


Searching for the Sphinx of the Lipid (Sphingolipid)

Nagoya University Graduate School
of Health Sciences

Takashi Murate M.D. Ph. D.

本日の講演内容

1. 名古屋大学医学部第一内科時代
1978～1998
2. 同 保健学科時代
(Searching for the sphinx of the lipid)
1998～2015



**第一内科第2研究室時代
(SKCC留学を含めて)
(1978. 4~1998. 3)**

2研の先輩



2001/7/11~12



Memorial Sloan Kettering Cancer Center

(1982-1985)

<http://www.mskcc.org/research/lab/paul-marks>



Dr. Paul A. Marks



<https://www.google.co.jp/maps/@40.764935,-73.958093,3a,75y,108.39h,84.73t/data=!3m4!1e1!3m2!1sb3Cgp3VF8qkveFHPafsewQ!2e0>



The York Ave side

https://www.google.co.jp/maps/@40.764141,-73.955733,3a,79.6y,223.32h,105.54t/data=!3m4!1e1!3m2!1s_9es4_Zm_rGv4pvrn1lclg!2e0

Memorial Hospital



<http://giving.mskcc.org/story/board>

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3394–3398, June 1984
Cell Biology

Inducer-mediated commitment of murine erythroleukemia cells to terminal cell division: The expression of commitment

(Friend virus/dexamethasone/hexamethylenebisacetamide/terminal cell division)

TAKASHI MURATE, TSUGUHIRO KANEDA, RICHARD A. RIFKIND, AND PAUL A. MARKS

DeWitt Wallace Research Laboratory and Sloan-Kettering Division Graduate School of Medical Sciences, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 5020–5024, August 1985
Cell Biology

Gene expression during terminal differentiation: Dexamethasone suppression of inducer-mediated α_1 - and β^{maj} -globin gene expression

(cell division/chromatin/hexamethylenebisacetamide/gene transcription/erythroleukemia)

TSUGUHIRO KANEDA, TAKASHI MURATE, MICHAEL SHEFFERY, KAREN BROWN, RICHARD A. RIFKIND, AND PAUL A. MARKS

DeWitt Wallace Research Laboratory of the Sloan-Kettering Institute, Sloan-Kettering Division of the Cornell University Graduate School of Medical Sciences, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

医学部保健学科時代 (1998. 4 ~ 2015. 3)



NAGOYA UNIVERSITY

保健学科での自分の研究路線

1. 大学院が出来て学生が入ってくれるまでは自分で研究を遂行し、筆頭者として論文を作成する。
2. 血液内科の研究テーマと競合しないように、独自の路線を設定し徐々に造血障害の病態解析から変更する。→ [Searching for the Sphinx of the Lipid](#)
3. しっかりと実験で裏づけの取れた解析を行う。

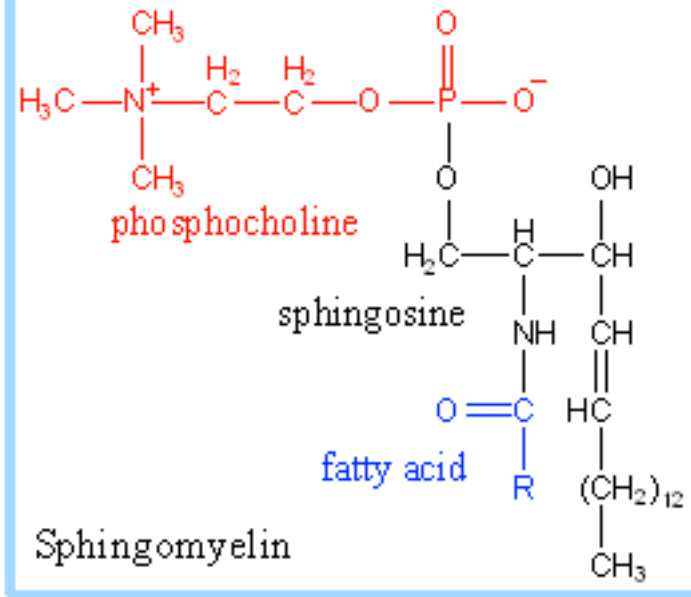
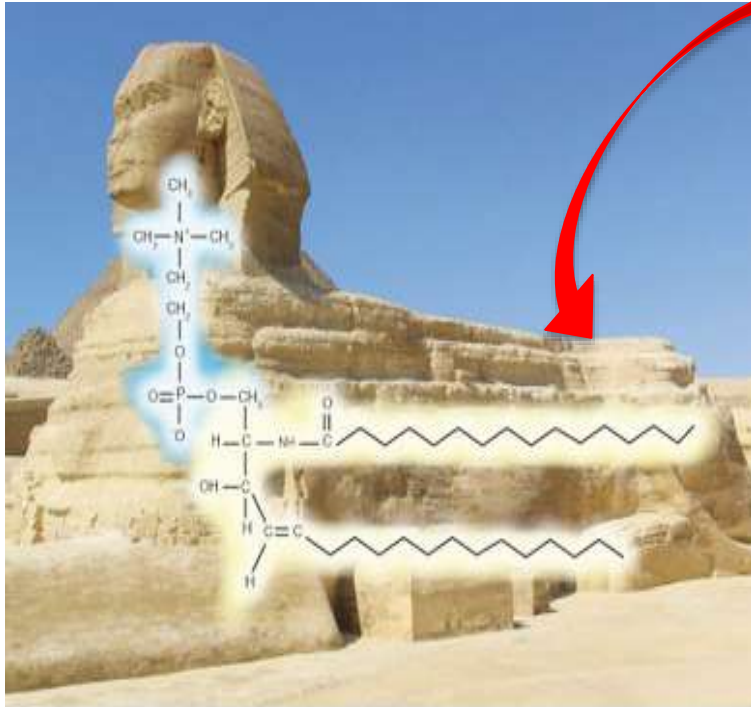
野澤先生

坂野先生

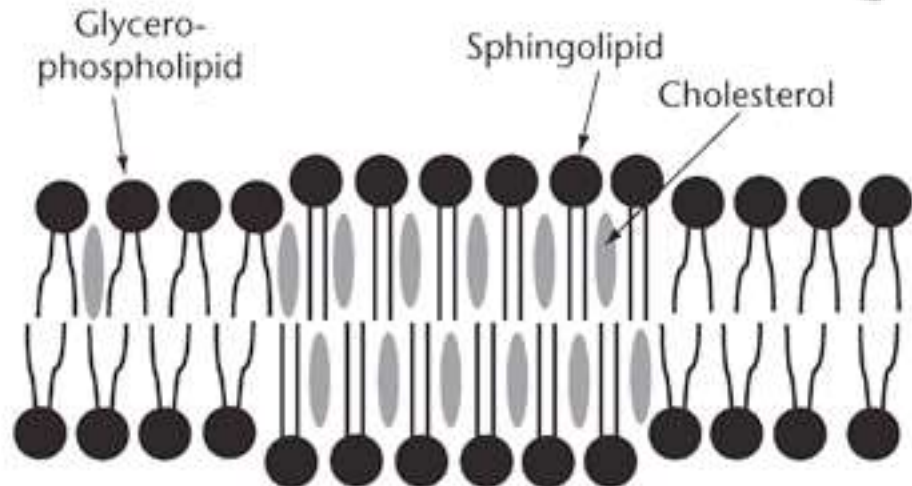
小泉先生



The Sphinx of the lipid (Sphingolipid)

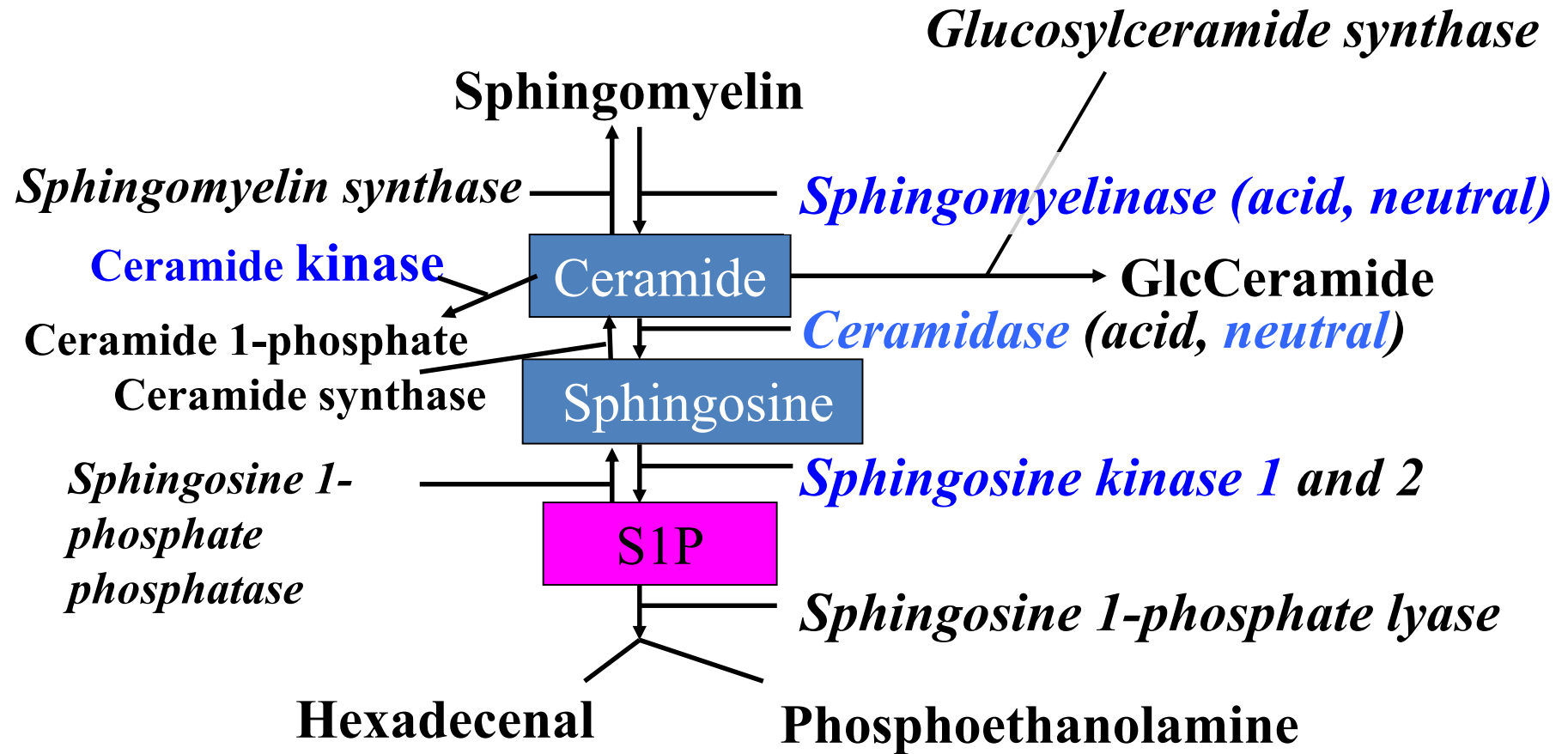


Lipid bilayer



<https://www.caymanchem.com/app/template/Article.vm/article/2142/figure/1;jsessionid=E9AC398983C8257FBEB62299147D008>

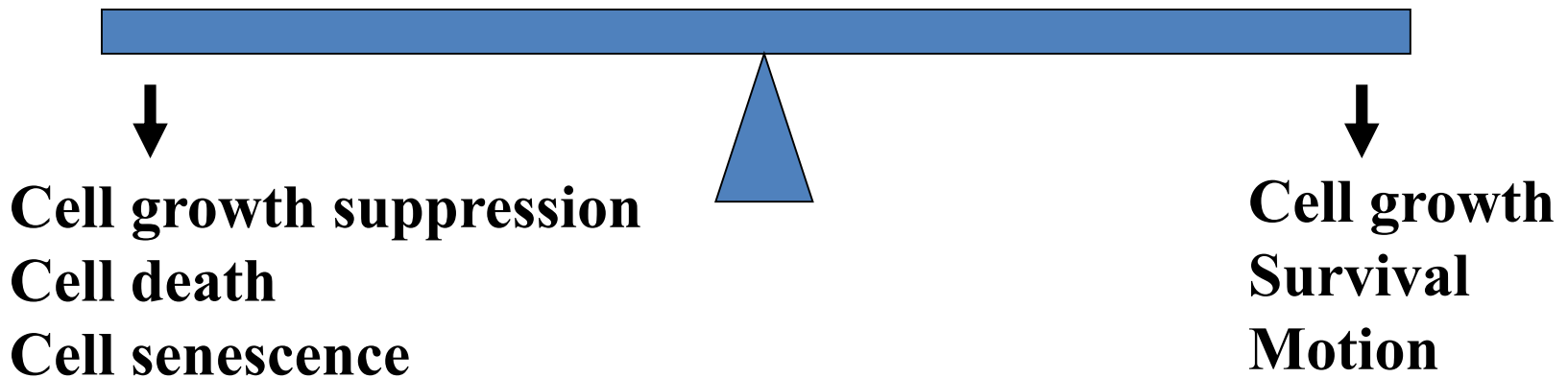
Sphingolipid metabolic pathway (mainly degradation)



Sphingolipid biostat (rheostat) model

Ceramide, Sphingosine

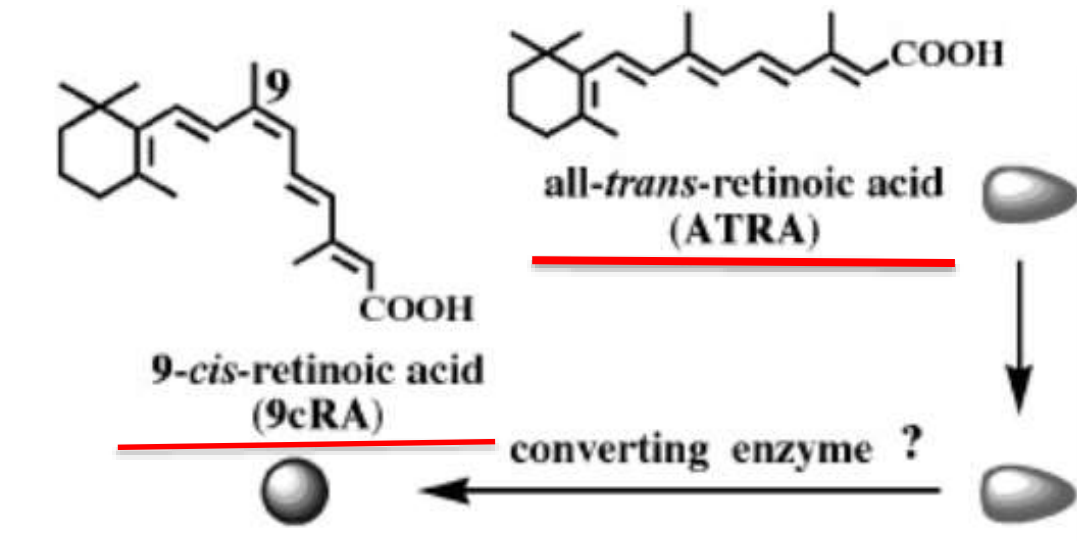
Sphingosine 1-phosphate



S1Pには細胞膜に特異的な
受容体が存在する。

(I) ATRA story

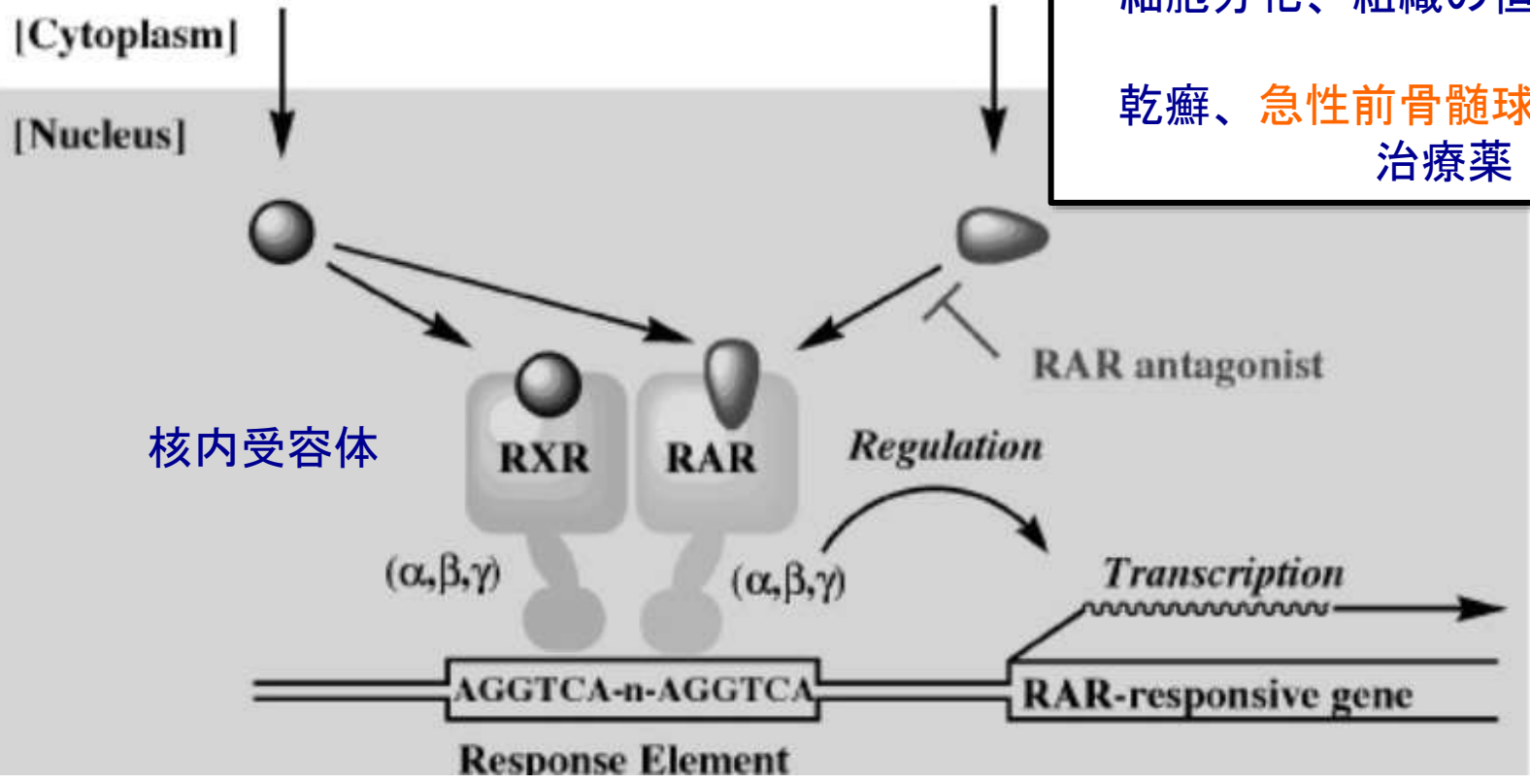
**All trans retinoic acid and sphingolipid
Metabolic pathways**



**RAR, RXRアゴニストの機能と
 生体への影響**

脊椎動物の視覚、形態形成、発生、
 細胞分化、組織の恒常性の維持

乾癬、急性前骨髄球性白血病の
 治療薬



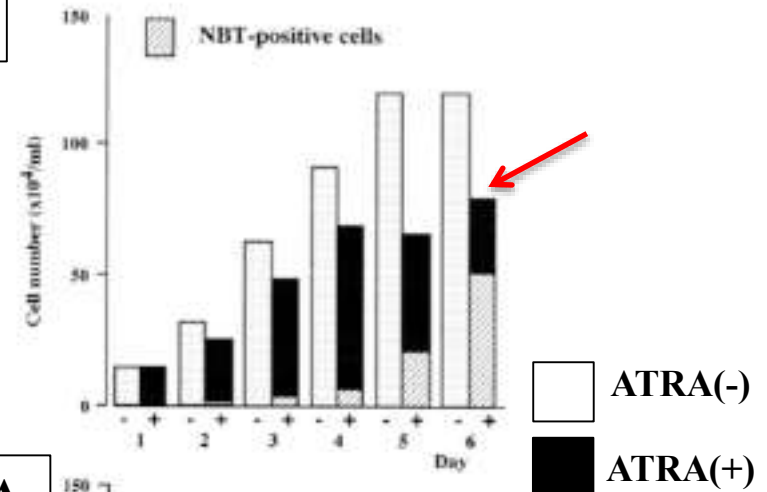
**(1) Up-regulation of acid
sphingomyelinase during retinoic acid-
induced myeloid differentiation of NB4, a
human acute promyelocytic leukemia cell
line**

Murate T. *et al.*

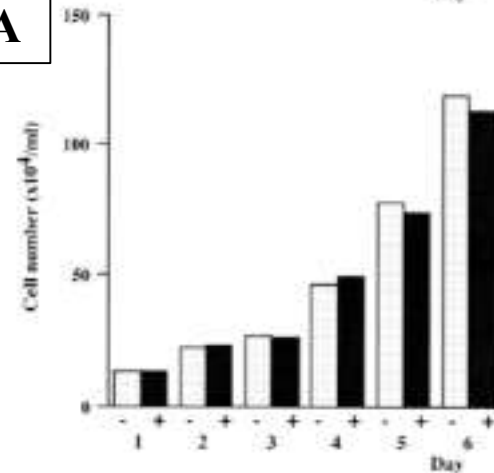
J Biol Chem 277:9936-9943, 2002 [1]

