

Supplementary Data S2- Cell profiler pipeline used for analysis for Colocalization mTOR and LAMP1 protein in PCCs.

SLC38A10 Regulate Glutamate Homeostasis and modulate the AKT/TSC2/mTOR Pathway in mouse cortex cells

CellProfiler data: plotting

Project: Rekha Tripathi - mTOR, Lamp

Data: 20200320_mTOR_LAMP_BIOVIS_2020_AT_63X

- Ch1: DAPI
- Ch2: mTOR: protein of interest
- Ch3: Lamp: lysosome marker
- Conditions: Basal, starved, refeed
- CP Pipeline: 2020421_imageInt_correlation.cproj

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Importing packages and data

```
In [1]: import pandas as pd
import re
import seaborn as sns
import matplotlib.pyplot as plt
```

```
In [2]: path_cpout = r"C:\Users\Anna\Documents\projects\Rekha_Tripathi\20200421_Imagei
ntensity_correlation_higherThres"
image = pd.read_csv(path_cpout + "\MyExpt_image.csv")
nuclei = pd.read_csv(path_cpout + "\MyExpt_nuclei.csv")
cytoplasm = pd.read_csv(path_cpout + "\MyExpt_Cytoplasm.csv")
```

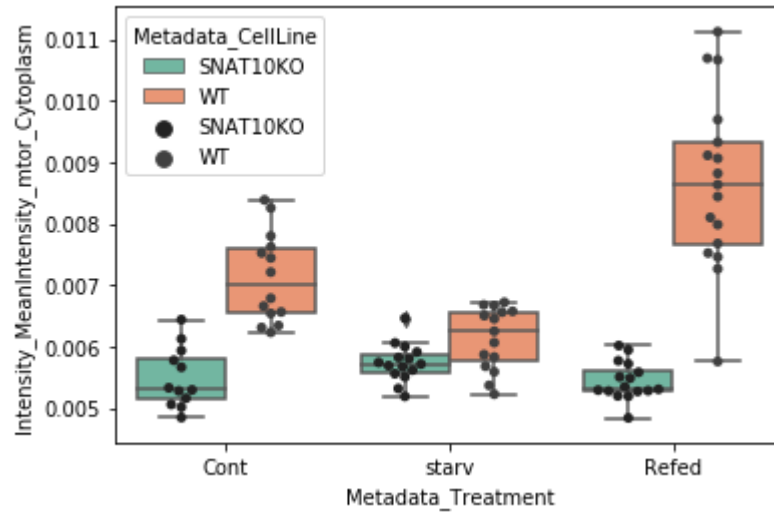
```
In [3]: #list(image.columns)
```

```
In [4]: image['average_areaPerCell_mTOR'] = image['AreaOccupied_AreaOccupied_mTOR_mask
ed_Cytoplasm']/image['Count_Cytoplasm']
image['average_areaPerCell_lamp'] = image['AreaOccupied_AreaOccupied_Lamp_mask
ed_Cytoplasm']/image['Count_Cytoplasm']
image['average_areaPerCellDonut_mTOR'] = image['AreaOccupied_AreaOccupied_mTOR
_masked_Cytoplasm_donut']/image['Count_Cytoplasm']
```

mTOR: mean intensity image within cytoplasm (Propagation, manual)

