Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Patients and tissue selection

The study was approved by the ethics review board of the National Health Group, Singapore. Sixteen formalin-fixed paraffin-embedded (FFPE) blocks containing lesional skin biopsies of patients with Henoch–Schönlein purpura (HSP) from the archives of the Histopathology Laboratory at the National Skin Centre, Singapore, were identified. The patients ($n = 16$; male (M)/ female (F): 7/9; age 14–73 years) were diagnosed between January 2013 and December 2015 and the diagnostic criteria were: (*i*) palpable purpura or petechiae clinically consistent with HSP, (*ii*) skin histology demonstrating leukocytoclastic vasculitis, and (*iii*) IgA deposition in vessel walls on immunohistochemical examination. The biopsies were all obtained before the patients underwent treatment for HSP.

The controls consist of 10 FFPE blocks of normal skin, as well as 11 blocks from patients with an inflammatory skin disease (pustular psoriasis 3, neutrophilic dermatoses 2, bacterial skin infections 6). From each block, 15–20 sections 10 μm thick were cut using a conventional microtome (Leica RM2125); a new sterile blade was used for each block to avoid contamination among the samples.

RNA extraction

All reactions were performed in an RNase-free environment and RNase-free tips and microtubes were used. Total RNA was extracted using RNeasy FFPE Kit from QIAGEN GmbH (Hilden, Germany) according to the manufacturer's instructions. RNA yield was assessed using Quant-iT Ribogreen RNA assay kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) following manufacturer's instruction and measured by Tecan Infinite M200 Monochromator microplate reader (Tecan, Switzerland).

Nanostring analysis

All RNAs were analysed on Agilent Bioanalyzer for quality assessment with RNA Integrity Number (RIN) range from 1.2 to 5.8 and median RIN 2.3. Direct mRNA expression levels of the samples were measured using the NanoString nCounter gene expression system. Total RNA of 11–100 ng were hybridized with probes from the nCounter Human Immunology v2 panel (NanoString Technologies, Seattle, WA, USA) at 65°C for 19 h according to the nCounter™ Gene Expression Assay Manual. Excess probes were washed away using a 2-step magnetic bead-based purification on the nCounter™ Prep Station (GEN1). The nCounter™ Digital Analyzer (GEN1) was used to count individual fluorescent barcodes and quantify target molecules present in each sample. For each assay, a high-density scan (600 fields of view) was performed.

Nanostring data were extracted as RCC files and processed using the R statistical language (version 3.3.1) following the procedure recommended by Nanostring. The geometrical means of the positive control probe counts were computed for each lane and a scaling factor computed for each lane being the mean of the geometrical means of all lanes divided by the geometrical mean of that particular lane. This lane specific scaling factor was then applied to all probe counts as a means to normalize for the technical variability of the platform.

The background threshold was determined for each lane using the negative controls. The threshold is the mean plus 2 standard deviations (SD) of the negative control probe counts.

The housekeeping genes *ABCF1, ALAS1, EEF1G, G6PD, GAPDH, GUSB, HPRT1, OAZ1, POLR1B, POLR2A, PPIA, RPL19, SDHA, TBP* and *TUBB* were then used to normalize for any RNA loading differences. This was performed in the same manner as the positive control probes, where the scaling factor was computed from the geometrical mean of the housekeeping genes.

The positive control and housekeeping gene normalized counts were then logarithmically transformed and used for all subsequent analysis and visualizations.

Statistical analysis

Logarithmically transformed counts of each gene were tested using ANOVAs to determine if there were any significant differences between normal, control inflammatory and HSP specimens (multiple testing correction was performed using the method of Benjamini and Hochberg). *Post-hoc t*-tests corrected for multiple testing using the method of Bonferroni were then used to determine if there were significant pairwise differences between the groups. All statistical analyses were done using the R statistical language and Excel.

Ingenuity pathway analysis

The full list of 575 genes with expression levels above background were analysed using the Ingenuity Pathway Analysis (IPA) software (March 2017 release) (Qiagen, Redwood City, CA, USA). The settings for the core analysis were as follows: user dataset; endogenous chemicals not included, direct and indirect relationships; molecules per network: 70; networks per analysis: 25. The false discovery rate or threshold was set at 0.05 to select for differentially expressed genes (DEGs) for testing. The settings were selected to compensate for the use of a targeted Nanostring panel, which has been enriched for immunological genes.

