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A CD3-specific Antibody Reduces Cytokine Production and Alters Phospho-protein Profiles in Intestinal Tissues from Patients with Inflammatory Bowel Disease

Short title: Anti-CD3 in inflammatory bowel disease tissue

Authors: Anna Vossenkämper[1], Christian Hundsrucker[2], Kevin Page[3], André van Maurik[3], Theodore J. Sanders[1], Andrew J. Stagg[1], Lisa Das[4], and Thomas T. MacDonald[1]

[1] Centre for Immunology and Infectious Disease, Barts and The London School of Medicine and Dentistry, Blizard Institute, E1 2AT London, UK. [2] Institute for Functional Genomics, Computational Diagnostics Group, University of Regensburg, 93053 Regensburg, Germany. [3] GlaxoSmithKline, pharmaceuticals R&D facility, Gunnels Wood Road, Stevenage Herts, SG1 2NY, UK. [4]Centre for Digestive Diseases, Barts and London School of Medicine and Dentistry, E1 2AT London, UK.

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Abbreviations: Ab, antibody; CD, Crohn's disease; FcR, Fc gamma Receptor; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; LPMCs, lamina propria mononuclear cells; TCR, T cell receptor; UC, ulcerative colitis

Correspondence:
Anna Vossenkämper, Centre for Immunology and Infectious Disease, Blizard Institute, Barts and The London School of Medicine and Dentistry, London E1 2AT, UK.
a.vossenkaemper@qmul.ac.uk; Tel +44-20-78822311; Fax +44-20-78822181

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Contributions: AV and TTM designed the study, performed most experiments and wrote the manuscript. CH analysed the array data and created heatmaps. TJS and AJS performed the qRT-PCR. LD provided intestinal specimens, analysed data and reviewed the manuscript. KP and AvM performed experiments and reviewed the manuscript. Otelixizumab was provided by GlaxoSmithKline.

Abstract

Background & Aims: T cells mediate the development of inflammation in inflammatory bowel disease (IBD). We investigated the effects of an antibody against CD3 called otelixizumab, which induces immune tolerance, in intestinal mucosa samples from patients. **Methods:** Intestinal tissues were isolated from individuals undergoing routine endoscopy or from patients undergoing intestinal surgery for colon cancer or IBD; healthy surrounding tissues were collected as controls. Isolated lamina propria mononuclear cells (LPMC) and mucosal tissue explants were incubated with otelixizumab for 24 or 48 hours. Production of inflammatory cytokines was determined by ELISA. Levels of 36 cytokines and chemokines and phosphorylation of 39 receptor tyrosine kinases and signaling molecules were measured using protein arrays. Immunoblot analysis was used to analyze T-cell transcription factors. **Results:** Incubation of intestinal tissues or LPMC with otelixizumab reduced production of interferon γ, interleukin (IL)17A, and other inflammatory cytokines and chemokines, simultaneously increasing production of IL10. Mucosal biopsies from patients with IBD retained inflammation-associated tyrosine phospho-protein profiles ex vivo. Incubation of the inflamed tissue with otelixizumab reduced phosphorylation of these proteins to levels observed in control tissues. Otelixizumab also markedly reduced phosphorylation of proteins associated with T-cell receptor activation. Neutralization of IL10 blocked the anti-inflammatory effects of otelixizumab. **Conclusions:** We observed anti-inflammatory effects of anti-CD3 in inflamed intestinal tissues from patients with IBD. The antibody appears to downregulate T-cell activation via IL10.

Keywords: Crohn's disease, ulcerative colitis, immune regulation, IFN

Introduction

Since the first anti-CD3 antibody, muromonab (OKT3), was developed more than 30 years ago to prevent transplant rejection and graft versus host disease [1], considerable progress has been made in the development and engineering of anti-CD3 antibodies for the treatment of immune disorders. OKT3 induces a cytokine storm, which obviates its use in the treatment of autoimmune conditions [2-4]. This cytokine storm is a consequence of the cross-linking of the CD3/T cell receptor (TcR) on T cells, coupled with Fc binding to the Fc gamma Receptor (FcR) on other immune cells. To reduce toxicity of anti-CD3 antibodies, $F(ab)_2$ fragments [5] and mutated, humanized Abs, which did not bind FcR, have been developed [6]. It is thought that these tolerising anti-CD3 antibodies function by driving the internalization of the TcR/CD3 complex so that signaling via peptide/MHC is abolished[7, 8]. In addition, the antibodies when binding to CD3/TcR may signal to the cell and drive differentiation along a tolerogenic pathway [7]. Finally, there is some evidence that tolerising anti-T cell antibodies can cause T cell apoptosis[9].

Tissue injury in inflammatory bowel disease is driven by T cells [10] and is therefore a disease target for T cell therapies. Initial studies in ulcerative colitis (UC) with the anti-CD3 antibody visilizumab showed clinical benefit [11, 12], however, a randomized, placebo-controlled trial gave negative results [13]. In Crohn's disease (CD), a phase I study of the anti-CD3 antibody NI-0401 showed no improvement in Crohn's disease activity index,

but there was significant endoscopic improvement[14]. Further clinical studies are clearly needed, however, what remains lacking is any evidence that anti-CD3 antibodies have an effect on pro-inflammatory T cells in the mucosa of patients with IBD.

Otelixizumab (TRX4 or ChAglyCD3), is an Fc-engineered, chimeric/humanized monoclonal IgG1 antibody against human CD3-epsilon which showed promising early results in Type I diabetes[15, 16] but was not superior to placebo in Phase 3 studies when given at a lower dose than the earlier work [17]. We have developed *ex vivo* assays where T cell activity in inflamed bowel tissue can be accurately studied[18-20] , and so we have investigated for the first time if anti-CD3 antibodies change T cell function in human tissues.

Materials and Methods

Reagents and Antibodies

All reagents were purchased from Sigma Aldrich (Gillingham, UK) unless otherwise stated. Anti-human CD28 was purchased from R&D systems (Abingdon, UK). Otelixizumab (Tolerx, Cambridge, MA, USA) is an aglycosylated chimeric/humanized monoclonal antibody (IgG1 lambda) directed against human CD3/epsilon (generously provided by GlaxoSmithKline). YTH12.5 is a mitogenic rat anti-human CD3 antibody (IgG2b lambda; provided by Tolerx) and the parental Ab of otelixizumab. Purified human IgG1 lambda was used for control purposes (Sigma Aldrich). IL-10 was neutralized with rat anti-IL-10 (used at 10µg/ml; BioLegend, Cambridge, UK).

Patient samples

Tissue was obtained during routine endoscopy or from surgical specimens of patients undergoing intestinal surgery because of colon cancer or IBD. Healthy intestinal tissue surrounding malignant tumors was sampled and used as control. All patients took part in this study after informed written consent. The study was approved by the local ethics committee. Patient characteristics are listed in supplementary table SI.

Isolation of peripheral blood mononuclear cells

Peripheral blood was obtained from healthy volunteers after informed consent, diluted with PBS 1:1 and layered on top of Ficoll Paque solution (GE Healthcare, Amersham, UK). After centrifugation at 21˚C for 30min, 300g without break, the buffy

coat was aspirated and washed once in PBS. Cells were cultured in RPMI/ 10% human serum/ Pen/Strep/ L-Glutamine or subjected to immunostaining.

Processing of tissue specimens

With surgical specimens, the mucosa was cut off the submucosa and cut into 2mm^2-sized pieces. Epithelial cells of biopsies or mucosal pieces were removed with 1 mM EDTA in HBSS containing 100 U/ml penicillin and 100 µg/ml streptomycin (Pen/Strep) for 30min. A single cell suspension was prepared in RPMI/ 10% FBS/ L-Glutamine/ Pen/Strep/ 50µg/ml gentamicin/ 5µg/ml amphotericin B with collagenase D (1mg/ml) and DNase (10U/ml; Roche, Burgess Hill, UK) for 1h. Cells were passed through a cell strainer and subjected to density centrifugation with Ficoll Paque (GE Healthcare, Amersham, UK) by layering the cell suspension on top of the Ficoll. After centrifugation at 21˚C for 30min, 300g without break, the buffy coat was aspirated and washed once in PBS. Cells were cultured in RPMI/ 10% human serum/ Pen/Strep/ L-Glutamine.

Organ culture of mucosal explants

The mucosa of intestinal surgical specimens was cut into 3mm^2 pieces and cultured in 24-well plates in 300µl serum-free HL1-medium (Lonza, Cambridge, UK) containing glutamine, Pen/Strep, and 50µg/ml gentamicin. Mucosal samples were emerged in liquid and culture was performed for up to 48h at 37°C, 5% CO_2. For antibody experiments, the respective antibody was added to the culture medium. Supernatants and tissue samples were snap-frozen and stored at -70C.

Flow cytometry

Antibodies were used at the concentrations recommended by the manufacturer: anti-CD4-FITC; anti-CD25-PE, CD2-APC, IFN-γ-PECy7, IL-10-PE, and matched isotype controls (BD Bioscience, Oxford, UK). PBS/20% human serum was used to block non-specific binding and cell surface staining was performed for 30min on ice. For intracellular cytokine staining, cells were cultured overnight with otelixizumab or human IgG. For the last 4h of culture, 2 mM monensin was added. After surface staining, cells were fixed in Leucoperm (AbD Serotec, Oxford, UK) solution A, followed by permeabilization in solution B and intracellularly stained for 30min. Intracellular staining of Foxp3 was performed with the Alexa Fluor 647 anti-human Foxp3 flow kit from BioLegend (London, UK). Cell viability was assessed by annexin V-FITC and propidium iodide (PI) staining (BD Bioscience) as previously described [21]. For multi-colour flow cytometry, all analyses included the appropriate fluorescence-minus-one controls (i.e. samples stained with every reagent except for the one of interest to guide compensation. Flow cytometry was performed using the LSRII analyzer (Becton Dickinson), data were analyzed with FACS Diva software.

Quantification of CD3/TCR modulation by flow cytometry

PBMC ($1x10^6$/mL) were cultured in RPMI + 10% AB serum in the presence or absence of otelixizumab. At indicated time points cells were removed, washed with FACS buffer (PBS + 1% FCS + sodium azide 0.1%) After Fc blocking for 5min with TruStain FcX (BioLegend) cells were stained with: CD4-PerCP-Cy5.5(clone RPA-T4), CD8-APC (clone RPA-T8), CD3-FITC(clone SK7), TCRab-PE (clone IP26) or anti-Human IgG Fc-PE (clone

HP6017) (all BioLegend). After washing with 3ml FACS buffer the cells were re-suspended in 300µl Cytofix (BD Biosciences). Lymphocytes were gated on FSC v SSC and 50,000 events were acquired. The Mean Fluorescence Intensity (MFI) data for PE and FITC fluorescence for gated CD4 and CD8 cells were converted to Molecules of Equivalent Soluble Fluorochrome (MESF) values using Quantum Simply Cellular beads (Bangs Laboratories) as per the manufacturer's instructions.

Western blotting

Whole cell lysates were subjected to SDS-PAGE and immunoblotting as previously described [21]. Anti-T-bet (Santa Cruz, Heidelberg, Germany), anti-RORgt and anti-β-actin (Abcam, Cambridge, UK) were used according to manufacturer's instructions. Intensities of the protein bands were measured using Image J software and relative values were calculated by dividing the IgG and otelixizumab values by the untreated control.

Signaling arrays and cytokine arrays

Phosphorylation status of receptor tyrosine kinases and signaling molecules was determined by employing PathScan RTK signaling arrays (Cell Signaling, Danvers, MA, USA). Seventy-five µg protein of whole cell lysates were probed onto the array. Cytokine arrays were purchased from R&D Systems ("Proteome Profiler Cytokine array kit, Panel A") and performed with 100 µl of supernatants according to instructions. The chemiluminescent signals of all arrays were detected on X-ray films and the pixel intensities measured using ImageJ software. Heatmaps of relative pixel intensities were generated with R (v. 2.15.0) and the compdiagTools package (v. 1.8.2) [22, 23] . For

consistency and easier data presentation, the intensity values >1700 were given the same dark red colour. When otelixizumab reduced cytokines to undetectable levels, the fold reduction was given an arbitrary value of 10.

ELISA

Cytokine concentrations in culture supernatants were determined by enzyme linked immunosorbent assay (ELISA) using kits from R&D Systems (IL-17A), ImmunoTools (Friesoythe, Germany; IFN-γ) and ebioscience (Hatfield, UK; IL-2) according to manufacturer's instructions.

