LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN
BACHELOR THESIS

Characterization of the potential phosphorylation site S678 in *Drosophila* Psidin

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Abbreviations

AL	antennal lobe
Amp	Ampicillin
AMP	antimicrobial peptide
AN	antennal nerve
CoIP	Co-immunoprecipitation
°C	degree Celsius
C-terminal	carboxy terminus
DNA	desoxyribonucleic acid
dNTPs	deoxyribonucleoside triphosphate
Dscam	Down Syndrome Cell Adhesion Molecule
FRT	Flippase recognition target
GFP	green fluorescent protein
HRP	horseradish peroxidase
MP	maxillary palp
NatB	N-acetyltransferase B
N-terminal	amino terminus
OR	olfactory receptor
ORN	olfactory receptor neuron
Sema-1a	Semaphorin-1a
TPR	tetratricopeptide repeat
UAS	upstream activation sequence

1 Summary

Psidin has been shown to be an actin binding protein (Kim, J. et al., 2011) and is predicted to be part of the N-acetyltransferase complex B (NatB). The main goal of this thesis was the characterization of a predicted phosphorylation site S678 in *Drosophila* Psidin (Trost, M. et al., 2009). I was able to demonstrate that this conserved serine is required for the regulation of NatB-complex formation. The NatB complex consists of one catalytic subunit and one auxiliary subunit. In *Drosophila* the Protein CG14222 represents the catalytic subunit and Psidin represents the auxiliary subunit. I was able to show that the phosphorylation of S678 prevents the interaction of Psidin and CG14222 in order to form the NatB complex. *In vivo*, overexpression of a phosphomimetic form of Psidin (S678D) failed to rescue the ORN cell number reduction in *psidin*^{1} mutants. This argues for a regulatory function of the serine 678 in NatB-complex formation. Contrary, the non-phosphorylatable form (S678A) was able to rescue the cell number.

Another aim of this project was to analyze the effect of the Psidin phosphomutants S678A and S678D *in vivo* to investigate the impact on targeting of Or59c neurons. Expression of both constructs, the phosphorylatable and the non-phosphorylatable construct, were able to rescue the Or59c targeting defect in *psidin*^{1} mutants.

The interaction domain of Psidin and CG14222 has only been predicted *in silico* so far. Deleting this entire domain in Psidin, I could confirm that CG14222 binds to Psidin in this region. Using deletions of different sizes, I was able to map a minimal interaction domain.

This thesis showed that Psidin harbors an interesting regulatory mechanism to perform two distinct functions using the conserved serine 678. The formation of the NatB complex is important for the survival of ORN neurons and seems to be regulated by the phosphorylation of Psidin. However ORN targeting seems to be phosphorylation independent.

This work was incorporated in the paper Stephan et al. (*"Drosophila Psidin is required for olfactory neuron viability and axon targeting through two distinct molecular mechanisms. Daniel Stephan, Natalia Sánchez-Soriano, Laura F. Loschek, Ramona Gerhards, Suanne Gutmann, Zuzana Storchova, Andreas Prokop and Ilona C. Grunwald Kadow")* which is currently under review at The Journal of Neuroscience.

Zusammenfassung

In vorhergehenden Studien wurde gezeigt, dass Psidin als Aktin-bindendes Protein (Kim, J. et al., 2011) fungiert und auch eine Rolle in dem N-Acetyltransferase-Komplex NatB spielt. Das Ziel dieser Arbeit war es, eine bisher vorhergesagte Phosphorylierungsstelle zu charakterisieren, welche an der Position S678 im Protein Psidin von *Drosophila* auftaucht (Trost, M. et al., 2009). Es konnte gezeigt werden, dass dieses konservierte Serin für die Regulation des NatB Komplexes verantwortlich ist. Der NatB Komplex besteht aus einer katalytischen Untereinheit und einer zusätzlichen Untereinheit die keine katalytische Funktion aufweist, sondern nur den Komplex unterstützt. In der Fruchtfliege ist die katalytische Domäne das Protein CG1422 und zusätzlich wird für die Formation des Komplexes das Protein Psidin benötig, welches keine katalytische Funktion aufweist. Die Phosphorylierung am Serin 678 in Psidin verhinderte die Interaktion mit CG14222 und somit auch die Bildung des NatB Komplexes. I*n vivo* konnte die Expression des phosphomimetischen Konstrukts von Psidin (S678D) in *psidin*^{1} Mutanten nicht den normalen Phänotyp der Anzahl an Neuronen (ORNs) wieder herstellen. Dies ist ein Hinweis dafür, dass für die Bildung des NatB Komplexes die Phosphorylierungsstelle S678 eine regulatorische Funktion übernimmt. Dagegen konnte das nicht phosphorylierte Psidin (S678A) die Anzahl an Neuronen wieder auf ein normales Level bringen. Des Weiteren wurden die Phosphomutanten S678A und S678D *in vivo* untersucht um die Auswirkungen auf die Konnektivität von Or59c-Neuronen zum Glomerulus im Antennallobus zu analysieren. Die Expression beider Proteine, ob phosphoryliert oder nicht, konnten den normalen Phänotyp in *psidin*^{1} Mutanten, welche einen Trageting Defekt der Neurone aufweisen, wiederherstellen.

In dieser Arbeit wurde die bisher nur *in silico* vorhergesagte Interaktionsdomäne von Psidin und CG14222 experimentell belegt. Dies wurde anhand einer kompletten Deletion dieser Domäne gezeigt. Durch weitere verschiedene Deletionen unterschiedlicher Größe wurde die Domäne gemappt.

Mit Hilfe dieser Arbeit konnte gezeigt werden, dass Psidin einen interessanten Regulationsmechanismus aufweist, um zwei verschiedenen Funktionen ausüben zu können. Die Ausbildung des NatB Komplexes spielt eine wichtige Rolle für das Überleben der ORNs und wird durch Phosphorylierung in Psidin reguliert. Dagegen ist das neuronale Targeting der ORNs zu den Glomeruli unabhängig von dieser Phosphorylierung.

2 Introduction

2.1 Olfactory system of *Drosophila*

The olfactory system of flies is required for many essential things such as finding food sources and mating partners or avoiding predators. Flies receive odors via two olfactory organs which are located on their head. One of those is the antenna which carries 1200 olfactory receptor neurons (ORNs) on the third antennal segment. The other olfactory organ is the maxillary palp (MP) where 120 ORNs are located (Stocker, 2001). Both olfactory organs are covered with different types of sensilla which differ in morphology and size. While the antennae are covered with basiconic, trichoid, and coeloconic sensilla, on the maxillary palps only basiconic sensilla can be found (Figure 2.1), (Vosshall, L. and Stocker, R., 2007).

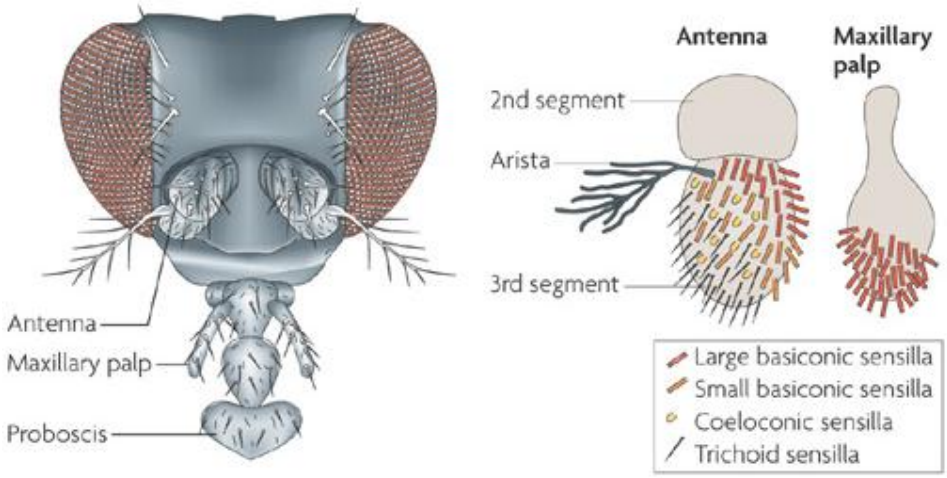


Figure 2.1 Overview of the olfactory system of the fly

The olfactory receptors which are responsible for the flies smell can be found in the antennae and the maxillary palps. Both organs are covered with thin hairs called sensilla. While the antennae harbor three different types, only one class is located on the MP (Kaupp, 2010).

The olfactory neurons are housed in these protecting sensilla surrounded by supporting cells which keep every ORN electrically isolated. Each single ORN expresses one type of olfactory receptor (OR) which is bound by odor molecules. Given that these molecules can also bind to multiple ORs, every odor activates a particular pattern of ORNs. Neurons expressing the same OR are clustered and target the same olfactory glomerulus in the antennal lobe (AL) in almost the same manner as mammalian olfactory neurons target the olfactory bulb (Fishilevich, E. and Vosshall, L., 2005). The AL of Drosophila has approximately 50 different glomeruli (Figure 2.2).

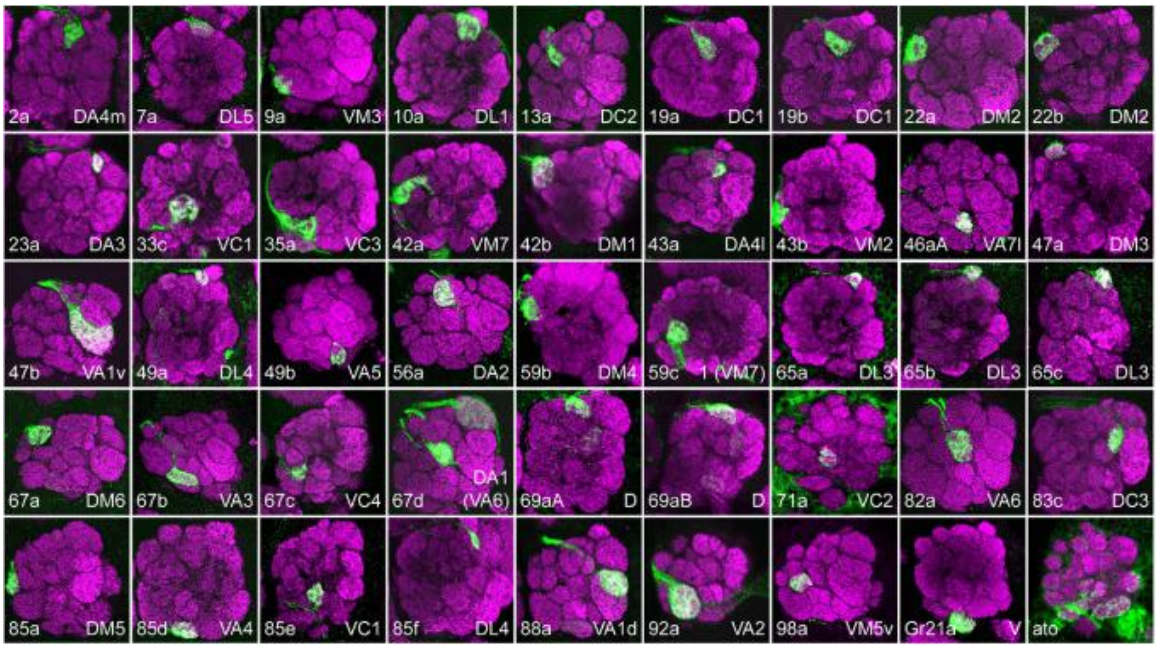


Figure 2.2 Expression pattern of the different ORNs

Several ORN classes are labeled using an Or X-GAL4 and UAS-mCD8GFP. The staining of these brains was carried out with α-GFP (green) and the nc82 antibody (magenta) to visualize the neuropil (Couto, A. et al., 2005).

