

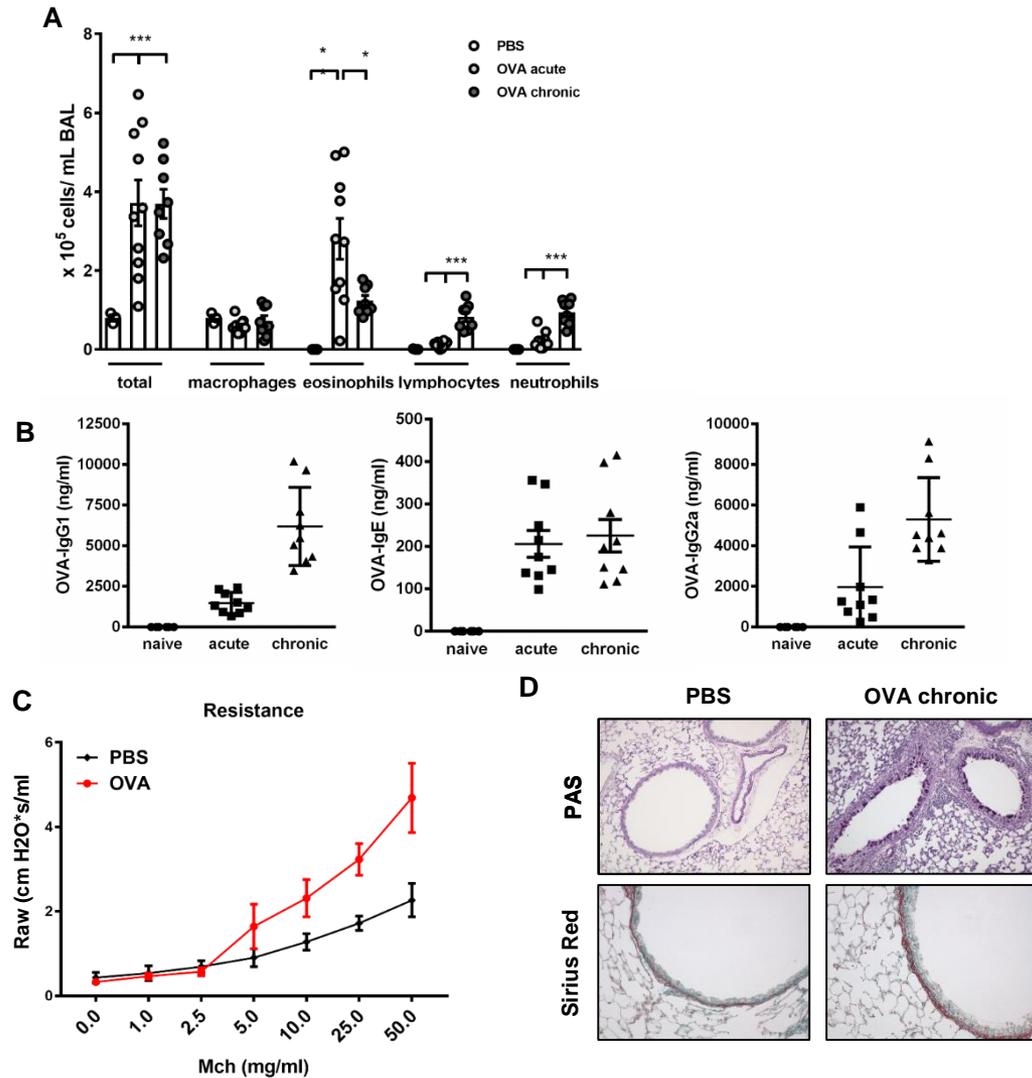
A systems immunology approach identifies the collective impact of five miRs in Th2 inflammation

Supplementary information

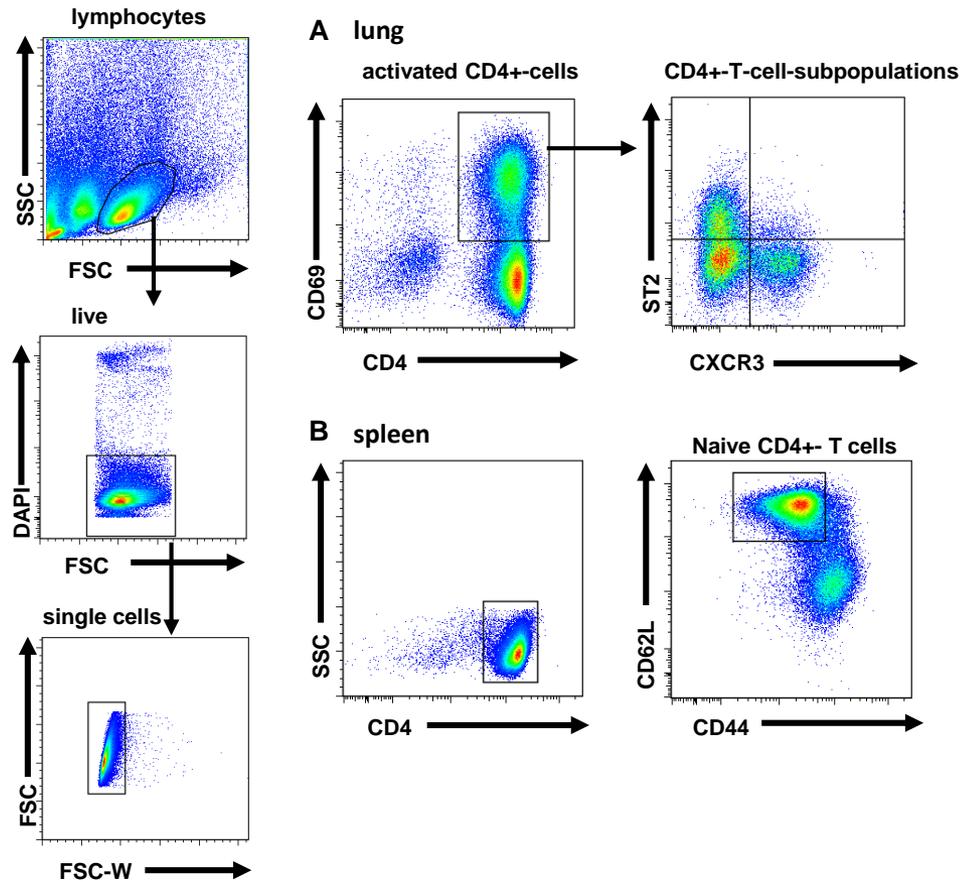
Ayşe Kılıç, Marc Santolini, Taiji Nakano, Matthias Schiller, Mizue Teranishi, Pascal Gellert, Yuliya Ponomareva, Thomas Braun, Shizuka Uchida, Scott T. Weiss, Amitabh Sharma and Harald Renz

