METABOLON
mView™

Metabolism of XXX gene expression in a human ovarian cell cancer line

XXXX-XX-11VW
January 30, 2012

Study Overview

Study Objective

The goal of this study was to better understand metabolic effects of the XXX gene in the human ovarian cell cancer line XXX via knockdown of XXX (using two different vectors), exogenous expression of wild-type XXX (with appropriate control), and comparison with parental control cells.

Study Design

Global biochemical profiles were compared across the following groups of XXX cells.

Group	n	Description
Parental	5	XXX parental cells
GR-2	5	XXX with non-targeted control shRNA vector
GR-3	5	XXX with XXX shRNA vector 2
GR-4	5	XXX with XXX shRNA vector 1
GR-5	5	XXX-GR-4 with empty control vector
GR-6	5	XXX-GR-4 with wild-type XXX expression

Statistical Summary

Welch's Two Sample *t*-tests

-used to determine whether the means of two populations are different.

***p*-value: evidence that the means are different**

- $p \leq 0.05$ was taken as significant
- p value trend of $0.05 < p < 0.10$ identified biochemicals approaching significance.

Statistical Comparisons Welch's Two-Sample t-Test								
Significantly Altered Biochemicals	GR-3 / GR-2	GR-4 / GR-2	GR-4 / GR-3	GR-2 / Parental	GR-3 / Parental	GR-4 / Parental	GR-6 / GR-5	GR-5 / GR-4
Total biochemicals $p \leq 0.05$	188	193	53	26	142	148	28	86
Biochemicals (↑|↓)	74|114	64|129	15|38	24|2	75|67	67|81	7|21	70|16
Total biochemicals $0.05 < p < 0.10$	19	28	28	29	32	33	25	34
Biochemicals (↑|↓)	9|10	10|18	8|20	26|3	13|19	11|22	5|20	24|10

