Supplementary file to Winkler et al. “Omi/HtrA2 and XIAP are components of platelet apoptosis signalling” (Thromb Haemost 2013; 109.3)

Suppl. Figure 1: Different expression levels of apoptotic proteins among healthy human donors. Western blot analyses of apoptosis protein expression in human platelets shown in Fig. 1 were quantified by densitometry. Apoptosis proteins levels are expressed relative to β-actin. For all proteins, data are expressed as mean values ± SEM of 3 independent analyses.
Suppl. Figure 2: Ucf-101 does not interfere with the release of mitochondrial intermembrane space (MIMS) proteins into the cytoplasm after induction of platelet apoptosis. Washed platelets (10^9/mL in Tyrodes-Hepes buffer) were first pretreated with Ucf-101 (10μM) as in Figure 3, and then stimulated with collagen (10 μg/mL) and thrombin (1 U/mL) (C + T) for 5 min, or Ca^{2+} ionophore A23187 (3 μM) for 10 min, or ABT-737 (3 μM) respectively buffer only (control) for 2 hr at 37°C. Release of MIMS Omi/HtrA2, Smac/Diablo and cytochrome c was assessed by Western blot analysis of the supernatant (SN) containing proteins released from mitochondria, and of the HM pellet containing intact mitochondria and platelet cytoskeleton, as described in Materials and Methods. As a positive control for induction of apoptosis, active caspase-3 and caspase-7 were analyzed in the HM (B). Representative Western blots of 3 different donors for C+T and A23187 and of 2 different donors for control and ABT-737. β-actin was included as loading control for HM fractions both in (A) and (B).