Decoding of Usher syndrome protein networks reveals insights in the molecular basis of the disease

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#USH2014

International Symposium on Usher Syndrome | July 10-11, 2014

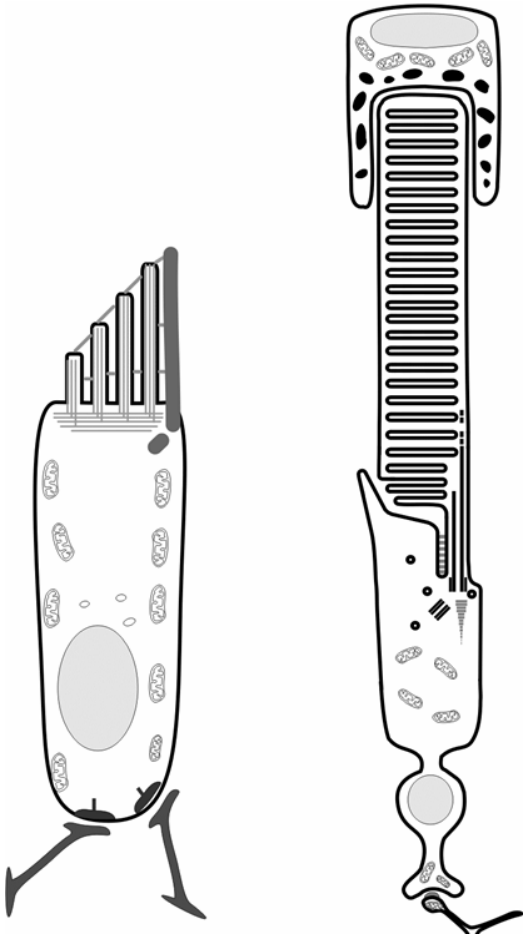
6th Annual Usher Syndrome Family Conference | July 12, 2014

BOSTON, MASSACHUSETTS, USA

JG|U

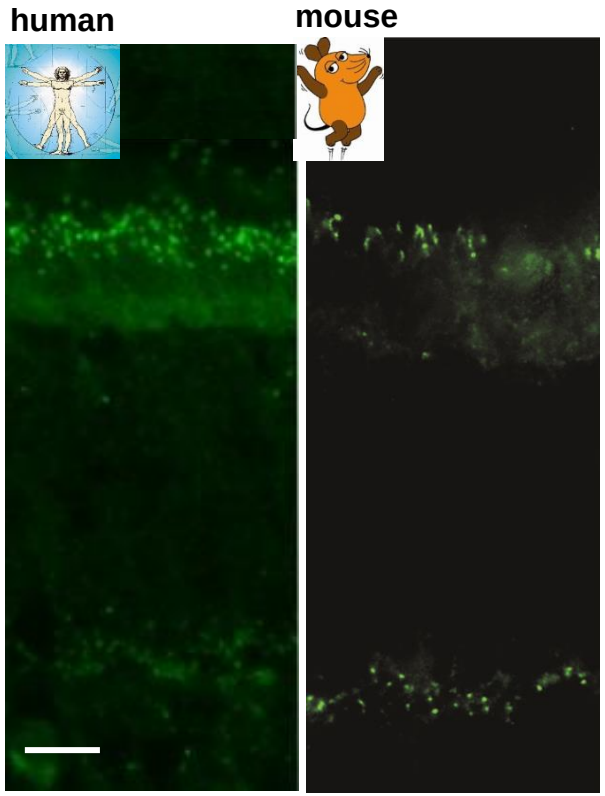
Human Usher syndrome (USH)

- USH is the most common form of combined hereditary deaf-blindness.
  ~ 1:10,000 to 1:6,000
  - Hearing impairment
  - Vestibular dysfunction
  - Vision loss - RP
- USH is a complex disease:
  - 3 clinical types (USH1, USH2, USH3);
  - > 12 genetic subtypes.
- 10 USH causing genes are identified, so far.
- USH proteins belong to diverse protein families
  molecular motors, cell adhesion
  & trans membrane molecules,
  scaffold proteins …

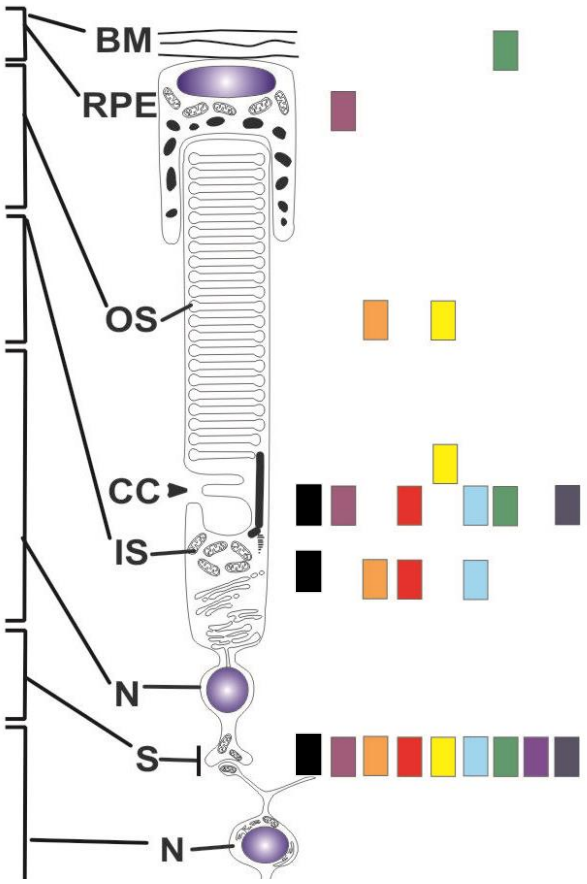


USH protein expression

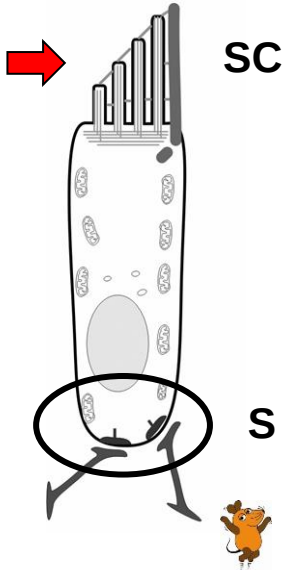
photoreceptor cells



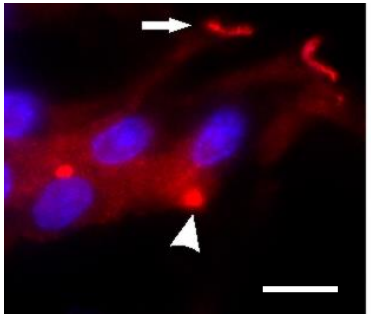
VLGR1/GPR98 (USH2C)



hair cells



VLGR1 (USH2C)



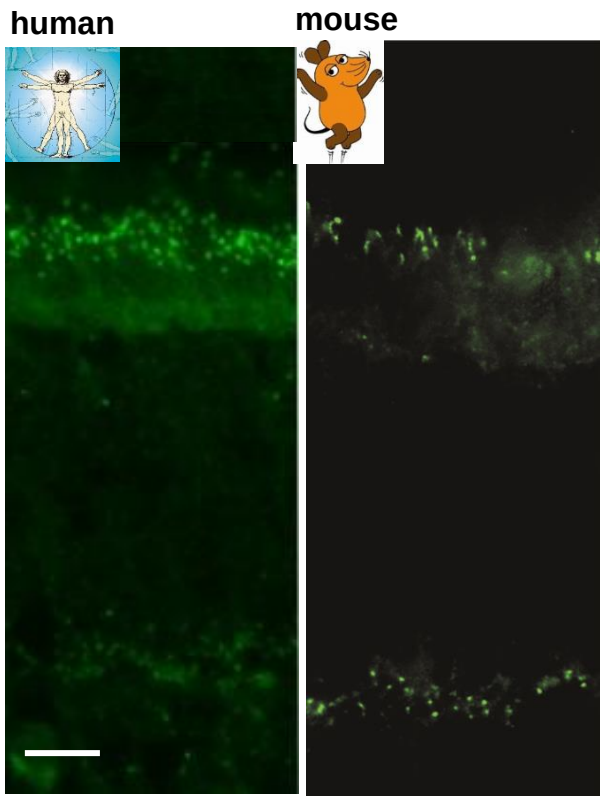
USH1: myosin VIIa | harmonin | Cdh23 | Pcdh15 | SANS

USH2: USH2A (usherin) | NBC3 ("USH2B") | VLGR1b (USH2C) | whirlin (USH2D)

Wolfrum (2011)

USH protein expression: synapse

photoreceptor cells



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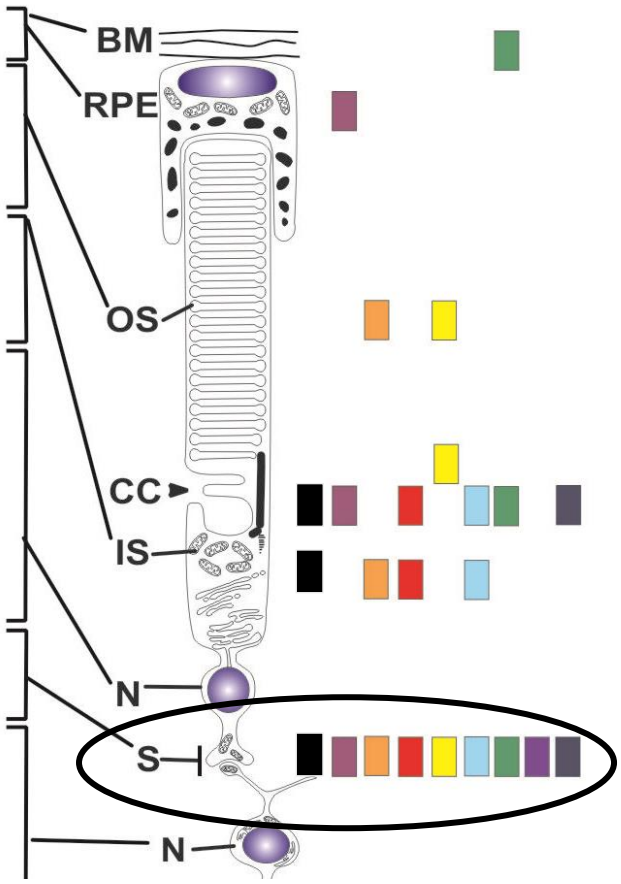
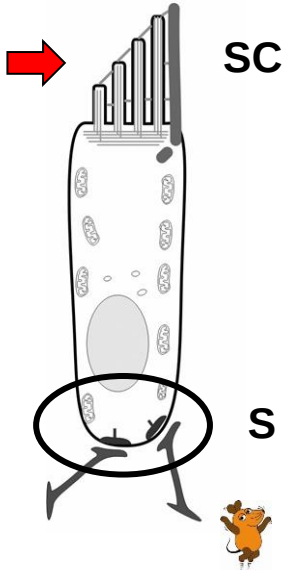


hair cells



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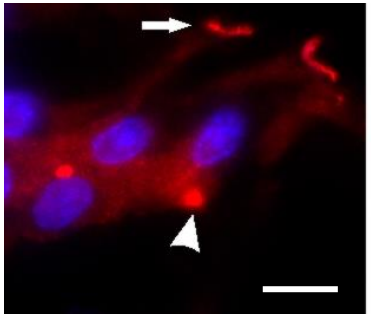


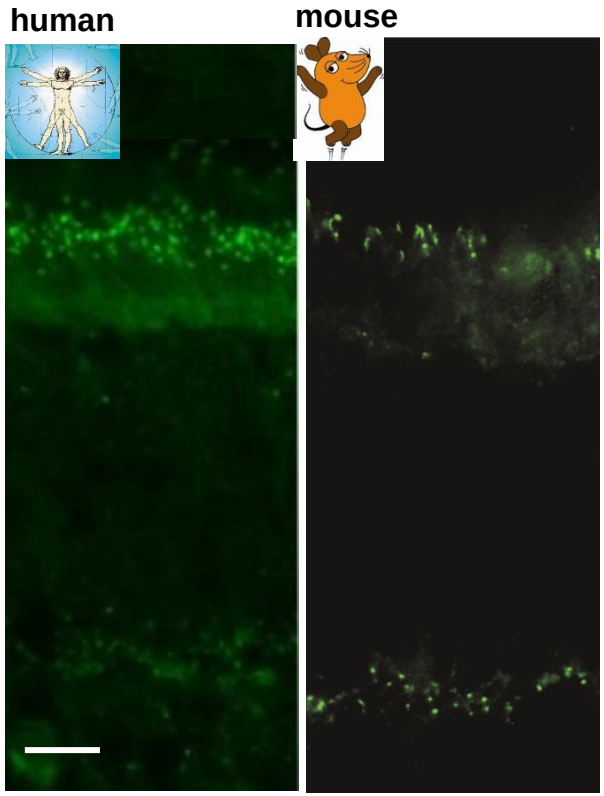
Harmonin (USH1C) binding regulates Ca^{2+} channel function (Gregory et al., 2013).

