

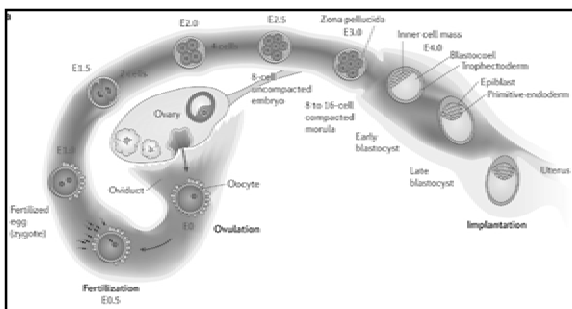
Immunophilin FKBP52 deficiency confers uterine-specific resistance to progesterone signaling during pregnancy

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Master regulators: Estrogen and Progesterone

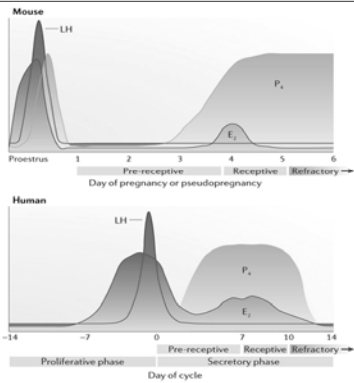
1. Prostaglandin-nuclear receptor-angiogenic signaling axis (cPLA2 α /Cox2/PPAR δ /Vegf/Flk1/Ang/Tie)
2. miRNA regulation of genes during implantation
3. Cytokine-growth factor-homeobox-morphogen signaling axis (Lif/Hb-Egf/Hoxa10/Msx1/lhh/Bmp/Wnt)
4. Ligand-receptor signaling with endocannabinoids (Anandamide-CB1)
5. Immunophilin/cochaperone signaling with PR (Fkbp52-PR)

Events of Early Pregnancy

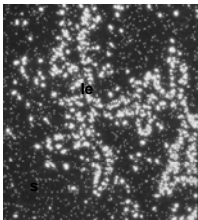


Nat Rev Genet, 7: 185, 2006

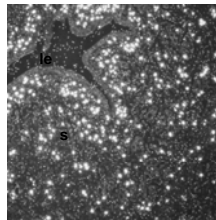
Coordinated effects of E_2 & P_4 determine the window for uterine receptivity



Proliferation patterns on days 1 and 4 of pregnancy

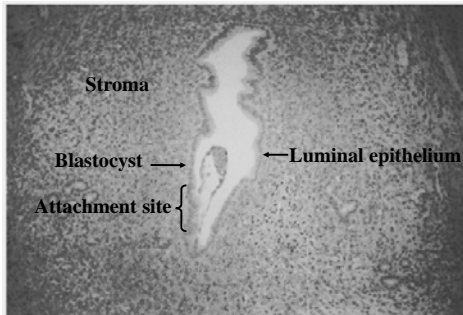


Under estrogen influence
Luminal epithelium proliferation
le, luminal epithelium
S, stroma



Under progesterone influence
Luminal epithelium differentiation
Stromal proliferation

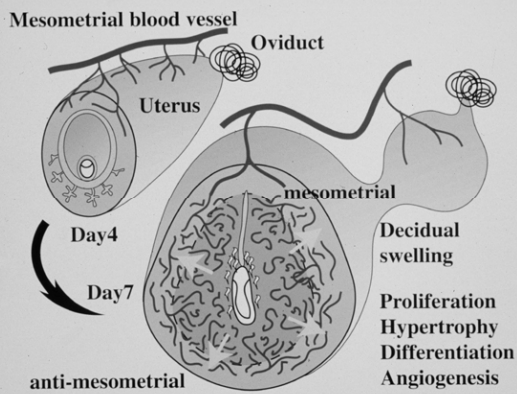
Attachment reaction between the blastocyst and uterine luminal epithelium occurs on day 4 midnight



Localized endometrial vascular permeability occurs with the onset of the attachment reaction



D4 night D8
Blue dye reaction



Embryo implantation is a powerful model system to study:

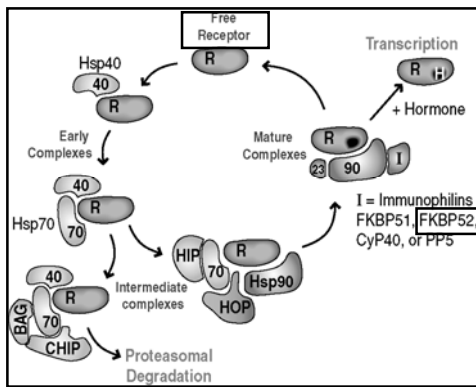
1. **Paracrine/juxtacrine interactions**
 - a. Trophoblast - uterine luminal epithelium interaction (Epithelial-epithelial)
 - b. Epithelial-mesenchymal interaction (luminal epithelium-stroma)
2. **Vascular permeability and angiogenesis**
3. **Regulated growth** (proliferation and differentiation):
decidualization

Progesterone: the “pregnancy” hormone

- Absolutely required for pregnancy success in most mammals studied
- Ovulation
- Fertilization
- Uterine receptivity
- Decidual response
- Maintenance of uterine quiescence until parturition

- Works via its nuclear steroid hormone receptor, *progesterone receptor* (PR)

Steroid Hormone-Receptor Complex



Cochaperones

- Tetratricopeptide repeat domain (TPR) binds Hsp90
- Two members of FK506 binding family of immunophilins: FKBP52/FKBP4 and FKBP51/FKBP5
- Cyclosporin-binding immunophilin: Cyclophilin 40 (Cyp40) and protein phosphatase PP5
- FKBP52 and FKBP51: peptidylprolyl cis/trans isomerase activity domain and catalyses conformational changes in protein substrates
- FKBP52 and FKBP51 have different functions toward steroid receptors

Hoxa10 null mice have reduced uterine P4 responsiveness

- Proteomic analysis of WT and *Hoxa10*^{-/-} uterine stromal cells
- Identified FKBP52 as a protein downregulated in *Hoxa10*^{-/-} stromal cells

FKBP52 is a cochaperone for PR

Mol Endo 19:683, 2005

PR null mice

- Complete failure of ovulation, fertilization, and implantation (Mulac-Jericevic et. al, *Science* 289: 1751, 2000)
- Precludes using this mouse model to examine the role of progesterone (P₄) in early pregnancy events

Fkbp52 null mice

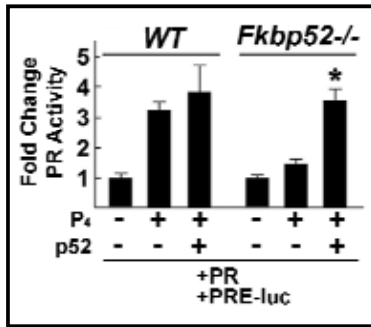
- Males have decreased androgen receptor responsiveness (Cheung-Flynn et. al, *Mol Endo* 19: 1654, 2005)
- Reason for female infertility remained unknown

Infertile Phenotype of *Fkbp52/C57* null females

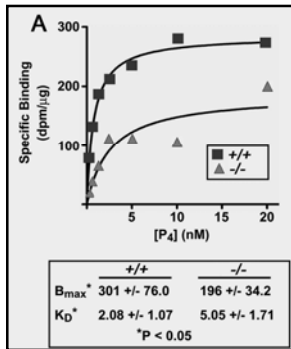
Genotypes (males x females)	No. of breeders	No. of litters	Average litter size
+/+ x ++	28	81	6.9 ± 3.0
+/- x +/-	63	195	6.7 ± 3.0
+/+ x -/-	53	0	0

Fkbp52 null (-/-) females cohabitated with wild-type (+/+) fertile males failed to produce any offspring, while the average litter sizes from *Fkbp52*+/+ and *Fkbp52*+/- females were comparable (mean ± SD).

Modulation of PR activation by FKBP52



P4 binding is decreased in uterine cytosol missing FKBP52

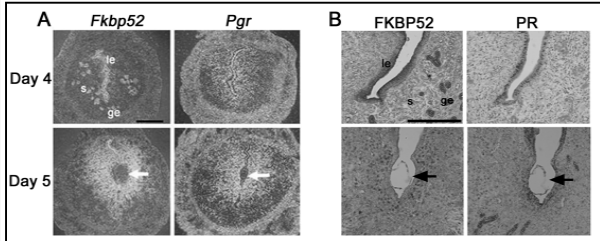


Examine various stages of early pregnancy in *Fkbp52*^{-/-} females

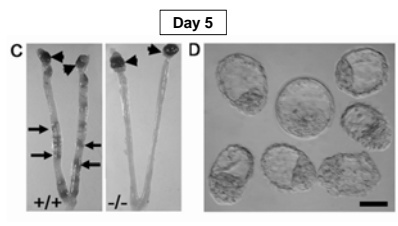
- Ovulation
- Fertilization
- Implantation

- Ovulation and fertilization are comparable to WT females
- What is the reason for infertility in *Fkbp52*^{-/-} females?

