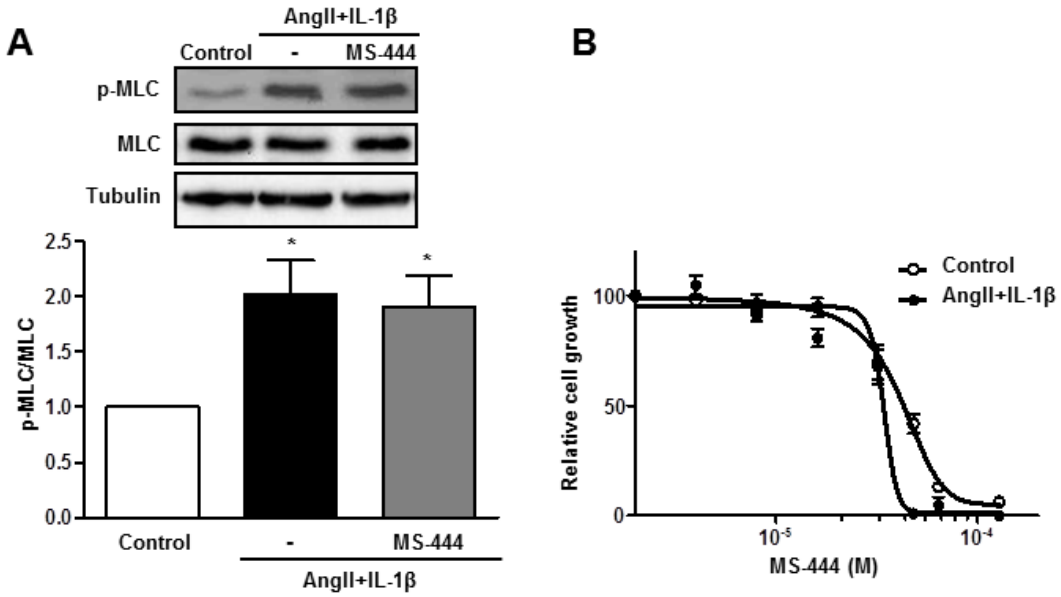
SUPPLEMENTARY MATERIAL

HuR is required for NOX-1 but not NOX-4 regulation by inflammatory stimuli in vascular smooth muscle cells

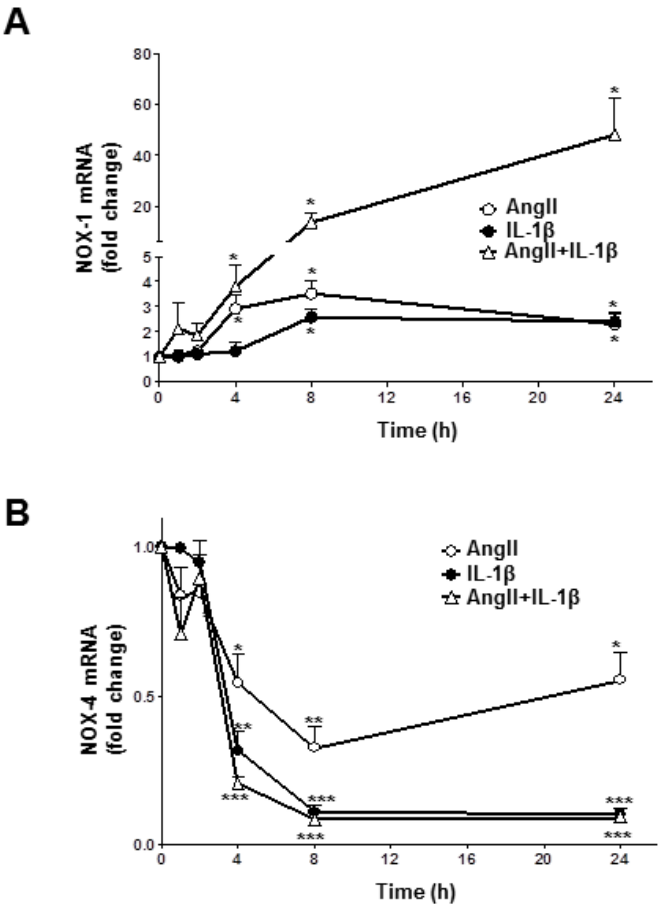
[1]Andrea Aguado, [2]Thierry Fischer, [3]Cristina Rodríguez, [4]Adrian Manea, [3]José Martínez-González, [5]Rhian M. Touyz, [6]Raquel Hernanz, [6]M. Jesús Alonso, [7]Dan A. Dixon, [1]Ana M. Briones, [1]Mercedes Salaices.

