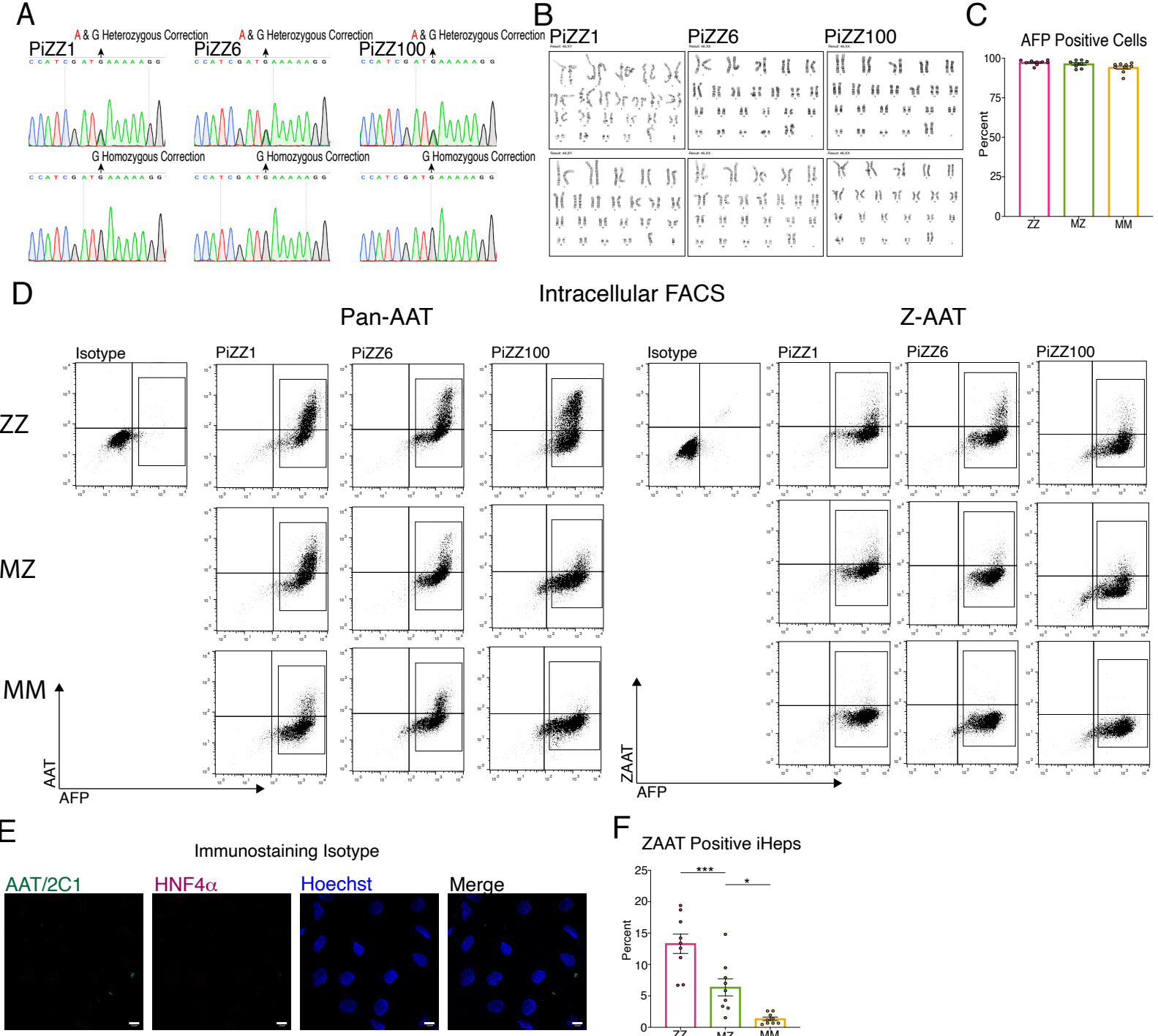


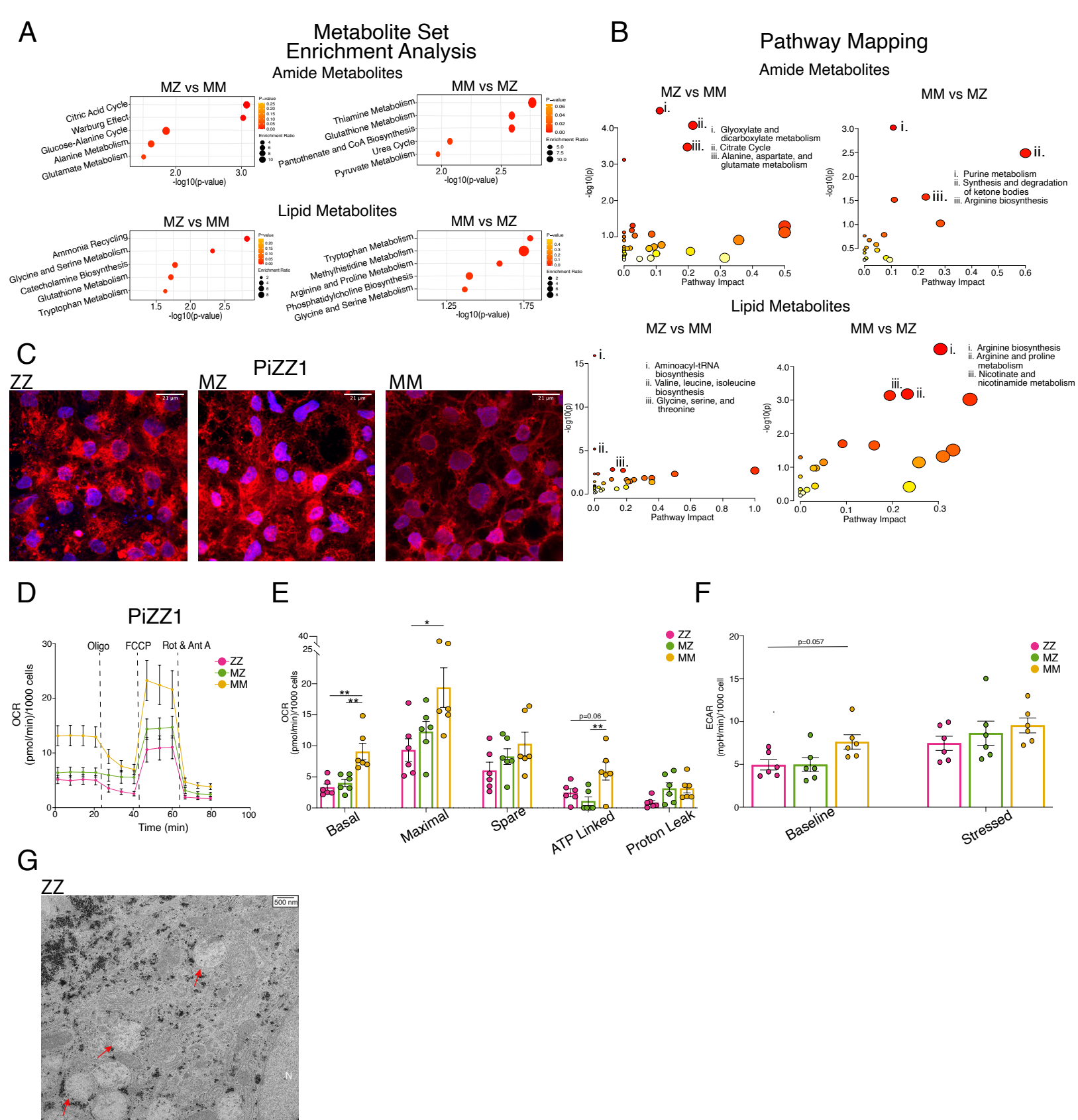
**Supplemental information**

**Human iPSC-hepatocyte modeling of alpha-1  
antitrypsin heterozygosity reveals metabolic  
dysregulation and cellular heterogeneity**

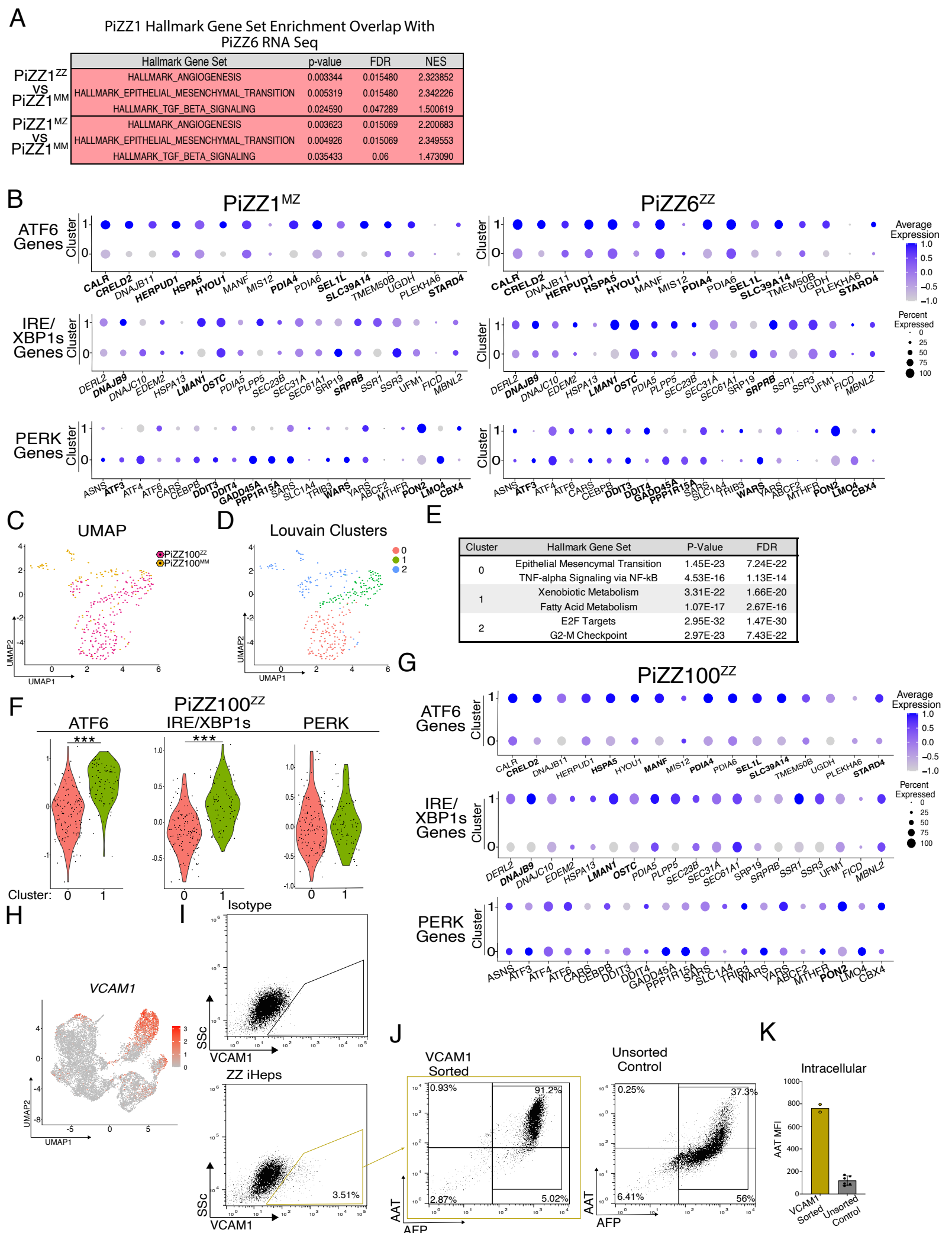
**Joseph E. Kaserman, Rhiannon B. Werder, Feiya Wang, Taylor Matte, Michelle I. Higgins, Mark Dodge, Jonathan Lindstrom-Vautrin, Pushpinder Bawa, Anne Hinds, Esther Bullitt, Ignacio S. Caballero, Xu Shi, Robert E. Gerszten, Nicola Brunetti-Pierri, Marc Liesa, Carlos Villacorta-Martin, Anthony N. Hollenberg, Darrell N. Kotton, and Andrew A. Wilson**



**Figure S1: Characterization of MZ and MM CRISPR/Cas9 generated iPSCs and iPSC derived hepatic cells, related to Figure 1.** A) Sanger sequencing for each of the MZ and MM iPSCs. B) Each line was confirmed to have retained a normal karyotype following CRISPR targeting. C) Percent of iHeps staining positive for AFP (Mean  $\pm$  SEM) D) Representative FACS plots for PiZZ1, PiZZ6, or PiZZ100 ZZ, MZ, or MM iHeps stained with pan-AAT or ZAAT antibodies together with antibodies against AFP. E) Immunostaining isotype controls for AAT, 2C1 and HNF4 $\alpha$  antibodies; scale bar, 10 $\mu$ m. F) Percent ZAAT positive iHeps identified in C (n=3 independent experiments from each of the syngeneic backgrounds. mean  $\pm$  SEM). \*p<0.05, \*\*\*p<0.001 by one-way anova with Dunnett's multiple comparisons.



