Supplemental material and methods:

Generation of transgenic ERβ-OE mice

A novel mouse strain allowing inducible expression of mouse ER beta (tetO-ERbeta) was generated by targeted transgenesis in the permissive *hprt* locus as previously described [1]. Briefly, the full length coding sequence of the mouse ERbeta (kindly provided by P. Furth) was subcloned into the EcoRV site located between the tetO promoter and the rabbit beta-globin polyadenylation sequence of the bidirectional tet-inducible pBI4 vector [2] and the transgene introduced as a single copy into the *hprt* locus by homologous recombination in embryonic stem (ES) cells. tetO-ERbeta targeted ES cells were then used to generate the tetO-ERbeta transgenic line. The tetO-ERbeta transgenic line was thereafter crossed with the previously characterized alpha MHC-tTA transactivator mouse strain [3] to generate alpha MHC-tTA / tetO-ERbeta double transgenic mice, allowing conditional and inducible cardiomyocyte-specific ERbeta overexpression (thereafter called ERβ-OE). Since cardiac phenotype and function of monotransgenic tetO-ERbeta and alpha MHC-tTA mice did not significantly differ from wild type-littermates (WT, data not shown), we did not include the monotransgenic mice in further analysis, and only the WT-littermates were used as control. ERβ-OE mice were fertile and survived to adulthood. Genotyping was performed routinely by PCR on DNA isolated from tail biopsies by Phire Animal Tissue Direct PCR Kit (Thermo Science) using specific primer sets:

mERβ:
FW: 5´- CTC TGT TTA CAG GCA AGG TGT GTT C - 3´
RV: 5´- AGC TCG GTA CCC GGG TCG A - 3´

mtTA (The Jackson Laboratory):
oIMR8746-FW: 5´- CGC TGT GGG GCA TTT TAC TTT AG - 3´
oIMR8747-RV: 5´- CAT GTC CAG ATC GAA ATC GTCC - 3´
Internal positive control (The Jackson Laboratory):
FW: 5´- CAA ATG TTG CTT GTC TGG TGC - 3´
RV: 5´ GTC AGT CGA GTG CAC AGT TT - 3´

Supplemental results

Estradiol (E2) levels in male and female WT and ERβ-OE mice were measured in blood samples (n=3-4 animals in each group and sex; data are mean ± standard error of the mean) as described earlier [4]. E2 levels in female mice ranged from 24 to 50 pg/ml with an average of 32.5±0.7 and 38±6 pg/ml for female WT and ERβ-OE mice, respectively. These values are in agreement with E2 levels reported by others [5-8]. Serum E2 levels in male mice ranged from 24 to 31 pg/ml with an average of 26.7±1.4 and 27.03±2.3 pg/ml for male WT and ERβ-OE mice, respectively, which is in range as reported by others [9].

Supplemental references

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Supplemental tables

Supplemental table 1: Primers used for Real-Time Polymerase Chain Reaction.

Species	Gene	Sequence (5`to 3`)
Mouse	*RPL0*	FW: CCA TCA TCA ATG GGT ACA AGC
		RV: CAG ATG GAT CAG CCA GGA AG
	Col1A2	FW: TGT AAA CAC CCC AGC GAA GAA
		RV: CTG AGT TGC CAT TTC CTT GGA
	Col3A1	FW: CTC ACC CTT CTT CAT CCC ACT CTT A
		RV: ACA TGG TTC TGG CTT CCA GAC AT
	NPPA	FW: GGG GGT AGG ATT GAC AGG AT
		RV: ACA CAC CAC AAG GGC TTA GG
	Myh6	FW: GCC AAG ACT GTC CGG AAT GA
		RV: TGG AAG ATC ACC CGG GAC TT
	Myh7	FW: CAA AGG CAA GGC AAA GAA AG
		RV: TCA CCC CTG GAG ACT TTG TC
	miR-21	TAGCTTATCAGACTGATGTTGA
	miR-24	GGCTCAGTTCAGCAGG
	miR-27a	CACAGTGGCTAAGTTCCG
	miR-106a	AAGT GCTAACAGTGCAGGTAG

	RNU6B	Hs_RNU6B_2, Qiagen
	RNU1A	Hs_RNU1A_1, Qiagen

Supplemental table 2:

Characteristics at echocardiographic baseline examination of female and male ERβ-OE and WT mice.

