

Supplementary Figures

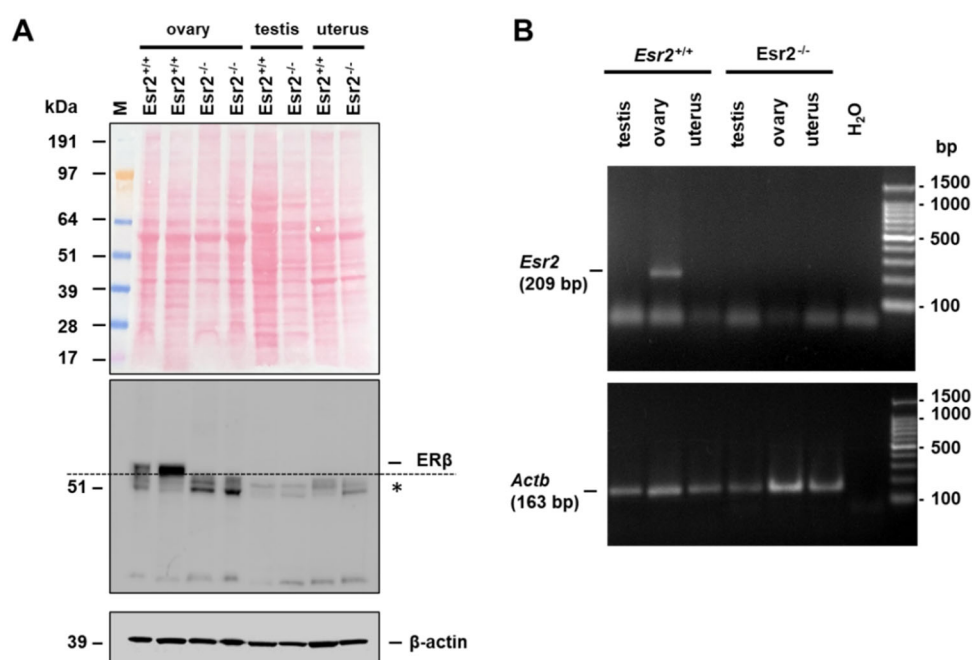
# Immunohistochemical Detection of Estrogen Receptor-Beta (ER $\beta$ ) with PPZ0506 Antibody in Murine Tissue: From Pitfalls to Optimization

