

Supplemental Methods and Figures with Legends

METHODS

VSMC proliferation assay: CRT floxed VSMCs were transfected via nucleofection using the primary VSMC Nucleofector Kit from Amaxa Biosystems (Amaxa GmbH, Lonza) in an Amaxa Nucleofector II using program P-024. Following transfection, 2,500 VSMCs were plated per well in 96 well plates containing DMEM (1g/L glucose) with 10% FBS for 24 hours. After 24 hours, media was switched to serum free media for 24 hours. After 24 hours, cells were treated with 100 pM TGF- β in DMEM or 10% FBS in DMEM. Cell number was determined using the Celltiter 96 AQueous cell proliferation assay according to the manufacturer's protocol on days 0 and at 24 and 48 hrs of treatment. Data are expressed as the mean absorbance of 3 wells +/- S.D. from one representative experiment which was repeated 3 times with similar results.

FIGURE LEGENDS

Supplemental Figure 1: Verification of LoxP sites flanking the CRT gene DNA was isolated from the tail of wild type or CRT floxed mice and PCR was performed to detect the presence of LoxP sites. The following primers were used to detect wild type mice (5'-GAGTGGAACACGTC AATTGACAACC-3' and 5'-CTTCTCTGATAAGTTTTCCTCTGACCTC - 3') or CRT floxed mice (5'-GAGTGGAACACGTC AATTGACAACC-3' and 5' - AGGGTTCCGGATCCGATGAAGTTCC - 3'). A ~400bp band is produced in floxed mice using floxed primers following PCR.

Supplemental Figure 2: Validation of Rabbit monoclonal anti-CRT antibody Laemmli cell lysates of wild type and CRT -/- mouse embryonic fibroblasts were immunoblotted for CRT using rabbit monoclonal anti-CRT antibody. This antibody detects one band of approximately 60 kDa in wild type cells and no bands in the CRT -/- cells.

Supplemental Figure 3: 11R-VIVIT blocks TGF- β stimulation of collagen transcript and protein in VSMCs. Rat VSMCs (p3) were pre-treated for 2 hr with 100 nM 11R-VIVIT peptide and then treated with 200 pM TGF- β for 24 hrs. Laemmli cell lysates were immunoblotted for collagen I and β -tubulin. VSMCs (p4) were treated with 1 μ M 11R-VIVIT and then treated with 200 pM TGF- β for 4 hrs. RNA was harvested for QRT-PCR of Col1A1. Results are the means \pm SD (n=3).

Supplemental Figure 4: *CRT knockdown does not affect VSMC proliferation* CRT floxed VSMCs were transfected with 1 μ g GFP or cre-recombinase-IRES-GFP plasmid, grown overnight in DMEM with 10% FBS, and then switched to serum free media for 24 hrs. Cells were treated with serum free DMEM, 10% FBS, or 100 pM TGF- β for 24 or 48 hours, refreshing daily. Cell number was determined using the CellTiter 96 AQueous cell proliferation assay. Data are the average of 3 technical replicates and are representative of experiments repeated on 3 separate occasions with similar results.