Supplementary Materials and Methods:

Bile duct ligation, laser capture microscopy and qRT-PCR array analysis. Common bile duct ligation was performed in DPPIV$^-$ F344 rats (2-3 months of age) as described previously (1). Liver tissue samples were harvested from BDL rats and age-matched normal rats (n = 3/3) after 1 month, and were used for laser capture microdissection. RNA extracts from laser-captured liver regions (fibrotic septa and surrounding parenchyma) were analyzed using amplified and subsequently pooled RNA samples, as described previously (1). Rat WNT Signaling Pathway RT² Profiler™ PCR arrays (SA Biosciences, a Qiagen company) containing 84 genes related to Wnt-mediated signal transduction (*www.sabiosciences.com*) were used to determine mRNA expression levels. The list of genes, as well as housekeeping genes can be found in *www.sabiosciences.com*. cDNA synthesis, qRT-PCR, and data analysis were performed according to the manufacturer's instructions. Data analysis was performed using the SA Bioscience web-based PCR Array Data Analysis tool (*www.sabiosciences.com/pcrarraydataanalysis.php*) with boundary set at 2 and *P* value at 0.05. Data from 3 independent PCR array analyses per group (normal liver/parenchyma/fibrotic septa) are expressed as fold differences in messenger RNA levels.

Cell culture. Hep3B cells were cultured in Eagle's Minimal Essential Medium (EMEM; ATCC) with 10% FBS (Atlanta Biologicals, Lawrenceville, GA). AML12 cells were cultured in Dulbecco Eagle's minimal essential medium (DMEM) / Ham's F-12 (1:1) mixed media supplemented with 10% FBS, 5 µg/ml Insulin, 5 µg/ml Transferrin, 5 µg/ml Sodium Selenite (Roche), and 100nM dexamethasone. 293T cells were cultured in DMEM supplemented with 10% FBS. All cells were grown at 37°C in a humidified 5% CO_2 incubator. The small mouse-SV40 cholangiocyte (sm-cc) cell line was obtained from Dr. Gianfranco Alpini (Texas A&M Health Science Center)(2), and was cultured in

minimal essential medium (MEM) supplemented with 10% FBS and 0.4 mM L-glutamine.

Histology and immunohistochemistry. Immnohistochemistry (IHC) and Sirius red staining on formalin-fixed, paraffin-embedded mouse liver sections was performed as previously described (3, 4). OCT-embedded frozen liver sections were dissected based on zonation after hematoxylin and eosin staining. Tissue dissection was performed manually under a light microscope as described (5). Antibodies used in this study are shown in Supplementary Table 1.

Western blotting. Protein lysates were prepared from transfected sm-cc cells or WT and Wls KO EpCAM+ cells as previously described (3, 6). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 20μg of protein resolved on Bio-Rad Protean precast gels, followed by transfer to a polyvinylidene difluoride (PVDF, Millipore, Bedford, MA) membrane. Membranes were blocked in 5% milk, followed by incubation with primary antibody in 5% milk overnight at 4°C. Antibodies used in this study are shown in Supplementary Table 1.

RNA extraction, synthesis of cDNA, and quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was extracted from frozen liver tissue, dissected liver sections, sorted cells, or cell lines by Trizol (Thermo Fisher Scientific, Pittsburgh, PA) and isolated using the mirVana microRNA isolation kit (Thermo Fisher Scientific) in accordance with the manufacturer's instructions. After DNase treatment and reverse transcription were performed using the SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific), qRT-PCR was performed on an ABI StepOnePlus Real-Time PCR System using 2× SYBR Green PCR Master Mix (Applied Biosystems, Foster, CA). Primer sequences are listed in Supplementary Table 2.

Wnt gene expression was measured from WT livers at baseline and after BDL using the mouse Wnt signaling pathway PCR array (Qiagen, Valencia, CA), as per the manufacturer’s instructions. Briefly, mRNA was isolated using TRIzol, and reverse

transcribed using the RT^2 First-Strand Kit (Qiagen). cDNA from each condition (baseline and BDL; n=3 per group) was then pooled and added to the plate containing primers for 84 genes related to Wnt-mediated signal transduction, along with RT^2 SYBR Green qPCR Master Mix (Qiagen). Fluorescence signal was measured during each step using an ABI StepOnePlus.

