Cell Reports, Volume *25*

Supplemental Information

Trisomy of a Down Syndrome Critical Region Globally Amplifies Transcription via HMGN1 Overexpression

Cody T. Mowery, Jaime M. Reyes, Lucia Cabal-Hierro, Kelly J. Higby, Kristen L. Karlin, Jarey H. Wang, Robert J. Kimmerling, Paloma Cejas, Klothilda Lim, Hubo Li, Takashi Furusawa, Henry W. Long, David Pellman, Bjoern Chapuy, Michael Bustin, Scott R. Manalis, Thomas F. Westbrook, Charles Y. Lin, and Andrew A. Lane

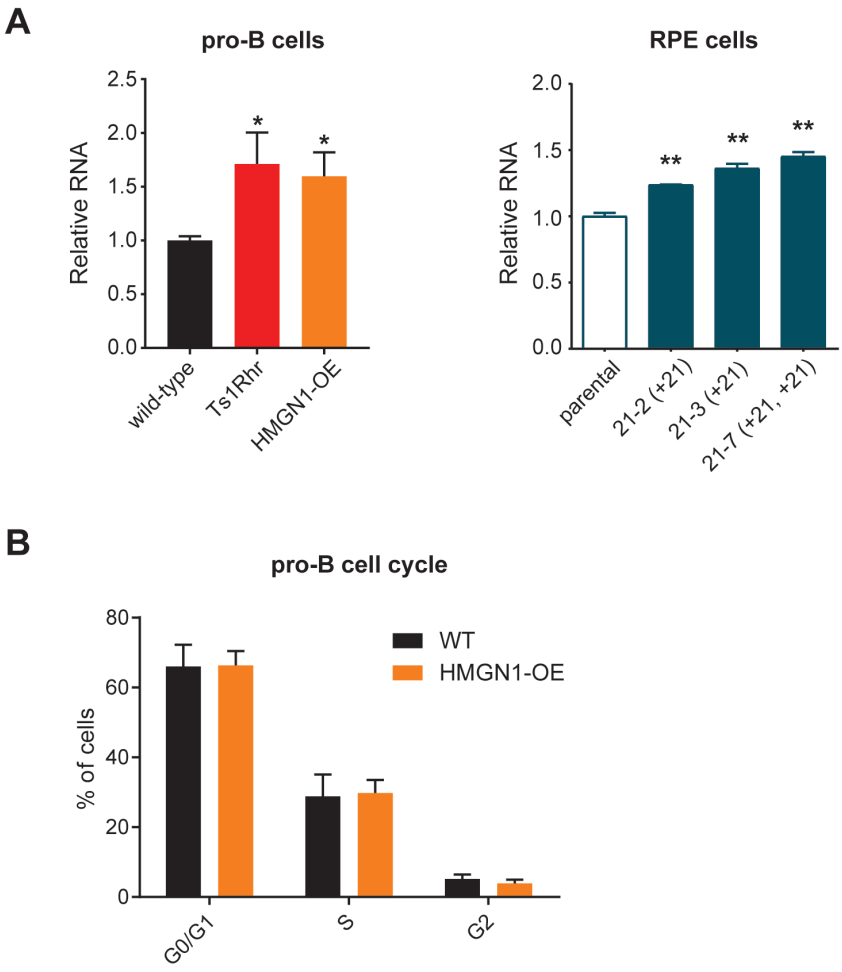

Figure S1. RNA content in cells modeling aneuploidy of chromosome 21 or HMGN1 overexpression, and cell cycle status of HMGN1 overexpressing progenitor B cells. Related to Figure 1.

(A) Relative RNA content per cell in primary mouse B cell progenitor colonies of the indicated genotypes (left) or parental retinal pigment epithelium (RPE) cells compared to RPE subclones (#21-2, 21-3, 21-7) harboring the indicated additional chromosomes (right). Each genotype or clone was measured in triplicate from independent culture wells, data compared by t test versus wild-type or parental cells (*P<0.05, **P<0.01). (B) Cell cycle analysis in primary B cell progenitor colonies derived from wild-type or HMGN1-OE transgenic mice (n=3 biological replicates).

