

Supplementary material

1. Transcriptome data

Table S1: Gene regulatory response of HUVECs stimulated with IL27 (10, 30, and 100 ng/mL) and calprotectin (1, 5, and 10 µg/mL) after 12 h. Multi-gene array TaqMan Immune human panel was used, genes with no indicated FC and p-values were not detected. (FC: Fold Change, p-val: p-value based on unpaired t.test).

Genes	IL27						Calp					
	10		30		100		1		5		10	
	FC	p-val	FC	p-val	FC	p-val	FC	p-val	FC	p-val	FC	p-val
18S	1.31	0.14	1.04	0.85	1.16	0.58	0.95	0.70	0.91	0.50	1.05	0.74
ACE	1.11	0.29	1.00	0.97	0.80	0.06	1.15	0.03 *	1.14	0.04 *	1.10	0.00 **
ACTB												
AGTR1												
AGTR2												
BAX	0.98	0.80	0.93	0.24	1.02	0.71	0.83	0.05	0.93	0.36	0.90	0.12
BCL2	0.79	0.48	0.95	0.88	0.77	0.46	1.02	0.71	1.04	0.63	1.13	0.03 *
BCL2L1	0.90	0.17	1.04	0.65	1.17	0.03 *	0.94	0.17	1.11	0.15	1.30	0.00 **
C3	1.68	0.02 *	1.71	0.02 *	1.17	0.50	0.95	0.71	1.15	0.35	0.84	0.32
CCL19												
CCL2	1.17	0.03 *	1.41	0.02 *	1.29	0.11	1.04	0.35	0.90	0.05 *	0.97	0.57
CCL3												
CCL5	1.03	0.87	1.23	0.23	1.18	0.41	4.83	0.02 *	0.83	0.44	1.52	0.52
CCR2												
CCR4	0.84	0.67	0.80	0.70	0.82	0.69	1.14	0.46	1.11	0.60	1.13	0.43
CCR5												
CCR7												
CD19												
CD28	0.94	0.86	1.52	0.34	0.62	0.29	0.90	0.84	1.14	0.70	1.11	0.73
CD34	0.99	0.90	0.85	0.03 *	0.81	0.02 *	0.99	0.85	0.96	0.60	0.97	0.41
CD38	2.17	0.08	2.52	0.00 ***	2.29	0.08	1.20	0.07	1.09	0.50	1.09	0.33
CD3E												
CD40	1.18	0.21	1.10	0.57	1.12	0.43	1.60	0.44	1.77	0.47	1.16	0.79
CD40LG												
CD4	0.82	0.47	0.95	0.89	0.91	0.80						
CD68	0.87	0.20	0.81	0.18	0.79	0.10	0.91	0.13	0.87	0.03 *	1.00	0.97
CD80												
CD86												
CD8A												

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Genes	IL27						Calp							
	10		30		100		1		5		10			
	FC	p-val		FC	p-val		FC	p-val		FC	p-val		FC	p-val
COL4A5	1.11	0.38		1.05	0.68		1.00	0.98		1.11	0.25		1.02	0.80
CSF1	1.55	0.01	**	1.78	0.00	**	1.64	0.04	*	1.00	0.93		0.98	0.37
CSF2	0.96	0.93		1.07	0.89		2.21	0.05	*	1.10	0.74		0.82	0.44
CSF3	0.64	0.27		2.26	0.08		0.91	0.84		1.13	0.48		1.35	0.23
CTLA4														
CXCL10	51.26	0.00	***	140.13	0.00	***	146.94	0.00	***	1.08	0.52		0.98	0.90
CXCL11	40.94	0.00	***	77.88	0.00	***	85.51	0.00	***	1.09	0.22		0.97	0.73
CXCR3														
CYP1A2														
CYP7A1	0.95	0.83		0.51	0.28		0.93	0.93		0.52	0.01	**	0.64	0.38
ECE1	0.91	0.51		0.98	0.89		1.00	0.99		1.12	0.22		0.97	0.67
EDN1	0.98	0.80		0.90	0.22		0.99	0.92		0.93	0.32		0.88	0.07
FAS	1.59	0.03	*	1.68	0.02	*	1.54	0.03	*	1.05	0.35		1.06	0.39
FASLG														
FN1	1.07	0.27		1.01	0.92		0.95	0.41		1.02	0.78		1.01	0.94
GAPDH														
GNLY														
GUSB														
GZMB														
HLA														
HLA														
HMOX1	0.89	0.42		0.89	0.43		1.03	0.85		0.98	0.78		0.95	0.57
ICAM1	1.28	0.03	*	1.65	0.03	*	2.08	0.00	***	1.05	0.51		0.94	0.48
ICOS														
IFNG														
IKBKB	0.86	0.26		0.89	0.34		0.70	0.02	*	1.00	0.93		0.88	0.05
IL10														
IL12A	1.98	0.00	***	1.92	0.05	*	1.95	0.04	*	1.14	0.21		0.84	0.12

