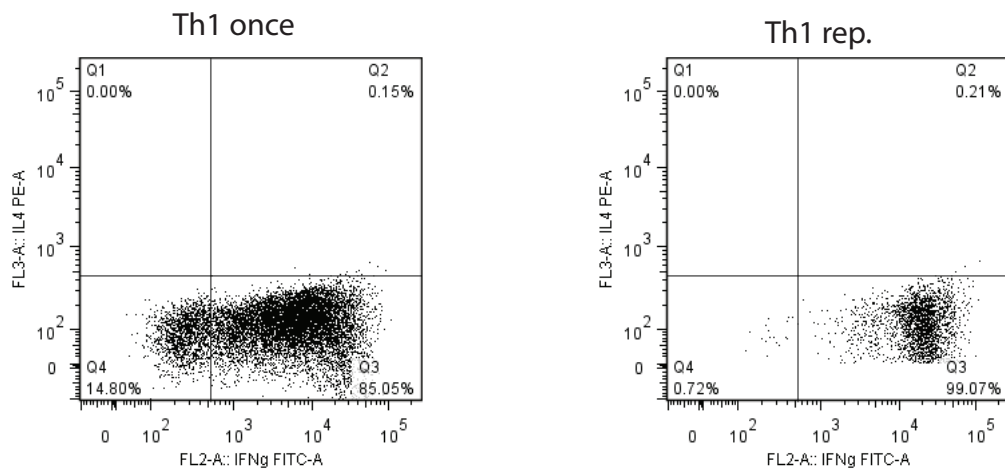
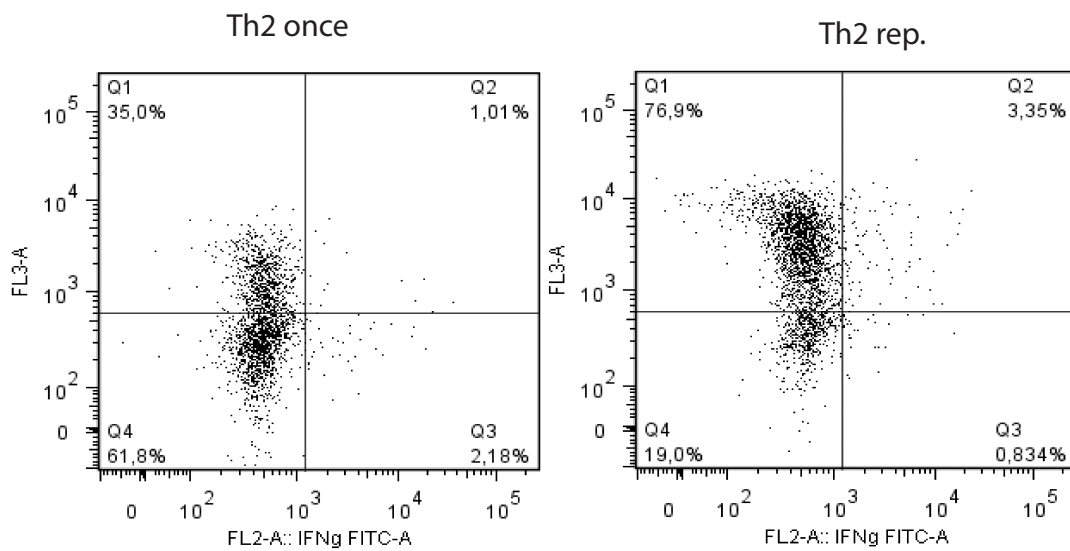


