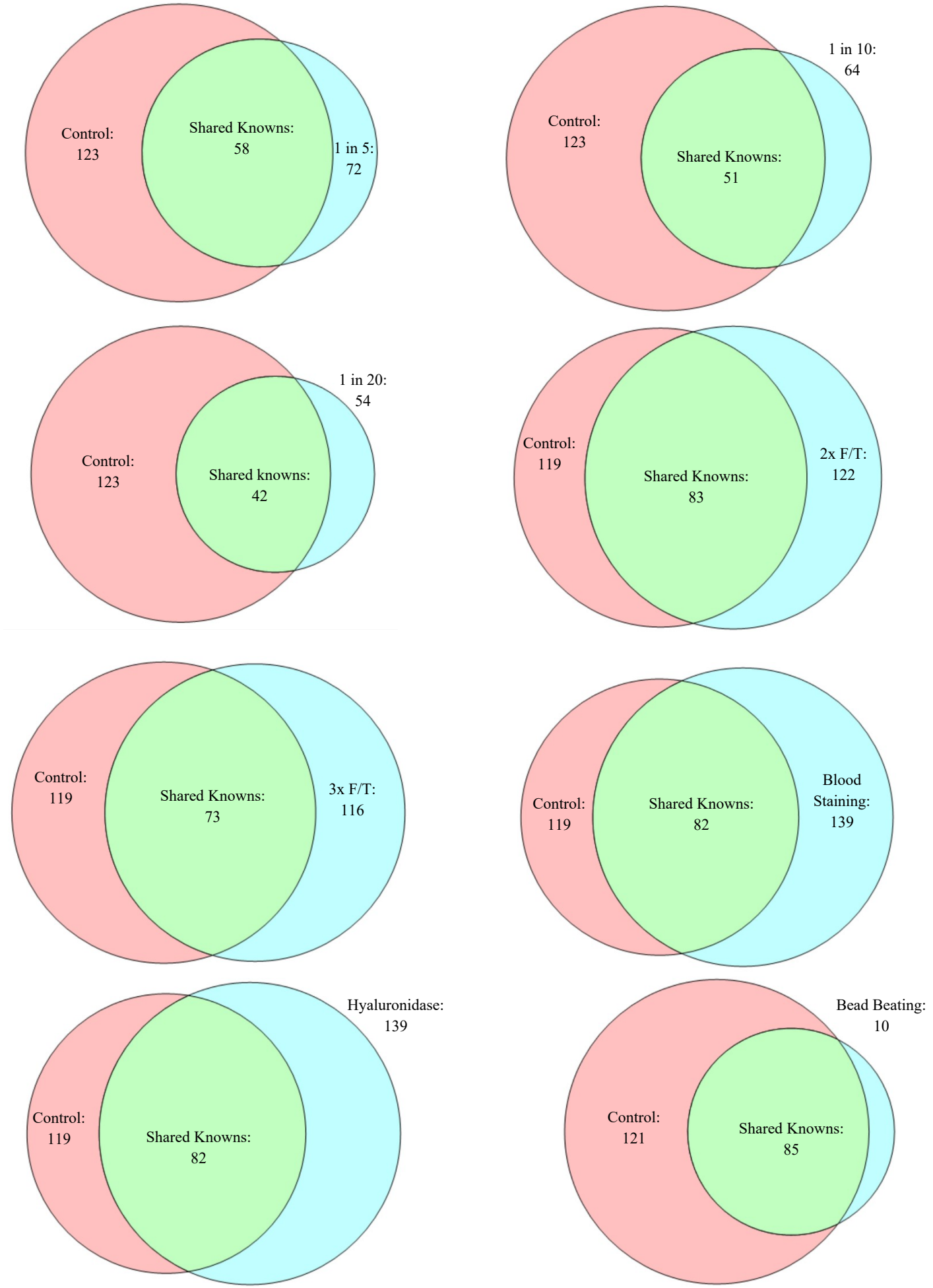

Figure S1. Venn diagrams illustrating overlapping and unique annotated metabolites identified across treatment groups compared to the control in the GC-ToF-MS analysis.