Preparation of plasmids. pReceiver-mWnt7A-HA-eGFP plasmid (EX-Mm06073-M45) was purchased from GeneCopoeia (MD, USA). The control vector was generated from this plasmid by cutting out Wnt7A. Wnt7B and Wnt10B were cloned and inserted into the control vector resulting in generation of plasmids pRec-Wnt7B and pRec-Wnt10A. Primers used for confirming the inserted genes were designed to recognize the coding region and are shown as *gene name*-cds in Supplementary Table 2.

Immunofluorescence staining. A6 immunofluorescence staining on frozen tissue sections was performed as previously described (3). Double immunofluorescence staining of Sox9 and CK19 was performed by modifying the above protocol as follows. Paraffin-embedded sections were deparaffinized in xylene, and dehydrated. Slides were steamed for 20 minutes in Tris-EDTA buffer pH 9 and cooled to room temperature. The sections were then incubated in 3% bovine serum albumin (BSA) for blocking nonspecific protein binding for 1 hour. For double-labeled immunofluorescence microscopy, sections were subsequently incubated overnight with both primary antibodies (polyclonal rabbit anti-Sox9 antibody at 1:500 and polyclonal rat anti-CK19 antibody at 1:200) in PBS containing 1% BSA. After three washes in PBS, the sections were incubated for 4 hours at room temperature in the dark with the appropriate secondary antibody (i.e., goat anti-rabbit IgG conjugated to either Alexa Fluor 555 or 488 diluted 1:500; or goat anti-rat IgG conjugated to either Alexa Fluor 555 or 488 diluted 1:500) in PBS containing 1% BSA. After two washes in PBS, sections were counterstained with Hoechst to detect nuclei. For double staining of CK19 and Ki67,

slides were incubated for 1 hour with both primary antibodies (monoclonal rabbit anti-Ki67 antibody at 1:100 and polyclonal rat anti-CK19 antibody at 1:100). Secondary antibodies were incubated for 1 hour and nuclear staining was performed as above. For double staining of A6 and HNF4α, slides were incubated overnight with both primary antibodies (polyclonal rabbit anti-HNF4α antibody at 1:100 and polyclonal rat anti-A6 antibody at 1:100). Secondary antibodies were incubated for 1 hour and nuclear staining was performed as above. Antibodies used for this protocol are shown in Supplementary Table 1.

Cell growth and viability assays. sm-cc cells at a concentration of 5×10^5 were transfected with Wnt7A, Wnt7B, or Wnt10A plasmids in the presence of Lipofectamine 3000 overnight. The next day, cells were trypsinized and 1×10^4 cells/well were added to 3 96-well plates in triplicate. A colorimetric cell viability kit (Promokine, Heidelberg, Germany) was then utilized to measure cell viability and proliferation. The tetrazolium salt Wst-8 was added to each well either immediately after plating, 24 hours after plating, or 48 hours after plating. Absorbance was measured at 460 nm on a spectrophotometer after 2 hours of incubation with Wst-8 at 37°C.

sm-cc cells transfected with 25 nM control or b-catenin siRNA were incubated in the presence of [^{3}H]thymidine for 48 hours, and incorporation of radiolabeled nucleotides was measured as described previously to assess cell proliferation (7). Viability of sm-cc cells transfected with 25 nM control or b-catenin siRNA for 48 hours was measured by (3,4.5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay (7).

Luciferase assay. Hep3Bs and sm-cc cells were transfected and measured for luciferase activity as previously described (8). Briefly, Hep3Bs were seeded onto 6-well plates and transfected with 0.8 μg of TOPflash reporter plasmid (Upstate Biotechnology) and 0.1 μg of *Renilla* plasmid in the presence of Lipofectamine 2000 reagent (Invitrogen)

and treated with recombinant human Wnt7A protein as described above. sm-cc cells were simultaneously transfected with TOPflash and Renilla reporters and either control or b-catenin siRNA at a final concentration of 25 nM. All cells were harvested 48 hours after transfection for luciferase assay. Luciferase assays were performed using the Dual Luciferase Assay System kit, in accordance with the manufacturer's protocols (Promega, Madison, WI). Relative luciferase activity (in arbitrary units) was reported as fold induction after normalization to Renilla for transfection efficiency.

