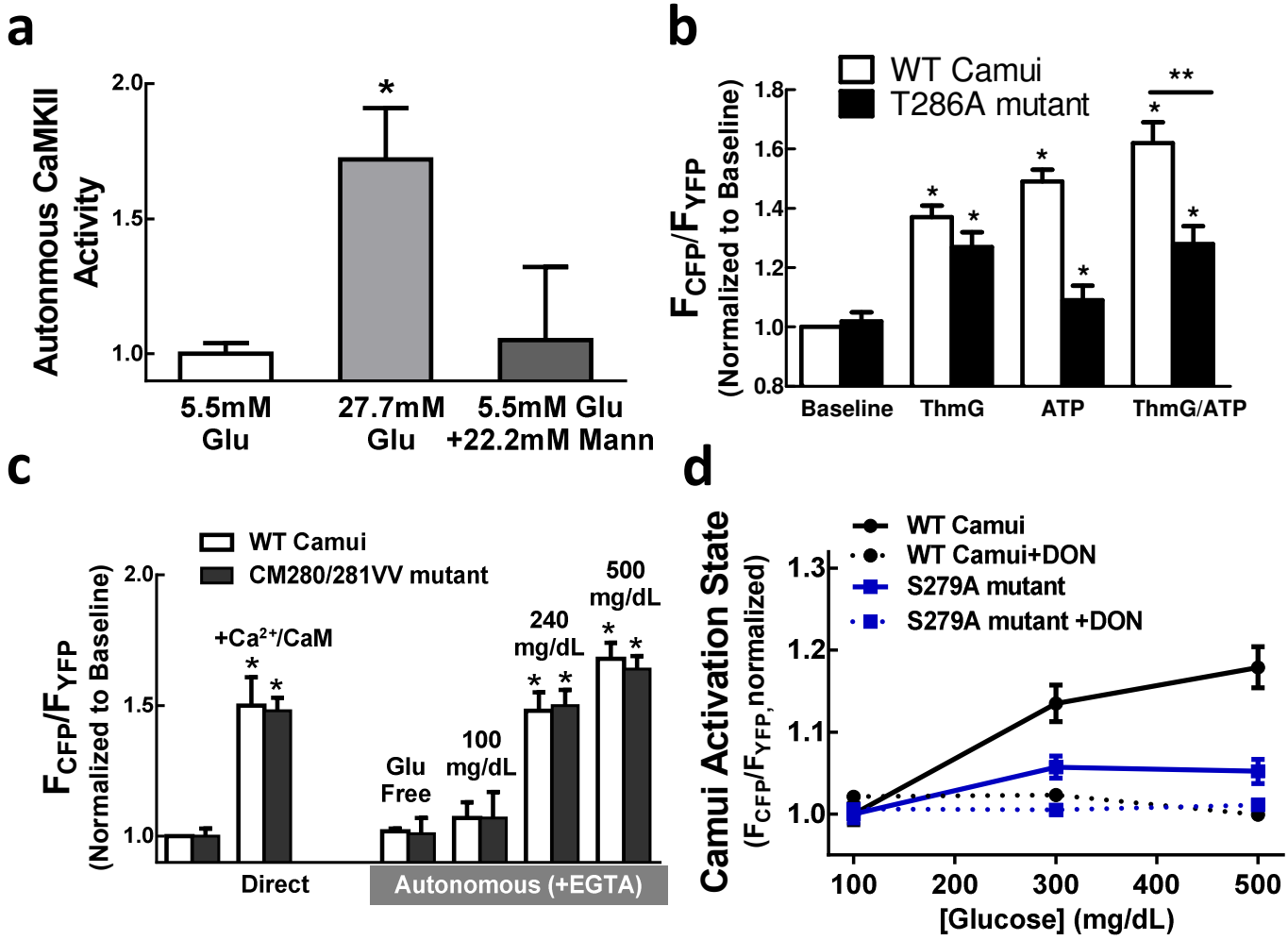
# Supplemental Figure 1




e

CaMKII regulatory domain alignment

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KHPWISHRSTVASCMHRQETVDCLKKFNARRKLKGAILTTMLATRNFSGGK – Mouse α
KHPWVCQRSTVASMMHRQETVECLKKFNARRKLKGAILTTMLATRNFSAKS – Mouse β
KHPWVCQRSTVASMMHRQETVECLRKFNARRKLKGAILTTMLVSRNFSAAK – Mouse γ
KHPWICQRSTVASMMHRQETVDCLKKFNARRKLKGAILTTMLATRNFSAAK – Mouse δ