ATRA はNB4 but not NB4/RA cellsの分化を誘導する

NB4



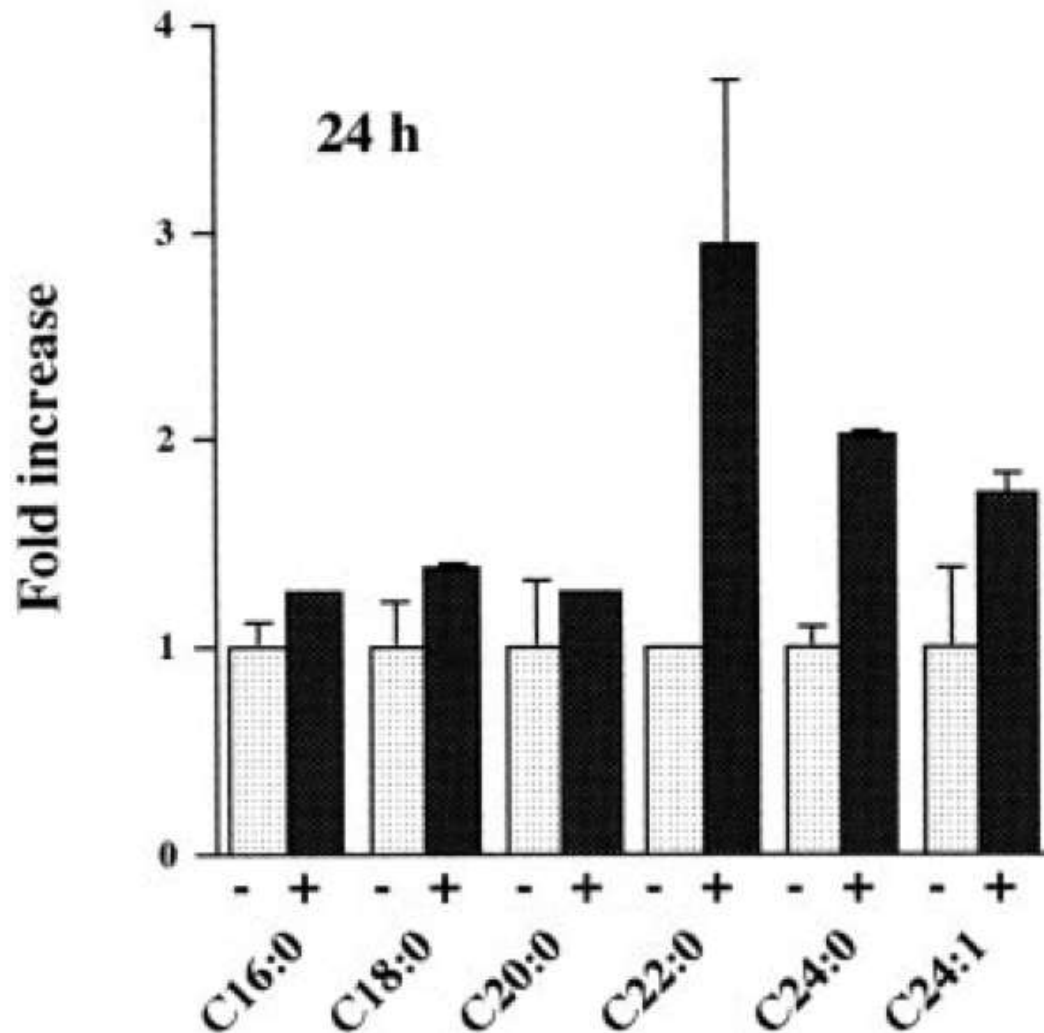
NB4/RA



出典 [1]

- (1) APL cell possesses chromosome abnormality t(15:17), producing the fusion protein of PML/RAR α
- (2) It can be differentiated by ATRA, a vitamin A derivative.
- (3) NB4 and NB4/RA are APL cell lines with or without ATRA sensitivity. NB4/RA cells have a mutation in its DNA binding domain of RAR.

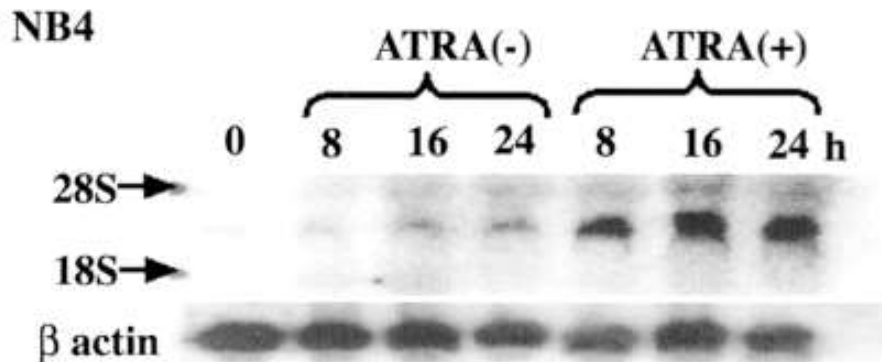
ATRAは細胞内セラミドを増加させる



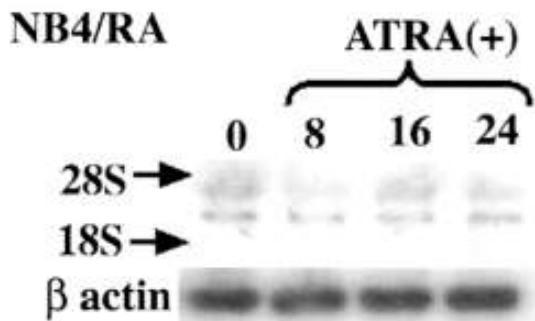
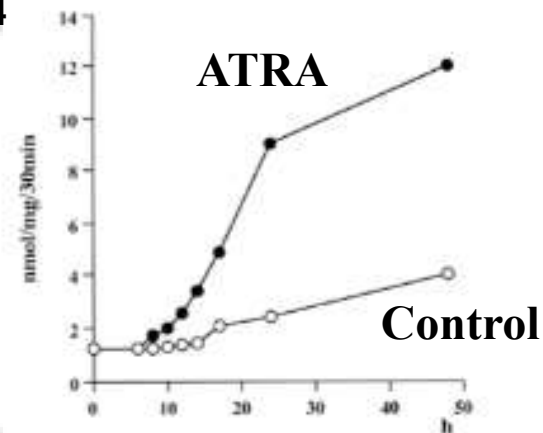
出典 [1]

ESI-MS/MSでの解析

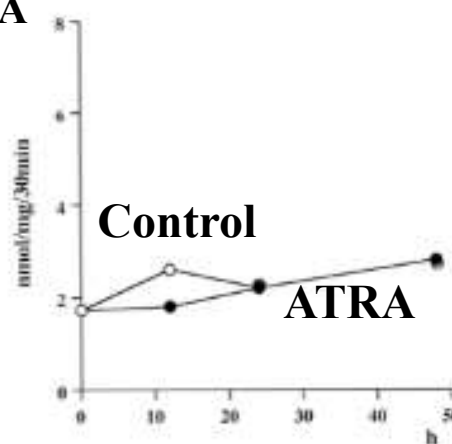
ATRA はASMase mRNAと 酵素活性を誘導する



NB4

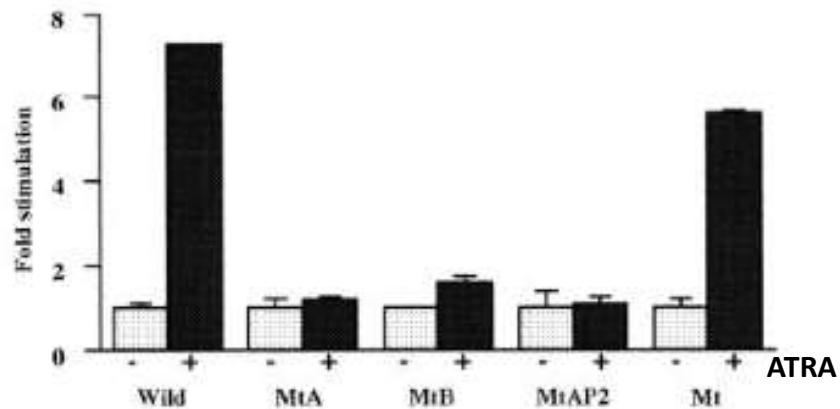
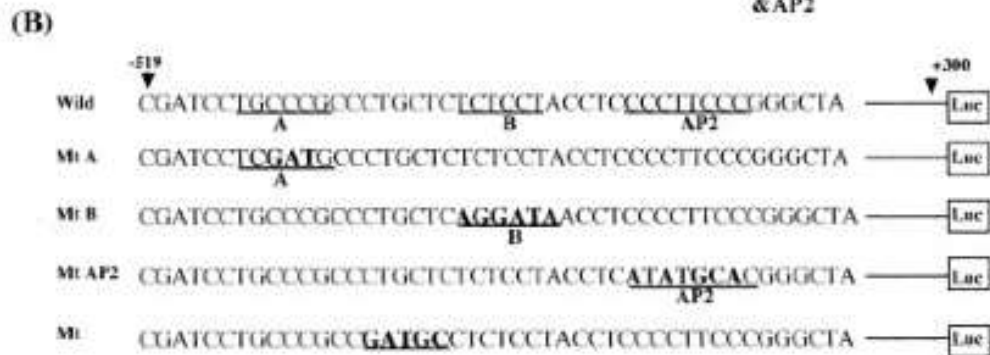
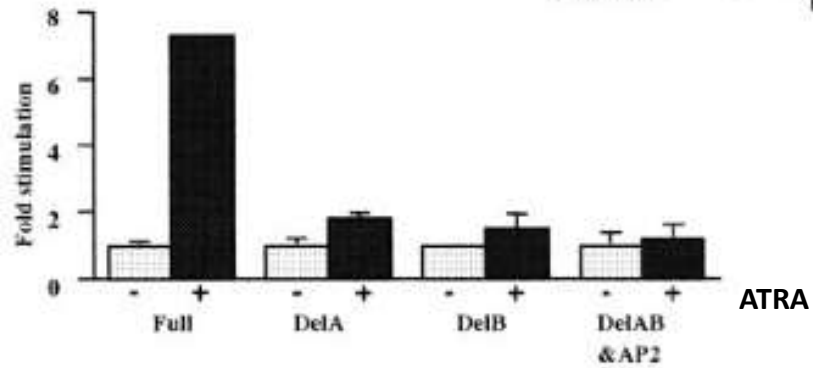
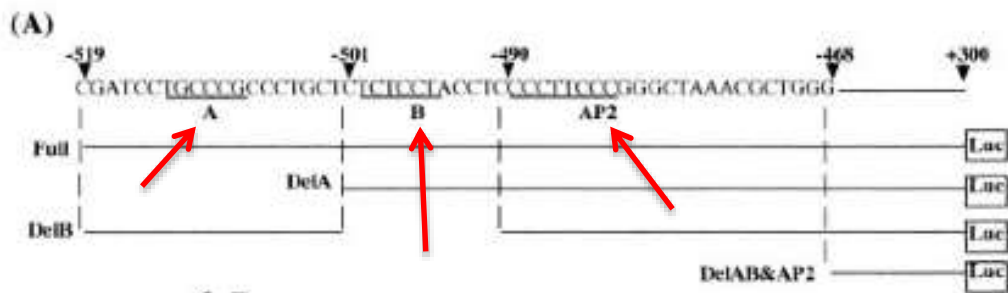


NB4/RA



出典 [1]

縦軸はASMase酵素活性



Intelligentes
 in Tradition. Mit besten Anleihen.
 gegen negative Rendite mit
 Aktien, Anleihen & Zinsen.
 Gegenwärtig 100% - 120%

Summer Urlaub
 ...

Achten Sie in dieser Ausgabe auf
 unsere Beiträge **Müller**

primaSonntag
 4. Ausgabe
 409.000
 WERBEBREITE WOCHEZEITUNG

dipeo
 Die besten Ideen
 gibt es im Sommer

**Traditionelles
 Stauertisch**

**Wetter am
 Wochenende**

**Saujagt beim großen
 PfälzerWettbewerb**

Wirtschaftskennzeichen
**Positiver
 Trend**

**Die Frankfurter Börse ist ein lebendiges Beispiel
 Die Welt zu Gast in Nürnberg**

Die Frankfurter Börse ist ein lebendiges Beispiel für die internationale Vernetzung der Finanzmärkte. In Nürnberg wird die Welt zu Gast sein.



Schorf
 ...

**Heizt mit
 den besten
 Preis!**

**ZAHNÄRZTLICHE
 TAGESKLINIK**
 Dr. J. Zschornig & Kollegen

Alle Kassen!

365 Tage für Sie da!

- Preiswerter Zahnersatz
- Kunststofffüllungen ohne Zuzahlung
- Günstige und moderne Zahnimplantate
- ... und vieles mehr

Schorf



The 50th ICBL at Regensburg (September, 2009)

Dr. Schmitz: 2000年にASMase プロモーター
ベクターを受領

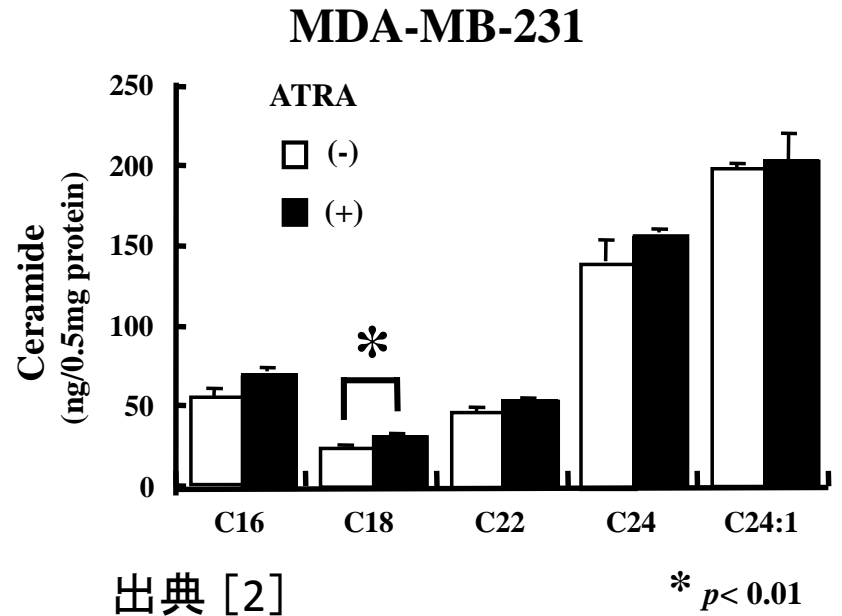
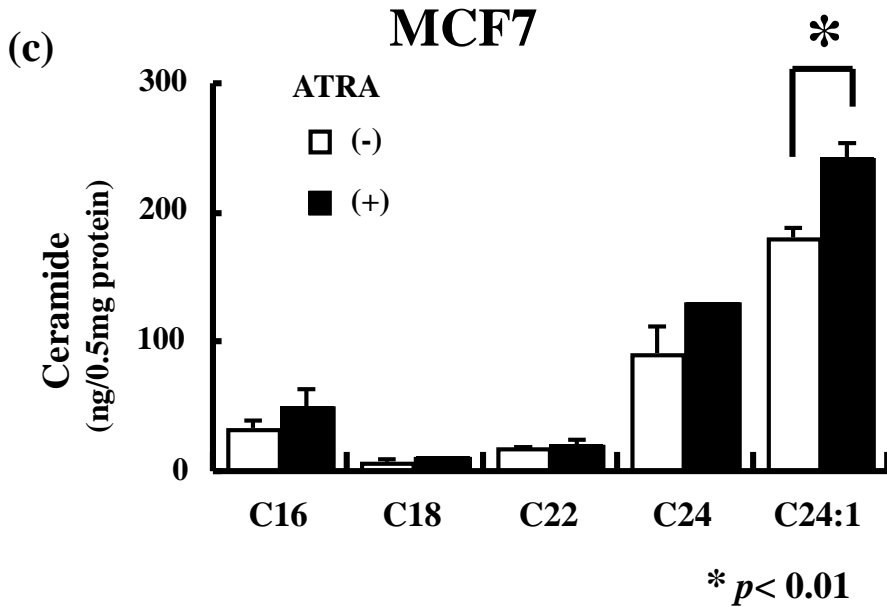
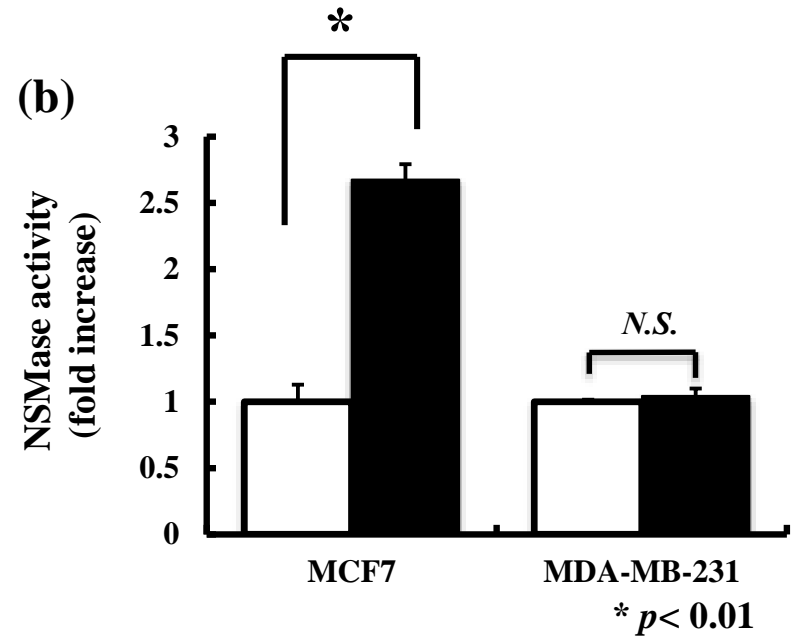
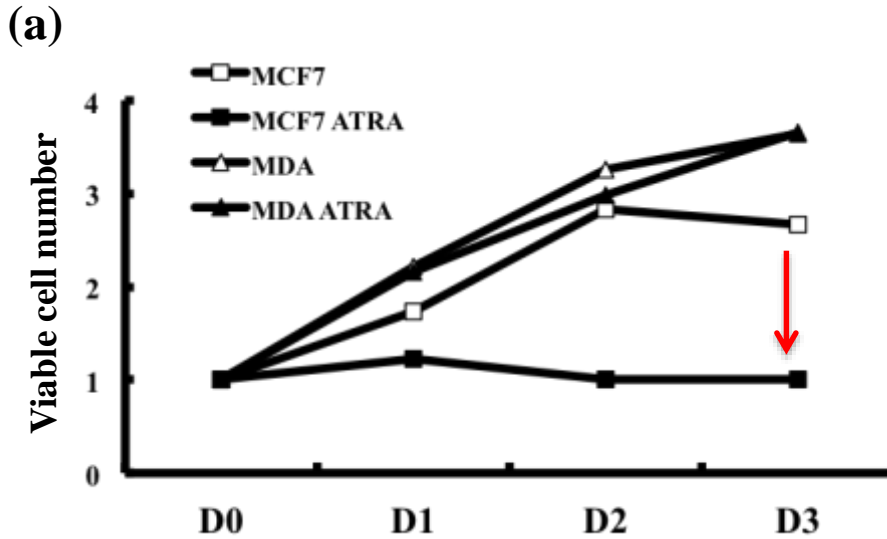
ニュルンベルグの日刊紙に紹介された

(2) Transcriptional regulation of neutral sphingoimyelinase 2 in all-trans retinoic acid-treated human breast cancer cell line, MCF-7

Ito H. *et al.*

J Biochem 151:599-610, 2012 [2]

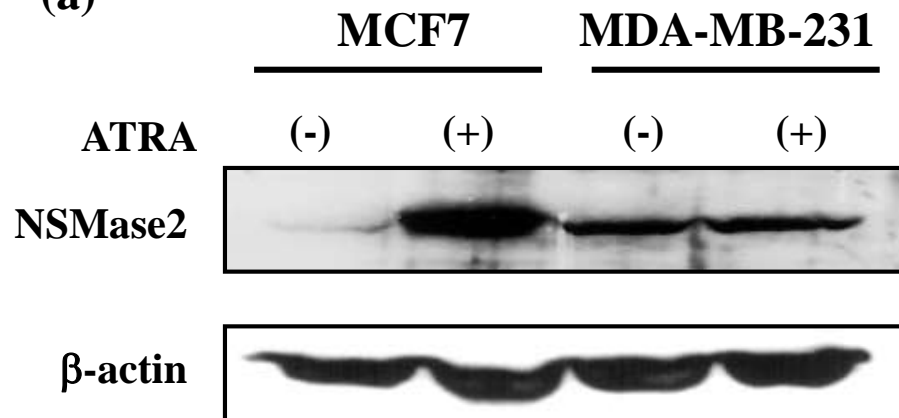
ATRA はMCF-7 but not MDA-MB-231の増殖を抑制する



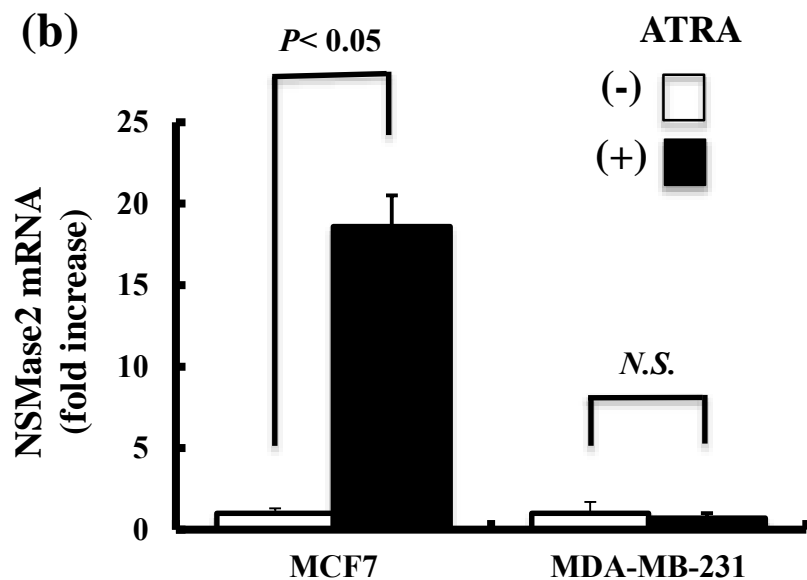
出典 [2]

