Supplemental Information

AID-Induced Genotoxic Stress Promotes B Cell Differentiation in the Germinal Center via ATM and LKB1

Mara H. Sherman, Ali I. Kuraishy, Chetan Deshpande, Jason S. Hong, Nicholas A. Cacalano, Richard A. Gatti, John P Manis, Michael A. Damore, Matteo Pellegrini, and Michael A. Teitell

Supplemental Experimental Procedures

ChIP-on-chip

Control and ChIP-enriched genomic DNA (500ng each) was heat fragmented at 95ºC for 30m. The heat fragmented DNA was labeled using BioPrime DNA Labeling System (Invitrogen) with some modifications. The control and enriched DNAs were labeled with 3ul Cy3 dCTP and Cy5 dCTP (PerkinElmer), respectively, and purified using a BioPrime Array CGH Purification Module (Invitrogen). Hybridizations to the Agilent 244K Human Promoter ChIP-on-chip Microarray Set (Agilent Technologies) were conducted using a Maui Hybridization Station (BioMicro Systems) on setting B. Formamide was added to the Agilent hybridization buffer to final concentration of 10% and the hybridization was conducted at 60ºC for 18h. The arrays were washed, scanned and data extracted using Agilent standard protocols, Agilent DNA Microarray Scanner, and Agilent Feature Extraction (version 9.5.1.1) software. For all subsequent analyses probes were averaged over 1kb windows centered at the probe. Each average was converted to a z-score by subtracting the array mean, dividing by the array standard deviation and multiplying by the square root of the number of probes in the window. The z-scores were then averaged for the two replicates and two different CRTC2 antibodies used in this study.

Gene Expression Arrays

Labeled cRNA was generated using 200ng of total RNA from each sample and the Agilent Low RNA Input Linear Amplification labeling kit. Each labeled treated sample was hybridized against its corresponding untreated sample in fluor-reversed pairs of arrays to an Agilent 4x44K Whole Human Genome Microarray. The arrays were scanned using the Agilent DNA Microarray Scanner, and data were extracted using the Agilent Feature Extraction (version 9.5.1.1) software using the standard Agilent protocol except with Lowess normalization.

For the expression arrays, the Agilent Feature Extracted log ratio values of each probe were averaged from fluor-reversed pair of arrays and three replicates for both conditions, anti-IgM and etoposide. The expression values were converted to z-scores by subtracting the array mean and dividing by the array standard deviation. To identify genes that were regulated by CRTC2 and showed changes in expression the maximum CRTC2 binding site was identified for each gene. Similarly, for genes that had multiple probes in the expression arrays the probe with the most significant z-score was used. A threshold of 2 for both CRTC2 binding and the absolute value of changes in expression in both α-IgM and Eto was used to identify a set of significantly bound and differentially expressed genes.

Flow Cytometry

B cell suspensions were labeled with fluorescein-conjugated goat anti-human IgG (Southern Biotech) or co-labeled with fluorescein-conjugated rat anti-mouse IgG1 and APC-conjugated rat anti-mouse B220. Stained samples were gated according to standard forward- and side-scatter values and analyzed on a Becton-Dickinson FACSscan fluorescence-activated cell sorter with CELLQuest software.

Magnetic Cell Separation

Four B cell subpopulations were isolated from whole tonsil by magnetic cell separation using the MidiMACS system (Miltenyi Biotech) as previously described (Klein et al., 2003; Said et al., 2001). Briefly, naïve B cells were isolated by depletion of GC B cells (CD10, CD27), memory B cells (CD27), plasma cells/blasts (CD27), and T cells (CD3), followed by a positive enrichment of IgD-positive cells. Centroblasts (CB) were isolated by magnetically labeling CD77-positive cells. To enrich for centrocytes (CC), tonsil mononuclear cells (MC) were depleted for CB (CD77), naïve, memory, and plasma cells/blasts (CD39), and T cells (CD3). CC were then enriched by CD10. To enrich for plasma cells, tonsil MC were depleted for T cells (CD3), followed by positive enrichment of CD138-positive cells. Sorting antibodies were obtained from BD Pharmingen.

Mouse Lymphocyte Culture System and Sorting

B lymphocytes from WT (C57BL/6) and *AID* knockout (Muramatsu et al., 2000) mice were isolated from spleens using CD43 microbeads (Miltenyi Biotech). Cells were cultured at 5 X 10^5 cells/ml in the presence of 2 μg/ml αCD40 Ab (BD Pharmingen) and 20 ng/ml IL-4 (R&D Systems) for 4 days prior to harvest. Flow cytometry for CSR and surface IgG1, subcellular fractionation, and immunoblotting were performed as described above.

Oligonucleotides

Oligo name	**Sequence**
TCL1 promoter F	TCCAGAAGAAGAAAGGGCCAAGGT
TCL1 promoter R	GTCTTGGTGGCAAGTGAGGGT
Intergenic region F	TCAGCATTACGTCTGCCAAGCCTA
Intergenic region R	TCCAATTTGGGCTGCTGGTTTCTG
BACH2 promoter F	TGGCACACTCCTGGCTTCT
BACH2 promoter R	TCCCTCAAGTTACCAAACTCGCCT
CDK6 promoter F	CAGACTGGACCGGGCCTTTA
CDK6 promoter R	AGAGAGAAGGTCTCTGTCCTCGG

AICDA promoter F	CCCTTTCTCTCATGTAACTGTCTGACTGA
AICDA promoter R	CAGAAAGAATTGGGCTGACAGCGT
SMARCA2 promoter F	GTTACCCTGCCCTCCCTCT
SMARCA2 promoter R	GGGAAGACGCGAAGCAAA
SMARCA4 promoter F	AGGGTCAAGAAGCCCAGCC
SMARCA4 promoter R	CTCGCGACACTGTGGCCTT
TCF3 promoter F	ATCAGACTTTCGCGGCTTCCGAT
TCF3 promoter R	TTGAAAGCTAAGGCAGAGCAGTCTGG
SMAD6 promoter F	AAAGCGTCCTGAGCTCCCTGTTT
SMAD6 promoter R	TTCCAGGGTTGAGCCTCTGAGGAG
MYC promoter F	GGTTCACTAAGTGCGTCTCCGAGATA
MYC promoter R	AGGGAGCACCCTTTCACCC
MTA3 promoter F	CCCACTCTAATTCTCTAACAGCCC
MTA3 promoter R	GCAGAGGCCTGCACTCAAAT
ACTB promoter F	GCTGGGTAGGTTTGTAGCCTTCATCA
ACTB promoter R	TAGCTAAATGTGCTGGGTGGGTCA
TCF3 expression F	TCCGTGACATCAACGAGGCCTTTA
TCF3 expression R	ATTCAGGTTCCGCTCTCGCACTT
SMARCA4 expression F	CAGATGCACAAGCCCATGGAGTC
SMARCA4 expression R	GTAACCTTGGGAGTGCTGGTCCAT
SMARCA2 expression F	CGTCAGAAACACCAGGAATACCTGAACA
SMARCA2 expression R	ATCAGTCGCCGCATTCTCTCCTTT
AICDA expression F	TTCGCAATAAGAACGGCTGCCAC
AICDA expression R	ATAATCTTTGAAGGTCATGATGGCTATTTGCAC
CDK6 expression F	CCCCAACGTGGTCAGGTTGTTT
CDK6 expression R	TGTGAATGAAGAAAGTCCAGACCTCGG
BACH2 expression F	CTTGCCTGAGGAGGTCACAGC
BACH2 expression R	AGCATCCTTCCGGCACACAAA
SMAD6 expression F	GTGAATTCTCAGACGCCAGCATGT
SMAD6 expression R	TGCCCTGAGGTAGGTCGTAGAAGAT
MYC expression F	AGCGACTCTGAGGAGGAACAAGAAGAT
MYC expression R	TTGGCAGCAGGATAGTCCTTCCG
MTA3 expression F	GTACCGGGTCGGAGATTATGTCTACTTTG
MTA3 expression R	CCTCAATTTCTTTAGCATGCTTATCTGCGAG
TCL1 expression F	CGATACCGATCCTCAGACTCCAGTT
TCL1 expression R	AAAGGAGACAGGTGCTGCCAAG
36B4 expression F	TGGCAGCATCTACAACCCTGAAGT
36B4 expression R	TGGGTAGCCAATCTGAAGACAGACA
OCAB expression F	TATGCCTCTCCGCCACTCATCA
OCAB expression R	TGAGGCCACGGGAAATAGGTGA
BCL6 expression F	TTGTCATTGTTGTGAGCCGTGAGC
BCL6 expression R	AAACTTCCGGCAAGTGTCCACAAC

