

Progesterone Receptor Membrane Component-1 regulates hepcidin biosynthesis

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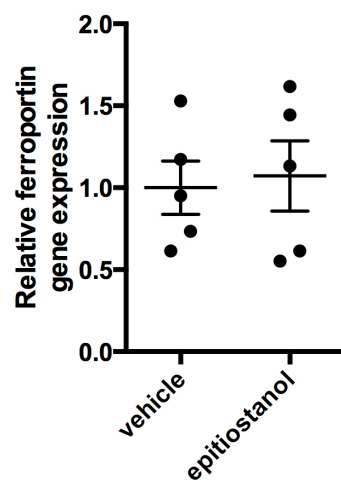
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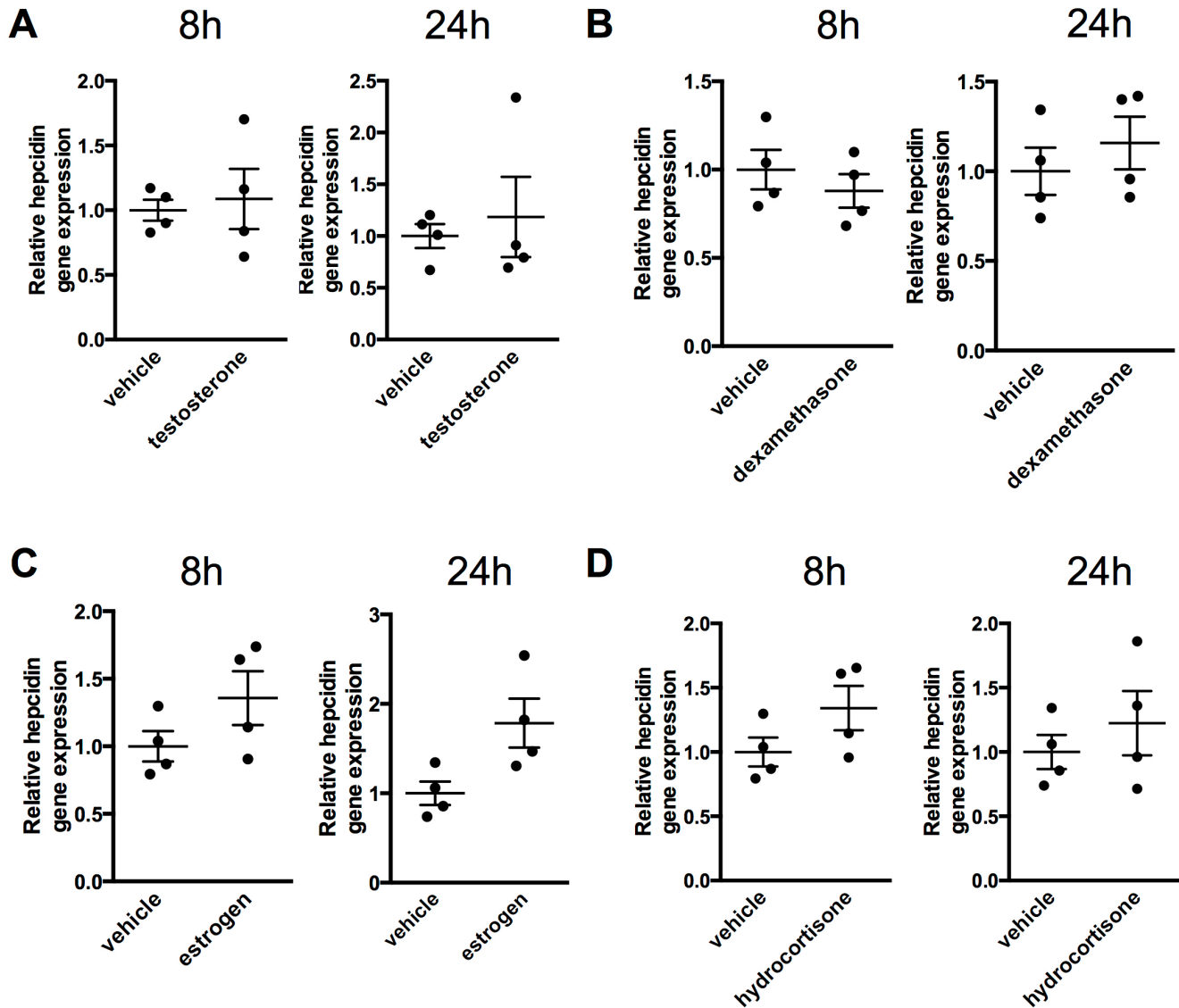
The authors declare no conflict of interest exists.