Supplementary Fig. S1



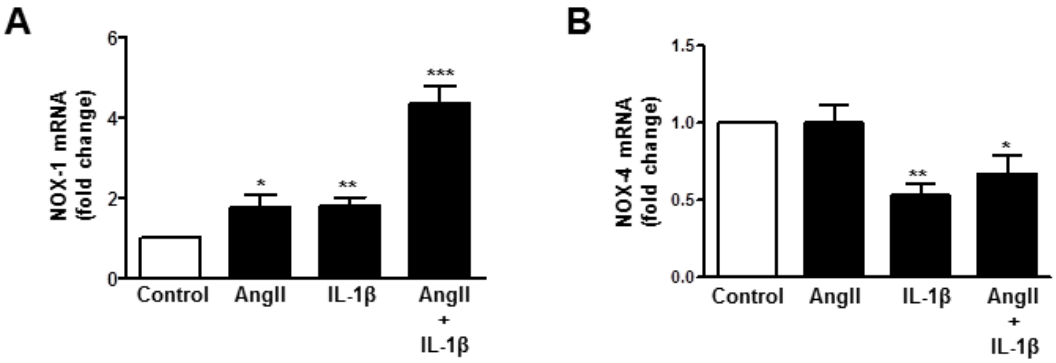
Supplementary Figure S1. (A) Effect of MS-444 on p-myosin light chain (p-MLC) and MLC expression in VSMC treated with AngII+IL-1β (24 h). Representative blots are also shown. (B) Concentration-dependent effect of MS-444 on cell viability in control or AngII+IL-1β-treated VSMC. Data are expressed as mean±SEM. *P<0.05, vs Control. n=5-6.

Supplementary Fig. S2



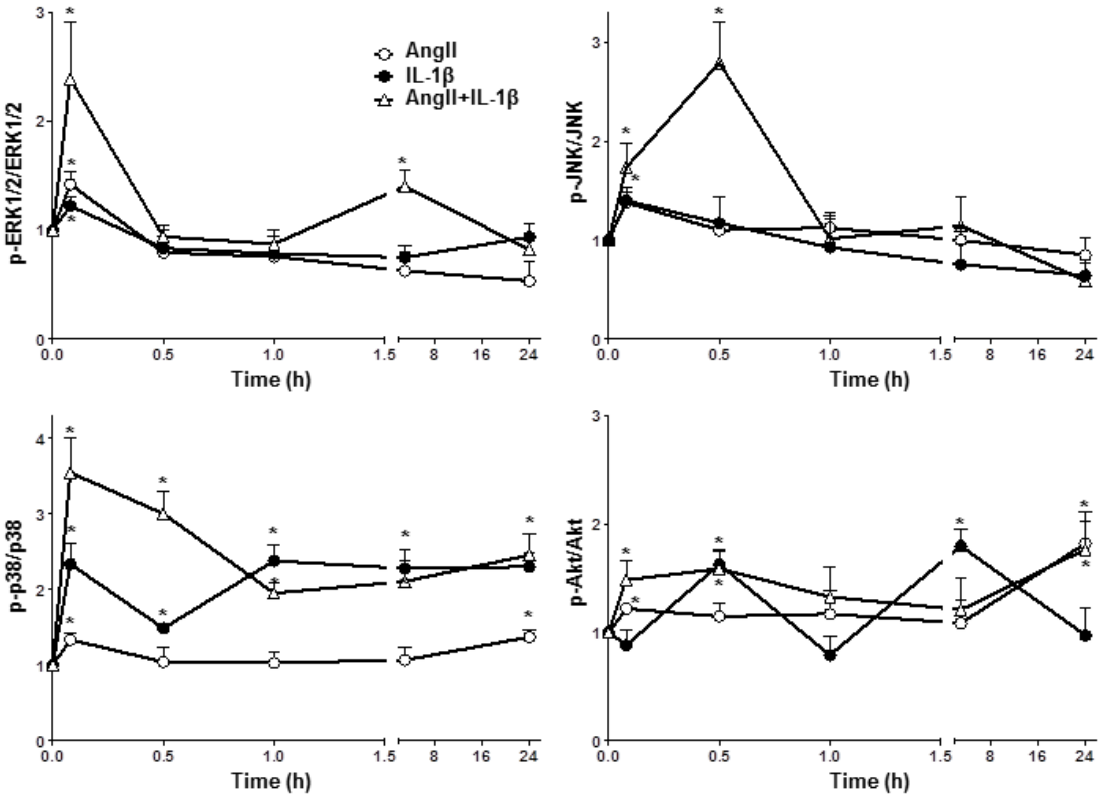
Supplementary Figure S2. Effect of AngII, IL-1β and AngII+IL-1β on NOX-1 and NOX-4 mRNA levels. Time course of NOX-1 (A) and NOX-4 (B) mRNA levels in rat VSMC. Data are expressed as mean±SEM. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.01$ vs Control. n=5-13.

