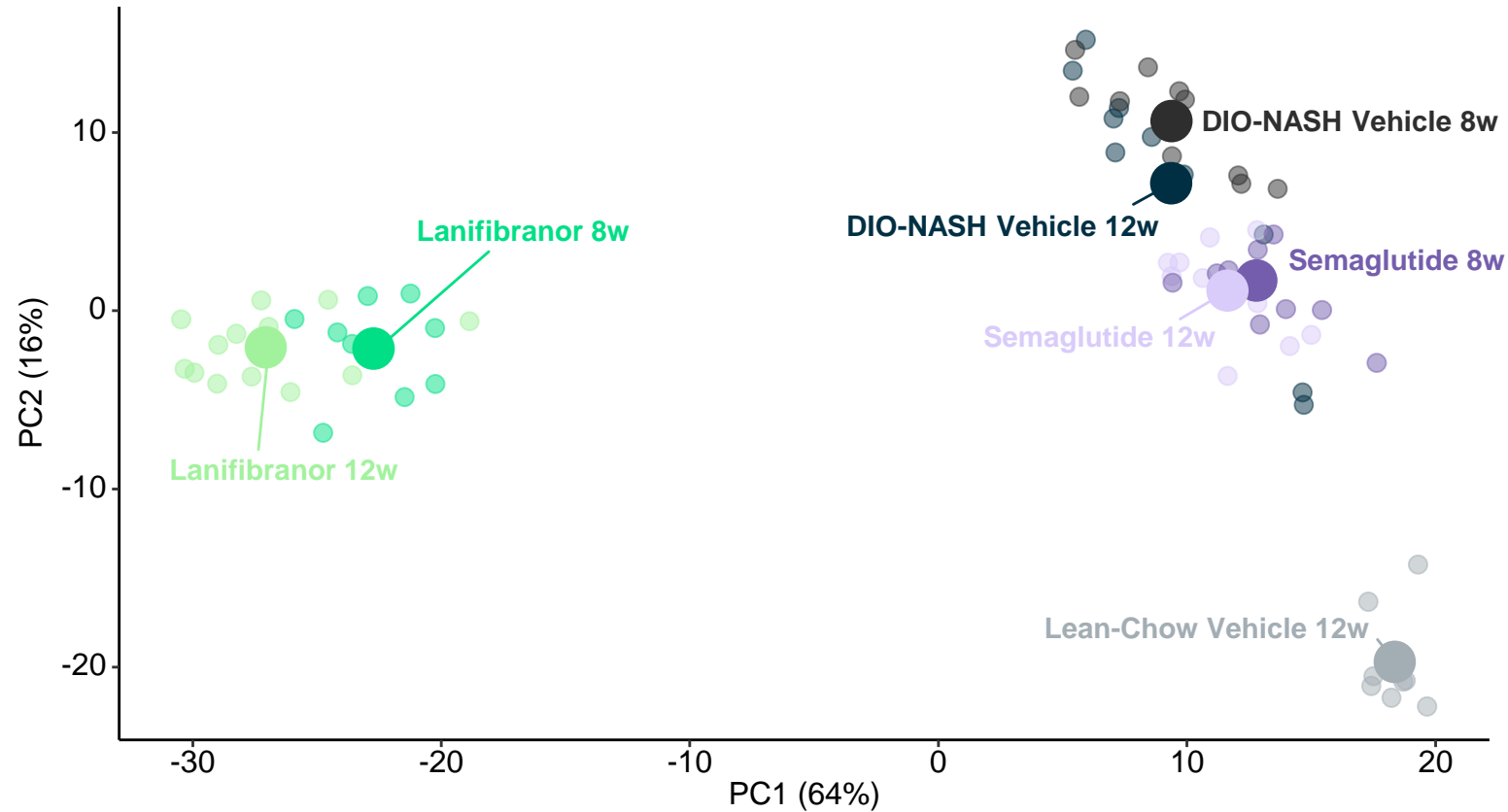


RNA-seq data package

Based on data published in [Møllerhøj et al.](#)

Principal component analysis

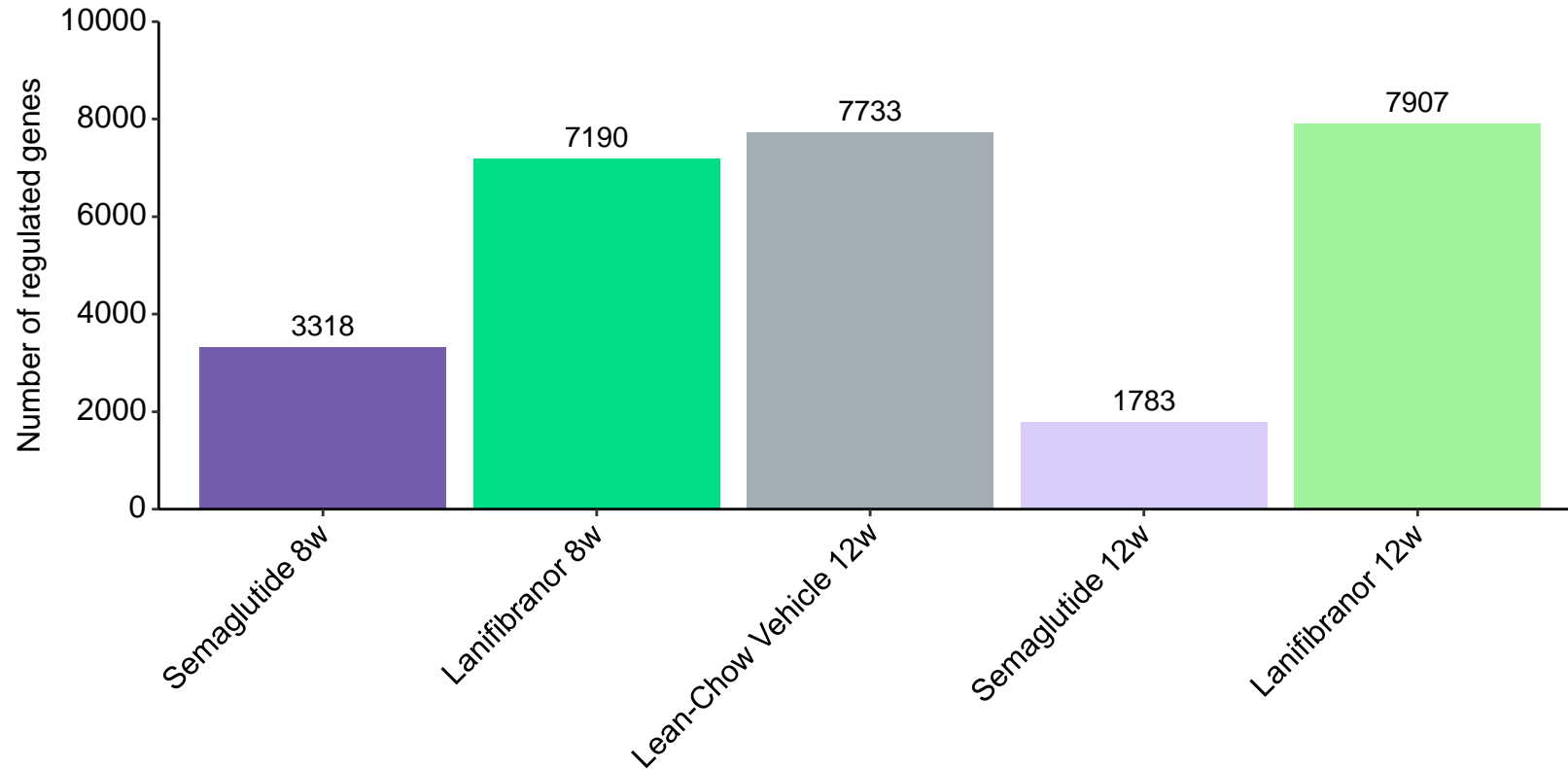
Sample variation within and between study groups



Principal component analysis based on the top 500 most varying genes across the dataset. The axes describe the percentage of variability explained by the principal component 1 (PC1) and principal component 2 (PC2). Each small point represents a sample, and each big point represents the study group average. Samples with similar gene expression profile will locate closer together than samples with different gene expression profiles. Thus, if there is pronounced gene expression differences between groups, the samples will cluster together with members of the same group and away from samples from other groups.

Differential gene expression analysis

Number of differentially expressed genes

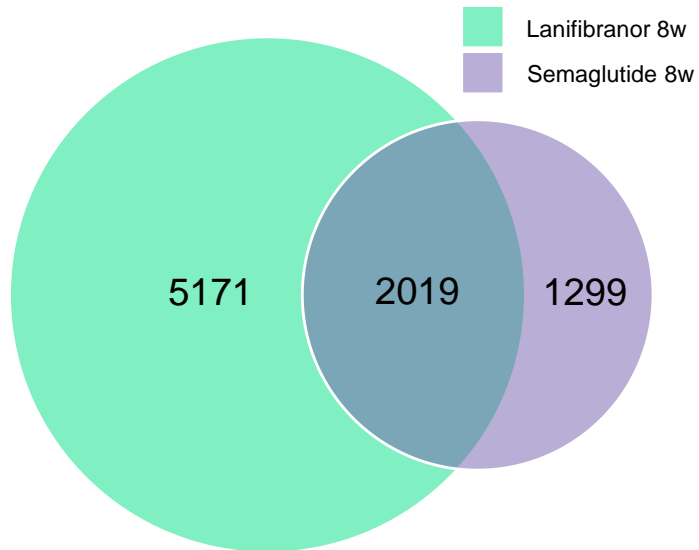


Number of differentially expressed genes for each study group compared to the corresponding DIO-NASH Vehicle at significance level $p_{adj} < 0.05$ after correcting for multiple testing. $n = 8 - 13$.

