

SUPPLEMENTAL MATERIAL

Supplementary Figure Legends

Supplementary Figure 1: *Outline of in vivo experimentation and establishment of pressure overload by tranverse aortic constriction.* A. Experimental timeline. B-C. Establishment of aortic stenosis in response to TAC surgery as noted by increases in flow velocity (B) and pressure gradient (C). n = 10, 10, 12, 14. Two-way ANOVA with repeated measures (time). D. Animal survival by treatment cohort. E. Body weight by treatment cohort. Two-way ANOVA with repeated measures (time). F. Systolic blood pressure by treatment cohort. Two-way ANOVA with repeated measures (time). * = p < 0.05 relative to saline. G. Resting heart rate by treatment cohort. Two-way ANOVA with repeated measures (time). H. Kidney weight/tibia length ratio. One-way ANOVA with repeated measures (time).

Supplementary Figure 2: Myocyte gene expression reveals that pathology-associated gene expression is decreased in SAC/VAL-treated mice, but not those treated with the equivalent dose of valsartan. One-way ANOVA for each gene, Bonferroni post-hoc. n = 5, 5, 6, 7. * p < 0.05 relative to saline. & = p < 0.05 relative to equimolar valsartan.

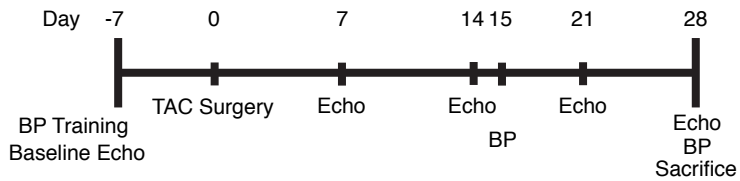
Supplementary Figure 3: *SAC/VAL does not exert obvious effects on the degree of vascularization of the heart.* A. IB4 staining reveals no major alterations in capillary structure in response to either SAC/VAL or valsartan treatment. B. Quantification of image fraction. One-way ANOVA, Bonferroni post-hoc. C. Quantification of inter-capillary distance, which further confirms myocyte hypertrophy (lower IC distance correlates to less hypertrophy). n = 5, 5, 6, 7. One-way ANOVA, Bonferroni post-hoc. * = p < 0.05 relative to saline.

Supplementary Figure 4: *Ces1, the enzyme responsible for converting Sacubitril to active Sacubitrilat, is expressed in nCF.* Ces1 expression suggests that CF might be able to directly

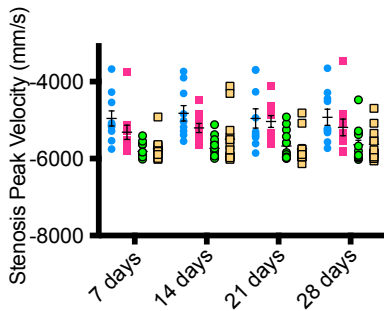
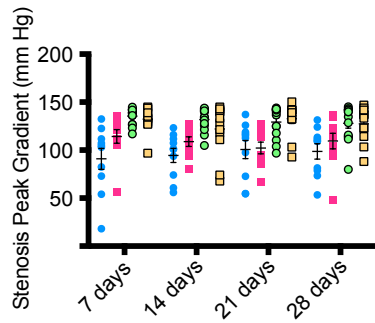
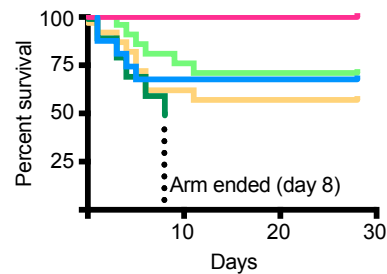
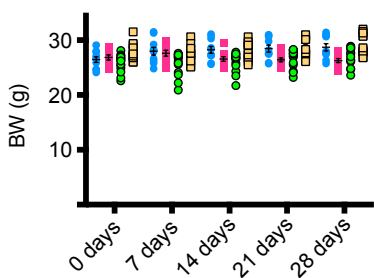
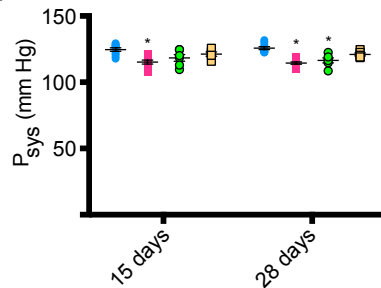
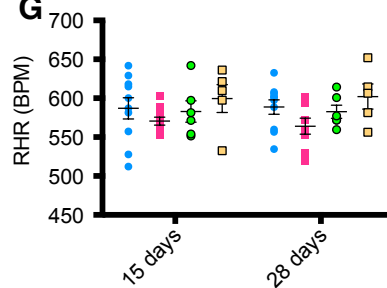
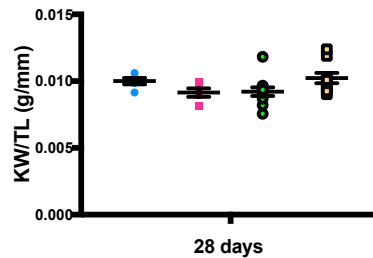
convert Sacubitril to Sacubitrilat *in vivo*. Its expression is insensitive to treatment regimen, suggesting that *Ces1* is still present in stressed CF.

