

**A Fermented Mistletoe (*Viscum album* L.) Extract Elicits markers characteristic for  
Immunogenic Cell Death Driven by Endoplasmic Reticulum Stress *in vitro***

**Ulrike Weissenstein<sup>1\*</sup>, Sibylle Tschumi<sup>1</sup>, Bettina Leonhard<sup>1</sup>, Stephan Baumgartner<sup>1,2</sup>**

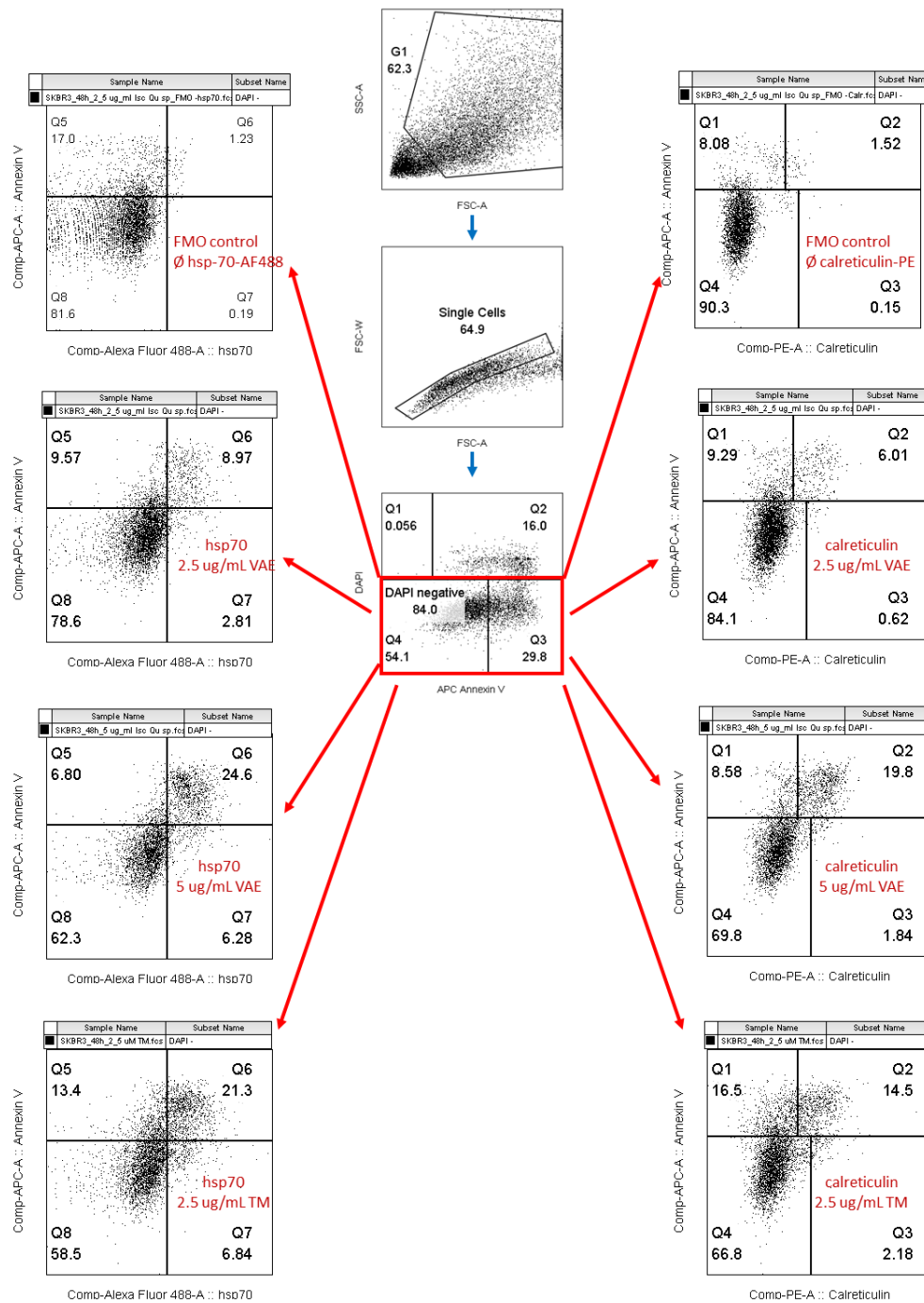
<sup>1</sup> Society for Cancer Research, Arlesheim, Switzerland

<sup>2</sup> Institute of Integrative Medicine, Witten/Herdecke University, Herdecke, Germany

