

Supplemental Information

Molecular Cell, Volume 39

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

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 95°C 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 3 μ l 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 60°C 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 CRT2 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 FACScan 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 5×10^5 cells/ml in the presence of 2 μ g/ml α CD40 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
<i>TCL1</i> promoter F	TCCAGAAGAAGAAAGGGCCAAGGT
<i>TCL1</i> promoter R	GTCTTGGTGGCAAGTGAGGGT
Intergenic region F	TCAGCATTACGTCTGCCAAGCCTA
Intergenic region R	TCCAATTTGGGCTGCTGGTTTCTG
<i>BACH2</i> promoter F	TGGCACACTCCTGGCTTCT
<i>BACH2</i> promoter R	TCCCTCAAGTTACCAAACCTCGCCT

<i>CDK6</i> promoter F	CAGACTGGACCGGGCCTTTA
<i>CDK6</i> promoter R	AGAGAGAAGGTCTCTGTCCTCGG
<i>AICDA</i> promoter F	CCCTTTCTCTCATGTAAGTGTCTGACTGA
<i>AICDA</i> promoter R	CAGAAAGAATTGGGCTGACAGCGT
<i>SMARCA2</i> promoter F	GTTACCCTGCCCTCCCTCT
<i>SMARCA2</i> promoter R	GGGAAGACGCGAAGCAAA
<i>SMARCA4</i> promoter F	AGGGTCAAGAAGCCCAGCC
<i>SMARCA4</i> promoter R	CTCGCGACACTGTGGCCTT
<i>TCF3</i> promoter F	ATCAGACTTTCGCGGCTTCCGAT
<i>TCF3</i> promoter R	TTGAAAGCTAAGGCAGAGCAGTCTGG
<i>SMAD6</i> promoter F	AAAGCGTCCTGAGCTCCCTGTTT
<i>SMAD6</i> promoter R	TTCCAGGGTTGAGCCTCTGAGGAG
<i>MYC</i> promoter F	GGTTCACTAAGTGCCTCCGAGATA
<i>MYC</i> promoter R	AGGGAGCACCCCTTTCACCC
<i>MTA3</i> promoter F	CCCCTCTAATTCTCTAACAGCCC
<i>MTA3</i> promoter R	GCAGAGGCCTGCACTCAAAT
<i>ACTB</i> promoter F	GCTGGGTAGGTTTGTAGCCTTCATCA
<i>ACTB</i> promoter R	TAGCTAAATGTGCTGGGTGGGTCA
<i>TCF3</i> expression F	TCCGTGACATCAACGAGGCCTTTA
<i>TCF3</i> expression R	ATTCAGGTTCCGCTCTCGCACTT
<i>SMARCA4</i> expression F	CAGATGCACAAGCCCATGGAGTC
<i>SMARCA4</i> expression R	GTAACCTTGGGAGTGCTGGTCCAT
<i>SMARCA2</i> expression F	CGTCAGAAACACCAGGAATACCTGAACA
<i>SMARCA2</i> expression R	ATCAGTCGCCGCATTCTCTCCTTT
<i>AICDA</i> expression F	TTCGCAATAAGAACGGCTGCCAC
<i>AICDA</i> expression R	ATAATCTTTGAAGGTCATGATGGCTATTTGCAC
<i>CDK6</i> expression F	CCCCAACGTGGTCAGGTTGTTT
<i>CDK6</i> expression R	TGTGAATGAAGAAAGTCCAGACCTCGG
<i>BACH2</i> expression F	CTTGCCTGAGGAGGTCACAGC
<i>BACH2</i> expression R	AGCATCCTTCCGGCACACAAA
<i>SMAD6</i> expression F	GTGAATTCTCAGACGCCAGCATGT
<i>SMAD6</i> expression R	TGCCCTGAGGTAGGTCGTAGAAGAT
<i>MYC</i> expression F	AGCGACTCTGAGGAGGAACAAGAAGAT
<i>MYC</i> expression R	TTGGCAGCAGGATAGTCCTTCCG
<i>MTA3</i> expression F	GTACCGGGTTCGGAGATTATGTCTACTTTG
<i>MTA3</i> expression R	CCTCAATTTCTTTAGCATGCTTATCTGCGAG
<i>TCL1</i> expression F	CGATACCGATCCTCAGACTCCAGTT
<i>TCL1</i> expression R	AAAGGAGACAGGTGCTGCCAAG
<i>36B4</i> expression F	TGGCAGCATCTACAACCCTGAAGT
<i>36B4</i> expression R	TGGGTAGCCAATCTGAAGACAGACA
<i>OCAB</i> expression F	TATGCCTCTCCGCCACTCATCA
<i>OCAB</i> expression R	TGAGGCCACGGGAAATAGGTGA
<i>BCL6</i> expression F	TTGTCATTGTTGTGAGCCGTGAGC
<i>BCL6</i> expression R	AAACTTCCGGCAAGTGTCCACAAC
<i>PRDM1</i> expression F	CAACAACCTTTGGCCTCTTCC
<i>PRDM1</i> expression R	GCATTCATGTGGCTTTTCTC
sh <i>LKB1</i> F	GATCCCCTGCTGAAAGGGATGCTTGATTCA
	AGAGATCAAGCATCCCTTTCAGCA TTTTTTGGAAC
sh <i>LKB1</i> R	TCGAGTTTCCAAAAAATGCTGAAAGGGATG
	CTTGATCTCTTGAATCAAGCATCCCTTTCAGCA GGG
sh <i>LKB1</i> 2F	TCCCAACGTGAAGAAGGAAATTCAACTCGAGTTGAA

