

Speciální metody fyziologie živočichů

Metody studia Wnt signalizace

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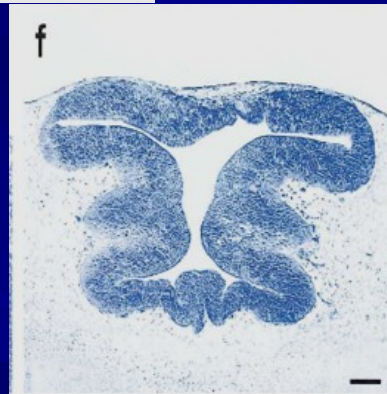
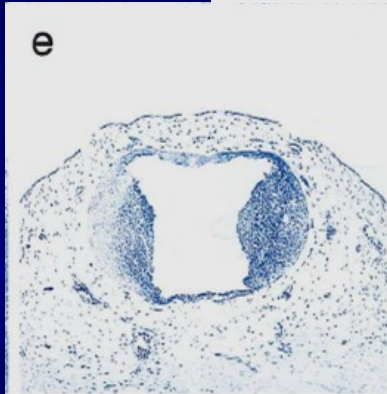
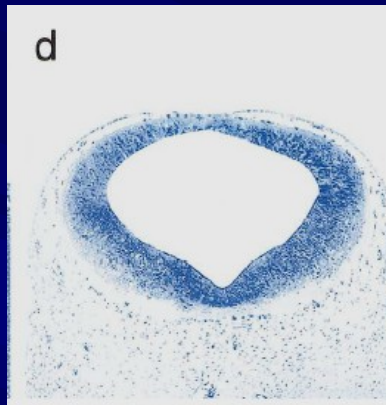
Wnt signalling

- eg. Wnt-1 or Wnt-3a



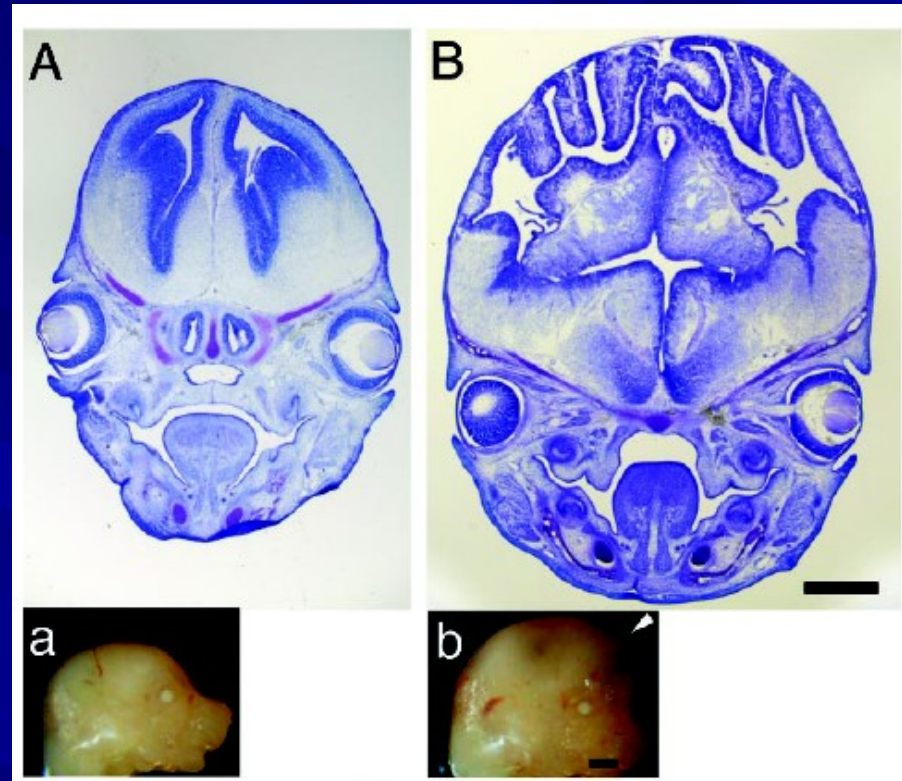
Consequences of β -catenin activation in the brain:

midbrain (Brn4-promotor)



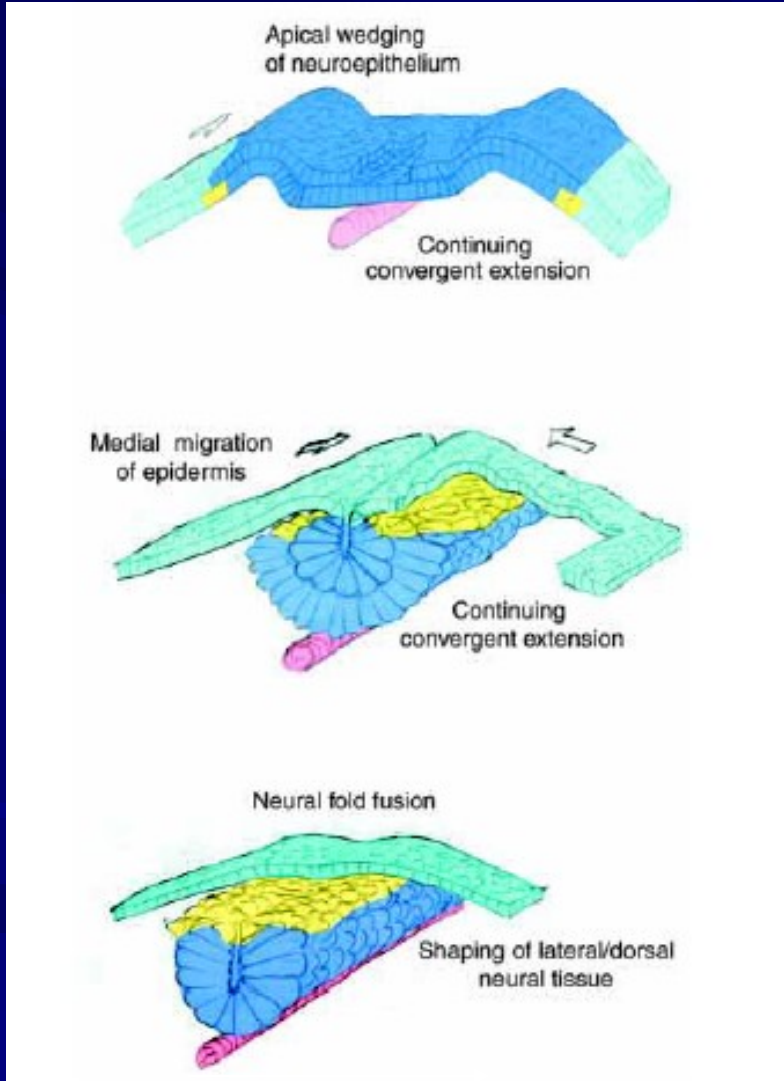
Zechner et al., 2003: Dev. Biol.;258:406-418.

cortex (nestin enhancer)

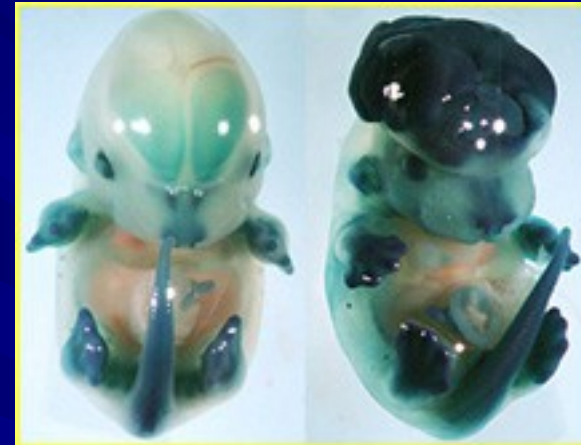


Chenn & Walsh, 2002: Science;297:365-369.

