Supplemental material:

STAT3/5 dependent IL-9 overexpression contributes to neoplastic cell survival in lesional skin of mycosis fungoides patients

Supplementary table 1: Primer list

IL-9
Forward 5'-CTCTGTTTGGGCATTCCCTCT-3',
Reverse 5'-GGGTATCTTGTTTGCATGGTGG-3’
IRF4
Forward 5'-GCGGTGCGCTTTGAACAAG-3',
Reverse 5'-ACACTTTGTACGGGTCTGAGA-3’
STAT3
Forward 5'-CAGCAGCTTGACACACGGTA-3',
Reverse 5'-GCCCAATCTTGACTCTCAATCC-3’
STAT5a
Forward 5'-TTACTGAAGATCAAGCTGGGG-3',
Reverse 5’-TCATTGTACAGAATGTGCCGG-3’
STAT5b
Forward 5’-GAACACCCGCAATGATTACAGT-3',
Reverse 5’-ACGGTCTGACCTCTTAATTCGT-3’
GAPDH
Forward 5'- ACCCACTCCTCCACCTTTGAC-3',
Reverse 5'- TGTTGCTGTAGCCAAATTCGTT-3’

Supplementary table 2: Set of samples from archived materials

Patient No.	Sex	Age (y) baseline biopsy	Diagnosis (EORTC-ISCL classification)	Type of skin lesion biopsied	**IHC** CD3/IL-9 (0-4)	**IHC** STAT3 (0-4)	**IHC** CD3/IRF4 (0-4)	**IHC** CD3/PU1 (0-4)	Type of treatment	Length of treatment (weeks)	Response
A1	M	95	Ib	Patch	DP, 2	3	DP 1	SP 3,2	311nm UVB	12	CR
A2	M	75	Ib	Patch	DP, 1	3	0	SP 3,2	PUVA, Bexarotene	18	CR
A3	M	60	Ib	Patch	DP, 2	3	DP, 2	SP 4,4	311nm UVB	2	CR
A4	M	47	Ib	Patch	DP, 3	2	DP, 2	SP 4,3	PUVA	48	CR
A5	M	90	III	Tumor	0	2	DP, 1	SP 4,2	PUVA, steroids	6	CR
A6	M	71	Ia	Patch	0	1	0	0	PUVA	45	CR
A7	M	69	Ia	Patch	DP, 1	3	DP, 1	SP 3,2	311nm UVB	6	CR
A8	F	59	Ia	Patch	DP, 1	1	0	SP 1,0	PUVA	6	CR

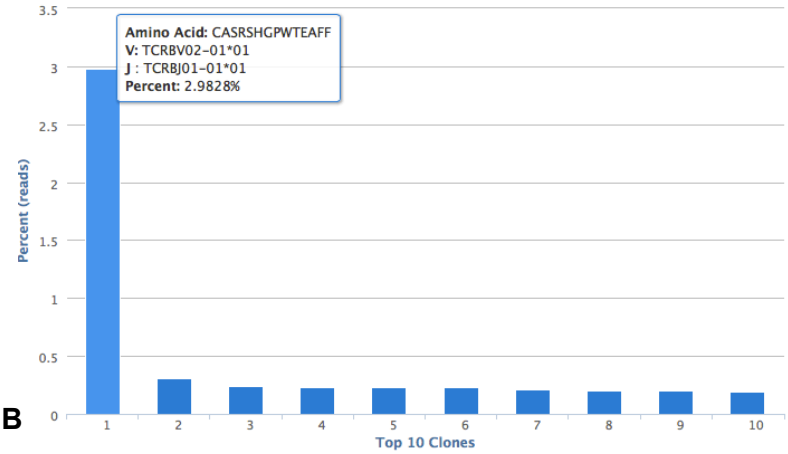
Patients were treated under daily life conditions with standard 311nm UVB phototherapy or photochemotherapy with 8- or 5-methoxypsoralen. Additional treatment with bexarotene or steroids was given as depicted. Single or double stains immunohistochemistry (IHC) were perfomed as indicated. The preparations were evaluated as containing single positive (SP) or double positive (DP) cells (mainly in the dermis) at baseline by a 0-4 score system of positivity with **0,** <10%; **1**, 10-25%; **2**, 25-50%; **3**, 50-75%; and **4**, 75-100%. No clinical signs of skin lesions and lack of infiltrate in histological examination was considered as complete response (CR).

