Supplementary Figure 1 - Analysis of H3K27me3 and H3K4me3 profiles in WT and $\rm Ik^{\rm L/L}$ DP cells.



(a) Coverage plots for H3K27me3 and H3K4me3 centered on peaks that were increased/decreased ($\geq 1.8x$) or unchanged (<1.8x) in lk^{L/L} cells, as indicated. Values in parentheses indicate the numbers of islands in each group. Y-axes show the average normalized tag numbers in 20 bp bins 10 kb around the peak centers. (b) Venn diagram show the overlap between genomic regions with increased H3K27me3 or decreased H3K4me3 in lk^{L/L} DP thymocytes. The blue-colored heatmap shows k-means clustering of the tag densities of the corresponding genomic regions for each group. z

Supplementary Figure 2 - Global changes of H3K27me3 in WT and $lk^{L/L}$ thymocytes during differentiation.



(a) Top: Western blot of H3K27me3 in total cell lysates of DN2 to DP thymocytes from WT and Ik^{L/L} mice. Histone H3 is shown as loading control. Numbers indicate the H3K27me3 signals relative to H3, as quantified by ImageJ (v1.44). Bottom: Relative H3K27me3 levels in DN2 to DP thymocytes from WT and Ik^{L/L} mice based on the quantification of n=5 Western blots. Error bars, SD (b) Coverage plots of H3K27me3 centered on peaks that are increased/decreased ≥1.8x or unchanged (<1.8x) in the indicated populations. LSK data represent the average of 2 experiments. Values in parentheses indicate the numbers of islands in each group. Y-axes show average normalized tag numbers in 20 bp bins 10 kb around the peak centers. (c) Number of H3K27me3 islands that were changed >1.8x in Ik^{L/L} LSK and DN1 to DP cells compared with WT. LSK corresponds to the mean of 2 independent experiments. DP data correspond to islands that were changed in both the sorted DP ChIP-seq experiments (Fig. 2, a and b) and the DP/CD8 experiment (Fig. 1, a-c). (d) Representative genome browser tracks of H3K27me3 ChIP-seq data from the indicated cell populations. WT DP input controls in green.

Supplementary Figure 3 - Expression of genes with decreased H3K27me3.



(a) Scatter plots comparing the changes in H3K27me3 levels (x-axis) and the expression of the corresponding genes in the DN3, DN4 and DP subsets (y-axis). Genes that exhibited decreased H3K27me3 levels >1.8x in DN3, DN4 or DP cells were selected, and the corresponding probe sets on the Affymetrix 430 2.0 array identified. Where there were multiple probe sets for a given gene, only the one with the highest absolute fold change was retained. (b) Gene set enrichment analysis for the group *a* genes from Fig. 2c, showing their higher expression in LSK cells vs. DN3 cells. Probe sets from the Affymetrix 430 2.0 array were ranked according to the fold change between LSK and DN3 cells.



Supplementary Figure 4 - Ikaros DNA binding profiles during T cell differentiation.

(a) Genome browser tracks of Ikaros ChIP-seq in WT DP cells. Input controls in green. Cd3d is shown as a positive control. (b) Ikaros protein expression in early thymocytes. Western blots of whole cell lysates of WT 3.5x10⁴ (DN1) or 7x10⁴ (DN2-4, DP, CD4, CD8) thymocytes. Numbers indicate the lkaros signals relative to β-actin, as quantified by ImageJ (v1.44). Representative of \geq 3 independent experiments. (c) Venn diagram of the 41,137 merged genomic regions bound by Ikaros in DN3, DN4 and DP cells (corresponding to either a single peak or multiple peaks per region, as each merged genomic region may comprise multiple overlapping binding sites from the different populations; thus these numbers may not match those in the corresponding text). (d) Pie charts depicting the distribution of Ikaros peaks from DN3 (37,050), DN4 (23,656) and DP (5,351) cells among promoter regions (from -10 kb to +1 kb of the TSS), gene bodies or intergenic regions. (e) Motif analysis of Ikaros peaks. (f) Scatter plots depicting Ikaros enrichment over input (x-axis, log₂) vs lk^{L/L}/WT H3K27me3 FC (y-axis, log₂) in the DN3 and DN4 populations at regions with overlapping Ikaros binding and H3K27me3. The horizontal lines show the threshold used for the selection of differential H3K27me3 levels $[\log_2(1.8)=0.84].$





(a) Flow cytometry analysis of thymocytes from 3-wee-old lk^{f/f} Lck-Cre⁻ and lk^{f/f} Lck-Cre⁺ mice. Numbers indicate percentage of the gated populations. Mutant mice display an increase in the proportion of mature CD4⁺CD3⁺ and CD8⁺CD3⁺ cells. (b) Analysis of the thymus, spleen and lymph nodes (LN) of moribund lk^{f/f} Lck-Cre⁺ mice with T cell leukemia and a healthy lk^{f/f} Lck-Cre⁻ mouse of similar age (~4 months). Numbers indicate percentage. (c) Western blots of total cell lysates from the indicated organs from the mice illustrated in (b), analyzed with an anti-Ikaros antibody specific for the non-targeted N-terminal part of Ikaros. (d) Survival curves of lk^{f/f} Lck-Cre⁻ and lk^{f/f} Lck-Cre⁺ mice.

Supplementary Figure 6 - Decreased Suz12 binding in Ik^{L/L} DN3 thymocytes at Ikaros target genes.





(a) Western blots of whole cell lysates for Ezh2 abundance in WT and lk^{L/L} thymocyte subsets. Representative of 3 independent experiments. (b) Coverage plots of Suz12 peaks from WT (black) and Ik^{L/L} (red) DN3 cells, and Ikaros peaks from WT DN3 cells, centered on 9,541 Suz12 peaks (±2 kb) which were divided into: (left) 216 Suz12 peaks overlapping with Ikaros ($p \le 10^{-7}$) and H3K27me3 in WT cells at positions where H3K27me3 was decreased in Ik^{L/L} thymocytes; (middle) 3,056 Suz12 peaks overlapping with Ikaros where H3K27me3 was not decreased in Ik^{L/L} thymocytes; (right) the rest of the Suz12 peaks (6,269) that did not overlap with Ikaros. (c) Scatter plots and box plot analysis of Affymetrix gene expression data corresponding to the 3 groups of Suz12 peaks in (b). Red corresponds to the 216 Suz12 peaks in (b) which mapped to 187 genes that were represented by 430 probe sets. Blue corresponds to the 3,056 Suz12 peaks in (b) which mapped to 2,500 genes that were represented by 4,868 probe sets. Green corresponds to the 6,269 Suz12 peaks in (b) which mapped to 3,380 genes that were represented by 5,423 probe sets. Gray shows all of the probe sets present on the Affymetrix 430 2.0 microarrays. In the box plots, dashed lines indicate means, dots indicate the 5th and 95th percentiles.



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(a) Cell surface expression of CD4, CD8, CD25 and CD44 on ILC87 cells. Staining of WT thymocytes is shown as control. (b) Western blot of cytoplasmic (c) and nuclear (n) extracts of ILC87-Ik1-ER cells treated with EtOH or 4OHT for 1-3d as indicated. Histone H3 was used as a loading control. Results are representative of ≥3 independent experiments. (c) ILC87-Ik1-ER cells were treated with EtOH or 4OHT for 1-3d, or left untreated as indicated and then analyzed. WT thymocytes were used as reference. Numbers indicate the percentages. Representative of 2 experiments.

Supplementary Figure 8 - Activation of Ikaros in ILC87-Ik1-ER cells induces H3K27 trimethylation.



