

**Regulation of rumen development in neonatal ruminants through microbial metagenomes
and host transcriptomes**

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Supplementary Figure Legends

Fig. S1. Composition of rumen microbiome in calves from birth (at birth) up to six weeks of life. Each data series represent mean relative abundance of microbial group as a % of total detected microbiota.

Fig. S2. Gene modules detected through WGCNA. (A) Number of transcripts detected and percentage of reads belonged in each gene module. B. The most relevant function of gene modules M10, M2, and M18 obtained through GO term enrichment.

Fig. S3. Protein coding genes belong to mitochondrial complex. Red color indicated genes co-expressed in M10 gene module identified via WGCNA.

Fig. S4. Level 2 microbial functions associated with host protein coding genes expression. (A) Level 2 microbial functions associated with ($P < 0.01$, $r^2 \geq 0.98$) host genes involved in rumen tissue carbohydrate metabolism (GO: 0005975, 20 genes). (B) Level 2 microbial functions associated with ($P < 0.01$, $r^2 \geq 0.98$) host genes involved in membrane transportation (GO: 0008643, 14 genes). Right side panel represents genes co-expressed in M10 gene module. FUCA1 – fucosidase alpha L-1; SLC16A1 – solute carrier family 16 member 1; SLC35A3 – solute carrier family 35 member 3.

Fig. S5. Level 2 microbial functions associated with ($P < 0.01$, $r^2 \geq 0.98$) host genes involved in tight junction protein genes (TJs) (GO: 0005923, 14 genes). Upper network represents TJs correlated with microbial carbohydrate metabolism. Lower network represents TJs correlated with other microbial functions. Left side panel represents genes co-expressed in M10 gene module. CLDN23 – claudin 23.

Table S1. Association between rumen papillae development, concentration of VFAs, and cellulolytic bacteria

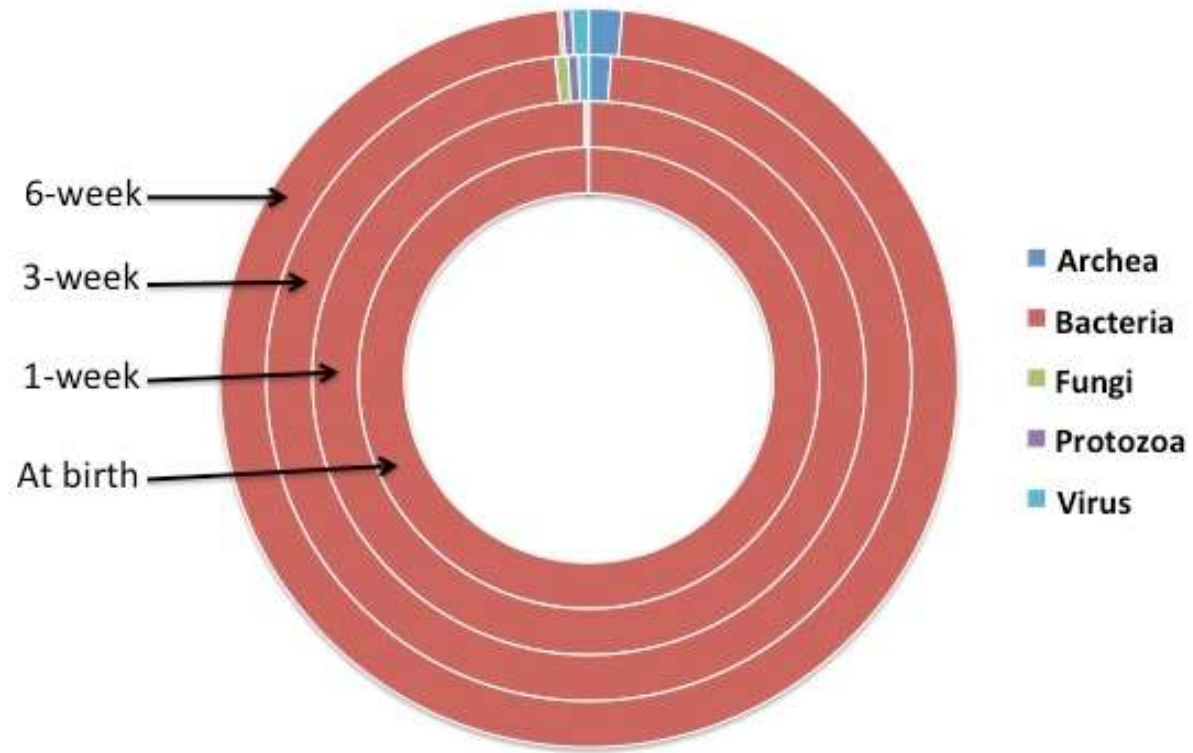
	Acetate	Propionate	Butyrate	Valerate	Isobutyrate	Isovalerate
Papillae length ¹	0.7***	0.6***	0.7***	0.6**	0.7***	0.6***
Papillae width	0.8***	0.8***	0.7***	0.7***	0.8***	0.7***
<i>R. flavefaciens</i>	0.6***	0.4	0.6***	0.4*	0.4*	0.3

