

## **A molecular mechanism underlying genotype-specific intrahepatic cholestasis resulting from MYO5B mutations**

Arend W. Overeem, Qinghong Li, Yi-ling Qiu, Fernando Carton-García, Changsen Leng, Karin Klappe, Just Dronkers, Nai-Hua Hsiao, Jian-She Wang, Diego Arango, Sven C. D. van IJzendoorn

### **Supplemental information**

#### **Supplemental figures:**

**Supplemental figure S1. Immunolabeling of BC transporters in PFIC6 liver tissue.** Immunolabeling of the canalicular bile acid transporter ABCB11 (green) and nuclei (blue) in liver biopsies of a non-MVID PFIC6 patient show mislocalization of the BC transporter to intracellular compartments when compared to a control subject.

#### **Supplemental figure S2. Sequencing results and (quantification of) immunolabeling of BC transporters in HepG2 cells expressing myoVb mutants.**

(A) Sequencing results of HepG2<sup>KO</sup> clone. Top depicts wildtype reference genomic sequence of exon 3 of *MYO5B*, with corresponding amino acid translation. Below depicts the sequence results corresponding to the two modified alleles. One allele contains a 19 nucleotide deletion, the other allele contains a 1 nucleotide insertion. Both modifications result in a frameshift, and thereby a premature stop-codon. (B) Quantification of BC formation (expressed as BC's per 100 cells) in HepG2<sup>KO</sup> cells

expressing myc-myoVb or myc-myoVb-P660L. (C) Immunofluorescent labeling of myc and ANO6, in HepG2<sup>KO</sup> or HepG2<sup>Par</sup> expressing myc-myoVb-P660L. White arrows indicate ANO6 accumulated intracellularly with myc-myoVb-P660L. Yellow arrowheads indicate BCs. (D) Quantification of BC formation (expressed as BC's per 100 cells) in HepG2<sup>KO</sup> cells or HepG2<sup>Par</sup> expressing myc-myoVb-P660L. (E). Quantification of the percentage of myc-positive cells that show intracellular clusters/accumulations of myc localized with ANO6, in HepG2<sup>KO</sup> or HepG2<sup>Par</sup> expressing myc-myoVb-P660L. (F) Quantification of the percentage of myc-positive cells that show subapical localization of myc, in HepG2<sup>KO</sup> or HepG2<sup>Par</sup> expressing myc-myoVb-P660L

**Supplemental figure S3. (Quantification of) immunolabeling of BC transporters in HepG2 cells expressing myoVb mutants.**

(A) Labeling of ANO6 and myc in HepG2<sup>KO</sup> cells expressing myoVb/ $\Delta$ 1-1195 showed intracellular colocalization of both markers (white arrows). (B) Quantification of the percentage of HepG2<sup>KO</sup> cells showing accumulation of ABCC2 (as shown in figure 3D, white arrows) upon expression of myoVb/ $\Delta$ 1-1195 compared to untreated control. (C) Quantification of BC formation (expressed as BC's per 100 cells) in HepG2<sup>KO</sup> cells expressing myc-myoVb/ $\Delta$ 1-1195, and untreated HepG2<sup>KO</sup> cells. (D,E) Labeling of ABCC2 with F-actin or ANO6 respectively, in HepG2<sup>KO</sup> cells expressing myoVb/ $\Delta$ 1-1195, compared to untreated control. White arrows indicate intracellular accumulation of ABCC2 (and ANO6 in figure E).

