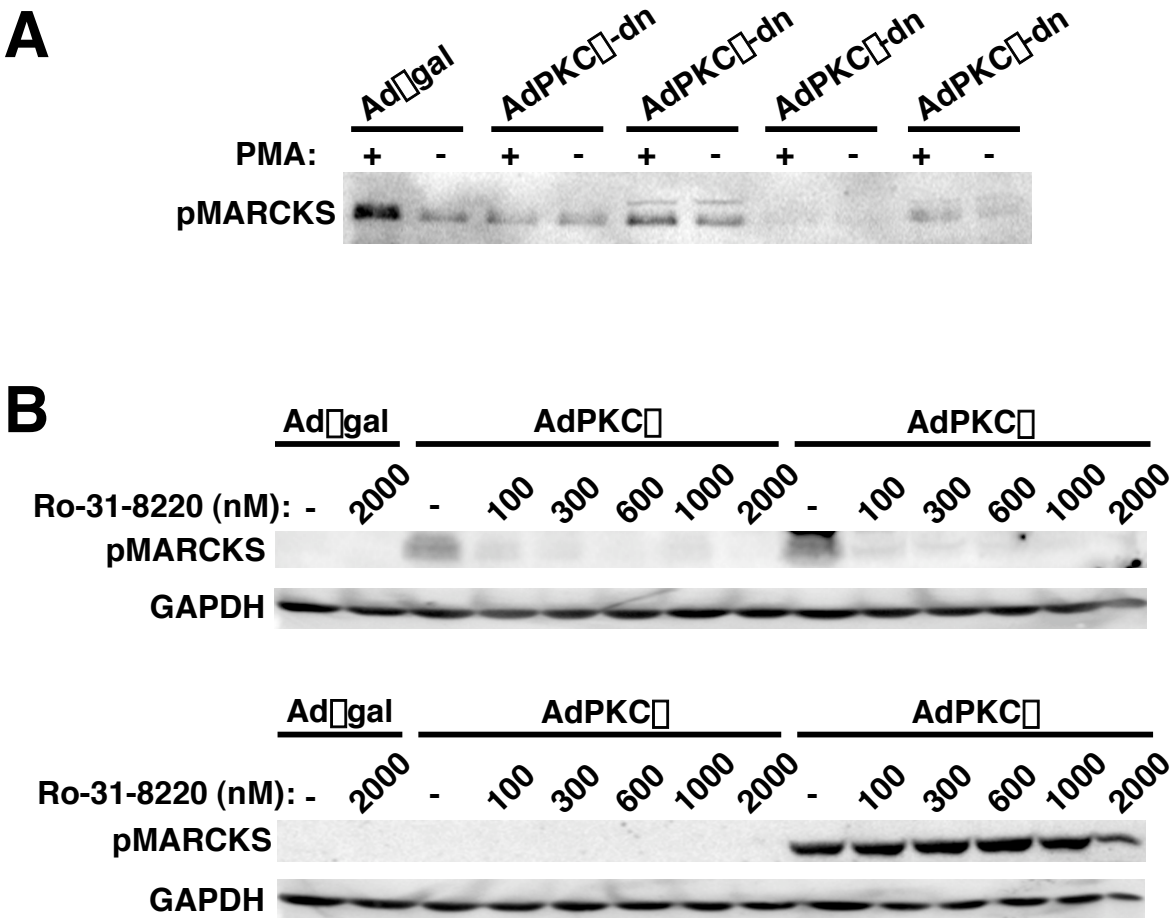
Supplemental Figure



Supplemental figure legend

Supplemental Figure. Analysis of PKC isoform inhibition with Ro-31-8220 in conjunction with MARCKS phosphorylation. (A) MARCKS phosphorylation has been reported to occur through multiple PKC isoforms, indicating that it is not selective for PKC. Indeed, endogenous MARCKS protein was activated by PMA (1 hr) through the action of endogenous PKC isoforms in neonatal cardiomyocyte cultures, as measured by western blotting with phospho-MARCKS specific antibody. However, prior infection (48 hr) with adenovirus expressing dominant-negative mutants of PKC, , , or each prevented MARCKS phosphorylation by PMA stimulation. These data indicate that MARCKS phosphorylation can occur through multiple endogenous PKC isoforms. (B) However, when wildtype PKC isoforms are overexpressed by adenoviral gene transfer (48 hrs) in neonatal cardiomyocytes they can mediate MARCKS phosphorylation without PMA stimulation, and this event is dependent only on the overexpressed isoform, allowing assessment of drug specificity. Indeed, Ro-31-8220 (Ro-32-0432 acted in a similar manner) inhibited PKC and PKC-dependent MARCKS phosphorylation at 100 nM, but PKC was not appreciably inhibited, while PKC was unable to even phosphorylate MARCKS in the first place, as assessed by western blotting. These data suggest that the Ro-31-8220 and Ro-32-0432 compounds are more specific for the conventional PKC isoforms.