels participating in pathways involved in the antiviral response in the jejunum, ileum, VAT and colon. VAT, visceral adipose tissue.

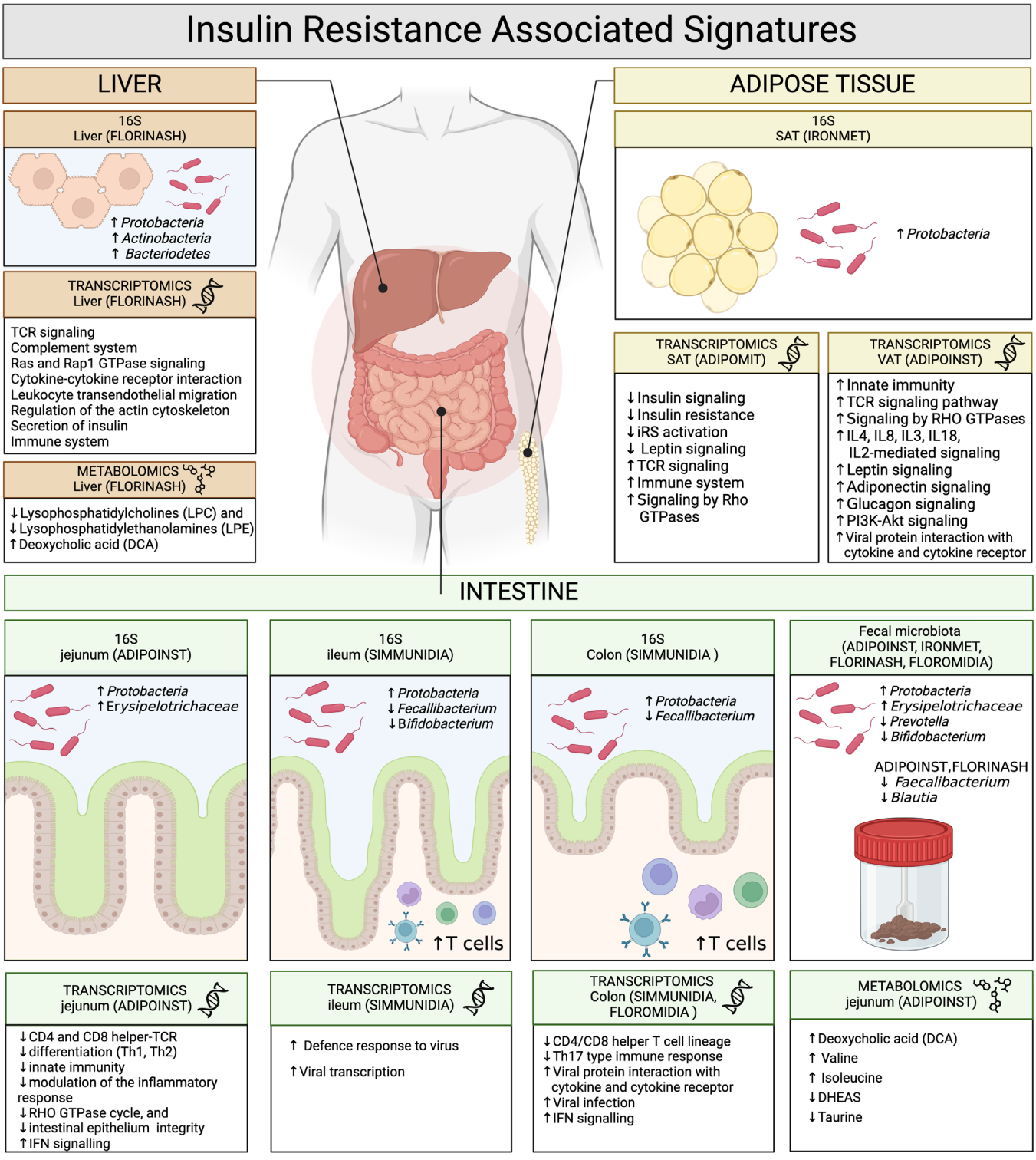

Figure 9 Functional analysis based on KEGG modules. Dot plots of the KEGG module-based pathway over-representation analyses (q value <0.1) mapping the KEGG orthologues molecular function significantly associated with insulin sensitivity in the (A) ADIPOINST, (B) IRONMET and (C) FLORINASH studies, respectively. Dots are coloured according to the q value. KEGG, Kyoto Encyclopaedia of Genes and Genomes.