(a) Venn diagram of the Ikaros peaks identified in 4OHT- and EtOH-treated ILC87-Ik1-ER cells by ChIP-seq. The intersection contains the 400 peaks that overlap. Box plots of the normalized tag count distributions of the 400 overlapping lkaros peaks (right) indicate small lkaros binding in the EtOH control at these sites which is strongly increased in the 4OHT-treated cells. Dashed lines indicate means, dots indicate the 5th and 95th percentiles. (b) Representative genome browser tracks showing ChIP-seq of Ikaros, Suz12 and H3K27me3 in ILC87-Ik1-ER cells treated with 4OHT (+) or EtOH (-) for 1 (Ikaros) or 3 days (Suz12 and H3K27me3). (c) ChIP-qPCR analysis of Ikaros, H3K27me3 and Suz12 at the Scn4b locus in ILC87-Ik1-ER cells treated with 4OHT or EtOH for the indicated times. Control samples (Mig) are ILC87 cells transduced with the empty MigR1 vector and treated with 4OHT for 3d. Negative control (ctrl) regions were measured with primers annealing to sequences 7.8 kb upstream of the TSS of Mpzl2 (middle panel) or to exon 6 of Cd4 (right panel). % input = [(ab ChIP)-(IgG ChIP)]/1% input. Error bars, SD; (*): p<0.05, (**): p<0.01, two-sample t-test; n=3-7. (d) ChIP-qPCR analysis of Ikaros, H3K27me3 and Suz12 at the Ctnnd1 and Scn4b loci in ILC87-Ik1-Bcl2 cells constitutively expressing non-tagged WT lkaros and ILC87-Mig-Bcl2 control cells. Negative control (ctrl) regions were measured with primers annealing to exon 6 of Cd4. % input = [(ab ChIP)-(IgG ChIP)]/1% input. Results are representative of three experiments. Error bars represent the SD of technical duplicates.



Supplementary Figure 9 - Mta2 and Mi2β depletion in ILC87-Ik1-Bcl2 cells.

Western blots of Mta2 and Mi2 β capture on the beads that were used in the 3 consecutive IP steps for the depletion of these proteins from the nuclear extracts of ILC87-Ik1-Bcl2 cells in Fig. 7g. 1/4 of the Protein A/G beads coupled to anti-Mta2 and anti-Mi2 β antibodies (lanes 4-6) or to IgG (lanes 7-9) were analyzed from each IP step (1st-3rd). 5% of the depleted (lane 3) and non-depleted (lane 2) supernatants and 5% input (lane 1) are also shown. An overexposed image of the Ikaros Western blot is shown at the bottom.

Supplementary Figure 10 - Original scans of Western blots in Figures 3, 6 and 7



(a) corresponds to Fig. 3c. (b) corresponds to Fig. 6c. (c-i) panels c-i correspond to Fig. 7 a-g, respectively. Boxed areas correspond to the parts of the scans shown in the figures. White arrowheads point to IgG heavy chains.

Supplementary Figure 11 - Original scans of Western blots in Supplementary Figures 2, 4-7 and 9



(a) corresponds to Supplementary Fig. 2a. (b) corresponds to Supplementary Fig. 4b. (c) corresponds to Supplementary Fig. 5c. (d) corresponds to Supplementary Fig. 6a. (e) corresponds to Supplementary Fig. 7b. (f) corresponds to Supplementary Fig. 9. Boxed areas correspond to the parts of the scans shown in the figures. White arrowheads point to IgG heavy chains.

Supplementary Table 1 - 583 genomic regions with >1.8-fold decreased H3K27me3.

Chromosome	Start	End	Gene
chr1	16095800	16097599	Rdh10
chr1	34291400	34296799	Mir5103
chr1	39119800	39121599	Intergenic
chr1	51969800	51975999	Myo1b
chr1	53007800	53010799	1700019D03Rik
chr1	55463200	55464599	Plc1
chr1	59536600	59544199	Fzd7
chr1	59817800	59825399	Bmpr2
chr1	65230800	65235199	ldh1
chr1	69872600	69873999	Spag16
chr1	78564000	78565599	Intergenic
chr1	82278000	82295999	lrs1
chr1	87830200	87831999	Intergenic
chr1	94719400	94734399	Gpc1
chr1	94742400	94748599	Mir149
chr1	107739800	107742399	Tnfrsf11a
chr1	121358000	121359399	Intergenic
chr1	121538800	121540399	Epb4.1l5
chr1	121542000	121545599	Epb4.1l5
chr1	133856800	133864399	Slc45a3
chr1	133961000	133967799	Mfsd4
chr1	135074200	135080999	Intergenic
chr1	135694400	135697799	Intergenic
chr1	135750800	135752799	Intergenic
chr1	135802000	135807999	Optc
chr1	136192800	136197999	Муод
chr1	136275600	136280199	4933406M09Rik
chr1	137477000	137485599	Nav1
chr1	138692800	138695599	Intergenic
chr1	154469400	154473999	Rgl1
chr1	158239000	158243599	Nphs2
chr1	173364400	173373599	F11r
chr1	174135400	174137199	Pea15a
chr1	174171000	174176999	Atp1a4
chr1	180204600	180208999	Intergenic
chr1	181595800	181602999	Sccpdh
chr1	181777400	181778799	Intergenic
chr1	181883600	181892799	Cdc42bpa
chr1	182723000	182725999	Intergenic

chr1	182823400	182825999	Lefty2
chr1	183849600	183853999	Lbr
chr1	188522000	188532799	Tgfb2
chr1	191547800	191558999	Ptpn14
chr1	192795600	192806599	Vash2
chr1	193914200	193916399	Traf5
chr1	194656800	194664399	1700065J18Rik
chr1	194671400	194691799	Sertad4
chr1	194856000	194866999	Syt14
chr2	21061000	21067199	Intergenic
chr2	25649400	25650999	Gm13539
chr2	27530000	27536199	Rxra
chr2	28301200	28305799	Ppp1r26
chr2	30458600	30463799	Cstad
chr2	30784000	30785599	Intergenic
chr2	31798600	31809599	Aif1l
chr2	32165600	32179399	Mir199b
chr2	32866200	32867799	Slc2a8
chr2	34088800	34114199	Pbx3
chr2	35473200	35480799	Dab2ip
chr2	35585800	35589999	Intergenic
chr2	50995200	51006599	Rnd3
chr2	58415600	58423399	Acvr1
chr2	59446600	59453999	Tanc1
chr2	60976400	60978199	Intergenic
chr2	70495000	70496399	Gorasp2
chr2	71463000	71470199	Intergenic
chr2	73790400	73792399	Intergenic
chr2	75441400	75451999	Intergenic
chr2	75864600	75874999	Ttc30a1
chr2	75946400	75948599	Ttc30a1
chr2	79230000	79231399	Cerkl
chr2	79338800	79340599	Intergenic
chr2	84488000	84491799	Ctnnd1
chr2	90622200	90624999	Agbl2
chr2	91033600	91038399	Nr1h3
chr2	91289000	91304399	Lrp4
chr2	92209400	92213199	Gyltl1b
chr2	92367400	92372599	Intergenic
chr2	92752600	92760799	Syt13
chr2	94281000	94281799	Api5
chr2	101345800	101349199	Intergenic

chr2	101634400	101641599	Prr5l
chr2	102024200	102027999	Ldlrad3
chr2	102274400	102310999	Fjx1
chr2	113237800	113239999	Fmn1
chr2	116947200	116949799	Spred1
chr2	119898000	119904399	Intergenic
chr2	130996200	131000599	Spef1
chr2	131176000	131178399	Rnf24
chr2	132297200	132302399	Intergenic
chr2	145498200	145504399	Intergenic
chr2	152235200	152241799	Zcchc3
chr2	152284000	152285399	Defb23
chr2	152548400	152552199	Intergenic
chr2	152558600	152568399	ld1
chr2	152736600	152740599	Mylk2
chr2	154230600	154236599	Snta1
chr2	155211800	155212999	Trp53inp2
chr2	155339200	155340799	Ggt7
chr2	156895400	156910399	9830001H06Rik
chr2	164610400	164612799	Snx21
chr2	165207800	165215199	Zfp334
chr2	166832000	166833399	Ddx27
chr2	167172400	167177199	B4galt5
chr2	167384000	167388799	Intergenic
chr2	169121600	169124599	Intergenic
chr2	169457000	169462199	Tshz2
chr2	172170400	172173999	2010011I20Rik
chr2	172675800	172678399	Intergenic
chr2	172686400	172689799	Intergenic
chr2	172759000	172767999	Bmp7
chr2	181402400	181413399	Sox18
chr3	9172200	9174799	Intergenic
chr3	9301600	9304199	Intergenic
chr3	27605600	27614599	Fndc3b
chr3	58439800	58443199	Fam194a
chr3	58806800	58814199	Med12l
chr3	68669600	68676999	1110032F04Rik
chr3	80603200	80607999	Gria2
chr3	81876400	81879599	Gucy1b3
chr3	83993200	83996999	Gm6525
chr3	84055600	84065999	Intergenic
chr3	86971000	86984599	Kirrel

