



## Data in Brief

## Identification of PRDM2 regulated genes in quiescent C2C12 myoblasts

Sirisha Cheedipudi <sup>a,b,1,\*</sup>, Hardik P. Gala <sup>a,b</sup>, Deepika Puri <sup>a,2</sup>, Jyotsna Dhawan <sup>a,b</sup><sup>a</sup> Institute for Stem Cell Biology and Regenerative Medicine, National Center for Biological Sciences, GKVK Post, Bellary Road, Bangalore 560065, India<sup>b</sup> Council of Scientific and Industrial Research-Centre for Cellular and Molecular Biology, Hyderabad 500 007, India

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## ABSTRACT

Quiescent stem cells contribute to tissue homeostasis and repair in adult mammals. We identified a tumor suppressor PRDM2, as an epigenetic regulator induced in quiescent muscle stem cells as well as in cultured quiescent myoblasts. To delineate the functions of PRDM2 in muscle cells, we compared the gene expression profiles of control and PRDM2 knockdown myoblasts in growing, differentiating and quiescent conditions (GEO accession number: GSE 58676). To identify the direct targets of PRDM2 and the promoters co-associated with H3K9me2 (mark catalyzed by PRDM2), ChIP-Chip analysis was performed (GSE58748). In this report we discuss in detail the methodology used to identify PRDM2 regulated genes and classify them into potential direct and indirect targets.

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## Specifications:

Organism/cell line/tissue	<i>Mus musculus</i> C2C12 myoblasts
Sex	Female
Sequencer or array type	NIA15k mouse array, Affymetrix Genome 430 2.0 array, Agilent 244 k promoter array.
Data format	Raw data as text and processed data as supplementary table for ChIP-Chip, Raw data for microarray as CEL and or txt files
Experimental features	Microarray analysis was performed with growing, quiescent and differentiated myoblasts stably expressing RNAi constructs—either control (GFPsh) or PRDM2sh. ChIP-Chip analysis was performed using chromatin isolated from quiescent myoblasts and immuno-precipitated using anti-PRDM2 and anti-H3K9me2 antibodies. The PRDM2 antibody recognizes both major isoforms of PRDM2 (Riz1 and Riz2).
Consent	N/A
Sample source collection	N/A

ChIP-Chip data link: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58748>.

## 2. Experimental design, materials and methods

## 2.1. Cell culture

C2C12 myoblasts originally obtained from H. Blau, Stanford were sub-cloned and screened to obtain clones that exhibited tight growth control and robust differentiation. Cells were maintained in growth medium containing DMEM [high glucose] + 20% fetal bovine serum + antibiotics. Differentiation was induced at 80–90% confluence by switching cultures to low mitogen medium containing DMEM + 2% horse serum. Growing C2C12 myoblasts were induced to arrest in G0 phase of cell cycle by suspension culture in 1.3% methyl-cellulose medium containing 20% fetal bovine serum + antibiotics.

## 2.2. Generation of stable myoblast pools expressing GFPsh or PRDM2sh

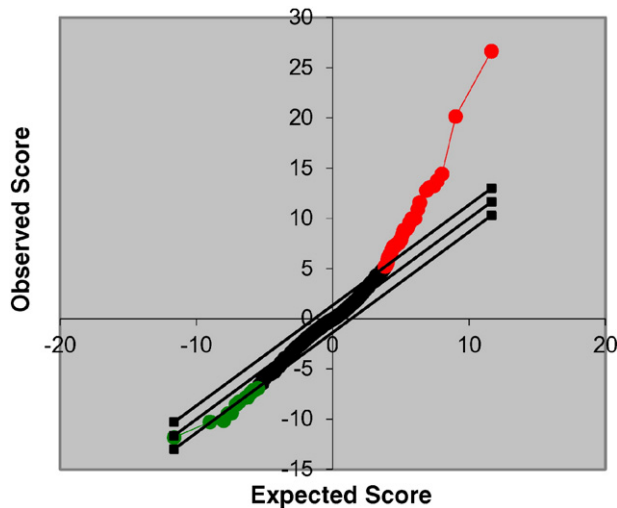
Small hairpin (sh) RNAs targeting PRDM2 and control GFP transcripts were obtained using Oligoretriever (URL: <http://www.cshl.org/public/SCIENCE/hannon.html>), sequences are given in [1] and cloned into mU6 vector. Growing C2C12 myoblasts were co-transfected with mU6 vector containing shRNA along with pSV2 neo plasmid at 5:1 ratio. To obtain stable pools, cells were selected in medium containing G418 (500 µg/ml) for 7 days with daily medium change. Stable pools were frozen after selection and

## 1. Direct link to deposited data

Microarray data series link: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58676>.

\* Corresponding author.

E-mail address: [sirisha.cheedipudi@gmail.com](mailto:sirisha.cheedipudi@gmail.com) (S. Cheedipudi).<sup>1</sup> Present address: Max Planck Institute for Heart and Lung Research, Bad Nauheim 61,231, Germany.<sup>2</sup> Present address: Max Planck Institute of Immunobiology and Epigenetics, Freiburg D-79,108, Germany.



