Fragmentation Evidence for Peptide

ITR FQDGDLTLYQSNTILR

| Residue	b	y
F	292.1778	2028.0518
Q	420.2363	1736.8814
D	535.2633	1608.8228
G	592.2847	1493.7958
D	707.3117	1436.7744
L	820.3957	1321.7474
T	921.4434	1208.6634
L	1034.5275	1107.6157
Y	1197.5908	994.5316
Q	1325.6494	831.4683
S	1412.6814	703.4097
N	1526.7243	616.3777
T	1627.7720	502.3348
I	1740.8561	401.2871
L	1853.9402	288.2030
R	2010.0413	175.1190

Supplementary Figure 1. Screenshot of ProteinPilot software showing MS/MS evidence for the 3+ peptide (676.7 m/z) #FQDGDLTLYQSNTILR from GST-pi, where # is an N-terminal iTRAQ moiety. The iTRAQ reagent reporter region of the MS/MS spectrum (114-117Da) is shown in profile mode. Integration of the iTRAQ reporter ions is used to calculate peptide ratios. The iTRAQ 115Da and 116Da reagent labeled CRC from patients PL132 and PL138 respectively, while the paired adjacent mucosa was labeled with 114Da and 117Da reagent for the respective patients.
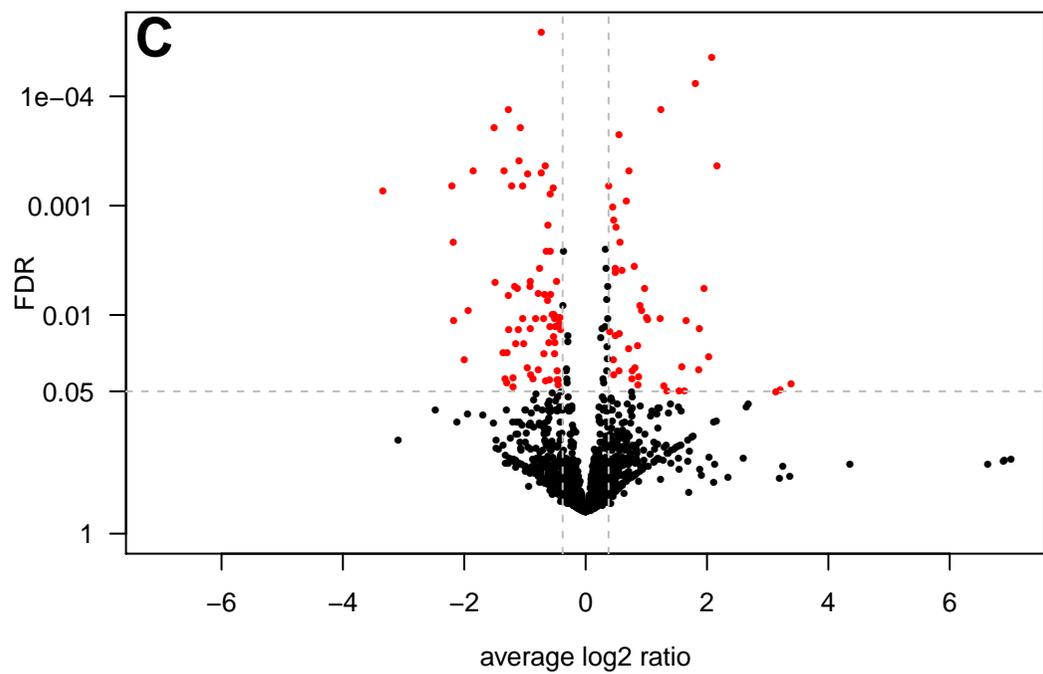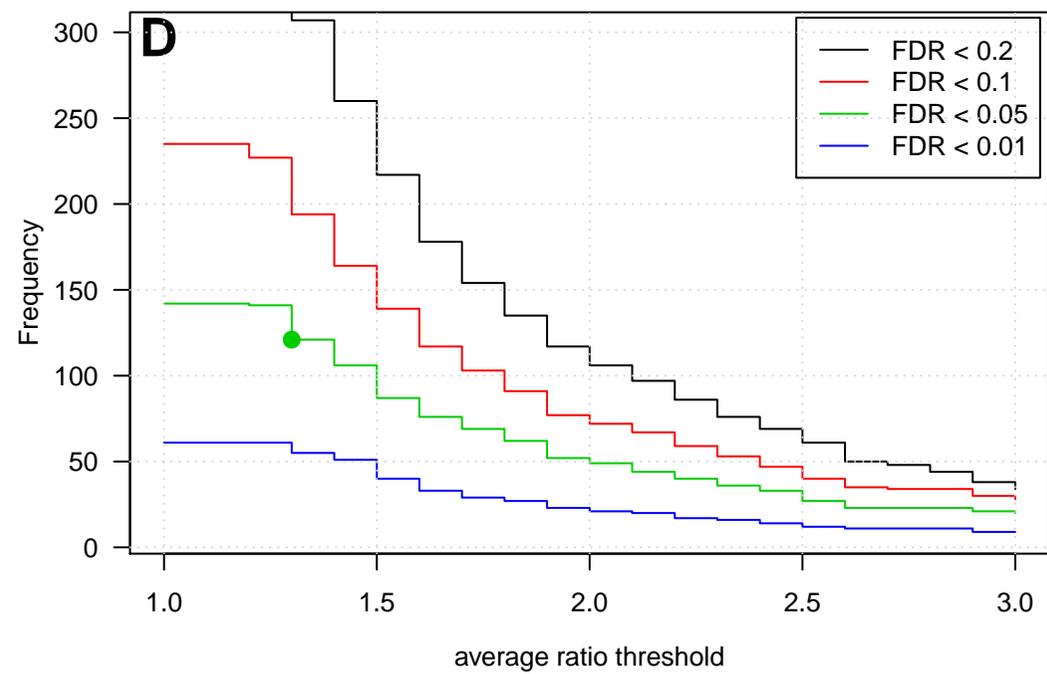
Histogram of unadjusted p-values
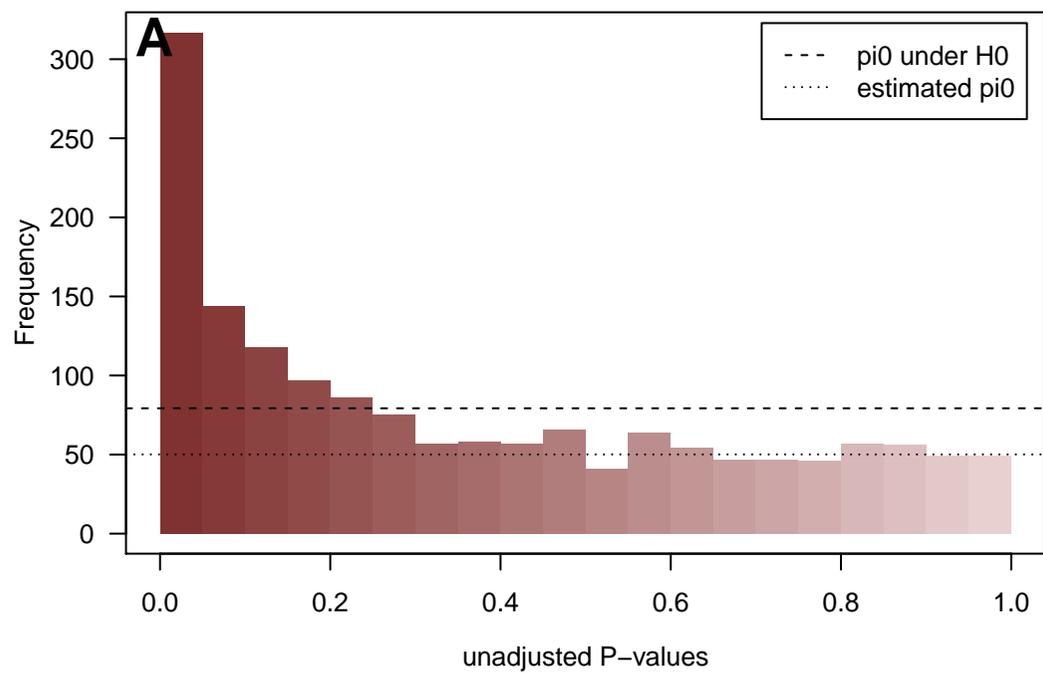
Histogram of q-values
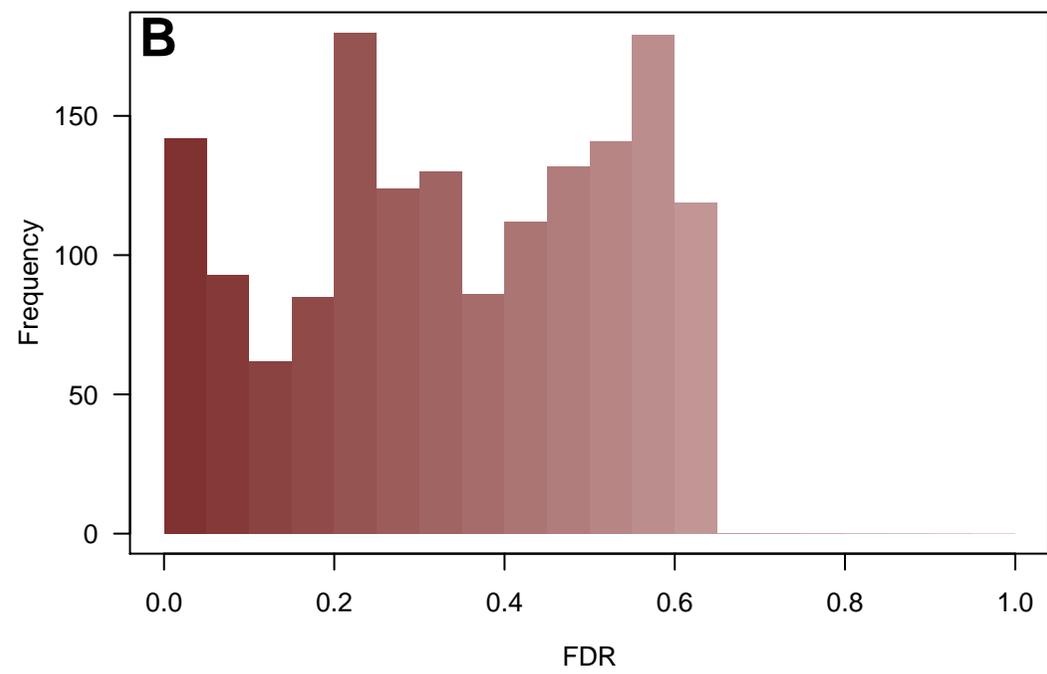
Volcano Plot