ATRAはMCF-7細胞のNSMase2発現を増強する

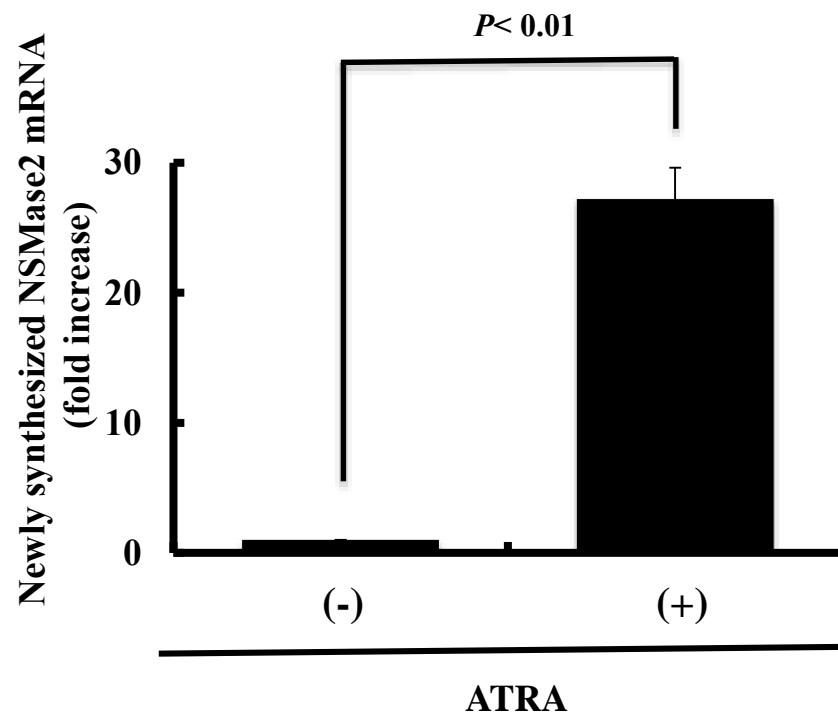
(a)



(b)

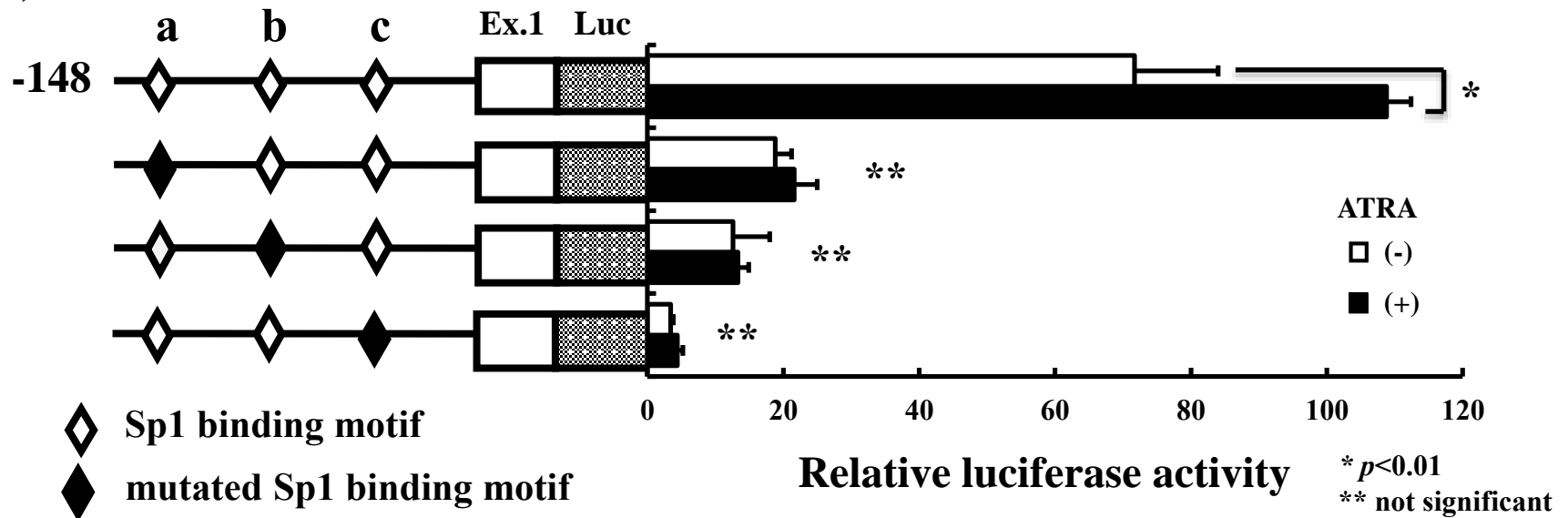


(c)

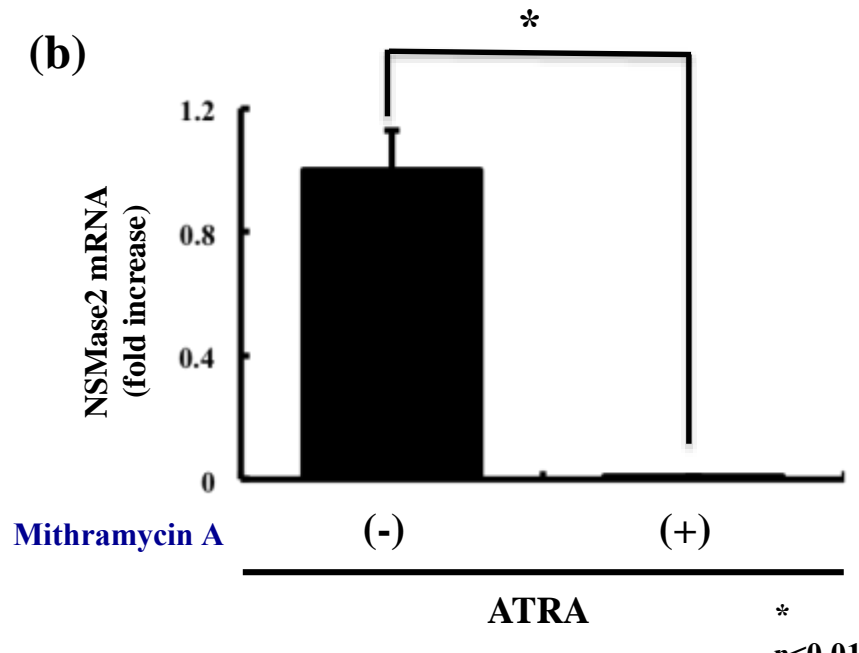


ATRAによるNSMase2 プロモーター活性の増強

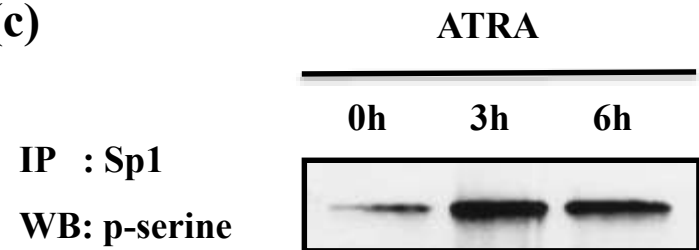
(a)



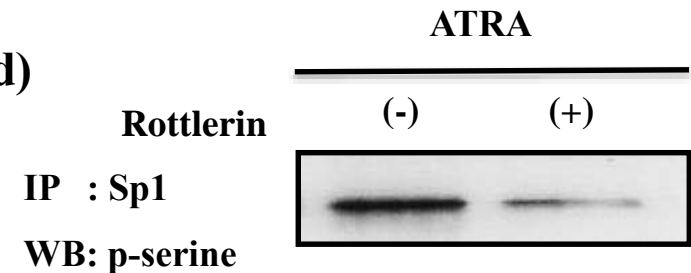
(b)



(c)



(d)



出典 [2]

In MCF-7 breast cancer cells

ATRA



PKC delta activation (RAR/RXR dependent)



Sp1 activation



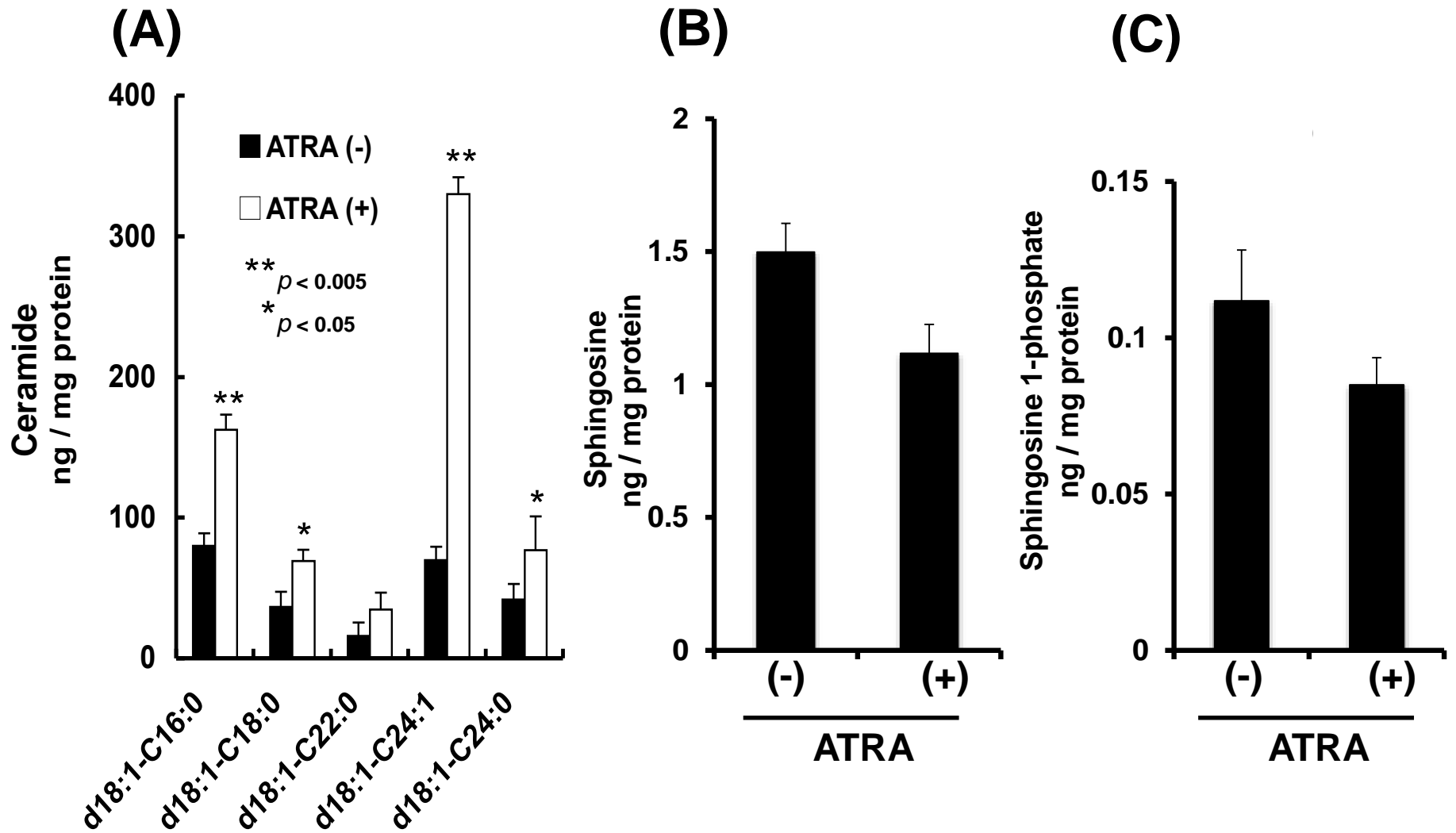
Increased NSMase2 expression

**(3) ATRA inhibits ceramide kinase
transcription in a human
neuroblastoma cell line, SH-SY5Y
cells: the role of COUP-TFI**

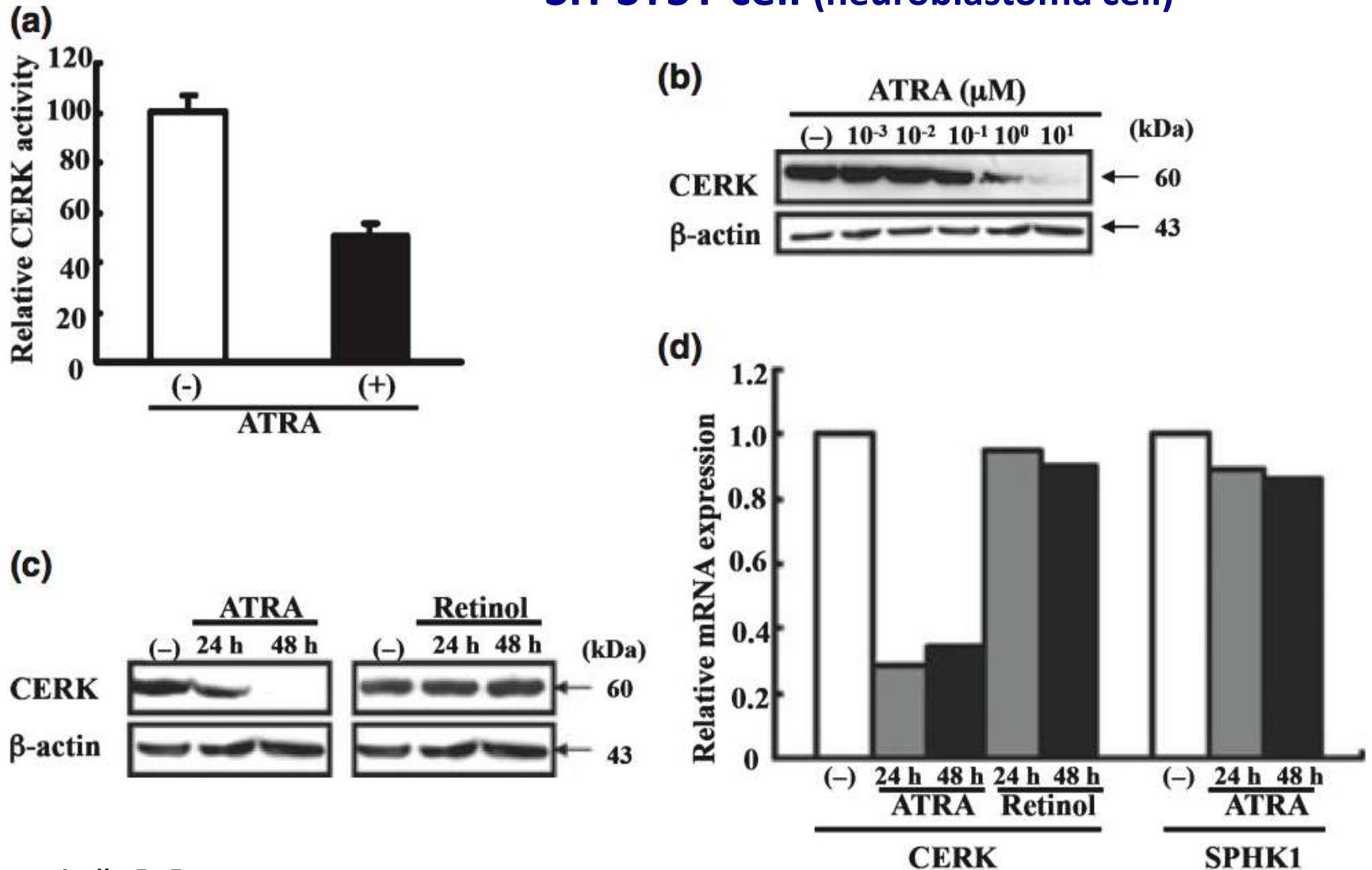
Murakami M. *et al.*

J Neurochem. 2010 Jan;112(2):511-520. [3]

ATRAによる細胞内セラミド、スフィンゴシン、S1Pの変化

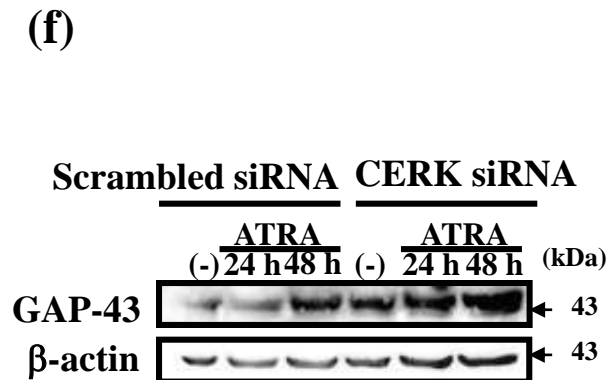
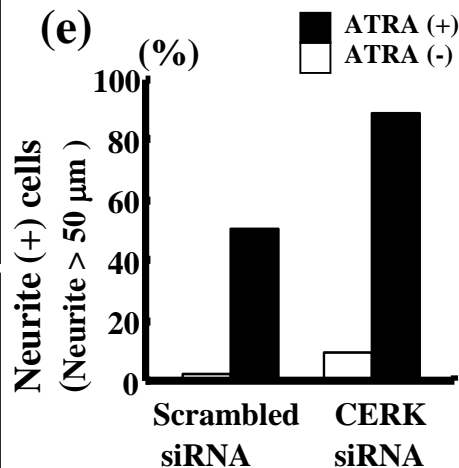
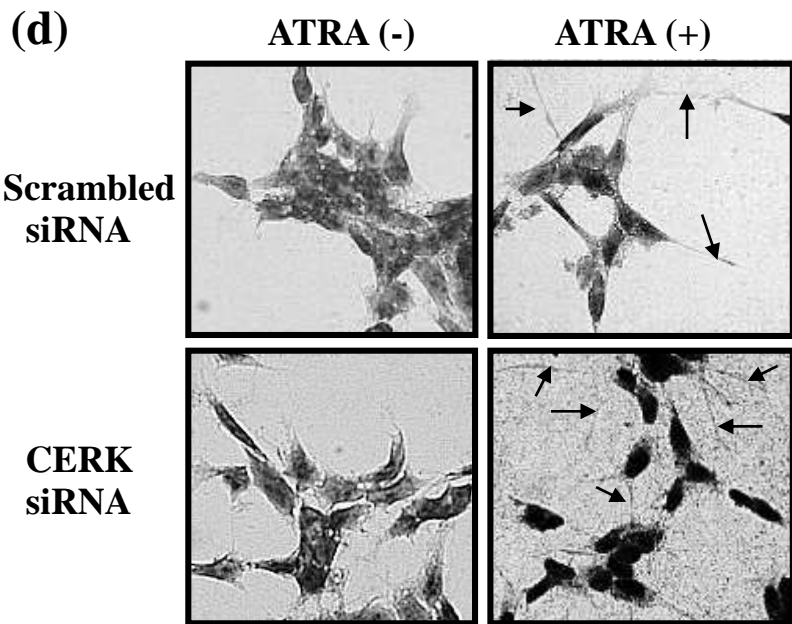
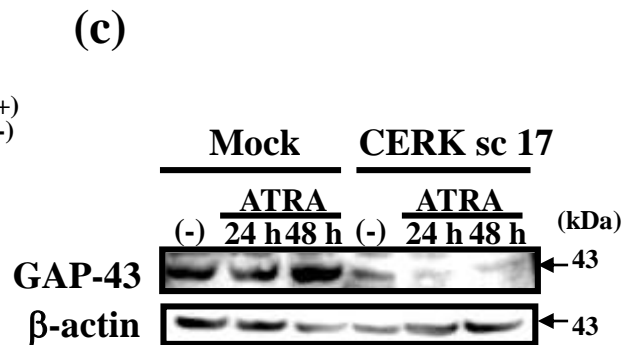
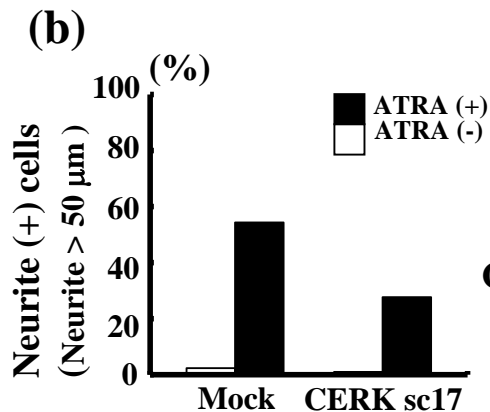
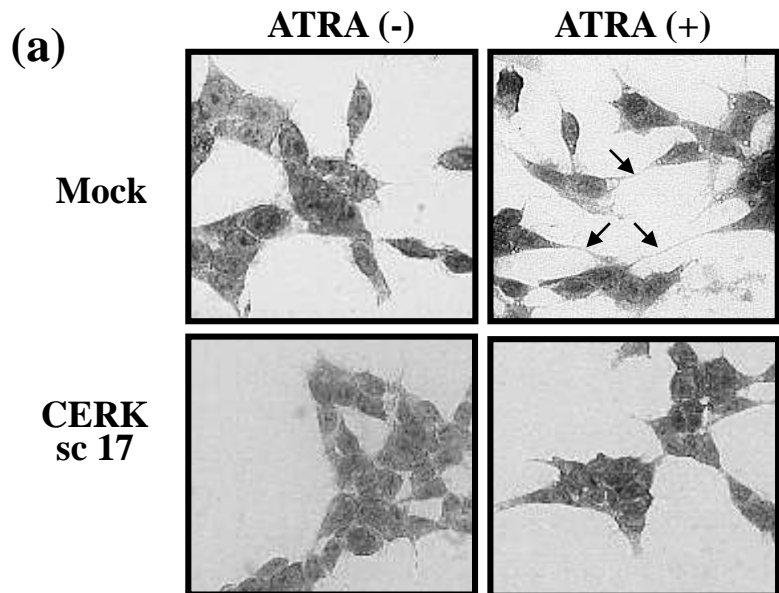


ATRA はセラミド キナーゼ(CERK) 発現を抑制する SH-SY5Y cell (neuroblastoma cell)

