

Application of Animal Models for Human Glucuronidation

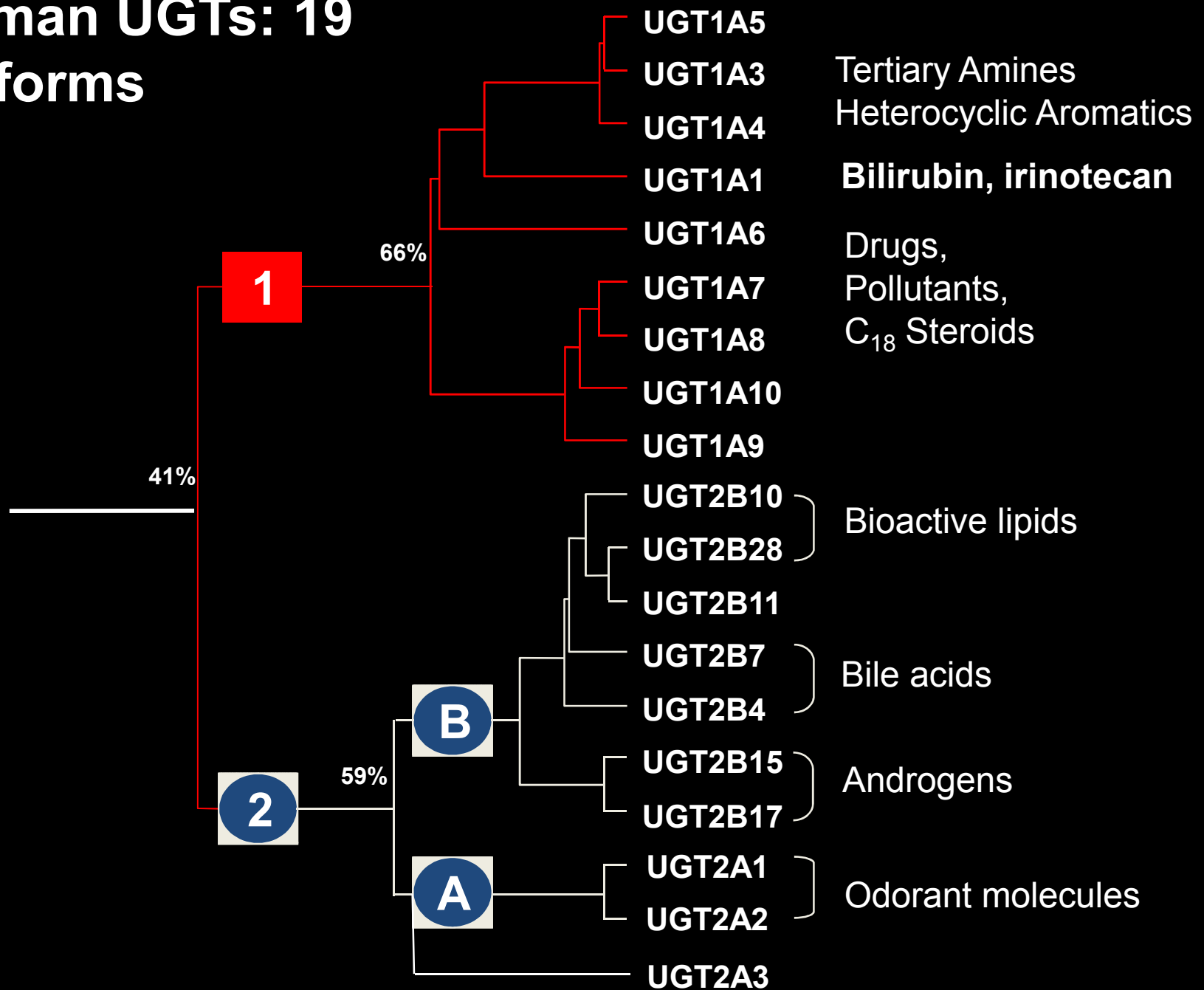
Shujuan Chen

Tukey Laboratory

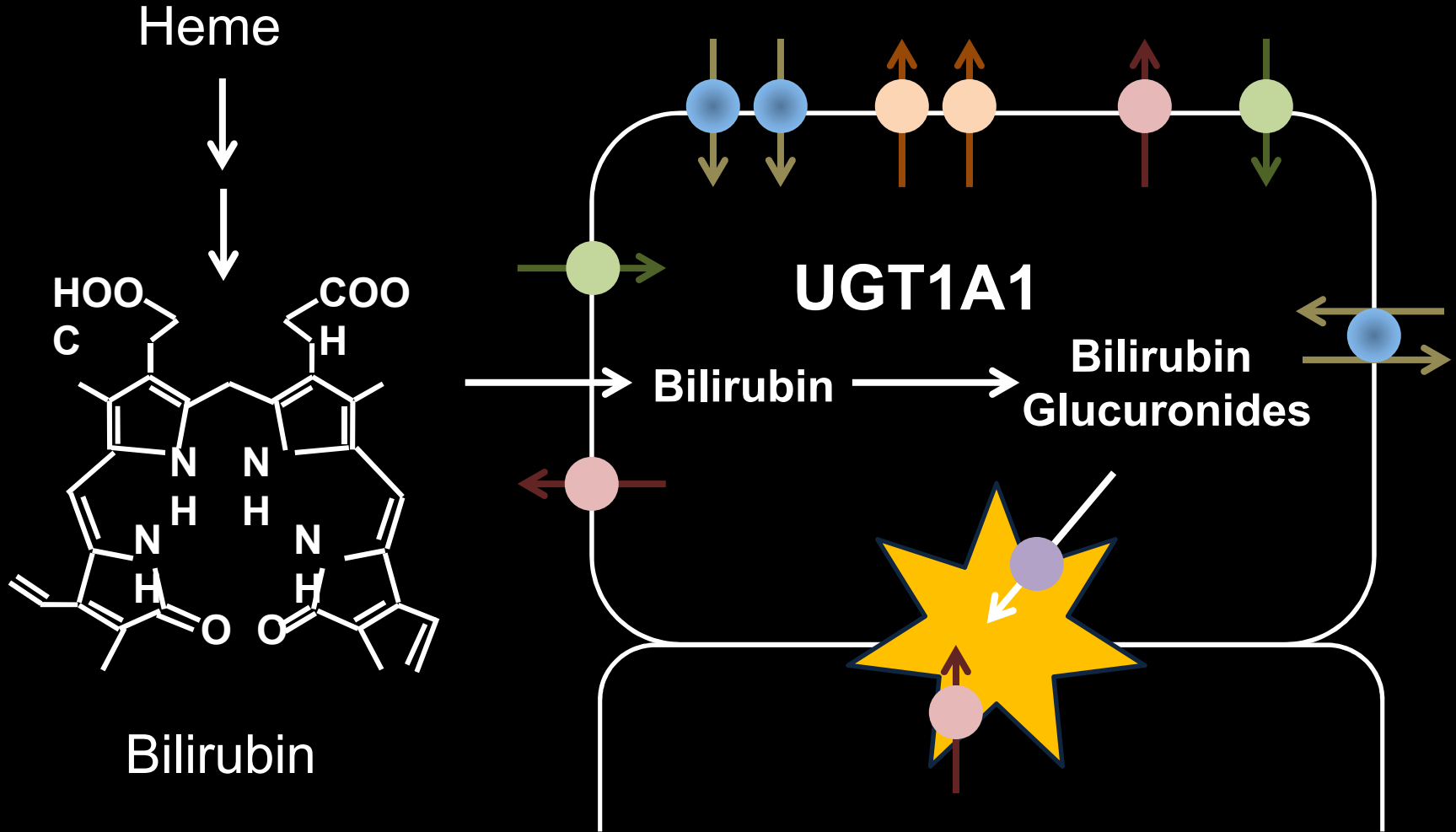
**Departments of Chemistry & Biochemistry
and Pharmacology, UCSD**

2012 SCDMDG

Human UGTs: 19 isoforms



Bilirubin Clearance



Neonatal Hyperbilirubinemia



Jaundice

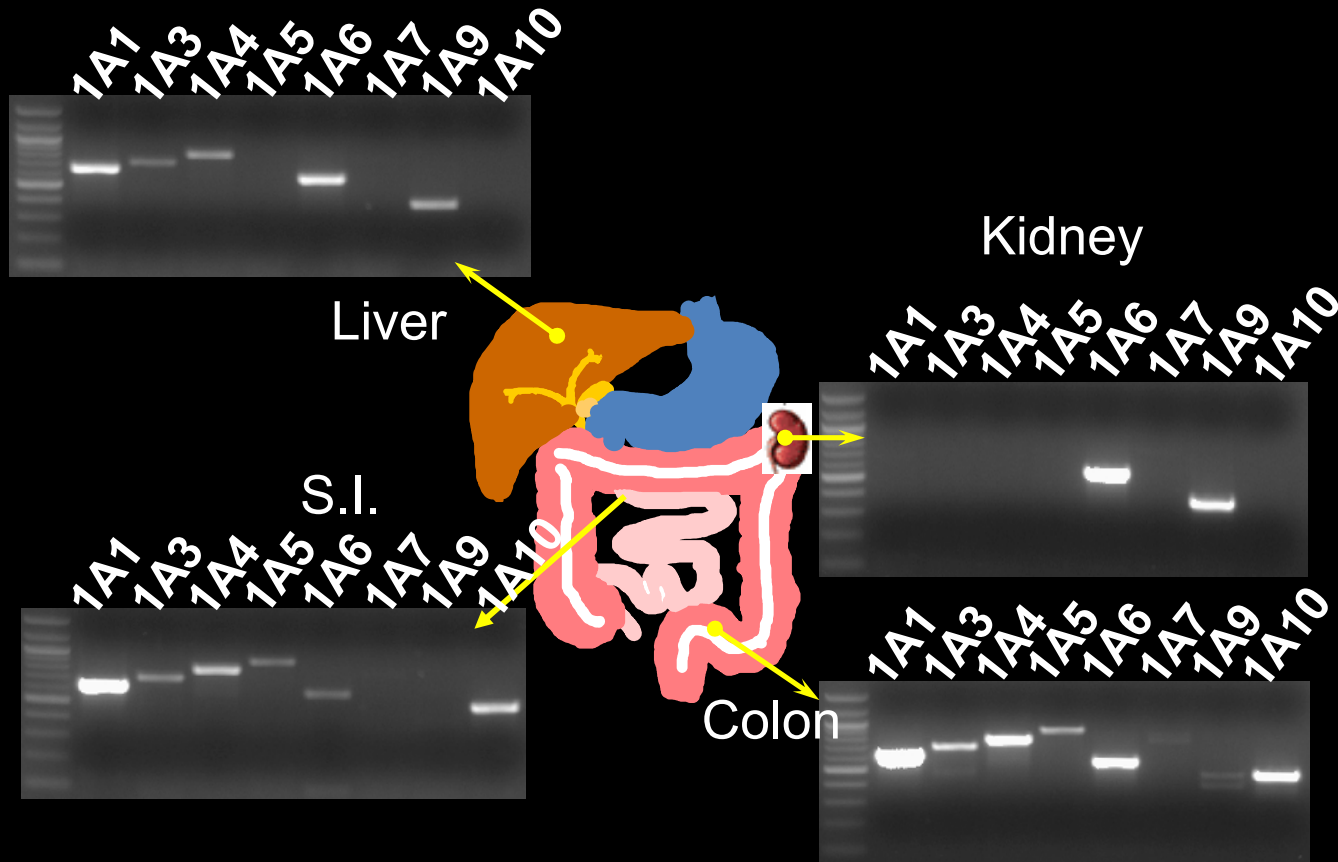
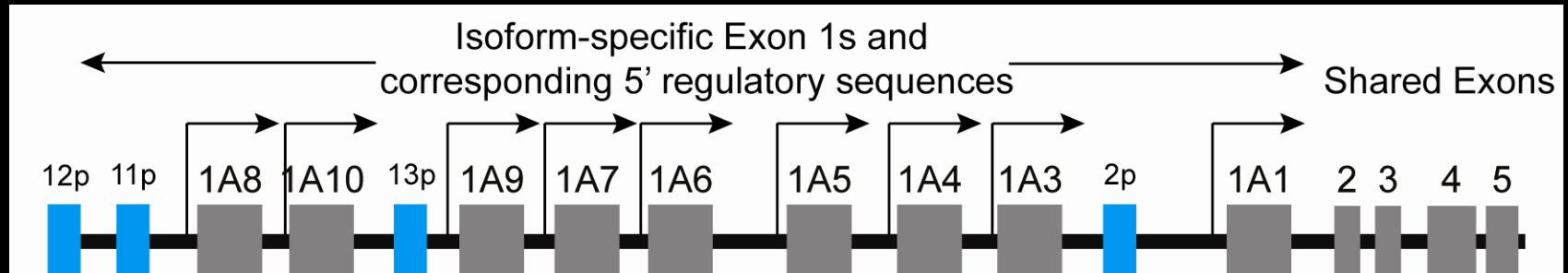


