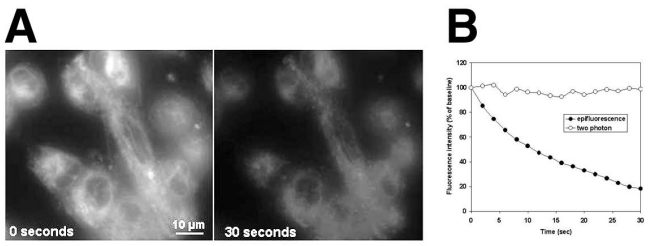

Figure S1. Limitations of conventional widefield UV excitation to examine the nucleoplasmic reticulum. **(A)** A field of SKHep1 cells loaded with ER-Tracker and visualized using conventional (one photon) epifluorescence rather than two photon microscopy. The nucleoplasmic reticulum is less distinct and quickly photobleaches. **(B)** Comparison of photobleaching of ER-Tracker dye visualized using two photon microscopy and epifluorescence microscopy. Fluorescence rapidly decreases with epifluorescence imaging, but not with two photon imaging. Values are the mean from 20 measurements in each group. These findings suggest that two photon excitation may facilitate identification of the nucleoplasmic reticulum, perhaps because of decreased photobleaching, or else because of decreased phototoxicity, increased axial resolution, or some combination of these factors [14,15].

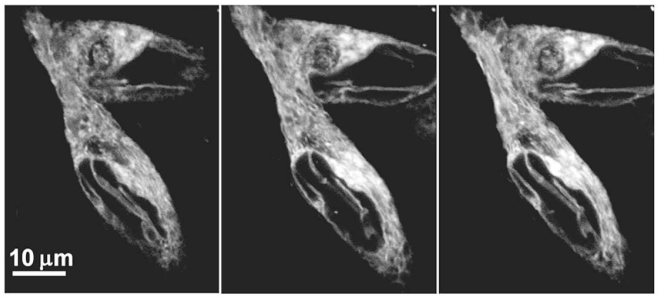

Figure S2. Visualization of the nucleoplasmic reticulum with mag-fluo-4. Three serial focal planes of an SKHep1 cell labeled with the low affinity Ca^{2+} dye mag-fluo-4/AM and visualized using confocal microscopy demonstrate that the nucleoplasmic reticulum stores Ca^{2+}. The nucleoplasmic reticulum can be followed from the ER and nuclear envelope into the nuclear interior. This is similar to the nuclear structures observed with ER-Tracker (Figure 1a, 1b), calreticulin (Figure 1c), and other Ca^{2+} dyes (Figure 1d).

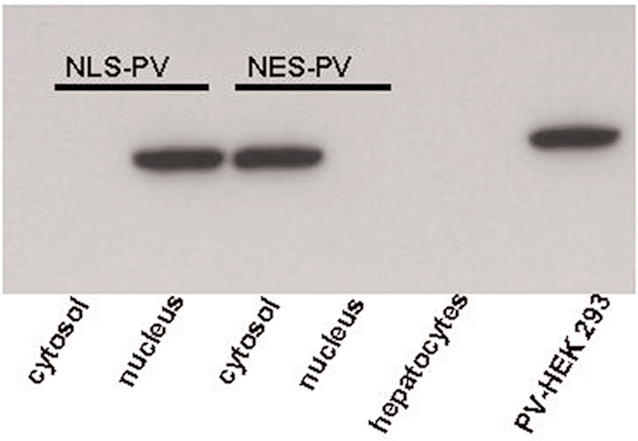

Figure S3. Demonstration of the purity of the cytosolic and nuclear fractions. SKHep1 cells were transfected with parvalbumin targeted either to the nucleus (NLS-PV) or the cytosol (NES-PV)[8], then cytosolic or nuclear fractions were probed for parvalbumin. Hepatocytes and PV-transfected HEK 293 cells were used as negative and positive controls, respectively. A specifically targeted protein was used rather than an ER marker because ER proteins would be found in either the cytosol or nucleus [16]. Parvalbumin was detected in the nuclear but not the cytosolic fraction of NLS-PV transfected cells, and in the cytosolic but not nuclear fraction of NES-PV transfected cells, which suggests the purity of nuclear and cytosolic fractions in this study. On the other hand, parvalbumin is a cytosolic protein, whereas the InsP3 receptor is an integral membrane protein of the ER. Thus, these fractionation studies could be limited by the possibility that adherent ER contaminates nuclear fractions. Although immunoelectron microscopic studies might be used to investigate this possibility, interpretation of such studies would be complicated by the fact that ER markers have also been identified within the nucleus [16].

