

# Cytokinesis inhibition in the liver drives polyploidization and HCC prevention

Hao Zhu, MD

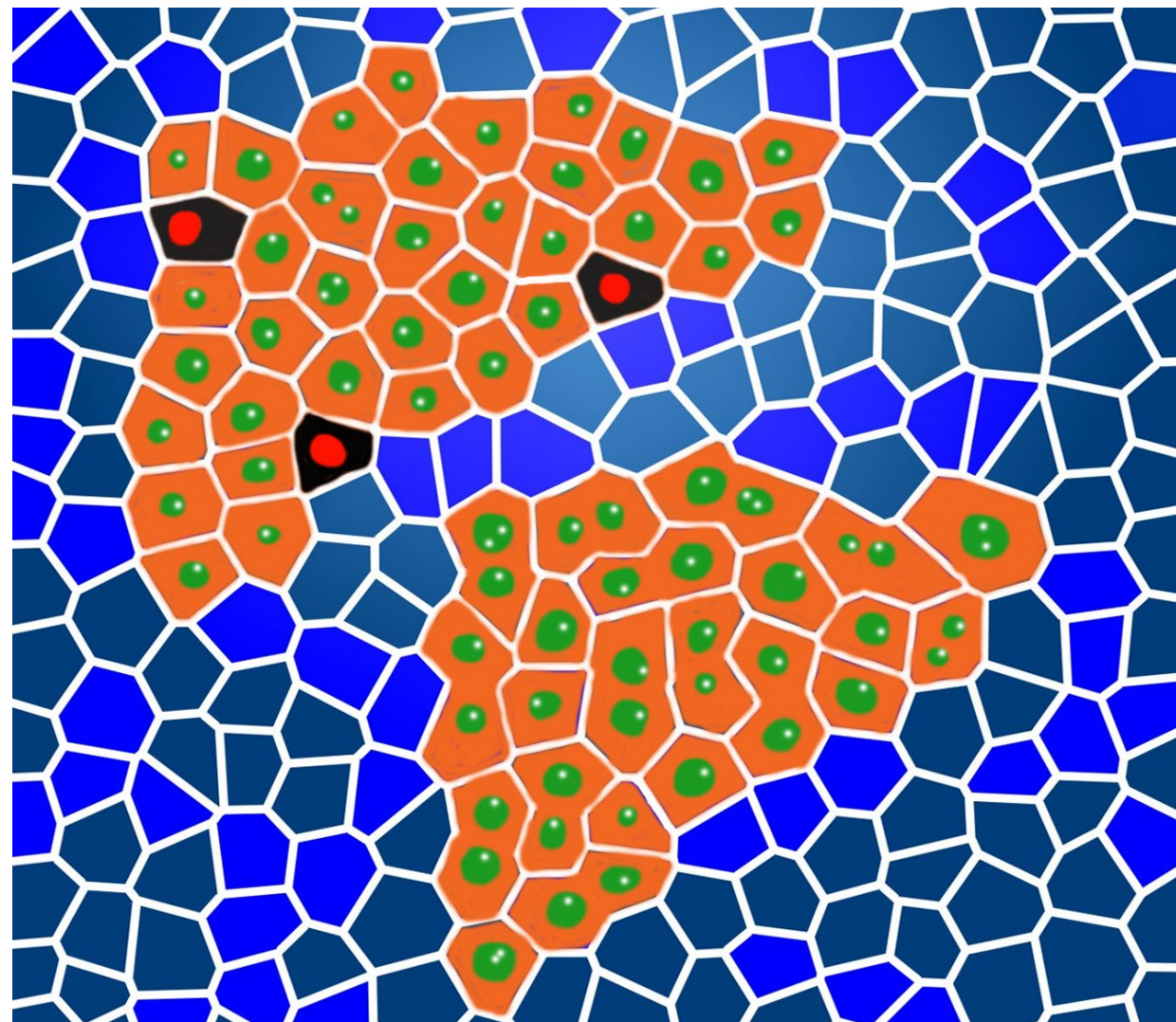
October 17, 2020

**UT Southwestern**  
Medical Center



CHILDREN'S MEDICAL CENTER  
**RESEARCH INSTITUTE**  
AT UT SOUTHWESTERN

*Relentless discovery toward the treatments of tomorrow*



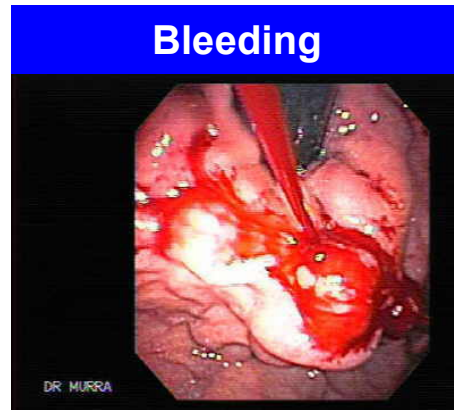
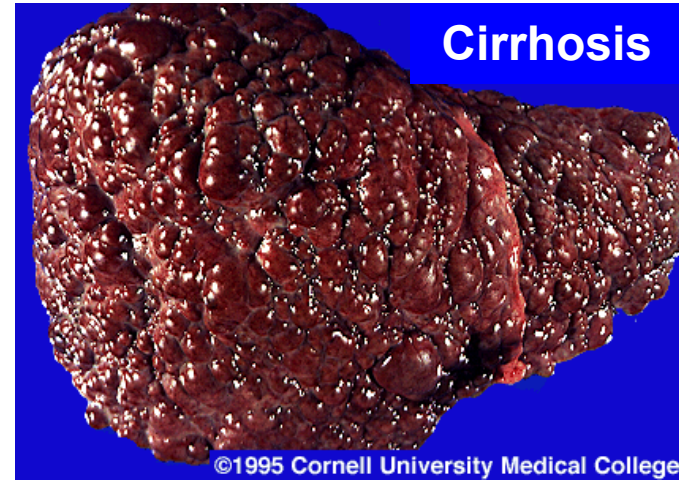
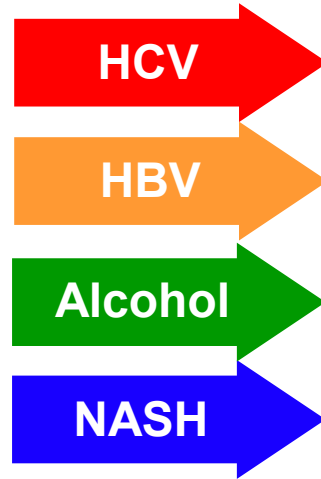
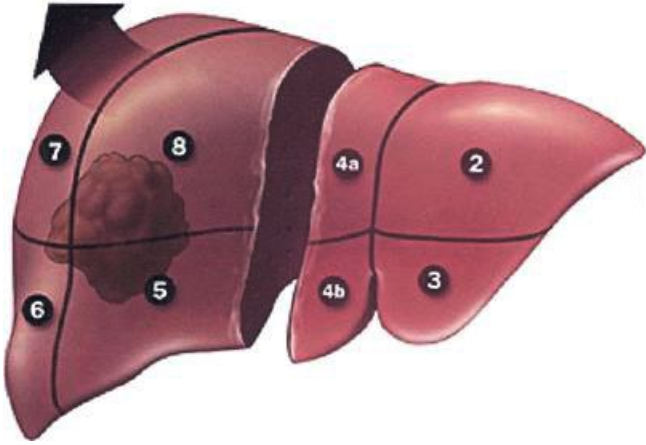
# **Conflicts of interest**

**I consult for 28-7 Therapeutics**

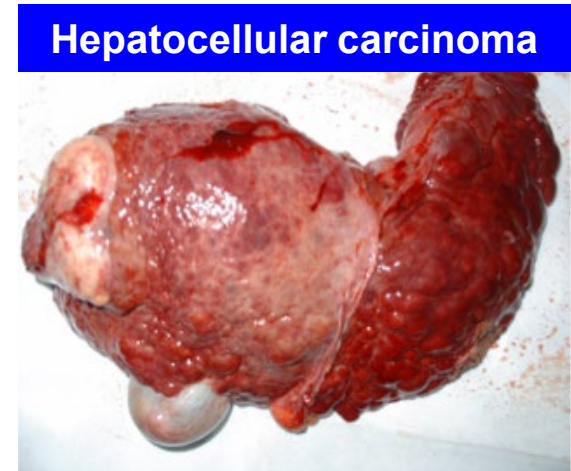
**I collaborate with Alynlam Therapeutics**

**I own stock in Ionis**

# Chronic liver disease from any cause ultimately results in cirrhosis

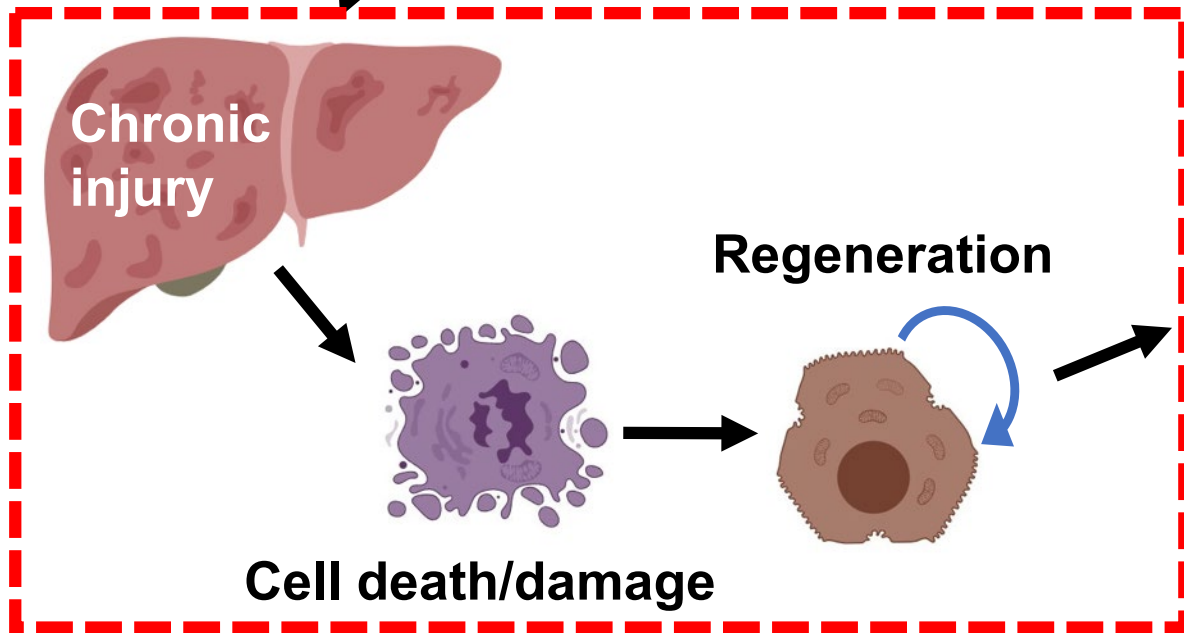
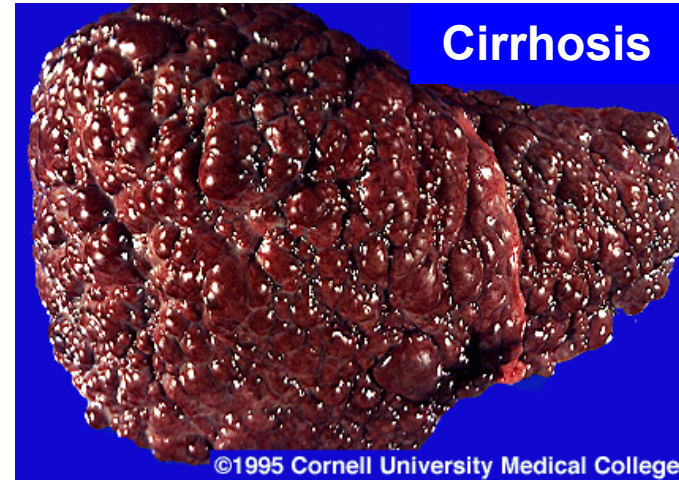
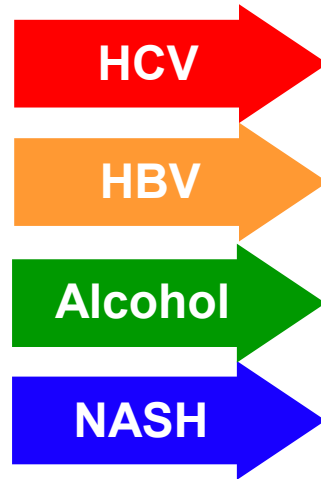
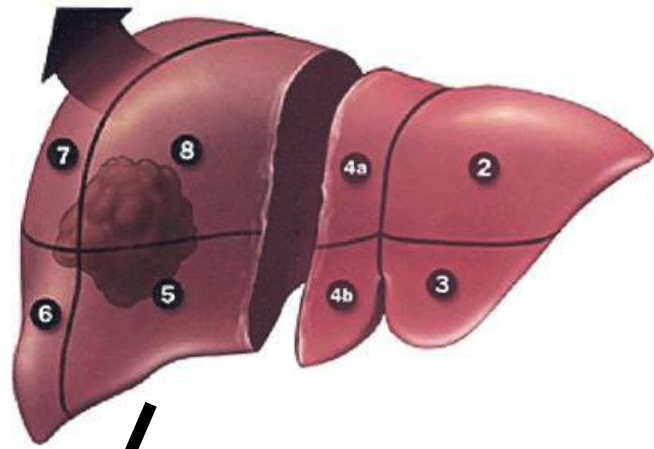