KHPWICQRSTVASMMHRQETVDCLKKFNARRKLKGAILTTMLATRNFSAAK – Rat δ
KHPWICQRSTVASMMHRQETVDCLKKFNARRKLKGAILTTMLATRNFSAAK – Human δ
RHPWICQRSTVASMMHRQETVDCLKKFNARRKLKGAILTTMLATRNFSAKS – Xenopus δ
KHPWICQRERVASVVHRQETVDCLKKFNARRKLKGAILTTMLATRNFSSRS – Drosophila δ
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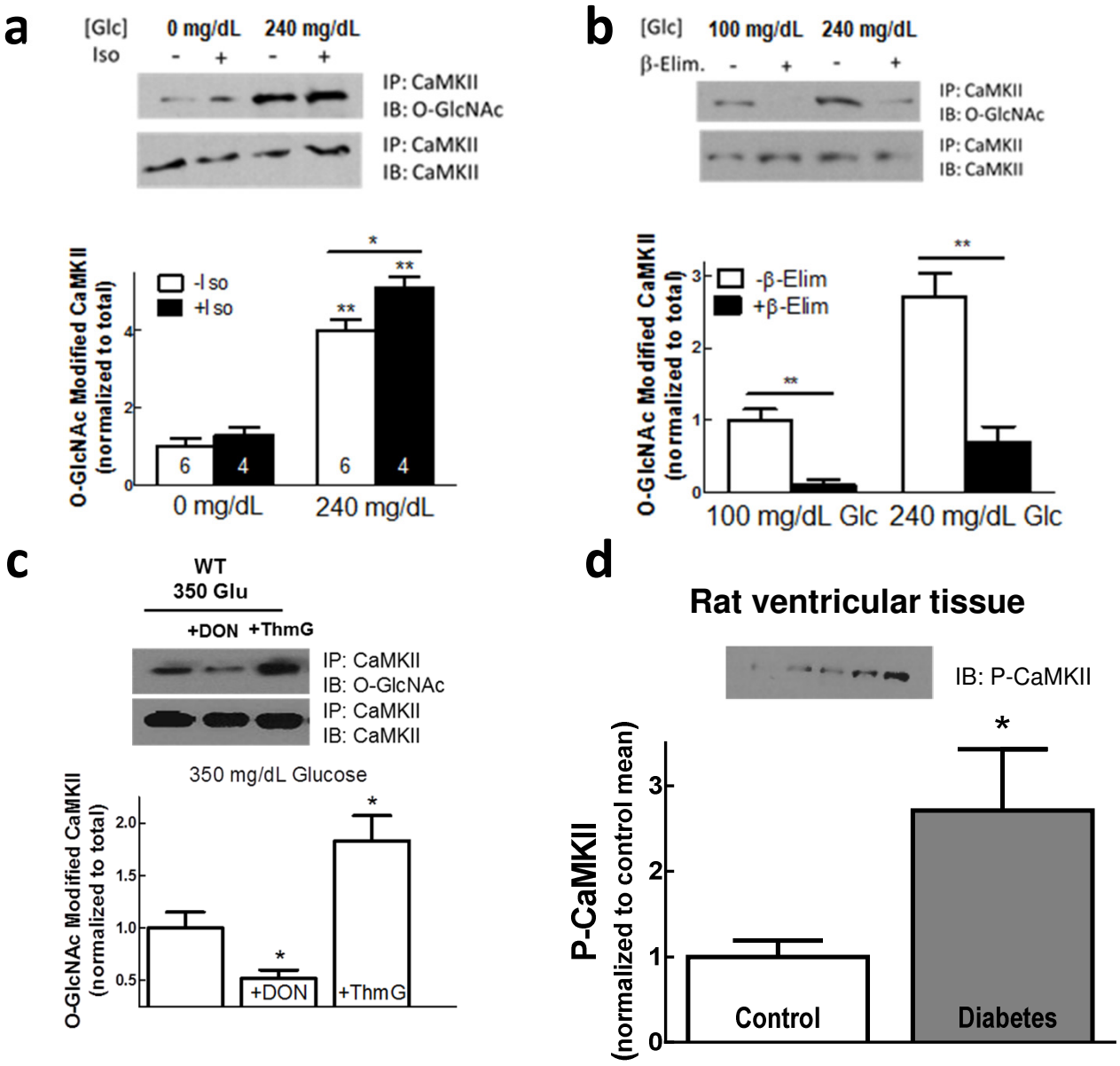
N – Non-conserved Amino Acid
N – Autophosphorylation Site
N – Oxidation Site
N – Potential O-glycosylation Site