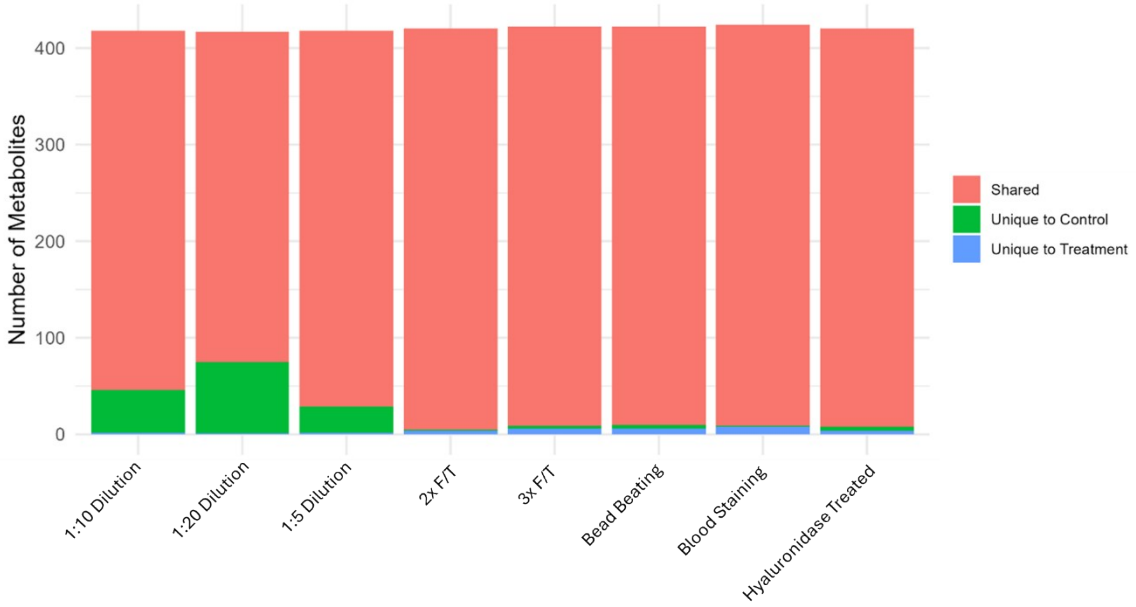

Figure S2. Bar plot illustrating overlapping and unique annotated metabolites identified across treatment groups compared to the control in the UHPLC-MS HILIC (ESI+) analysis.

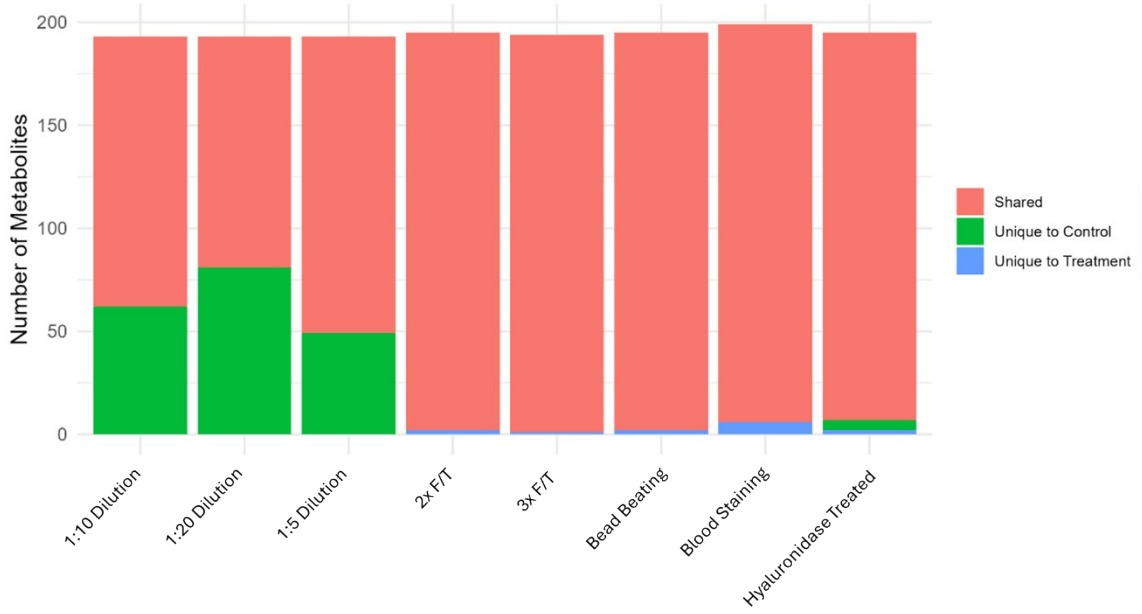

Figure S3. Bar plot illustrating overlapping and unique annotated metabolites identified across treatment groups compared to the control in the UHPLC-MS HILIC (ESI-) analysis.

