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Unravelling the desmoid-type fibromatosis at the cellular level: GSK-3beta, a new piece of the puzzle

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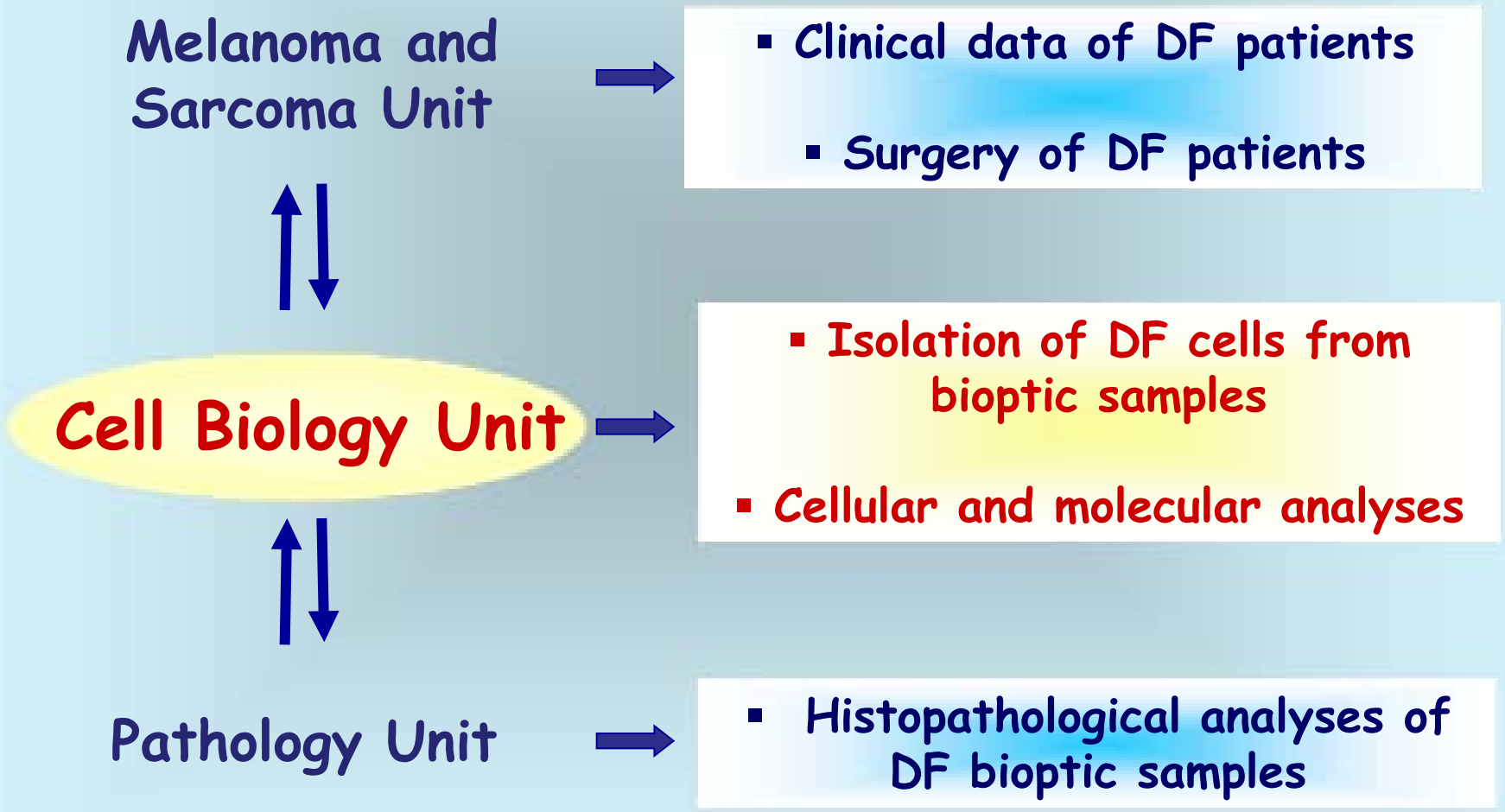
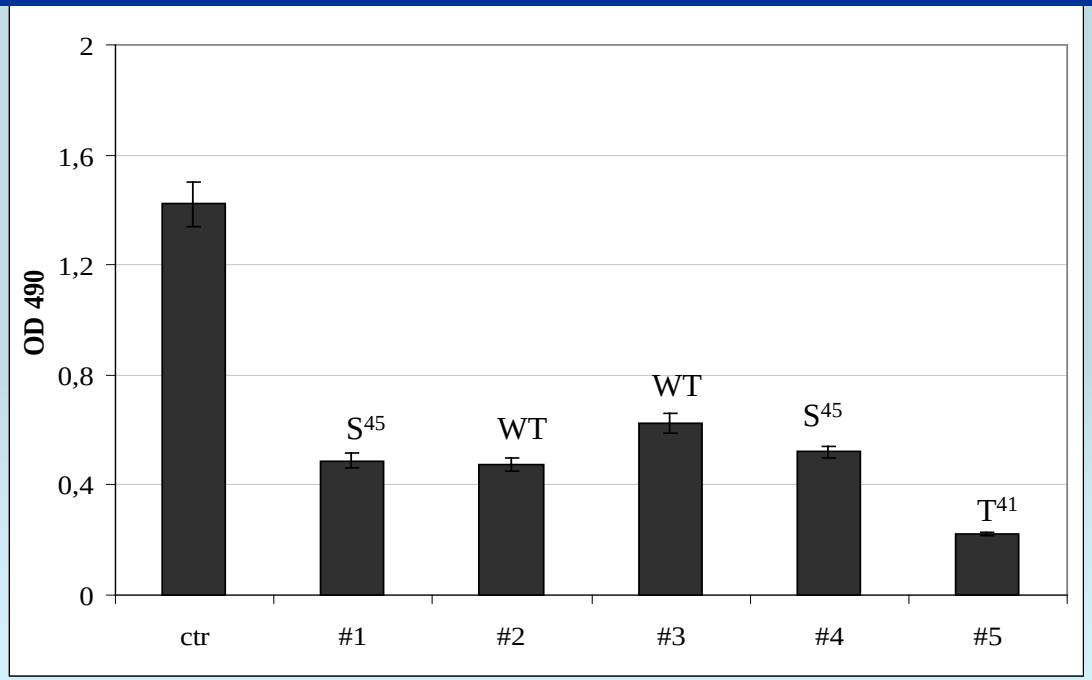
COLLECTION OF DESMOID-TYPE FIBROMATOSIS (DF) BIOPTIC SAMPLES



