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Supplemental Information

High Levels of Canonical Wnt Signaling Lead to Loss of Stemness and Increased Differentiation in Hematopoietic Stem Cells

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Supplementary Experimental Procedures

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Supplemental Experimental procedures

Mouse bone marrow (BM) cells were isolated from femurs and tibiae, which were crushed in a mortar and filtered through 70 µm filters. The cells were stained using biotinylated lineage antibodies (MAC-1/CD11b, B220/CD45R, CD3e, CD4, NK1.1, Gr1, Ter119), Streptavidin PE, CD117 APC and Sca1 PECy7. LSK cells were isolated using a BD Aria II SORP cell sorter (Beckton-Dickinson) and were collected in Stemspan (Stem Cell Technologies), supplemented with mFlt3L (50 ng/ml), rmSCF (100 ng/ml) and rmTPO (10 ng/ml, all cytokines purchased from R&D sytems. The cells were incubated for 16 hr at 37°C and 5% CO_2. LSKs from Apc 15 Lox heterozygous mice with mildly elevated Wnt levels were shown to perform better in reconstitution experiments but are not integral part of the current study, as only subtle changes in gene expression were found.

Retroviral Production and Transduction

MSCV-Cre-IRES-GFP plasmid was kindly provided by H. Nakauchi (Institute of Medical Science, University of Tokyo, Japan) and viruses were generated with the Phoenix-packaging cell line. 40,000–70,000 sorted LSKs were stimulated overnight in serum-free medium (StemCell Technologies) supplemented with cytokines (100 ng/ml rmSCF, 10 ng/ml rmTPO, and 50 ng/ml rmFlt3L; from R&D) and transduced by spinoculation (800 x g, 2 hours, 32°C) with titrated amounts of virus with Retronectin (Takara Bio Inc.). Cells were cultured for 2 additional days. Subsequently, Cre-GFP expressing LSK cells were isolated using flow cytometic cell sorting and collected for RNA expression. For in vitro assays including apoptosis, proliferation and differentiation assays bulk of transduced and untransduced cells were used.

RNA amplification

RNA was isolated from the sorted transduced cells using Qiagen RNEasy micro columns (Qiagen, Hilden, Germany). RNA of more than 10,000 cells were then amplified using the Ovation RNA amplification system v2(Nugen Inc., San Carlos, CA, USA), processed using the Encore Biotin module (Nugen) and hybridized to Affymetrix mouse 430 2.0 Genechip arrays.
Data is available at the NCBI Gene Expression Omnibus (GEO), accession number GSE79495

Gene expression normalization. Gene expression data was measured in two batches. Raw data is normalized per batch with Robust Multi-Array Average (RMA), and batch correction is applied using Combat. Intensity values were mean centered per probe set. Gene symbols are mapped using MM9. As a result of the normalization, probe-intensity values follow a normal distribution for which intensities higher than 0 are up-regulated, and intensities lower than 0 are down-regulated. Principal component analysis and pairwise correlations across the 21 samples showed the expected results; wild-type and mutants, t1572, n1638, and Knock-Out samples are different from each other in the PCA-space and correlation map.

Gene expression analysis. Differential expressed genes for the APC samples are determined by using Limma, and genes are considered to be differential expressed between the two selected groups if mRNA levels differ with $P \leq 0.05$ after multiple test correction using Holm.

ChIP-Seq normalization. In this study we used massively parallel sequenced DNA-fragments bound by the transcription factors, TCF1, TCF7, and β-catenin. All the sequencing data is aligned using Burrows-Wheeler transformation (BWA), according MM9. We used several literature sources (Li et al., 2013a; Steinke et al., 2014; Zhang and Li, 2008; Zhang et al., 2000) (Wu et al., 2012).

ChIP-Seq analysis. Binding of transcription factors is determined by utilizing Hypergeometric Analysis of Tilling arrays (HATSEQ). A binding event was called when fragments are enriched based on default parameter settings, i.e., FWER significance level < 0.05, and a bandwidth (fragment size) of 300bp. We mapped the significantly detected binding sites to RefSeq genes in UCSC mm9 database (genome.ucsc.edu). A gene was designated as the target gene if the peak was present within 5000bp upstream of the transcription start site or inside of the gene.

For TCF1 (in mature CD8 T cells, accession number GSM1258235), we detected 591 significantly enriched regions (ranges between 104bp-1048bp, median: 233bp) by comparing it to control IgG using sorted post-select DP and CD4^{+}8^{lo} thymocytes[1] (accession number GSM1258236). The detected regions could subsequently be mapped to 116 unique genes. For the two TCF1 experiments in murine thymocytes (GSM1285796 for TCF1-CAT and GSM1133644 for TCF1), we detected 732 (size ranges between 102bp-2632bp, median: 237bp), and 2600 (102bp-2632bp, median: 237bp) significant binding regions respectively after comparing to control TCF1-CAT-INPUT (GSM1285797) and TCF1-INPUT (GSM1133645) respectively. The detected regions could subsequently be mapped to respectively 131, and 653 unique genes (Table S2). The third analyzed ChIP-Seq data set was the binding of TCF7 (GSM773994). For TCF7 we detected 6395 significant binding regions (size ranges between 103bp-5840bp, median: 341bp) by comparing it to one control (input DNA of TCF7). These regions are subsequently mapped to 2015 genes (Table S2). The fourth public data set that we analyzed were three Beta-Catenin experiments, two with biotinylation and one based on FLAG-tag technology. As a background four different controls are used per experiment (2 with Beta-Catenin biotin without GSK and two GSK input samples). This resulted in respectively 990, 385, and 671 significant binding regions for Beta-Catenin-Biotin-rep1, Beta-Catenin-Biotin-rep2, and Beta-Catenin-Flag-rep1 and were mapped to 121, 49, and 79 genes (Table S2). Binding sites have median size of 336bp, 385bp, and 320bp.

To test the validity of the detected binding regions of each experiment, we expected an overrepresentation of WNT-associated genes. To test this, we overlaid the mapped genes with known WNT-associated genes (n=1136) from the Molecular Signature Database (MSigDB, v4.0), and detected that all seven ChIP-seq experiment showed a significant enrichment for binding in close vicinity of WNT-associated genes ($P \leq 0.05$, Table S1) based on the hypergeometric test. As an example, all seven experiments showed binding of in the transcriptional start site of *Axin2* (Figure S4A), whereas TCF1, and Beta-Catenin experiments showed also binding for *Lef1* (Figure S4A).

Pathway Analysis. Pathway analysis is performed by utilizing the Molecular Signature Database (MSigDB, v4.0) for the detection of enriched curated gene sets (C2), motif gene sets (C3), computational gene sets (C4), GO gene sets (C5), oncogenic signatures (C6), and immunologic signatures (C7). Gene sets and signatures are considered statistically significant when the P-value, derived from the hypergeometric test, is less or equal than 0.05 after correcting for multiple testing using Holm.

Mice

Mice were bred and maintained in the animal facilities of Leiden University Medical Center, in accordance with legal regulations in The Netherlands and with the approval of the Dutch animal ethical committee. C57Bl/6-CD45.1 (Ly5.1) and C57Bl/6-CD45.2 (Ly5.2) mice were obtained from the Jackson Laboratory. Mice carrying targeted mutations on Apc were previously described (Fodde et al., 1994; Robanus-Maandag et al., 2010; Smits et al., 1999) and continuously backcrossed to C57Bl/6 background.

Flow Cytometry

The following antibodies were obtained from BD Biosciences (San Diego, CA): anti CD11b-PE (M1/70), anti CD19-APC (ID3) and anti CD117 (2B6). For Lineage depletion these markers were used: CD3 (145-2C11), CD4 (L3T4), CD8 (53-6.7), CD11b (M1/70), Gr1 (RB6-8C5), B220 (Ra3-6B2), Ter119 (Ly76) and Nk1.1 (PK136) biotin and subsequently were stained with streptavidin eFluor 450 (48-4317) from eBioscience. The following antibodies were also purchased from eBiosiences: B220 PE-Cy7 (RA3-6B2), Gr1 eFluor 450 (RB6-8C5) and Sca1 PE-Cy7 (D7). Cells were stained in Fluorescenceactivated cell sorter (FACS) buffer (PBS, 2% bovine serum albumin, 0.1% sodium azide) for 30 min at 4 °C. Ultimately, Cells were washed and measured either on a Canto I, or an Aria (BD Biosciences). Data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

Prolferation, apoptosis and differentiation assays

5 × 10^4 sorted BM LSKs from APC WT and APC 15lox/15lox mice were transduced with titrated amount of CRE viruses in stemspan with FTS cytokines as previously described. For apoptosis assay harvested cells after 2 days (Day 0) or 5 days (Day 3) of culture, cells were stained with either 7AAD/AnnexinV (BD Bioscience), or PE-Active caspase-3 apoptosis kit (BD pharmingen) according to the manufacturer’s instruction. For proliferation assay, cells were labelled with 5 uM Cell Proliferation Dye eFluor® 670 (eBioscience) at Day 0. Subsequently, cells were harvested at Day 3 and were assessed by flow cytometry for proliferation.