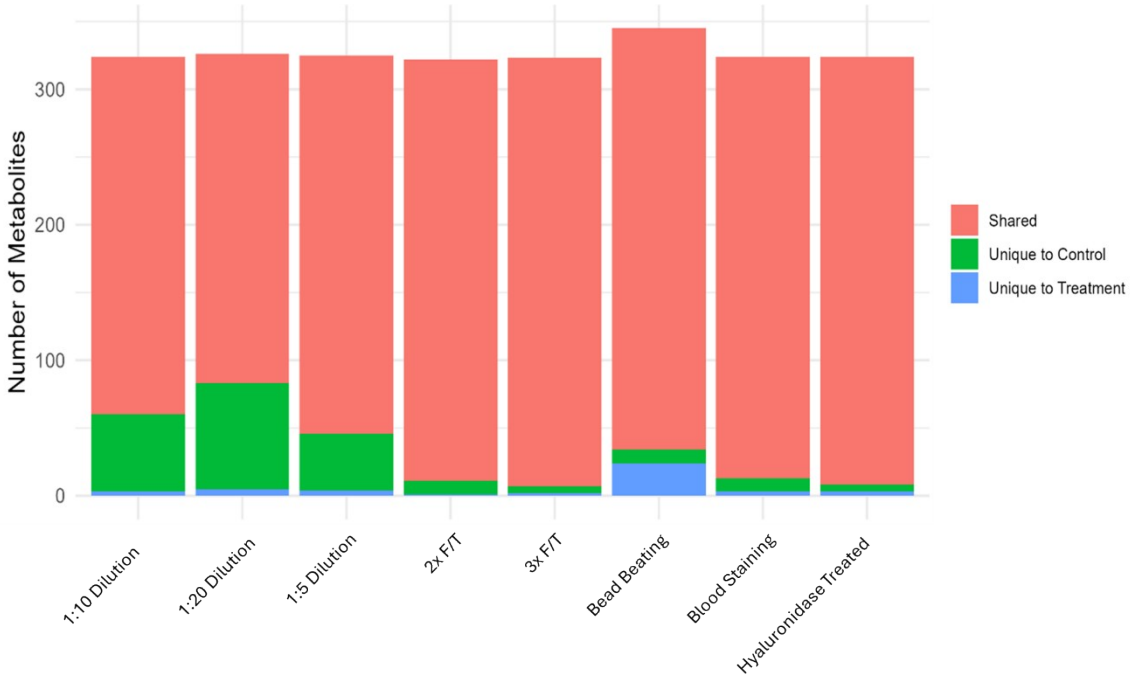

Figure S4. Bar plot illustrating overlapping and unique annotated metabolites identified across treatment groups compared to the control in the UHPLC-MS Lipid (ESI+) analysis.

