***SUPPLEMENTAL DATA***

*for the manuscript by*

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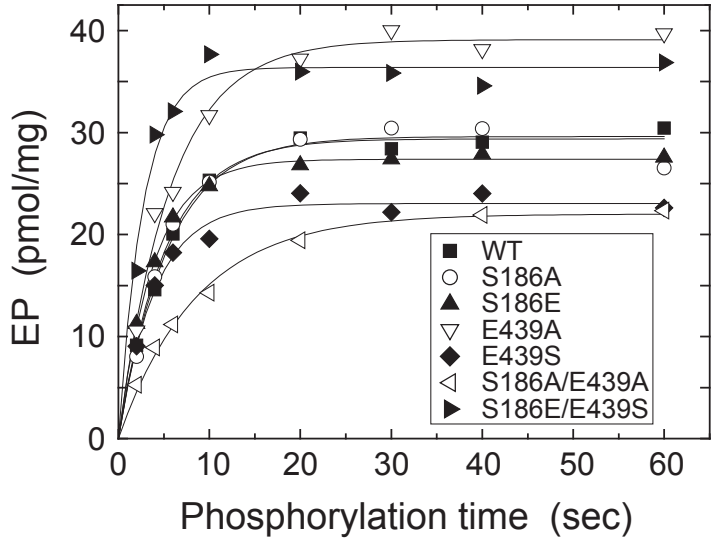
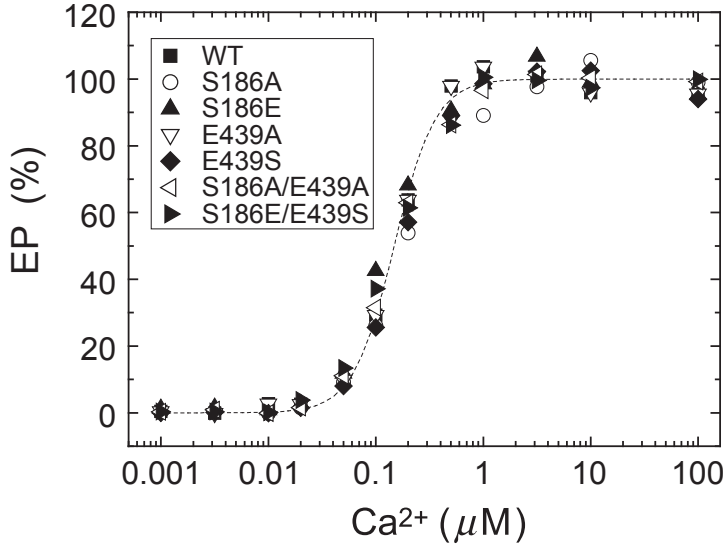
# Roles of Interaction between Actuator- and Nucleotide Binding-domains of Sarco(endo)plasmic Reticulum $Ca^{2+}$-ATPase as Revealed by Single and Swap Mutational Analyses of Serine186 and Glutamate439

