

Table S1. MALDI-TOF signals provided by the predictive model generation. Effect of the mother condition (NW vs OW/Ob).

| Algorithm                 | m/z      | weight |
|---------------------------|----------|--------|
| Quick classifier          | 4322.43  | 1.895  |
| Supervised neural network | 4041.4   | 0.123  |
|                           | 14281.32 | 0.103  |
|                           | 4162.71  | 0.097  |
|                           | 10516.64 | 0.089  |
|                           | 8131.44  | 0.066  |
|                           | 8838.71  | 0.058  |
|                           | 9127.24  | 0.052  |
|                           | 15203.42 | 0.039  |
|                           | 5867.56  | 0.038  |
|                           | 6294.35  | 0.032  |
|                           | 7144.01  | 0.028  |
|                           | 8558.49  | 0.025  |
|                           | 11301.63 | 0.025  |
|                           | 8420.52  | 0.024  |
|                           | 8459.07  | 0.024  |
|                           | 8245.42  | 0.021  |
|                           | 14488.11 | 0.021  |
|                           | 26114.9  | 0.017  |
| Genetic algorithm         | 4162.71  | 0.687  |
|                           | 8838.71  | 1.035  |
|                           | 10308.53 | 0.432  |
|                           | 11960.16 | 0.440  |
|                           | 6021.39  | 0.129  |
|                           | 4162.71  | 0.687  |

Table S2. Identified peptides matching sequences previously observed in the gastrointestinal content of newborns or a rat pup model after human milk consumption.

| Sequence             | Range <sup>1</sup> | Protein <sup>2</sup> | <i>In vivo</i> occurrence  |
|----------------------|--------------------|----------------------|--|
| RETIESL              | 1 - 7              | CASB_HUMAN           | Nielsen et al (2018)   |
| VKHEDQQQGEDEHQDK     | 24 - 39            | CASB_HUMAN           | Nielsen et al (2018)   |
| VKHEDQQQGEDEHQDKIYPS | 24 - 43            | CASB_HUMAN           | Nielsen et al (2018)   |
| HEDQQQGEDEHQDK       | 26 - 39            | CASB_HUMAN           | Nielsen et al (2018)   |
| GEDEHQDKIYP          | 32 - 42            | CASB_HUMAN           | Nielsen et al (2018)   |
| VEPIPY               | 54 - 59            | CASB_HUMAN           | Wada et al (2014)  |
| GFLPQNILP            | 60 - 68            | CASB_HUMAN           | Su et al (2017), Nielsen et al (2018)  |
| AQPAVVLVPVPQPE       | 70 - 82            | CASB_HUMAN           | Wada et al (2017)  |
| QPAVVLVPVPQPE        | 71 - 82            | CASB_HUMAN           | Wada et al (2017)  |
| QPAVVLVPVPQPEIMEVPK  | 71 - 88            | CASB_HUMAN           | Dallas et al (2014), Nielsen et al (2018).   |
| VVLPVPQP             | 74 - 81            | CASB_HUMAN           | Nielsen et al (2018)   |
| VVLPVPQPE            | 74 - 82            | CASB_HUMAN           | Nielsen et al (2018), Wada et al (2017)  |
| VVLPVPQPEIM          | 74 - 84            | CASB_HUMAN           | Dallas et al (2014), Nielsen et al (2018), Wada et al (2017)                       |
| VVLPVPQPEIMEVPK      | 74 - 88            | CASB_HUMAN           | Nielsen et al (2018), Wada et al (2017)  |
| VVLPVPQPEI           | 74 - 83            | CASB_HUMAN           | Dallas et al (2014), Nielsen et al (2018), Wada et al (2017)                       |
| LPVPQPE              | 76 - 82            | CASB_HUMAN           | Wada et al (2017)  |
| LPVPQPEIM            | 76 - 84            | CASB_HUMAN           | Dallas et al (2014), Nielsen et al (2018)  |
| LPVPQPEIMEVPK        | 76 - 88            | CASB_HUMAN           | Dallas et al (2014), Nielsen et al (2018), Dingess et al (2017), Wada et al (2017) |
| PVPQPEIM             | 77 - 84            | CASB_HUMAN           | Dallas et al (2014), Nielsen et al (2018)  |
| PQPEIMEVPK           | 79 - 88            | CASB_HUMAN           | Nielsen et al (2018)   |
| PEIMEVPK             | 81 - 88            | CASB_HUMAN           | Nielsen et al (2018)   |
| MEVPK                | 84 - 88            | CASB_HUMAN           | Wada et al (2017)  |
| MPVLKSPTIPFFD        | 100 - 112          | CASB_HUMAN           | Nielsen et al (2018)   |
| VLKSPTIPFFD          | 102 - 112          | CASB_HUMAN           | Nielsen et al (2018)   |

