Supporting information for:

In-depth mass spectrometry-based proteomics of formalin-fixed, paraffin embedded tissues with a spatial resolution of 50–200 μm
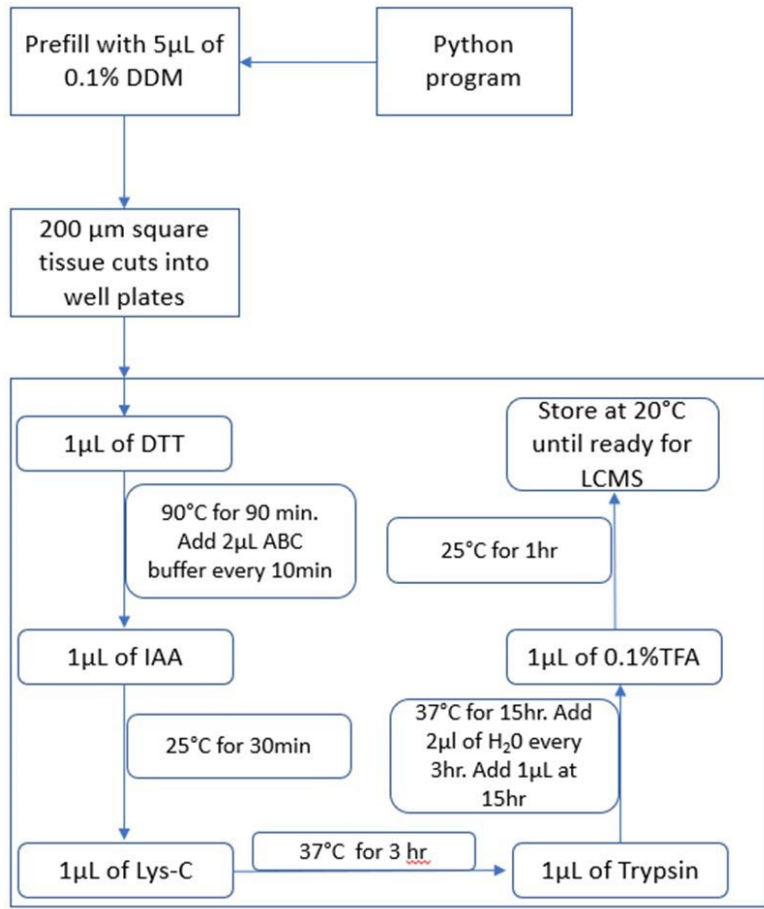
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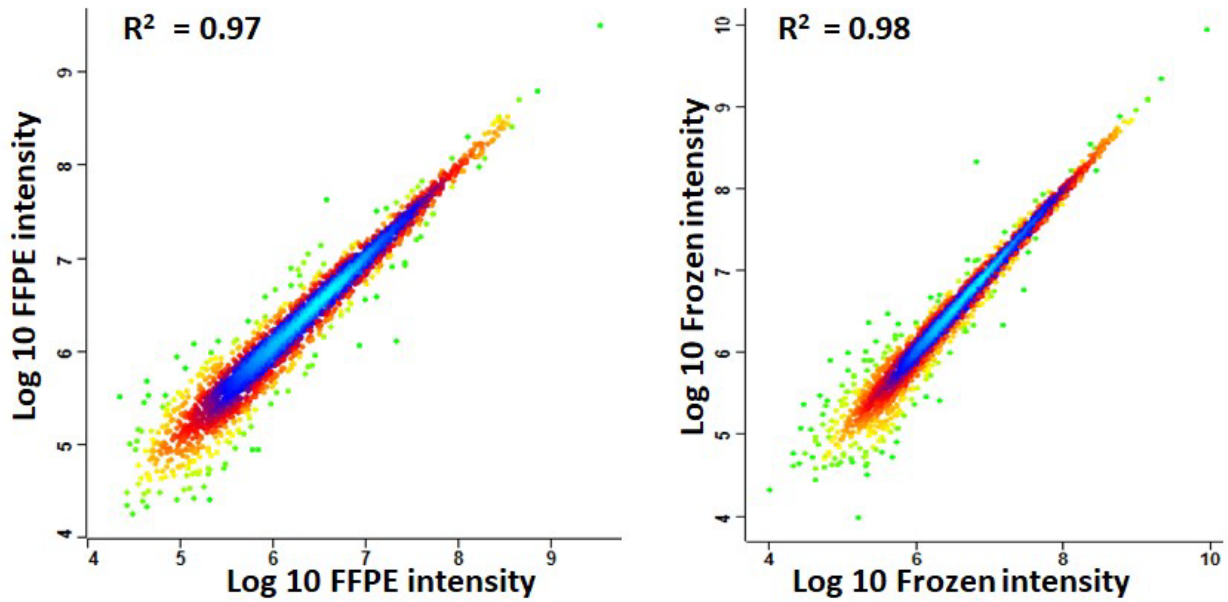Thomas, Terry Morgan, John C. Price, Ryan T. Kelly*

