Supplemental Methods, Figures and Tables

Gene-expression profiling of CD8 T-cells predicts prognosis in Crohn's disease and ulcerative colitis

James C. Lee[1,2,3], Paul A. Lyons[1,2], Eoin F. McKinney[1,2], John M. Sowerby[1,2], Edward J. Carr[1,2], Francesca Bredin[2,3], Hannah M. Rickman[1,2], Huzefa Ratlamwala[1,2], Alexander Hatton[1,2], Tim F. Rayner[1,2], Miles Parkes[2,3], Kenneth G.C. Smith[1,2*]

[1] Cambridge Institute for Medical Research and [2] Department of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Hills Rd, Cambridge, CB2 0XY, UK. [3] NHS Department of Gastroenterology, Addenbrooke's Hospital, Hills Rd, Cambridge, CB2 0QQ, UK.

Contents

Supplementary Methods

Consensus clustering

Following independent quality control assessment, variance stabilisation normalisation and correction of batch effects in R, we investigated whether any substructure was present in the CD4 and CD8 T-cell gene expression data from both disease cohorts using an unsupervised classification method. Unlike standard unsupervised classification procedures, which provide no information as to whether clusters are stable and reproducible or simply reflect random sampling variation, we used iterative consensus clustering (1). This is a generalised, model independent, resampling methodology that can assess cluster stability by providing a consensus classification output across multiple runs of a clustering algorithm using subsampling of the data. Samples that consistently cluster together are, by definition, reliable cluster members, whereas those whose co-clustering is dependent upon the presence of other samples are less reliable. We performed 5000 iterations of consensus clustering using two different clustering methods (k-means and hierarchical clustering) with an 80% subsampling ratio. We also employed an extension of the standard consensus clustering methodology, which enables visualisation of the merged consensus results between the different clustering methodologies (2). This can improve the confidence in any apparent clusters by demonstrating that similar results are produced using methodologically different clustering algorithms. All clustering algorithms used a Euclidean distance metric and average-linkage clustering.

Assessment of clustering significance

To further confirm that any clusters were real and not due to overfitting of the data, we used a another independent method to evaluate the validity and statistical significance of these results (3). This method, implemented using the *sigclust* package in R, tests the null hypothesis that the entire data can be modelled as coming from a single, multivariate

Gaussian distribution. The rationale for this approach is that if a dataset can be appropriately modelled by such a Gaussian distribution, then any clustering algorithm that would split this data into distinct clusters is not significant. This method therefore enables direct quantification of the significance of any given clustering.

Classifier generation

We investigated whether simple subgroup classifiers, consisting of smaller numbers of genes, could be generated that would enable prediction of subgroup membership in either disease cohort without the necessity and expense of whole-genome gene-expression analysis by microarray. We used two different classification algorithms, weighted-voting (4) and random forests (5) to generate classifiers of various sizes (between 4 and 100 genes). To do this we partitioned our overall dataset into two independent cohorts, each containing both UC and CD patients, with preservation of the ratio between IBD1 and IBD2 patients. Classifiers were generated in one of the cohorts (training set) and tested upon the other cohort to independently assess their performance. The performance of classifiers was found to be relatively insensitive to their size and the method that was used to generate them.

PBMC gene-expression analysis

To assess whether the IBD1/2 subgroups were detectable in PBMC we initially performed a principal component analysis (implemented in MEV (6)) to examine the distribution of the samples. We then performed iterative consensus clustering to examine whether the subgroups that might exist within the data bore any resemblance to the IBD1/2 subgroups (Supplemental Figure 2). We also applied the classifier detailed in Supplemental Figure 4 to the PBMC data to determine if this could ascribe subgroup designation – as it had in the CD8 T-cell datasets.

R code for analysis

The R code used for analyses following preprocessing is shown below, where "CD_analysis" is CD dataset. Analysis of the UC dataset is not shown but was performed using identical commands:

```
# Consensus clustering (packages = clusterCons, affy)
>  cd_data.frame=data.frame(t(exprs(CD_analysis)))
>  cd_consensus_clustering=cluscomp(cd_data.frame,
   algorithms=list('kmeans', "hclust"), clmin=2, clmax=5, reps=5000,
   prop=0.8, merge=1)

# To assess optimal number of groups
>  mergeAUC=aucs(cd_consensus_clustering)
>  mergeDK=deltak(mergeAUC)
>  dkplot(mergeDK)

# To visualise results (shown for 2 groups) (package=heatmap.2)
>  cd_kmeans=cd_consensus_clustering$e1_kmeans_k2
>  cd_hclust=cd_consensus_clustering$e2_hclust_k2
>  cd_merge=cd_consensus_clustering$merge_k2
>  heatmap.2(cd_merge, col=bluered, key=F, trace='none', rowsep=c(1:35),
   colsep=c(1:35), sepcolor="black", sepwidth=c(0.03,0.03), labRow="",
   labCol=CD_analysis$patient_number)

# Add column to phenoData of expression set indicating group designation
>  CD_analysis$group=c(1,1,2,1,2,1,1,etc)

# To compare distribution of data with multivariate Gaussian distribution
(package=sigclust)
>  cd_for_sigclust=t(exprs(CD_analysis))
>  sigclust(cd_for_sigclust, nsim=500, nrep=1, labflag=1,
   label=CD_analysis$group, icovest=1)

# To determine differentially-expressed genes between subgroups
(package=limma)
>  CDdesign=model.matrix(~-1+factor(CD_analysis$group))
>  colnames(CDdesign)=c('A', 'B')
>  CDfit=lmFit(CD_analysis, CDdesign)
>  CDmatrix=makeContrasts(A-B, levels=CDdesign)
>  CDfit2=contrasts.fit(CDfit, CDmatrix)
>  CDfit3=eBayes(CDfit2)
>  CD_results=decideTests(CDfit3, adjust.method="holm", p=0.05)

# To subset this (or any other) expression set by this signature
>  CD_diff.exprs.genes.only=CD_analysis[as.logical(CD_results),]

# Classifier generation for object "All_Samples" (method shown using
Random Forests; package=MLInterfaces)
>  group=as.factor(All_samples$group)
```

```
> classifier=MLearn(group~., All_samples, randomForestI, 1:34,
  importance=T)
> confuMat(classifier)
> varImpPlot(All_samples(classifier))
```

References

1. Monti S, Tamayo P, Mesirov J, Golub T Consensus clustering: A resampling-based method for class discovery and visualization of gene expression microarray data. *Machine Learning* 2003; 52: 91-118.

2. Simpson TI, Armstrong JD, Jarman AP Merged consensus clustering to assess and improve class discovery with microarray data. *BMC Bioinformatics* 2010; 11: 590.

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4. Golub TR, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 1999; 286: 531-537.