Quantitative real-time PCR

Tissue was sonicated in Trizol reagent (Life Technologies, Paisley, UK) and RNA isolated with the Direct-Zol kit (Zymo Research, Irvine, CA, USA). Reverse transcription was performed using QuantiTect Reverse Transcription Kit (Qiagen, Manchester, UK). Quantitative RT- PCR was performed using QuantiFast SYBR Green PCR Kit (Qiagen) on a 7500 Real-Time PCR System (Applied Biosystems, Paisley, UK). IL-10 expression was determined as the geometric mean of IL10 normalized to GAPDH, RPL30 and PGK1 using the $2^{-\Delta Ct}$ method [24]. Primers: IL10 (QT00041685), PGK1 (QT00013776), RPL30 (QT00056651) (Qiagen). GAPDH: (forward) TGCACCACCAACTGCTTAGC; (reverse) GCATGGACTGTGGTCATGAG.

Statistical testing

Statistical analysis was performed with GraphPad Prism or InStat software. Two-tailed Student t-test was used to compare two independent values and the One-way ANOVA with post-test was used for multiple comparisons. Array data were statistically

analysed by calculating the average of all individual proteins in the individual groups and the sums of the resulting two data sets were then compared to each other with the Wilcoxon rank-sum test (in GraphPad Prism). For example, the intensity values of the individual cytokines in the group "CD treated with IgG" were averaged and the whole set of values then statistically compared to all cytokine averages in group "CD treated with otelixizumab". Tables used for calculations are shown in the supplementary material. A p value < 0.05 was considered significant.

Results

Otelixizumab dramatically reduces surface CD3 in blood and mucosal T cells.

We aimed to use concentrations of otelixizumab which caused internalization of surface CD3. With one million blood T cells, a concentration of 1µg/ml otelixizumab reduced CD3 sites from around 200,000 per cell to < 5,000 **(Fig 1A)**. This effect persisted for 24h. Otelixizumab caused the internalization of surface CD3 as well as disappearance of the αβ T cell receptor from the cell surface. Otelixizumab bound to CD3 blocked the binding of all available anti-TCR and anti-CD3 detection antibodies we have tested. As a consequence down-modulation of the CD3/TCR complex could only be measured by detection of bound otelixizumab with secondary detection antibodies. Down-modulation was evident after 4h (data not shown) and by 24h less than 50% of CD3/TCR molecules remained **(Fig 1A)**. By day 4 almost 90% of the complexes had been internalized (data not shown). When biopsies from UC patients were cultured with 1µg/ml otelixizumab for 16h, rapidly dispersed without collagenase treatment between

two glass slides and incubated with FITC-anti-CD3, CD3 expression was greatly reduced on the cells from biopsies treated with otelixizumab. Strong CD3 expression was observed in cells isolated from biopsies treated with a control IgG **(Fig 1B)**.

Otelixizumab is non-mitogenic and does not affect cell viability

T cells in both normal and inflamed human gut are in an activated state [10], therefore the first experiments were designed to ensure that otelixizumab was neither mitogenic nor increased pro-inflammatory cytokine production by normal and IBD lamina propria mononuclear cells (LPMC). The rat YTH12.5 antibody, from which otelixizumab was derived, induced proliferation of T cells from normal human gut and from IBD mucosa. In contrast, otelixizumab was non-mitogenic, even in the presence of anti-CD28 **(Fig 1C)**. In terms of cytokine production, YTH12.5 induced high levels of IFN-γ and IL-17A, both being important cytokines in IBD[25], by both normal and IBD LPMC. While there was some spontaneous IFN-γ and IL-17A production by IBD LPMC, cytokine production was reduced by otelixizumab **(Fig 1D)**. Crohn's disease and control LPMCs were next cultured with plate-bound anti-CD3 Ab and anti-CD28 Ab in the culture medium. When otelixizumab was added at the onset of the cultures, IFN-γ and IL-17A production were significantly reduced. We also added otelixizumab after 16h to αCD3/CD28 stimulated LPMC. IFN-γ and IL-17A concentrations were reduced, but significance was only reached for control LPMCs **(Fig 1E)**. To test if otelixizumab treatment can reduce the stimulatory capacity of a subsequent YTH12.5 treatment, we

cultured LPMCs from normal and inflamed mucosa for 3h with otelixizumab, changed the medium, then added YTH12.5 for 21h. Pre-treatment with otelixizumab before YTH12.5 was added strongly diminished IFN- γ production by LPMCs. We observed that LPMCs pre-treated with YTH for 3h, then cultured with otelixizumab show a reduced IFN- γ response **(Fig 1E,** for IL-17A and IL-2 data see **supplementary figure S1).**

We determined if the inhibitory effects of otelixizumab were due to T cell death since an earlier *in vitro* study had indicated that the anti-CD3 antibody visilizumab rapidly induced apoptosis in gut T cells in IBD [9]. LPMCs were cultured with otelixizumab for 48h and stained with annexin V and propidium iodide. Otelixizumab at concentrations of up to 10μg/ml did not increase the number of apoptotic or dead cells in LPMCs from healthy gut or from CD and UC mucosa (**Fig 1F**). Crohn's disease and UC LPMCs were more resistant to cell death than cells from healthy colon, confirming a previous study [26].

Otelixizumab decreases proinflammatory cytokine production in inflamed mucosa

We next examined T cell responses in CD and UC mucosal biopsies cultured *ex vivo*. Otelixizumab reduced the concentrations of IFN-γ and IL-17A in CD and UC explant culture supernatants compared to the IgG control (**Fig 2A**). The transcription factors T-bet and RORγT, the main regulators of IFN-γ and IL17 expression, respectively, were also reduced in CD explants treated with otelixizumab **(Fig 2B).** Supernatants of inflamed CD and UC explants were further subjected to a protein array to measures relative levels of

36 cytokines and chemokines **(Fig 2C, D, E**). Otelixizumab in CD and UC organ cultures had strong global effects. For Crohn's disease there was a 2-5 fold decrease in C5a, sCD40L, G-CSF, GRO-α, ICAM-1, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-12, IL-13, IL-16, IL-27, CXCL11, CCL2, CCL3, TNF-α, RANTES, CCL4, sTREM-1, and a 5-10 fold decrease in GM-CSF, CCL-1, IL-6, IL-17A, IL-23, IL-32α, CXCL10, CXCL12. For ulcerative colitis there was a 2-5 fold decrease in C5a, ICAM-1, IFN-γ, IL-12, IL-13, IL-27, CXCL11, CCL2, CCL3, CCL4, RANTES, a 5-10 fold decrease in GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-17A, IL-32α, CXCL10, CXCL12, and sTREM-1, and a greater than ten-fold decrease in TNF-α and IL-1α. Cytokines which changed less than two-fold were IL-1RA, IL-8, MIF, and Serpin E1, in CD, and sCD40L, G-CSF, GRO-α, IL-1RA, IL-16, IL-8, IL-23, MIF, and Serpin E1 in UC. The average value of all cytokines of IgG-treated vs. otelixizumab-treated samples were significantly different for CD as well as for UC (**Figures 2C, D** showing representative cases). IgG (green bars), otelixizumab (red bars). **Fig 2E** shows heatmaps of all 12 cases. The individual values are listed in **supplementary table SII and III**. Otelixizumab significantly increased the levels of IL-10 in IBD mucosa supernatants **(supplementary tables II and III)**.

Otelixizumab reduces phosphorylation in IBD tissue to levels observed in healthy mucosa

Because phosphorylation of receptors and signaling molecules is an important mechanism for cells to respond to inflammation and other extracellular stimuli, we analysed the phosphorylation status of 39 receptor tyrosine kinases and signaling

molecules in freshly isolated colonic mucosa of healthy individuals and IBD patients by employing a phosphorylation-specific protein array. Molecules on the array included growth factor receptors, Ephrin family members, ERK, Zap70, Lck, Src, and Stat1 and Stat3. Very few phosphorylation events were detectable in lysates of freshly isolated healthy colonic mucosa **(Fig 3A,** single representative cases**)**. In contrast, inflamed CD mucosa and UC mucosa showed high relative intensity of phosphorylation of the majority of analysed kinases (**Fig 3A, B**). There was variation between individual patients, especially in Crohn's disease, probably reflecting the patchy nature of the lesions. Average intensity values of phospho-proteins of IgG-treated vs. otelixizumab-treated samples were significantly different for CD and UC.

When phosphorylation status was measured in lysates of CD mucosal explants cultured with IgG for 48h, the strong level of phosphorylation was maintained, indicating the continuing persistence of the inflammatory process *ex vivo* **(Fig 3C** showing single representative cases**)**. Addition of otelixizumab to CD explants for 48h strongly reduced the phosphorylation of the majority of kinases (green bars in **Fig 3C**). Similar results were observed for UC explants cultured with IgG or otelixizumab for 48h (green bars in **Fig 3C**). By 48h, the phosphorylation status of IBD biopsies cultured with otelixizumab had returned to about the same level as fresh normal mucosa**.** Notably, otelixizumab strongly inhibited phospho-proteins associated with T cell receptor signaling. **Fig 3D** shows a heatmap of the relative intensities of the phosphorylation status of receptor tyrosine kinases in biopsies from 6 CD and 6 UC cases, cultured with IgG or otelixizumab for 48h. Relative intensity values are shown in **supplementary table**

SIV, V and VI. While there was some variation between patients, overall the effects at 48h were strong. For example in Crohn's disease patient 1, only 1 of the 39 kinases had a value of zero when cultured with IgG whereas in the biopsy cultured with otelixizumab, 26 of 39 kinases gave no signal. Likewise in ulcerative colitis patient 2, the biopsy treated with IgG gave a positive signal for all 39 kinases while the biopsy treated with otelixizumab gave no signal for 26 kinases. The average intensity values of phospho-proteins of IgG-treated vs. otelixizumab-treated samples were significantly different for the 48h values.

Otelixizumab's effects are dependent on interleukin-10

Next, we focused on the role of IL-10 since in the array it was the only cytokine which increased with otelixizumab treatment. Supernatants of IBD LPMCs and explants cultured with otelixizumab showed increased IL-10 concentrations **(Fig 4A)**. Biopsies of inflamed mucosa cultured with otelixizumab had higher relative IL-10 mRNA expression than the IgG treated biopsies as measured by RT-PCR **(Fig 4A)**. When CD explants were cultured with otelixizumab and IL-10-neutralizing Ab, the IFN-γ concentration exceeded basal levels **(Fig 4B).** Unstimulated Crohn's disease LPMCs cultured with otelixizumab showed reduced intracellular IFN-γ and increased intracellular IL-10 **(Fig 4C)**. We next determined if the reduction in the phospho-protein profile in IBD mucosa cultured *ex-vivo* with otelixizumab was IL-10-dependent. Consistent with our earlier experiments, otelixizumab treatment reduced the phosphorylation levels in CD explants **(Fig 4D** showing one representative experiment**);** however, adding IL-10 neutralizing antibody along with otelixizumab inhibited this effect and the tissue maintained a high

phosphorylation status, far in excess of IgG control (**Fig 4D, E showing the heatmaps of 5 cases; supplementary table VII**). We confirmed the importance of IL-10 in our explant culture system by adding exogenous IL-10 to CD biopsies. IFN-γ and IL-17A levels were reduced in cultures treated with IL-10 (**Fig 4F**).

Discussion

Our results show that otelixizumab is profoundly anti-inflammatory when added to IBD LPMC and mucosal tissues from IBD patients. We must emphasize that this study was designed to show proof of principal that tolerising anti-CD3 antibodies have effects on T cells in inflamed tissue, and did not attempt to address why visilizumab, another tolerising anti-CD3 antibody, was not effective in ulcerative colitis in a randomized placebo controlled study[13]. We were well aware of the issues regarding the potential effects of dose of anti-CD3 antibodies and so we chose a dose of 1μg/ml for most of our studies since at this dose there was almost complete internalization of surface CD3 in blood T cells and in T cells in biopsies. The functional effects of otelixizumab were evidenced by the broad down-regulatory effects on pro-inflammatory cytokine and chemokine production, and even more remarkably, the reduction in phospho-protein levels in pathways unrelated to immune cells. For example, TrkA and TrkB are receptors for nerve growth factor and neurotrophins, ephrin receptors are involved in cell migration in many cell types, FGF receptors are involved in angiogenesis and wound healing, and HER2, HER3 and EGFR are involved in epithelial renewal. Since we can be

completely sure otelixizumab only targets T cells, these data show that T cell activation drives these myriad increased signaling pathways in IBD.

We also show that otelixizumab increases IL-10 production by IBD biopsies and that the inhibitory effects of otelixizumab on phospho-protein reduction are IL-10 dependent. This is very similar to previous studies where patients with type I diabetes treated with a tolerising anti-CD3 antibody showed increases in serum IL-10, and activation of blood T cells also increased IL-10 production [27]. The new data that we present here show that the same effect occurs in activated T cells in tissues. It is very well established that IL-10 is a potent inhibitor of antigen presentation and the fact that we were able to show that otelixizumab reduced phosphorylated Lck and Zap70 in explants is consistent with both a decrease in antigen presentation and a loss of TcR signaling caused by the internalization of the TcR/CD3 complex. However, we must emphasize that we do not know if there is some signaling when otelixizumab binds to the CD3/TcR complex which delivers a signal to the T cell to shut down pro-inflammatory cytokines and produce IL-10, similar to the effects of anti-CD46 and anti-CD55 [28, 29].

An obvious question is whether otelixizumab is activating nascent IL-10 secreting regulatory T cells or whether it is driving Th1 or Th17 cells to produce IL-10. Our intracellular staining suggests the latter. However, we did examine if otelixizumab increased the number of CD4+, CD25+, FoxP3+ cells (**Supplementary Figure 1**), and indeed this was the case. Further studies are needed to define exactly the pathways by which otelixizumab is having such powerful effects and importantly, whether the effects

we have identified here are seen at lower doses of antibody. Although systemic and mucosal delivery of IL-10 for IBD therapy failed [30, 31], our study does re-inforce the important role of IL-10 in controlling inflammation in the human gut, and is consistent with reports on children with mutations in IL-10R who develop severe gut inflammation [32, 33]. In addition, studies in humanized mice have shown that treatment with the tolerising anti-CD3 antibody teplizumab induces T cells which home to the gut and secrete IL-10 [34]. This study, however, was markedly different to ours because we investigated resident cells in human tissues.

Our present work also builds on animal models of inflammatory disease where anti-CD3 antibodies have proved to be beneficial. For example, in mouse models of systemic lupus erythematosus, nasal and oral administration of anti-CD3 antibody results in decreased numbers of IL17+ follicular T helper cells and an increase in IL10-producing CD4+CD25+ T cells [35, 36]. Likewise, intravenous administration of anti-CD3 antibodies in murine experimental autoimmune encephalomyelitis (EAE) is helpful by the induction of regulatory T cells [37]; and intraperitoneal treatment with an Fc-engineered anti-CD3 antibody showed beneficial effects with regard to symptoms, cytokine levels and T cell numbers in EAE[38].

We appreciate that a limitation of our study was the inability to track the fate and responsiveness of mucosal T cells treated with otelixizumab for prolonged periods of time because of the need to activate T cells in long-term culture to prevent cell death. Also, the often limited availability of biopsies and surgical specimens prevented us from working with a larger number of cases. Further, we used only one dose of antibody

throughout our study. Therefore the focus of future studies will be to evaluate the effect of different otelixizumab doses on mucosal T cells as the issue of the optimal anti-CD3 in patients dose remains. For example, with regard to the failed clinical studies with visilizumab, it is not at all clear that 5 μg/kg for two days is a high enough dose to drive the internalization of the TcR/CD3 complex on all T cells in the tissues [11, 12]. Our *in vitro* studies had on the other hand the advantage that we could interrogate closely how otelixizumab affects cytokines and phosphorylation patterns in the inflamed mucosa; something which would be difficult to do during a clinical trial.

In conclusion, we show here by various experimental approaches that the anti-CD3 antibody otelixizumab dampens inflammation in human tissue by a mechanism involving the induction of IL-10. Overall, we consider it premature to think tolerising anti-CD3 antibodies may not be a potential therapy for IBD on the basis of the previously failed clinical studies. Since otelixizumab can be given safely at much higher doses than other anti-CD3 antibodies, consideration should be given as to whether a clinical trial of this particular antibody in IBD may be justified.

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Legends to figures

Fig 1. Otelixizumab leads to CD3/ TCR internalization, but does not induce proliferation, inflammatory cytokine production, or cell death. A. Normal PBMCs were cultured with/ without otelixizumab for indicated times, stained with αCD4, αCD3, αTCRα/β or αhuman IgG Fc-PE and expression determined by flow cytometry. Culture with otelixizumab reduced expression of CD3 and TCRα/β on blood T cells. Graphs show the results of two experiments. **B.** UC biopsies were cultured with otelixizumab or IgG (1μg/ml) for 16h, tissue was manually disintegrated by rubbing it between frosted glass slides and repeated aspiration through a needle. Cells were stained and expression of CD3 analysed by flow cytometry. Data from one of two experiments with similar results. **C.** Normal LPMCs, CD LPMCs, and UC LPMCs were labeled with CFSE and cultured with indicated treatments for 96h. Histograms show CFSE dilutions indicating cell proliferation (gate set on CD2+ cells). Histograms show one of three experiments with similar results. **D.** IFN-γ and IL-17A concentrations in supernatants of LPMCs from healthy, inflamed CD and UC mucosa that were cultured with otelixizumab or YTH12.5. N=3 samples per group; Two-tailed paired t-test. Mean + SEM. **E.** Normal LPMCs and CD LPMCs were cultured in the presence of plate-bound αCD3 and soluble αCD28. Otelixizumab and IgG (1μg/ml) were added to the cultures immediately or after 16h of culture. IFN-γ concentration in the supernatants was measured after 48 hours of culture. Even when added at 16 hours, otelixizumab decreased IFN-γ production although statistical significance was only achieved for the former. n=3 per group, two-

tailed paired Student t-test. Mean + SEM. In addition, normal LPMCs and CD LPMCs were cultured in the presence of otelixizumab, IgG and YTH (all 1µg/ml) as indicated. The second antibody was added after 3h and a wash-out of the first Ab. IFN-γ in the supernatant was measured by ELISA. HC n=4; CD n=3. One-way ANOVA with post-test. Mean + SEM. **F.** Percentages of apoptotic and dead LPMCs (from normal, CD and UC mucosa) after culture with indicated treatments for 48h. Normal LPMCs n=4, CD LPMCs n=5, and UC LPMCs n=5.

Fig 2. Otelixizumab modulates the inflammatory cytokine response in inflamed IBD tissue. A. IFN-γ and IL-17 concentrations in supernatants of mucosal explants of inflamed CD colon and UC colon. Explants were cultured with IgG or otelixizumab (both 1µg/ml) for 16h. CD, n=20; UC, n=14. Two-tailed paired t-test. Mean + SEM. **B.** Mucosal explants from active CD colon were cultured with IgG or otelixizumab for 16 h and then lysed. T-bet, RORγt and ß-actin (loading control) were determined by western blot. Shown is one representative blot of 5 experiments with similar result. The graph shows the relative optical density of the bands (n=5 blots). Values are the ratios between IgG or otelixizumab-treated samples and the untreated control. Mean +SD. The * indicates $p<0.0001$. Two-tailed paired Student's t-test. **C, D**. Inflamed CD mucosal explants (**C**) and UC colon (**D**) explants were cultured with IgG or otelixizumab for 16h. Supernatants were probed onto a multiplex cytokine array. Relative pixel intensities of cytokine levels of one representative array of a single Crohn's patient and a single UC patient are shown. **E**. Heatmaps showing the relative signal intensities for the analysed cytokines. Plotted are the results from 6 inflamed CD samples and 6 UC inflamed samples which

had been cultured with IgG or otelixizumab for 16h; the data of the 6 cases each were averaged for each cytokine and all average values were analysed by comparing them with the Wilcoxon rank-sum test (i.e. all cytokine averages of CD IgG vs all cytokine averages of CD otelix). CD n=6, UC n=6. CD IgG vs. CD otelix p=0.0004; UC IgG vs UC otelix $p=3.4x\ 10^{-5}$.

Fig 3. Otelixizumab alters the kinase phosphorylation status in inflamed Crohn's disease and ulcerative colitis tissue. **A**. Representative phosphorylation levels of various receptor-tyrosine kinases and signaling molecules in lysates of healthy colonic mucosa, inflamed CD mucosa, and inflamed UC mucosa as analysed by a multiplex phosphorylation array. Biopsies were immediately snap frozen after being taken from the patient. **B**. Heatmap showing the relative signal intensities of the phospho-proteins in 4 normal colon samples, 7 CD and 6 UC samples. The relative values between 1700-4000 were given the same red colour to simplify the heatmap. The phospho-intensities of all values of the 4 controls, 7 CD, and 6 UC, respectively, were averaged for the particular group and analysed by comparing them with the Wilcoxon rank-sum test. Control vs. CD $p=1.08x10^{-13}$; Control vs. UC $p=2.910^{-14}$. **C**. Representative phosphorylation levels of kinases in lysates of one inflamed CD explants and one inflamed UC explants cultured for 48h with either IgG or otelixizumab (both 1µg/ml). **D**. Heatmap showing the relative signal intensities for the analysed phosphorylated kinases. Plotted are the results of inflamed samples from six Crohn's patients (CD1, CD2, etc.) and six UC patients (UC1, UC2, etc.) at 48h culture with otelixizumab. The relative

values between 1700-4000 were given the same red colour to simplify the heatmap. All samples show a reduction in phosphoproteins at 48h; Wilcoxon rank-sum test, n=6. CD IgG vs otelix $p=1.08x10^{-7}$; UC IgG vs. otelix $p=1.4 x10^{-6}$.

Fig 4. Otelixizumab promotes an anti-inflammatory response *via* IL-10. A. LPMCs from normal, CD and UC mucosa were cultured with IgG or otelixizumab (both 1µg/ml) for 48h as indicated. CD and UC explants were cultured for 16h with IgG or otelixizumab. IL-10 concentrations in supernatants were measured. LPMCs n=4; explants n=18 (CD) and n=14 (UC). Two-tailed t-test. Mean + SEM. To measure the relative expression of IL-10 mRNA by qRT-PCR, mucosal biopsies of inflamed CD and UC colon were cultured with IgG, otelixizumab (both 1µg/ml) for 24h. UC cases in blue n=6; CD cases in black n=5. p=0.04, Wilcoxon rank sum test. **B.** Mucosal biopsies of inflamed CD colon were cultured with IgG, otelixizumab (1µg/ml), neutralizing anti-IL-10 (10µg/ml) or the matching isotype Ab for 16h. IL-10 in supernatants was measured by ELISA. Otelixizumab abolished the spontaneous IFN-γ production, but this effect was reversed by anti-IL-10. Shown are the means + SEM of three independent experiments. One way ANOVA with post-test. **C.** Unstimulated CD LPMCs were cultured as indicated for 16h, then for further 4h with monensin before intracellular staining of IFN-γ and IL-10 was performed. Plots show cytokine expression in CD2+ LPMCs and demonstrate one of two experiments. **D**. Explants of cultures like shown in (**A**) were subjected to the RTK-phospho-array. Shown is the result of one of five independent experiments. **E.** The heatmap summarizes the data of the five separate samples. Wilcoxon rank-sum test,

otelix + anti-IL10 vs. otelix + isotype, p= 6.6x 10^{-8}. **F**. CD explants were cultured for 16h as indicated. Recombinant IL-10 was used at 10ng/ml, antibodies at 1µg/ml. IFN-γ and IL-17A were determined by ELISA in supernatants. Exogenously added IL-10 clearly diminishes IFN-γ and IL-17A production. N=3. One way ANOVA with post-test. Mean +SEM.

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Author names in bold designate shared co-first authors.

Figure 1

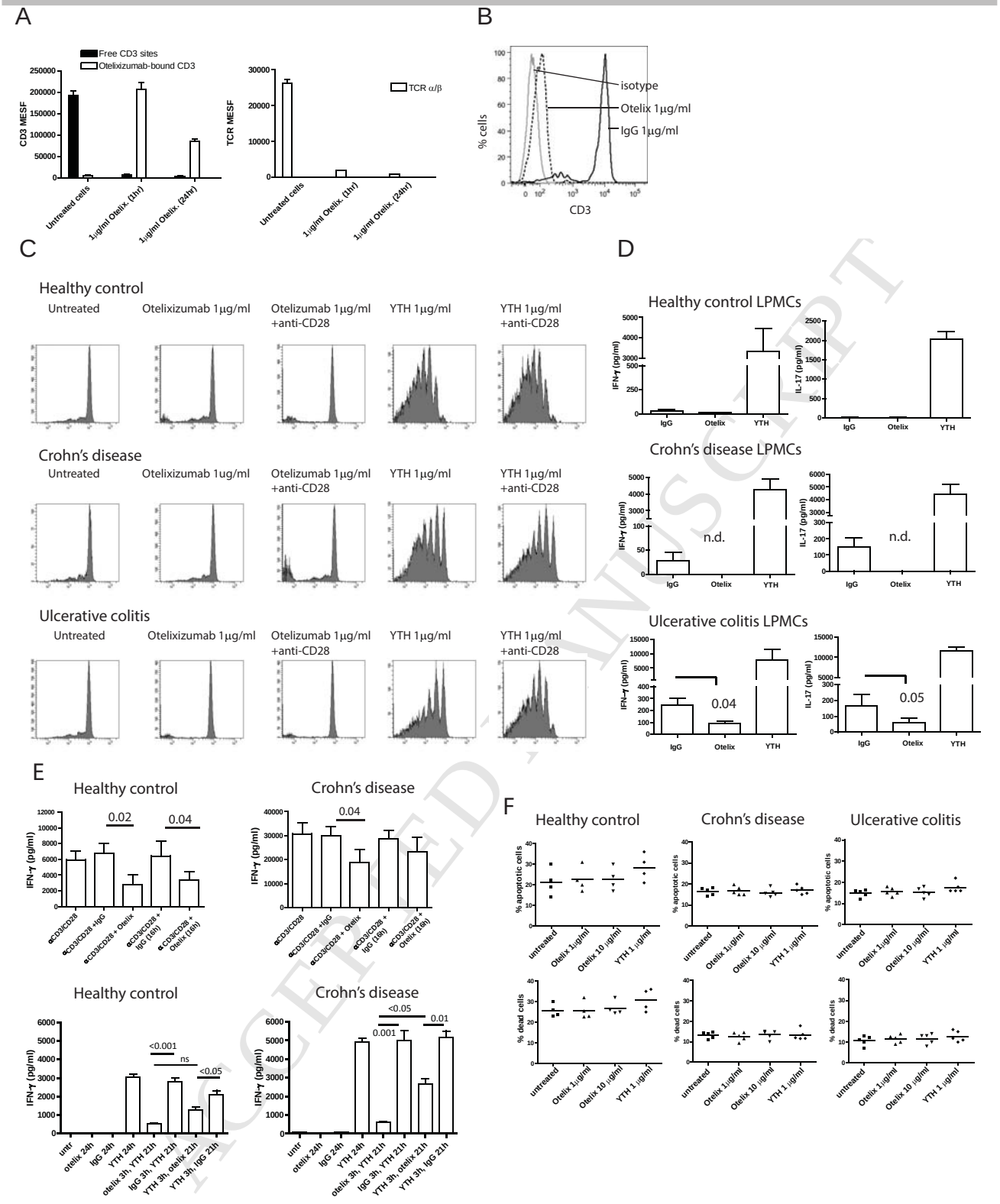


Figure 2

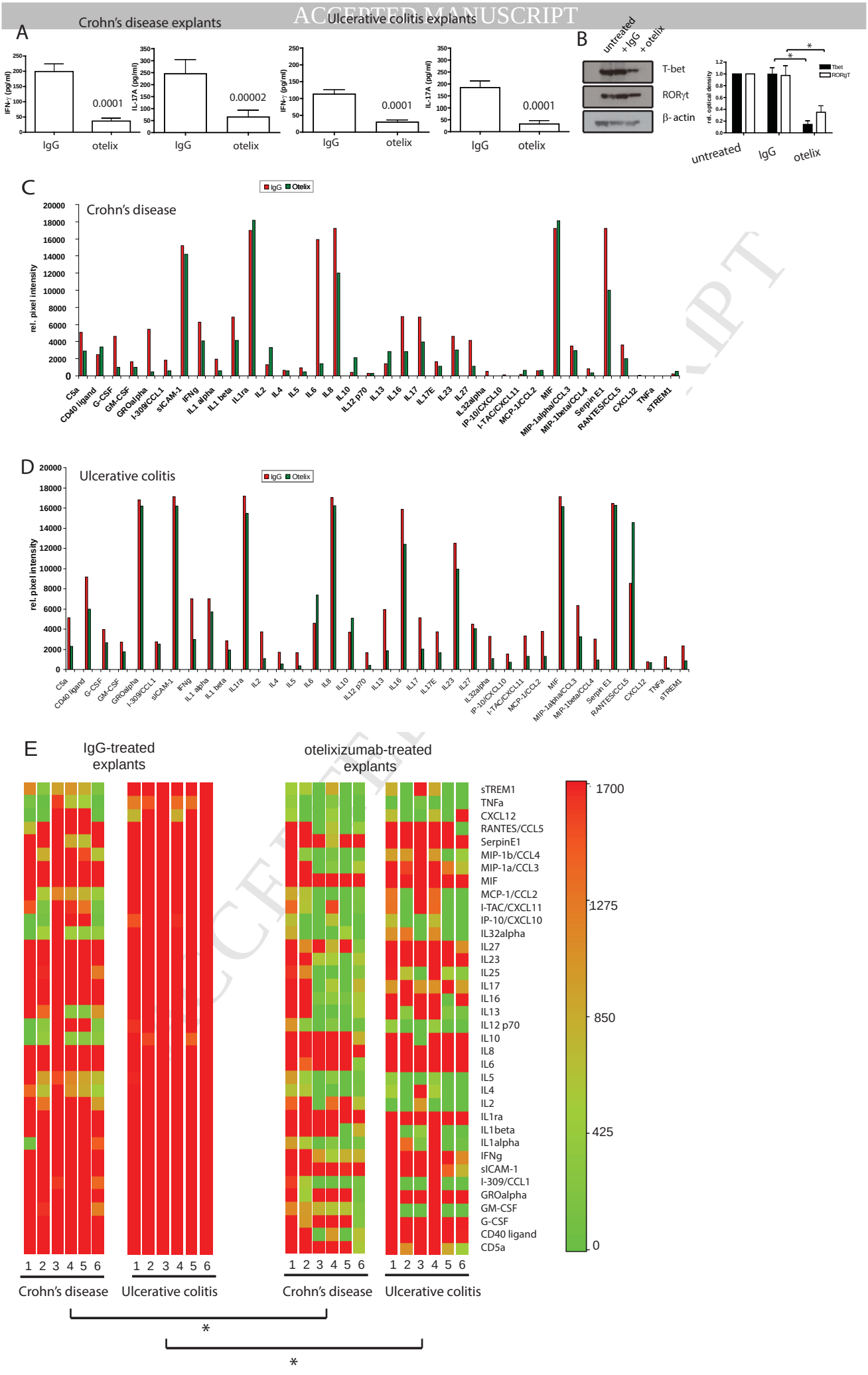
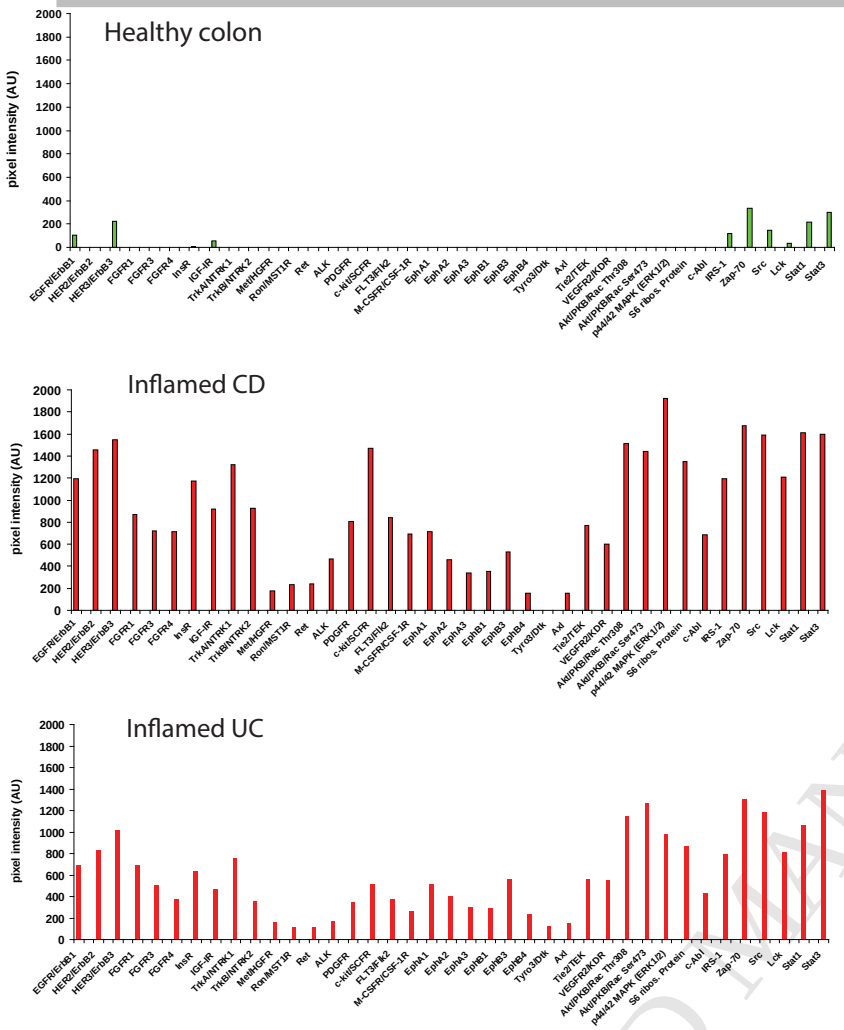
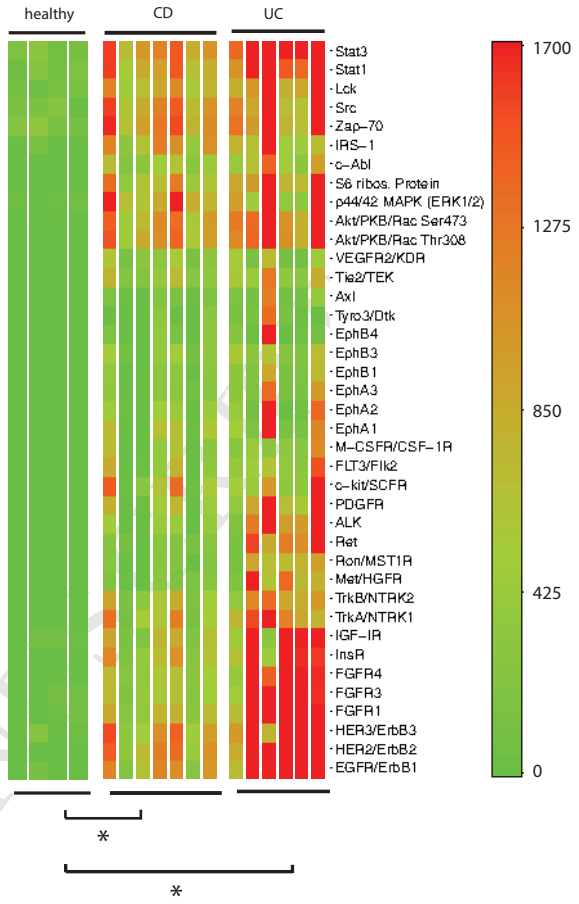
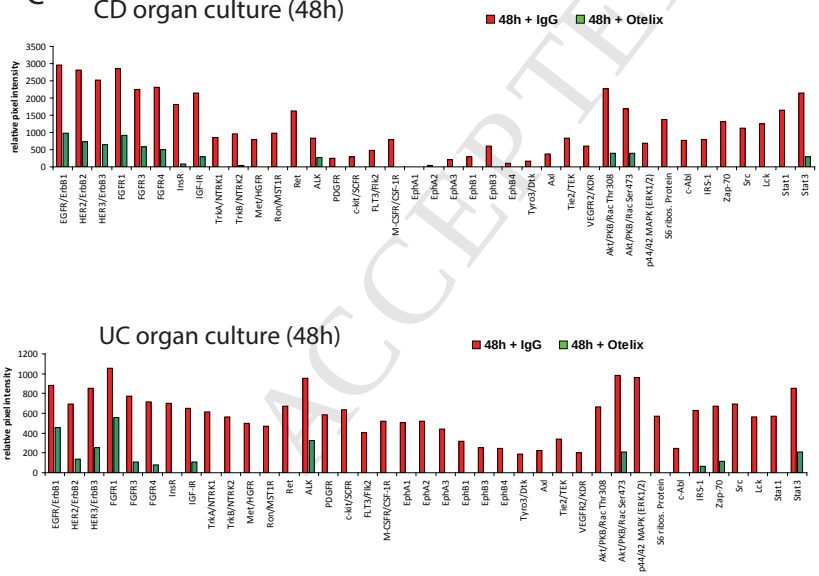
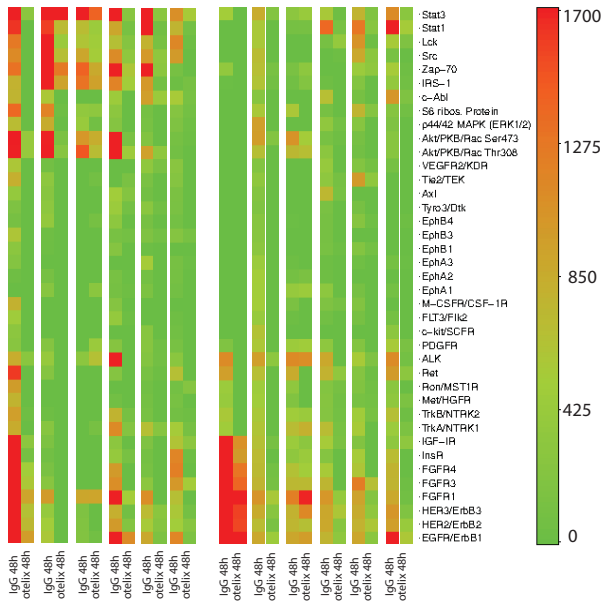


Figure 3





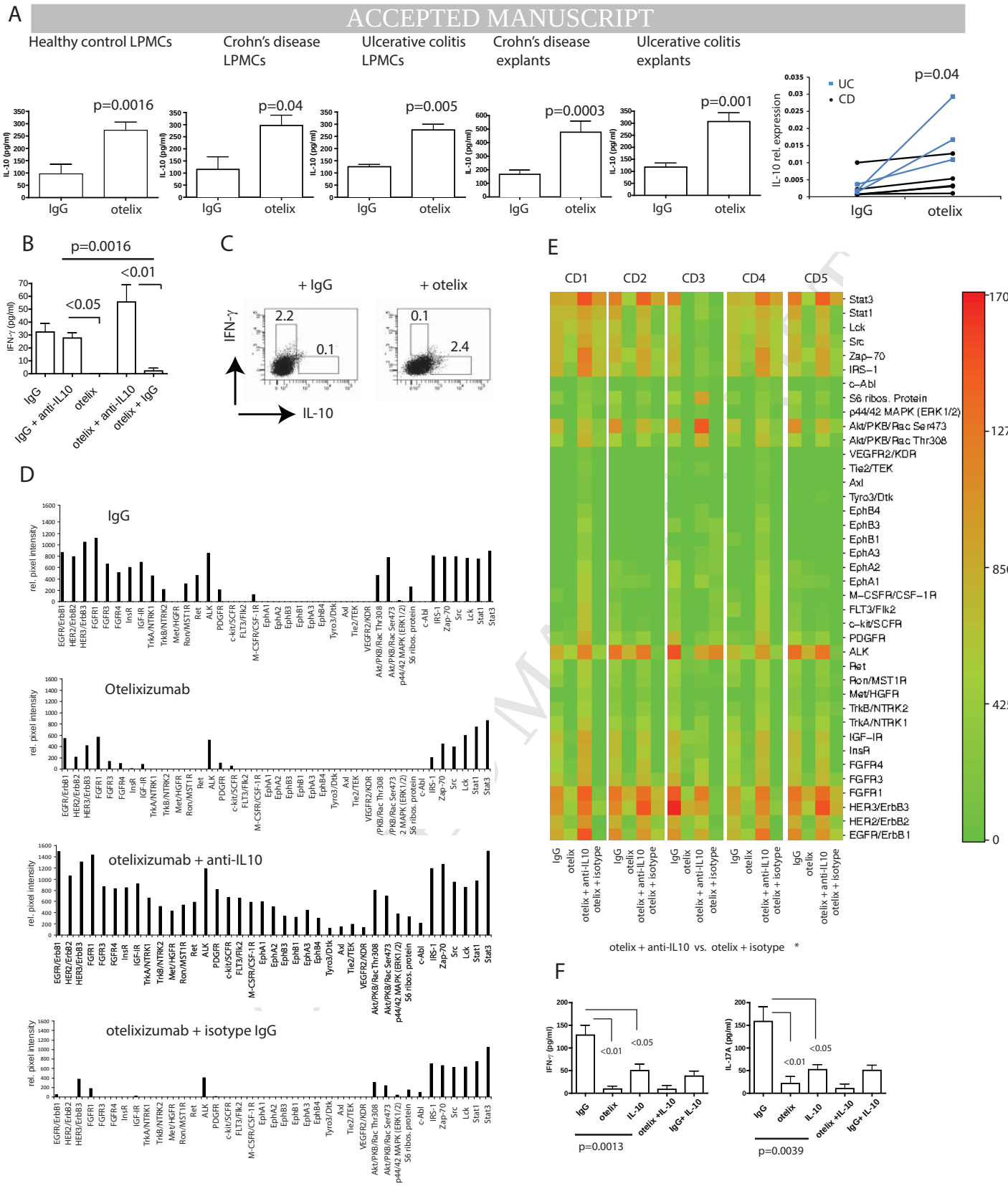




Crohn's
IgG 48h vs. otelix 48h *

UC
IgG 48h vs. otelix 48h *

Figure 4



Legends to Supplementary Materials

Supplementary Figure 1.

A. Normal LPMCs and **B.** CD LPMCs were cultured in the presence of otelixizumab, IgG and YTH (1µg/ml) as indicated. The second antibody was added after 3h and a wash-out of the first Ab. IL-17A and IL-2 levels in the supernatants were measured by ELISA. HC n=4; CD n=3. One-way ANOVA with post-test. Mean + SD.

Supplementary Figure 2. A. LPMCs from CD mucosa were cultured with IgG or otelixizumab for 24h. Foxp3 was stained intracellularly. Histograms show Foxp3 signal in the CD4+ CD25+ population. Histograms show one of six experiments. **B, C**. Graphs show percentage (**B**) of CD4+CD25+Foxp3+ cells and mean fluorescence intensity (**C**) in three CD as well as three healthy LPMCs cultured with either IgG or otelixizumab (both 1µg/ml) for 24h. Mann-Whitney-test. Depicted is the median.

Supplementary Table I

Patient and disease characteristics of the CD and UC patients recruited for this study.

Supplementary Table II

Relative cytokine values that were measured in the explant supernatants by protein array. Shown are the relative pixel intensities of the dots on the array (mean of duplicates per analyte). Explants of six Crohn's disease patients had been cultured for 16h with IgG or otelixizumab. Shown is also the table used for statistical testing.

Supplementary Table III

Relative cytokine values that were measured in the explant supernatants by protein array. Shown are the relative pixel intensities of the dots on the array (mean of dublicates per analyte). Explants of six ulcerative colitis patients had been cultured for 16h with IgG or otelixizumab. Shown is also the table used for statistical testing.

Supplementary Table IV

Relative phosphorylation levels that were measured in the explant lysates by phospho-protein array. Analysed were four healthy colon specimens, seven inflamed CD and six inflamed UC specimens. Specimens were immediately snap-frozen and untreated. Shown is also the table used for statistical testing.

Supplementary Table V

Relative phosphorylation levels that were measured in the explant lysates by phospho-protein array. Explants of six Crohn's disease patients had been cultured for 48h with IgG or otelixizumab. Lysates were subjected to the phospho-protein array and relative intensities of the dots (in duplicates) were measured. Shown is also the table used for statistical testing.

Supplementary Table VI

Relative phosphorylation levels that were measured in the explant lysates by phospho-protein array. Explants of six ulcerative colitis patients had been cultured for 48h with IgG or otelixizumab. Lysates were subjected to the phospho-protein array and relative intensities of the dots (in duplicates) were measured. Shown is also the table used for statistical testing.

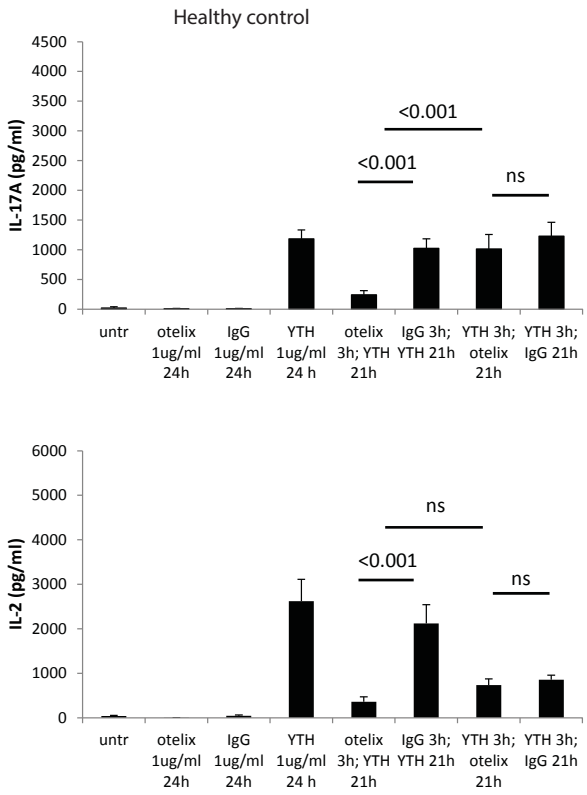
Supplementary Table VII

Relative phosphorylation levels that were measured in the explant lysates by phospho-protein array. Explants of five Crohn's disease patients had been cultured for 24h as indicated. Lysates

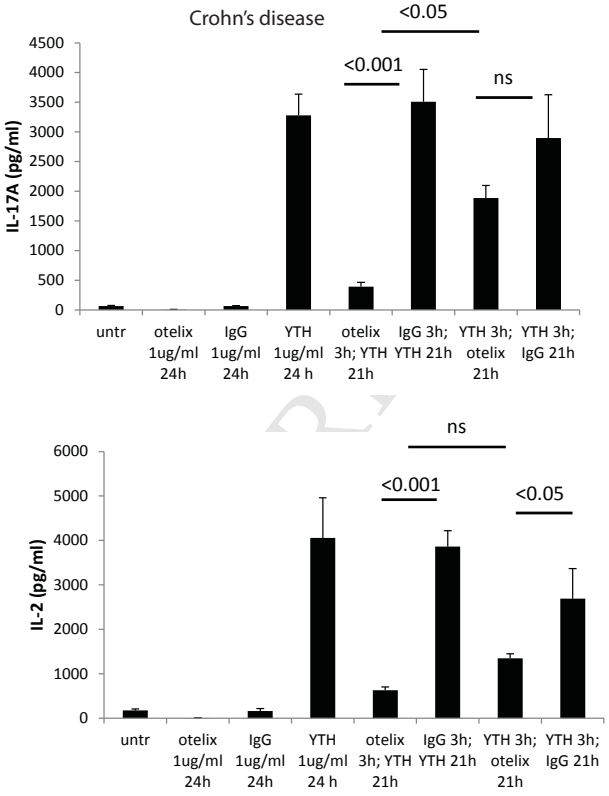
were subjected to the phospho-protein array and relative intensities of the dots (in duplicates) were measured. Shown is also the table used for statistical testing.

