

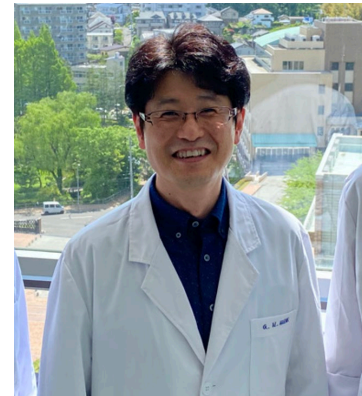
メチル水銀による 脳内炎症応答を介した 毒性発現機構の解析

本発表に関連し、開示すべきCOI関係にある企業等はありません。



環境衛生学教室
東北医科薬科大学薬学部

ふあん ぎうく
黄 基旭



CHEMICALS

known to disrupt

BRAIN DEVELOPMENT

90%

OF PREGNANT
U.S. WOMEN

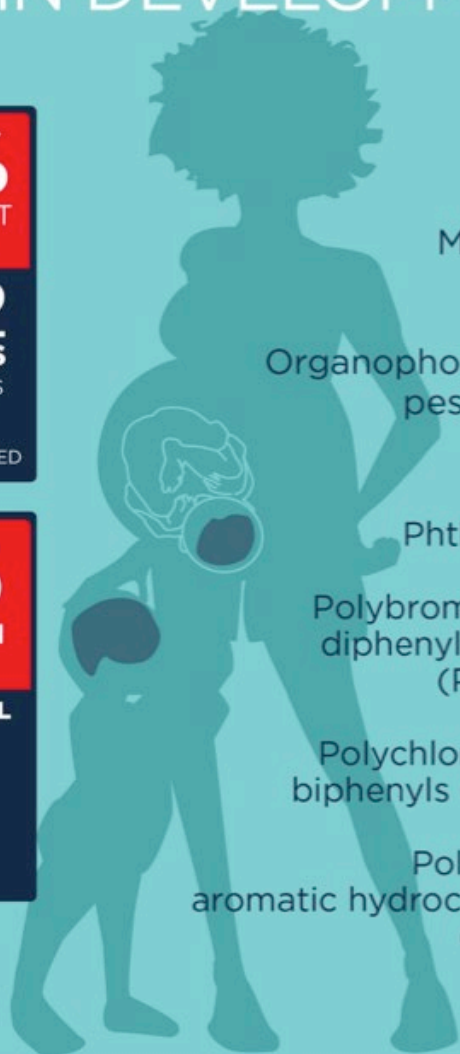
HAVE
DETECTABLE
LEVELS OF
62
CHEMICALS
IN THEIR BODIES
OUT OF **163**
CHEMICALS SCREENED

1 in 6

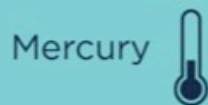
U.S. CHILDREN
HAVE A

**DEVELOPMENTAL
DISABILITY**

INCLUDING: ADHD,
AUTISM, LEARNING
DISABILITIES AND
DEVELOPMENTAL
DELAYS



Lead



Mercury

Organophosphate
pesticides



Phthalates



Polybrominated
diphenyl ethers
(PBDEs)



Polychlorinated
biphenyls (PCBs)



Polycyclic
aromatic hydrocarbons
(PAHs)



Project TENDR:

Targeting Environmental Neuro-Developmental Risks

These chemicals are commonly found in our environment as well as in the bodies of pregnant women, where many can cross the placenta and are often found in cord blood or other fetal tissue.

~Source: Health Care Without Harm HP~

化学物質による健康影響が
懸念されており、大きな社
会問題となっている

メチル水銀 (MeHg)



メチル水銀は水俣病の原因物質として知られ
脳組織選択的な障害を引き起こす



中枢神経障害

食物連鎖によるメチル水銀の魚類中への濃縮

プランクトン

小型魚

大型魚

ヒト

海水



1

5×10^3

1×10^5

1×10^7 : 濃縮度

メチル水銀

マグロ過食に注意 妊婦から胎児へ影響、低濃度でも 東北大調査（東北地方沿岸地域での調査）

毎日新聞 2016年11月28日 東京朝刊

マグロやメカジキなどメチル水銀を比較的多く含む魚介類を妊婦が食べ過ぎると、生まれた子の運動機能や知能の発達に悪影響が出るリスクが増すことが、東北大チームの疫学調査で分かった。メチル水銀は水俣病の原因物質だが、一般的な食用に問題のない低濃度の汚染でも胎児の発達に影響する可能性があることが明らかになるのは、日本人対象の調査では初めて。

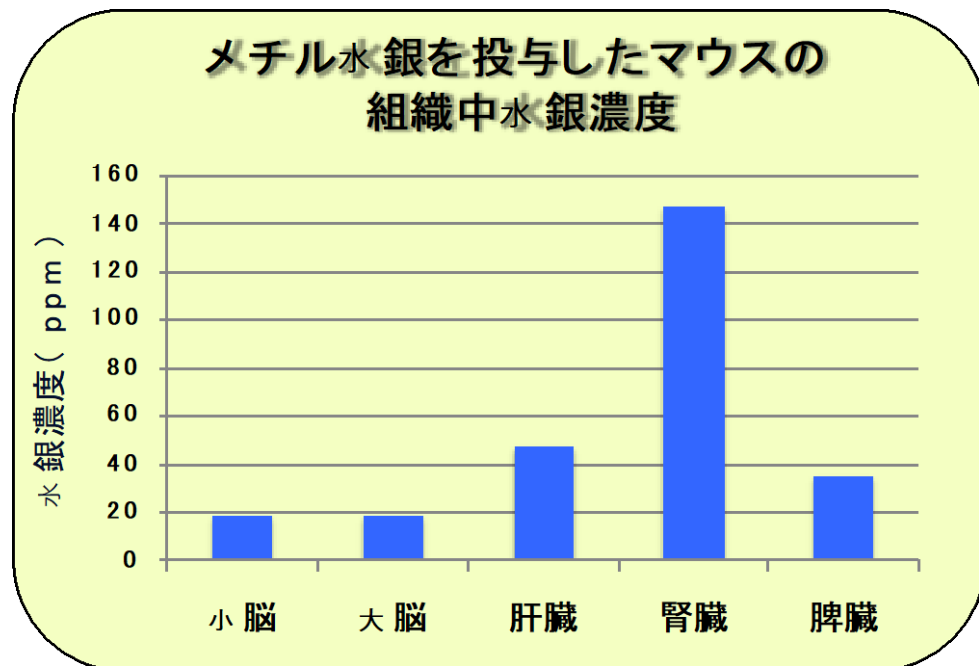
現在、日本においても、メチル水銀が胎児に引き起こす健康影響は大きな社会問題となっている

メチル水銀毒性発現のメカニズムは？

- 我々を含め世界中の研究者が毒性発現機構の解明に盛んに取り組んできた



- 水俣病の発症確認から約70年になる現在も未解明のまま



これまでの謎：

メチル水銀は脳よりも肝臓・腎臓に高濃度に蓄積するが、障害は脳にしか出ない

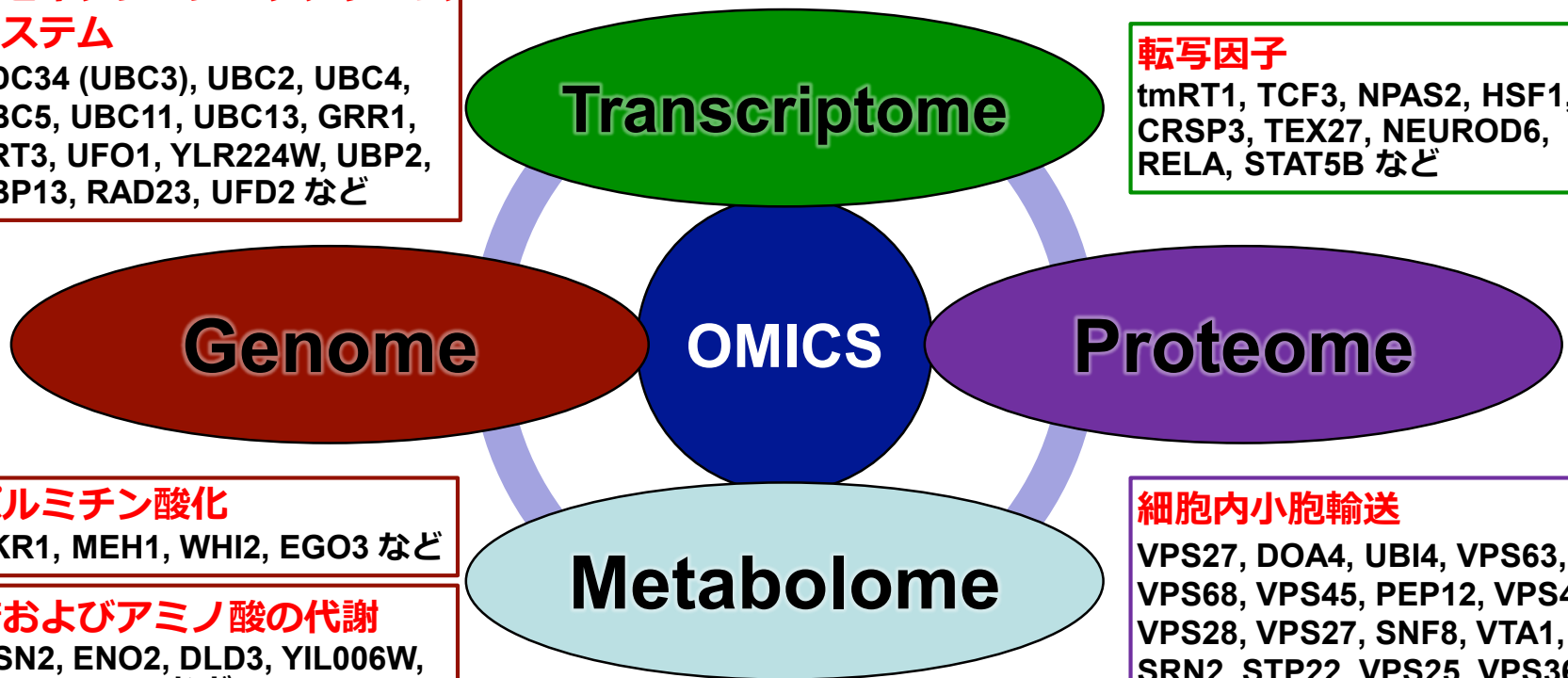
OMICS技術を応用した 網羅的スクリーニング

ユビキチン・プロテアソーム システム

CDC34 (UBC3), UBC2, UBC4,
UBC5, UBC11, UBC13, GRR1,
HRT3, UFO1, YLR224W, UBP2,
UBP13, RAD23, UFD2 など

転写因子

tmRT1, TCF3, NPAS2, HSF1,
CRSP3, TEX27, NEUROD6,
RELA, STAT5B など



パルミチン酸化

AKR1, MEH1, WHI2, EGO3 など

糖およびアミノ酸の代謝

MSN2, ENO2, DLD3, YIL006W,
RIP1, MET25 など

サイトカイン類

TNF-a, OSM, IL-6, CCL2, CCL3,
CCL4 など

細胞内小胞輸送

VPS27, DOA4, UBI4, VPS63,
VPS68, VPS45, PEP12, VPS4,
VPS28, VPS27, SNF8, VTA1,
SRN2, STP22, VPS25, VPS36,
DID4, VAM3, VAM7 など

低分子代謝物

プトレシン、ハイポタウリン
など

Effects of MeHg on TNFSF mRNA levels in the brains of mice

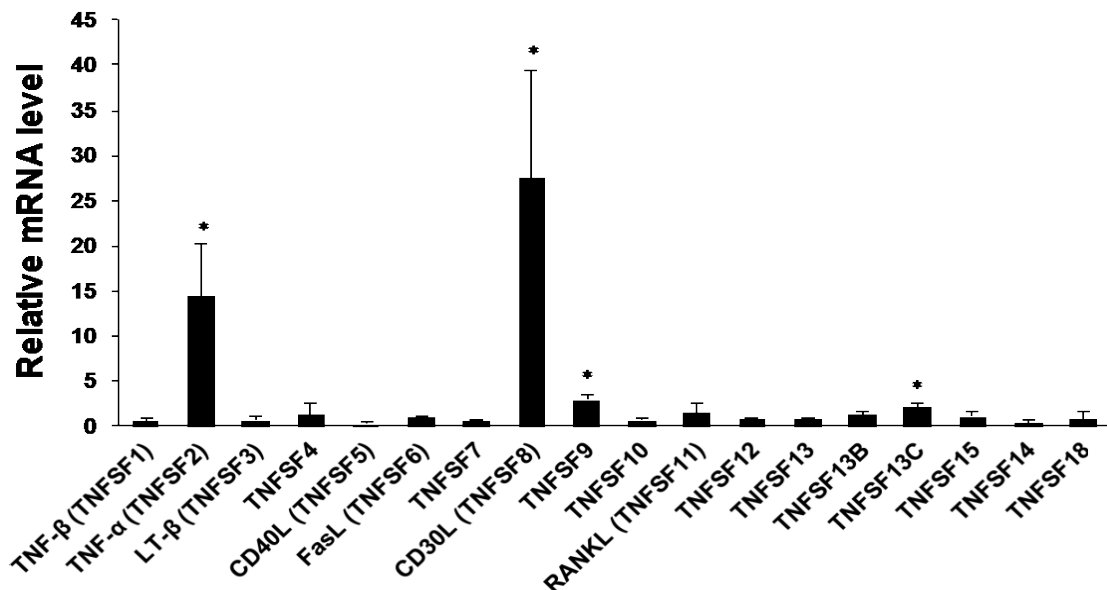


Methylmercury
(25 mg/kg, one shot)