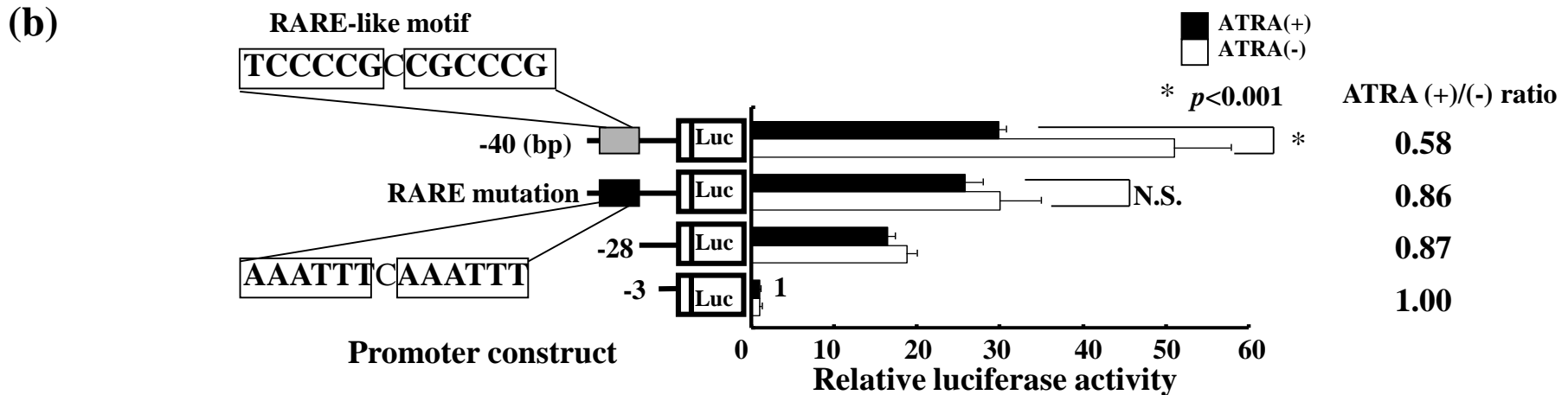
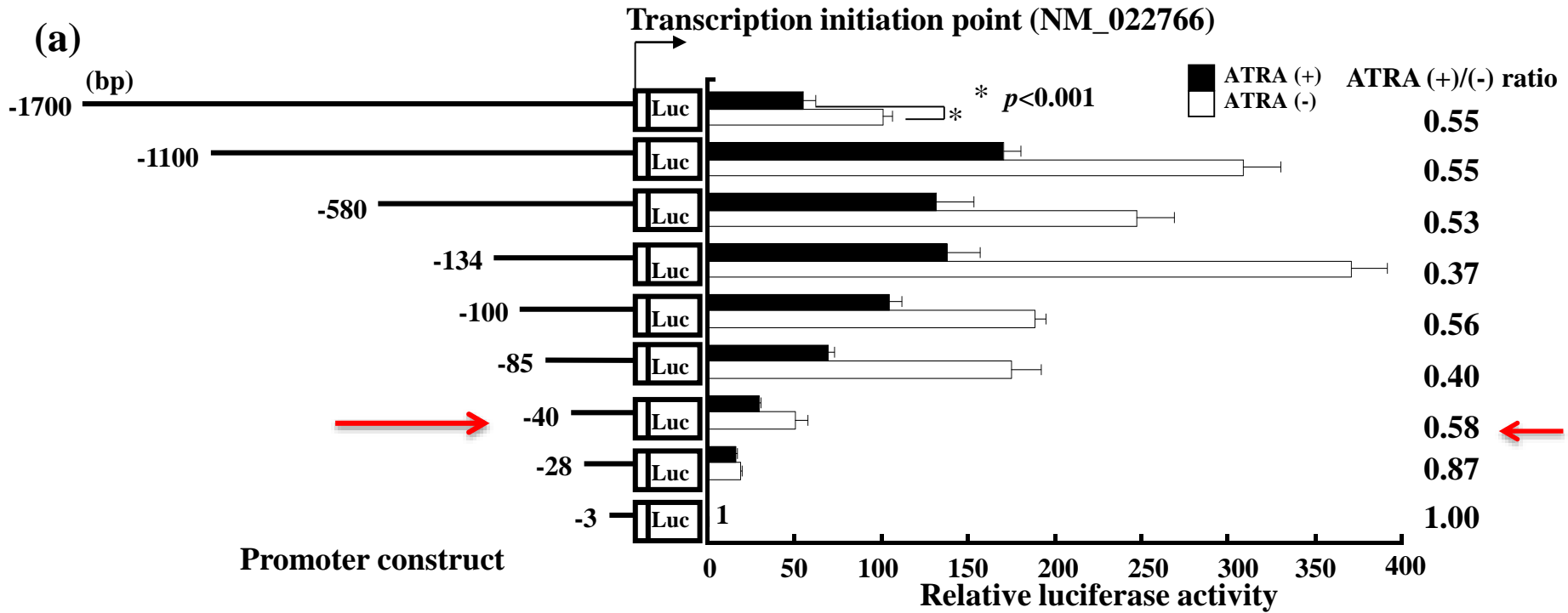


出典 [3]

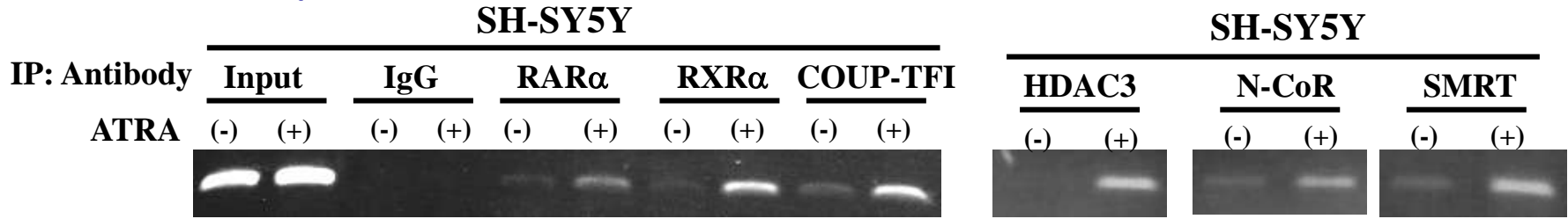
ATRA はSH-SY5Y cellの神経系分化を誘導する



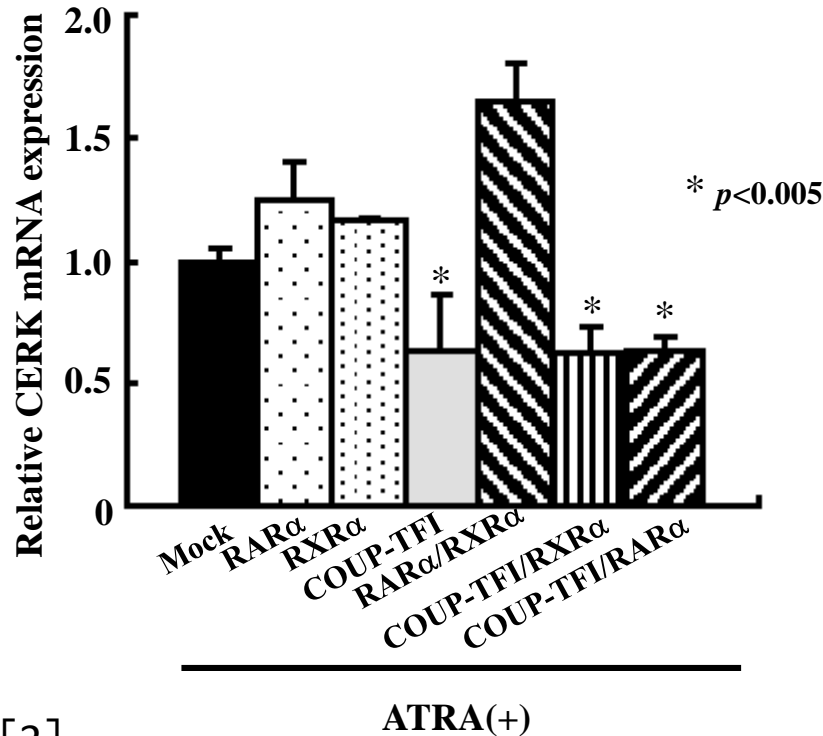
プロモーター解析



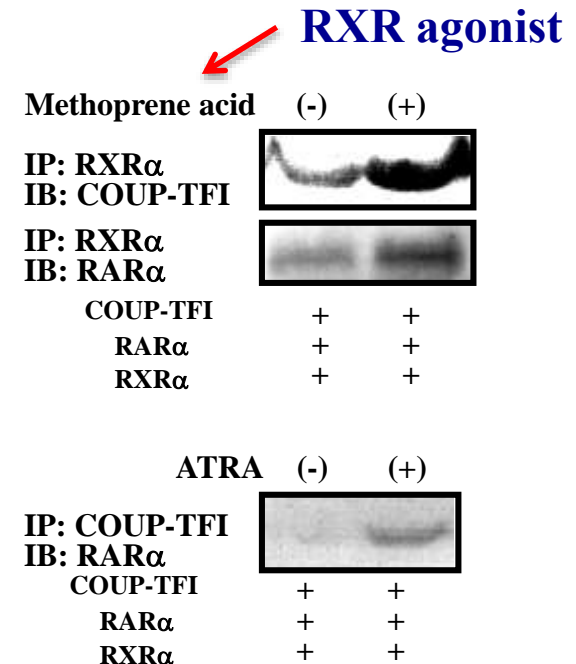
ChIP assay



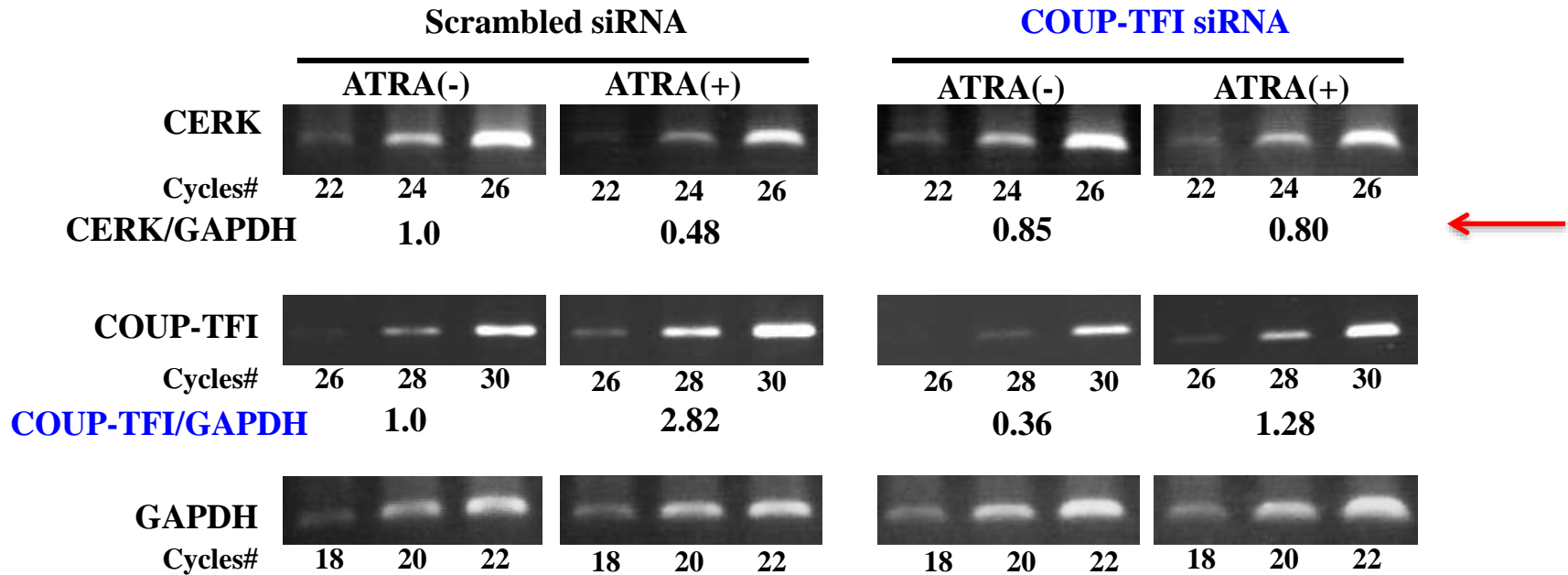
cDNA overexpression



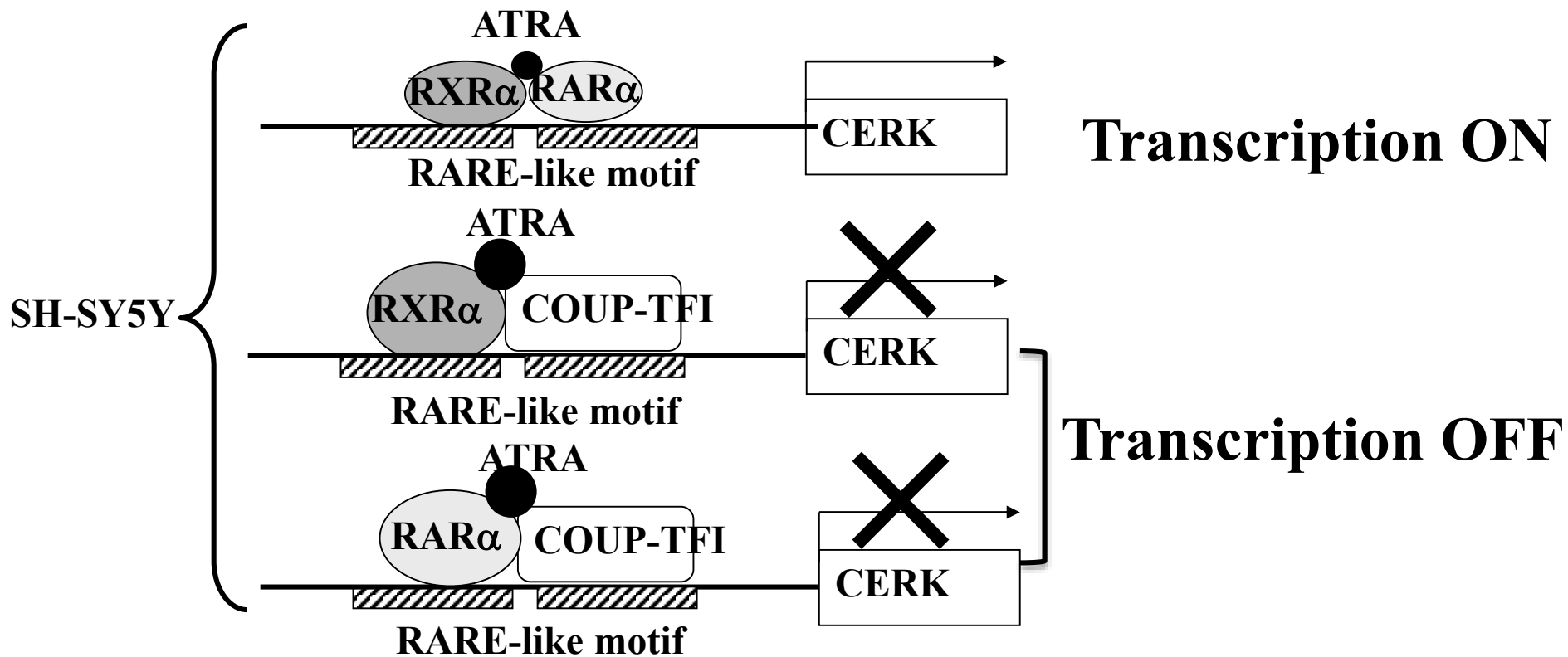
Immunoprecipitation and WB



COUP-TFI siRNAの効果



我々の解析の結果から



In case of ATRA (-), further analysis is needed

Co-repressor and co-activator omitted

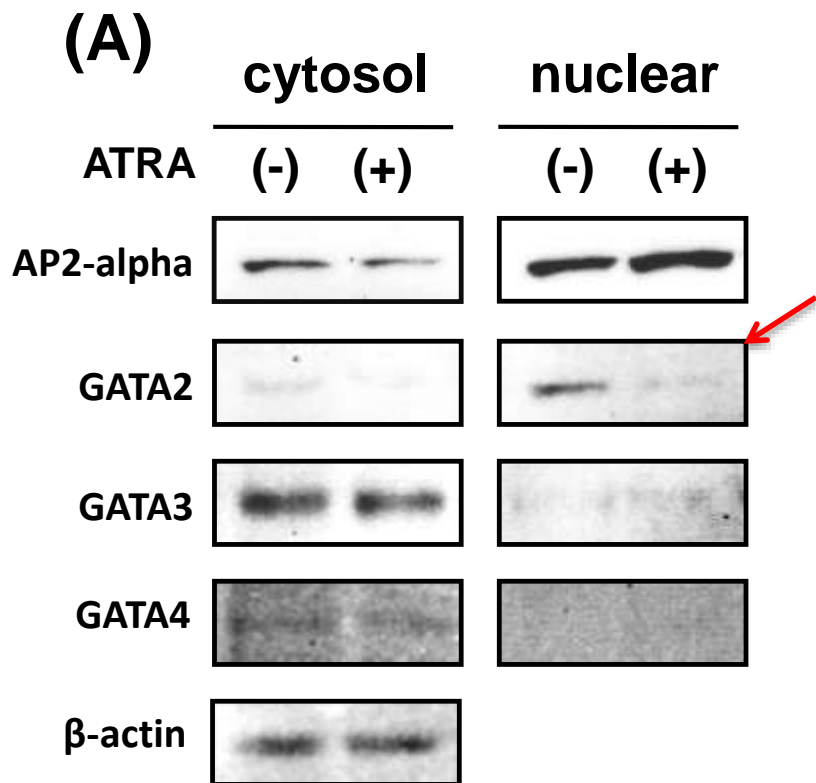
(4) Role of down-regulated neutral ceramidase during all-trans retinoic acid-induced neuronal differentiation in SH-SY5Y neuroblastoma cells

Tanaka K. et al.

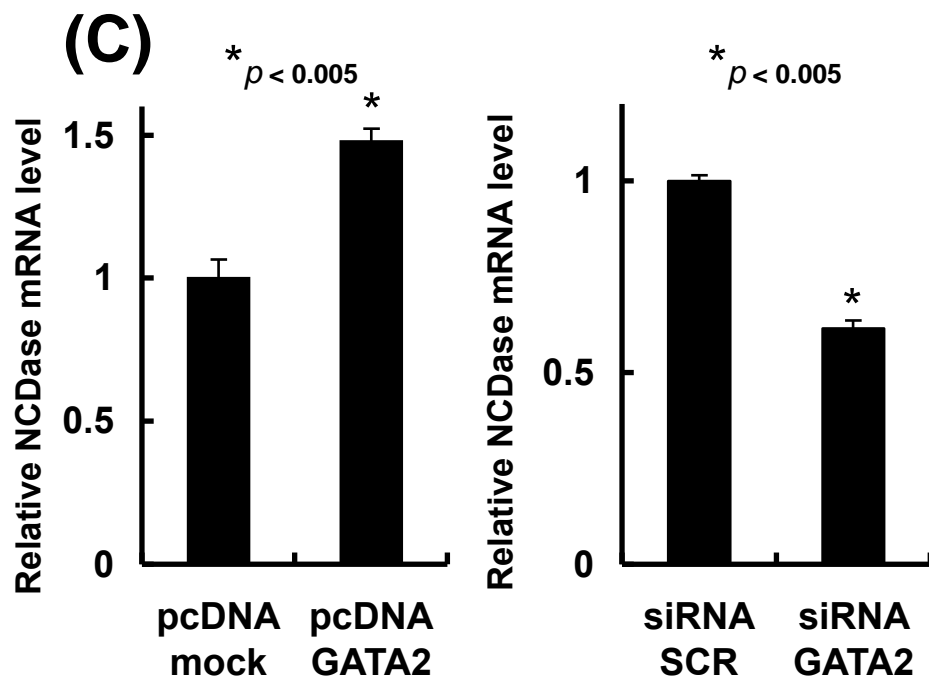
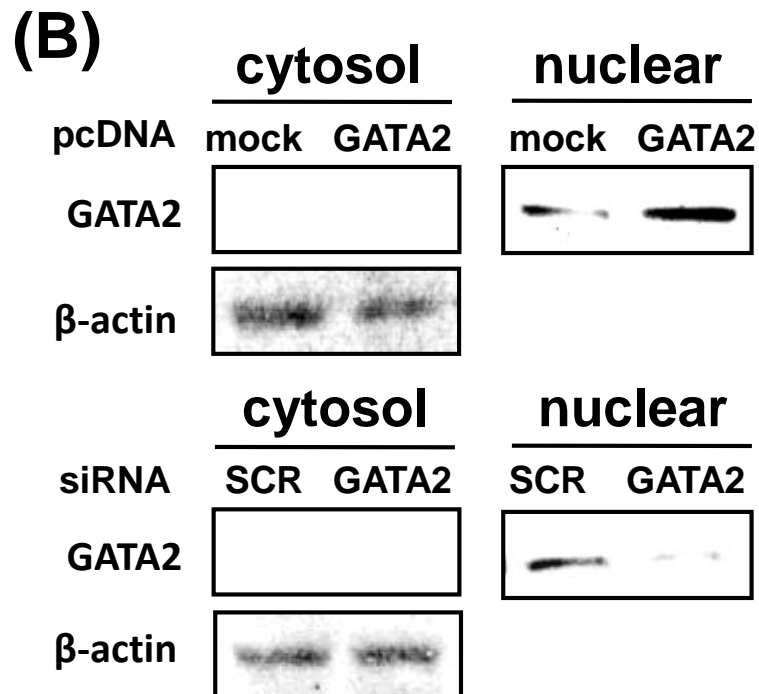
J. Biochem. 2012;151(6):611-620 [4]