For differentiation assay 2 × 10^4 BM LSKs were used and transduced cells at Day 0 were transferred onto confluent monolayers of OP9 WT and cocultured for additional 14 days with AlphaMEM 10% FCS containing 50 ng/ml rmSCF, 10 ng/ml rmFlt3L and 10 ng/ml rmIL-7 (all cytokines from R&D). After 7 days cells were harvested and transferred onto new monolayer of OP9 cells, and half of the medium were replaced every 3-4 days. Finally, after 14 days of coculture cells were harvested and assessed by flow cytometry for B and myeloid lineage differentiation.

Supplemental Figure Legends

Suppl. Fig 1a: Validation of differential gene expression by Q-PCR. Sorted LSK cells were cultured and transduced with CRE-GFP as described in the supplemental experimental procedures. RNA was isolated and used for analysis by Q-PCR for the indicated Wnt target genes.
Suppl. Fig 1b: Biological processes associated with clusters 1 and 2. For details see text

Suppl. Fig 2: High Wnt signaling is associated with differentiation into monocytes and B lymphocytes based on published gene sets.

Suppl. Fig 3: No differences in apoptosis and cell cycle in high Wnt signature when compared to published gene sets.

Suppl. Fig 4a: Tcf and beta catenin binding sites in the Lef1 and Axin2 promoters based on literature data mining of CHIP-Seq data

Suppl. Fig 4b: Differentially expressed genes from the gene expression profiles of heterozygote samples against 1572T,1638N, and full KO, and detected in A. 157 differential expressed genes with $P<0.05$ and B. the representation of 55 unique gene sets (see also Table S3)

Figure S1

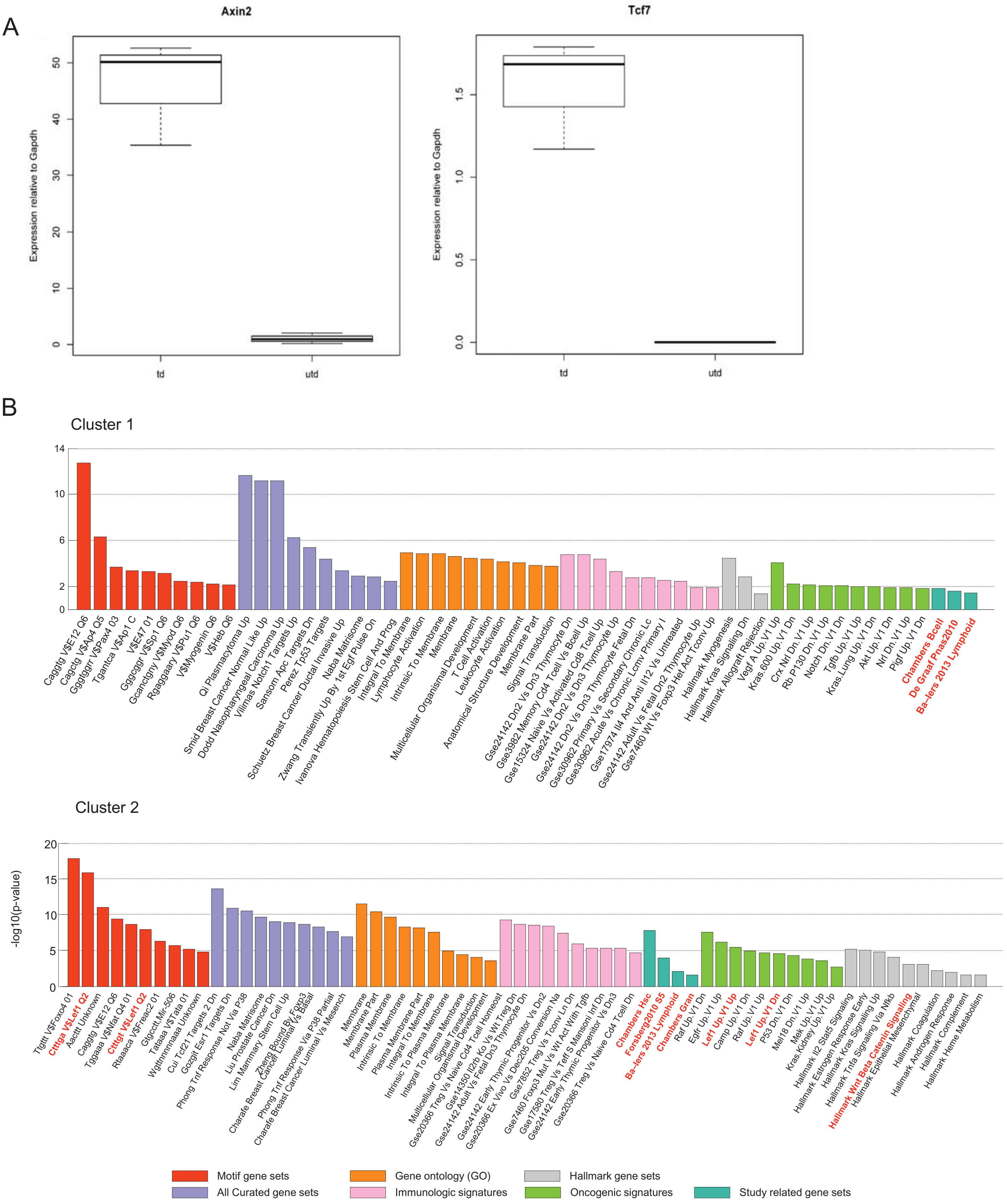
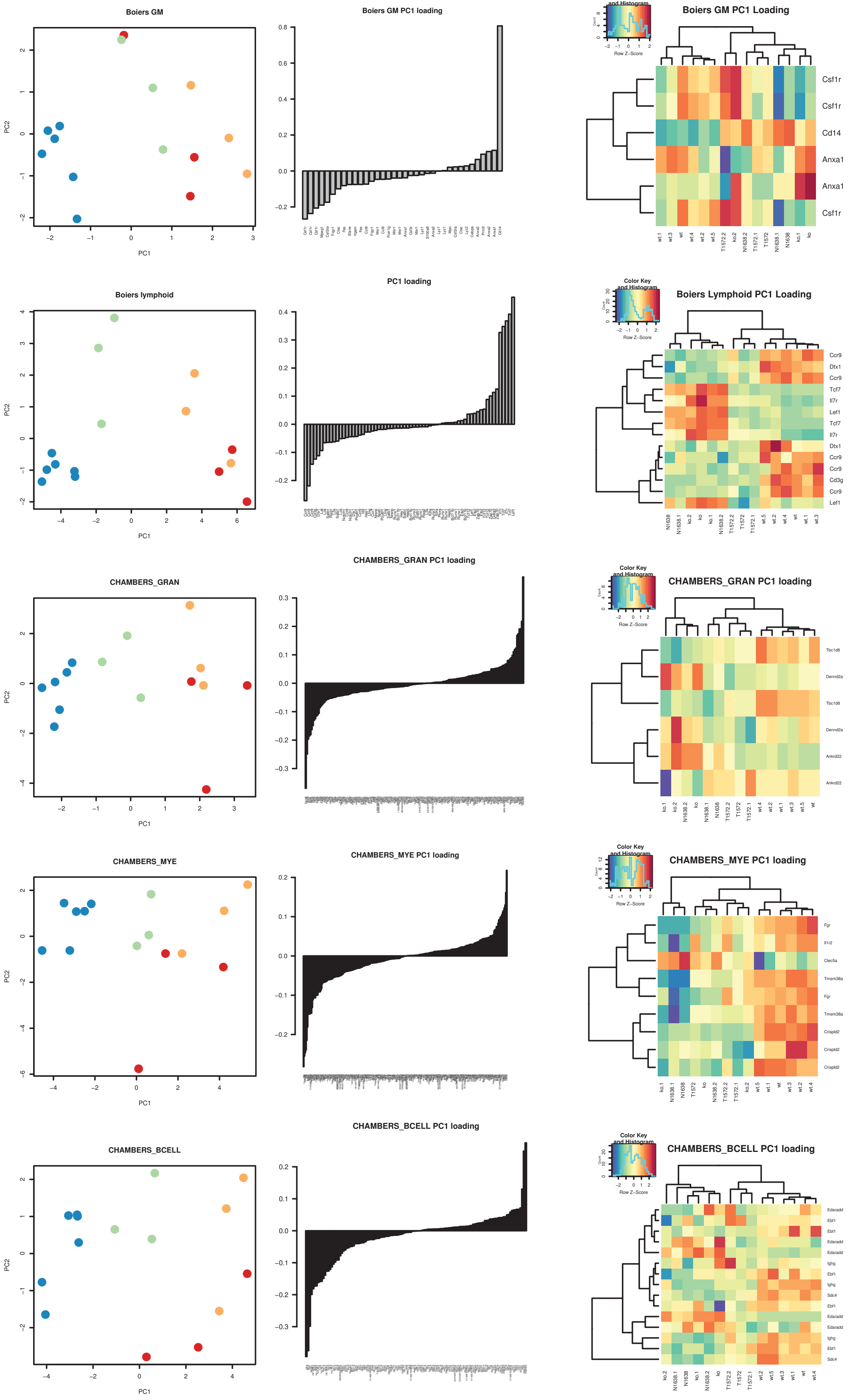

