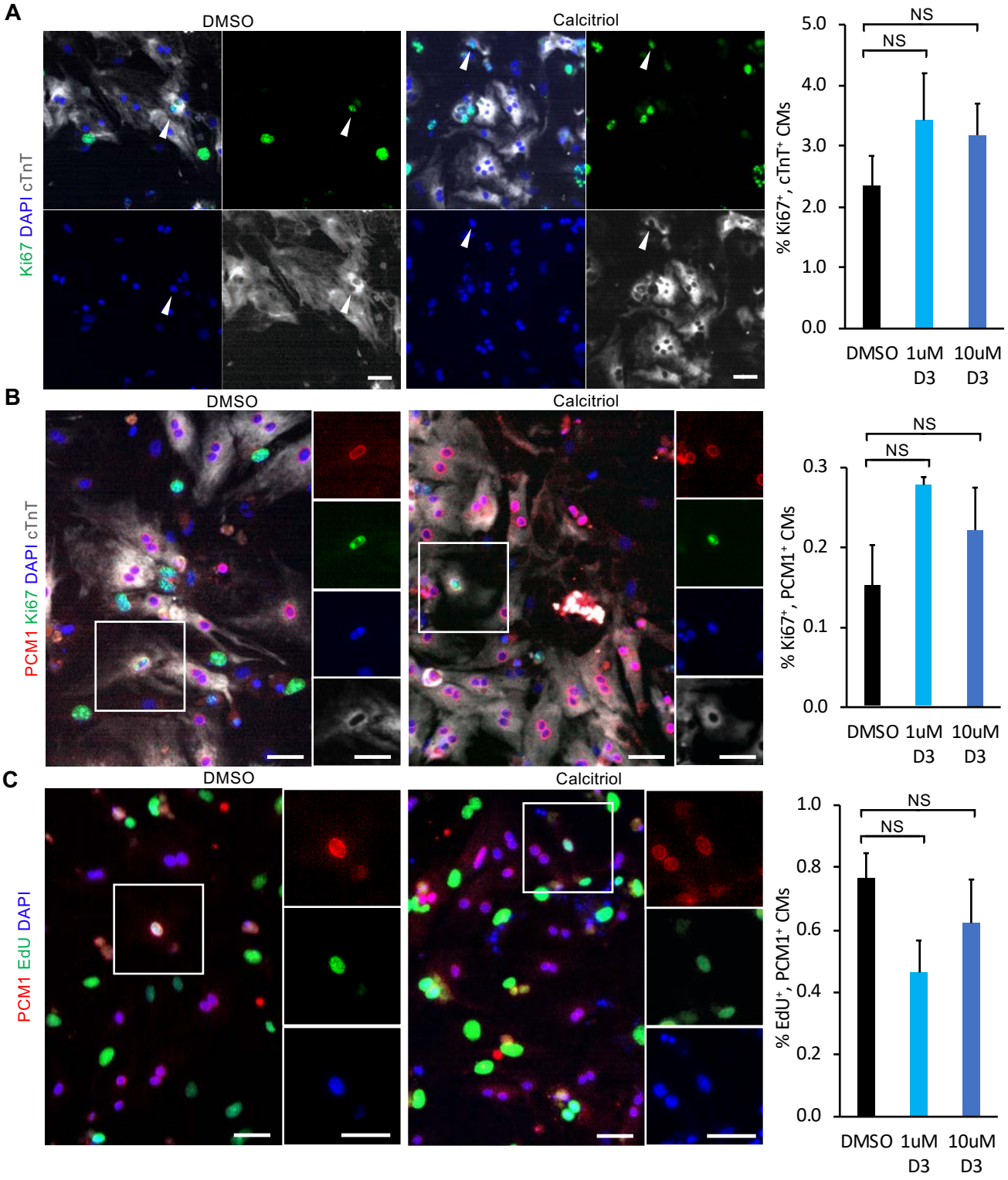

Figure S1. Effect of VDR activation on P7 cardiomyocyte (CM) proliferation *in vitro*. Representative images and quantifications of CM proliferation. Cells were isolated from P7 mouse hearts and incubated with calcitriol (D3) or DMSO for 72 hr. Three different CM staining and proliferation standards were utilized. (**A**) Cardiac troponin-T (cTnT) is a marker for CM cell bodies and Ki67 is a nuclear marker of cell cycle progression. Co-staining of Ki67 and DAPI inside of cTnT indicated proliferating CMs. Using this method, VDR agonist D3 did not significantly increase CM proliferation *in vitro*. (**B**) PCM1 localizes to the nuclear membrane in CMs, forming a shell around CM nuclei. Co-staining of Ki67, DAPI, and a PCM1 shell indicated proliferating CMs. D3 did not significantly increase CM proliferation *in vitro* using this quantification method. (**C**). Using EdU as a proliferation marker, D3 