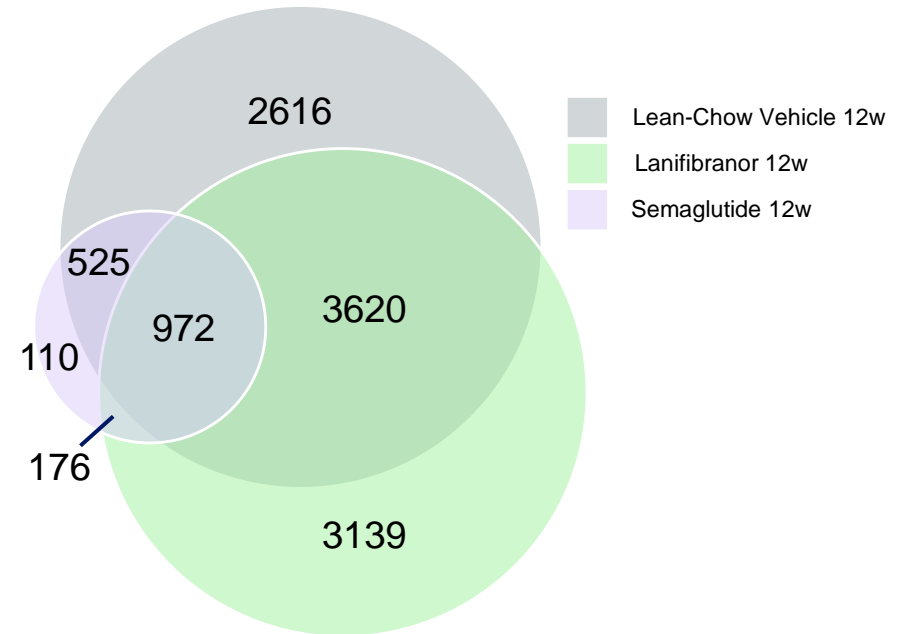
Gene regulation overlaps

Overview of shared regulations

Week 8



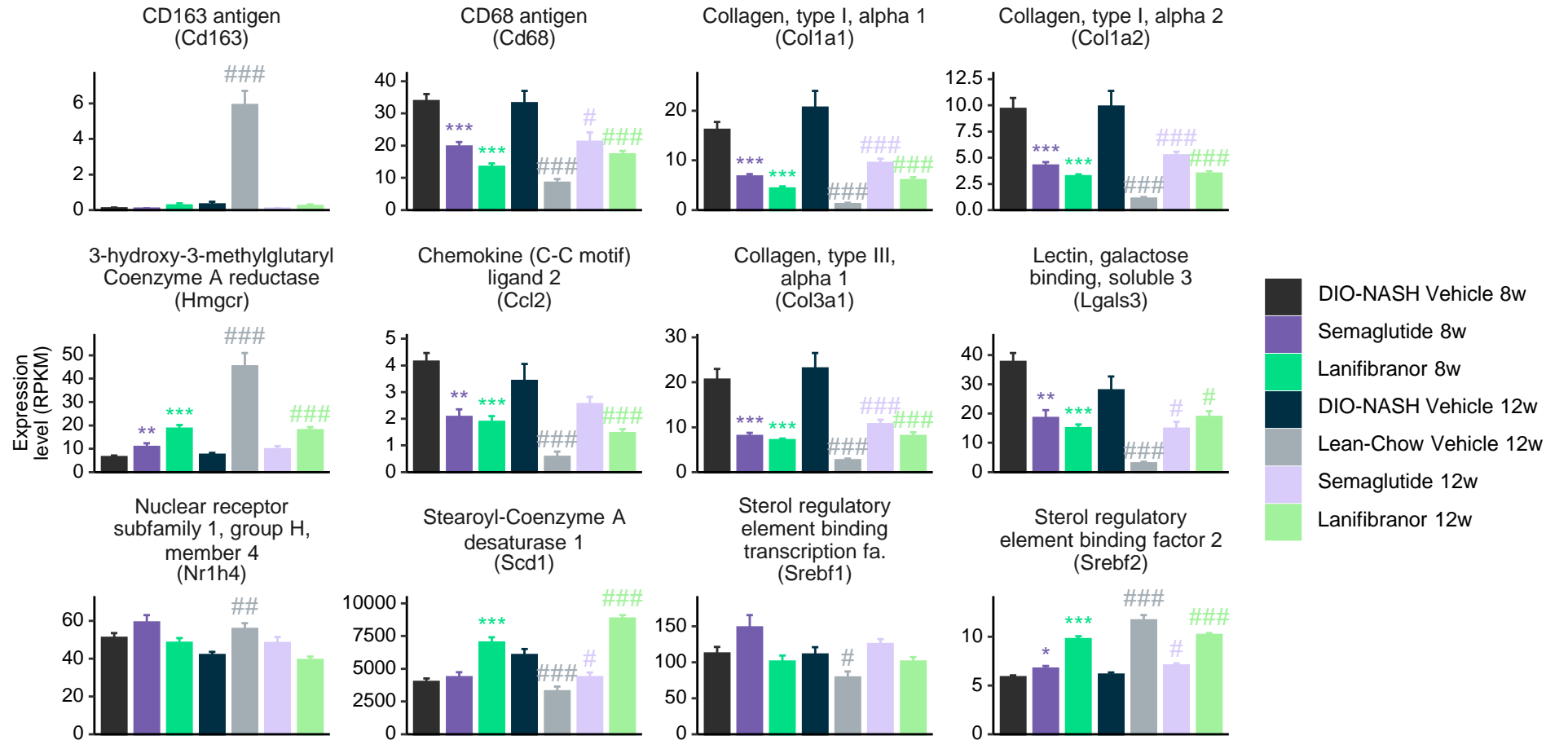
Week 12



Number of differentially expressed genes regulated by either one or more study groups when compared to the corresponding DIO-NASH Vehicle. n = 8 - 13.

MASH: Genes of interest

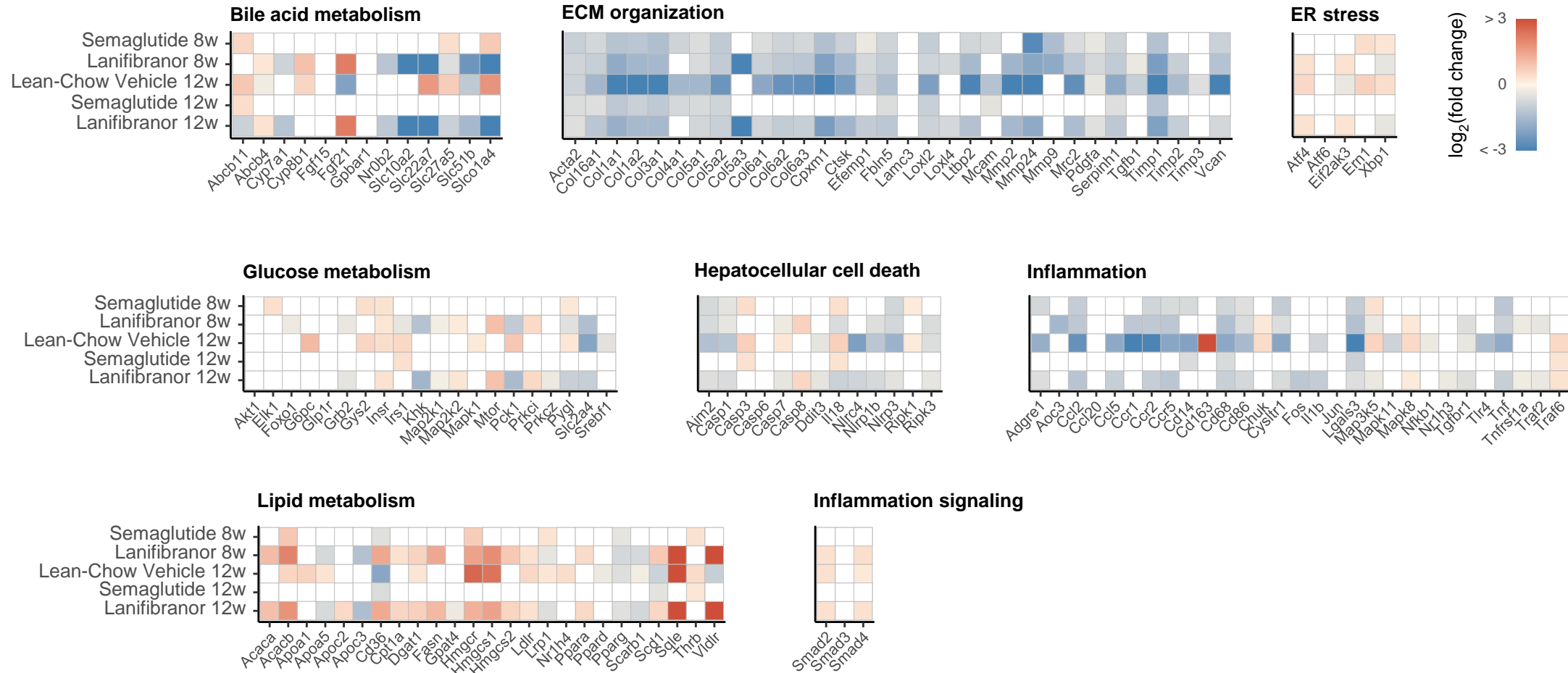
Selected genes associated with MASH biology



Gene expression of selected genes associated with MASH biology. Values are expressed as study group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Gubra curated gene panels

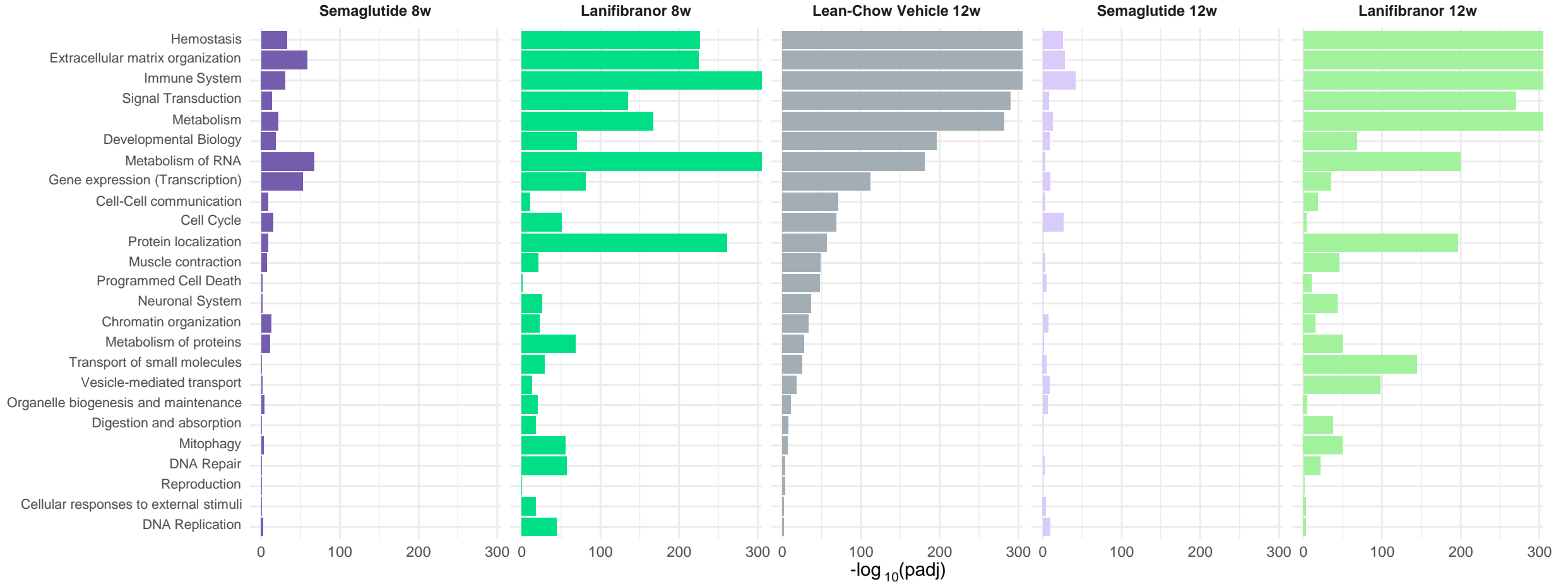
Regulation of disease-related genes



Heatmap of gene regulation in Gubra curated gene panels. \log_2 fold change is shown for significantly regulated genes ($\text{padj} < 0.05$ after correcting for multiple testing). Red and blue colours indicate up- and down-regulation, respectively, when compared to the corresponding DIO-NASH Vehicle. Non-significantly regulated genes for a given comparison are shown in white. $n = 8 - 13$.