(figure 6C, online supplemental figure 5A and online supplemental table 40). Importantly, we found again a negative association of DCA with insulin sensitivity (figure 6C and online supplemental figure 5A). We also analysed the microbiota of the liver of a subset of participants from Spain (n=17) and Italy (n=20) in the FLORINASH study. Consistent with our previous results in faeces, ileum, jejunum and colon, we found that genera from the Proteobacteria phylum in the liver exhibited the strongest and/or most significant negative associations with insulin sensitivity, after controlling for age, BMI, sex, dyslipidaemia and hypertension medication and diabetes both in Spain (figure 6D and online supplemental table 41) and Italy (online supplemental figure 5B and online supplemental table 42). A MB-sPLS-based multiomics integration of the

faecal microbiota and liver lipids and genes (only the 50 most relevant using an L1 regularisation) associated with insulin sensitivity revealed the presence of a cluster of highly interconnected features including genera from the Proteobacteria phylum (*Deltaproteobacteria*_uc, *Desulfovibrionaceae*_uc, *Bilophila* and *Desulfovibrionales*_uc) and the *Erysipelotrichaceae* family, primary (taurocholic acid) and secondary (DCA) bile acids and a transcriptomic signature negatively associated with insulin sensitivity (figure 6E).

Consistent with our previous results in the jejunum and VAT, a pathway enrichment analysis on this transcriptome identified an over-representation of the Rap1 signalling pathway, the leucocyte transendothelial migration, the regulation of the actin cytoskeleton, the chemokine signalling pathway, the immune system, the cytokine signalling in the immune system and the secretion of insulin, among others (figure 6F,G and online supplemental table 43). Again, a GO-BP analysis highlighted an enrichment of pathways mainly involved in lymphocyte activation, leucocyte cell–cell adhesion, regulation of leucocyte migration and T cell activation (online supplemental table 44).

HFD and mono associations with *Enterobacter cloacae* induce transcriptional changes associated with insulin resistance in *D. melanogaster* intestine and fat body

To further explore the multiorgan transcriptional changes associated with insulin sensitivity, we used the model organism *D. melanogaster*. To model insulin resistance in *Drosophila*, we administer a HFD that causes an obese-like phenotype in flies, including increased circulating glucose levels and insulin-like resistance.[46] To further assess the role of Proteobacteria in insulin resistance, *E. cloacae* was selected for monocolonisation experiments in *D. melanogaster* due to its dual relevance as both a commensal of the Proteobacteria phylum in *Drosophila*[47 48] and its established association with obesity-related phenotypes in humans and animal models (figure 7A).[49–51] We depicted the *Drosophila* orthologues of the jejunal genes identified in cluster 2 involved in the regulation of the immune system *CR2* (*hig*), *DOCK8* (*Zir*), *GRAP2* (*drk*), *CYFIP2* (*Cyflip*), *SPN* (*Deaf*) and *RHOH* (*Rac1*) (figure 5D) and assessed relative gene expression by qRT-PCR in the fly's intestine fed with standard diet (SD) or HFD either raised in axenic conditions or mono-associated with *E. cloacae*. HFD-fed flies exhibited a significant decrease in the expression of *hig, Rac1, Cyflip* and *Deaf*, which aligns with our findings in humans, as these genes were positively associated with insulin sensitivity in the jejunum (figure 7B-G). Similarly, SD-fed flies mono-associated with *E. cloacae* showed a significant reduction in the intestinal expression of *hig*, *Rac1* and a tendency for *Deaf* (figure 7B-G). These findings collectively suggest that both HFD and mono-associations with *E. cloacae* lead to alterations in intestinal gene expression patterns reminiscent of those observed in insulin-resistant humans.

Next, we conducted gene expression analysis in the *Drosophila* fat body, which serves as the equivalent of human adipose tissue. We assessed the expression of Cluster 1 genes, *Past1*, *ssh*, *siz*, *cta*, *Dsor1* and *Col4a1*, which correspond to the human homologues of *EHD2*, *SSH1*, *IQSEC1*, *GNA12*, *MAP2K1* and *COL4A2*, respectively (figure 5F,G). HFD-fed flies displayed a significant increase in the expression of *Col4a1, siz* and *Past1* compared with SD-fed flies (figure 7H-M). In humans, these genes were negatively associated with insulin sensitivity in the VAT (figure 5F,G). Mono-associations with *E. cloacae* did not affect FB expression of the selected genes.

