Supplemental Tables and Figures:

Supplemental Table 1: Details of parent proteins from which peptides were derived

Paret Protein names	**Gene name**	**Peptides (n)**	**Mol. Weight, Parent Protein [kDa]**	**Sequence length, Parent Protein**
β-casein	CSN2	568	25.38	226
polymeric immunoglobulin receptor	PIGR	155	83.28	764
α_{S1}-casein	CSN1S1	128	21.67	185
osteopontin	SPP1	92	35.42	314
κ-casein	CSN3	47	20.31	182
bile salt-activated lipase	CEL	42	79.32	753
clusterin	CLU	16	52.49	449
lactotransferrin	LTF	15	78.18	710
actin, cytoplasmic 1	ACTB	14	41.74	375
butyrophilin subfamily 1 member A1	BTN1A1	12	58.96	526
α-lactalbumin	LALBA	11	16.23	142
histone H1.2	HIST1H1C	2	21.37	213
thymosin β-4	TMSB4	2	5.05	44
14-3-3 protein zeta/delta	YWHAZ	2	27.75	245
protein O-linked-mannose beta-1,4-N-acetylglucosaminyltransferase 2	POMGNT2	2	66.62	580
14-3-3 protein epsilon	YWHAE	1	29.17	255

Supplemental Tabel 2: Fold change of peptide intensity by GA and LS indicated from volcano plots

	Sequence	Protein Group	Fold Change	p-value
Gestational Age	ADGSRASVDSGSSEEQGGSS	PIGR	-0.61	0.0003
	ADGSRASVDSGSSEEQGGSSRA	PIGR	-0.45	0.0001
	EESITEYKQKV	β-casein	1.25	0.0012
	ESLSSSEESITEYK	β-casein	1.20	0.0013
	ETIESLSSSEESITEY	β-casein	0.92	0.0011
	ETIESLSSSEESITEYK	β-casein	0.74	0.0014
	ETIESLSSSEESITEYKQ	β-casein	1.21	< 0.0001
	ETIESLSSSEESITEYKQK	β-casein	0.99	0.0012
	ETIESLSSSEESITEYKQKV	β-casein	1.00	0.0010
	LSSSEESITEYK	β-casein	1.06	0.0003
	PAVVLPVPQPEI	β-casein	-0.61	0.0004
	PKAKDTVYT	β-casein	-0.72	0.0009
	RETIESLSSSEESITEYKQK	β-casein	1.08	0.0048
	SEESITEYK	β-casein	1.04	0.0001
	SLSSSEESITE	β-casein	0.83	0.0003
	SLSSSEESITEYK	β-casein	0.88	0.0002
	SSEESITEYK	β-casein	1.17	0.0034
	SSSEESITEYK	β-casein	0.69	0.0011
	SSSEESITEYKQKV	β-casein	0.94	0.0007
	GAKTTFDVYTESWAQDPSQENK	bile salt-activated lipase	0.85	0.0031
Lactational Stage				
	ADTRDQADGSRASVD	PIGR	-0.71	0.0007
	DSGSSEEQGGSSRALVSTLV	PIGR	-1.14	0.0001
	VDSGSSEEQGGSSRA	PIGR	-0.76	0.0020
	AKDTVYTKGRVM	β-casein	-0.65	0.0006
	EESITEYK	β-casein	0.97	< 0.0001
	ESLSSSEESITEYK	β-casein	1.39	0.0006
	ETIESLSSSEESITEY	β-casein	1.21	0.0000
	ETIESLSSSEESITEYKQ	β-casein	1.03	0.0024
	ETIESLSSSEESITEYKQK	β-casein	1.33	< 0.0001
	IESLSSSEESITEYK	β-casein	1.19	0.0007
	IMEVPKAKDTVYT	β-casein	0.74	0.0005
	LLNPTHQIYPVTQPLAP	β-casein	-1.36	< 0.0001
	LLNPTHQIYPVTQPLAPVHNPIS	β-casein	-0.97	< 0.0001
	LLNPTHQIYPVTQPLAPVHNPISV	β-casein	-0.80	0.0013
	LPIPQQVVPYP	β-casein	0.83	0.0025
	LPVPQPEIMEVPK	β-casein	0.68	0.0005

