SUPPLEMENTAL MATERIALS AND METHODS

Cardiomyocyte-specific estrogen receptor alpha increases angiogenesis, lymphangiogenesis and reduces fibrosis in the female mouse heart post myocardial infarction

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I) Materials and Methods:

Generation of the ER-alpha conditional mouse model (ERα-OE mice)

The previously described tetO-ERα mouse strain [1] carrying a transgene composed of a coding sequence for murine ERα placed under the regulatory control of a tet-operator promoter (tet-op-ERα mice) (kindly provided by PA Furth, University of Maryland, Baltimore, USA) was crossed with the previously described α-MHCtTA transactivator mouse strain [2] (kindly provided by GI Fishman, Columbia University, NY). This allows to obtain MHCtTA/tetO-ERα double transgenic (ERα-OE) mice with conditional, cardiomyocyte-specific ERα expression. The "control" genotype refers to the ERα-OE littermates (including wild type and mono-transgenic α-MHCtTA and tetO-ERa mice). Genomic DNA was isolated from tail biopsies using Phire animal tissue direct PCR kit (Finnzyme, Thermo Scientific, USA) according the manufacturer's instructions. The presence of the ERα- and tTA-transgenes was detected using following primers by PCR.

<u>mERα:</u>

FW: 5´- CGA GCT CGG TAC CCG GGT CG - 3

RV: 5´- GAA CAC AGT GGG CTT GCT TGT TG - 3´

<u>mtTA (The Jackson Laboratory):</u>

oIMR8746-FW: 5´- CGC TGT GGG GCA TTT TAC TTT AG - 3´

oIMR8747-RV: 5´- CAT GTC CAG ATC GAA ATC GTCC - 3´

<u>Internal positive control (The Jackson Laboratory):</u>

FW: 5´- CAA ATG TTG CTT GTC TGG TGC - 3´

RV: 5´ GTC AGT CGA GTG CAC AGT TT - 3´

The Sizes of PCR-products are as followed: ERα: 350 bp; mtTA: 450 bp: Internal positive control: 200 bp.

II) Table S1:

List of all primers used in this study:

Primers for quantitative Real-Time polymerase Chain Reaction (qRT-PCR): the qRT-PCR was performed with gene-specific, intron-spanning primers. The annealing temperature was 60°C.

Gene	Size (bp)	Sequence
m-VEGFa	94	FW: 5-GCA GCT TGA GTT AAA CGA ACG-3
		FW: 5-GGT TCC CGA AAC CCT GAG-3
m-Col I (procollagen, type I, alpha 2)	124	FW: 5-TAG TGG TCC TCA AGG CAT CC-3
		RV: 5-ACC ATG TAG GCC AGC AAG AC-3
m-Col III (Col3a1)	117	FW: 5-CTC ACC CTT CTT CAT CCC ACT CTT A-3
		RV: 5-ACA TGG TTC TGG CTT CCA GAC AT-3
m-Nppa	140	FW: 5-GGG GGT AGG ATT GAC AGG AT-3
		RV: 5-ACA CAC CAC AAG GGC TTA GG-3
m-Nppb	144	FW: 5-GCA CAA GAT AGA CCG GAT CG-3
		RV: 5-CTT CAA AGG TGG TCC CAG AG-3
m-Myh6	131	FW: 5-GCC AAG ACT GTC CGG AAT GA-3
		RV: 5-TGG AAG ATC ACC CGG GAC TT-3
m-Myh7	149	FW: 5-CAA AGG CAA GGC AAA GAA AG-3
		RV: 5-TCA CCC CTG GAG ACT TTG TC-3
m-Lyve1	91	FW: 5-GAA GCA GCT GGG TTT GGA-3
		RV: 5-CGT AGC AAA CAG CCA GCA C-3
m-Pecam-1 (CD31)	117	FW: 5-GGA CCA CGT GTT AGT GTT TCG-3
		RV: 5-TTG GGA GTG GAG AAG GAC TC-3
m-Hprt	78	FW: 5-GCT TTC CCT GGT TAA GCA GTA CA-3
		RV: 5-ACA CTT CGA GAG GTC CTT TTC AC-3

III) References:

1 Hruska KS, Tilli MT, Ren S, Cotarla I, Kwong T, et al. (2002) Conditional over-expression of estrogen receptor alpha in a transgenic mouse model. Transgenic Res 11: 361-372.

2 Yu Z, Redfern CS, Fishman GI (1996) Conditional transgene expression in the heart. Circ Res 79: 691-697.