Supplementary Figure 5: *Gene expression in response to Sacubitrilat and valsartan treatment of patient-derived hfCF lines.* qPCR from LVAD-derived CF reveals that CF from four selected patients are inducible by TGF- β 1/AngII as revealed by increased expression of (A) *Acta2* and (B) *Postn*, and decreased expression of *Tcf21*(C). Proliferation-associated gene expression of (D) *Ccnb1* and (E) *Cdkn1a* is less conclusive despite serum starvation prior to stimulation to synchronize cell cycle. n = 3 per condition. * = p < 0.05 relative to vehicle. & = p < 0.05 relative to TGF- β 1/AngII/ANF.

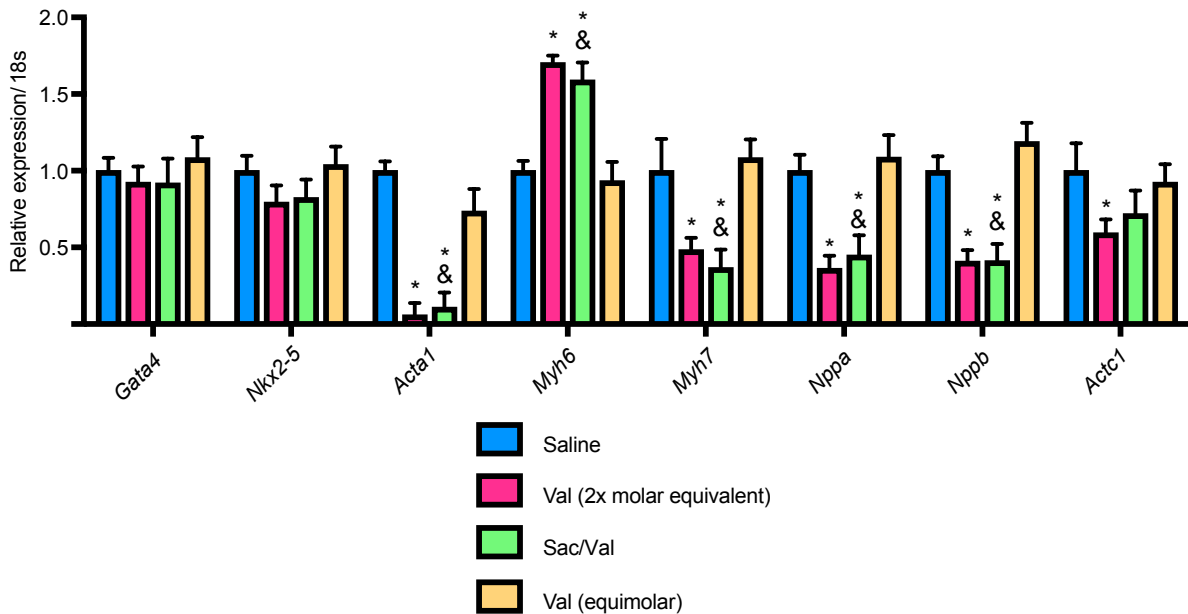
Supplementary Figure 6: *Protein expression in response to Sacubitrilat and valsartan treatment of patient-derived hfCF lines.* A. Myofibroblast activation by TGF- β 1/AngII/ANF is confirmed at the protein level for ACTA2 for each of these patient-derived lines. As predicted by literature, NPRA levels decrease in response to stress, and are generally not recoverable by treatment with valsartan, Sacubitrilat, or the combination. PKG1A protein levels remain constant for all patient-derived lines in response to treatment, confirming that PKG activity, not expression level, is the defining variable for ablation of myofibroblast activation. B-E. Quantification of (A). n = 3 each condition. * = p < 0.05 relative to vehicle. & = p < 0.05 relative to TGF- β 1/AngII/ANF.

