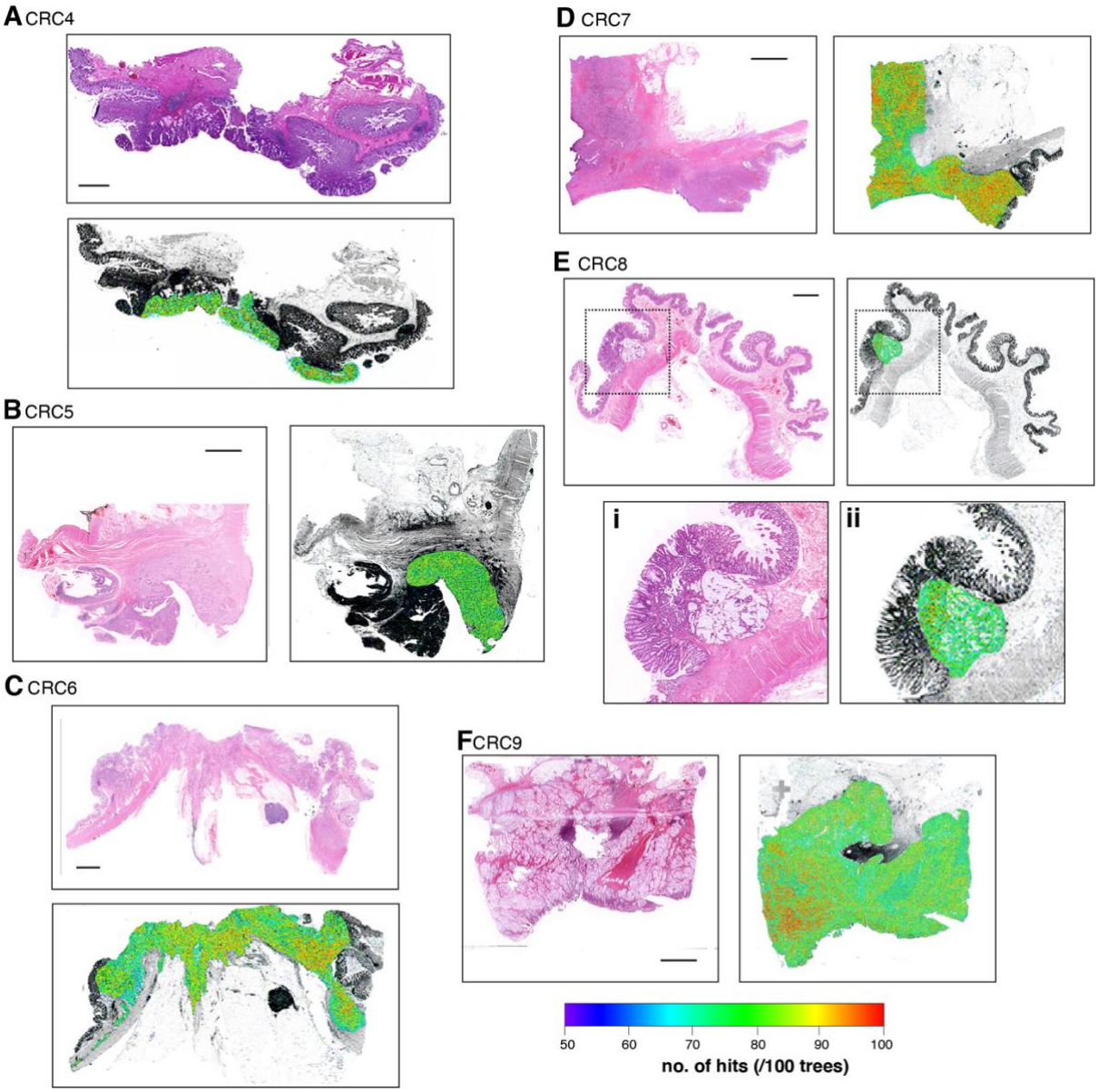
Electronic Supplementary Information for:

An integrated mass spectrometry imaging and digital pathology workflow for objective detection of colorectal tumours by unique atomic signatures†

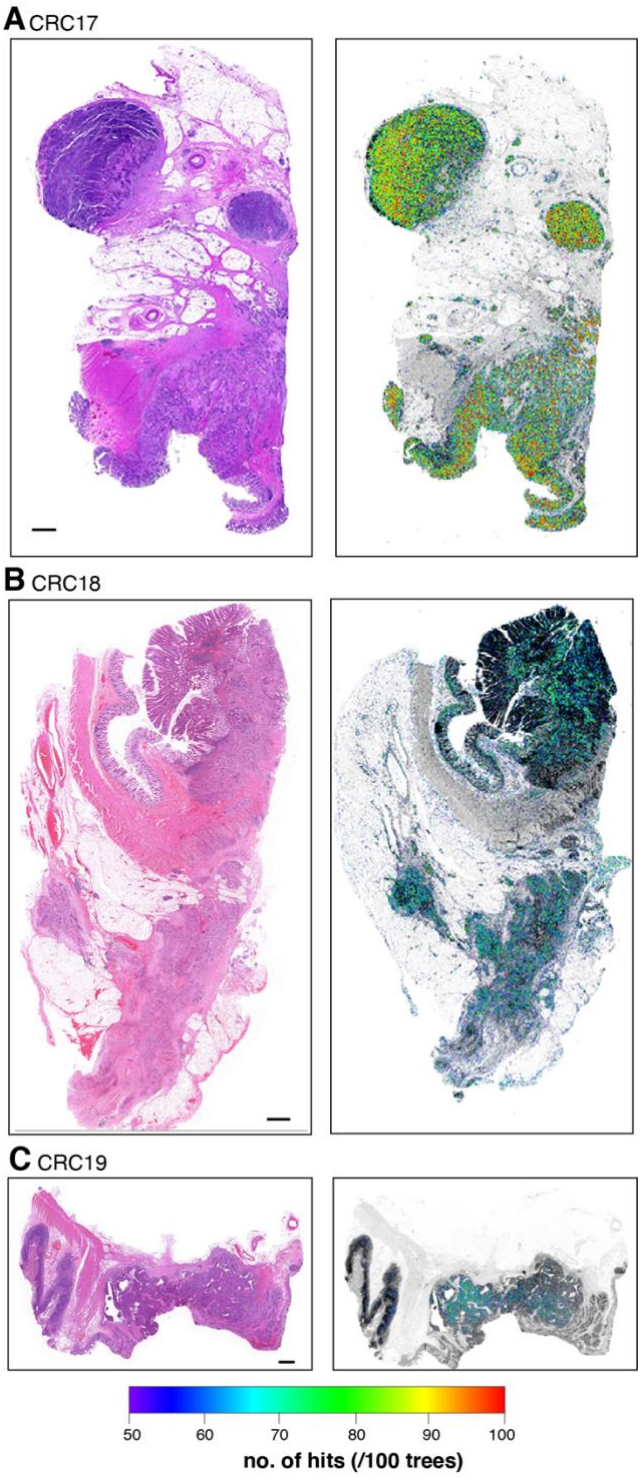
Bence Paul[a], Kai Kysenius[b], James B. Hilton[b], Michael W.M. Jones,[c] Robert W. Hutchinson[d], Daniel D. Buchanan[efg], Christophe Rosty[hij], Fred Fryer[k], Ashley I. Bush[l], Janet M. Hergt[a], Jon D. Woodhead[a], David P. Bishop[m], Philip A. Doble[m], Michelle M. Hill[no], Peter J. Crouch[b]* and Dominic J. Hare[lmp]*

