Supplementary Materials for

Microbiotas from extremely preterm infants with growth faltering impair postnatal growth and metabolism in mice

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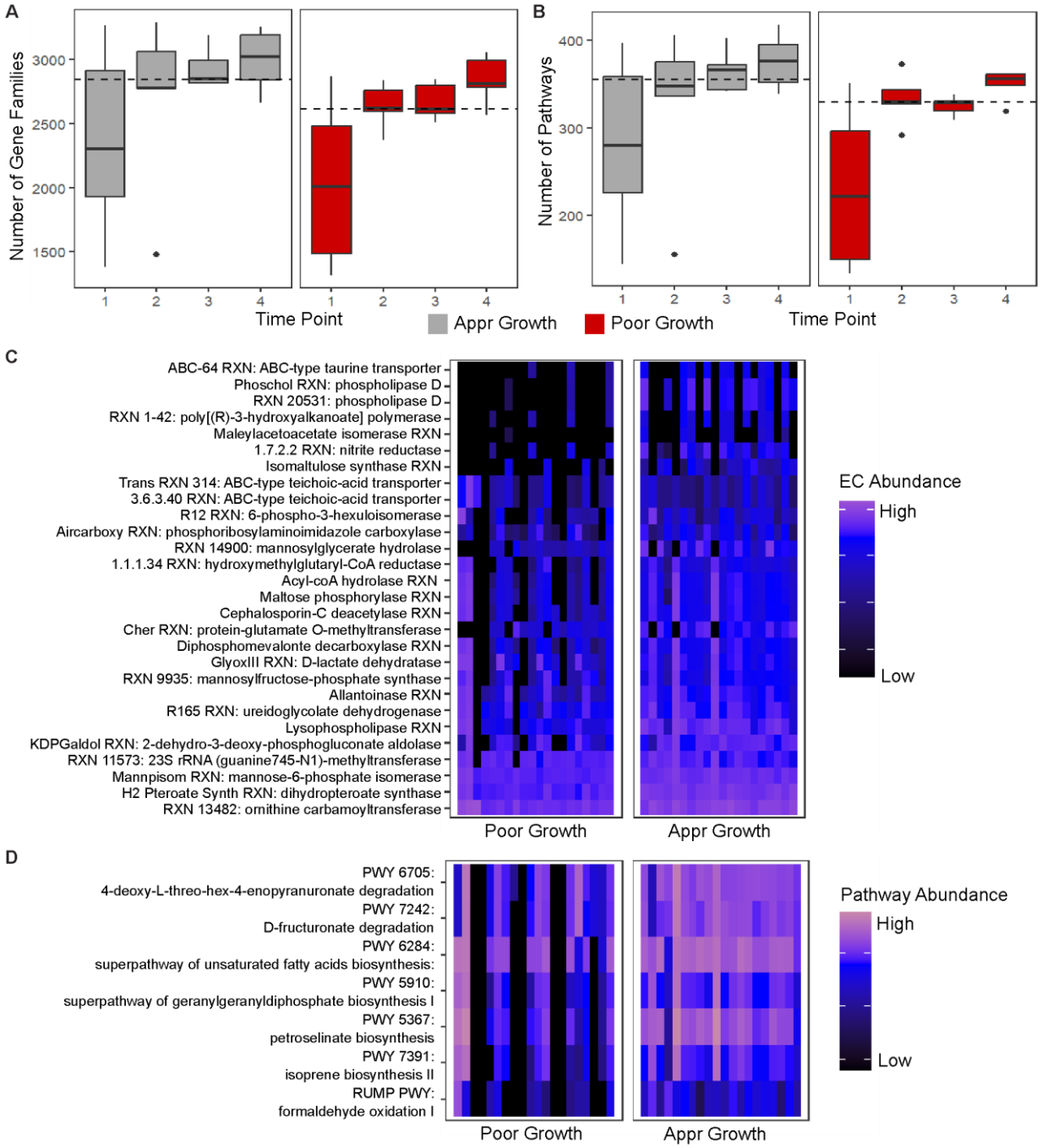


Figure S1. Functional metagenomic profiles of infant fecal microbiomes. Number of Enzyme Commission (EC, level 4) assigned gene families (**A**) and functional MetaCyc pathways present per sample by group and time point. ECs (**C**) and pathways (**D**) that differed between groups in mixed-effects models accounting for repeated measures from individual subjects.