Supplementary table 3: Set of samples from clinical PUVA trial (NCT0168659)

Patient No.	Sex	Age (y) baseline biopsy	Diagnosis (EORTC-ISCL classification)	Type of skin lesion biopsied	Type of treatment	Length of treatment (weeks)	Response
CS1	M	77	Ia	Patch	PUVA	6	PR
CS2	M	75	Ib	Patch	PUVA	6	PR
CS3	M	63	Ia	Patch	PUVA	6	PR
CS4	M	35	Ia	Patch	PUVA	6	PR
CS5	M	71	Ib	Patch	PUVA	6	PR
CS6	M	31	Ib	Patch	PUVA	6	PR
CS7	M	59	Ia	Patch	PUVA	6	PR
CS8	F	61	Ia	Patch	PUVA	6	PR

Patients from the clinical PUVA trial (ClinicalTrials.gov no. NCT01686594) were treated with oral 8-MOP (10mg per 20kg of body weight) and UVA (PUVA) two times per week. The patients were started on treatment with 50% of the minimal phototoxic dose (MPD) and UVA dose was increased thereafter by 0-30% per week, depending on the individual preceding erythema response. A decrease of more than 50% in the mSWAT was considered as partial response (PR). IL-9, STAT3, STAT5a/b and IRF4 were determined by qPCR, as shown in respective figures.

A

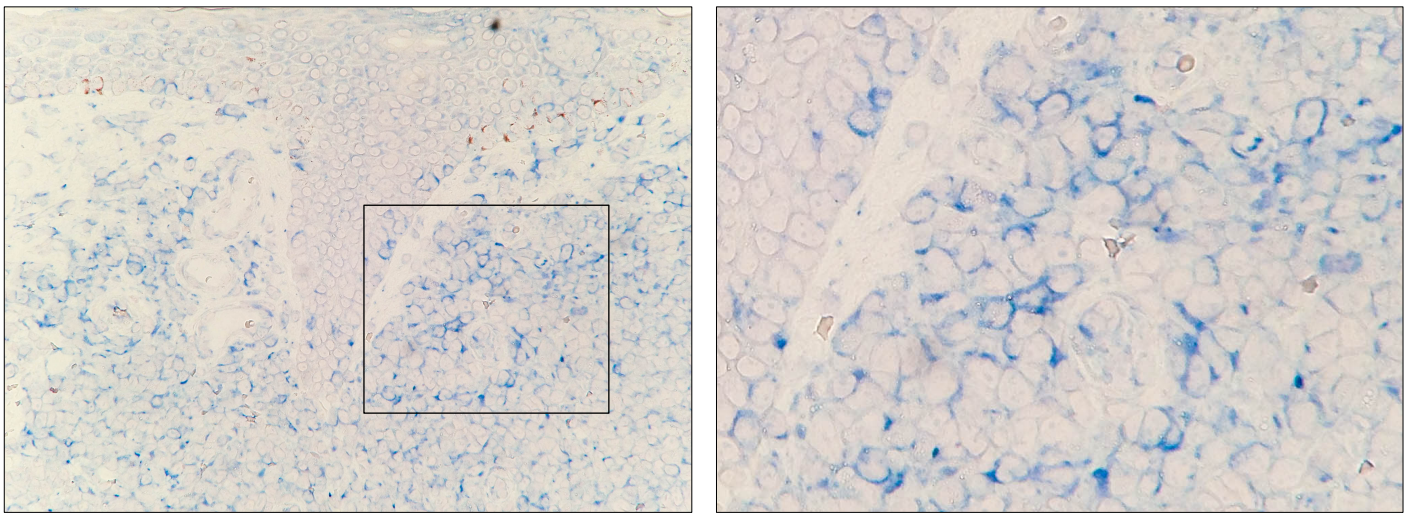


TCRβV02-01 (Vb22): 25,933 reads

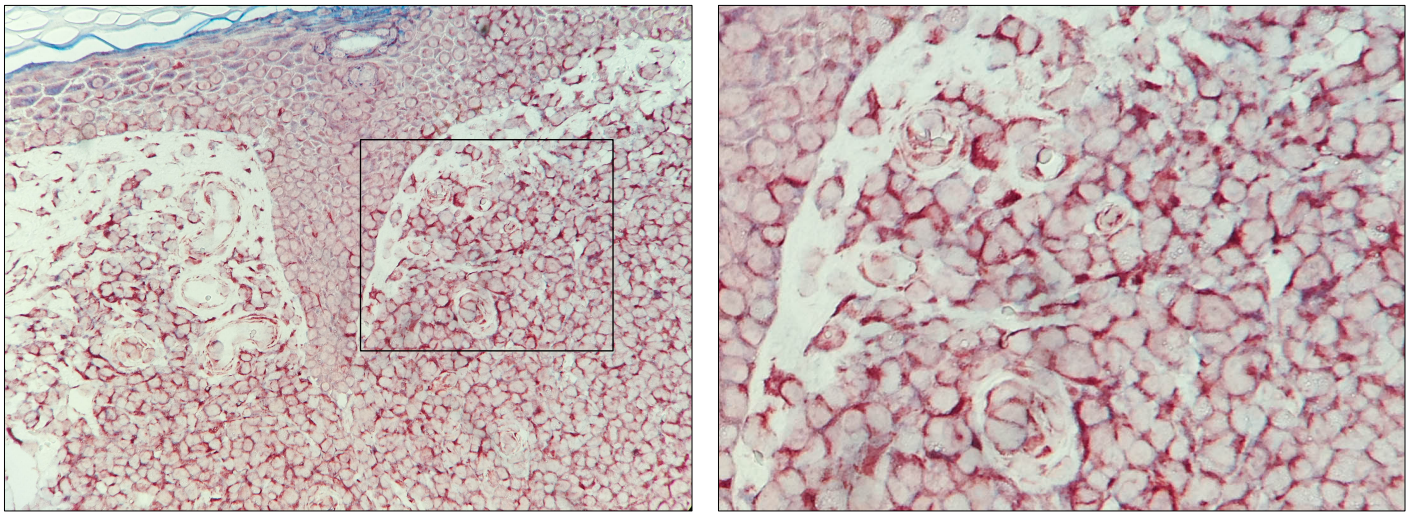
TCRβV02-01*01
TCRβJ01-01*01
CASRHGPWTEAFF clone: 9,804 reads

