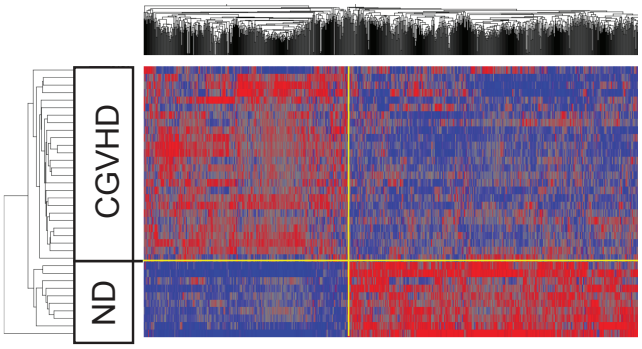
Supplemental Figure 1

A



B

Ingenuity Canonical Pathways	p value
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	6.9E-06
Role of PKR in Interferon Induction and Antiviral Response	9.3E-06
Interferon Signaling	3.7E-05
Toll-like Receptor Signaling	5.5E-04
Activation of IRF by Cytosolic Pattern Recognition Receptors	1.9E-03

C

Geneset	Number of Genes	Proportion Up	P Value	FDR
Moserle: IFNA Response	33	0.848	0.004	0.036
Reactome: Inflammasomes	16	0.75	0.002	0.017
Browne: IFN Responsive Genes	76	0.724	0.002	0.017
Bennett: Systemic Lupus Erythematosus	36	0.722	0.002	0.017
Urosevic: Response to imiquimod	25	0.64	0.004	0.036
Bosco: IFN-induced Antiviral Module	83	0.566	0.004	0.036
Reactome: Regulation of IFNg signaling	15	0.533	0.002	0.016
Geiss: Response to dsRNA UP	42	0.524	0.004	0.036

Ingenuity Upstream Regulators	Activation Proportion	Overlap p value
IFNα	.875	5.5E-05 ↑
IFNγ	.734	9.5E-04 ↑

Supplemental Figure S1. Microarray analysis of monocytes from CGVHD patients and healthy normal controls. A. Supervised hierarchical cluster analysis of 1146 genes differentially expressed between the normal control donors and CGVHD patients, based on t test with both p value and FDR less than 0.05. B. Ingenuity Canonical Pathways identified by analysis of differentially expressed genes and Ingenuity Upstream Regulators identified by analysis of up and down regulated genes. For IFNα, 21 of 24 genes in the set had a direction consistent with activation; for IFNγ, 47 of 64 genes were consistent with activation, whereas for IL-13, 30 of 51 had a direction consistent with inhibition of the pathway. C. Gene Set Enrichment Analysis identified upregulation of genes associated with pathways involving IFN response and activation of NLR and TLR pathways.

Supplemental Figure S2. Main Factors Contributing to BAFF Levels in CGVHD

Linear Regression Summary vs Log BAFF	Std Coefficient	t-Value	P-Value
Intensity of Immunosuppression[a]	.140	1.563	.1206
Time since alloHSCT[b]	-.190	-2.150	.0335
Log B Cell Count[c]	-.493	-6.278	<.0001
Log CXCL10[c]	.430	5.289	<.0001

Regression Summary	
Count[d]	125
R	.656
R^2	.430
Adjusted R^2	.411

Variables in the model	Coefficient	S.E.	*β*	*t*	*p*
Constant	2.843	.185	2.843	15.39	<.0001
Intensity of immunosuppression[a]	0.022	0.024	0.066	.925	.3570
Days since transplant[b]	-2.08E-5	2.73E-5	-0.054	-0.763	.4468
B Cell Count[c]	-.177	0.027	-.466	-6.574	<.0001
CXCL10[c]	0.397	0.066	0.421	6.018	<.0001

[a] Intensity of current immunosuppressive regimen scoring: (1) none, (2) mild (defined as single agent prednisone <0.5 mg/kg/day), (3) moderate (defined as single agent/modality +/- prednisone ≥ 0.5 mg/kg/day) or (4) high (defined as 2 or more agents/modalities +/- prednisone ≥ 0.5 mg/kg/day).

