SUPPLEMENTAL DATA

MATERIALS & METHODS.

***Skeletal muscle incubations and [^{3}H]-2-deoxyglucose uptake*.** Muscle incubation experiments were performed as previously described (1)**.** Briefly, mice were fasted overnight and killed by cervical dislocation. The soleus muscles were removed and placed in Krebs-Ringer-Bicarbonate (KRB) solution containing (in mM): 117 NaCl, 4.7 KCl, 2.5 $CaCl_2 \bullet 2H_2O$, 1.2 KH_2PO_4, 1.2 $MgSO_4 \bullet 7H_2O$, 24.6 $NaHCO_3$) supplemented with 2 mM pyruvic acid.

For the basal state, muscles were incubated in KRB, 2 mM pyruvic acid, DMSO (0.2%), and EtOH (0.2%) for 20 min. All other muscles were incubated in KRB, 2 mM pyruvic acid, DMSO (0.2%), EtOH (0.2%), and 50 mU/ml insulin (Eli Lilly) for 20 min to stimulate the movement of GLUT4 to the plasma membrane. After 20 min, muscles were transferred to vials containing KRB supplemented with 1.5 μCi/ml [^{3}H]-2-deoxyglucose, 1 mM deoxyglucose, 0.45 μCi/ml [^{14}C]-mannitol, and 7 mM cold-mannitol. For the basal state, muscles were incubated in the radioactive KRB buffer. The insulin-stimulated muscles were divided into one of four groups as follows: 1] 50 mU/ml insulin (insulin only); 2] wortmannin (100 nM, Sigma-Aldrich); 3] cytochalasin-B (10 μM, Sigma-Aldrich); or 4] KN-62 (10 μM, Sigma-Aldrich) for 10 min. Appropriate amounts of DMSO and/or EtOH were added to the radioactive buffer to maintain concentrations at 0.2% for all conditions. Muscles were frozen in liquid N_2, solubilized in 1 M NaOH at 80°C and neutralized with 1 M HCl. Samples were centrifuged at 11,000 x g for 1 min. Aliquots were removed for scintillation counting of the [^{3}H] and [^{14}C] labels, and [^{3}H]-deoxyglucose uptake calculated.

Immunoblot analysis. Immunoblots were performed using standard procedures as described in (1). Primary antibodies were obtained from sources as follows: phospho-ACC1/2 ($Ser^{79/212}$) from Fisher Scientific**;** ACC (streptavidin-HRP) from Pierce Biotechology.

REFERENCES.

1. **Witczak CA, Fujii N, Hirshman MF and Goodyear LJ**. CaMKK{alpha} Regulates Skeletal Muscle Glucose Uptake Independent of AMPK and Akt Activation. *Diabetes* 56: 1403-1409, 2007.

FIGURE LEGENDS.

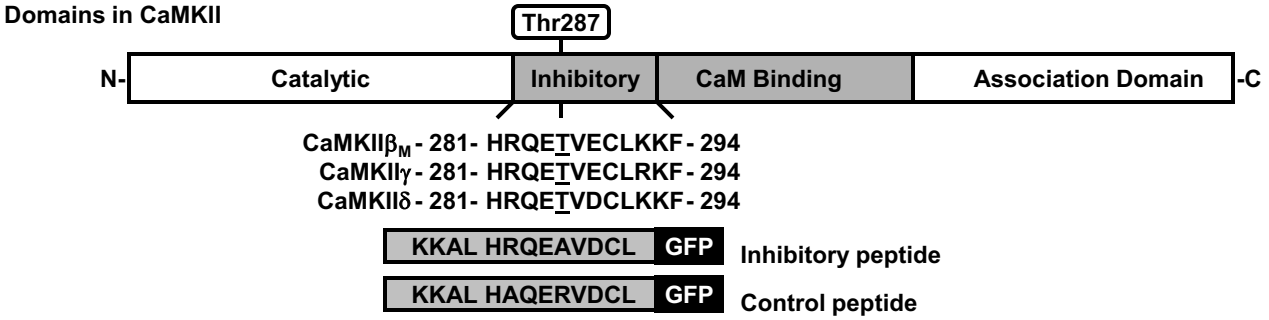
Supplemental FIG S1. The CaMKII inhibitory peptide, AC3-I, exhibits near identical homology with the inhibitory domain of CaMKII. Sequence of the CaMKII inhibitory and control peptides, and their alignment with the activating phosphorylation site (i.e. Thr287) of the three main isoforms of CaMKII in mouse skeletal muscle, CaMKIIβ_M, CaMKIIγ, and CaMKIIδ.

