

UTERINE SELECTION OF HUMAN EMBRYOS AT IMPLANTATION

Jan J. Brosens^{a,1}, Madhuri S. Salker^{a,b}, Gijs Teklenburg^c, Jaya Nautiyal^b, Scarlett Salter^a, Emma S. Lucas^a, Jennifer H. Steel^b, Mark Christian^a, Yi-Wah Chan^d, Carolien M. Boomsma^c, Jonathan D. Moore^d, Geraldine M. Hartshorne^a, Sandra Šučurović^e, Biserka Mulac-Jericević^e, Cobi J. Heijnen^c, Siobhan Quenby^a, Marian J Groot Koerkamp^f, Frank C.P. Holstege^f, Anatoly Shmygol^a, Nick S. Macklon^{c,g}

Supplementary Figures and Data

Supplementary Figures

Fig. S1. Gene Ontology (GO) network was generated using GOTermFinder for genes significantly altered ($P < 0.01$) in mouse uteri 24 h after exposure to culture medium conditioned by developmentally competent human embryos (DCEs).

Fig. S2. Gene Ontology (GO) network was generated using GOTermFinder for genes significantly regulated ($P < 0.01$) in mouse uteri 24 h after exposure to conditioned media from developmentally impaired human embryos (DIEs).

Fig. S3. Total protein lysates from snap-frozen murine uterine samples obtained 24 h after exposure to pooled unconditioned embryo culture medium (ECM) or conditioned media from DIEs or DCEs were subjected to Western blot analysis and immunoprobed for the indicated proteins. A representative result of three biological repeat experiments is shown. β -actin served as a loading control.

Fig. S4. Mining of the murine embryonic transcriptome throughout pre-implantation development for genes involved in trypsinogen activation and trypsin inhibition. The *in silico* analysis was performed on publicly available datasets from the Gene Expression Omnibus¹, accession number GSD813². Transcript levels of *Prss28*, coding for implantation serine proteinase 1 (IPS1), increase during the later stages of pre-implantation embryo development in the mouse. By contrast, mRNA levels for enterokinase (*Tmprss15*), the enzyme that converts the proenzyme trypsinogen to trypsin, remained unchanged. This is also the case for *Ambp*, which encodes a protein that is cleaved into trypstatin and bikunin, the light chain of Inter- α -trypsin inhibitor (α I). Trypstatin is a trypsin inhibitor and α I inhibits trypsin, plasmin, and lysosomal granulocytic elastase (<http://www.uniprot.org/uniprot/P02760>). While a single gene codes the light chain of α I (*Ambp*), the heavy chain consists of different polypeptides encoded by at least 4 genes (*Itih1-4*)³. Modest fluctuations in embryonic *Itih1*, *Itih2*, and *Itih3* transcript levels were noted throughout pre-

implantation development in mice. Different letters above the error bars indicate that those groups are significantly ($P < 0.05$) different from each other.

Fig. S5. Mining of human embryonic transcriptome throughout pre-implantation development for genes involved in trypsinogen activation and trypsin inhibition. The *in silico* analysis was performed on publicly available datasets from the Gene Expression Omnibus¹, accession number GSD3959⁴. Pre-implantation development of human embryos was not associated with significant changes in expression of *PRSSI*, the primary trypsinogen gene. However, the later stages of development were characterized by marked induction and inhibition of *TMPRSS15* and *AMBP*, respectively. *TMPRSS15* codes the enzyme that converts the proenzyme trypsinogen to trypsin; whereas *AMBP* codes trypstatin and bikunin, the light chain of I α I. This expression profile in human embryos differs from that their murine counterparts. Embryonic transcript levels of *ITIH2*, *ITIH3*, and *ITIH4*, but not *ITIH1*, declined more gradually upon progression to the blastocyst stage. Different letters above the error bars indicate that those groups are significantly ($P < 0.05$) different from each other.

Fig. S6. Human embryos and trypsin induce $[Ca^{2+}]_i$ oscillations in Ishikawa cells via a common signal transduction pathway. (A) Application of 10 nM trypsin elicited $[Ca^{2+}]_i$ responses similar to those induced by conditioned embryo culture medium (ECM). (B) pre-treatment of cells with ECM greatly reduced their responses to trypsin. (C) trypsin inhibitor diminished the response to ECM. (D) Averaged values of $[Ca^{2+}]_i$ responses (expressed as area under the curve) to trypsin (n=8), ECM followed by trypsin (n=8), and ECM with trypsin inhibitor (n=8). Different letters above the error bars indicate that those groups are significantly different from each other at $P < 0.01$.

Supplementary Tables

Table S1: Patient demographics and embryo characteristics.

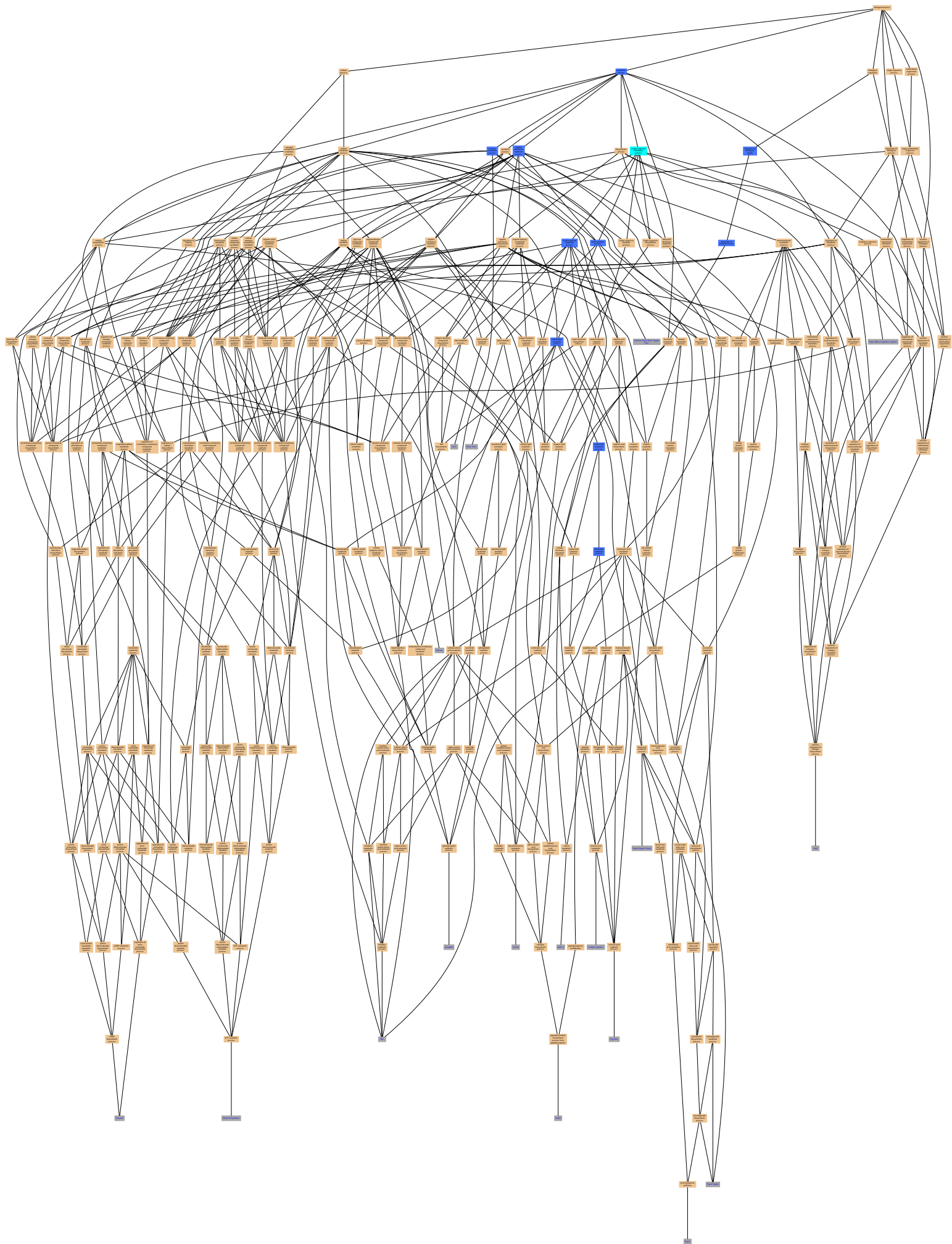
Table S2: Genes regulated in decidualizing human endometrial stromal cells upon incubation of culture medium from developmentally impaired human embryos (DIE).

Table S3: Significantly Regulated Transcripts in Mouse Uterus in Response to Developmentally Competent Embryo Conditioned Media

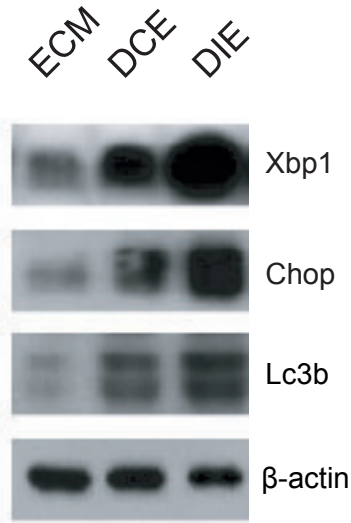
Supplementary References

- 1 Edgar, R., Domrachev, M. & Lash, A. E. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* **30**, 207-210 (2002).
- 2 Xie, D. *et al.* Rewirable gene regulatory networks in the preimplantation embryonic development of three mammalian species. *Genome Res* **20**, 804-815, doi:10.1101/gr.100594.109 (2010).
- 3 Zhuo, L., Hascall, V. C. & Kimata, K. Inter-alpha-trypsin inhibitor, a covalent protein-glycosaminoglycan-protein complex. *J Biol Chem* **279**, 38079-38082, doi:10.1074/jbc.R300039200 (2004).
- 4 Zeng, F., Baldwin, D. A. & Schultz, R. M. Transcript profiling during preimplantation mouse development. *Developmental biology* **272**, 483-496, doi:10.1016/j.ydbio.2004.05.018 (2004).