chr3	88304600	88309799	Lmna
chr3	90024400	90025399	Rab13
chr3	90317000	90318599	S100a1
chr3	95908800	95909799	Otud7b
chr3	95962400	95969199	Mtmr11
chr3	100906000	100921999	Ptgfrn
chr3	100963600	100970799	Intergenic
chr3	101179800	101207199	lgsf3
chr3	102905000	102907799	Intergenic
chr3	103427200	103429399	Syt6
chr3	104847000	104859599	Cttnbp2nl
chr3	104880000	104881599	4930564D02Rik
chr3	107984400	107991199	Amigo1
chr3	108086200	108090799	Sort1
chr3	114606800	114608199	Olfm3
chr3	116414000	116416599	Slc35a3
chr3	127407800	127410599	Neurog2
chr3	129878400	129887999	Col25a1
chr3	130780400	130791399	Intergenic
chr3	132605600	132615399	Npnt
chr3	142056600	142059199	Pdlim5
chr3	142361200	142366999	Ccbl2
chr3	145769600	145773799	Wdr63
chr3	146066600	146068999	Ssx2ip
chr3	148618000	148624199	Lphn2
chr3	153385800	153388599	St6galnac3
chr4	9593000	9597399	Asph
chr4	48550000	48557399	5730528L13Rik
chr4	53171400	53172599	Abca1
chr4	53453600	53456199	Slc44a1
chr4	57793600	57797199	Intergenic
chr4	62333400	62337399	Rgs3
chr4	86873000	86877799	Slc24a2
chr4	94715800	94718399	Jun
chr4	97986400	97988799	Intergenic
chr4	98784400	98790999	Dock7
chr4	107835600	107842599	Echdc2
chr4	108073600	108081599	Gpx7
chr4	116268600	116271399	C530005A16Rik
chr4	116766400	116771399	Ptch2
chr4	118905600	118907999	Lepre1
chr4	119406400	119408999	Intergenic

chr4	119819000	119824799	Intergenic
chr4	119912200	119915999	Intergenic
chr4	119921600	119964399	Foxo6
chr4	122909000	122918799	Heyl
chr4	129053800	129055999	Zbtb8a
chr4	132309800	132317599	Smpdl3b
chr4	132564000	132567399	Ahdc1
chr4	133958000	133959399	Pafah2
chr4	134318800	134328399	Ldlrap1
chr4	135686400	135692599	Intergenic
chr4	138468600	138469599	Otud3
chr4	141150400	141158999	Fblim1
chr4	141792600	141796599	Kazn
chr4	142886000	142893399	Pdpn
chr4	147309000	147313199	Plod1
chr4	147511200	147514199	Mad2l2
chr4	155408200	155410599	Tnfrsf18
chr4	155559800	155561999	Agrn
chr5	5514000	5515199	Cldn12
chr5	13396800	13400799	Sema3a
chr5	20208600	20210399	Phtf2
chr5	20559400	20561199	Ptpn12
chr5	23179200	23181199	Intergenic
chr5	31246400	31249399	Cgref1
chr5	36098800	36102999	Ablim2
chr5	42096200	42099399	Rab28
chr5	44485800	44494599	Prom1
chr5	52906600	52908799	Intergenic
chr5	66913000	66916999	Apbb2
chr5	76756400	76757399	Pdcl2
chr5	77444000	77446599	1700023E05Rik
chr5	77959400	77962399	Intergenic
chr5	93432400	93437799	Intergenic
chr5	103769400	103770799	Intergenic
chr5	114624000	114626599	Acacb
chr5	114962800	114965199	Intergenic
chr5	114985200	114990199	Intergenic
chr5	116738600	116740199	Ccdc60
chr5	117570400	117572199	Taok3
chr5	122295400	122296399	Fam109a
chr5	124134400	124136199	Clip1
chr5	128111200	128117399	Glt1d1

chr5	135723200	135729399	Fzd9
chr5	137055800	137058599	Intergenic
chr5	137370600	137372799	Intergenic
chr5	137787200	137796199	Ephb4
chr5	151988600	151995999	Stard13
chr5	152032600	152039399	Intergenic
chr6	8725600	8730799	lca1
chr6	17312200	17314799	Intergenic
chr6	30409800	30415799	Intergenic
chr6	34403600	34407399	Intergenic
chr6	38826400	38829599	Hipk2
chr6	39504400	39512799	Dennd2a
chr6	42641200	42645199	Fam115a
chr6	45009400	45011399	Cntnap2
chr6	48394600	48399999	Sspo
chr6	51559800	51565599	Intergenic
chr6	54273200	54286799	Prr15
chr6	70960600	70968999	Intergenic
chr6	70997600	71000799	Intergenic
chr6	87927600	87933199	H1fx
chr6	89307800	89314199	Plxna1
chr6	100237800	100239999	Rybp
chr6	100670000	100672999	Gxylt2
chr6	112220800	112227599	Lmcd1
chr6	114992000	114993999	Tamm41
chr6	115162000	115164199	Timp4
chr6	116143600	116145199	Tmcc1
chr6	119398400	119404999	Fbxl14
chr6	119491000	119496199	Wnt5b
chr6	119796800	119801399	3110021A11Rik
chr6	122246400	122249999	Intergenic
chr6	122288200	122289399	Phc1
chr6	124704600	124711599	Atn1
chr6	124804200	124810399	Leprel2
chr6	124857800	124863199	Lag3
chr6	124940000	124955599	Pianp
chr6	125262600	125264599	Ltbr
chr6	125328200	125332999	Plekhg6
chr6	125441600	125446999	Cd9
chr6	125551000	125556799	Vwf
chr6	128304000	128306399	Tulp3
chr6	135014600	135017599	Gprc5a

chr6	140370000	140374599	Plekha5
chr6	145690800	145699799	Intergenic
chr7	4114600	4118399	Cdc42ep5
chr7	17202600	17204799	Grlf1
chr7	19945200	19950799	Ppp1r13l
chr7	25854800	25859999	Grik5
chr7	51690200	51704599	Lrrc4b
chr7	52228600	52230999	Cpt1c
chr7	53037400	53056999	Lmtk3
chr7	63096000	63099999	Cyfip1
chr7	75089200	75104399	lgf1r
chr7	78616400	78618399	Intergenic
chr7	86931400	86939199	Intergenic
chr7	86986200	86995199	Anpep
chr7	87078600	87083999	2610034B18Rik
chr7	87169200	87171399	Zfp710
chr7	89076200	89083199	Hdgfrp3
chr7	89318400	89321799	Sh3gl3
chr7	97574400	97576599	Ccdc89
chr7	105258600	105261599	Муо7а
chr7	105504800	105512199	Tsku
chr7	107756600	107758799	Mrpl48
chr7	108041800	108044599	Mir3102
chr7	108981000	108989799	Inppl1
chr7	116813200	116818999	Intergenic
chr7	123448800	123453399	Intergenic
chr7	125735600	125741799	Tmc5
chr7	134779400	134781599	Intergenic
chr7	135559400	135563599	Rgs10
chr7	135665400	135668599	Bag3
chr7	135755200	135756799	Inpp5f
chr7	141860200	141865199	Dock1
chr7	149556000	149559999	lfitm10
chr7	150235200	150243799	R74862
chr8	13952200	13967799	2610019F03Rik
chr8	28368800	28372799	Eif4ebp1
chr8	32222400	32224199	Rnf122
chr8	32296600	32298999	Fut10
chr8	35035200	35044199	Rbpms
chr8	35792800	35797599	Intergenic
chr8	35866600	35874399	Dusp4
chr8	47235600	47237199	Intergenic

chr8	49073200	49078599	Wwc2
chr8	86715800	86717599	Intergenic
chr8	97412200	97414399	Intergenic
chr8	107676200	107680199	Intergenic
chr8	107872200	107875199	Slc9a5
chr8	109453600	109463999	Sntb2
chr8	118964600	118967199	Intergenic
chr8	119257400	119260199	Cdyl2
chr8	119385800	119389999	Intergenic
chr8	122784800	122790999	Intergenic
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chrX	35533000	35534799	Zbtb33
chrX	70917600	70920999	SIc6a8
chrX	91785400	91788399	Maged1
chrX	92220000	92222399	Spin4
chrX	96081200	96087399	Ophn1
chrX	162674200	162679199	Gpm6b

Supplementary Table 2 - Genes with 4OHT-dependent increase of H3K27me3 in ILC87-Ik1-ER cells.

