

Supplemental Table S1: Glycoproteoforms detected between both donors across lactation for both low and high resolutions LC-MS experiments

Detected proteoform	Theoretical mass (kDa)		Experimental mass (kDa; average \pm stdev)		Retention time apex (min; average \pm stdev)
	Monoisotopic	Average	Monoisotopic	Average	
β -casein 0P	23842.82	23857.50	23843.05 \pm 0.02	23857.36 \pm 0.19	16.71 \pm 0.11
β -casein 1P	23922.79	23937.48	23923.00 \pm 0.20	23937.27 \pm 0.16	16.83 \pm 0.12
β -casein 2P	24002.75	24017.46	24002.97 \pm 0.19	24017.28 \pm 0.10	17.06 \pm 0.07
β -casein 3P	24082.72	24097.44	24082.94 \pm 0.02	24097.25 \pm 0.09	17.28 \pm 0.08
β -casein 4P	24162.69	24177.42	24162.90 \pm 0.01	24177.27 \pm 0.09	17.51 \pm 0.06
β -casein 5P	24242.65	24257.40	24242.88 \pm 0.01	24257.22 \pm 0.08	17.81 \pm 0.05
β -casein 0P N ₁	24045.90	24060.69	24047.00 \pm 0.03	24062.20 \pm 0.29	17.12 \pm 0.09
β -casein 0P N ₁ H ₁	24207.95	24222.84	24208.25 \pm 0.47	24223.32 \pm 0.62	17.51 \pm 0.19
β -casein 1P N ₁	24125.87	24140.67	24126.42 \pm 0.73	not detected	17.33 \pm 0.05
β -casein 1P N ₁ H ₁	24287.92	24302.82	24288.31 \pm 0.50	24302.97 \pm 0.57	17.72 \pm 0.42
β -casein 2P N ₁	24205.83	24220.65	24205.03 \pm 0.34	not detected	17.53 \pm 0.08
β -casein 2P N ₁ H ₁	24367.89	24382.80	24368.69 \pm 0.60	24383.22 \pm 0.45	16.57 \pm 0.14
β -casein 3P N ₁	24285.80	24300.63	24284.89 \pm 0.01	not detected	17.88 \pm 0.00
β -casein 3P N ₁ H ₁	24447.85	24462.78	not detected	24464.17 \pm 0.01	16.52 \pm 0.21
β -casein 4P N ₁ H ₁	24527.82	24542.76	not detected	24542.32 \pm 0.73	17.01 \pm 0.12
β -casein 4P N ₂ H ₂	24892.95	24908.09	not detected	24907.34 \pm 0.08	16.96 \pm 0.00

*P is phosphorylation, H = hexose, N = N-acetylhexosamine

Supplemental Table S2: Curation criteria used to determine proper score settings reducing the FDR

Filtering conditions	Proteome derived data				Peptidome derived data			
	NM* PSMs	Glyco PSMs	False NM PSMs	False Glyco PSMs	NM PSMs	Glyco PSMs	False NM PSMs	False Glyco PSMs
None	73,278	18,488	11,839	6,783	86,174	33,747	6,701	5,864
Percent of total	25		16	37	39		8	17
First AA > 15	71,747	17,285	10,943	6,274	79,612	27,235	6,230	5,448
Percent of total	24		15	36	34		8	20
Score \geq 150	50,047	61,89	310	26	58,367	10,444	87	32
Percent of total	12		0.6	0.4	18		0.1	0.3
Score + log probability \geq 1.5	49,091	5,895	100	0	57,490	99,50	14	7
Percent of total	12		0.2	-	17		0.02	0.07
Score + log probability + Delta modification \geq 5	44,667	2,286	97	0	48,295	2,241	6	1
Percent of total	5		0.2	-	5		0.01	0.04

*Non-modified (NM)

Supplemental Table S3: PSM counts of phospho- and glycosite modifications by donor and time in the peptidome and proteome derived data

Donor one peptidome derived data												
Peptidome Phosphorylation	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18	21	42	78	43	49	70	80	50	75	508	56
	Ser21	405	278	460	319	345	398	429	405	428	3467	385
	Ser23	521	347	544	381	440	513	581	574	577	4478	498
	Ser24	607	462	664	526	568	708	783	826	826	5970	663
	Ser25	610	477	656	512	594	681	788	828	839	5985	665
	Ser28		4	1	2	4	2	7	4	5	29	4
	Thr30		1								1	1
	Thr207	11	8			9	3	1			32	6
	Thr214	1	5			3					9	3
	Ser225										0	0
	*Sum	2164	1606	2402	1781	1996	2370	2661	2683	2745		
	*Average	433	321	480	356	399	474	532	537	549		
Peptidome Glycosylation	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18			1			3	1	2	2	9	2
	Ser21	1		3		4	7	1	4	8	28	4
	Ser23		1	3	1	2	5	3	6	8	29	4
	Ser24		1	2	1	1		4	4	7	20	3
	Ser25			2			1	3	6	8	20	4
	Ser28	4	1	1	2	1	3	9	5	14	40	4
	Thr30	5	2	1	2	1	2	8	6	11	38	4
	Thr207	149	103	20	17	171	23	39	46	51	619	69
	Thr214	182	166	46	34	217	37	50	57	63	852	95
	Ser225	31	29	7	5	31	6	5	9	11	134	15
	*Sum	362	298	73	56	419	66	94	112	125		
	*Average	121	99	24	19	140	22	31	37	42		
Peptidome non-modified	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18	583	444	590	486	587	698	765	864	836	5853	650
	Ser21	215	229	233	244	330	411	476	575	544	3257	362
	Ser23	106	187	163	205	277	323	364	440	449	2514	279
	Ser24	25	79	46	64	159	141	171	201	216	1102	122
	Ser25	26	72	59	89	142	176	180	207	223	1174	130
	Ser28	639	553	720	612	738	871	974	1052	1067	7226	803
	Thr30	639	555	724	615	744	875	984	1057	1075	7268	808
	Thr207	765	733	259	203	721	228	247	286	381	3823	425
	Thr214	727	653	270	226	660	249	267	318	403	3773	419
	Ser225	737	688	312	253	709	279	309	342	424	4053	450
	Sum	6987	6124	4097	3555	7139	4859	5630	6319	7167		
	Average	388	340	228	198	397	270	313	351	398		

* The Indicated Sum and Average were calculated using on sites that showed evidence of the respective PTM. For phosphosites, only Thr18-Ser25 were considered; for *O*-glycosylation, only sites Thr207-Ser225 were considered. Serine (Ser), Threonine (Thr), weeks (W)