Identification of Wnt transcriptional regulators. The ChEA software and ChIP-X database was utilized to profile the predicted binding of transcription factors to Wnt promoters as described previously (9). All regulators with at least 3 Wnt targets are shown. The data was compiled in a heatmap that displays hierarchical clustering of regulators according to similarity in their target Wnts. Potential regulators of all 3 Wnts of interest - 7A, 7B, and 10A (top 3 rows) - appear on the left side of the heatmap.

Supplementary Figure 1. Wnt7A and Wnt10A protein is expressed primarily in cholangiocytes. IHC shows absence of Wnt7A expression in livers of mice on normal diet, while after 28D DDC diet, cholangiocytes and a few periportal hepatocytes begin to express Wnt7A. Wnt10A is present in cholangiocytes at baseline; however, after 28D DDC diet, expression of Wnt10A is greatly enhanced, with expansion into surrounding periportal hepatocytes. Arrows: Wnt-expressing cholangiocytes; arrowheads: Wnt-expressing hepatocytes. Magnification: 200x.

Supplementary Figure 2. Confirmation of Wls deletion in hepatocytes from liver-specific Wls KO. (A) PCR shows deletion of the Wls allele from genomic DNA extracted from liver tissue. (B) Parenchymal cells (hepatocytes (HC) and hepatic stellate cells (HSC)) were separated from non-parenchymal cells (Kupffer cells (Kp), endothelial cells (EC), and cholangiocytes/biliary epithelial cells (BEC)) by collagenase perfusion of the liver. (C) Expression of Wls in the PC and NPC fractions of WT and KO mice as determined by qRT-PCR. Right panel shows magnification of Wls expression in PC.

Supplementary Figure 3. Confirmation of Wls deletion in cholangiocytes from liver-specific Wls KO. (A) Decreased Wls expression in EpCAM+ cells obtained from KO is shown by qRT-PCR. (B) Expression of Wls is decreased in EpCAM+, CK-19 expressing cells from KO, as shown by WB.

Supplementary Figure 4. The heatmap shows potential interactions between Wnts and transcriptional regulators as compiled from different ChIP-X experiments (see Supplementary Materials and Methods). The Wnts of interest - Wnt7A, Wnt7B, and Wnt10A - are in the top 3 rows and the regulators are hierarchically clustered according to similarity in their target Wnts.

Supplementary Table 1. Primary antibodies used in this study

Molecule	Cat number	Company	Species	Application	Dilution
α-SMA	M0851	DAKO	Ms	IHC	× 200
A6	gift‡		Rat	IF	× 100
Actin	MAB1501	Millipore	Ms	Western	× 2000
CK19	TROMA-III	DSHB	Rat	Western	× 1000
				IHC	× 200
				IF	× 100
b-catenin	610154	BD	Mouse	Western	× 500
CD31	102404	Biolegend	Rat	Cell separation	× 10
CD45	103104	Biolegend	Rat	Cell separation	× 10
EpCAM	118204	Biolegend	Rat	Cell separation	× 10
F4/80	123105	Biolegend	Rat	Cell separation	× 10
HNF4 α	sc8987	Santa Cruz	Rb	IF	× 100
Ki67	NM-SP6	DAKO	Rb	IF	× 200
Sox9	AB5535	Millipore	Rb	IF	× 500
	Sc20095	Santa Cruz	Rb	IHC	× 100
Wls	MABS87	Millipore	Ms	Western	× 1000

Wls: wntless; DSHB: Developmental Studies Hybridoma Bank; BD: BD Biosciences; α-SMA: alpha-smooth muscle actin; HNF4α: hepatocyte nuclear factor 4α

‡ A6 antibody was a kind gift from Dr. Valentina Factor, National Cancer Institute, Bethesda, MD

