

## Supplementary Results

### MTF1 binds to metal-responsive element e within the *ATP7B* promoter and is a strong candidate in regulating the *ATP7B* expression

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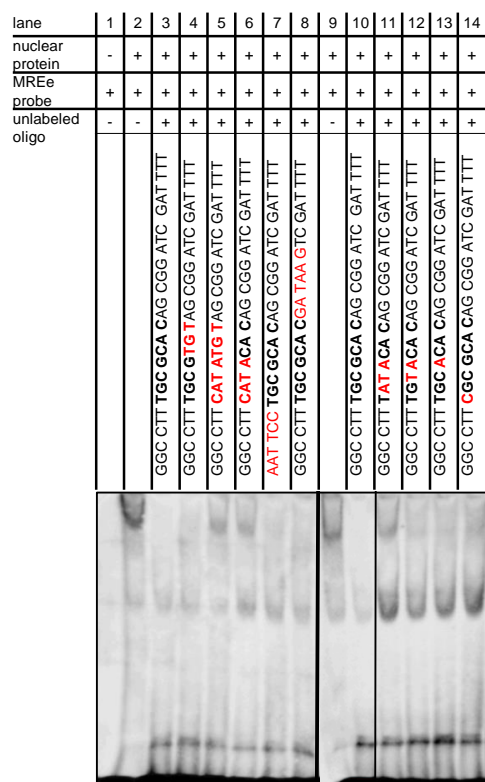
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**Fig. S1: Full size EMSA pictures for binding site determination of MREe, MREc and MREd and detailed output of MatInspector analysis**

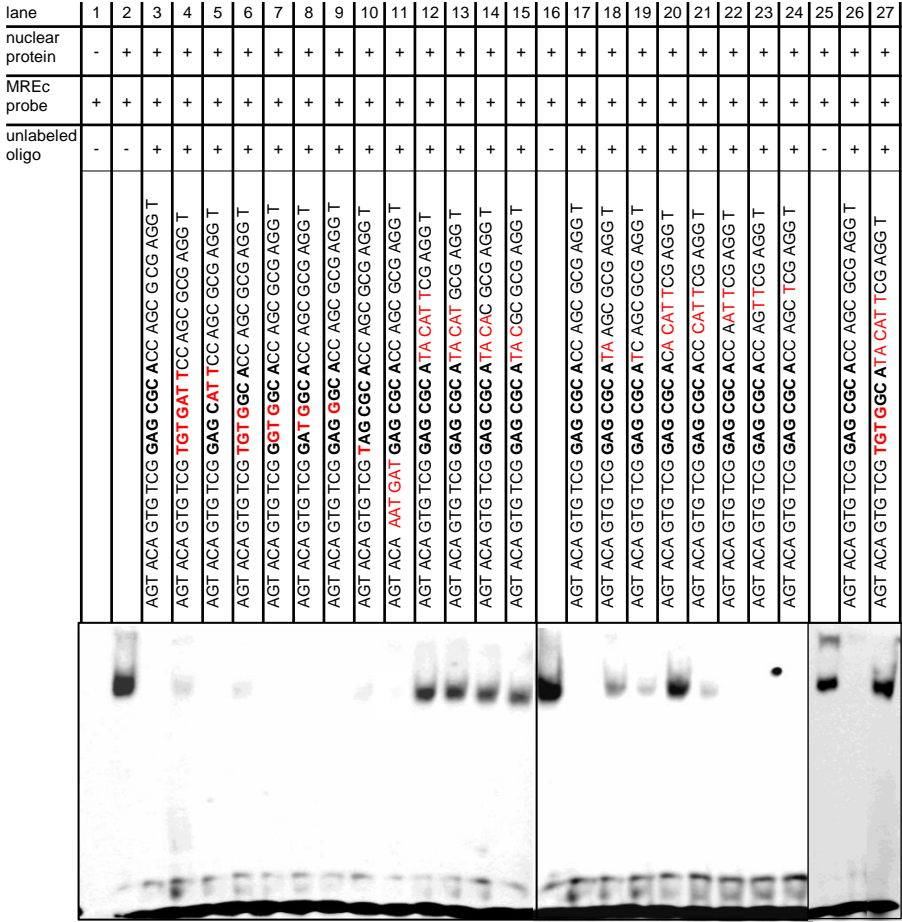
**Fig. S2: Luciferase reporter assays for MREc and MREd**