FKBP52 and PR expression overlaps in periimplantation uteri



Implantation fails in *Fkbp52/C57* null mice as examined on day 5 of pregnancy

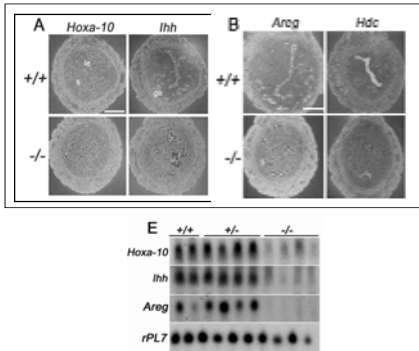


Embryo transfer experiments show that wild-type blastocysts fail to implant in *Fkbp52/C57* null females

Genotype		No. of blastocysts transferred	No. of recipients	No. of mice with IS	No. of IS (%)	No. of IS	No. of blastocysts recovered
Blastocysts	Recipients						
+/+	+/+ or +/-	178	10	8 (80%)	74 (41%)	7.3 ±4.0*	2 ^a
+/+	-/-	79	4	0 (0%)	0	0	14 ^b

^a2 mice without IS yielded one blastocyst each
^b14 blastocysts were recovered from 3 recipients
 *mean ±SD

Decreased expression of PR-regulated genes in uteri on day 4 of pregnancy in *Fkbp52* null females



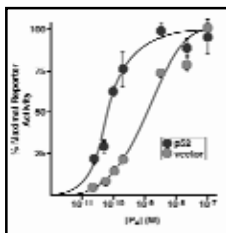
FKBP52 is critical to uterine receptivity and implantation in mice

***C57Fkbp52*/null mice:**

- Normal ovulation
- Implantation failure
 - Reduced P4 responsiveness
 - Exaggerated estrogenic influence

PNAS 102: 14326, 2005

Ovulation is not impaired suggesting uterine responsiveness to PR signaling differs from ovarian responsiveness



Fkbp52^{-/-} MEFs

Locally high P4 levels in the ovary may enhance basal PR activity sufficient for ovulation

Determine whether exogenous P₄ treatment rescues the infertile phenotype of *Fkbp52* null mice

- Use silastic implants placed on day 2 of pregnancy (day 1 = vaginal plug)
- Examine implantation on day 5 of pregnancy

P₄ fails to rescue implantation of transferred WT blastocysts in C57 *Fkbp52* null females

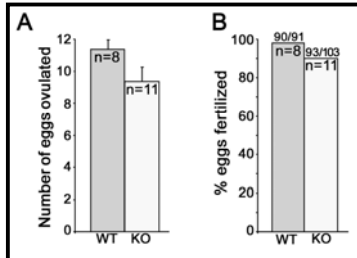
Genotype		No. of blastocysts transferred	No. of Recipients	No. of IS (%)	No. of embryos recovered
Blastocyst	Recipient				
WT	WT	82	6	49/82 (59%)	n/a
WT	KO + P ₄	83	5	6/83 (7%)	45

P₄ supplementation fails to restore expression of P₄-regulated genes (*Hoxa-10*, *Ihh* and *Areg*) critical for uterine receptivity.

