SUPPLEMENTAL FIGURE CAPTIONS

Supplemental Figure S1. Extracted ion chromatograms for the K4-trimethylated H3 T3-R8 peptide and the H3 K9-R17 peptide derivatized at their n-termini by either propionylation (top graph) or by phenyl isocyanate (bottom graph).

Supplemental Figure S2. Comparison of the workflow of the standard Prop-x2 method with two rounds of double-propionylation (pre- and post-digest with trypsin, top) and the Prop-PIC hybrid method with pre-digest single-round propionylation and post-digest PIC-labeling (bottom).

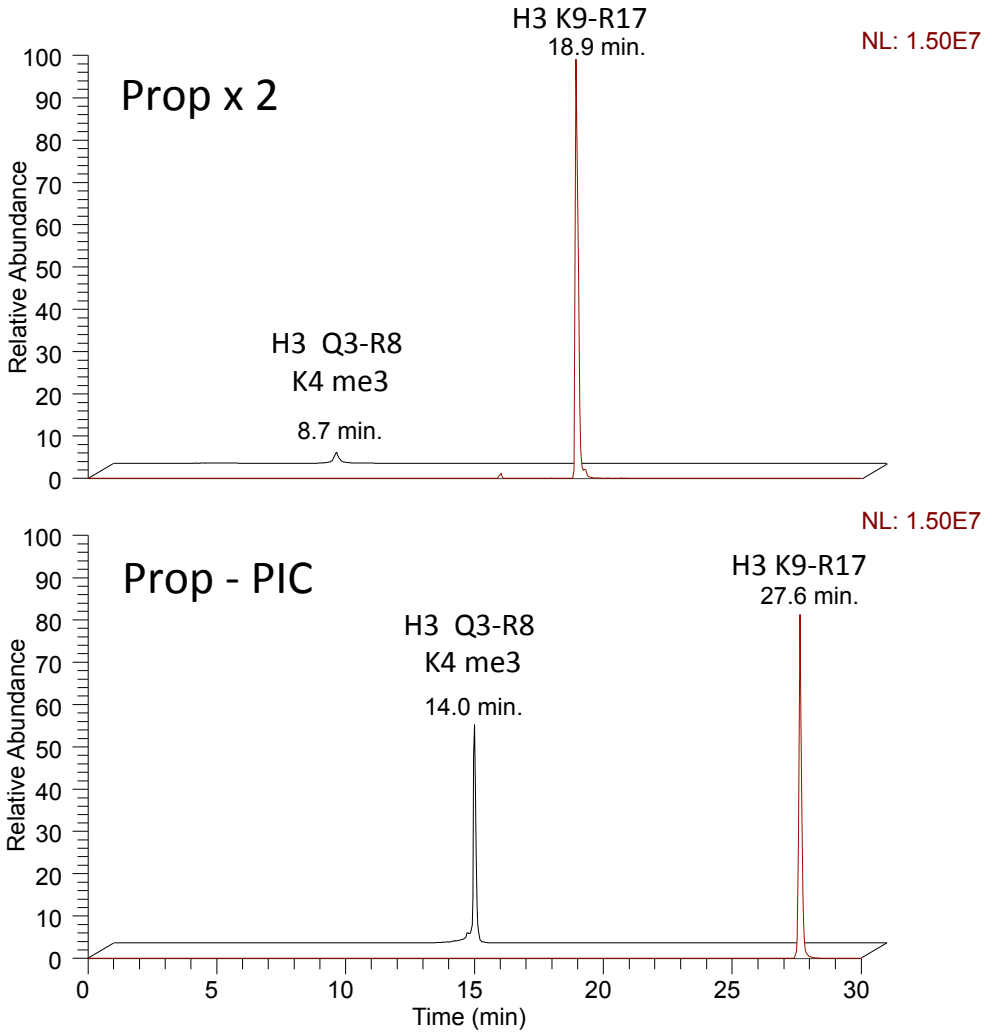
Supplemental Figure S3. Workflow schematic of peptide peak identification and quantification with the Fishtones program (more documentation at http://research-pub.gene.com/fishtones-js/howto): MS1-browser window (**A**) with peak areas for the K4-dimethyl H3 T3-R8 peptide from purified PC9 cell histones (see Figure 5A). Blue and red traces correspond to the light and heavy channels in this SILAC experiment for each peptide's charge state. Single MS/MS events are displayed as pie-charts, depicting matched product ion intensities as a heatmap. Selecting the pie-charts opens up a mini-heatmap and annotated product ion spectra are obtained by a mouse-click. This spectrum can be expanded to a separate browser window (**B**) in which peptide identity and specific fragment ions (e.g. loss of water for the Y1-ion) can be analyzed interactively to explore alternative sequence or PTM assignments. Chromatographic peaks can be selected for quantitation (**C**), bringing up a display of the calculated areas under the curves and other relevant data.