Decreased NCDase transcription by ATRA-induced GATA2 suppression

関連する転写因子の検索

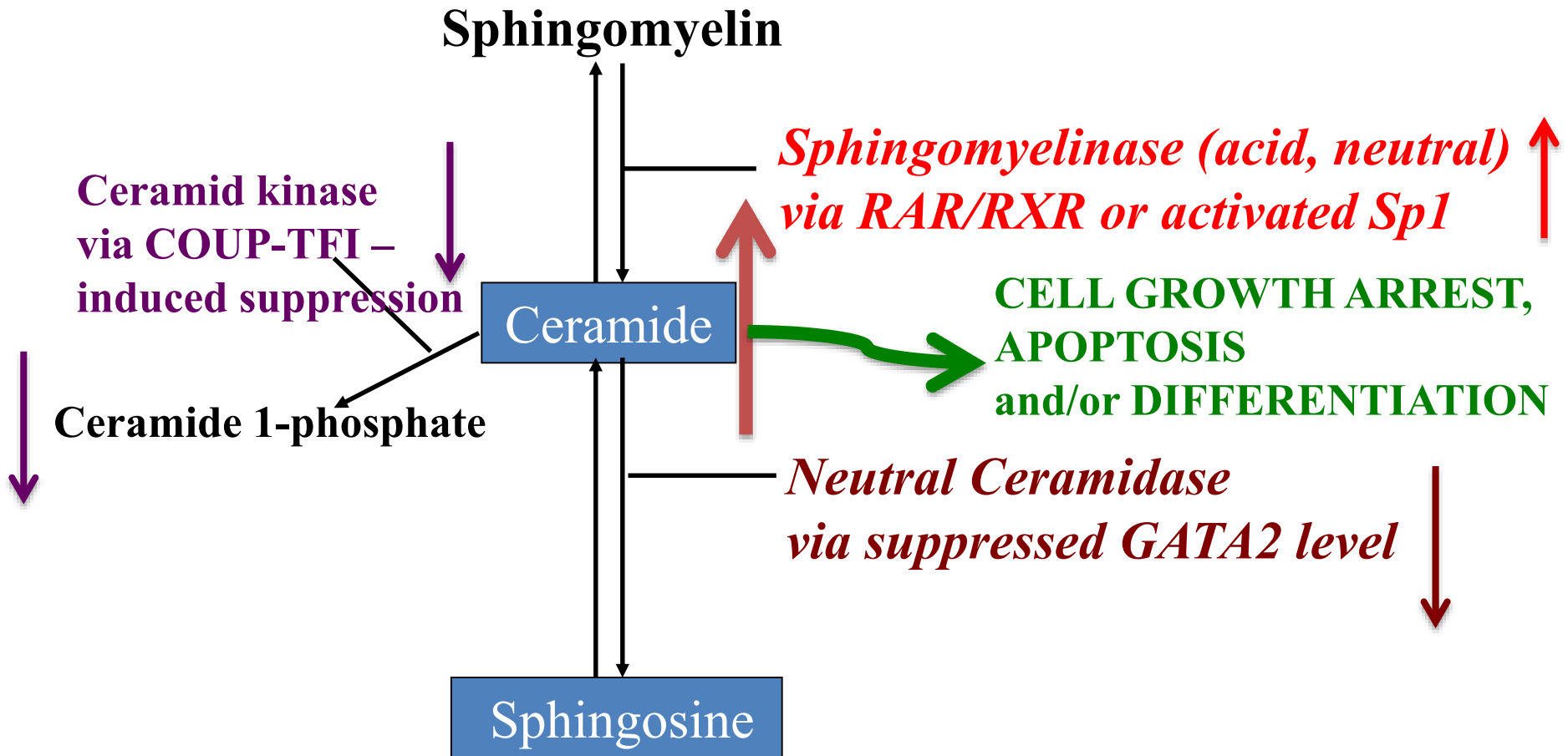


出典 [4]



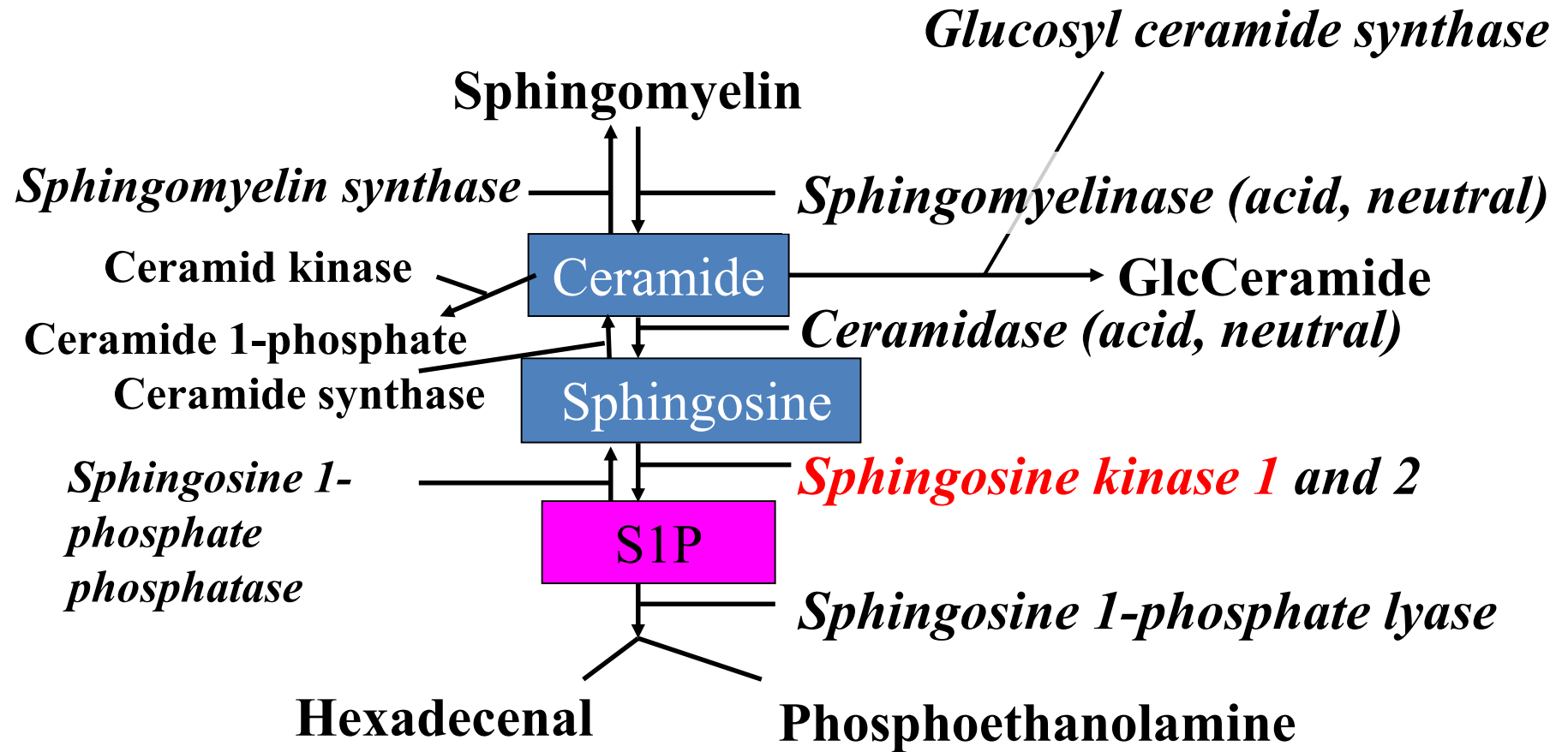
Summary of ATRA story

ATRA modulates sphingolipid metabolic pathway, leading to increased ceramide levels (context-dependent).



(II) Sphingosine kinase 1 (SPHK1) story

Sphingolipid metabolic pathway (mainly degradation)



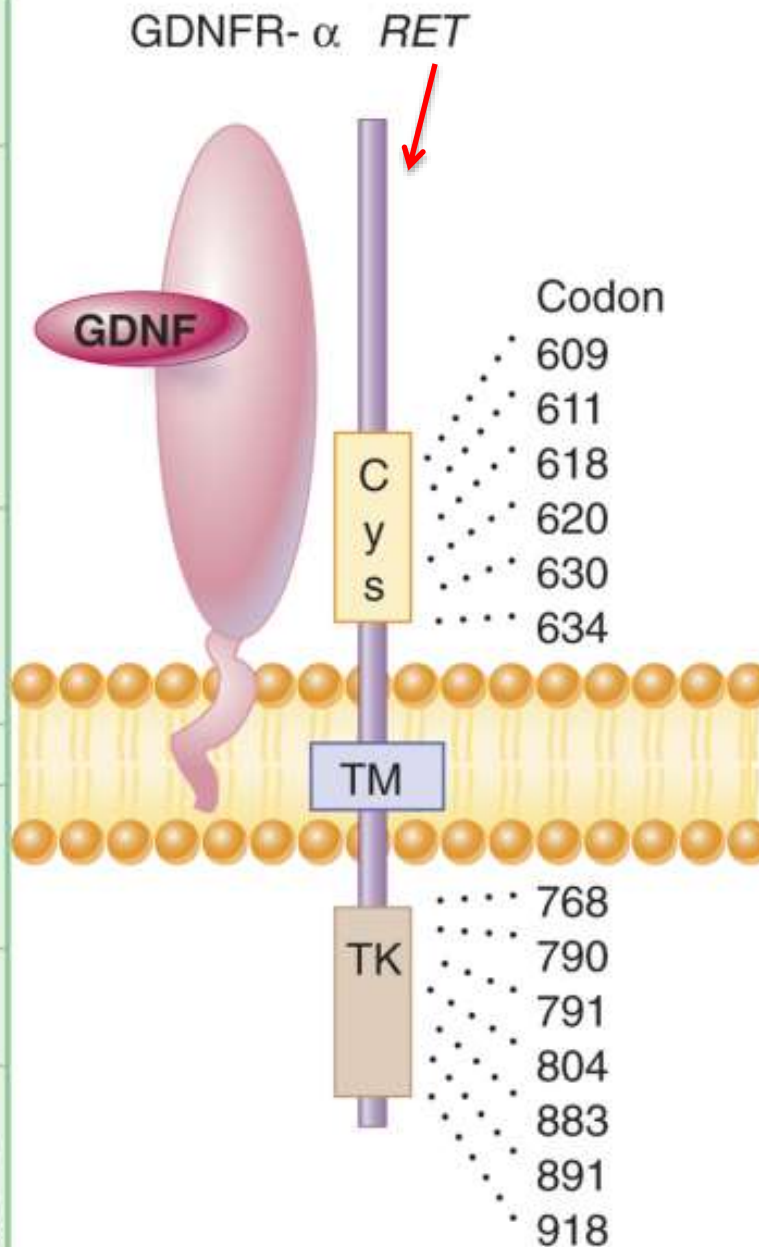
(5) RET signaling-induced SPHK1 gene expression plays a role in both GDNF-induced differentiation and MEN2-type oncogenesis

Murakami M. *et al.*

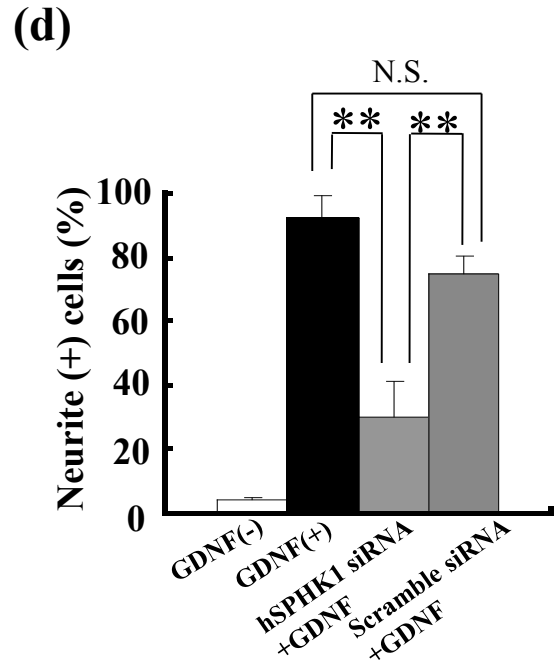
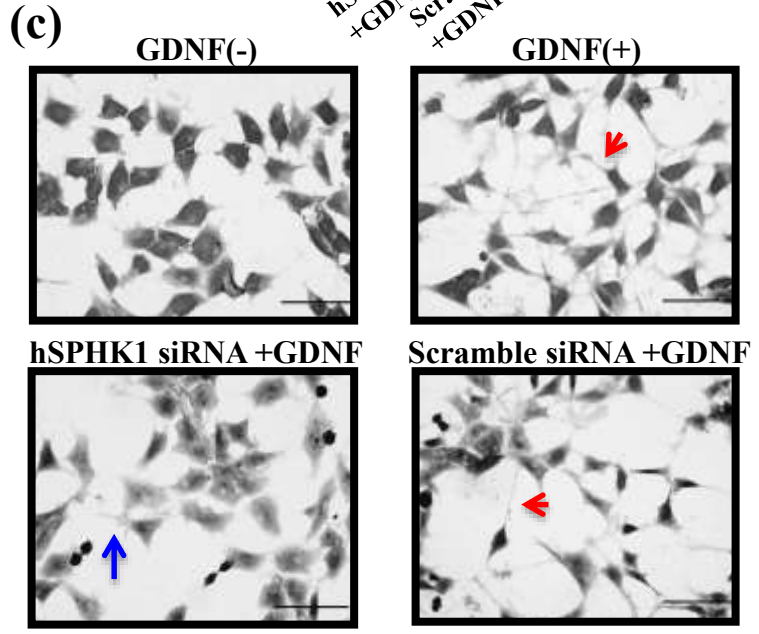
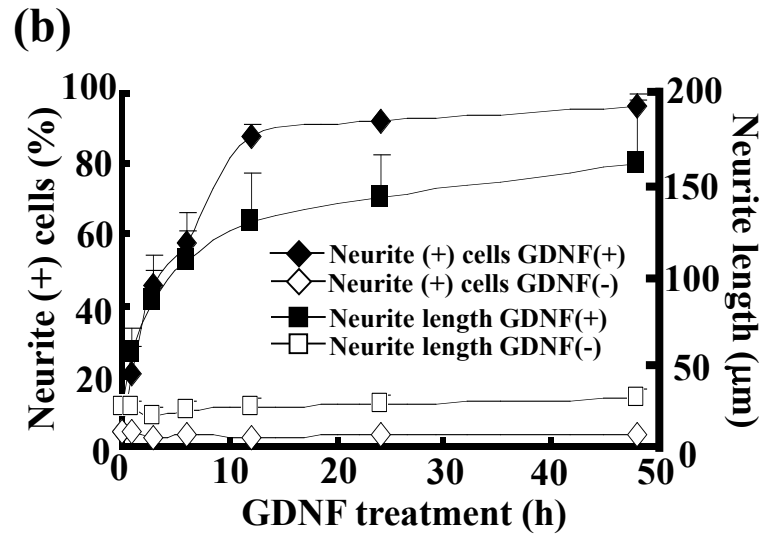
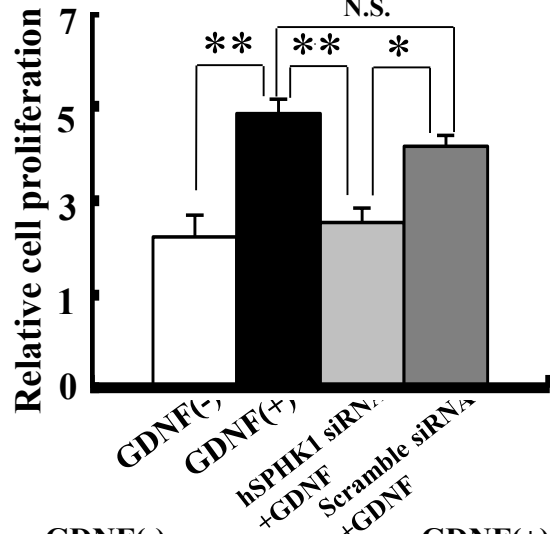
J Neurochem 102:1585-1594, 2007 [5]

**RET is the GDNF receptor subunit
TGW cell: RET(+) cell line**

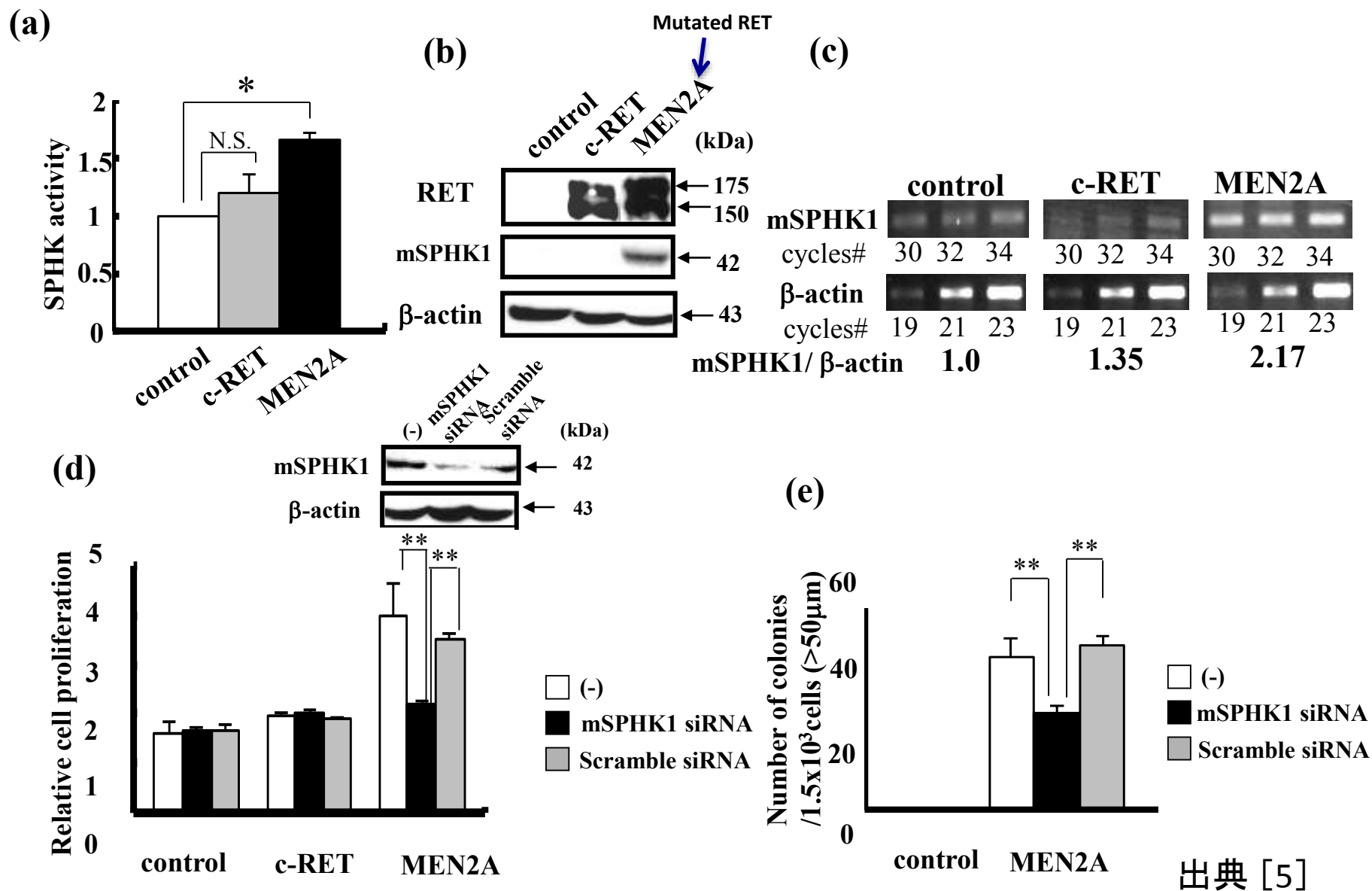
| Clinical syndrome | Codon of <i>RET</i> mutated |
|------------------------------|-----------------------------|
| MEN 2a FMTC | 609 |
| | 611 |
| | 618 |
| | 620 |
| | 630 |
| | 634 |
| FMTC | 768 |
| | 791 |
| | 804 |
| | 891 |
| MEN 2a/CLA | 634 |
| MEN 2/ Hirschsprung | 609 |
| | 618 |
| MEN 2b | 620 |
| | 634 |
| Sporadic MTC (Somatic) | 883 |
| | 918 |
| | 630(rare) |
| | 768 (rare) |
| | 883 (rare) |
| | 918 (25%) |



