# Supplementary materials

Metabolic Pathways Underlying GATA6 Regulating Trastuzumab Resistance in Gastric Cancer Cells Based on Untargeted Metabolomics

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Fig. S1 PCA-X one-dimensional line plots from QC samples were calculated to assess the experiment reproducibility of trastuzumab resistant groups and GATA6 knock out groups in ESI+ and ESI− respectively.

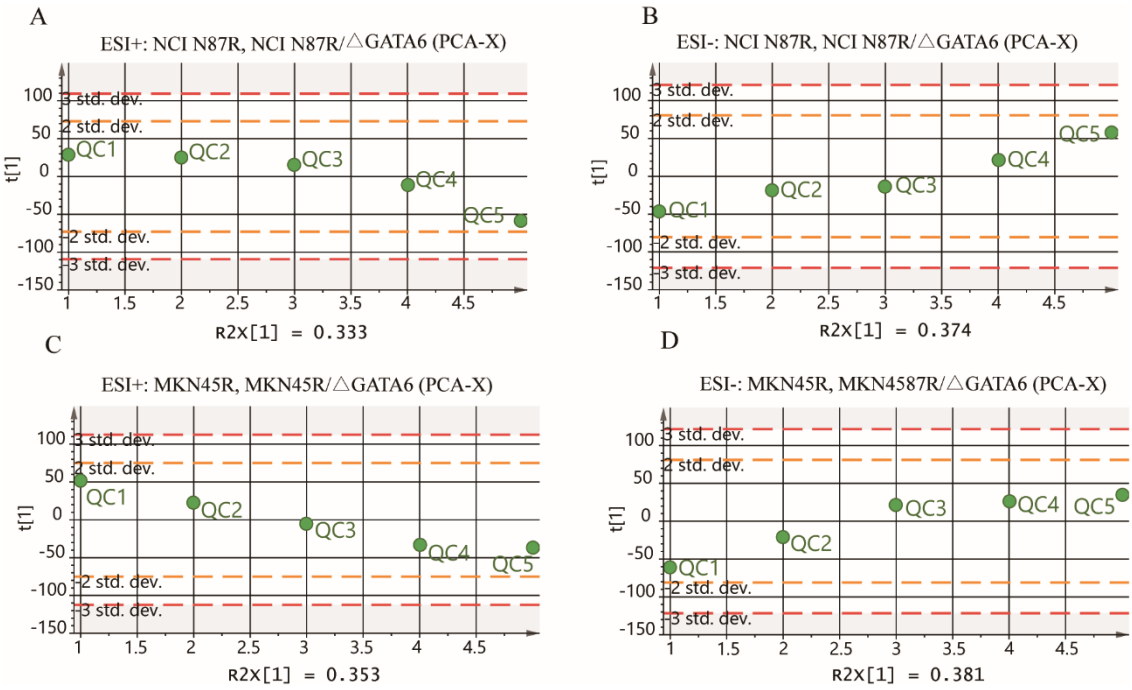

Fig. S2 Spearman's correlation coefficients from QCs samples were calculated to assess the experiment reproducibility of NCI N87R and NCI N87R/ΔGATA6 cells in ESI+ (A) and ESI– (B), and MKN45R and MKN45R/ΔGATA6 cells in ESI+ (C) and ESI– (D) respectively. The lower-left half shows pairwise scatter plots of QCs samples. The upper-right half shows pairwise Spearman's correlation coefficients from the same comparison.