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



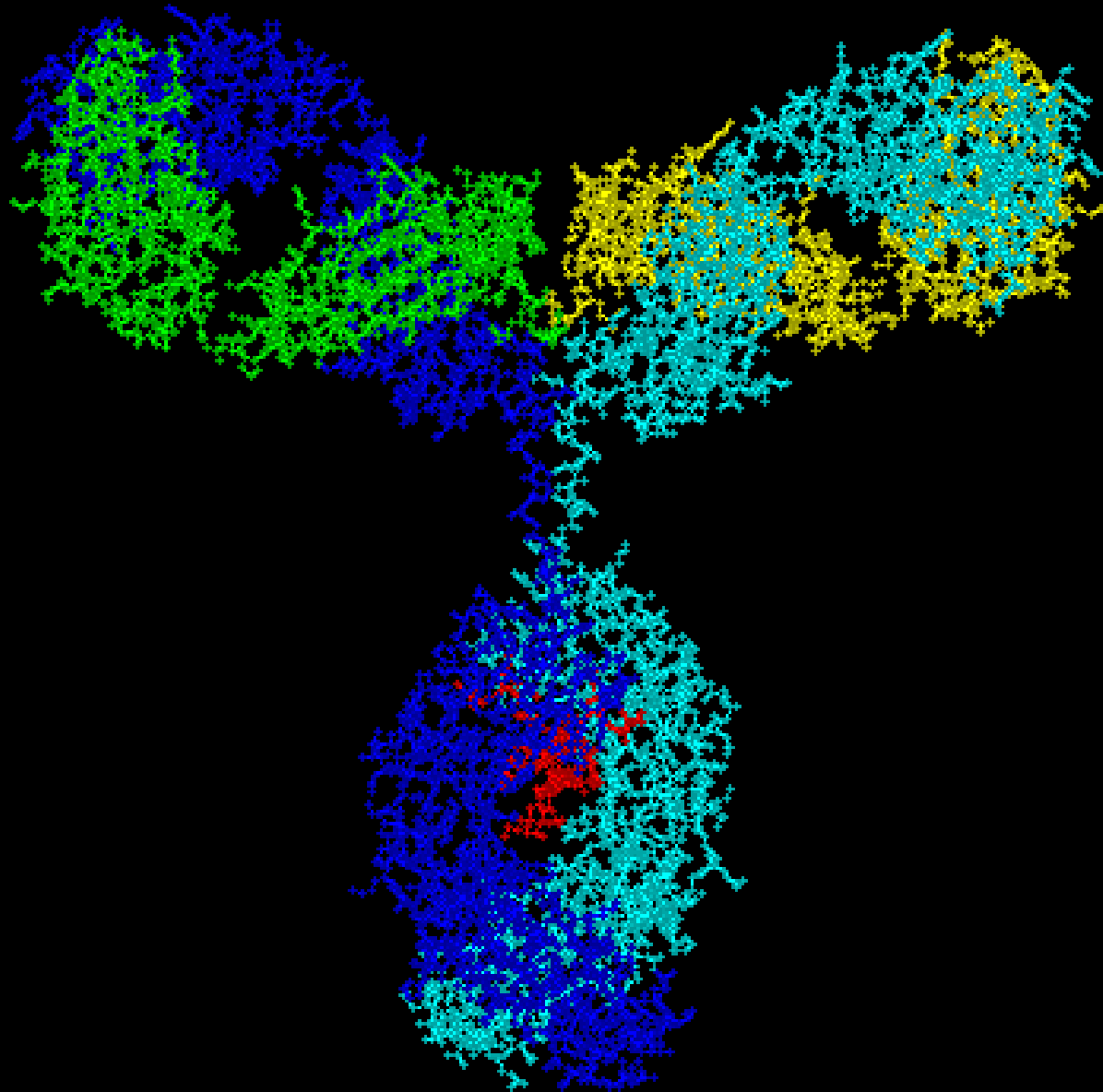
Exencephaly



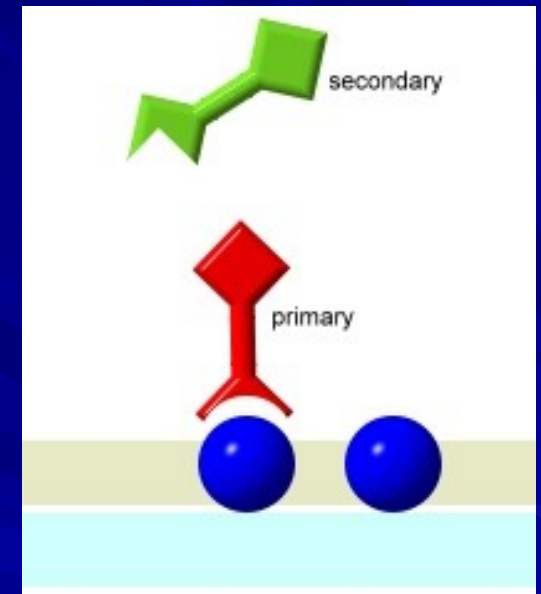
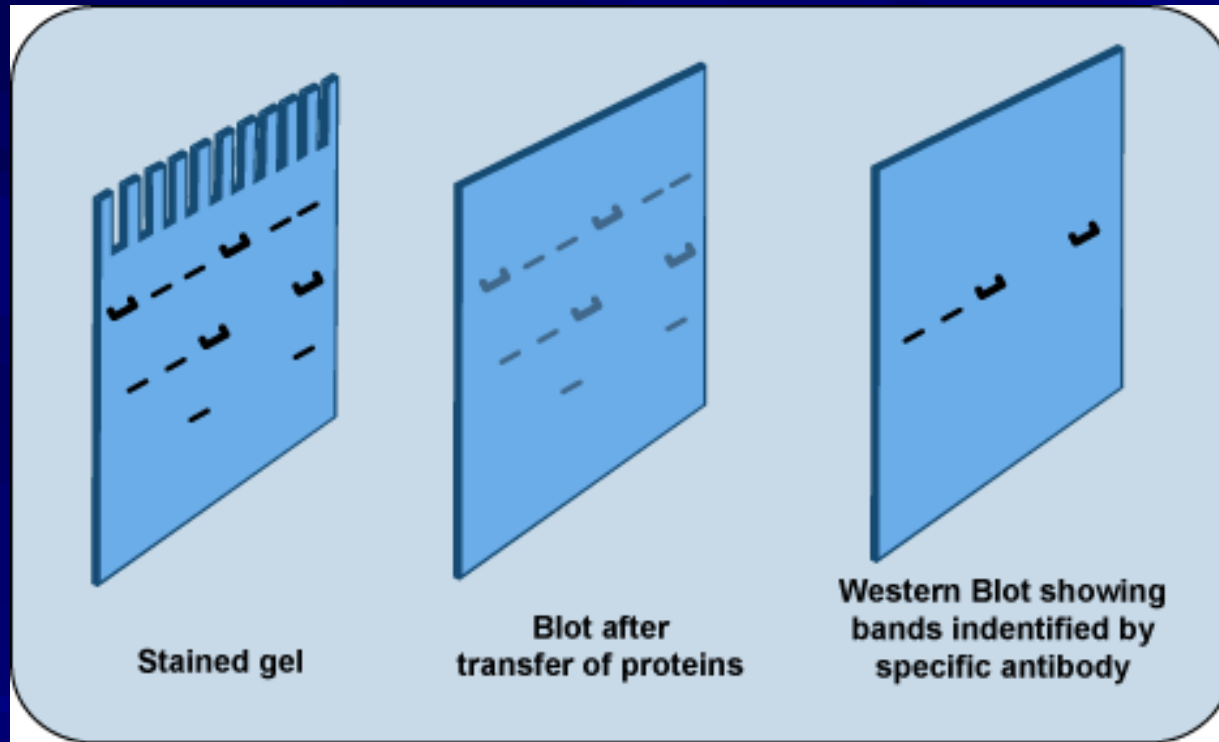
Open neural tube