Rescue implantation failure in *Fkbp52* null uteri on a different genetic background

- CD1: outbred, more robust reproduction
- Changed background of mice to CD1 background to F10 generation
- Can P₄ supplementation rescue implantation failure in *Fkbp52/CD1* null mice?
 - First characterize reproductive phenotype

***Fkbp52*/CD1 null females have normal ovulation and fertilization**

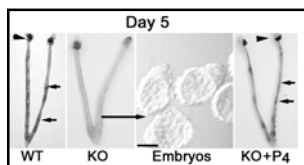


Implantation fails in CD1 *Fkbp52* null females

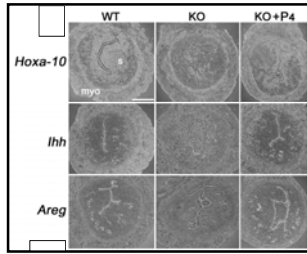
Genotype	Day of Pregnancy	No. of Mice	No. of Mice with IS (%)	No. of IS	No. of embryos recovered
WT	5	16	16 (100%)	12.2 ± 0.3	n/a
KO	5	14	2 (14%)	7.0 ± 0.3	66

P4 implant rescues implantation failure in CD1 *Fkbp52* null females

Genotype	Day of Pregnancy	No. of Mice	No. of Mice with IS (%)	No. of IS	No. of embryos recovered
WT	5	16	16 (100%)	12.2 ± 0.3	n/a
KO	5	14	2 (14%)	7.0 ± 0.3	66
KO+P4	5	11	9 (81%)	10.3 ± 0.5	17

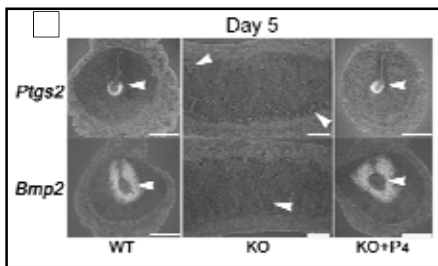


P4 supplementation restores P4-regulated gene expression in CD1 *Fkbp52* null uteri on day 4



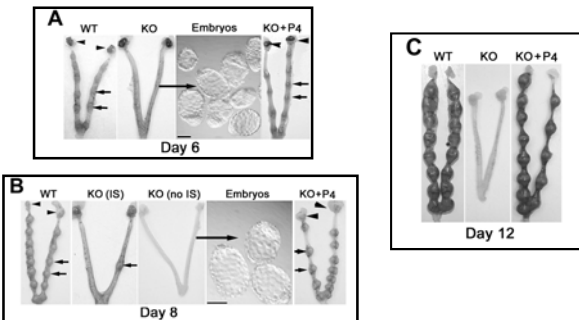
P4-regulated genes

P4 supplementation restores implantation-specific gene expression in CD1 *Fkbp52* null uteri on day 5

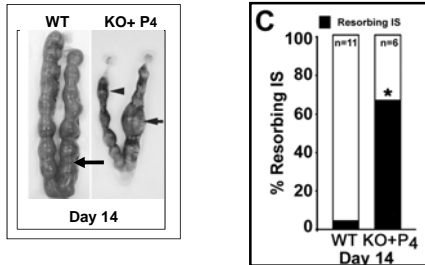


Arrow heads indicate the location of blastocysts

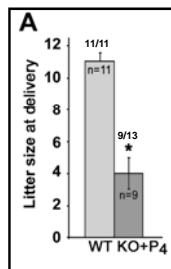
P4 supplementation rescues pregnancy through day 12 in *Fkbp52/CD1* null females



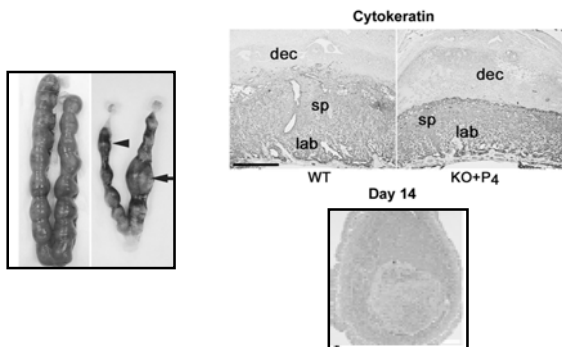
P4 supplementation via implants fails to restore normal pregnancy examined on day 14 in CD1 *Fkbp52*^{-/-} females



P4 supplementation via implant cannot restore normal pregnancy to term



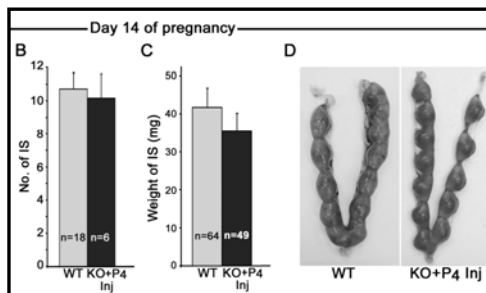
Defective placental development in P4-treated CD1 *Fkbp52*^{-/-} females



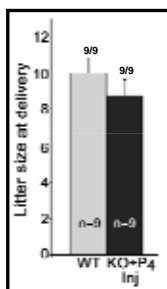
Does daily P4 injections rescue pregnancy to full-term in CD1 *Fkbp52*^{-/-} females?