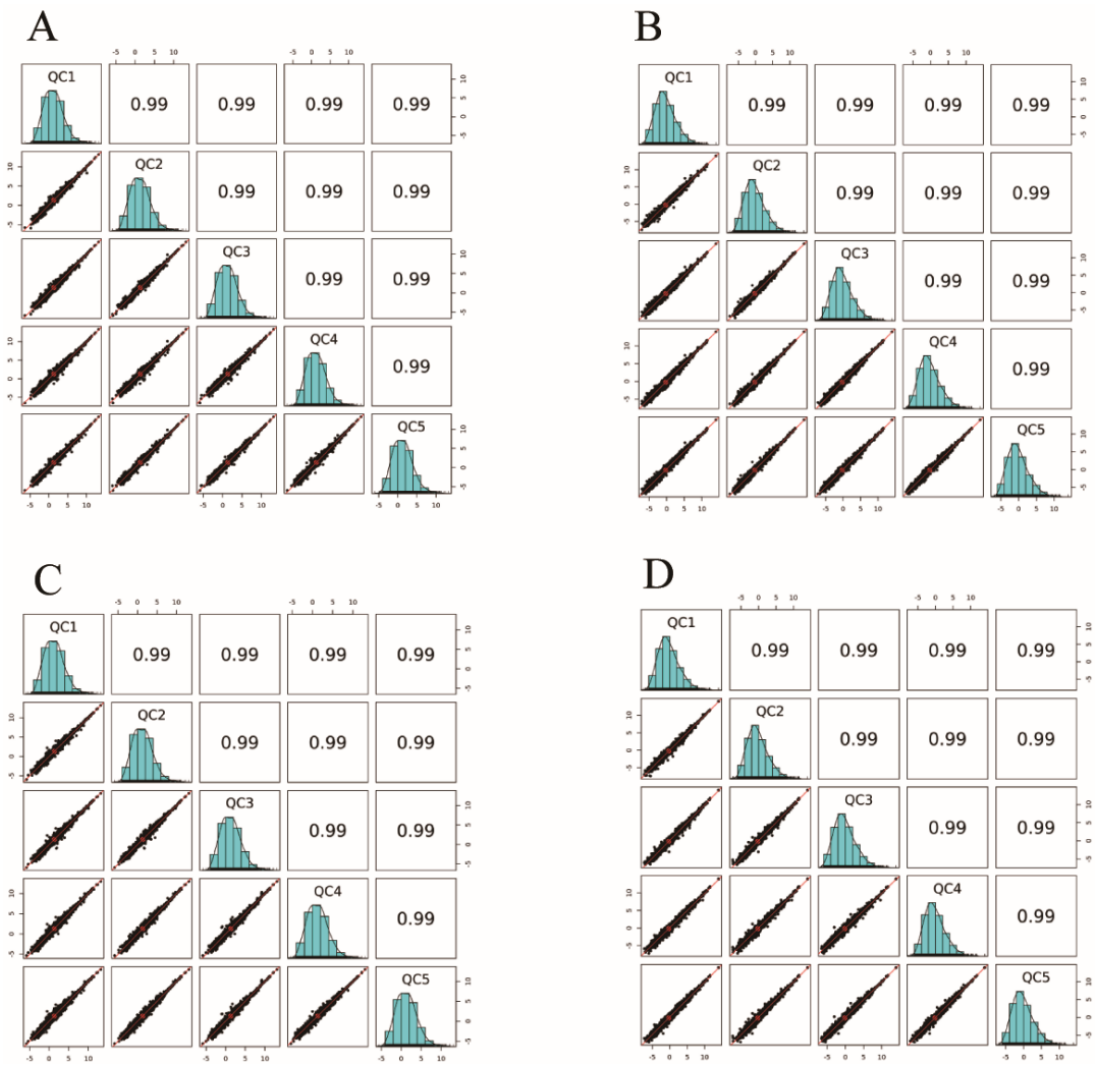

Fig. S3 Hierarchical clustering analysis of NCI N87R and NCI N87R/ΔGATA6 cells (A, B), MKN45R and MKN45R/ΔGATA6 cells (C, D) in ESI+ and ESI– respectively. The increased and decreased features are represented by range of red and blue intensities, respectively.