Light Therapy

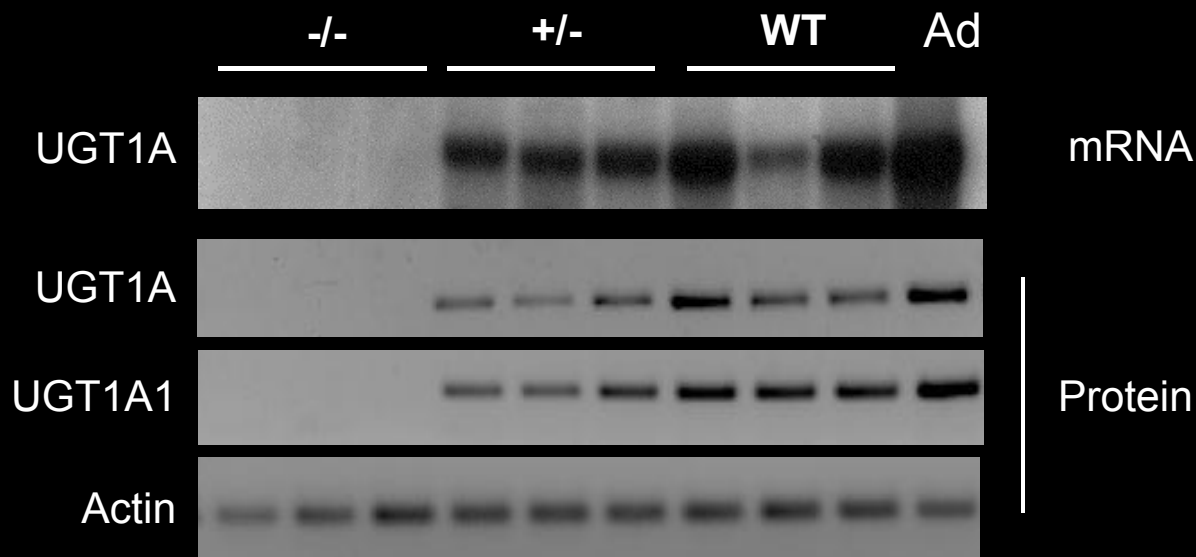
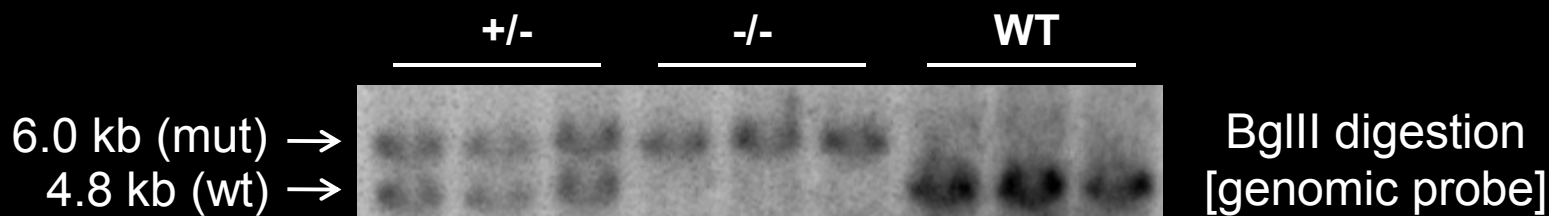
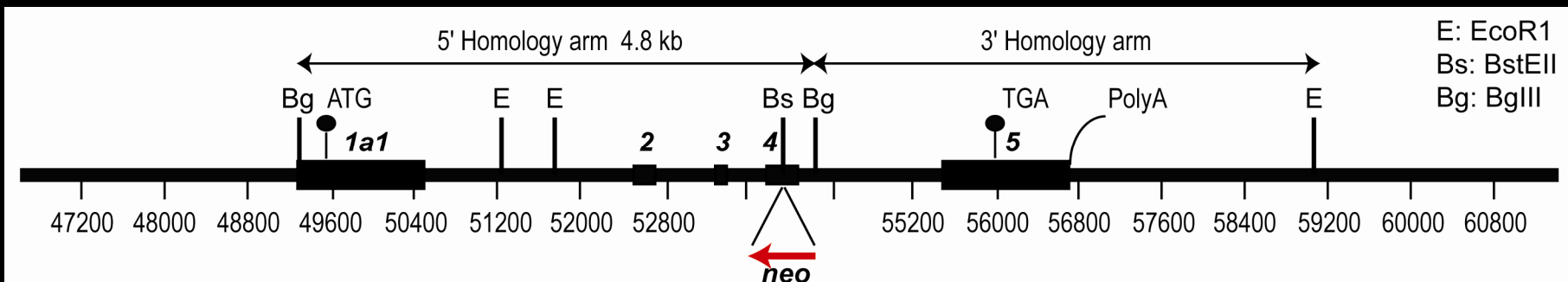