A
Axin2
Expression relative to Gapdh
0
10
20
30
40
50
td
utd
Tcf7
Expression relative to Gapdh
0.0
0.5
1.0
1.5
td
utd
B
Cluster 1
14
10
6
2
0
Caggtg V$E12 Q6
Cagctg V$Ap4 Q5
Gggtggrr V$Pax4 03
Tgantca V$Ap1 C
V$E47 01
Gggcggr V$Sp1 Q6
Gcanctgny V$Myod Q6
Rgaggaary V$Pu1 Q6
V$Myogenin Q6
V$Heb Q6
Qi Plasmacytoma Up
Smid Breast Cancer Normal Like Up
Dodd Nasopharyngeal Carcinoma Up
Vilimas Notch1 Targets Up
Sansom Apc Targets Dn
Perez Tp53 Targets
Schuetz Breast Cancer Ductal Invasive Up
Naba Matrisome
Zwang Transiently Up By 1st Egf Pulse On
Ivanova Hematopoiesis Stem Cell And Prog
Integral To Membrane
Lymphocyte Activation
Intrinsic To Membrane
Membrane
Multicellular Organismal Development
T Cell Activation
Leukocyte Activation
Anatomical Structure Development
Membrane Part
Signal Transduction
Gse24142 Dn2 Vs Dn3 Thymocyte Dn
Gse3982 Memory Cd4 Tcell Vs Bcell Up
Gse15324 Naive Vs Activated Cd8 Tcell Up
Gse24142 Dn2 Vs Dn3 Thymocyte Up
Gse24142 Dn2 Vs Dn3 Thymocyte Fetal Dn
Gse30962 Primary Vs Secondary Chronic Lc
Gse30962 Acute Vs Chronic Lcmv Primary I
Gse17974 Il4 And Anti Il12 Vs Untreated
Gse24142 Adult Vs Fetal Dn2 Thymocyte Up
Gse7460 Wt Vs Foxp3 Het Act Tconv Up
Hallmark Myogenesis
Hallmark Kras Signaling Dn
Hallmark Allograft Rejection
Vegf A Up.V1 Up
Kras.600 Up.V1 Dn
Crx Nrl Dn.V1 Up
Rb P130 Dn.V1 Up
Notch Dn.V1 Dn
Tgfb Up.V1 Up
Kras.Lung Up.V1 Dn
Akt Up.V1 Dn
Nrl Dn.V1 Up
Pigf Up.V1 Dn
Chambers Bcell
De Graaf Pnas2010
Ba–lers 2013 Lymphoid
Cluster 2
-log10(p-value)
20
15
10
5
0
Ttgttt V$Foxo4 01
Ctttga V$Lef1 Q2
Aacttt Unknown
Caggtg V$E12 Q6
Tggaaa V$Nfat Q4 01
Ctttgt V$Lef1 Q2
Rtaaaca V$Freac2 01
Gtgcctt,Mir-506
Tataaa V$Tata 01
Wgttnnnnnaaa Unknown
Cui Tcf21 Targets 2 Dn
Gozgit Esr1 Targets Dn
Phong Tnf Response Not Via P38
Naba Matrisome
Liu Prostate Cancer Dn
Lim Mammary Stem Cell Up
Zheng Bound By Foxp3
Charafe Breast Cancer Luminal Vs Basal
Phong Tnf Response Via P38 Partial
Charafe Breast Cancer Luminal Vs Mesench
Membrane
Membrane Part
Plasma Membrane
Intrinsic To Membrane
Plasma Membrane Part
Integral To Membrane
Intrinsic To Plasma Membrane
Integral To Plasma Membrane
Signal Transduction
Multicellular Organismal Development
Gse20366 Treg Vs Naive Cd4 Tcell Homeost
Gse14350 Il2rb Ko Vs Wt Treg Dn
Gse24142 Adult Vs Fetal Dn3 Thymocyte Dn
Gse24142 Early Thymic Progenitor Vs Dn2
Gse20366 Ex Vivo Vs Dec205 Conversion Na
Gse7852 Treg Vs Tconv Ln Dn
Gse7460 Foxp3 Mut Vs Wt Act With Tgfb
Gse17580 Treg Vs Teff S Mansoni Inf Dn
Gse24142 Early Thymic Progenitor Vs Dn3
Gse20366 Treg Vs Naive Cd4 Tcell Dn
Chambers Hsc
Forsberg2010 S5
Ba–lers 2013 Lymphoid
Chambers Gran
Raf Up.V1 Dn
Egfr Up.V1 Up
Lef1 Up.V1 Up
Camp Up.V1 Dn
Raf Up.V1 Up
Lef1 Up.V1 Dn
P53 Dn.V1 Dn
Mel18 Dn.V1 Up
Mek Up.V1 Up
Kras.Kidney Up.V1 Up
Hallmark Il2 Stat5 Signaling
Hallmark Estrogen Response Early
Hallmark Kras Signaling Up
Hallmark Tnfa Signaling Via Nfkb
Hallmark Wnt Beta Catenin Signaling
Hallmark Epithelial Mesenchymal
Hallmark Coagulation
Hallmark Androgen Response
Hallmark Complement
Hallmark Heme Metabolism
🟥 Motif gene sets
🟪 All Curated gene sets
🟧 Gene ontology (GO)
▨ Immunologic signatures
⬜ Hallmark gene sets
🟩 Oncogenic signatures
▨ Study related gene sets

Figure S2



Differentiation signatures as determined by Boiers (Cell Stem Cell 2013) and Chambers (Cell Stem Cell 2007).

Stem cell signature, such as proposed by Ivanova (Science 2002), Forsberg (PLoS One 2010), Chambers (Cell Stem Cell 2007) and de Graaf (PNAS 2010) and Ridell (Cell 2014) were mostly driven by only a few genes.

