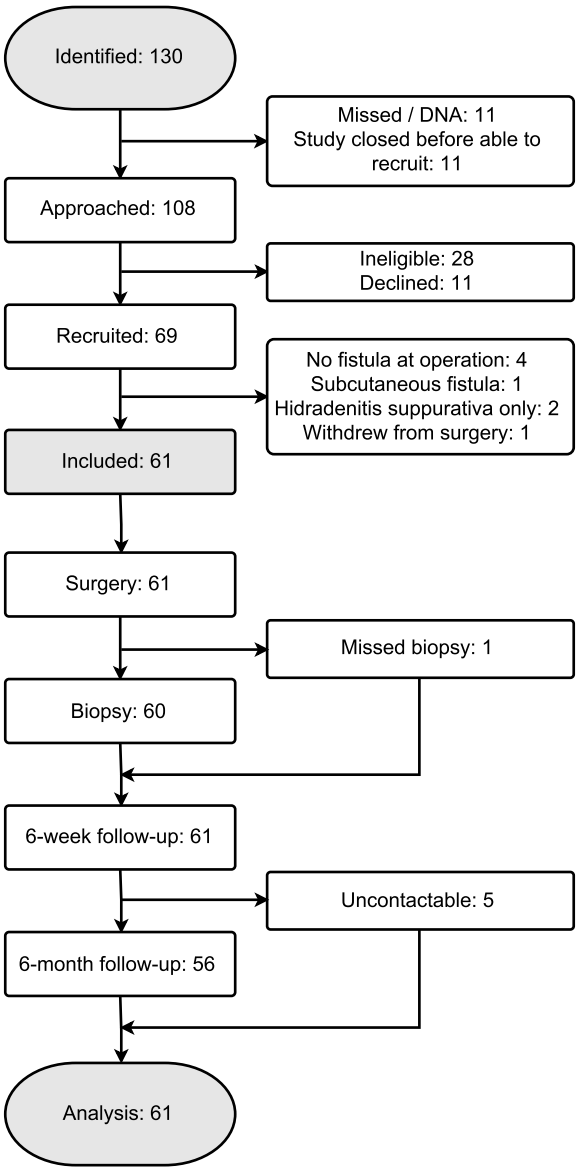
SUPPLEMENTARY MATERIAL

Supplementary Figure 1 Patient flow diagram.



Supplementary Table 1 Characteristics of patients.

	Idiopathic	Crohn's Disease
n	48	13
Male	38 (79%)	8 (62%)
Mean age (SD), years	41.1 (11.6)	36.1 (18.5)
Ethnic origin:		
White	11 (23%)	5 (38%)
Asian	26 (54%)	4 (31%)
Black	3 (6%)	1 (8%)
Other	7 (15%)	3 (23%)
Unanswered	1 (2%)	0
Median fistula duration (IQR), months	35 (14–60)	48 (17–66)
Previously received antibiotics	39 (81%)	3 (23%)
Median previous operations (IQR)	1 (0–4)	0 (0–4)
Previously had surgery with curative intent	9 (19%)	1 (8%)
Current smoker	22 (46%)	4 (31%)
Imaging diagnostic for Crohn's disease		13
Histological features of Crohn's disease		12
Presence of granulomata		6
Crohn's disease diagnosed during study		2
Highest order therapy received:		
Steroids		1
Mesalazine		1
Azathioprine		2
Anti-TNF-α		6
Receiving anti-TNF-α at recruitment		5
Previous abdominal fistulae		6
Previous abdominal surgery		6

Standard deviation (SD), interquartile range (IQR), tumour necrosis factor (TNF)

Supplementary Table 2 Characteristics of healthy controls.

