

TCL1A and ATM are co-expressed in chronic lymphocytic leukemia cells without deletion of 11q

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Online Supplementary Design and Methods

Modeling and statistics

Gene expression profiling data were quantile normalized and single genes ranked according to the highest standard deviation between stimulated (murine and human feeder cells) versus unstimulated culture and the highest standard deviation between time points. This identified genes most deregulated upon co-culture compared to unstimulated culture. The 0.5% most deregulated genes (243 of 48600) were tested with Ingenuity Pathway Analysis (IPA, Ingenuity Systems, www.ingenuity.com) and DAVID⁷ (<http://david.abcc.ncifcrf.gov>) for functional annotation with *cell death*, *apoptosis* and *cell survival*. This identified 35 genes in common between IPA and DAVID, from which the 23 most deregulated genes were selected by visual inspection for mod-

eling in a 2-step approach. First, candidate genes were further grouped into clusters according to their gene expression kinetics using the Partitioning Around Medoids (PAM) clustering method⁸ to yield a number of genes small enough for subsequent network modeling. Here, genes (medoids) were selected from a cluster that displays a gene expression time course kinetic representative of the whole cluster of genes. The resulting 8 medoids were the genes corresponding to the network nodes in the second step of the statistical analysis. Network estimation was made using the dynamic Bayesian network approach,⁹ where probabilities of interdependencies of genes (*nodes*) are displayed also dependent on kinetics (time). The analysis employs a Markov Chain Monte Carlo algorithm to obtain the posterior edge probabilities of the network. These 8 genes were also used as controls for analysis of transcription factor binding (Figure 2J).

References

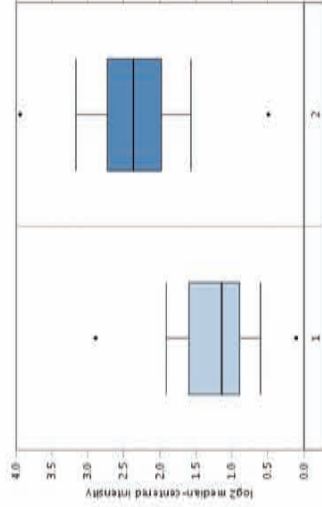
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Online Supplementary Table S1. Primer sequences.

TBP_for:	5'-GCCCGAAACGCCGAATAT-3'
TBP_rev:	5'-CCGTGGTTCGTGGCTCTCT-3'
SDHA_for:	5'-TGGGAACAAGAGGGCATCTG-3'
SDHA_rev:	5'-CCACCCTGCATCAAAATTCATG-3'
TCL1A_for:	5'-TCCAGTTTCTGGCGCTTAGT-3'
TCL1A_rev:	5'-ACATCAGTCATCTGGCAGCA-3'
ATM_for:	5'-AGAGGCCGGAAGATGAAACT-3'
ATM_rev:	5'-TGCCTTCTTCCACTCCTTTC-3'

A**Häflerlach Leukemia Statistics**

Over-expression Gene Rank: 1785 (in top 10%)
 Reporters: 211058_A_at
 P-value: 4.31E-30
 t-Test: 15.907
 Fold Change: 3.206

**Legend**

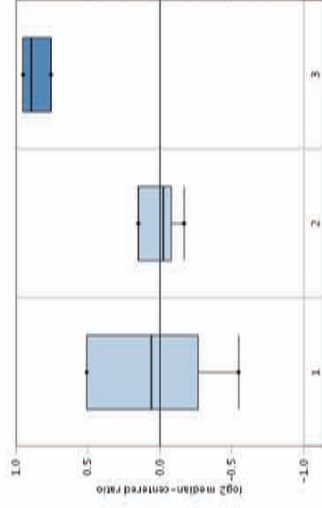
1. Peripheral Blood Mononuclear Cell (74)
2. Chronic Lymphocytic Leukemia (448)

Häflerlach Leukemia

J Clin Oncol 2010;15:120
 2,096 samples
 15,574 measured genes
 ATM information
 Reporter information
 Platform not pre-defined in
 OncoPrint