|                 |           |            |   |
|-----------------|-----------|------------|---|
| LKSPTIPF        | 103 - 110 | CASB_HUMAN | Nielsen et al (2018)  |
| SPTIPFFDPQIPK   | 105 - 117 | CASB_HUMAN | Dallas et al (2014), Su et al (2017), Nielsen et al (2018), Wada et al (2017) |
| PFFDPQIPK       | 109 - 117 | CASB_HUMAN | Nielsen et al (2018)  |
| PFFDPQIPKL      | 109 - 118 | CASB_HUMAN | Nielsen et al (2018)  |
| FDPQIPK         | 111 - 117 | CASB_HUMAN | Nielsen et al (2018), Wada et al (2017)                                       |
| DPQIPK          | 112 - 117 | CASB_HUMAN | Wada et al (2017)   |
| LTDLENLHLPLP    | 118 - 129 | CASB_HUMAN | Nielsen et al (2018), Wada et al (2017)                                       |
| LENLHLPLP       | 121 - 129 | CASB_HUMAN | Dallas et al (2014), Nielsen et al (2018), Wada et al (2017)                  |
| ENLHLPLPL       | 122 - 130 | CASB_HUMAN | Su et al (2017), Nielsen et al (2018)   |
| HLPLP           | 125 - 129 | CASB_HUMAN | Wada et al (2014)   |
| MQQVPQPIPQT     | 135 - 145 | CASB_HUMAN | Dallas et al (2014), Nielsen et al (2018)                                     |
| QQVPQPIPQ       | 136 - 144 | CASB_HUMAN | Dallas et al (2014), Nielsen et al (2018)                                     |
| QQVPQPIPQT      | 136 - 145 | CASB_HUMAN | Dallas et al (2014), Su et al (2017), Nielsen et al (2018)                    |
| VPQPIPQT        | 138 - 145 | CASB_HUMAN | Dallas et al (2014)   |
| LPPQPL          | 148 - 153 | CASB_HUMAN | Wada et al (2017)   |
| LPPQPLWSVP      | 148 - 157 | CASB_HUMAN | Wada et al (2017)   |
| WSVPQPK         | 154 - 160 | CASB_HUMAN | Su et al (2017), Nielsen et al (2018), Wada et al (2014)                      |
| VPYPQ           | 169 - 173 | CASB_HUMAN | Wada et al (2014)   |
| LLNQELL         | 182 - 188 | CASB_HUMAN | Nielsen et al (2018)  |
| NQELLLNPT       | 184 - 192 | CASB_HUMAN | Nielsen et al (2018), Wada et al (2017)                                       |
| LLLNPTHQIY      | 187 - 196 | CASB_HUMAN | Dallas et al (2014), Nielsen et al (2018)                                     |
| LNPTHQIYPV      | 189 - 198 | CASB_HUMAN | Nielsen et al (2018), Wada et al (2017)                                       |
| HQIYPVTQPL      | 193 - 202 | CASB_HUMAN | Wada et al (2017)   |
| HQIYPVTQPLAPV   | 193 - 205 | CASB_HUMAN | Wada et al (2017)   |
| QIYPVTQPLAPV    | 194 - 205 | CASB_HUMAN | Wada et al (2017)   |
| YPVTQPLAPV      | 196 - 205 | CASB_HUMAN | Wada et al (2017)   |
| YPVTQPLAPVHNPIS | 196 - 210 | CASB_HUMAN | Nielsen et al (2018)  |
| PTQPLAPVH       | 197 - 206 | CASB_HUMAN | Dallas et al (2014), Nielsen et al (2018)                                     |