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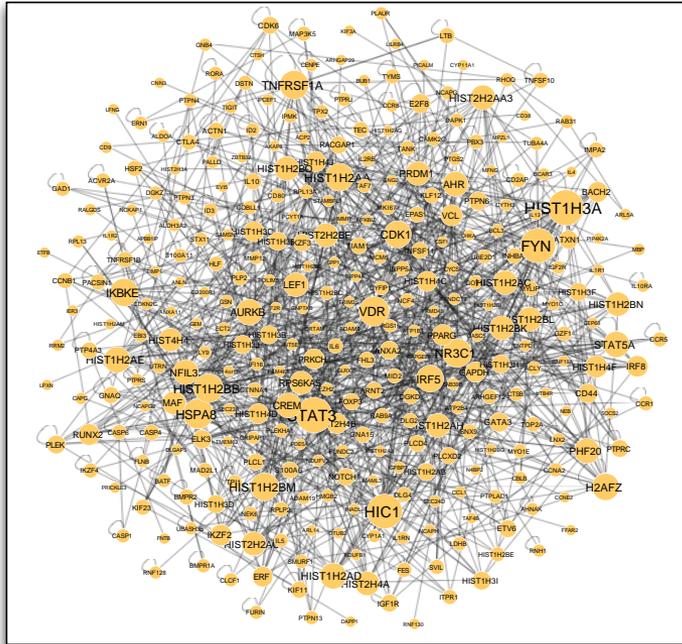
Supplementary figure 1: Characteristic phenotype of the acute and chronic allergic airway inflammatory response in the OVA-induced mouse model. (a) Total and differential cell counts determined in BAL and cytopins show a dominant influx of eosinophils. **(b)** Serum titers of OVA-specific immunoglobulins are elevated. **(c)** Representative measurement of airway reactivity to increasing methacholine (Mch) responsiveness measured by head-out body plethysmography. **(d)** Representative lung section staining depicting airway inflammation and mucus production (PAS, original magnification x 10) and airway remodeling (SiriusRed; original magnification x 20). Data are presented as mean ± SEM and are representative for at least 3 independent experiments with n=8-10 animals per group. Statistical analyses have been performed with one-way ANOVA and Tukey's post-test and show *p<0.05, **p<0.01 and ***p<0.001.



Supplementary figure 2. Gating strategy for the isolation of live CD4⁺ T cell subsets using FACS-sorting. Representative dot-plots, depicting the gating strategy for the isolation of activated Th2 cells as CD4⁺ CD69⁺ T1/ST2⁺ CXCR3⁻ from inflamed lung tissue. **(A)** Activated/tissue resident Th1 and Th2 cells were sorted from the CD4⁺CD69⁺ fraction and based on surface expression of CXCR3⁺ (Th1) and T1/ST2⁺ (Th2). **(B)** Naïve CD4⁺ T cells were isolated from spleens of sham immunized and sham challenged mice by sorting CD4⁺CD62L⁺CD44⁻ cells. (C)

