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IDENTIFICATION OF TARGET GENES FOR A PROLACTIN FAMILY PARALOG IN MOUSE DECIDUA

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Abstract

Prolactin family 8, subfamily a, member 2 (PRL8A2; also called decidual prolactin-related protein; dPRP) is a member of the expanded prolactin family. PRL8A2 is expressed in the uterine decidua and contributes to pregnancy-dependent adaptations to hypoxia. The purpose of this study was to identify gene targets for PRL8A2 action within the uteroplacental compartment. Affymetrix DNA microarray analysis was performed for RNA samples from wild type and *Prl8a2* null tissues. Validation of the DNA microarray was performed using quantitative RT-PCR. Nine genes were confirmed with decreased expression in *Prl8a2* null tissues (e.g. *Klk7, Rimklb, Arhgef6, Calm4, Sprr2h, Prl4a1, Ccl27, Lipg*, and *Htra3*). These include potential decidual, endothelial, and trophoblast cell targets positively regulated by PRL8A2. A significant upregulation of *Derl3, Herpud1, Creld2, Hsp90b1, Ddit3*, and *Hspa5* was identified in *Prl8a2* null tissues, reflecting an increased endoplasmic reticulum (ER) stress response. ER stress genes were prominently expressed in the uterine decidua. We propose that PRL8A2 is a mediator of progesterone-dependent modulation of intrauterine responses to physiological stressors.

Keywords

Decidua; prolactin; pregnancy; ER stress

INTRODUCTION

The mouse possesses an expanded prolactin (PRL) gene family that encodes hormones/cytokines (Wiemers *et al*. 2003; Soares *et al*. 2007). In some species the expansion was robust such as occurred in the mouse, rat, guinea pig, and cow (Wiemers *et al*. 2003; Alam *et al*. 2006, 2010; Ushizawa & Hashizume 2006), whereas evidence for an expansion in other species such as the human and dog is lacking (Cooke & Liebhaber 1995; Lindblad-Toh *et al*. 2005). These hormones and cytokines are associated with pregnancy and are

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

produced by the anterior pituitary, uterine decidua, and/or trophoblast cells (Soares 2004). The biological activities of PRL are well described and include profound effects on the reproductive axis and lactation (Horseman *et al*. 1997; Bole-Feysot *et al*. 1998; Horseman & Gregerson 2014); however, the actions of the remaining PRL family paralogs are less well appreciated. Roles for these PRL related proteins in regulating blood vessel and hematopoietic cell development have been demonstrated (Jackson *et al*. 1994; Lin & Linzer 1999; Bittorf *et al*. 2000). Based on mouse mutagenesis experiments, the biological activities of at least some expanded PRL family paralogs include modulation of uteroplacental adaptations to physiological stressors (Ain *et al*. 2004; Alam *et al*. 2007; Soares *et al*. 2007). PRL also participates homeostatic responses to stress (Dorshkind & Horseman 2001).

Hemochorial placentation is associated with differentiation of uterine stromal cells into epithelial-like cells called decidual cells possessing extensive secretory capabilities and essential roles in the establishment and maintenance of pregnancy (Aplin 2000; Gellersen *et al*. 2007; Herington & Bany 2009; Teklenburg *et al*. 2010a,b). Decidual cells effectively create an environment within the uterus compatible with development of the placenta and fetus. Among the factors secreted by decidual cells are members of the PRL family (Orwig *et al*. 1997c; Jabbour & Critchley 2001). Human decidual cells produce PRL, while the mouse and rat produce PRL and an additional three PRL family paralogs (Soares 2004; Soares *et al*. 2007).

Biological roles for decidual PRL family hormones/cytokines are not well understood (Jabbour & Critchley 2001). Among the decidual PRL family paralogs in the mouse and rat is a protein referred to as PRL family 8, subfamily a, member 2 (PRL8A2; also referred to as decidual PRL-related protein dPRP; Roby *et al*. 1993). PRL8A2 is abundantly expressed in the uterine decidua (Roby *et al*. 1993; Gu *et al*. 1994; Lin *et al*. 1997; Orwig *et al*. 1997a,b,c, 1999; Rasmussen *et al*. 1996; 1997; Bany and Cross 2006; Alam et al. 2008), binds to heparin, and although it is structurally similar to PRL it does not appear to signal through the PRL receptor (Rasmussen *et al*. 1996; Wang et al. 2000; Alam *et al*. 2008). PRL8A2 deficiency interferes with pregnancy-dependent adaptations to hypoxia resulting in pregnancy failure (Alam *et al*. 2007).

The purpose of this study was to identify candidate targets for PRL8A2 action within the uteroplacental compartment.

MATERIALS AND METHODS

Animals and tissue preparation

C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed in an environmentally controlled facility, with lights on from 0600-2000 h, and allowed free access to food and water. Timed matings of animals were conducted by placing females with fertile males. The day when a seminal plug was found in the vagina of female mice was designated as day 0.5 of pregnancy. Placentation sites, including uterus, decidual, and placental tissues, were dissected from pregnant animals. Harvested tissues were snap-frozen in liquid nitrogen for RNA and protein analyses. For in situ hybridization analyses,

tissues were frozen in dry ice-cooled heptane. All tissue samples were stored at –80°C until used. Protocols for the above procedures have been described (Deb *et al*. 2006; Ain *et al*. 2006; Alam et al. 2007, 2008). The University of Kansas Medical Center Animal Care and Use Committee approved all procedures for handling and experimentation with rodents.

DNA microarray

Wild type and *Prl8a2* null mice were mated and sacrificed on gestation d7.5. Decidual-placental-embryonic tissues were dissected from implantation sites and homogenized. Total RNA was extracted using TRIzol reagent according to the manufacturer's protocol (Invitrogen, Carlsbard, CA). RNA extractions were pooled to form three groups of three for each group in nuclease-free water at a concentration of 1.0 μg/μl. RNA samples were hybridized to the Affymetrix 430 2.0 DNA microarray chip using the GeneChip® Hybridization Oven 640 (Affymetrix, Santa Clara, CA). Washing and staining of the hybridized chips were conducted using the GeneChip® Fluidics Station 450 (Affymetrix). Chips were scanned using the Affymetrix GeneChip® Scanner 3000 (Affymetrix) with autoloader by the KUMC Biotechnology Support Facility. Hybridization signals were normalized with internal controls. Expression data sets were analyzed using the expression analysis software GeneSpring 7.0 and R statistics software (http://www.r-project.org/) with BioConductor software (http://www.bioconductor.org/) packages. The RMA method from the BioConductor software was used for background correction, normalization, and summarization of the DNA microarray data. Statistical comparisons of expression values between two groups were determined with a moderated t-test. Pathway analysis was performed with AltAnalyze (http://altanalyze.org) and PathVisio (http://www.pathvisio.org).

