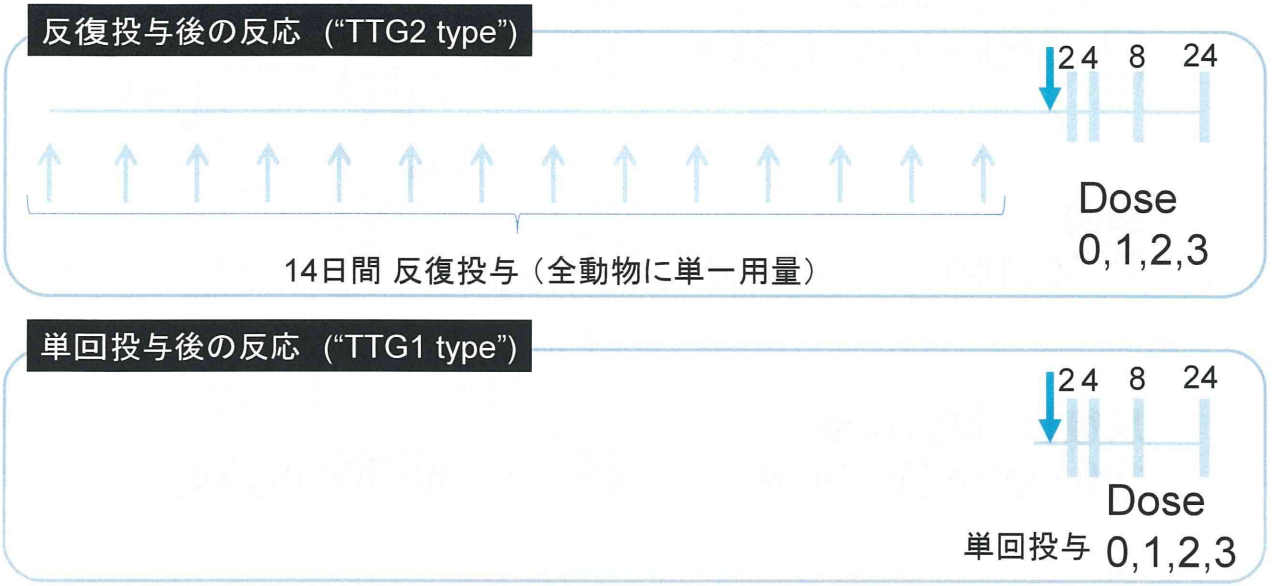
# 単回投与 vs 反復投与後の反応

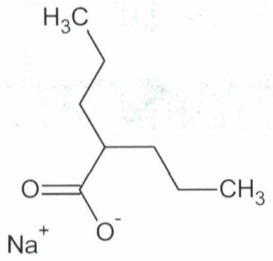
A+A’ protocol　バルプロ酸 と 四塩化炭素




## 前年度

A+A’ protocol 実験で示唆されたこと

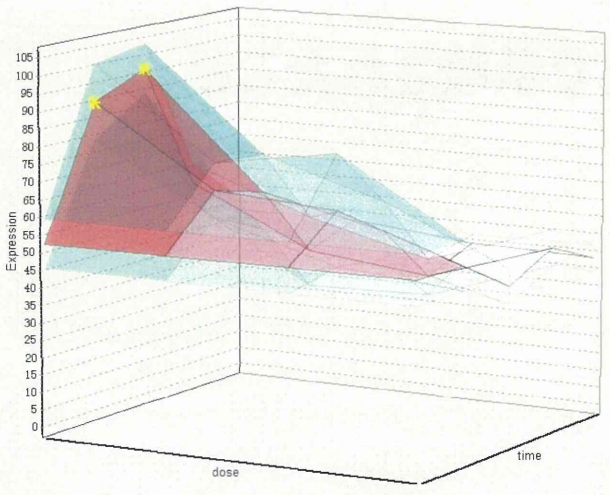
# バルプロ酸ナトリウム
## [Valproic acid sodium salt]




単回投与
[0, 50, 150, 500 mg/kg]

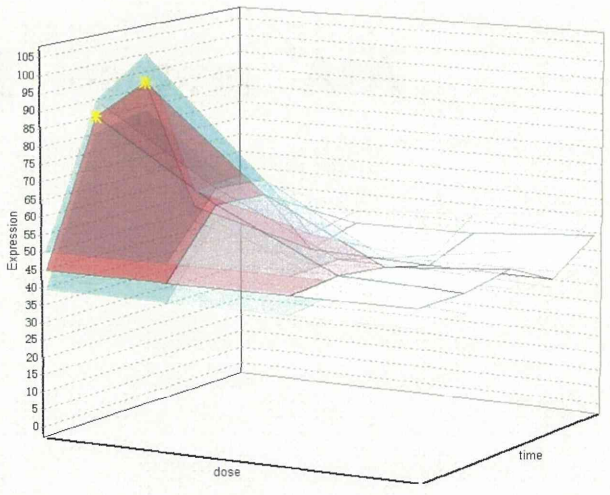
反復後　単回投与
100 mg/kg (for 14 days) +　[0, 50, 150, 500 mg/kg]

TTG157-L_SpNC_0　1423309_at　VPA 肝 単回




VPA single

TTG151-L_SpNC_0　1423309_at　VPA 肝 反復



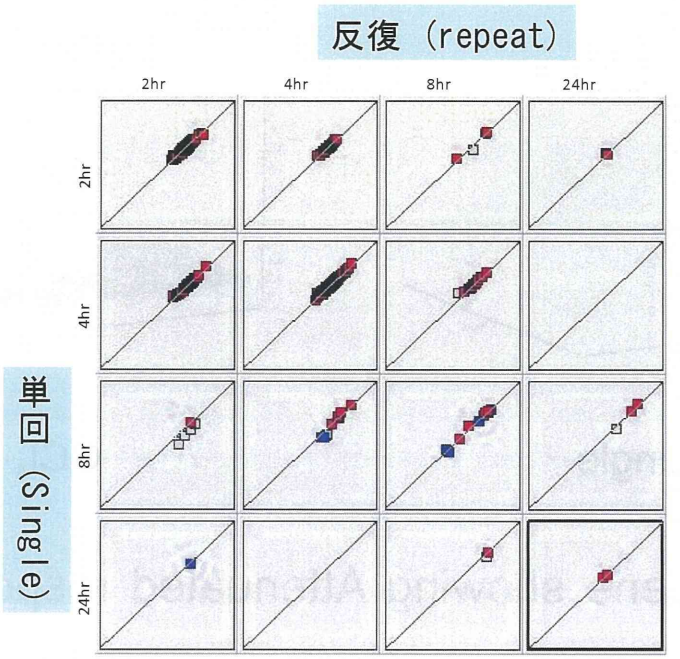

VPA repeat

Tgoln1 /// Tgoln2
trans-golgi network protein /// trans-golgi network protein 2

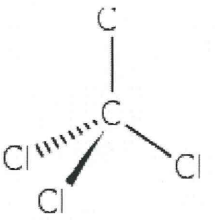
## Percellome Explorer解析

## バルプロ酸ナトリウム
## [Valproic acid sodium salt]

・PDBEx_RSort101008_Std-Med.PDBEx
・Target*Candidate



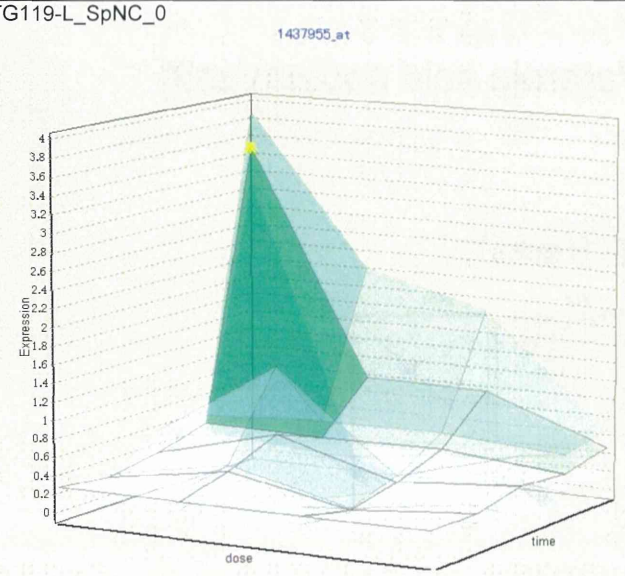

## 四塩化炭素
## [Carbon tetrachloride]



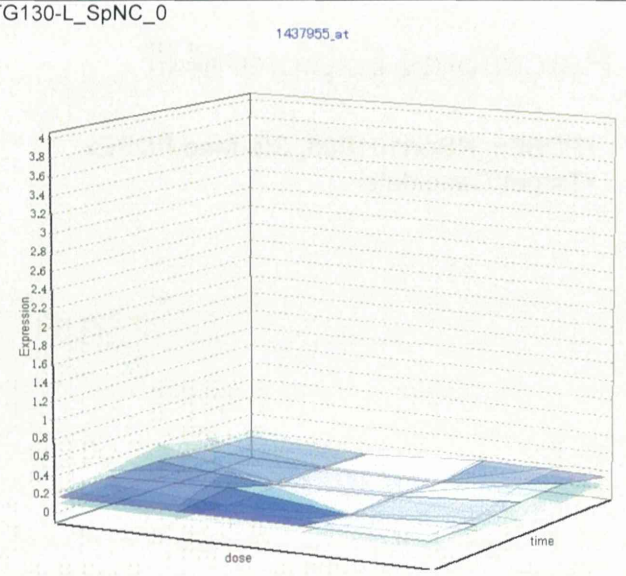

単回投与
[0, 0.7, 2, 7 mg/kg]

反復後　単回投与
5 mg/kg (for 14 days) +　[0, 0.7, 2, 7 mg/kg]