**Fig. 1.** Scatter plot generated using SAM tools shows genes whose expression is significantly changed between proliferating PRDM2sh and GFPsh myoblasts; duplicate arrays were used for the analysis. Up-regulated genes are indicated in red, black indicates unchanged genes and green indicates down-regulated genes.

minimal expansion. Frozen aliquots were thawed for expansion and used for one passage only.

### 2.3. RNA extraction

G0 arrested myoblasts from suspension culture were collected by diluting in PBS followed by centrifugation. Cells were washed with PBS and collected into TRIzol (Invitrogen) on ice. Growing and 28 h differentiated myoblasts were washed briefly with PBS on the culture dish and TRIzol added onto the cells directly followed by scraping to collect lysates. Total RNA from samples in TRIzol was isolated according to the manufacturer's instructions.

### 2.4. Confirmation of the extent of knockdown

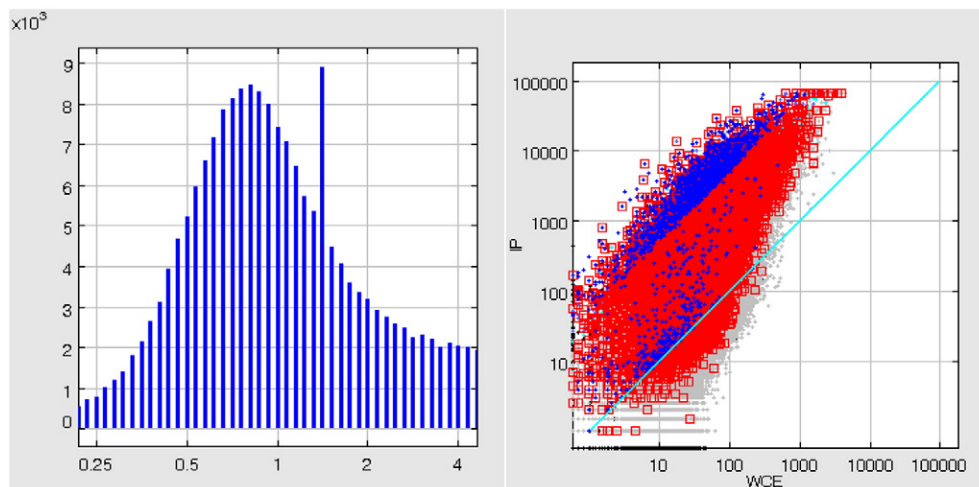
RNA samples were analyzed by real time q-RT-PCR on an ABI7700 cyclor to confirm the suppression of PRDM2 expression in PRDM2sh myoblasts. Knockdown of Riz1 and Riz2 transcripts were observed [1].

### 2.5. Microarray profiling of growing GFPsh and PRDM2sh myoblast using NIA15k mouse array

PRDM2 knock down leads to precocious expression of muscle differentiation markers even in growth inducing conditions [1]. To understand the global gene deregulation, microarray analysis was performed by competitive hybridization of Cy3 and Cy5 labeled cDNA prepared using Superscript II® (Invitrogen) with 20 µg of total RNA obtained from GFPsh and PRDM2sh myoblasts. Equimolar (25–30 picomolar) quantities of purified labeled cDNA was hybridized competitively to NIA15K mouse spotted cDNA arrays at 42 °C for 16 h (University Health Network/Ontario Cancer Institute) according to protocols provided by Amersham/GE. The arrays were washed with  $1 \times$  SSC and 0.2% SDS followed by  $0.1 \times$  SSC and 0.2% SDS and  $0.1 \times$  SSC. Slides were dried and scanned using a Molecular Dynamics scanner. Array Vision software was used for feature extraction, background subtraction, assigning detection limit and for spot intensity calculations. Lowess normalization was done using TIGR's Microarray Data Analysis System (TIGR MIDAS) [2]. Two independent hybridizations including a dye reversal for each sample and two biological replicates were performed. SAM (Significance Analysis of Microarrays) tools were used to obtain differentially expressed genes with  $FDR \leq 0.05$  and 1.5 fold cutoff. A graphic representation of scatter plot obtained with SAM tools was shown in Fig. 1. Raw data is deposited in GEO with accession number GSE58675.

### 2.6. Microarray profiling of G0 arrested and 28 h differentiated myoblasts

Proliferating PRDM2sh myoblasts showed subtle changes in gene expression compared to GFPsh myoblasts suggesting that the major function of this chromatin remodeler could be at other cell cycle stages. To better understand the functions of PRDM2, we performed microarray analysis with PRDM2sh myoblasts under quiescent and differentiating conditions with triplicate samples including biological replicates. 8 µg of total RNA was converted into cDNA using Affymetrix (Santa Clara, CA) one cycle labeling kit. cDNA was column purified and further subjected to RNA amplification using an Affymetrix IVT labeling kit. Equal amount of biotin labeled cRNA was fragmented (into 50–200 bp fragments) and hybridized to Affymetrix Genome 430 2.0 arrays, further slides were washed, stained and scanned as per Affymetrix protocols.



