THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

# sLats1 Involvement in Cellular Proliferation and the Hippo Cell Signaling Pathway

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## ABSTRACT

Many cell signaling pathways exist in which one or more components are directly implicated in tumor suppression or tumorigenesis. The Hippo pathway for control of cellular proliferation and apoptosis is one of these pathways, and several of the component proteins are linked to overall cell proliferation control. Two of these proteins, LATS1 and YAP, are directly involved in cell-cell inhibition of proliferation and tumor suppression, yet the exact mechanisms of these mammalian proteins and their *Drosophila* homologs are not fully known. The mechanisms of regulation of the Hippo pathway are not yet entirely known, but a LATS1 variant protein, short LATS1 (sLats1) may be involved in the regulation of the proteins LATS1 and YAP, and could therefore be a potential target for the development of novel tumor suppressing therapies. The role of sLats1 in the regulation of the Hippo pathway in *Drosophila melanogaster* and human cells is herein explored, and sLats1 overexpression is found to result in decreased cellular proliferation in *Drosophila* wing plates, indicating a possible tumor suppressive function. The dominant effect of sLats1 overexpression in HEK293A cells has yet to be determined, but results suggest several possible outcomes related to sLats1 regulation of the proteins LATS1 and YAP.

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# Chapter 1

## Hippo Tumor Suppressor Pathway

There are many biochemical pathways in living organisms for the regulation of cellular growth and proliferation. Mistakes and mutations in the components of these pathways can lead to unusual patterns of cell growth and proliferation such as those seen in malignant cancer cells, or result in increased cell death, called apoptosis, as seen in many neurodegenerative disorders. Many of these biochemical pathways are highly conserved between species, with the proteins involved in the pathway either existing in a similar sequence form between two species, or existing as similarly functioning but unique analogs. The Hippo tumor suppressor pathway is one such pathway that was originally identified in *Drosophila melanogaster* and has since been characterized by its implicated components and mechanisms in *Drosophila* and other higher eukaryotes, including mammals (Meng et al. 2016).

## Core Components of the Hippo Pathway in Drosophila

The Hippo pathway primarily consists of a kinase cascade, transcriptional co-activators, and DNA-binding partners, with regulation by intrinsic cellular machinery (Meng et al. 2016). The pathway is summarized graphically in Figure 1. In *Drosophila*, the core pathway involves Hippo (Hpo) kinase phosphorylating and activating Warts (Wts). In turn, Wts phosphorylates and restricts the transcriptional activating activity of Yorkie (Yki). Activated Yki can translocate into the nucleus to bind Scalloped (Sd) transcription factor, inducing expression of a range of

genes involved in proliferation and apoptosis. Inactive, phosphorylated Yki is retained in the cytoplasm by binding 14-3-3, and transcription via Sd is restricted by the binding of Tgi in the absence of competition by Yki (Meng et al. 2016).

The Hippo kinase cascade is initiated by the phosphorylation and activation of Hpo by Tao kinase, and also requires the adaptor protein Merlin (Mer) and scaffold proteins Salvador (Sav) and Mob as tumor suppressor (Mats) in order to activate Wts. Two other kinases, Misshapen (Msn) and Happyhour (Hppy) work to phosphorylate Wts independently of Hpo, and thus independently inactivate Yki (Meng et al. 2016).

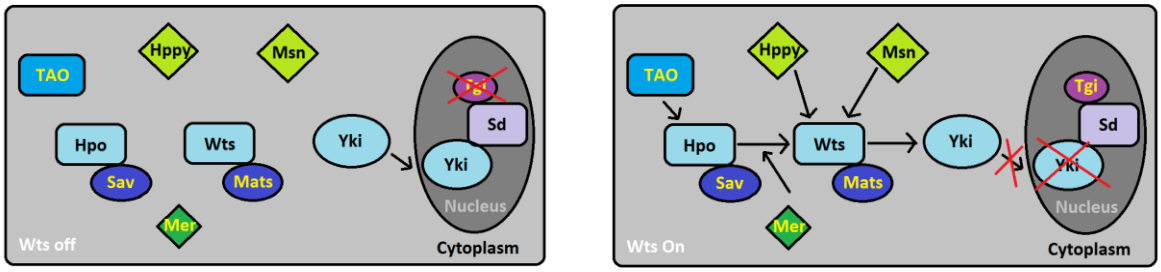


**Figure 1 - Hippo Core Pathway in *Drosophila***

## Core Components of Hippo Pathway in Mammals

In mammals, the core of the Hippo pathway is highly similar to that in *Drosophila*, but with some additional complexity due to the existence of multiple homologs for single fly proteins. The pathway is summarized graphically in Figure 2. In mammals, the mammalian Ste20-like kinases 1 and 2 (MST1/2) act as homologs for the *Drosophila* Hpo, and phosphorylate and activate large tumor suppressors 1 and 2 (LATS1/2) as homologs of *Drosophila* Wts. In turn, LATS1/2 phosphorylate and restrict the activity of the transcriptional co-activators Yes-associated protein (YAP) and Transcriptional co-activator with PDZ-binding motif (TAZ), both

homologs of *Drosophila* Yki. Active YAP and TAZ are able to translocate to the nucleus where they bind the TEAD transcription factor family, homologs of *Drosophila* Sd. Inactive, phosphorylated YAP/TAZ is retained in the cytoplasm by 14-3-3 or targeted for degradation (Meng et al. 2016).