Estimated clone freq: 37.80%

B

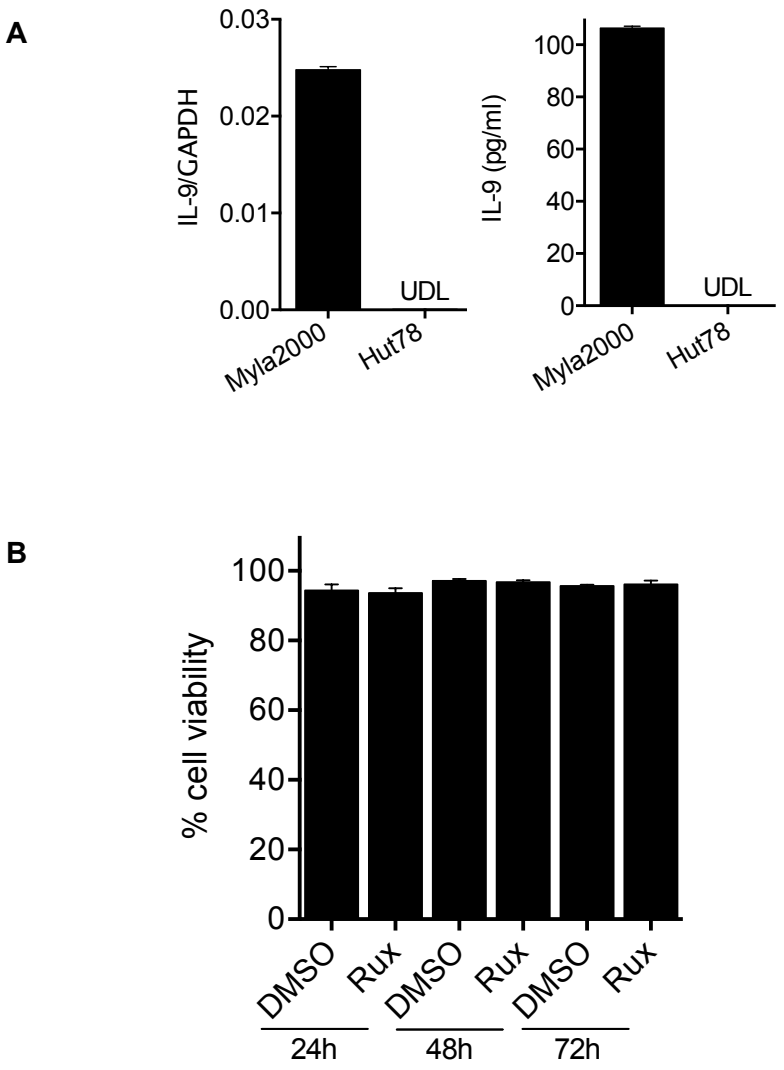


Vb22 / Isotype

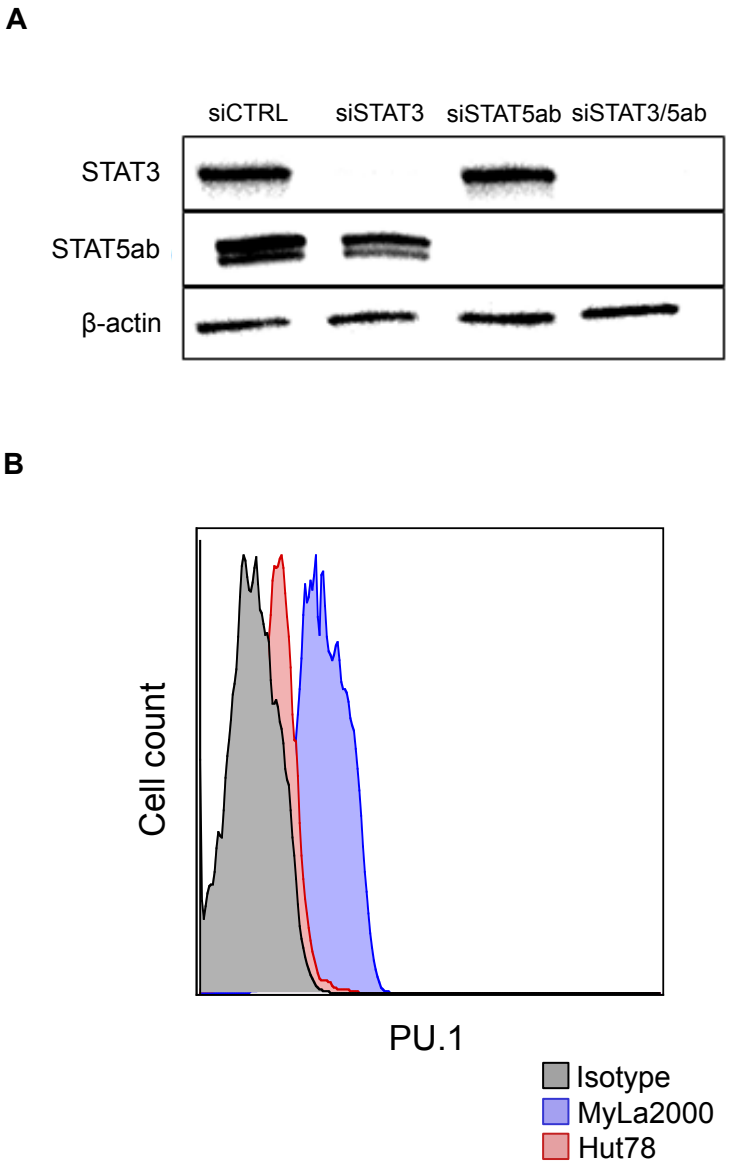


Vb3 / IL-9

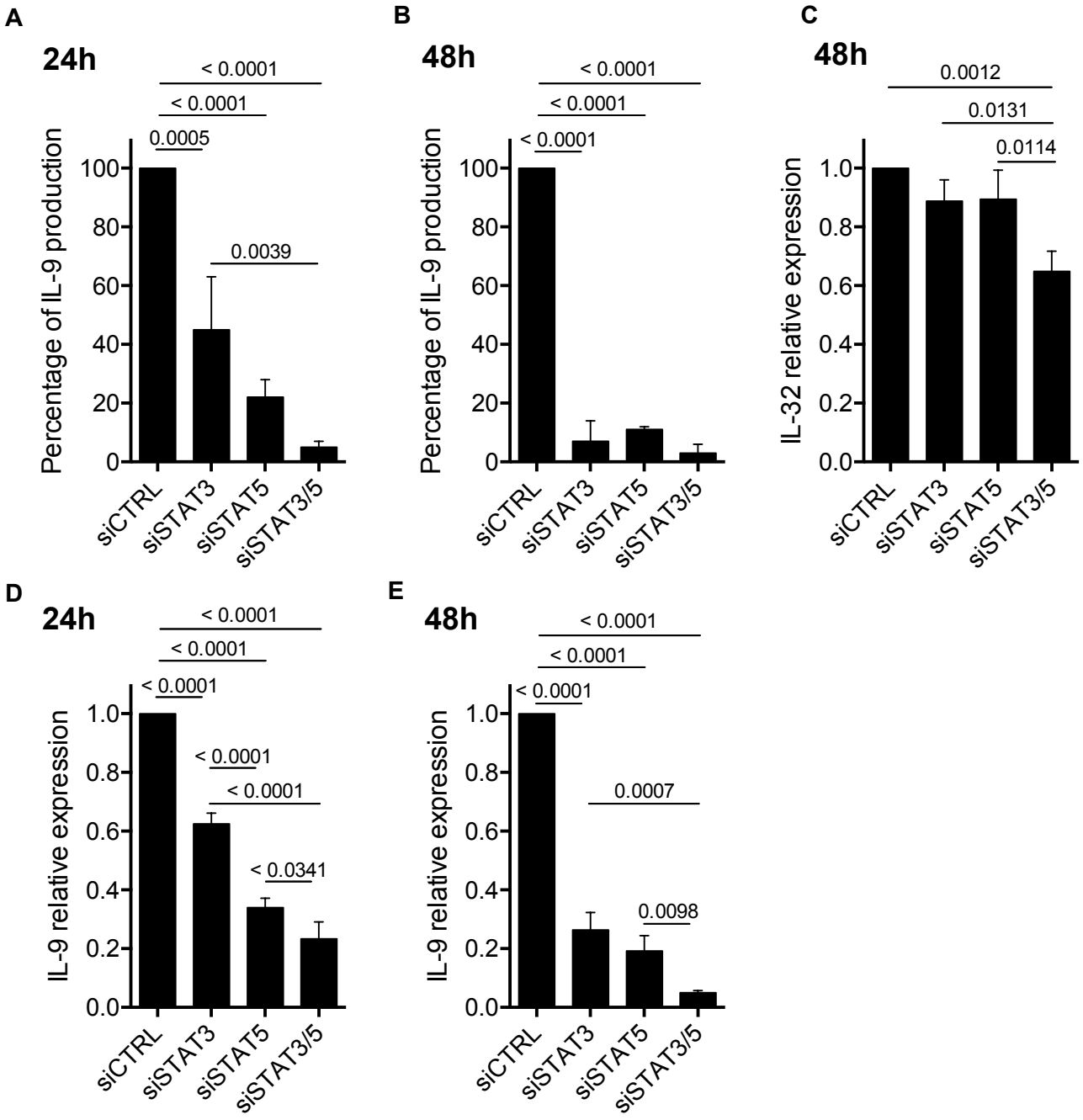
Supplementary Figure 1. A) Analysis of the top 10 Functional TCR sequences in a biopsy sample of a MF patient; the amino acid sequence of the CDR3 is shown (CASRHGPWTEAFF TCRBV02-01*01, TCRBJ01-01*01); frequency of this clone was 3.5%. B) Immunohistochemical double stain; Vb22 (blue) and mouse isotype control (red) (upper panel) and TCR specificity control stain: Vb3/IL-9 (blue/red).



