Supplemental Materials

Top-down Targeted Proteomics for Deep Sequencing of Tropomyosin Isoforms

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Running title: *Top-down proteomics for characterizing Tm*

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Supplemental Tables:

Supplemental Table 1. Peak list and fragment assignment of one ECD spectrum of un-phosphorylated α–Tm isoform (precursor ion at m/z 936, 35+, searched with MASH Suite version 1.0 and manually validated)

Observed monoisotopic m/z	Charge state	Observed monoisotopic mass (Da)	Fragment assignment	Calculated Monoisotopic mass (Da)	Error (ppm)
618.330	1	617.323	c_{5}	617.321	3.4
746.425	1	745.418	c_{6}	745.416	3.5
373.718	2	745.421	c_{6}	745.416	7.7
503.284	2	1004.554	c_{8}	1004.551	3.2
567.314	2	1132.614	c_{9}	1132.610	3.4
632.835	2	1263.656	c_{10}	1263.650	4.5
689.376	2	1376.739	c_{11}	1376.734	3.6
1377.750	1	1376.743	c_{11}	1376.734	6.4
753.424	2	1504.835	c_{12}	1504.829	3.7
540.313	3	1617.919	c_{13}	1617.913	3.7
809.967	2	1617.920	c_{13}	1617.913	3.9
578.655	3	1732.946	c_{14}	1732.940	3.1
466.267	4	1861.041	c_{15}	1861.035	3.3
621.689	3	1861.042	c_{15}	1861.035	3.9
664.703	3	1990.086	c_{16}	1990.078	4.0
702.717	3	2104.129	c_{17}	2104.121	3.7
726.397	3	2175.167	c_{18}	2175.158	4.3
764.091	3	2288.251	c_{19}	2288.242	3.8
602.077	4	2403.277	c_{20}	2403.269	3.2

802.434	3	2403.279	c_{20}	2403.269	4.3
513.083	5	2559.378	c_{21}	2559.370	3.3
641.102	4	2559.379	c_{21}	2559.370	3.6
527.290	5	2630.415	c_{22}	2630.407	3.0
658.862	4	2630.416	c_{22}	2630.407	3.6
691.122	4	2759.459	c_{23}	2759.450	3.4
553.099	5	2759.460	c_{23}	2759.450	3.6
578.711	5	2887.512	c_{24}	2887.508	4.0
723.138	4	2887.521	c_{24}	2887.508	4.3
592.918	5	2958.556	c_{25}	2958.545	3.5
740.897	4	2958.557	c_{25}	2958.545	4.0
773.157	4	3087.597	c_{26}	3087.588	3.0
618.727	5	3087.599	c_{26}	3087.588	3.6
632.935	5	3158.6375	c_{27}	3158.625	3.9
655.940	5	3273.664	c_{28}	3273.652	3.5
819.674	4	3273.668	c_{28}	3273.652	4.8
851.948	4	3401.759	c_{29}	3401.747	3.6
681.760	5	3401.760	c_{29}	3401.747	3.7
601.489	6	3600.887	c_{31}	3600.879	2.1
721.587	5	3600.894	c_{31}	3600.879	4.2
613.329	6	3671.925	c_{32}	3671.916	2.5
735.794	5	3671.931	c_{32}	3671.916	4.1
634.836	6	3800.972	c_{33}	3800.959	3.5
761.602	5	3800.973	c_{33}	3800.959	3.8

951.753	4	3800.980	c_{33}	3800.959	5.6
784.607	5	3915.993	c_{34}	3915.986	1.9
654.008	6	3916.000	c_{34}	3915.986	3.5
815.828	5	4072.100	c_{35}	4072.087	3.3
680.0245	6	4072.101	c_{35}	4072.087	3.5
858.854	5	4287.228	c_{37}	4287.214	3.4
715.879	6	4287.231	c_{37}	4287.214	3.9
632.049	7	4415.288	c_{38}	4415.272	3.5
737.222	6	4415.288	c_{38}	4415.272	3.6
648.203	7	4528.372	c_{39}	4528.356	3.4
756.070	6	4528.373	c_{39}	4528.356	3.7
666.638	7	4657.415	c_{40}	4657.399	3.4
777.577	6	4657.417	c_{40}	4657.399	3.9
683.071	7	4772.441	c_{41}	4772.426	3.3
955.897	5	4772.446	c_{41}	4772.426	4.3
796.749	6	4772.447	c_{41}	4772.426	4.4
818.422	6	4901.487	c_{42}	4901.468	3.7
731.955	7	5113.631	c_{44}	5113.621	1.9
744.389	7	5200.671	c_{45}	5200.653	3.5
651.467	8	5200.672	c_{45}	5200.653	3.5
868.287	6	5200.674	c_{45}	5200.653	4.0
681.608	8	5441.806	c_{47}	5441.796	1.9
908.477	6	5441.811	c_{47}	5441.796	2.9
778.839	7	5441.816	c_{47}	5441.796	3.8

697.621	8	5569.904	c_{48}	5569.891	2.3
797.1381	7	5569.912	c_{48}	5569.891	3.8
713.633	8	5698.004	c_{49}	5697.986	3.2
634.453	9	5698.009	c_{49}	5697.986	4.1
815.438	7	5698.011	c_{49}	5697.986	4.5
727.768	8	5811.081	c_{50}	5811.070	2.0
680.371	9	6111.271	c_{53}	6111.250	3.5
765.292	8	6111.273	c_{53}	6111.250	3.8
874.476	7	6111.279	c_{53}	6111.249	4.8
694.709	9	6240.314	c_{54}	6240.292	3.5
781.423	8	6240.318	c_{54}	6240.292	4.2
707.490	9	6355.341	c_{55}	6355.319	3.5
909.343	7	6355.343	c_{55}	6355.319	3.8
795.801	8	6355.344	c_{55}	6355.319	3.9
721.828	9	6484.386	c_{56}	6484.361	3.7
812.057	8	6484.388	c_{56}	6484.361	4.1
661.154	10	6597.462	c_{57}	6597.446	2.5
734.504	9	6597.468	c_{57}	6597.446	3.5
826.192	8	6597.470	c_{57}	6597.446	3.7
840.569	8	6712.491	c_{58}	6712.472	2.7
645.979	11	7090.687	c_{61}	7090.3	3.4
710.477	10	7090.690	c_{61}	7090.663	3.8
789.307	9	7090.693	c_{61}	7090.663	4.3
887.845	8	7090.695	c_{61}	7090.663	4.5

723.381	10	7219.727	$\boldsymbol{c}_{62}$	7219.705	3.0
803.645	9	7219.736	$\boldsymbol{c}_{62}$	7219.705	4.2
811.537	9	7290.761	$\boldsymbol{c}_{63}$	7290.742	2.6
730.485	10	7290.772	$\boldsymbol{c}_{63}$	7290.742	4.0
674.448	11	7403.845	$\boldsymbol{c}_{64}$	7403.827	2.6
741.792	10	7403.846	$\boldsymbol{c}_{64}$	7403.827	2.6
824.1027	9	7403.847	$\boldsymbol{c}_{64}$	7403.827	2.7
754.602	10	7531.948	$\boldsymbol{c}_{65}$	7531.921	3.6
686.0944	11	7531.954	$\boldsymbol{c}_{65}$	7531.921	4.4
766.105	10	7646.973	$\boldsymbol{c}_{66}$	7646.948	3.2
773.209	10	7718.016	$\boldsymbol{c}_{67}$	7717.986	3.9
859.010	9	7718.019	$\boldsymbol{c}_{67}$	7717.986	4.3
703.010	11	7718.024	$\boldsymbol{c}_{67}$	7717.986	4.9
982.268	8	7846.075	$\boldsymbol{c}_{68}$	7846.044	4.0
786.016	10	7846.078	$\boldsymbol{c}_{68}$	7846.044	4.4
873.239	9	7846.079	$\boldsymbol{c}_{68}$	7846.044	4.4
1122.449	7	7846.082	$\boldsymbol{c}_{68}$	7846.044	4.8
836.042	10	8345.336	$\boldsymbol{c}_{72}$	8345.308	3.4
760.130	11	8345.345	$\boldsymbol{c}_{72}$	8345.308	4.4
706.293	12	8458.422	$\boldsymbol{c}_{73}$	8458.392	3.5
770.410	11	8458.423	$\boldsymbol{c}_{73}$	8458.392	3.6
847.351	10	8458.427131	$\boldsymbol{c}_{73}$	8458.392	4.1
776.868	11	8529.458	$\boldsymbol{c}_{74}$	8529.429	3.3
854.454	10	8529.459	$\boldsymbol{c}_{74}$	8529.429	3.4

867.359	10	8658.508	c_{75}	8658.472	4.1
733.641	12	8786.593	c_{76}	8786.567	3.0
800.245	11	8786.603	c_{76}	8786.567	4.0
758.656	12	9086.774	c_{79}	9086.747	3.0
827.534	11	9086.787	c_{79}	9086.747	4.5
700.376	13	9086.789	c_{79}	9086.747	4.6
910.187	10	9086.794	c_{79}	9086.747	5.1
709.223	13	9201.795	c_{80}	9201.774	2.3
768.241	12	9201.801	c_{80}	9201.774	2.9
837.991	11	9201.813	c_{80}	9201.774	4.2
714.688	13	9272.843	c_{81}	9272.811	3.4
774.162	12	9272.845	c_{81}	9272.811	3.7
784.914	12	9401.871	c_{82}	9401.853	1.9
856.181	11	9401.901	c_{82}	9401.853	5.0
790.835	12	9472.925	c_{83}	9472.891	3.7
678.004	14	9472.950	c_{83}	9472.891	6.3
821.932	12	9845.083	c_{87}	9845.055	2.9
767.482	13	9958.158	c_{88}	9958.139	1.9
712.734	14	9958.170	c_{88}	9958.139	3.1
906.843	11	9958.179	c_{88}	9958.139	4.0
997.427	10	9958.183	c_{88}	9958.139	4.6
776.255	13	10072.218	c_{89}	10072.182	3.5
720.881	14	10072.218	c_{89}	10072.182	3.6
1008.830	10	10072.219	c_{89}	10072.182	3.7