Fruit flies do not contain an organ equivalent to the liver; its functions are assumed by the fat body.[52 53] Therefore, we assessed fat body gene expression of *vib, Moe, Ac76E* and *mys* (figure 7N-Q), the *Drosophila* orthologues of *PITPNA*, *MSN*, *ADCY7*, and *ITGB2,* all of them associated with the hyperinsulinaemic-euglycaemic clamp in liver transcriptomics (figure 6G). HFD-fed flies mono-associated with *E. cloacae* exhibited a significant increase of *vib* expression in the FB (figure 7N). Remarkably, we observed that *PITPNA*, its corresponding human orthologue, was depicted as one of the genes associated with insulin resistance together with species from the Proteobacteria phylum (figure 6E). Furthermore, *Moe*, the *Drosophila* orthologue of *MSN*, which displayed an inverse association with insulin sensitivity in the human liver, exhibited a significant upregulation in HFD-fed flies (figure 7O).

Finally, in order to investigate the role of Proteobacteria in insulin resistance and validate our model, we assessed the expression of *dilp2* (*Drosophila* insulin-like peptide 2), one of the *Drosophila* orthologues of human insulin. We observed increased expression of *dilp2* in HFD-fed flies compared with SD-fed flies, resembling insulin resistance as previously described.[46] Mono-association of flies with *E. cloacae* further elevated the levels of *dilp2* expression of SD-fed flies, indicating that this bacterium exacerbates insulin resistance also in *D. melanogaster* (figure 7R). Additionally, flies monocolonised with *E. cloacae* showed a significant decrease in the expression of hydroxyphenylpyruvate dehydrogenase (*Hpd*) compared with axenic flies (figure 7S). *Hpd* expression is regulated by the transcription factor dFOXO, which is activated downstream of PI3K in response to insulin.[54] In conditions of insulin resistance, there is a decrease in dFOXO signalling and the expression of its downstream genes[55] (figure 7U). Finally, when challenged for glucose tolerance,[56] larvae monocolonised with *E. cloacae* did not clear glucose from haemolymph as efficiently as sterile larvae. Ninety min after ingesting a glucose solution, the *E. cloacae*-colonised larvae had significantly higher levels of glucose in the haemolymph (figure 7T). Given that *Drosophila* larvae primarily use trehalose as a circulating sugar,[56] we also measured the levels of circulating trehalose in addition to glucose. Our findings indicated that larvae monocolonised with *E. cloacae* consistently exhibited higher levels of trehalose and glucose in the haemolymph compared with sterile larvae (online supplemental figure 6).

Overall, these experiments align with the transcriptional changes observed in the human intestine, VAT and liver, correlating with insulin sensitivity and further supporting the role of the Proteobacteria in the exacerbation of insulin resistance pathophysiology when present in the intestine.

Fasting glucose levels are linked to antiviral response genes

Our results identified consistent associations between insulin sensitivity and glycaemic state with specific microbiota and T cell numbers and signalling in different tissues. Given the importance of T cells in responding to viral infections, and our observation of intriguing associations between insulin sensitivity and viral infections such as Epstein-Barr virus infection, viral myocarditis and human cytomegalovirus infection (figure 2B and figure 6F), we also analysed the associations of fasting glucose with the transcriptome in the jejunum, ileum, VAT and liver (figure 8A-C,G and online supplemental tables 45–48). Strikingly, a GP-BP analysis revealed the defence response to the virus (figure 8D,H and online supplemental table 49) and viral transcription (figure 8E,H and online supplemental table 50) as