	MEVPKAKDTVYTKG	β-casein	-0.80	0.0006
	PLMQQVPQPIPQTL	β-casein	-0.61	0.0004
	RETIESLSSSEESITEYK	β-casein	1.59	0.0035
	SEESITEYK	β-casein	1.15	0.0001
	SLSSSEESITE	β-casein	0.81	0.0017
	SLSSSEESITEY	β-casein	0.75	0.0007
	SLSSSEESITEYK	β-casein	1.15	< 0.0001
	SLSSSEESITEYKQKV	β-casein	0.77	0.0005
	SSSEESITEYK	β-casein	0.87	0.0001
	TEYKQKVEKVKHED	β-casein	-0.85	< 0.0001
	VLPIPQQVVPYPQR	β-casein	0.80	0.0013
	VPQPEIMEVPK	β-casein	0.84	< 0.0001
	IPQRQYLPNSHPPTVV	κ-casein	-0.94	0.0014
	NEYNQLQLQAAHAQEQIR	α-S1-casein	1.22	0.0003

Positive values indicate an upregulation and negative values indicate a downregulation in fold change peptide intensity. Bold peptide sequences were significant upregulated in both GA and LS groups.

Supplemental Table 3: Differing peptide sequences by GA, LS and the interaction of GA and LS

Group	**Sequence**	**Protein Group**	**ANOVA p-value**
Preterm vs Term Gestational Age	ETIESLSSSEESITEYKQ	β-casein	< 0.0001
	ADGSRASVDSGSSEEQGGSSRA	PIGR	0.0001
	SEESITEYK	β-casein	0.0001
	PKAKDTVYT	β-casein	0.0002
	SLSSSEESITEYK	β-casein	0.0002
	SLSSSEESITE	β-casein	0.0002
	ADGSRASVDSGSSEEQGGSS	PIGR	0.0003
	LSSSEESITEYK	β-casein	0.0004
	ESLSSSEESITEYK	β-casein	0.0005
	SSSEESITEYKQKV	β-casein	0.0006
	ETIESLSSSEESITEYKQKV	β-casein	0.0009
	SSSEESITEYK	β-casein	0.0010
	AVADTRDQADGSRASVDSGSSEEQ	PIGR	0.0011
	SRASVDSGSSEEQGGSSRA	PIGR	0.0012
	ETIESLSSSEESITEYK	β-casein	0.0012
	AVADTRDQADGSRASVDSG	PIGR	0.0012
	ETIESLSSSEESITEYKQK	β-casein	0.0013
	RYPERLQNPSE	α_{S1}-casein	0.0018
	EESITEYKQKV	β-casein	0.0021
	GSRASVDSGSSEEQGGSSRA	PIGR	0.0025
	ETIESLSSSEESITEY	β-casein	0.0026
	NQELLLNPTHQIYPVT	β-casein	0.0027
	LSSSEESITEYKQKV	β-casein	0.0029
	SSEESITEYK	β-casein	0.0029
	EESITEYK	β-casein	0.0029
	DQADGSRASVDSGSSEEQG	PIGR	0.0031
	ADTRDQADGSRASVDSGSSEEQGGSS	PIGR	0.0036
	PQIPKLTD	β-casein	0.0038
	GAKTTFDVYTESWAQDPSQENK	bile salt-activated lipase	0.0039
	SLSSSEESITEYKQKV	β-casein	0.0040
Early vs. Late Lactational Stage	SLSSSEESITEYK	β-casein	< 0.0001
	LLNPTHQIYPVTQPLAP	β-casein	< 0.0001
	VPQPEIMEVPK	β-casein	< 0.0001
	LLNPTHQIYPVTQPLAPVHNPIS	β-casein	< 0.0001
	EESITEYK	β-casein	< 0.0001

