Supplementary materials

PELP1 and SRC kinase as important molecules in the estrogen-mediated pathway in human testis and epididymis

Izabela Skibińska[1*](ORCID: 0000-0002-7684-028X), Mirosław Andrusiewicz[1] (ORCID: 0000-0002-8781-3447), Magdalena Jendraszak[1] (ORCID: 0000-0001-8139-9532), Aleksandra Żbikowska[1] (ORCID: 0000-0002-6891-8569), Czesław Żaba[2] (ORCID: 0000-0001-7522-4568) and Małgorzata Kotwicka[1] (ORCID: 0000-0002-9802-374X)

[1] Chair and Department of Cell Biology, Poznan University of Medical Sciences, Rokietnicka 5D, Poznan 60-806, Poland; iskibinska@ump.edu.pl (IS); andrus@ump.edu.pl (MA); jendraszak@ump.edu.pl (MJ); azbikowska@ump.edu.pl (AŻ); mkotwic@ump.edu.pl (MK)
[2] Chair and Department of Forensic Sciences, Poznan University of Medical Sciences, Rokietnicka 5D, Poznan 60-806, Poland; czaba@ump.edu.pl (CZ)
* Correspondence: iskibinska@ump.edu.pl; Tel.: +48618547167 (IS)

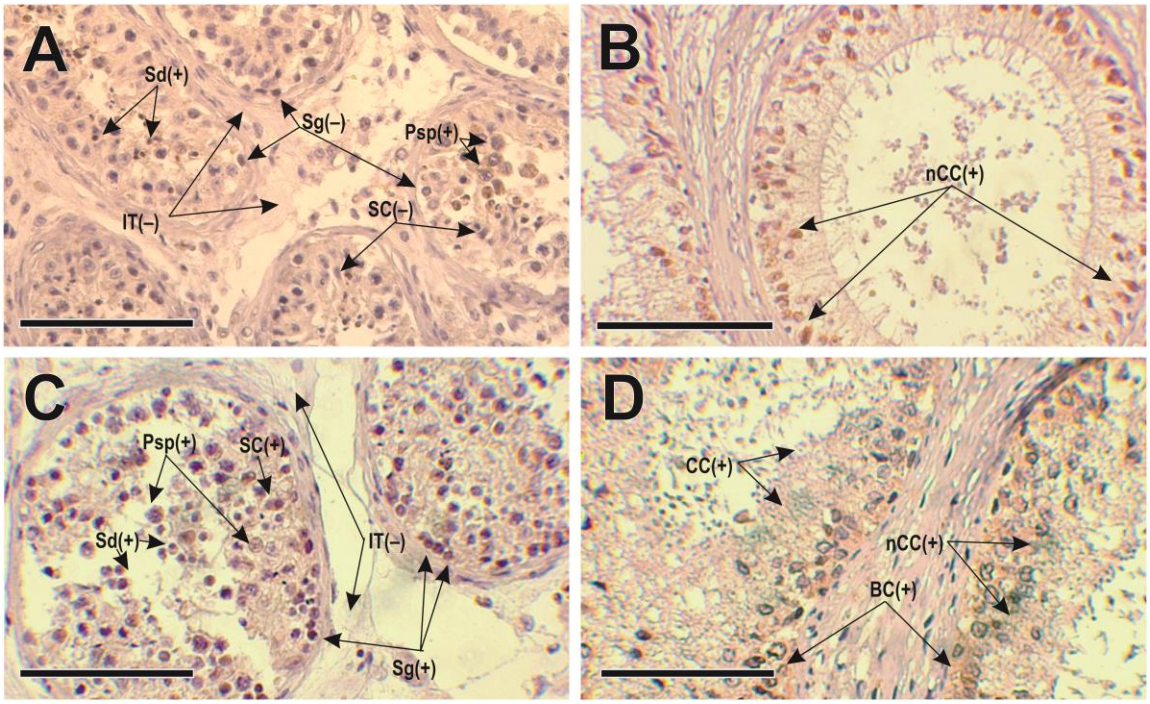
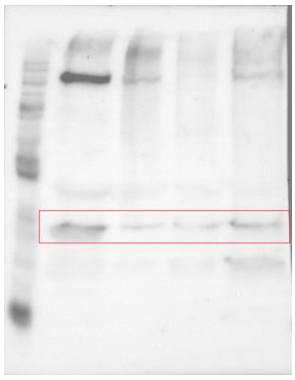