the most over-represented processes in the jejunum and ileum. It also highlighted the type I interferon (IFN) production and the cytoplasmic pattern recognition receptor pathway in response to the virus (figure 8D). A KEGG over-representation analysis in the colon and VAT identified a significant enrichment of the viral protein interaction with cytokine and cytokine receptor (figure 8F,H and online supplemental table 51), and the viral infection, including the hepatitis C, influenza A and cytomegalovirus infection, which have been associated with an increased risk for the development of prediabetes and diabetes.[57] A pathway over-representation analysis in the jejunum also highlighted an enrichment of the IFN signalling, the antiviral mechanism by IFN-stimulated genes and the oligoadenylate synthetase antiviral response (online supplemental table 52), while the type II IFN signalling was also over-represented in the colon (figure 8H and online supplemental table 53). A recent study also identified that downregulation of glucose metabolism promoted retinoic acid-inducible gene I-like receptor (RLR)-induced type I IFN production along with a reduction in virus replication and identified elevated lactate dehydrogenase and/or glycolysis as a potential mechanism for viruses to evade host defence by inhibiting RLR-triggered type I IFN production.[58] Type I IFNs play a crucial role in protecting cells from viral pathogens. A screening of over >380 human IFN-stimulated genes revealed eight broadly acting effectors, including IRF7 and IFIH1,[59] which we also found associated with glucose levels in the ileum (figure 8H). Genes identified in the jejunum included *OASL,* a gene involved in targeted antiviral specificity and well-known antiviral effectors such as *EIF2AK2*, or the IFN-induced genes, *OAS1, OAS2* and *OAS3,* which play a key role in innate cellular antiviral response and have recently been identified as associated with critical illness caused by COVID-19.[60]

CONCLUSIONS

Our study sought to uncover the complex interactions among the gut microbiota, intestine, adipose tissue and liver and their effects on systemic insulin action. Using a multiomics (metagenomics, metabolomics, transcriptomics and lipidomics), multitissue (jejunum, ileum, adipose tissue and liver) approach across six different studies, combined with gold-standard measurements of insulin sensitivity using the hyperglycaemic-euglycaemic clamp and other measurements of glucose metabolism and insulin resistance, we provide the first comprehensive analysis of its kind. This integrative approach offers an unprecedented comprehensive view into the intricate microbiome-gut-adipose-liver crosstalk influencing insulin sensitivity (figure 9).

However, our study has several limitations. Although it includes multiomics measurements in multiple tissues across multiple studies, its nature is mainly observational. In addition, some studies had a small sample size, which is a limitation for the analysis of high-dimensional omics data sets and their integration. Thus, data from large-scale longitudinal studies would have further supported the current findings. Finally, not all omics data were available in all tissues and all studies. For instance, faecal shotgun metagenomics data was only available in three studies, while gold standard measurements of insulin sensitivity were only performed in four studies.

Despite this, it is the first study to offer comprehensive insights into the microbiome-gut-adipose-liver axis and its impact on systemic insulin action in humans. Our approach was crucial in underscoring the pivotal role of Proteobacteria, bile acids and T cell-related genes in influencing insulin sensitivity and identified a positive association between insulin sensitivity and SCFA-producing species from the *Blautia* and *Faecalibacterium* genera. Thus, our single- and integrated-omics analyses unveiled faecal microbial signatures comprising taxa from the Proteobacteria phylum and DCA in the jejunum negatively associated with insulin sensitivity, along with intestine (jejunum, ileum and colon) T cell-related signatures that were linked to better insulin sensitivity. Transcriptomics analyses of the SAT and VAT also revealed upregulated and downregulated T cell-related signatures linked to HbA1c and insulin sensitivity, respectively, while Proteobacteria in the VAT were negatively associated with insulin sensitivity. This opposite association of T cell signatures in the intestine and VAT in relation to insulin sensitivity might indicate asynchronous responses to environmental stimuli. Increased intestinal immune response might prevent gut dysbiosis-induced insulin resistance, while increased adipose immune response could indicate insulin resistance-associated adipose tissue dysfunction, for which chronic activation could be detrimental. Multiomics analyses in the liver revealed again negative associations of liver deoxycholic acid with insulin sensitivity as well as a liver transcriptomic signature involved in insulin and T cell signalling. Notably, the involvement of *Enterobacteria* through several of the identified genes in the jejunum, VAT and liver was validated in the intestine and fat body of *D. melanogaster* models. Lastly, we identified a consistent link between glucose metabolism and IFN-induced genes involved in the antiviral response. In fact, recent evidence supports a bidirectional relationship between metabolic disease and viral infection.[57] Hence, the immune system may induce transient changes in glucose metabolism as a strategy against viral infection.[61] Our study emphasises the significance of considering microbial functionality and adopting a multiomics integration approach to thoroughly understand the mechanisms driving the complex interplay among tissues and the gut microbiota within the context of obesity and insulin resistance. The novo insights gained from our findings may lead to the development of innovative therapeutic strategies targeting the gut microbiota and the intestine-adipose-liver axis to enhance insulin sensitivity and improve metabolic health.

