Table S1. Genes differentially expressed in once- versus four-time stimulated Th1 cells

Affymetr_No	Gene symbol	Gene name	Fold change	*t* test P value
97994_at	Tcf7	Transcription factor 7, T cell specific	−50.7	6.47485E-09
104578_f_at	3110023F10Rik	RIKEN cDNA 3110023F10 gene	−11.9	7.10526E-09
102282_g_at	Tnfrsf7	TNF receptor superfamily, member 7	−7.2	3.15546E-05
160667_at	Evl	Ena-vasodilator stimulated phosphoprotein	−6.6	3.20332E-07
96672_at	2300002F06Rik	RIKEN cDNA 2300002F06 gene	3.4	0.000107159
95333_at	Il18rap	IL-18 receptor accessory protein	3.4	9.00834E-06
102914_s_at	Bcl2a1b	B cell leukemia/lymphoma 2 related protein A1b	3.5	2.70088E-07
97885_at	1810009M01Rik	RIKEN cDNA 1810009M01 gene	4.2	1.42213E-07
97949_at	Fgl2	fibrinogen-like protein 2	4.4	9.86764E-07
96605_at	0610011I04Rik	RIKEN cDNA 0610011I04 gene	4.7	9.8031E-07
99370_at	Klrc1	Killer cell lectin-like receptor subfamily C, member 1	7.5	4.1522E-07
103024_at	Adam8	A disintegrin and metalloprotease domain 8	9.9	7.20938E-06
99051_at	S100A4	S100 calcium binding protein A4	10.5	1.27979E-06
97519_at	Spp1	Secreted phosphoprotein 1	12.9	1.84635E-06
99957_at	Mmp9	Matrix metalloproteinase 9	19.0	9.75999E-07
98028_at	Twist1	Twist gene homolog, (*Drosophila*)	38.8	7.14961E-05

Naïve DO11.10 cells were activated under Th1-polarizing conditions. The transcriptional profiles of resting 6-d-old Th1 cells and resting 24-d-old Th1 cells were compared using Affymetrix Murine Genome (MG) U74Av2 GeneChip arrays. The Affymetrix probe set ID (Affymetr_No), mean fold change, significance of differential expression (*t* test), and mean signal intensity (Th1 6 d, Th1 24 d) of three arrays per group representing three independent cultures are shown. Genes were filtered according to the following criteria: fold change ≥ 2; difference of means ≥ 200; P value ≤ 0.05.

Table S2. Clinical characteristics of patients in the study

Patient ID	Relative *twist1* mRNA	Sex (male/female)	Age (yr)	Sampling Date (mo/yr)	Disease duration (yr)	ESR	CRP (mg/l)
Endoscopic biopsies from ulcerative colitis (UC) patients							
UC1	414.8	m	48	01/04	3	103	0.4
UC1	195.7			04/04		88	0.3
UC2	47.6	m	30	05/04	9	63	1.7
UC3	32.8	f	38	11/04	8	94	9.13
UC4	27.3	f	33	12/04	6	NA	19.6
UC5	2.7	m	26	12/04	<1	NA	49.8
UC6	83.0	m	42	7/05	6	NA	3.5
UC7	47.4	m	44	8/05	5	NA	14.9
UC8	40.7	m	18	11/05	2	30	10
UC9	10.9	m	67	12/05	15	88	25
Endoscopic biopsies from Crohn's disease patients							
CD1	72.9	m	25	12/04	4	NA	43
CD2	27.9	f	45	2/05	11	NA	NA
CD3	23.8	f	41	3/05	<1	NA	114.4
CD4	31.8	f	22	7/05	3	NA	31.3
CD5	12.7	f	34	8/05	5	NA	53.4
CD6	13.3	f	21	11/05	5	27	10
CD7	27.8	f	39	12/05	10	95	18
Synovial fluid from rheumatoid arthritis patients							
RA1	30.8	m	68	03/04	1	NA	39.3
RA2	12.5	f	40	02/05	13	10	12.3
RA2	10.1			06/05		12	6
RA3	6.0	f	51	04/05	11	70	39
RA4	15.9	m	68	08/05	1	30	14
RA4	25.5			11/05		NA	NA
Synovial fluid from reactive arthritis patients							
ReA1	30.0	m	28	03/04	1	25	35
ReA2	7.4	m	43	06/05	<1	50	116.4
ReA3	9.2	m	31	11/05	1	10	0.3
Synovial fluid of psoriatic arthritis patients							
PsA1	54.9	m	55	03/04	10	9/22	0.3
PsA2	14.2	m	68	02/05	2	39	34
PsA3	32.7	m	50	07/05	19	NA	NA
PsA3	24.1			09/05		NA	NA
Synovial fluid from ankylosing spondylitis patients							
AS1	296.1	m	38	03/04	18	42	49
AS1	171.1			08/04		20	3
AS1	130.1			09/04		NA	NA
AS1	168.3			03/05		30	14.6
AS1	138.6			09/05		20	3.1
AS2	13.6	m	73	07/04	8	65	NA
AS2	9.4			12/04		NA	NA
AS3	2.4	m	20	12/04	5	70	86
AS4	61.3	m	27	02/05	18	NA	NA
AS5	5.5	m	21	10/05	1.5	80	4.5
Not included in Fig. 5 B							
Crohn's disease	15.5	NA	NA	08/04	surgical specimen		
Crohn's disease	45.9	NA	NA	11/05	surgical specimen		
Rheumatoid arthritis	40.8	NA	NA	08/04	NA	NA	NA
Rheumatoid arthritis	14.8	NA	NA	09/04	NA	NA	NA
undifferentiated SpA	37.9	f	37	02/05	1.5	20	2
undiff. SpA	12.8	m	44	09/06	NA	NA	NA
undiff. arthritis	3.0	m	48	8/05	1	18	6
Lyme arthritis	15.4	m	37	02/05	<1	38	7
Lyme arthritis	3.5	m	21	06/05	2	NA	NA
Juvenile RA	42.8	f	20	11/05	6	18	1.3

