

Speciální metody fyziologie živočichů

# Metody studia Wnt signalizace

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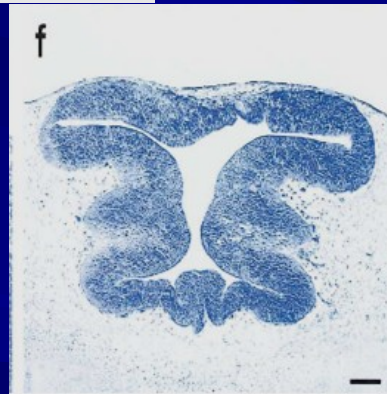
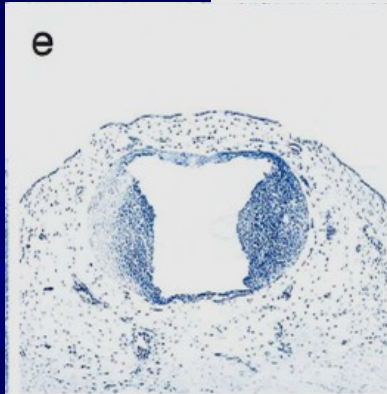
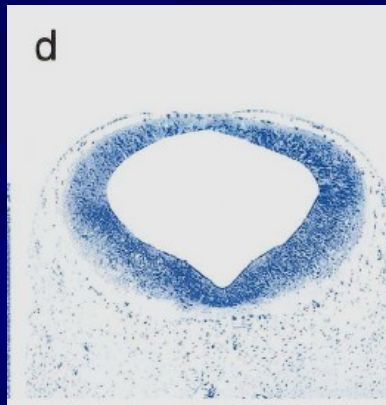
# Wnt signalling

- eg. Wnt-1 or Wnt-3a



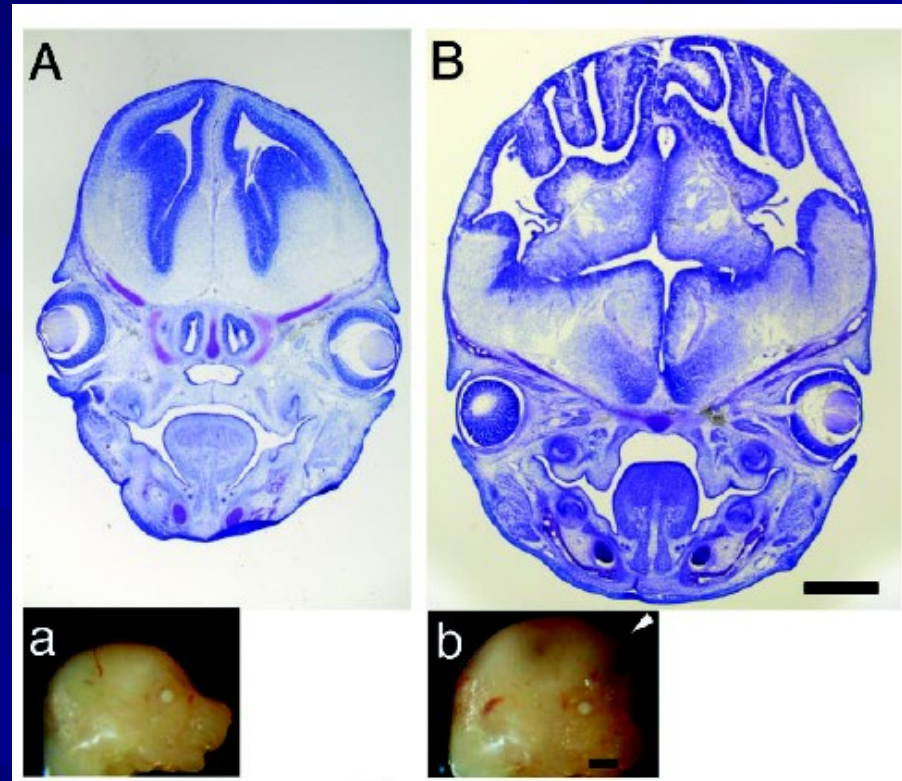
# Consequences of $\beta$ -catenin activation in the brain:

## midbrain (Brn4-promotor)



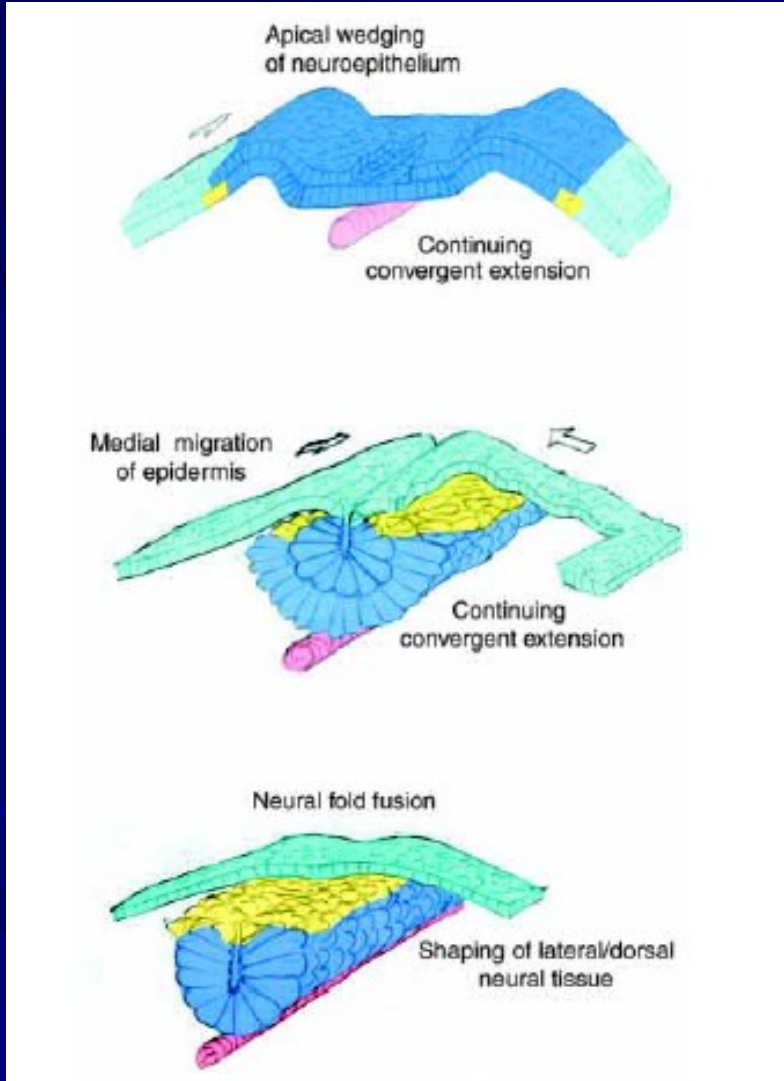
Zechner et al., 2003: Dev. Biol.;258:406-418.

## cortex (nestin enhancer)

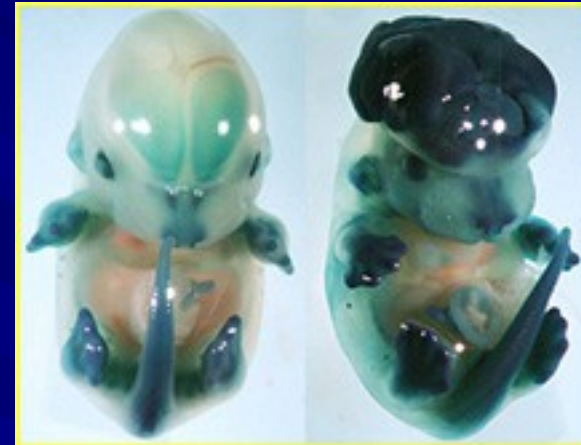


Chenn & Walsh, 2002: Science;297:365-369.

# Non-canonical/PCP (Planar cell polarity) pathway defects cause neural tube closure phenotypes in mouse (and human)



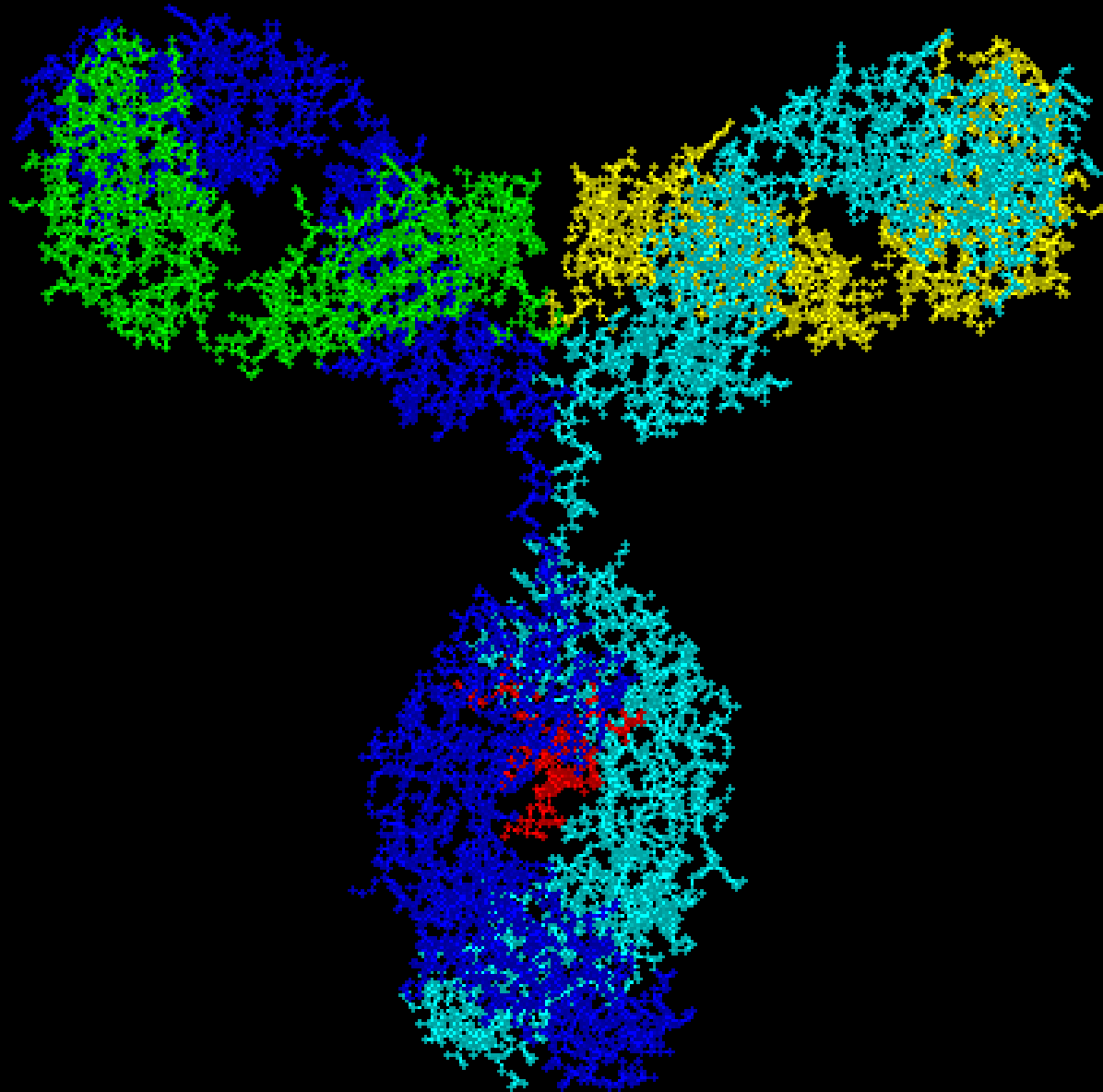
## Exencephaly



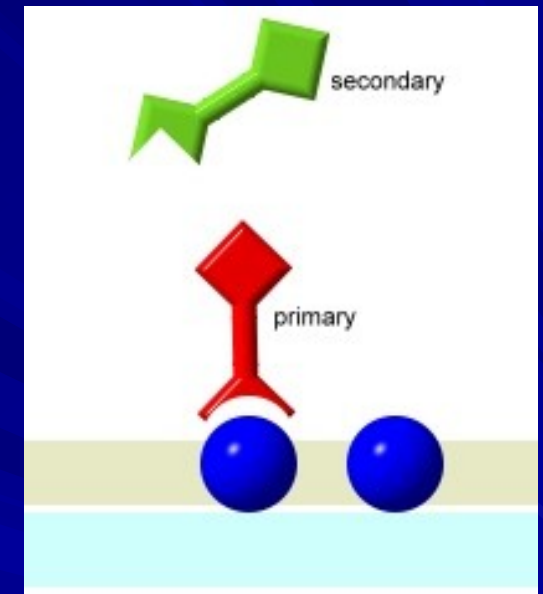
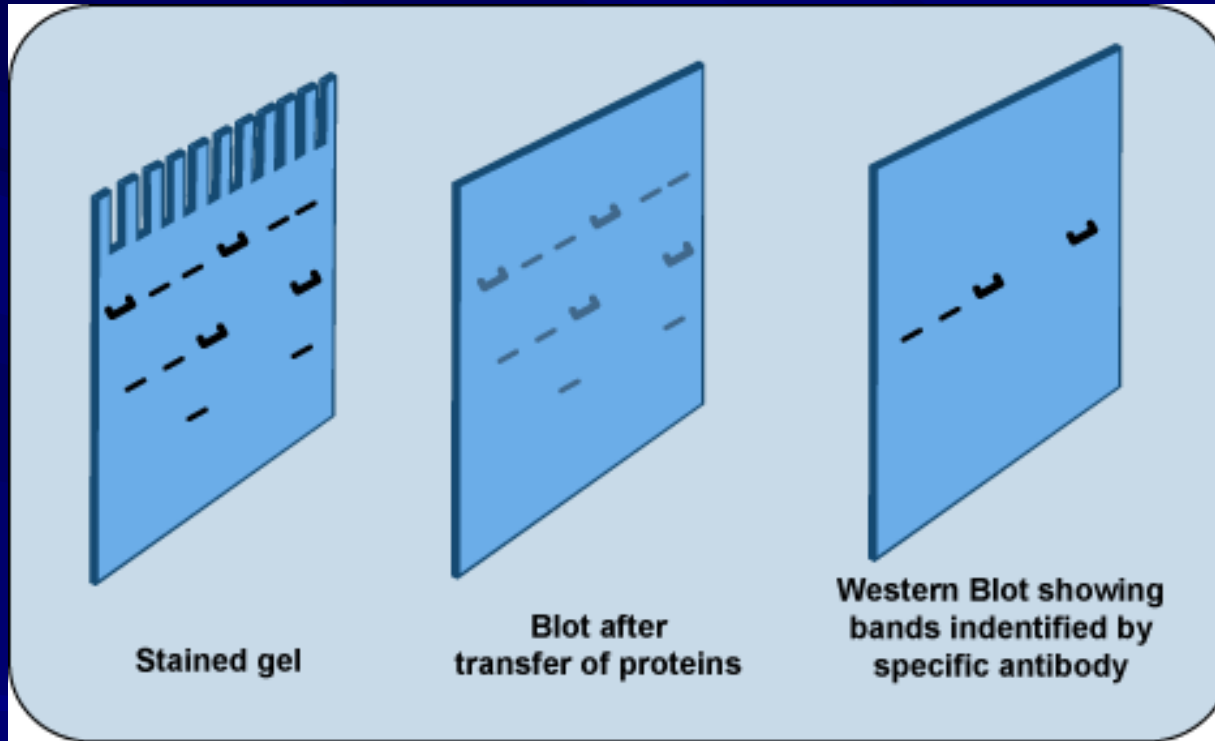
## Open neural tube

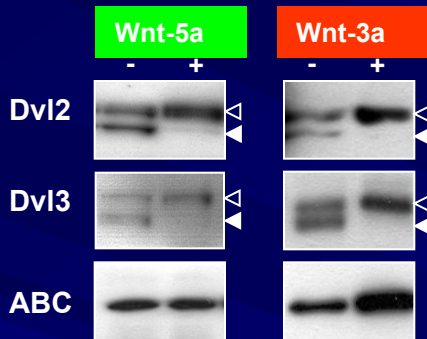


Protilátka  
(imunoglobulin)



# Metoda 1: Western blotting





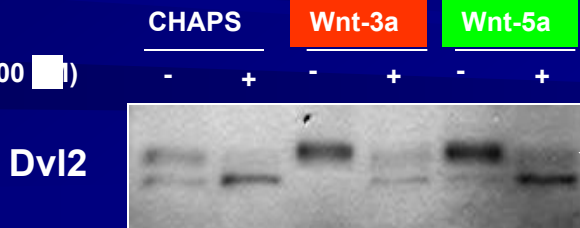
ABC – active  $\blacksquare$  catenin =  $\blacksquare$  catenin  
dephosphorylated on GSK3  $\blacksquare$  target sites

Dvl – Dishevelled – activated by phosphorylation  
detected as phosphorylation dependent mobility  
shift

◁ PS-Dvl

Compound	Target	Concn	Activity
PTX	Galpha i/o	100 ng/ml	No
PDBu	PKC activator	1 $\mu$ M	No
Wortmannin	PI3K	50 nM	No
LY294002	PI3K	50 $\mu$ M	No
PD98059	MEK1/2	10 $\mu$ M	No
UO126	MEK1/2	10 $\mu$ M	No
SB203580	p38	10 $\mu$ M	No
JNKII inhib	JNK	6 $\mu$ M	No
Genistein	PKC	50 $\mu$ M	No
chelerythrine	PKC	10 $\mu$ M	No
Ro-31 8220	PKC	1 $\mu$ M	No
BIM I	PKC	500 nM	No
KN93	CamKII	10 $\mu$ M	No
I3M	GSK-3	2 $\mu$ M	No
Kenpauullone	GSK-3	6 $\mu$ M	No
H89	PKA	10 $\mu$ M	No
8-Br-cAMP	cAMP pathway activator	10 $\mu$ M	No
8CPT-2Me-cAMP	EPAC activator	30 $\mu$ M	No
SQ22536	Adenylyl cyclase	100 $\mu$ M	No
MDL12330	Adenylyl cyclase	10 $\mu$ M	No
PP2	Src-like	10 $\mu$ M	No
AG1276	EGFR	10 $\mu$ M	No
ET-18-OCH3	PLC	10 $\mu$ M	No
<b>D4476</b>	<b>Casein kinase 1</b>	<b>100 <math>\mu</math>M</b>	<b>Yes</b>
staurosporin	Ser/Thr kinases, PKC	2 $\mu$ M	No

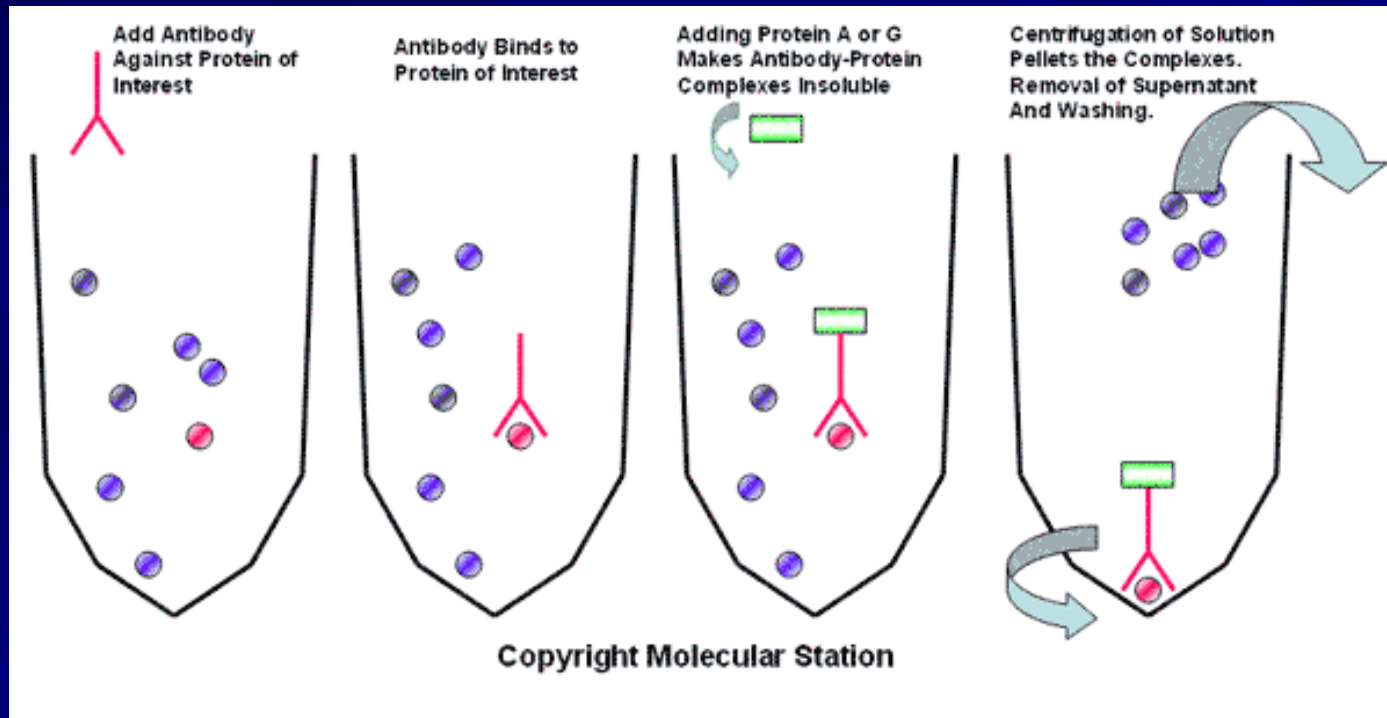
D4476 (100  $\mu$ M)



Both **Wnt-3a** and **Wnt-5a** activate Dvl2  
and Dvl3 via casein kinase 1 (CK1)

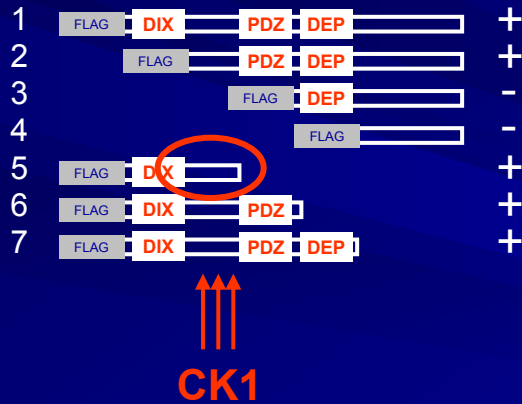


# Metoda 2: Immunoprecipitace



# $\beta$ -arrestin binds Dishevelled

Flag-Dvl3 constructs

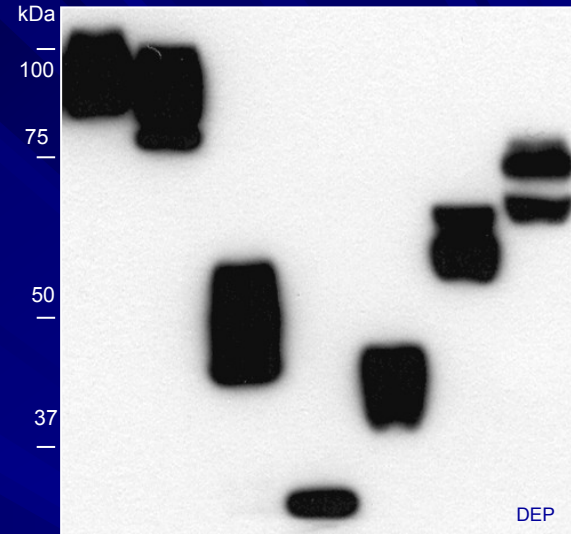


Flag-Dvl3 mutants

1 2 3 4 5 6 7

TCL:

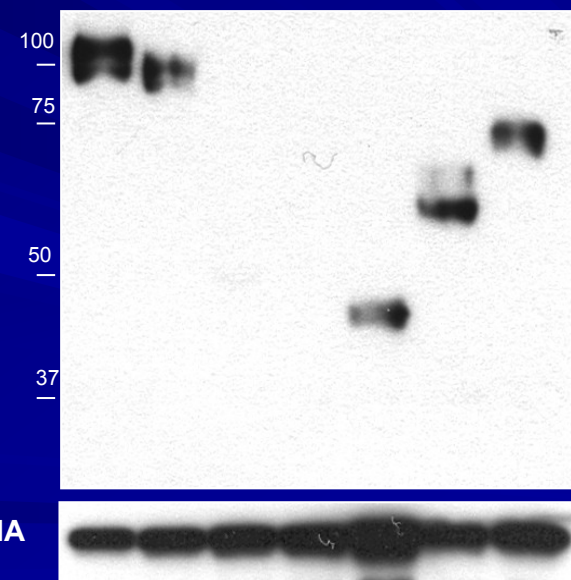
WB:  
Flag



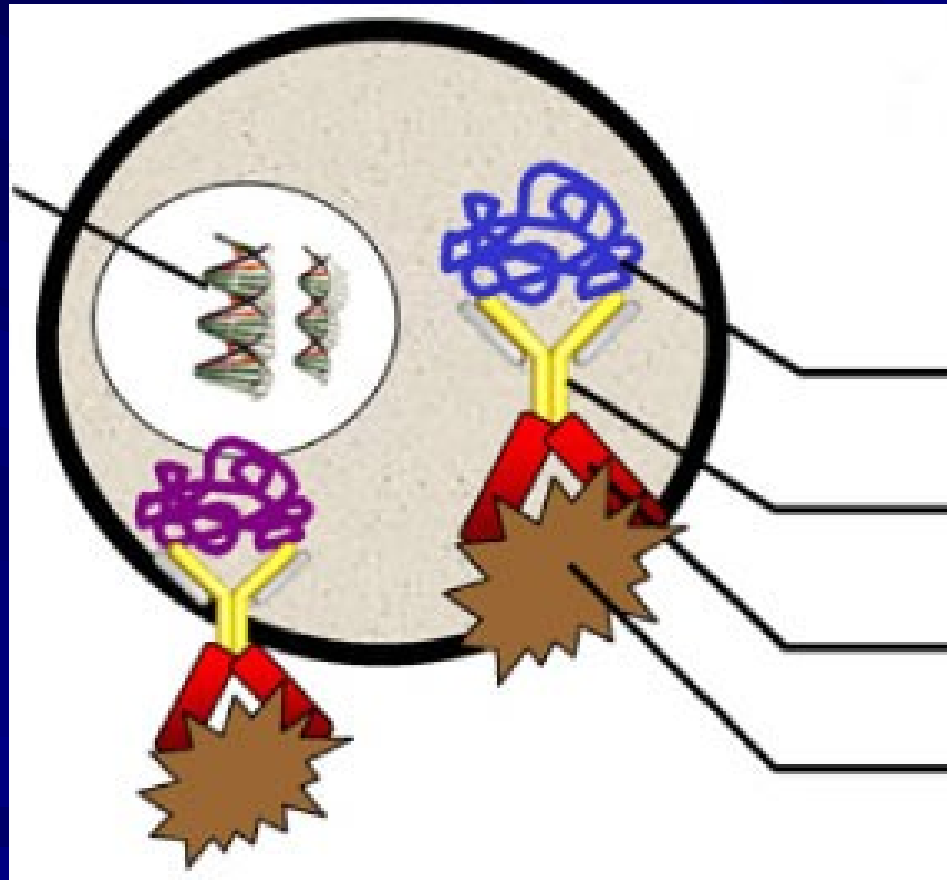
IP: HA- $\beta$ -arrestin

WB:  
Flag

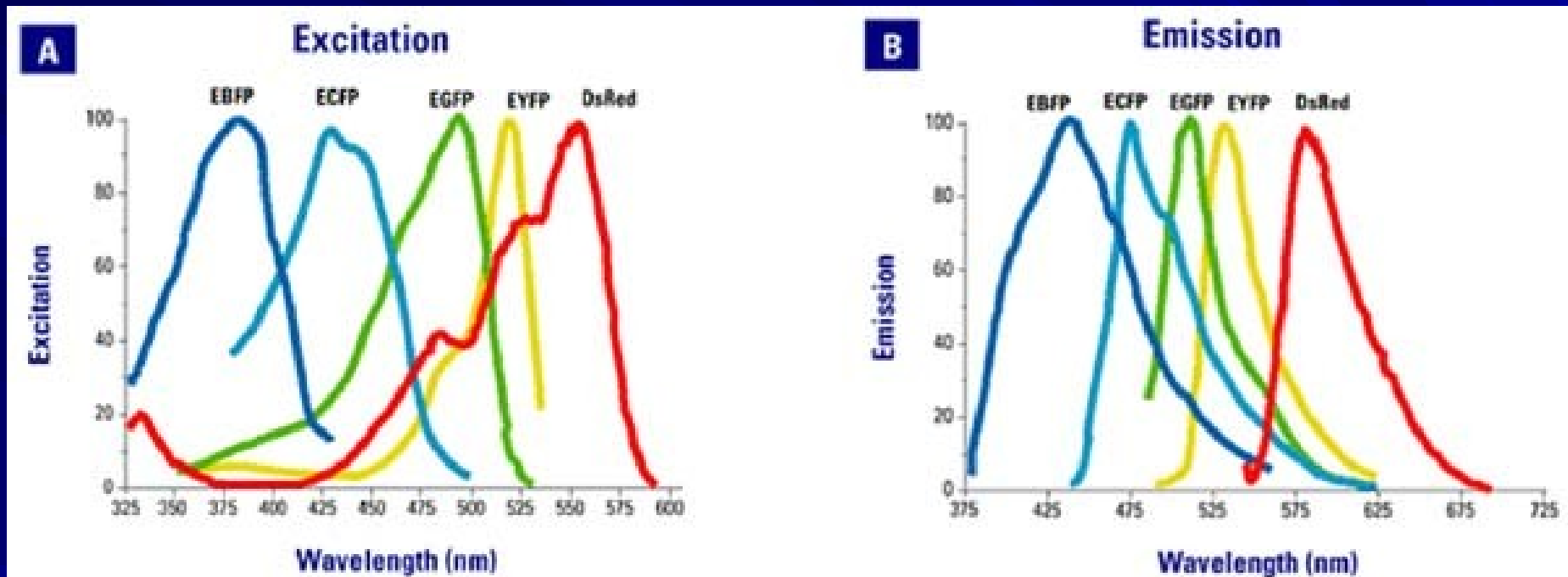
WB: HA



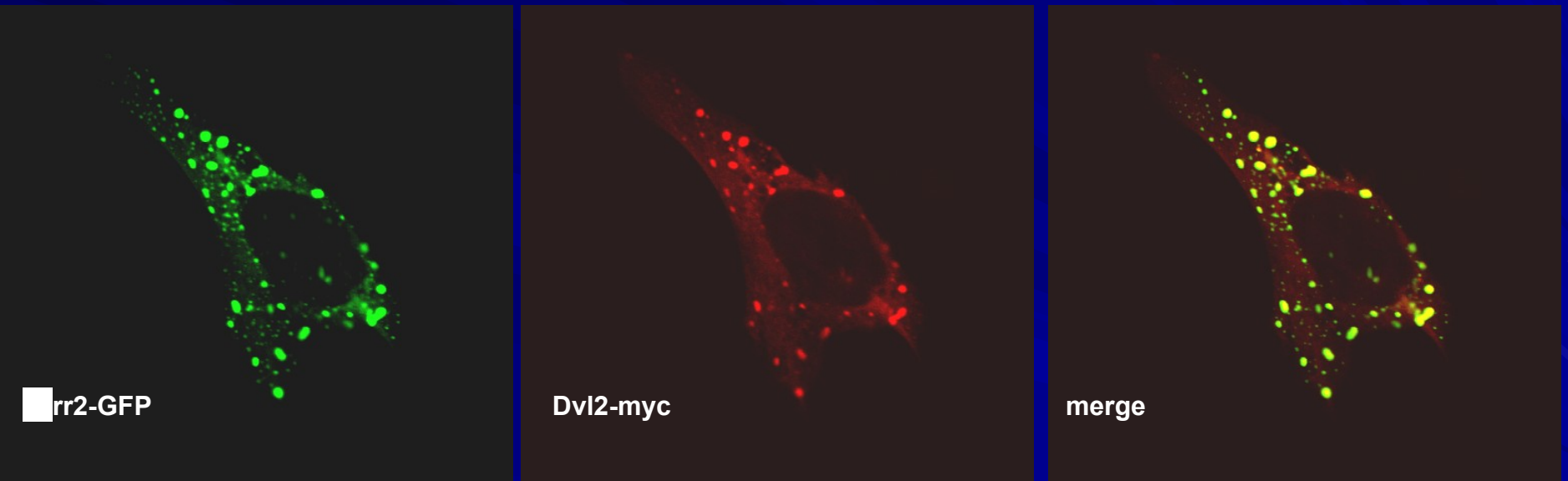
# Metoda 3: Immunocytochemie



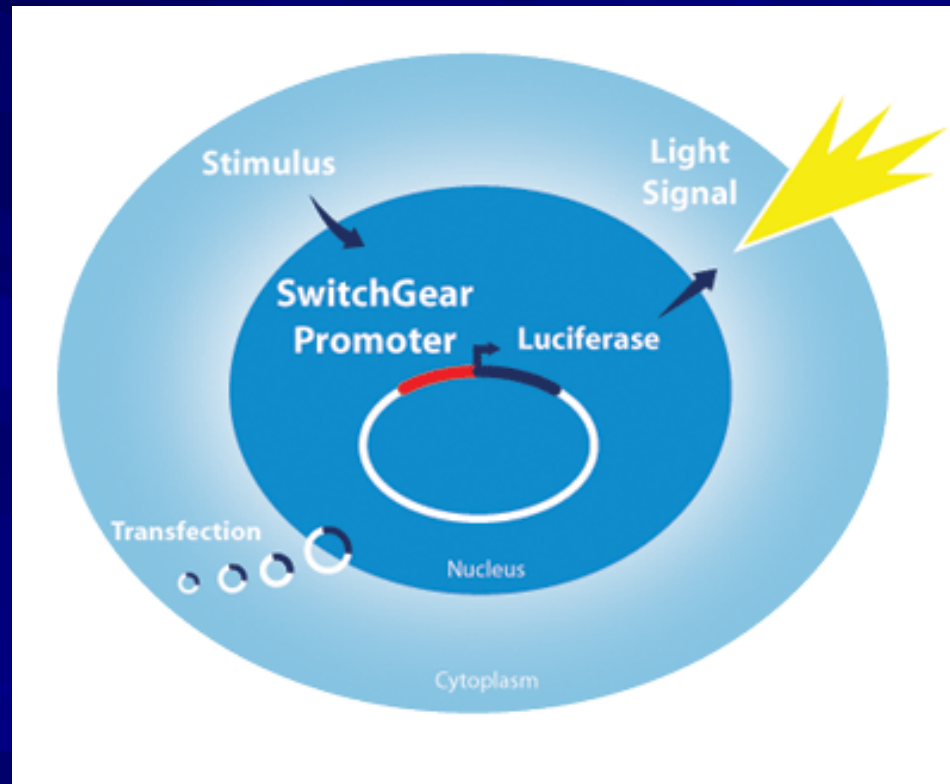
# Fluorescenční proteiny



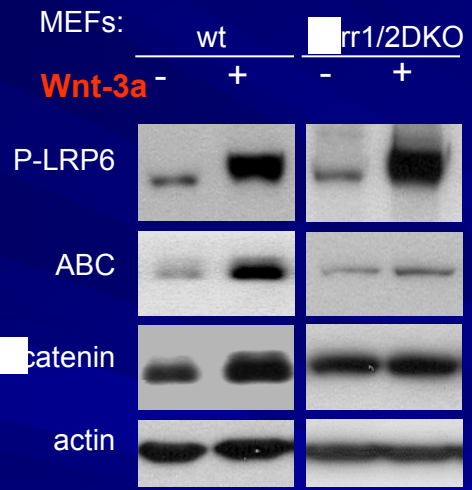
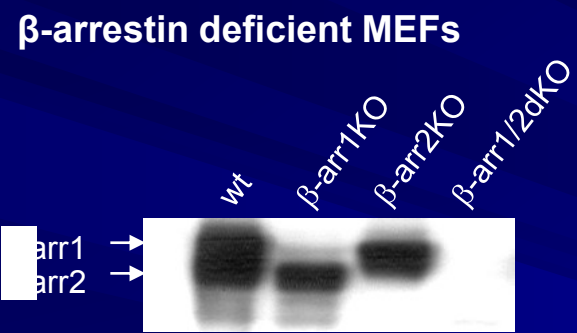
# $\beta$ -arrestin co-localizes with Dvl in the cytoplasm



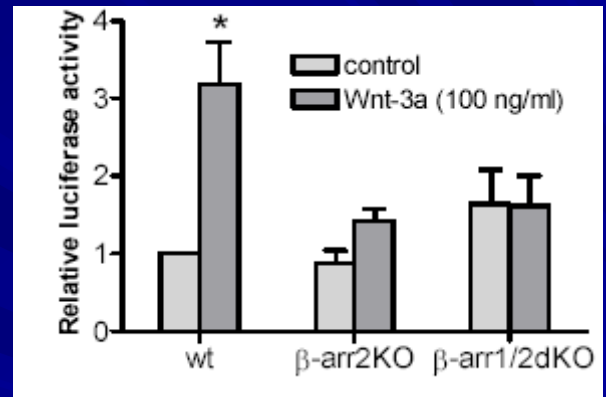
# Metoda 4: Reportérové eseje



1.  $\beta$ -arrestin is required for  $\beta$ -catenin activation in vitro

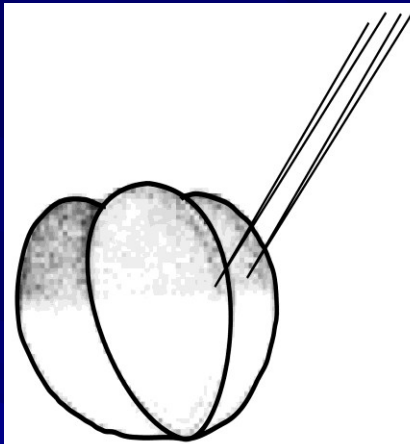


TopFlash reporter -  $\beta$ -catenin transcriptional activity

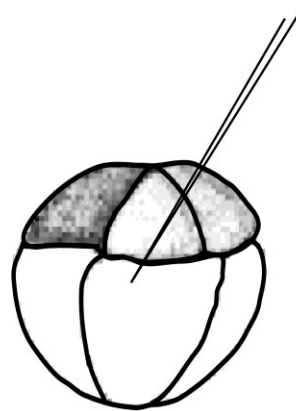


Is this relevant for Wnt signal transduction in vivo?

# Metoda č. 5: Analýza in vivo - drápatka



injection in 4-cell stage embryo  
targets Dorsal Marginal Zone  
affects primary axis formation (ventralization) and CE movements  
Figures 2, 3, and Suppl Figure 1

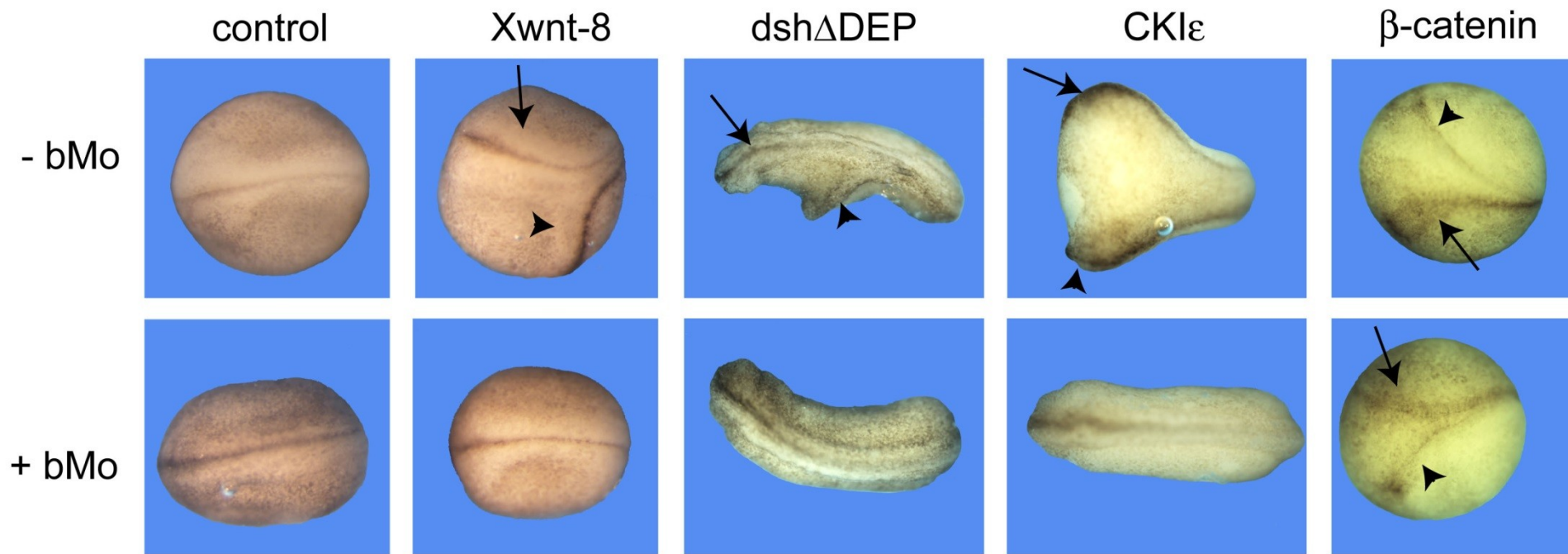


injection in 8-cell stage embryo  
targets presumptive cardiac mesoderm  
affects cardiac development, but avoids earlier effects on primary axis and CE  
Figure 4

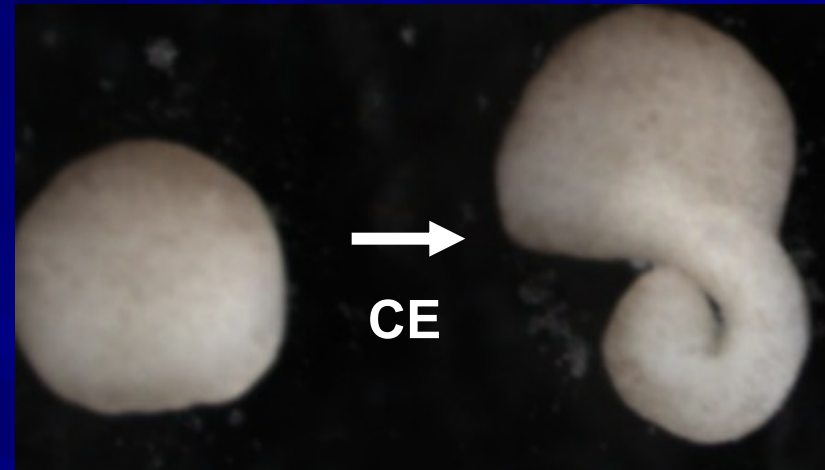
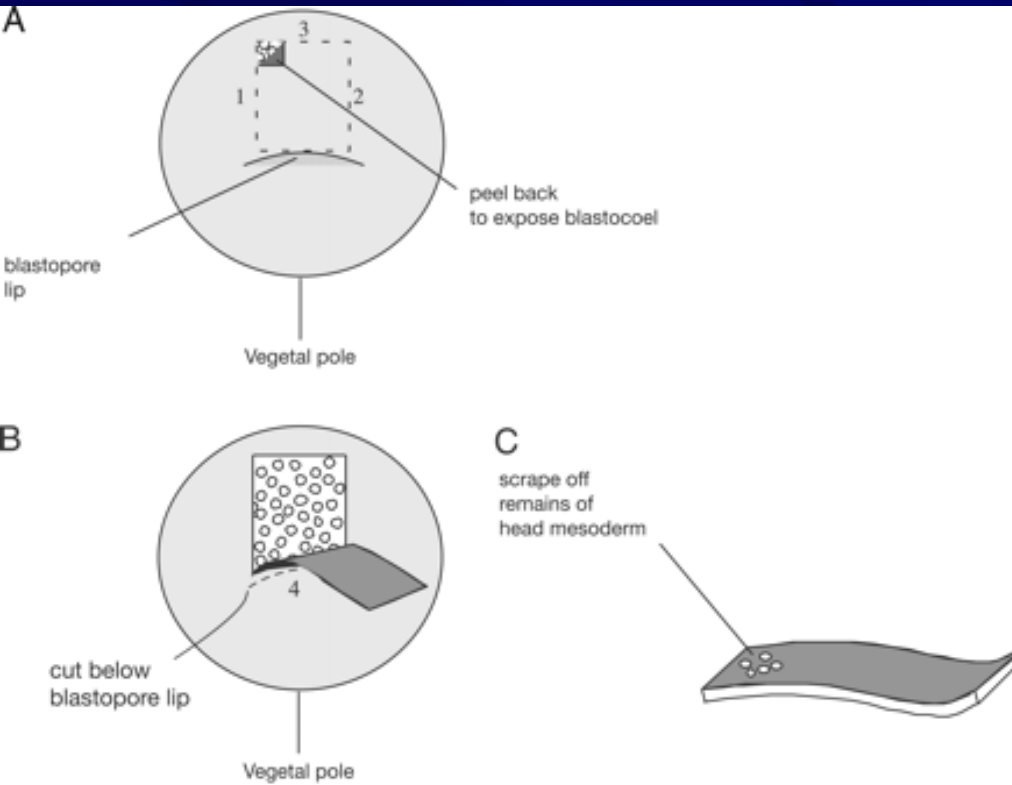


# Is $\beta$ -arrestin important for the Wnt/ $\beta$ -catenin signalling in vivo?

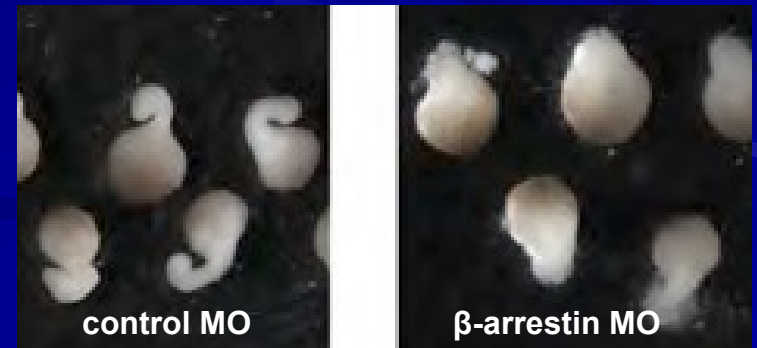
$\beta$ -arrestin knockdown in *Xenopus* (**axis duplication assay**):



# Keller explants (Xenopus)



$\beta$ -arrestin regulates convergent extension movements in vivo



# Metody č. 6: Genetické modifikace myši

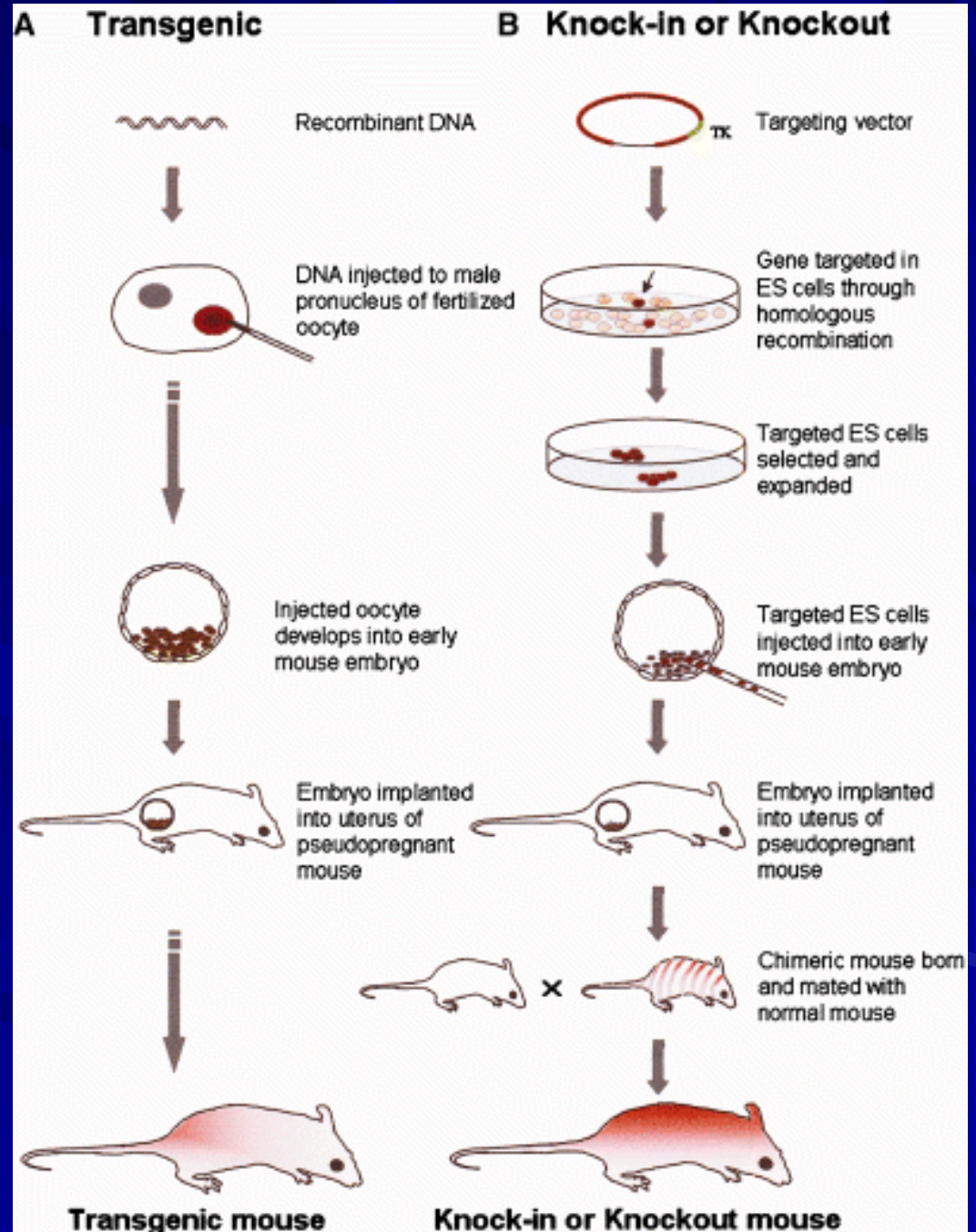
# Transgenní myš

Nobelova cena 2007

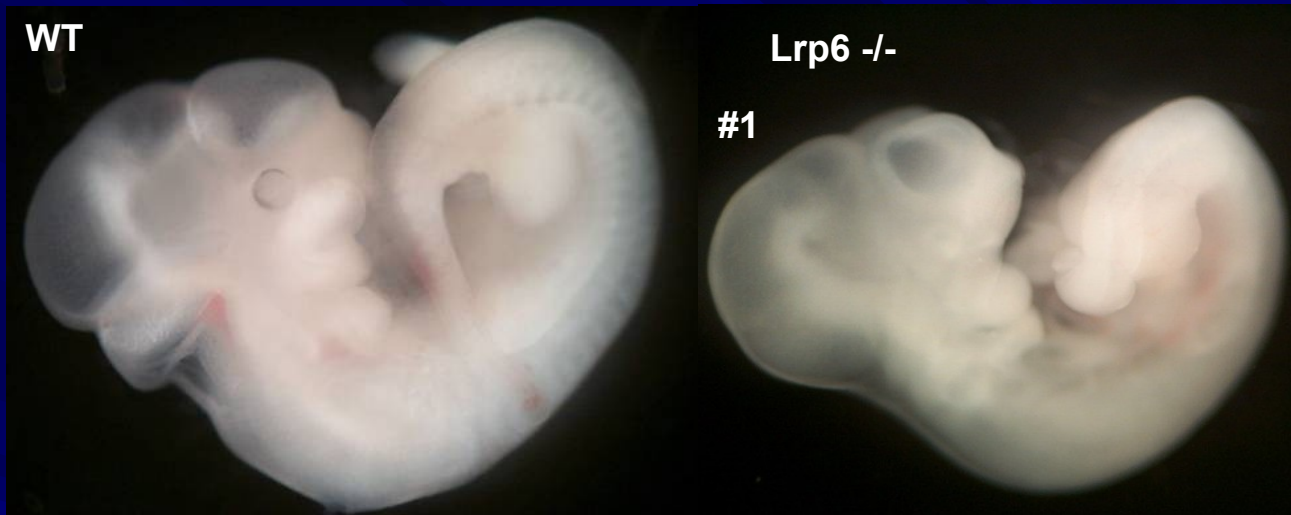
**Mario R. Capecchi,**  
**Martin J. Evans** and  
**Oliver Smithies**

za

„principles for  
introducing specific  
gene modifications in  
mice by the use of  
embryonic stem cells“