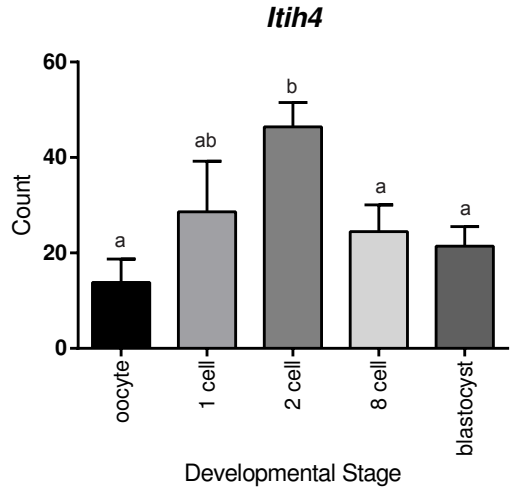
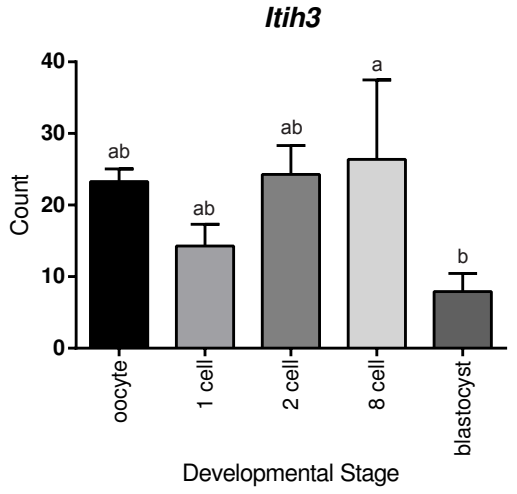
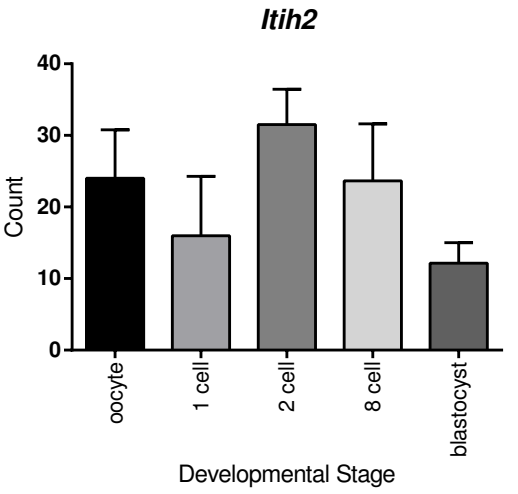
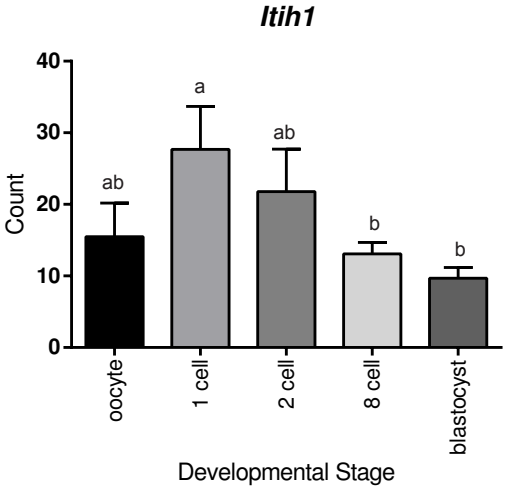
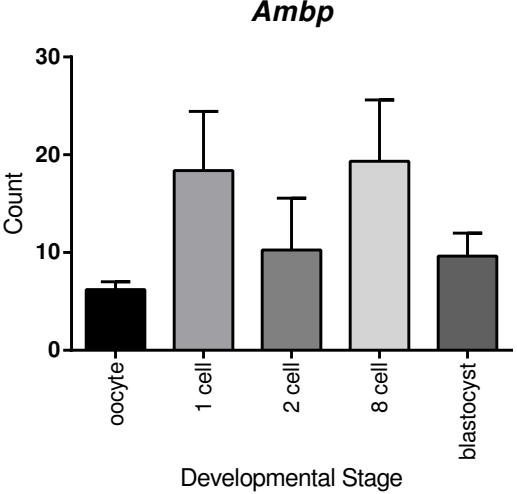
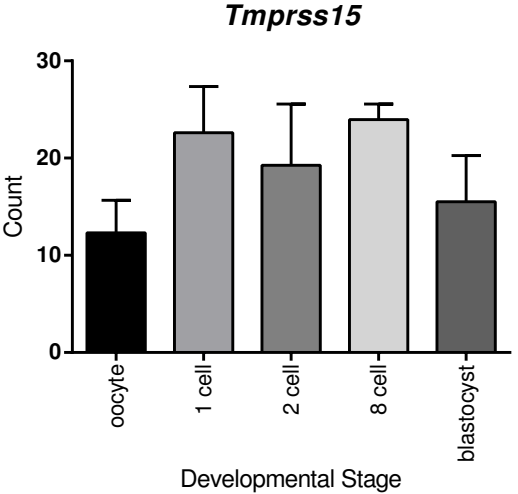
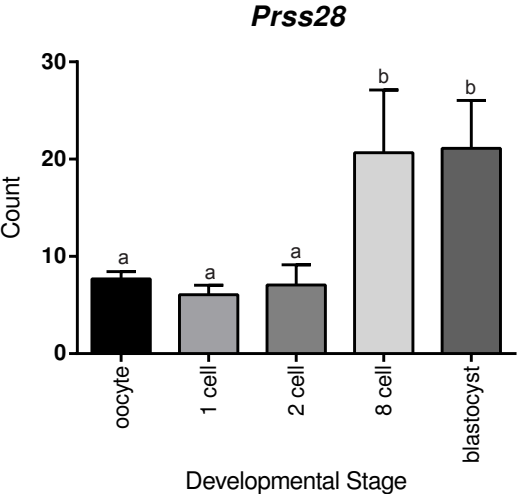
Supplementary Fig. 1



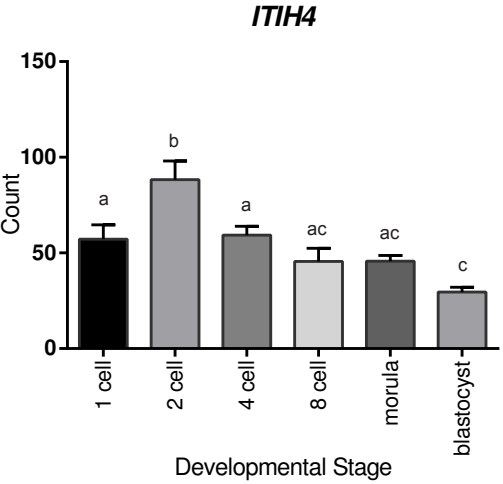
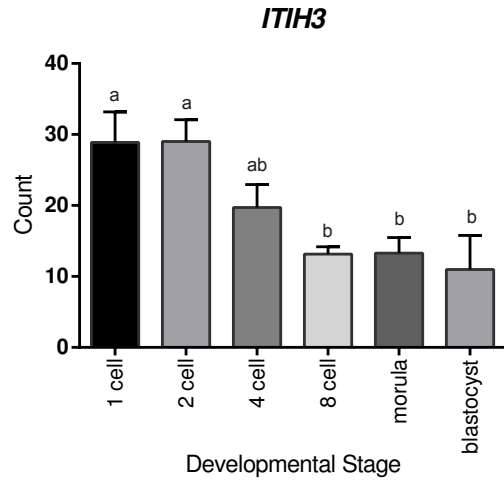
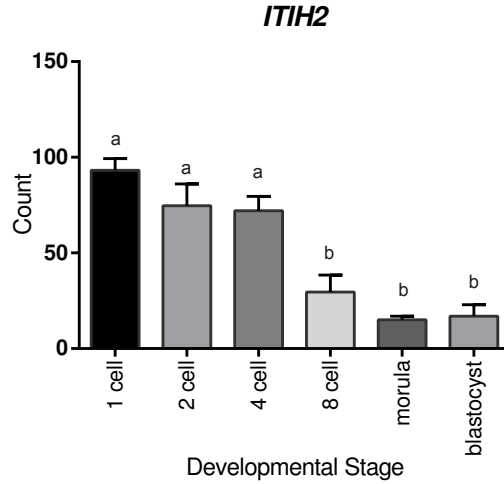
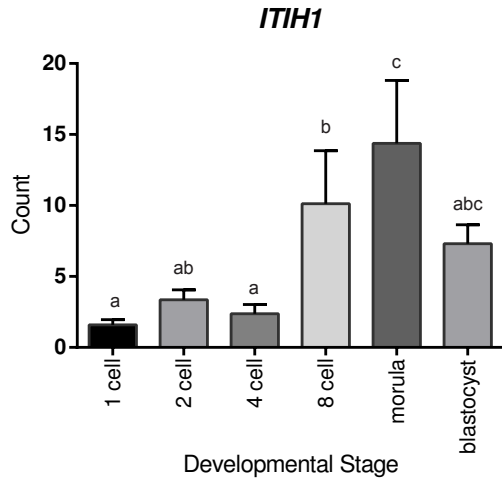
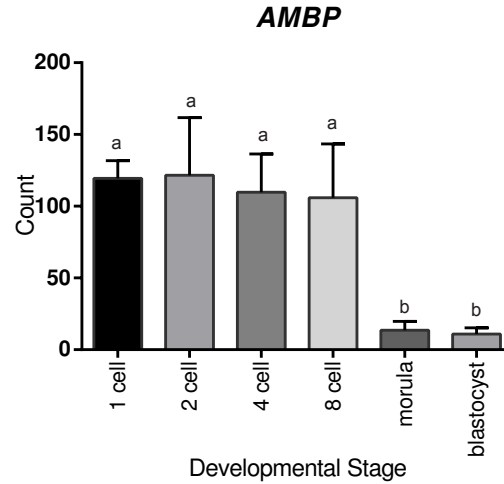
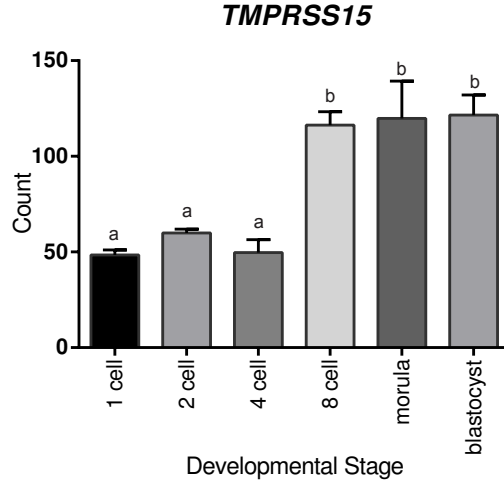
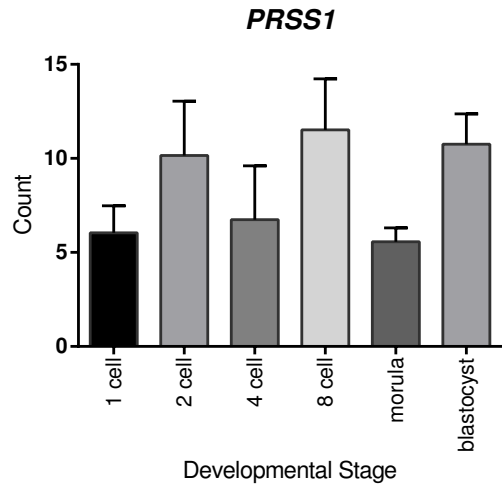
Supplementary Fig. 3