sensitivity analysis

Supplementary Figure 2: Differentially expressed proteins in colorectal tumours relative to non-neoplastic mucosa. Differences in protein abundance between tumour and normal mucosa across 16 patients was assessed using a moderated t-test, with adjustment for multiple correction by the positive FDR[20]. A) histogram of the unadjusted p-values resulting from testing 1483 proteins for differential expression. Dashed line = expected proportion if all proteins were not differentially expressed; dotted line = actual proportion of differentially expressed proteins determined by the positive FDR method. B) histogram of FDR values, indicating a large spike of proteins with FDR<0.05. This histogram stops at 0.633, since that is the maximum FDR of this dataset. C) volcano plot of average log2 ratio (x-axis) vs FDR (y-axis, -log10 scale) for each protein. Red dots are differentially expressed with FDR<0.05 (above horizontal dashed line) and fold change > 1.3 (either side of vertical dashed lines). D) sensitivity analysis indicating the number of DE proteins across a range of fold change thresholds (x-axis) and a number of FDR thresholds (coloured lines). Green dot = FDR<0.05 and FC>1.3.

Supplementary Figure 3. TGM2 expression is diminished in tumour cells compared to adjacent normal mucosa as shown by immunohistochemistry on conventional tissue sections from the same patient samples that were used for immunoblotting (Fig.3). The images show strong positive staining of cells in the crypts of the normal mucosa in each case compared to negative staining of tumour cells in sample PL140 (A); weak staining of tumour cells in sample PL12 (B); negative staining of tumour cells in PL36 (C); and weak staining of tumour cells in PL97(D) (original magnification 200x).
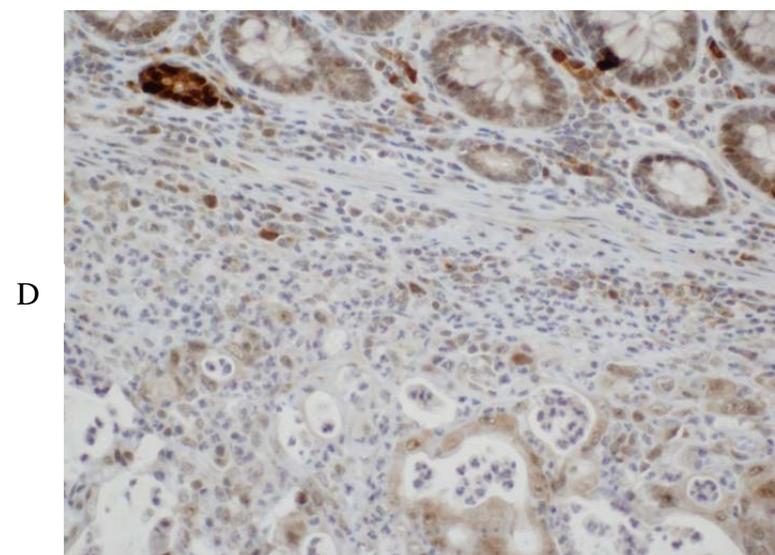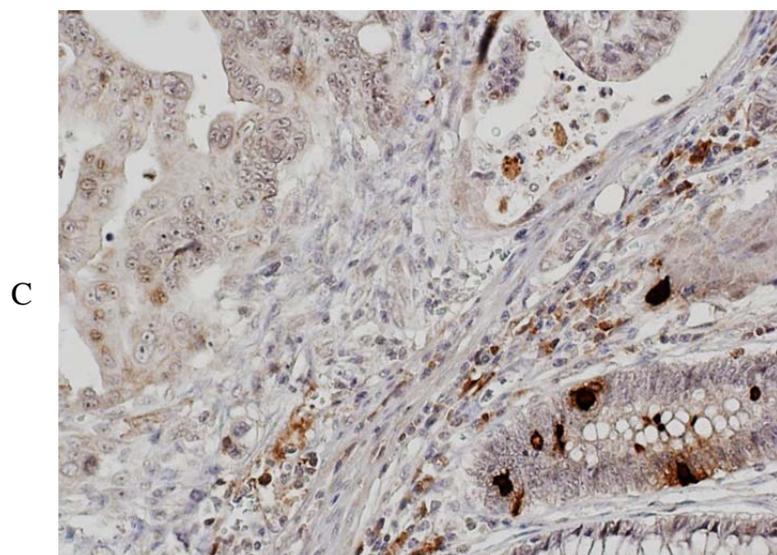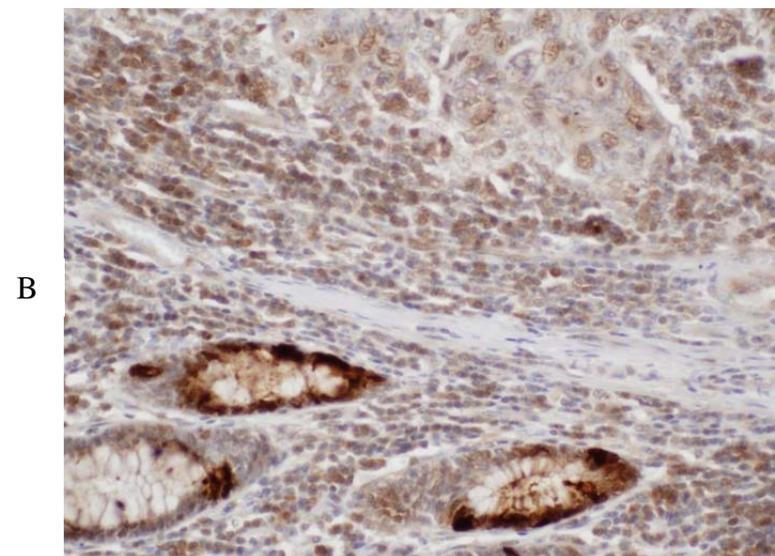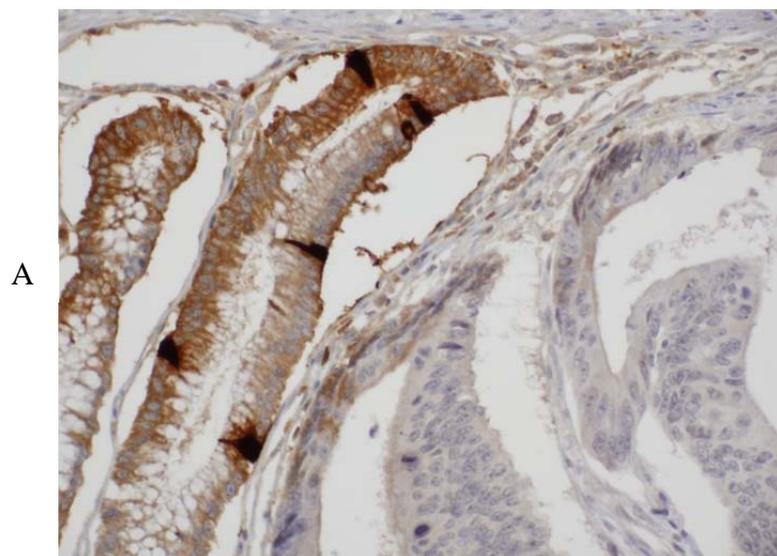
Supplementary Table 1. Clinicopathological data of CRC patients.