A

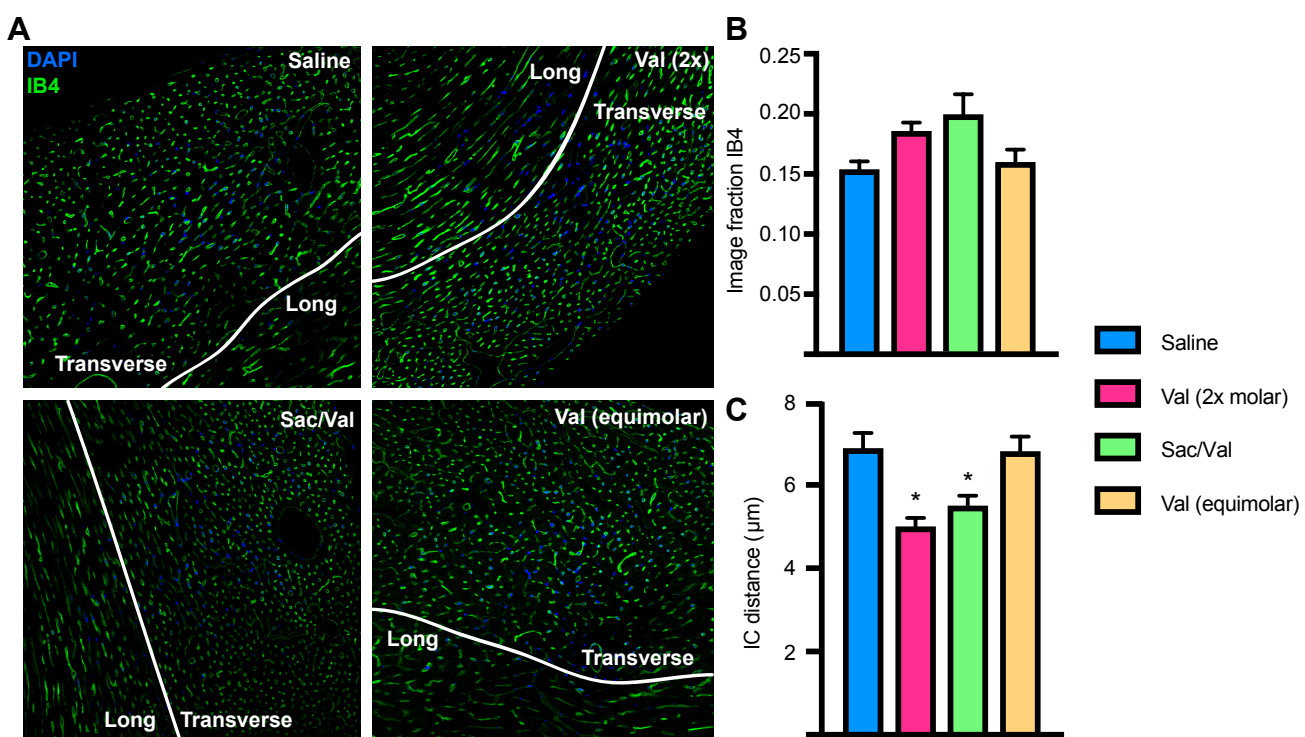
- Saline
- Valsartan (52 mg/kg)
- LCZ696 (57 mg/kg)
- Valsartan (26 mg/kg)