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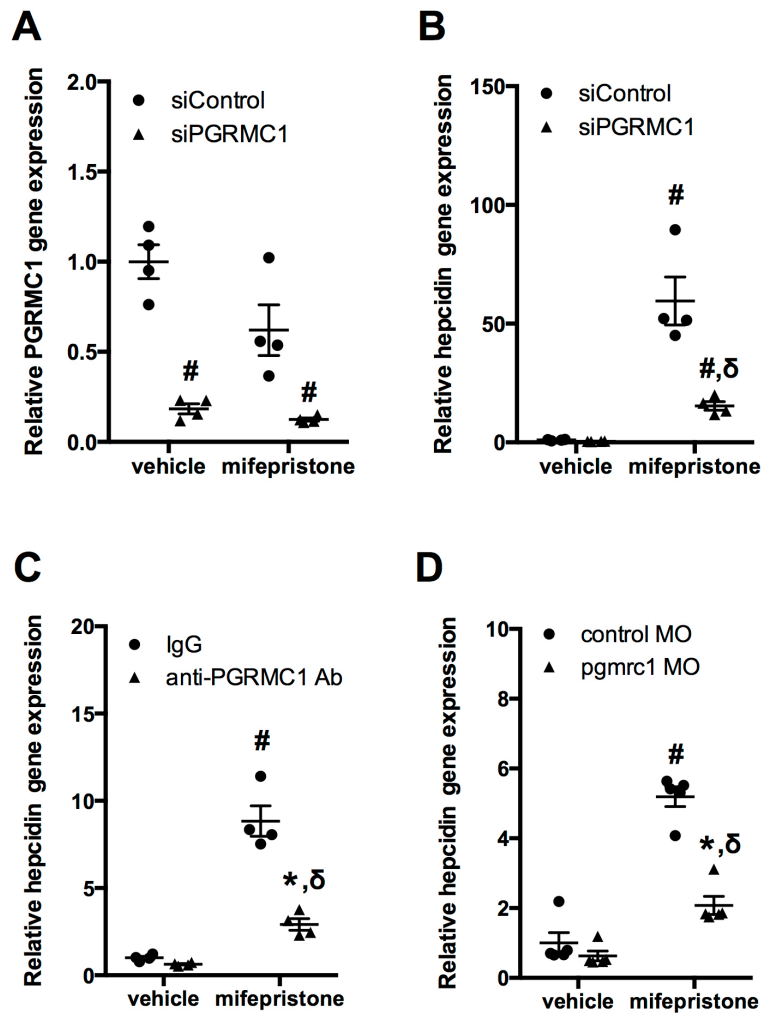


Supplemental Figure 1. Epitiostanol does not alter ferroportin gene expression in wild-type zebrafish.

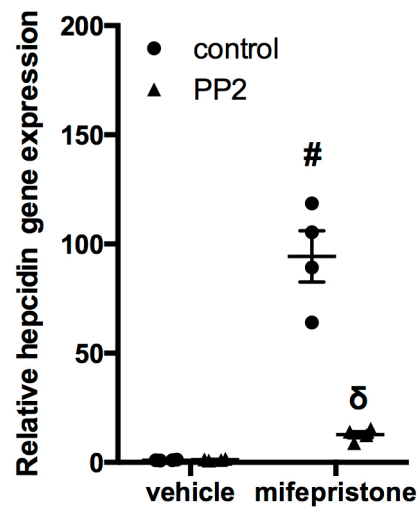
qPCR for ferroportin gene expression in 3-day old wild-type zebrafish larvae treated with 10 μ M epitiostanol or vehicle for 12 hours. Zebrafish *gapdh* is used as internal reference and gene expression was normalized to vehicle treated group. (2-tailed t-test, n=5 per group). Results expressed as mean $\pm$ SEM.



