SUPPLEMENTAL FIGURES AND LEGENDS

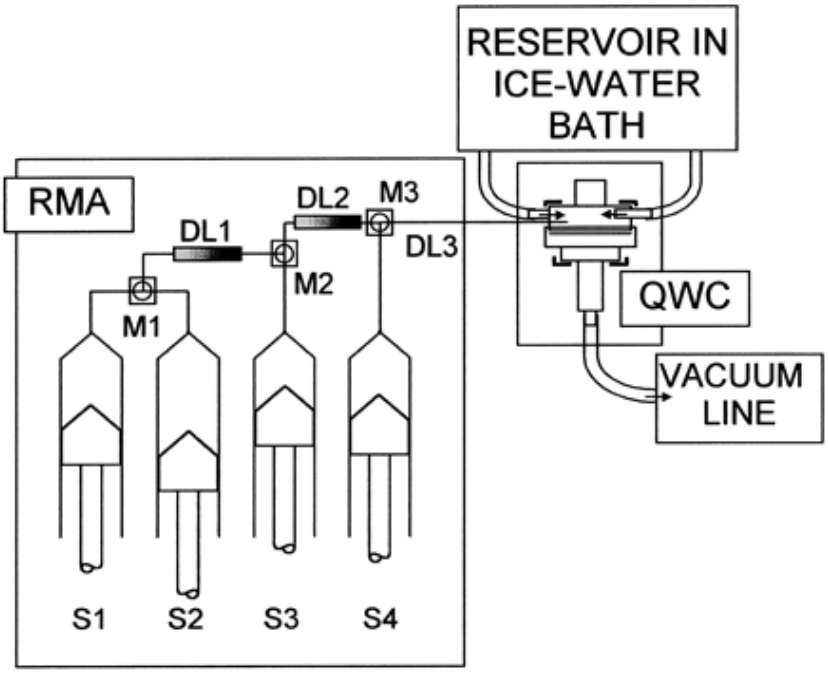

Figure S1. A scheme of the arrangement used to measure occluded Ca^{2+}. The arrangement of the rapid-mixing apparatus (RMA) and of the quenching-and-washing chamber (QWC). The RMA has four syringes (S1 to S4) to hold the reactants, three mixers (M1 to M3), and three delay lines (DL1 to DL3) in which the reactions are allowed to proceed. These components are contained in a water-tight compartment whose temperature is kept constant by circulating water from a bath. After reacting for preset lengths of time in one or more delay lines, the incubation mixture is squirted from DL3 into the stream of an ice-cold (1–2°C) washing solution flowing through the QWC. The washing solution comes from a reservoir which is kept in an ice-water bath. During a run, this solution flows along two flexible tubings into the QWC, where it can receive the incubation mixture, traverses the Millipore filter, and is drained out. The flow is driven by the vacuum generated by a water-jet pump, connected to a 2-liter sided-arm flask.