TTG119-L_SpNC_0




TTG130-L_SpNC_0



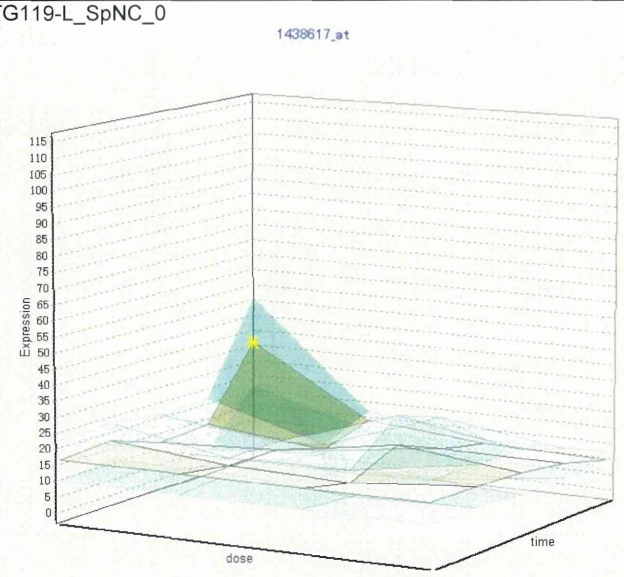

$CCl_4$ single

$CCl_4$ repeat

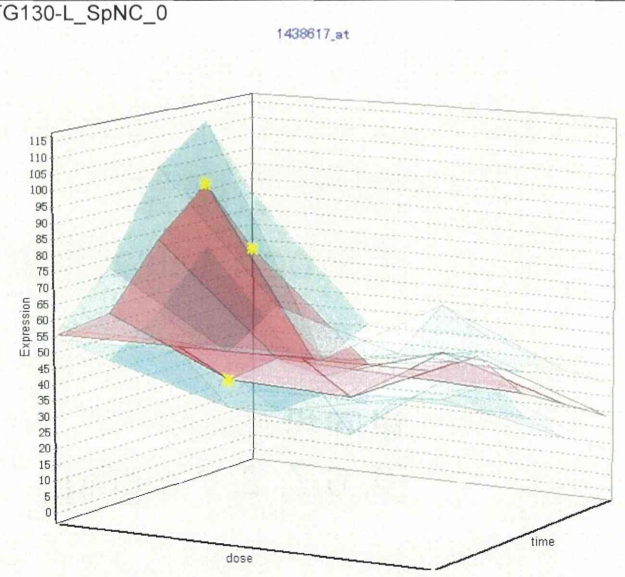
## Gene showing Attenuated response

UbqlnI
ubiquilin-like

TTG119-L_SpNC_0




TTG130-L_SpNC_0




$CCl_4$ single

$CCl_4$ repeat

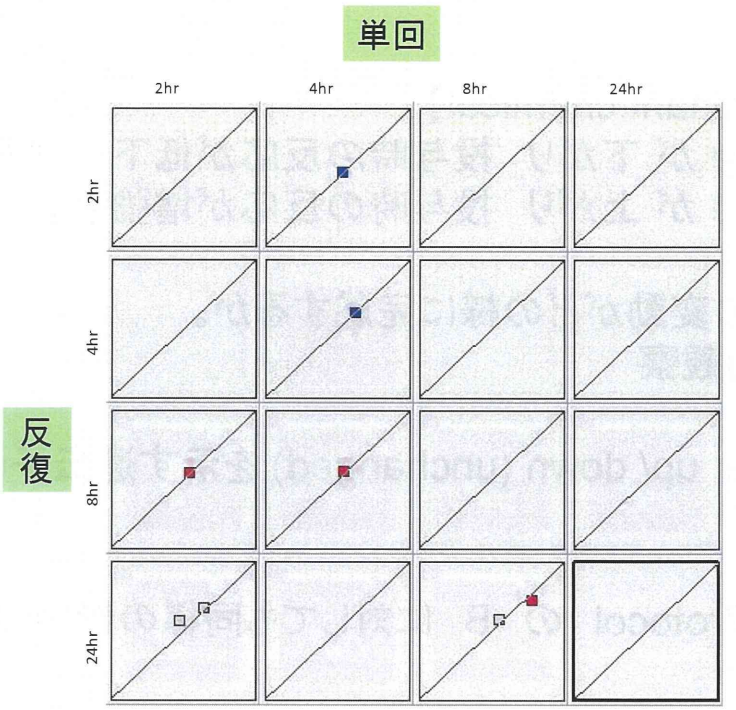
## Gene showing Enhanced response

Serpina7
serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7

## Percellome Explorer解析

## 四塩化炭素 [Carbon tetrachloride]

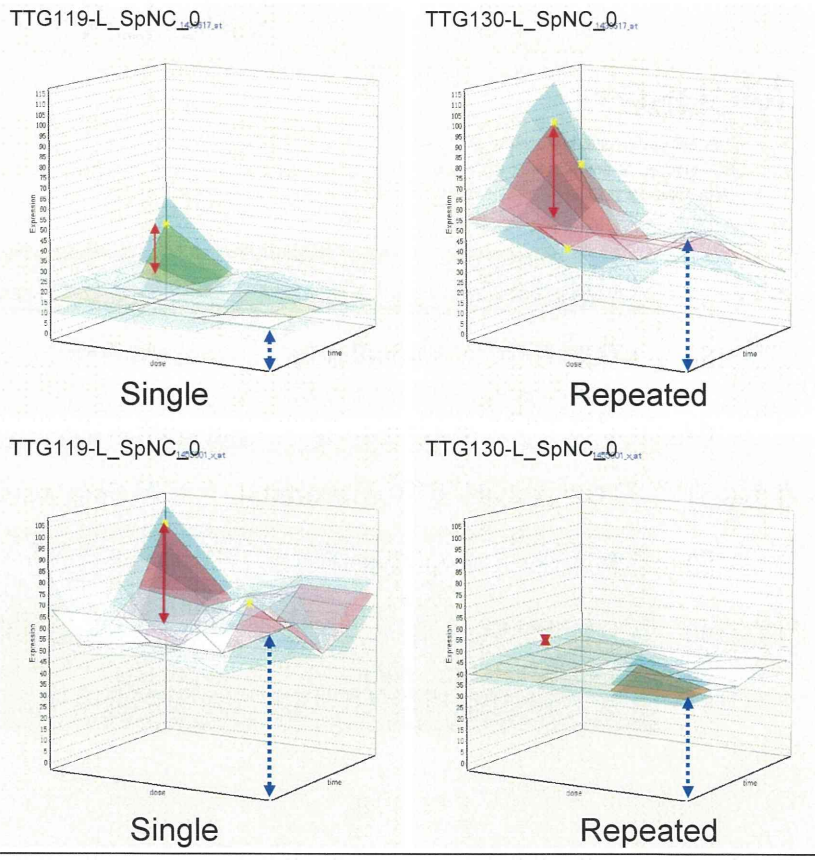
・PDBEx_RSort101008_Std-Med.PDBEx
・Target*Candidate