Whirlin (USH12D) may also contribute (Kersten et al., 2010).

Wolfrum (2011)

USH protein expression: cilium

photoreceptor cells



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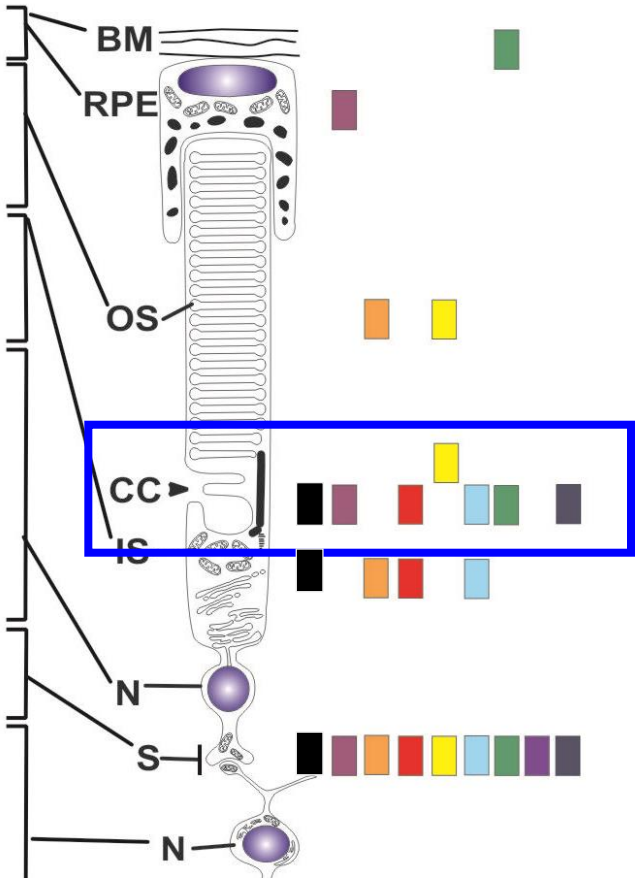
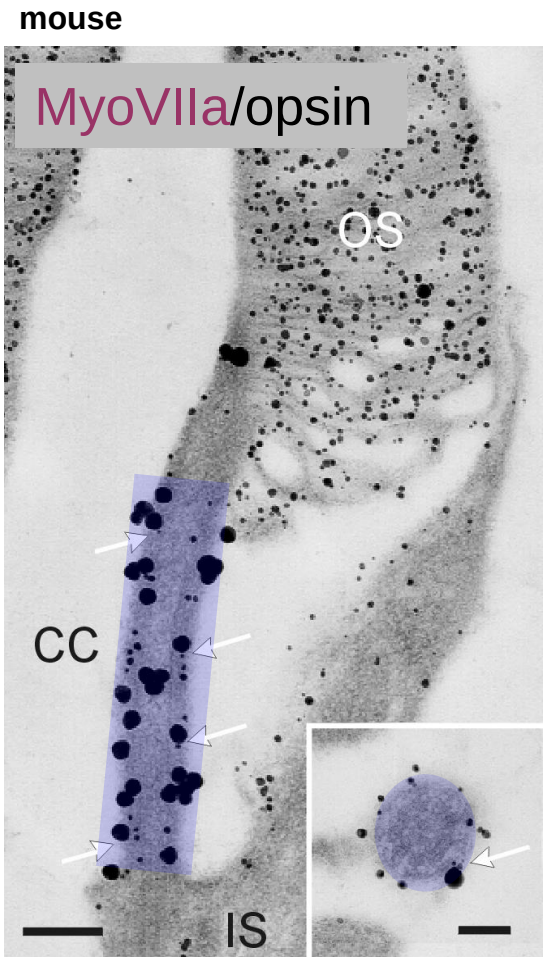




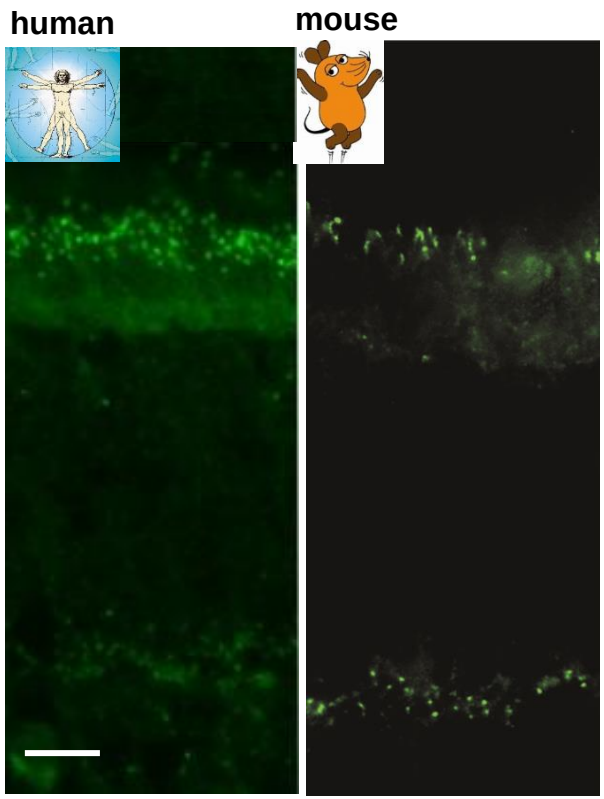
Most USH1 and USH2 proteins are associated with the photoreceptor cilium.

Wolfrum (2011)

Wolfrum & Schmitt (2000)

USH protein expression: calycal processes

photoreceptor cells



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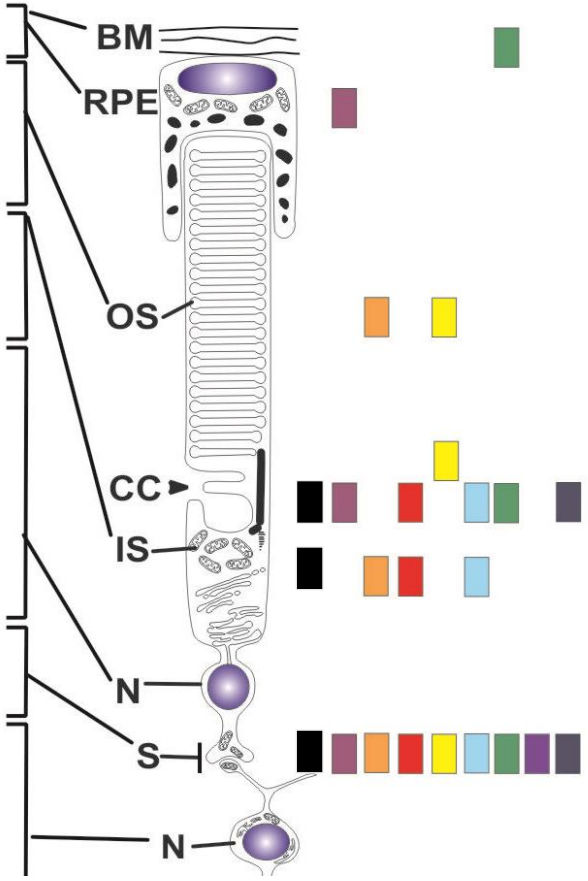
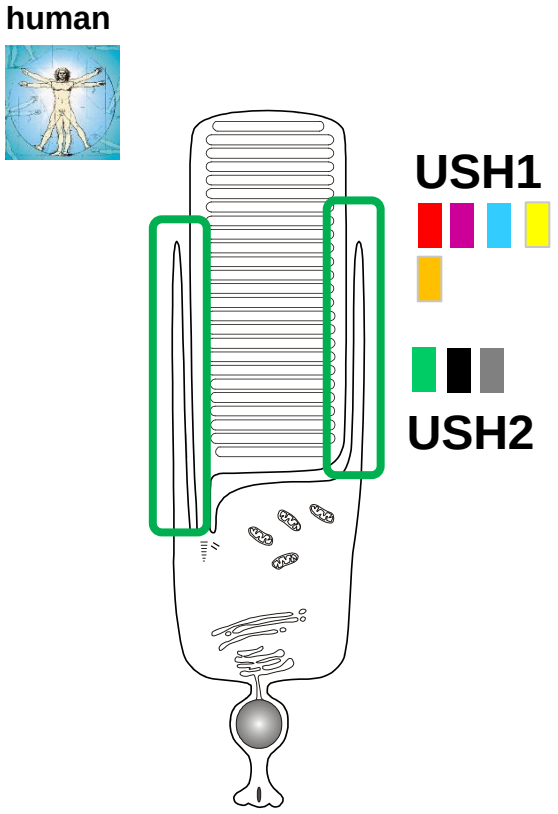




Sahly et al. 2012, JCB

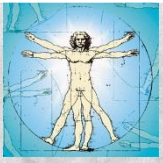
Wolfrum 2010, ARVO

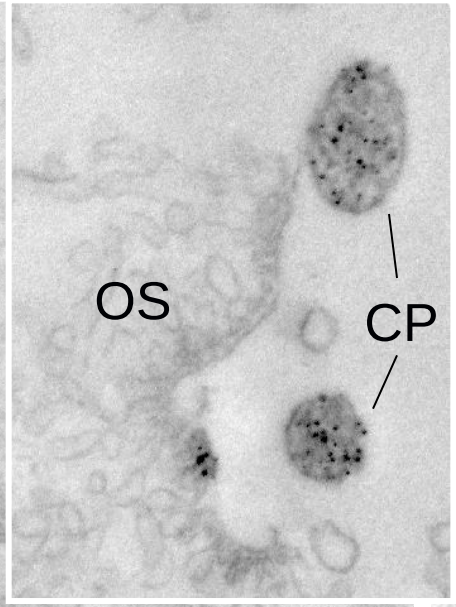
USH1 and USH2 proteins are associated with the calycal processes in human photoreceptor cells, not present in rodents.