¹ Pearson correlation coefficient.

* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$.

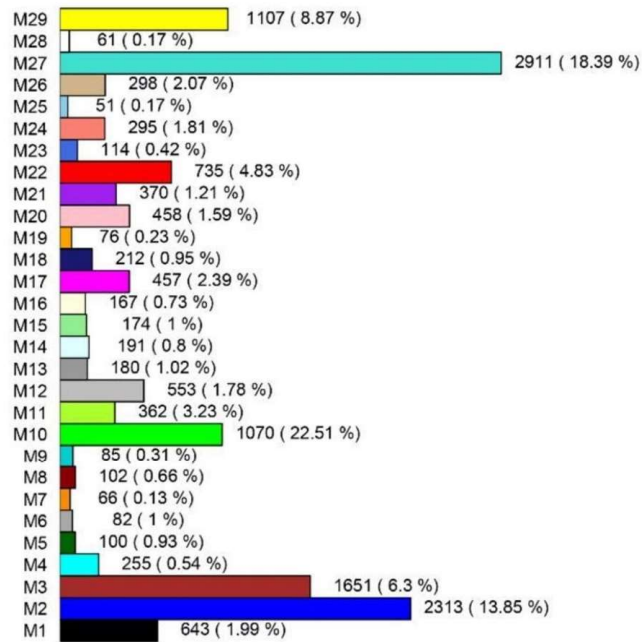
Table S2. Primers used in qPCR to estimate bacterial/archaeal densities

	Forward primer (5'-3')	Reverse primer (5'-3')	Source
Total bacteria	actcctacgggaggcag	gactaccagggtatctaacc	Stevenson & Weimer, 2007
<i>E. ruminantium</i>	ctcccgagactgaggaagcttg	gtccatctcacaccaccgga	Stevenson & Weimer, 2007
<i>P. ruminicola</i>	gaaagtcggattaatgctctatggtg	catcctatagcggtaaactttgg	Stevenson & Weimer, 2007
<i>R. albus</i>	gttaacagaggggaagcaaagca	tgcagcctacaatccgaactaa	Stevenson & Weimer, 2007
<i>R. flavefaciens</i>	tggcggacgggtgagtaa	ttaccatccgttccagaagct	Stevenson & Weimer, 2007
Total archaea	ccggagatggaacctgagac	cggcttgcccagctcttattc	Zhou et al., 2010