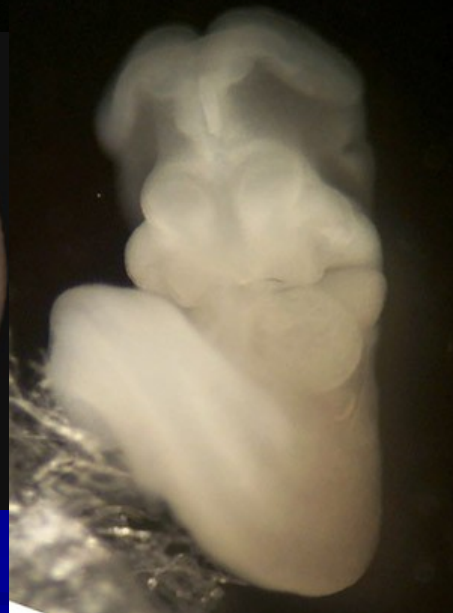


# Lrp6 KO embryos display exencephaly....

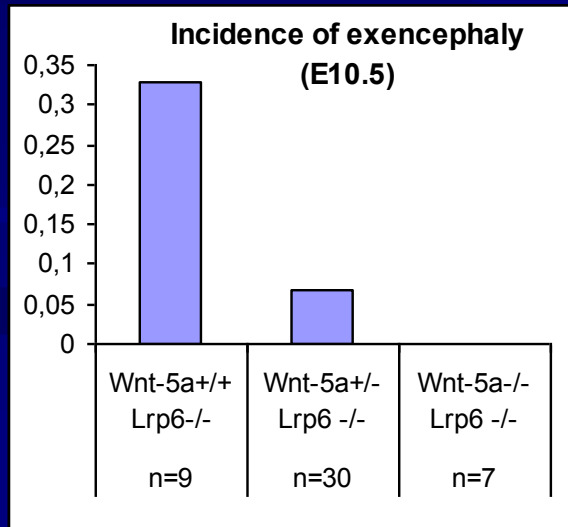


#1

#2

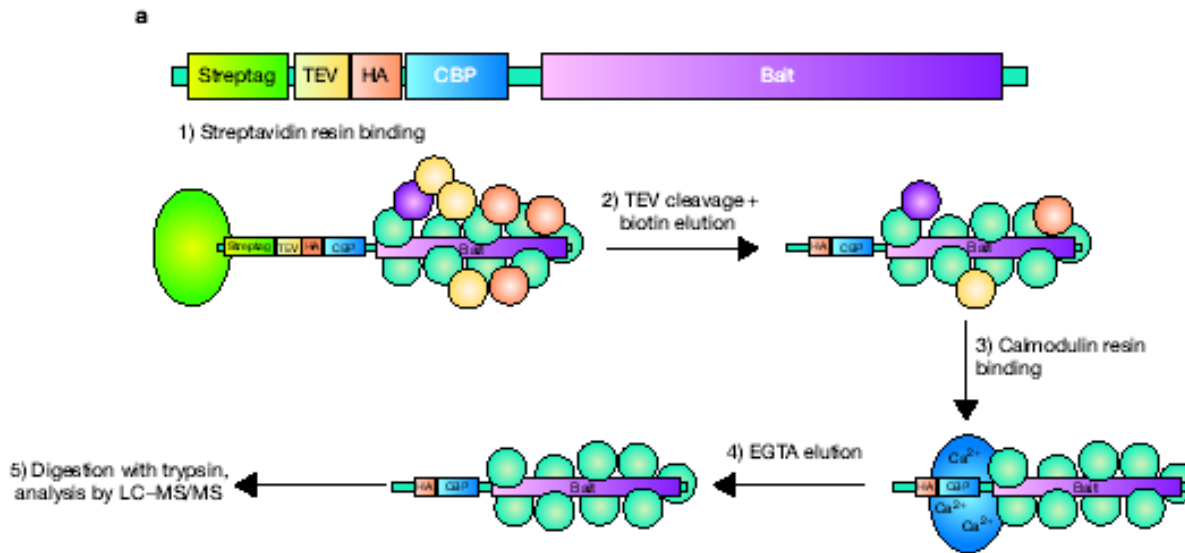


....which is rescued by loss of Wnt5a

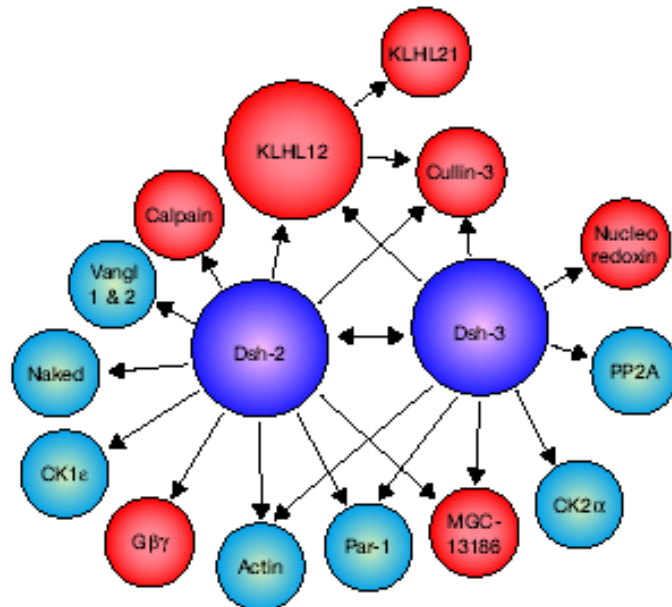


# Metody č. 7: Afinity purifikace a hmotnostní spektroskopie

# Afinitní purifikace



**b**

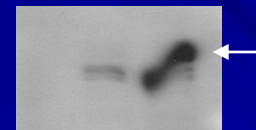


pGlue  
pGlue-Dvl2  
pGlue-Dvl3

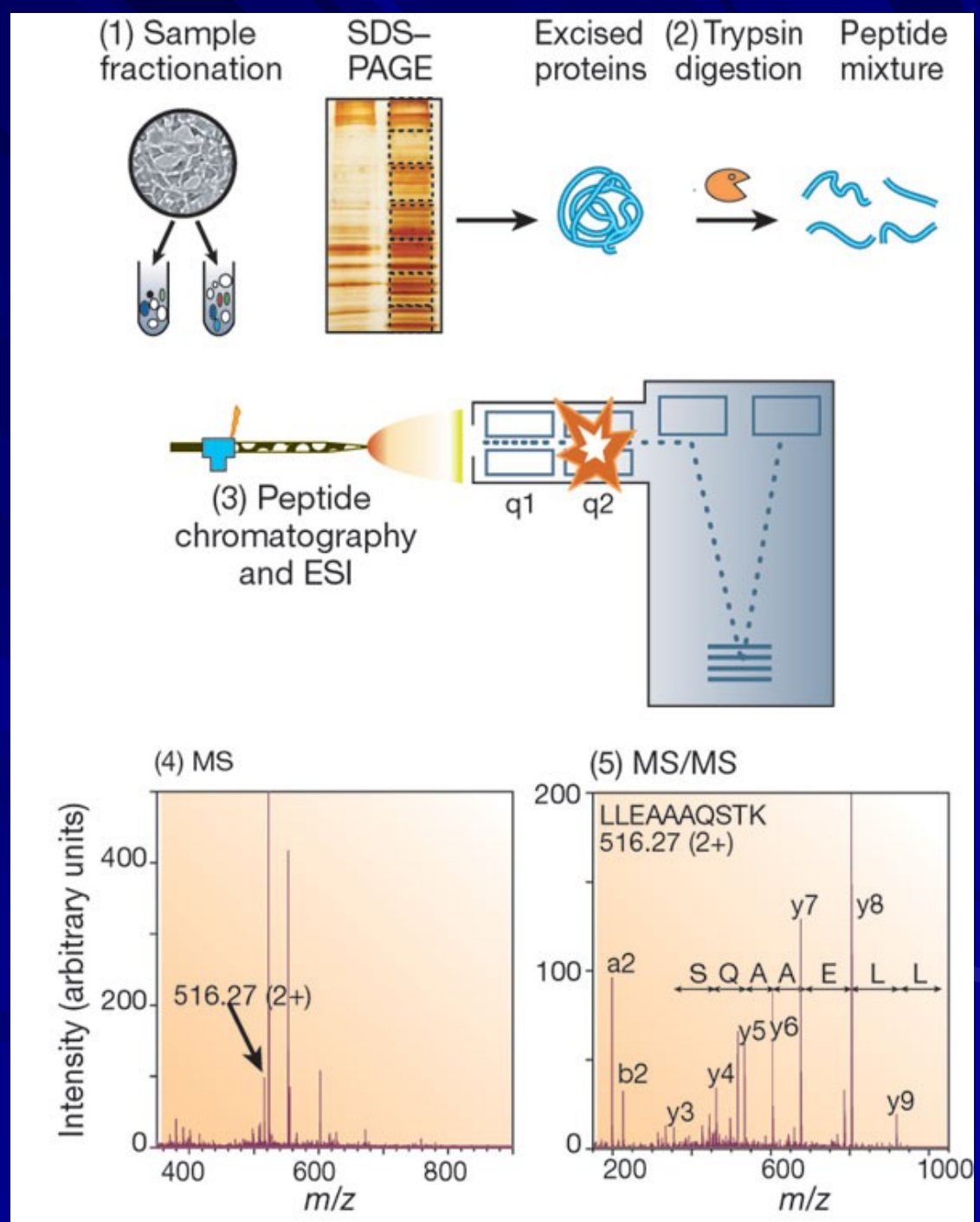
Dvl2



Dvl3



# Hmotnostní spektroskopie (Mass Spec)





# Děkuji za pozornost!

Celogenomové  
techniky

Molekulární  
mechanismus

Celoproteomové  
techniky