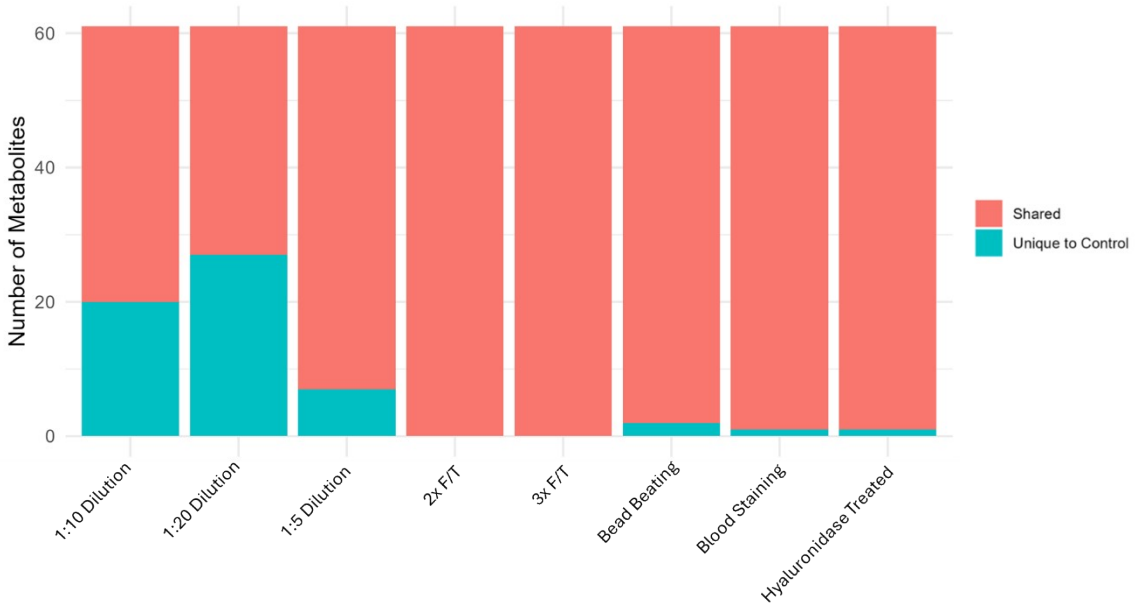

Figure S5. Bar plot illustrating overlapping and unique annotated metabolites identified across treatment groups compared to the control in the UHPLC-MS Lipid (ESI-) analysis.

Table S1. Number of metabolites per treatment for UHPLC-MS showing a > ± 30 % change in abundance relative to the control group. The ±30 % threshold represents a descriptive effect-size measure (fold-change > 1.3 or < 0.77) and is not FDR-corrected.

Treatment	Number of Metabolites changed ±30%
Lipid (ESI+)	
1in5 Dilution	1458
1in10 Dilution	1537
1in20 Dilution	1542
2x F/T	1272
3x F/T	1218
Blood Staining	1071
Hyaluronidase Treated	784
Bead Beating	1341
Lipid (ESI-)	
1in5 Dilution	785
1in10 Dilution	790
1in20 Dilution	794
2x F/T	561
3x F/T	514
Blood Staining	432
Hyaluronidase Treated	485
Bead Beating	525
HILIC (ESI+)	
1in5 Dilution	739
1in10 Dilution	737
1in20 Dilution	754
2x F/T	589
3x F/T	627
Blood Staining	593
Hyaluronidase Treated	180
Bead Beating	225
HILIC (ESI-)	
1in5 Dilution	471
1in10 Dilution	482
1in20 Dilution	473
2x F/T	461
3x F/T	386
Blood Staining	315
Hyaluronidase Treated	172
Bead Beating	78