Supplemental Figure S4. Comparison of peak areas for 53 H3.1 N-terminal PTMs between Prop-2x and Prop-PIC method as measured using the Fishtones application. Methylation marks are color coded blue, acetylation marks and their respective methyl-mark combinations are green, phosphorylation marks and combinations are orange. Products of propionylation side- reactions are color coded in grey. A. H3 Y41-R49. B. H3 T3-R8. C. H3 K18-R27. D. H3 K9-R17. **E**. H3.1 K27-R40 peptides.

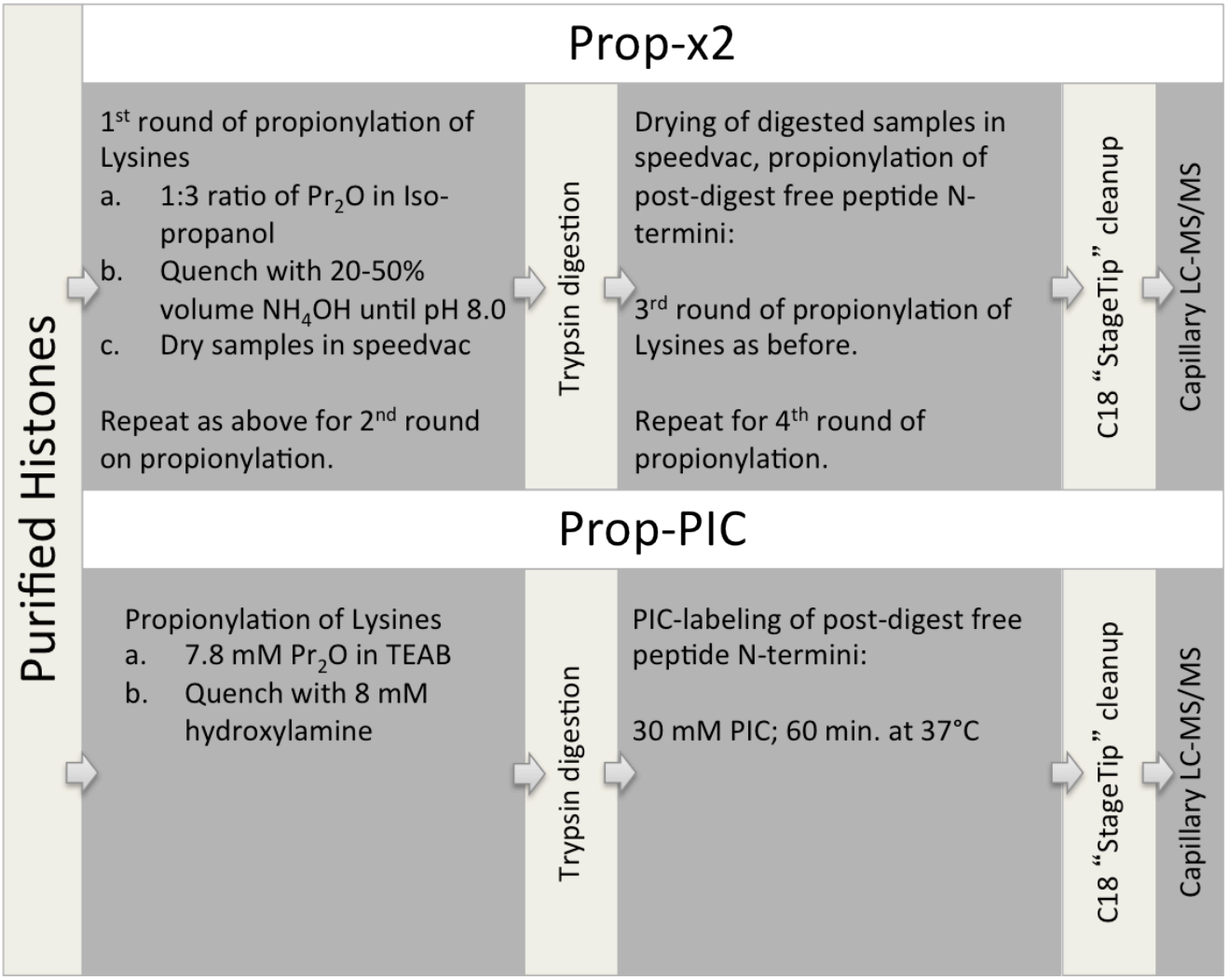
Supplemental Figure S5. Product ion spectrum of the H3 T3-R8 phospho-T3 peptide, annotated by Fishtones. The arrow designates the neutral loss of H_3PO_4 (calculated m/z = 431.22, detected at m/z = 431.23) from the precursor ion (calculated m/z = 480.22, detected at m/z = 480.28). The immonium ion of the propionylated K4 lysine residue is indicated by an asterisk.