Gene	Ikaros binding	Suz12 increase	Decreased H3K27me3 in primary thymocytes
Ahcyl2	+	+	-
Aif1I	+	-	+ ^a
Alcam	+	-	+ ^a
Apbb2	+	+	+ ^a
Arhgap26	+	-	+ ^b
Arhgef10	+/-	+	-
B3gnt6	+	+	-
C77080	+	+	+ ^b
Cacnb3	+	+	-
Chd7	+	+	+ °
Coro2a	+	+/-	-
Ctdsp2	+	-	- . a
Ctnna1	+/-	-	_
Cuedci	+	+	- . a
Cylip I Dook1	+	-	+
DUCKI	+	+	+
Eam163h	+	τ/-	- + ^b
Fndc3h	+ -		т + ^а
Fnin2	+	- r	- ·
Fosl2	+	+/-	-
Gfra2	+	+	-
Gli1	+	+	+ ^a
Gls2	+	+	+ ^b
Gpr83	+	-	+ ^a
H1f0	+	+	+ ^a
Норх	+	+/-	-
ler3	+/-	+	+ ^a
lgf1r	+	+	+ ^a
ll17rd	+	+/-	-
ltga11	+	+/-	-
Jun	+	+	+ ^a
Kctd1	+	+	-
Kctd12	+	+	+ *
Lhx2	+/-	-	- b
Lipg	+/-	-	+
Lpcati	+	+	+
LZISZ Maat4b	+	+/-	+
Nah2	+	+	T a
Nfia	+		+ b
Nkd1	+	+	
Parva	+	+	-
Pcaf2	+	+	+ ^b
Ppp1r9b	+	+	-
Pvrl1	+	+	+ ^b
Rbpms	+	+	+ ^a
Rnf182	+	-	+ b
Scamp5	+	+/-	+ ^a
Scn4b	+	+	+ ^a
Sertad4	+	+	+ ^a
Shb	+	+	+
Slc45a3	+	+	+ "
Smpdl3b	+	-	+ "
I bkbp1	+	+	+ [•]
	+	+	+-
	+	+	- . a
Upta1	+/-	-	+ ^b
VaSIII Wacf1	+ ,	- د	+
VVdSLL Xv/H1	+	+	- + ^b
	+	+	T

Genes with increased H3K27me3 and Ikaros binding in 4OHT-treated ILC87-Ik1-ER cells (see Fig. 5a and Supplementary Fig. 8b for specific examples). Genes in italics were identified through visual assessment of the data. The rest were selected bioinformatically as having an Ikaros peak in 4OHT-treated cells which overlapped with a H3K27me3 island that increased >1.8x between EtOH- and 4OHT-treated cells. The second column documents Ikaros binding that coincides with H3K27me3: (+) strong binding; (+/-) small peak but clearly above background. The third column documents increased Suz12 binding after 3d of 4OHT treatment: (+) clear increase; (+/-) small peak close to background level; (-) absent or unchanged Suz12 peak. The last column indicates whether H3K27me3 is decreased at the corresponding regions in primary Ik^{L/L} thymocytes: ^a identified among the 583 regions in Fig. 2b; ^b visual inspection of the data confirmed clear decrease of H3K27me3 at the corresponding region though it did not pass the bioinformatic selection threshold.

Supplementary Table 3- Biological functions associated with genes whose H3K27 trimethylation is regulated by Ikaros.

Biological		
Process	p-value	Genes NAB2 MYH10 PLD2 INHA SI C6A9 ATP2B1 SGK1 CDC42EP2 HI ADOB1 RAPH1 AMD1
Cell Morphology	1.41x10 ⁻⁶	TSC22D3,CLIP1,SCN4B,TBC1D4,RORA,SEZ6,ARHGEF11,GFAP,WNT5B,GOLPH3,RND 2,OSBPL1A,GJA1,ACP2,LMNA,FLOT1,RHOJ,INHBA,DDR1,OPHN1,DOCK1, RHOQ,CD9,CYFIP1,Dst,IRS1,ALCAM,HDGFRP3,RXRA,RAP2A,MMP14,PTPN14, PTPN12,EIF4EBP1,KLF9,CCDC88A,CSPG4,PDLIM5,IGF1R,PPARGC1B,SORT1, SEMA3F,PEX11A,AKAP5,Pmaip1,IRF4,PTPRK,SH3GL3,DAB2IP,GNAQ,MYOZ1,ERBB3, DOCK7,ID3,GRN,APLP2,MAPK14,PLEKHG5,SPRY2,NFIA,ATP1B2,PEA15,AMIGO1, APBB2 DSCAM,JAK3 Hydin L RP1, CTNND1
Cellular Assembly and Organization	1.41x10 ⁻⁶	MYH10,FH0D1,PLD2,SGK1,ATP2B1,CDC42EP2,RAPH1,CLIP1,SCN4B,SEZ6,Cstad, GFAP,ARHGEF11,HIPK2,GOLPH3,RND2,GJA1,ACP2,LMNA,FLOT1,RHOJ,ARHGEF17,I NHBA,DDR1,SHROOM4,DOCK1,OPHN1,RHOQ,CD9,Dst,CYFIP1,HDGFRP3,RXRA,RAP 2A,CTNNA1,EIF4EBP1,KLF9,CCDC88A,CSPG4,IGF1R,PPARGC1B,SORBS3,SEMA3F,P EX11A,AKAP5,PTPRK,RTKN,DAB2IP,GNAQ,DOCK7,GRN,MAPK14,INPP5J,NFIA,SPRY 2,AMIGO1,APBB2,ALS2CL,DSCAM,Hydin,LRP1,AKAP1,CTNND1
Cellular Development	1.41x10 ⁻⁶	NAB2,MYH10,PLD2,INHA,MAGED1,RDH10,SGK1,FNDC3B,RAPH1,TSC22D3,CLIP1,SC N4B,RORA,MCOLN3,SEZ6,GPC1,HIPK2,WNT5B,RND2,AXIN2,GJA1,PDE2A,PPP1R1B, LMNA,FLOT1,RHOJ,MYOF,DDR1,INHBA,DOCK1,OPHN1,RHOQ,CD9, Dst,IRS1,RXRA,SCD,RAP2A,RAB28,MMP14,BMPR2,EIF4EBP1,KLF9,SH3PXD2B,IGF1R ,PPARGC1B,SMOX,CUL9,PEX11A,AKAP5,PTPRK,RTKN,DAB2IP,ACVR1,GNAQ,ERBB3 ,DOCK7,ID3,GRN,MAPK14,PLEKHG5,SPRY2,NFIA,AMIGO1,DSCAM,CTNND1
Cellular Function and Maintenance	1.41x10 ⁻⁶	FHOD1,MYH10,PLD2,SGK1,ATP2B1,CDC42EP2,RAPH1,CLIP1,SCN4B,SEZ6,Cstad,AR HGEF11,GFAP,HIPK2,GOLPH3,RND2,GJA1,ACP2,FLOT1,RHOJ,ARHGEF17,MYOF,DD R1,INHBA,SHROOM4,DOCK1,OPHN1,RHOQ,CD9,Dst,CYFIP1,IRS1,HDGFRP3,RAP2A, CTNNA1,EIF4EBP1,KLF9,CCDC88A,IGF1R,PPARGC1B,SORBS3,SORT1,PEX11A,AKA P5,PTPRK,RTKN,DAB2IP,GNAQ,ERBB3,DOCK7,ID3,GRN,MAPK14,INPP5J,SPRY2,NFI A,PEA15,AMIGO1,ALS2CL,DSCAM,JAK3,Hydin,LRP1,AKAP1,CTNND1
Nervous System Development and Function	1.41x10 ⁻⁶	NAB2,MYH10,PLD2,MAGED1,SLC6A9,SGK1,ADAM11,RAPH1,SCN4B,RORA,SEZ6,MC OLN3,ZBTB20,CUX2,GFAP,HIPK2,WNT5B,RND2,GJA1,ACP2,PPP1R1B,GRIA2,FLOT1, LMNA,DDR1,DOCK1,OPHN1,RHOQ,CD9,Dst,RAI1,ARHGAP35,HDGFRP3,RAP2A,EIF4 EBP1,FXR2,KLF9,CSPG4,IGF1R,SORT1,SEMA3F,AKAP5,KLC2,PTPRK,SH3GL3,DAB2I P,GNAQ,ERBB3,DOCK7,ID3,GRN,APLP2,PBX3,SPRY2,NFIA,ATP1B2,PEA15,AMIGO1, APBB2,DSCAM
Tissue Development	1.41x10 ⁻⁶	NAB2,FHOD1,MYH10,PLD2,SGK1,RDH10,ESRRB,CDC42EP2,RAPH1,CLIP1,SCN4B,SE Z6,MCOLN3,ZBTB20,ARHGEF11,GFAP,GPC1,HIPK2,RND2,AXIN2,GJA1,PDE2A, BCOR,ACP2,LMNA,FLOT1,RHOJ,ARHGEF17,MYOF,DDR1,INHBA,DOCK1, OPHN1,TSHZ1,CD9,Dst,RAI1,IRS1,RXRA,FOXP4,RAP2A,RAB28,MMP14,CTNNA1, BMPR2,PTPN12,EIF4EBP1,KLF9,SH3PXD2B,GF1R,PPARGC1B,SORBS3,LUZP1, AKAP5,PTPRK,RTKN,DAB2IP,ACVR1,GNAQ,ERBB3,DOCK7,ID3,GRN,APLP2,MAPK14, INPP5J,PLEKHG5,NFIA,SPRY2,AMIGO1,APBB2,DSCAM,CHD7,LRP1,AKAP1,CTNND1
Embryonic Development	6.37 x10 ⁻⁵	NAB2,MYH10,RDH10,ESRRB,RAPH1,SCN4B,ARL13B,RORA,MCOLN3,ZBTB20,ARHGE F11,HIPK2,GRSF1,RND2,AXIN2,GJA1,ACP2,BCOR,LMNA,MYOF,INHBA,DDR1, SHROOM4,DOCK1,OPHN1,TSHZ1,CD9,PPP2R3A,RAI1,IRS1,ARHGAP35,PLTP, RXRA,FOXP4,RAP2A,MMP14,CTNNA1,BMPR2,PTPN12,EIF4EBP1,KLF9,CSPG4, IGF1R,PPARGC1B,SORT1,SEMA3F,LUZP1,AKAP5,DAB2IP,ACVR1,GNAQ,ERBB3, ID3,GRN,APLP2,MAPK14,NFIA,SPRY2,ATP1B2,PEA15,APBB2,DSCAM,CHD7,CTNND1
Organ Morphology	2.05 x10⁻⁴	NAB2,MYH10,INHA,MMP14,BMPR2,HLADQB1,TSC22D3,PDLIM5,IGF1R,CUX2,PPARG, C1B,GFAP,ARHGEF11,SEMA3F,AKAP5,GJA1,GNAQ,LMNA,MYOZ1,ERBB3,ID3,GPX7,I NHBA,DDR1,TSHZ1,DOCK1,OPHN1,PLEKHG5,IRS1,ATP1B2,NFIA,RAI1,APBB2, ARHGAP35,DSCAM,JAK3,RXRA,CHD7,FOXP4
Reproductive System Development and Function	2.05 x10 ⁻⁴	NAB2,MYH10,INHA,GJA1,SLC6A9,ACP2,PPP1R1B,RDH10,MMP14,GNAQ,ESRRB,ERB B3,DDR1,INHBA,TSC22D3,ASB1,GRN,KLF9,MAPK14,IRS1,IGF1R,DSCAM,RXRA
Lipid Metabolism	2.23 x10 ⁻⁴	SCD,PLD2,INHA,SGK1,BAG3,TSC22D3,IDH1,TBC1D4,RORA,CSPG4,IGF1R,ZBTB20,P PARGC1B,SORT1,LDLRAP1,PEX11A,GOLPH3,DAB2IP,CREB3L3,GNAQ,LMNA,INHBA, CD9,RAI1,IRS1,RBMS1,PLTP,RXRA,LRP1
Molecular Transport	2.23 x10 ⁻⁴	MYH10,SCD,PLD2,INHA,SLC6A9,SGK1,BAG3,TSC22D3,IDH1,TBC1D4,CSPG4,RORA, IGF1R,ZBTB20,PPARGC1B,SORT1,LDLRAP1,PEX11A,GOLPH3,SLC6A8,AKAP5,GJA1, CREB3L3,DAB2IP,GNAQ,LMNA,ERBB3,SLC44A1,INHBA,RHOQ,RAI1,IRS1,PEA15, RBMS1,PLTP,RXRA,LRP1
Small Molecule Biochemistry	2.23 x10 ⁻⁴	SCD,PLD2,INHA,SLC6A9,SGK1,BAG3,AMD1,TSC22D3,IDH1,TBC1D4,CSPG4,RORA, IGF1R,ZBTB20,PPARGC1B,SMOX,SORT1,B4GALT5,GPC1,LDLRAP1,PEX11A,GOLPH3 ,SLC6A8,AKAP5,GJA1,PDE2A,PPP1R1B,CREB3L3,DAB2IP,GNAQ,LMNA,ERBB3, SLC44A1,INHBA,RHOQ,CD9,RAI1,IRS1,PEA15,RBMS1,PLTP,RXRA,LRP1
Cardiovascular System Development	2.26 x10 ⁻⁴	NAB2,MYH10,PLD2,RAB28,RDH10,MMP14,BMPR2,HLADQB1,PTPN12,RORA,CSPG4, PDLIM5,IGF1R,PPARGC1B,ARHGEF11,GPC1,HIPK2,LUZP1,AKAP5,GJA1,PDE2A, BCOR,DAB2IP,ACVR1,GNAQ,LMNA,ERBB3,RHOJ,MYOF,GPX7,ID3,INHBA,DOCK1,