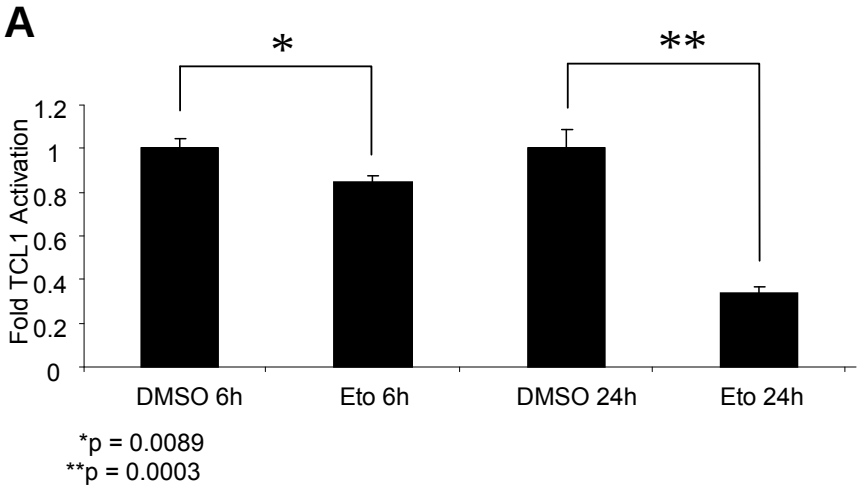
PRDM1 expression F	CAACAACTTTGGCCTCTTCC
PRDM1 expression R	GCATTCATGTGGCTTTTCTC
sh*LKB1* F	GATCCCCTGCTGAAAGGGATGCTTGATTCA AGAGATCAAGCATCCCTTTCAGCA TTTTTTGGAAC
sh*LKB1* R	TCGAGTTTCCAAAAAATGCTGAAAGGGATG CTTGATCTCTTGAATCAAGCATCCCTTTCAGCA GGG
sh*LKB1* 2F	TCCCAACGTGAAGAAGGAAATTCAACTCGAGTTGAA TTTCCTTCTTCACGTTTTTTTG
sh*LKB1* 2R	AAAACAAAAAAACGTGAAGAAGGAAATTCAACTCGAGTTGAA TTTCCTTCTTCACGTT
sh*ATM* F	TCCCCCCGGAACATACTACTCAAAGACATTTTC AAGAGAAATGTCTTTGAGTAGTATGTTCCTTTT TTGGAAAC
sh*ATM* R	AAAAGTTTCCAAAAAAGGAACATACTACTCAA AGACATTTCTCTTGAAAATGTCTTTGAGTAGTA TGTTCCGGG
sh*ATM* 2F	TCCCCCCGGAATGATCTGCTTATCTGCTGCCGTTCAAGA GACGGCAGCAGATAAGCAGATCATTCCTTTTTTGGAAAC
sh*ATM* 2R	AAAAGTTTCCAAAAAAGGAATGATCTGCTTATCTGCTG CCGTCTCTTGAACGGCAGCAGATAAGCAGATCATTCCGGG
Scramble F	TTTGGTCTACCCTACACGCGTTACACGACAATG CGTGTAGAGTAGAGGCTTTTTT
Scramble R	AAAAAAGCCTCTACTCTACACGCATTGTCGTGT AACGCGTGTAGGGTAGACGATC
FLAG-CRTC2 F	GCTCTAGAGCCACCATGGACTACAAG
FLAG-CRTC2 R	GCGTCTAGATCACTGTAGCCGATCAC
CRTC2 sequencing primer F	ATTGACAGCTCTCCCTATAGTCCTGC
CRTC2 sequencing primer R	AGGCTGGTCAGGAGATGGAAAGAT
LKB1 expression F	CAGCTGATTGACGGCCTGGA
LKB1 expression R	CACGCCCAGGTCGGAGATTT
ATM expression F	GCGCCTGATTCGAGATCCTGAAACAATTA
ATM expression R	TGGAGGCTTGTGTTGAGGCTGATA
Ig var. region heavy chain F	GACACGGCTGTGTATTACTGTGCG
Ig Cμ R	CCGAATTCAGACGAGGGGGAAAAGGGTT
Ig Cγ1 R	GTTTTGTCACAAGATTTGGGCTC
Ig Cγ2 R	GTGGGCACTCGACACAACATTTGCG
Ig Cγ3 R	TTGTGTCACCAAGTGGGGTTTTGAGC
Ig Cγ4 R	ATGGGCATGGGGGACCATTTGGA

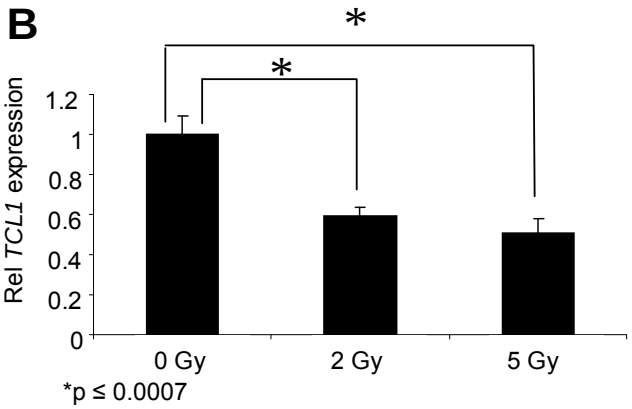
Supplemental References

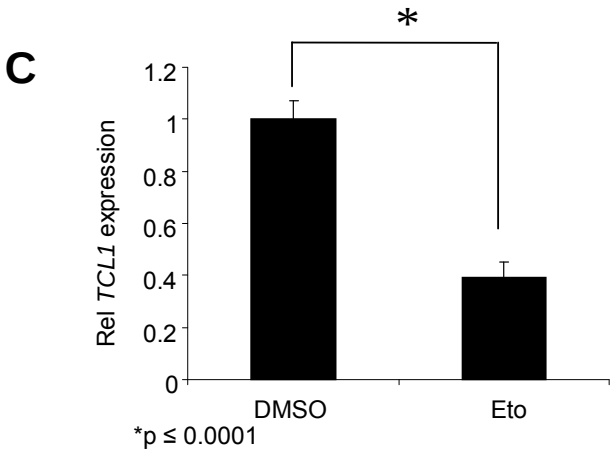
Klein, U., Tu, Y., Stolovitzky, G. A., Keller, J. L., Haddad, J., Jr., Miljkovic, V., Cattoretti, G., Califano, A., and Dalla-Favera, R. (2003). Transcriptional analysis of the B cell germinal center reaction. Proc Natl Acad Sci U S A *100*, 2639-2644.