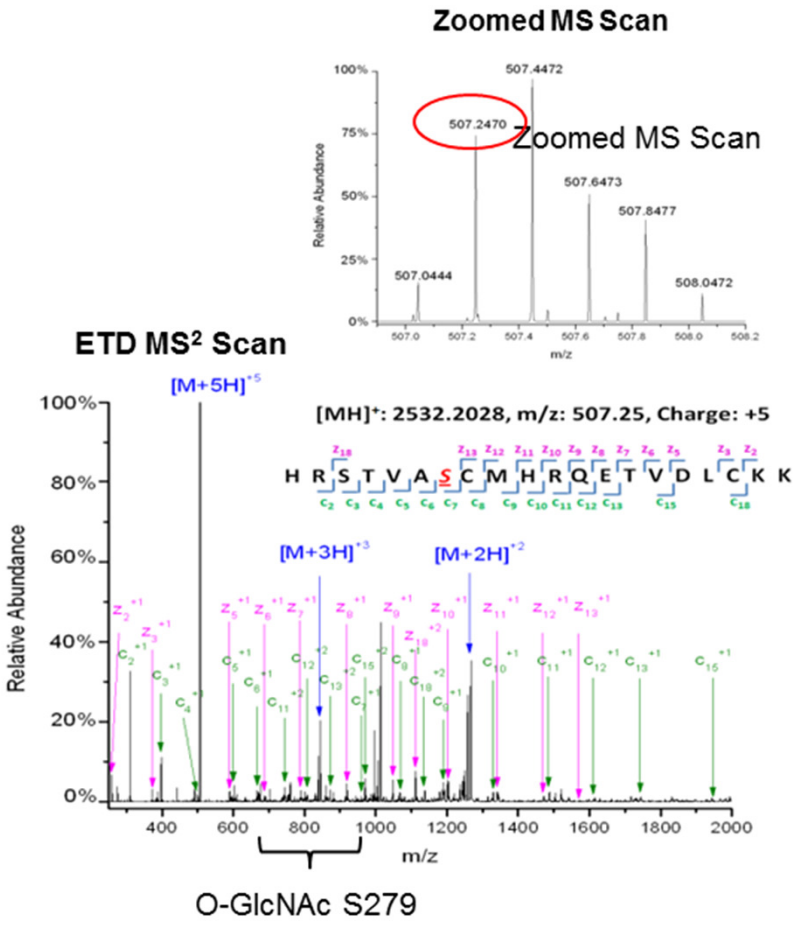
**Supplemental Figure 1 - O-GlcNAc effect is not abolished by T286A or CM280/1VV mutation and CaMKII regulatory domain contains consensus O-GlcNAc modification sites. a,** Increased [glucose], but not osmolarity matched [mannitol], activates CaMKII in HEK cells (n=9). **b,** O-GlcNAc dependent CaMKII activation is reduced but still present in T286A mutant Camui (n=9) **c,** Glucose dependent CaMKII activation is preserved in CM280/281VV mutant Camui expressed in HEK cell lysates (n=9). **d,** Activation of Camui by increased [glucose] is blunted in the S279A mutant and ablated entirely by DON. (n values: WT = 100, WT+DON = 72, S279A = 57, S279A+DON = 44 cells) **e,** These sites are conserved in all known isoforms of CaMKII and in a wide variety of mammalian species. Data are mean±s.e.m. * indicates $p<0.05$, ** indicates $p<0.01$ vs. control.