also did not increase $PCM1^+$ CM proliferation. Three biological replicate experiments were performed with three technical repeats for each quantification condition. Arrows indicate proliferating CM nuclei. Values are reported as mean ± SEM (n=3 hearts). NS, not significant. Scale bars: 50 μm.

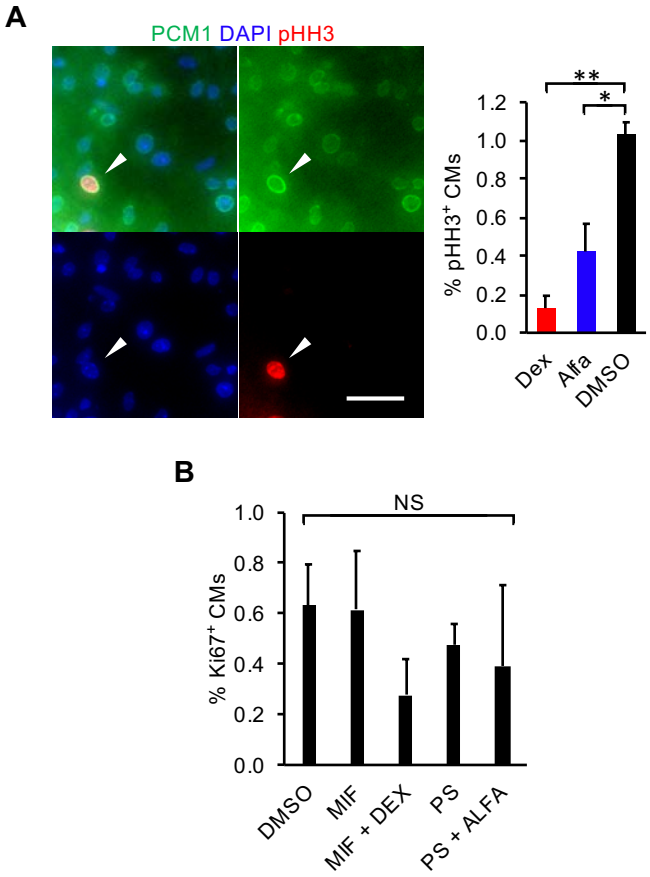

Figure S2. P1 CM proliferation in various conditions. (**A**) Proliferating *in vitro* P1 CMs cultured with 100 nM dexamethasone (Dex), 1 μM alfacalcidol (Alfa), and DMSO. Mitotic cells were stained positive for phospho-histone H3 (pHH3). Arrowheads indicate proliferating CM nuclei. (**B**) *In vitro* P1 CM proliferation ($Ki67^+$) in response to 100 nM mifepristone (MIF), 100 nM mifepristone and 100 nM dexamethasone (MIF + DEX), 1 μM PS121912 (PS), and 1 μM PS121912 and 1 μM alfacalcidol (PS + ALFA). Values are reported as mean ± SEM (n=3). NS, not significant. *$p < 0.05$, **$p < 0.01$. Scale bar: 50 μm.