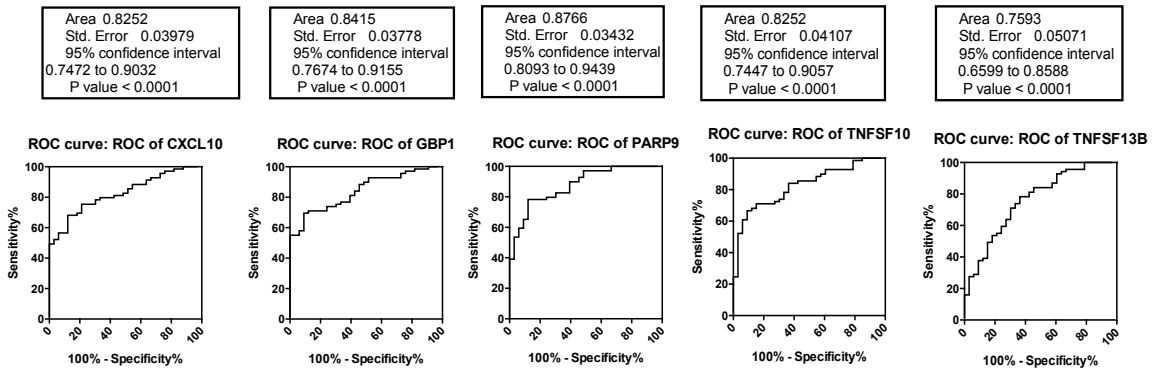
[b] measured in days following allogeneic hematopoietic stem cell transplantation

[c] values were log transformed prior to analysis; the value 0.1 was substituted for B cell values ≤ 0.1 to support the log transformation in 12 patients

[d] Patients were assayed based on availability of plasma, including 107 of the first 145 patients serially enrolled and 18 additional later patients with severe oral or cutaneous CGVHD. A total of 37 of 69 Nanostring patients were assessed, including 21 of the 26 microarray patients.

