Supplementary Information for:

NOX1 loss-of-function genetic variants in patients with inflammatory bowel disease

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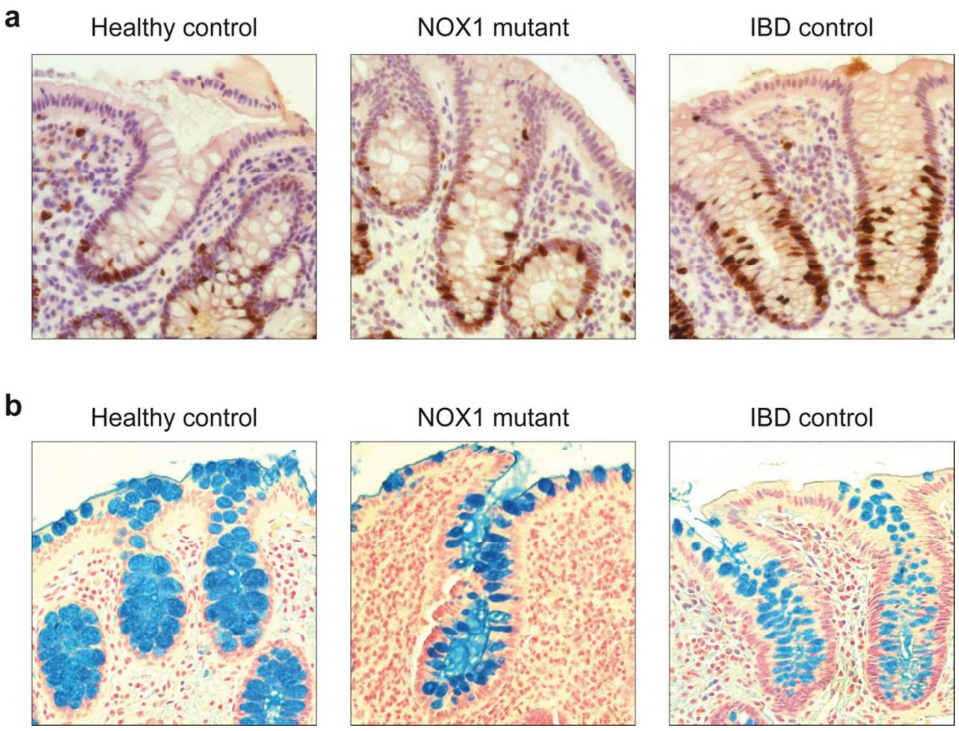
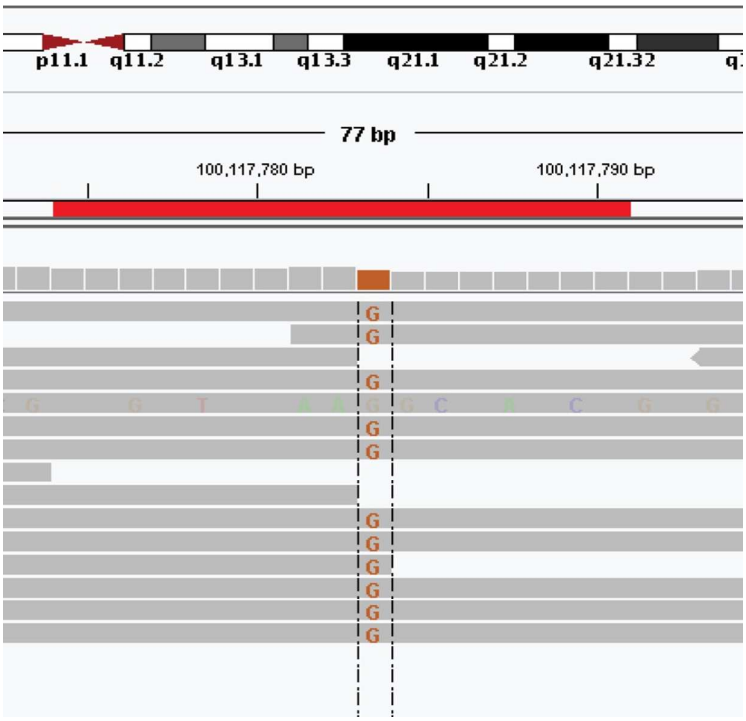
The PDF file includes:

Supplementary Figure S1



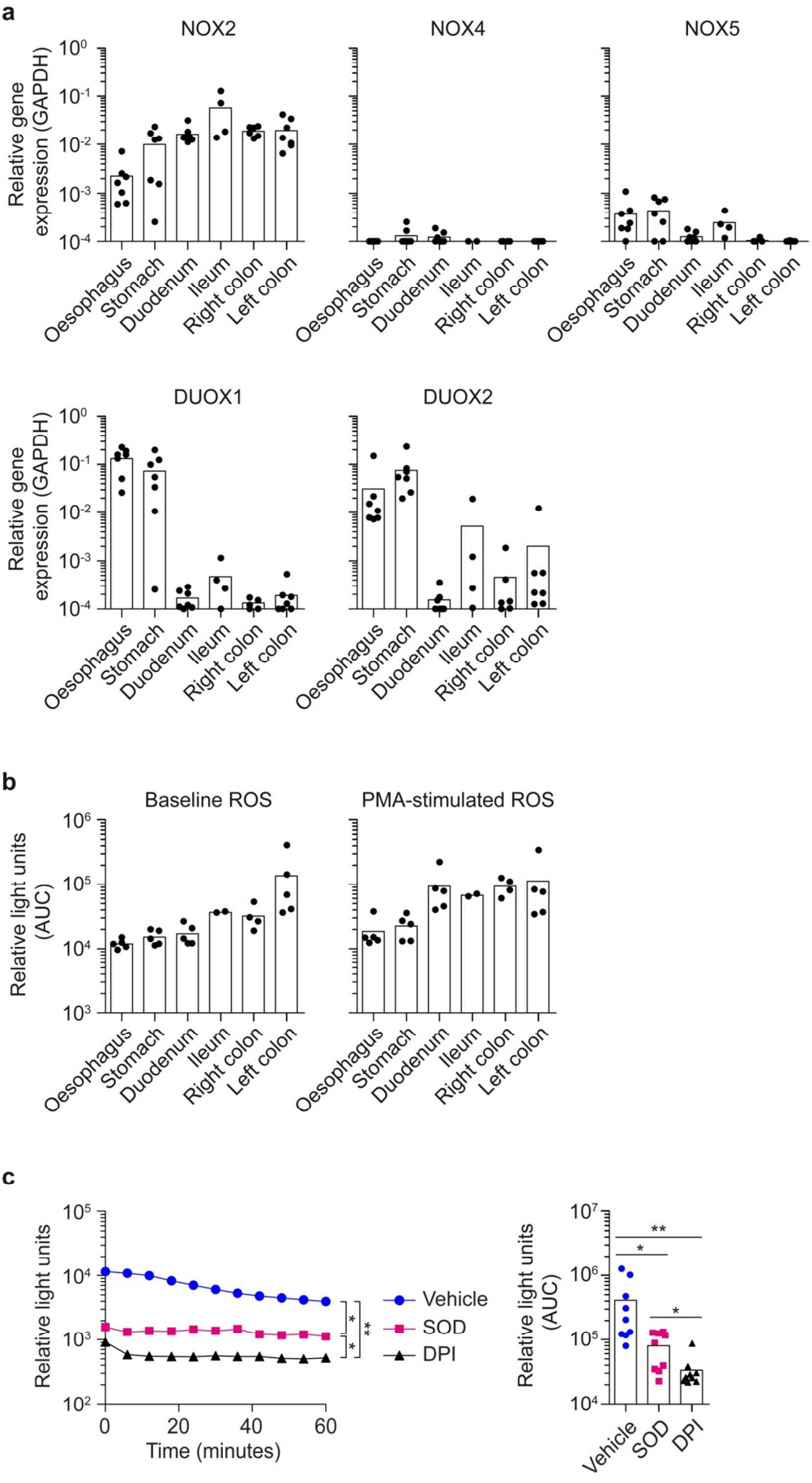
Supplementary Figure S1. **(a)** Representative images of immunohistochemistry staining of Ki67 in colonic crypts of a healthy control, the NOX1 mutant patient p.N122H and an IBD control patient. Histology for NOX1 and IBD patient was obtained during disease quiescence. **(b)** Representative images of goblet cells in colonic crypts stained with Alcian blue. Healthy control biopsy showing regular, plump goblet cells lining the colonic epithelial crypt. NOX1 patient p.N122H during active disease showing relative paucity of goblet cells and retention of mucin within goblet cells. IBD control patient during active disease showing increased number of goblet cells which are of relatively reduced size having extruded mucin onto the surface epithelium.

