

## **Supplementary Figure Legends:**

**Supplementary Figure 1 PDE4D isoforms display indiscriminate interaction with  $\beta_2$ AR in HEK 293 cells.** Cells expressing flag- $\beta_2$ AR and GFP-PDE4D isoforms (N-terminal fragments from 4D1 to 4D9) are lysed and immunoprecipitated with anti-flag M2 beads. The bound  $\beta_2$ AR and PDE4D isoforms are detected with indicated antibodies respectively. The total immunoprecipitates are loaded, and the lysate loading represents 5% of the total.

**Supplementary Figure 2 PDE4D isoforms display subcellular localization in wild-type and  $\beta_1$ AR-KO neonatal myocytes.** Wild-type and  $\beta_1$ AR-KO myocytes are infected with viruses to express GFP-PDE4D5, 4D8, or 4D9. After 24hours, the cells are fixed; the images are acquired to examine the localization of PDE4D5, 4D8, or 4D9 in myocytes.

**Supplementary Figure 3 PDE4D9 and  $\beta_2$ AR display co-localization in  $\beta_1$ AR-KO neonatal myocytes.**  $\beta_1$ AR-KO myocytes are infected with viruses to express flag- $\beta_2$ AR together with GFP-PDE4D5, 4D8, or 4D9. After 24hours, cells are fixed for immunofluorescence staining of flag- $\beta_2$ AR with M1 antibody followed by goat-anti-mouse IgG Alexa-594. The images from green channel of GFP-PDE4Ds and red channel of flag- $\beta_2$ AR are acquired and overlapped. Inserts show the overlap staining between GFP-PDE4D9 and flag- $\beta_2$ AR.

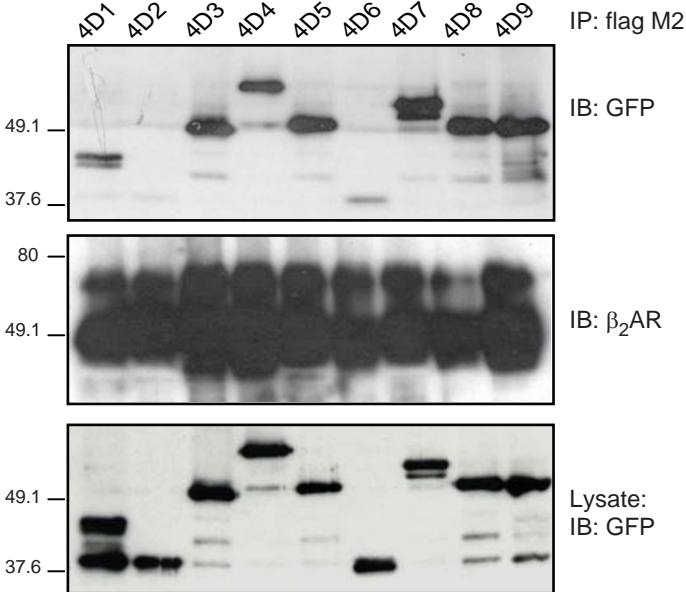
**Supplementary Figure 4 PDE4D8 isoform binds to  $\beta_1$ AR in myocytes.** Wild-type myocytes are infected with viruses to express HA- $\beta_1$ AR together with full length mcherry-PDE4D8 (A) or mcherry-PDE4D9 (B). Cells are treated with 10 $\mu$ M of isoproterenol for 0 or 10 minutes before being lysed for immunoprecipitation. The immunoprecipitated HA- $\beta_1$ AR and the bound PDE4D proteins are blotted with indicated

antibodies. The total immunoprecipitates are loaded, and the lysate loading represents 5% of the total.