Unbiased pathway analysis

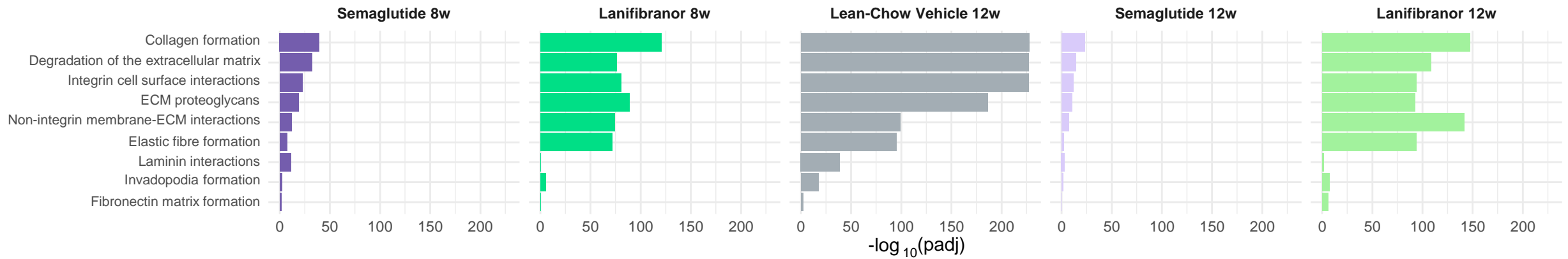
Top-level pathways



Enrichment of top-level pathways (Reactome database) for the shown study groups compared to the corresponding DIO-NASH Vehicle. The x-axis illustrates the level of enrichment depicted as the statistical significance for each gene-set analysis after correcting for multiple testing. n = 8 - 13.

Unbiased pathway analysis

Extracellular matrix organization sub-pathways



Enrichment of *Extracellular matrix organization* sub-pathways (Reactome database, level 2) for the shown study groups compared to the corresponding DIO-NASH Vehicle. The x-axis illustrates the level of enrichment depicted as the statistical significance for each gene-set analysis after correcting for multiple testing. n = 8 - 13.

Gene expression data in GubraView

We believe that the best way to fully exploit the potential of an RNA-seq dataset is to enable our customers to explore and raise questions to the data. For this purpose, we have developed a panel of interactive functionalities in GubraView.

An instructional video showcasing how to use the interactive RNA-seq module in GubraView can be found here:



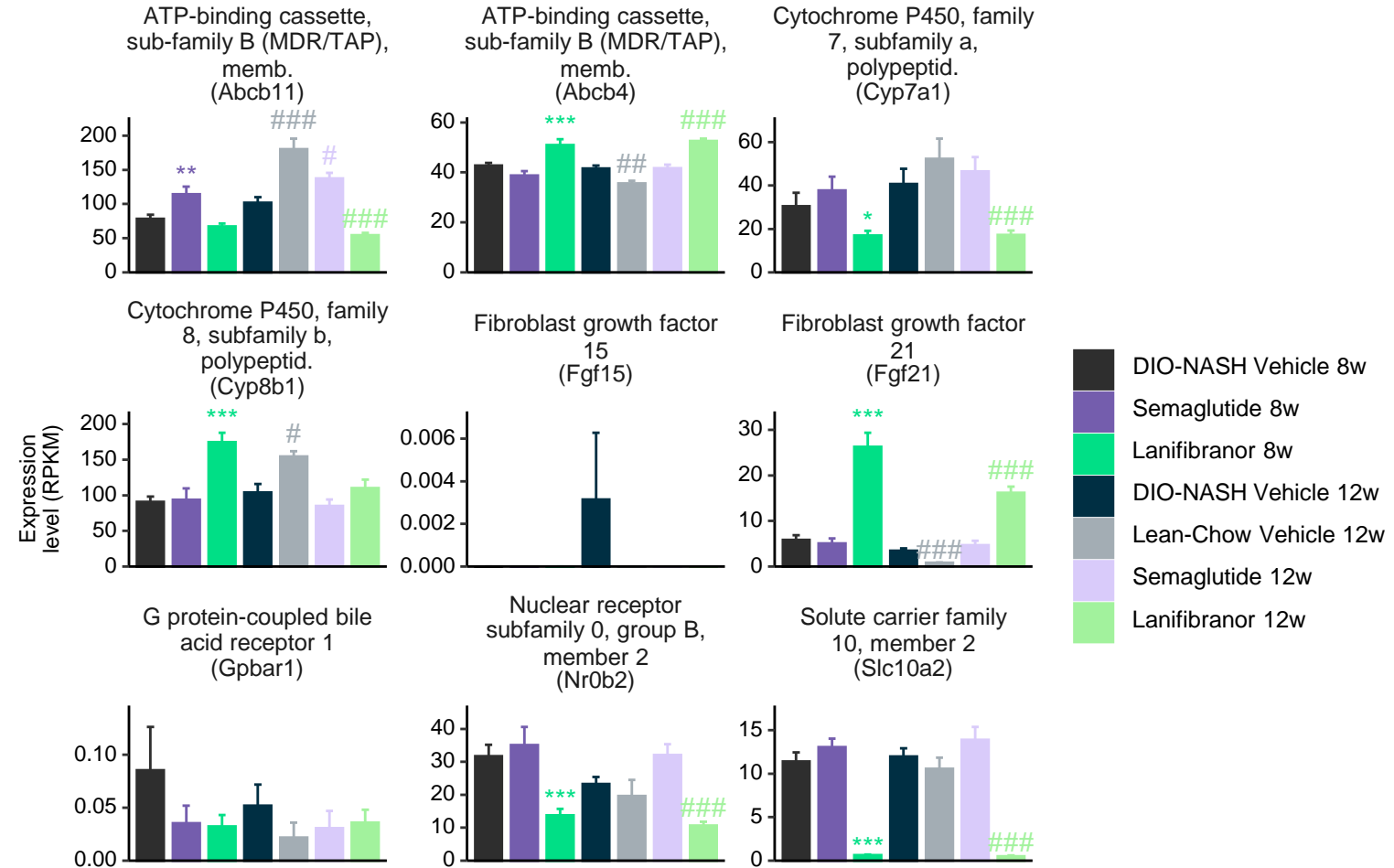
[Video: RNA-seq in GubraView](#)



Appendix

Bile acid metabolism

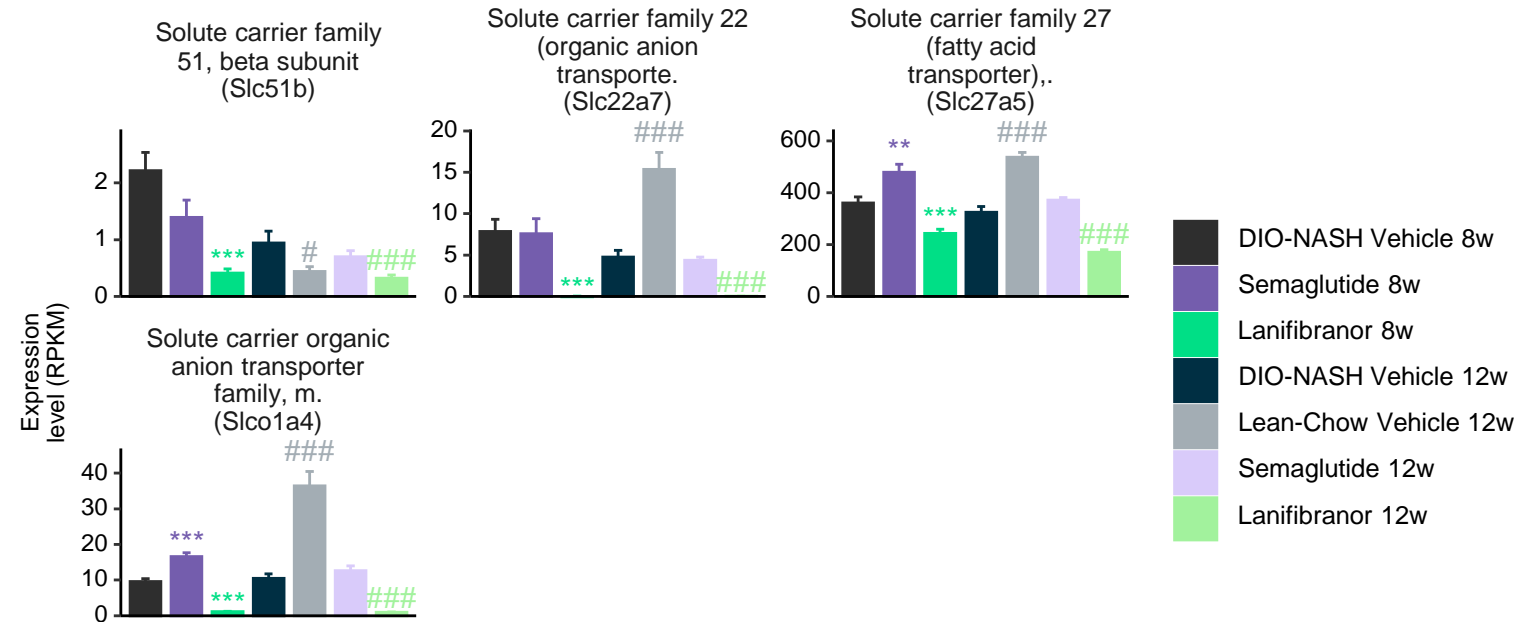
Bile acid metabolism - 1/2



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Bile acid metabolism

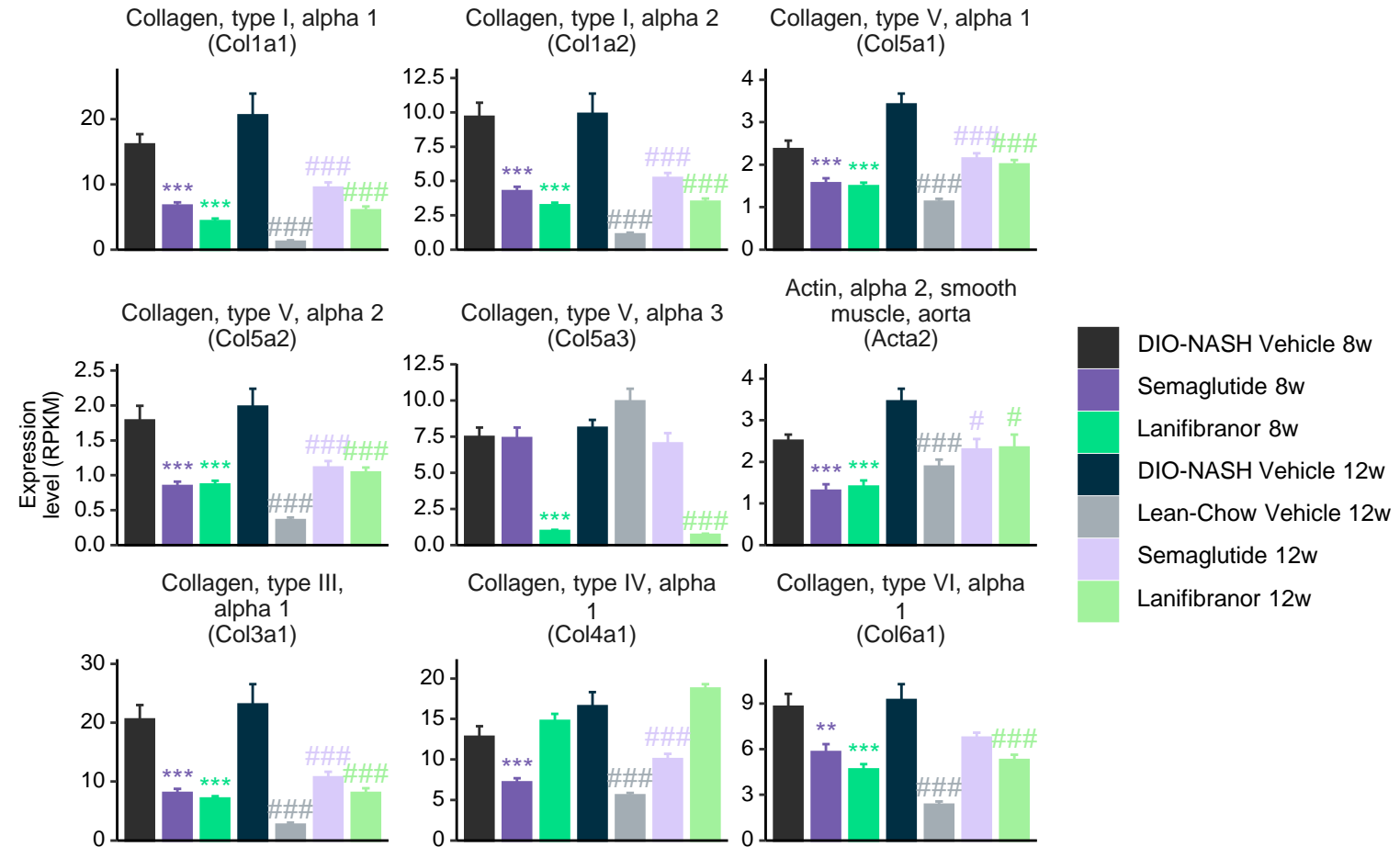
Bile acid metabolism - 2/2



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. #:padj < 0.05, **:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