and Function		MAPK14,CD9,PLEKHG5,IRS1,SPRY2,ALCAM,RXRA,CHD7,FOXP4
		NAB2,ST6GAL1,MYH10,INHA,SLC6A9,RDH10,ESRRB,ARL13B,RORA,MCOLN3,CUX2,
		ZBTB20,ARHGEF11,GPC1,HIPK2,GRSF1,PDE2A,AXIN2,GJA1,ACP2,BCOR,GRIA2,
		LMNA,RHOJ,MYOF,GPX7,INHBA,DDR1,SHROOM4,OPHN1,DOCK1,TSHZ1,CD9,
		PPP2R3A,RAI1,IRS1,ALCAM,ARHGAP35,PLTP,RXRA,FOXP4,SCD,RAB28,MMP14,
		CTNNA1,BMPR2,PTPN1,EIF4EBP1,KLF9,CSPG4,PDLIM5,IGF1,PPARGC1B,SORT1,
Organismal	4	CUL9,SEMA3F,LUZP1,AKAP5,SH3GL3,DAB2IP,GNAQ,ACVR1,MYOZ1,ERBB3,ID3,GRN
Development	2.38 x10 ⁻	,APLP2,MAPK14,PLEKHG5,
		NFIA,SPRY2,ATP1B2,PEA15,APBB2,DSCAM,JAK3,CHD7,CTNND1
		ST6GAL1,INHA,ESRRB,,GNB4,RORA,EXOC2,ARHGEF11,TRAF5,HIPK2,WNT5B,
Cell-To-Cell	4	GOLPH3,RND2,GJA1,PPP1R1B,GRIA2,P2RX5,LAG3,MYOF,INHBA,OPHN1,DOCK1,
Signaling and	2.69 x10 ⁻	RPS6KA6,IRS1,ALCAM,GPR125,RAP2A,MOK,MMP14,CTNNA1,WNT8B,ZBTB33,
Interaction		EIF4EBP1,GSTT1,CSPG4,IGF1R,SORT1,LDLRAP1,PEX11A,AKAP5,KLC2,PTPRK,
		SH3GL3,RTKN,GNAQ,ACVR1,ERBB3,ID3,MAPK14,PBX3,SPRY2,PEA15,LRP1
		FHOD1,ST6GAL1,MYH10,PLD2,FNDC3B,AMD1,TSC22D3,RORA,GFAP,ARHGEF11,B4
		GALT5,GPC1,HIPK2,WNT5B,GJA1,PDE2A,GRIA2,LAG3,LMNA,FLOT1,INHBA,DDR1,
Cellular	1	DOCK1,RPS6KA6,CD9,CYFIP1,IRS1,ALCAM,ARHGAP35,PLTP,RXRA,RAP2A,RAB28,
Movement	5.26 x10 ⁻	MMP14,PTPN14,CTNNA1,BMPR2,PTPN12,CCDC88A,SH3PXD2B,CSPG4,IGF1R,SORB
		S3,SORT1,LDLRAP1,SEMA3F,PTPRK,ACVR1,ERBB3,ID3,GRN,MAPK14,PLEKHG5,NFI
	4	A,SPRY2,APBB2,JAK3,LRP1,CTNND1
Cellular	5.99 x10 ^{-₄}	MYH10,OSBPL1A,GJA1,SGK1,RTKN,GRIA2,LMNA,ESRRB,GPX7,MAPK14,RHOQ,CSP
Compromise		G4,SGIP1,IGF1R,HIPK2
		NAB2,GJA1,SLC6A9,RDH10,MMP14,GNAQ,BMPR2,TSHZ1,MAPK14,CD9,PBX3,RAI1,S
Respiratory		PRY2,PPARGC1B,DSCAM,RXRA
System	8.4 x10 ⁻⁴	
Development		
and Function		

Ingenuity Pathway Analysis of the biological functions associated with genes that had decreased H3K27me3 in $Ik^{L/L}$ DN or DP cells (392 genes represented on the Affymetrix 430 2.0 array). The biological functions with the most significant enrichment are shown, as well as the associated genes.