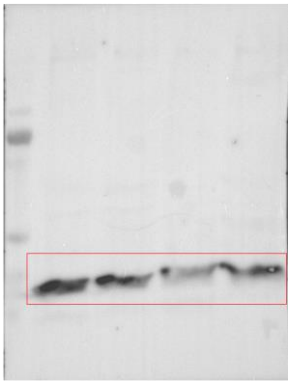
Figure S1. Immunohistochemical localization of ESR1 (A) and ESR2 (C) in the testis and ESR1 (B) and ESR2 (D) in the epididymis. Legend: BC – basal cells, CC – ciliated cells, nCC – non-ciliated cells, IT – interstitial tissue, Psp – primary spermatocytes, SC – Sertoli cells, Sd – spermatids, Sg – spermatogonia, (+) – positive immunostaining, (–) – negative immunostaining. Scale bar: 200 μm.

Additional information: Full-length blots. Note that the cropped areas displayed in the composite manuscript figures are marked in red.

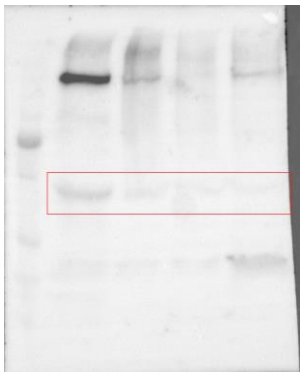
Original gels for Figure 5:



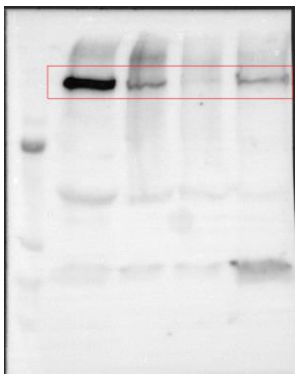
GAPDH



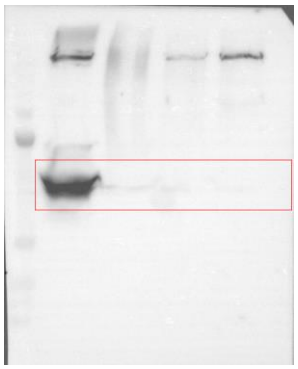
ESR1



ESR2



PELP1



SRC

pSRC

MIQE checklist and data set

ITEM TO CHECK	IMPORTANCE	Comments
EXPERIMENTAL DESIGN		
Definition of experimental and control groups	**E**	Materials and methods
Number within each group	**E**	Materials and methods
Assay carried out by core lab or investigator's lab?	D	Yes
Acknowledgement of authors' contributions	D	Yes
SAMPLE		
Description	**E**	Materials and methods
Volume/mass of sample processed	D	Materials and methods
Microdissection or macrodissection	**E**	NA
Processing procedure	**E**	Materials and methods
If frozen - how and how quickly?	**E**	Materials and methods
If fixed - with what, how quickly?	**E**	Materials and methods
Sample storage conditions and duration (especially for FFPE samples)	**E**	Materials and methods
NUCLEIC ACID EXTRACTION		
Procedure and/or instrumentation	**E**	Materials and methods
Name of kit and details of any modifications	**E**	Materials and methods
Source of additional reagents used	D	Materials and methods
Details of DNase or RNAse treatment	**E**	Materials and methods
Contamination assessment (DNA or RNA)	**E**	Materials and methods
Nucleic acid quantification	**E**	Materials and methods
Instrument and method	**E**	Materials and methods
Purity (A260/A280)	D	Materials and methods
Yield	D	Materials and methods
RNA integrity method/instrument	**E**	Materials and methods
RIN/RQI or Cq of 3' and 5' transcripts	**E**	NA
Electrophoresis traces	D	Materials and methods
Inhibition testing (Cq dilutions, spike or other)	**E**	Materials and methods/Standard curves
REVERSE TRANSCRIPTION		
Complete reaction conditions	**E**	Materials and methods
Amount of RNA and reaction volume	**E**	Materials and methods
Priming oligonucleotide (if using GSP) and concentration	**E**	NA
Reverse transcriptase and concentration	**E**	Materials and methods
Temperature and time	**E**	Materials and methods
Manufacturer of reagents and catalogue numbers	D	Materials and methods
Cqs with and without RT	D*	Materials and methods
Storage conditions of cDNA	D	-20˚C (not longer than one week) or immediate use
qPCR TARGET INFORMATION		