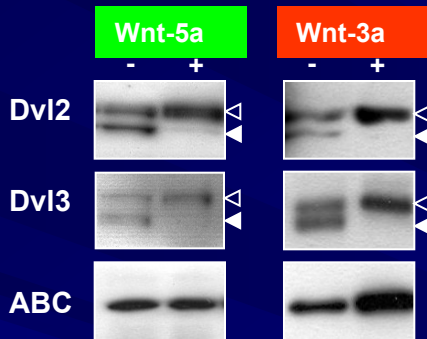


Protilátka
(imunoglobulin)



Metoda 1: Western blotting





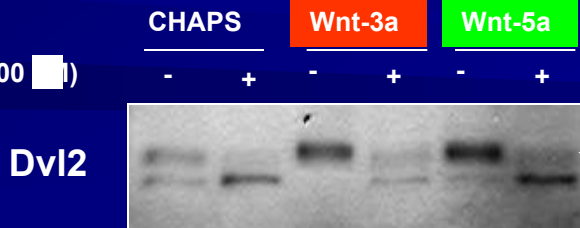
ABC – active \blacksquare catenin = \blacksquare catenin
dephosphorylated on GSK3 \blacksquare target sites

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

◁ PS-Dvl

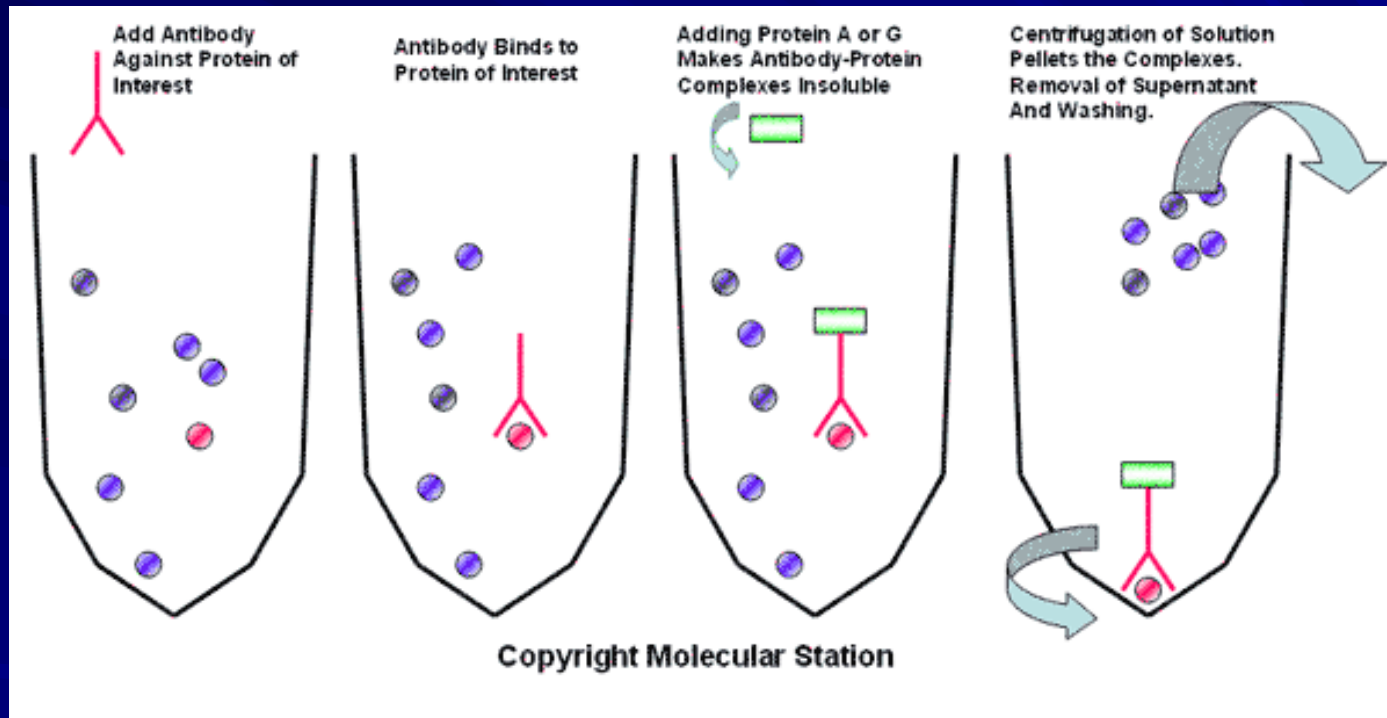
Compound	Target	Concn	Activity
PTX	Galpha i/o	100 ng/ml	No
PDBu	PKC activator	1 μ M	No
Wortmannin	PI3K	50 nM	No
LY294002	PI3K	50 μ M	No
PD98059	MEK1/2	10 μ M	No
UO126	MEK1/2	10 μ M	No
SB203580	p38	10 μ M	No
JNKII inhib	JNK	6 μ M	No
Genistein	PKC	50 μ M	No
chelerythrine	PKC	10 μ M	No
Ro-31 8220	PKC	1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

D4476 (100 μ M)



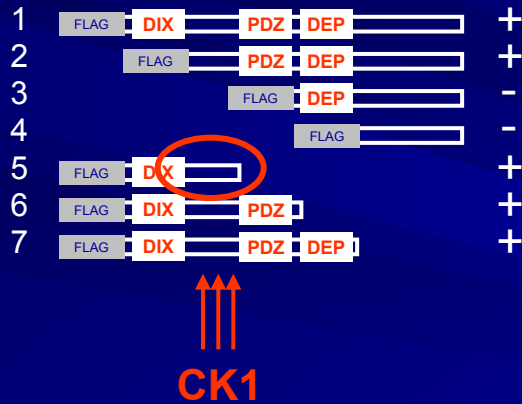
Both **Wnt-3a** and **Wnt-5a** activate Dvl2
and Dvl3 via casein kinase 1 (CK1)

Metoda 2: Immunoprecipitace



β -arrestin binds Dishevelled

Flag-Dvl3 constructs

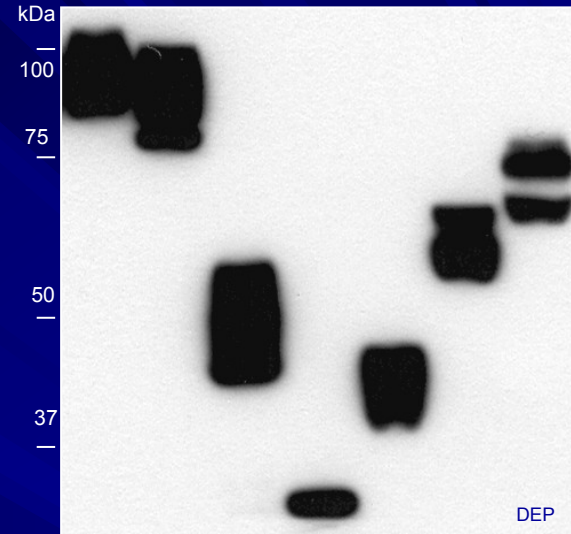


Flag-Dvl3 mutants

1 2 3 4 5 6 7

TCL:

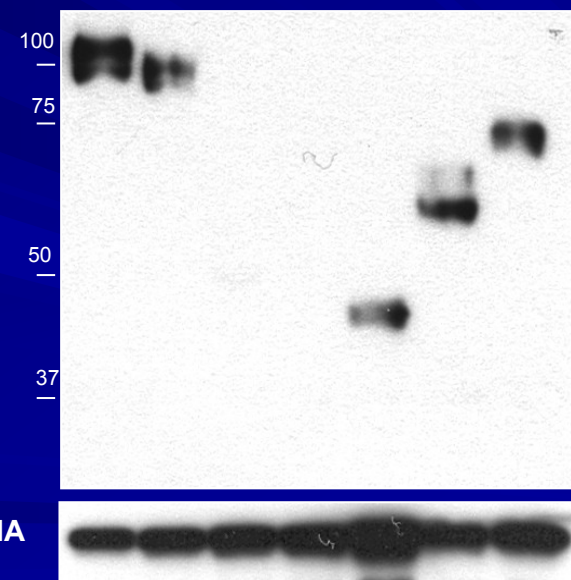
WB:
Flag



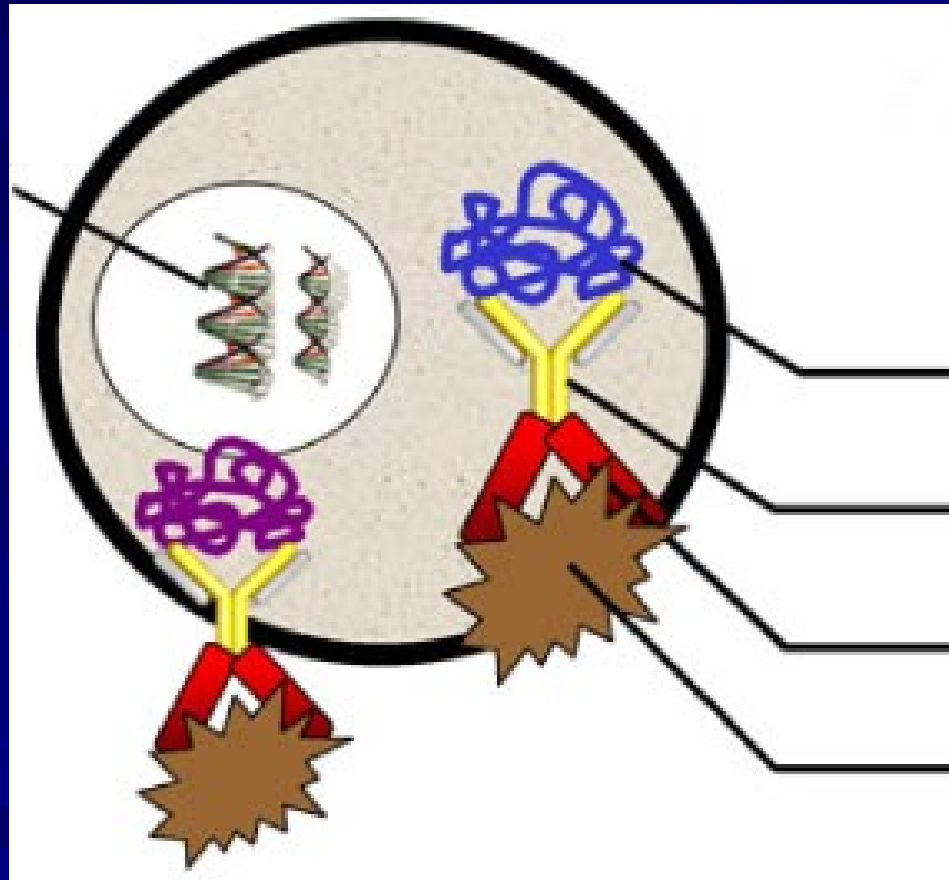
IP: HA- β -arrestin

WB:
Flag

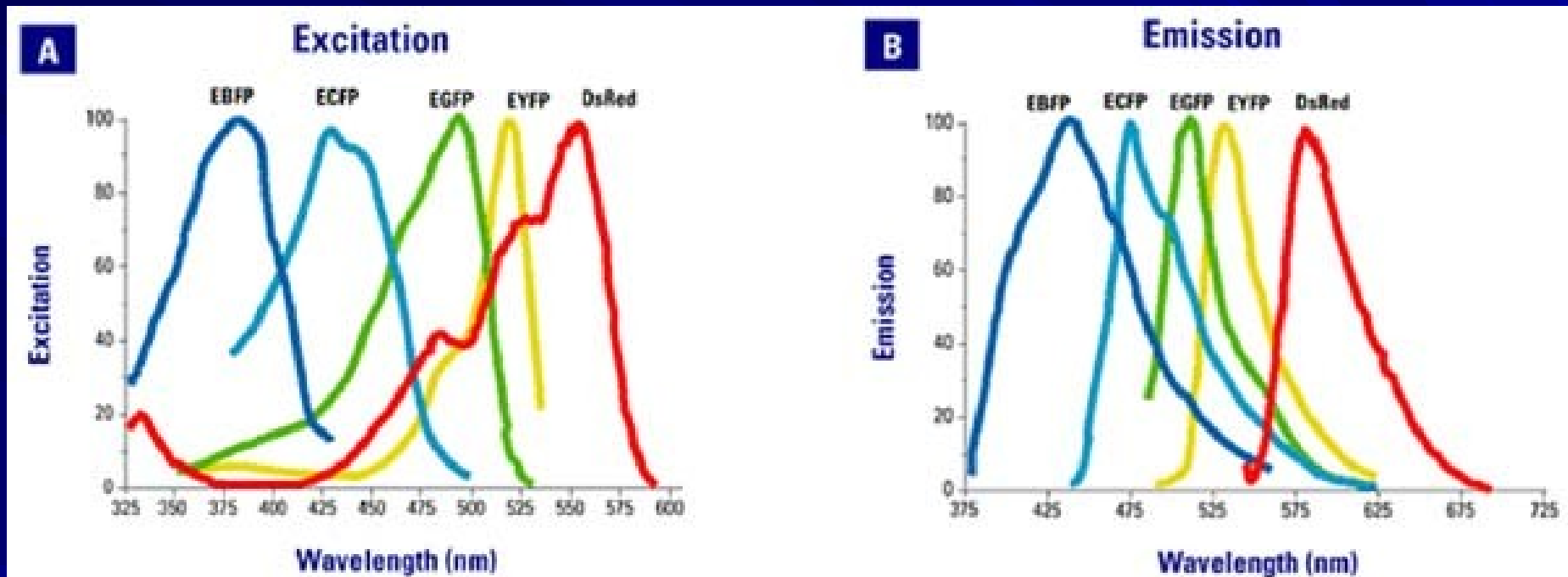
WB: HA



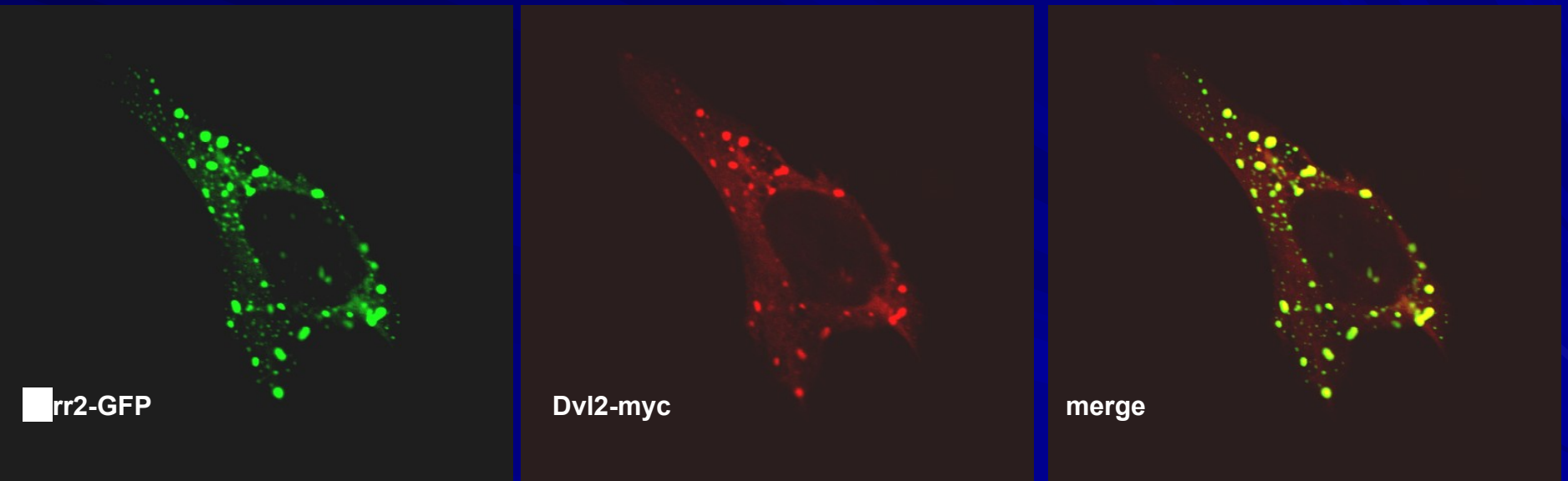
Metoda 3: Immunocytochemie



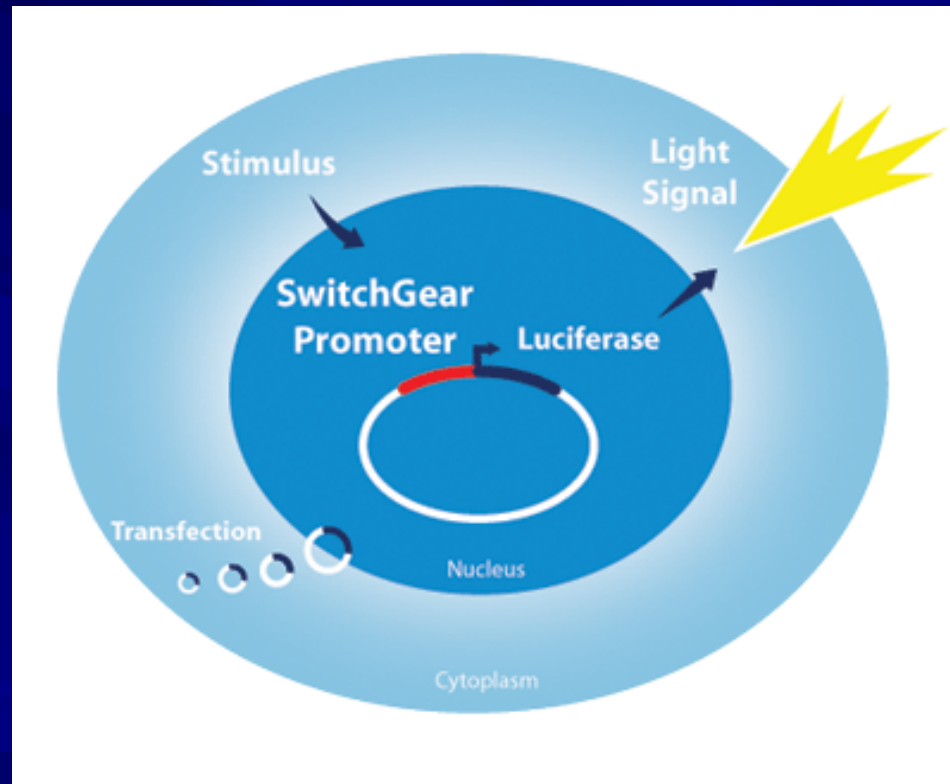
Fluorescenční proteiny



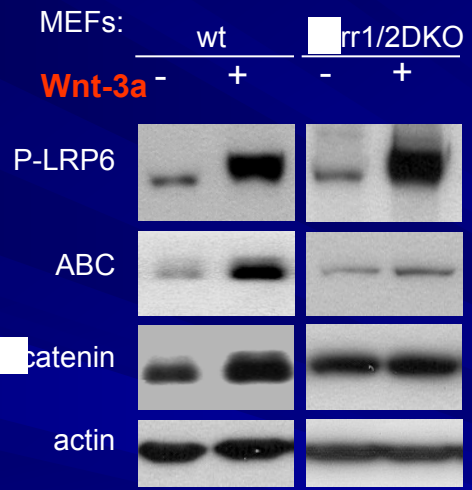
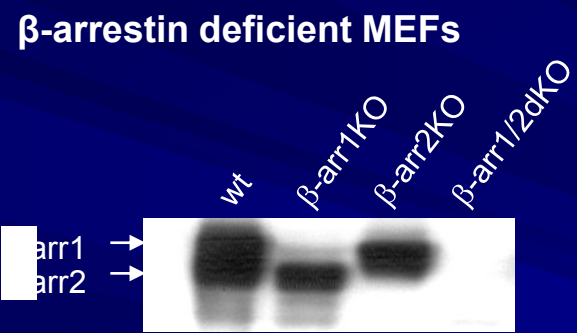