From analysis of the dataset total 338 named biochemicals detected

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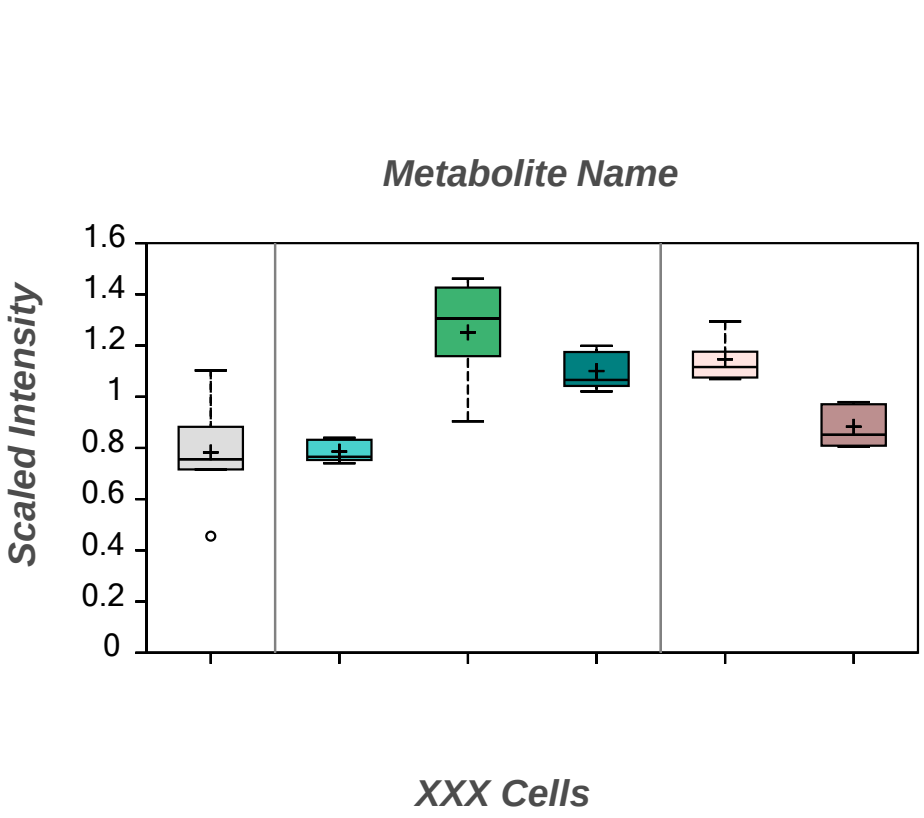
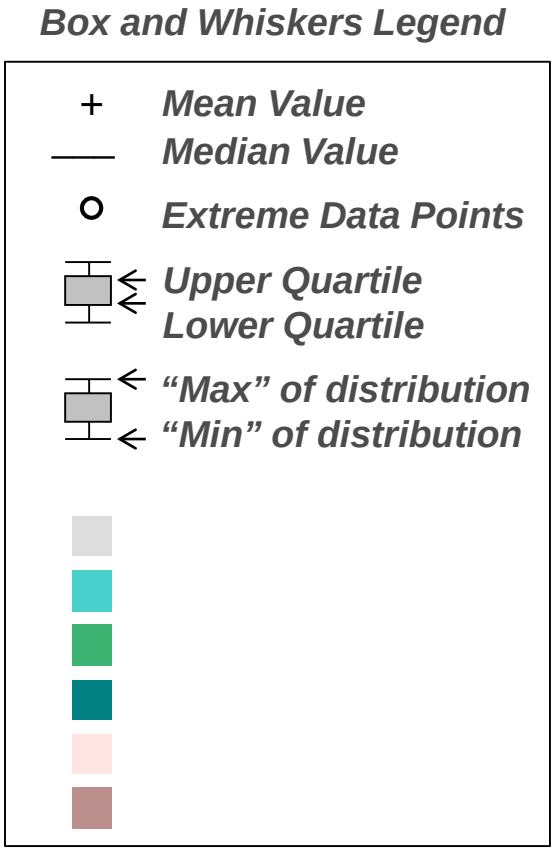
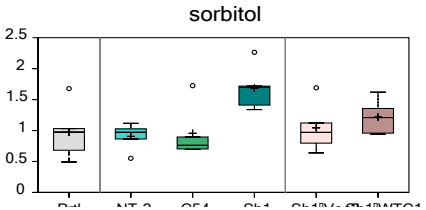
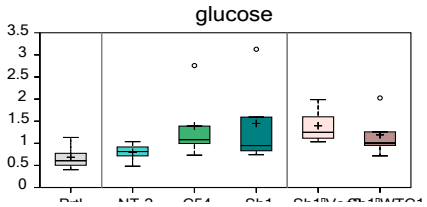
Data Display