Donor one proteome derived data												
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome Phosphorylation	Thr18				3	1					4	2
	Ser21	15	3	67	81	6		23	7	12	214	27
	Ser23	50	51	140	150	47	42	78	76	82	716	80
	Ser24	103	125	253	287	187	138	221	235	226	1775	197
	Ser25	93	117	257	279	186	140	222	233	228	1755	195
	Ser28								1		1	1
	Thr30										0	0
	Thr207	1	6	1	5			3	2	1	19	3
	Thr214		1	1							2	1
	Ser225		1	1	2		1		2	2	9	2
	*Sum	261	296	717	800	427	320	544	551	548		
	*Average	261	74	179	160	85	107	136	138	137		
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome Glycosylation	Thr18			1							1	1
	Ser21			2							2	2
	Ser23			2							2	2
	Ser24			2	2	1	1	1	2	1	10	1
	Ser25			1	2	1	1	1	2	1	9	1
	Ser28			1					1		2	1
	Thr30			1					1		2	1
	Thr207	55	66	88	119	74	96	99	105	111	813	90
	Thr214	100	103	115	148	88	141	125	124	138	1082	120
	Ser225	9	16	13	24	14	23	14	18	26	157	17
	*Sum	164	185	216	291	176	260	238	247	275		
	*Average	55	62	72	97	59	87	79	82	92		
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome non-modified	Thr18	100	129	264	303	203	149	234	272	266	1920	213
	Ser21	99	143	218	250	216	166	238	285	271	1886	210
	Ser23	67	101	151	192	184	134	189	226	211	1455	162
	Ser24	16	27	39	55	44	39	46	68	69	403	45
	Ser25	26	36	38	65	45	37	46	72	69	434	48
	Ser28	124	157	298	348	236	181	274	308	301	2227	247
	Thr30	124	157	298	348	236	181	274	309	301	2228	248
	Thr207	573	735	755	808	505	679	593	656	668	5972	664
	Thr214	494	655	697	760	414	611	526	589	607	5353	595
	Ser225	493	618	712	788	346	647	559	609	635	5407	601
	Sum	3212	4268	5193	5655	3484	4183	4162	5029	4903		
	Average	178	237	289	314	194	232	231	279	272		

* The Indicated Sum and Average were calculated using on sites that showed evidence of the respective PTM. For phosphosites, only Thr18-Ser25 were considered; for *O*-glycosylation, only sites Thr207-Ser225 were considered. Serine (Ser), Threonine (Thr), weeks (W)

Donor two peptidome derived data												
Peptidome Phosphorylation	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18	107	52	59	13	79	62	44	28	10	454	50
	Ser21	508	368	390	209	454	382	339	210	208	3068	341
	Ser23	631	486	520	357	629	568	495	339	295	4320	480
	Ser24	769	695	797	743	973	932	945	676	751	7281	809
	Ser25	775	685	770	846	926	867	908	664	738	7179	798
	Ser28	8	6	6	12	7	21	6	12	6	84	9
	Thr30							1		1	2	1
	Thr207	3									3	3
	Thr214										0	0
	Ser225		1								1	1
	*Sum	2790	2286	2536	2168	3061	2811	2731	1917	2002		
	*Average	2691	457	507	434	612	562	546	383	400		
Peptidome Glycosylation	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18	5	5	3	7		3	5	7	4	39	5
	Ser21	6	10	6	8	1	3	8	11	6	59	7
	Ser23	6	9	6	8	1	3	9	11	9	62	7
	Ser24	1	7	3	6	3	4	7	6	6	43	5
	Ser25	1	4	4	4	3	2	7	3	7	35	4
	Ser28	2	4	6	7	6	5	10	3	8	51	6
	Thr30	2	5	4	5	6	5	10	3	6	46	5
	Thr207	144	151	43	29	57	45	40	11	26	546	61
	Thr214	184	177	58	44	87	61	54	21	37	723	80
	Ser225	13	17		6	13	9	3	1	2	64	8
	*Sum	341	345	101	79	157	115	97	33	65		
	*Average	114	115	51	26	52	38	32	11	22		
Peptidome non-modified	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18	655	692	776	1271	1044	1062	1145	925	1135	8705	967
	Ser21	308	436	512	1161	745	817	943	831	1002	6755	751
	Ser23	209	352	426	1062	639	688	859	739	969	5943	660
	Ser24	81	155	166	701	309	342	428	425	532	3139	349
	Ser25	78	175	206	617	373	419	483	457	565	3373	375
	Ser28	851	865	985	1483	1311	1274	1407	1124	1313	10613	1179
	Thr30	859	870	998	1500	1319	1296	1417	1139	1324	10722	1191
	Thr207	571	653	296	201	380	295	279	168	235	3078	342
	Thr214	553	652	315	217	378	298	291	177	255	3136	348
	Ser225	610	652	340	258	412	328	320	184	286	3390	377
	Sum	7671	7903	6201	9508	8561	8020	8855	7030	8536		
	Average	426	439	345	528	476	446	492	391	474		

* The Indicated Sum and Average were calculated using on sites that showed evidence of the respective PTM. For phosphosites, only Thr18-Ser25 were considered; for *O*-glycosylation, only sites Thr207-Ser225 were considered. Serine (Ser), Threonine (Thr), weeks (W)

Donor two proteome derived data												
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome Phosphorylation	Thr18	2								2	4	2
	Ser21	150	73	49	22	1	1	2	1	3	302	34
	Ser23	207	147	114	80	44	84	53	49	49	827	92
	Ser24	286	249	280	234	155	282	241	261	259	2247	250
	Ser25	282	247	273	229	155	283	240	267	251	2227	247
	Ser28			2	1				1		4	1
	Thr30										0	0
	Thr207	1	1	1		1	5				9	2
	Thr214		1								1	1
	Ser225			1							1	1
	*Sum	927	716	716	565	355	650	536	578	564		
	*Average	925	179	179	141	89	163	134	145	113		
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome Glycosylation	Thr18										0	0
	Ser21	1									1	1
	Ser23	1					1				2	1
	Ser24			1	1	2	2	1	1	1	9	1
	Ser25			1	1	2	2	1	1	1	9	1
	Ser28	1					2		1	1	5	1
	Thr30	1					2		1	1	5	1
	Thr207	44	77	64	50	55	66	36	34	39	465	52
	Thr214	74	100	98	78	82	97	57	53	70	709	79
	Ser225	13	20	17	11	18	10	14	13	10	126	14
	*Sum	131	197	179	139	155	173	107	100	119		
	*Average	44	66	60	46	52	58	36	33	40		
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome non-modified	Thr18	295	273	315	263	189	364	308	354	335	2696	300
	Ser21	157	221	284	263	208	382	324	375	360	2574	286
	Ser23	104	156	228	216	176	309	286	340	329	2144	238
	Ser24	27	56	64	63	66	114	101	130	121	742	82
	Ser25	32	59	73	70	68	113	105	125	132	777	86
	Ser28	318	309	349	304	229	400	350	392	384	3035	337
	Thr30	318	309	351	305	229	400	350	393	384	3039	338
	Thr207	435	503	514	476	445	612	299	390	421	4095	455
	Thr214	397	482	478	446	411	582	273	367	390	3826	425
	Ser225	402	480	504	463	429	588	295	378	411	3950	439
	Sum	3379	4022	4348	4011	3529	5268	3700	4421	4414		
	Average	188	223	242	223	196	293	206	246	245		

