Phospholamban as a Crucial Determinant of the Inotropic Response of Human Pluripotent Stem Cell–Derived Ventricular Cardiomyocytes and Engineered 3-Dimensional Tissue Constructs

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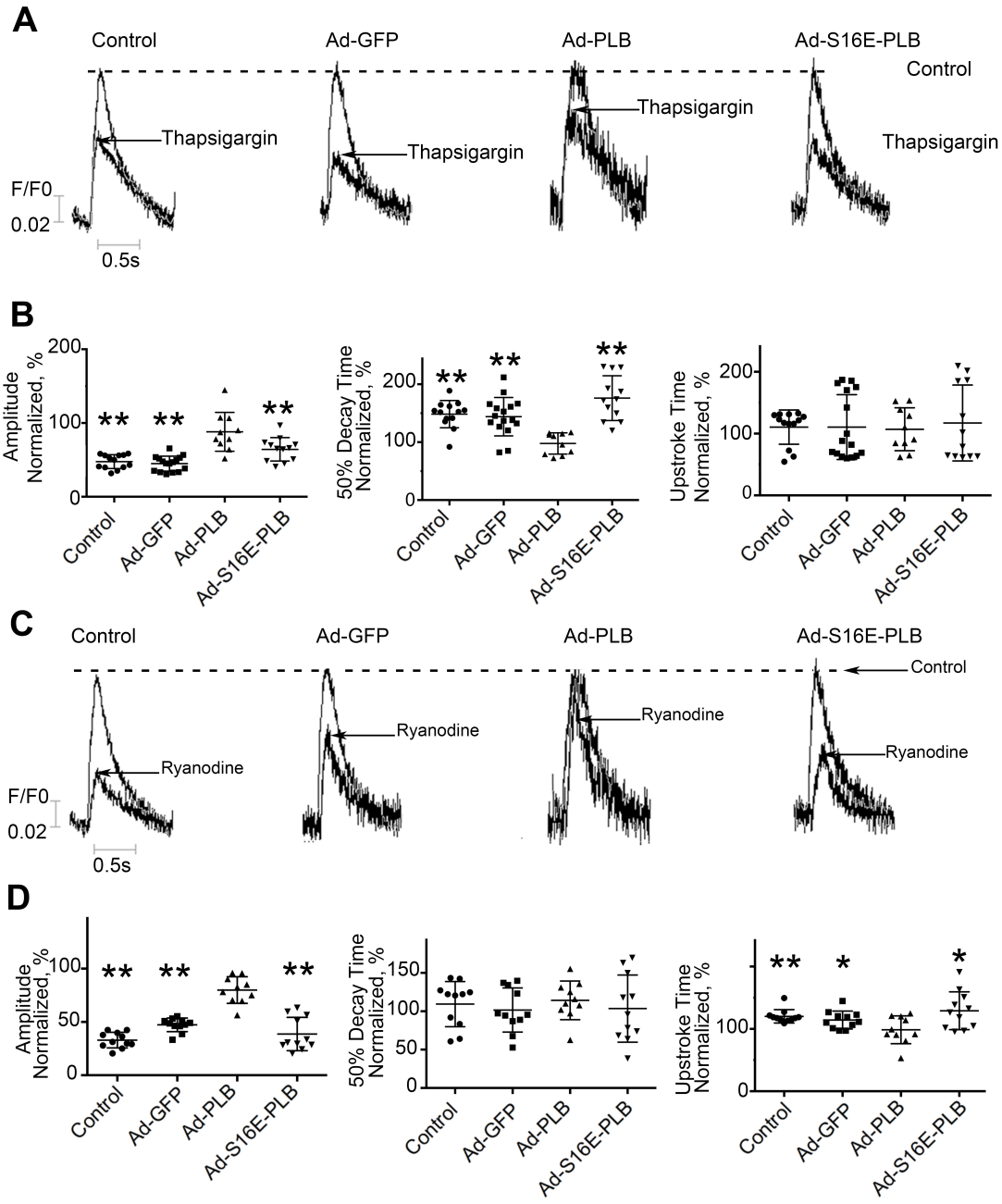
SUPPLEMENTAL MATERIAL