**Figure S2: MZ and ZZ iHeps Demonstrate Metabolic and Mitochondrial Dysregulation, related to Figure 3 and Figure 4.** A) Summary plots for metabolite set enrichment analysis from MZ vs MM iHeps showing the top 5 pathways as ranked by p-value. B) Metabolome projection of pathway enrichment analysis for MZ vs MM iHeps with top 3 pathways as ranked by FDR annotated. C) MitoTracker staining of PiZZ1 syngeneic iHeps. D) Mitochondrial oxygen consumption rate (OCR) for PiZZ1 syngeneic iHeps. E) Quantification of OCR components from (D) (n=6 independent measurements. Mean +/- SEM). F) Extracellular acidification rate (ECAR) quantification at basal and stressed states (n=6 independent measurements. Mean +/- SEM). G) Transmission electron microscopy identifies globular inclusions (red arrows) in ZZ iHeps. \*p < 0.05, \*\*p < 0.01 by one-way anova with Tukey's multiple comparison test.



**Figure S3: Transcriptional Heterogeneity and Branch-Specific Activation of the UPR In MZ and ZZ iHeps, related to Figure 3 and Figure 5.** A) GSEA analysis of PiZZ1 syngeneic iHeps grouped by original identity displaying overlapping Hallmark pathway enrichments from bulk RNA-seq (Figure 2). B) PiZZ1<sup>MZ</sup> and PiZZ6<sup>ZZ</sup> UPR branch specific gene dot plot projections. Genes that are differentially expressed are bolded (FDR p<0.05). C) UMAP projection by original identity for PiZZ100<sup>ZZ</sup> and PiZZ100<sup>MM</sup> scRNA-seq experiment using the Fluidigm C1 platform. D) Louvain clustering demonstrates 3 clusters. E) Top two Hallmark gene sets by cluster as ranked by FDR using Enrichr analysis of all DEGs (FDR <0.05). F) Violin plots for UPR branch specific module scores for PiZZ100<sup>ZZ</sup> clusters 0 and 1. G) PiZZ100<sup>ZZ</sup> UPR branch specific gene dot plot projections. Genes that are differentially expressed are bolded (FDR p<0.05). H) UMAP of VCAM1 expression. I) Representative fluorescence-activated cell sorting plots demonstrating identification and isolation of VCAM1 positive ZZ iHeps. J) Representative flow cytometry plots of VCAM1 sorted iHeps fixed and stained for AAT versus an unsorted control. K) MFI of intracellular AAT protein from VCAM1 sorted iHeps versus unsorted controls from PiZZ1 and PiZZ6 iHeps. n=1 from each genetic background for VCAM1 sorted iHeps and n = 3 for unsorted controls from each genetic background. \*\*\*p<0.001.