The Hippo kinase cascade in mammals is initiated by the Tao family of kinases (TAOK1/2/3), which phosphorylate MST1/2, in turn phosphorylating and activating LATS1/2. The cascade also involves the adaptor protein NF2/Merlin and scaffold proteins SAV1 and MOB1A/B, homologs of *Drosophila* Sav and Mats, respectively. Two groups of mitogen-activated protein kinase kinase kinase kinase (MAP4Ks), MAP4K1/2/3/5 and MAP4K4/6/7, homologs of *Drosophila* Hppy and Msn, can directly phosphorylate and activate LATS1/2 independently of MST1/2, and thus independently inactivate YAP/TAZ (Meng et al. 2016).

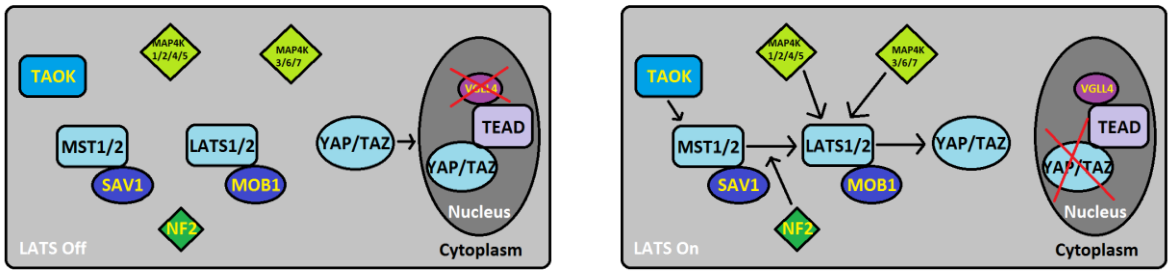


**Figure 2 - Hippo Core Pathway in Mammals**

## LATS1 and YAP

The two major proteins examined in the course of this experiment are the mammalian proteins LATS1 and YAP, and their associated *Drosophila* homologs. LATS1 in humans is a 1130 amino acid long polypeptide, and contains a kinase domain between amino acids 705 and 1013 (Avruch et al. 2012). The protein YAP functions as an oncoprotein, and is inhibited by cell

density via the Hippo pathway (Zhao et al. 2007). Phosphorylation of the protein results in its export into and retention in the cytoplasm, where it is unable to activate transcription of genes for cellular proliferation (Meng et al. 2016). Overexpression of YAP is able to overcome the inhibition of cellular proliferation by cell-cell contact, implicating the protein in several types of cancers (Zhao et al. 2007). The protein Yorkie in *Drosophila* performs these same functions as a fly ortholog of YAP (Huang et al. 2005). LATS1 overexpression in mammalian cells suppresses YAP phenotypes while similar expression of LATS2 has not demonstrated the same effect (Zhang et al. 2008). Further, LATS1 works to inactivate YAP by suppressing its transcription regulation of several proliferative genes through sequestering phosphorylated YAP in the cytoplasm, effectively preventing the protein's entry into the nucleus (Hao et al. 2008).

## sLats1

The protein sLats1 is a short, truncated version of the full cellular protein LATS1 without its kinase domain and therefore lacking its kinase function. This protein likely has regulatory effects on several proteins in the Hippo pathway, including the proteins LATS and YAP, given that N-terminal truncated LATS1 induces downregulation of LATS2 and results in stable accumulation of YAP protein in mammalian cells, further resulting in tumorigenesis from these cells (Yabuta et al. 2013). sLats1 also possess the ability to interact with LATS1, and so could have regulatory effects on proteins downstream, namely the transcriptional coactivator activity of YAP. As a shorter version of LATS1, sLats1 could result in an inhibitory effect on LATS1 and therefore promote YAP activity, leading to over-proliferation and tumorigenesis activity in cells overexpressing sLats1. The exact mechanism of sLats1 action on the various proteins of the

Hippo pathway is not yet known, but the overall effect of sLats1 overexpression on cellular proliferation and apoptosis can be demonstrated in specific fly tissues, as shown in the following chapters. The dominant effect of overexpression of this protein on the overall proliferation of human cells promises to be experimentally determined by developing cell lines that overexpress the sLats1 protein, as discussed in later chapters.

# Chapter 2

## Generating sLats1 Overexpression in *Drosophila*

There are several techniques used to generate mutant fly lines in *Drosophila melanogaster*, including random mutagenesis and microinjection. In this experiment, a fly line for the overexpression of the gene of interest, sLats1, was generated through microinjection of a plasmid construct containing the sLats1 gene into a fly line, then crossing the newly established stable line to flies containing genes for the cellular machinery to selectively express sLats1. The resulting fly line was allowed to mature, and the adult phenotype was analyzed in comparison to a control fly line.