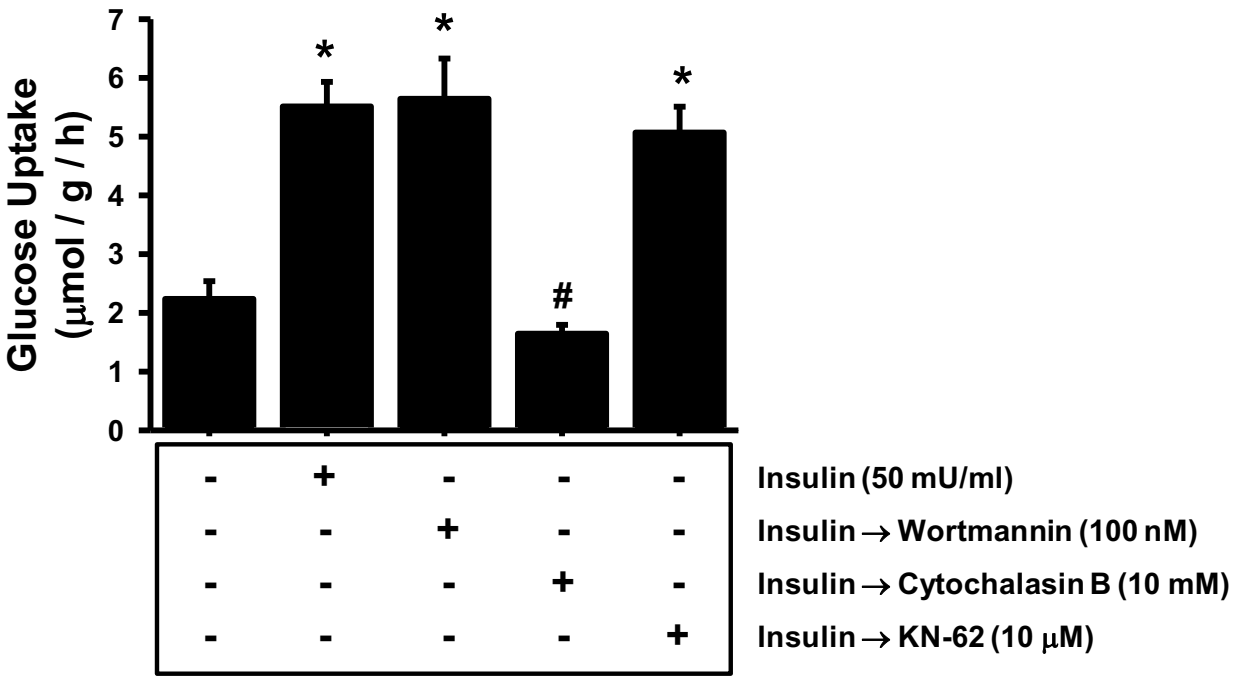
Supplemental FIG S2. KN-62 does not compete for the glucose binding site on glucose transporters. Mouse soleus muscles with stimulated with insulin (50 mU/ml, 20 min) to induce translocation of GLUT4 to the plasma membrane, and then muscles were incubated with either the phosphoinositide-3-kianse inhibitor, wortmannin (100 nM), the glucose transporter inhibitor,

cytochalasin B (10 μM) or the Ca^{2+}/calmodulin-competitive inhibitor, KN-62 (10 μM). KN-62 does not inhibit muscle glucose uptake via the same mechanism as cytochalasin B, which competes with glucose for the glucose binding site on glucose transporters. Statistical significance was defined as $P<0.05$ where * *vs.* basal, and # *vs.* insulin only. N = 4-6 muscles/group.

Supplemental FIG S3. The GFP-CaMKII inhibitory peptide enhances contraction-induced increases in acetyl-CoA carboxylase (ACC) phosphorylation. Mouse tibialis anterior muscles were transfected with DNA vectors containing either the GFP-CaMKII inhibitory peptide or the GFP-control peptide. After 1 wk, mice were anesthetized and then stimulated by contraction for 15 min. Muscles were harvested to assess signaling proteins by immunoblot analysis. The contraction-induced phosphorylation of ACC1/2 ($Ser^{79/212}$) was enhanced in muscles expressing the GFP-CaMKII inhibitory peptide compared to the control peptide. There was no difference in ACC1/2 protein expression. Statistical significance was defined as $P<0.05$ where * *vs.* sham, and # *vs.* control peptide. (N=6-8 muscles/group)

Witczak et al., *AJP-Endo* – Supplemental Figure S1




Glucose Uptake
(μmol / g / h)
0
1
2
3
4
5
6
7
*
*
#
*
- + - - -
- - + - -
- - - + -
- - - - +
Insulin (50 mU/ml)
Insulin → Wortmannin (100 nM)
Insulin → Cytochalasin B (10 mM)
Insulin → KN-62 (10 μM)

Witczak et al., *AJP-Endo* – Supplemental Figure S3