**Table S2: Top 20 GO terms enriched in MM iHeps compared with either MZ or ZZ iHeps as ranked by NES, related to Figure 2.**

MZ vs MM					ZZ vs MM				
GO TERM	p-value	FDR	NES		GO TERM	p-value	FDR	NES	
GO_STEROL_HOMEOSTASIS	0.00074	0.03346	-2.39231		GO_STEROL_HOMEOSTASIS	0.00074	0.02275	-2.45276	
GO_LIPID_HOMEOSTASIS	0.00069	0.03346	-2.27034		GO_LIPID_HOMEOSTASIS	0.00069	0.02275	-2.37286	
GO_TRIGLYCERIDE_METABOLIC_PROCESS	0.00073	0.03346	-2.20907		GO_PROTEIN_LIPID_COMPLEX_SUBUNIT_ORGANIZATION	0.00080	0.02275	-2.35827	
GO_PROTEIN_CONTAINING_COMPLEX_REMODELING	0.00083	0.03346	-2.18950		GO_PROTEIN_CONTAINING_COMPLEX_REMODELING	0.00082	0.02275	-2.35273	
GO_PROTEIN_ACTIVATION_CASCADE	0.00083	0.03346	-2.18073		GO_REGULATION_OF_STEROID_METABOLIC_PROCESS	0.00070	0.02275	-2.33170	
GO_PROTEIN_LIPID_COMPLEX_SUBUNIT_ORGANIZATION	0.00081	0.03346	-2.17964		GO_STEROL_BIOSYNTHETIC_PROCESS	0.00075	0.02275	-2.32424	
GO_ORGANIC_HYDROXY_COMPOUND_CATABOLIC_PROCESS	0.00076	0.03346	-2.16887		GO_STEROL_METABOLIC_PROCESS	0.00067	0.02275	-2.31082	
GO_BRUSH_BORDER_MEMBRANE	0.00079	0.03346	-2.16585		GO_REGULATION_OF_PLASMA_LIPOPROTEIN_PARTICLE_LEVELS	0.00074	0.02275	-2.30332	
GO_STEROID_ESTERIFICATION	0.00086	0.03346	-2.15333		GO_STEROL_TRANSPORT	0.00074	0.02275	-2.26510	
GO_CELLULAR_BIOGENIC_AMINE_METABOLIC_PROCESS	0.00078	0.03346	-2.14896		GO_ORGANIC_ACID_CATABOLIC_PROCESS	0.00063	0.02275	-2.25546	
GO_DIGESTION	0.00071	0.03346	-2.11228		GO_REGULATION_OF_CHOLESTEROL_METABOLIC_PROCESS	0.00076	0.02275	-2.24717	
GO_CHOLESTEROL_EFFLUX	0.00080	0.03346	-2.10765		GO_STEROID_BIOSYNTHETIC_PROCESS	0.00066	0.02275	-2.24666	
GO_MICROVILLUS	0.00075	0.03346	-2.10161		GO_MONOCARBOXYLIC_ACID_CATABOLIC_PROCESS	0.00069	0.02275	-2.22795	
GO_REGULATION_OF_STEROID_METABOLIC_PROCESS	0.00071	0.03346	-2.09505		GO_BILE_ACID_METABOLIC_PROCESS	0.00078	0.02275	-2.22404	
GO_STEROL_TRANSPORTER_ACTIVITY	0.00082	0.03346	-2.09457		GO_REGULATION_OF_TRIGLYCERIDE_METABOLIC_PROCESS	0.00083	0.02275	-2.21593	
GO_NEUTRAL_LIPID_BIOSYNTHETIC_PROCESS	0.00080	0.03346	-2.08586		GO_PROTEIN_LIPID_COMPLEX_ASSEMBLY	0.00082	0.02275	-2.21311	
GO_STEROL_METABOLIC_PROCESS	0.00068	0.03346	-2.08177		GO_STEROID_METABOLIC_PROCESS	0.00062	0.02275	-2.20904	
GO_MYELIN_ASSEMBLY	0.00083	0.03346	-2.07211		GO_TRIGLYCERIDE_METABOLIC_PROCESS	0.00074	0.02275	-2.20839	
GO_NEUTRAL_LIPID_METABOLIC_PROCESS	0.00071	0.03346	-2.06520		GO_REGULATION_OF_LIPID_BIOSYNTHETIC_PROCESS	0.00066	0.02275	-2.20832	
GO_STEROL_TRANSPORT	0.00073	0.03346	-2.04958		GO_REGULATION_OF_LIPID_CATABOLIC_PROCESS	0.00076	0.02275	-2.19853	

