Wnt 신호전달의 개요 및 연구 동향

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Historical Background

• The Wnt signaling pathway plays a critical and evolutionarily conserved role in directing cell fates during embryogenesis. In addition, inappropriate activation of the Wnt signal transduction pathway plays a role in a variety of human cancers.

•The first Wnt gene was cloned in 1982 as a proto-oncogene (int-1) whose expression was up-regulated in breast tumor and was found adjacent to the integration site of mouse mammary tumor virus(MMTV). (Nusse et al,Cell,1982)

•*Wnt* gene family encode secreted-,cystein-rich glycoproteins.

(Bradley et al,EMBO,1990/ Papkoff et al, MCB,1987)

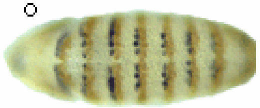
•The wingless (wg) gene of Drosophila is homolog of mouse int.

wg + int = Wnt (Nusse et al, Cell,1991)

•Highly conserved in human, mouse, *Drosophila*, *C.elegans* , Hydra.

http://www.stanford.edu/~rnusse/wntwindow.html

Wnt genes - Microsoft Internet Explorer
파일(F) 편집(E) 보기(V) 즐겨찾기(A) 도구(T) 도움말(H)
뒤로 앞으로 중지 새로고침 홈 검색 즐겨찾기 미디어 기록 메일 인쇄 편집 토론 리서치
주소(D) http://www.stanford.edu/~rnusse/wntwindow.html 이동
연결 링크 사용자 정의 무료 Hotmail 연결 사용자 정의 Windows Windows Media

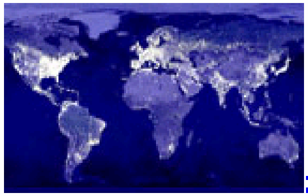


The Wnt Homepage

© 1997-2005 Roel Nusse

UPDA December 1, 2004.

See History for timeline additions



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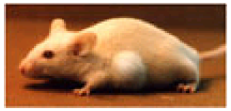
Wnt proteins form a family of highly conserved secreted signaling molecules that regulate cell-to-cell interactions during embryogenesis. Wnt genes and Wnt signaling are also implicated in cancer. Insights into the mechanisms of Wnt action have emerged from several systems: genetics in *Drosophila* and *Caenorhabditis elegans*; biochemistry in cell culture and ectopic gene expression in *Xenopus* embryos. Many Wnt genes in the mouse have been mutated, leading to very specific developmental defects. As currently understood, Wnt proteins bind to receptors of the Frizzled family on the cell surface. Through several cytoplasmic relay components, the signal is transduced to beta-catenin, which then enters the nucleus and forms a complex with TCF to activate transcription of Wnt target genes. These pages contain some diagrams of the pathway. Wnt signaling has been discussed in several reviews, listed here.



Wnt genes, proteins

- Vertebrates April 2003
- Mouse June 2004
- Human Dec 2004
- Xenopus August 2002
- Chicken Feb 2001
- Zebrafish October 2003
- Drosophila April 2003

Frizzleds, SFRP

- Frizzleds in Mammals June 2004
- Frizzleds in Drosophila and C. elegans Oct 2002
- SFRP/FrzB genes March 2004
- Structure April 2002
- Annotated Alignment
- Alignment mouse/human

TCF

- TCF/Lef June 2004
- Alignment March 2000
- Structure April 2001

Other genes

페이지에 오류가 있습니다. 인터넷

- Wnt alignments All
- Wnt alignment Fewer
- InterPro Entry

Dishevelled

- Dsh January 2003
- Alignment
- Structure of the Dep domain
- InterPro DIX
- InterPro Dsh

LRP

- LRP/arrow Dec 2004
- Alignment

APC

- APC April 2002
- Alignment
- Structure April 2001

Axin

- Axin June 2004
- Alignment
- Structure April 2001

beta-catenin

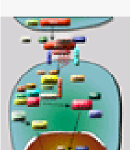
- Beta-catenin/armadillo Dec 2004
- mutations in cancer Sept 2000
- alignment
- Structure April 2001
- InterPro Entry

- Naked Dec 2004
- Porcupine April 2002
- FRAT/GBP March 2003
- Wnt components in primitive organisms March 2004
- Human diseases Dec 2004

Methods, Resources, Reagents

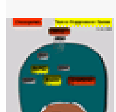
- Where to obtain reagents Dec 2004
- How to activate the Wnt pathway Dec 2004 NEW
- How to inhibit Wnt signaling June 2002
- Isolation of Wnt protein July 2003
- How to visualize Wnt signaling in animals October 2003
- Assays Nov 2000
- Small molecules in Wnt signaling Dec 2004

all components signaling



Protein interactions

Wnt signaling in cancer



2 state model

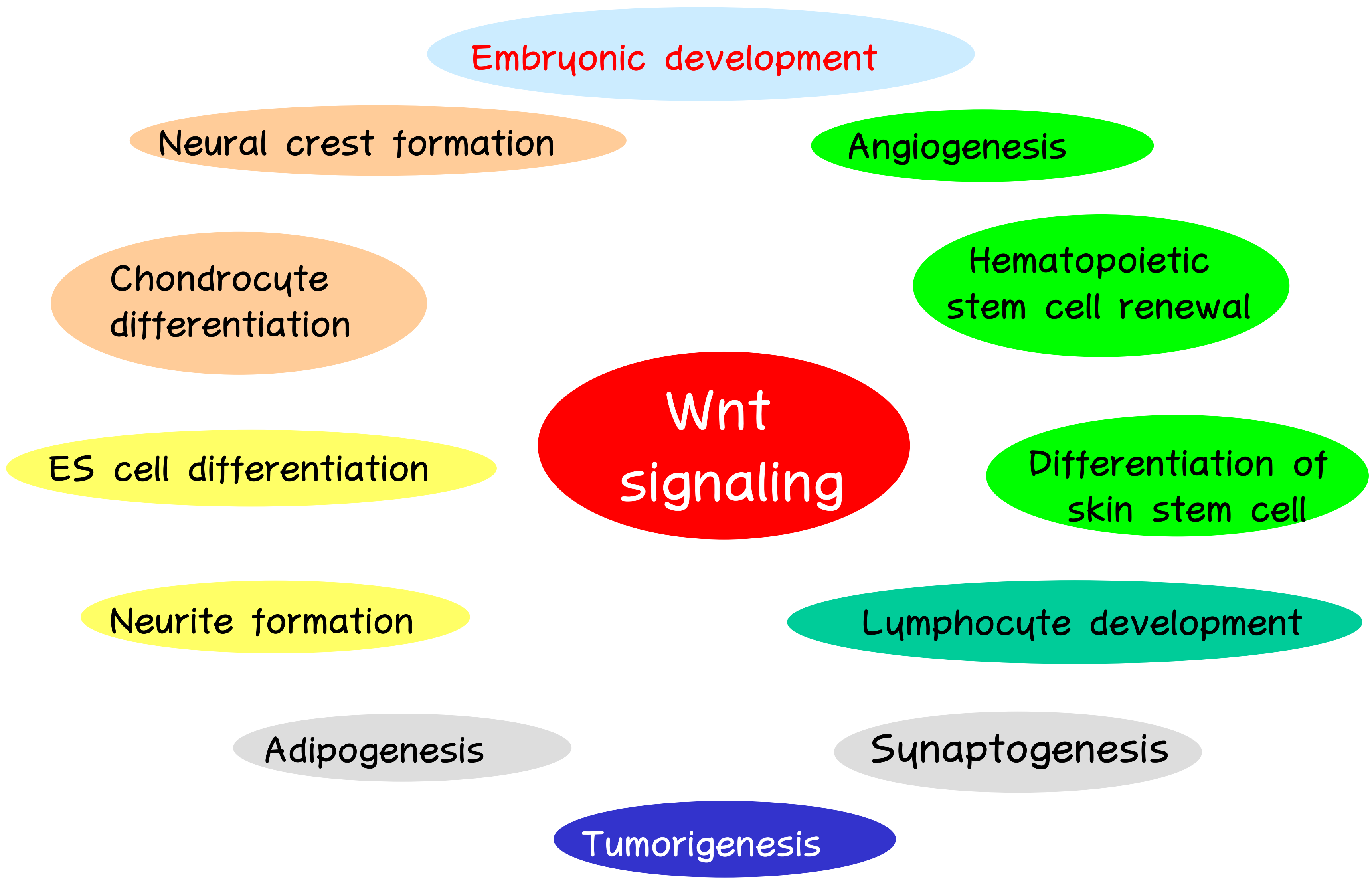
June 2004

Comparison Wnt-Hedgehog

October 2002

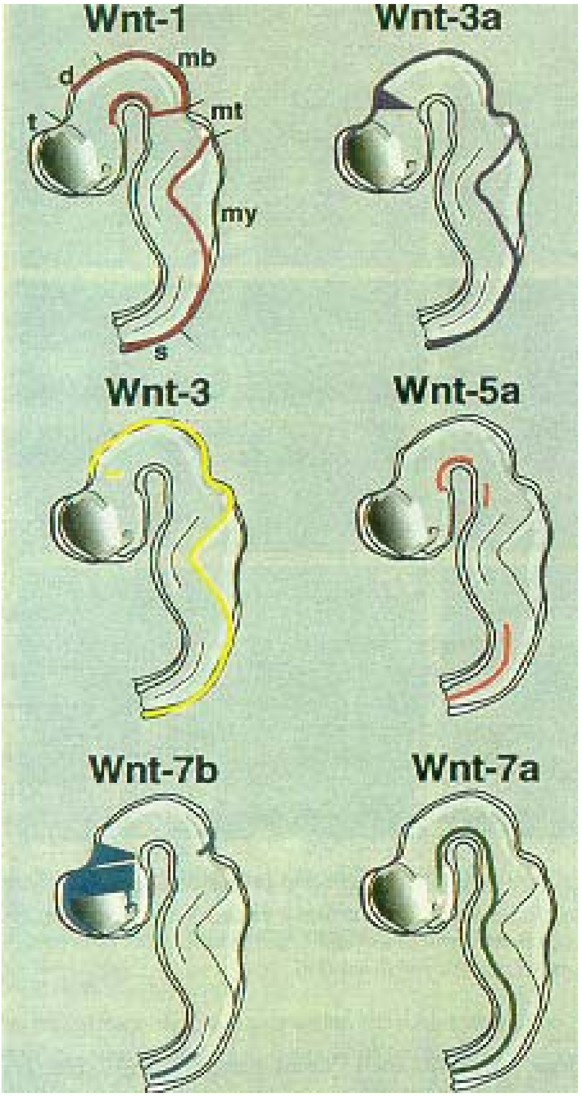
Wnt signaling in C. elegans

Target Genes Dec 2004



19 Wnts and 10 Frizzleds

Parr et al. Development 119, 247



Wnt 1 -/-

- loss midbrain, loss cerebellum

Wnt 3a -/-

- deficiency in neural crest derivatives (together with Wnt 1-/-)
- Loss hippocampus

Wnt 3 -/-

- early gastrulation defect; Axis formation

Wnt 5a -/-

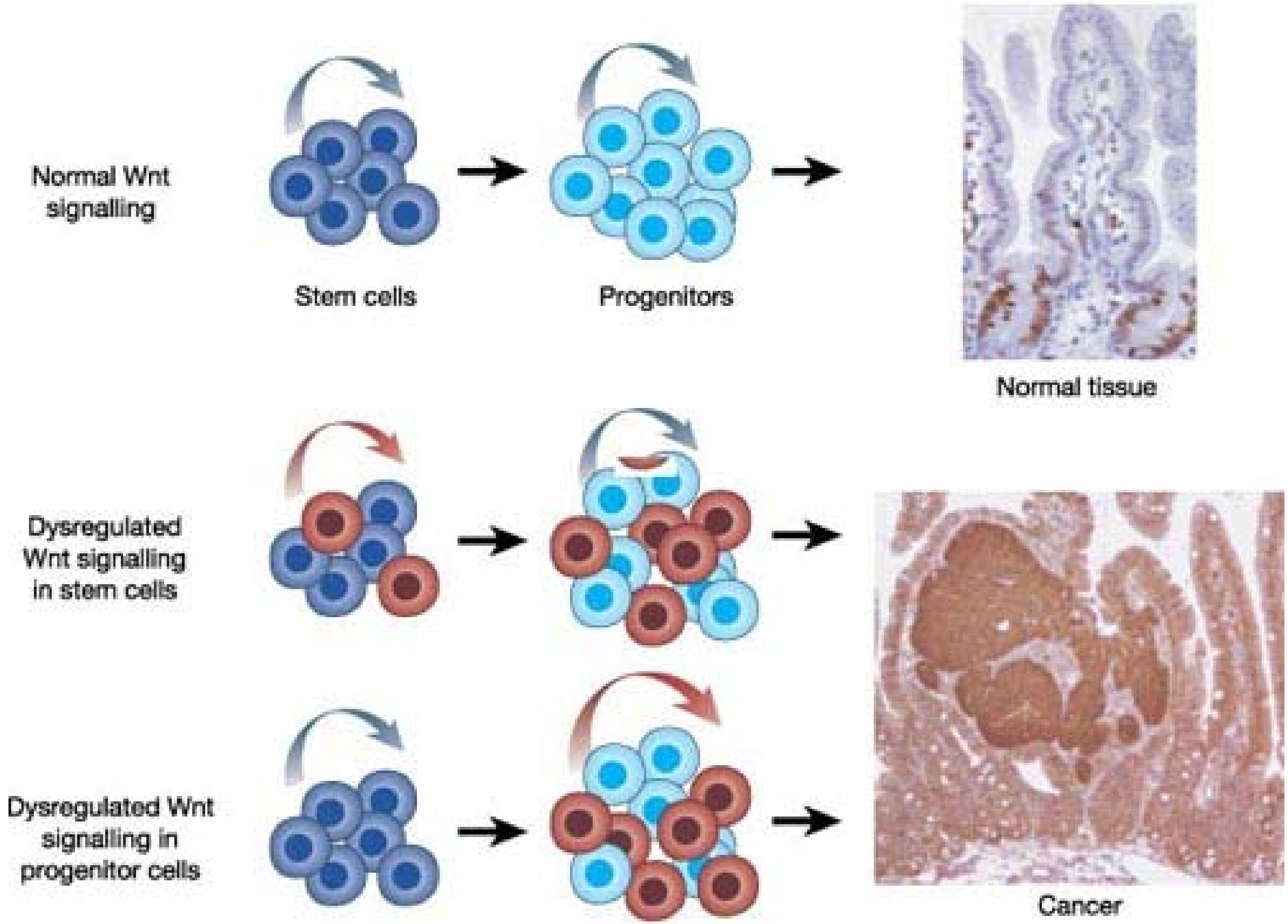
- truncated limbs, truncated AP axis, reduced number proliferating cells

Wnt 7a -/-

- Delayed maturation synapses in Cerebellum

http://www.stanford.edu/~rnusse/wntwindow.html

Dysregulation of Wnt signaling leads to Cancer formation



Reya and Clevers (2005) *Nature* 434: 843-850

Target Genes of Wnt signaling

Gene	Organism/system	Direct/Indirect	up/down	Ref.
c-myc	human colon cancer	yes	up	He 1998
Cyclin D	human colon cancer	yes	up	Tetsu 1999 Shtutman 1999
Tcf-1	human colon cancer	yes	up	Roose 1999
LEF1	human colon cancer	yes	up	Hovanes, 2001 Filali 2002
Siamois	Xenopus	yes	up	Brannon 1997
fibronectin	Xenopus	yes	up	Gradl 1999
BMP4	Xenopus	?	down	Baker 1999
myogenic bHLH	Xenopus	?	up	Munsterberg 1995
engrailed-2	Xenopus	yes	up	McGrew 1999
Xnr3	Xenopus	yes	up	McKendry 1997
connexin43	Xenopus, Mouse	yes	up	van der Heyden 1999
twin	Xenopus	yes	up	Laurent 1997
connexin 30	Xenopus	?		McGrew 1999
retinoic acid receptor gamma	Xenopus	?		McGrew 1999
dharma/bozozok	Zebrafish	yes	up	Ryu 2001
Cdx4	Zebrafish HSC	?	up	Davidson 2003

http://www.stanford.edu/~rnusse/wntwindow.html

Wnt signaling을 연구하는 연구자들

Nobel 상 수상자들

- Dr. Harold Varmus (1982) : Tumorigenesis
- Dr. David Baltimore (1975) : 최근 Wnt co-receptor 인 Ryk cloning
- Dr. Eric Wieschaus (1995) : Wnt signaling의 전달체계를 재조명
- Dr. Paul Greengard (2000) : Wnt signaling을 조절하는 small molecule

Howard Hughes Medical Institute (HHMI) investigators 들

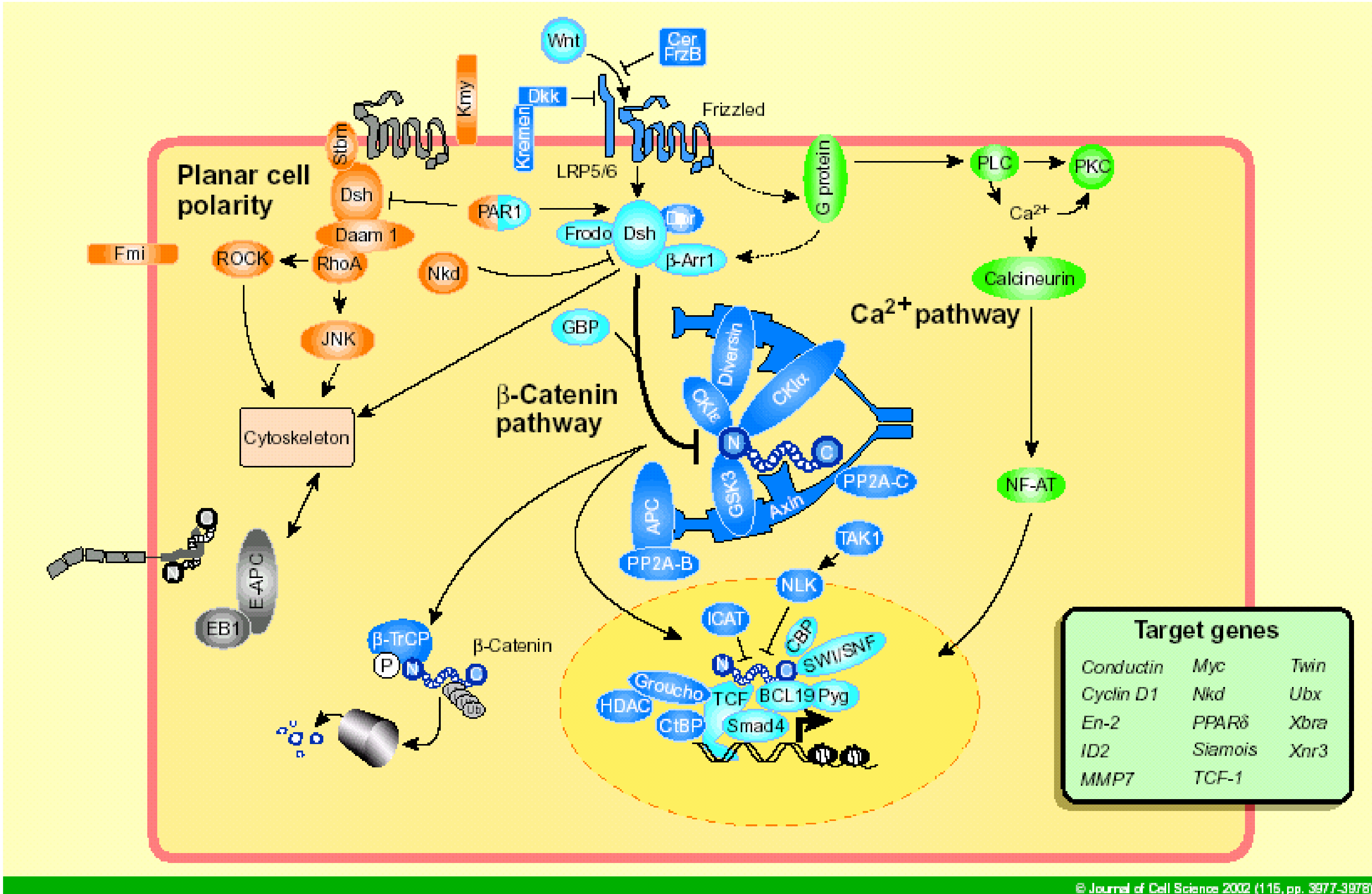
- Dr. Roel Nusse : Wnt Homepage, Stem cell
- Dr. Doug Melton : Development, Embryonic Stem cell
- Dr. Randall Moon : Development, non-cannonical Wnt siganling
- Dr. Nobert Perrimon : Wnt modification and secretion

등 13 명의 HHMI members (HHMI member의 총 수는 301 명)

Wnt 신호전달의 연구 방향

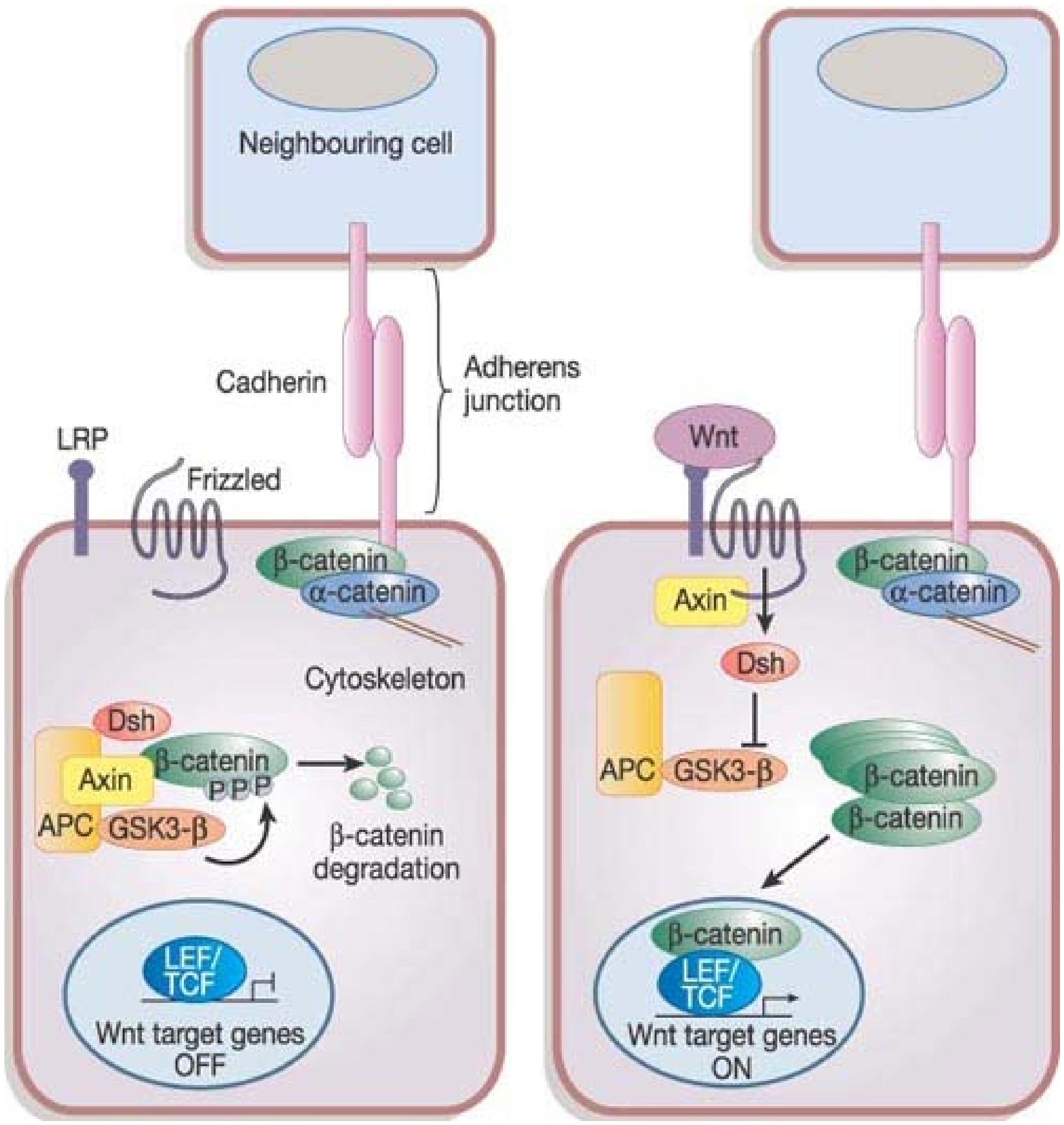
1. Wnt signaling의 신호전달에 관련하는 새로운 component의 발굴 및 기능 연구.
2. 모델 시스템을 이용한 Wnt signaling의 이상에 따른 질병과의 연관성 연구.
3. Wnt signaling을 조절할 수 있는 small molecule의 발굴 및 target identification.
4. Wnt signaling의 조절에 의한 directed 세포 분화의 연구

