# Supplemental Material

## PDE4B mediates local feedback regulation of $\beta_1$-adrenergic cAMP signaling in a sarcolemmal compartment of cardiac myocytes

Mika et al., PDE4B controls subsarcolemmal cAMP

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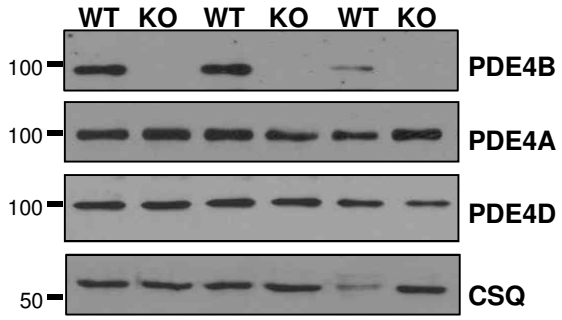


**Fig. S1.** ***PDE4B ablation does not affect the levels of protein expression of PDE4A and PDE4D.*** Detergent extracts prepared from Wild Type (WT) and PDE4BKO (KO) neonatal cardiomyocytes (NCMs) were probed by Western blot with anti-PDE4A, anti-PDE4B or anti-PDE4D antibodies. Calsequestrin (CSQ) was used as a loading control.

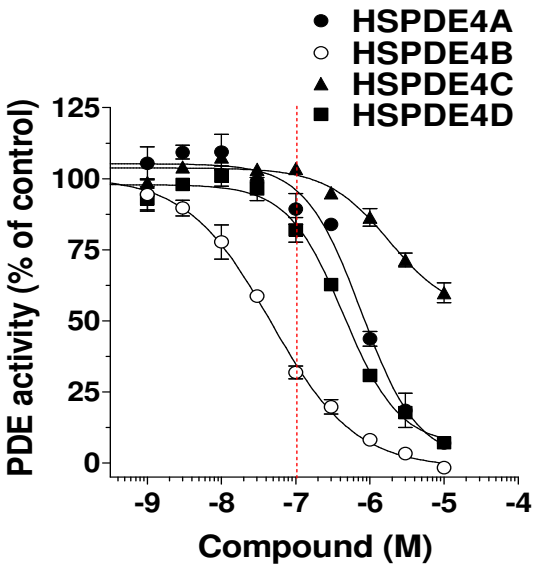


**Fig. S2. Properties of the PDE4B-selective inhibitor, GSK16.** Recombinant PDE4A4, PDE4B3, PDE4C2 and PDE4D3 proteins were expressed in HEK293 cells by transfection. Soluble extracts from the transfected cells were assayed for PDE activity with 1 μM cAMP in the absence or presence of increasing concentrations of the GSK16 compound. Data were fitted with a four parameter logistic equation and the following $EC_{50}$ calculated: PDE4A = 770 nM, PDE4B = 46 nM, PDE4C = 1.9 μM, PDE4D = 500 nM. Each point is the average of two experiments each done in triplicate.