Supplementary Table 2. Primer sequences for quantitative real-time PCR

Albumin	S: 5'- tga ccc agt gtt gtg cag ag -3'
	AS: 5'- ttc tcc ttc aca cca tca agc -3'
CK19	S: 5'- agt ccc agc tca gca tga a-3'
	AS: 5'- taa cgg gcc tcc gtc tct -3'
EpCAM	S: 5'- gat tct gca cgt gag acct g -3'
	AS: 5'- gat acc aag tca aac cga gaa ctt -3'
H-GAPDH	S: 5'- ccc ttc ata ccc tca cgt att c -3'
	AS: 5'- atg aca agc ttc ccg ttc tc -3'
M-GAPDH	S: 5'- acc cag aag act gtg gat gg -3'
	AS: 5'- cac att ggg ggt agg aac ac -3'
GS	S: 5'- ctc gct ctc ctg acc tgt tc -3'
	AS: 5'- ttc aag tgg gaa ctt gct ga -3'
Human Sox9	S: 5'- gct cag gtc aga ctg caa taa -3'
	AS: 5'- ctc tgc aca caa ctc cat ctt -3'
Mouse Sox9	S: 5'- gta ccc gca tct gca caa c -3'
	AS: 5'- ctc ctc cac gaa ggg tct ct -3'
Wls	S: 5'- ccc agc cat gag caa agt -3'
	AS: 5'- gca tga gga act tga acc tga -3'
Wnt7a	S: 5'- gga tgc tca cag gga aag aac -3'
	AS: 5'- gca gga aac cca gaa tac cc -3'
Wnt7a-cds	S: 5'- cgc tgg gag agc gta ctg -3'
	AS: 5'- cga taa tcg cat agg tga agg -3'
Wnt7b	S: 5'- gct cct tcc tac tcg ctc tgt -3'
	AS: 5'- ggt ccc ttt gtg gtt cac ttt -3'
Wnt7b-cds	S: 5'- tca tga acc ttc aca aca atg a -3'
	AS: 5'- tgg tcc agc aag ttt tgg t -3'
Wnt10a	S: 5'- tcc tgt tct tcc tac tgc tgc t -3'
	AS: 5'- acg cac aca cac ctc cat c -3'