**Supplemental figure S4. (Quantification of) immunolabeling of canalicular proteins in HepG2 cells expressing myoVb mutants.** (A) Quantification of BC formation (expressed as BCs per 100 cells) in HepG2<sup>Par</sup> cells expressing myc-myovb/ $\Delta$ 1-1195, HepG2<sup>Par</sup> cells transduced with empty pLenti-Puro plasmid, or untreated HepG2<sup>Par</sup>. (B) Immunofluorescent images of HepG2 cells co-expressing myc-myovb/ $\Delta$ 1-1195 and DPPIV-mCherry (or DPPIV-mCherry only as control), stained for ABCC2. DPPIV-mCherry colocalized with ABCC2 intracellular accumulations (white arrows), but not exclusively (yellow arrowheads indicate lack of colocalization). (C) Immunofluorescent images of HepG2 cells co-expressing myc-myovb/ $\Delta$ 1-1195 and DPPIV-mCherry (or DPPIV-mCherry only as control), stained for myc. White arrows indicate colocalization of myc and DPPIV-mCherry, yellow arrowheads indicate lack of colocalization. (D) Wildtype HUES9 derived human induced hepatocytes (hiHeps), expressing myc- myovb/ $\Delta$ 1-1195, labeled for ANO6, myc and hepatic lineage marker HNF4 $\alpha$ . In hiHeps lacking myc- myovb/ $\Delta$ 1-1195 expression, ANO6 is present at bile canaliculi (yellow arrowhead) and faintly at basolateral membranes. In hiHeps expressing myc- myovb/ $\Delta$ 1-1195, ANO6 colocalized with myc inside the cells (white arrows).

**Supplemental figure S5. (Quantification of) immunolabeling of BC transporters and organelle markers in HepG2 cells expressing myoVb mutants.**

(A) Labeling of radixin in HepG2 cells expressing myc- myovb/ $\Delta$ 1-1195 (white arrows), compared to untreated control. Yellow arrowheads indicate BCs. (B,C) In both HepG2<sup>Par</sup> and HepG2<sup>KO</sup>, rip11 and rab11a localized as subapical rings surrounding the BC (labeled with ABCC2 and ANO6 respectively). (D) Labeling of LAMP1 In proteins in HepG2 expressing myc- myovb/ $\Delta$ 1-1195 (white arrows), compared to untreated

control. White arrows indicate lack of colocalization. (E) Microscopy images of untreated and myc- myoVb/ $\Delta$ 1-1195 expressing HepG2, fixed after 30 minutes incubation ( $t= 0h$ ) with fluorescently labeled transferrin (388Tf), and after a 2 hour chase period. Cells were stained for transferrin receptor (TfR) and ANO6.

**Supplemental figure S6. Quantification of BC formation HepG2 cells expressing different myoVb mutants.** (A) Quantification of BC formation upon expression of myoVb tail domain variants, compared to untreated control. (B) Quantification of BC formation upon expression of myc-myoVb/ $\Delta$ 1-1460, or its Y1714E mutant variant, compared to untreated control. (C) Relative (over)expression levels of myc-myoVb/ $\Delta$ 1-1460 and myc-myoVb/ $\Delta$ 1-1460-Y1614E as determined by qPCR, compared to endogenous myoVb expression in untreated control.

### Supplemental Table T1.

List of antibodies

Name	Supplier	Cat no.
ABCC2 / MRP2 (mouse)	Millipore	MAB4150
ABCC2 / MRP2 (rabbit)	Sigma	M8316
Radixin	Sigma	R3653 1:200
HNF4a	Santa Cruz	sc-6556
Golgin97	Thermo Fisher	A-21270
LAMP1	BD Biosciences	611042
BSEP	Santa Cruz	sc-17292
ANO6	Sigma Aldrich	HPA038958
TGN46	Biorad	AHP500

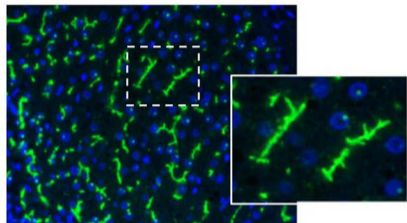
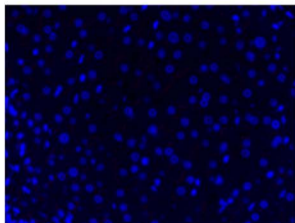
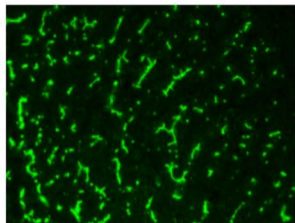
Actin	Sigma	A5441
myc	Clontech	631206
Rip11	Gift from dr. R. Prekeris, University of Colorado Anschutz Medical Campus, Aurora/CO, USA.	
Rab8a	Abnova	M02
Giantin	Biologend	PRB114C
Ap1y	Abcam	ab220251
BSEP	Santa Cruz	sc-74500
Rab11a	Biosciences	610656
Myosin Vb	NOVUS	NBP1-87746
Transferrin receptor	Invitrogen	13-6800