Supporting Information 1

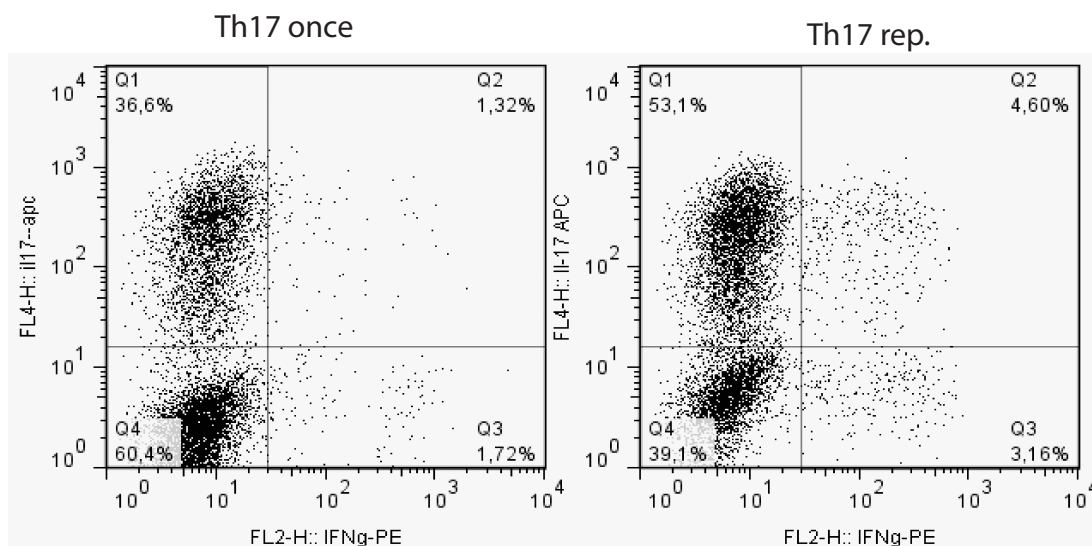
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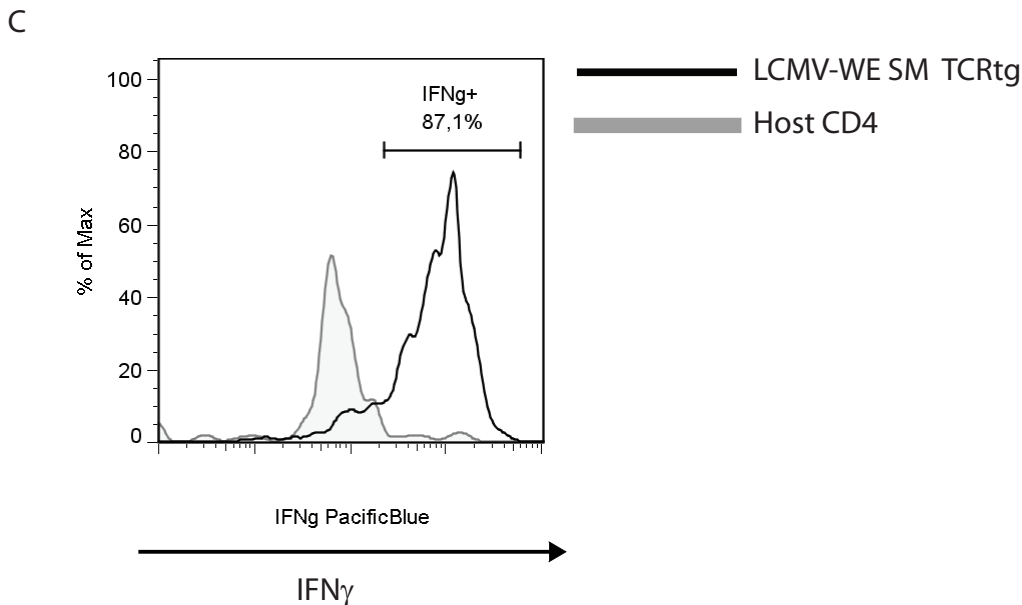
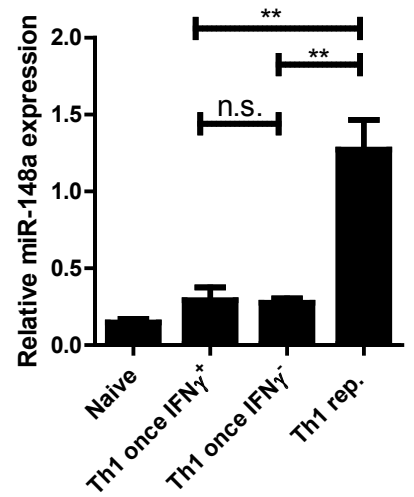
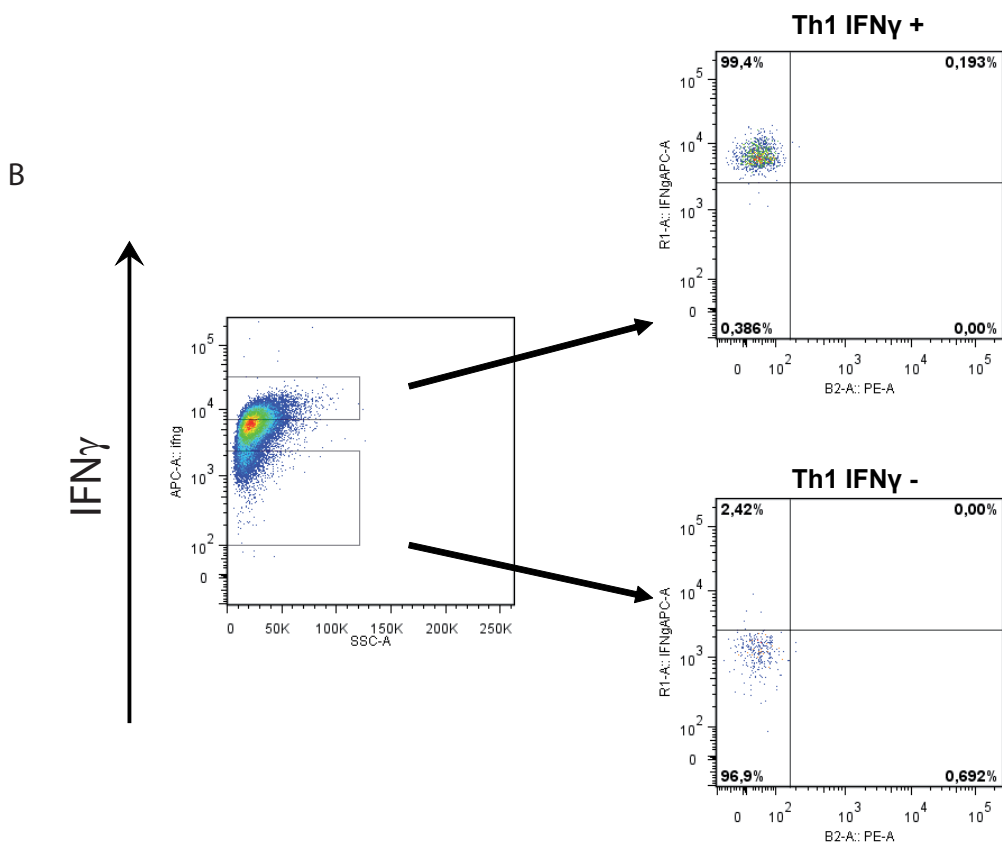
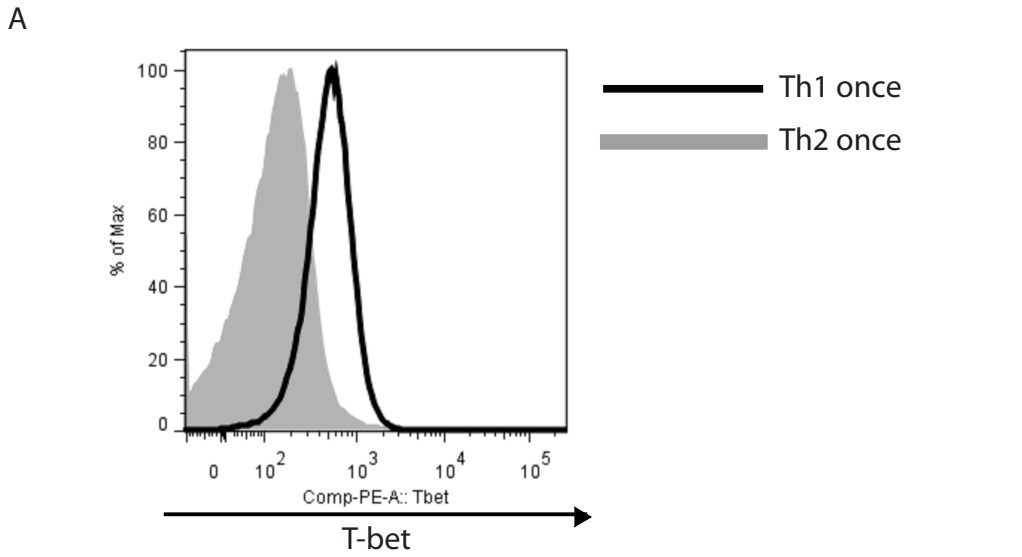
B