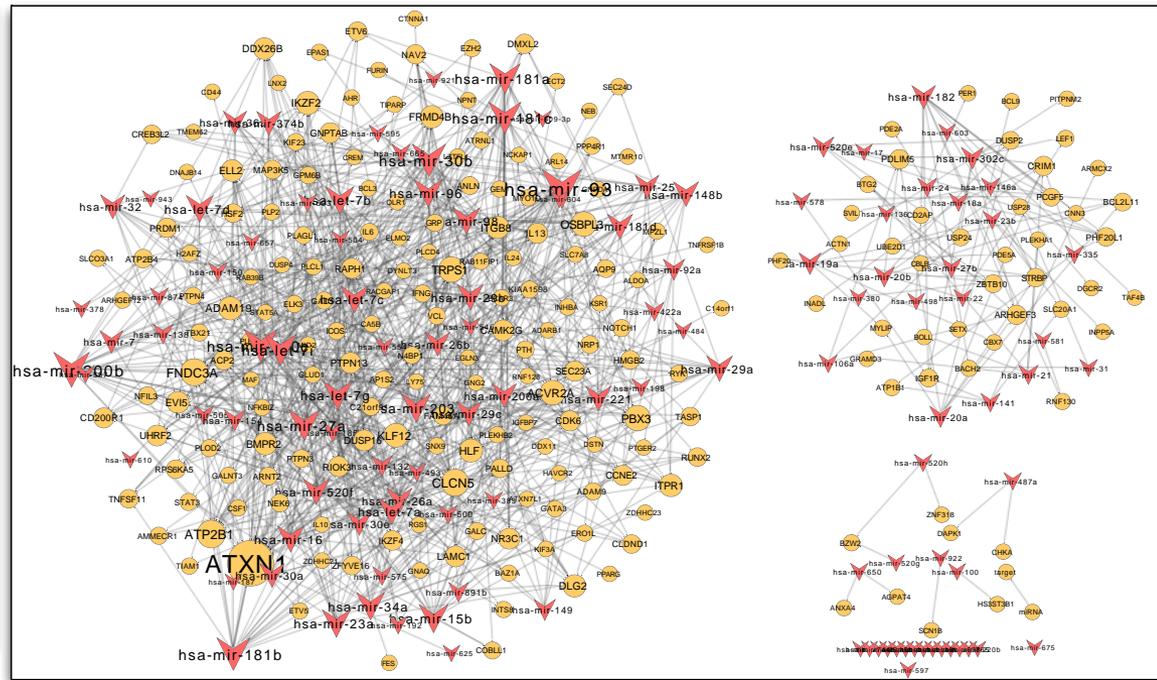
A

mRNA-mRNA network



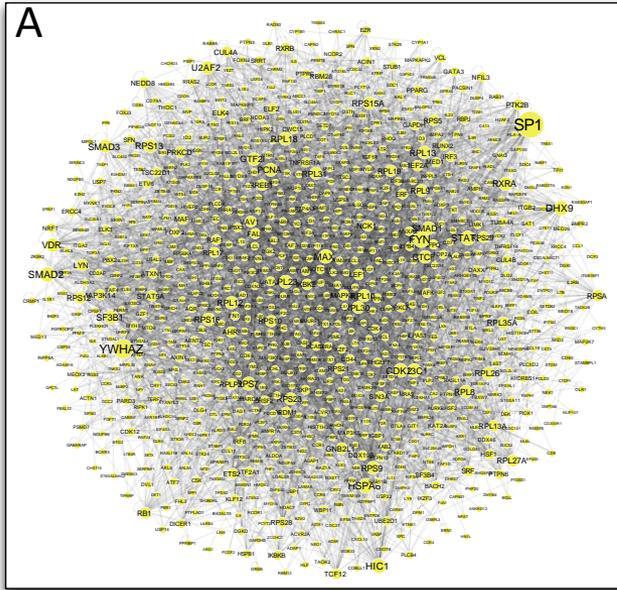
B

miRNA-mRNA network

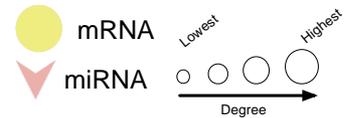
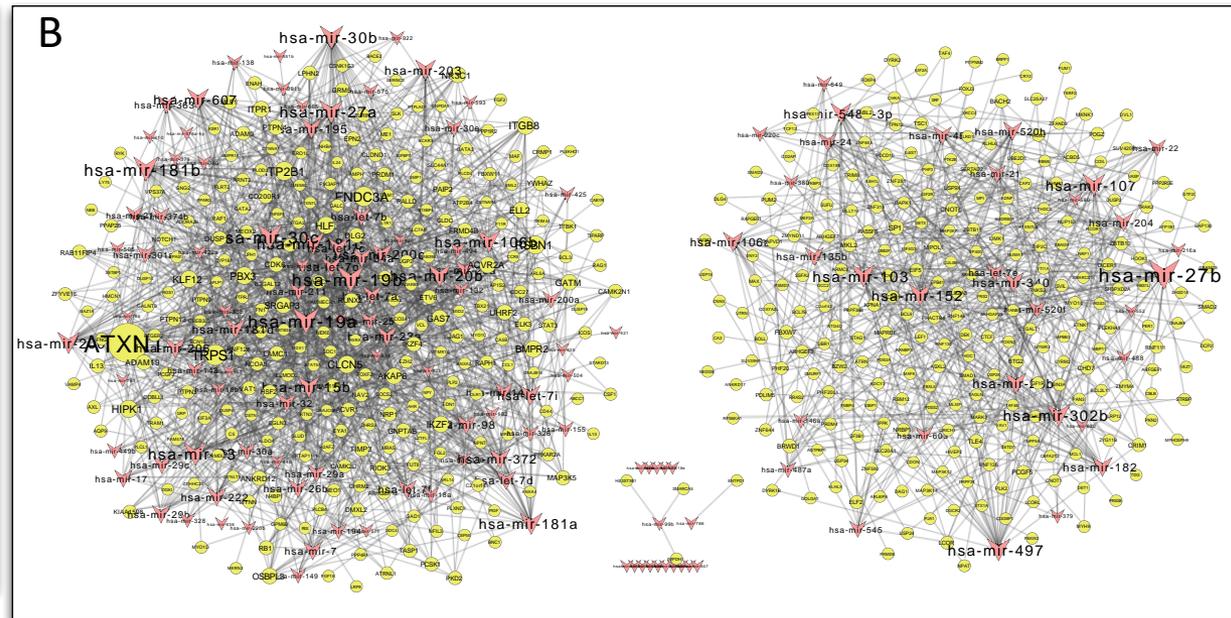


Supplementary Figure 3. (A) The connectivity of mRNA/genes coding proteins in interactome in early Th2 cells. **(B)** The connectivity of miRNA and their targets in miRNA-mRNA network (mirDIP). We applied force-directed layout in Cytoscape for visualization of the networks.

mRNA-mRNA network



miRNA-mRNA network



Supplementary figure 3. (A) The connectivity of mRNA/genes coding proteins in Interactome in stable Th2 cells. The module comprised of 4104 edges between 1059 genes encoding proteins. **(B)** The connectivity of miRNA and their targets in miRNA-mRNA network (mirDIP). The network consisted of the connection between 511 genes and 142 miRNAs. We applied force-directed layout in Cytoscape for visualization of the networks.

CTCF

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212:5' ucucugagggUUACUGUGAa 3' mCtcf 3'-UTR WT
      ::| | | | | | | |
213:5' ucccugagggUUACUGUGAa 3' hCTCF 3'-UTR WT
      ::| | | | | | | |
3' cgucuuugaaucGGUGACACUu 5' miR-27b
      ::| | | | | | | |
213:5' ucucugagggUU-----a 3' hCTCF 3'-UTR ΔmiR-27b

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NCOA3

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637:5' aggaauaggugaaccACAUUCcu 3' mNcoa3 3'-UTR WTT
      | | | | | | | |
763:5' gggaaauagugaaacACAUUCcu 3' hNCOA3 3'-UTR WT
      | | | | | | | |
3' ggugugugaaggaaUGUAAGGu 5' hsa-miR-206
      | | | | | | | |
763:5' gggaaauagugaaac-----u 3' hNCOA3 3'-UTR ΔmiR-206

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ELK3

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116:5' gUUCAUUUUGGUUGCACUUUu 3' mElk3 3'-UTR WT
      | | | | | | | |
100:5' guuaauuugGUUGCACUUUu 3' hELK3 3'-UTR WT
      | | | | | | | |
3' uagacgugaCAGUCGUGAAAU 5' hsa-miR-106b
      | | | | | | | |
100:5' guuaauuugGUUU-----u 3' hELK3 3'-UTR ΔmiR-106b

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AHR

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1091:5' uuaagcagagcacuCAUUUCAc 3' mAhr 3'-UTR WT
      | | | | | | | |
1041:5' uuaagcaaagcaccCAUUUCAa 3' hAHR 3'-UTR WT
      | | | | | | | |
3' gaucaccaggauuuGUAAGUGg 5' hsa-miR-203
      | | | | | | | |
1041:5' uuaagcaaagcacc-----a 3' mAHR 3'-UTR ΔmiR-203 (1)

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2021:5' acaacuGUCUAAAACAUUUCAc 3' mAhr 3'-UTR WT
      | | | | | | | |
2395:5' aaAUUUGUCUGAAAACAUUUCAu 3' hAHR 3'-UTR WT
      | | | | | | | |
3' gaUCACCAGGAUUUUGUAAGUGg 5' hsa-miR-203
      | | | | | | | |
2395:5' aaAUUU-----u 3' mAHR 3'-UTR ΔmiR-203 (2)

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ELF2

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458:5' acaGGUAUCUAG-AAUGUGAa 3' mElf2 3'-UTR WT
      | | | | | | | |
470:5' auaGGUAUUUUAGAAUGUGAa 3' hELF2 3'-UTR WT
      ::| | | | | | | |
3' ccaUUAGGGACCGUUACACUa 5' hsa-miR-23b
      ::| | | | | | | |
470:5' auaGGUAUUUUAG-----a 3' hELF2 3'-UTR ΔmiR-23b

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Supplementary figure 3. Conservation of miRNA-seed sequences among mouse and human. Alignment of the conserved miR target site in wildtype human and mouse target 3'-UTRs. To assess sequence specificity deletion of the seed sequence was performed and is highlighted in red color.