840.860	12	10072.222	c_{89}	10072.182	4.0
917.210	11	10072.225	c_{89}	10072.182	4.2
1120.813	9	10072.239	c_{89}	10072.182	5.6
788.264	13	10228.323	c_{90}	10228.283	3.9
800.271	13	10384.424	c_{91}	10384.384	3.8
866.878	12	10384.435	c_{91}	10384.384	4.9
808.970	13	10497.508	c_{92}	10497.468	3.8
709.779	15	10625.565	c_{93}	10625.527	3.6
818.821	13	10625.566	c_{93}	10625.527	3.8
886.9727	12	10625.572	c_{93}	10625.527	4.3
760.406	14	10625.581	c_{93}	10625.527	5.1
723.922	15	10837.715	c_{95}	10837.679	3.3
904.651	12	10837.718	c_{95}	10837.679	3.5
775.559	14	10837.721	c_{95}	10837.679	3.8
835.141	13	10837.727	c_{95}	10837.679	4.4
686.805	16	10966.755	c_{96}	10966.722	3.0
732.525	15	10966.757	c_{96}	10966.722	3.2
784.777	14	10966.762	c_{96}	10966.722	3.6
845.067	13	10966.767	c_{96}	10966.722	4.1
915.406	12	10966.776	c_{96}	10966.722	4.9
793.994	14	11095.813	c_{97}	11095.765	4.3
854.994	13	11095.814	c_{97}	11095.765	4.4
926.161	12	11095.832	c_{97}	11095.765	6.1
749.798	15	11224.851	c_{98}	11224.807	3.9

803.283	14	11224.852	c_{98}	11224.807	4.0
864.997	13	11224.854	c_{98}	11224.807	4.2
882.542	13	11452.946	c_{100}	11452.918	2.4
819.577	14	11452.962	c_{100}	11452.918	3.8
900.0172	13	11680.120	c_{102}	11680.056	5.4
747.520	16	11937.196	c_{104}	11937.158	3.3
854.165	14	11937.200	c_{104}	11937.158	3.6
797.288	15	11937.203	c_{104}	11937.158	3.8
996.360	12	11937.220	c_{104}	11937.158	5.2
940.494	13	12206.317	c_{106}	12206.343	-2.1
885.683	14	12378.449	c_{108}	12378.427	1.8
775.100	16	12378.475	c_{108}	12378.427	3.8
826.707	15	12378.480	c_{108}	12378.427	4.2
831.440	15	12449.486	c_{109}	12449.465	1.7
890.759	14	12449.515	c_{109}	12449.465	4.1
779.540	16	12449.519	c_{109}	12449.465	4.4
898.837	14	12562.601	c_{110}	12562.549	4.2
908.056	14	12690.671	c_{111}	12690.607	5.1
863.666	15	12931.865	c_{113}	12931.786	6.1
817.812	16	13060.871	c_{114}	13060.829	3.2
769.765	17	13060.873	c_{114}	13060.829	3.4
872.267	15	13060.886	c_{114}	13060.829	4.4
781.536	17	13260.970	c_{116}	13260.909	4.6
838.383	16	13390.002	c_{117}	13389.951	3.8

958.009	14	13390.013	c_{117}	13389.951	4.6
894.209	15	13390.020	c_{117}	13389.951	5.2
726.438	19	13775.178	c_{121}	13775.147	2.2
811.784	17	13775.197	c_{121}	13775.147	3.6
766.741	18	13775.197	c_{121}	13775.147	3.6
862.459	16	13775.210	c_{121}	13775.147	4.6
870.523	16	13904.234	c_{122}	13904.190	3.2
819.375	17	13904.247	c_{122}	13904.190	4.1
785.911	18	14120.305	c_{124}	14120.264	2.9
884.027	16	14120.309	c_{124}	14120.264	3.1
744.603	19	14120.312	c_{124}	14120.264	3.4
832.086	17	14120.325	c_{124}	14120.264	4.3
1010.17	14	14120.332	c_{124}	14120.264	4.8
859.924	17	14592.578	c_{128}	14592.5228	3.8
865.752	17	14691.653	c_{129}	14691.591	4.2
823.992	18	14804.717	c_{130}	14804.675	2.8
872.403	17	14804.717	c_{130}	14804.675	2.9
780.678	19	14804.723	c_{130}	14804.675	3.2
926.867	16	14804.735	c_{130}	14804.675	4.0
879.994	17	14933.769	c_{131}	14933.718	3.4
787.470	19	14933.786	c_{131}	14933.718	4.6
885.115	17	15020.818	c_{132}	15020.750	4.6
792.051	19	15020.820	c_{132}	15020.750	4.7
906.008	17	15376.001	c_{135}	15375.946	3.6

855.731	18	15376.004	c_{135}	15375.946	3.8
810.746	19	15376.029	c_{135}	15375.946	5.4
739.727	21	15504.100	c_{136}	15504.041	3.8
817.487	19	15504.108	c_{136}	15504.041	4.3
913.546	17	15504.137	c_{136}	15504.0413	6.2
869.237	18	15619.128	c_{137}	15619.068	3.8
823.541	19	15619.128	c_{137}	15619.068	3.8
782.414	20	15619.130	c_{137}	15619.068	4.0
920.311	17	15619.147	c_{137}	15619.068	5.0
830.385	19	15748.163	c_{138}	15748.111	3.3
876.463	18	15748.187	c_{138}	15748.111	4.8
833.287	20	16635.586	c_{145}	16635.517	4.2
877.092	19	16635.594	c_{145}	16635.517	4.7
850.998	20	16989.799	c_{148}	16989.743	3.2
895.736	19	16989.828	c_{148}	16989.743	5.0
816.622	21	17117.903	c_{149}	17117.838	3.8
857.404	20	17117.918	c_{149}	17117.838	4.7
896.373	20	17896.288	c_{156}	17896.236	2.9
853.737	21	17896.313	$c1_{56}$	17896.236	4.4
905.6756	20	18082.3456	c_{158}	18082.300	2.5
828.662	22	18197.397	c_{159}	18197.327	3.9
868.075	21	18197.400	c_{159}	18197.327	4.0
959.345	19	18197.402	c_{159}	18197.327	4.2
911.429	20	18197.424	c_{159}	18197.327	5.4

875.508	21	18353.504	$\boldsymbol{c}_{160}$	18353.428	4.2
944.216	21	19795.371	$\boldsymbol{c}_{172}$	19795.293	3.9
901.344	22	19795.384	$\boldsymbol{c}_{172}$	19795.293	4.6
856.745	24	20524.693	$\boldsymbol{c}_{178}$	20524.623	3.4
865.082	24	20724.782	$\boldsymbol{c}_{180}$	20724.702	3.9
1342.614	1	1341.607	$\boldsymbol{z}^{\bullet}_{12}$	1341.600	5.6
871.185	4	3478.708	$\boldsymbol{z}^{\bullet}_{30}$	3478.694	3.9
1161.246	3	3478.711	$\boldsymbol{z}^{\bullet}_{30}$	3478.694	4.9
1308.994	3	3921.955	$\boldsymbol{z}^{\bullet}_{34}$	3921.932	5.9
1352.008	3	4050.999	$\boldsymbol{z}^{\bullet}_{35}$	4050.974	6.0
1389.702	3	4164.079	$\boldsymbol{z}^{\bullet}_{36}$	4164.059	4.9
1074.551	4	4292.170	$\boldsymbol{z}^{\bullet}_{37}$	4292.154	3.8
1432.402	3	4292.181	$\boldsymbol{z}^{\bullet}_{37}$	4292.154	6.4
1528.120	3	4579.333	$\boldsymbol{z}^{\bullet}_{40}$	4579.302	6.8
1185.364	4	4735.424	$\boldsymbol{z}^{\bullet}_{41}$	4735.403	4.6
1235.635	4	4935.505	$\boldsymbol{z}^{\bullet}_{43}$	4935.482	4.6
1272.406	4	5082.590	$\boldsymbol{z}^{\bullet}_{44}$	5082.551	7.6
1304.664	4	5211.621	$\boldsymbol{z}^{\bullet}_{45}$	5211.593	5.3
907.968	6	5438.758	$\boldsymbol{z}^{\bullet}_{47}$	5438.732	4.8
1361.451	4	5438.769	$\boldsymbol{z}^{\bullet}_{47}$	5438.732	6.8
1149.583	5	5739.874	$\boldsymbol{z}^{\bullet}_{50}$	5739.859	2.6
1436.732	4	5739.893	$\boldsymbol{z}^{\bullet}_{50}$	5739.859	5.9
1175.395	5	5868.931	$\boldsymbol{z}^{\bullet}_{51}$	5868.902	5.0
1468.993	4	5868.936	$\boldsymbol{z}^{\bullet}_{51}$	5868.902	5.9

1272.258	5	6353.250	$z^{\bullet}_{55}$	6353.203	7.5
1590.072	4	6353.252	$z^{\bullet}_{55}$	6353.203	7.8
1312.482	5	6553.363	$z^{\bullet}_{57}$	6553.319	6.8
1131.761	6	6780.517	$z^{\bullet}_{59}$	6780.482	5.2
1193.625	6	7151.698	$z^{\bullet}_{62}$	7151.651	6.5
1215.132	6	7280.742	$z^{\bullet}_{63}$	7280.694	6.6
1282.828	6	7686.918	$z^{\bullet}_{66}$	7686.879	5.0
1136.444	7	7944.052	$z^{\bullet}_{68}$	7944.017	4.4
1325.684	6	7944.053	$z^{\bullet}_{68}$	7944.017	4.5
1010.646	8	8072.099	$z^{\bullet}_{69}$	8072.075	3.0
1154.883	7	8072.118	$z^{\bullet}_{69}$	8072.075	5.3
1347.197	6	8072.125	$z^{\bullet}_{69}$	8072.075	6.2
1190.610	7	8322.210	$z^{\bullet}_{71}$	8322.171	4.7
1388.878	6	8322.217	$z^{\bullet}_{71}$	8322.171	5.6
1208.911	7	8450.316	$z^{\bullet}_{72}$	8450.266	6.0
1410.230	6	8450.327	$z^{\bullet}_{72}$	8450.266	7.3
1227.345	7	8579.355	$z^{\bullet}_{73}$	8579.308	5.5
1237.493	7	8650.387	$z^{\bullet}_{74}$	8650.345	4.9
1098.936	8	8778.423	$z^{\bullet}_{75}$	8778.404	2.2
1255.789	7	8778.460	$z^{\bullet}_{75}$	8778.404	6.5
1107.818	8	8849.479	$z^{\bullet}_{76}$	8849.441	4.3
1265.940	7	8849.517	$z^{\bullet}_{76}$	8849.441	8.6
1284.370	7	8978.530	$z^{\bullet}_{77}$	8978.483	5.2
1123.950	8	8978.533	$z^{\bullet}_{77}$	8978.483	5.6