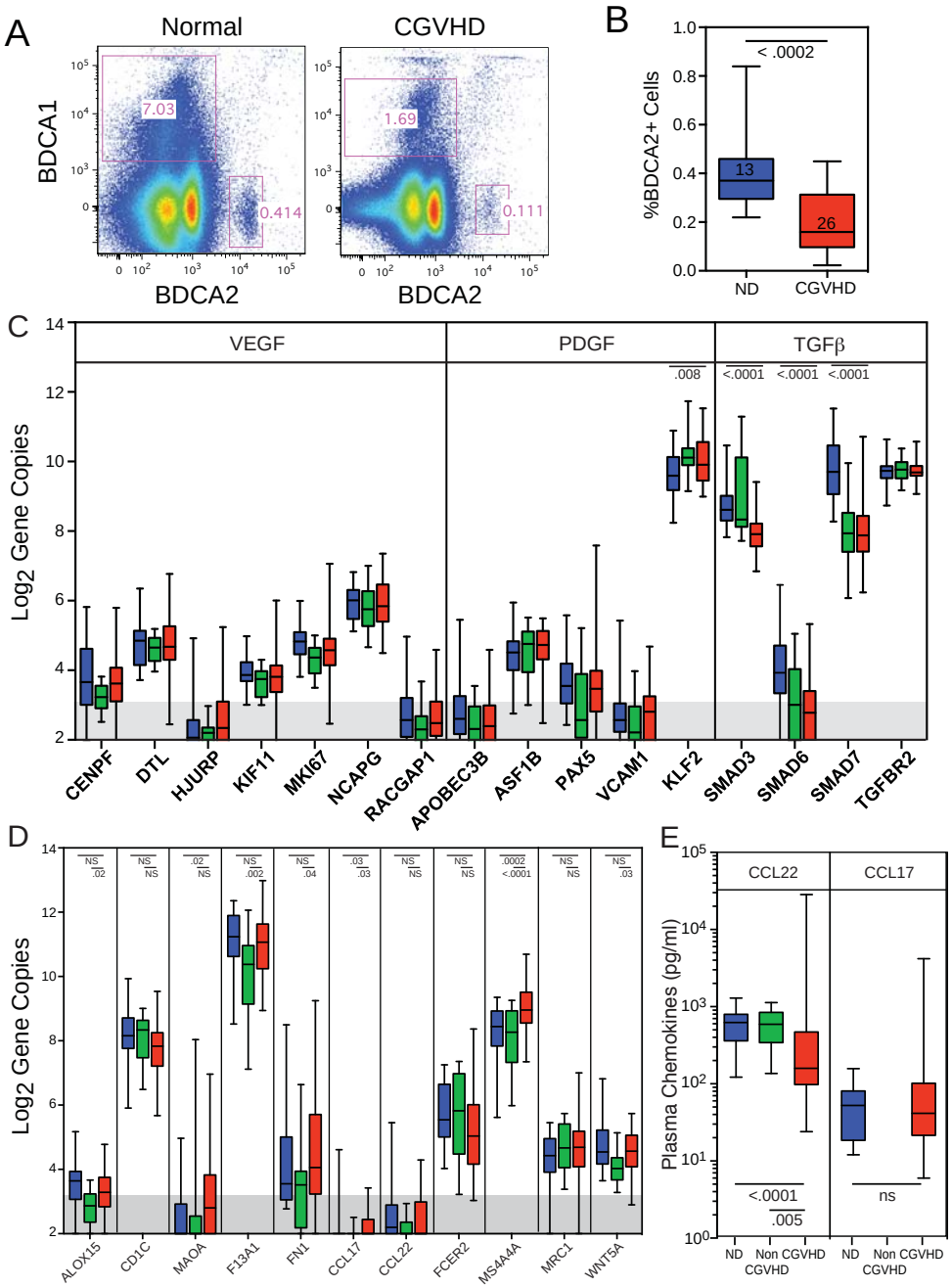
Supplemental Figure S2. BAFF interactions with IFN-induced genes. Univariate regression and multiple regression analyses of plasma BAFF levels in CGVHD, comparing the effects of immune suppression, time from transplant, B cells/μl and plasma levels of IFN-inducible chemokine CXCL10.

Supplemental Figure S3



Supplemental Figure S3. Receiver operating characteristic (ROC) curves for five IFN-inducible genes within the Nanostring cohort of 69 patients with established CGVHD, compared with 33 normal controls and non-CGVHD patients.

Supplemental Figure S4



Supplemental Figure S4. A. Flow cytometry panels gated on lymphocytes showing the frequency of BDCA2+ pDC and BDCA1+ mDC in normal and CGVHD patient. B. Box and whisker plot comparing the frequency of BDCA2+ pDC in the lymphocyte population in normal controls and CGVHD patients. C - D. Nanostring analysis of expression of additional pathways in CGVHD monocytes. C. Expression of VEGF, PDGF and TGFβ inducible genes. Among the TGFβ inducible genes, CGVHD levels were significantly lower than normal for SMAD3, SMAD6 and SMAD7. D. Expression of IL-4 and IL-13 inducible genes. Only MS4A4A, which has also been found to be induced by glucocorticoids, showed a significant increase over normal levels. Gene expression was assessed in monocytes sorted from 19 normal controls (blue), 14 nonCGVHD (green) and 69 CGVHD patients (red). Gene copy data was normalized by log2 transformation.D. Plasma levels of the Th2-recruiting chemokines CCL22 (MDC) and CCL17 (TARC) in 10 normal controls (blue), 14 nonCGVHD (green) and 122 CGVHD (red) patients.