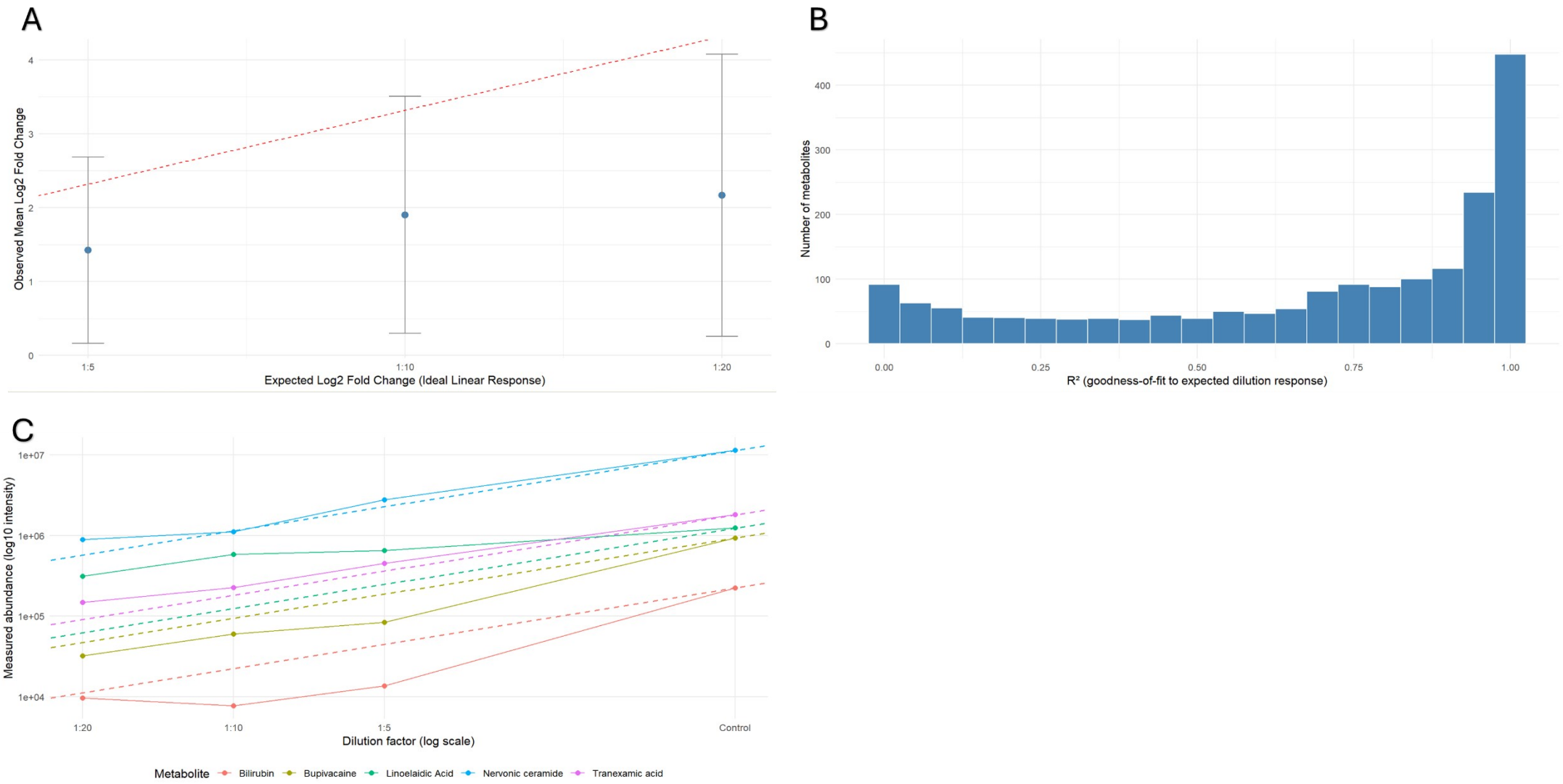

Figure S6. Evaluation of dilution linearity and response behaviour across metabolite features (UHPLC-MS Lipid (ESI+)). (A) Observed versus expected $\log_2$ fold change for serially diluted samples (1:5, 1:10, 1:20) relative to control. The dashed red line indicates the ideal linear response expected under perfect proportionality; points represent the mean observed response ± SD across metabolites. (B) Distribution of linearity (R^2) values calculated from individual metabolite regressions against the theoretical dilution series, illustrating that most metabolites exhibited strong linear responses ($R^2 > 0.8$). (C) Representative metabolite abundance responses across dilutions plotted on a $\log_{10}$ intensity scale. Solid lines denote measured values; dashed lines indicate the theoretical dilution response assuming linear behaviour.