The olfactory receptor system of Drosophila is a powerful tool, because the entire range of the different ORs can be used for genetic manipulations. The pattern of those ORNs is very stereotyped, so that targeting defects in mutants can be easily analyzed.

The moment of entering the antennal lobe is different between the ORNs of the antennae and the maxillary palps. During the development the ORNs of the antennae reach the AL first. Afterwards the ORNs of the MP reach the AL (Sweeney, L. et al., 2007). In the antennae the outgrowth of the different ORNs occurs in three sensilla typical bundles which form together with other sensory neurons the antennal nerve (AN). This nerve bundle grows towards the glomeruli in the AL. In a similar manner ORNs from the MP grow as the labial nerve towards the AL (Rodrigues, V. and Hummel, T., 2008). In the glomerulus ORN axons form synapses with the dendrites of projection neurons (PN) which grow to higher centers of the brain like the Mushroom body or the Lateral horn (Jefferis, G. and Hummel, T., 2006).

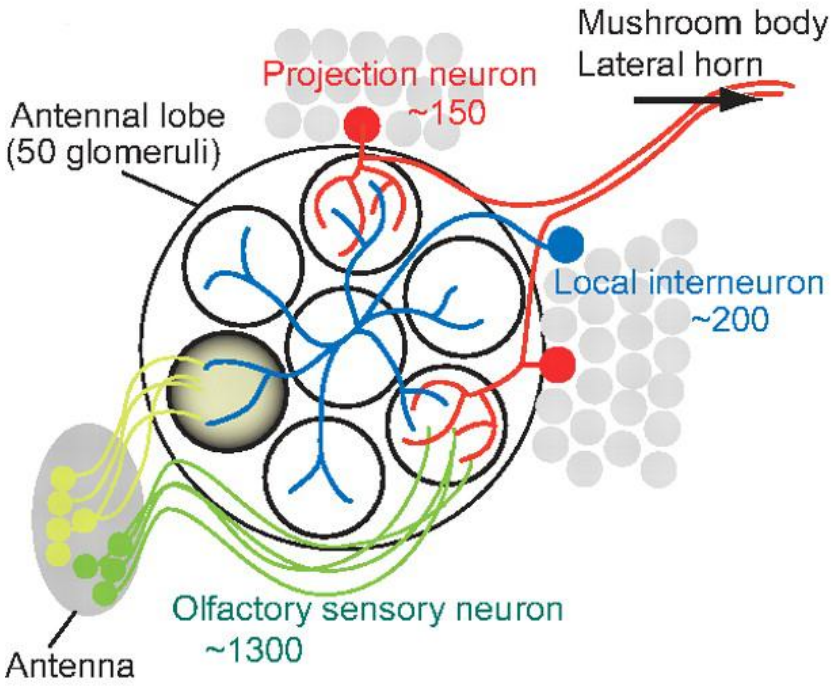


Figure 2.3 Wiring from the ORNs to the brain

If an odor binds to an OR which is expressed in a specific ORN the chemical signal is converted to an electrical axon potential. Then the ORNs expressing the same OR target the same glomerulus and form synapses to the dendrites of the projection neurons which target higher centers of the brain. The local interneurons are inhibitory neurons among the glomeruli (Seki, Y. et al., 2010).

The inhibitory local interneurons form connections between the distinct glomeruli. The estimated 200 cell bodies of these cells as well as the 150 cell bodies of the PN form three different clusters around the antennal lobe (Figure 2.3), (Seki, Y. et al., 2010). Several guidance molecules have already been identified. For example, for correct separation of neurons in the glomeruli a protein called Semaphorin-1a (Sema-1a) is required. The knock down of this protein results in a merge of distinct neighboring ORNs classes (Lattemann, M. et al., 2007). The guidance molecule Semaphorin can act either as repellent or as attractant (Sánchez-Soriano, N. et al., 2007). The essential receptor PlexinA binds the protein Sema-1a and this complex mediates repulsion by which early-arriving ORNs affect the targeting of late-arriving ORNs (Sweeney, L. et al., 2007). Another protein called Dscam (Down Syndrome Cell Adhesion Molecule) is responsible for providing neuronal identity. This molecule ensures that neurons only form synapses with other neurons to avoid self – innervation (Hummel, T. et al., 2003). Mutations in the *dscam* gene don't affect the targeting towards the AL but axons often stop and form ectopic glomeruli (Hummel, T. et al., 2003).

2.2 The protein Psidin

Psidin was first identified as a lysosomal protein in blood cells activating *Defensin* and degrading coated bacteria (Brennan, A. et al., 2007). In the immune system of *Drosophila* the blood cells are required for the elimination of pathogens. These immune cells are able to engulf and digest bacteria if Psidin is present. Mutations in the *psidin* gene lead to the engulfment of bacteria but not to the digestion and clearance. Furthermore, the expression of antimicrobial peptides (AMP) such as *Defensin* is defective in larvae (Brennan, A. et al., 2007).

In another study Psidin´s effect on the border cell migration in *Drosophila* oocytes was characterized (Kim, J. et al., 2011). Normally these cells are required for the separation of nursing cells from the oocyte by migrating from the tip of the ovary towards the center. But if *psidin* is mutated the migration of the border cells is defective (Kim, J. et al., 2011). Also Psidin´s function as an F-actin binding protein was pointed out. Tropomyosin competes with Psidin for the F-actin binding site (Kim, J. et al., 2011). Psidin plays therefore as an actin-binding proteine also a big role in the neuronal cytoskeleton and axon guidance (Stephan et al., 2012 under review). Mutations like *psidin*^{1} cause loss of function of Psidin due to the stop of translation at lysine around the position 441 (Brennan, A. et al., 2007). Flies carrying the *psidin*^{1} allele show complete mistargeting of the axons because the ORNs innervate the entire antennal lobe instead of one single glomerulus (Stephan et al., 2012 under review). Psidin is not just required for axon targeting but also for survival of the cell bodies of ORNs in the maxillary palps. Here the allele *psidin*^{1} cause a reduction of the cell number (Stephan et al., 2012 under review). Given that Psidin is the homologue of the yeast protein Mdm20 with 7 % identity and 22 % similarity (Brennan, A. et al., 2007) it is possible that Psidin acts as non-catalytic subunit of the N-acetyltransferase B complex (NatB). If this complex is build survival of the cells in the maxillary palps is enhanced. The NatB complex consists of a non-catalytic protein such as Mdm20 and a catalytic protein Nat3 in yeast. This nomenclature is used for *Saccharomyces cerevisiae* but in *Drosophila* the putative catalytic domain is currently named CG14222 and the homologue of Mdm20 is Psidin, as already mentioned. There are still two additional Nat complexes, NatA and NatC (Polevoda, A. at al., 2009). Together with NatB they acetylate the N-terminus of at least 60 % of all proteins like Tropomyosin in yeast (Singer, J. ans Shaw J., 2003) but few target proteins are identified so far.

This interaction domain in Psidin is just predicted so far but the goal of this thesis was to characterize the binding domain of Psidin where the interaction to the catalytic subunit of NatB takes place.

To find out how the two distinction functions of Psidin, especially the formation of the NatB complex are regulated, a potential phosphorylation site identified in human Psidin was considered. The motif IRSLMLR was found to be phosphorylated *in vitro* (Trost, M. et al., 2009). This phosphorylatable serine exists in human Psidin at position S691 and in *Drosophila melanogaster* the corresponding motif VRSLMLR occurs around the serine 678 of the protein. This region is highly conserved from *C. elegans* to humans (Figure 2.4).

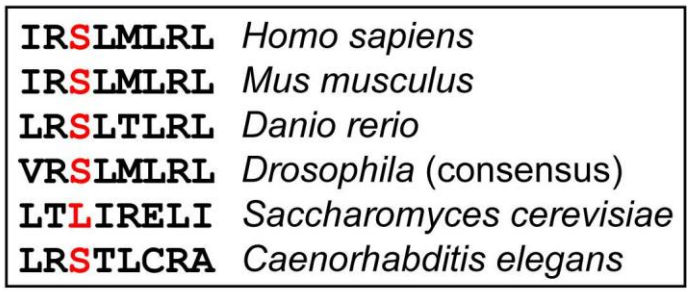


Figure 2.4 Conserved region S678 in Psidin

The entire sequence stretch next to the serine 678 is conserved from *C. elegans* to mammals. It was shown that this motif containing a serine is phosphorylated *in vitro*.

To address if this position is regulated by phosphorylation two mutant flies were generated. The first mutant mimics the phosphorylation by exchanging the serine for the amino acid aspartate and the second one is a non phosphorylatable mutant which carries a non-phosphorylatable alanine at position S678. Those alleles were named *psidin*^{S678A} and *psidin*^{S678D}. Furthermore, the alleles *psidin*^{IG978} and *psidin*^{1} were used. The *psidin*^{IG978} allele carries a mutation E320K of the Psidin protein and was generated in the lab and *psidin*^{1} reveals the loss of function of Psidin as I already mentioned.

Both of these mutations are located within the putative subunit of the NatB complex while *psidin*^{S678A} and *psidin*^{S678D} is downstream of this 948 amino acids long protein. The coiled coil domains on the N-terminus are responsible for building homodimers (Kim, J. et al., 2011), (Figure 2.5). At the C-terminus a TPR domain is located, which acts as docking site for proteins and allows protein-protein interactions (Iyer, S. and Hartl, G., 2003).