## Plasmid Construction

The technique of microinjection involves the construction of a plasmid containing the gene for the protein of interest as well as any necessary associated cellular machinery and the injection of this plasmid into the embryos of a pre-existing cell line. This experiment utilized the UAS-GAL4 system for the controlled expression of the gene of interest in *Drosophila melanogaster*. GAL4 is a yeast transcriptional activator that selectively binds to a specific set of DNA sequences referred to as Upstream Activating Sequences (UAS) and initiates transcription (Duffy 2002). Because this transcription factor is not native to *Drosophila*, sequences containing the UAS and any gene of interest can be inserted into fly embryos and remain un-transcribed

until the sequence encoding the GAL4 protein is also transposed into the *Drosophila* genome. This mechanism is highly useful in fly genetics, as UAS-GAL4 can be used to express novel genes, overexpress endogenous proteins, or even silence protein activity through introduction of sequences encoding hairpin RNA to create RNAi for the targeted knockdown of a protein of interest.

The plasmid construct in this experiment utilized a pUAST vector (vector map in Figure 3) and flag-sLats1 insert (sequence in Appendix A), which when combined result in a plasmid containing the sLats1 gene, a p-element, a flag antibody tag, a fly eye-color marker (white), and an ampicillin resistance site, as well as many targets for enzyme digestion. The sLats1 insert (2.2 kb) was amplified by PCR (30 cycles, 10 sec at 98°C denaturation, 30 sec at 55°C annealing, 75 sec at 72°C extension) using Phusion polymerase. The PCR product underwent gel electrophoresis (agarose 10%) to confirm successful amplification of the insert, then was purified (OMEGA E.Z.N.A.® MicroElute DNA Clean-Up Kit). Both insert and vector were digested with enzymes Kpn1 and Xba1, then run through gel electrophoresis for purification (agarose 10%) and extracted (OMEGA E.Z.N.A.® Gel Extraction Kit). Insert and vector were ligated using T4 DNA ligase, then transformed into competent bacteria and spread on ampicillin plates (50 ug/mL). Colonies were then selected and placed into ampicillin broth and amplified using PCR with Phusion polymerase (same as above) to confirm presence of plasmid. Successful colonies were inoculated into ampicillin broth (50 ug/mL). Successful colony cultures were purified through miniprep (Promega Wizard® Plus SV Minipreps DNA Purification System) and digested with the enzymes Kpn1 and Xba1. Digestion products then underwent gel electrophoresis (10% agarose) to confirm presence of sLats1 insert, and successful plasmids were sent to the Huck Institute Genomics Core Facility to confirm proper plasmid construct.

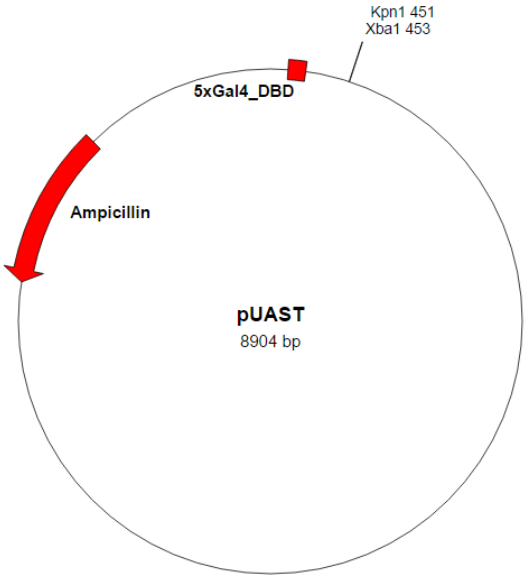


**Figure 3 - pUAST Vector, based on Brand and Perrimon (1993), drawn with Savvy**

## Plasmid Expression in Fly Cells

After confirmation via sequencing, successful plasmids were expressed in S2 fly cell cultures. S2 fly cells cultured in media (Schneider's Insect Medium, Fetal Bovine Serum) for several days prior were transfected with PUAST-flag-sLats1 plasmid DNA and Gal4 (150 ng Gal4 + 150 ng empty pUAST/150 ng pUAST-flag-sLats1/300 ng pUAST-flag-sLats1), then allowed to culture (3 days). Protein was then extracted from cells using lysis buffer on ice for protein analysis via Western Blot. The Western Blot gel was stained using an anti-flag primary antibody (Sigma-Aldrich ANTI-FLAG™ antibody produced in rabbit F7425) and anti-rabbit secondary antibody (Sigma-Aldrich Anti Rabbit IgG Peroxidase A0545). The resultant chemiluminescent staining was visualized under photospectometry to prove expression of sLats1 protein, and then compared to endogenous expression levels of cells transfected with an empty pUAST vector to determine base overexpression level.