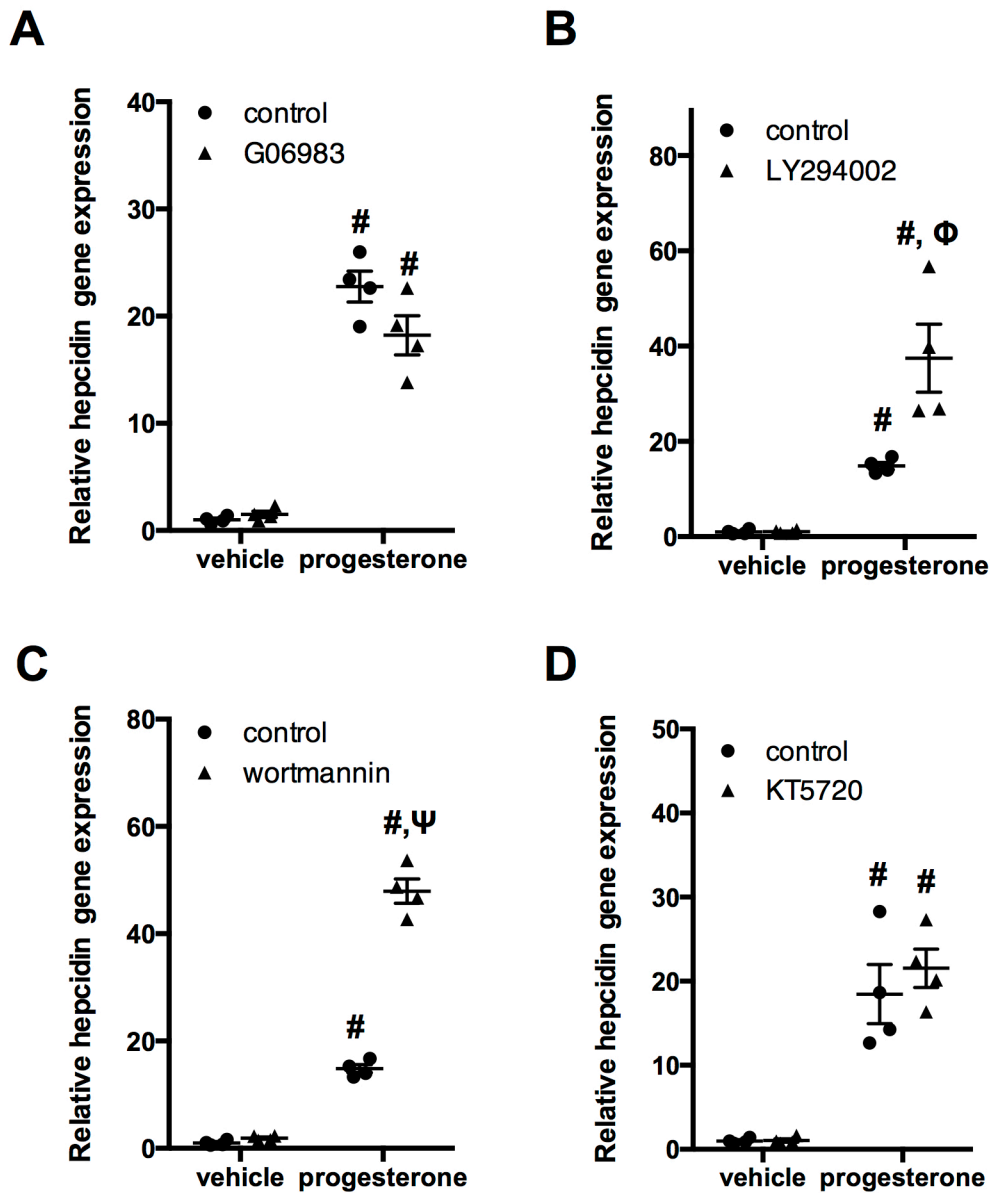
Supplemental Figure 2. Testosterone, dexamethasone, estrogen, and hydrocortisone do not increase hepcidin gene expression. (A-D) qPCR for hepcidin gene expression in HepG2 cells serum starved for 18 hours and then exposed to (A) testosterone (30 μ M), (B) dexamethasone (30 μ M), (C) estrogen (30 μ M), or hydrocortisone (30 μ M), for 8 or 24 hours. (2-tailed t-test, n=4 per group). All results expressed as mean $\pm$ SEM.