C



Supporting Information 2



Supporting Information 3

Bim bs (7mer-1a, nt 4183 - 4189; 8mer, 4273 - 4280)

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TTCTGTACCCAGACCTGTCAGGTATCCTTTAGTGAACAG**GCACTGAT**CTCTGTATAGTCCCATCACTAA
TGTCAGAACCCAGCGTATGTAGCATTGTATTGCAGTTTCCCTGGCTTCCCTTTACGTTTT**GCACTGATG**
AATTTTGACAGGGTAATTGCCACTGTACCTGTG
miR-148a
miR-148a

Bim^{MUT} bs

CTCAAGTTC~~CC~~CAGCAAAGTACTTTGATCTCTTTCAAATGTGTGTTATTGTCTAGGTTCTGTGACCACTGGT
TTCTGTACCCAGACCTGTCAGGTATCCTTTAGTGAACAG**GCGGTCG**CTCTGTATAGTCCCATCACTAA
TGTCAGAACCCAGCGTATGTAGCATTGTATTGCAGTTTCCCTGGCTTCCCTTTACGTTTT**GCGGCCATG**
AATTTTGACAGGGTAATTGCCACTGTACCTGTG
miR-148a*
miR-148a*

Pten bs (8mer, nt 3146 - 3153)

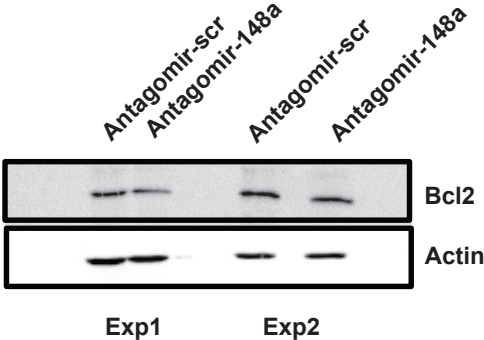
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GGATACACAAATATGACGTGTACAGGATAATGCCTCATACCAATCAGATGTCCATTTGTTACTGTGTTTG
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GTGGGTG
miR-148a

Pten^{MUT} bs

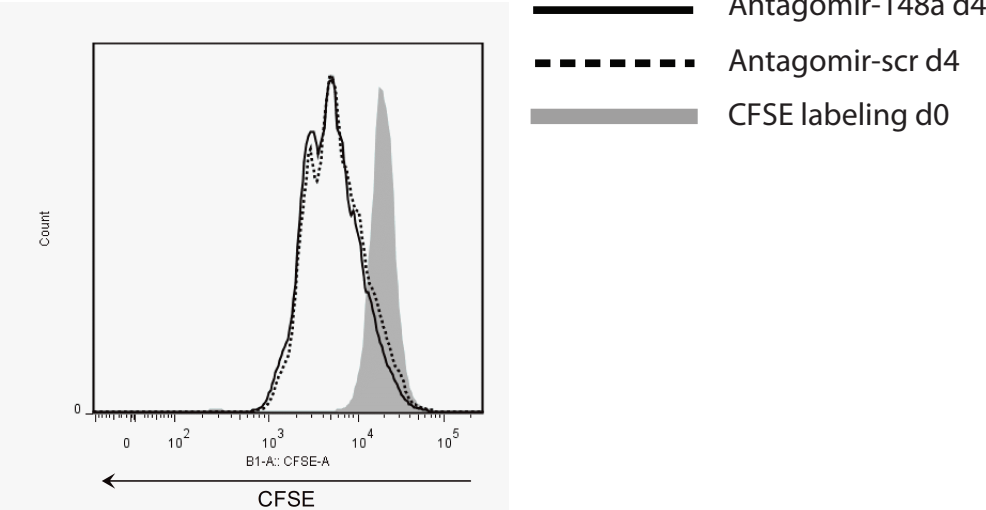
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miR-148a*