## Microinjection of Plasmid into Fly Larvae

After examining sLats1 overexpression via transformation of S2 fly cells with plasmid construct, fly embryos were microinjected with the pUAST-flag-sLats1 plasmid. Collected embryos were glued to microscope slides and injected with enough of the PUAST-flag-sLats1 plasmid to enter embryo without swelling (4 ug plasmid per 10uL). Injected embryos were permitted to hatch, then were transferred to fresh fly vials and allowed to mature. Progeny of the injected embryos expressing the plasmid as demonstrated by the eye color marker were removed to a fresh fly vial. Virgin females from this fly line as well as CantonS were collected in preparation for the test cross. A full procedure of this microinjection process is detailed in "The Use of P-Element Transposons to Generate Transgenic Flies" (Bachmann and Knust, 2008).

## Test Cross and Wing Preparation

In this experiment, a previously established fly line with a GAL4 driver and double balancer chromosomes, en-GAL4/SM1, Sb-/TM6 was crossed with the microinjected sLats1 fly line and the CantonS control line. The engrailed GAL4 driver selectively expresses GAL4 in only cell lines where engrailed is expressed. This particular cross resulted in progeny in which the sLats1 gene had been overexpressed in the posterior portion of the wing disc. Successful progeny were collected and allowed to mature. The flies were separated by sex, and the wings of mature progeny were removed (one wing per fly) and fixed with clear glue to a microscope slide.

# Chapter 3

## sLats1 in Adult *Drosophila*

Many techniques exist to quantify the differences in fly lines when examining the impact of a gene overexpression or knockout. For genes implicated in cellular proliferation and apoptosis, these techniques often involve quantifying organ size. As sLats1 overexpression in particular is associated with a pathway involved in cellular proliferation, its action in vivo can be quantified in such a manner.

### Sensitized Wing Size Assay

One such technique to quantify organ size is a sensitized assay for wing size, given that sLats1 was overexpressed in the posterior portion of the wing. In this specific assay, the size of the posterior portion of the adult fly wing was compared to the overall size of that same wing as measured by area using ImageJ imaging software. The proportional wing size (posterior area/entire area as measured in pixels) of female and male flies of each the CantonS control and UAS-sLats1 experimental lines was averaged, then quantified relative to the CantonS control. The standard errors of these averages were calculated, then the T-test for significance in a difference of means (14 degrees of freedom) was calculated between the control and experimental lines. These numbers are summarized in Tables 1 and 2 below. The relative wing size in comparison to the CantonS control expressed as a percentage was expressed graphically in a chart for visualization of the difference in wing sizes between the two lines, as separated by

sex (Figures 4 and 5 below). A full listing of the measurements taken can be seen in Appendix B. The experimental UAS-sLats1 fly line was found to have a highly significant difference in wing size for both male and female flies in comparison to the control, with the experimental line exhibiting a decreased posterior wing size in relation to the overall size of the wing.

**Table 1: Female Wing Size Statistical Summary**

| en-Gal4/SM1, Sb-/TM6 | CantonS | UAS-sLats1 |
|---|---|---|
| Average | 0.483196682 | 0.449780827 |
| Relative | 100 | 93.08441961 |
| ST error | 0.503388582 | 0.322972331 |
| T-test against CantonS | x | 2.89966E-11 |

**Table 2: Male Wing Size Statistical Summary**

| en-Gal4/SM1, Sb-/TM6 | CantonS | UAS-sLats1 |
|---|---|---|
| Average | 0.467000359 | 0.432499508 |
| Relative | 100 | 92.61224317 |
| ST error | 0.325582011 | 0.703208101 |
| T-test against CantonS | x | 7.93635E-09 |

