

SUPPLEMENTAL MATERIAL

Isolated Nuclei from Atrial Fibroblasts

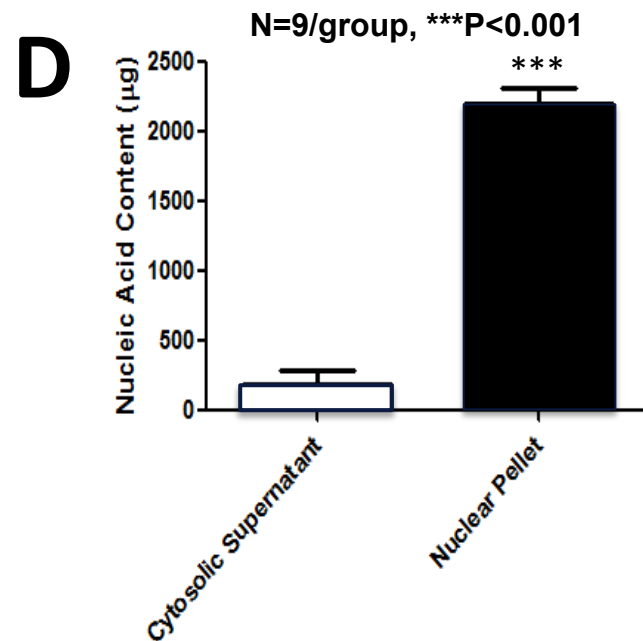
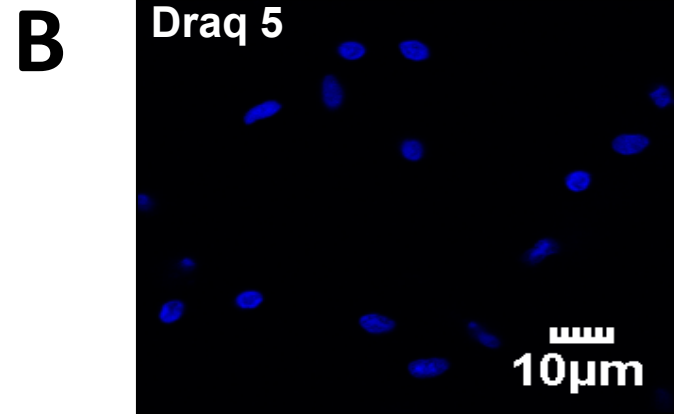
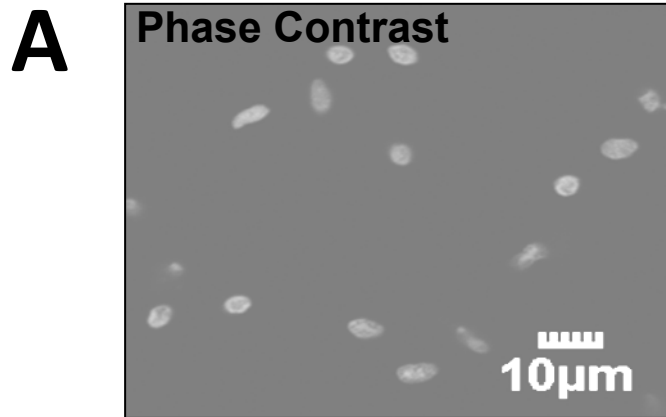


Figure S1. Isolation of nuclei from atrial fibroblasts. Images show isolated nuclei in phase contrast (**A**) or labelled with either with the DNA stain DRAQ5 (**B**) or Alexa Fluor 594-conjugated transferrin, a marker of cell surface membranes (**C**). The histogram (**D**) shows the total nucleic acid content of the cytosolic and the nuclear fractions. Mean±SEM ***P<0.001, N=9.