\* Corresponding Author

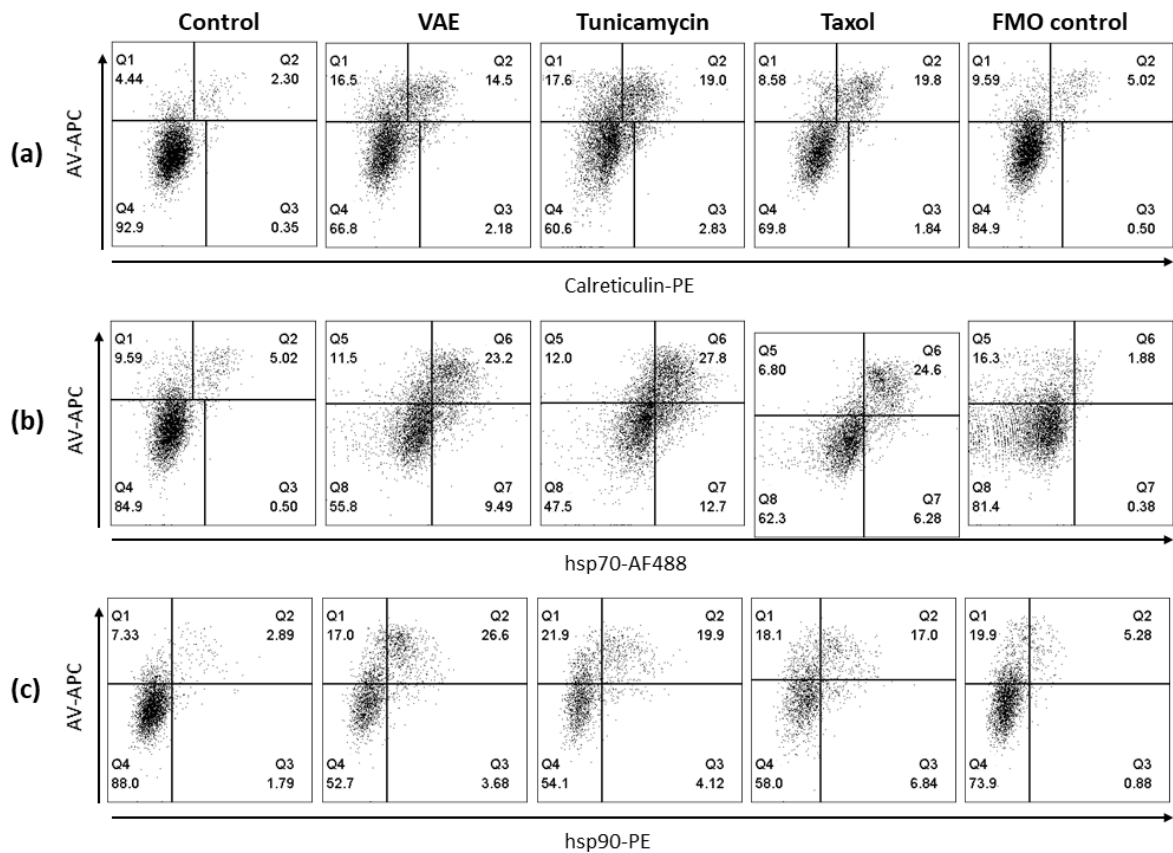
***Supplementary Material***

## Gating strategy for DAMP analysis



**Supplementary Figure 1.** Representative flow cytometry plots illustrating the gating strategy for FACS analysis of cancer cells. In the FSC/SSC dot blot, G1 encompasses the cancer cell population, single cells were further gated. Dapi negative cells were then analyzed for their expression of Annexin V and the respective DAMPs. Separation between DAMP-positive and DAMP-negative populations was performed using FMO (fluorescence minus one) controls.

Flow cytometry analysis of DAMP exposure



**Supplementary Figure 2.** Representative dot blots of the flow cytometry analysis of DAMP exposure on the surface of 7-AAD/DAPI-negative SKBR3 cells after 48h treatment with VAE, tunicamycin or Taxol compared to untreated control and FMO control. (a) Calreticulin, (b) hsp70 and (c) hsp90.