- Inject mice with P4 (2 mg/0.1ml oil/mouse) sc from day 2 of pregnancy until sacrifice (D14) or D17 to allow labor to occur

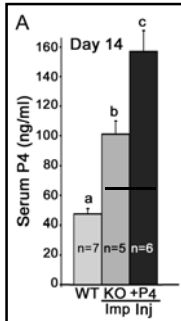
Daily P4 injection rescues pregnancy through day 14



Daily P4 injection restores full-term pregnancy in CD1 *Fkbp52*^{-/-} females

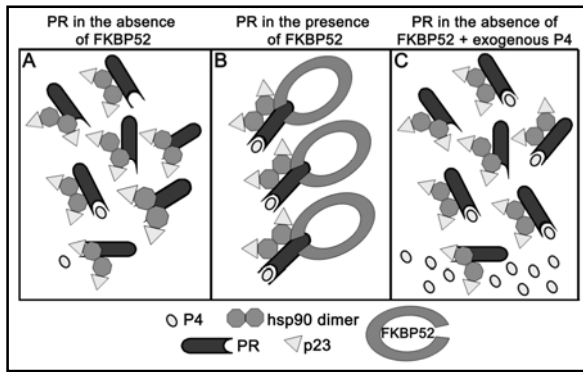


Serum P4 levels in *Fkbp52* null mice treated with implant or daily injection



Day 14 WT: 45 ng/ml
 Day 14 KO Imp: 100 ng/ml
 Day 14 KO Inj: 156 ng/ml

Proposed model for pregnancy rescue in the presence of high P4 levels



Conclusions

- The major reproductive phenotype in mice missing *Fkbp52* is unique to uterine deficiency in the context of implantation
- P4-PR-FKBP52 signaling is a function of genetic makeup
- More robust P4-PR signaling is required for pregnancy maintenance than is required for uterine receptivity and implantation in *Fkbp52* null mice
- Blastocyst's presence determines the ability of P4 to rescue decidualization
- FKBP52 may have a unique PR-independent role during pregnancy (placenta/embryo?)

JCI 117: 1824, 2007

Clinical Implications

- Infertility and P4 resistance

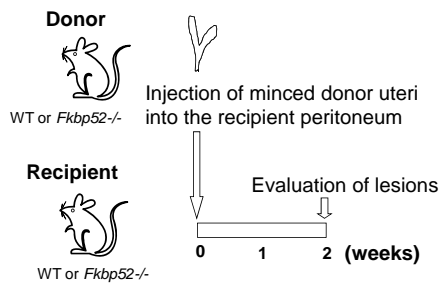
Exogenous P4 treatment results in a decrease in miscarriages in women with history of pregnancy loss

- Endometriosis and P4 resistance

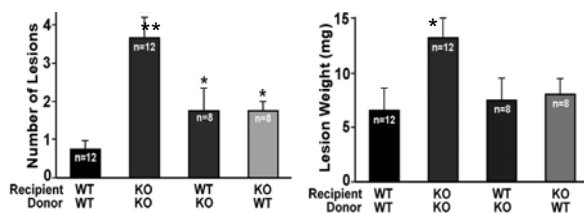
P4 resistance promotes this disease process

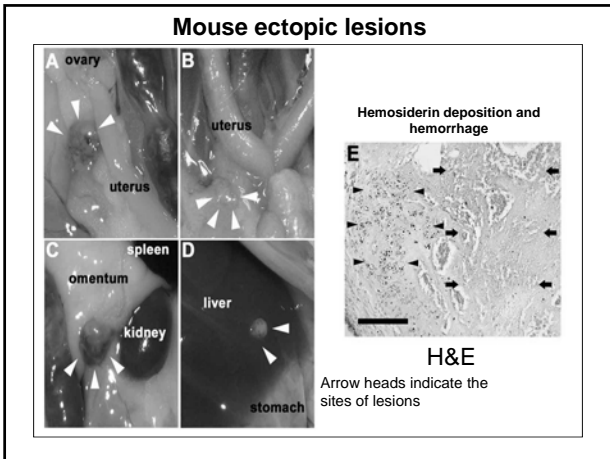
Endometriosis model using *Fkbp52*^{-/-} mice