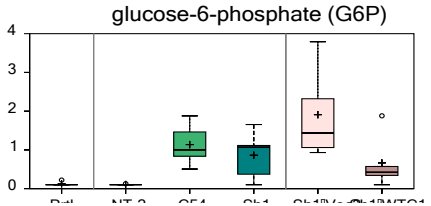


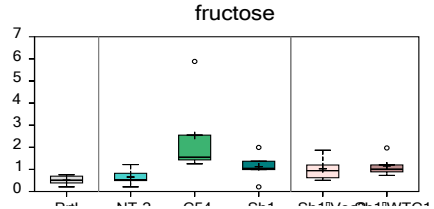


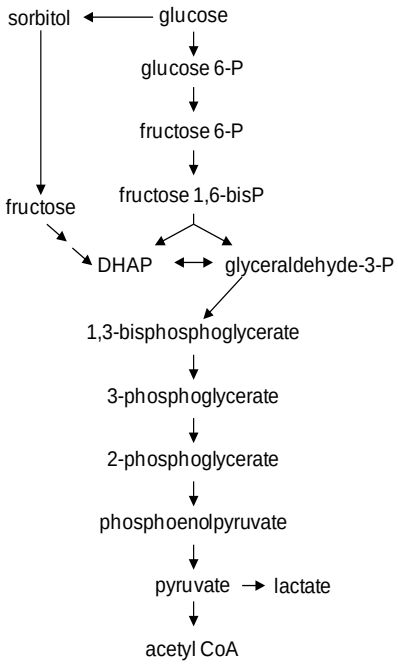
Warburg Effect

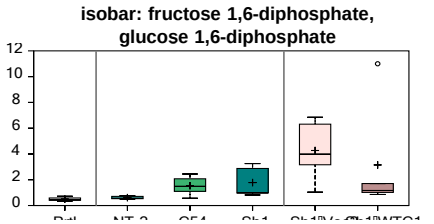


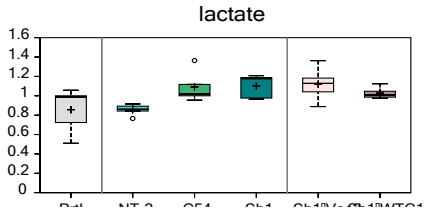


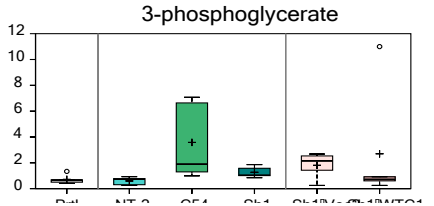


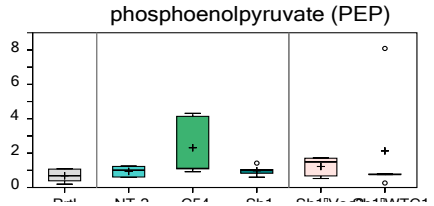












- Glucose utilization and conversion to lactate was increased in XXX cells with downregulation of XXX expression.
  - Expression of wild-type XXX partially reversed this phenotype, although transduction with a CMV-driven vector increased levels of glycolytic intermediates.

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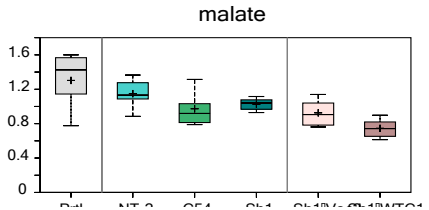
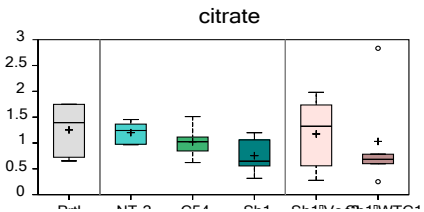
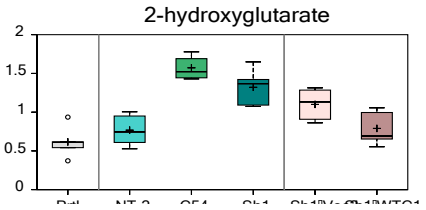
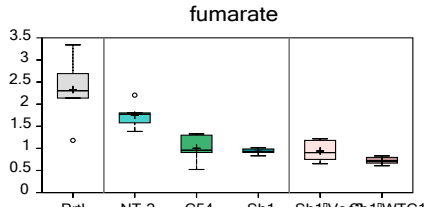
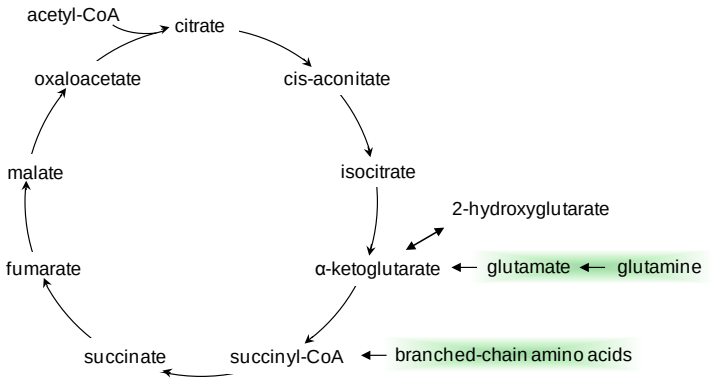
TCA Cycle and 2-Hydroxyglutarate