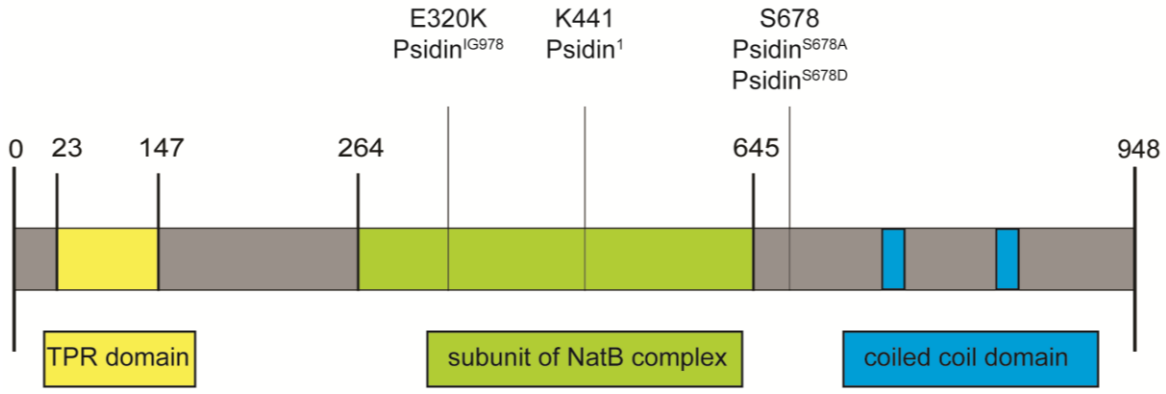


Figure 2.5 Domains of Psidin

The protein Psidin contains a TPR (tetratricopeptide repeat) domain which is used for protein-protein interactions and a putative subunit of the NatB complex. This subunit might be necessary for the interaction with CG14222. The coiled coil domains are important for the dimerization of Psidin.

3 Aims of the thesis

The aim of this work was to map the interaction domain between Psidin and CG1422 and to find putative mechanisms of regulation of this complex. Therefore the potential phosphorylation site S678 in *Drosophila* Psidin was investigated due to the predicted motif VRSLMLR (Trost, M. et al., 2009), in which the serine is regulated by an unknown kinase. The targeting pattern of the two Psidin isoforms carrying either the phosphomimetic amino acid aspartate or the non phosphorylatable amino acid alanine was analyzed. Also the rescue of the *psidin* null background by these two Psidin mutants was a main part of this work. The second part of the project dealt with the influence of the two Psidin isoforms on the neuron number in the maxillary palps. Furthermore, the role of Psidin in the NatB complex was of big interest. Here, Psidin together with the catalytic subunit CG14222 forms the NatB complex. The characterizations of the interaction domain provided information about the position were CG14222 binds to Psidin. The final goal was to analyze which role Psidin plays during development dependent on NatB and NatB-independent. To conclude, this thesis had following main goals:

(i) Investigation of a potential phosphorylation site in *Drosophila* Psidin

(ii) Effect of this site on targeting and ORN survival in *psidin* mutants

(iii) Analysis of the predicted Psidin/CG14222 interaction domain

4 Materials and Methods

4.1 Materials

This section summarizes the solutions and materials that were used for the different experiments.

4.1.1 Solutions

Table 4.1 List of buffers, solutions and media

Solution	Ingredients
Cloning	
LB medium (1 L)	– 10 g NaCl – 10 g tryptone – 5 g yeast extract – pH 7.5 ➢ dissolve ingredients in distilled H_2O to final volume of 1 L
TAE 50x (Tris base, acetic acid and EDTA)	– Tris base 242 g – Glacial acetic acid 57.1 ml – EDTA (0.5 M, pH 8.0) 100 ml ➢ dissolve ingredients in distilled H_2O to final volume of 1 L
Mutagenesis	
NZY^+ medium (1 L)	– 10 g NZ amine – 5 g yeast extract – 5 g NaCl – pH 7.5 (NaOH) ➢ autoclaving ➢ add following sterile solutions – 12.5 ml of 1M $MgCl_2$ – 12.5 ml of 1M $MgSO_4$

	– 10 ml of 2M glucose
Dissection of adult fly brains	
Phosphate buffered saline (1xPBS)	– 137 mM NaCl – 8 mM Na_2HPO_4 – 2.7 mM KCl – 1.5 mM KH_2PO_4 – pH 7.4
Phosphate buffered saline - 0.5 % Triton (PBT)	– PBS – 0.5 % Triton-X100
Periodate-Lysine-Paraformaldehyde (PLP)-4 % PFA	– 2 ml of 8 % PFA – 2 ml PBL
Blocking solution	– 10 % Donkey serum in PBT – 5 % BSA in TBT
Co-immunoprecipitation (Co-IP)	
S2 cell media	– Schneider's *Drosophila* medium – 1 % of Penicillin/Streptomycin mixture – 10 % of heat inactivated FBS
Lysis Buffer (50 ml)	– 50 mM Tris – 150 mM NaCL – 2 mM EDTA – one tablet of phosphatase inhibitor (Roche) – one tablet of protease inhibitor (Roche) – 1 % Triton
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS – PAGE)	
Separating gel 10 % (10 ml)	– 4.05 ml H_2O – 2.6 ml Buffer 1 (1.5 M Tris, 0.4 % SDS, pH 8.8) – 3.3 ml 30 % Acrylamide/Bis – 50 µl Ammonium persulfate (APS) – 50 µl TEMED
Stacking gel 4 % (10 ml)	– 6.1 ml H_2O – 2.6 ml Buffer 2 (0.5 M Tris, 0.4 % SDS, pH 6.8) – 1.3 ml 30 % Acrylamide/Bis – 100 µl Ammonium persulfate (APS)

	– 100 µl TEMED
SDS running buffer (1 L)	– 15.45 g Tris base – 72.1 g Glycine – 5 g SDS – fill up with distilled H_2O to final volume of 1 L
Western Blot	
Blotting buffer	– Fast Semi-Dry Transfer Buffer 10x (Thermo Scientific) was diluted to 1x
TBST (10x)	– 150 mM NaCl – 100 mM Tris (pH 7.5) – 0.1 % Tween
Blocking solution (50 ml)	– 2.5 g milk powder – 5 ml TBST (10x) – fill up to 50 ml with distilled H_2O

4.1.2 Enzymes and DNA/protein standards

- Restriction enzymes and corresponding buffers
- *Dpn* I
- Antarctic Phosphatase
- T4 DNA Ligase
- *Pfu* Ultra II Fusion
- 1 kb ladder
- Protein standard 10-250 kDa

4.1.3 Commercial Kits

Table 4.2 List of the commercial Kits

Supplier	Name of the Kit
Quiagen	Maxi prep Kit
	Spin Miniprep Kit
	Gel extraction Kit
	PCR purification Kit
	Effectene Transfection Reagent
Agilent Technologies	Quick Change Lightning Site-Directed Mutagenesis Kit

4.1.4 Plasmids

Table 4.3 List of plasmids

Plasmid	Antibiotic resistance
pUAST	Amp
pBluescript KS^+	Amp
pUAST-UAS-Psidin-HA	Amp
pUAST-KO	Amp
pUAST-CG14222-myc	Amp
pUAST-KO	Amp

4.1.5 Primer

Table 4.4 List of primers

Primer	Sequence 5'- 3'
Mutagenesis	
Psidin^{S678A} forward	AAGTTGAGGTGCTTCAAGTACGTGCGCTGATGCTTC
Psidin^{S678A} reverse	GAAGCATCAGCGCACGTACTTGAAGCACCTCAACTT
Psidin^{S678D} forward	GTTGAGGTGCTTCAAGTACGTGATCTGATGCTTCGACTCTTTGCC
Psidin^{S678D} reverse	GGCAAAGAGTCGAAGCATCAGATCACGTACTTGAAGCACCTCAAC
Deletions	
Psidin$^{\Delta NatB1}$ forward	GATTAATTAACAGCAAGTTGTTGTTGGAGAG
Psidin$^{\Delta NatB1}$ reverse	GTTAATTAAACGATCACGATCTGCG
Psidin$^{\Delta NatB2}$ forward	TTTAATTAACCAGATTCAGCTGGACTCCATG
Psidin$^{\Delta NatB2}$ reverse	ATTAATTAAGCTCTCCAACAACAACTTG
Psidin$^{\Delta NatB23}$ forward	TTTAATTAACGGGGCCATTATCCGATG
Psidin$^{\Delta NatB23}$ reverse	TTTAATTAAGCTCTCCAACAACAACTTGCT
Psidin$^{\Delta NatBfull}$ forward	TTTAATTAACGGGGCCATTATCCGATGG
Psidin$^{\Delta NatBfull}$ reverse	TTTAATTAAACGATCACGATCTGCGTCC

4.1.6 Bacteria

One Shot Top10 (Invitrogen) chemical competent cells were used to amplify the respective plasmids.

XL10-Gold Ultracompetent Cells (Agilent) were used for mutagenesis reactions. Those cells are lacking the recA and endA1 gene leading to a higher DNA yield and stability.

4.1.7 Antibodies

Table 4.5 List of primary and secondary antibodies

Antibody	Dilution	Supplier
Primary antibodies		
rat α-HA	1:1000	Roche (Switzerland)
rabbit α-myc	1:1000	Abcam (UK)
rabbit α-GFP	1:1000	Clonetech
mouse α-disclarge	1:200	DSHB (USA)
Secondary antibodies		
α-rat HRP	1:1000	Jackson (USA)
α-rabbit HRP	1:1000	Jackson (USA)
α-rabbit-488	1:200	Dianova (Germany)
α-mouse-CY5	1:200	Dianova (Germany)

4.1.8 Fly stocks

Flies were raised in vials containing standard fly food consisting of yeast and other ingredients. The incubator was set to 25° C at around 60–70 % humidity.