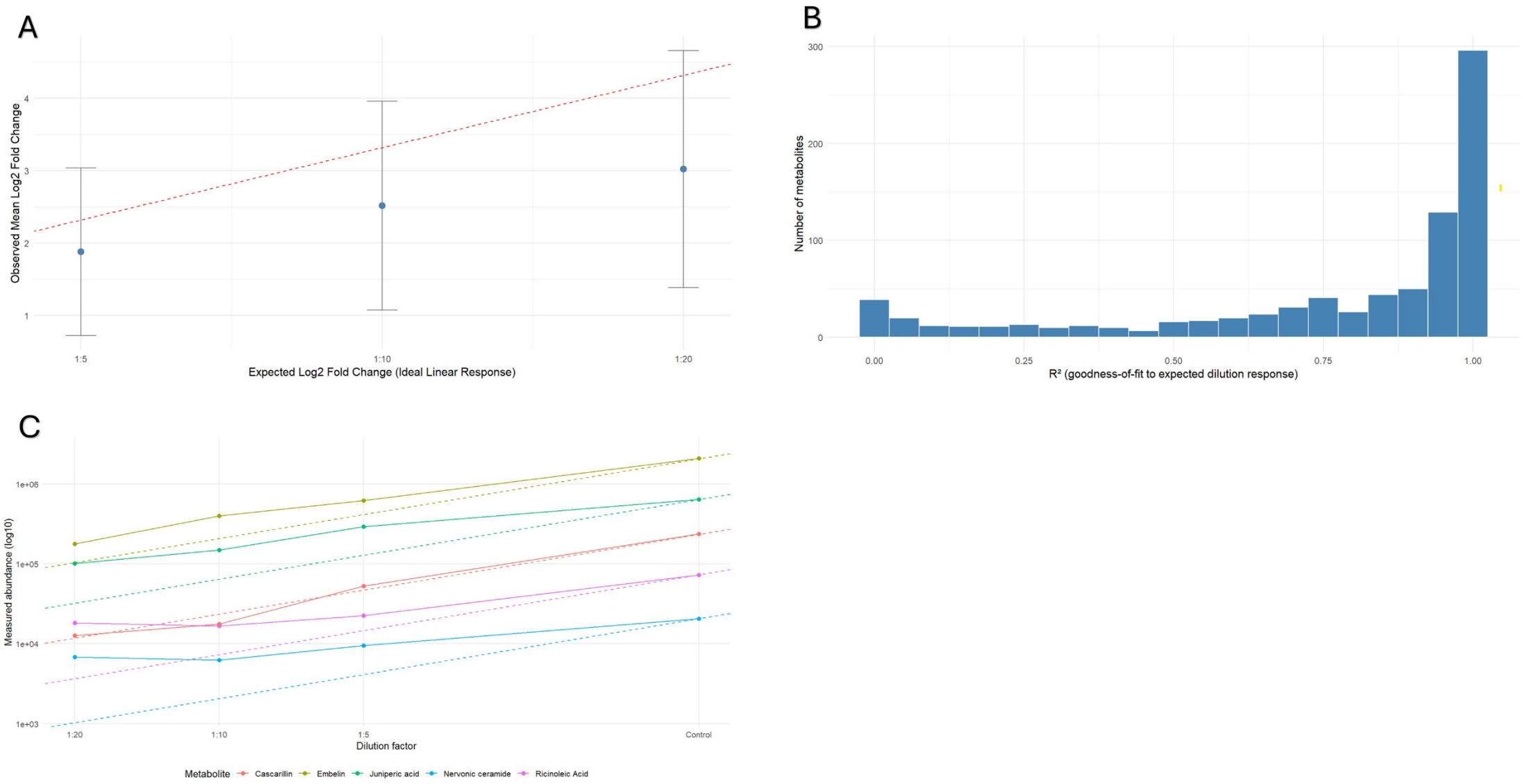

Figure S7. Evaluation of dilution linearity and response behaviour across metabolite features (UHPLC-MS Lipid (ESI-)). (A) Observed versus expected $\log_2$ fold change for serially diluted samples (1:5, 1:10, 1:20) relative to control. The dashed red line indicates the ideal linear response expected under perfect proportionality; points represent the mean observed response ± SD across metabolites. (B) Distribution of linearity (R^2) values calculated from individual metabolite regressions against the theoretical dilution series, illustrating that most metabolites exhibited strong linear responses ($R^2 > 0.8$). (C) Representative metabolite abundance responses across dilutions plotted on a $\log_{10}$ intensity scale. Solid lines denote measured values; dashed lines indicate the theoretical dilution response assuming linear behaviour.

