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online data

## SUPPLEMENTARY ONLINE DATA

# Neutral sphingomyelinase 2 (nSMase2) is the primary neutral sphingomyelinase isoform activated by tumour necrosis factor-$\alpha$ in MCF-7 cells

Christopher J. CLARKE, Emily A. CLOESSNER, Patrick L. RODDY and Yusuf A. HANNUN[1]

Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425, U.S.A.

## MATERIALS AND METHODS

### N-SMase assay buffer compositions

The composition of the buffer in the standard assay [1] is 200 mM Tris/HCl (pH 7.4), 5 mM $MgCl_2$, 2.5 mM dithiothreitol, 2 mM EDTA, 0.2 % Triton X-100, 1 mM PMSF, 50 $\mu$M phosphatidylserine, 100 $\mu$M SM and $1 \times 10^5$ c.p.m. [$^{14}$C]SM.

The composition of the buffer in the alternative assay [2] is 20 mM Hepes (pH 7.4), 10 mM $MgCl_2$, 2 mM EDTA, 0.1 mM sodium orthovanadate, 0.1 mM molybdic acid (sodium salt), 30 mM *p*-nitrophenylate, 10 mM 2-glycerophosphate, 750 $\mu$M ATP, 1 mM PMSF, 10 $\mu$M leupeptin, 10 $\mu$M pepstatin, 0.5 % CHAPS and $1 \times 10^6$ c.p.m. [$^{14}$C]SM.

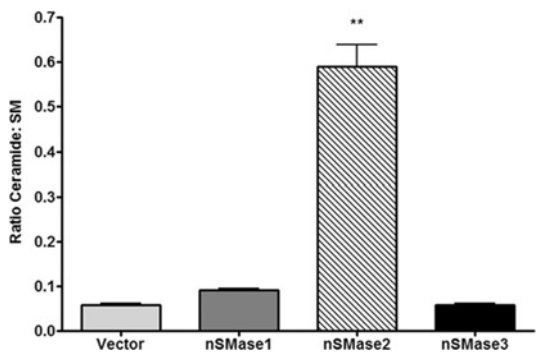


**Figure S1 Transient overexpression of nSMase2, but not nSMase1 or nSMase3, in HEK-293 cells alters the ceramide/SM ratio**

HEK-293 cells were transiently transfected with nSMase1, nSMase2 or nSMase3 for 48 h. Cellular ceramide levels were analysed by LC-MS/MS as described in the Materials and methods section of the main text. Results, expressed as the ceramide/SM ratio, are means $\pm$ S.E.M. (**$P < 0.01$, one-way ANOVA with Dunnet's post-hoc test, $n = 4$).

## REFERENCES

1 Marchesini, N., Luberto, C. and Hannun, Y. A. (2003) Biochemical properties of mammalian neutral sphingomyelinase 2 and its role in sphingolipid metabolism. J. Biol. Chem. **278**, 13775–13783

2 Krut, O., Wiegmann, K., Kashkar, H., Yazdanpanah, B. and Kronke, M. (2006) Novel TNF-responsive mammalian neutral sphingomyelinase-3 is a C-tail-anchored protein. J. Biol. Chem. **281**, 13784–13793

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[1] To whom correspondence should be addressed (email hannun@musc.edu).