Supplementary Table 1: Genes upregulated in early Th2 cells versus naive T cells

Gene Symbol	Gene Name	early Th2 vs naive (log2-fold)
Il5	interleukin 5	7.5
Il10	interleukin 10	6.9
Il13	interleukin 13	6.8
Il3	interleukin 3	6.3
Il1rl1	interleukin 1 receptor-like 1	6.2
Il9	interleukin 9	6.2
Gp49a	glycoprotein 49 A	5.8
Mt2	metallothionein 2	5.6
Il4	interleukin 4	5.5
Hamp	hepcidin antimicrobial peptide	5.4
Cxcr6	chemokine (C-X-C motif) receptor 6	5.4
Penk	preproenkephalin	5.0
Tigit	T cell immunoreceptor with Ig and ITIM domains	5.0
Gzmb	granzyme B	4.9
Ctla4	cytotoxic T-lymphocyte-associated protein 4	4.9
Lilrb4	leukocyte immunoglobulin-like receptor, subfamily B, member 4	4.8
Ifitm3	interferon induced transmembrane protein 3	4.7
Ccr4	chemokine (C-C motif) receptor 4	4.6
Il17f	interleukin 17F	4.4
Perp	PERP, TP53 apoptosis effector	4.3
Gcnt1	glucosaminyl (N-acetyl) transferase 1, core 2	4.2
Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog	4.1
Ly6a	lymphocyte antigen 6 complex, locus A	4.1
Fgl2	fibrinogen-like protein 2	4.0
Ccr2	chemokine (C-C motif) receptor 2	4.0
Syt13	synaptotagmin-like 3	4.0
Ptpn22	protein tyrosine phosphatase, receptor type, J	4.0
Ttc39c	tetratricopeptide repeat domain 39C	4.0
Ccr8	chemokine (C-C motif) receptor 8	3.9
Atp2b4	ATPase, Ca ⁺⁺ transporting, plasma membrane 4	3.8
Icos	inducible T-cell co-stimulator	3.8
Cyp11a1	cytochrome P450, family 11, subfamily a, polypeptide 1	3.7
Cysltr2	cysteinyl leukotriene receptor 2	3.7
Osbpl3	oxysterol binding protein-like 3	3.7
Tnfrsf8	tumor necrosis factor receptor superfamily, member 8	3.7
Areg	amphiregulin	3.7
Cpd	carboxypeptidase D	3.6
Mid2	midline 2	3.6
Mmp13	matrix metalloproteinase 13	3.6
Ccl1	chemokine (C-C motif) ligand 1	3.6
Cysltr1	cysteinyl leukotriene receptor 1	3.6
Vdr	vitamin D receptor	3.6

Il21	interleukin 21	3.6
Prdm1	PR domain containing 1, with ZNF domain	3.6
Itgb8	integrin beta 8	3.6
Il1r2	interleukin 1 receptor, type II	3.5
Havcr2	hepatitis A virus cellular receptor 2	3.5
Olr1	oxidized low density lipoprotein (lectin-like) receptor 1	3.4
Nek6	NIMA (never in mitosis gene a)-related expressed kinase 6	3.3
Car5b	carbonic anhydrase 5b, mitochondrial	3.3
Ero1l	ERO1-like (S, cerevisiae)	3.3
Glrx	glutaredoxin	3.3
Bmpr2	bone morphogenic protein receptor, type II (serine/threonine kinase)	3.3
Casp4	caspase 4, apoptosis-related cysteine peptidase	3.3
4930506M07Rik	RIKEN cDNA 4930506M07 gene	3.3
Pparg	peroxisome proliferator activated receptor gamma	3.3
Mt1	metallothionein 1	3.2
Plin2	perilipin 2	3.2
Egln3	EGL nine homolog 3 (C, elegans)	3.1
Entpd1	ectonucleoside triphosphate diphosphohydrolase 1	3.1
Zbtb32	zinc finger and BTB domain containing 32	3.1
Raph1	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1	3.1
2010002N04Rik	RIKEN cDNA 2010002N04 gene	3.0
Slc15a3	solute carrier family 15, member 3	3.0
Spp1	secreted phosphoprotein 1	3.0
Gata3	GATA binding protein 3	3.0
Klrg1	killer cell lectin-like receptor subfamily G, member 1	3.0
Adam19	a disintegrin and metallopeptidase domain 19 (meltrin beta)	3.0
Plek	pleckstrin	2.9
Tnfsf10	tumor necrosis factor (ligand) superfamily, member 10	2.9
Ptpn13	protein tyrosine phosphatase, non-receptor type 13	2.9
Asb2	ankyrin repeat and SOCS box-containing 2	2.9
Ptpn4	protein tyrosine phosphatase, non-receptor type 4	2.9
Igfbp7	insulin-like growth factor binding protein 7	2.9
AW112010	expressed sequence AW112010	2.9
Zdhhc2	zinc finger, DHHC domain containing 2	2.8
Nfil3	nuclear factor, interleukin 3, regulated	2.8
Rgs1	regulator of G-protein signaling 1	2.8
Furin	furin (paired basic amino acid cleaving enzyme)	2.8
Lgals1	lectin, galactose binding, soluble 1	2.8
Lamc1	laminin, gamma 1	2.8
Tnfsf8	tumor necrosis factor (ligand) superfamily, member 8	2.8
Olfir60	olfactory receptor 60	2.8
Pth	parathyroid hormone	2.8
Wisp1	WNT1 inducible signaling pathway protein 1	2.8
Etv6	ets variant gene 6 (TEL oncogene)	2.8
Myo1e	myosin IE	2.8
Lag3	lymphocyte-activation gene 3	2.8

Ell2	elongation factor RNA polymerase II 2	2.8
Epas1	endothelial PAS domain protein 1	2.7
Il17a	interleukin 17A	2.7
Creb3l2	cAMP responsive element binding protein 3-like 2	2.7
Stom	stomatin	2.7
Mmp10	matrix metalloproteinase 10	2.7
Aqp9	aquaporin 9	2.7
Hdc	histidine decarboxylase	2.7
Ptpn3	protein tyrosine phosphatase, non-receptor type 3	2.7
Il10ra	interleukin 10 receptor, alpha	2.7
Stx11	syntaxin 11	2.6
Ccna2	cyclin A2	2.6
G530011O06Rik	RIKEN cDNA G530011O06 gene	2.6

Supplementary Table 2: Genes downregulated in early Th2 cells versus naive T cells

