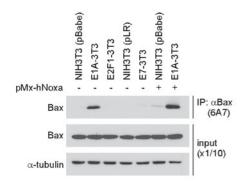
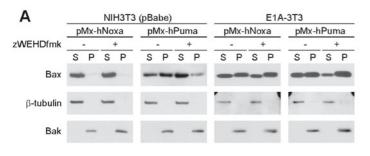
Supplementary Figure 3

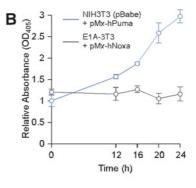


Supplementary Figure 3 Activation-related conformational change of Bax.

Total cell lysates were prepared from the derivatives of NIH3T3 cells either or not infected with the Noxa-expressing retrovirus, using CHAPS lysis buffer that has a marginal effect on the conformation of Bax (Ref: Hsu and Youle, J Biol Chem 273, 10777, 1998). Immunoprecipitation was performed using the anti-Bax monoclonal antibody 6A7, which specifically recognizes conformationally active Bax molecules (Ref: Hsu and Youle, J Biol Chem 272, 13829, 1997), followed by immunoblotting with the anti-Bax polyclonal antibody N-20, which recognizes Bax molecules regardless of their conformation. Cell lysates of 50 µg, which corresponds to 1/10 the amount of each of the samples analyzed by immunoprecipitation, were separately analyzed by the direct immunoblotting (input (x1/10)).

Supplementary Figure 4





Supplementary Figure 4 Involvement of WEHDase activity in Puma-induced apoptosis of NIH3T3 cells.

(A) Effect of zWEHDfmk on Noxa- and Puma-induced Bax membrane insertion in control NIH3T3 cells and E1A-3T3 cells.

(B) Changes in WEHDase activity during Puma-induced apoptosis of control NIH3T3 cells and Noxa-induced apoptosis of E1A-3T3 cells. In control NIH3T3 cells, Puma expression resulted in up to 197% increase (24 h after Puma-expressing retrovirus infection) in the absorbance of free p-nitroaniline (pNa) at 405 nm, which is released from WEHD-pNa upon WEHD sequence cleavage. Values shown are means \pm S.D. from triplicate samples.