ABCB11/BSEP

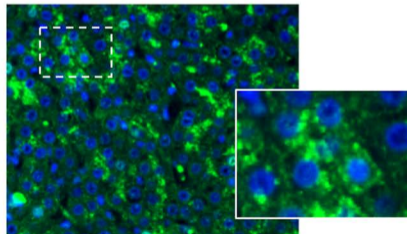
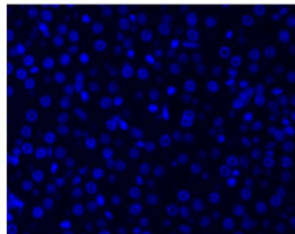
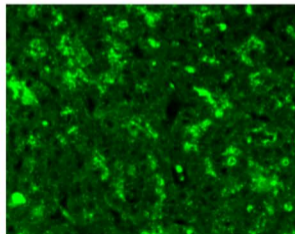
nuclei

merge

control



PFIC6



**A**

Wildtype  
Myosin Vb  
genomic  
sequence

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I L E Y P I D V Q R N Q L P F L R N P D I L V G E N D L T

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HepG2-KO

Allele 1

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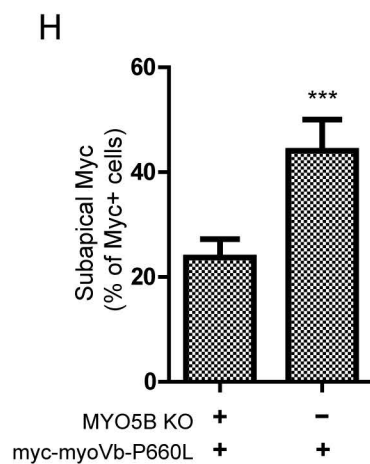
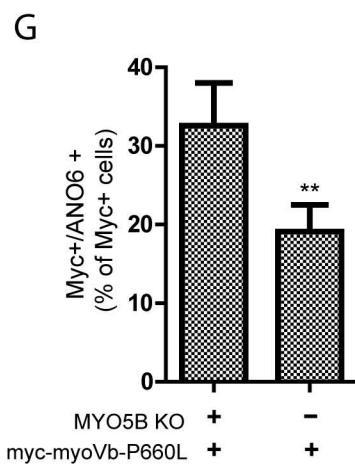
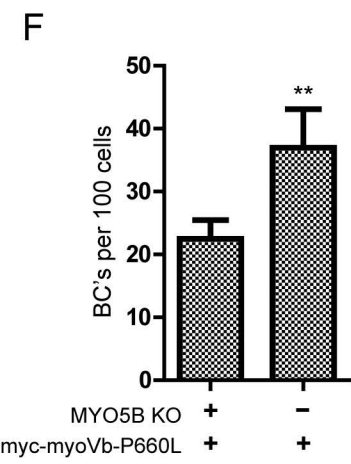
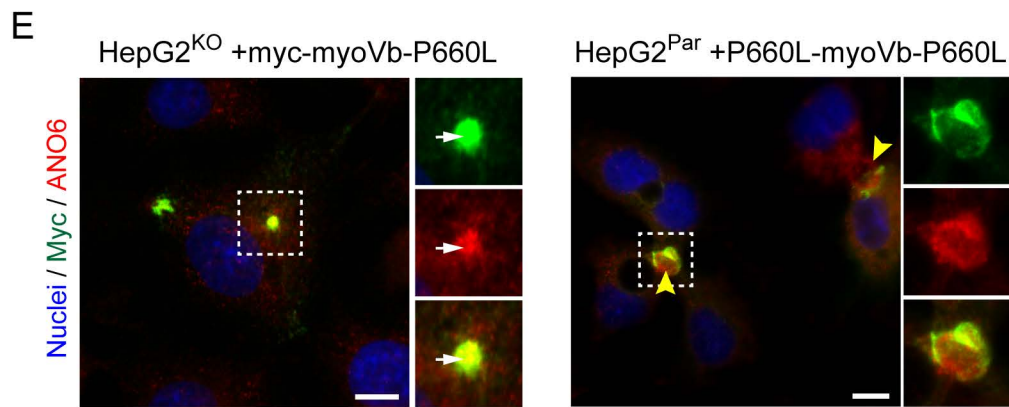
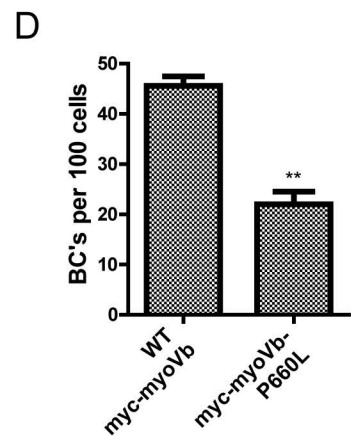
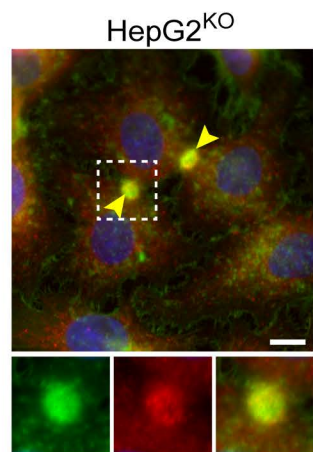
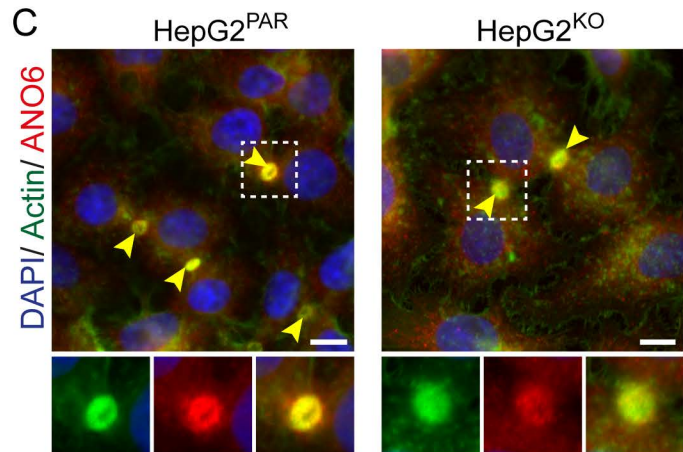