qRT-PCR

cDNAs were synthesized with total RNA (1 μg) from each sample using M-MLV reverse transcriptase (Invitrogen), diluted five times with water, and subjected to qRT-PCR to quantify mRNA levels of the genes identified from the DNA microarray. Primers were designed using Primer Express 2.0 (Applied Biosystems, Foster City, CA). Primer sequences can be found in Table 1. Real-time PCR amplification of cDNAs was carried out in a reaction mixture (10 μl) containing SYBR GREEN PCR Master Mix (Applied Biosystems) and primers (600 nM each). Amplification and fluorescence detection were carried out using the ABI Prism 7500 Real Time PCR System (Applied Biosystems). Cycling conditions included an initial hold step (95 °C for 10 min) and 40 cycles of a 2-step PCR (92 °C for 15 s, then 60 °C for 1 min), followed by a dissociation step (95 °C for 15 s, 60 °C for 15 s, and then 95 °C for 15 s). qRT-PCR for each query mRNA was validated, including determining amplification efficiencies and co-linearity of the query mRNAs and 18S rRNA. The comparative CT method was used for relative quantification of the amount of mRNA for each sample normalized to 18S RNA.

In situ hybridization

The localization of mRNAs within tissues was performed as described previously (Ain *et al*. 2003; Weimers *et al*. 2003). Cryosections (10 μm) of tissues were prepared and stored at −80°C until used. Plasmids containing cDNAs for mouse *Rimklb, Derl3, Hspa5, and*

Hsp90b1 were used as templates to synthesize sense and antisense digoxigenin labeled riboprobes according to the manufacturer's instructions (Roche Molecular Biochemicals, Indianapolis, IN). Images were captured using a Leica MZFIII stereomicroscope (Leica Microsystems GmbH, Welzlar, Germany) or a Nikon Eclipse 55i microscope (Nikon Instruments Inc., Melville, NY), both equipped with Leica CCD cameras (Leica).

Statistical Analysis

Statistical analyses were performed using the R statistical software (http://www.r-project.org). Statistical comparisons between two means were determined with Student's t-test or Welch's t-test, depending on the homogeneity of variances.

RESULTS

Mice possessing null mutations at the *Prl8a2* locus reproduce within the normal range but unlike wild type mice do not effectively adapt when exposed to hypoxic conditions during pregnancy (Alam *et al*. 2007). This mutant mouse model was used as a tool to identify downstream actions of PRL8A2 signaling. We used DNA microarray analysis to examine the consequences of PRL8A2 deficiency on gene expression at gestation day 7.5. Gestation day 7.5 is associated with robust *Prl8a2* expression and represents a pivotal time point in decidual development and the establishment of the placenta. Probe sets for fifty-seven transcripts exhibited a ≥2 fold change in expression between *Prl8a2* null and wild type tissues. Thirty-four transcripts were significantly downregulated and 23 transcripts were significantly upregulated in the *Prl8a2* null tissues ($P<0.05$, Tables 2 and 3). The complete dataset has been deposited at the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/; accession number GSE60220). Pathway analyses of the transcriptome data were not informative.

Nine genes were confirmed with decreased expression in gestation day 7.5 *Prl8a2* null implantation sites (e.g. *Klk7*, *Rimklb*, *Ccl27*, *Calm4*, *Prl4a1*, *Lipg*, *Sprr2h*, *Htra3*, *Arhgef6*; Table 2, Fig. 1). These include potential decidual, endothelial, and trophoblast cell targets positively regulated by PRL8A2. *Rmklb* transcripts were localized to a subset of cells within the anti-mesometrial decidual compartment of the gestation day 7.5 implantation site (Fig. 2).

Six genes were confirmed with increased expression in gestation day 7.5 *Prl8a2* null implantation sites (*Derl3*, *Herpud1*, *Creld2*, *Hsp90b1*, *Ddit3*, and *Hspa5*; Table 3, Fig. 3). Each of these transcripts encodes proteins that participate in the endoplasmic reticulum (ER) stress response. *Derl3*, *Hspa5*, and *Hsp90b1* transcripts were localized to the anti-mesometrial decidual compartment and were dramatically upregulated in the *Prl8a2* null mouse (Fig. 4).

DISCUSSION

The uterine deciduum is a transitory tissue with the responsibilities of modulating hemochorial placentation. A PRL-related cytokine, PRL8A2, is expressed in a temporally- and spatially-precise pattern within the uterine deciduum during the establishment of

pregnancy. PRL8A2 facilitates pregnancy-associated uterine adaptations to physiological stressors (Alam *et al*. 2007). In this report, we identified potential targets of PRL8A2 action and determined that PRL8A2 acts in a pathway that restrains activation of decidual cell ER stress.

The ER stress response is a cellular process facilitating adaptations to harmful conditions, including cellular damage, and if severe or prolonged leads to cell death (Xu et al. 2005; Yoshida 2007; Zhang and Kaufman 2008). Implantation of the embryo within the uterus elicits many of the hallmarks of an inflammatory response (Finn 1986; Mor et al. 2011). Inflammation leads to cellular injury and activation of ER stress (Zhang and Kaufman 2008). An assortment of pregnancy-related disorders, including early pregnancy loss, preeclampsia, and intrauterine growth restriction are associated with increased decidual cell ER stress responses (Lian et al. 2011; Liu et al. 2011; Loset et al. 2011; Gao et al. 2012). Pregnancy related disease occurs when the harmful inflammatory stimuli are excessive or the decidual cell adaptations are inadequate. Consequently, during the establishment of a successful pregnancy mechanisms must exist to thwart excessive or prolonged ER stress responses, which could compromise embryo survival.

The PRL family is part of a conserved decidual cell adaptation regulatory pathway. PRL and a subgroup of PRL related genes are expressed in decidua cells of the rat, mouse, and human (Orwig et al. 1997c; Telgmann & Gellersen 1998). PRL has a decidua-protective role in the rat and mouse. It inhibits the expression of decidual genes that interfere with the maintenance of pregnancy (Tessier et al. 2001; Bao et al. 2007). Complementary observations are apparent in the human. PRL is produced by decidua and its production is impaired in decidua from patients with recurrent pregnancy loss (Salker et al. 2010; Teklenburg et al. 2010a,b) and correlates with failures in optimal embryo recognition (Brosens & Gellersen 2010; Weimar et al. 2012). PRL8A2, a PRL-related protein, is abundantly expressed in decidua of the mouse and rat, especially within anti-mesometrial decidua (Rasmussen et al. 1997; Orwig et al. 1997a,b,c). In the absence of PRL8A2, transcripts associated with ER stress are significantly upregulated in the anti-mesometrial decidua. Insights into the mechanism of PRL8A2 action are modest. PRL8A2 is a secreted heparin-binding cytokine (Rasmussen et al. 1996; Wang et al. 2000; Alam et al. 2008). Although PRL8A2 is structurally related to PRL, it does not bind the PRL receptor (Rasmussen et al. 1996). Collectively, the results suggest that the decidua-protective functions associated with PRL may extend to other members of the PRL family, including PRL8A2.