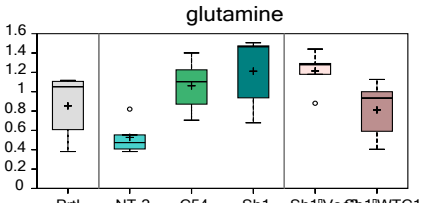


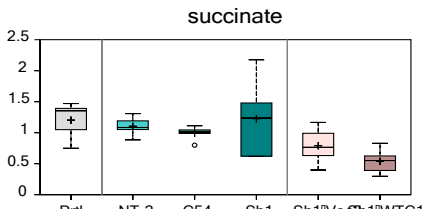


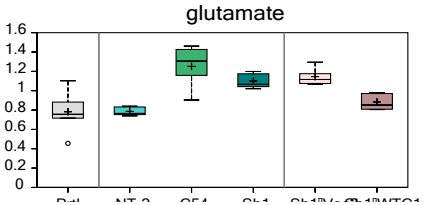












- Consistent with the Warburg effect, levels of TCA cycle intermediates were reduced with downregulation of XXX expression in XXX cells.
  - Rescue with wild-type XXX expression did not reverse this decrease in TCA cycle activity.
- Glutamine/glutamate and levels of the “onco-metabolite” 2-hydroxyglutarate were significantly increased in XXX cells with decreased XXX expression and this was reversed with wild-type XXX expression.
- Transduction with a CMV-driven vector may affect anaplerotic contribution of branched-chain amino acids to the TCA cycle.

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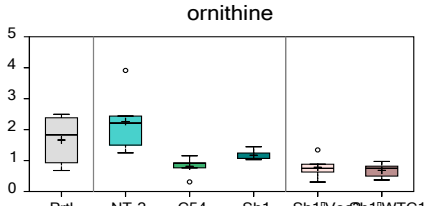
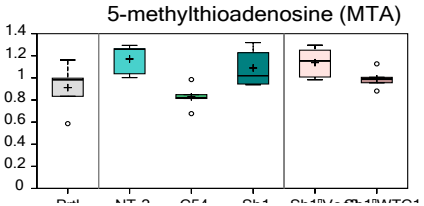
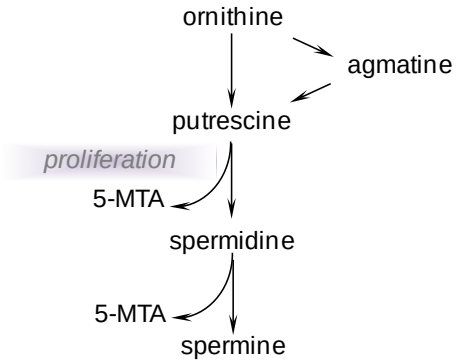
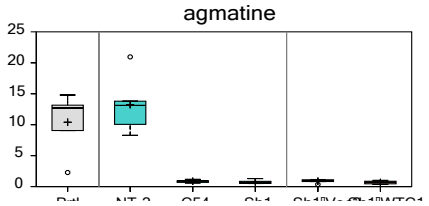
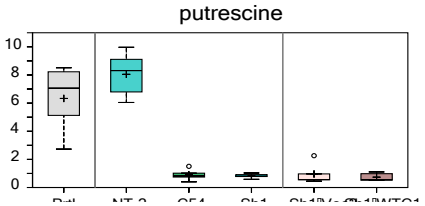
Polyamines

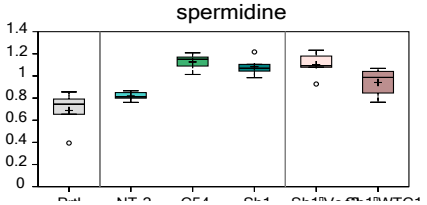


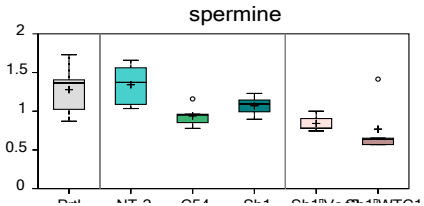












- Polyamines are associated with cellular growth and proliferation.
  - Spermidine was significantly increased with knockdown of XXX expression in XXX cells and this was reversed with expression of wild-type XXX.
  - Transduction with a CMV-driven vector may reduce synthesis of spermine.