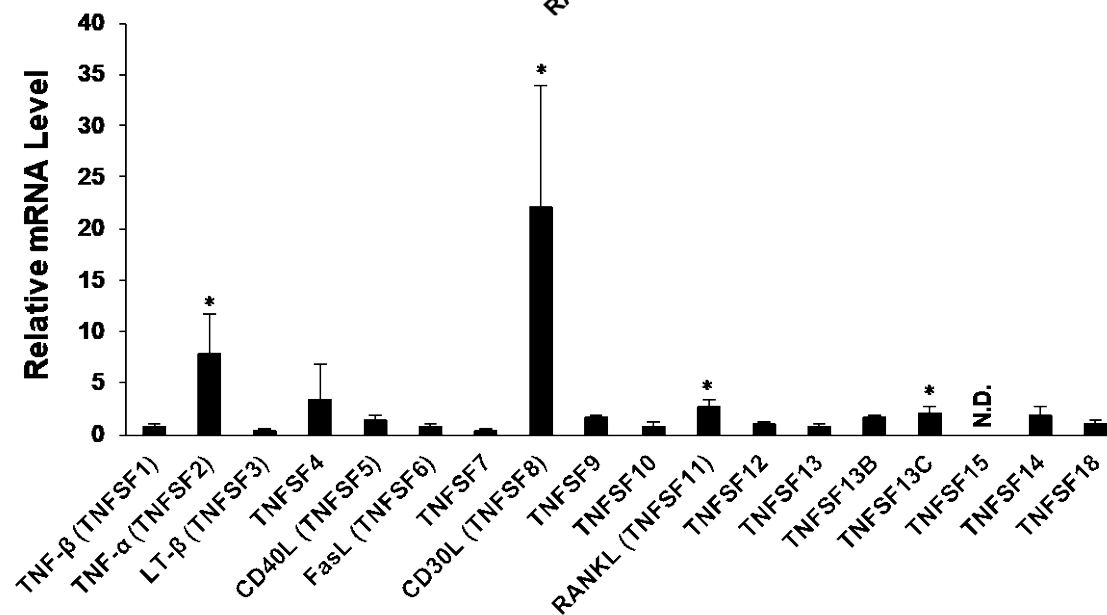


Quantitative PCR

Cerebrum

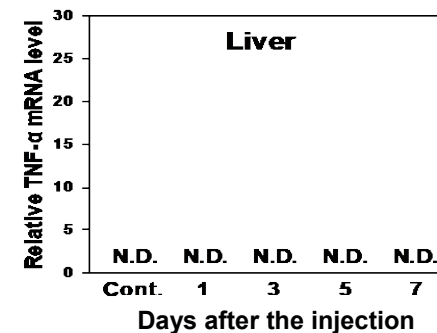
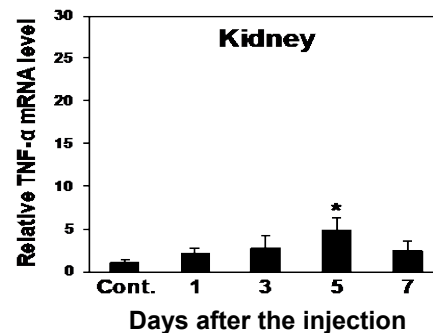
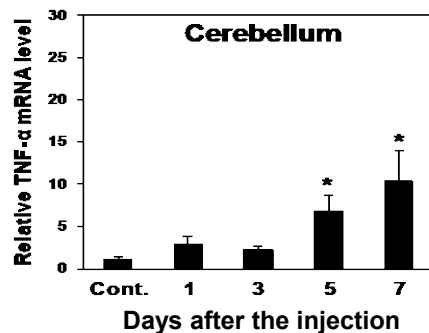
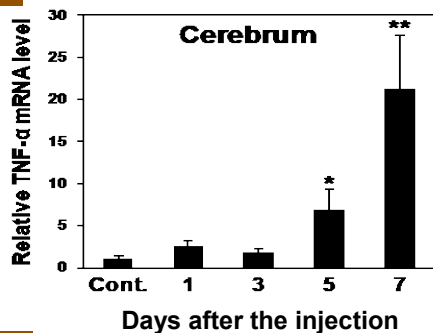


Cerebellum

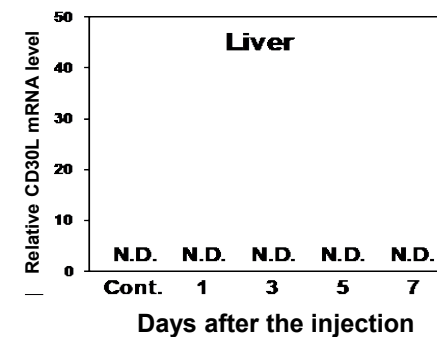
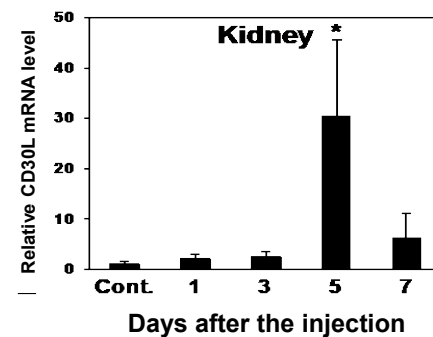
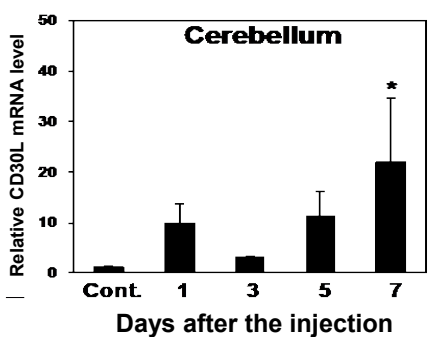
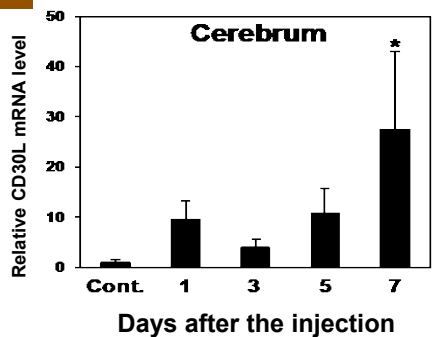


Effects of MeHg on the levels of TNF- α and CD30L mRNA in different mouse tissues

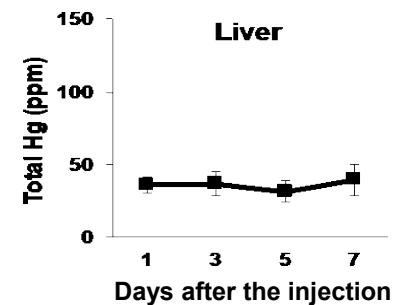
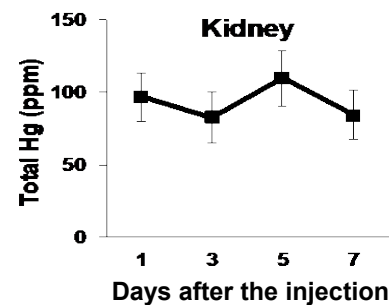
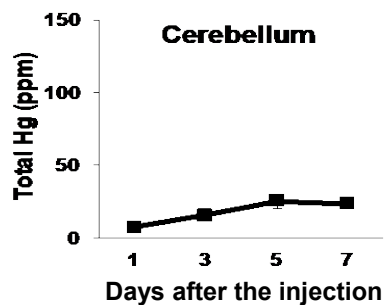
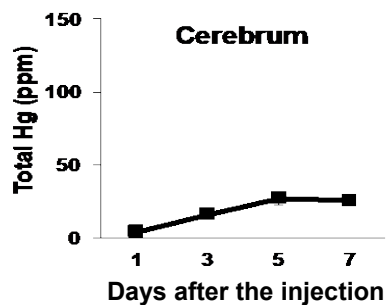
TNF- α



CD30L



Mercury concentration



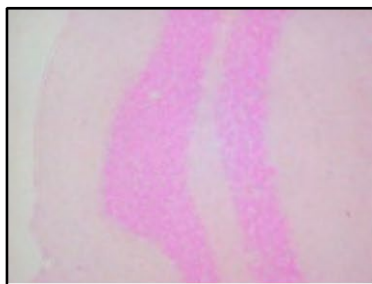
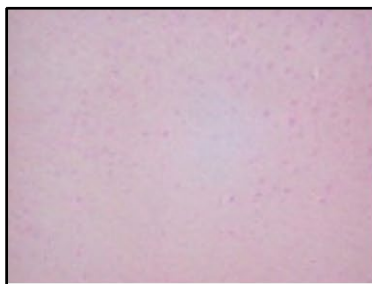
TNF- α expressing cells in the brains of mice treated with MeHg

TNF- α mRNA

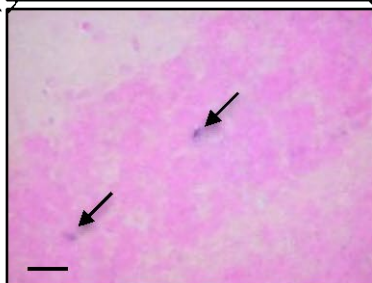
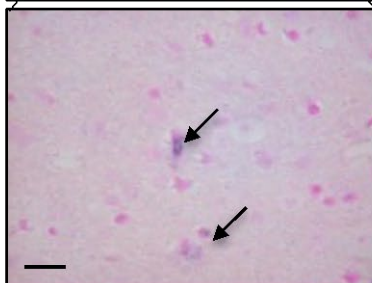
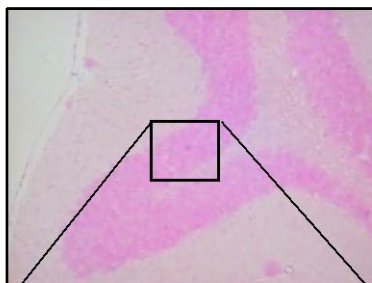
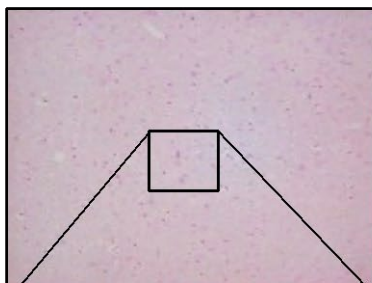
Cerebrum
cortex

Cerebellum

Vehicle



MeHgCl (7 days)



GFAP & Iba1

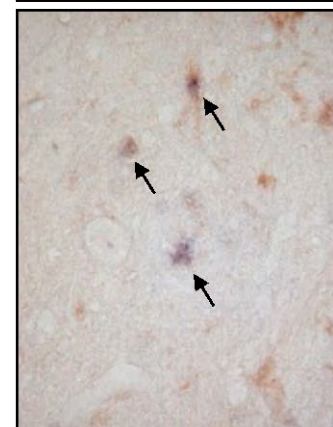
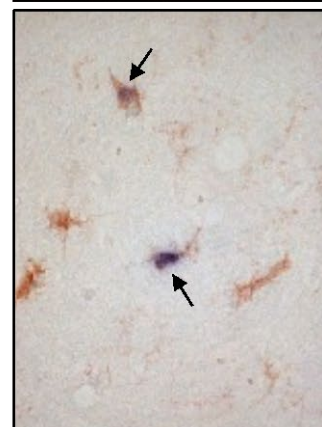
Cerebrum
cortex

Cerebellum

TNF- α (ISH) &
GFAP (IHC)



TNF- α (ISH) &
Iba1 (IHC)

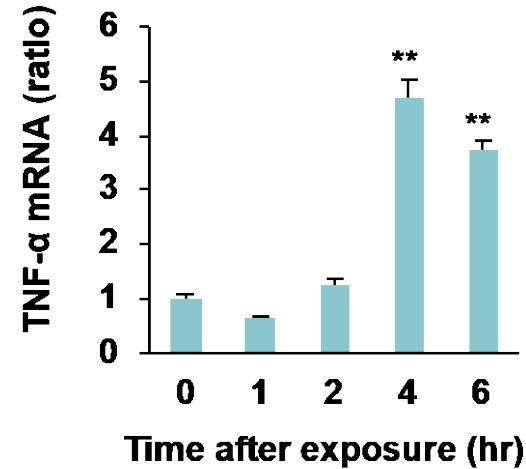
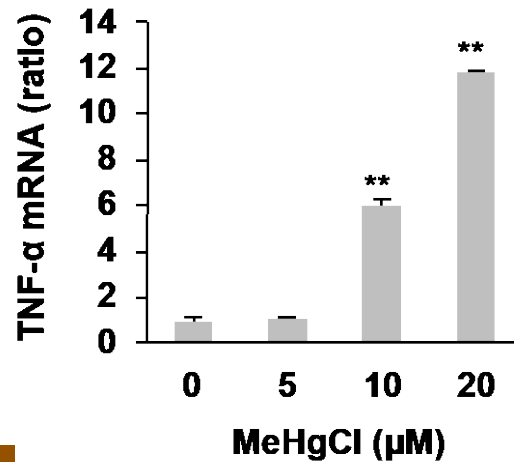