Encephalopathy

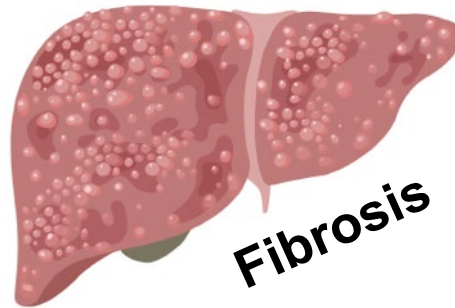




# We dissect the cellular + genetic events culminating in cirrhosis, liver cancer



Inflammation

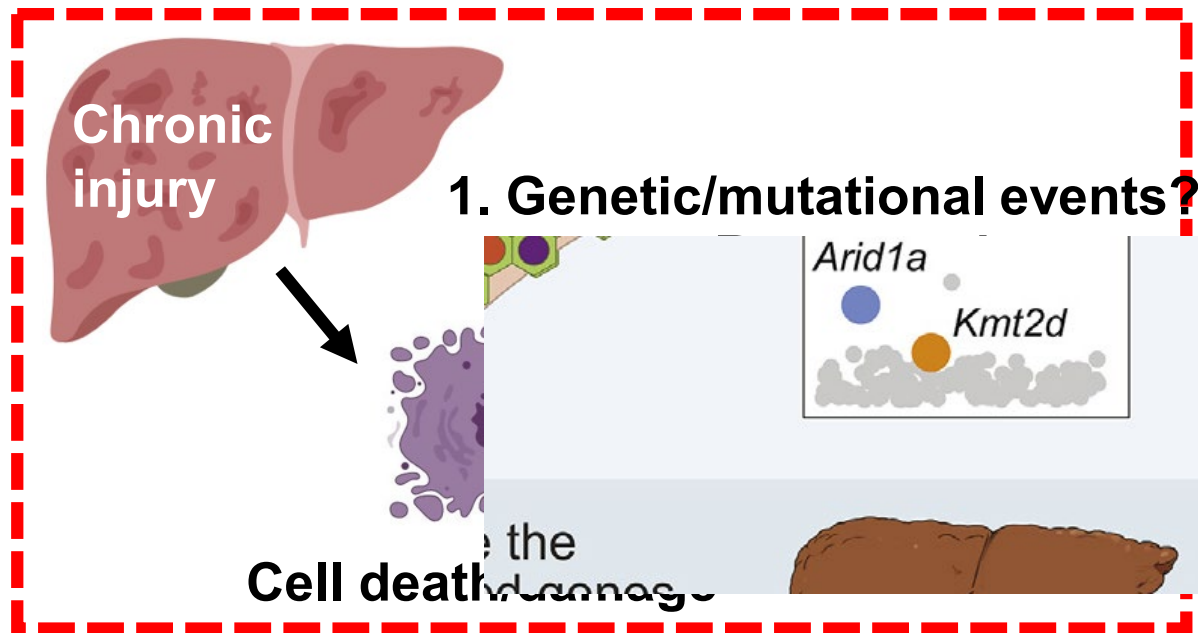


Hepatocellular carcinoma

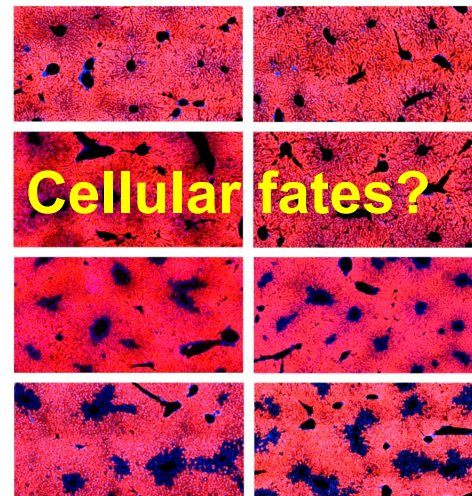




# We dissect the cellular + genetic events culminating in cirrhosis, liver cancer



2. Cellular fates?





Wheat



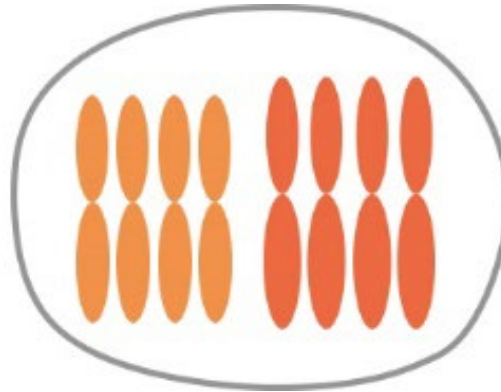
Coffee



Yeast



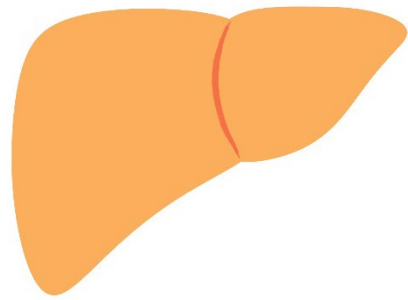
Diploid cell



Polyploid cell



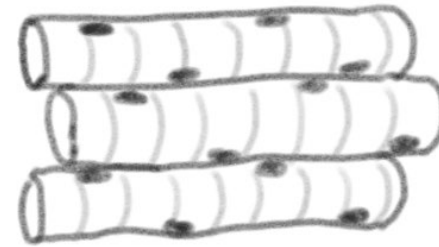
Salmon



Liver



Megakaryocyte



Skeletal muscle



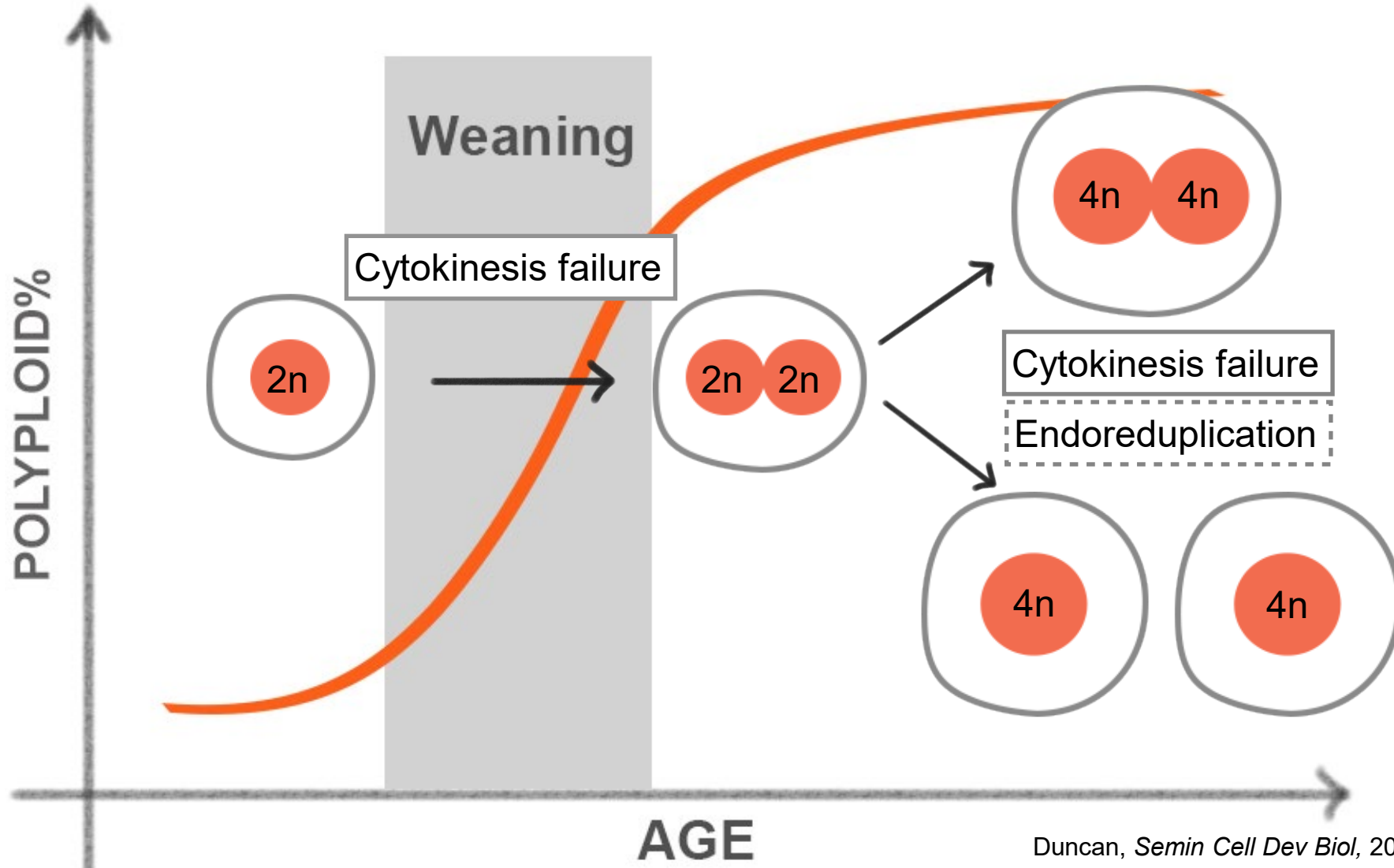


**Hypothesis:**

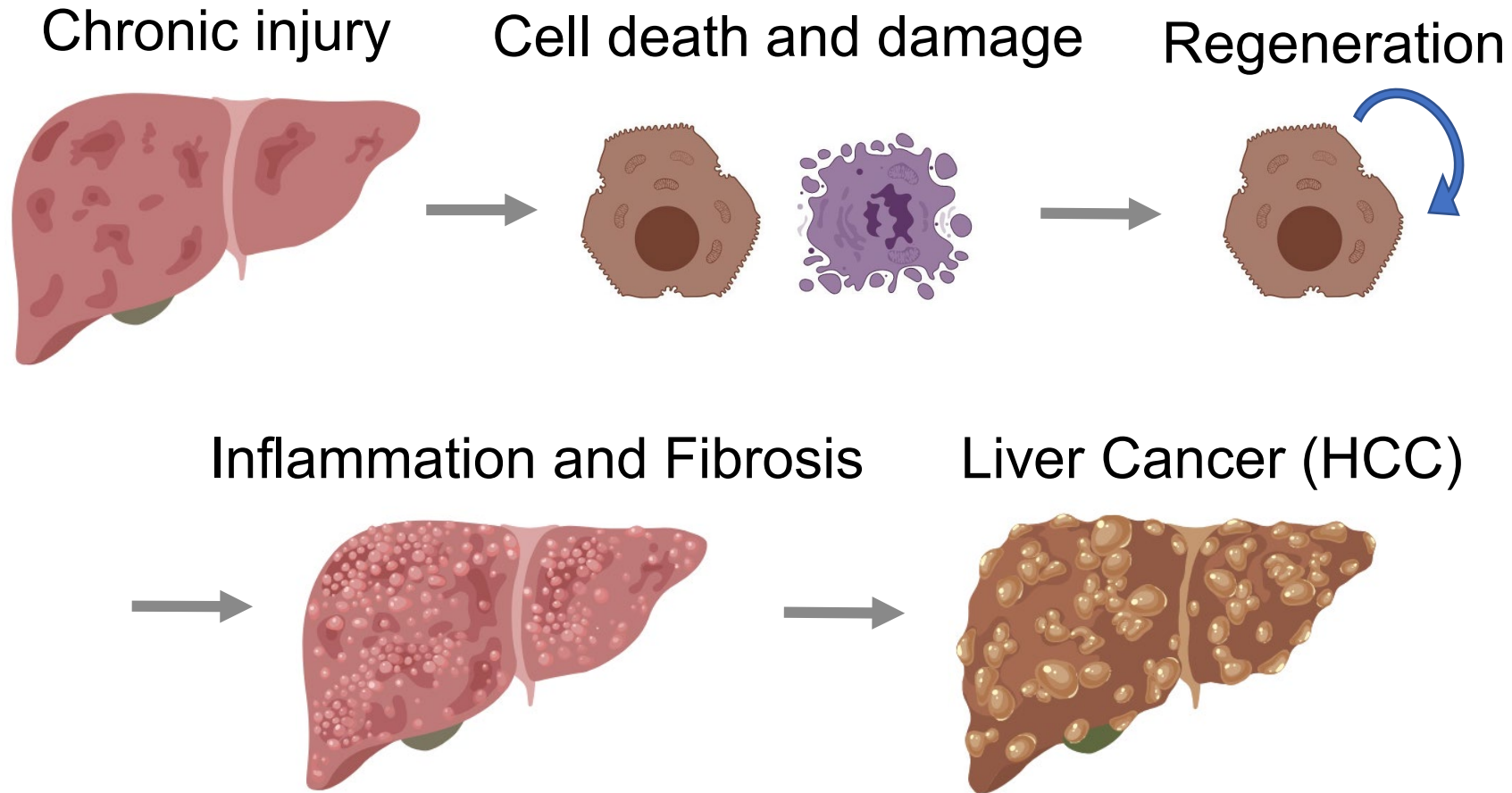
**Polyploidy in hepatocytes allows the liver to safely sustain mutagenesis (w/o carcinogenesis) during wound healing**



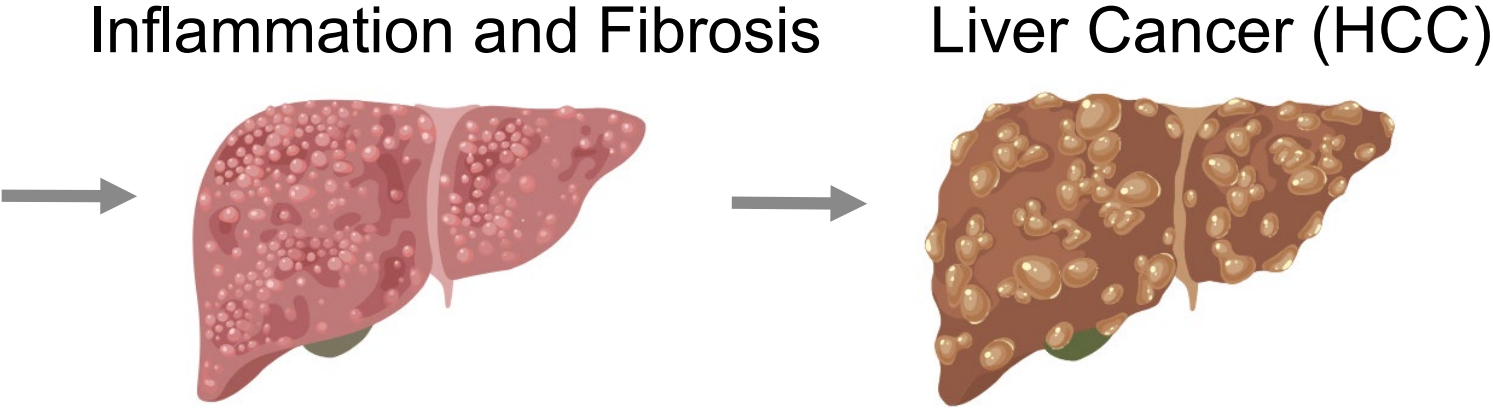
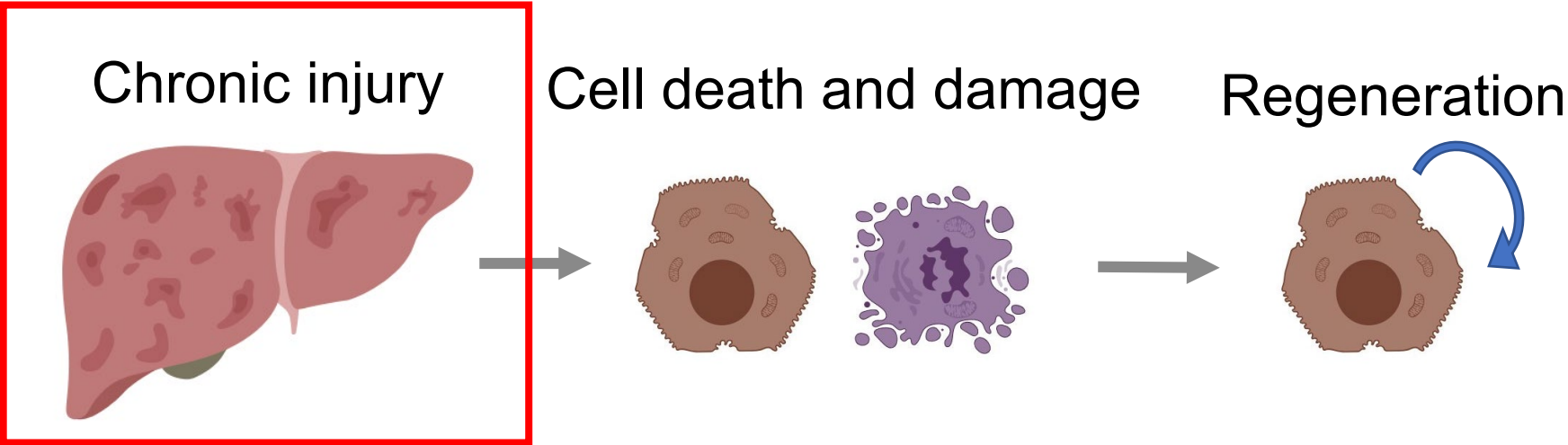
# Up to 90% of mouse and 50% of human hepatocytes are polyploid



# Liver diseases involve chronic injury, eventually leading to HCC



# How does chronic injury affect the ploidy in normal liver?

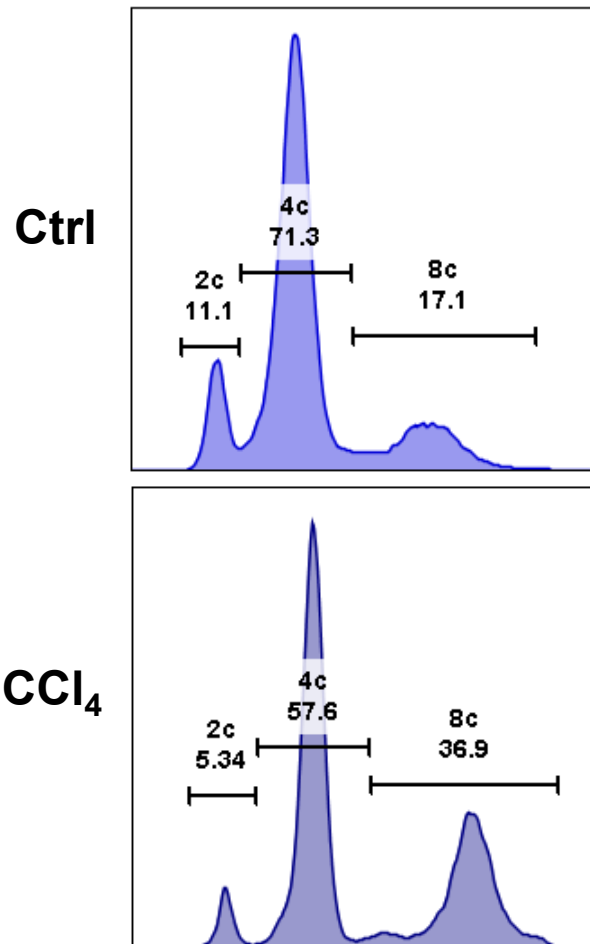




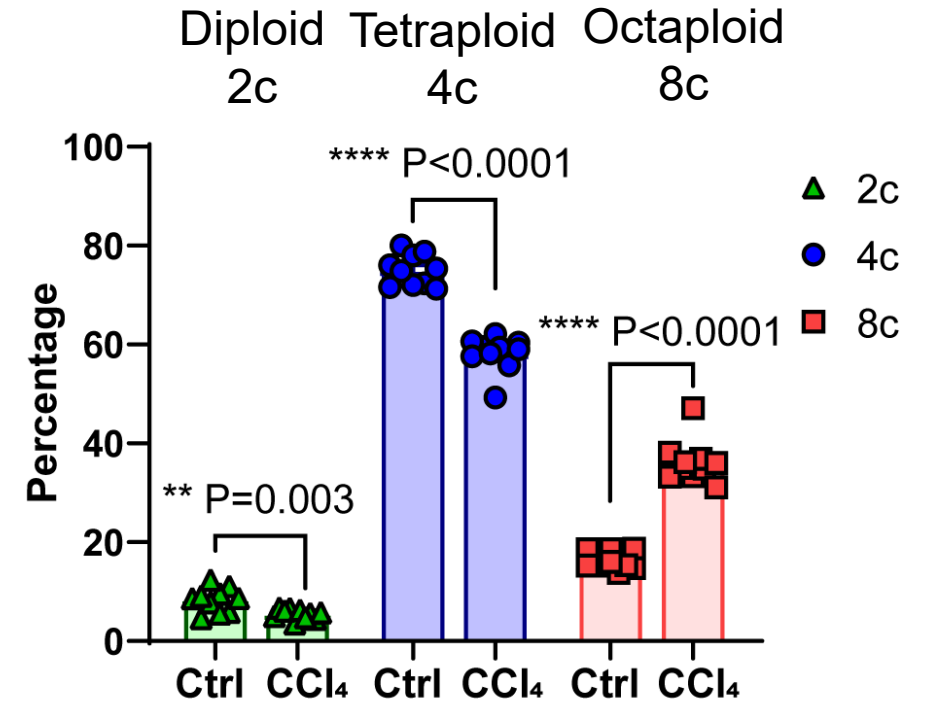
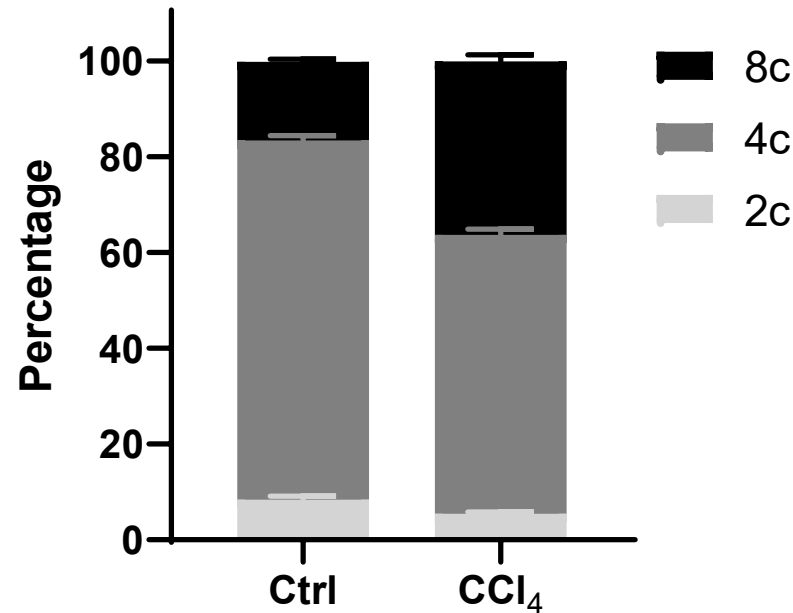
# Chronic injury increases hepatic polyploidy