Supplementary Figure 1

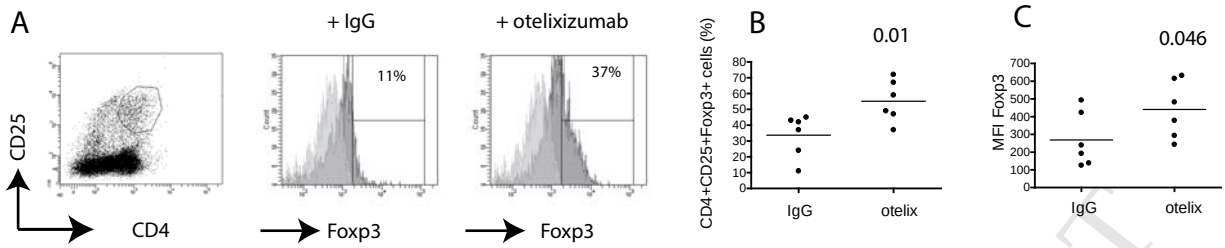
A



B



Supplementary Figure 2



Supplementary Table I

Patient characteristics

Crohn's disease n=85	Nr	Median (range)
Age	85	31.1 (15-57)
Female	45	
Male	40	
Disease location		
- Ileum & colon	38	
- colon only	47	
Treatment		
- 5-AZAs	21	
- 5-AZAs & steroids	35	
- 5-AZAs & azathioprine/methotrexate/other	29	

Ulcerative colitis n=61	Nr	Median (range)
Age	61	34.5 (13-65)
Female	29	
Male	32	
Disease location		
- distal colon	39	
- pan-colitis	22	
Treatment		
- 5-AZAs	16	
- 5-AZAs & steroids	30	
- 5-AZAs & azathioprine/methotrexate/other	15	

Note: patients on anti-TNF drugs were not recruited for this study

Supplementary Table II

cytokines **Crohn's disease explant supernatants**

	CD1		CD2		CD3		CD4		CD5		CD6		AVERAGE	
	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix
C5a	23656.5	23574.5	5079.5	2877	8577.5	2111.5	10224	5679	8179.5	1689.5	3809.5	575.5	9921.08333	6084.5
CD40 ligand	4518	3388	2483.5	3354.5	8167.5	0	5546	1023	4436.5	0	1862.5	670.5	4502.33333	1406
G-CSF	25618	10045.5	4608	1024.5	12575.5	7290.5	8221.5	2346	6577	5832	3456	204.5	10176	4457.16667
GM-CSF	5713	1183.5	1638	1000	7002.5	806.5	6798.5	631	5438	645	1228.5	200	4636.41667	744.333333
GROalpha	25507	10396.5	5466.5	486.5	14656.5	17691.5	18996.5	9446	151970	14153	4099.5	97	36782.6667	8711.75
I-309/CCL1	2213	1600.5	1844.5	574	1616.5	0	9765	27	7812	0	1383.5	114	4105.75	385.916667
sICAM-1	23166.5	26668.5	15217.5	14182	17553	2080.5	14665	2289.5	11732	1664	11413	2836	15624.5	8286.75
IFNg	8544.5	6704	6250	4110	6331	1033.5	7746	550	6196.5	826.5	4687.5	822	6625.91667	2341
IL1 alpha	67.5	976.5	1934	586	11186.5	0	5679	225	4543	0	1450.5	117	4143.41667	317.416667
IL1 beta	19663	4999.5	6878.5	4162.5	11185.5	7065.5	16447	2346	131570	0	5158	832.5	31817	3234.33333
IL1ra	25463	28341	16973	18143.5	21239	17516	15446.5	10166	123570	14012	12729.5	3628.5	35903.5	15301.1667
IL2	2900	1421	1311.5	3292.5	2367.5	0	4022	1344	32170	5652	983	658.5	7292.33333	2061.33333
IL4	1382.5	655	680	621	2279	0	1079	0	863.5	0	510	124.5	1132.33333	233.416667
IL5	2277.5	1002	968.5	448	1532.5	0	1163	267	930	0	726	89.5	1266.25	301.083333
IL6	25298	6631.5	15938	1424	20742.5	15264	22679	6021.5	18143	12211	11953.5	284	19125.6667	6972.66667
IL8	25394.5	28285	17219.5	12031.5	21378	18580	26449	4990	21159	14864	12914.5	2406	20752.4167	13526.0833
IL10	0	3964	396.5	2129.5	2774.5	3681.5	336	1977.5	268	2945	297.5	825	678.75	2587.08333
IL12 p70	0	1070	300	292	3948.5	0	4679	223	3743	0	225	58	2149.25	273.833333
IL13	3261.5	3478.5	1393	2857	5972	0	279	0	223	0	1044.5	571	2028.83333	1151.08333
IL16	7644	4285.5	6917	2862	6287.5	168.5	5467.5	344	4374	134	5187.5	572	5979.58333	1394.33333
IL17	10795	3246	6884	3950	7237.5	0	8877	578.5	7101	0	5163	790	7676.25	1427.41667
IL25	3870.5	1639	1668.5	1151	3043.5	0	2264	0	1811	0	1251.5	230	2318.16667	503.333333
IL23	12422	5479	4600.5	3021.5	8224.5	600	4479	548	3583	0	3450.5	604	6126.58333	1708.75
IL27	18485.5	9713	4167.5	1140.5	14732	7209	16344	805	13075	5767.2	3125.5	228	11654.9167	4143.78333
IL32alpha	0	848	521.5	0	4152.5	0	468	0	374	480	391	0	984.5	221.333333
IP-10/CXCL10	0	684	107	0	2629.5	0	5590	664	4472	0	80	0	2146.41667	224.666667
I-TAC/CXCL11	1492.5	1394.5	153.5	634.5	2113	0	1645	1554	1316	0	115	126.5	1139.16667	618.25
MCP-1/CCL2	2240.5	892	583	659	1195	0	997	0	797	0	437	131.5	1041.58333	280.416667
MIF	25220	28171.5	17226.5	18094	21262.5	18416	23445.5	19778	18756	14732.5	12919	3618	19804.9167	17135
MIP-1alpha/CCL3	5144.5	1843.5	3502.5	2951.5	2499.5	0	2246	244	1796	0	2626	590	2969.08333	938.166667
MIP-1beta/CCL4	3783	2005	852	332	2488.5	0	1922	134	1537	0	639	66	1870.25	422.833333
Serpin E1	25454.5	27976.5	17198.5	10010	21277	18333.5	879	746	703	14666	12898.5	2002	13068.4167	12289
RANTES/CCL5	736	2462	3624.5	2017	6609	0	8799	640.5	7039.5	0	2718.5	403	4921.08333	920.416667
CXCL12	0	498	39	0	3033.5	0	6643	446	5314.5	0	29.5	0	2509.91667	157.333333
TNFa	111	398	0	0	1594.5	0	645	213	516	0	0	0	477.75	101.833333
sTREM1	1146.5	479.5	255.5	560.5	937	0	1044	879	835	0	191.5	112.5	734.916667	338.583333

U-test **0.0004**

Supplementary Table III

cytokines **Ulcerative colitis explant supernatants**

	UC1		UC2		UC3		UC4		UC5		UC6		AVERAGE	
	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix	16h + IgG	16h + Otelix
C5a	5140.5	2283	5060.5	1100.5	11644.5	7747	5654.5	2385.5	4554.0	880.0	8849	484	6817.2	2480.0
CD40 ligand	9186.5	5959.5	6730.5	3807	4295.5	1863	10105	6227.5	6057.0	3045.0	3264.5	1675	6606.5	3762.8
G-CSF	3946.5	2655	11759	6272	19103.5	11453	4341.5	2774.5	10583.0	5017.0	14518	2759	10708.6	5155.1
GM-CSF	2701	1760	4909.5	0	12435	0	2971	1839	4418.0	0.0	9450	0	6147.4	599.8
GROalpha	16799.5	16179.5	11498.5	7859	13439	8866.5	18479.5	16907	10348.5	6287.0	10213	3457.5	13463.0	9926.1
I-309/CCL1	2757.5	2522.5	4927.5	0	9478.5	0	3033.5	2636	4434.5	0.0	7203	0	5305.8	859.8
sICAM-1	17107.5	16181	9139	1765.5	16714	10819	18818	16909	8225.0	1412.0	12702.5	776	13784.3	7977.1
IFNg	7015.5	2986.5	8998	2593	11553	7481	7717	3120	8098.0	2074.0	8780	1140	8693.6	3232.4
IL1 alpha	7030.5	5719.5	5129	1365	6360.5	178	7733	5976	4616.0	0.0	4833	55.5	5950.3	2215.7
IL1 beta	2814	1947.5	3371.5	0	2540.5	356	3095	2035	3034.5	0.0	1930	0	2797.6	723.1
IL1ra	17163	15458	8460.5	5863	16278	11361	18879	16153	7614.5	4690.0	12371	2579	13461.0	9350.7
IL2	3726	0	2225.5	0	3863.5	1032.5	4098	0	2002.5	0.0	2936	0	3141.9	172.1
IL4	1709.5	529.5	3833.5	0	4918	2551	1880.5	553	3450.5	0.0	3737.5	0	3254.9	605.6
IL5	1676.5	350.5	4852	0	5985	0	1844	366.5	4366.0	0.0	4548	0	3878.6	119.5
IL6	4592.5	7362.5	11814.5	6833	20816.5	14639.5	5051.5	7693.5	10633.0	5466.0	15820.5	3006.5	11454.8	7500.2
IL8	17035	16205.5	11577.5	7448.5	24541	17286.5	18738.5	16934.5	10419.5	5958.0	18651	3277	16827.1	11185.0
IL10	3676	5092	1566.5	4173	3613	0	4043	6321	1409.5	4338.0	1836	3745	2842.2	3293.3
IL12 p70	1644.5	386	2499	0	4212	0	1808.5	403	2249.1	0.0	3201	0	2602.4	131.5
IL13	5924.5	1856	2645.5	274.5	3012.5	2073.5	6516.5	1939	2380.5	219.0	2289	120	3794.8	1080.3
IL16	15861.5	12426.5	5060.5	7491	14839.5	13397.5	17447.5	12985.5	4554.5	0.0	11278.02	3296	11506.9	8266.1
IL17	5117	1035.5	6656.5	2213.5	7282	1037.5	5628	1082	5990.0	1770.0	5534	973	6034.6	1351.9
IL25	3745	1685	6520	669.5	8072.5	0	4119	1760.5	5868.0	535.0	6135.5	294.5	5743.3	824.1
IL23	12505	9936	9175.5	4480.5	12674	5736.5	13755.5	10383	8257.0	3584.0	9632	1971	10999.8	6015.2
IL27	4496	4066.5	10304	2510.5	20048.5	12106.5	4945	4249.4925	9273.0	2008.0	15236	1104	10717.1	4340.8
IL32alpha	3266.5	1081.5	5335.5	1285	11438	0	3593.5	1130.5	4801.5	0.0	8692	0	6187.8	582.8
IP-10/CXCL10	1507	699.5	2361.5	0	4284.5	0	1657	730	2125.5	0.0	3256	0	2531.9	238.3
I-TAC/CXCL11	3310	1318	2514.5	0	3043	6249	3641	1377	2263.0	0.0	2312.5	0	2847.3	1490.7
MCP-1/CCL2	3773.5	1293	2407.5	14.5	6218	4869	4150.5	1351.5	2166.0	11.0	4725	6	3906.8	1257.5
MIF	17143.5	16149	11298	7667	26003	21605	18857	16875.5	10168.0	6133.0	19762.5	3373.5	17205.3	11967.2
MIP-1alpha/CCL3	6330	3241.5	5947	1585	16469.5	4527	6963	3387	5352.0	1268.0	12516	697.5	8929.6	2451.0
MIP-1beta/CCL4	2995	960.5	6221	1022.5	18286.5	5039	3294.5	1003.5	5598.0	0.0	13897	449	8382.0	1412.4
Serpin E1	16434	16263.5	11656.5	7125.5	31253.5	17033	18077	16995	10490.5	5700.0	23752	3135	18610.6	11042.0
RANTES/CCL5	8521.5	14547.5	9559	4502.5	12788.5	3041.5	9373.5	15202	8603.0	3602.0	9719	0	9760.8	6815.9
CXCL12	767.5	695	3422	0	4972	0	844.5	726	3079.0	0.0	3778	1981	2810.5	567.0
TNFa	1237.5	0	1489	0	3784	97.5	1361.5	0	1340.0	0.0	2875	0	2014.5	16.3
sTREM1	2344	839.5	2050.5	0	6983	6093.5	2578	877	1845.0	0.0	5307	0	3517.9	1301.7