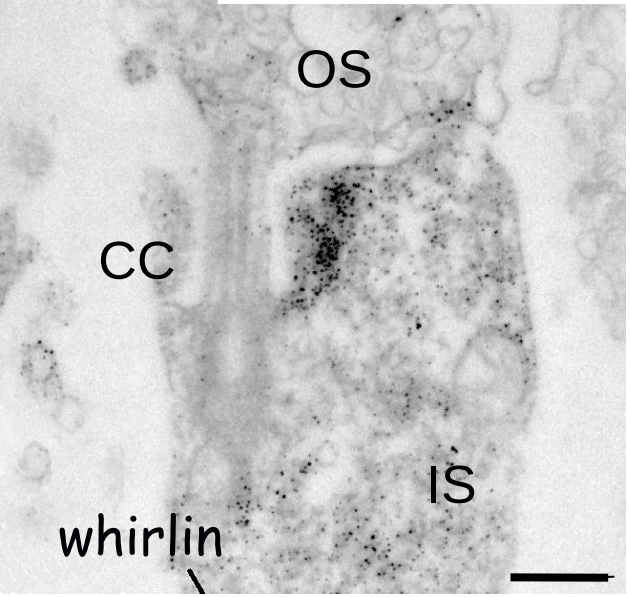
Wolfrum (2011)

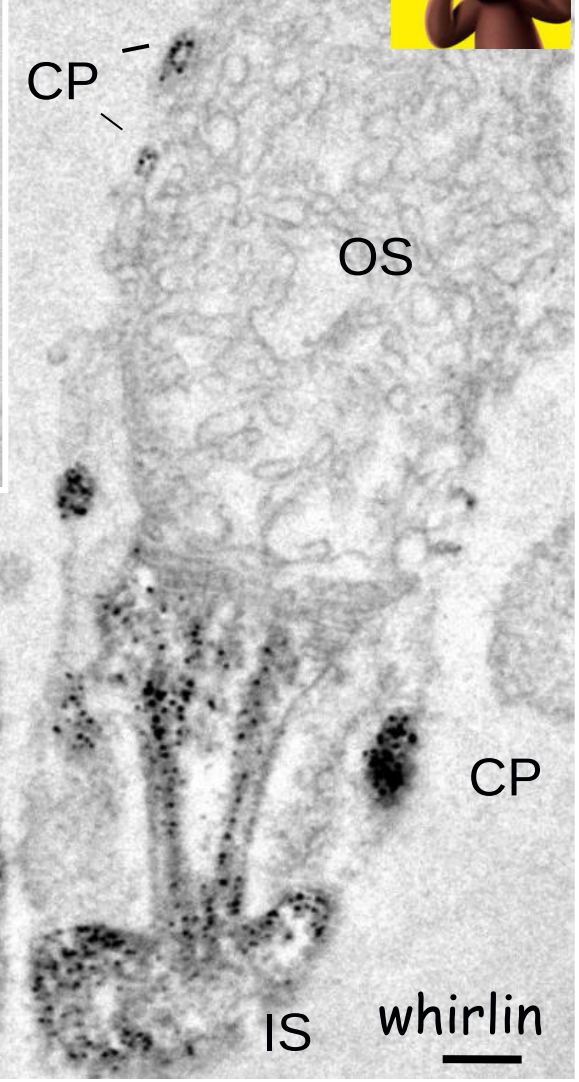
Nagel-Wolfrum et al., submitted

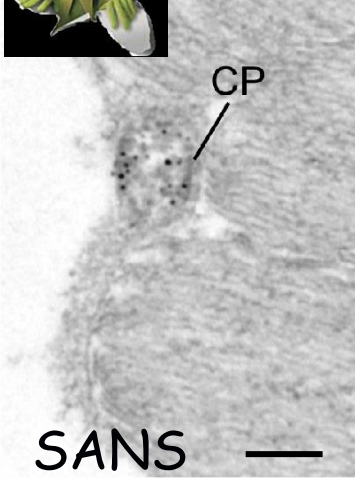
Evolutionary conservation of USH proteins in calycal processes of photoreceptors

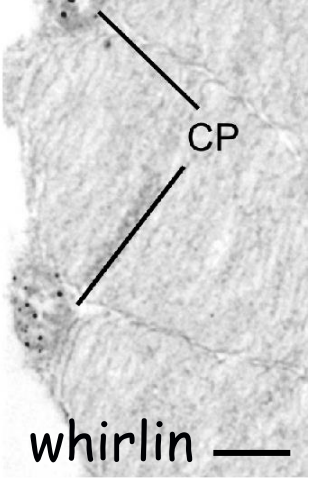


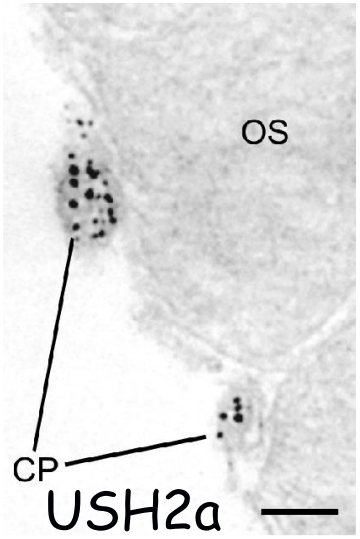


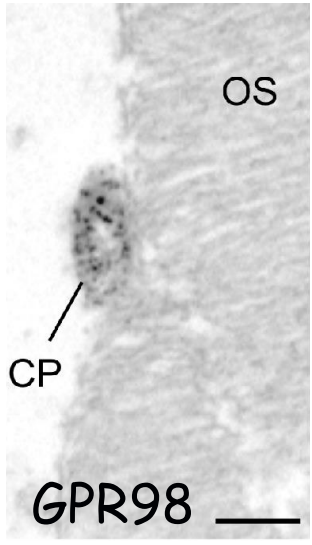












Nagel-Wolfrum et al., submitted

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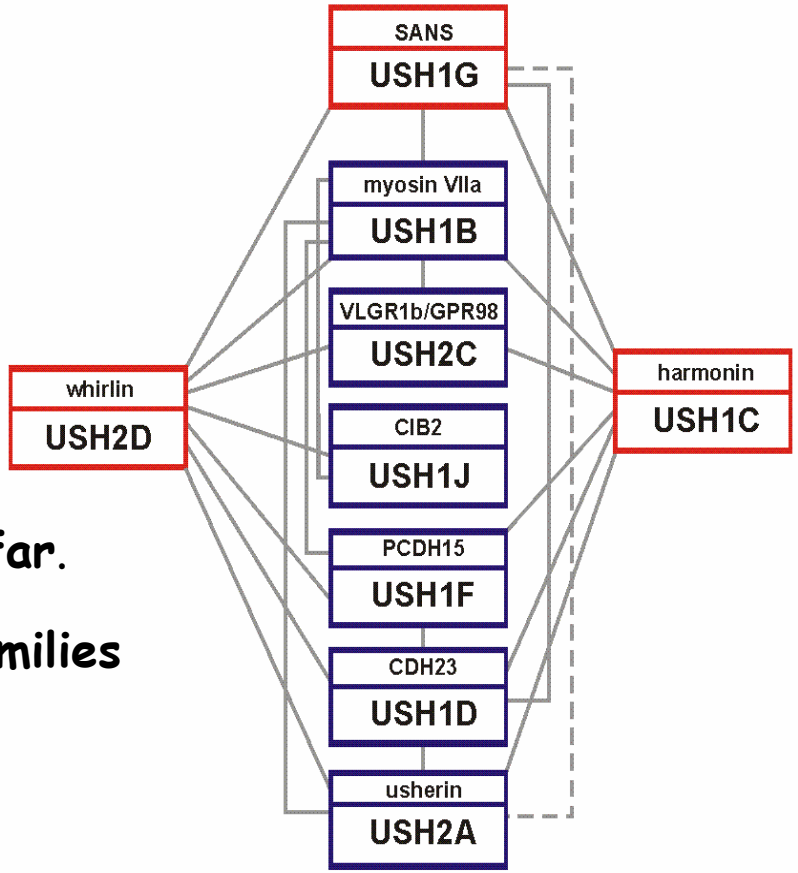
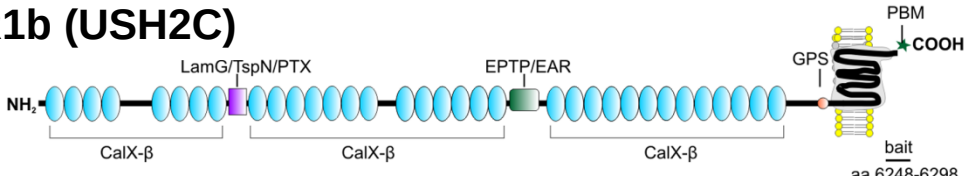
USH protein network

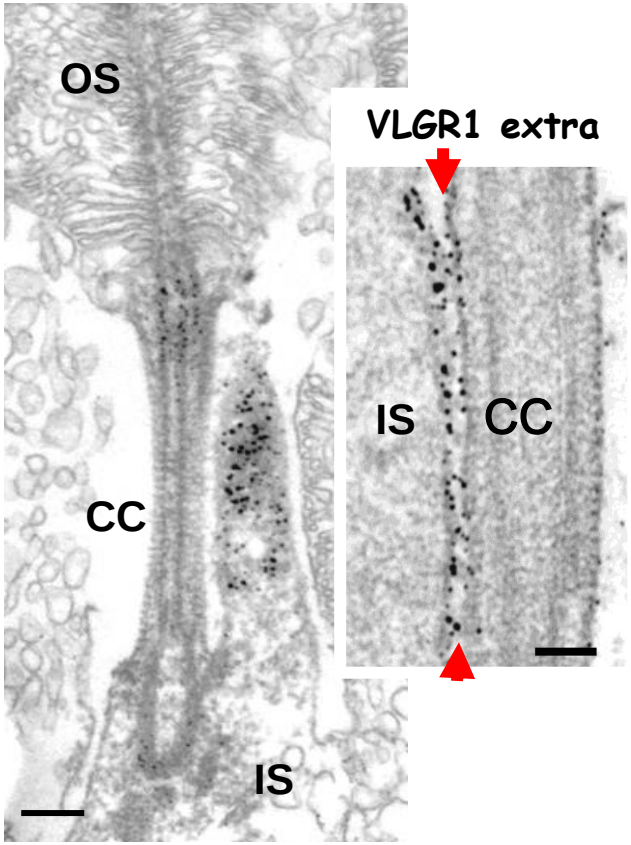


All USH1 and USH2 proteins are integrated into protein networks - the USH interactome.

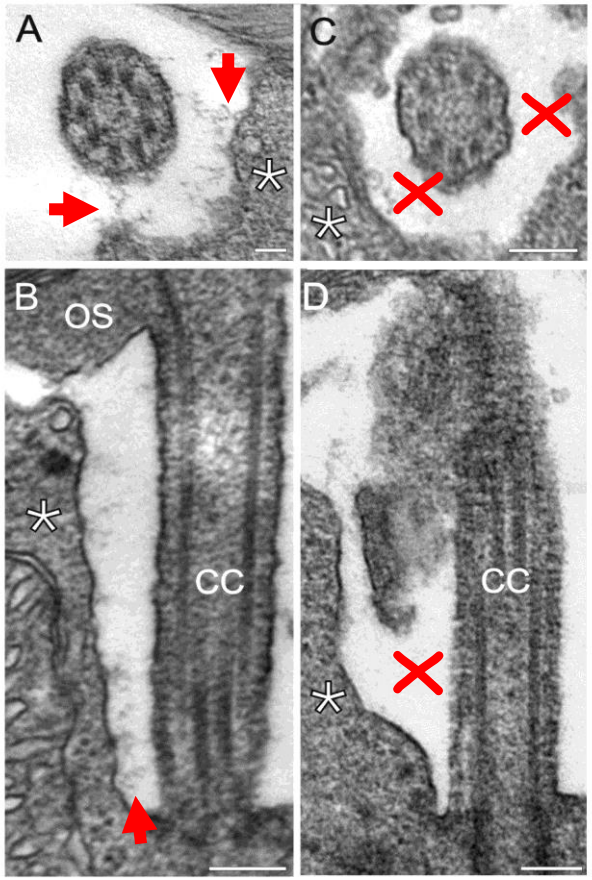
Periciliary USH protein complex

VLGR1b (USH2C)