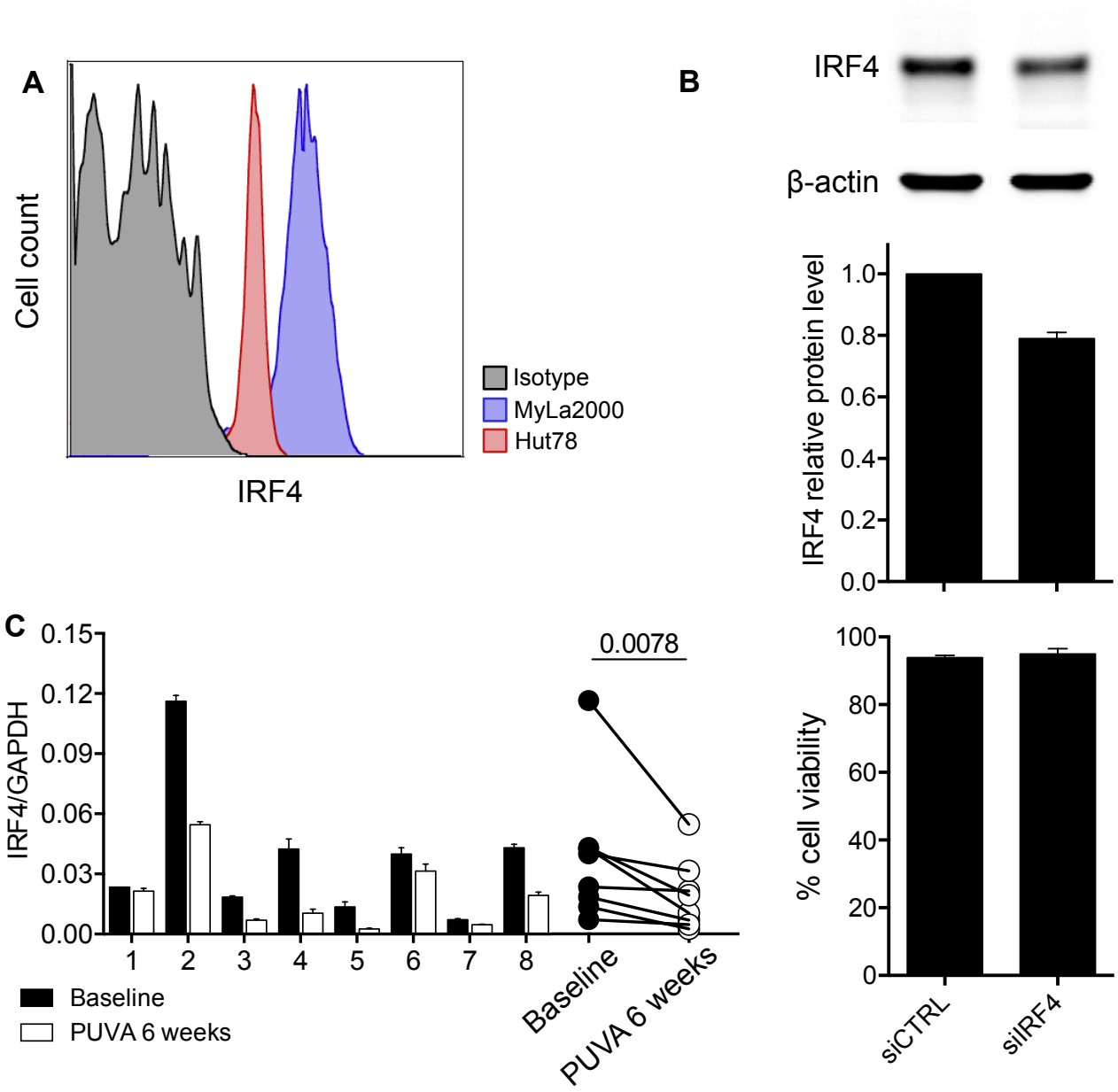
Supplementary Figure 2. (A) Expression of IL-9 in MyLa2000 cells and Hut78 quantified by qPCR and ELISA. Values under detection limit (UDL) results are indicated. (B) MyLa2000 cells treated with ruxolitinib (180nM) or DMSO as control. Viability was assessed by exclusion of propidium iodide at indicated time points. Experiments were done in triplicates.



