The DNA binding factor Hmg20b is a repressor of erythroid differentiation

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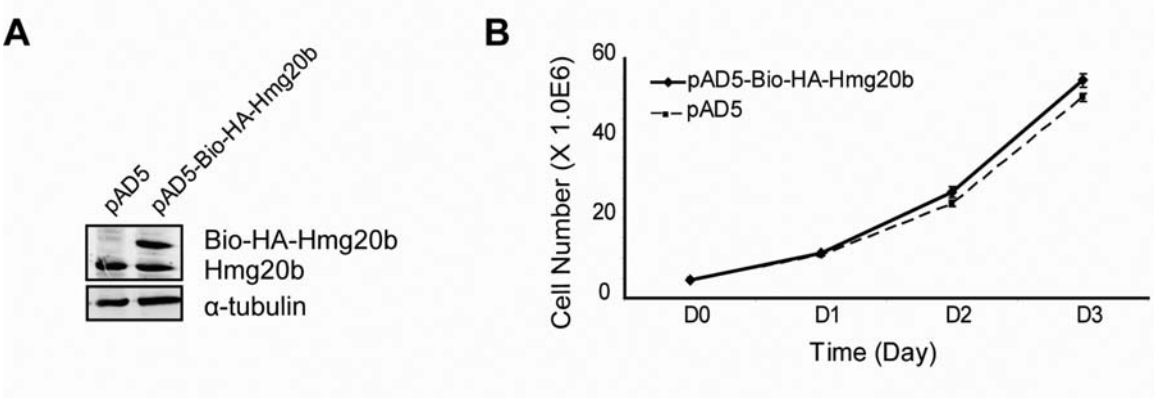
Online Supplementary Design and Methods

Cell cycle analysis

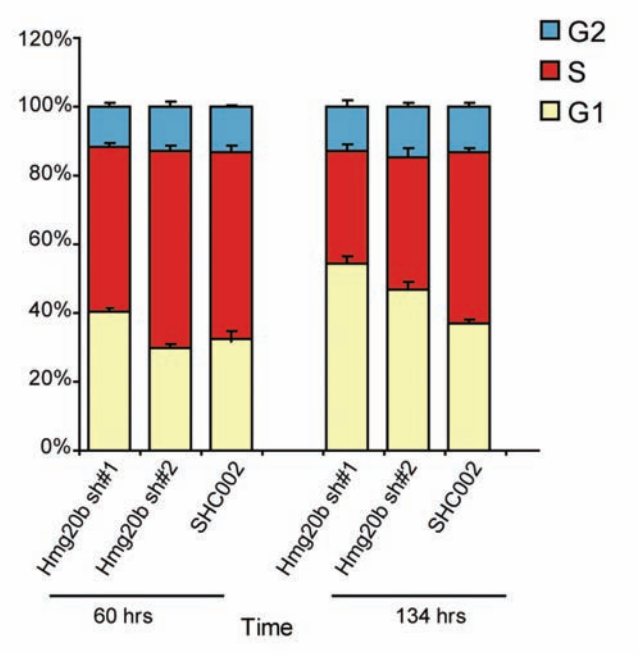
To analyze cell cycle profiles, at different time points after virus transduction I/11 cells were washed once with cold PBS and fixed in 70% ethanol. Fixed cells were pelleted and stained with a propidium iodide (PI) solution (50 g/mL, Invitrogen) containing 1U/l RNase A and 0.1% Triton X-100 (Sigma-Aldrich) for 30 min at room temperature. Flow cytometric quantification of DNA was performed using a FACScan (Becton Dickinson).[1]

Reference

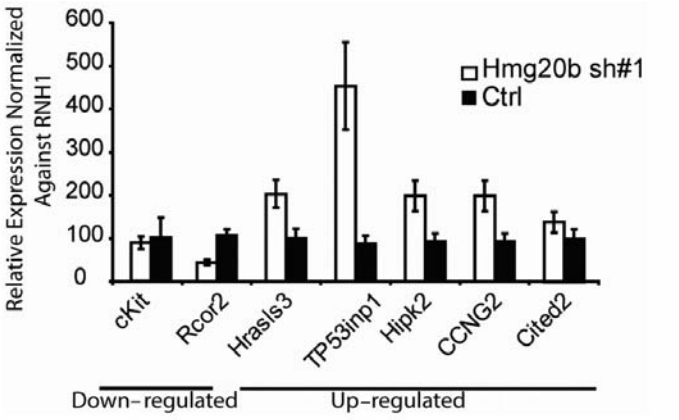
1. Vindelov LL, Christensen IJ, Nissen NI. A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. Cytometry. 1983;3(5):323-7.