H: human; M: mouse

References

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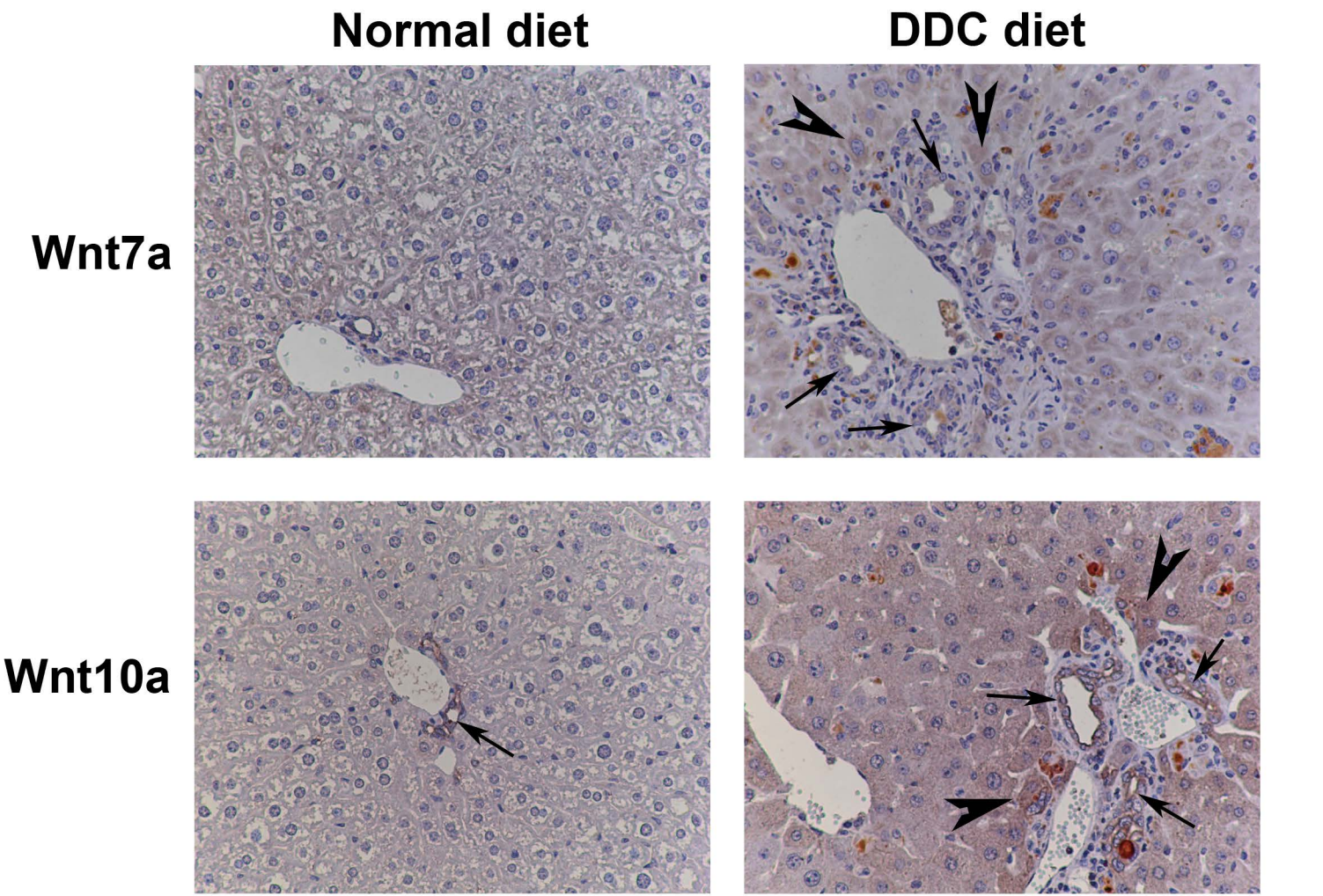
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