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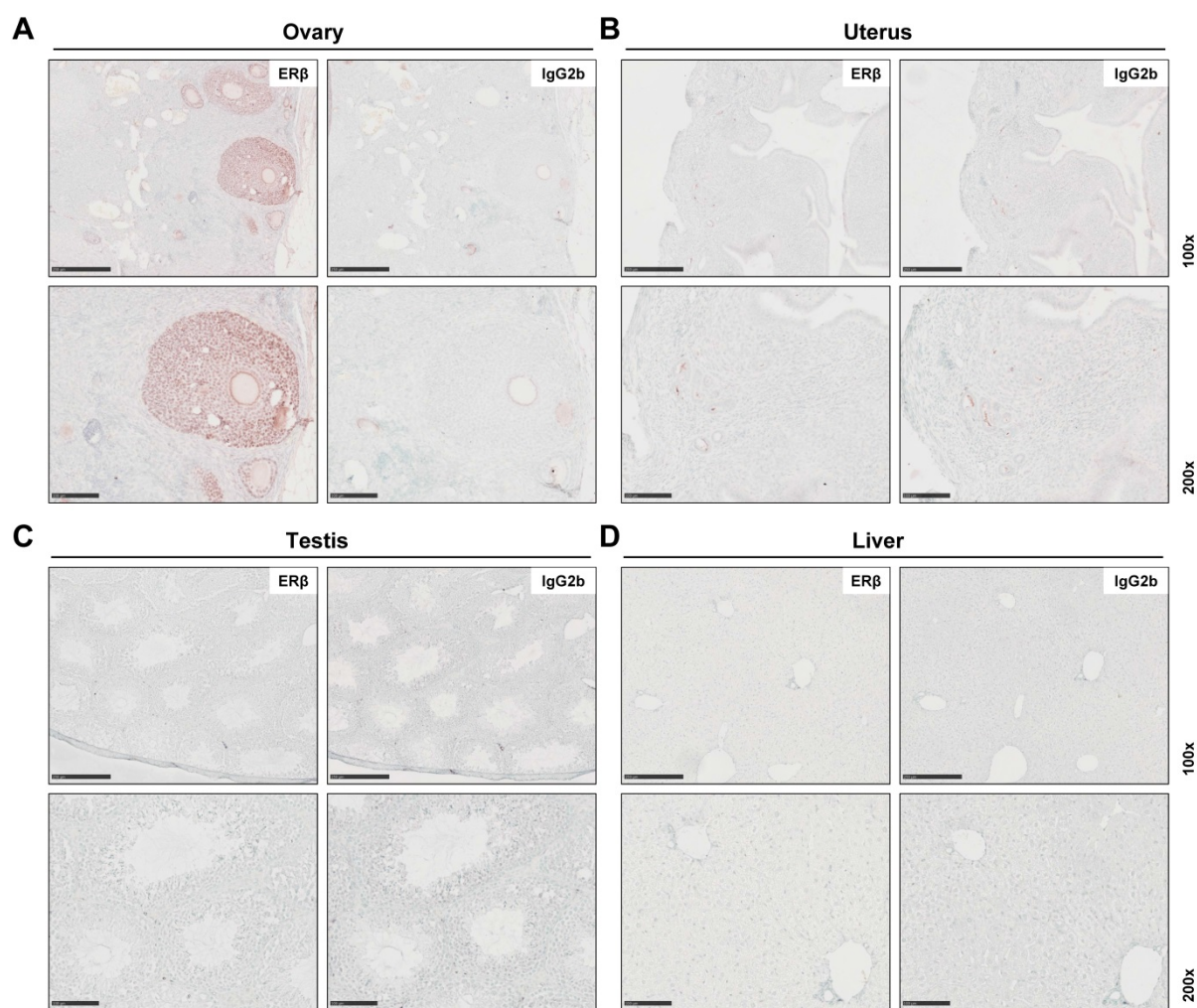
		region targeted by PPZ0506		
hER $\beta$	1	MDIKNSPSSSLNSPSSYNCSQSILPLEHGSIIYIPSSYVDSHHEYFAMTFYSPAVMNYSTPS	60	
mER $\beta$	1	MEIKNSPSSSLTSPASYNCSQSILPLEHGPDIYIPSSYVESRHEYSAMTFYSPAVMNYSVPS	60	
rER $\beta$	1	MEIKNSPSSLSPPASYNCSQSILPLEHGPDIYIPSSYVDNRHEYSAMTFYSPAVMNYSVPC	60	
hER $\beta$	61	NVTNLEGGPCROTTSFNVLWPTSGHLSPLLVVHRQLSHLYAEPQKSPWCEARSLEHTLPVN	120	
mER $\beta$	61	STCNLEGGPVROTASPNVLWPTSGHLSPLATHCQSSLLYAEPQKSPWCEARSLEHTLPVN	120	
rER $\beta$	61	STSNLDGGPVRLSTSPNVLWPTSGHLSPLATHCQSSLLYAEPQKSPWCEARSLEHTLPVN	120	
hER $\beta$	121	RETLKRKVSNGRCASPVTGPGSKRDAHFCAVCSGYHYGVWSCEGCKAFFKRSIQGH	180	
mER $\beta$	121	RETLKRKLGGSGCASPVTSPAKRDAHFCAVCSGYHYGVWSCEGCKAFFKRSIQGH	180	
rER $\beta$	121	RETLKRKLSGSSCASPVTSPNAKRDAHFCAVCSGYHYGVWSCEGCKAFFKRSIQGH	180	
hER $\beta$	241	CAGKAKRSGGHAPRVRELLDALSPQLVLTLLAEPPHVLISRPSAPFTEASMMMSLTk	300	
mER $\beta$	241	CLNKAKRTSGHTPRVKELLNSLSPEQLVLTLLAEPPNVLSRPSMPFTEASMMMSLTk	300	