|               |           |             |  |
|---------------|-----------|-------------|--|
| APVHNPI       | 203 - 209 | CASB_HUMAN  | Wada et al (2017)  |
| CAVSQPEAT     | 9 - 17    | TRFL_HUMAN  | Wada et al (2017)  |
| GPPVSC        | 31 - 36   | TRFL_HUMAN  | Wada et al (2017)  |
| FPNLC         | 166 - 170 | TRFL_HUMAN  | Wada et al (2017)  |
| EDLSDEAERDE   | 216 - 226 | TRFL_HUMAN  | Dallas et al (2014), Su et al (2017), Nielsen et al (2018) |
| ELLCPDNT      | 228 - 235 | TRFL_HUMAN  | Wada et al (2017)  |
| FKDSAIGF      | 300 - 307 | TRFL_HUMAN  | Dallas et al (2014), Su et al (2017), Nielsen et al (2018) |
| CVDRPVEG      | 427 - 434 | TRFL_HUMAN  | Wada et al (2017)  |
| CIGDEQGEN     | 507 - 515 | TRFL_HUMAN  | Wada et al (2017)  |
| CLAENAGDVA    | 534 - 543 | TRFL_HUMAN  | Wada et al (2017)  |
| NGSDCPDKF     | 623 - 631 | TRFL_HUMAN  | Wada et al (2017)  |
| NGSDCPDKFC    | 623 - 632 | TRFL_HUMAN  | Wada et al (2017)  |
| TKCELSQ       | 4 - 10    | LALBA_HUMAN | Wada et al (2017)  |
| KDIDGYGGIAL   | 13 - 23   | LALBA_HUMAN | Dallas et al (2014)  |
| GGIALPELI     | 19 - 27   | LALBA_HUMAN | Wada et al (2017)  |
| DTQAIVENNESTE | 37 - 49   | LALBA_HUMAN | Nielsen et al (2018)                                       |
| AIVENNESTEY   | 40 - 50   | LALBA_HUMAN | Dallas et al (2014). Nielsen et al (2018)                  |
| AIVENNESTEYG  | 40 - 51   | LALBA_HUMAN | Nielsen et al (2018)                                       |
| IVENNESTEY    | 41 - 50   | LALBA_HUMAN | Dallas et al (2014), Su et al (2017), Nielsen et al (2018) |
| IVENNESTEYG   | 41 - 51   | LALBA_HUMAN | Su et al (2017), Nielsen et al (2018)                      |
| GLFQISNK      | 51 - 58   | LALBA_HUMAN | Nielsen et al (2018)                                       |
| NICDISCD      | 71 - 78   | LALBA_HUMAN | Wada et al (2017)  |
| NICDISCDK     | 71 - 79   | LALBA_HUMAN | Wada et al (2017)  |
| CDISCDK       | 73 - 79   | LALBA_HUMAN | Wada et al (2017)  |
| DDITDDIM      | 82 - 90   | LALBA_HUMAN | Wada et al (2017)  |
| ALCTEKLEQ     | 109 - 117 | LALBA_HUMAN | Wada et al (2017)  |
| CTEKLEQ       | 111 - 117 | LALBA_HUMAN | Wada et al (2017)  |
| CTEKLEQWL     | 111 - 119 | LALBA_HUMAN | Wada et al (2017)  |