* The Indicated Sum and Average were calculated using on sites that showed evidence of the respective PTM. For phosphosites, only Thr18-Ser25 were considered; for *O*-glycosylation, only sites Thr207-Ser225 were considered. Serine (Ser), Threonine (Thr), weeks (W)

Supplemental Table S4: PSM percentage of phospho- and glycosite modifications by donor and time in the peptidome and proteome derived data

Donor one peptidome												
Peptidome Phosphorylation	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18	3	9	12	8	8	9	9	5	8	72	8
	Ser21	65	55	66	57	51	49	47	41	44	476	53
	Ser23	83	65	77	65	61	61	61	57	56	587	65
	Ser24	96	85	94	89	78	83	82	80	79	767	85
	Ser25	96	87	92	85	81	79	81	80	79	760	84
	Ser28		1			1		1			4	0
	Thr30										0	0
	Thr207	1	1			1	1				5	1
	Thr214		1								1	0
	Ser225										0	0
	*Sum	344	301	340	304	279	283	282	264	267		
	*Average	69	60	68	61	56	57	56	53	53		
Peptidome Glycosylation	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18										1	0
	Ser21			1		1	2		1	1	7	1
	Ser23		1	2		1	2	1	1	2	9	1
	Ser24		1	4	2	1		2	2	3	15	2
	Ser25			3			1	2	3	3	12	1
	Ser28	1						1		1	4	0
	Thr30	1						1	1	1	4	0
	Thr207	16	12	7	8	19	9	14	14	12	111	12
	Thr214	20	20	15	13	25	13	16	15	14	150	17
	Ser225	4	4	2	2	4	2	2	3	3	25	3
	*Sum	40	37	24	23	48	24	31	32	28		
	*Average	13	12	8	8	16	8	10	11	9		

* The Indicated Sum and Average were calculated using on sites that showed evidence of the respective PTM. For phosphosites, only Thr18-Ser25 were considered; for *O*-glycosylation, only sites Thr207-Ser225 were considered. Serine (Ser), Threonine (Thr), weeks (W)

Donor one proteome derived data												
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome Phosphorylation	Thr18				1						1	0
	Ser21	13	2	24	24	3		9	2	4	81	9
	Ser23	43	34	48	44	20	24	29	25	28	295	33
	Ser24	87	82	87	84	81	78	83	78	77	735	82
	Ser25	78	76	87	81	81	79	83	76	77	718	80
	Ser28										0	0
	Thr30										0	0
	Thr207		1		1			1			3	0
	Thr214										0	0
	Ser225										1	0
	*Sum	221	194	245	234	185	181	204	182	186		
	*Average	44	39	49	47	37	36	41	36	37		
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome Glycosylation	Thr18										0	0
	Ser21			1							1	0
	Ser23			1							1	0
	Ser24			5	4	2	3	2	3	1	20	2
	Ser25			3	3	2	3	2	3	1	17	2
	Ser28										1	0
	Thr30										1	0
	Thr207	9	8	10	13	13	12	14	14	14	108	12
	Thr214	17	14	14	16	18	19	19	17	19	152	17
	Ser225	2	3	2	3	4	3	2	3	4	26	3
	*Sum	27	24	26	32	34	35	36	34	37		
	*Average	9	8	9	11	11	12	12	11	12		

* The Indicated Sum and Average were calculated using on sites that showed evidence of the respective PTM. For phosphosites, only Thr18-Ser25 were considered; for *O*-glycosylation, only sites Thr207-Ser225 were considered. Serine (Ser), Threonine (Thr), weeks (W)

Donor two peptidome derived data												
Peptidome Phosphorylation	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18	14	7	7	1	7	6	4	3	1	49	5
	Ser21	62	46	43	15	38	32	26	20	17	300	33
	Ser23	75	58	55	25	50	45	37	31	23	399	44
	Ser24	90	82	83	51	76	73	69	61	59	644	72
	Ser25	91	80	79	58	71	67	65	59	57	627	70
	Ser28	1	1	1	1	1	2		1		7	1
	Thr30										0	0
	Thr207	1									1	0
	Thr214										0	0
	Ser225										0	0
	*Sum	333	272	267	151	242	223	201	175	157		
	*Average	67	54	53	30	48	45	40	35	31		
Peptidome Glycosylation	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
	Thr18	1	1		1				1		4	0
	Ser21	2	2	1	1			1	1	1	9	1
	Ser23	3	2	1	1			1	1	1	11	1
	Ser24	1	4	2	1	1	1	2	1	1	14	2
	Ser25	1	2	2	1	1		1	1	1	11	1
	Ser28			1				1		1	4	0
	Thr30		1					1			4	0
	Thr207	20	19	13	13	13	13	13	6	10	119	13
	Thr214	25	21	16	17	19	17	16	11	13	153	17
	Ser225	2	3		2	3	3	1	1	1	15	2
	*Sum	47	43	28	32	35	33	29	17	23		
	*Average	16	14	9	11	12	11	10	6	8		

* The Indicated Sum and Average were calculated using on sites that showed evidence of the respective PTM. For phosphosites, only Thr18-Ser25 were considered; for *O*-glycosylation, only sites Thr207-Ser225 were considered. Serine (Ser), Threonine (Thr), weeks (W)