Twist1 transcripts in $CD3^+CD4^+$ cells purified from patient material were quantified after 3 h of restimulation with PMA/ionomycin. The mean expression of *twist1* mRNA in total peripheral $CD3^+CD4^+$ cells from healthy donors was set to 1. ESR, erythrocyte sedimentation rate (ESR); CRP, C-reactive protein.

Table S3. Genes differentially expressed upon ectopic *twist1* overexpression

Affymetr_No	Gene symbol	Gene name	Fold change	*t* test P value	Vector	*Twist1*
1449216_at	Itgae	integrin, αE, epithelial-associated	5.8	0.003675582	45	257
1432466_a_at	Apoe	apolipoprotein E	3.5	0.000333012	173	425
1419532_at	Il1r2	IL-1 receptor, type II	2.8	1.90721E-13	784	2,192
1415812_at	Gsn	gelsolin	2.4	0.000427877	541	1,282
1427076_at	Mpeg1	macrophage expressed gene 1	2.1	0.027175171	168	377
1456393_at	Pdcd4	programmed cell death 4	2.0	0.000960162	800	1,573
1423089_at	Tmod3	tropomodulin 3	2.0	2.63289E-06	422	875
1426519_at	P4ha1	procollagen-proline, 2-oxoglutarate 4-dioxygenase	1.9	0.030113345	233	445
1415947_at	Creg	cellular repressor of E1A-stimulated genes	1.9	2.00686E-06	832	1,571
1448710_at	Cxcr4	chemokine (C-X-C motif) receptor 4	1.8	0.029381152	237	529
1455976_x_at	Dbi	diazepam binding inhibitor	1.7	0.001908118	1,340	2,276
1450194_a_at	Myb	myeloblastosis oncogene	1.7	0.006922509	221	443
1417302_at	Rcor	RE1-silencing transcription factor co-repressor	1.7	0.013680996	173	384
1428301_at	2610042L04Rik	RIKEN cDNA 2610042L04 gene	1.7	4.57606E-05	394	707
1438390_s_at	Pttg1	pituitary tumor-transforming 1	1.7	0.001249245	625	1,044
1418126_at	Ccl5	chemokine (C-C motif) ligand 5	1.6	0.014298725	937	1,699
1448021_at	ESTmz98f08.r1	ESTmz98f08.r1	1.6	0.015998701	806	1,337
1418744_s_at	Tesc	tescalcin	1.6	6.11991E-05	841	1,507
1424112_at	Igf2r	insulin-like growth factor 2 receptor	1.6	0.010457946	731	1,132
1460419_a_at	Prkcb	protein kinase C, β	1.6	0.006315009	487	815
1419550_a_at	Stk39	serine/threonine kinase 39, STE20/SPS1 homologue	1.6	0.002358995	321	528
1425923_at	Nmyc1	neuroblastoma myc-related oncogene 1	1.6	0.003631455	7,548	11,408
1449109_at	Socs2	suppressor of cytokine signaling 2	1.6	0.001896075	805	1,319
1426397_at	Tgfbr2	TGFβ receptor II	1.6	0.000741228	2,286	3,306
1426970_a_at	Ube1l	ubiquitin-activating enzyme E1-like	1.6	0.030406184	297	511
1422414_a_at	Calm2	calmodulin 2	1.6	1.80435E-08	1,587	2,338
1433504_at	Pygb	brain glycogen phosphorylase	1.5	0.00183523	514	757
1450662_at	Tesk1	testis specific protein kinase 1	1.5	0.000560519	766	1,187
1450446_a_at	Socs1	suppressor of cytokine signaling 1	1.5	0.000193353	936	1,366
1455065_x_at	Gnpda1	glucosamine-6-phosphate deaminase 1	-1.5	0.00169187	1,430	956
1448548_at	Tulp4	tubby like protein 4	-1.5	2.19399E-05	1,144	820
1452026_a_at	Pla2g12a	phospholipase A2, group XIIA	-1.5	0.013979562	1,228	826