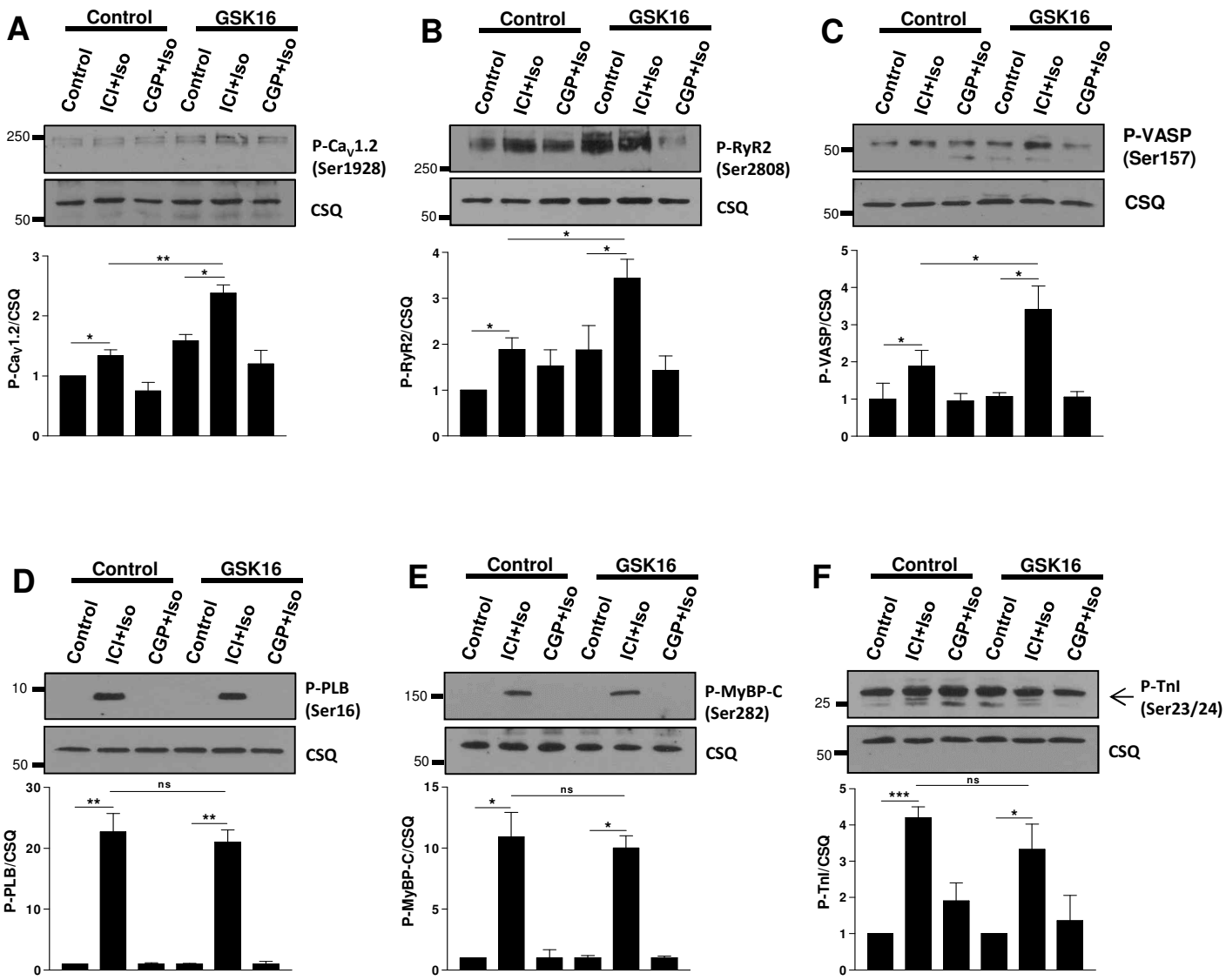


**Fig. S3.** ***PDE4B-selective inhibition increases $\beta_1$AR-, but not $\beta_2$AR-induced-PKA activity in a subsarcolemmal compartment.*** Wild Type myocytes pretreated for 5 minutes with or without a PDE4B-selective inhibitor (GSK16, 100 nM), were treated for 10 min with or without Iso (10 nM) in the presence of ICI-118551 (ICI, $\beta_2$AR antagonist, 1 μM) or CGP-20712A (CGP, $\beta_1$AR antagonist, 1 μM). Detergent extracts were probed for phospho-$Ca_V1.2$ (P-$Ca_V1.2$, Ser1928) (**A**), phospho-ryanodin receptor 2 (P-RyR2, Ser2808) **(B)**, phospho-VASP (P-VASP, Ser157) (**C**), phospho-phospholamban (P-PLB, Ser16) (**D**), phospho-myosine-binding protein C (P-MyBP-C, Ser282) (**E**) and phospho-troponin I (P-TnI, Ser23/24) (**F**). Calsequestrin (CSQ) was used as a loading control. Shown for each figure is a representative Western blot of three performed. For quantification, the ratios of the immunoblot intensity for the phosphorylated proteins over that for CSQ were normalized to untreated cells and expressed as mean ± s.e.m. Statistical significance (unpaired *t*-test) is indicated as: ns, not significant; *, p<0.05; **, p<0.01; ***, p<0.001. Iso, isoproterenol.

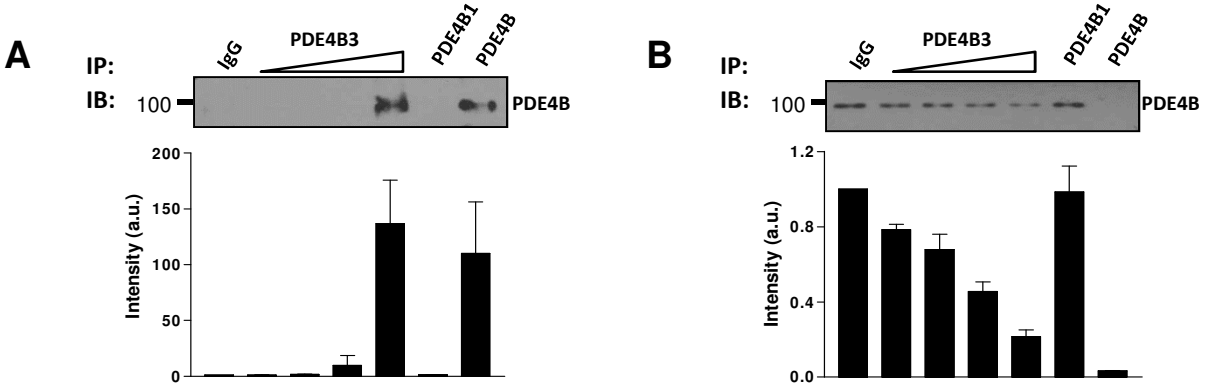


**Fig. S4.** ***PDE4B3, but not PDE4B1, is expressed in isolated mouse neonatal cardiomyocytes.*** Total extracts from Wild Type neonatal cardiomyocytes (NCMs) were subjected to immunoprecipitation (IP) with anti-PDE4B3 (0.5, 1.5, 5 or 15 µg), anti-PDE4B1 (5 µg), anti-PDE4B (10 µg) antibodies or control IgG. IP pellets (**A**) and post-IP supernatants (**B**) were probed by Western blot with anti-PDE4B antibodies. Shown is a representative Western blot of two performed. For quantification, immunoblot intensities (a.u.) are expressed as mean ± range.