sh <i>LKB1</i> 2R	TTTCCTTCTTCACGTTTTTTTTG AAAACAAAAAACGTGAAGAAGGAAATTCAACTCGAGTTGAA TTTCCTTCTTCACGTT
sh <i>ATM</i> F	TCCCCCGGAACATACTACTCAAAGACATTTTC AAGAGAAATGTCTTTGAGTAGTATGTTCCTTTT TTGGAAAC
sh <i>ATM</i> R	AAAAGTTTCCAAAAAAGGAACATACTACTCAA AGACATTTCTCTTGAAAATGTCTTTGAGTAGTA TGTTCGGG
sh <i>ATM</i> 2F	TCCCCCGGAATGATCTGCTTATCTGCTGCCGTTCAAGA GACGGCAGCAGATAAGCAGATCATTCTTTTTTGGAAAC
sh <i>ATM</i> 2R	AAAAGTTTCCAAAAAAGGAATGATCTGCTTATCTGCTG CCGTCTTTGAACGGCAGCAGATAAGCAGATCATTCCGGG
Scramble F	TTTGGTCTACCCTACACGCGTTACACGACAATG CGTGTAGAGTAGAGGCTTTTTT
Scramble R	AAAAAAGCCTCTACTCTACACGCATTGTCGTGT AACGCGTGTAGGGTAGACGATC
<i>FLAG-CRTC2</i> F	GCTCTAGAGCCACCATGGACTACAAG
<i>FLAG-CRTC2</i> R	GCGTCTAGATCACTGTAGCCGATCAC
<i>CRTC2</i> sequencing primer F	ATTGACAGCTCTCCCTATAGTCCTGC
<i>CRTC2</i> sequencing primer R	AGGCTGGTCAGGAGATGGAAAGAT
<i>LKB1</i> expression F	CAGCTGATTGACGGCCTGGA
<i>LKB1</i> expression R	CACGCCCAGGTCGGAGATTT
<i>ATM</i> expression F	GCGCCTGATTGAGATCCTGAAACAATTA
<i>ATM</i> expression R	TGGAGGCTTGTGTTGAGGCTGATA
Ig var. region heavy chain F	GACACGGCTGTGTATTACTGTGCG
Ig C μ R	CCGAATTCAGACGAGGGGGAAAAGGGTT
Ig Cy1 R	GTTTTGTCACAAGATTTGGGCTC
Ig Cy2 R	GTGGGCACTCGACACAACATTTGCG
Ig Cy3 R	TTGTGTCACCAAGTGGGGTTTTGAGC
Ig Cy4 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., Sullur, 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.