Gene Symbol	Gene Name	early Th2 vs naive (log2-fold)
Dapl1	death associated protein-like 1	-3.9
Ms4a4b	membrane-spanning 4-domains, subfamily A, member 4B	-3.9
Itm2a	integral membrane protein 2A	-3.8
Atp1b1	ATPase, Na ⁺ /K ⁺ transporting, beta 1 polypeptide	-3.7
Actn1	actinin, alpha 1	-3.7
Sell	selectin, lymphocyte	-3.6
Cmah	cytidine monophospho-N-acetylneuraminic acid hydroxylase	-3.5
St8sia6	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 6	-3.4
Tcf7	transcription factor 7, T-cell specific	-3.0
Tmem71	transmembrane protein 71	-3.0
Ipcef1	interaction protein for cytohesin exchange factors 1	-2.8
Lef1	lymphoid enhancer binding factor 1	-2.8
Gm16591	predicted gene, 16591	-2.8
Ms4a6b	membrane-spanning 4-domains, subfamily A, member 6B	-2.6
Qser1	glutamine and serine rich 1	-2.6
Ms4a6c	membrane-spanning 4-domains, subfamily A, member 6C	-2.6
Parp8	poly (ADP-ribose) polymerase family, member 8	-2.6
E430029J22Rik	RIKEN cDNA E430029J22 gene	-2.6
Ms4a4c	membrane-spanning 4-domains, subfamily A, member 4C	-2.5
Gramd3	GRAM domain containing 3	-2.5
Cd55	CD55 antigen	-2.5
Sidt1	SID1 transmembrane family, member 1	-2.5
D130062J21Rik	RIKEN cDNA D130062J21 gene	-2.5
Txk	TXK tyrosine kinase	-2.4
Fam49a	family with sequence similarity 49, member A	-2.4
Adamts6	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 6	-2.4
BC094916	cDNA sequence BC094916	-2.4
Pyhin1	pyrin and HIN domain family, member 1	-2.3
Sepp1	selenoprotein P, plasma, 1	-2.3
Tagap	T-cell activation Rho GTPase-activating protein	-2.3
Ephx1	epoxide hydrolase 1, microsomal	-2.2
Il21r	interleukin 21 receptor	-2.2
Rapgef4	Rap guanine nucleotide exchange factor (GEF) 4	-2.2
2610019F03Rik	RIKEN cDNA 2610019F03 gene	-2.2
1700025G04Rik	RIKEN cDNA 1700025G04 gene	-2.2
Sgk3	serum/glucocorticoid regulated kinase 3	-2.1
Bach2	BTB and CNC homology 2	-2.1
Klhl6	kelch-like 6 (Drosophila)	-2.1
A430078G23Rik	RIKEN cDNA A430078G23 gene	-2.1
Pde5a	phosphodiesterase 5A, cGMP-specific	-2.0
Trem12	triggering receptor expressed on myeloid cells-like 2	-2.0
Fam101b	family with sequence similarity 101, member B	-2.0
Atp8b4	ATPase, class I, type 8B, member 4	-2.0

Crtam	cytotoxic and regulatory T cell molecule	-2.0
Arhgef3	Rho guanine nucleotide exchange factor (GEF) 3	-2.0
St8sia1	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1	-2.0
Ccr7	chemokine (C-C motif) receptor 7	-1.9
Itga6	integrin alpha 6	-1.9
Mctp2	multiple C2 domains, transmembrane 2	-1.9
Boll	bol, boule-like (Drosophila)	-1.9
Trav14-3	T cell receptor alpha variable region 14-3	-1.9
Ifi203	interferon activated gene 203	-1.9
Slc44a2	solute carrier family 44, member 2	-1.9
Gm16452	predicted gene 16452	-1.9
Trav14d-1	T-cell receptor alpha variable region 14D-1	-1.8
Samd9l	sterile alpha motif domain containing 9-like	-1.8
Ltb	lymphotoxin B	-1.8
Arhgef18	rho/rac guanine nucleotide exchange factor (GEF) 18	-1.8
Gm4759	GTPase, very large interferon inducible 1 pseudogene	-1.8
Clec2i	C-type lectin domain family 2, member i	-1.8
Cnn3	calponin 3, acidic	-1.8
Cd9	CD9 antigen	-1.8
Ttyh3	tweety homolog 3 (Drosophila)	-1.7
Igf1r	insulin-like growth factor I receptor	-1.7
Utrn	utrophin	-1.7
Il1rl2	interleukin 1 receptor-like 2	-1.7
Pacsin1	protein kinase C and casein kinase substrate in neurons 1	-1.7
Gm13892	predicted gene 13892	-1.7
Dgka	diacylglycerol kinase, alpha	-1.7
B230325K18Rik	RIKEN cDNA B230325K18 gene	-1.7
LOC547323	similar to RIKEN cDNA A430107P09 gene	-1.7
Usp28	ubiquitin specific peptidase 28	-1.7
Pik3ip1	phosphoinositide-3-kinase interacting protein 1	-1.7
Lfng	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	-1.7
Cd27	CD27 antigen	-1.7
Ctsl	cathepsin L	-1.7
Dusp2	dual specificity phosphatase 2	-1.7
Snora75	small nucleolar RNA, H/ACA box 75	-1.6
Rassf2	Ras association (RalGDS/AF-6) domain family member 2	-1.6
Il6ra	interleukin 6 receptor, alpha	-1.6
Per1	period homolog 1 (Drosophila)	-1.6
Aff3	AF4/FMR2 family, member 3	-1.6
Gimap4	GTPase, IMAP family member 4	-1.6
Scarna17	small Cajal body-specific RNA 17	-1.6
Zbtb20	zinc finger and BTB domain containing 20	-1.6
Znrf3	zinc and ring finger 3	-1.6
Cblb	Casitas B-lineage lymphoma b	-1.6
H2-Ob	histocompatibility 2, O region beta locus	-1.5
Ift80	intraflagellar transport 80 homolog (Chlamydomonas)	-1.5

Plekhg2	pleckstrin homology domain containing, family G (with RhoGef domain) member 2	-1.5
Sik1	salt inducible kinase 1	-1.5
Klf2	Kruppel-like factor 2 (lung)	-1.5
Gm13969	predicted gene 13969	-1.5
Esy2	extended synaptotagmin-like protein 2	-1.5
Myo10	myosin X	-1.5
Hsd11b1	hydroxysteroid 11-beta dehydrogenase 1	-1.5
Gm614	predicted gene 614	-1.5
Iqcb1	IQ calmodulin-binding motif containing 1	-1.5
Chchd10	coiled-coil-helix-coiled-coil-helix domain containing 10	-1.5
Mef2d	myocyte enhancer factor 2D	-1.5
Dnajb4	DnaJ (Hsp40) homolog, subfamily B, member 4	-1.5

Supplementary Table 3: Top 100 Genes upregulated in stable Th2 cells versus naive T cells