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Figure S1. AutoPOTS sample preparation workflow using the Opentrons OT2.

Figure S2. Representative Pearson correlation plots for replicate analyses of 200 μm × 200 μm sections of FFPE (left) and frozen (right) tissues.

Figure S3. Figure S3. Gene ontology plots with respect to (a) cellular component, (b) molecular function and (c) biological process for proteins identified in frozen and FFPE tissues.

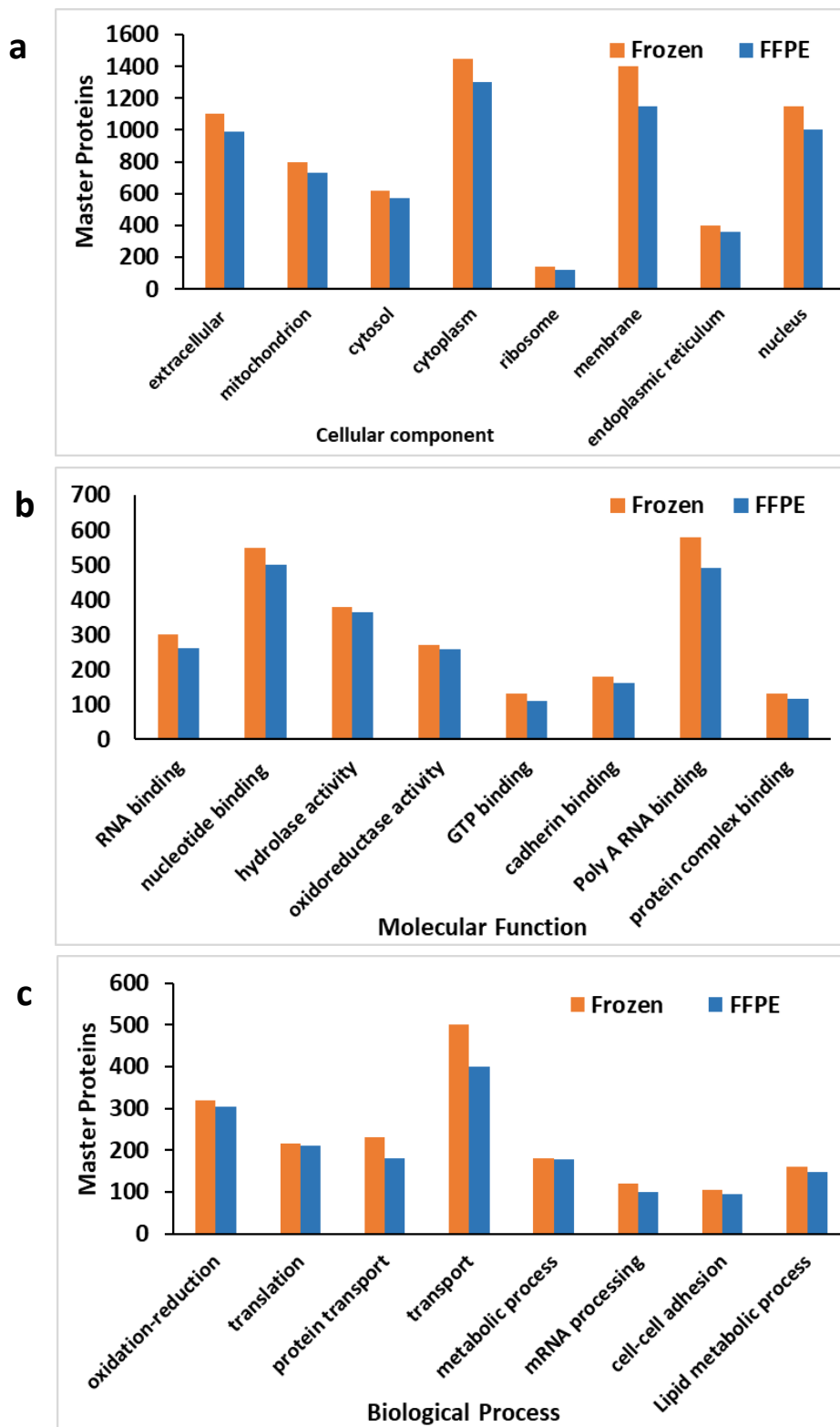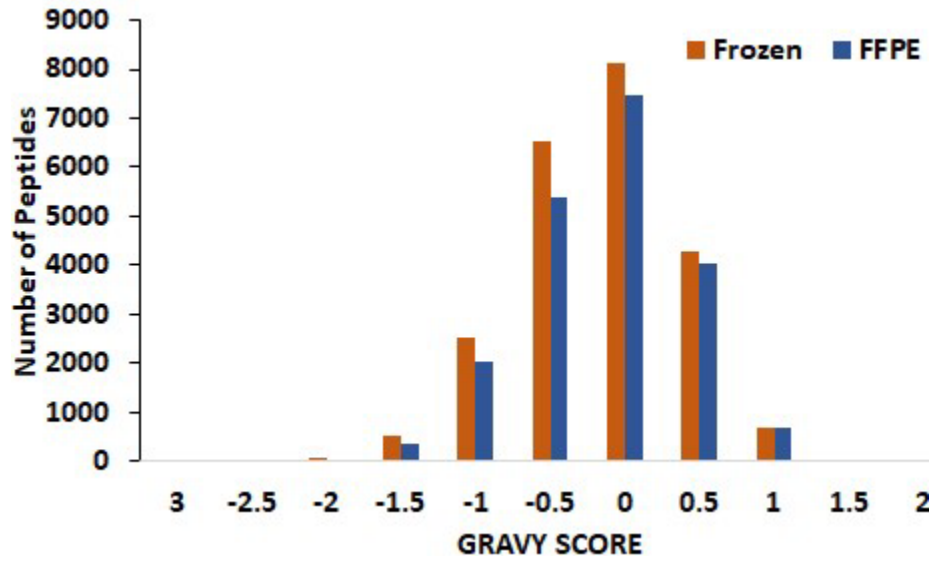**Figure S4.** Gravy score showing the relative hydrophobicity of unique peptides from 200 μm × 200 μm frozen and FFPE tissue squares.