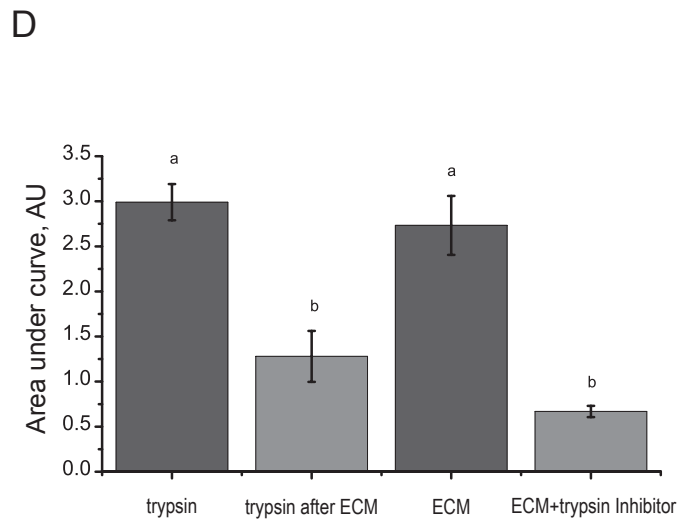
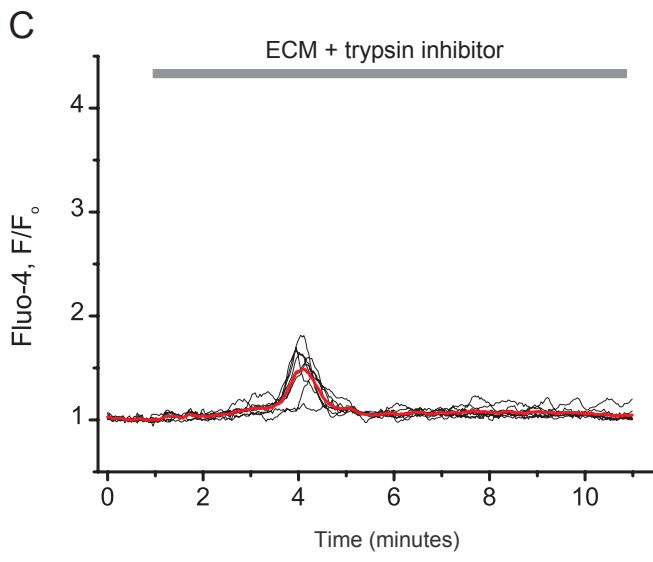
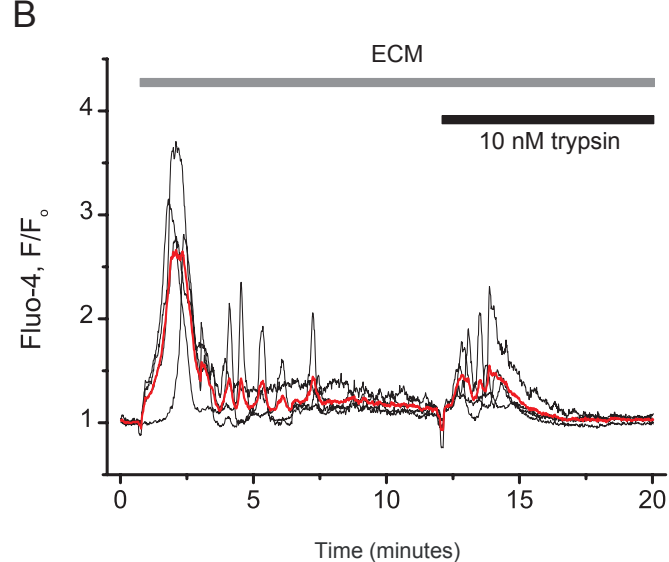
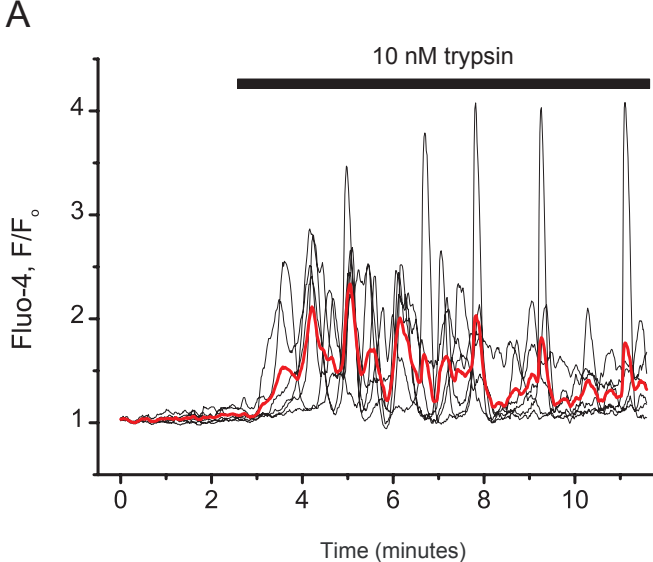
Supplementary Fig. 4



Supplementary Fig. 5



Supplementary Fig. 6



Supplementary Table 1

Patient demographics and embryo characteristics.

	DCE (n = 40)	DIE (n = 49)
Age (years):	33.5 ± 4.2	35.2 ± 4.3
Duration of infertility (years)	2.6 ± 1.9	3.3 ± 2.4
Primary infertility (%):	64.3%	50%
No. of oocytes collected:	10.3 ± 6.0	9.5 ± 4.2
No. of developing embryos:	4.6 ± 2.7	4.4 ± 2.4
Top quality embryos (%):	53.6%	0% *

DIE: developmentally impaired embryos; **DCE:** developmentally competent embryos. The data are expressed as mean ± standard deviation. * $P < 0.001$. Embryos were scored on standard morphological criteria (2) with top quality embryos defined as morula stage embryos with less than 10% fragmentation on day 4 after oocyte collection.

Supplementary Table 2

Genes regulated in decidualizing human endometrial stromal cells upon incubation of culture medium from developmentally impaired human embryos (DIE).

A. Decidual genes significantly ($P < 0.01$) down-regulated after familywise error correction by a factor ≥ 1.2

Gene symbol	Gene name	Factor down
HSPA8	Heat shock 70 protein	1.88
THBS1	Thrombospondin 1	1.76
RPS3A	Small nucleolar RNA. C/D box 73A	1.69
PDGFC	Platelet derived growth factor C	1.66
SBDS	Shwachman-Bodian-Diamond syndrome	1.59
CTGF	Connective tissue growth factor	1.56
ST3GAL5	ST3 beta-galactoside alpha-2.3-sialyltransferase 5	1.51
XIST	X (inactive)-specific transcript (non-protein coding)	1.51
CALD1	Caldesmon 1	1.49
ERICH1	Glutamate-rich 1	1.42
TMEM200A	Transmembrane protein 200A	1.42
EIF4G2	Eukaryotic translation initiation factor 4 gamma. 2	1.41
UBE2D3	Ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog. yeast)	1.4
LAMB1	Laminin. beta 1	1.38
ACTR2	ARP2 actin-related protein 2 homolog (yeast)	1.38
FXR1	Fragile X mental retardation. autosomal homolog 1	1.37
BPGM	2.3-bisphosphoglycerate mutase	1.37
SSB	Sjogren syndrome antigen B (autoantigen La)	1.35
SUB1	SUB1 homolog (S. cerevisiae)	1.34
ZFAND5	Zinc finger. AN1-type domain 5	1.34
PTP4A1	Protein tyrosine phosphatase type IVA. member 1	1.33
HNRNPM	Heterogeneous nuclear ribonucleoprotein M	1.33
ZNF107	Zinc finger protein 107	1.32
EPS8	Epidermal growth factor receptor pathway substrate 8	1.32
TMEM212	Transmembrane protein 212	1.32
COL8A1	Collagen. type VIII. alpha 1	1.32
ZNF549	Zinc finger protein 549	1.31

MUC16	Mucin 16. cell surface associated	1.31
PHLDA1	Pleckstrin homology-like domain. family A. member 1	1.31
UACA	Uveal autoantigen with coiled-coil domains and ankyrin repeats	1.3
DIO2	Deiodinase. iodothyronine. type II	1.3
KLF5	Kruppel-like factor 5 (intestinal)	1.3
UBE2E3	Ubiquitin-conjugating enzyme E2E 3 (UBC4/5 homolog. yeast)	1.29
hsa-mir-568	Hsa-mir-568	1.29
ZNF565	Zinc finger protein 565	1.29
NDFIP1	Nedd4 family interacting protein 1	1.29
AC108938.4	LIM and senescent cell antigen-like-containing domain protein 3	1.29
ECHDC1	Enoyl CoA hydratase domain containing 1	1.28
DOCK4	Dedicator of cytokinesis 4	1.28
SERINC1	Serine incorporator 1	1.27
ADAMTS5	ADAM metallopeptidase with thrombospondin type 1 motif. 5	1.27
SCOC	Short coiled-coil protein	1.27
RHBDD1	Rhomboid domain containing 1	1.27
IGSF9	Immunoglobulin superfamily. member 9	1.26
LIMS1	LIM and senescent cell antigen-like domains 1	1.26
SLC38A2	Solute carrier family 38. member 2	1.26
PNN	Pinin. desmosome associated protein	1.26
FBXL3	F-box and leucine-rich repeat protein 3	1.26
PPM1B	Protein phosphatase. Mg ²⁺ /Mn ²⁺ dependent. 1B	1.26
JMJD1C	Jumonji domain containing 1C	1.26
DNAJA1	DnaJ (Hsp40) homolog. subfamily A. member 1	1.26
THOC7	THO complex 7 homolog (Drosophila)	1.26
EIF2A	Eukaryotic translation initiation factor 2A. 65kDa	1.25
CHML	Choroideremia-like (Rab escort protein 2)	1.25
FAM20C	Family with sequence similarity 20. member C	1.24
MRFAP1L1	Morf4 family associated protein 1-like 1	1.24
LEPROT	Leptin receptor overlapping transcript	1.24
MAPK13	Mitogen-activated protein kinase 13	1.24
RAB9A	RAB9A. member RAS oncogene family	1.24
NMD3	NMD3 homolog (S. cerevisiae)	1.23

CBX3	Chromobox homolog 3	1.23
NEK7	NIMA (never in mitosis gene a)-related kinase 7	1.23
OGFRL1	Opioid growth factor receptor-like 1	1.23
TANK	TRAF family member-associated NFKB activator	1.22
BCAR3	Breast cancer anti-estrogen resistance 3	1.22
CCNG1	Cyclin G1	1.22
TNFSF15	Tumor necrosis factor (ligand) superfamily. member 15	1.22
CCT6A	Chaperonin containing TCP1. subunit 6A (zeta 1)	1.22
CCNG2	Cyclin G2	1.22
HCG11	HLA complex group 11	1.21
PRDX4	Peroxiredoxin 4	1.21
MED10	Mediator complex subunit 10	1.21
NDUFB6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex. 6. 17kDa	1.21