Supplemental Results

We investigated the pharmacological responses of the various groups of hES2-**v**CMs to thapsigargin and ryanodine, specific SERCA and RyR inhibitors, respectively. Application of 1μmol/L thapsigargin to control hES2-**v**CMs (un- and Ad-GFP-transduced) significantly decreased the transient amplitude and slowed the decay ($P<0.05$; Supplemental Figure 1). Likewise, ryanodine application (10μmol/L) significantly reduced the electrically evoked Ca^{2+} transient amplitude and slowed the upstroke ($P<0.01$; Supplemental Figure 1). Interestingly, while similar inhibitory effects on the peak amplitude, decay and upstroke by thapsigargin or ryanodine were seen with Ad-PLB-transduced hES2-**v**CMs, the changes were significantly less compared to the controls, indicating that Ad-PLB transduction rendered cells less sensitive to these pharmacological agents (Supplemental Figure 1). As anticipated from pseudo-phosphorylation, the effects of Ad-PLB-S16E on the responses to thapsigargin and ryanodine resembled those of controls (Supplemental Figure 1).

Supplemental Figure and Legends

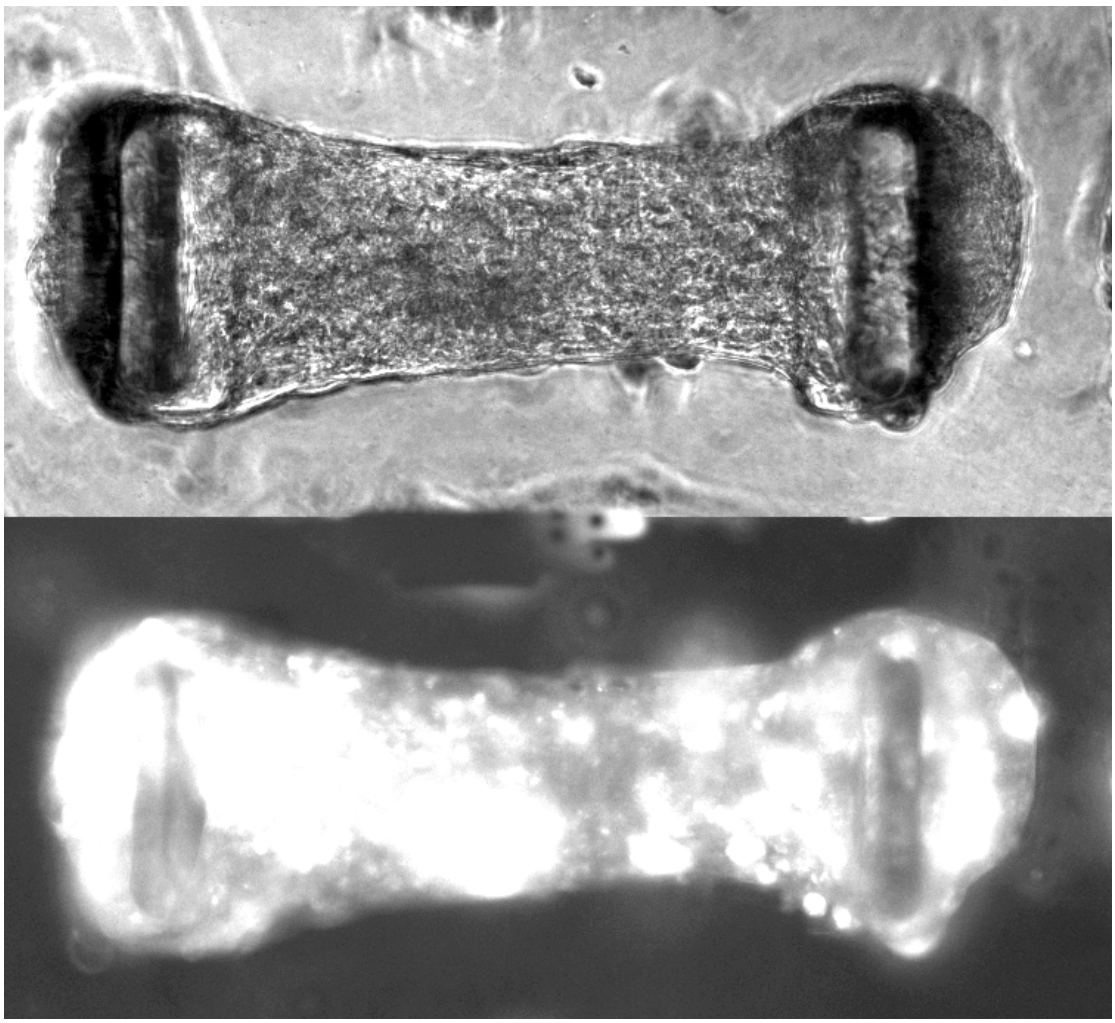


Supplemental Figure 1

*Effects of thapsigargin or ryanodine on electrically-evoked Ca2+ transient of un-, Ad-GFP-, Ad-PLB-, Ad-S16E-PLB-transduced hES2-**v**CMs.*