ITEM TO CHECK	IMPORTANCE	Comments
If multiplex, efficiency and LOD of each assay.	E	NA
Sequence accession number	E	Materials and methods
Location of amplicon	D	NA
Amplicon length	E	Materials and methods
In silico specificity screen (BLAST, etc)	E	Materials and methods
Pseudogenes, retropseudogenes or other homologs?	D	NA
Sequence alignment	D	NA
Secondary structure analysis of amplicon	D	NA
Location of each primer by exon or intron (if applicable)	E	Materials and methods
What splice variants are targeted?	E	Materials and methods
qPCR OLIGONUCLEOTIDES		
Primer sequences	E	Materials and methods or NA
RTPrimerDB Identification Number	D	NA
Probe sequences	D	Commercial pre-designed assays
Location and identity of any modifications	E	Materials and methods
Manufacturer of oligonucleotides	D	Materials and methods
Purification method	D	HPLC
qPCR PROTOCOL		
Complete reaction conditions	E	Materials and methods
Reaction volume and amount of cDNA/DNA	E	Materials and methods
Primer, (probe), Mg++ and dNTP concentrations	E	Materials and methods
Polymerase identity and concentration	E	Materials and methods
Buffer/kit identity and manufacturer	E	Materials and methods
Exact chemical constitution of the buffer	D	NA
Additives (SYBR Green I, DMSO, etc.)	E	NA
Manufacturer of plates/tubes and catalog number	D	Materials and methods
Complete thermocycling parameters	E	Materials and methods
Reaction setup (manual/robotic)	D	Manual
Manufacturer of qPCR instrument	E	Materials and methods
qPCR VALIDATION		
Evidence of optimization (from gradients)	D	NA
Specificity (gel, sequence, melt, or digest)	E	NA
For SYBR Green I, Cq of the NTC	E	NA
Standard curves with slope and y-intercept	E	Materials and methods
PCR efficiency calculated from slope	E	Materials and methods/Standard curve
Confidence interval for PCR efficiency or standard error	D	Materials and methods/Standard curve
r2 of standard curve	E	Standard curve
Linear dynamic range	E	NA
Cq variation at lower limit	E	Materials and methods/Duplicates

ITEM TO CHECK	IMPORTANCE	Comments
Confidence intervals throughout range	D	NA
Evidence for limit of detection	**E**	Yes (Decimal dilutions)
If multiplex, efficiency and LOD of each assay	**E**	NA
DATA ANALYSIS		
qPCR analysis program (source, version)	**E**	Materials and methods
Cq method determination	**E**	Yes
Outlier identification and disposition	**E**	Yes
Results of NTCs	**E**	Yes
Justification of number and choice of reference genes	**E**	Materials and methods
Description of normalization method	**E**	Materials and methods
Number and concordance of biological replicates	D	Materials and methods
Number and stage (RT or qPCR) of technical replicates	**E**	Materials and methods
Repeatability (intra-assay variation)	**E**	Yes
Reproducibility (inter-assay variation, %CV)	D	NA
Power analysis	D	NA
Statistical methods for result significance	**E**	Throughout Manuscript, Supplementary materials

E – essential information, D – desirable information, NA – not applicable or not available