DDIT3 is a component of the ER stress response targeted by PRL8A2 and may represent a critical modulator of the integrity of decidual cells. DDIT3 is also known as CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) and is a negative modulator of C/EBP transcriptional regulation (Ron & Habener 1992; Tang & Lane 2000). C/EBPβ is a key transcriptional mediator of the actions of progesterone on decidual cell differentiation (Bagchi et al. 2006; Mantena et al. 2006; Wang et al. 2010; Ramathal et al. 2011). Uterine stromal cells of C/EBPβ null female mice fail to undergo decidualization and are unresponsive to the actions of progesterone (Bagchi et al. 2006; Mantena et al. 2006). Progesterone signaling and C/EBPβ also synergize in the differentiation of primate

endometrial stromal cells to decidual cells (Pohnke et al. 1999; Christian et al. 2002a,b; Kannan et al. 2010). This leads us to speculate that by restraining DDIT3 expression, PRL8A2 effectively facilitates the actions of progesterone and C/EBPβ on decidual cell development and integrity.

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REFERENCES

Ain R, Canham LN, Soares MJ. Gestation stage-dependent intrauterine trophoblast cell invasion in the rat and mouse: novel endocrine phenotype and regulation. Developmental Biology. 2003; 260:176–190. [PubMed: 12885563]

Ain R, Dai G, Dunmore JH, Godwin AR, Soares MJ. A prolactin family paralog regulates reproductive adaptations to a physiological stressor. Proceedings of the National Academy of Sciences USA. 2004; 101:16543–16548.

Ain R, Konno T, Canham LN, Soares MJ. Phenotypic analysis of the rat placenta. Methods in Molecular Medicine. 2006; 121:295–313. [PubMed: 16251750]

Alam SMK, Ain R, Konno T, Ho-Chen JK, Soares MJ. The rat prolactin gene family locus: species-specific gene family expansion. Mammalian Genome. 2006; 17:858–877. [PubMed: 16897344]

Alam SMK, Konno T, Dai G, Lu L, Wang D, Dunmore JH, Godwin AR, Soares MJ. A uterine decidual cell cytokine ensures pregnancy-dependent adaptations to a physiological stressor. Development. 2007; 134:407–415. [PubMed: 17166917]

Alam SM, Konno T, Rumi MA, Dong Y, Weiner CP, Soares MJ. Prolactin family of the guinea pig, *Cavia porcellus*. Endocrinology. 2010; 151:3918–3928. [PubMed: 20534723]

Alam SMK, Konno T, Sahgal N, Lu L, Soares MJ. Decidual cells produce a heparin-binding prolactin family cytokine with putative intrauterine regulatory actions. Journal of Biological Chemistry. 2008; 283:18957–18968. [PubMed: 18467328]

Aplin J. Maternal influences on placental development. Seminars in Cell and Developmental Biology. 2000; 11:115–125. [PubMed: 10873708]

Bagchi MK, Mantena SR, Kannan A, Bagchi IC. Control of uterine cell proliferation and differentiation by C/EBPβ. Cell Cycle. 2006; 5:922–925. [PubMed: 16687914]

Bany BM, Cross JC. Post-implantation mouse conceptuses produce paracrine signals that regulate the uterine endometrium undergoing decidualization. Developmental Biology. 2006; 294:445–456. [PubMed: 16616120]

Bao L, Tessier C, Prigent-Tessier A, Li F, Buzzio OL, Callegari EA, Horseman ND, Gibori G. Decidual prolactin silences the expression of genes detrimental to pregnancy. Endocrinology. 2007; 148:2326–2334. [PubMed: 17255200]

Bittorf T, Jaster R, Soares MJ, Seiler J, Brock J, Friese K, Müller H. Induction of erythroid proliferation and differentiation by a trophoblast-specific cytokine involves activation of the JAK/STAT pathway. Journal of Molecular Endocrinology. 2000; 25:253–262. [PubMed: 11013351]

Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocrine Reviews. 1998; 19:225–268. [PubMed: 9626554]

Brosens JJ, Gellersen B. Something new about early pregnancy: decidual biosensoring and natural embryo selection. Ultrasound in Obstetrics & Gynecology. 2010; 36:1–5. [PubMed: 20582930]

Christian M, Pohnke Y, Kempf R, Gellersen B, Brosens JJ. Functional association of PR and CCAAT/enhancer-binding protein beta isoforms: promoter-dependent cooperation between PR-B and liver-

enriched inhibitory protein, or liver-enriched activatory protein and PR-A in human endometrial stromal cells. Molecular Endocrinology. 2002a; 16:141–154. [PubMed: 11773445]

Christian M, Zhang X, Schneider-Merck T, Unterman TG, Gellersen B, White JO, Brosens JJ. Cyclic AMP-induced forkhead transcription factor, FKHR, cooperates with CCAAT/enhancer-binding protein beta in differentiating human endometrial stromal cells. Journal of Biological Chemistry. 2002b; 277:20825–20832. [PubMed: 11893744]

Cooke NE, Liebhaber SA. Molecular biology of the growth hormone-prolactin gene system. Vitamins and Hormones. 1995; 50:385–459. [PubMed: 7709603]

Deb K, Reese J, Paria BC. Methodologies to study implantation in mice. Methods in Molecular Medicine. 2006; 121:9–34. [PubMed: 16251731]

Dorshkind K, Horseman ND. Anterior pituitary hormones, stress, and immune system homeostasis. Bioessays. 2001; 23:288–294. [PubMed: 11223886]

Faria TN, Deb S, Kwok SC, Talamantes F, Soares MJ. Ontogeny of placental lactogen-I and placental lactogen-II expression in the developing rat placenta. Developmental Biology. 1990; 141:279–291. [PubMed: 2210037]

Finn CA. Implantation, menstruation and inflammation. Biological Reviews. 1986; 61:313–328. [PubMed: 3542071]

Gao H-J, Zhu Y-M, He W-H, Liu A-X, Dong M-Y, Jin M, Sheng J-Z, Huang H-F. Endoplasmic reticulum stress induced by oxidative stress in decidual cells: a possible mechanism of early pregnancy loss. Molecular Biology Reports. 2012; 39:9179–9186. [PubMed: 22733488]

Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. Seminars in Reproductive Medicine. 2007; 25:445–453. [PubMed: 17960529]

Gu Y, Soares MJ, Srivastava RK, Gibori G. Expression of decidual prolactin-related protein in the rat decidua. Endocrinology. 1994; 135:1422–1427. [PubMed: 7925104]

Herington JL, Bany BM. Do molecular signals from the conceptus influence endometrium decidualization in rodents? Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution. 2009; 312:797–816.

