**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 InsP3R (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** Fixed-time observation of the [Ca\(^{2+}\)]\(_e\) elevation during Puma-induced apoptosis.

(A) [Ca\(^{2+}\)]\(_e\) 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 pMr (control vector), pMr-hPuma or hPuma, 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 xestospongin C (xestC) treatment on Puma-induced [Ca\(^{2+}\)]\(_e\) elevation. [Ca\(^{2+}\)]\(_e\) of Puma-expressing NIH3T3 cells were measured as described in (A), in the presence and absence of xestC. (*2); p < 0.001

(C) Puma-induced [Ca\(^{2+}\)]\(_e\) elevation in the presence of zVADfmk. [Ca\(^{2+}\)]\(_e\) 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.