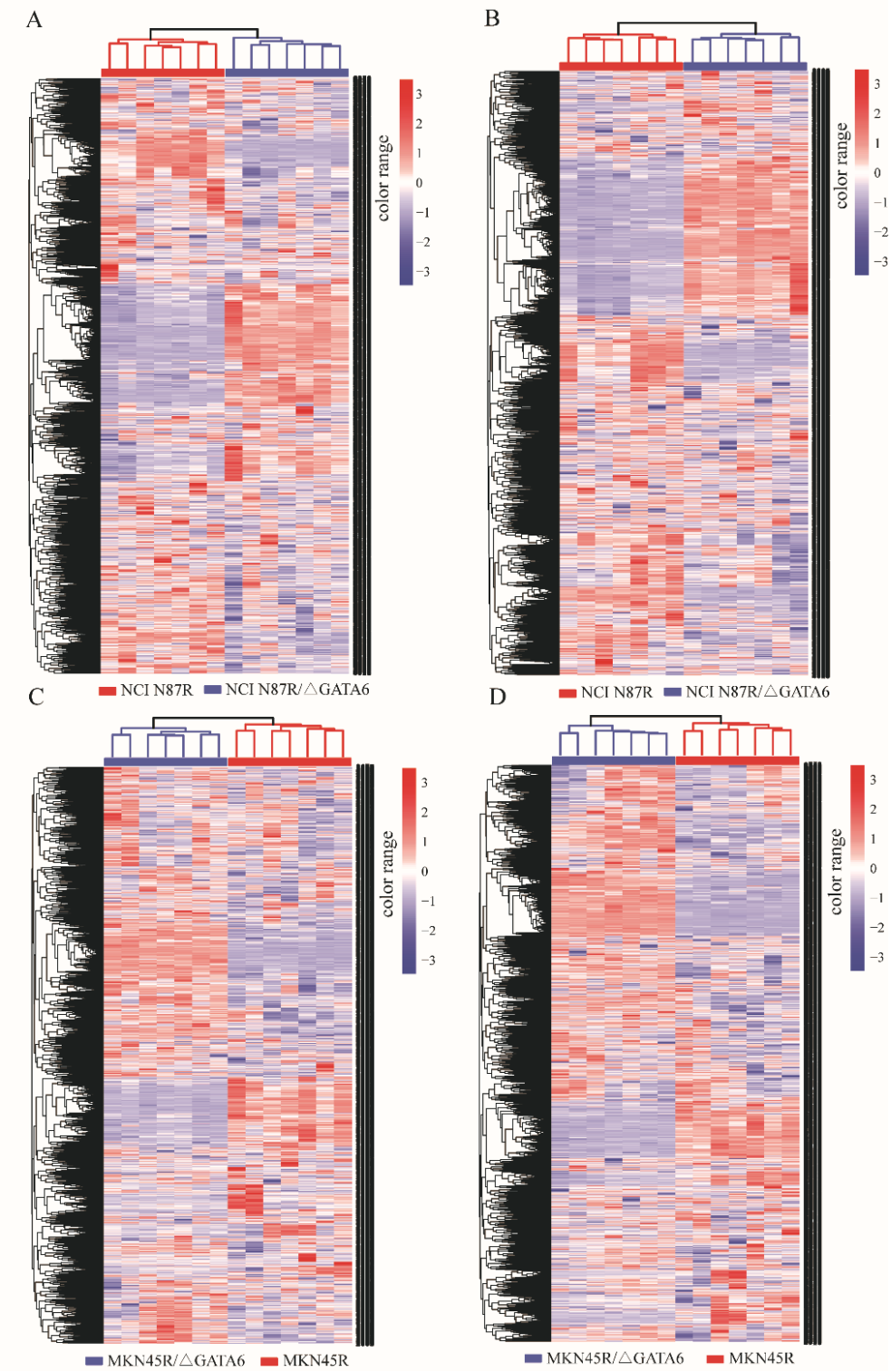

Fig. S4 (A-D) Differential metabolites among NCI N87R, NCI N87R/ΔGATA6, MKN45R and MKN45R/ΔGATA6 cells are illustrated by volcano plots in ESI+ and ESI-, respectively. The mean ratios of seven biological repeats were plotted in log2 scale (x-axis) against the corresponding-log10 *p* value (y-axis). The vertical dotted lines mark 1.2 and 0.83 fold change while horizontal dotted lines represent cutoff p=0.05. Metabolites with fold changes greater than 1.2 or less than 0.83 and $p<0.05$ are regarded as increased or decreased and marked in red and blue, respectively. The gray dots are considered as having no significant change.