Horseman ND, Gregerson KA. Prolactin actions. Journal of Molecular Endocrinology. 2014; 52:R95–R106. [PubMed: 24130130]

Horseman ND, Zhao W, Montecino-Rodriguez E, Tanaka M, Nakashima K, Engle SJ, Smith F, Markoff E, Dorshkind K. Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. EMBO Journal. 1997; 16:6926–6935. [PubMed: 9384572]

Jabbour HN, Critchley HOD. Potential roles of decidual prolactin in early pregnancy. Reproduction. 2001; 121:197–205. [PubMed: 11226044]

Jackson D, Volpert OV, Bouck N, Linzer DI. Stimulation and inhibition of angiogenesis by placental proliferin and proliferin-related protein. Science. 1994; 266:1581–1584. [PubMed: 7527157]

Jung E-M, An B-S, Choi K-C, Jeung E-B. Apoptosis – and endoplasmic reticulum stress-related genes were regulated by estrogen and progesterone in the uteri of calbindin-D9k and –D28k knockout mice. Journal of Cellular Biochemistry. 2012; 113:194–203. [PubMed: 21882229]

Kannan A, Fazleabas AT, Bagchi IC, Bagchi MK. The transcription factor C/EBPβ is a marker of uterine receptivity and expressed at the implantation site in the primate. Reproductive Sciences. 2010; 17:434–443. [PubMed: 20224108]

Lian IA, Loset M, Mundal SB, Fenstad MH, Johnson MP, Eide IP, Bjorge L, Freed KA, Moses EK, Austgulen R. Increased endoplasmic reticulum stress in decidual tissue from pregnancies complicated by fetal growth restriction with and without pre-eclampsia. Placenta. 2011; 32:823–829. [PubMed: 21907405]

Lin J, Linzer DI. Induction of megakaryocyte differentiation by a novel pregnancy-specific hormone. Journal of Biological Chemistry. 1999; 274:21485–21489. [PubMed: 10409714]

Lin J, Poole J, Linzer DIH. Three new members of the mouse prolactin/growth hormone family are homologous to proteins expressed in the rat. Endocrinology. 1997; 138:5541–5549. [PubMed: 9389542]

Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, Chang JL, Kulbokas EJ 3rd, Zody MC, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature. 2005; 438:803–819. [PubMed: 16341006]

Liu A-X, He W-H, Yin L-J, Lv P-P, Zhang Y, Sheng J-Z, Leung PCK, Huang H-F. Sustained endoplasmic stress as a cofactor of oxidative stress in decidual cells from patients with early pregnancy loss. Journal of Clinical Endocrinology and Metabolism. 2011; 96:E493–E497. [PubMed: 21177787]

Loset M, Mundal SB, Johnson MP, Fenstad MH, Freed KA, Lian IA, Eide IP, Bjorge L, Blangero J, Moses EK, Austgulen R. A transcriptional profile of the decidua in preeclampsia. American Journal of Obstetrics & Gynecology. 2011; 204:e1–e27. [PubMed: 20934677]

Mantena SR, Kannan A, Cheon YP, Li Q, Johnson PF, Bagchi IC, Bagchi MK. C/EBPbeta is a critical mediator of steroid hormone-regulated cell proliferation and differentiation in the uterine epithelium and stroma. Proceedings of the National Academy of Sciences USA. 2006; 103:1870–1875.

Mor G, Cardenas I, Abrahams V, Guller S. Inflammation and pregnancy: the role of the immune system at the implantation site. Annals of the New York Academy of Sciences. 2011; 1221:80–87. [PubMed: 21401634]

Orwig KE, Dai G, Rasmussen CA, Soares MJ. Decidual/trophoblast prolactin related protein: characterization of gene structure and cell-specific expression. Endocrinology. 1997a; 138:2491–2500. [PubMed: 9165040]

Orwig KE, Ishimura R, Müller H, Liu B, Soares MJ. Identification and characterization of a mouse homolog for decidual/trophoblast prolactin-related protein. Endocrinology. 1997b; 139:5511–5517. [PubMed: 9389538]

Orwig KE, Rasmussen CA, Soares MJ. Decidual signals in the establishment of pregnancy: the prolactin family. Trophoblast Research. 1997c; 10:329–343.

Orwig KE, Soares MJ. Transcriptional activation of the decidual/trophoblast prolactin-related protein gene. Endocrinology. 1999; 140:4032–4039. [PubMed: 10465273]

Pohnke Y, Kempf R, Gellersen B. CCAAT/enhancer-binding proteins are mediators in the protein kinase A-dependent activation of the decidual prolactin promoter. Journal of Biological Chemistry. 1999; 274:24808–24818. [PubMed: 10455153]

Ramathal C, Wang W, Hunt E, Bagchi IC, Bagchi MK. Transcription factor CCAAT enhancer-binding protein beta (C/EBPbeta) regulates the formation of a unique extracellular matrix that controls uterine stromal differentiation and embryo implantation. Journal of Biological Chemistry. 2011; 286:19860–19871. [PubMed: 21471197]

Rasmussen CA, Orwig KE, Vellucci S, Soares MJ. Dual expression of prolactin-related protein in decidua and trophoblast tissues during pregnancy. Biology of Reproduction. 1997; 55:647–654. [PubMed: 9047009]

Rasmussen CA, Hashizume K, Orwig KE, Xu L, Soares MJ. Decidual prolactin-related protein: heterologous expression and characterization. Endocrinology. 1996; 137:5558–5566. [PubMed: 8940384]

Roby KF, Deb S, Gibori G, Szpirer C, Levan G, Kwok SCM, Soares MJ. Decidual prolactin related protein: identification, molecular cloning and characterization. Journal of Biological Chemistry. 1993; 268:3136–3142. [PubMed: 7679108]

Ron D, Habener JF. CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription. Genes and Development. 1992; 6:439–453. [PubMed: 1547942]

Salker M, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, et al. Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. PLoS One. 2010; 5:e10287. [PubMed: 20422017]

Soares MJ. The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. Reproductive Biology and Endocrinology. 2004; 2:51. [PubMed: 15236651]

Soares MJ, Konno T, Alam SMK. The prolactin family: effectors of pregnancy-specific adaptations. Trends in Endocrinology and Metabolism. 2007; 18:114–121. [PubMed: 17324580]

Tang Q-Q, Lane MD. Role of C/EBP homologous protein (CHOP-10) in the programmed activation of CCAAT/enhancer-binding protein-β during adipogenesis. Proceedings of the National Academy of Sciences USA. 2000; 97:12446–12450.