Supplementary Figure S2



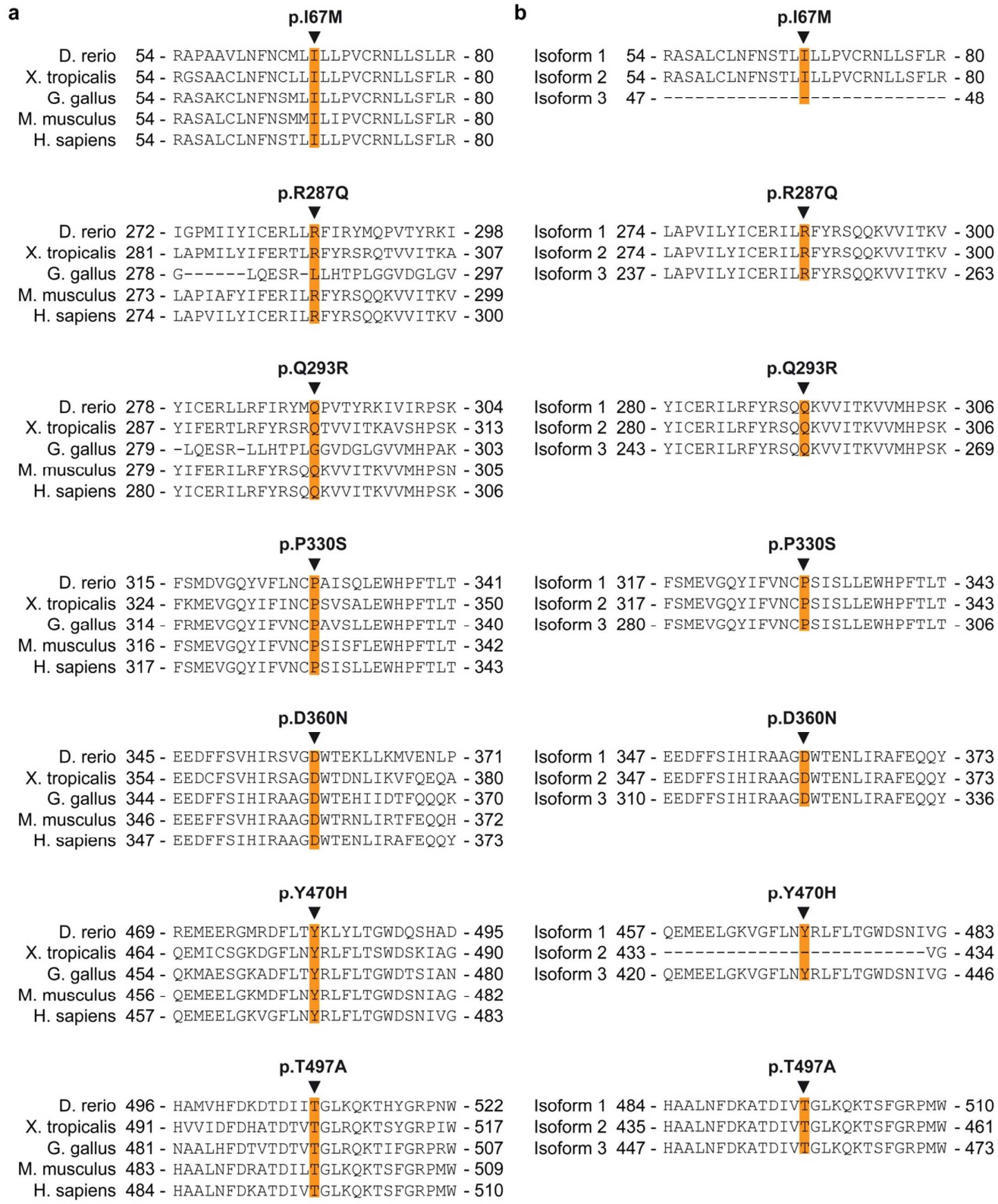
Supplementary Figure S2. Coverage of *NOX1* gene by whole genome sequencing. The reverse strand is shown.

