A new therapeutic target for the prevention of heart failure due to aortic stenosis

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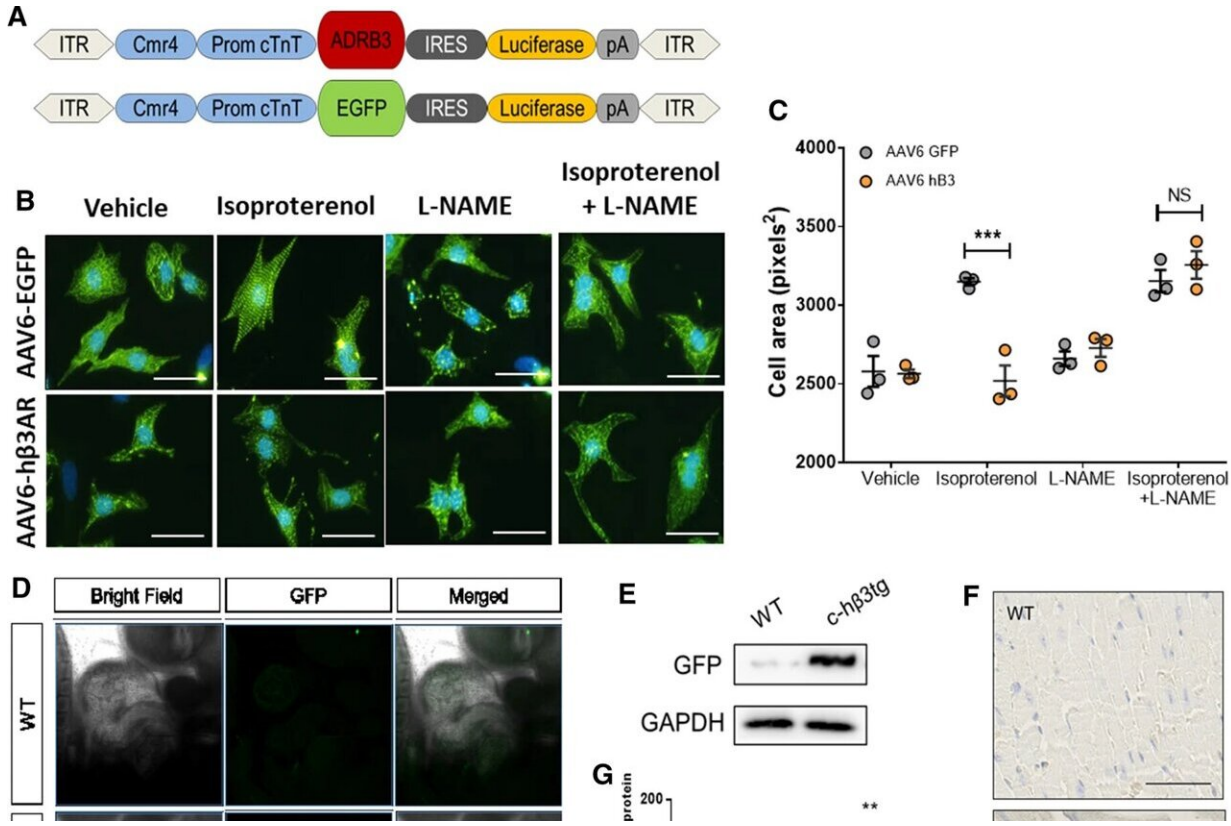

Cardiomyocyte-specific human β3AR overexpression prevents cardiomyocyte hypertrophy upon catecholamine challenge via NO/cGMP pathway. **A** Genetic constructs for adeno-associated virus (AAV) vectors encoding hβ3AR and control EGFP. ITR, recognition site for AVV packaging; Cmr4, enhancer sequence; Prom cTnT, troponin T promoter sequence for cardiomyocyte-specific expression; ADRB3, human β3AR receptor cDNA sequence, *EGFP* enhanced green fluorescent protein sequence, *IRES* internal ribosome entry site, Luciferase, firefly luciferase sequence; pA, simian virus 40 polyadenylation

signal. **B** Representative images of neonatal rat ventricular myocytes (NRVM) transduced with control (AAV6-EGFP) or human β3AR adeno-associated virus (AAV6-hβ3AR) for 72 h and incubated for 24 h with isoproterenol (10 μM), L-NAME (100 μM) or both. Nucleus is stained in blue with DAPI and α-actin is stained in green to differentiate myocytes from other cells. Scale bar, 60 μm. **C** Size assessment of NVRM treated as above (40 cells/condition in each preparation; 3 independent preparations). The isoproterenol-induced hypertrophic response is blunted in hβ3AR myocytes and NOS inhibition by L-NAME restores the hypertrophy. **D** Confocal microscopy images of E9.5 $cTnT^{+/+}$;$R26ADRB3^{tg/tg}$ (control) and $cTnT^{Cre/+}$;$R26ADRB3^{tg/tg}$ (c-hβ3tg) embryos, showing cardiac expression of GFP in an E9.5 embryo. **E** Immunoblot showing GFP expression in cardiomyocytes isolated from adult c-hβ3tg mice. **F** Immunostaining analysis for GFP in cardiac tissue. Scale bar, 50 μm. **G** β3AR protein levels is increased in c-hβ3tg mice. β3AR density (Bmax) in fmol of [3H]-CGP12177 specifically bound/ mg protein in homogenates from c-hβ3tg (red, $n = 3$) and WT (black, $n = 3$) hearts. **H** Mice with cardiomyocyte-specific overexpression of human β3AR (c-hβ3tg, red) and littermate controls (WT, black) were subjected to transaortic constriction (TAC) surgery (to induce supravalvular AS) or sham surgery and were followed for 2 weeks. **I** Supravalvular AS was confirmed by echocardiography as an increase in the descendant aortic velocity blood flow. **J** ATP levels were increased in hearts from c-hβ3tg 2 weeks after supravalvular AS induction ($n = 5$/condition). **K** Cyclic GMP:AMP levels ratio was boosted in hearts from c-hβ3tg mice, thus suggesting an enhancing effect of human β3 overexpression in cardiomyocytes on NO/cGMP signaling ($n = 5$/condition). Data are means ± SEM. Mann–Whitney or Student's *t* test for non-normally or normally distributed data, and Kruskal–Wallis test with Dunn's multiple comparisons test. *p