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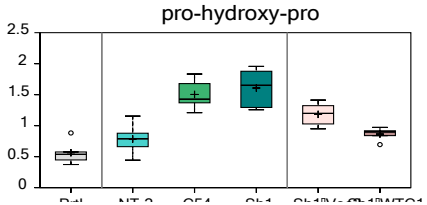
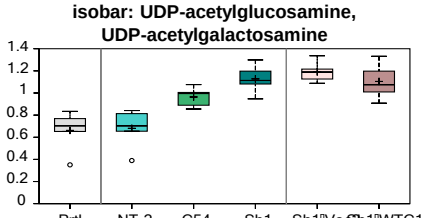
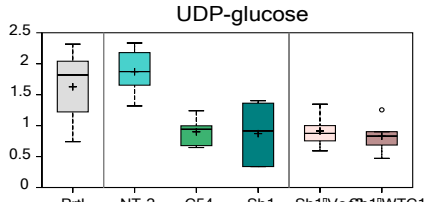
Extracellular Matrix Remodeling

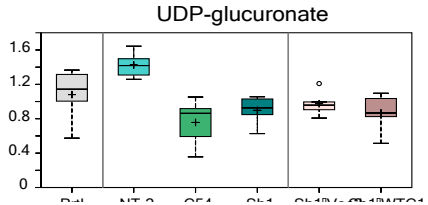
- XXX modulates activity of XXX in the extracellular matrix.
- Prolylhydroxyproline (pro-hydroxy-pro) is a dipeptide and a marker of collagen and ECM degradation.
    - Pro-hydroxy-pro was significantly increased with knockdown of XXX expression and significantly reduced with expression of wild-type XXX.

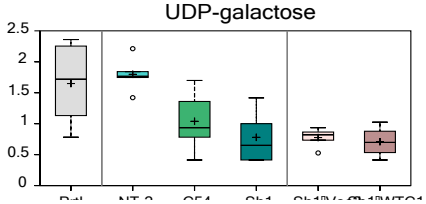


- Several UDP-glycosylation moieties were altered by reduced XXX expression but were unchanged with wild-type XXX expression.









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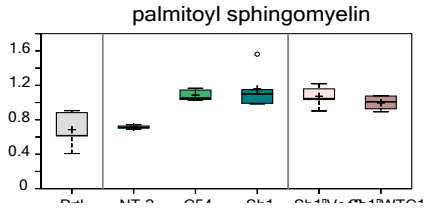
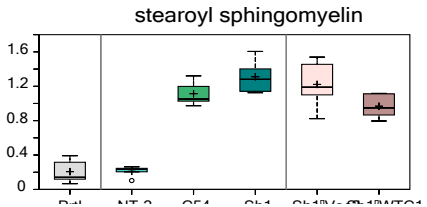
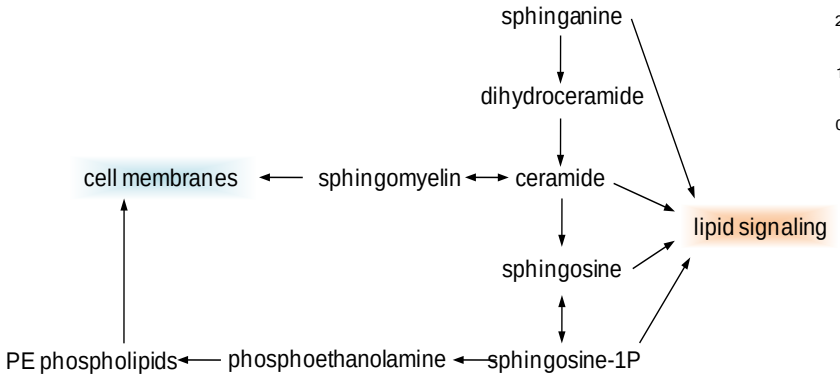
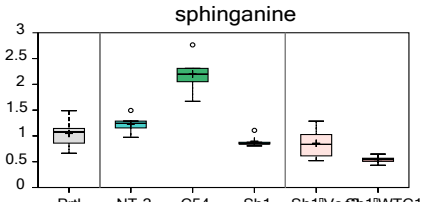
Sphingolipids

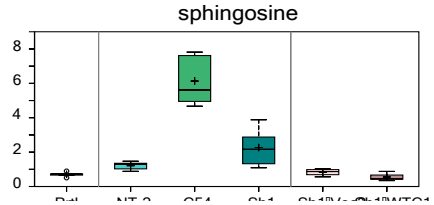
- Increased sphingolipid production was observed with downregulation of XXX expression, suggestive of changes in lipid signaling and/or membrane remodeling in XXX cells.
  - This effect was partially reversed with expression of wild-type XXX.
- Transduction with lentiviral and CMV-driven vectors may affect sphingosine levels.