[a] *School of Geography, Earth and Atmospheric Sciences, The University of Melbourne, Parkville, Victoria, 3010, Australia.*
[b] *Department of Biochemistry and Pharmacology, School of Biomedical Sciences, The University of Melbourne, Parkville, Victoria, 3010, Australia. Email: pjcrouch@unimelb.edu.au*
[c] *Central Analytical Research Facility, Queensland University of Technology, Brisbane, Queensland, 4000, Australia*
[d] *Elemental Scientific Lasers, Huntingdon, Cambridgeshire, PE29 6XS, United Kingdom.*
[e] *Department of Clinical Pathology, Melbourne Medical School, The University of Melbourne, Parkville, Victoria, 3010, Australia.*
[f] *University of Melbourne Centre for Cancer Research, The University of Melbourne, Parkville, Victoria, 3010, Australia.*
[g] *Genomic Medicine and Family Cancer Clinic, Royal Melbourne Hospital, Melbourne, Victoria, 3000, Australia.*
[h] *Envoi Pathology, Brisbane, Queensland, 4000, Australia.*
[i] *Faculty of Medicine, The University of Queensland, Brisbane, Queensland, 4000, Australia.*
[j] *Department of Clinical Pathology, The University of Melbourne, Parkville, Victoria, 3010, Australia.*
[k] *Agilent Technologies Australia, Mulgrave, Victoria, 3170, Australia.*
[l] *Melbourne Dementia Research Centre at the Florey Institute of Neuroscience and Mental Health and The University of Melbourne, Parkville, Victoria, 3010, Australia.*
[m] *Atomic Medicine Initiative, University of Technology Sydney, Broadway, NSW, 2007, Australia. Email. dominic.hare@uts.edu.au*
[n] *Centre for Clinical Research, Faculty of Medicine, The University of Queensland, Herston, Qld, 4006, Australia*
[o] *QIMR Berghofer Medical Research Institute, Herston, Queensland, 4006, Australia*
[p] *School of BioSciences, The University of Melbourne, Parkville, Victoria, 3010, Australia.*

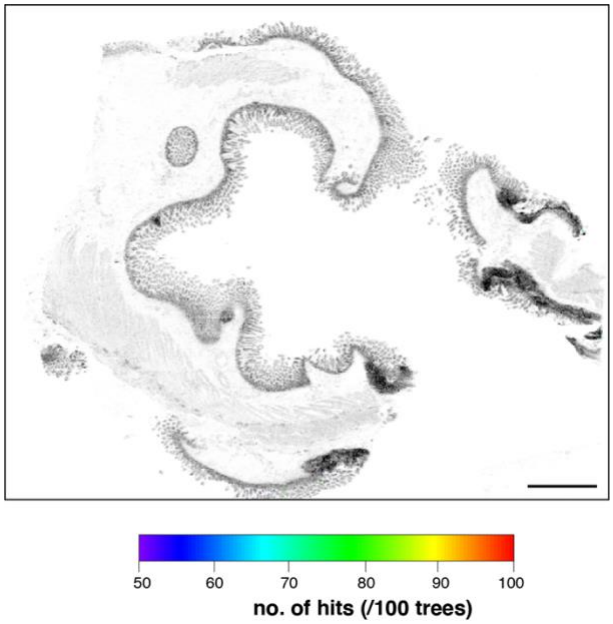
Supplementary Figure 1: A sample decision tree showing each leaf and branch. The 40× zoomed section is shown in red in the lower panel, which was originally 3.16 m wide.



Supplementary Figure 2: H&E stain micrographs and corresponding atomic signature images for random forest regression model training. Primary tumours included T classifications of: (A) T1, (B) T3, (C) T4, (D) T3, (E) T1, and (F) T3. LA-ICP-MS imaging of CRC8 revealed invasion of adenocarcinoma into adjacent mucosal tissue (E; i-ii), highlighting the need for higher resolution imaging studies in the future. Scale bar = 2 mm.



Supplementary Figure 3: Blind testing of random forest model for automated detection of colorectal tumours by atomic signature. Primary tumours were classified according to TNM staging as: (A) T3, (B) T3, and (C) T3. Scale bars = 2 mm.



Supplementary Figure 4: Blind testing of random forest model eliminated false positive pixels in NIW. Scale bar = 2 mm.