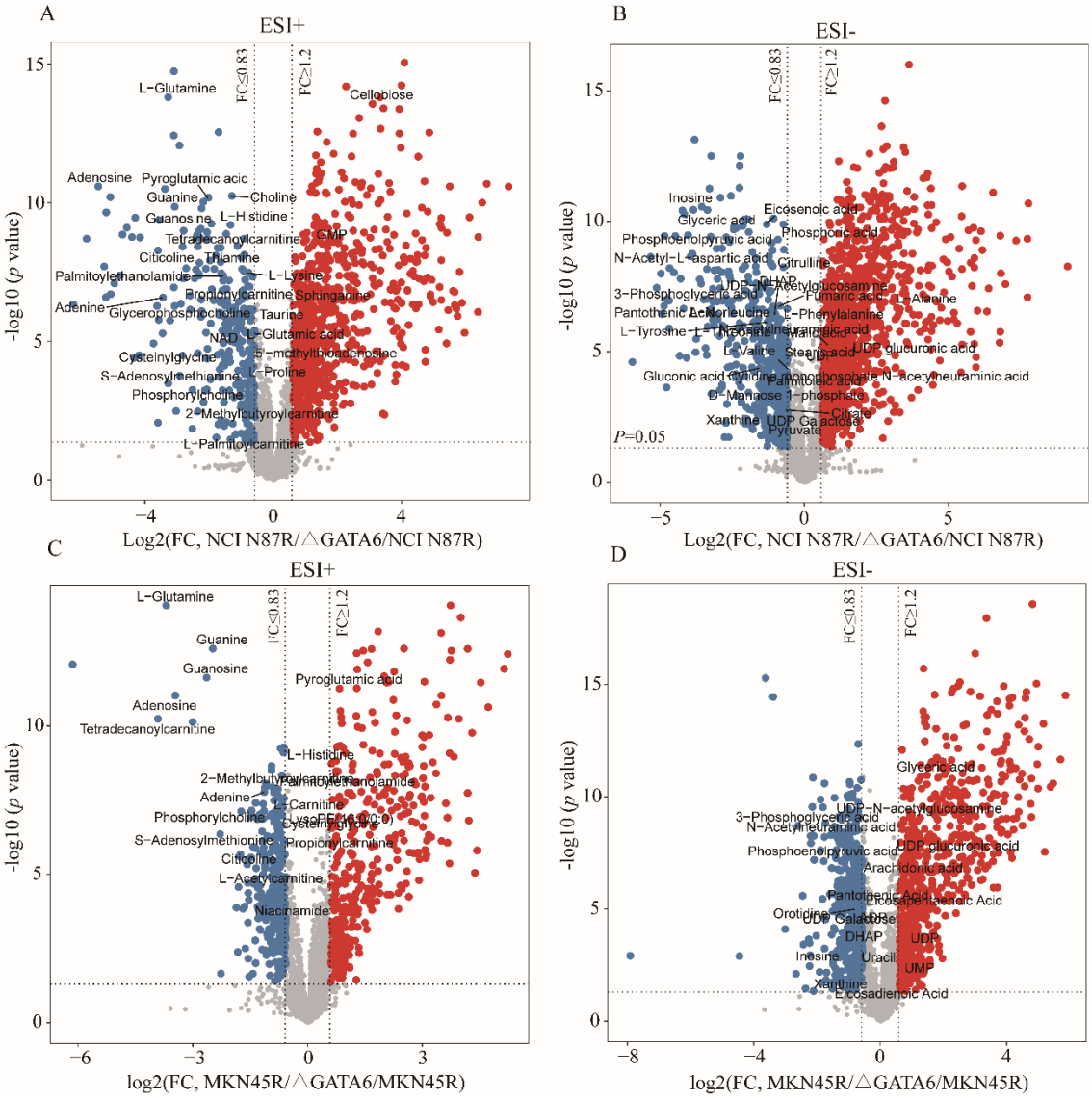

Fig. S5 Violin plots of the differential metabolites were showed in NCI N87R/ΔGATA6 *vs*. NCI N87R cells.

