Supplementary Figure 2 PKA-dependent activation of PDE4D upon βAR stimulation. (A-C) Cultured neonatal cardiac myocytes were stimulated with 10 µM Isoproterenol before cells were lysed and subjected to IP with α-PAN-PDE4D (M3S1) antibody. Shown is the PDE activity recovered in the IP pellet. (A) Time-dependent activation of endogenous PDE4D by Isoproterenol. (B) PDE4D activation is blocked by the PKA inhibitor, H89 (20 µM). (C) Isoproterenol-induced PDE4D activation is ablated in myocytes deficient in β1AR and β2AR. (D) A shift in migration in SDS/PAGE of exogenous PDE4D3 indicates that Isoproterenol treatment produces partial phosphorylation whereas treatment with Forskolin (Fsk) results in complete phosphorylation of the PDE. (E) Activation of endogenous PDE4D splice variants in myocytes deficient in β2AR after stimulation of β1AR with 10 µM Isoproterenol. All graphs show the means ± S.E.M. of at least three experiments performed.