Mod2 Nr ACTGCTGTTAGAGGCCGTAGAGGCC 7800 Fig. 1d. Fig. 38c Nr ACTGCCATAGTCACTAGGCCACTAGTTA 2500 Fig. 1d Nr ACTGCCATGTCACTGCAGTTA 2500 Fig. 1d Nr CAGCTCGTCTGTGGTGTGCCCATTAC 2500 Fig. 1d Nr CAGCTCGTGTGTGTGTGGCCGTGTAGC -000 Fig. 1d, Fig. 3c Nr TGTGCAAGAGAAAGGCTG -000 Fig. 1d Nr CAGCACAGACAAAGGCAAGCCTAG 500 Fig. 1d Nr CAGCAGAGGCAAGGCAAGGCAAG 100 Fig. 1d Nr CAGCAAAGACCTGCAAGGCTG 3000 Fig. 1d Nr CAGCAAAGACTGCAAGCAAG 100 Fig. 1d Nr CAGCAAAGACTGCAAGGCAGA 100 Fig. 1d Nr CAGCAGACAAGCAGA 100 Fig. 1d Nr CAGCAGAGACAA 100 Fig. 1d Nr CAGCAGAGCAAA 1000 Fig. 1d Nr TAGCTGCAGGCAGAAACTCAC 1000 Fig. 1d Nr TAGCTGCAGGCGGGAAGAAATTCAC 1000 Fig. 1d <t< th=""><th>ChIP primers gene</th><th></th><th>Sequence</th><th>Amplicon's approximate mapping position relative to TSS [bp]</th><th>Used in</th></t<>	ChIP primers gene		Sequence	Amplicon's approximate mapping position relative to TSS [bp]	Used in
rev TTTGSCATAGTSCATAGGTTCC 1000 Fig. 1d. N GTTTGSCATAGTSCATA 2500 Fig. 1d N CACCCCACTTCCTTCCACTTA 2500 Fig. 1d N CACCCCCCACAACCCAAACCCTG -100 Fig. 1d N CACCCCCCCAACAACCCAAG -000 Fig. 1d. N CACCCACCAAACCCAAG -000 Fig. 1d. N CACCACACAACCCAAG 100 Fig. 1d. N CACCACAAACCCACCAGG 500 Fig. 1d. N CACCACAAACCCACCCACAGG 100 Fig. 1d. N CACCACAAACCCACCCACAACCACCCAAGA 100 Fig. 1d. N CACCACAAACCCACCCACAACCACCCAAGA 100 Fig. 1d. N CACCACAAACCCACACCAAACCACCAACCACCAACCACC	Mpzl2	fw	ACTGCTGTTAGGAGGCCGTAGAGA	7800	Fig. 1d: Fig. Sto
Nm GTTTGATTGANGTGGCCCACAGC -2600 Fg. 1d Nm CAGCTTGCTGTGTGGCCTTACC -100 Fg. 1d Nm CAGCTTGCTGTGTGGCCTTACC -500 Fg. 1d. Nm TGTGGCACAGCACAAGCAAAGCAGG -500 Fg. 1d. Nm TGTGGCAGAGAAAGCAGG -500 Fg. 1d. Nm TGGCTTTCGTGATGTATGGATGGA 100 Fg. 1d. Nm CGGAAGAAAGCCAGG -500 Fg. 1d. Nm CGGAAGAAAGCCAGG 500 Fg. 1d. Nm CGGAAGAAAGCCAGG 4100 Fg. 1d. Nm CGGAAGAAAGCCAGGAGGAAGGA 4100 Fg. 1d. Nm TGAGCGAAGAAAGCAGGA 42000 Fg. 1d. Nm TGAGCAAGAAGAAGGA 2200 Fg. 1d. Nm TGAGCAAGACAAATCCCAGGAGGAAGGA -500 Fg. 1d. Nm TGAGCAAGCCCAAGAACTCAG -500 Fg. 1d. Nm TGAGCAAGCCCAAGAACTCAG -500 Fg. 1d. Nm TGAGCAAGCCCAAGAACTCAG -500 Fg. 1d. Nm TGAGCAGCCCAGAG		rev	TTTGGCCATAGTCACATAGGTTCC	-7000	
rev ACCCCCATCCTCTACC 100 Fig. 14 rev CTGCCCACAACCAAACCCTTACC 100 Fig. 14 rev CTGCCCACAACCAAACCCACC 500 Fig. 16 rev ACTCACCCCAAACCAACCACC 500 Fig. 17 rev ACTCACCCCAAACCAACCAACCACC 500 Fig. 14 rev GCCATTGCTTACCTGCTATAGG 500 Fig. 14 rev GCCATTGCTTACCGTCTACGG 500 Fig. 14 rev GCCAATGGTGGGGAAGCAACGGG 100 Fig. 14 rev CGTGCAATGGTGGGGAAGCAACGGG 100 Fig. 14 rev CGTGCAATGGTGGGGAAGCAAGGG 2200 Fig. 14 rev TACTTCTGTGGGAATGCAAGGGG 2200 Fig. 14 rev TACTTCTGTGGGAATGCAAGGG 2200 Fig. 14 rev TACATGTGGGAAGCAACACCAACGGG 1000 Fig. 14 rev TACATGTGGGAAGCAAGATGGGAGGGAAGGGA 500 Fig. 14 rev CATGGGGGGGGGAAGAGGA 500 Fig. 14 rev CTGGCGGGGGGGAGGAAGGAG 500 Fig. 14		fw	GTTTTGATTGAAGTTCCGCACAC	-2500	Fia. 1d
M CAGCTTGCTGTGTGTGCCCTTGTACC 100 Fig. 1d M TGTGGGGGAAAAGCAAACCAAACCAACCAACCAACCAACC		rev	ACGCCCAGTTCCTTCCAGTTA		
rw CTGGCCACAMAGACTACC -500 Fig. 16, Fig. 3c rw ACTCAGCAGAAACAACCAAG -500 Fig. 16, Fig. 3c rw ACTCAGCAGAAACAACCAAG 100 Fig. 14 rw GGCATTGGTAGAAATCCTAGA 100 Fig. 14 rw AGGCATTGGTAGAAATCCTAGA 100 Fig. 14 rw CAGACAAATCCTCCCACGG 500 Fig. 14 rw CAGACAAATCCTCCCACGG 100 Fig. 14 rw CAGACAAACTCACCCCCACTCAGC 4000 Fig. 14 rw CAGCCATTGCTTCACCCCCCACTCAG 4000 Fig. 14 rw TAGCCCTACCTCTCACCCCC 4000 Fig. 14 rw TAGCCCTACCCCTCATTCAG 4000 Fig. 14 rw TAGCCCTACCCCAGACAC -1000 Fig. 14 rw TAGACTCCTGACACTCCAGACA -1000 Fig. 14 rw TAGACTCCTGACACTCCAGACA -1000 Fig. 14 rw TAGACTCCTGACACTCCAGACAC -1000 Fig. 14 rw TAGACTCCTGACACTCCAGACAC -100 Fig. 14 rw <td></td> <td>fw</td> <td>CAGCTTGCTGTTGTGCCTCTTACC</td> <td>-100</td> <td>Fig. 1d</td>		fw	CAGCTTGCTGTTGTGCCTCTTACC	-100	Fig. 1d
Nm Initial indexides/addebace/addeb		rev	CTGCCCACAAACCAAAAGCCTG		
Inv ACCONSTRUCTOR 100 Fig. 1d Inv GGCANTGGTAGAAAATCCTAGA 100 Fig. 1d Inv GGCANTGGTTACAGATCCAGG 500 Fig. 1d Inv CASACAAATCCTCAGGT 500 Fig. 1d Inv CASACAAATCCTCAGGT 100 Fig. 1d Inv CASACAAATCCTCAGCCCTCATGG 4100 Fig. 1d Inv CACCCTATCGTCACCCCTCATCAG 8000 Fig. 1d Inv TACCTCCTAGACCCTCATCAGC 8000 Fig. 1d Inv TACCTCCTAGACCACAAAAACAAA 2200 Fig. 1d Inv TAGACATGCTAAACTCCAAAAA 2200 Fig. 1d Inv TAGACATGCTGAACAATCCACAAAA 2200 Fig. 1d Inv TAGACATGCTGAACAATCCACAAAA 100 Fig. 1d Inv TGAACATGCTGAACAATCCACAAAA 0 Fig. 1d Inv TGAACATGCTGAACAACTCACAC 1000 Fig. 1d Inv TGAACATGCTGAACACTCACACT 1000 Fig. 1d Inv TGAACATGCTGAACACACTCACACC 7800 Fig. 1d		fW		-500	Fig. 1d; Fig. 3c
Image of the constraint o		fev			