Gene Symbol	Gene Name	stable Th2 vs naïve (log2-fold)
Il10	interleukin 10	7.4
Il5	interleukin 5	7.2
Il13	interleukin 13	6.5
Il1rl1	interleukin 1 receptor-like 1	6.4
Il9	interleukin 9	6.2
Il4	interleukin 4	5.9
Gp49a	glycoprotein 49 A	5.9
Penk	preproenkephalin	5.7
Mt2	metallothionein 2	5.6
Cxcr6	chemokine (C-X-C motif) receptor 6	5.5
Tigit	T cell immunoreceptor with Ig and ITIM domains	5.3
Ctla4	cytotoxic T-lymphocyte-associated protein 4	5.2
Lilrb4	leukocyte immunoglobulin-like receptor, subfamily B, member 4	4.8
Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog	4.7
Ccr2	chemokine (C-C motif) receptor 2	4.6
Il1r2	interleukin 1 receptor, type II	4.6
Hamp	hepcidin antimicrobial peptide	4.6
Fgl2	fibrinogen-like protein 2	4.5
Ccr4	chemokine (C-C motif) receptor 4	4.5
Gzmb	granzyme B	4.5
Ly6a	lymphocyte antigen 6 complex, locus A	4.4
Syt13	synaptotagmin-like 3	4.3
Areg	amphiregulin	4.3
Perp	PERP, TP53 apoptosis effector	4.3
Il3	interleukin 3	4.2
4930506M07Rik	RIKEN cDNA 4930506M07 gene	4.2
Itgb8	integrin beta 8	4.2
Ccr8	chemokine (C-C motif) receptor 8	4.2
Atp2b4	ATPase, Ca ⁺⁺ transporting, plasma membrane 4	4.2
Ttc39c	tetratricopeptide repeat domain 39C	4.1
Icos	inducible T-cell co-stimulator	4.1
Ptpnj	protein tyrosine phosphatase, receptor type, J	4.1
Vdr	vitamin D receptor	4.0
Gcnt1	glucosaminyl (N-acetyl) transferase 1, core 2	3.9
Prdm1	PR domain containing 1, with ZNF domain	3.9
Ifitm3	interferon induced transmembrane protein 3	3.7
Mid2	midline 2	3.6
Tnfrsf8	tumor necrosis factor receptor superfamily, member 8	3.6
Cpd	carboxypeptidase D	3.5
Ccr1	chemokine (C-C motif) receptor 1	3.5
Glrx	glutaredoxin	3.4
Havcr2	hepatitis A virus cellular receptor 2	3.4

Nek6	NIMA (never in mitosis gene a)-related expressed kinase 6	3.4
Osbp13	oxysterol binding protein-like 3	3.4
Ahr	aryl-hydrocarbon receptor	3.3
Bmpr2	bone morphogenic protein receptor, type II (serine/threonine kinase)	3.3
Cysltr2	cysteinyl leukotriene receptor 2	3.3
Lag3	lymphocyte-activation gene 3	3.3
Raph1	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1	3.3
Cysltr1	cysteinyl leukotriene receptor 1	3.3
Pparg	peroxisome proliferator activated receptor gamma	3.3
Cyp11a1	cytochrome P450, family 11, subfamily a, polypeptide 1	3.2
Tnip3	TNFAIP3 interacting protein 3	3.2
Il21	interleukin 21	3.2
Calca	calcitonin/calcitonin-related polypeptide, alpha	3.2
AW112010	expressed sequence AW112010	3.2
Il17f	interleukin 17F	3.2
Epas1	endothelial PAS domain protein 1	3.2
Asb2	ankyrin repeat and SOCS box-containing 2	3.1
Nfil3	nuclear factor, interleukin 3, regulated	3.1
Olfir60	olfactory receptor 60	3.0
2010002N04Rik	RIKEN cDNA 2010002N04 gene	3.0
Ptpn4	protein tyrosine phosphatase, non-receptor type 4	3.0
Neb	nebulin	3.0
Gata3	GATA binding protein 3	3.0
Tnfsf8	tumor necrosis factor (ligand) superfamily, member 8	2.9
Ero1l	ERO1-like (S, cerevisiae)	2.9
Rgs1	regulator of G-protein signaling 1	2.9
Erc1	ELKS/RAB6-interacting/CAST family member 1	2.9
Adam8	a disintegrin and metallopeptidase domain 8	2.9
Adam19	a disintegrin and metallopeptidase domain 19 (meltrin beta)	2.9
Igfbp7	insulin-like growth factor binding protein 7	2.8
Lgals1	lectin, galactose binding, soluble 1	2.8
Entpd1	ectonucleoside triphosphate diphosphohydrolase 1	2.8
Etv6	ets variant gene 6 (TEL oncogene)	2.8
Ccl1	chemokine (C-C motif) ligand 1	2.8
Ell2	elongation factor RNA polymerase II 2	2.7
Zbtb32	zinc finger and BTB domain containing 32	2.7
Casp4	caspace 4, apoptosis-related cysteine peptidase	2.7
Stx11	syntaxin 11	2.7
Runx2	runt related transcription factor 2	2.7
Klrg1	killer cell lectin-like receptor subfamily G, member 1	2.7
Arnt2	aryl hydrocarbon receptor nuclear translocator 2	2.7
Car5b	carbonic anhydrase 5b, mitochondrial	2.7
Egln3	EGL nine homolog 3 (C, elegans)	2.7
Gas2l3	growth arrest-specific 2 like 3	2.7
Tnfsf10	tumor necrosis factor (ligand) superfamily, member 10	2.7
Il10ra	interleukin 10 receptor, alpha	2.7

Tmtc2	transmembrane and tetratricopeptide repeat containing 2	2.7
Mt1	metallothionein 1	2.6
Slc15a3	solute carrier family 15, member 3	2.6
Ptpn13	protein tyrosine phosphatase, non-receptor type 13	2.6
Ptpn3	protein tyrosine phosphatase, non-receptor type 3	2.6
Plek	pleckstrin	2.6
Wisp1	WNT1 inducible signaling pathway protein 1	2.6
Lamc1	laminin, gamma 1	2.5
Cd200r1	CD200 receptor 1	2.5
Galnt3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3	2.5
Ier3	immediate early response 3	2.5
Cytip1	cytoplasmic FMR1 interacting protein 1	2.5
Trps1	trichorhinophalangeal syndrome I (human)	2.5

Supplementary Table 4: Top 100 Genes down regulated in stable Th2 cells versus naive T cells