Supporting Information 4

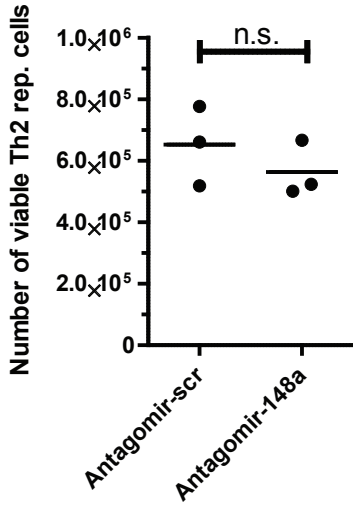
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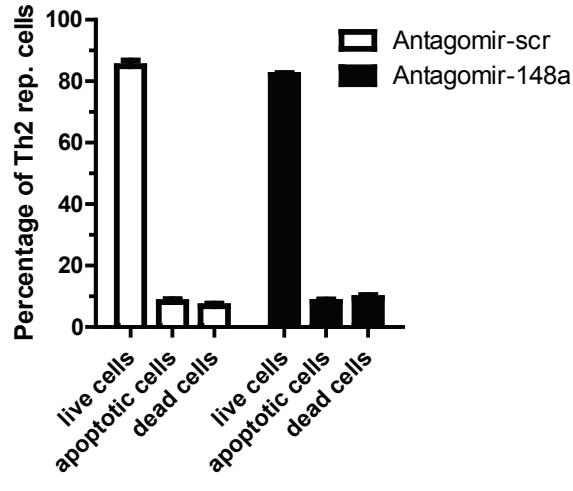
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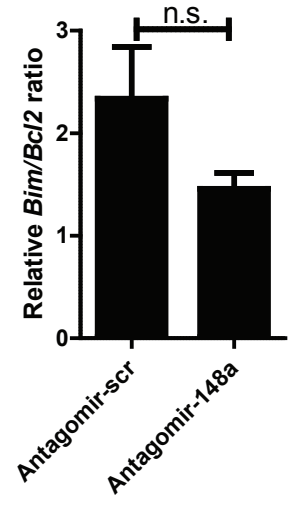
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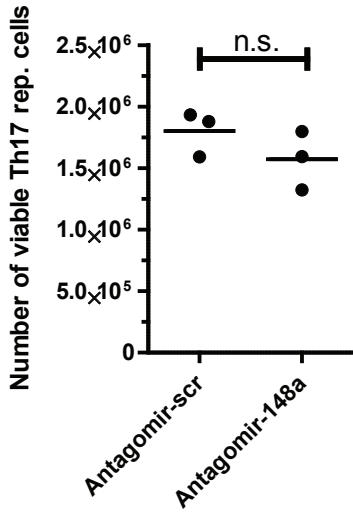
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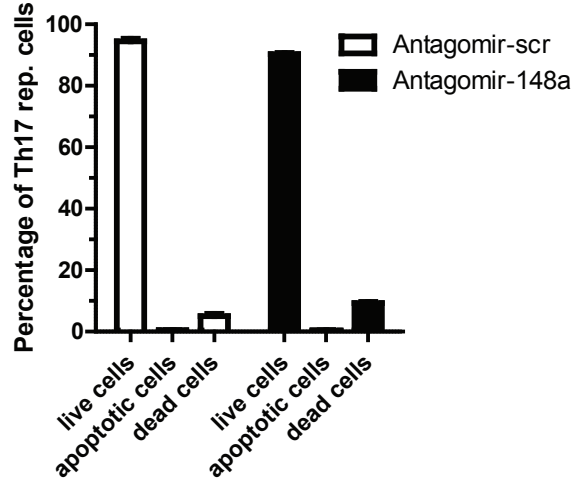
C