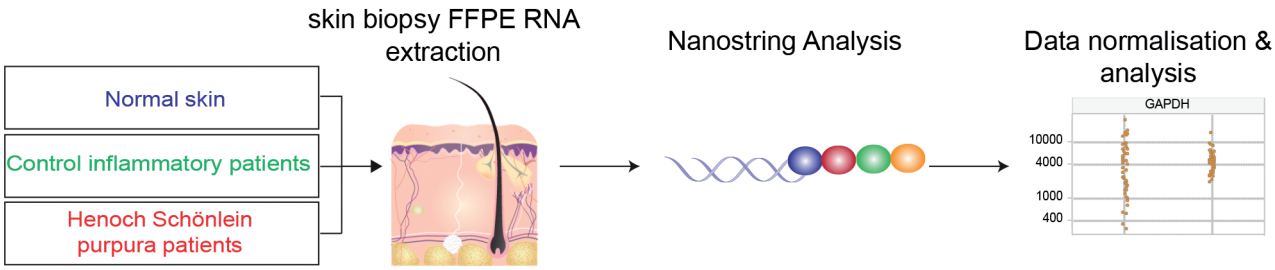

Fig. S1. Formalin-fixed paraffin-embedded (FFPE) Nanostring analysis workflow. RNA extraction was first carried out on FFPE samples from normal skin, control inflammatory patients and Henoch Schönlein purpura (HSP) lesions, followed by Nanostring gene expression analysis and data normalization and analysis.

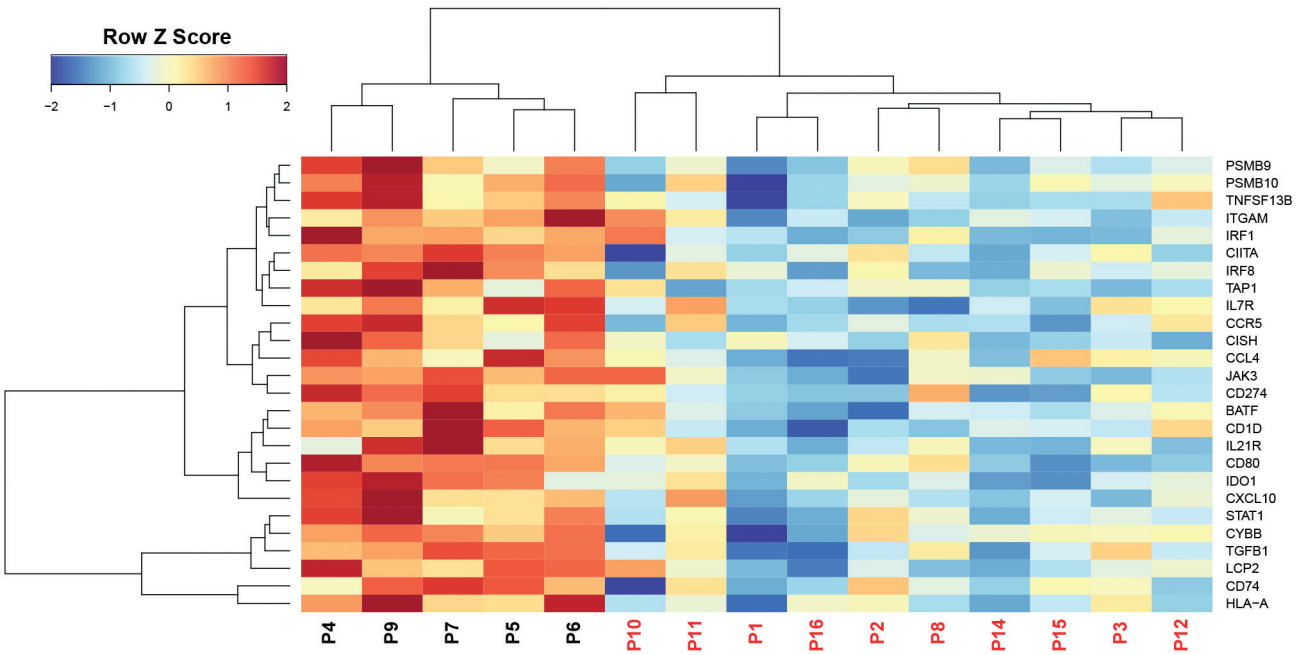

Fig. S2. Dendrogram of genes identified by Ingenuity Pathway Analysis in the 2 HSP patient clusters. HSP-normal samples are labelled in black and HSP-inflamed samples are labelled in red. The log10 normalized counts were transformed into row z-scores for each gene and depicted in a colour scale with blue as the minimum value and red as the maximum value. The samples and genes are clustered using hierarchical clustering of the Euclidean distance with average linkage.