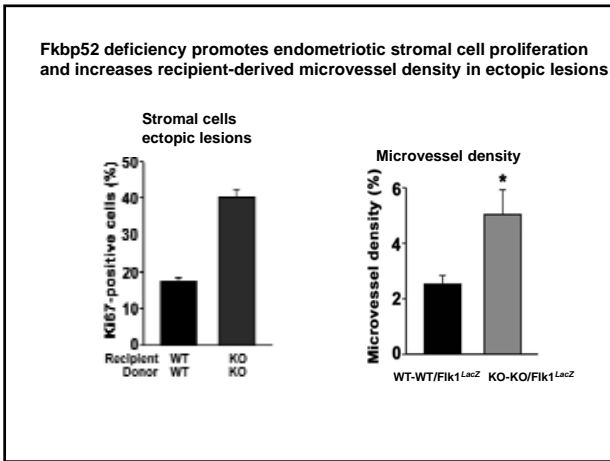
CD1 female mice (7-10 wk old)
Estrous cycle: Diestrus

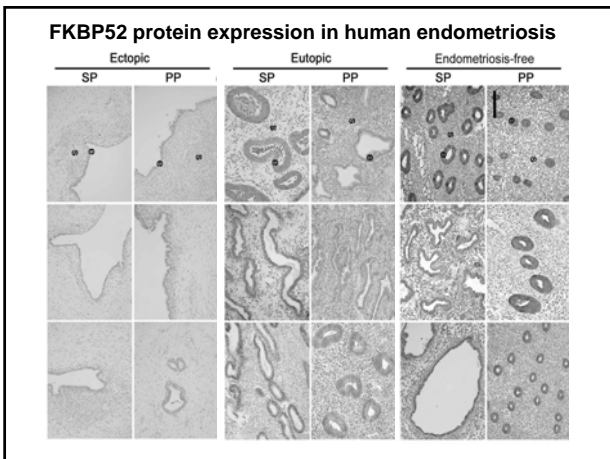


Fkbp52 deficiency promotes endometriotic lesions









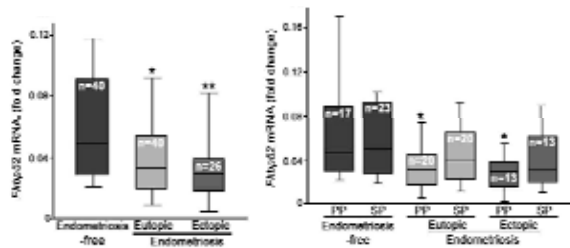
FKBP52 deficiency promotes endometriosis
(AM J Path 173:1747-1757, 2008)

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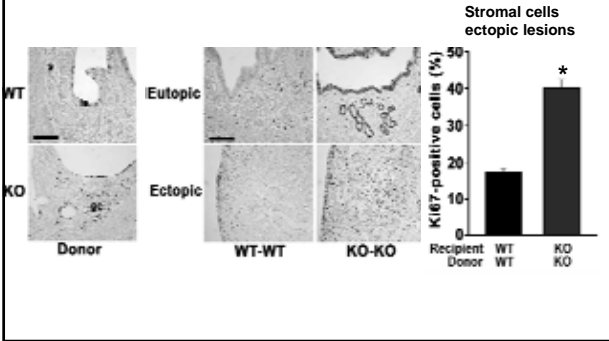
NRSA/NIDA, NICHD, NIDA

Fkbp52 mRNA expression in human endometriosis



PP: Proliferative Phase
SP: Secretory Phase

Fkbp52 deficiency promotes endometriotic stromal cell proliferation



P4 supplementation fails to restore expression of P4-regulated genes critical for uterine receptivity

Hoxa-10, Ihh and Areg

P4 supplementation restores P4- and estrogen-target gene expression in CD1 Fkbp52 null uteri on day 4