β -arrestin co-localizes with Dvl in the cytoplasm



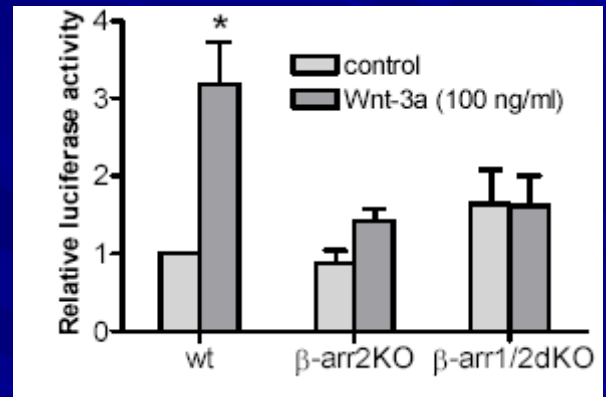
Metoda 4: Reportérové eseje



1. β -arrestin is required for β -catenin activation in vitro

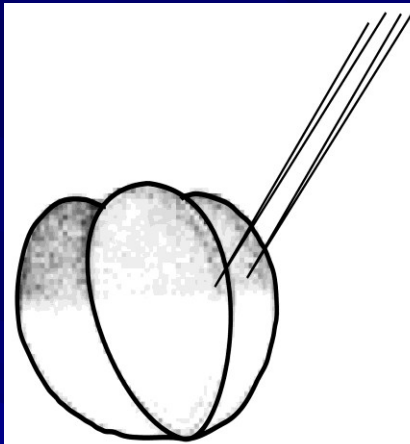


TopFlash reporter - β -catenin transcriptional activity

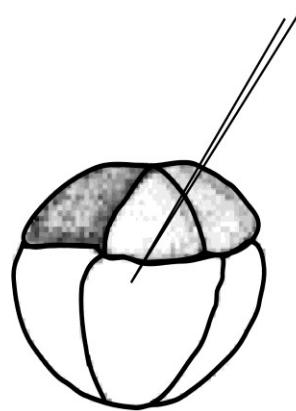


Is this relevant for Wnt signal transduction in vivo?

