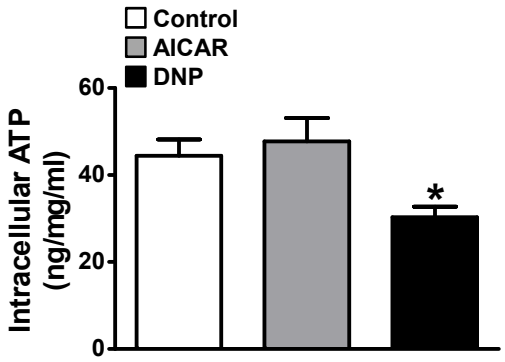
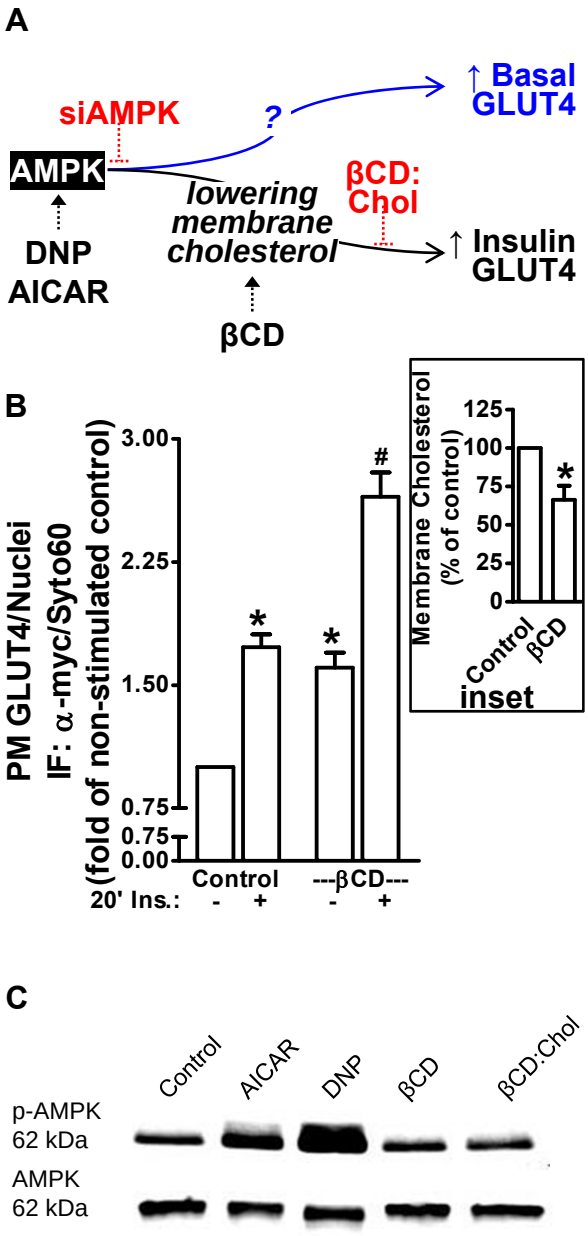


Supplemental Fig. 1 AMPK activation by ATP-independent and ATP-dependent mechanism. Myotubes were left untreated or treated with AICAR (1 mM, 45 min) or DNP (200 μM, 30 min). Intracellular ATP content was measured using a luminescence ATP detection assay system as described in *Materials and Methods*. ATP concentrations were normalized for protein concentration determined by the Bradford method. Values are means ±SE of 4 independent experiments. *P<0.05 vs. control group.



Supplemental Fig. 2 Proposed model of divergent membrane cholesterol-independent and dependent AMPK pathways regulating basal and insulin-stimulated PM GLUT4 content. Proposed model of divergent AMPK pathways highlighting that the insulin-like action of AICAR and DNP on mobilizing GLUT4 to the cell surface results from a cholesterol-independent mechanism; whereas membrane cholesterol lowering by AICAR and DNP contributes to the enhancement of insulin regulation of the transporter (A). Basal and insulin-stimulated (100 nM, 20 min) plasma membrane GLUT4 contents (B) were determined in myotubes treated as described in Fig. 2 in the absence or presence of βCD (2.5 mM, 30 min). Membrane cholesterol contents (panel B inset) were determined as described in Fig. 4. Myotubes were left untreated or treated with AICAR (1 mM, 45 min), DNP (200 μM, 30 min), βCD (2.5 mM, 30 min) or βCD:Chol (1 mM 8:1 molar ratio, 45 min). Cell lysates were subjected to SDS-PAGE and immunoblot analyses using anti-phospho AMPK and anti-pan AMPK antibodies (C). Representative immunoblots are shown from 3 independent experiments. Values are means SE of 4-5 independent experiments. *P<0.05 vs. control (or control-basal) groups; #P<0.05 vs. control-insulin group.