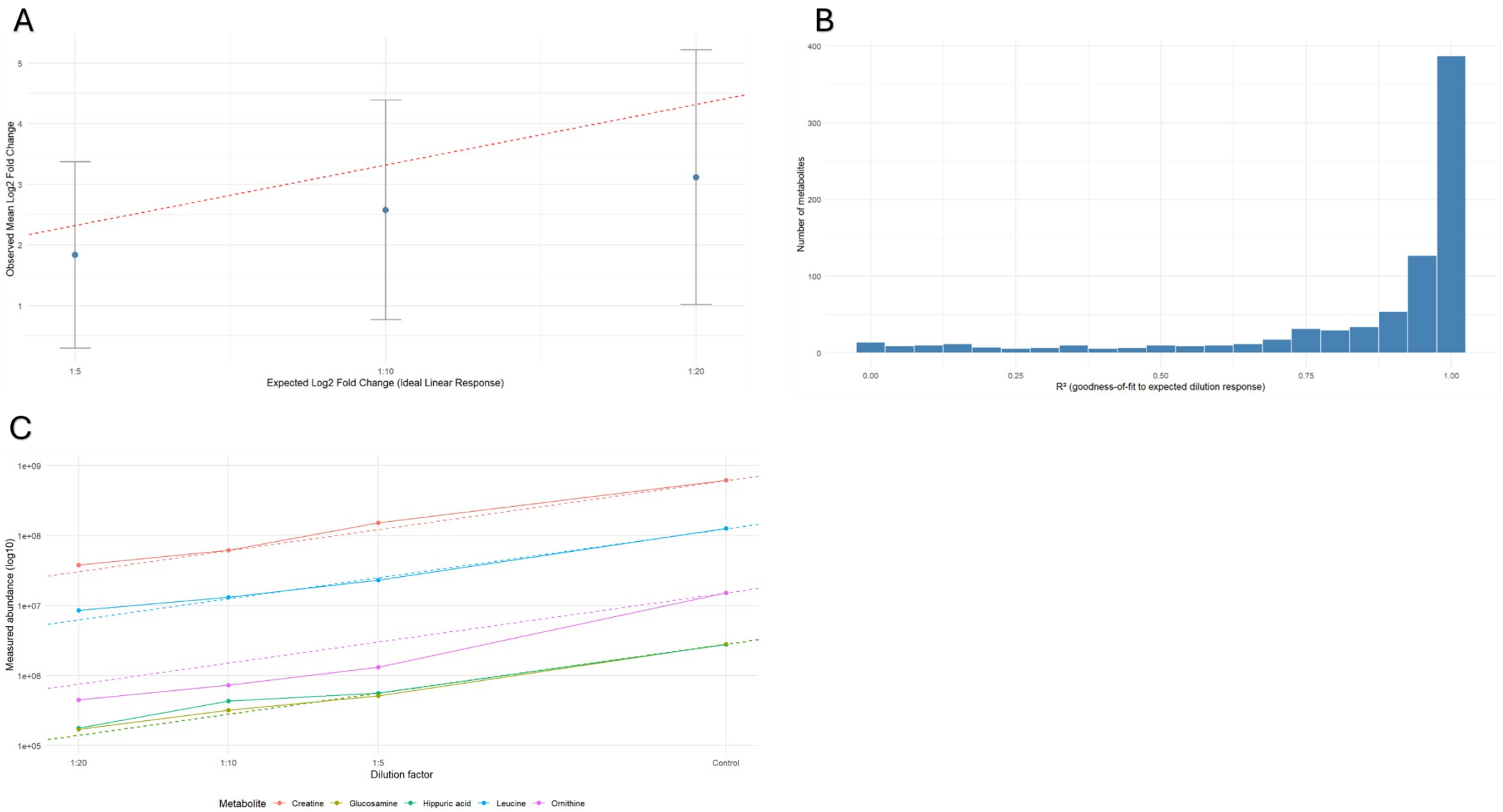

Figure S8. Evaluation of dilution linearity and response behaviour across metabolite features (UHPLC-MS HILIC (ESI+)). (A) Observed versus expected $\log_2$ fold change for serially diluted samples (1:5, 1:10, 1:20) relative to control. The dashed red line indicates the ideal linear response expected under perfect proportionality; points represent the mean observed response ± SD across metabolites. (B) Distribution of linearity (R^2) values calculated from individual metabolite regressions against the theoretical dilution series, illustrating that most metabolites exhibited strong linear responses ($R^2 > 0.8$). (C) Representative metabolite abundance responses across dilutions plotted on a $\log_{10}$ intensity scale. Solid lines denote measured values; dashed lines indicate the theoretical dilution response assuming linear behaviour.