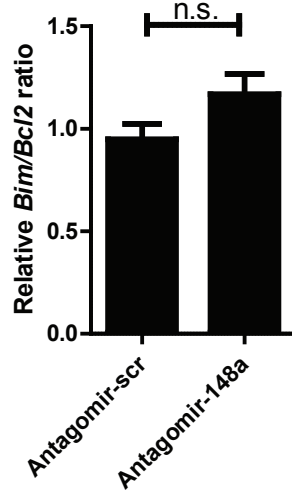
D



E

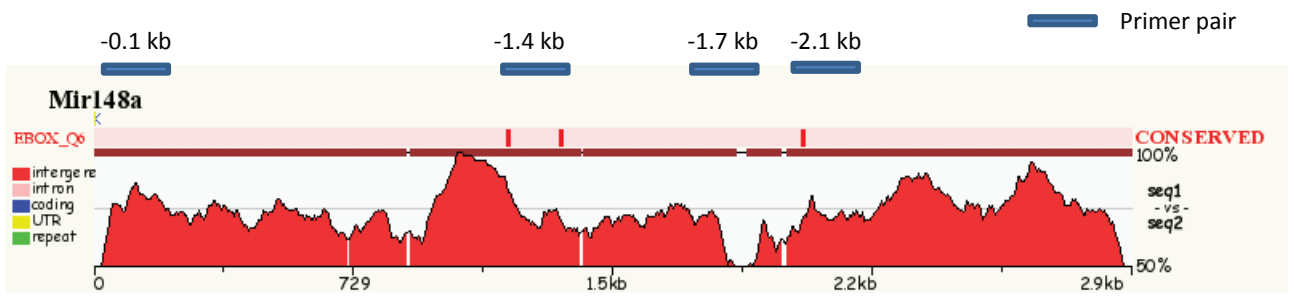


F

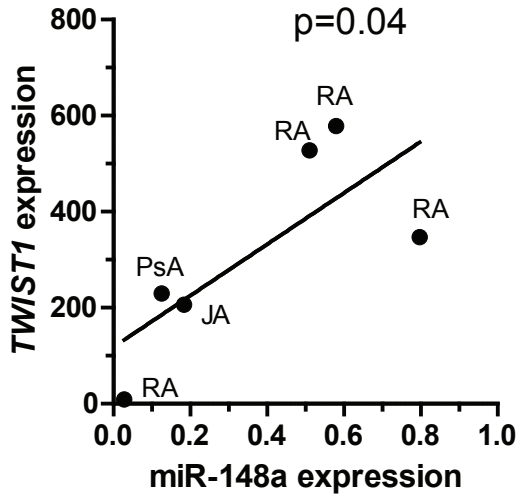


Supporting Information 6

A

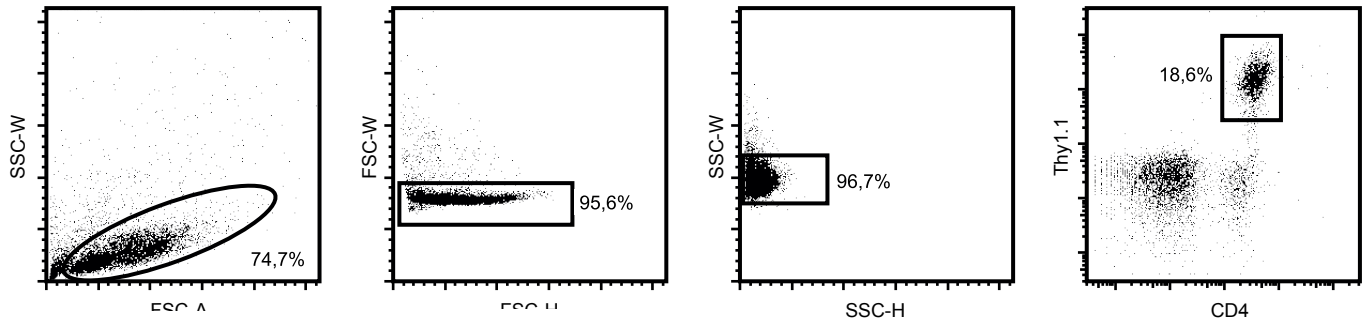


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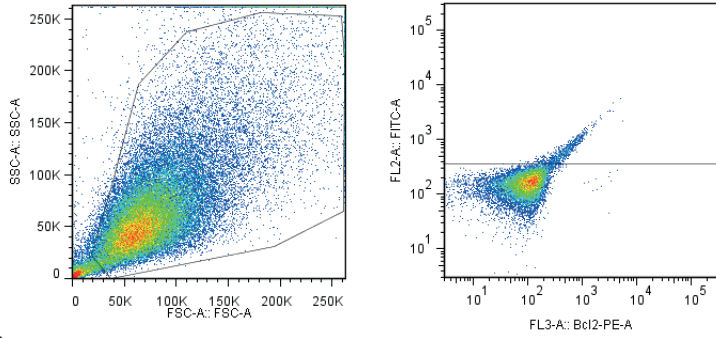


Supporting Information 7

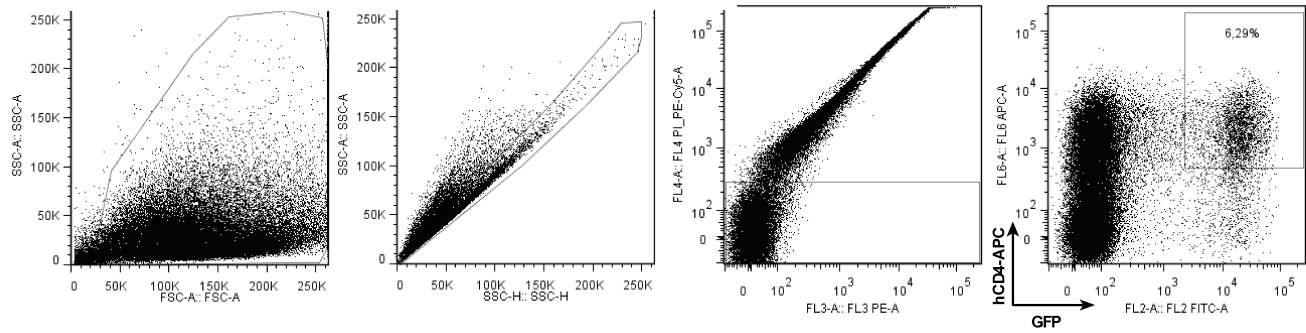
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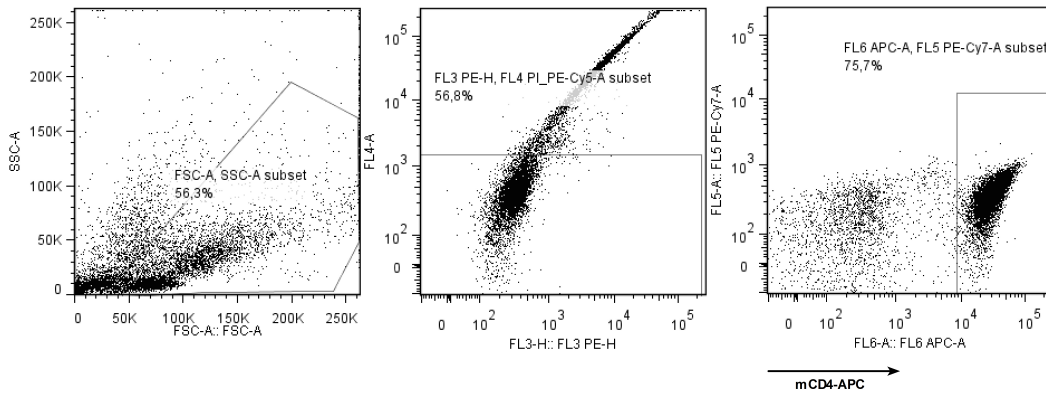
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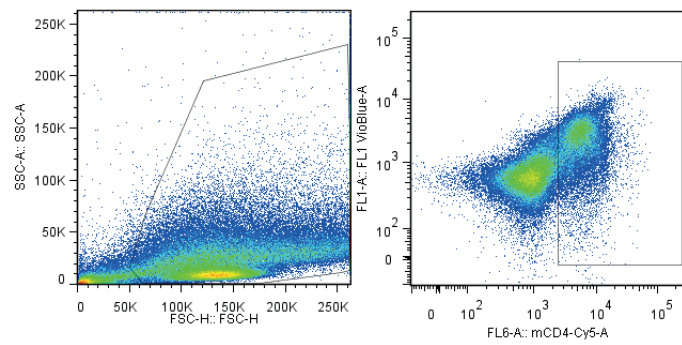
C