|               |           |             |                      |
|---------------|-----------|-------------|----------------------|
| TEKLEQW       | 112 - 118 | LALBA_HUMAN | Wada et al (2017)    |
| LQNPSSESEPIPL | 12 - 24   | CASA1_HUMAN | Nielsen et al (2018) |
| CAEQFC        | 84 - 89   | CASA1_HUMAN | Wada et al (2017)    |
| SHVQVPFQLL    | 115 - 124 | CASA1_HUMAN | Nielsen et al (2018) |
| GGFVEGVNK     | 10 - 18   | CEL_HUMAN   | Nielsen et al (2018) |
| ASIDMPAINK    | 325 - 334 | CEL_HUMAN   | Nielsen et al (2018) |
| EDITSHME      | 171 - 178 | OSTP_HUMAN  | Nielsen et al (2018) |
| ELDSASSEVN    | 289 - 298 | OSTP_HUMAN  | Nielsen et al (2018) |
| NNPYVPR       | 52 - 58   | CASK_HUMAN  | Wada et al (2017)    |
| LPNSHPPT      | 79 - 86   | CASK_HUMAN  | Wada et al (2017)    |

<sup>1</sup>Without signal peptide

<sup>2</sup>Uniprot entry name

## References

Dallas, D.C.; Guerrero, A.; Khaldi, N.; Borghese, R.; Bhandari, A.; Underwood, M.A.; Lebrilla, C.B.; German, J.B.; Barile, D. A Peptidomic Analysis of Human Milk Digestion in the Infant Stomach Reveals Protein-Specific Degradation Patterns. *J. Nutr.* 2014, **144**, 815–820, doi:10.3945/jn.113.185793.

Nielsen, S.D.; Beverly, R.L.; Underwood, M.A.; Dallas, D.C. Release of functional peptides from mother's milk and fortifier proteins in the premature infant stomach. *PLoS One* 2018, **13**, e0208204, doi:10.1371/journal.pone.0208204.

Beverly, R.L.; Woonnimani, P.; Scottoline, B.P.; Lueangsakulthai, J.; Dallas, D.C. Peptides from the Intestinal Tract of Breast Milk-Fed Infants Have Antimicrobial and Bifidogenic Activity. *Int. J. Mol. Sci.* 2021, **22**, 2377, doi:10.3390/ijms22052377.

Wada, Y.; Phinney, B.S.; Weber, D.; Lönnadal, B. In vivo digestomics of milk proteins in human milk and infant formula using a suckling rat pup model. *Peptides* 2017, **88**, 18–31, doi:10.1016/j.peptides.2016.11.012.

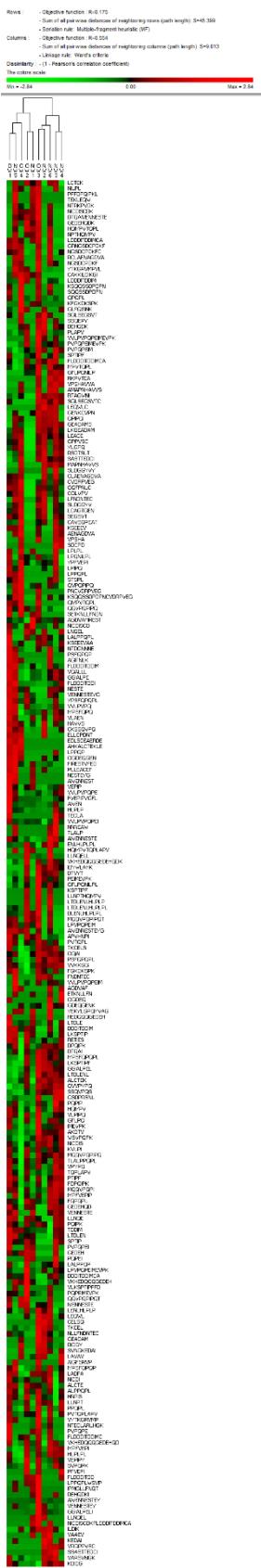


Figure S1. Complete heat map representation of the hierarchical clustering analysis of peptides identified after in vitro digestion of breast milk. Columns correspond to subjects included in the overweight/obese group or normal weight group. Rows correspond to intensity of sequences derived from  $\beta$ -casein,  $\alpha$ -lactalbumin and lactoferrin identified in at least 3 subjects.