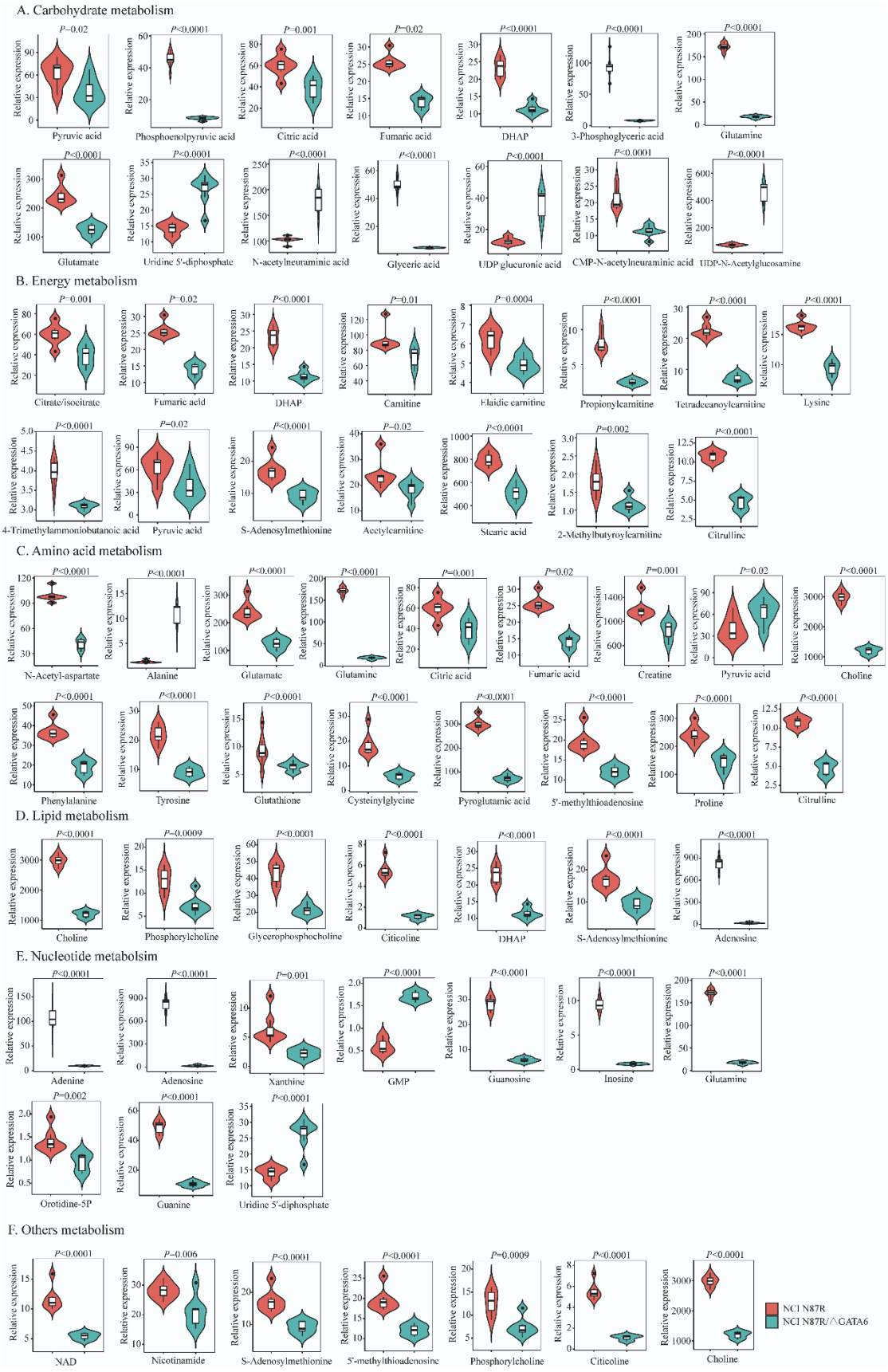

Fig. S6 Violin plots of the differential metabolites were showed in MKN45R/ΔGATA6 *vs.* MKN45R cells.