Kernicterus

Tg-UGT1 Mice



Ugt1^{-/-} Mice

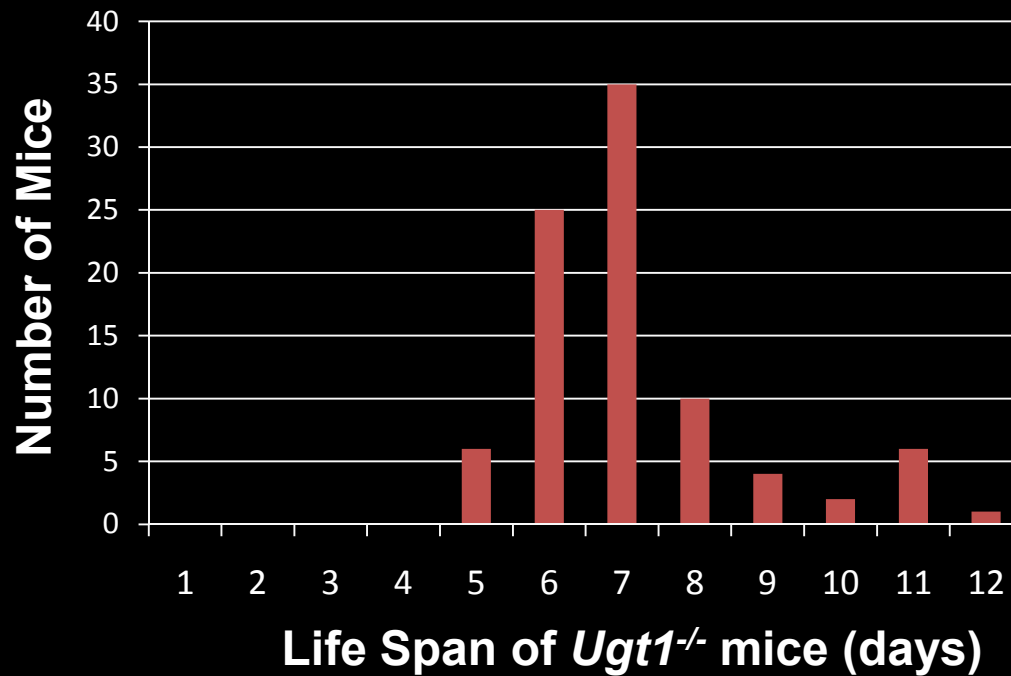


Longevity of *Ugt1*^{-/-} Mice

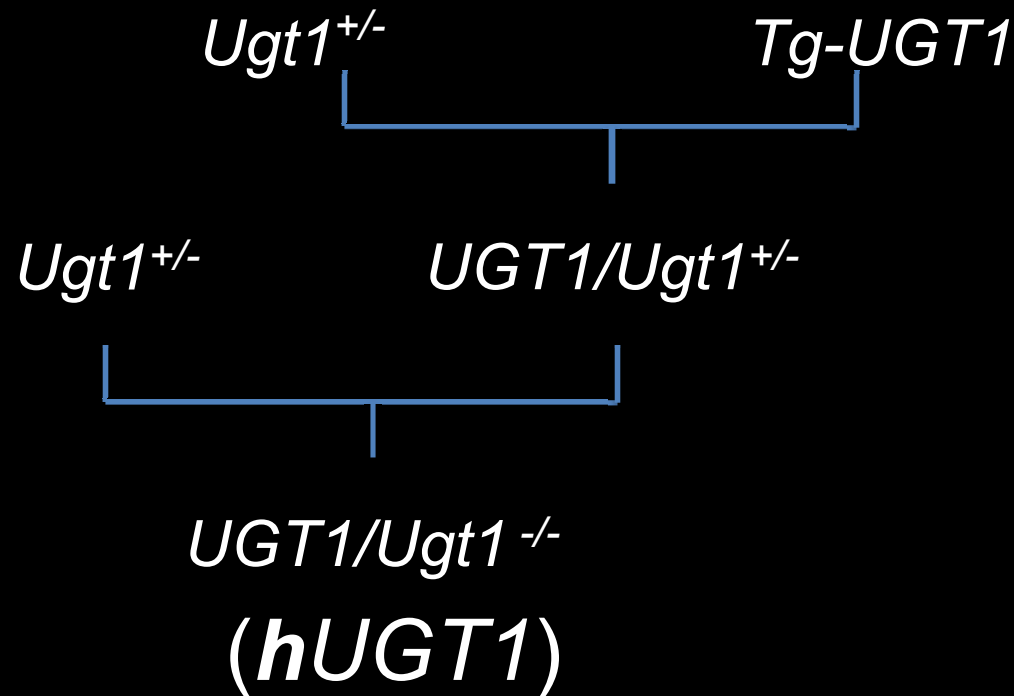


Ugt1^{+/-}

Ugt1^{-/-}



Generation of Humanized *UGT1* Mice



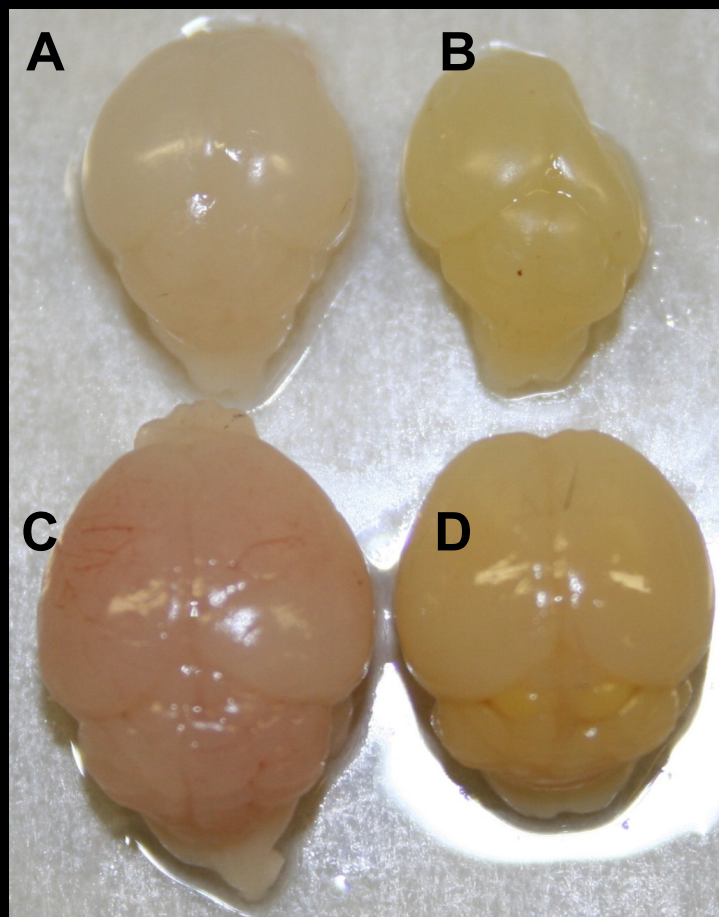
Humanized *UGT1* Mice as a Model for Hyperbilirubinemia and Brain Toxicity



7-day
Ugt1^{+/-}

15-day
hUGT1

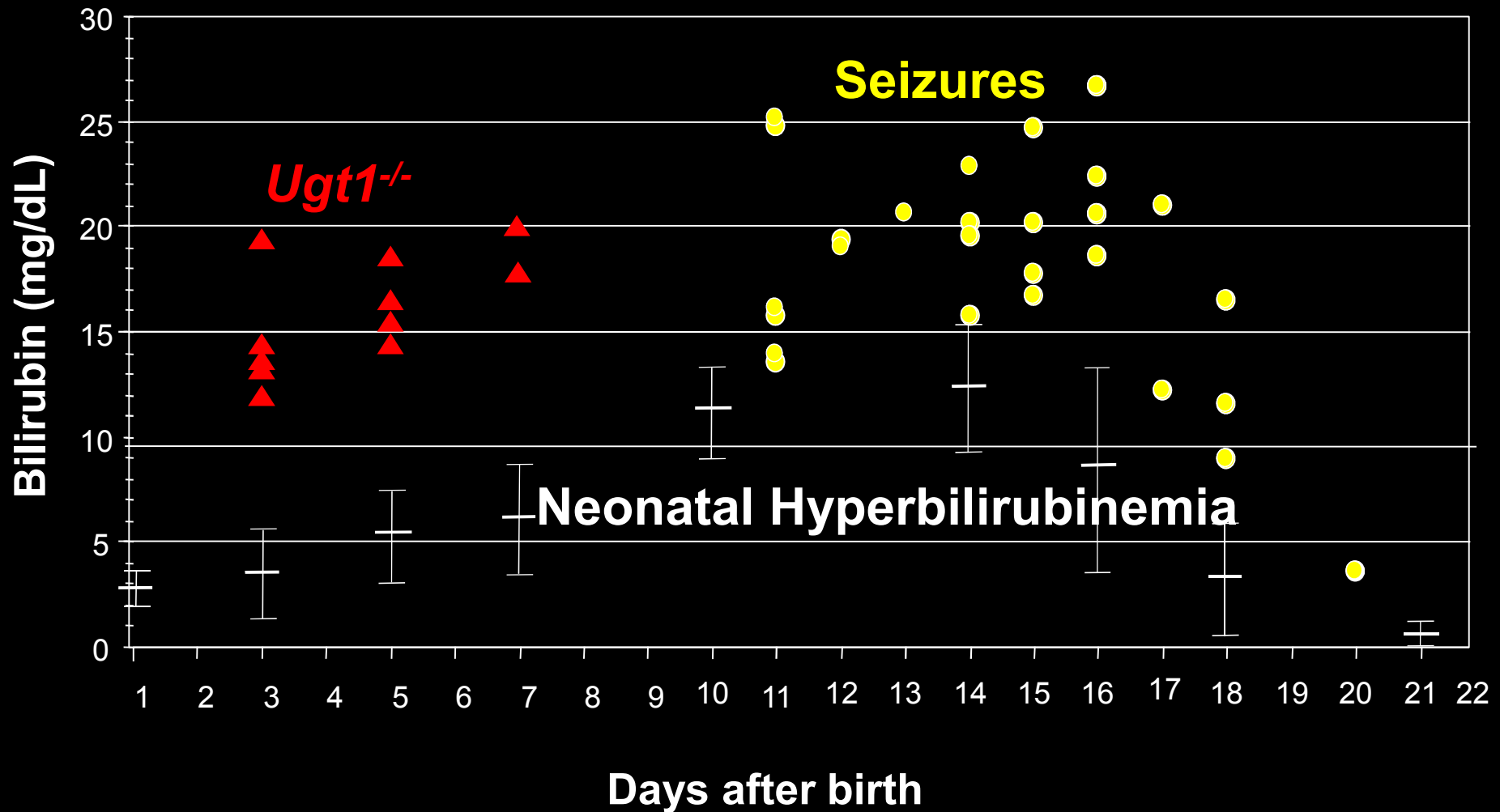
Normal Kernicterus



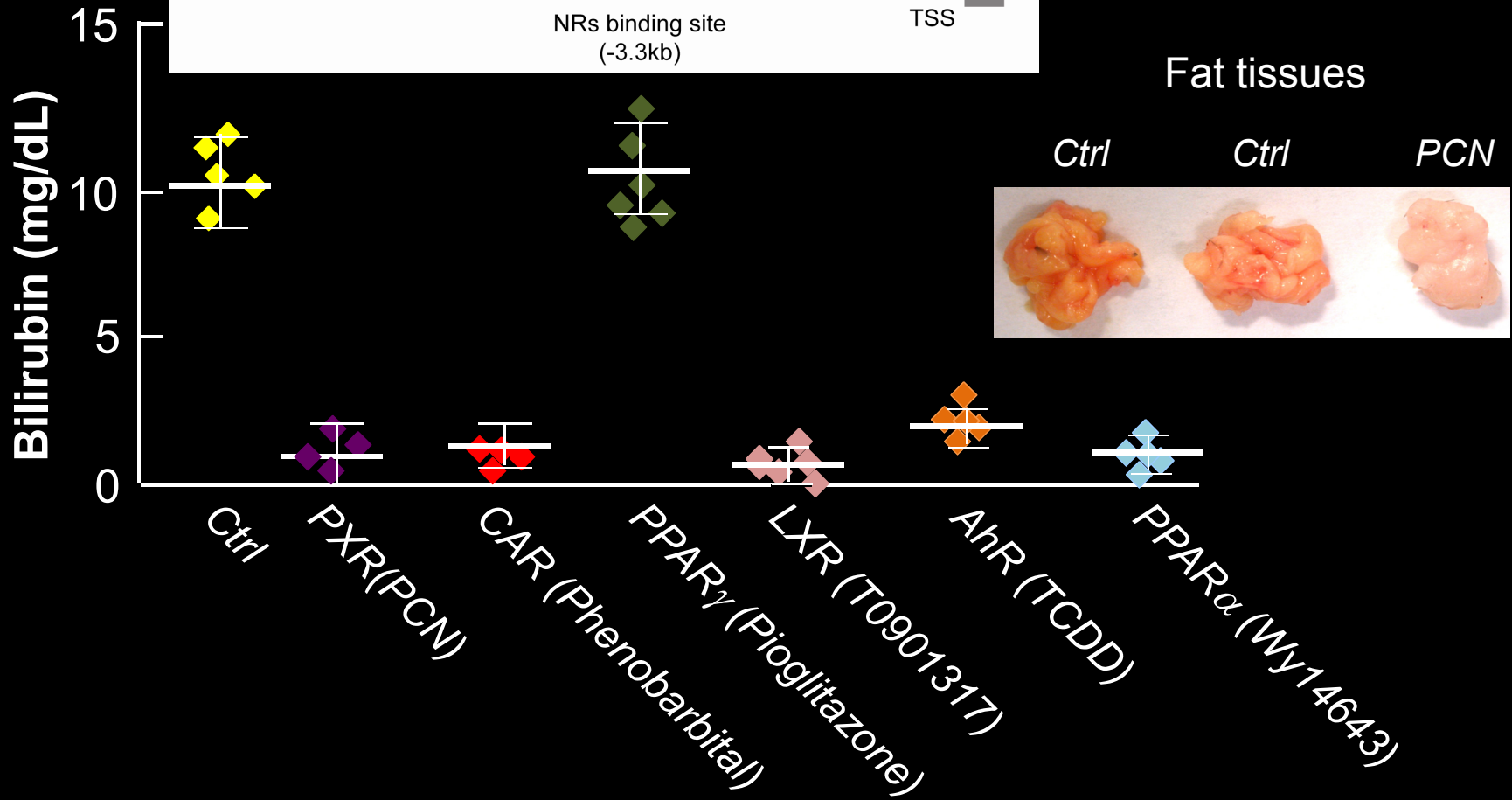
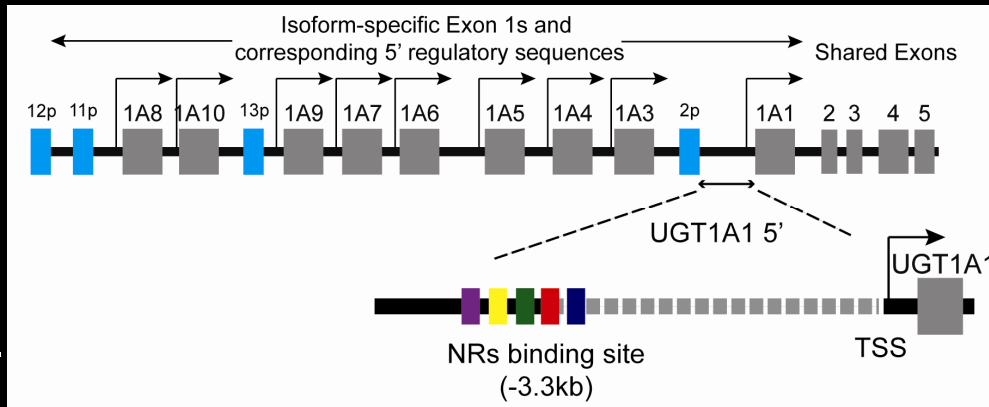
7-day
Ugt1^{-/-}