## ●反復投与が毎回の投与による反応に大きな影響を与える物質

Transient Response (T-Res)
過渡反応

Baseline Response (B-Res)
基線反応



# 前年度

## A+A’ protocol 実験で示唆されたこと

■【B-Res-dominant chemical】

Baseline が 下がり 投与時の反応が低下～消失する
Baseline が 上がり 投与時の反応が増強する

★ Baseline の変動がどの様に完成するか。
・経時的観察

★★ Baseline: up/ down (unchanged) を示す遺伝子の上流は？

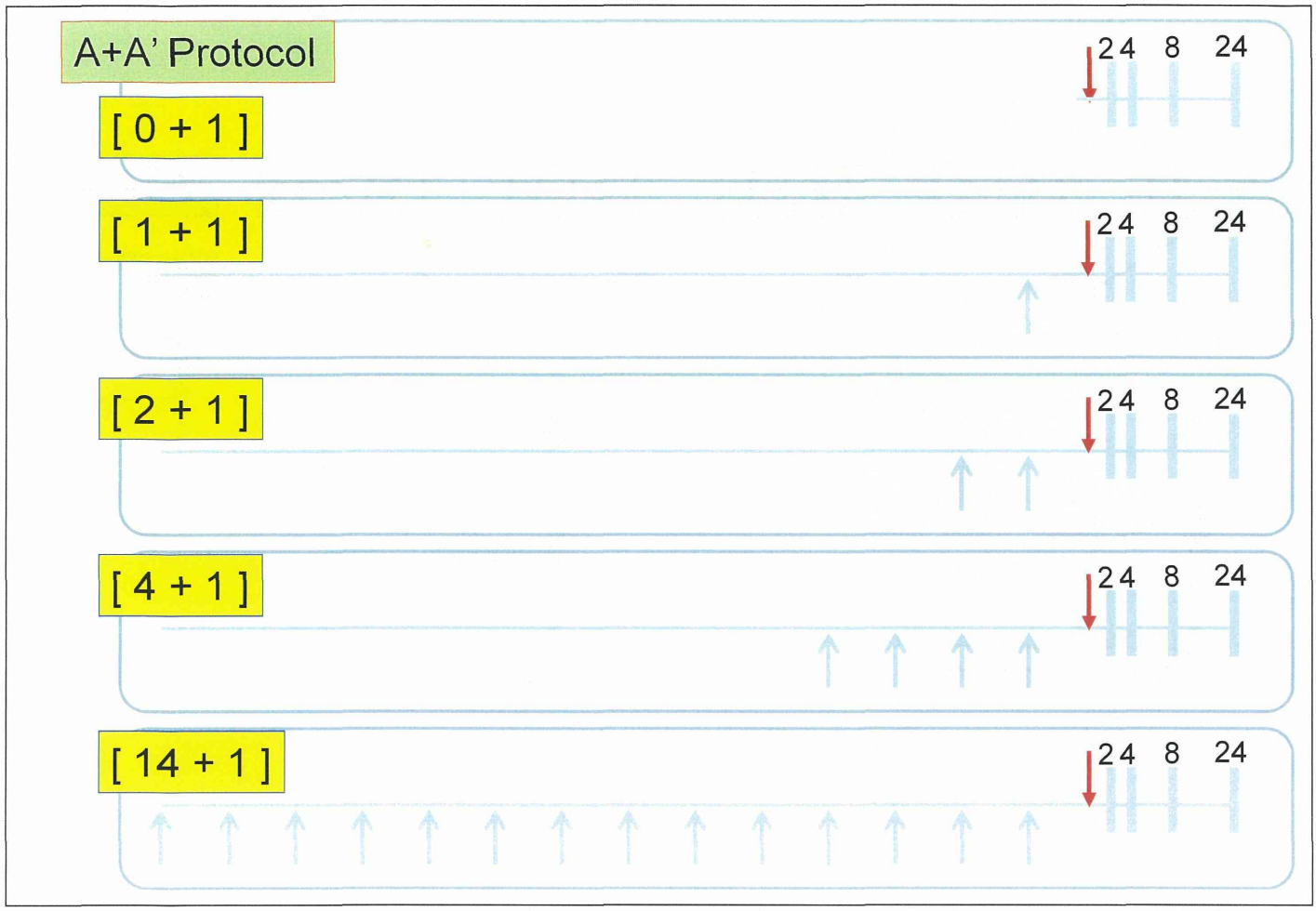
★★★ A+B Protocol の B に対しても同様の影響が及ぶか

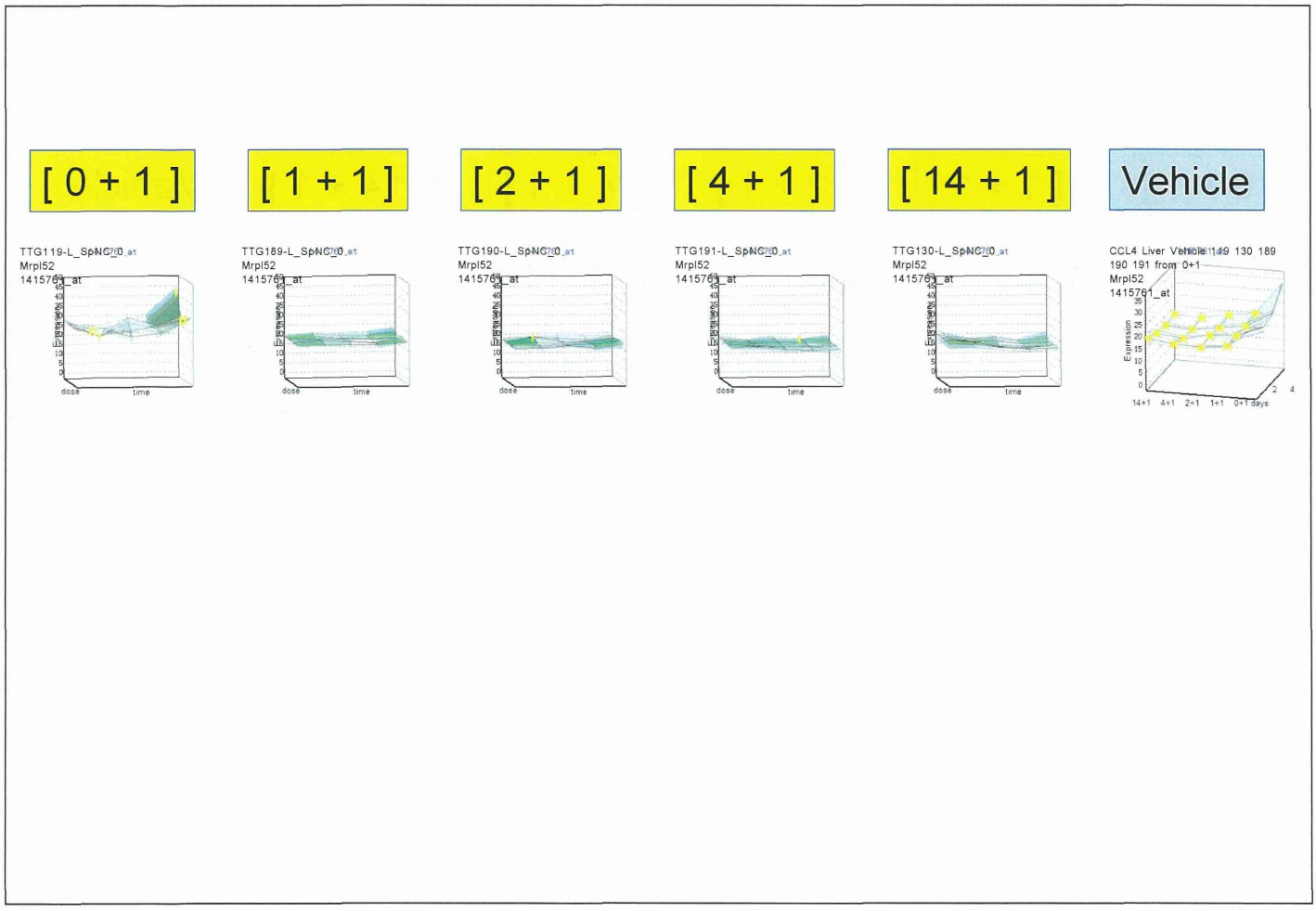
# CCL4

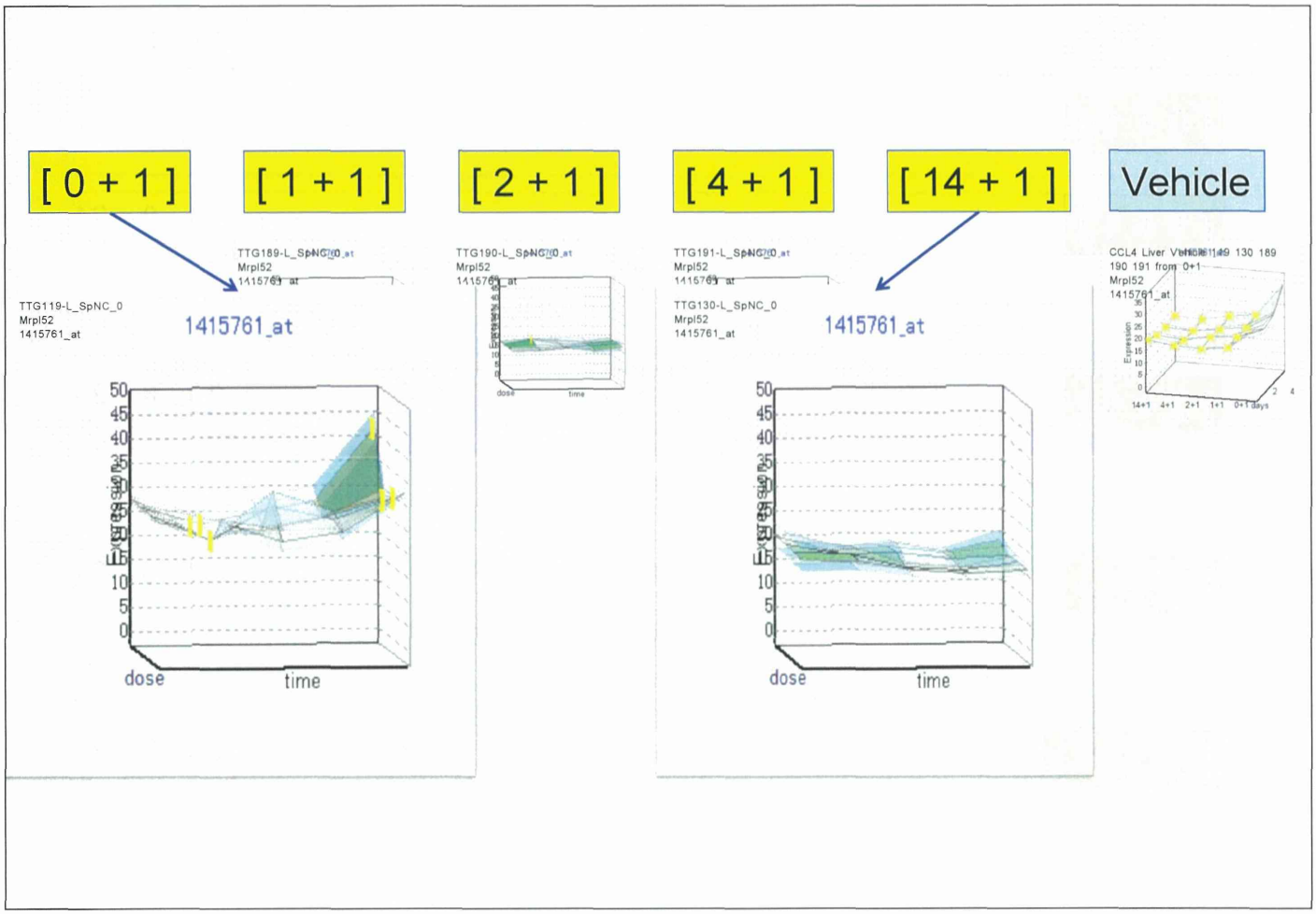
## Mouse:

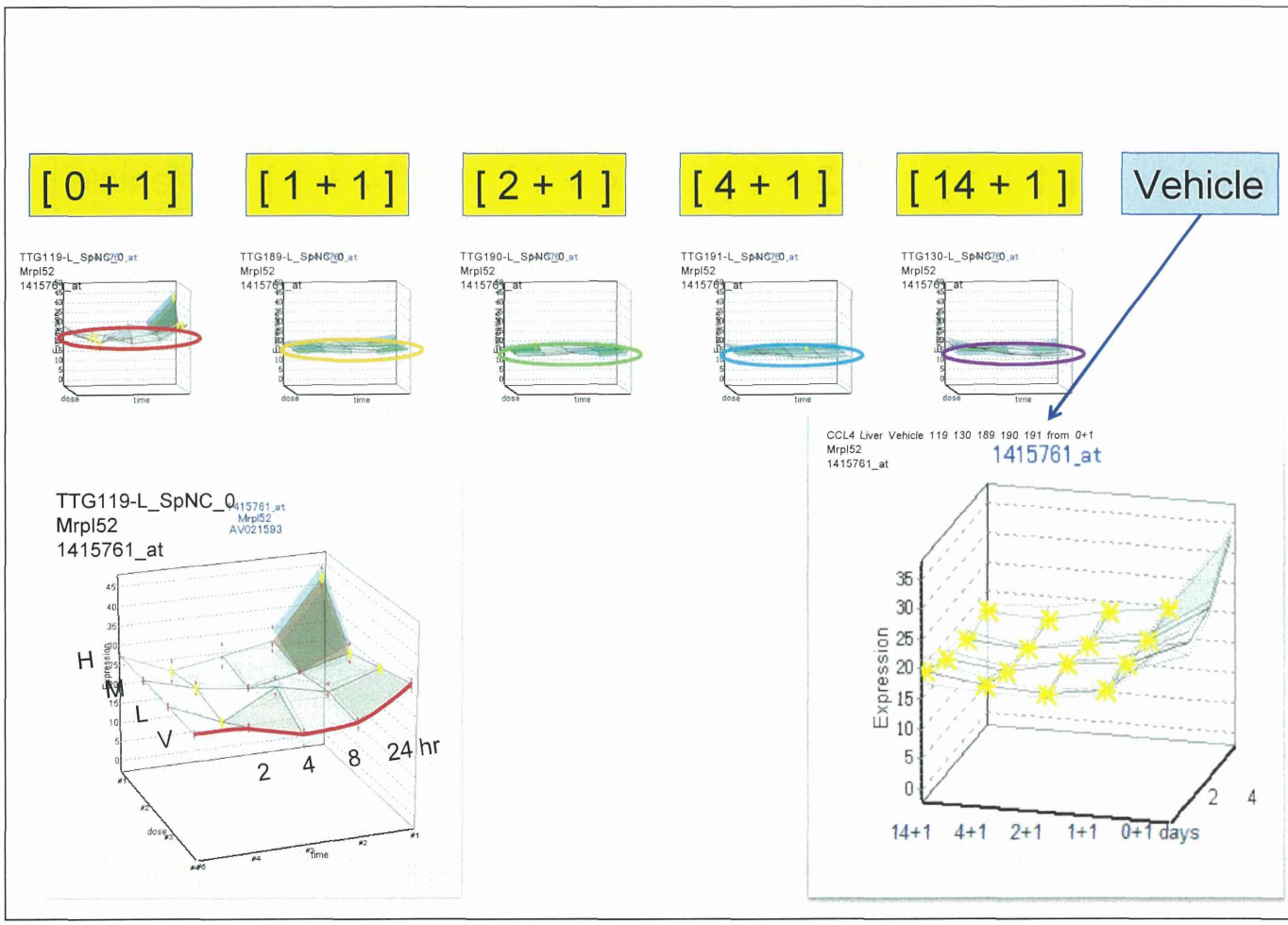
- [PSs induced at any protocol] & BR Down
- [PSs induced at any protocol] & BR Up
- [Regardless of induction] & BR Down

