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## Data in Brief

## Transcriptomic profiling of splenic B lymphomas spontaneously developed in B cell-specific TRAF3-deficient mice

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## ABSTRACT

TRAF3, a critical regulator of B cell survival, was recently recognized as a tumor suppressor gene in B lymphocytes. Specific deletion of TRAF3 from B lymphocytes leads to spontaneous development of marginal zone lymphomas (MZL) or B1 lymphomas in mice. To identify novel oncogenes and tumor suppressive genes involved in malignant transformation of TRAF3-deficient B cells, we performed a microarray analysis to identify genes differentially expressed in TRAF3<sup>-/-</sup> mouse splenic B lymphomas. We have identified 160 up-regulated genes and 244 down-regulated genes in TRAF3<sup>-/-</sup> B lymphomas as compared to littermate control splenocytes. Here we describe the samples, quality control assessment, as well as the data analysis methods in detail for the transcriptomic profiling study. Data are archived at NIH GEO with accession number GSE48818.

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## Specifications

Organism/cell line/tissue	Mus musculus
Sex	Male and female
Sequencer or array type	Illumina Sentrix MouseRef-8 24 K
Data format	Raw and processed
Experimental factors	TRAF3-deficient splenic B lymphomas and littermate control splenocytes
Experimental features	Microarray data of TRAF3-deficient splenic B lymphomas and littermate control splenocytes
Consent	Not applicable
Sample source location	Piscataway, New Jersey, USA

## Experimental design, materials, and methods

## Sample collection and preparation

Spleens were harvested from B cell-specific TRAF3-deficient mice with B lymphomas or tumor-free littermate control mice (Table 1). In the three selected TRAF3<sup>-/-</sup> splenic B lymphoma samples (mouse ID: 6983-2, 7041-10, and 7060-8), B lymphoma cells are >70% of B cells as assessed by FACS analysis of B cell populations and Southern blot analysis of IgH gene rearrangements [1]. Spleens were separated into single cell suspensions by mechanic dissociation, and red blood cells were depleted using 1X ACK solution as described [2,3]. The resulting splenocytes were collected for total cellular RNA extraction using TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. RNA samples were purified using an RNeasy MinElute Cleanup Kit (QIAGEN, Valencia, CA). RNA concentration and quality were assessed using a NanoDrop spectrometer (NanoDrop Products, Wilmington, DE) (Table 1). RNA integrity was further analyzed on an RNA Nano Chip using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), and the results are shown in Fig. 1.

## Gene expression analysis

The mRNA was amplified with a TotalPrep RNA amplification kit with a T7-oligo(dT) primer according to the manufacturer's instructions