	ETIESLSSSEESITEYKQK	β-casein	< 0.0001
	LPIPQQVVPYP	β-casein	< 0.0001
	SEESITEYK	β-casein	0.0001
	TEYKQKVEKVKHED	β-casein	0.0001
	ETIESLSSSEESITEY	β-casein	0.0001
	DSGSSEEQGGSSRALVSTLV	PIGR	0.0001
	SSSEESITEYK	β-casein	0.0001
	NEYNQLQLQAAHAQEQIR	α_{S1}-casein	0.0001
	DLENLHLP	β-casein	0.0002
	ESLSSSEESITEYK	β-casein	0.0002
	SLSSSEESITEYKQKV	β-casein	0.0002
	SLSSSEESITEY	β-casein	0.0003
	MEVPKAKDTVYTKG	β-casein	0.0004
	IMEVPKAKDTVYT	β-casein	0.0004
	LNPTHQIYPVTQ	β-casein	0.0004
	ETIESLSSSEESITEYKQ	β-casein	0.0006
	LPVPQPEIMEVPK	β-casein	0.0006
	ADTRDQADGSRASVD	PIGR	0.0008
	PLMQQVPQPIPQTL	β-casein	0.0009
	EDQQQGEDEHQDKIYPS	β-casein	0.0010
	LLNPTHQIYPVTQPLAPVHNPISV	β-casein	0.0011
	VDSGSSEEQGGSSRA	PIGR	0.0012
	AKDTVYTKGRVM	β-casein	0.0012
	VLPIPQQVVPYPQR	β-casein	0.0013
	IESLSSSEESITEYK	β-casein	0.0013
	TEYKQKVEKV	β-casein	0.0014
	SGSSEEQGGSSRAL	PIGR	0.0015
	ADTRDQADGSRASVDSGSSEEQGGSS	PIGR	0.0017
	LAQPAVVLPVPQPEIMEVPK	β-casein	0.0021
	NPTAHENYEKNNVML	α_{S1}-casein	0.0027
	ETIESLSSSEESITEYK	β-casein	0.0028
	AVADTRDQADGSRASVD	PIGR	0.0030
	IPQRQYLPNSHPPTVV	κ-casein	0.0031
	PLAPVHNPISV	β-casein	0.0031
	AVADTRDQADGSRA	PIGR	0.0032
	SLSSSEESITE	β-casein	0.0032
Interaction of GA and LS	SLSSSEESITEYK	β-casein	0.0018
	SEESITEYK	β-casein	0.0045
	LLNPTHQIYPVTQPLAPVHNPIS	β-casein	0.0052
	ETIESLSSSEESITEYKQ	β-casein	0.0085

	ETIESLSSSEESITEYKQK	β-casein	0.0088
	LLNPTHQIYPVTQPLAP	β-casein	0.0088
	EESITEYK	β-casein	0.0096
	VPQPEIMEVPK	β-casein	0.0131
	SSSEESITEYK	β-casein	0.0137
	ESLSSSEESITEYK	β-casein	0.0144
	ETIESLSSSEESITEY	β-casein	0.0160
	DLENLHLP	β-casein	0.0186
	TEYKQKVEKVKHED	β-casein	0.0211
	LPIPQQVVPYP	β-casein	0.0232
	ADGSRASVDSGSSEEQGGSSRA	PIGR	0.0246
	SLSSSEESITE	β-casein	0.0260
	PKAKDTVYT	β-casein	0.0260
	DSGSSEEQGGSSRALVSTLV	PIGR	0.0262
	SLSSSEESITEYKQKV	β-casein	0.0273
	SLSSSEESITEY	β-casein	0.0334
	DQADGSRASVDSGSSEEQGGSSRALVSTL	PIGR	0.0345
	MEVPKAKDTVYTKG	β-casein	0.0348
	NEYNQLQLQAAHAQEQIR	α_{S1}-casein	0.0360
	ADGSRASVDSGSSEEQGGSS	PIGR	0.0384
	LNPTHQIYPVTQ	β-casein	0.0403
	ETIESLSSSEESITEYK	β-casein	0.0424
	ADTRDQADGSRASVD	PIGR	0.0429
	LSSSEESITEYK	β-casein	0.0477