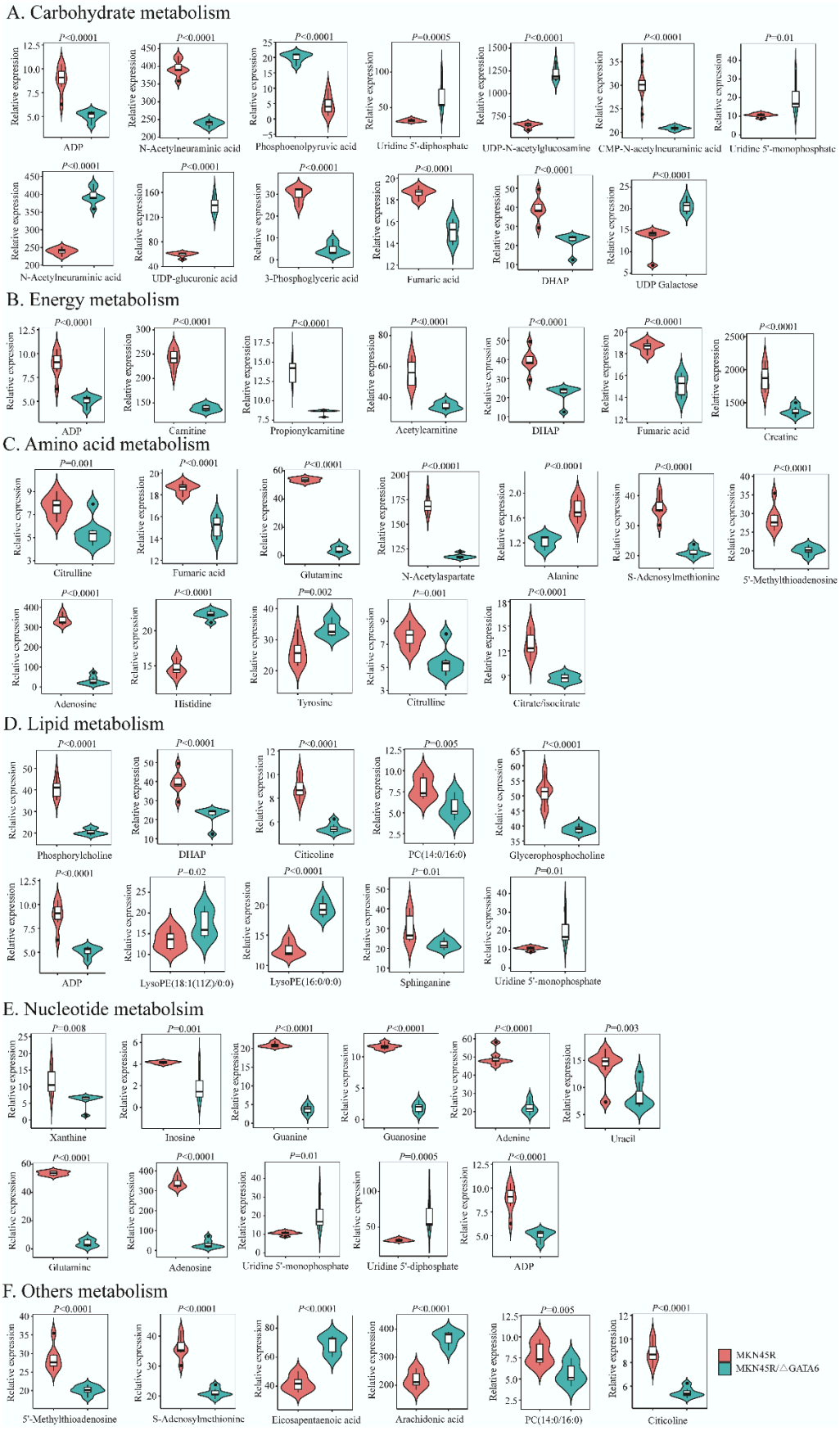

Table. S1 Response stability of internal standard from QC samples

| Internal standard | ESI+ | | | ESI− | | |
|---|---|---|---|---|---|---|
| 2-chloro-L-phenylalanine | m/z | RT(s) | RSD | m/z | RT(s) | RSD |
| | 200.0663 | 175.5319 | 3.24% | 198.142 | 165.825 | 4.79% |