Table 4.6 List of fly stocks

w-; Bl/CyO; TM2/TM6B
eyFlp; Bl/CyO; FRT82/TM6B
eyFlp; Bl/CyO; FRT82 psidin^{IG978}/TM2
eyFlp; Bl/CyO; FRT82-psidin^{1}/TM2
w- ; OR59c-mCD8-GFP, act gal4/CyO; FRT82 Cl gal80/TM2
w- ; UAS-Psidin-HA/CyO; TM2/TM6B
w-; UAS-Psidin^{S678A}-HA/CyO; TM2/TM6B
w-; UAS-Psidin^{S678D}-HA/CyO; TM2/TM6B

4.1.9 Cell line

Drosophila Schneider S2 cells were cultured in Schneider's Drosophila medium. To complete this medium 1 % of Penicillin/Streptomycin mixture and 10 % of heat inactivated FBS were added. S2 cells were incubated in 250 ml flasks at 25°C without CO_2 as a semi adherent culture and split weekly in a 1:20 ratio.

4.2 Methods

The following methods were used to execute the experiments for the thesis.

4.2.1 Preparation of Plasmid DNA

Single colonies were picked and incubated in 2 ml or 250 ml, for a Mini- or Maxi-prep, respectively. The LB medium contained 100 µg/ml Ampicillin. After o/n incubation at 37°C cells were purified with the protocol provided from the manufacturer (Qiagen). DNA concentration was measured using a NanoDrop.

4.2.2 Transformation of chemical competent *E. coli* cells

Competent *E.coli* cells (25 µl) were thawed on ice. Subsequently, 1-5 µl of plasmid DNA was added and incubated for 30 min on ice. Heat shock was given at 42°C for 45 sec. Finally cells recovered in 1 ml plain LB medium for 1 h and were plated on LB plates containing 100 µg/ml Ampicillin.

4.2.3 Molecular Cloning

Digest of plasmids which led to sticky ends was conducted with restriction enzymes and buffers from NEB. The samples were incubated at 37°C for 1 h.

Standard reaction mixture

1 µl restriction enzyme

1 µl suitable buffer

1 µl BSA (10x)

300 – 400 ng DNA template

fill up with ddH_2O to a total volume of 10 µl

Dephosphorylation of the vector was conducted with 1 µl Antarctic Phosphatase (NEB) and 1/10 volume of Antarctic Phosphatase Reaction Buffer (10x). The Mixture was incubated for 30 min at 37°C. Afterwards this enzyme was heat inactivated at 65°C for 5 min.

Ligation was carried out with T4 DNA Ligase from NEB at 16 °C o/n. The vector-insert ratio was 1:3 with 100 ng of the vector.

Calculation of insert – vector ratio 1:3

$$\text{amount of insert (ng)} = \frac{100\ \text{ng vector} \times \text{size of insert (kb)}}{\text{size of vector (kb)}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}}$$

Standard reaction mixture

100 ng vector (9kb)

300 ng insert (3kb)

1 µl T4 ligase buffer

1 µl T4 ligase

fill up with ddH_2O to a total volume of 10 µl

4.2.4 Site-Directed Mutagenesis

Site-directed mutagenesis was performed according to the manufacturer's guidelines. PCR-mediated mutagenesis is based on the principle that the used primer pairs introduce single mutation (single base pairs up to multiple base pair) into a template plasmid. In the beginning the mutant strand was PCR-amplified using primer pairs introducing a specific point mutation. PCR was carried out in a total volume of 50 µl and contained 125 ng of each mutagenic primer, 1x reaction buffer, 25-100 ng of plasmid DNA, 2.5 mM dNTPs, 1.5 µl QuikSolution reagent and 1 µl of QuikChange Lightning Enzyme.

Cycling conditions

Table 4.7 PCR program for site directed mutagenesis

Segment	Cycles	Temperature	Time
1	1	95°C	2 min
2	18	95°C 60°C 68°C	20 sec 10 sec 6 min (30 sec/kb)
3	1	68°C	5 min

The amplified products were treated with 2 µl *Dpn* I restriction enzyme for 10 min in order to digest the wild type plasmid template. For the transformation 45 µl of XL10-Gold ultracompetent cells were thawed on ice. Cells were incubated for 2 min on ice after addition of 2 µl of β-mercaptoethanol. Then 10 µl of the *Dpn* I-treated DNA was transferred to the cells and after 30 min on ice, a heat shock was given for 30 s at 42°C.

The tubes were placed back on ice for 2 min and afterwards cells recovered with 0.5 ml preheated NZY+ broth at 250 rpm for 1 h. Bacteria were plated on LB-Ampicillin agar plates to select positive clones.

4.2.5 GAL4/UAS system

This system was used to drive the expression of distinct genes. It consists of the yeast transcription activator protein (GAL4) and the upstream activation sequence (UAS). If the GAL4 protein is activated by a specific promoter such as the olfactory receptor neuron marker Or59c, it binds to the enhancer UAS (Brand, A. and Perrimon, N., 1993). Then the transcription of the genes upstream of the UAS sequence will start. Therefore different *psidin* isoforms which were under the control of the GAL4/UAS system were used (Figure 4.1).

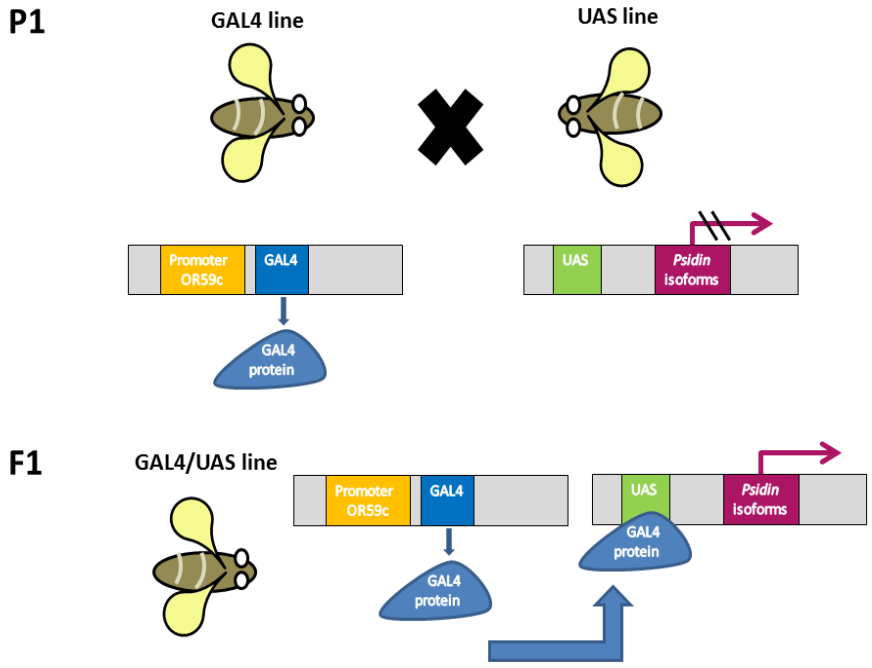


Figure 4.1 Mechanism of the GAL4/UAS system

The GAL4/UAS system was used to express the different isoforms under the promotor of the olfactory receptor OR59c. The *psidin* isoforms were also under the control of a UAS element.

4.2.6 eyFlp system

The eyeless Flippase system mediates recombination of sequences between two FRT (Flippase recognition target) sites (Newsome, T. et al., 2000). The FRT sequence was taken from *Saccharomyces cerevisiae*. Since the expression of the Flippase recombination enzyme is under the tissue specific promoter eyeless the recombination will only occur in the eye-antennal disc. This system is useful to avoid lethality created by the loss of gene function because the protein of interest can be knock-out in a tissue specific manner rather than in the entire animal (Wu, J. and Luo, L., 2006).

With the aid of this system cell mosaics will arise due to the recombination during mitosis. The parental cells are heterozygous and if recombination occurs the two daughter cells are either homozygous for the mutation or homozygous wild type. Due to the expression of GAL80 in wild type cells, the GAL4/UAS expression is repressed in these cells. On the other hand, the lack of GAL80 in homozygous mutant cells allows the expression of constructs under the control of the GAL4/UAS system (Figure 4.2).

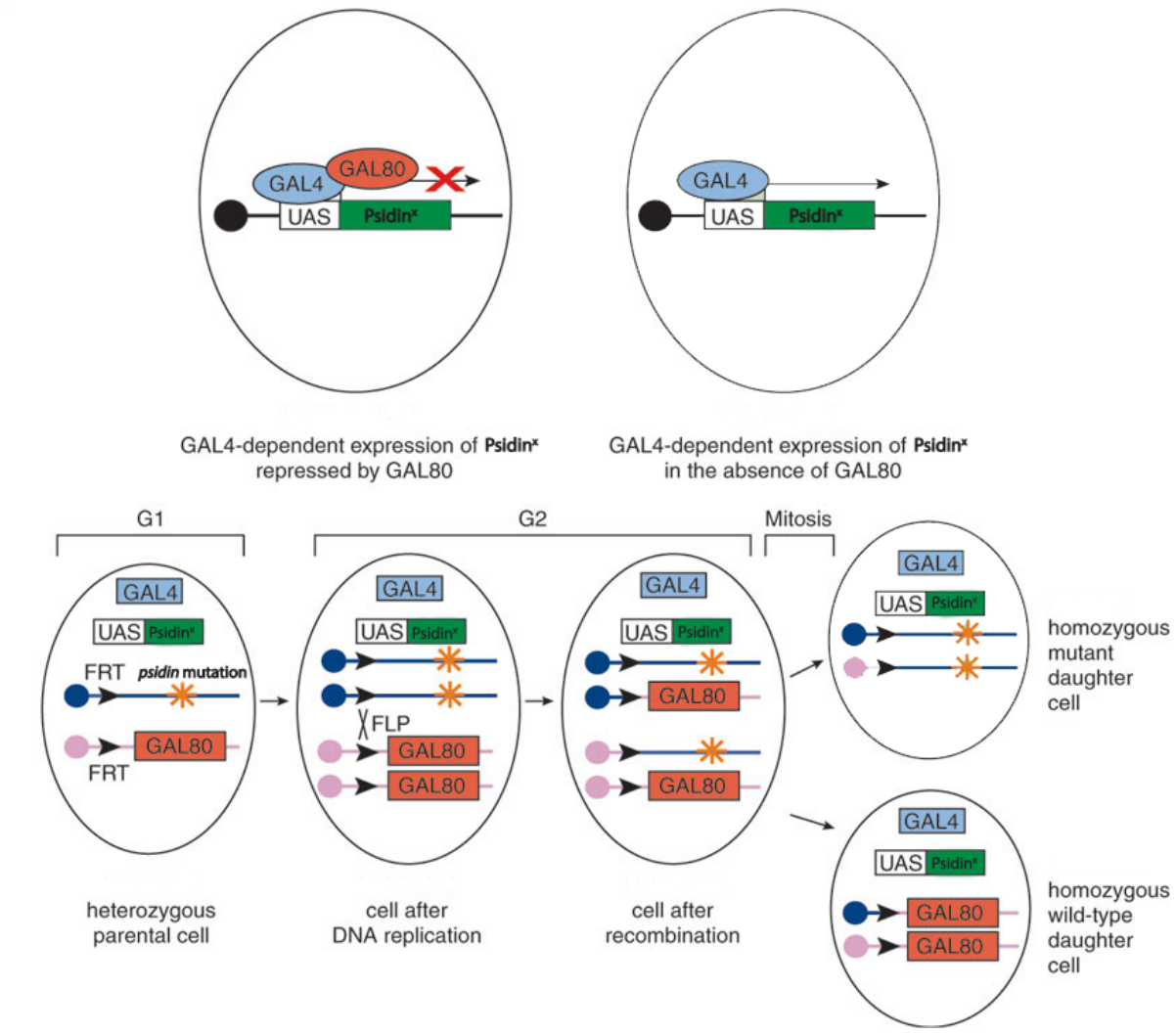


Figure 4.2 Mechanism of the FRT-FLP system

This system shows how homozygous wild type daughter cells will not express the construct under the control of the GAL4/UAS system because GAL4 is inhibited by GAL80. In this experiment Psidin^{wt}, Psidin^{1} or Psidin^{IG978} (represented as Psidin^{X}) were under the control of the GAL4/UAS system (adapted from Wu, J. and Luo, L., 2006).