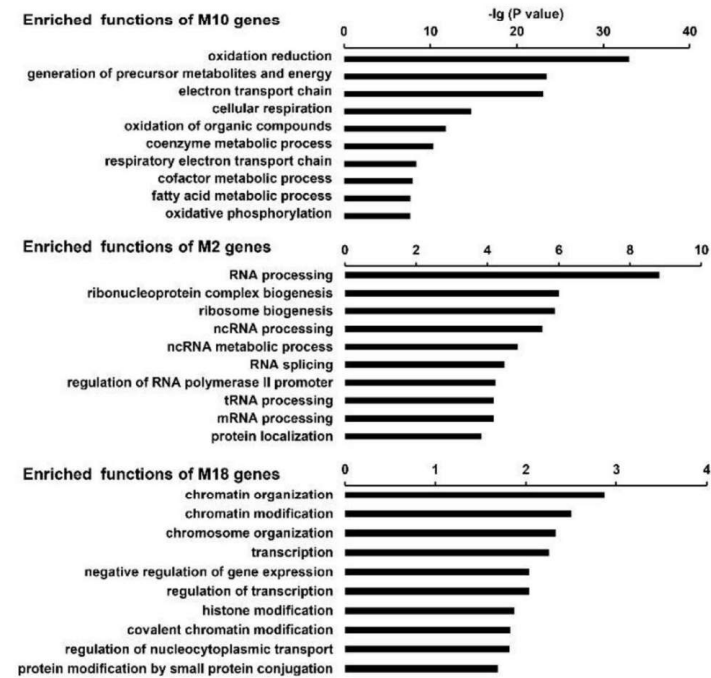


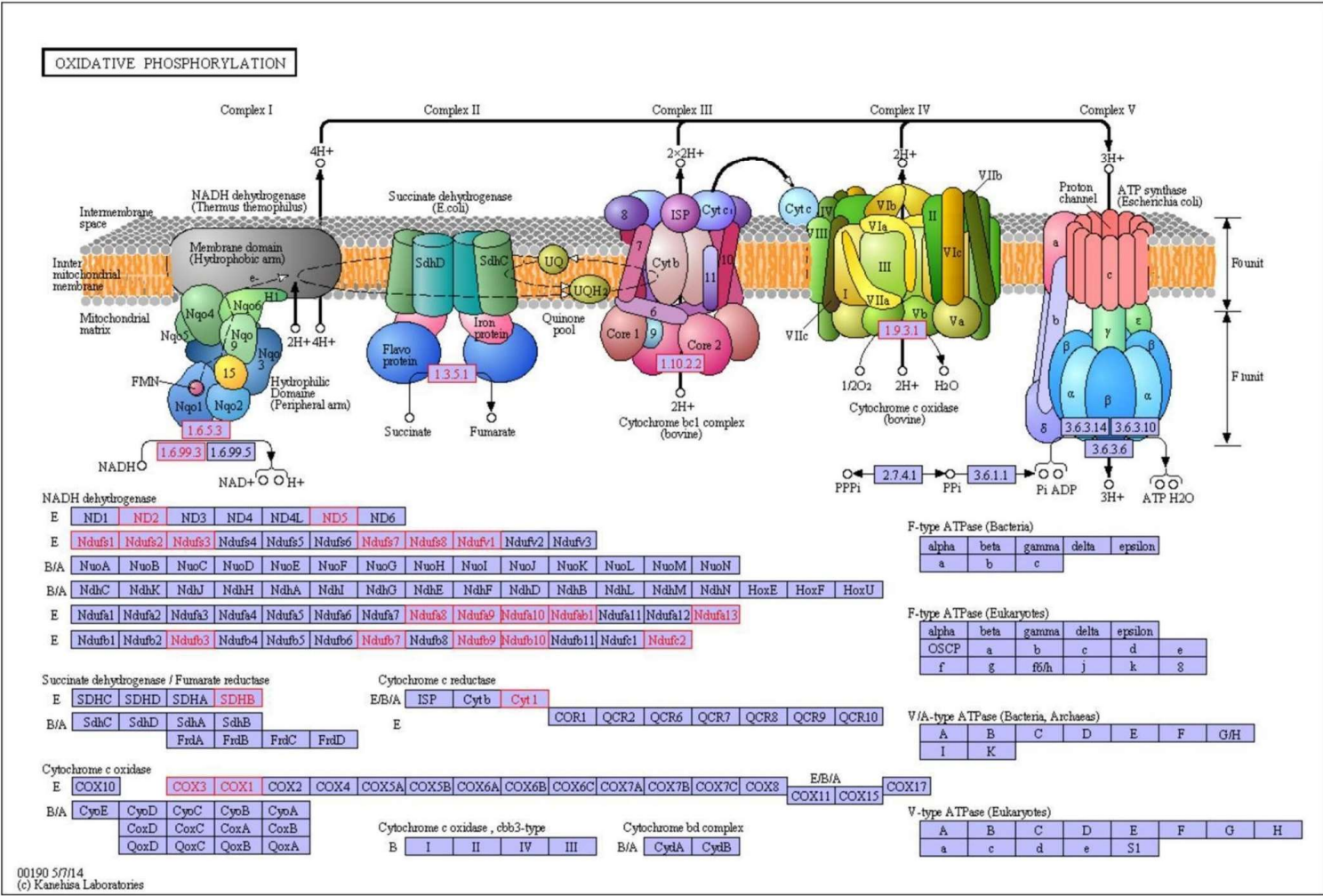
Malmuthuge et al., Fig. S1

A

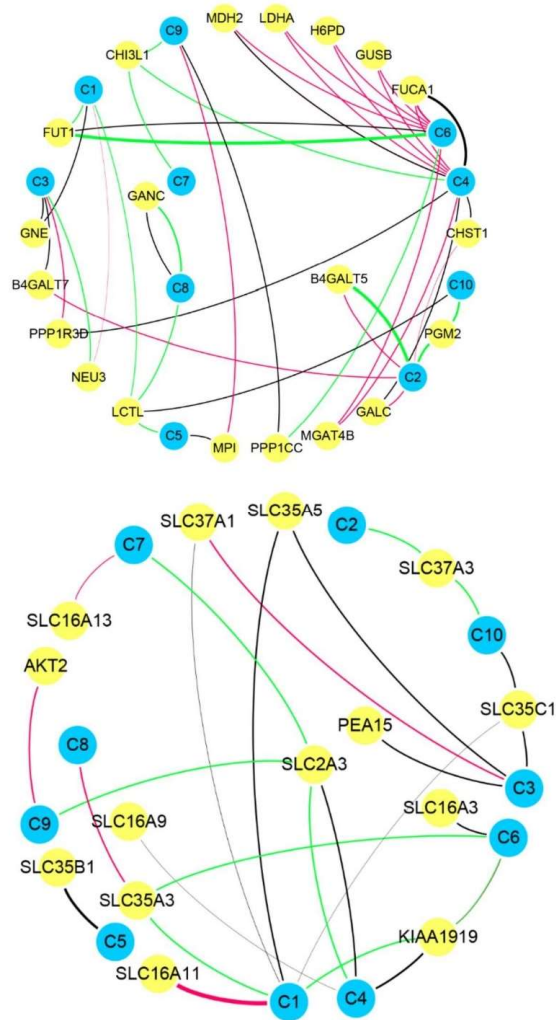


B

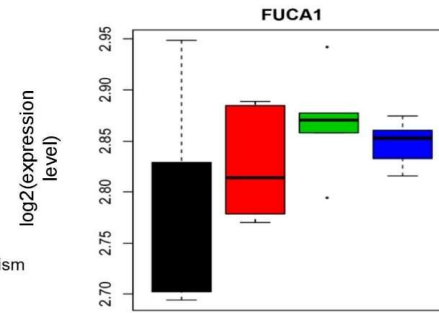




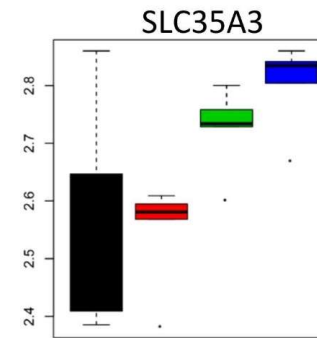
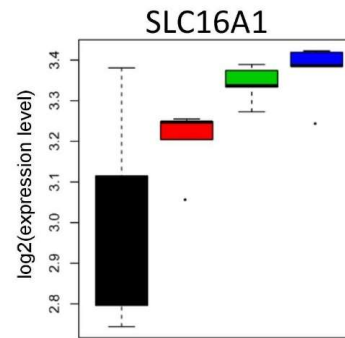
Malmuthuge et al., Fig. S3