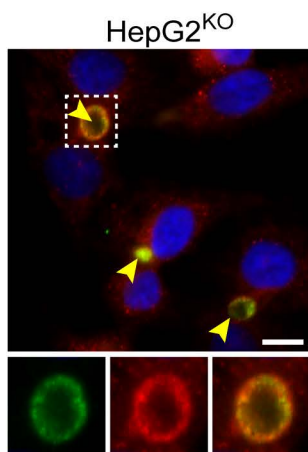
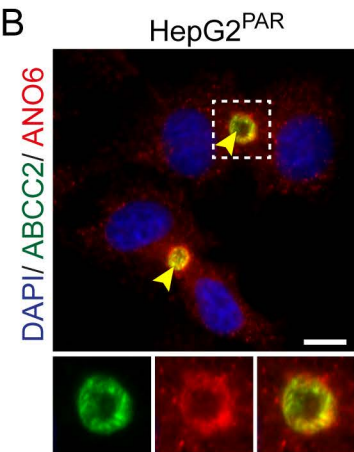
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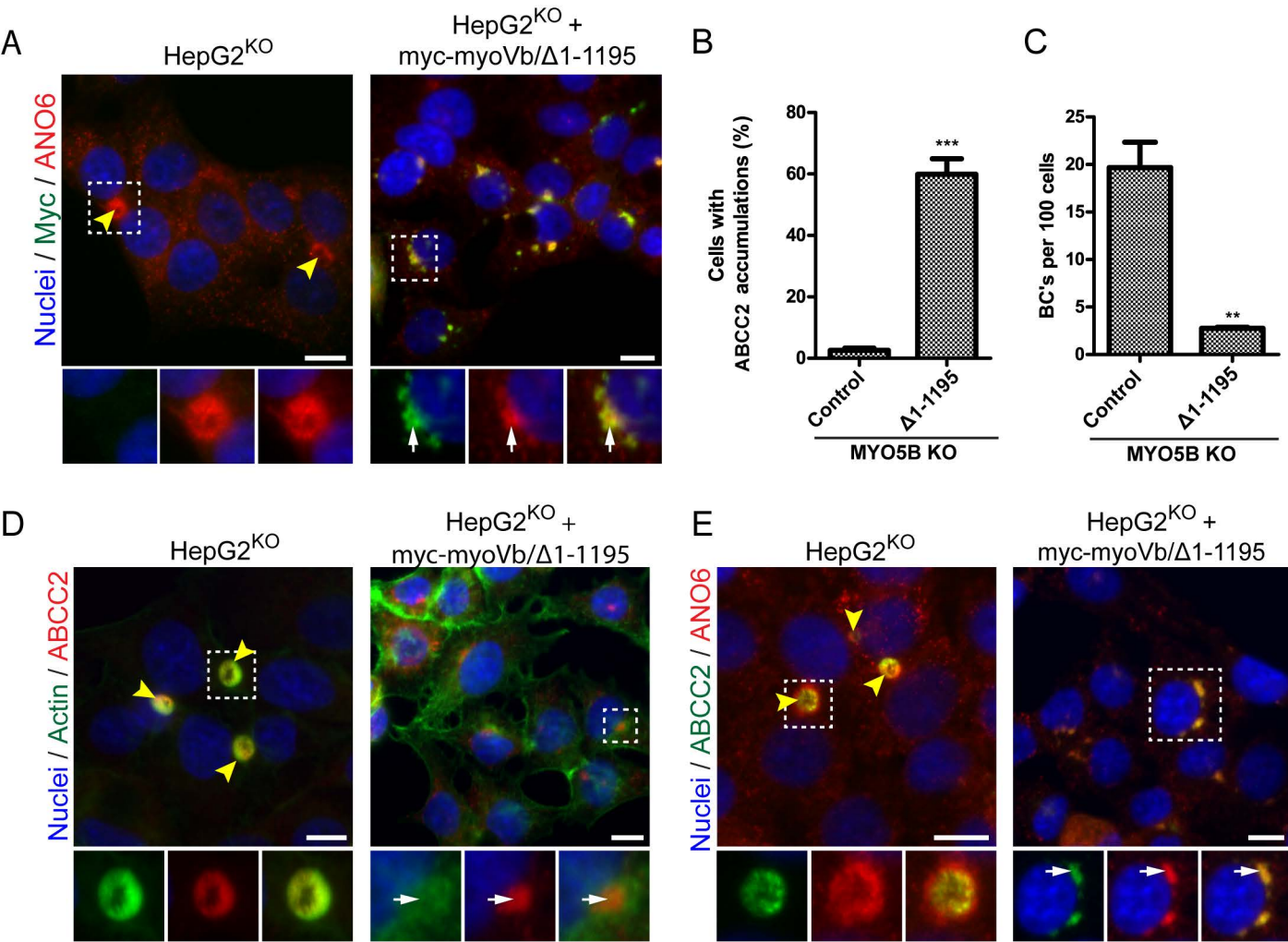
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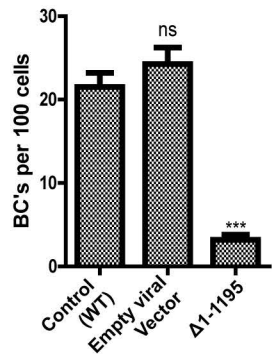
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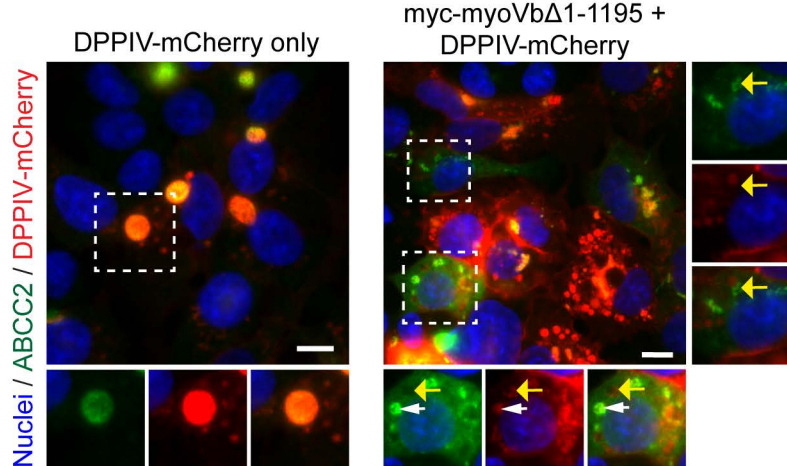