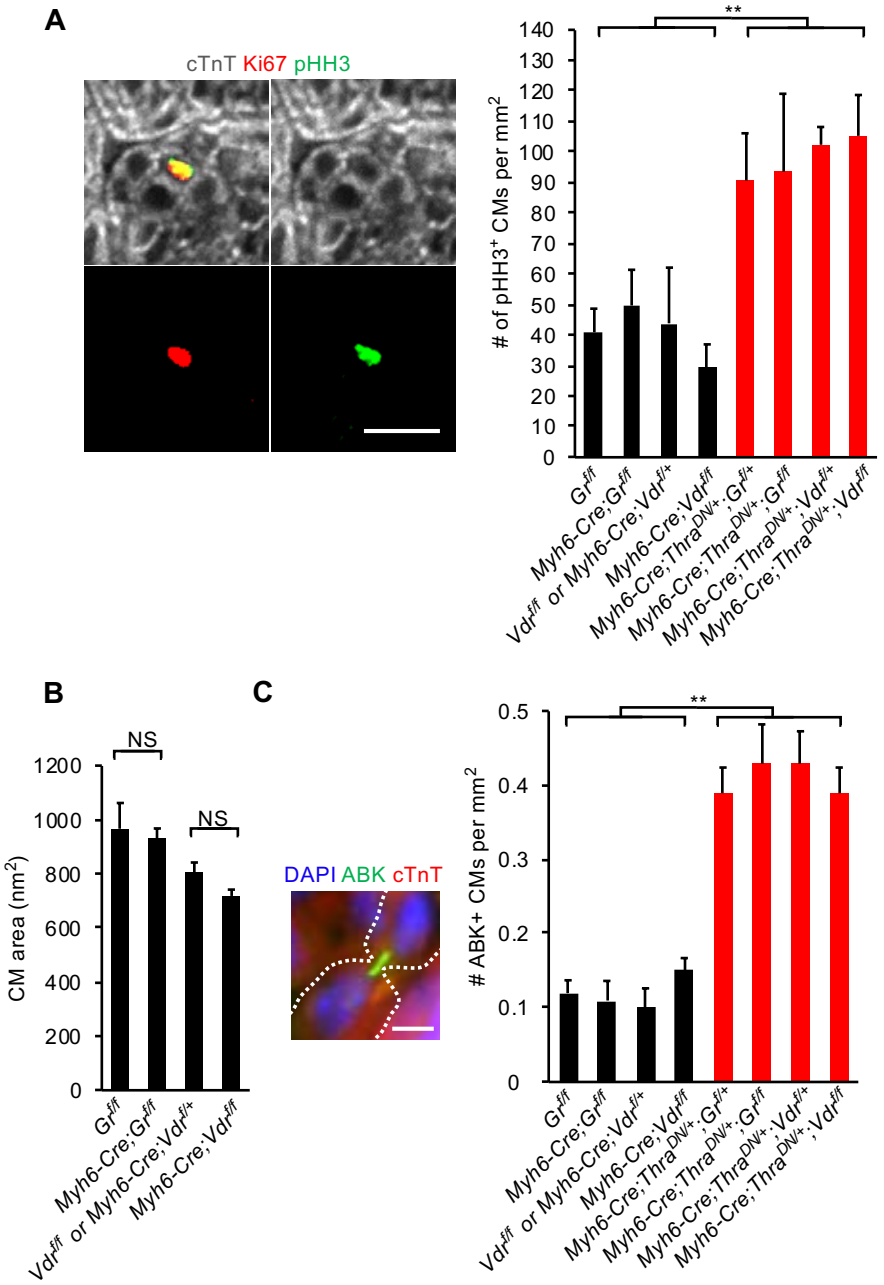

Figure S3. CM-specific loss of glucocorticoid receptor (GR) and vitamin D receptor (VDR) signaling do not affect *in vivo* CM size or proliferation at P14. (**A**) The average number of phosphohistone-H3$^+$ (pHH3$^+$) positive CMs per mm^2 area was not affected by *Gr* or *Vdr* loss under any conditions, but was significantly increased in all mice with CM-specific deficiency of thyroid hormone signaling (Thra$^{DN/+}$). A representative pHH3$^+$, Ki67$^+$ double-positive CM nucleus from a P14 *Myh6-Cre;Thra$^{DN/+}$;Vdr$^{f/f}$* mouse is shown. (**B**) Average cell area of dissociated CMs did not significantly differ between *Myh6-Cre;Gr$^{f/f}$* mice and littermate controls at P14, nor between *Myh6-Cre;Vdr$^{f/f}$* mice and littermate controls at P14. (**C**) CM cytokinesis analysis of aurora-B kinase (ABK)$^+$ at the cleavage furrow. A representative ABK$^+$ CM from a P14 *Myh6-Cre;Thra$^{DN/+}$;Gr$^{f/f}$* mouse is shown. Values are reported as mean ± SEM (n=3 animals). NS, not significant. *p < 0.05. **p < 0.01. Scale bars: 25 μm (A), 5 μm (B).