**Fig. S3: Full size EMSA pictures of validation experiments of MTF1-MREe binding within the *ATP7B* promoter**

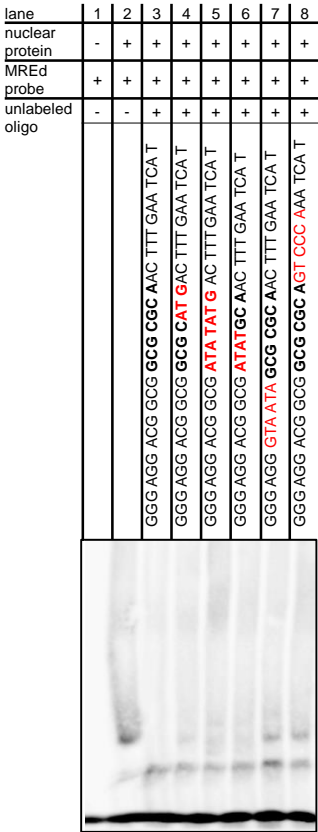
**A**



B



C

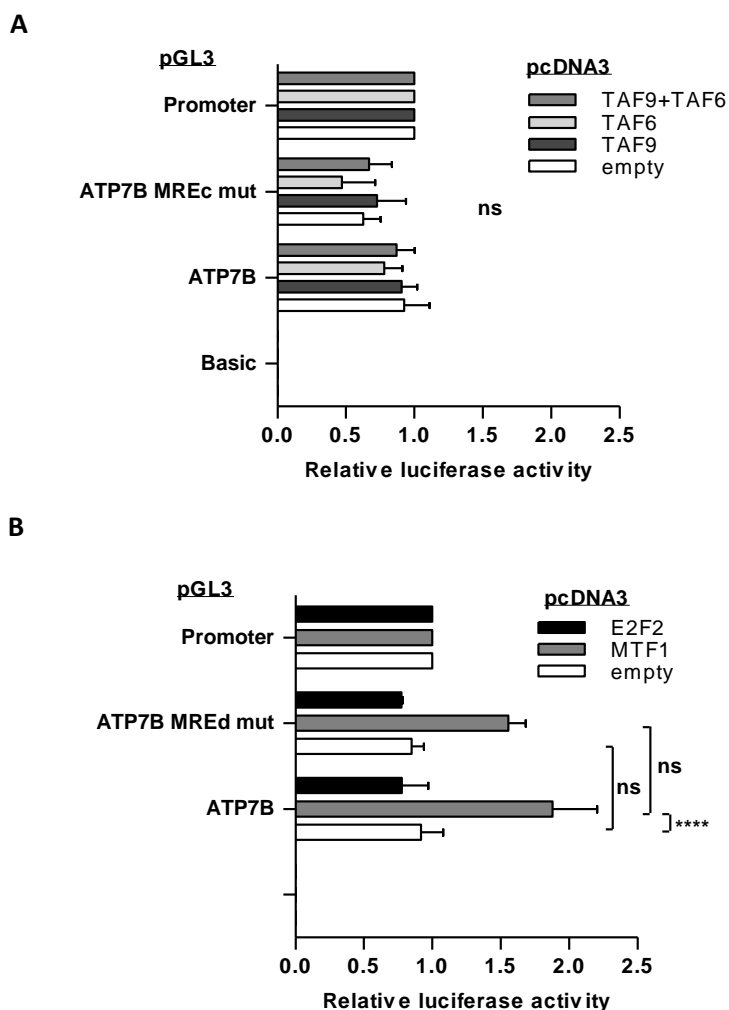


D

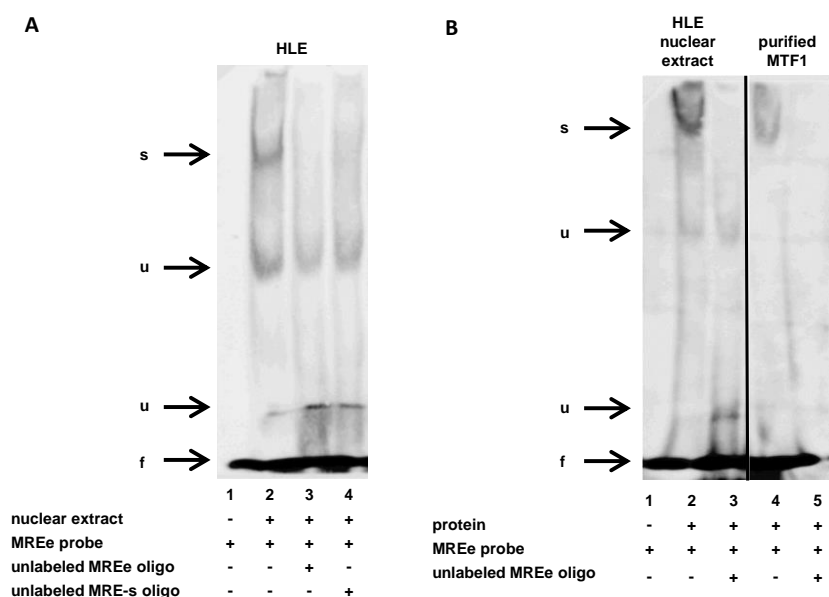
Seq. name	Matrix Family	Detailed Family Information	Matrix	Detailed Matrix Information	Tissue	Opt. position	Start position	End position	Anchor position	Strand	Core sim.	Matrix sim.	Mat. sim. - opt.	Evidence #	Sequence
MREc	OSMTEN	Core promoter motif ten elements	OSDMTE.01	Drosophila motif ten element		0,77	7	27	17	+	1	0,819	0,049	0	gtgtcggAGGcaccacgcgcg
MREc	V\$NRF1	Nuclear respiratory factor 1	V\$NRF1.01	Nuclear respiratory factor 1 (NRF1), bZIP transcription factor that acts on nuclear genes encoding mitochondrial proteins	Adipose Tissue Connective Tissue Muscle, Skeletal Muscles	0,78	13	29	21	+	0,75	0,828	0,048	0	gagcGCACcagcgcgag
MREc	V\$NRF1	Nuclear respiratory factor 1	V\$NRF1.01	Nuclear respiratory factor 1 (NRF1), bZIP transcription factor that acts on nuclear genes encoding mitochondrial proteins	Adipose Tissue Connective Tissue Muscle, Skeletal Muscles	0,78	14	30	22	-	1	0,817	0,037	0	cctcGGCtggcgct
MREd	V\$E2FF	E2F-myc activator/cell cycle regulator	V\$E2F2.01	E2F transcription factor 2	ubiquitous	0,85	7	23	15	-	1	0,88	0,03	0	aagthGGCgcccgcgt
MREd	V\$MTF1	Metal induced transcription factor	V\$MTF-1.01	Metal transcription factor 1, MRE		0,88	8	22	15	-	0,949	0,89	0,01	0	agthGGCgcccgcg