1179.111	8	9419.824	z_{81}	9419.779	4.8
1048.211	9	9419.828	z_{81}	9419.779	5.3
1347.414	7	9419.837	z_{81}	9419.779	6.2
1363.847	7	9533.864	z_{82}	9533.822	4.5
1193.494	8	9533.884	z_{82}	9533.822	6.5
1207.748	8	9647.916	z_{83}	9647.864	5.3
1380.142	7	9647.931	z_{83}	9647.864	6.9
1133.926	9	10190.259	z_{88}	10190.207	5.0
1148.264	9	10319.299	z_{89}	10319.250	4.8
1291.671	8	10319.300	z_{89}	10319.250	4.9
1033.539	10	10319.303	z_{89}	10319.250	5.1
939.673	11	10319.315	z_{89}	10319.250	6.3
1132.479	10	11307.706	z_{98}	11307.667	3.5
1141.184	10	11394.753	z_{99}	11394.699	4.8
1280.435	9	11507.839	z_{100}	11507.783	4.8
1152.493	10	11507.841	z_{100}	11507.783	5.1
1001.012	12	11993.049	z_{104}	11993.006	3.6
1091.923	11	11993.060	z_{104}	11993.006	4.5
1334.349	9	11993.063	z_{104}	11993.006	4.7
1201.015	10	11993.069	z_{104}	11993.006	5.2
1110.113	11	12193.149	z_{106}	12193.086	5.2
1221.024	10	12193.149	z_{106}	12193.086	5.2
1156.864	11	12706.405	z_{110}	12706.341	5.1
1176.504	11	12922.450	z_{112}	12922.415	2.7

1078.548	12	12922.474	z_{112}	12922.415	4.5
1294.059	10	12922.505	z_{112}	12922.415	6.9
1186.788	11	13035.573	z_{113}	13035.500	5.6
1305.366	10	13035.575	z_{113}	13035.500	5.8
1197.068	11	13148.651	z_{114}	13148.584	5.1
1188.028	12	14236.235	z_{123}	14236.186	3.4
1198.786	12	14364.330	z_{124}	14364.281	3.4
1307.679	11	14364.372	z_{124}	14364.281	6.3
1118.659	13	14520.450	z_{125}	14520.382	4.6
1211.796	12	14520.452	z_{125}	14520.382	4.8
1038.827	14	14520.458	z_{125}	14520.382	5.2
1236.887	12	14821.544	z_{128}	14821.473	4.8
1141.820	13	14821.552	z_{128}	14821.473	5.3
1301.755	12	15599.962	z_{135}	15599.871	5.8
1201.698	13	15599.962	z_{135}	15599.871	5.9
1125.083	14	15728.050	z_{136}	15727.966	5.4
1050.146	15	15728.058	z_{136}	15727.966	5.9
1150.456	14	16082.262	z_{139}	16082.192	4.3
1342.035	12	16082.315	z_{139}	16082.192	7.6
1132.985	15	16969.646	z_{146}	16969.598	2.9
1062.238	16	16969.673	z_{146}	16969.598	4.4
1141.588	15	17098.699	z_{147}	17098.641	3.4
1070.303	16	17098.712	z_{147}	17098.641	4.2
1223.060	14	17098.721	z_{147}	17098.641	4.7

1007.404	17	17098.725	$\mathfrak{z}_{147}$	17098.641	5.0
1317.068	13	17098.765	$\mathfrak{z}_{147}$	17098.641	7.2
1231.278	14	17213.776	$\mathfrak{z}_{148}$	17213.668	6.3
1240.498	14	17341.852	$\mathfrak{z}_{149}$	17341.763	5.2
1157.867	15	17341.873	$\mathfrak{z}_{149}$	17341.763	6.4
1171.136	15	17540.909	$\mathfrak{z}_{151}$	17540.858	2.9
1034.814	18	18597.505	$\mathfrak{z}_{160}$	18597.444	3.2
1241.576	15	18597.509	$\mathfrak{z}_{160}$	18597.444	3.4
1115.990	17	18942.680	$\mathfrak{z}_{163}$	18942.562	6.2
967.999	20	19327.811	$\mathfrak{z}_{167}$	19327.758	2.7
1138.645	17	19327.827	$\mathfrak{z}_{167}$	19327.758	3.6
1075.444	18	19327.837	$\mathfrak{z}_{167}$	19327.758	4.1
1018.895	19	19327.855	$\mathfrak{z}_{167}$	19327.758	5.0
1015.024	20	20268.306	$\mathfrak{z}_{175}$	20268.244	3.0

Supplemental Table 2. Peaklist and fragment assignments of one ECD spectrum of mono-phosphorylated α–Tm isoform (precursor ion at m/z 887, 37+, searched with MASH Suite version 1.0 with no phosphorylation or mono-phosphorylation on *Ser*^{283} and validated manually, monophosphorylated ions were labeled as "$_p$")

Observed monoisotopic m/z	Charge state	Observed monoisotopic mass (Da)	Fragment assignment	Calculated Monoisotopic mass (Da)	Error (ppm)
618.328	1	617.322	c_5	617.321	1.7
746.424	1	745.417	c_6	745.416	2.3
503.283	2	1004.553	c_8	1004.551	1.4
1005.561	1	1004.554	c_8	1004.551	3.1
567.312	2	1132.611	c_9	1132.610	1.5
632.832	2	1263.651	c_{10}	1263.650	1.0
689.375	2	1376.737	c_{11}	1376.734	2.1
1377.748	1	1376.741	c_{11}	1376.734	4.9
753.423	2	1504.833	c_{12}	1504.829	2.7
809.965	2	1617.917	c_{13}	1617.913	2.6
578.654	3	1732.943	c_{14}	1732.940	1.5
867.480	2	1732.946	c_{14}	1732.940	3.4
621.687	3	1861.039	c_{15}	1861.035	2.0
932.029	2	1861.041	c_{15}	1861.035	2.9
664.702	3	1990.082	c_{16}	1990.078	2.1
996.552	2	1990.087	c_{16}	1990.078	4.8
702.715	3	2104.122	c_{17}	2104.121	0.5
1053.573	2	2104.130	c_{17}	2104.121	4.5
764.090	3	2288.247	c_{19}	2288.242	2.4
1145.635	2	2288.254	c_{19}	2288.242	5.1

602.076	4	2403.274	c_{20}	2403.269	2.2
802.434	3	2403.278	c_{20}	2403.269	3.8
1203.149	2	2403.281	c_{20}	2403.269	5.0
513.082	5	2559.373	c_{21}	2559.370	1.4
641.101	4	2559.375	c_{21}	2559.370	2.0
854.467	3	2559.378	c_{21}	2559.370	3.3
658.861	4	2630.414	c_{22}	2630.407	2.7
878.147	3	2630.419	c_{22}	2630.407	4.4
691.121	4	2759.455	c_{23}	2759.450	2.0
921.161	3	2759.459	c_{23}	2759.450	3.3
723.137	4	2887.517	c_{24}	2887.508	3.0
963.848	3	2887.520	c_{24}	2887.508	4.0
592.917	5	2958.549	c_{25}	2958.545	1.3
740.895	4	2958.551	c_{25}	2958.545	2.1
987.527	3	2958.559	c_{25}	2958.545	4.6
618.726	5	3087.593	c_{26}	3087.588	1.7
773.156	4	3087.595	c_{26}	3087.588	2.3
1030.542	3	3087.602	c_{26}	3087.588	4.5
632.933	5	3158.631	c_{27}	3158.625	1.8
790.917	4	3158.637	c_{27}	3158.625	3.7
655.939	5	3273.658	c_{28}	3273.652	1.9
819.673	4	3273.661	c_{28}	3273.652	2.9
1092.562	3	3273.662	c_{28}	3273.652	3.1
681.558	5	3401.754	c_{29}	3401.747	2.2

851.948	4	3401.758	$\boldsymbol{c}_{29}$	3401.747	3.1
707.378	5	3529.849	$\boldsymbol{c}_{30}$	3529.842	2.1
883.970	4	3529.848	$\boldsymbol{c}_{30}$	3529.842	1.8
721.585	5	3600.886	$\boldsymbol{c}_{31}$	3600.879	1.8
901.732	4	3600.895	$\boldsymbol{c}_{31}$	3600.879	4.5
735.792	5	3671.921	$\boldsymbol{c}_{32}$	3671.916	1.2
919.490	4	3671.929	$\boldsymbol{c}_{32}$	3671.916	3.5
761.602	5	3800.969	$\boldsymbol{c}_{33}$	3800.959	2.7
951.751	4	3800.972	$\boldsymbol{c}_{33}$	3800.959	3.5
654.006	6	3915.992	$\boldsymbol{c}_{34}$	3915.986	1.7
784.607	5	3915.996	$\boldsymbol{c}_{34}$	3915.986	2.7
980.507	4	3915.997	$\boldsymbol{c}_{34}$	3915.986	2.9
815.827	5	4072.097	$\boldsymbol{c}_{35}$	4072.087	2.6
715.878	6	4287.223	$\boldsymbol{c}_{37}$	4287.214	2.2
858.854	5	4287.229	$\boldsymbol{c}_{37}$	4287.214	3.6
737.221	6	4415.278	$\boldsymbol{c}_{38}$	4415.272	1.4
648.203	7	4528.368	$\boldsymbol{c}_{39}$	4528.356	2.7
756.069	6	4528.369	$\boldsymbol{c}_{39}$	4528.356	2.7
666.637	7	4657.403	$\boldsymbol{c}_{40}$	4657.399	0.9
777.576	6	4657.411	$\boldsymbol{c}_{40}$	4657.399	2.5
932.891	5	4657.414	$\boldsymbol{c}_{40}$	4657.399	3.2
683.070	7	4772.436	$\boldsymbol{c}_{41}$	4772.426	2.0
796.747	6	4772.438	$\boldsymbol{c}_{41}$	4772.426	2.5
955.896	5	4772.441	$\boldsymbol{c}_{41}$	4772.426	3.1