ECM organization

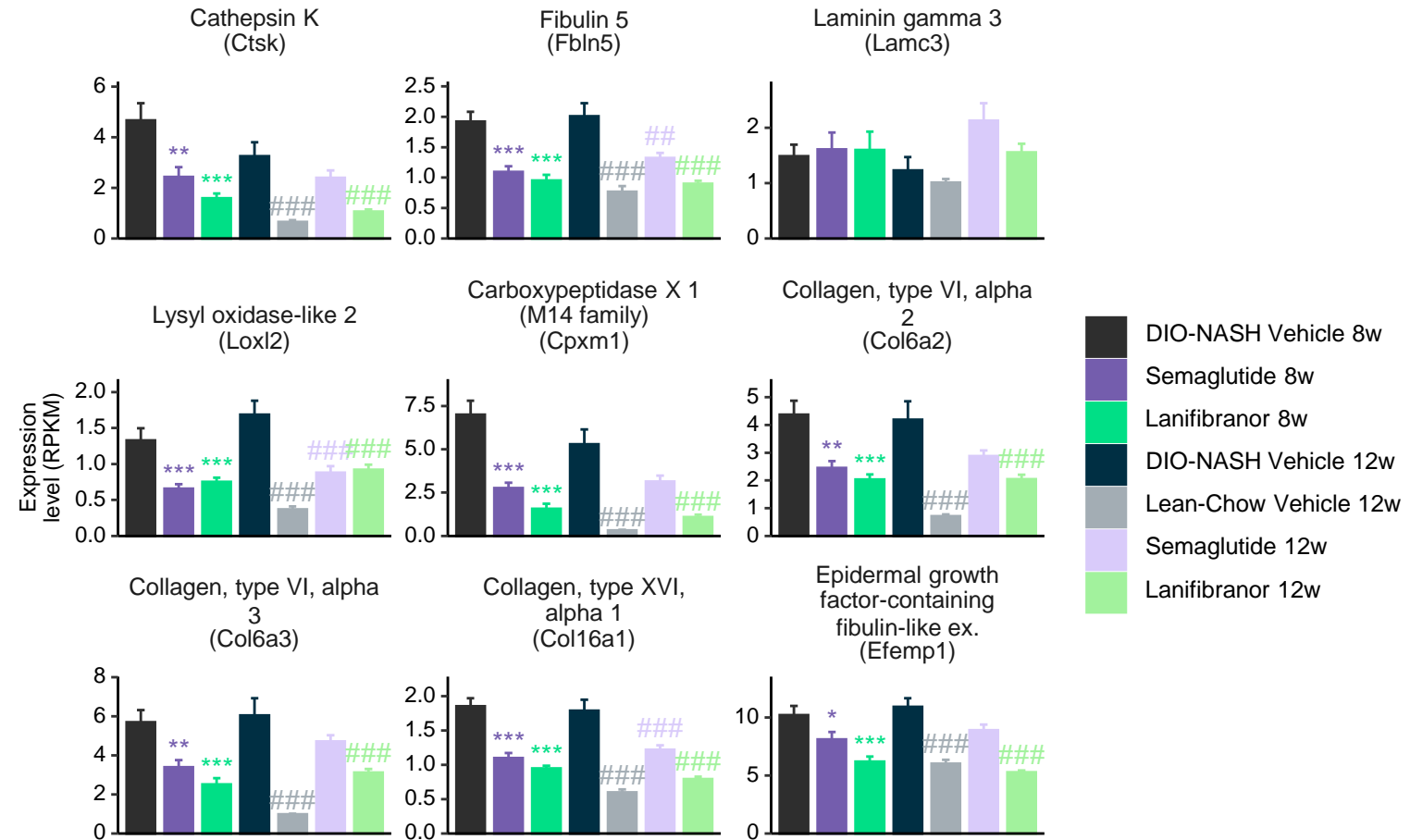
ECM organization - 1/4



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. #:padj < 0.05, **:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

ECM organization

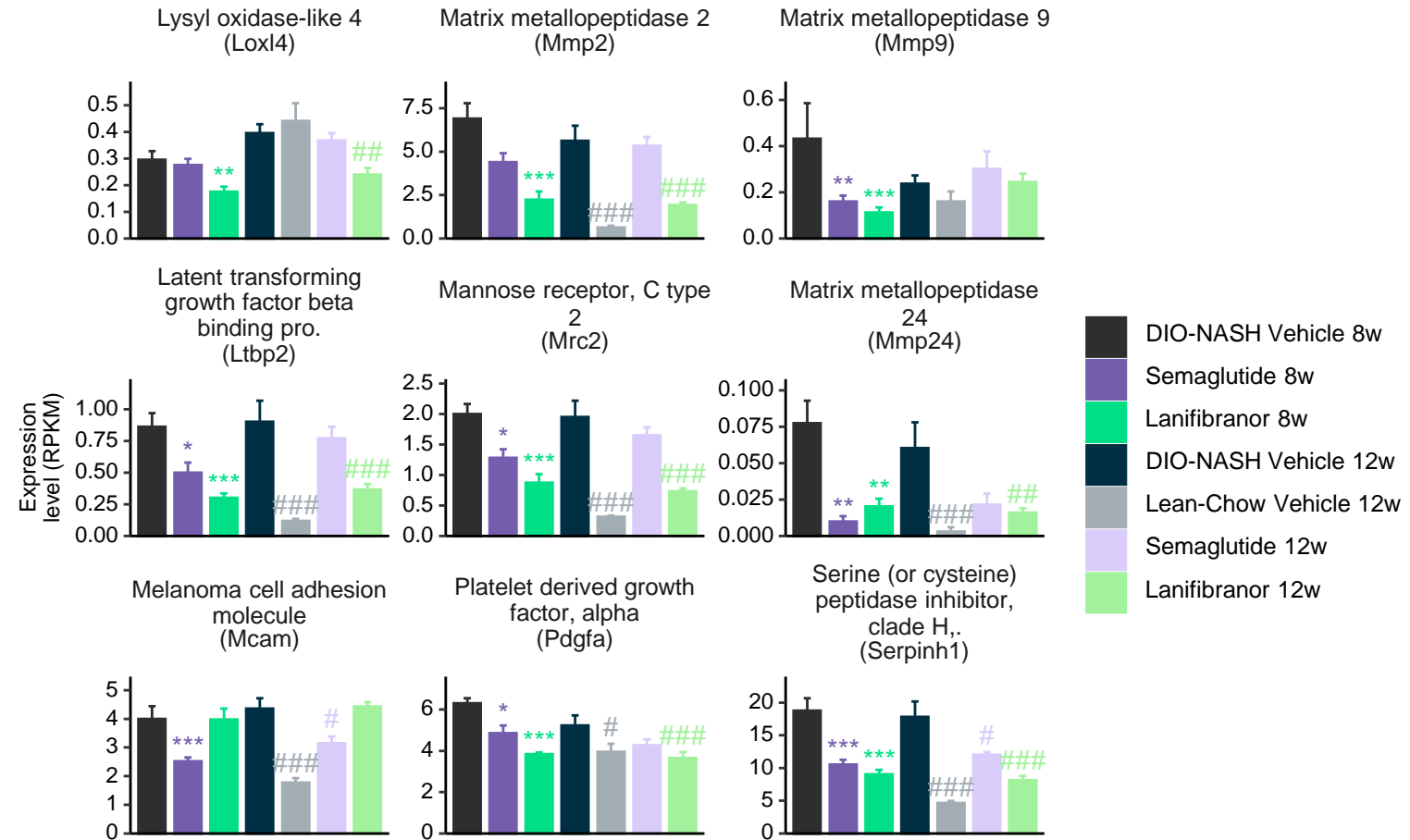
ECM organization - 2/4



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. *:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