GFAP: Astrocyte marker

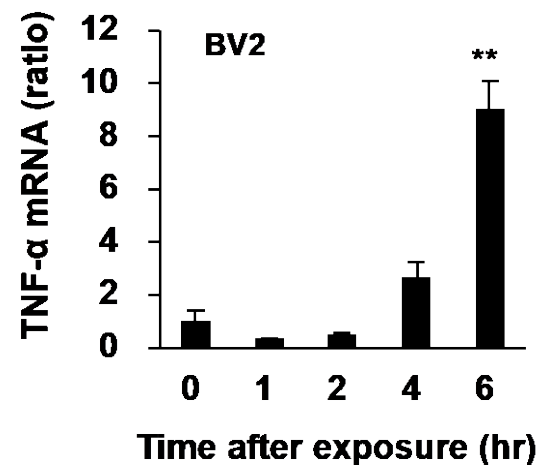
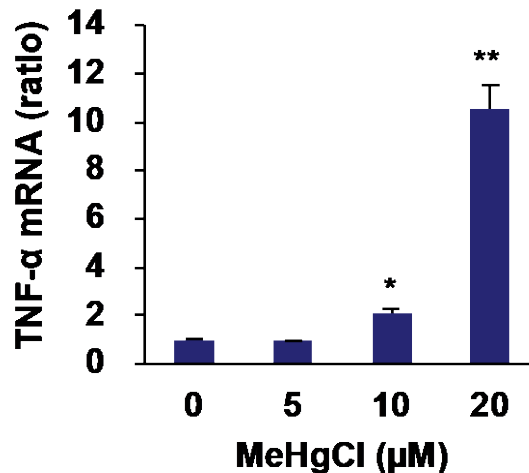
Iba1: Microglia marker

Effects of MeHg on TNF- α expression in primary microglia and BV2 cells

Primary microglia

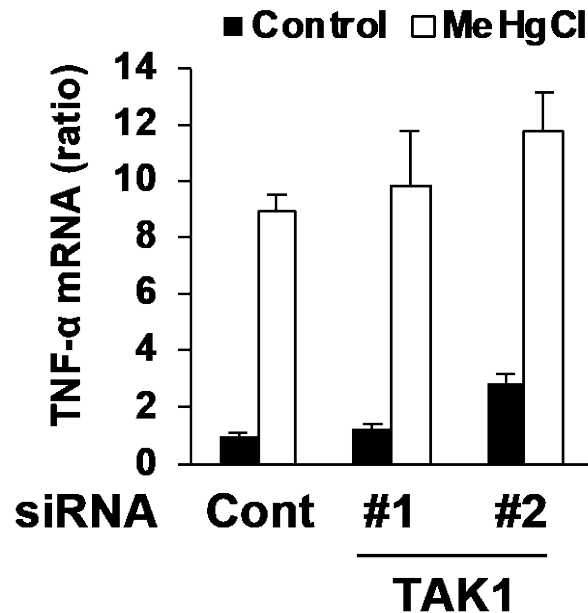
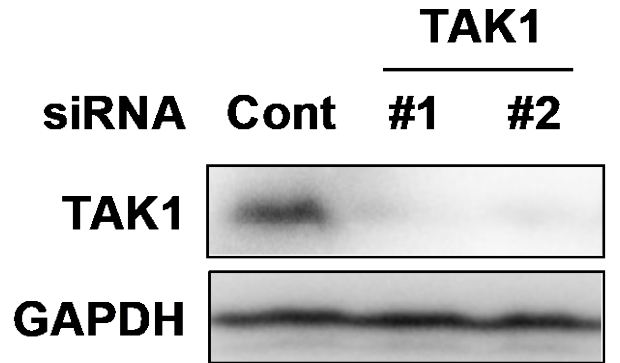


BV2 cells

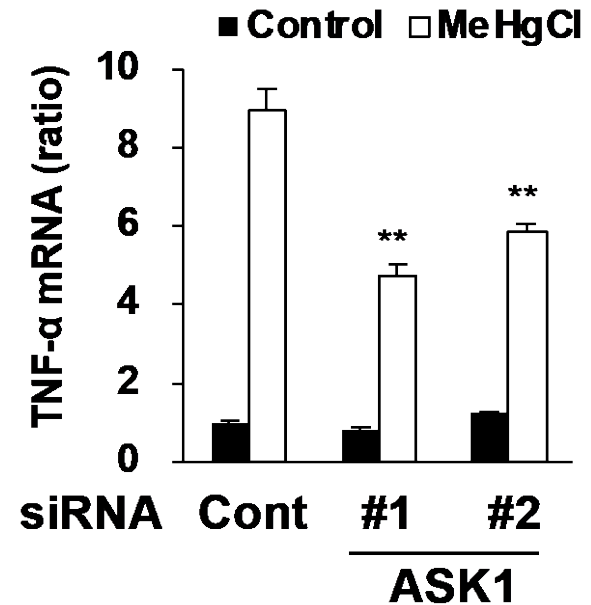
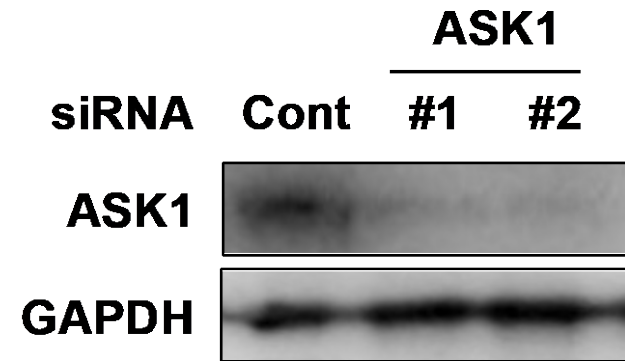


Involvement of MAP kinase pathways in MeHg-induced TNF- α expression in BV2 cells

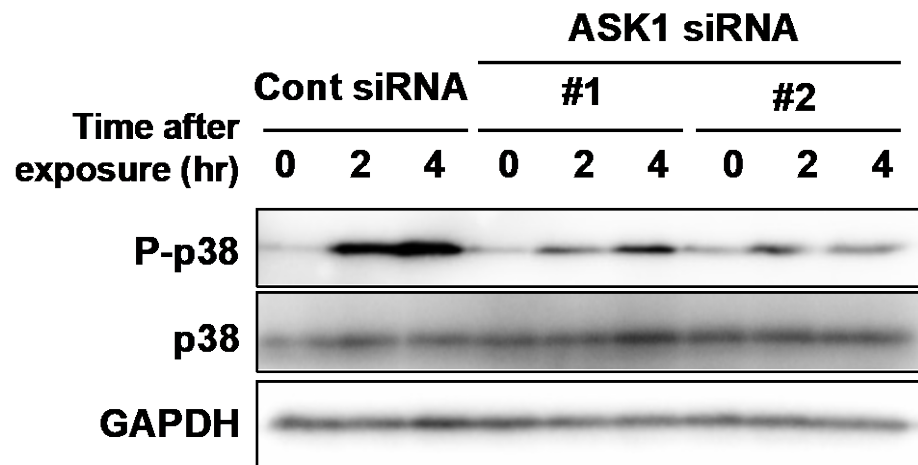
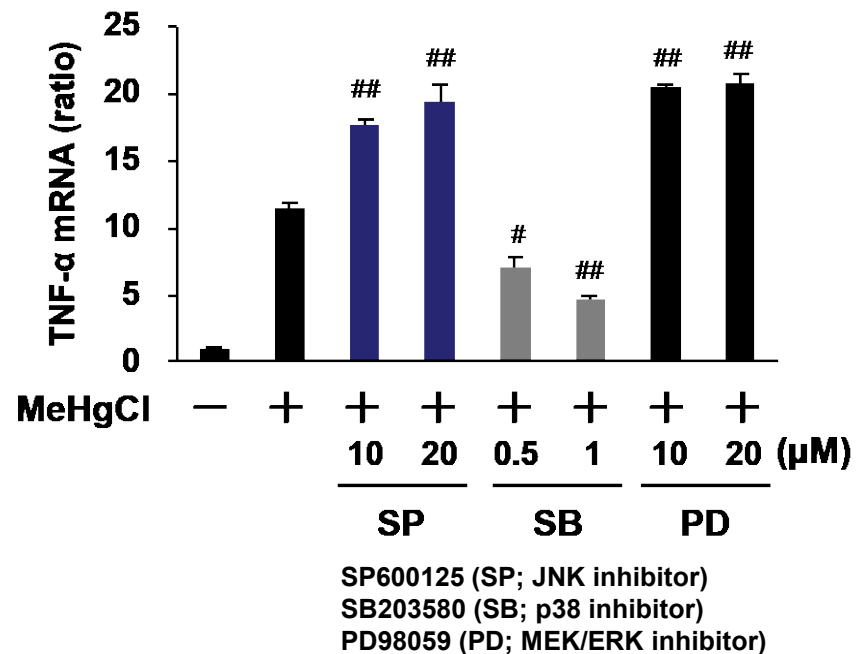
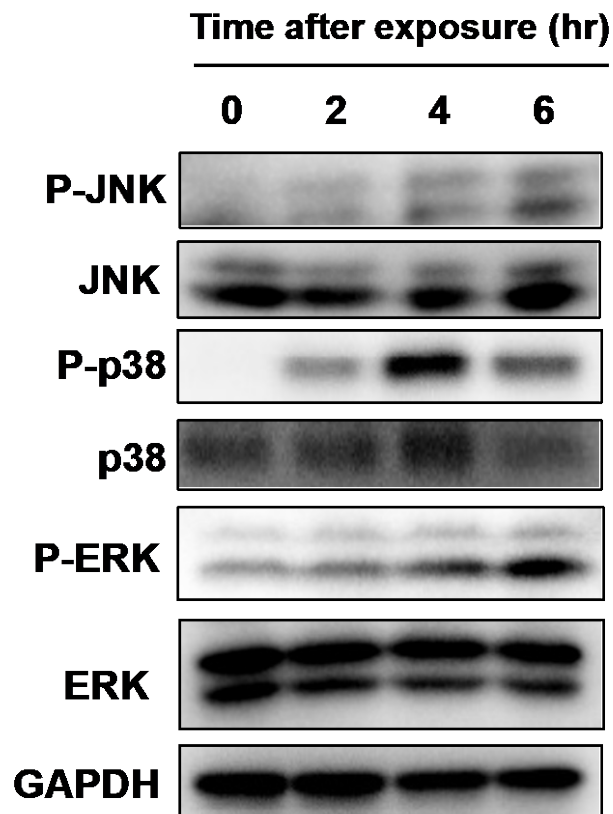
TAK1



ASK1

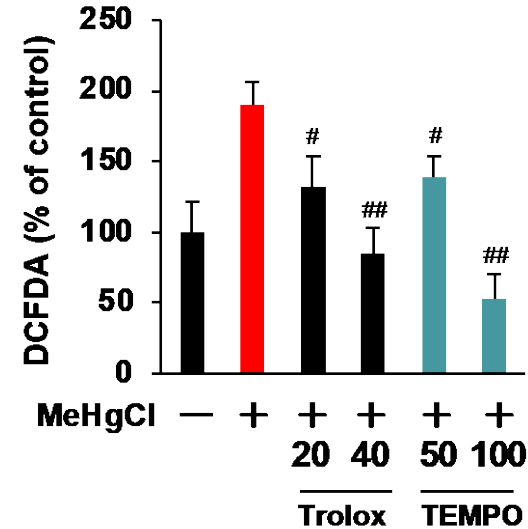
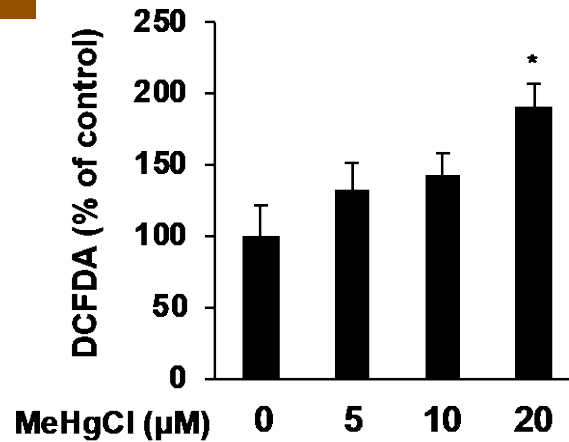


Involvement of MAP kinase pathways in MeHg-induced TNF- α expression in BV2 cells