15-day
hUGT1

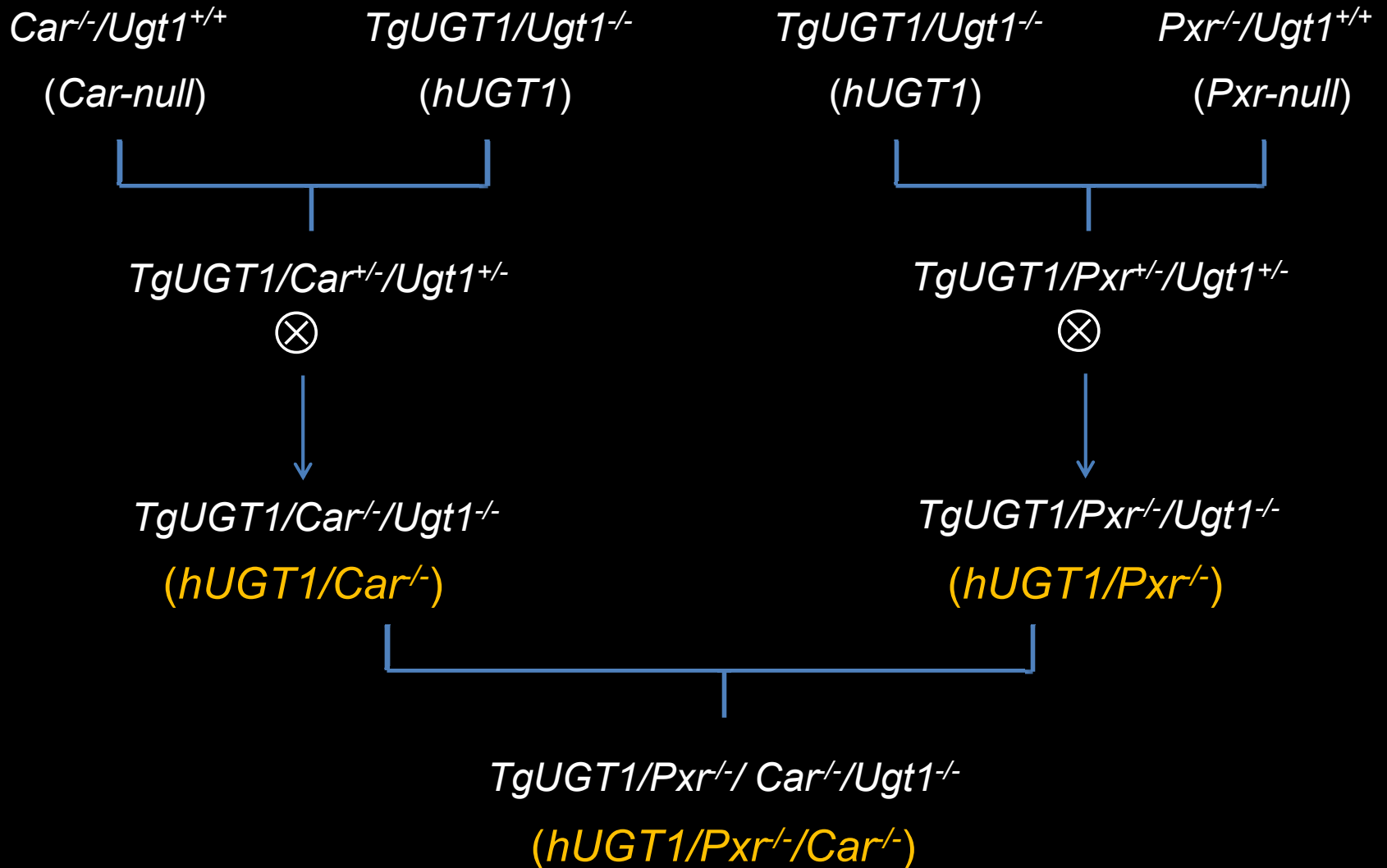
TSB Levels in *Ugt1*-null and Humanized *UGT1* Mice



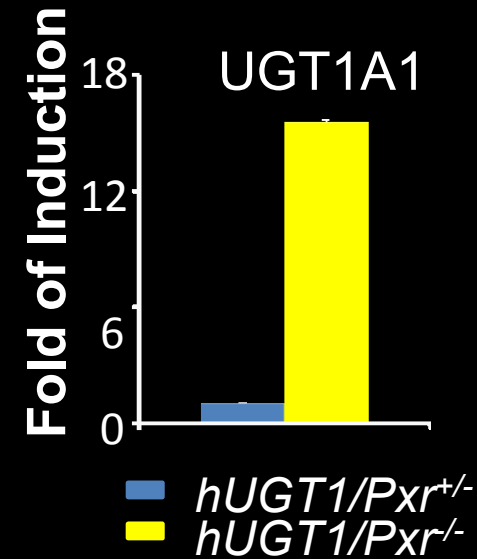
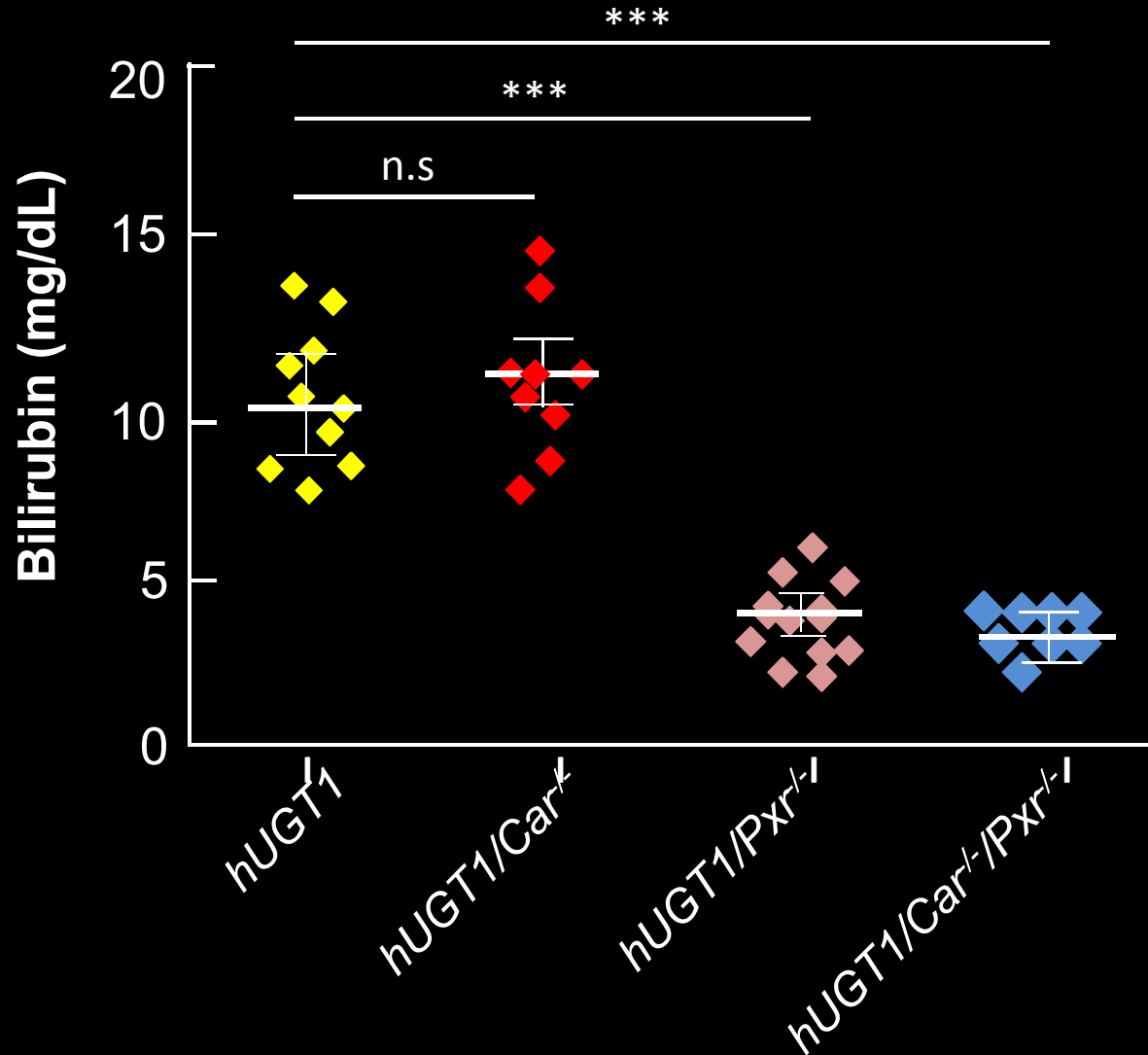
NRs Agonists v.s. Bilirubin Levels



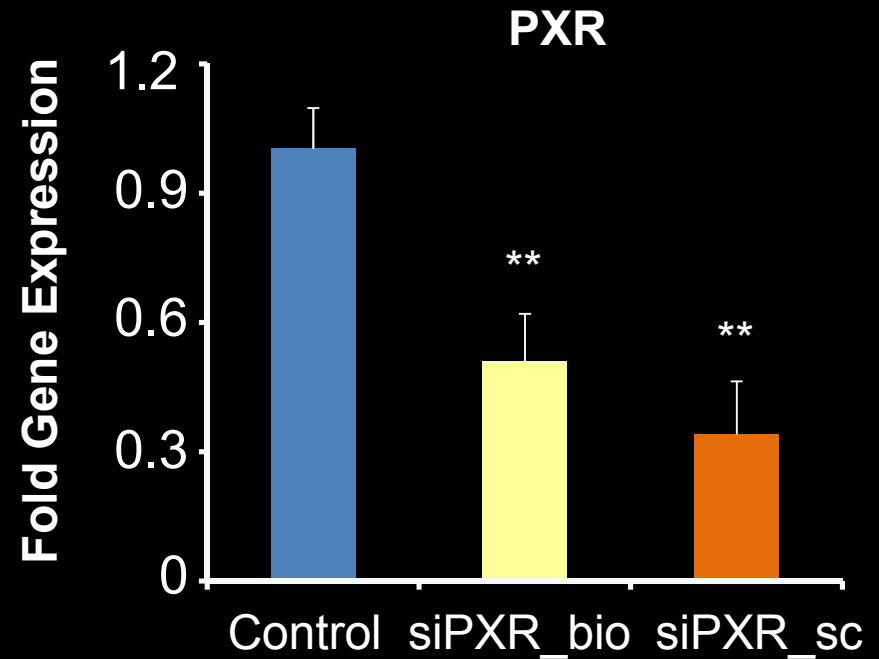
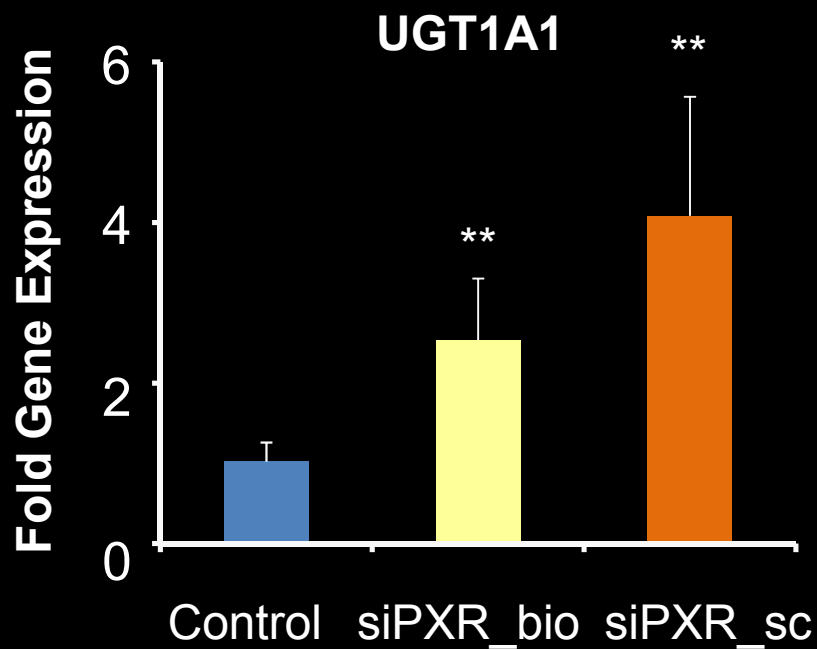
Generation of *hUGT1* Mice with *NR-null* background



