

SUPPLEMENTAL SECTION

A novel mitochondrial complex of P450c11AS, StAR and Tom22

metabolizes aldosterone in the heart

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Journal of Pharmacology and Experimental Therapeutics

SUPPLEMENTAL FIGURES

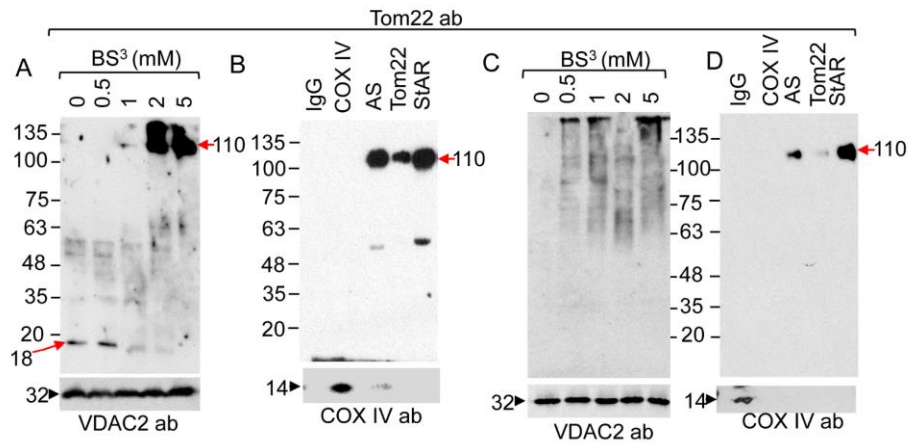


Figure S1

Supplementary Fig. S1

Identification of Tom22 interaction with StAR and P450c11AS by chemical crosslinking following AngII stimulation of H9c2 cells without and with StAR knockdown. (A) *In vivo* chemical crosslinking with the indicated concentrations of BS³ and 20 μ g of mitochondrial protein isolated from AngII-stimulated H9c2 cells. The crosslinking reactions were visualized by immunoblotting with anti-Tom22; 2 mM BS³ gave optimal results. (B) Immunoprecipitation of the crosslinking products obtained with 2 mM BS³ from panel A with IgG, anti-COX IV (COX IV) anti-P450c11AS (AS), anti-Tom22 (Tom22) and anti-StAR (StAR) antibodies independently. The interaction was detected by immunoblotting with anti-Tom22. Bottom, immuno-blot with COX IV antibody. (C) Identification of interactions in mitochondria from H9c2 cells incubated with AngII and siRNA against StAR. *In vivo* chemical crosslinking with the indicated concentrations of BS³ and 20 μ g of mitochondrial protein, seen by immunoblotting with anti-Tom22 antibody. (D) Immunoprecipitation of the crosslinked products obtained with 2 mM BS³ from panel C with IgG, anti-COX IV (COX IV) anti-P450c11AS (AS), anti-Tom22 (Tom22) and anti-StAR (StAR) antibodies independently, followed by immunoblotting with anti-Tom22. Bottom, immuno-blot with COX IV antibody.

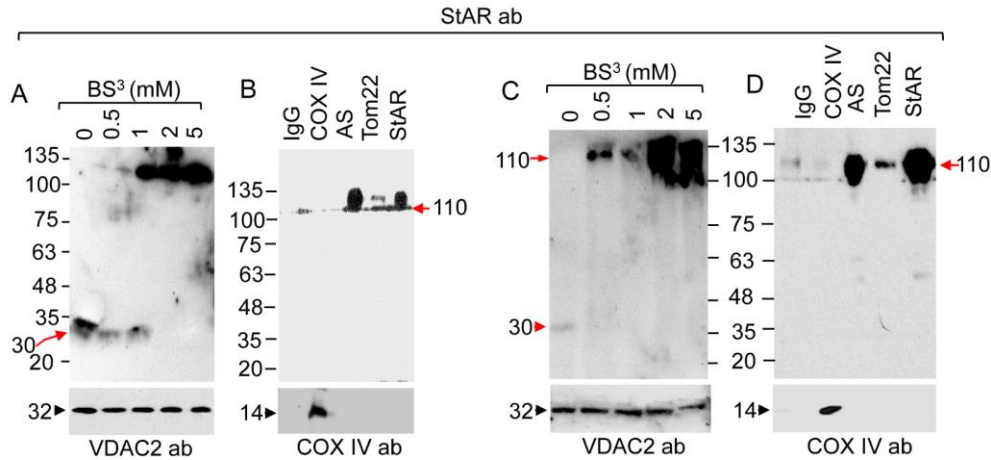


Figure S2

Supplementary Fig. S2

Identification of StAR interaction with P450c11AS and Tom22 by chemical crosslinking following AngII stimulation in presence and absence of siRNA against StAR. (A) *In vivo* chemical crosslinking with the indicated concentrations of BS³ and 20 µg mitochondrial protein isolated from AngII-stimulated H9c2 cells. The crosslinking reactions were visualized by immunoblotting with anti-StAR; 2mM BS³ gave optimal results. (B) Immunoprecipitation of the crosslinking products obtained with 2 mM BS³ from panel A with IgG, anti-COX IV (COX IV) anti-P450c11AS (AS), anti-Tom22 (Tom22) and anti-StAR (StAR) antibodies independently. The interaction was detected by immunoblotting probed with anti-StAR. Bottom, immuno-blot with COX IV antibody. (C) Identification of interactions following stimulation of AngII and StAR knockdown by siRNA. *In vitro* chemical crosslinking with the indicated concentrations of BS³ and 20 µg of mitochondrial protein, seen by immunoblotting with anti-StAR antibody. (D) Immunoprecipitation of the crosslinking products obtained with 2 mM BS³ from panel C with IgG, anti-COX IV (COX IV) anti-P450c11AS (AS), anti-Tom22 (Tom22) and anti-StAR (StAR) antibodies independently, followed by immunoblotting with anti-StAR. Bottom, immuno-blot with COX IV antibody.