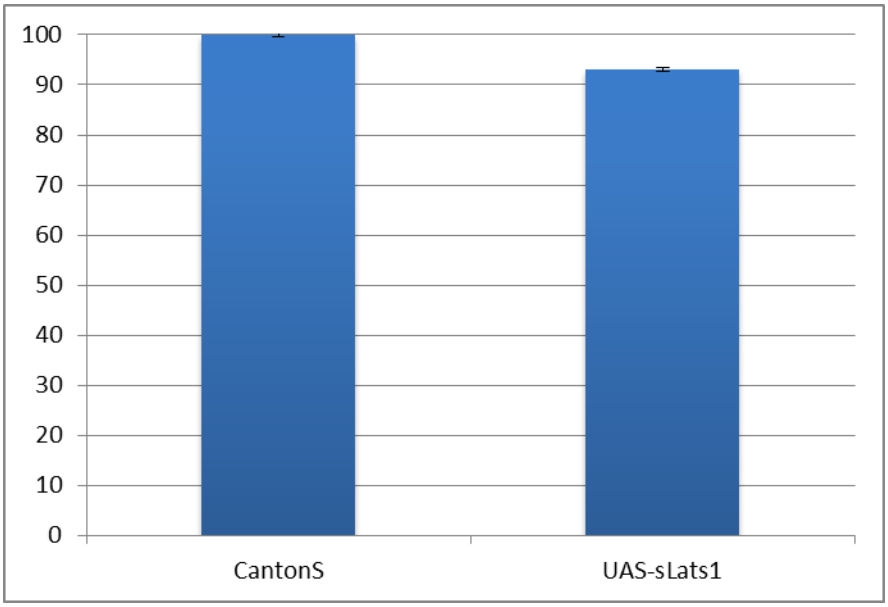


**Figure 4 - Female Wing Size Comparison**

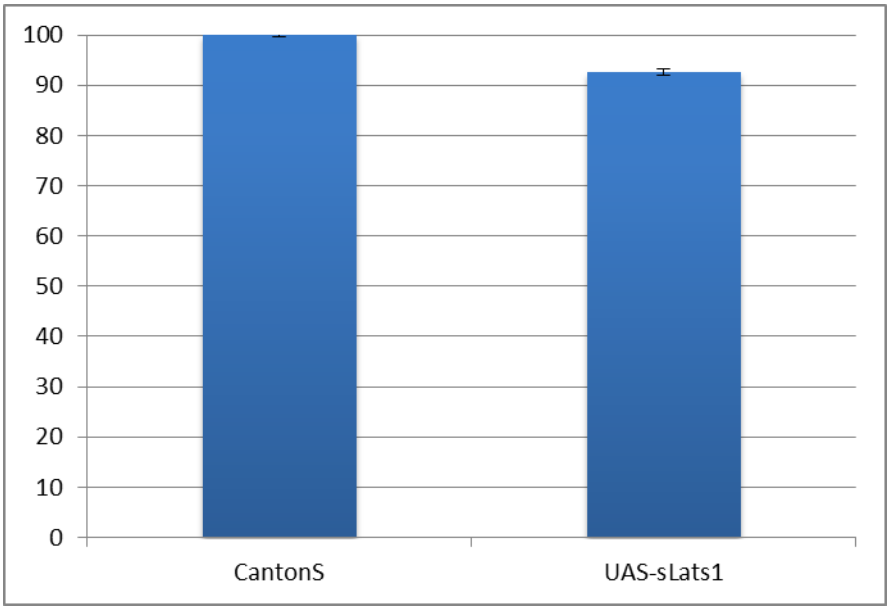


**Figure 5 - Male Wing Size Comparison**

## Discussion

The results of the sensitized assay demonstrate a distinct difference in the ratio of the posterior portion of the fly wing to the overall wing size in both male and female flies when the

sLats1 overexpression line is compared to the control CantonS, with the UAS-sLats1 line exhibiting a decreased size ratio phenotype. This gives evidence that the overexpression of sLats1 in the posterior wing disc results in an undergrowth phenotype when compared to a control, countering the hypothesis that sLats1 regulation results in increased Yki/YAP activity, given that increased Yki/YAP activity should result in an overgrowth, rather than undergrowth, phenotype. This in turn gives evidence for the action of sLats1 as a regulator of the transcription of genes for cellular proliferation through its action in the Hippo cell signaling pathway, though it is still unclear whether this action is through direct interaction with LATS1 or through some other mechanism. In either case, further investigation is necessary.

# Chapter 4

## Generating sLats1 Overexpression in Human Cells

While the ability of sLats1 overexpression to restrict cellular proliferation in *Drosophila* has been demonstrated, the exact mechanism of action and overall effect on proliferation of sLats1 overexpression in human cells is not known. The two major candidates for activation via the activity of sLats1 in mammalian cells are LATS1 and YAP, which have contradictory impacts on cellular proliferation when activated in the Hippo signaling pathway. In order to determine the dominant effect of sLats1 in human cells, the overall effect of sLats1 overexpression on cellular proliferation must be examined. The following sections outline a procedure for generating sLats1 overexpression in HEK293A cells and discuss both the current status and future directions of this experiment, as well as possible results and their implications.

## Plasmid Construction

In order to induce the overexpression of sLats1 in a human cell line, a plasmid construct must be produced and transfected into human cells. In this experiment, the plasmid pSin-EF2-flg-sLats1 was constructed using the vector pSin-EF2-MCS and the insert pSin-flg-sLats1 (insert sequence listed in Appendix A). This vector was previously constructed with an overexpression vector using lentivirus pSin-EF2-Sox2-Pur (vector plasmid map pictured in Figure 6) and pSin-MCS F/R primers (primer sequences in Appendix A). The insert was amplified via Phusion polymerase PCR (30 cycles, 10 sec at 98°C denaturation, 30 sec at 55°C annealing, 70 sec at 72°C extension) using the template pBiFC-flg-sLats1-VN and pSin-flg-sLats1 F/R primers (primer sequences in Appendix A). The PCR product underwent gel electrophoresis (agarose

