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## The inducible KPC:APC mouse organoid model for colon carcinogenesis studies

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## Introduction

Every day, 500,000 CT-scans are performed worldwide, and this number is on the rise<sup>1</sup>.

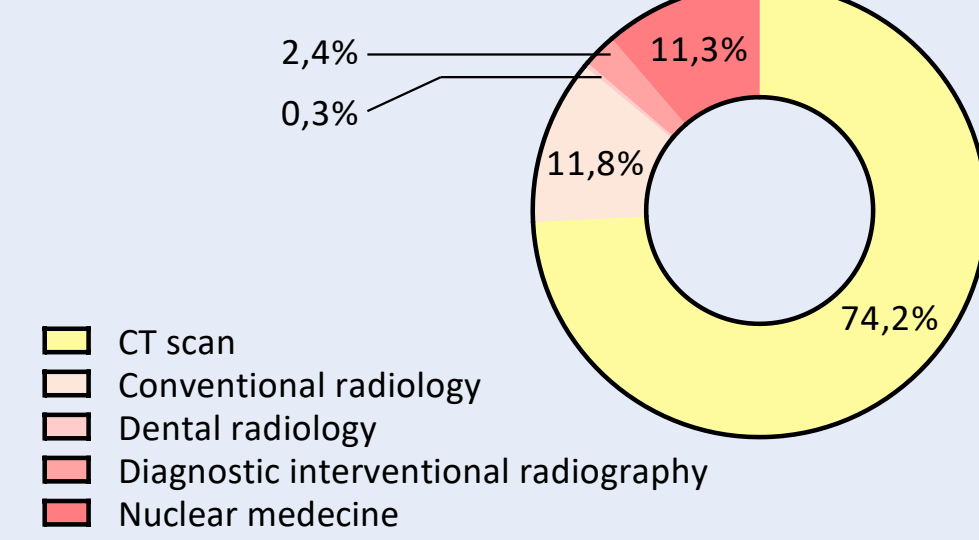
Abdominal and pelvic CT-scans have the highest LAR (Lifetime Attributable Risk) of solid cancer. They deliver 8–31 mGy per scan on average<sup>2,3</sup>. An increasing number of patients are exposed to doses >100 mSv<sup>1</sup>.

### Ionizing radiation and colon cancer

Epidemiological studies show an increased risk of colorectal cancer after low dose medical radiation exposure (LDR) but benefits still outweigh the risk of radio-induced cancer<sup>4</sup>. However, LDR increase tumor multiplicity and interact with carcinogenesis-related pathways through gene expression modulation, DNA hypomethylation or protein interactions<sup>5,6,7</sup>.

The *Apc*<sup>min</sup> mouse is a widely used model of colorectal cancer but inducible models are more suitable for colon carcinogenesis studies.

Origins of the collective effective dose attributable to the diagnostic medical imaging sector



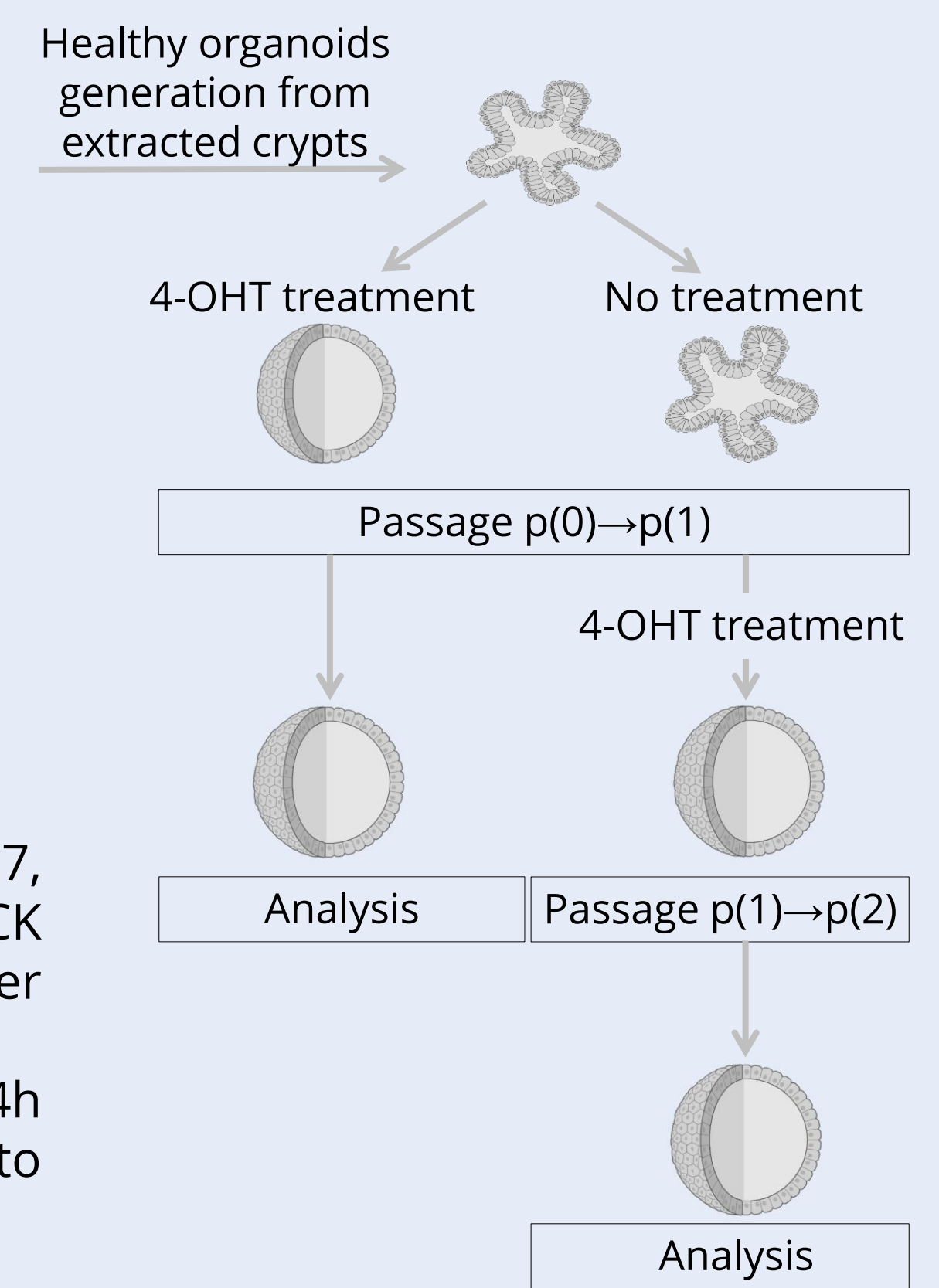
**Objectives** → To develop and characterize suitable *in vivo* and organoid models to study colon carcinogenesis after medical diagnostic radiation exposure.

