Investigating the real role of HIF-1 and HIF-2 in iron recycling by macrophages

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FIGURE S1

A

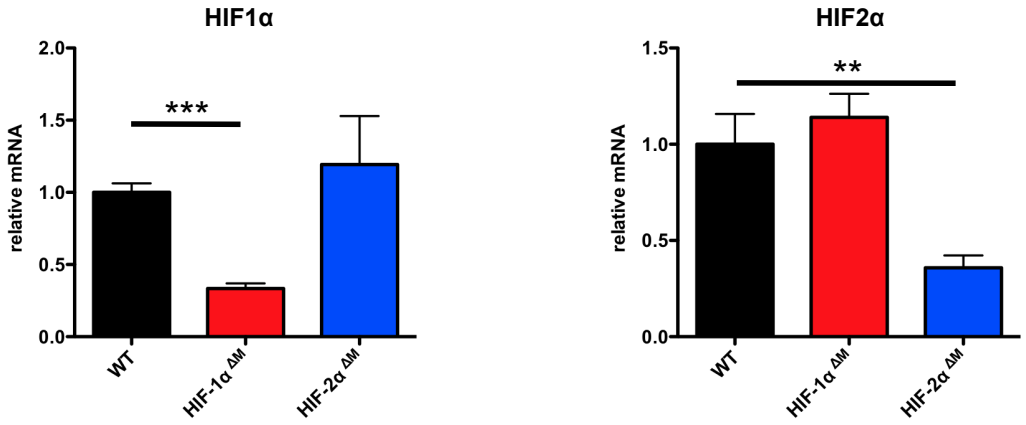

Figure S1A : HIF-1 and HIF-2 mRNA expression in F4/80 macrophages of the spleen of WT and HIF KO mice : Spleen macrophages were isolated from WT and HIF KO littermates (n=3 per group). To isolate F4/80^{+} cells from WT and HIF KO mice, spleen were treated with biotin-labeled anti-Ter119, anti-CD3, and anti-CD45R mAbs (BD) and then incubated with anti-biotin microbeads (Invitrogen) to deplete these populations. These depleted cells were stained with an anti–F4/80-mAb and macrophage fractions were collected using a FACSJazz (BD). Statistical significance was evaluated by one-way ANOVA analysis followed by a Bonferroni posttest; **p < 0,01; ***p < 0,001.