Wnt signaling pathway



Joerg Huelsken and Juergen Behrens (2002) J. of Cell Science 115: 3977

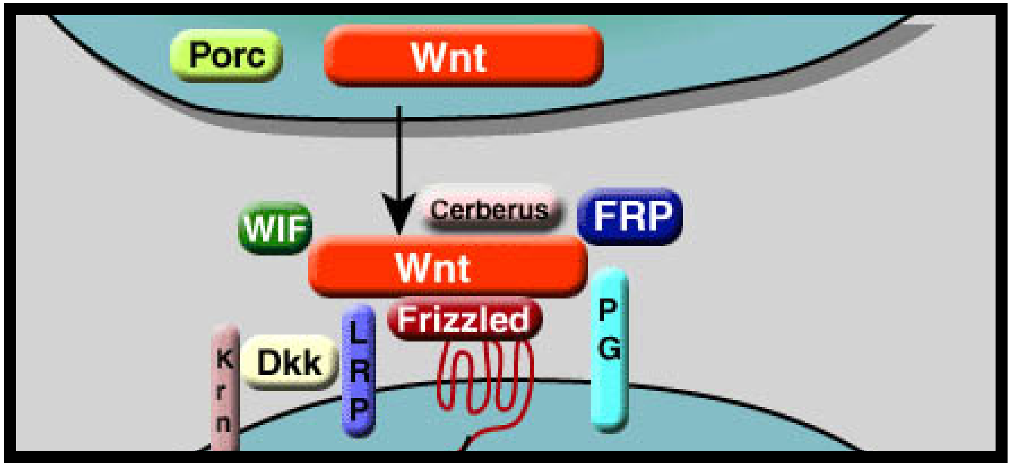
The canonical Wnt signaling pathway



Reya and Clevers (2005) *Nature* 434: 843-850

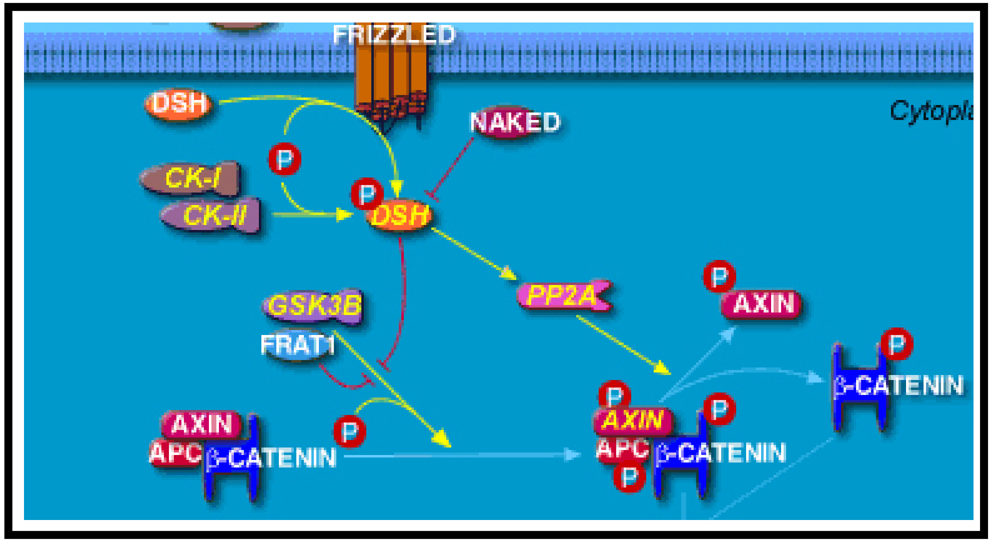
Wnt – Frizzled

- *Wnt* gene family : humans(19), mouse (19) xenopus(15),zebrafish(9),*Drosophila* (4), *C.elegans(5)* , Hydra(at least 1).
- 350~400 a.a
- the difficulty in preparing purified, biologically active forms : conditioned medium



- Frizzled proteins (10) are seven-transmembrane receptors
- Wnts can bind the the CRD (cysteine-rich domain) of Frizzled, an extracellular part of the receptor
- Little is known about the mechanism of Frizzled signaling.
- The structure of the CRD has been solved.(Dann et al,nature, 2001)
- Some but not all Frizzleds stimulate Ca 2+release and PKC activity

Frizzled-Dishevelled (Cytoplasm)



•The Wnt signal leads, through its receptor to activation of Dishevelled (Dsh), while the mechanism of activation of Dsh is not known

•Dsh interacts with Casein Kinase 1 (Peters ,1999), Casein Kinase 2 (Willert, 1997) and GBP/Frat1 (Li, 1999, Salic, 2000.)

•Dsh also interacts with GBP (Yost et al) and can bind to Axin (Smalley, 1999), an interaction that may lead to the next step in Wnt signaling, the accumulation of b-catenin.

•Dsh can also bind to the **Phosphatase PP2C** (PP2C) which is able to dephosphorylate **Axin** (Strovel, 1999) and to **Frodo** (Gloy, 2002)

•In *Drosophila*, the **naked cuticle gene (naked)** acts as an inducible inhibitor of Wingless signaling (Zeng et al, 2000) The naked protein can directly bind to the Dsh protein (Rousset et al, 2001)

•**Par-1** (Sun et al, 2001) kinase acts as a positive regaulator of Wnt signaling in Drosophila and in other systems and can phosphorylate Dsh directly.

•**Dapper (Dpr)** interacts with Dsh, to counteract its activity (Cheyette, 2002)

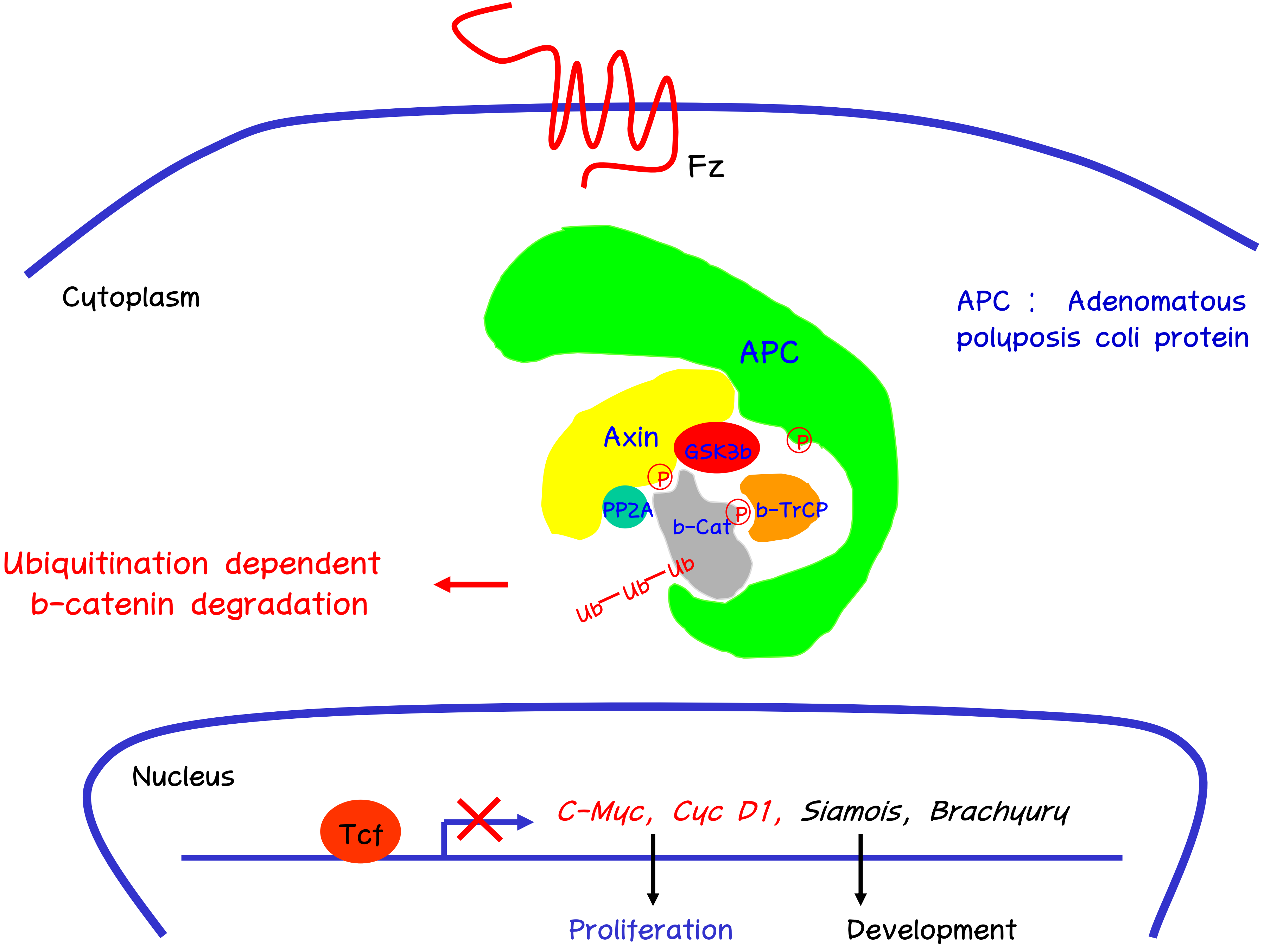
Cytoplasmic complex for β-catenin degradation

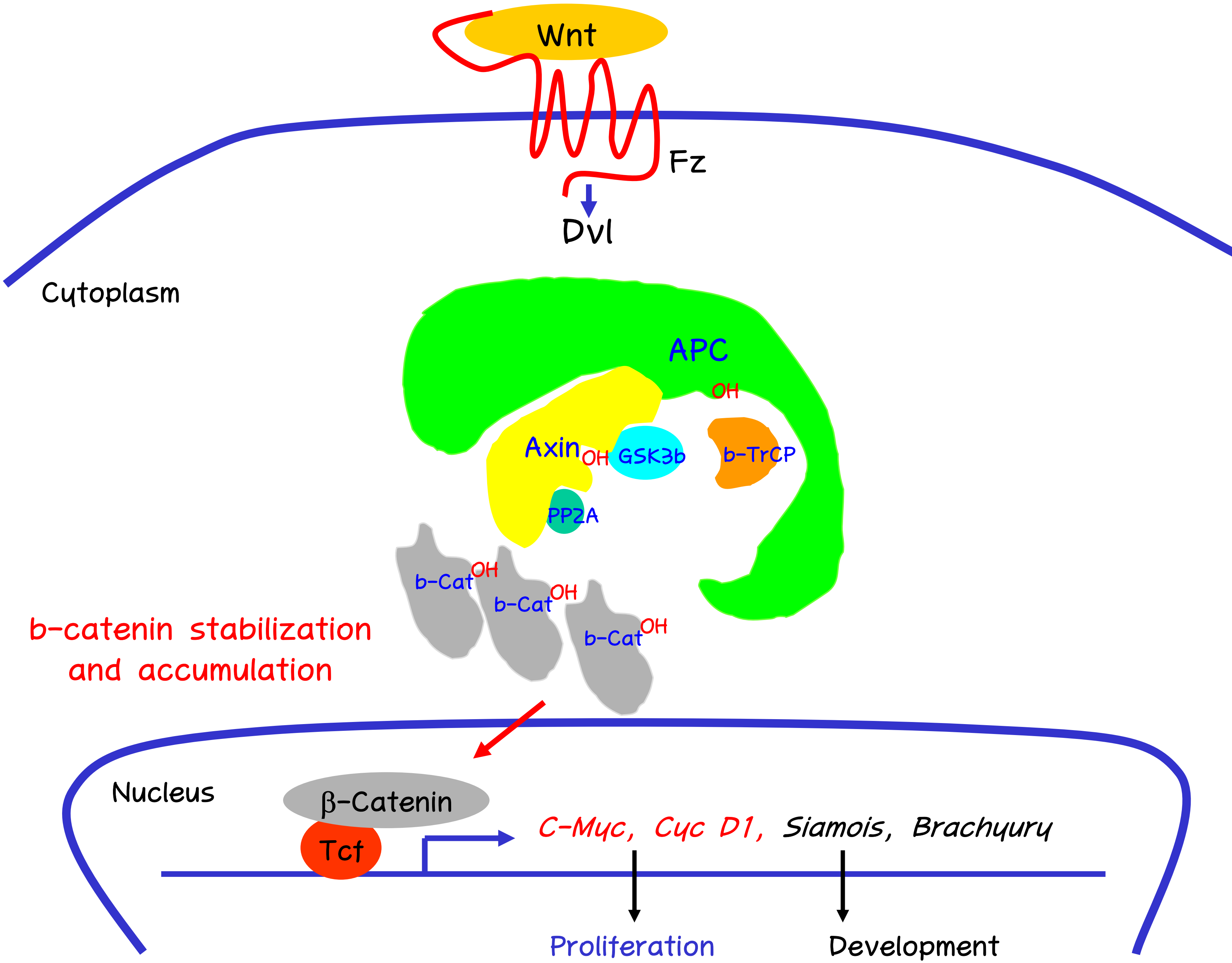
- Without the Wnt signal, b-catenin levels are kept low through interactions with the protein kinase **zw3/GSK-3b**, **CK1a**, **APC (Adenomatous polyposis coli protein)** and **Axin** (reviewed in Kikuchi, 2003)

- b-catenin is degraded, after phosphorylation by GSK-3 and CK1 alpha (Yanagawa 2002, Liu 2002), through the ubiquitin pathway (Aberle 1997.), involving interactions with **Slimb/b-TrCP** (reviewed in Maniatis 1999)

- Axin also binds to the **phosphatase PP2A**. (Hsu 1999), while the **B56 subunit of PP2A** interacts with APC (Seeling 1999). According to Li, 2001, PP2A activity inhibits Wnt signaling. The binding between Axin and **Diversin** brings **CK1epsilon** to this complex (Schwarz-Romond 2002)

- Loss of **APC** in mammalian cells can also lead to a critical loss over b-catenin control, leading to cell transformation (reviewed in Polakis, 2000). **APC** has a specific function in keeping b-catenin out of the nucleus (Henderson, 2000)


Fz
Cytoplasm
APC : Adenomatous polyposis coli protein
APC
Axin
GSK3b
P
P
PP2A
b-Cat
P
b-TrCP
Ubiquitination dependent b-catenin degradation
Ub—Ub—Ub
Nucleus
Tcf
C-Myc, Cyc D1, Siamois, Brachyury
Proliferation
Development


Wnt
Fz
Dvl
Cytoplasm
APC
OH
Axin
OH
GSK3b
b-TrCP
PP2A
b-Cat
OH
b-Cat
OH
b-Cat
OH
b-catenin stabilization
and accumulation
Nucleus
β-Catenin
Tcf
C-Myc, Cyc D1, Siamois, Brachyury
Proliferation
Development

Phenotypic effect of mutant *Axin* alleles

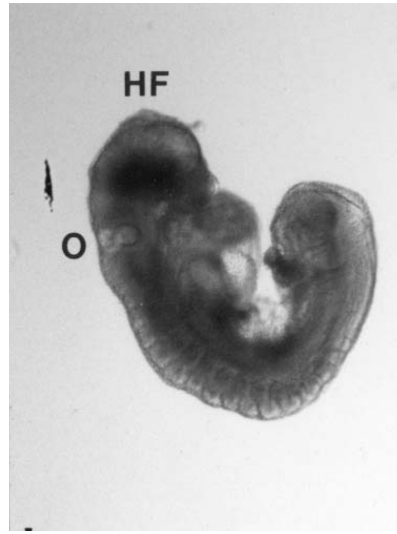
	Fused ($Axin^{Fu}$)	*Kinky* ($Axin^{Ki}$)	*Knobbly* ($Axin^{Kb}$)	transgenic ($Axin^{Tg1}$)	deletion (t^{h20})
Dominant: Skeletal defects, Neurological defects	✓	✓	✓	–	–
Recessive: Embryonic lethality Axial Duplications Neuroectodermal defects Cardiac defects	–	✓	✓	✓	(✓)

Axis duplication in a *Knobbly* embryo

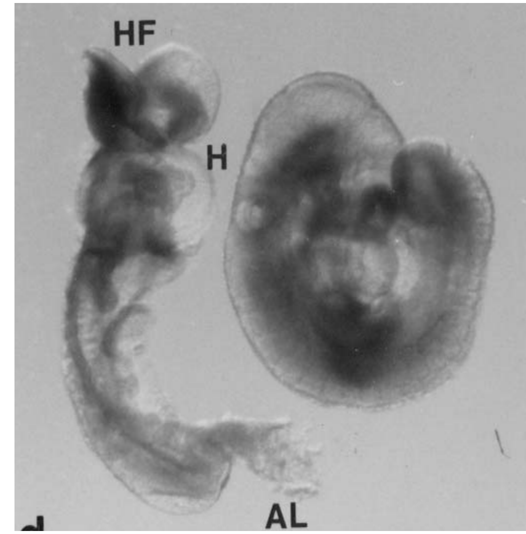
Kinky tail in heterozygote mice

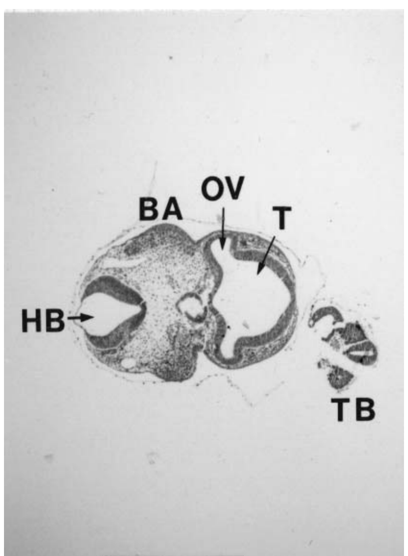
WILD TYPE

TRANSGENIC

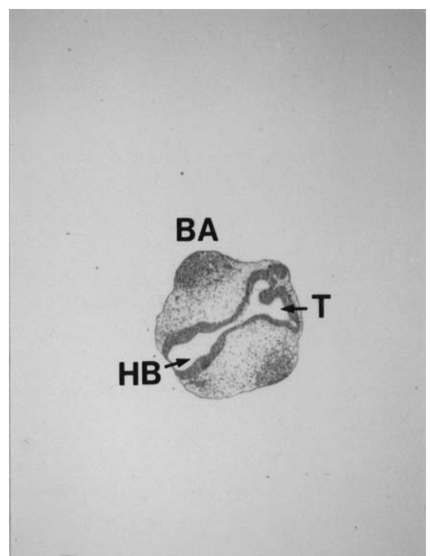


TRANSGENIC WILD TYPE





WILD TYPE



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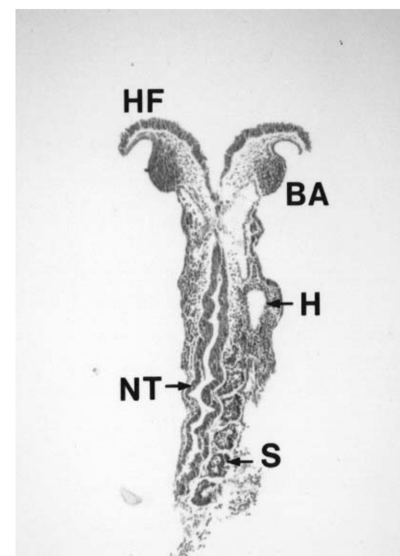
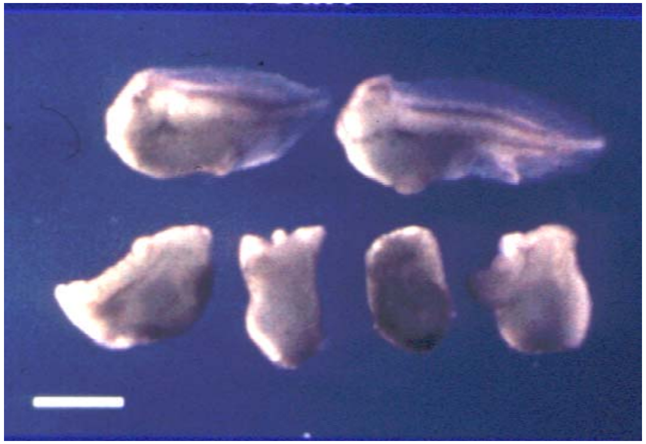


TRANSGENIC

Role of Wnt signaling in early embryonic axis formation

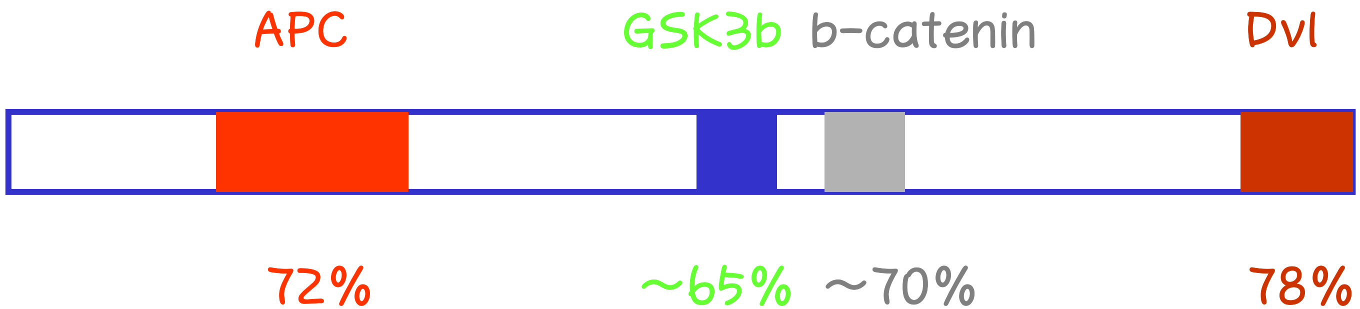
b-galactosidase



Axin

Inhibition of body axis formation by dorsal injection of Axin

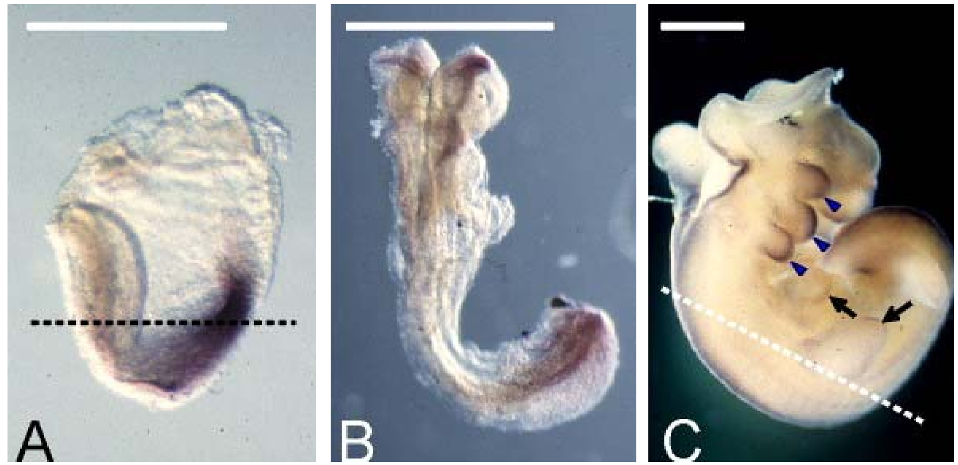
Similarity between Axin and Axin2 amino acid sequences