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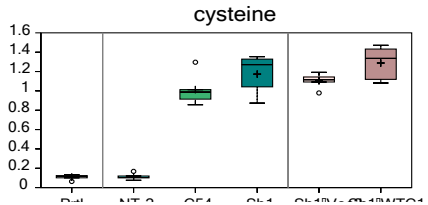
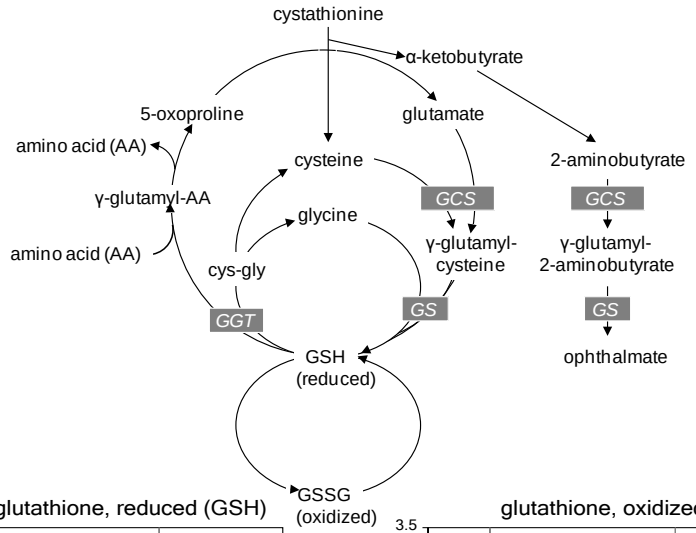
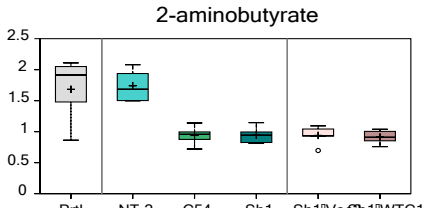
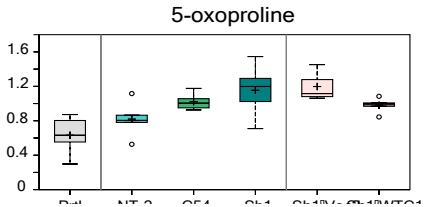
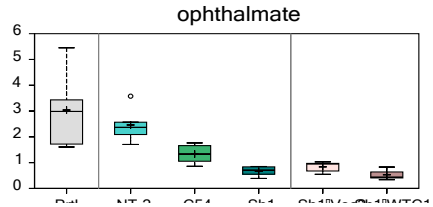
Glutathione and Redox Status

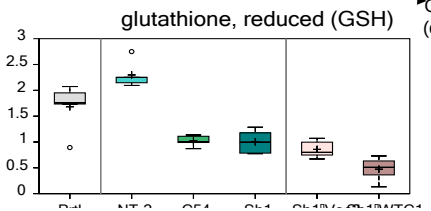


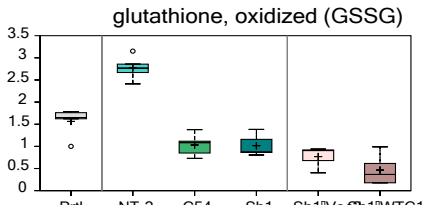


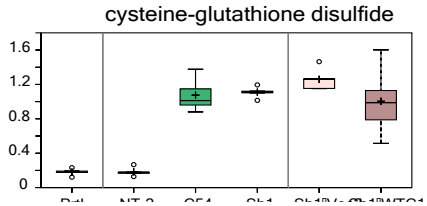


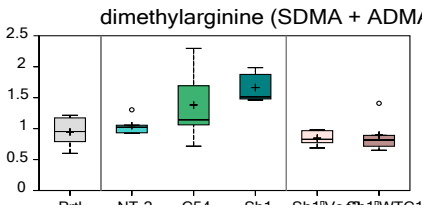












- There was a reduction in glutathione and increase in markers of oxidative stress with downregulation of XXX expression in XXX cells.
  - Expression of wild-type XXX did not increase glutathione synthesis or improve redox status.