5. Breiman L Random forests. *Machine Learning* 2001; 45: 5-32.

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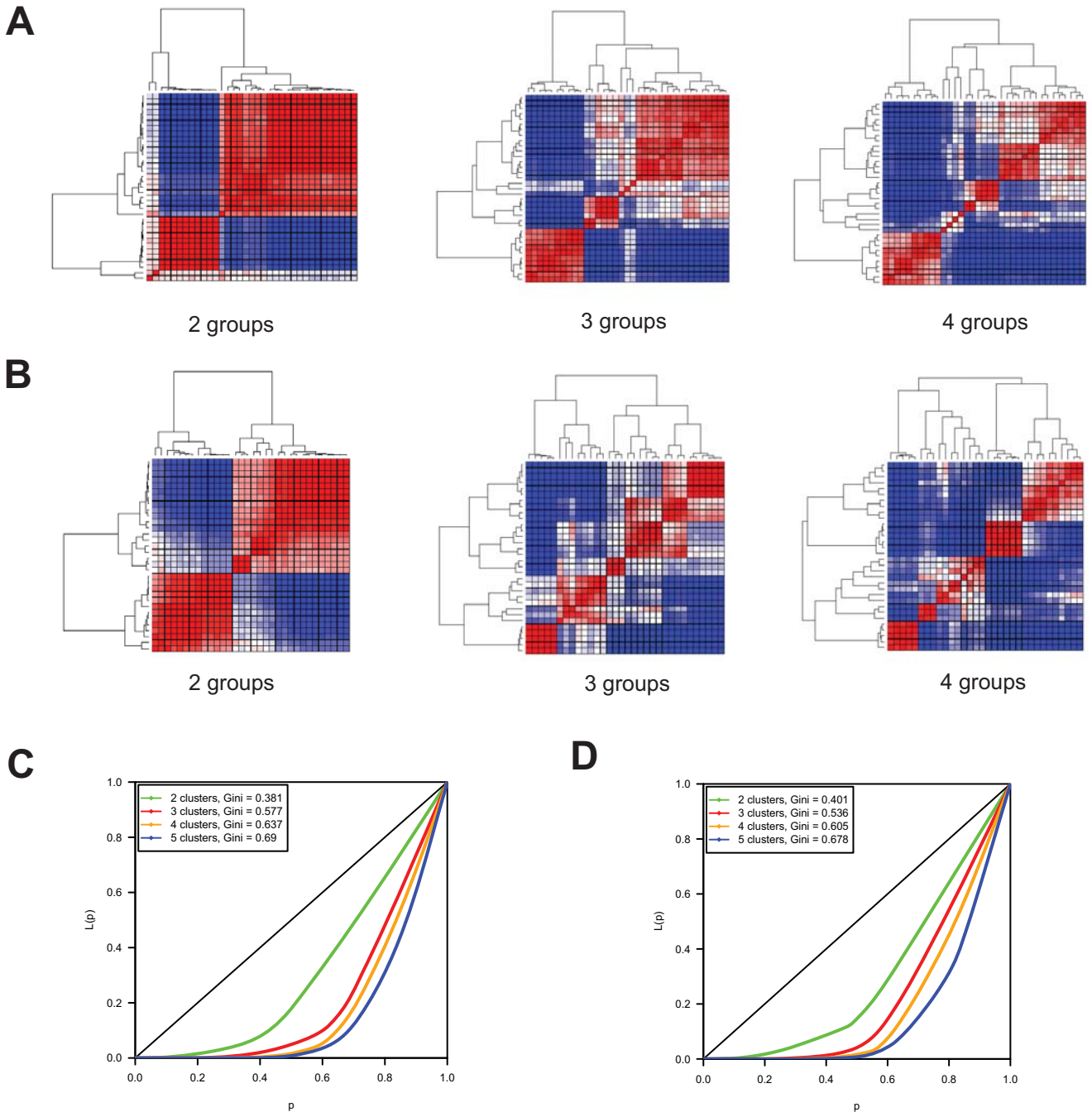
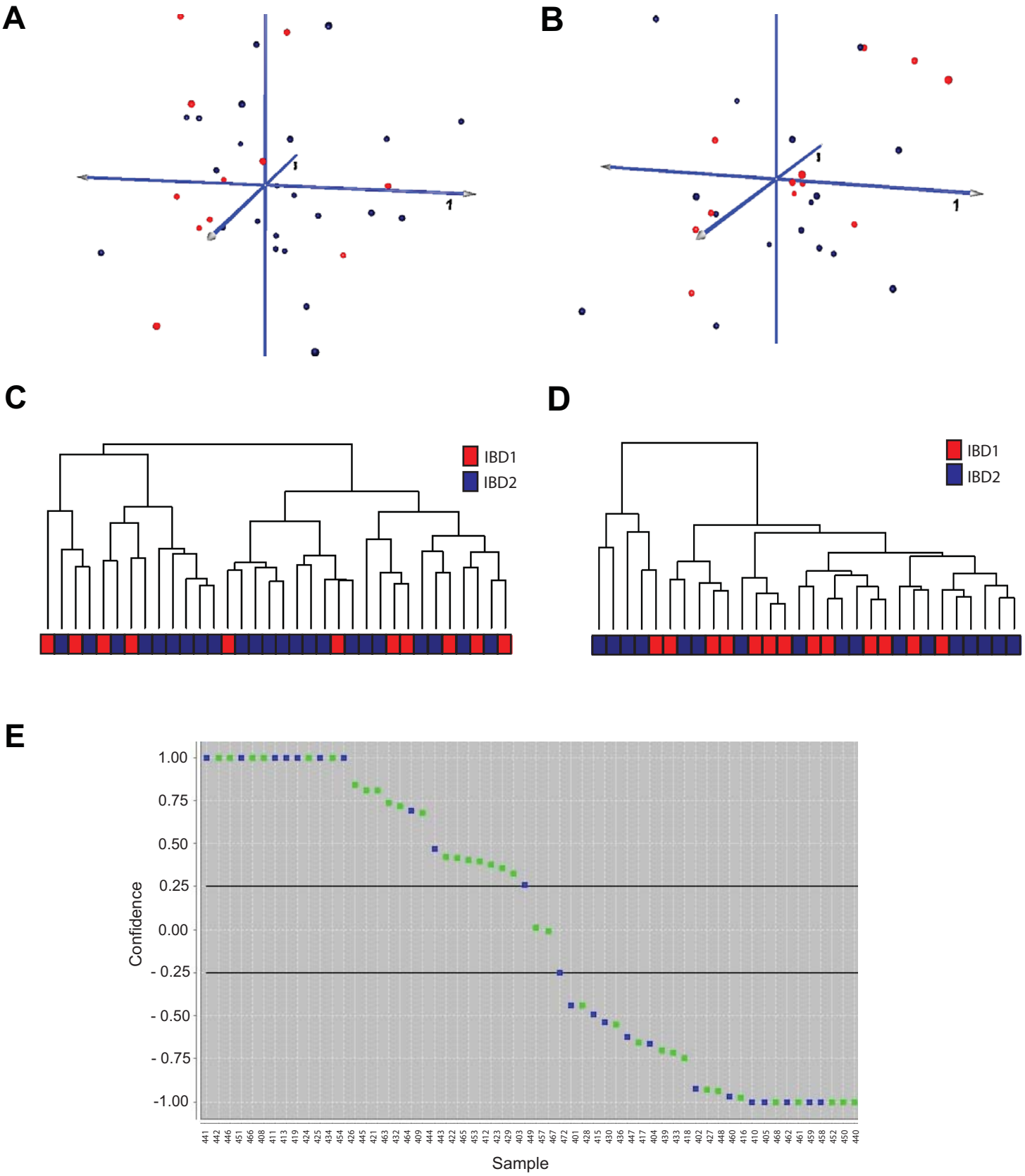
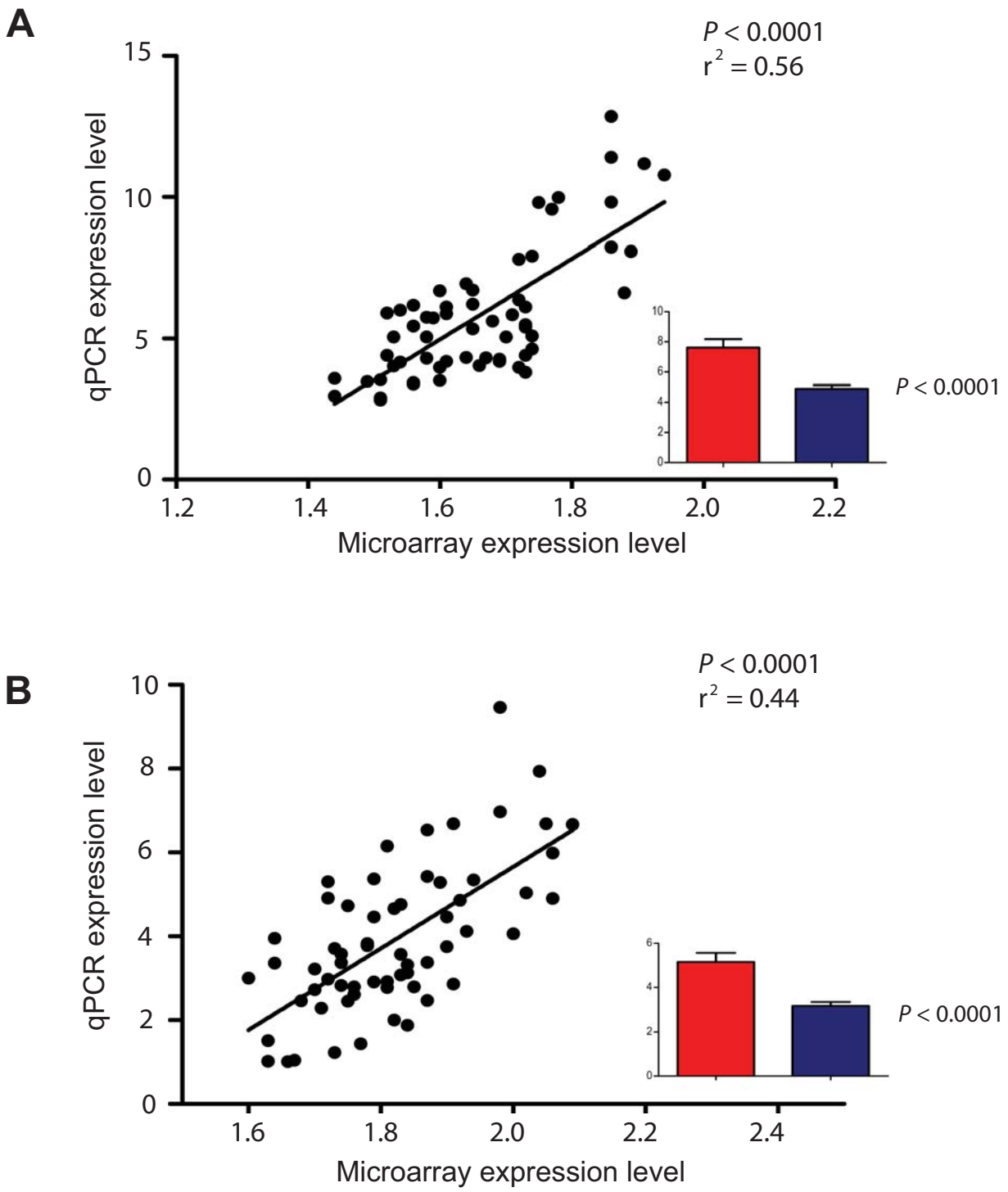