ECM organization

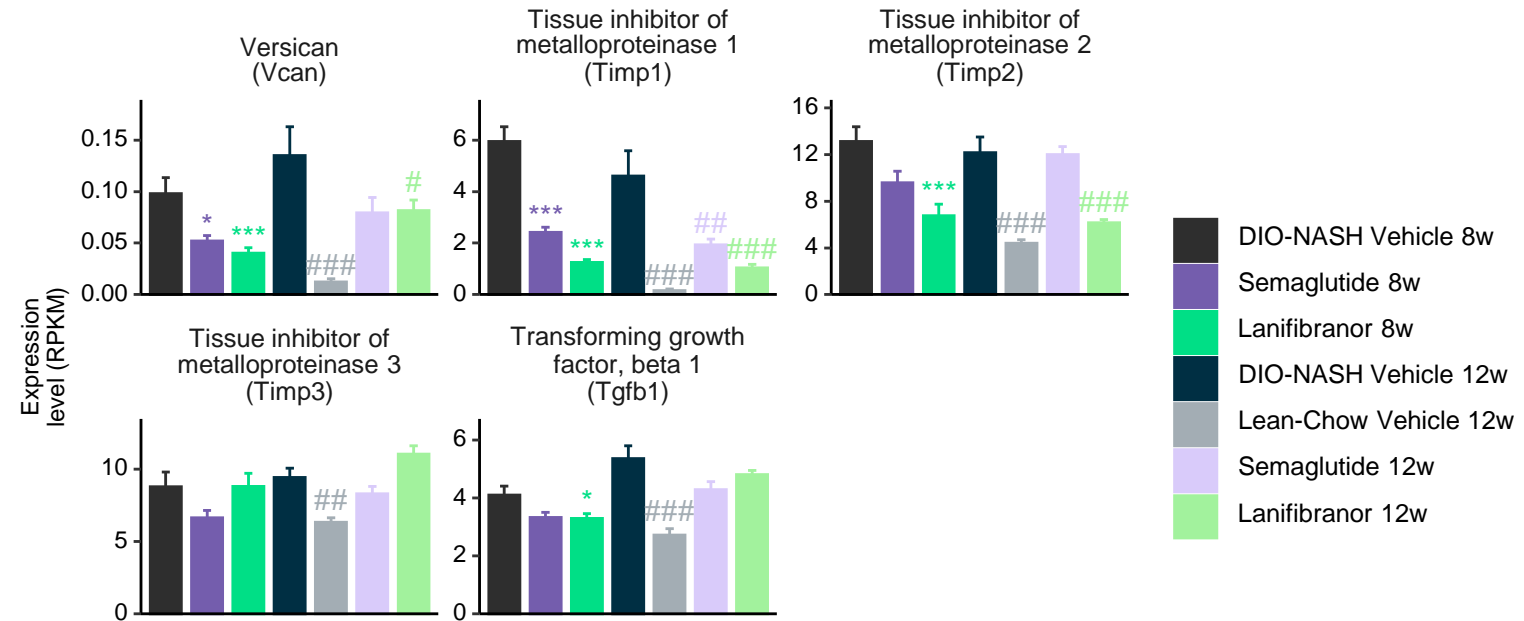
ECM organization - 3/4



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

ECM organization

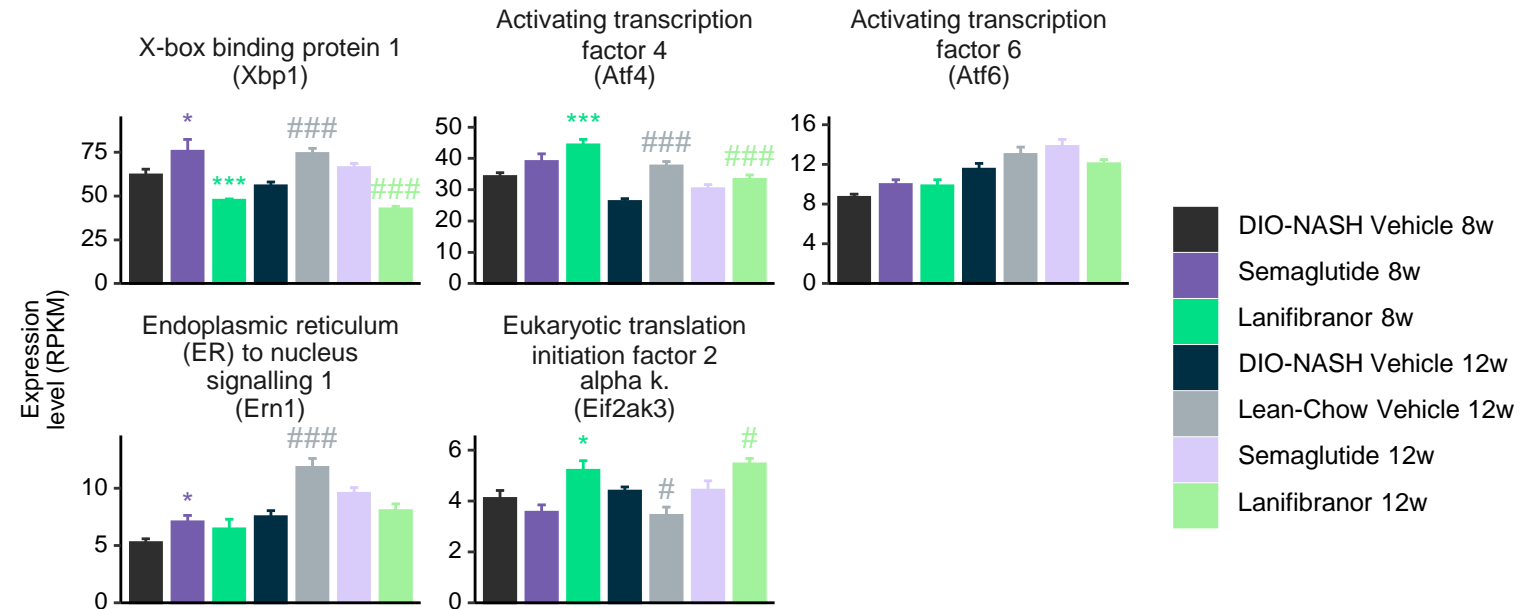
ECM organization - 4/4



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, ##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