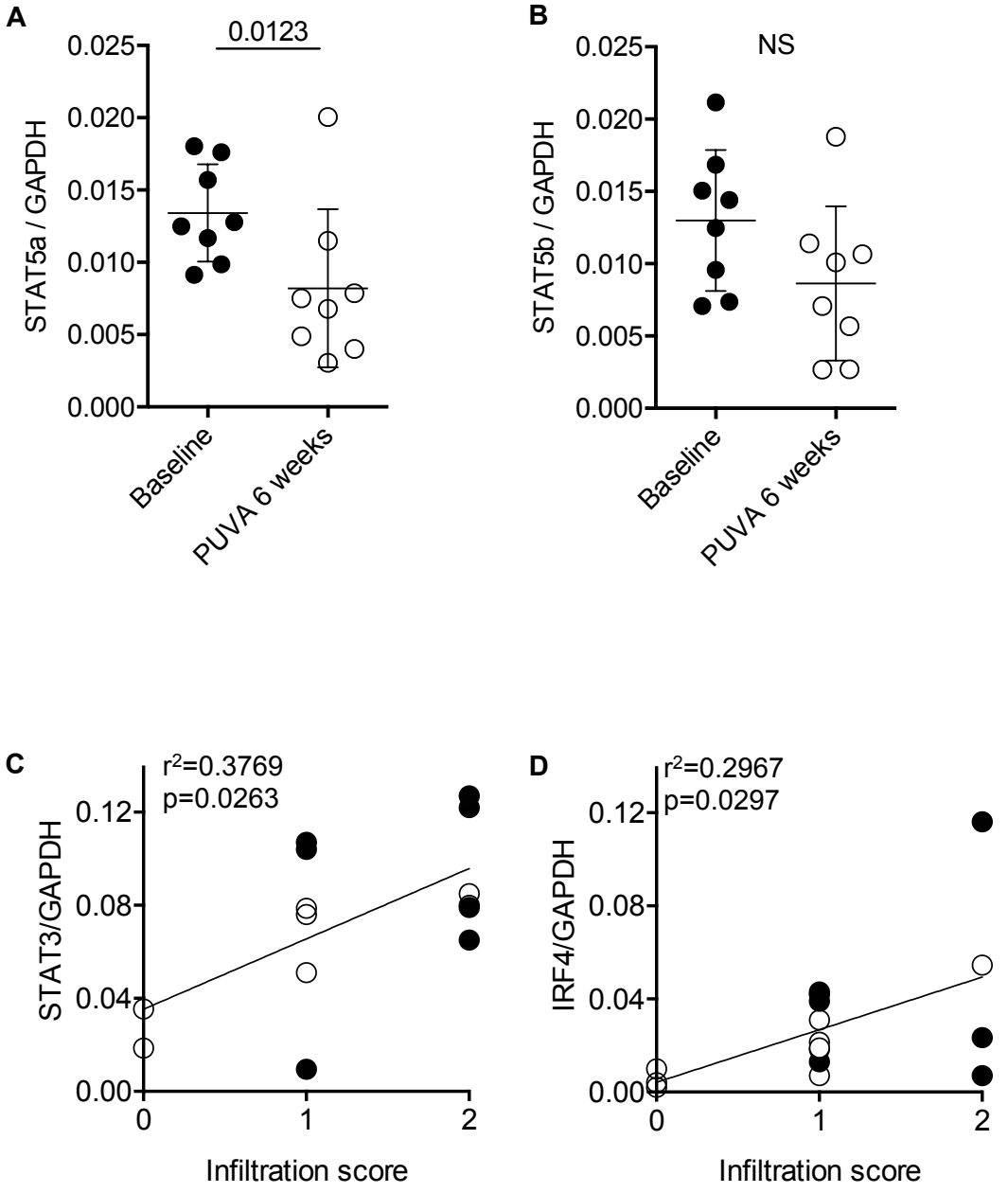
Supplementary Figure 3. (A) Representative western blot of MyLa2000 cells after 48 hours of transfection with siRNA targeting STAT3, STAT5ab or STAT3 and STAT5ab together. Similar results were obtained at 24h (data not shown). (B) Intracellular stain of PU.1 on MyLa2000 and Hut78 cells by flow cytometry.