Metoda č. 5: Analýza in vivo - drápatka



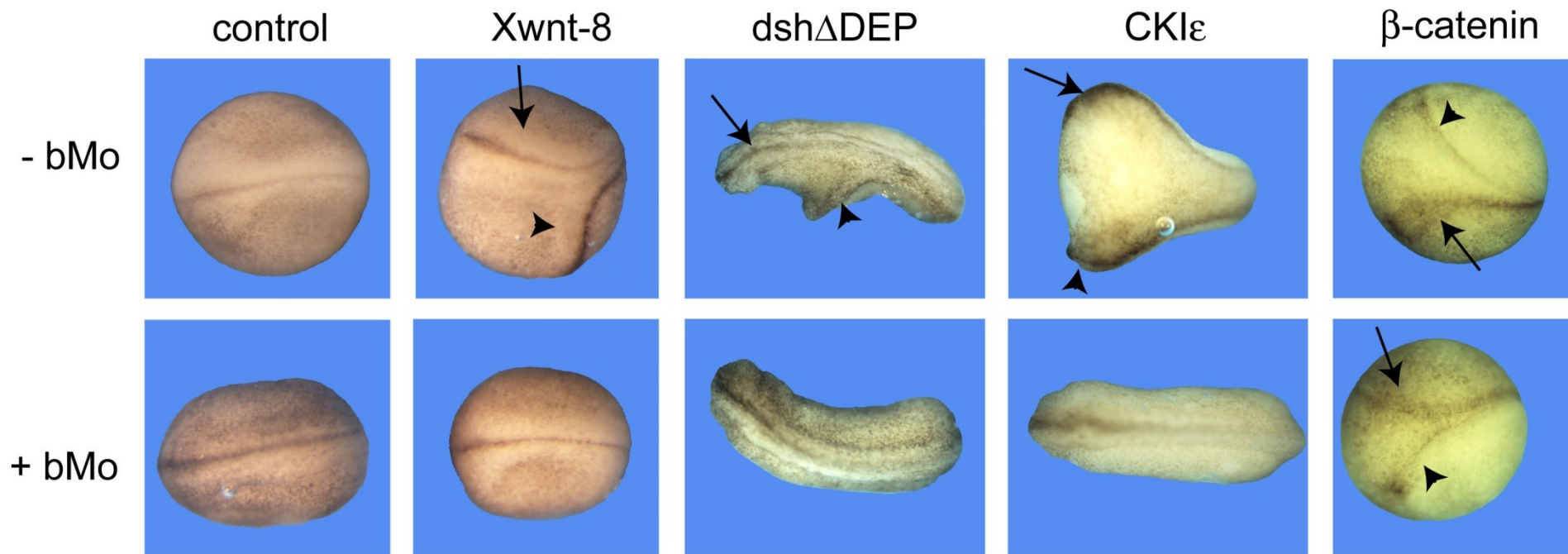
injection in 4-cell stage embryo
targets Dorsal Marginal Zone
affects primary axis formation (ventralization) and CE movements
Figures 2, 3, and Suppl Figure 1



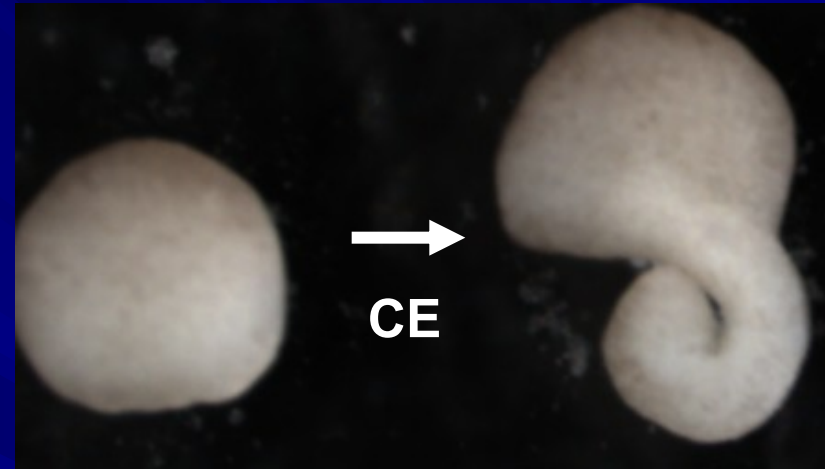
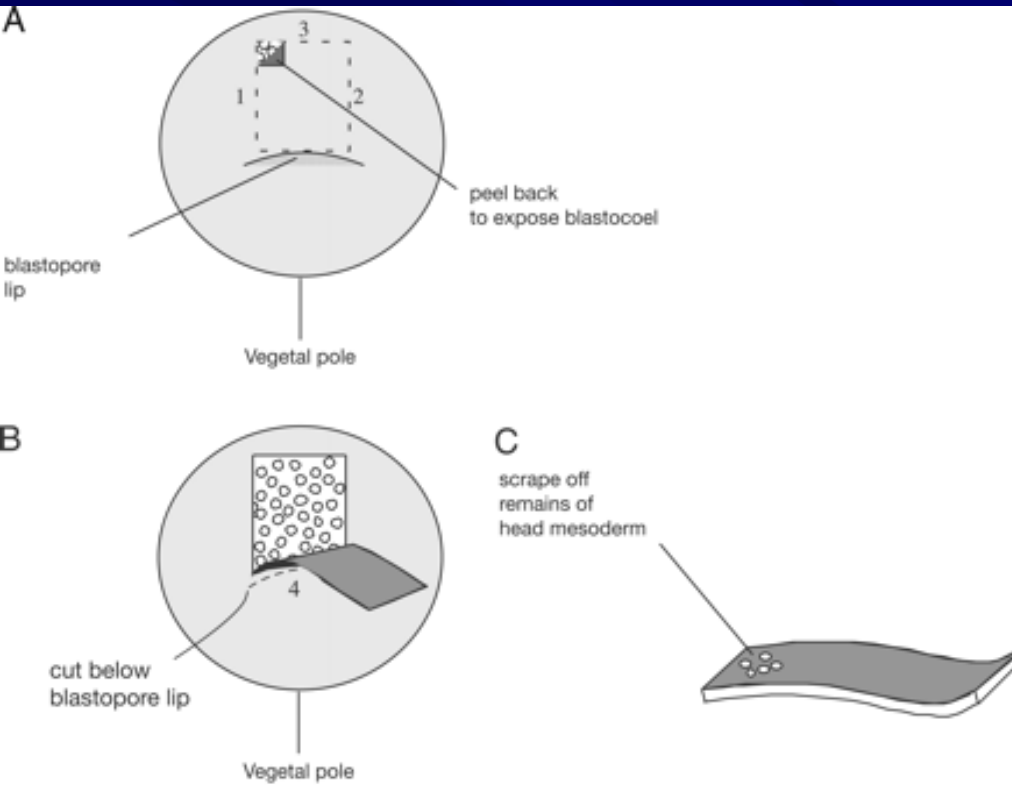
injection in 8-cell stage embryo
targets presumptive cardiac mesoderm
affects cardiac development, but avoids earlier effects on primary axis and CE
Figure 4

Is β -arrestin important for the Wnt/ β -catenin signalling in vivo?

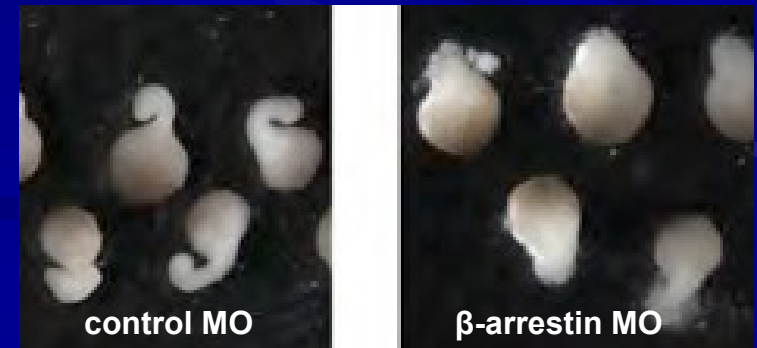
β -arrestin knockdown in *Xenopus* (**axis duplication assay**):



Keller explants (Xenopus)