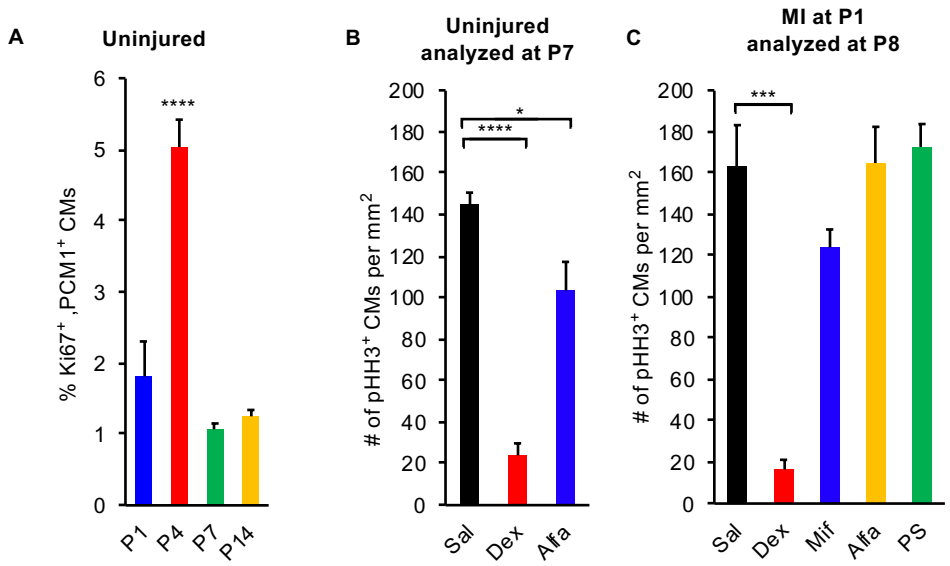

Figure S4. Analysis of neonatal cardiomyocyte (CM) proliferation at different developmental stages and under different drug treatments. (**A**) Developmental time course analysis of neonatal CM proliferation *in vivo*. The percentage of Ki67$^+$ CMs (PCM1$^+$ rings) was quantified at P1, P4, P10, and P14 of wild-type mice. (**B**) Co-staining of pHH3 and Ki67 within cardiac troponin-T (cTnT) indicated proliferating CMs. The average number of proliferating CMs per mm^2 in uninjured P7 neonatal mice injected daily with either Saline (Sal), Dexamethasone (Dex), or Alfacalcidol (Alfa) from birth. (**C**). Co-staining of pHH3 and Ki67 within cTnT indicated proliferating CMs. The average number of proliferating CMs per mm^2 in P8 neonatal mice given surgically-induced myocardial infarction (MI) at P1 and subsequently injected daily with either Sal, Dex, Alfa, Mifepristone (Mif), or PS121912 (PS). Values are reported as mean ± SEM (n = 5-7). NS, not significant. *p < 0.05, **p < 0.01, ****p < 0.0001.