	Female Wild type (n=30)	Male Wild type (n=32)	Female ERβOE (n=36)	Male ERβOE (n=25)	Sex effect	ERβ effect
Clinical characteristics						
Age (weeks)	10.4±0.2	10.7±0.2	10.5±0.2	10.4±0.3	NS	NS
Body weight (g)	21.2±0.2	26.7±0.3	21.1±0.2	26.6±0.6	<0.0001	NS
Heart Rate (bpm)	456±2	458±2	458±2	455±2	NS	NS
LV Morphology M-Mode						
IVSD (mm)	0.62±0.01	0.67±0.01	0.63±0.01	0.67±0.01	<0.0001	NS
PWTD (mm)	0.61±0.01	0.67±0.02	0.62±0.02	0.67±0.02	<0.001	NS
LVDD (mm)	3.79±0.05	4.21±0.06	3.86±0.03	4.17±0.06	<0.0001	NS
LV Morphology Long Axis View						
Average wall thickness (mm)	0.62±0.01	0.65±0.02	0.63±0.01	0.66±0.01	<0.001	NS
LVEDV (µl)	52.1±1.4	67.3±1.7	51.8±0.9	67.1±1.7	<0.0001	NS
LVEDV / TL (µl/mm)	3.12±0.09	3.92±0.10	3.14±0.05	3.90±0.09	<0.0001	NS
LVESV (µl)	17.4±0.6	23.5±1.1	16.9±0.4	23.7±0.8	<0.0001	NS
LV mass (µg)	106±1	125±3	107±2	126±3	<0.0001	NS
LV mass/TL (µg/mm)	6.33±0.09	7.40±0.16	6.38±0.10	7.44±0.15	<0.0001	NS
LV systolic function						
Ejection fraction (%)	66.1±0.8	65.5±1.0	66.0±0.9	66.8±0.9	NS	NS
Tissue Doppler S ($cm.s^{-1}$)	25.3±0.6	25.7±0.7	26.5±0.6	25.7±0.8	NS	NS
LV diastolic function						
Mitral E ($cm.s^{-1}$)	842±23	892±31	857±27	879±30	NS	NS
Mitral A ($cm.s^{-1}$)	420±29	404±21	422±18	428±27	NS	NS
Mitral E/A ratio	2.26±0.26	2.25±0.14	2.16±0.12	2.21±0.20	NS	NS
E wave deceleration time (ms)	26.8±1.0	27.4±1.2	27.3±0.9	27.8±1.6	NS	NS
Tissue Doppler E' ($cm.s^{-1}$)	33.6±1.1	31.8±1.6	34.7±0.9	33.3±1.3	NS	NS
E/E' ratio	26.3±1.0	28.4±1.5	25.3±1.1	27.1±1.0	=0.05	NS

Data are presented as mean ± SEM. ANOVA analysis for sex effect (male vs female) and ERβ effect (ERβOE vs WT). IVSD: interventricular septum diastole; PWTD: posterior wall thickness diastole; LVDD: left ventricular diastolic diameter; LVEDV: left ventricular enddiastolic volume; LVESD: left ventricular endsystolic diameter; TL: tibia length

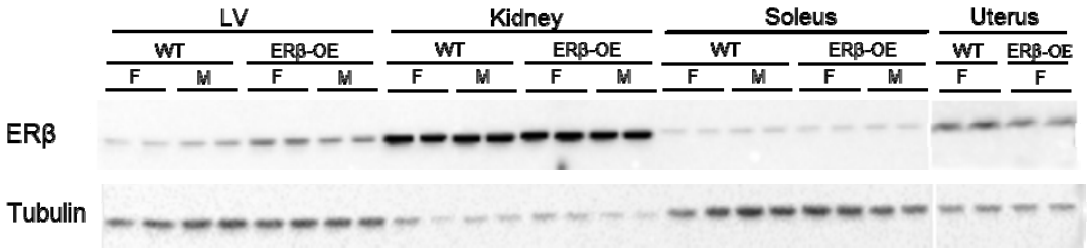
Supplemental figure legends

Figure 1: **Characterization of ERβ-OE mice.** Representative Western blot demonstrate that ERβ protein is overexpressed only in the LV tissue but not in kidney, soleus muscle and uterus of ERβ-OE mice. Uterus was used as positive control.