| | | |
|---|---|---|
| TTG119 | TTG2(0d+1d): CCl4 | 0, 0.7, 2.0, 7.0 mg/kg |
| TTG129 | TTG2: A)CCl4, B)Clofibrate | A) 5mg/kg, B) 0, 10, 30, 100mg/kg |
| TTG130 | TTG2(14d+1d): A)CCl4, B)CCl4 | A) 5mg/kg, B) 0, 0.7, 2.0, 7.0mg/kg |
| TTG131 | TTG2: A)CCl4, B)Phenobarbital | A) 5mg/kg, B) 0, 15, 50, 150mg/kg |
| TTG189 | TTG4(1d+1d): A)CCl4, B)CCl4 | A) 5mg/kg, B) 0, 0.7, 2.0, 7.0mg/kg |
| TTG190 | TTG4(2d+1d): A)CCl4, B)CCl4 | A) 5mg/kg, B) 0, 0.7, 2.0, 7.0mg/kg |
| TTG191 | TTG4(4d+1d): A)CCl4, B)CCl4 | A) 5mg/kg, B) 0, 0.7, 2.0, 7.0mg/kg |

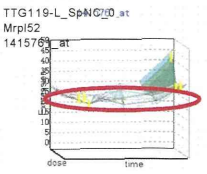

A+A' Protocol
[ 0 + 1 ]
[ 1 + 1 ]
[ 2 + 1 ]
[ 4 + 1 ]
[ 14 + 1 ]
24 8 24

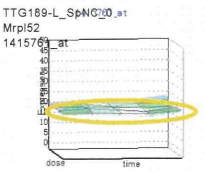

[ 0 + 1 ]
[ 1 + 1 ]
[ 2 + 1 ]
[ 4 + 1 ]
[ 14 + 1 ]
Vehicle
Mrpl52
1415761_at
dose
time

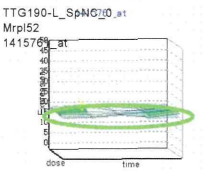

[ 0 + 1 ]
[ 1 + 1 ]
[ 2 + 1 ]
[ 4 + 1 ]
[ 14 + 1 ]
Vehicle
TTG119-L_SpNC_0
Mrpl52
1415761_at
1415761_at
dose
time
TTG130-L_SpNC_0
Mrpl52
1415761_at
1415761_at
dose
time

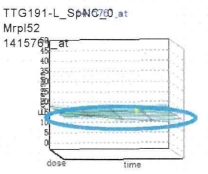

[ 0 + 1 ]
[ 1 + 1 ]
[ 2 + 1 ]
[ 4 + 1 ]
[ 14 + 1 ]
Vehicle
TTG119-L_SpNC_0
Mrpl52
1415761_at
AV021593
H
M
L
V
2
4
8
24 hr
dose
time
CCL4 Liver Vehicle 119 130 189 190 191 from 0+1
Mrpl52
1415761_at
1415761_at
Expression
14+1
4+1
2+1
1+1
0+1 days

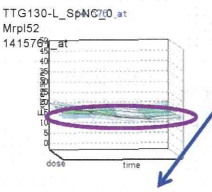
[ 0 + 1 ] [ 1 + 1 ] [ 2 + 1 ] [ 4 + 1 ] [ 14 + 1 ] Vehicle

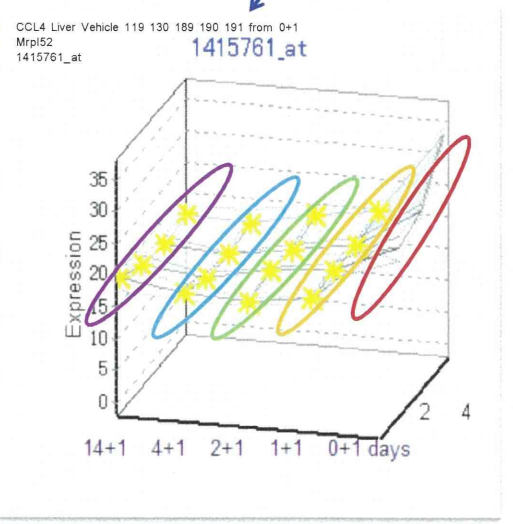


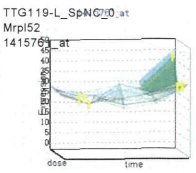



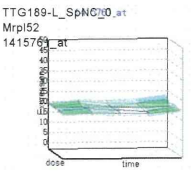



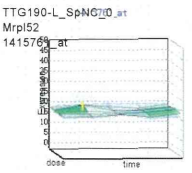



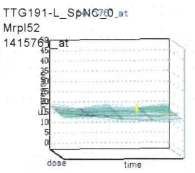



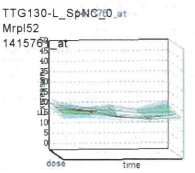

[ 0 + 1 ] [ 1 + 1 ] [ 2 + 1 ] [ 4 + 1 ] [ 14 + 1 ] Vehicle

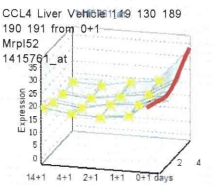


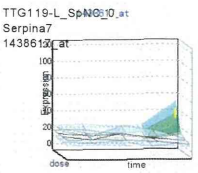



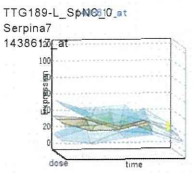



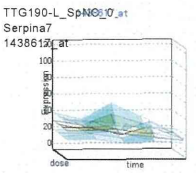



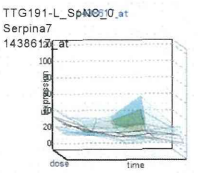



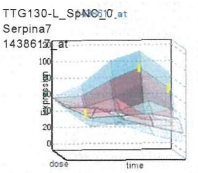



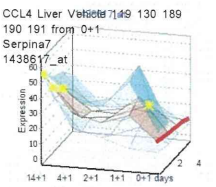



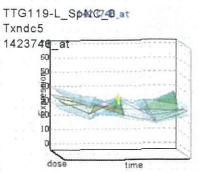



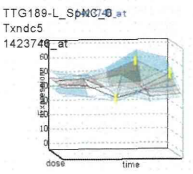



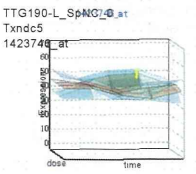



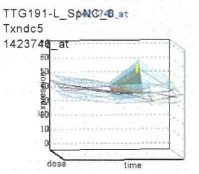



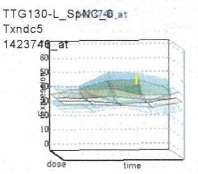



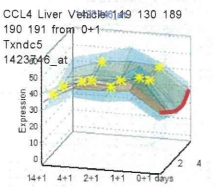



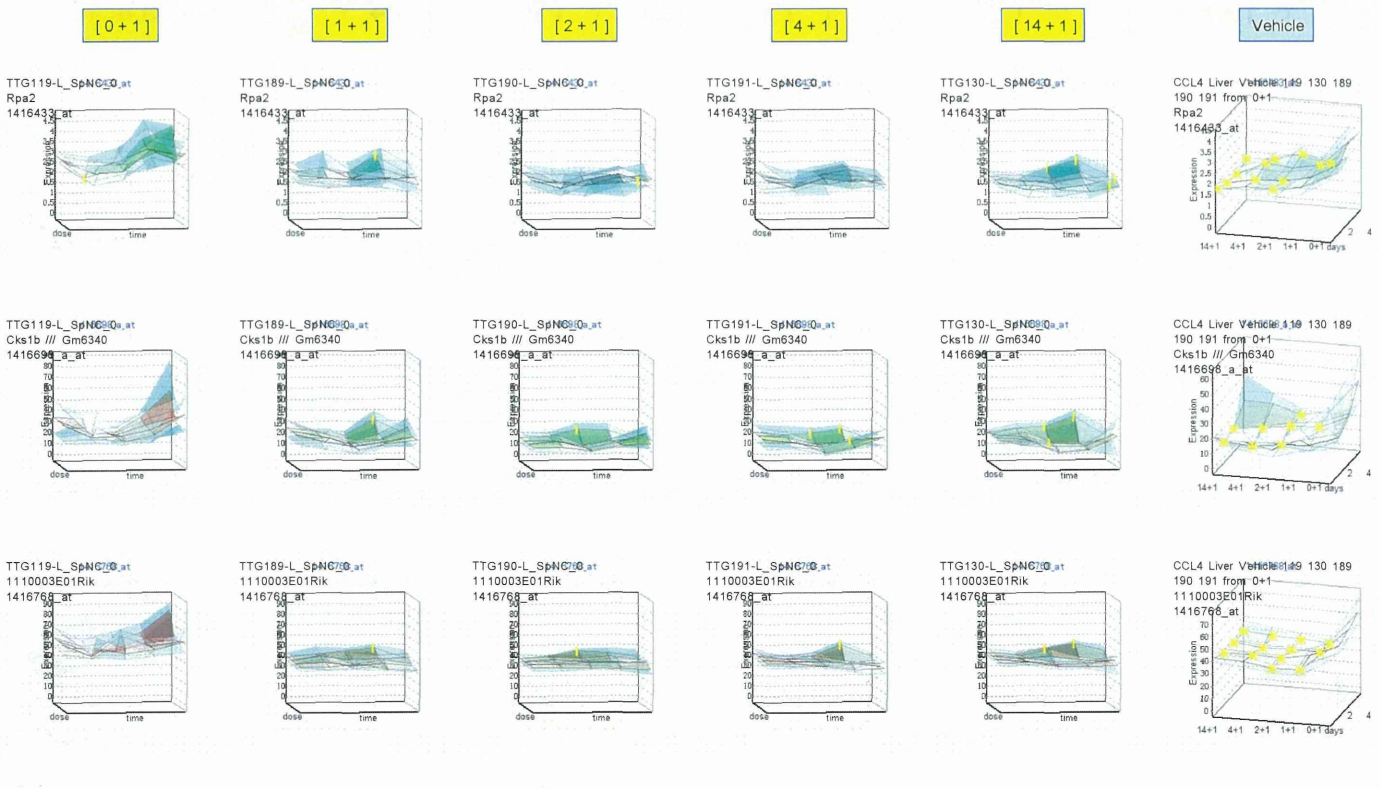



# CCL4

Mouse:

- BR Down



# CCl4

Summary of Analysis - Selected DownLiver M1 Copy AND Or of ttest001 - 2015-01-06 08:20

## Top Canonical Pathways

| Name | p-value | Ratio |
|---|---|---|
| EIF2 Signaling | 1.43E-37 | 72/185 (0.389) |
| Oxidative Phosphorylation | 1.22E-31 | 51/109 (0.468) |
| Mitochondrial Dysfunction | 1.34E-28 | 60/171 (0.351) |
| Regulation of eIF4 and p70S6K Signaling | 8.63E-14 | 38/146 (0.26) |
| Acute Phase Response Signaling | 2E-10 | 36/169 (0.213) |

## Top Upstream Regulators

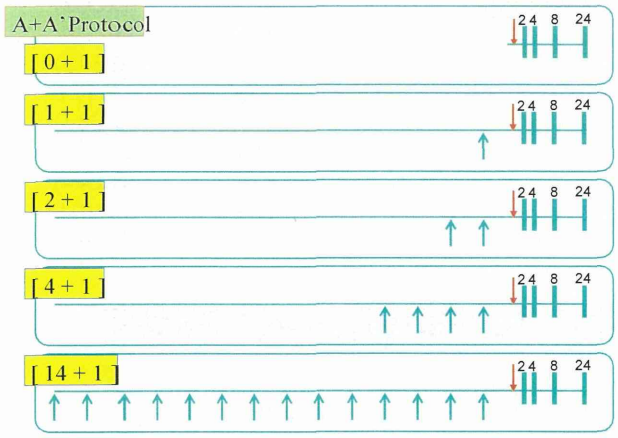
| Upstream Regulator | p-value of overlap | Predicted Activation State |
|---|---|---|
| RICTOR | 7.17E-75 | Activated |
| HNF4A | 1.21E-45 | Inhibited |
| MYCN | 6.44E-35 | Inhibited |
| CD 437 | 1.70E-32 | Activated |
| 5-fluorouracil | 8.72E-30 | Activated |

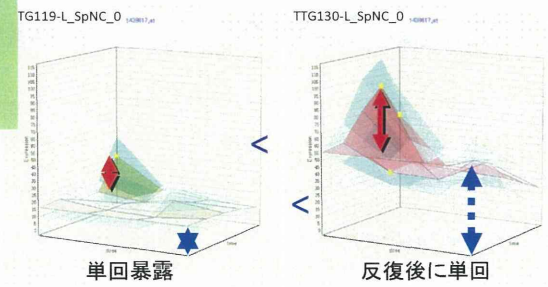
【新型反復暴露：H25に2物質追加】

- 四塩化炭素
- クロフィブレート
- トリブチル錫
- バルプロ酸

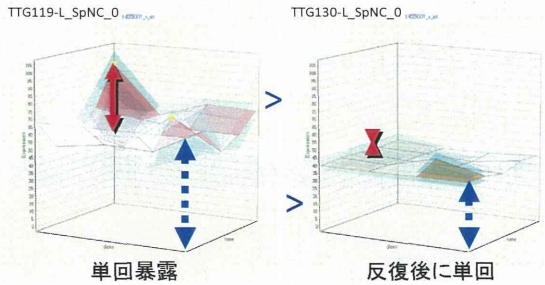
Transient Response (T-Res) 過渡反応

Baseline Response (B-Res) 基線反応



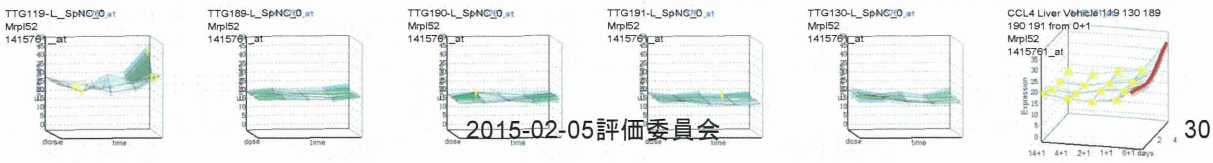




単回暴露　　反復後に単回




単回暴露　　反復後に単回

[ 0 + 1 ]　[ 1 + 1 ]　[ 2 + 1 ]　[ 4 + 1 ]　[ 14 + 1 ]　Vehicle



# Clofibrate

Summary of Analysis - Clofibrate Vehicle Baseline SUPpression rev IPA - 2015-01-06 08:20

## Top Canonical Pathways

| Name | p-value | Ratio |
|---|---|---|
| EIF2 Signaling | 9.61E-57 | 89/185 (0.481) |
| Mitochondrial Dysfunction | 5.19E-39 | 70/171 (0.409) |
| Oxidative Phosphorylation | 4.74E-38 | 56/109 (0.514) |
| Regulation of eIF4 and p70S6K Signaling | 8.84E-27 | 53/146 (0.363) |
| mTOR Signaling | 6.5E-21 | 53/188 (0.282) |

## Top Upstream Regulators

| Upstream Regulator | p-value of overlap | Predicted Activation State |
|---|---|---|
| RICTOR | 5.16E-99 | Activated |
| HNF4A | 3.50E-52 | Inhibited |
| 1,2-dithiol-3-thione | 3.76E-52 | Inhibited |
| 5-fluorouracil | 4.77E-45 | Activated |
| sirolimus | 3.59E-43 | Activated |

# Tri-butyl-Tin (TBT)

Summary of Analysis - AND ratio L0.8 And AND AND of All time ttest 0.01 And - 2015-01-12 06:35

## Top Canonical Pathways

| Name | p-value | Ratio |
|---|---|---|
| EIF2 Signaling | 6.52E-47 | 56/185 (0.303) |
| Oxidative Phosphorylation | 1.03E-34 | 38/109 (0.349) |
| Mitochondrial Dysfunction | 7.38E-29 | 40/171 (0.234) |
| FXR/RXR Activation | 3.29E-23 | 31/127 (0.244) |
| LXR/RXR Activation | 1.03E-22 | 30/121 (0.248) |

## Top Upstream Regulators

| Upstream Regulator | p-value of overlap | Predicted Activation State |
|---|---|---|
| RICTOR | 3.86E-77 | |
| sirolimus | 9.52E-46 | |
| MYCN | 1.81E-41 | |
| 5-fluorouracil | 1.00E-38 | |
| HNF4A | 5.08E-34 | |

# Deet

Summary of Analysis - AND ratio L0.8 And AND AND of All time ttest 0.01 And - 2015-01-12 05:50

**Top Canonical Pathways**

| Name | p-value | Ratio |
|---|---|---|
| EIF2 Signaling | 6.52E-47 | 56/185 (0.303) |
| Oxidative Phosphorylation | 1.03E-34 | 38/109 (0.349) |
| Mitochondrial Dysfunction | 7.38E-29 | 40/171 (0.234) |
| FXR/RXR Activation | 3.29E-23 | 31/127 (0.244) |
| LXR/RXR Activation | 1.03E-22 | 30/121 (0.248) |

**Top Upstream Regulators**

| Upstream Regulator | p-value of overlap | Predicted Activation State |
|---|---|---|
| RICTOR | 3.86E-77 | Activated |
| sirolimus | 9.52E-46 | Activated |
| MYCN | 1.81E-41 | Inhibited |
| 5-fluorouracil | 1.00E-38 | Activated |
| HNF4A | 5.08E-34 | Inhibited |