Donor two proteome derived data												
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome Phosphorylation	Thr18	1								1	1	0
	Ser21	49	25	15	8			1		1	99	11
	Ser23	67	49	33	27	20	21	16	13	13	258	29
	Ser24	91	82	81	79	70	71	70	67	68	680	76
	Ser25	90	81	79	77	70	71	70	68	66	670	74
	Ser28			1							1	0
	Thr30										0	0
	Thr207						1				2	0
	Thr214										0	0
	Ser225										0	0
	*Sum	297	236	208	190	160	164	156	148	148		
	*Average	59	47	42	38	32	33	31	30	30		
	Location	W1	W2	W3	W4	W6	W8	W10	W12	W16	Sum	Average
Proteome Glycosylation	Thr18										0	0
	Ser21	1									1	0
	Ser23	1									1	0
	Ser24			2	2	3	2	1	1	1	10	1
	Ser25			1	1	3	2	1	1	1	10	1
	Ser28										1	0
	Thr30										1	0
	Thr207	9	13	11	10	11	10	11	8	8	91	10
	Thr214	16	17	17	15	17	14	17	13	15	141	16
	Ser225	3	4	3	2	4	2	5	3	2	29	3
	*Sum	28	34	31	27	32	26	33	24	26		
	*Average	9	11	10	9	11	9	11	8	9		

* The Indicated Sum and Average were calculated using on sites that showed evidence of the respective PTM. For phosphosites, only Thr18-Ser25 were considered; for *O*-glycosylation, only sites Thr207-Ser225 were considered. Serine (Ser), Threonine (Thr), weeks (W)

Supplemental Table S5: Observed Skyline MS1 glycopeptide percentages of the ladder peptide from (¹⁹⁰AVPVQALLLNQ... to ²⁰⁰QELLLNPTHQIYPVTQPLAPVHNPISV₂₂₆) for donor one over lactation in the proteome and peptidome derived data

Proteome derived data									
	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8	Week 10	Week 12	Week 16
¹⁹⁰ AVPVQALLLNQELLLNPTHQIYPVTQPLAPVHNPISV ₂₂₆									
AVPVQALLLNQELLLNPT[N ₁ H ₁]HQIYPVTQPLAPVHNPISV	1	2	2	3	1	2	2	2	3
AVPVQALLLNQELLLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁]QPLAPVHNPISV	1	1	2	4	1	3	3	3	3
¹⁹⁶ LLLNQELLLNPTHQIYPVTQPLAPVHNPISV ₂₂₆									
LLLNQELLLNPT[N ₁ H ₁]HQIYPVTQPLAPVHNPISV	1	1	1	1	1	1	1	1	1
LLLNQELLLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁]QPLAPVHNPISV	0	0	0	1	0	0	0	1	0
¹⁹⁷ LLLNQELLLNPTHQIYPVTQPLAPVHNPISV ₂₂₆									
LLLNQELLLNPT[N ₁ H ₁]HQIYPVTQPLAPVHNPISV	1	1	1	1	1	1	1	1	1
LLLNQELLLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁]QPLAPVHNPISV	0	0	0	1	0	1	1	0	1

Weeks (W)

Peptidome derived data									
	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8	Week 10	Week 12	Week 16
¹⁹⁰ AVPVQALLLNQELLNPTHQIYPVTQPLAPVHNPISV ₂₂₆									
AVPVQALLLNQELLNPT[N ₁ H ₁]HQIYPVTQPLAPVHNPISV	3	3	3	2	2	1	1	1	1
AVPVQALLLNQELLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁]QPLAPVHNPISV	5	5	8	8	7	5	5	4	5
¹⁹⁵ ALLLNQELLNPTHQIYPVTQPLAPVHNPISV ₂₂₆									
ALLLNQELLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁]QPLAPVHNPISV	5	3	4	12	8	5	3	6	5
¹⁹⁶ LLLNQELLNPTHQIYPVTQPLAPVHNPISV ₂₂₆									
LLLNQELLNPT[N ₁ H ₁]HQIYPVTQPLAPVHNPISV	6	6	0	1	25	1	2	2	4
LLLNQELLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁]QPLAPVHNPISV	4	7	2	1	27	2	4	3	5
LLLNQELLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁ S ₁]QPLAPVHNPISV	10	11	0	0	9	1	1	1	1
¹⁹⁷ LLNQELLNPTHQIYPVTQPLAPVHNPISV ₂₂₆									
LLNQELLNPT[N ₁ H ₁]HQIYPVTQPLAPVHNPISV	9	9	1	1	8	0	1	1	2
LLNQELLNPT[N ₁ H ₁ S ₁]HQIYPVTQPLAPVHNPISV	4	3	0	0	1	0	0	0	0
LLNQELLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁]QPLAPVHNPISV	10	11	2	2	16	1	2	3	3
LLNQELLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁ S ₁]QPLAPVHNPISV	8	8	1	0	2	0	0	0	0
¹⁹⁸ LNQELLNPTHQIYPVTQPLAPVHNPISV ₂₂₆									
LNQELLNPT[N ₁ H ₁ S ₁]HQIYPVTQPLAPVHNPISV	1	1	0	0	0	0	0	0	0
LNQELLNPT[N ₁ H ₁]HQIYPVT[N ₁ H ₁]QPLAPVHNPISV	4	7	1	1	7	1	1	1	2
LNQELLNPT[N ₁ H ₁ S ₁]HQIYPVT[N ₁ H ₁]QPLAPVHNPISV	3	3	0	0	1	0	0	0	0
¹⁹⁹ NOELLNPTHQIYPVTOPLAPVHNPISV ₂₂₆									

NQELLLNPT[N ₁ H ₁]HQ IYPVTQPLAPVHNPI SV	1	2	0	0	2	0	0	1	1
200QELLLNPTHQIYPVTQPLAPVHNPI SV ₂₂₆									
QELLLNPT[N ₁]HQIYP VT[N ₁]QPLAPVHNPI SV	1	2	2	1	37	2	1	1	6

Weeks (W)

Supplemental Table S6: Comparative average glycosylation occupancy percentage data from Byonic PSM counts and Skyline MS1 integration of the peptidome and proteome data.