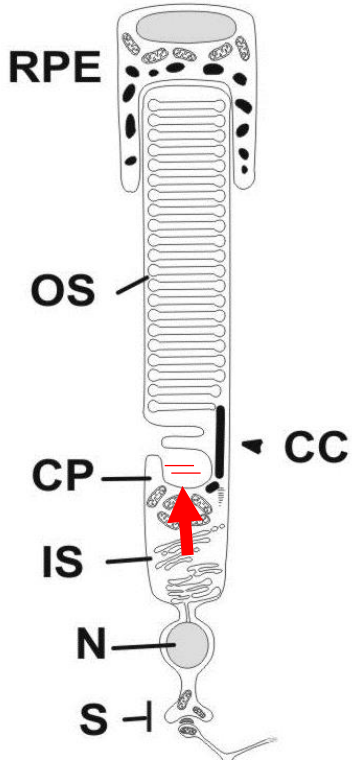




VLGR1 cyto



wild type Vlgr1/del7TM

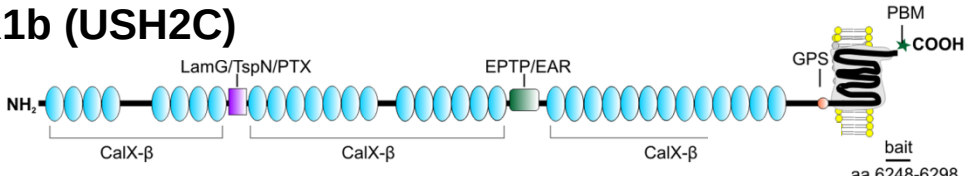


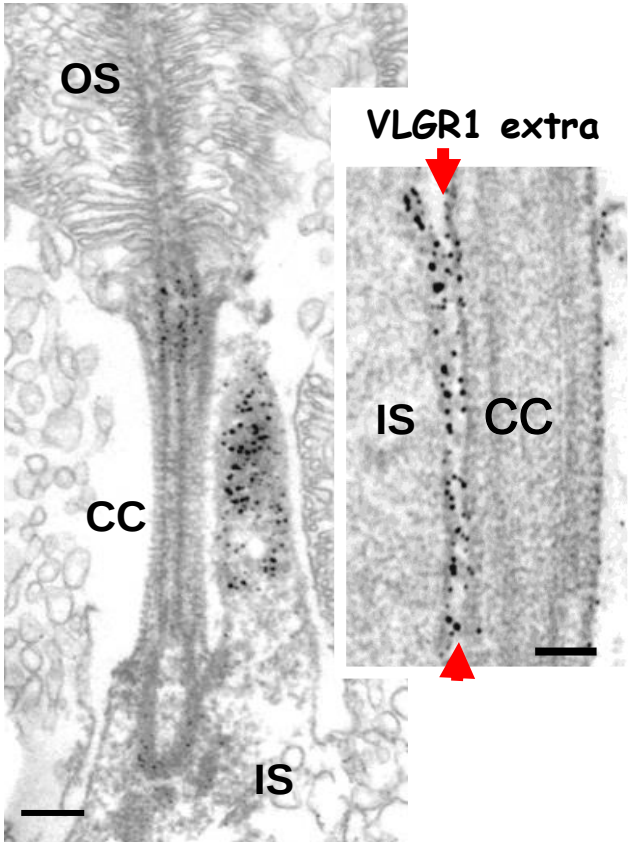
= fibrous links

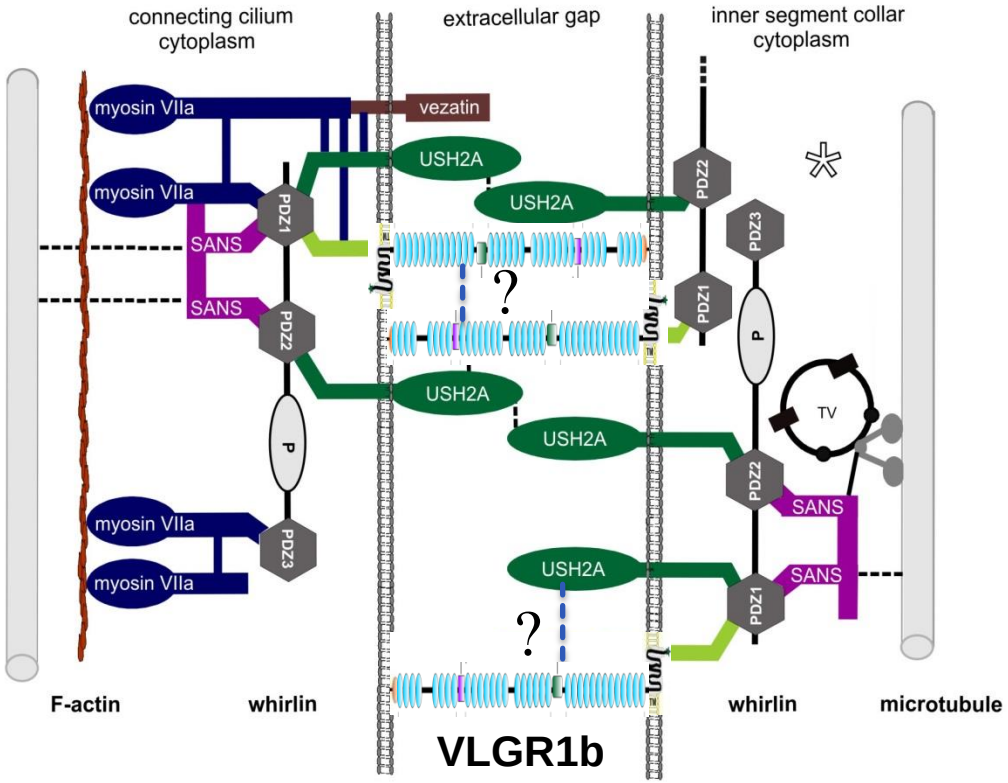
Maerker et al. 2008

Periciliary USH protein complex

VLGR1b (USH2C)

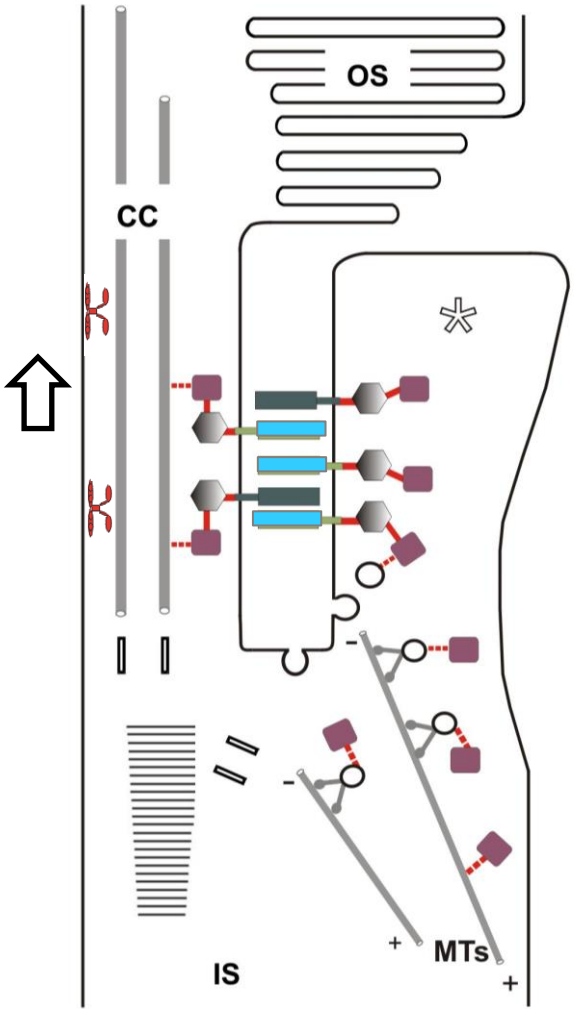


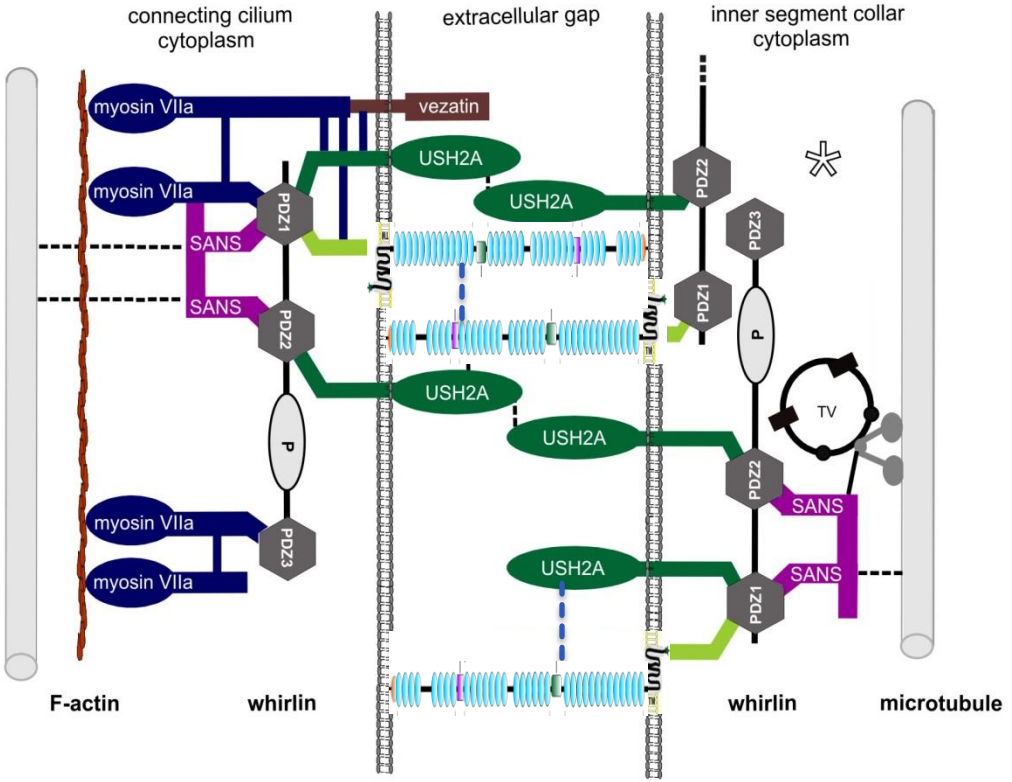




Maerker et al. 2008

USH protein complexes: ciliary cargo transport





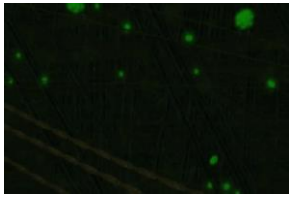
Maerker et al. 2008

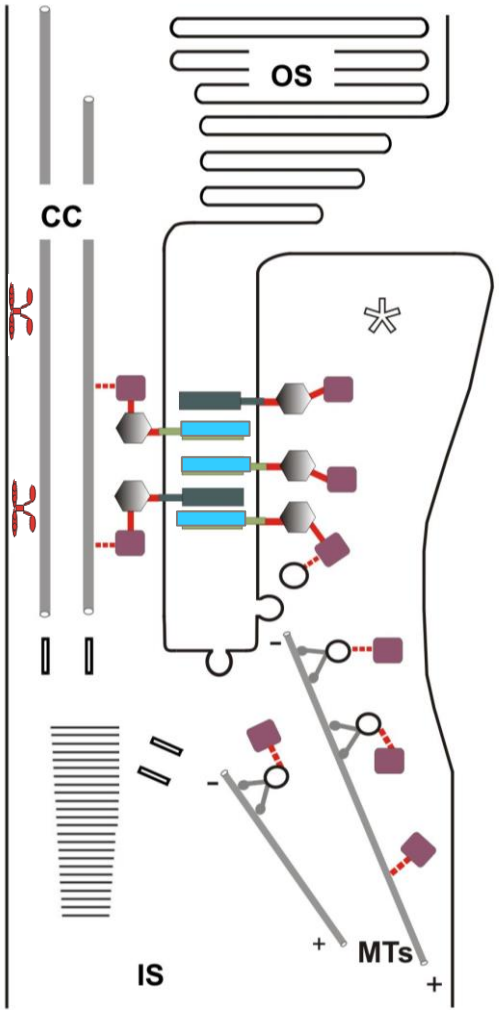
USH protein complexes: ciliary cargo transport

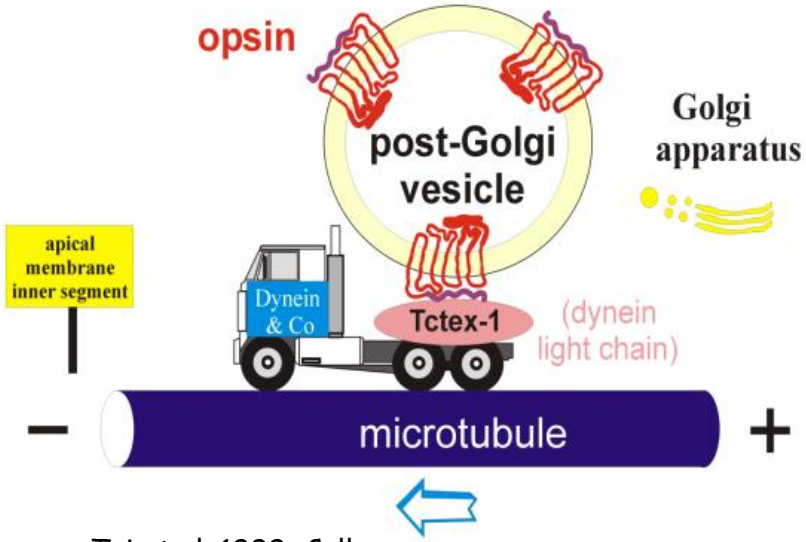
Myosin7a (USH1B)