Supplemental Table 1. Histone H3 peptides analyzed in the course of this study, displaying their sequences, molecular masses, and m/z values for their various charge states upon Prop-PIC labeling. The m/z values for peaks detected in LC-MS experiments are highlighted by color to indicate the predominant charge state(s) observed (darker colors indicate the more abundant charge states found).

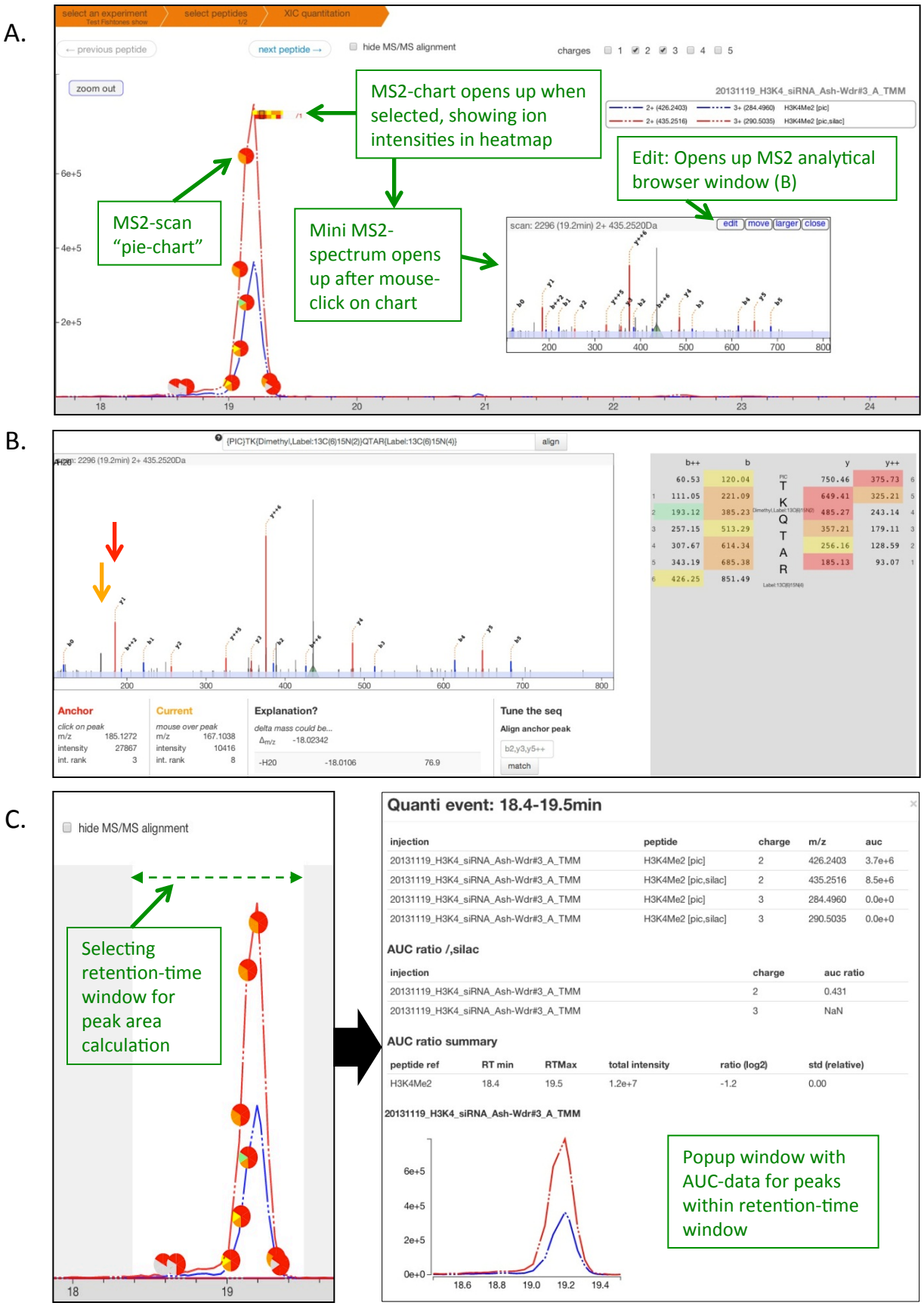
Supplemental Figure S1. Extracted ion chromatograms for the K4-trimethylated H3 T3-R8 peptide and the H3 K9-R17 peptide derivatized at their n-termini by either propionylation (top graph) or by phenyl isocyanate (bottom graph).