B**Rosenwald Lymphoma Statistics**

Over-expression Gene Rank: 23 (in top 1%)
 Reporters: AAD76254_2
 P-value: 6.30E-6
 t-Test: 7.643
 Fold Change: 1.807

**Legend**

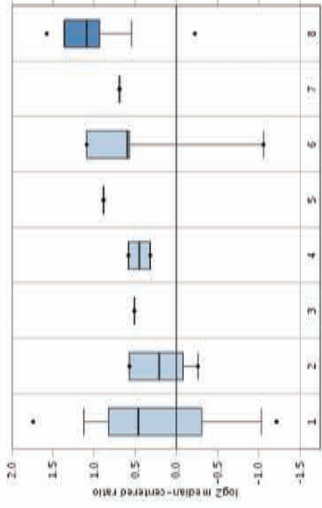
1. B-Lymphocyte (6)
2. Germinal Center B-Lymphocyte (4)
3. Chronic Lymphocytic Leukemia (2)

Rosenwald Lymphoma

N Engl J Med 2002;28:20
 293 samples
 2,615 measured genes
 ATM information
 Reporter information
 Platform not pre-defined in
 OncoPrint

C**Alizadeh Lymphoma Statistics**

Over-expression Gene Rank: 231 (in top 9%)
 Reporters: IMAGE126598
 P-value: 3.54E-4
 t-Test: 3.803
 Fold Change: 1.630

**Legend**

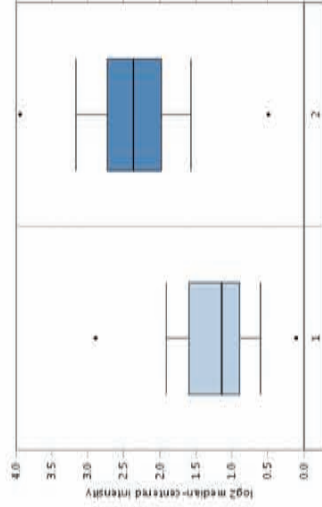
1. B-Lymphocyte (16)
2. CD4-Positive T-Lymphocyte (6)
3. Centroblast (1)
4. Germinal Center B-Lymphocyte (2)
5. Lymph Node (1)
6. Memory B-Lymphocyte (4)
7. T-cell (1)
8. Chronic Lymphocytic Leukemia (12)

Alizadeh Lymphoma

Nature 2000;403:83
 130 samples
 2,677 measured genes
 ATM information
 Reporter information
 Platform not pre-defined in
 OncoPrint

D**Rosenwald Multi-cancer Statistics**

Over-expression Gene Rank: 317 (in top 8%)
 Reporters: AAD74260_A_at
 P-value: 7.32E-4
 t-Test: 3.633
 Fold Change: 1.530

**Legend**

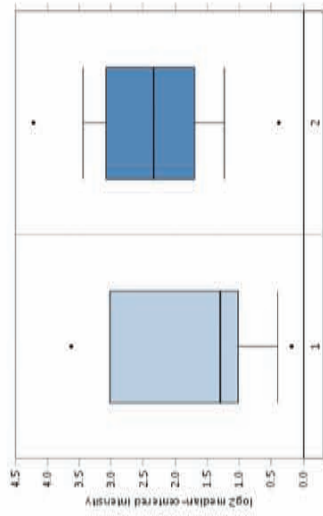
1. B-Lymphocyte (5)
2. CD4-Positive T-Lymphocyte (5)
3. Germinal Center B-Lymphocyte (1)
4. Memory B-Lymphocyte (1)
5. Umbilical Cord B-Lymphocyte (1)
6. Umbilical Cord T-Lymphocyte (1)
7. Chronic Lymphocytic Leukemia (37)

Rosenwald Multi-Cancer

J Exp Med 2001;171:103
 102 samples
 4,202 measured genes
 ATM information
 Reporter information
 Platform not pre-defined in
 OncoPrint