## Direct link to deposited data

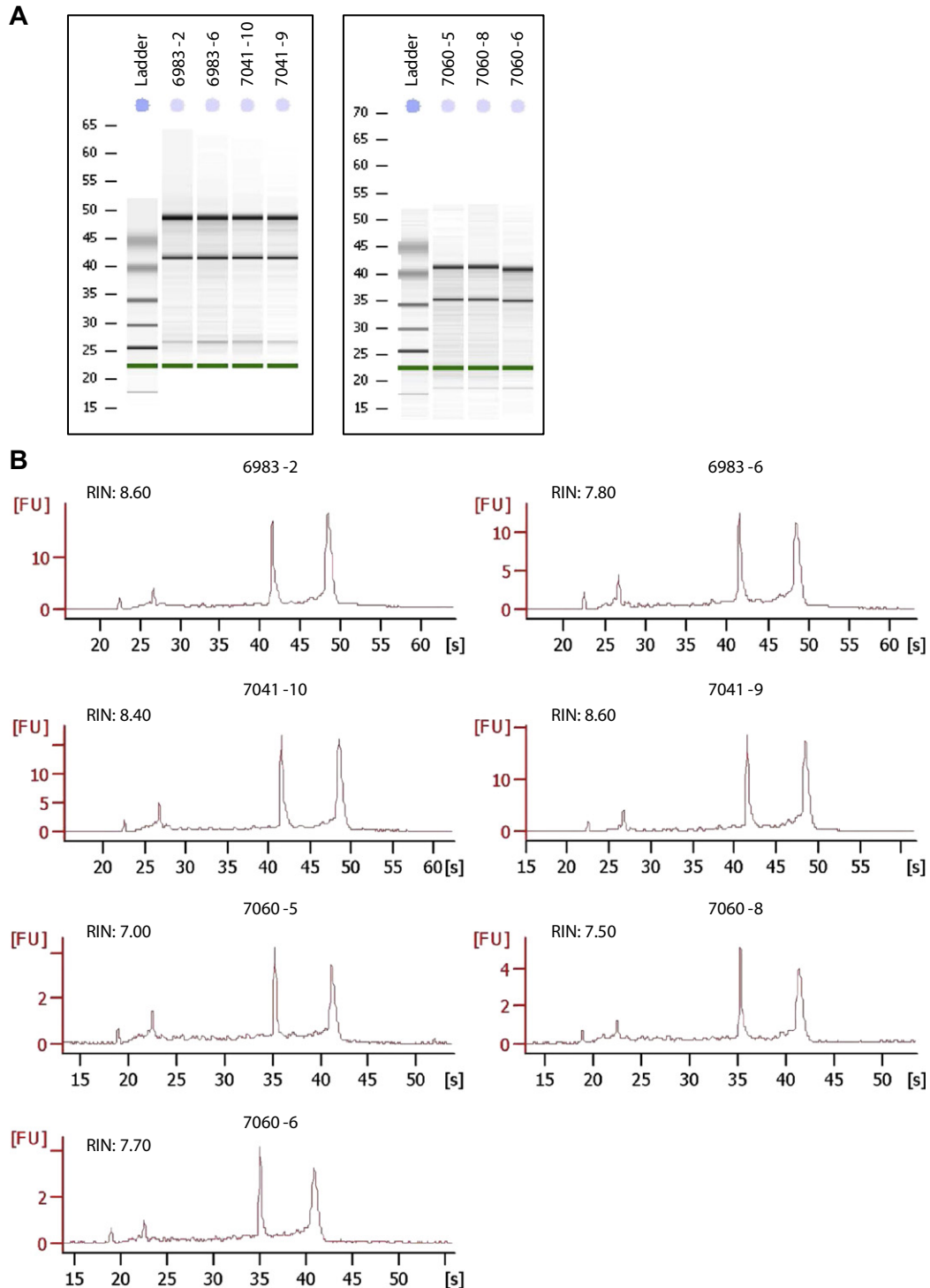
Deposited data can be found at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48818>.

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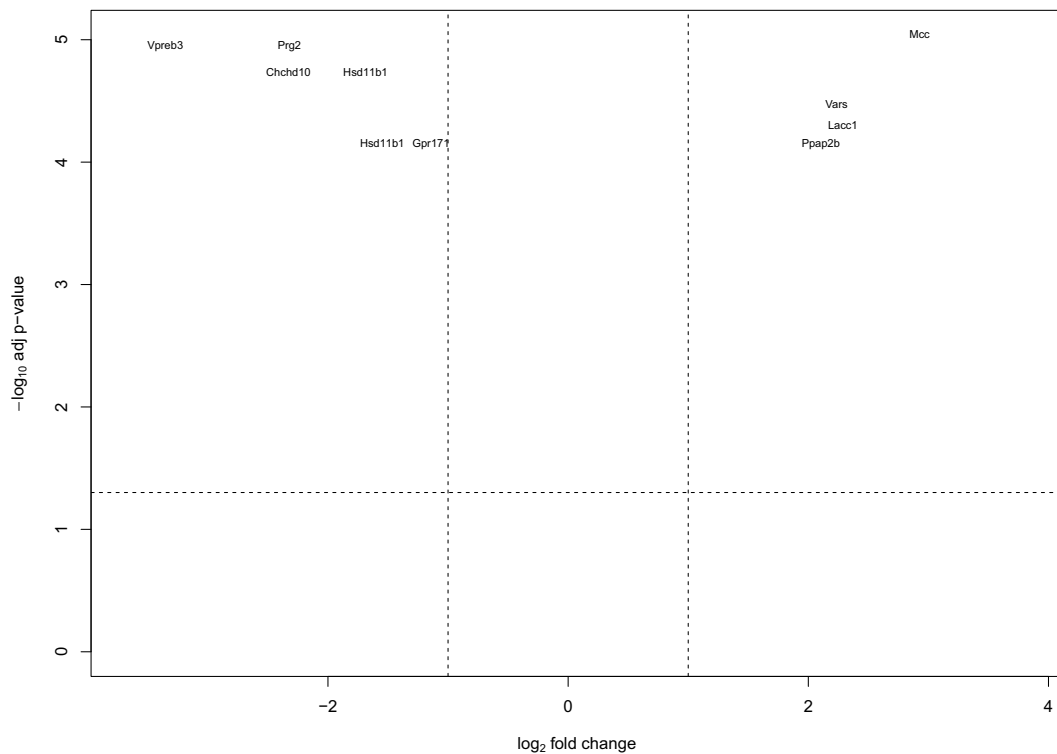
E-mail address: [rhart@rutgers.edu](mailto:rhart@rutgers.edu) (R.P. Hart).