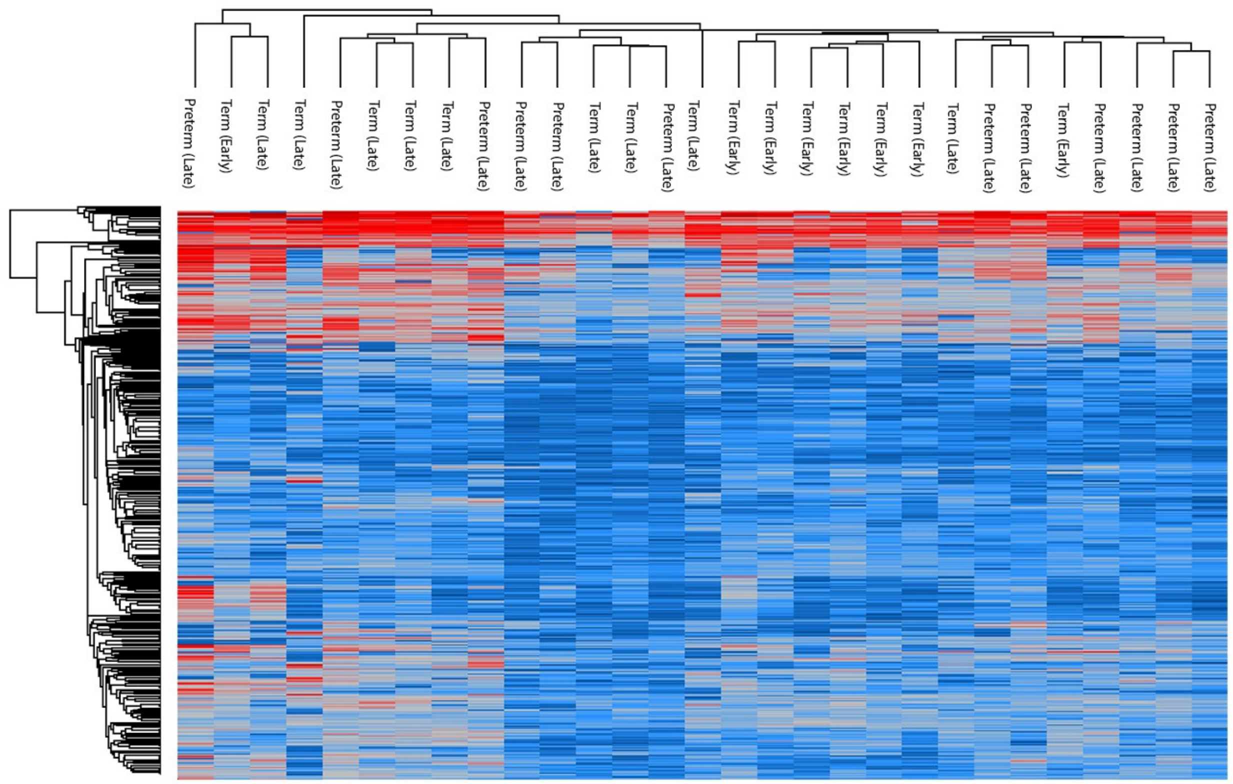
Bold peptide sequences were uniquely significant for the designated group, and were not found significant in other groups.

Supplemental Table 4: Prediction of the enzymes responsible for cleavage of human milk parent proteins, ranked by total number of cleavages