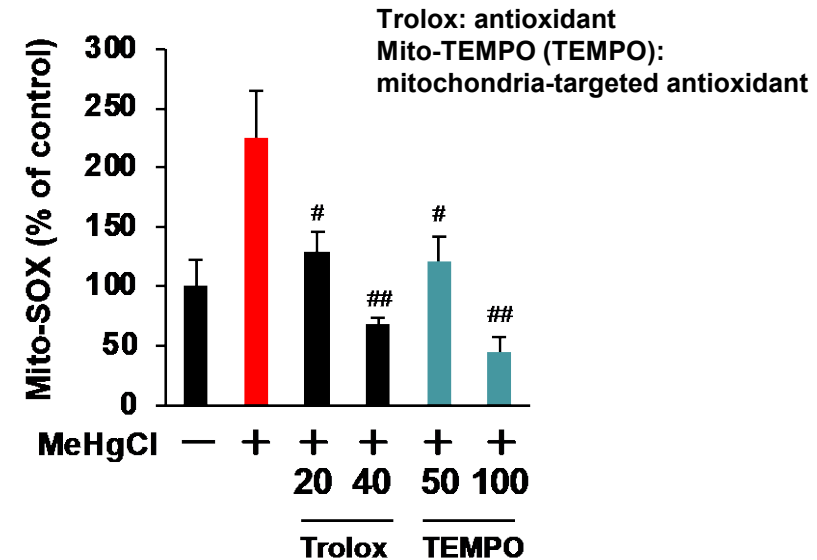
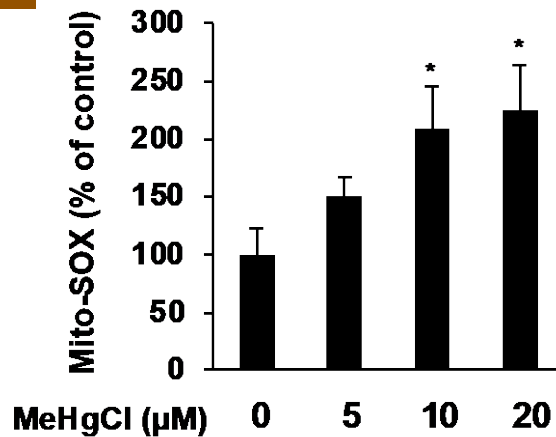


Effects of antioxidants on the induction of ROS by MeHg in BV2 cells

Intracellular ROS

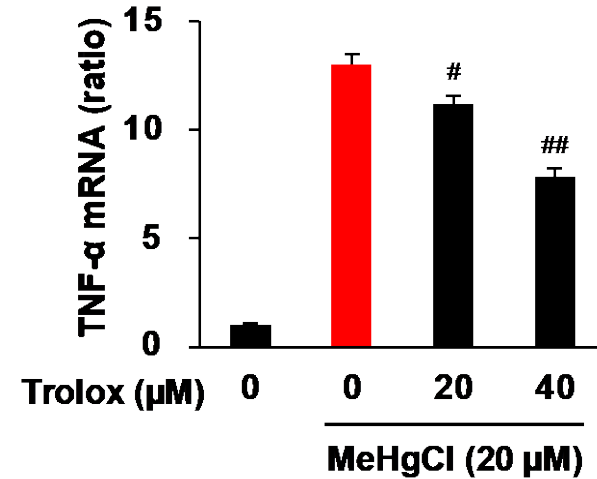
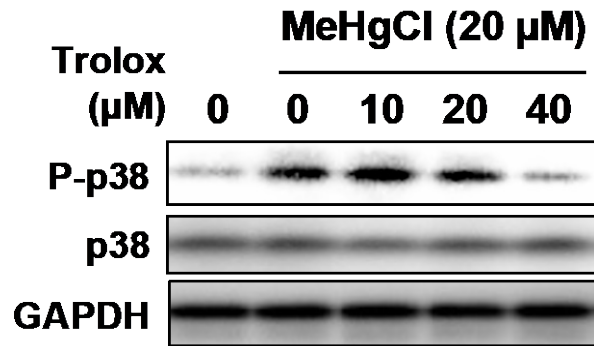


Mitochondrial ROS

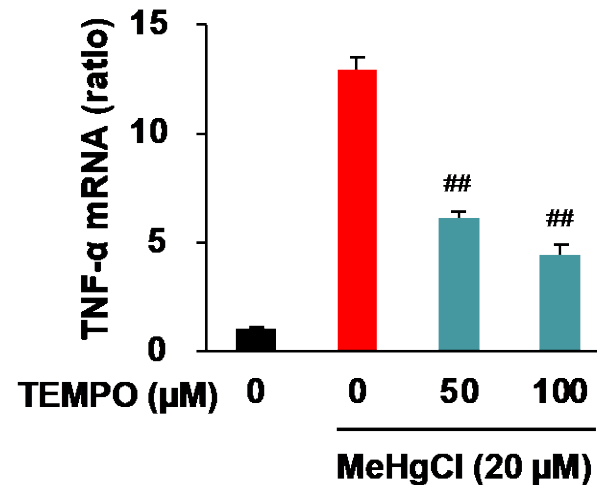
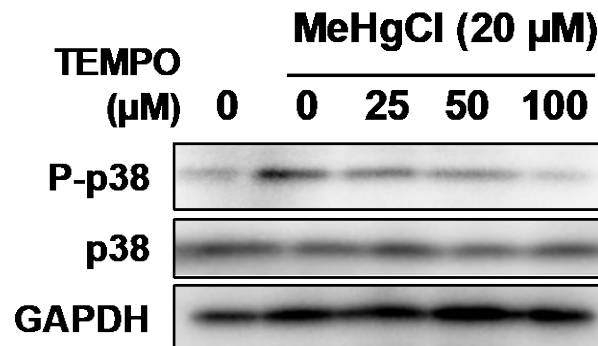


Effects of antioxidants on the induction of TNF- α expression by MeHg via the ASK1/p38 pathway in BV2 cells

Intracellular ROS



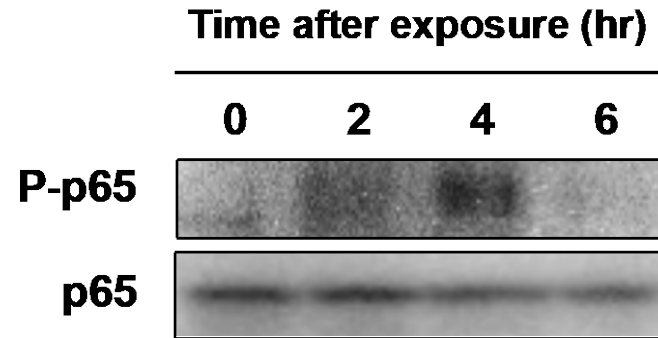
Mitochondrial ROS



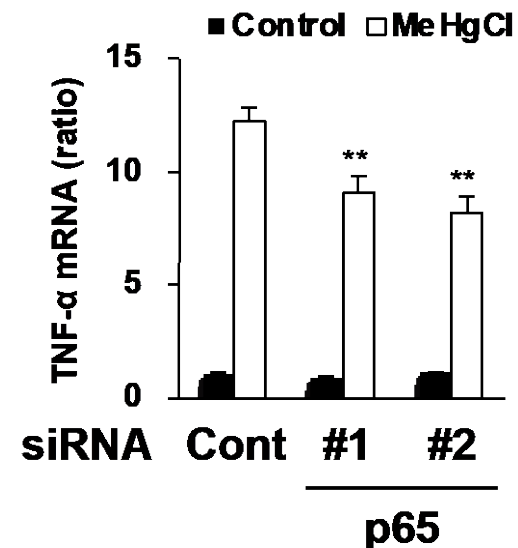
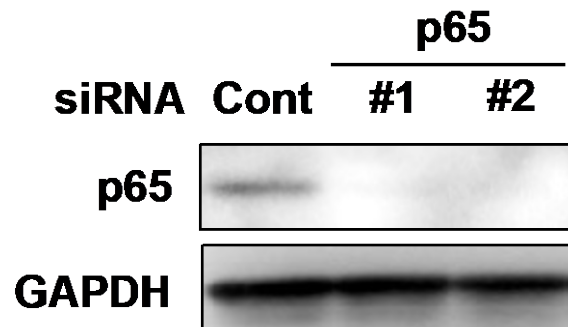
Trolox: antioxidant
Mito-TEMPO (TEMPO): mitochondria-targeted antioxidant

Involvement of NF- κ B activation in MeHg-induced TNF- α expression in BV2 cells

NF- κ B activation

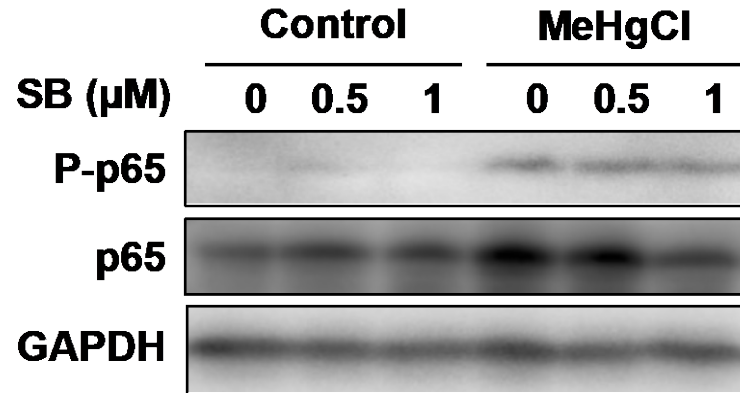


Knockdown effect



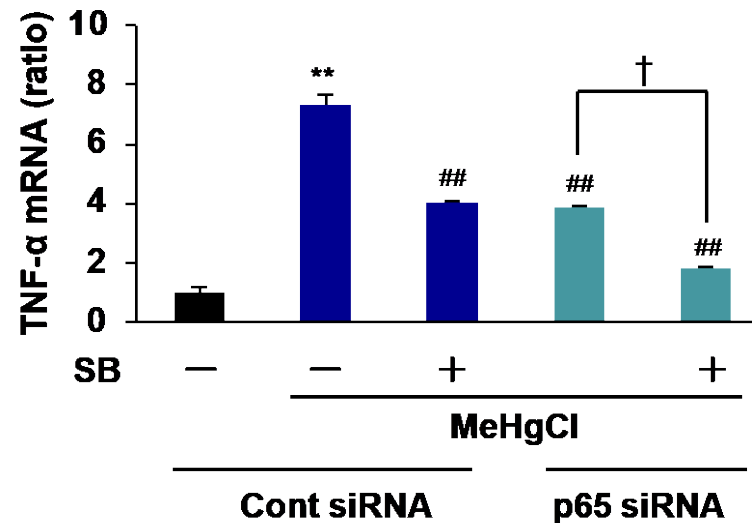
Involvement of NF- κ B in MeHg-induced TNF- α expression via p38 in BV2 cells

NF- κ B activation



SB203580
(SB; p38 inhibitor)

Knockdown effect

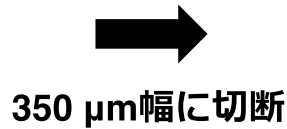


Morphology of microglia in the cerebral cortices of adult mice and the brain slice cultures

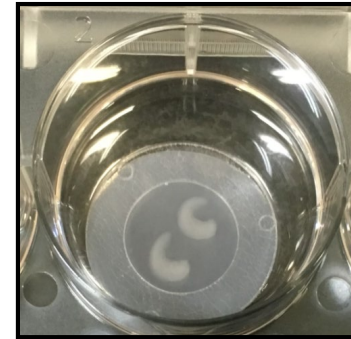
生後7日



大脳皮質を採取



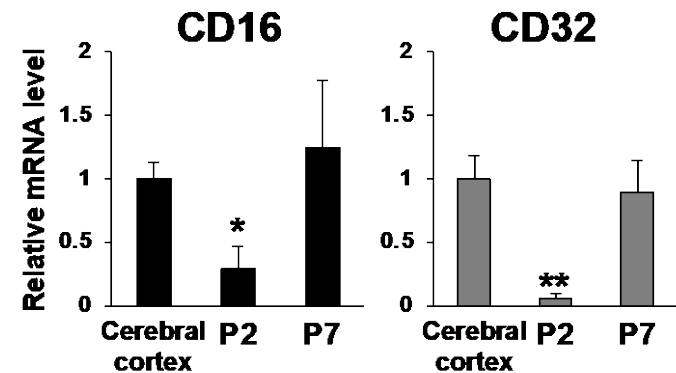
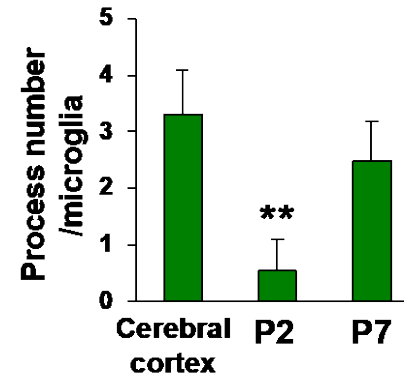
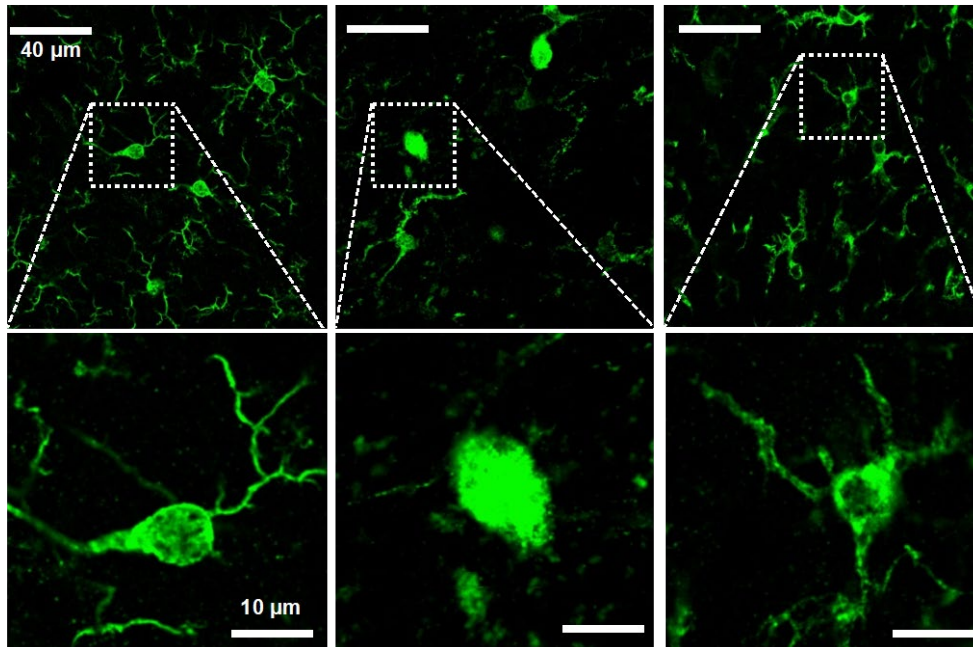
培養