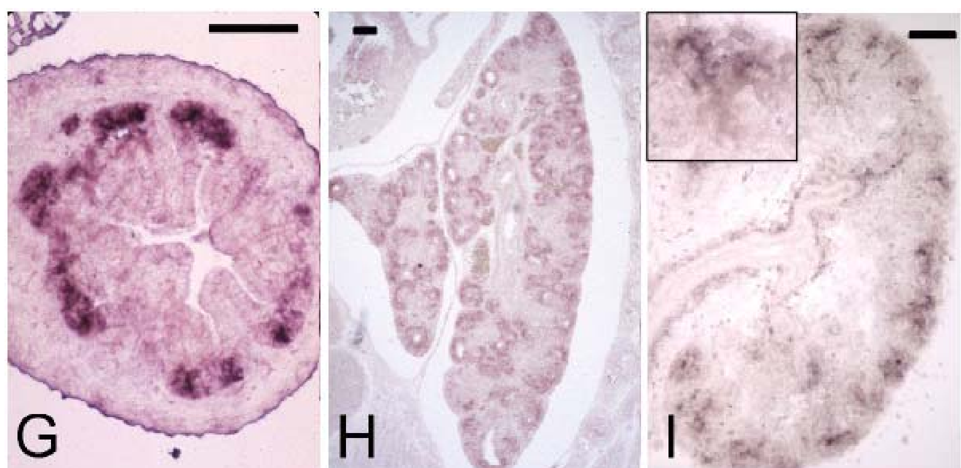
Overall similarity : ~54 %

Expression of Axin2 during Embryogenesis and Organogenesis

E7.5



E7.5 E8.5 E10.5



Gut Lung Kidney

E14.5

AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of *AXIN1*
Seiji Satoh *et al.*

Nature Genetics 24, 245 – 250 (2000)

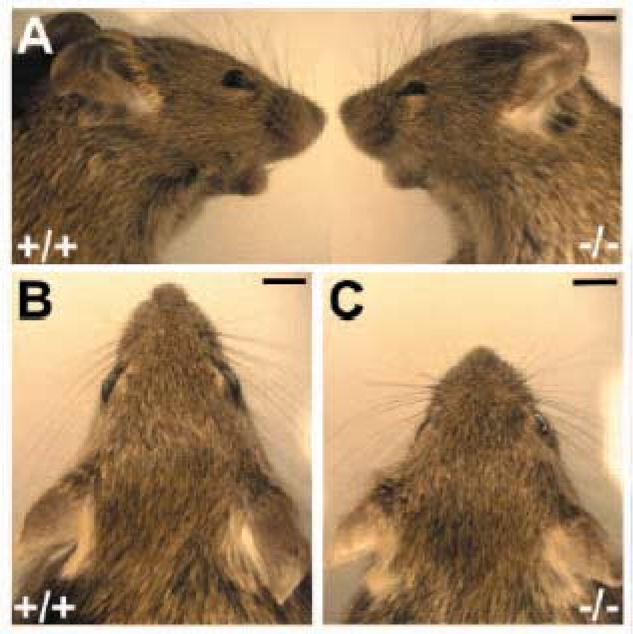
Mutations in *AXIN2* cause colorectal cancer with defective mismatch repair by activating b-catenin/TCF signalling
Wanguo Liu *et al.*

Nature Genetics 26, 146 – 147 (2000)

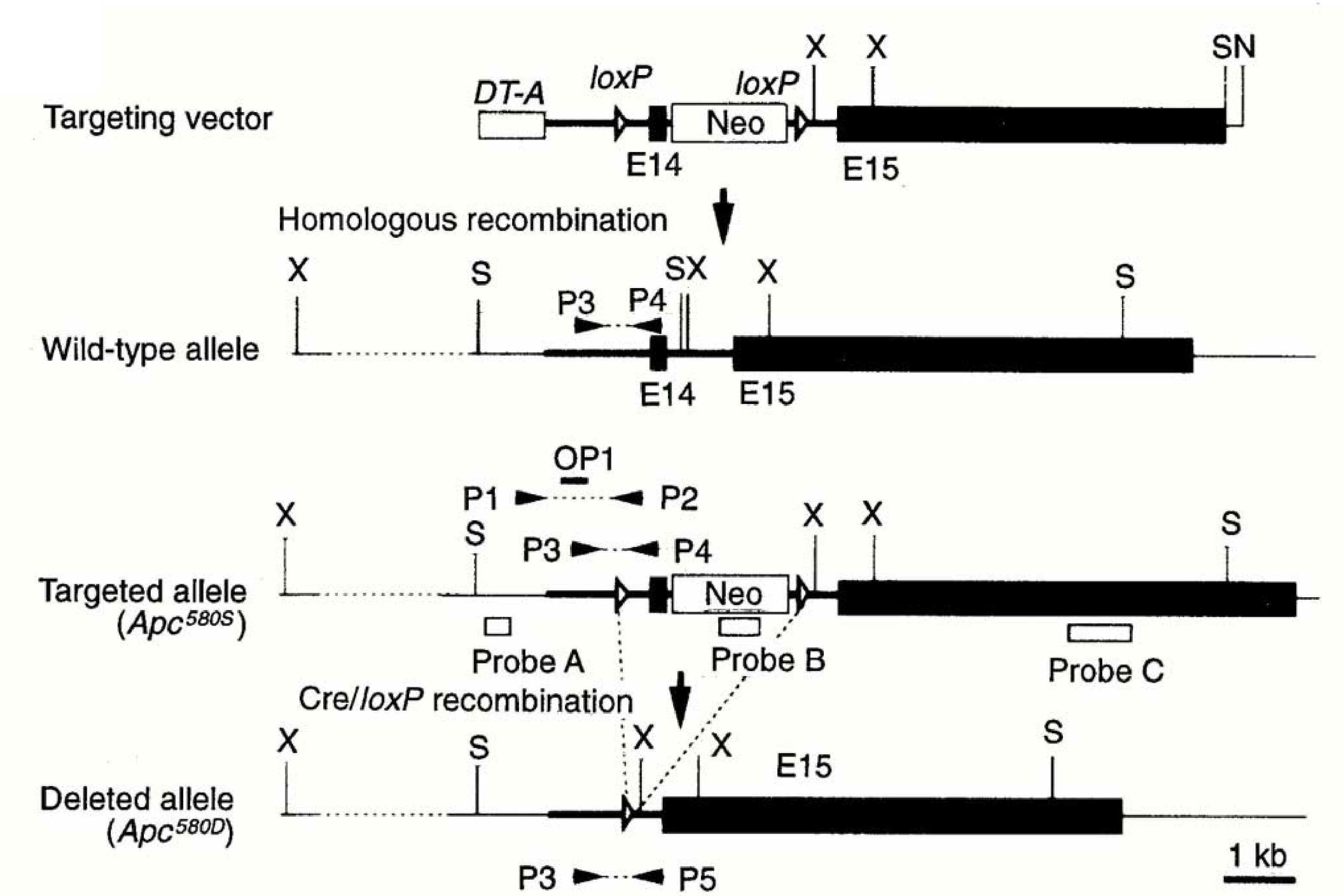
Axin vs. Axin 2

Chia IV and Costantini F (2005) Mouse axin and axin2/conductin proteins are functionally equivalent in vivo. Mol Cell Biol. Jun;25(11):4371-6.

Yu et al. (2005) The role of Axin2 in calvarial morphogenesis and craniosynostosis. Development. 132:1995-2005.

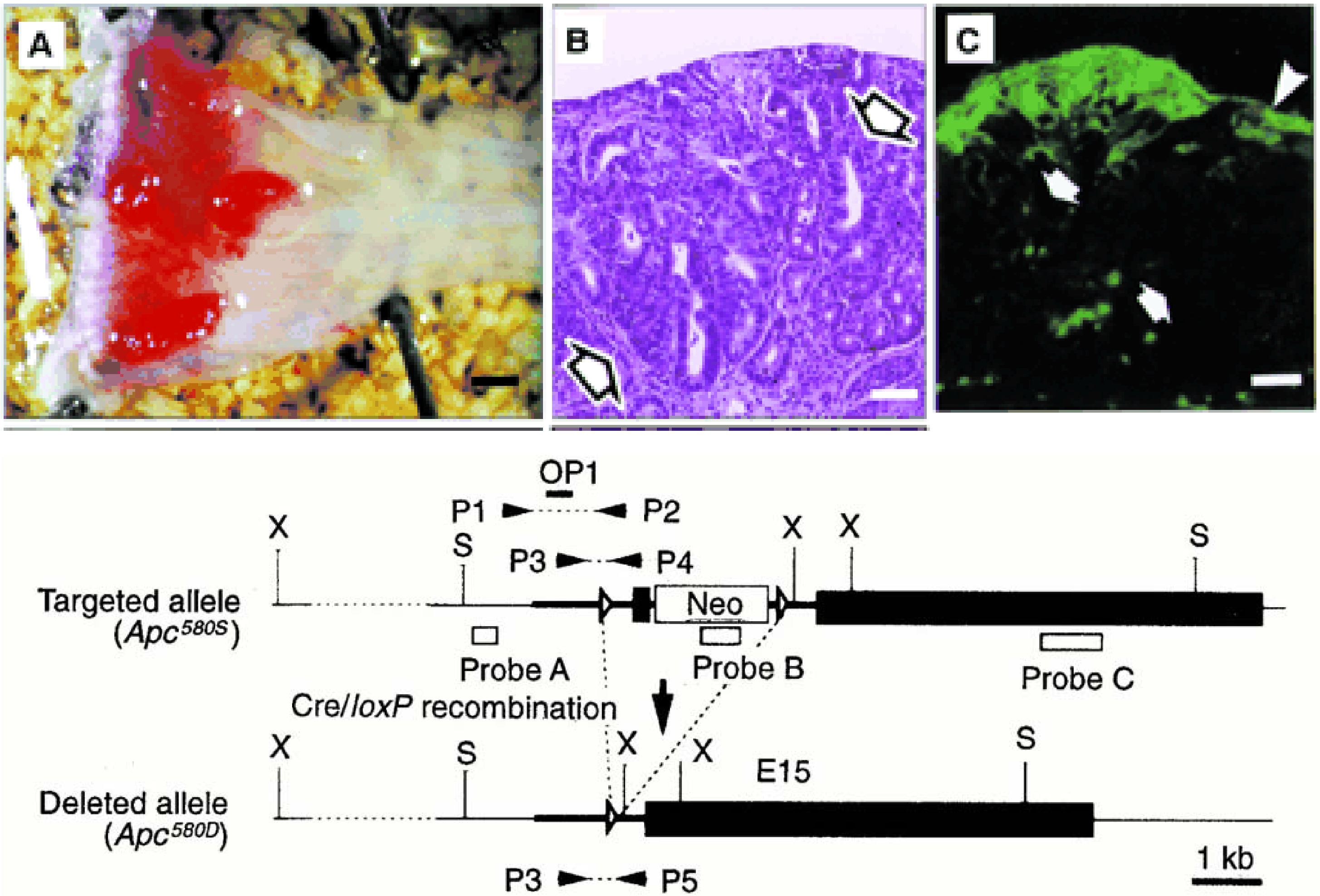


Cre/*loxP* conditional Knock-out of APC



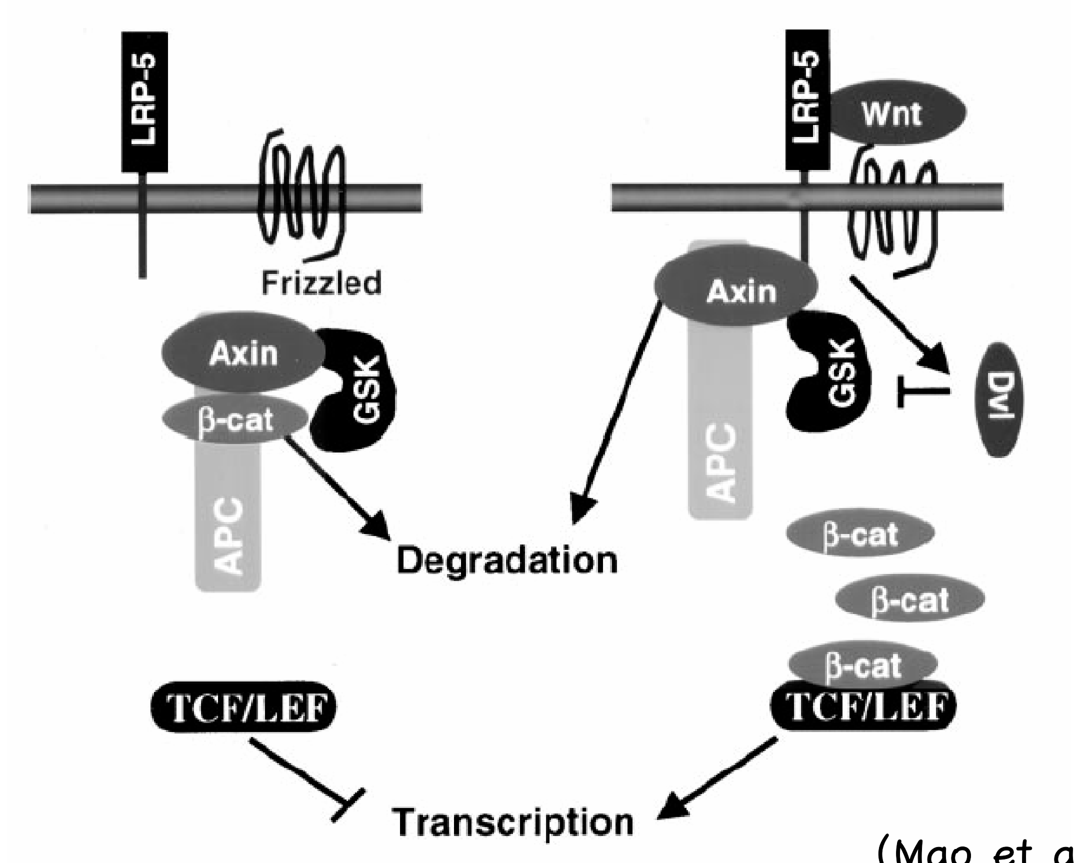
Shibata et al. (1997) *Science* 278:120-3.

Analysis of the colorectal region of mice infected with recombinant adenoviruses.



Shibata et al. (1997) *Science* 278:120-3.

Regulation of the level of Axin upon Wnt signal



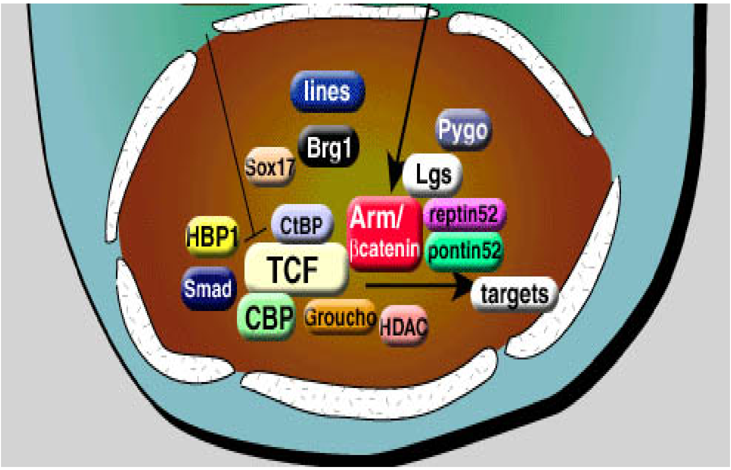
(Mao et al., 2001)

Rethinking WNT signaling.....

We propose that the control of Axin stability, rather than the control of GSK3b phosphorylation of b-catenin, is the key step in signaling.

Nobel Laureate, Eric Wieschaus TIG 2004

Nuclear (b-catenin - TCF)



b-catenin can convert TCF into a transcriptional activator of the same genes that are repressed by TCF alone (reviewed in Nusse, 1999).

In Drosophila, TCF interacts with CBP (Waltzer 1998.) repressing gene transcription when Wnt signaling is inactive. In mammalian cells, CBP/P300 can however behave as a co-activator of TCF-b-catenin (Hecht, 2000, Takemaru, 2000).

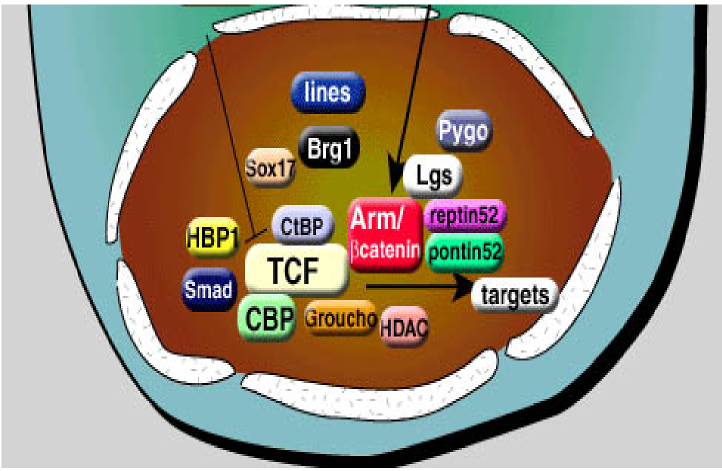
•In the nucleus, in the absence of the Wnt signal, TCF acts as a repressor of Wnt/Wg target genes (Brannon 1997, Bienz 1998).

CtBP acts as another co-repressor binding to TCF (Brannon, 1999) and so does the HMG box protein HBP1 (Sampson, 2001)

•TCF can form a complex with Groucho (Cavallo 1998). The repressing effect of Groucho is mediated by interactions with Histone Deacetylases (HDAC, Chen 1999).

b-catenin activity in the nucleus may also be regulated by interactions with other members of the HMG-box family (to which TCF belongs) including XSox17 (Zorn et al, 1999)

Nuclear (b-catenin - TCF)



•Tcf is antagonized by phosphorylation, and the protein kinase **Lit-1/Nemo/ NLK** is implicated in direct phosphorylation

•The kinase activity of Lit1/NLK/Nemo is stimulated by another kinase, **TAB1/TAK1** (or **MOM-4** in the worm). (Rocheleau .; Ishitani ; Meneghini, 1999

•Specificity of activation of target genes can be achieved by interaction with other factors, for example the **Smad4**. (Nishita et al, 2000).

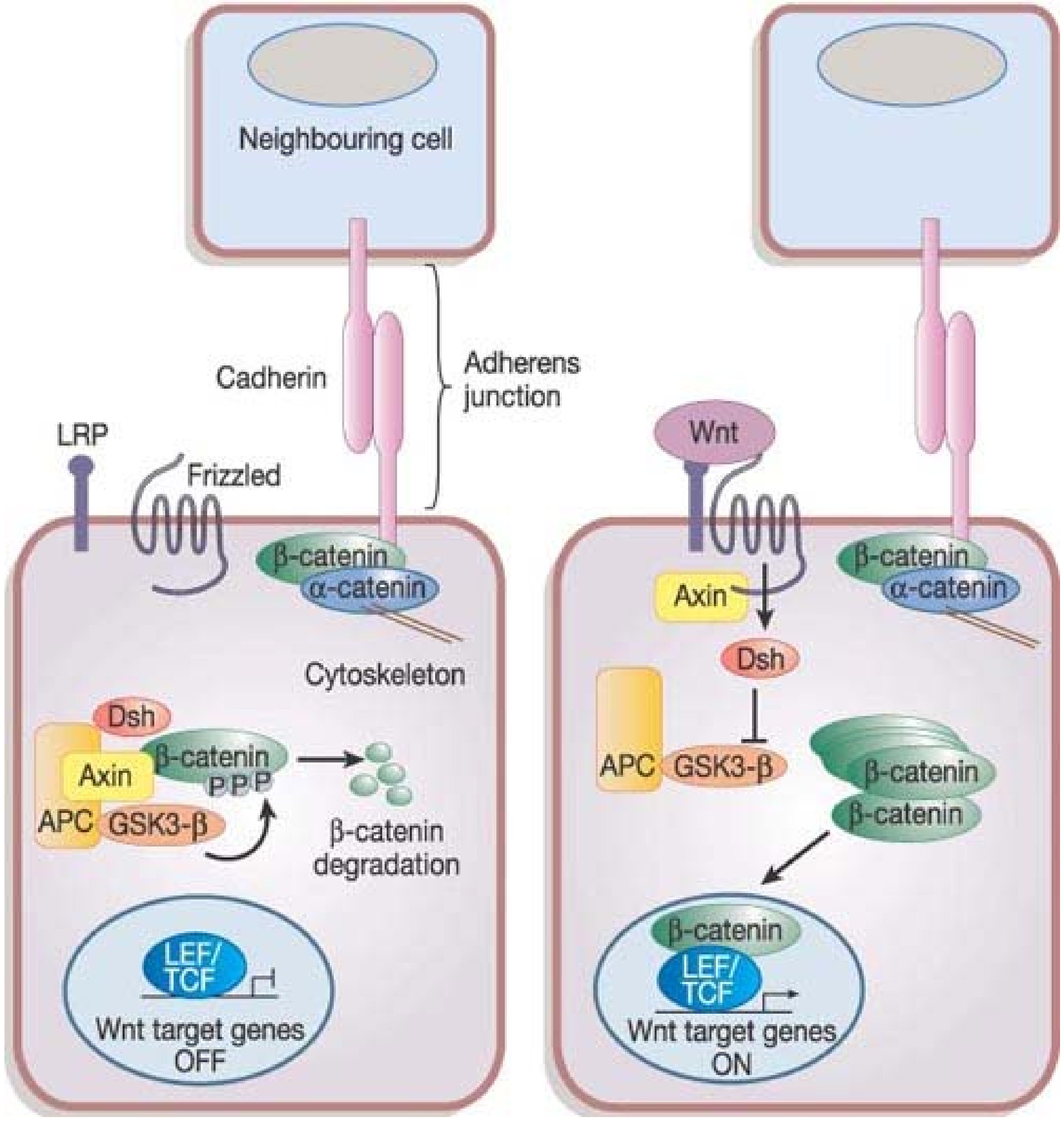
•**ICAT** is a b-catenin binding protein that inhibits b-catenin function (Tago et al, 2000)

•Two other key players in this complex are **Legless (Bcl9)** and **Pygopos** (Kramps 2002, Thompson 2002, Parker 2002)

•**Pontin52** and **Reptin52** are proteins that are related to each other and interact with b-catenin as antagonist (Bauer, EMBO, 2000).