Figure S3

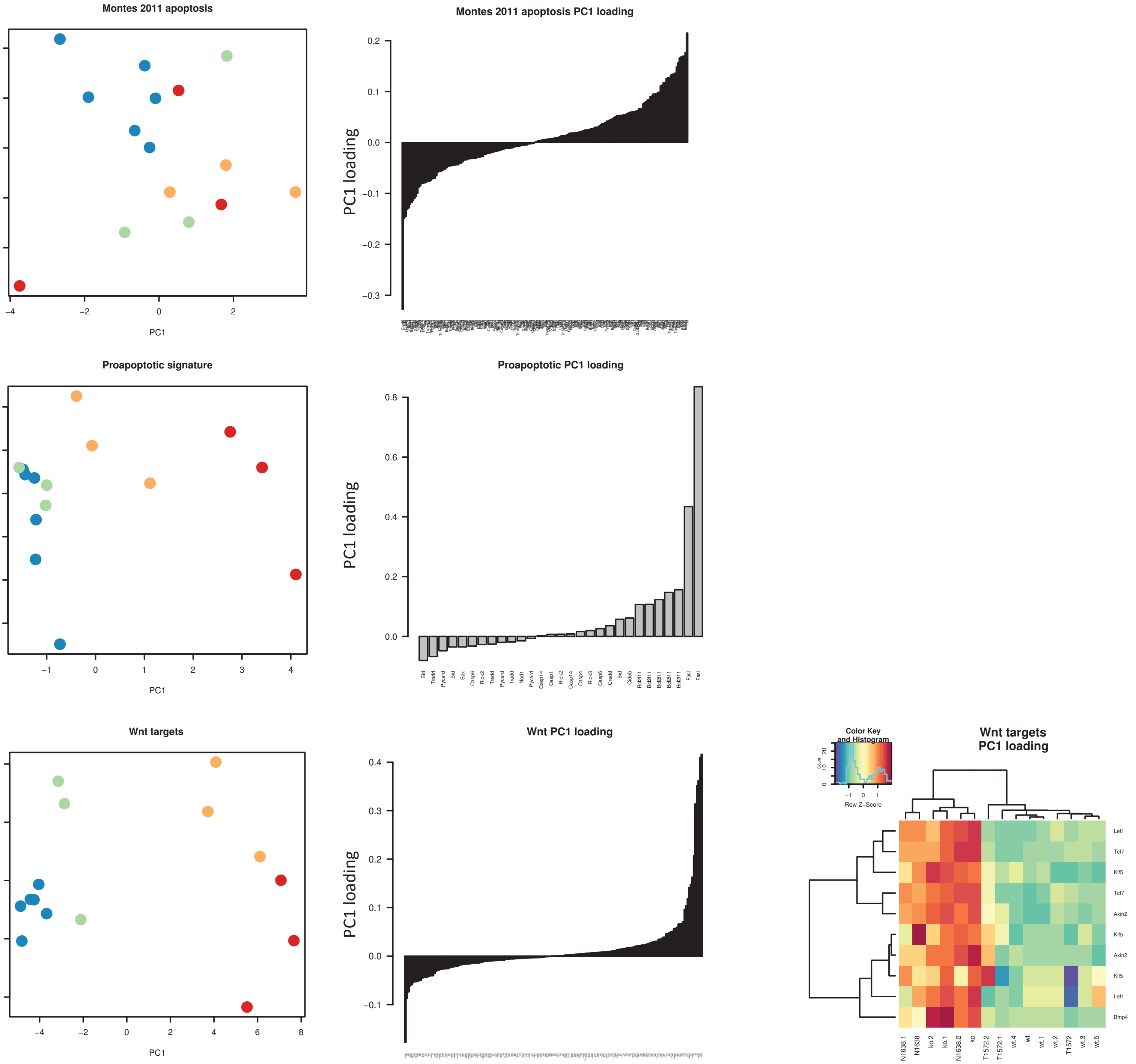

Montes 2011 apoptosis
−4
−2
0
2
PC1
Montes 2011 apoptosis PC1 loading
0.2
0.1
0.0
−0.1
−0.2
−0.3
PC1 loading
Proapoptotic signature
−1
0
1
2
3
4
PC1
Proapoptotic PC1 loading
0.8
0.6
0.4
0.2
0.0
PC1 loading
Bid
Tradd
Pycard
Bid
Bax
Casp6
Ripk2
Tradd
Pycard
Tradd
Nod1
Pycard
Casp14
Casp1
Ripk2
Casp14
Casp4
Ripk3
Casp6
Cradd
Bid
Cideb
Bcl2l11
Bcl2l11
Bcl2l11
Bcl2l11
Bcl2l11
Fasl
Fasl
Wnt targets
−4
−2
0
2
4
6
8
PC1
Wnt PC1 loading
0.4
0.3
0.2
0.1
0.0
−0.1
PC1 loading
Color Key
and Histogram
Count
0
10
20
−1
0
1
Row Z−Score
Wnt targets
PC1 loading
Lef1
Tcf7
Klf5
Tcf7
Axin2
Klf5
Axin2
Klf5
Lef1
Bmp4
N1638.1
N1638
ko.2
ko.1
N1638.2
ko
T1572.2
T1572.1
wt.4
wt
wt.1
wt.2
T1572
wt.3
wt.5

Figure S4

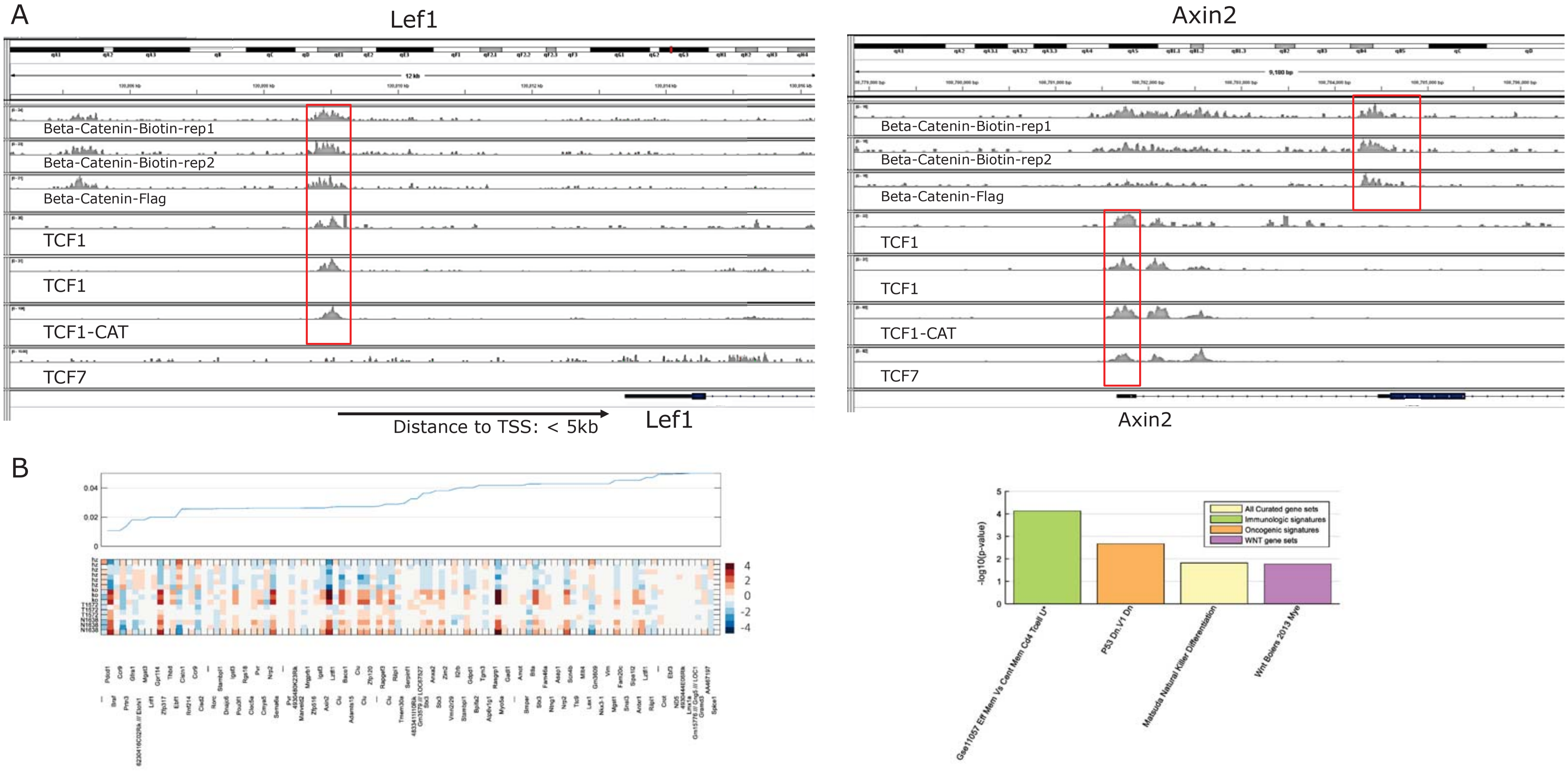

A
Lef1
Beta-Catenin-Biotin-rep1
Beta-Catenin-Biotin-rep2
Beta-Catenin-Flag
TCF1
TCF1
TCF1-CAT
TCF7
12 kb
Distance to TSS: < 5kb
Lef1
Axin2
Beta-Catenin-Biotin-rep1
Beta-Catenin-Biotin-rep2
Beta-Catenin-Flag
TCF1
TCF1
TCF1-CAT
TCF7
9,180 bp
Axin2
B
0.04
0.02
0
4
2
0
-2
-4
-log10(p-value)
5
4
3
2
1
0
All Curated gene sets
Immunologic signatures
Oncogenic signatures
WNT gene sets
Gse11057 Eff Mem Vs Cent Mem Cd4 Tcell U*
P53 Dn.V1 Dn
Matsuda Natural Killer Differentiation
Wnt Boiers 2013 Mye