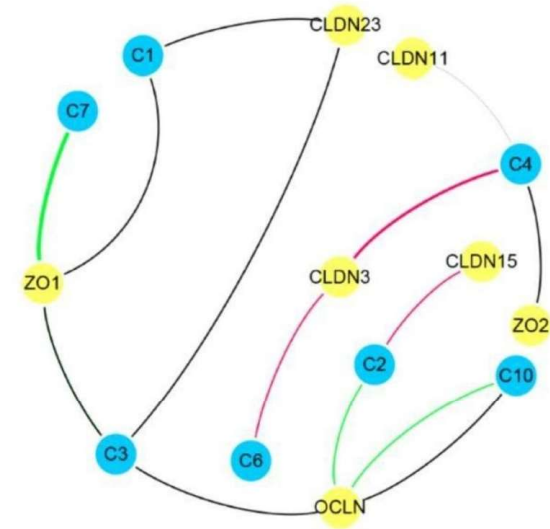
- Host genes
- Level 2 functions
- C1: Aminosugars
- C2: Carbohydrates
- C3: Central carbohydrate metabolism
- C4: CO₂ fixation
- C5: Di- and oligosaccharides
- C6: Monosaccharides
- C7: One-carbon Metabolism
- C8: Organic acids
- C9: Polysaccharides
- C10: Sugar alcohols
- 1-week
- 3-week
- 6-week



- 0D
- 1W
- 3W
- 6W



Malmuthuge et al., Fig. S4



● Host genes

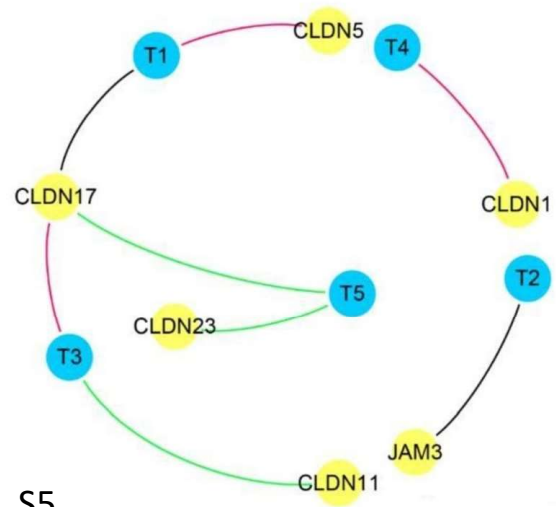
● Level 2 functions

- C1: Aminosugars
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- C3: Central carbohydrate metabolism
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- C5: Di- and oligosaccharides
- C6: Monosaccharides
- C7: One-carbon Metabolism
- C8: Organic acids
- C9: Polysaccharides
- C10: Sugar alcohols
- T1: Gram-Negative cell wall components
- T2: Heat shock
- T3: Regulation and Cell signaling
- T4: Ribosome-related cluster
- T5: Transposable elements

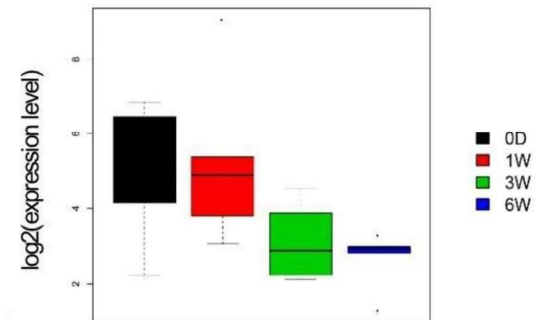
— 1-week

— 3-week

— 6-week



CLDN23



Malmuthuge et al., Fig. S5