Gene Symbol	Gene Name	stable Th2 vs naive (log2-fold)
Ms4a4b	membrane-spanning 4-domains, subfamily A, member 4B	-4.2
Sell	selectin, lymphocyte	-4.1
Atp1b1	ATPase, Na ⁺ /K ⁺ transporting, beta 1 polypeptide	-3.7
Iitm2a	integral membrane protein 2A	-3.7
Actn1	actinin, alpha 1	-3.7
Dapl1	death associated protein-like 1	-3.7
Cmah	cytidine monophospho-N-acetylneuraminic acid hydroxylase	-3.4
Lef1	lymphoid enhancer binding factor 1	-3.4
Tcf7	transcription factor 7, T-cell specific	-3.4
St8sia6	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 6	-3.4
Ipcef1	interaction protein for cytohesin exchange factors 1	-3.2
Gm16591	predicted gene, 16591	-3.1
Tmem71	transmembrane protein 71	-3.1
Qser1	glutamine and serine rich 1	-3.1
Parp8	poly (ADP-ribose) polymerase family, member 8	-3.0
Gramd3	GRAM domain containing 3	-2.9
Cd55	CD55 antigen	-2.8
Txk	TXK tyrosine kinase	-2.7
5830405N20Rik	RIKEN cDNA 5830405N20 gene	-2.7
Klhl6	kelch-like 6 (Drosophila)	-2.7
Adamts6	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 6	-2.7
Arhgef3	Rho guanine nucleotide exchange factor (GEF) 3	-2.7
Tagap	T-cell activation Rho GTPase-activating protein	-2.7
Gramd1b	GRAM domain containing 1B	-2.7
Ms4a6b	membrane-spanning 4-domains, subfamily A, member 6B	-2.5
Boll	bol, boule-like (Drosophila)	-2.5
Gm13949	predicted gene 13949	-2.5
Ms4a6c	membrane-spanning 4-domains, subfamily A, member 6C	-2.5
Atp8b4	ATPase, class I, type 8B, member 4	-2.5
Sidt1	SID1 transmembrane family, member 1	-2.5
Pyhin1	pyrin and HIN domain family, member 1	-2.5
Trav14-3	T cell receptor alpha variable region 14-3	-2.5
Sepp1	selenoprotein P, plasma, 1	-2.4
Gimap4	GTPase, IMAP family member 4	-2.4
LOC547323	similar to RIKEN cDNA A430107P09 gene	-2.3
Fam49a	family with sequence similarity 49, member A	-2.3
2610019F03Rik	RIKEN cDNA 2610019F03 gene	-2.3
E430029J22Rik	RIKEN cDNA E430029J22 gene	-2.3
BC094916	cDNA sequence BC094916	-2.3
Arhgap15	Rho GTPase activating protein 15	-2.2
D130062J21Rik	RIKEN cDNA D130062J21 gene	-2.2
Itga6	integrin alpha 6	-2.2

Ephx1	epoxide hydrolase 1, microsomal	-2.2
Ifi203	interferon activated gene 203	-2.2
Gm16452	predicted gene 16452	-2.2
Rasgrp2	RAS, guanyl releasing protein 2	-2.2
Gm8721	predicted gene 8721	-2.2
1700025G04Rik	RIKEN cDNA 1700025G04 gene	-2.2
Ccr7	chemokine (C-C motif) receptor 7	-2.2
Slc44a2	solute carrier family 44, member 2	-2.2
Bach2	BTB and CNC homology 2	-2.1
Myo10	myosin X	-2.1
Ltb	lymphotoxin B	-2.1
Ms4a4c	membrane-spanning 4-domains, subfamily A, member 4C	-2.1
Utrn	utrophin	-2.1
Mctp2	multiple C2 domains, transmembrane 2	-2.1
Il1rl2	interleukin 1 receptor-like 2	-2.1
Rassf2	Ras association (RalGDS/AF-6) domain family member 2	-2.0
Rasa3	RAS p21 protein activator 3	-2.0
Dgka	diacylglycerol kinase, alpha	-2.0
Fam101b	family with sequence similarity 101, member B	-2.0
Gm13892	predicted gene 13892	-2.0
Sgk3	serum/glucocorticoid regulated kinase 3	-2.0
Il6ra	interleukin 6 receptor, alpha	-2.0
Tmsb10	thymosin, beta 10	-2.0
Fam65b	family with sequence similarity 65, member B	-2.0
Trem12	triggering receptor expressed on myeloid cells-like 2	-2.0
Aff3	AF4/FMR2 family, member 3	-2.0
Ikbke	inhibitor of kappaB kinase epsilon	-2.0
Ly9	lymphocyte antigen 9	-2.0
Pde5a	phosphodiesterase 5A, cGMP-specific	-1.9
Rapgef4	Rap guanine nucleotide exchange factor (GEF) 4	-1.9
A430078G23Rik	RIKEN cDNA A430078G23 gene	-1.9
Usp28	ubiquitin specific peptidase 28	-1.9
Il21r	interleukin 21 receptor	-1.9
Samd9l	sterile alpha motif domain containing 9-like	-1.9
St8sia1	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1	-1.9
Crtam	cytotoxic and regulatory T cell molecule	-1.9
Trav14d-1	T-cell receptor alpha variable region 14D-1	-1.9
Spnb2	spectrin beta 2	-1.9
Cd9	CD9 antigen	-1.8
Smc6	structural maintenance of chromosomes 6	-1.8
Tle4	transducin-like enhancer of split 4, homolog of Drosophila E(spl)	-1.8
Ift80	intraflagellar transport 80 homolog (Chlamydomonas)	-1.8
Gm4759	GTPase, very large interferon inducible 1 pseudogene	-1.8
Gm13969	predicted gene 13969	-1.8
Arhgef18	rho/rac guanine nucleotide exchange factor (GEF) 18	-1.8
Cnn3	calponin 3, acidic	-1.8
5033414D02Rik	RIKEN cDNA 5033414D02 gene	-1.8

Znrf3	zinc and ring finger 3	-1.8
Snora34	small nucleolar RNA, H/ACA box 34	-1.8
Ecm1	extracellular matrix protein 1	-1.8
Apbb1ip	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein	-1.7
Sesn1	sestrin 1	-1.7
Gm6907	predicted gene 6907	-1.7
Per1	period homolog 1 (Drosophila)	-1.7
Cd52	CD52 antigen	-1.7
Esyt2	extended synaptotagmin-like protein 2	-1.7
Dnajb4	DnaJ (Hsp40) homolog, subfamily B, member 4	-1.7
Pvr	poliovirus receptor	-1.7
Prkcq	protein kinase C, theta	-1.7