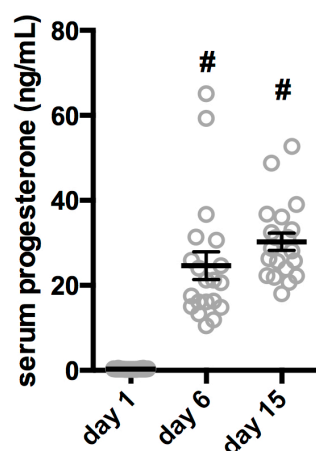
Supplemental Figure 3. PGRMC1 is required for mifepristone to increase hepcidin gene expression in HepG2 cells and zebrafish. (A) qPCR for PGRMC1 gene expression in HepG2 cells transfected with scrambled siRNAs (siControl) or siRNAs directed against PGRMC1 (siPGRMC1) and exposed to vehicle or mifepristone (30 μ M for 8 hours) (# $P < 0.001$ compared to siControl transfected cells treated with vehicle, 2-way ANOVA, $n=4$ per group). (B) qPCR for hepcidin gene expression in HepG2 cells transfected with scrambled siRNAs (siControl) or siRNAs directed against PGRMC1 (siPGRMC1) and treated with vehicle or mifepristone (30 μ M for 8h). (C) qPCR for hepcidin gene expression in HepG2 cells pre-incubated with control IgG or anti-PGRMC1 antibodies (PGRMC1 Ab) for 3 hours and then exposed to vehicle or mifepristone (30 μ M for 8h). (# $P < 0.001$, * $P < 0.01$ compared to siControl transfected cells or IgG treated cells exposed to vehicle, $\delta P < 0.001$ compared to siControl transfected cells or IgG treated cells exposed to mifepristone, 2-way ANOVA, $n=4$ per group). (D) qPCR for hepcidin gene expression in 3-day old wild-type zebrafish embryos injected with control MO or *pgrmc1* MO and treated with vehicle or mifepristone (5 μ M for 12h) (# $P < 0.001$ compared to control MO injected embryos treated with vehicle, * $P < 0.01$ compared to control MO injected embryos treated with vehicle, $\delta P < 0.001$ compared to control MO injected embryos treated with mifepristone, 2-way ANOVA, $n=5$ per group). All results expressed as mean $\pm$ SEM.



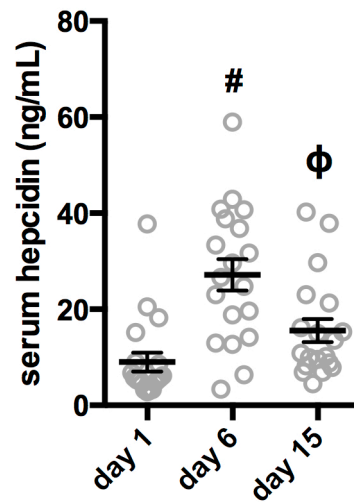
Supplemental Figure 4. Mifepristone requires SKF activity to increase hepcidin gene expression. qPCR for hepcidin gene expression in HepG2 cells serum starved for 18 hours and then pre-incubated with the SKF inhibitor PP2 (10 μ M) or control (DMSO) for 30 minutes and then exposed to vehicle or mifepristone (30 μ M for 8 hours) (# $P < 0.001$ compared to control treated cells exposed to vehicle, δ $P < 0.001$ compared to control treated cells exposed to mifepristone, 2-way ANOVA, $n=4$ per group). All results expressed as mean $\pm$ SEM.



Supplemental Figure 5. PKC, PI3 Kinase, and PKA, signaling pathways are not required by progesterone to induce hepcidin gene expression. (A-D) qPCR for hepcidin gene expression in HepG2 cells serum starved for 18 hours and then incubated for 30 minutes with the PKC inhibitor G06983 (1 μ M), the PI3 kinase inhibitors LY294002 (40 μ M) or wortmannin (1 μ M), or the PKA inhibitor KT5720 (1 μ M) and then exposed to vehicle or 30 μ M of progesterone for 8 hours (# $P < 0.001$ compared to control treated cells exposed to vehicle, Φ $P < 0.001$ compared to control treated cells exposed to progesterone; Ψ $P < 0.01$ compared to control treated cells exposed to progesterone, 2-way ANOVA, $n=4$ per group). All results expressed as mean $\pm$ SEM.



Supplemental Figure 6. Serum progesterone levels in women undergoing in vitro fertilization with frozen embryo transfer. Serum progesterone levels in women prior to progesterone treatment (day 1) or after receiving progesterone daily (day 6 and day 15) during a standard in vitro fertilization protocol using frozen embryo transfer. (# $P < 0.001$ compared to day 1 sample, paired t-test with bonferroni adjustment for multiple comparisons, $n=30$ per group). All results expressed as mean $\pm$ SEM.



Supplemental Figure 7. Serum hepcidin levels in women undergoing *in vitro* fertilization with frozen embryo transfer. Serum hepcidin levels in women prior to progesterone treatment (day 1) or after receiving progesterone daily (day 6 and day 15) during a standard *in vitro* fertilization protocol using frozen embryo transfer. Serum hepcidin levels were measured using a competitive ELISA kit from Peninsula Laboratories (formerly sold by Bachem). Please note that a single patient (at all three time points) had serum hepcidin levels that were below the detection limit of this ELISA kit and this patient was excluded from the analysis. (# $P < 0.001$, $\Phi P < 0.05$ compared to day 1 sample, paired t-test with bonferroni adjustment for multiple comparisons, $n=19$ per group). All results expressed as mean $\pm$ SEM.