**Table S3: Differential Metabolites ranked by FDR, related to Figure 3.**

Metabolite	p-value	FDR	Metabolite	p-value	FDR
<b>Amide Metabolites</b>			<b>Lipid Metabolites</b>		
Glycocholic acid	3.11E-12	1.54E-10	kynurenine	4.50E-16	5.36E-14
Kynurenine	3.27E-12	1.54E-10	proline	3.46E-15	2.06E-13
Pantothenic acid	4.71E-12	1.54E-10	asparagine	3.30E-12	1.31E-10
Glycerol-3-phosphate	7.80E-12	1.91E-10	serine	7.31E-12	1.76E-10
N-Acetyl-L-Glutamic acid	6.47E-10	1.27E-08	N-carbomoyl-beta-alanine	8.84E-12	1.76E-10
Glutathione_reduced	1.00E-09	1.63E-08	5-Adenosyl-Methionine (SAME)	8.85E-12	1.76E-10
UDP-GlcNAc	1.29E-09	1.80E-08	alpha-glycerophosphocholine	4.16E-11	6.61E-10
D-Gluconic acid	1.50E-09	1.84E-08	citrulline	4.59E-11	6.61E-10
UDP-glucose /-galactose	3.74E-09	4.07E-08	phosphoethanolamine	5.49E-11	6.61E-10
Glycochenodeoxycholic acid	9.84E-09	9.39E-08	N-Acetyl-Lysine	5.56E-11	6.61E-10
Hypoxanthine	1.05E-08	9.39E-08	kynurenic acid	7.15E-11	7.74E-10
Pyruvic acid	1.42E-08	1.16E-07	Ala-Leu	1.98E-10	1.94E-09
Pseudouridine	1.58E-07	1.17E-06	citicholine	2.12E-10	1.94E-09
ATP	1.79E-07	1.17E-06	C9-carnitine	6.37E-10	5.41E-09
Glyceric acid	3.46E-07	2.12E-06	serotonin	8.88E-10	7.04E-09
AMP	5.10E-07	2.77E-06	betaine	1.69E-09	1.17E-08
N-Acetyl-L-Methionine	8.00E-07	4.12E-06	5-HIAA	1.75E-09	1.17E-08
Citrulline	1.36E-06	6.68E-06	creatine	1.77E-09	1.17E-08
Kynurenic acid	2.18E-06	1.02E-05	N-Acetyl-L-Methionine	2.27E-09	1.42E-08
ADP	3.31E-06	1.47E-05	ADMA/SDMA	4.20E-09	2.50E-08
Uric acid	3.69E-06	1.57E-05	carnitine	6.12E-09	3.47E-08
Oxypurinol	6.97E-06	2.84E-05	C14-carnitine	1.34E-08	7.25E-08
Xanthine	1.05E-05	4.12E-05	N-Carbamoyl-BAIBA	1.63E-08	8.13E-08
3-Hydroxybutyric acid	1.31E-05	4.94E-05	C3-malonyl-carnitine	1.64E-08	8.13E-08
Glutathione Disulfide	2.58E-05	9.36E-05	N1-Methylnicotinamide	1.80E-08	8.55E-08
Taurodeoxycholic acid			acetylcholine	2.67E-08	1.22E-07
Taurochenodeoxycholic acid	3.69E-05	0.0001	anthranilic acid	5.14E-08	2.27E-07
2-ketoisovaleric acid KIV	8.88E-05	0.0003	C4-methylmalonyl-carnitine	7.58E-08	3.22E-07
Arachidonic acid	0.0002	0.0005	C4-butyryl-carnitines	8.89E-08	3.65E-07
2-Hydroxyglutaric acid	0.0002	0.0005	C5-valeryl-carnitines	1.50E-07	5.96E-07
Uridine	0.0002	0.0006	C3-carnitine	2.32E-07	8.91E-07
cyclic-AMP	0.0002	0.0006	Cystine	2.58E-07	9.61E-07
Oxalic acid	0.0002	0.0006	N-Acetyl-L-Phenylalanine	2.76E-07	9.97E-07
1,5-AG / 1-deoxyglucose	0.0002	0.0006	N-Acetyl-L-Alanine	3.44E-07	1.21E-06
Glucose/Fructose/Galactose_waterloss	0.0003	0.0007	C16-carnitine	1.13E-06	3.84E-06