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Genes	IL27						Calp									
	10		***	30		**	100		**	1		**	5		**	10
FC	p-val	FC		p-val	FC		p-val	FC		p-val	FC		p-val	FC		p-val
IL12B																
IL13																
IL15	6.53	0.00	***	6.22	0.00	**	5.75	0.00	**	0.87	0.48		0.80	0.23	0.83	0.43
IL17																
IL18	1.45	0.32		0.88	0.71		0.84	0.65		0.97	0.91		1.19	0.47	1.02	0.94
IL1A	0.83	0.37		0.84	0.53		0.79	0.27		1.09	0.38		0.92	0.55	1.01	0.96
IL1B	0.92	0.58		1.47	0.23		2.26	0.01	**	0.90	0.46		0.83	0.20	1.06	0.52
IL2																
IL2RA																
IL3																
IL4																
IL5																
IL6	1.53	0.08		2.23	0.01	**	2.19	0.02	*	1.13	0.46		1.00	1.00	1.12	0.36
IL7	7.42	0.00	***	6.80	0.00	***	4.40	0.03	*	2.19	0.01	**	1.42	0.37	1.41	0.29
IL8	0.73	0.27		0.76	0.44		0.92	0.72		1.06	0.64		0.90	0.48	1.06	0.64
IL9																
LRP2																
LTA	1.20	0.37		1.09	0.58		0.85	0.02	*	1.13	0.29		0.66	0.07	0.88	0.59
MYH6																
NFKB2	0.72	0.02	*	0.85	0.28		0.75	0.03	*	0.95	0.48		0.89	0.16	1.02	0.79
NOS2A	0.67	0.07		1.37	0.11		1.16	0.36		1.03	0.94		0.80	0.21	1.52	0.05
PGK1	0.92	0.56		1.00	0.98		0.94	0.69		0.84	0.03	*	0.89	0.20	0.78	0.01
PRF1																
PTGS2	1.13	0.64		0.99	0.97		1.26	0.48		1.07	0.01	*	0.92	0.01	**	1.14
PTPRC															0.00	**
REN																
RPL3L																
SELE	0.53	0.22		0.89	0.79		0.62	0.38		1.10	0.31		0.78	0.04	*	0.80

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Genes	IL27						Calp					
	10		30		100		1		5		10	
	FC	p-val	FC	p-val	FC	p-val	FC	p-val	FC	p-val	FC	p-val
SELP	1.10	0.12	0.99	0.81	1.02	0.64	1.26	0.01 **	1.17	0.02 *	1.29	0.00 **
SKI	1.04	0.56	1.00	0.98	0.96	0.62	1.12	0.22	1.03	0.78	1.10	0.27
SMAD3	0.93	0.70	1.10	0.64	0.97	0.89	1.08	0.23	1.09	0.39	1.25	0.01 **
SMAD7	1.09	0.51	0.98	0.85	0.98	0.86	1.01	0.79	1.05	0.44	0.99	0.76
STAT3	1.16	0.22	1.19	0.16	1.13	0.45	0.96	0.23	0.89	0.03 *	1.00	0.97
TBX21												
TFRC	1.04	0.69	1.03	0.78	0.94	0.55	1.05	0.34	0.99	0.90	0.93	0.19
TCFB1	1.08	0.14	1.02	0.58	1.05	0.41	0.91	0.37	0.86	0.05	0.97	0.58
TNF												
TNFRSF18	0.63	0.55	2.82	0.29	6.55	0.10	0.60	0.41	1.49	0.74	0.63	0.48
VEGF	0.95	0.71	0.89	0.39	0.91	0.60	0.96	0.64	0.89	0.11	0.94	0.34

2. Analysis and normalization of proteome data

Assessment of protein abundance densities was performed to compare the individual replicates of each stimulatory condition and time point (Figure S1). Some replicates showed differences in protein abundance densities compared to the majority of overlapping protein abundance densities (Figure S1 A-C). Therefore, a further comparison by calculation of the peak sums for every sample was performed. Figure S2 shows the peak sums of protein abundances for every replicate per condition and time point. A median of all peak sums per time point was calculated and is shown as black lines. Application of a threshold standard deviation (sd) of 1.25 caused the elimination of replicates representing a different protein abundance density compared to the majority. Using this approach, we excluded 2 replicates for the 6 h time point, 6 replicates for the 12 h time point, and 7 replicates for the 24 time point. Analysis of the data distribution revealed to be variable and asymmetric by representing the sd over mean of protein abundances per time. Therefore, a variance stabilization and normalization (VSN) was applied and sd over mean of transformed protein abundances remained stable (Figure S3).