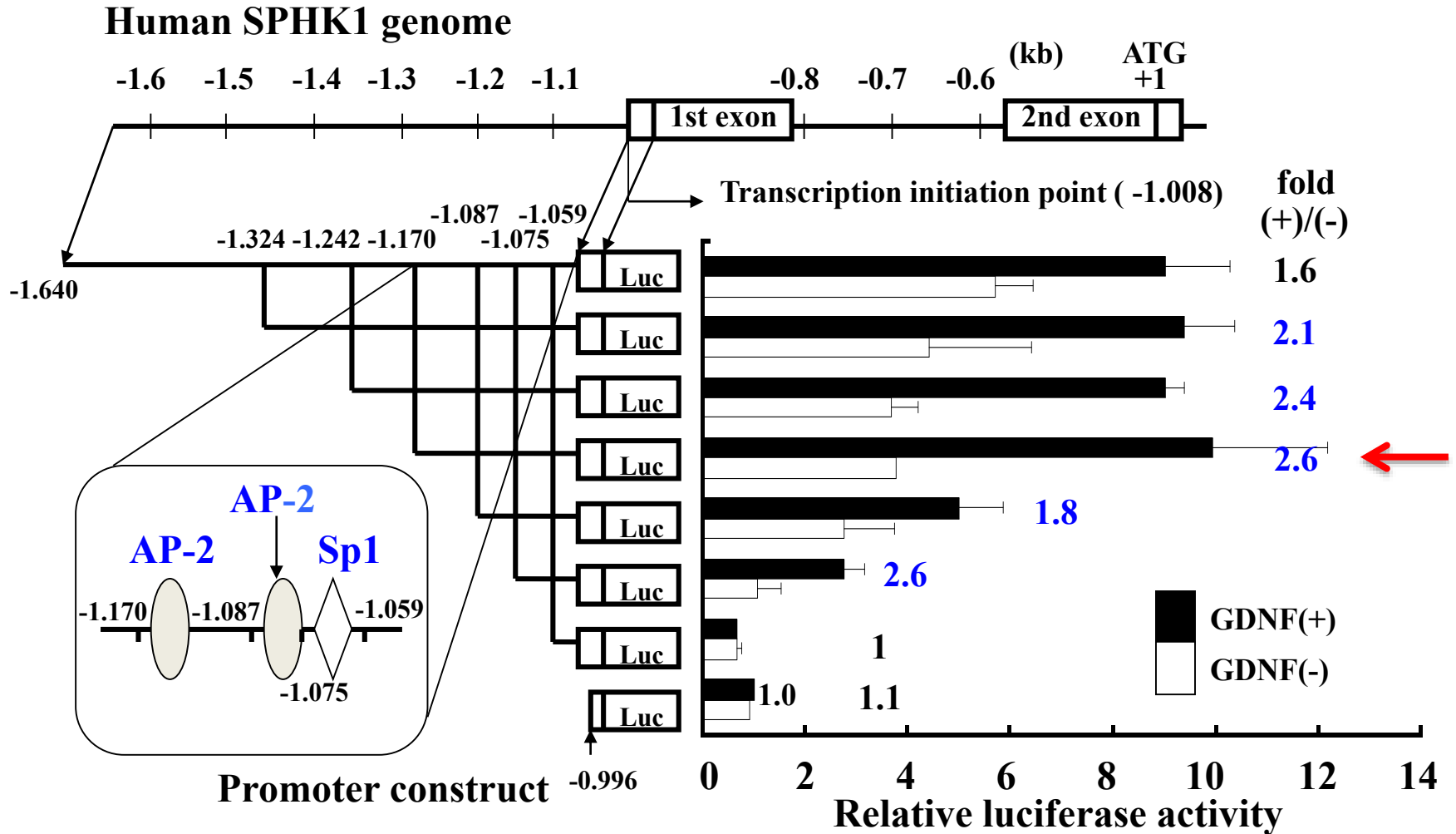
GDNF and SPHK1 in TGW cell line



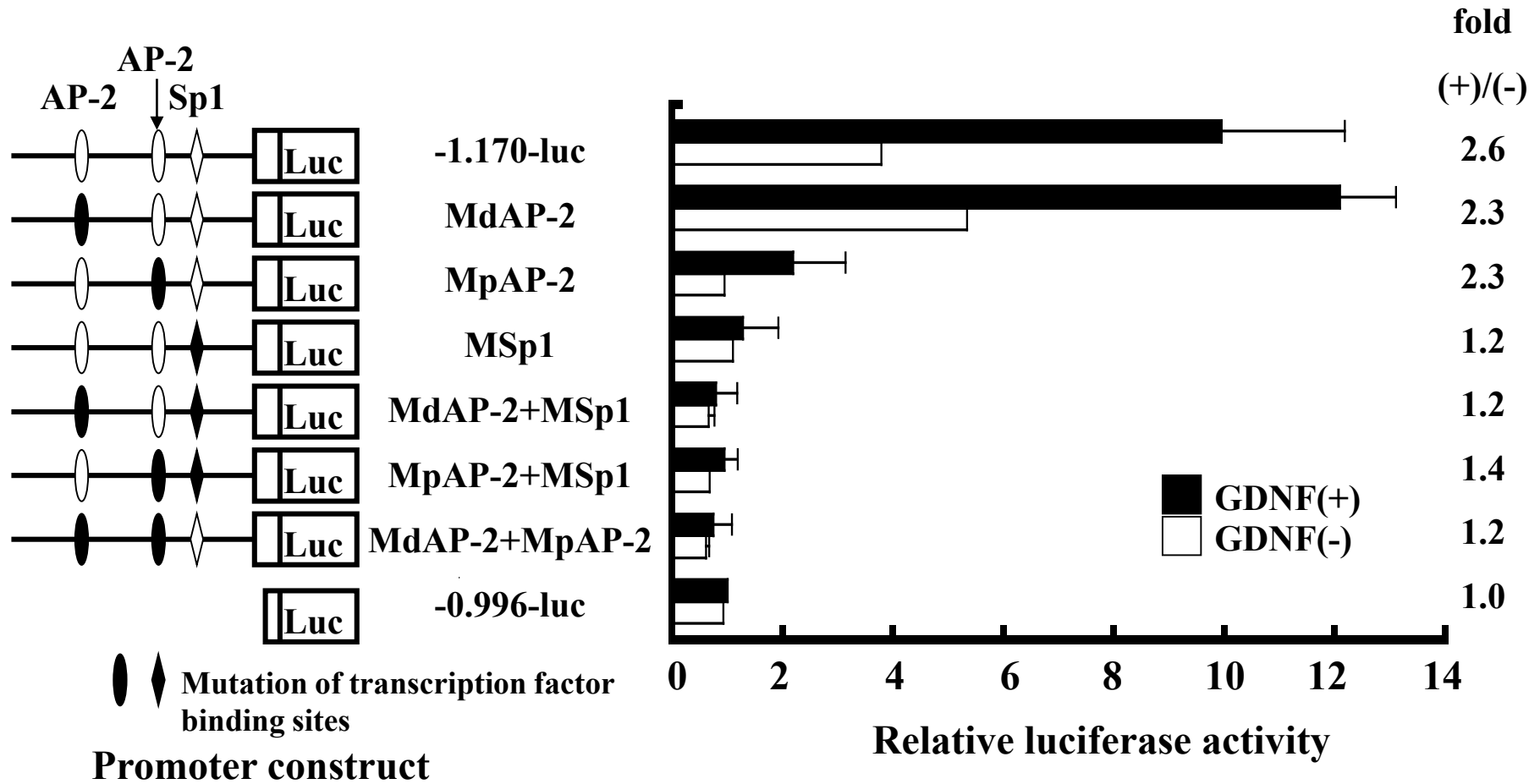
GDNF-receptor, RET, and SPHK1 (using mouse NIH3T3 cell)



SHK1 promoterのGDNF反応領域の同定



SPHK1 プロモーター領域の解析

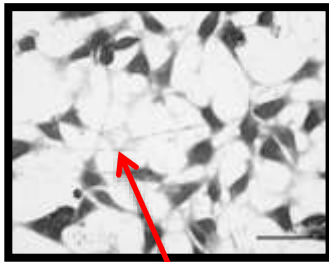


(6) Sphingosine kinase 1/S1P pathway involvement in the GDNF-induced GAP43 transcription

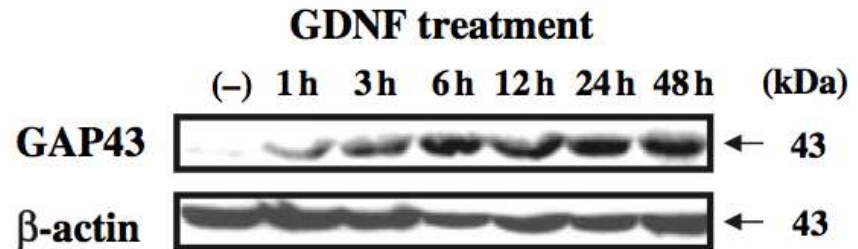
Murakami N. *et al.*

J Cell Biochem 112:3449-3468, 2011

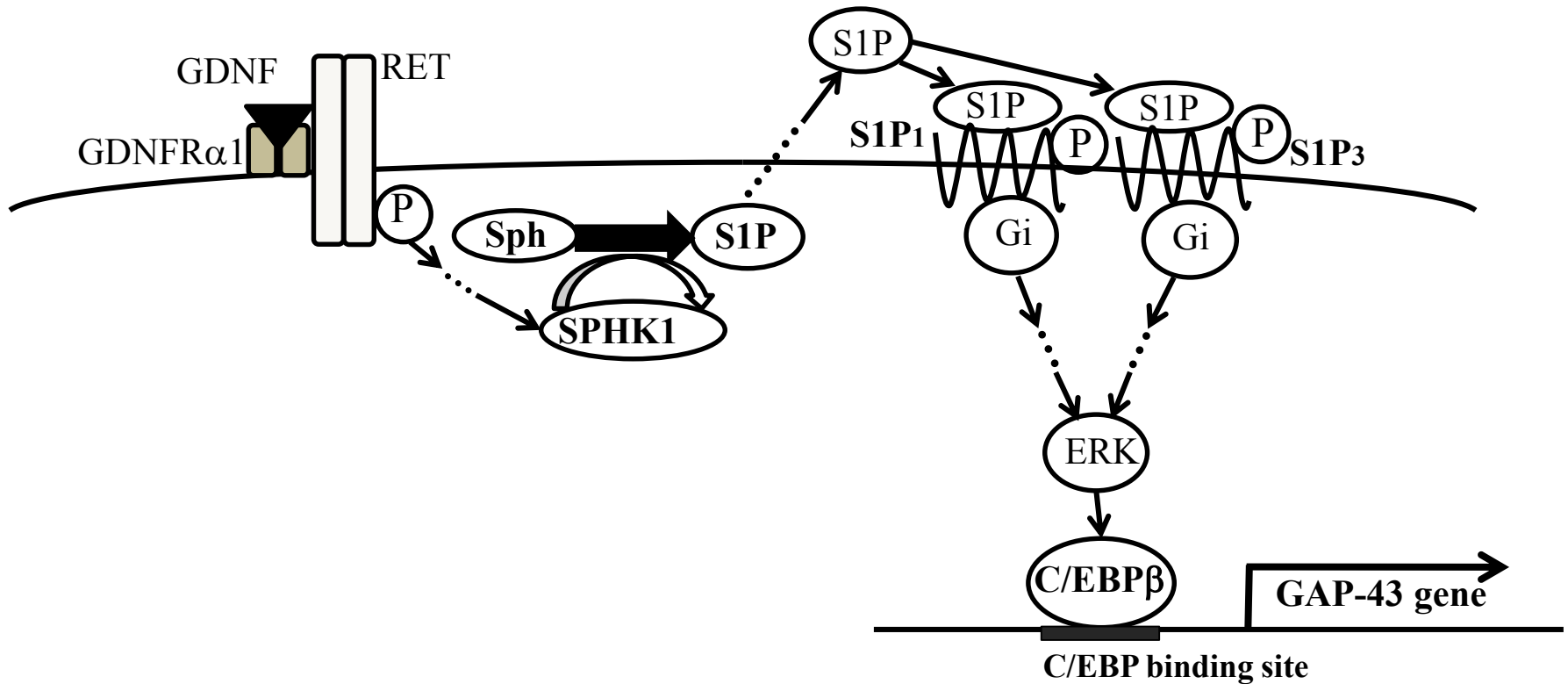
GDNF treatment



Accumulated GAP43 protein



Schematic presentation of GDNF/RET/SPHK/S1P/GAD43 pathway



**Cell Proliferation and
Neuronal Differentiation**

(7) Transcription factor specificity protein 1 (Sp1) is the main regulator of nerve growth factor-induced sphingosine kinase 1 gene expression of the rat pheochromocytoma cell line, PC12.

Sobue S. *et al.*

J Neurochem 95:940-949, 2005

**(8) Aberrant expression of SPHK1 in
hematological malignancies :
Myelodysplastic syndrome and AML**

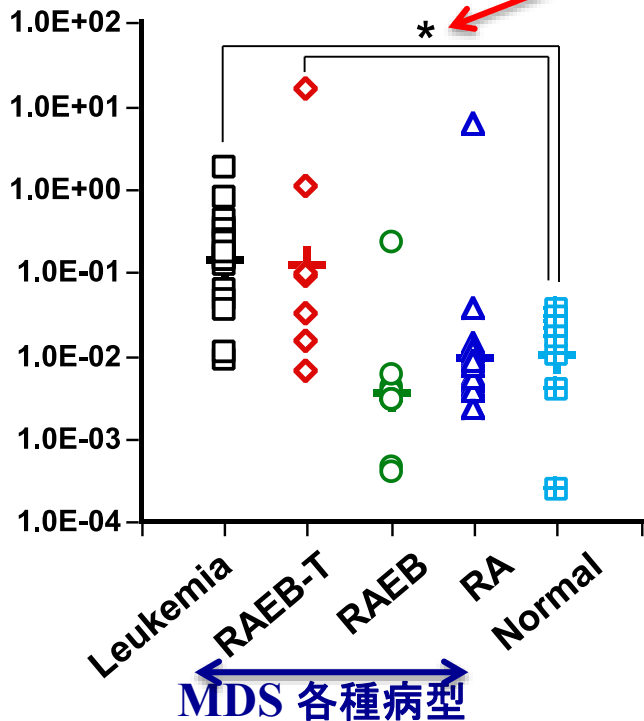
SPHK1 is oncogenic

Sobue S. *et al.* Leukemia 20: 2042-2046, 2006 [6]

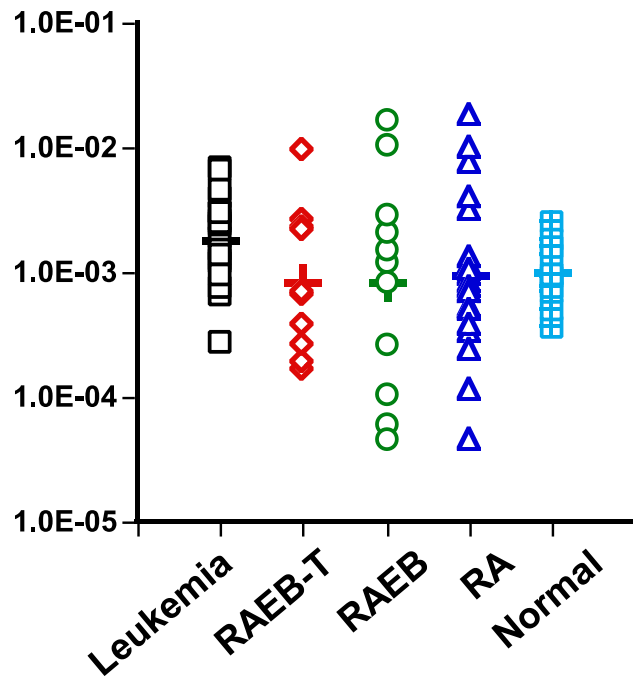
骨髄異形成症候群(Myelodysplastic Syndromes, MDS)の特徴

- 高齢者に発症頻度が高い。
- 通常の貧血治療に不応性の慢性貧血
- 汎血球減少を認める。
- 骨髄は細胞髄で血球形態異常を伴う。
- 急性白血病に移行する頻度が高い。

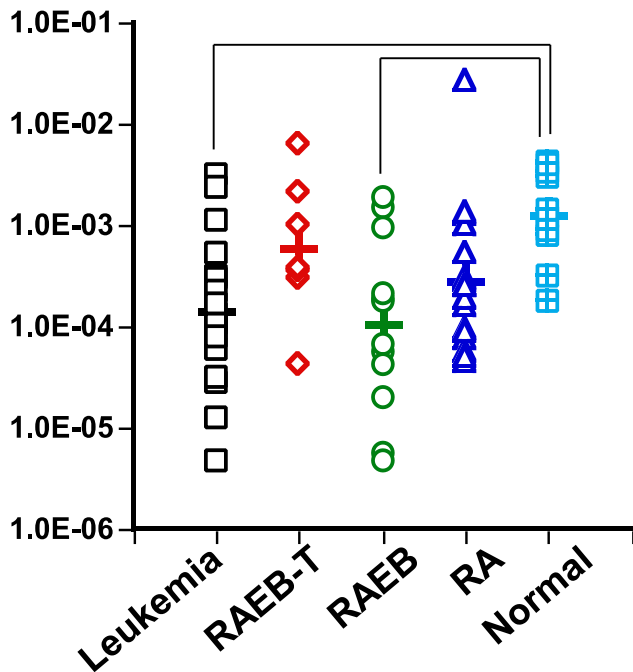
SPHK1



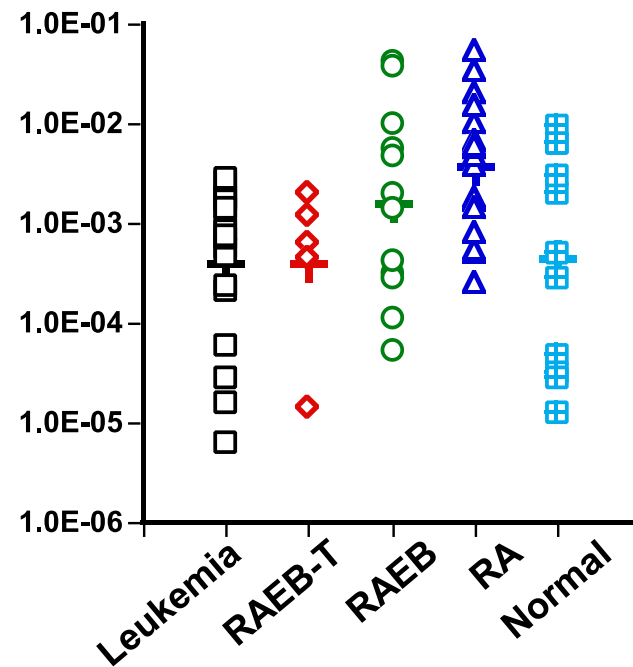
SPHK2



NSMase2



GlcCer synthase



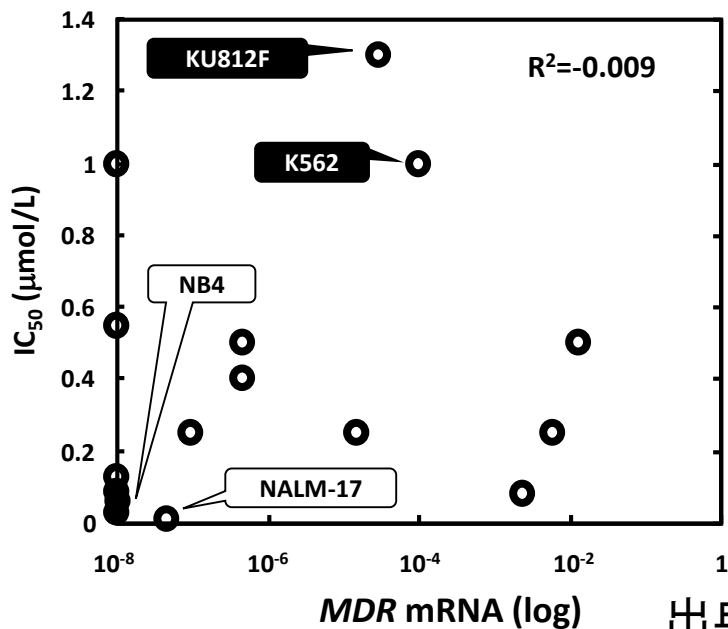
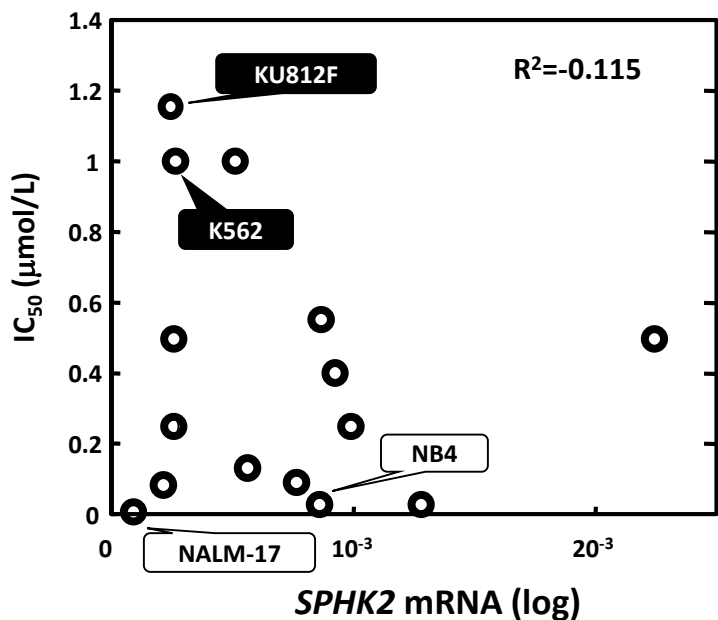
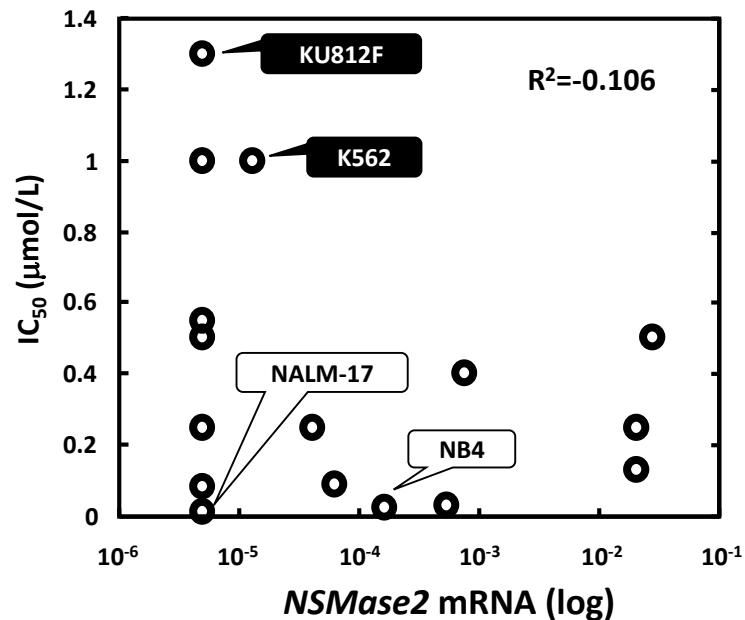
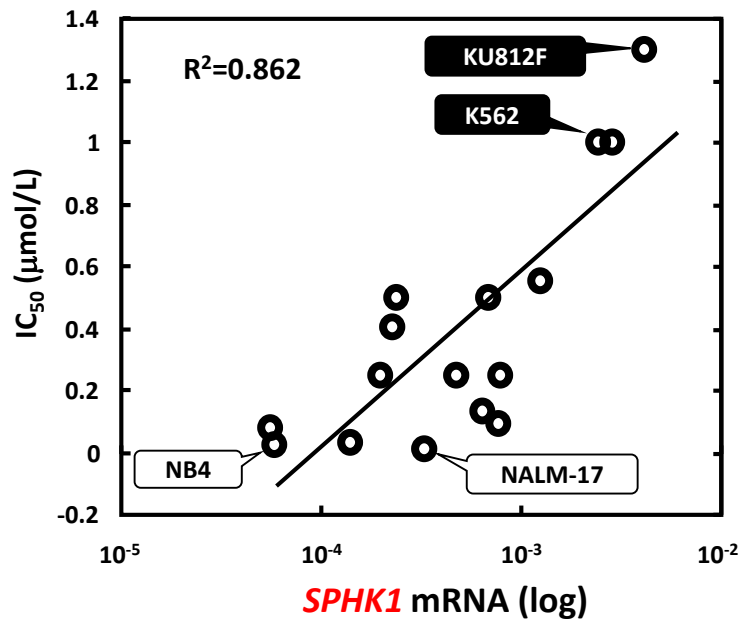
**(9) The relevance of SPHK1 as a marker for
Daunorubicin sensitivity of leukemia cells**

