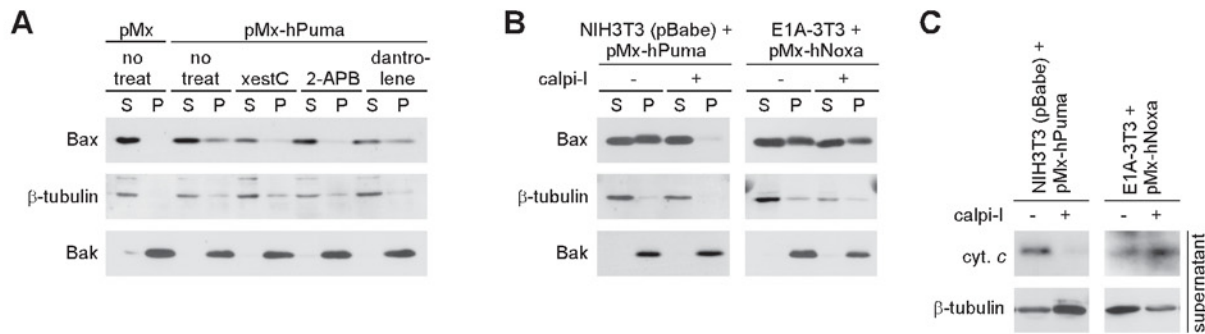


Supplementary Figure 5

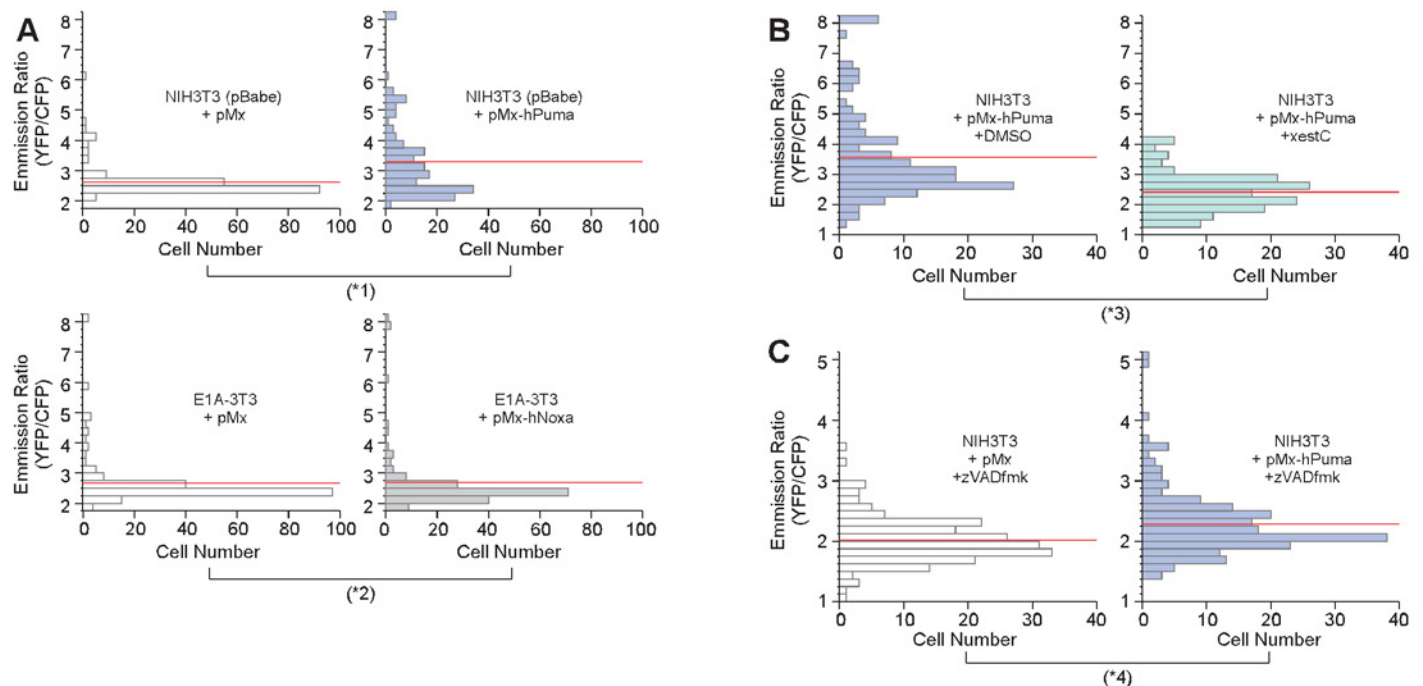


Supplementary Figure 5 Involvement of Ca^{2+} /calpain system in Puma-induced apoptosis of NIH3T3 cells.

(A) Puma-induced Bax membrane insertion under the blockade of ER calcium channels. Puma-induced Bax membrane insertion in NIH3T3 cells was analyzed in the presence of the blockers of InsP_3R (xestC and 2-APB) or the blocker of RyR (dantrolene).

(B, C) Role of calpain in the upstream of MOMP in Puma-induced apoptosis. The effects of calpain inhibitor I (calpi-I) treatment on Bax membrane insertion (B) and cytochrome *c* (cyt. *c*) release (C) during Puma-induced apoptosis of control NIH3T3 cells and Noxa-induced apoptosis of E1A-3T3 cells were analyzed.

Supplementary Figure 6



Supplementary Figure 6 Fixed-time observation of the $[\text{Ca}^{2+}]_c$ elevation during Puma-induced apoptosis.

(A) $[\text{Ca}^{2+}]_c$ changes in Puma-expressing control NIH3T3 cells and Noxa-expressing E1A-3T3 cells. Control NIH3T3 cells and E1A-3T3 cells were nucleofected with the YC3.60 expression vector, together with pMx (control vector), pMx-hPuma or -hNoxa, and 10 h later, analyzed by fluorescence microscopy. The emission ratio (YFP/CFP) of the fluorescence intensity of CFP and YFP within the region drawn around an individual cell are calculated and plotted on the histograms (see Materials and methods). (*1): $p < 0.001$; (*2): $p > 0.3$

(B) Effect of xestospongins C (xestC) treatment on Puma-induced $[\text{Ca}^{2+}]_c$ elevation. $[\text{Ca}^{2+}]_c$ of Puma-expressing NIH3T3 cells were measured as described in (A), in the presence and absence of xestC. (*3): $p < 0.001$

(C) Puma-induced $[\text{Ca}^{2+}]_c$ elevation in the presence of zVADfmk. $[\text{Ca}^{2+}]_c$ of Puma-expressing NIH3T3 cells and that of control NIH3T3 cells were measured as described in (A), in the presence of zVADfmk. (*4): $p < 0.001$

In (A)-(C), red line indicates the average value, and the top bars in the histograms represent the numbers of cells with emission ratios of more than 8 (A, B) or 5 (C). PI-positive cells, which were supposed to have lost the plasma membrane integrity, were excluded from counting.