B. Decidual genes significantly ($P < 0.01$) up-regulated after familywise error correction by a factor ≥ 1.2

Gene symbol	Gene name	Factor up
HMOX1	Heme oxygenase (decycling) 1	1.54
ZNRF3	Zinc and ring finger 3	1.46
TMEM87B	Transmembrane protein 87B	1.44
IGSF1	Immunoglobulin superfamily. member 1	1.39
KHSRP	KH-type splicing regulatory protein	1.37
GNA11	Guanine nucleotide binding protein (G protein). alpha 11 (Gq class)	1.34
FCHO1	FCH domain only 1	1.32
ARHGDI1	Rho GDP dissociation inhibitor (GDI) alpha	1.31
BMP5	Bone morphogenetic protein 5	1.28
LIMK1	LIM domain kinase 1	1.26
MAP1LC3A	Microtubule-associated protein 1 light chain 3 alpha	1.26
SRC	V-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	1.26
WASH6P	WAS protein family homolog 6 pseudogene	1.24
RHOC	Ras homolog gene family. member C	1.24
LRRN1	Leucine rich repeat neuronal 1	1.24

FOSL2	FOS-like antigen 2	1.23
INPP5K	Inositol polyphosphate-5-phosphatase K	1.23
NAB1	NGFI-A binding protein 1 (EGR1 binding protein 1)	1.23
MICAL2	Microtubule associated monooxygenase	1.22
SUPT5H	Suppressor of Ty 5 homolog (S. cerevisiae)	1.22
S100A11	S100 calcium binding protein A11	1.22
CDK4	Cyclin-dependent kinase 4	1.22
LDB2	LIM domain binding 2	1.22
TRIM17	Tripartite motif-containing 17	1.22
BSG	Basigin (Ok blood group)	1.22
VPS37B	Vacuolar protein sorting 37 homolog B (S. cerevisiae)	1.21
COLEC12	Collectin sub-family member 12	1.21
H2AFX	H2A histone family. member X	1.2

Genes significantly significantly down-regulated ($P < 0.01$) after familywise error correction by a factor ≥ 1.2 in decidualizing human endometrial stromal cells upon incubation with culture medium from developmentally competent human embryos (DCE).

Gene symbol	Gene name	Factor down
MEG3	Maternally expressed 3 (non-protein coding)	1.49
MAGT1	Magnesium transporter 1	1.26
COL7A1	Collagen. type VII. alpha 1	1.24
IGSF9	Immunoglobulin superfamily. member 9	1.23
SULT1A3	Sulfotransferase family. cytosolic. 1A. phenol-preferring. member 3	1.23
C1orf63	UPF0471 protein C1orf63	1.22
ZKSCAN1	Zinc finger with KRAB and SCAN domains 1	1.22

Supplementary Table 3:**Significantly Regulated Transcripts in Mouse Uterus in Response to Developmentally Competent Embryo Conditioned Media**

Gene Symbol	Gene Name	Fold Change	References
Depp	Decidual protein induced by progesterone (RIKEN cDNA 8430408G22 gene)	94.43	(Chen et al., 2011, Watanabe et al., 2005)
Prss28	protease serine28	67.63	(O'Sullivan et al., 2004, Sharma et al., 2006)
Pinlyp	phospholipase A2 inhibitor and LY6/PLAUR domain containing	56.36	
Atp6v0a4	ATPase H+ transporting lysosomal V0 subunit A4	45.77	
Spink3	serine peptidase inhibitor Kazal type 3	36.76	(Chen et al., 2010, Nuno-Ayala et al., 2012)
Guca2b	guanylate cyclase activator 2B (uroguanylin)	31.23	(McConaha et al., 2011)
Spp1	secreted phosphoprotein 1	28.75	(Chaen et al., 2012, Wei et al., 2009)
Tll1	tolloid-like	22.69	
E230016K23Rik	RIKEN cDNA E230016K23 gene	16.69	
Cyp26a1	cytochrome P450 family 26 subfamily a polypeptide 1	16.18	(Han et al., 2010, Ma et al., 2012)
Plin1	perilipin 1	15.72	
Cidec	cell death-inducing DFFA-like effector c	15.30	
Adipoq	adiponectin C1Q and collagen domain containing	13.67	(Dos Santos et al., 2012, Kim et al., 2011)
Slc5a8	solute carrier family 5 (iodide transporter) member 8	13.21	
Car3	carbonic anhydrase 3	12.88	
Nsd1	nuclear receptor binding SET domain protein 1	11.48	
Efhd1	EF-hand domain family member D1	10.91	
Cyp2e1	cytochrome P450 family 2 subfamily E polypeptide 1	10.86	
Gcnt4	glucosaminyl (N-acetyl) transferase 4 core 2 (beta-16-N-acetylglucosaminyltransferase)	10.68	
Aqp8	aquaporin 8	10.66	(Sha et al., 2011)
Adar	adenosine deaminase RNA-specific	10.40	
Retn	resistin	10.01	(Di Simone et al., 2009)
Ergic2	ERGIC and golgi 2	8.68	
Fabp4	fatty acid binding protein 4 adipocyte	8.57	(Tian et al., 2011)
Abcb1b	ATP-binding cassette sub-family B (MDR/TAP) member 1B	7.22	(Kalabis et al., 2005)
Sncg	synuclein gamma (breast cancer-specific protein 1)	7.12	
Gm17147	GM17147 gene product from transcript GM17147-RA	6.56	
Hp	haptoglobin	6.51	(Berkova et al., 2001, Gloria-Bottini et al., 2009)
Hpgds	hematopoietic prostaglandin D synthase	6.49	(Michimata et al., 2002)
Prss29	protease serine29	6.29	(Huang et al., 2004, Sharma et al., 2006)

Rdh12	retinol dehydrogenase 12 (all-trans/9-cis/11-cis)	5.93	
Ptgs2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	5.52	(Banerjee et al., 2013, Dorniak et al., 2012)
Smpd13b	sphingomyelin phosphodiesterase acid-like 3B	4.32	
Hdc	histidine decarboxylase	4.25	(Paria et al., 1998)
Lrp2	low density lipoprotein receptor-related protein 2	3.79	(Pan et al., 2006)
Ctgf	connective tissue growth factor	3.70	(Surveyor et al., 1998, Waddell et al., 2011)
C2cd4a	C2 calcium-dependent domain containing 4A	3.58	
Tmem213	transmembrane protein 213	3.57	
Sult1d1	sulfotransferase family 1D member 1	3.24	
Gsn	gelsolin	3.13	
Igfbp3	insulin-like growth factor binding protein 3	3.04	(Liu et al., 1995, Sherwin et al., 2004)
Hsp90aa1	heat shock protein 90kDa alpha (cytosolic) class A member 1	2.88	
Padi2	peptidyl arginine deiminase type II	-2.60	
Klk1	kallikrein 1	-3.69	(Chan et al., 1999, Rajapakse et al., 2007)
Slc4a2	solute carrier family 4 (anion exchanger) member 2	-4.41	
Irf6	interferon regulatory factor 6	-4.56	(Fleming et al., 2009)
Crabp2	cellular retinoic acid binding protein 2	-4.64	(Lee et al., 2007, Lee et al., 2011)
Slc1a5	solute carrier family 1 (neutral amino acid transporter) member 5	-4.77	(Dorniak et al., 2012)
Spink12	serine peptidase inhibitor Kazal type 11	-5.00	
Sema4d	sema domain immunoglobulin domain (Ig) transmembrane domain (TM) and short cytoplasmic domain (semaphorin) 4D	-5.18	(Lorenzi et al., 2012)
Gak	cyclin G associated kinase	-6.29	
Nedd4	neural precursor cell expressed developmentally down-regulated 4 E3 ubiquitin protein ligase	-8.33	
Plch1	phospholipase C eta 1	-9.18	
Zfp369	zinc finger protein 369	-10.13	
3110040N11Rik	RIKEN cDNA 3110040N11 gene	ND	
Adcy9	adenylate cyclase 9	ND	
Add1	adducin 1 (alpha)	ND	
Arf5b	ADP-ribosylation factor-like 5B	ND	
Arv1	ARV1 homolog (S. cerevisiae)	ND	
Begain	brain-enriched guanylate kinase-associated homolog (rat)	ND	
Rbm48	RNA binding motif protein 48	ND	
Ccdc117	coiled-coil domain containing 117	ND	
Ccdc77	coiled-coil domain containing 77	ND	
Cdc25a	cell division cycle 25 homolog A (S. pombe)	ND	

Clec2d	C-type lectin domain family 2 member D	ND	(Kusumi et al., 2006)
Cpsf6	cleavage and polyadenylation specific factor 6 68kDa	ND	
Dpy19l3	dpy-19-like 3 (C. elegans)	ND	
Fip11	FIP1 like 1 (S. cerevisiae)	ND	
Focad	focadhesin	ND	
Gcap14	granule cell antiserum positive 14	ND	
Gls	glutaminase	ND	
Gpr144	G protein-coupled receptor 144	ND	
Ppfibp1	PTPRF interacting protein binding protein 1 (liprin beta 1)	ND	
Ppp4r1l-ps	protein phosphatase 4 regulatory subunit 1-like pseudogene	ND	
Rfx5	regulatory factor X 5 (influences HLA class II expression)	ND	
Rictor	RPTOR independent companion of MTOR complex 2	ND	
Rit1	Ras-like without CAAX 1	ND	
Slc39a1	solute carrier family 39 (zinc transporter) member 1	ND	
Slc4a4	solute carrier family 4 sodium bicarbonate cotransporter member 4	ND	
Snapc4	small nuclear RNA activating complex polypeptide 4 190kDa	ND	
Sort1	sortilin 1	ND	
Tdrp	testis-development related protein (RIKEN cDNA 2610019F03 gene)	ND	
Trappc9	trafficking protein particle complex 9	ND	
Tro	trophinin	ND	(Tamura et al., 2011)
Usp9x	ubiquitin specific peptidase 9 X-linked	ND	
Vmp1	vacuole membrane protein 1	ND	
Wdsub1	WD repeat sterile alpha motif and U-box domain containing 1	ND	
Zc3h6	zinc finger CCCH type containing 6	ND	
Zfp558	zinc finger protein 558	ND	
Fkrp	fukutin related protein	DCE only	