	Demographics	Indication	Endoscopic result
1	Female, 59 years	Survey for extra-mammary Paget's disease	Ileocolonoscopy revealed left sided diverticulosis only
2	Female, 71 years	Loose stools and bloating	Ileocolonoscopy revealed one small polyp only
3	Female, 30 years	Constipation	Normal flexible sigmoidoscopy
4	Male, 65 years	Anorectal bleeding	Normal flexible sigmoidoscopy
5	Female, 21 years	Constipation	Normal ileocolonoscopy
6	Female, 75 years	Iron deficiency anaemia	Normal colonoscopy

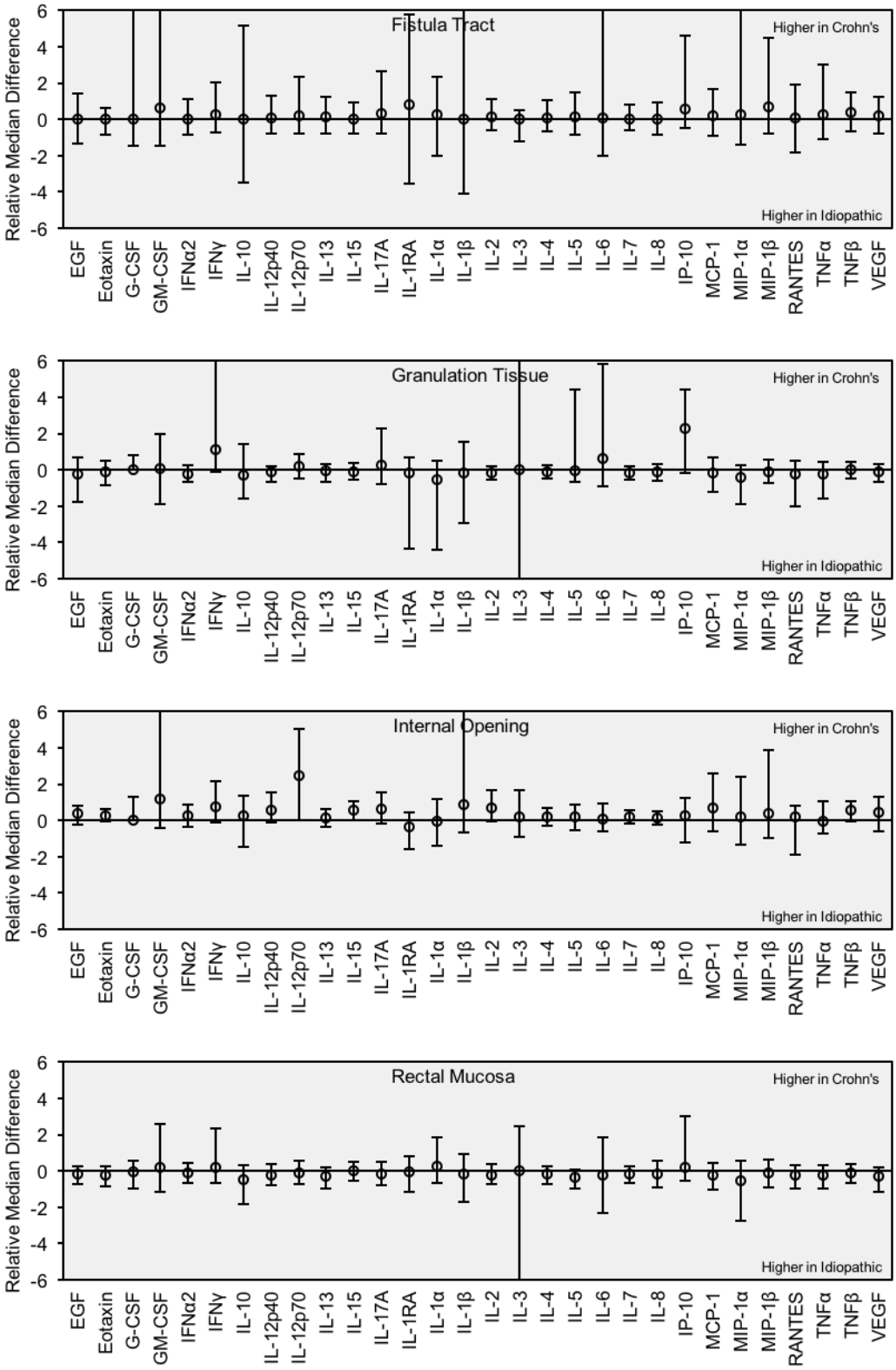
Supplementary Table 3 List of 30 cytokines and chemokines measured by the 30-plex Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore, Billerica, Massachusetts).