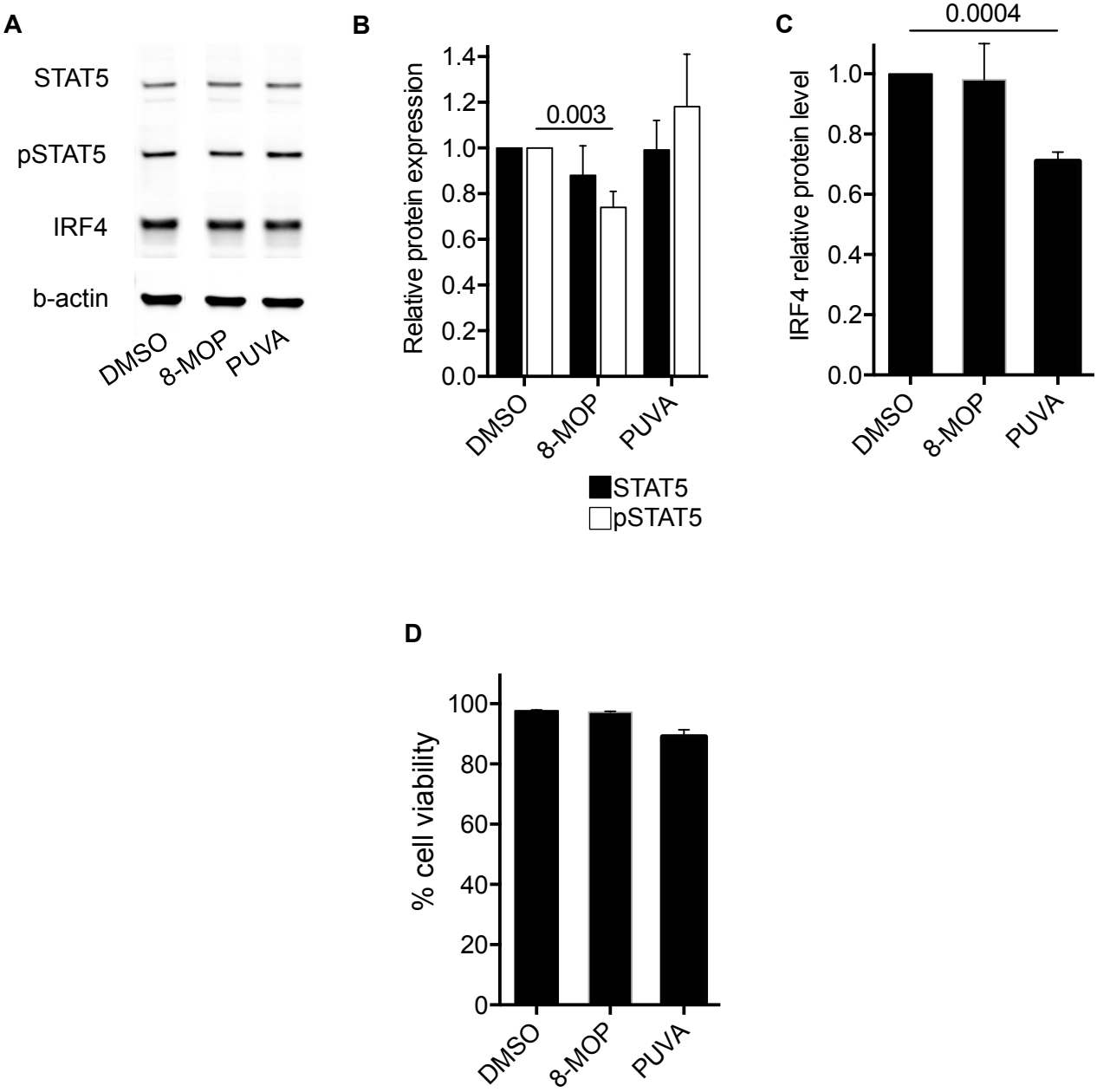
Supplementary Figure 4. (A) PB2B cells were transfected with siRNA targeting STAT3 and STAT5 separately or together. IL-9 expression was quantified by ELISA (A,B) and qPCR (D,E) 24 and 48 hours after transfection. Unspecific siRNA treated cells were used as controls. (C) IL-32 expression was quantified by qPCR at 48h of transfection as control to examine the specificity of the silencing effect on IL-9 expression. Experiments were done in triplicates; statistical significance was assessed by Dunnet post test; p values are shown.



Supplementary Figure 5. (A) Expression of IRF4 in MyLa2000 cells and Hut78 determined by flow cytometry. (B) Representative western blot of MyLa2000 cells after 96 hours of transfection with siRNA targeting IRF4. A 20% reduction of IRF4 expression was observed (data from two independent experiments were analyzed). Viability was assessed by exclusion of propidium iodide. (C) Expression of IRF4 quantified by qPCR on biopsies from the clinical PUVA trial (ST3). Data from baseline and 6 weeks after treatment are presented. Statistical significance was assessed by Student paired t test



Supplementary Figure 6. Biopsies from the clinical PUVA trial (ST3) were analyzed for expression of STAT5a (A) and STAT5b (B) by qPCR. Data from baseline and 6 weeks after treatment are presented. Statistical significance was assessed by Student paired t test. Correlation of infiltrate score with expression of STAT3 (C) or IRF4 (D) at baseline (filled circles) and 6 weeks of PUVA (empty circles), as assessed by qPCR. Infiltration scoring of the skin was performed by one of the investigators (L. C.) on a scale from 0 to 2; with 0, as no infiltrate; 1, light infiltrate; and 2, moderate to dense infiltrate. Data from eight patients is presented (circles of two patients overlap). Linear regression analyses were performed, statistical values are given.



Supplementary Figure 7. (A) Western blot of MyLa2000 cells after 48 hours of PUVA treatment (1μM 8-MOP, 0.8J/cm^2), 8-MOP or DMSO control. Relative protein quantification of STAT5, pSTAT5 (B) and IRF4 (C). Experiments were done in duplicates. Viability was assessed by exclusion of propidium iodide in three independent experiments (D). Statistical significance was assessed by unpaired t-test; p values are shown.