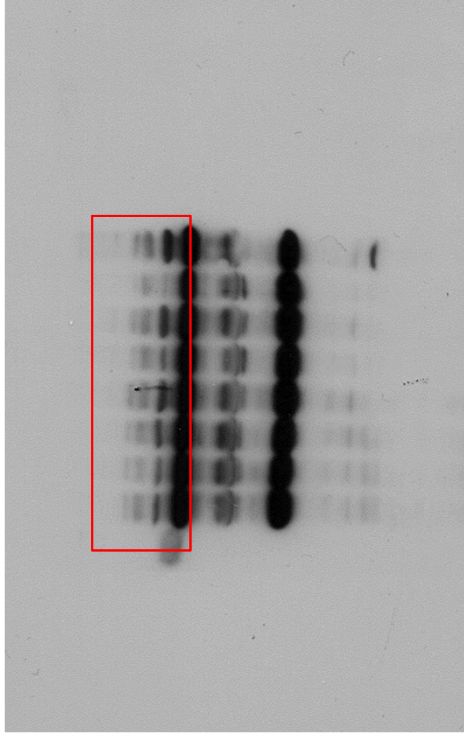
ND = not detected

References

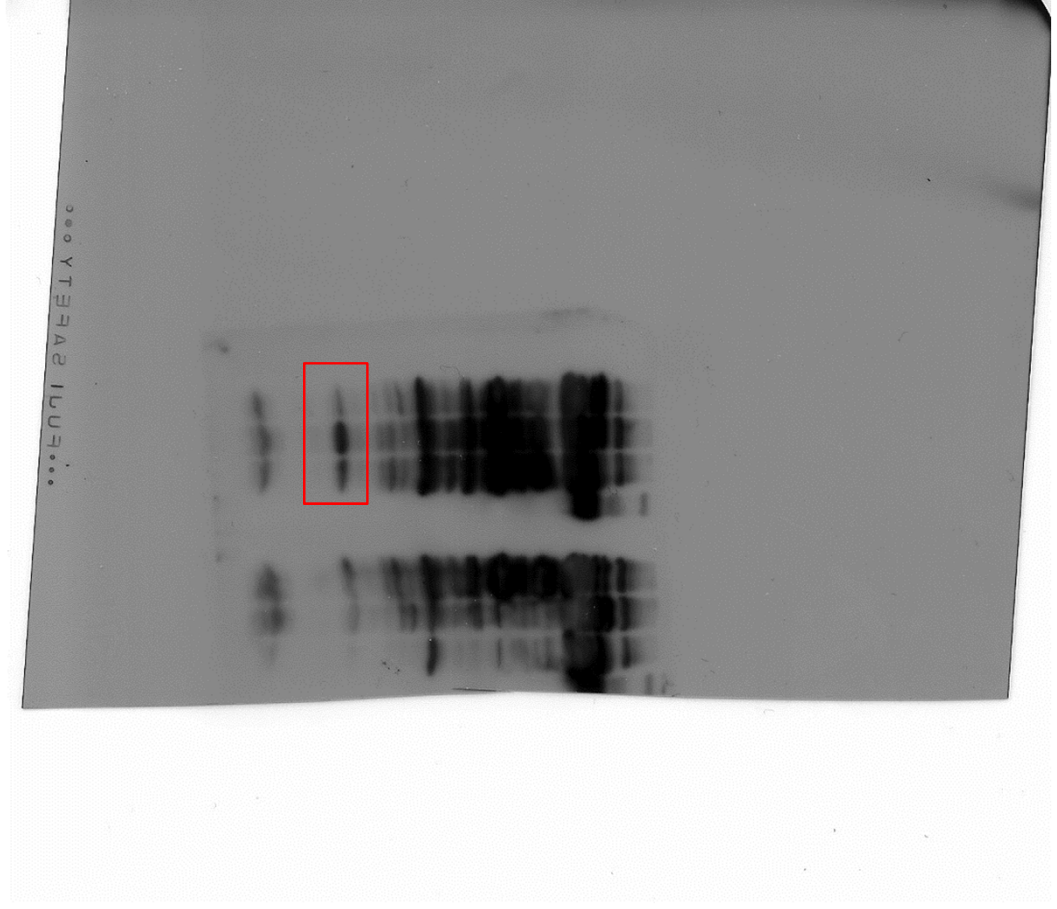
- Banerjee, P., Jana, S. K., Pasricha, P., Ghosh, S., Chakravarty, B. & Chaudhury, K. 2013. Proinflammatory cytokines induced altered expression of cyclooxygenase-2 gene results in unreceptive endometrium in women with idiopathic recurrent spontaneous miscarriage. *Fertil Steril*, 99, 179-87.
- Berkova, N., Lemay, A., Dresser, D. W., Fontaine, J.-Y., Kerizit, J. & Goupil, S. 2001. Haptoglobin is present in human endometrium and shows elevated levels in the decidua during pregnancy. *Molecular Human Reproduction*, 7, 747-754.
- Chaen, T., Konno, T., Egashira, M., Bai, R., Nomura, N., Nomura, S., Hirota, Y., Sakurai, T. & Imakawa, K. 2012. Estrogen-dependent uterine secretion of osteopontin activates blastocyst adhesion competence. *PLoS One*, 7, e48933.
- Chan, C. S., Harvey, M. B. & Clements, J. A. 1999. Temporal and tissue-specific expression of kallikrein (Klk) genes and identification of a novel Klk messenger ribonucleic acid transcript during early development in the mouse. *Biol Reprod*, 61, 621-8.
- Chen, S. M., Gai, J. F., Wang, Y. Y. & Li, H. 2011. FoxO regulates expression of decidual protein induced by progesterone (DEPP) in human endothelial cells. *Febs Letters*, 585, 1796-1800.
- Chen, W., Han, B. C., Wang, R. C., Xiong, G. F. & Peng, J. P. 2010. Role of secretory protease inhibitor SPINK3 in mouse uterus during early pregnancy. *Cell and Tissue Research*, 341, 441-451.
- Di Simone, N., Di Nicuolo, F., Marzioni, D., Castellucci, M., Sanguinetti, M., D'ippolito, S. & Caruso, A. 2009. Resistin modulates glucose uptake and glucose transporter-1 (GLUT-1) expression in trophoblast cells. *Journal of Cellular and Molecular Medicine*, 13, 388-397.
- Dorniak, P., Bazer, F. W., Wu, G. & Spencer, T. E. 2012. Conceptus-derived prostaglandins regulate endometrial function in sheep. *Biol Reprod*, 87, 9, 1-7.
- Dos Santos, E., Serazin, V., Morvan, C., Torre, A., Wainer, R., De Mazancourt, P. & Dieudonne, M. N. 2012. Adiponectin and leptin systems in human endometrium during window of implantation. *Fertility and Sterility*, 97, 771-U281.
- Fleming, J. A., Song, G., Choi, Y., Spencer, T. E. & Bazer, F. W. 2009. Interferon regulatory factor 6 (IRF6) is expressed in the ovine uterus and functions as a transcriptional activator. *Mol Cell Endocrinol*, 299, 252-60.
- Gloria-Bottini, F., Magrini, A., Amante, A., Nicotra, M. & Bottini, E. 2009. Haptoglobin phenotype and reproductive success in repeated spontaneous abortion. *Eur J Obstet Gynecol Reprod Biol*, 144, 153-6.
- Han, B. C., Xia, H. F., Sun, J., Yang, Y. & Peng, J. P. 2010. Retinoic Acid-Metabolizing Enzyme Cytochrome P450 26a1 (Cyp26a1) Is Essential for Implantation: Functional Study of Its Role in Early Pregnancy. *Journal of Cellular Physiology*, 223, 471-479.
- Huang, Z. P., Yu, H., Yang, Z. M., Shen, W. X., Wang, J. & Shen, Q. X. 2004. Uterine expression of implantation serine proteinase 2 during the implantation period and in vivo inhibitory effect of its antibody on embryo implantation in mice. *Reprod Fertil Dev*, 16, 379-84.
- Kalabis, G. M., Kostaki, A., Andrews, M. H., Petropoulos, S., Gibb, W. & Matthews, S. G. 2005. Multidrug Resistance Phosphoglycoprotein (ABCB1) in the Mouse Placenta: Fetal Protection. *Biology of Reproduction*, 73, 591-597.
- Kim, S. T., Marquard, K., Stephens, S., Loudon, E., Allsworth, J. & Moley, K. H. 2011. Adiponectin and adiponectin receptors in the mouse preimplantation embryo and uterus. *Human Reproduction*, 26, 82-95.
- Kusumi, M., Yamashita, T., Fujii, T., Nagamatsu, T., Kozuma, S. & Taketani, Y. 2006. Expression patterns of lectin-like natural killer receptors, inhibitory CD94/NKG2A, and activating CD94/NKG2C on decidual CD56bright natural killer cells differ from those on peripheral CD56dim natural killer cells. *J Reprod Immunol*, 70, 33-42.
- Lee, J., Oh, J., Choi, E., Park, I., Han, C., Kim Do, H., Choi, B. C., Kim, J. W. & Cho, C. 2007. Differentially expressed genes implicated in unexplained recurrent spontaneous abortion. *Int J Biochem Cell Biol*, 39, 2265-77.
- Lee, J., Oh, J. S. & Cho, C. 2011. Impaired expansion of trophoblast spheroids cocultured with endometrial cells overexpressing cellular retinoic acid-binding protein 2. *Fertil Steril*, 95, 2599-601.
- Liu, H. C., Mele, C., Catz, D., Noyes, N. & Rosenwaks, Z. 1995. Production of insulin-like growth factor binding proteins (IGFBPs) by human endometrial stromal cell is stimulated by the presence of embryos. *J Assist Reprod Genet*, 12, 78-87.