rER $\beta$	241	CLSKAKRNGGHAPRVKELLLSTLSPEQLVLTLLAEPPNVLSRPSMPFTEASMMMSLTk	300	
hER $\beta$	301	LADKELVHMISWAKKIPGFVELSLFDQVRLLESCWMEVLMGLMWRSIDHPGKLI FAPDL	360	
mER $\beta$	301	LADKELVHMIGWAKKIPGFVELSLDQVRLLESCWMEVLMVGLMWRSIDHPGKLI FAPDL	360	
rER $\beta$	301	LADKELVHMIGWAKKIPGFVELSLDQVRLLESCWMEVLMVGLMWRSIDHPGKLI FAPDL	360	
hER $\beta$	361	VLDRDEGKCVEGILEIFDMLLATTSRFRELKLQHKYLCVKAMILLNSSMYPLVTATQDA	420	
mER $\beta$	361	VLDRDEGKCVEGILEIFDMLLATTSRFRELKLQHKYLCVKAMILLNSSMYPLATASQEA	420	
rER $\beta$	361	VLDRDEGKCVEGILEIFDMLLATTSRFRELKLQHKYLCVKAMILLNSSMYPLASANQEA	420	
hER $\beta$	421	DSSRKLHLLNAVTDALVWVIAKSGISSQQQSMRLANLLMLLSHVRHASNKGMEHLLNMK	480	
mER $\beta$	421	ESSRKLTHLLNAVTDALVWVIAKSGISSQQQSVRLANLLMLLSHVRHISNKGMEHLLSMK	480	
rER $\beta$	421	ESSRKLTHLLNAVTDALVWVIAKSGISSQQQSVRLANLLMLLSHVRHISNKGMEHLLSMK	480	
hER $\beta$	481	CKNVVPVYDILLEMLNAHVLRGCKSSITGSECSPAEDSKSKEGSQNLQSQ	530	
mER $\beta$	481	CKNVVPVYDILLEMLNAHTLRGYKSSISGSECCSTEDSKSKEGSQNLQSQ	530	
rER $\beta$	481	CKNVVPVYDILLEMLNAHTLRGYKSSISGSECSSTEDSKNKESSQNLQSQ	530	

Protein sequence identity	Percentage identity to entire hER $\beta$	Percentage identity to hER $\beta$ (2-88 AA)
mER $\beta$	88.68%	83.91%
rER $\beta$	88.30%	80.46%

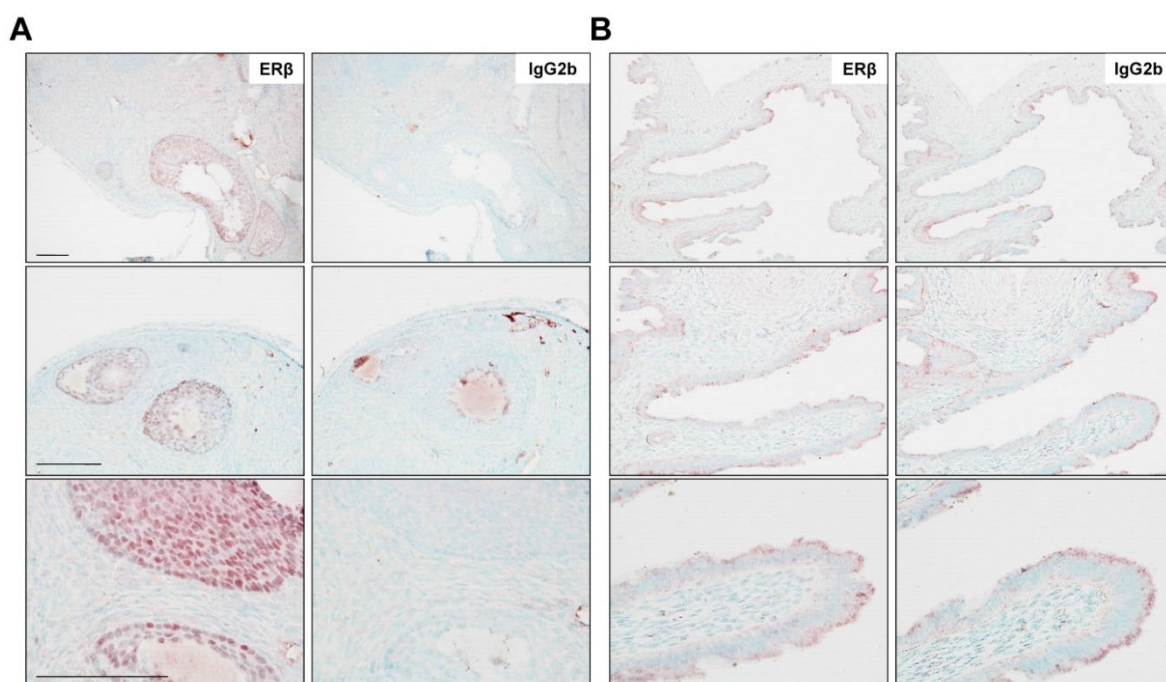
**Figure S1.** Alignment of human and rodent estrogen receptor beta (ER $\beta$ ) protein sequences. The entire protein sequences of mER $\beta$  and rER $\beta$  were aligned to hER $\beta$  using BLAST software. The N-terminal region containing the epitope recognized by the PPZ0506 antibody in hESR2 (2-88 amino acids (AA)) is labeled. The colors show the properties of the respective amino acid groups, as negatively charged at pH 7.0 (yellow), polar (green), non-polar (grey), affecting structure of the peptide (red), and positively charged at pH 7.0 (turquoise) in the antibody binding region. In addition, the percentages of identity of rodent ER $\beta$  to human ER $\beta$  (hER $\beta$ ) are displayed.



**Figure S2.** Estrogen receptor beta (ERβ) expression in reproductive tissues of *Esr2*<sup>+/+</sup> and *Esr2*<sup>-/-</sup> animals. Tissues were collected and processed for protein or mRNA analysis as described in Material and Methods. **(A)** ERβ protein expression was analyzed by western blot analysis in ovarian, uterine and testis tissue extracts using monoclonal PPZ0506 antibody. Ponceau S stain was used to confirm the successful protein transfer and re-probing with β-actin served to document equal protein loading in each lane. ERβ protein could only be detected in *Esr2*<sup>+/+</sup> ovaries but not in *Esr2*<sup>-/-</sup>. The asterisk (\*) indicates non-specific bands, proven by knockout animals where entire *Esr2* has been removed. **(B)** In line, RT-PCR amplified a 206-bp amplicon for *Esr2* in *Esr2*<sup>+/+</sup> ovary that was absent in all other tissues analyzed. Amplification of a 163-bp *Actb* fragment served as a control to document integrity of cDNA used as template.



**Figure S3.** Estrogen receptor beta (ER $\beta$ ) protein expression in different tissues. The expression of ER $\beta$  protein was studied in murine ovarian (A), uterine (B), testis (C) and liver tissue (D) with monoclonal PPZ0506 (1:6000) antibody or with isotype-specific IgG2b negative control. Staining was performed using the optimized protocol and includes a HIER step as described in Materials and Methods. Please note that only in ovarian tissue (A) ER $\beta$ -positive cells were observed. Scale bars: 250  $\mu$ m (100x) or 100  $\mu$ m (200x).



**Figure S4.** Mouse monoclonal IgG<sub>2b</sub> was used as a negative control for PPZ0506 antibody. Murine ovaries (A) or uterine tissue (B) were processed as described in Material and Methods using the optimized protocol including HIER. Slices were incubated either with PPZ0506 (diluted 1:6000) or with IgG<sub>2b</sub> as a negative control (same concentration as primary antibody). Please note that ovaries, incubated with IgG<sub>2b</sub> do not show any specific nuclear staining for estrogen receptor beta (ERβ) in the granulosa cells. In uterine tissue, there is no specific staining and slight off-target signals are only visible. Scale bars 100 μm.