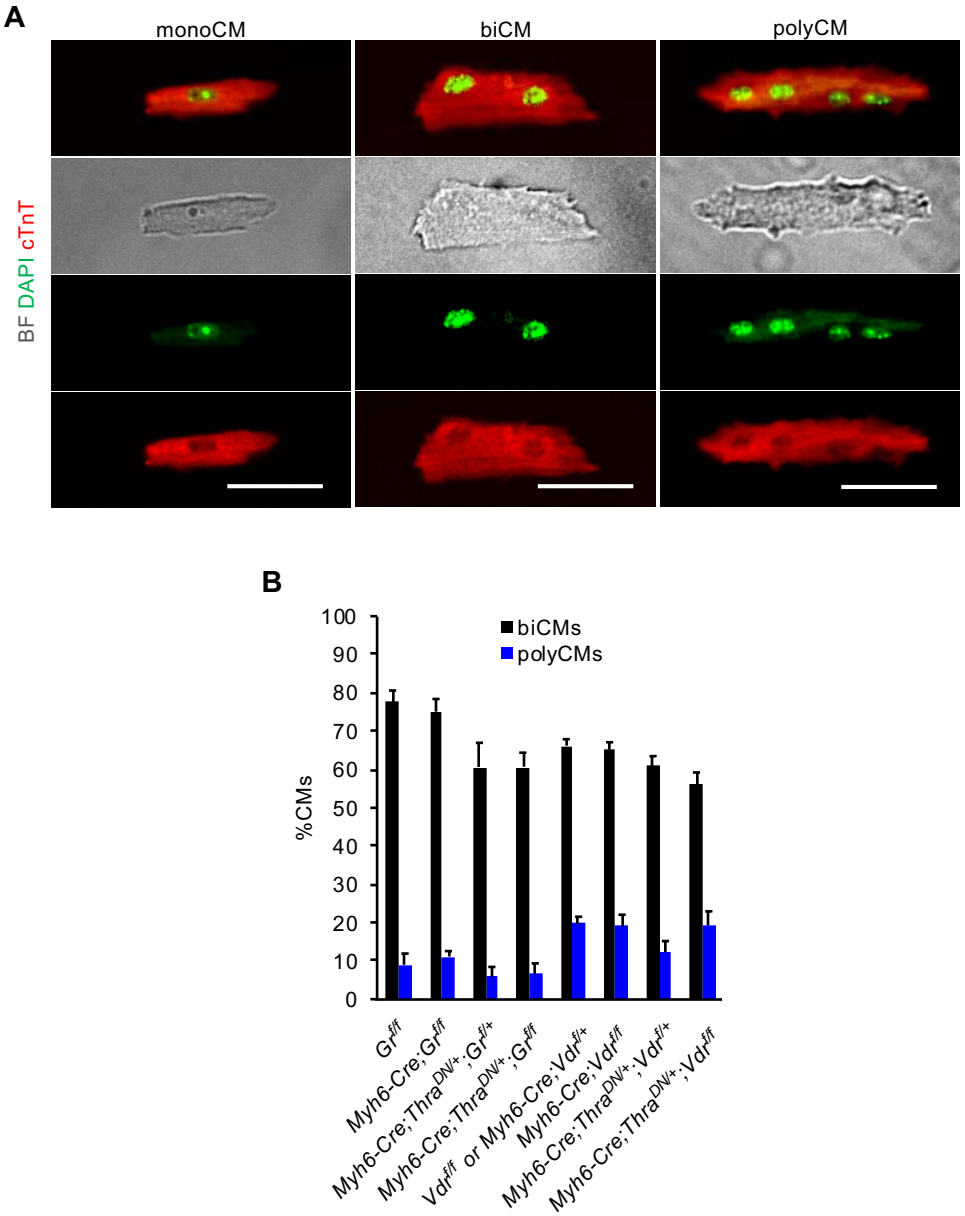

Figure S5. CM nucleation at P14. (**A**) Representative images of a dissociated mononuclear (monoCM), binuclear (biCM), and polynuclear (polyCM) CM at P14. (**B**) P14 biCM% and polyCM% of the control and mutant cohorts described in Fig. 2-5. Values are reported as mean ± SEM (n=3 animals). Scale bars: 50 μm.