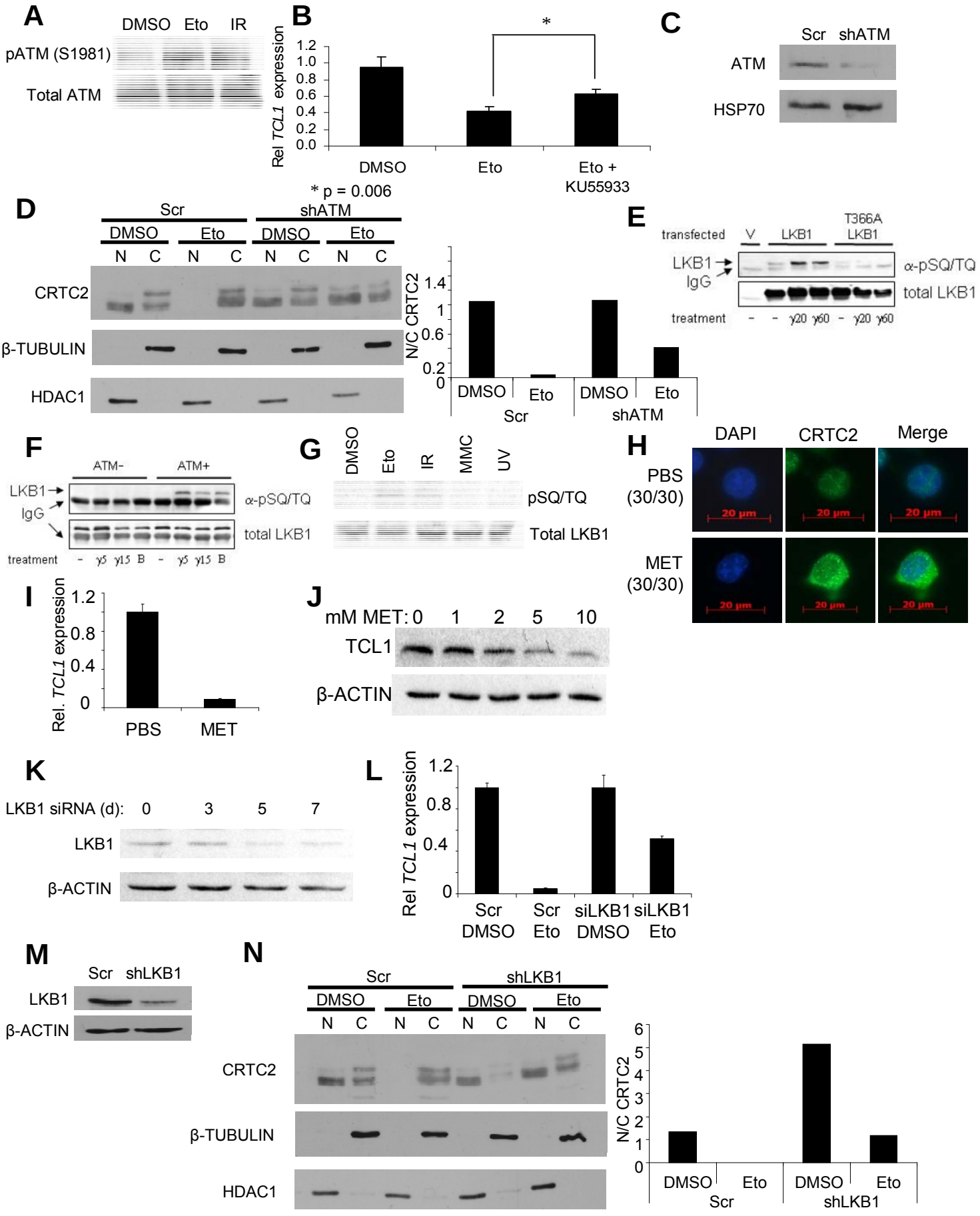
Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., and Honjo, T. (2000). Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell *102*, 553-563.

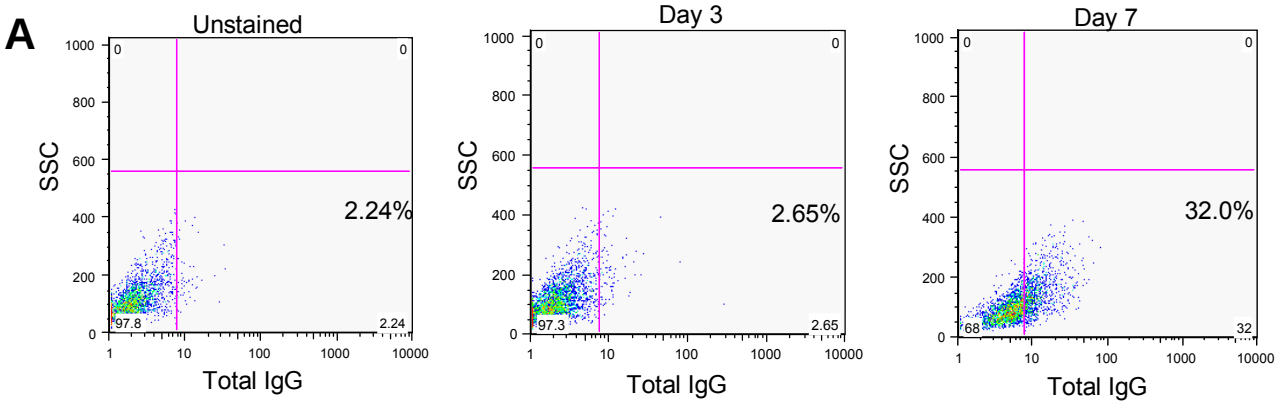
Said, J. W., Hoyer, K. K., French, S. W., Rosenfelt, L., Garcia-Lloret, M., Koh, P. J., Cheng, T. C., Sulur, G. G., Pinkus, G. S., Kuehl, W. M.*, et al.* (2001). TCL1 oncogene expression in B cell subsets from lymphoid hyperplasia and distinct classes of B cell lymphoma. Lab Invest *81*, 555-564.

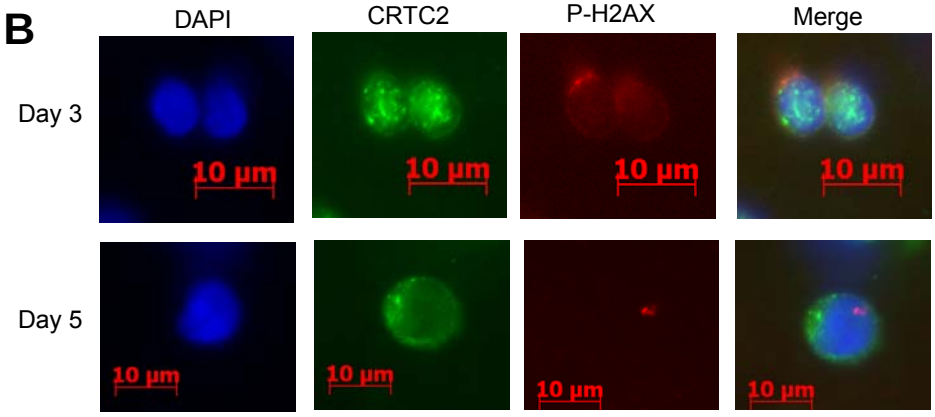

A
*
**
Fold TCL1 Activation
1.2
1
0.8
0.6
0.4
0.2
0
DMSO 6h
Eto 6h
DMSO 24h
Eto 24h
*p = 0.0089
**p = 0.0003