4.2.7 Rescue experiment

The GAL4/UAS system was used to drive the expression of UAS-Psidin^{S678A}-HA or UAS-Psidin^{S678D}-HA. If the GAL4 protein binds to UAS then the transcription of the gene downstream will start. The GAL4 element was under the control of actin promoter (act-GAL4).

Transgenes were only expressed in eyFlp induced clones. Olfactory neurons were labeled using a direct fusion of Or59c and mCD8-GFP. This allows the labeling of a single class of ORNs. The two Psidin isoforms carrying either the phosphomimetic amino acid aspartate or the non phosphorylatable amino acid alanine were overexpressed in the background of *psidin*^{IG978} or *psidin*^{1}.

4.2.8 Dissection and staining of adult fly brains

Once flies were anesthetized with CO_2 they were placed into ice-cold ethanol (100 %) and then transferred into ice-cold PBS. The brains were dissected at room temperature and afterwards fixed in in PLP (4 % PFA) for 1 hour (Hartl, M. et al., 2011). Then they were washed 3x in PBT each time for 15 min and incubated in blocking solution (10 % donkey serum) for 15 min. The primary antibody, diluted in blocking solution was applied to the tissue and after one day at 4°C the brains were washed again 3x with PBT in the same manner. At last they were transferred into a dilution of the secondary antibody in blocking solution and after 2 h at room temperature washed 3x in PBT in the same way.

Afterwards the brains were mounted with Vectashield and images were taken with the confocal microscopy (Olympus FV1000, Leica SP2). The different samples were stored at 4°C in the dark. All images were edited with ImageJ and Adobe Photoshop.

4.2.9 PCR mediated deletion

The UAS-Psidin-HA construct was subcloned into pBluescript KS^{+}. The primers were designed to amplify the entire plasmid except the region with the desired deletion. Each 5' end of the primer was attached to a *Pac* I restriction site (Figure 4.3).

In addition, the primer consisted of one base overhang and one additional base to keep the reading frame. About 15 to 21bp of the primer were designed to bind the template plasmid (Pérez-Pinera, P. et al., 2006).

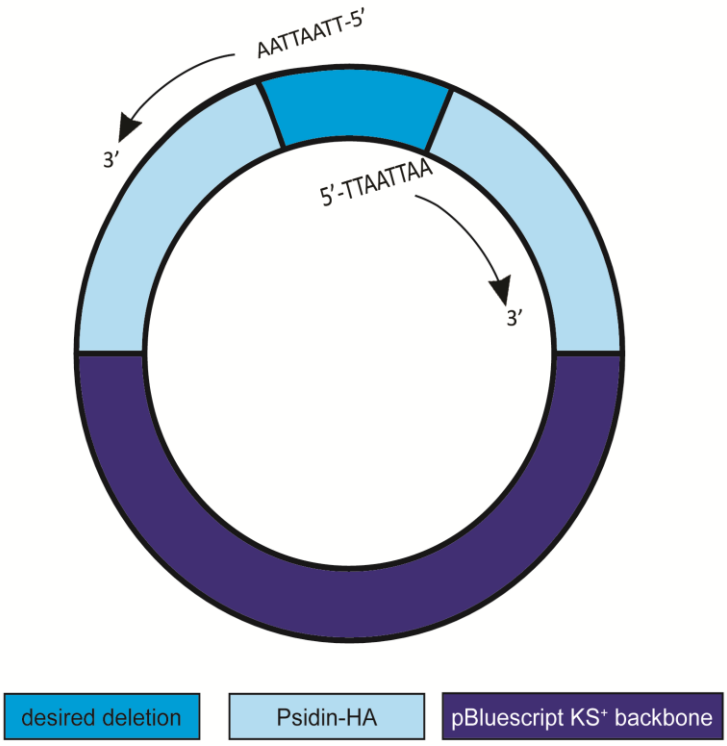


Figure 4.3 pBluescript-Psidin HA

The vector pBluescript was used for the PCR mediated deletions. Several small and big deletions were engineered into the Psidin-HA coding region.

Table 4.8 Primer annealing temperatures

	Primer annealing temperature (T_A)
Psidin$^{\Delta NatB123}$	61°C
Psidin$^{\Delta NatB1}$	56°C
Psidin$^{\Delta NatB2}$	57°C
Psidin$^{\Delta NatB23}$	58°C

PCR mixture

1 µl primer #1

1 µl primer #2

1 µl *Pfu* Ultra II Fusion

5 µl *Pfu* Ultra II Fusion buffer (10x)

100 mM dNTP mix

30 ng DNA template

fill up with ddH_2O to a total volume of 50 µl

Cycling conditions

Table 4.9 PCR program for deletions in NatB domain

Segment	Cycles	Temperature	Time
1	1	95°C	2 min
2	30	95°C T_A 72°C	20 sec 20 sec (15 sec/kb)
3	1	72°C	3 min

The following figure shows the entire work flow for the distinct deletions in Psidin which were first made in the vector pBluescript and after verifying by sequencing, these constructs were cloned back into the vector pUAST.

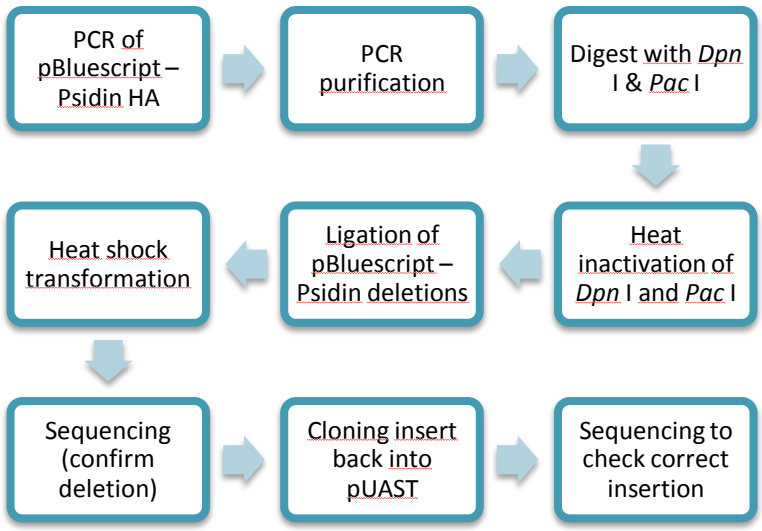


Figure 4.4 Steps for deletions in pUAST-Psidin HA

UAS-Psidin-HA was subcloned into pBluescript KS^+ and afterwards the deletions were made by PCR. Then several purification steps followed. Deletions were verified by sequencing and cloned back into pUAST.

4.2.10 Transfection

The Effectene Transfection kit from Qiagen was used to transfect the S2 cells. According to the provided protocol the cells were seeded one day before with a density of 10^6 cells in 6 cm dishes containing 4 ml of Schneider's Drosophila medium. The transfected constructs were expressed using the GAl4/UAS system (Table 4.10).

Table 4.10 Scheme of different transfection conditions

	Cell dish #1	Cell dish #2	Cell dish #3	Cell dish #4	Cell dish #5
ub–GAL4	+	+	+	+	+
UAS–KO	+	-	-	-	-
UAS–Psidin–HA	-	-	+	-	-
UAS–Psidin$^{\Delta NatBX}$–HA	-	+	-	+	-
UAS–CG14222–myc	-	+	+	-	+

4.2.11 Coimmunoprecipitation

After 2-3 days incubation, the transfected S2 cells were centrifuged for 10 min at 3300 rpm and the pellet was suspended in 300 µl lysis buffer. In addition cells were lysed using a homogenizer for 1 min at full speed. Cells were incubated at 4°C for 30 min followed by a centrifugation step at 3300 rpm for 5 min. Afterwards the samples were diluted 1:20 in lysis buffer and 3 µl of the desired antibody was added and samples were incubated at 4°C for 2 h on the rotary shaker. Subsequently, the protein A sepharose beads were prepared.

The powder was filled up to the 0.1 ml mark of an eppendorf tube and then washed 3x with 1 ml PBS. The centrifugation was carried out at 3000 rpm for 5 min each washing step. Then the beads were dissolved in lysis buffer in the same volume as the beads obtained. Afterwards 40 µl of washed beads were applied to each sample and then they were placed back on the rotary shaker at 4°C for 2 h. After that, beads were washed 3x at 3000 rpm for 5 min.

In the first step the beads were treated with 400 µl ice cold lysis buffer then with 400 µl of a 1:1 mixture PBS and lysis buffer and in the third step the beads were washed with 400 µl lysis buffer. The supernatant was always removed. At last the beads were boiled in 6 µl SDS (6x) for 10 min.

4.2.12 SDS Gelelectrophoresis

Samples (10 µl) were loaded on a 7.5 % or 10 % separating gel, respectively. The gelelectrophoresis was carried out in 1x SDS running buffer at 200 V and 160 mA for 45 min.