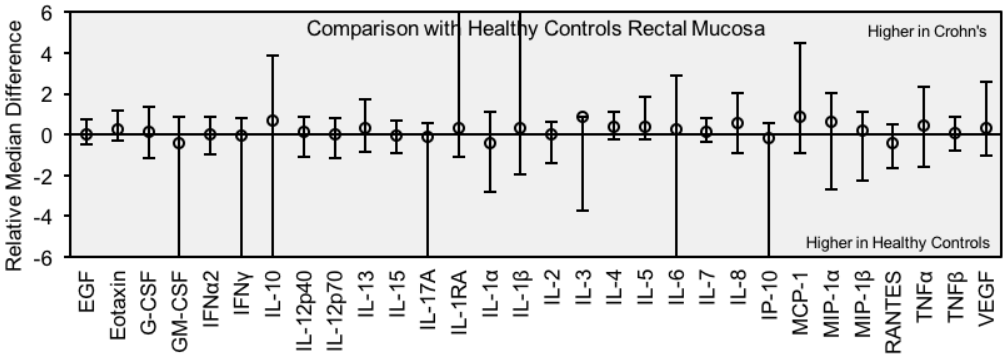
	Cytokine	Name
1	EGF	Epidermal growth factor
2	Eotaxin	Human epidermal growth factor receptor 2
3	G-CSF	Granulocyte-colony stimulating factor
4	GM-CSF	Granulocyte-macrophage- colony stimulating factor
5	IFNα2	Interferon alpha 2
6	IFNγ	Interferon gamma
7	IL-10	Interlukin-10
8	IL-12p40	Interlukin-12 p40 homodimer
9	IL-12p70	Interlukin-12 p70 heterodimer
10	IL-13	Interlukin-10
11	IL-15	Interlukin-15
12	IL-17	Interlukin-17
13	IL-1RA	Interlukin-1 receptor agonist
14	IL-1α	Interlukin-1 alpha
15	IL-1β	Interlukin-1 beta
16	IL-2	Interlukin-2
17	IL-3	Interlukin-3
18	IL-4	Interlukin-4
19	IL-5	Interlukin-5
20	IL-6	Interlukin-6
21	IL-7	Interlukin-7
22	IL-8	Interlukin-8
23	IP-10	Interferon-gamma-inducible protein-10
24	MCP-1	Monocyte chemoattractant protein-1
25	MIP-1α	Macrophage inflammatory protein-1 alpha
26	MIP-1β	Macrophage inflammatory protein-1 beta
27	RANTES	Regulated on activation normal T cell expressed and secreted
28	TNFα	Tumour necrosis factor alpha
29	TNFβ	Tumour necrosis factor beta
30	VEGF	Vascular endothelial growth factor

Supplementary Table 4 List of the 39 phosphoproteins measured by the PathScan RTK Signaling Antibody Array, Chemiluminescent Readout (Cell Signaling Technology, Danvers, Massachusetts).

