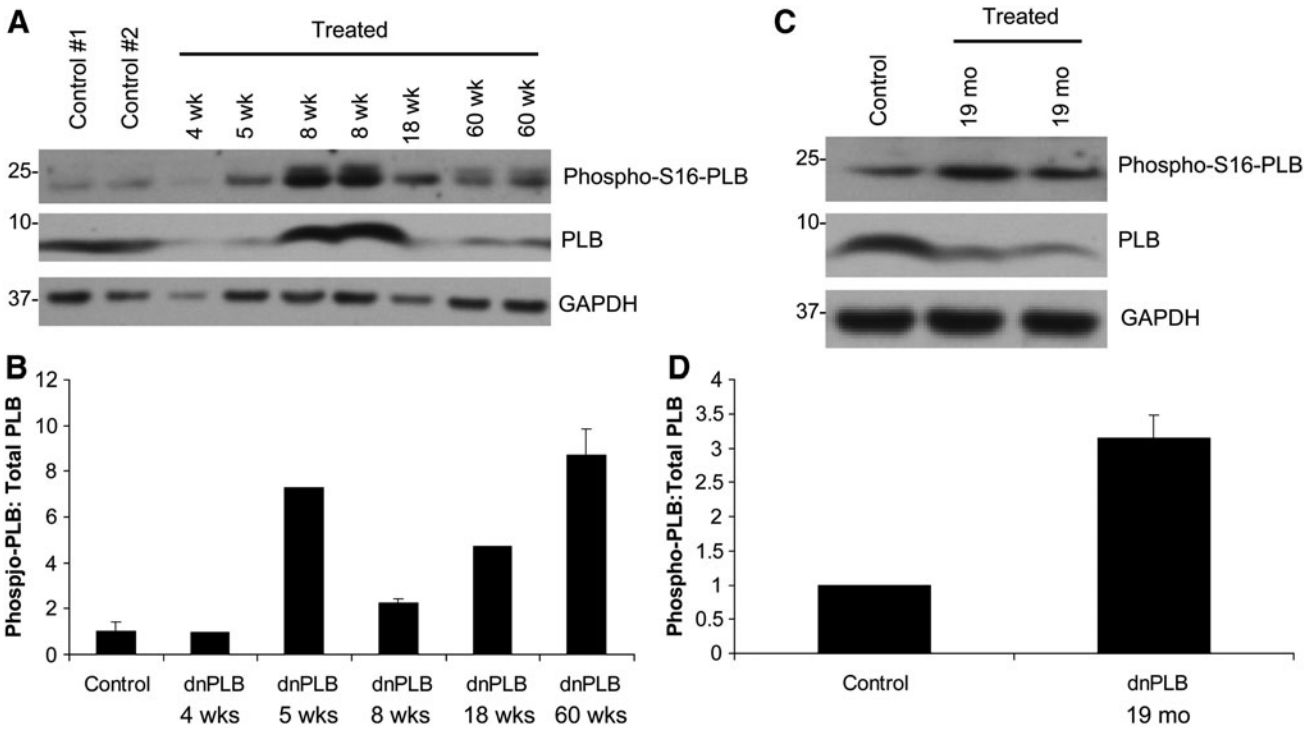
Supplementary Methods

Western Blot Analysis

Cardiac biopsies obtained for Western blotting were snap-frozen in liquid nitrogen. Specimens were pulverized, homogenized in 10 volumes of triple-detergent lysis buffer [50mM Tris, pH 8.0, 0.1% SDS, 1.0% Triton X-100, 0.5% DOC, 5mM EDTA, 50mM DTT, 0.4 tab/10 mL Complete protease inhibitor (Roche, Indianapolis, IN)], and centrifuged at 13,000 rpm for 5 minutes. Protein concentration of the supernatant was then determined using the BioRad Protein Assay (Hercules, CA). 50 ug of each sample were electrophoresed on a 4-20% SDS-polyacrylamide gel (Lonza, Rockland, ME) following the addition of 2X sample loading buffer (130mM Tris, pH = 8.0, 20% glycerol, 4.6% SDS, 2% DTT, 0.02% bromophenol blue) and 5 minutes of denaturation at 100°C. Proteins were then transferred to Immobilon-P (Millipore, Bedford, MA) using the iBlot transfer apparatus (Invitrogen, Carlsbad, CA). The membrane was subsequently blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.05% Tween 20. Immunoblotting was performed to detect phospholamban (1:500, Millipore, Temecula, CA), phospho-S16-phospholamban (1:500, Upstate, Lake Placid, NY), and GAPDH as loading control (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA). Detection was performed using the Super Signal West Pico Chemiluminescent Substrate kit (Pierce, Rockford, IL). Since the dnPLB being expressed via AAV is a pseudo-phosphorylated mutant, the ratio of phospho-PLB to total PLB (normalized to GAPDH as a loading control) was calculated as previously described (Kaye *et al.*, 2007).

Reference

Kaye, D.M., Preovolos, A., Marshall, T., Byrne, M., Hoshijima, M., Hajjar, R., Mariani, J.A., Pepe, S., Chien, K.R., and Power, J.M. (2007). Percutaneous cardiac recirculation-mediated gene transfer of an inhibitory phospholamban peptide reverses advanced heart failure in large animals. J. Am. Coll. Cardiol. 50, 253–260.



SUPPLEMENTARY FIG. S1. Western blot demonstrating cardiac expression of PLB in canines treated with scAAV6-CB-dnPLB. Since the dnPLB being expressed via AAV is a pseudo-phosphorylated mutant, the ratio of phospho-PLB to total PLB (normalized to GAPDH as a loading control) was calculated as previously described (Kaye *et al.*, 2007). Expression of PLB was confirmed both (A, B) in canines prior to its use in this study, and (C, D) in one of the long-term canines from this study at 19 months vs. AAV capsid control.