Supplementary Figure S3



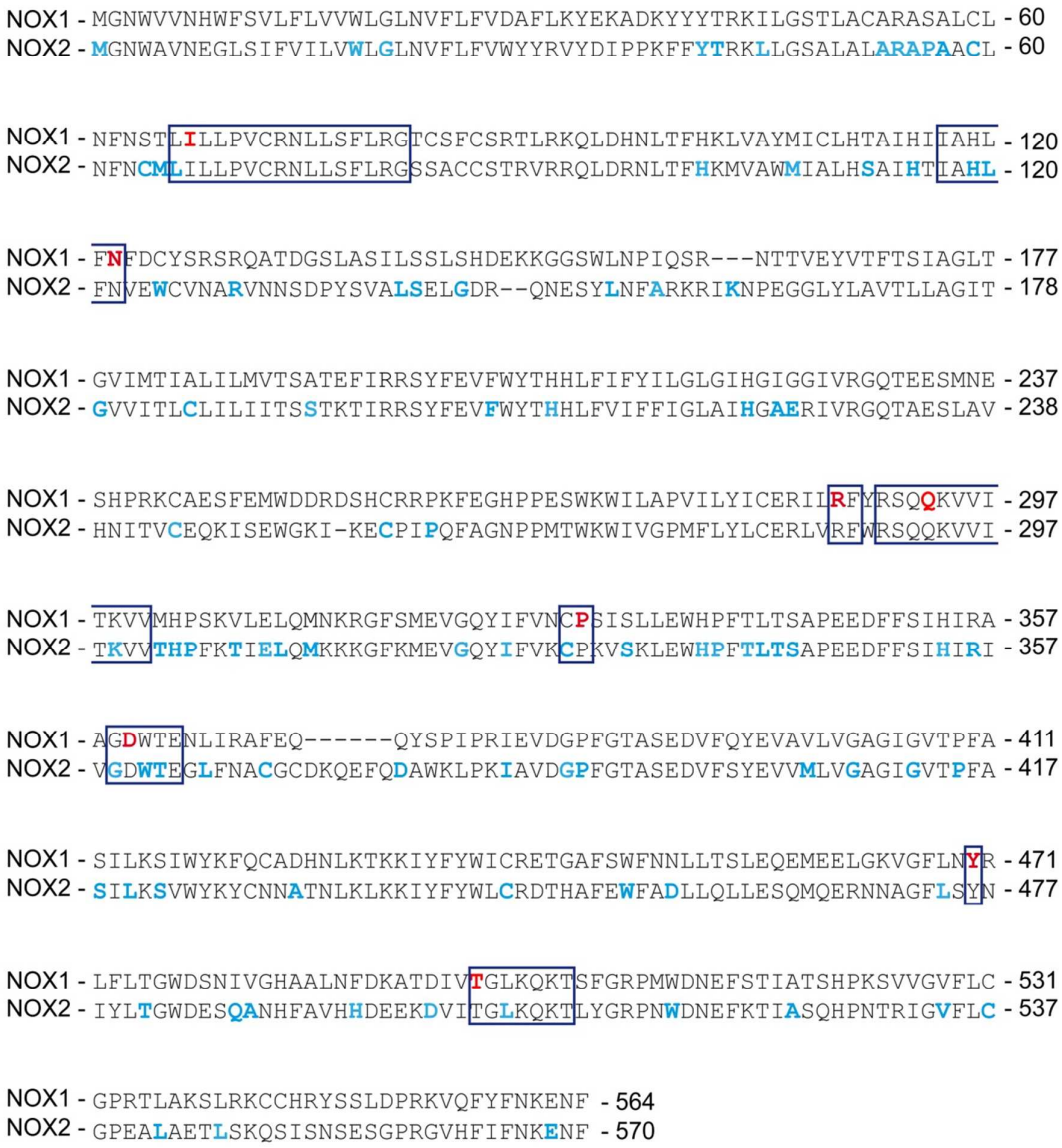
Supplementary Figure S3. **(a)** Expression of NADPH oxidase (NOX) family subunits in pan-enteric biopsies was determined by quantitative PCR, as indicated. Single biopsies per segment were obtained from 5 non-inflamed controls. Bars represent mean. **(b)** L-012-enhanced chemiluminescence on pan-enteric biopsy specimens obtained from 5 non-inflamed controls and stimulated with (right) or without (left) PMA. ROS was measured over 60 minutes and results are depicted as area under the curve (AUC). Where more than one biopsy per segment was available for a patient, the mean AUC was calculated. **(c)** SOD significantly attenuates the L-012 chemiluminescence signal, confirming specificity of the L-012 probe for superoxide rather than hydrogen peroxide. DPI however, further abrogated the chemiluminescence signal. Pan-colonic epithelial biopsies from three healthy control patients were co-incubated with L-012 alone, or together with 5 μM SOD or 10 μM DPI. **(c left)** ROS production measured in relative light units (RLU) from L-012 chemiluminescence was plotted as a kinetic curve over 60 minutes. **(c, right)** AUC of RLU was calculated. Experiment performed in triplicates for each condition (three biopsies per patient per condition); the kinetic curve plotted as mean of measurements, symbols on AUC graph represent results. Statistical analysis was performed using unpaired nonparametric Mann Whitney test.

Supplementary Figure S4



Supplementary Figure S4. **(a)** NOX1 protein sequence alignment of different genera showing the evolutionary conserved position 67, 287, 293, 330, 360, 470 and 497. Alignment was performed using ClustalW. **(b)** Corresponding amino acid sequence alignment of three NOX1 isoforms using ClustalW.

Supplementary Figure 5



Supplementary Figure S5. Protein sequence alignment of NOX1 and NOX2. Amino acids marked in blue indicate missense mutations in NOX2 responsible for Chronic Granulomatous Disease (CGD) according to the Human Gene Mutation Database (HGMD). Conserved regions around NOX1 variants (in red) are boxed in dark blue.

Supplementary Table 1 Minor allele frequency of NOX1 variants

Patient ID	NOX1 variants*	Position X chromosome	rs #	Minor allele frequency (ExAC v0.3)	NHLBI Exome Variant Server	INTERVAL cohort	UK10K consented exomes	1000 Genomes phase I
P1	c.A364C p.N122H	100117783	Novel	No MAF data	No MAF data	No MAF data	No MAF data	No MAF data
P2	c.T1408C p.Y470H	100104304	Novel	0.00001155	No MAF data	No MAF data	No MAF data	No MAF data
P3	c.C201G p.I67M	100118525	Novel	No MAF data	No MAF data	No MAF data	No MAF data	No MAF data
P4, P5, P6, P12, P13.1, P13.2	c.G860A p.R287Q	100106259	rs143127702	0.0007329	0.000663	0.0001558	No MAF data	No MAF data
P7	c. A878G p.Q293R	100106241	Novel	0.00008563	No MAF data	No MAF data	No MAF data	No MAF data
P9	c.C988T p.P330S	100105285	Novel	No MAF data	No MAF data	No MAF data	No MAF data	No MAF data
P8	c.A1489G p.T497A	100103698	Novel	0.0001597	No MAF data	No MAF data	No MAF data	No MAF data
P10, P11	c.G1078A p.D360N	100105195	rs34688635	0.01782	0.019881	0.026	0.029	0.022414
P14	splice donor - exon 9 c.1133+1G>A near R378	100105139	Novel	0.00008487	No MAF data	No MAF data	No MAF data	No MAF data
P15	splice acceptor - exon 7 c.672-1G>C near G224	100117293	Novel	0.00002283	No MAF data	No MAF data	No MAF data	No MAF data
P16	Splice region variant c.142-4G>A	100118588	rs201776721	0.0007563				

* Based on NOX1 transcript variant 1.

Supplementary Table 2 In silico analysis of NOX1 variants

Patient ID	NOX1 variants	SIFT prediction (cutoff = 0.05)	PolyPhen-2 prediction	PROVEAN prediction (cutoff = -2.5)	CADD score
P1	c.A364C p.N122H	Damaging (0.0)	Probably damaging (1.0)	Deleterious (-4.68)	25.4
P2	c.T1408C p.Y470H	Damaging (0.001)	Benign (0.347)	Deleterious (-3.79)	25.2
P3	c.C201G p.I67M	Damaging (0.0)	Probably damaging (1.0)	Neutral (-2.42)	23.8
P4, P5, P6, P12, P13.1, P13.2	c.G860A p.R287Q	Damaging (0.0)	Probably damaging (1.0)	Deleterious (-3.31)	33
P7	c. A878G p.Q293R	Tolerated (0.144)	Possibly damaging (0.635)	Deleterious (-2.65)	23.6
P9	c.C988T p.P330S	Tolerated (0.051)	Probably damaging (0.995)	Deleterious (-6.27)	23.7
P8	c.A1489G p.T497A	Damaging (0.019)	Probably damaging (1.0)	Deleterious (-4.75)	24.7
P10, P11	c.G1078A p.D360N	Damaging (0.042)	Benign (0.446)	Deleterious (-4.37)	25.1
P14	splice donor - exon 9 c.1133+1G>A near R378	NA	NA	NA	23.4
P15	splice acceptor - exon 7 c.672-1G>C near G224	NA	NA	NA	24.2
P16	Splice region variant c.142-4G>A	NA	NA	NA	3.7

Note: The severity of amino acid substitutions was predicted using SIFT (http://sift.jcvi.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), PROVEAN (http://provean.jcvi.org/index.php), or using CADD scores (http://cadd.gs.washington.edu/)[1]. NA, not applicable

Supplementary Table 3 Rare hemizygous, homozygous and compound heterozygous variants identified by WGS in p.N122H patient

Chrom	Pos	Ref	Alt	Fct	Ef	Gene	Amino acid
X	11160419	G	T	exonic	nonsynonymous_SNV	ARHGAP6	NM_013423:c.C1582A:p.P528T
X	69424828	A	T	exonic	nonsynonymous_SNV	DGAT2L6	NM_198512:c.A886T:p.I296F
X	132092293	G	A	exonic	nonsynonymous_SNV	HS6ST2	NM_001077188:c.C338T:p.A113V
X	100117783	T	G	exonic	nonsynonymous_SNV	NOX1	NM_007052:c.A364C:p.N122H
X	46466524	C	T	exonic	nonsynonymous_SNV	SLC9A7	NM_032591:c.G2041A:p.A681T
5	115782730	G	A	exonic	nonsynonymous_SNV	SEMA6A	NM_020796:c.C2672T:p.P891L
11	100999245	G	A	exonic	nonsynonymous_SNV	PGR	NM_000926:c.C557T:p.P186L
17	27414024	C	T	exonic	nonsynonymous_SNV	MYO18A	NM_078471:c.G5642A:p.R1881Q
19	40732460	C	T	exonic	nonsynonymous_SNV	CNTD2	NM_024877:c.G89A:p.S30N
20	42694558	C	CCCG	exonic	nonframeshift_insertion	TOX2	NM_001098798:c.1113_1114insCCG:p.S371delinsSP
20	2844619	G	A	exonic	nonsynonymous_SNV	VPS16	NM_080413:c.G1069A:p.E357K
1	228430941	C	T	exonic	nonsynonymous_SNV	OBSCN	NM_001098623:c.C2987T:p.A996V
1	228553159	G	A	exonic	nonsynonymous_SNV	OBSCN	NM_001098623:c.G18961A:p.G6321R
1	145112420	C	T	exonic	stopgain_SNV	SEC22B	NM_004892:c.C394T:p.R132X
1	145115810	A	G	exonic	nonsynonymous_SNV	SEC22B	NM_004892:c.A569G:p.H190R
6	47846793	T	G	exonic	nonsynonymous_SNV	C6orf138	NM_001013732:c.A1787C:p.K596T
6	48036121	G	A	exonic	nonsynonymous_SNV	C6orf138	NM_001013732:c.C271T:p.L91F
6	90365641	C	G	exonic	nonsynonymous_SNV	MDN1	NM_014611:c.G15332C:p.G5111A
6	90372547	G	T	exonic	nonsynonymous_SNV	MDN1	NM_014611:c.C14376A:p.D4792E
7	45121241	C	T	exonic	nonsynonymous_SNV	NACAD	NM_001146334:c.G4216A:p.A1406T
7	45125429	G	A	exonic	nonsynonymous_SNV	NACAD	NM_001146334:c.C350T:p.P117L
7	149482585	C	T	exonic	nonsynonymous_SNV	SSPO	NM_198455:c.C3005T:p.P1002L
7	149484527	T	C	exonic	nonsynonymous_SNV	SSPO	NM_198455:c.T3454C:p.W1152R
17	21318773	G	A	exonic	nonsynonymous_SNV	KCNJ12_KCNJ18	NM_001194958:c.G119A:p.R40H

17	21318952	A	G	exonic	nonsynonymous_SNV	KCNJ12_KCNJ18	NM_001194958:c.A298G:p.I100V
17	21319399	A	G	exonic	nonsynonymous_SNV	KCNJ12_KCNJ18	NM_001194958:c.A745G:p.I249V

Note: Filtering revealed 277 rare variants (non-synonymous AA variants/potential splice sites/ncRNA-exonic/rare <0.001 MAF variants). This included six genes with homozygous variants (SEMA6A, PGR, MYO18A, CNTD2, TOX2, VPS16), five genes with hemizygous variants (ARHGAP6, DGAT2L6, HS6ST2, NOX1, SLC9A7) and seven genes with potentially compound heterozygous variants (OBSCN, SEC22B, C6orf138, MDN1, NACAD, SSPO, KCNJ12_KCNJ18). None of these variants have been described as causative for IBD or EBV-induced hemophagocytosis. No variants in the Crohn's disease associated gene *NOD2* were identified.

Supplementary Table 4 Patient characteristics of published hemi- and heterozygous NOX1 variants and splice variants without functional testing

Patient ID	NOX1 variants	Gender	Age of diagnosis (of symptoms) in years	Diagnosis Paris class.[2]	Family history for IBD	Intestinal and extra-intestinal symptoms/findings on examination (age in years)	Histology	Treatment for IBD
P9*	c.C988T p.P330S	M	1.8 (1.5)	IBDU; E4	Grandmother (UC)	Severe pancolitis; Bloody stools (1.5); developed PSC (7.1); mild psoriasis (8.9).	Granuloma	AB, FTx, SALZ, NR
P10*	c.G1078A p.D360N	F	5.3	UC	No data provided	Pancolitis	No data provided	No data provided
P11*	c.G1078A p.D360N	M	4.7	IBDU	No data provided	Pancolitis	No data provided	No data provided
P12	c.G860A p.R287Q	F	8.8	CD; L3L4ab, B1, G0	Father (UC)	Presented with abdominal pain, fatigue, fevers, and oral ulcers, blood per rectum after painful bowel movements, perianal skin tags and mild joint pain.	Loose histiocytic aggregates, severely active colitis with ulcerations	Oral CS, 6-MP, ASA, IFX, ADA, MTX
P13.1	c.G860A p.R287Q	F	1.7	CD; L3, B2, G1	Mother P13.2 (CD)	Presented with bloody diarrhea and failure to thrive (1.7); initial colonoscopy with severe pancolitis, developed colonic stricture and ultimately required colectomy; severe failure to thrive.	Cryptitis, crypt abscesses, crypt distortion, multiple granulomas	ASA, AZA, MTX, CSA, IFX, ADA, AB
P13.2	c.G860A p.R287Q	F	Unknown	CD	Unknown	Unknown	Unknown	Unknown
P14	splice donor - exon 9 c.1133+1G>A near R378	M	11.9 (11.2)	CD; L2, B1	Father (IBDU, ankylosing spondylitis)	Large joint arthritis (10.6). Presented with diarrhea and abdominal pain (11.2). Mild pancolitis at diagnostic scope (11.9).	Acute and chronic inflammatory infiltrate, crypt architectural distortion (shortening, branching), no granulomas.	5-ASA, CS, AZA

Table 4 continued

Patient ID	NOX1 variants	Gender	Age of diagnosis (of symptoms) in years	Diagnosis Paris class.[2]	Family history for IBD	Intestinal and extra-intestinal symptoms/findings on examination (age in years)	Histology	Treatment for IBD
P15	splice acceptor - exon 7 c.672-1G>C near G224	M	12.7 (12.4)	IBDU; E4	No	Presented with bloody diarrhea and abdominal pain (12.4). Diagnostic scope reveals pancolitis (12.7). Hypertension secondary to CS, along with discovery of small right kidney (differential function: left kidney 64%; right kidney 36%).	Crypt abscesses, heavy cell infiltrates, branching glands. No giant cells or granulomas.	CS, AB, IFX.
P16	splice region variant c.142-4G>A	M	5	CD; B1p	No	Oral and perianal CD only	No data provided	IFX, ADA

Note: AB, oral or i.v. antibiotics given for treatment of colitis, bowel decontamination, fistula-treatment; ADA, adalimumab; ASA, 5-aminosalicylic acid; AZA, azathioprine; CD, Crohn's Disease, CS, corticosteroids; EIMS, extra-intestinal manifestations; F, female; FTx, fecal transplant; IBDU, indeterminate colitis; IFX, infliximab; M, male; MTX, methotrexate; NR, natural remedies (e.g. curcumin); NUT, polymeric/elemental diet; PR, per rectal; PSC, primary sclerosing cholangitis SALZ, sulfasalazine; ***Patients P9-11** are described in Hayes et al.[3]

Supplementary table 5 **Genetic association testing in males**

Males control		C(HOM A1)	C(HET)	C(HOM A2)	C(HAP A1)	C(HAP A2)	C(MISSING)	MAF	Allele numbers	p-value ALLELIC Test
	European ancestry exome chip dataset	0	0	0	71	2741	0	0.0252	2812	
	INTERVAL cohort	0	0	0	55	1877	0	0.0285	1932	
	Summed counts	0	0	0	126	4618	0	0.0266	4744	
Males IBD		C(HOM A1)	C(HET)	C(HOM A2)	C(HAP A1)	C(HAP A2)	C(MISSING)			
	European ancestry exome chip dataset	0	0	0	147	5093	3	0.0281	5240	0.5047
	Oxford IBD study	0	0	0	4	244	0	0.0161	248	
	COLORS in IBD	0	0	0	1	60	0	0.0164	61	
	Summed counts	0	0	0	152	5397	3	0.0274	5549	0.8425
Males UC		C(HOM A1)	C(HET)	C(HOM A2)	C(HAP A1)	C(HAP A2)	C(MISSING)			
	European ancestry exome chip dataset	0	0	0	84	2299	3	0.0352	2383	0.04243
	Oxford IBD study	0	0	0	1	60	0	0.0164	61	
	COLORS in IBD	0	0	0	0	18	0	0.0000	18	
	Summed counts	0	0	0	85	2377	3	0.0345	2462	0.0675
Males CD		C(HOM A1)	C(HET)	C(HOM A2)	C(HAP A1)	C(HAP A2)	C(MISSING)			
	European ancestry exome chip dataset	0	0	0	61	2691	3	0.0222	2752	0.5045
	Oxford IBD study	0	0	0	1	114	0	0.0087	115	
	COLORS in IBD	0	0	0	1	27	0	0.0357	28	
	Summed counts	0	0	0	63	2832	3	0.0218	2895	0.2173

Supplementary table 6 **Genetic association testing in females**

Female controls	C(HOM A1)	C(HET)	C(HOM A2)	C(HAP A1)	C(HAP A2)	C(MISSING)	MAF	Allele numbers	p-value ALLELIC Test
European ancestry exome chip dataset	2	137	2775	0	0	0	0.0242	5828	
INTERVAL cohort	1	90	1764	0	0	0	0.0248	3710	
Summed counts	3	227	4539	0	0	0	0.0244	9538	
Female IBD	**C(HOM A1)**	**C(HET)**	**C(HOM A2)**	**C(HAP A1)**	**C(HAP A2)**	**C(MISSING)**			
European ancestry exome chip dataset	7	271	5001	0	0	1	0.0270	10560	0.3044
Oxford IBD study	2	15	248	0	0	0	0.0358	530	
COLORS in IBD	0	6	37	0	0	0	0.0698	86	
Summed counts	9	292	5286	0	0	1	0.0277	11176	0.1486
Female UC	**C(HOM A1)**	**C(HET)**	**C(HOM A2)**	**C(HAP A1)**	**C(HAP A2)**	**C(MISSING)**			
European ancestry exome chip dataset	1	131	2067	0	0	1	0.0302	4400	0.06982
Oxford IBD study	0	5	43	0	0	0	0.0521	96	
COLORS in IBD	0	0	16	0	0	0	0.0000	32	
Summed counts	1	136	2126	0	0	1	0.0305	4528	0.04142
Female CD	**C(HOM A1)**	**C(HET)**	**C(HOM A2)**	**C(HAP A1)**	**C(HAP A2)**	**C(MISSING)**			
European ancestry exome chip dataset	5	135	2847	0	0	0	0.0243	5974	1
Oxford IBD study	1	5	127	0	0	0	0.0263	266	
COLORS in IBD	0	5	13	0	0	0	0.1389	36	
Summed counts	6	145	2987	0	0	0	0.0250	6276	0.8567

Supplementary References

1. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nature genetics* 2014; **46**(3)**:** 310-315.

2. Levine A, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK *et al.* Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflammatory bowel diseases* 2011; **17**(6)**:** 1314-1321.

3. Hayes P, Dhillon S, O'Neill K, Thoeni C, Hui KY, Elkadri A *et al.* Defects in NADPH Oxidase Genes and in Very Early Onset Inflammatory Bowel Disease. *Cellular and molecular gastroenterology and hepatology* 2015; **1**(5)**:** 489-502.