10%) to ensure successful amplification of the insert (2.1 kb), and was then purified (OMEGA E.Z.N.A.® MicroElute DNA Clean-Up Kit). Both insert and vector were digested with enzymes Nde1 and BamH1, then underwent gel electrophoresis for purification (agarose 10%) and were extracted from the gel (OMEGA E.Z.N.A.® Gel Extraction Kit). Insert and vector were ligated using T4 DNA ligase, then transformed into competent bacteria and spread on 5x ampicillin plates. Colonies were selected and placed into ampicillin broth, then amplified by PCR with Phusion polymerase (same as above) to confirm presence of pSin-EF2-flg-sLats1 plasmid (~10 kb). Successful colonies were inoculated into ampicillin broth to culture. Successful colony cultures were then cleaned up (Promega Wizard® Plus SV Minipreps DNA Purification System) and digested with the enzymes Nde1 and BamH1. Digestion products underwent gel electrophoresis to confirm presence of sLats1 insert (2.1 kb) and pSin vector (8.5 kb), and successful plasmids were sent to the Huck Institute Genomic Core Facility to confirm proper plasmid construct. At this point in time, sequencing has not been able to confirm the successful construction of the plasmid, and so this experiment is ongoing. The following chapter describes the remainder of the planned procedure, possible results, and a discussion of the significance of possible results.

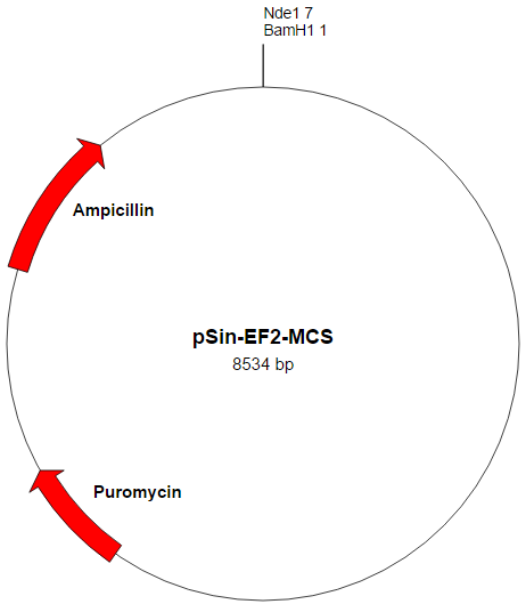


**Figure 6 - pSin-EF2-MCS Vector, based on pSin from Yu et al. (2007), drawn with Savvy**

# Chapter 5

## Future Directions

This experiment was intended to determine the dominant protein activation effect of sLats1 overexpression in human cells by using the HEK293A cell line, given that sLats1 action could activate two proteins with opposing impacts on overall cell proliferation and apoptosis. There exist several possible results to this experiment, each with implications as to the dominant effect of sLats1 in these cell lines. The remainder of the planned procedure to establish sLats1 overexpression in HEK293A cells and the possible results of the experiment are discussed in the following sections.

## Establishing sLats1 Overexpression Cell Line

Establishing the impact of protein overexpression on the proliferation of human cell lines involves the creation of a stable cell line overexpressing the protein of interest and a method of quantifying the cellular proliferation over time. The simplest method to express a plasmid construct in a cell line is through the use of a lentiviral vector to insert and incorporate a plasmid containing the gene of interest into a stable human cell line. The constructed plasmid containing the sLats1 gene, as well as an empty vector, will be transfected into HEK293T cells, and the stable cell protein extracts will then be run on Western Blot to demonstrate sLats1 overexpression using the flag tag. The viruses will then be purified from the cell extract and used to infect both wildtype and Lats1/2 double knockout HEK293A cells. Samples of the HEK293A cell lines will then be puromycin selected for successfully infected cells, then lysed and the cell

extracts analyzed by Western Blot to quantify overexpression of sLats1 in the cells lines infected with the constructed plasmid. Six stable cell lines will be established in all; infected for sLats1 overexpression, infected with the empty plasmid virus, and uninfected each for the wildtype and Lats1/2 double knockout HEK293A cell lines. Three rounds of cell counting via hemocytometer and staining for living and dead cells will be carried out, with four wells of each cell line for each of ten days all started in the same day. For the first round, cells from each day will be extracted for Western Blot analysis of sLats1 expression. Growth curves tracking the number of living cells per day will be constructed for each cell line and compared, as well as proportion of cells that die off per day in comparison to the total number of cells for that day. Each of the three rounds of cell counting will be averaged for rate of proliferation at each time point for comparison to the other cell lines.

## sLats1 Overexpression in Double Knockout Cell Line

The expected outcome of a growth curve analysis of the overexpression of sLats1 in the Lats1/2 double knockout cell line would be an increase in proliferation and decrease in apoptosis as compared to the double knockout line without sLats1 overexpression. This is based on the hypothesis that sLats1 could result in the activation of both LATS1 and YAP. Given that this hypothesis is true, sLats1 cannot work in this double knockout cell line to activate LATS1, preventing the regulation of YAP by LATS1 that would suppress proliferation. Therefore, YAP would both be unhindered by LATS1 activity and activated by sLats1, resulting in the increased transcription of proliferation and anti-apoptotic genes.

## No sLats1 Overexpression in Double Knockout Cell Line

The expected outcome of a growth curve analysis of the Lats1/2 double knockout cell line without sLats1 overexpression would be increased proliferation and decreased apoptosis in comparison to the wildtype cell line. This is because LATS1 is not present in this cell line to regulate YAP, but sLats1 is also not present in large quantities to further activate YAP, resulting in levels of active YAP above those of a wildtype cell line but below those of the double-knockout Lats1/2 overexpression cell line.