# Supplemental Figure 2



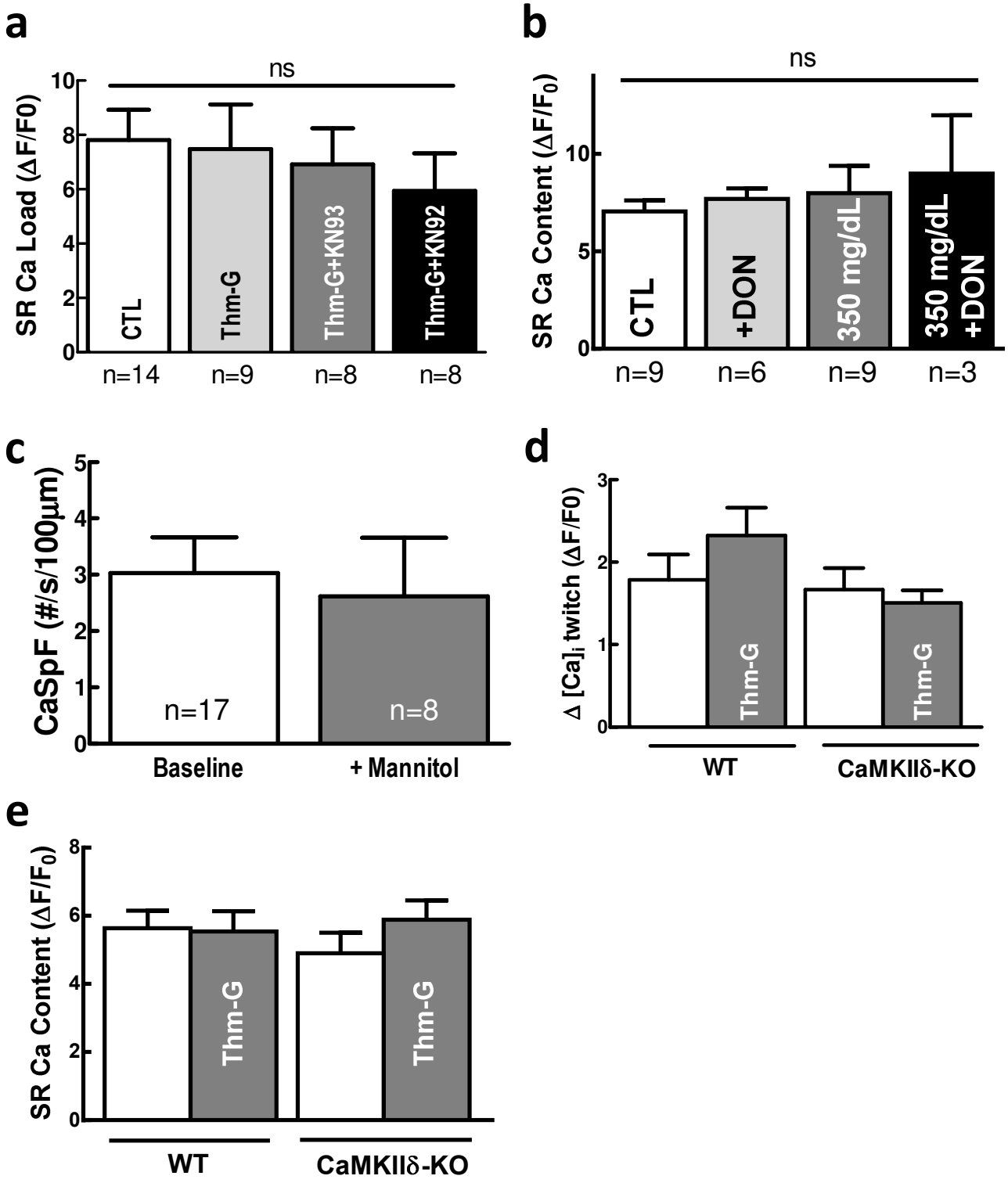

**Supplemental Figure 2 - O-GlcNAc modification of CaMKII is enhanced in hyperglycemic conditions. a,** Immunoblot with an O-GlcNAc specific antibody shows O-GlcNAc modification of CaMKII is inducible by increased glucose availability and is enhanced by Iso treatment. (n values indicated) **b,** O-GlcNAc modification of CaMKII is reversed by β-elimination reaction prior to immunoblot. **c,** O-GlcNAc modification of CaMKII is ablated by DON and enhanced by Thm-G. **d,** Autophosphorylation of cardiac CaMKII is significantly increased in a rat model of diabetes. n=3 for all immunoblots except where indicated. Data are mean±s.e.m. * indicates $p<0.05$, ** indicates $p<0.01$ vs. control.