Author affiliations
[1]Department of Diabetes, Endocrinology and Nutrition, Dr. Josep Trueta University Hospital, Girona, Spain
[2]Nutrition, Eumetabolism and Health Group, Girona Biomedical Research Institute (IDIBGI-CERCA), Girona, Spain
[3]Integrative Systems Medicine and Biology Group, Girona Biomedical Research Institute (IDIBGI-CERCA), Parc Hospitalari Martí i Julià, Edifici M2, Salt, Spain
[4]CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain
[5]Department of Medical Sciences, School of Medicine, University of Girona, Girona, Spain
[6]Department of Systems Medicine, University of Rome Tor Vergata, Via Montpellier 1, Rome, Italy
[7]Institut National de la Santé et de la Recherche Médicale (INSERM), Toulouse, France
[8]Université Paul Sabatier (UPS), Unité Mixte de Recherche (UMR), Toulouse, France
[9]Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), Team 2: 'Intestinal Risk Factors, Diabetes, Dyslipidemia, and Heart Failure', F-31432, Toulouse, France
[10]Team "Immunité et ALTernatives aux Antibiotiques (IALTA)", Laboratory of host to pathogens Interactions (IHAP), UMR INRAE 1225 / ENVT, Toulouse, France
[11]Department of Diabetology, metabolic Diseases and Nutrition, CHU de Toulouse, Toulouse, France
[12]Institute of Metabolic and Cardiovascular Diseases, UMR1297 I2MC, INSERM, Toulouse 3 University, Toulouse, France
[13]Department of Endocrinology & Nutrition, Clínica Universidad de Navarra, IdiSNA, Pamplona, Spain
[14]Fisiopatología Endocrina Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Complejo Hospitalario Universitario de Santiago de Compostela (CHUS/SERGAS), Santiago de Compostela, Spain

[15]Department of Internal Medicine, Hospital Universitario Reina Sofía, Maimonides Institute for Biomedical Research in Cordoba (IMIBIC), Universidad de Córdoba, Córdoba, Spain
[16]Virgen de la Victoria Hospital, Department of Endocrinology, Instituto de Investigación Biomédica de Málaga (IBIMA), University of Málaga, Málaga, Spain
[17]Department of Physiology, CIMUS, University of Santiago de Compostela, Instituto de Investigación Sanitaria, Santiago de Compostela, Spain
[18]Section of Biomolecular Medicine, Division of Systems Medicine, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK
[19]Section of Genomic and Environmental Medicine, National Heart & Lung Institute, Imperial College London, London, UK
[20]European Genomic Institute for Diabetes, CNRS UMR 8199, INSERM UMR 1283, Institut Pasteur de Lille, Lille University Hospital; University of Lille, Lille, France
[21]McGill Genome Centre, Mc Gill University, Montréal, Quebec, Canada
[22]Department of Genomics and Health, Foundation for the Promotion of Health and Biomedical Research of Valencia Region (FISABIO-Public Health), Valencia, Spain
[23]Biomedical Research Networking Center for Epidemiology and Public Health (CIBERESP), Madrid, Spain
[24]Institute for Integrative Systems Biology (I2SysBio), University of Valencia, Spanish National Research Council (CSIC-UVEG), Valencia, Spain
[25]Department of Internal Medicine III, University Hospital Carl Gustav Carus, TU Dresden, Paul Langerhans Institute Dresden (PLID), Helmholtz Center Munich, Dresden, Germany
[26]Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Rome, Italy
[27]General and Digestive Surgery Service, Dr. Josep Trueta University Hospital, Girona, Spain

X Andrés Moya @moyasimarro1

Contributors AC-N performed part of statistical analysis and wrote the manuscript. JMM-N researched the data and partially wrote the manuscript. MA-R contributed to data collection for the IRONMET and ADIPOINST studies. AC-N, LdlV-C and IP performed Drosophila experiments. RB, LG-P, PG and LC contributed to the SIMMUNIDIA cohort. GF, LMS, JL-M, FT and CD contributed to the ADIPOMIT cohort. MF contributed to the FLOROMIDIA cohort. RB, M-ED, MF and JMF-R contributed to the FLORINASH cohort. VP-B and AM contributed to the shotgun metagenomics analysis of faecal samples. All authors contributed to the discussion and reviewed the manuscript. JMF-R and JM-P carried out the conception and coordination of the study, performed statistical analysis and wrote the manuscript. JIR-H and EC contributed to the collection of adipose and intestine tissue samples. JMF-R and JM-P are the guarantors.