Supplementary Fig. S3



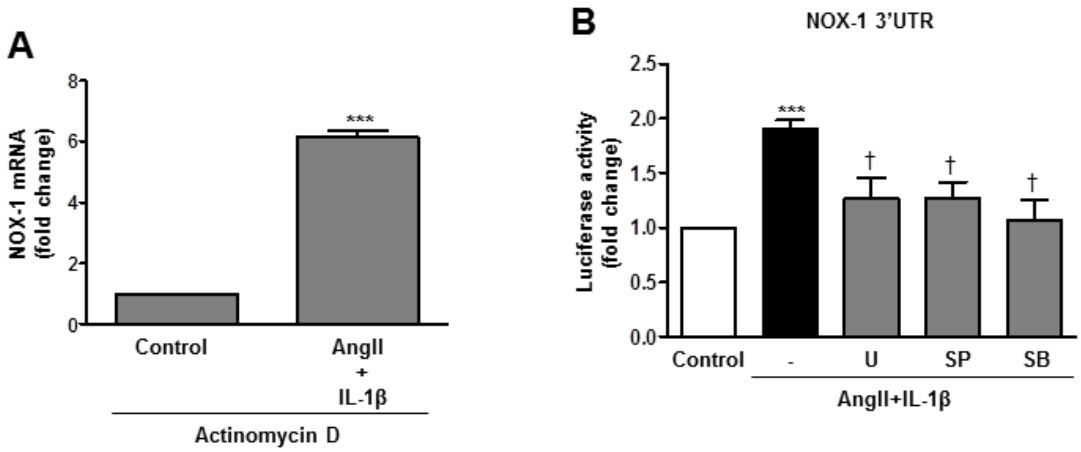
Supplementary Figure S3. AngII potentiates IL-1β-induced NOX-1 expression and does not modify IL-1β-dependent NOX-4 decrease expression in human VSMC. Effect of AngII, IL-1β and AngII+IL-1β on NOX-1 (A) and NOX-4 (B) mRNA levels in human VSMC. Data are expressed as mean±SEM. *P <0.05, **P<0.01, ***P<0.001 vs Control. n=4-8.