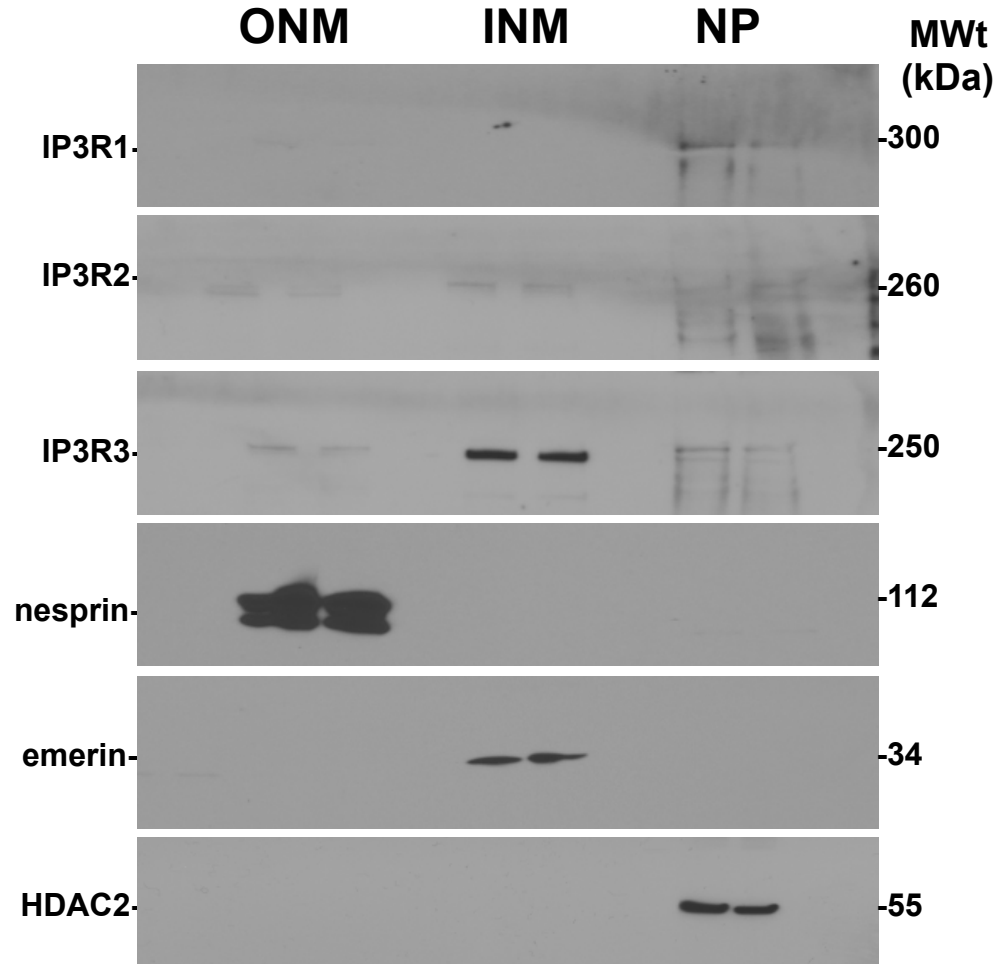


Figure S2. Detection of IP3R isoforms in fibroblast nuclei subfractions. Freshly isolated fibroblast nuclei were separated into outer nuclear membrane (ONM), inner nuclear membrane (INM), and nucleoplasm (NP) as described in Methods. Nesprin, emerlin and HDAC2 immunoreactivity served as markers of the ONM, INM/lamina and NP, respectively. IP3R isoform immunoreactivity was detected in nuclear subfractions.