Online Supplementary Figure S1. Hmg20b expression in I/11 cells. (A) Hmg20b cDNA N-terminally tagged with Bio-HA was cloned in 5pRRLsin.sPPT.CMV.GFP.Wpre (pAD5) and exogenously expressed in BirA expressing I/11 cells (Hmg20b-Bio-HA-Hmg20b). (B) Bio-HA-Hmg20b overexpression does not affect I/11 cell proliferation rate compared with control cells.



Online Supplementary Figure S2. Cell cycle analysis of Hmg20b knockdown I/11 cells. Cell cycle analysis using PI[1] in Hmg20b knockdown I/11 cells at day 3 and 5 after transduction showed higher percentage of Hmg20b-depleted cells arrested in G1 compared with control SHC002 transduced cells.



Online Supplementary Figure S3. Validation of microarray data by QRT-PCR. QRT-PCR analysis of deregulated genes according to the microarray analysis. Gene expression has been normalized against mouse Ribonuclease Inhibitor1.

Online Supplementary Table S1. Oligonucleotides used in this study.

Name	Sequence	Purpose
Ccng2-S	AAGCAAGACCATCTGTATTAGCTC	qRT-PCR
Ccng2-A	GTGTCGCTGAGCTTCAAATGT	qRT-PCR
Cited2-S	AAGCTCAACAACCAGTATTTCAAC	qRT-PCR
Cited2-A	ATCTCGGAAGTGCTGGTTTGT	qRT-PCR
Hipk2-S	GATTGAGAACACAAGCAGCGT	qRT-PCR
Hipk2-A	TTCACTGTTGGAGCCACTGTT	qRT-PCR
Hmg20b-S	CACGGGGCCTTTGTAGTG	qRT-PCR
Hmg20b-A	CAGCCTCGCTTCTTCACTG	qRT-PCR
Hrasls3-S	TCCAAGTGAAATCGCAGGAG	qRT-PCR
Hrasls3-A	TACTCCTCGTCATGTTTGTTATTG	qRT-PCR
Rcor2-S	GCTATAACATTGAGCAGGCACT	qRT-PCR
Rcor2-A	CAGCACCTTGTCCTCCACC	qRT-PCR
Trp53inp-S	ACTTCATAGATACCTGCCCTGG	qRT-PCR
TRP53inp-A	TTCCAAAGATGCAGGTAAACAG	qRT-PCR
Hmg20b-S	AAGTGGAATGTGAGAATGGTTT	ChIP
Hmg20b-A	AAACAAAAATCAGAAAGAAAAGAGA	ChIP
Amylase-S	CTCCTTGTACGGGTTGGT	ChIP
Amylase-A	AATGATGTGCACAGCTGAA	ChIP
Gfi1b-S	CGCCAGATTTTGACACAAATAA	ChIP
Gf1b-A	CTGCACAGACAGACACTTCTCC	ChIP
Nm_026-S	AAACACGTAGGAACACCAGCTC	ChIP
Nm_026-A	CATCCCCTGACAAGCATAAAA	ChIP

Online Supplementary Table S2. Correlation of QRT-PCR and microarray data.

Name	Probe set	Fold change in microarray	Correlation
Trp53inp1	10503259	3.88	0.69
Hrasls3	10461093	1.60	0.80
Ccng2	10523297	2.31	0.89
Cited2	10361828	1.89	0.55
Hipk2	10544114	1.86	0.85
Rcor2	10461057	0.66	0.98
cKit	10522530	0.49	0.30

Online Supplementary Table S3. SEE XLS FILE