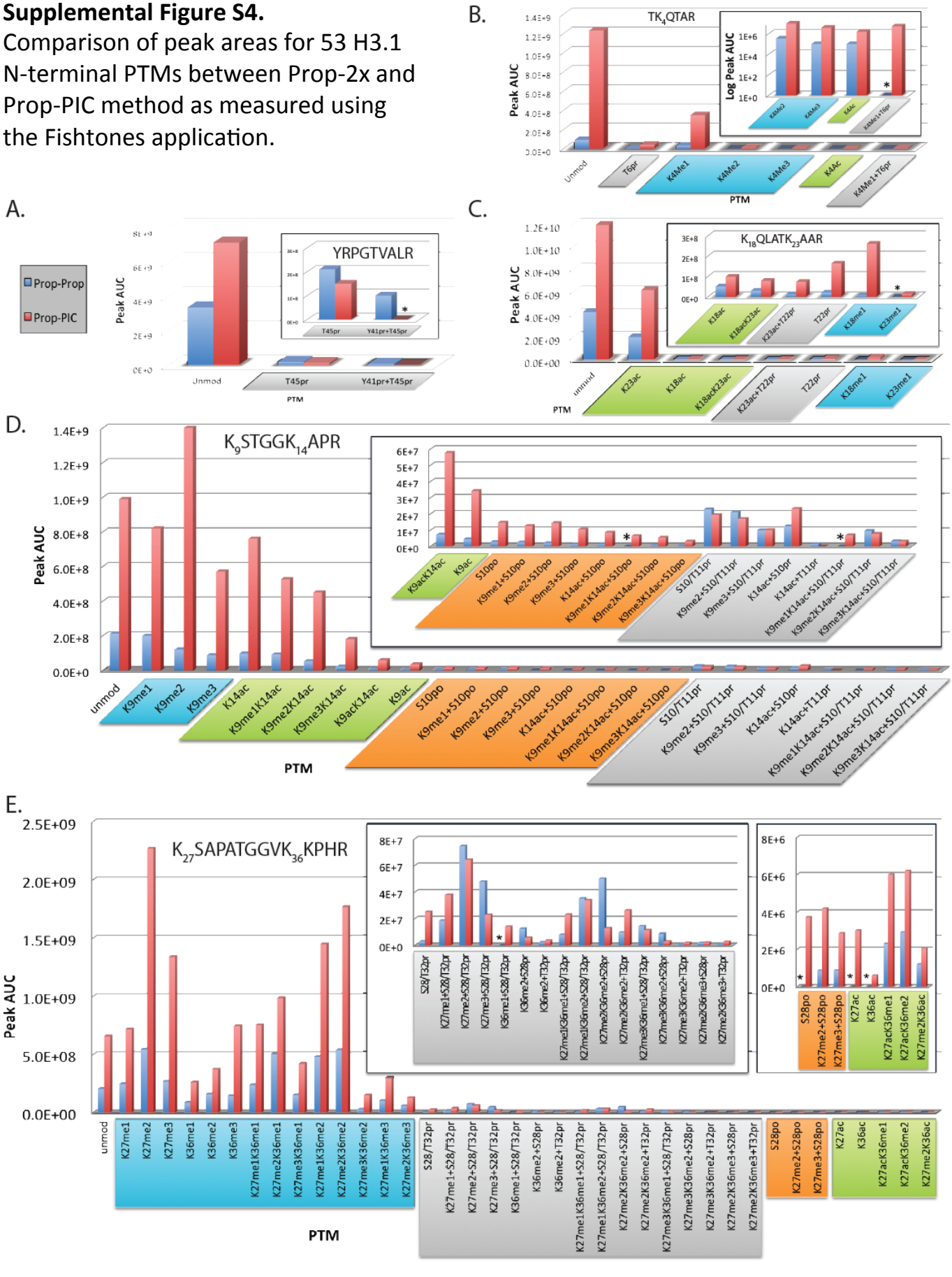
Supplemental Figure S2. Comparison of Prop-x2 and Prop-PIC workflows.



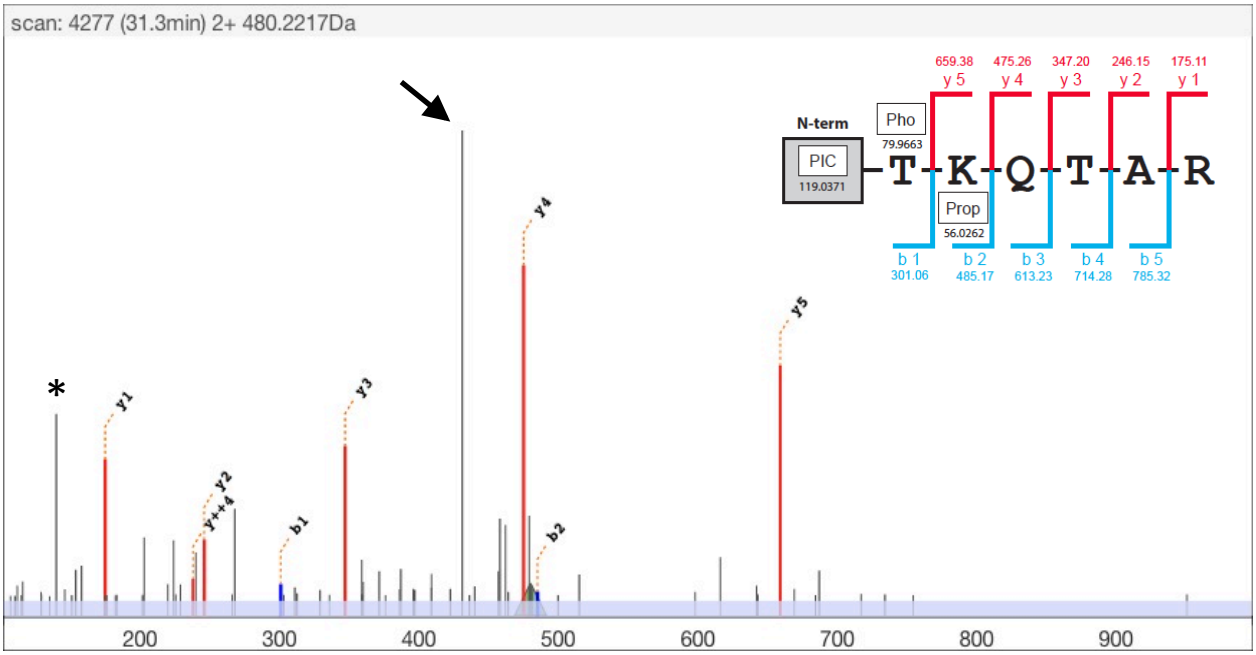
Supplemental Figure S3. Workflow schematic of peptide peak identification and quantification with the Fishtones program.



Supplemental Figure S4.
Comparison of peak areas for 53 H3.1 N-terminal PTMs between Prop-2x and Prop-PIC method as measured using the Fishtones application.



Supplemental Figure S5. Product ion spectrum of the H3 T3-R8 phospho-T3 peptide, annotated by Fishtones.



Supplemental Table 1: Histone H3 N-terminal tail peptide masses

	Sequence	M	[M+H]+	[M+2H]2+	[M+3H]3+	[M+4H]4+
H3T 3-R8	TK(Me2)QTAR	850.46595	851.4732265	426.2402515	284.4959265	213.623764
	TK(Ac)QTAR	864.44525	865.4525265	433.2299015	289.1556931	217.118589
	TK(Me3)QTAR	864.48165	865.4889265	433.2481015	289.1678265	217.127689
	TK(un)QTAR	878.46085	879.4681265	440.2377015	293.8275598	220.622489
	TK(Me1)QTAR	892.47655	893.4838265	447.2455515	298.4994598	224.126414
	T(ph)KQTAR	958.4273235	959.4346	480.221	320.4831	240.6141
H3K 9-R17	K(Me2)STGGK(Ac)APR	1089.593	1090.600276	545.8037765	364.2049431	273.4055265
	K(Ac)STGGK(Ac)APR	1103.5723	1104.579576	552.7934265	368.8647098	276.9003515
	K(Me2)STGGK(un)APR	1103.6086	1104.615876	552.8115765	368.8768098	276.9094265
	K(Me3)STGGK(Ac)APR	1103.6087	1104.615976	552.8116265	368.8768431	276.9094515
	K(un)STGGK(Ac)APR	1117.5879	1118.595176	559.8012265	373.5365765	280.4042515
	K(Ac)STGGK(un)APR	1117.5879	1118.595176	559.8012265	373.5365765	280.4042515
	K(Me3)STGGK(un)APR	1117.6243	1118.631576	559.8194265	373.5487098	280.4133515
	K(un)STGGK(un)APR	1131.6035	1132.610776	566.8090265	378.2084431	283.9081515
	K(Me1)STGGK(Ac)APR	1131.6036	1132.610876	566.8090765	378.2084765	283.9081765
	K(Me1)STGGK(un)APR	1145.6192	1146.626476	573.8168765	382.8803431	287.4120765
	K(un)S(Ph)TGGK(un)APR	1211.570024	1212.5773	606.7923	404.8639	303.8998
	K(Me1)S(Ph)TGGK(un)APR	1225.585524	1226.5928	613.8	409.5358	307.4037
	K(Me2)S(Ph)TGGK(un)APR	1183.575024	1184.5823	592.7948	395.5323	296.901
	K(Me3)S(Ph)TGGK(un)APR	1197.590624	1198.5979	599.8026	400.2042	300.4049
	K(un)S(Ph)TGGK(Ac)APR	1197.554324	1198.5616	600.0677	400.192	300.3959
	K(Me1)S(Ph)TGGK(Ac)APR	1211.569924	1212.5772	606.7922	404.8639	303.8998
	K(Me2)S(Ph)TGGK(Ac)APR	1169.559424	1170.5667	585.787	390.8604	293.3971
	K(Me3)S(Ph)TGGK(Ac)APR	1183.575024	1184.5823	592.7948	395.5323	296.901
H3K 18-R26	K(Ac)QLATK(Ac)AAR	1188.66145	1189.668726	595.3380015	397.2277598	298.172639
	K(un)QLATK(Ac)AAR	1202.67705	1203.684326	602.3458015	401.8996265	301.676539
	K(Ac)QLATK(un)AAR	1202.67705	1203.684326	602.3458015	401.8996265	301.676539