B**C****D****E****F****G****H**

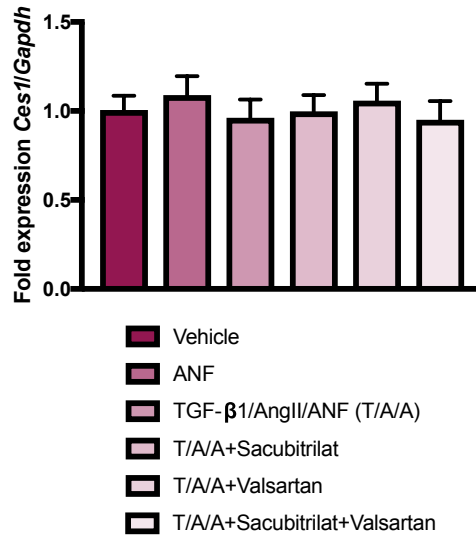
Supplemental Figure 1



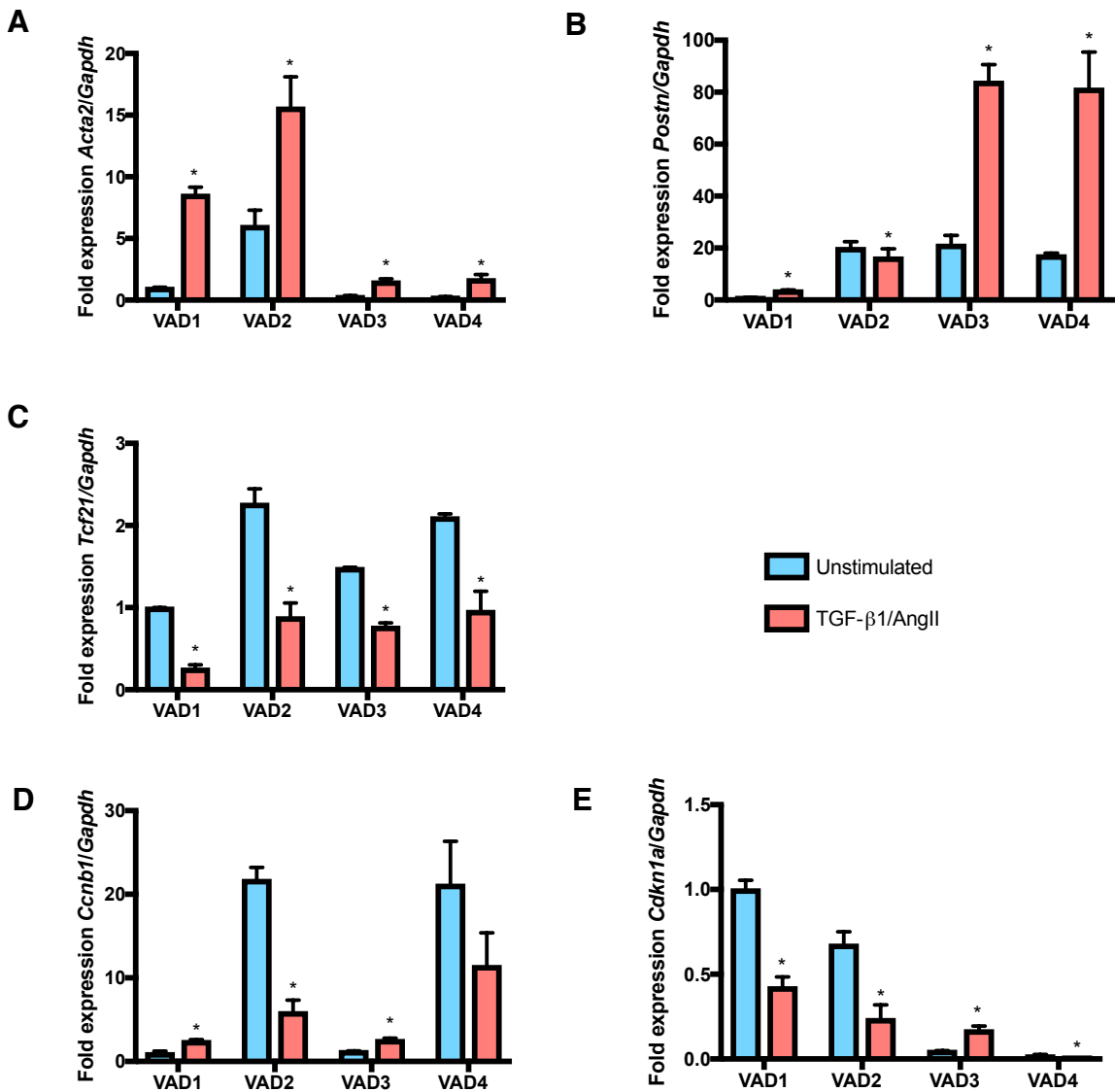
Supplemental Figure 2



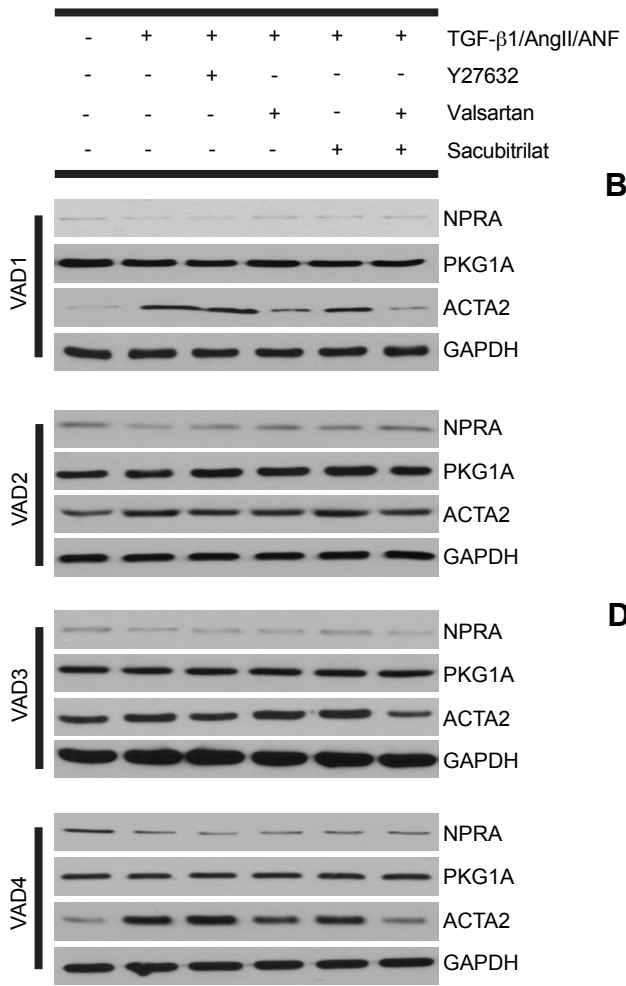
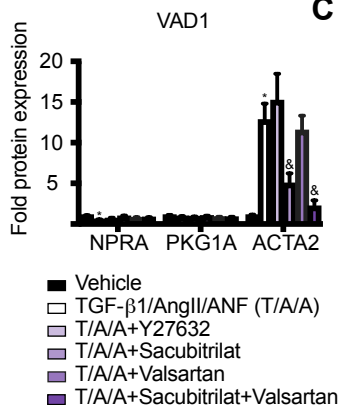
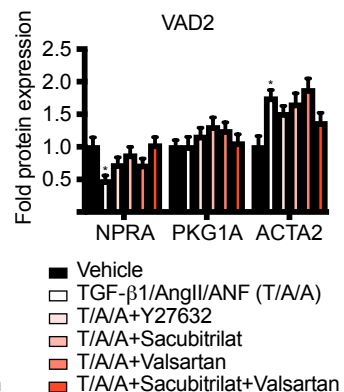
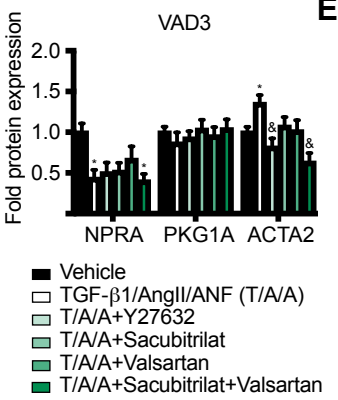
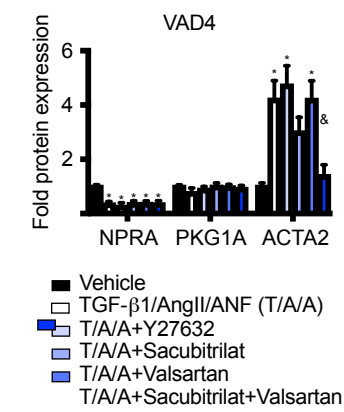
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5

A**B****C****D****E**

Supplemental Figure 6

Supplementary Table 1 – Primary Antibodies

Antibody	Manufacturer	Catalog No.	Application	Dilution
Vimentin	Abcam	ab45939	IF-Paraffin	1:100
WGA-A647	Thermo-Fisher	W32466	IF-Paraffin	1:100
IsolectinB4-FITC	Sigma-Aldrich	L2895	IF-Paraffin	1:50
Acta2	Sigma-Aldrich	A5228	IB	1:1000
GAPDH	EMD-Millipore	AB2302	IB	1:30000
VASP-pS239	Cell Signaling	3114S	IB	1:1000
RhoA-pS188	Abcam	ab41435	IB	1:1000
Rho (total)	Cell Signaling	2117S	IB	1:1000
NPRA	Abcam	ab116680	IB	1:1000
PKG1A	Cell Signaling	13511S	IB	1:1000
RhoA pulldown assay	Cytoskeleton, Inc.	BK036	IP-IB	Manufacturer Instructions
PDGFRα	R&D Systems	AF1062	IF-Paraffin	1:100