ER stress

ER stress

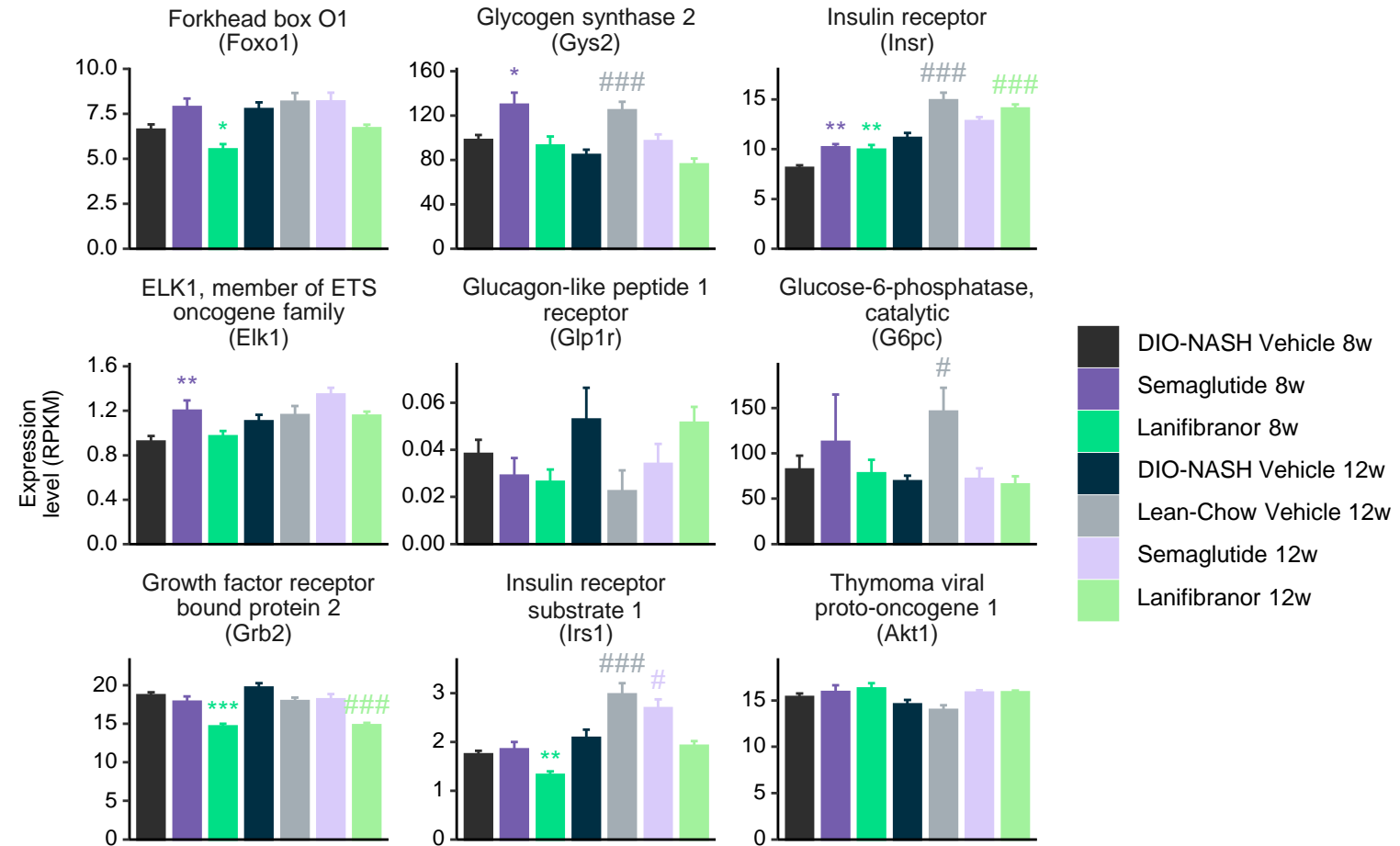


Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Glucose metabolism



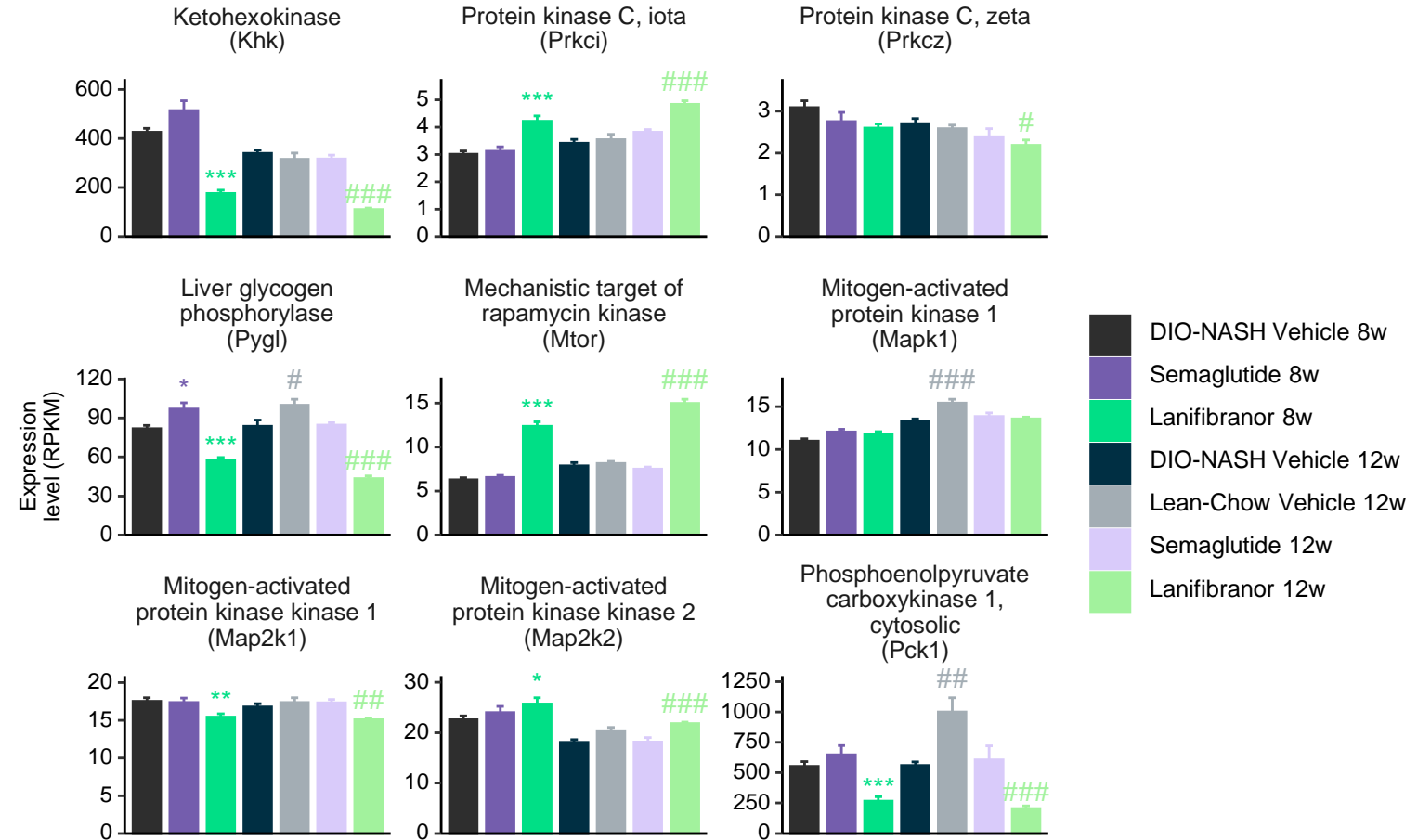
Glucose metabolism - 1/3



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Glucose metabolism

Glucose metabolism - 2/3



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Glucose metabolism

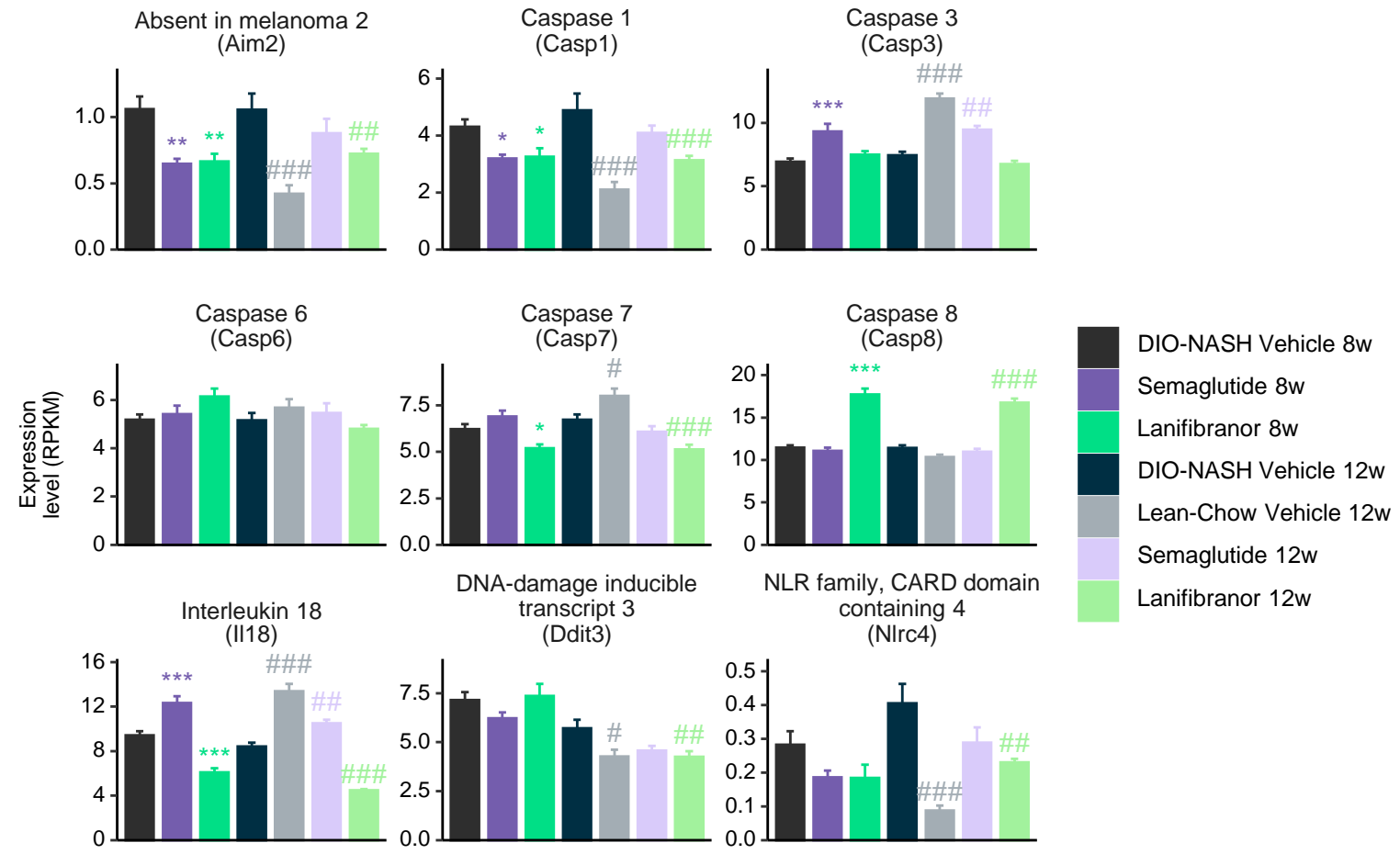
Glucose metabolism - 3/3



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. #:padj < 0.05, ***/####:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Hepatocellular cell death