Supplementary Fig. S4



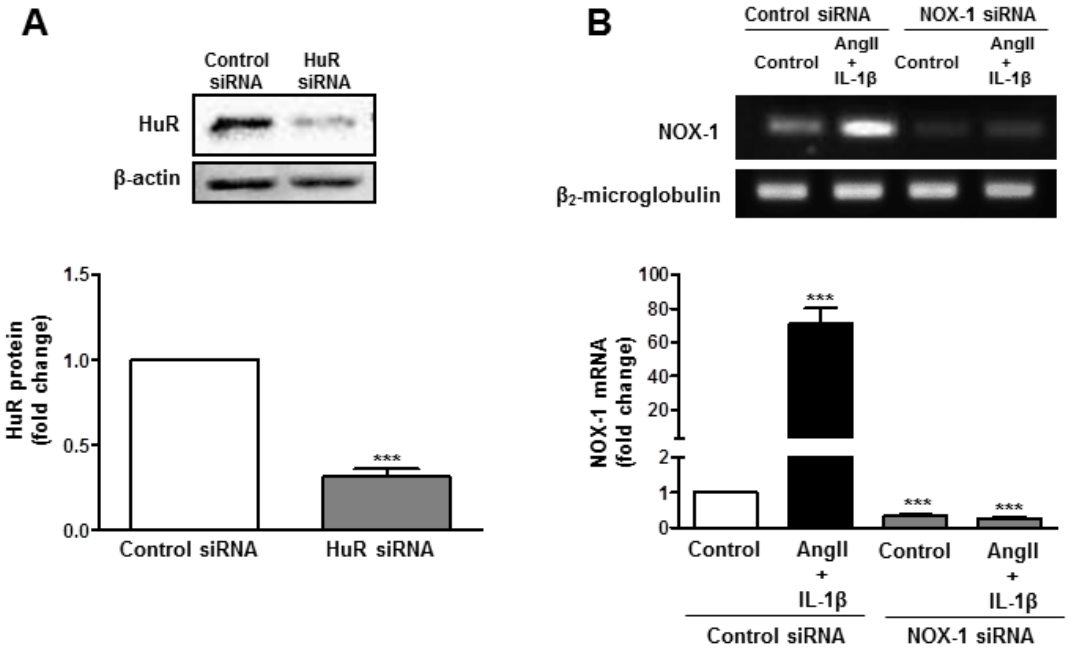
Supplementary Figure S4. Effect of AngII, IL-1β and AngII+IL-1β on ERK1/2, JNK, p38 and Akt activation. Time course of p-ERK1/2, p-JNK, p-p38 MAPK and p-Akt levels in rat VSMC. Data are expressed as mean±SEM. $^{*}P<0.05$, $^{***}P<0.001$ vs unstimulated cells. n=4-7.

Supplementary Fig. S5



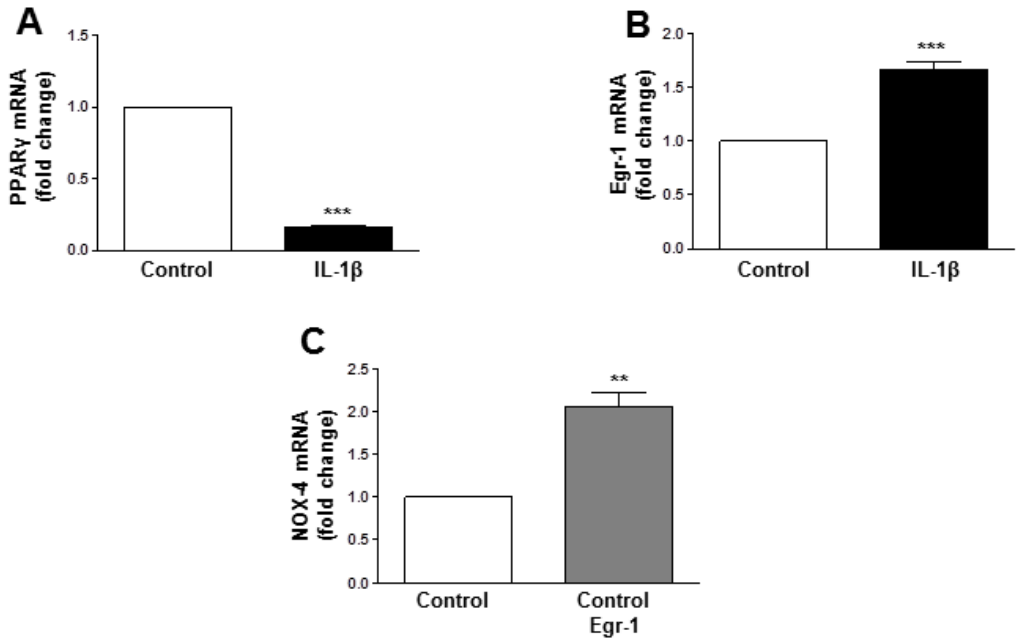
Supplementary Figure S5. (A) NOX-1 mRNA levels in VSMC preincubated with Actinomycin D (30 min) and treated with AngII+IL1β for 24 h. (B) Effect of inhibitors of ERK1/2 (U, U0126), JNK (SP, SP600125) and p38 MAPK (SB, SB203580) on NOX-1 3'UTR luciferase activity induced by AngII+IL-1β (24 h). Data are expressed as mean±SEM. $^{*}P<0.05$, $^{***}P<0.001$ vs unstimulated cells. n=5-6.