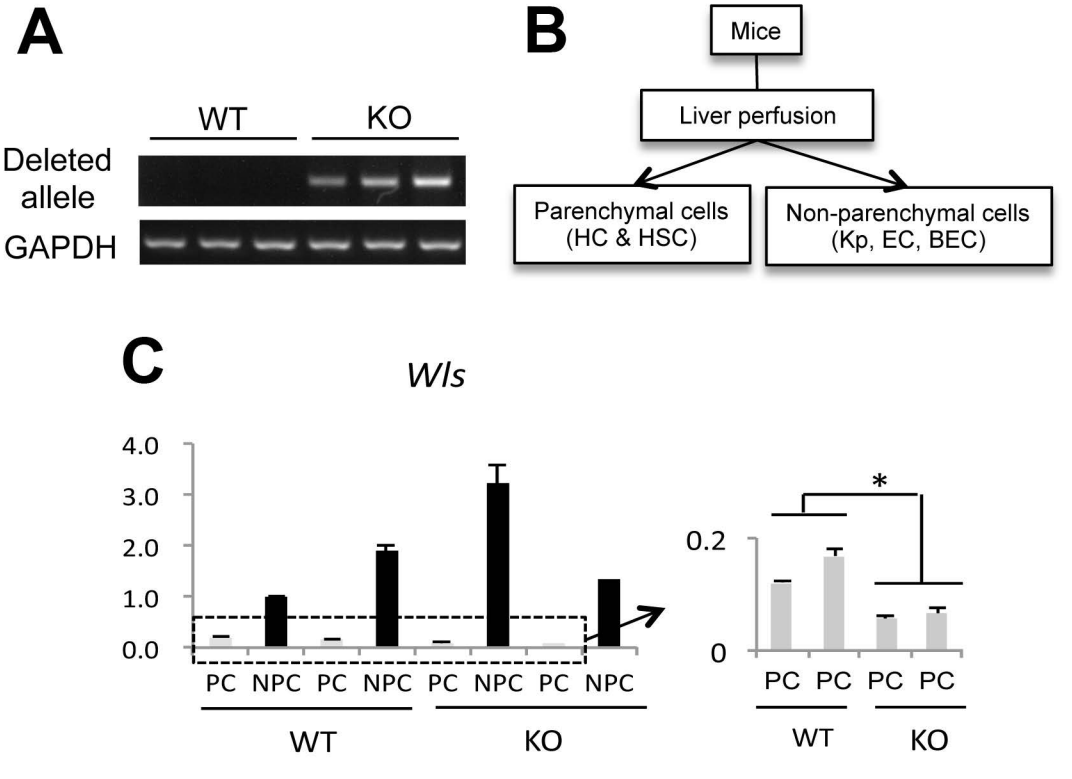
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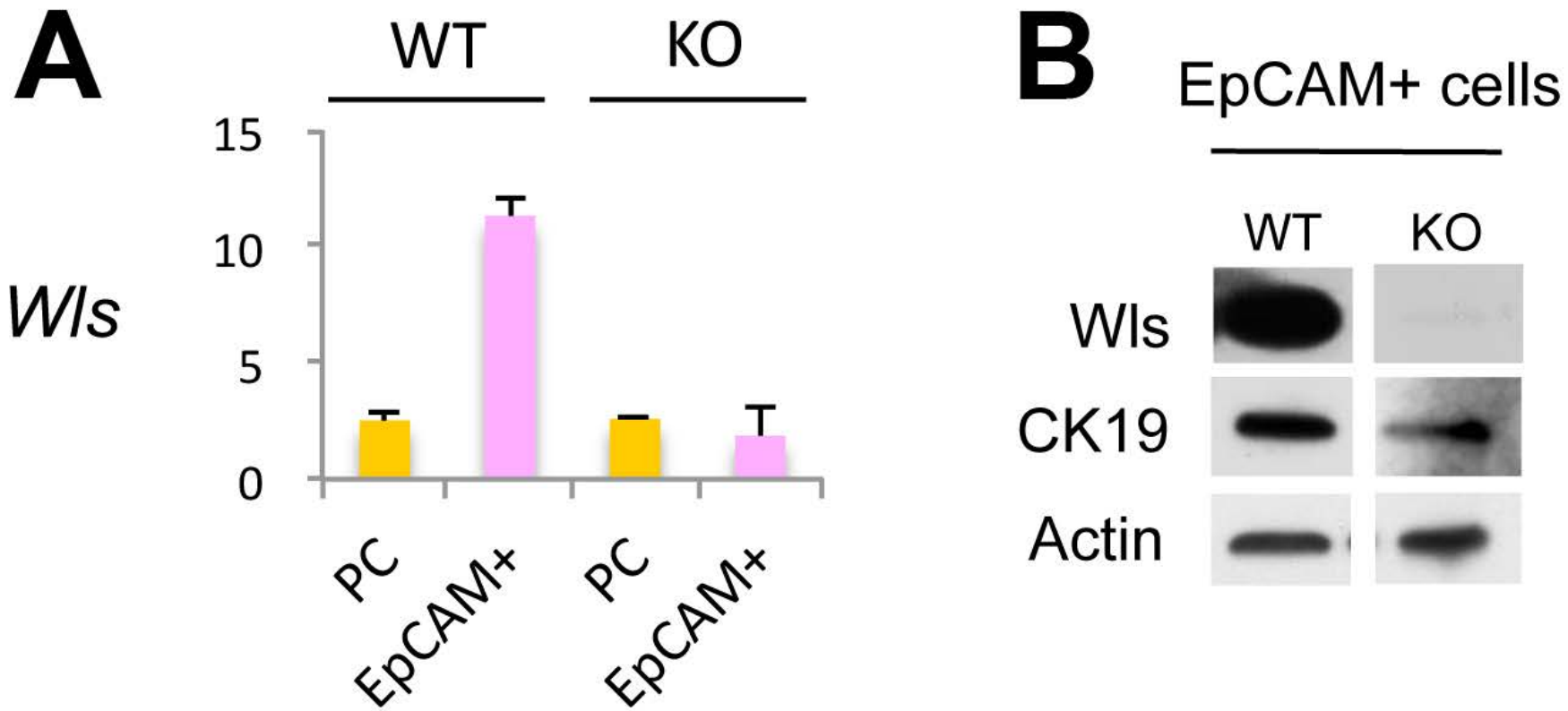