## sLats1 Overexpression in Wildtype Cell Line

There are three possible expected effects of sLats1 overexpression in the wildtype cell line based upon which of the two proteins LATS1 and YAP are activated in a more dominant manner by sLats1. If neither protein is activated at a higher level than the other, the effects of sLats1 overexpression on cell proliferation should be minimal and insignificant when compared with a wildtype HEK293A line not overexpressing sLats1. If YAP activation is dominant, it is expected that proliferation should increase and apoptosis should increase, as active YAP results in the increased transcription of proliferative and anti-apoptotic genes. If LATS1 activation is dominant, it is expected that proliferation should decrease and apoptosis should remain at normal levels, as YAP would be down-regulated, resulting in the lack of expression of anti-apoptotic genes and a lower than normal level of expression of proliferation genes.

If YAP activation is found to be the dominant effect, it would contradict the likely therapeutic use of sLats1 as a tumor suppressive gene therapy, as overexpression of sLats1 would be more likely to grow a tumor. However, if LATS1 activation is found to be the dominant effect, it could give power to the possibility of the use of sLats1 as an anti-tumor therapy, given the ability to decrease cellular proliferation in human cells. In either case, more research would be necessary to determine any differential effects of sLats1 overexpression in varying cell types to ensure that the effect holds true in any human tissue.

# Appendix A

**Primer Sequences**:

pSin_flag_sLats1_F: ATC CCA TAT GAA GCA GAG CTC GTT TAG TG
pSin_flag_sLats1_R: AGG CGG ATC CTT AAA ATA AAC ACC AAG C

pSin_MCS_F: AAT TCC ATA TGG CTA GCT TAA TTA AAT CGA TGC TAG CG
pSin_MCS_R: GGC TAG CAT CGA TTT AAT TAA GCT AGC CAT ATG CCT AG

**Flag-sLats1 Insert sequence**:
AAGCAGAGCTCGTTTAGTGAACCGTCAGAATTGATCTACCATGGACTACAAAGACG
ATGACGACAAGCTTGCGGCCGCGAATTCATCGATAGATCTGAAGAGGAGTGAAAAG
CCAGAAGGATATAGACAAATGAGGCCTAAGACCTTTCCTGCCAGTAACTATACTGTC
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AGTGAAAAGAGTTATGAAAATGTTGATAGTGGGGATAAAGAAAAGAAACAGATTAC

AACTTCACCTATTACTGTTAGGAAAAACAAGAAAGATGAAGAGCGAAGGGAATCTC
GTATTCAAAGTTATTCTCCTCAAGCATTTAAATTCTTTATGGAGCAACATGTAGAAA
ATGTACTCAAATCTCATCAGCAGCGTCTACATCGTAAAAAACAATTAGAGAATGAA
ATGATGCGGGTAAAACCTTTTAAAATGTCCATTTTTATACTTAATCATCTGTTTGCTT
GGTGTTTATTTTAA

# Appendix B

## Supplementary Data

**Table 3 - Wing Measurements, CantonS**

| CantonS | Female | | | Male | | |
|---|---|---|---|---|---|---|
| | Posterior | Whole | Ratio | Posterior | Whole | Ratio |
| 1 | 713471 | 1471180 | 0.48496513 | 518116 | 1112730 | 0.465626 |
| 2 | 714405 | 1496395 | 0.477417393 | 526947 | 1140867 | 0.461883 |
| 3 | 705778 | 1468291 | 0.48067992 | 522112 | 1111539 | 0.46972 |
| 4 | 727820 | 1606081 | 0.453165189 | 513848 | 1110209 | 0.462839 |
| 5 | 690865 | 1420998 | 0.48618295 | 491518 | 1072979 | 0.458087 |
| 6 | 703635 | 1419485 | 0.495697383 | 513239 | 1077676 | 0.476246 |
| 7 | 706671 | 1464884 | 0.482407481 | 524494 | 1101919 | 0.475982 |
| 8 | 692806 | 1440924 | 0.48080676 | 529386 | 1124456 | 0.470793 |
| 9 | 715991 | 1464557 | 0.48887889 | 507100 | 1079971 | 0.46955 |
| 10 | 713714 | 1457459 | 0.48969748 | 496563 | 1079404 | 0.460034 |
| 11 | 706560 | 1455364 | 0.485486792 | 527670 | 1135893 | 0.464542 |
| 12 | 682027 | 1411047 | 0.48334818 | 530426 | 1130975 | 0.468999 |
| 13 | 685584 | 1401621 | 0.489136507 | 522334 | 1113981 | 0.46889 |
| 14 | 683340 | 1413089 | 0.483578883 | 514954 | 1088611 | 0.473038 |
| 15 | 689799 | 1417877 | 0.486501297 | 533072 | 1161942 | 0.458777 |