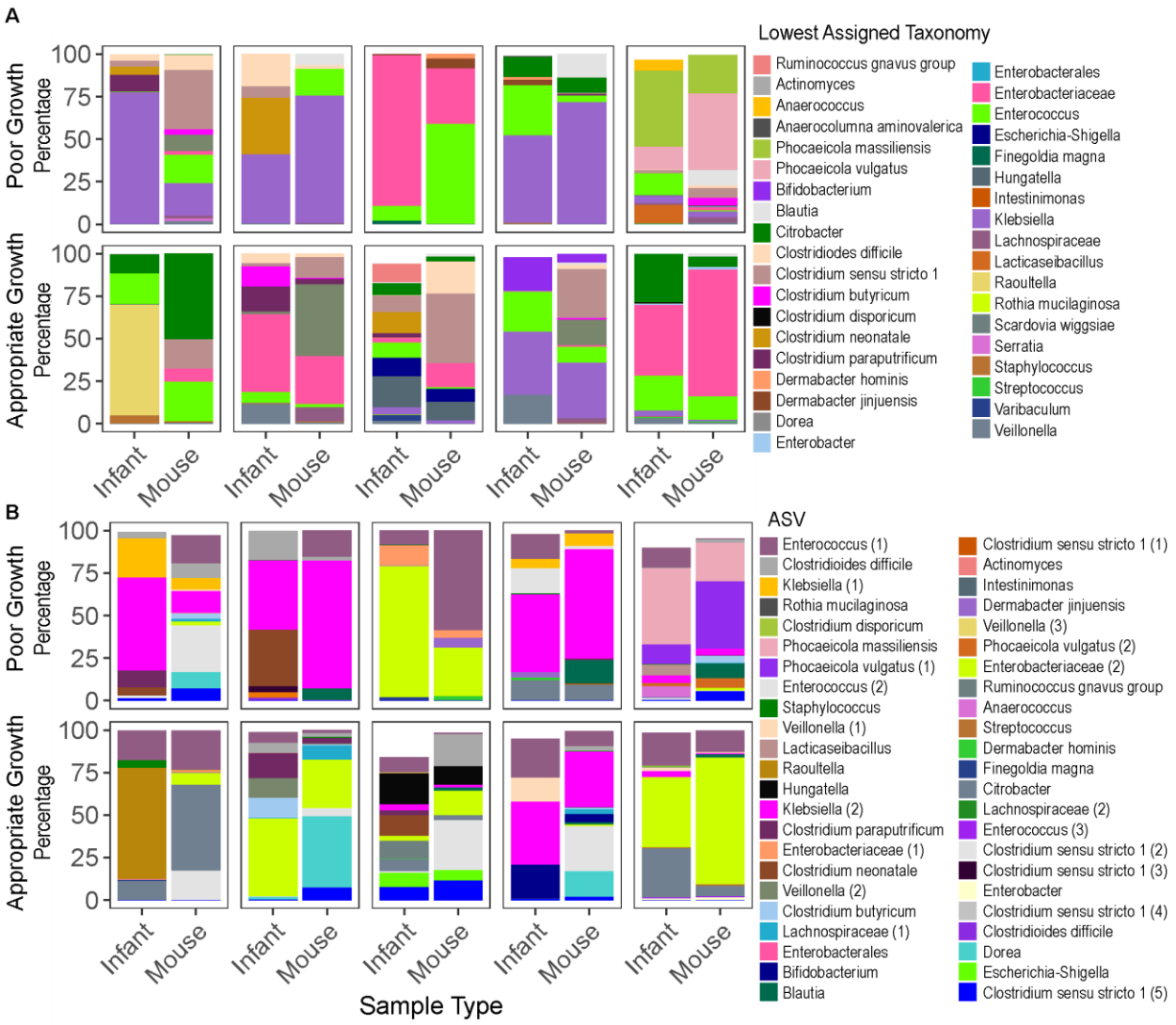


Figure S2. Fecal microbiota profiles of extremely preterm infant donors and recipient neonatal mice. A) Relative abundances of top bacterial taxa grouped at the level of the lowest taxonomic assignment. **B)** Relative abundances of top amplicon sequence variants (ASV). Colors in individual bars are arranged from top to bottom in the same order as shown in the legend.