Teklenburg G, Salker M, Heijnen C, Macklon NS, Brosens JJ. The molecular basis of recurrent pregnancy loss: impaired natural embryo selection. Molecular Human Reproduction. 2010a; 16:886–895. [PubMed: 20847090]

Teklenburg G, Salker M, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, et al. Natural selection of human embryos: decidualizing endometrial stromals cells serve as sensors of embryo quality upon implantation. PLoS One. 2010b; 5:e10258. [PubMed: 20422011]

Telgmann R, Gellersen B. Marker genes of decidualization: activation of the decidual prolactin gene. Human Reproduction Update. 1998; 4:472–479. [PubMed: 10027598]

Tessier C, Prigent-Tessier A, Ferguson-Gottschall S, Gu Y, Gibori G. PRL antiapoptotic effect in the rat decidua involves the PI3K/protein kinase B-mediated inhibition of caspase-3 activity. Endocrinology. 2001; 142:4086–4094. [PubMed: 11517188]

Ushizawa K, Hashizume K. Biology of the prolactin family in bovine placenta. II. Bovine prolactin-related proteins: their expression, structure, and proposed roles. Animal Science Journal. 2006; 77:18–27.

Wang D, Ishimura R, Walia DS, Müller H, Dai G, Hunt JS, Lee NA, Lee JJ, Soares MJ. Eosinophils are cellular targets of the novel uteroplacental heparin-binding cytokine, decidual/trophoblast prolactin-related protein. Journal of Endocrinology. 2000; 167:15–29. [PubMed: 11018749]

Wang W, Li Q, Bagchi IC, Bagchi MK. The CCAAT/enhancer binding protein beta is a critical regulator of steroid-induced mitotic expansion of uterine stromal cells during decidualization. Endocrinology. 2010; 151:3929–3940. [PubMed: 20501671]

Weimar CHE, Kavelaars A, Brosens JJ, Gellersen B, de Vreeden-Elbertse JMT, Heijnen CJ, Macklon NS. Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low-quality human embryos. PLoS One. 2012; 7:e41424. [PubMed: 22848492]

Wiemers DO, Shao L-J, Ain R, Dai G, Soares MJ. The mouse prolactin gene family locus. Endocrinology. 2003; 144:313–325. [PubMed: 12488360]

Xu C, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. Journal of Clinical Investigation. 2005; 115:2656–2664. [PubMed: 16200199]

Yoshida H. ER stress and diseases. FEBS Journal. 2007; 274:630–658. [PubMed: 17288551]

Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. Nature. 2008; 454:455–462. [PubMed: 18650916]

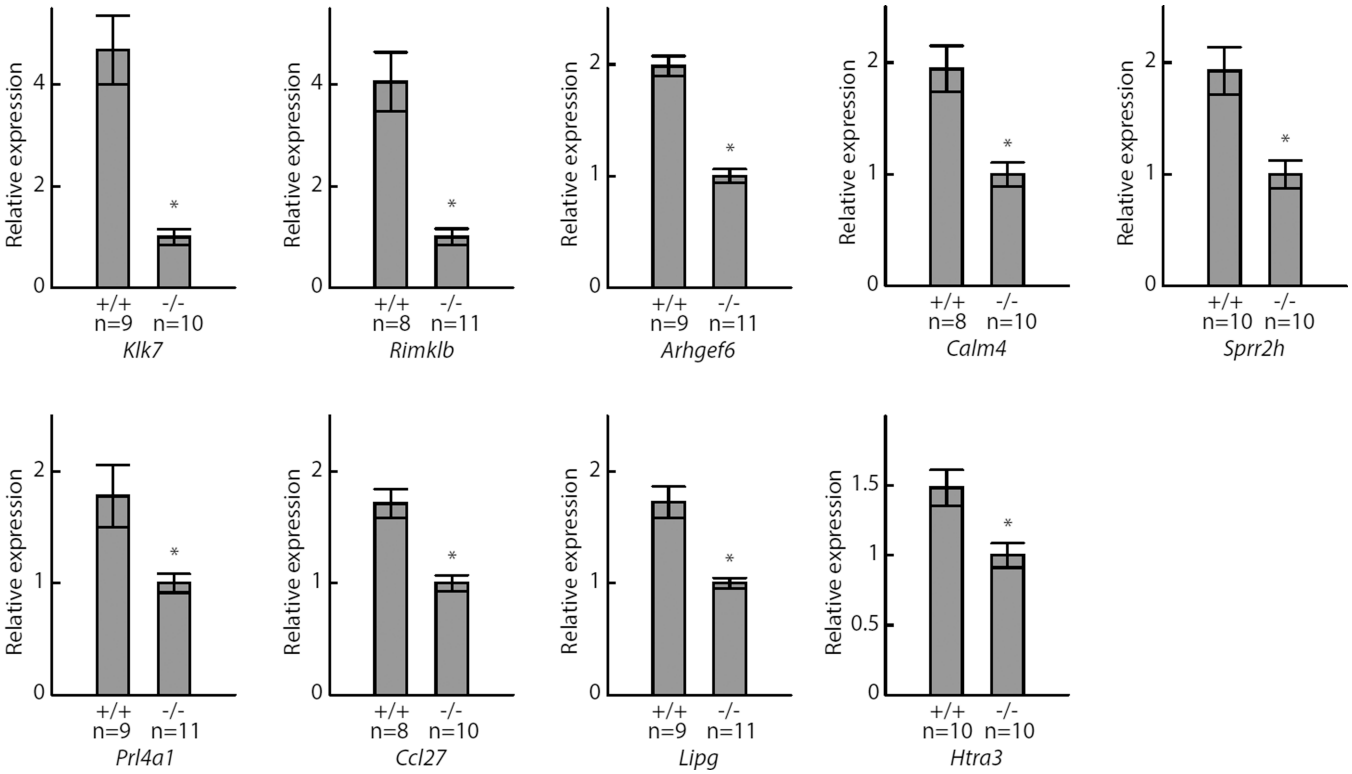

Fig. 1. Validation of expression profiles of genes downregulated in *Prl8a2* null conceptus tissues

Total RNA samples from wild type (+/+) and *Prl8a2* (−/−) null gestation day 7.5 implantation sites were subjected to quantitative RT-PCR (SYBR Green, ΔΔCt method) with transcript specific primer sets. Reactions were performed in duplicate. 18S rRNA served as an internal control. Please note the significant downregulation genes in the *Prl8a2* null tissues. Asterisks denote significant differences between wild type and *Prl8a2* null samples, $P<0.05$.

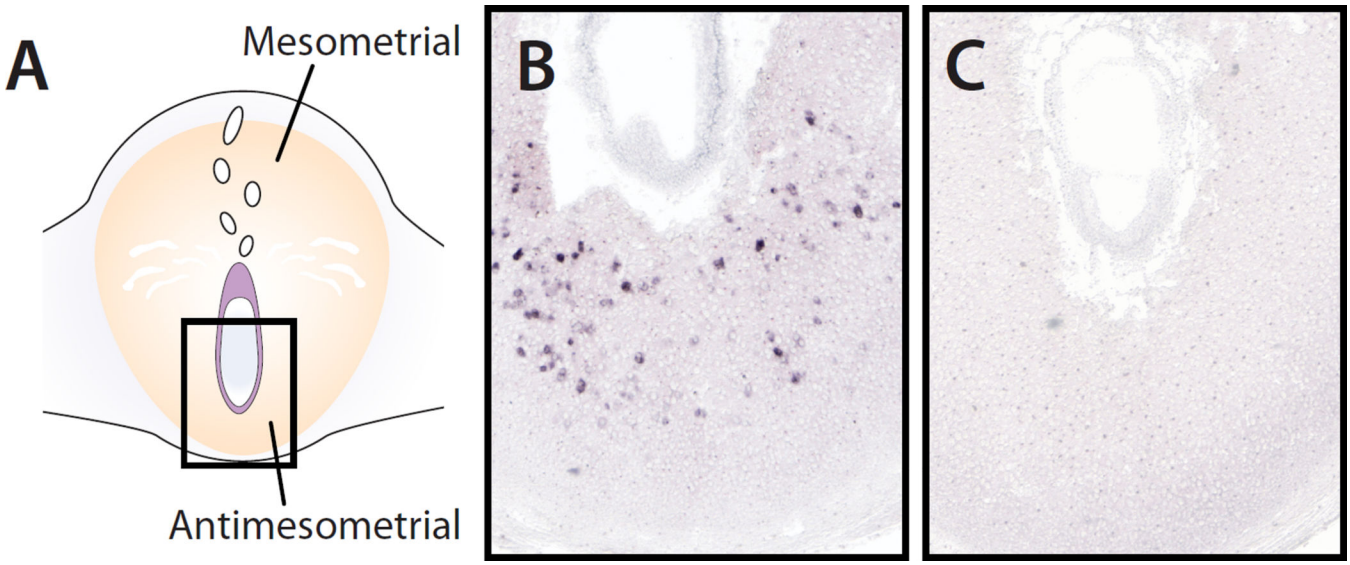

Fig. 2. In situ detection of *Rimklb* mRNA within implantation sites on gestation day 7.5

Panel A, schematic representation of implantation sites from day 7.5 of gestation. The black box indicates the region of images shown in panels B and C. Gestation day 7.5 implantation sites of both wild type (+/+, B) and *Prl8a2* null (−/−, C) mice were subjected to in situ hybridization with a *Rimklb* specific antisense RNA probe. Please note the significant downregulation of *Rimklb* mRNA in the anti-mesometrial compartments of *Prl8a2* null tissues.

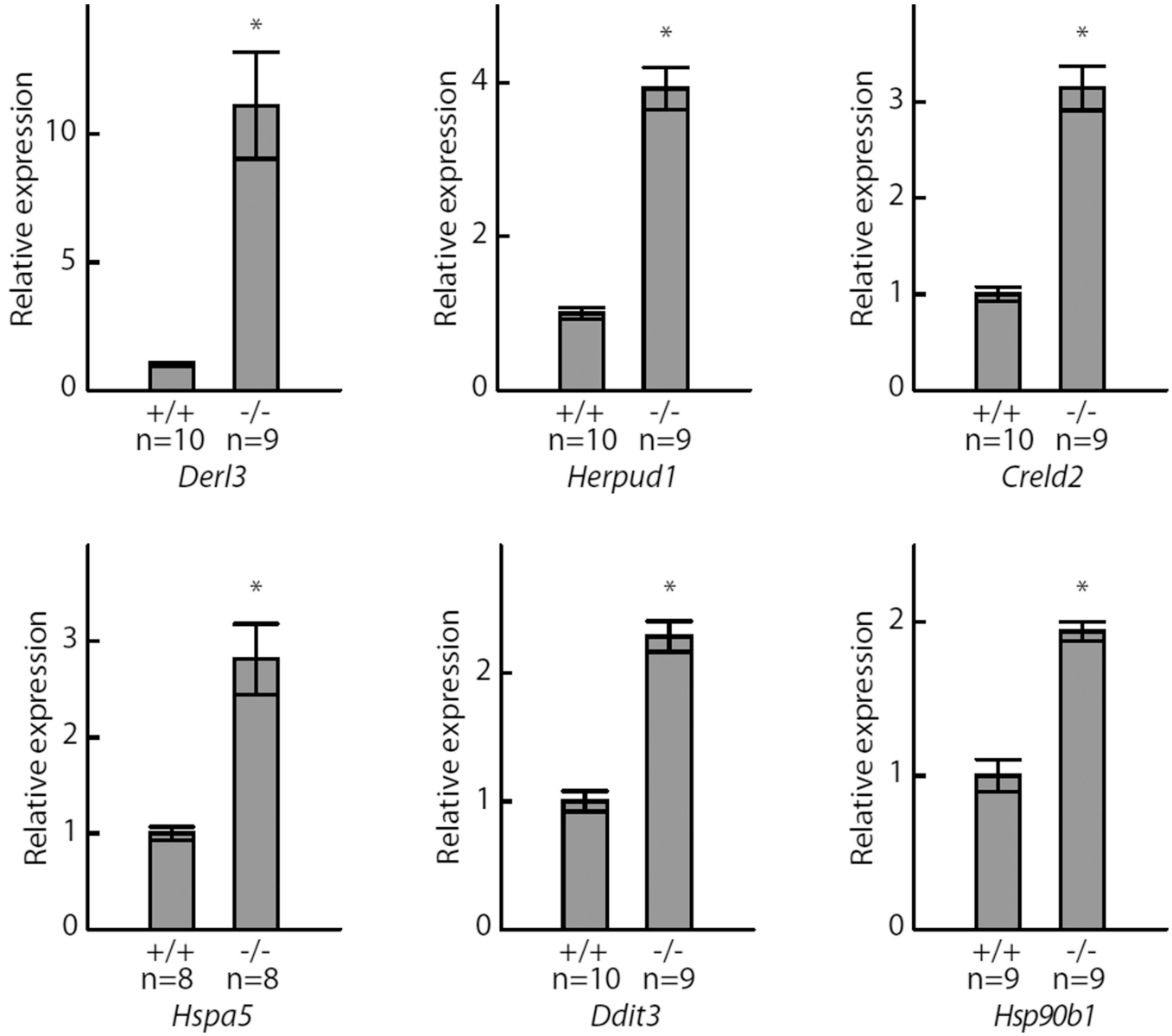

Fig. 3. Validation of expression profiles of genes upregulated in *Prl8a2* null conceptus tissues

Total RNA samples from wild type (+/+) and *Prl8a2* (−/−) null gestation day 7.5 implantation sites were subjected to quantitative RT-PCR (SYBR Green, ΔΔCt method) with transcript specific primer sets. Reactions were performed in duplicate. 18S rRNA served as an internal control. Please note the significant upregulation genes in the *Prl8a2* null tissues. Asterisks denote significant differences between wild type and *Prl8a2* null samples, $P<0.05$.

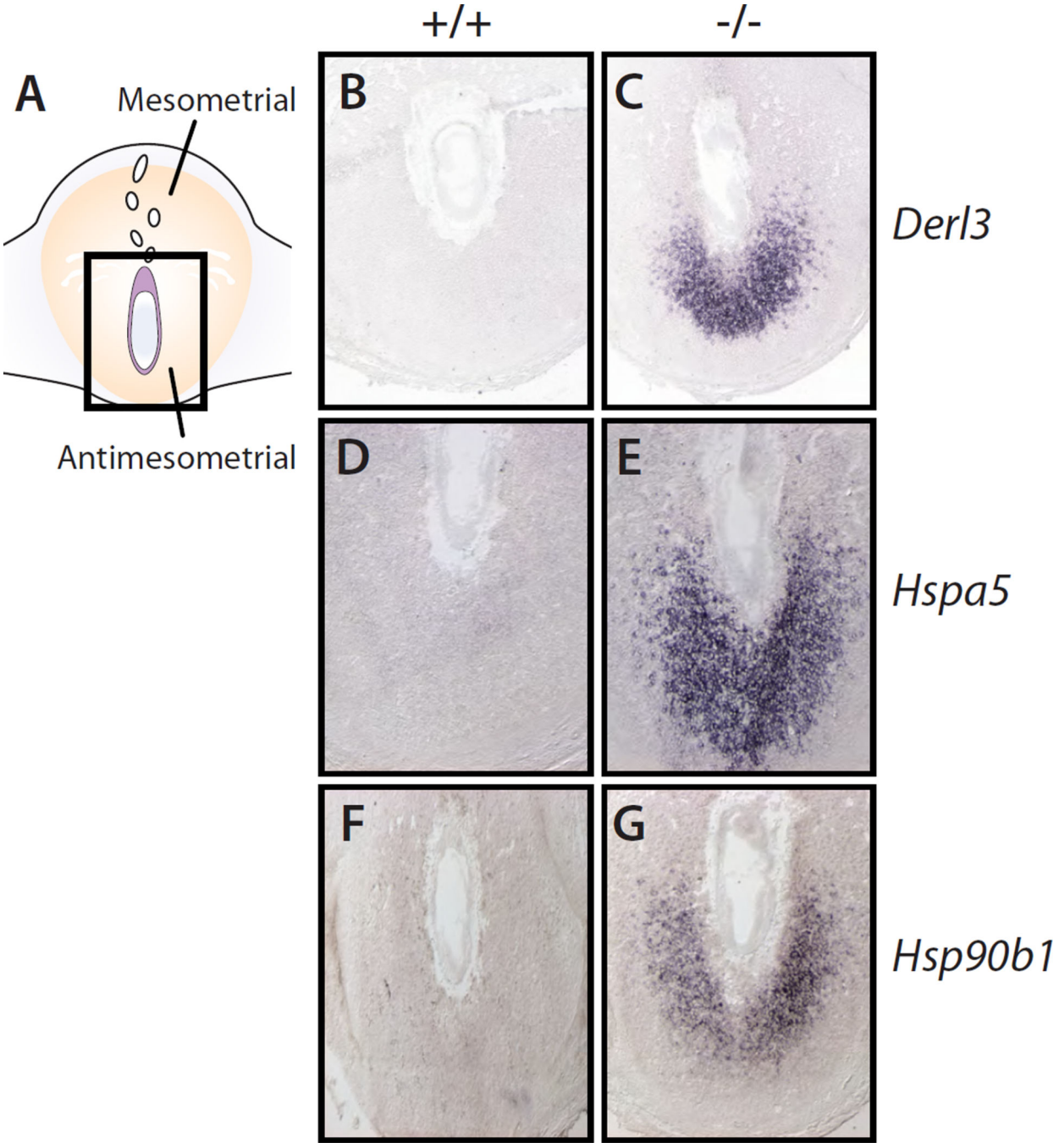

Fig. 4. In situ detection of *Derl3, Hspa5, Hsp90b1* mRNA within implantation sites on gestation day 7.5

Panel A, schematic representation of implantation sites from day 7.5 of gestation. The black box indicates the region of images shown in panels B and C. Serial cryosections from gestation day 7.5 implantation sites of both wild type (+/+, B) and *Prl8a2* null (−/−, C) mice were subjected to in situ hybridization with a gene specific antisense RNA probes. Please note the significant upregulation of *Derl3, Hspa5, and Hsp90b1* in the anti-mesometrial compartment of the *Prl8a2* null uterus.

Table 1

Primer sequences for transcripts regulated by PRL8A2.

Gene	GenBank Accession No.	Forward primer	Reverse primer
Rimklb	NM_027664	TGAAGGCCAAATGTTGTGAA	TCTCCACTGATCCGAAGACC
Klk7	NM_011872	TCTGGCTCCTTTCCCTGATA	GGTGCGAGCCTTCTTTACAT
Ccl27	NM_001048179	GACTGTCACCTCCAGGCTGT	CTTTTCCCTTGGCGTTCTAA
Calm4	NM_020036	CAGAGATGTCTCACGGGTTT	GTTCCTCGACGCTGATATGG
Prl4a1	NM_011165	GGAGACCATAGAGAAGATT	GCAAGAGTTCCAATTCAGA
Lipg	NM_010720	CCAAACCAAAAACCTGCTTG	CGCCGGGAAGTAACAATAGA
Htra3	NM_001042615	CCGATGTGGTGGAGAAGATT	ACTGGACAGCGGCACATT
Sprr2h	NM_011474	ACACTTGGTACTCAAGCTCT	AAGGCTGCTTGCACTGCT
Arhgefb	NM_152801	TCCCCTAAGGCTATCAAAGGA	GGCATATTCTTTTTCAGTGTCC
Derl3	NM_024440	GGGATTCGGCTTCTTTTTCAA	CATGAAAACGAAGTCAGCCTT
Herpud1	NM_022331	CCTCAGCATCCTTTACTTCT	CTCTGTCTGAACGGAAACCA
Creld2	NM_029720	ACTGCACAGACGGCTTCTTC	CTTGGACCAGAGCAGGTCTT
Hsp90b1	NM_011631	ATGGCACAGTGGAAGAGGAC	TGCGTTTAACCCATCCAACT
Ddit3	NM_007837	CACCTATATCTCATCCCCA	GGATGTGCGTGTGACCTCT
Hspa5	NM_0011634	TGCAGCAGGACATCAAGTTC	TTTCTTCTGGGGCAAATGTC
18S	NR_003278	GCAATTATTCCCCATGAACG	GGCCTCACTAAACCATCCAA

Table 2

List of transcripts downregulated (≥2 fold) in implantation sites of the PRL8A2 deficient mouse.

Gene name	Symbol	GenBank Accession No.	Function	Ratio (null/wild type)
Prolactin family 8, subfamily A, member 2	*Prl8a2*	NM_010088	Hormone/cytokine	0.00
Ribosomal modification protein rimK-like family member B	*Rimklb*	AV271892	ATP binding, amino acid ligase activity, glutathione synthase activity	0.10
Kallikrein related peptidase 7	*Klk7*	BB283507	Trypsin-like serine protease	0.22
Midline 1	*Mid1*	BG073178	Microtubule associated	0.25
Chemokine (C-C motif) ligand 27a	*Ccl27a*	NM_011336	Chemokine, leukocyte recruitment	0.28
Predicted gene, EG633640	*EG633640*	BG068672	Unknown	0.28
Proline rich 9	*A030004J04Rik*	BB150166	Unknown	0.30
Orosomucoid 1	*Orm1*	BE628912	Transporter activity/immune-related	0.30
Calmodulin 4	*Calm4*	NM_020036	Calcium signaling	0.35
Porcupine homolog	*Porcn*	AB036749	Wnt signaling pathway	0.35
Predicted gene 9780	*MGI:3710532*	AI508243	Unknown	0.36
Expressed sequence tag	---	AV271189	Unknown	0.40
Orosomucoid 2	*Orm2*	NM_011016	Transporter activity/immune-related	0.40
Cellular retinoic acid binding protein	*Crabp2*	BC018397	Retinoic acid transport	0.40
Expressed sequence tag	---	BG083989	Unknown	0.40
Lipase, endothelial	*Lipg*	BC020991	Lipid metabolism	0.41
A disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 5	*Adamts5*	BB658835	Integrin-mediated signaling, metalloproteinase	0.41
Prolactin family 4, subfamily A, member 1	*Prl4a1*	NM_011165	Hormone/cytokine	0.42
HtrA serine peptidase 3	*Htra3*	NM_030127	Serine protease	0.43
Expressed sequence tag	---	BM115786	Unknown	0.43
Small proline-rich protein 2H	*Sprr2h*	NM_011474	Epithelial barrier	0.43
Neuromedin U	*Nmu*	NM_019515	Neuropeptide signaling	0.43
PR domain containing 16	*Prdm16*	BB356786	Transcription coregulator	0.44
Endogenous retroviral sequence 3	*Erv3*	AK005451	Unknown	0.44
Carcinoembryonic antigen-related cell adhesion molecule 9	*Ceacam9*	NM_011927	Immune-related	0.44
Expressed sequence tag	---	AU067772	Unknown	0.45
Expressed sequence tag	---	BB712583	Unknown	0.45
Guanylate cyclase activator 2a	*Guca2a*	NM_008190	Activator of guanylate cyclase	0.45
Calmodulin-like 3	*Calml3*	NM_027416	Calcium signaling	0.46
Shisa homolog 3	*Shisa3*	AV277495	FGF and WNT signaling	0.46
Histidine ammonia lyase	*Hal*	L07645	Histidine catabolism	0.46
LRRN4 C-terminal like	*Lrrn4cl*	BB783125	Unknown	0.46

Gene name	Symbol	GenBank Accession No.	Function	Ratio (null/wild type)
Predicted gene 9746	*D14Ertd449e*	BG072279	Unknown	0.48
Rac/Cdc42 guanine nucleotide exchange factor 6	*Arhgef6*	NM_152801	Rho GTPase guanine nucleotide exchange factor	0.50

Table 3

List of transcripts upregulated (≥2 fold) in implantation sites of the PRL8A2 deficient mouse.

Gene name	Symbol	GenBank Accession No.	Function	Ratio (null/wild type)
Platelet-derived growth factor receptor-like	*Pdgfrl*	Ak004179	Similarity to ligand binding domain of Pdgfr	11.48
Der1-like domain family, member 3	*Derl3*	AK007348	Endoplasmic reticulum stress response	6.29
Expressed sequence tag	---	AK007420	Unknown	4.65
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	*Smarcb1*	BB820473	Chromatin remodeling	3.84
Nicotinamide nucleotide transhydrogenase	*Nnt*	BB205930	Mitochondrial enzyme, production of NADPH	3.20
CDC14 cell division cycle 14 homolog B	*Cdc14b*	AK013228	Protein tyrosine phosphatase, cell cycle control	3.10
Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	*Herpud1*	NM_022331	Endoplasmic reticulum stress response	3.00
EF-hand calcium binding domain 7	*Efcab7*	BC020077	Calcium binding	2.91
Expressed sequence tag	---	BB629079	Unknown	2.84
Cysteine-rich with EGF-like domains 2	*Creld2*	AK017880	Endoplasmic reticulum stress response/calcium binding	2.75
Expressed sequence tag	---	AK007420	Unknown	2.69
Rab9 effector protein with kelch motifs	*Rabepk*	AA217054	Facilitates transport of mannose 6-phosphate receptor	2.60
Arrestin domain containing 3	*Arrdc3*	AW556597	Associated with G protein-coupled receptor signaling	2.44
Gamma-aminobutyric acid A receptor, subunit alpha 2	*Gabra2*	BB339336	GABA-A receptor, ligand-gated chloride channel	2.39
Immunoglobulin kappa constant	*Igkc*	AV057155	Light chain of antibodies	2.38
Hemochromatosis	*Hfe*	AJ306425	Iron transport	2.38
Predicted gene, EG665955	*EG665955*	BF580235	Unknown	2.23
DNA segment, Chr 13, ERATO Doi 666, expressed	*D13Ertd666e*	BG070282	Unknown	2.17
DNA-damage inducible transcript 3	*Ddit3*	NM_007837	Endoplasmic reticulum stress response	2.13
Heat shock protein 5	*Hspa5*	AJ002387	Endoplasmic reticulum stress response	2.07
Uroplakin 1B	*Upk1b*	BB427704	Member of the tetraspanin family, signal transduction	2.06
Expressed sequence tag	---	BG862223	Unknown	2.04
Heat shock protein 90, beta, member 1	*Hsp90b1*	NM_011631	Endoplasmic reticulum stress response	2.00