Table S2. Differential metabolites overlapped in NCI N87R, NCI N87R/ΔGATA6 and MKN45R, MKN45R/ΔGATA6 cells

| No | Metabolites | HMDB | KEGG | Tread (NCI N87R/ΔGATA6/NCI N87R) | Tread (MKN45R/ΔGATA6/MKN45R) |
|---|---|---|---|---|---|
| 1 | 5'-Methylthioadenosine | HMDB0001173 | C00170 | Down | Down |
| 2 | Gluconic acid | HMDB0000625 | C00257 | Down | Up |
| 3 | Xanthine | HMDB0000292 | C00385 | Down | Down |
| 4 | L-Tyrosine | HMDB0000158 | C00082 | Down | Up |
| 5 | Sphinganine | HMDB0000269 | C00836 | Up | Down |
| 6 | Creatine | HMDB0000064 | C00300 | Down | Down |
| 7 | L-Acetylcarnitine | HMDB0000201 | C02571 | Down | Down |
| 8 | Glycerophosphocholine | HMDB0000086 | C00670 | Down | Down |
| 9 | Fumaric acid | HMDB0000134 | C00122 | Down | Down |
| 10 | Guanosine | HMDB0000133 | C00387 | Down | Down |
| 11 | Pyroglutamic acid | HMDB0000267 | C01879 | Down | Up |
| 12 | Niacinamide | HMDB0001406 | C00153 | Down | Down |
| 13 | Cysteinylglycine | HMDB0000078 | C01419 | Down | Down |
| 14 | S-Adenosylmethionine | HMDB0001185 | C00019 | Down | Down |
| 15 | Glyceric acid | HMDB0000139 | C00258 | Down | Up |
| 16 | Palmitoylethanolamide | HMDB0002100 | C16512 | Down | Up |
| 17 | Tetradecanoylcarnitine | HMDB0005066 | — | Down | Down |
| 18 | Phosphorylcholine | HMDB0001565 | C00588 | Down | Down |
| 19 | Citrulline | HMDB0000904 | C00327 | Down | Down |
| 20 | L-Histidine | HMDB0000177 | C00135 | Down | Up |
| 21 | N-Acetyl-L-aspartic acid | HMDB0000812 | C01042 | Down | Down |
| 22 | Adenosine | HMDB0000050 | C00212 | Down | Down |
| 23 | Adenine | HMDB0000034 | C00147 | Down | Down |
| 24 | UDP Galactose | HMDB0000302 | C00052 | Up | Up |
| 25 | Inosine | HMDB0000195 | C00294 | Down | Down |
| 26 | Phosphoenolpyruvic acid | HMDB0000263 | C00074 | Down | Down |
| 27 | Pantothenic acid | HMDB0000210 | C00864 | Down | Down |
| 28 | 2-Methylbutyroylcarnitine | HMDB0000378 | — | Down | Down |
| 29 | L-Alanine | HMDB0000161 | C00041 | Up | Up |
| 30 | L-Glutamine | HMDB0000641 | C00064 | Down | Down |
| 31 | Malic acid | HMDB0000744 | C03668 | Down | Down |
| 32 | L-Threonine | HMDB0000167 | C00188 | Down | Up |
| 33 | Orotidine | HMDB0000788 | C01103 | Down | Down |
| 34 | 3-Phosphoglyceric acid | HMDB0000807 | C00597 | Down | Down |
| 35 | CMP-Neu5Ac | HMDB0001176 | C00128 | Down | Down |
| 36 | L-Carnitine | HMDB0000062 | C00318 | Down | Down |
| 37 | Guanine | HMDB0000132 | C00242 | Down | Down |
| 38 | UDP glucuronic acid | HMDB0000935 | C00167 | Up | Up |
| 39 | N-Acetylneuraminic acid | HMDB0000230 | C19910 | Up | Down |