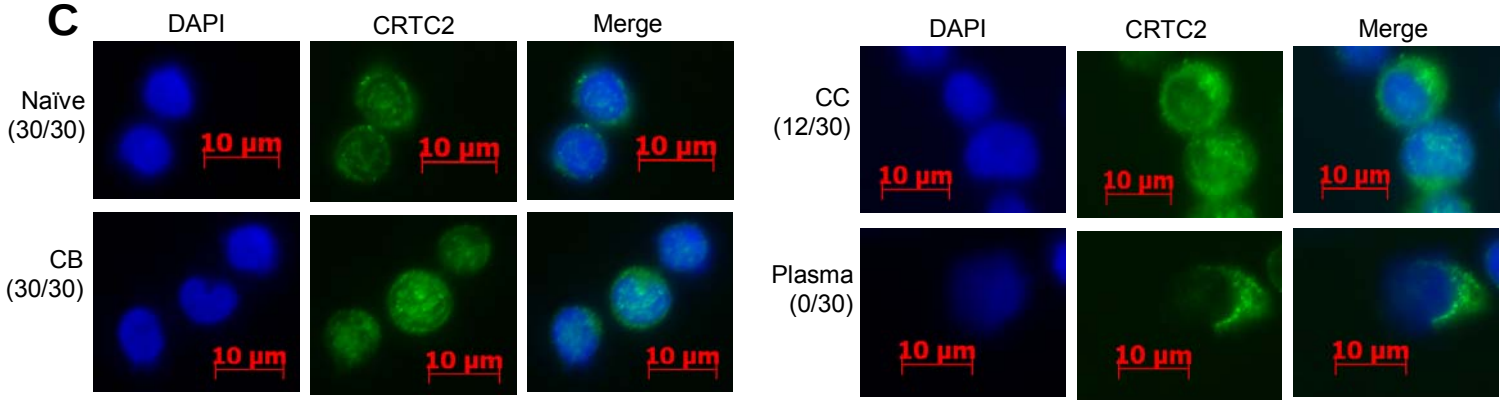

B
*
*
Rel TCL1 expression
1.2
1
0.8
0.6
0.4
0.2
0
0 Gy
2 Gy
5 Gy
*p ≤ 0.0007

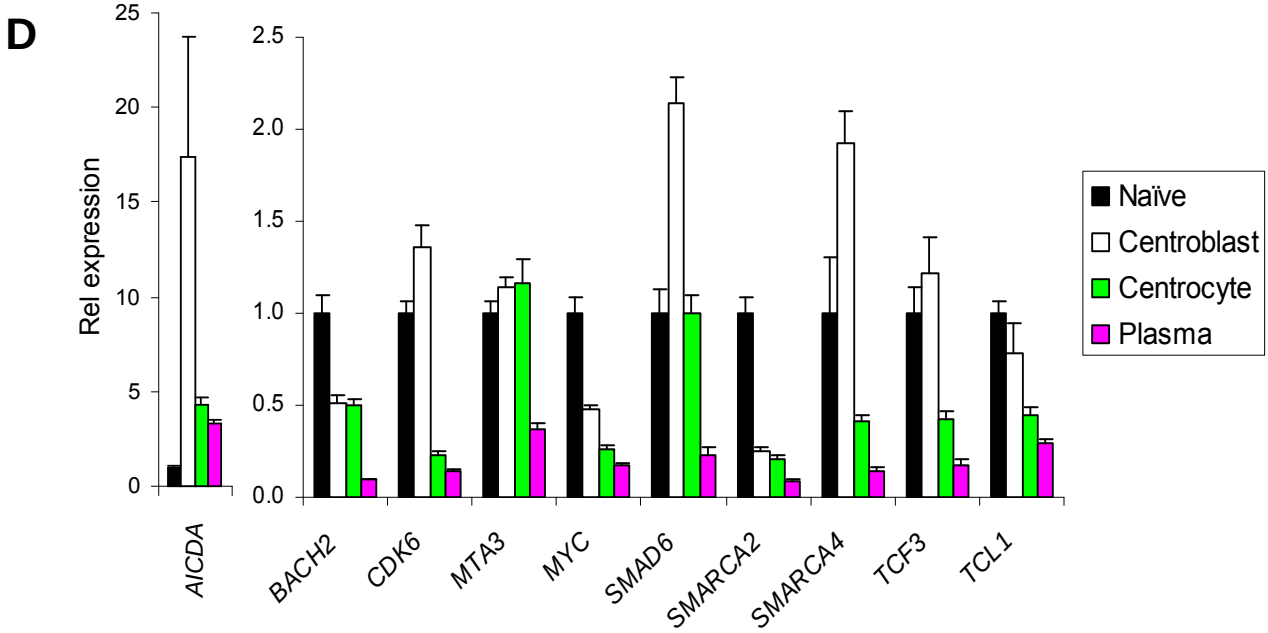

C
*
Rel TCL1 expression
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
Eto
*p ≤ 0.0001