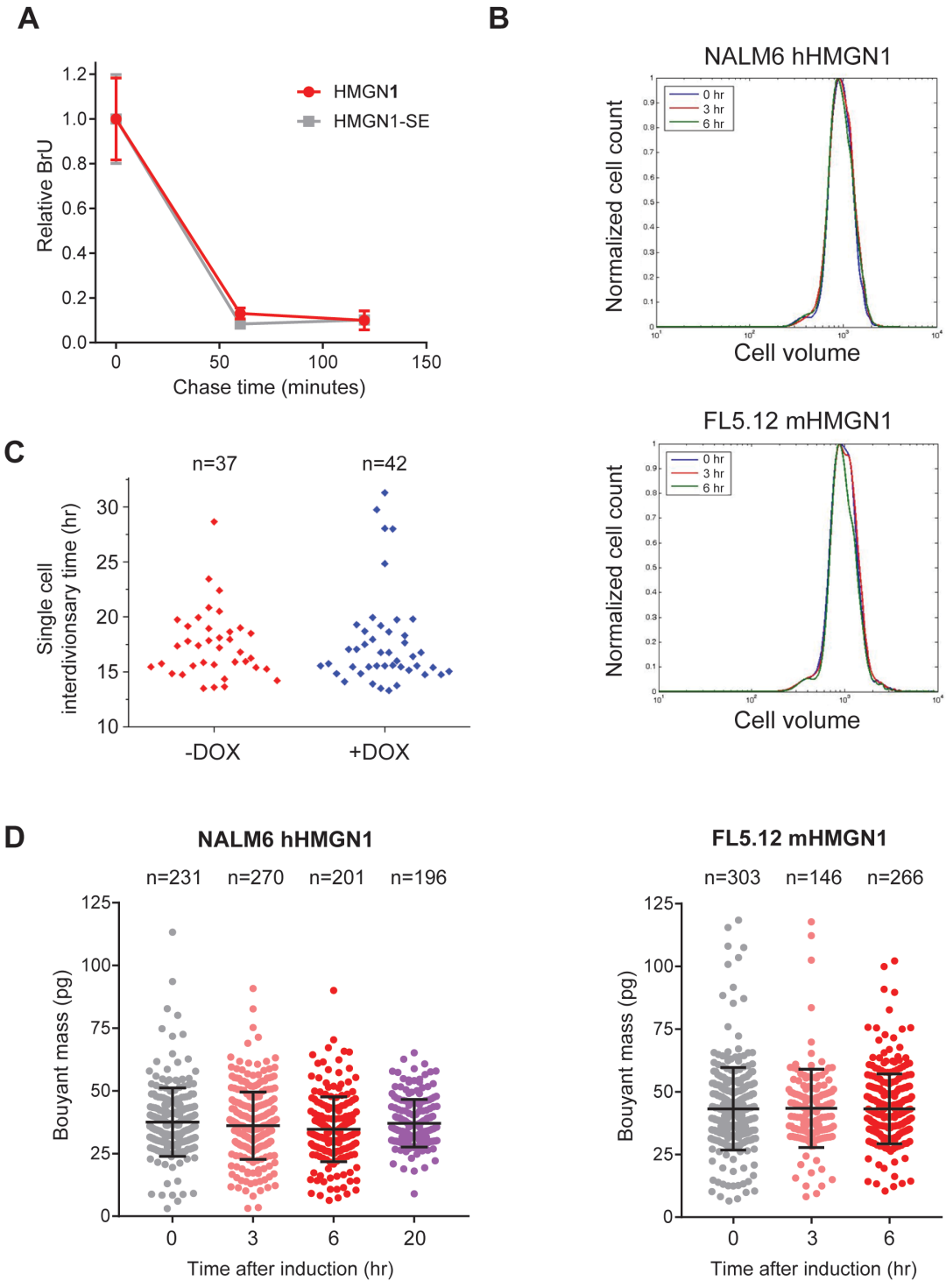

Figure S2. Cells overexpressing HMGN1 have no difference in rate of RNA turnover, cell volume, single cell interdivisionary time, or buoyant mass compared to cells overexpressing an HMGN1 mutant that is unable to bind nucleosomes. Related to Figure 3.

(A) Pulse-chase analysis of RNA decay in Nalm6 cells 6 hours after induction of HMGN1 or HMGN1-SE expression. Doxycycline was added to the cells and 5 hours later BrU labeling was performed for one hour. Cells

were then washed and relative BrU was measured at the indicated time points. N=3 biological replicates. (B) Cell volume measured in Nalm6 human B-ALL cells (top) or FL5.12 mouse B cells (bottom) at baseline and at the indicated time points after induction of human or mouse HMGN1, respectively. Representative plots shown from 3 biological replicates. (C) Single cell interdivisionary time measured using a microfluidic trap assay in Nalm6 cells stably harboring a doxycycline-inducible HMGN1 over 24 hours in the absence or presence of doxycycline. (D) Buoyant mass measured using a SMR in single Nalm6 and FL5.12 cells at the indicated time points after induction of human or mouse HMGN1, respectively (n = number of single cells analyzed at the indicated time point).

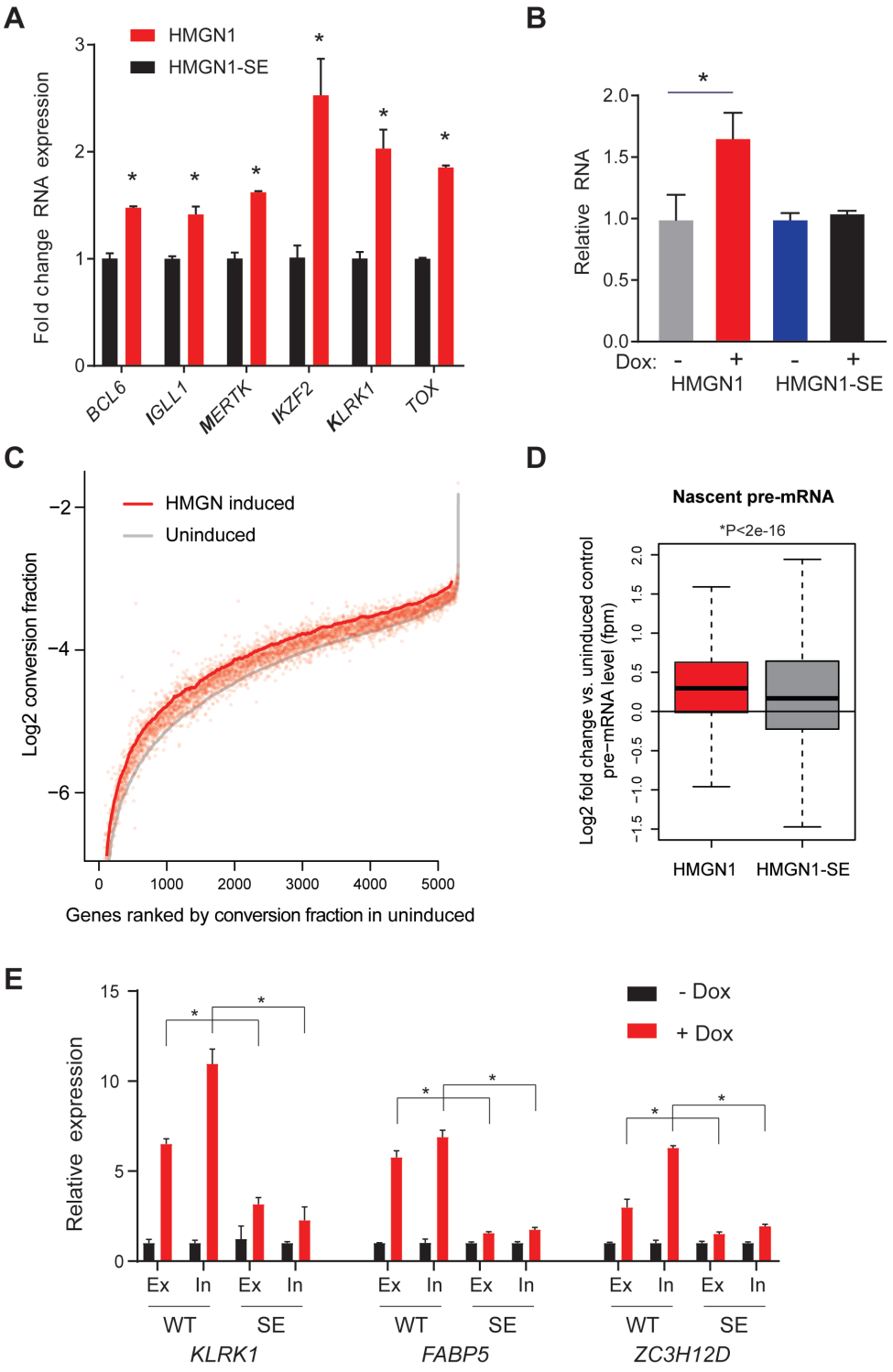

Figure S3. HMGN1 overexpression affects global and gene-specific expression and nascent transcription. Related to Figure 3.

(A) Real-time quantitative RT-PCR to confirm single gene expression differences detected in Nalm6 cells 6 hours after induction of HMGN1 or the HMGN1-SE mutant (n=technical triplicates, *P<0.05). (B) Relative total RNA

quantitated by Hoechst/Pyronin Y flow cytometry in Nalm6 cells before and after induction of the indicated HMGN1 genes (n=biological triplicates, *P<0.05). (C) SLAM-seq conversion fraction comparing Nalm6 cells labeled with 4-thiouridine, either at baseline or after addition of doxycycline to induce HMGN1. The x-axis represents genes ranked by conversion fraction (nascent reads transcribed during the labeling period over total reads) in uninduced cells, plotted versus the conversion fraction (log_2) in uninduced (smoothed gray line) or HMGN1-induced (smoothed red line; individual genes as red dots) on the y-axis. The upward shift of the red curve suggests that per gene nascent transcription is increased after induction of HMGN1. N=3 biological replicates for induced, n=2 biological replicates for uninduced. (D) SLAM-seq quantification of nascent pre-mRNA levels after induction of HMGN1 or the HMGN1-SE mutant compared to no induction. Distributions were compared by Welch's t test. (E) Real-time quantitative RT-PCR of exon (Ex) or intron (In) regions for the indicated genes after induction (+Dox) of HMGN1 (WT) or HMGN1-SE (SE) relative to uninduced (-Dox). Relative expression compared by t test (n=3 technical replicates, *P<0.05). Controls also included qPCR reactions using cDNA prepared without reverse transcriptase ("no RT control"), which showed no amplification in any samples, suggesting there was no DNA contamination of the RNA preparation.

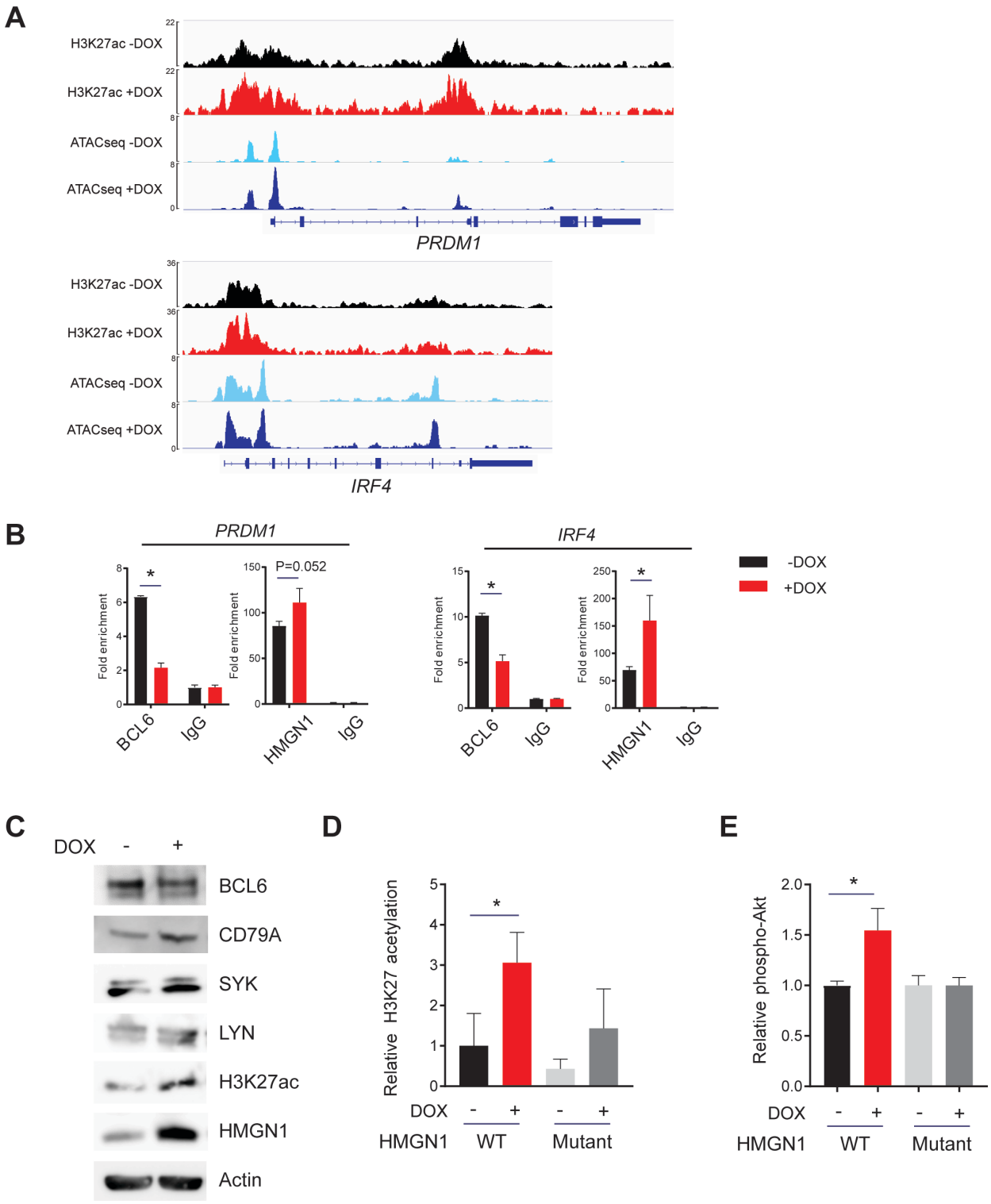

Figure S4. Chromatin, transcriptional, and protein changes occur in Nalm6 preB-ALL cells after induction of HMGN1. Related to Figures 4 and 5.