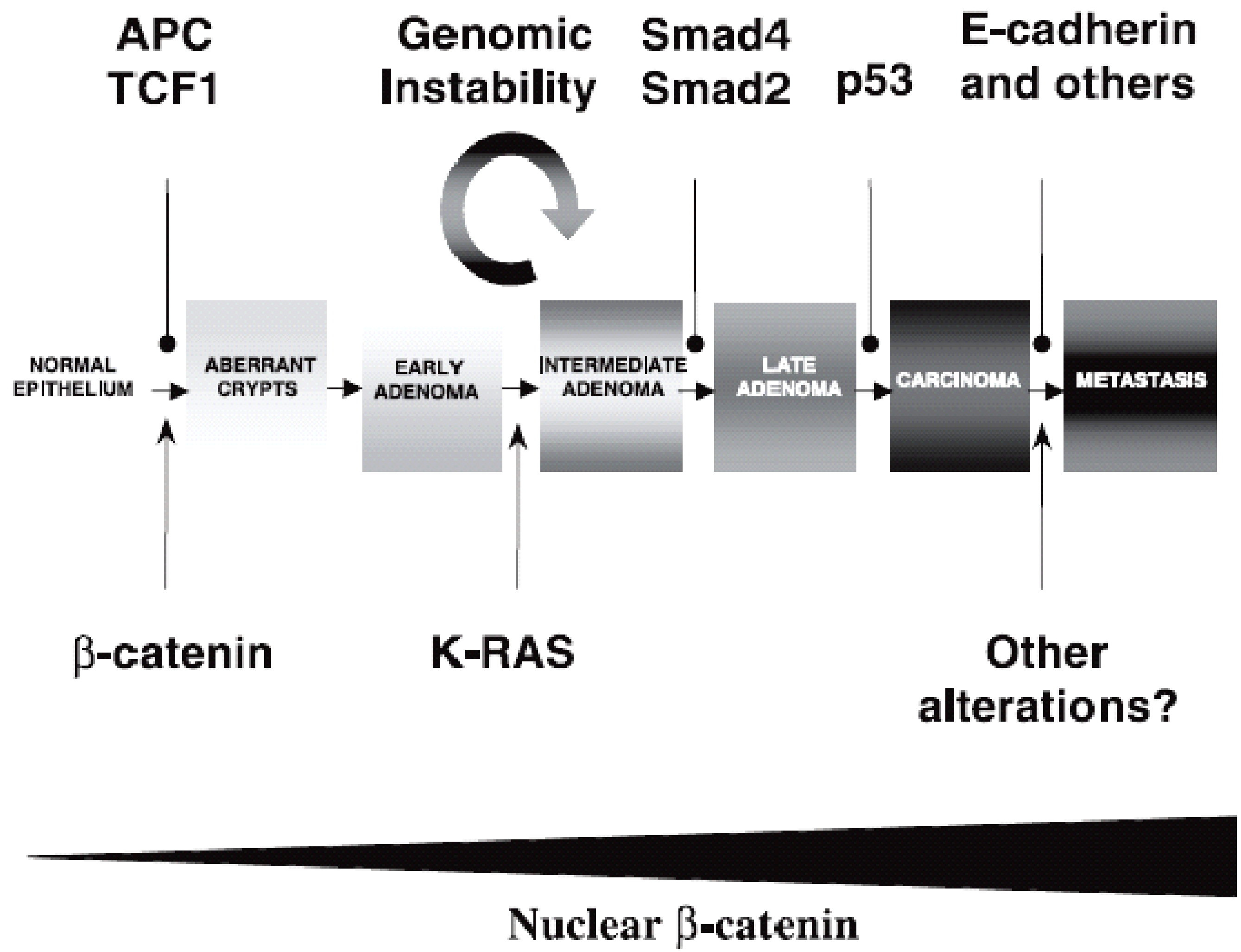
•**Brg-1** is a mammalian SWI/SNF and Rsc chromatin-remodelling complex protein binding to b-catenin and promoting activity (Barker, EMBO, 2001)

The canonical Wnt signaling pathway

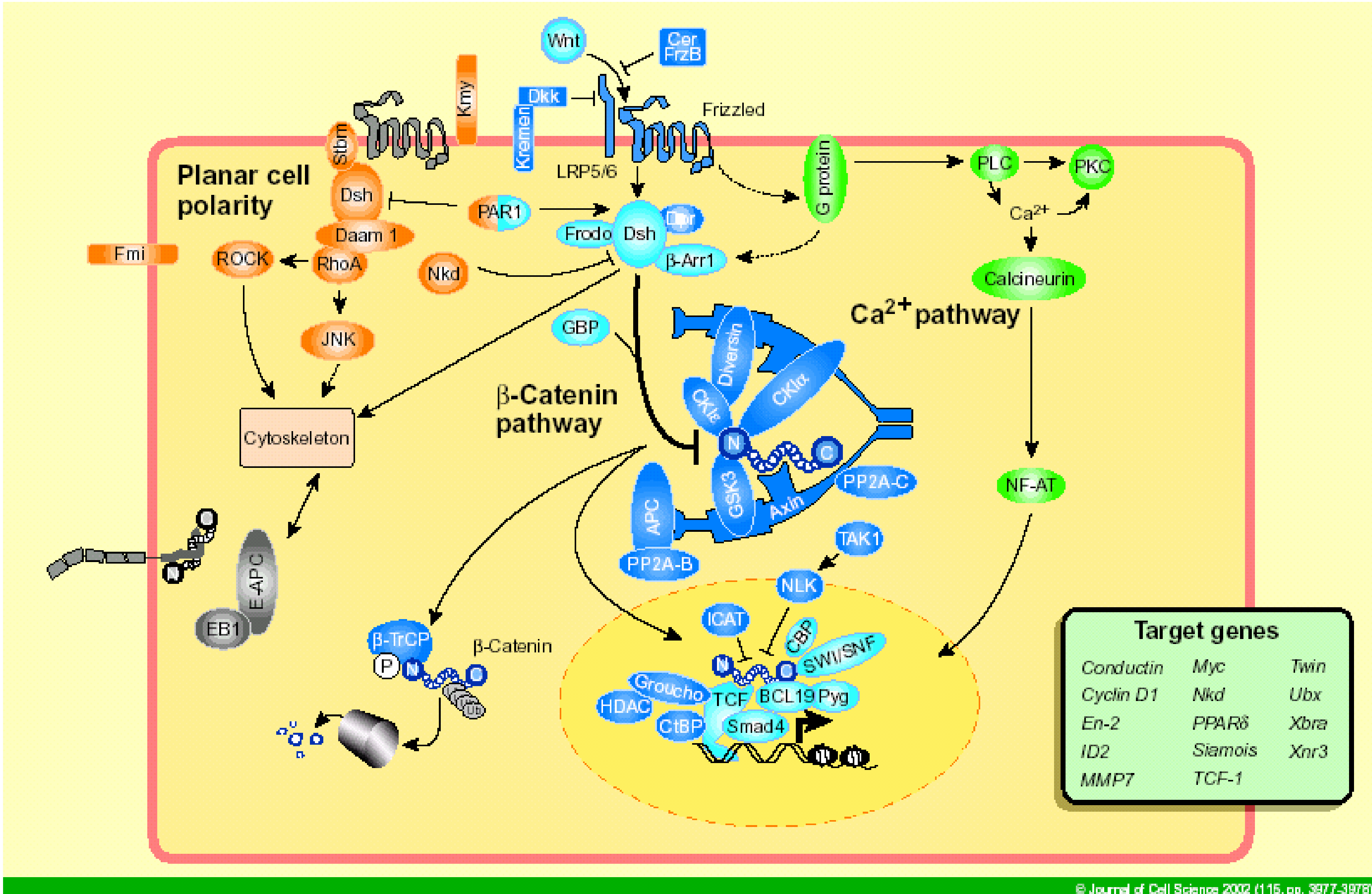


Reya and Clevers (2005) *Nature* 434: 843-850

Progression of Cancer Formation

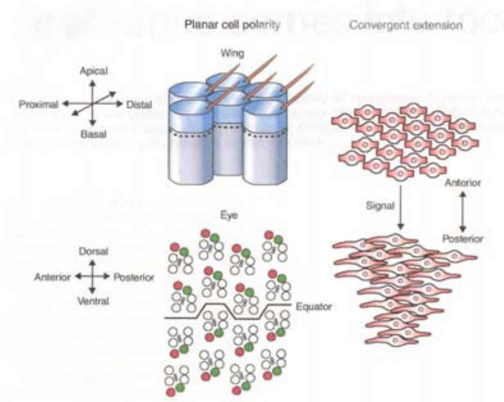


Wnt signaling pathway

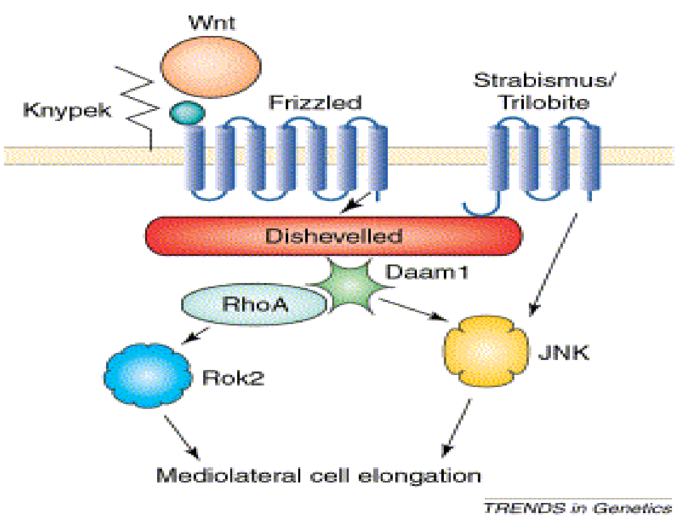


Joerg Huelsken and Juergen Behrens (2002) J. of Cell Science 115: 3977

Wnt/PCP pathway



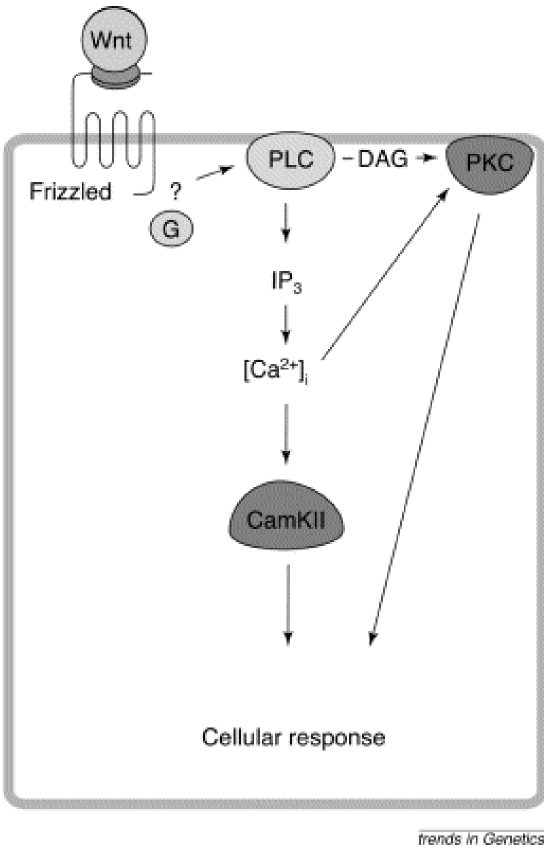
planer cell polarity
(Alexlrod,Nature, 2001)



(Myers et al, Triends in Genetics, 2002)

- In vertebrates, Wnt-mediated **Fz/Dsh** PCP signaling is essential for cell polarity and movement during gastrulation.
  (Sokol, Nat. cell biology, 2000).
- **vang/stbm** modulating convergent extention movement can activate JNK, but inhibit Wnt/b-catenin signaling.
  (Park et al, Nat. cell biology, 2002)
- Wnt/Fz signaling directly activates small G-protein **RhoA** via **Dsh** and **Daam1**
  (He et al, cell, 2001)
- Zebrafish **rho kinase 2** acts downstream of wnt11 to mediate cell polarity and effective convergence and extension movements.
  (Marlow et al, Curr Biol, 2002)
- JNK functions in the non-canonical Wnt pathway to regulate convergent extension movements in vertebrates.
- (Yamanaka et al, EMBO report, 2002)

Wnt/Ca^{2+} pathway



(Moon et al, TIG, 2000)

•Depending on ligand(Wnt), Wnt signaling stimulate CamKII and PKC via up-regulation of intracellular Ca2+ level.

Wnt 1 Group :Wnt 1,3A,8,8B

-induction of secondary axis

Wnt 5A Group : Wnt 4,5A,11

-alternation of cell movement and reduction of cell adhesion

•The signaling specificities are probably defined the receptor with which the Wnts interact

(RFz-1,mFz-7,-8, XFz-1,DFz,Dfz-2 : b-catenin
RFz-2,mFz-3,-4,-6, :PKC)

•CamkII is stimulated by Wnt/Fz homologue and promote ventral cell fate in Xenopus.

(Kuhl et al, JBC, 2000)

•Wnt/calcium pathway activates NF-AT and promotes ventral cell fate in Xenopus embryos

(Saneyshl et al, nature,2002)

Wnt/Ca2+ pathway ? Wnt/b-catenin pathway

Wnt signaling & Development 1

Canonical Wnt signaling components play important roles in multiple cell fate decision

• **Body axis formation and mesodermal patterning** in Xonepus, Zebrafish and mouse

-overexpressed b-catenin :axis-duplication

-knock-out b-catenin : no mesoderm and head structure

(Moon et al, Bioessays,1998 and Huelsen et al, JCB, 2000)

•**Brain & CNS development**

-Inhibition of Wnt signaling is crucial at later stages of bodyplan formation in vertebrates.

- ctopic expression of Wnt antagonists (FrzB-1, Dkk-1,Cerberus) promotes **head formation** (J Cell Biol, 1997)

-Wnt signaling is important in **neural development** and maintenance (*Development*, 2000)

•**Cardigenesis**

-Wnt signaling also plays a negative role in **heart formation** (Cell, 1999)

•**Limb development**

-Wnt signaling plays essential roles **in limb** initiation and induction of **the apical ectodermal ridge(AER)** in vertebrates. (Cell, 2000)

Wnt signaling & Development 2

Wnt signaling and stem cell control.

•Wnt signaling pathway is implicated in the control of skin epithelial stem cell specification and Hair Follicle Cell Fate (cell, 2001,105: 533-545)

•Wnt signaling controls stem cell proliferation in the intestine.

(Nat Genet, 1998,19:379-383)

•Wnts may also play a major role in regulating hematopoietic cell fate.

Adipogenesis

synaptic modulation.

Wnt signaling & Cancer

- Colorectal cancer

: Activation of b-catenin Tcf signaling in colon cancer by mutation in b-catenin and APC (Morin et al, science, 1997)

- Melanoma

: Mutant b-catenin with a single amino acid substitution at the N-terminus was identified as a melanoma-specific antigen.(Robbine et al, , J Exp Med,1996)

- Prostate cancer

: b-catenin mutation in human prostate cancer.(Voeller et al, cancer Res.,1998)

- Hepatocellular carcinoma and hepatoblastomas

:b-catenin mutation in Hepatocellular carcinoma and hepatoblastomas (Miyoshi et al, cancer Res,1998)

- Uterine Endometrial carcinomas

:Mutations in the b-catenin gene were reported in 10 of 76 cases (13%) of endometrial carcinomas studied.(Fukuchi et al , Cancer Res, 1998)

- Medulloblastoma(brain tumor)

:germ line mutation of APC, small percentage of b-catenin and Axin sporadic mutation

- Ovarian cancer, Pilomatricomas(skin cancer)

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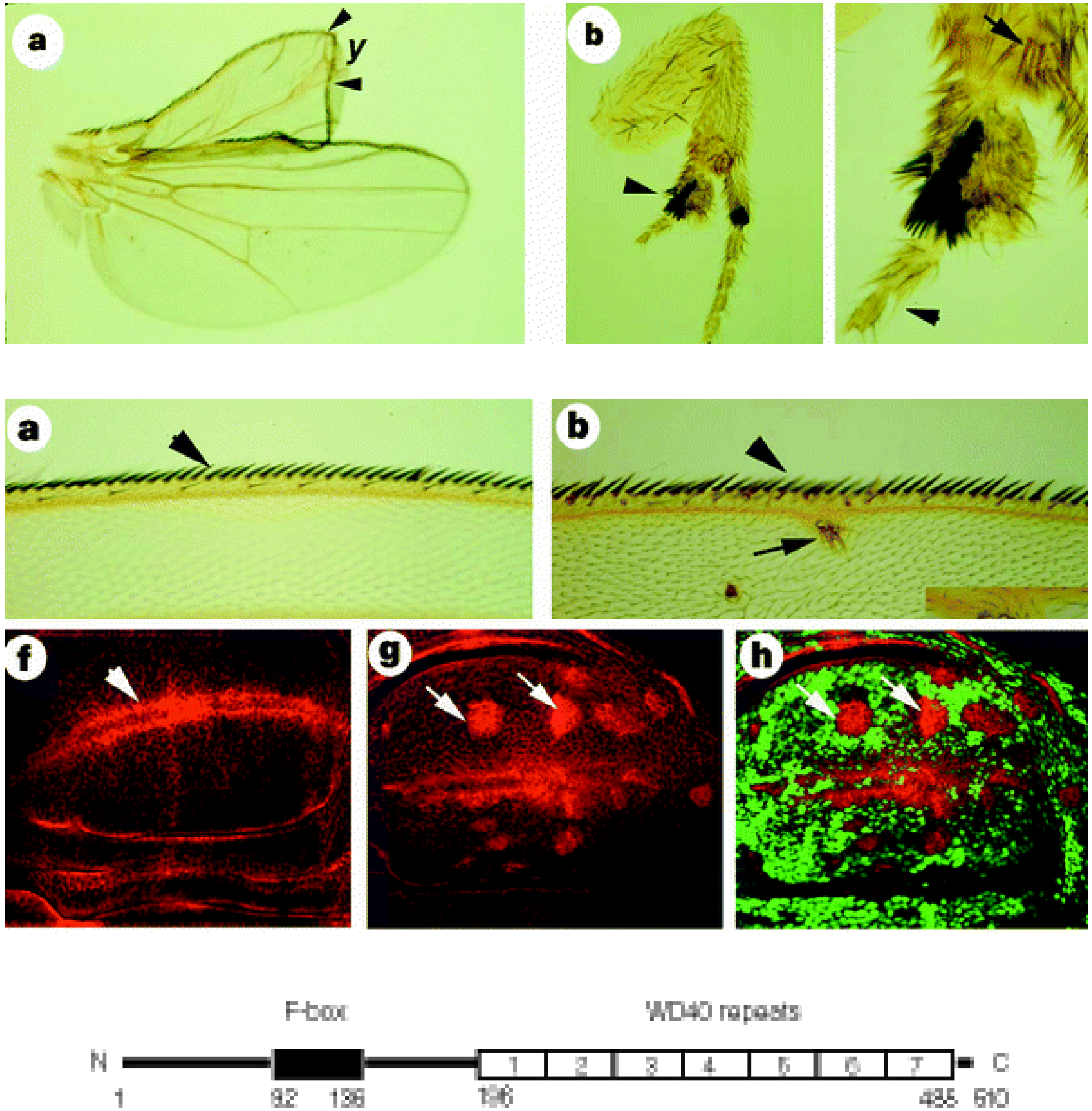
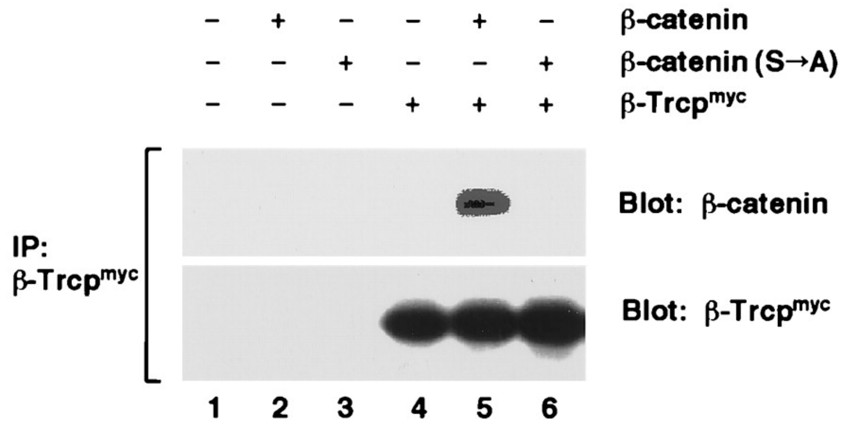
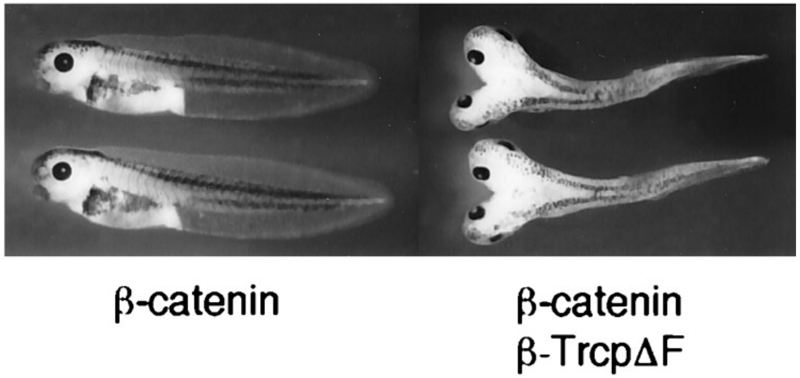
slimb (for supernumerary limbs, bTrCP)

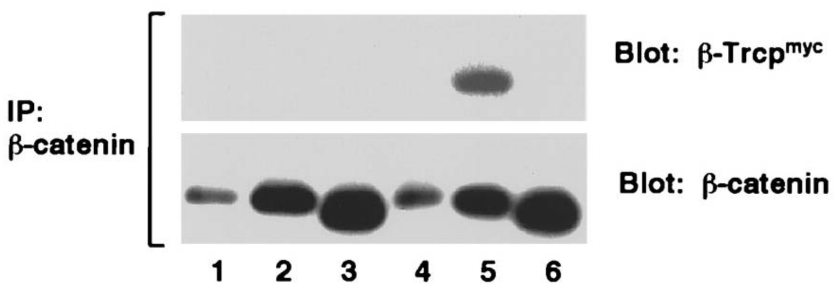


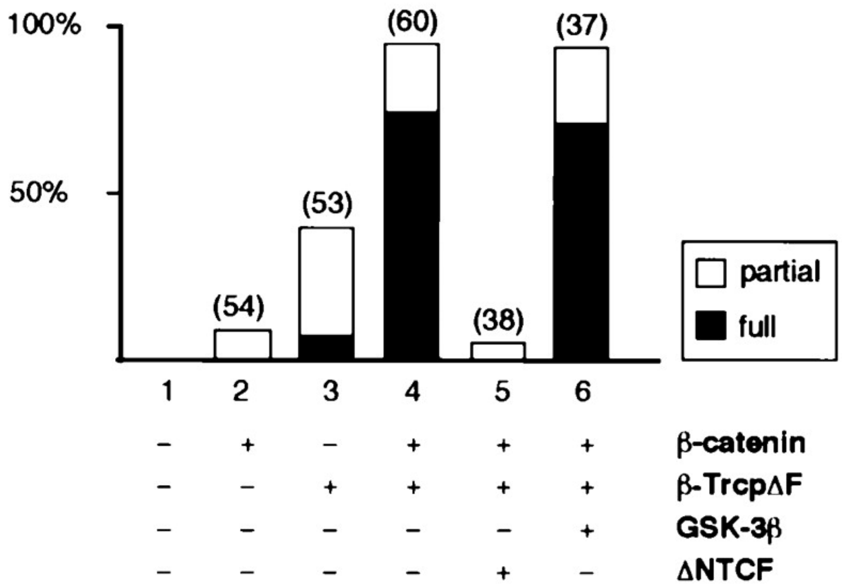
Jiang J and Struhl G. (1998) Nature 391:493-6.

b-Trcp couples beta-catenin phosphorylation-degradation and regulates Xenopus axis formation.









Liu et al. (1999) PNAS 96:6273-8.

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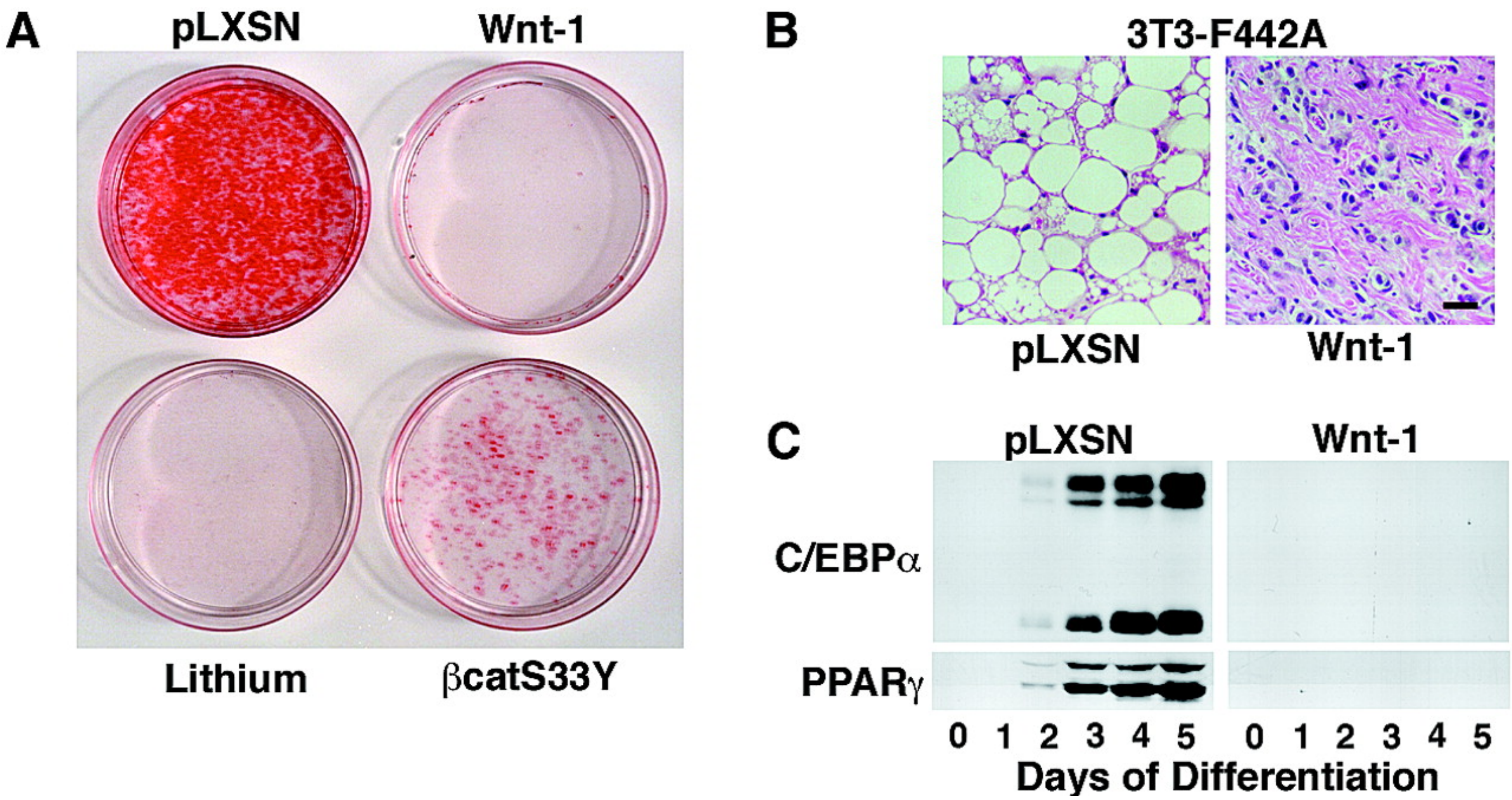
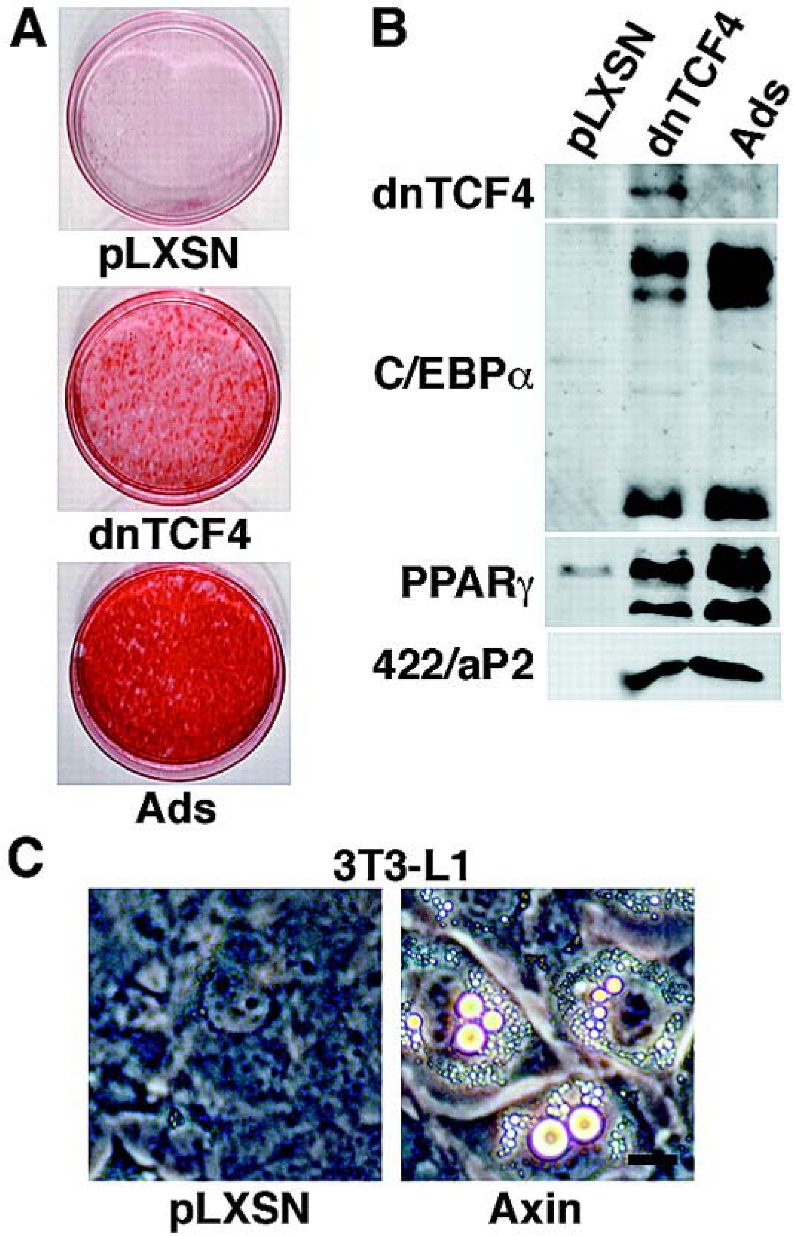
Inhibition of adipogenesis by Wnt signaling

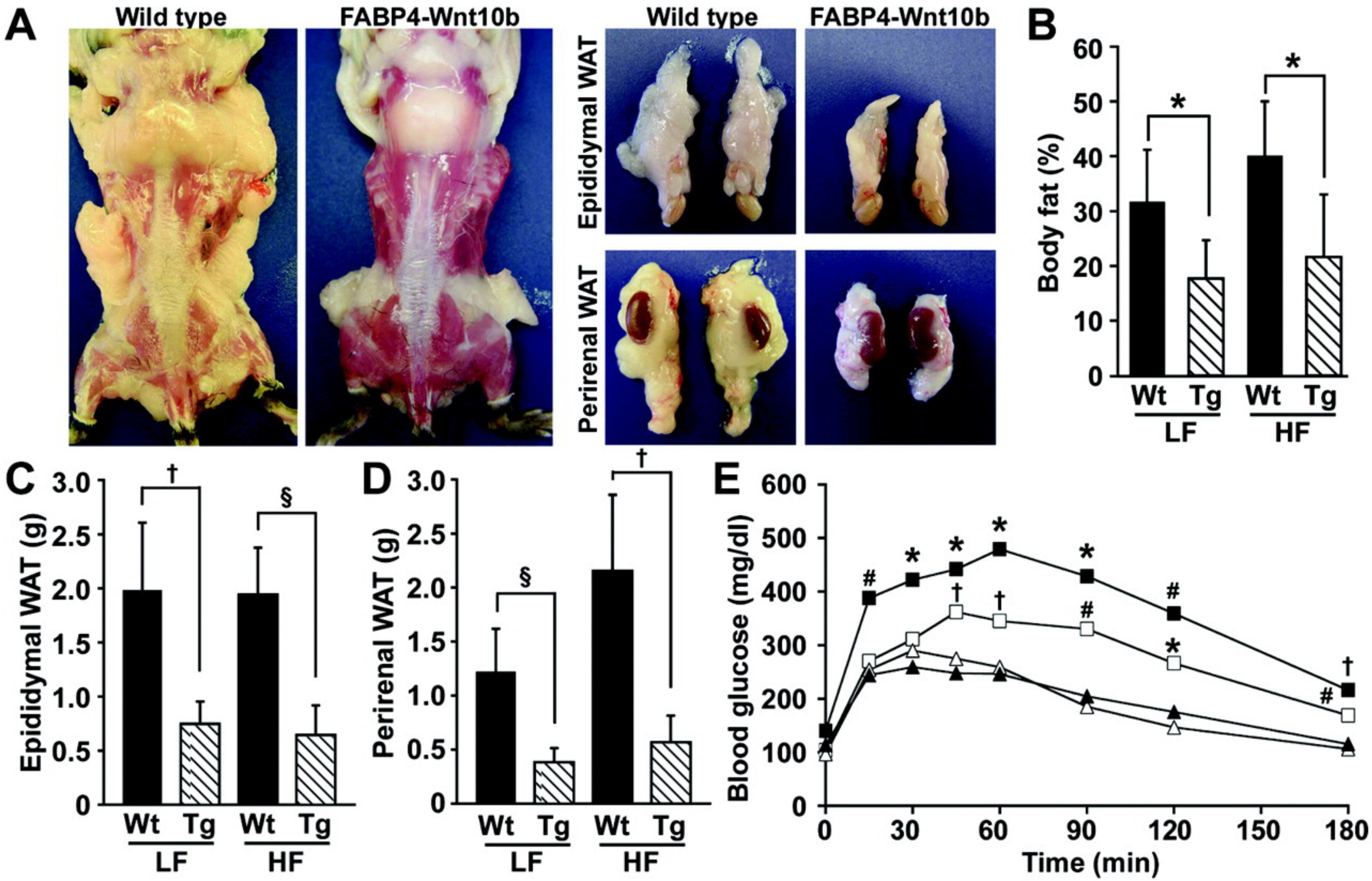


Ross et al. (2000) Science. 289:950-3.



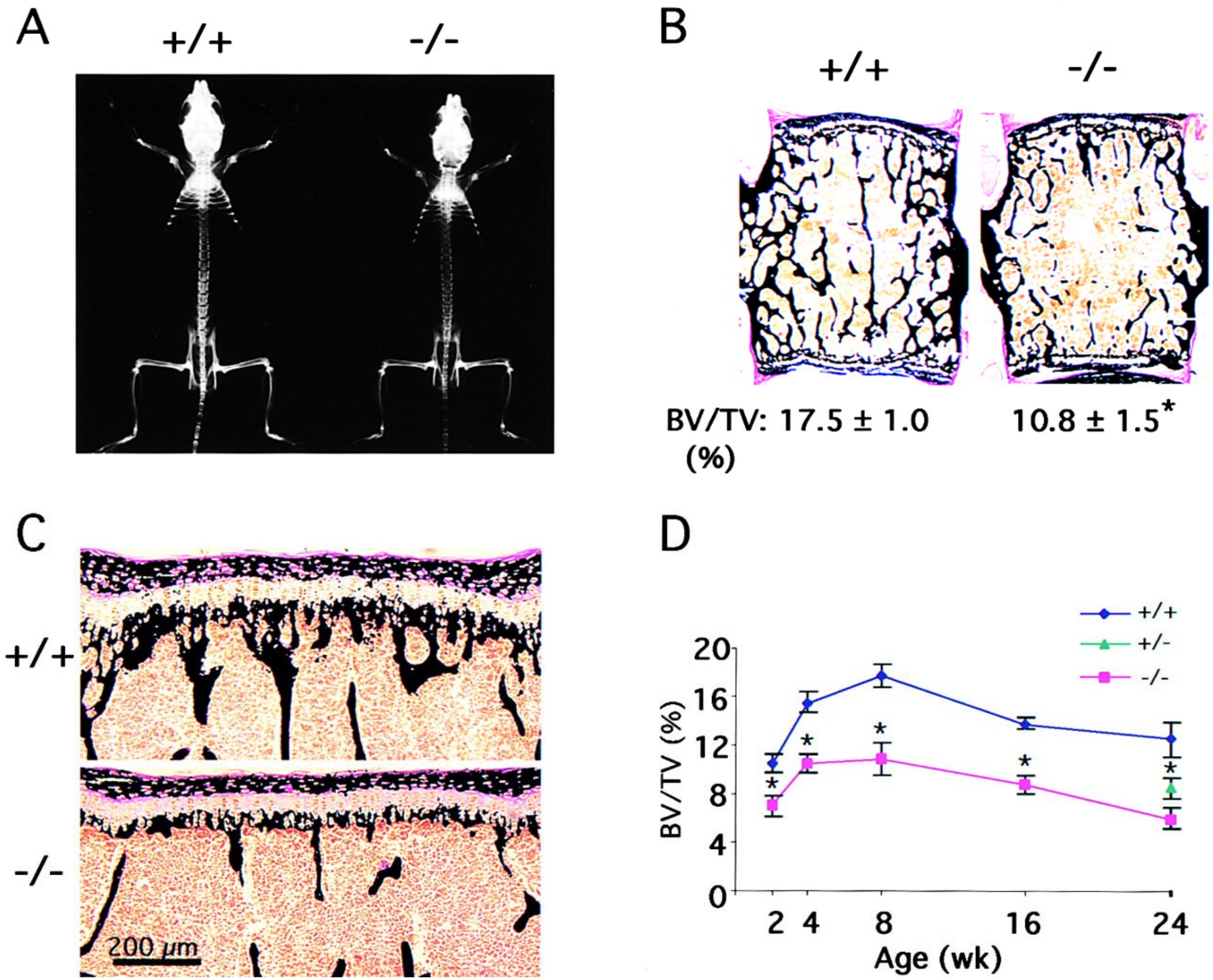
Ross et al. (2000) Science. 289:950-3.

Wnt10b inhibits body fat accumulation and improves glucose tolerance in FABP4-Wnt10b transgenic mice



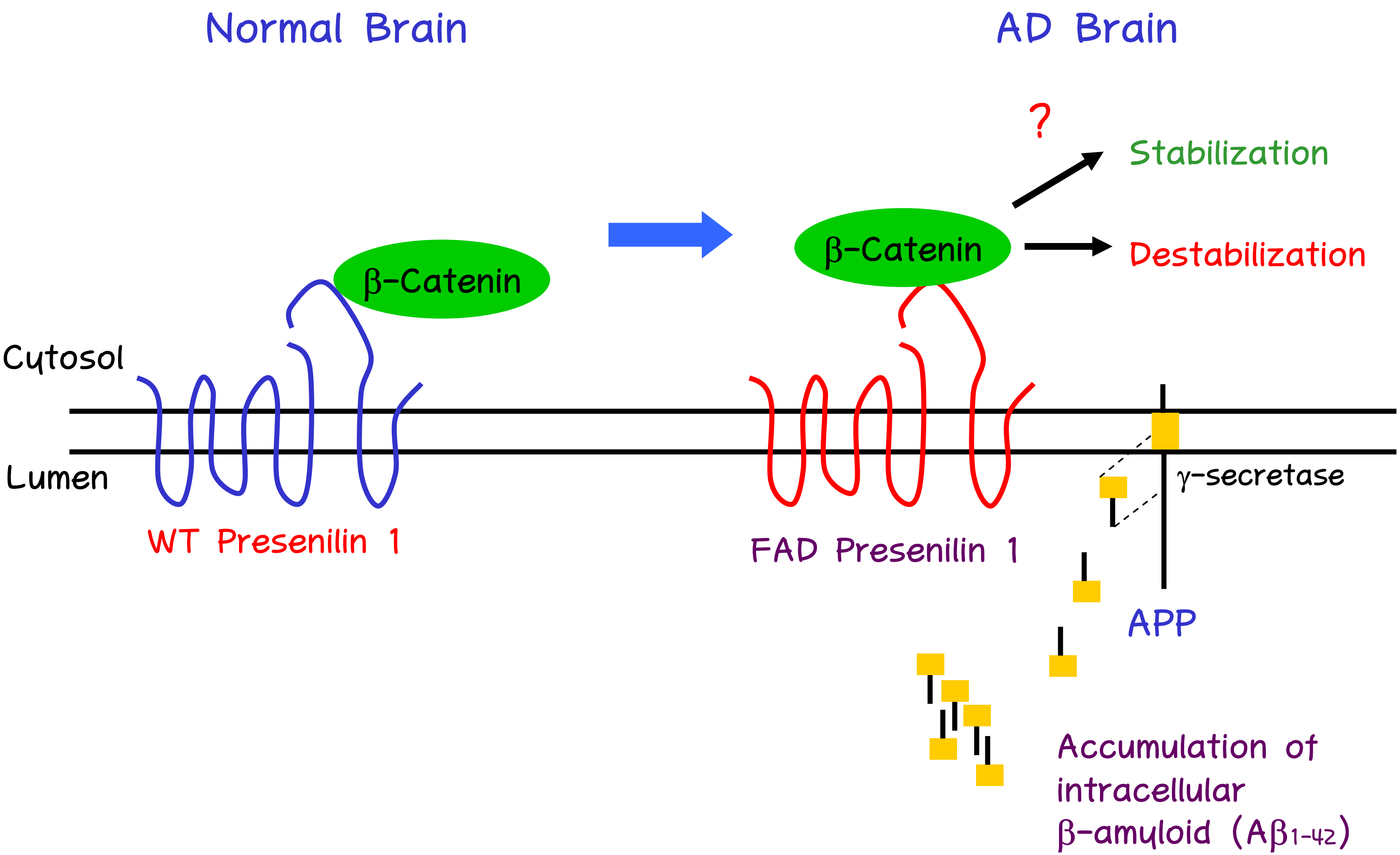
Kenneth et al. (2004) J. Biol. Chem., 279, 35503-35509

Low bone mass in *Lrp5-/-* mice.