Cerebral cortex

P2

P7

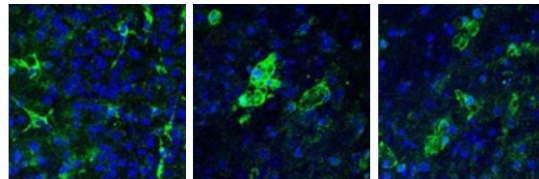


Role of microglial activation in MeHg-induced neuronal cell death using the brain slice cultures

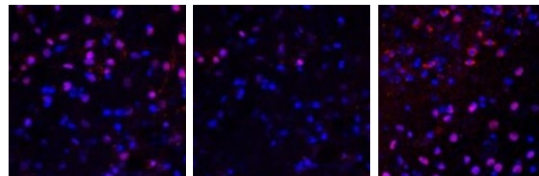
TNF- α inhibitor

WP9QY	-	-	+
MeHgCl	-	+	+

Iba1



NeuN



WP9QY	-	-	+
MeHgCl	-	+	+

NeuN



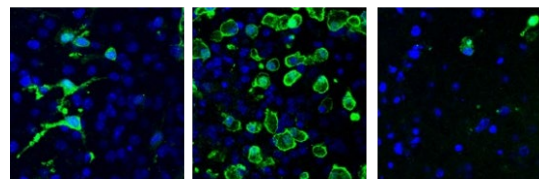
GAPDH



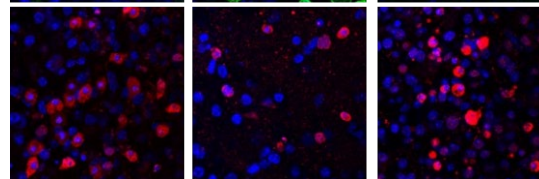
Microglia removal

Clo-lip	-	-	+
MeHgCl	-	+	+

Iba1

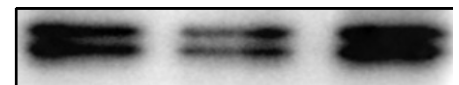


NeuN

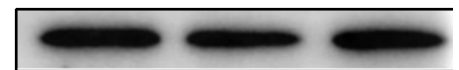


Clo-lip	-	-	+
MeHgCl	-	+	+

NeuN



GAPDH



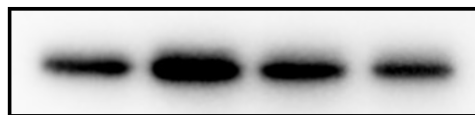
Role of p38 activation in MeHg-induced neuronal cell death using the brain slice cultures

Microglia removal

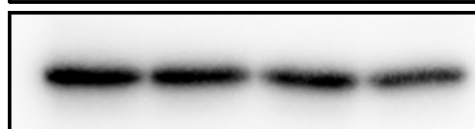
Clo-lip (μM) 0 0 100 200

MeHgCl - + + +

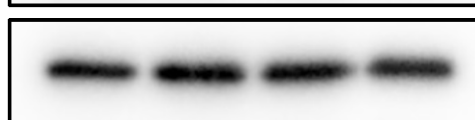
P-p38



p38



GAPDH

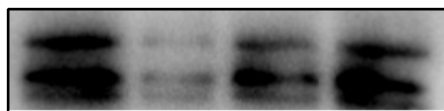


p38 inhibitor

SB203580 0 0 1 2

MeHgCl - + + +

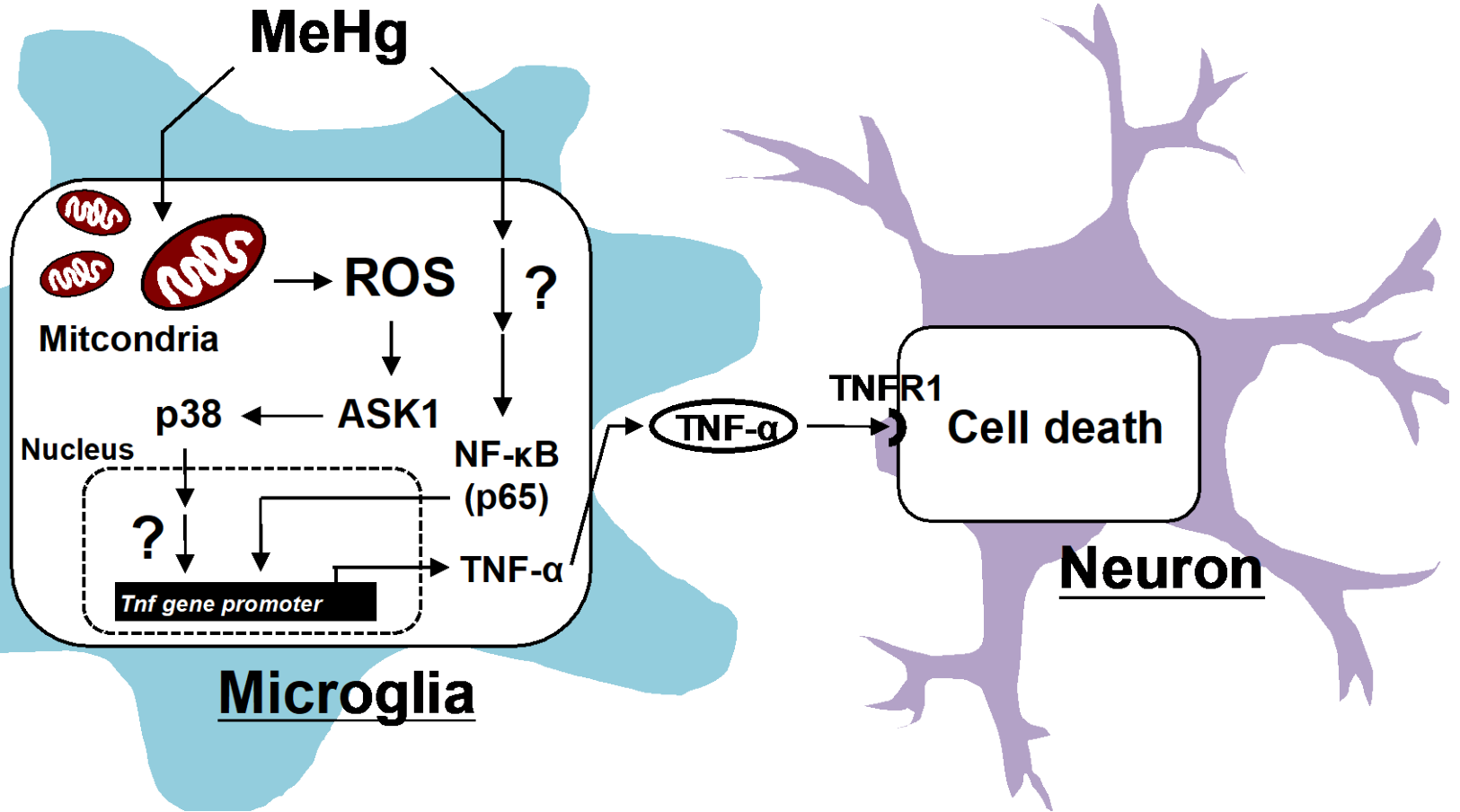
NeuN



GAPDH



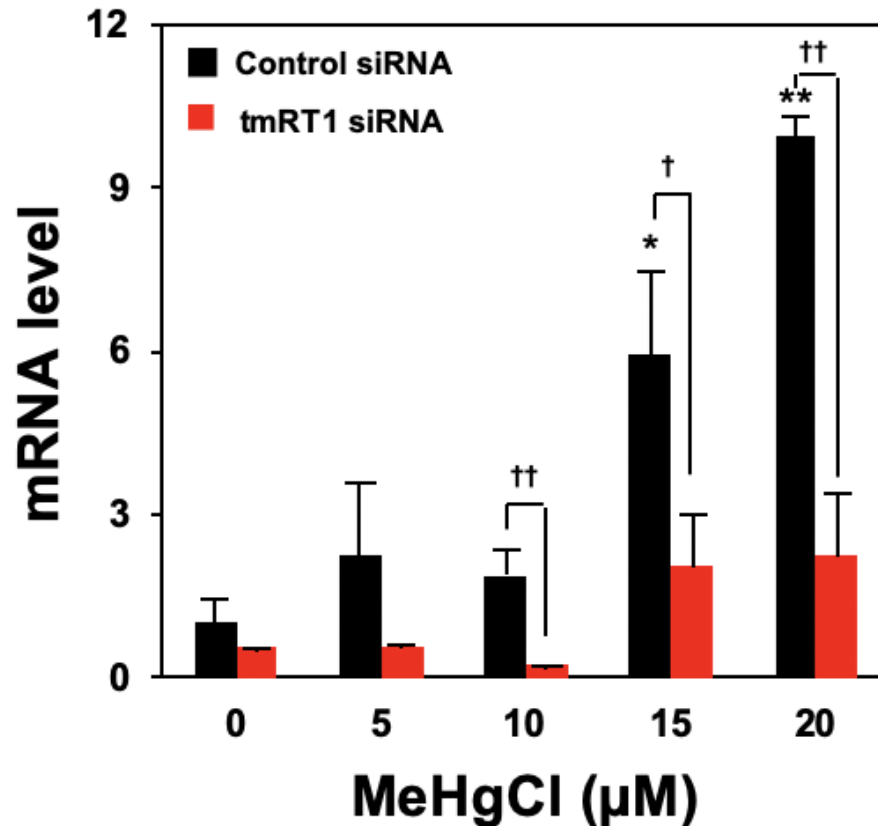
Summary_1



Effects of tmRT1 knockdown on the induction of oncostatin M expression by MeHg

Oncostatin M (OSM): interleukin (IL)-6 family cytokine

tmRT1: TNF- α related methylmercury-responsive transcription factor 1

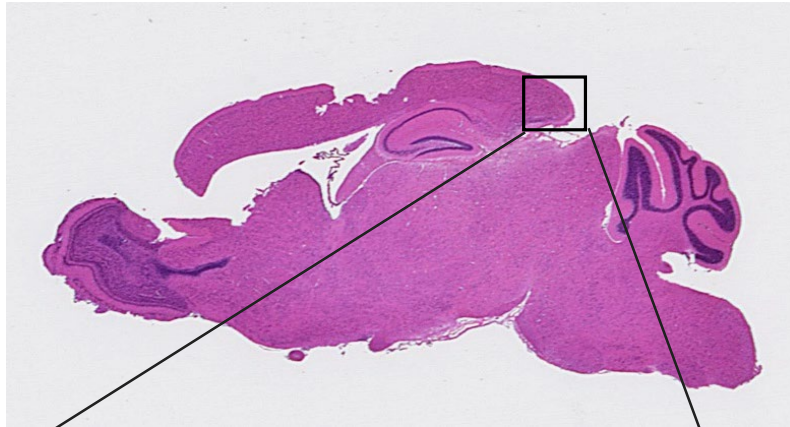


* $p < 0.05$ vs. control, ** $p < 0.01$ vs. control

† $p < 0.05$ vs. control siRNA, †† $p < 0.01$ vs. control siRNA

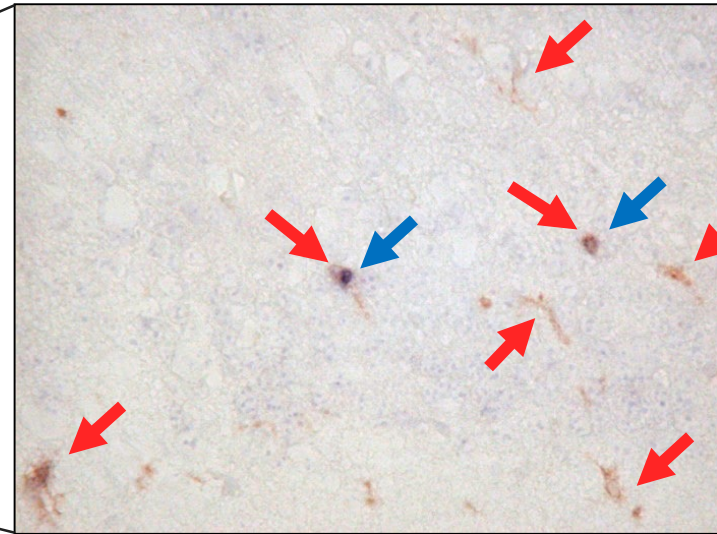
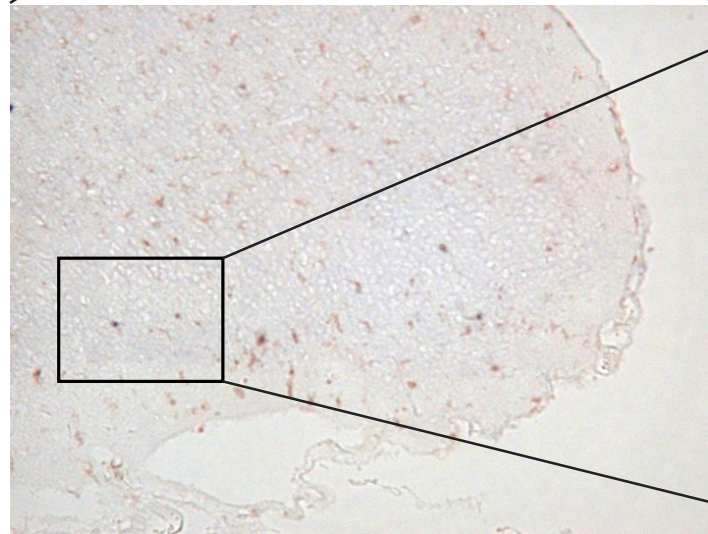
Oncostatin M expressing cells in the brains of mice treated with MeHg