U-test **0.000034**

Supplementary Table IV

Phosphorylation in untreated explants

	Healthy1	Healthy2	Healthy 3	Healthy 4	CD1	CD2	CD3	CD4	CD5	CD6	CD7	UC1	UC2	UC3	UC4	UC5	UC6
EGFR/ErbB1	0	105	0	0	1195	202	636	1168	1102.5	247	1032.5	691.5	3177	2736.5	2478	2451.5	2536.5
HER2/ErbB2	0	0	0	0	1457.5	339	710	1219	1344.5	414	1077	837.5	2877	1696.5	2244	2351.5	2295.5
HER3/ErbB3	0	220.5	0	35	1551	343.5	495.5	1102	1430	465	974	1017	2798	752	2182.5	2150	2356
FGFR1	0	0	37.5	69	872.5	243.5	352.5	780.5	804	298.5	689	691.5	2951.5	3649.5	2302.5	2359	2392.5
FGFR3	0	0	85.5	57.5	720	193	382.5	568	664	236.5	502	512	2443.5	1996.5	1905	2018	1656
FGFR4	0	0	0	0	710.5	247.5	358.5	588	655	302.5	519	376	3074	1432.5	2397	2018	1670
InsR	0	5.5	0	0	1172	192.5	381.5	700	1080	246	618	637	2897	462.5	2259	1660.5	1615.5
IGF-IR	0	58	66	23.5	918	147	188	749.5	846.5	179.5	662	464	2347	285.5	1830.5	1962.5	1718
TrkA/NTRK1	0	0	0	0	1321.5	101	554	596	1218.5	123.5	526	756	1544	1943.5	1204	889.5	778
TrkB/NTRK2	0	0	0	0	928	140	321.5	579	855	171.5	511	355	1127.5	1346	879	838	1018
Met/HGFR	0	0	0	0	176.5	14	0	267	162	167.5	236	163	1770.5	634.5	1380	737	803
Ron/MST1R	0	0	0	0	236.5	0	0	255	218	0	225	113	910	673	709	780	959.5
Ret	0	0	0	0	241	105	0	293.5	222	128.52	259	115	1573	847.5	1226	1117	1814.5
ALK	0	0	0	0	469.5	0	0	435	433	0	384.5	175	1264	3349	985	987.5	2858
PDGFR	0	0	0	0	807	0	30.5	499	744.5	0	441	351	875	2242	682.5	564.5	1901
c-kit/SCFR	0	0	0	0	1468	25.5	258	620.5	1353.5	31.5	548.5	519	430	1035.5	335.4	345	1729
FLT3/Flk2	0	0	0	0	844.5	0	27	386.5	778	0	341	375.5	472.5	617.5	368.5	311.5	1527
M-CSFR/CSF-1R	0	0	0	0	691	0	0	355	637	0	313	263.5	411.5	289	320	260.5	1137
EphA1	0	0	0	0	713.5	0	0	673.5	658.5	24.5	595	518	180	2291	140	134.5	1158
EphA2	0	0	0	0	461	20	0	477	425	0	421	404	75	1747.5	0	64	1394
EphA3	0	0	0	0	338.5	0	0	296.5	312	76.5	262	302.5	187	1374	145	138	994.5
EphB1	0	0	0	0	352.5	0	0	304	325	0	268.5	290	190	866	148	156	727.5
EphB3	0	0	0	0	527.5	80	0	386.5	486.5	97.5	341	566	299	604.5	233	189	678.5
EphB4	0	0	0	0	156.5	3	0	363.5	144	3.5	321.5	233	25	1920.5	0	67	108.35
Tyro3/Dtk	0	0	0	0	0	0.5	0	316.5	0	0	279.5	122	144	1346.5	112	33	0
Axl	0	0	0	0	159	28	0	184	146	34.5	162	152	101	1227	78	90	396
Tie2/TEK	0	0	0	0	773	208.5	227	385	712	255.5	340	561.5	428	1270.5	333.5	290	857
VEGFR2/KDR	0	0	0	0	598.5	118	282	319.5	551.5	134	282.5	552	119.5	726.5	93	73	444
Akt/PKB/Rac Thr308	0	0	0	0	1513	475.5	843.5	1097	1395	582	969.5	1147.5	1396	3874	1088	824	2743
Akt/PKB/Rac Ser473	0	0	0	0	1443.5	460.5	725.5	1117	1331	563	987	1262.5	1374	3815.5	1071	821	2960
p44/42 MAPK (ERK1/2)	32.5	0	33.5	64.5	1923	682	617.5	856	1773.5	834	756	980.5	466	3597	363	332	1955
S6 ribos. Protein	0	0	0	0	1349	358.5	618.5	677.5	1244	438	598	867.5	991.5	3998	773	717	2160.5
c-Abl	0	0	0	0	686	214.5	314	492.5	632	279	435	434.5	639	1409	498	304	963.5
IRS-1	0	120.5	0	0	1197	304	641.5	1226.5	1103	372	1084	793	646.5	2803	504.5	453.5	696.5
Zap-70	220	333.5	115.5	87.5	1673.5	629	972.5	1280	1543	769	1131.5	1311.5	987	3304.5	769	676.5	1801.5
Src	131.5	147	203	0	1591.5	590.5	845	1211	1467.5	722	1070.5	1186	876	3113	683	667.5	2020
Lck	75	34	110	112	1207.5	454	677.5	879	1113	555.5	777	815.5	1023	2854	797.5	912.5	2223
Stat1	37.5	213.5	67	179	1610	547	862.5	946.5	1484	669	836.5	1068.5	1899	3533	1481.5	1382.5	2555
Stat3	173	301	69	98.5	1598.5	686.5	1023.5	1202.5	1474	840.5	1063	1390.5	2448	3976	1909	1817.5	2811.5

average CD	average HC
797.5714286	26.25
937.2857143	0
908.7142857	63.875
577.2142857	26.625
466.5714286	35.75
483	0
627.1428571	1.375
527.2142857	36.875
634.3571429	0
500.8571429	0
146.1428571	0
133.5	0
178.4314286	0
246	0
360.2857143	0
615.0714286	0
339.5714286	0
285.1428571	0
380.7142857	0
257.7142857	0
183.6428571	0
178.5714286	0
274.1428571	0
141.7142857	0
85.21428571	0
101.9285714	0
414.4285714	0
326.5714286	0
982.2142857	0
946.7857143	0
1063.142857	32.625
754.7857143	0
436.1428571	0
846.8571429	30.125
1142.642857	189.125
1071.142857	120.375
809.0714286	82.75
993.6428571	124.25
1126.928571	160.375

U test **1.085326e-13***

average UC	average HC
2345.166667	26.25
2050.333333	0
1875.916667	63.875
2391.083333	26.625
1755.166667	35.75
1827.916667	0
1588.583333	1.375
1434.583333	36.875
1185.833333	0
927.25	0
914.6666667	0
690.75	0
1115.5	0
1603.083333	0
1102.666667	0
732.3166667	0
612.0833333	0
446.9166667	0
736.9166667	0
614.0833333	0
523.5	0
396.25	0
428.3333333	0
392.3083333	0
292.9166667	0
340.6666667	0
623.4166667	0
334.6666667	0
1845.416667	0
1884	0
1282.25	32.625
1584.583333	0
708	0
982.8333333	30.125
1475	189.125
1424.25	120.375
1437.583333	82.75
1986.583333	124.25
2392.083333	160.375

U test **2.9e-014***

Supplementary Table V

Phosphorylation levels in treated Crohn's disease explants

	CD1 IgG48h	CD1 Ote 48h	CD2 IgG48h	CD2 Ote 48h	CD3 IgG48h	CD3 Ote 48h	CD4 IgG48h	CD4 Ote 48h	CD5 IgG48h	CD5 Ote 48h	CD6 IgG48h	CD6 Ote 48h
EGFR/ErbB1	2948	985	574.5	0	201	56	2734	1123	873	153	1172	153
HER2/ErbB2	2809.5	730	512.5	0	190	0	997	210	553	234	505	243
HER3/ErbB3	2523	647	388	0	174.5	0	823	55	705	275.5	577	79.5
FGFR1	2849	916	997.5	0	898.5	915.5	2661	522	1101	0	661	0
FGFR3	2255	580	259	0	0	0	1105	0	314	0	1025	531
FGFR4	2305	503.5	128.5	0	0	0	1002	0	115	0	1221	0
InsR	1817	90	47	0	0	0	396	110	75	0	1175.5	27
IGF-IR	2144.5	300.5	155.5	0	0	0	301	0	177.5	0	620	305
TrkA/NTRK1	856.5	0	242.5	0	0	101.5	1132	215.5	663	237	535	104
TrkB/NTRK2	954	41	27	0	0	0	775.5	101	113	0	67.5	37
Met/HGFR	782	0	0	0	0	0	51	0	75.5	0	115.5	0
Ron/MST1R	979	0	0	0	0	0	0	0	53	0	70.5	225
Ret	1616	0	223.5	0	0	0	223	0	117	27	663	0
ALK	843	267.5	398.5	0	297	647.5	3011	0	205	67.5	273	0
PDGFR	253	0	219.5	0	0	432.5	231	0	245	51.5	117	0
c-kit/SCFR	301	0	0	0	0	0	0	0	237	0	51	0
FLT3/Flk2	478	0	0	0	0	0	373.5	22	0	0	31.5	0
M-CSFR/CSF-1R	801.5	0	0	0	0	0	225	0	0	0	0	0
EphA1	0	0	249	0	0	266	93	32	145	0	67.5	0
EphA2	31.5	0	101	0	0	0	113	11	79.5	0	88.5	0
EphA3	215	0	0	0	0	0	0	0	533	0	103.5	23.5
EphB1	294.5	0	35.5	0	0	0	227.5	0	24	0	0	0
EphB3	602.5	0	43	0	0	0	115.5	77.5	179	72	225	113
EphB4	107.5	0	369	0	0	87	318.5	0	105.5	0	73	0
Tyro3/Dtk	158	0	324	0	0	0	443	72	279	51	124	0
Axl	375.5	0	264.5	0	0	0	501	213	66.5	0	137.5	0
Tie2/TEK	826.5	0	318.5	0	0	69.5	0	135	129	0	220	0
VEGFR2/KDR	594	0	67	0	0	0	0	0	345	125	257.5	0
Akt/PKB/Rac Thr308	2276	392	1874	250	1489	693	1992	459.5	943	337	69	0
Akt/PKB/Rac Ser473	1696.5	401	1688.5	137	1034.5	836.5	1883.5	123	201	0	88.5	27
p44/42 MAPK (ERK1/2)	695	0	843.5	0	412	59	223	0	115	0	175	45.5
S6 ribos. Protein	1384	0	1189.5	42	354	332	307	0	93	0	223	73
c-Abl	766	0	579.5	0	0	0	453.5	0	958	432.5	555	110
IRS-1	793	0	2383.5	928	1313.5	938	1195	504	1025	72	35.5	103
Zap-70	1317.5	0	2518.5	1251	1422.5	865.5	2013	588	2537	304	103	0
Src	1131.5	0	1903	662	901	529.5	1122.5	337	1010	335	993	0
Lck	1259	0	1886.5	433	614	483.5	657	228.5	793	110	1150	23
Stat1	1636	0	1595.5	709.5	700	443	895	115	1887.5	62	773	72
Stat3	2144	295.5	3374.5	2220	2283	1413.5	2245.5	259	2310	275	1095	554

AVERAGE

Crohn's IgG48h	Crohn's Ote 48h
1417.08333	411.666667
927.833333	236.166667
865.083333	176.166667
1528	392.25
826.333333	185.166667
795.25	83.9166667
585.083333	37.8333333
566.416667	100.916667
571.5	109.666667
322.833333	29.8333333
170.666667	0
183.75	37.5
473.75	4.5
837.916667	163.75
177.583333	80.6666667
98.1666667	0
147.166667	3.66666667
171.083333	0
92.4166667	49.6666667
68.9166667	1.83333333
141.916667	3.91666667
96.9166667	0
194.166667	43.75
162.25	14.5
221.333333	20.5
224.166667	35.5
249	34.0833333
210.583333	20.8333333
1440.5	355.25
1098.75	254.083333
410.583333	17.4166667
591.75	74.5
552	90.4166667
1124.25	424.166667
1651.91667	501.416667
1176.83333	310.583333
1059.91667	213
1247.83333	233.583333
2242	836.166667

U-test **1.08e-07***

Supplementary Table VI
Phosphorylation levels in treated Ulcerative colitis explants