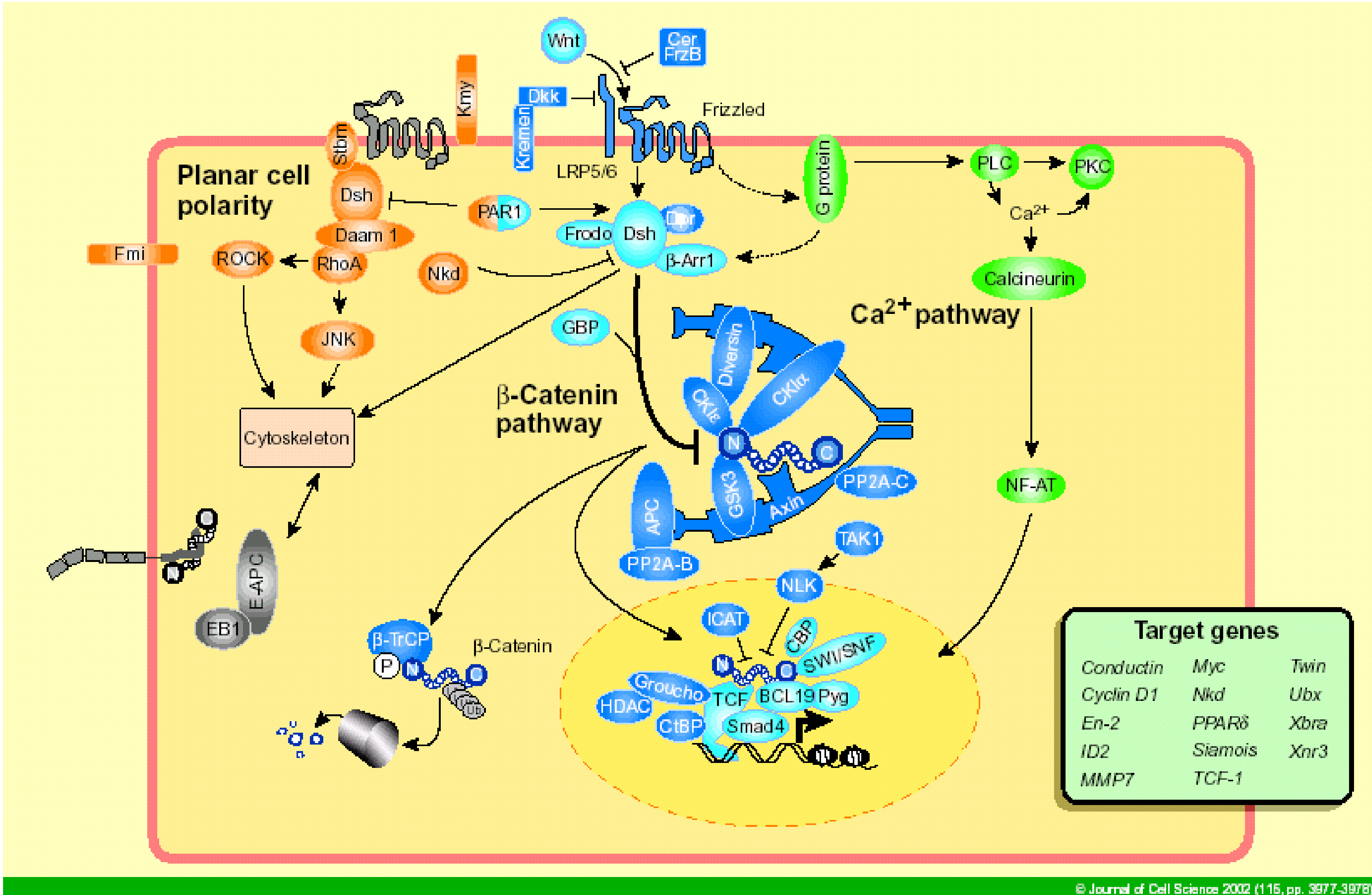


Kato et al. (2002) J Cell Biol., 157:303-14

Mis-regulation of Wnt signaling in the pathogenesis of Alzheimer's disease

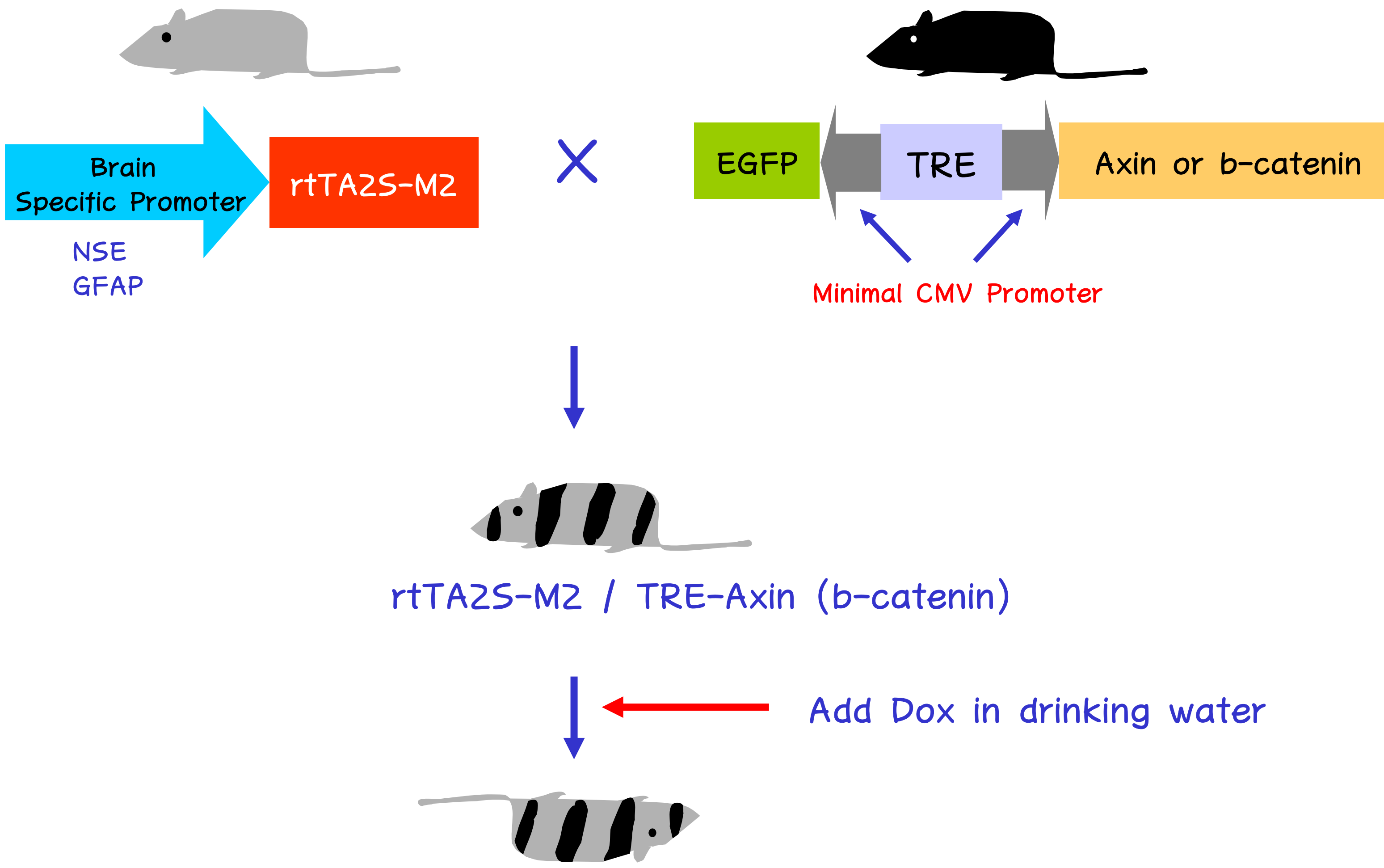


Wnt signaling pathway



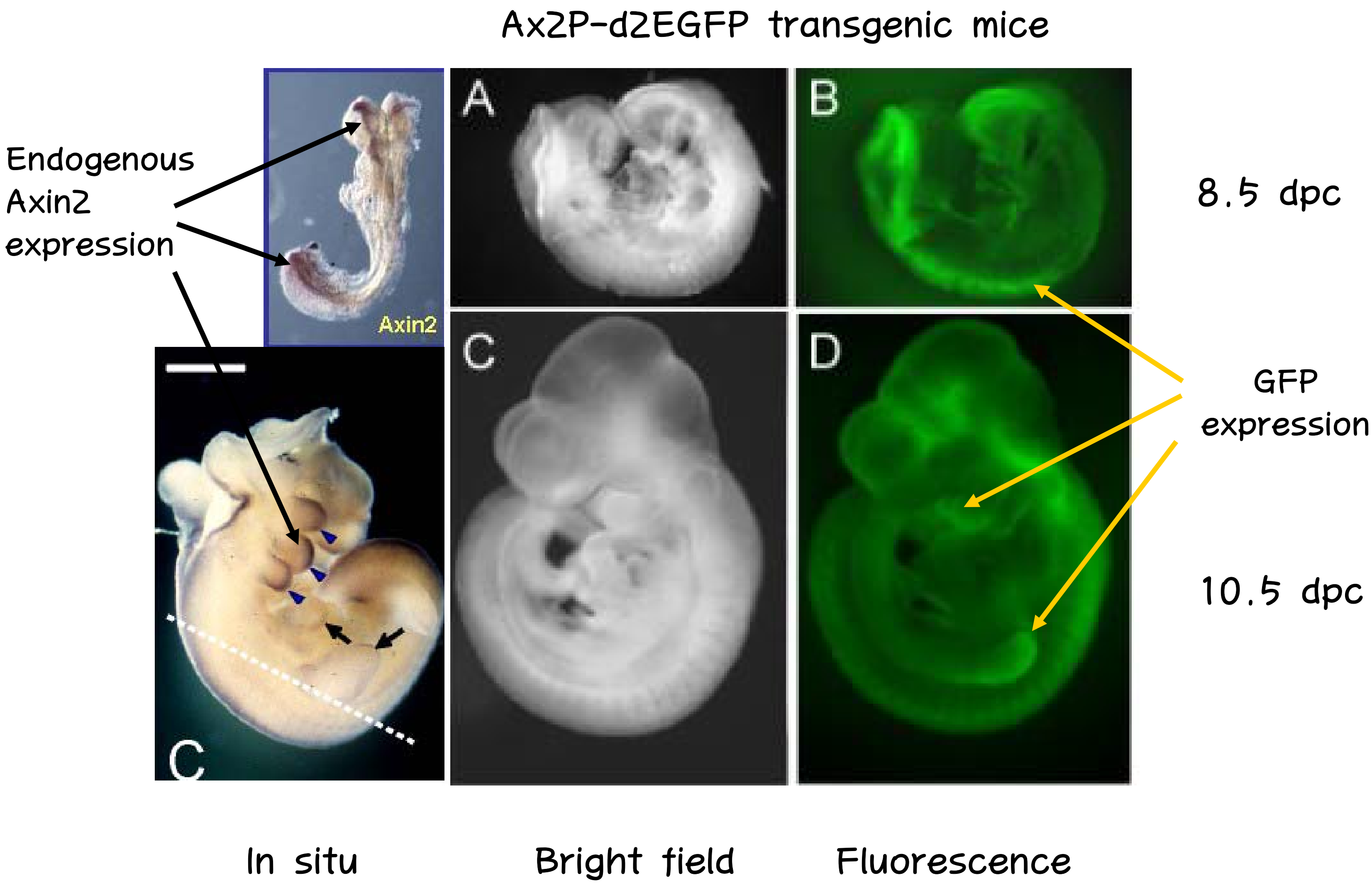
Joerg Huelsken and Juergen Behrens (2002) J. of Cell Science 115: 3977

Regulation of Axin or b-catenine level at neuronal or glial cells in transgenic mice



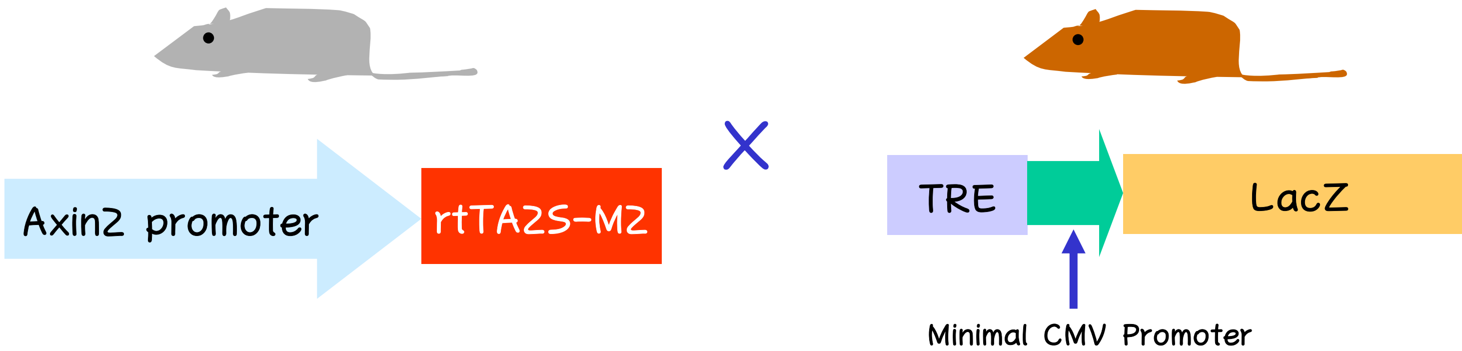
(Die due to defect in neuronal maturation, brain tumor or AD ?)

GFP expression by Axin2 promoter in Transgenic mice recapitulate endogenous Axin2 expression



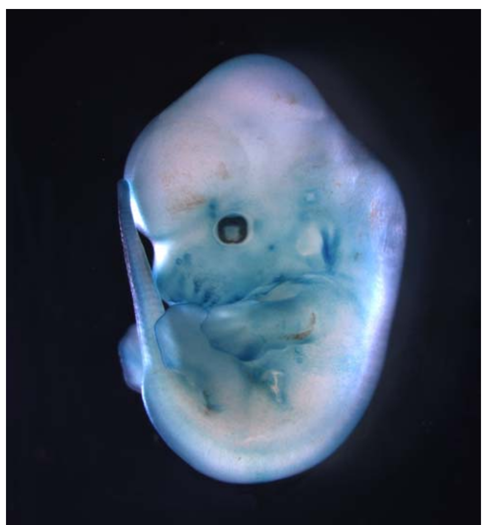
Jho et al. Mol. Cell. Biol. (2002)

Specific reporter gene expression by inducible system *in vivo*

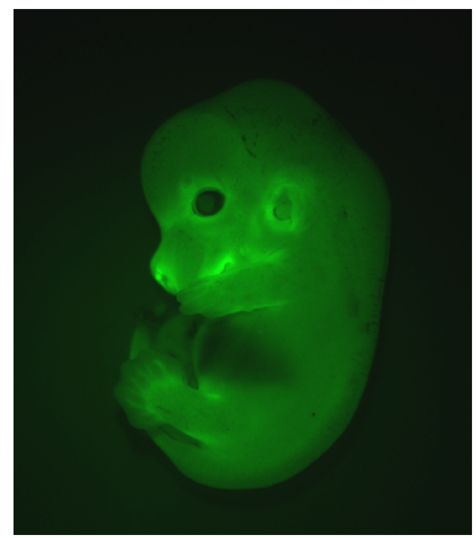


Add Dox after 8.5 days of pregnancy and isolate embryos in 13.5dpc

Ax2P-rtTA
+
TRE-LacZ



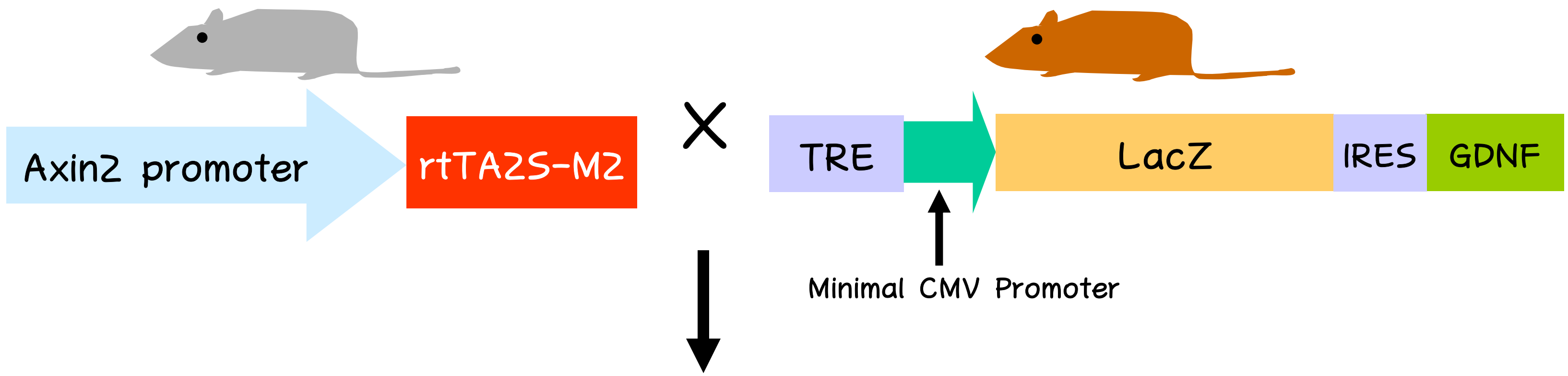
LacZ staining



Fluorescence

Ax2P-d2EGFP

Ax2P-rtTA driven expression of TRE-lacZ-GDNF in mouse embryonic kidney causes abnormal phenotype



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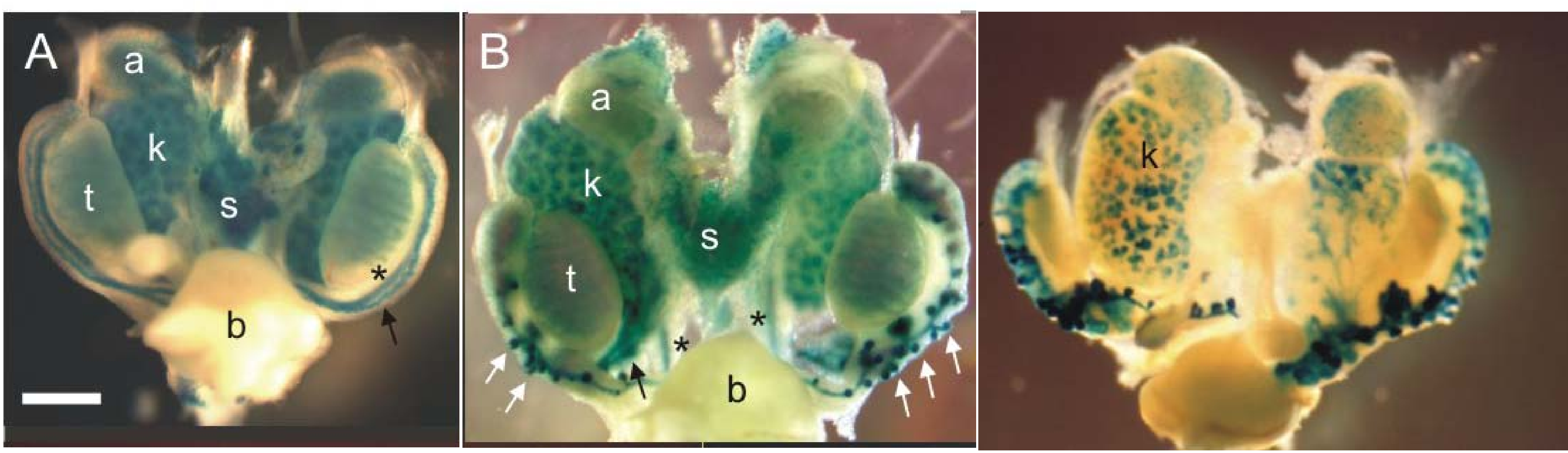
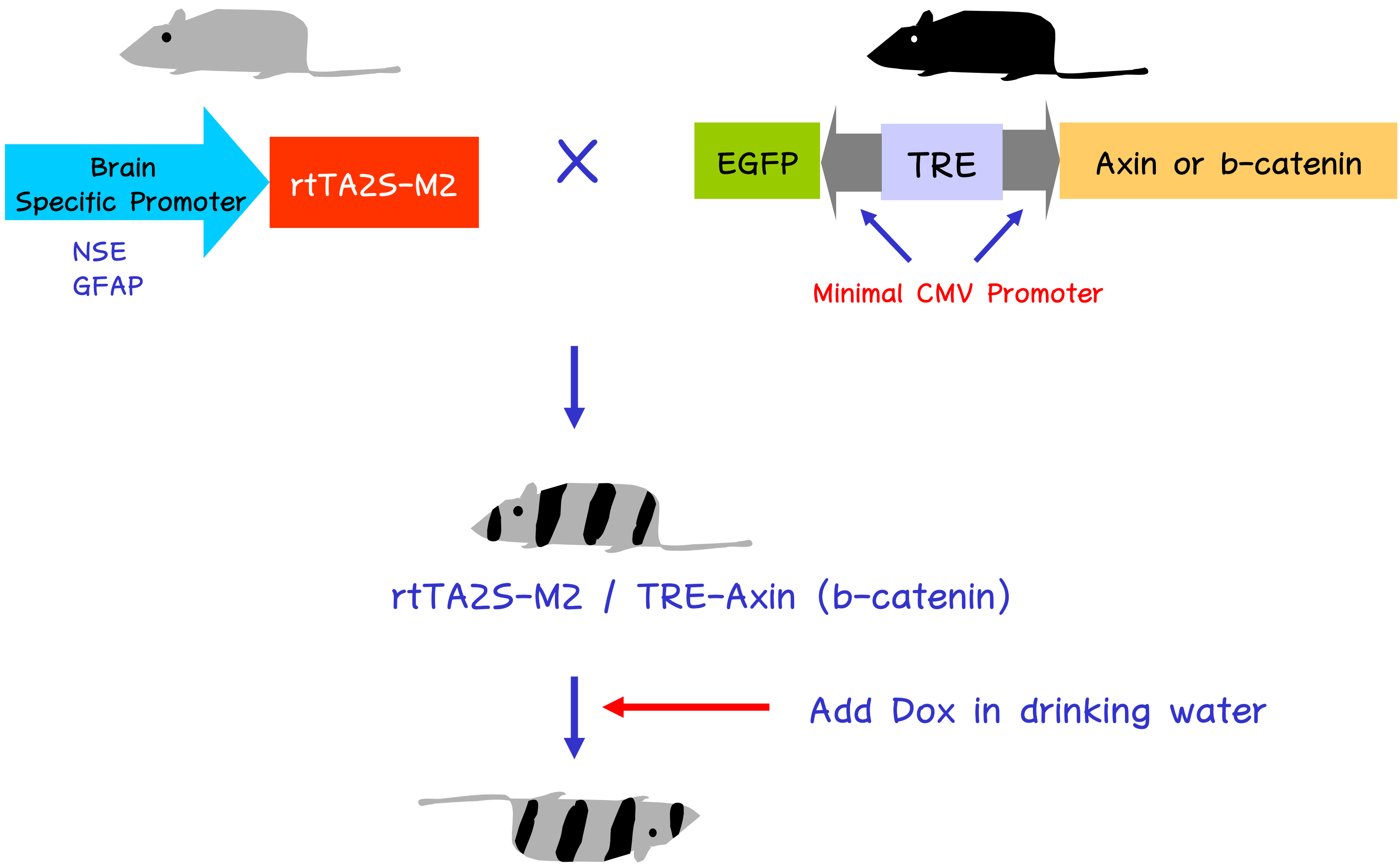


Shakya et al. 2005 *Dev Biol.* (in press)

Regulation of Axin or b-catenine level at neuronal or glial cells in transgenic mice



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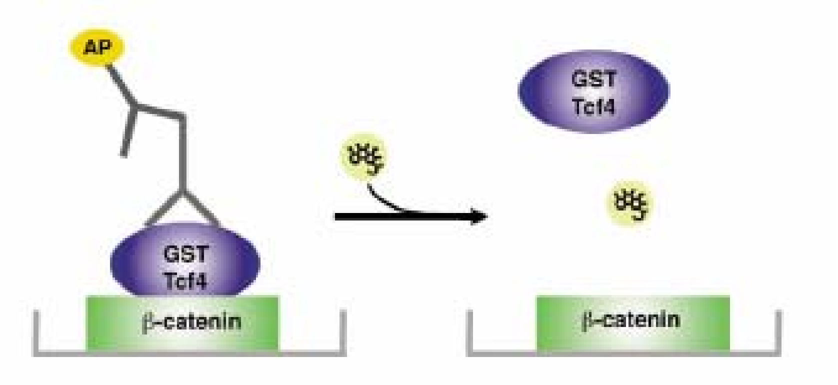
Antagonists of the Tcf4/ b-catenin association isolated in a high-throughput screen

1. b-catenin을 membrane에 coating 시키고,

2. bacteria에서 purify 한 GST-Tcf를 b-catenin에 binding 시킴.