Table S1

Gene-set	Pathway	$P_{BY}\leq 0.05$	Genes
All Curated gene sets	MATSUDA_NATURAL_KILLER_DIFFERENTIATION	6.58E-06	ANXA1,APCDD1,CCR9,CD160,CDC23,GPR34,IL2RB,MYO5A,NTRK3,PDCD1,PLAGL1,PRSS23,PTPRF,PVR,SH3BGRL2,SYTL2,TCF7,TULP3,XCL1,ZC3H12C
All Curated gene sets	PICCALUGA_ANGIOIMMUNOBLASTIC_LYMPHOMA_UP	0.0002209	ANTXR1,CD93,CLU,DCLK1,FN1,FSTL1,IL18,LAMC1,PLA2G4C,RAI14,TMEM163,TNS1
All Curated gene sets	LIU_PROSTATE_CANCER_DN	0.0002209	BEND5,CHST2,CLU,CRISPLD2,EPAS1,GPR155,ITGA2,PHLDA1,PLAGL1,PRSS23,RHOJ,ROBO1,TFCP2L1,TMEM35,TNS1,WIF1,ZCCHC14
All Curated gene sets	ONDER_CDH1_TARGETS_2_DN	0.0002209	ALDH1A3,CD83,CDK5R1,EPAS1,FGD6,FST,GJB5,IGSF3,IL18,ITGA2,KLF5,PTPRF,ROBO1,TFCP2L1,THBD,TNFRSF25,TPD52L1
All Curated gene sets	DELYS_THYROID_CANCER_UP	0.0006965	ALDH1A3,ANXA1,CHST2,DPP4,ENTPD1,FN1,IGSF3,ITGA2,MED13,NRP2,NT5E,P4HA2,PRSS23,PTPRF,S100A5,STX3
All Curated gene sets	ST_WNT_BETA_CATENIN_PATHWAY	0.001009	APC,AXIN2,DKK2,FSTL1,NKD1,WIF1
All Curated gene sets	SANA_TNF_SIGNALING_DN	0.001009	ANTXR1,ANXA1,CLU,EPAS1,GIMAP6,NT5E,PHLDA1,RHOJ
All Curated gene sets	GOZGIT_ESR1_TARGETS_DN	0.001305	ABHD2,CLU,DCLK1,FETUB,GFRA1,GPC4,MB21D2,MYO5A,PPAP2A,PRSS23,RASGRP1,RNF144B,SDK1,SH3BGRL2,SHROOM3,SIPA1L2,SYTL2,THBD,THSD4
All Curated gene sets	CUI_TCF21_TARGETS_2_UP	0.001305	ANTXR1,APCDD1,ARSB,BMP4,BMPER,CLU,DCLK1,EMID1,FN1,GAS2L3,HUNK,KLF5,LYPD6B,NKD1,NRP2
All Curated gene sets	GAVIN_PDE3B_TARGETS	0.001305	ENTPD1,IL18,LAMC1,NT5E,SYTL2
All Curated gene sets	NABA_MATRISOME	0.003449	ADAM22,ANXA1,BMP4,BMPER,CRISPLD2,ELFN1,EMID1,FN1,FREM2,FST,FSTL1,GPC4,IL18,ISM1,KY,LAMC1,P4HA2,S100A5,SCUBE3,THSD4,WIF1,XCL1
All Curated gene sets	KEGG_WNT_SIGNALING_PATHWAY	0.00359	APC,AXIN2,CAMK2D,DKK2,LEF1,NFATC2,NKD1,TCF7,WIF1
All Curated gene sets	CERVERA_SDHB_TARGETS_1_UP	0.00359	CAND2,CCDC109B,FSTL1,IL18,LYPD6B,PACSIN1,PRSS23,TNFRSF19
All Curated gene sets	KINSEY_TARGETS_OF_EWSR1_FLII_FUSION_DN	0.005222	CAMK2D,DCLK1,DLC1,FAM63A,FN1,FSTL1,LAMC1,MB21D2,NT5E,PHLDA1,PRSS23,SIPA1L2
All Curated gene sets	CHARAFE_BREAST_CANCER_LUMINAL_VS_BASAL_DN	0.005292	ADA,ALDH1A3,ANTXR1,ANXA1,CD14,FST,FSTL1,IL18,IL7R,KLF5,LAMC1,NT5E,PHLDA1,ZC3H12C
All Curated gene sets	RIGGI_EWING_SARCOMA_PROGENITOR_DN	0.007768	ABHD2,ALDH1A3,BACE1,BMP4,CLU,FST,NRP2,PHLDA1,TNFRSF19
All Curated gene sets	SANSOM_WNT_PATHWAY_REQUIRE_MYC	0.008652	AXIN2,LEF1,NKD1,TCF7,TNFRSF19,WIF1
All Curated gene sets	PASQUALUCCI_LYMPHOMA_BY_GC_STAGE_UP	0.008714	ADA,ANTXR1,ENTPD1,IRF4,NUDT4,OSBPL1A,PHLDA1,PVR,SH3BGRL2,SHROOM3,TULP3
All Curated gene sets	BYSTRYKH_HEMATOPOIESIS_STEM_CELL_QTL_TRANS	0.008714	AXIN2,CD14,CHST2,EFHD1,EXTL3,FAM63A,GFRA1,GPC4,IL2RB,KIF5C,LEF1,MYO5A,PDCD1,STX3,SULT1A1,TEK,TFCP2L1,THBD,TULP3,XCL1
All Curated gene sets	GAUSSMANN_MLL_AF4_FUSION_TARGETS_F_UP	0.009692	ARHGAP28,ARSB,BMP4,BMPER,FST,GPC4,IL18,NT5E,TEK
All Curated gene sets	GAVIN_FOXP3_TARGETS_CLUSTER_P4	0.01055	CCDC109B,CD83,EPAS1,IL2RB,LYPD6B,PLAGL1,SH3BGRL2
All Curated gene sets	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	0.01299	CCR2,CCR9,EDAR,IL17RB,IL18,IL2RB,IL7R,TNFRSF19,TNFRSF25,XCL1
All Curated gene sets	DODD_NASOPHARYNGEAL_CARCINOMA_UP	0.01525	ABHD2,ALDH1A3,ANXA1,APCDD1,ATP13A4,BACE1,BDH1,BEND5,CAND2,CLU,EDAR,EPAS1,FAM63A,FMN1,IL18,KLF5,LYPD6B,PRKAA2,PRSS23,SH3BGRL2,SMPD3,SNX31,SYTL2,TNFRSF19,TNS1,TUBB3
All Curated gene sets	CREIGHTON_ENDOCRINE_THERAPY_RESISTANCE_1	0.01646	BMPER,CCDC101,CRISPLD2,DLC1,EFHD1,FREM2,GFRA1,ITGA2,MB21D2,MYO5A,PRSS23,SYTL2,THSD4,TPD52L1
All Curated gene sets	SMID_BREAST_CANCER_NORMAL_LIKE_UP	0.01763	CCR2,CD3G,CLU,DPP4,GIMAP6,IL7R,LEF1,NT5E,SNCAIP,THBD,TNFRSF25,WIF1,XCL1
All Curated gene sets	SMIRNOV_CIRCULATING_ENDOTHELIOCYTES_IN_CANCER_UP	0.01851	B4GALT5,CD14,CD93,CLU,EPAS1,PRSS23,THBD,TNS1