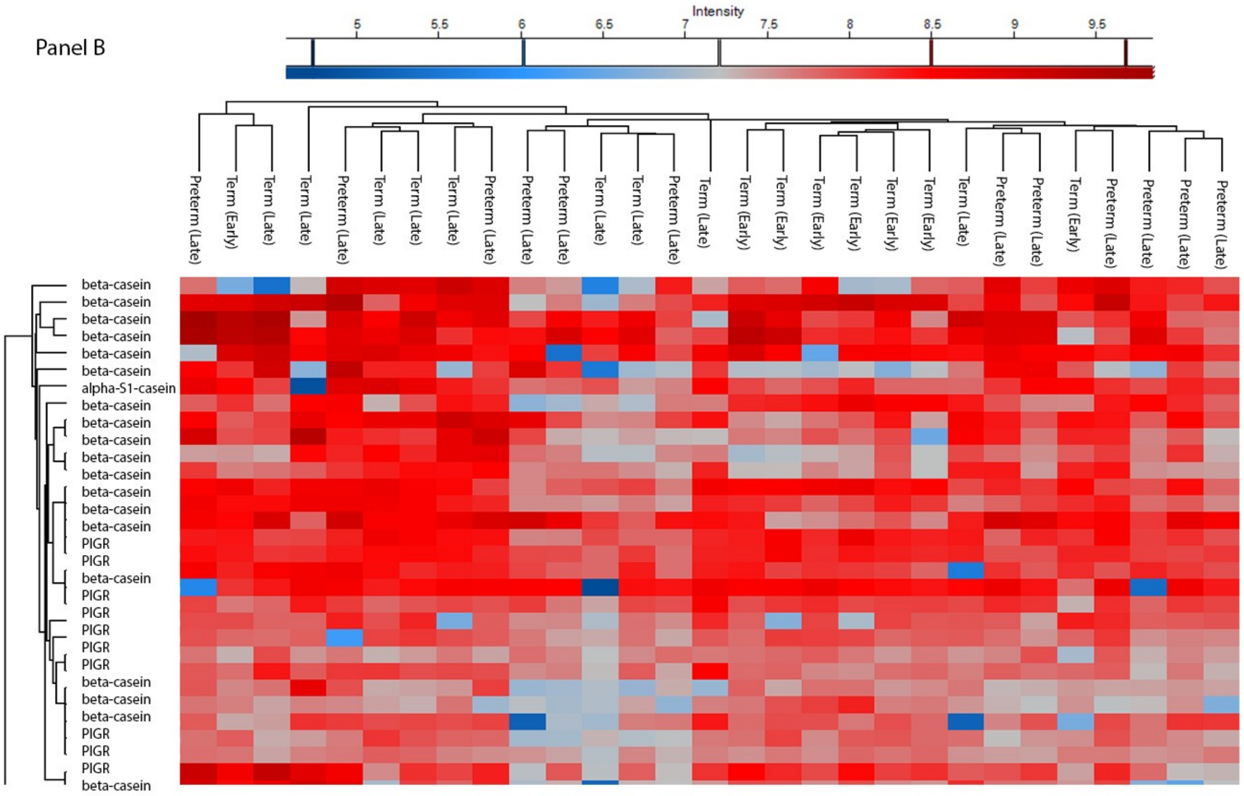
Enzymes	**N-terminal cleavage count**	**C-terminal cleavage count**	**Total cleavages**	**Unique cleavage**	**Number of expected cleavages within the peptide**	**Number of proteins cleaved**	**Odds Ratio**	**Standard Error**
trypsin 1[a]	130	89	219	0	419	7	1.96	1.09
plasmin	130	89	219	0	426	7	1.93	1.09
cathepsin D	93	88	181	0	828	6	0.76	1.09
LysC	77	64	141	0	220	6	2.38	1.12
pepsin (pH 1.3) 1	38	91	129	0	292	8	1.61	1.11
elastase	60	63	123	0	570	5	0.76	1.11
modified chymotrypsin	79	43	122	0	343	8	1.28	1.11
chymotrypsin low 1	76	43	119	0	338	8	1.27	1.12
pepsin (pH > 2) 1	34	82	116	0	209	6	2.04	1.13
Pepsin (pH 1.3) 2	50	30	80	0	262	8	1.09	1.14
AspN endopeptidase	68	11	79	0	329	7	0.85	1.14
argcproteinase	53	25	78	0	206	6	1.36	1.14
clostripain	53	25	78	0	206	6	1.36	1.14
proline endopeptidase diff	23	46	69	0	494	5	0.48	1.14
pepsin (pH > 2) 2	40	21	61	0	130	7	1.69	1.17
formic acid	19	37	56	0	351	4	0.56	1.16
staphprotease	28	24	52	0	537	6	0.33	1.16
glutamyl endopeptidase[a]	28	24	52	0	537	6	0.33	1.16
staphylococcal peptidase I	26	21	47	0	409	6	0.40	1.17
chymotrypsin high 1	21	15	36	0	128	7	1.00	1.21
chymotrypsin low 4	8	5	13	0	125	3	0.37	1.34
CNBr	9	3	12	0	68	2	0.63	1.37
cyanogen bromide	9	3	12	0	68	2	0.63	1.37
chymotrypsin low 3	8	2	10	0	47	2	0.76	1.42
proline endopeptidase	4	0	4	0	9	1	1.59	1.82
iodosobenzoic acid	3	0	3	0	5	1	2.14	2.08
iodosobenzoate	3	0	3	0	5	1	2.14	2.08
chymotrypsin low 2	3	0	3	0	5	1	2.14	2.08
chymotrypsin high 2	3	0	3	0	5	1	2.14	2.08
NTCB	0	2	2	0	0	2	17.85	4.71
thrombin 1	1	0	1	1	1	1	3.57	4.11

[a] Enzymes that do not have expression in milk, but data indicate the presence of other enzymes with similar activity