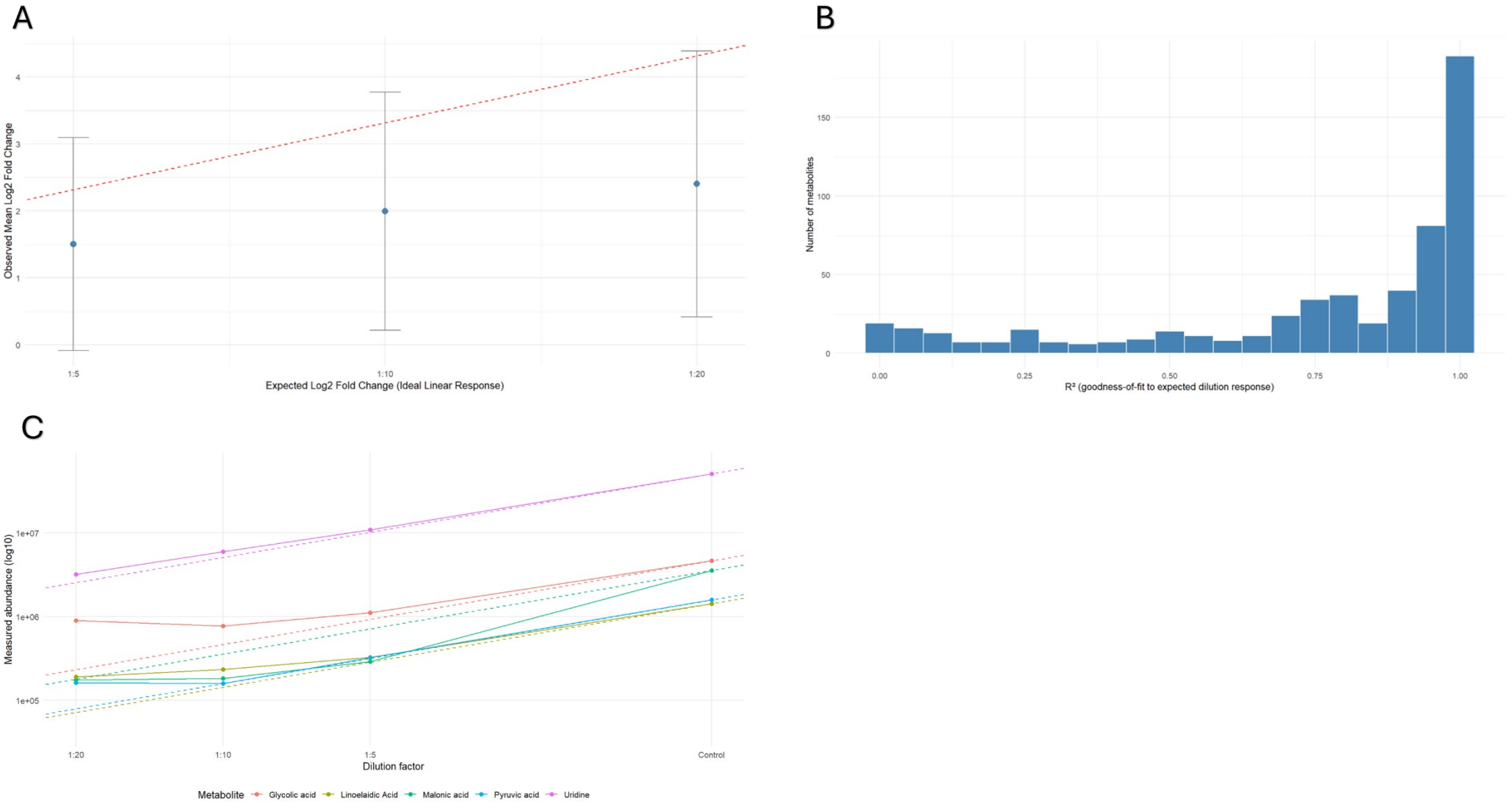

Figure S9. Evaluation of dilution linearity and response behaviour across metabolite features (UHPLC-MS HILIC (ESI-)). (A) Observed versus expected $\log_2$ fold change for serially diluted samples (1:5, 1:10, 1:20) relative to control. The dashed red line indicates the ideal linear response expected under perfect proportionality; points represent the mean observed response ± SD across metabolites. (B) Distribution of linearity (R^2) values calculated from individual metabolite regressions against the theoretical dilution series, illustrating that most metabolites exhibited strong linear responses ($R^2 > 0.8$). (C) Representative metabolite abundance responses across dilutions plotted on a $\log_{10}$ intensity scale. Solid lines denote measured values; dashed lines indicate the theoretical dilution response assuming linear behaviour.