OSM & Iba1



In situ hybridization
and immunostaining

→ : OSM
→ : Iba1
(Microglia Marker)

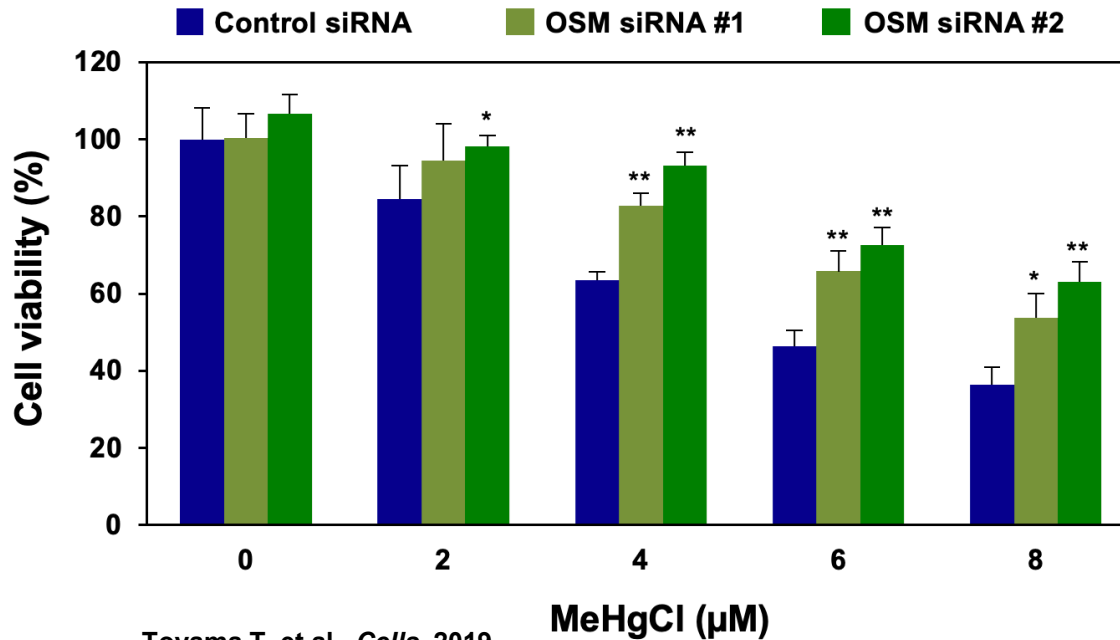


- マウス脳内でのメチル水銀によるOSMの発現誘導に主に免疫細胞であるミクログリアが関与している

Effects of oncostatin M knockdown on cytotoxicity caused by MeHg

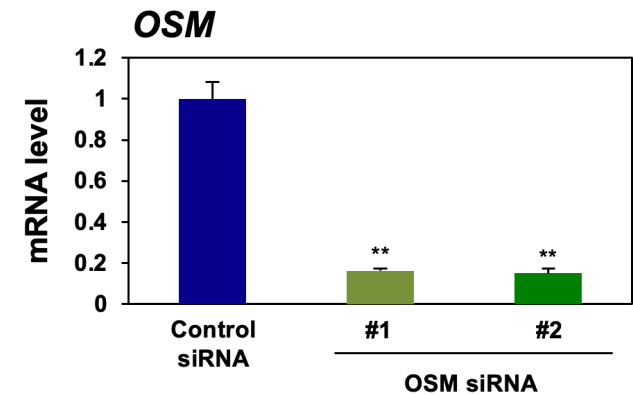
Oncostatin M (OSM): interleukin (IL)-6 family cytokine

生存率



Toyama T. et al., *Cells*, 2019

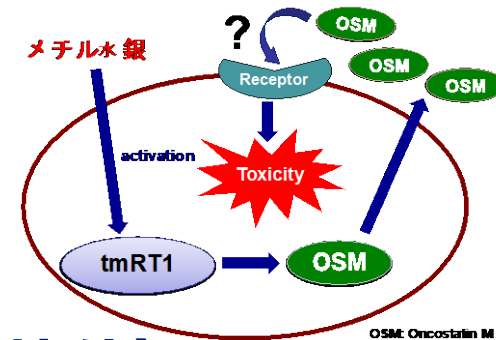
発現抑制の確認



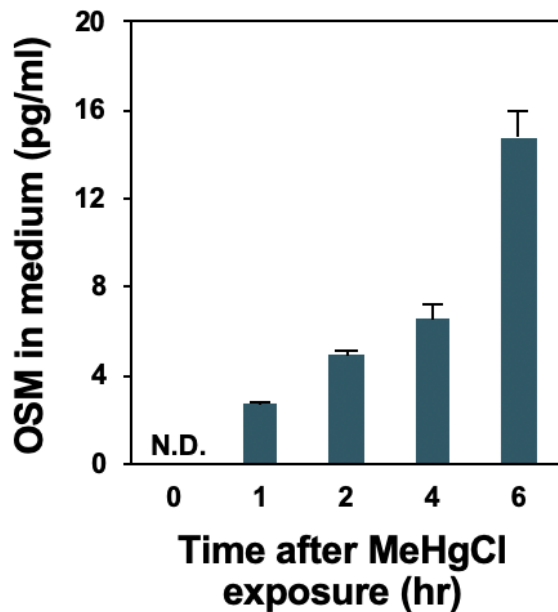
* p < 0.05 vs. control siRNA, ** p < 0.01 vs. control siRNA

Role of oncostatin M in MeHg toxicity

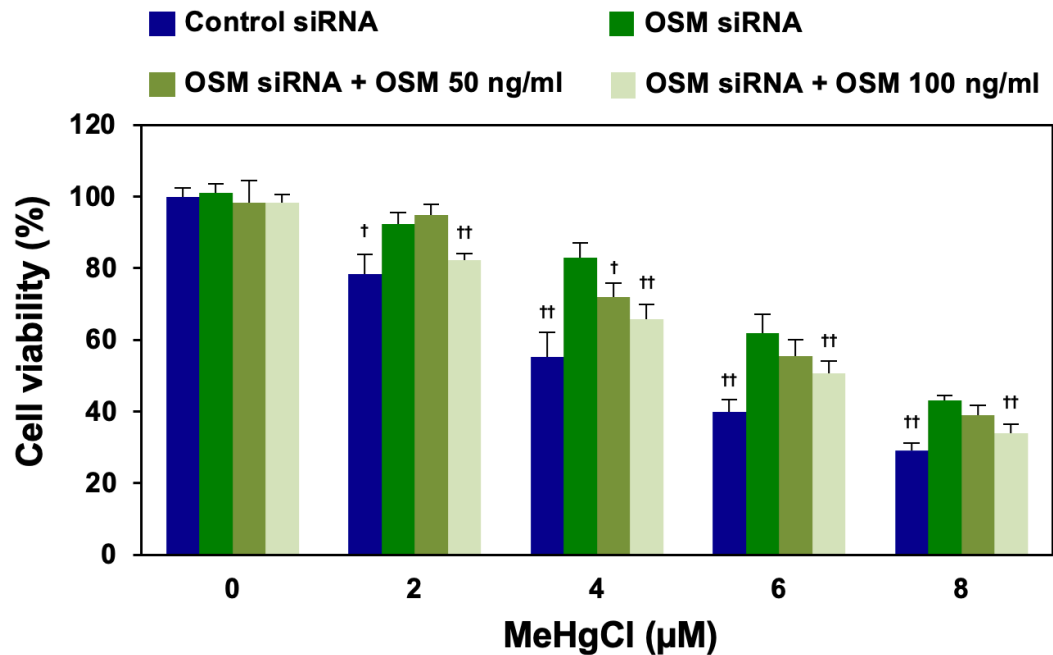
Oncostatin M (OSM): interleukin (IL)-6 family cytokine



培地に放出された OSM量

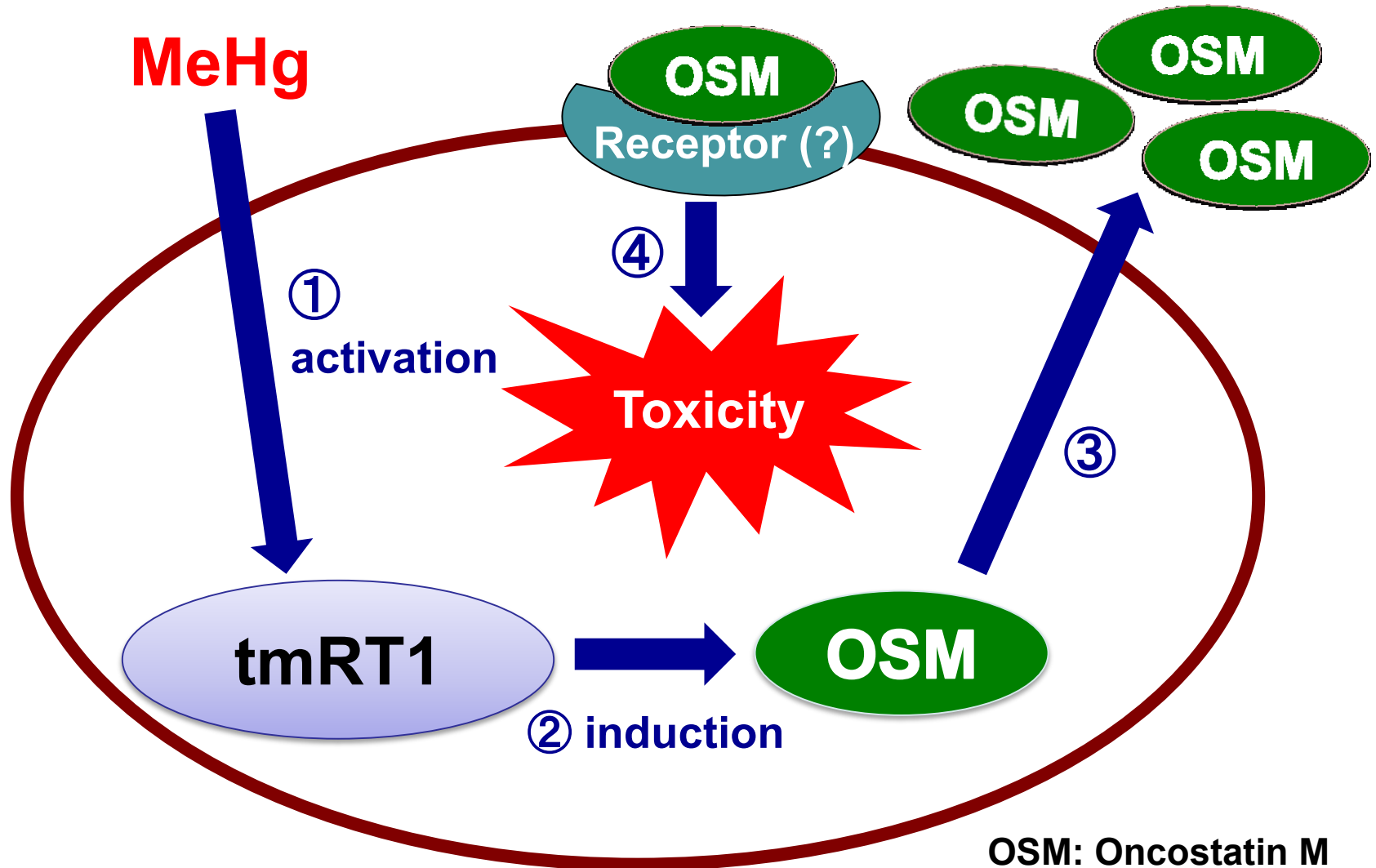


生存率 (OSM中和抗体)