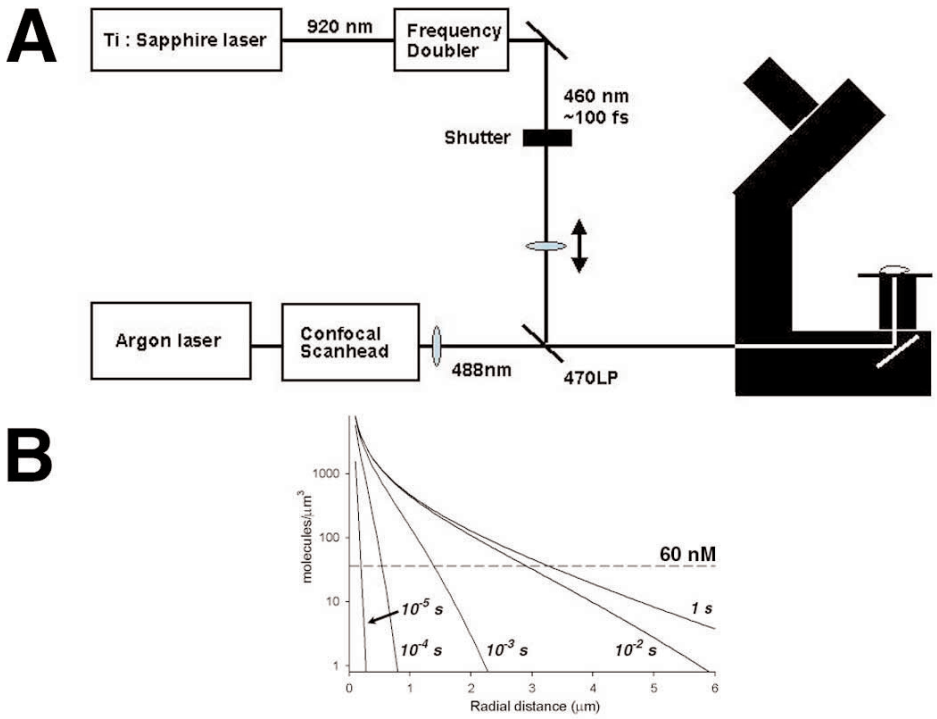

Figure S4. A method for efficient localized photolysis of NPE-InsP3 using two photon excitation. **(A)** A practical strategy for localized subcellular release of caged InsP3 by two-photon flash photolysis. An LBO crystal is used to double the frequency of the femtosecond-pulsed beam, then a moveable lens is used to assure that uncaging occurs in the plane of focus of the confocal image. **(B)** Spatial gradients of the InsP3 concentration formed by photorelease of NPE-InsP3 by two-photon excitation. It was assumed that NPE-InsP3 diffuses into and free InsP3 diffuses out of the focal volume, and that free InsP3 is degraded at a constant rate. Calculations based on 1 mW of 460 nm, frequency-doubled light focused through a 1.4 NA lens show that photolysis of all NPE-InsP3 within the focal volume would occur within a few ?sec. The two-photon illumination point spread function at 460 nm has a lateral full-width half-maximum (FWHM) of 130 nm and 310 nm in the axial direction, corresponding to an excitation volume of ~10^{-2} ?m^3 (10^{-17} liters). Under our experimental conditions, the intracellular concentration of NPE-InsP3 is between 50-500 ?M, corresponding to 300-3000 molecules in the focal volume. After the molecules initially within the focal volume are photolysed, additional InsP3 is produced by diffusion of fresh NPE-InsP3 into the volume. To determine the local intranuclear concentration of free InsP3, a diffusion-reaction equation was formulated and numerically solved that includes diffusion of fresh NPE-InsP3 into the focal volume, instantaneous photorelease, and then diffusion away from the focal volume. The calculation was based on diffusion constants of 280 μm^2/sec for InsP3 [38] and 200 μm^2/sec for NPE-InsP3, an InsP3 degradation rate of 2/sec [39], an initial intracellular concentration of 50 ?M NPE-InsP3, and a cell diameter of 12 μm. Figure S4b shows the concentration profile of free InsP3 for uncaging intervals ranging from 10^{-5} to 1 sec. An uncaging interval of ~1 sec was required to form a region with radius 3 μm in which the concentration of free InsP3 exceeds 60 nM, the EC_{50} for the type II InsP3 receptor [40]. Because the type II InsP3R is relatively enriched in the nucleus of SKHep1 cells, a photolysis time of 1 sec was used to insure that this InsP3 concentration was reached throughout the region of the nucleoplasmic reticulum.