**Supplementary information for:**

# Characterization of porcine milk oligosaccharides over lactation between primiparous and multiparous female pigs

AUTHORS

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**Details for Structural characterization and quantification of PMOs by HPLC-ESI-MS/MS**

Main text figure 1 is to show the details of identification of specific oligosaccharide structures. All twin isotope peaks in the spectra were further analyzed using the "GlycoWorkBench" software (version 2.1, https://code.google.com/archive/p/glycoworkbench/).

Structural analyses and annotation of the chemical structures of the PMOs were carried out as shown in Figure 1E by analysing the MS spectrum (top panel), HPLC chromatographic profile (middle panel) and the MS/MS spectrum (bottom panels) for each candidate PMOs peak observed in the mass spectra. For example, the MS spectrum and LC chromatographic profile for each PMOs peak, e.g. F566 (m/z 566.17), was examined as shown in the flow diagram (Fig. 1E). Isomers identified as twin isotope peaks were differentiated based on their retention times (Fig. 1E). The MS/MS profile for each isoform of the candidate PMOs peaks were then analyzed. Each sialic acid or neutral isomer was then annotated with the "GlycoWorkBench" software. The composition and proposed structures of the PMOs confirmed by both isotopic labelled twin peaks and MS/MS profile analyses during course of lactation are designated in Table 1 as either "known" or "unknown" PMO structures.

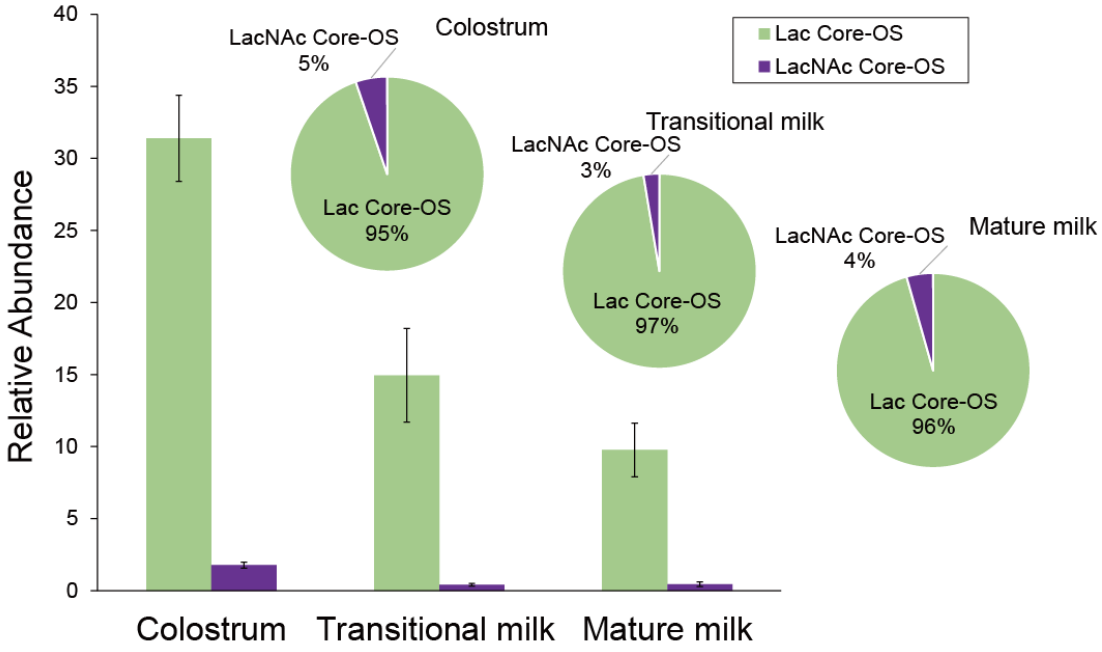


**Figure S1**. Change of Lac-Core- and LacNAc-Core PMOs during the developmental stages of lactation. Inserted triangular pie structures in the diagram show the ratio of both core PMOs in total PMOs during each stage of lactation.