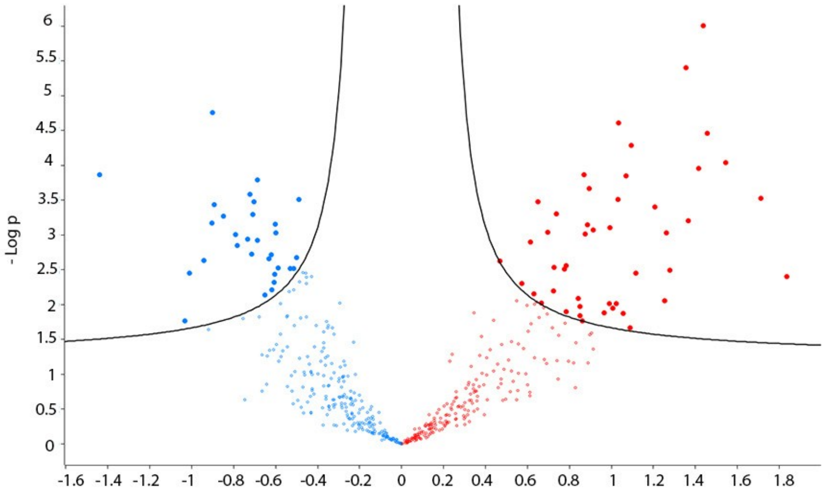
Panel A



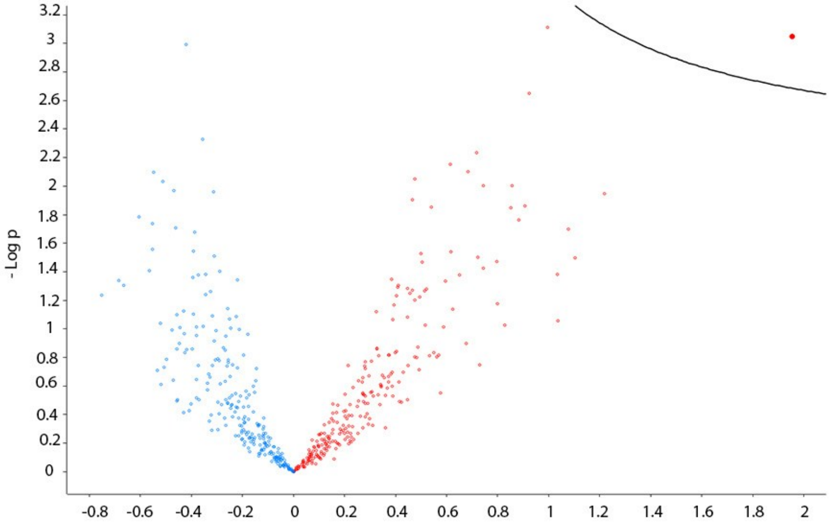
Panel B



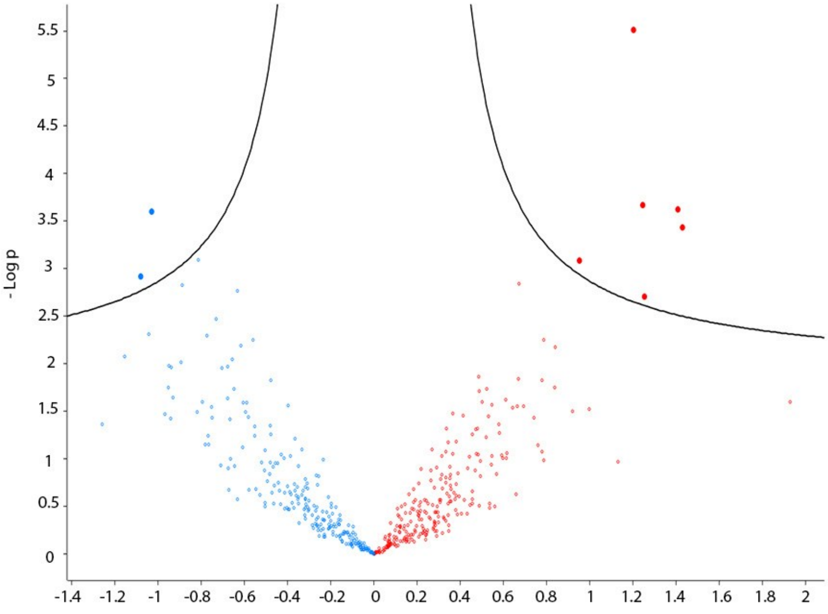
Supplemental Figure 1: Panel A: Quantitative hierarchical k-means clustering shows samples as the column tree and peptide sequences as the row tree, with lower intensities plotted as dark blue with an increasing color scale from gray to dark red as the log10 fold change of the peptide intensity increases. Panel B shows the zoomed in portion of the top of Panel A where peptide intensities were highest, the row tree indicateds the partent protein of the most intense peptides.



Panel A



Panel B



Panel C

Supplemental Figure 2**:** Volcano plots depicting log 10 fold change in peptide intensity (x axis) and -log p value (y-axis). All significant peptides are represented as filled circles and non-significant peptides are unfilled. Panel A, peptide intensity distribution between preterm late LS (blue) and term early LS milk (red). Panel B, peptide intensity distribution between preterm late LS (blue) and term late LS milk (red). Panel C, peptide intensity distribution between term early LS (blue) and term late LS milk (red).