Peptidome Glycosylation Data									
	W1	W2	W3	W4	W6	W8	W10	W12	W16
Donor one PSM counts									
Average percentage	10	11	9	8	13	2	7	3	1
Donor one MS1 intensities									
Average percentage	5	5	2	2	10	1	1	2	2
Proteome Glycosylation Data									
	W1	W2	W3	W4	W6	W8	W10	W12	W16
Donor one PSM counts									
Average percentage	13	9	8	9	5	12	7	7	10
Donor one MS1 intensities									
Average percentage	5	5	7	11	4	8	7	8	9

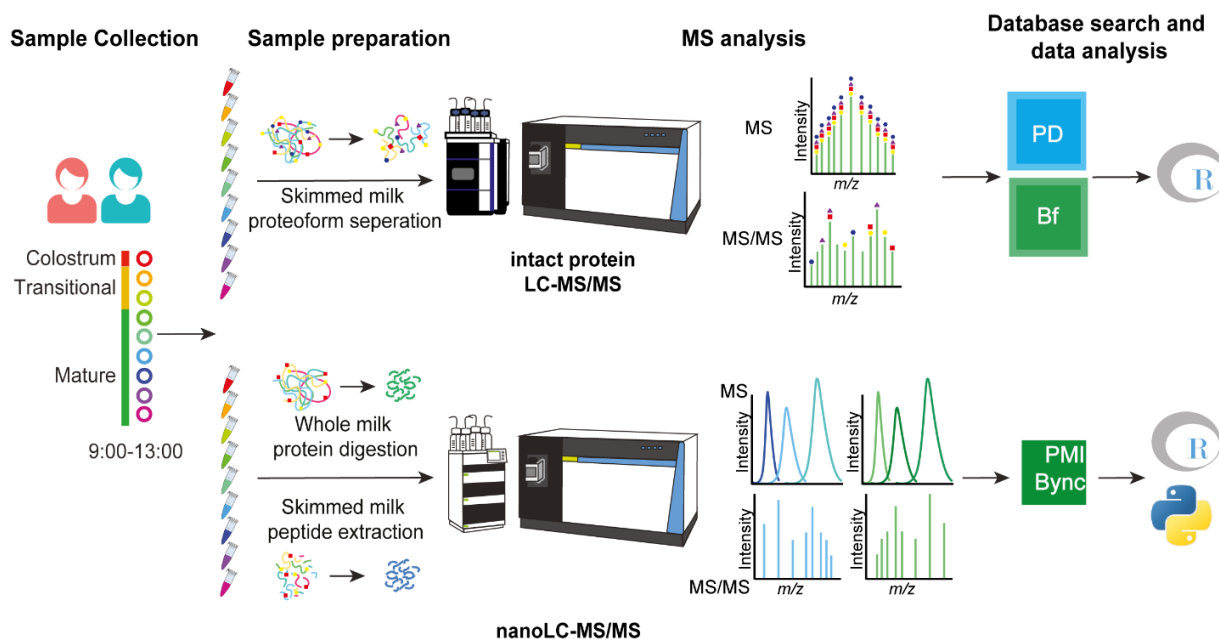
* PSM count percentages and MS1 intensity percentages were curated from all possible PSMs from modified peptides with glycosylation at sites Thr207 or Thr214, from Supplemental Table 7. Data was matched by donor one, weeks 1-16, peptidome or proteome data, peptide sequences (ladder peptides from 190-226 to 200-226), glycan species (N₁, N₁H₁, N₁H₁S₁), and fragmentation type (EThcD). The only differences in the curation of the datasets was the score filtering, where PSM counts had Byonic Score \geq 150, log probability \geq 1.5 and Delta Mod Score > 5 and Skyline data had idot values \geq 0.85.

Supplemental Table S7: Oxonium ions for mass spectrometry identification

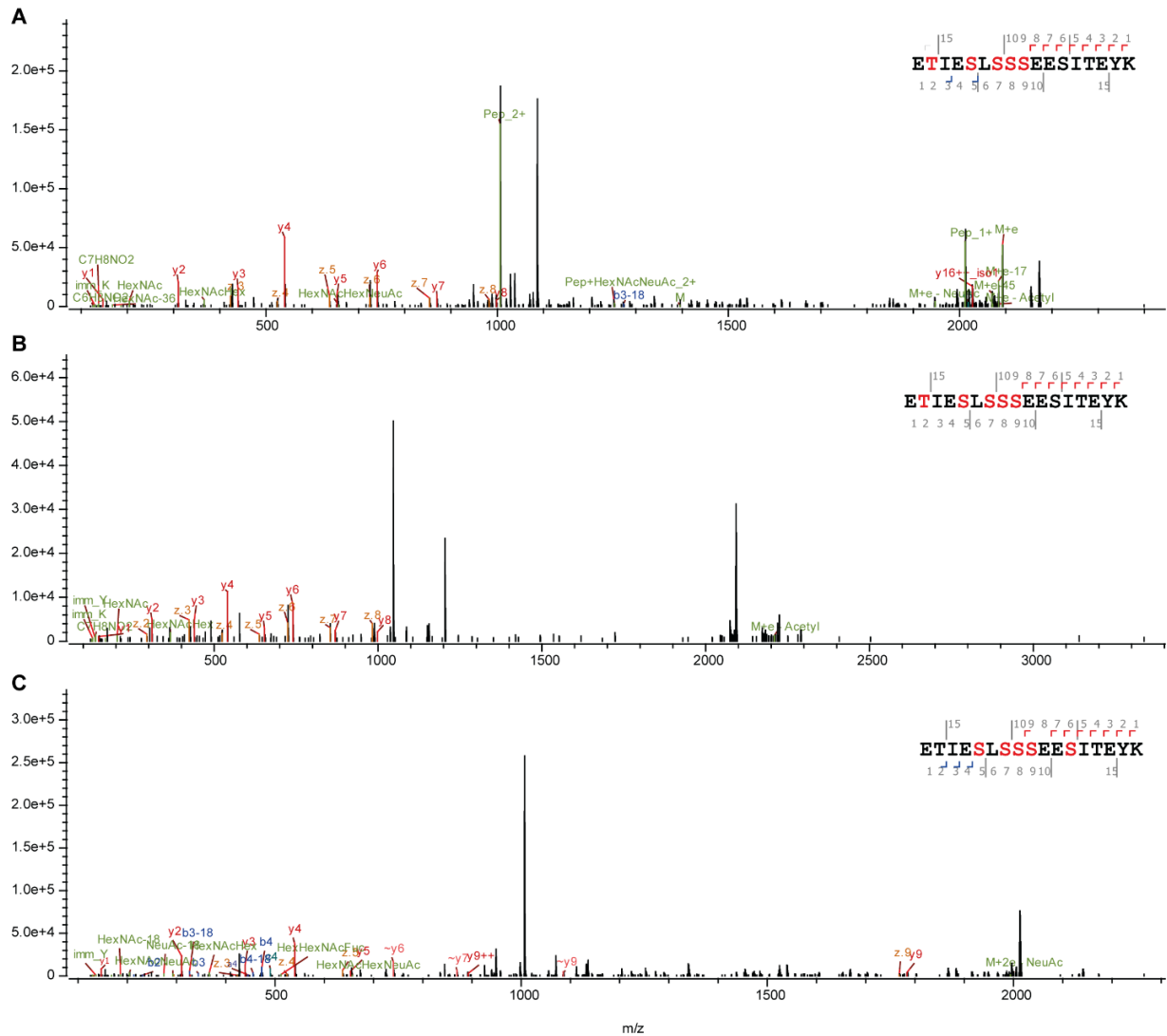
Oxonium ions	m/z
Hex	127.0390
Hex	145.0495
Hex	163.0601
PHex	243.0264
PHex	405.0793
HexNAc	138.0550
HexNAc	168.0655
HexNAc	186.0761
HexNAc	204.0867
Neu5Ac	274.0921
Neu5Ac	292.1027
HexHexNAc	366.1395
HexNAcHexNAc	407.1660
HexHexNAcdHex	512.1974
HexHexNAcNeu5Ac	657.2349