1449990_at	Il2	IL-2	-1.5	0.000138665	8,582	5,654
1449273_at	Cyfip2	cytoplasmic FMR1 interacting protein 2	-1.5	0.000719924	1,325	983
1417240_at	Zyx	zyxin	-1.5	0.000445837	876	601
1425787_a_at	Sytl3	synaptotagmin-like 3	-1.5	6.82725E-06	2,239	1,478
1426245_s_at	Mapre2	microtubule-associated protein, RP/EB family, 2	-1.5	0.022816609	1,224	791
1417679_at	Gfi1	growth factor independent 1	-1.5	8.3341E-05	2,506	1,619
1423467_at	Ms4a4b	membrane-spanning 4-domains A, member 4B	-1.5	0.001242527	4,717	3,005
1456226_x_at	Ddr1	discoidin domain receptor family, member 1	-1.6	0.004473965	593	367
1425947_at	Ifng	interferon γ	-1.6	0.018605196	6,646	4,323
1426816_at	Ccdc64	coiled-coil domain containing 64	-1.6	0.000603641	705	438
1422473_at	Pde4b	phosphodiesterase 4B, cAMP specific	-1.6	1.66039E-05	1,133	627
1420965_a_at	Enc1	ectodermal-neural cortex 1	-1.7	0.001533107	1,510	894
1421065_at	Jak2	Janus kinase 2	-1.8	1.41758E-07	4,700	2,734
1419561_at	Ccl3	chemokine (C-C motif) ligand 3	-1.8	0.03228739	3,034	1,616
1450387_s_at	Ak3l1	adenylate kinase 3 alpha-like 1	-1.8	5.09427E-06	744	427
1415995_at	Casp6	caspase 6	-1.8	0.000232808	1,137	573
1419412_at	Xcl1	chemokine (C motif) ligand 1	-1.9	0.001140306	2,274	1,208
1422812_at	Cxcr6	chemokine (C-X-C motif) receptor 6	-1.9	0.000118139	739	375
1416593_at	Glrx1	glutaredoxin 1 (thioltransferase)	-2.0	0.000130173	853	444
1449911_at	Lag3	lymphocyte-activation gene 3	-2.0	0.000660697	1,455	638
1416899_at	Utf1	undifferentiated embryonic cell transcription factor 1	-2.0	0.000102794	663	378
1416926_at	Trp53inp1	transformation related p53 inducible nuclear protein 1	-2.1	0.00167533	2,514	1,087
1421578_at	Ccl4	chemokine (C-C motif) ligand 4	-2.1	0.000600675	7,169	3,053
1419247_at	Rgs2	regulator of G-protein signaling 2	-2.2	8.12651E-06	830	385
1416871_at	Adam8	a disintegrin and metalloprotease domain 8	-2.5	0.000112885	716	281
1417256_at	Mmp13	matrix metalloproteinase 13	-3.4	1.70802E-06	689	190

Splenic DO11.10 cells were activated in vitro with the cognate peptide $OVA_{327-339}$ in the presence of 1 ng/ml IL-12 and 1 ng/ml IL-2. On day 2, cells were infected with control virus or *twist1*-encoding virus. On day 5, cells were sorted according to expression of the viral marker gene *gfp*. Cells were restimulated for 4 h with PMA/ionomycin. The transcriptional profiles of duplicates of cultures were compared using Affymetrix Murine Genome (MG) 430A 2.0 GeneChip arrays. The Affymetrix probe set ID (Affymetr_No), mean fold change, significance of differential expression (*t* test), and mean signal intensity (Vector, Twist1) of two arrays per group are shown. Genes were filtered according to the following criteria: fold change ≥ 1.5; difference of means ≥ 200; P value ≤ 0.05; and excluding immunoglobulin genes.