D



E



Supporting Information Table 1

Refseq_id	log2_fold_change	gene_symbol	description
NM_007521	-2.463407345	Bach2	BTB and CNC homology 2 Gene;
NM_007901	-2.426255141	S1pr1	sphingosine-1-phosphate receptor 1 Gene;
NM_001012330	-2.234328784	Zfp238	zinc finger protein 238 Gene;
NM_028709	-1.742821873	Btbd11	BTB (POZ) domain containing 11 Gene;
NM_022022	-1.585208591	Ube4b	ubiquitination factor E4B, UFD2 homolog (S. cerevisiae) Gene;
NM_027629	-1.503036811	Pgm2l1	phosphoglucomutase 2-like 1 Gene;
NM_001033299	-1.501165737	Zfp217	zinc finger protein 217 Gene;
NM_001081049	-1.400389119	Mll1	myeloid/lymphoid or mixed-lineage leukemia 1 Gene;
NM_021310	-1.389553256	Jmy	junction-mediating and regulatory protein Gene;
NM_198022	-1.377309773	Tnrc6c	trinucleotide repeat containing 6C Gene;
NM_001081216	-1.211219049	Phip	pleckstrin homology domain interacting protein Gene;
NM_198164	-1.206423796	Cdk19	cyclin-dependent kinase 19 Gene;
NM_172397	-1.128515187	Limd2	LIM domain containing 2 Gene;
NM_007841	-1.113071527	Ddx6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6 Gene;
NM_001029934	-1.097488542	Usp32	ubiquitin specific peptidase 32 Gene;
NM_001013367	-1.014331971	Prkaa1	protein kinase, AMP-activated, alpha 1 catalytic subunit Gene;
NM_010569	-1.011400192	Invs	inversin Gene;
NM_144812	-0.97734212	Tnrc6b	trinucleotide repeat containing 6b Gene;
NM_025347	-0.976965068	Ypel3	yippee-like 3 (Drosophila) Gene;
NM_009754	-0.968422247	Bcl2l11	BCL2-like 11 (apoptosis facilitator) Gene;
NM_144925	-0.94918819	Tnrc6a	trinucleotide repeat containing 6a Gene;
NM_138599	-0.906715518	Tomm70a	translocase of outer mitochondrial membrane 70 homolog A (yeast) Gene;
NM_010657	-0.880405262	Hivep3	human immunodeficiency virus type I enhancer binding protein 3 Gene;
NM_199299	-0.864163563	Phf15	PHD finger protein 15 Gene;
NM_028148	-0.863476326	Sfrs2lp	splicing factor, arginine/serine-rich 2, interacting protein Gene;
NM_023646	-0.835092099	Dnaja3	DnaJ (Hsp40) homolog, subfamily A, member 3 Gene;
NM_008997	-0.831673812	Rab11b	RAB11B, member RAS oncogene family Gene;
NM_024288	-0.828748808	Rmnd5a	required for meiotic nuclear division 5 homolog A (S. cerevisiae) Gene;
NM_172894	-0.812626865	Saps1	SAPS domain family, member 1 Gene;
NM_001008550	-0.794489981	Zfyve26	zinc finger, FYVE domain containing 26 Gene;
NM_010804	-0.782682142	Mllt10	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10 Gene;
NM_172790	-0.777063755	Ankrd52	ankyrin repeat domain 52 Gene;
NM_148948	-0.750150654	Dicer1	Dicer1, Dcr-1 homolog (Drosophila) Gene;
NM_172516	-0.717531982	Dstyk	dual serine/threonine and tyrosine protein kinase Gene;
NM_001093752	-0.705582159	Sfrs11	splicing factor, arginine/serine-rich 11 Gene;
NM_181405	-0.700933541	Rnpep11	arginyl aminopeptidase (aminopeptidase B)-like 1 Gene;
NM_001159964	-0.662655461	Eps15	epidermal growth factor receptor pathway substrate 15 Gene;