Table S1. Proteome coverage for 50 µm, 100 µm and 200 µm fresh frozen and FFPE tissue squares. Peptide and protein coverage reported in parentheses include identifications based on MBR. Identifications are expressed as the mean ±1 standard deviation (n = 4).

Tissue square size	Tissue sample & workflow	Peptide ID (with MBR in parentheses)	Protein ID (with MBR in parentheses)
50 µm	Frozen nanoPOTS	5000±1200 (7000±2000)	900±200 (1300±300)
50 µm	FFPE nanoPOTS	4700±100 (6100±300)	850±10 (1100±80)
50 µm	FFPE autoPOTS	2100±200 (4200±200)	480±40 (920±30)
100 µm	Frozen nanoPOTS	18000±3000 (27000±2000)	2300±300 (2800±200)
100 µm	FFPE nanoPOTS	13000±1000 (19600±1100)	1990±80 (2490±50)
100 µm	FFPE autoPOTS	9100±1900 (14000±2000)	1400±200 (1830±150)
200 µm	Frozen nanoPOTS	25000±5000 (32000±4000)	2880±140 (3180±90)
200 µm	FFPE nanoPOTS	20000±400 (23800±300)	2570±40 (2770±20)
200 µm	FFPE autoPOTS	15800±1600 (19100±900)	1970±120 (2150±50)