β -arrestin regulates convergent extension movements in vivo



Metody č. 6: Genetické modifikace myši

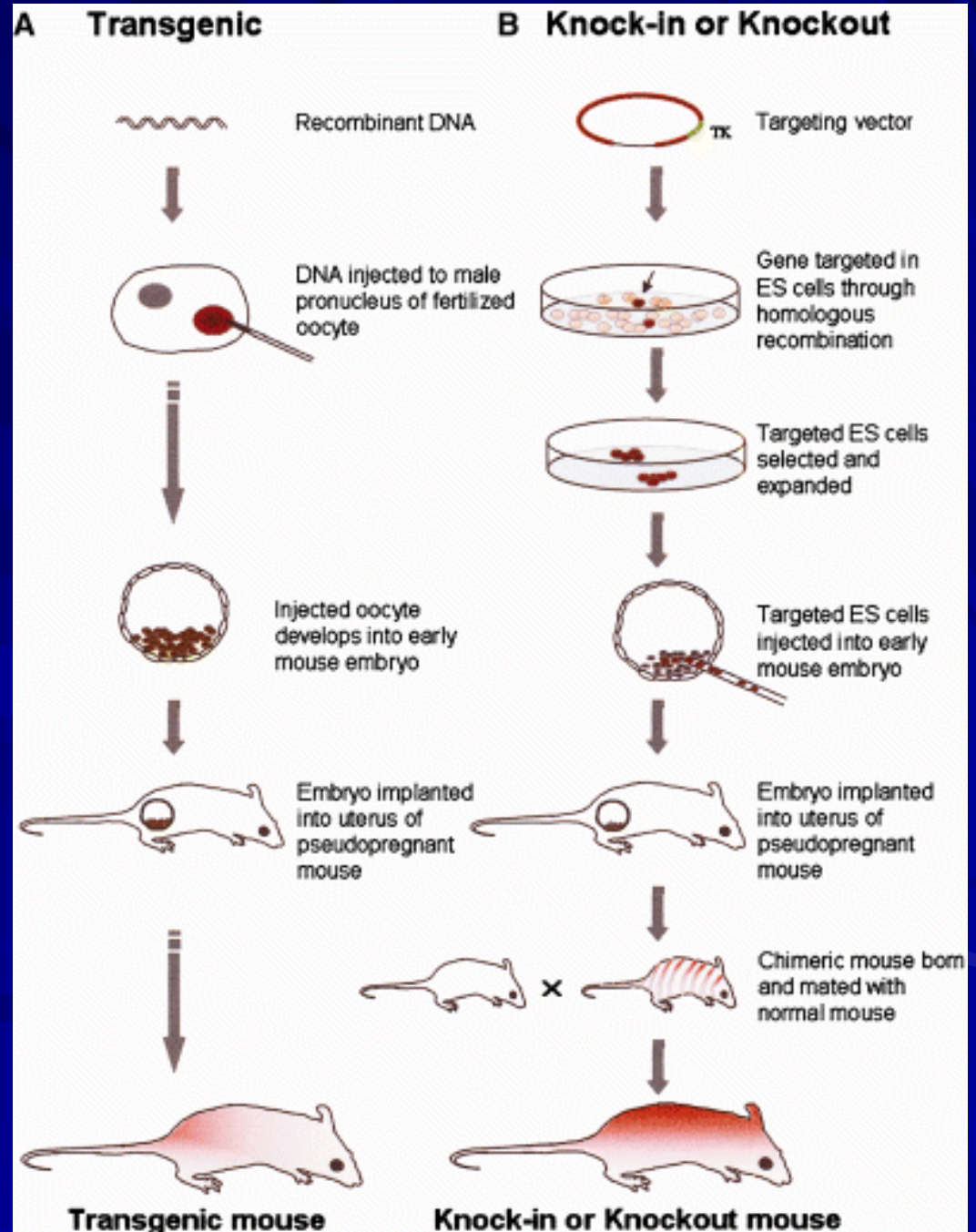
Transgenní myš

Nobelova cena 2007

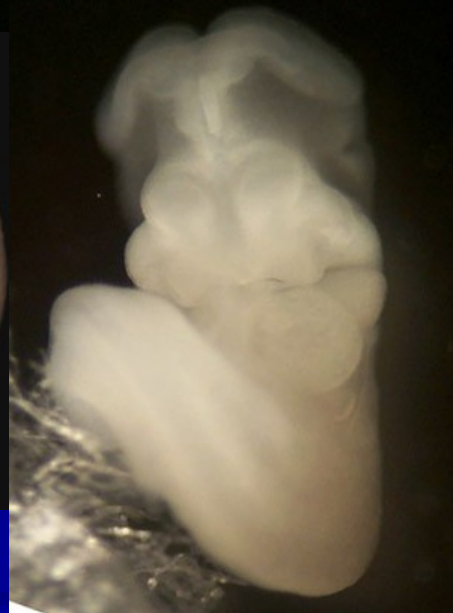
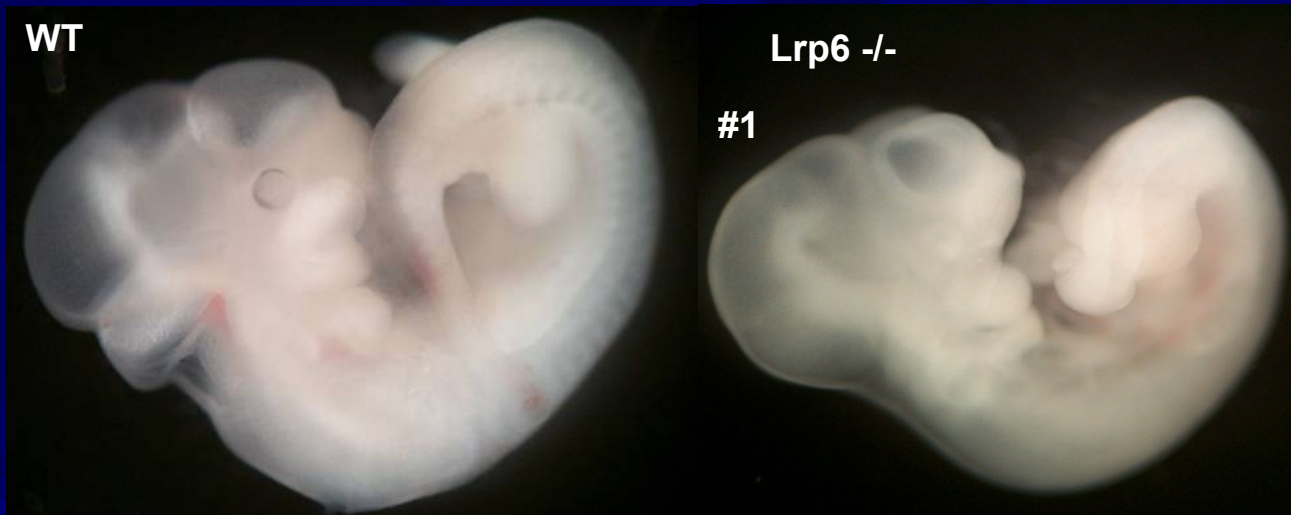
Mario R. Capecchi,
Martin J. Evans and
Oliver Smithies

za

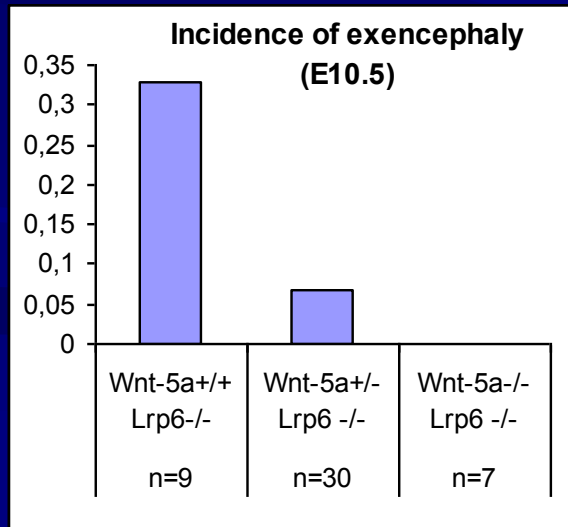
„principles for
introducing specific
gene modifications in
mice by the use of
embryonic stem cells“



Lrp6 KO embryos display exencephaly....