(A) Representative gene tracks in the *PRDM1* and *IRF4* loci before (-DOX) and after (+DOX) induction of HMGN1. Red and black tracks are S2 ChIP-Rx exogenous spike-in normalized H3K27acetyl ChIP-seq. Light and

dark blue tracks are ATAC-sequencing. (B) ChIP PCR in promoter regions of *PRDM1* and *IRF4* before and after induction of HMGN1 using antibodies targeting BCL6 or HMGN1, compared to control IgG antibodies (n=3 technical replicates, *P < 0.05). (C) Western blot for the indicated proteins before and after induction of HMGN1. (D) Relative H3K27 acetylation quantitated by intracellular flow cytometry in Nalm6 cells before and after induction of the indicated HMGN1 genes (n=3 technical replicates, *P<0.05). (E) Relative AKT phosphorylation quantitated by intracellular flow cytometry in Nalm6 cells before and after induction of the indicated HMGN1 genes (n=3 technical replicates, *P<0.05).

Primer	Sequence
Human qRT-PCR	
BCL6 For	GGAGTCGAGACATCTTGACTGA
BCL6 Rev	ATGAGGACCGTTTTATGGGCT
IGLL1 For	ACCCAGCTCACCGTTTTAAGT
IGLL1 Rev	GGTCACCGTCAAGATTCCCG
MERTK For	CTCTGGCGTAGAGCTATCACT
MERTK Rev	AGGCTGGGTTGGTGAAAACA
IKZF2 For	TCACCCGAAAGGGAGCACT
IKZF2 Rev	CATGGCCCCTGATCTCATCTT
TOX For	TATGAGCATGACAGAGCCGAG
TOX Rev	GGAAGGAGGAGTAATTGGTGGA
GAPDH For	GGAGCGAGATCCCTCCAAAAT
GAPDH Rev	GGCTGTTGTCATACTTCTCATGG
KLRK1 For	GAGTGATTTTTCAACACGATGGC
KLRK1 Rev	ACAGTAACTTTCGGTCAAGGGAA
KLRK1 intron For	TTCTGGACTAATAGCAAAAATGTGA
KLRK1 intron Rev	GACAACAGAGAAGTAGATTGCAACA
FABP5 For	TGAAGGAGCTAGGAGTGGGAA
FABP5 Rev	TGCACCATCTGTAAAGTTGCAG
FABP5 intron For	GACCTTTGTCACAGGCCACT
FABP5 intron Rev	ACCGGGTTAACATTTGTGGA
ZC3H12D For	AGTTCTCTGCGACCCATAGTG
ZC3H12D Rev	AACAGCCAGCTTGATTCCCC
ZC3H12D intron For	CCTGGGCAACAGAGCTAGAC
ZC3H12D intron Rev	GGGGTGAAACACAGGAGAAA
Human ChIP-PCR	
PRDM1 CHIP For	CAGTCCGAGGAAGGCTAACT
PRDM1 CHIP Rev	GGGTTCCATCGCTCTTGTACT
IRF4 CHIP For	CCATCGCAGTGGAACTCAG
IRF4 CHIP Rev	AGGGGAGAACAATCGTAACAAC
Control intragenic For	GGCTCCTGTAACCAACCACTACC
Control intragenic Rev	CCTCTGGGCTGGCTTCATTC
Mouse qRT-PCR	
Gapdh For	AGGTCGGTGTGAACGGATTTG
Gapdh Rev	TGTAGACCATGTAGTTGAGGTCA
Hmgn1 For	GGACGCGAACCGAAAAAGG
Hmgn1 Rev	CAGCCACGTCAGCCTGTTT
Kit For	GCCTGACGTGCATTGATCC

Kit Rev	AGTGGCCTCGGCTTTTTCC
Fgf13 For	CTCATCCGGCAAAAGAGACAA
Fgf13 Rev	TTGGAGCCAAAGAGTTTGACC

Table S1. Oligonucleotide primer sequences. Related to Figure 3, 4, and 5, S3, and S4.