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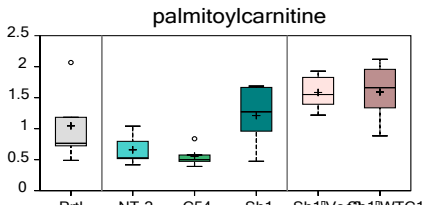
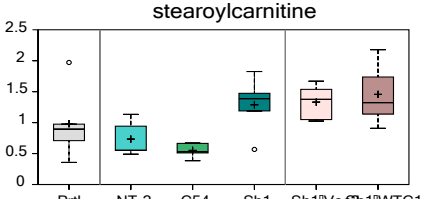
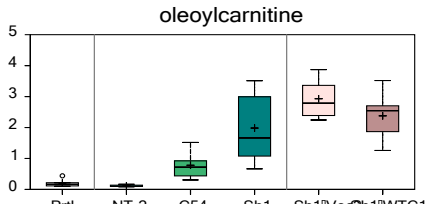
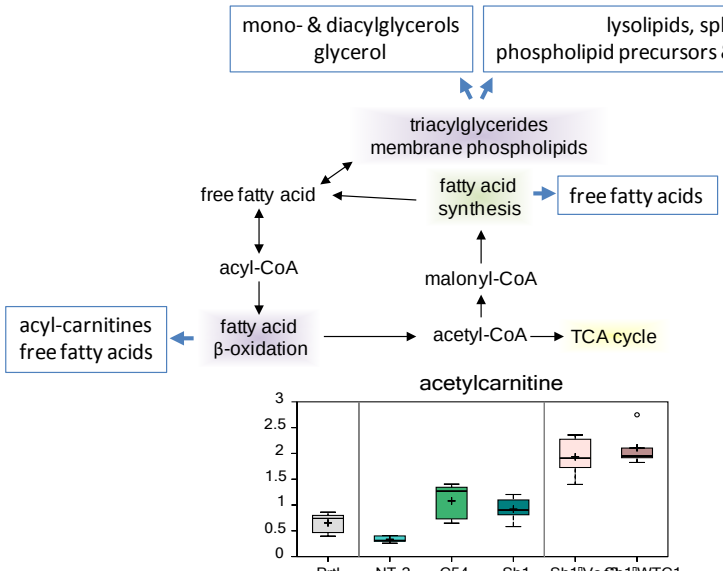
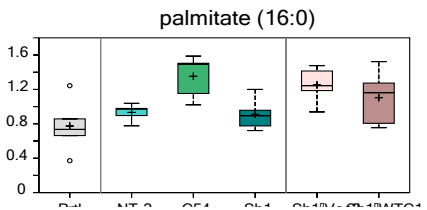
Lipid Metabolism

