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Sandow, JJ; Dorstyn, L; O'Reilly, LA; Tailler, M; Kumar, S; Strasser, A; Ekert, PG. ER stress does not cause upregulation and activation of caspase-2 to initiate apoptosis. *Cell Death & Differentiation* (2014) **21,** 475–480; doi:<u>10.1038/cdd.2013.168</u>

http://www.nature/cdd/journal/v21/n3/suppinfo/cdd2013168s1.html?url=/cdd/journal/v 21/n3/full/cdd2013168a.html

#### Sandow et al. Figure 1



Sandow et al. Figure 2

















Sandow et al. Supplementary Figure 1



#### Sandow et al. Supplementary Figure 2



## Sandow et al. Supplementary Figure 3



### **Supplementary information**

Supplementary Figure 1. Thymocytes from wt and *caspase-2<sup>-/-</sup>* mice are equally susceptible to ER stress inducing drugs. Primary thymocytes from wild-type and *caspase-2<sup>-/-</sup>* mice (WT n=6, *caspase-2<sup>-/-</sup>* n=6) were treated with BFA, TG (at the indicated doses) or vehicle over the indicated time course and Annexin V negative/PI negative (i.e. surviving) cells quantified by flow cytometry. P-values were calculated using unpaired t-test, (ns) signifies p>0.05.

## Supplementary Figure 2. Caspase-2 protein levels are not upregulated following ER stress in thymocytes.

Primary thymocytes from wild-type and *caspase-2*<sup>-/-</sup> (negative control for antibodies to caspase-2) mice were treated with BFA (1  $\mu$ g/ml), TG (100 nM) or vehicle over the indicated time course. Cell lysates were analysed by immunoblotting using antibodies against caspase-2 (clone 10C6), cleaved (i.e. activated) caspase-3 and  $\beta$ -actin (loading control).

# Supplementary Figure 3. Caspase-2 protein levels are not upregulated following ER stress or vehicle treatment in several human leukemia and lymphoma derived cell lines.

NALM-6, RAJI, MV4-11 and U-937 cells were treated with TM (2.5  $\mu$ g/ml), TG (2  $\mu$ M) or BFA (2.5  $\mu$ g/ml) or their respective vehicle controls over 24 h. Cell lysates were analysed by immunoblotting using antibodies against caspase-2 (clone 10C6), cleaved (i.e. activated) caspase-3 and  $\beta$ -actin (loading control). Full-length proteins are designated (FL) with cleavage products following ER stress indicated (C).