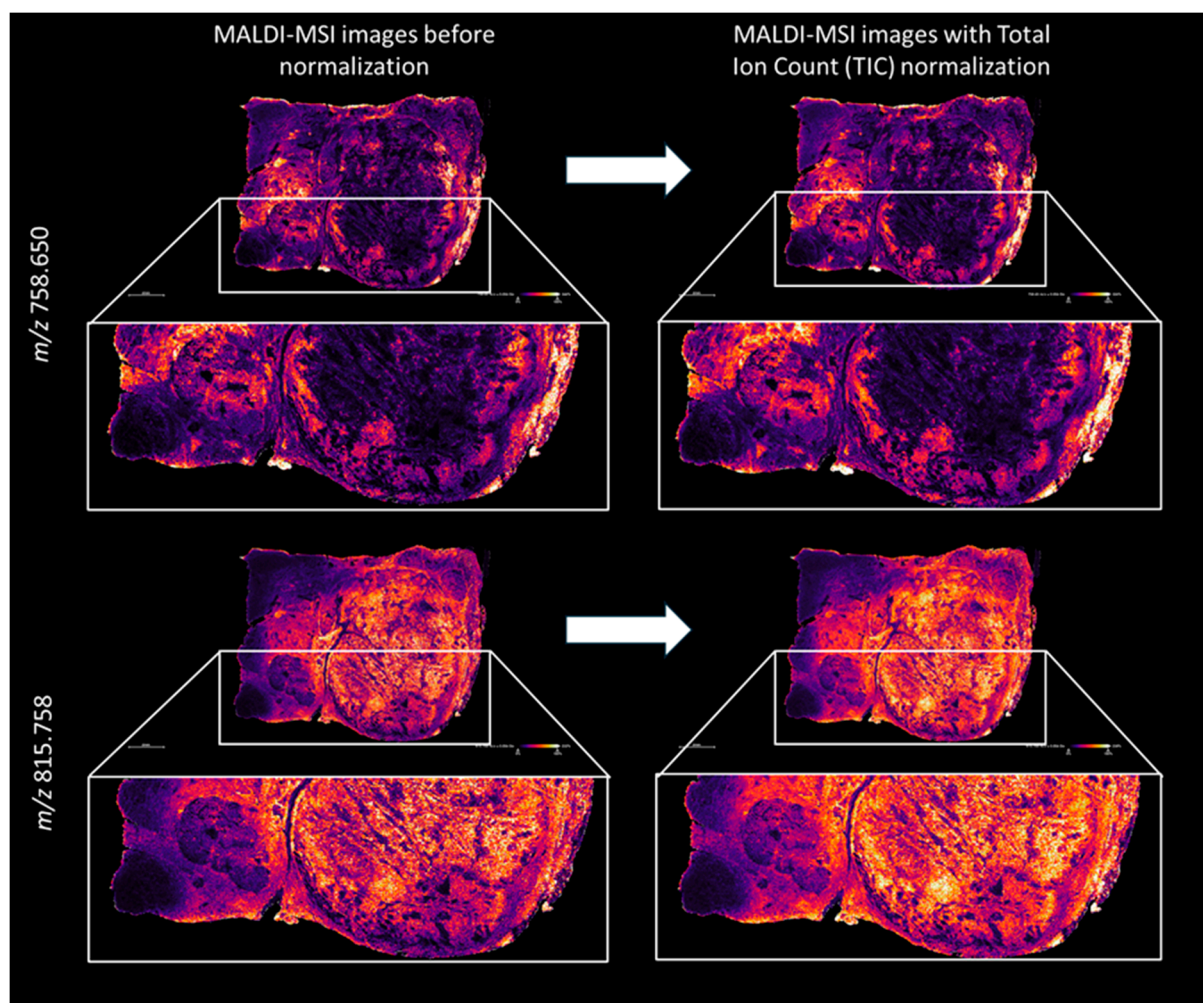
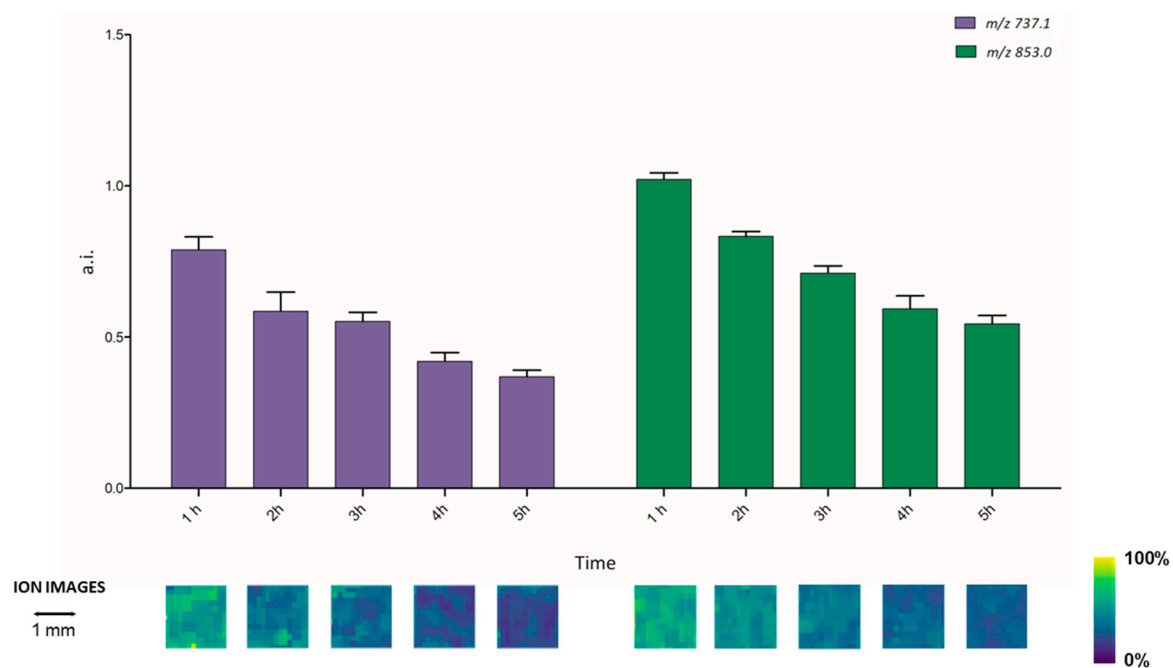