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Ethics approval This study involves human participants. All participants gave written informed consentto participate in the study before taking part and all procedures were performed in accordance with the Declaration of Helsinki. The studies IRONMET (2015.111; 2022.184 and 2023.239), ADIPOMIT (2019.062) and ADIPOINST (2016.051 and 2019.143) were approved by Ethics Committee of the Hospital Dr Josep Trueta. FLORINASH was approved by the Ethics Committee of the Hospital Dr Josep Trueta (200904, 2022.187, 2022.066) and the ethical committee of Policlinico Tor Vergata University of Rome (49/09). FLOROMIDIA was validated and approved by the ethical committee of Policlinico Tor Vergata University of Rome (Comitato Etico Indipendente, approval number 113/15). SIMMUNIDIA was approved by the Ethics Committee of the Rangueil Hospital, France (RC31/16/8217). Registration number IDRCB/EudraCT (reference CPP-ANSM): 2016-A01213-48. ClinicalTrials.gov Identifier: NCT02811575.

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ORCID iDs
José Maria Moreno-Navarrete http://orcid.org/0000-0002-2883-511X
Massimo Federici http://orcid.org/0000-0003-4989-5194
Remy Burcelin http://orcid.org/0000-0002-7942-8346
Luisa Maria Seoane http://orcid.org/0000-0003-1004-9898
Marc-Emmanuel Dumas http://orcid.org/0000-0001-9523-7024
Andrés Moya http://orcid.org/0000-0002-2867-1119
Jose Manuel Fernández-Real http://orcid.org/0000-0002-7442-9323
Jordi Mayneris-Perxachs http://orcid.org/0000-0003-3788-3815

REFERENCES

1 Canfora EE, Meex RCR, Venema K, *et al*. Gut microbial metabolites in obesity, NAFLD and T2DM. *Nat Rev Endocrinol* 2019;15:261–73.
2 Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol* 2021;19:55–71.
3 Cani PD, Amar J, Iglesias MA, *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;56:1761–72.
4 Cani PD, Bibiloni R, Knauf C, *et al*. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008;57:1470–81.
5 Konrad D, Wueest S. The gut-adipose-liver axis in the metabolic syndrome. *Physiology (Bethesda)* 2014;29:304–13.
6 de Groot P, Scheithauer T, Bakker GJ, *et al*. Donor metabolic characteristics drive effects of faecal microbiota transplantation on recipient insulin sensitivity, energy expenditure and intestinal transit time. *Gut* 2020;69:502–12.
7 Vrieze A, Van Nood E, Holleman F, *et al*. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012;143:913–6.
8 Vrieze A, Out C, Fuentes S, *et al*. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. *J Hepatol* 2014;60:824–31.
9 Kootte RS, Levin E, Salojärvi J, *et al*. Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition. *Cell Metab* 2017;26:611–9.