NM_173870	-0.659950936	Mgat4a	mannoside acetylglucosaminyltransferase 4, isoenzyme A Gene;
NM_008739	-0.646751993	Nsd1	nuclear receptor-binding SET-domain protein 1 Gene;
NM_001115076	-0.642632633	Camsap1	calmodulin regulated spectrin-associated protein 1 Gene;
NM_032000	-0.63498975	Trps1	trichorhinophalangeal syndrome I (human) Gene;
NM_178638	-0.616754113	Tmem108	transmembrane protein 108 Gene;
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NM_011543	-0.593752791	Skp1a	S-phase kinase-associated protein 1A Gene;
NM_021548	-0.591582601	Arpp19	cAMP-regulated phosphoprotein 19 Gene;
NM_001110832	-0.590380834	Nfya	nuclear transcription factor-Y alpha Gene;
NM_057172	-0.588056442	Fubp1	far upstream element (FUSE) binding protein 1 Gene;
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NM_175127	0.511537054	Fbxo28	F-box protein 28 Gene;
NM_007597	0.517631329	Canx	calnexin Gene;
NM_026444	0.524698758	Cs	citrate synthase Gene;
NM_001009935	0.533204878	Txnlp	thioredoxin interacting protein Gene;
NM_009145	0.534987022	Nptn	neuropilin Gene;
NM_172734	0.540204134	Stk38l	serine/threonine kinase 38 like Gene;
NM_001014288	0.545443843	Ptprd	protein tyrosine phosphatase, receptor type, D Gene;
NM_018753	0.54978732	Ywhab	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide Gene;
NM_001122675	0.568267936	Zcchc2	zinc finger, CCHC domain containing 2 Gene;
NM_008960	0.571787216	Pten	phosphatase and tensin homolog Gene;
NM_009071	0.578945322	Rock1	Rho-associated coiled-coil containing protein kinase 1 Gene;
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NM_001145209	0.593145891	Hbs1l	Hbs1-like (S. cerevisiae) Gene;

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NM_008155	0,640910888	Gpi1	glucose phosphate isomerase 1 Gene;
NM_175318	0,644904149	Spty2d1	SPT2, Suppressor of Ty, domain containing 1 (<i>S. cerevisiae</i>) Gene;
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NM_052993	0,648475312	C1galt1	core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase, 1 Gene;
NM_009231	0,6622741	Sos1	son of sevenless homolog 1 (<i>Drosophila</i>) Gene;
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NM_001160139	0,800452117	Kcnq5	potassium voltage-gated channel, subfamily Q, member 5 Gene;
NM_025773	0,804393448	Ube2w	ubiquitin-conjugating enzyme E2W (putative) Gene;
NM_145501	0,83483292	Pi4k2a	phosphatidylinositol 4-kinase type 2 alpha Gene;
NM_009727	0,83535192	Atp8a1	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1 Gene;
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NM_054078	0,880903005	Baz2a	bromodomain adjacent to zinc finger domain, 2A Gene;
NM_173396	0,882927784	Tgif2	TGFB-induced factor homeobox 2 Gene;
NM_009443	0,889056429	Tgoln2Tgoln1	trans-golgi network protein Gene;trans-golgi network protein 2 Gene;
NM_024195	0,935774368	Cyb5r4	cytochrome b5 reductase 4 Gene;
NM_007952	0,964839004	Pdia3	protein disulfide isomerase associated 3 Gene;
NM_175465	0,978116465	Sestd1	SEC14 and spectrin domains 1 Gene;
NM_008408	0,979710707	Stt3a	STT3, subunit of the oligosaccharyltransferase complex, homolog A (<i>S. cerevisiae</i>) Gene;
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NM_010118	1,114124273	Egr2	early growth response 2 Gene;
NM_019425	1,117266323	Gnpat1	glucosamine-phosphate N-acetyltransferase 1 Gene;
NM_177327	1,183406414	Wwp1	WW domain containing E3 ubiquitin protein ligase 1 Gene;
NM_001081364	1,242237543	Arhgap21	Rho GTPase activating protein 21 Gene;
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NM_001145820	1,523372817	Gpd2	glycerol phosphate dehydrogenase 2, mitochondrial Gene;
NM_133700	1,573588222	Btbd10	BTB (POZ) domain containing 10 Gene;
NM_010637	1,702288528	Klf4	Kruppel-like factor 4 (gut) Gene;
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NM_011308	1,781899308	Ncor1	nuclear receptor co-repressor 1 Gene;
NM_133753	1,82666196	Errf1	ERBB receptor feedback inhibitor 1 Gene;
NM_011631	1,993378872	Hsp90b1	heat shock protein 90, beta (Grp94), member 1 Gene;
NM_011803	2,165836215	Klf6	Kruppel-like factor 6 Gene;
NM_009828	2,284540398	Ccna2	cyclin A2 Gene;
NM_011400	2,310311918	Slc2a1	solute carrier family 2 (facilitated glucose transporter), member 1 Gene;
NM_001113529	2,539577361	Csf1	colony stimulating factor 1 (macrophage) Gene;
NM_172449	2,599745998	Bzrap1	benzodiazapine receptor associated protein 1 Gene;
NM_011170	3,006809045	Prnp	prion protein Gene;
NM_001039081	3,029309033	Mier1	mesoderm induction early response 1 homolog (Xenopus laevis) Gene;
NM_023794	3,451911978	Etv5	ets variant gene 5 Gene;

Supplementary Figure legends

Figure 1 Representative IFN γ , IL-4 and IL-17 production in once and repeatedly activated Th1 (A), Th2 (B) and Th17 cells (C) of at least seven independent experiments. For gating strategies see Supporting Information Fig.7B.

Figure 2 (A) Representative intracellular protein staining of T-bet in once activated Th1 cells compared to once activated Th2 cells, assessed by flow cytometry. (B) Gating strategy for separation of IFN γ producing and non-producing Th1 cells after one round of activation. The expression of miR-148a in Naive (n=2), Th1 once IFN γ^+ (n=5), Th1 once IFN γ^- (n=5) and Th1 rep. (n=7). In addition the Th1 subsets were restimulated with PMA/Ionomycin for 3h to allow IFN γ secretion. miR-148a expression was normalized to snU6 and presented relative to values obtained with unsorted Th1 (Wilcoxon-Test for paired data when comparing Th1 once IFN γ^+ with Th1 once IFN γ^- cells and Mann-Whitney-Test for unpaired data when comparing IFN γ^+ and IFN γ^- with Th1 rep. cells, mean, error bars, s.e.m., $p \leq 0.01$ (**), independent experiments, depicted are pools of two independent experiments). (C) Representative IFN γ production of SM TCRtg Th cells and Host CD4 $^+$ Th cells on day 6 post infection with 200 pfu of LCMV-WE after four hours of mitogenic restimulation with PMA/Ionomycin and assessed by flow cytometry, representative for three independent experiments.