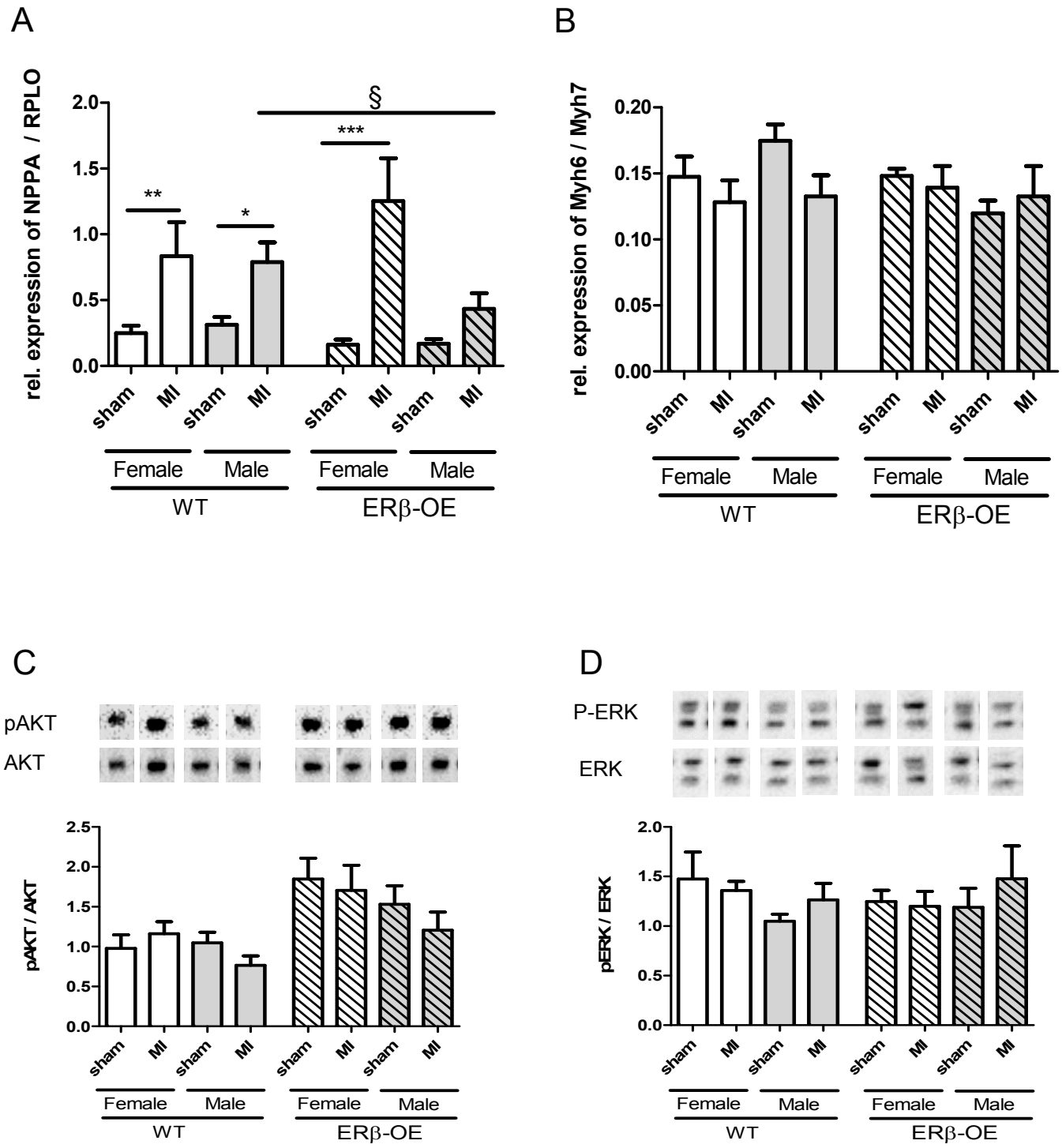
Figure 2: mRNA expression of *NPPA* **(A)**, *Myh6/Myh7* ratio **(B),** and protein levels of p-AKT, AKT **(C)** and p-ERK1/2, ERK1/2 **(D)** in LV tissues of female and male WT and ERβ-OE mice. For all analyses: 2-way ANOVA: MI-effect independent of genotype *p<0.05, **p<0.01, and ***p<0.001; genotype effect independent of sex §p<0.05. Data are mean ± SEM of n≥8/group.

Figure 3: mRNA expression of miR-24 **(A)**, miR-27a **(B)**, and miR-106a **(C)** in LV tissues of female and male WT and ERβ-OE mice. Mean ± SEM of n>8/group.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3