Supplemental Table S8: Glycan species used for Byonic identifications

Glycan name	Short name	Chemical name	Mass (Da)
HexNAc(1)	N ₁	H ₁₃ C ₈ N ₁ O ₅	203.08
HexNAc(1)dHex(1)	N ₁ F ₁	H ₂₃ C ₁₄ N ₁ O ₉	349.14
HexNAc(1)Hex(1)	N ₁ H ₁	H ₂₃ C ₁₄ N ₁ O ₁₀	365.13
HexNAc(1)NeuAc(1)	N ₁ S ₁	H ₃₀ C ₁₉ N ₂ O ₁₃	494.17
HexNAc(1)Hex(1)dHex(1)	N ₁ H ₁ F ₁	H ₃₃ C ₂₀ N ₁ O ₁₄	511.19
HexNAc(1)Hex(1)NeuAc(1)	N ₁ H ₁ S ₁	H ₄₀ C ₂₅ N ₂ O ₁₈	656.23
HexNAc(1)Hex(1)dHex(2)	N ₁ H ₁ F ₂	H ₄₃ C ₂₆ N ₁ O ₁₈	657.25
HexNAc(1)Hex(1)dHex(1)NeuAc(1)	N ₁ H ₁ F ₁ S ₁	H ₅₀ C ₃₁ N ₂ O ₂₂	802.29
HexNAc(1)Hex(1)NeuAc(2)	N ₁ H ₁ S ₂	H ₅₇ C ₃₆ N ₃ O ₂₆	947.32