Image: Add Construction Constructin Constructin Construction Construction Construction Construction		rov	GGCAATGGTAGAAAATCCTAGA	100	Fig. 1d
nv CARACAMATICCTOCACCG 500 Fig. 1d Nv CTGCATTGAGGCTOTANGGC 100 Fig. 1d Nv CTGCATTGAGGCTANGGC 100 Fig. 1d Nv CATGCATTGAGGCTANGGC 4100 Fig. 1d Nv CATGCATGCACCTATCAGC 48000 Fig. 1d Nv TAGCCCATGCTACCCCCTATCAGC 48000 Fig. 1d Nv TAGCAGAGCCAAACGAAAGAG 2200 Fig. 1d Nv TAGCAGAGCCAAACGAAAGAG 2200 Fig. 1d Nv TAGCAGAGCCGAACAGAAAGAG 2200 Fig. 1d Nv TAGCAGAGCCGAACAGAAGAGG 2200 Fig. 1d Nv TGAGCATACCCAAGGCAAACGAAAGAG 2200 Fig. 1d Nv TGAGCATCCCAAGGCAAGAAGAAGAG 200 Fig. 1d Nv TGAGCATCCCAAGCAGAGAAGATAA 0 Fig. 1d Nv TGAGCATCCCAAGGCAGAGAAGATAA 0 Fig. 1d Nv TGAGCATCCAAGCAGAGAAGATAA 0 Fig. 1d Nv TGAGCAGCGAGCAGAAGAAGAAGA 0 Fig. 1d Nv		fw	AGGCTTTCTGATCTCAGGT		
In Control Con		rv	CAGACAAAATCCTCCCACGG	500	Fig. 1d
rv CTGCATGGAGGGAAGGAAGGA 100 Fig. 1d Nm ATCGGACGTGAGGGGCTTATGG 4100 Fig. 1d Nm TAGCCCTATCACCCCCTATTCAG 4000 Fig. 1d Nm TAGCCCTATCCTCTCACACTC 40000 Fig. 1d Nm TAGCCATACCCTCAACGCAAAGAA 2200 Fig. 1d Nm TAGCACATCCTGAACACTCCAACGAAAGAAGA 2200 Fig. 1d Nm TAGCACATCCTGAACTCCAAGAAA -1000 Fig. 1d Nm GATACATTGCTGAACTCCAAGAAA 0 Fig. 1d Nm TAGCGACAGGGAGGGAGACAAA 0 Fig. 1d Nm CGGACGGGCGGAGGAACAAA 0 Fig. 1d Nm CGGACGGGCGGGAGGACAAA 0 Fig. 1d Nm CGGACGGCGCGGAGGAACAA 0 Fig. 1d Nm CGGACGGGCGGGAGGAACAA 0 Fig. 1d Nm CGGACGGGCGGGAGGAACAA 0 Fig. 1d Nm CGGACGGGCGCGGGAGGAACAC 2000 Fig. 1d Nm CGGACGGGGCGGGAGGACACA 7800 Fig. 1d Nm CGG		fw	CGGTAAGGCTGTGAAGAAAGTGAG		——————————————————————————————————————
Iw ATCTGAACGTTGGGGCTTATGG 4100 Fig. 1d curva1 Iw TAGCCTATCACCTTGTTCACCTC -8000 Fig. 1d rv TAGCCAAACAGAAACAGTCAC -8000 Fig. 1d rv TAGCCAAACAGAAACAGTCAC -1000 Fig. 1d rv TAGCCAAACAGAACAGTCACAA -1000 Fig. 1d rv TAACATGCCTAATCCCTAAGCCT -1000 Fig. 1d rv TAACATGCTAATCCTAAGCTCAAAGG -2200 Fig. 1d rv TAACATGCTAATCCTAATCCTAAGCT -500 Fig. 1d rv CAGCTGGGCGGAGGAACAAA 0 Fig. 1d		rv	CTGCAATGGAGGGAAGGAAGAG	100	Fig. 1d
rv CAACCCTACCCTCATCAGC 100 Fig. 1d Chna1 IW TAGCCTATCATTCAACATC -0000 Fig. 1d rv TAGCTAACATTCACACATC -0000 Fig. 1d rv TAGCAGAAGCCAAACAGAAGAG -2200 Fig. 1d rv TAAACATCCTAACTCCACACAC -1000 Fig. 1d rv TAAACATCCTAACTCCACACAC -5000 Fig. 1d rv TAAACATCCTAACTCCAACACACTTCAC -5000 Fig. 1d rv CAGAGGGAGGAACAAAT 0 Fig. 1d rv CGGAGGGGCGGAGACAAA 0 Fig. 1d rv CGGAGGGGCGGAGACAAAA 0 Fig. 1d rv CGGAGGGGCGGAGACAAA 0 Fig. 1d rv CGGAGGGGCGGAGACAAAA 0 Fig. 1d rv CGGAGGGGCGGAGGACAAAA 0 Fig. 1d rv CGGAGGGGCGGGAGGACAAA 0 Fig. 1d rv CGACGGGCGGGAGGACACA 2000 Fig. 1d rv TGGCGGGCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG		fw	ATCTGGACGTTGGGGCTTATGG	4100	Fig. 1d
Nm TAGECCTATGCTTTCTACACTC -8000 Fig. 1d Nr TAGECTATGCTTTCTACCTC -8000 Fig. 1d Nr TAGCAGAAGCAAACGAAAGAG -2200 Fig. 1d Nr TAGCAGAAGCCAAACGAAAGAC -1000 Fig. 1d Nr TAGCAGTACCTAGCC -1000 Fig. 1d Nr TAGCAGTACCTAGCCAAGC -500 Fig. 1d Nr TAGCACTGCGGCGGGGGAGCAAA 0 Fig. 1d Nr CGGACGGGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGG		rv	CAACCCTACTCACCCCTCATTCAG	4100	rig. ru
IN TACTITICIGGAATIGCTOCIG Intervention Intervention IN TAGCAGAAGCCAAACGAAAGA 2200 Fig. 1d IN GAACATGCTAAACCGAAAGA -1000 Fig. 1d IN GAACATGCTAATCCTCAACACA -1000 Fig. 1d IN GAGAGTCCGTTCTACTCT -500 Fig. 1d IN TGAGGATCCCGTTCTACTCT -500 Fig. 1d IN TGAGGATCCCGTCAACACACA 0 Fig. 1d IN CGGAGGGGGCGGGAGGAAATG 0 Fig. 1d IN CGGAGGGGGCGGGAGGAAGTA 0 Fig. 1d IN CGGGGCCGGGAGCAGGAAGTA 0 Fig. 1d IN CGGGGGCGGGAGGAGGGGTGTTGTAGG 2000 Fig. 1d IN TGACGTAATCACACACACAC 2000 Fig. 1d IN CGTGGGCCGGAGAGAGGAGCACAC 7800 Fig. 1d IN CGTGGGCCGGAGAGAGAGACACC 7800 Fig. 1d, Fig. 3c IN TGGGCATACACCCAGACAC 7800 Fig. 1d, Fig. 3c IN TGGGCATACACCCAGATACACC 0 Fig. 1d, Fig. 3c	Ctnna1	fw	TAGCCCTATGCTTTCTACACTC	-8000	Fig. 1d
M TAGCAGAAGCCAAACAGAAGAGA -2200 Fig. 1d M GATACAGTGGCAAACAACTCCAC -1000 Fig. 1d M GATACAGTGGCAAACACTCCAC -1000 Fig. 1d M TGAGGGCAACAACTCCAC -500 Fig. 1d M TGAGGGCAGCAACA 0 Fig. 1d M CCGAGGGGCGGCAACAA 0 Fig. 1d M GGCGGCGGGGAACAAA 0 Fig. 1d, Fig. 3c M GGCGGCGGCGGCAGCAACAA 0 Fig. 1d, Fig. 3c M GGTCGGCGCGGGCAGCAAGAATTA 0 Fig. 1d M GGTCGGCCGGCGGCAGCAAGAATTA 0 Fig. 1d M GGTCGGCCGGCGCGCGCGCGCGCAGCAATGA 0 Fig. 1d M GGTCGGCCGCGCGCGCGCAGCAATGA 2000 Fig. 1d M GATCTGCCTAATCCACCCCA 7800 Fig. 1d M TGGGGATAGCAACCA 7800 Fig. 1d, Fig. 3c M GATCGCCCCGAATCACC 0 Fig. 1d, Fig. 3c M GATCGCCCCAGATCACCA 0 Fig. 1d, Fig. 3c M GATCGC		rv	TACTTCTCTGGAATTGTCTCCTG		