## Methods

**KPC:APC**  
Inducible colon cancer mouse model  
B6.Cg-*Kras*<sup>tm4Tyj</sup> *Apc*<sup>tm1Tno</sup> Tg(CD<sub>2</sub>-cre/ERT2)<sup>752Erf/Maraj</sup><sup>8</sup>  
o CreLox system  
o Under control of the CD<sub>2</sub> promoter  
*Kras*<sup>+/+</sup> *Apc*<sup>+/+</sup> Cre<sup>ERT2+</sup> (*ApcKras*)  
*Kras*<sup>+/+</sup> *Apc*<sup>+/+</sup> Cre<sup>ERT2+</sup> (*Apc*)  
*Kras*<sup>+/+</sup> *Apc*<sup>+/+</sup> (Control)

Organoids were grown in complemented Matrigel (B27, N2, ROCK inhibitor, EGF and N-acetylcysteine). ROCK inhibitor was added to the medium for 3 days after culture initiation and organoid passaging. Organoids were treated with 4-OHT (0,125–1µM) for 24h two days after culture initiation at passage 0 or 1 to induce *Apc* and/or *Kras* mutations.

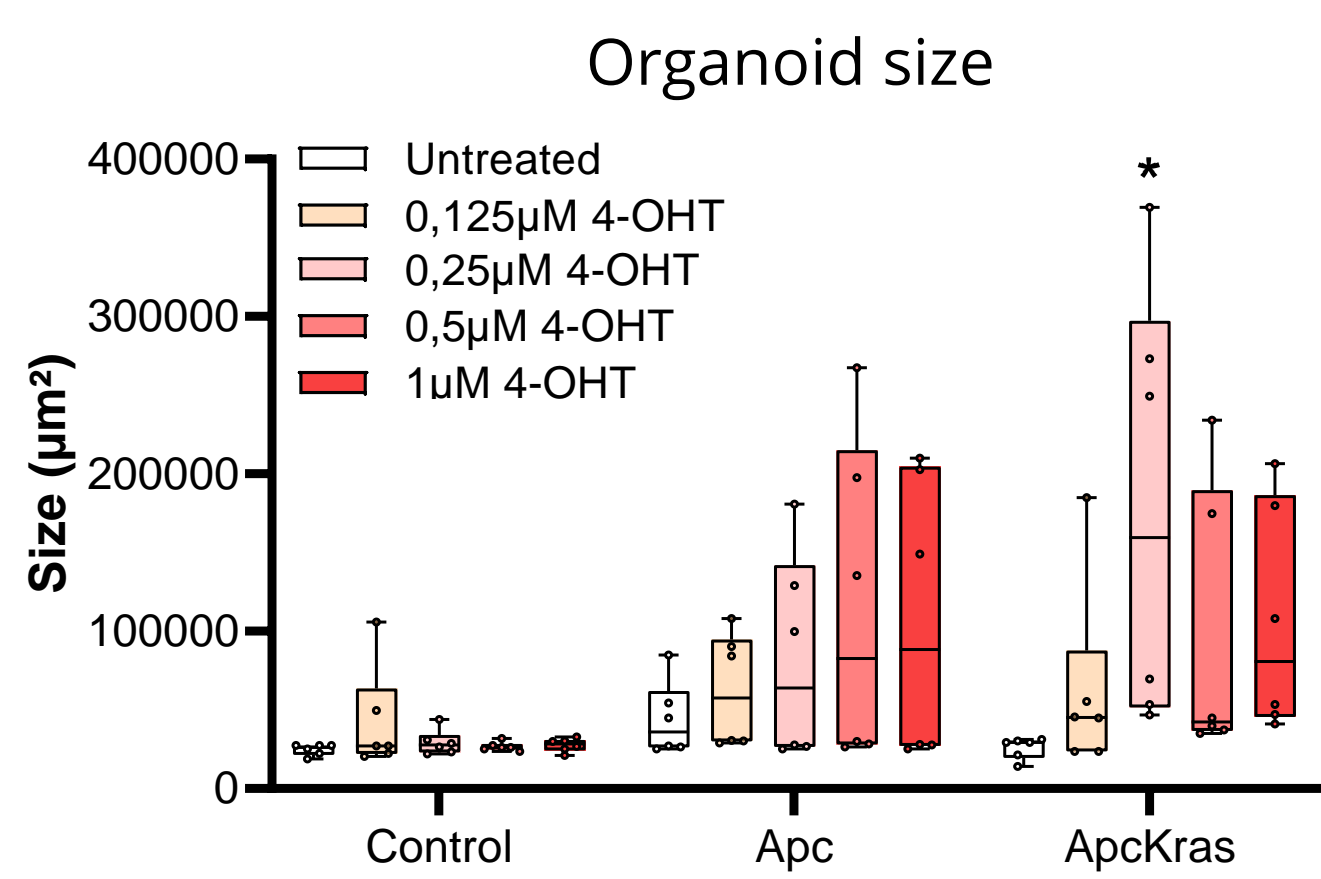
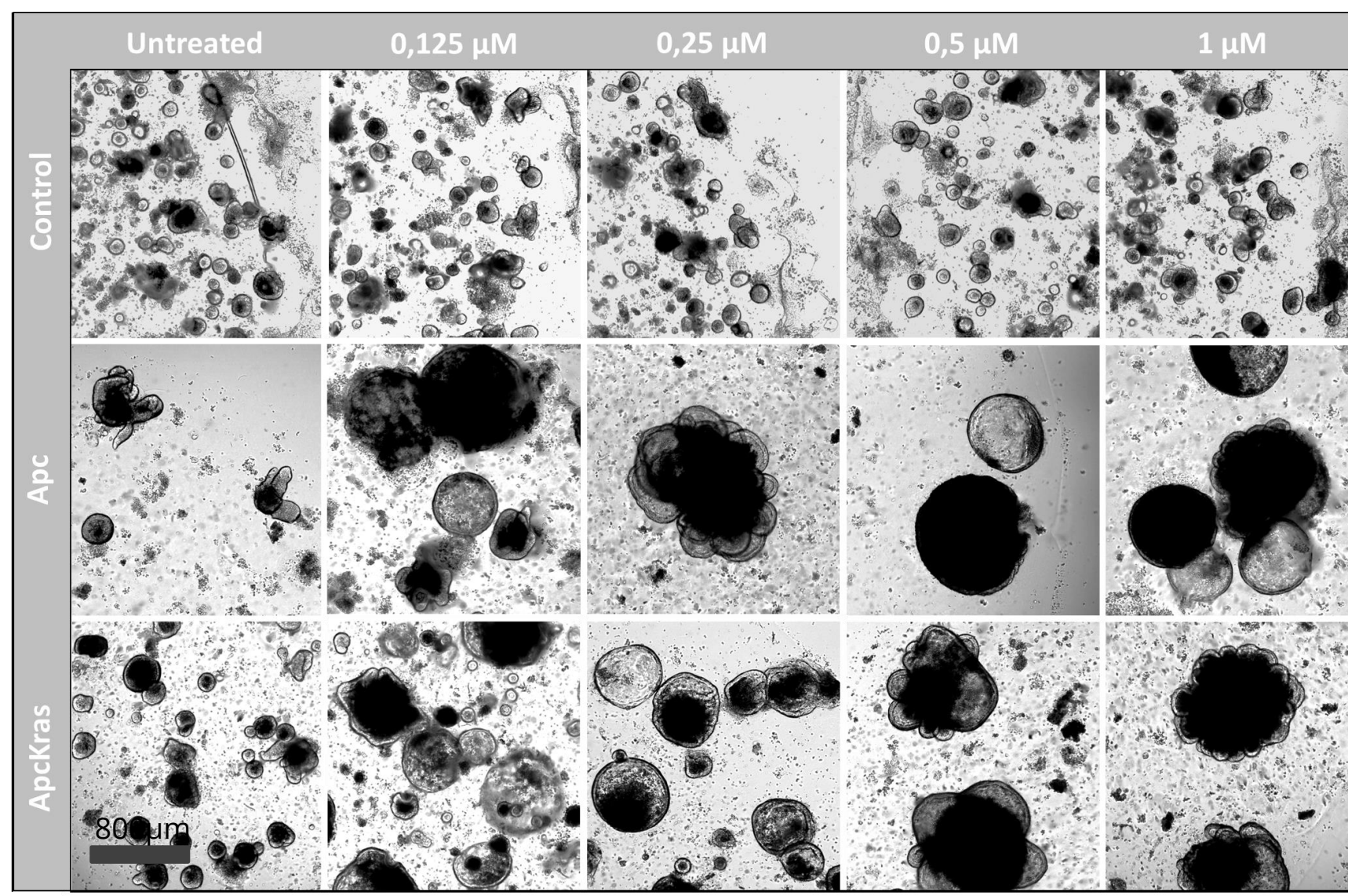
### Organoid generation and carcinogenesis induction