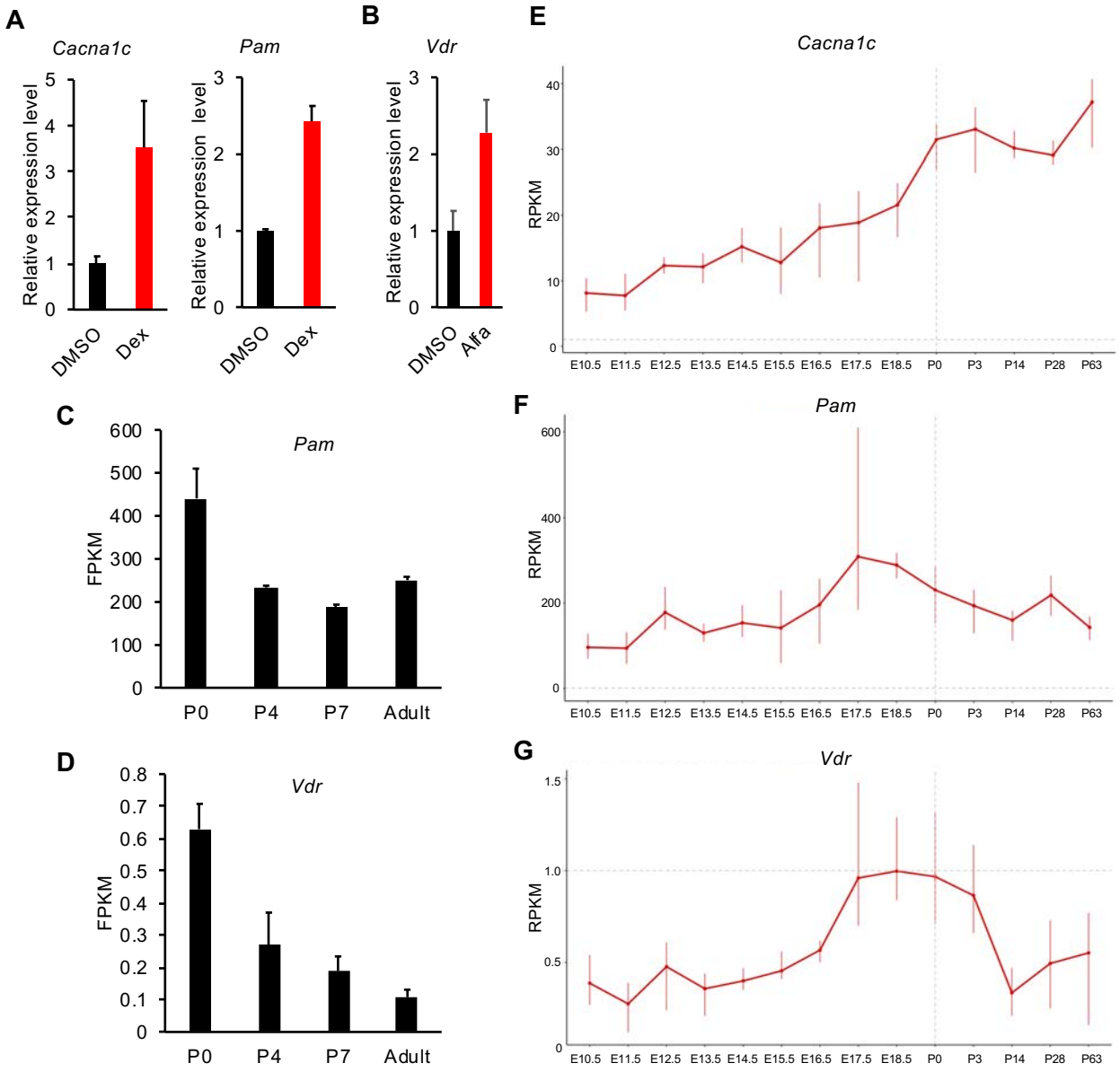

Figure S6. Expression of glucocorticoid receptor (GR) and vitamin D receptor (VDR) downstream targets in neonatal CMs. (**A**) Quantitative PCR analysis of *in vitro* P1 CMs treated with 100 nM dexamethasone (Dex) showed upregulation of GR targets *Cacna1c* and *Pam*. Values are reported as mean ± SEM (n=3). (**B**) Quantitative PCR analysis of *in vitro* P1 CMs cultured with 1 μM alfacalcidol (Alfa) showed upregulation of a VDR target *Vdr*. (**C**-**G**) Developmental changes of GR and VDR target gene expression in the mouse heart. Raw RNA-seq data are derived from [46] for C and D, and from [48] for E-G.