| | | | | | |
|---|---|---|---|---|---|
| 40 | Propionylcarnitine | HMDB0000824 | C03017 | Down | Down |
| 41 | D-Mannose 1-phosphate | HMDB0006330 | C00636 | Down | Up |
| 42 | Uridine 5'-diphosphate | HMDB0000295 | C00015 | Up | Up |
| 43 | Dihydroxyacetone phosphate | HMDB0001473 | C00111 | Down | Down |
| 44 | Eicosenoic acid | HMDB0002231 | C16526 | Down | Up |
| 45 | Citicoline | HMDB0001413 | C00307 | Down | Down |
| 46 | UDP-N-Acetylglucosamine | HMDB0000290 | C00043 | Up | Down |
| 47 | Citrate/isocitrate | HMDB0000094 | C00158 | Down | Down |

Table S3. Differential metabolites in NCI N87R/ΔGATA6 *vs*. NCI N87R cells

| No | Metabolites | HMDB | KEGG | Tread (NCI N87R/*Δ*GATA6/NCI N87R) |
|---|---|---|---|---|
| 1 | Citrate/isocitrate | HMDB0000094 | C00158 | Down |
| 2 | L-Glutamic acid | HMDB0000148 | C00025 | Down |
| 3 | Cholesterol sulfate | HMDB0000653 | C18043 | Up |
| 4 | NAD | HMDB0000902 | C00003 | Down |
| 5 | 4-Trimethylammoniobutanoic acid | HMDB0001161 | C01181 | Down |
| 6 | Palmitoleic acid | HMDB0003229 | C08362 | Up |
| 7 | L-Valine | HMDB0000883 | C00183 | Down |
| 8 | Thiamine | HMDB0000235 | C00378 | Down |
| 9 | L-Proline | HMDB0000162 | C00148 | Down |
| 10 | Phosphoric acid | HMDB0002142 | C00009 | Down |
| 11 | L-Norleucine | HMDB0001645 | C01933 | Down |
| 12 | Taurine | HMDB0000251 | C00245 | Down |
| 13 | GMP | HMDB0001397 | C00144 | Up |
| 14 | Pyruvic acid | HMDB0000243 | C00022 | Down |
| 15 | Glutathione | HMDB0000125 | C02471 | Down |
| 16 | L-Palmitoylcarnitine | HMDB0000222 | C02990 | Down |
| 17 | Cellobiose | HMDB0000055 | C06422 | Up |
| 18 | Stearic acid | HMDB0000827 | C01530 | Down |
| 19 | Choline | HMDB0000097 | C00114 | Down |
| 20 | L-Phenylalanine | HMDB0000159 | C00079 | Down |
| 21 | Elaidic carnitine | HMDB0006464 | — | Down |
| 22 | L-Lysine | HMDB0000182 | C00047 | Down |

Table S4. Differential metabolites in MKN45R/ΔGATA6 *vs*. MKN45R cells

| No | Metabolites | HMDB | KEGG | Tread (MKN45R/*Δ*GATA6/MKN45R) |
|---|---|---|---|---|
| 1 | Eicosapentaenoic acid | HMDB0001999 | C06428 | Up |
| 2 | Arachidonic acid | HMDB0001043 | C00219 | Up |
| 3 | LysoPE (18:1(11Z)/0:0) | HMDB0011505 | — | Up |
| 4 | PC (14:0/16:0) | HMDB0007869 | C00157 | Down |
| 5 | Lauroyl diethanolamide | HMDB0032358 | — | Down |
| 6 | Uracil | HMDB0000300 | C00106 | Down |
| 7 | Niacinamide | HMDB0001406 | C00153 | Down |
| 8 | Eicosadienoic acid | HMDB0005060 | C16525 | Up |
| 9 | Glyceraldehyde | HMDB0001051 | C02154 | Down |
| 10 | Uridine 5'-monophosphate | HMDB0000288 | C00105 | Up |
| 11 | LysoPE (16:0/0:0) | HMDB0011503 | — | Up |
| 12 | ADP | HMDB0001341 | C00008 | Down |