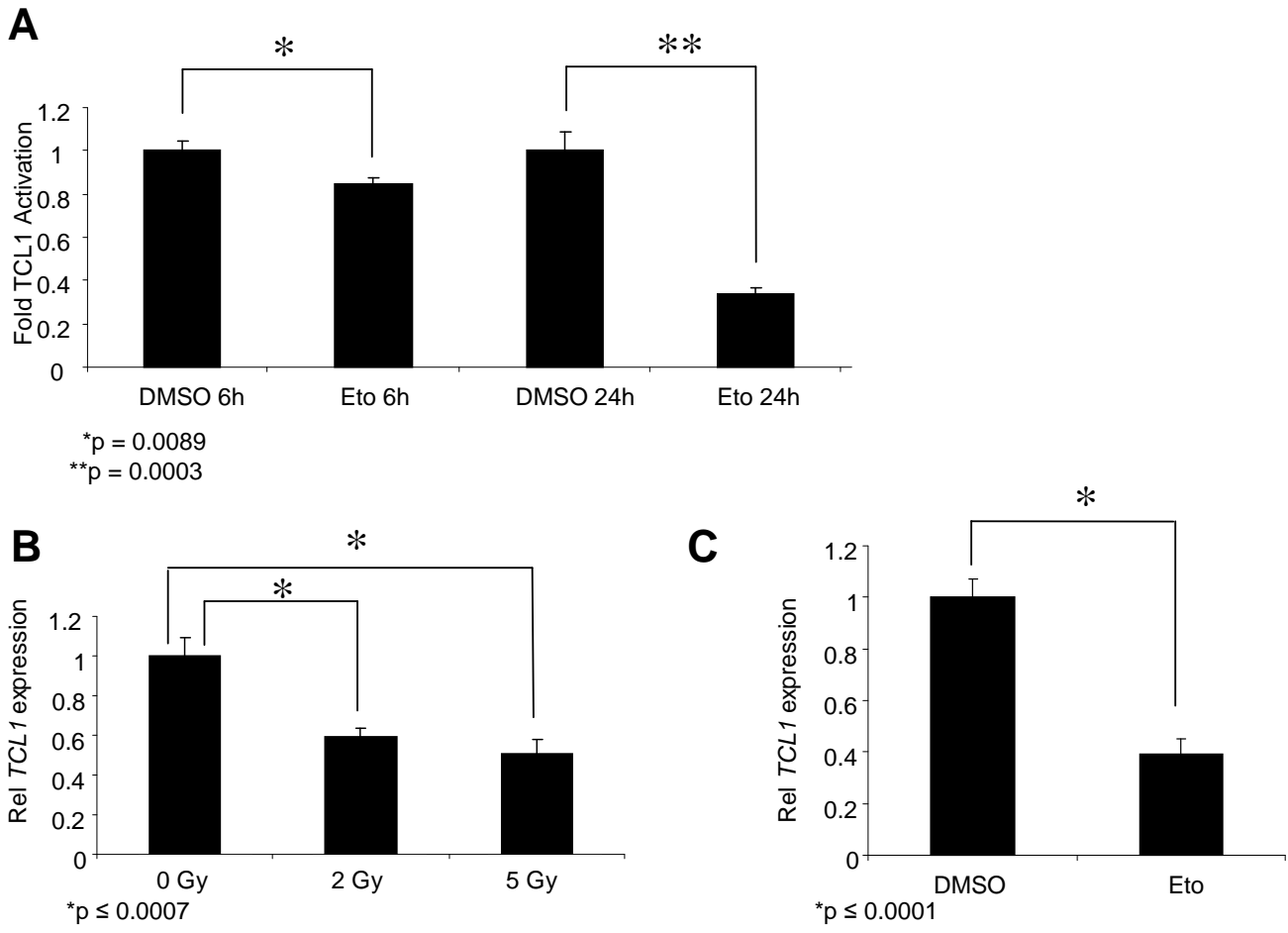


Figure S1

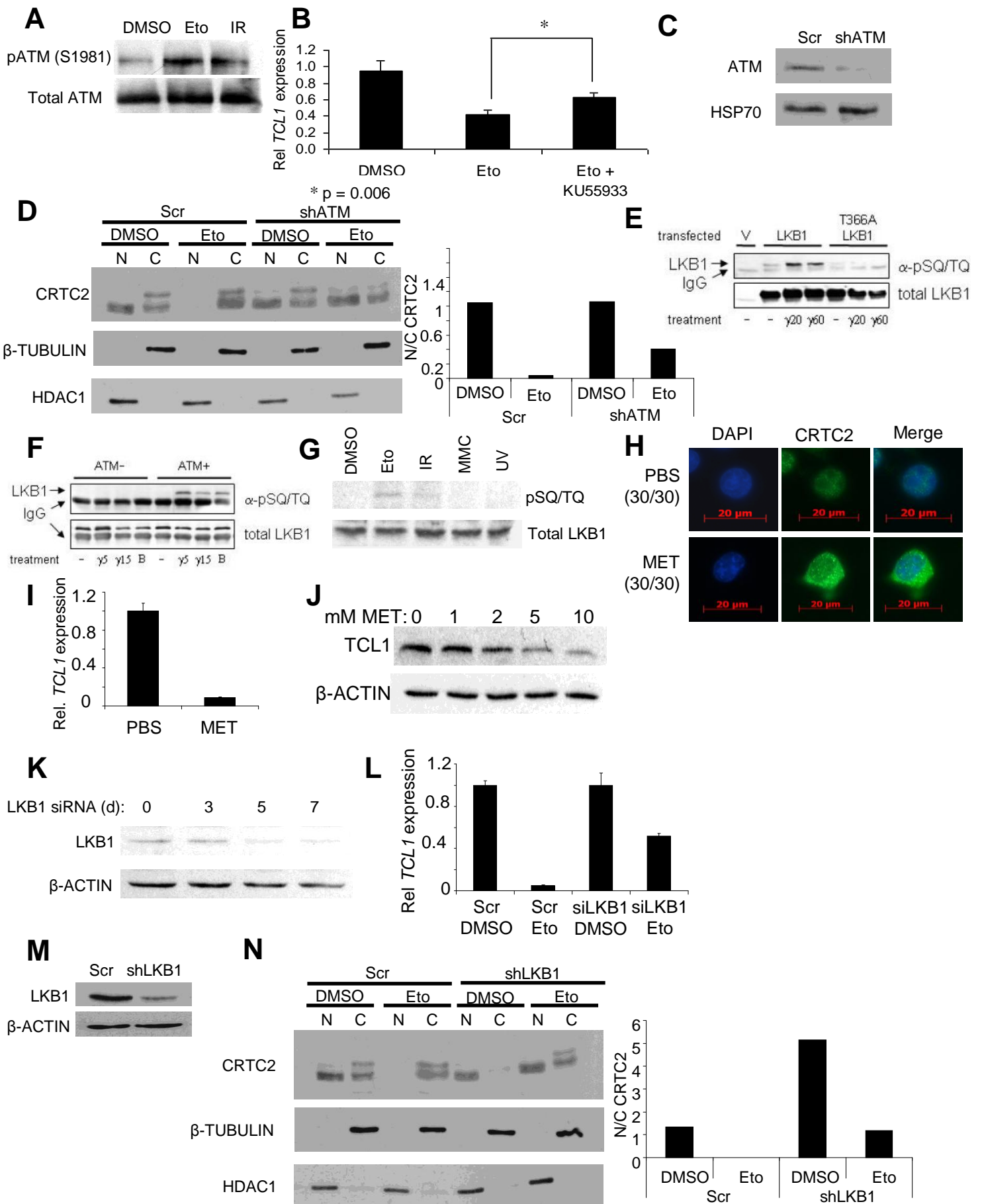


Figure S2

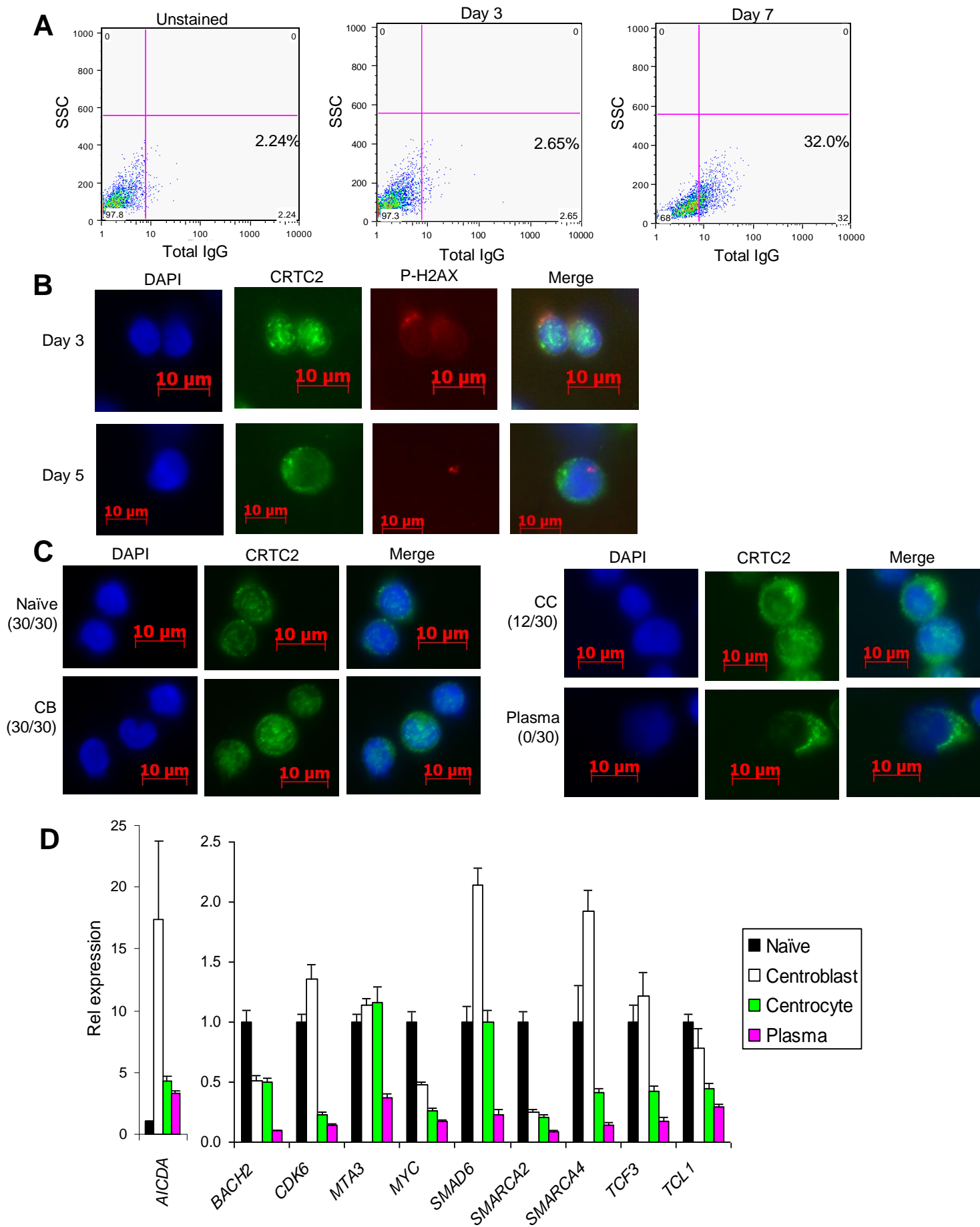


Figure S3

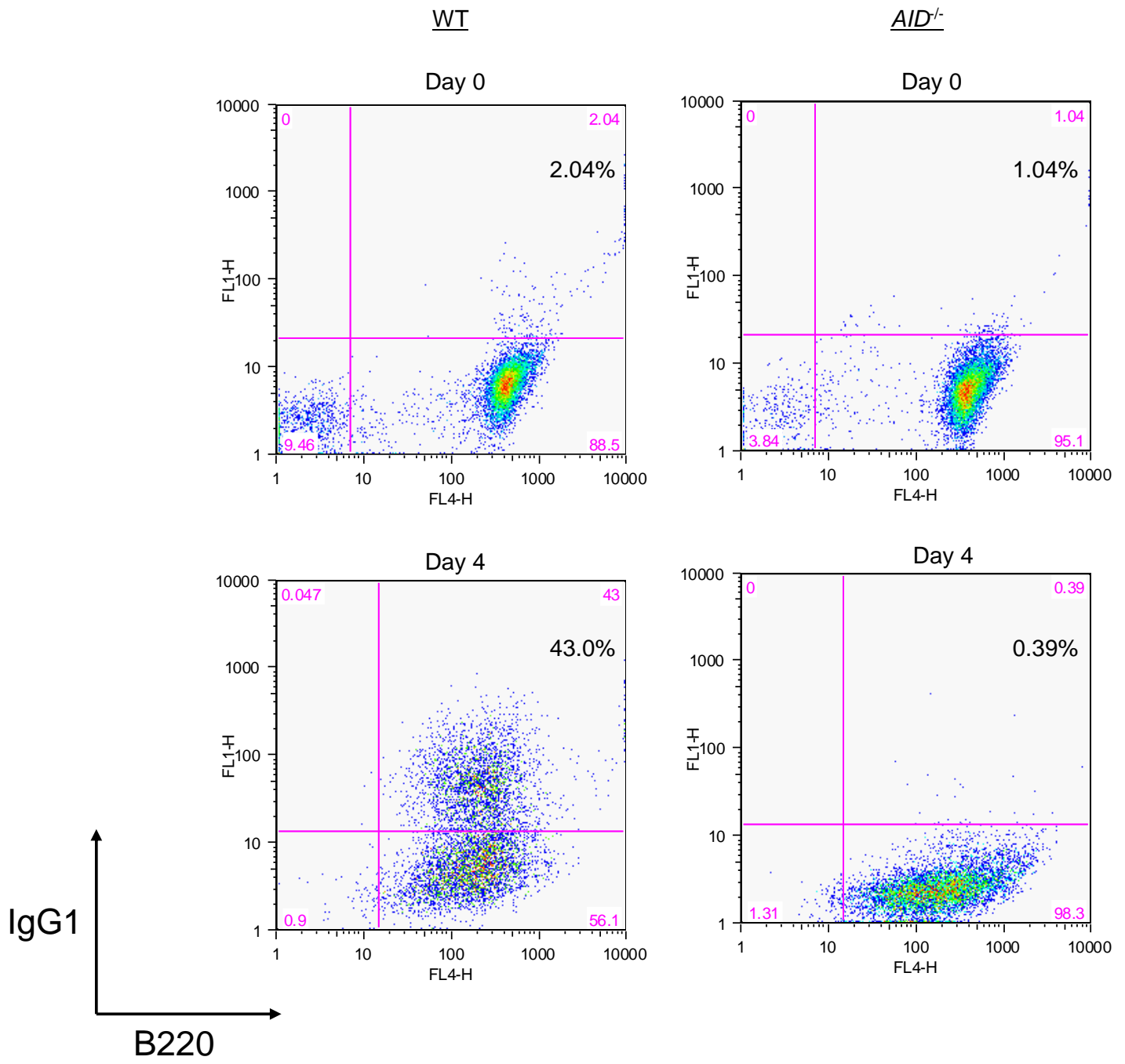


Figure S4

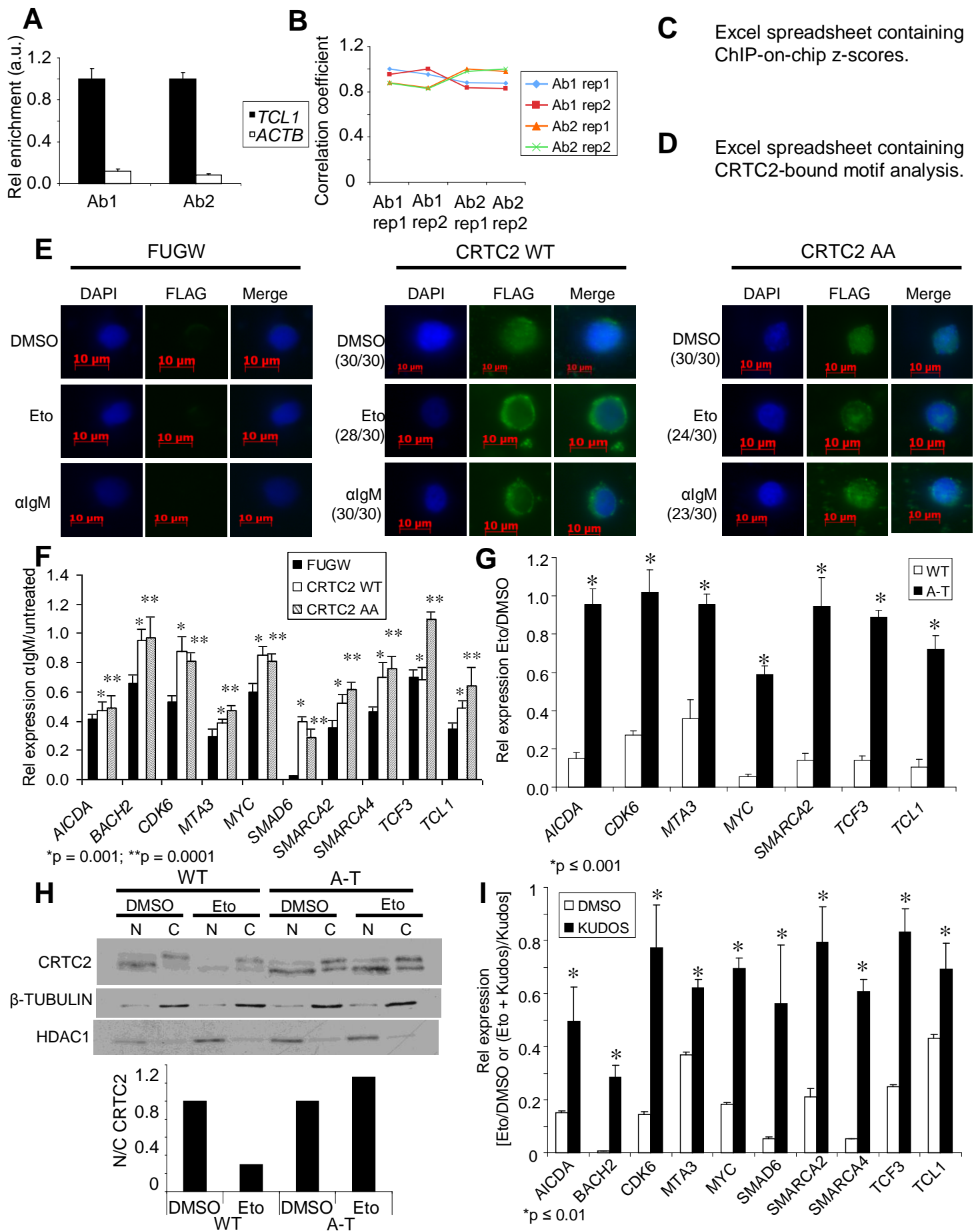


Figure S5

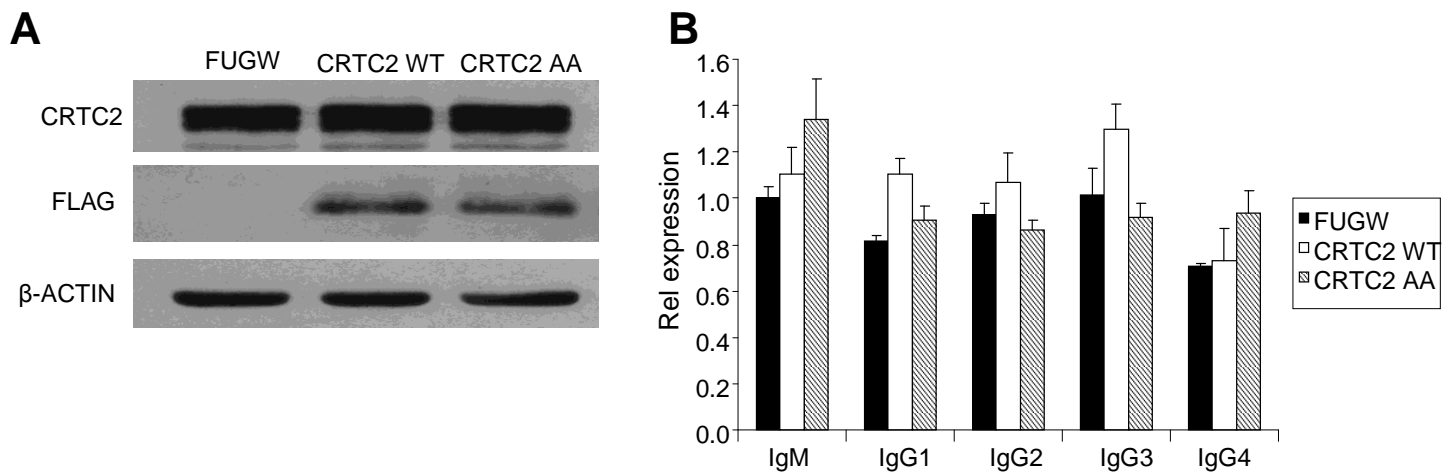


Figure S6

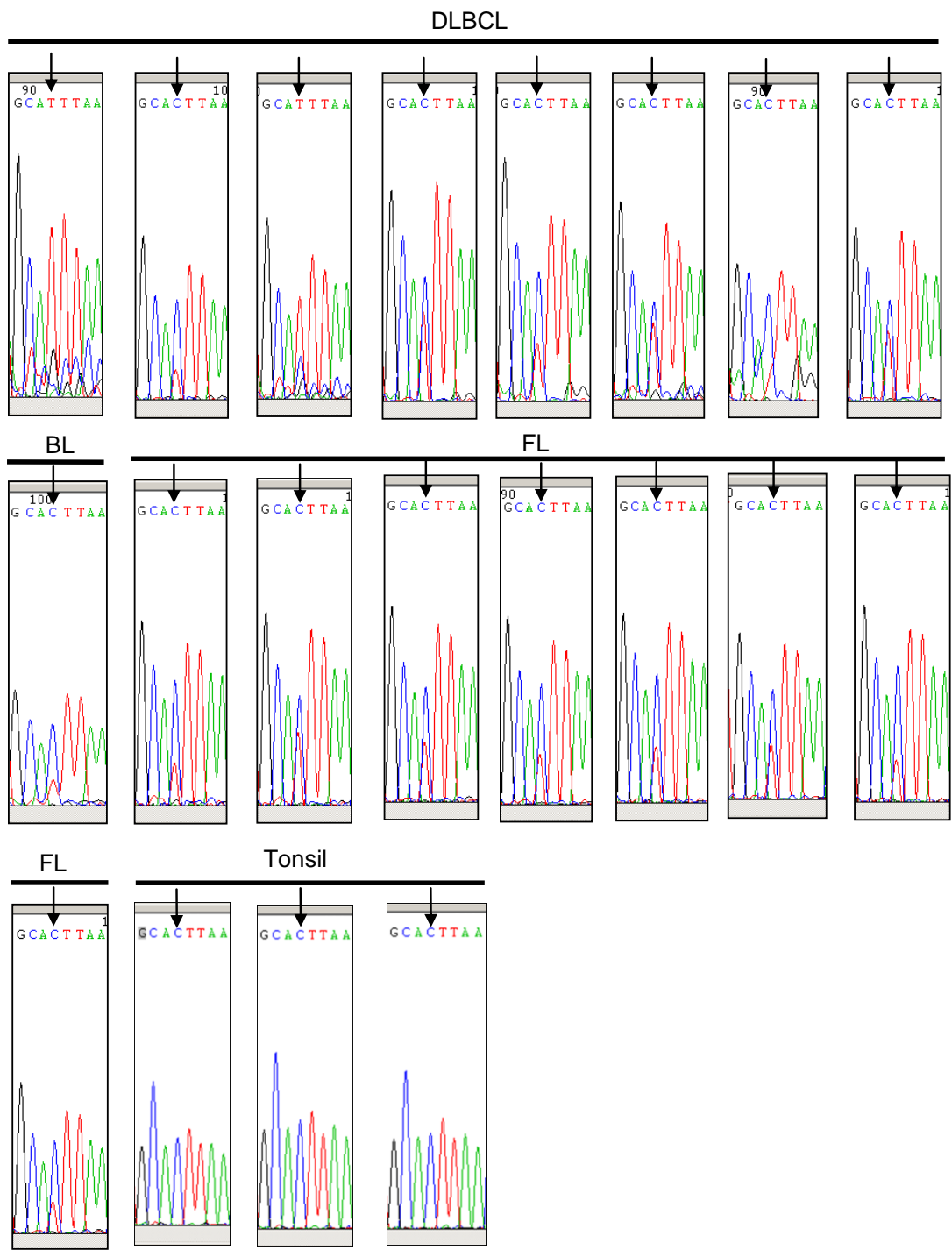


Figure S7

Table S1

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

<i>FBXL20</i>	2.011966	0.340605	0.281296