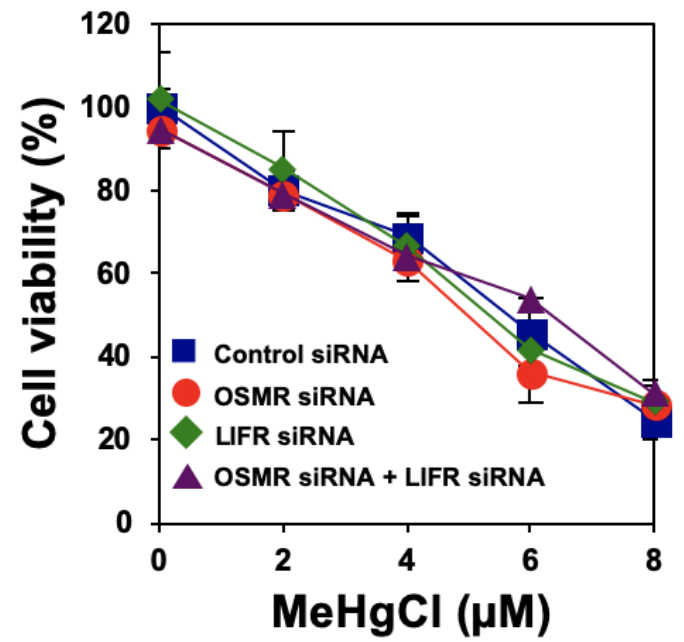
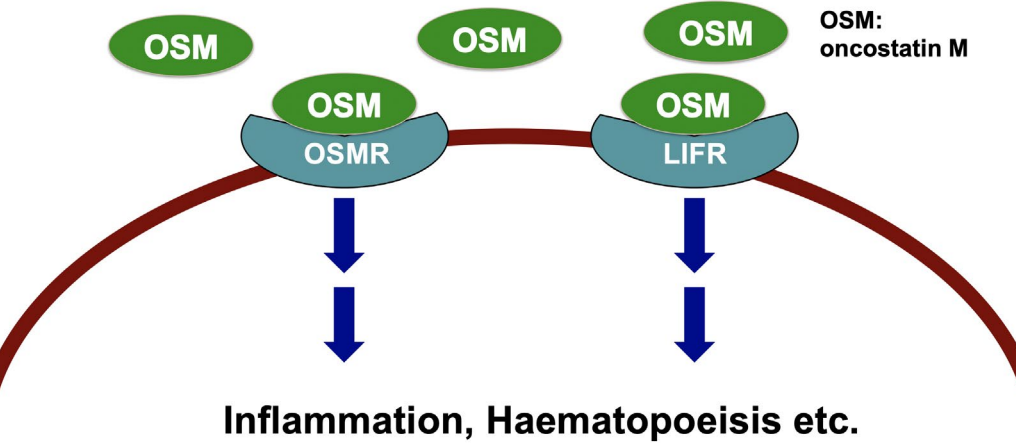


* $p < 0.05$ vs. control siRNA, ** $p < 0.01$ vs. control siRNA
 † $p < 0.05$ vs. MeHgCl-treated control siRNA
 †† $p < 0.01$ vs. MeHgCl-treated OSM siRNA. N.D. indicates not detected.

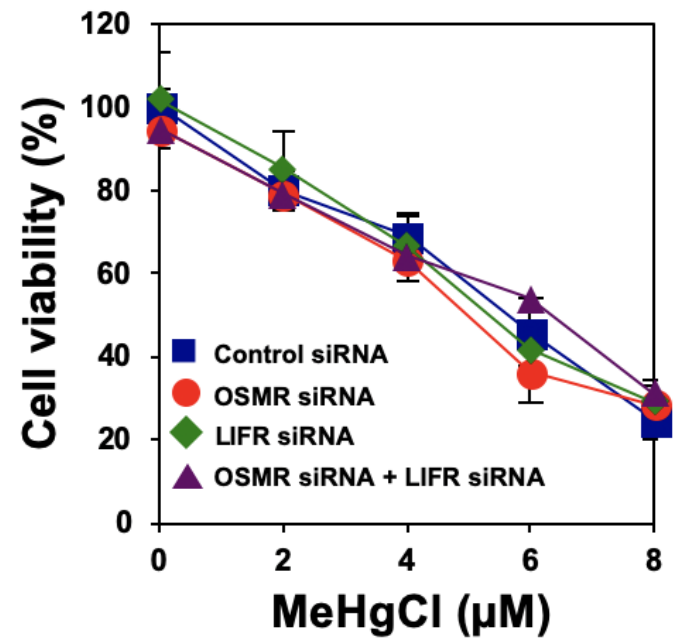
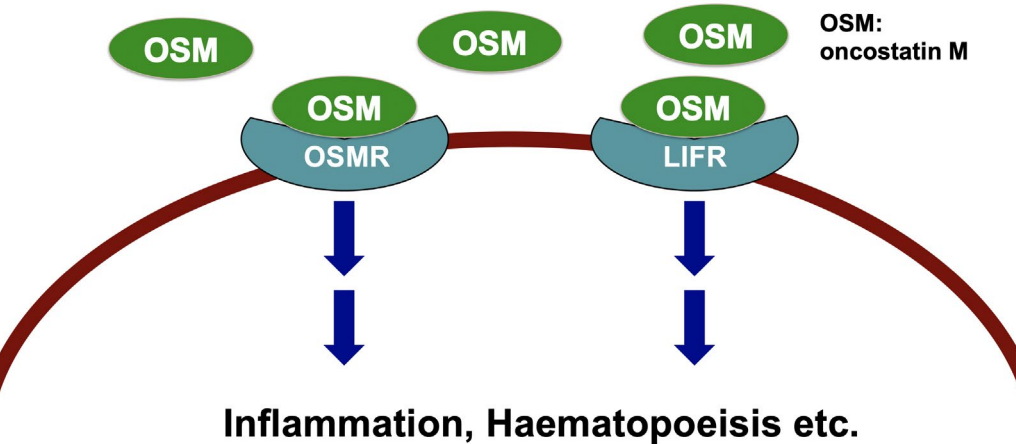
Mechanisms involved in oncostatin M-mediated MeHg toxicity



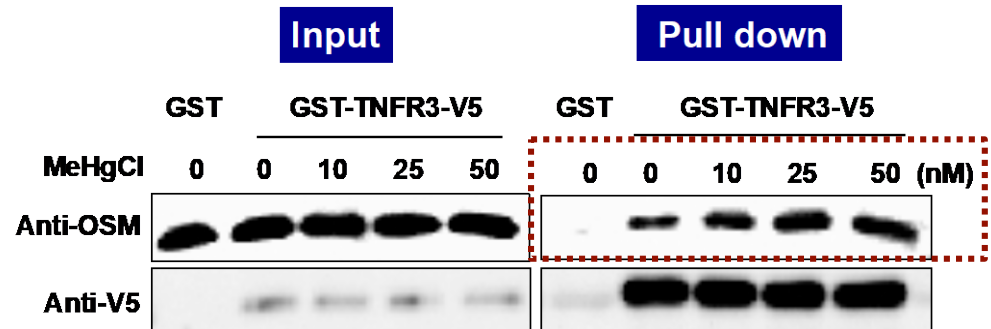
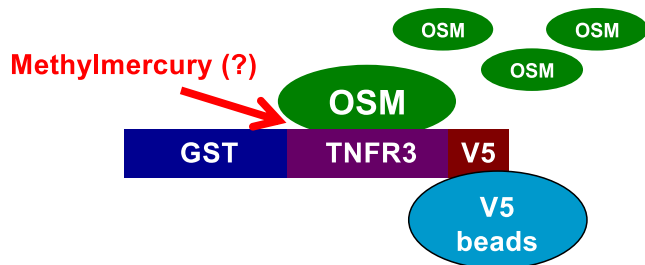
OSMによるシグナル伝達



OSMによるシグナル伝達



TNFR3: TNF receptor 3



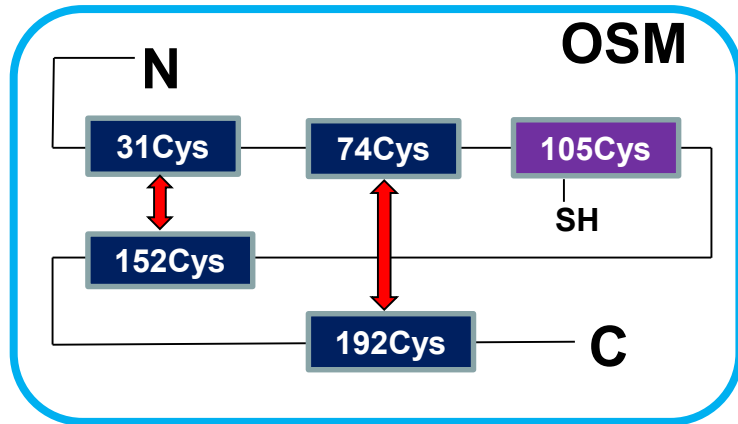
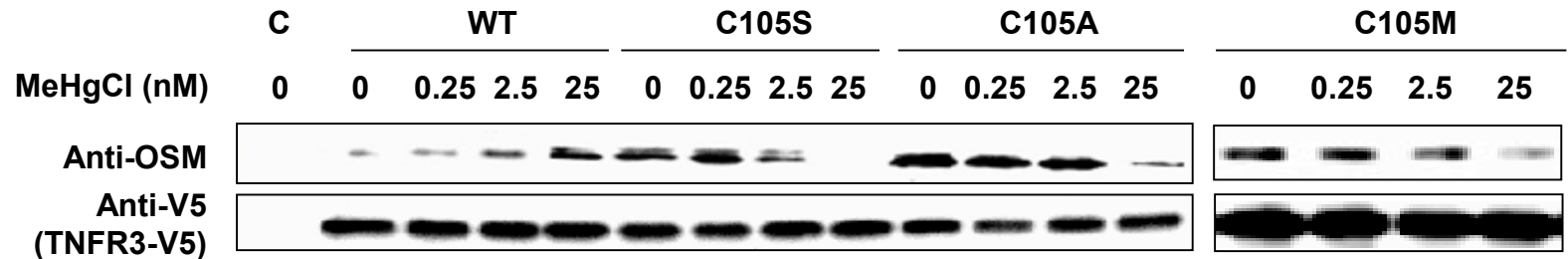
* メチル水銀は、OSMのCys105に結合する

- メチル水銀は、OSMのCys105に結合することでTNFR3との結合を促進する

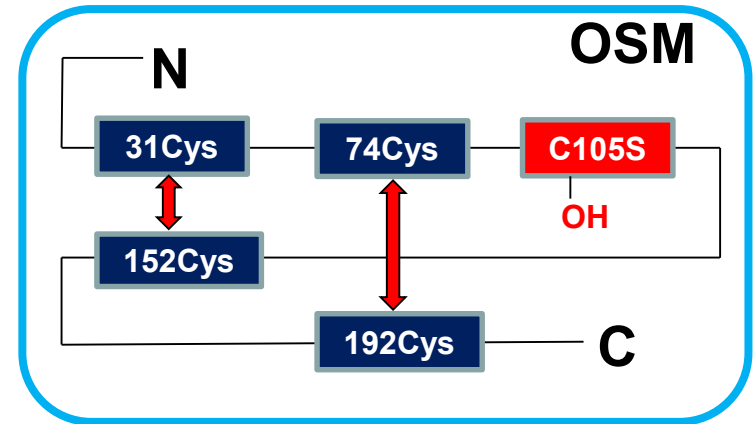
Effects of mutation of cysteine 105 in oncostatin M on binding to TNFR3

C105A: システインをアラニンに置換した変異体
C105S: システインをセリンに置換した変異体
C105M: システインをメチオニンに置換した変異体

C: Control
WT: Wild-type



TNFR3



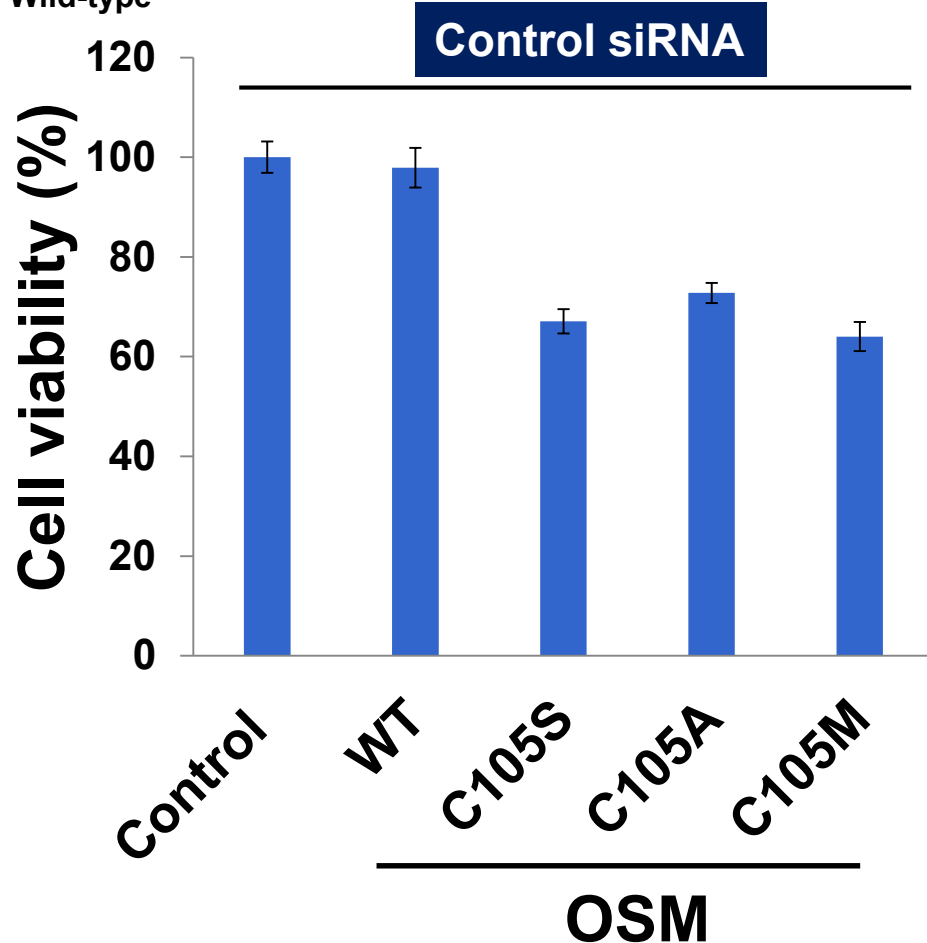
TNFR3

Effects of addition of OSM cysteine mutants to the culture medium on cell proliferation