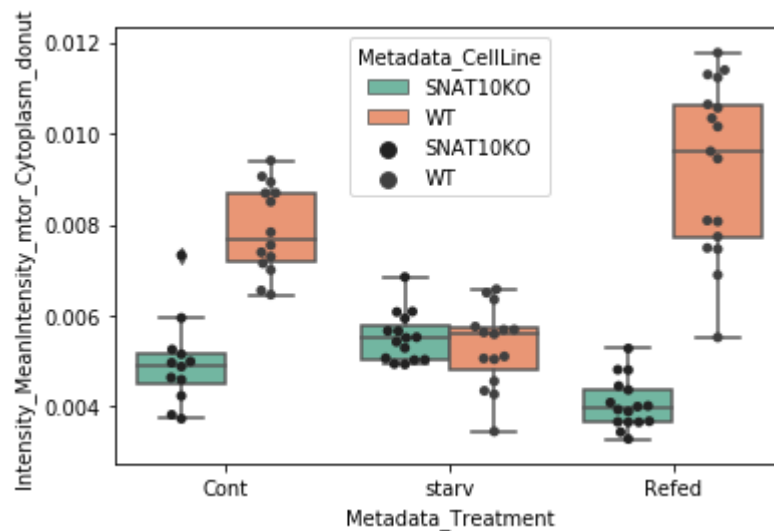
The level of mTOR stays the same under starvation in the KOKO mutant

```
In [5]: sns.boxplot(x='Metadata_Treatment', y= 'Intensity_MeanIntensity_mtor_Cytoplasm', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine', data=image, palette="Set2")
ax = sns.swarmplot(x='Metadata_Treatment', y= 'Intensity_MeanIntensity_mtor_Cytoplasm', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine', data=image, color=".25", dodge=True)
```



control: mTOR: mean intensity image within "donut" cytoplasm (Distance-N, 100px)

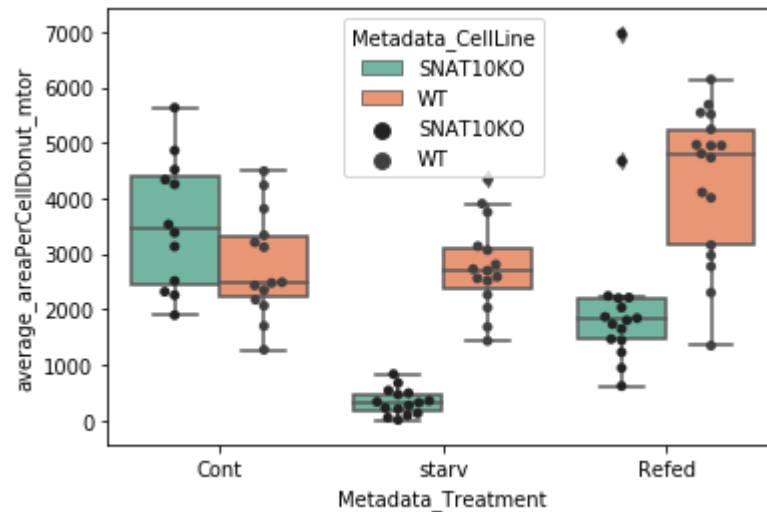
```
In [6]: sns.boxplot(x='Metadata_Treatment', y= 'Intensity_MeanIntensity_mtor_Cytoplasm_donut', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine', data=image, palette="Set2")
ax = sns.swarmplot(x='Metadata_Treatment', y= 'Intensity_MeanIntensity_mtor_Cytoplasm_donut', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine', data=image, color=".25", dodge=True)
```



mTOR-area over threshold, cytoplasm "donut-cells"

KOKO has less aggregates under starved condition, recovery when refed. WT is always in aggregates

```
In [16]: sns.boxplot(x="Metadata_Treatment", y='average_areaPerCellDonut_mtor', order=
["Cont", "starv", "Refed"], hue= 'Metadata_CellLine',
data=image, palette="Set2")
ax = sns.swarmplot(x='Metadata_Treatment', y= 'average_areaPerCellDonut_mtor',
order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine',
data=image, color=".25", dodge=True)
```