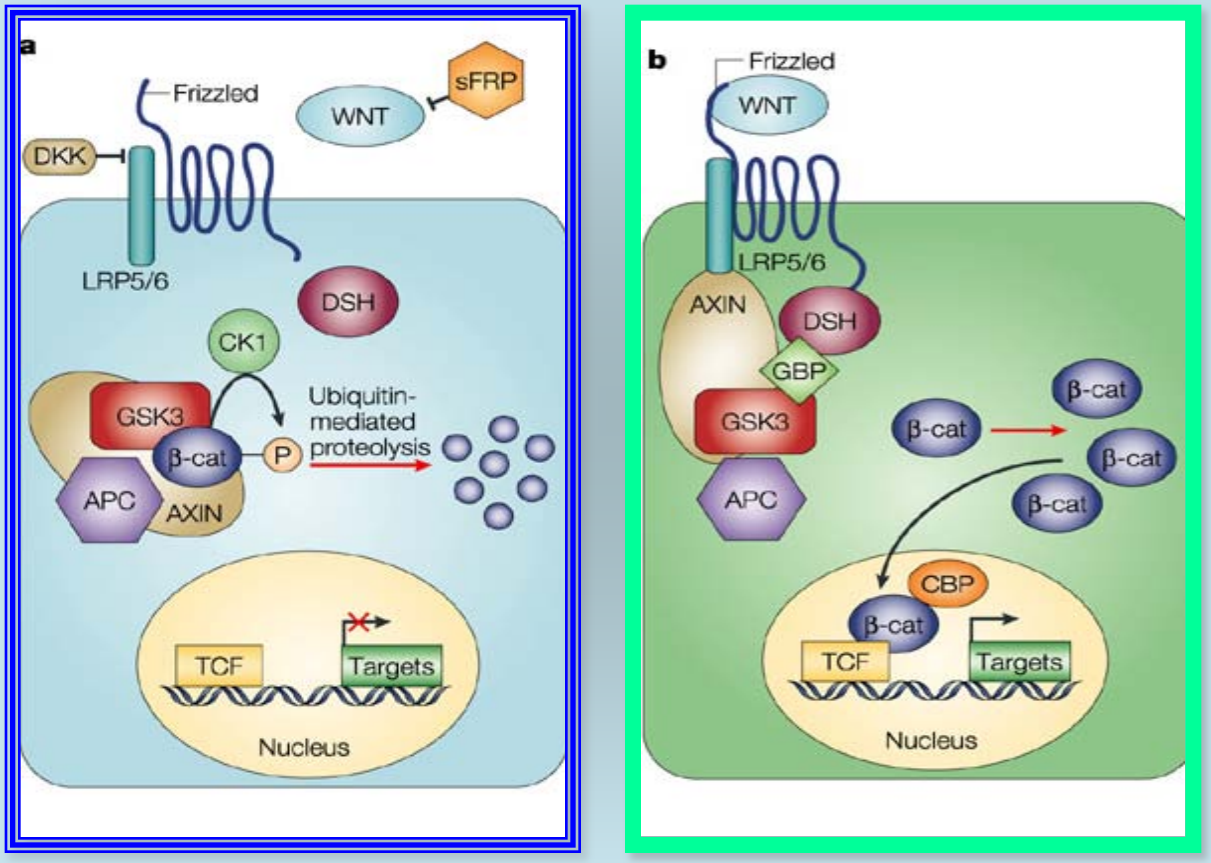
ISOLATION AND PROLIFERATION OF DESMOID-TYPE FIBROMATOSIS (DF) CELLS

- Fragmented bioptic fresh tissue were cultured in CHANG medium (50%FBS) for 1 week
- Primary DF cell coltures were grown and expanded in CHANG medium (10% FBS)

LOW DF CELLS PROLIFERATION RATE



WNT/β-CATENIN PATHWAY IS INVOLVED IN THE PATHOGENESIS OF DESMOID-TYPE FIBROMATOSIS



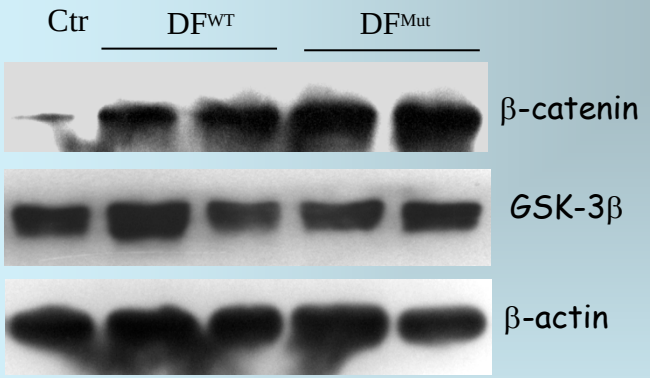
β-catenin is the common marker used for desmoid-type fibromatosis diagnosis

CHARACTERIZATION OF β-CATENIN IN DESMOID-TYPE FIBROMATOSIS CELLS

	Female	Male	Total
Number	15	7	22
Age (yrs)	33,5	53,3	40

	Female	Male	Total
CTNNB1 mut (%)	46,6	43	45,5
CTNNB1 wt (%)	53,4	57	54,5

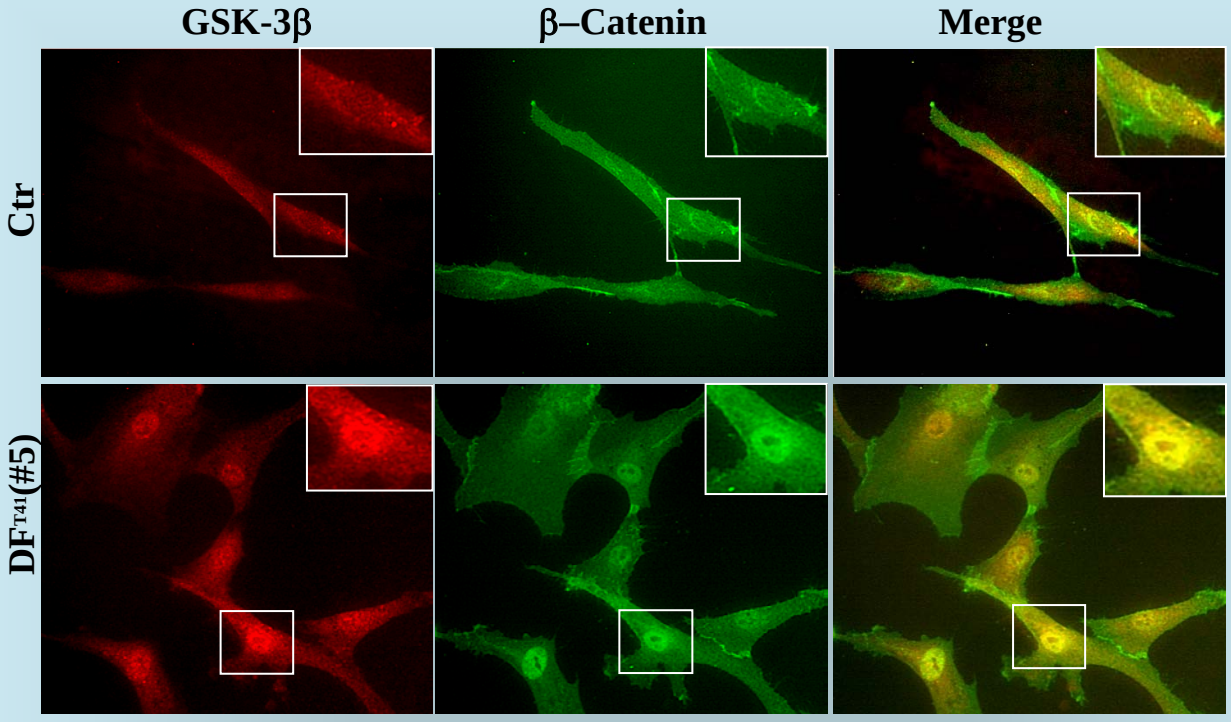
- The female patients are younger than male patients
- Only 45% of the DF cases have mutations of the exon 3 of CTNNB1 gene, with no difference between females and males

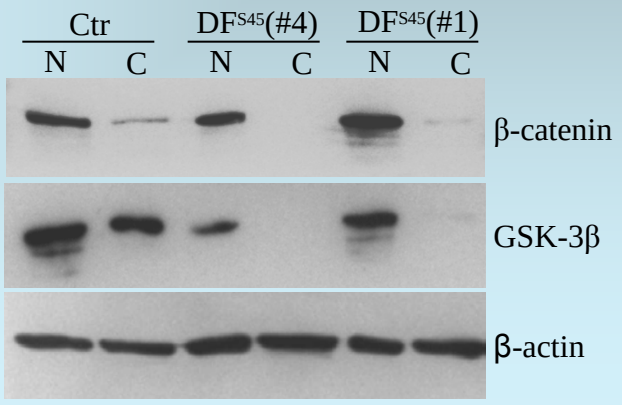


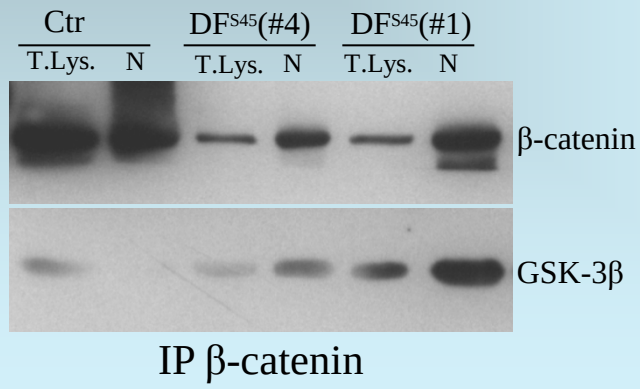
- β-catenin is highly expressed in DF samples in comparison to the control
- The expression level of GSK-3β is comparable in DF and control samples