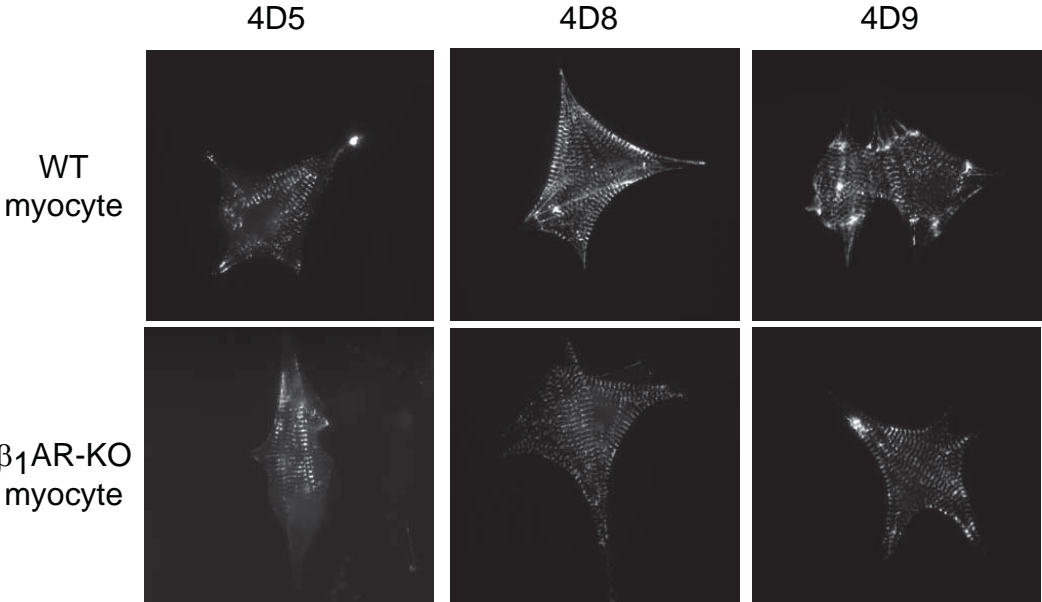
**Supplementary Figure 5 Activation of  $\beta_2$ AR recruits both PDE4D5 and PDE4D8 via  $\beta$  arrestin-dependent mechanism in MEF cells.** Flag- $\beta_2$ AR together with full length mcherry-PDE4D5 or mcherry-PDE4D8 is coexpressed in wild-type or  $\beta$  arrestin1 and 2 knockout ( $\beta$ ARR1/2-KO) MEF cells. Alternatively, GFP- $\beta$ arrestin2, flag- $\beta_2$ AR, and mcherry-PDE4D8 are coexpressed in  $\beta$ ARR1/2-KO MEF cells (the right two lanes). Cells are treated with 10 $\mu$ M of isoproterenol for 0 or 10 minutes before being lysed for immunoprecipitation. The immunoprecipitated flag- $\beta_2$ AR and the bound PDE4D proteins are blotted with indicated antibodies. The total immunoprecipitates are loaded, and the lysate loading represents 5% of the total.

**Supplementary Figure 6 Activation of  $\beta_2$ AR does not recruit PDE4D9 in MEF cells.** Flag- $\beta_2$ AR and full-length mcherry-PDE4D9 is co-expressed in wild-type or  $\beta$ ARR1/2-KO MEF cells. Cells are treated with 10 $\mu$ M of isoproterenol for 0 or 10 minutes before being lysed for immunoprecipitation. The immunoprecipitated flag- $\beta_2$ AR and the bound PDE4D proteins are blotted with indicated antibodies. The total immunoprecipitates are loaded, and the lysate loading represents 5% of the total.