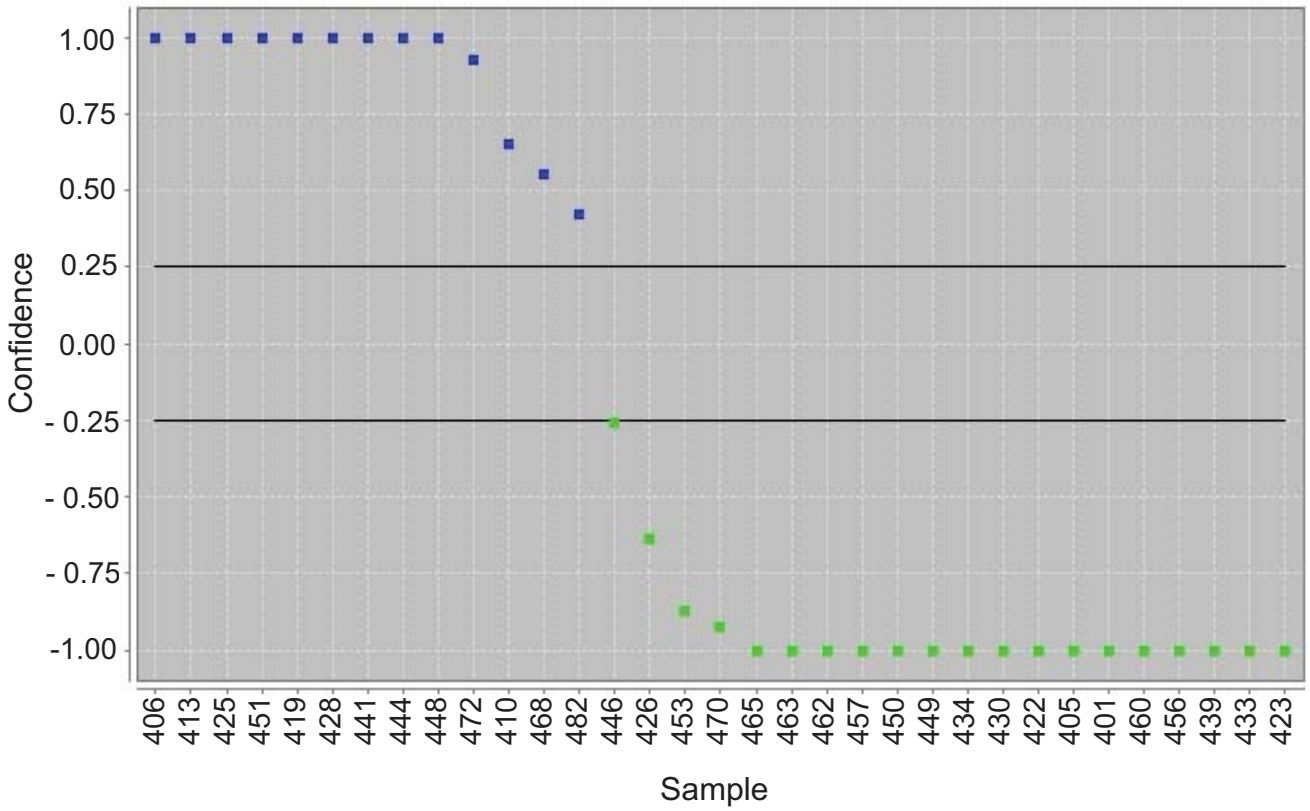
Supplemental Figure 1. The substructure within the CD8 T-cell gene-expression data from both UC and CD is best explained by the presence of two distinct subgroups.
Consensus clustering heatmaps demonstrating the merged output of 5000 iterations of hierarchical and k-means clustering of (A) CD patients and (B) UC patients for the specified number of groups. Patient samples are arranged in the same order along the x and y axes. The colours of the intersecting squares represent the frequency with which samples cluster together both within individual consensus clustering analyses and also between analyses using different methods of clustering. The colour ranges from red (patients always cluster together) to blue (patients never cluster together). The optimal number of subgroups can be assessed by visual inspection of consensus heatmaps and by deriving the Gini coefficient from Lorenz plots relating to the respective number of clusters – shown for the (C) CD patients and (D) UC patients.



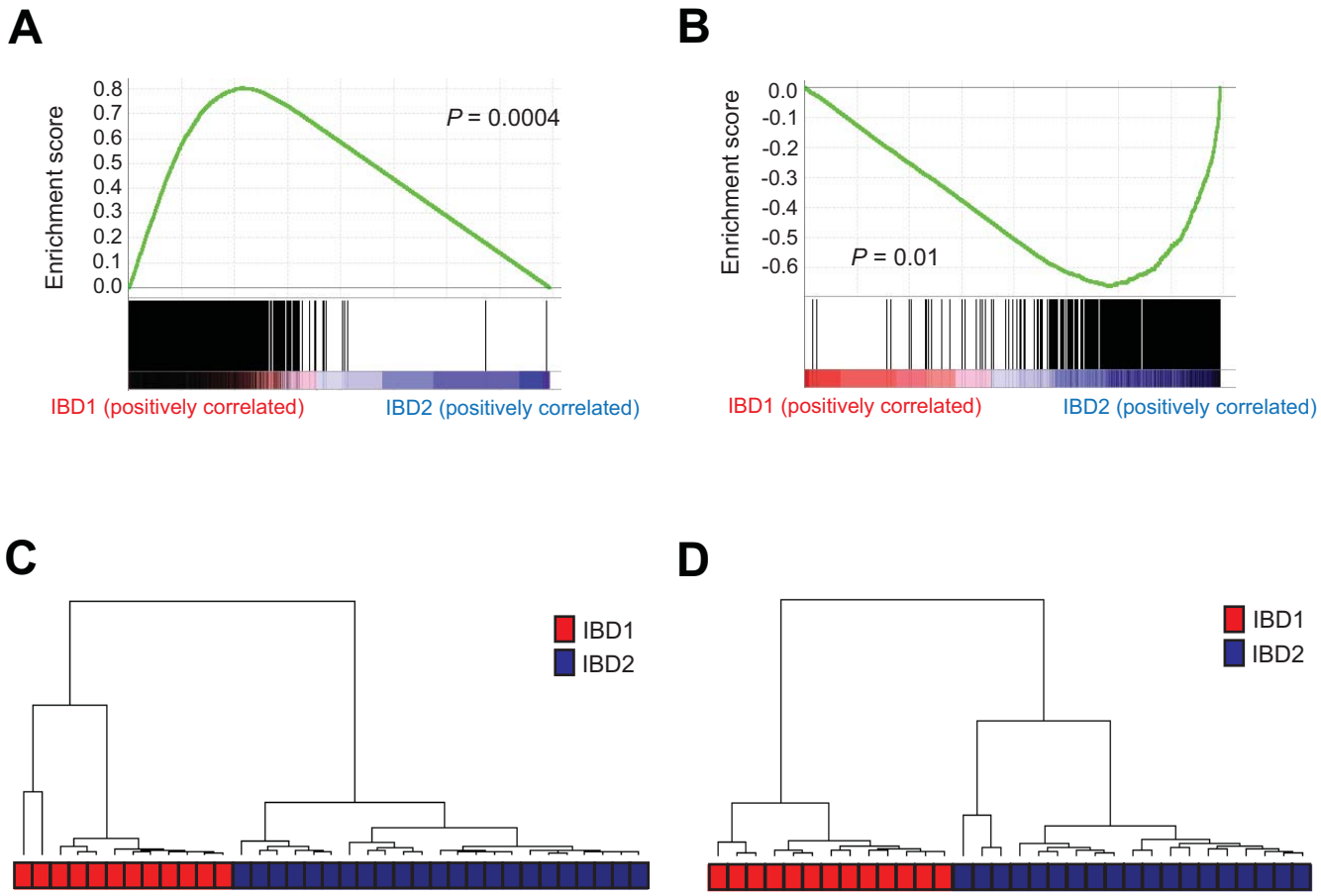
Supplemental Figure 2. The subgroups identified within CD8 T-cells cannot be detected within PBMC. Principal component analysis of PBMC gene expression data from CD (A) and UC (B). Colours indicate CD8 T-cell subgroup designation (red - IBD1, blue - IBD2). (B) The clusters of CD patients (C) and UC patients (D) that were produced by unsupervised k-means consensus clustering of the PBMC gene-expression data from the IBD cohorts. The coloured bar beneath each dendrogram corresponds to the original IBD1/2 subgroup membership. (E) Application of the 4 gene classifier generated and validated in CD8 T-cell expression datasets to PBMC gene-expression data. IBD1 and IBD2 patients are shown as blue and green dots respectively. Confidence of assignment is shown on the y axis from +1 (definitely IBD2) to -1 (definitely IBD1). Confidence threshold set at ±0.25. 2 samples were unclassified and 32 samples were misclassified. Genes used in this classifier: interleukin enhancer binding factor 2 (ILF2), GDP dissociation inhibitor 2 (GDI2), cluster of differentiation 44 (CD44) and interleukin-10 receptor, beta (IL10RB).



Supplemental Figure 3. Validation of array results with quantitative PCR
Quantitative PCR and microarray assessment of mRNA abundance demonstrated a statistically significant, positive correlation. Example data are shown for (A) IL2RG and (B) ILF2, which were both significantly upregulated in subgroup IBD1 in the microarray analysis. The scatter plot demonstrates the correlation between the transcript expression level as determined by quantitative PCR with the microarray probe signal. The raw microarray data was normalised for this comparison using a housekeeping gene (ST6GALNAC3). Significance of correlation was assessed using a Pearson goodness-of-fit test. The inset bar chart demonstrates the mean qPCR expression levels of each gene, stratified by IBD1/2 subgroup (IBD1; red, IBD2; blue, error bars indicate standard error of the mean). Statistical significance was determined using a Mann-Whitney test (2-tailed).

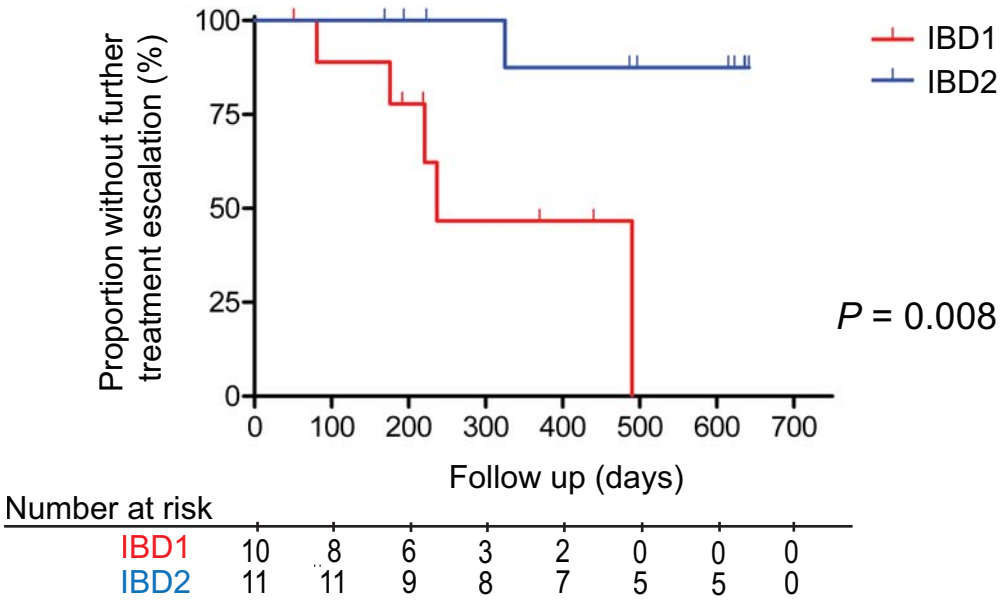


Supplemental Figure 4. Subgroup designation can be accurately ascribed using a smaller gene list
Classifiers were generated in a training-set of patients using different prediction algorithms and were then tested in an independent test-set of patients to assess their performance. An example is shown of the performance of a four gene classifier, which was generated using a weighted-voting algorithm. IBD1 and IBD2 patients, defined using whole CD8 T-cell microarray data, are shown as blue and green dots respectively. Confidence of assignment is shown on the y axis from +1 (definitely IBD1) to -1 (definitely IBD2). Confidence threshold set at ±0.25. No samples were unclassified or misclassified. The genes used in this classifier were interleukin enhancer binding factor 2 (ILF2), GDP dissociation inhibitor 2 (GDI2), cluster of differentiation 44 (CD44) and interleukin-10 receptor, beta (IL10RB).

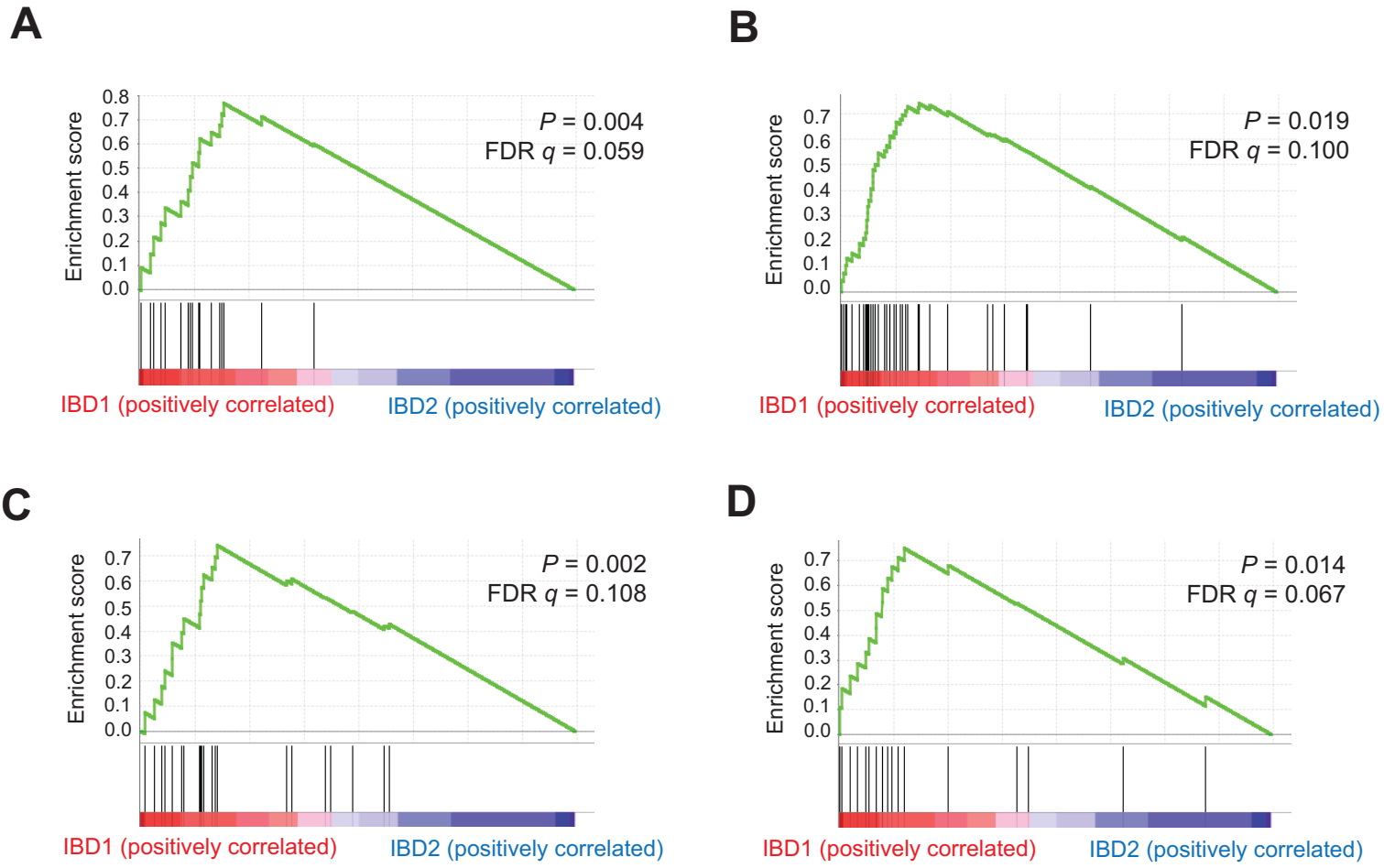


Supplemental Figure 5. The IBD1/2 signature is analogous to the prognostic signature previously described in SLE and AAV

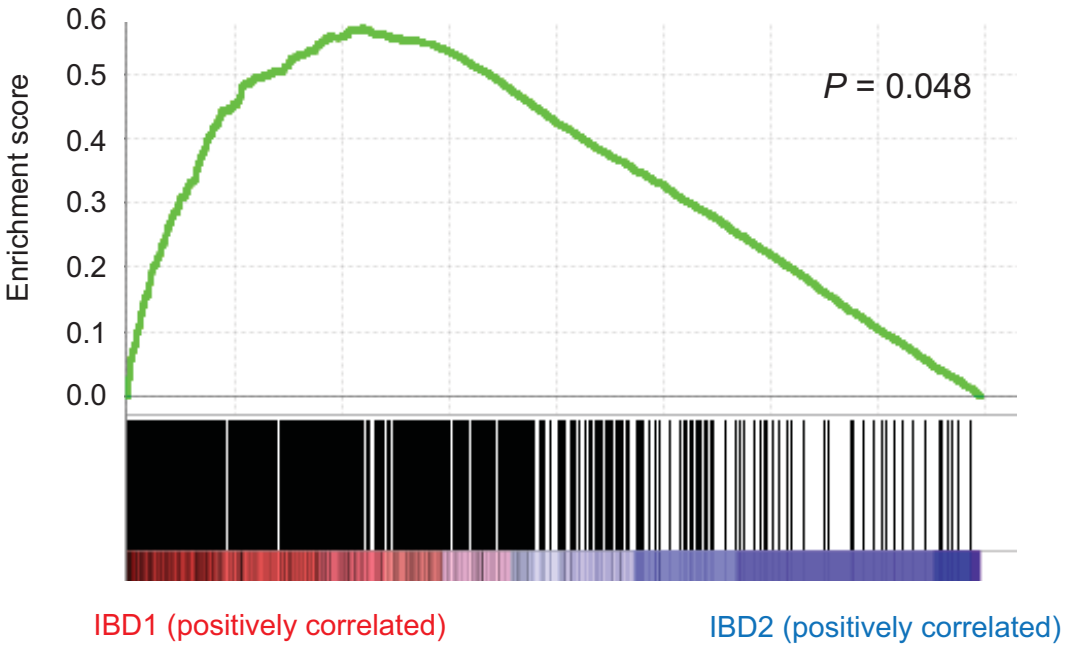
Gene-set enrichment profiles of the (A) upregulated and (B) downregulated SLE/AAV signature genes within the IBD1/2 signature. Genes are ranked in order (by signal-to-noise ratio) along the x axis from the most up-regulated in IBD1 to the most upregulated in IBD2 (downregulated in IBD1). The relative position of each of the SLE/AAV signature genes within the IBD1/2 signature is indicated by the vertical black lines along the x axis. The significance of the enrichment score is determined by comparison with a null distribution which is permuted from the data according to a standard method (Subramanian et al. (2005) Proc Natl Acad Sci U S A 102: 15545-15550). The clusters of (C) CD patients and (D) UC patients that were produced by k-means clustering of the IBD cohorts using the SLE/AAV signature. The coloured bar beneath each dendrogram corresponds to the original IBD1/2 subgroup membership.



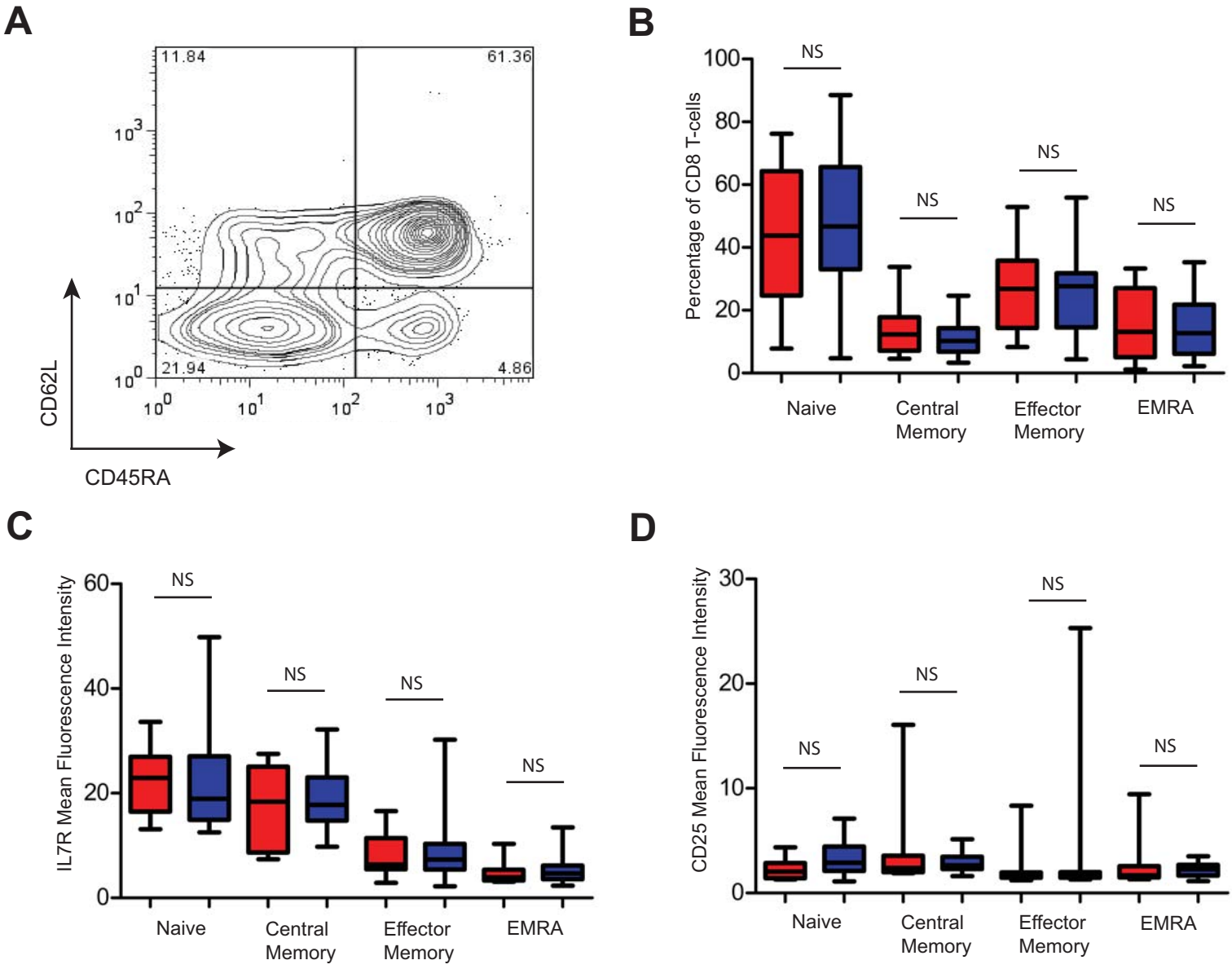
Supplemental Figure 6. The subsequent disease course of CD patients following an initial treatment escalation. Kaplan-Meier survival curve demonstrating the proportion of CD patients who, having received one treatment escalation, subsequently required a second escalation (due to unremitting, persistent disease activity). All CD patients who required a treatment escalation at any time were included in this analysis. The patients are stratified according to IBD1/2 subgroup. Statistical significance was determined using Log-rank test (1df).



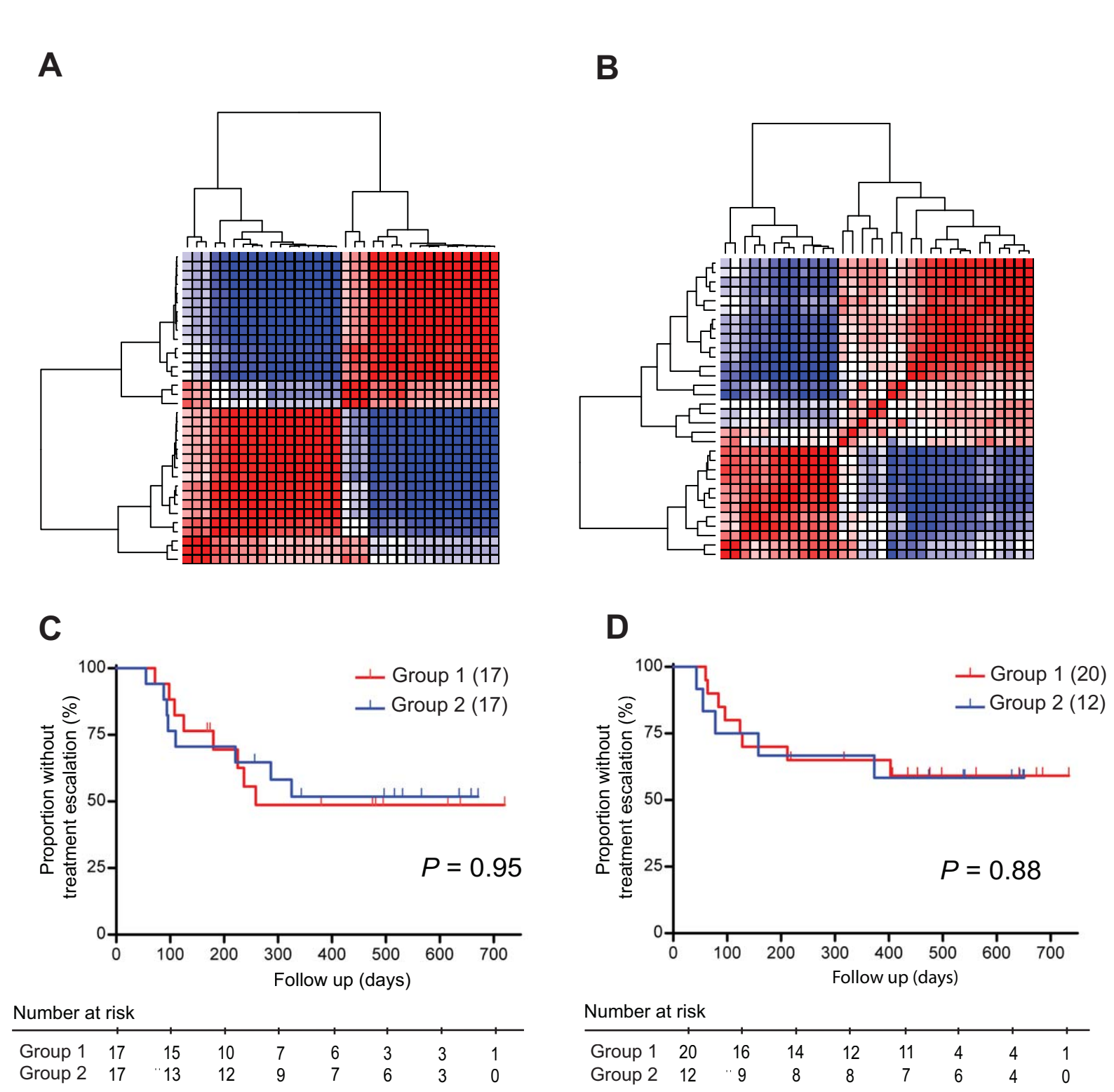
Supplemental Figure 7. Gene-set enrichment profiles of pathways enriched within the IBD1/2 signature. Gene-set enrichment plots demonstrating the enrichment of (A) IL-7 signalling pathway, (B) T-cell receptor signalling pathway, (C) IL-2 signalling pathway and (D) CD28 co-stimulatory signalling, within the genes that are differentially expressed between subgroups IBD1 and IBD2. Genes are ranked in order (by signal-to-noise ratio) along the x axis from the most up-regulated in IBD1 to the most upregulated in IBD2 (downregulated in IBD1).The relative position of each gene within a pathway is indicated by the vertical black lines along the x axis. The significance of the enrichment score is determined by comparison with a null distribution, which is permuted from the data according to a standard method. The nominal *P* value and false-discovery rate (FDR) *q* value shown were calculated by assessing the enrichment of selected pathways in the IBD1/2 signature in half of the patients, where the pathways under investigation were pre-selected due to enrichment ($P < 0.05$) in an independent analysis using the other half. Standard significance cut-off $P < 0.05$, $q < 0.25$.



Supplemental Figure 8. Gene-set enrichment profile demonstrating the enrichment of a CD8 T-cell activation signature within the IBD1/2 signature.
Gene-set enrichment plot demonstrating the enrichment of a genes that are known to be differentially expressed upon CD8 T-cell activation by CD3/CD28 signalling, within the genes that are differentially expressed between subgroups IBD1 and IBD2 in the entire dataset (n=67). 738 gene signature derived from Wang et al. (2008, BMC Genomics) by cross-platform mapping the list of genes that were significantly altered upon CD8 T-cell stimulation to Affymetrix probe annotations. 520 transcripts did not have an equivalent probe and were excluded as they could not be mapped (mostly unannotated ESTs). Genes are ranked in order (by signal-to-noise ratio) along the x axis from the most up-regulated in IBD1 to the most upregulated in IBD2 (downregulated in IBD1). The relative position of each gene within a pathway is indicated by the vertical black lines along the x axis. The significance of the enrichment score is determined by comparison with a null distribution, which is permuted from the data according to a standard method.



Supplemental Figure 9. Contemporaneous immunophenotyping of CD8 T-cell memory compartments.
(A) Representative FACS plot demonstrating CD8 T-cell memory compartments. Gated on CD3+CD8+ cells. Populations shown are naive (CD45RA+CD62L+), central memory (CD45RA-CD62L+), effector memory (CD45RA-CD62L-) and EMRA (CD45RA+CD62L-). (B) Relative sizes of CD8 T-cell memory compartments, expressed as a percentage of all CD8 T-cells. (C) IL7R mean fluorescence intensity. Geometric mean fluorescence intensity of interleukin-7 receptor (IL7R) on CD8 T-cell memory compartments. (D) IL2RA (CD25) mean fluorescence intensity. Geometric mean fluorescence intensity of interleukin-2 receptor alpha (IL2RA/CD25) on CD8 T-cell memory compartments. The 'box' and 'whiskers' of each 'box and whiskers' plot indicate the lower and upper quartiles and the range respectively. The colour of each 'box' refers to IBD1/2 subgroup (IBD1: red, IBD2; blue). Statistical significance (P<0.05) in panels b-d was determined using a Mann-Whitney test (2-tailed). NS; not significant. n=67.



Supplemental Figure 10. Transcriptional differences in CD4 T-cells do not predict disease course
Consensus clustering heatmaps demonstrating the clustering of CD4 samples from (A) CD patients and (B) UC patients. Patients are arranged in the same order along the x and y axes. The colours of the intersecting squares represent the frequency with which patients cluster together. The colour ranges from red (always cluster together) to blue (never cluster together). The correlation between the resulting subgroups and subsequent disease course is shown by the Kaplan-Meier survival curves, which demonstrate the proportions of (C) CD and (D) UC patients who did not require a subsequent treatment escalation (immunomodulator or surgery) after enrolment. Statistical significance was determined using a Log-rank test (1df).

Supplemental Table 1. Crohn's disease phenotype data

ID	IBD1 or IBD2	Age at entry	Follow up (days)	Previously diagnosed	Disease distribution	HBSI at entry	Treatment at entry	Response to initial treatment	ASCA (at entry)	Smoking Status	Subsequent disease course
411	IBD1	30	693	Yes	L1	11	Prednisolone	Partial response. Fully responded to Infliximab[A] (induction only)	Negative	Never	Flare (221 days). HBSI 11. - Treated with Prednisolone and commenced on regular Infliximab[A]. Fully responded.
416	IBD1	32	677	Yes	L3	8	Budesonide	Partial response. Fully responded to Prednisolone.	Positive	Never	Flare (180 days). HBSI 11. - Treated with Elemental diet and commenced Azathioprine (2mg/kg)[A]. Fully responded. Flare (284 days). HBSI 10. - Treated with Prednisolone. Azathioprine increased (2.5mg/kg). Fully responded. Flare (356 days). HBSI 25. - Admitted to hospital, treated with intravenous steroids and antibiotics. - Commenced Infliximab[A]. Fully responded.
419	IBD1	18	671	No	L1	6	Budesonide	Partial response. Fully responded to Prednisolone.	Negative	Never	Flare (134 days). HBSI 7. - Treated with Budesonide. Declined Azathioprine. Fully responded. (*NOT counted as treatment escalation*).
428	IBD1	40	639	Yes	L3	14	Prednisolone	Partial response only	Positive	Current	Chronic continuous symptoms. Ileocaecal resection[A] (88 days). Fully responded. Flare (578 days). HBSI 12. - Treated with Prednisolone and commenced Azathioprine (2mg/kg)[A]. Fully responded.
441	IBD1	60	565	Yes	L3	7	Budesonide	Partial response. Fully responded to Prednisolone.	Negative	Ex	Flare (125 days). HBSI 7. - Treated with Prednisolone and commenced Azathioprine (2mg/kg)[A]. Fully responded. Flare (351 days). HBSI 22. - Admitted to hospital, treated with intravenous corticosteroids and antibiotics. Fully responded.
444	IBD1	26	539	Yes	L3	19	Prednisolone 6-Mercaptopurine[A]	Partial response only	Positive	Ex	Chronic continuous symptoms initially, with significant deterioration (55 days). HBSI 14. - Commenced Adalimumab[A]. Fully responded. Flare (136 days). HBSI 14. - Treated with Prednisolone and Adalimumab interval shortened to weekly[A]. - Chronic continuous symptoms thereafter. Elective panproctocolectomy[A] (510 days)
448	IBD1	22	512	No	L2, perianal	8	Infliximab[A]	Partial response only	Positive	Never	Chronic continuous symptoms (predominantly perianal). - Required 2 operations for perianal disease (seton x2) - *not counted as treatment escalations*. - Infliximab dose doubled due to inadequate response[A] (237 days).
451	IBD1	25	495	No	L2	22	Prednisolone	Complete remission	Negative	Current	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
454	IBD1	27	478	No	L2	12	Intravenous steroids	Complete remission	Negative	Current	Flare (108 days). HBSI 10. - Treated with Prednisolone and commenced on Azathioprine (2mg/kg)[A]. Fully responded.
459	IBD1	35	470	No	L3	10	Prednisolone	Complete remission	Negative	Never	Flare (287 days). HBSI 9. - Treated with Prednisolone and commenced on Azathioprine (2mg/kg)[A]. Fully responded.
467	IBD1	27	315	No	L3	12	Budesonide	Partial response only Responded fully to Prednisolone	Positive	Current	Flare (96 days). HBSI 12. - Treated with Prednisolone and commenced on Azathioprine (2mg/kg)[A]. Fully responded.

482	IBD1	28	161	No	L2, perianal	9	Prednisolone	Partial response only Responded fully to extended course	Negative	Current	Flare (110 days). HBSI 13. - Treated with Prednisolone and commenced on Azathioprine (2mg/kg)[A]. Fully responded.
401	IBD2	24	735	No	L3	10	Budesonide	Partial response. Fully responded to Prednisolone.	Negative	Current	Flare (98 days). HBSI 9. - Treated with Prednisolone and commenced on Azathioprine (2mg/kg)[A]. Fully responded.
405	IBD2	64	720	Yes	L1, L4	7	Budesonide	Complete remission	Positive	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
408	IBD2	37	714	Yes	L1	9	Prednisolone	Partial remission	Positive	Ex	Chronic continuous obstructive symptoms. Ileocaecal resection[A] (72 days). Fully responded.
409	IBD2	57	712	No	L1	7	Elemental diet	Complete remission	Negative	Current	Flare (225 days). HBSI 7. - Persistent stricture in terminal ileum. Ileocaecal resection[A]. Fully responded.
412	IBD2	48	687	Yes	L3	14	Prednisolone	Complete remission	Positive	Never	Flare (64 days). HBSI 9. - Treated with Prednisolone and commenced on Azathioprine (2mg/kg)[A]. Fully responded.
421	IBD2	29	659	Yes	L3, perianal	10	Budesonide Azathioprine[A]	Complete remission	Positive	Never	Flare (325 days). HBSI 10. - Commenced on Adalimumab[A]. Fully responded.
422	IBD2	19	658	No	L3	10	Budesonide	Complete remission	Positive	Current	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
429	IBD2	22	638	No	L2	12	Prednisolone	Complete remission	Positive	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
430	IBD2	47	636	Yes	L1, L4	9	Prednisolone Azathioprine[A]	Complete remission	Positive	Current	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
434	IBD2	30	615	Yes	L3	11	Prednisolone Azathioprine[A]	Complete remission	Positive	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
440	IBD2	30	566	No	L3	18	Budesonide	Complete remission	Negative	Current	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
446	IBD2	40	531	No	L2	9	Topical therapy	Complete remission	Negative	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
447	IBD2	34	516	No	L3	13	Prednisolone	Partial remission Fully responded with extended course	Negative	Current	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
450	IBD2	40	497	Yes	L1	12	Surgery[A] (declined steroids)	Complete remission	Positive	Current	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
452	IBD2	27	482	No	L2	9	Prednisolone	Complete remission	Negative	Never	Flare (259 days). HBSI 10. - Treated with Prednisolone and commenced on Azathioprine (2mg/kg)[A]. Fully responded.
453	IBD2	23	481	No	L3	8	Budesonide	Complete remission	Negative	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
457	IBD2	55	475	No	L3	20	Prednisolone	Complete remission	Negative	Current	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
463	IBD2	31	380	No	L3	10	Prednisolone	Complete remission	Positive	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
465	IBD2	68	343	No	L2	7	Topical therapy	Complete remission	Negative	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
470	IBD2	21	288	No	L3	9	Intravenous steroids	Complete remission	Positive	Current	Flare (94 days). HBSI 10. - Treated with Prednisolone and commenced on Azathioprine (2mg/kg)[A]. Fully responded.

476	IBD2	41	257	No	L1	8	Prednisolone	Partial remission Responded fully to extended course	Negative	Current	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
480	IBD2	35	174	No	L2, perianal	11	Prednisolone	Complete remission	Positive	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
481	IBD2	30	169	No	L3	12	Surgery[A]	Complete remission	Positive	Current	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*

(A) denotes treatment escalation.

Abbreviations

HBSI Harvey Bradshaw Severity Index
ASCA anti-*Saccharomyces cerevisiae* antibodies
L1 Ileal disease
L2 Colonic disease
L3 Ileocolonic disease
L4 Upper GI disease

Supplemental Table 2. Ulcerative colitis phenotype data

ID	IBD1 or IBD2	Age at entry	Follow up (days)	Previously diagnosed	Disease distribution	SCCAI at entry	Treatment at entry	Response to initial treatment	Smoking Status	Subsequent disease course
403	IBD1	50	723	Yes	E2	10	Prednisolone	Complete remission	Never	Flare (212 days). SCCAI 11 - Treated with Prednisolone and commenced on Azathioprine (2mg/kg)†. Chronic continuous symptoms. Flare (463) - Treated with Prednisolone and switched to Methotrexate†. Fully responded.
406	IBD1	55	719	No	E3	14	Intravenous steroids	Partial response Complete remission with Cyclosporin	Ex	Commenced Azathioprine (2mg/kg)[A] (60 days).
410	IBD1	51	707	Yes	E1	11	Topical therapy	Complete remission	Current	Flare (124 days). SCCAI 8 - Failed to respond to Prednisolone. Completely responded to Acetarsol suppositories. Offered Azathioprine but declined – elected to use cyclical Acetarsol[A]. Flare (404 days). SCCAI 12 - Treated with Prednisolone and Acetarsol suppositories. Fully responded. commenced Methotrexate[A].
413	IBD1	56	685	Yes	E3	11	Intravenous steroids Azathioprine[A]	Complete remission	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
415	IBD1	50	678	Yes	E3	8	Prednisolone	Complete remission	Ex	Flare (212 days). SCCAI 11 - Treated with Prednisolone and commenced on 6-Mercaptopurine (1mg/kg)[A]. Fully responded. Flare (284 days). SCCAI 7. - Treated with high dose 5-ASA. 6-Mercaptopurine increased (1.5mg/kg). Fully responded.
417	IBD1	36	674	Yes	E2	11	Prednisolone	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
418	IBD1	43	673	Yes	E1	10	Prednisolone	No response Complete remission with intravenous steroids	Ex	Flare (43 days). SCCAI 7 - Treated with increased Prednisolone dose and commenced Methotrexate[A]. Fully responded.
425	IBD1	27	646	Yes	E3	8	Prednisolone	Complete remission	Ex	Flare (64 days). SCCAI 9 - Treated with Prednisolone and commenced Azathioprine (2mg/kg)[A]. Fully responded. Flare (238 days). SCCAI 10. - Treated with Prednisolone. Azathioprine increased (2.5mg/kg). Fully responded.
436	IBD1	45	594	Yes	E2	8	Prednisolone	Partial remission Complete remission with extended course	Ex	Flare (403 days). SCCAI 12 - Treated with Prednisolone and commenced Azathioprine (2mg/kg)[A]. Fully responded.
461	IBD1	50	435	No	E1	6	Topical therapy	Complete remission	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
464	IBD1	48	362	No	E2	7	Prednisolone	Partial response Complete remission with Cyclosporin (following partial response to intravenous steroids)	Ex	Commenced Azathioprine (2mg/kg)[A] (55 days).

468	IBD1	26	307	No	E3	6	Prednisolone	Complete remission	Never	Flare (78 days). SCCAI 12 - Treated with Prednisolone and commenced Azathioprine (2mg/kg)[A]. Fully responded.
472	IBD1	31	268	No	E3	11	Intravenous steroids	Complete response	Never	Flare (158). SCCAI 13 - Admitted to hospital and treated with intravenous steroids. Fully responded. Commenced Azathioprine (2mg/kg) [A]
402	IBD2	23	734	Yes	E2	6	Topical therapy	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
404	IBD2	65	721	Yes	E2	8	Prednisolone	Complete remission	Ex	Flare (96 days). SCCAI 8 - Treated with Prednisolone and commenced Azathioprine (2mg/kg)[A]. Fully responded.
423	IBD2	46	651	Yes	E3	6	Prednisolone 6-Mercaptopurine[A]	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
424	IBD2	40	649	No	E2	8	Budesonide	Partial response Complete remission with Prednisolone	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
426	IBD2	18	643	No	E3	7	Prednisolone	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
427	IBD2	40	642	Yes	E1	11	Topical therapy	Complete response	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
432	IBD2	24	628	Yes	E3	10	Budesonide Azathioprine[A]	Complete remission	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
433	IBD2	43	624	Yes	E2	8	High dose 5-ASA	Complete remission	Never	Flare (373 days). SCCAI 8 - Treated with Prednisolone and commenced 6-Mercaptopurine (1mg/kg)[A]. Fully responded.
439	IBD2	70	566	Yes	E2	11	Prednisolone	Complete remission	Ex	Flare (127 days). SCCAI 7 - Treated with Prednisolone and commenced Azathioprine (2mg/kg)[A]. Fully responded.
442	IBD2	22	562	No	E2	9	High dose 5-ASA	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
443	IBD2	84	541	No	E3	8	Prednisolone	Complete remission	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
445	IBD2	22	538	No	E3	9	Prednisolone	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
449	IBD2	64	498	Yes	E2	13	High dose 5-ASA	Complete remission	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
456	IBD2	41	476	No	E2	7	High dose 5-ASA	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
458	IBD2	38	474	No	E1	7	Topical therapy	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
460	IBD2	38	453	No	E1	6	Topical therapy	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*

462	IBD2	34	407	No	E2	8	Topical therapy	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
466	IBD2	52	317	No	E3	9	Prednisolone	Complete remission	Ex	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*
477	IBD2	43	218	No	E3	8	Prednisolone	Complete remission	Never	*No subsequent disease activity necessitating treatment escalation (until end of follow up.)*

(A) denotes treatment escalation

Abbreviations
SCCAI Simple Clinical Colitis Activity Index
E1 Proctitis
E2 Left-sided disease
E3 Extensive disease

Supplemental Table 3. Gene-set enrichment analysis results

Pathway	Subgroup in which pathway is enriched	Nominal *P* value (Primary cohort)	Nominal *P* value (Replication cohort)	FDR *q* value
IL-2 pathway	**IBD1**	**0.0267**	**0.0020**	**0.1078**
IL-7 pathway	**IBD1**	**0.0102**	**0.0041**	**0.0586**
CD28 co-stimulation pathway	**IBD1**	**0.0430**	**0.0142**	**0.0667**
T-cell receptor pathway	**IBD1**	**0.0060**	**0.0185**	**0.1001**
IL-2 receptor beta pathway	**IBD1**	**0.0183**	**0.0328**	**0.1654**
Calcineurin pathway	IBD1	0.0104	0.0522	0.0928
Fas pathway	IBD1	0.0331	0.0541	0.1359
Gleevec pathway	IBD1	0.0336	0.0547	0.1159
CTCF pathway	IBD1	0.0143	0.0604	0.1205
Growth hormone pathway	IBD1	0.0183	0.0890	0.1260
TNF receptor 1 pathway	IBD1	0.0388	0.1129	0.1344
CCR3 pathway	IBD1	0.0370	0.1237	0.1782
EIF pathway	IBD1	0.0323	0.1461	0.1784
RAC1 pathway	IBD1	0.0426	0.2134	0.2215
Complement pathway	IBD2	0.0271	0.0586	0.1538

Nominal *P* value – the statistical significance of the enrichment of a list of genes (pathway) within the IBD1/2 signature. FDR *q* value - the probability that the enrichment observed for a specific list of genes (pathway) represents a false positive based upon correction for multiple testing (standard significance cut-off; 0.25). The *q* value shown corresponds to the analysis of the replication cohort. Pathways were considered enriched (shown in bold) if the enrichment in the primary cohort ($P<0.05$) was reproduced in an independent replication cohort ($P<0.05$, FDR $q<0.25$).