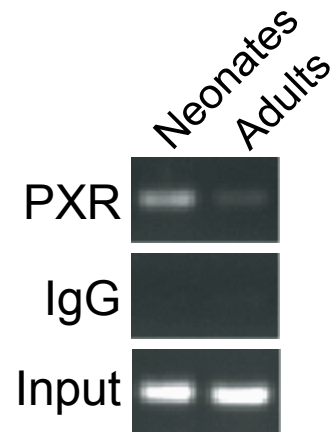
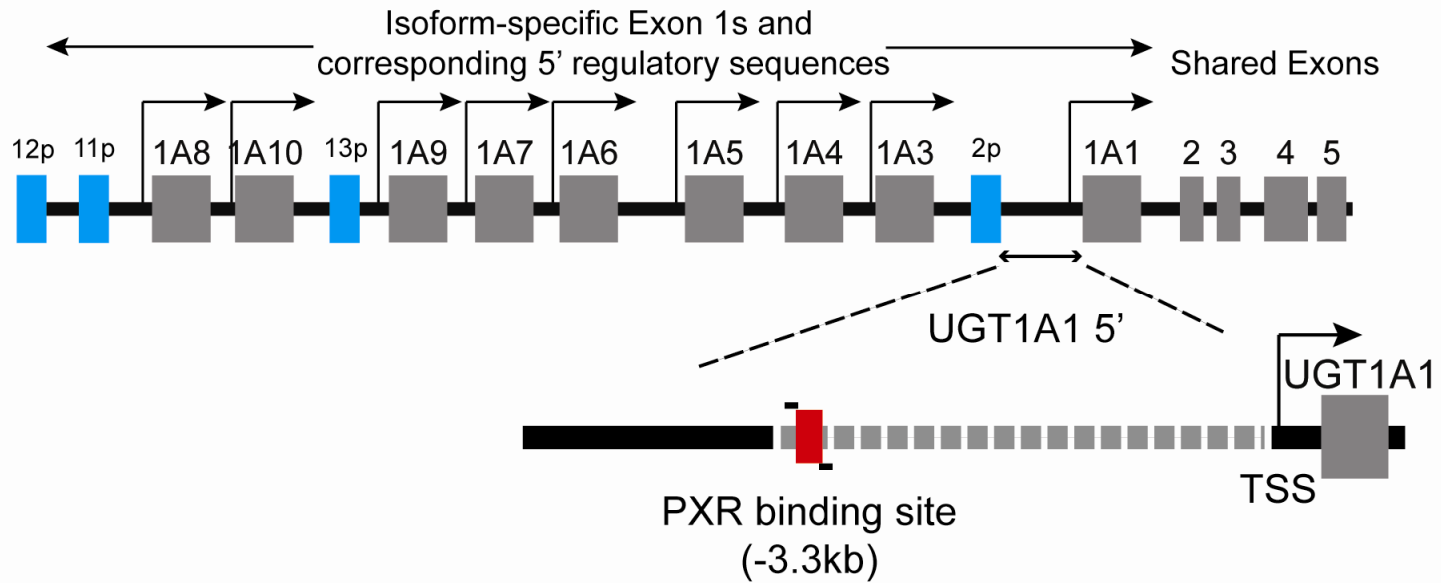
hUGT1 Mice with *Pxr*-null and/or *Car*-null



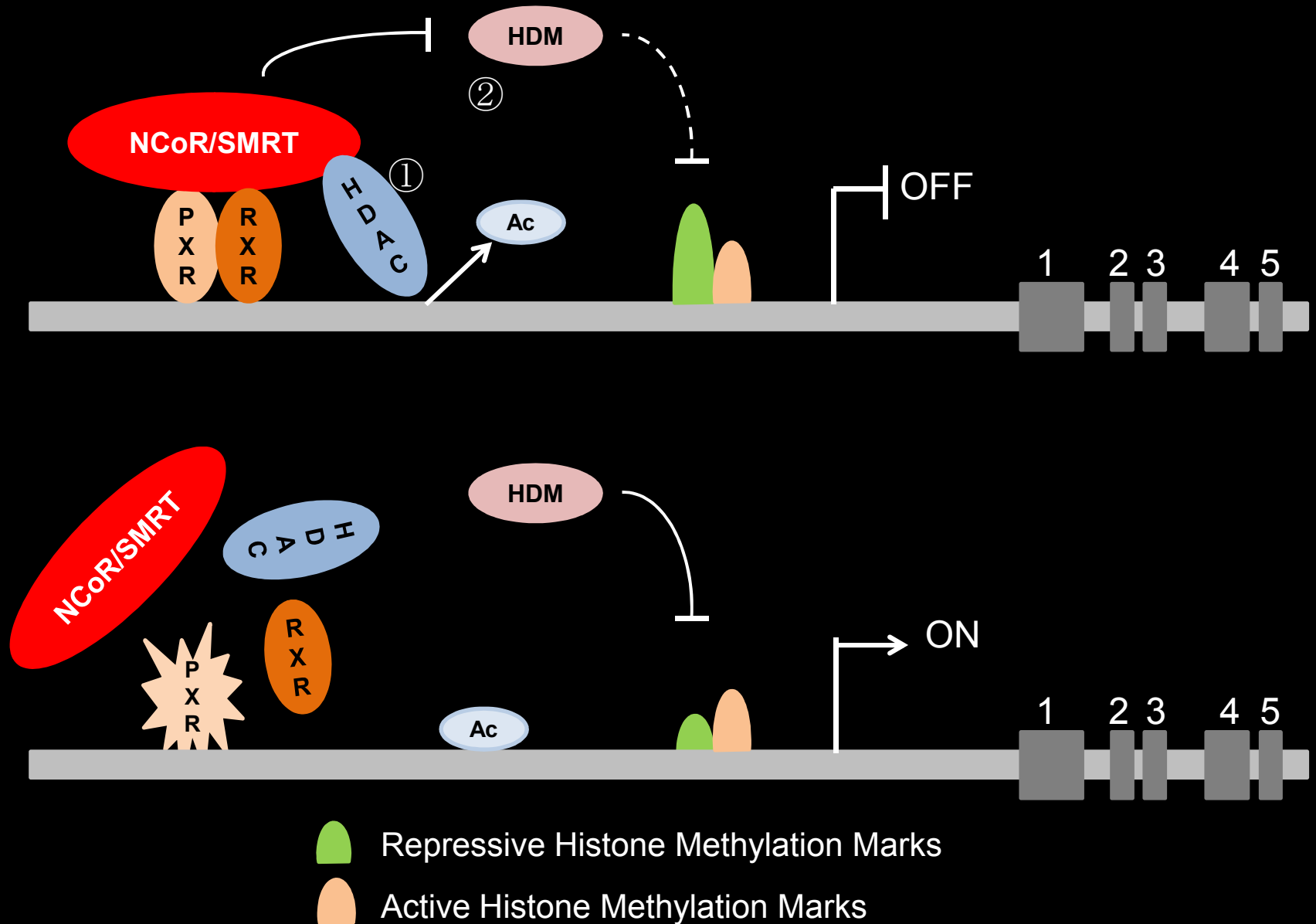
Neonatal *hUGT1* Hepatocyte Treated with siPXR



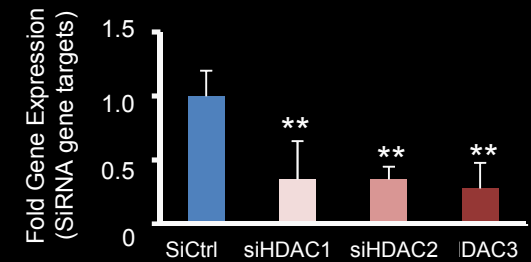
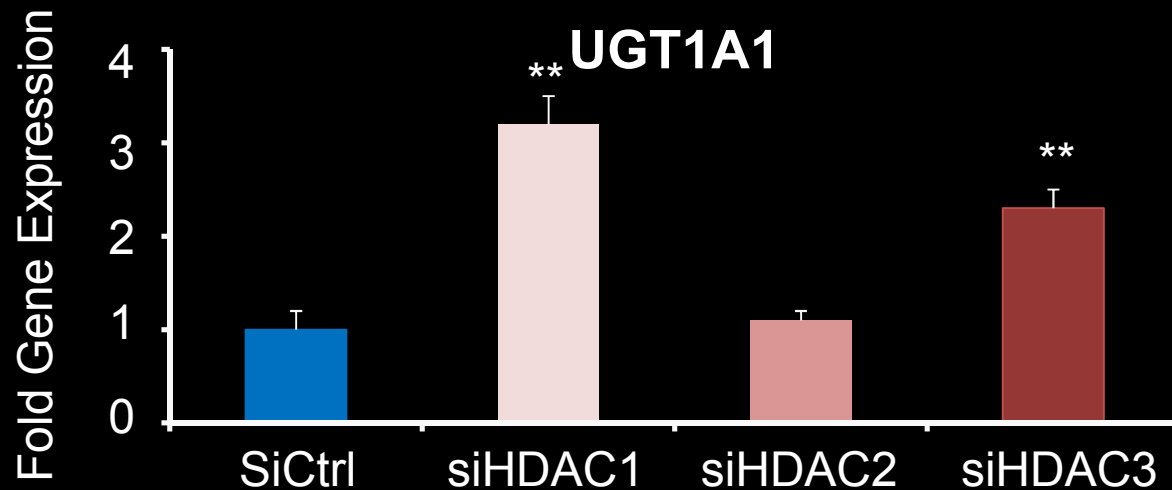
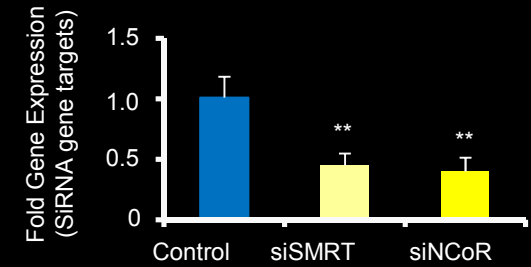
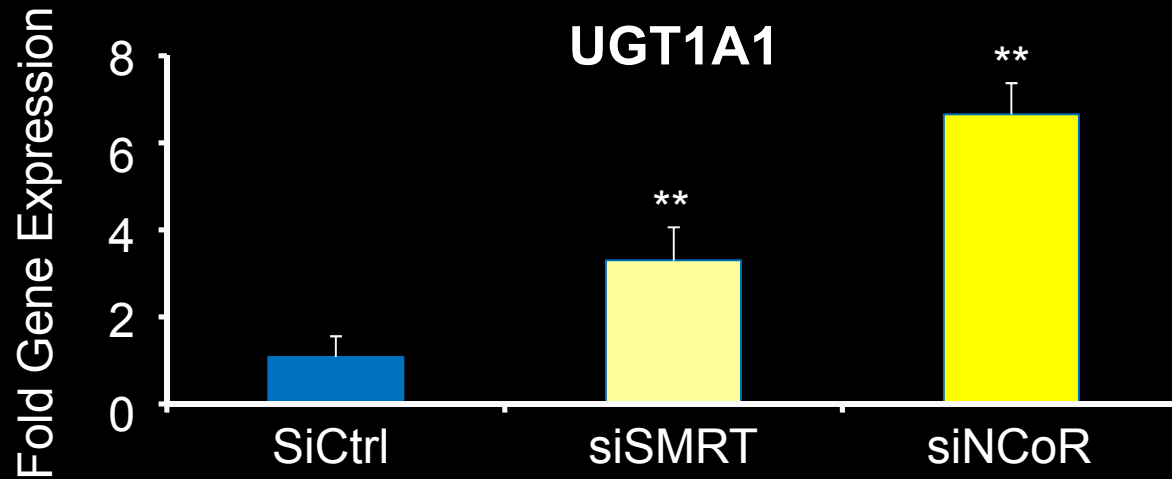
ChIP Assay