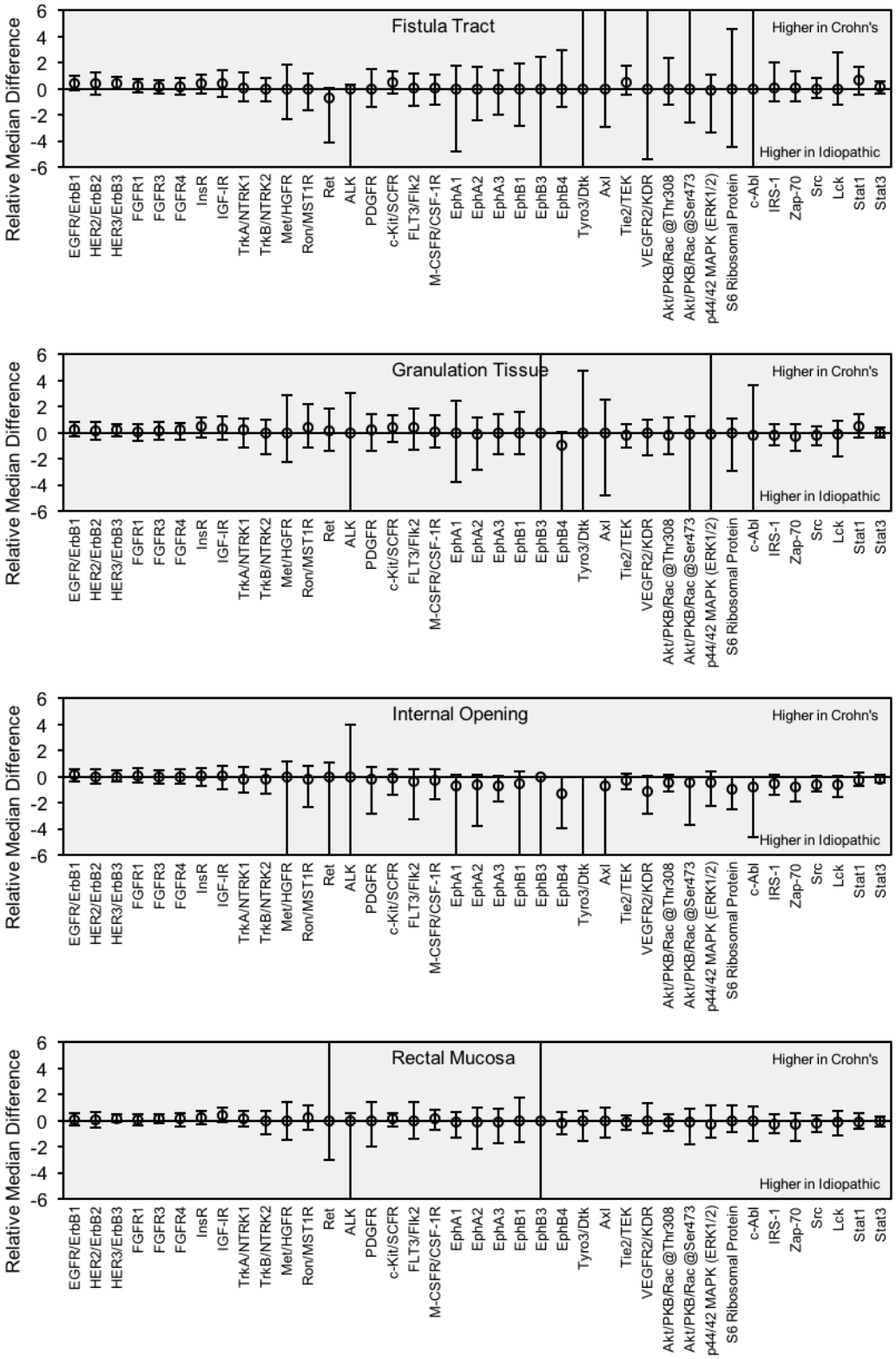
	Protein (phosphorylation site)	Name
Receptor tyrosine kinases		
1	EGFR/ErbB1	Epidermal growth factor receptor
2	HER2/ErbB2	Human epidermal growth factor receptor 2
3	HER3/ErbB3	Human epidermal growth factor receptor 3
4	FGFR1	Fibroblast growth factor receptor 1
5	FGFR3	Fibroblast growth factor receptor 3
6	FGFR4	Fibroblast growth factor receptor 4
7	InsR	Insulin receptor
8	IGF-IR	Insulin-like growth factor receptor 1
9	TrkA/NTRK1	Tyrosine kinase receptor A
10	TrkB/NTRK2	Tyrosine kinase receptor B
11	Met/HGFR	Hepatocyte growth factor receptor
12	Ron/MST1R	Macrophage stimulating 1 receptor
13	Ret	Ret proto-oncogene receptor tyrosine kinase
14	ALK	Anaplastic lymphoma kinase
15	PDGFR	Platelet-derived growth factor receptor
16	c-Kit/SCFR	Stem cell growth factor receptor
17	FLT3/Flk2	Foetal liver kinase 2
18	M-CSFR/CSF-1R	Macrophage colony-stimulating factor 1 receptor
19	EphA1	Ephrin type-A receptor 1
20	EphA2	Ephrin type-A receptor 2
21	EphA3	Ephrin type-A receptor 3
22	EphB1	Ephrin type-B receptor 1
23	EphB3	Ephrin type-B receptor 3
24	EphB4	Ephrin type-B receptor 4
25	Tyro3/Dtk	Tyro3 tyrosine-protein kinase
26	Axl	AXL receptor tyrosine kinase
27	Tie2/TEK	Tunica interna endothelial cell kinase
28	VEGFR2/KDR	Vascular endothelial growth factor receptor 2
Signalling Nodes		
29	Akt/PKB/Rac (Thr308)	Proto-oncogene c-Akt / protein kinase B
30	Akt/PKB/Rac (Ser473)	Proto-oncogene c-Akt / protein kinase B
31	p44/42 MAPK	Mitogen-activated protein kinase isoform p44
32	S6 Ribosomal Protein	S6 Ribosomal Protein
33	c-Abl	Abelson murine leukaemia viral oncogene homolog 1
34	IRS-1	Insulin receptor substrate 1
35	Zap-70	70 kDa zeta-associated protein
36	Src	Rous sarcoma
37	Lck	Lymphocyte cell-specific protein-tyrosine kinase
38	Stat1	Signal transducer and activator of transcription 1
39	Stat3	Signal transducer and activator of transcription 3