<i>FCHSD2</i>	2.667797	0.969865	0.388468
<i>FCRLM2</i>	3.056374	0.541384	0.352928
<i>FGD6</i>	2.697788	0.445747	0.327159
<i>FGF12</i>	3.036584	0.471456	0.365337
<i>FLI1</i>	3.058955	0.498507	0.330382
<i>FLJ10781</i>	2.644249	0.531821	0.417518
<i>FLJ43339</i>	2.524056	0.609436	0.326945
<i>GAS2L1</i>	2.371583	0.362618	0.507901
<i>GPR146</i>	2.258408	0.445487	0.739014
<i>GTF2I</i>	2.103	0.515733	0.278886
<i>HERC3</i>	2.624257	0.429328	0.354839
<i>IBRDC2</i>	2.185693	0.785971	0.751008
<i>IQCE</i>	2.129561	0.356021	0.381282
<i>JDP2</i>	2.621162	0.528779	0.363568
<i>KCNH8</i>	2.122059	0.34674	0.713449
<i>KCNMB4</i>	2.101346	0.351969	0.533397
<i>KHDRBS2</i>	2.448985	0.64085	0.592891
<i>KIAA0182</i>	2.455095	0.348313	0.295321
<i>KIAA0774</i>	2.34963	0.801442	0.858986
<i>KIAA1622</i>	2.415789	0.467571	0.352437
<i>KIAA1900</i>	2.198882	0.451565	0.473353
<i>KIAA1914</i>	2.369715	0.672084	0.51291
<i>KLF15</i>	2.430034	0.536293	0.441149
<i>KLHL14</i>	2.029478	0.407345	0.520742