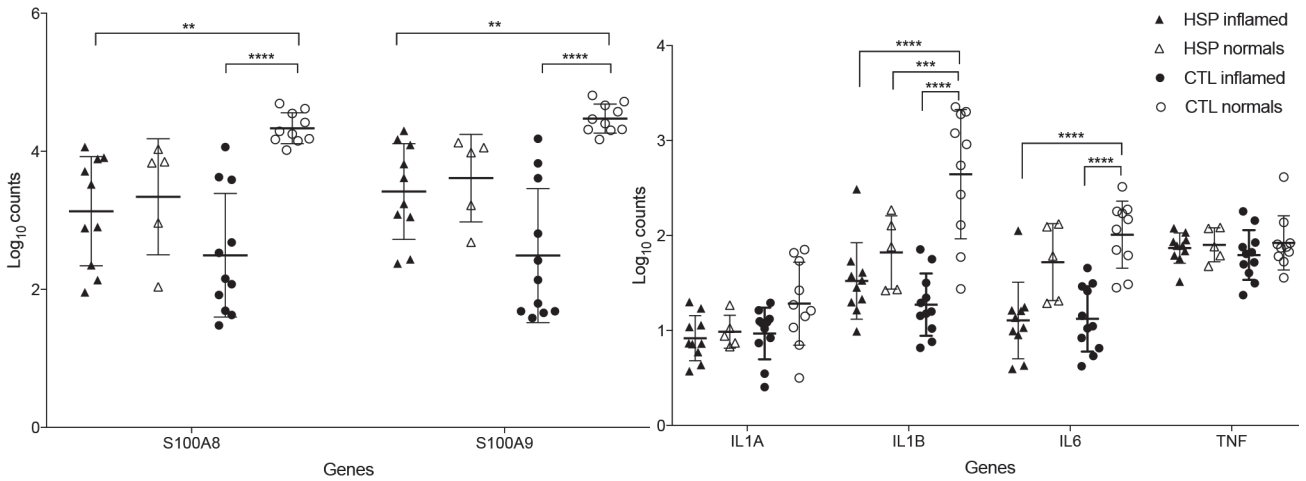

Fig. S3. Dot plots of selected proinflammatory cytokines and genes linked with neutrophil recruitment in Henoch Schönlein purpura (HSP) patient clusters (HSP-normal and HSP-inflamed) and control (normal skin and control inflammatory) samples. The left-hand panel shows genes linked with neutrophil recruitment and the right-hand panel shows classical proinflammatory cytokines. Log10 normalized counts were used. **p* adjusted < 0.05; ***p* adjusted < 0.01; ****p* adjusted < 0.001; *****p* adjusted < 0.0001 using the Bonferroni correction.