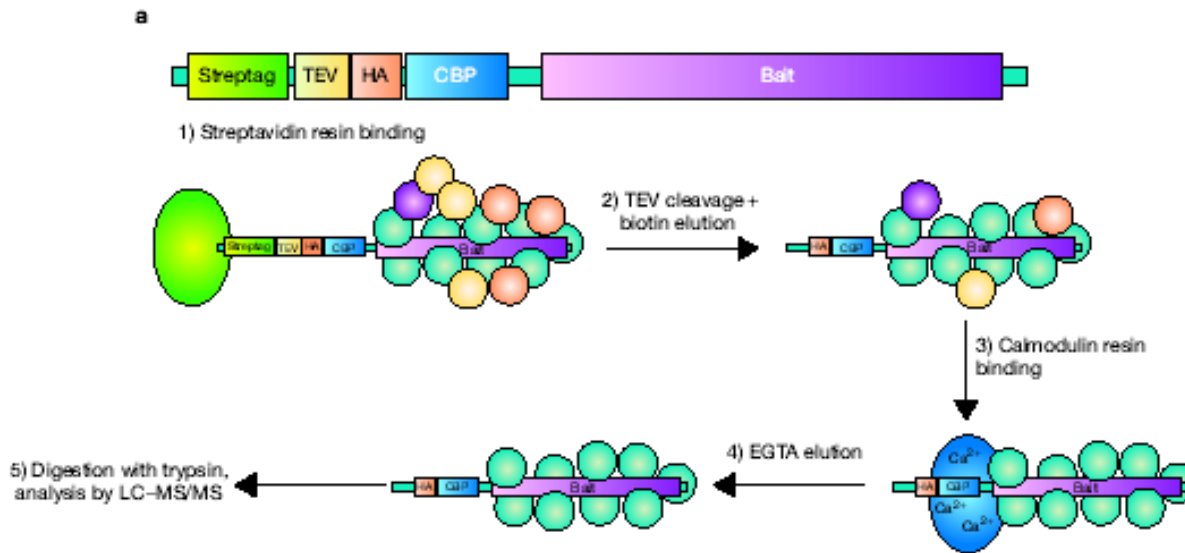


....which is rescued by loss of Wnt5a

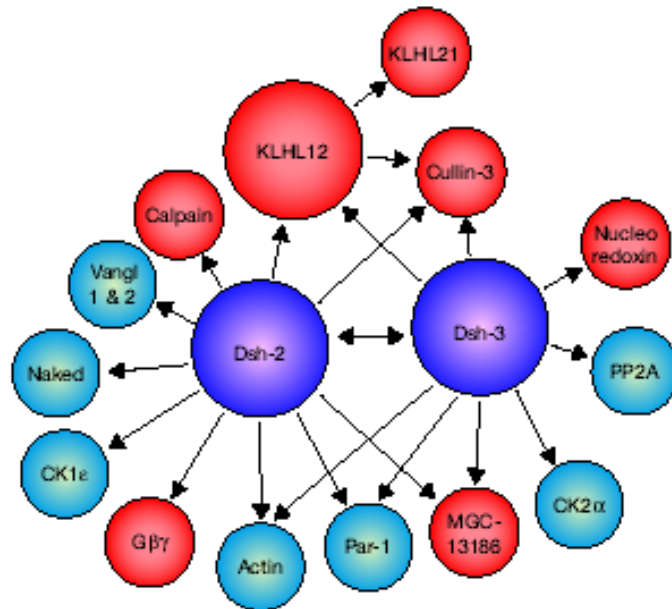


Metody č. 7: Afinity purifikace a hmotnostní spektroskopie

Afinitní purifikace



b

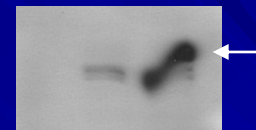


pGlue
pGlue-Dvl2
pGlue-Dvl3

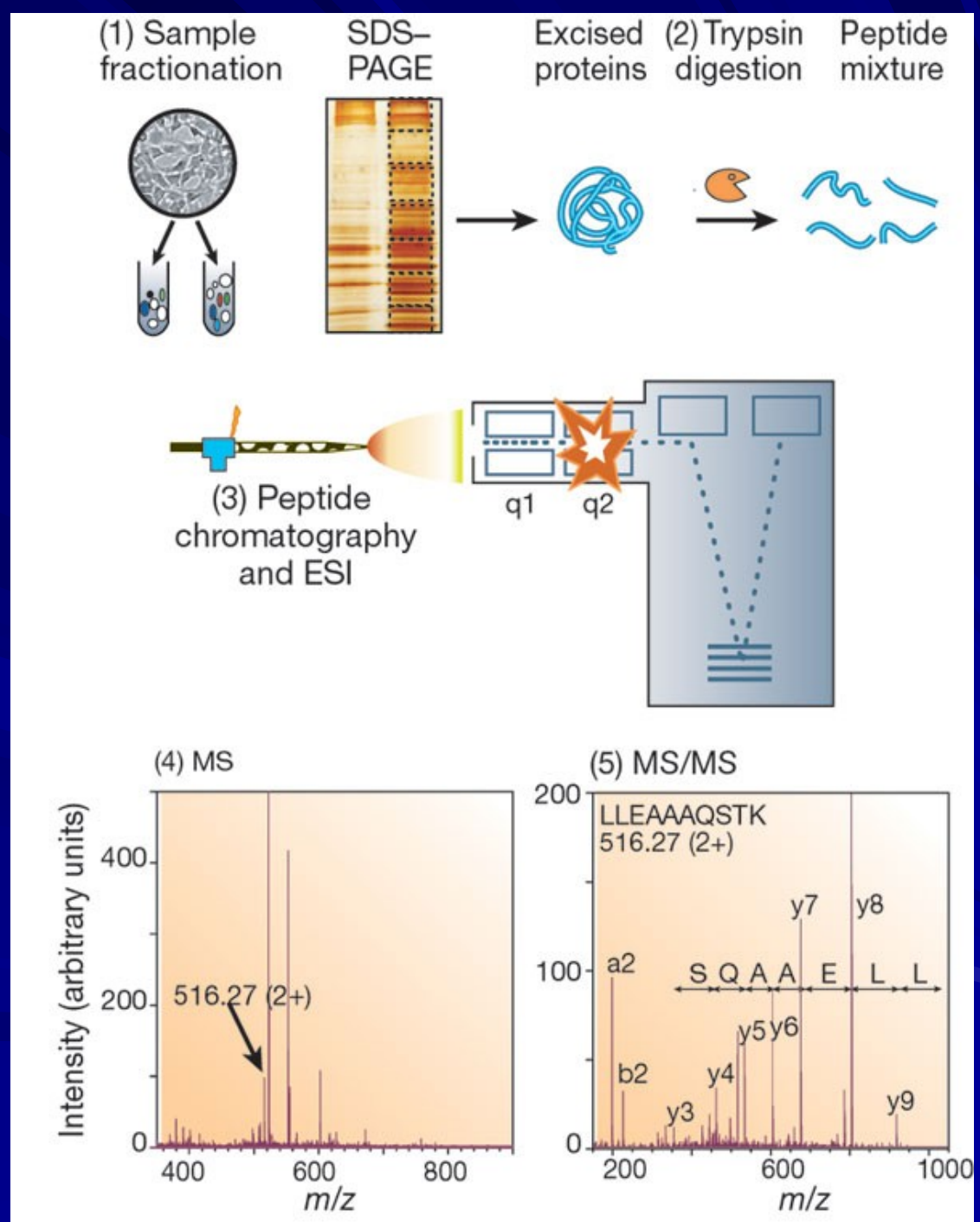
Dvl2



Dvl3



Hmotnostní spektroskopie (Mass Spec)



Děkuji za pozornost!

Celogenomové
techniky

Molekulární
mechanismus

Celoproteomové
techniky