All Curated gene sets	WALLACE_PROSTATE_CANCER_RACE_UP	0.01852	CCDC109B,CD83,CD93,CLU,DLC1,GIMAP6,IL7R,RASGRP1,THBD,TMEM35
All Curated gene sets	QI_PLASMACYTOMA_UP	0.02286	CARD11,CCR2,CD3G,CLU,DPP4,IL17RB,IL18,IL2RB,TUBB3,XCL1
All Curated gene sets	AMIT_EGF_RESPONSE_480_HELA	0.02337	ABHD2,DCLK1,FST,ITGA2,NUDT4,PTPRF,PVR,TUBB3
All Curated gene sets	NABA_MATRISOME_ASSOCIATED	0.03018	ADAM22,ANXA1,BMP4,ELFN1,FREM2,FST,FSTL1,GPC4,IL18,ISM1,KY,P4HA2,S100A5,SCUBE3,WIF1,XCL1
All Curated gene sets	REACTOME_IMMUNE_SYSTEM	0.03141	BTLA,CAMK2D,CARD11,CCR2,CD14,CD160,CD3G,CDC23,IL18,IL2RB,IL7R,IRF4,OSBPL1A,PDCD1,PVR,RAP1GAP2,RASGRP1,RNF144B
All Curated gene sets	FULCHER_INFLAMMATORY_RESPONSE_LECTIN_VS_LPS_UP	0.03141	ABHD2,CD93,CHST2,FN1,IL7R,IRF4,MB21D2,MYO5A,NRIP3,P4HA2,PHLDA1,RAI14,RASGRP1,THBD
All Curated gene sets	SCHAEFFER_PROSTATE_DEVELOPMENT_48HR_UP	0.03313	ALDH1A3,ANXA1,BDH1,CLU,CRISPLD2,EDARADD,GPR155,NT5E,PPFIBP2,SULT1A1,TFCP2L1,TPD52L1,WIF1
All Curated gene sets	KIM_MYC_AMPLIFICATION_TARGETS_DN	0.03336	DCLK1,GAS2L3,IL17RB,KLF5,NFATC2,SHROOM3
All Curated gene sets	LIM_MAMMARY_STEM_CELL_UP	0.04392	ANTXR1,EDARADD,EPAS1,FST,ISM1,LAMC1,NRP2,NT5E,PPAP2A,RHOJ,THSD1,TNS1,WIF1
All Curated gene sets	KEGG_BASAL_CELL_CARCINOMA	0.04862	APC,AXIN2,BMP4,LEF1,TCF7
All Curated gene sets	TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_8D_DN	0.04862	ANTXR1,CD14,CLU,ENTPD1,EPAS1,GPR34,IL7R,TMEM163
All Curated gene sets	LINDGREN_BLADDER_CANCER_CLUSTER_2B	0.0488	CRISPLD2,EFHD1,ENTPD1,IL7R,LEF1,MYO5A,NRP2,TBC1D8,TCF7,THBD,TNS1
All Curated gene sets	NUYTTEN_EZH2_TARGETS_UP	0.0488	ANXA1,AXIN2,B4GALT5,BACE1,CCDC109B,CD83,FGD6,FN1,GPR155,NT5E,P4HA2,PLAGL1,PRRG1,PTPRF,ROBO1,STX3,TCF7,THSD1,ZC3H12C
All Curated gene sets	SCHAEFFER_PROSTATE_DEVELOPMENT_48HR_DN	0.0488	ANTXR1,CD83,CHDH,DKK2,GAS2L3,HUNK,LYPD6B,NRP2,P4HA2,PRTG,RHOJ,SIPA1L2
Computational gene sets	MODULE_46	2.83E-06	ADA,CCR2,CCR9,CD14,CD3G,CD83,CDK5R1,CLU,DPP4,ENTPD1,FN1,IL18,IL2RB,IL7R,P4HA2,PDCD1,XCL1
Computational gene sets	MODULE_75	2.83E-06	ADA,CCR2,CCR9,CD14,CD3G,CD83,CDK5R1,CLU,DPP4,FN1,IL18,IL2RB,IL7R,P4HA2,PDCD1,TEK,XCL1
Gene ontology (GO)	PLASMA_MEMBRANE_PART	0.000438	ACTN2,APC,BACE1,CACNA1B,CACNA1D,CARD11,CCR2,CCR9,CD160,CD83,DCLK1,ENTPD1,GPC4,GPR34,IL17RB,IL2RB,ITGA2,NTRK3,PRRG1,PTPRF,ROBO1,SHROOM3,STX3,SYTL2,TEK,THBD,TNFRSF25
Gene ontology (GO)	MEMBRANE	0.0006684	ACTN2,APC,BACE1,CACNA1B,CACNA1D,CARD11,CCR2,CCR9,CD14,CD160,CD83,CDK5R1,DCLK1,ENTPD1,GPC4,GPR114,GPR34,IL17RB,IL2RB,ITGA2,NTRK3,PLA2G4C,PPAP2A,PRRG1,PTPRF,PVR,RNF144B,ROBO1,SHROOM3,SLC7A4,STX3,SYTL2,TBC1D8,TEK,THBD,TNFRSF25
Gene ontology (GO)	PLASMA_MEMBRANE	0.0007698	ACTN2,APC,BACE1,CACNA1B,CACNA1D,CARD11,CCR2,CCR9,CD14,CD160,CD83,DCLK1,ENTPD1,GPC4,GPR34,IL17RB,IL2RB,ITGA2,NTRK3,PPAP2A,PRRG1,PTPRF,ROBO1,SHROOM3,STX3,SYTL2,TEK,THBD,TNFRSF25
Gene ontology (GO)	MEMBRANE_PART	0.001722	ACTN2,APC,BACE1,CACNA1B,CACNA1D,CARD11,CCR2,CCR9,CD160,CD83,DCLK1,ENTPD1,GPC4,GPR114,GPR34,IL17RB,IL2RB,ITGA2,NTRK3,PRRG1,PTPRF,PVR,RNF144B,ROBO1,SHROOM3,SLC7A4,STX3,SYTL2,TEK,THBD,TNFRSF25
Gene ontology (GO)	SIGNAL_TRANSDUCTION	0.007247	ANXA1,AXIN2,CCR2,CCR9,CD14,CD160,CD3G,CD83,CDK5R1,DLC1,EDARADD,EPAS1,FGD6,GPR34,HUNK,IL2RB,IL7R,KCNIP2,MED13,NTRK3,NUDT4,PPAP2A,PRKAA2,PTPRF,RASGRP1,TEK,TNFRSF25,TPD52L1,XCL1
Gene ontology (GO)	RESPONSE_TO_EXTERNAL_STIMULUS	0.01613	AHSG,ANXA1,CCR2,CCR9,CHST2,ENTPD1,ITGA2,PGLYRP2,SHROOM3,THBD,XCL1
Gene ontology (GO)	RECEPTOR_ACTIVITY	0.01613	CD14,CD160,CD3G,GFRA1,GPR114,IL2RB,IL7R,MED13,NRP2,PGLYRP2,PTPRF,PVR,ROBO1,TEK,TNFRSF25
Hallmark gene sets	HALLMARK_ESTROGEN_RESPONSE_EARLY	0.004103	ABHD2,DLC1,FAM63A,GFRA1,IL17RB,PRSS23,RASGRP1,THSD4,TPD52L1