## Chronic Carbon tetrachloride (CCl<sub>4</sub>) injury

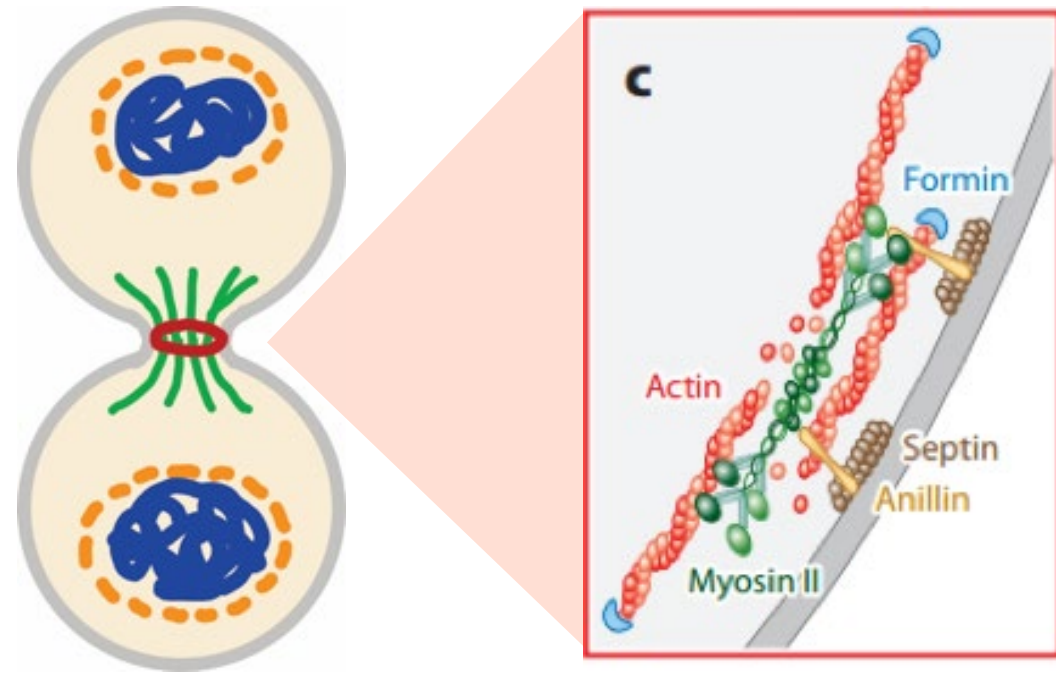
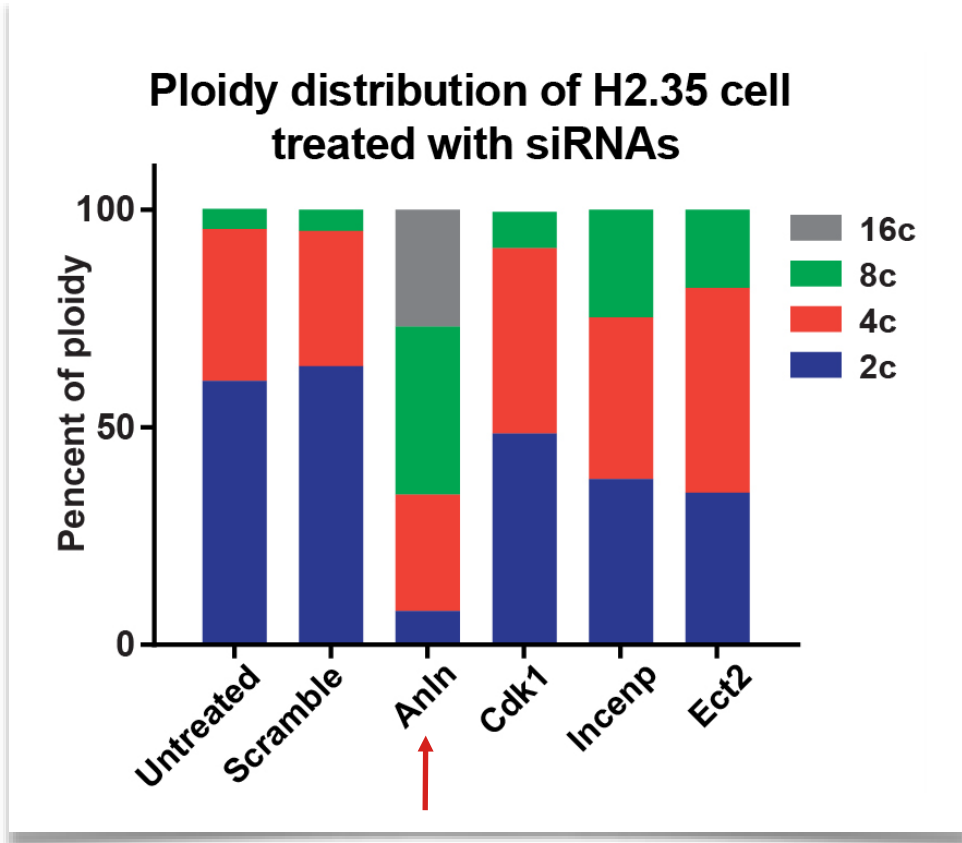
Wildtype mice



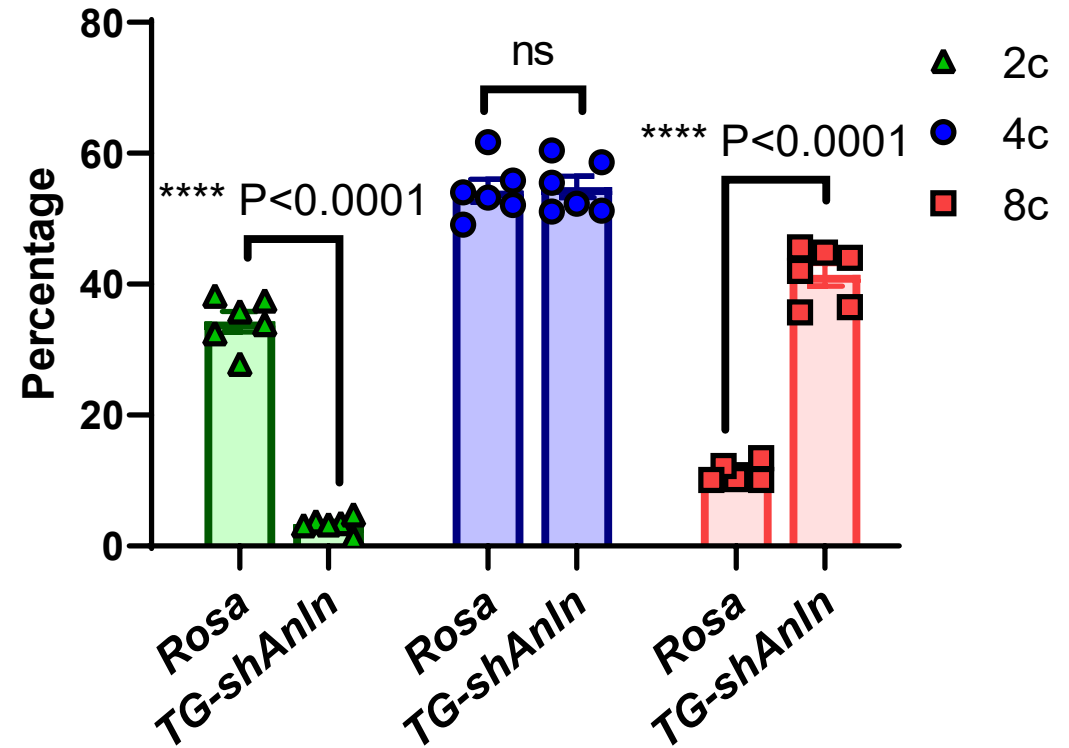
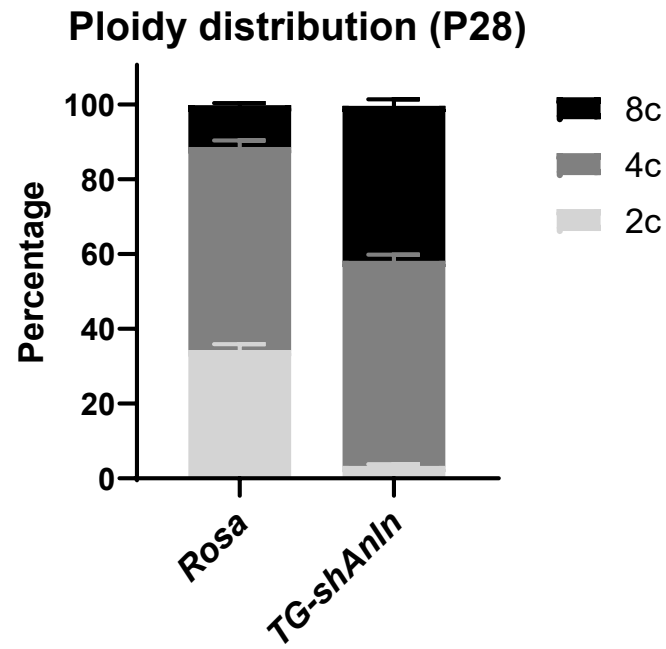
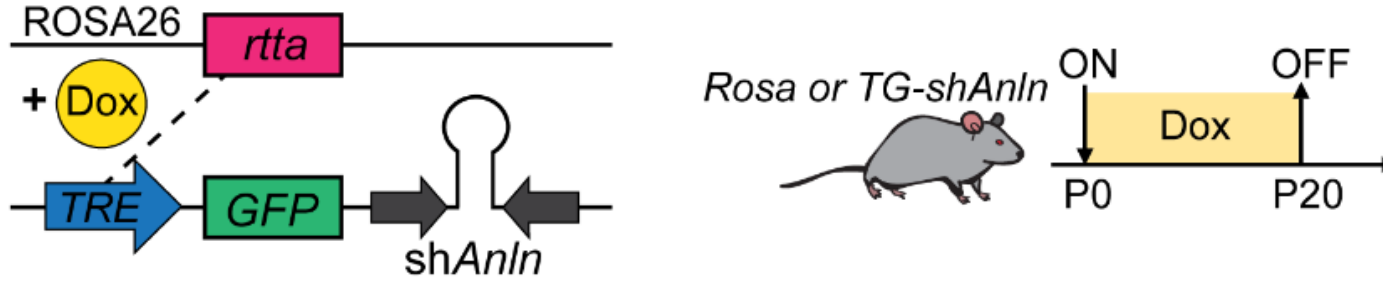
*Rosa*



# A genetic switch to study ploidy: ANLN (Anillin) cytokinesis protein



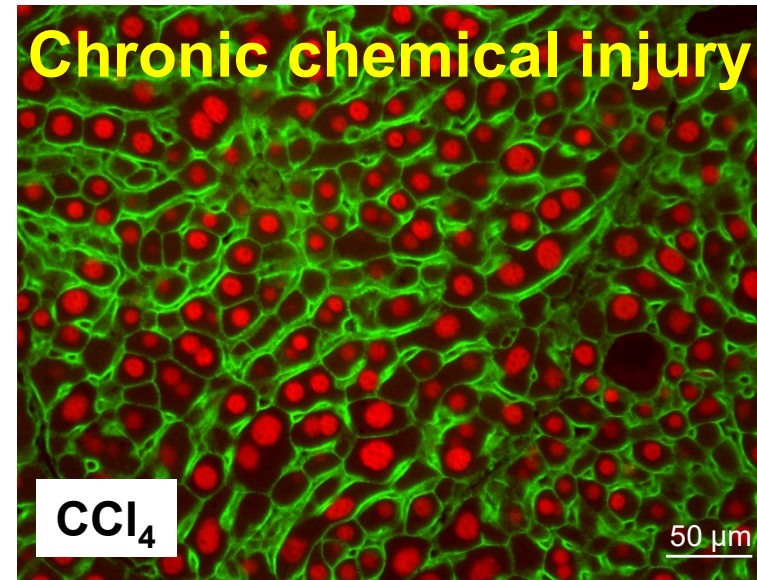
# Inducible *TG-shAnln* mice are a tool to increase polyploidy



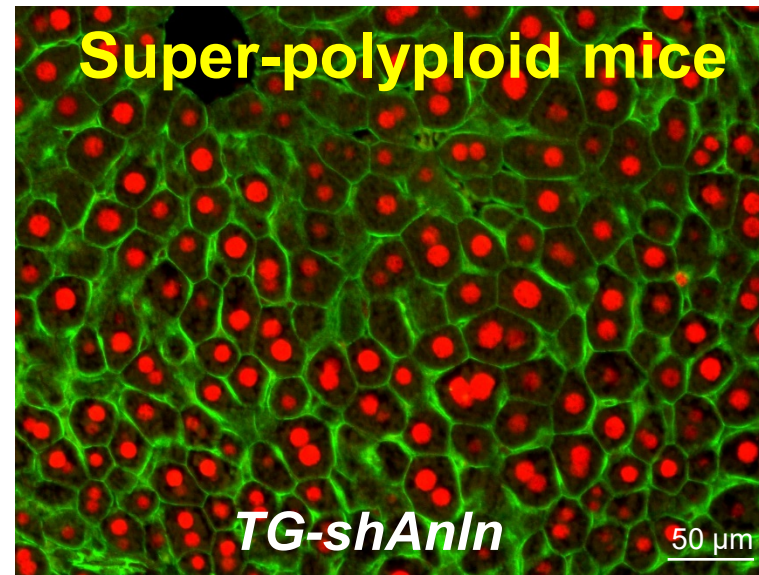
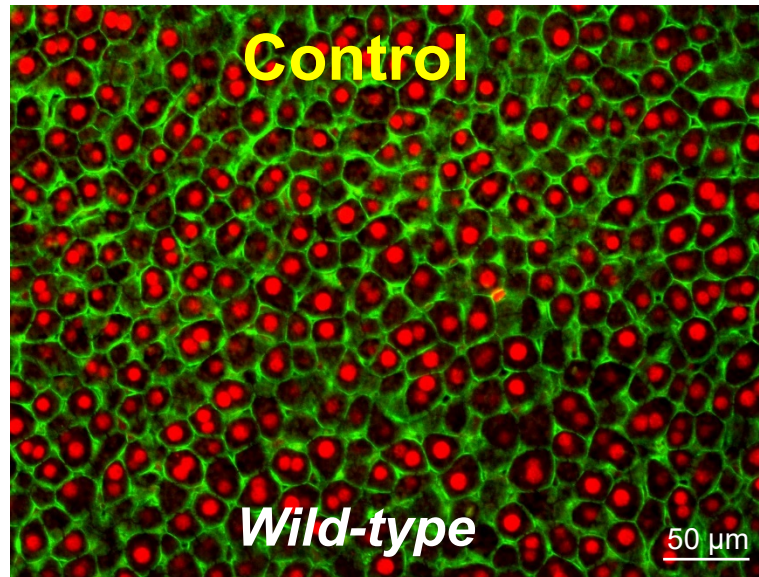


# Knocking down ANLN in mice increases polyploidy

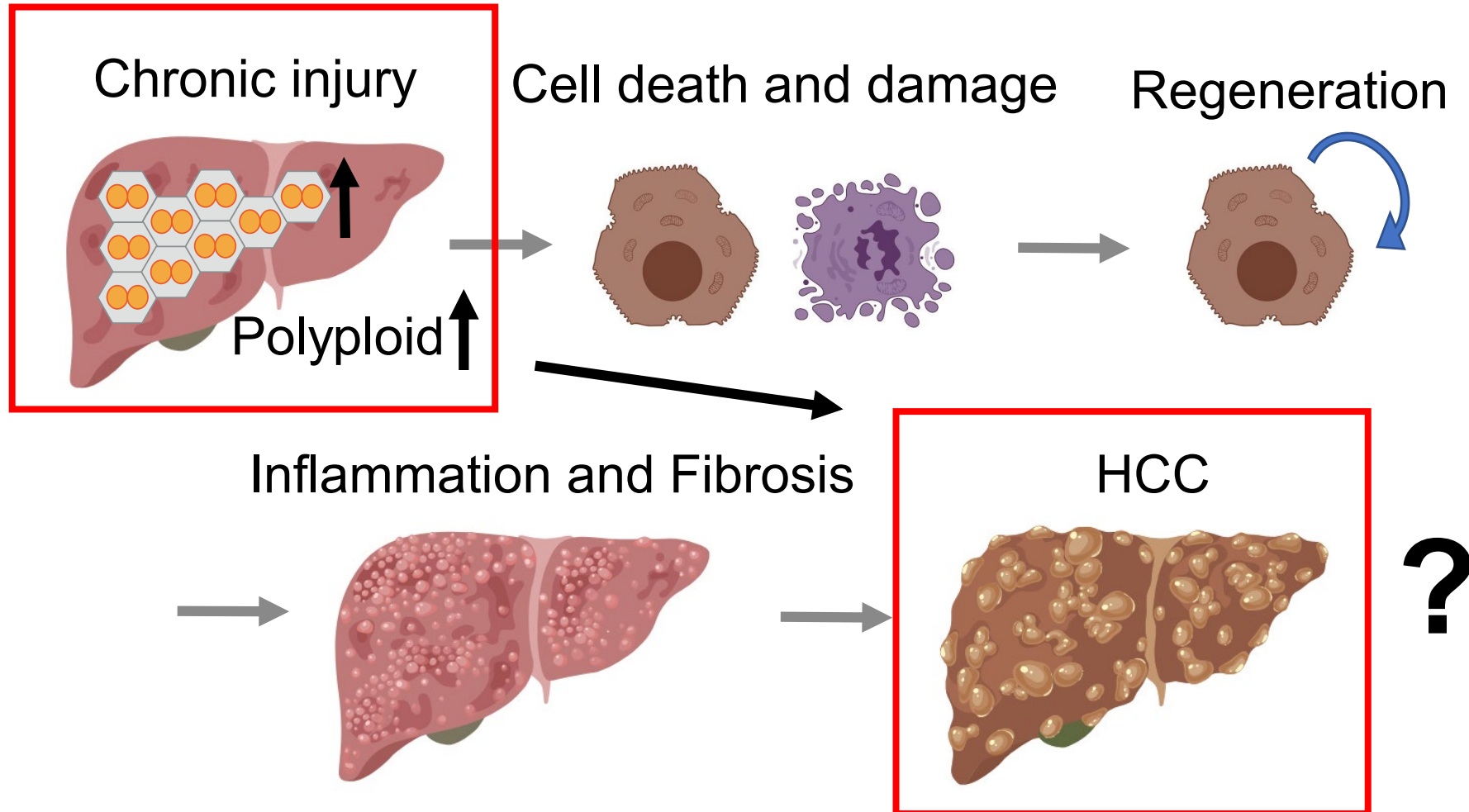
CTNNB1 HNF4A



CTNNB1 HNF4A



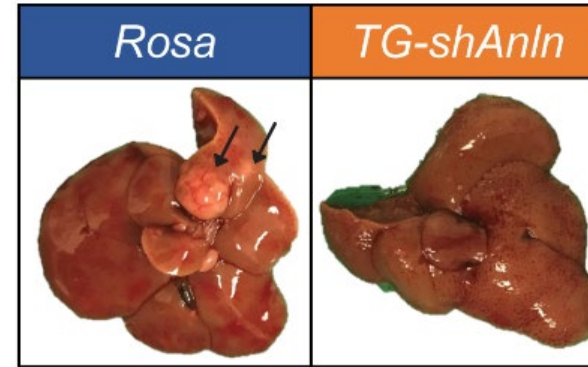
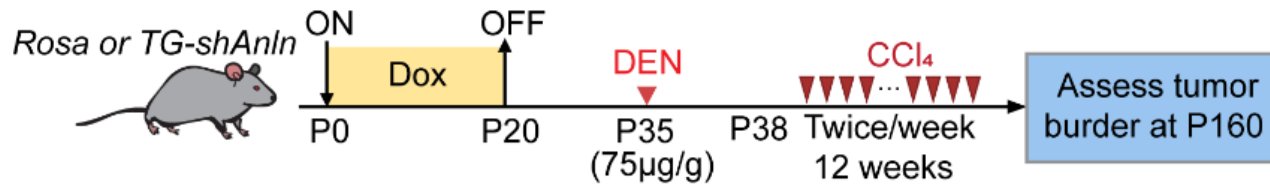
# Does increased ploidy influence chronic injury-induced HCC?