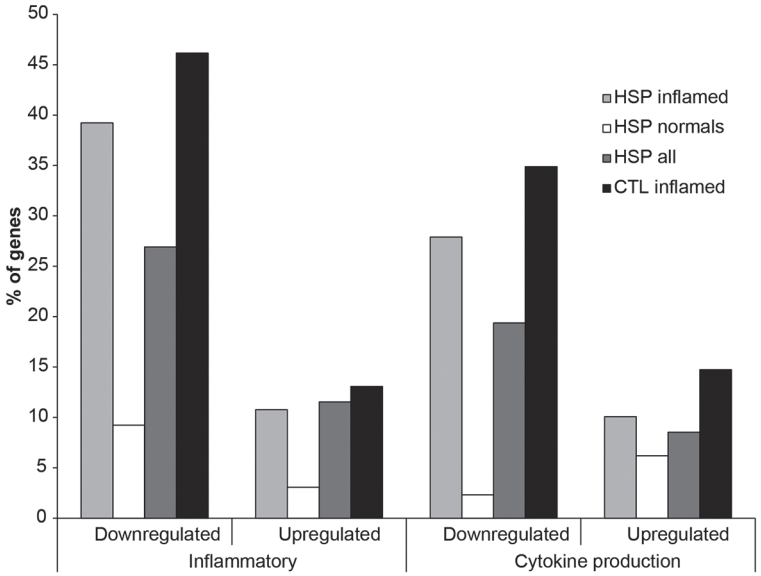

Fig. S4. Overview of differentially regulated genes (DEGs) in pathways linked with inflammatory response and cytokine production in the different sample groups. The percentage of genes in the panel that fulfilled $p<0.05$, and were upregulated or downregulated with reference to normal skin that were linked with inflammatory response (GO: 0006954) and with positive regulation of cytokine production (GO: 0001819) were identified and plotted.

Table SI. Differentially expressed genes associated with Henoch–Schönlein purpura (HSP) patients. Log2 fold changes are described in relation to the respective gene expression in normal skin

Gene	Description	Function	Log_2 fold change	*p*-value	Confidence intervals	Related vasculitis disorder	Reference
Upregulated genes							
CCL26	C-C motif chemokine ligand 26	Chemokine for eosinophils & basophils	1.053	2.30E-02	(0.307, 1.80)	Elevated CCL26 in EGPA patients	3
CFP	Complement factor properdin	Positive regulator of alternative complement pathway	0.705	3.01E-02	(0.130, 1.28)	Elevated CFP levels in AAV patients in remission	7
LGALS3	Galectin-3	Galactose-specific lectin which binds IgE	0.639	1.88E-04	(0.367, 0.911)	Elevated serum galectin-3 in Behcet's disease	8
STAT 6	Signal transducer and activator of transcription 6	Transcription factor, activates IL-3 & IL-4 pathway	0.287	2.45E-03	(0.142, 0.431)	Not known	
Downregulated genes							
ARG2	Arginase 2	Nitric oxide and polyamine metabolism	–0.67	1.64E-02	(–1.12, –0.217)	Not known	
CTLA4_all	Cytotoxic T-lymphocyte associated protein 4	Inhibitory receptor, negatively regulates T cell responses	–1.1	4.31E-02	(–1.94,–0.257)	Polymorphism on CTLA-4 allele linked with AAV	4
CXCR6	C-X-C motif chemokine receptor 6	Receptor for CXCL16, found on T and NKT cells	–1.015	4.85E-02	(–1.82,–0.213)	CXCL16 upregulated in acute HSP patients	9
DPP4	Dipeptidyl peptidase 4	Positive regulator of T cell co-activation	–0.872	3.88E-02	(–1.54, –0.207)	Sitagliptin-associated vasculitis	10
ETS1	ETS proto-oncogene 1, transcription factor	Controls cytokine and chemokine genes	–0.587	2.83E-02	(–1.00, –0.174)	Polymorphism on allele 7 associated with vasculitis in SLE patients	11
GBP1	Guanylate binding protein 1	Induced by interferon, hydrolyses GTP	–1.621	4.81E-02	(–2.88, –0.358)	Part of predictive gene set for MPA treatment	12
IL21R	Interleukin 21 receptor	Receptor for IL21, important for proliferation of T, B and NK cells	–1.018	1.69E-02	(–1.70, –0.338)	Polymorphism associated with Kawasaki disease	13
MALT1	Mucosa associated lymphoid tissue lymphoma translocation gene 1	Enhances BCL10-induced activation of NF-kB	–0.418	7.42E-03	(–0.665, –0.171)	Not known	
PSMB5	Proteasome subunit beta 5	Involved in peptide cleavage	–0.351	1.72E-03	(–0.525, –0.177)	Bortezomib-associated cutaneous vasculitis	14
XBP1	X-Box binding protein 1	Transcription factor that regulates MHC class II genes	-0.353	3.66E-02	(–0.655, –0.050)	Vasculitis reduced in XBP-1 deficient mice	15

EGPA: eosinophilic granulomatosis with polyangiitis; AAV: ANCA-associated vasculitis; SLE: systemic lupus erythematous; MPA: microscopic polyangiitis.