**Table 4 - Wing Measurements, UAS-sLats1**

| UAS-sLats1 | Female | | | Male | | |
|---|---|---|---|---|---|---|
| | Posterior | Whole | Ratio | Posterior | Whole | Ratio |
| 1 | 676928 | 1481350 | 0.456967 | 463708 | 1057830 | 0.438358 |
| 2 | 647804 | 1481728 | 0.437195 | 465241 | 1063598 | 0.437422 |
| 3 | 633977 | 1390168 | 0.456043 | 444590 | 1047986 | 0.424233 |
| 4 | 651553 | 1450653 | 0.449145 | 439798 | 1013049 | 0.434133 |
| 5 | 628706 | 1385805 | 0.453676 | 436555 | 1027087 | 0.425042 |
| 6 | 630879 | 1415154 | 0.445802 | 490183 | 1118147 | 0.438389 |
| 7 | 607660 | 1367372 | 0.4444 | 494984 | 1123824 | 0.440446 |
| 8 | 628421 | 1414286 | 0.444338 | 479535 | 1084413 | 0.442207 |
| 9 | 615791 | 1353113 | 0.455092 | 484859 | 1103557 | 0.43936 |
| 10 | 620605 | 1362867 | 0.455367 | 459461 | 1070735 | 0.429108 |
| 11 | 661463 | 1463414 | 0.452 | 474718 | 1080221 | 0.439464 |
| 12 | 659432 | 1477389 | 0.44635 | 485781 | 1116637 | 0.435039 |
| 13 | 632702 | 1420053 | 0.445548 | 487787 | 1107385 | 0.440485 |
| 14 | 636226 | 1424230 | 0.446716 | 458537 | 1059805 | 0.432662 |
| 15 | 609479 | 1330526 | 0.458074 | 336894 | 861302 | 0.391145 |

## BIBLIOGRAPHY

Avruch J., Zhou D., Fitamant J., Bardeesy N., Mou F., Barrufet L. (2012) Protein kinases of the Hippo pathway: Regulation and substrates. *SEMINARS IN CELL & DEVELOPMENTAL BIOLOGY*, 23, 770-784.

Bachmann A., and Knust E. (2008) The Use of P-Element Transposons to Generate Transgenic Flies. *Methods in Molecular Biology*: Drosophila: *Methods and Protocols*, 61-77.

Brand A.H., Perrimon N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*. 118, 401-415.

Duffy J. (2002) GAL4 System in Drosophila: A Fly Geneticist's Swiss Army Knife. *GENESIS*. 34,1-15.

Hao Y., Chun A., Cheung K., Rashidi B., Yang X.. (2008:2007)Tumor Suppressor LATS1 Is a Negative Regulator of Oncogene YAP. *Journal of Biological Chemistry*. 283, 5496-5509.

Huang J., Wu S., Barrera J., Matthews K., Pan D. (2005) The Hippo Signaling Pathway Coordinately Regulates Cell Proliferation and Apoptosis by Inactivating Yorkie, the Drosophila Homolog of YAP. *Cell*. 122, 421-434.

Meng Z., Moroishi T., Guan K. (2016) Mechanisms of Hippo pathway regulation. *GENES & DEVELOPMENT*. 30, 1-17.

Yabuta N., Mukai S., Okamoto A., et al. (2013) N-terminal truncation of Lats1 causes abnormal cell growth control and chromosomal instability. *Journal of cell science*. 126, 508-520.

Yu J., Vodyanik M.A., Smuga-Otto K., et al. (2007) Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. *Science*. 318, 1917-1920.

Zhang J., Smolen G., Haber D. (2008) Negative regulation of YAP by LATS1 underscores evolutionary conservation of the Drosophila Hippo pathway. *CANCER RESEARCH*. 68, 2789-2794.

Zhao B., Wei X., Li W., et al. (2007) Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *GENES & DEVELOPMENT*. 21, 2747-2761.

# ACADEMIC VITA

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## EDUCATION

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Thesis Supervisor: Zhi-Chun Lai

**Awards and Honors**
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## RESEARCH AND EXPERIENCE

**The Pennsylvania State University** *5/2015 – 12/2016*
*Undergraduate Researcher – Department of Life Sciences* *University Park, PA*
- Performed research on the Hippo cell signaling pathway for cell apoptosis and proliferation in *Drosophila melanogaster* under Dr. Zhi-Chun Lai

**The Pennsylvania State University** *6/2013 – 8/2013*
*Summer Research Assistant - SE Agricultural Research & Extension Center* *Manheim, PA*
- Maintained numerous field research projects over several weeks, including planting, harvesting, administering chemical treatments, data collection and entry, supervised by Dr. Alyssa Collins and Dr. Steven Bogash

**Susquehannock High School** *8/2012 – 6/2013*
*Senior Aide to Biology Instructor* *Glen Rock, PA*
- Prepared and maintained experiments and research projects for high school students in higher level biology courses, supervised by Mrs. Barbara Nealon

## LEADERSHIP AND ACTIVITIES

**No Refund Theatre** *9/2015 – Present*
*Technical Chair, Member* *University Park, PA*
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