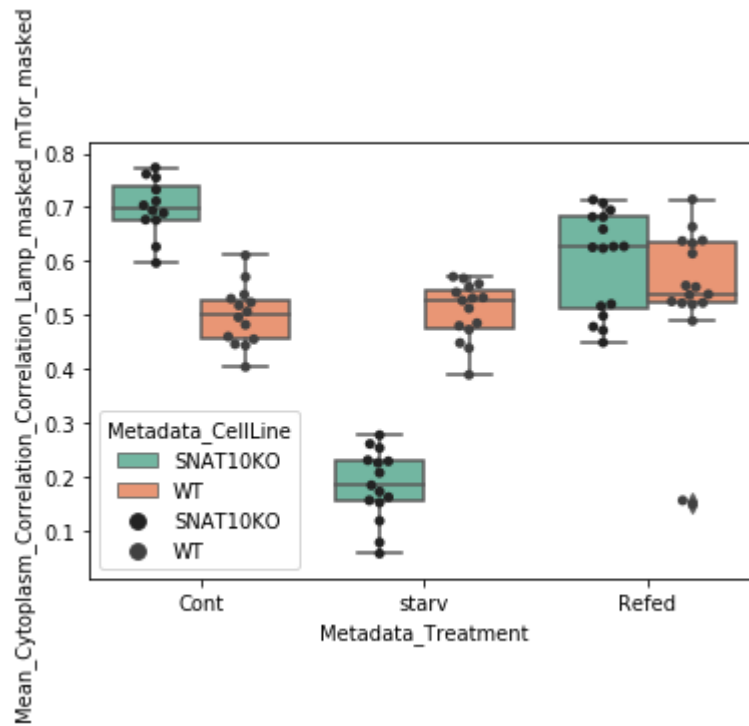


Co-localisation correlation

Pearson

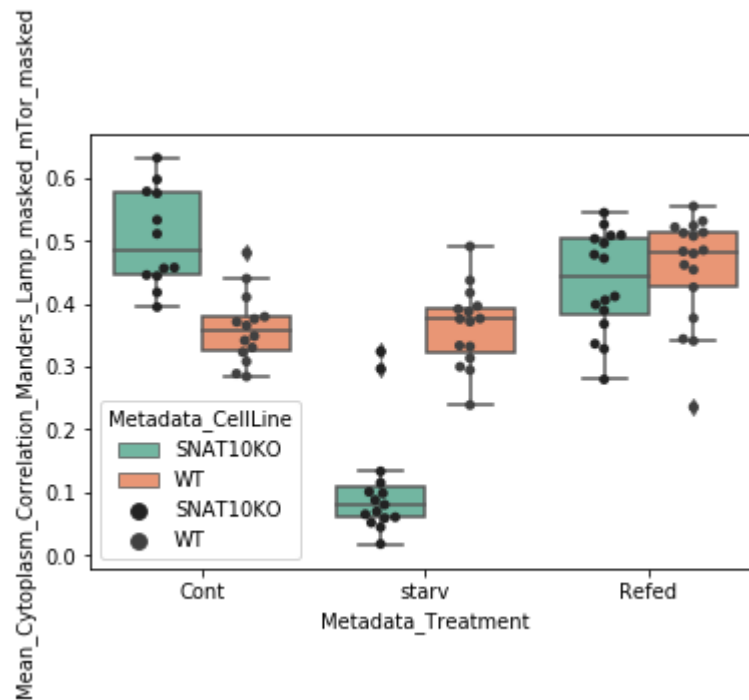
SNATKO: SNATKO starved: few mTOR aggregates do not overlap with lamp; SNATKO refed: fewer objects that exist overlapping with lamp WT. mTOR aggregates always overlap with lamp.

```
In [8]: sns.boxplot(x="Metadata_Treatment", y='Mean_Cytoplasm_Correlation_Correlation_Lamp_masked_mTor_masked', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine',
                  data=image, palette="Set2")
ax = sns.swarmplot(x='Metadata_Treatment', y= 'Mean_Cytoplasm_Correlation_Correlation_Lamp_masked_mTor_masked', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine',
                  data=image, color=".25", dodge=True)
```