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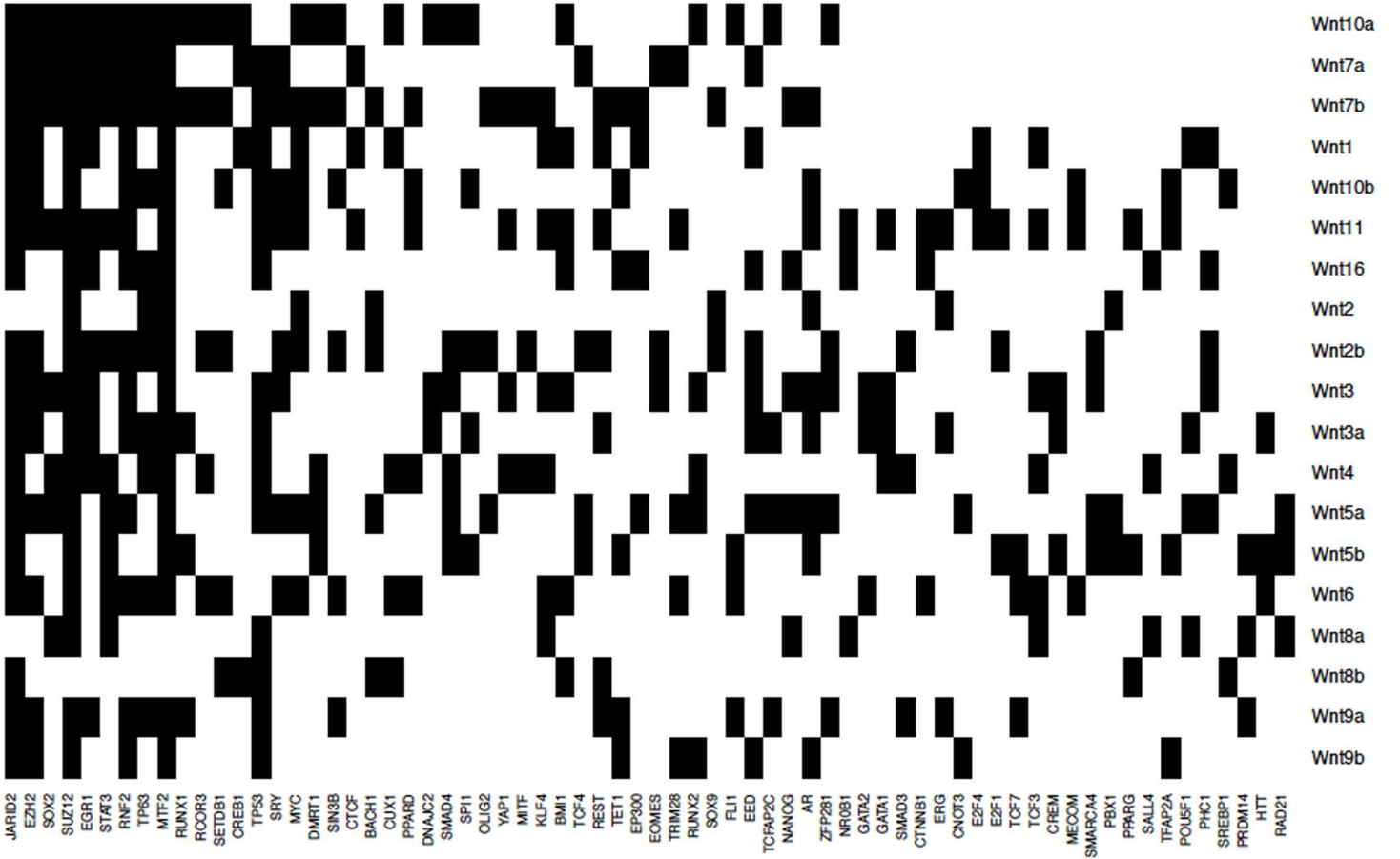
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