# Supplemental Figure 1



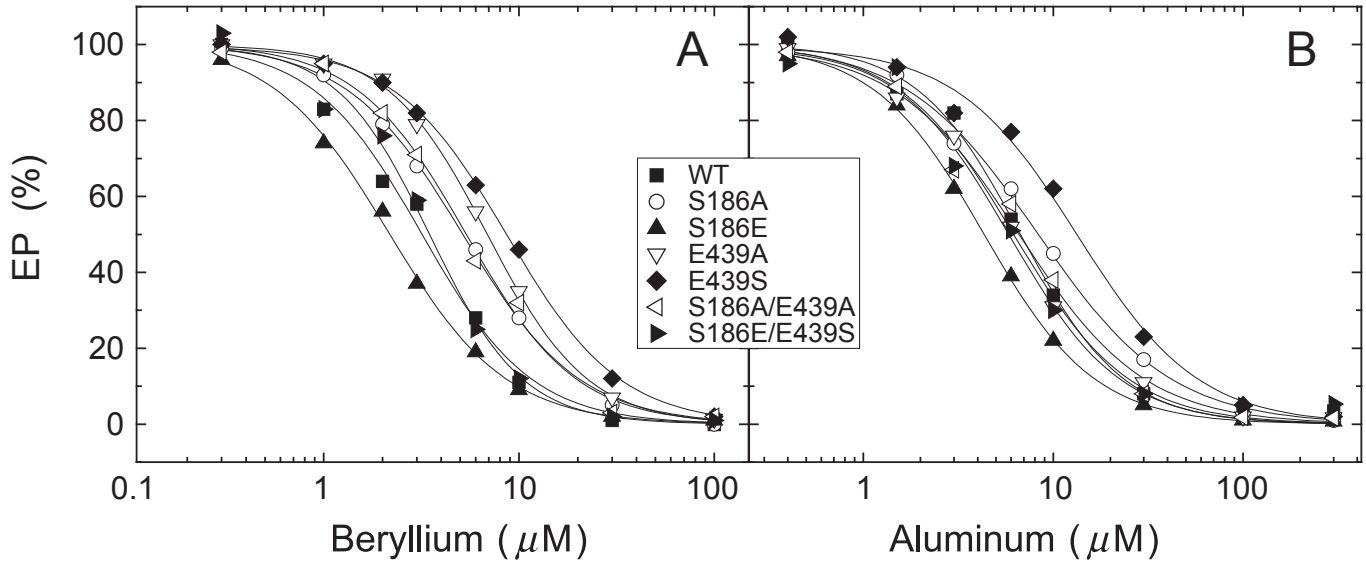

Supplemental Figure 1. **Phosphorylation upon simultaneous addition of ATP and $Ca^{2+}$.** Microsomes expressing the wild-type or mutant SERCA1a (2 $\mu$g of microsomal protein) were preincubated in the absence of $Ca^{2+}$ at 0 °C for 5 min in 50 $\mu$l of a buffer containing 1 mM EGTA, 1 $\mu$M A23187, 0.1 M KCl, 7 mM $MgCl_2$, and 50 mM MES/Tris (pH 6.0). At zero time, an equal volume of the buffer containing 20 $\mu$M [$\gamma$-$^{32}$P]ATP and 1.2 mM $CaCl_2$ in place of EGTA otherwise as above, was added to the microsome suspension. At various times after this addition, the amount of *E*P formed was determined and shown as pmol/mg of microsomal protein.

# Supplemental Figure 2




Supplemental Figure 2. **$Ca^{2+}$ dependence of *E*P formation from ATP.** Microsomes expressing the wild-type or mutant SERCA1a were preincubated with various concentrations of $Ca^{2+}$ as indicated at 25 °C for 15 min in 50 $\mu$l of a mixture containing 2 $\mu$g microsomal protein, 1 $\mu$M A23187, 0.1 M KCl, 7 mM $MgCl_2$, 50 mM MOPS/Tris (pH 7.0), and various concentrations of $CaCl_2$ with 2 mM EGTA, and then cooled and phosphorylated at 0 °C for 15 s by addition of a small volume of [$\gamma$-$^{32}$P]ATP to give 10 $\mu$M. The amount of *E*P formed was determined as described under "Experimental Procedures." The data were best fitted with the Hill equation, and the $EP_{max}$ obtained in the fitting in each mutant was normalized to 100%. The dissociation constant for $Ca^{2+}$ ($K_{0.5}$) and Hill coefficient ($n_H$) thus obtained are given in Table 1. The *solid line* shows the least squares fit for the wild type.

# Supplemental Figure 3




Supplemental Figure 3. **$BeF_3^-$ and $AlF_4^-$ binding to the *E*2-state ATPase.** Microsomes expressing the wild-type or mutant SERCA1a were incubated for 30 min at 25 °C in 50 $\mu$l of a mixture containing 50 mM MOPS/Tris (pH 7.0), 0.1 M LiCl, 2 mM EGTA, 200 $\mu$M $MgCl_2$, 2 mM KF, and the indicated concentrations of $BeSO_4$ (*A*) or $AlCl_3$ (*B*). The samples were then cooled and phosphorylated by ATP at 0 °C by the addition of an equal volume of a buffer containing 4.2 mM $CaCl_2$, 2 mM EGTA, 0.1 M LiCl, 9.8 mM $MgCl_2$, 2 mM KF and 20 $\mu$M [$\gamma$-$^{32}$P]ATP. The reaction was quenched by acid at 15 s. Solid lines show the least squares fit to the Hill equation, and $K_{0.5}$ values are given in Table 1.