818.422	6	4901.481	$\boldsymbol{c}_{42}$	4901.468	2.7
981.906	5	4901.488	$\boldsymbol{c}_{42}$	4901.468	3.9
853.780	6	5113.634	$\boldsymbol{c}_{44}$	5113.621	2.5
744.389	7	5200.666	$\boldsymbol{c}_{45}$	5200.653	2.6
868.286	6	5200.669	$\boldsymbol{c}_{45}$	5200.653	3.1
778.837	7	5441.805	$\boldsymbol{c}_{47}$	5441.796	1.8
908.476	6	5441.810	$\boldsymbol{c}_{47}$	5441.796	2.7
1089.971	5	5441.811	$\boldsymbol{c}_{47}$	5441.796	2.8
797.137	7	5569.905	$\boldsymbol{c}_{48}$	5569.891	2.6
929.828	6	5569.919	$\boldsymbol{c}_{48}$	5569.891	5.1
1115.594	5	5569.929	$\boldsymbol{c}_{48}$	5569.891	7.0
713.632	8	5697.992	$\boldsymbol{c}_{49}$	5697.986	1.1
815.437	7	5698.002	$\boldsymbol{c}_{49}$	5697.986	2.8
849.891	7	5939.181	$\boldsymbol{c}_{51}$	5939.165	2.8
765.291	8	6111.266	$\boldsymbol{c}_{53}$	6111.249	2.7
874.475	7	6111.271	$\boldsymbol{c}_{53}$	6111.249	3.5
781.421	8	6240.303	$\boldsymbol{c}_{54}$	6240.292	1.8
707.489	9	6355.337	$\boldsymbol{c}_{55}$	6355.319	2.8
795.800	8	6355.336	$\boldsymbol{c}_{55}$	6355.319	2.7
909.343	7	6355.343	$\boldsymbol{c}_{55}$	6355.319	3.8
811.930	8	6484.379	$\boldsymbol{c}_{56}$	6484.361	2.7
734.503	9	6597.458	$\boldsymbol{c}_{57}$	6597.446	1.8
826.191	8	6597.466	$\boldsymbol{c}_{57}$	6597.446	3.1
944.076	7	6597.471	$\boldsymbol{c}_{57}$	6597.446	3.9

710.475	10	7090.674	c_{61}	7090.663	1.6
789.305	9	7090.678	c_{61}	7090.663	2.2
1014.535	7	7090.690	c_{61}	7090.663	3.8
803.645	9	7219.730	c_{62}	7219.705	3.4
903.974	8	7219.725	c_{62}	7219.705	2.7
811.537	9	7290.764	c_{63}	7290.742	2.9
824.103	9	7403.853	c_{64}	7403.827	3.6
838.335	9	7531.945	c_{65}	7531.921	3.1
859.008	9	7718.005	c_{67}	7717.986	2.5
786.015	10	7846.075	c_{68}	7846.044	3.9
873.237	9	7846.064	c_{68}	7846.044	2.5
847.350	10	8458.416	c_{73}	8458.392	2.8
854.453	10	8529.449	c_{74}	8529.429	2.3
827.533	11	9086.773	c_{79}	9086.747	2.9
862.637	11	9472.915	c_{83}	9472.891	2.6
917.209	11	10072.205	c_{89}	10072.182	2.2
775.558	14	10837.702	c_{95}	10837.679	2.1
1422.586	1	1421.580	$\mathrm{p}z^{\bullet}_{12}$	1421.566	9.5
895.107	3	2681.297	$\mathrm{p}z^{\bullet}_{23}$	2681.290	2.6
949.462	3	2844.364	$\mathrm{p}z^{\bullet}_{24}$	2844.353	3.7
1068.515	3	3201.522	$\mathrm{p}z^{\bullet}_{27}$	3201.507	4.7
1187.902	3	3558.682	$\mathrm{p}z^{\bullet}_{30}$	3558.660	6.0
1226.244	3	3673.705	$\mathrm{p}z^{\bullet}_{31}$	3673.687	4.9
1335.649	3	4001.921	$\mathrm{p}z^{\bullet}_{34}$	4001.898	5.7

1378.666	3	4130.971	pz• 35	4130.941	7.3
1416.360	3	4244.055	pz• 36	4244.025	7.1
1459.059	3	4372.152	pz• 37	4372.120	7.4
964.483	5	4815.376	pz• 41	4815.369	1.5
1205.356	4	4815.390	pz• 41	4815.369	4.3
1255.626	4	5015.471	pz• 43	5015.449	4.4
1292.395	4	5162.544	pz• 44	5162.517	5.2
1324.657	4	5291.591	pz• 45	5291.560	6.0
1105.353	5	5518.725	pz• 47	5518.698	4.9
1381.441	4	5518.729	pz• 47	5518.698	5.6
1125.562	5	5619.766	pz• 48	5619.746	3.6
1165.579	5	5819.856	pz• 50	5819.825	5.2
992.991	6	5948.900	pz• 51	5948.868	5.4
1191.387	5	5948.893	pz• 51	5948.868	4.3
1073.708	6	6433.199	pz• 55	6433.169	4.7
1288.249	5	6433.203	pz• 55	6433.169	5.4
1145.087	6	6860.471	pz• 59	6860.448	3.3
1034.671	7	7231.640	pz• 62	7231.618	3.1
1111.134	7	7766.878	pz• 66	7766.845	4.1
1004.636	8	8024.021	pz• 68	8023.983	4.8
1148.011	7	8024.020	pz• 68	8023.983	4.6
1339.178	6	8024.017	pz• 68	8023.983	4.2
1020.644	8	8152.082	pz• 69	8152.041	5.0
1166.307	7	8152.087	pz• 69	8152.041	5.6

1360.525	6	8152.097	$pz^{\bullet}_{69}$	8152.041	6.8
1084.047	8	8659.312	$pz^{\bullet}_{73}$	8659.274	4.3
1133.944	8	9058.482	$pz^{\bullet}_{77}$	9058.450	3.5
1057.206	9	9499.778	$pz^{\bullet}_{81}$	9499.745	3.4
1189.227	8	9499.746	$pz^{\bullet}_{81}$	9499.745	0.1
1041.532	10	10399.238	$pz^{\bullet}_{89}$	10399.216	2.1
1007.676	12	12073.011	$pz^{\bullet}_{104}$	12072.973	3.2
974.968	15	14600.397	$pz^{\bullet}_{125}$	14600.349	3.3

Supplemental Table 3. Peak list and fragment assignment of one ECD spectrum of un-phosphorylated β–Tm isoform (precursor ion at m/z 889, 37+, searched with MASH Suite version 1.0 and manually validated)

Observed monoisotopic m/z	Charge state	Observed monoisotopic mass (Da)	Fragment assignment	Calculated Monoisotopic mass (Da)	Error (ppm)
618.327	1	617.321	c_5	617.321	0.2
746.423	1	745.416	c_6	745.416	0.2
503.282	2	1004.551	c_8	1004.551	0.0
567.312	2	1132.610	c_9	1132.610	0.1
632.832	2	1263.651	c_{10}	1263.650	0.7
1377.744	1	1376.737	c_{11}	1376.734	2.1
689.374	2	1376.734	c_{11}	1376.734	0.1
753.421	2	1504.829	c_{12}	1504.829	0.1
809.963	2	1617.913	c_{13}	1617.913	0.1
540.311	3	1617.913	c_{13}	1617.913	-0.3
867.477	2	1732.941	c_{14}	1732.940	0.3
578.654	3	1732.941	c_{14}	1732.940	0.4
932.026	2	1861.036	c_{15}	1861.035	0.6
621.682	3	1861.022	c_{15}	1861.035	-7.0
621.686	3	1861.036	c_{15}	1861.035	0.4
466.265	4	1861.034	c_{15}	1861.035	-0.6
996.546	2	1990.076	c_{16}	1990.078	-0.7
664.701	3	1990.079	c_{16}	1990.078	0.5
1053.570	2	2104.125	c_{17}	2104.121	1.8
702.715	3	2104.122	c_{17}	2104.121	0.6

764.088	3	2288.242	c_{19}	2288.242	0.0
1203.142	2	2403.267	c_{20}	2403.269	-0.8
802.431	3	2403.271	c_{20}	2403.269	0.8
602.075	4	2403.270	c_{20}	2403.269	0.3
641.100	4	2559.371	c_{21}	2559.370	0.3
513.082	5	2559.372	c_{21}	2559.370	0.7
658.859	4	2630.407	c_{22}	2630.407	-0.2
691.120	4	2759.451	c_{23}	2759.450	0.4
553.096	5	2759.446	c_{23}	2759.450	-1.4
963.845	3	2887.511	c_{24}	2887.508	0.8
723.134	4	2887.505	c_{24}	2887.508	-1.2
987.525	3	2958.551	c_{25}	2958.545	2.1
740.894	4	2958.544	c_{25}	2958.545	-0.4
592.916	5	2958.546	c_{25}	2958.545	0.1
1030.539	3	3087.592	c_{26}	3087.588	1.5
773.154	4	3087.587	c_{26}	3087.588	-0.2
618.725	5	3087.589	c_{26}	3087.588	0.5
632.932	5	3158.625	c_{27}	3158.625	0.1
1092.558	3	3273.651	c_{28}	3273.652	-0.3
819.671	4	3273.654	c_{28}	3273.652	0.6
655.938	5	3273.653	c_{28}	3273.652	0.2
851.946	4	3401.750	c_{29}	3401.747	0.8
681.557	5	3401.750	c_{29}	3401.747	0.8
883.970	4	3529.846	c_{30}	3529.842	1.1

707.377	5	3529.844	c_{30}	3529.842	0.6
732.987	5	3657.893	c_{31}	3657.900	-1.9
966.003	4	3857.980	c_{33}	3857.980	-0.1
773.005	5	3857.984	c_{33}	3857.980	1.0
644.338	6	3857.984	c_{33}	3857.980	1.0
1123.578	4	4488.279	c_{38}	4488.271	1.7
658.630	7	4601.358	c_{39}	4601.355	0.7
854.264	6	5116.535	c_{43}	5116.541	-1.2
1050.529	5	5244.605	c_{44}	5244.600	0.9
875.608	6	5244.601	c_{44}	5244.600	0.3
750.665	7	5244.602	c_{44}	5244.600	0.5
760.814	7	5315.640	c_{45}	5315.637	0.7
906.295	6	5428.724	c_{46}	5428.721	0.6
776.969	7	5428.725	c_{46}	5428.721	0.7
758.152	8	6054.150	c_{51}	6054.149	0.2
777.910	8	6212.219	c_{53}	6212.218	0.2
907.330	7	6341.256	c_{54}	6341.260	-0.7
794.040	8	6341.259	c_{54}	6341.260	-0.3
923.763	7	6456.288	c_{55}	6456.287	0.1
808.420	8	6456.294	c_{55}	6456.287	1.0
718.706	9	6456.289	c_{55}	6456.287	0.3
956.494	7	6684.400	c_{57}	6684.398	0.3
837.058	8	6684.402	c_{57}	6684.398	0.5
744.163	9	6684.398	c_{57}	6684.398	-0.1