**Fig. 2.** Example of probe intensity and distribution of signal (A) Histogram showing distribution of normalized Log<sub>2</sub> ratios for all probes in PRDM2 ChIP-Chip data set. B. Scatter plot of normalized intensities of IP vs Input showing probe enrichment.

## 2.7. Data analysis

The data was analyzed by using the Gene Chip operating software (GCOS) of Affymetrix to generate CEL (cell intensity) files. Further CEL files were uploaded to Avadis™ software version 4.3 (an Affymetrix approved software manufactured by Strand Life Sciences, Bangalore, India) for further analysis. The CEL files of GFPsh samples were treated as control group and PRDM2sh samples were treated as experimental group in G0 and D28. The data normalization was performed using PLIER algorithm and genes with differential expression of  $\geq 1.5$  fold, either upregulated or downregulated with  $p \leq 0.05$  were processed for further analysis. Raw data files were deposited in GEO with accession numbers for quiescent PRDM2sh and GFPsh myoblasts GSE58674 and D28 PRDM2sh and control GFPsh myoblasts with GSE58673.

## 2.8. ChIP-Chip analysis of quiescent myoblasts

C2C12 myoblasts were recovered from non-adherent G0 conditions by dilution of methocel in PBS followed by centrifugation. Crosslinking was performed by re-suspending cells in growth medium containing 1% formaldehyde (Sigma) at 37 °C for 10 min. Crosslinking was terminated by adding 0.125 M glycine followed by washes with ice-cold PBS. Around  $1 \times 10^6$  cells were lysed in 200  $\mu$ l of SDS lysis buffer, followed by sonication with 45 s on/off for 25 cycles at high power (BioRuptor® sonicator) to obtain chromatin fragments of size ranging from 200 to 600 bp. Fragmented chromatin was diluted 10 times with ChIP dilution buffer and pre-cleared with Protein A/G beads containing salmon sperm DNA. Cleared supernatants were incubated with 5  $\mu$ g of PRDM2 polyclonal antibody (that detects both isoforms RIZ1 and RIZ2 #Ab3790) and 2.5  $\mu$ g H3K9me2 (Ab1220) for 16 h at 4 °C on a rotary shaker. Immune complexes were collected by adding Protein A/G agarose beads + salmon sperm DNA mix (Millipore). The beads were washed sequentially with low salt, high salt, LiCl, and TE wash buffers (buffer compositions were from Millipore Chromatin Immunoprecipitation assay kit 17–295) at 4 °C on a rotary shaker followed by centrifugation at  $3200 \times g$  for 5 min. Immune complexes were eluted at RT for 15 min twice followed by reverse crosslinking at 65 °C with 5 M NaCl for 16 h and proteinase K digestion. DNA purification was carried out using Qiagen nucleotide removal kit.

## 2.9. Hybridization

Purified DNA sample of IP and Input were blunt ended using T4 DNA polymerase followed by ligation and amplification. The linker sequences used were JW102GCGGTGACCCGGGAGATCTGAATTC and JW103GAATTCAGATC. Amplified IP and input DNA was recovered by ethanol precipitation followed by labeling with Cy3 and Cy5 dyes using Agilent genomic DNA labeling kit plus (Agilent p/n 5188–5309). Labeled samples were purified using Microcon YM-30 filter and quantified by Nano-Drop ND-1000 UV–Vis spectrophotometer. 2.5  $\mu$ g to 5  $\mu$ g

of Cy3 and Cy5 labeled DNA was used for hybridization at 65 °C for 40 h, 20 RPM on to a 1X244k promoter array from Agilent technologies. Post hybridization slides were washed with wash buffers (Agilent) according to manufacturer instructions.

## 2.10. Data Analysis

Slides were scanned on an Agilent scanner and images were analyzed using Agilent feature extraction software. Text files generated by Agilent feature extraction software were normalized by median blank subtraction, inter-array median normalization and dye bias normalization by DNA Analytics software. Whitehead per-array neighborhood algorithm was used to detect robust peaks corresponding to the DNA binding. Enrichment values for all probes were obtained by normalized log IP/Input and probes with  $p$  value  $\leq 0.05$  were considered for further analysis (Fig. 2). ChIP-Chip with PRDM2 antibody was performed with two biological replicates and one array was used for H3K9me2. The data were deposited in GEO with the accession number GSE58748.

## 3. Conclusions

Here we describe complete methodology used to perform microarray and ChIP-Chip to identify the genes regulated by PRDM2. We used this data set recently in a study describing the role of PRDM2 in regulating quiescence by repressing myogenesis and preserving the cell cycle genes from permanent silencing [1].

## Conflict of interest

None.

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