4.2.13 Western Blot and immunohistochemistry

Filter and Whatmann paper were soaked in Fast Semi-Dry Transfer Buffer (1x) for 15 min. After the electrophoresis the gels were equilibrated in distilled water for 10 min. The blotting lasted 15 min at 25 V and 400 mA. Then the blots were blocked in milk powder solution (20 %) for 15 min. This step was followed by the incubation in a primary antibody solution overnight.

On the next day the blots were washed 3x with TBST (1x) 15 min each. Then they were incubated with the secondary antibody for 2 h and again washed three times with TBST (1x). Afterwards the blots were developed in the 1:1 mixture of the Western Blot Detection Reagents from GE Healthcare.

4.2.14 Quantification

The quantification of the targeting phenotype was carried out in three categories. The first one was strong mistargeting, followed by mild mistargeting and the third category was the wild type phenotype (Figure 4.5).

To get the binding efficiency between Psidin deletion mutants and CG14222, the intensity of bound CG14222 was divided by the intensity of Psidin in the Western blot bands. The experimental lanes were normalized to the wild type.

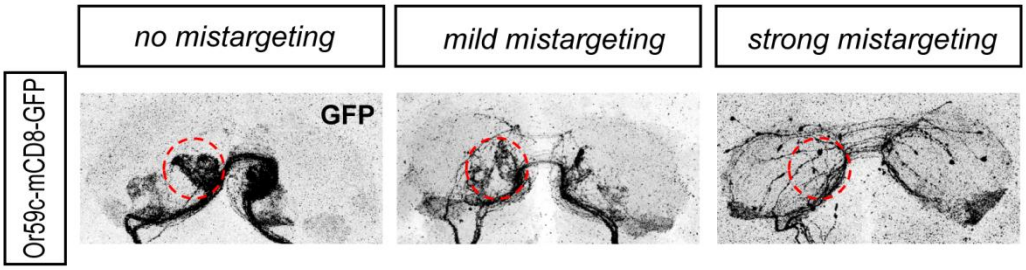


Figure 4.5 Three targeting categories

The first category looks like the wild type phenotype where the glomerulus is innervated normally. The second category shows a mild mistargeting where the glomerulus is innervated in a weaker manner. In addition, some axons innervate across the entire AL. Finally, the third group shows a strong mistargeting defect where the glomerulus is not visible (adapted from Stephan et al., 2012 under review).

5 Results

5.1 Analysis of Psidin mutants S678A and S678D *in vivo*

In order to address if the phosphorylation site S678 affects the targeting pattern of ORNs projecting from the periphery of the antennae to the Or59c glomerulus in the antennal lobe, two different constructs *psidin*^{S678A} and *psidin*^{S678D} were generated and injected into flies. Also effects on the cell number in the maxillary palps were pointed out.

5.1.1 The Targeting phenotype is rescued by both Psidin phosphomutants

To visualize the targeting phenotype of ORNs in various *psidin* mutant backgrounds, UAS-Psidin constructs were expressed by the strong driver act-GAL4. The ORNs target towards the antennal lobe and form there the glomerulus Or59c (Figure 5.1, A). In *psidin*^{1} mutant background, a complete loss of innervation at the respective target glomerulus could be observed. The ORNs were all spread over the entire antennal lobe (Figure 5.1, E). Only 25 % showed the wild type targeting, 45 % revealed strong mistargeting and 30 % showed mild mistargeting (Figure 5.2, E).

In flies with the mutated allele *psidin*^{IG978} the ORNs showed a strong mistargeting, but compared to *psidin*^{1} considerably milder. By showing 55 % (Figure 5.2, I) mild mistargeting, not all of the ORNs reached the glomerulus in a correct manner but some did and so the glomerulus was visible but not as much as in the wild type (Figure 5.1, I). To quantify the targeting of the Psidin phosphomutants in those three backgrounds a rescue experiment with Psidin^{wt} was carried out first. The targeting defect was rescued in all three backgrounds (Figure 5.2, B, F and J).

All ORNs reached the Or59c glomerulus (Figure 5.1, B, F, and J). Phosphomutant isoforms Psidin^{S678A} and Psidin^{S678D} were expressed in eyFlp clones in *wild type* background and had there no effect on the targeting of Or59c axons (Figure 5.1, C, D and Figure 5.2, C, D), while they failed to rescue the mild mistargeting in *psidin^{IG978}* background (Figure 5.1, K and L). Still 40-50 % mild mistargeting (Figure 5.2, K and L) was observed. This unsuccessful rescue has something to do with the dimerization of Psidin. Psidin^{wt} could rescue this phenotype successful due to identical phosphorylation level of both proteins which might affect the formation of homodimers. But in contrast Psidin^{S678A} and Psidin^{S678D} were able to rescue the strong mistargeting phenotype of *psidin^{1}* (Figure 5.1, G and H). Psidin^{S678A} could reduce the strong mistrageting in *psidin^{1}* background to a level of 10 % mild mistargeting (Figure 5.2, G) and Psidin^{S678D} reduced it to 8 % strong mistargeting (Figure 5.2, H).

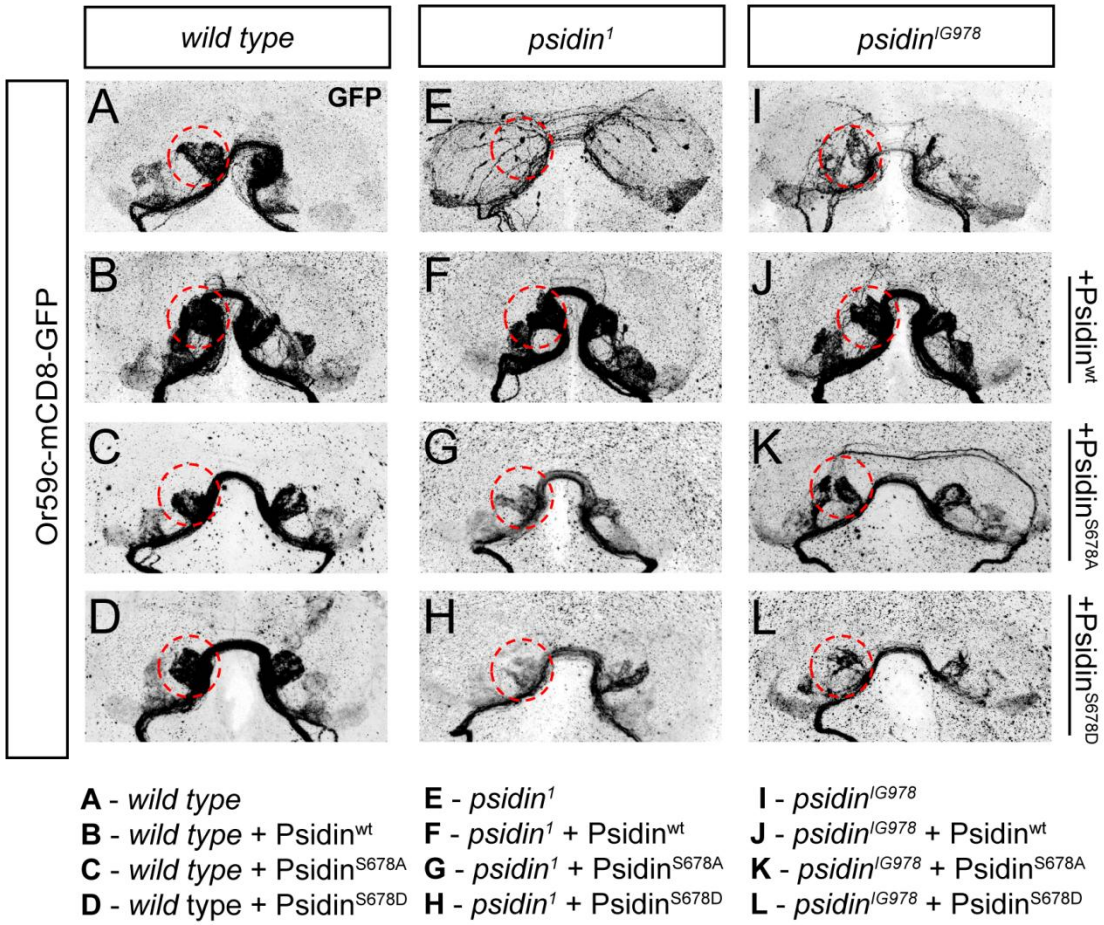


Figure 5.1 Targeting pattern in adult fly brains

To visualize the constructs in the antennal lobe the flies were crossed to an olfactory receptor (OR) marker stock. In this study the construct OR59c-mCD8-GFP was used to drive the expression of ORNs projecting from the antennae into the OR59c glomerulus in the antennal lobe.

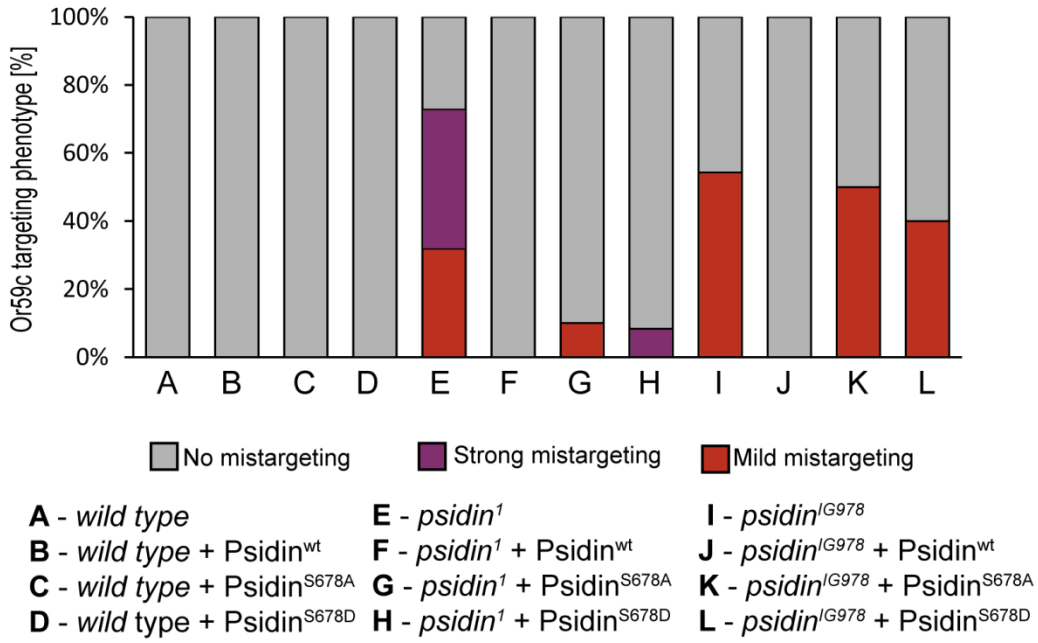


Figure 5.2 Quantification of the targeting pattern in adult fly brains

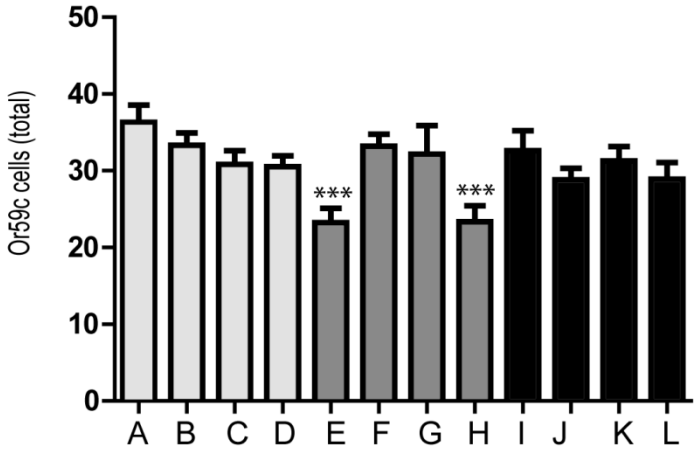
The rescue of the two phosphomutant isoforms of Psidin in the *wild type*, *psidin*^{1} and *psidin*^{IG978} background led to different categories of the targeting phenotype. While the wild type phenotype is colored grey, strong mistargeting is colored pink and the quantification of mild mistargetin is colored red.