B

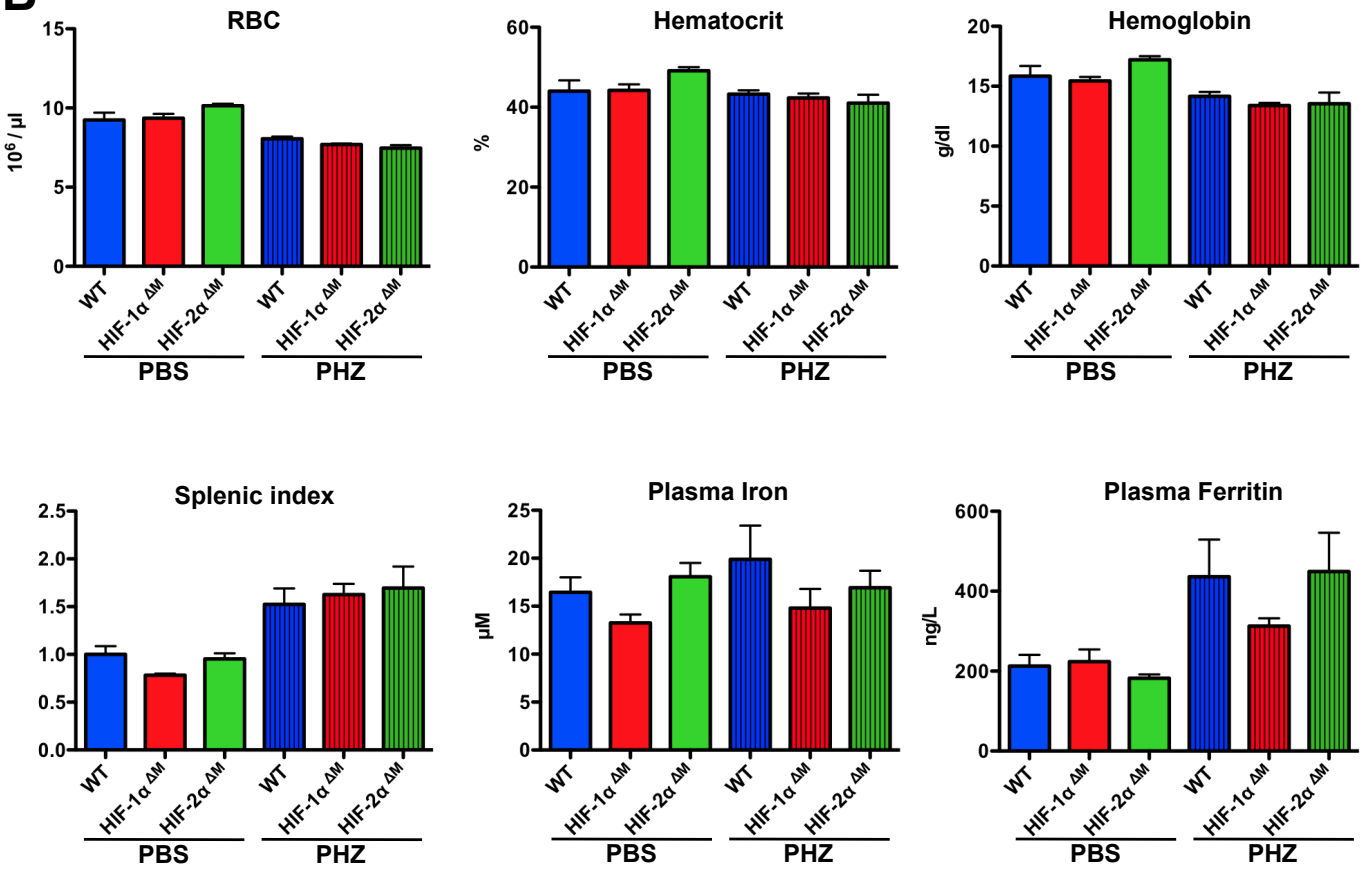

Figure S1B : Iron homeostasis parameters 9 days after PHZ treatment : 12 week-old WT, HIF-1$\alpha^{\Delta M}$ and HIF-2$\alpha^{\Delta M}$ male mice (n ≥ 3) were injected for 2 consecutive days with 50 mg/kg of PHZ or PBS and killed 9 days after the last injection. The mice were maintained on an iron deficient diet immediately after the first injection.

FIGURE S2

A

TfR1

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	*	ns
8	ns	ns
24	***	ns

FPN

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	***	ns
8	***	ns
24	ns	ns

HO-1

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	ns
1	***	ns
4	***	ns
8	***	ns
24	ns	ns

DMT-1+IRE

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	ns
1	***	ns
4	***	ns
8	***	ns
24	ns	ns

Nramp1

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	***	ns
8	***	*
24	ns	ns

DMT-1-IRE

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	ns
1	*	ns
4	***	ns
8	***	ns
24	ns	ns

TfR1

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	*	ns
8	ns	ns
24	***	***

FPN

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	***	ns
8	***	ns
24	ns	ns

HO-1

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	ns	ns
1	***	ns
4	***	ns
8	***	ns
24	ns	ns

DMT-1+IRE

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	**	ns
1	***	ns
4	***	ns
8	**	ns
24	**	ns

Nramp1

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	***	ns
8	***	ns
24	***	ns

DMT-1-IRE

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	***	ns
8	***	ns
24	ns	ns

B

HIF-1α

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	***
1	***	***
4	***	***
8	***	***
24	ns	***

HIF-2α

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	***	ns
8	**	ns
24	*	ns

PGK1

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	ns
1	ns	*
4	ns	***
8	***	***
24	**	***

Arginase

Time (h)	WT RBC vs WT	HIF-1α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	*	ns
8	ns	ns
24	*	ns

HIF-1α

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	***	ns
8	**	ns
24	ns	ns

HIF-2α

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	ns	***
1	ns	***
4	***	ns
8	***	ns
24	**	***

PGK1

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	ns	ns
1	ns	ns
4	ns	ns
8	***	ns
24	**	ns

Arginase

Time (h)	WT RBC vs WT	HIF-2α KO + RBC vs WT + RBC
0	ns	*
1	ns	ns
4	*	ns
8	ns	*
24	***	***

Figure S2 : Statistical values of Figure 2. Analysis was performed using GraphPad Prism 5.0 and statistical significance was evaluated by two-way ANOVA analysis followed by a Bonferroni posttest; ns: not significant; *p < 0,05; **p < 0,01; ***p < 0,001