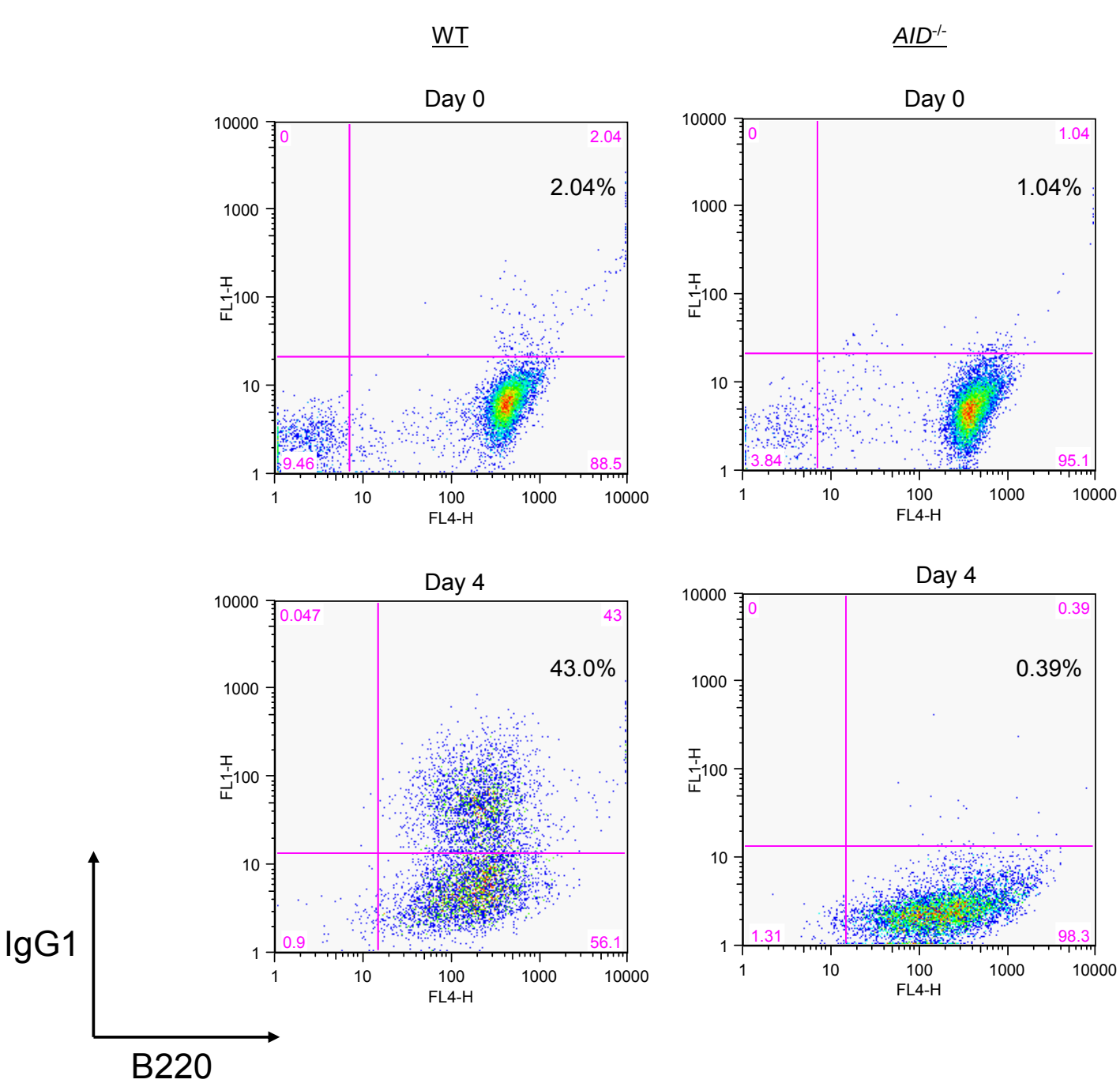

A
DMSO Eto IR
pATM (S1981)
Total ATM
B
Rel TCL1 expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
DMSO
Eto
Eto + KU55933
* p = 0.006
C
Scr shATM
ATM
HSP70
D
Scr
shATM
DMSO Eto DMSO Eto
N C N C N C N C
CRTC2
β-TUBULIN
HDAC1
N/C CRTC2
1.4
1
0.6
0.2
0
DMSO Eto DMSO Eto
Scr
shATM
E
transfected V LKB1 T366A LKB1
LKB1
IgG
α-pSQ/TQ
total LKB1
treatment - - γ20 γ60 - γ20 γ60
F
ATM- ATM+
LKB1
IgG
α-pSQ/TQ
total LKB1
treatment - γ5 γ15 B - γ5 γ15 B
G
DMSO Eto IR MMC UV
pSQ/TQ
Total LKB1
H
DAPI CRTC2 Merge
PBS (30/30)
MET (30/30)
20 μm
I
Rel. TCL1 expression
1.2
0.8
0.4
0
PBS MET
J
mM MET: 0 1 2 5 10
TCL1
β-ACTIN
K
LKB1 siRNA (d): 0 3 5 7
LKB1
β-ACTIN
L
Rel TCL1 expression
1.2
0.8
0.4
0
Scr DMSO
Scr Eto
siLKB1 DMSO
siLKB1 Eto
M
Scr shLKB1
LKB1
β-ACTIN
N
Scr
shLKB1
DMSO Eto DMSO Eto
N C N C N C N C
CRTC2
β-TUBULIN
HDAC1
N/C CRTC2
6
5
4
3
2
1
0
DMSO Eto DMSO Eto
Scr
shLKB1

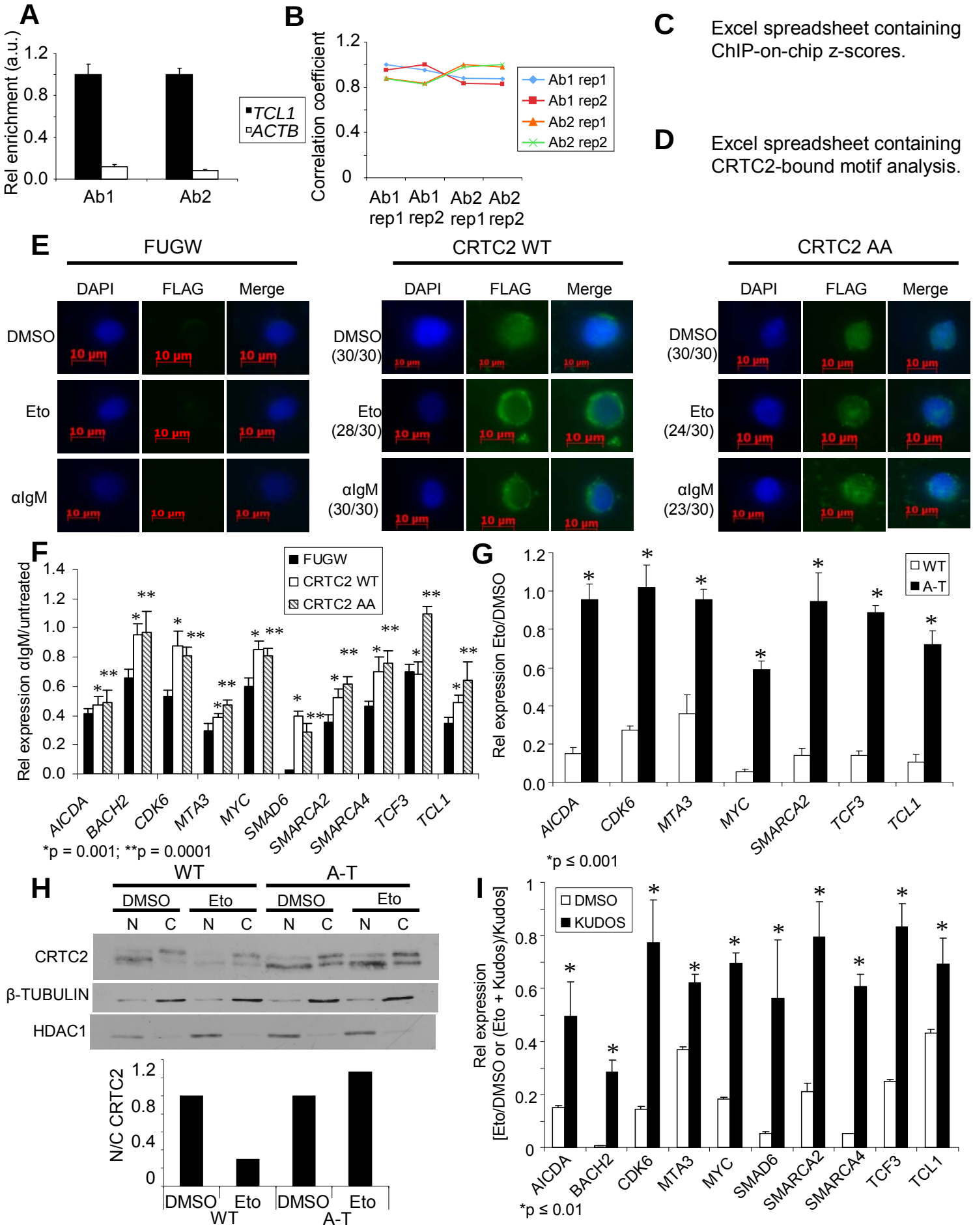

A
Unstained
Day 3
Day 7
SSC
Total IgG
2.24%
2.65%
32.0%
97.8
2.24
97.3
2.65
68
32