These experiments pointed out that the phosphorylation at serine S678 has no impact on the targeting of the ORNs towards the Or59c glomerulus. Both constructs, the phosphomimic protein Psidin^{S678D} as well as the non phosphorylateable protein Psidin^{S678A}, showed the same results.

5.1.2 Cell number is rescued only by Psidin^{S678A}

In this experiment the cell bodies of the ORNs which target from the periphery of the maxillary palp into the Or59c glomerulus in the antennal lobe were counted and the effect of both phosphomutants on the cell number was observed. In wild type flies around 37 cell bodies could be counted (Figure 5.3, A). *Psidin*^{1} mutant flies showed a significantly reduced cell number of 23 (Figure 5.3, E) while flies carrying the *psidin*^{IG978} allele, cell numbers were not significantly decreased (Figure 5.3, I).

UAS-Psidin^{wt} rescue didn't affect the cell number in the wild type and *psidin*^{IG978} background (Figure 5.3, B and J) but this protein was able to rescue the null background *psidin*^{1} to wild type levels (Figure 5.3, F). Psidin^{S678A} restored wild type cell numbers in *psidin*^{1} background and had no impact in *psidin*^{wt} and *psidin*^{IG978} background (Figure 5.3, C and K) but full rescue in the loss of function mutant *psidin*^{1} (Figure 5.3, G). In contrast, Psidin^{S678D} couldn't rescue the cell number loss in the *psidin*^{1} background (Figure 5.3, H) and had also no negative effect on *psidin*^{IG978} or wild type background (Figure 5.3, D and L).



A - *wild type* **E** - *psidin*^{1} **I** - *psidin*^{IG978}
B - *wild type* + Psidin^{wt} **F** - *psidin*^{1} + Psidin^{wt} **J** - *psidin*^{IG978} + Psidin^{wt}
C - *wild type* + Psidin^{S678A} **G** - *psidin*^{1} + Psidin^{S678A} **K** - *psidin*^{IG978} + Psidin^{S678A}
D - *wild* type + Psidin^{S678D} **H** - *psidin*^{1} + Psidin^{S678D} **L** - *psidin*^{IG978} + Psidin^{S678D}

Figure 5.3 Quantification of the number of neuronal cell bodies in the MP

All types of overexpression in the three different backgrounds led to a normal number of cell bodies in the maxillary palp. Except in *psidin*^{1} background the cell number to wild type level is significantly different and also Psidin^{S678D} rescue failed in this background.
Bar graphs: One-way ANOVA, Bonferroni Post-test (* p<0.05, ** p<0.01, *** p<0.001). Error bars ± SEM

While the phosphorylation at S678 had an impact on the neuron number, the non-phosphorylated protein carrying S678A rescued the *psidin* null background like Psidin^{wt}. Given that the phosphorylation at this particular site entailed a reduced cell number something is regulated by it.

5.2 Analysis of Psidin mutants S678A and S678D *in vitro*

In order to address the effect of the phosphomimic construct Psidin^{S678D} and the non phosphorylatable construct Psidin^{S678A}, those two proteins were expressed in S2 cells for doing a co-immunoprecipitation.

5.2.1 Psidin interacts with CG14222

First of all I checked out if Psidin^{wt} is able to bind CG14222. So after the transfection of S2 cells with Psidin-HA the protein was immunoprecipitated and analyzed on a Western blot where it showed a size of about 118 kDa. In CG14222-myc single transfected S2 cells, a single band at 20 kDa was visible. Using co-immunoprecipitation it was possible to show the interaction of Psidin with CG14222 (Figure 5.4).

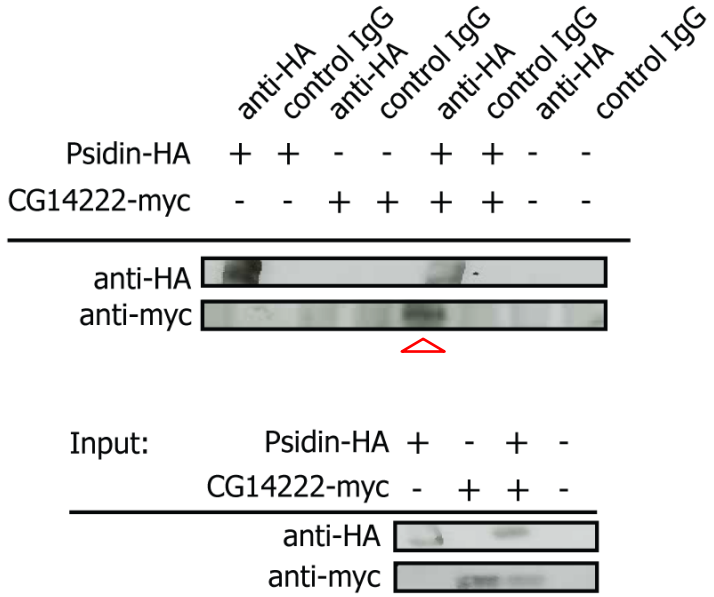


Figure 5.4 CoIP of Psidin^{wt} with CG14222

S2 cells were transfected either with Psidin-HA or CG14222-myc. Also a co-transfection was carried out. Red triangle marks pulled down CG14222 together with wild type Psidin *in vitro*.

5.2.2 Co-immunoprecipitation of Psidin^{S678D} with CG14222 showed reduced interaction

In order to investigate if the two phosphomutant isoforms of Psidin interact with equal intensity with the protein CG14222 a co-immunoprecipitation was carried out. In the IP against the HA tag of the non phoshorylatable mutant, Psidin^{S678A} pulled down CG14222 similar to the wild type level. The phosphomimic mutant Psidin^{S678D} showed a reduced binding of GC14222 (Figure 5.5, A).

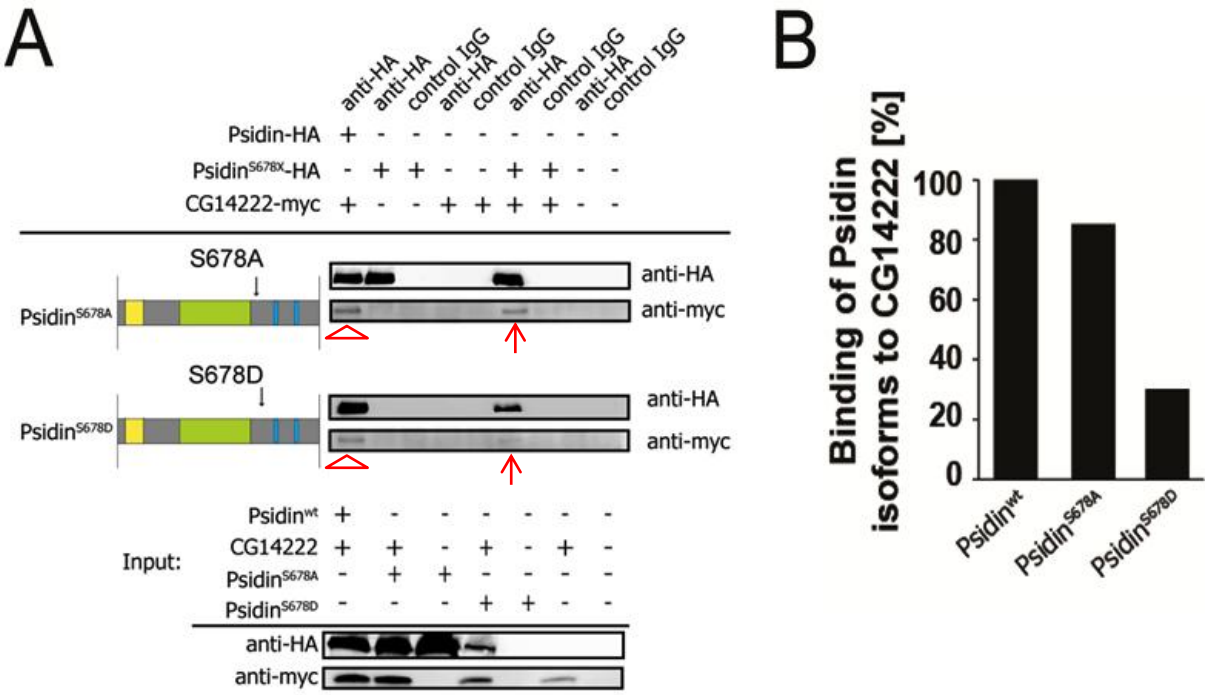


Figure 5.5 CoIP of Psidin^{S678A} or Psidin^{S678D} with CG14222 and quantification of the binding efficiency

(A) Psidin^{S678D} pull down with CG14222 (red arrow) showed a weak band compared to wild type (red triangle) or Psidin^{S678A} pull down. (B) Psidin^{wt} was normalized to 100 %. Psidin^{S678A} interacted in 85 % with CG14222 however Psidin^{S678D} in 30 %.