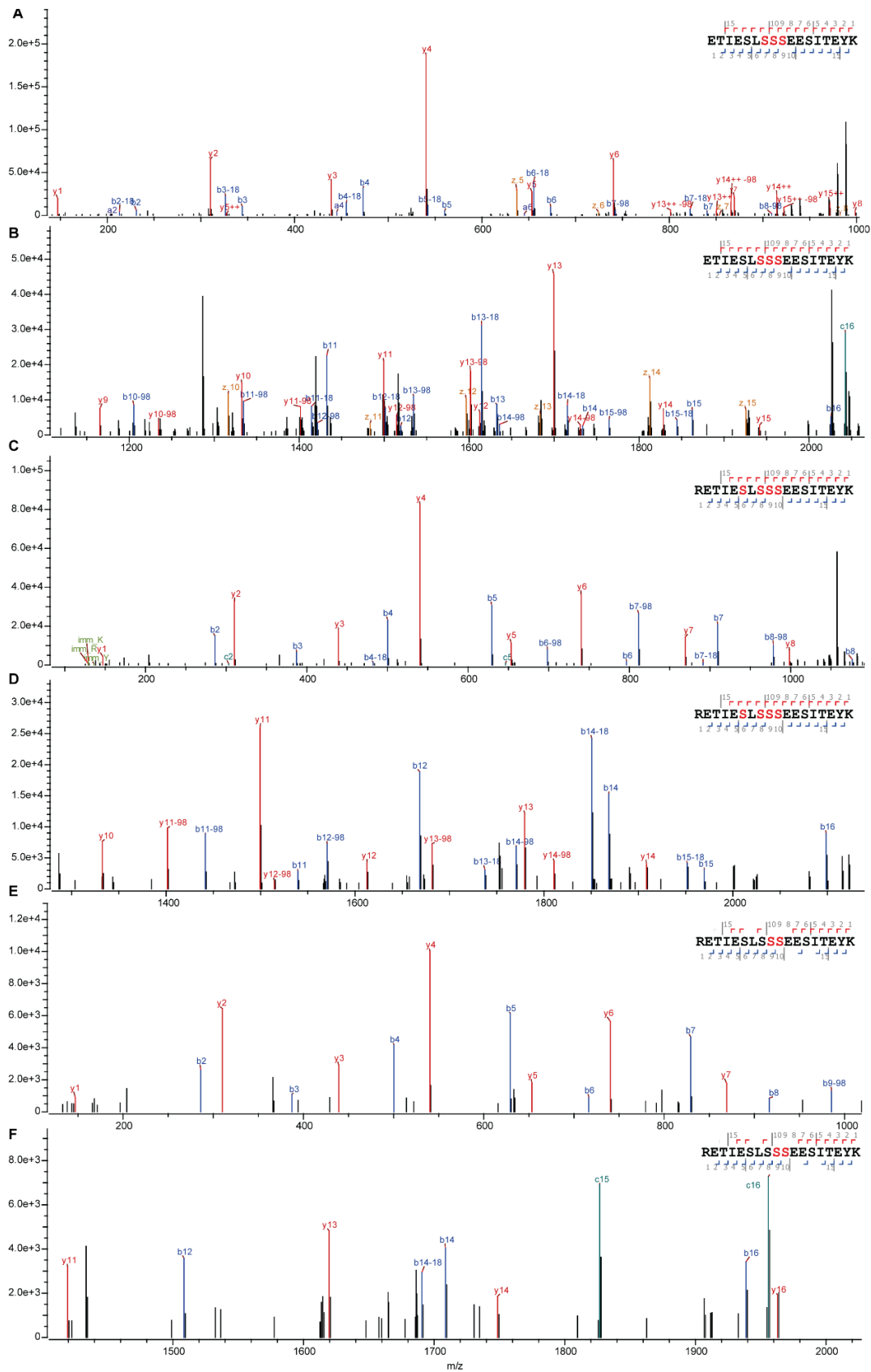


Supplemental Figure 1: Experimental scheme for personalized human milk PTM profiling. Samples from two individual donors were collected across lactation from weeks 1 to 16. Human milk proteoforms were first extracted and analyzed by intact protein LC-MS/MS, followed by protein and endogenous peptide extraction and analysis by label free quantification (LFQ) using LC-MS/MS in triplicate. All LFQ samples were ran as MS triplicates with varying MS/MS fragmentation types as higher-energy collisional dissociation (HCD), HCD-product-dependent stepping collision energy HCD (HCD-pd-sHCDs) and HCD-product-dependent electron-transfer/higher-energy collision dissociation (HCD-pd-ET_hcD). For intact proteoform analysis, isotopically resolved and unresolved spectra were deconvoluted with the BioPharma Finder software using Xtract or ReSpect algorithms respectively. For high-resolution proteoform MS/MS experiments, automatic searches were made in Thermo Proteome Discoverer software using ProSightPD nodes. All LFQ raw files obtained for the glycopeptide identification were processed in Byonic searching against a human β -casein protein database. For glycan analysis a database of 9 core 1 glycans was used. All data processing was done with Anaconda3 distribution of Python or R.



Supplemental Figure 2: EThcD fragmentation spectra of the N-terminal β -casein peptide

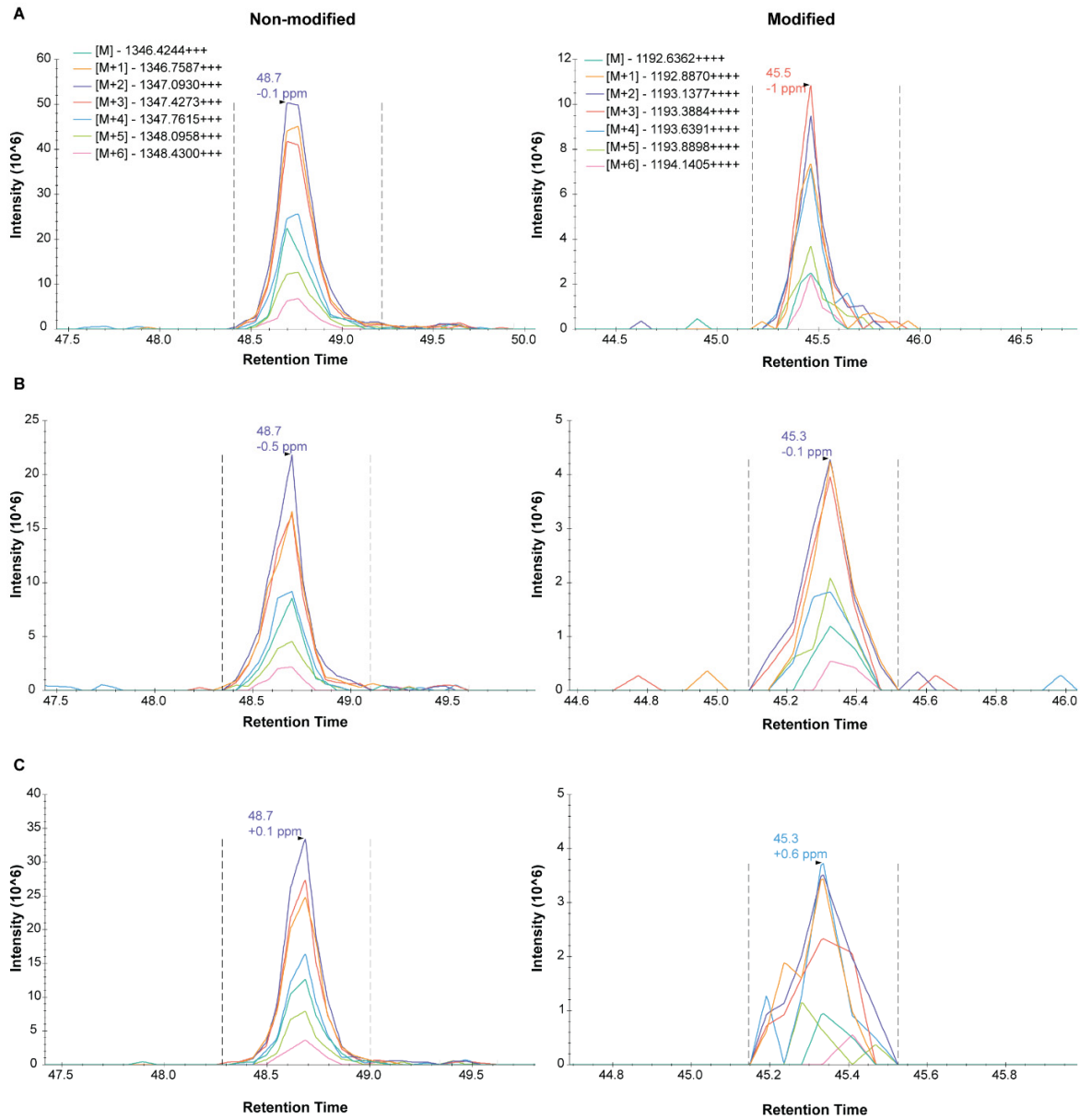
${}_{17}\text{ETIESLSSEESITEYK}_{33}$ with negative O-glycan characteristics. All spectra are from donor one, week 16 peptidome data and passed through the strict filtering criteria. **A-C)** EThcD spectra with major abundant peaks unannotated, large indicated glycans on more than three sites (A) $\text{N}_1\text{H}_1\text{S}_2$, N_1S_1 , N_1H_1 , P , $\text{N}_1\text{H}_1\text{F}_2$ B) $\text{N}_1\text{H}_1\text{S}_2$ on 5 sites C) $\text{N}_1\text{H}_1\text{S}_2$, N_1F_1 on 4 sites) without corresponding oxonium ions and an absence of peptide fragments with retained phospho-/glyco- modifications.