SPHK1 as the sensor of leukemia chemosensitivity

Sobue S. *et al.* Int J Hematol 87:266-275, 2008 [7]

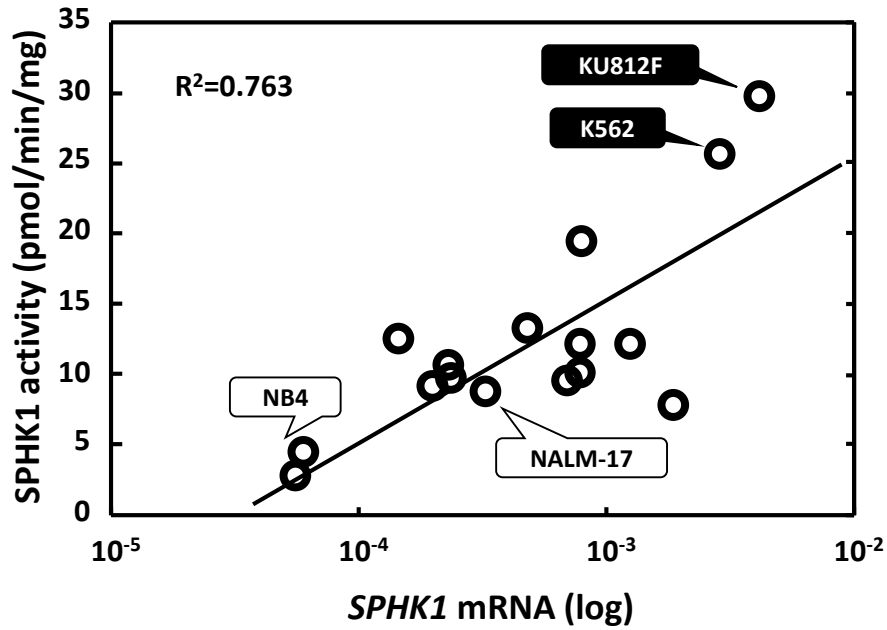
このフィールドでの最初の報告

抗がん剤(ダウノルビシン)のIC50 とスフィンゴ脂質代謝酵素 mRNA との相関

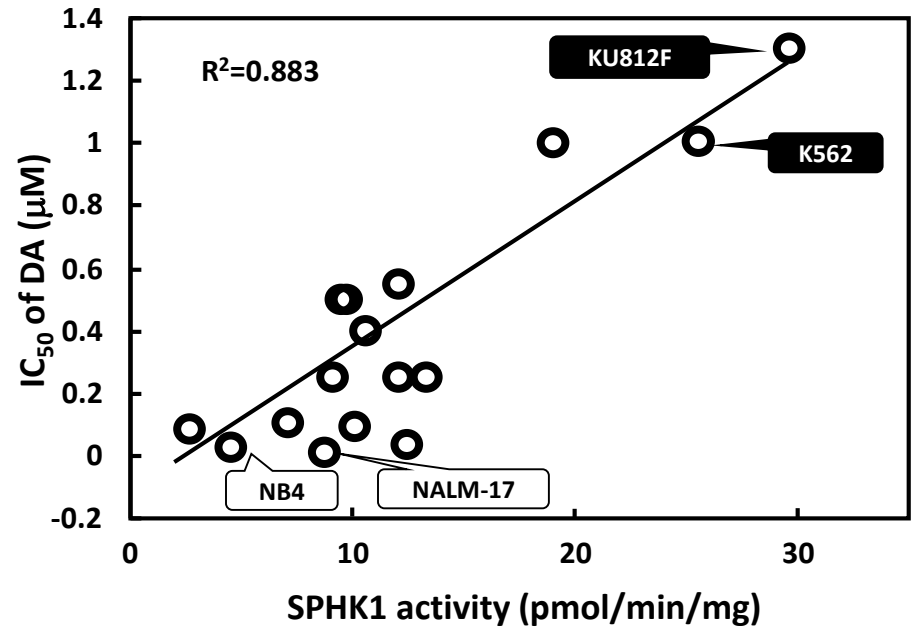


白血病細胞株におけるSPHK1とダウノルビン IC50 との関連

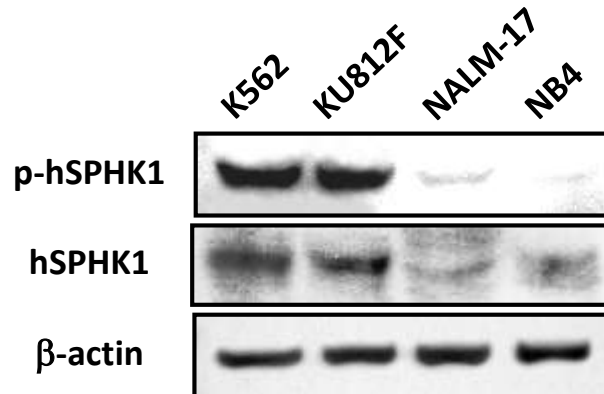
A



B



C



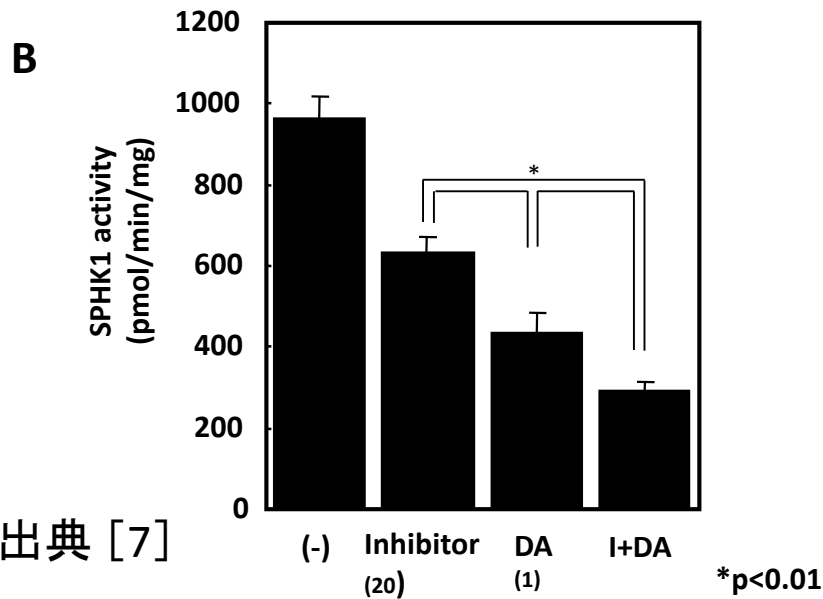
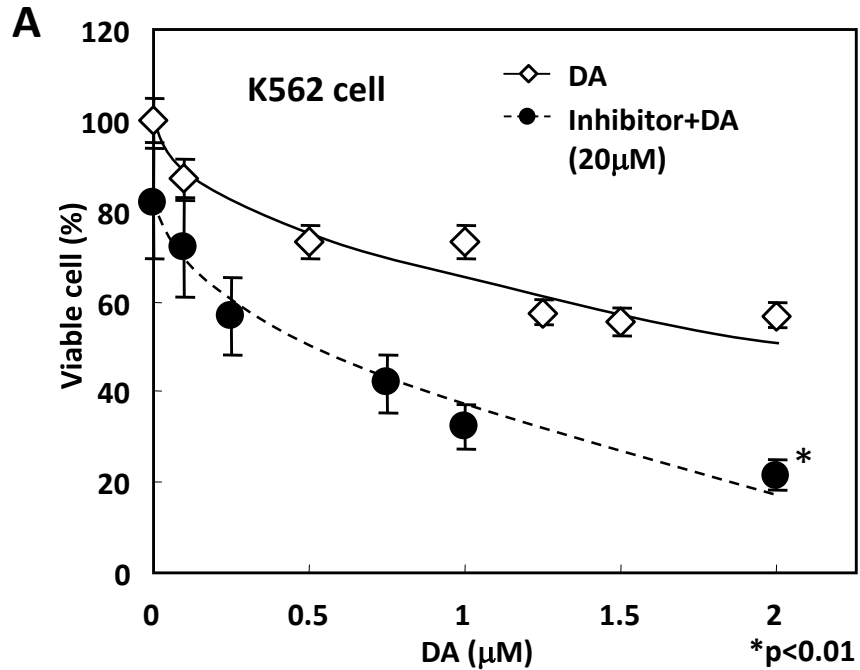
Changes in Cellular Ceramides and S1P Content after Daunorubicin Treatment

| | Cell | Ceramide | | | | | | S1P | |
|-----------------------|----------------|----------|------|------|------|-------------|-------------|-------------|-------------|
| | | C16 | | C18 | | C24 | | | |
| | | - | + | - | + | - | + | - | + |
| Daunorubicin | | - | + | - | + | - | + | - | + |
| (pmol/0.5 mg protein) | | | | | | | | | |
| DA-resistant | K562 | 375 | 464 | 19.6 | 34.8 | <u>726</u> | <u>715</u> | <u>6.1</u> | <u>5.4</u> |
| | +/-SD | 75.7 | 159 | 3.8 | 11.7 | 12.8 | 43 | 1.1 | 1.2 |
| | KU812F | 120 | 185 | 7.2 | 12.5 | <u>757</u> | <u>848</u> | <u>10.2</u> | <u>12.3</u> |
| | +/-SD | 2.4 | 9.3 | 1.7 | 0.6 | 103 | 241 | 0.9 | 1.9 |
| DA-sensitive | NALM-17 | 1118 | 1678 | 498 | 1047 | <u>1760</u> | <u>4692</u> | <u>24.3</u> | <u>11.5</u> |
| | +/-SD | 508 | 214 | 16.6 | 66.2 | 238 | 739 | 4.3 | 1.7 |
| | NB4 | 579 | 703 | 141 | 208 | <u>670</u> | <u>1436</u> | <u>8.2</u> | <u>3.5</u> |
| | +/-SD | 80.7 | 41.5 | 35.5 | 8.2 | 109 | 254 | 3.3 | 1.3 |

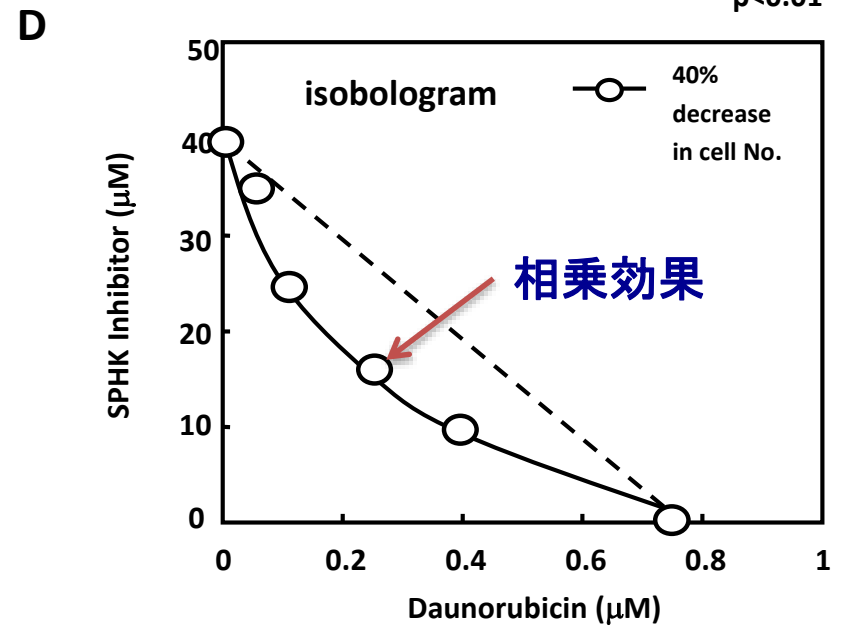
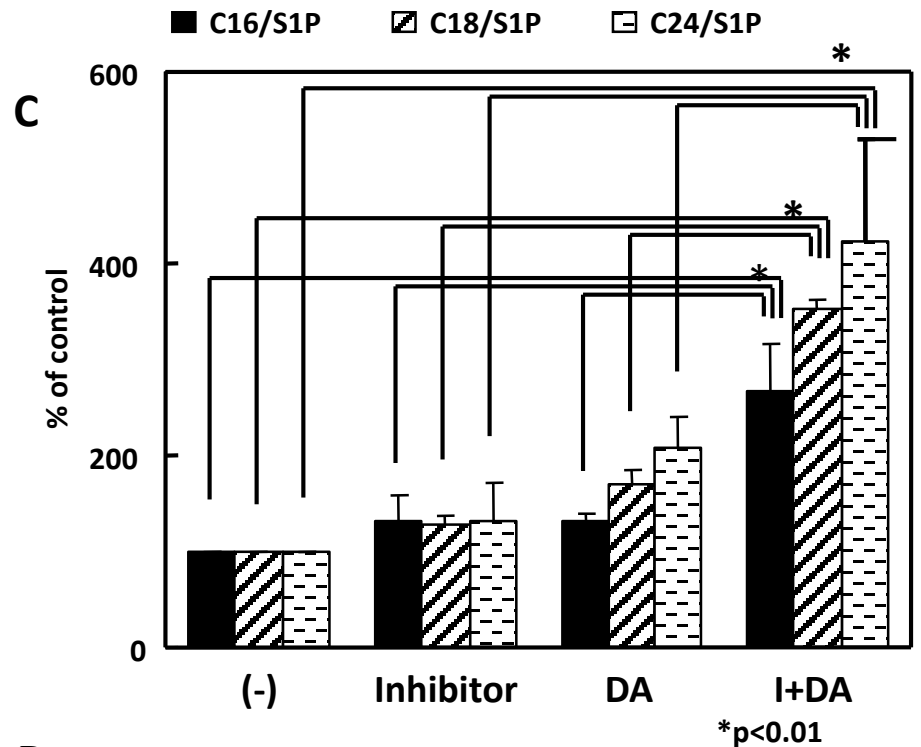
出典 [7]

DA: Daunorubicin
Sphingolipid: Measured by LC-MS/MS

抗がん剤としてのSPHK 阻害剤



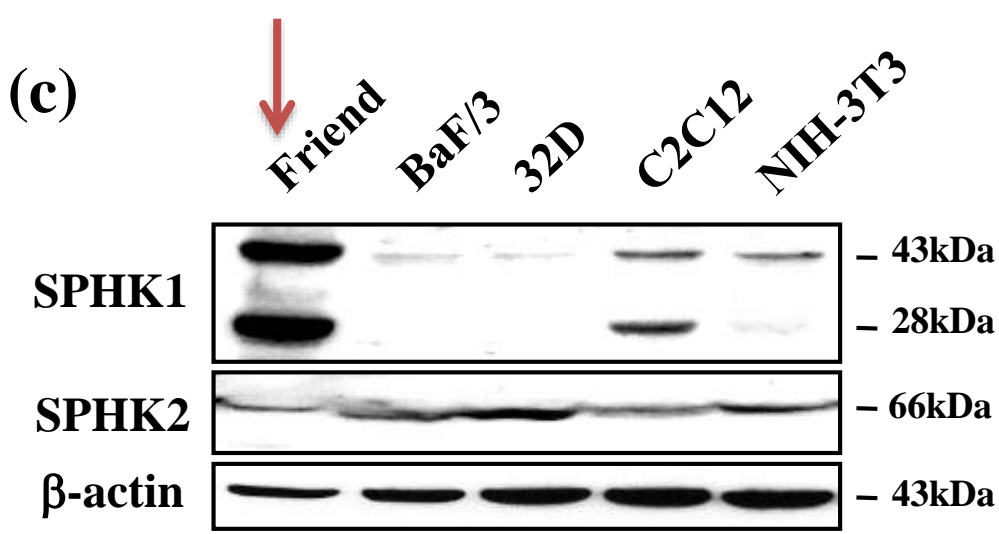
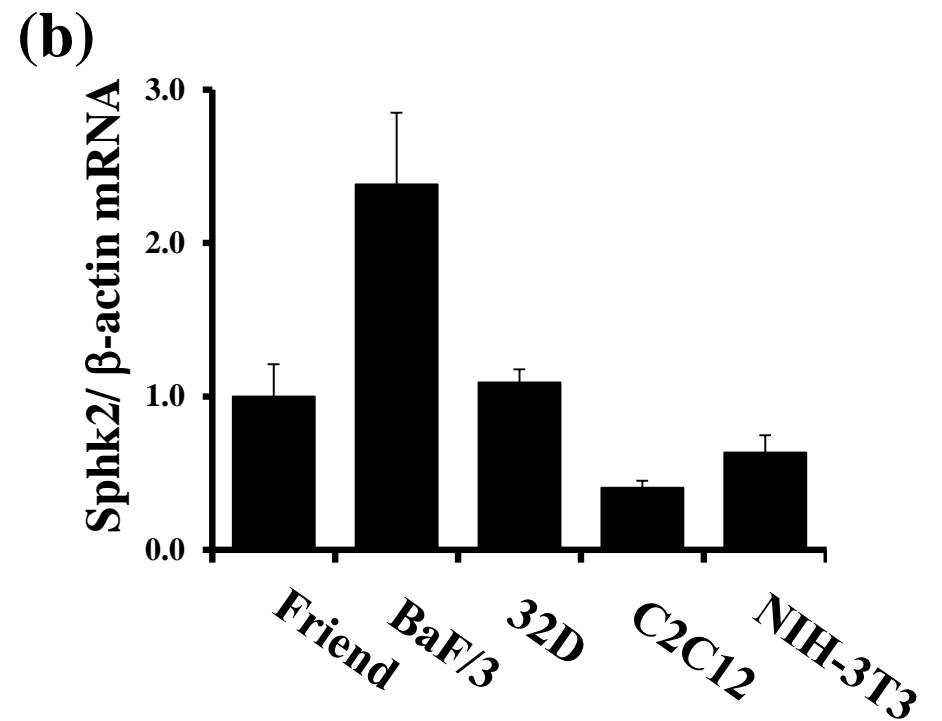
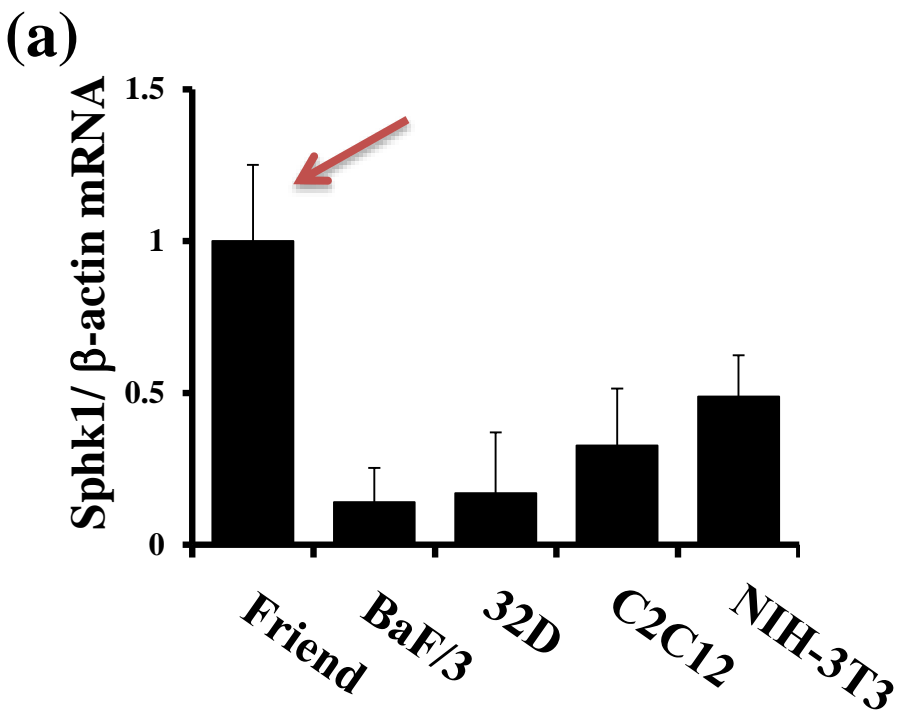
出典 [7]