- Lorenzi, T., Turi, A., Lorenzi, M., Paolinelli, F., Manciola, F., La Sala, L., Morroni, M., Ciarmela, P., Mantovani, A., Tranquilli, A. L., Castellucci, M. & Marzioni, D. 2012. Placental expression of CD100, CD72 and CD45 is dysregulated in human miscarriage. *PLoS One*, 7, e35232.
- Ma, J. J., Han, B. C., Yang, Y. & Peng, J. P. 2012. Retinoic acid synthesis and metabolism are concurrent in the mouse uterus during peri-implantation. *Cell and Tissue Research*, 350, 525-537.
- McConaha, M. E., Eckstrum, K., An, J., Steinle, J. J. & Bany, B. M. 2011. Microarray assessment of the influence of the conceptus on gene expression in the mouse uterus during decidualization. *Reproduction*, 141, 511-527.
- Michimata, T., Tsuda, H., Sakai, M., Fujimura, M., Nagata, K., Nakamura, M. & Saito, S. 2002. Accumulation of CRTH2-positive T-helper 2 and T-cytotoxic 2 cells at implantation sites of human decidua in a prostaglandin D(2)-mediated manner. *Mol Hum Reprod*, 8, 181-7.
- Nuno-Ayala, M., Guillen, N., Arnal, C., Lou-Bonafonte, J. M., De Martino, A., Garcia-De-Jalon, J. A., Gascon, S., Osaba, L., Osada, J. & Navarro, M. A. 2012. Cystathionine beta-synthase deficiency causes infertility by impairing decidualization and gene expression networks in uterus implantation sites. *Physiol Genomics*, 44, 702-16.
- O'sullivan, C. M., Ungarian, J. L., Singh, K., Liu, S., Hance, J. & Rancourt, D. E. 2004. Uterine secretion of ISP1 & 2 tryptases is regulated by progesterone and estrogen during pregnancy and the endometrial cycle. *Mol Reprod Dev*, 69, 252-9.
- Pan, H., Zhu, L., Deng, Y. & Pollard, J. W. 2006. Microarray analysis of uterine epithelial gene expression during the implantation window in the mouse. *Endocrinology*, 147, 4904-16.
- Paria, B. C., Das, N., Das, S. K., Zhao, X., Dileepan, K. N. & Dey, S. K. 1998. Histidine Decarboxylase Gene in the Mouse Uterus Is Regulated by Progesterone and Correlates with Uterine Differentiation for Blastocyst Implantation. *Endocrinology*, 139, 3958-3966.
- Rajapakse, S., Yamano, N., Ogiwara, K., Hirata, K., Takahashi, S. & Takahashi, T. 2007. Estrogen-dependent expression of the tissue kallikrein gene (Kik1) in the mouse uterus and its implications for endometrial tissue growth. *Mol Reprod Dev*, 74, 1053-63.
- Sha, X. Y., Xiong, Z. F., Liu, H. S., Zheng, Z. & Ma, T. H. 2011. Pregnant phenotype in aquaporin 8-deficient mice. *Acta Pharmacologica Sinica*, 32, 840-844.
- Sharma, N., Liu, S., Tang, L., Irwin, J., Meng, G. & Rancourt, D. E. 2006. Implantation Serine Proteinases heterodimerize and are critical in hatching and implantation. *BMC Dev Biol*, 6, 61.
- Sherwin, J. R., Freeman, T. C., Stephens, R. J., Kimber, S., Smith, A. G., Chambers, I., Smith, S. K. & Sharkey, A. M. 2004. Identification of genes regulated by leukemia-inhibitory factor in the mouse uterus at the time of implantation. *Mol Endocrinol*, 18, 2185-95.
- Surveyor, G. A., Wilson, A. K. & Brigstock, D. R. 1998. Localization of connective tissue growth factor during the period of embryo implantation in the mouse. *Biol Reprod*, 59, 1207-13.
- Tamura, N., Sugihara, K., Akama, T. O. & Fukuda, M. N. 2011. Trophinin-mediated cell adhesion induces apoptosis of human endometrial epithelial cells through PKC-delta. *Cell Cycle*, 10, 135-43.
- Tian, Z., Zhao, Z. A., Liang, X. H., Zhang, X. H., Sha, A. G., Zhang, Z. R., Yu, Y. S. & Yang, Z. M. 2011. Expression and function of fatty acid-binding protein 4 during mouse decidualization. *Fertil Steril*, 95, 2749-52 e1-5.
- Waddell, J. M., Evans, J., Jabbour, H. N. & Denison, F. C. 2011. CTGF expression is up-regulated by PROK1 in early pregnancy and influences HTR-8/Svneo cell adhesion and network formation. *Hum Reprod*, 26, 67-75.
- Watanabe, H., Nonoguchi, K., Sakurai, T., Masuda, T., Itoh, K. & Fujita, J. 2005. A novel protein Depp, which is induced by progesterone in human endometrial stromal cells activates Elk-1 transcription factor. *Molecular Human Reproduction*, 11, 471-476.
- Wei, Q., St Clair, J. B., Fu, T., Stratton, P. & Nieman, L. K. 2009. Reduced expression of biomarkers associated with the implantation window in women with endometriosis. *Fertil Steril*, 91, 1686-91.

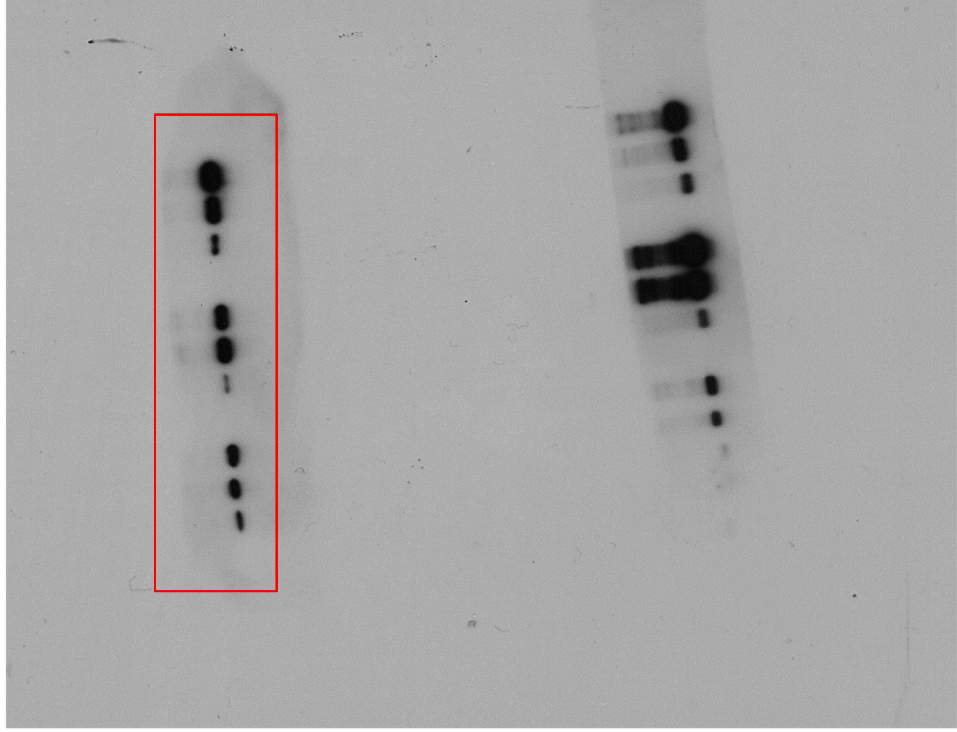
Timecourse (Figure 1C)



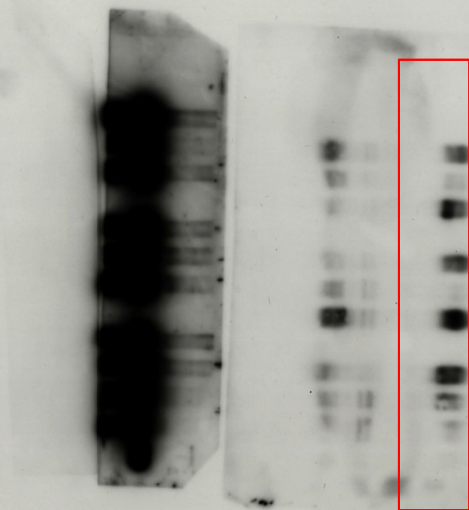
Knock down (Figure 1D)



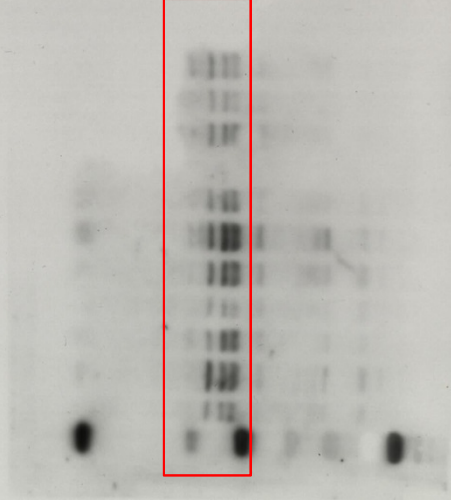
XBP1 (Suppl. Fig 3)



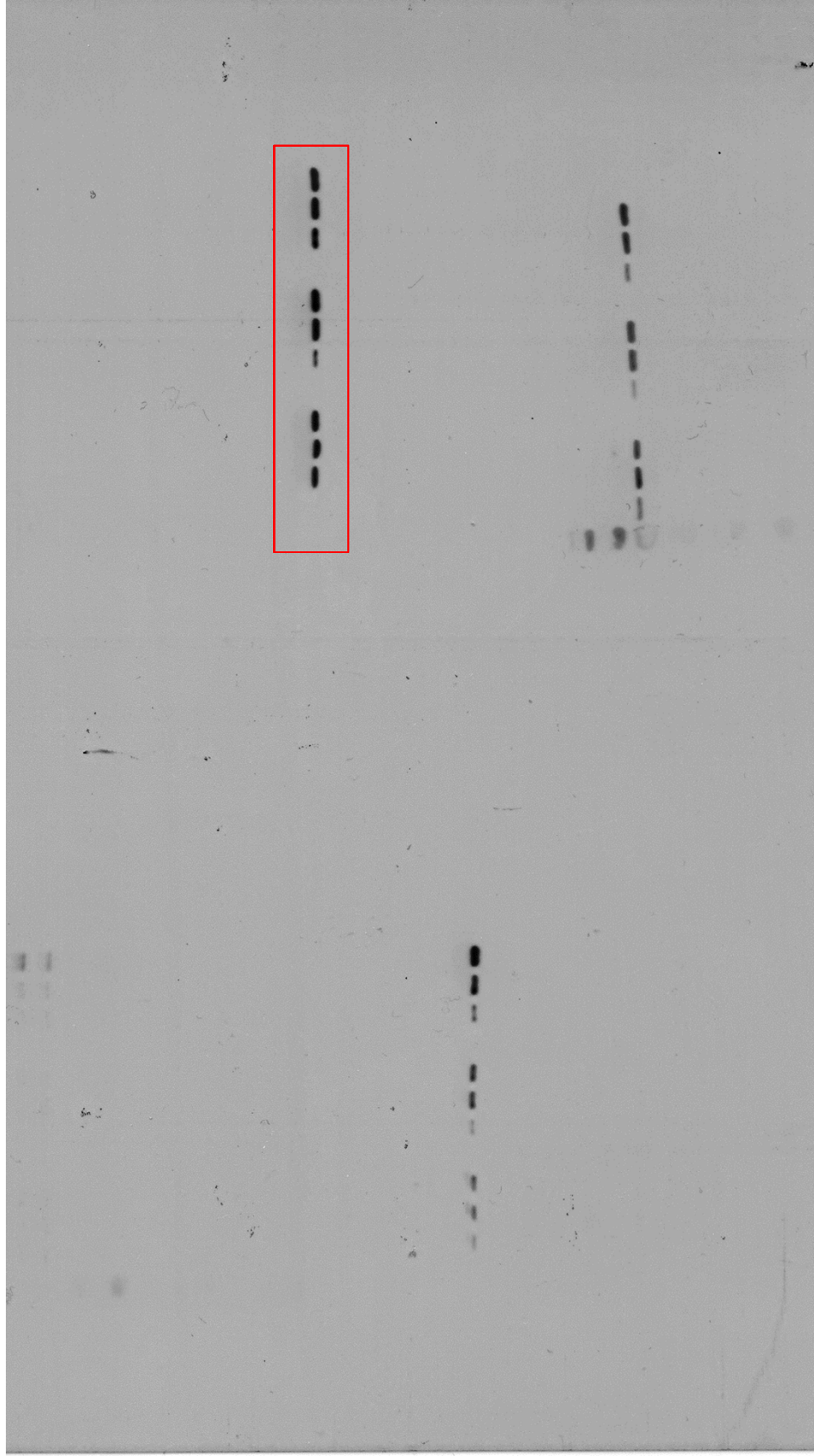
CHOP (Suppl. Fig 3)