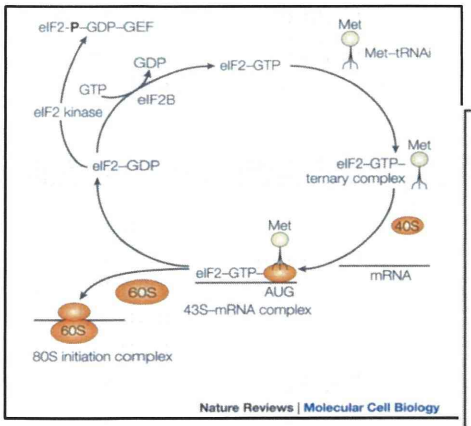
●基線反応のメカニズムに、『エピジェネティックな制御』を想定

**基線反応が低下した化合物**

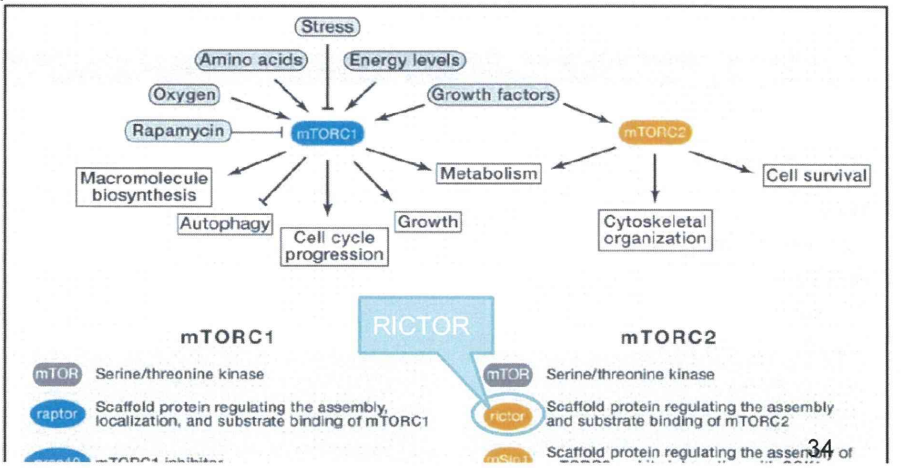
- 四塩化炭素
- クロフィブレート
- トリブチル錫

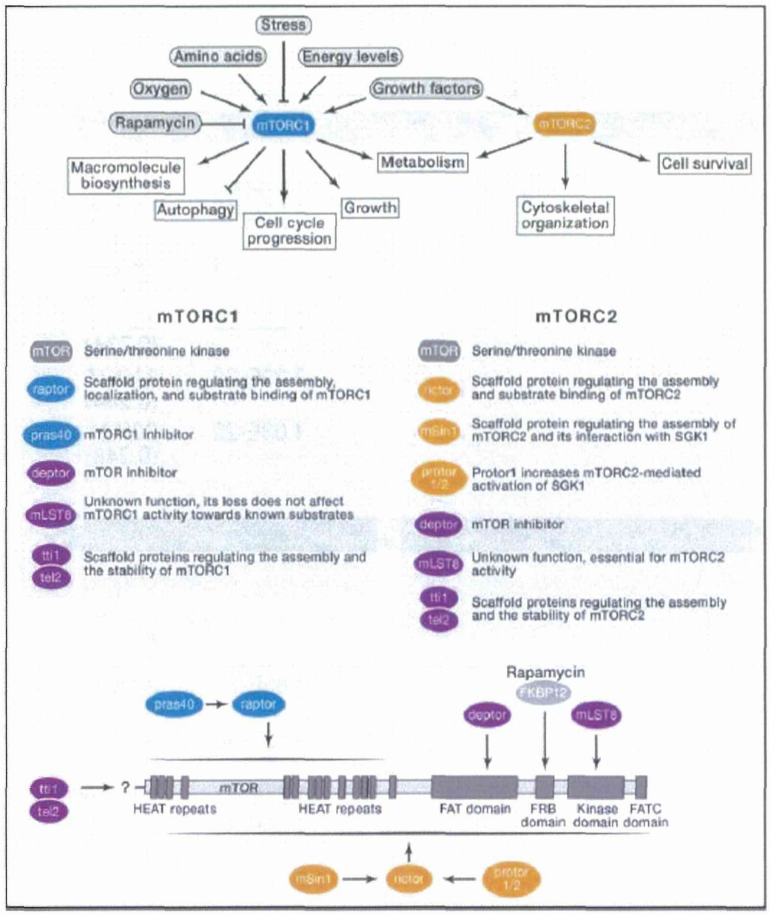
**低下した遺伝子のリストが共通であった。**

- eIF2回路/ 酸化的リン酸化/ ミトコンドリア
- 上流にRICTOR/ HNF4A










# TGP Rat CCL4

Summary of Analysis - DOWN TGP0066-0067_RSort - 2015-01-25 04:06

## Top Canonical Pathways

| Name | p-value | Ratio |
|---|---|---|
| Protein Ubiquitination Pathway | 3.72E-17 | 21/255 (0.082) |
| Unfolded protein response | 1.63E-06 | 6/54 (0.111) |
| Endoplasmic Reticulum Stress Pathway | 3.61E-04 | 3/21 (0.143) |
| CMP-N-acetylneuraminate Biosynthesis I (Eukaryotes) | 4.42E-04 | 2/5 (0.4) |
| Lipid Antigen Presentation by CD1 | 6.88E-04 | 3/26 (0.115) |

## Top Upstream Regulators

| Upstream Regulator | p-value of overlap | Predicted Activation State |
|---|---|---|
| XBP1 | 9.56E-31 | Inhibited |
| 1,2-dithiol-3-thione | 5.38E-26 | Inhibited |
| NFE2L2 | 1.09E-15 | Inhibited |
| RICTOR | 3.62E-15 | Activated |
| RAB1B | 3.31E-11 | Inhibited |

## Mouse – Rat comparison

| CCL4 | | | | | |
|---|---|---|---|---|---|
| | Mouse (Max 20mg/kg) | | | Rat (Max 300mg/kg) | |
| | Down (induction +) | Up (induction +) | Down (induction -) | Down (induction +) | Up (induction +) |
| | 89 | 13 | 1798 | 173 | 65 |
| Canonical Pathway | Protein Ubiquitination Pathway | | EIF2 Signaling | Protein Ubiquitination Pathway | EIF2 Signaling |
| | | | Oxidative Phosphorylation | | |
| | | | Mitochondrial Dysfunction | | |
| | | | Acute Phase Response Signaling | | |
| | | | | | |
| Upstream TF | XBP1 | (PAX6) | HNF4A | XBP1 | MYCN |
| | NFE2L2 | (XBP1) | MYCN | NFE2L2 | MYC |
| | | (SIRT1) | MYC | HNF4A | |
| | | | NFE2L2 | ATF6 | |

# H26年度

VPAの[1＋1]データを加えての解析
Clofibrate の解析
Tributyltin
Deet
TGP-Rat VPAおよびClofibrateデータの解析

★組み合わせデータの解析
- $CCl_4$ & $CCl_4$、Clof、PB、
- VPA & VPA * 、Asp、Thalidomide
- Clofibrate & Clof、PCN、ATRA
- TBT & TBT、PB、Clof

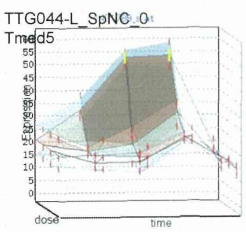
# CCL4

## Mouse:

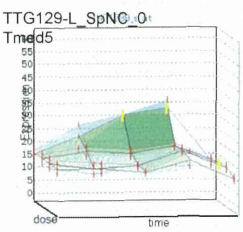
- [PSs induced at any protocol] & BR Down
- [PSs induced at any protocol] & BR Up
- [Regardless of induction] & BR Down

| | | |
|---|---|---|
| TTG119 | TTG2(0d+1d): CCl4 | 0, 0.7, 2.0, 7.0 mg/kg |
| TTG129 | TTG2: A)CCl4, B)Clofibrate | A) 5mg/kg, B) 0, 10, 30, 100mg/kg |
| TTG130 | TTG2(14d+1d): A)CCl4, B)CCl4 | A) 5mg/kg, B) 0, 0.7, 2.0, 7.0mg/kg |
| TTG131 | TTG2: A)CCl4, B)Phenobarbital | A) 5mg/kg, B) 0, 15, 50, 150mg/kg |
| TTG189 | TTG4(1d+1d): A)CCl4, B)CCl4 | A) 5mg/kg, B) 0, 0.7, 2.0, 7.0mg/kg |
| TTG190 | TTG4(2d+1d): A)CCl4, B)CCl4 | A) 5mg/kg, B) 0, 0.7, 2.0, 7.0mg/kg |
| TTG191 | TTG4(4d+1d): A)CCl4, B)CCl4 | A) 5mg/kg, B) 0, 0.7, 2.0, 7.0mg/kg |