There is no correlation between β-catenin mutations and its expression in desmoid-type fibromatosis cells

NUCLEAR LOCALIZATION OF GSK-3β AND ITS COLOCALIZATION WITH β-CATENIN







IP β-catenin

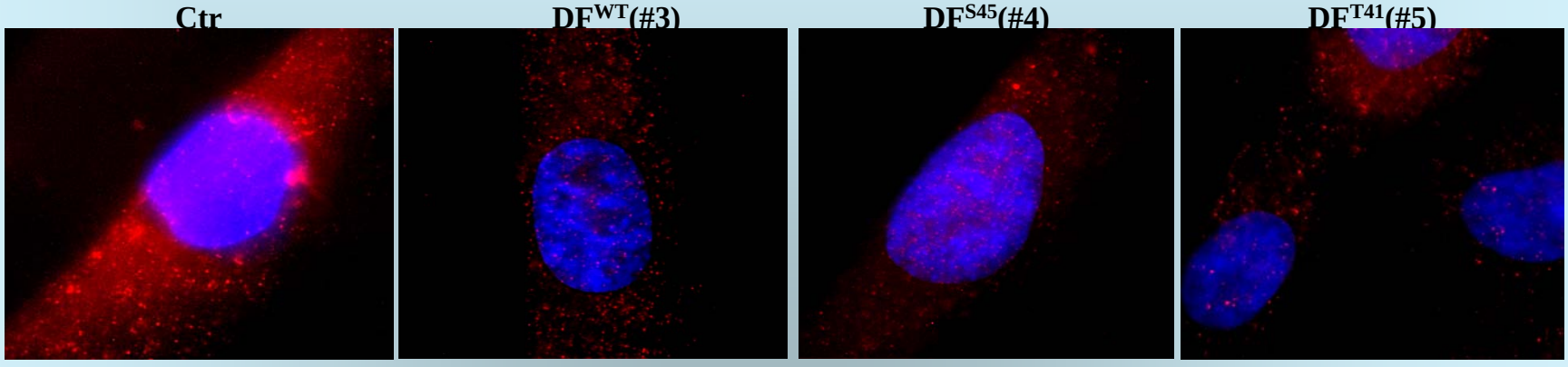
β-CATENIN AND GSK-3β NUCLEUS-POSITIVE DF CELLS

	β-catenin		GSK-3β	
Samples	**Nucleus %**	**Cytoplasm %**	**Nucleus %**	**Cytoplasm %**
DF#1^{S45}	**88**	**12**	**97**	**3**
DF#2^{WT}	**80**	**20**	**95**	**5**
DF#3^{WT}	**72,7**	**27,3**	**90**	**10**
DF#4^{S45}	**84**	**16**	**85**	**15**
DF#5^{T41}	**84**	**16**	**95**	**5**
Ctr	**0**	**100**	**0**	**100**

- The number of β-catenin nucleus-positive cells is reduced of 10% in *CTNNB1* **non-mutated** DF cells compared to *CTNNB1* **mutated** DF cells
- The number of GSK-3β nucleus-positive cells is equivalent in *CTNNB1* **non-mutated** and **mutated** DF cells

PHOSPHORYLATED BETA-CATENIN IS ALWAYS ABSENT IN DF CELLS

A.



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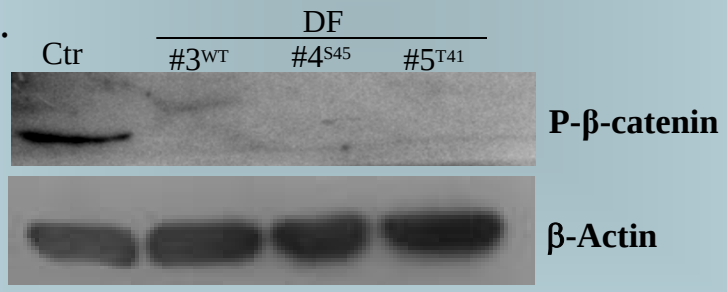
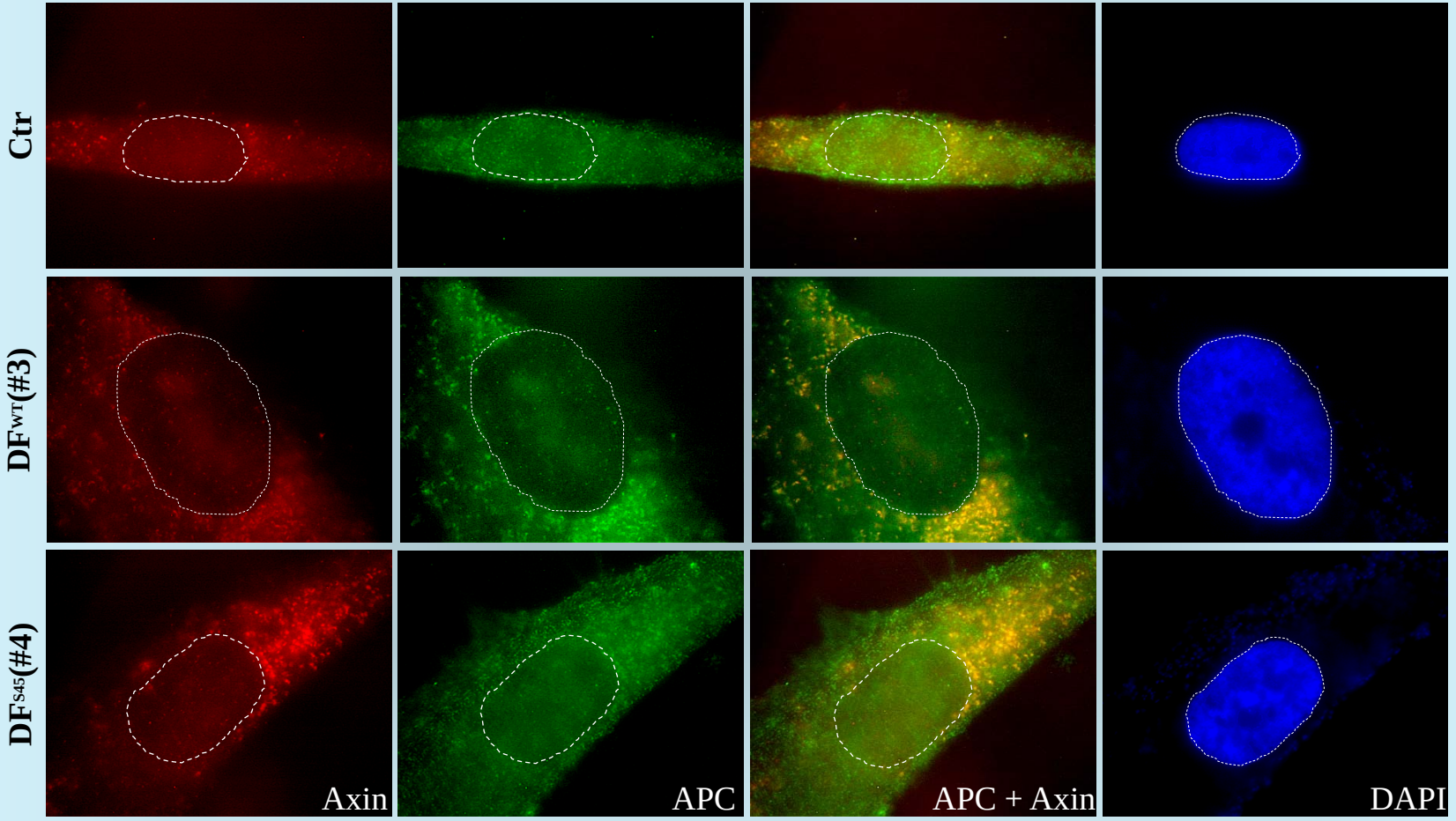