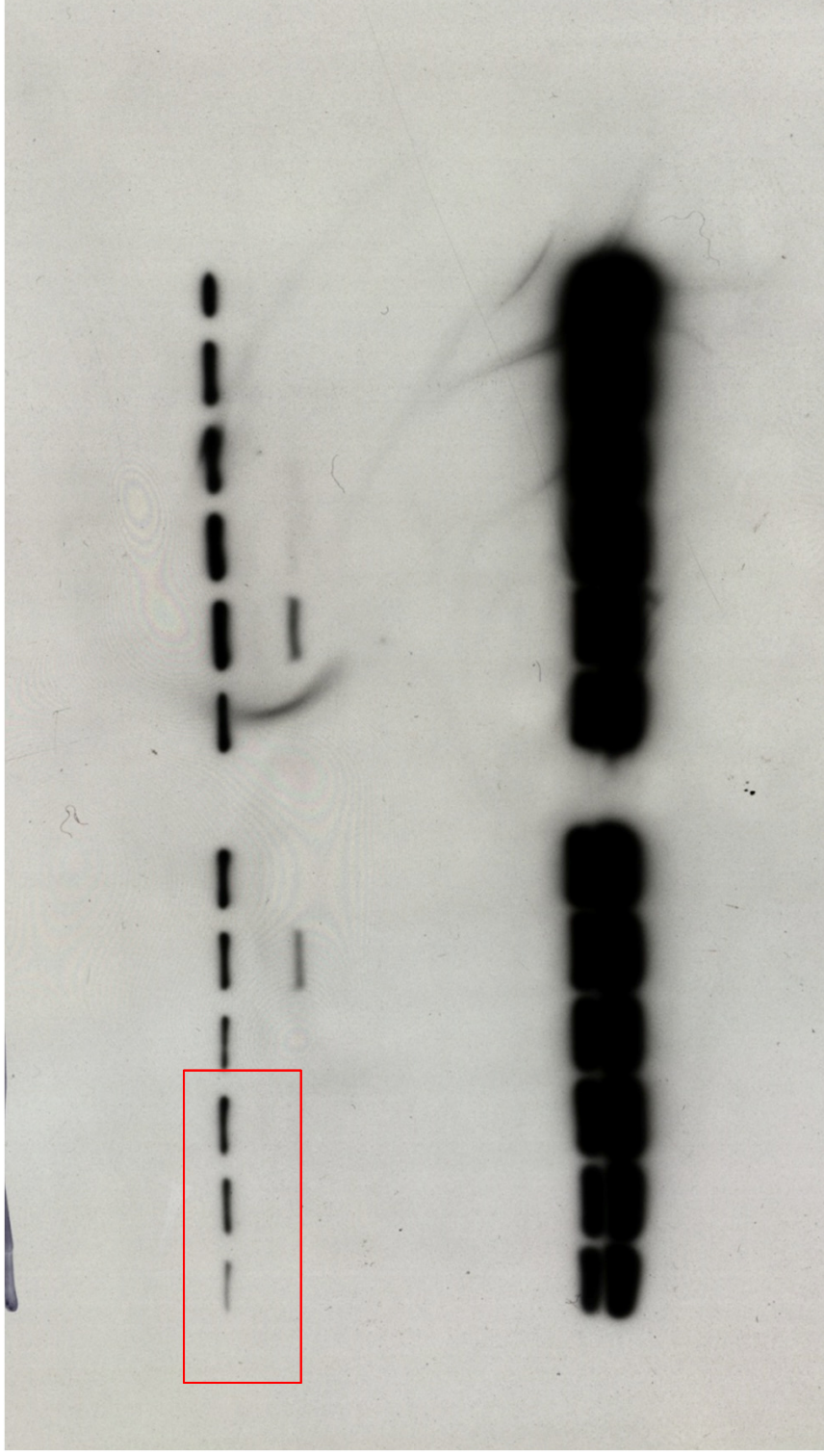
LC3B (Suppl. Fig 3)



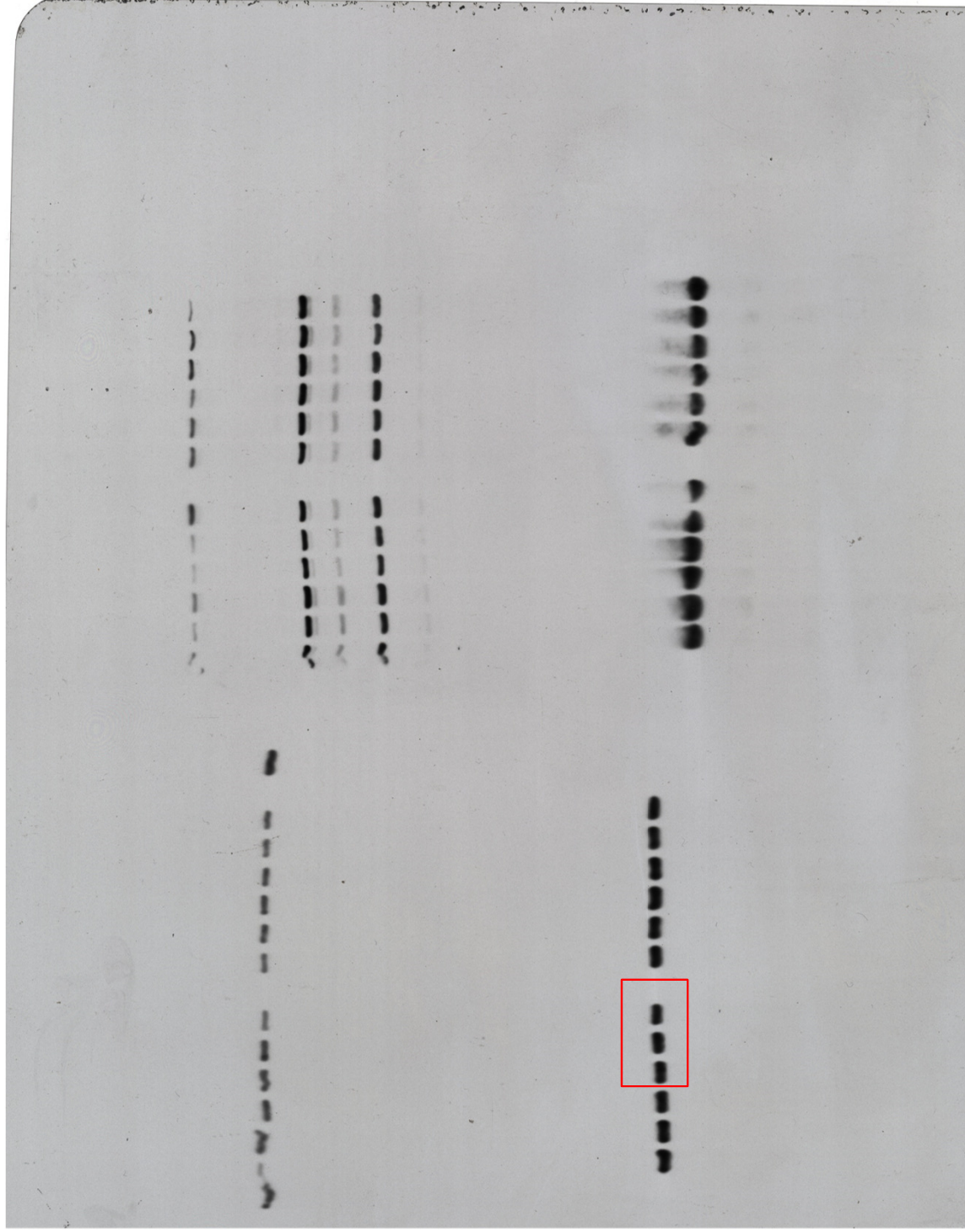
B-Actin(Suppl. Fig 3)



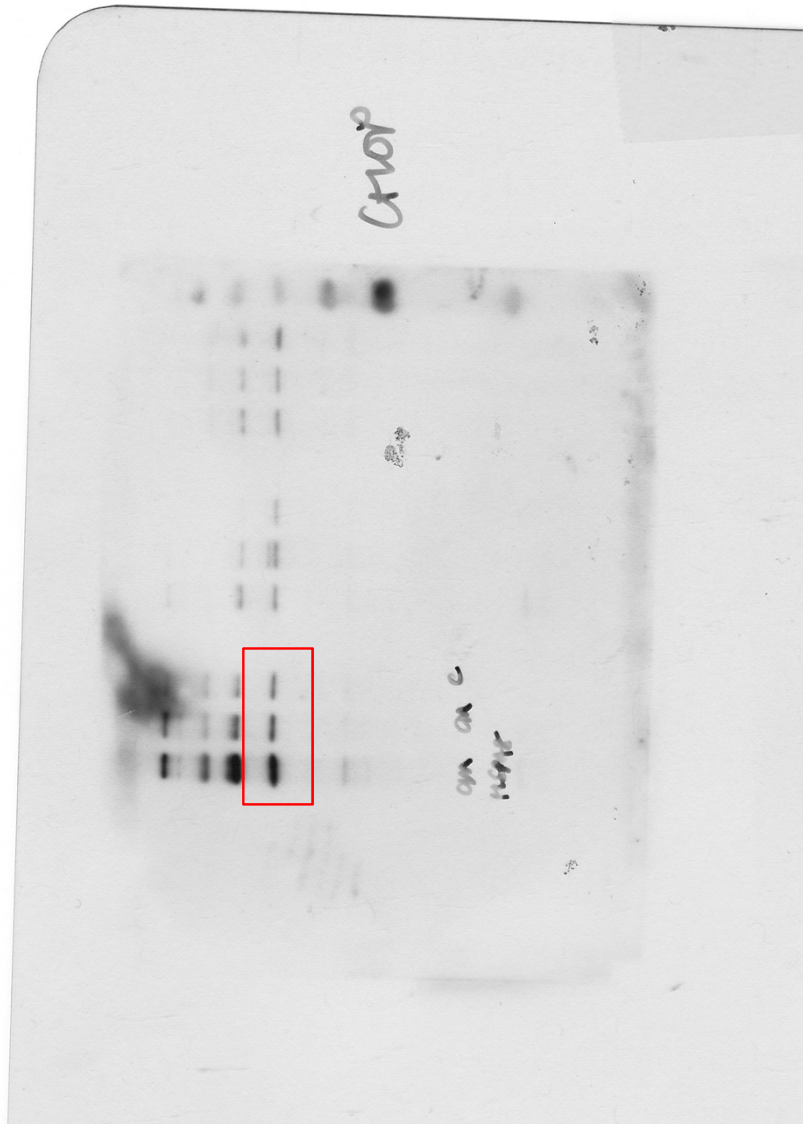
Bip (Figure 2)