Ciliary delivery of opsin

Liu et al. 1997, 1999
Wolfrum et al. 1998
Wolfrum & Schmidt 2000



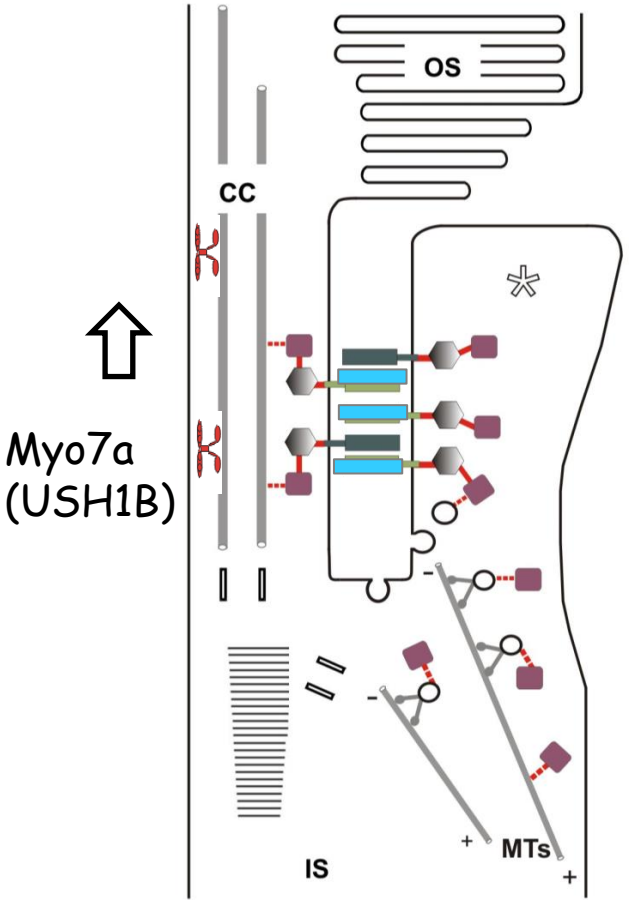




Tai et al. 1999, *Cell*

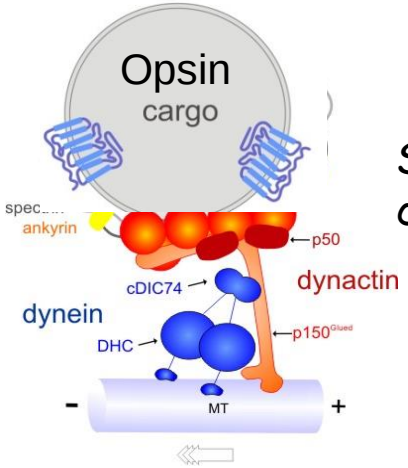
Opsin cargo binds via tctex-1 to the cytoplasmic dynein motor complex.

USH protein complexes: ciliary cargo transport



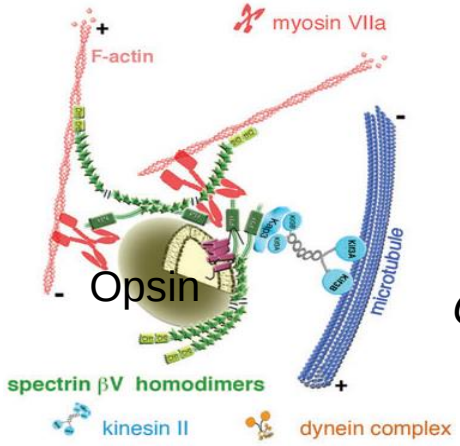
USH protein association with transport vesicles:

Overlack et al. 2011; Zallocchi … Cosgrove 2010, 2012; Papal et al. 2013; Bauß et al. 2014



SANS (USH1G) is part of the cytoplasmic dynein complex.

Sorusch et al. in prep.



Microtubules serve as transport tracks from Golgi to ciliary base, in association with myomegalin - SANS (SUH1G).

Overlack et al. 2011, BBA

Opsin cargo vesicles association with F-actin – spectrinβV – myosin7a

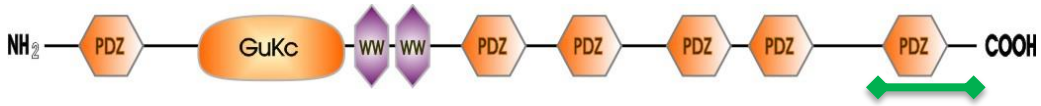
Papal et al. 2013, HMG

Identification of SANS binding partners

SANS

MAGI2

membrane-associated guanylate kinase inverted-2

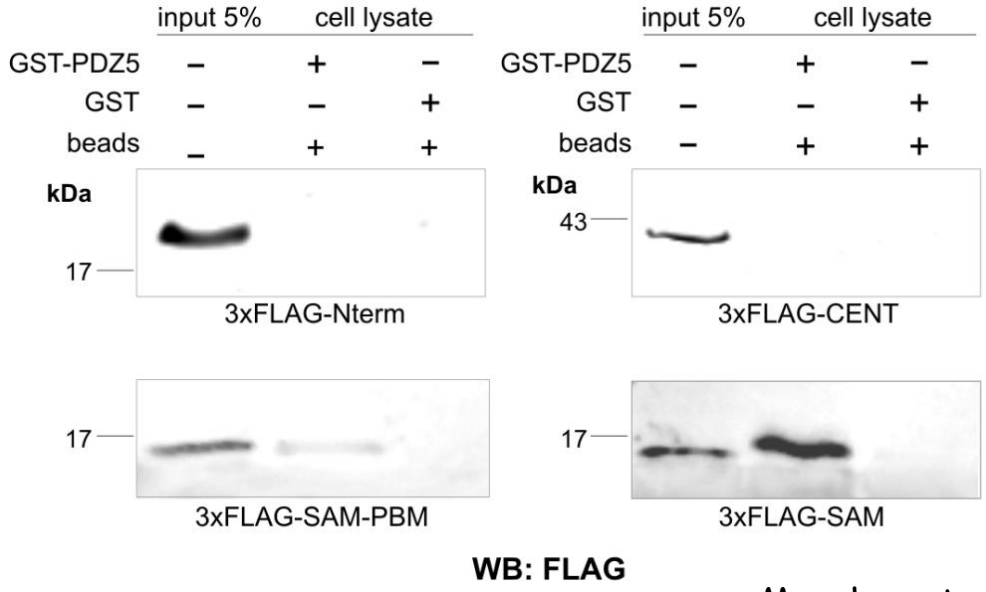


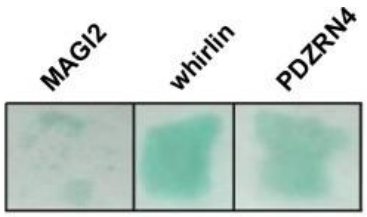
prey aa 1039-1112

Yeast-2-Hybrid Screen

bait aa 385-461

Valitation by GST-pull down





SANS interactors (SAMPBM domain)	n	function
whirlin (USH2D)	33	scaffold protein
MAGI2 (membrane-associated guanylate kinase inverted-2)	3	scaffold protein
PDZRN4 (PDZ domain containig Ring finger protein 4)	2	ubiquitin protein ligase

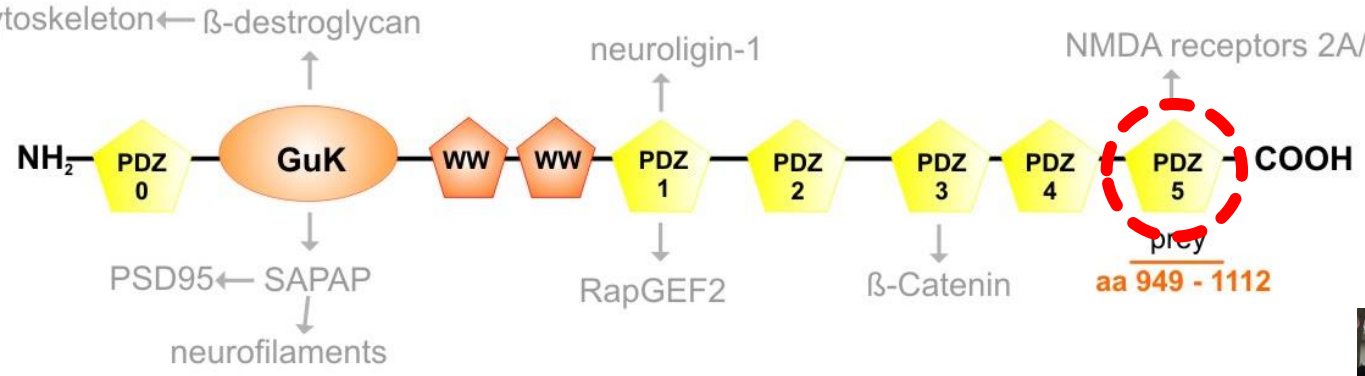
Maerker et al. (2008), Overlack et al. (2011); Bauß et al. (2014)

Summary SANS-MAGI2 complex

SANS

- SANS-SAM domain binds MAGI2-PDZ5.
- POW predicts internal PDZ binding motif: **SDLDL** motif aa422-426)*.
- CK2 mediates phosphorylation of SANS S422.

MAGI2



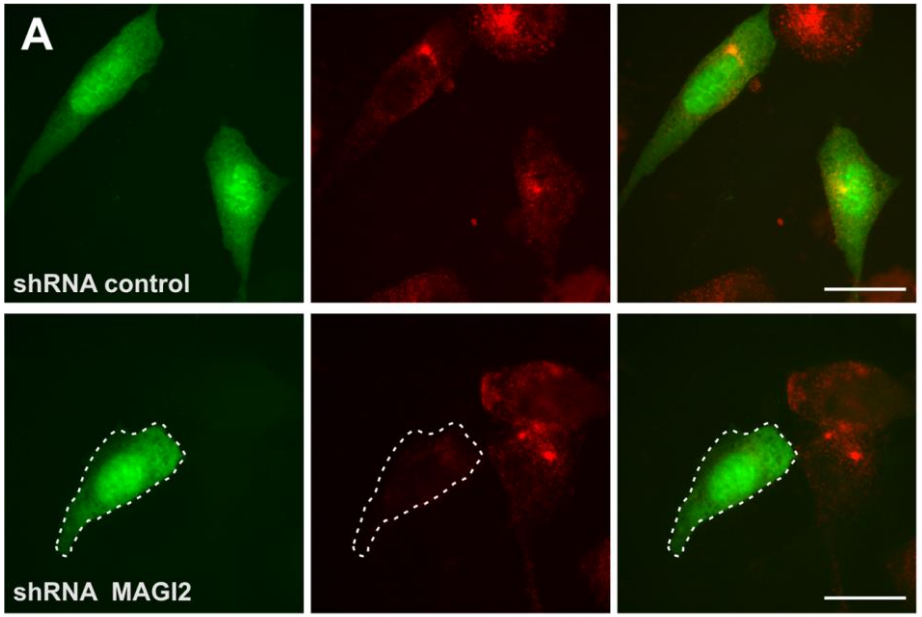
- MAGI2 = S-SCAM (synaptic scaffolding molecule)
- assembly of post-synaptic protein complexes
- endocytosis of AMPA receptor