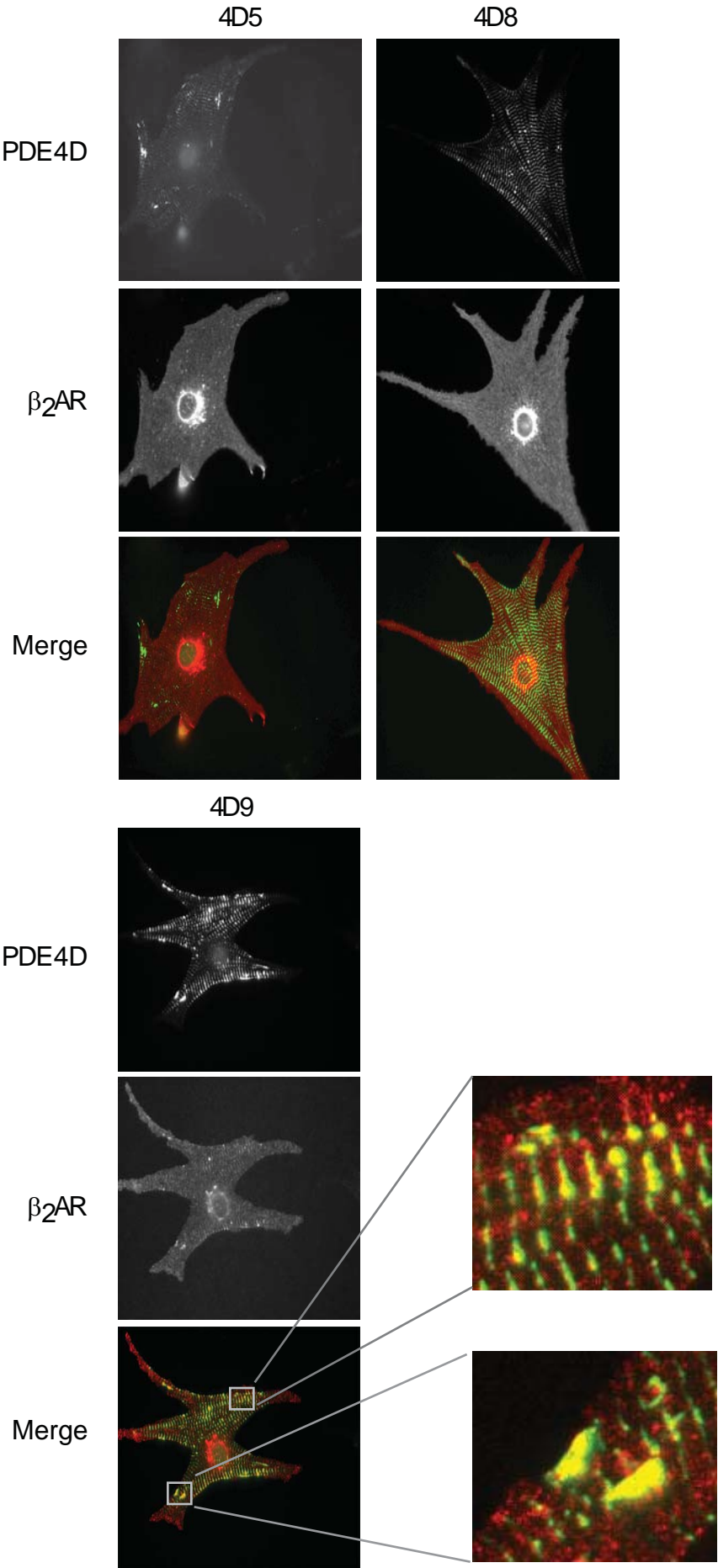
Supplementary Figure 1



Supplementary Figure 2

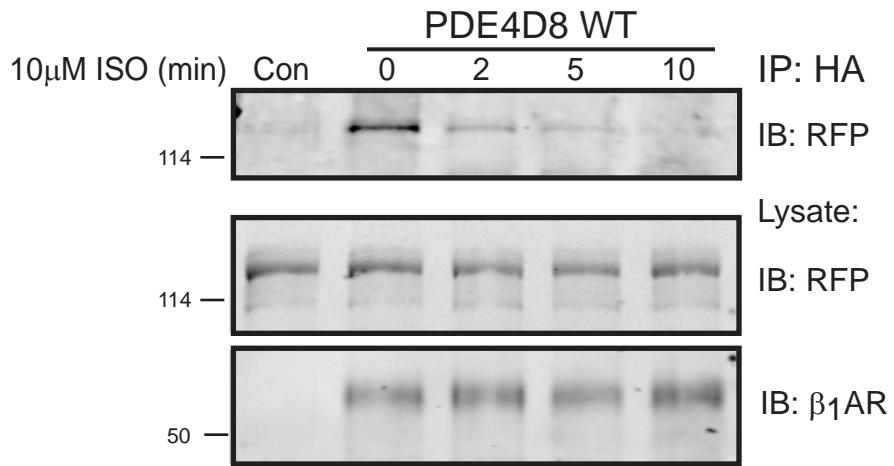


Supplementary Figure 3