MREd	V\$ZF5F	ZF5 POZ domain zinc finger	V\$ZF5.01	Zinc finger / POZ domain transcription factor		0,95	8	22	15	-	1	0,953	0,003	0	agthcGGCgcccgcg
MREd	V\$E2FF	E2F-myc activator/cell cycle regulator	V\$E2F.03	E2F involved in cell cycle regulation, interacts with Rb p107 protein	ubiquitous	0,85	8	24	16	+	1	0,947	0,097	0	cgcgcGGCgcaactt
MREd	V\$HESF	Vertebrate homologues of enhancer of split complex	V\$HES1.01	Drosophila hairy and enhancer of split homologue 1 (HES-1)	Cardiovascular System Central Nervous System Ear, Germ Cells Embryonic Structures Heart, Muscles Myocardium, Neurons Nervous System Urogenital System	0,92	9	23	16	-	0,944	0,941	0,021	0	aagthcGGCgcccgcg
MREd	V\$ZF5F	ZF5 POZ domain zinc finger	V\$ZF5.02	ZF5 POZ domain zinc finger, zinc finger protein 161		0,83	9	23	16	+	1	0,851	0,021	0	ggcgcGGCgcaactt
MREd	V\$BARB	Barbiturate-inducible element box from pro-eukaryotic genes	V\$BARBIE.01	Barbiturate-inducible element		0,88	14	28	21	-	1	0,881	0,001	0	attcAAAGthgcgcg
MREd	V\$CHRF	Cell cycle regulators: Cell cycle homology element	V\$CHRF.01	Cell cycle gene homology region (CDE/CHR tandem elements regulate cell cycle dependent repression)		0,92	19	31	25	+	1	0,943	0,023	0	aactTTGAatcat
MREe	V\$MTF1	Metal induced transcription factor	V\$MTF-1.02	Metal-regulatory transcription factor 1		0,85	4	18	11	+	0,758	0,85	0	0	ctftgcGCACgcccgcg
MREe	V\$RBP2	Retinoblastoma-binding proteins with demethylase activity	V\$PLU1_A RD1B.01	Jumonji, AT rich interactive domain 1B		0,96	10	18	14	+	1	0,973	0,013	0	GCACgcccgcg