Table S4. *Twist1* knock-down results in a higher inflammatory response in murine arthritis

	Acute inflammation				Chronic inflammation				Sum	
Mouse	Exudate	Gran. inf. SM	Fibrin	Periart. gran.	Hyperpl.	Mono. inf SM	Peri. mono.	Cartilage destr.	Acute	Chronic
scrT1	0	1	0	0	0	0.5	0	0	1	0.5
scrT2	0	1	0	0	1	1	2	0	1	4
scrT3	0	1	1	0	0	0.5	0	0	2	0.5
scrT4	1	1	1	0	0	1	0	0	3	1
scrT5	0	1	1	0	0	1	0	0	2	1
Twist1	1	1	1	1	0	1.5	1	0	4	2.5
Twist2	1	2	1	1	1	2	1	0	5	4
Twist3	1	2	1	1	1	1.5	1	0	5	3.5
Twist4	2	3	1	1	1	2	1	1	7	4
Twist5	2	3	1	1	0	2	2	1	7	4

After i.v. cell transfer of 12-d-old GFP^{+} DO11.10 Th1 cells expressing *twist1*-targeting shRNA (Twist1–5) or scrambled *twist1* control shRNA (scrT1–5), arthritis was induced in the recipient SCID mice by intraarticular injection of cationized OVA into the knee joint. 21 d later, knee joint sections were stained for hematoxylin/ eosin and scored for exudates, granulocyte infiltration (gran. inf. SM), hyperplasia (hyperpl.), fibroblast proliferation/ mononuclear cell infiltration (mono. inf. SM), periarticular mononuclear cell infiltration (peri. mino.; each scoring 0–3), bone/cartilage destruction (scoring 0–4), and an additional score of 1 for fibrin deposition and periarticular granulocyte infiltration (periart. gran), resulting in a maximum score of 21.

Table S5. Primer sequences

	Construction of shRNA-expression retrovirus
XbaI EF1aGFP forward	TTCTAGAGACGATAAGCTTTGCAAAGATG
EcoR5 EF1aGFP reverse	TGATATCCATATGACTAGTCCCCGAAGTTG
shTwist1 forward	TGCTGAGCAAGATTCAGACCTTCAAGAGAGGTCTGAATCTTGCTCAGCTTTTTTC
shTwist1 reverse	TCGAGAAAAAAGCTGAGCAAGATTCAGACCTCTCTTGAAGGTCTGAATCTTGCTCAGCA
scr shTwist1 forward	TGCTATCGAGAAGATCAGCCTTCAAGAGAGGCTGATCTTCTCGATAGCTTTTTTC
scr shTwist1 reverse	TCGAGAAAAAAGCTATCGAGAAGATCAGCCTCTCTTGAAGGCTGATCTTCTCGATAGCA
XhoI U6shRNA forward	TATCTCGAGCAGAGATCCAGTTTGGTTAGTACC
U6shRNA reverse	TAGGTCCCTCGACCTGCTGG
	Construction of protein expression retrovirus
BglII mTbet forward	ATGGAAGATCTATGGGCATCGTGGAGCC
XhoI mTbet reverse	ATCCGCTCGAGTCAGTTGGGAAAATAATTATAAAAC
BglII mTwist1 forward	GAAGATCTATGATGCAGGACGTGTCCAGC
XhoI mTwist reverse	ATCCGCTCGAGCTAGTGGGACGCGGACATGG
BglII hMIkBa forward	ATGGAAGATCTATGTTCCAGGCGGCCGA
SalI hMIkBa reverse	TTCGTCGACTCATAACGTCAGACGCTGGC
	Real-time PCR
Murine HPRT forward	GCTGGTGAAAAGGACCTCT
Murine HPRT reverse	CACAGGACTAGAACACCTGC
Murine Twist2 forward	GCATCCTGGCCAACGTGC
Murine Twist2 reverse	TCCATGCGCCACACGGAG
Murine Twist1 forward	CGCACGCAGTCGCTGAACG
Murine/hum Twist1 reverse	GACGCGGACATGGACCAGG
Human Twist1 forward	GGCACCCAGTCGCTGAACG
Human UbcH5 forward	TCTTGACAATTCATTTCCCAACAG
Human UbcH5 reverse	TCAGGCACTAAAGGATCATCTGG