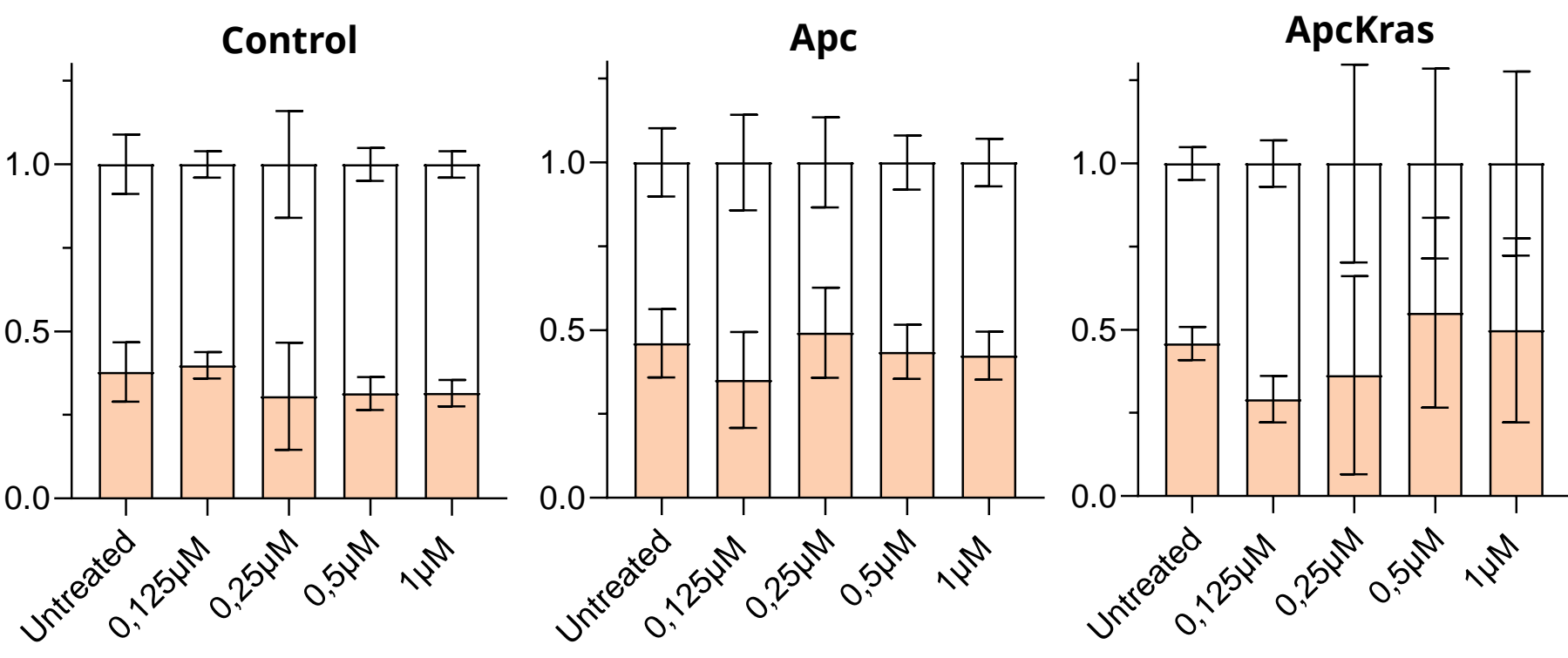
## Results

### Treatment on the second week of culture p(1)

Dose effect at p(2)



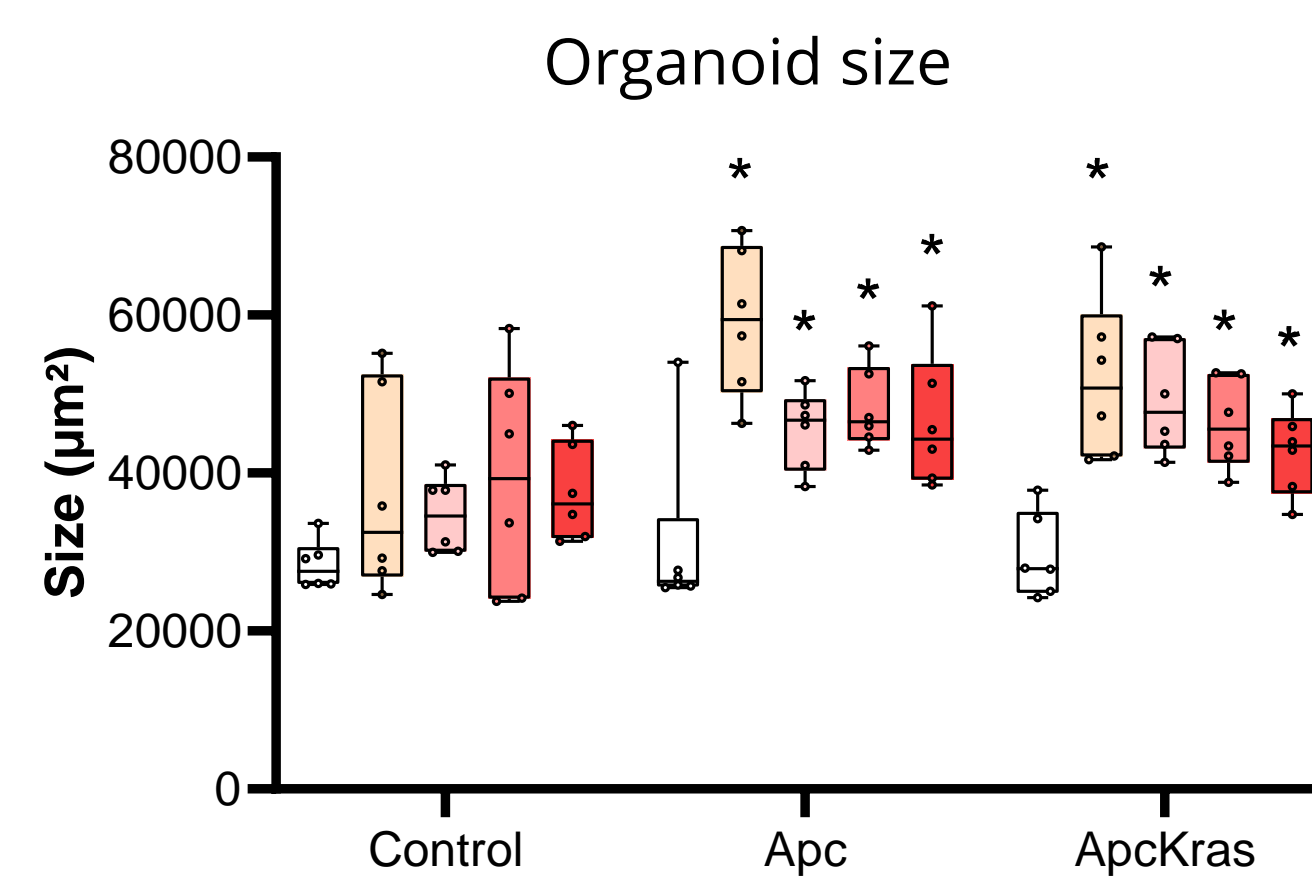
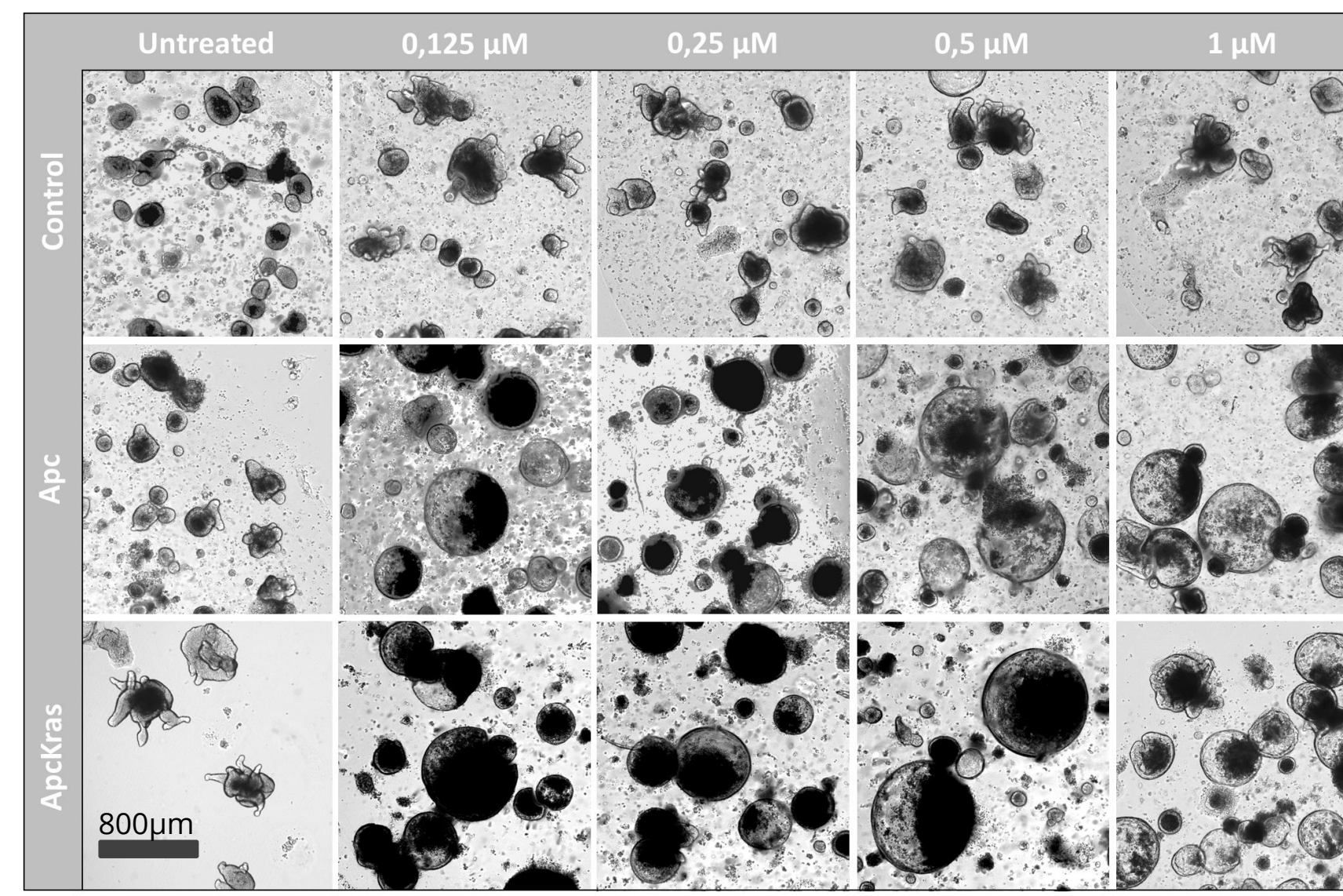
### Proportion of spheroids and organoids for each genotype



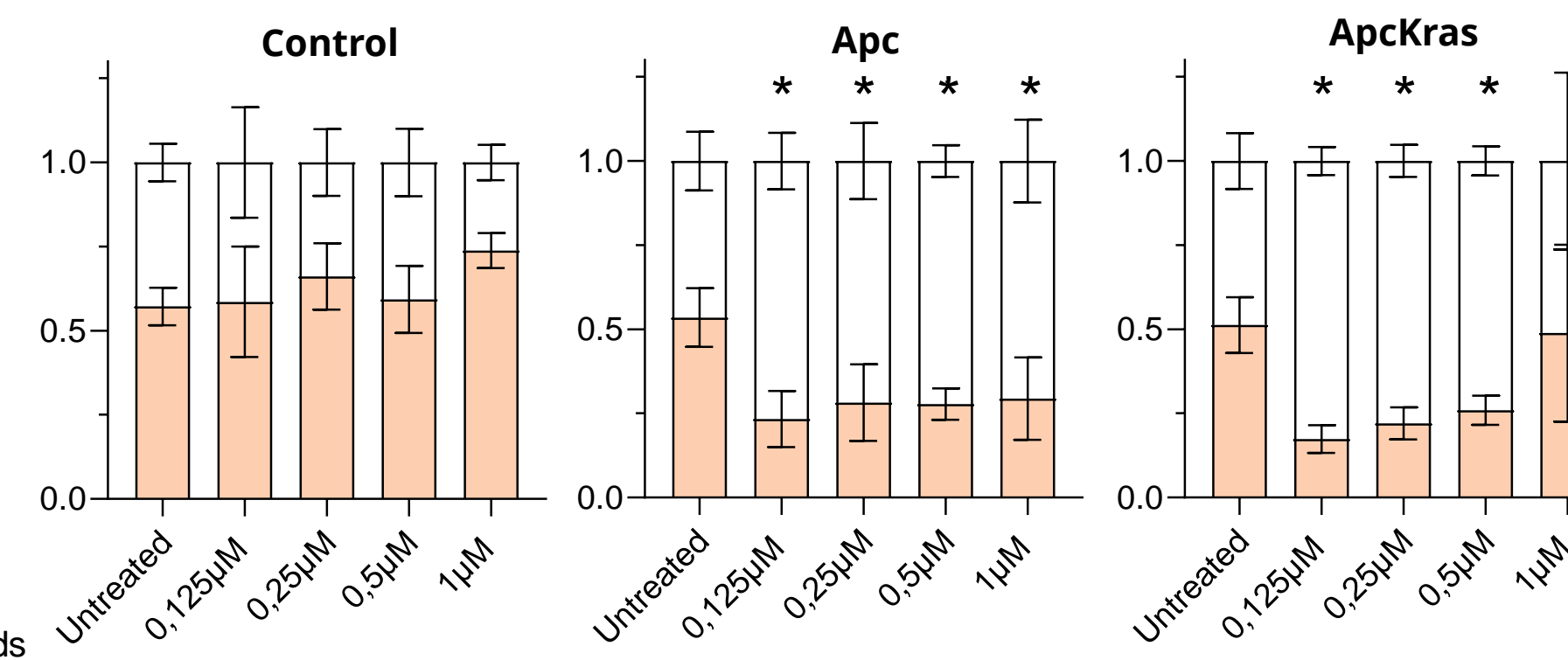
N=2; n=3 – Dunnett's multiple comparison test, each treated group was compared to the corresponding untreated genotype, \*: p-value < 0,05

### Treatment on the first week of culture p(0)

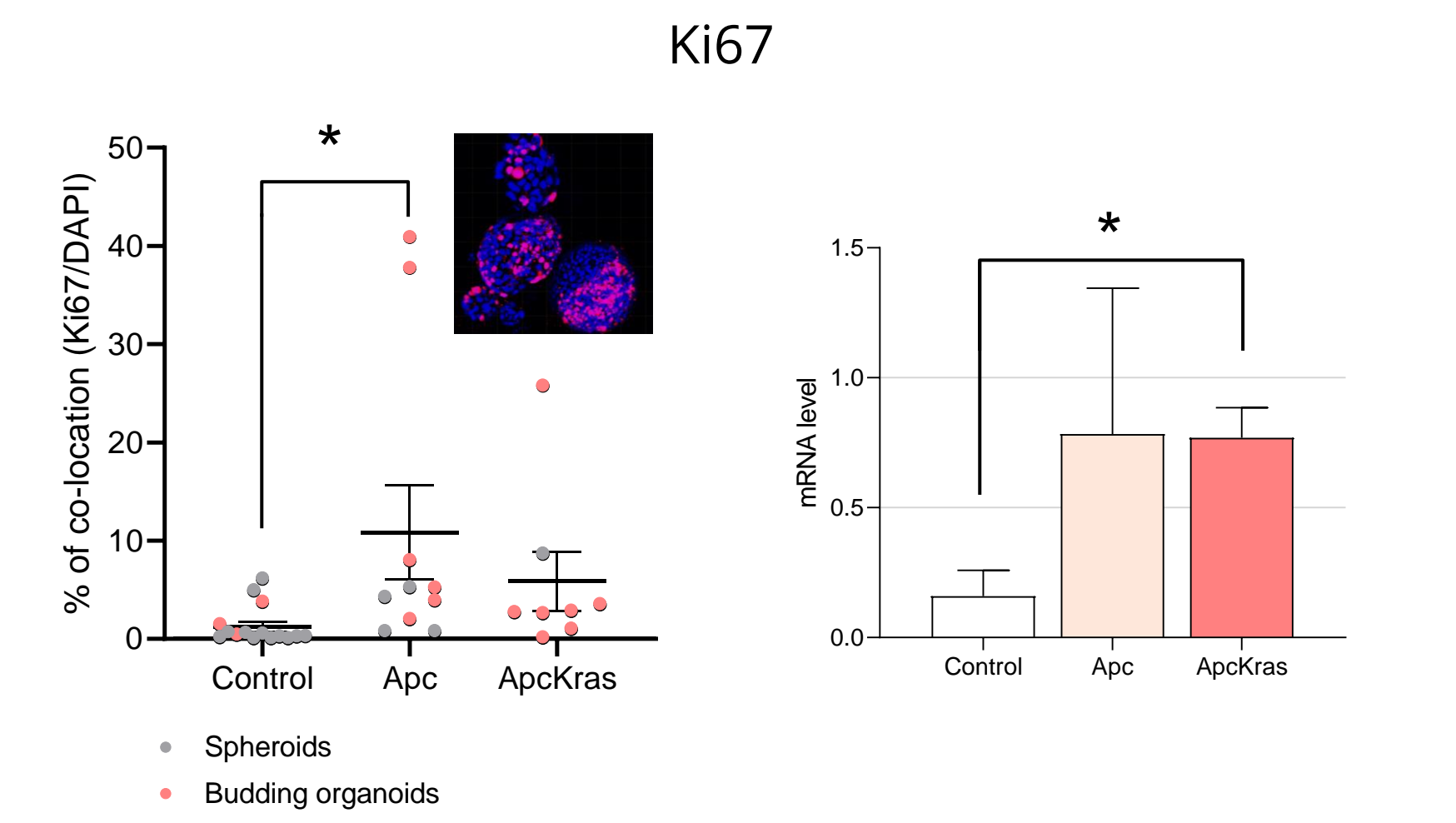
Dose effect at p(1)



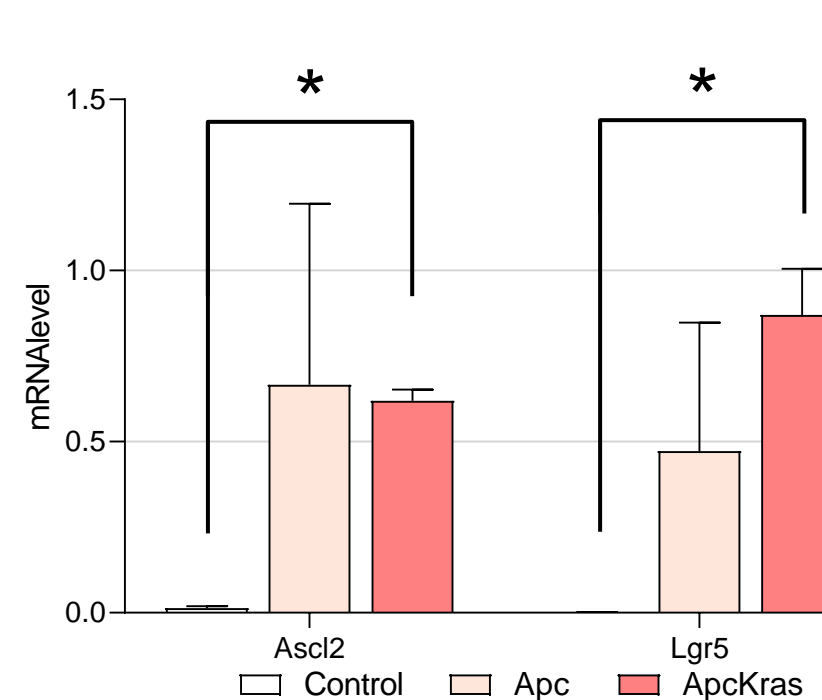
### Proportion of spheroids and organoids for each genotype



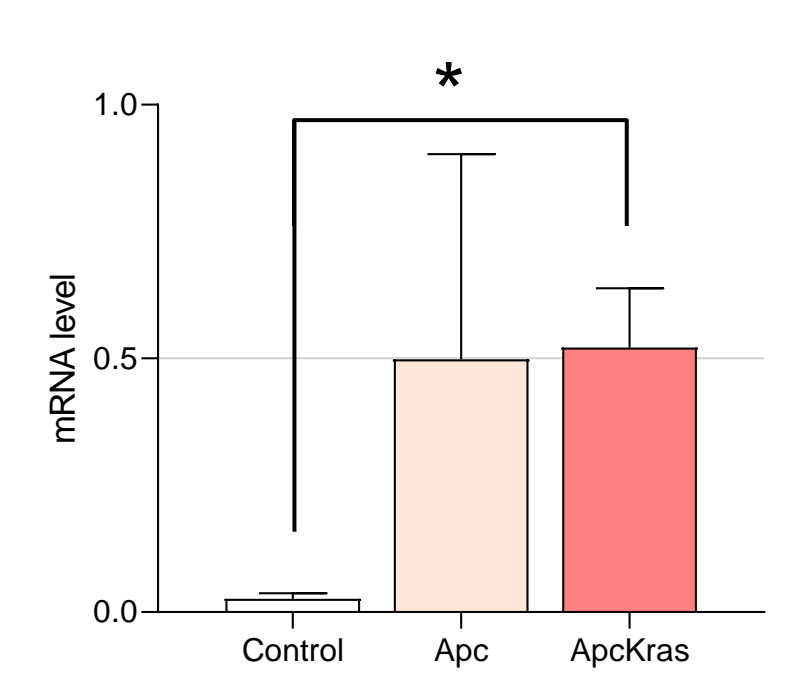
### Characterization after treatment with 0,125µM of 4-OHT



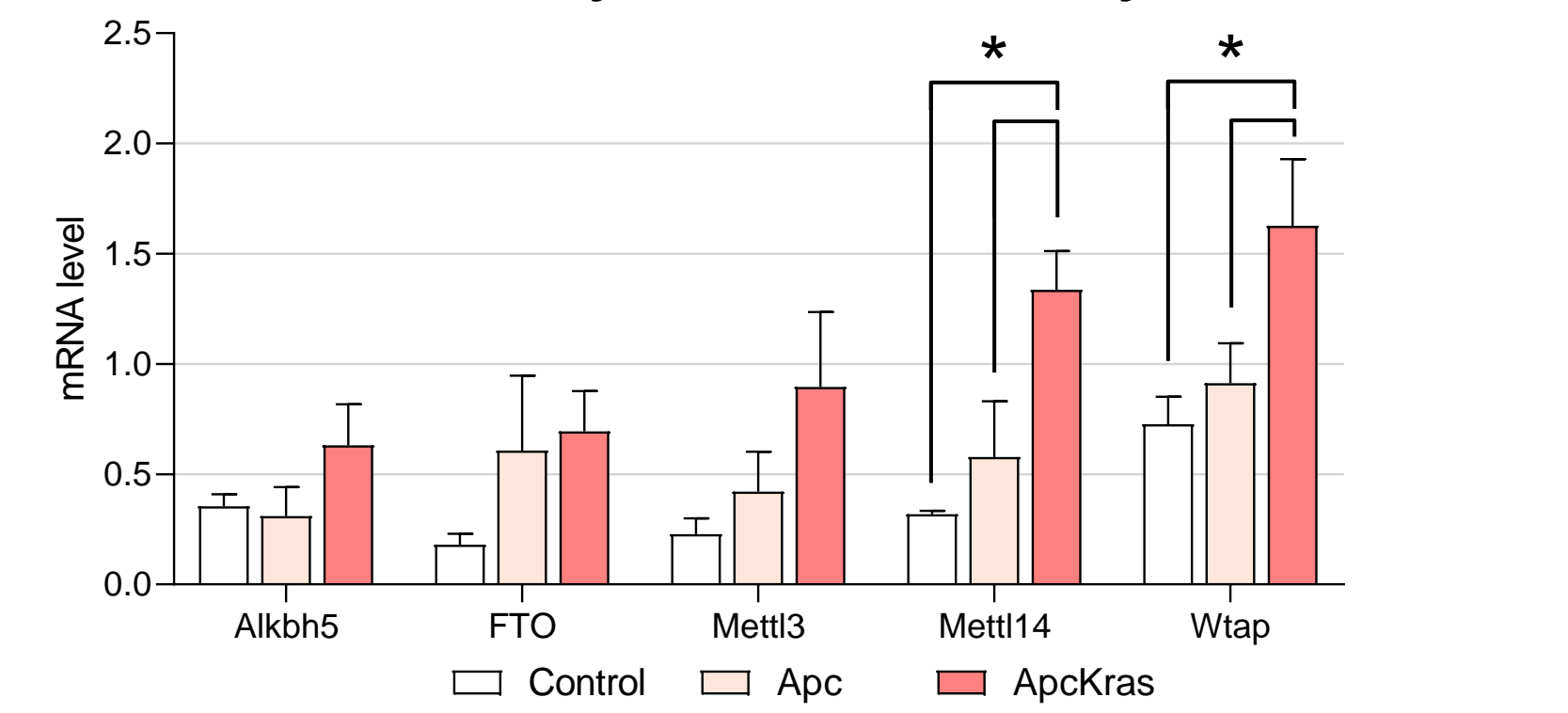
### Stem cell markers



### Wnt pathway activation



### RNA methylases and demethylases



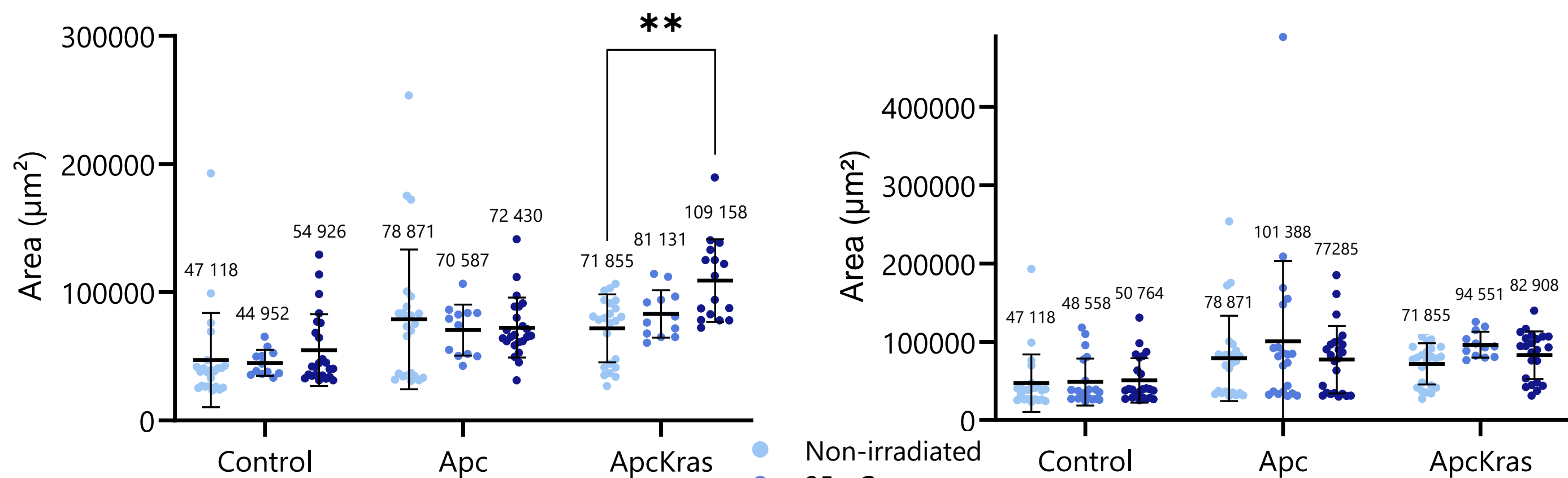
n=3, Standard deviation, Two-way ANOVA with Tukey multiple comparison. \*: p-value < 0,05

## Application

Organoids were irradiated with 25 or 250mGy X-rays. The irradiation was performed on the 5<sup>th</sup> day of p(0) (early irradiation), or on the 5<sup>th</sup> day of p(1) (late irradiation).

### Effect of early irradiation on organoids size

### Effect of late irradiation on organoids size



N>2; n=8 – Dunnett's multiple comparison test, \*: p-value < 0,05

## Conclusions

We have developed a new KPC:APC dynamic organoid model which allows the induction and study of colon carcinogenesis *in vitro*. After inducing *Kras* and/or *Apc* mutations, the organoids exhibited increased size and a characteristic cystic morphology. Markers for proliferation, stemness and Wnt pathway activation were also increased, suggesting that this model may be able to recapitulate colon carcinogenesis *in vitro*.

Organoids derived from KPC:APC mice therefore provide the opportunity to perform parallel *in vivo* histopathological and *in vitro* mechanistic studies to advance research on colon carcinogenesis and to investigate the effects of low-dose radiation exposures.

## References

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