**Fig. S1: Full size EMSA pictures for binding site determination of MREe, MREc and MREd and detailed output of MatInspector analysis.** (A-C) EMSA assays with mutated unlabeled oligonucleotides which were preincubated with HLE nuclear extracts to narrow down the protein binding sites of MREe, MREc and MREd, respectively. Letters in bold indicate MRE consensus sequence, red letters indicate inserted mutations. Lines between lanes indicate independent EMSA experiments (A: lines between lanes 15-16 and 24-25; D: lines between lanes 8-9) or not directly

neighbored lanes of the same EMSA experiment (C: lines between lanes 10-11). (D) Transcription factors (TF) predicted to bind MREc, MREd and MREe within the *ATP7B* promoter by MatInspector algorithm<sup>1</sup>. Provided are optimized matrix similarity (opt.), start and end position of sequence, anchor position, information if match is localized on (+) or (-) strand, core similarity (core sim.), difference of matrix similarity and optimized matrix similarity (mat. sim.-opt.) and the identified sequence. Position data are relative to first base of input sequence. Anchor position marks the center position of the matrix. Capital letters designate the core sequence. Bases marked in red show a high conservation profile within the matrix (ci-value > 60).



**Fig. S2: Luciferase reporter assays for MREc and MREd.** (A+B) 48 h after cotransfection of HepG2 cells with indicated pGL3 luciferase reporter vectors and pcDNA3 expression vectors firefly luciferase activity was measured and normalized to Renilla luciferase activity. 1-way ANOVA/Bonferroni's multiple comparison test, ns: not significant, \*\*\*\* $P \leq 0.0001$ .



**Fig. S3: Full size EMSA pictures of validation experiments of MTF1-MREe binding within the *ATP7B* promoter.** (A) MREe-containing biotin-labeled probes were incubated with HLE nuclear extract. In lane 4 nuclear extract was preincubated with an unlabeled oligonucleotide containing a known MTF1 binding site (MRE-s)<sup>2,3</sup>. (B) 50 ng purified human MTF1 protein was used (lane 4+5) instead of HLE nuclear extract (lane 2+3). Lines between lanes 3 and 4 indicate not directly neighbored lanes of the same EMSA experiment.

[1] Cartharius, K., Frech, K., Grote, K., Klocke, B., Haltmeier, M., Klingenhoff, A. et al., MatInspector and beyond: promoter analysis based on transcription factor binding sites, *Bioinformatics* **21**, 2933-2942 (2005).

[2] Radtke, F., Heuchel, R., Georgiev, O., Hergersberg, M., Gariglio, M., Dembic, Z. et al., Cloned transcription factor MTF-1 activates the mouse metallothionein I promoter., *EMBO J.* **12**, 1355-1362 (1993).

[3] Brugnera, E., Georgiev, O., Radtke, F., Heuchel, R., Baker, E., Sutherland, G. R. et al., Cloning, chromosomal mapping and characterization of the human metal-regulatory transcription factor MTF-1., *Nucleic Acids Res.* **22**, 3167-3173 (1994).