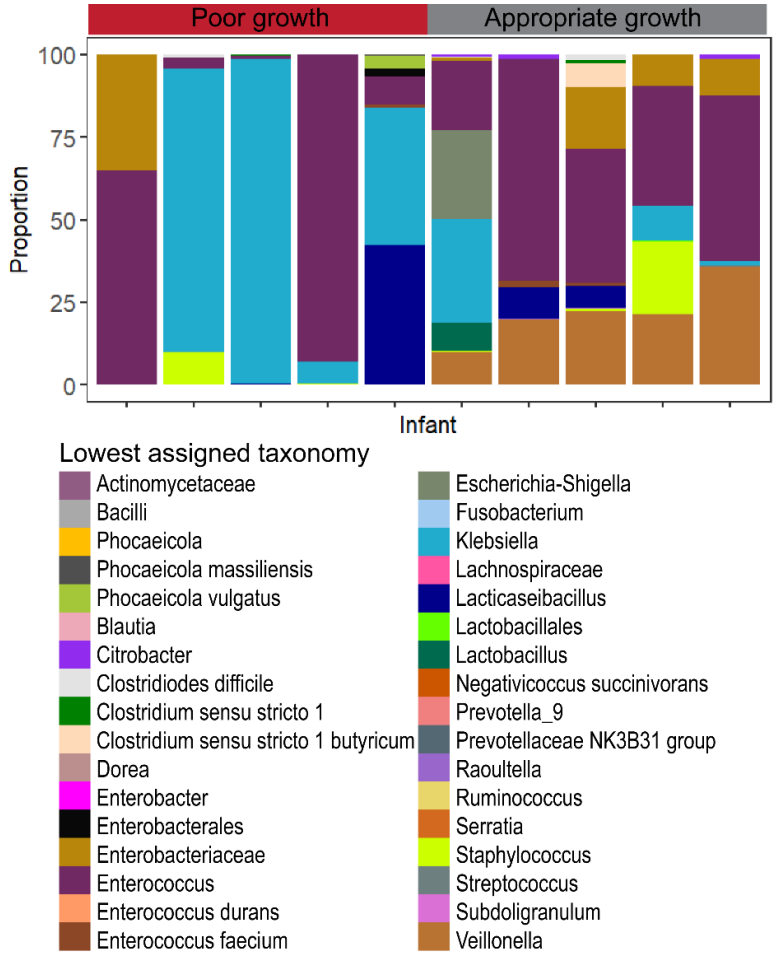


Figure S3. Relative abundance of bacterial taxa in the cultured microbial communities from preterm infant fecal samples as determined by 16S rRNA gene sequencing. Each column represents a cultured microbial community from an individual infant.

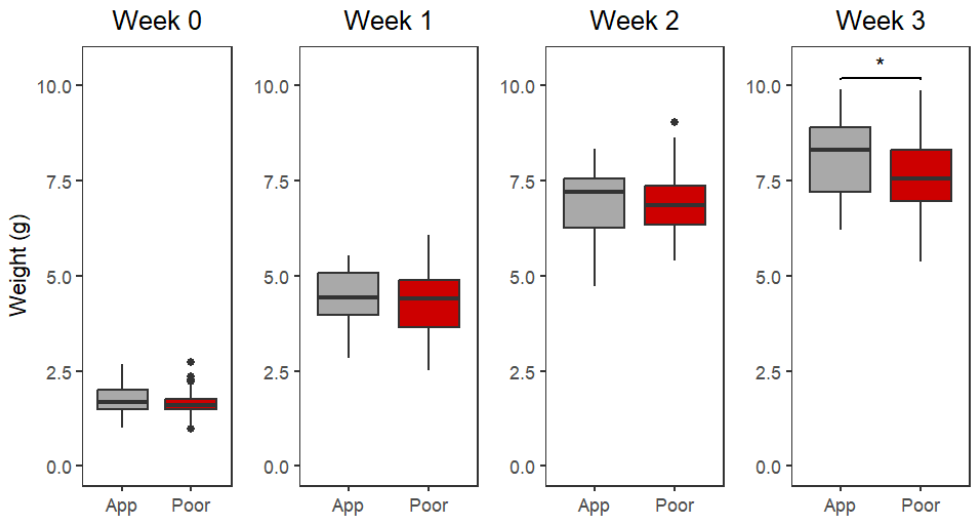


Figure S4. Neonatal mouse weights over time. Body weights (in grams) at Week 0 (postnatal day 2-3), Week 1 (postnatal day 7-8), Week 2 (postnatal day 14-15), and Week 3 (postnatal day 20) of neonatal mice colonized with microbiotas from extremely preterm infants with appropriate (App) growth (gray) or poor growth (red). *P<0.01.