<i>LMO3</i>	2.59422	0.846254	0.574315
<i>LRRC34</i>	2.59429	0.683172	0.350709
<i>LRRCC1</i>	2.085134	0.548395	0.336318
<i>LZTS1</i>	2.532856	0.533674	0.729731
<i>MCF2L2</i>	3.33377	0.782938	0.393772
<i>MEGF11</i>	2.018422	0.390101	0.613916
<i>MFSD4</i>	2.174939	0.354325	0.49376
<i>MLLT3</i>	2.409267	0.7309	0.561898
<i>MTA3</i>	2.309794	0.432268	0.327869
<i>MTSS1</i>	2.203541	0.584444	0.574611
<i>MYC</i>	3.296981	0.566382	0.310759
<i>NAV2</i>	2.420738	0.33487	0.290352
<i>NFIA</i>	2.818319	0.893464	0.629842
<i>NOD3</i>	2.296451	0.753535	0.357265
<i>NR3C2</i>	2.711054	0.834021	0.523465
<i>NTHL1</i>	2.180005	0.34279	0.429389
<i>PAPSS1</i>	2.598499	0.351185	0.411316
<i>PARN</i>	2.542358	0.614113	0.289197
<i>PITPNC1</i>	2.397147	1.045999	0.339616
<i>PITPNM2</i>	2.12482	1.009885	0.30657
<i>PMS1</i>	2.776674	0.33346	0.280531
<i>PPP2R3B</i>	2.921845	0.359545	0.295142
<i>PRDM10</i>	2.080148	0.90388	0.327894
<i>PRKCA</i>	2.332082	0.762065	0.388994