Table 5: Top 10 KEGG*-pathways enriched in early Th2 cells versus naive CD4+ T cells

term	fold enrichment	p-value	Genes
mmu05321:Inflammatory bowel disease (IBD)	9.5	2.58E-10	MAF; IL4; IL6; IL18RAP; IL5; TBX21; IL13; RORA; IL21; IL10; STAT3; IL17A; IL12RB1; IL17F; IFNG
mmu05310:Asthma	9.4	3.54E-04	IL4; IL3; IL5; IL9; IL13; IL10
mmu04060:Cytokine-cytokine receptor interaction	4.9	2.20E-13	IL1R2; IL9R; CSF1; CCR1; BMPR2; TNFRSF8; IL13; TNFRSF4; IL10; IL17RB; TNFRSF1B; IL17A; IL12RB1; IL10RA; IFNG; CXCR6; IL4; IL3; IL2RB; IL6; IL5; IL18RAP; IL9; IL21; TNFSF8; CCR8; ACVR2A; TNFSF10; CCR4; CCR3; CCR2; BMPR1A
mmu04630:Jak-STAT signaling pathway	4.7	2.46E-07	IL4; IL3; IL2RB; IL6; IL9R; IL5; SOCS2; STAT5A; IL9; IL13; IL24; IL21; IL10; STAT3; CISH; IL12RB1; IL10RA; IFNG
mmu04672:Intestinal immune network for IgA production	4.5	2.46E-02	IL4; IL6; IL5; ICOS; IL10
mmu04640:Hematopoietic cell lineage	3.6	6.76E-03	IL4; IL1R2; IL3; IL6; IL9R; IL5; CD44; CSF1
mmu05203:Viral carcinogenesis	3.2	1.10E-05	CDK1; HIST1H2BB; RBL2; DDB1; STAT5A; HIST1H2BH; CDK6; MAPKAPK2; STAT3; HIST2H4; CCR8; HIST1H2BM; HIST2H2BB; CCR4; GSN; CCR3; CREB3L2; HIST1H4F; RBPJ; CCNA2
mmu05202:Transcriptional misregulation in cancer	3.2	4.52E-04	MAF; IL3; IL1R2; IL6; IL2RB; ARNT2; PPARG; GZMB; NUPR1; ID2; CDKN2C; ETV6; ETV5; RUNX2
mmu04350:TGF-beta signaling pathway	3.1	2.51E-02	ACVR2A; INHBA; ID2; ZFYVE16; IFNG; BMPR2; BMPR1A
mmu05133:Pertussis	3.0	4.63E-02	IL6; LY96; HC; IRF8; CASP1; IL10
mmu05200:Pathways in cancer	1.7	3.47E-02	PTGER2; IL6; PTGS2; EPAS1; STAT5A; PPARG; ARNT2; EGLN3; CDK6; CTNNA1; ARHGEF12; STAT3; RAD51; HIF1A; GNAQ; GNG2; GNB4; LAMC1

*KEGG- Pathway analysis was performed using DAVID Bioinformatics Resources 6.8.

Table 6: Top 10 KEGG*-pathways enriched in stable Th2 cells versus naive CD4+ T cells

Term	fold enrichment	p-Value	Genes
mmu05310:Asthma	11.7	1.25E-04	IL4; IL3; IL5; IL9; IL13; IL10
mmu05321:Inflammatory bowel disease (IBD)	10.3	2.66E-09	MAF; IL4; IL18RAP; IL5; TBX21; IL13; RORA; IL21; STAT3; IL10; IL12RB1; IL17F; IFNG
mmu04630:Jak-STAT signaling pathway	5.5	5.98E-08	IL4; IL3; IL2RB; IL5; SOCS2; SOCS3; STAT5A; IL9; IL13; IL24; IL21; IL10; STAT3; CISH; IL12RB1; IL10RA; IFNG
mmu04060:Cytokine-cytokine receptor interaction	5.0	4.53E-11	IL1R2; CSF1; CCR1; BMPR2; TNFRSF8; IL13; IL10; IL17RB; IL12RB1; IL10RA; CXCR6; IFNG; IL4; IL3; IL2RB; IL18RAP; IL5; IL9; IL21; TNFSF8; CCR8; ACVR2A; TNFSF10; CCR4; CCR3; CCR2
mmu05330:Allograft rejection	4.2	3.07E-02	IL4; IL5; IFNG; GZMB; IL10
mmu04640:Hematopoietic cell lineage	3.3	3.28E-02	IL4; IL1R2; IL3; IL5; CD44; CSF1
mmu04512:ECM-receptor interaction	3.2	3.89E-02	CD44; ITGB8; ITGAV; NPNT; LAMC1; SPP1
mmu04062:Chemokine signaling pathway	3.1	9.17E-04	CCL1; LYN; CCR1; CXCL2; STAT3; CCR8; TIAM1; CCR4; CCR3; CXCR6; CCR2; GNG2; GNB4
mmu05202:Transcriptional misregulation in cancer	3.1	2.75E-03	MAF; IL1R2; IL3; IL2RB; ID2; CDKN2C; PPARG; ARNT2; GZMB; ETV6; RUNX2
mmu05146:Amoebiasis	2.8	3.81E-02	IL1R2; GNA15; GNAQ; IFNG; LAMC1; IL10; VCL

*KEGG- Pathway analysis was performed using DAVID Bioinformatics Resources 6.8.

Table 1: Top 10 pathways formed by IDEAL miRNAs in the molecular interactome

Immediate targets of the IDEAL miRNAs and their first neighbors in the disease module were subjected to pathway enrichment analysis using the Enrich method (see Methods section). The selection was done according to a high combined score.

miRNA	Term	Overlap ¹	Adjusted p-value ²	Genes
miR-27b	Regulation of nuclear SMAD2/3 signaling	13/81	2.78E-08	IL10;SMAD2;HSPA8;SMAD3;VDR;MAX;GATA3;NR3C1;RUNX2;SP1;CDK4;SIN3A;CDK2
miR-206	mTOR signaling pathway	8/69	5.57E-05	IKBKB;CDK2;TSC2;TSC1;SFN;RAF1;YWHAZ; MTOR
miR-106b	TRIF-mediated TLR3/TLR4 signaling	9/97	7.39E-04	MEF2A;IKBKB;MAPK8;IRF3;FBXW11;RIPK1;MAP2K7; IKBKE;SKP1
miR-203	IL-5 Signaling Pathway	7/68	1.31E-04	LYN;STAT5A;PRKCD;STAT3;PTK2B;YWHAZ;VAV1
miR-23b	EGFR1 Signaling Pathway	16/171	4.0399E-08	SMAD2;STAT5A;SMAD3;STAT3;ASAP1;PTPN12;VAV1;ELK4;SP1;SIN3A;PTK2B;PTPN6; RAF1;RALGDS;GIT1

¹ Overlap: number of genes in Th2 disease module overlapping with genes annotated to respective pathway

² Adjusted p-value: p-value was calculated using the Fisher exact test and adjustment was done using Bonferroni correction.