Hallmark gene sets	HALLMARK_INFLAMMATORY_RESPONSE	0.006397	CD14,CHST2,IL18,IL2RB,IL7R,PVR,RASGRP1,RNF144B
Hallmark gene sets	HALLMARK_COAGULATION	0.006397	ANXA1,CLU,DPP4,FN1,ITGA2,PRSS23,THBD
Hallmark gene sets	HALLMARK_IL2_STAT5_SIGNALING	0.006397	CD83,IL2RB,IRF4,NT5E,PHLDA1,PLAGL1,PPAP2A,SH3BGRL2
Hallmark gene sets	HALLMARK_WNT_BETA_CATENIN_SIGNALING	0.01107	AXIN2,LEF1,NKD1,TCF7
Hallmark gene sets	HALLMARK_COMPLEMENT	0.01636	ACTN2,CDK5R1,CLU,DPP4,FN1,KCNIP2,RASGRP1
Immunologic signatures	GSE20366_EX_VIVO_VS_DEC205_CONVERSION_NAIVE_CD4_TCELL_UP	0.001054	ACTN2,CCR2,CD160,EPAS1,GPR114,GPR34,IL17RB,RASGRP1,THBD,XCL1,XKRX
Immunologic signatures	GSE24142_EARLY_THYMIC_PROGENITOR_VS_DN2_THYMOCYTE_DN	0.001054	ADA,AXIN2,CCDC109B,CD3G,DPP4,EDARADD,IL17RB,IL7R,PDCD1,PTPRF,TUBB3
Immunologic signatures	GSE7852_TREG_VS_TCONV_LN_UP	0.001054	CCR2,CD83,ENTPD1,FGD6,IRF4,LAMC1,NT5E,PLAGL1,PPAP2A,ZC3H12C,ZDHHC23
Immunologic signatures	GSE10325_LUPUS_CD4_TCELL_VS_LUPUS_BCELL_UP	0.003344	ANXA1,CCDC109B,CD3G,DPP4,GIMAP6,IL7R,LEF1,TCF7,TNFRSF25,ZCCHC14
Immunologic signatures	GSE30962_PRIMARY_VS_SECONDARY_CHRONIC_LCMV_INF_CD8_TCELL_	0.003344	AHSG,ANXA1,CD93,ENTPD1,EPAS1,GPR114,GPR34,PRKAA2,RASGRP1,TMEM163
Immunologic signatures	GSE24142_EARLY_THYMIC_PROGENITOR_VS_DN2_THYMOCYTE_ADULT_	0.003403	AXIN2,CCDC109B,CD3G,EDARADD,IL17RB,IL7R,LEF1,PPAP2A,PTPRF,SYTL2
Immunologic signatures	GSE7460_TCONV_VS_TREG_LN_DN	0.004089	CD83,DPP4,ENTPD1,IL2RB,IRF4,NT5E,PPAP2A,SH3BGRL2,ZC3H12C,ZDHHC23
Immunologic signatures	GSE20366_EX_VIVO_VS_DEC205_CONVERSION_NAIVE_CD4_TCELL_DN	0.0137	ANXA1,CCDC109B,CD83,DCLK1,IL18,PLAGL1,RNF144B,SNX31,STX3
Immunologic signatures	GSE10325_CD4_TCELL_VS_BCELL_UP	0.01559	ANXA1,CCR2,CD3G,DPP4,GIMAP6,IL2RB,LEF1,RASGRP1,TNFRSF25
Immunologic signatures	GSE24142_EARLY_THYMIC_PROGENITOR_VS_DN3_THYMOCYTE_DN	0.01559	ADA,CCDC109B,GFRA1,IL7R,LEF1,PDCD1,PTPRF,TUBB3,TULP3
Immunologic signatures	GSE39820_CTRL_VS_IL1B_IL6_IL23A_CD4_TCELL_UP	0.01559	CD83,GAS2L3,GPR34,LYPD6B,PACSIN1,PHLDA1,PLAGL1,RASGRP1,SYTL2
Immunologic signatures	GSE7460_TCONV_VS_TREG_THYMUS_DN	0.01559	CCDC109B,CD83,IGSF3,IL2RB,KIF5C,NRP2,PLAGL1,PPAP2A,SH3BGRL2
Immunologic signatures	GSE7852_TREG_VS_TCONV_THYMUS_UP	0.01634	CCDC109B,CCR2,CD83,IGSF3,KIF5C,PDCD1,PLAGL1,PPAP2A,SH3BGRL2
Immunologic signatures	GSE24142_DN2_VS_DN3_THYMOCYTE_DN	0.01649	CCR9,CD3G,GFRA1,IRF4,LEF1,PHLDA1,PTPRF,TBC1D8,TULP3
Immunologic signatures	GSE3982_BCELL_VS_CENT_MEMORY_CD4_TCELL_DN	0.03801	DLC1,DPP4,IL7R,ITGA2,PHLDA1,PRKAA2,TCF7,TNS1
Immunologic signatures	GSE3982_MEMORY_CD4_TCELL_VS_BCELL_UP	0.04586	ANXA1,CAND2,CD3G,DPP4,GIMAP6,IL2RB,KIF5C,PHLDA1
Motif gene sets	TTGTTT_V$FOXO4_01	0.0001493	ANXA1,APC,AXIN2,BDH1,BMP4,CCDC109B,CD83,DLC1,EDAR,ENTPD1,EXTL3,FAM63A,FN1,FST,FSTL1,GFRA1,IL18,IL7R,IRF4,ITGA2,KCNIP2,KLF5,LAMC1,NKD1,NRP2,NTRK3,RNF214,ROBO1,SCUBE3,SDK1,SMPD3,SNCAIP,SYTL2,TEK,TNFRSF19,XKRX,ZCCHC14
Motif gene sets	CTTTGA_V$LEF1_Q2	0.0001493	ABHD2,ATP13A4,BACE1,CD160,CPB1,DLC1,EMID1,FAM63A,FST,GFRA1,GPC4,KIF7,KY,LEF1,MB21D2,MED13,NKD1,NRP2,ROBO1,SESTD1,SLC22A23,SNCAIP,SYTL2,TBC1D8,TCF7,TNFRSF19,XKRX
Motif gene sets	TGGAAA_V$NFAT_Q4_01	0.006435	ANTXR1,B4GALT5,BMP4,CHDH,DCLK1,DKK2,DLC1,EFHD1,FN1,FST,FSTL1,GALNT7,IGSF3,IL17RB,IL7R,IRF4,ITGA2,KCNIP2,KLF5,MED13,NFATC2,SCUBE3,SH3BGRL2,SMPD3,SNCAIP,TBC1D8,TMEM163,TMEM35,TNFRSF19,TNS1,XKRX
Motif gene sets	V$TCF4_Q5	0.009151	ABHD2,FAM63A,FST,GPC4,KY,NKD1,NRP2,SYTL2,TCF7,TNFRSF19
Oncogenic signatures	CAMP_UP.V1_DN	1.97E-05	ANXA1,BACE1,CAMK2D,CCR9,CD160,CD83,CHST2,FSTL1,IL7R,NFATC2,TUBB3,ZCCHC14
Oncogenic signatures	MEL18_DN.V1_UP	0.03587	CD83,CHST2,CRISPLD2,IL7R,NRP2,NT5E,TEK