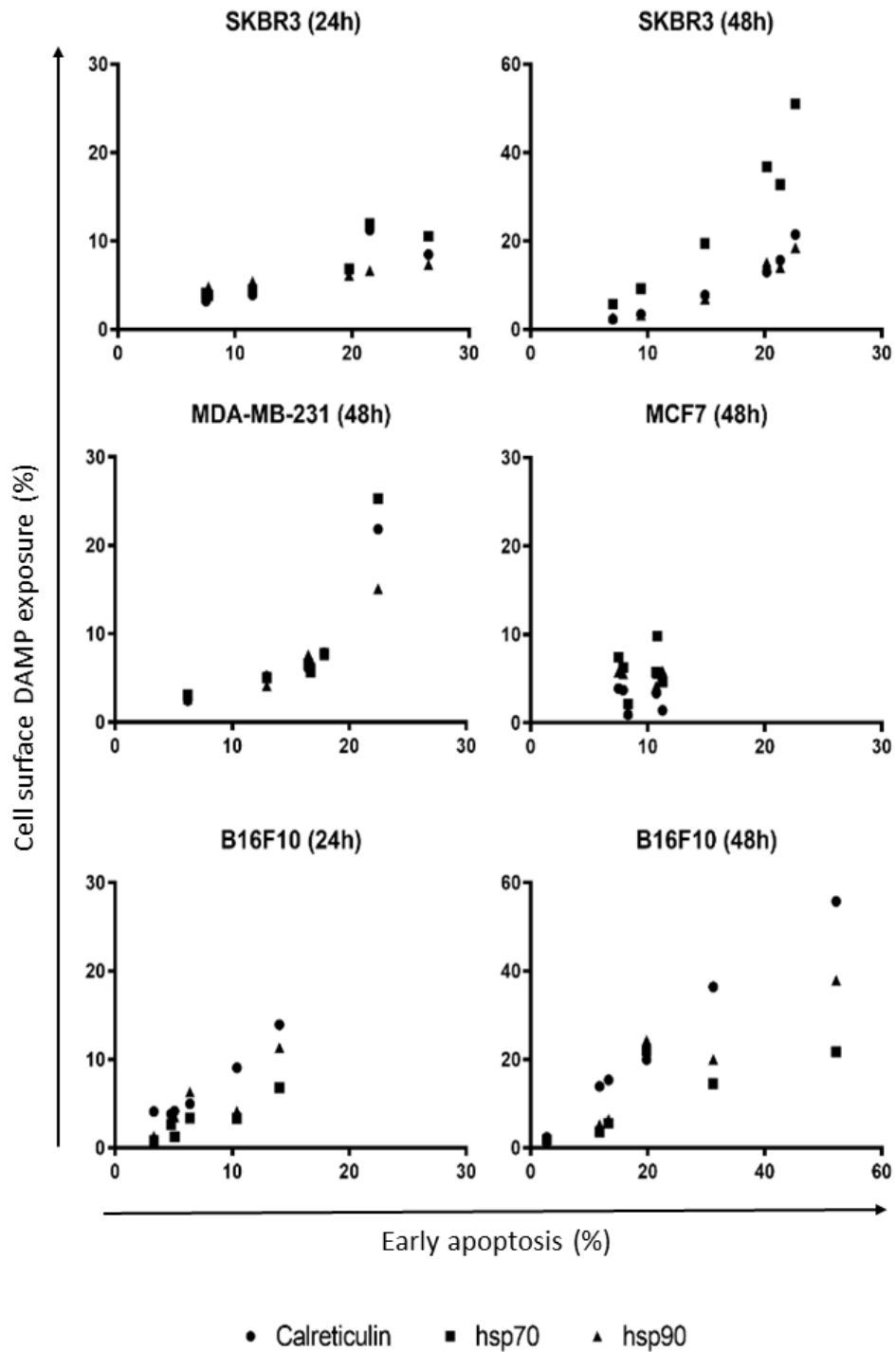
# **Spearman nonparametric correlation between early apoptosis and surface expositions of DAMPs**

**Supplementary Table 1:** Spearman nonparametric correlation between early apoptosis and surface expositions of DAMPs after 24h treatment (SKBR3 and B16F10) and 48h treatment (SKBR3, MDA-MB-231, MCF-7 and B16F10) with ICD inducing drugs

		<b>AV+/DAPI- - vs. CAL+</b>	<b>AV+/DAPI- vs. hsp70+</b>	<b>AV+/DAPI- vs. hsp90+</b>
<b>SKBR3 24h</b>	Spearman r	0.9429	0.8857	1.0
	P value (two-tailed)	0.0167	0.0333	0.0028
	Exact or approximate P value	Exact	Exact	Exact
	Significance level (alpha = 0.05)	Yes	Yes	Yes
<b>SKBR3 48h</b>	Number of XY Pairs	6	6	6
	Spearman r	1.000	0.9429	0.9429
	P value (two-tailed)	0.0028	0.0167	0.0167
	Exact or approximate P value	Exact	Exact	Exact
	Significance level (alpha = 0.05)	Yes	Yes	Yes
	Number of XY Pairs	6	6	6
<b>MDA-MB-231 48h</b>	Spearman r	1.000	0.9429	0.9429
	P value (two-tailed)	0.0028	0.0167	0.0167
	Exact or approximate P value	Exact	Exact	Exact
	Significance level (alpha = 0.05)	Yes	Yes	Yes
	Number of XY Pairs	6	6	6
<b>MCF-7 48h</b>	Spearman r	-0.2000	-0.2000	0.2571
	P value (two-tailed)	0.7139	0.7139	0.6583
	Exact or approximate P value	Exact	Exact	Exact
	Significance level (alpha = 0.05)	No	No	No
	Number of XY Pairs	6	6	6