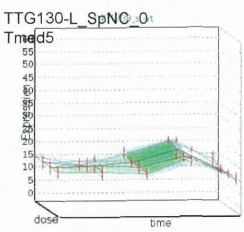
None + Clofibrate 1



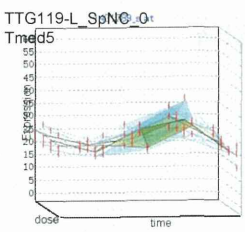

$CCl_4$ 14 + Clofib 1



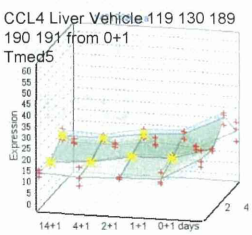

$CCl_4$ 14 + $CCl_4$ 1

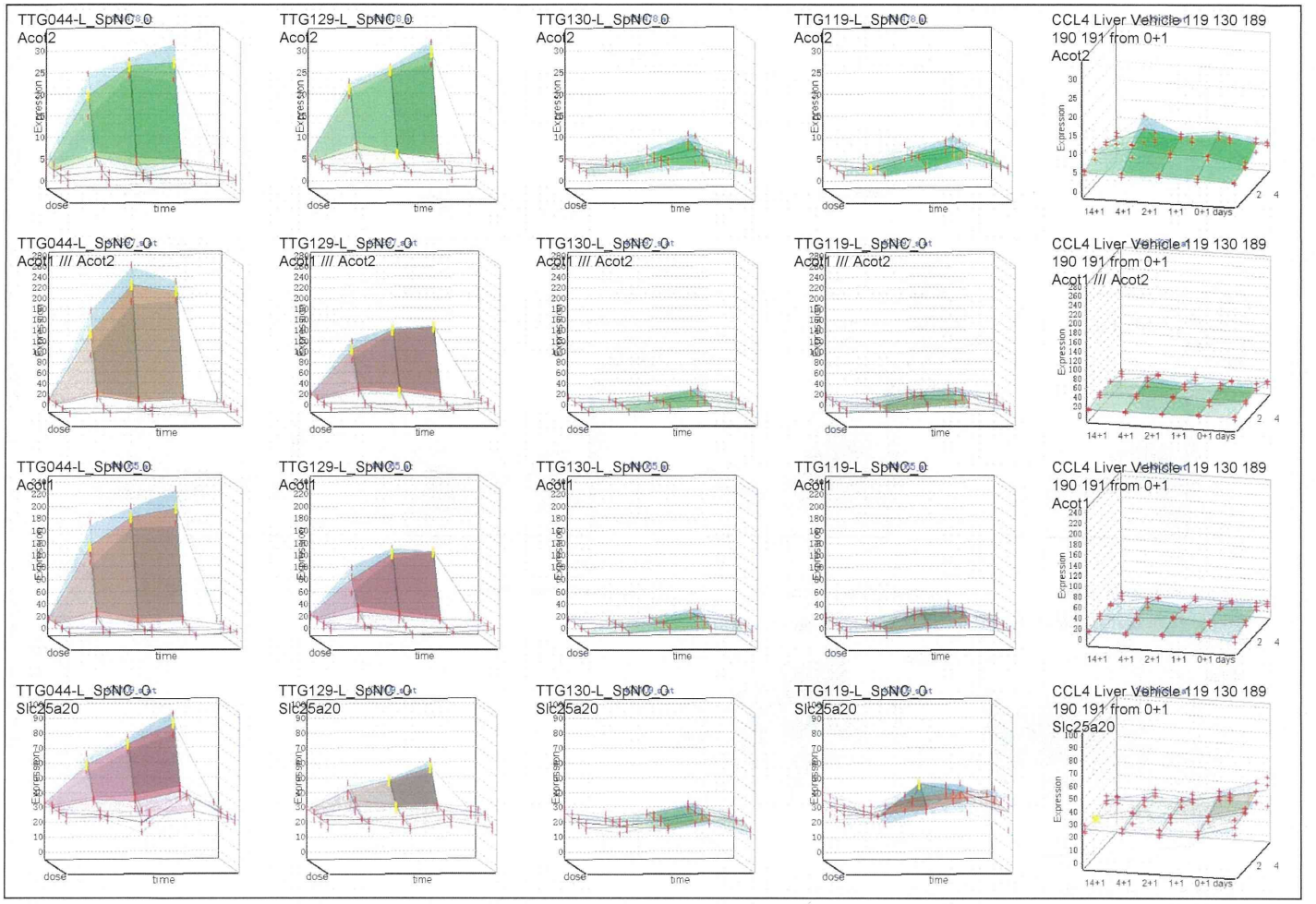



None + $CCl_4$ 1



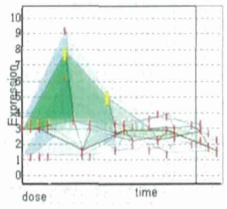

$CCl_4$ Vehicle





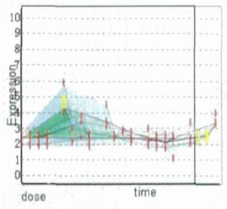

[14+1]
TBT + Clofibrate
CCl4 + Clofibrate

TTG044-L_SpNC-0 1437300_at
Clofibrate
Mef2d



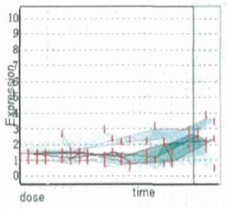

myocyte enhancer factor 2D

TTG141-L_SpNC-0 1437300_at
Tributyltin chloride x Clofibrate
Mef2d



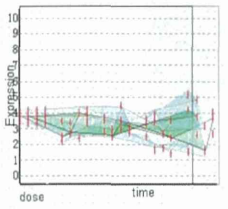

myocyte enhancer factor 2D

TTG085-L_SpNC-0 1437300_at
Trybutyltin
Mef2d



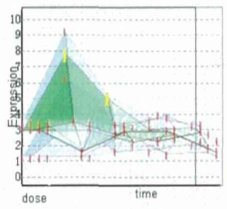

myocyte enhancer factor 2D

TTG145-L_SpNC-0 1437300_at
Tributyltin chloride x Tributyltin chloride
Mef2d



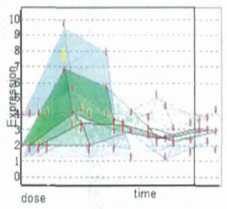

myocyte enhancer factor 2D

TTG044-L_SpNC-0 1437300_at
Clofibrate
Mef2d



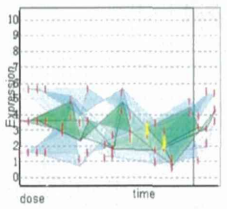

myocyte enhancer factor 2D

TTG129-L_SpNC-0 1437300_at
CCl4 x Clofibrate
Mef2d



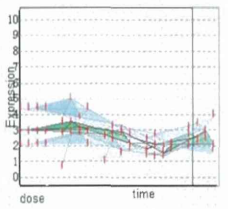

myocyte enhancer factor 2D

TTG119-L_SpNC-0 1437300_at
Carbon tetrachloride
Mef2d



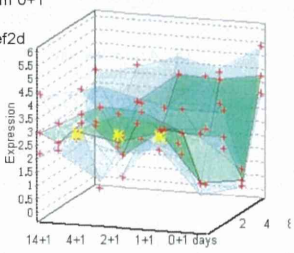

myocyte enhancer factor 2D

TTG130-L_SpNC-0 1437300_at
CCl4 x CCl4
Mef2d



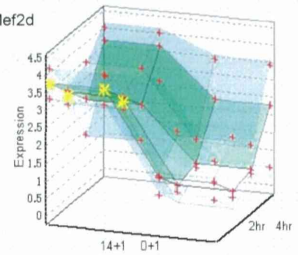

myocyte enhancer factor 2D

CCL4 Liver Vehicle 119 130 189 190 191 from 0+1 1437300_at
Mef2d



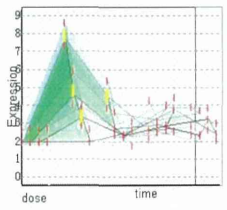

myocyte enhancer factor 2D

TBT 0+1 14+1 Baseline 085 145 1437300_at
Mef2d



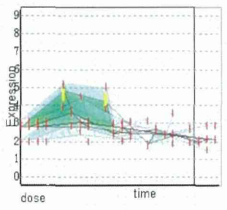

myocyte enhancer factor 2D

TTG044-L_SpNC-0 1455082_at
Clofibrate
Cblb



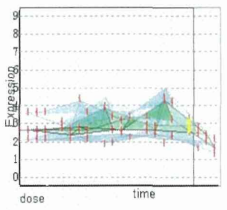

Casitas B-lineage lymphoma b

TTG141-L_SpNC-0 1455082_at
Tributyltin chloride x Clofibrate
Cblb



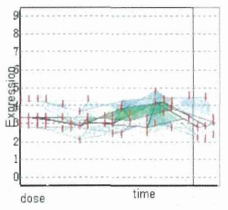

Casitas B-lineage lymphoma b

TTG085-L_SpNC-0 1455082_at
Trybutyltin
Cblb



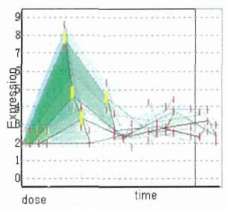

Casitas B-lineage lymphoma b

TTG145-L_SpNC-0 1455082_at
Tributyltin chloride x Tributyltin chloride
Cblb



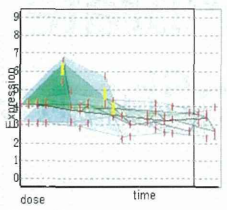

Casitas B-lineage lymphoma b

TTG044-L_SpNC-0 1455082_at
Clofibrate
Cblb



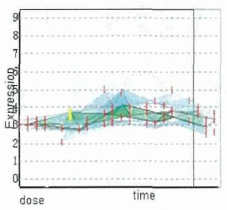

Casitas B-lineage lymphoma b

TTG129-L_SpNC-0 1455082_at
CCl4 x Clofibrate
Cblb



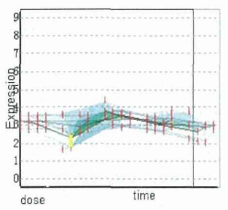

Casitas B-lineage lymphoma b

TTG119-L_SpNC-0 1455082_at
Carbon tetrachloride
Cblb



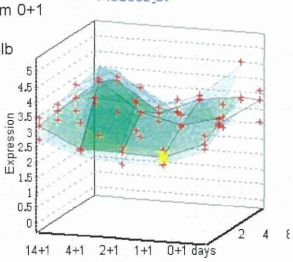

Casitas B-lineage lymphoma b

TTG130-L_SpNC-0 1455082_at
CCl4 x CCl4
Cblb



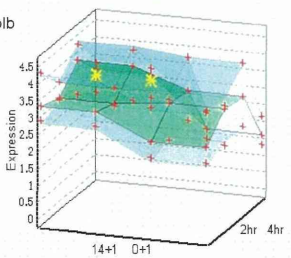

Casitas B-lineage lymphoma b

CCL4 Liver Vehicle 119 130 189 190 191 from 0+1 1455082_at
Cblb



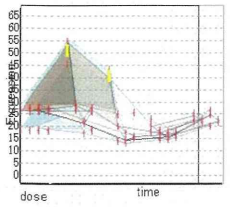

Casitas B-lineage lymphoma b

TBT 0+1 14+1 Baseline 085 145 1455082_at
Cblb



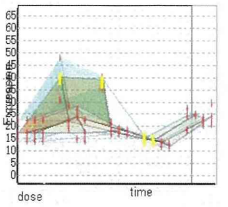

Casitas B-lineage lymphoma b

TTG044-L_SpNC_0 1439438_at
Clofibrate
Pxmp4



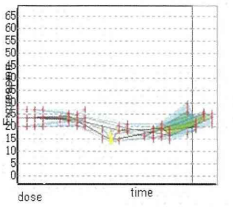

peroxisomal membrane protein 4

TTG141-L_SpNC_0 1439438_at
Tributyltin chloride x Clofibrate
Pxmp4



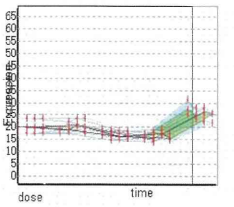

peroxisomal membrane protein 4

TTG085-L_SpNC_0 1439438_at
Trybutyltin
Pxmp4



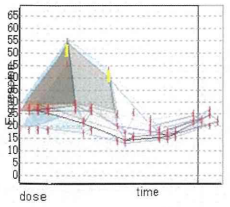

peroxisomal membrane protein 4

TTG145-L_SpNC_0 1439438_at
Tributyltin chloride x Tributyltin chloride
Pxmp4



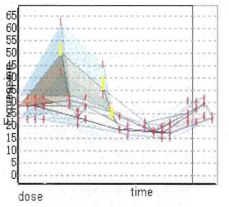

peroxisomal membrane protein 4

TTG044-L_SpNC_0 1439438_at
Clofibrate
Pxmp4



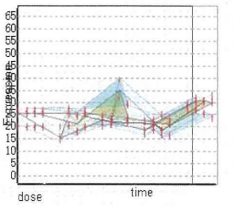

peroxisomal membrane protein 4

TTG129-L_SpNC_0 1439438_at
CCl4 x Clofibrate
Pxmp4



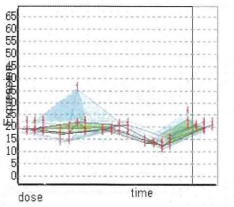

peroxisomal membrane protein 4

TTG119-L_SpNC_0 1439438_at
Carbon tetrachloride
Pxmp4



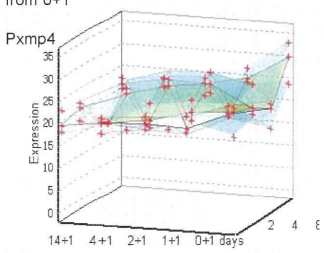

peroxisomal membrane protein 4

TTG130-L_SpNC_0 1439438_at
CCl4 x CCl4
Pxmp4



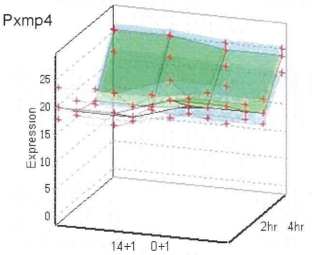

peroxisomal membrane protein 4

CCL4 Liver Vehicle 119 130 189 190 191 from 0+1 1439438_at
Pxmp4



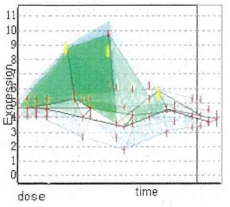

peroxisomal membrane protein 4

TBT 0+1 14+1 Baseline 085 145 1439438_at
Pxmp4



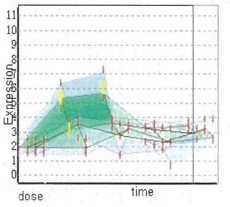

peroxisomal membrane protein 4