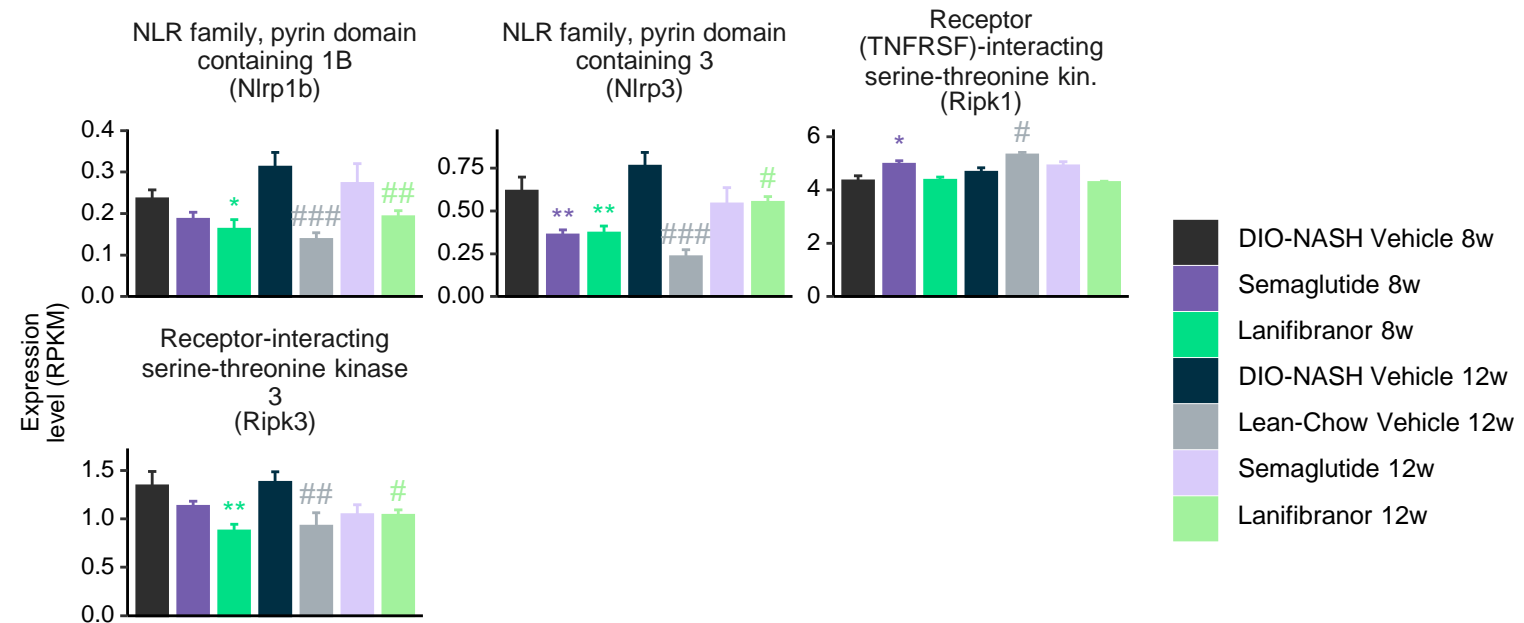
Hepatocellular cell death - 1/2



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Hepatocellular cell death

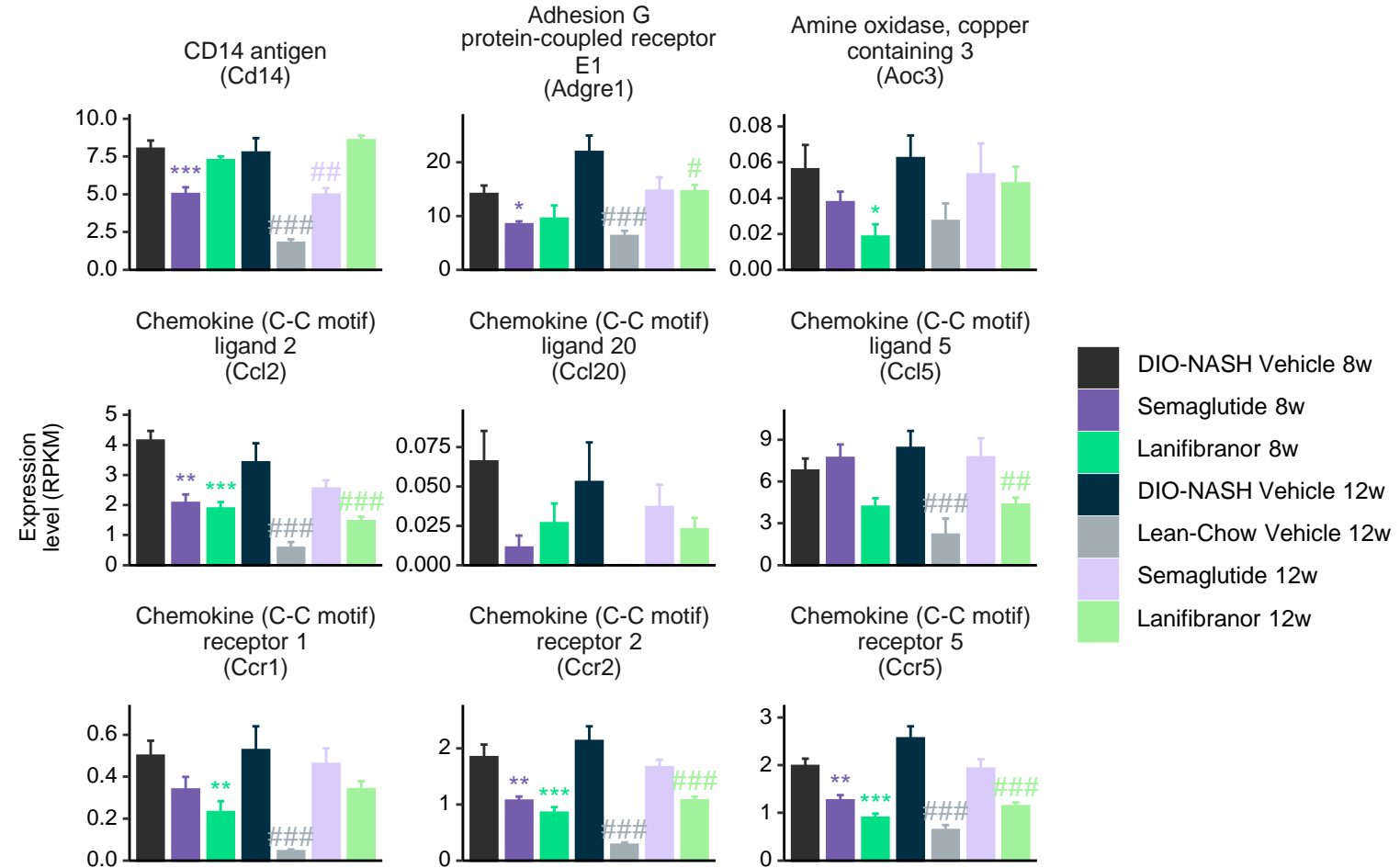
Hepatocellular cell death - 2/2



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Inflammation

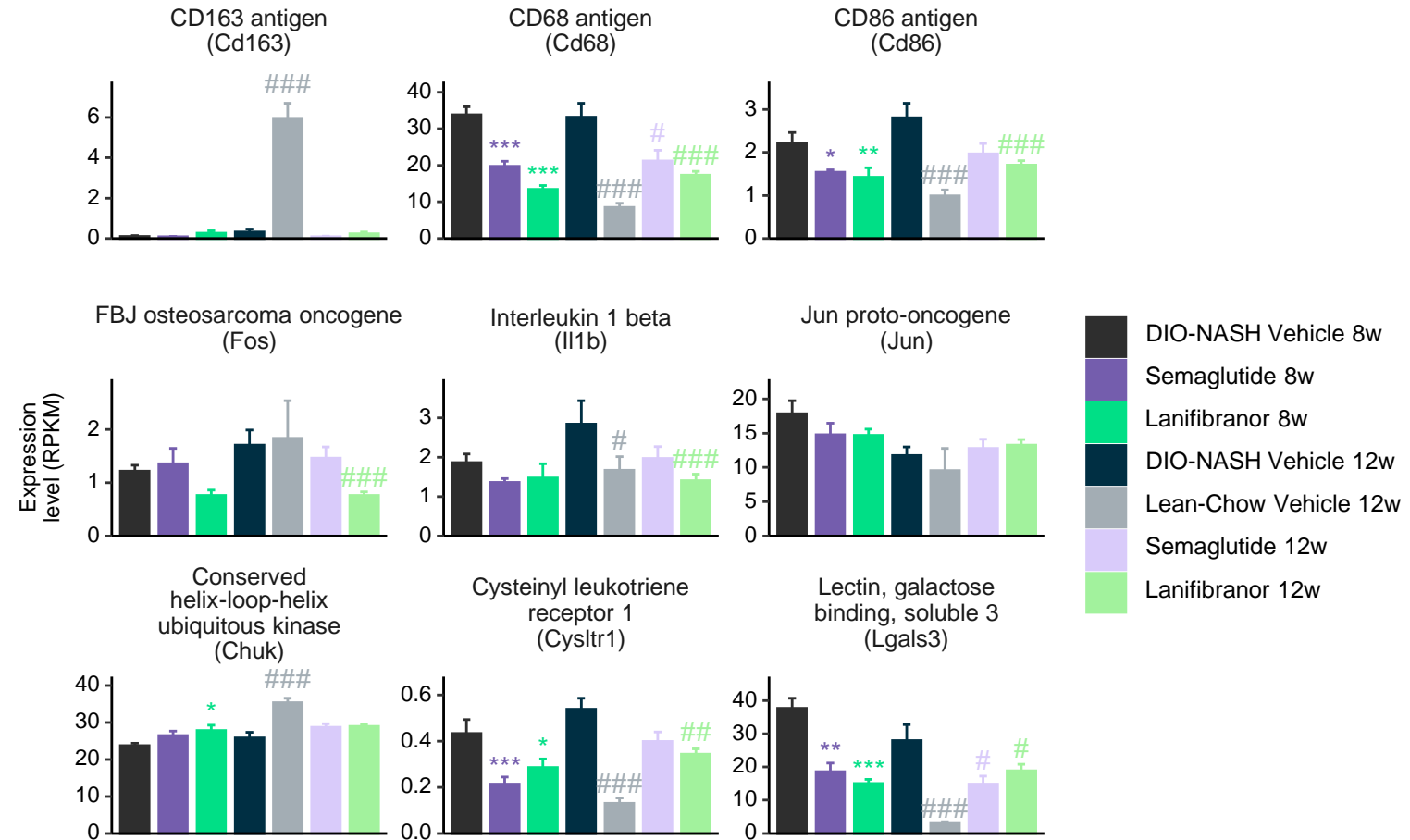
Inflammation - 1/4



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Inflammation

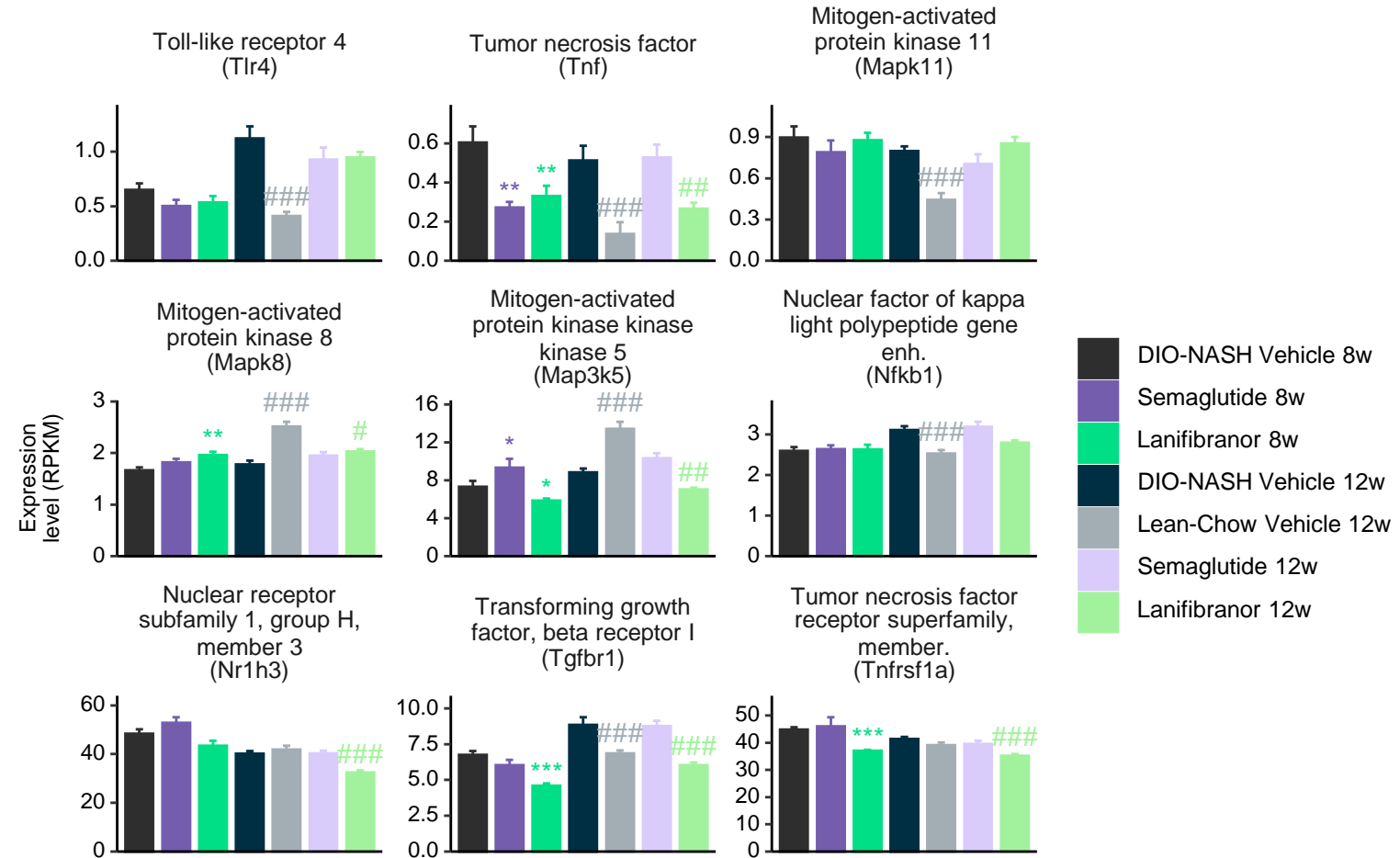
Inflammation - 2/4



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Inflammation

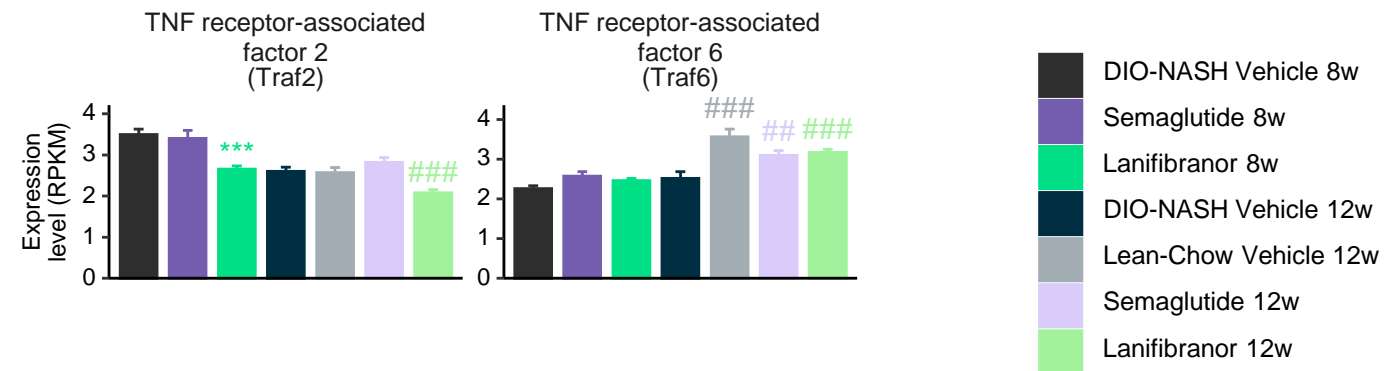
Inflammation - 3/4



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Inflammation

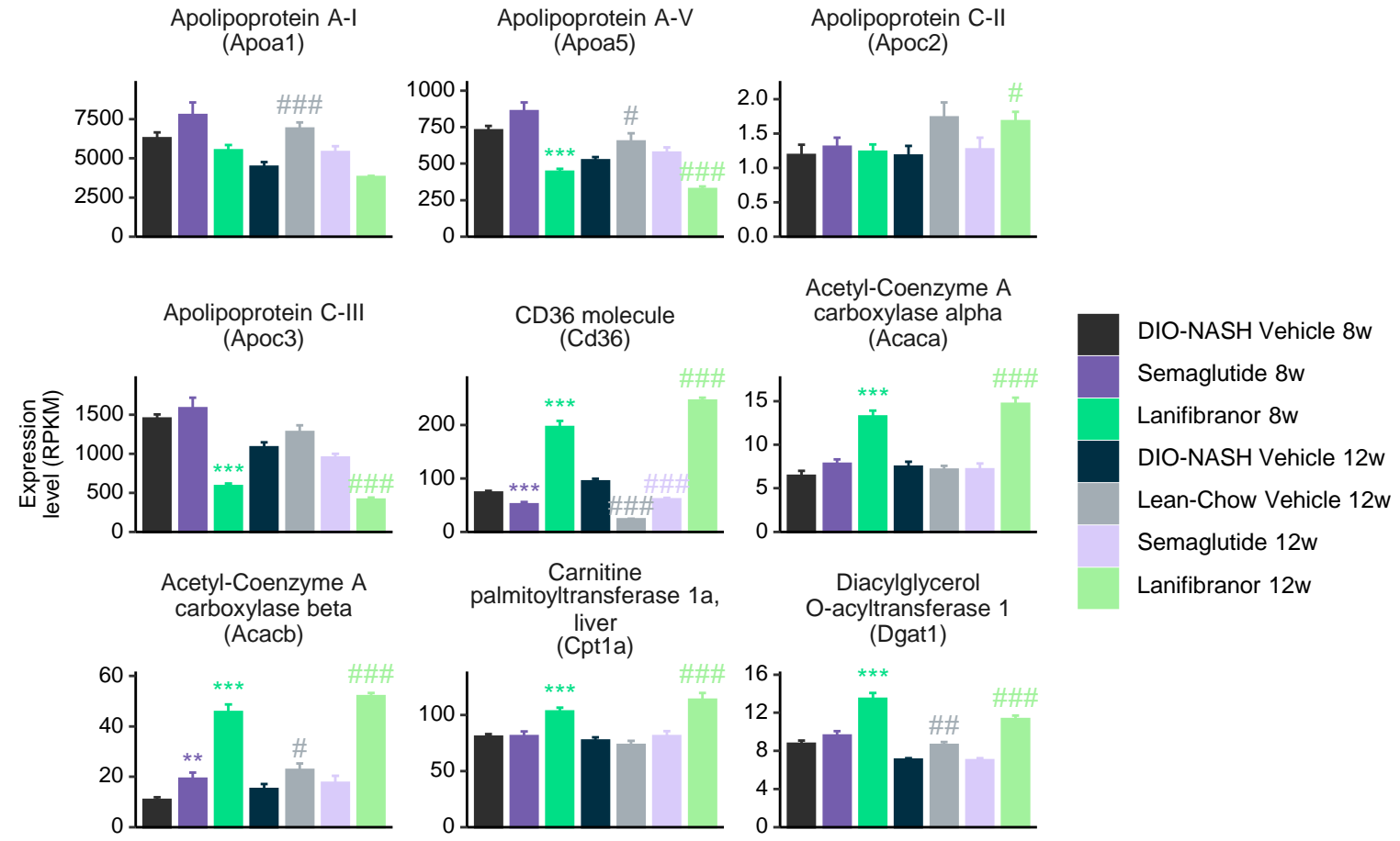
Inflammation - 4/4



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. #:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Lipid metabolism