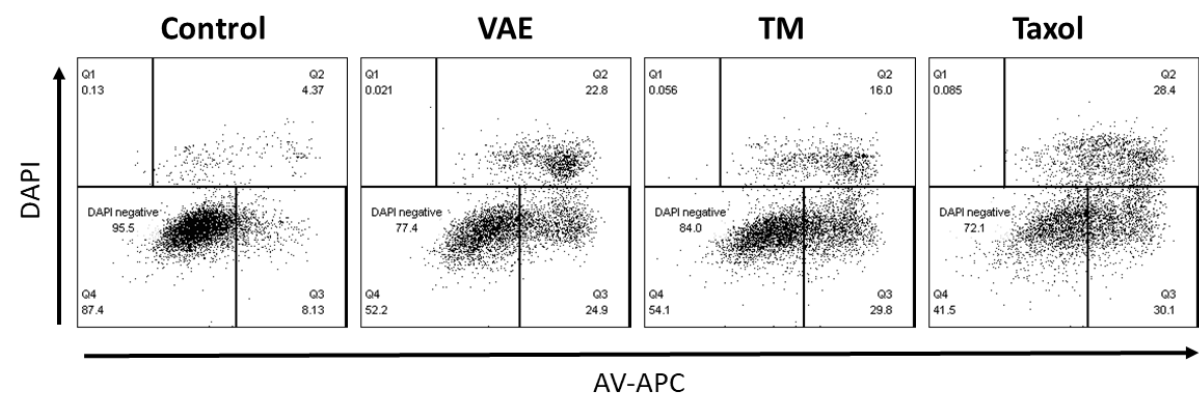
<b>B16F10 24h</b>	Spearman r	0.9429	0.8857	0.9429
	P value (two-tailed)	0.0167	0.0333	0.0167
	Exact or approximate P value	Exact	Exact	Exact
	Significance level (alpha = 0.05)	Yes	Yes	Yes
	Number of XY Pairs	6	6	6
<b>B16F10 48h</b>	Spearman r	1.000	0.8286	0.9429
	P value (two-tailed)	0.0028	0.0583	0.0167
	Exact or approximate P value	Exact	Exact	Exact
	Significance level (alpha = 0.05)	Yes	No	Yes
	Number of XY Pairs	6	6	6

### Correlation of Apoptosis and DAMP surface expression



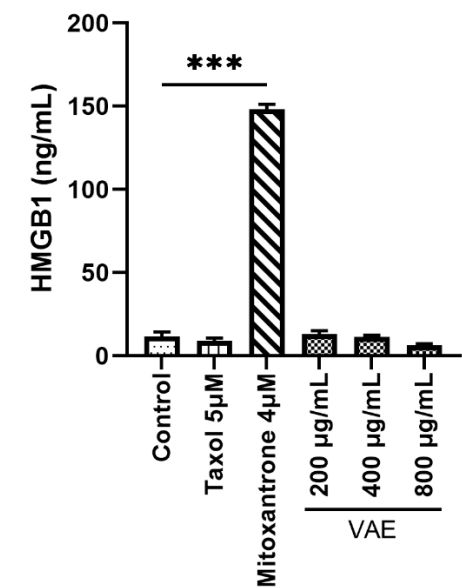
**Supplementary Figure 3.** Scatter plot of representative correlation between the cell surface DAMP exposure and early apoptosis detected in cancer cell lines

Flow cytometric analysis of apoptosis



**Supplementary Figure 4.** Representative dot blots of flow cytometric analysis of apoptosis in SKBR3 cells treated for 48h with VAE, tunicamycin or Taxol compared to untreated control. Cells were labeled with Annexin V-APC and DAPI. The numbers in the graphs represent the percentages of cells in each quadrant. Q1 and Q2: late apoptotic/necrotic cells, Q3: early apoptotic cells, Q4: living cells. Cells in Q3 and Q4 were referred to as DAPI negative.

HMGB1 release by MDA-MB-231 cells



**Supplementary Figure 5.** HMGB1 release by MDA-MB-231 cells. Cells treated with indicated concentrations of Taxol, Mitoxantrone or VAE for 24h. Results are presented as mean  $\pm$  SE from at least 3 independent experiments. Significance values are given relative to the untreated control (\*\*\*) $p < 0.001$ .