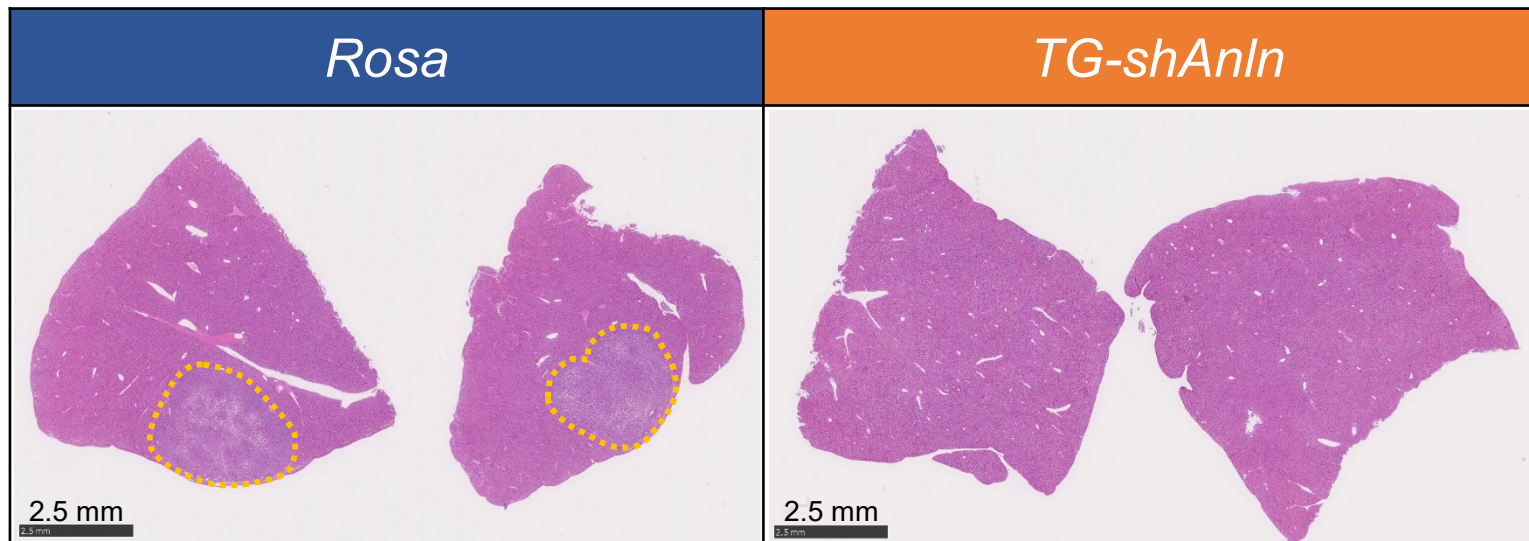
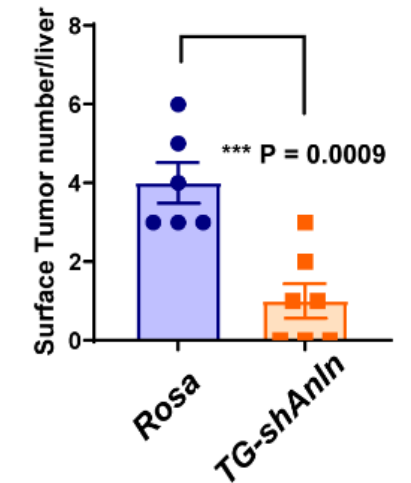


# Polyploidy prevents chronic injury-induced HCC development

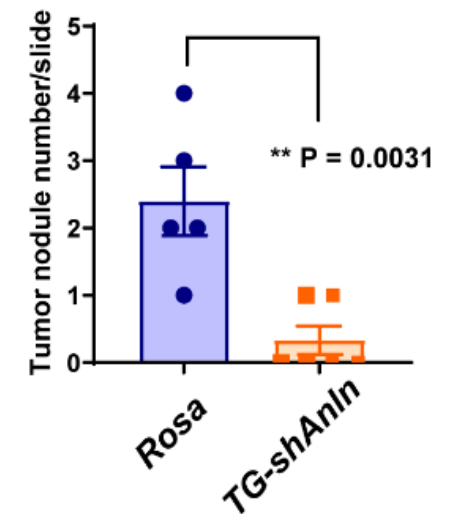
Control or Super-polyploid



Tumor quantification

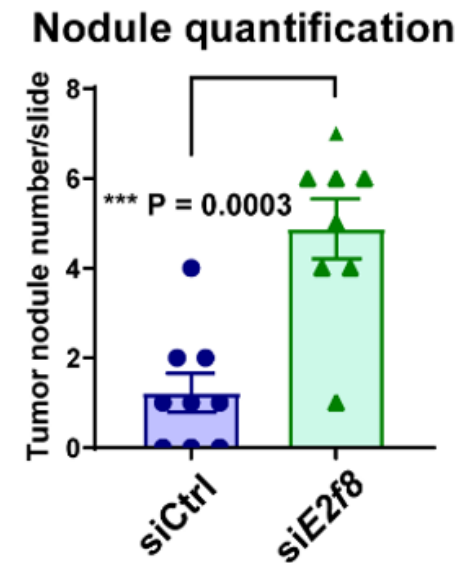
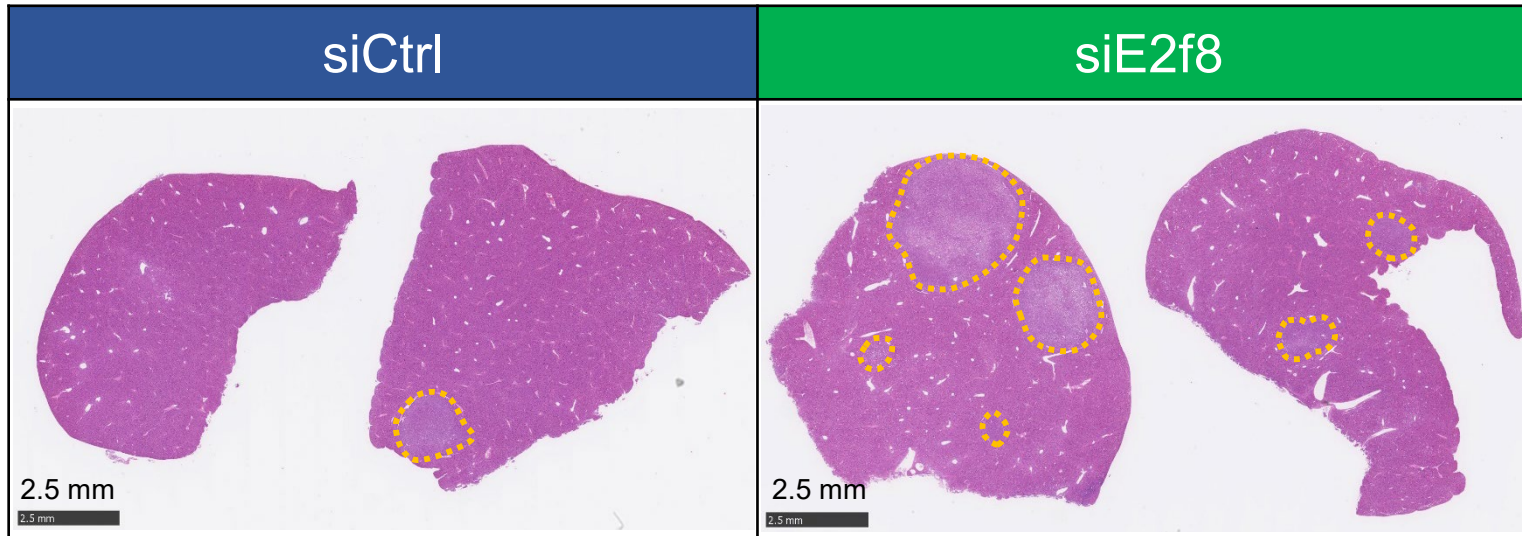
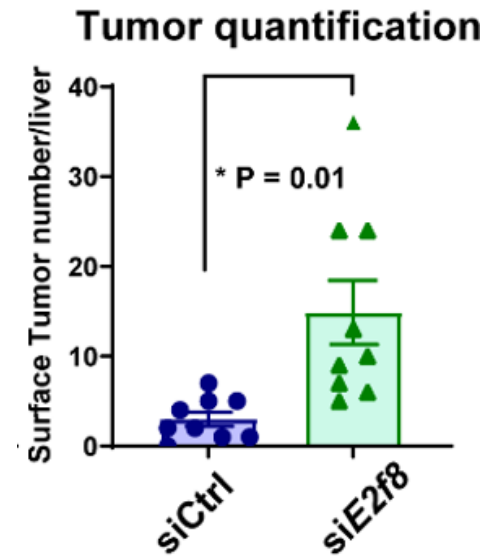
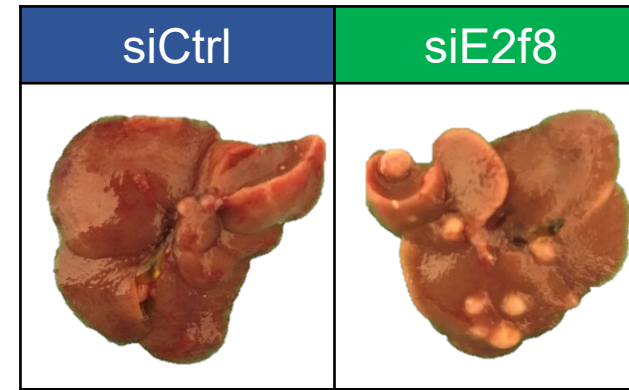
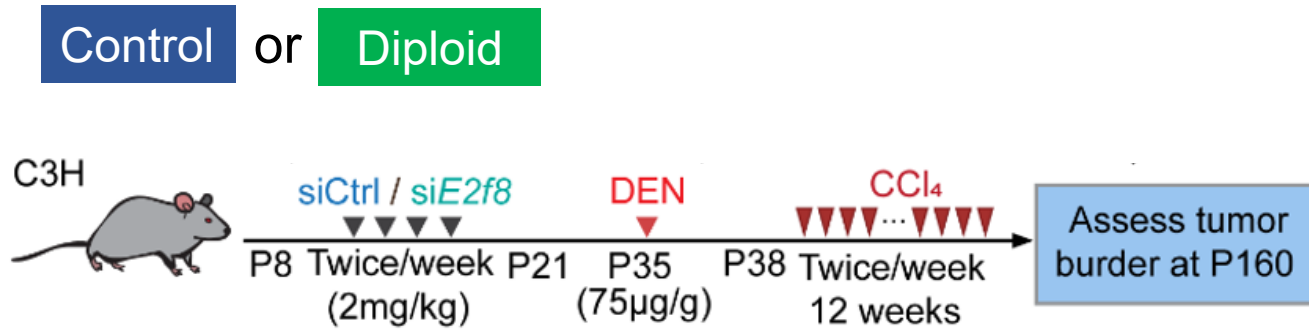


Nodule quantification

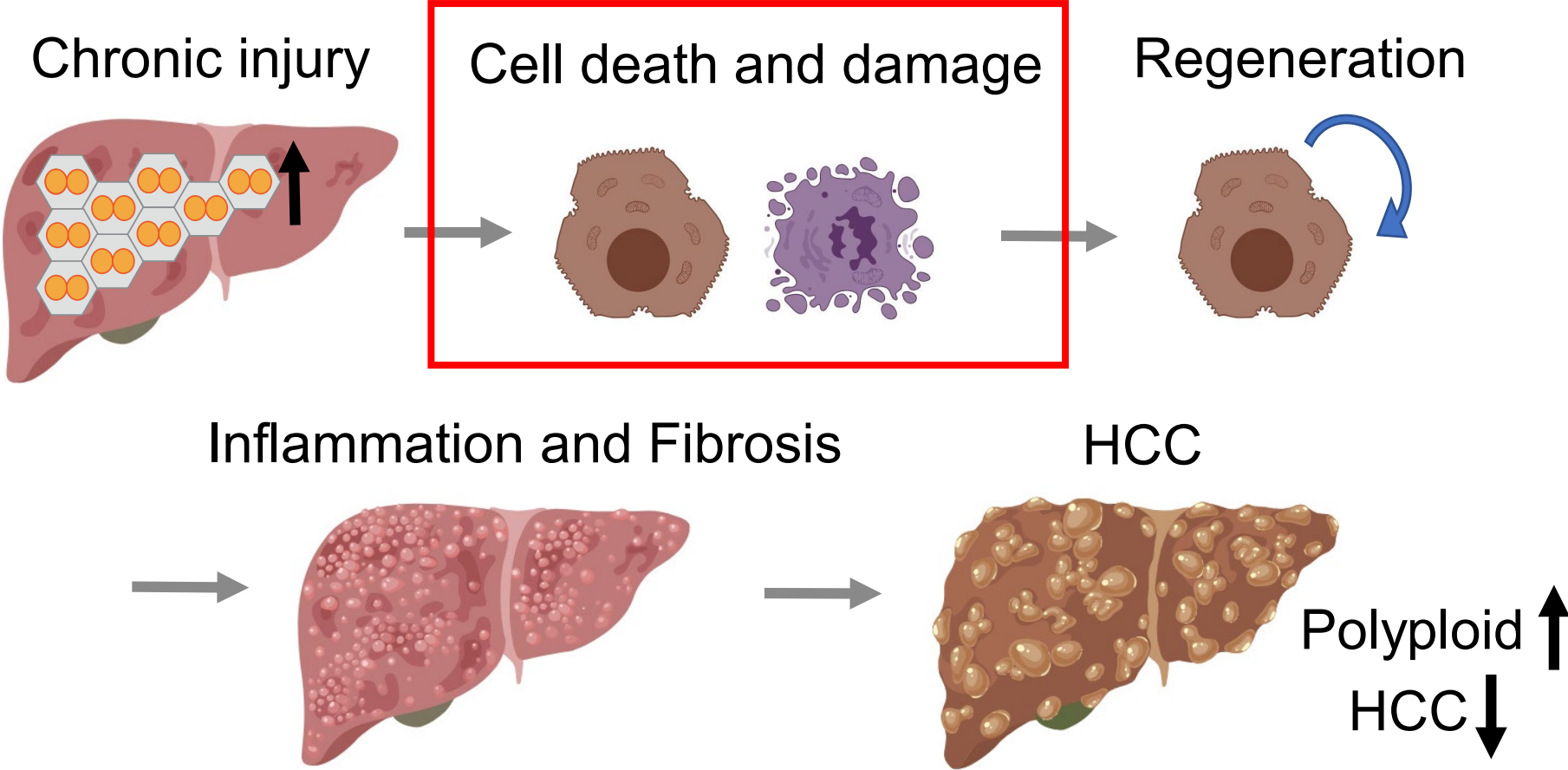




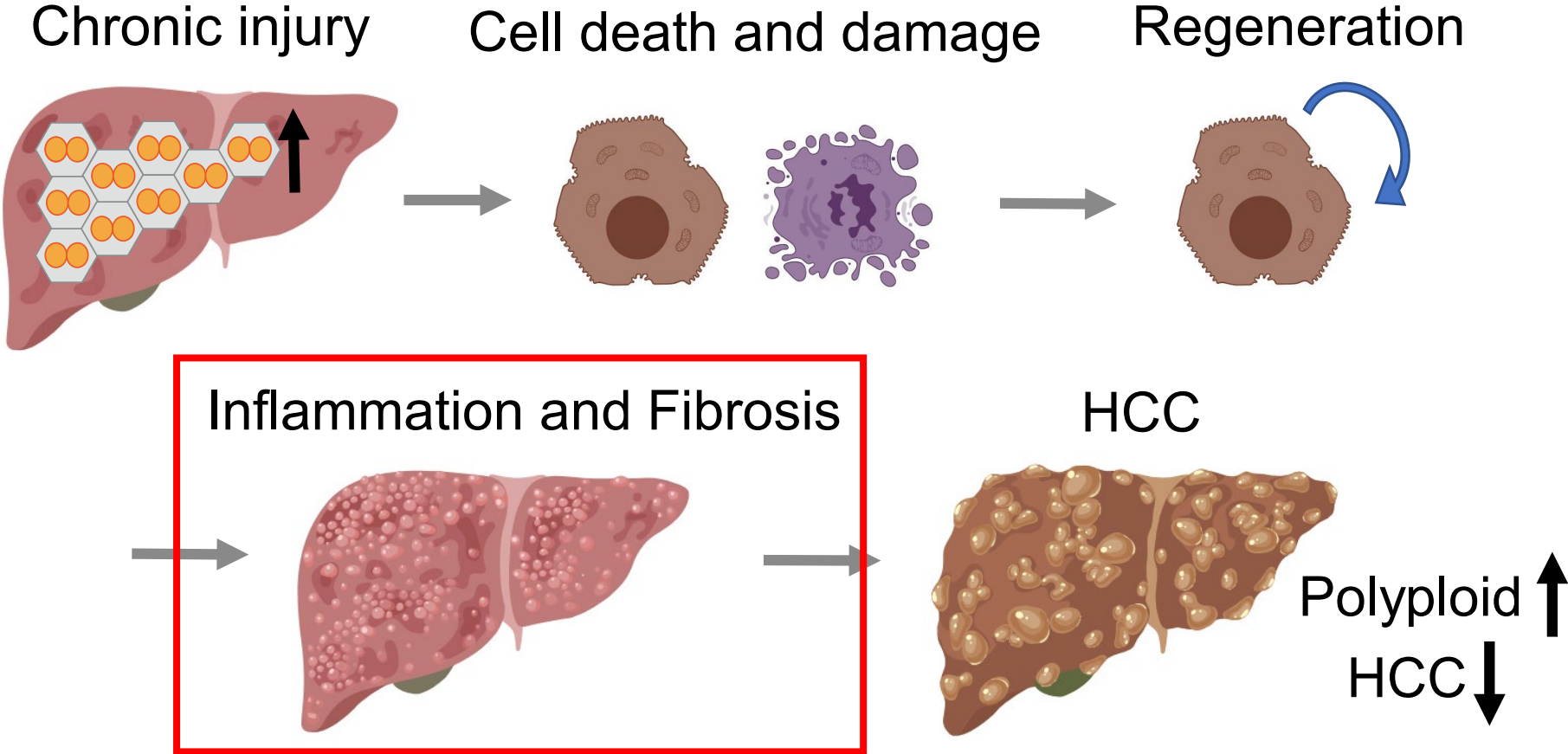
# Diploidy increases chronic injury-induced HCC development



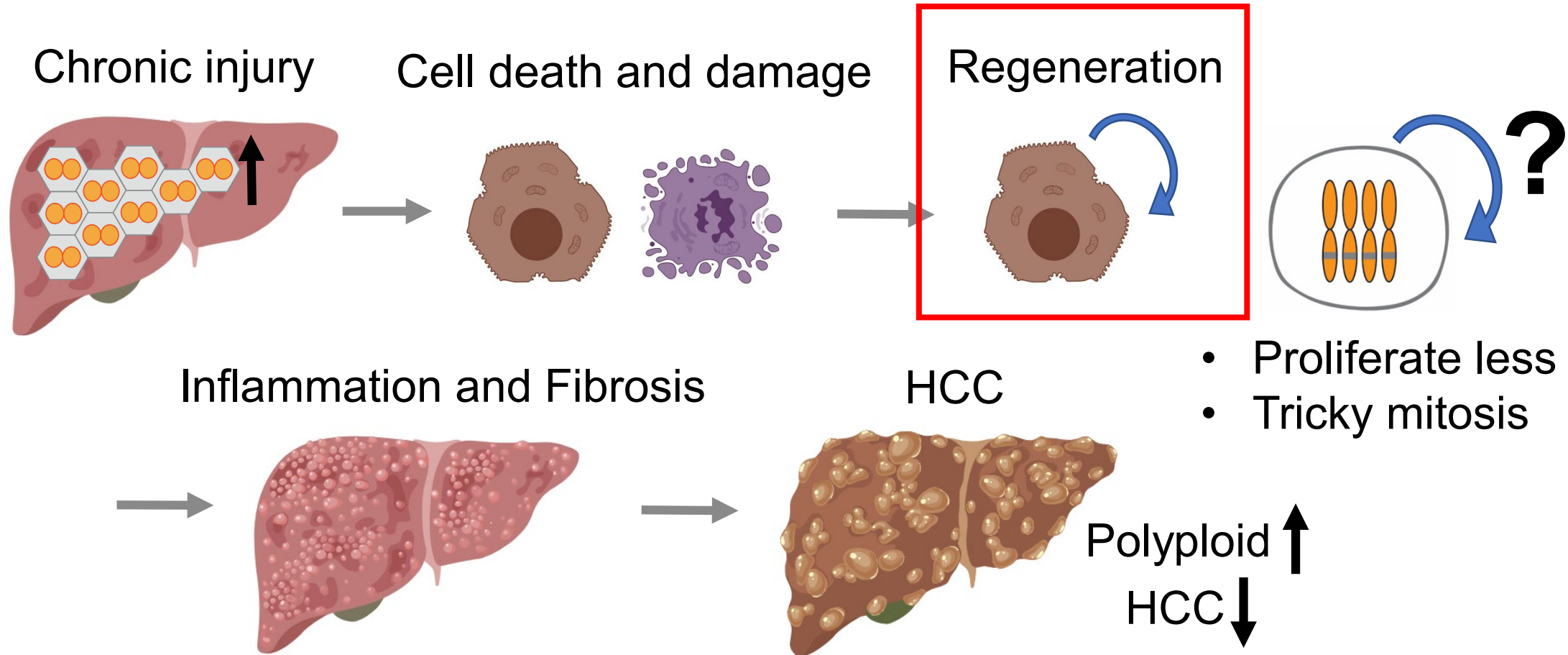
# Does polyploidy influence pathogenic steps to cancer?