Significance was calculated based on Storey's positive false discovery rate also known as q.value. A comparative study between q.value and p-value revealed that a p-value ≤ 0.05 result in a q.value of 0.175 (Figure S4).

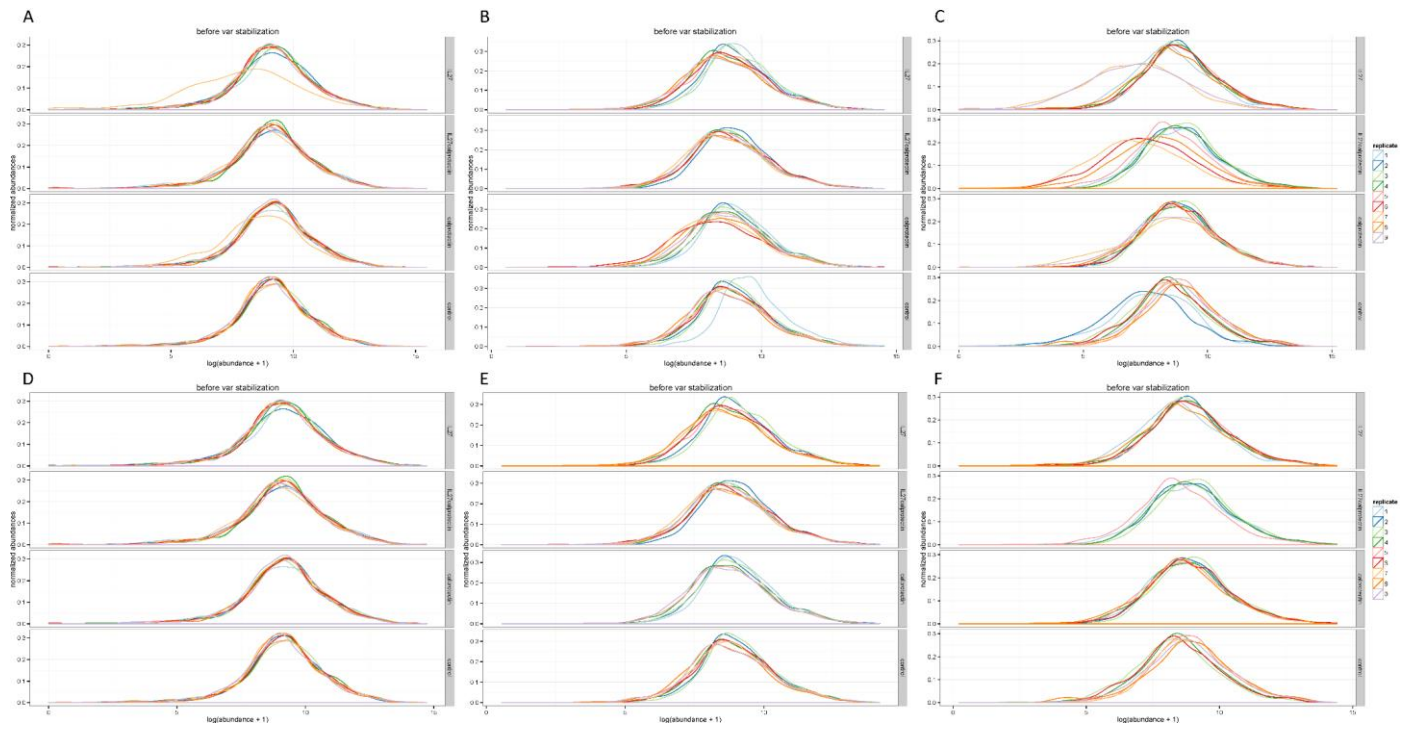


Figure S1: Protein abundances presented as densities of time points 6, 12, and 24 h. A, B, and C: before elimination of replicates at 6, 12, and 24 h respectively. D, E, and F: after elimination of replicates.