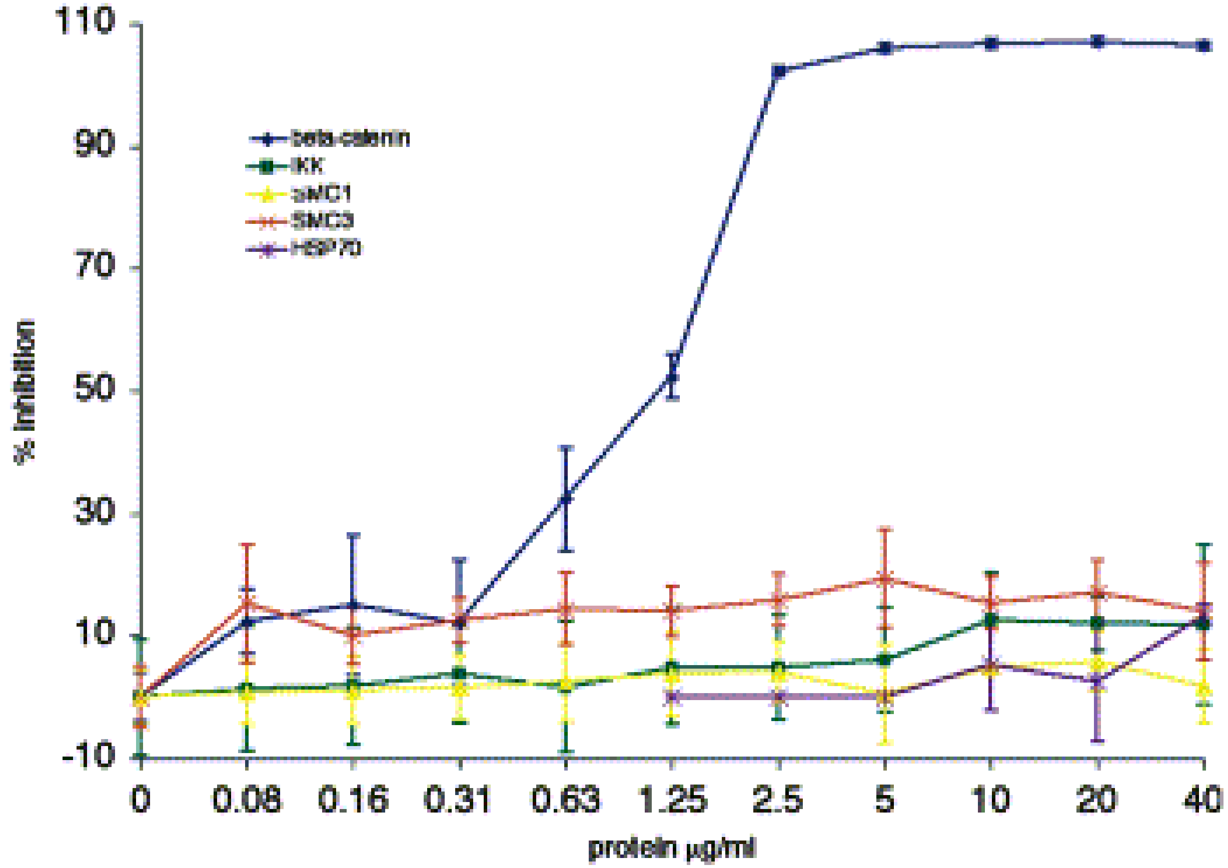
3. drug이 없는 상태에서는 AP conjugated GST antibody가 GST에 binding 하고, drug이 b-catenin과 Tcf의 interaction을 blocking할 경우는 antibody가 결합할 수 없게 된다.

4. 이때 AP의 substrate를 넣어 주어서 발색이 되지 않는 drug을 선택



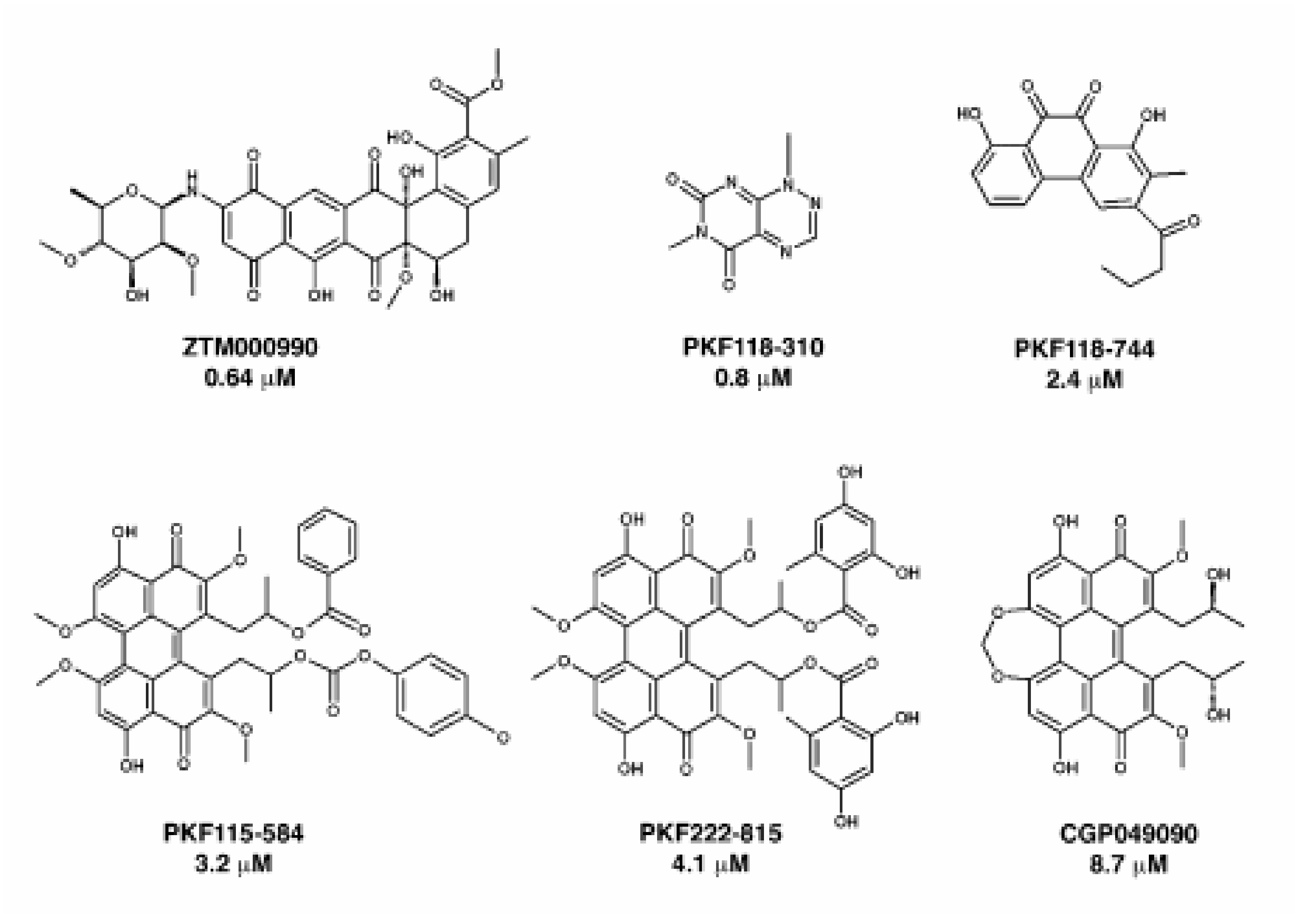
Lepourcelet et al. (2004) *Cancer Cell* 5,91-102

Inhibition of Tcf4/b-catenin association in the presence of various concentrations of recombinant fusion proteins



Lepourcelet et al. (2004) *Cancer Cell* 5,91-102

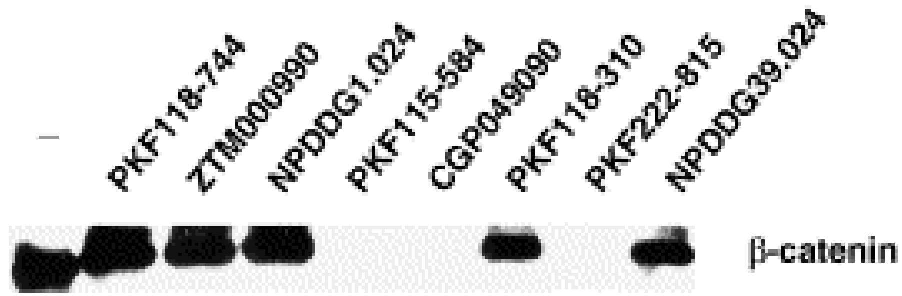
Chemical structures of inhibitors of the Tcf4/b-catenin interaction



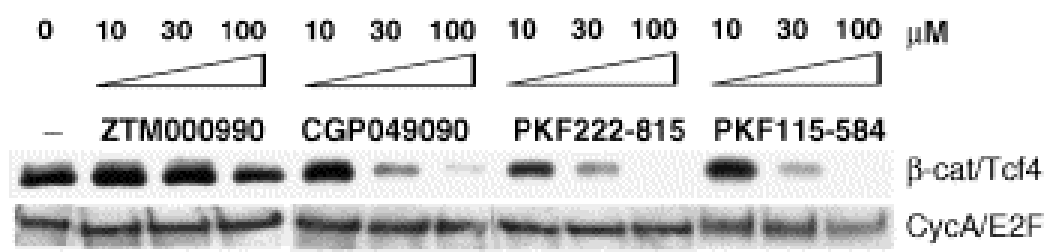
The activity of six compounds as the concentration (μM) of compound required to inhibit the Tcf4/ b-catenin association by 50% (IC_{50}).

Compounds isolated in the HTS inhibit Tcf4/ b-catenin interaction in independent assays

Precipitation of cellular b-catenin by a GST-tethered Tcf4 fragment.



Inhibition of the interaction with GST-Tcf4 is dose dependent.



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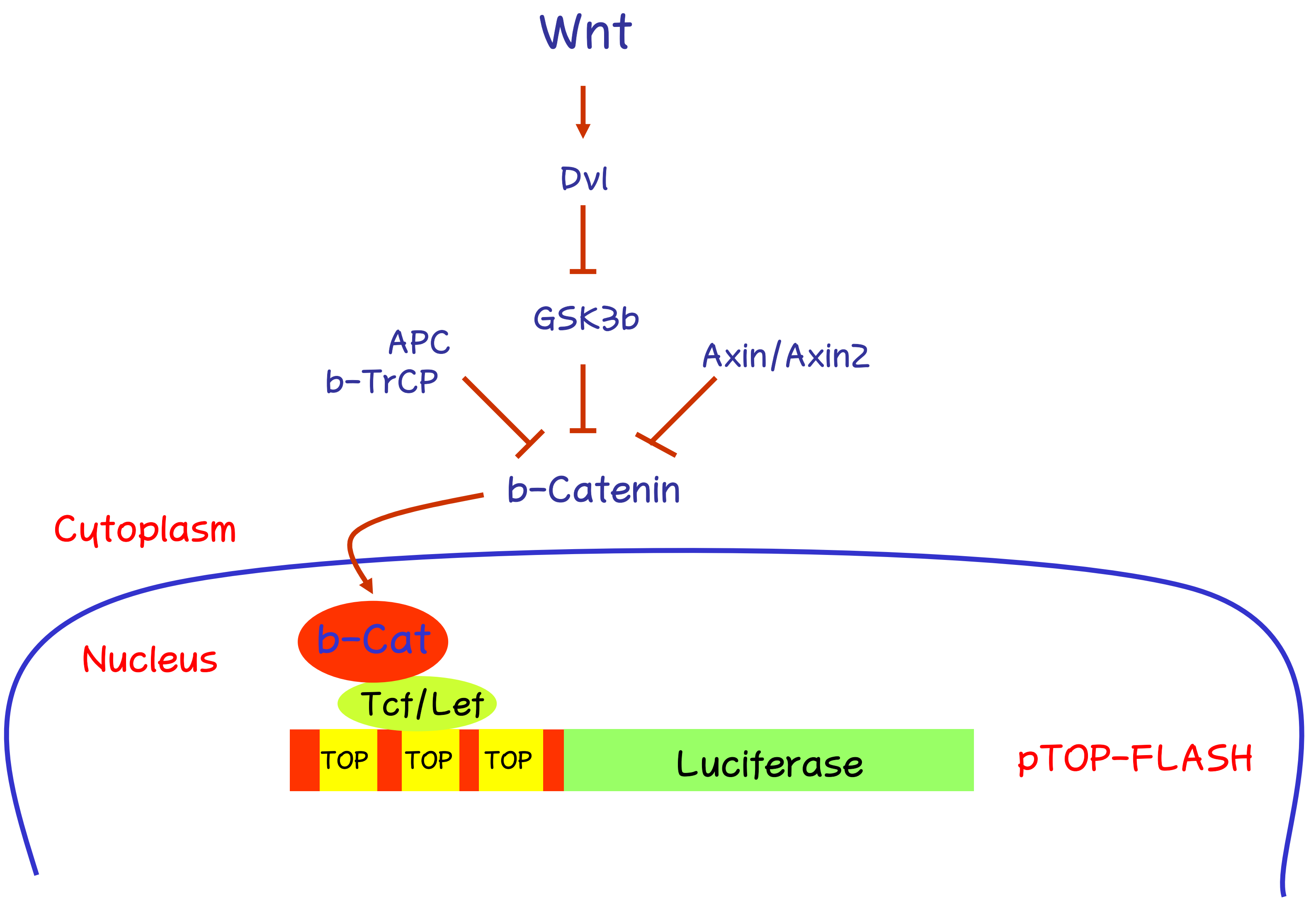
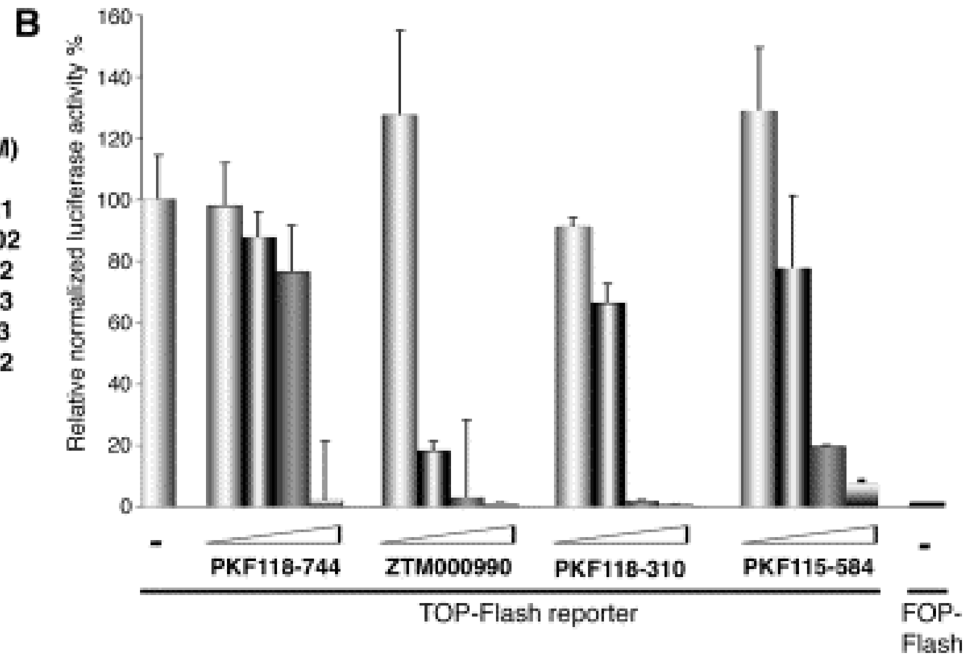


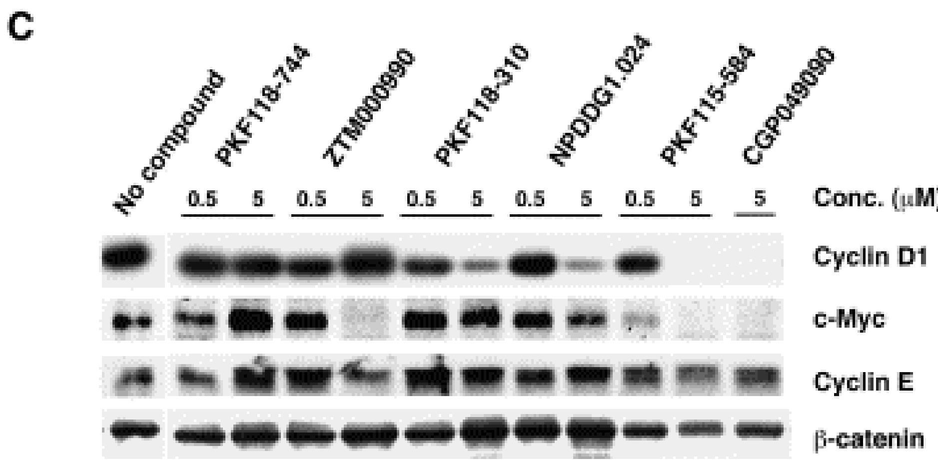
Wnt
Dvl
GSK3b
APC
b-TrCP
Axin/Axin2
b-Catenin
Cytoplasm
Nucleus
b-Cat
Tcf/Lef
TOP
TOP
TOP
Luciferase
pTOP-FLASH

Inhibition of biological markers of Tcf4/ b-catenin transactivation

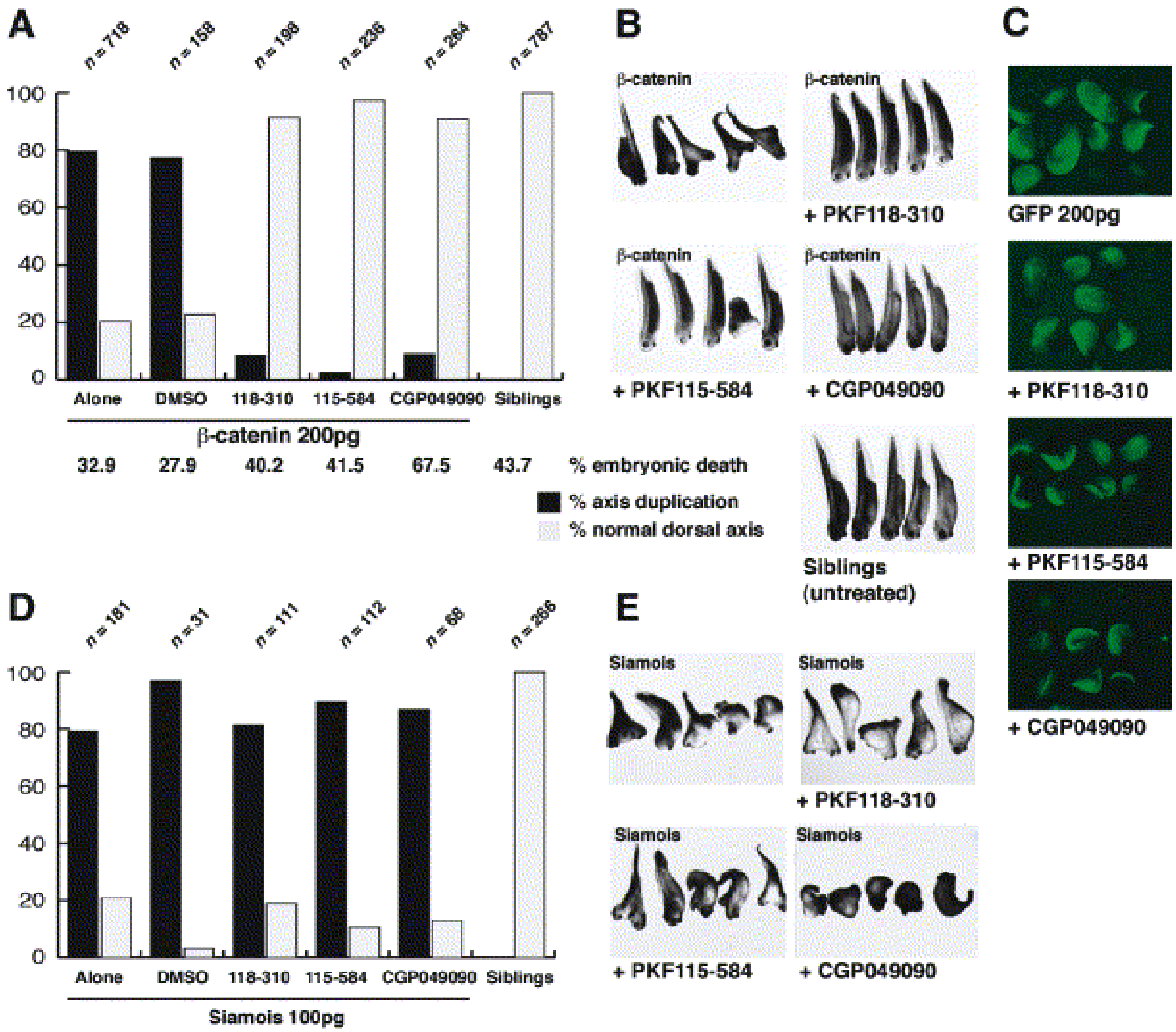
A

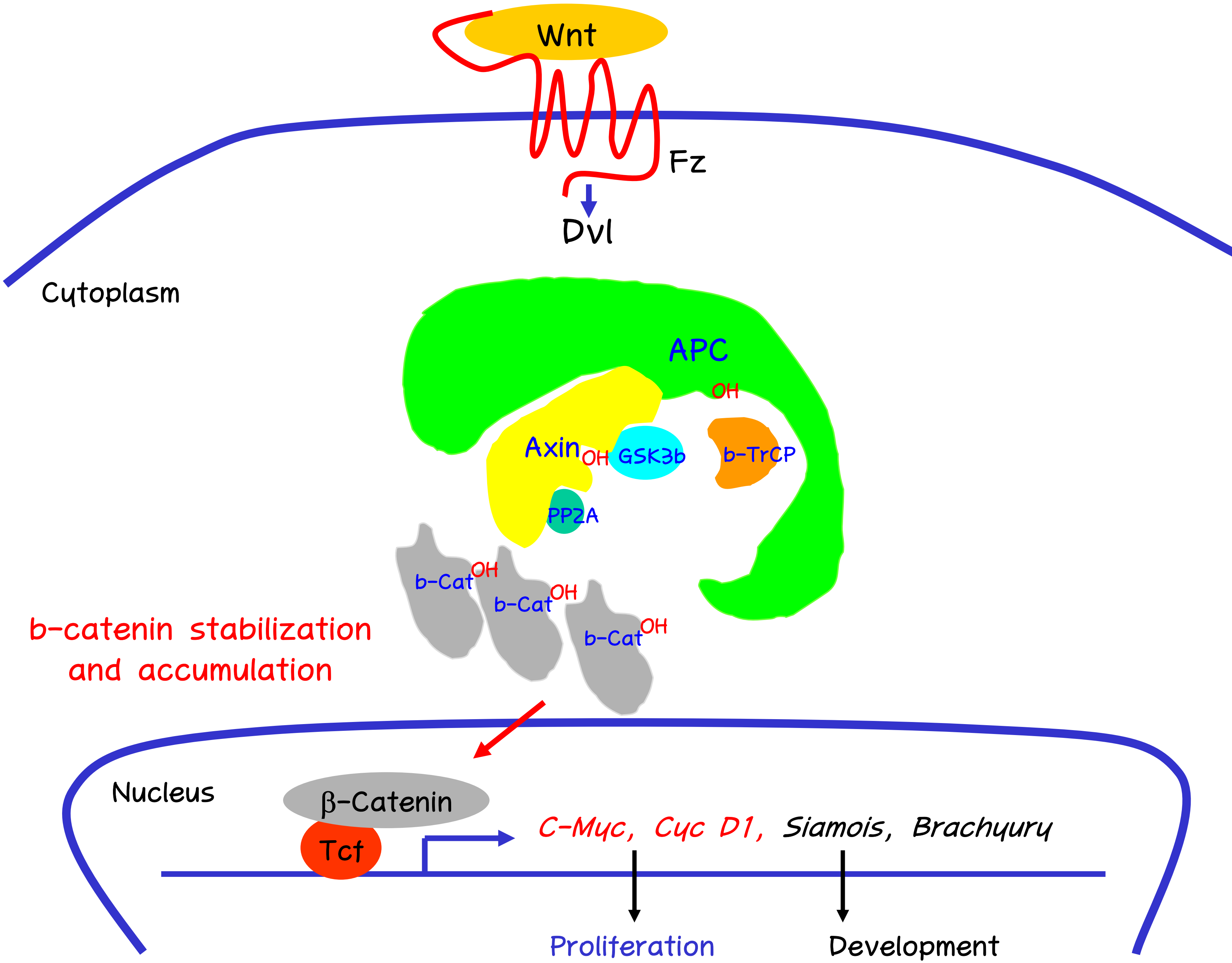
Compound	IC50 (μM)
ZTM000990	0.3 ± 0.1
PKF118-310	0.3 ± 0.02
PKF118-744	0.5 ± 0.2
PKF115-584	0.4 ± 0.3
CGP049090	1.4 ± 0.3
NPDD1.024	0.7 ± 0.2
PKF222-815	3
NPDD39.024	3
ZTM002377	>10
CGP039329	>10