# Does polyploidy influence pathogenic steps to cancer?



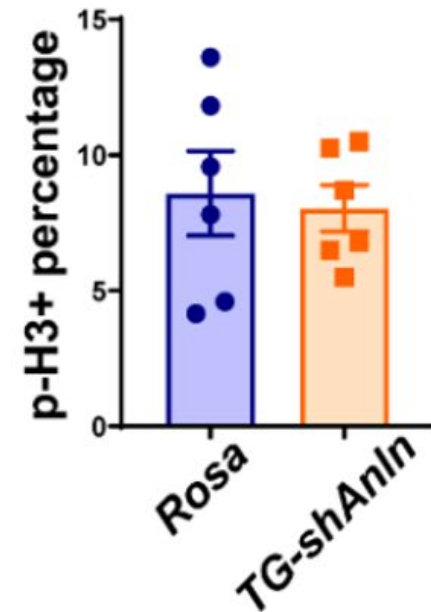
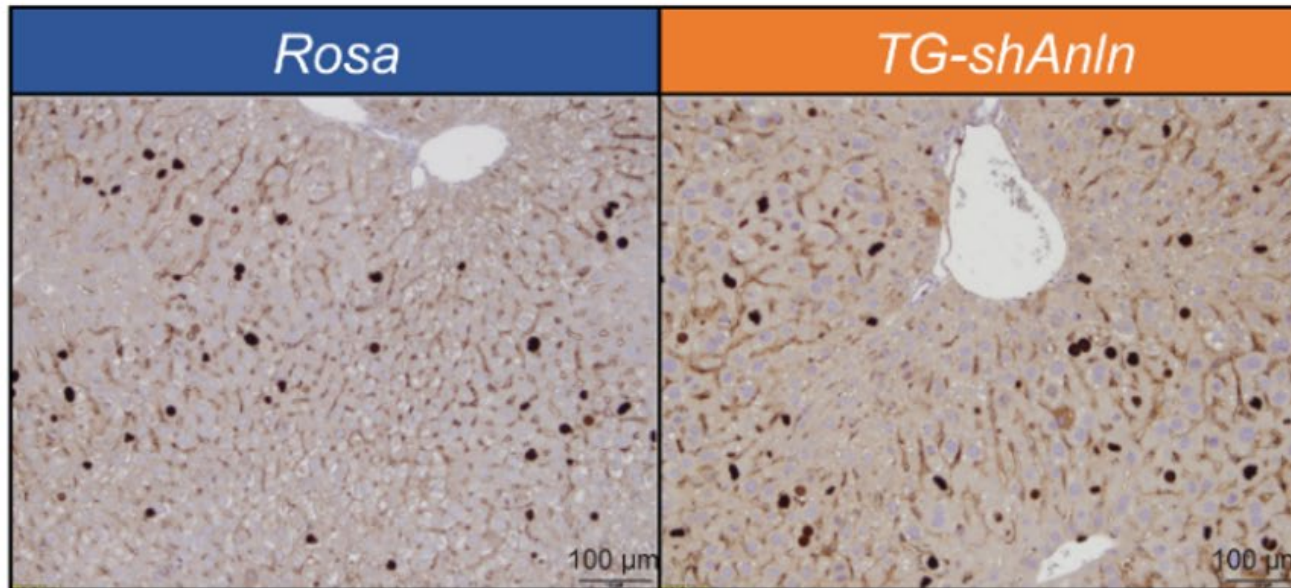
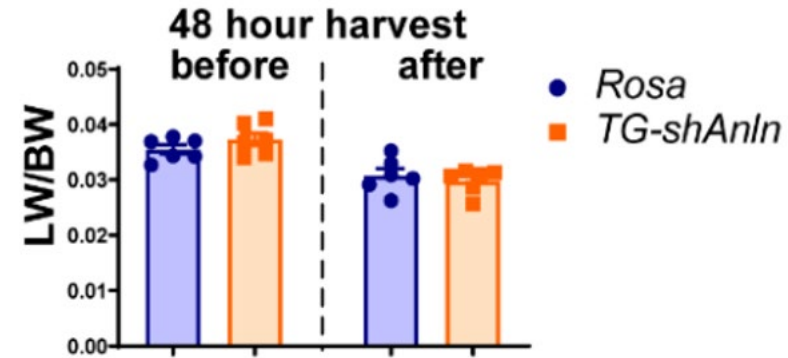
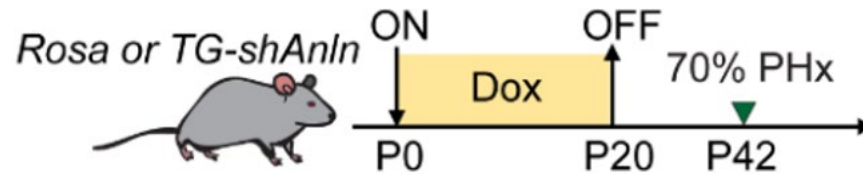
# Can polyploid cells proliferate and regenerate the liver?





# Polyploid cells can regenerate the liver after acute injury

Control or Super-polyploid



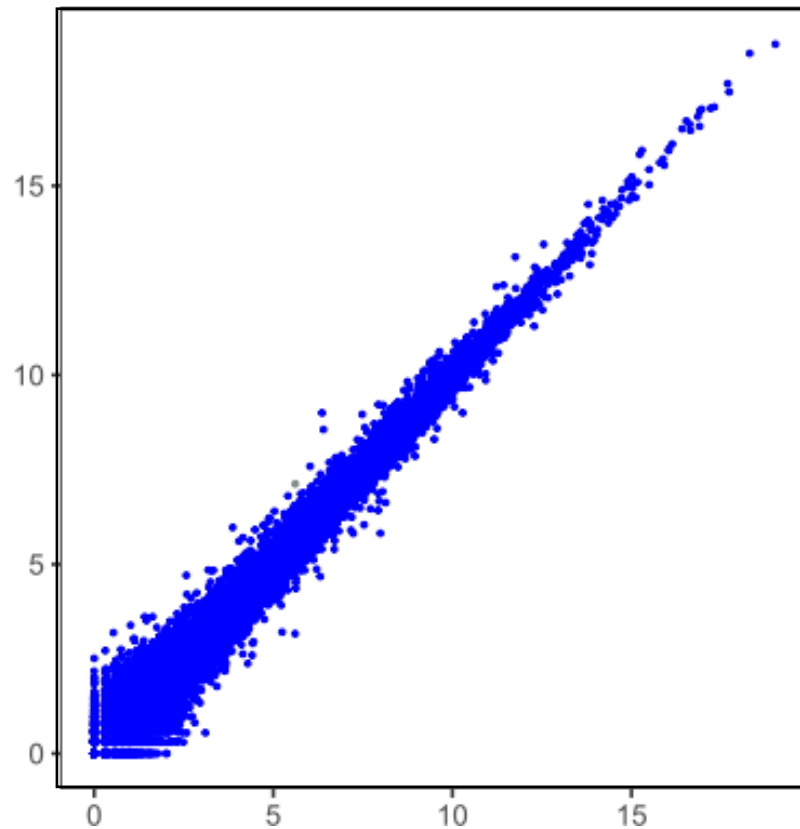


# Polyploidy does not affect gene expression in regeneration

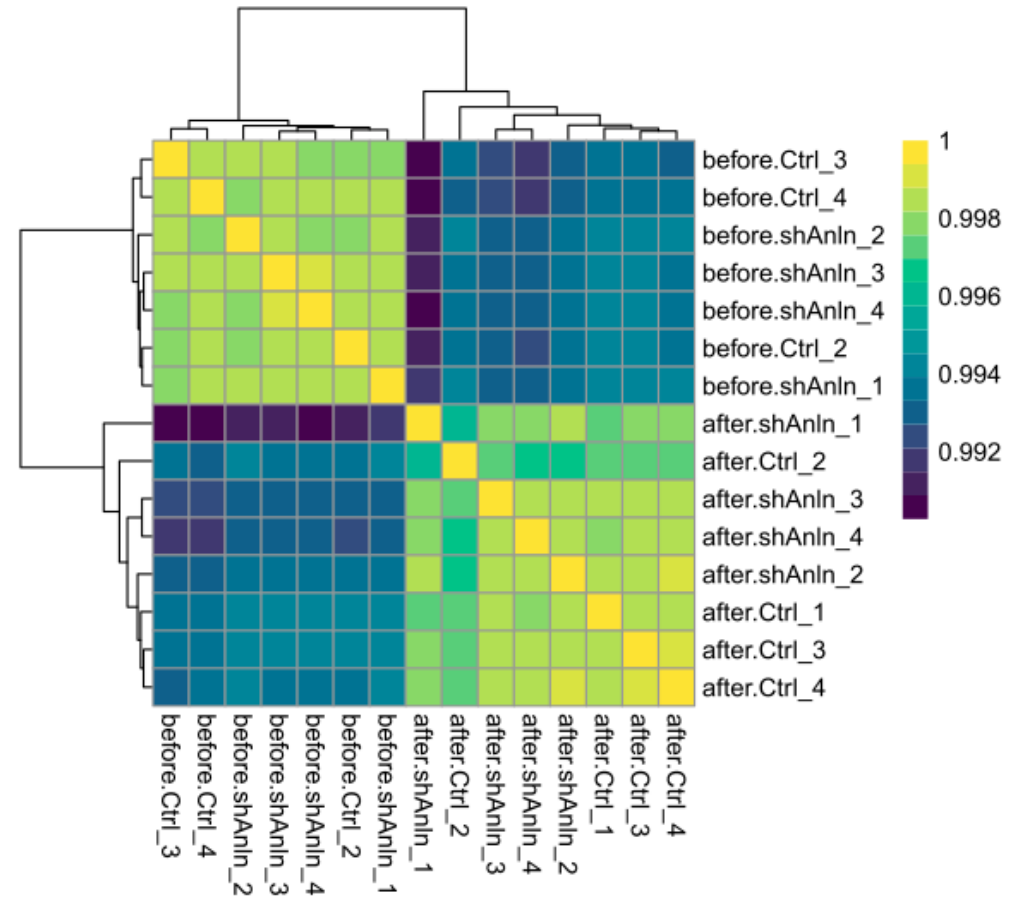
RNA-seq  
→

During regeneration

Super-polyploid

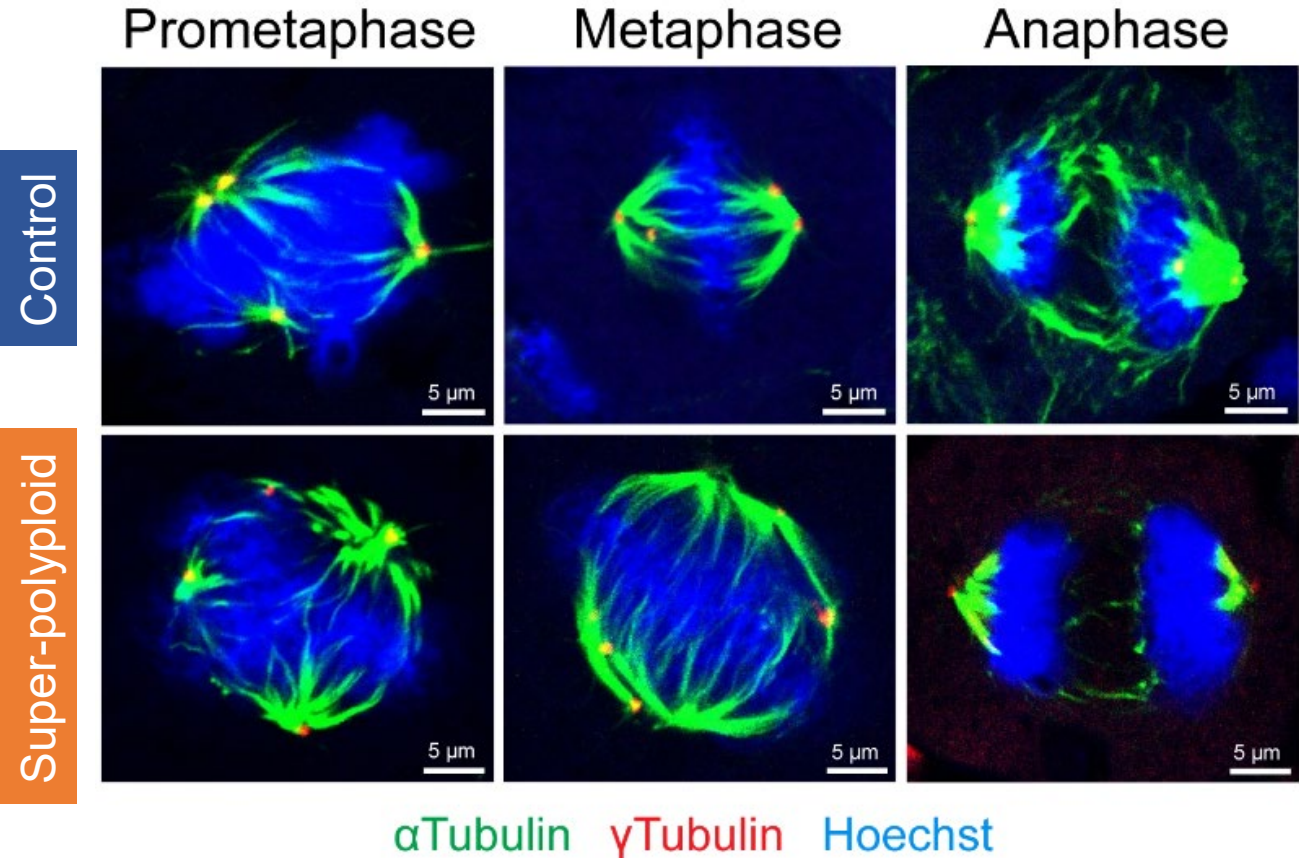
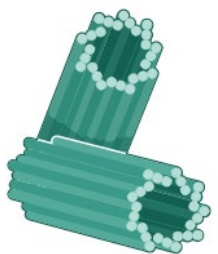
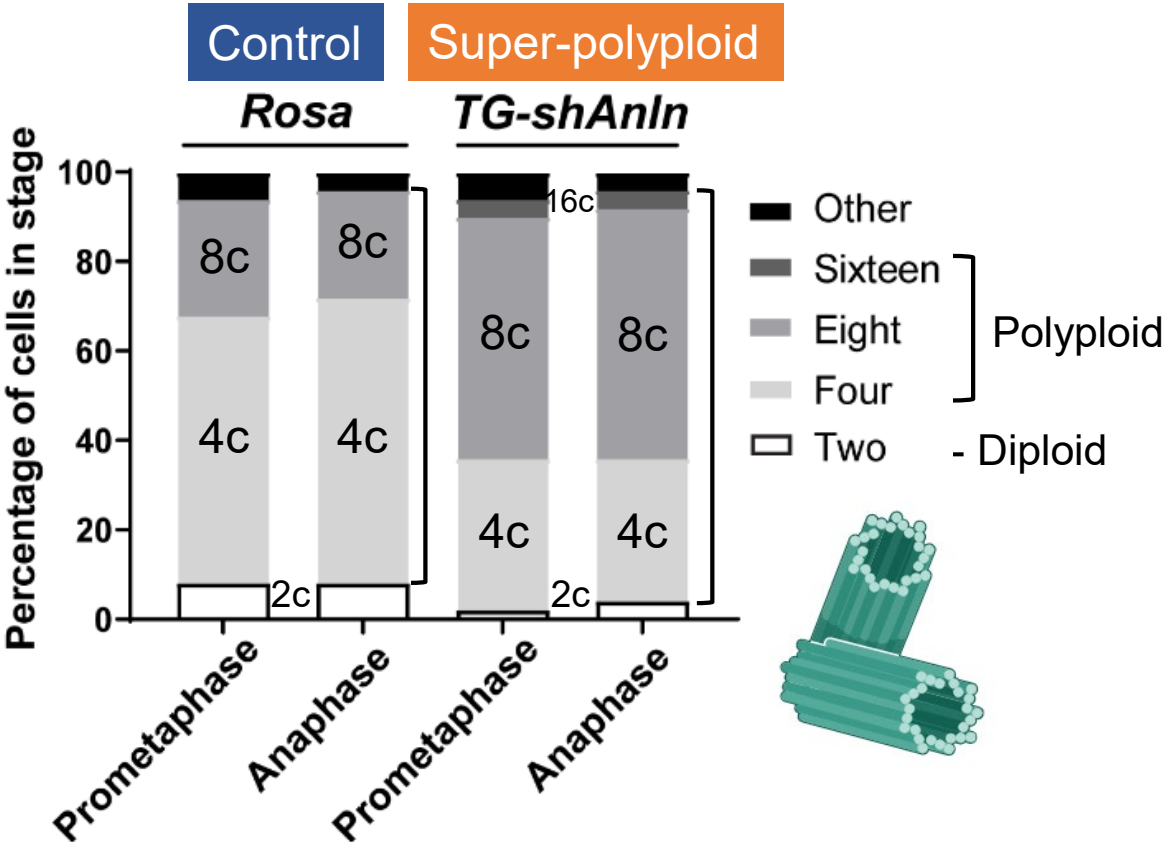