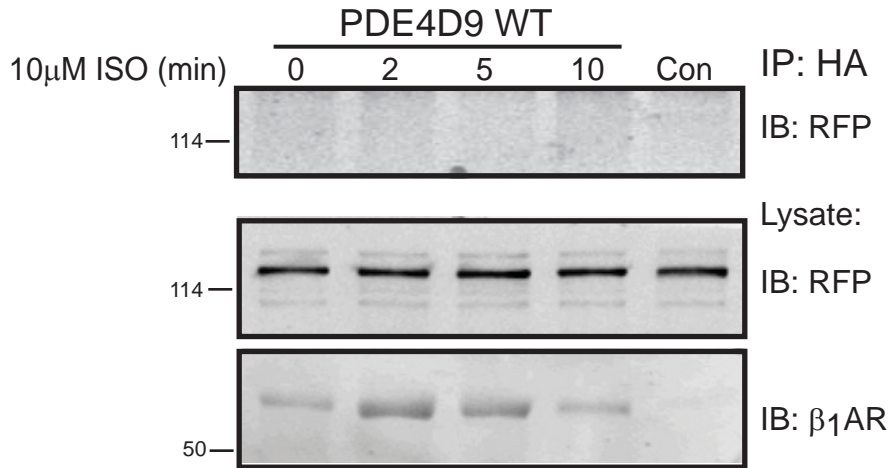


Supplementary Figure 4

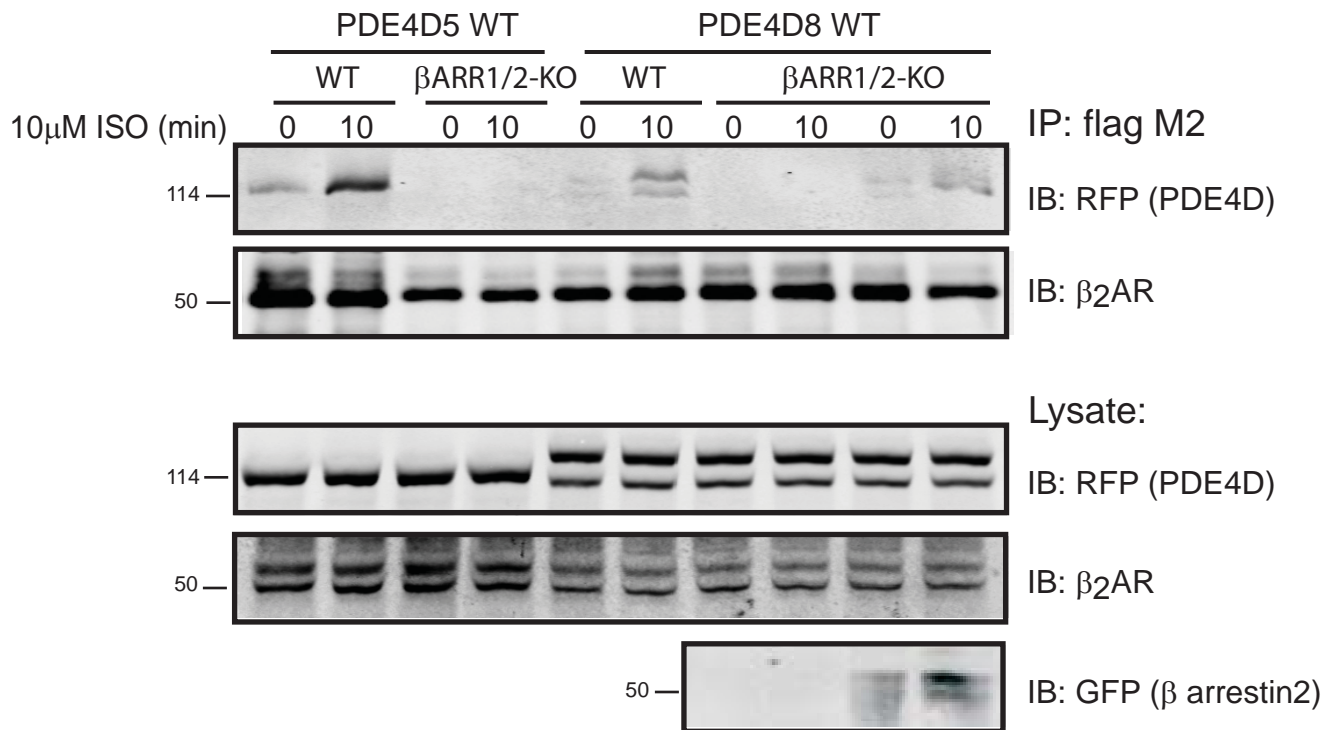
A



B



# Supplementary Figure 5



# Supplementary Figure 6