10 Naderpoor N, Mousa A, Gomez-Arango LF, *et al*. Faecal Microbiota Are Related to Insulin Sensitivity and Secretion in Overweight or Obese Adults. *J Clin Med* 2019;8:452.
11 Hermes GDA, Reijnders D, Kootte RS, *et al*. Individual and cohort-specific gut microbiota patterns associated with tissue-specific insulin sensitivity in overweight and obese males. *Sci Rep* 2020;10:7523.
12 Lin H, Peddada SD. Analysis of compositions of microbiomes with bias correction. *Nat Commun* 2020;11:3514.
13 Qin J, Li Y, Cai Z, *et al*. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature New Biol* 2012;490:55–60.
14 Larsen N, Vogensen FK, van den Berg FWJ, *et al*. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010;5:e9085.
15 Reijnders D, Goossens GH, Hermes GDA, *et al*. Effects of Gut Microbiota Manipulation by Antibiotics on Host Metabolism in Obese Humans: A Randomized Double-Blind Placebo-Controlled Trial. *Cell Metab* 2016;24:63–74.
16 Furet J-P, Kong L-C, Tap J, *et al*. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* 2010;59:3049–57.
17 Liou AP, Paziuk M, Luevano J-M Jr, *et al*. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Sci Transl Med* 2013;5:178ra41.
18 Guadagnini D, Rocha GZ, Santos A, *et al*. Microbiota determines insulin sensitivity in TLR2-KO mice. *Life Sci* 2019;234:116793.
19 Vich Vila A, Collij V, Sanna S, *et al*. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat Commun* 2020;11:362.
20 Forslund SK, Chakaroun R, Zimmermann-Kogadeeva M, *et al*. Combinatorial, additive and dose-dependent drug-microbiome associations. *Nature New Biol* 2021;600:500–5.
21 Mayneris-Perxachs J, Fernández-Real JM. Exploration of the microbiota and metabolites within body fluids could pinpoint novel disease mechanisms. *FEBS J* 2020;287:856–65.
22 Kamenova P. Improvement of insulin sensitivity in patients with type 2 diabetes mellitus after oral administration of alpha-lipoic acid. *Hormones (Athens)* 2006;5:251–8.
23 Jacob S, Ruus P, Hermann R, *et al*. Oral administration of RAC-alpha-lipoic acid modulates insulin sensitivity in patients with type-2 diabetes mellitus: a placebo-controlled pilot trial. *Free Radic Biol Med* 1999;27:309–14.
24 Jacob S, Henriksen EJ, Tritschler HJ, *et al*. Improvement of insulin-stimulated glucose-disposal in type 2 diabetes after repeated parenteral administration of thioctic acid. *Exp Clin Endocrinol Diabetes* 1996;104:284–8.
25 Koh A, Molinaro A, Ståhlman M, *et al*. Microbially Produced Imidazole Propionate Impairs Insulin Signaling through mTORC1. *Cell* 2018;175:947–61.
26 Molinaro A, Bel Lassen P, Henricsson M, *et al*. Imidazole propionate is increased in diabetes and associated with dietary patterns and altered microbial ecology. *Nat Commun* 2020;11:5881.
27 Johnson AMF, Olefsky JM. The origins and drivers of insulin resistance. *Cell* 2013;152:673–84.
28 Lema I, Araújo JR, Rolhion N, *et al*. Jejunum: The understudied meeting place of dietary lipids and the microbiota. *Biochimie* 2020;178:124–36.
29 Jung C, Hugot J-P, Barreau F. Peyer's Patches: The Immune Sensors of the Intestine. *Int J Inflam* 2010;2010:823710.
30 Winer S, Chan Y, Paltser G, *et al*. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med* 2009;15:921–9.
31 McLaughlin T, Liu L-F, Lamendola C, *et al*. T-cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. *Arterioscler Thromb Vasc Biol* 2014;34:2637–43.
32 Tilg H, Zmora N, Adolph TE, *et al*. The intestinal microbiota fuelling metabolic inflammation. *Nat Rev Immunol* 2020;20:40–54.
33 Gutiérrez-Repiso C, Moreno-Indias I, Martín-Núñez GM, *et al*. Mucosa-associated microbiota in the jejunum of patients with morbid obesity: alterations in states of insulin resistance and metformin treatment. *Surg Obes Relat Dis* 2020;16:1575–85.
34 Garidou L, Pomié C, Klopp P, *et al*. The Gut Microbiota Regulates Intestinal CD4 T Cells Expressing RORγt and Controls Metabolic Disease. *Cell Metab* 2015;22:100–12.
35 Xu M, Cen M, Shen Y, *et al*. Deoxycholic Acid-Induced Gut Dysbiosis Disrupts Bile Acid Enterohepatic Circulation and Promotes Intestinal Inflammation. *Dig Dis Sci* 2021;66:568–76.