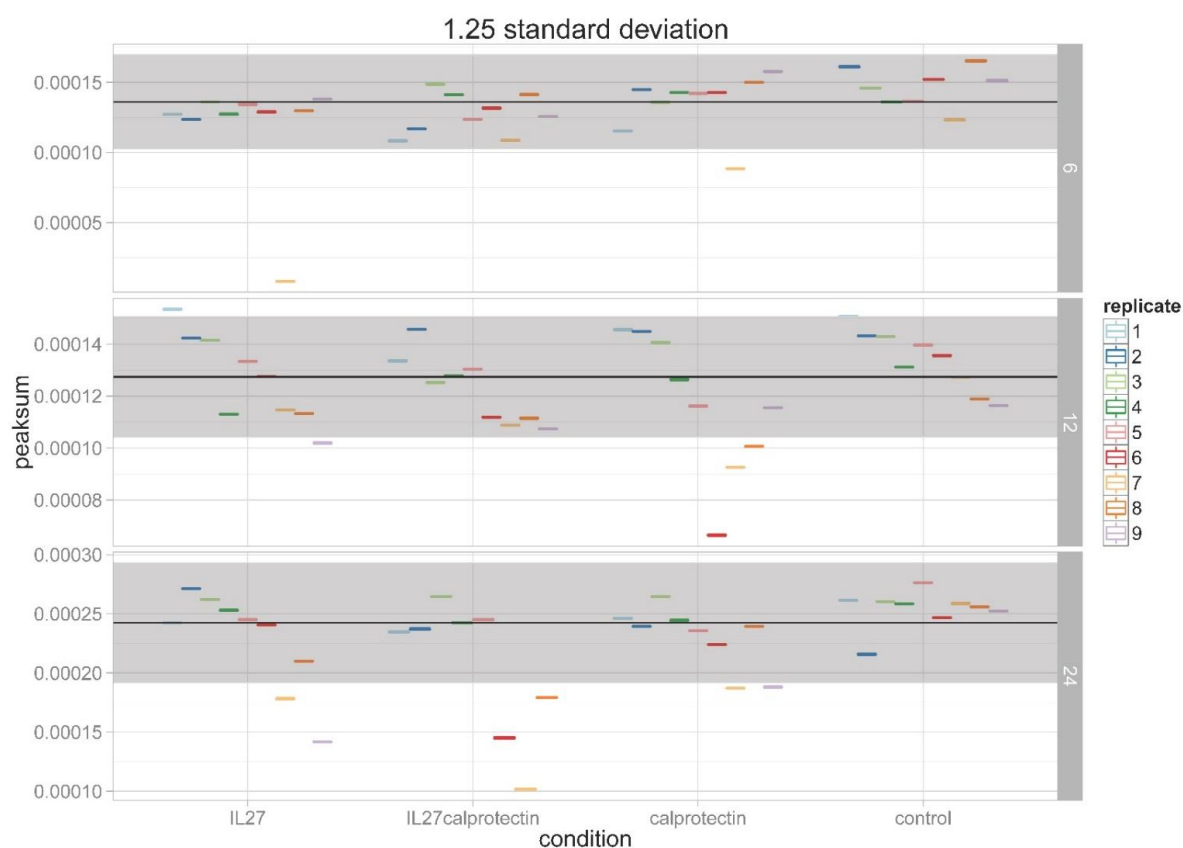


Figure S2: Elimination of replicates based on peak sums of protein abundances and application of a threshold standard deviation (sd) of 1.25 (indicated in grey). Time points 6, 12, and 24 h are presented separately and the black lines indicate the median of all peak sums for each time point. Peak sum per replicates is represented by a colour code.