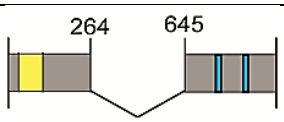
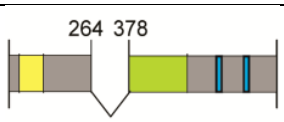
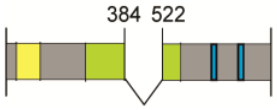
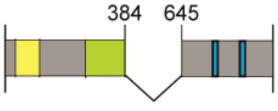
The quantification of this experiment illustrates the results. Binding of Psidin isoforms was normalized to Psidin^{wt} pull-down of CG14222. Psidin^{S678A} pulled down CG14222 (85 %) at comparable levels as wild type Psidin.

In contrast, Psidin^{S678D} showed a reduced pull-down of CG14222 (30 %), (Figure 5.5, B). This experiment pointed out the role of the phosphorylation site S678 for the interaction with CG14222. If this site is phosphorylated in Psidin, the interaction with CG14222 was intensely inhibited.

5.3 Mapping of the putative NatB domain in Psidin

The putative NatB interaction domain in Psidin is predicted from amino acid 264 to 645 while the entire protein consists of 948 amino acids. Distinct fragments of this domain were deleted (Table 5.1) and so the binding efficiency to CG14222 of the resulting Psidin variants could be tested in order to determine the binding region within Psidin.

Table 5.1 Overview of the deletions in NatB domain

	Position of deletion in the protein (aa)	Size of deletion (bp)	Size of Protein (kDa)
Psidin$^{\Delta NatB123}$		1146	74
Psidin$^{\Delta NatB1}$		339	105
Psidin$^{\Delta NatB2}$		417	102
Psidin$^{\Delta NatB23}$		786	88

The result of the co-immunoprecipitation against the HA tag of Psidin showed different binding efficiency to CG14222 caused by distinct deletions in the protein Psidin. Psidin^{wt} interaction with CG14222 was normalized to 100 %. If the entire subunit of the NatB domain was deleted in Psidin$^{\Delta NatB123}$ no band of pulled down CG14222 was visible (Figure 5.6, A). The deletion Psidin$^{\Delta NatB23}$ led also to a highly reduced CG14222 – binding efficiency of less than 10 % (Figure 5.6, B). Psidin$^{\Delta NatB2}$ remained with 40 % binding a bigger impact on the binding efficiency than Psidin$^{\Delta NatB1}$ but not as much as Psidin$^{\Delta NatB23}$. In contrast the deletion Psidin$^{\Delta NatB1}$ didn't affect the binding very much – only a small reduction of 20 % was observed (Figure 5.6, A and B).

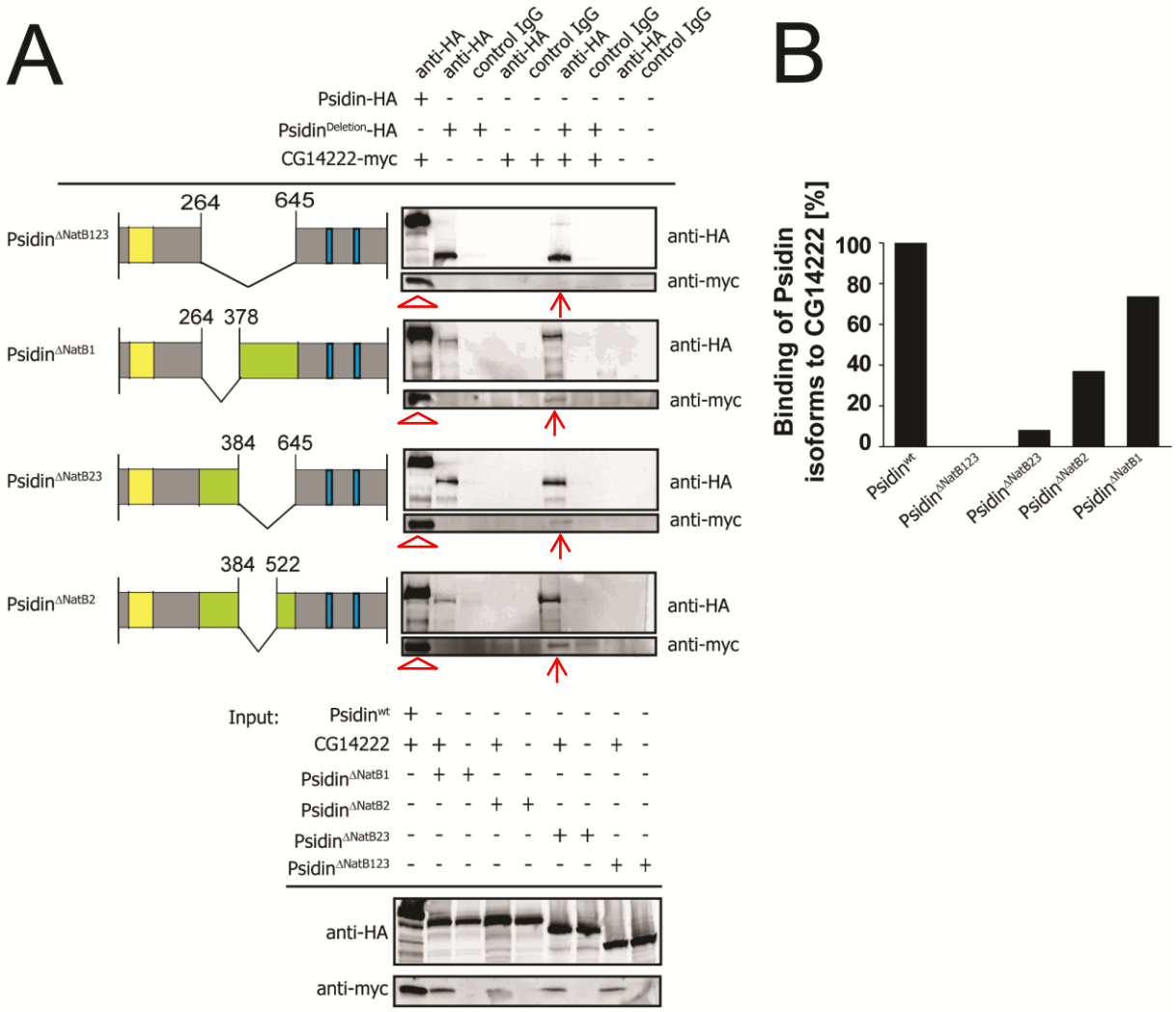


Figure 5.6 CoIP and binding efficiency quantification of Psidin deletion mutants with CG14222

(A) CoIP was against the HA tag of Psidin. Red triangles show CG14222 pull down by Psidin^{wt} and red arrows mark CG14222 pull down by Psidin$^{\Delta NatBX}$. **(B)** The deletion of the entire NatB domain in the mutant Psidin$^{\Delta NatB123}$ led to the complete loss of the binding to CG14222. However the absence of the first part hardly reduced the interaction. Psidin$^{\Delta NatB2}$ was less affected than Psidin$^{\Delta NatB23}$.

This experiment confirmed the predicted subunit of Psidin for the interaction with CG14222. While the deletion of the entire domain resulted in a complete loss of the binding efficiency to CG14222 the deletion of the first part didn't affect the interaction that much. However the absence of the middle part of this domain had a bigger impact. Accordingly, the C-terminal region of the subunit of the NatB complex is more important for the interaction than the N-terminus.

6 Discussion

Guidance and targeting of neurons have a big effect on the development of organisms. If something in these several pathways goes wrong, it can have enormous impact on animal or human. Especially defects in neuronal targeting in the olfactory system of humans can result in different diseases such as the Kallman syndrome (Lutz, B. et al., 1993).

This thesis showed on the one hand a putative mechanism of regulation of the protein Psidin, which was previously shown to be required for neuronal targeting in the olfactory system of the fruit fly. A particular serine S678 appears involved in the regulation of Psidin's two distinct functions. The motif VRSLMLR containing the serine 678 was shown to be phosphorylated in human homologue of Psidin (Trost, M. et al., 2009).

The phosphorylation at this site didn't appear to play a role in the targeting pattern of Or59c neurons. Both proteins the phosphomimetic and the phospho-ablative could rescue the strong mistargeting phenotype in the *psidin* null background. In contrast, they failed to rescue the mild mistargeting in $psidin^{IG978}$ background but there is no solid and persuasive reasoning so far. It might be that the dimerization of Psidin which is executed via the coiled coil domain (Kim, J. et al., 2011) could be affected. Perhaps, expression of wild type Psidin rescued $psidin^{IG978}$ background successful due to identical phosphorylation level of both proteins which might affect the formation of homodimers. To get a conclusive explanation for this observation more studies are required to clarify this aspect.

On the other hand this thesis revealed the role of the phosphorylation site serine 678 for the formation of the NatB complex. This complex consists of the auxiliary unit Psidin and the catalytic subunit CG14222. If this position is phosphorylated, fly Psidin as non – catalytic subunit can merely interact at very low levels with the catalytic subunit CG14222 of the NatB complex.

Furthermore, the absence of this complex generated by Psidin^{S678D} caused a reduction of the cell bodies of the ORNs in the MP. Accordingly, the interaction of Psidin with CG14222 in order to build the NatB complex supports the survival of the neurons since defects show disruptions in cell cycle progression or cell survival (Starheim, K. et al., 2008). So the NatB complex is a matter of survival for the neurons and can be built in the absence of the phosphorylation at S678 to acetylate proteins. Additionally, with this thesis the interaction domain between Psidin and CG14222 which had been predicted *in silico* so far could be characterized. Via the deletion of the entire domain in Psidin it has been shown that this site from amino acid 264 to 645 is essential for the binding of Psidin to CG14222. Additionally, the experiments ensured that the C-terminal region of this interaction domain is more important for the interaction with CG14222 because deletions of the posterior part had more impact on the binding efficiency. Accordingly, the N-terminal region of the subunit of the NatB complex is less important for the interaction.

It was observed that that co-expression and mutual binding of CG14222 and Psidin positively affects their expression levels. This provides an indication that protein levels are down regulated, if the formation of the NatB complex is disturbed. A similar phenomenon was observed for the human homologues (Starheim, K. et al., 2008).

Taken together Psidin harbors an interesting regulator to function in two distinct ways. For the formation of NatB complex which affected positively the survival of neurons, the absence of the phosphorylation at S678 is required. However the status of phosphorylation at S678 didn't affect the neuronal targeting in the fruit fly's brain. Since the phosphorylation site was found in human MDM20 which is Psidin's homologue this mechanism regards humans equally.

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