Actin (Figure 2)



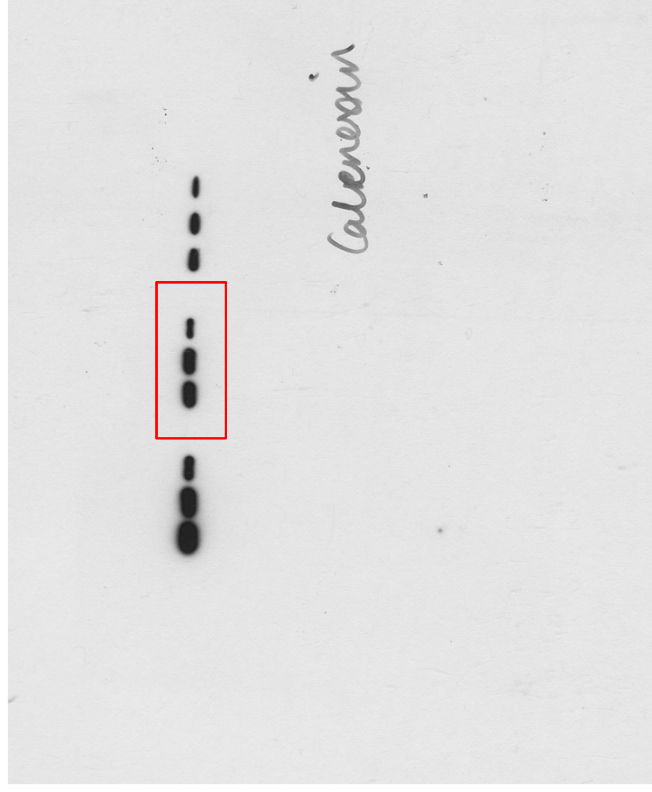
Chop (Figure 2)



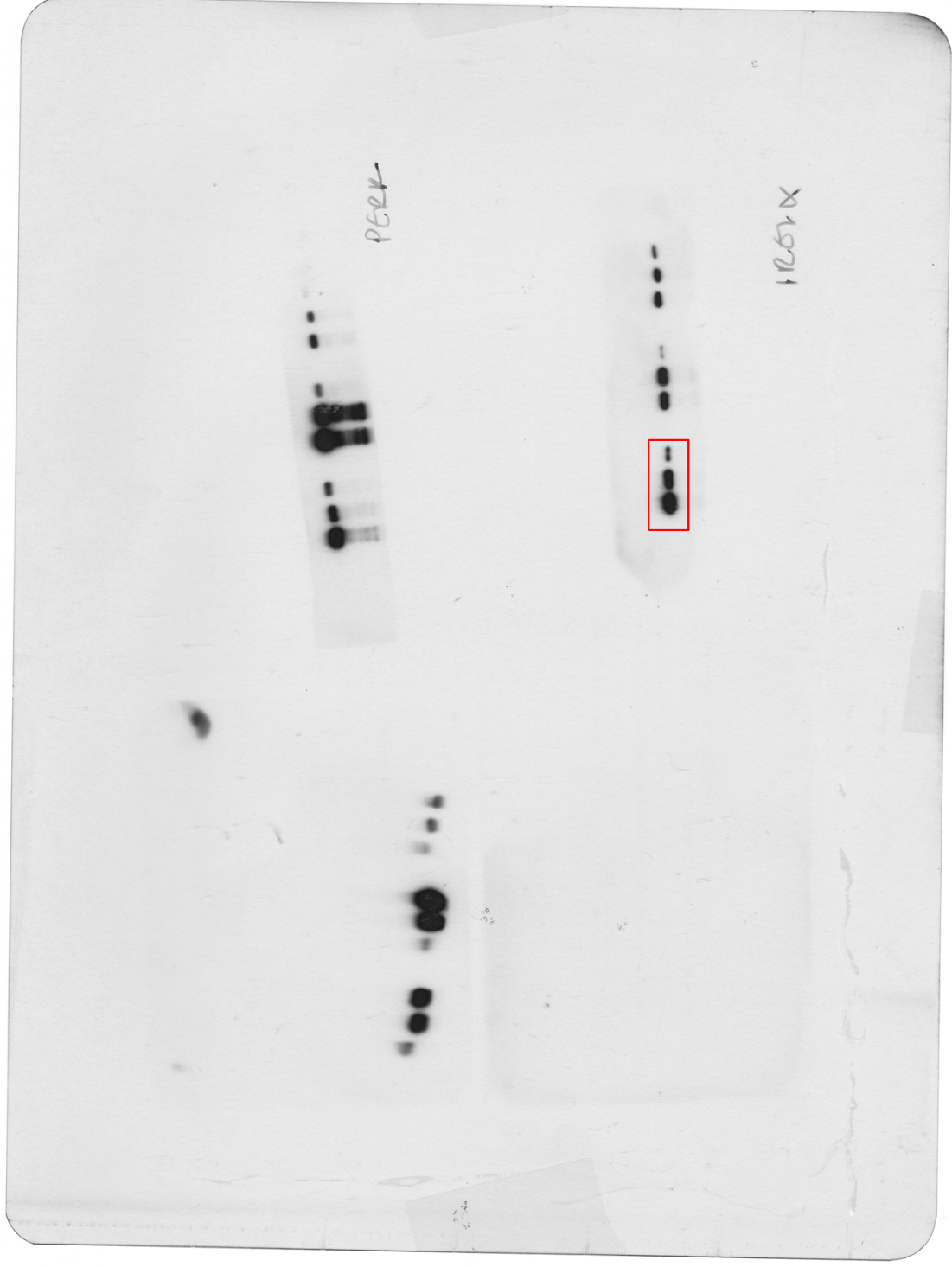
PDI (Figure 2)



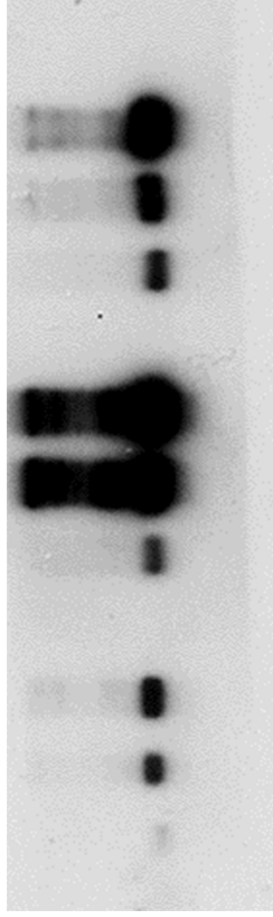
Calnexin (Figure 2)



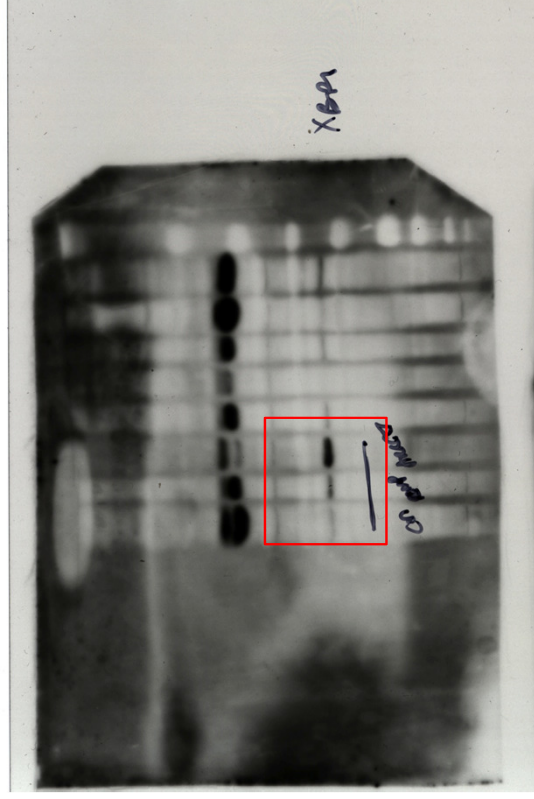
IRE1a (Figure 2)



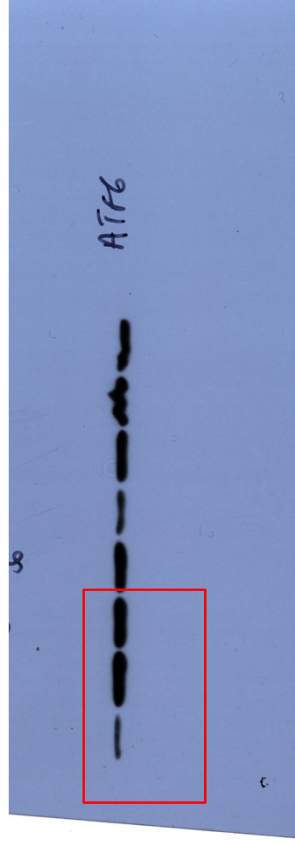
PERK (Figure 2)



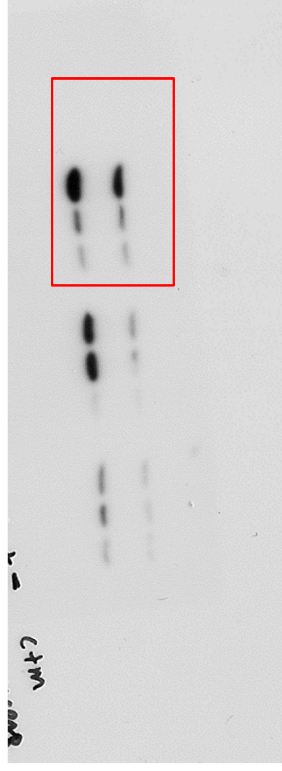
Xbp1 (Figure 2)



Atf6 (Figure 2)

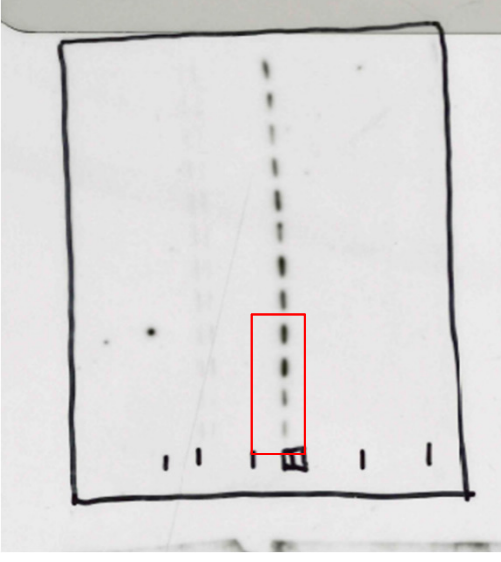


Lc3b (Figure 2)



COX2 and B-ACTIN (Figure 3)

COX2



B-ACTIN