Figure 3 Sequence of Bim 3'-UTR with bs for miR-148a (Bim bs) and mutated miR-148a bs (Bim^{MUT} bs). Sequence of Pten 3'-UTR with bs for miR-148a (Pten bs) and mutated miR-148a bs (Pten^{MUT} bs).

Figure 4 (A) Bcl-2 expression in repeatedly activated Th1 cells after treatment with antagomir-148a or antagomir-scr on day 3 post restimulation with α CD3/ α CD28 validated by immunoblotting, independent experiments, n=2. The filter shown in Fig. 3D was probed with anti-Bcl2, same actin WB as shown in Figure 3D. (B) Representative CFSE staining of repeatedly activated Th1 prior to antagomir treatment (CFSE labeling d0) and after treatment with antagomir-148a or antagomir-scr, assessed by flow cytometry on day 4 post restimulation with α CD3/ α CD28, representative for two independent experiments. For gating strategies see Supporting Information Fig.7D

Figure 5 Numbers of viable repeatedly activated Th2 cells (Th2 rep.) (A) after antagomir treatment and restimulation with α CD3/ α CD28, assessed by flow cytometry, pools of three independent antagomir treatments for day 3 (Mann-Whitney-Test for unpaired data, error bars, s.e.m.). For gating strategies see Supporting Information Fig.7D (B) frequencies of live Th2 rep. cells (AnnexinV $^-$ PI $^-$), apoptotic Th2 rep. cells (AnnexinV $^+$ PI $^-$) and dead Th2 rep. cells (AnnexinV $^+$ PI $^+$) after antagomir treatment assessed by Annexin/PI staining followed by flow cytometry on day 3 post restimulation with α CD3/ α CD28. Statistical evaluation of pools of three independent antagomir treatments (Mann-Whitney-Test for unpaired data, error bars, s.e.m.). For gating strategies see Supporting Information Fig.7E (C) Statistical evaluation of the *Bim/Bcl2* mRNA ratio in Th2 rep. cells after treatment with antagomir-148a presented relative to values obtained with antagomir-scr. Depicted are pools of three independent antagomir treatments (Mann-Whitney-Test for unpaired data, error bars, s.e.m.).

Numbers of viable repeatedly activated Th17 cells (Th17 rep.) (D) after antagomir treatment and restimulation with α CD3/ α CD28, assessed by flow cytometry, pools of three independent antagomir treatments for day 3 (Mann-Whitney-Test for unpaired data, error bars, s.e.m.). For gating strategies see Supporting Information Fig.7D (E) frequencies of live Th17 rep.

cells (AnnexinV⁻PI⁻), apoptotic Th17 rep. cells (AnnexinV⁺PI⁻) and dead Th17 rep. cells (AnnexinV⁺PI⁺) after antagomir treatment assessed by Annexin/PI staining followed by flow cytometry on day 3 post restimulation with α CD3/ α CD28. Statistical evaluation of pools of three independent antagomir treatments (Mann-Whitney-Test for unpaired data, error bars, s.e.m.). For gating strategies see Supporting Information Fig.7E (F) Statistical evaluation of the *Bim/Bcl2* mRNA ratio in Th17 rep. cells after treatment with antagomir-148a presented relative to values obtained with antagomir-scr. Depicted are pools of three independent antagomir treatments (Mann-Whitney-Test for unpaired data, error bars, s.e.m.).

Figure 6 (A) *in silico* analysis of Twist1 bs (Ebox consensus sequence) in transcriptional region of miR-148a. Primer pairs were designed for sequences harboring putative Ebox bs (-1.4kb and -2.1 kb) or not harboring Ebox bs (-1.7kb), conservation comparing tool rVista based on the professional V10.2 library of the TRANSFAC database. (B) Correlation of miR-148a expression (qRT-PCR) and *TWIST1* expression in ex vivo isolated restimulated CD3⁺CD4⁺CD14⁻CD45RO⁺ T cells of patients with RA, psoriatic arthritis (PsA), and juvenile idiopathic arthritis (JA) (Pearson correlation analysis, $r=0.76$, one-tailed, $p=0.04$).

Figure 7 Gating strategies for (A) intracellular protein staining of T-bet, (B) intracellular protein staining of Bim and Bcl-2 and intracellular cytokine staining of signature cytokines of Th1, Th2 and Th17 cells, (C) reporter assays in Th1 cells as assessed by flow cytometry of the MFI of human CD4, (D) investigation of numbers of viable cells and CFSE dilution assays, (E) apoptosis assays by AnnexinV staining.

Table 1 Table containing 130 putative target genes of miR-148a, which were differentially expressed in Th1 cells with a foldchange ≥ 1.4 in once versus repeatedly activated Th1 cells with 61 genes being down- and 69 genes being upregulated. Candidate miR-148a targets in repeatedly activated Th1 cells were identified by target screens with PicTar (<http://pictar.mdc-berlin.de/>; [20]) and TargetScan (<http://www.targetscan.org/>; [21]), in combination with global transcriptome data of once versus repeatedly activated Th1 cells.