E**Hasslinger Leukemia Statistics**

Over-expression Gene Rank: 2011 (in top 24%)
 Reporters: 2000_A_at
 P-value: 0.021
 t-Test: 2.295
 Fold Change: 1.495

**Legend**

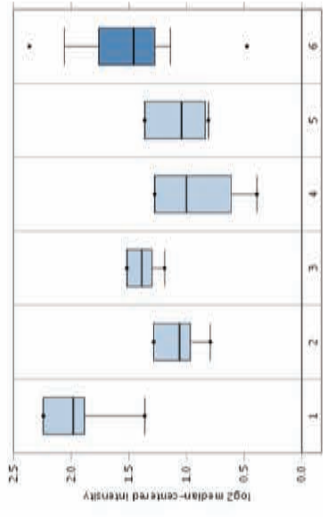
1. B-Lymphocyte (11)
2. Chronic Lymphocytic Leukemia (100)

Hasslinger Leukemia

J Clin Oncol 2004;17:101
 111 samples
 8,602 measured genes
 ATM information
 Reporter information
 Platform not pre-defined in
 OncoPrint

F**Basso Lymphoma Statistics**

Over-expression Gene Rank: 1807 (in top 22%)
 Reporters: 1863_A_at
 P-value: 0.021
 t-Test: 2.081
 Fold Change: 1.179

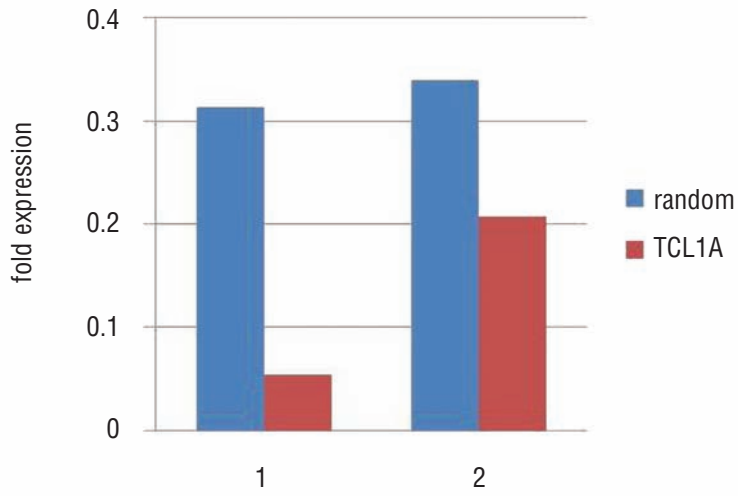
**Legend**

1. B-Lymphocyte (5)
2. Centroblast (5)
3. Memory B-Lymphocyte (5)
4. Naive Pregerminal Center B-Lymphocyte (5)
5. Small Cleaved Follicle Center Cell (5)
6. Chronic Lymphocytic Leukemia (24)

Basso Lymphoma

Nat Genet 2005;34:401
 336 samples
 6,603 measured genes
 ATM information
 Reporter information
 Platform not pre-defined in
 OncoPrint

Online Supplementary Figure 1. The tumor suppressor gene ATM is up-regulated in CLL. Visualization of ATM expression in microarray gene expression datasets using the OncoPrint tool (OncoPrint™ Compendia Bioscience, Ann Arbor, MI, USA) uncovered a significant overexpression of ATM in 6/6 publications available for analysis. Shown in box plots is the median value (thick line), the 90th/10th percentile (whiskers) and the 75th percentile (box) and maximum/minimum values as dots. Light blue: non-malignant tissue, dark blue: CLL cells. Significance levels are calculated using Student's t-test (A-F).



Online Supplementary Figure 2. siRNA knockdown of TCL1a causes 40-80% reduction of transcript levels *MEC2* cells were transfected with 1 μ g non-target siRNA (*random*) or a siRNA specifically targeting *TCL1A* using Amaxa in two independent experiments. TCL1A expression was measured by qRT-PCR and normalized to *LMNB1* expression.