Supplementary Fig. S6



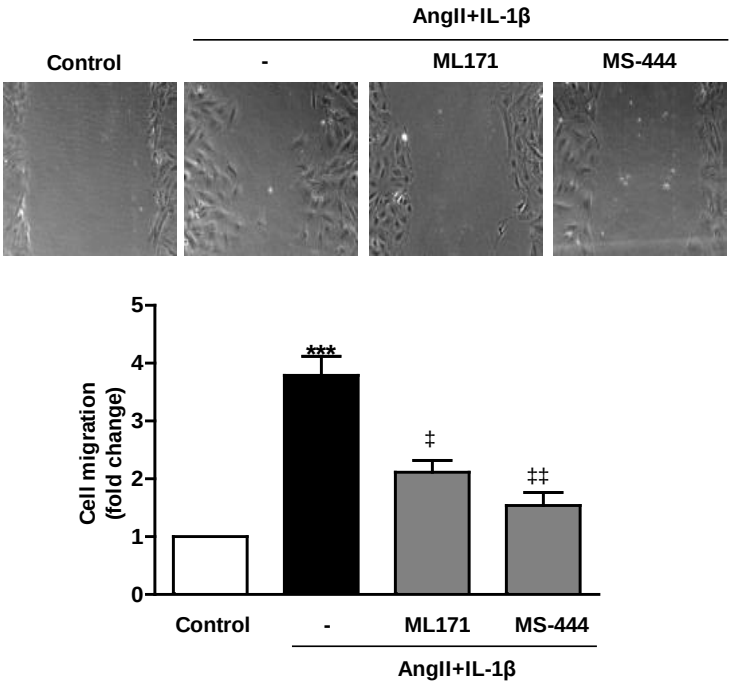
Supplementary Figure S6. (A) HuR protein levels in cells transfected with control or HuR siRNA. (B) NOX-1 mRNA levels in cells transfected with control or NOX-1 siRNA stimulated or not with AngII+IL-1β (24 h). Data are expressed as mean±SEM. ***P<0.001 vs Control or Control siRNA. n=5-7.

Supplementary Fig. S7



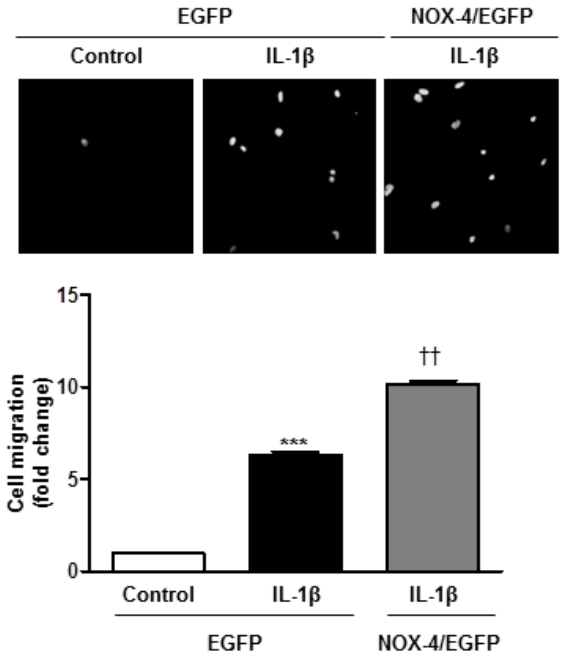
Supplementary Figure S7. Transcription factors that might be involved in NOX-4 repression. Effects of IL-1β (24 h) on PPAR-γ (A) and Egr-1 (B) mRNA levels in rat VSMC. (C) NOX-4 mRNA levels in VSMC transfected with pcDNA3.1 (Control) or Egr-1. Data are expressed as mean±SEM. $^{**}P<0.01$, $^{***}P<0.001$ vs Control. n=6-11.

Supplementary Fig. S8



Supplementary Figure S8. NOX-1 and HuR participate in cell migration induced by AngII+IL-1β. Effect of ML171 and MS-444 on cell migration induced by AngII+IL-1β measured by wound healing assay. Data are expressed as mean±SEM. ***P<0.001 vs Control. ‡P<0.05, ‡‡P<0.01 vs AngII+IL-1β. n=5.

Supplementary Fig. S9



Supplementary Figure S9. NOX-4 participates in cell migration induced by IL-1β. Effect of NOX-4/EGFP overexpression on cell migration induced by IL-1β measured by transwell assays. Data are expressed as mean±SEM. ***P<0.001 vs Control. ††P<0.01 vs EGFP-IL-1β. n=3-5.