Patient ID	Sub-stage*	Tumour location	Age	Gender
PL 145	A2	Ascending colon	64	Female
PL 67	A3	Rectum	76	Female
PL 144	A3	Ascending colon	76	Female
PL 12	B1	Transverse colon	80	Female
PL 140	B1	Ascending colon	84	Male
PL 284	B1	Ascending colon	60	Male
PL 318	B1	Ascending colon	83	Male
PL 226	B1	Cecum	60	Female
PL 97	C1	Hepatic flexure	72	Male
PL 23	C1	Sigmoid colon	65	Female
PL 36	C1	Splenic flexure	78	Male
PL 32	C1	Rectum	63	Male
PL 47	C1	Ascending colon	75	Female
PL 132	D2	Cecum	69	Male
PL 138	D2	Cecum	87	Male
PL 173	D2	Cecum	92	Male

*Reference[36]

Supplementary Table 3. IHC quantitation of tumour compared to normal mucosa. Numbers refer to observations from 16 samples.

Protein	# elevated in tumour	# no change	# reduced in tumour
AGR2	1	6	9
Beta-catenin	1	11	4
Caldesmon	16	-	-
CEA	12	3	1
CK-20	-	3	13
Galectin-3	-	5	11
HLA-DR	7	5	4
Maspin	13	2	1
S100A8/A9	16	-	-
Stat-1	3	4	9
TGM2	-	-	16