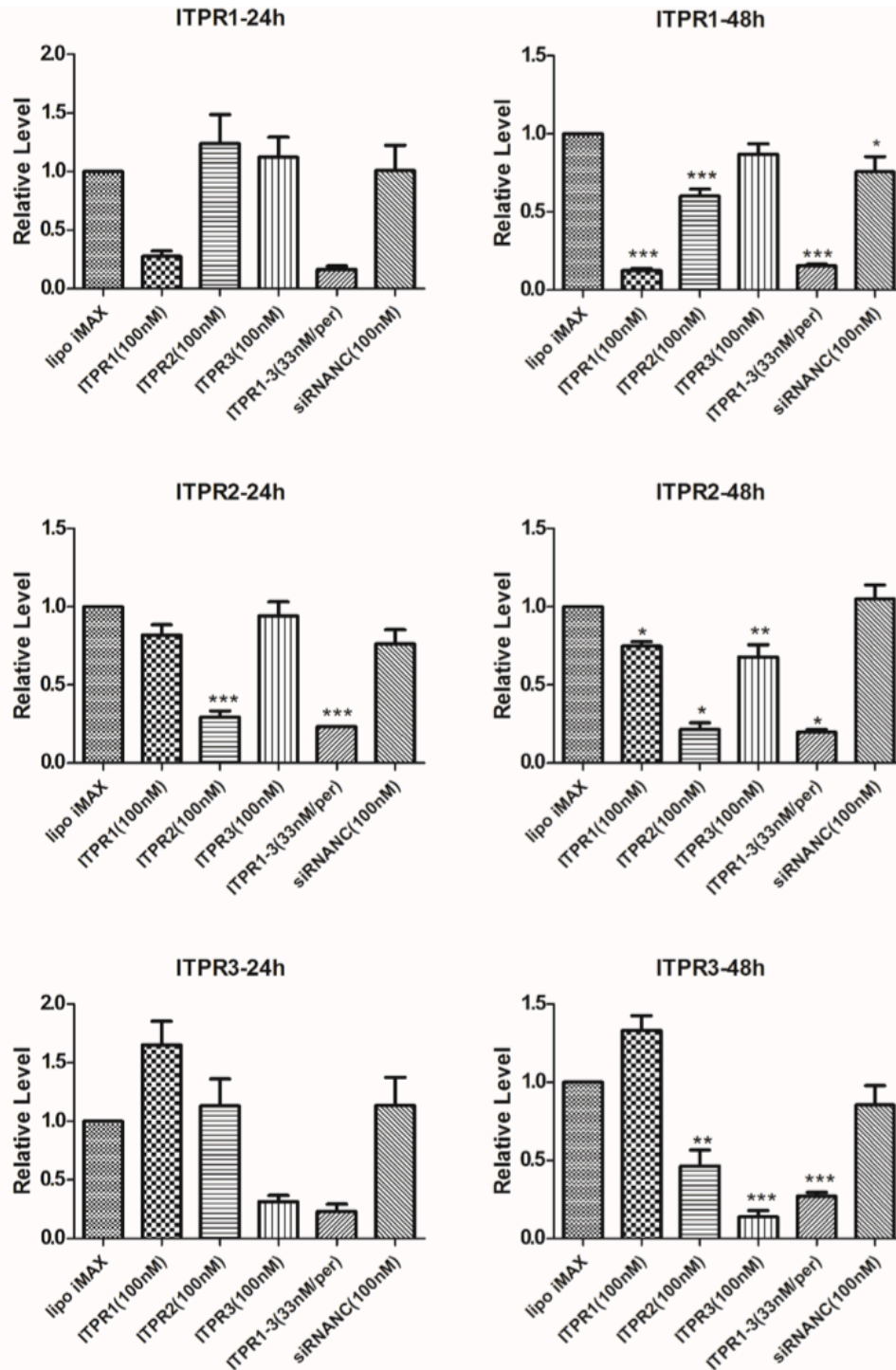


Figure S3. Efficiency of IP3R isoform knockdown by siRNA. Quantification of mRNA encoding IP3R1, IP3R2 and IP3R3 following transfection (24 and 48 hours) of cultured fibroblasts with siRNA negative control (si-NC), or siRNAs targeting the *ITPR1*, *ITPR2*, *ITPR3* or all 3 genes (*si-ITPR1*, *si-ITPR2*, *si-ITPR3*, *si-ITPR1-3* respectively) normalized to transfection with the vector alone. Mean±SEM, *P<0.05, **P<0.01, ***P<0.001 compared to vector alone.

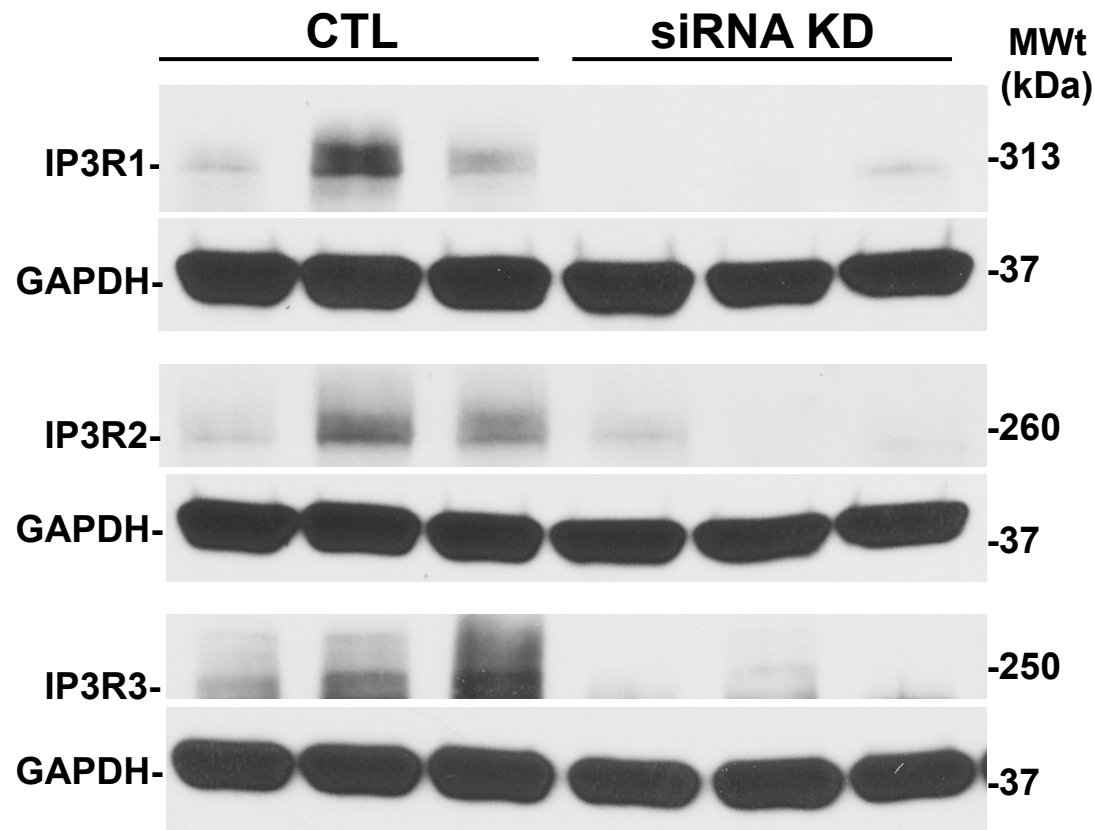


Figure S4. Validation of IP3R siRNA knockdown by immunoblotting. After 48 hours of transfection with si-*ITPR1*, si-*ITPR2* or si-*ITPR3* cells were collected, lysed and run on 7.5% Mini-PROTEAN® TGX™ Precast Protein Gels. Immunoblotting was performed using isoform specific antibodies for IP3R. GAPDH was used as a loading control.