1338.695	5	6684.433	$\boldsymbol{c}_{57}$	6684.398	5.1
1115.746	6	6684.424	$\boldsymbol{c}_{57}$	6684.398	3.8
853.186	8	6813.424	$\boldsymbol{c}_{58}$	6813.441	-2.5
900.462	8	7191.631	$\boldsymbol{c}_{61}$	7191.631	-0.1
800.522	9	7191.631	$\boldsymbol{c}_{61}$	7191.631	0.0
720.570	10	7191.617	$\boldsymbol{c}_{61}$	7191.631	-1.9
814.860	9	7320.671	$\boldsymbol{c}_{62}$	7320.674	-0.3
835.538	9	7506.770	$\boldsymbol{c}_{64}$	7506.774	-0.5
752.087	10	7506.797	$\boldsymbol{c}_{64}$	7506.774	3.0
849.771	9	7634.872	$\boldsymbol{c}_{65}$	7634.869	0.3
796.308	10	7949.006	$\boldsymbol{c}_{68}$	7948.992	1.8
809.312	10	8078.035	$\boldsymbol{c}_{69}$	8078.034	0.0
940.259	9	8448.258	$\boldsymbol{c}_{72}$	8448.256	0.2
787.585	11	8647.344	$\boldsymbol{c}_{74}$	8647.352	-0.9
879.147	10	8776.392	$\boldsymbol{c}_{75}$	8776.394	-0.2
855.166	11	9390.735	$\boldsymbol{c}_{81}$	9390.733	0.2
827.889	13	10743.459	$\boldsymbol{c}_{93}$	10743.449	0.9
1061.548	2	2120.079	$\boldsymbol{z}^{\bullet}_{19}$	2120.076	1.4
1127.067	2	2251.118	$\boldsymbol{z}^{\bullet}_{20}$	2251.117	0.6
1191.112	2	2379.208	$\boldsymbol{z}^{\bullet}_{21}$	2379.212	-1.5
860.778	3	2578.310	$\boldsymbol{z}^{\bullet}_{23}$	2578.307	0.9
1421.728	2	2840.439	$\boldsymbol{z}^{\bullet}_{25}$	2840.439	0.1
948.154	3	2840.440	$\boldsymbol{z}^{\bullet}_{25}$	2840.439	0.3
1486.253	2	2969.489	$\boldsymbol{z}^{\bullet}_{26}$	2969.482	2.4

991.169	3	2969.483	$z^{\bullet}_{26}$	2969.482	0.3
1029.512	3	3084.512	$z^{\bullet}_{27}$	3084.509	0.9
1148.898	3	3441.669	$z^{\bullet}_{30}$	3441.662	1.9
1187.238	3	3556.688	$z^{\bullet}_{31}$	3556.689	-0.3
1301.315	3	3898.919	$z^{\bullet}_{34}$	3898.916	0.8
1344.329	3	4027.961	$z^{\bullet}_{35}$	4027.959	0.5
1382.025	3	4141.050	$z^{\bullet}_{36}$	4141.043	1.8
1424.724	3	4269.145	$z^{\bullet}_{37}$	4269.138	1.8
1448.401	3	4340.177	$z^{\bullet}_{38}$	4340.175	0.4
1510.436	3	4526.282	$z^{\bullet}_{40}$	4526.275	1.6
1172.104	4	4682.384	$z^{\bullet}_{41}$	4682.376	1.6
1204.364	4	4811.426	$z^{\bullet}_{42}$	4811.419	1.4
963.692	5	4811.421	$z^{\bullet}_{42}$	4811.419	0.4
1629.498	3	4882.465	$z^{\bullet}_{43}$	4882.456	1.9
1222.374	4	4882.461	$z^{\bullet}_{43}$	4882.456	1.0
1678.522	3	5029.539	$z^{\bullet}_{44}$	5029.524	2.9
1259.142	4	5029.532	$z^{\bullet}_{44}$	5029.524	1.6
1291.400	4	5158.566	$z^{\bullet}_{45}$	5158.567	-0.3
1348.189	4	5385.722	$z^{\bullet}_{47}$	5385.705	3.1
1078.751	5	5385.714	$z^{\bullet}_{47}$	5385.705	1.6
1373.452	4	5486.772	$z^{\bullet}_{48}$	5486.753	3.4
1098.961	5	5486.763	$z^{\bullet}_{48}$	5486.753	1.8
1124.765	5	5615.786	$z^{\bullet}_{49}$	5615.795	-1.7
813.847	7	5686.877	$z^{\bullet}_{50}$	5686.832	7.8

1455.729	4	5815.881	$z^{\bullet}_{51}$	5815.875	1.1
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1075.382	6	6443.241	$z^{\bullet}_{56}$	6443.234	1.1
1335.690	5	6669.408	$z^{\bullet}_{58}$	6669.402	0.8
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988.807	7	6910.592	$z^{\bullet}_{60}$	6910.581	1.5
1007.241	7	7039.627	$z^{\bullet}_{61}$	7039.624	0.5
1196.454	6	7168.675	$z^{\bullet}_{62}$	7168.667	1.1
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1102.138	7	7703.906	$z^{\bullet}_{66}$	7703.894	1.5
1307.164	6	7832.933	$z^{\bullet}_{67}$	7832.937	-0.5
1120.568	7	7832.918	$z^{\bullet}_{67}$	7832.937	-2.5
1328.517	6	7961.052	$z^{\bullet}_{68}$	7961.032	2.6
1138.871	7	7961.040	$z^{\bullet}_{68}$	7961.032	1.1
996.638	8	7961.043	$z^{\bullet}_{68}$	7961.032	1.3
1207.477	7	8440.276	$z^{\bullet}_{72}$	8440.270	0.7
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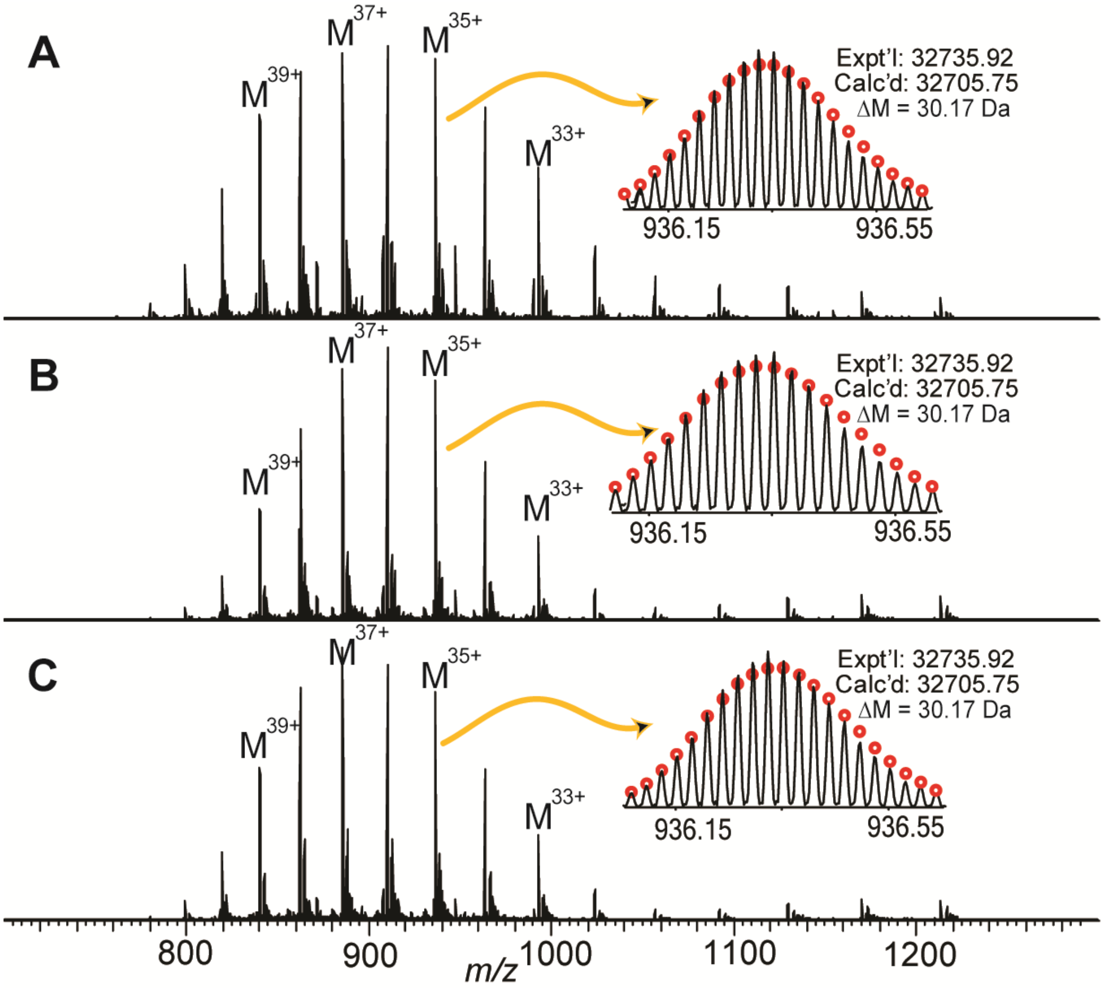
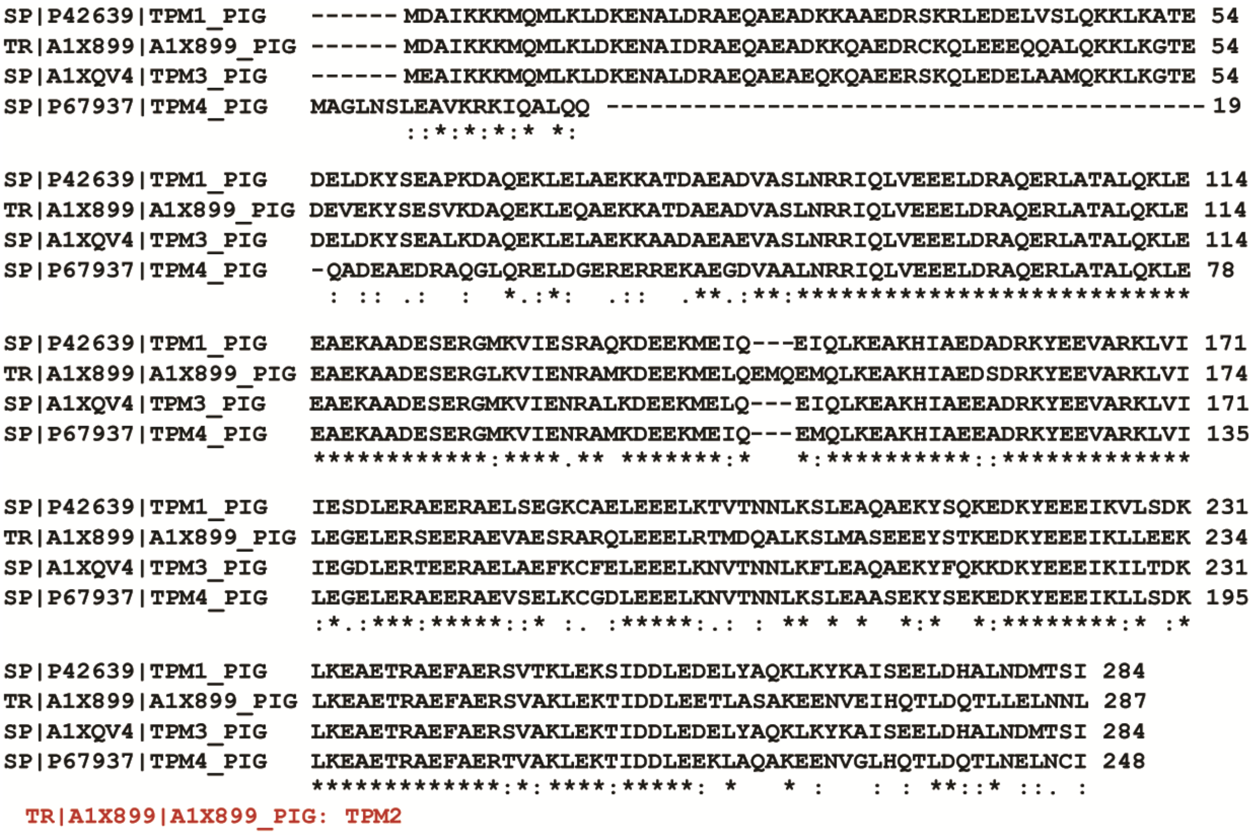
Supplemental Figures