Control

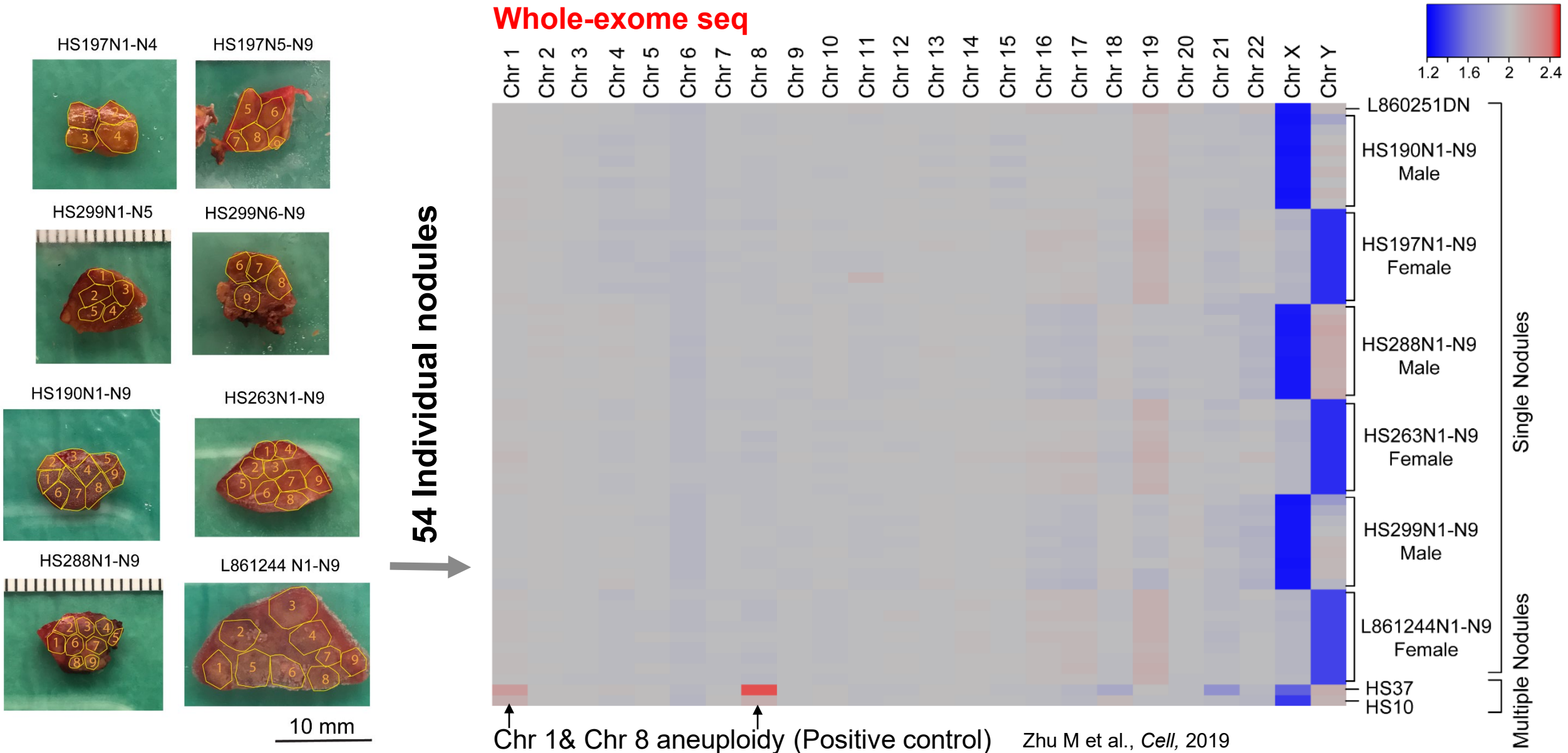


# Polyploid hepatocytes readily divide, with high fidelity

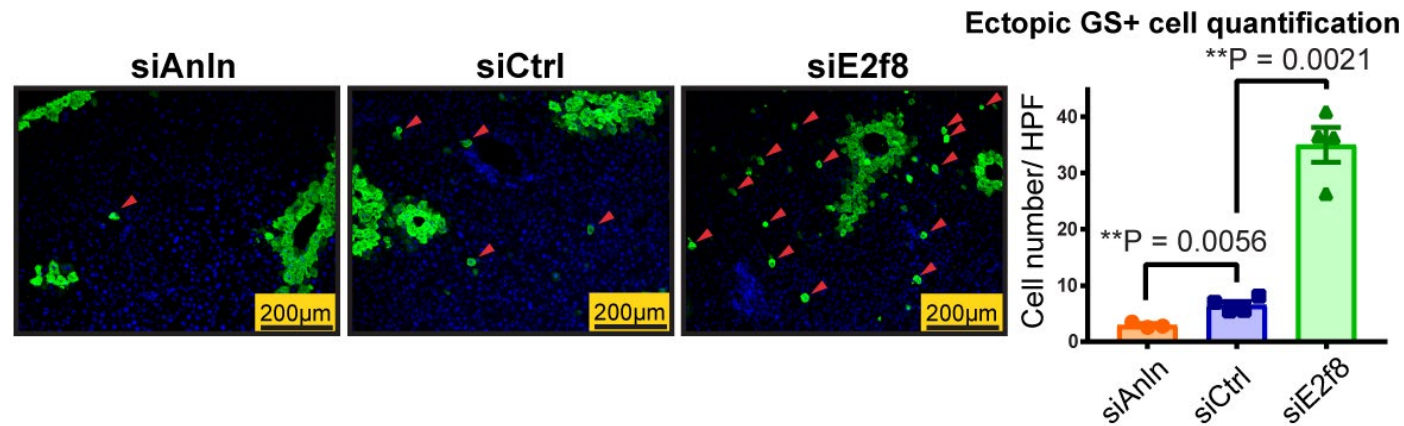
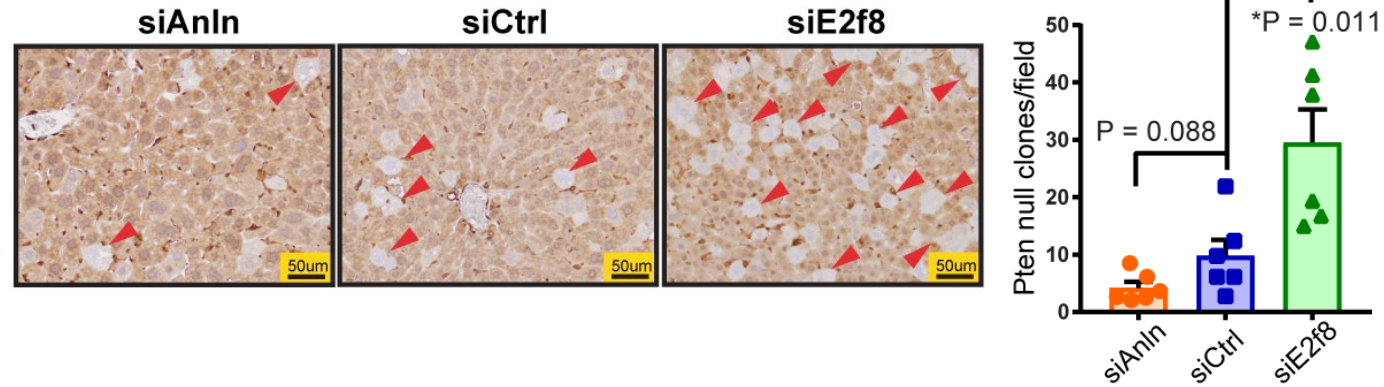
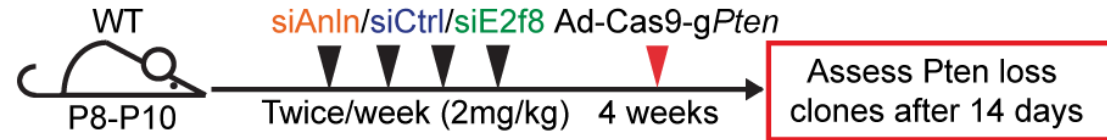
## Mitosis staining in the regenerating liver



# In human livers, aneuploid nodules are rare



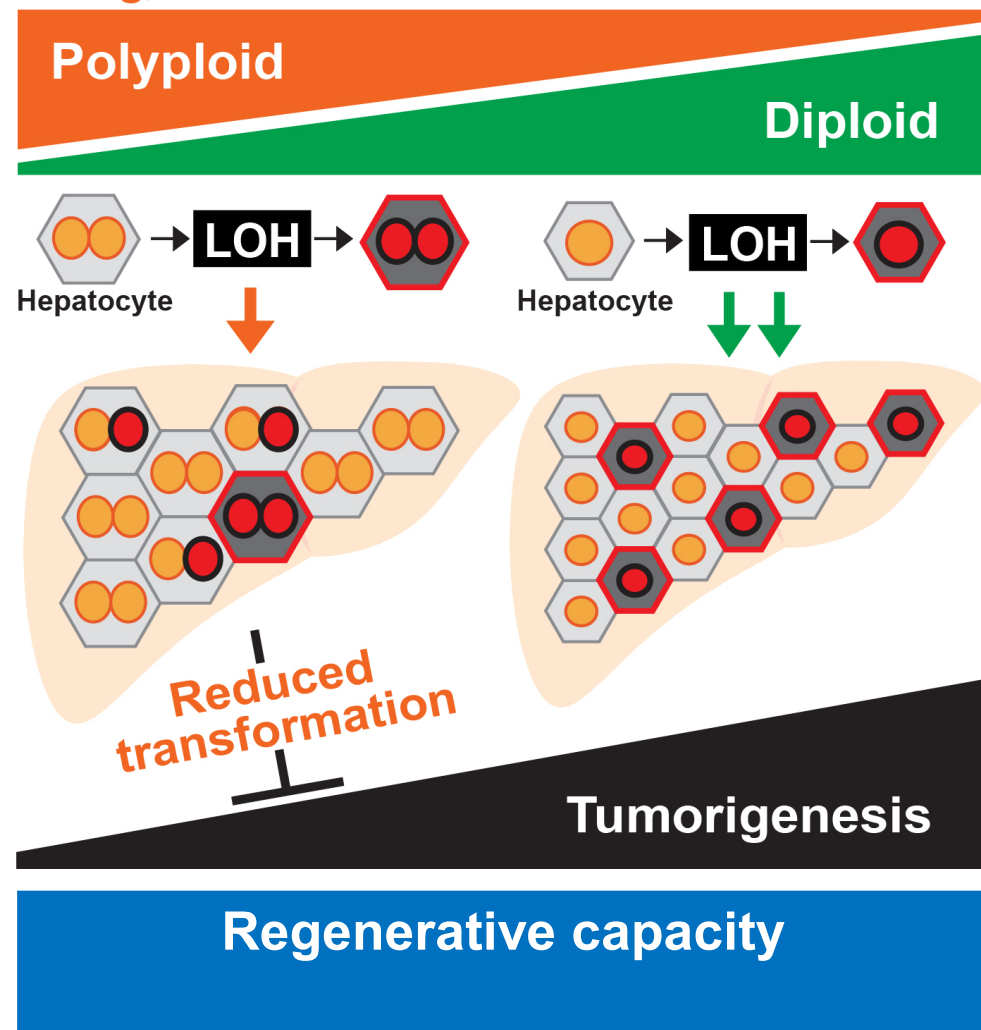
# Tumor suppressor loss of heterozygosity reduced in polyploids



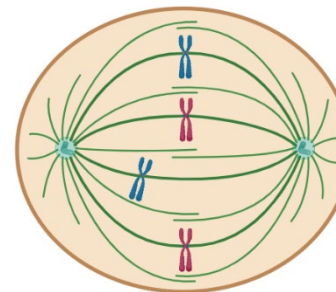


# Polyploid hepatocytes protect from cancer while maintaining regenerative capacity in chronic liver disease

Transient, reversible manipulations of liver ploidy  
*Weaning, Anln knockdown*      *E2f8 knockdown*



## DOES NOT CAUSE



Chromosome  
missegregation

Aneuploidy

Polyploidy could be an  
adaptation to buffer  
against TSG mutations.

How do polyploids  
ensure segregational  
fidelity during mitosis?

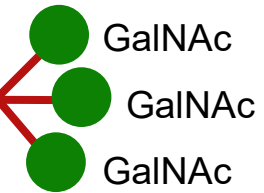
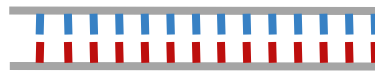


# Cytokinesis inhibition and polyploidization with *ANLN* siRNAs for HCC prevention?

Cirrhotic patients with higher risk for HCC



GaINAc-siRNA

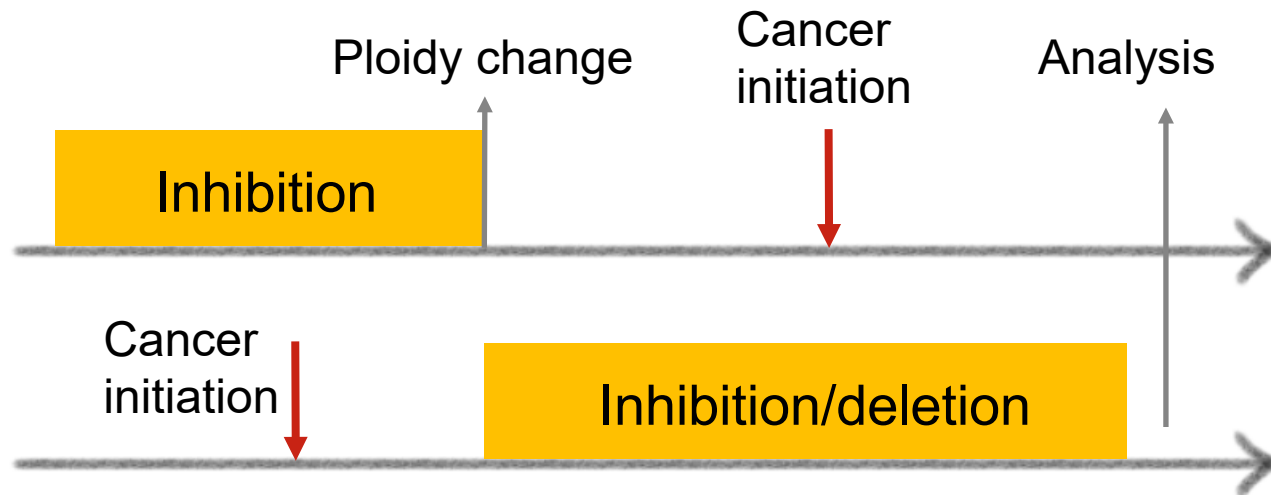


PROS of hepatocyte-specific siRNA:

- Achievable, FDA approved
- Less toxic than systemic small molecules

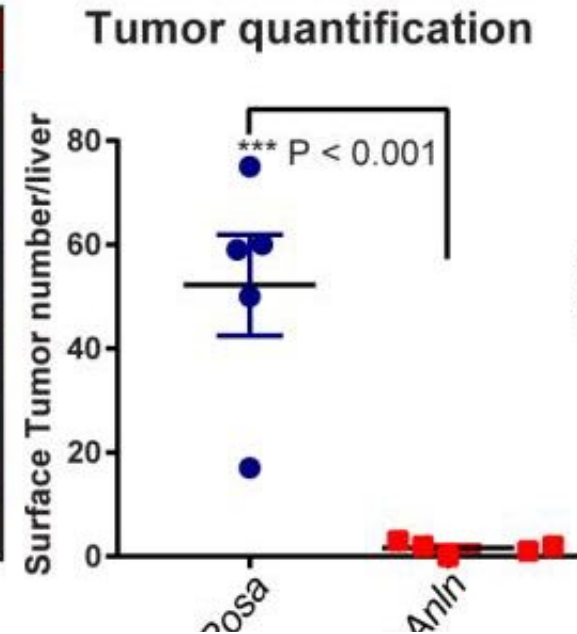
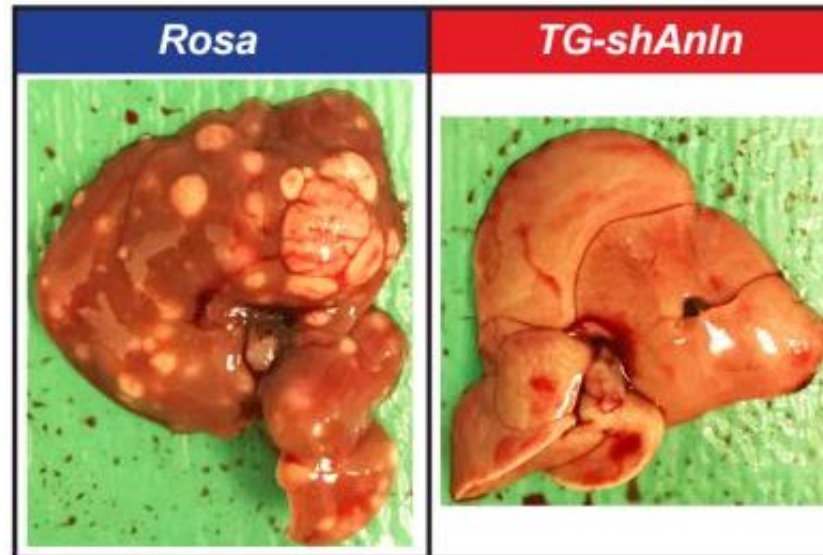
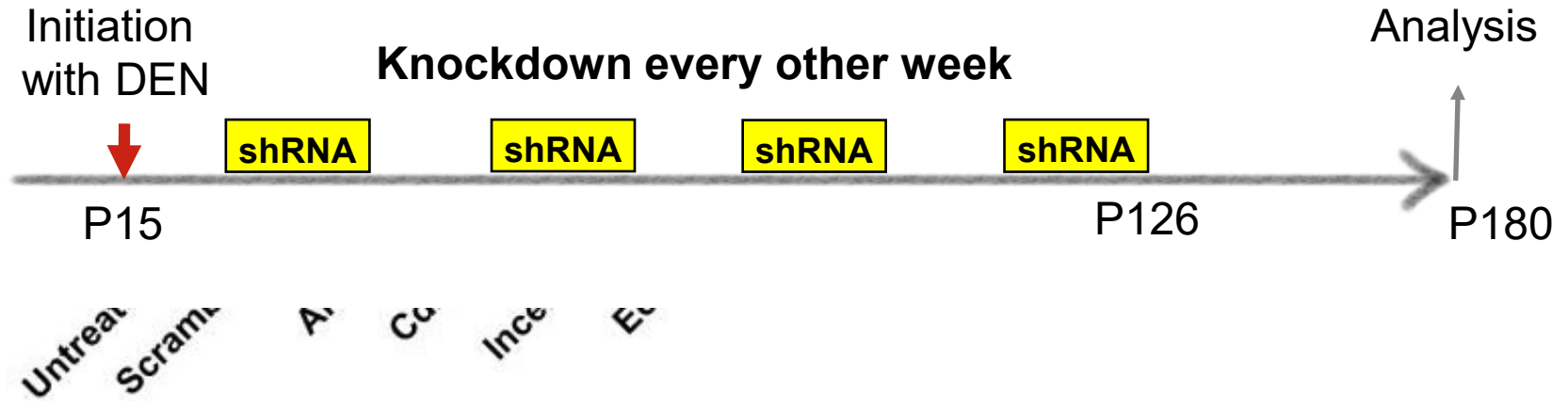
**Transient inhibition:  
Ploidy**

**Continuous  
inhibition**

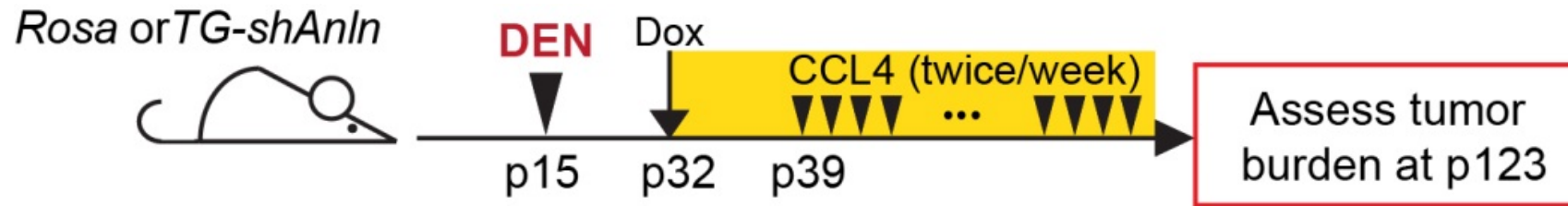


# Persistent ANLN shRNA inhibition: DEN mutagen HCC model

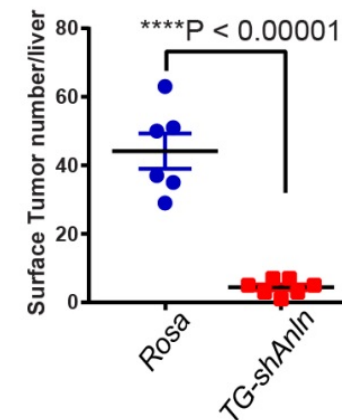
Intermittent ANLN inhibition



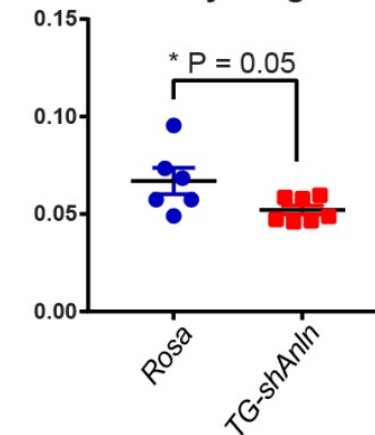
# Persistent ANLN shRNA inhibition: chronic DEN + CCl4 injury