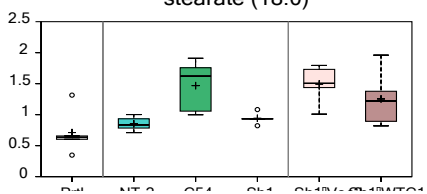


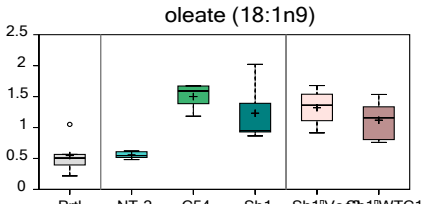






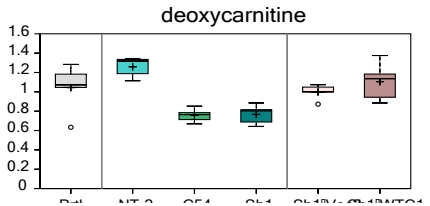


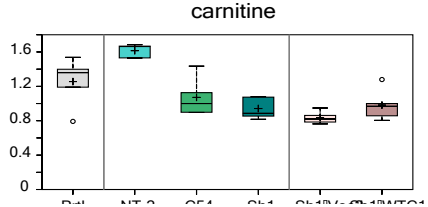




- Levels of many free fatty acids were increased with reduction of XXX expression, suggestive of increased uptake from the media and/or synthesis.
- Expression of wild-type XXX did not significantly affect free fatty acids
- Transduction with lentiviral and CMV-driven vectors had significant effects on levels of free fatty acids and acylcarnitines.
- Carnitine synthesis was also affected by modulation of XXX expression and vector transduction.

deoxycarnitine → carnitine





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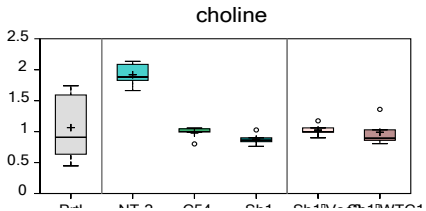
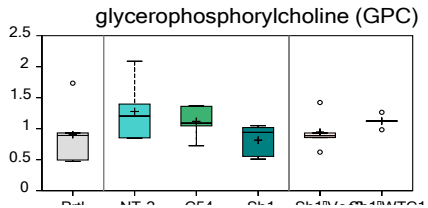
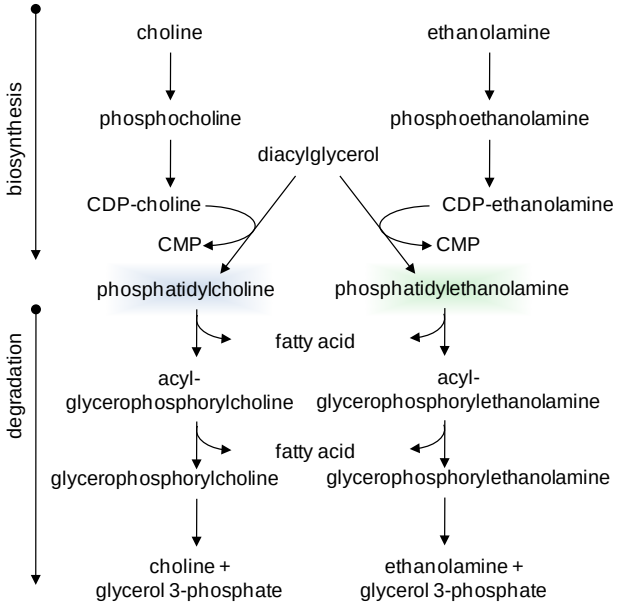
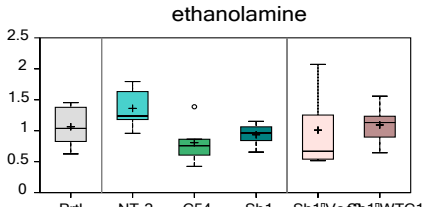
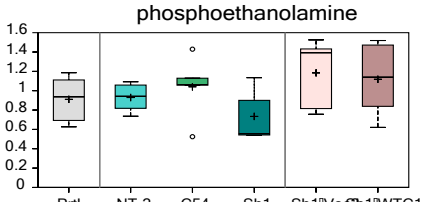
Membrane Lysolipids

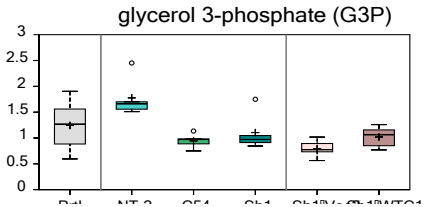












- Downregulation of XXX in XXX cells resulted in alteration of membrane lysophosphocholines and lysophosphoethanolamines.
  - Changes were suggestive of increased synthesis/reduced degradation of membrane lysolipids, possibly related to cellular growth and proliferation.
- There were non-significant trends for expression of wild-type XXX to reduce levels of lysolipids and increase products of lysolipid degradation, indicative of decreased membrane remodeling and/or changes in β-oxidation of fatty acids.
- Transduction with a CMV-driven promoter increased many lysolipids.

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