

Maternal prolactin or estrogen signaling in hepatocytes does not regulate iron homeostasis during pregnancy

Authors

Vida Zhang,¹ Allison L. Fisher,² S. Marguerite Hewitt,¹ Tomas Ganz,¹ Elizabetha Nemeth¹ and Veena Sangkhae¹

¹UCLA Center for Iron Disorders, UCLA Department of Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, David Geffen School of Medicine, Los Angeles, CA and ²Nephrology Division and Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Correspondence:

V. SANGKHAЕ - VSangkhae@mednet.ucla.edu

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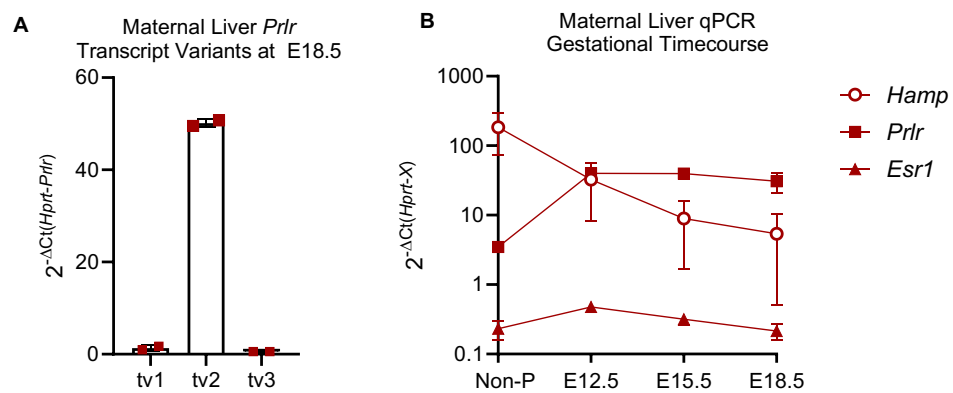
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Supplemental Table 1: Primers, Antibodies and Other Reagents			
Primers			
Species	Gene	Sequence	Product size
Mouse	Hprt	Fwd: 5'-CTGGTTAAGCAGTACAGCCCCAA-3'	65bp
		Rev: 5'-CAGGAGGTCCTTTTCACCAGC-3'	
	Prlr all	Fwd: 5'-AAGCAGTACCTCGGATCCAC-3'	129bp
		Rev: 5'-GGCAACCATTTTACCCACAG-3'	
	Prlr tv1	Fwd: 5'-CCTGCATCTTTCCACCAGTT-3'	96bp
		Rev: 5'-CCAAGGCACTCAGCAGTTCT-3'	
	Prlr tv2	Fwd: 5'-CCTGCATCTTTCCACCAGTT-3'	147bp
		Rev: 5'-CAAGTTCCTTCATTGTCC-3'	
	Prlr tv3	Fwd: 5'-CCTGCATCTTTCCACCAGTT-3'	107bp
		Rev: 5'-TGGAATCTTCACCAGGGAAGT-3'	
	Esr1	Fwd: 5'-AGAACCGGAGGAAGAGTTGC-3'	82bp
		Rev: 5'-TCTTCCGTATGCCGCCTTT-3'	
	Esr2	Fwd: 5'-GTCCACCCGCTAGGCATTC-3'	105bp
		Rev: 5'-TGTCTTGCGTAGGTCTCAGC-3'	
	Ebag9	Fwd: 5'-ATGCAGATCTGGCAGAGGAA-3'	102bp
		Rev: 5'-CACTCCTCCACATCTGTCTGC-3'	
Ctsd	Fwd: 5'-TCAAAAACCTACCTGGATGCCCA-3'	74bp	
	Rev: 5'-GACGACTGTGAAACACTGCG-3'		
Gper1	Fwd: 5'-TAACGCCACGGCACAGATCA -3'	120bp	
	Rev: 5'-TGGCAGAAATGACACCCTGG-3'		
Antibodies			
Target Protein		Primary Antibody	
Prolactin receptor		Mouse Monoclonal Prolactin Receptor Antibody (U5): MA1-610, ThermoFisher Scientific	
Estrogen receptor α		Mouse Monoclonal Estrogen Receptor alpha Antibody (6F11): MCA1799T, Bio-Rad	
β-Actin		Mouse Monoclonal β-actin antibody-peroxidase (AC-15): A3854, Sigma-Aldrich	
Secondary antibodies			
Anti-mouse IgG HRP: #7076 Cell Signaling			
TrueBlot Anti-mouse Ig HRP: 18-8817-31 Rockland			
Other Reagents			
Assay		Product	Protocol
Mouse Hepcidin ELISA		In-house	1
Tissue Iron		Seksui Iron Total SL #157-30	1 or 2
Animals			
Experiments were conducted in accordance with guidelines by the Animal Research Committee and were approved by UCLA. Prolactin Receptor-flox animals were kindly provided by Dr. Ronadip Banerjee ³ . Estrogen Receptor alpha-flox (strain #032173) and Albumin-Cre (strain #0035574) animals were purchased from The Jackson Laboratory. All mice were on a C57BL/6 background.			

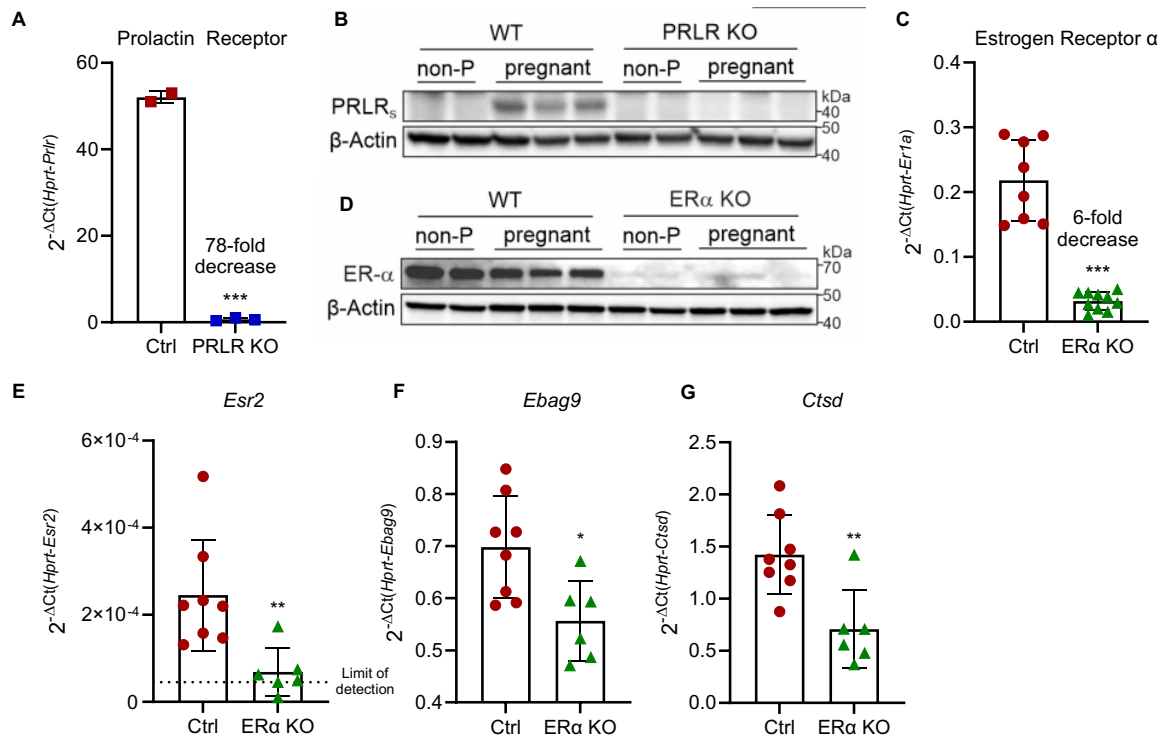
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Supplemental Figure 1



Supplemental Figure 1. Liver expression of *Prlr*, *Esr1* and *Hamp* in wild-type females. (A) Maternal liver *Prlr* mRNA transcript variant expression. **(B)** Liver mRNA expression of *Hamp*, *Prlr*, and *Esr1* in non-pregnant females and over gestation.

Supplemental Figure 2



Supplemental Figure 2. Validation of hepatocyte-specific PRLR KO and ERα KO. Prolactin receptor (**A**) mRNA and (**B**) protein expression in WT and PRLR KO whole dam livers. Estrogen receptor (**C**) mRNA and (**D**) protein expression in WT and ERα KO whole dam livers. (**E**) *Esr2*, (**F**) *Ebag9* and (**G**) *Ctsc* mRNA expression in WT and ERα KO whole dam livers at E18.5. Statistical differences between groups were determined by unpaired two-tailed t-test (*** $P < 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$). Ctrl: control; Prlr: prolactin receptor; ERα: estrogen receptor α; Non-P: non-pregnant; Esr2: estrogen receptor β; Ebag9: estrogen receptor binding site associated 9; Ctsc: cathepsin D. ● Alb-Cre⁺; ■ Prlr^{fl/fl}; ▲ Esr1^{fl/fl}; ■ Prlr^{fl/fl} Alb-Cre⁺; ▲ Esr1^{fl/fl} Alb-Cre⁺