a-Ketoglutaric acid	0.0004	0.0010	creatinine	1.23E-06	4.08E-06
Indole-3-lactic acid	0.0007	0.0019	glycine	1.48E-06	4.75E-06
Acetoacetic acid	0.0022	0.0056	glutamate	1.69E-06	5.29E-06
Butyric acid	0.0024	0.0058	valine	1.83E-06	5.57E-06
Inosine	0.0030	0.0072	NMMA	2.14E-06	6.37E-06
Anthranilic acid	0.0039	0.0091	C6-carnitine	3.29E-06	9.48E-06
Keto-isocaproic acid KIC	0.0040	0.0091	threonine	3.34E-06	9.48E-06
Keto-methylvalerate KMV	0.0042	0.0093	Norepinephrine-updated	6.14E-06	1.69E-05
Methylmalonic acid	0.0056	0.0117	C2-carnitine	6.29E-06	1.69E-05
Taurine	0.0056	0.0117	histidine	6.41E-06	1.69E-05
Adipic acid	0.0067	0.0138	C18-carnitine	8.97E-06	2.32E-05
N-Acetyl-L-Aspartic acid	0.0071	0.0142	aminoisobutyric acid	1.11E-05	2.81E-05
N-Acetyl-L-Glutamine	0.0096	0.0188	1-methylhistamine	1.24E-05	3.06E-05
Oleoyl Glycine	0.0113	0.0214	N-Methyl-4-Pyridone-3-Carboxamide	1.68E-05	4.08E-05
Lactic acid	0.0117	0.0216	cis/trans hydroxyproline	2.10E-05	5.00E-05
Glutamic acid	0.0126	0.0228	N-Acetyl-L-Ornithine	2.36E-05	5.52E-05
Succinic acid	0.0138	0.0245	C18:1-carnitine	2.79E-05	6.38E-05
Aconitic acid	0.0190	0.0332	2'-deoxycytidine	3.08E-05	6.91E-05
Oleoyl Phenylalanine	0.0213	0.0367	arginine	3.14E-05	6.91E-05
Citric acid/Isocitric acid	0.0233	0.0393	2'-deoxyadenosine	5.03E-05	0.0001
Deoxycholic acid	0.0251	0.0416	uridine	6.11E-05	0.0001
Hippuric acid			N-Acetyl-L-Glutamic acid	6.74E-05	0.0001
			C8-carnitine	8.40E-05	0.0002
			3-hydroxyanthranilic acid	8.95E-05	0.0002
			5'-adenosylhomocysteine	9.78E-05	0.0002
			Dihydrouracil T1	0.0001	0.0002
			carosine	0.0001	0.0002
			tryptophan	0.0001	0.0003
			taurine	0.0002	0.0003
			glutamine	0.0002	0.0003
			aspartate	0.0002	0.0003
			phenylalanine	0.0003	0.0005
			histamine	0.0003	0.0005
			lysine	0.0004	0.0007
			GABA	0.0005	0.0008
			glycerol	0.0005	0.0009
			C12-carnitine	0.0008	0.0014
			dimethylglycine	0.0011	0.0018
			leucine	0.0012	0.0020
			xanthurenate	0.0014	0.0023
			ornithine	0.0015	0.0024
			choline	0.0016	0.0025
			Ala-Gly	0.0017	0.0027
			methyl-hydroxyisobutyric acid	0.0018	0.0027
			methionine	0.0021	0.0031
			DMGV T1	0.0030	0.0043
			cAMP	0.0044	0.0064
			thiamine	0.0071	0.0101
			spermidine	0.0099	0.0141
			C5-glutaryl-carnitine	0.0110	0.0154
			niacinamide	0.0112	0.0155
			tyrosine	0.0129	0.0176
			alanine	0.0146	0.0197
			Sarcosine	0.0158	0.0211
			thymidine	0.0166	0.0220
			C7-carnitine	0.0199	0.0260
			isoleucine	0.0228	0.0292
			N-Methyl-2-Pyridone-5-Carboxamide	0.0228	0.0292
			phosphocholine	0.0236	0.0299
			Dopamine-updated	0.0268	0.0336