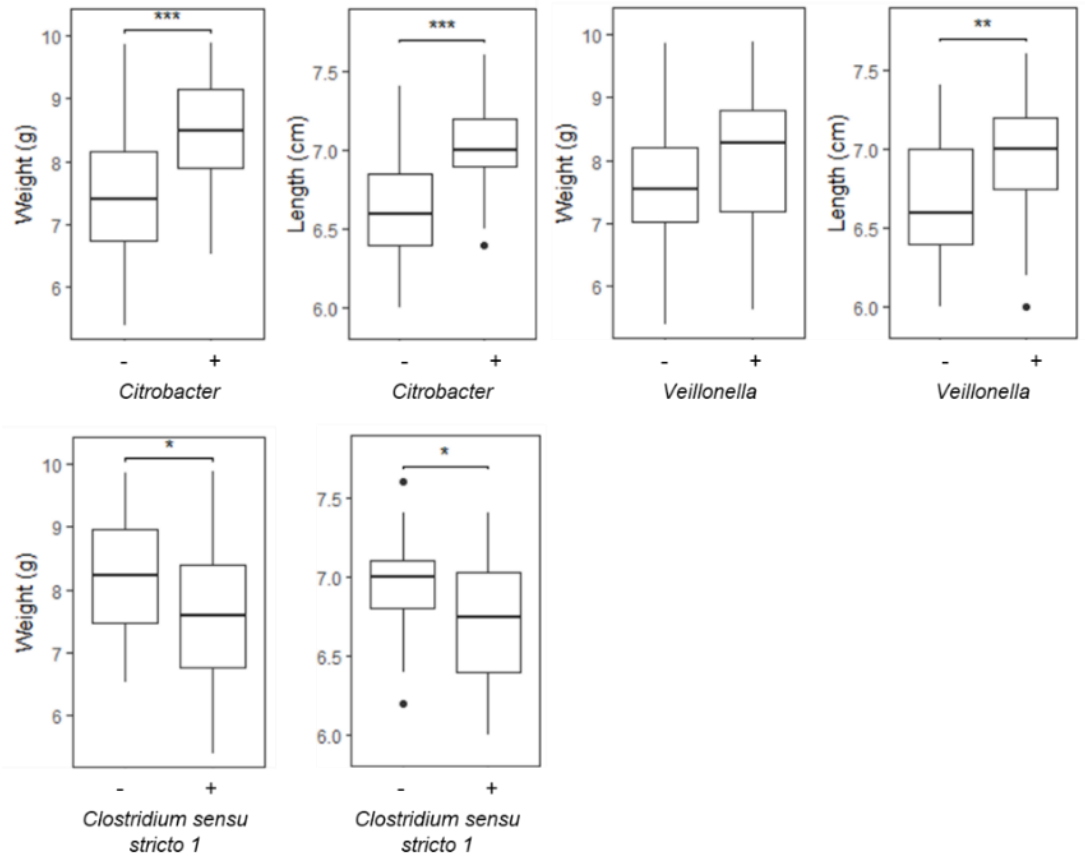


Figure S5. Preterm infant taxa associated with postnatal growth in mice. Weight and length (P20) were compared between mice based on the presence or absence of bacterial taxa in the donor preterm infant samples. Only taxa present in ≥2 donor infant samples were included in these comparisons. The number of litters with each taxa present were: *Citrobacter*, n=11; *Clostridium sensu stricto* 1, n=14; and *Veillonella*, n=16 of 25 total litters. *p_{adj}<0.05, ** p_{adj}<0.01, *** p_{adj}<0.001.

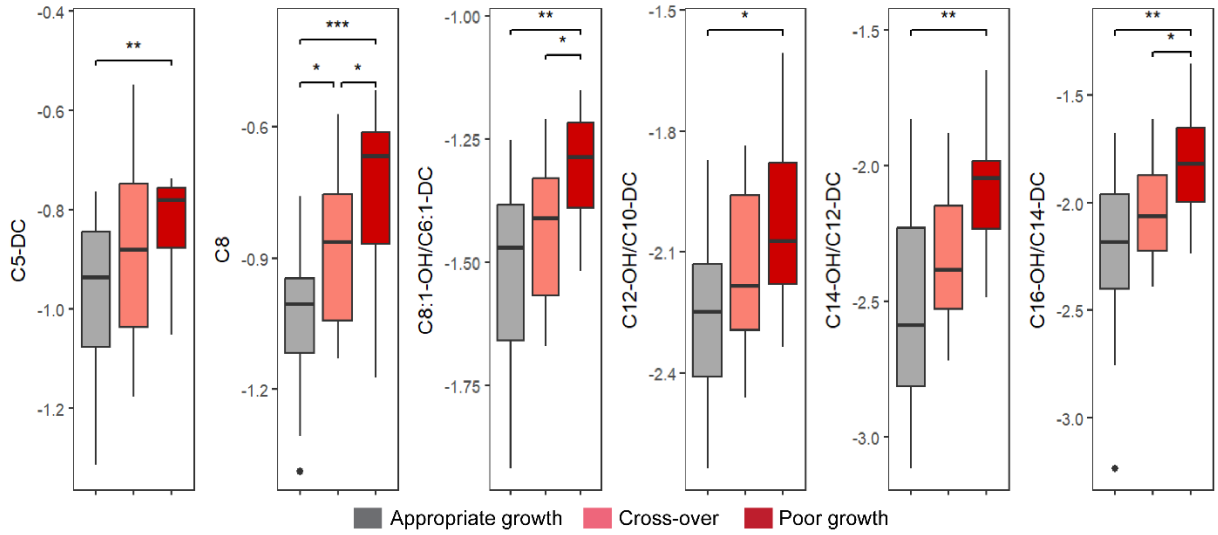


Figure S6. Comparison of select acylcarnitines in the livers of mice in the appropriate growth, cross-over and poor growth groups at P20. *p<0.05, **p<0.01, ***p<0.001