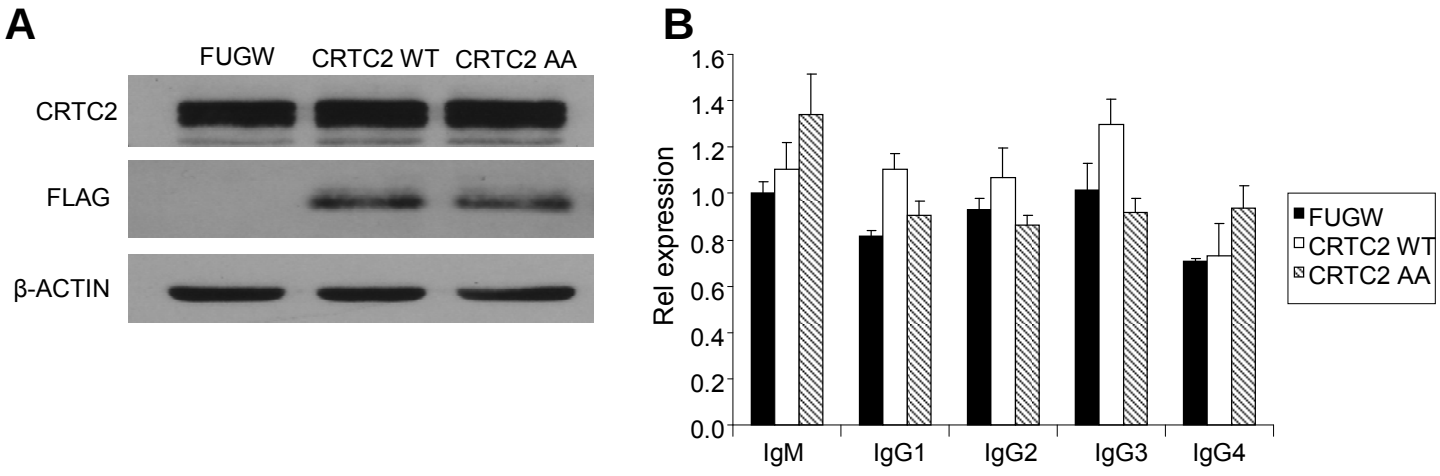

B
DAPI
CRTC2
P-H2AX
Merge
Day 3
Day 5
10 µm

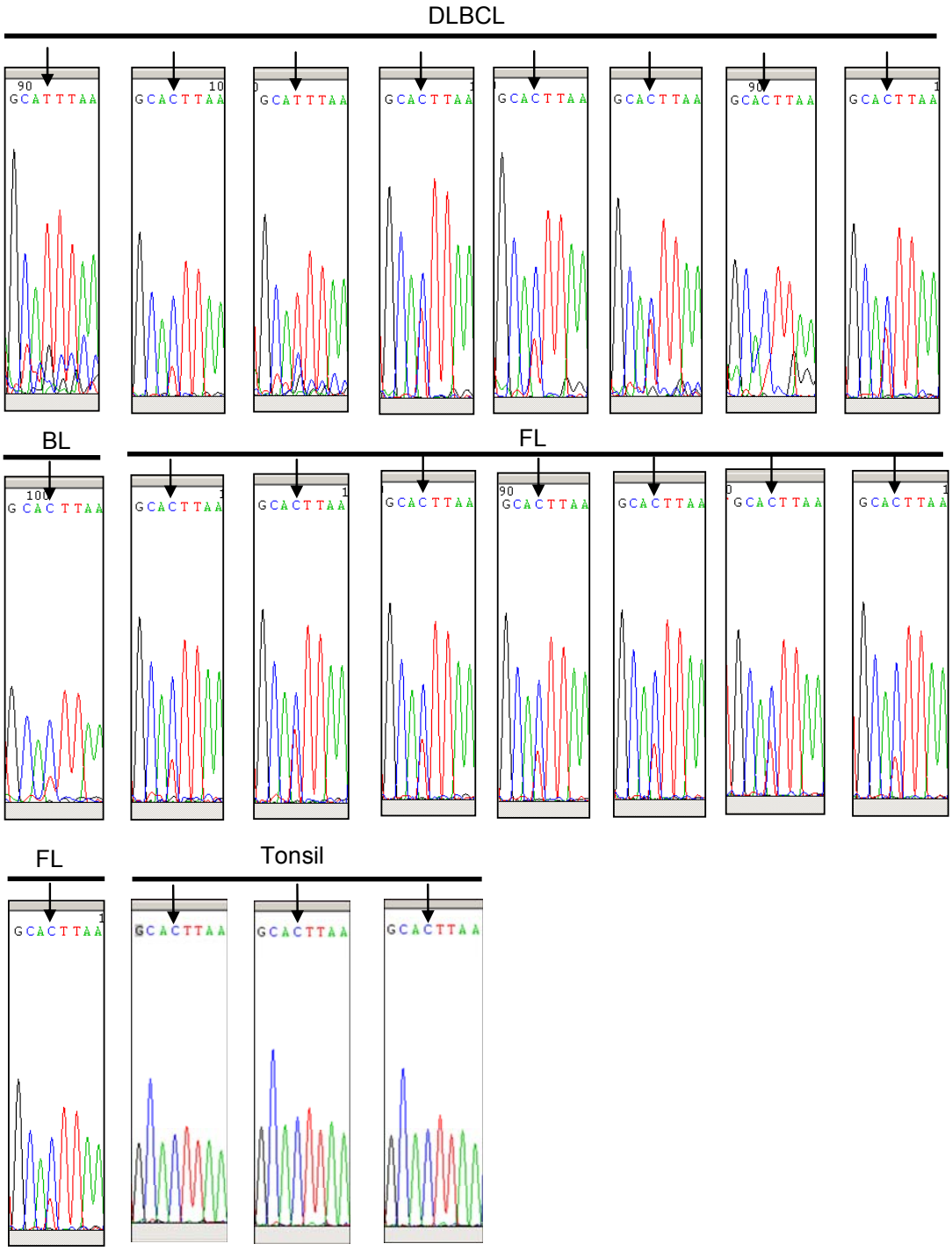

C
DAPI
CRTC2
Merge
Naïve
(30/30)
CB
(30/30)
CC
(12/30)
Plasma
(0/30)
10 µm


D
Rel expression
Naïve
Centroblast
Centrocyte
Plasma
AICDA
BACH2
CDK6
MTA3
MYC
SMAD6
SMARCA2
SMARCA4
TCF3
TCL1


WT
AID-/-
Day 0
Day 0
2.04%
1.04%
0
2.04
9.46
88.5
0
1.04
3.84
95.1
Day 4
Day 4
43.0%
0.39%
0.047
43
0.9
56.1
0
0.39
1.31
98.3
FL1-H
FL4-H
10000
1000
100
10
1
IgG1
B220


A
Rel enrichment (a.u.)
1.2
0.8
0.4
0.0
Ab1
Ab2
TCL1
ACTB
B
Correlation coefficient
1.2
0.8
0.4
0
Ab1 rep1
Ab1 rep2
Ab2 rep1
Ab2 rep2
Ab1 rep1
Ab1 rep2
Ab2 rep1
Ab2 rep2
C
Excel spreadsheet containing ChIP-on-chip z-scores.
D
Excel spreadsheet containing CRTC2-bound motif analysis.
E
FUGW
CRTC2 WT
CRTC2 AA
DAPI
FLAG
Merge
DMSO
Eto
αIgM
DMSO (30/30)
Eto (28/30)
αIgM (30/30)
DMSO (30/30)
Eto (24/30)
αIgM (23/30)
10 μm
F
Rel expression αIgM/untreated
FUGW
CRTC2 WT
CRTC2 AA
AICDA
BACH2
CDK6
MTA3
MYC
SMAD6
SMARCA2
SMARCA4
TCF3
TCL1
*p = 0.001; **p = 0.0001
G
Rel expression Eto/DMSO
WT
A-T
AICDA
CDK6
MTA3
MYC
SMARCA2
TCF3
TCL1
*p ≤ 0.001
H
WT
A-T
DMSO
Eto
N
C
CRTC2
β-TUBULIN
HDAC1
N/C CRTC2
WT
A-T
I
Rel expression [Eto/DMSO or (Eto + Kudos)/Kudos]
DMSO
KUDOS
AICDA
BACH2
CDK6
MTA3
MYC
SMAD6
SMARCA2
SMARCA4
TCF3
TCL1
*p ≤ 0.01


A
FUGW
CRTC2 WT
CRTC2 AA
CRTC2
FLAG
β-ACTIN
B
Rel expression
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
IgM
IgG1
IgG2
IgG3
IgG4
FUGW
CRTC2 WT
CRTC2 AA


DLBCL
BL
FL
FL
Tonsil

Gene	Z-score ChIP-on-chip	Log ratio anti-IgM	Log ratio etoposide
ABCA1	2.533062	0.613317	0.566003
ABR	2.613673	0.908852	0.320111
ADA	2.35635	0.345962	0.386043
AFF2	2.680356	0.692824	0.444513
AGPAT7	2.634599	0.401922	0.282317
AICDA	1.873877	0.620055	0.512977
AIM1	2.369449	0.324286	0.340401
AK5	3.317455	0.440185	0.27711
AMN	2.533098	0.685571	0.569847
ANK1	2.495314	0.669897	0.438766
APBB1IP	3.117517	0.652551	0.421887
ARL15	2.689234	0.595505	0.643995
ASB13	2.337807	0.596578	0.52969
ASPH	2.304926	0.346	0.308866
BACH2	2.380462	1.099336	0.279499
BCL11A	2.769602	0.765287	0.364046
BCR	2.788809	0.758508	0.466938
BICD2	2.470839	0.768101	0.292209
BMP7	2.373191	0.486419	0.308128
BMPR2	2.087795	0.383258	0.500501
BTLA	2.204403	0.597689	0.387213
C10orf137	2.9013	0.368824	0.392849
C2orf34	2.814604	0.430071	0.35837
C6orf167	3.092857	0.34254	0.271094
C9orf93	2.845509	0.649877	0.388685
CDK6	2.448405	0.635971	0.285365
COL9A3	2.395174	0.585344	0.346924
CORO2B	2.641635	0.355005	0.484163
CUL3	2.620073	0.993322	0.303674
CUTL2	2.212787	0.744418	0.448459
CYP26A1	2.687213	0.583931	0.509085
DCK	2.203917	0.536555	0.472156
DEPDC6	2.020862	0.942209	0.932246
DFFB	2.322888	0.56659	0.320518
DFNA5	3.223839	0.610006	0.818488
DIXDC1	2.062898	0.604093	0.297909
DST	2.924647	0.663497	0.380811
E2F5	2.727275	0.546192	0.329692
EAF2	3.156663	0.602801	0.308079
EDG6	2.342687	0.528694	0.409731
EHHADH	2.448008	0.382506	0.63437
ERGIC1	2.216364	0.444332	0.332314
EVC2	2.298478	0.608452	0.380892
FAM59A	2.10581	0.436556	0.499527
FBXL16	2.080073	0.487178	0.353613
FBXL17	2.827795	0.330237	0.47957
FBXL20	2.011966	0.340605	0.281296