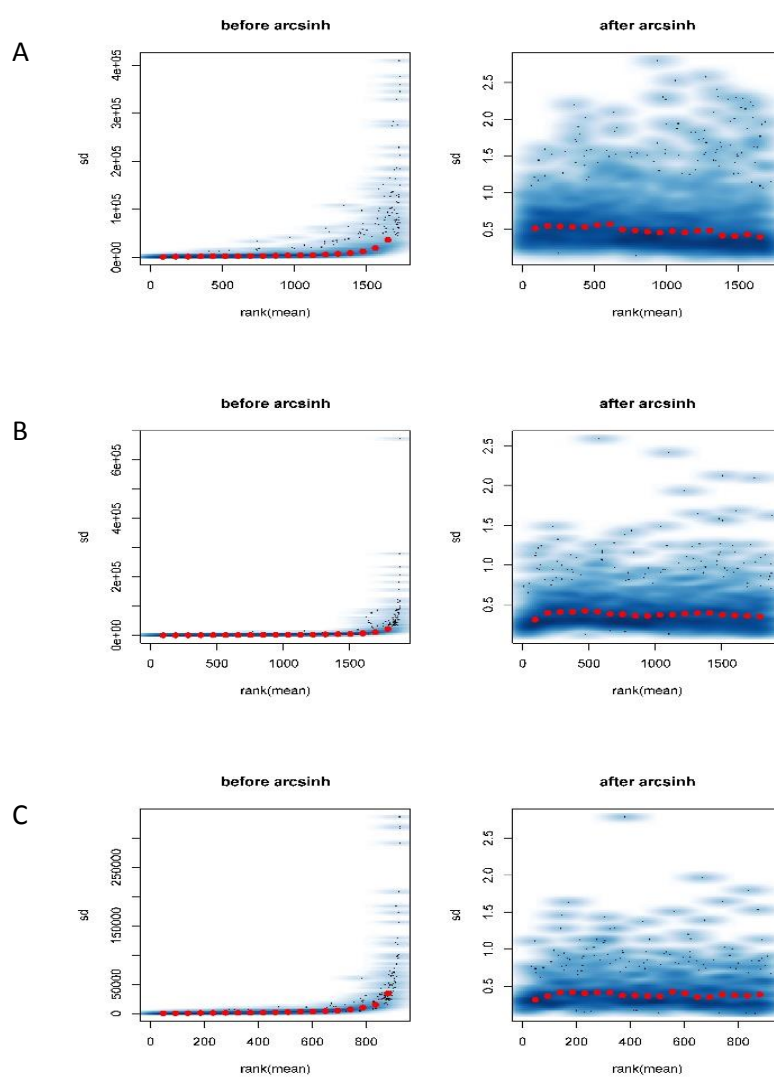


Figure S3: Standard deviation over mean before and after applying arcsinh function (variance stabilization and normalization (VSN)) of 6, 12, and 24 h time points.