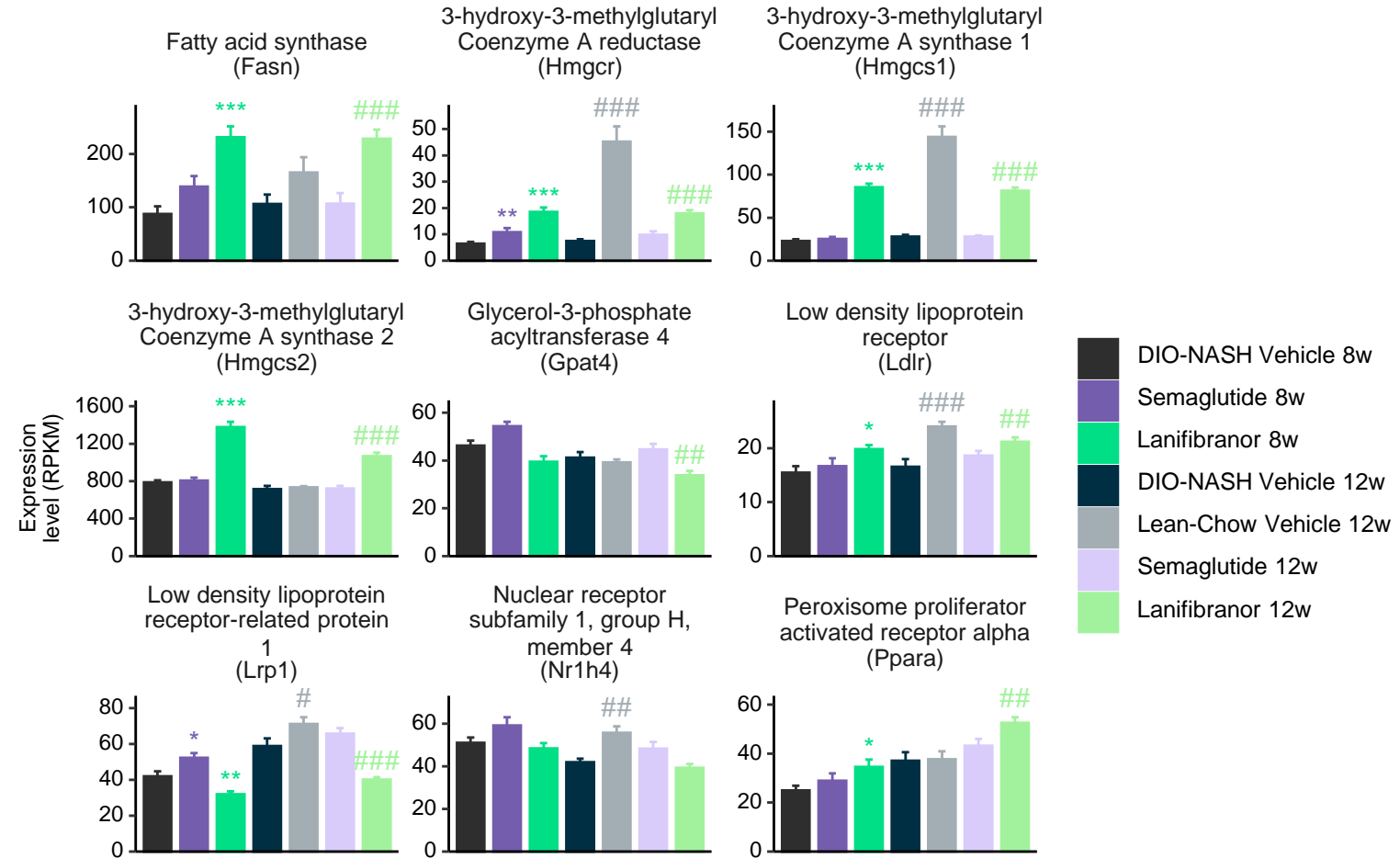
Lipid metabolism - 1/3



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. #:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Lipid metabolism

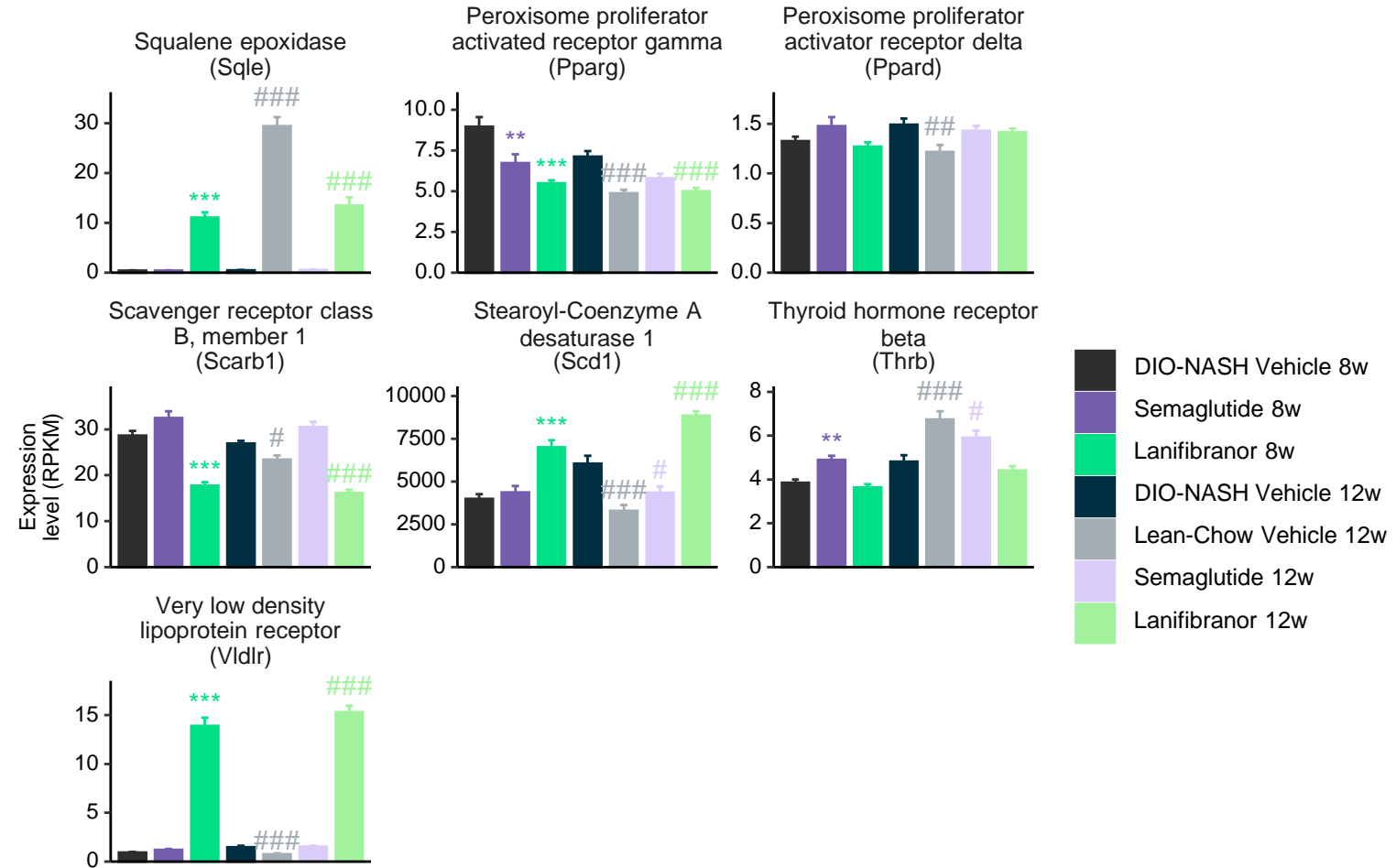
Lipid metabolism - 2/3



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. */#:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Lipid metabolism

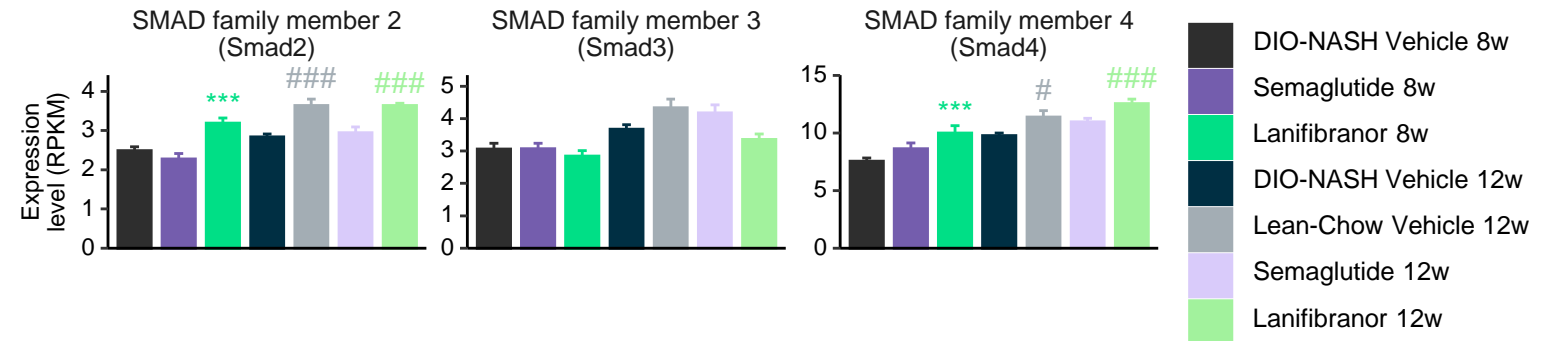
Lipid metabolism - 3/3



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. #:padj < 0.05, **/##:padj < 0.01, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.

Inflammation signaling

Inflammation signaling



Gene expression of genes found in Gubra curated gene panels. Values are expressed as group mean + SEM. #:padj < 0.05, ***/###:padj < 0.001 compared to the corresponding DIO-NASH Vehicle after correcting for multiple testing. RPKM: Reads Per Kilobase Million. n = 8 - 13.



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