**Figure S1.** Bisecting K-Means segmentation cluster tree of the thyroid tissues analysed with ATT and CHCA, based on the proteomic data, allowed the distinction of different regions throughout the tissues.



**Figure S2.** MALDI-MSI images of FFPE ccRCC tissue analysed with ATT matrix using RapifleX MALDI TissueTyper mass spectrometer before (left panel) and after (right panel) Total Ion Count normalisation. Time of analysis ~ 7.5h.



**Figure S3.** On the top, graphical representation of ATT sublimation – considering two ATT matrix peaks  $m/z$  737.1 and 853.0 – on tissues up to 5 hours after matrix deposition. Time and absolute intensity (a.i) are presented in the X and Y axes respectively. On the bottom, ion images of the tissues section corresponding to the time of the analyses are shown.

Observed <i>m/z</i> (MALDI-MSI)	Expected <i>m/z</i>	Protein ID	Sequence	$\Delta$ ppm	$\Delta$ Da	
944.53	944.53	H2A1_HUMAN	r.AGLQFPVGR.v	2	0.00	*
1105.57	1105.58	CO1A1_HUMAN	r.VQGPPGPAGPR.g(Hydroxylated)	-9	-0.01	**
1428.71	1428.71	VIME_HUMAN	r.SLYASSPGGVYATR.s	-1	0.00	*
2215.00	2215.07	ACTB_HUMAN / ACTG_HUMAN	k.DLYANTVLSGGTTMYPGIADR.m	-32	-0.07	*
2869.43	2869.41	CO1A1_HUMAN	r.GLTGPIGPPGPAGAPGDKGESGPSGPAGPTGAR.g (Hydroxylated)	7	0.02	**
2959.43	2959.41	CO2A1_HUMAN	r.GPPGAAGAPGPFQFGPAGEPGEFGQTGPAGAR.g (Hydroxylated)	8	0.02	**
2959.40	2959.66	RRBP1_HUMAN	R.APAVAVAPTPVQPPPIIVAPVATVPAMPQEK.L	-87	-0.26	*

\* identity confirmed with internal libraries nanoLC-MS/MS  
and or in situ MS/MS

\*\* confirmed with literature data [1]

## Table S1.

## References

1. Groseclose MR, Massion PP, Chaurand P, Caprioli RM. High-throughput proteomic analysis of formalin-fixed paraffin-embedded tissue microarrays using MALDI imaging mass spectrometry. *Proteomics*. 2008;8: 3715–3724.