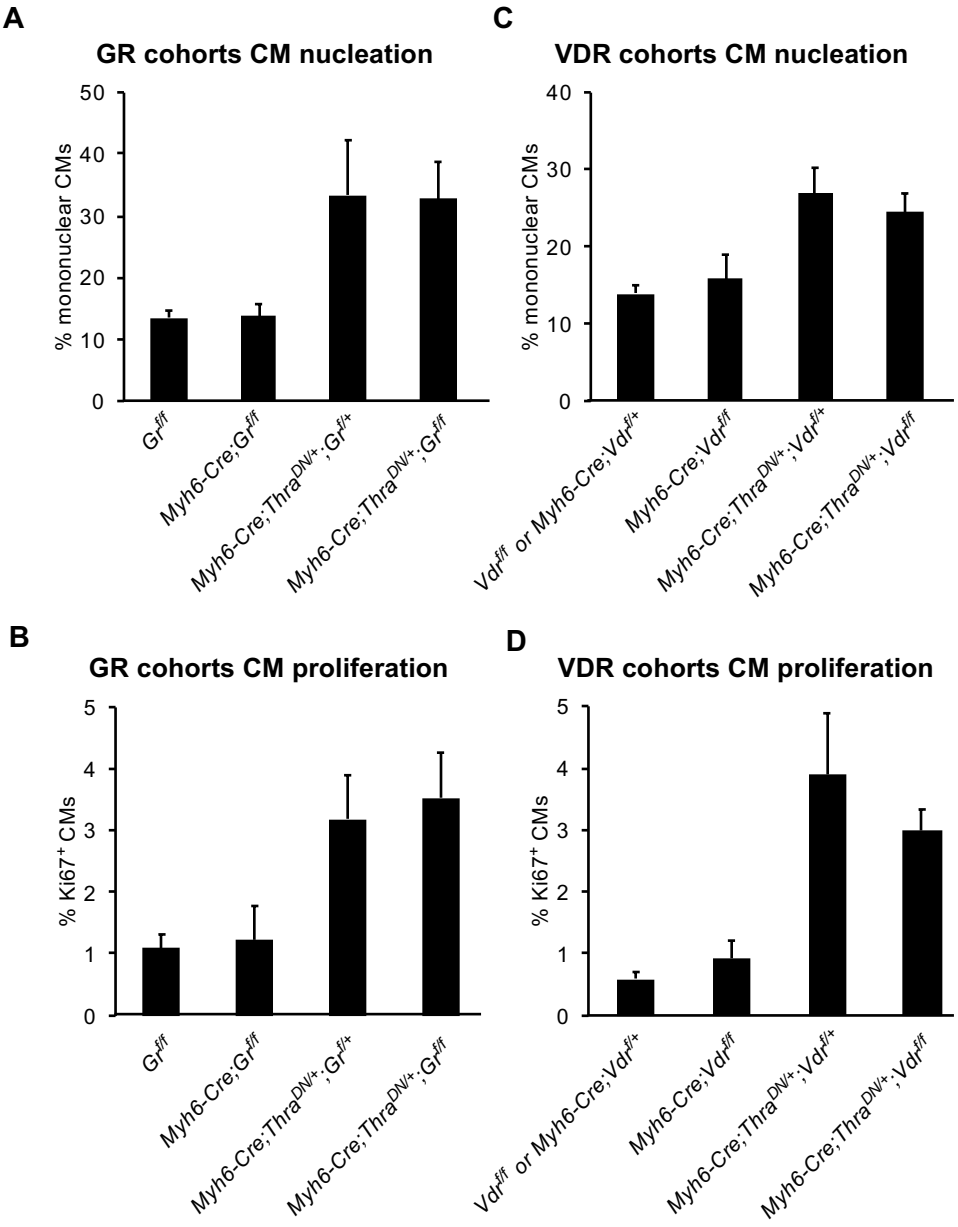

Figure S7. P14 cardiomyocyte (CM) nucleation and proliferation in mice with CM-specific deficiency of glucocorticoid receptor (GR), vitamin D receptor (VDR) and thyroid hormone receptor signaling. (**A**) CM nucleation data for all GR cohorts examined. (**B**) CM proliferation data for all GR cohorts examined. (**C**) CM nucleation data for all VDR cohorts examined. (**D**) CM proliferation data for all GR cohorts examined. Values are reported as mean ± SEM (n=3 animals).