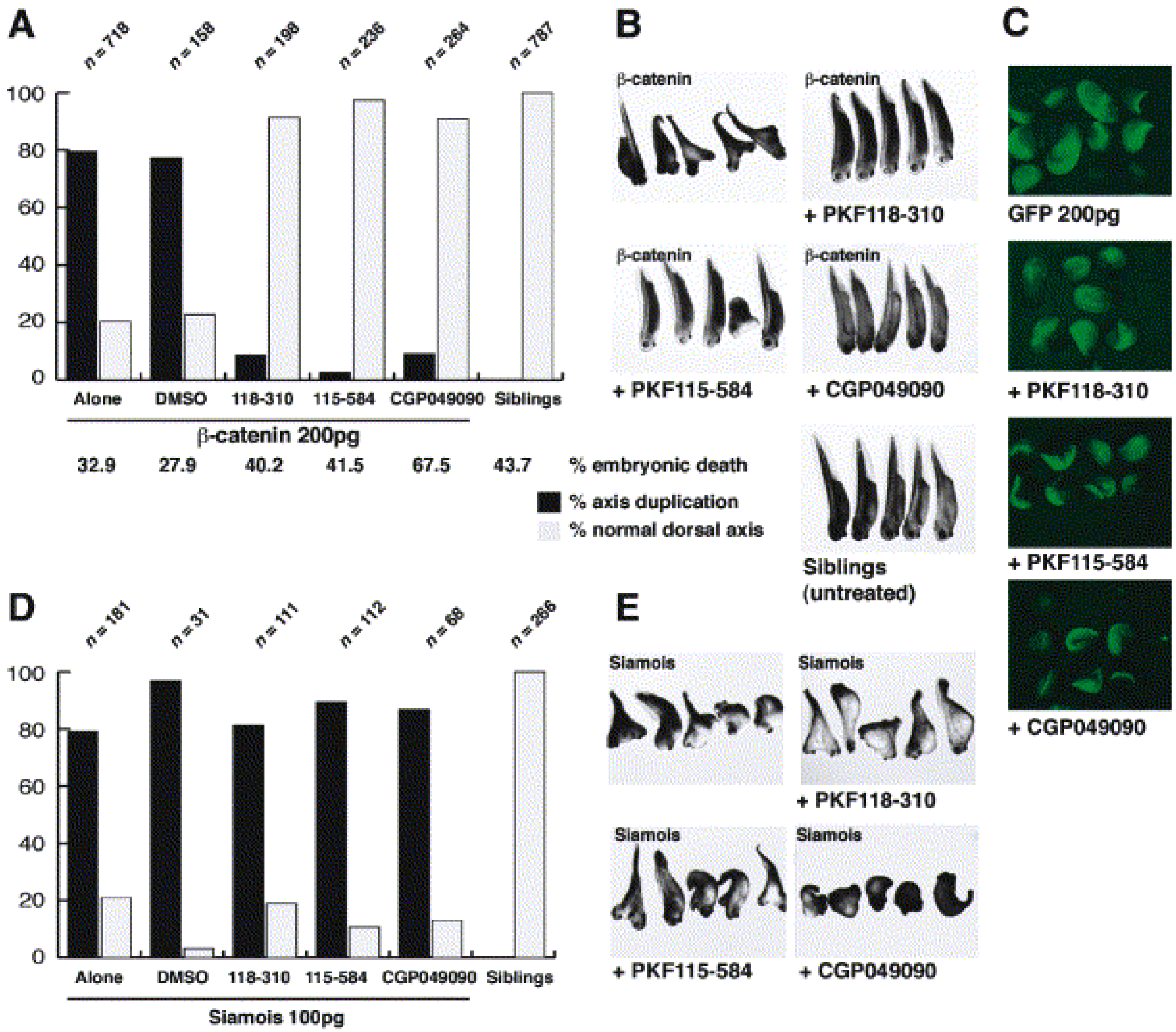
Validation of inhibitor activity in vivo



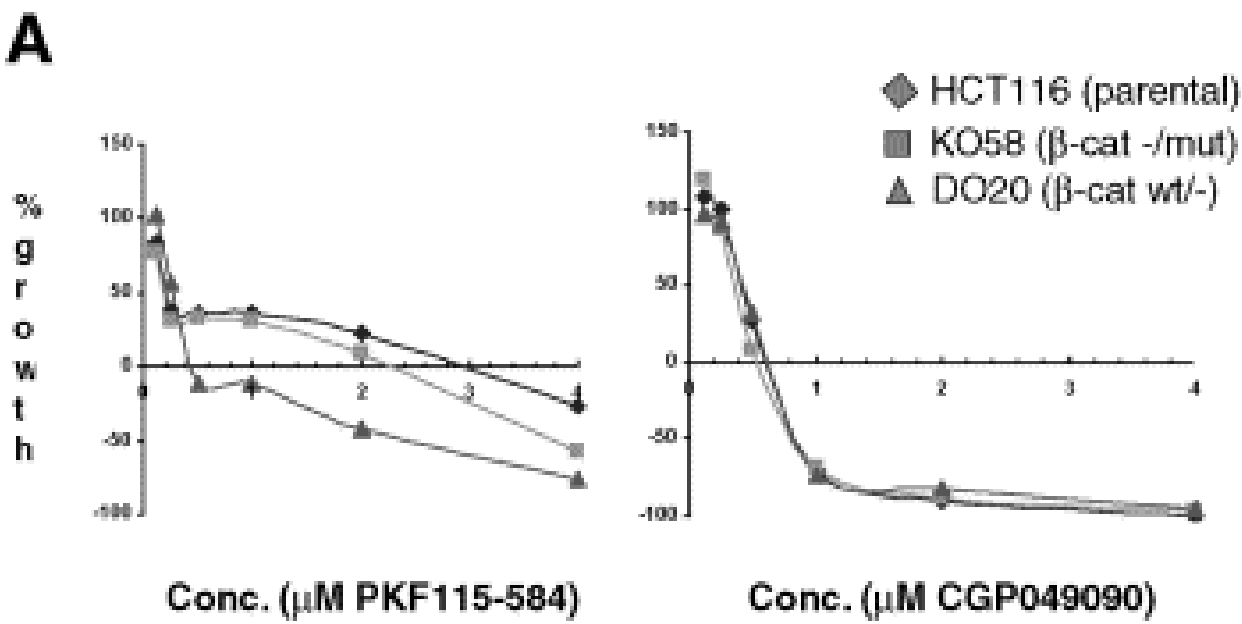


Wnt
Fz
Dvl
Cytoplasm
APC
OH
Axin
OH
GSK3b
b-TrCP
PP2A
b-Cat
OH
b-Cat
OH
b-Cat
OH
b-catenin stabilization
and accumulation
Nucleus
β-Catenin
Tcf
C-Myc, Cyc D1, Siamois, Brachyury
Proliferation
Development

Validation of inhibitor activity in vivo



Activity of compounds against colon cancer cells



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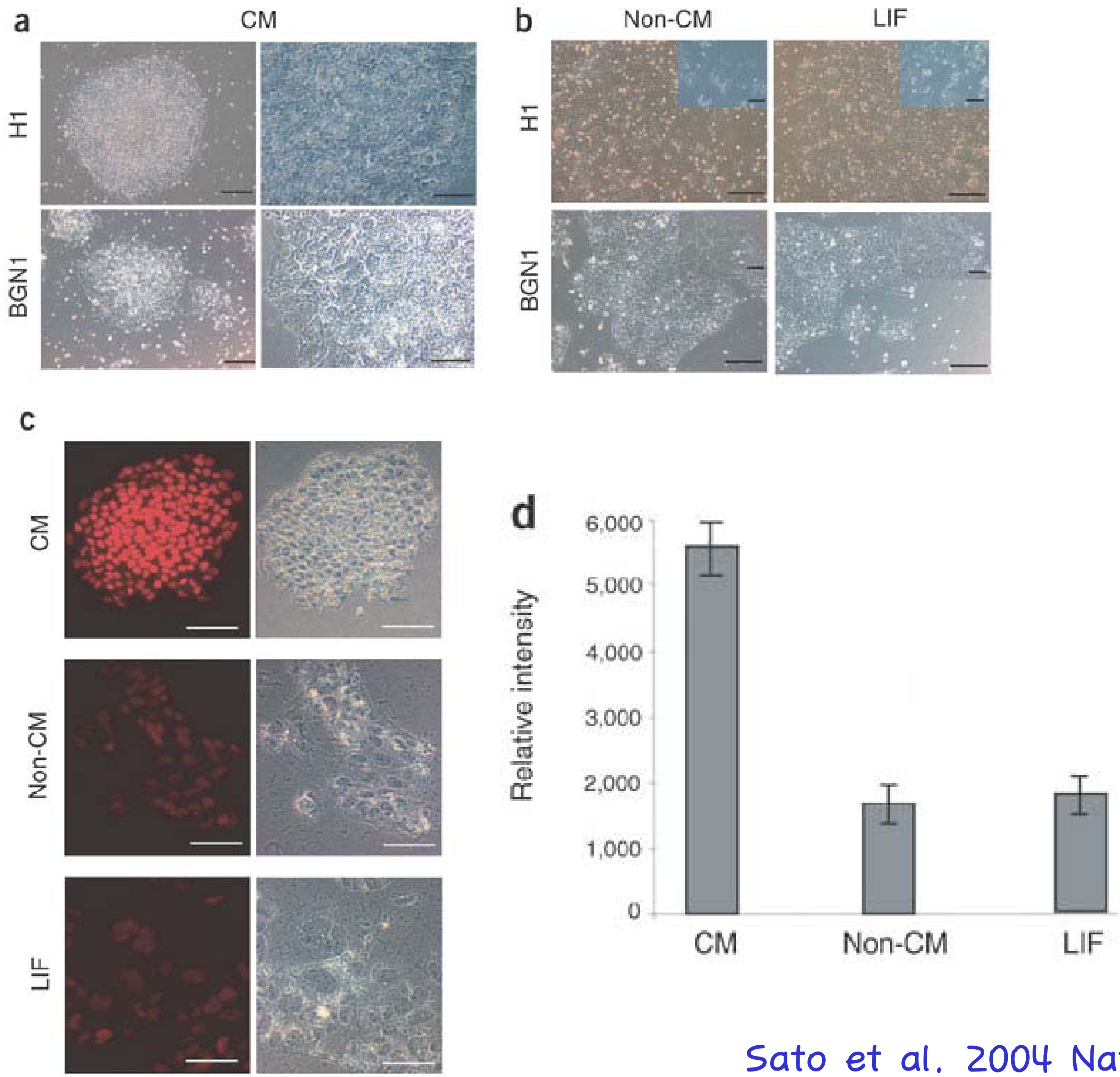
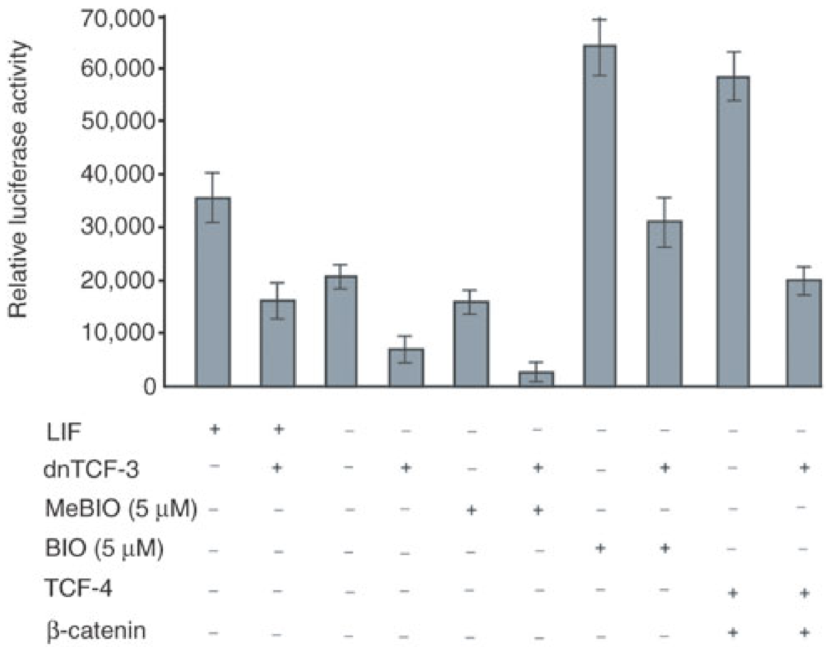
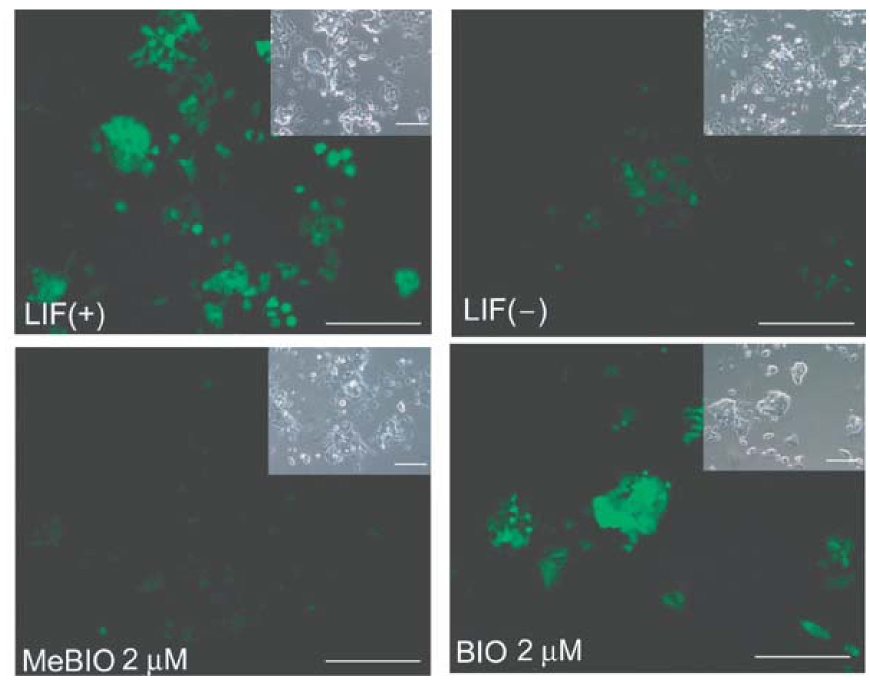
LIF-induced Stat-3 activation is not sufficient to maintain the undifferentiated state of HESCs



Sato et al. 2004 Nat Med. 10:55-63.

Activation of Wnt signaling by BIO maintains the undifferentiated state of MESCs





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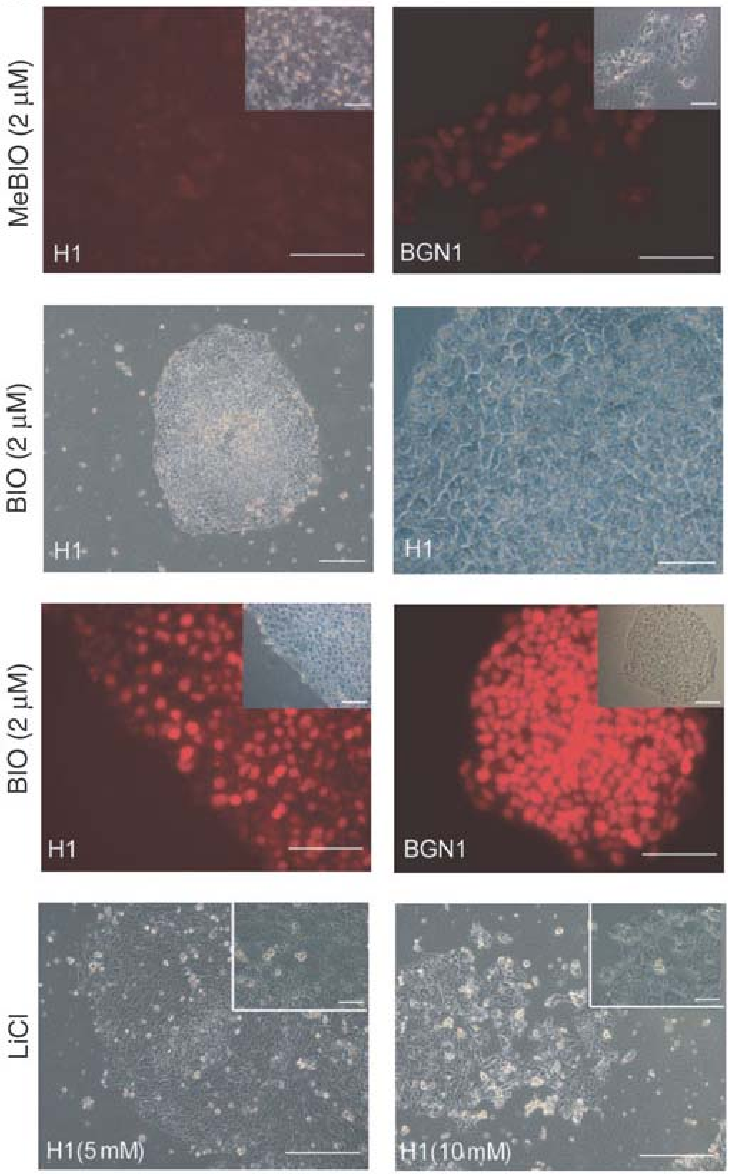

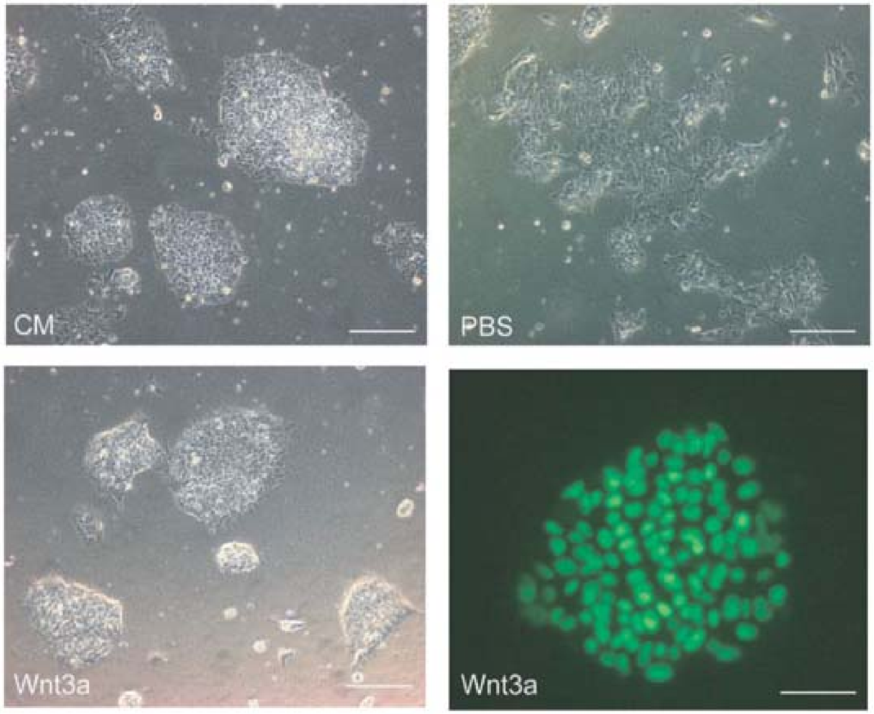

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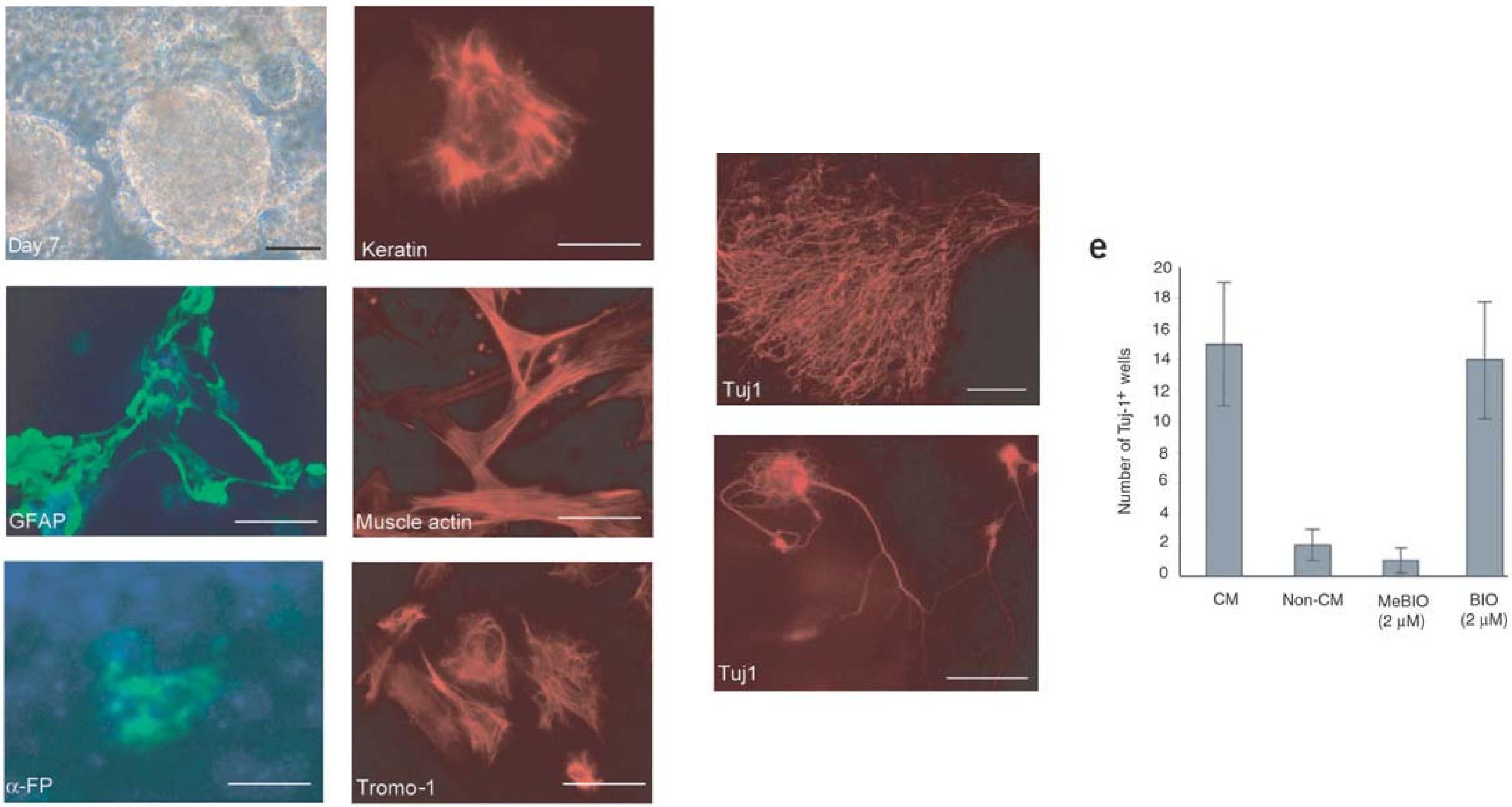
Wnt activation of HESCs by BIO preserves normal multidifferentiation potential



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