C105A: シス테인をアラニンに置換した変異体
C105S: シス테인をセリンに置換した変異体
C105M: シス테인をメチオニンに置換した変異体

メチル水銀非存在下

WT: Wild-type

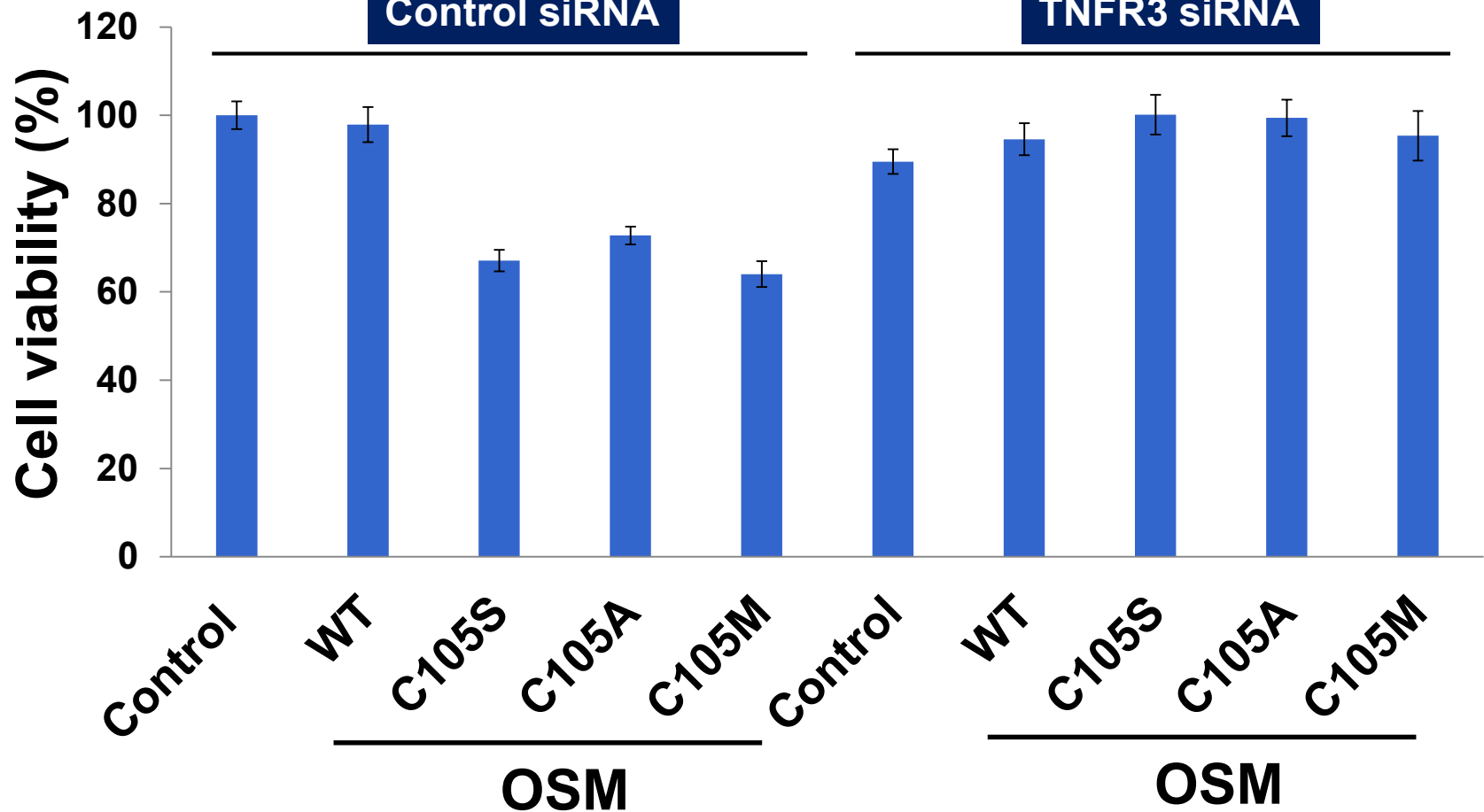


Effects of addition of OSM cysteine mutants to the culture medium on cell proliferation

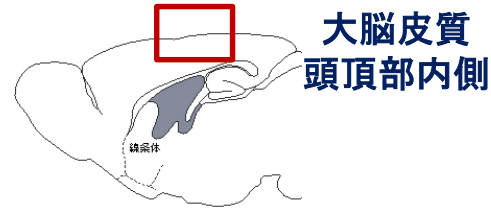
C105A: システインをアラニンに置換した変異体
C105S: システインをセリンに置換した変異体
C105M: システインをメチオニンに置換した変異体

メチル水銀非存在下

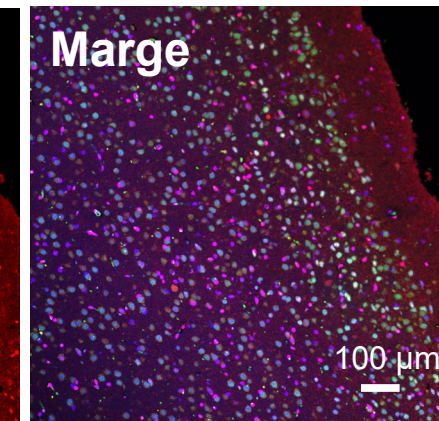
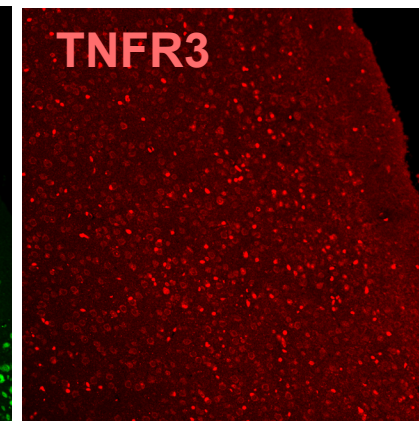
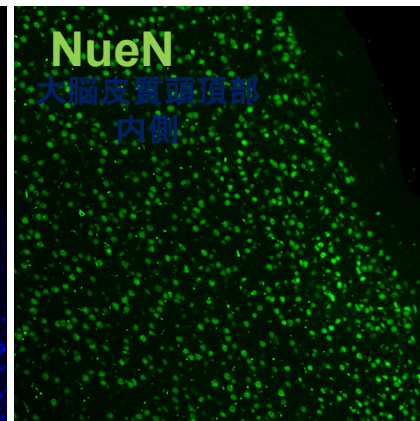
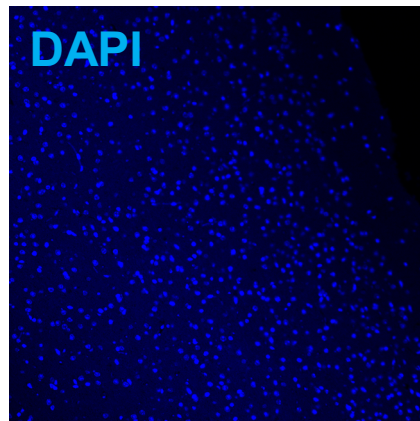
WT: Wild-type



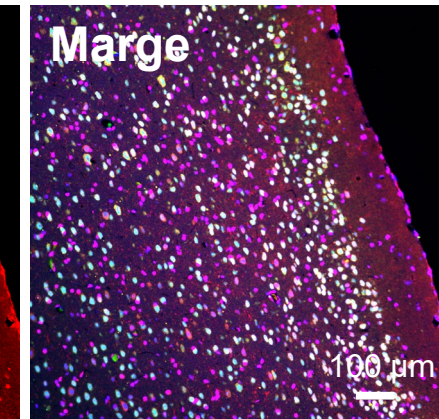
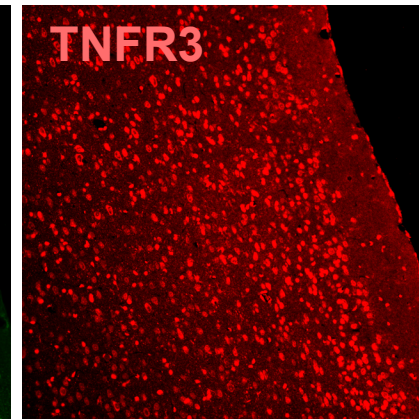
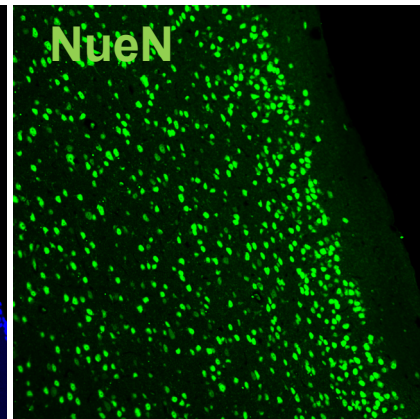
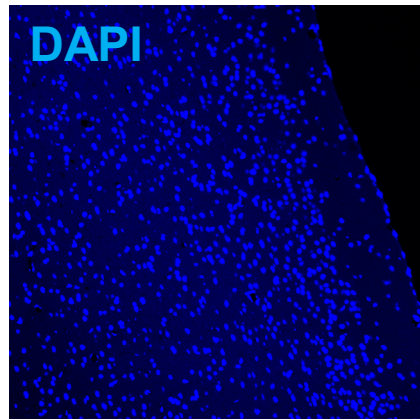
Effects of MeHg on TNFR3 expression in the brains of mice



Vehicle



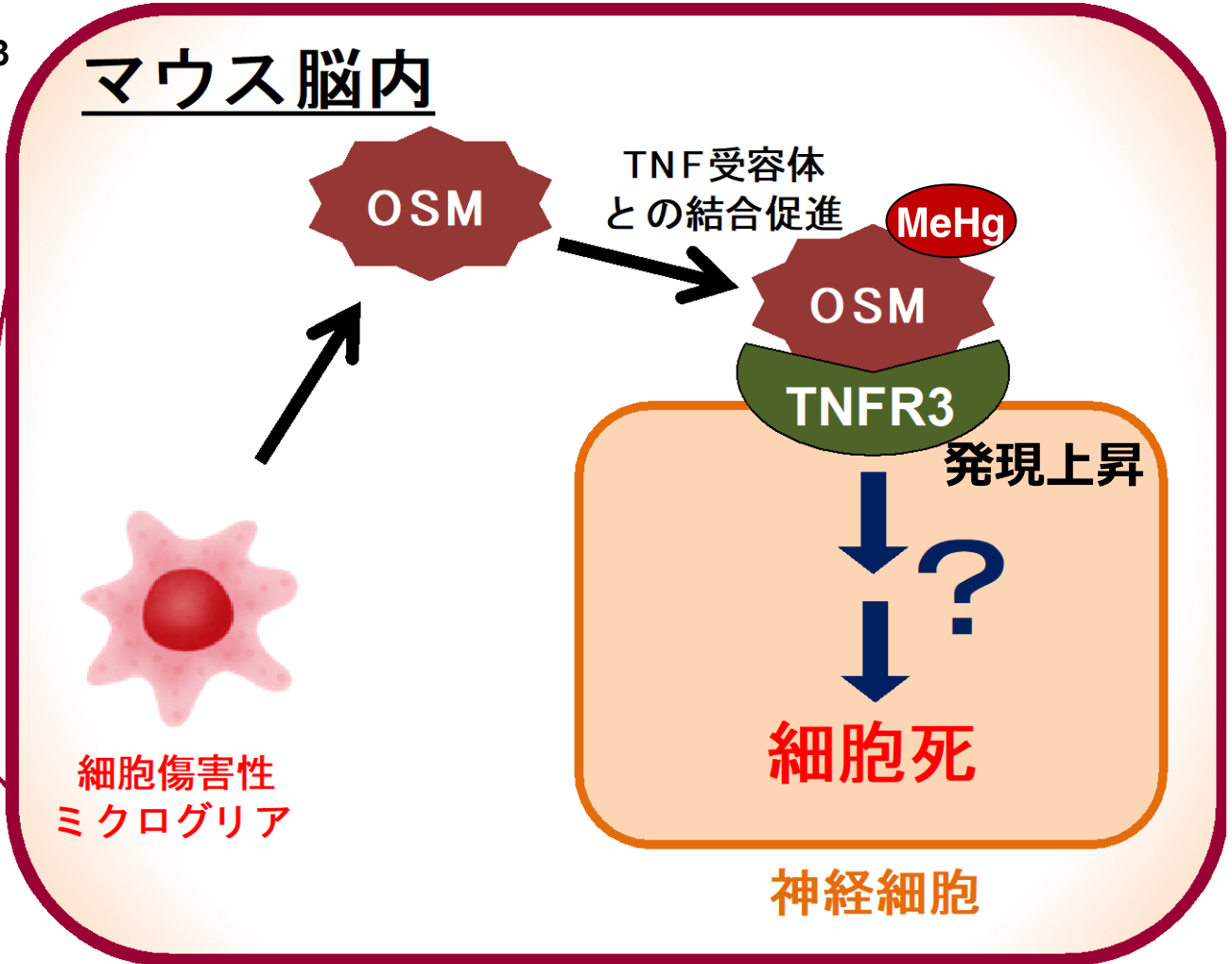
MeHg



DAPI: nuclear staining NeuN: neuronal marker

Summary_2

OSM: oncostatin M
TNFR3: TNF receptor 3



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2022.7.8

