Supplementary table 1. Sequence of epitope detection in multiplex immunofluorescence staining.

Cell types to be detected	Target (Antibody clone)	Secondary antibody enzyme-conjugate	Opal fluorochrome
Melanocytes	Melan-A (A103)	Poly-HRP Ms + Rb	Opal 620
Keratinocytes	pan Cytokeratin (C11)	Poly-HRP Ms + Rb	Opal 570
T cell subset	CD8 (C8/144B)	Poly-HRP Ms + Rb	Opal 520
Mast cells	Mast cell tryptase (AA1)	Poly-HRP Ms + Rb	Opal 690
Leukocytes	CD45 (2B11+PD7/26)	Poly-HRP Ms + Rb	Opal 480
T cells	CD3 (polyclonal)	Poly-HRP Ms + Rb	Opal 780
Nuclear cells	DAPI		

Abbreviations: Ms, mouse; Rb, rabbit; HRP, horseradish peroxidase.

Supplementary figure 1. Repetitive high-temperature heating leads to tissue damage, while tissues maintain their morphology after multiple rounds of treatment with stripping buffer. (A-C) On top is shown, hematoxylin and eosin staining of human kidney (A), melanoma (B), and colon (C) tissue prior to heat-induced epitope retrieval (HIER). At the bottom is shown, treatment of kidney (A), melanoma (B), and colon (C) tissue after 1 treatment (left) and 5 subsequent treatments (right) with either HIER in citrate buffer, HIER in Tris-EDTA buffer, or β-mercaptoethanol-containing stripping buffer. (D) Hematoxylin and eosin staining of gallbladder, ileum, pancreas, thymus and intestine tissue after 1 treatment (top), followed by 5 subsequent treatments with HIER in Tris-EDTA buffer (middle) or β-mercaptoethanol-containing stripping buffer (bottom). Bars equal 1 mm.

Supplementary figure 1