Table S2				
Experiment	**Study**	**Significantly detected binding regions**	**Mapped to genes within 5Kb from TSS**	**P-value, Significance with WNT-associated genesets from MsigDB**
TCF1	GSE52070	591	116	0.033
TCF1 (n=2)	GSE46662	732 in Sample 1	131	0.050
		2600 in Sample 2	653	9.395E-04
TCF7	GSE31221	6395	2015	0.017
Beta-Catenin (n=3)	GSE43565	990 in Sample 1	121	2.273E-04
		385 in Sample 2	49	9.564E-04
		671 in Sample 3	79	0.004

Table S3

Gene-set	Pathway	$P_{BY} \leq 0.05$	Genes
WNT gene sets	WNT_BOIERS_2013_LYMPHOID	0.01053	CCR9,LEF1,TCF7
All Curated gene sets	ST_WNT_BETA_CATENIN_PATHWAY	0.0001483	APC,AXIN2,DKK2,FSTL1,NKD1,WIF1
All Curated gene sets	RIGGI_EWING_SARCOMA_PROGENITOR_DN	0.0006029	ALDH1A3,BACE1,BMP4,CLU,EBF1,FST,NRP2,PHLDA1,TNFRSF19
All Curated gene sets	SANSOM_WNT_PATHWAY_REQUIRE_MYC	0.001274	AXIN2,LEF1,NKD1,TCF7,TNFRSF19,WIF1
All Curated gene sets	LIU_PROSTATE_CANCER_DN	0.001855	BEND5,CHST2,CLU,EPAS1,ITGA2,NDNF,PHLDA1,PRSS23,ROBO1,TFCP2L1,WIF1,ZCCHC14
All Curated gene sets	MATSUDA_NATURAL_KILLER_DIFFERENTIATION	0.001855	ANXA1,APCDD1,CCR9,CD160,EBF1,NTRK3,PDCD1,PRSS23,SH3BGRL2,TCF7,TULP3,ZC3H12C
All Curated gene sets	KINSEY_TARGETS_OF_EWSR1_FLII_FUSION_DN	0.001979	DCLK1,EBF1,FAM63A,FSTL1,IL17RD,MB21D2,NT5E,PHLDA1,PRSS23,SIPA1L2
All Curated gene sets	KUMAR_TARGETS_OF_MLL_AF9_FUSION	0.001979	ANXA1,CCR9,CD83,EBF1,EXTL3,GPC4,IL7R,IRF4,LEF1,TCF7,TNFRSF19
All Curated gene sets	CUI_TCF21_TARGETS_2_UP	0.003067	ANTXR1,APCDD1,ARSB,BMP4,CLU,DCLK1,HUNK,KLF5,LYPD6B,NKD1,NRP2
All Curated gene sets	CHARAFE_BREAST_CANCER_LUMINAL_VS_BASAL_DN	0.003672	ADA,ALDH1A3,ANTXR1,ANXA1,FST,FSTL1,IL7R,KLF5,NT5E,PHLDA1,ZC3H12C
All Curated gene sets	DODD_NASOPHARYNGEAL_CARCINOMA_UP	0.003672	ALDH1A3,ANXA1,APCDD1,ATP13A4,BACE1,BDH1,BEND5,CLU,EBF1,EDAR,EPAS1,FAM63A,KLF5,LYPD6B,PRKAA2,PRSS23,SH3BGRL2,TNFRSF19,TUBB3,UST,WWC1
All Curated gene sets	DELYS_THYROID_CANCER_UP	0.003672	ALDH1A3,ANXA1,CHST2,DPP4,IGSF3,ITGA2,MED13,NRP2,NT5E,PRSS23,STX3
All Curated gene sets	ONDER_CDH1_TARGETS_2_DN	0.004116	ALDH1A3,CD83,EPAS1,FST,IGSF3,ITGA2,KLF5,ROBO1,TFCP2L1,THBD,WWC1
All Curated gene sets	SANA_TNF_SIGNALING_DN	0.004116	ANTXR1,ANXA1,CLU,EPAS1,NT5E,PHLDA1
All Curated gene sets	KEGG_WNT_SIGNALING_PATHWAY	0.004871	APC,AXIN2,DKK2,LEF1,NKD1,TCF7,WIF1
All Curated gene sets	KEGG_BASAL_CELL_CARCINOMA	0.005932	APC,AXIN2,BMP4,LEF1,TCF7
All Curated gene sets	CUI_TCF21_TARGETS_2_DN	0.006368	ANXA1,ARHGAP28,BACE1,DKK2,DPP4,EBF1,EPAS1,MED13,NT5E,PPAP2A,SH3BGRL2,SHROOM3,SNCAIP,THBD
All Curated gene sets	BYSTRYKH_HEMATOPOIESIS_STEM_CELL_QTL_TRANS	0.01287	AXIN2,CHST2,EFHD1,EXTL3,FAM63A,GPC4,KIF5C,LEF1,PDCD1,STX3,SULT1A1,TFCP2L1,THBD,TULP3
All Curated gene sets	GAUSSMANN_MLL_AF4_FUSION_TARGETS_F_UP	0.0141	ARHGAP28,ARSB,BMP4,FST,GPC4,IL17RD,NT5E
All Curated gene sets	CHARAFE_BREAST_CANCER_LUMINAL_VS_MESENCHYMAL_DN	0.01879	ANTXR1,ANXA1,CHST2,FST,FSTL1,IL7R,NT5E,PHLDA1,RAI14,ZC3H12C
All Curated gene sets	GOZGIT_ESR1_TARGETS_DN	0.03962	CLU,DCLK1,FETUB,GPC4,MB21D2,PPAP2A,PRSS23,RASGRP1,SH3BGRL2,SHROOM3,SIPA1L2,THBD
All Curated gene sets	ENK_UV_RESPONSE_EPIDERMIS_DN	0.04184	ANXA1,APC,CD83,ITGA2,PHLDA1,PPAP2A,PRSS23,RAI14,ROBO1,THBD
All Curated gene sets	WNT_SIGNALING	0.04194	APC,LEF1,NKD1,TCF7,WIF1
All Curated gene sets	PID_PS1_PATHWAY	0.04605	APC,DKK2,NKD1,WIF1
All Curated gene sets	SENESE_HDAC1_AND_HDAC2_TARGETS_UP	0.04605	DCLK1,DKK2,EXTL3,IL7R,NRIP3,PHLDA1,WWC1
All Curated gene sets	KIM_MYC_AMPLIFICATION_TARGETS_DN	0.04605	DCLK1,IL17RB,IL17RD,KLF5,SHROOM3
Motif gene sets	TTGTTT_V$FOXO4_01	3.15E-05	ANXA1,APC,AXIN2,BDH1,BMP4,CCDC109B,CD83,EBF1,EDAR,EXTL3,FAM63A,FST,FSTL1,IL7R,IRF4,ITGA2,KCNIP2,KLF5,NKD1,NRP2,NTRK3,RNF214,ROBO1,SNCAIP,TNFRSF19,XKRX,ZCCHC14
Motif gene sets	CTTTGA_V$LEF1_Q2	0.0003071	ATP13A4,BACE1,CD160,CPB1,FAM63A,FST,GPC4,KY,LEF1,MB21D2,MED13,NKD1,NRP2,ROBO1,SLC22A23,SNCAIP,TCF7,TNFRSF19,XKRX
Motif gene sets	CAGGTG_V$E12_Q6	0.001973	ACTN2,AXIN2,BACE1,BMP4,CD83,CPB1,EBF1,EDAR,EPAS1,EXTL3,FST,IGSF3,ITGA2,KCNIP2,LEF1,LYPD6B,MB21D2,NRIP3,NRP2,NTRK3,SH3BGRL2,SNCAIP,TCF7,UNC45B,UST,WWC1
Motif gene sets	V$TCF4_Q5	0.005048	FAM63A,FST,GPC4,KY,NKD1,NRP2,TCF7,TNFRSF19
Motif gene sets	TGGAAA_V$NFAT_Q4_01	0.006748	ANTXR1,BMP4,DCLK1,DKK2,EBF1,EFHD1,FST,FSTL1,IGSF3,IL17RB,IL7R,IRF4,ITGA2,KCNIP2,KLF5,MED13,SH3BGRL2,SNCAIP,TMEM163,TNFRSF19,XKRX
Motif gene sets	TATTATA,MIR-374	0.01408	ARHGAP28,BACE1,CHST2,EDAR,MED13,RNF214,UST,ZCCHC14
Motif gene sets	TGCCAAR_V$NF1_Q6	0.01468	AHSG,AXIN2,DCLK1,KY,LEF1,MB21D2,MED13,NRP2,NTRK3,RAI14,ROBO1,XKRX
Motif gene sets	RTAAACA_V$FREAC2_01	0.02911	AXIN2,BMP4,FST,FSTL1,IRF4,KY,NTRK3,ROBO1,SNCAIP,TCF7,TNFRSF19,UNC45B,UST
Oncogenic signatures	CAMP_UP.V1_DN	5.78E-06	ANXA1,BACE1,CCR9,CD160,CD83,CHST2,FSTL1,IL7R,TUBB3,ZCCHC14
Oncogenic signatures	AKT_UP.V1_DN	0.04519	AXIN2,EDARADD,TNFRSF19,TULP3,WIF1,ZC3H12C
Immunologic signature	GSE24142_EARLY_THYMIC_PROGENITOR_VS_DN2_THYMOCYTE_DN	0.001121	ADA,AXIN2,CCDC109B,DPP4,EDARADD,IL17RB,IL7R,PDCD1,TUBB3
Immunologic signature	GSE20366_EX_VIVO_VS_DEC205_CONVERSION_NAIVE_CD4_TCELL_UP	0.007534	ACTN2,CD160,EPAS1,GPR114,IL17RB,RASGRP1,THBD,XKRX
Immunologic signature	GSE10325_LUPUS_CD4_TCELL_VS_LUPUS_BCELL_UP	0.01556	ANXA1,CCDC109B,DPP4,IL7R,LEF1,TCF7,ZCCHC14
Immunologic signature	GSE14350_IL2RB_KO_VS_WT_TREG_DN	0.01556	CCDC109B,CD160,CD83,KY,NT5E,PDCD1,ZC3H12C
Immunologic signature	GSE24142_EARLY_THYMIC_PROGENITOR_VS_DN3_THYMOCYTE_DN	0.01556	ADA,CCDC109B,IL7R,LEF1,PDCD1,TUBB3,TULP3
Immunologic signature	GSE24142_EARLY_THYMIC_PROGENITOR_VS_DN2_THYMOCYTE_ADULT_DN	0.01556	AXIN2,CCDC109B,EDARADD,IL17RB,IL7R,LEF1,PPAP2A
Immunologic signature	GSE26495_NAIVE_VS_PD1HIGH_CD8_TCELL_UP	0.01556	BDH1,BEND5,EDAR,EFHD1,LEF1,NT5E,PPAP2A
Immunologic signature	GSE26495_NAIVE_VS_PD1LOW_CD8_TCELL_UP	0.01556	BDH1,BEND5,EDAR,EFHD1,LEF1,NT5E,PPAP2A
Immunologic signature	GSE30962_PRIMARY_VS_SECONDARY_CHRONIC_LCMV_INF_CD8_TCELL_DN	0.01556	AHSG,ANXA1,EPAS1,GPR114,PRKAA2,RASGRP1,TMEM163
Immunologic signature	GSE3982_BCELL_VS_CENT_MEMORY_CD4_TCELL_DN	0.01556	DPP4,IL7R,ITGA2,NDNF,PHLDA1,PRKAA2,TCF7
Immunologic signature	GSE7460_TCONV_VS_TREG_LN_DN	0.01556	CD83,DPP4,IRF4,NT5E,PPAP2A,SH3BGRL2,ZC3H12C
Immunologic signature	GSE7460_TCONV_VS_TREG_THYMUS_DN	0.01556	CCDC109B,CD83,IGSF3,KIF5C,NRP2,PPAP2A,SH3BGRL2
Immunologic signature	GSE7852_TREG_VS_TCONV_THYMUS_UP	0.01556	CCDC109B,CD83,IGSF3,KIF5C,PDCD1,PPAP2A,SH3BGRL2
Hallmark gene sets	HALLMARK_WNT_BETA_CATENIN_SIGNALING	0.002609	AXIN2,LEF1,NKD1,TCF7
Hallmark gene sets	HALLMARK_COAGULATION	0.002609	ANXA1,CLU,DPP4,ITGA2,PRSS23,THBD
Hallmark gene sets	HALLMARK_IL2_STAT5_SIGNALING	0.007102	CD83,IRF4,NT5E,PHLDA1,PPAP2A,SH3BGRL2
Hallmark gene sets	HALLMARK_KRAS_SIGNALING_DN	0.007102	CHST2,CPB1,EDAR,EFHD1,PDCD1,TFCP2L1
Hallmark gene sets	HALLMARK_ESTROGEN_RESPONSE_EARLY	0.04111	FAM63A,IL17RB,PRSS23,RASGRP1,WWC1
Hallmark gene sets	HALLMARK_COMPLEMENT	0.04111	ACTN2,CLU,DPP4,KCNIP2,RASGRP1