Supplemental Figure 1. Broadband ESI/FTMS spectra of swine Tm purified from three different domestic swine hearts (A), (B) and (C). Isotopically resolved molecular ions of the predominant swine Tm from three swine hearts were demonstrated in the spectra with highly accurate molecular weights measured. Circles: the theoretical isotopic distribution of isotopomer peaks. Calc'd, calculated most abundant molecular weight. Expt'l, experimental most abundant molecular weight. Molecular weights were calculated based on DNA-prdicted sequence of Sus scrofa Tm (UnitprotKB/Swiss-Prot P42639, TPM1_PIG) without modification.



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SP|P67937|TPM4_PIG   MAGLNSLEAVKRKIQALQQ----------------------------------------- 19
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TR|A1X899|A1X899_PIG DEVEKYSESVKDAQEKLEQAEKKATDAEADVASLNRRIQLVEEELDRAQERLATALQKLE 114
SP|A1XQV4|TPM3_PIG   DELDKYSEALKDAQEKLELAEKKAADAEAEVASLNRRIQLVEEELDRAQERLATALQKLE 114
SP|P67937|TPM4_PIG   -QADEAEDRAQGLQRELDGERERREKAEGDVAALNRRIQLVEEELDRAQERLATALQKLE 78
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TR|A1X899|A1X899_PIG LEGELERSEERAEVAESRARQLEEELRTMDQALKSLMASEEEYSTKEDKYEEEIKLLEEK 234
SP|A1XQV4|TPM3_PIG   IEGDLERTEERAELAEFKCFELEEELKNVTNNLKFLEAQAEKYFQKKDKYEEEIKILTDK 231
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                     :*.:***:*****::* :. :*****:.: : ** * *  *:*  *:********:* :*