<i>PRKCH</i>	2.550004	0.463826	0.314377
<i>PROC</i>	2.038438	0.32483	0.384179
<i>PTPN18</i>	2.913371	0.406896	0.303096
<i>PYCARD</i>	2.89214	0.41603	0.368077
<i>RAB37</i>	2.813755	0.593259	0.441196
<i>RCSD1</i>	2.156679	0.585655	0.364485
<i>SATB2</i>	2.826687	0.576078	0.594463
<i>SCARB1</i>	2.676037	0.712547	0.378365
<i>SH2D1A</i>	2.149962	0.812706	0.472465
<i>SIPA1L3</i>	3.013577	1.221007	0.281492
<i>SLC2A5</i>	2.247339	0.714206	0.529839
<i>SLC39A10</i>	2.655167	0.462249	0.282513
<i>SLC7A7</i>	2.122767	0.32907	0.379662
<i>SMAD1</i>	2.371985	0.610308	0.513803
<i>SMAD6</i>	3.265875	0.579633	0.495518
<i>SMARCA2</i>	2.517652	0.335236	0.415966
<i>SMARCA4</i>	1.87733	0.631448	0.303977
<i>SNTB1</i>	2.193671	0.676703	0.429238
<i>SPG3A</i>	2.016751	0.669465	0.537908
<i>SSBP2</i>	2.81366	0.916858	0.306627
<i>STOX2</i>	2.710594	0.421158	0.716616
<i>STS</i>	2.347684	0.550401	0.315803
<i>SUSD3</i>	2.729927	0.795396	0.617332
<i>TBC1D10C</i>	2.000706	0.712491	0.318883
<i>TCF3</i>	2.733384	0.696164	0.449621
<i>TCL1A</i>	2.062001	0.522095	0.587388
<i>TCL6</i>	2.483377	0.943989	0.684595
<i>TFAP4</i>	3.07121	0.362593	0.392455
<i>TLL1</i>	2.080367	0.479435	0.651101
<i>TNFSF8</i>	2.01981	0.505971	0.338815
<i>TRERF1</i>	2.119094	0.620778	0.47355
<i>TRIB2</i>	2.293439	0.600313	0.35314
<i>UNC5C</i>	2.769456	0.442902	0.328565
<i>UNQ739</i>	2.704115	0.677697	0.504866
<i>VPREB1</i>	2.797564	0.530493	0.383741
<i>WNK2</i>	2.376737	0.468577	0.571981
<i>WNT2B</i>	2.506678	0.801435	0.8741
<i>WNT6</i>	2.300466	0.528299	0.375488
<i>ZNF143</i>	2.849458	0.584438	0.478842
<i>ZNF533</i>	2.718106	1.256506	0.758396
<i>ZNF575</i>	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 μ M, 6 or 24h). Values were normalized to a co-transfected *Renilla*-luciferase expression construct. Values are expressed as mean \pm 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 \pm SEM of three independent experiments.

(C) QPCR measuring *TCL1* expression in peripheral blood B lymphocytes (PBL) cells after DMSO or Eto (20 μ M, 6h) exposure. Values are normalized to *36B4*. Values are expressed as mean \pm 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 μ M, 1h) or IR (5 Gy).

(B) QPCR for *TCL1* expression in Ramos cells after 6h of DMSO, Eto (20 μ M), or Eto (20 μ M) plus the ATM-inhibitor KU55933 (10 μ M) exposure. Values are normalized to *36B4*. Values are expressed as mean \pm 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 μ M, 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 μ M, 1h), IR (5 Gy), mitomycin C (MMC, 0.225 μ M, 1h), or UV irradiation (1000 mJ/cm²), 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 \pm 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 (Amaza 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 μ M, 6h). Values are normalized to *36B4*. Values are expressed as mean \pm 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 μ M, 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 \pm 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 α CD40 (2 μ g/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 \pm 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 μ M, 1h), or α IgM Ab (10 μ g/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 α IgM Ab (10 μ g/ml, 6h). Values are

normalized to *36B4* and presented as ratios of α M Ab exposed vs. untreated controls. Values are expressed as mean \pm 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 μ M, 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 \pm 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 μ M, 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 μ M). Values are normalized to *36B4*. Values are expressed as mean \pm 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 α FLAG 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 \pm 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 α lgM expression arrays (biological triplicate plus dye swap), and average log ratio from 6 Eto expression arrays (biological triplicate plus dye swap).