Hypothesis of PXR Repression & De-repression



UGT1A1 Gene Expression v.s. siRNA



Conclusions

1. Humanized *UGT1* mice develop neonatal hyperbilirubinemia and serve as a model for brain toxicity.
2. Humanized *UGT1* mice are subject to regulation by nuclear receptor agonists.
3. Deletion of the *Pxr* gene, but not the *Car* gene, lowers serum bilirubin in *hUGT1* mice.
4. PXR binds to the *UGT1A1* promoter, and the genetic deletion of *Pxr* leads to the upregulation of *UGT1A1* in *hUGT1/Pxr^{-/-}* mice.

Irinotecan Metabolism and Toxicities

Blood

Liver

GI Epithelium

GI Lumen

Neutropenia

← Blood

CPT-11

CE

→ SN-38

↓ UGT1A1

SN-38G

SN-38G

↑ UGT1A1

SN-38

SN-38G
(Bile)

↓ Glucuronidase

SN-38

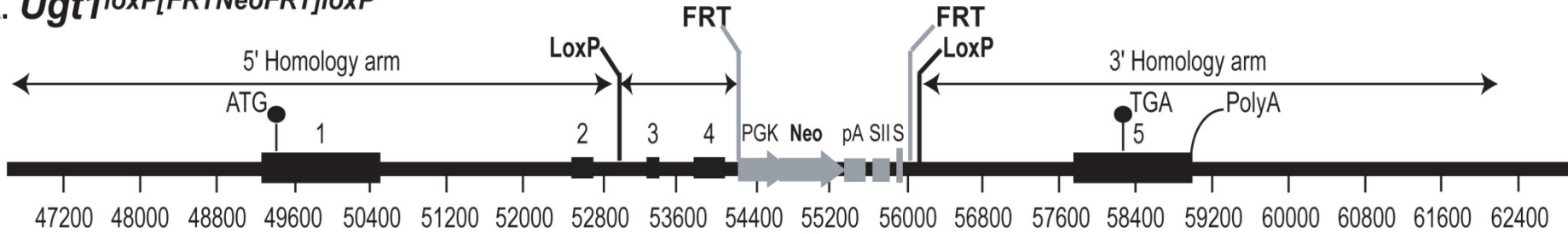
Late Diarrhea

Table 1. SN-38 glucuronic activity studies in mouse liver microsomes (MLM) and small intestine microsomes (MSIM).

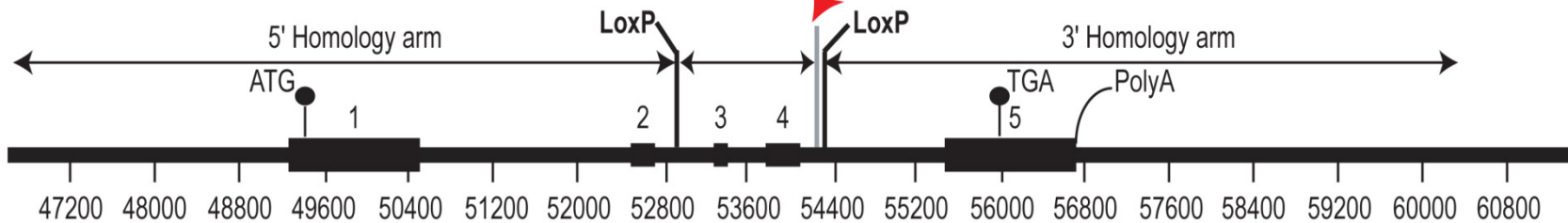
Michaelis-Menten	MLM	MSIM
Km (μM)	10.9±0.3	8.22±1.22
Vmax (pmol/min/mg)	69.1±1.0	217±13

Target Constructs

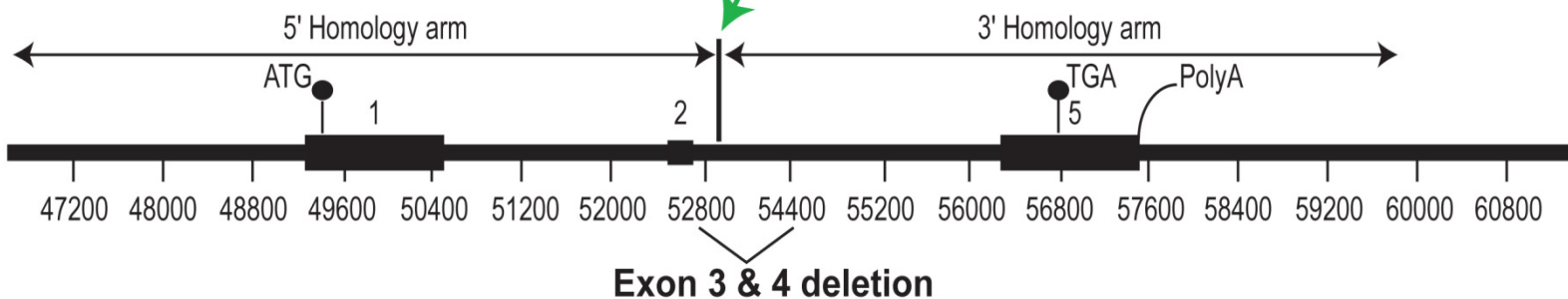
A. *Ugt1^{loxP[FRTNeoFRT]loxP}*



B. *Ugt1^{F/F}*

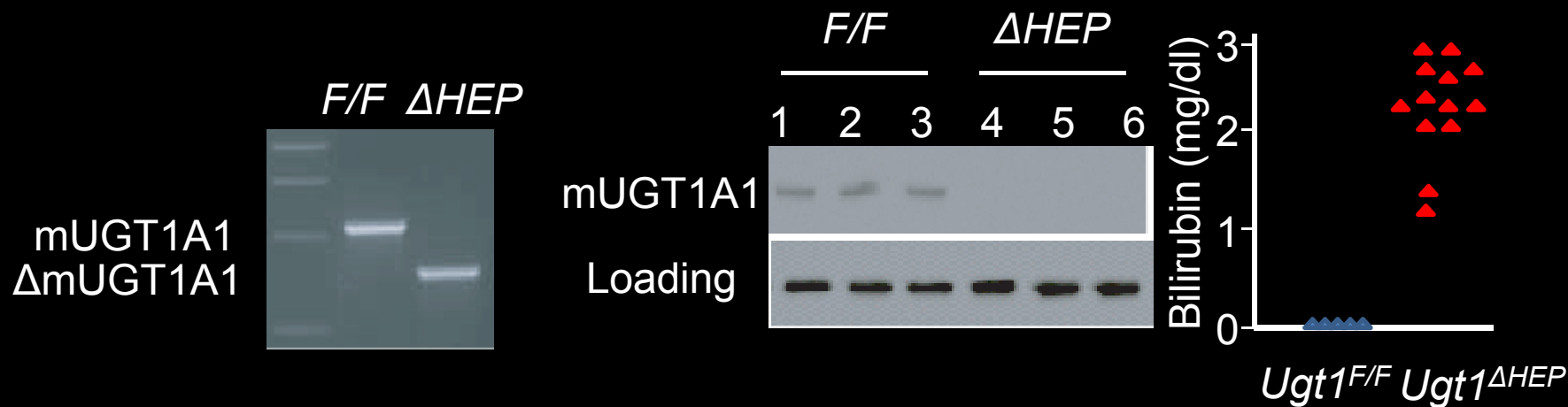


c. *Cre-Ugt1^{F/F}*

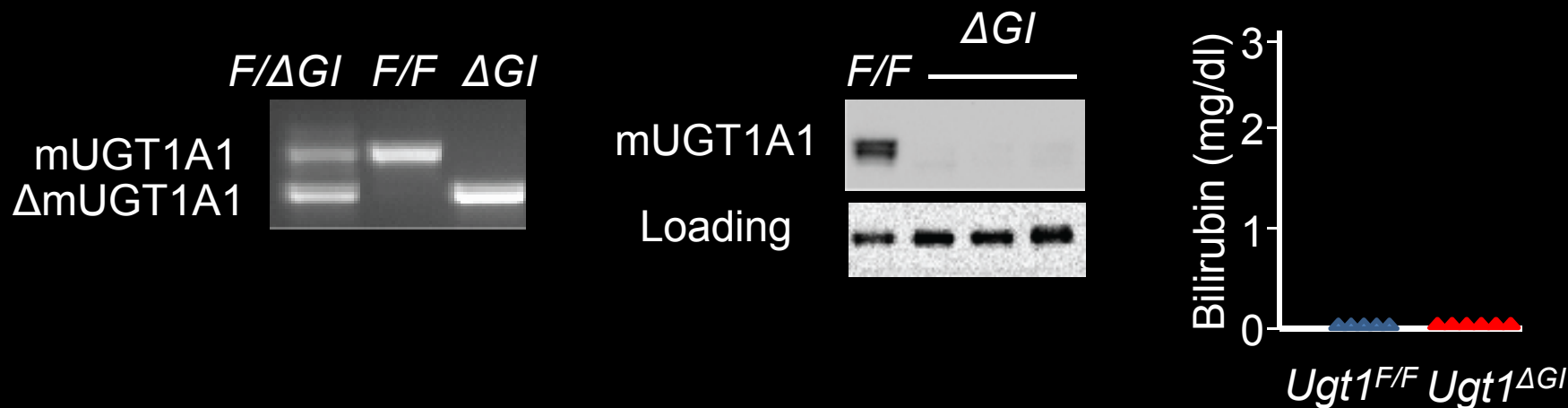


Conditional Deletion of the *Ugt1* Locus

Hepatocytes deletion (*Alb-Cre*)

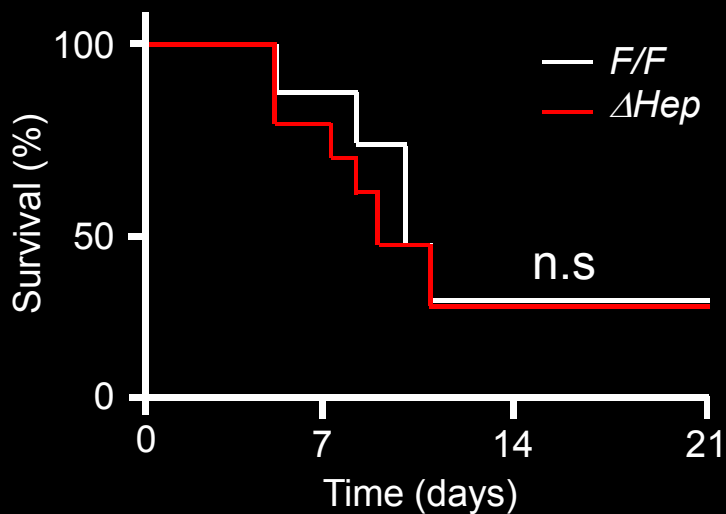


Intestinal Deletion (*Vil-Cre*)

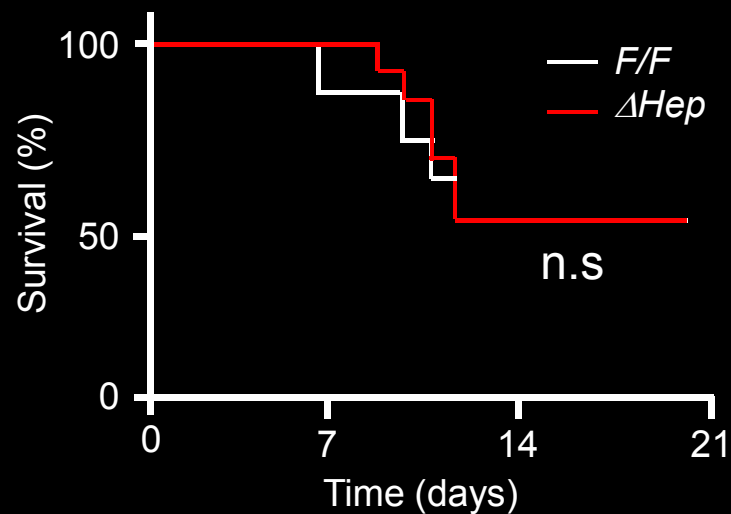


Δ Hep v.s. Δ GI Survival

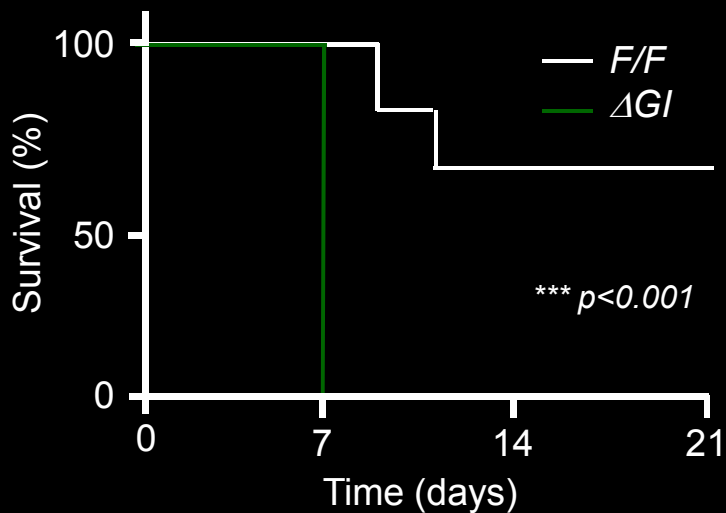
CPT-11_100mg/kg



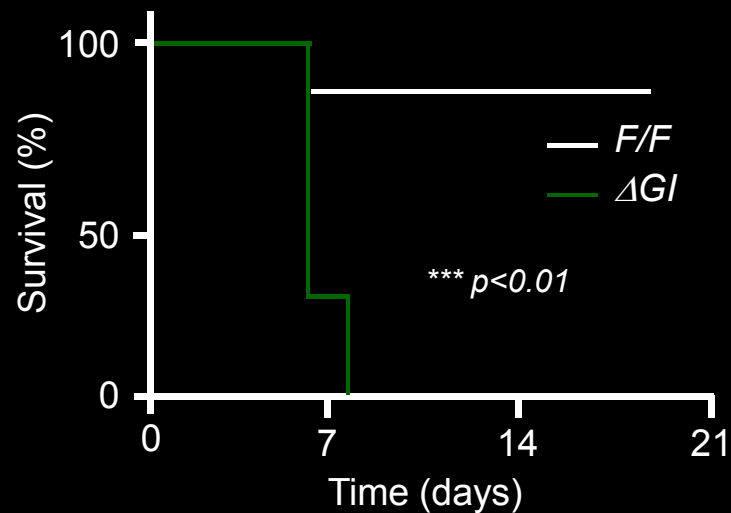
CPT-11_75mg/kg



CPT-11_50mg/kg

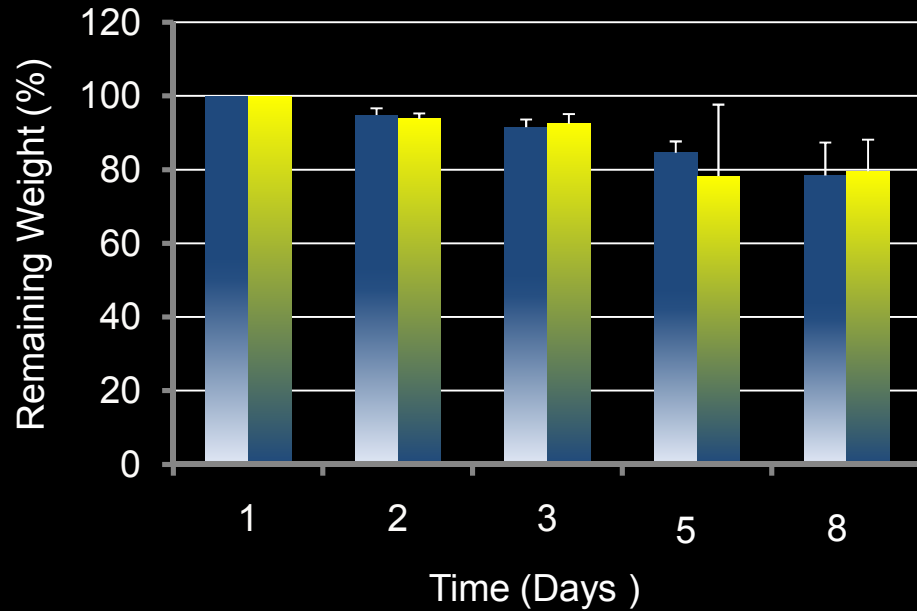


CPT-11_25mg/kg



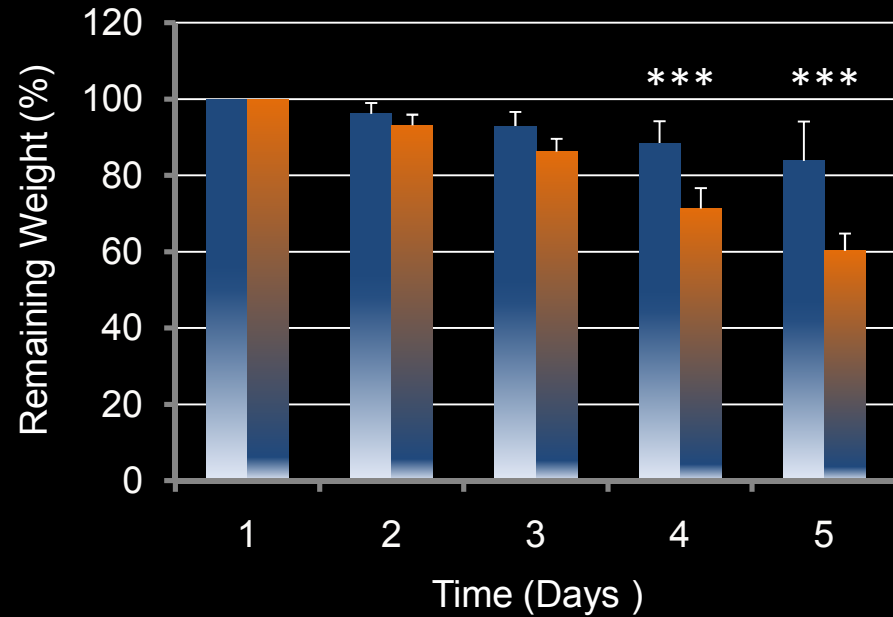
Δ Hep v.s. Δ GI Weight Loss

CPT-11_75 mg/kg



F/F
 Δ Hep

CPT-11_50 mg/kg



F/F
 Δ GI

*** $P < 0.001$

Irinotecan-induced Intestinal Toxicity

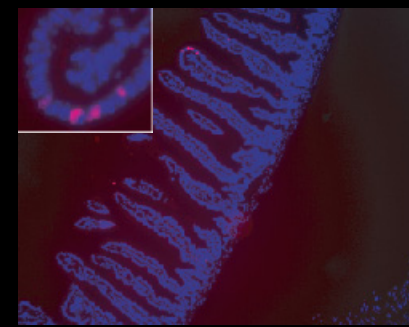
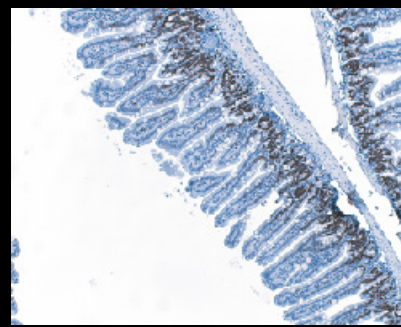
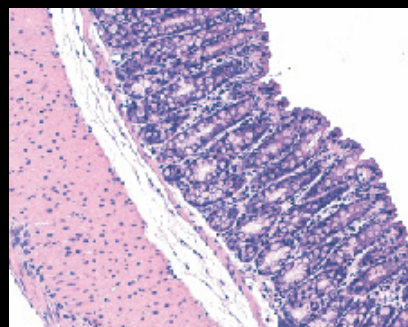
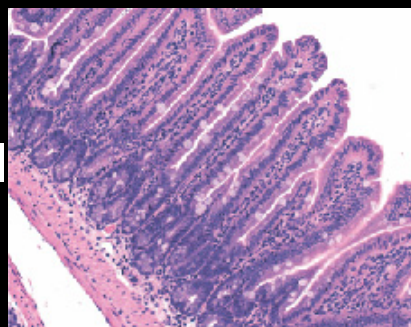
Jejunum