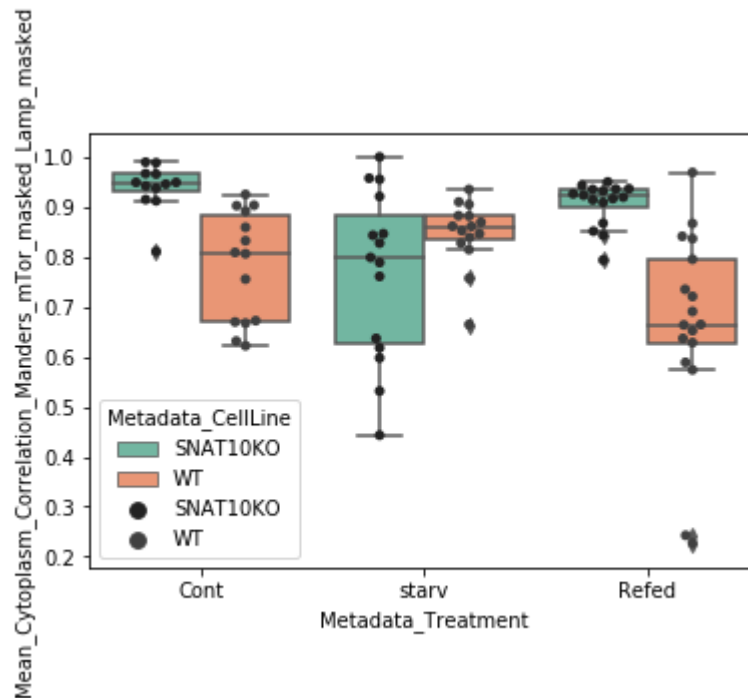
Manders M1

```
In [12]: sns.boxplot(x="Metadata_Treatment", y='Mean_Cytoplasm_Correlation_Manders_Lamp_masked_mTor_masked', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine',  
                  data=image, palette="Set2")  
ax = sns.swarmplot(x='Metadata_Treatment', y= 'Mean_Cytoplasm_Correlation_Manders_Lamp_masked_mTor_masked', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine',  
                  data=image, color=".25", dodge=True)  
# 'Mean_Cytoplasm_Correlation_Manders_mTor_masked_Lamp_masked',
```



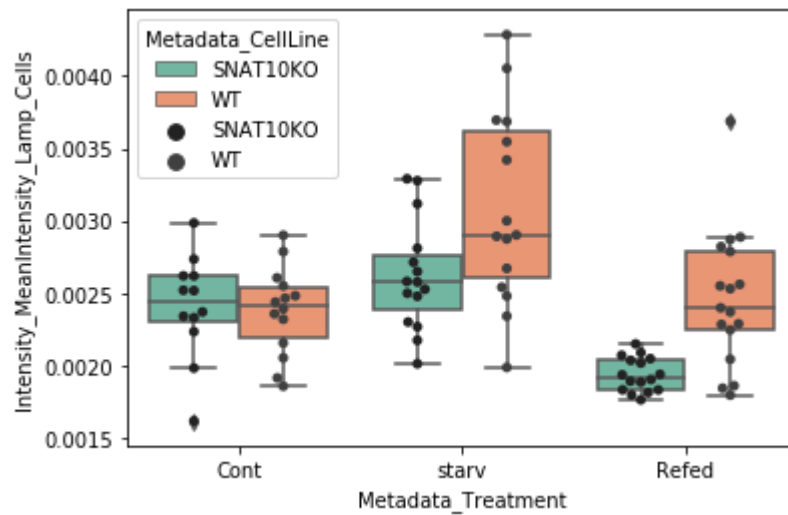
Manders M2

```
In [13]: sns.boxplot(x="Metadata_Treatment", y='Mean_Cytoplasm_Correlation_Manders_mTor_masked_Lamp_masked', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine', data=image, palette="Set2")  
ax = sns.swarmplot(x='Metadata_Treatment', y= 'Mean_Cytoplasm_Correlation_Manders_mTor_masked_Lamp_masked', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine', data=image, color=".25", dodge=True)  
# 'Mean_Cytoplasm_Correlation_Manders_mTor_masked_Lamp_masked'
```



Lamp: mean intensity within cells

```
In [14]: ax = sns.boxplot(x='Metadata_Treatment', y= 'Intensity_MeanIntensity_Lamp_Cells', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine', data=image, palette="Set2")
ax = sns.swarmplot(x='Metadata_Treatment', y= 'Intensity_MeanIntensity_Lamp_Cells', order=["Cont", "starv", "Refed"], hue= 'Metadata_CellLine', data=image, color=".25", dodge=True)
```



In []: