

# **Notch pathway inhibition with DAPT diminishes tumor growth and hormone secretion in GH3 xenografted NUDE/NUDE mice.**

Zubeldia Brenner L, Carolina Cristina y Damasia Becú-Villalobos.

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# Notch pathway inhibition with DAPT diminishes tumor growth and hormone secretion in GH3 xenografted NUDE/NUDE mice.

Lautaro Zubeldia-Brenner <sup>1</sup>, Carolina Cristina<sup>2</sup> and Damasia Becú-Villalobos<sup>1</sup>.

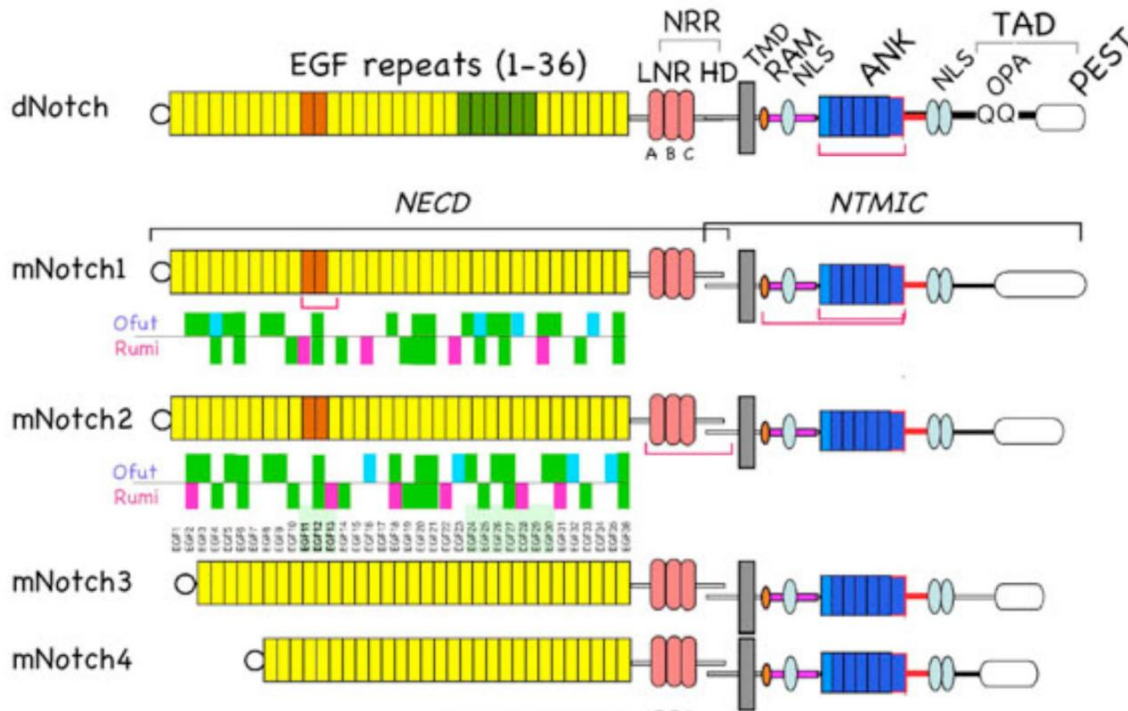
<sup>1</sup>Instituto de Biología y Medicina Experimental (IBYME-CONICET).

<sup>2</sup>CITNOBA (UNNOBA-CONICET), Universidad Nacional del Noroeste de la Provincia de Buenos Aires.

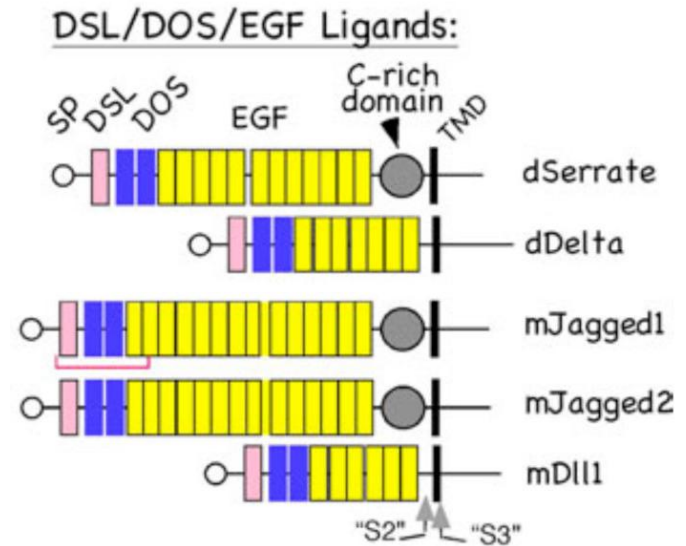


# Notch System

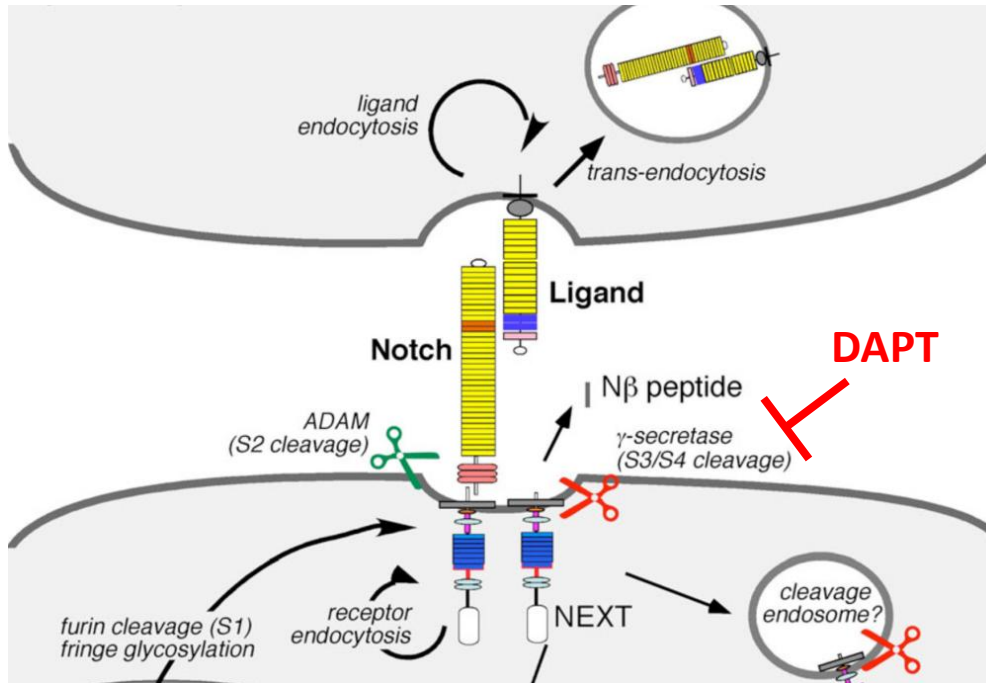
## Receptors



## Ligands



# Notch pathway and tumor development.



- Cell-cell signaling system that primarily **governs cell-fate choices.**

- In a context dependent manner: **promotes or suppresses proliferation.**

- Controls the balance between commitment to differentiated state and repression of differentiation, **promoting proliferation of the undifferentiated cells. Also, has a critical role in angiogenesis.**

- Misregulated Notch signaling has been demonstrated in various human cancers.**

**Notch aberrant signaling has been described with critical roles in:**

1. Breast cancer.
2. Colon cancer.
3. Pancreas cancer.
4. Lung adenocarcinoma.
5. T-cell acute lymphoblastic leukemia (T-ALL).
6. Others.

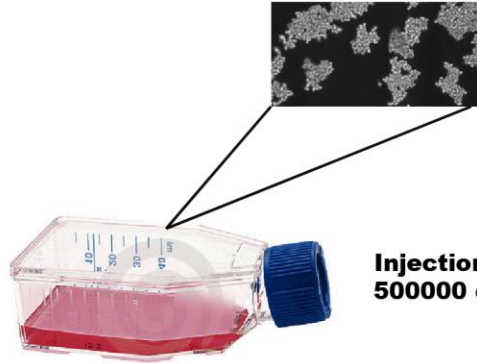
**In some cases **stimulating** and in **others inhibiting** proliferation.**

# **Main goal of the work**

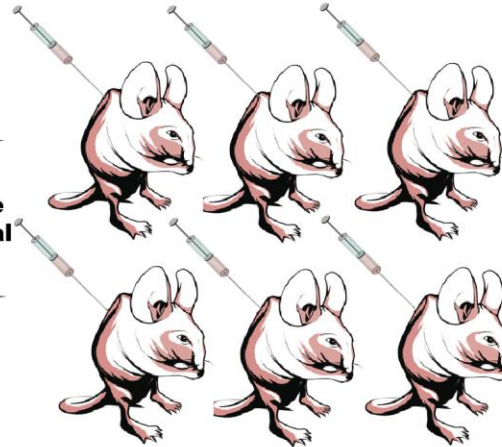
- **Determine the role of Notch signaling in pituitary tumor generation.**

# Experimental procedures: GH3 Xenografted tumors.

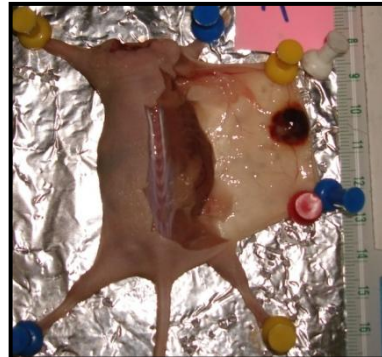
GH3 somatolactotropic cells (Rat)



Injection in NUDE mice  
500000 cells per animal



1 month of tumor growing  
25 mm<sup>3</sup> aprox.

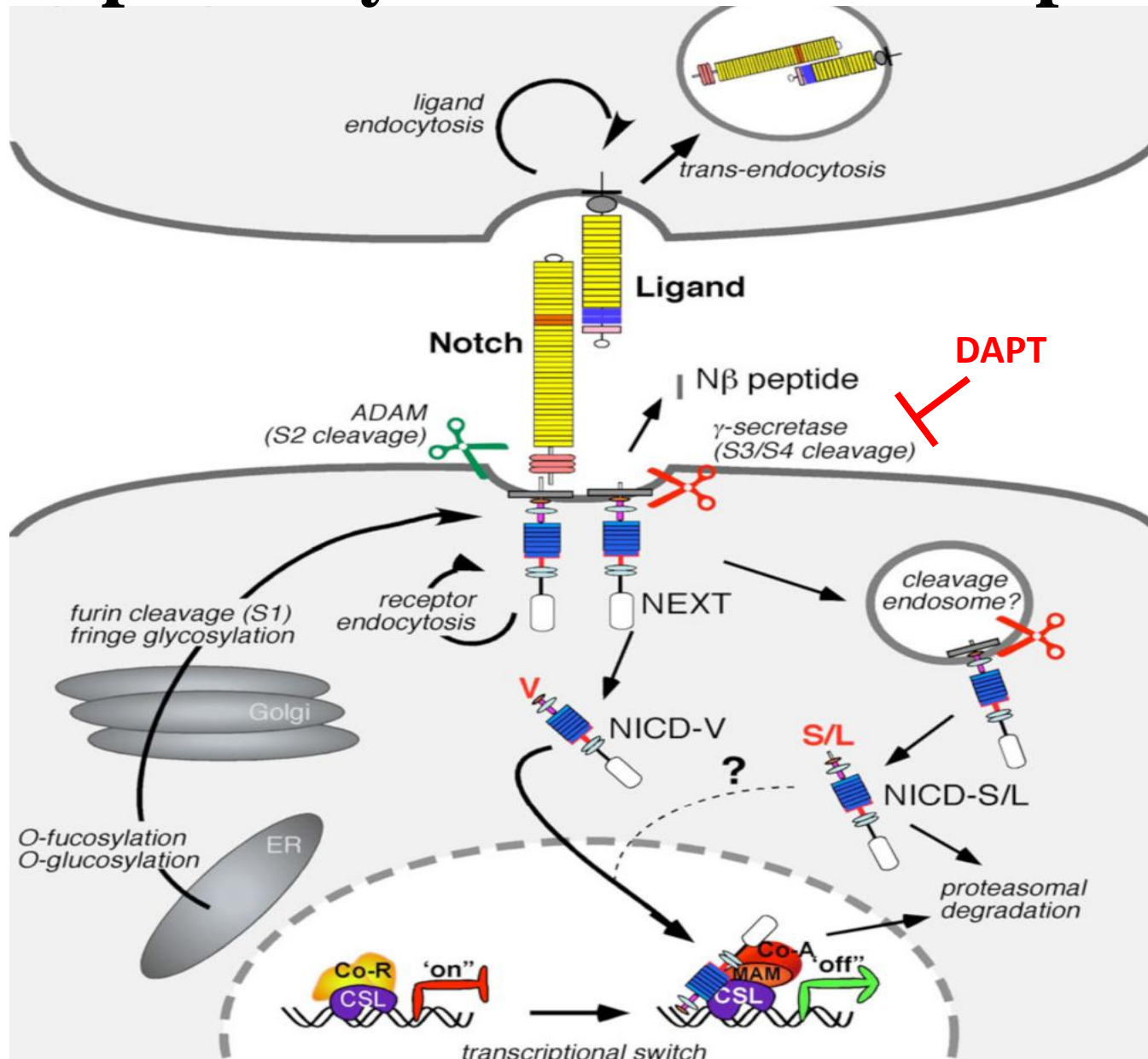


Control

**DAPT i.p**

Mice treated with 8mg per Kg  
8 injections in 2 weeks

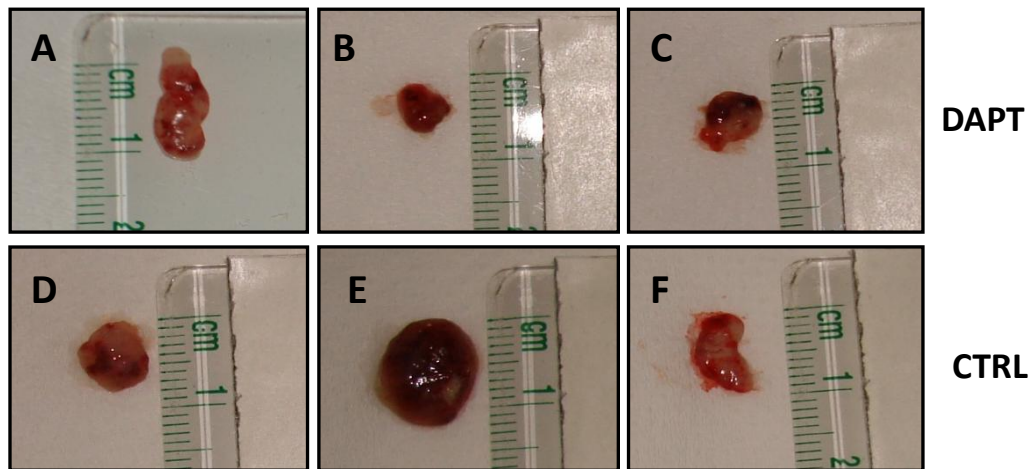
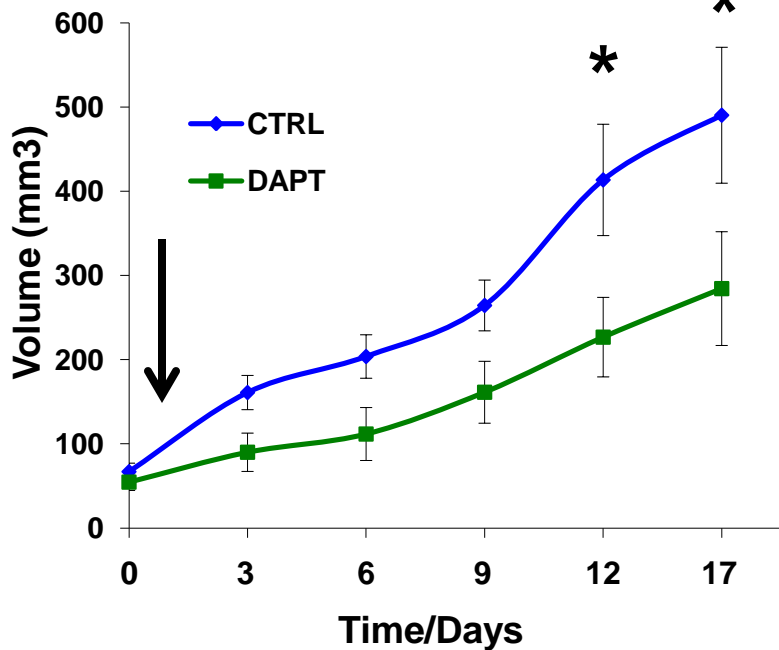
# Notch pathway and tumor development.



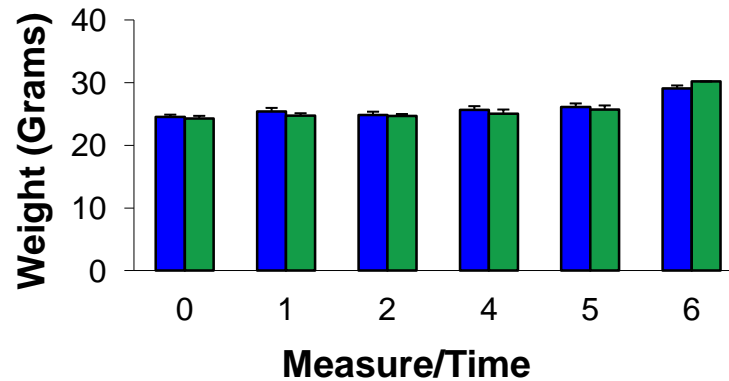


# Notch pathway inhibition with DAPT blocks tumor growth.

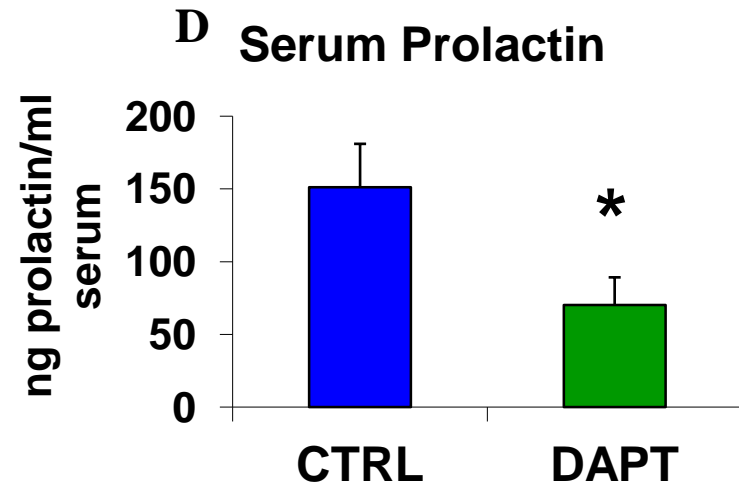
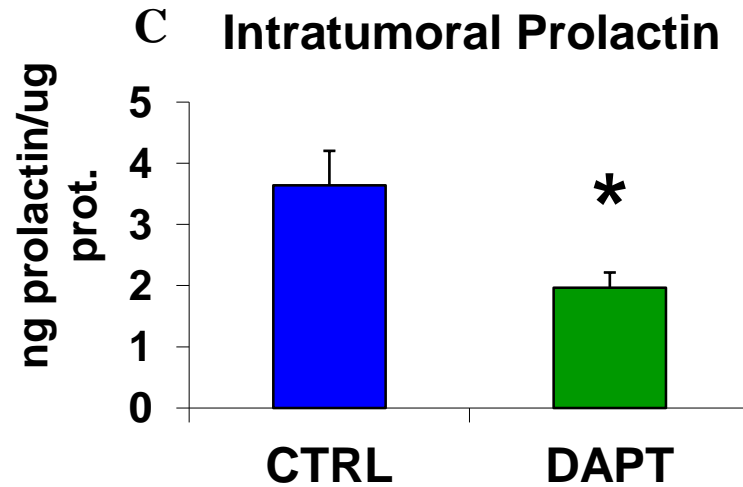
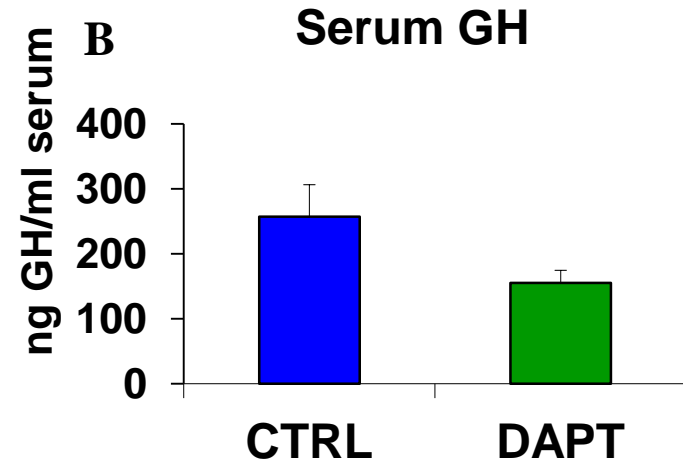
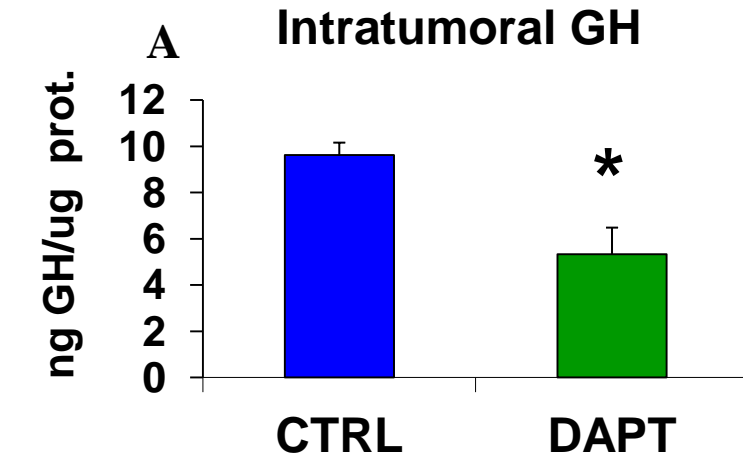
### Xenograft tumor volume



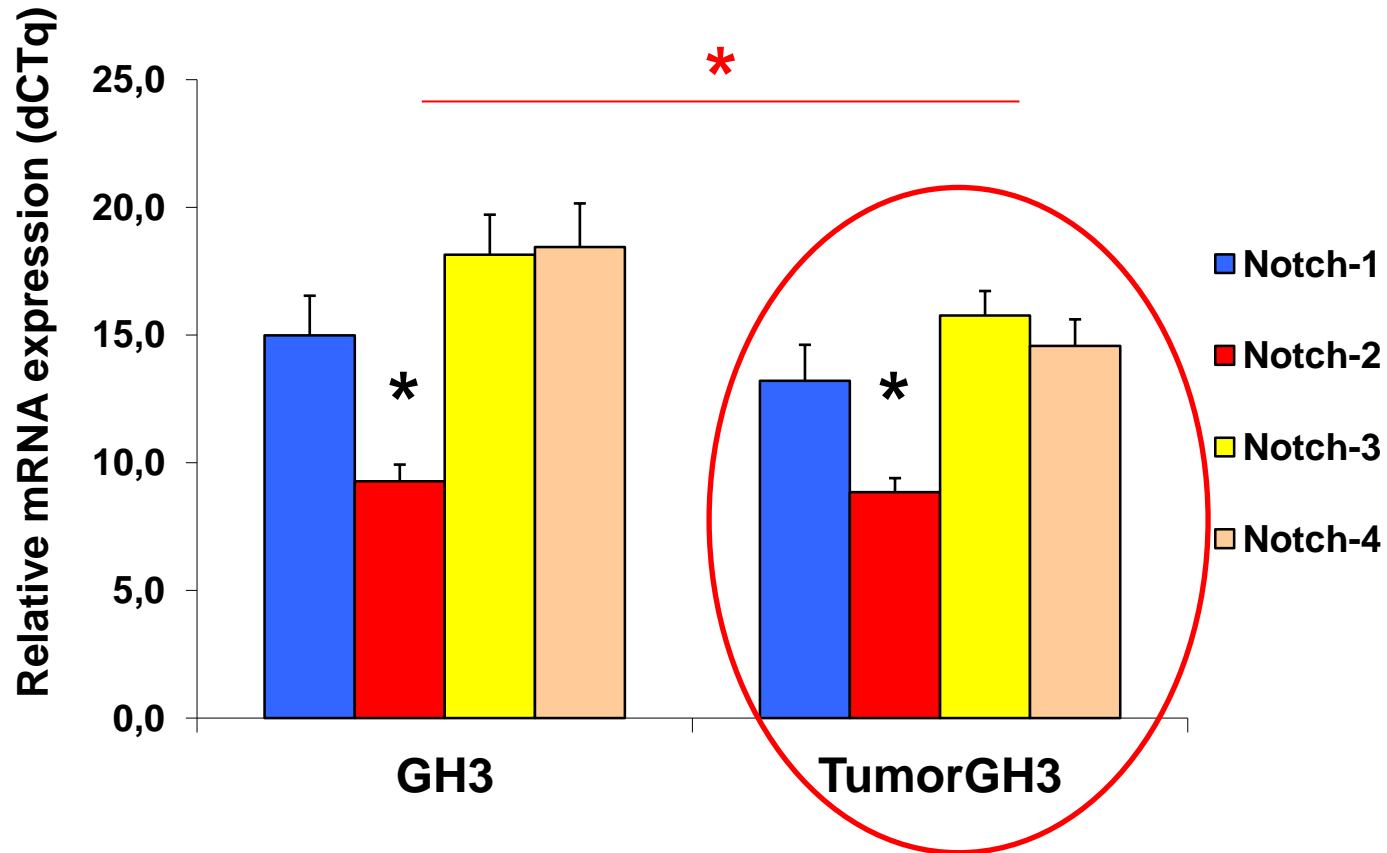
### Body Weight



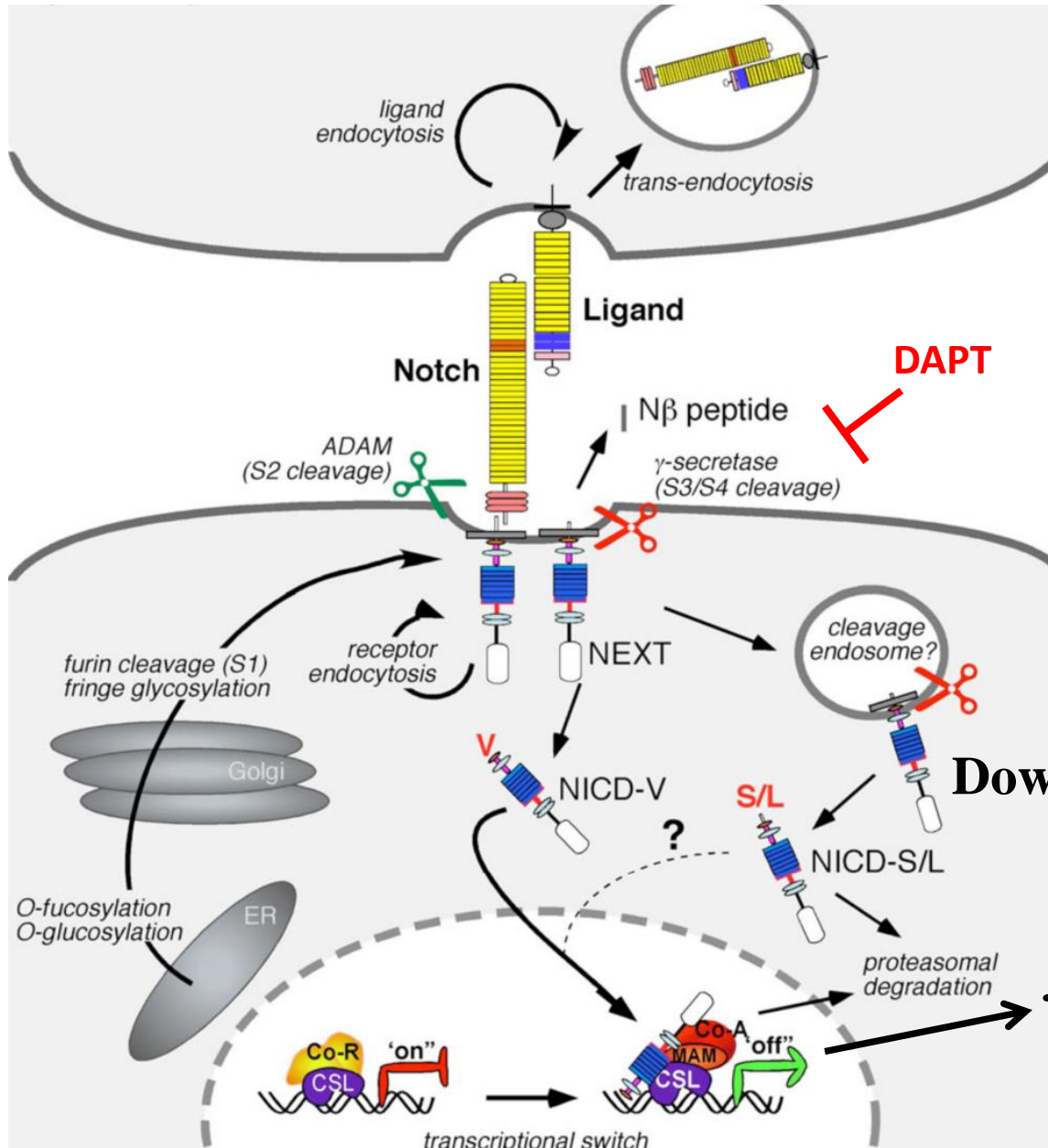
# DAPT treatment modifies the tumor secretory activity.



# Notch mRNA expression in the parental line and xenografted tumor.



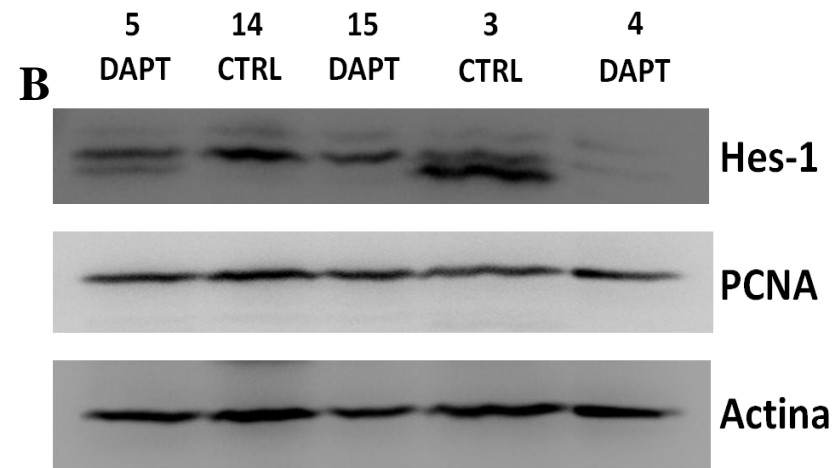
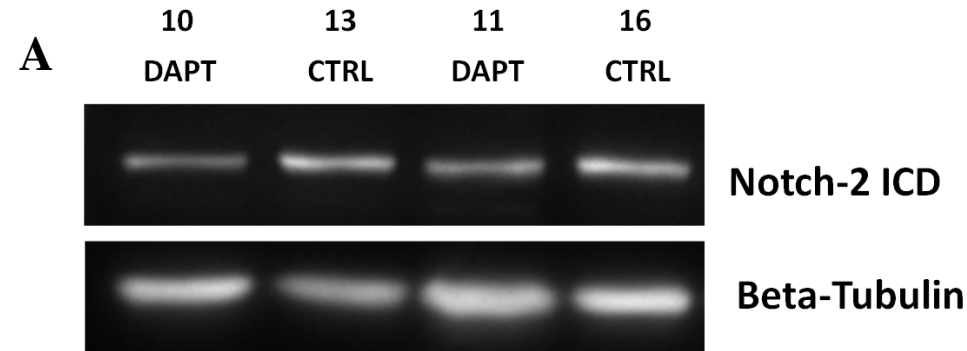
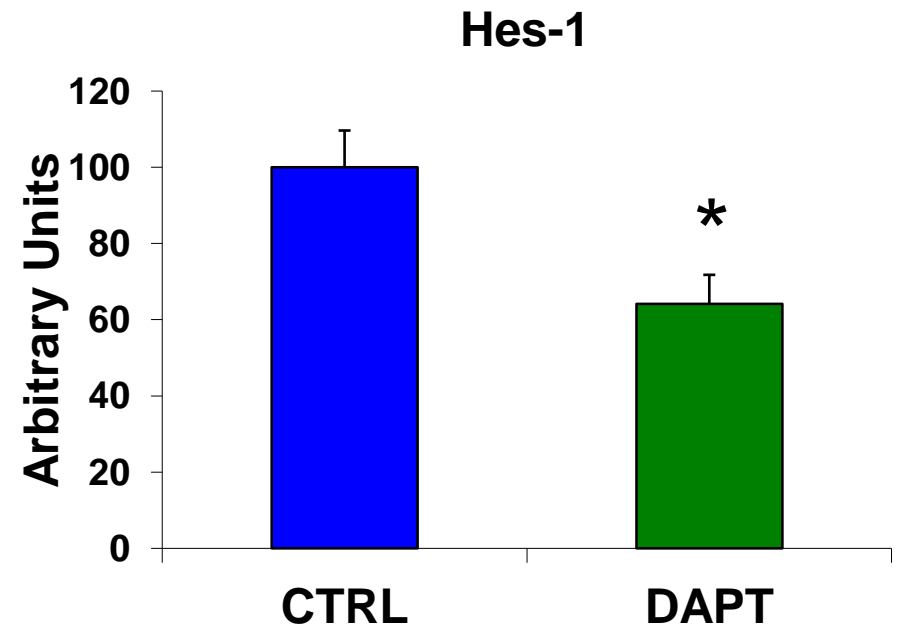
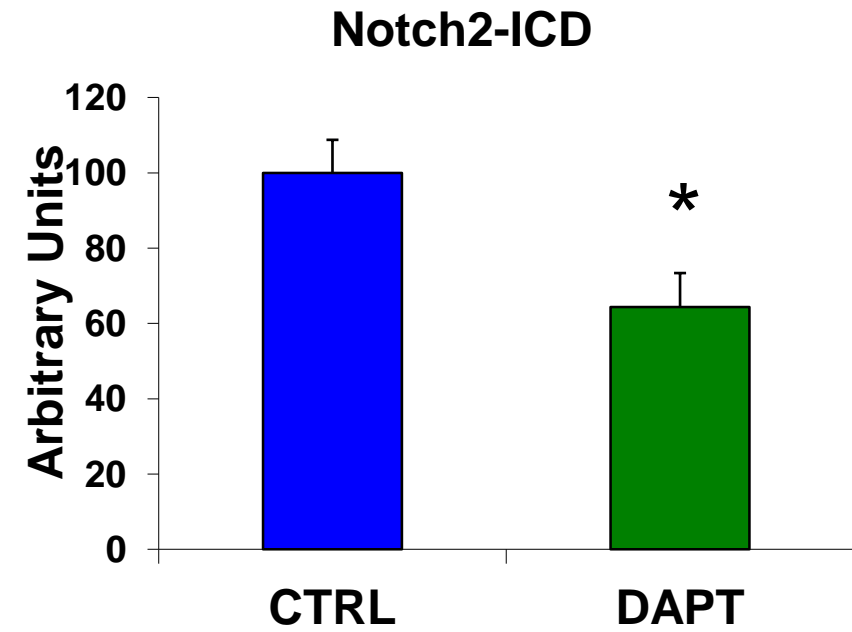
# Notch pathway and its targets.



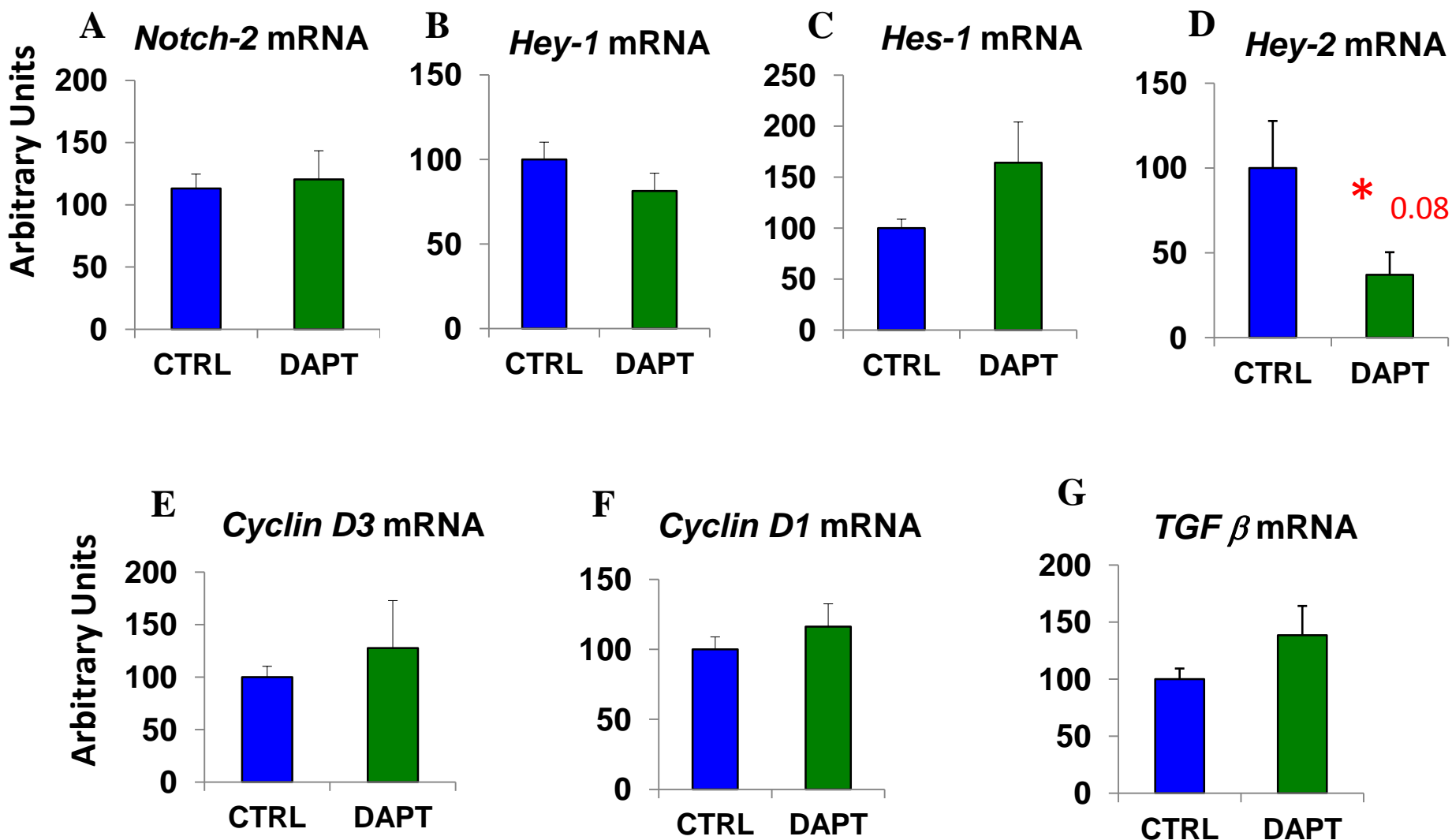
## Downstream Effectors of Notch

- Hes-1**
- Hey-1**
- Hey-2**
- Cyclin D1**
- Cyclin D3**
- TGF beta**

# DAPT treatment decreases cleaved Notch-2, and Hes-1.

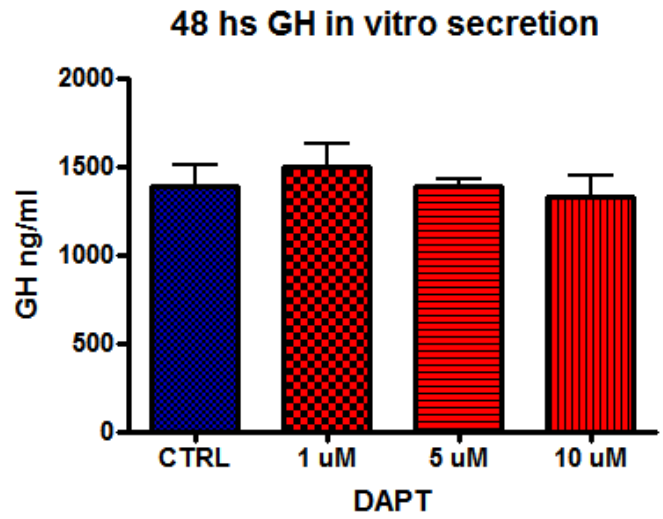
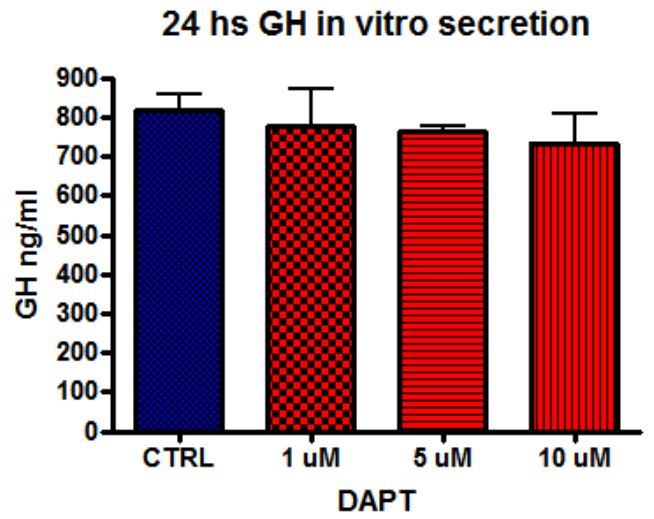
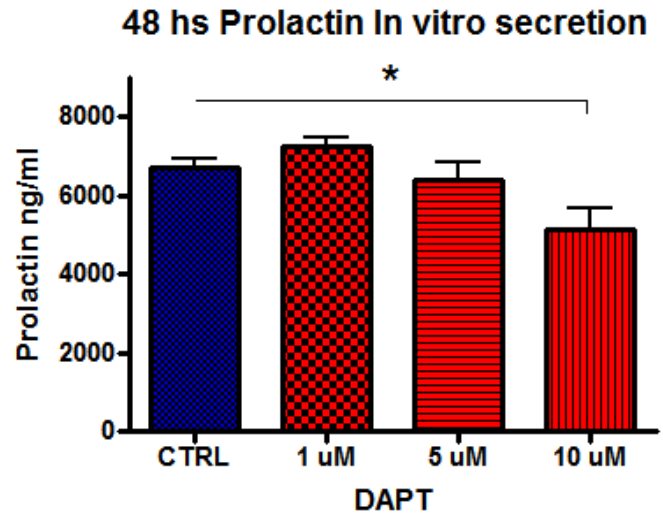
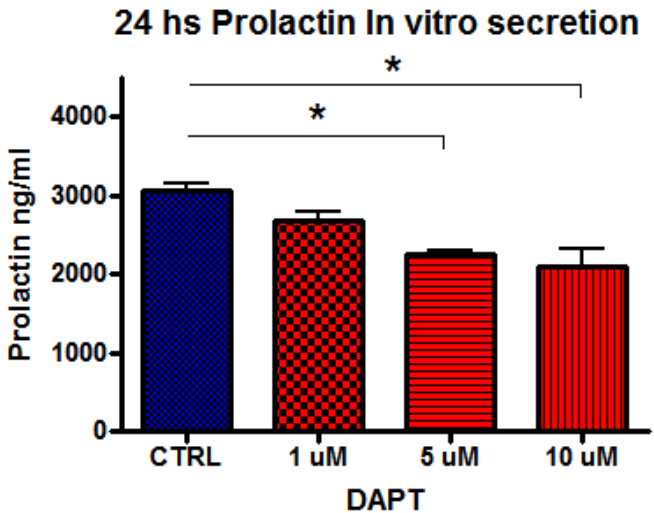


# DAPT treatment modifies Notch pathway mRNA levels.



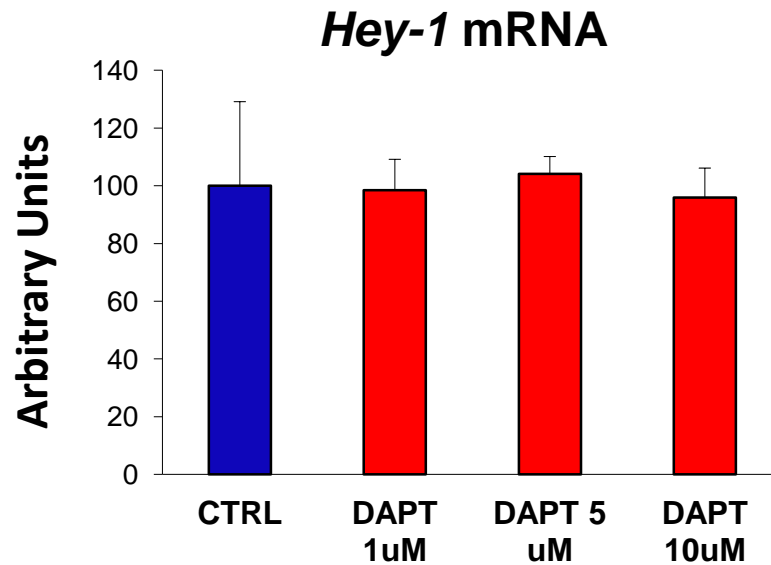
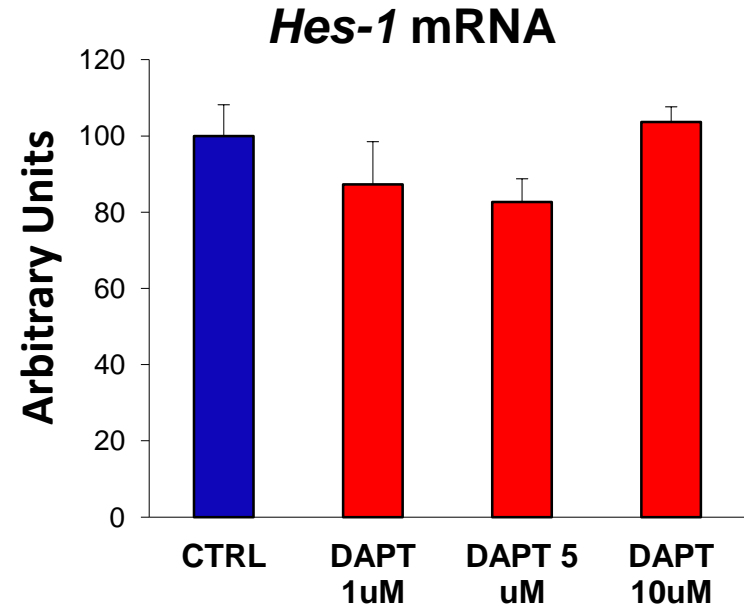
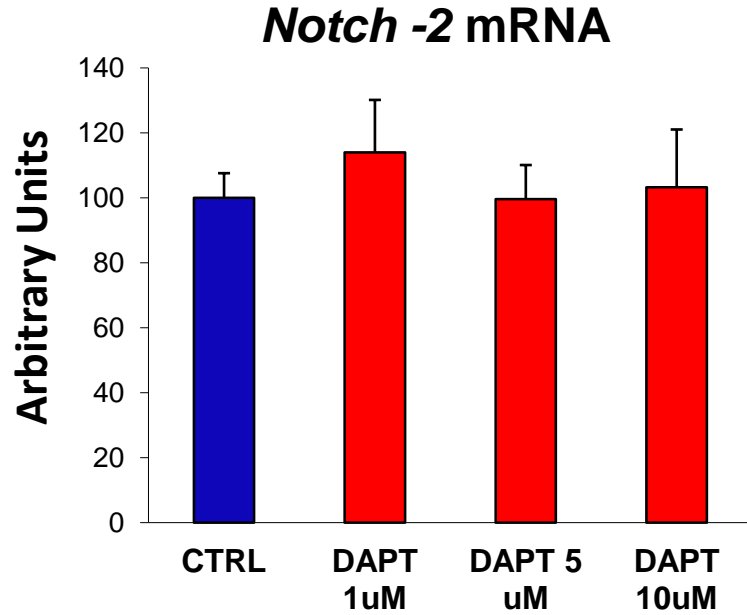
**Effect of DAPT in Notch pathway  
in GH3 cells *in vitro*.**

# DAPT treatment modifies the secretory activity of GH3 cells *in vitro*.



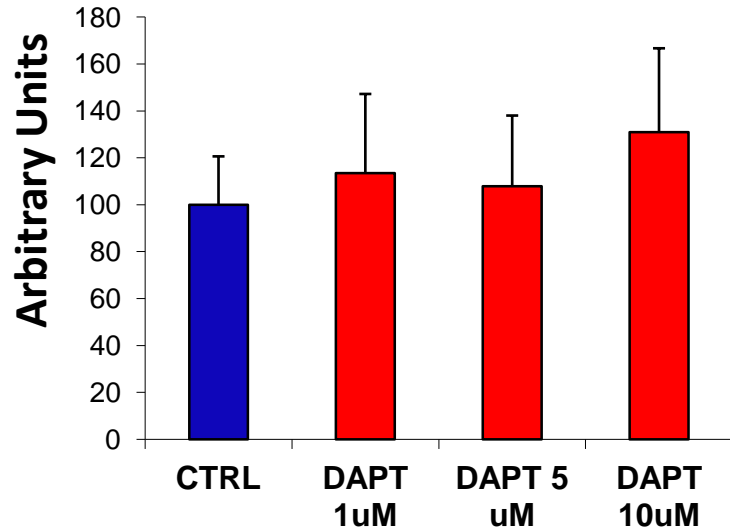


# Effect of DAPT treatment for 48 hs on Notch pathway mRNA levels in GH3 cells in vitro.

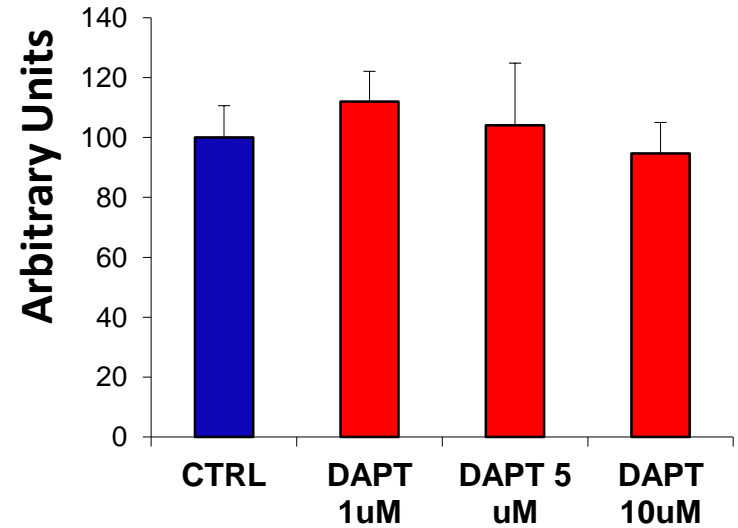


# Effect of DAPT treatment for 48 hs on Notch pathway mRNA levels in GH3 cells in vitro.

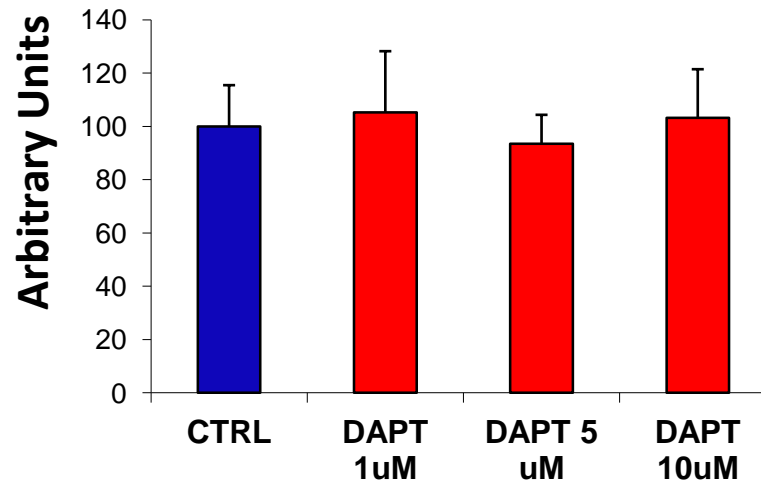
## *TGF- $\beta$* mRNA



## *Cyclin D3* mRNA

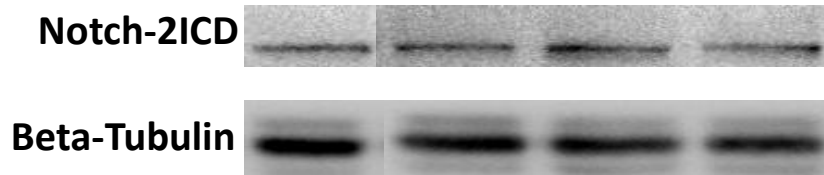
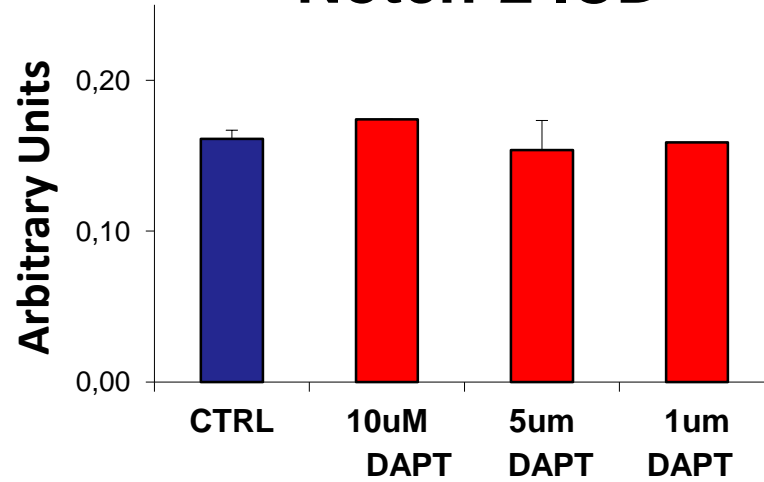


## *Cyclin-D1* mRNA

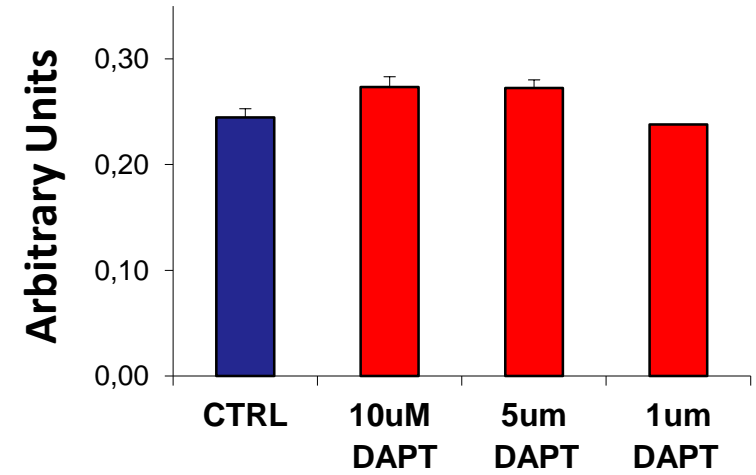


# DAPT treatment did not modify Notch pathway protein levels in GH3 cells in vitro.

## Notch-2 ICD

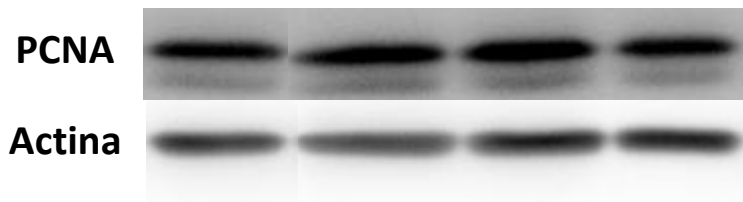
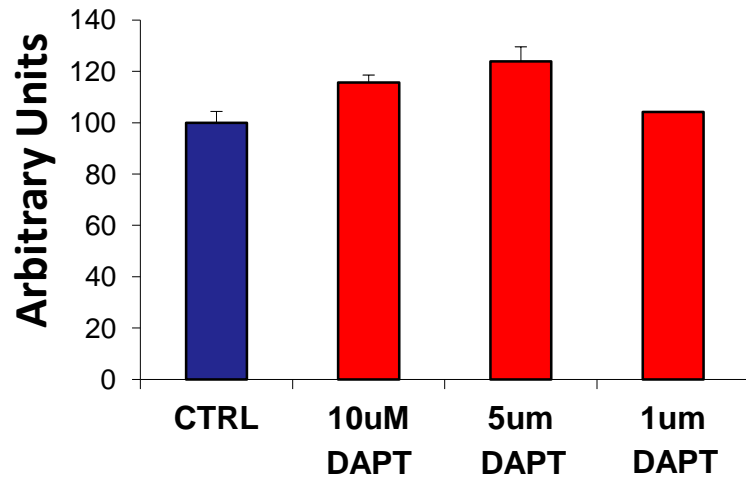


## Hes-1

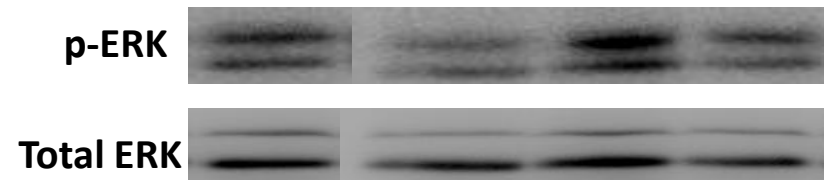
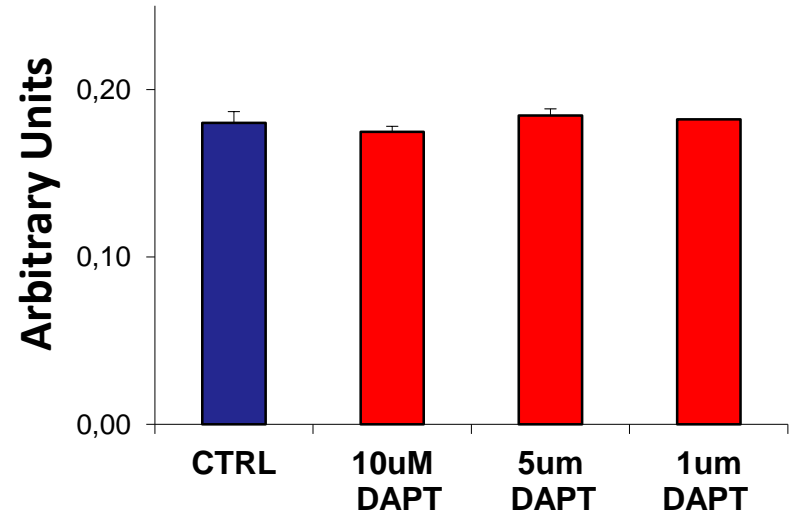


# DAPT treatment did not modify Notch pathway protein levels in GH3 cells in vitro.

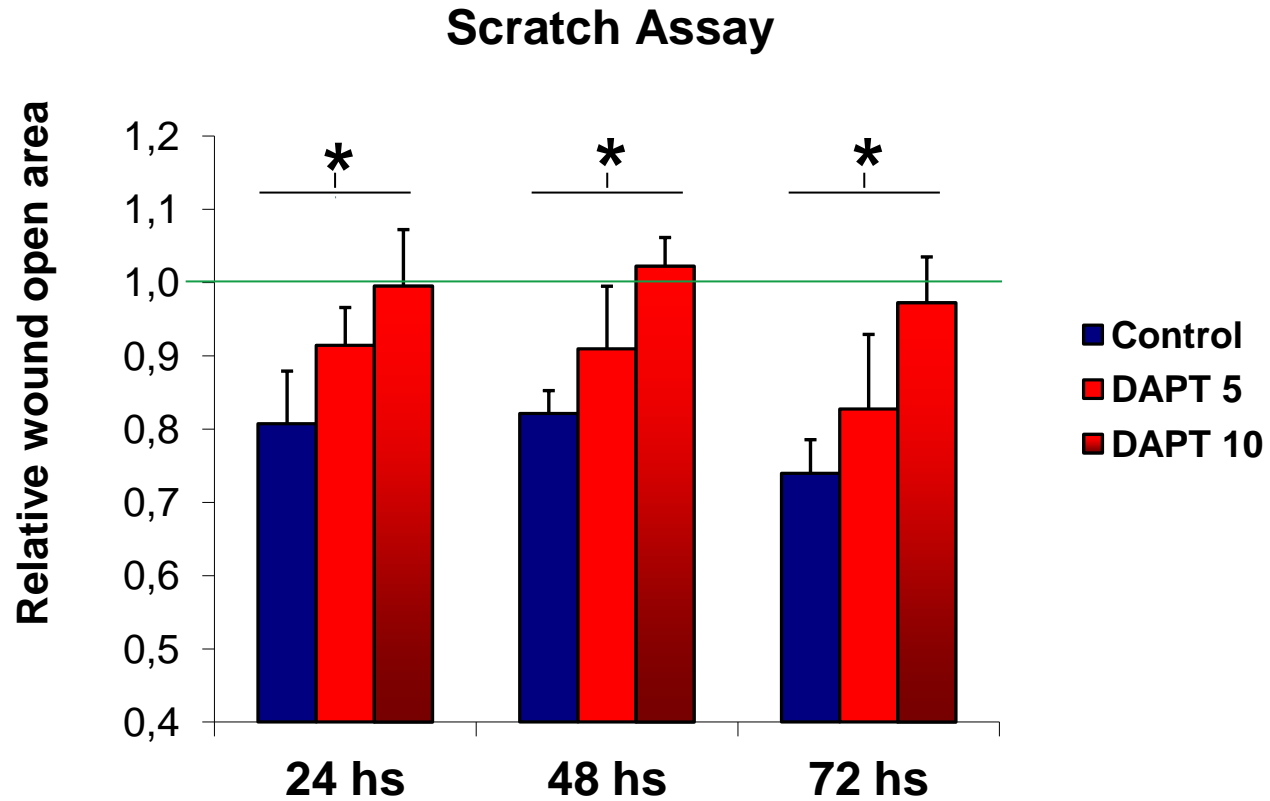
## PCNA



## pERK/ERK



# DAPT decreased migratory activity of GH3 cells in a Scratch assay.

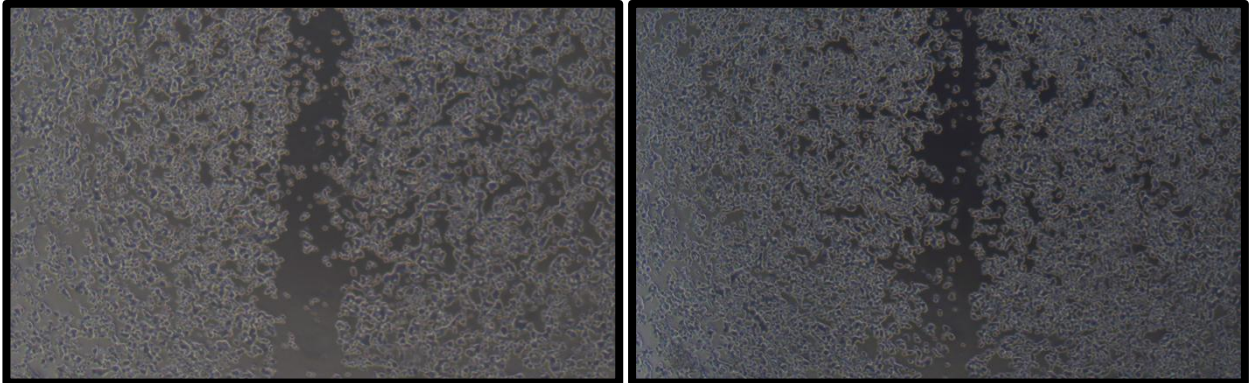


# DAPT blocks wound closure in GH3 cells in a Scratch assay.

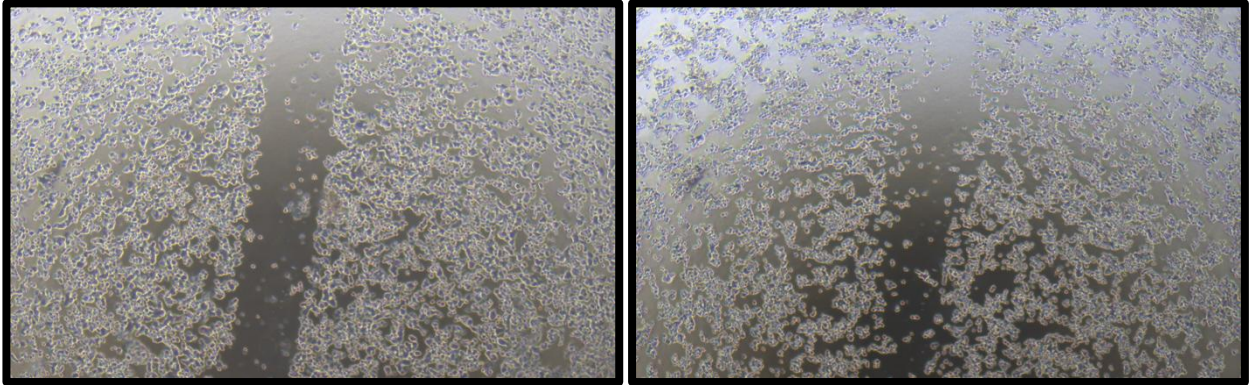
0 hs

72 hs

CTRL



10  $\mu$ M  
DAPT



# Conclusions

## In vivo Notch inhibition reduced:

- Xenograft tumor growth.
- Hormone secretory activity.
- Cleaved Notch-2 and Hes-1 protein expression.
- And Hey-2 mRNA Expression.

## In vitro Notch inhibition reduced:

- GH3 cell secretory activity.
- And wound healing, or migratory activity.

**These data highlight a fundamental role of Notch signaling in pituitary tumor generation.**

**;;Thank You Very Much!!**