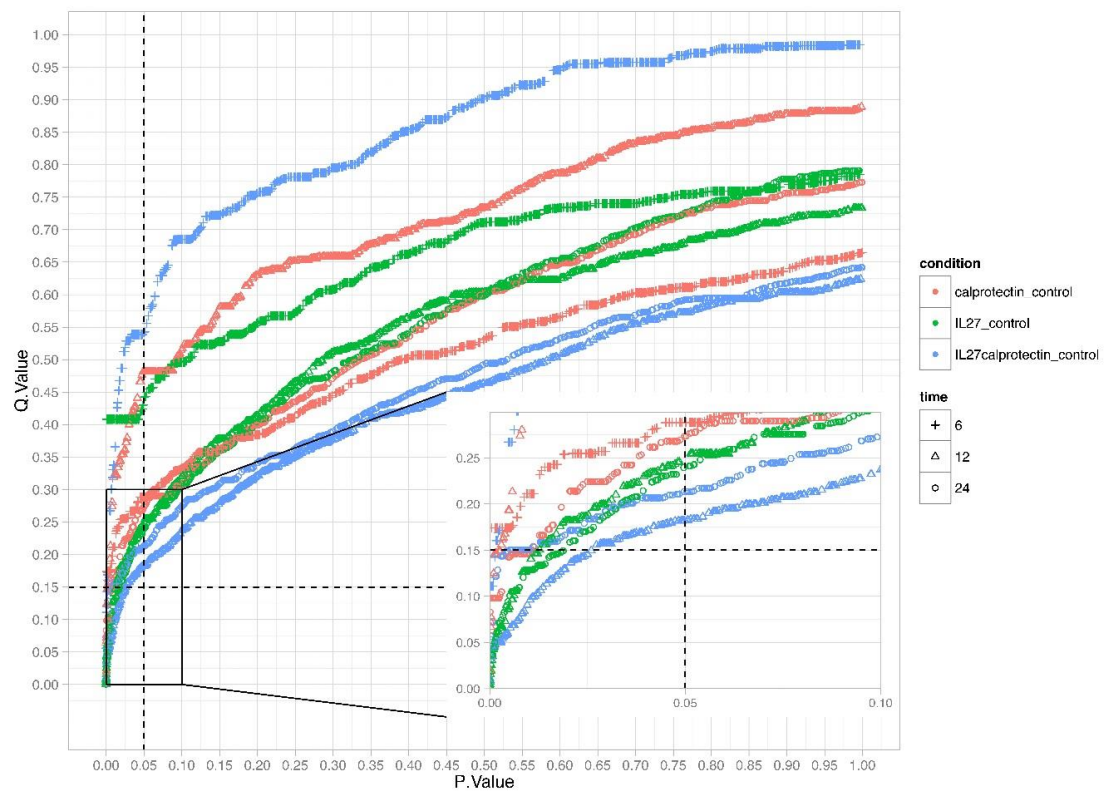


Figure S4: Comparison of p-values and q-values to identify a significance threshold for the q-values. A q.value of 0.15 was chosen as significance threshold.

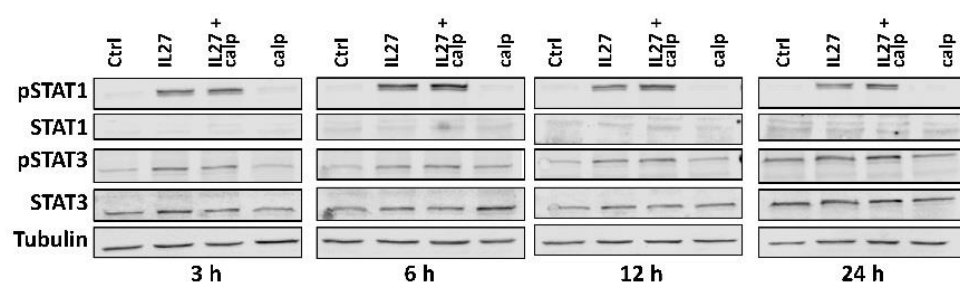


Figure S5: Western blot of LI-COR detected pSTAT1, STAT1, pSTAT3, STAT3, and tubulin in HUVECs stimulated with IL27 (30 ng/mL) \pm calprotectin (1 μ g/mL) for 3, 6, 12, and 24 h.

Table S2: Core analysis performed by IPA showing the top canonical pathways after stimulation with IL27 (30 ng/mL) \pm calprotectin (1 μ g/mL) for each stimulus vs Ctrl and also the comparison between IL27 and IL27 + calprotectin. Abbreviation: not a number (NAN).

IL27			
Name	p-value	z-score	Proteins
Signalling by Rho Family GTPases	$6.30 \cdot 10^{-7}$	0	12
Remodelling of Epithelial Adherens Junction	$1.44 \cdot 10^{-6}$	0	7
Protein Ubiquitination Pathway	$1.68 \cdot 10^{-6}$	NAN	12
RhoGDI Signalling	$2.40 \cdot 10^{-6}$	0.707	10
Actin Cytoskeleton Signalling	$1.54 \cdot 10^{-6}$	NAN	10
IL27+calprotectin			
Name	p-value	z-score	Proteins
Protein Ubiquitination Pathway	$2.77 \cdot 10^{-7}$	NAN	11
EIF2 Signalling	$7.29 \cdot 10^{-4}$	NAN	6
mTOR Signalling	$8.58 \cdot 10^{-4}$	NAN	6
Antigen Presentation Pathway	$1.21 \cdot 10^{-3}$	NAN	3
Regulation of eIF4 and p70S6K Signalling	$1.68 \cdot 10^{-3}$	NAN	5
calprotectin			
Name	p-value	z-score	Proteins
EIF2 Signalling	$1.30 \cdot 10^{-10}$	-2	11
mTOR Signalling	$5.19 \cdot 10^{-8}$	NAN	9
Regulation of EIF4 and p70S6K Signalling	$1.02 \cdot 10^{-7}$	NAN	8
Germ Cell-Sertoli Cell Junction Signalling	$3.90 \cdot 10^{-4}$	NAN	5
14-3-3-mediated Signalling	$1.09 \cdot 10^{-3}$	NAN	4
IL27 vs IL27+calprotectin			
Name	p-value	z-score	Proteins
EIF2 Signalling	$9.26 \cdot 10^{-17}$	1.155	25
Regulation of EIF4 and p70S6K Signalling	$1.20 \cdot 10^{-11}$	1.342	18
Protein Ubiquitination Pathway	$2.53 \cdot 10^{-9}$	NAN	20
mTOR Signalling	$6.16 \cdot 10^{-9}$	2.449	17
Remodelling of Epithelial Adherens Junction	$7.70 \cdot 10^{-7}$	NAN	9