36 Liu L, Dong W, Wang S, *et al*. Deoxycholic acid disrupts the intestinal mucosal barrier and promotes intestinal tumorigenesis. *Food Funct* 2018;9:5588–97.
37 Zeng H, Safratowich BD, Cheng W-H, *et al*. Deoxycholic Acid Modulates Cell-Junction Gene Expression and Increases Intestinal Barrier Dysfunction. *Molecules* 2022;27:723.
38 Wang L, Gong Z, Zhang X, *et al*. Gut microbial bile acid metabolite skews macrophage polarization and contributes to high-fat diet-induced colonic inflammation. *Gut Microbes* 2020;12:1–20.
39 Haeusler RA, Astiarraga B, Camastra S, *et al*. Human insulin resistance is associated with increased plasma levels of 12α-hydroxylated bile acids. *Diabetes* 2013;62:4184–91.
40 Tsakiridis T, Tong P, Matthews B, *et al*. Role of the actin cytoskeleton in insulin action. *Microsc Res Tech* 1999;47:79–92.
41 Kanzaki M, Pessin JE. Insulin-stimulated GLUT4 translocation in adipocytes is dependent upon cortical actin remodeling. *J Biol Chem* 2001;276:42436–44.
42 Wong S-W, Ting Y-W, Yong Y-K, *et al*. Chronic inflammation involves CCL11 and IL-13 to facilitate the development of liver cirrhosis and fibrosis in chronic hepatitis B virus infection. *Scand J Clin Lab Invest* 2021;81:147–59.
43 Fan Z, Sun X, Chen X, *et al*. C-C motif chemokine CCL11 is a novel regulator and a potential therapeutic target in non-alcoholic fatty liver disease. *JHEP Rep* 2023;5:100805.
44 Tamehiro N, Nishida K, Sugita Y, *et al*. Ras homolog gene family H (RhoH) deficiency induces psoriasis-like chronic dermatitis by promoting T_H17 cell polarization. *J Allergy Clin Immunol* 2019;143:1878–91.
45 Crequer A, Troeger A, Patin E, *et al*. Human RHOH deficiency causes T cell defects and susceptibility to EV-HPV infections. *J Clin Invest* 2012;122:3239–47.
46 Miao Y, Chen R, Wang X, *et al*. *Drosophila melanogaster* diabetes models and its usage in the research of anti-diabetes management with traditional Chinese medicines. *Front Med (Lausanne)* 2022;9:953490.
47 Adair KL, Wilson M, Bost A, *et al*. Microbial community assembly in wild populations of the fruit fly Drosophila melanogaster. *ISME J* 2018;12:959–72.
48 Cox CR, Gilmore MS. Native microbial colonization of Drosophila melanogaster and its use as a model of Enterococcus faecalis pathogenesis. *Infect Immun* 2007;75:1565–76.
49 Fei N, Zhao L. An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. *ISME J* 2013;7:880–4.
50 Yan H, Fei N, Wu G, *et al*. Regulated Inflammation and Lipid Metabolism in Colon mRNA Expressions of Obese Germfree Mice Responding to *Enterobacter cloacae* B29 Combined with the High Fat Diet. *Front Microbiol* 2016;7:1786.
51 Broderick NA, Lemaitre B. Gut-associated microbes of Drosophila melanogaster. *Gut Microbes* 2012;3:307–21.
52 Moraes KCM, Montagne J. *Drosophila melanogaster*: A Powerful Tiny Animal Model for the Study of Metabolic Hepatic Diseases. *Front Physiol* 2021;12:728407.
53 Arquier N, Léopold P. Fly foie gras: modeling fatty liver in Drosophila. *Cell Metab* 2007;5:83–5.
54 Musselman LP, Fink JL, Narzinski K, *et al*. A high-sugar diet produces obesity and insulin resistance in wild-type Drosophila. *Dis Model Mech* 2011;4:842–9.
55 Biglou SG, Bendena WG, Chin-Sang I. An overview of the insulin signaling pathway in model organisms Drosophila melanogaster and Caenorhabditis elegans. *Peptides* 2021;145:170640.
56 Tennessen JM, Barry WE, Cox J, *et al*. Methods for studying metabolism in Drosophila. *Methods* 2014;68:105–15.
57 Perakakis N, Harb H, Hale BG, *et al*. Mechanisms and clinical relevance of the bidirectional relationship of viral infections with metabolic diseases. *Lancet Diabetes Endocrinol* 2023;11:675–93.
58 Zhang W, Wang G, Xu Z-G, *et al*. Lactate Is a Natural Suppressor of RLR Signaling by Targeting MAVS. *Cell* 2019;178:176–89.
59 Schoggins JW, Wilson SJ, Panis M, *et al*. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature New Biol* 2011;472:481–5.
60 Pairo-Castineira E, Clohisey S, Klaric L, *et al*. Genetic mechanisms of critical illness in COVID-19. *Nature New Biol* 2021;591:92–8.
61 Šestan M, Marinović S, Kavazović I, *et al*. Virus-Induced Interferon-γ Causes Insulin Resistance in Skeletal Muscle and Derails Glycemic Control in Obesity. *Immunity* 2018;49:164–77.