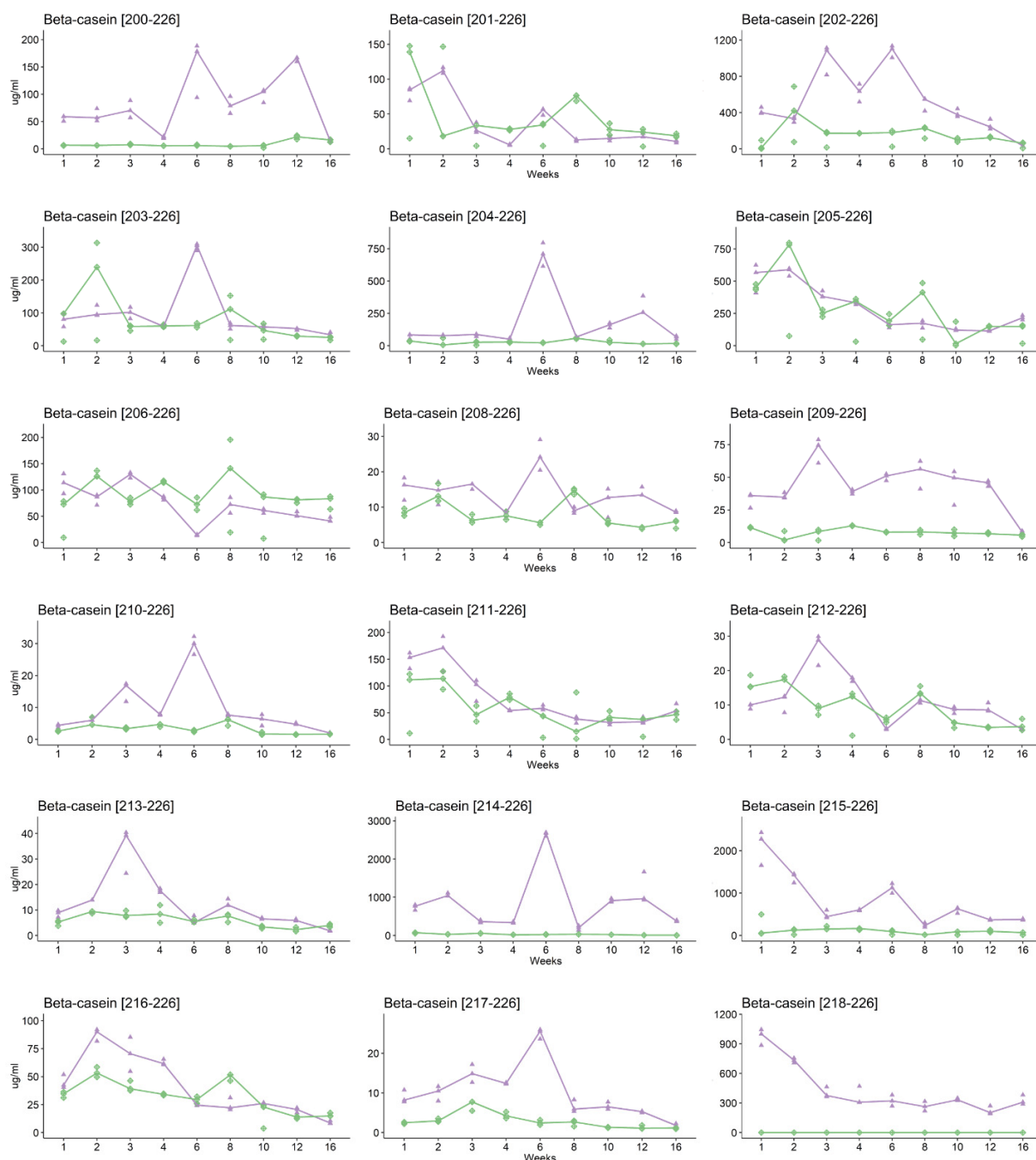
Supplemental Figure 3: Illustrative EThcD fragmentation spectra of β -casein phosphopeptides zoomed in regions. The N-terminal phospho peptides in figure 3 contain low intensity regions relative to the most intense ions, here these low intensity regions are displayed. **A)** Zoomed in region from m/z 100 to 1000 of the peptidome peptide with 3 phosphorylated Ser residues. **B)** Zoomed in region from m/z 1100 to 2000 of the peptidome peptide with 3 phosphorylated Ser residues. **C)** Zoomed in region from m/z 100 to 1000 of the peptidome peptide with 4 phosphorylated Ser residues. **D)** Zoomed in region from m/z 1300 to 2000 of the peptidome peptide with 4 phosphorylated Ser residues. **E)** Zoomed in region from m/z 100 to 1000 of the proteome peptide with 2 phosphorylated Ser residues. **F)** Zoomed in region from m/z 1400 to 2000 of the proteome peptide with 2 phosphorylated Ser residues. Designated phosphosites are indicated as red amino acids in the peptide sequence. Confidence in the phosphosite annotations are evident from the precursor mass with the neutral loss of phosphorylation from Ser ($M-98$ Da) upon fragmentation, with additional b- and y-ions from the peptide backbone placing the -98 Da at specific Ser residues.



Supplemental Figure 4: PSM-based comparison of phosphorylated and glycosylated sites in the peptidome and proteome across lactation. The PTMs across lactation are displayed for the peptidome and proteome at the indicated Ser and Thr sites across the protein backbone for donors one (left) and two (right). PTMs are displayed as stacked bars of non-modified to modified PSMs per site across all points in lactation. Each stacked bar represents the number of identified PSMs for non-modified peptides (grey), modified phosphopeptides (yellow) and glycopeptides (purple). Donor specific and lactations trends indicate that PTMs are highly individual specific, though overall the peptidome displays a greater relative abundance of phosphorylated and glycosylated amino acids than the proteome.



Supplemental Figure 5: MS1 ion traces of non-modified and modified C-terminal β -casein peptide ${}_{190}\text{AVPVQALLNQELLNPTHQIYPVTQPLAPVHNPISV}{}_{226}$ to validate glycan species quantification. All ion traces were extracted using Skyline software suite and are from donor one EThcD spectra across weeks 1, 6 and 16 of lactation for the non-modified (left) and glycosylated (right) peptide. The glycopeptide depicted at every time point has both sites Thr207 and Thr214 occupied with N_1H_1 . The depicted ion traces show confident and reproducible integration of both the non-modified and glycosylated peptide with highly accurate retention times and low ppm error. **A)** The C-terminal β -casein peptide at week 1 of lactation. **B)** Peptide at week 6 of lactation **C)** Peptide at week 16 of lactation.



Supplemental Figure 6: Abundances in the peptidome of putative antimicrobial C-terminal β -casein peptides. Non-modified peptides were detected in both donors. Data for donor one and two are depicted in purple and green, respectively. Data points indicate the values of each technical replicate, lines are linked by the median of the data points in each week for each donor. The data show that every

ladder peptide has a different concentration in human milk and a distinctive longitudinal pattern. These ladder peptides may potentially also exhibit different functionality.