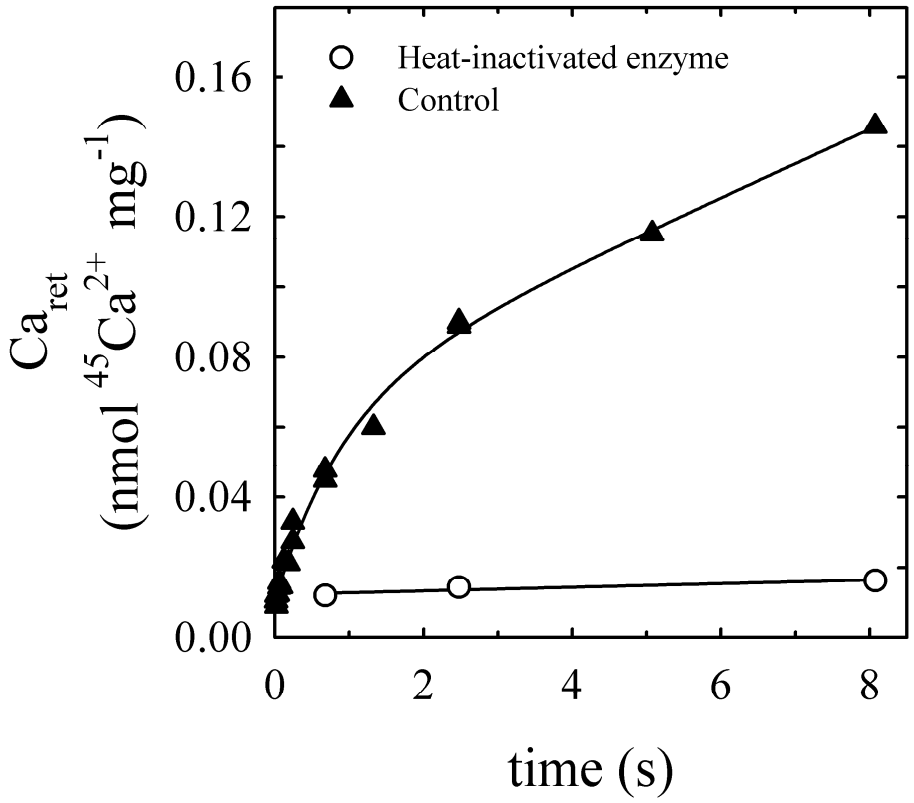

Figure S2. Calcium retained as a function of time. We measured the amount of retained calcium as a function of time in the presence of inactive enzyme (○) and control enzyme (▲).The retained calcium was measured at 25°C in reaction medium containing 30 mM MOPS (pH 7.4 at 25°C); 60 µg/ml total protein, 3 mM $MgCl_2$; 0.2 mM thapsigargin; 25 mM ATP, enough (^{45}Ca)$CaCl_2$ to give concentrations of 60 µM free Ca^{2+}. The heat-inactivated enzyme was treated with 24 µg/ml alamethicin. Figure S3 shows that the retained Ca^{2+} for control enzyme is exponentially dependent on time while that for the inactivated enzyme is minimal and shows a linear time dependence. These results suggest that the inactivated enzyme retains small amounts of calcium nonspecifically and this condition was therefore used as a blank for measurements of specifically retained calcium.

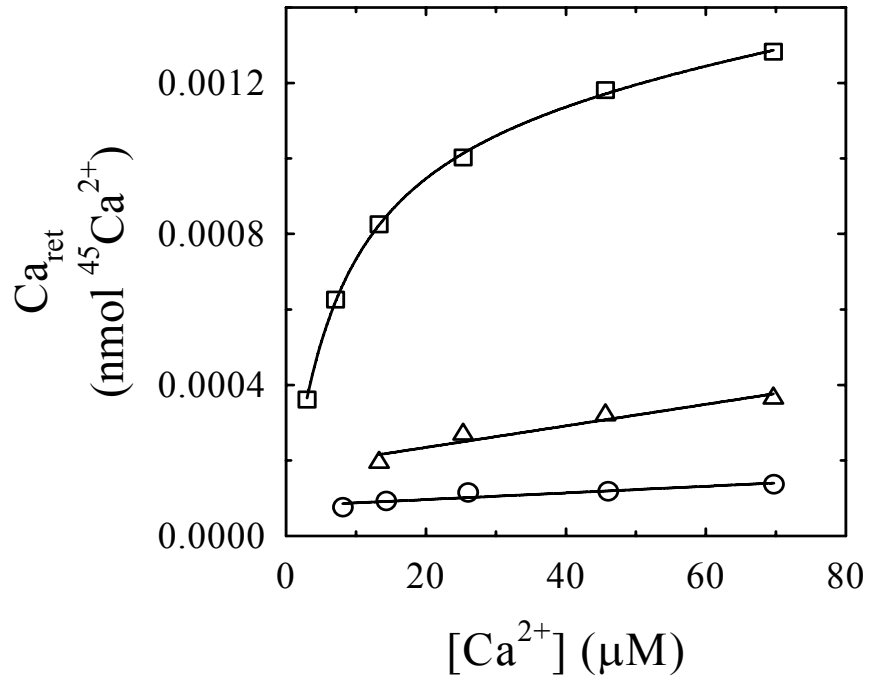

Figure S3. Calcium retained as a function of $[Ca^{2+}]$. We measured the amount of retained calcium as a function of $[Ca^{2+}]$ in the presence of inactived enzyme (2 h at 50 ºC) (Δ), and with (□) and without active control enzyme (○). The retained calcium was measured at 25°C during 1 second in the reaction medium containing 60 μg/ml total protein, 3 mM $MgCl_2$; 0.2 mM thapsigargin; 2000 μM ATP, 12 μg/ml alamethicin and enough (^{45}Ca)$CaCl_2$ to give different concentrations of free Ca^{2+}. The active control enzyme shows a hyperbolic dependence of Ca^{2+} retained on $[Ca^{2+}]$ while the inactivated enzyme and no enzyme control show a linear dependence. These results suggest that the inactivated enzyme retains some calcium nonspecifically and this condition should therefore be used as a blank for measurements of specific calcium retained.