Katharina Bauß

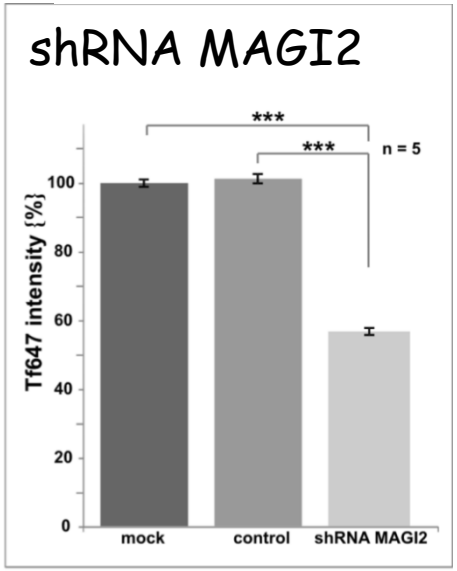
*POW: PDZ domain-peptide interaction prediction website

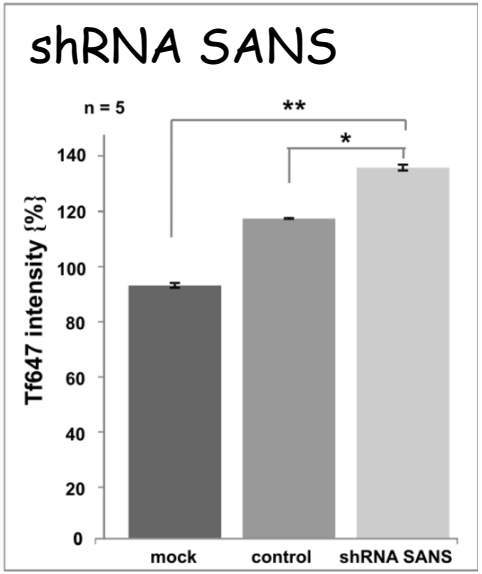
Bauß et al., Hum Mol Gen, 2014

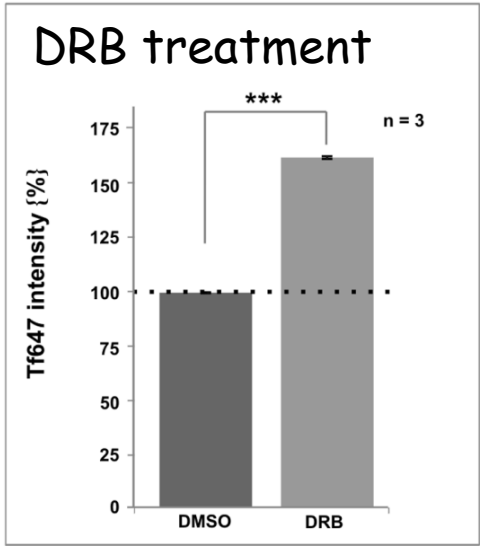
Transferrin (Tf647) up-take/endocytosis assays



Endocytosis is mediated by MAGI2, negatively regulated by SANS and by phosphorylation.

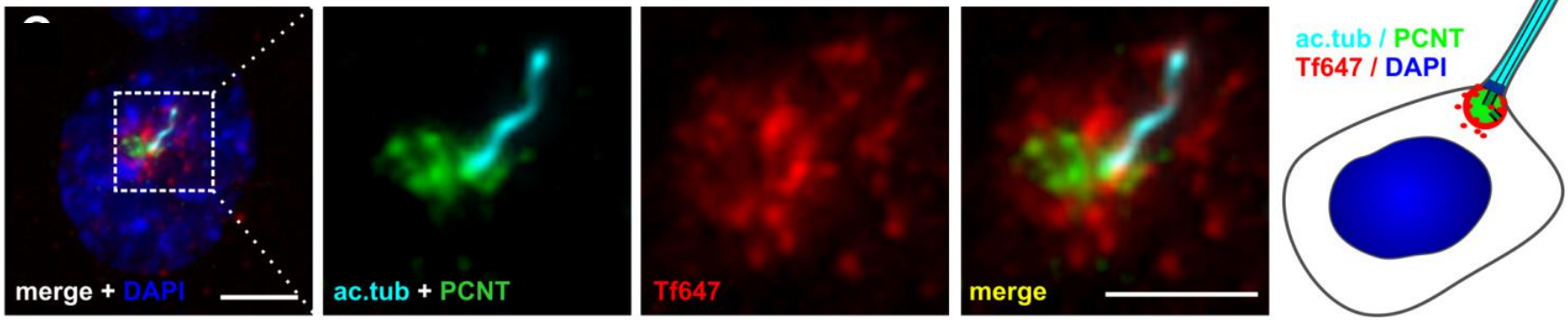




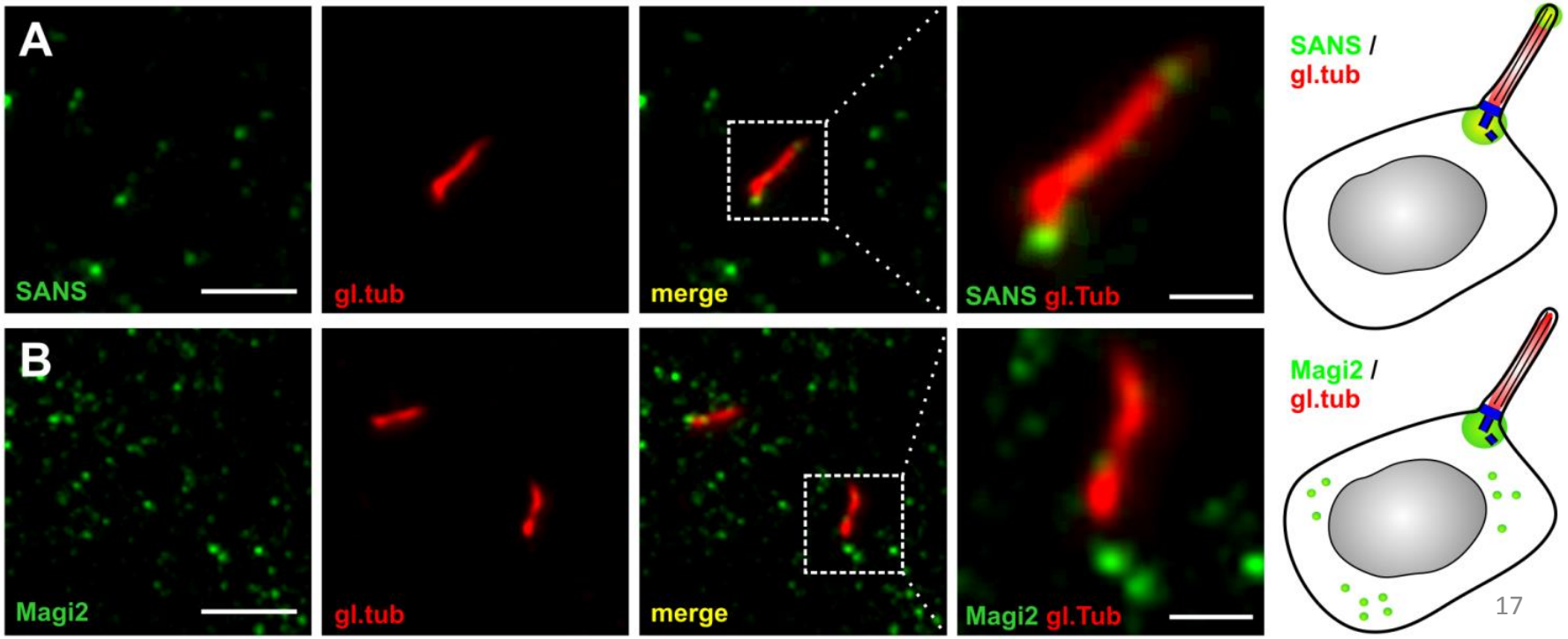


Bauß et al., HMG, 2014

Endocytosis at the base of primary cilia

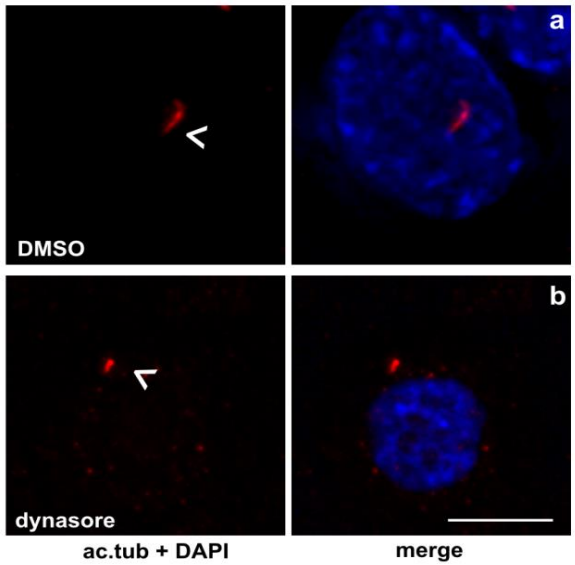


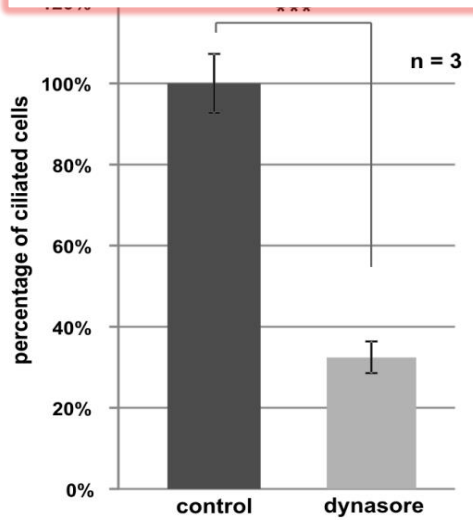
MAGI2-SANS complex at the base of primary cilia



Bauß et al. 2014

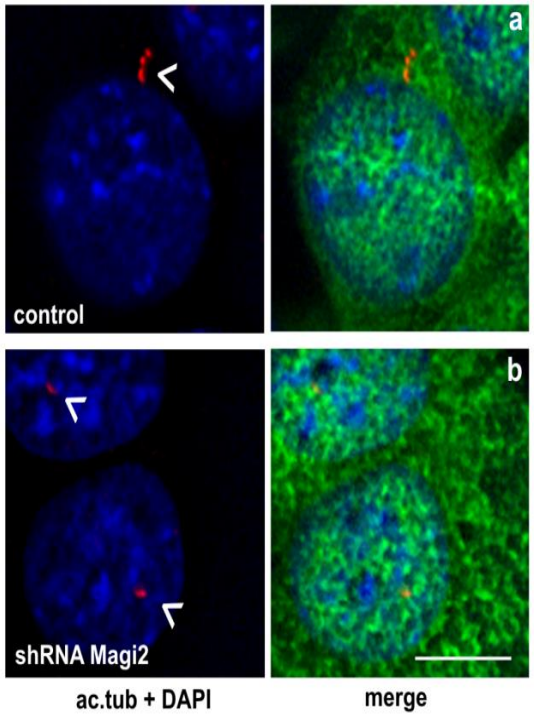
Dynasore treatment

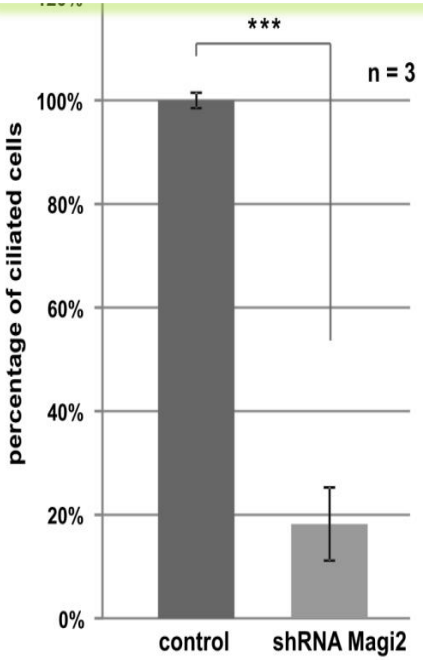




… both drastically reduces ciliogenesis.