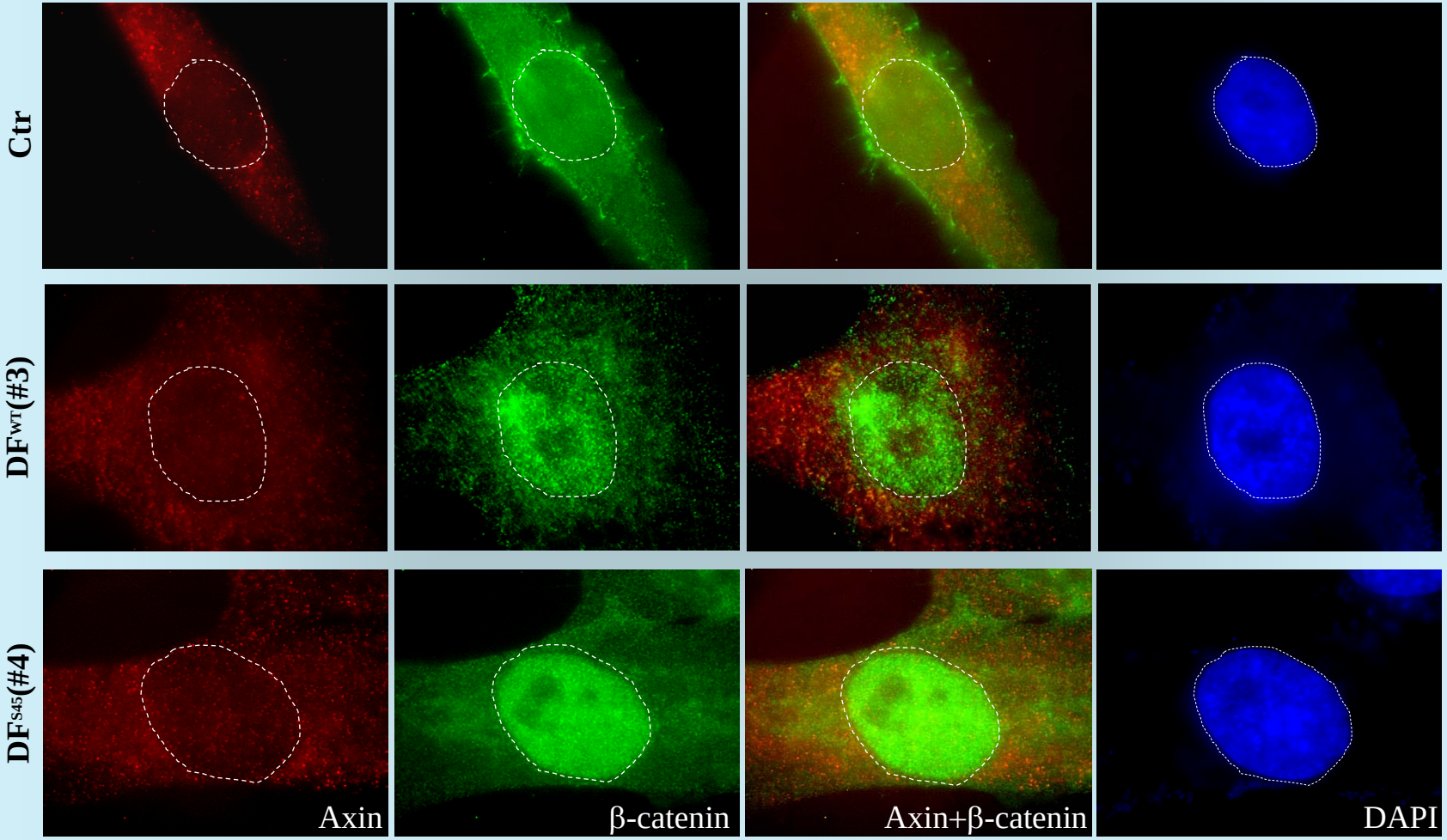
- β-catenin phosphorylation is not associated to mutations in *CTNNB1* gene
- In DF cells β-catenin is not phosphorylated and consequently not degraded

Nuclear accumulation of β-catenin

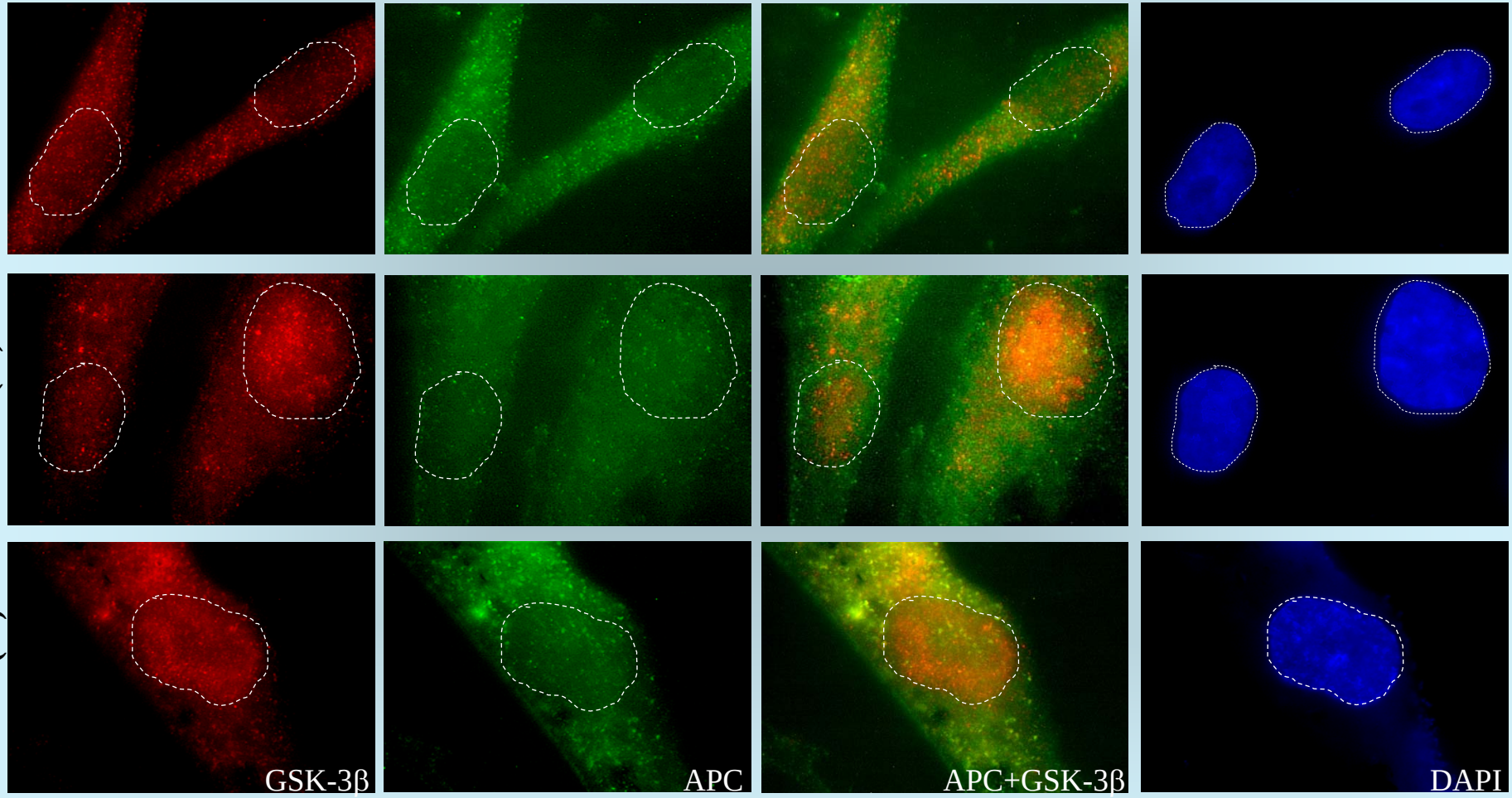
APC AND AXIN COLOCALIZE IN THE CYTOPLASMIC COMPARTMENT OF DF CELLS



BETA-CATENIN AND AXIN ARE LOCALIZED IN DIFFERENT CELL COMPARTMENTS IN DF CELLS



GSK-3B AND APC ARE LOCALIZED IN DIFFERENT CELL COMPARTMENTS IN DF CELLS



CONCLUSIONS 1

β-catenin is not the sole component of the multiprotein complex accumulated in the nucleus

GSK-3β is exclusively nuclear and it is complexed with β-catenin

Nuclear translocation of β-catenin and GSK-3β is not associated to *CTNNB1* mutations

Proteins of the Wnt pathway have different cells compartmentalization

The multiprotein complex, responsible for β-catenin phosphorylation, cannot be assembled

GSK-3β

GSK-3β binds to an altered β-catenin that cannot be phosphorylated

GSK-3β binds to β-catenin but it cannot be phosphory-lated because the complex is not assembled

Nuclear migration of β-catenin/GSK-3β

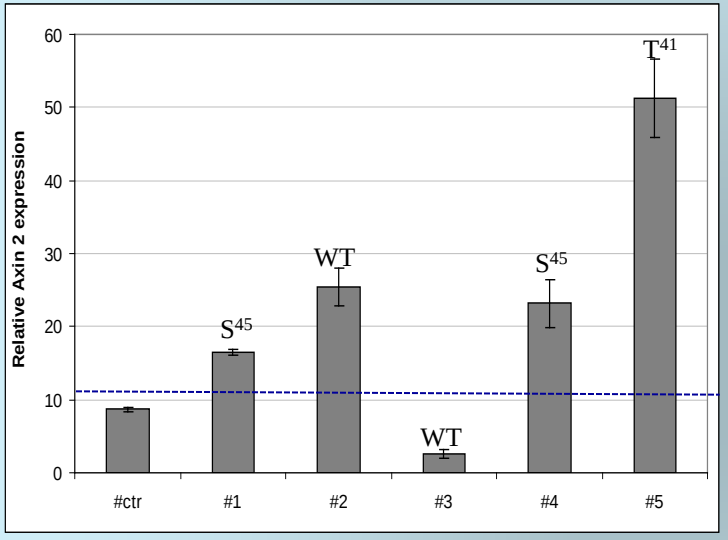
NUCLEAR GSK-3β AS ADDITIONAL MARKER FOR DF CELLS

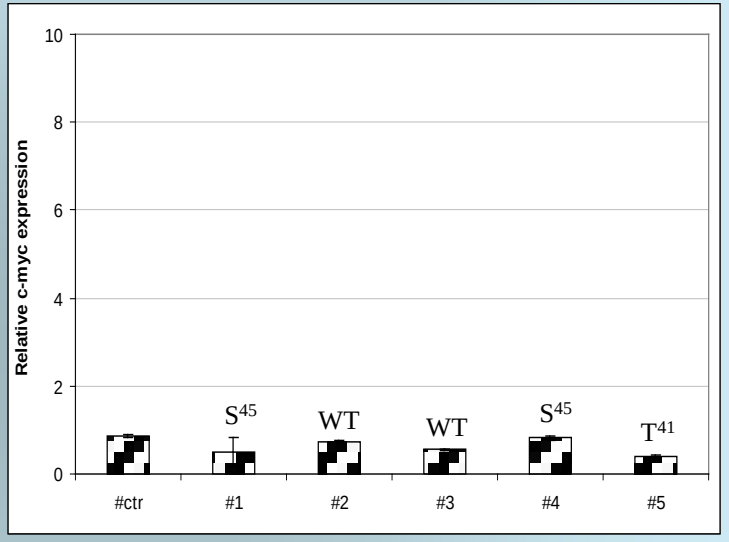
…… AND NOW THE AIMS ARE ……

- To identify the key molecules of the Wnt pathway leading the alteration of β-catenin and GSK-3β localization

- To investigate the effect of nuclear GSK-3β

GENE EXPRESSION OF Wnt TARGETS: *AXIN2* AND *C-MYC*

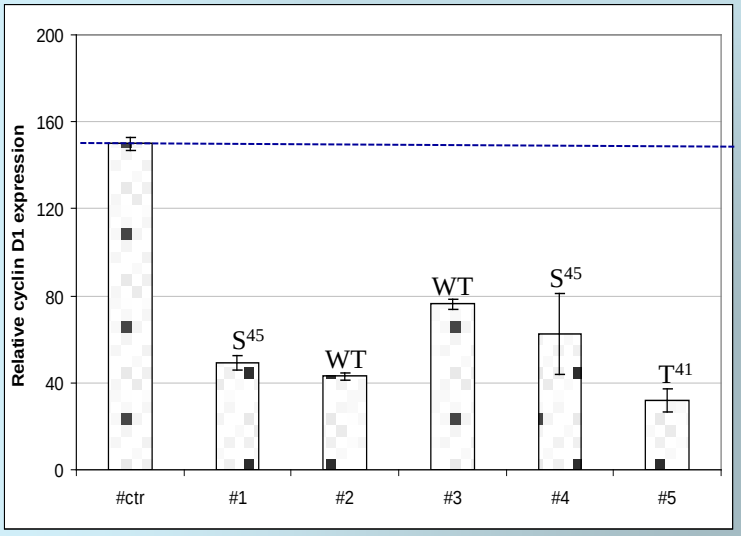


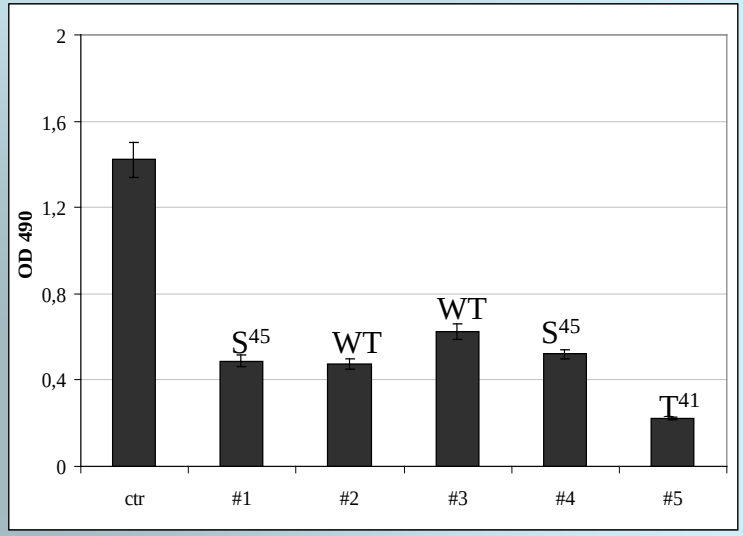


AXIN2 gene expression was increased two to six-fold in DF cells

c-myc gene is not expressed in DF cells

GENE EXPRESSION OF Wnt TARGETS: *CCND1*



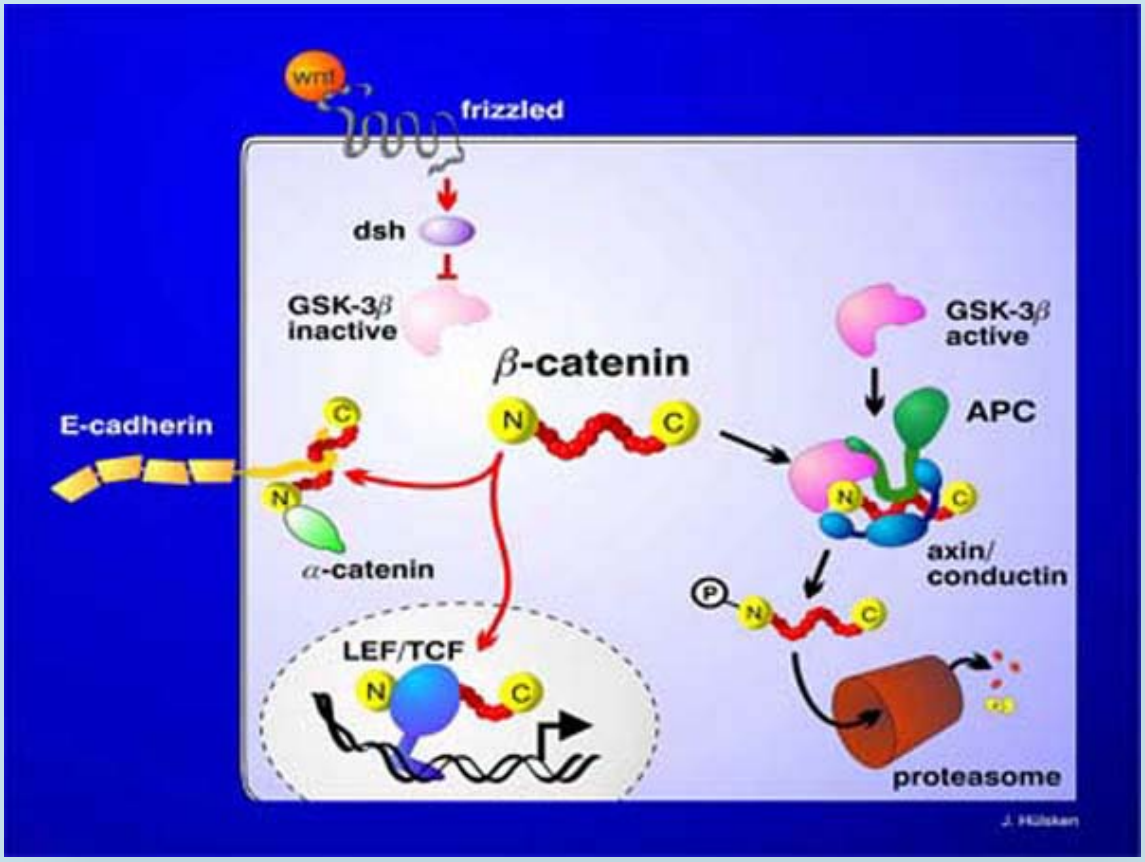


CCND1 gene expression is downregulated in DF cells

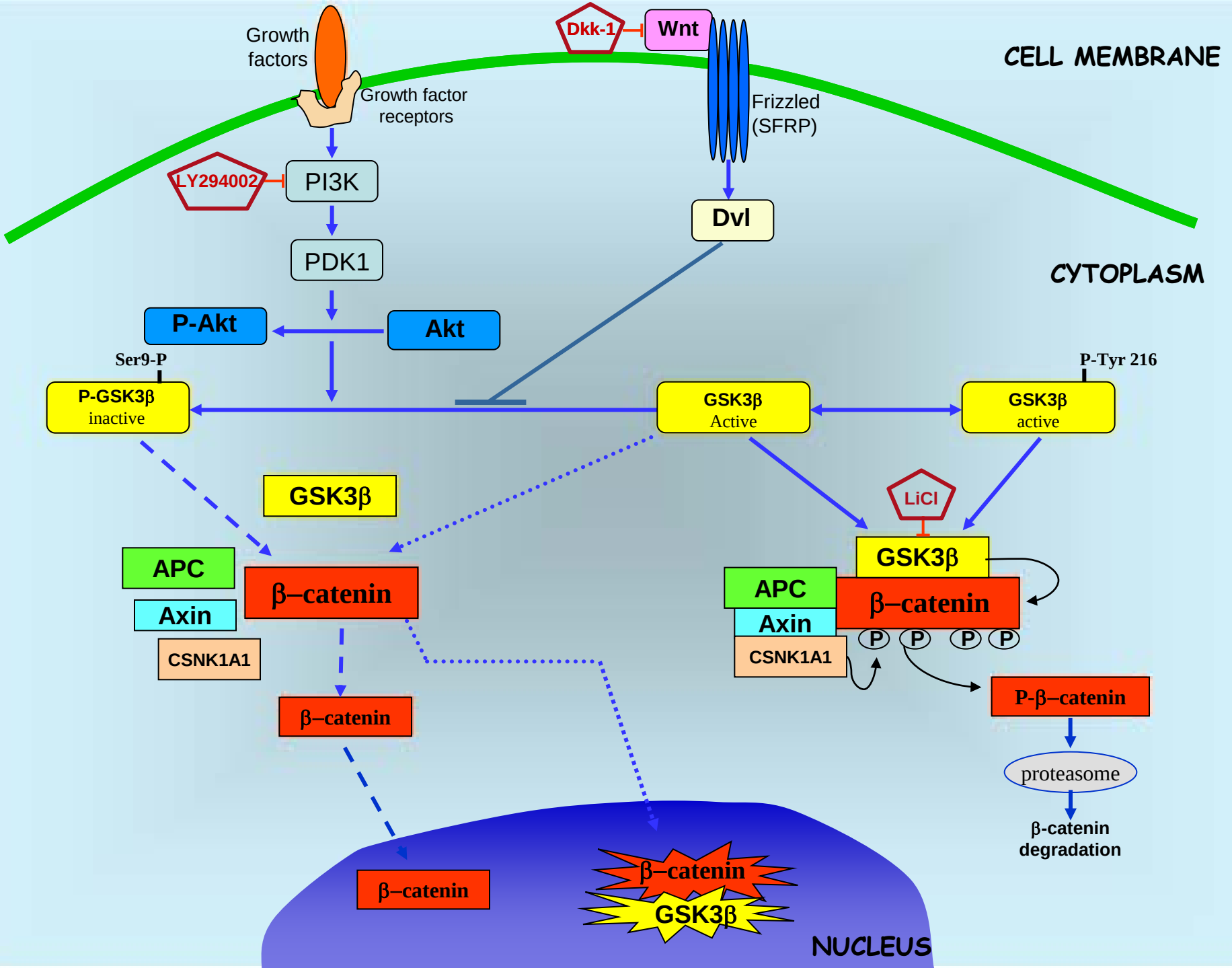
Possible reason of low DF cells proliferation rate

GSK-3β might play a role in Cyclin D1 degradation

ACTIVE GSK-3β PHOSPHORYLATES β-CATENIN

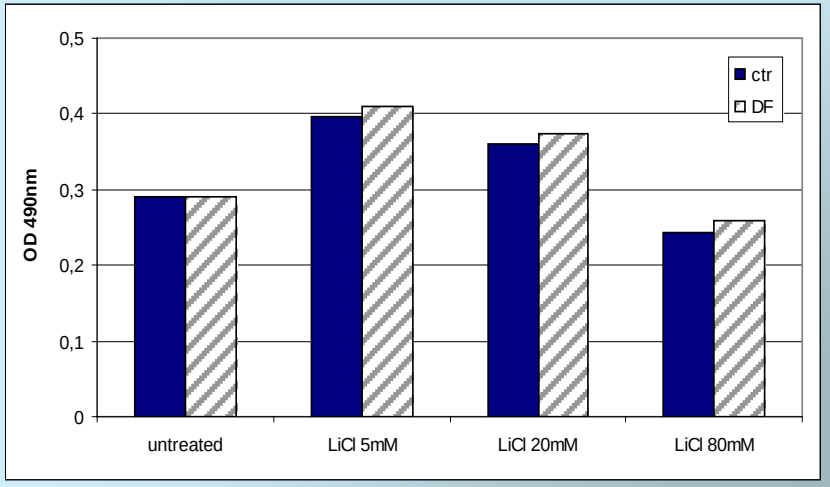


- Activation of Wnt pathway results in an inactivation of GSK-3β
- Akt phosphorylates and inactives GSK-3β

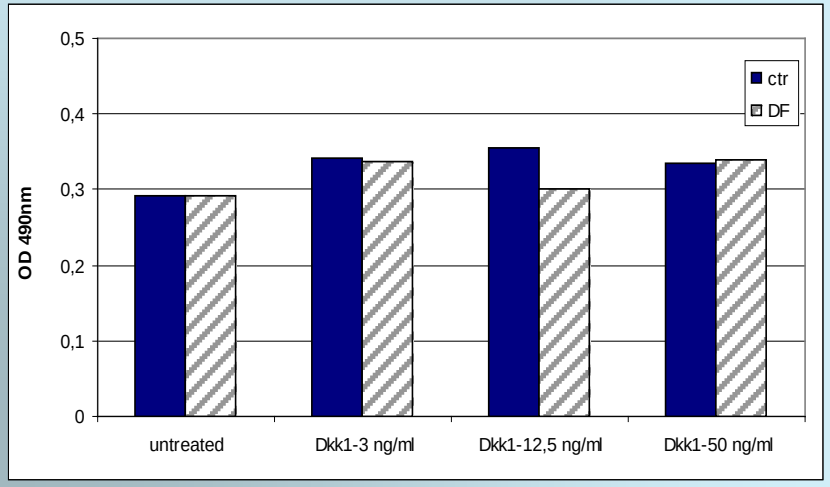

CELL MEMBRANE
Growth factors
Growth factor receptors
Dkk-1
Wnt
Frizzled (SFRP)
LY294002
PI3K
PDK1
Dvl
CYTOPLASM
P-Akt
Akt
Ser9-P
P-Tyr 216
P-GSK3β inactive
GSK3β Active
GSK3β active
GSK3β
LiCl
APC
Axin
CSNK1A1
β–catenin
GSK3β
APC
Axin
CSNK1A1
β–catenin
P
P
P
P
P-β–catenin
proteasome
β-catenin degradation
β–catenin
β–catenin
GSK3β
NUCLEUS

CELLS VIABILITY AFTER DRUGS TREATMENT

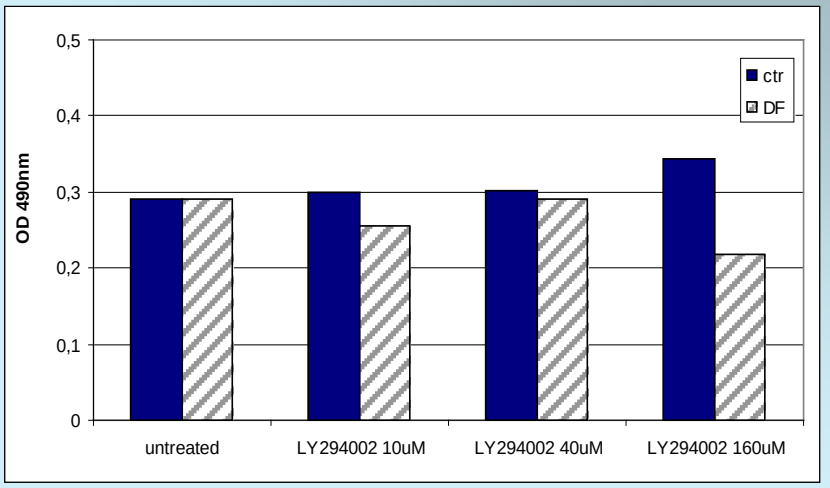
LiCl: inhibitor of GSK-3β



Dkk1: antagonist of the Wnt signalling



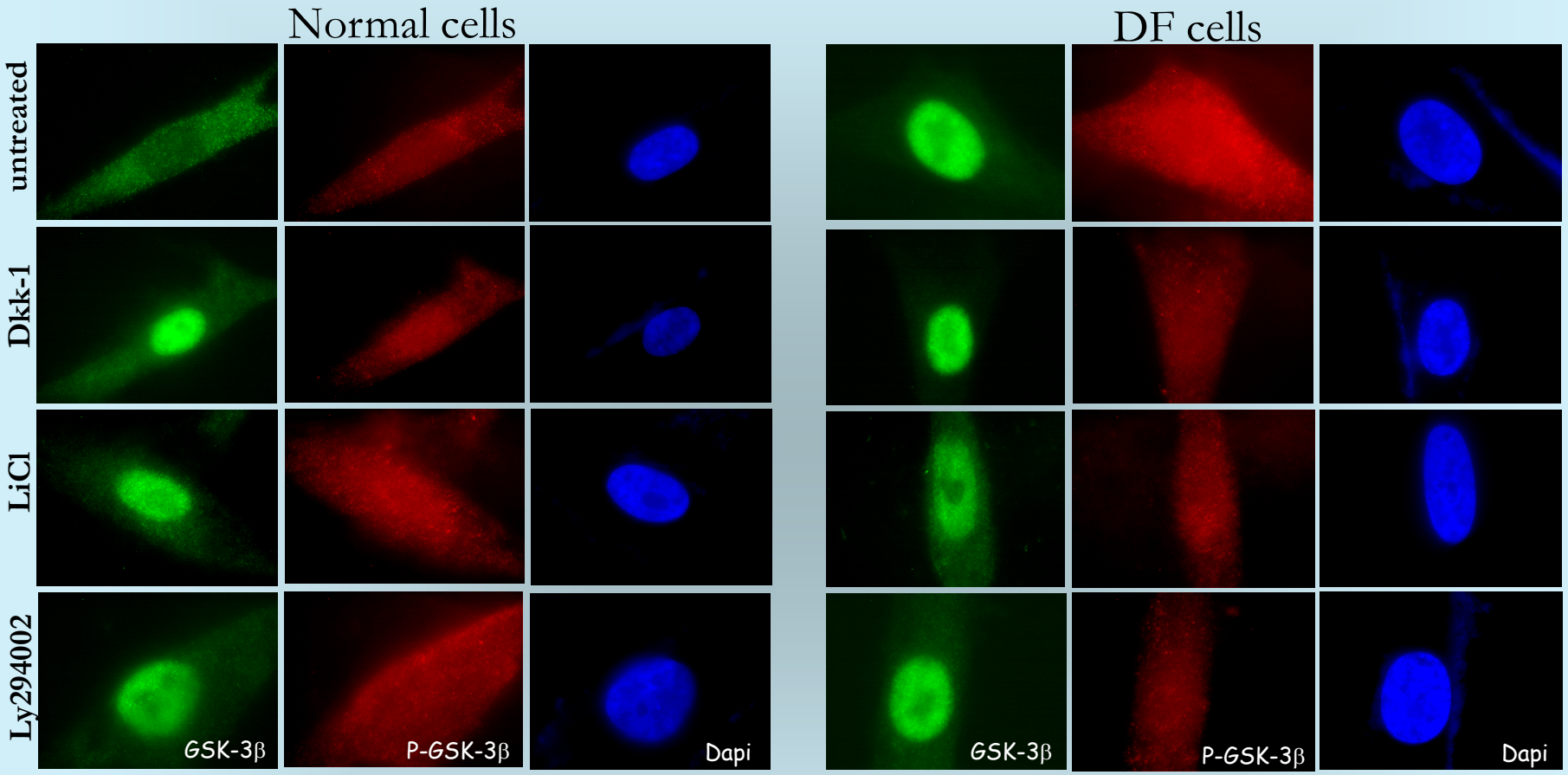
LY294002: inhibitor of PI3 kinase



The optimal drug concentrations compatible with cells viability are:

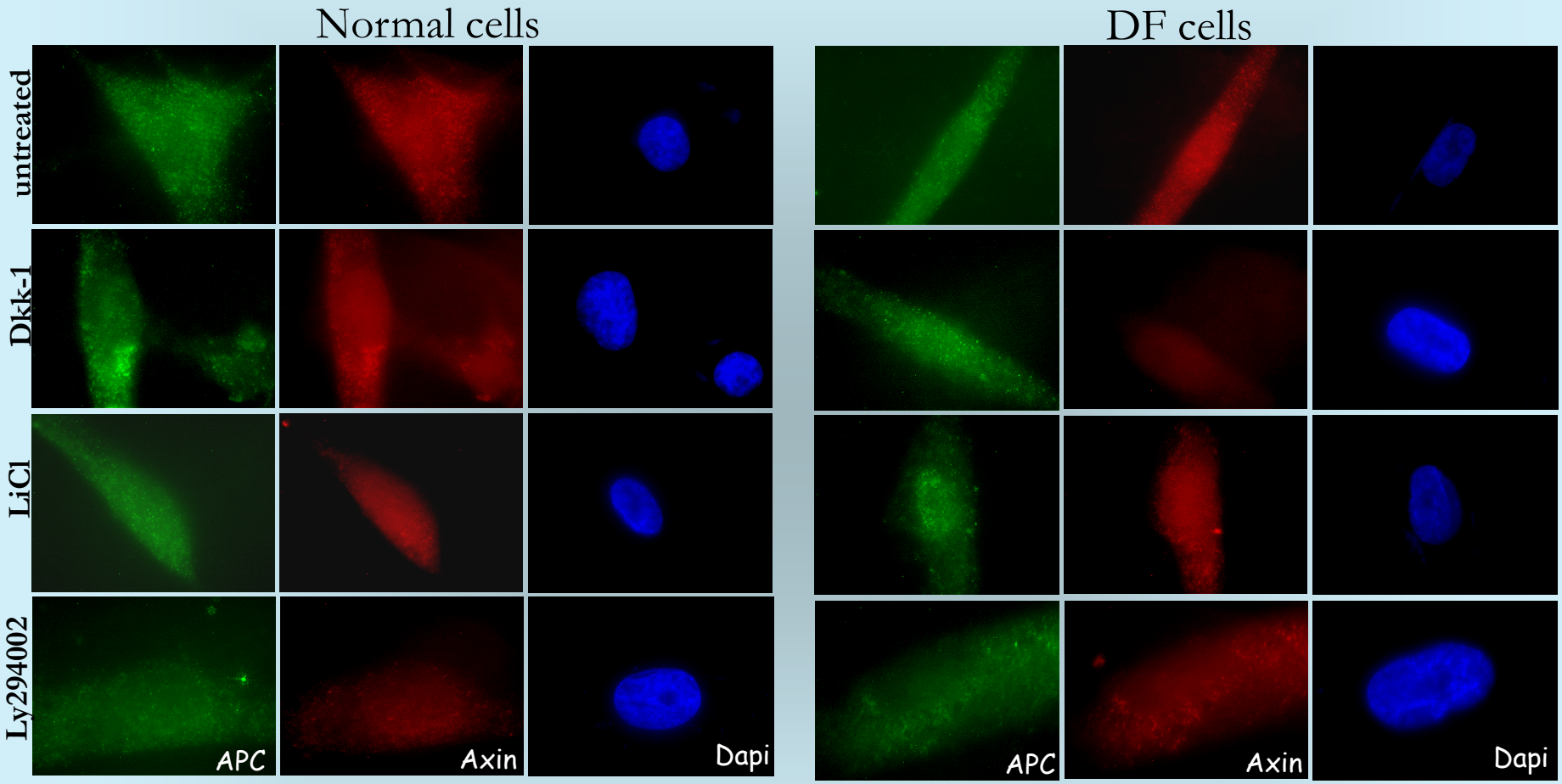
LiCl:	20mM
Dkk1:	50ng/ml
LY294002:	40mM

EXPRESSION AND LOCALIZATION OF TOTAL AND PHOSPHORYLATED GSK-3β IN NORMAL AND DF CELLS



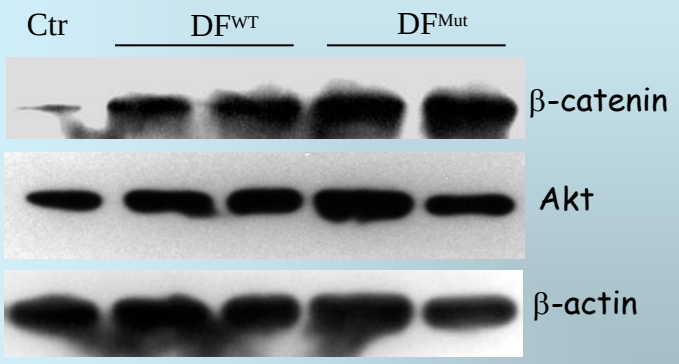
- Normal and DF cells treated with drugs lead to nuclear GSK-3β translocation
- Substantial loss of P-GSK-3β in DF cells treated with drugs

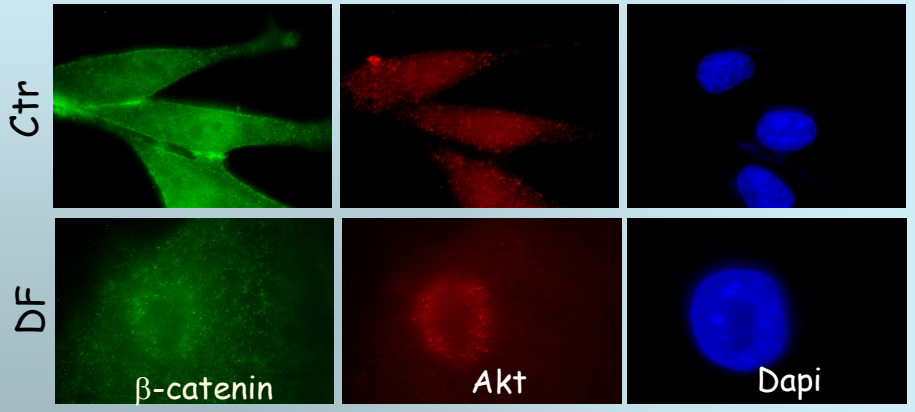
EXPRESSION AND LOCALIZATION OF APC AND AXIN IN NORMAL AND DF CELLS



- Normal and DF cells treated with Lithium lead to nuclear APC and Axin translocation
- Substantial loss of Axin in DF cells treated with Dkk-1 inhibitor

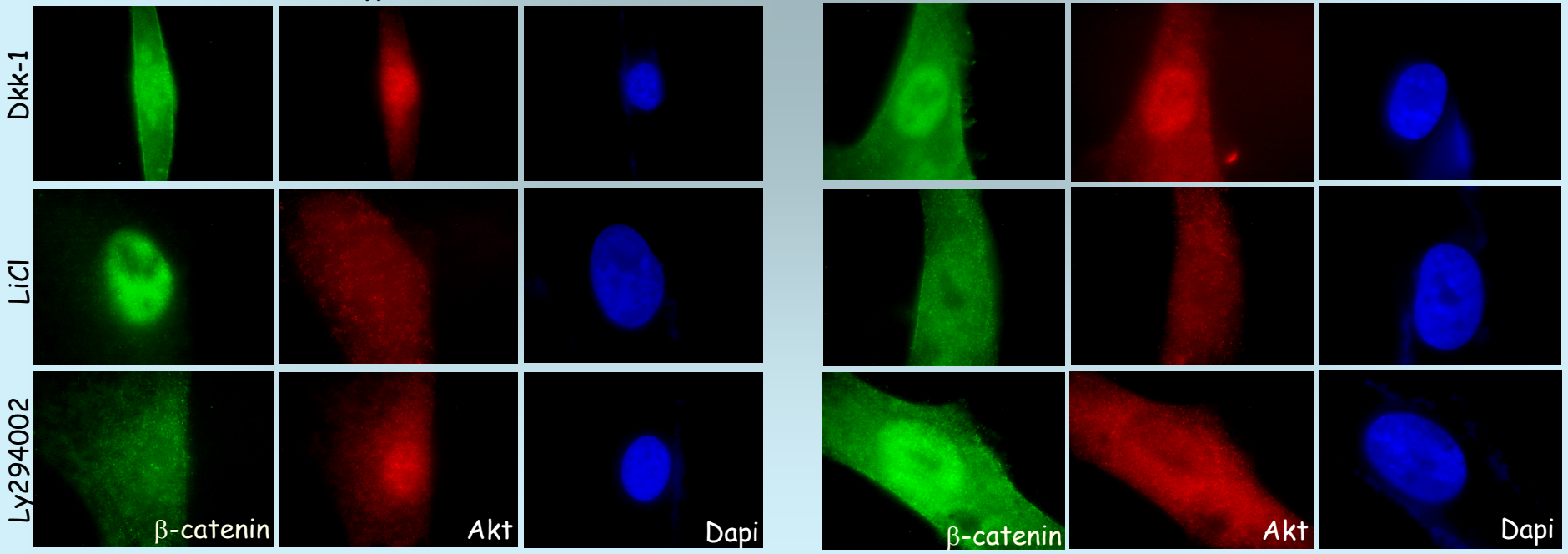
EXPRESSION AND LOCALIZATION OF AKT AND β-CATENIN IN NORMAL AND DF CELLS





Normal cells

DF cells



SUMMARY

LiCl: inhibitor of GSK-3β

- Nuclear translocation of β-catenin, GSK-3β, APC and Axin in normal and DF cells
- Loss of P-GSK-3β expression in DF cells

Dkk1: antagonist of the Wnt signalling

- Nuclear translocation of GSK-3β and P-GSK-3β in normal cells
- Nuclear translocation of Akt in normal and DF cells
- Loss of Axin and P-GSK-3β expression in DF cells

LY294002: inhibitor of PI3 kinase

- Nuclear translocation of GSK-3β and Akt in normal cells
- Loss of P-GSK-3β expression in DF cells
- Increase cytoplasmic Akt in DF cells

THERE IS STILL A LOT OF WORK TO BE DONE …

To validate of nuclear GSK-3β as a novel clinical marker for desmoid-type fibromatosis

To deeply investigate the role of inhibitor molecules on the expression and localization of the Wnt proteins in DF cells

To identify the factors responsible for the DF cells growth and aggressiveness

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