Tumor quantification

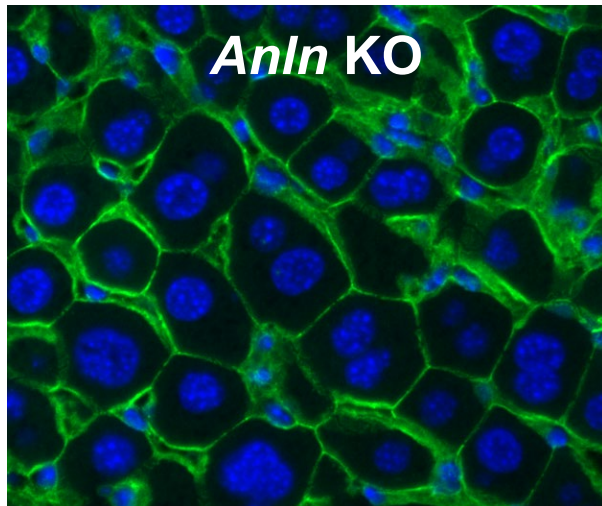
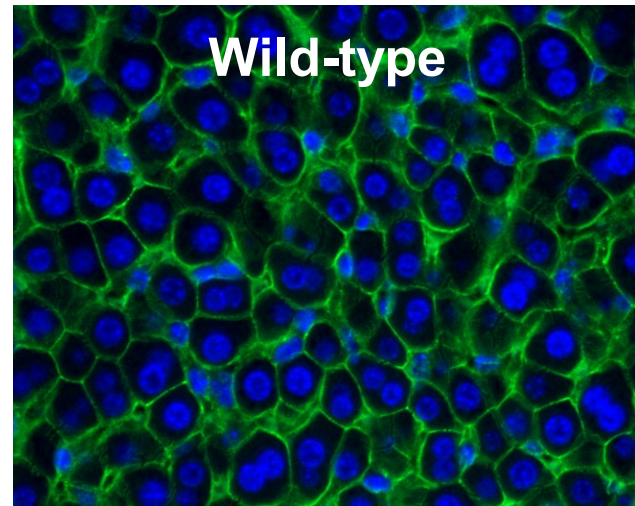


Liver to body weight ratio



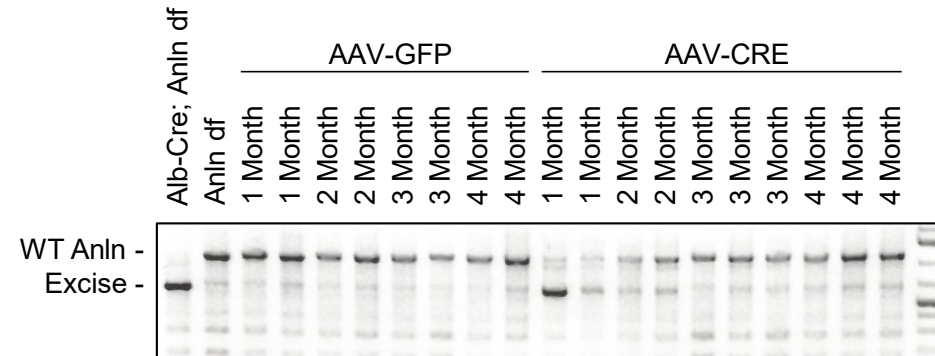
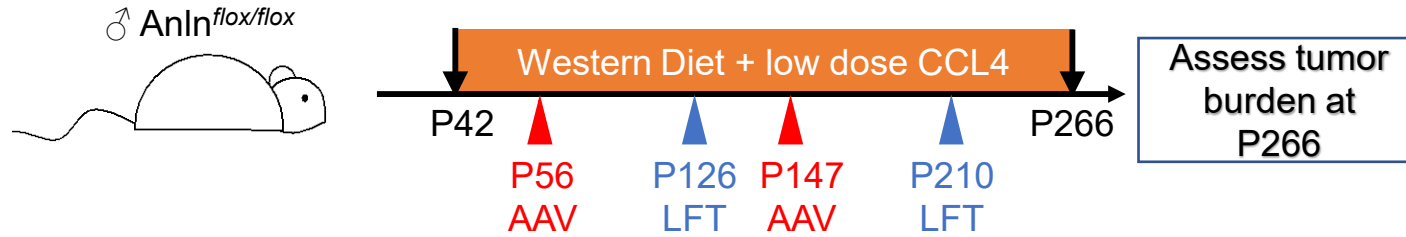


# Floxed ANLN genetic models



ANLN knockout livers appear grossly normal, regenerate after hepatectomy, and are highly polyploid.

# Assessing ANLN knockout efficiency using AAV-Cre in NASH



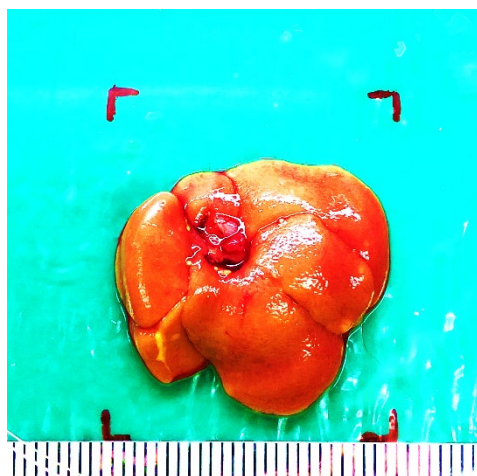
AAV-Cre effect disappears in 3 months.  
Second dose has no effect (possibly due to immunogenicity)

# Despite poor KO at 3 months, NASH related HCC is suppressed at 9 months of age

AAV-GFP

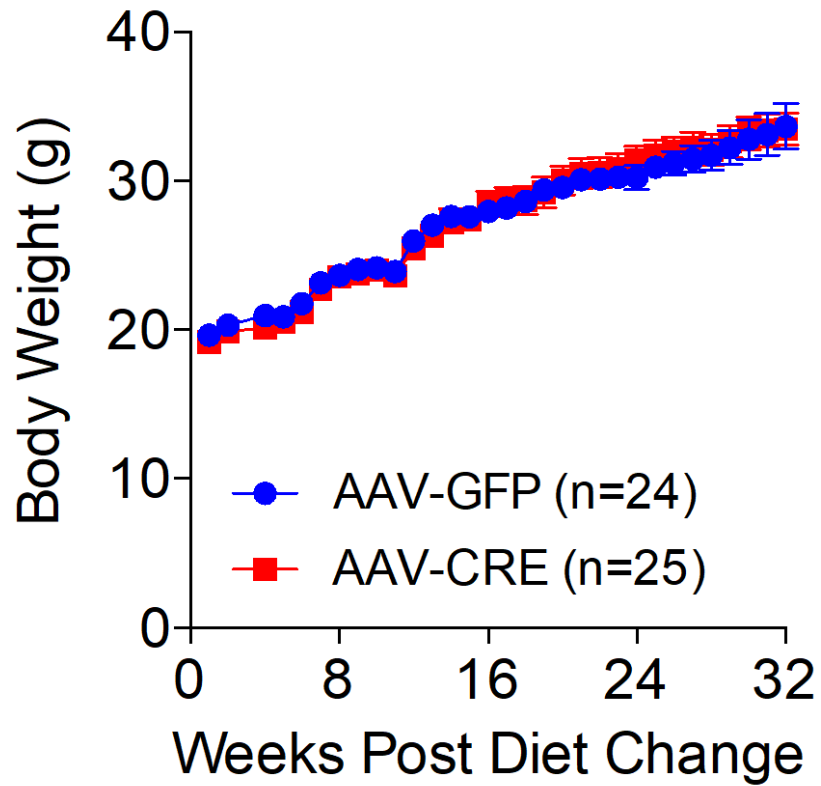


AAV-CRE

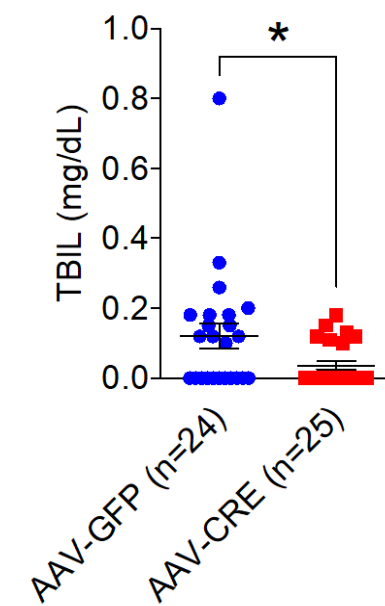
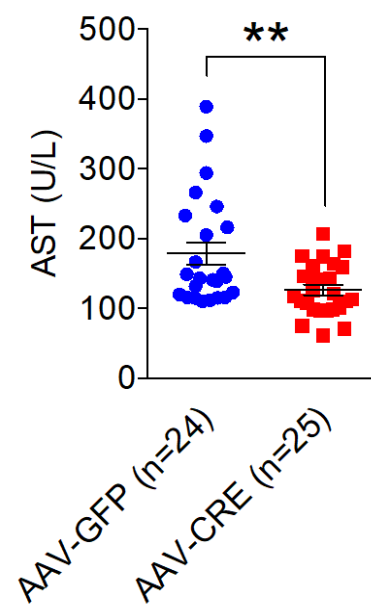
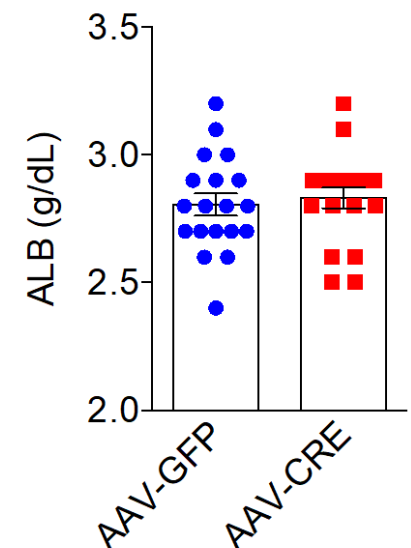
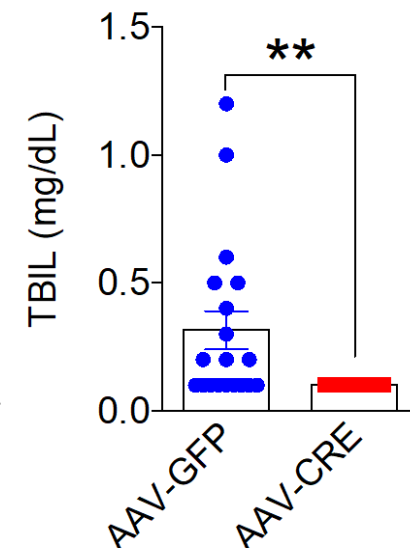
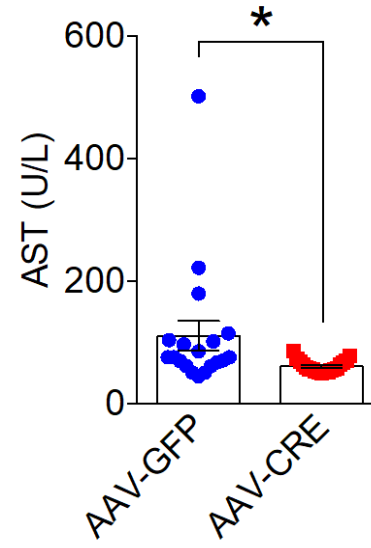




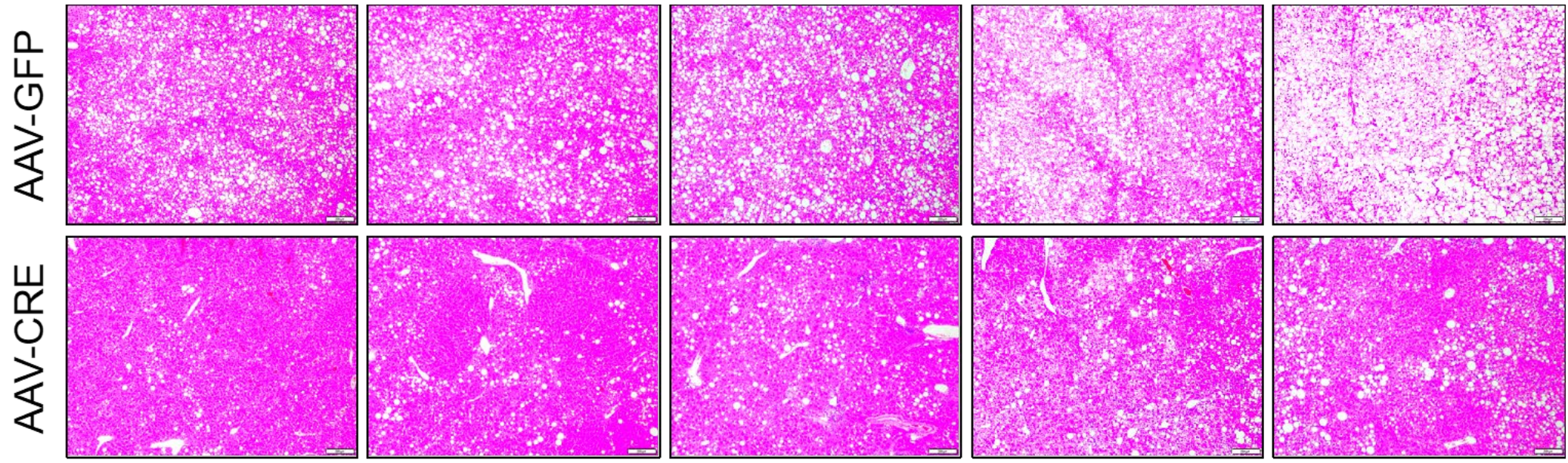
# ANLN KO via AAV-Cre prevents liver damage in NASH model



12 weeks post NASH diets  
24 weeks post NASH diets



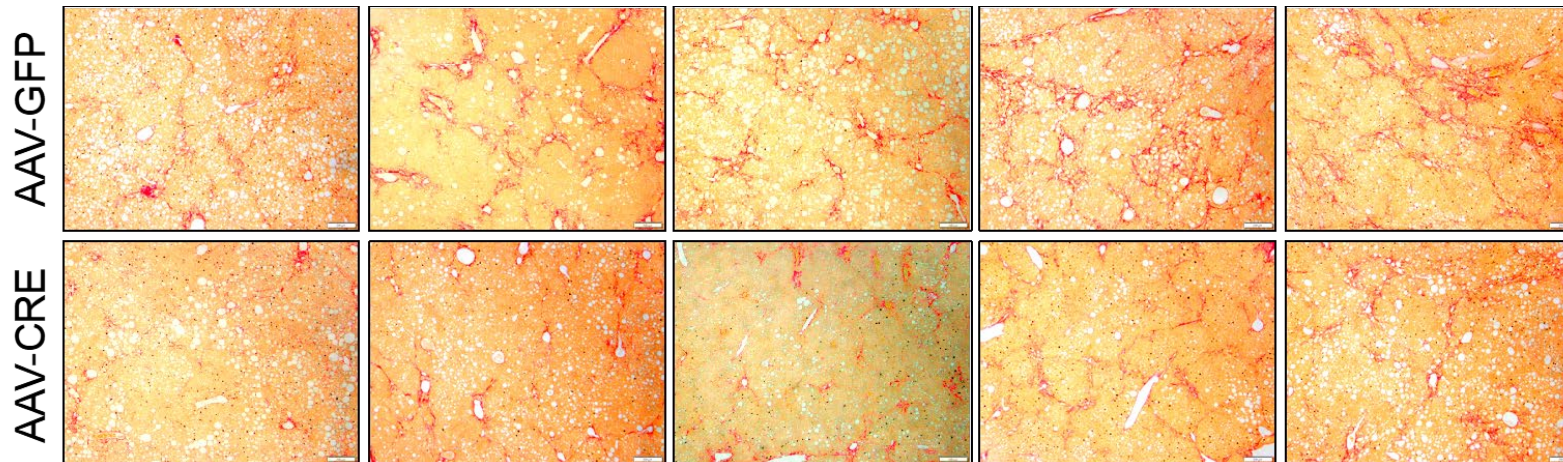
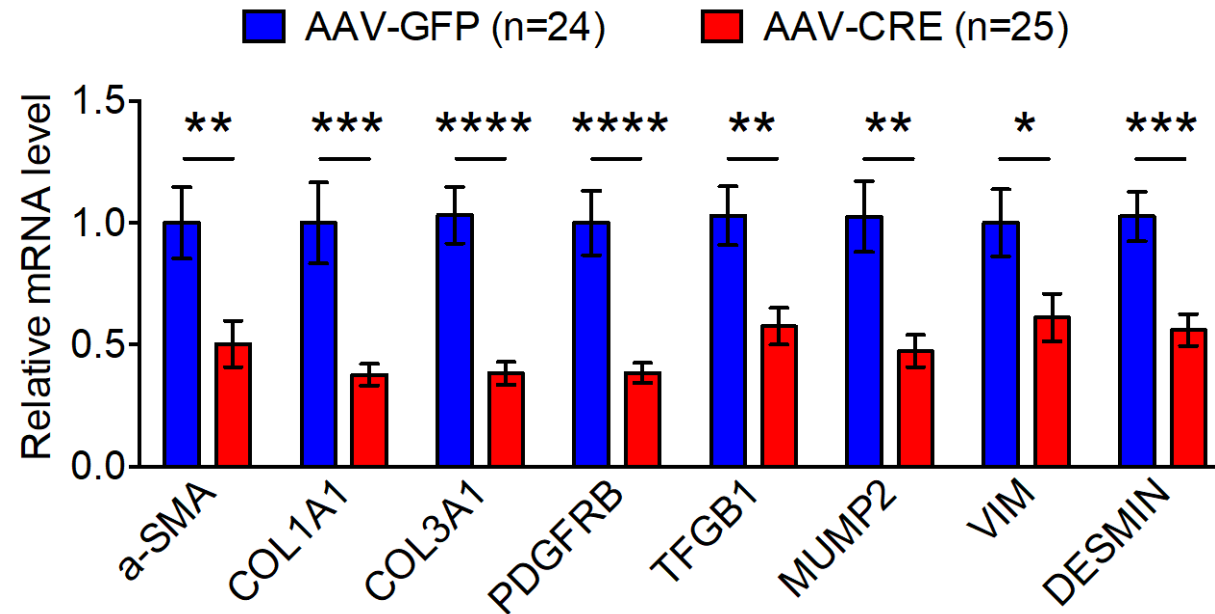
# ANLN KO via AAV-Cre prevents steatosis in NASH model