	K(un)QLATK(un)AAR	1216.69265	1217.699926	609.3536015	406.5714931	305.180439
	K(un)QLATK(Me1)AAR	1230.70835	1231.715626	616.3614515	411.2433931	308.684364
	K(Me1)QLATK(un)AAR	1230.70835	1231.715626	616.3614515	411.2433931	308.684364
H3.1 K27-R40	K(Me2)SAPATGGVK(Me2)K(un)PHR	1663.95209	1664.959366	832.9833215	555.6579731	416.995299
	K(Me2)SAPATGGVK(Ac)K(un)PHR	1677.93139	1678.938666	839.9729715	560.3177398	420.490124
	K(Ac)SAPATGGVK(Me2)K(un)PHR	1677.93139	1678.938666	839.9729715	560.3177398	420.490124
	K(Me2)SAPATGGVK(Me3)K(un)PHR	1677.96779	1678.975066	839.9911715	560.3298731	420.499224
	K(Me3)SAPATGGVK(Me2)K(un)PHR	1677.96779	1678.975066	839.9911715	560.3298731	420.499224
	K(un)SAPATGGVK(Me2)K(un)PHR	1691.94699	1692.954266	846.9807715	564.9896065	423.994024
	K(Me2)SAPATGGVK(un)K(un)PHR	1691.94699	1692.954266	846.9807715	564.9896065	423.994024
	K(un)SAPATGGVK(Ac)K(un)PHR	1705.92629	1706.933566	853.9704215	569.6493731	427.488849
	K(Ac)SAPATGGVK(un)K(un)PHR	1705.92629	1706.933566	853.9704215	569.6493731	427.488849
	K(Me1)SAPATGGVK(Me2)K(un)PHR	1705.96269	1706.969966	853.9886215	569.6615065	427.497949
	K(un)SAPATGGVK(Me3)K(un)PHR	1705.96269	1706.969966	853.9886215	569.6615065	427.497949
	K(Me2)SAPATGGVK(Me1)K(un)PHR	1705.96269	1706.969966	853.9886215	569.6615065	427.497949
	K(Me3)SAPATGGVK(un)K(un)PHR	1705.96269	1706.969966	853.9886215	569.6615065	427.497949
	K(un)SAPATGGVK(un)K(un)PHR	1719.94189	1720.949166	860.9782215	574.3212398	430.992749
	K(Me1)SAPATGGVK(Ac)K(un)PHR	1719.94199	1720.949266	860.9782715	574.3212731	430.992774
	K(Ac)SAPATGGVK(Me1)K(un)PHR	1719.94199	1720.949266	860.9782715	574.3212731	430.992774
	K(Me1)SAPATGGVK(Me3)K(un)PHR	1719.97839	1720.985666	860.9964715	574.3334065	431.001874
	K(Me3)SAPATGGVK(Me1)K(un)PHR	1719.97839	1720.985666	860.9964715	574.3334065	431.001874
	K(un)SAPATGGVK(Me1)K(un)PHR	1733.95759	1734.964866	867.9860715	578.9931398	434.496674
	K(Me1)SAPATGGVK(un)K(un)PHR	1733.95759	1734.964866	867.9860715	578.9931398	434.496674
	K(Me1)SAPATGGVK(Me1)K(un)PHR	1747.97329	1748.980566	874.9939215	583.6650398	438.000599
	K(un)S(Ph)APATGGVK(un)K(un)PHR	1799.908324	1800.9156	900.9614	600.9767	450.9844
	K(Me2)S(Ph)APATGGVK(un)K(un)PHR	1771.913424	1772.9207	886.964	591.6451	443.9856
	K(Me3)S(Ph)APATGGVK(un)K(un)PHR	1785.929024	1786.9363	893.9718	596.3169	447.4895
H3 K41-R49	YRPGTVALR	1150.624724	1151.632	576.3196	384.5488	288.6635