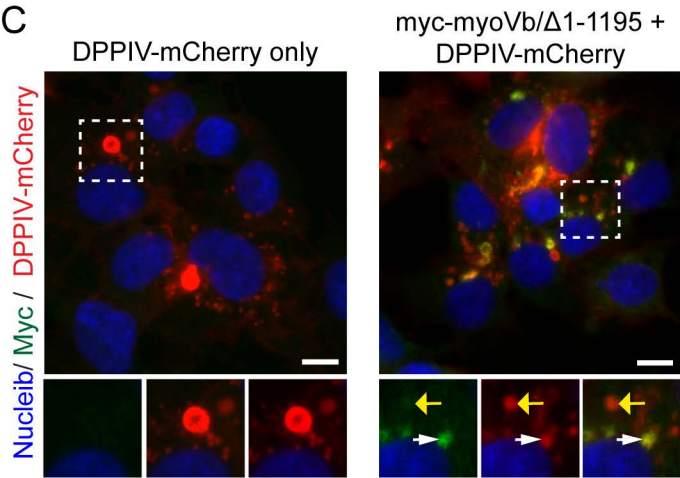
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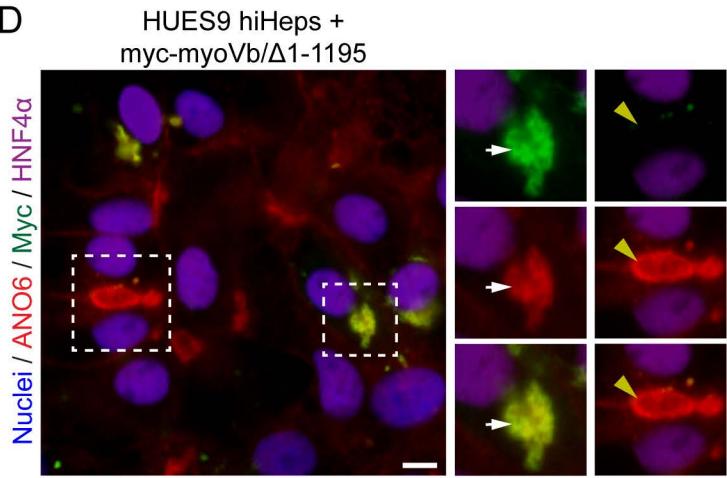
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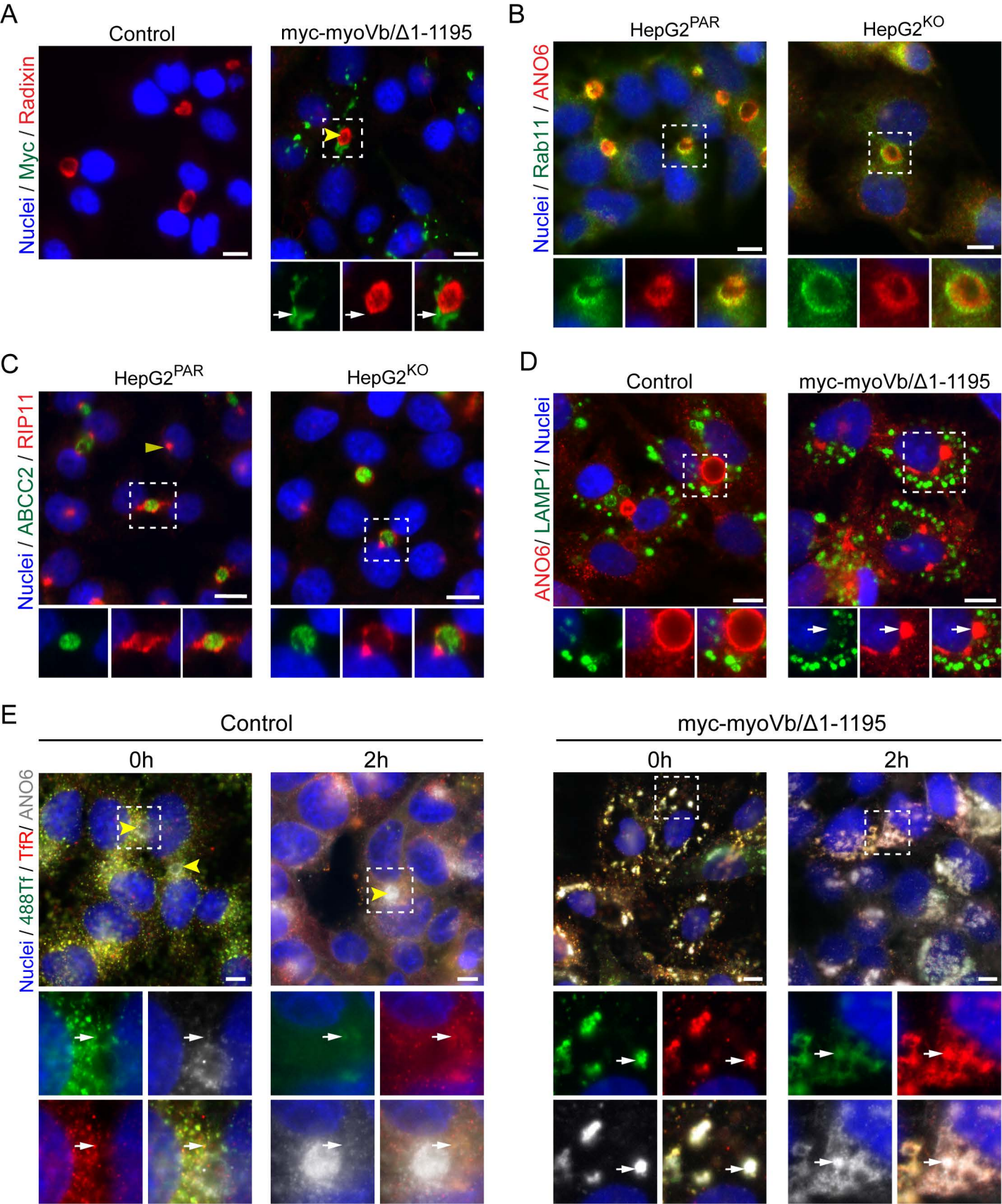