Colon

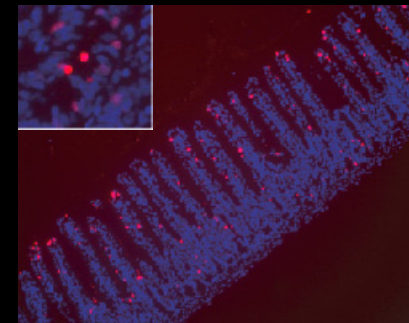
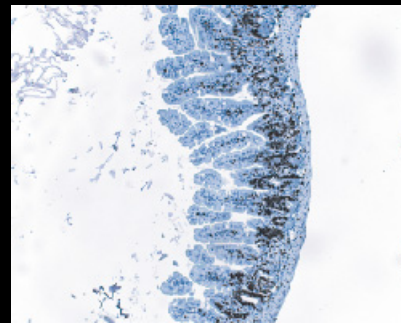
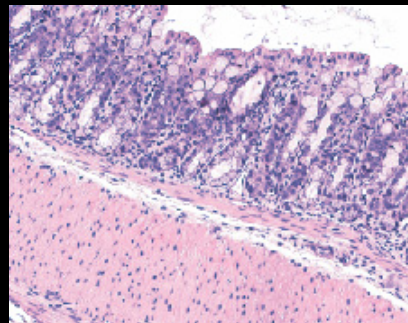
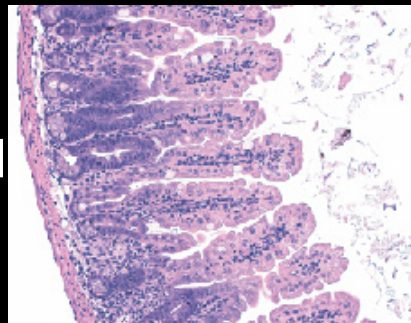
Ki67

TUNEL

Control

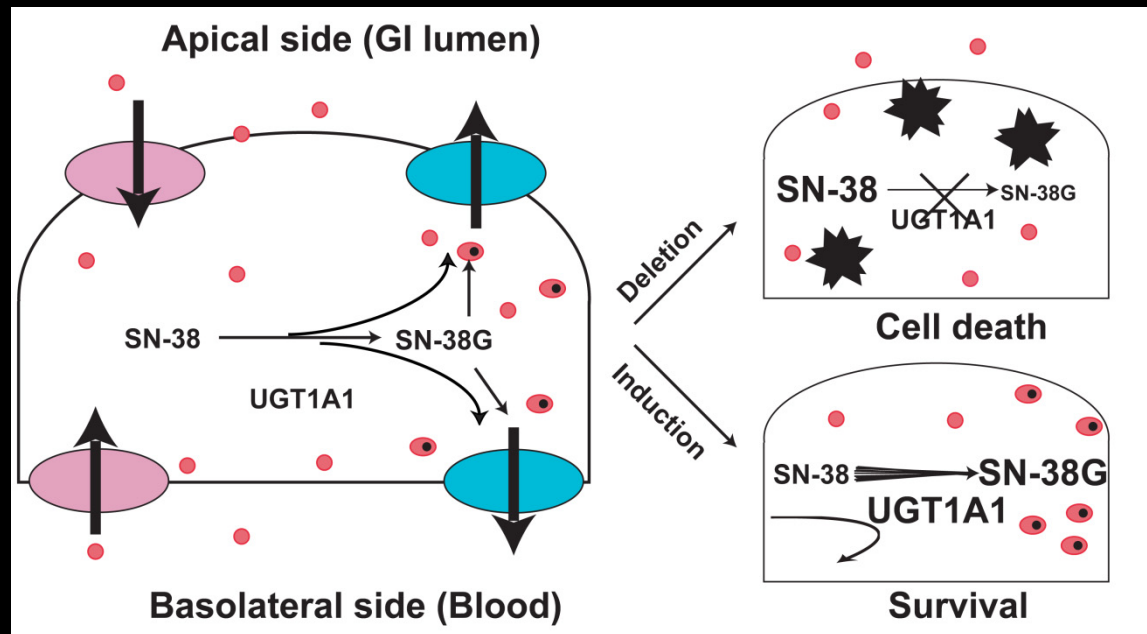


CPT-11



Summary

1. Tissue specific *Ugt1* deletion were generated by adopting *Cre/loxP* recombination system.
2. Extrahepatic clearance of bilirubin occurs in the absence of functional hepatic *Ugt1a1*.
3. Intestinal *Ugt1a1* plays an important role in preventing CPT-11 induced toxicity.



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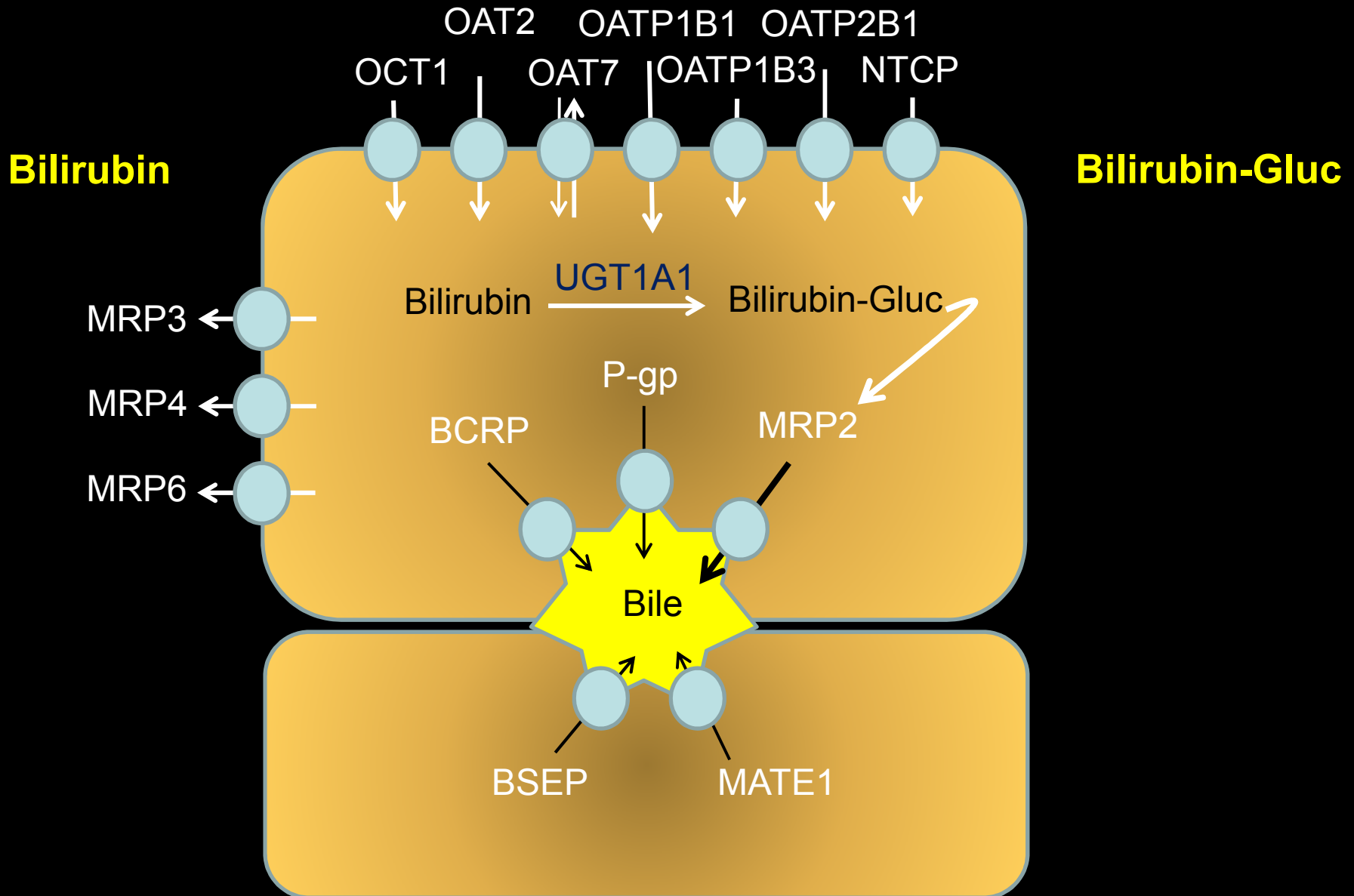
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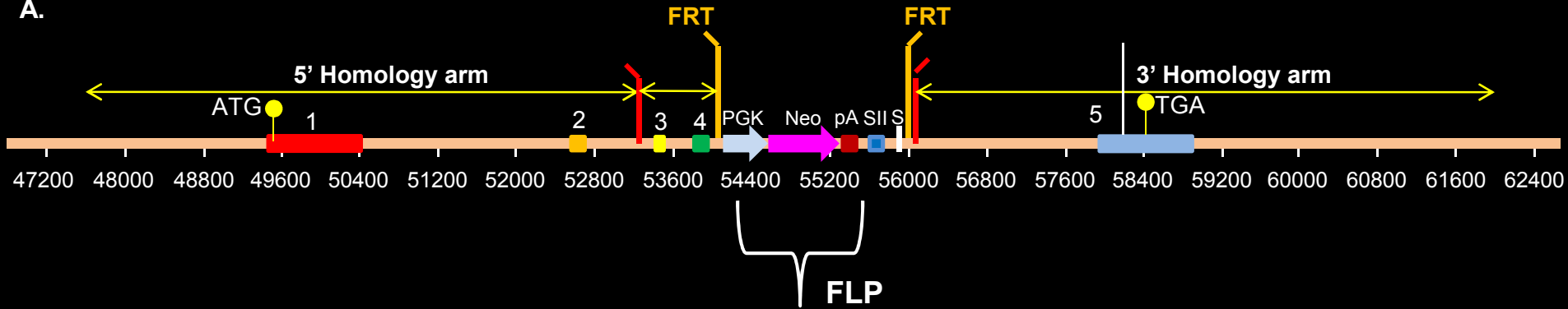


Bilirubin Transport and Metabolism in Hepatocytes

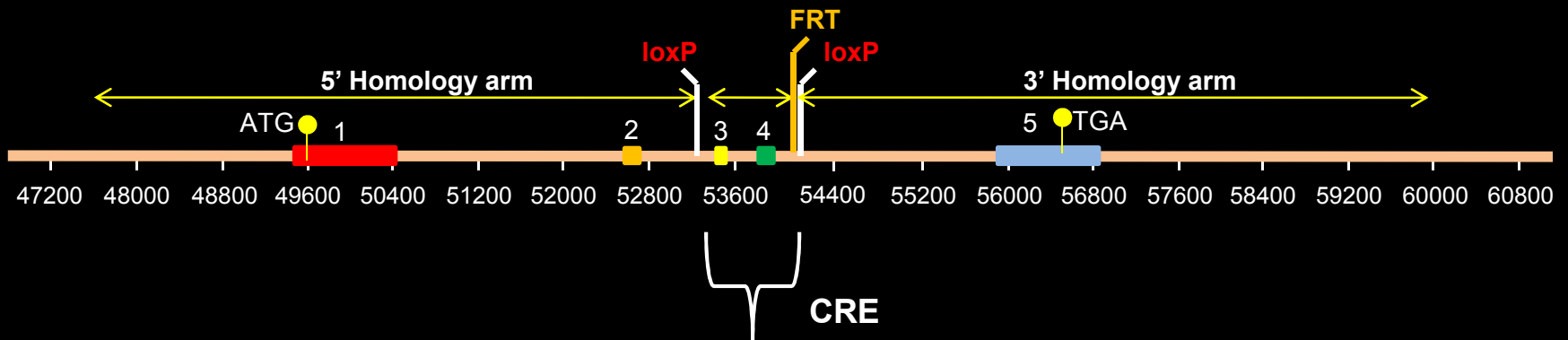


Ugt1 gene locus and targeting constructs

A.



B.



C.

