

SUPPLEMENTAL INFORMATION

Supplemental Table 1: Primer Sequences used in RT-PCR
F, forward; R, Reverse

| Primer | Sequence |
|--------------------|---------------------------------|
| CerS1 (F) | 5'-TCTGTCTTCTATGACTGGAGGT-3' |
| CerS1 (R) | 5'-ATAGATGGAGTGGCAGTAGAAAC-3' |
| CerS2 (F) | 5'-CCTGCTCTTTCTCGTCATTTCG-3' |
| CerS2 (R) | 5'-CTTGCCGCTGGTCTGGTAG-3' |
| CerS4 (F) | 5'-CTATACAAAAATCCGAGATAGAAAC-3' |
| CerS4 (R) | 5'-AAATCTATCTCATCTCACTACCTTG-3' |
| CerS5 (F) | 5'-CTACTGGTCTCTTATGTTTTCTCAG-3' |
| CerS5 (R) | 5'-TAGGAGAAAGTGGTGAGCATA-3' |
| CerS6 (F) | 5'-TTCCTTTTACCTCTATGTATTTAGC-3' |
| CerS6 (R) | 5'-AGGATGTAATAGTAGTGAAGGTCAG-3' |
| β -actin (F) | 5'-TCCTCCCTGGAGAAGAGCTA-3' |
| β -actin (R) | 5'-CCAGACAGCACTGTGTTGGC-3' |

Supplemental Table 2: in vitro CerS activity (pmole C16-C17-ceramide/ h)
parentheses indicate predicted activities based on the sum of the individual components

| BAK KO (μ g) | WT (μ g) | 0 | 1 | 5 | 10 | 20 |
|-------------------|---------------|------------------|-----------------------------|-----------------------------|------------------------------|-----------------|
| 0 | | - | 74.9 \pm 7.7 | 360.3 \pm 21.3 | 717.6 \pm 37.2 | 1440 \pm 54.1 |
| 10 | | 83.8 \pm 7.5 | - | - | 1233.1 \pm 31.8 (801.4) | - |
| 15 | | 118.9 \pm 9.1 | - | 823.6 \pm 26.1 (479.2) | - | - |
| 19 | | 143.2 \pm 7.9 | 372.0 \pm 23.8 (218.0) | - | - | - |
| 20 | | 161.7 \pm 18.6 | - | - | - | - |

Supplemental Figure Legends:

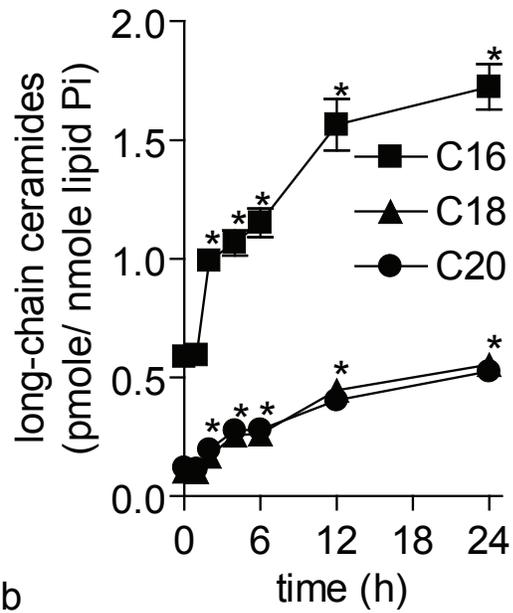
Supplemental Figure 1: Absolute values for ceramide elevations in WT BMK cells following UV-C irradiation. Wild-type (WT) BMK cells were untreated or UV-C irradiated (10 mJ/cm²) and (a) long-chain ceramides and (b) very long-chain ceramides measured and normalized to total lipid phosphate. Data are the mean of three independent experiments. Error bars, SD. For statistical analyses a two-way ANOVA was performed with a Bonferroni post-test. * indicates $p \leq 0.05$ statistically significant difference between treatment and its corresponding control.

Supplemental Figure 2: The requirement of Bax/Bak for ceramide generation was confirmed in multiple clones. WT or DKO BMK cells were UV-C irradiated (panel a; 10 mJ/cm²) or treated with cisplatin (panel b; 25µM) and long-chain ceramides measured. Data were normalized to total lipid phosphates and expressed as the percentage of the control untreated cells. Data are the average of three independent experiments. Error bars, SD. For statistical analyses a two-way ANOVA was performed with a Bonferroni post-test. * indicates p≤ 0.001 statistically significant difference between treatment and untreated control.

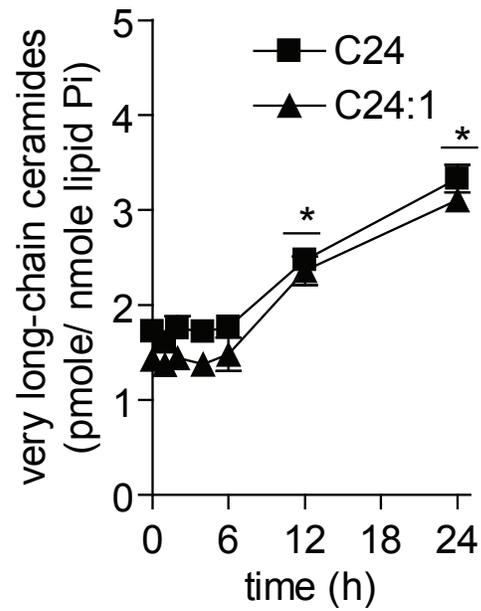
Supplemental Figure 3: Absolute values of ceramide elevations in WT hematopoietic cells treated with apoptotic stimuli. Long-chain ceramides were measured and normalized to total lipid phosphate in WT hematopoietic cells 8h (a) following removal of IL-3 (GFW, growth factor withdrawal) or (b) treatment with cisplatin (cis; 10µM). Data are the average of three independent experiments. Error bars, SD. For statistical analyses a two-way ANOVA was performed with a Bonferroni post-test. * indicates p≤ 0.05 statistically significant difference between treatment and untreated control.