**Supplementary Table 1.** Number of Lac Core- and LacNAc Core- OS structures through lactation

| | Number of OS Structures | | |
|---|---|---|---|
| | Colostrumm | Transitional milk | Mature milk |
| Lac Core-OS | 38 | 35 | 30 |
| LacNAc Core-OS | 17 | 18 | 17 |

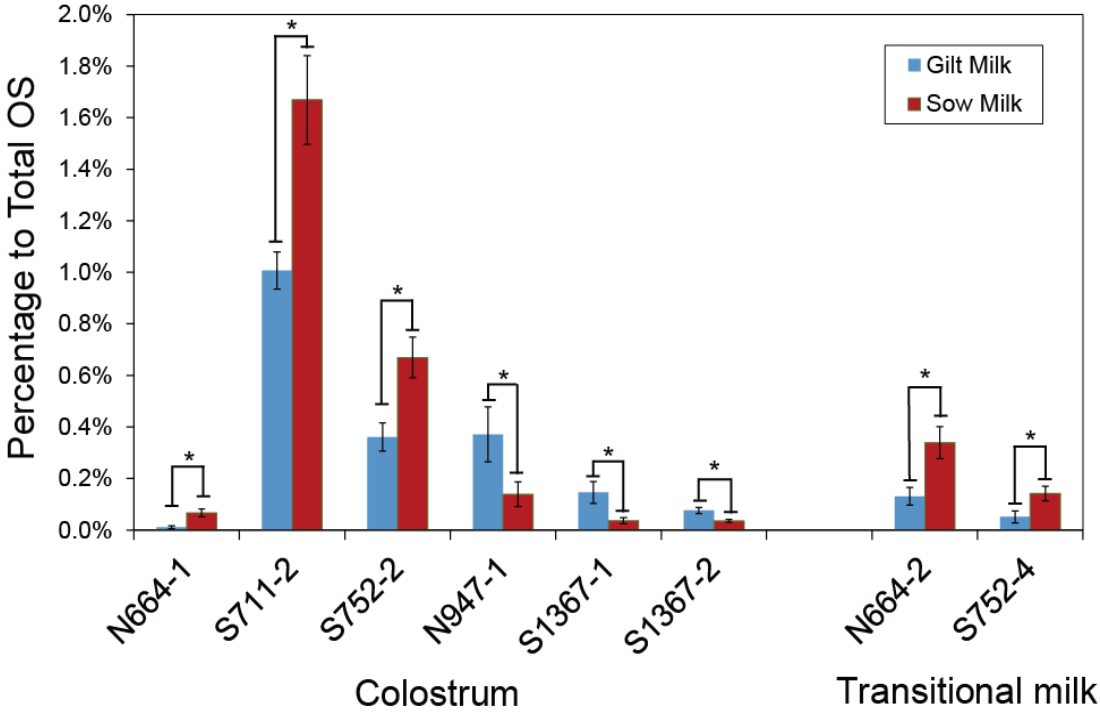


**Figure S2**. PMO structures with significantly different abundance between gilt and sow in samples of colostrum and transitional milk. Asterisk represents statistical significance, P<0.05.

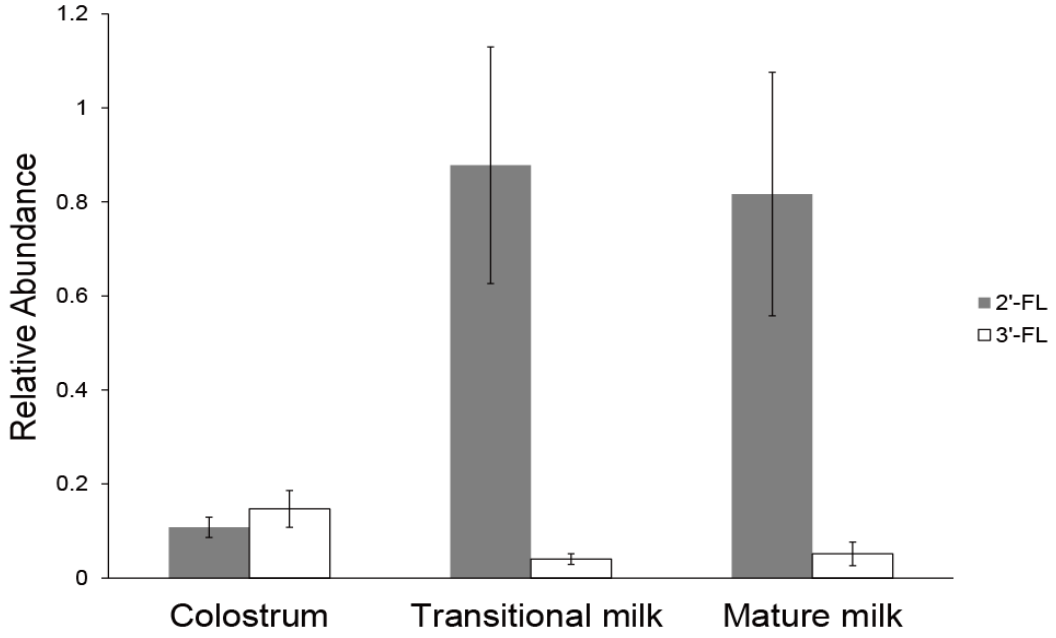


**Figure S3**. Change in relative abundance of 2'-FL (F566-1) and 3'-FL (F566-2) through lactation.

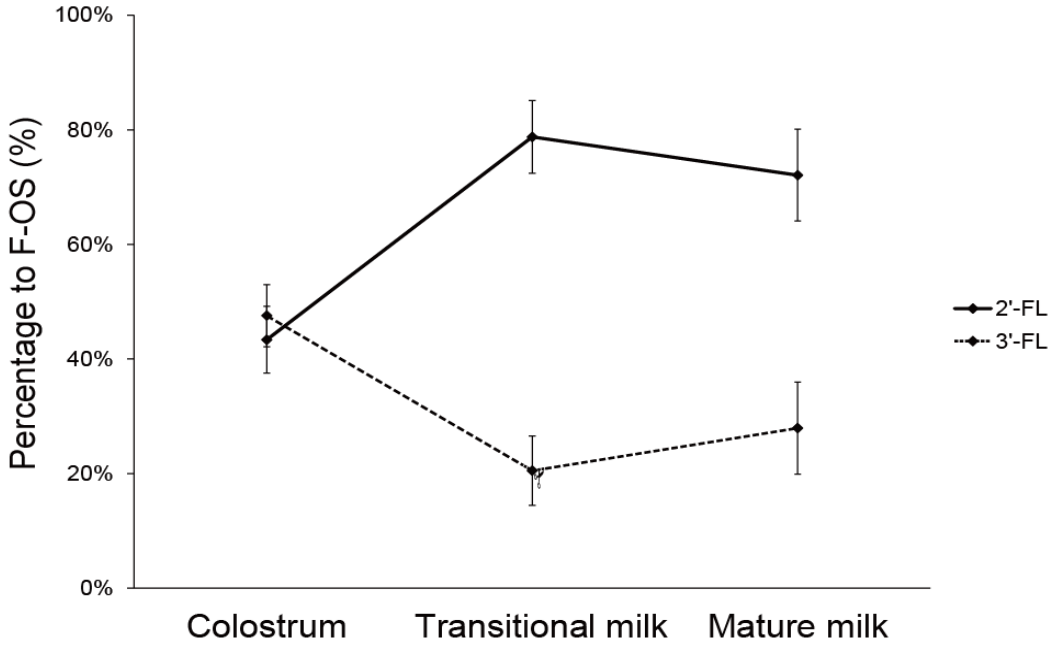


**Figure S4**. Change in percentage to total F-OS of 2'-FL (F566-1) and 3'-FL (F566-2) through lactation.