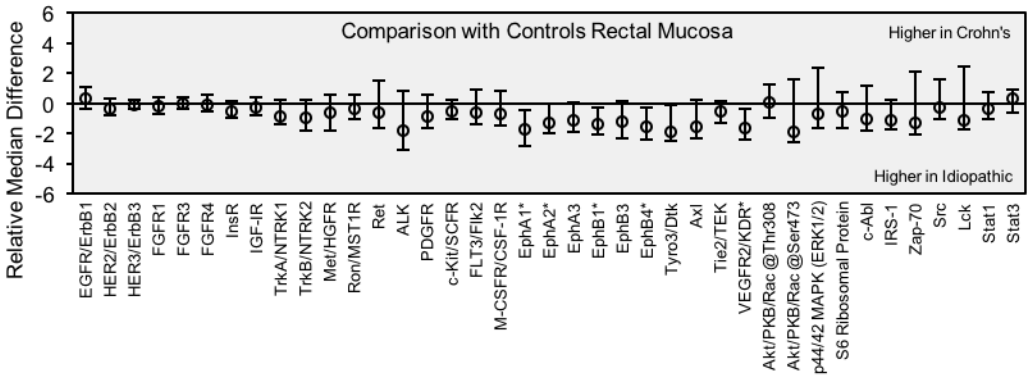
Supplementary Figure 2 Relative median difference in cytokine concentrations between idiopathic and Crohn's disease groups, and between the Crohn's disease group and healthy controls. Error bars show 99% confidence intervals estimated using Hodges-Lehman method.




Comparison with Healthy Controls Rectal Mucosa
Higher in Crohn's
Higher in Healthy Controls
Relative Median Difference
6
4
2
0
-2
-4
-6
EGF
Eotaxin
G-CSF
GM-CSF
IFNα2
IFNγ
IL-10
IL-12p40
IL-12p70
IL-13
IL-15
IL-17A
IL-1RA
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IP-10
MCP-1
MIP-1α
MIP-1β
RANTES
TNFα
TNFβ
VEGF

Supplementary Figure 3 Relative median difference in phosphoprotein pixel intensities between idiopathic and Crohn's disease groups, and between the Crohn's disease group and healthy controls. Error bars show 99% confidence intervals estimated using Hodges-Lehman method.




Comparison with Controls Rectal Mucosa
Higher in Crohn's
Higher in Idiopathic
Relative Median Difference
6
4
2
0
-2
-4
-6
EGFR/ErbB1
HER2/ErbB2
HER3/ErbB3
FGFR1
FGFR3
FGFR4
InsR
IGF-IR
TrkA/NTRK1
TrkB/NTRK2
Met/HGFR
Ron/MST1R
Ret
ALK
PDGFR
c-Kit/SCFR
FLT3/Flk2
M-CSFR/CSF-1R
EphA1*
EphA2*
EphA3
EphB1*
EphB3
EphB4*
Tyro3/Dtk
Axl
Tie2/TEK
VEGFR2/KDR*
Akt/PKB/Rac @Thr308
Akt/PKB/Rac @Ser473
p44/42 MAPK (ERK1/2)
S6 Ribosomal Protein
c-Abl
IRS-1
Zap-70
Src
Lck
Stat1
Stat3