FCHSD2	2.667797	0.969865	0.388468
FCRLM2	3.056374	0.541384	0.352928
FGD6	2.697788	0.445747	0.327159
FGF12	3.036584	0.471456	0.365337
FLI1	3.058955	0.498507	0.330382
FLJ10781	2.644249	0.531821	0.417518
FLJ43339	2.524056	0.609436	0.326945
GAS2L1	2.371583	0.362618	0.507901
GPR146	2.258408	0.445487	0.739014
GTF2I	2.103	0.515733	0.278886
HERC3	2.624257	0.429328	0.354839
IBRDC2	2.185693	0.785971	0.751008
IQCE	2.129561	0.356021	0.381282
JDP2	2.621162	0.528779	0.363568
KCNH8	2.122059	0.34674	0.713449
KCNMB4	2.101346	0.351969	0.533397
KHDRBS2	2.448985	0.64085	0.592891
KIAA0182	2.455095	0.348313	0.295321
KIAA0774	2.34963	0.801442	0.858986
KIAA1622	2.415789	0.467571	0.352437
KIAA1900	2.198882	0.451565	0.473353
KIAA1914	2.369715	0.672084	0.51291
KLF15	2.430034	0.536293	0.441149
KLHL14	2.029478	0.407345	0.520742
LMO3	2.59422	0.846254	0.574315
LRRC34	2.59429	0.683172	0.350709
LRRCC1	2.085134	0.548395	0.336318
LZTS1	2.532856	0.533674	0.729731
MCF2L2	3.33377	0.782938	0.393772
MEGF11	2.018422	0.390101	0.613916
MFSD4	2.174939	0.354325	0.49376
MLLT3	2.409267	0.7309	0.561898
MTA3	2.309794	0.432268	0.327869
MTSS1	2.203541	0.584444	0.574611
MYC	3.296981	0.566382	0.310759
NAV2	2.420738	0.33487	0.290352
NFIA	2.818319	0.893464	0.629842
NOD3	2.296451	0.753535	0.357265
NR3C2	2.711054	0.834021	0.523465
NTHL1	2.180005	0.34279	0.429389
PAPSS1	2.598499	0.351185	0.411316
PARN	2.542358	0.614113	0.289197
PITPNC1	2.397147	1.045999	0.339616
PITPNM2	2.12482	1.009885	0.30657
PMS1	2.776674	0.33346	0.280531
PPP2R3B	2.921845	0.359545	0.295142
PRDM10	2.080148	0.90388	0.327894
PRKCA	2.332082	0.762065	0.388994
PRKCH	2.550004	0.463826	0.314377

PROC	2.038438	0.32483	0.384179
PTPN18	2.913371	0.406896	0.303096
PYCARD	2.89214	0.41603	0.368077
RAB37	2.813755	0.593259	0.441196
RCSD1	2.156679	0.585655	0.364485
SATB2	2.826687	0.576078	0.594463
SCARB1	2.676037	0.712547	0.378365
SH2D1A	2.149962	0.812706	0.472465
SIPA1L3	3.013577	1.221007	0.281492
SLC2A5	2.247339	0.714206	0.529839
SLC39A10	2.655167	0.462249	0.282513
SLC7A7	2.122767	0.32907	0.379662
SMAD1	2.371985	0.610308	0.513803
SMAD6	3.265875	0.579633	0.495518
SMARCA2	2.517652	0.335236	0.415966
SMARCA4	1.87733	0.631448	0.303977
SNTB1	2.193671	0.676703	0.429238
SPG3A	2.016751	0.669465	0.537908
SSBP2	2.81366	0.916858	0.306627
STOX2	2.710594	0.421158	0.716616
STS	2.347684	0.550401	0.315803
SUSD3	2.729927	0.795396	0.617332
TBC1D10C	2.000706	0.712491	0.318883
TCF3	2.733384	0.696164	0.449621
TCL1A	2.062001	0.522095	0.587388
TCL6	2.483377	0.943989	0.684595
TFAP4	3.07121	0.362593	0.392455
TLL1	2.080367	0.479435	0.651101
TNFSF8	2.01981	0.505971	0.338815
TRERF1	2.119094	0.620778	0.47355
TRIB2	2.293439	0.600313	0.35314
UNC5C	2.769456	0.442902	0.328565
UNQ739	2.704115	0.677697	0.504866
VPREB1	2.797564	0.530493	0.383741
WNK2	2.376737	0.468577	0.571981
WNT2B	2.506678	0.801435	0.8741
WNT6	2.300466	0.528299	0.375488
ZNF143	2.849458	0.584438	0.478842
ZNF533	2.718106	1.256506	0.758396
ZNF575	2.438773	0.456804	0.319495

Supplemental Figure Legends

Figure S1, related to Figure 1- DNA Double-Strand Breaks Inactivate CRTC2

(A) Luciferase assay measuring the activity of the *TCL1* promoter 48h post-transfection of HEK293T cells. Cells were exposed to DMSO or Eto (20 μM, 6 or 24h). Values were normalized to a co-transfected *Renilla*-luciferase expression construct. Values are expressed as mean ± SEM of three independent experiments.

(B) QPCR measuring *TCL1* expression in PBL cells 6h post-IR. Values were normalized to *36B4*. Values are expressed as mean ± SEM of three independent experiments.

(C) QPCR measuring *TCL1* expression in peripheral blood B lymphocytes (PBL) cells after DMSO or Eto (20μM, 6h) exposure. Values are normalized to *36B4*. Values are expressed as mean ± SEM of three independent experiments.

Figure S2, related to Figure 2- DSB-induced CRTC2 Inactivation Requires Activation of ATM and LKB1

(A) Immunoblots of phospho-S1981 ATM (pATM) and total ATM in Ramos cells exposed to Eto (20 μM, 1h) or IR (5 Gy).

(B) QPCR for *TCL1* expression in Ramos cells after 6h of DMSO, Eto (20 μM), or Eto (20μM) plus the ATM-inhibitor KU55933 (10 μM) exposure. Values are normalized to *36B4*. Values are expressed as mean ± SEM of three independent experiments.

(C) Immunoblot showing shATM knockdown in Ramos cells 4d post-transduction. HSP70 is a loading control.

(D) (Left) Immunoblot showing CRTC2 protein expression in the nucleus (N) and cytoplasm (C) of Ramos B cells transduced with control (Scr) shRNA or shATM after exposure to DMSO or Eto (20 μM, 1h). Upper bands in the cytoplasm lanes indicate

phosphorylated CRTC2. β-TUBULIN (cytoplasm) and HDAC1 (nucleus) are loading controls. (Right) Nuclear/cytoplasmic ratios for CRTC2 are plotted from densitometry.