# Supplemental Figure 3



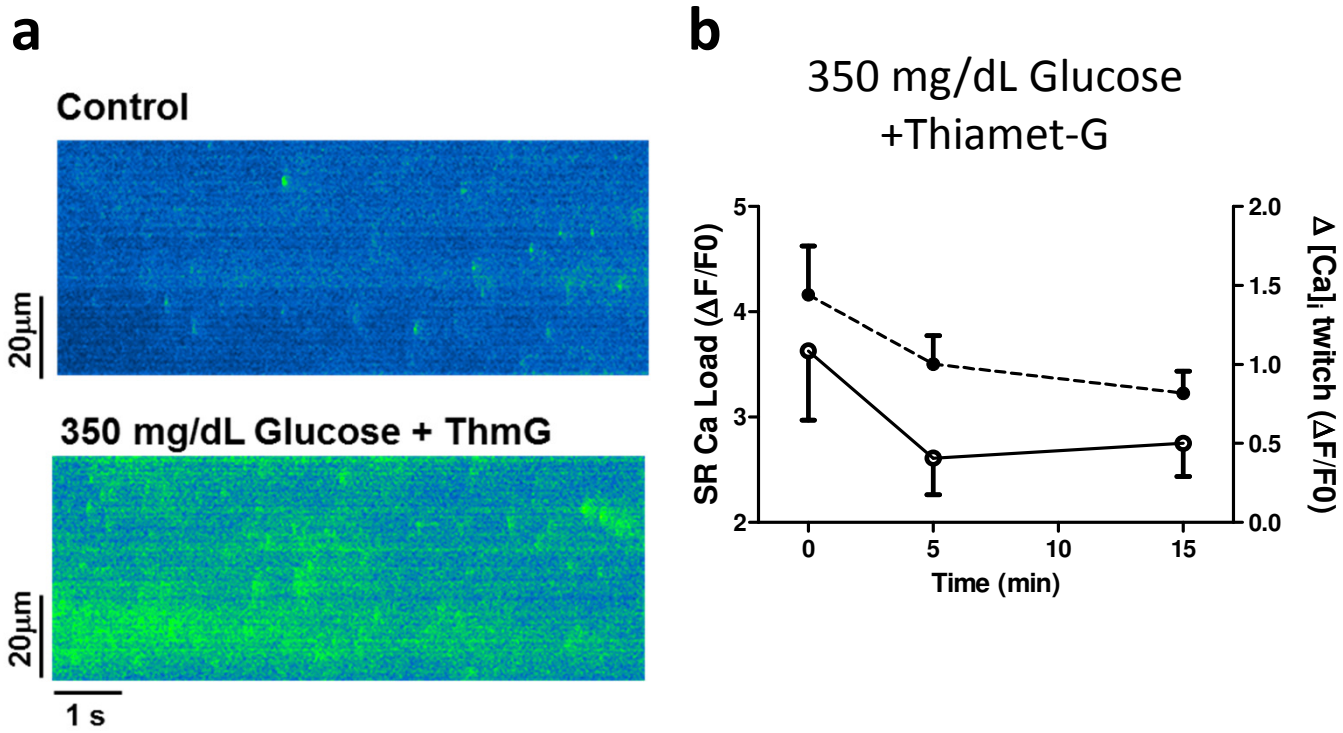

**Supplemental Figure 3 – ETD-MS analysis confirms O-GlcNAc modification at S279A.** A synthetic peptide encoding the regulatory domain of CaMKII was subjected to in vitro O-GlcNAc labeling followed by ETD-MS analysis. Examination of the 507.25 *m/z* peptide fragment (upper right inset) indicates the presence of an O-GlcNAc modification at S279 (*c6* to *c7* fragmentation).