N = 5 and 5 mice shown here



# ANLN KO via AAV-Cre suppresses fibrogenesis in NASH

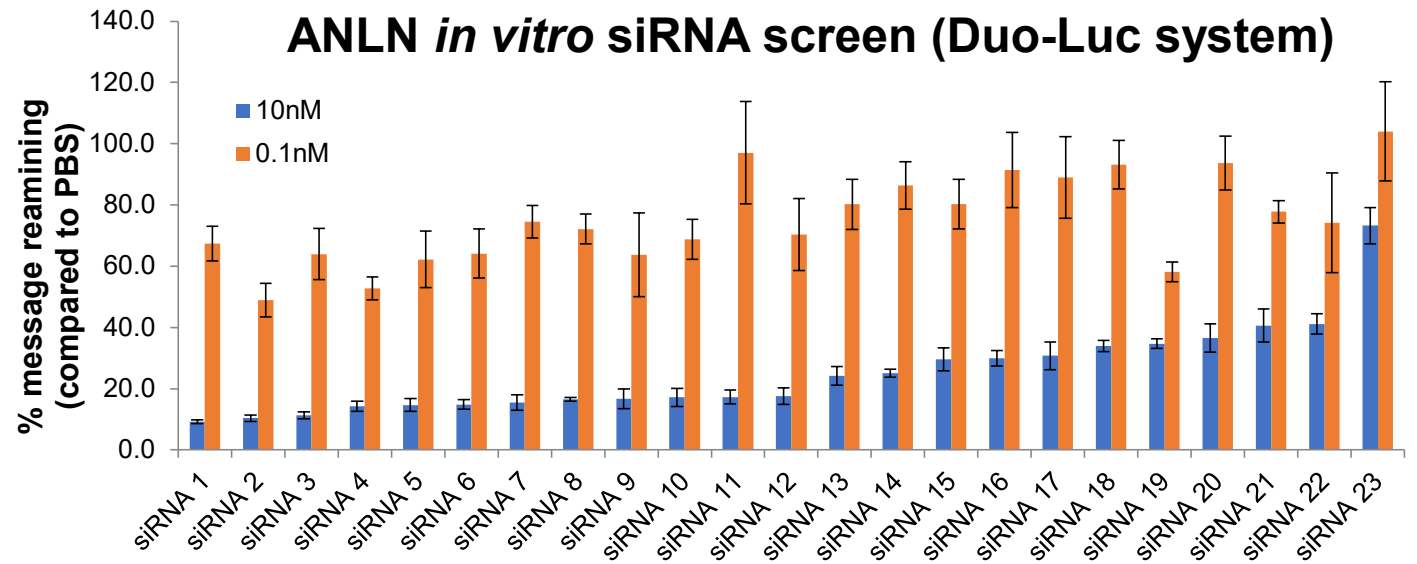


**Can this be replicated with Alnylam siRNAs?**

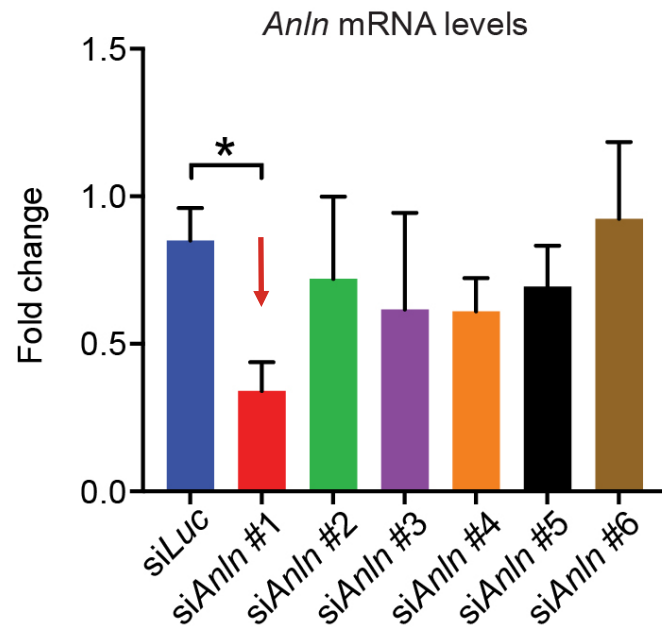


# Knockdown efficiencies of GalNAc conjugated siAnIns

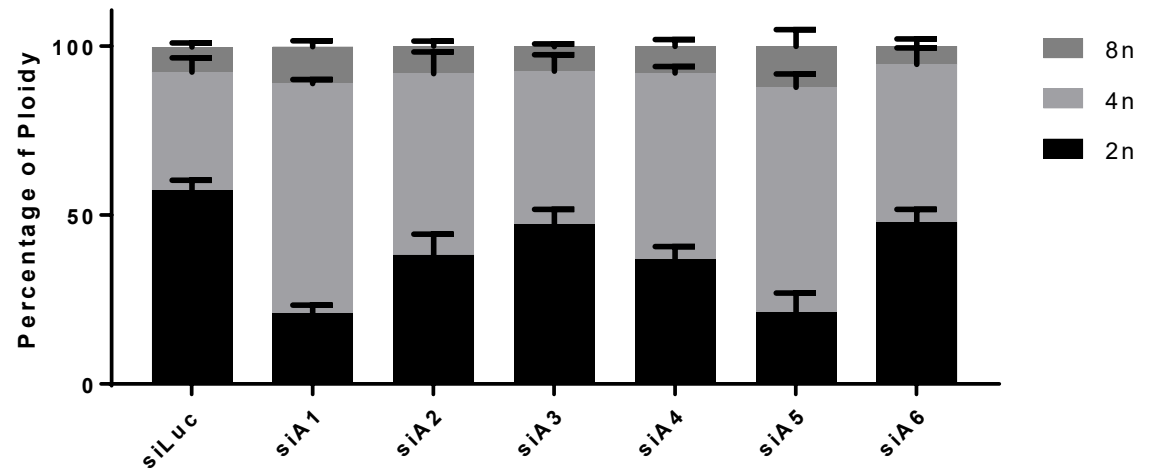
*in vitro:*



*in vivo:*

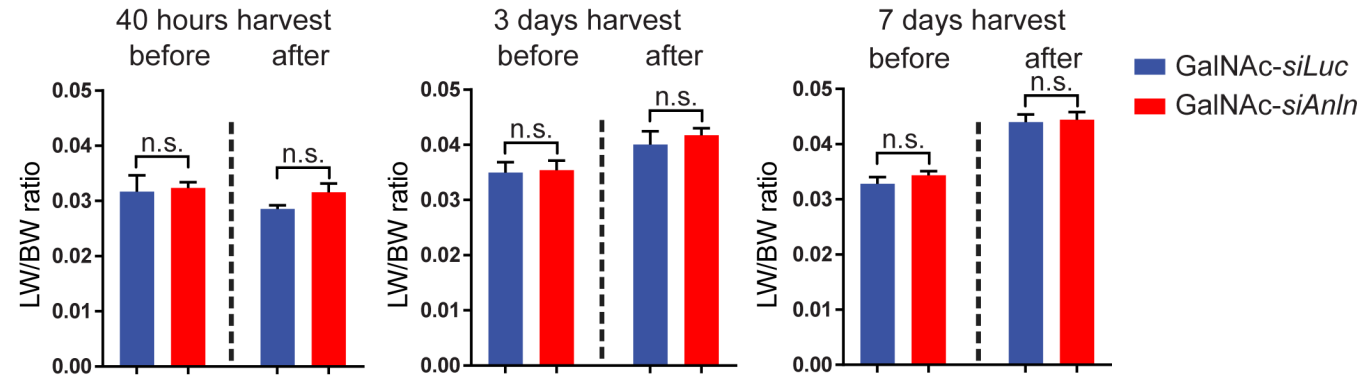


Ploidy distribution of C3h mice treated with Alnylam siAnIn

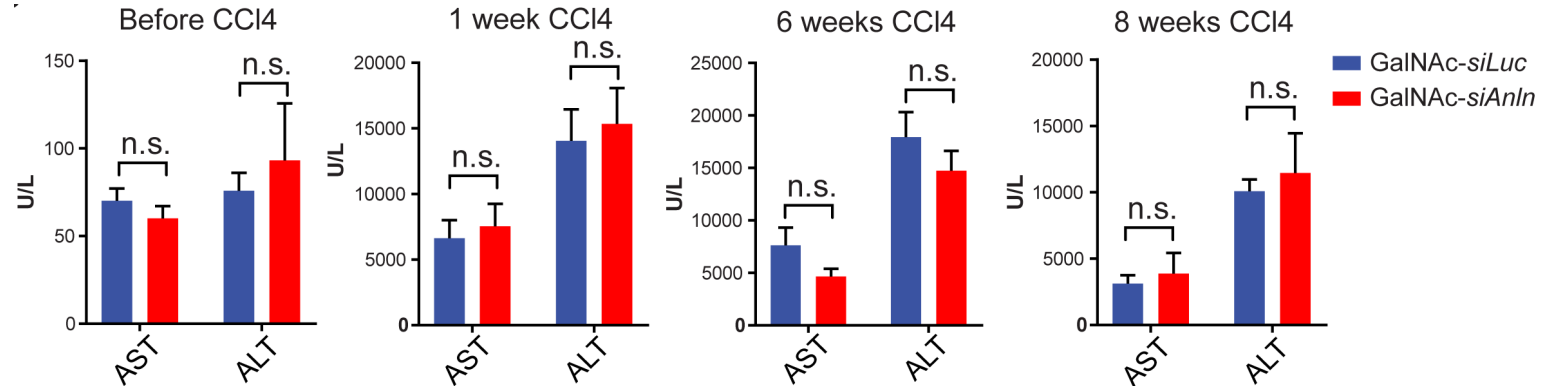


# GalNAc si*Anln* did not affect acute or chronic tissue repair

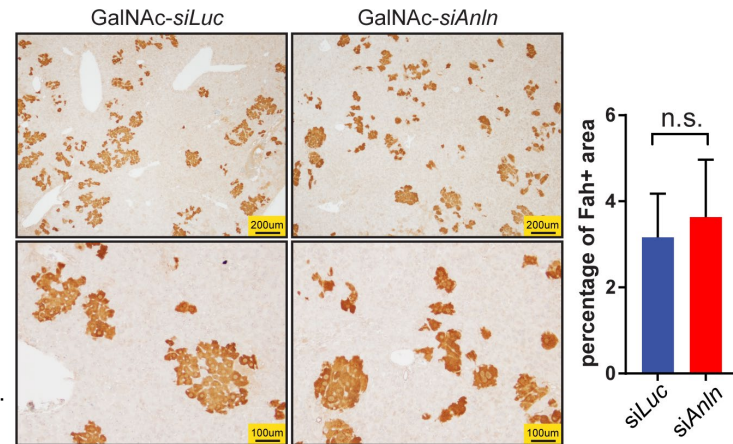
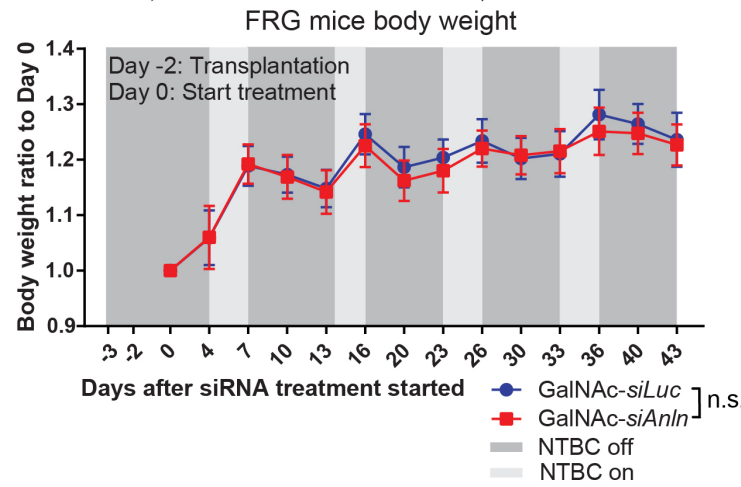
Hepatectomy



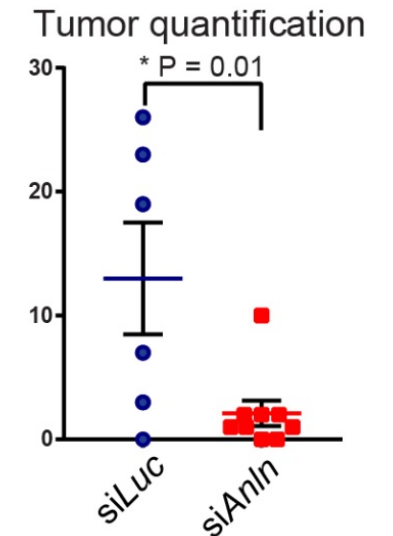
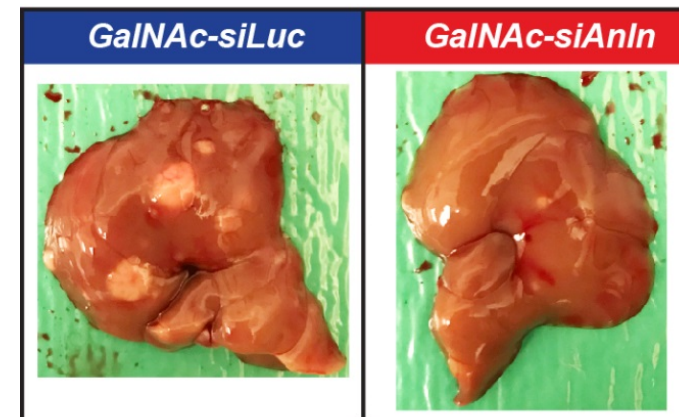
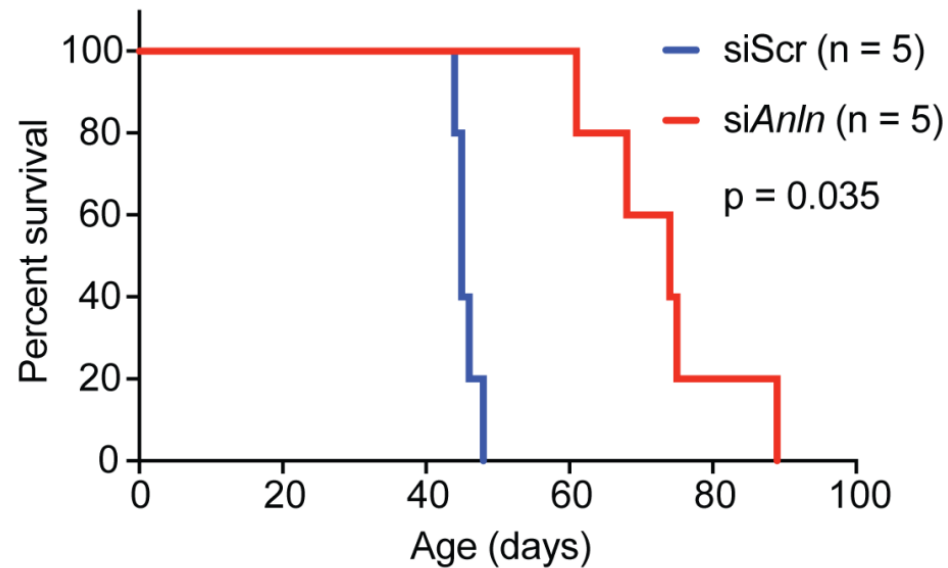
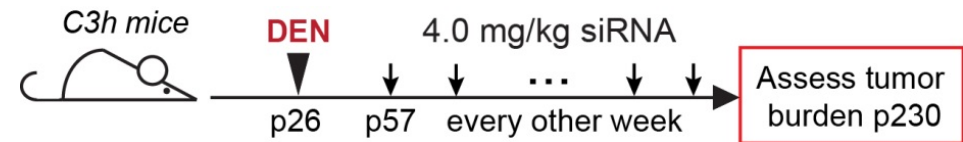
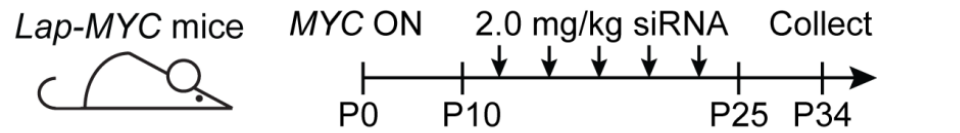
CCI4



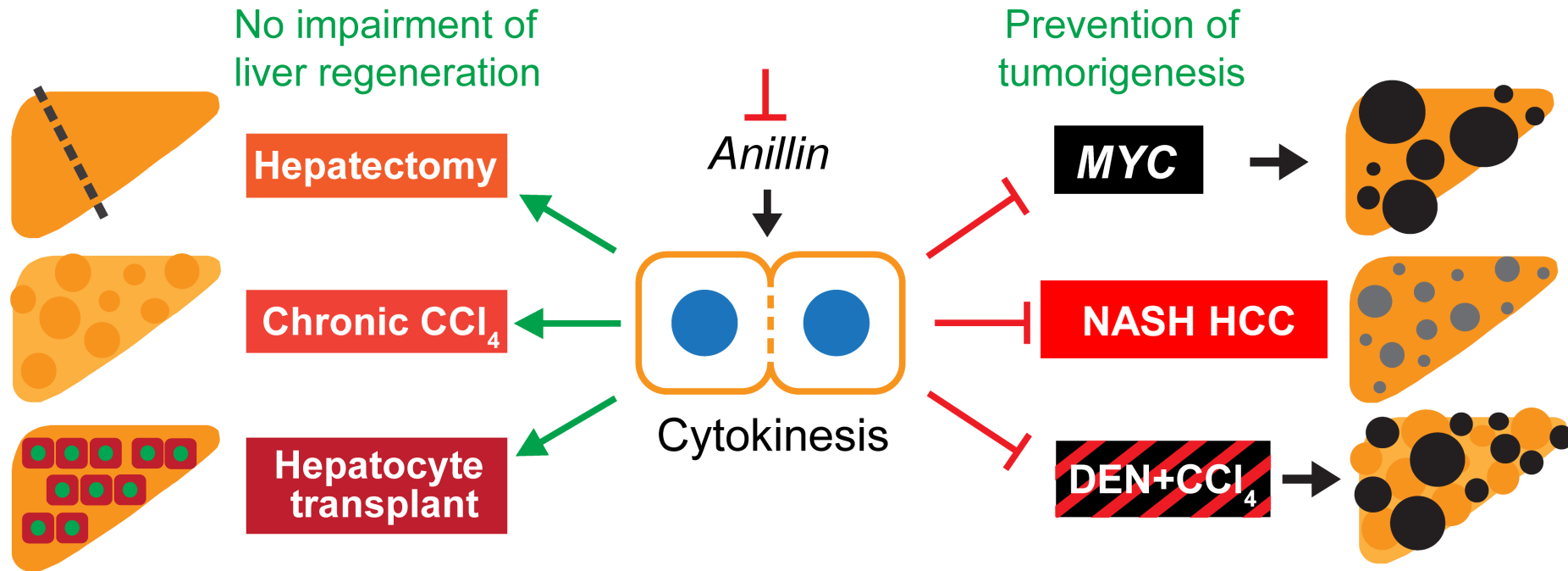
hepatocyte transplant



# Multiple HCC models are prevented by GalNAc-siANLN



# Summary: safe, and effective for HCC prevention



## Prospective clinical use:

Patient with cirrhosis and small Lirads/hcc lesion <2 cm.

Patient with early HCC resected or ablated.



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