	UC1 IgG48h	UC1 Ote 48h	UC2 IgG48h	UC2 Ote 48h	UC3 IgG48h	UC3 Ote 48h	UC4 IgG48h	UC4 Ote 48h	UC5 IgG48h	UC5 Ote 48h	UC6 IgG48h	UC6 Ote 48h
EGFR/ErbB1	2591	2024.5	881.5	457	903	980.5	773	331	905	278	2103	587
HER2/ErbB2	2403.5	1564	695.5	134	755	656.5	885	238.5	793	589	953.5	423
HER3/ErbB3	2567	1584.5	855	252.5	793	1148.5	893	593.5	845	347	1037	182
FGFR1	2449	1916.5	1052	560	1215.5	1686.5	1034	378	925	214	883	55
FGFR3	1851.5	1389.5	772.5	109	728	717.5	753	0	1235.5	663	766	32
FGFR4	1891	1293.5	714	79.5	639.5	507.5	554	0	598.5	0	778	0
InsR	1892	987	704	0	342	433.5	225	0	538	0	701	0
IGF-IR	1943	1080.5	648	106.5	447	308.5	543	138	201.5	0	331	325.5
TrkA/NTRK1	493.5	0	613.5	0	688	810	689	110.5	553	0	449	143
TrkB/NTRK2	568	0	566.5	0	491	432	223	73	493.5	113	661	37.5
Met/HGFR	407	0	495.5	0	196.5	0	239.5	65.5	117	72	338.5	145
Ron/MST1R	445	0	469	0	339	0	210	103	193	51.5	278	0
Ret	961.5	0	669.5	0	860	0	793	347	225	22.5	993	289
ALK	1124	0	957.5	323.5	1131	1103.5	885.5	0	554	173.5	1120	79
PDGFR	182.5	0	586	0	456	487.5	331	0	203	0	557.5	123
c-kit/SCFR	0	0	633	0	0	0	0	0	0	0	301	0
FLT3/Flk2	0	0	408	0	27	0	55	0	101	0	75	0
M-CSFR/CSF-1R	0	0	518	0	139	0	98.5	173	201	0	0	0
EphA1	0	0	506	0	404.5	447	320	0	102.5	0	71.5	0
EphA2	0	0	517.5	0	80	138	43	0	0	0	0	0
EphA3	0	0	444.5	0	35	0	27	0	45	0	75	11
EphB1	0	0	314.5	0	0	0	115	27.5	203	73	0	0
EphB3	0	0	252.5	0	0	0	105	0	0	0	0	0
EphB4	0	0	246.5	0	12	5	98	0	235	43.5	0	23
Tyro3/Dtk	0	0	185.5	0	0	0	128	0	98	0	0	0
Axl	0	0	224.5	0	0	0	745	114	0	0	0	0
Tie2/TEK	0	0	337	0	0	0	332	88	1001	338.5	0	0
VEGFR2/KDR	0	0	206	0	0	0	405	0	205	75	88	0
Akt/PKB/Rac Thr308	0	0	666.5	0	739	674.5	287.5	0	67	0	237	0
Akt/PKB/Rac Ser473	0	0	983	211.5	1057	528.5	118.5	31	103.5	0	244	0
p44/42 MAPK (ERK1/2)	0	0	964	0	0	0	345.5	70.5	245	0	178	0
S6 ribos. Protein	0	0	568	0	574.5	0	101	0	773	220	240	53
c-Abl	0	0	243	0	0	0	668.5	0	109.5	0	1041	178
IRS-1	9.5	0	626	64	50	212.5	31	0	211	0	370.5	57
Zap-70	362.5	0	671	112.5	133.5	320.5	0	0	443	345	538	37.5
Src	0	0	694	1	0	0	0	28.5	887	287	110	0
Lck	0	0	565.5	0	0	0	102.5	375	1057	37	553	175
Stat1	0	0	569	0	93	100.5	1380	112	1288	244.5	2780	534
Stat3	522	0	852.5	206.5	307.5	425	558	37	775.5	184	1123	268

AVERAGE

UC IgG48h	UC Ote 48h
1359.41667	776.333333
1080.91667	600.833333
1165	684.666667
1259.75	801.666667
1017.75	485.166667
862.5	313.416667
733.666667	236.75
685.583333	326.5
581	177.25
500.5	109.25
299	47.0833333
322.333333	25.75
750.333333	109.75
962	279.916667
386	101.75
155.666667	0
111	0
159.416667	28.8333333
234.083333	74.5
106.75	23
104.416667	1.83333333
105.416667	16.75
59.5833333	0
98.5833333	11.9166667
68.5833333	0
161.583333	19
278.333333	71.0833333
150.666667	12.5
332.833333	112.416667
417.666667	128.5
288.75	11.75
376.083333	45.5
343.666667	29.6666667
216.333333	55.5833333
358	135.916667
281.833333	52.75
379.666667	97.8333333
1018.33333	165.166667
689.75	186.75

U-test **1.4 -06***

Supplementary Table VII

Phosphorylation levels in anti-IL-10 treated Crohn's disease explants

	CD1				CD2				CD3			
	IgG	otelix	otelix +anti-IL10	otelix + isotype	IgG	otelix	otelix +anti-IL10	otelix + isotype	IgG	otelix	otelix +anti-IL10	otelix + isotype
EGFR/ErbB1	869.5	542	1490.5	44.5	1118.5	243	1029	564	1070.5	278.5	858.5	115
HER2/ErbB2	790.5	215	1056.5	0	820.5	74.5	885.5	284.5	883.5	300	582	33.5
HER3/ErbB3	1050.5	411.5	1310	373.5	1153	594.5	1486.5	795	1677	830	984	119.5
FGFR1	1115.5	566.5	1436.5	177.5	1281	831	1324	749	1483	426.5	1236	115
FGFR3	663.5	137.5	867	0	707.5	145.5	731	227.5	820	32.5	592	0
FGFR4	514.5	95.5	833	0	620	7.5	707.5	3	634	108	532.5	0
InsR	601	11	848.5	0	690	0	581	0	722.5	176	235	0
IGF-IR	694	84	916.5	17	714	0	672.5	0	775.5	362.5	312.5	0
TrkA/NTRK1	453.5	0	658	0	450	51.5	466	0	513	0	343.5	0
TrkB/NTRK2	217.5	0	510.5	0	286	0	333	0	381	0	128	0
Met/HGFR	0	0	423.5	0	240.5	0	167	0	308.5	0	38	0
Ron/MST1R	314.5	0	539.5	0	338	0	293	0	444.5	0	57.5	60.5
Ret	460.5	0	587	0	401.5	0	330	0	494	83	160	0
ALK	848.5	516	1182.5	408	1312	793	1254	123.5	1508.5	135.5	931.5	1159.5
PDGFR	206	103.5	811.5	12	486	139	377	0	767	0	184	367.5
c-kit/SCFR	0	51.5	668.5	0	258	0	68.5	0	379.5	0	0	241.5
FLT3/Flk2	1	0	662	0	224.5	0	42.5	0	322	0	0	191.5
M-CSFR/CSF-1R	121	0	586.5	0	87.5	0	0	0	264	31	0	242.5
EphA1	0	0	595	0	188.5	159	112.5	0	523.5	0	404	147.5
EphA2	0	0	502.5	0	225.5	53	0	0	480.5	0	170.5	226
EphA3	0	0	338	0	11.5	0	0	0	84	0	133.5	209
EphB1	0	0	315.5	0	17.5	0	0	0	0	0	0	233.5
EphB3	0	0	447	0	0	0	0	0	247	0	178.5	263
EphB4	0	0	302.5	0	0	0	0	0	0	0	160	0
Tyro3/Dtk	0	0	115.5	0	0	0	0	0	0	0	0	0
Axl	0	0	140	0	0	0	0	0	0	0	0	0
Tie2/TEK	0	0	192.5	0	0	0	0	0	85.5	0	124.5	41.5
VEGFR2/KDR	0	0	132.5	0	0	0	0	0	26.5	0	0	12
Akt/PKB/Rac Thr308	460	0	795	302	527	6	454.5	59.5	446.5	0	828	0
Akt/PKB/Rac Ser473	778	0	697.5	236	1083	67.5	772	339	965	0	1474	5
p44/42 MAPK (ERK1/2)	18	0	376	39	319.5	0	143.5	0	207	0	105.5	0
S6 ribos. Protein	256	0	330	144	454.5	0	304.5	0	508	0	951.5	31.5
c-Abl	0	0	209.5	100	14	0	86	0	128.5	0	72.5	12.5
IRS-1	811.5	202.5	1187.5	702.5	611	210	889	364.5	669.5	0	251	88.5
Zap-70	783	449	1262	658.5	800	465	914	416	739.5	0	245.5	31.5
Src	792.5	392	949.5	626.5	760	176.5	814	254	690.5	0	127.5	0
Lck	758	598.5	852	628	646	115	691.5	72.5	561	0	140	0
Stat1	747.5	748	962	740.5	890	138	800.5	124	624.5	0	356.5	67.5
Stat3	893.5	864	1501	1047	1210	514	1410	899.5	1207	19.5	318	0

	CD4				CD5				AVERAGE	
	IgG	otelix	otelix +anti-IL10	otelix + isotype	IgG	otelix	otelix +anti-IL10	otelix + isotype	otelix +anti-IL10	otelix + isotype
EGFR/ErbB1	753.5	439	1292.5	38.5	1140	247	1049.5	575.5	1144	267.5
HER2/ErbB2	685.5	174	915.5	0	836	75	903	290	868.5	121.6
HER3/ErbB3	910.5	333.5	1135	323.5	1176	606	1516	810	1286.3	484.3
FGFR1	967	458	1245.5	153.5	1306	847	1350	763	1318.4	391.6
FGFR3	575	111.5	751	0	721	148	745	232.5	737.2	92
FGFR4	446	77.5	722	0	632	7.5	721.5	3	703.3	1.2
InsR	521	8	735.5	0	703	0	592	0	598.4	0
IGF-IR	601	68	794.5	14	728	0	685.5	0	676.3	6.2
TrkA/NTRK1	393	0	570	0	459	52.5	475	0	502.5	0
TrkB/NTRK2	188	0	442.5	0	291	0	339	103	350.6	20.6
Met/HGFR	0	0	367.5	0	245	0	170	0	233.2	0
Ron/MST1R	272	0	467	0	344.5	0	298	54	331	22.9
Ret	399	0	508	0	409.5	0	336	0	384.2	0
ALK	735	417	1025.5	353	1338	808	1279	125	1134.5	433.8
PDGFR	178	83.5	703	10	495	141	384.5	0	492	77.9
c-kit/SCFR	0	41.5	579	0	263	0	69	0	277	48.3
FLT3/Flk2	346	0	573	0	228	0	43.5	0	264.2	38.3
M-CSFR/CSF-1R	104	0	508.5	0	89.5	0	0	0	219	48.5
EphA1	0	0	515	22	192	162	114.5	0	348.2	33.9
EphA2	0	0	435.5	0	230	54	0	22	221.7	49.6
EphA3	0	0	293	0	11	0	0	0	152.9	41.8
EphB1	144	0	273	0	17.5	0	0	0	117.7	46.7
EphB3	0	0	387	0	0	0	0	0	202.5	52.6
EphB4	0	0	262.5	0	0	0	0	0	145	0
Tyro3/Dtk	0	0	100.5	0	36	0	49.5	0	53.1	0
Axl	0	0	121	0	0	0	0	0	52.2	0
Tie2/TEK	0	0	166.5	0	0	0	0	0	96.7	8.3
VEGFR2/KDR	0	0	114.5	0	0	0	0	0	49.4	2.4
Akt/PKB/Rac Thr308	398	0	689	261	537.5	6.5	463	60	645.9	136.5
Akt/PKB/Rac Ser473	674	0	604	204	1104	68.5	787	345	866.9	225.8
p44/42 MAPK (ERK1/2)	15	0	325	33	325	0	146	0	219.2	14.4
S6 ribos. Protein	221	0	286	124	463.5	0	310.5	0	436.5	59.9
c-Abl	0	0	181	86	14	0	87	0	127.2	39.7
IRS-1	703	164.5	1029.5	609	623	214	906	371	852.6	427.1
Zap-70	678	363	1094	570.5	816	474	932.5	424	889.6	420.1
Src	687.5	317.5	823.5	543.5	775	180	830	259	708.9	336.6
Lck	657	484.5	738	544	658	117	705	73.5	625.3	263.6
Stat1	648.5	605	834	642	907	140	816	126	753.8	340
Stat3	774.5	699	1301	907	1234	524	1438	917	1193.6	754.1

U-test **6.61144e-08***