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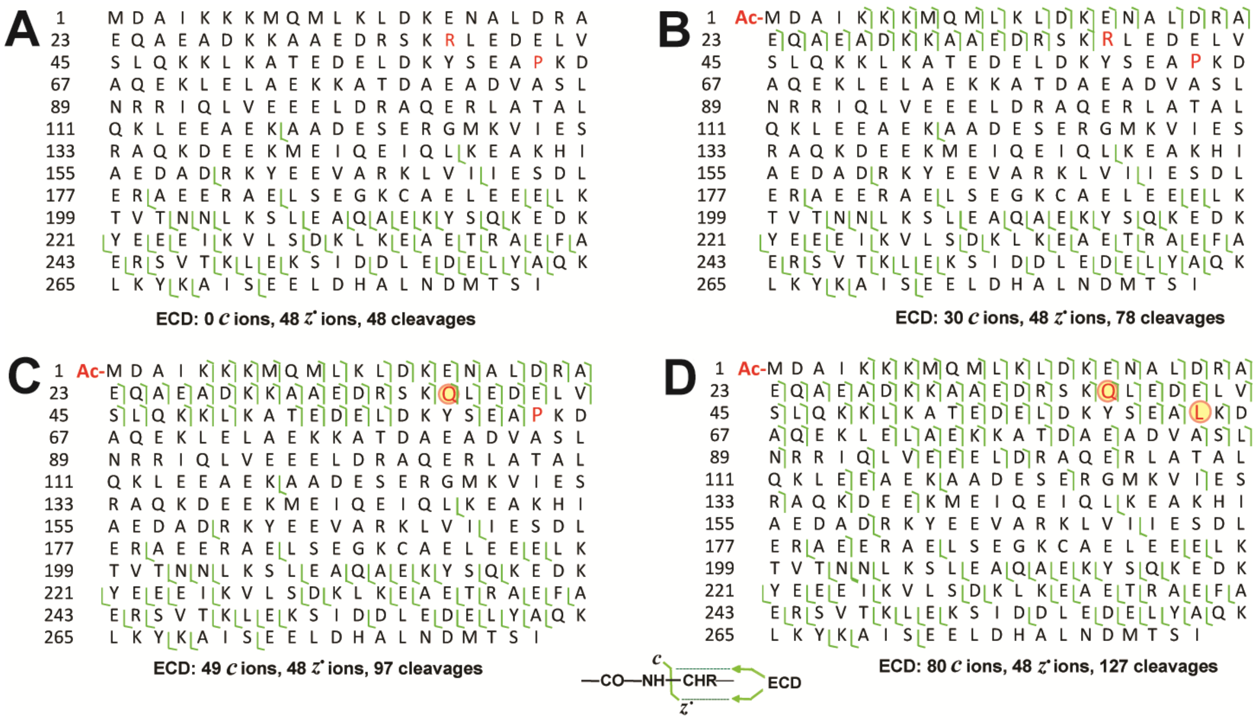
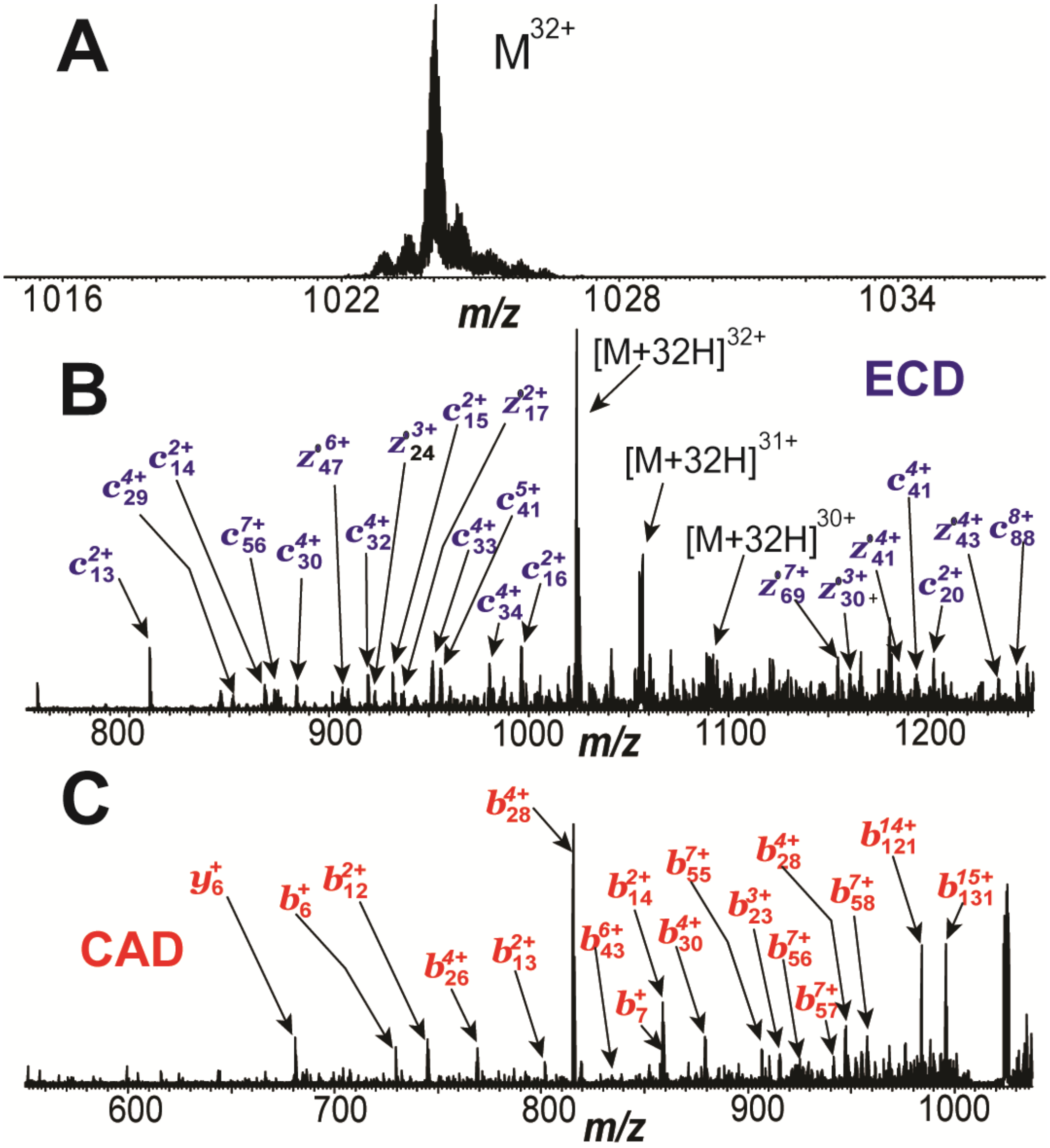
SP|A1XQV4|TPM3_PIG   LKEAETRAEFAERSVAKLEKTIDDLEDELYAQKLKYKAISEELDHALNDMTSI 284
SP|P67937|TPM4_PIG   LKEAETRAEFAERTVAKLEKTIDDLEEKLAQAKEENVGLHQTLDQTLNELNCI 248
                     *************:*:****:*****: *   * :   : : **::* ::. :
  TR|A1X899|A1X899_PIG: TPM2
```

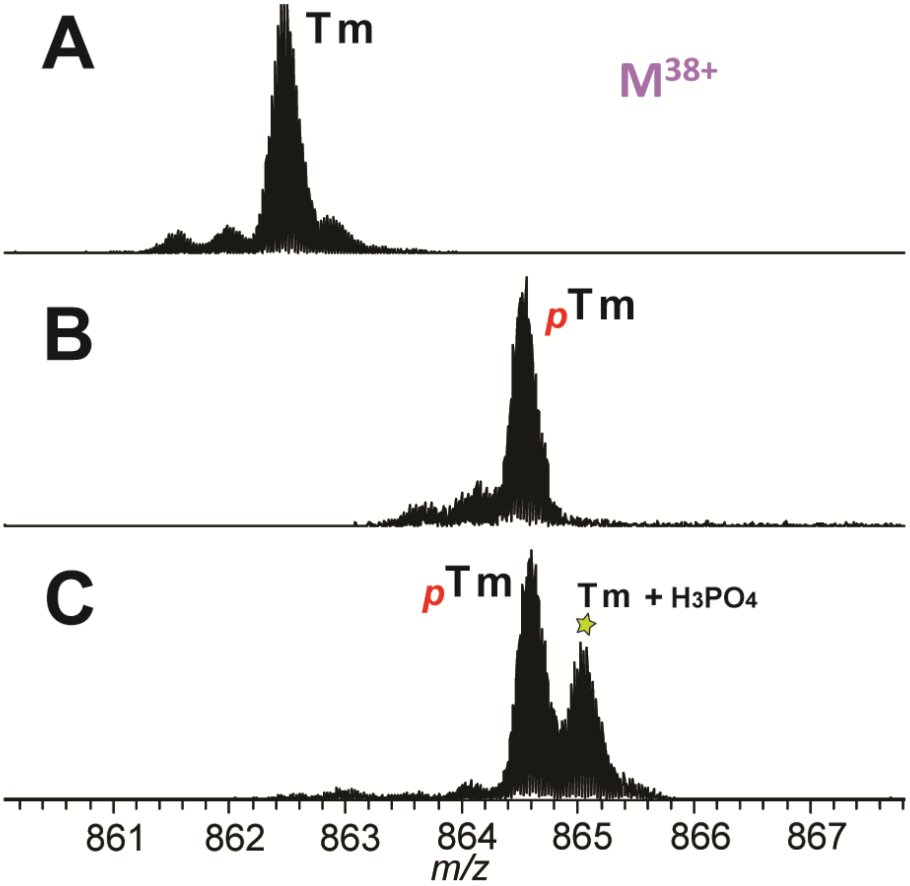
Supplemental Figure 2. Protein sequence homology alignment of multiple swine Tm isoforms. The swine isoforms presented here are encoded by *TPM1*, *TPM2*, *TPM3* and *TPM4* genes, respectively (UniprotKB/Swiss-Prot database). Asterisks indicate the conserved sequence regions. A1X899_PIG is encoded by *TPM2* gene but the sequence is marked as "unreviewed" in the SwissProt database.



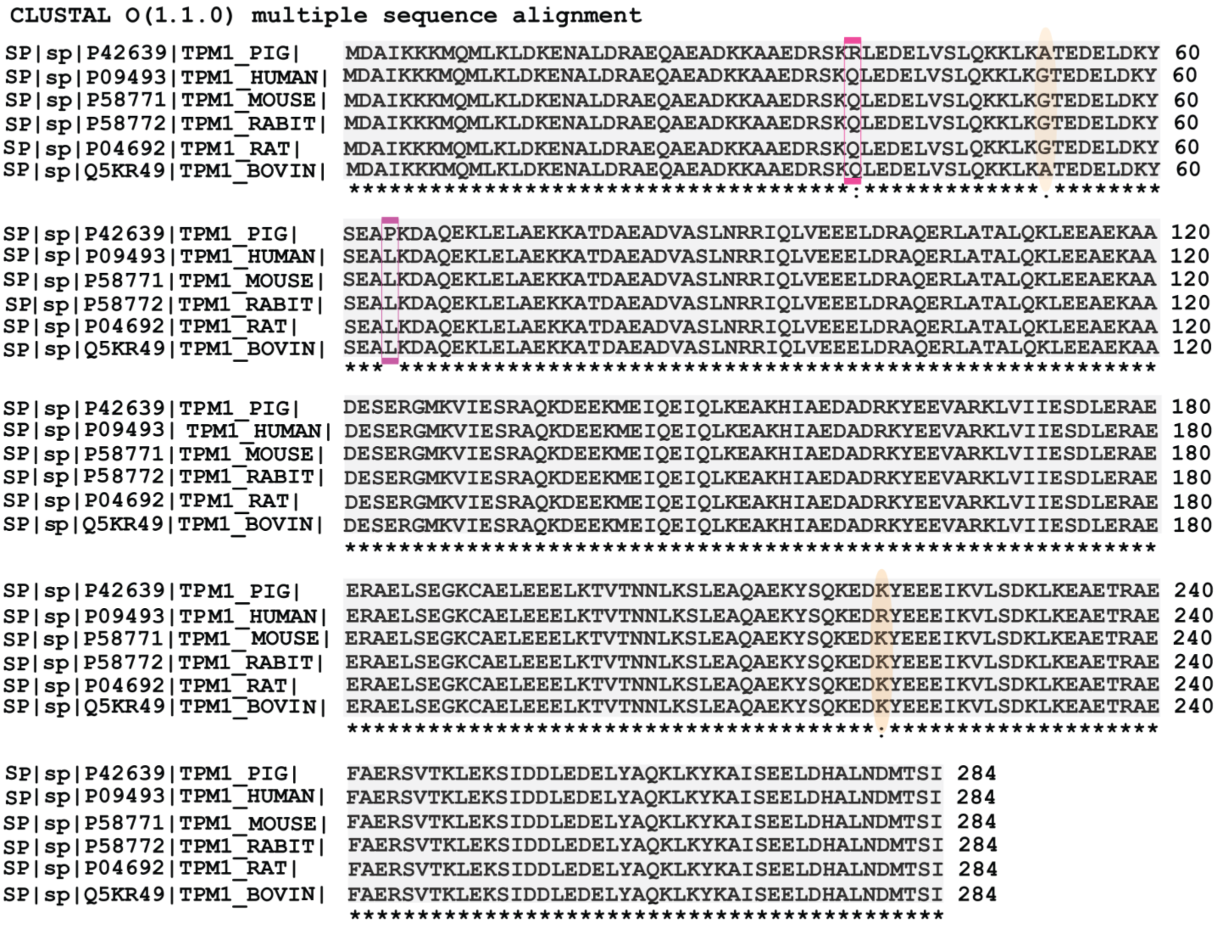
Supplemental Figure 3. MS/MS product ion map from one ECD spectrum of predominant swine Tm for assignment to un-phosphorylated swine α–Tm. (A) Fragment assignments were made to the DNA-predicted sequence of *Sus scrofa* (UnitprotKB/Swiss-Prot P42639, TPM1_PIG) without any modifications. (B) Fragment assignments were made to the *Sus scrofa* Tm sequence with acetylation of the first N-terminal amino acid. (C) Fragment assignments were made to the Sus scrofa Tm sequence with acetylation and one amino acid polymorphism R38→Q. (D) Fragment assignments were made to the *Sus scrofa* Tm sequence with acetylation and two amino acid polymorphisms R38→Q and P64→L. Two amino acid polymorphisms were highlighted by orange circles.



Supplemental Figure 4. (A) Isolation of single charge state (M^{32+}) of α–Tm precursor ions, (B) ECD and (C) CAD spectra of the isolated α–Tm ions. The product ions in (B) and (C) were assigned according to the sequence of swine α–Tm (UnitprotKB/Swiss-Prot P42639, TPM1_PIG) with acetylation of the first amino acid at the N-terminus and two amino acid polymorphisms.



Supplemental Figure 5. Gas-phase separation (purification) of swine Tm. Isolation of a single charge state (M$^{38+)}$ of (A) un-phosphorylated swine α–Tm, (B) mono-phosphorylated swine α–Tm, and (C) mixture of un-phosphorylated (phosphoric acid adduct) and mono-phosphorylated swine Tm. $_{p}$Tm, mono-phosphorylated pig α–Tm. H_3PO_4, non-covalent adduct of phosphoric acid.



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CLUSTAL O(1.1.0) multiple sequence alignment

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SP|sp|P09493|TPM1_HUMAN|   MDAIKKKMQMLKLDKENALDRAEQAEADKKAAEDRSKQLEDELVSLQKKLKGTEDELDKY 60
SP|sp|P58771|TPM1_MOUSE|   MDAIKKKMQMLKLDKENALDRAEQAEADKKAAEDRSKQLEDELVSLQKKLKGTEDELDKY 60
SP|sp|P58772|TPM1_RABIT|   MDAIKKKMQMLKLDKENALDRAEQAEADKKAAEDRSKQLEDELVSLQKKLKGTEDELDKY 60
SP|sp|P04692|TPM1_RAT|     MDAIKKKMQMLKLDKENALDRAEQAEADKKAAEDRSKQLEDELVSLQKKLKGTEDELDKY 60
SP|sp|Q5KR49|TPM1_BOVIN|   MDAIKKKMQMLKLDKENALDRAEQAEADKKAAEDRSKQLEDELVSLQKKLKATEDELDKY 60
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SP|sp|P58771|TPM1_MOUSE|   SEALKDAQEKLELAEKKATDAEADVASLNRRIQLVEEELDRAQERLATALQKLEEAEKAA 120