supplemental figure 1

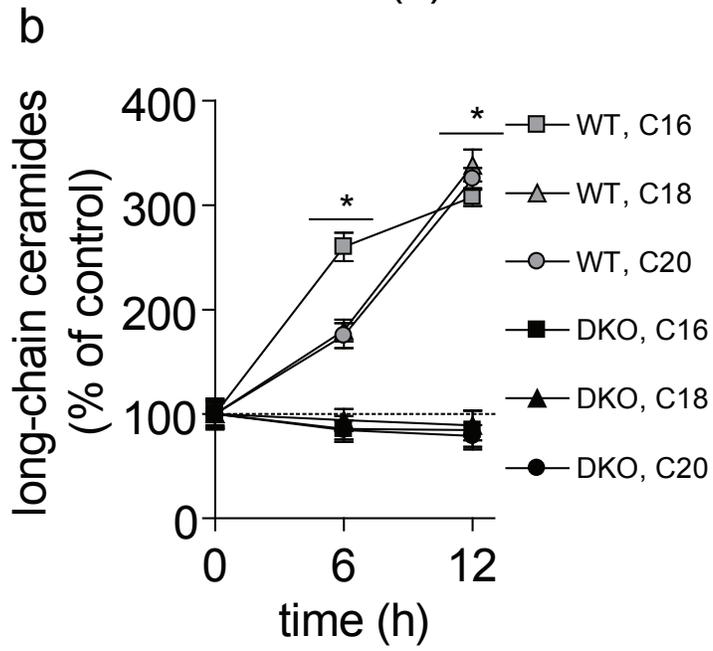
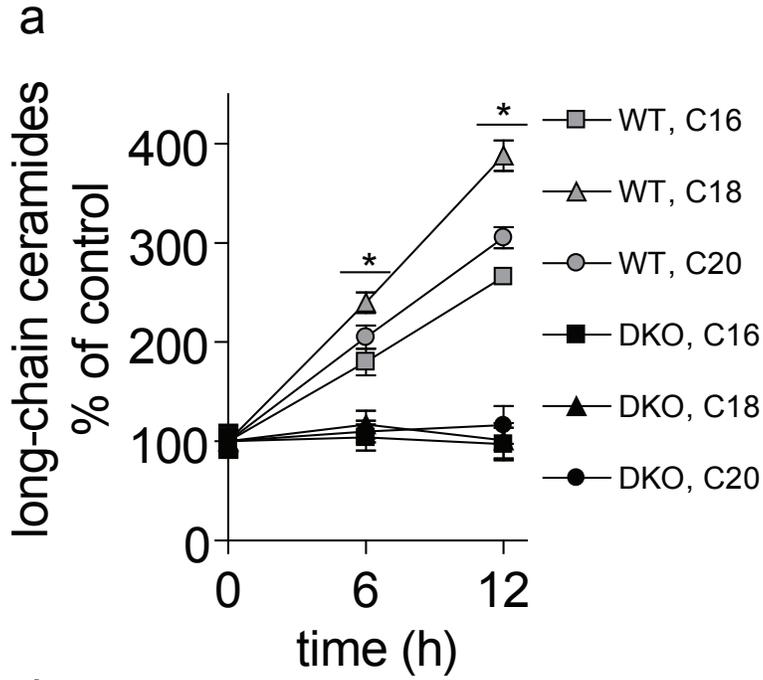
a



b

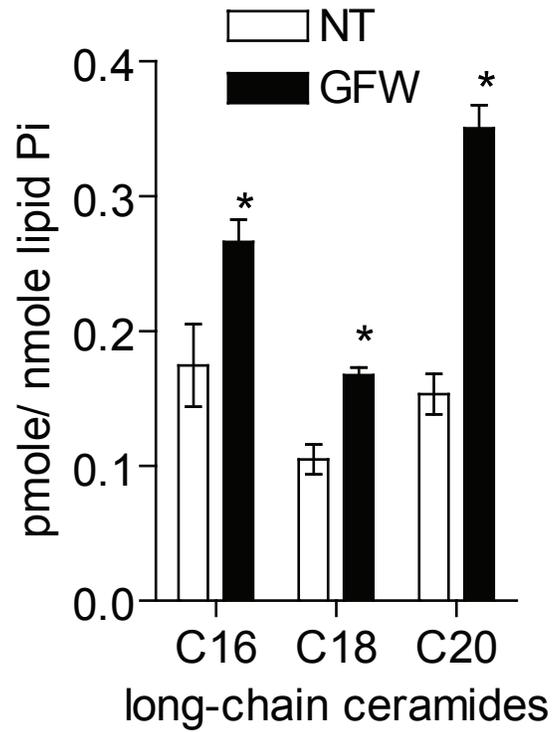


Supplemental figure 2



Supplemental figure 3

a



b