TTG044-L_SpNC_0 1439488_at
Clofibrate
Dot1l



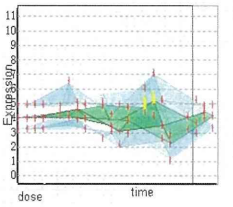

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

TTG141-L_SpNC_0 1439488_at
Tributyltin chloride x Clofibrate
Dot1l



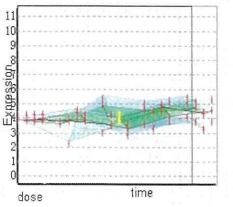

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

TTG085-L_SpNC_0 1439488_at
Trybutyltin
Dot1l



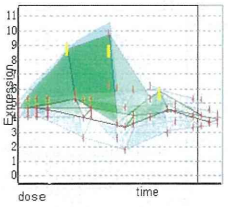

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

TTG145-L_SpNC_0 1439488_at
Tributyltin chloride x Tributyltin chloride
Dot1l



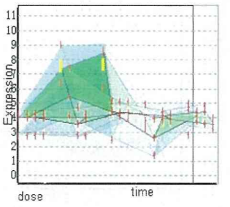

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

TTG044-L_SpNC_0 1439488_at
Clofibrate
Dot1l



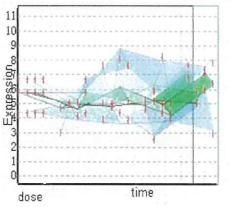

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

TTG129-L_SpNC_0 1439488_at
CCl4 x Clofibrate
Dot1l



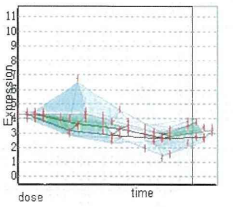

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

TTG119-L_SpNC_0 1439488_at
Carbon tetrachloride
Dot1l



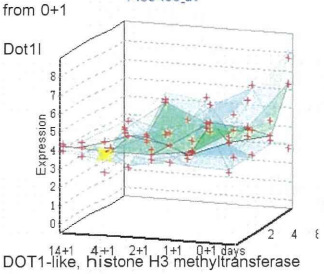

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

TTG130-L_SpNC_0 1439488_at
CCl4 x CCl4
Dot1l



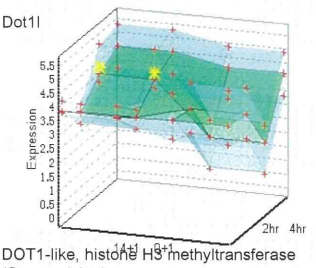

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

CCL4 Liver Vehicle 119 130 189 190 191 from 0+1 1439438_at
Dot1l



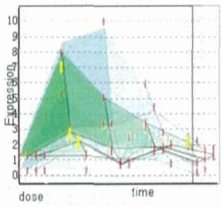

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

TBT 0+1 14+1 Baseline 085 145 1439438_at
Dot1l



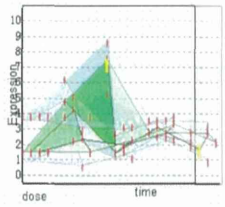

DOT1-like, histone H3 methyltransferase (S. cerevisiae)

TTG044-L_SpNC_0 1435312_at
Clofibrate
Paqr7



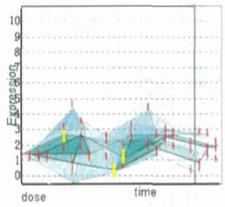

progestin and adipoQ receptor family member VII

TTG141-L_SpNC_0 1435312_at
Tributyltin chloride x Clofibrate
Paqr7



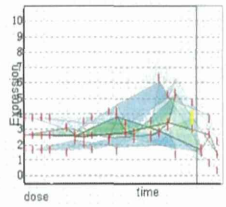

progestin and adipoQ receptor family member VII

TTG085-L_SpNC_0 1435312_at
Trybutyltin
Paqr7



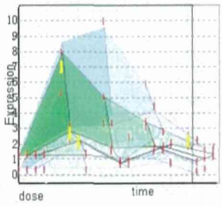

progestin and adipoQ receptor family member VII

TTG145-L_SpNC_0 1435312_at
Tributyltin chloride x Tributyltin chloride
Paqr7



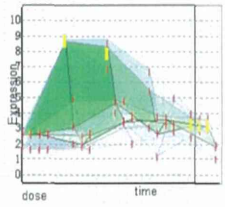

progestin and adipoQ receptor family member VII

TTG044-L_SpNC_0 1435312_at
Clofibrate
Paqr7



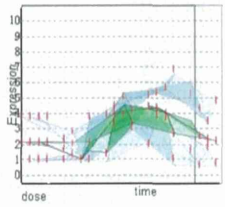

progestin and adipoQ receptor family member VII

TTG129-L_SpNC_0 1435312_at
CCl4 x Clofibrate
Paqr7



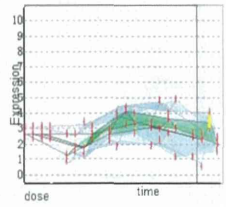

progestin and adipoQ receptor family member VII

TTG119-L_SpNC_0 1435312_at
Carbon tetrachloride
Paqr7



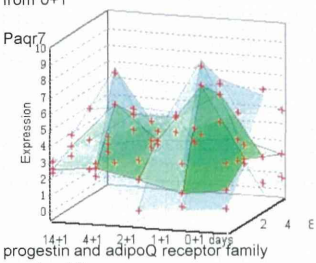

progestin and adipoQ receptor family member VII

TTG130-L_SpNC_0 1435312_at
CCl4 x CCl4
Paqr7



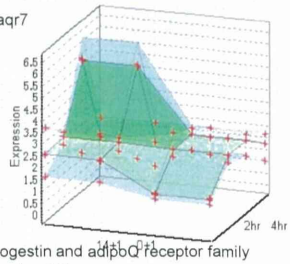

progestin and adipoQ receptor family member VII

CCL4 Liver Vehicle 119 130 189 190 191 from 0+1 1435312_at
Paqr7



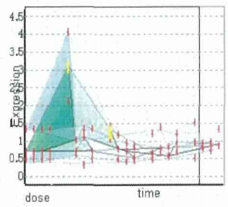

progestin and adipoQ receptor family member VII

TBT 0+1 14+1 Baseline 085 145 1435312_at
Paqr7



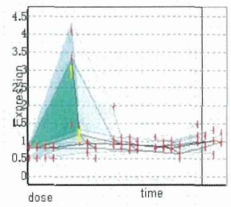

progestin and adipoQ receptor family member VII

TTG044-L_SpNC_0 1434808_at
Clofibrate
Palb2



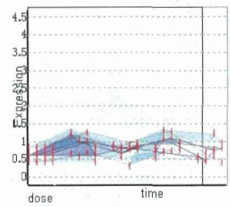

partner and localizer of BRCA2

TTG141-L_SpNC_0 1434808_at
Tributyltin chloride x Clofibrate
Palb2



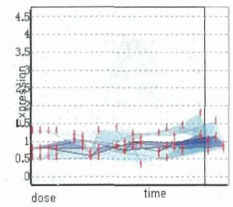

partner and localizer of BRCA2

TTG085-L_SpNC_0 1434808_at
Trybutyltin
Palb2



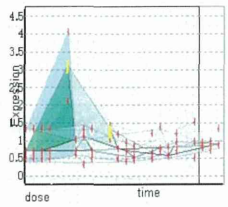

partner and localizer of BRCA2

TTG145-L_SpNC_0 1434808_at
Tributyltin chloride x Tributyltin chloride
Palb2



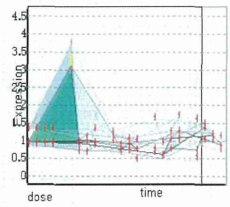

partner and localizer of BRCA2

TTG044-L_SpNC_0 1434808_at
Clofibrate
Palb2



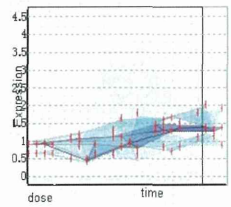

partner and localizer of BRCA2

TTG129-L_SpNC_0 1434808_at
CCl4 x Clofibrate
Palb2



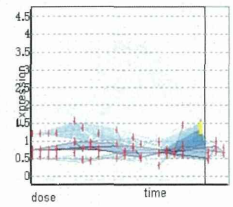

partner and localizer of BRCA2

TTG119-L_SpNC_0 1434808_at
Carbon tetrachloride
Palb2



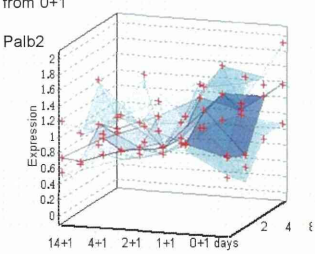

partner and localizer of BRCA2

TTG130-L_SpNC_0 1434808_at
CCl4 x CCl4
Palb2



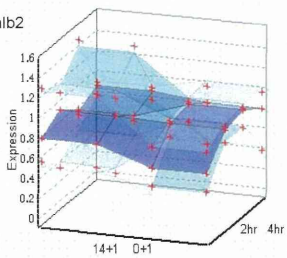

partner and localizer of BRCA2

CCL4 Liver Vehicle 119 130 189 190 191 from 0+1 1434808_at
Palb2




partner and localizer of BRCA2

TBT 0+1 14+1 Baseline 085 145 1434808_at
Palb2




partner and localizer of BRCA2