SP|sp|P58772|TPM1_RABIT|   SEALKDAQEKLELAEKKATDAEADVASLNRRIQLVEEELDRAQERLATALQKLEEAEKAA 120
SP|sp|P04692|TPM1_RAT|     SEALKDAQEKLELAEKKATDAEADVASLNRRIQLVEEELDRAQERLATALQKLEEAEKAA 120
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SP|sp|P09493|TPM1_HUMAN|   DESERGMKVIESRAQKDEEKMEIQEIQLKEAKHIAEDADRKYEEVARKLVIIESDLERAE 180
SP|sp|P58771|TPM1_MOUSE|   DESERGMKVIESRAQKDEEKMEIQEIQLKEAKHIAEDADRKYEEVARKLVIIESDLERAE 180
SP|sp|P58772|TPM1_RABIT|   DESERGMKVIESRAQKDEEKMEIQEIQLKEAKHIAEDADRKYEEVARKLVIIESDLERAE 180
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SP|sp|P04692|TPM1_RAT|     FAERSVTKLEKSIDDLEDELYAQKLKYKAISEELDHALNDMTSI 284
SP|sp|Q5KR49|TPM1_BOVIN|   FAERSVTKLEKSIDDLEDELYAQKLKYKAISEELDHALNDMTSI 284
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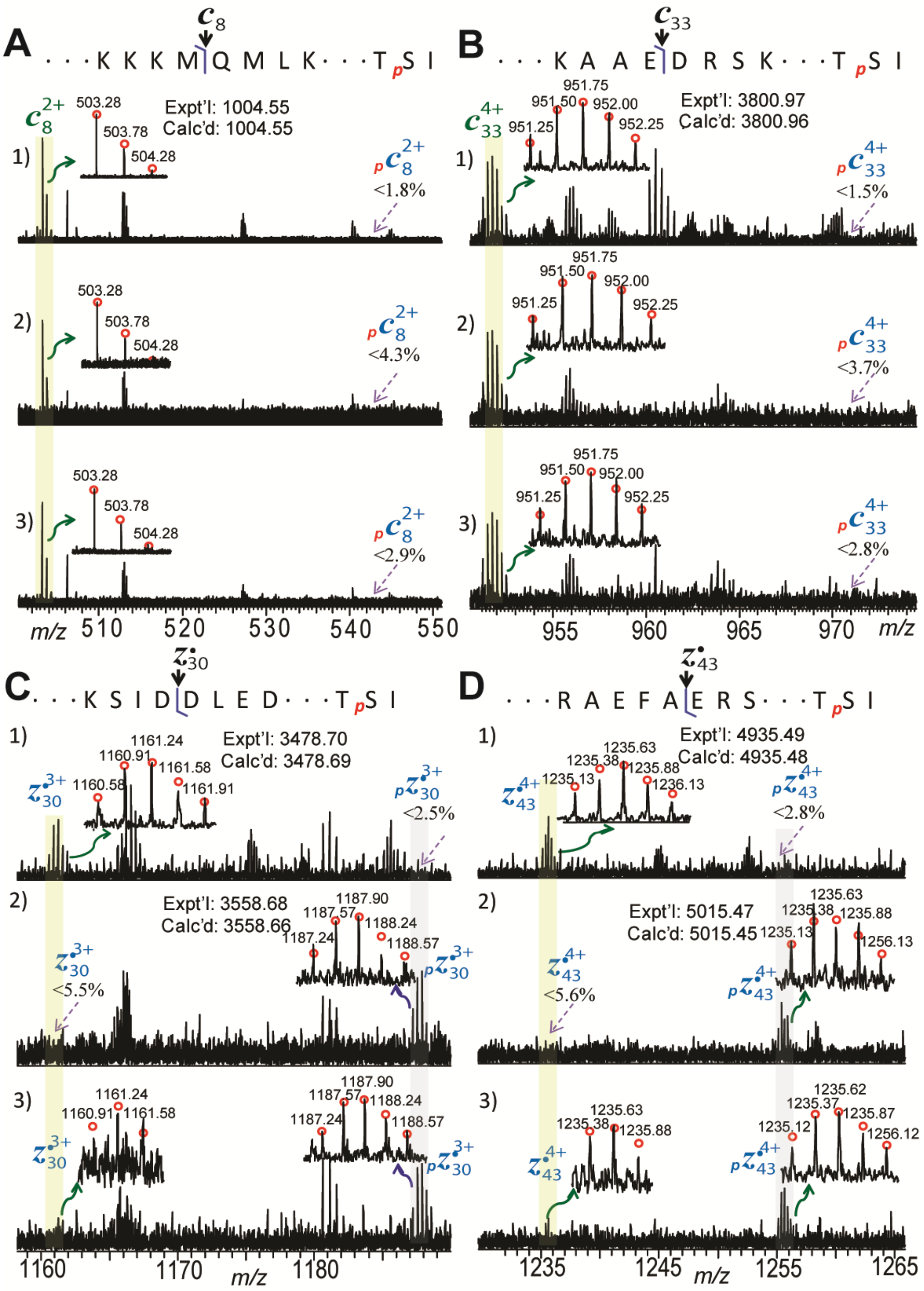
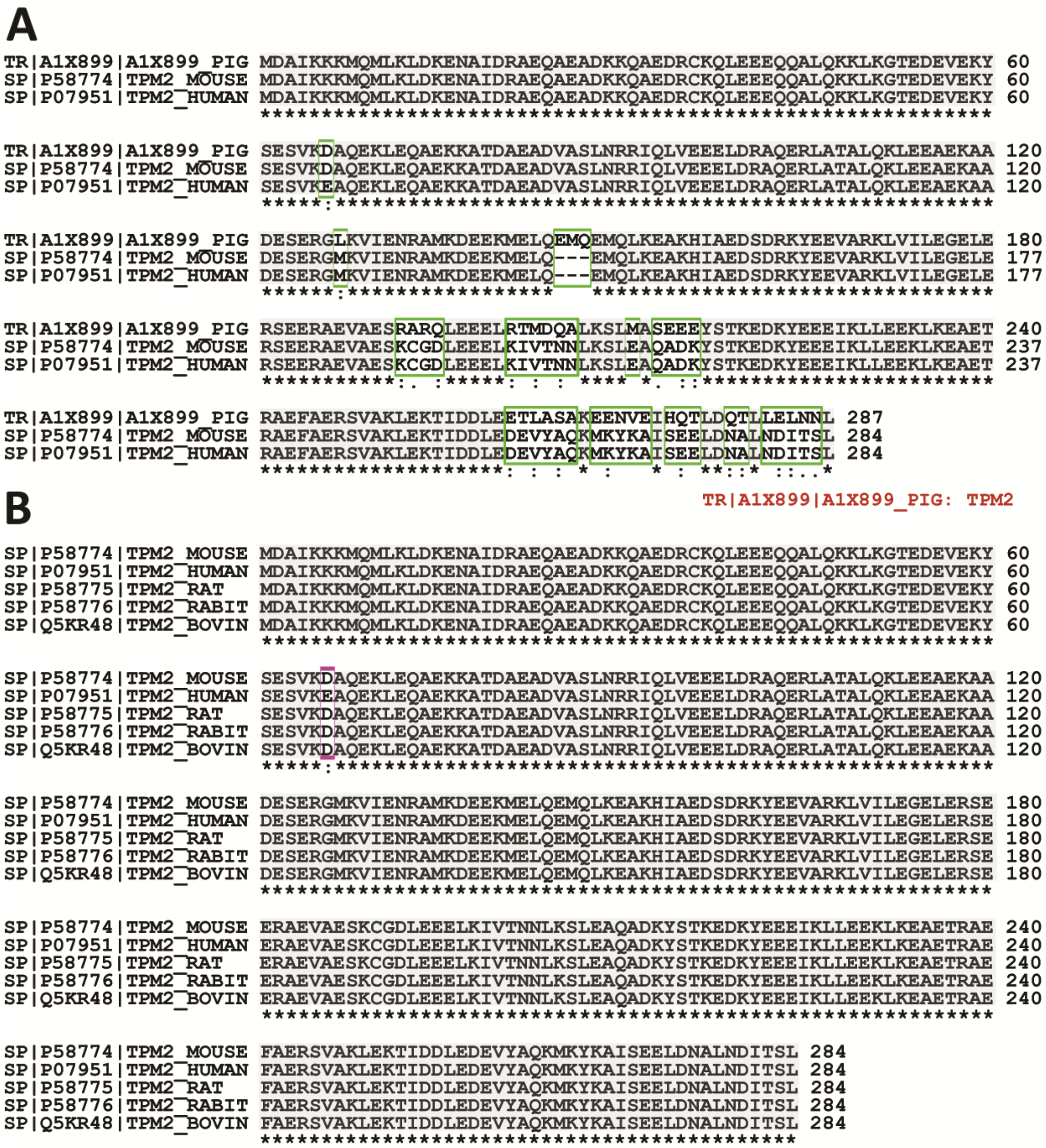
Supplementary Figure 6. Protein sequence homology alignment of α–Tm encoded by gene *TPM1*. The highlighted area in purple indicates different amino acid sequences across the α–Tm chains between mouse and human (UniprotKB/Swiss-Prot database). Asterisks indicate the conserved sequence regions. The four common amino acid substitutions were highlighted in purple rectangles (for R38→Q and P64→L) and orange ovals (for G52→A and K220→R), respectively.



Supplemental Figure 7. Representative ECD fragment ions for mapping the mono-phosphorylation site of swine α–Tm. (A)-(D), MS/MS spectra of ***c*** and ***z***˙ ions from ECD spectra of (1) un-, (2) mono-phosphorylated Tm, and (3) mixture of un-phosphorylated (phosphoric acid adduct) and mono-phosphorylated swine Tm. Circles: the theoretical isotopic distribution of isotopomer peaks. Calc'd, calculated most abundant molecular weight. Expt'l, experimental most abundant molecular weight.



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SP|P58774|TPM2_MOUSE ERAEVAESKCGDLEEELKIVTNNLKSLEAQADKYSTKEDKYEEEIKLLEEKLKEAETRAE 240
SP|P07951|TPM2_HUMAN ERAEVAESKCGDLEEELKIVTNNLKSLEAQADKYSTKEDKYEEEIKLLEEKLKEAETRAE 240
SP|P58775|TPM2_RAT   ERAEVAESKCGDLEEELKIVTNNLKSLEAQADKYSTKEDKYEEEIKLLEEKLKEAETRAE 240
SP|P58776|TPM2_RABIT ERAEVAESKCGDLEEELKIVTNNLKSLEAQADKYSTKEDKYEEEIKLLEEKLKEAETRAE 240
SP|Q5KR48|TPM2_BOVIN ERAEVAESKCGDLEEELKIVTNNLKSLEAQADKYSTKEDKYEEEIKLLEEKLKEAETRAE 240
                     ************************************************************

SP|P58774|TPM2_MOUSE FAERSVAKLEKTIDDLEDEVYAQKMKYKAISEELDNALNDITSL 284
SP|P07951|TPM2_HUMAN FAERSVAKLEKTIDDLEDEVYAQKMKYKAISEELDNALNDITSL 284
SP|P58775|TPM2_RAT   FAERSVAKLEKTIDDLEDEVYAQKMKYKAISEELDNALNDITSL 284
SP|P58776|TPM2_RABIT FAERSVAKLEKTIDDLEDEVYAQKMKYKAISEELDNALNDITSL 284
SP|Q5KR48|TPM2_BOVIN FAERSVAKLEKTIDDLEDEVYAQKMKYKAISEELDNALNDITSL 284
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Supplementary Figure 8. Protein sequence homology alignment of β–Tm encoded by gene *TPM2*. The highlighted area in purple indicates different amino acid sequences across the β–Tm chains among various species (UniprotKB/Swiss-Prot database). Asterisks indicate the conserved sequence regions. A1X899_PIG is encoded by *TPM2* but the sequence is marked as “unreviewed” in the SwissProt database.