IV TGAACATGCTGACAATCACTTACC -1000 Fig. 1d IV GATACAGTGGCAAACAACTTACC -1000 Fig. 1d IV TAAACTCCTAATCCTCAGGAC -500 Fig. 1d IV CGGACGGCAGGAAGAACTTA 0 Fig. 1d IV CGGACGGCAGGAAGAATA 0 Fig. 1d IV CGGACGGCAGGAAGAATA 0 Fig. 1d IV GGTCGGGCAGGAAGATG 0000 Fig. 1d, Fig. 3c IV GGTCGGGCAGGAAGATG 10000 Fig. 1d IV AAATTCCAATACAACACCACCACTC 2000 Fig. 1d IV AAATTCCAATACAACACCACCACT 7800 Fig. 1d IV CTGACATACAAGGCATGAACACC 7800 Fig. 3c IV TCGACATACAAGGCATGAACCAC 7800 Fig. 3c IV TCGACATACAAGCCAGAATACAC 7800 Fig. 3c IV TCGACATACAAGCACACCAC 7800 Fig. 3c IV TCGACATACAAGCCAGAATACAC 7800 Fig. 3c IV TCGACATACAAGCCAGACATGG 0 Fig. 3c IV <t< td=""><td></td><td>fw</td><td>TAGCAGAAGCCAAACAGAAAGAG</td><td>-2200</td><td>Fig. 1d</td></t<>		fw	TAGCAGAAGCCAAACAGAAAGAG	-2200	Fig. 1d
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$\begin{array}{ c c c c c c } \hline Fig. ATAGETTICTETCECCECTENTITATEGCC & 0 & Fig. 1d; Fig. S8d \\ \hline rv & TTCCCCTCAGATCCAACAC & 0 & Fig. 1d; Fig. S8d \\ \hline rv & TTTCCCCTCAGATCCAACAC & -200 & Fig. 1d; Fig. 3c \\ \hline rv & TTTCGGGACTTCCGTTTCAC & 0 & Fig. 1d \\ \hline rv & TTTCTGGGACTCCGTTTCAC & 0 & Fig. 1d \\ \hline rv & CCTCACGGGTCCATCCTC & 0 & Fig. 1d \\ \hline rv & CCTCACGGGGTCCATCCTC & -600 & Fig. 1d; Fig. 3c; Fig. S8c \\ \hline rev & TTGTTTCTGTTGGTTCCAG & -600 & Fig. 1d; Fig. 3c; Fig. S8c \\ \hline rev & TGGACGAGAGAGGGGCTG & -300 & Fig. 3c; Fig. 6d; Fig. S8c, d \\ \hline rv & CGGAGCTGAAGGAGGGCTG & -300 & Fig. 3c; Fig. 6d; Fig. S8c, d \\ \hline rv & CGGAGCTGAAGGGAGCC & -300 & Fig. 3c; Fig. 6d; Fig. 3c; Fig. 6d; Fig. 3c; Fig. 6d; Fig. 3c; Fig. 6d; Fig. 3c; Fig. 3c \\ \hline rv & CGGAGCTGAAGGGAGCC & -300 & Fig. 3c \\ \hline rv & GAGAGCGGACTGCTAGCA & 1400 & Fig. 3c \\ \hline rv & CCTCACGTGTGCCGGGGGCC & -100 & Fig. 3c \\ \hline Tb21 & fw & CAGGTGGTTGCTGAGCGCC & -100 & Fig. 3c \\ \hline rv & CGCGGGGCTCTACCCAGGGC & -100 & Fig. 3c \\ \hline rv & CGCGGGCCTCTACCCAGGGC & -100 & Fig. 3c \\ \hline rv & CTCCTGCAGCAACGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCAGCAACGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCAGCAACGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCGCGCAAAGGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCGCGCAAAGGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCGCGCAAAGGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCGCGCAAAGGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCGCGCAAAGGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCGCGCAAAGGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCGCGCAAAGGGCCC & -100 & Fig. 3c \\ \hline rv & CTCCTGCGCGCAAACTGAGCACTC & 17200 & Fig. 3c \\ \hline Tubb2a & fw & GACAGAGGAAACTGAGCACCC & 3300 & Fig. 3c \\ \hline Tubb2a & fw & GACAGAGGCAAACTGAGCACCC & -3300 & Fig. 3c \\ \hline Tubb2a & fw & GACAGAGGCAACTGAGCACCC & -3300 & Fig. 3c \\ \hline Tubb2a & fw & GACAGAGGCAACTGAGCACCC & -3300 & Fig. 3c \\ \hline Tubb2a & fw & GACAGAGGCAACTGAGCCCC & -3300 & Fig. 3c \\ \hline Tubb2a & fw & GACAGAGGCAACTGAGCACCC & -3300 & Fig. 3c \\ \hline Tubb2a & fw & GACAGAGGCCAACTGAGCCCC & -3300 & Fig. 3c \\ \hline Tubb2a & fw & GACAGAGGCCAACTGAGCCCC & -3000 & Fig. 3c \\ \hline Tubb2a & fw & GACAGAGGCCAACTGAGCCCC & -3000 & Fig. 3c \\ \hline Tubb2a & f$	Clinia	fw	GATTCTGCCTATTGTCCCAG	-600	Fig. 3c
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rv CTTCCTGCAGCTACTGCTTC 1200 Fig. 3c Cd4 fw CACAACGAGAATGGACTAAT 17200 (exon6) Fig. S8c,d rv TGGGTACTGGAGGACATC 17200 (exon6) Fig. 3c Tubb2a fw GACAGAGGCAAACTGAGCACC 3300 Fig. 3c rv CAACGTCAAGACGGCCGTGTG Fig. 3c Fig. 3c	Prom1	fw	GCCAGCAACCGCCCACAAGA	4000	E. A
Cd4 fw CACAACGAGAATGGACTAAT 17200 (exon6) Fig. S8c,d rv TGGGTACTGGAGGACATC 17200 (exon6) Fig. S8c,d Tubb2a fw GACAGAGGCAAACTGAGCACC 3300 rv CAACGTCAAGACGGCCGTGTG 3300 Fig. 3c		rv	CTTCCTGCAGCTACTGCTTC	1200	⊢ıg. 3c
rv TGGGTACTGGAGGACATC 17200 (exotio) Fig. S8c,d Tubb2a fw GACAGAGGGCAAACTGAGCACC 3300 Fig. 3c rv CAACGTCAAGACGGCCGTGTG 3300 Fig. 3c	Cd4	fw	CACAACGAGAATGGACTAAT	17200 (2005)	Eig Sood
Tubb2a fw GACAGAGGCAAACTGAGCACC 3300 Fig. 3c rv CAACGTCAAGACGGCCGTGTG 3300 Fig. 3c		rv	TGGGTACTGGAGGACATC	17200 (8X010)	r iy. 300,0
rv CAACGTCAAGACGGCCGTGTG	Tubb2a	fw	GACAGAGGCAAACTGAGCACC	3300	Fig. 3c
		rv	CAACGTCAAGACGGCCGTGTG		J

Supplementary Table 4 - PCR primers

RT-qPCR primers

gene			
Scn4b	fw	CGAGAGAAGAAGAAGGAGTG	Fig. 6b. e
	rv	CAGATAGGACTTGGCATCAG	r ig. 00, e
Hprt	fw	GTTGGATACAGGCCAGACTTTGTTG	Fig. 6b. e
	rv	GATTCAACTTGCGCTCATCTTAGGC	1 lg. 00, e