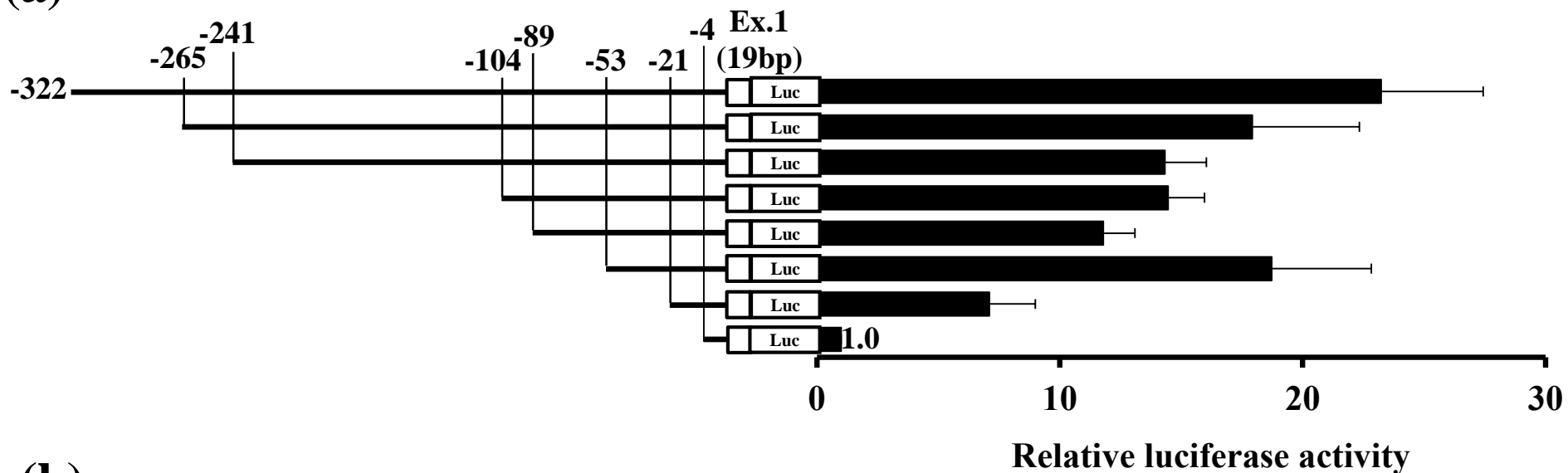
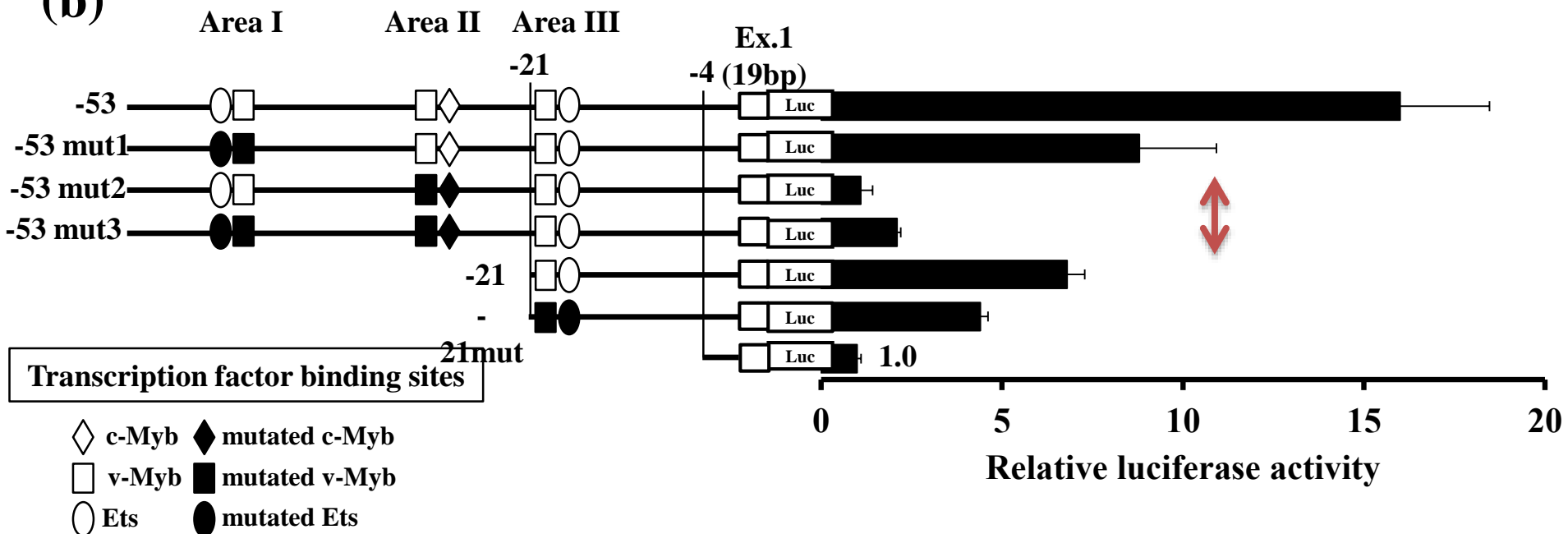


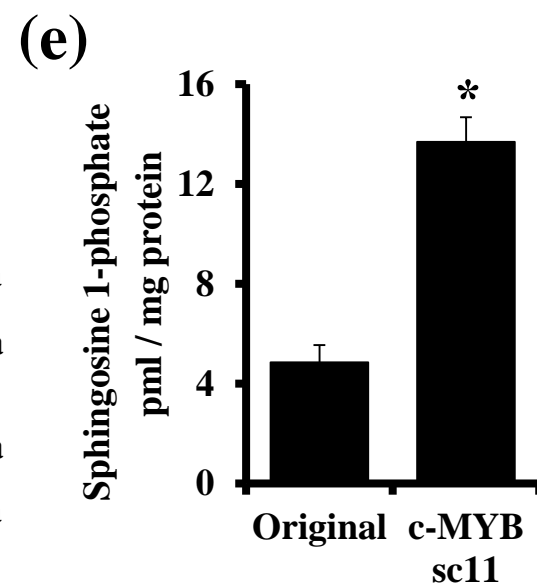
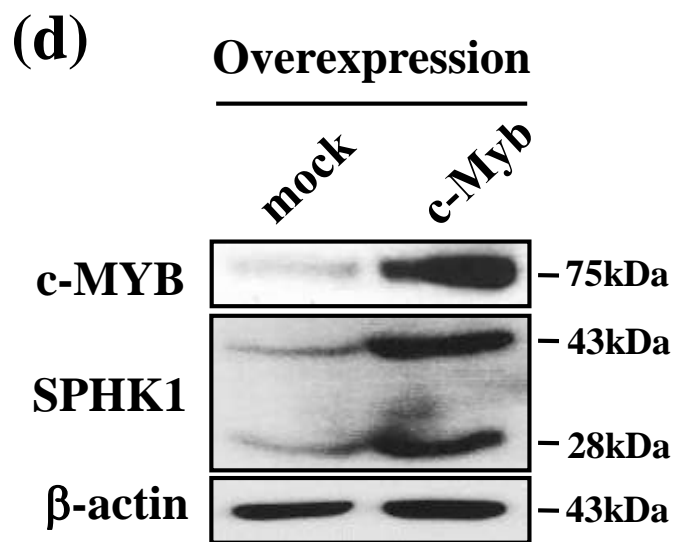
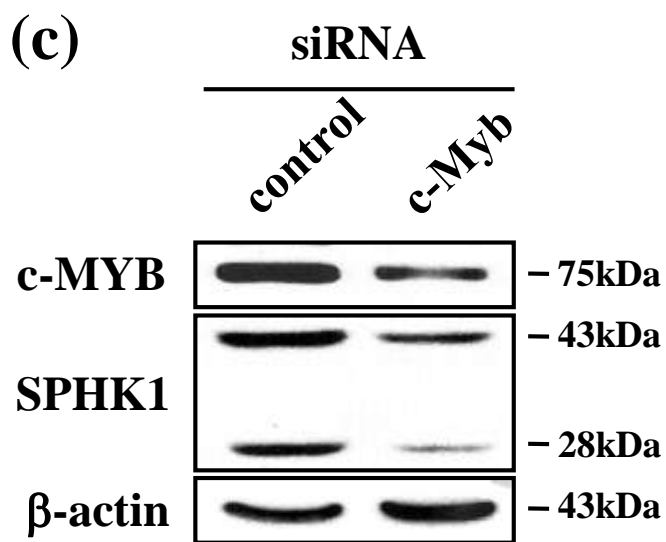
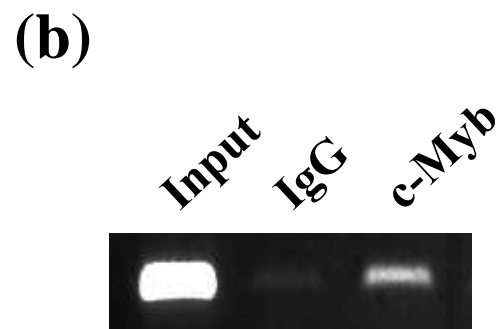
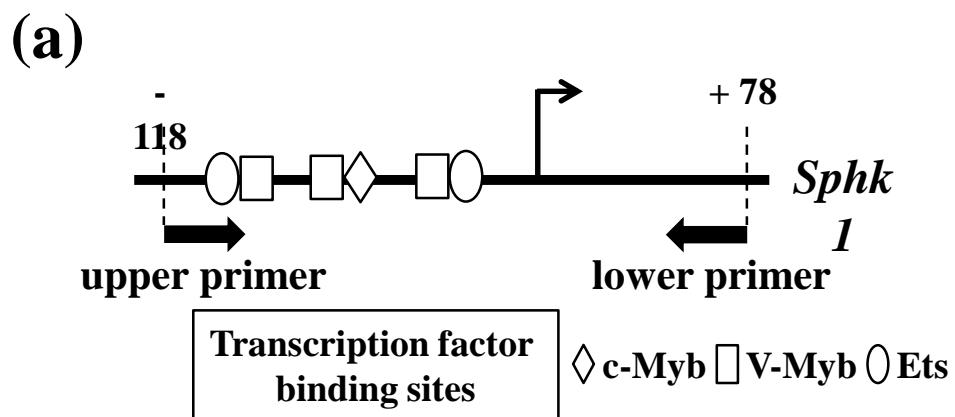
(10) Sphingosine kinase 1 expression is downregulated during differentiation of Friend cells due to decreased c-MYB

Mizutani N. et al.

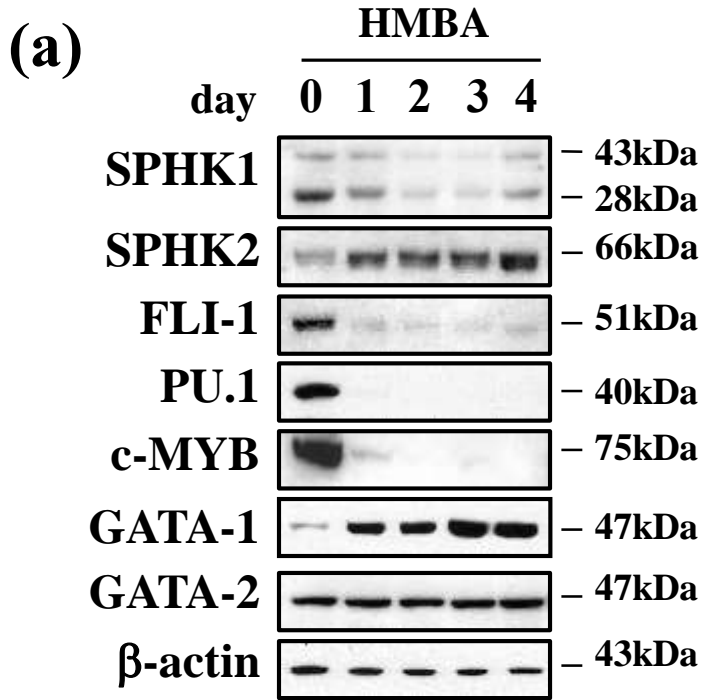
Biophys Biophys Acta 2013 [8]



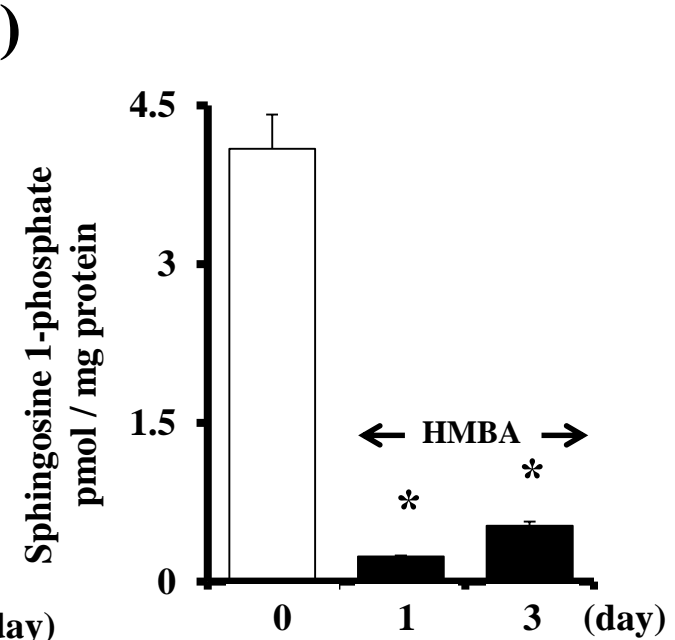
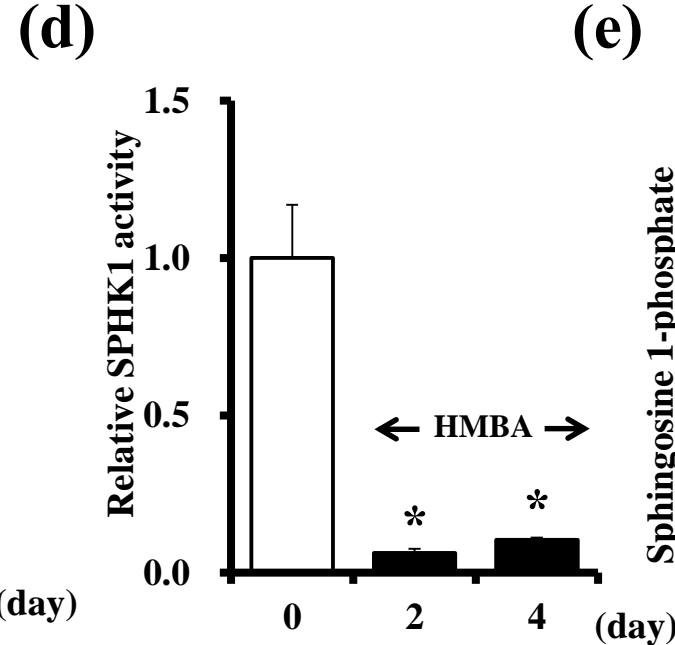
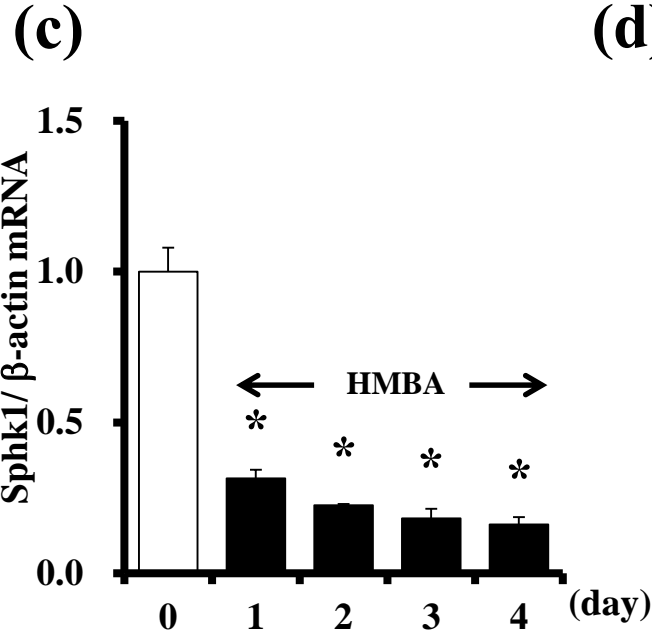
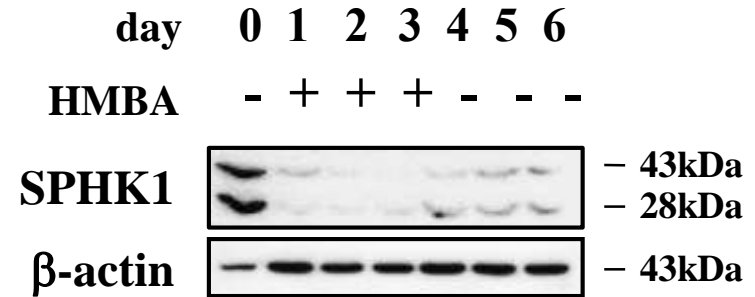
(a)**(b)**



HMBAによる赤血球系分化とSPHK1



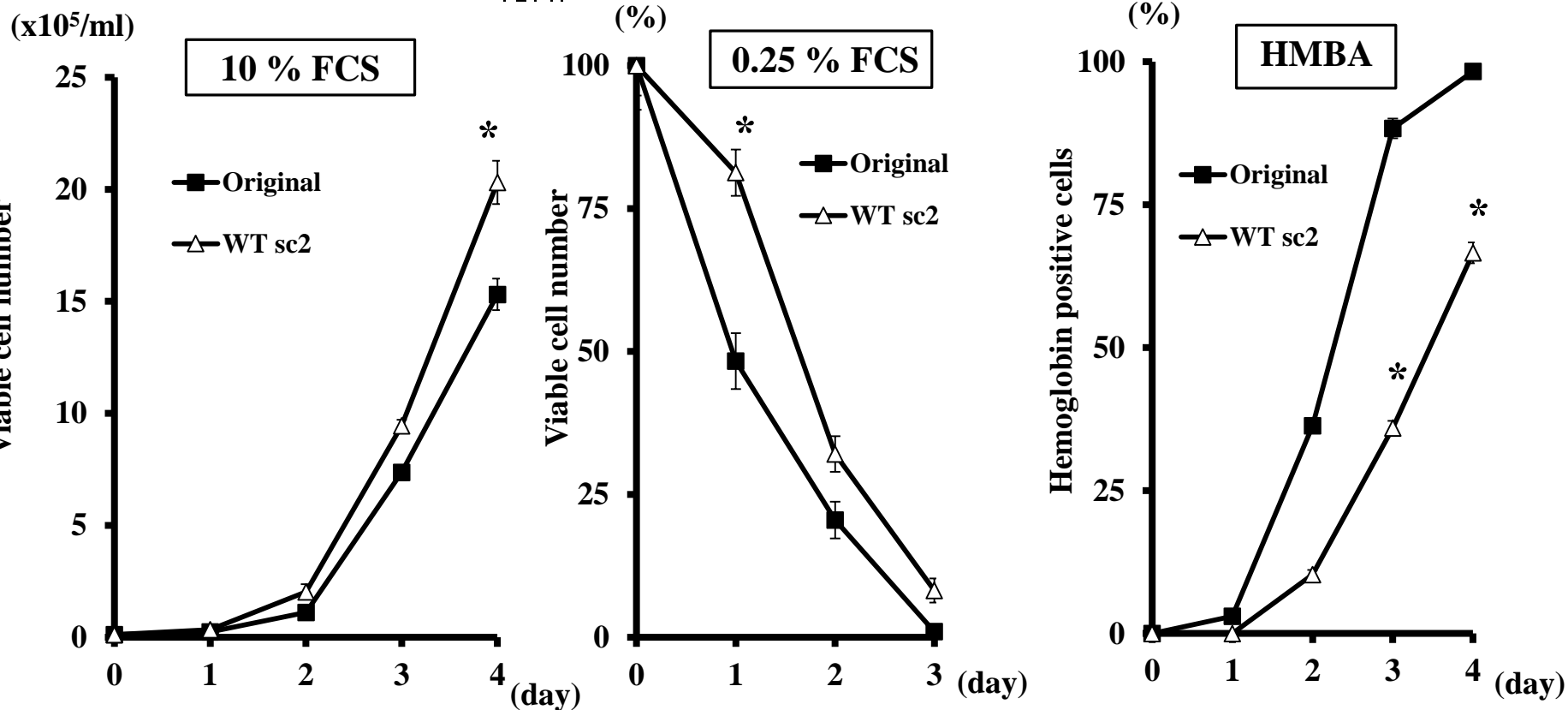
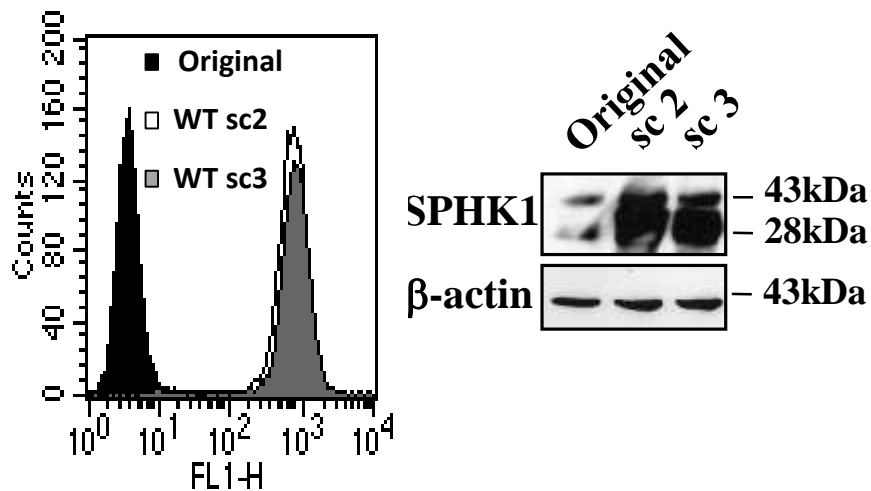
(b)



出典 [8]

SPHK1過剰発現はフレンド細胞の細胞死抵抗性および細胞分化抵抗性を獲得する。

出典[8]



My journey to the sphinx of the lipids

- **Cellular sphingolipid metabolites are finely regulated by a series of metabolic enzymes**
- **The aberrant expression of sphingolipid metabolic enzymes leads to the abnormal cellular behavior, such as oncogenesis or apoptosis.**
- **The modulation of respective sphingolipid metabolic enzyme is the promising strategy in the field of oncology, immunology, metabolic diseases and degenerative disorders.**



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岩崎卓識（社会人）、水谷直貴、
MC: 中出祐介、菊池亮介、高四強、古畑彩子、木村有美、柴山修司（社会人）、
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