**Table 1**  
RNA samples for transcriptome profiling by microarray analysis.

Sample ID	Sample Name	Mouse ID	Genotype	Tissue	Concentration	O.D. 260/280	O.D. 260/230	Total volume
GSM1185225	XP1	6983-2	TRAF3flox/flox, CD19 +/-Cre	Spleen	200 ng/μl	2.09	2.01	5 μl
GSM1185226	XP2	7041-10	TRAF3flox/flox, CD19 +/-Cre	Spleen	100 ng/μl	2.08	2.08	10 μl
GSM1185227	XP3	7060-8	TRAF3flox/flox, CD19 +/-Cre	Spleen	200 ng/μl	2.09	1.83	5 μl
GSM1185228	XP5	6983-6	TRAF3flox/flox	Spleen	100 ng/μl	2.09	2.09	10 μl
GSM1185229	XP6	7060-5	TRAF3flox/flox	Spleen	200 ng/μl	2.07	2.39	5 μl
GSM1185230	XP7	7060-6	TRAF3flox/flox	Spleen	200 ng/μl	2.03	2.16	5 μl
GSM1185231	XP9	7041-9	TRAF3flox/flox	Spleen	200 ng/μl	2.08	2.01	5 μl



**Fig. 1.** Quality control assay of RNAs used for microarrays. (A) Bioanalyzer output as gel images for all seven samples as identified by the Mouse ID (see Table 1). (B) Bioanalyzer output as traces with RIN (RNA integrity number) shown for each sample. Results are plotted as fluorescence units [FU] over time [s].



**Fig. 2.** Volcano plot of *limma*-modeled microarray data. The data for all genes are plotted as  $\log_2$  fold change versus the  $-\log_{10}$  of the adjusted  $p$ -value. Thresholds are shown as dashed lines. Genes selected as significantly different are highlighted as blue dots. The top ten genes (sorted by adjusted  $p$ -value) are labeled with gene symbols. Note the prominent position of the MCC gene, which was chosen for further analysis [8].

(Ambion), and microarray analysis was carried out with the Illumina Sentrix MouseRef-8 24K Array at the Burnham Institute (La Jolla, CA).

#### Data processing and normalization

Results were extracted with Illumina GenomeStudio v2011.1 and exported as the sample probe profile format without background correction or normalization. This file, along with a matching control table output from GenomeStudio, was loaded into a *lumi* object in R/Bioconductor [4–6]. Gene probes were tracked using the nulD system [4]. Background correction and quantile normalization was performed using the *lumiExpresso* function. Expression data above detection limits (using the *detectionCall* function) were selected for modeling. Target data were used to extract RNA group names as factors and assembled into a model matrix. Extracted expression values, the model design, and the array weights were used to model data in the *limma* package [7]. The contrast of “B lymphoma—control” was selected and used to generate contrasts using the *eBayes* function. Finally, gene annotation was added using the *lumiMouseAll.db* and *annotate* packages. Results were selected using the *topTable* function (with  $n = \text{Inf}$  to output all contrasts) and saved as in csv format. This table was reviewed using Excel to select significantly different genes with a minimum mean fold change (Supplemental Table 1). A volcano plot of the modeled data clearly shows large numbers of significantly different genes with an adjusted  $p$ -value  $\leq 0.05$  and a  $\log_2$  fold change  $\geq 1$  (Fig. 2).

Microarray data are available from NIH GEO Accession GSE48818 and described by Edwards et al. [8].

#### Statistics

Statistical analyses were performed using *limma* modeling (ANOVA with empirical Bayes moderation of standard errors). Adjusted  $p$ -values less than 0.05 with a fold-change greater than 2 are considered significant.

#### Discussion

Results of the microarray analysis have identified 160 up-regulated genes and 244 down-regulated genes in TRAF3<sup>-/-</sup> B lymphomas as compared to LMC spleens (2-fold up or down fold-change, adjusted  $p < 0.05$ ) (NCBI GEO accession number: GSE48818).

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gdata.2014.10.017>.

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