(E) Mouse embryonic fibroblasts (MEFs) expressing FLAG-tagged wild-type LKB1 or T366A LKB1 were exposed to IR for 20m or 60m, followed by FLAG-LKB1 immunoprecipitation and immunoblot for ATM-target SQ/TQ phosphorylation substrates.

(F) MEFs expressing or lacking ATM were treated with IR for 5m, 15m, or with bleomycin to induce DSBs, followed by FLAG-tagged LKB1 immunoprecipitation and immunoblot for ATM-target SQ/TQ phosphorylation substrates.

(G) PBLs expressing FLAG-tagged WT LKB1 were exposed to DMSO, Eto (20 μM, 1h), IR (5 Gy), mitomycin C (MMC, 0.225 μM, 1h), or UV irradiation (1000 mJ/cm^2), followed by FLAG-LKB1 immunoprecipitation and immunoblot for ATM-target SQ/TQ phosphorylation substrates.

(H) Immunofluorescence microscopy (63X) showing CRTC2 localization in Ramos cells exposed to PBS (control) or metformin (MET; 10mM, 24h). Nuclei are stained with DAPI (blue). Ratio indicates the number of scored cells with the displayed phenotype.

(I) QPCR for *TCL1* expression in Ramos cells grown in the presence or absence of MET (10mM, 24h). Values are normalized to *36B4*. Values are expressed as mean ± SEM of three independent experiments.

(J) Immunoblot for TCL1 protein expression in Ramos cells after 72h exposure to the indicated doses of MET. β-ACTIN is a loading control.

(K) Immunoblot showing LKB1 and β-ACTIN protein expression in Nalm-6 pre-B cells at the indicated number of days following electroporation (Amaxa Nucleofector I, Germany) of an LKB1 siRNA oligonucleotide. Control electroporation of a scrambled siRNA oligonucleotide into Nalm-6 cells did not affect LKB1 or β-ACTIN protein expression (data not shown).

(L) QPCR for *TCL1* expression in Nalm-6 cells with scrambled or LKB1 siRNA oligonucleotides exposed to DMSO or Eto (20 μM, 6h). Values are normalized to *36B4*. Values are expressed as mean ± SEM of three independent experiments.

(M) Immunoblot showing shLKB1 knockdown in Ramos cells 4d post-transduction. β-ACTIN is a loading control.

(N) (Left) Immunoblot showing CRTC2 protein expression in the nucleus (N) and cytoplasm (C) of Ramos B cells transduced with control (Scr) shRNA or shLKB1 after exposure to DMSO or Eto (20 μM, 1h). β-TUBULIN (cytoplasm) and HDAC1 (nucleus) are loading controls. (Right) Nuclear/cytoplasmic ratios for CRTC2 are plotted from densitometry.

Figure S3, related to Figure 3- CRTC2 Inactivation Occurs During CSR in GC B Cells

(A) Flow cytometry detection of total surface IgG in isolated human tonsil B cells on day 3 or 7 of differentiation.

(B) Immunofluorescence microscopy showing CRTC2 localization and phospho-H2AX (pH2AX) focus formation in isolated human tonsil B cells on day 3 or 5 of culture. Nuclei are stained with DAPI. pH2AX foci appeared in 13/30 cells scored; all B cells with pH2AX foci displayed a cytoplasmic shift of CRTC2.

(C) Immunofluorescence microscopy showing CRTC2 localization in sorted naïve B cells, centroblasts (CB), centrocytes (CC), and plasma cells from fresh human tonsil. Ratios indicate the number of scored cells with nuclear CRTC2.

(D) QPCR measuring CRTC2 target gene expression in tonsil B cell fractions. Values are normalized to *36B4*. Values are expressed as mean ± SEM of three independent experiments.

Figure S4, related to Figure 4- AID-Dependent ATM to LKB1 Signaling in GC B Cells Inactivates CRTC2

Flow cytometry detection of cell-surface IgG1 and B220 in CD43-negative spleen cells from WT and *AID* knockout mice on days 0 and 4 of stimulation with αCD40 (2 μg/ml) and IL-4 (20 ng/ml) exposure.

Figure S5, related to Figure 5- CRTC2 Regulates a Gene Program that Controls B Cell Development

(A) ChIP was performed on chromatin fractions from untreated Ramos cells using two different antibodies (Ab1: EMD Biosciences; Ab2: Cell Signaling Technologies) which recognize distinct epitopes on the CRTC2 protein. Immunoprecipitates were analyzed by QPCR for the *TCL1* promoter (positive control) and the *ACTB* promoter (negative control). Values are normalized to an IgG isotype control and shown as arbitrary units (a.u.). Values are expressed as mean ± SEM of three independent experiments.

(B) Plot demonstrating the correlation coefficient for 4 ChIP-on-chip experiments, including 2 Abs (Ab1 or Ab2) used for each of 2 replicates (rep1 or rep2).

(C) Spreadsheet listing z-scores for genes whose promoters were considered "CRTC2-bound" by our criteria. Z-scores are listed from left to right as follows: Ab1 rep1; Ab1 rep2; Ab2 rep1; Ab2 rep2; average of 4 experiments.

(D) Spreadsheet containing p values for CRTC2 binding to all possible 6-mer motifs.

(E) Immunofluorescence microscopy showing FLAG-CRTC2 localization in Ramos cells 48h after transduction with the indicated lentiviruses. Cells were exposed to DMSO, Eto (20 μM, 1h), or αIgM Ab (10 μg/ml, 1h) prior to staining.

(F) QPCR measuring the expression of CRTC2 target genes in Ramos B cells infected with the aforementioned lentiviruses and exposed to αIgM Ab (10μg/ml, 6h). Values are

normalized to *36B4* and presented as ratios of αIgM Ab exposed vs. untreated controls. Values are expressed as mean ± SEM of three independent experiments.

(G) QPCR measuring the expression of CRTC2 target genes in WT or ATM-deficient (A-T) lymphoblastoid cells exposed to DMSO or Eto (20 μM, 6h). Values are normalized to *36B4*. Three candidate genes (*BACH2*, *SMAD6*, and *SMARCA4*) were not expressed at detectable levels in these cells. Values are expressed as mean ± SEM of three independent experiments.

(H) (Top) Immunoblot showing CRTC2 protein expression in the nucleus (N) and cytoplasm (C) of WT and A-T lymphoblastoid cells after exposure to DMSO or Eto (20 μM, 1h). β-TUBULIN (cytoplasm) and HDAC1 (nucleus) are loading controls. (Bottom) Nuclear/cytoplasmic ratios for CRTC2 are plotted from densitometry.

(I) QPCR measuring the expression of CRTC2 target genes in Ramos cells exposed for 6h to DMSO or Eto in the absence (DMSO) or presence (KUDOS) of the ATM inhibitor KU55933 (10 μM). Values are normalized to *36B4*. Values are expressed as mean ± SEM of three independent experiments.

Figure S6, related to Figure 6- CRTC2 Inactivation is Required for Plasma Cell Differentiation

(A) Immunoblot showing CRTC2 expression in human tonsil B cells 72h post-transduction with the listed lentiviruses. A band that reacted with an αFLAG Ab was detected at the molecular weight of CRTC2, confirming successful transduction.

(B) QPCR for productive *IG* transcripts in human B cells infected with the indicated lentiviruses after 7 days of differentiation. Values are normalized to *36B4*. Values are expressed as mean ± SEM of three independent experiments.

Figure S7, related to Figure 7- CRTC2 Inactivation is Disrupted in GC-derived B Cell Lymphomas

Chromatograms demonstrate results of sequencing a 500-base pair fragment amplified from human lymphoma cDNA. Arrows point to base pair 496 in the CRTC2 coding sequence.

Table S1, related to Figure 5- CRTC2 Regulates a Gene Program that Controls B Cell Development

Listed are, from left to right, gene names, average z-score for 4 ChIP-on-chip experiments, average log ratio from 6 αIgM expression arrays (biological triplicate plus dye swap), and average log ratio from 6 Eto expression arrays (biological triplicate plus dye swap).