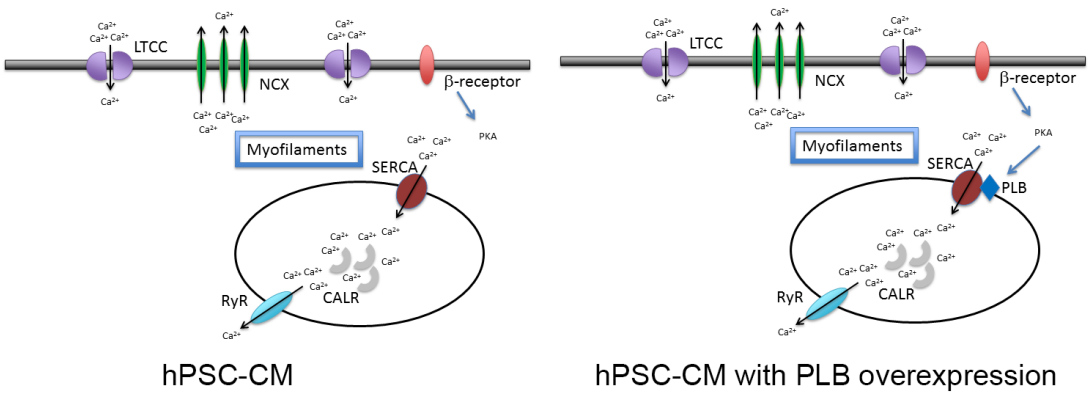
A) Representative raw tracings of electrically-induced Ca^{2+} transients of un-, Ad-GFP-, Ad-PLB-, Ad-S16E-PLB-transduced hES2-**v**CMs recorded in the absence

and presence of 1μM thapsigargin as indicated. Peaks recorded under control drug-free condition of all groups have been normalized for comparison. **B)** Bar graphs summarizing the transient parameters of the same groups from A. At 1μmol/L, thapsigargin significantly decreased the amplitude of control, Ad-GFP and Ad-S16E-PLB- but not Ad-PLB-transduced hES2-**v**CMs. 50% decay time was affected in all groups except Ad-PLB-transduced hES2-vCMs. Upstroke time was not affected in any of the groups. (N=13, 16, 10 and 12 for control, Ad-GFP, Ad-PLB and Ad-S16E-PLB respectively.) **C)** Representative raw tracings of electrically-induced Ca2+ transients of un-, Ad-GFP-, Ad-PLB-, Ad-S16E-PLB-transduced hES2-**v**CMs recorded in the absence and presence of 10μmol/L ryanodine as indicated. Peaks recorded under drug-free condition of all groups have been normalized. **D)** Bar graphs summarizing the transient parameters of the same groups from A. At 10μmol/L, ryanodine significantly decreased the amplitude of control, Ad-GFP and Ad-S16E-PLB- but not Ad-PLB-transduced hES2-**v**CMs. 50% decay time was unaffected in all groups. Upstroke time was affected in control, Ad-GFP and Ad-S16E-PLB- but not Ad-PLB-transduced hES2-**v**CMs (N=11, 11, 10 and 11 for control, Ad-GFP, Ad-PLB and Ad-S16E-PLB respectively). *P<0.05, **P<0.01, compared to parameters under drug-free condition.



Supplemental Figure 2

*Bright and fluorescence images of h**v**CMT after Ad-GFP transduction demonstrated that the h**v**CMT could be fully transduced by Ad.*



Supplemental Figure 3

Role of PLB in Ca^{2+} handling of hPSC-CM.

In adult CM, stimulation of β-adrenergic receptors leads to phosphorylation of PLB by PKA, which is a fundamental mechanism of the positive inotropic effect of β-adrenergic stimulation. However, PLB is missing in hPSC-CM. Overexpression of PLB in hPSC-CMs restored the positive inotropic response of hPSC-CM.