# Supplemental Figure 4



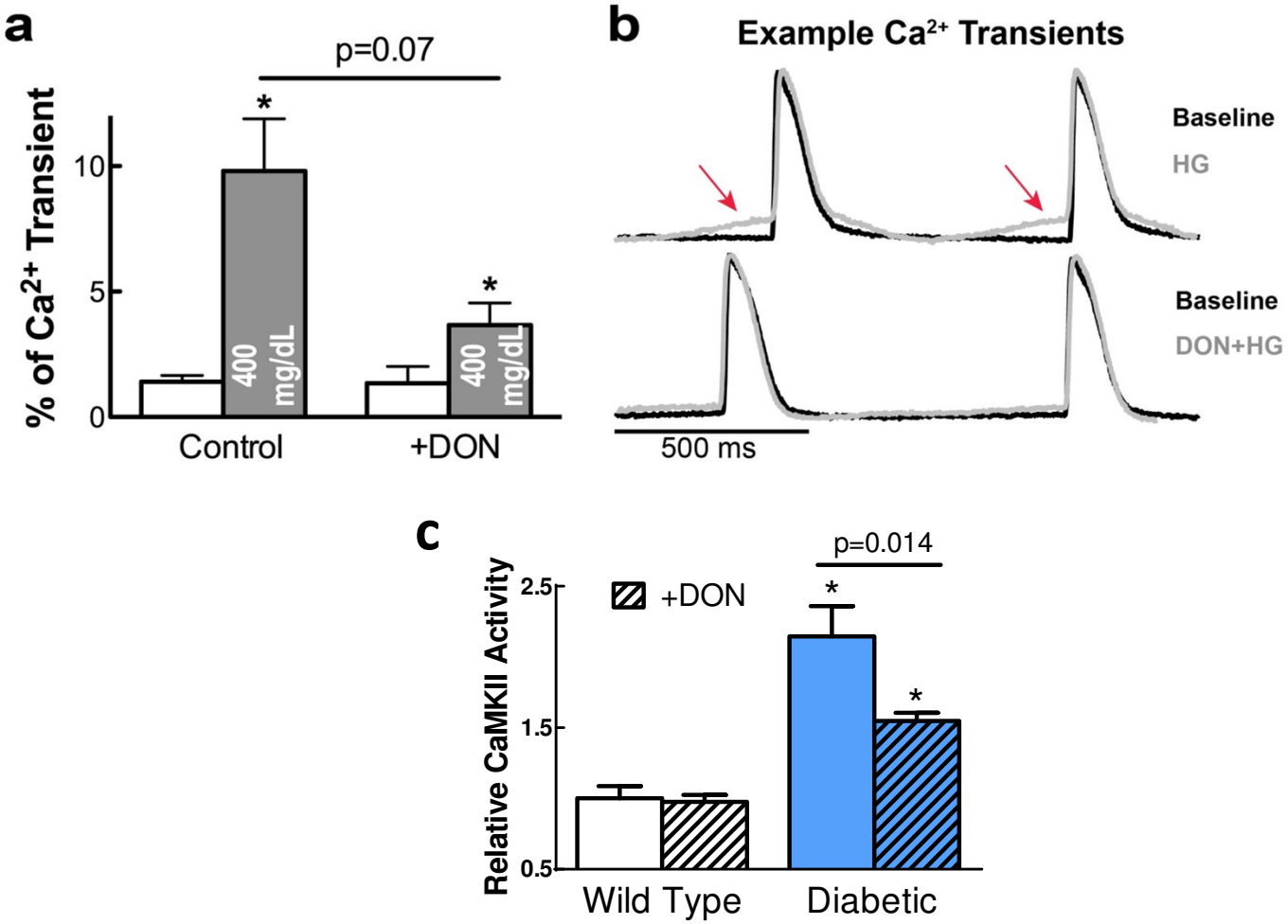

**Supplemental Figure 4 -** SR content is unaffected by Thm-G (**a**) or DON (**b**) in isolated rat myocytes (n values indicated). **c,** Mannitol does not enhance $Ca^{2+}$ spark frequency in isolated rat myocytes. $Ca^{2+}$ transient amplitude (**d**, n=13) and SR content (**e**, n=13) content are unaffected by Thm-G treatment in isolated myocytes from wild type (WT) or CaMKIIδ knockout mice. Data are mean±s.e.m. ns indicates no significant difference.

# Supplemental Figure 5




**Supplemental Figure 5 -** Simultaneous treatment with 350 mg/dL glucose and ThmG greatly enhances $Ca^{2+}$ spark frequency **a,** and SR $Ca^{2+}$ depletion **b,** in isolated rat myocytes (n=6). Data are mean±s.e.m.

# Supplemental Figure 6




**Supplemental Figure 6 - Diastolic $[Ca^{2+}]_i$ elevation under high glucose (HG) is suppressed by pre-treatment with 50 mM DON. a,** Average diastolic $Ca^{2+}$ elevation at baseline and following treatment with either HG or DON+HG. $Ca^{2+}$ elevation was measured as the percent increase in the diastolic $Ca^{2+}$ signal relative to the amplitude of the following $Ca^{2+}$ transient. (n=3) **b,** Example $Ca^{2+}$ transients during baseline conditions (black) and following treatment with either HG or DON+HG (gray). Minimal diastolic $Ca^{2+}$ elevation occurs following pre-treatment with DON. n=3-4 rats for all data points. **c,** CaMKII activity is enhanced in heart lysate from diabetic rats (n=3), and this effect is blunted by treatment with DON. Data are mean±s.e.m. * indicates $p<0.05$ vs. control.