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Suppressor of fused is an essential repressor of the Hedgehog signaling pathway


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Functional genomics of the mouse – The toolbox

- Forward genetics
 - Chemical
 - ENU, chlorambucil
 - Radiation
 - X-ray
- Reverse genetics
 - Insertional mutagenesis
 - Gene trapping, transposon
 - Transgenic technologies
 - Pronuclear DNA microinjection
 - Gene targeting technologies
 - Homologous recombination in ESCs
 - Targeted genome editing
 - ZFN, TALENs, CRISPR/Cas9



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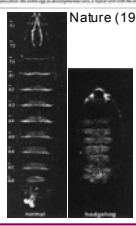

The *Drosophila* segment polarity mutant hedgehog (*hh*)

Mutations affecting segment number and polarity in *Drosophila*
Christiane Nüsslein-Volhard & Eric Wieschaus


In 1983, mutations that affect the number of *Drosophila* segments as well as the polarity of the segments were identified. The discovery of the *hedgehog* gene, which encodes a secreted signaling molecule, was the first step in the identification of the genetic control of early embryonic development. The discovery of the *hedgehog* gene was the first step in the identification of the genetic control of early embryonic development.

The Nobel prize in Physiology or Medicine 1995 "for their discoveries concerning the genetic control of early embryonic development"

Nature (1980) 287:795-801





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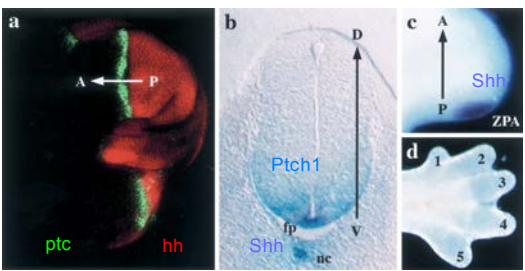
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THE HEDGEHOG PATHWAY IN DEVELOPMENT


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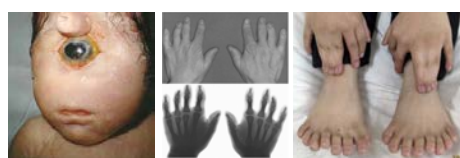
Hedgehog activity is central for morpho-genetic events during embryo development



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Developmental disorders due to aberrant Hedgehog pathway activity




Too **high** or too **low** Hedgehog signaling activity

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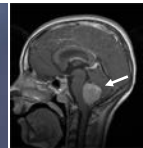
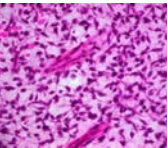
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THE HEDGEHOG PATHWAY IN CANCER DEVELOPMENT

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Cancer due to aberrant Hedgehog pathway activity

Basal cell carcinoma

- *PTCH1* & *SMO* mutations

Medulloblastoma

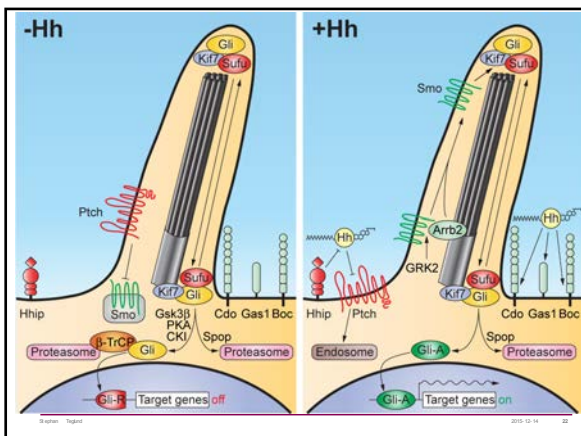
- *PTCH1* & *SUFU* mutations


Rhabdomyosarcoma

- *PTCH1* mutations

Aberrantly high Hedgehog signaling activity due to **activating** or **inactivating** mutations


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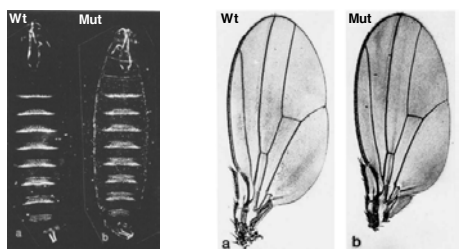
Studies on the SUPPRESSOR OF FUSED (SUFU) KNOCKOUT MOUSE

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Suppressor of Fused is dispensable in *Drosophila*

Wt **Mut**





Préat(1992) Genetics 132:725-736

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Sufu^{-/-} embryos die at ~E9.5 with head and neural tube defects

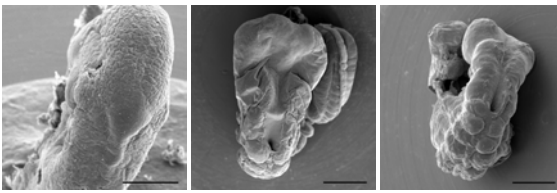



Svard *et al.* (2006) Dev Cell 10:187-197

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***Sufu*^{-/-} and *Ptch1*^{-/-} embryos display similar neural tube defects**



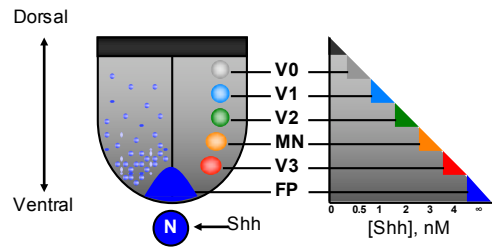
Wt *Sufu*^{-/-} *Ptch1*^{-/-}

Svärd *et al.* (2006) *Dev Cell* 10:187-197

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Ventral neural tube progenitor domains




Dorsal
↑
V0
V1
V2
MN
V3
FP
↓
Ventral

N ← Shh

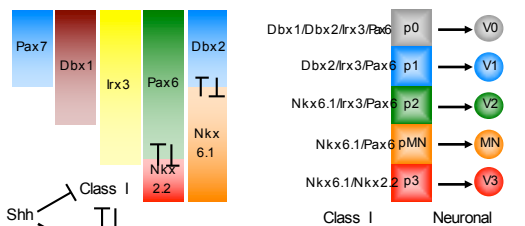
[Shh], nM

Adapted from Jacob and Briscoe (2003) *EMBO Reports* 4:761

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Homeodomain proteins specify neuronal fate




Shh → Class I
 → Class II

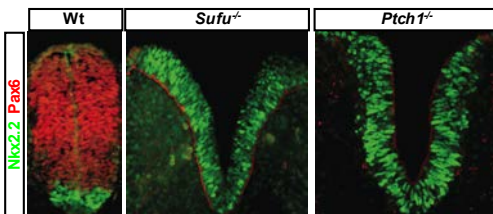
Class I → Neuronal fate
Class II →

Adapted from Jacob and Briscoe (2003) *EMBO Reports* 4:761

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Ventralization of the *Sufu*^{-/-} and *Ptch1*^{-/-} neural tube




Wt *Sufu*^{-/-} *Ptch1*^{-/-}

Nkx2.2 Pax6

Svärd *et al.* (2006) *Dev Cell* 10:187-197


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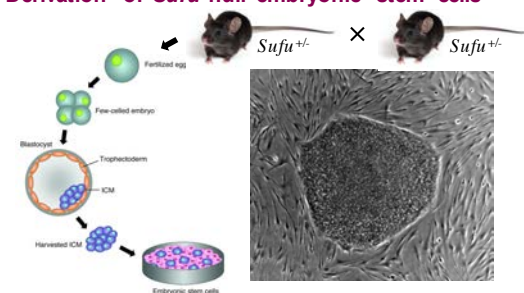
Studies on

SUFU NULL EMBRYONIC STEM CELLS

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Derivation of *Sufu* null embryonic stem cells



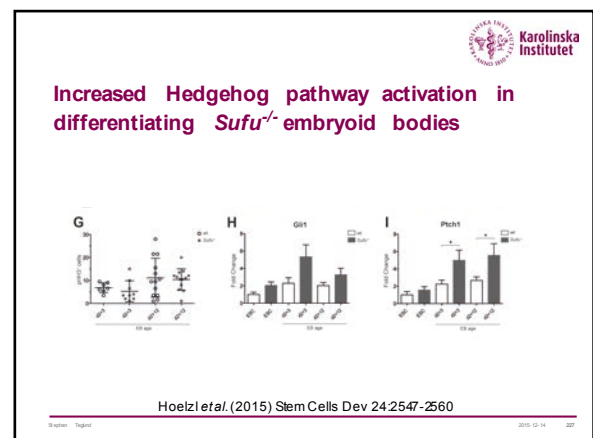
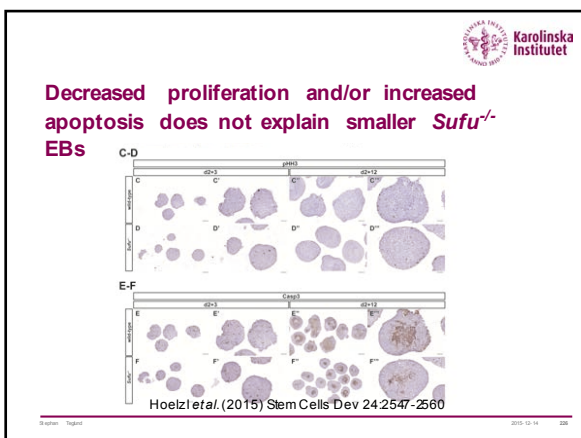
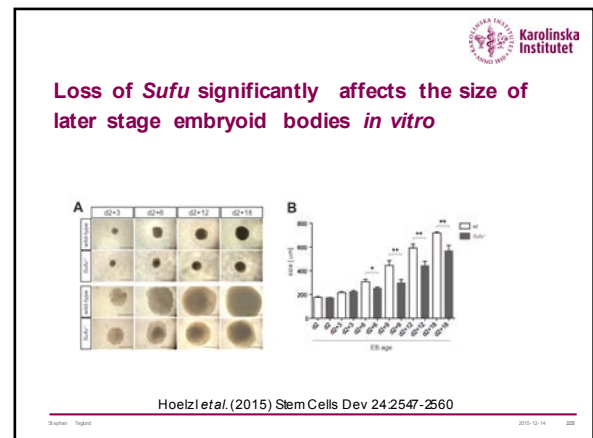
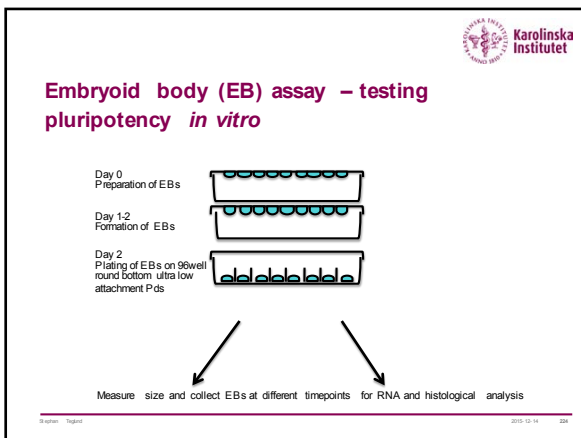
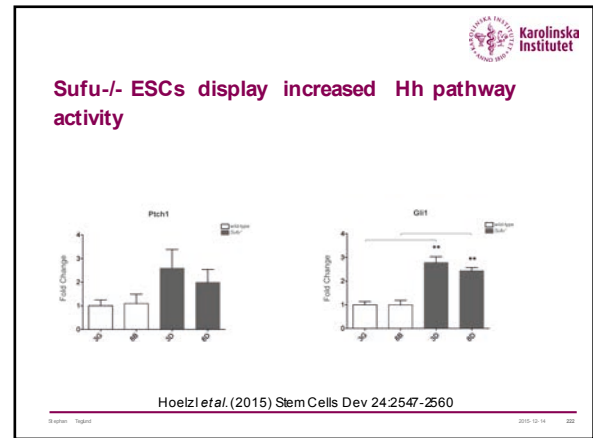
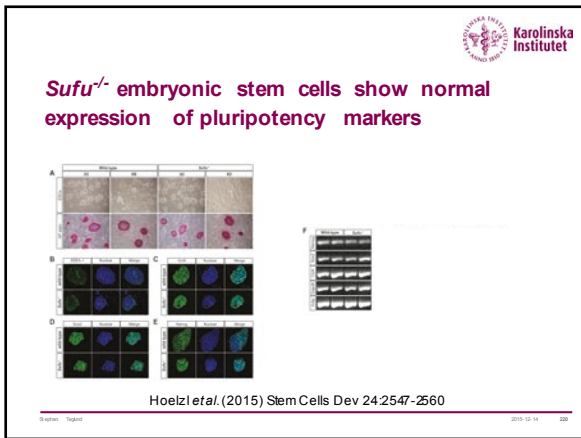
Fertilized egg × *Sufu*^{+/-} × *Sufu*^{+/-}


Free-cell embryo

Blastocyst Trophoblast ICM

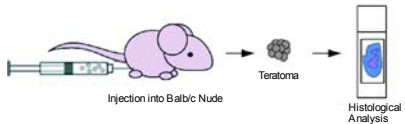
Harvested ICM Embryonic stem cells

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Teratoma assay: testing pluripotency *in vivo*



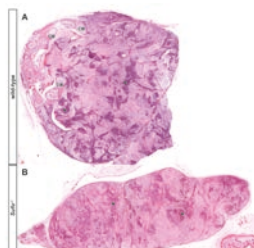
Injection into Balb/c Nude Teratoma Histological Analysis



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Sufu*^{-/-} ESCs are able to form teratomas *in vivo

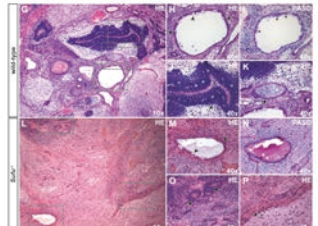


Hoelzl *et al.* (2015) *Stem Cells Dev* 24:2547-2560

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Restricted mesodermal differentiation in teratomas lacking *Sufu*

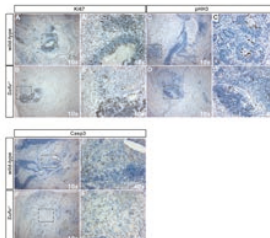


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Loss of *Sufu* does not affect proliferation or apoptosis in teratomas

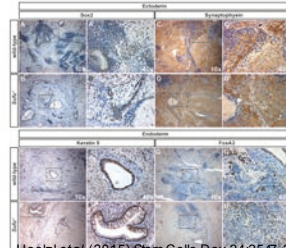


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Ectodermal and endodermal differentiation appear normal in *Sufu*^{-/-} teratomas

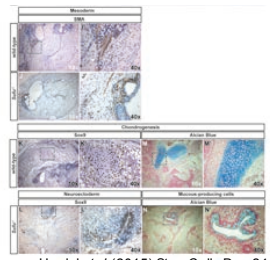


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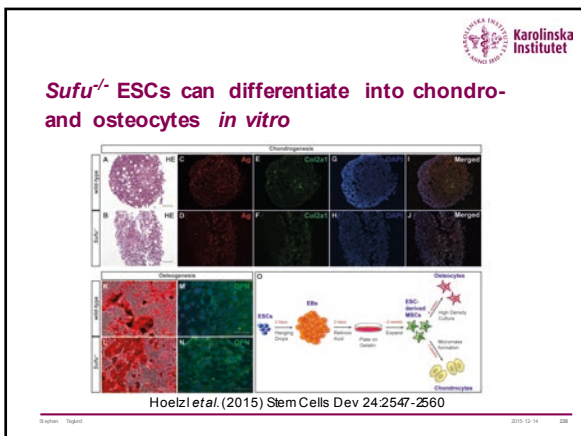
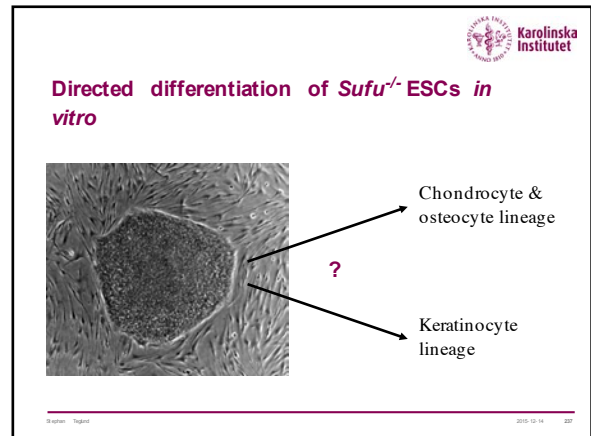
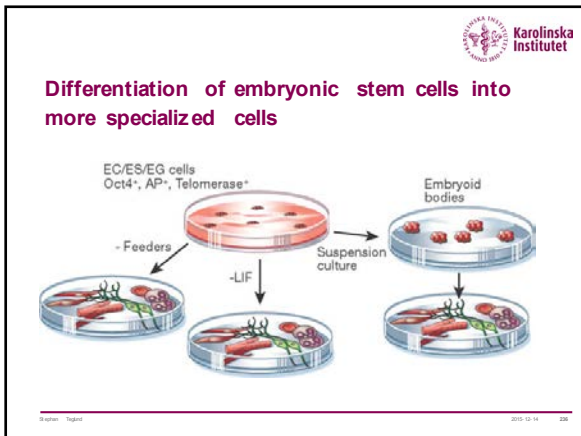
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Teratomas from *Sufu*^{-/-} ESCs do not show chondrogenic differentiation



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- Summary**
- *Sufu*^{-/-} mutant embryos die at ~E9.5
→ Open and ventralized neural tube
 - *Sufu*^{-/-} mice display skin phenotype
→ Basaloid follicular hamartomas, alopecia, pigmentation
 - *Sufu*^{-/-} ESCs have normal pluripotency marker expression
 - *Sufu*^{-/-} later stage EBs are smaller in size
→ Increased Hh pathway activity
 - *Sufu*^{-/-} teratomas lack cartilage and bone tissue
 - Directed differentiation of *Sufu*^{-/-} ESCs towards the chondrogenic, osteogenic, and keratinocyte lineages reveal no changes

Acknowledgements

- **Group**



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 Vetenskapsrådet, PEOPLE, HEALING
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