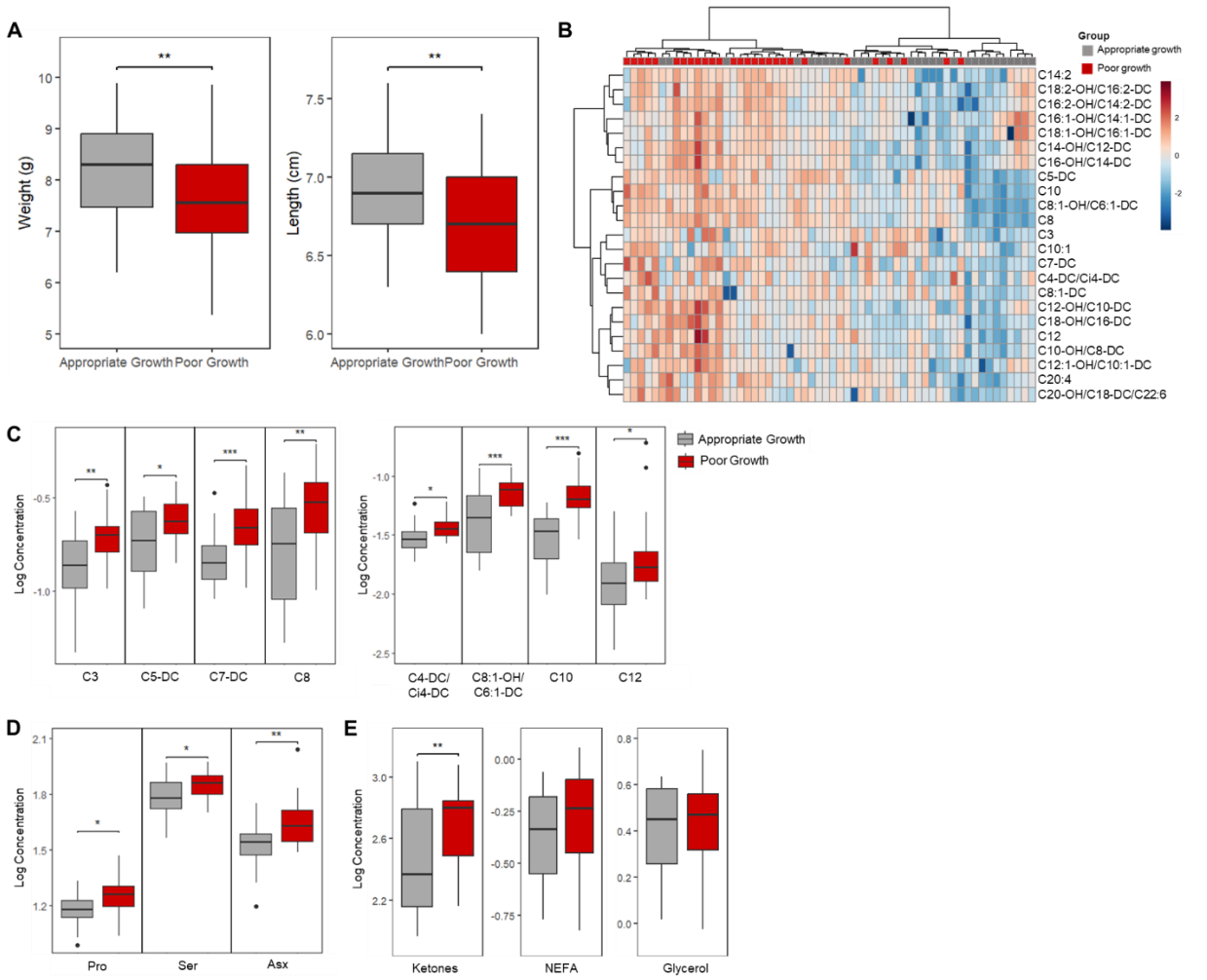
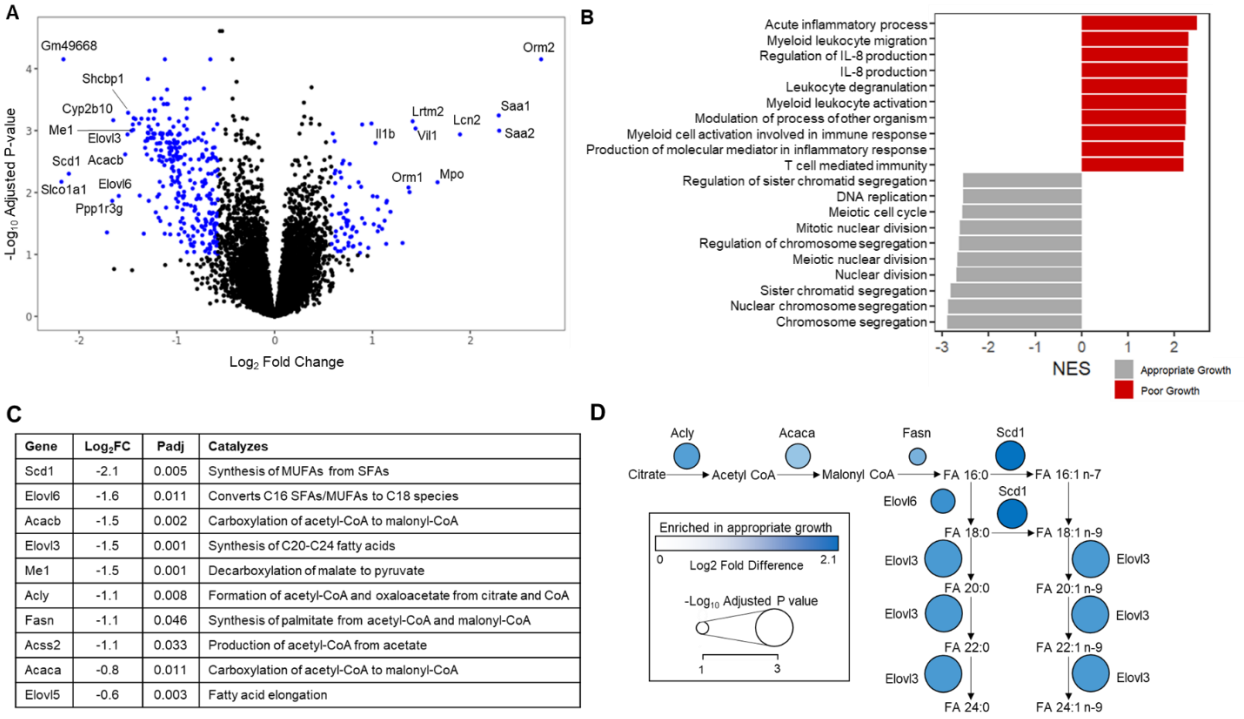


Figure S7. Effect of the extremely preterm infant microbiota on postnatal growth and metabolism excluding litters with *Blautia* in mouse fecal microbiota only. **A)** Mice colonized microbial communities of extremely preterm infants with poor growth (n=11 litters) had significantly lower weight and length at P20 than mice colonized with microbial communities of extremely preterm infants with appropriate growth (n=11 litters). **B)** Heatmap of hepatic acylcarnitine profiles in neonatal mice colonized with microbial communities of preterm infants with poor growth (red) and appropriate growth (gray). **C)** Log-transformed concentrations of select acylcarnitines that were significantly elevated in the livers of poor growth microbiota recipient mice compared to appropriate growth microbiota recipient mice. Groups were compared by linear regression, adjusted for sex. **D)** Log-transformed concentrations of select amino acids in the liver. **E)** Log-transformed serum ketones, NEFA, and glycerol concentrations in the appropriate growth and poor growth groups. Asx, aspartic acid/asparagine; NEFA, non-esterified fatty acid; Pro, proline; Ser, serine. *p<0.05, **p<0.01, ***p<0.001



Gene	Log_2FC	Padj	Catalyzes
Scd1	-2.1	0.005	Synthesis of MUFAs from SFAs
Elovl6	-1.6	0.011	Converts C16 SFAs/MUFAs to C18 species
Acacb	-1.5	0.002	Carboxylation of acetyl-CoA to malonyl-CoA
Elovl3	-1.5	0.001	Synthesis of C20-C24 fatty acids
Me1	-1.5	0.001	Decarboxylation of malate to pyruvate
Acly	-1.1	0.008	Formation of acetyl-CoA and oxaloacetate from citrate and CoA
Fasn	-1.1	0.046	Synthesis of palmitate from acetyl-CoA and malonyl-CoA
Acss2	-1.1	0.033	Production of acetyl-CoA from acetate
Acaca	-0.8	0.011	Carboxylation of acetyl-CoA to malonyl-CoA
Elovl5	-0.6	0.003	Fatty acid elongation

Figure S8. Effect of the extremely preterm infant microbiota on hepatic gene expression, excluding litters with *Blautia* in mouse fecal microbiota only. A) Volcano plot of gene expression in the livers of mice colonized with fecal microbial communities from infants with poor growth (positive Log_2 fold change values) or appropriate growth (negative Log_2 fold change values). Statistically significant differences are highlighted in blue (Log_2 fold change>0.58 and p_{adj}<0.1)**. B)** Top GSEA pathways enriched in each group of significantly differentially expressed genes. **C)** Lipogenesis and fatty acid elongation genes upregulated in the livers of mice colonized with bacteria from infants with appropriate growth. **D)** Schematic of the de novo lipogenesis pathway showing all enzymes were upregulated in mice colonized with bacteria from infants with appropriate growth. NES, normalized enrichment score.

Table S1. Extremely preterm infant characteristics.

	Subject ID	Weight z-score change	Birth gestational age, weeks	Birth weight, g (z-score)	Sex	Delivery mode	Diet*	First day of enteral feeds	First day full feeds	Cumulative antibiotic days**	Antibiotics
Appropriate Growth	AG1	0.3	27	1080 (0.7)	Male	Vaginal	fMBM/DBM	2	14	11	A/G/Az/PT
	AG2	0.3	27	1135 (0.5)	Male	Vaginal	fMBM	4	13	2	A/G
	AG3	-0.2	23	580 (0.5)	Female	Vaginal	fDBM	7	36	17	A/G/PT
	AG4	-0.5	24	550 (-0.6)	Female	Vaginal	fMBM/DBM	2	21	8	A/G/V/C3
	AG5	-0.7	26	1020 (1.1)	Male	Cesarean	fMBM	1	9	9	A/G
Poor Growth	PG1	-1.0	25	790 (0.8)	Female	Vaginal	fMBM	4	20	6	A/G
	PG2	-1.4	24	680 (0.3)	Male	Vaginal	fDBM	3	19	25	A/G/C1/C4/V/PT
	PG3	-1.9	27	1075 (0.7)	Male	Cesarean	fMBM	2	16	7	A/G/V
	PG4	-2.6	27	1000 (0.3)	Male	Cesarean	fDBM	2	17	9	A/G
	PG5	-3.5	27	720 (-0.9)	Female	Cesarean	fMBM/DBM	2	48	24	A/G/V/C1/AS

*All infants received enteral feedings of fortified maternal breast milk (fMBM), fortified donor breast milk (fDBM), or a combination (fMBM/DBM) during the neonatal course. Infants were commonly transitioned to partial or full feedings of infant formula prior to discharge.
**Cumulative antibiotic days represents the total number of days the infant received antibiotics from birth to 40 weeks' postmenstrual age.
A, ampicillin; AS, ampicillin-sulbactam; Az, azithromycin; C1, first-generation cephalosporin; C3, third-generation cephalosporin; C4, fourth-generation cephalosporin; G, gentamicin; PT, piperacillin-tazobactam; V, vancomycin

Table S2. Acylcarnitine and amino acid concentrations.

Metabolite*, median (IQR)	Appropriate Growth Microbiota Recipients	Poor Growth Microbiota Recipients	P value	Adjusted P value
Liver acylcarnitines (nM)	N=36	N=28		
C3	138 (99, 180)	200 (162, 223)	*0.0004*	*0.004*
C4-DC/Ci4-DC	29 (25, 34)	36 (31, 41)	*0.001*	*0.008*
C8	179 (107, 263)	299 (205, 382)	*0.0002*	*0.002*
C5-DC	187 (133, 265)	236 (203, 293)	*0.003*	*0.013*
C8:1-OH/C6:1-DC	44 (26, 67)	76 (56, 88)	*<0.0001*	*0.0007*
C10:3	4 (3, 5)	5 (3, 7)	*0.0496*	0.117
C10:1	7 (6, 8)	9 (7, 11)	*0.004*	*0.019*
C10	34 (21, 44)	63 (54, 82)	*<0.0001*	*<0.0001*
C7-DC	149 (120, 184)	218 (177, 275)	*<0.0001*	*0.0007*
C12	12 (10, 17)	17 (13, 23)	*0.005*	*0.021*
C12:1-OH/C10:1-DC	3 (2, 4)	4 (3, 7)	*0.025*	0.068
C12-OH/C10-DC	7 (5, 8)	9 (7, 13)	*0.001*	*0.008*
C14:2	4 (1, 7)	7 (4, 9)	*0.0005*	*0.005*
C14	21 (13, 29)	26 (17, 41)	*0.038*	0.097
C14-OH/C12-DC	8 (4, 11)	13 (8, 16)	*0.002*	*0.010*
C16:2-OH/C14:2-DC	4 (3, 6)	9 (5, 11)	*0.015*	0.052
C16:1-OH/C14:1-DC	9 (6, 11)	11 (9, 18)	*0.018*	0.052
C16-OH/C14-DC	18 (12, 24)	24 (18, 37)	*0.003*	*0.013*
C18:2-OH/C16:2-DC	11 (9, 15)	19 (13, 22)	*0.0001*	*0.001*
C18:1-OH/C16:1-DC	15 (11, 24)	25 (19, 32)	*0.002*	*0.010*
C18-OH/C16-DC	6 (5, 10)	9 (6, 14)	*0.009*	*0.033*
C20:4	12 (6, 14)	16 (11, 20)	*0.018*	0.052
C20-OH/C18-DC/C22:6	2 (2, 3)	3 (2, 4)	*0.045*	0.111
C22	1 (0.5, 2)	0.7 (0.2, 1)	*0.020*	0.056
Plasma acylcarnitines (nM)	N=35	N=30		
C3	616 (491, 826)	798 (611, 1020)	*0.003*	0.135
C8:1-OH/C6:1-DC	11 (9, 15)	14 (10, 21)	*0.046*	0.891
Liver amino acids (µM)	N=36	N=28		
Proline	15 (13, 17)	18 (16, 20)	*0.002*	*0.011*
Serine	60 (54, 72)	72 (63, 79)	*0.002*	*0.011*
Asparagine/Aspartic acid	35 (29, 38)	43 (35, 52)	*0.0001*	*0.002*
Plasma amino acids	N=36	N=29		
Phenylalanine	93 (84, 101)	103 (92, 109)	*0.044*	0.317
Glutamine/Glutamic acid	136 (124, 167)	109 (99, 125)	*0.013*	0.201

*Only metabolites that differed between groups ($p<0.05$) are shown.

Table S3. Serum analyte values by study group and mouse sex

	Ketones (umol/L) Median (IQR)	**NEFA (mmol/L)** Median (IQR)	**Glycerol (mg/dL)** Median (IQR)
Group			
Poor growth	610 (243, 684)	0.56 (0.36, 0.78)	2.77 (1.86, 3.55)
Appropriate growth	283 (144, 522)	0.52 (0.27, 0.68)	3.08 (1.92, 3.77)
Sex			
Male littermate pair	543 (297, 590)	0.67 (0.52, 0.69)	2.52 (2.43, 3.08)
Female littermate pair	583 (264, 1070)	0.65 (0.42, 0.69)	3.3 (1.87, 3.84)
Mixed-sex littermate pair	264 (174, 635)	0.44 (0.28, 0.71)	2.77 (1.82, 3.52)

Table S4. Select lipogenesis and fatty acid elongation genes upregulated in the livers of mice colonized with microbial communities from infants with appropriate growth

Gene	Log_2FC	Padj	Catalyzes
Scd1	-1.9	0.005	Synthesis of MUFAs from SFAs
Elovl6	-1.5	0.009	Converts C16 SFAs/MUFAs to C18 species
Acacb	-1.4	0.002	Carboxylation of acetyl-CoA to malonyl-CoA
Elovl3	-1.4	<0.001	Synthesis of C20-C24 fatty acids
Me1	-1.4	<0.001	Decarboxylation of malate to pyruvate
Fasn	-1.1	0.04	Synthesis of palmitate from acetyl-CoA and malonyl-CoA
Acly	-1.1	0.007	Production of acetyl-CoA and oxaloacetate from citrate and CoA
Acss2	-1.0	0.02	Production of acetyl-CoA from acetate
Acaca	-0.7	0.007	Carboxylation of acetyl-CoA to malonyl-CoA

Table S5. Liver acylcarnitine and amino acid concentrations in the cross-over experiment.

Metabolite*, median (IQR)	**Appropriate Growth Microbiota Recipients** N=19 samples	**Poor Growth Microbiota Recipients** N=16 samples	**Cross-Over Group** N=14 samples
Liver acylcarnitines (nM)			
C3	116 (81, 170)	151 (114, 173)	205 (177, 254)$^{**,+}$
C4-DC/Ci4-DC	21 (18, 24)	25 (22, 27)$^{++}$	35 (27, 37)$^{**,+}$
C8	100 (83, 113)	200 (121, 241)$^{++}$	137 (91, 176) $^{**,+}$
C5-DC	116 (80, 146)	165 (126, 175)$^{++}$	132 (92, 179)
C8:1-OH/C6:1-DC	35 (21, 41)	51 (38, 60)$^{++}$	39 (27, 47)**
C10	38 (25, 46)	56 (41, 60)$^{++}$	38 (31, 64)
C12-OH/C10-DC	6 (4, 7)	8 (6, 13)$^{++}$	7 (5, 11)
C14	33 (21, 78)	53 (36, 94)$^{++}$	42 (32, 69)
C14-OH/C12-DC	3 (2, 6)	8 (5, 10)$^{++}$	4 (3, 7)
C16-OH/C14-DC	8 (4, 11)	14 (10, 21)$^{++}$	9 (6, 13)**
Liver amino acids (μM)			
Proline	10 (8, 12)	12 (11, 15)	17 (13, 19)$^{**,+}$
Phenylalanine	4 (4, 5)	4 (4, 5)	6 (5, 6)$^{**,+}$
Tyrosine	5 (4, 6)	6 (5, 7)$^{++}$	6 (5, 8)$^{+}$
Glutamate/Glutamine	61 (48, 74)	70 (62, 80)	93 (70, 104)$^{**,+}$
Arginine	1.0 (0.9, 1.1)	0.7 (0.5, 0.9)$^{++}$	1.2 (0.9, 1.4)**

*Only metabolites that differed between groups ($p<0.05$) are shown. **Cross-over vs. poor growth, $p<0.05$. $^{+}$Cross-over vs. appropriate growth, $p<0.05$. $^{++}$Appropriate growth vs. poor growth, $p<0.05$.