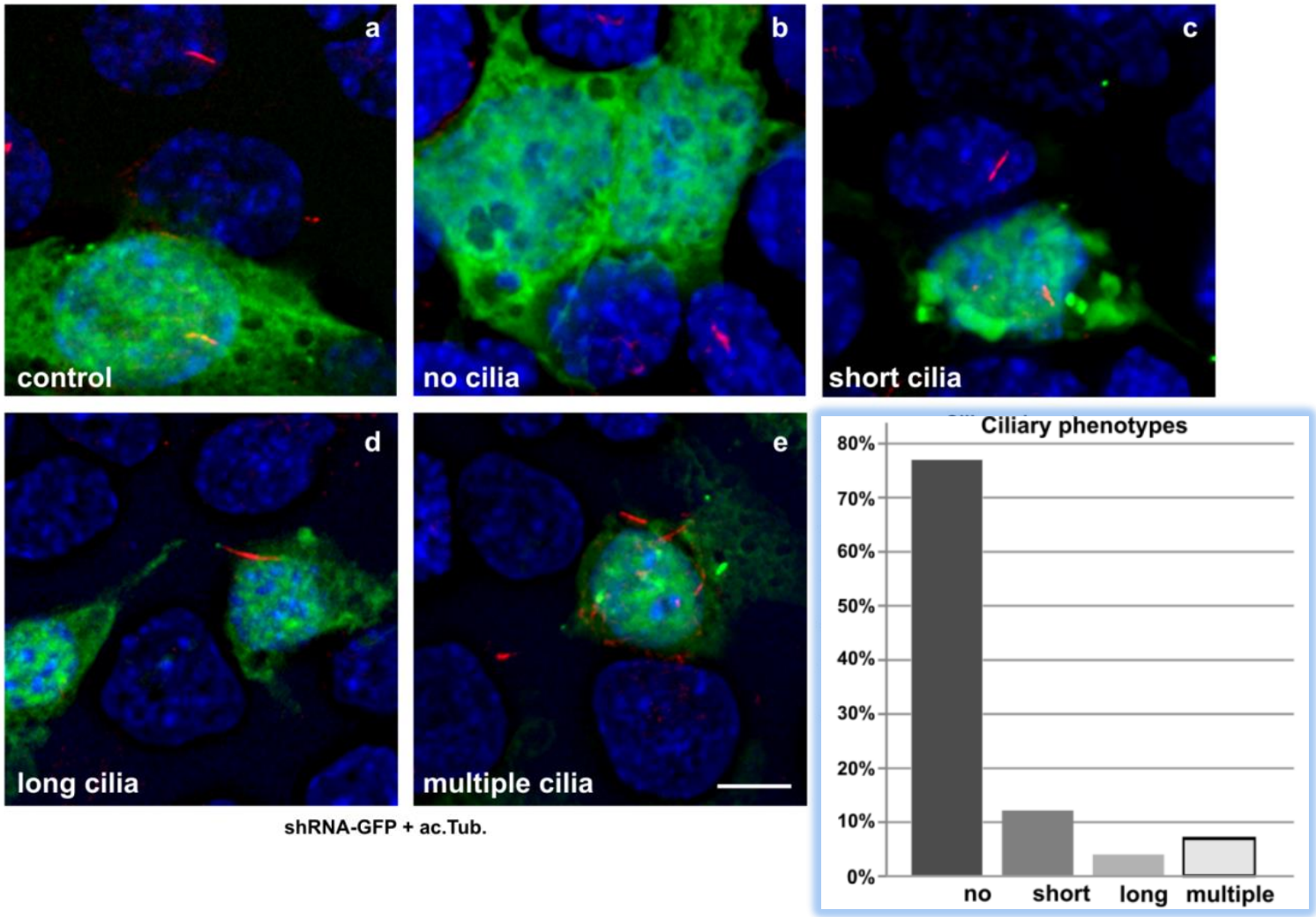
Magi2 depletion





Bauß et al., Hum Mol Gen, 2014

SANS depletion causes multiple phenotypes in primary cilia

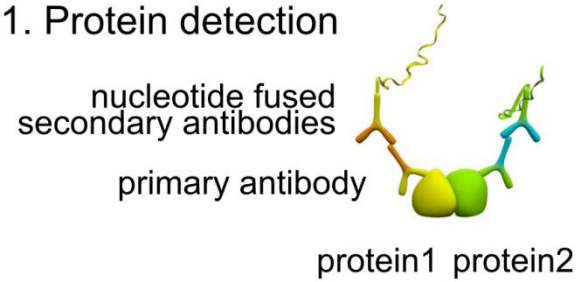


Bauß et al., Hum Mol Gen, 2014

In situ localization of MAGI2/SANS and CK2/SANS complexes in photoreceptor cells

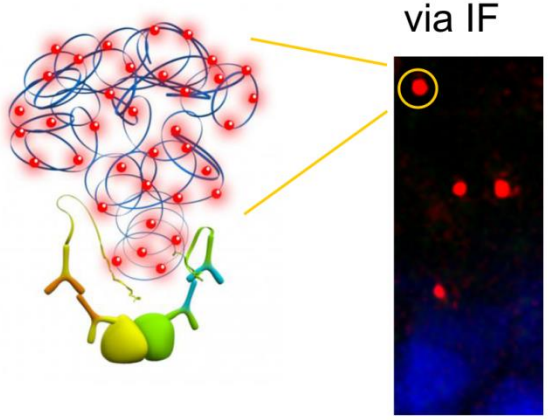
PLA: proximity ligation assay

1. Protein detection

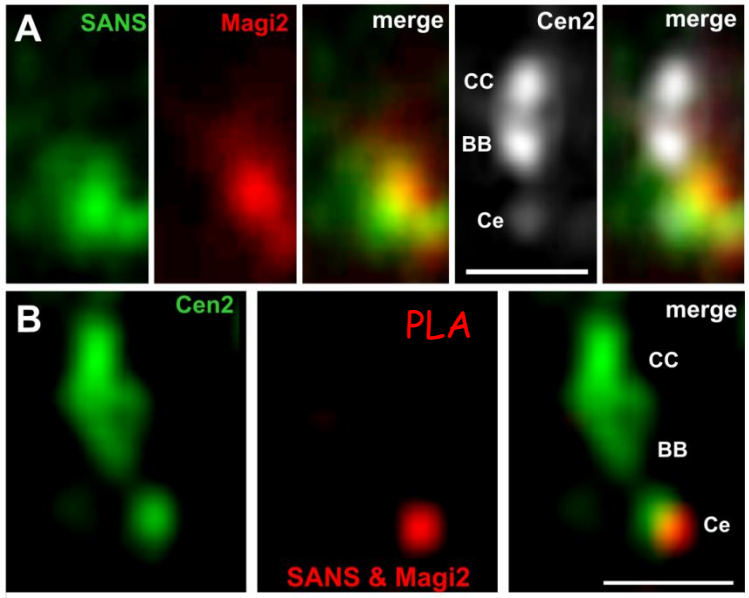


2. Signal amplification and labeling

3. signal detection via IF

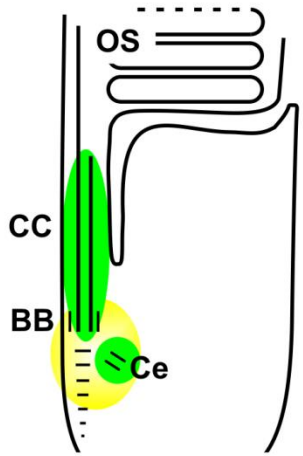


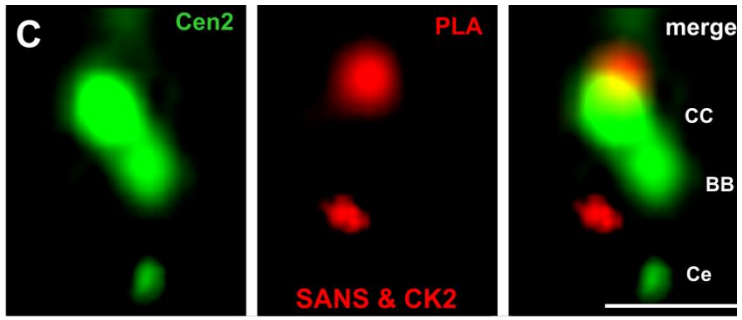
modified from http://www.olink.com



MAGI2/SANS

IHC



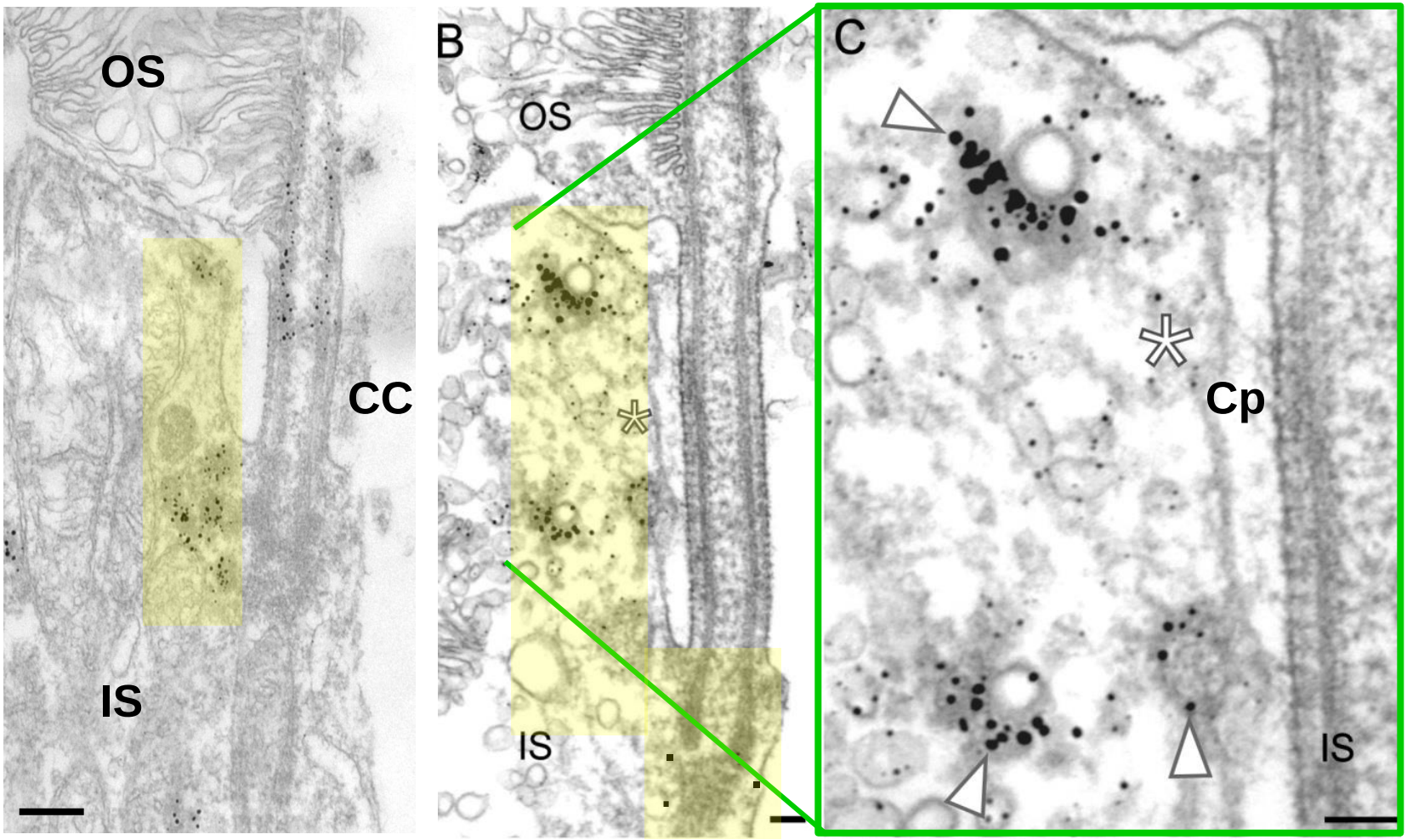


CK2/SANS

PLA

Bauß et al., Hum Mol Gen, 2014

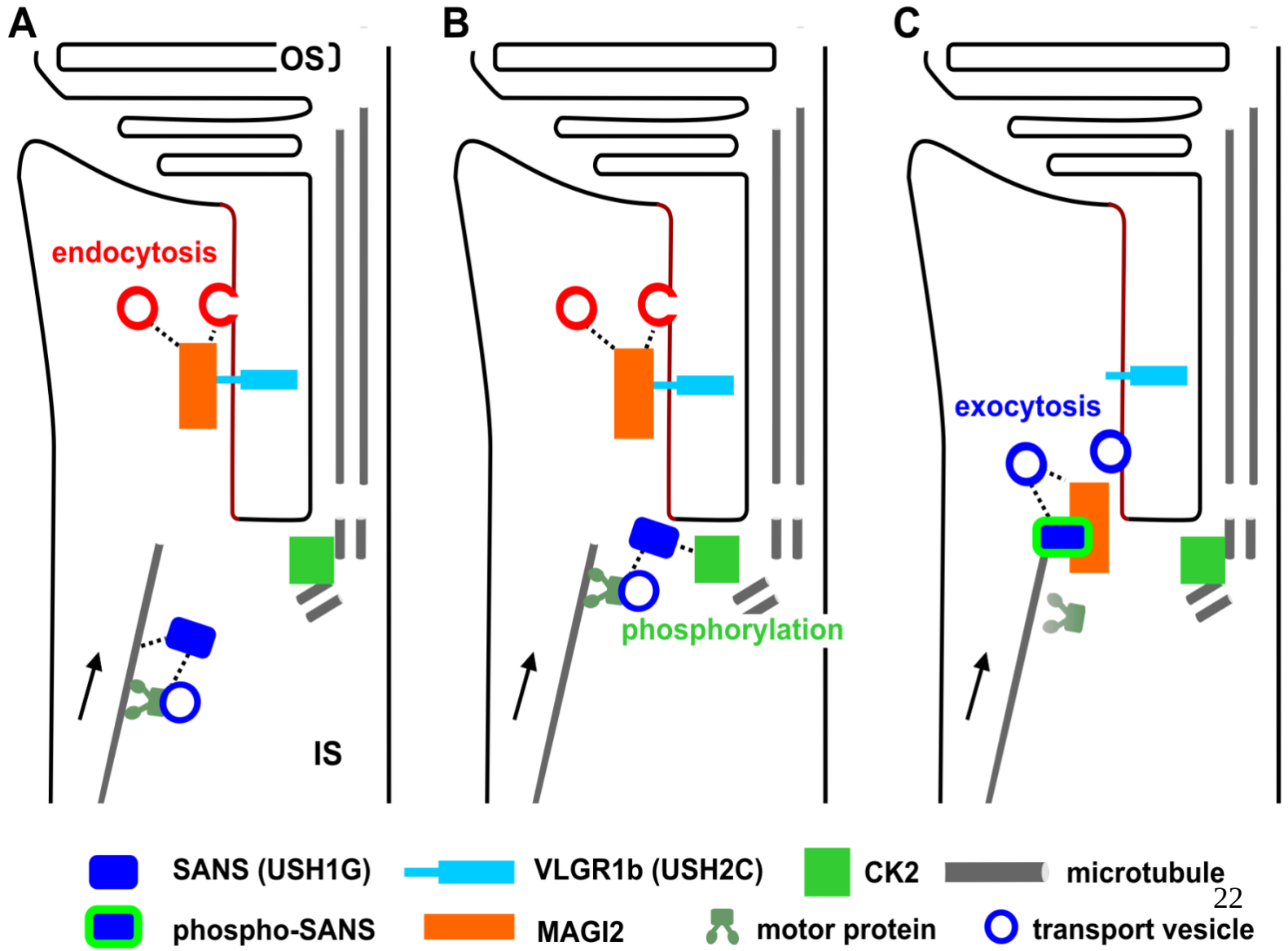
Sucellular localization of SANS and MAGI2 in the periciliary compartment of rods

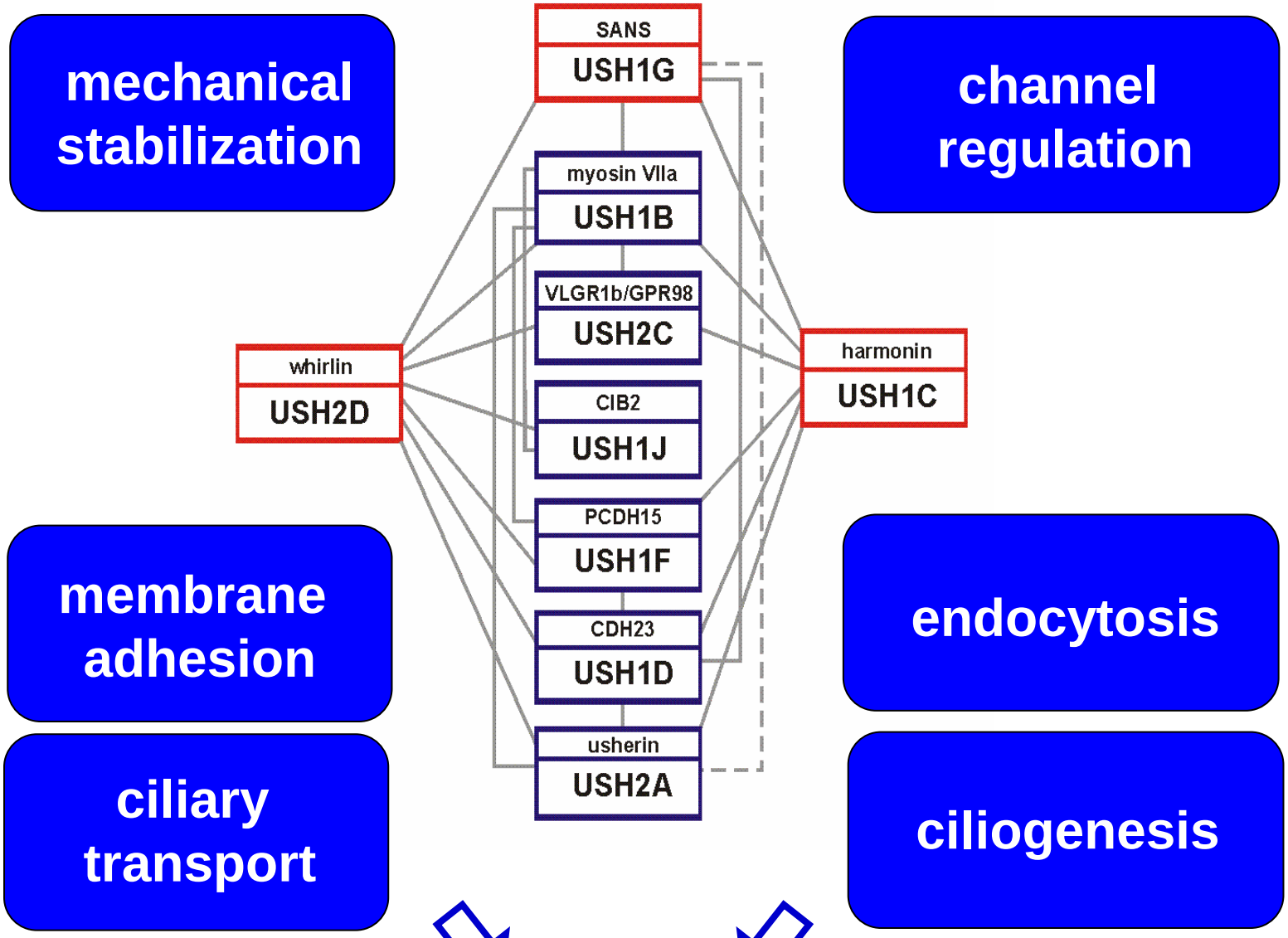


MAGI2 mediates endocytosis from the ciliary pocket (Cp).

Bauß et al. (2014) Hum Mol Gen

Putative SANS-MAGI2 complex function



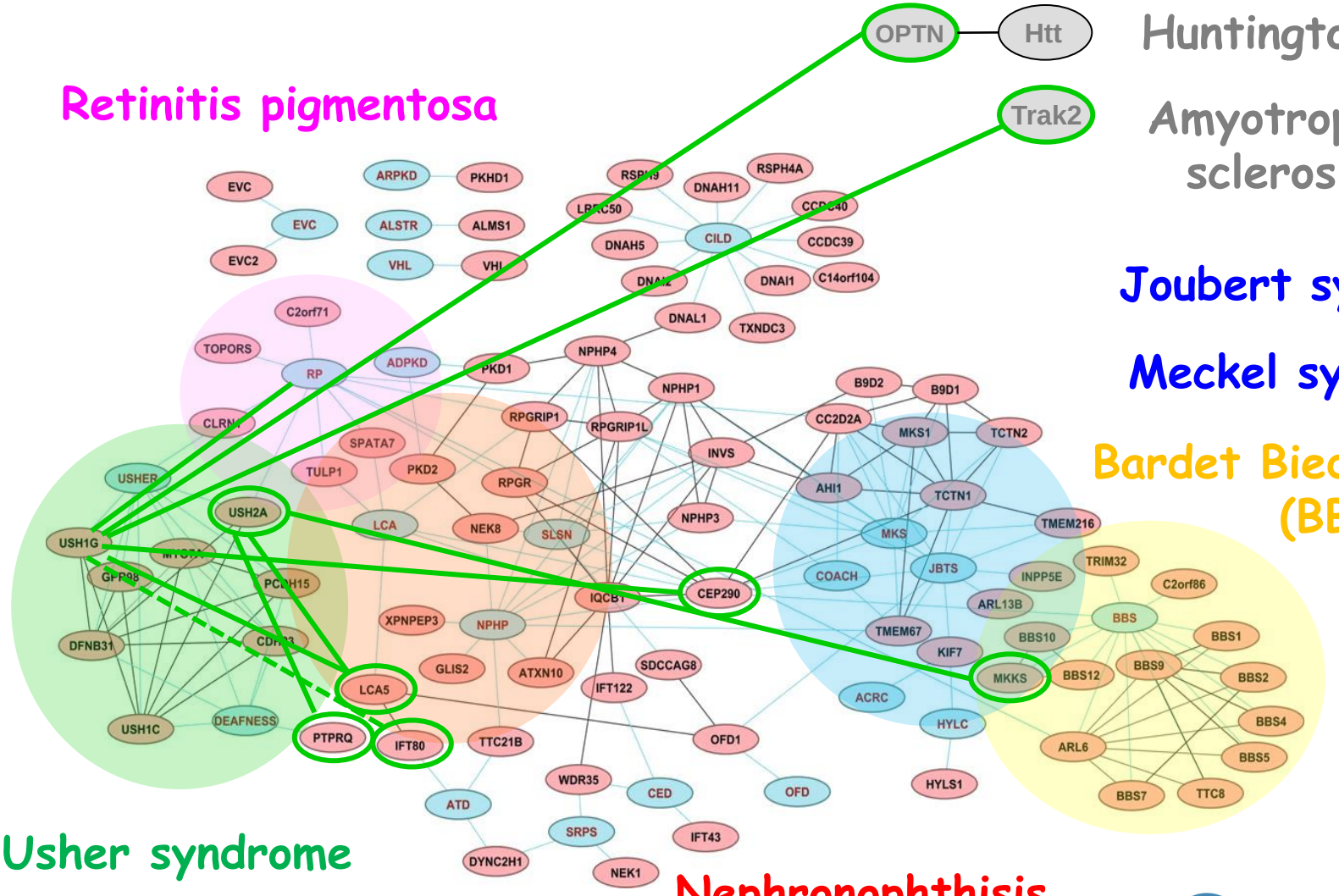


Ciliopathy genes

Nasrin Sorusch, Poster # 20

Lars Tebbe, Poster # 23

Ciliopathy disease protein interaction network



Huntington disease

Amyotrophic lateral sclerosis (ALS)

Retinitis pigmentosa

Joubert syndrome

Meckel syndrome

Bardet Biedl syndrome (BBS)

Usher syndrome

Nephronophthisis

Leber congenital amaurosis (LCA)

Syscilia

modified from van Reeuwijk et al. (2011) Hum Mol Genet

Wolfrum Lab 2014

Network functions

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- M. Becker
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- F. Möller Poster #17
- A. Samanta

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… many more

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Thank you!