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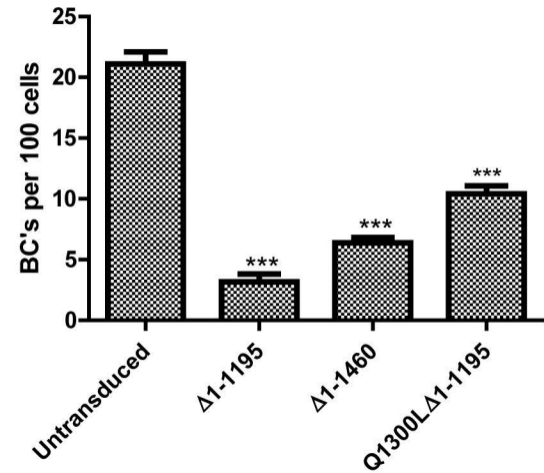


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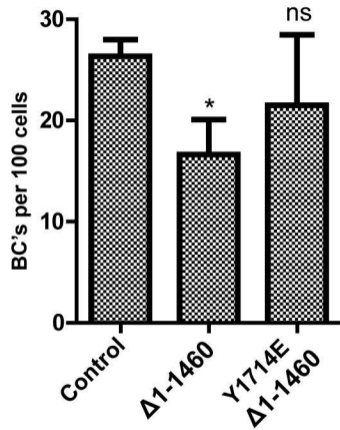




E



F



G