Abbreviations:
FDR; False discovery rate
IL-7; Interleukin-7
IL-2; Interleukin-2
CTCF; CCCTC-binding factor
TNF; Tumour necrosis factor
CCR3; C-C chemokine receptor type 3
EIF; Eukaryotic translation initiation factor
RAC1; Ras-related C3 botulinum toxin substrate 1

Supplemental Table 4. Significantly enriched gene-sets

IL-7 Pathway *(nominal P = 0.004, FDR q = 0.059)*

Gene Symbol	Affymetrix Probe ID	Subgroup associated with increased expression	Disease cohort	CD adjusted *p* value	UC adjusted *p* value
BCL2	8023646	IBD1	CD	8.15E-03	ns
CREBBP	7999044	IBD1	CD and UC	4.33E-07	1.45E-05
EP300	8073345	IBD1	CD and UC	1.69E-06	4.21E-06
FYN	8128956	IBD1	CD and UC	9.42E-04	9.64E-05
IL2RG	8173444	IBD1	CD and UC	8.05E-07	6.78E-06
IL7	8104901	Neither	na	ns	ns
IL7R	8104901	IBD1	CD and UC	3.44E-04	3.98E-02
JAK1	7916747	IBD1	CD and UC	4.37E-07	2.44E-05
JAK3	8035351	IBD1	CD and UC	3.27E-05	4.65E-06
LCK	7899753	IBD1	CD and UC	3.47E-06	1.05E-04
NMI	8055702	IBD1	CD and UC	3.32E-04	1.38E-06
PIK3CA	8084016	IBD1	CD and UC	7.95E-05	8.12E-06
PIK3CG	8135363	IBD1	CD and UC	5.48E-05	1.33E-04
PIK3R1	8105778	IBD1	CD and UC	4.90E-05	1.71E-04
PTK2B	8145490	IBD1	CD and UC	1.34E-07	3.61E-05
STAT5A	8007212	IBD1	CD and UC	1.80E-05	7.63E-05
STAT5B	8015590	IBD1	CD and UC	3.96E-06	8.36E-07

TCR Pathway *(nominal P = 0.019, FDR q = 0.100)*

Gene Symbol	Affymetrix Probe ID	Subgroup associated with increased expression	Disease cohort	CD adjusted *p* value	UC adjusted *p* value
CALM1	7976200	IBD1	CD and UC	7.23E-07	4.69E-07
CALM2	8052010	IBD1	CD and UC	2.54E-06	3.36E-07
CALM3	8029831	IBD1	CD and UC	2.74E-07	9.45E-07
CD247	7922040	IBD1	CD and UC	1.40E-04	1.29E-03
CD3D	7952056	IBD1	CD and UC	1.83E-05	4.93E-05
CD3E	7944179	IBD1	CD and UC	6.11E-06	7.08E-06
CD3G	7944185	IBD1	CD and UC	9.08E-06	8.17E-06
CYCSP35	U/A	na	na	na	na
ELK1	8172345	Neither	na	ns	ns
FOS	7975779	Neither	na	ns	ns
FYN	8128956	IBD1	CD and UC	9.42E-04	9.64E-05
GRB2	8018364	IBD1	CD and UC	5.84E-07	1.08E-07
HRAS	7945436	Neither	na	ns	ns
JUN	7916609	Neither	na	ns	ns
LAT	7994541	IBD1	CD and UC	1.99E-05	3.44E-06
LCK	7899753	IBD1	CD and UC	3.47E-06	1.05E-04
MAP2K1	7984319	IBD1	CD and UC	1.03E-05	1.72E-06
MAP2K4	8005029	IBD1	CD and UC	5.61E-07	1.95E-06
MAP3K1	8105436	IBD1	CD and UC	9.48E-03	2.54E-04
MAPK3	8000811	IBD1	CD and UC	9.16E-03	1.57E-02
MAPK8	7927389	IBD1	CD and UC	1.58E-05	1.06E-04
NFATC1	8021866	Neither	na	ns	ns
NFATC2	8067040	IBD1	CD and UC	1.26E-03	4.11E-04
NFATC3	7996744	IBD1	CD and UC	1.86E-06	2.26E-06
NFATC4	7973709	Neither	na	ns	ns
NFKB1	8096635	IBD1	CD and UC	7.46E-05	8.78E-03
NFKBIA	7978644	IBD1	CD and UC	5.86E-05	1.93E-03
PIK3CA	8084016	IBD1	CD and UC	7.95E-05	8.12E-06
PIK3CG	8135363	IBD1	CD and UC	5.48E-05	1.33E-04
PIK3R1	8105778	IBD1	CD and UC	4.90E-05	1.71E-04
PLCG1	8062623	IBD1	CD and UC	1.35E-05	8.33E-03
PPP3CA	8101971	IBD1	CD and UC	9.94E-04	4.59E-07
PPP3CB	7934393	IBD1	CD and UC	2.79E-06	1.33E-07
PPP3CC	8145136	IBD1	CD and UC	2.45E-05	5.67E-07
PRKCA	8009301	Neither	na	ns	ns
PRKCB	7994131	IBD1	CD and UC	3.98E-05	2.75E-06
PTPN7	7923406	Neither	na	ns	ns
RAC1	8180343	IBD1	CD and UC	2.94E-07	1.59E-06
RAF1	8085374	IBD1	CD and UC	1.39E-07	7.79E-07
RASA1	8106784	IBD1	CD and UC	4.37E-04	4.64E-05
RELA	7949465	IBD1	CD and UC	3.42E-04	2.60E-05
SHC1	7920600	IBD1	CD and UC	1.93E-02	3.62E-02
SOS1	8051670	IBD1	CD and UC	7.12E-05	1.11E-07
TRA@	7973303	IBD1	UC	ns	3.20E-02
TRB@	7973298	Neither	na	ns	ns
VAV1	8025076	IBD1	CD and UC	9.30E-08	3.87E-06
ZAP70	8043725	IBD1	CD and UC	5.56E-07	4.07E-06

IL-2 Pathway *(nominal P = 0.002, FDR q = 0.108)*

Gene Symbol	Affymetrix Probe ID	Subgroup associated with increased expression	Disease cohort	CD adjusted *p* value	UC adjusted *p* value
CSNK2A1	8064351	IBD1	CD and UC	3.08E-07	1.37E-06
ELK1	8172345	Neither	na	ns	ns
FOS	7975779	Neither	na	ns	ns
GRB2	8018364	IBD1	CD and UC	5.84E-07	1.08E-07
HRAS	7945436	Neither	na	ns	ns
IL2	8102697	Neither	na	ns	ns
IL2RA	7931914	Neither	na	ns	ns
IL2RB	8075886	IBD1	UC	ns	2.29E-03
IL2RG	8173444	IBD1	CD and UC	8.05E-07	6.78E-06
JAK1	7916747	IBD1	CD and UC	4.37E-07	2.44E-05
JAK3	8035351	IBD1	CD and UC	3.27E-05	4.65E-06
JUN	7916609	Neither	na	ns	ns
LCK	7899753	IBD1	CD and UC	3.47E-06	1.05E-04
MAP2K1	7984319	IBD1	CD and UC	1.03E-05	1.72E-06
MAPK3	8000811	IBD1	CD and UC	9.16E-03	1.57E-02
MAPK8	7927389	IBD1	CD and UC	1.58E-05	1.06E-04
RAF1	8085374	IBD1	CD and UC	1.39E-07	7.79E-07
SHC1	7920600	IBD1	CD and UC	1.93E-02	3.62E-02
SOS1	8051670	IBD1	CD and UC	7.12E-05	1.11E-07
STAT5A	8007212	IBD1	CD and UC	1.80E-05	7.63E-05
STAT5B	8015590	IBD1	CD and UC	3.96E-06	8.36E-07
SYK	8156321	Neither	na	ns	ns

CD28 co-stimulation *(nominal P = 0.014, FDR q = 0.067)*

Gene Symbol	Affymetrix Probe ID	Subgroup associated with increased expression	Disease cohort	CD adjusted *p* value	UC adjusted *p* value
CD247	7922040	IBD1	CD and UC	1.40E-04	1.29E-03
CD28	8047677	IBD1	CD and UC	3.72E-03	1.88E-03
CD3D	7952056	IBD1	CD and UC	1.83E-05	4.93E-05
CD3E	7944179	IBD1	CD and UC	6.11E-06	7.08E-06
CD3G	7944185	IBD1	CD and UC	9.08E-06	8.17E-06
CD80	8089771	Neither	na	ns	ns
CD86	8082035	Neither	na	ns	ns
CTLA4	8047692	Neither	na	ns	ns
GRB2	8018364	IBD1	CD and UC	5.84E-07	1.08E-07
HLA-DRA	8178193	IBD1	CD and UC	2.98E-02	2.43E-02
HLA-DRB1	U/A	na	na	na	na
ICOS	8047702	IBD1	CD and UC	3.36E-03	2.15E-03
ICOSLG	8070720	Neither	na	ns	ns
IL2	8102697	Neither	na	ns	ns
ITK	8109507	IBD1	CD and UC	9.51E-05	2.41E-03
LCK	7899753	IBD1	CD and UC	3.47E-06	1.05E-04
PIK3CA	8084016	IBD1	CD and UC	7.95E-05	8.12E-06
PIK3R1	8105778	IBD1	CD and UC	4.90E-05	1.71E-04
PTPN11	7958846	IBD1	CD and UC	2.71E-07	2.25E-06
TRA@	7973303	IBD1	UC	ns	3.20E-02
TRB@	7973298	Neither	na	ns	ns

Gene-set enrichment of publically-available pathways curated from BioCarta by the Molecular Signatures database. The significance of each pathway's enrichment is shown, together with the significance of each gene annotated within that pathway. *P* values were adjusted where indicated using false-discovery rate (FDR) as is the standard for GSEA. Nominal *P* values and FDR *q* values are as described in Supplementary Figure 5. Abbreviations: FDR; False-discovery rate, TCR; T-cell receptor, IL-7; Interleukin-7, IL-2; Interleukin-2, na; Not appropriate, ns; Not significant, U/A; Unannotated