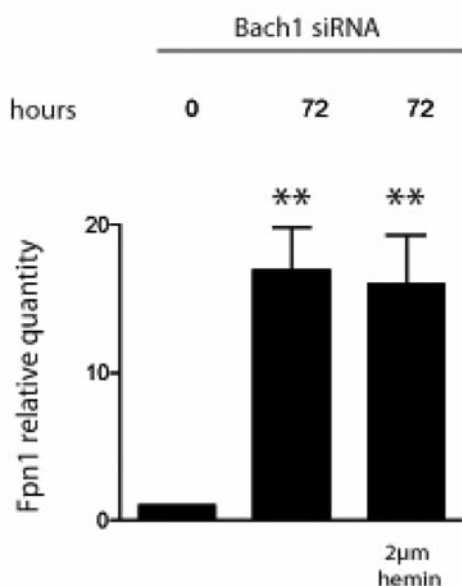


# Heme controls ferroportin1 (FPN1) transcription involving Bach1, Nrf2 and a MARE/ARE sequence motif at position -7007 of the FPN1 promoter

Samuele Marro,<sup>1,2</sup> Deborah Chiabrando,<sup>2</sup> Erika Messina,<sup>2</sup> Jens Stolte,<sup>1</sup> Emilia Turco,<sup>2</sup> Emanuela Tolosano,<sup>2</sup> and Martina U. Muckenthaler<sup>1</sup>

<sup>1</sup>Department of Pediatric Oncology, Hematology and Immunology, University of Heidelberg, Heidelberg, Germany, and <sup>2</sup>Molecular Biotechnology Center, University of Torino, Torino, Italy

Citation: Marro S, Chiabrando D, Messina E, Stolte J, Turco E, Tolosano E, and Muckenthaler MU. Heme controls ferroportin1 (FPN1) transcription involving Bach1, Nrf2 and a MARE/ARE sequence motif at position -7007 of the FPN1 promoter. *Haematologica* 2010;95(8):1261-1268. doi:10.3324/haematol.2009.020123



**Online Supplementary Figure S1.** siRNA mediated knock-down of Bach1 in the presence or absence of heme. FPN1 mRNA expression was analyzed by qPCR 72 h after transfection with specific siRNAs directed against Bach1. Cells were treated with 2 microM hemin or 1%DMSO 64 h after transfection as a control. Data were normalized to mRNA expression of GAPDH and presented as fold changes whereby the untreated control was set to 1. \*\*P<0.01.

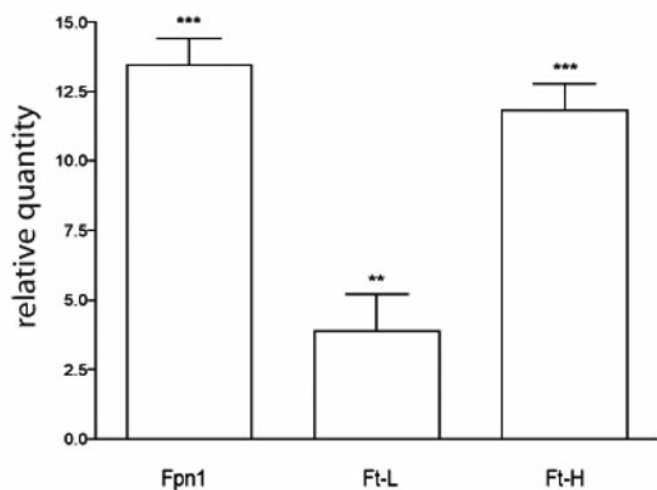
IUPAC matrix	Position from TSS	Score	Sequence
GCNNNRTCAY	-1848/-1857	1.000	GCTCAGTCAC
GCNNNRTCAY	-3105/-3114	1.000	GCATCTCAC
GCNNNRTCAY	-5385/-5394	1.000	GCAATATCAC
GCNNNRTCAY	-7007/-7016	1.000	GCCAGATCAT

**Online Supplementary Figure S2.** Bioinformatic analysis identifies four putative MARE elements within the FPN1 promoter. We have indicated the matrix that was used as a query; the positions of the identified putative MARE elements (bp from transcription start site), the score as well as the identified sequence motif.

**Alignment block 1 of 1 in window, 45989417 - 45989451, 35 bps**

```
B D Mouse taac--tgtgtgtcaggctcctcgaaggccagatcat
B D Rat taac--tgtgtgtcaggctcctcaaagagcagatcct
B D Human --gt--tgtggttcagtcct-tcataagggtggaccat
B D Orangutan --at--tgtggttcagtcct-tcataagggtggaccat
B D Dog --ac--tgtgtgtcagctct-tcatagggtgggcctt
B D Horse --at--tgtgtgtcagctct-tcatagggtggaccat
B D Opossum --at--tgtctcctattcct-tcacagggaagaccct
```

Online Supplementary Figure S3. Alignment of the murine genomic DNA region containing the -7007/-7016 MARE sequence (in box).



Online Supplementary Figure S4. Fpn1 and Ferritin expression after hemoglobin treatment. FPN1, Ferritin L and Ferritin H mRNA expression was analyzed by qPCR in RAW264.7 cells treated 12 h with 0.5 $\mu$ M hemoglobin. Data were normalized to mRNA expression of GADPH and presented as fold changes whereby the untreated control was set to 1. \*\* $P < 0.01$  and \*\*\*  $P < 0.001$ .