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Supplementary Materials for

Liver-expressed *Cd302* and *Cr1l* limit hepatitis C virus cross-species transmission to mice

Richard J. P. Brown*, Birthe Tegtmeyer, Julie Sheldon, Tanvi Khera, Anggakusuma, Daniel Todt, Gabrielle Vieyres, Romy Weller, Sebastian Joecks, Yudi Zhang, Svenja Sake, Dorothea Bankwitz, Kathrin Welsch, Corinne Ginkel, Michael Engelmann, Gisa Gerold, Eike Steinmann, Qinggong Yuan, Michael Ott, Florian W. R. Vondran, Thomas Krey, Luisa J. Ströh, Csaba Miskey, Zoltán Ivics, Vanessa Herder, Wolfgang Baumgärtner, Chris Lauber, Michael Seifert, Alexander W. Tarr, C. Patrick McClure, Glenn Randall, Yasmine Baktash, Alexander Ploss, Viet Loan Dao Thi, Eleftherios Michailidis, Mohsan Saeed, Lieven Verhoye, Philip Meuleman, Natascha Goedecke, Dagmar Wirth, Charles M. Rice, Thomas Pietschmann*

*Corresponding author. Email: richard.brown@pei.de (R.J.P.B.); thomas.pietschmann@twincore.de (T.P.)

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Supplementary Materials

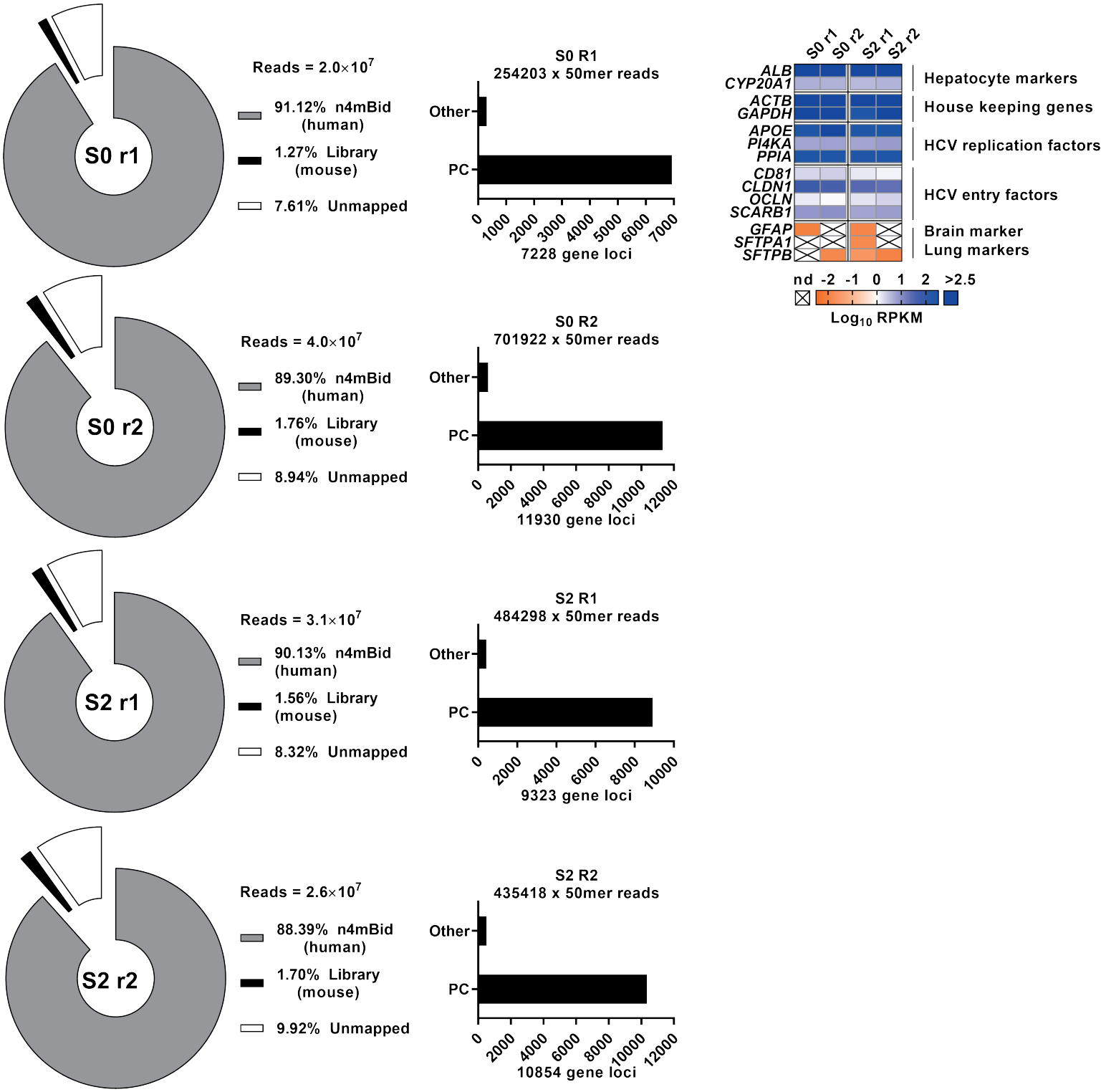

Fig. S1. Quantification of murine library delivery. Left panels. Species-specificity of RNA-seq mapping for S0 and S2 n4mbid cells. Grey segments indicate the percentage of reads mapping to hg19 human genomic scaffold and represent the n4mbid cellular background. Black segments indicate the percentage of reads mapping to the *Mus musculus* mm9 genomic scaffold and represents the integrated murine library. Middle panels. RNA biotype of integrated murine genes. PC: protein-coding genes; Other: 3' overlapping ncRNA, antisense RNA, immunoglobulin gene RNA, lincRNA, polymorphic pseudogene, processed transcript, pseudogene, sense intronic, sense overlapping and T-cell receptor gene. Right panel. No depletion of HCV host factors. Log_{10} RPKM mRNA expression values for selected gene subsets present in S0 and S2 n4mBid cells. Cells with X represent genes with no detectable expression (RPKM = 0).

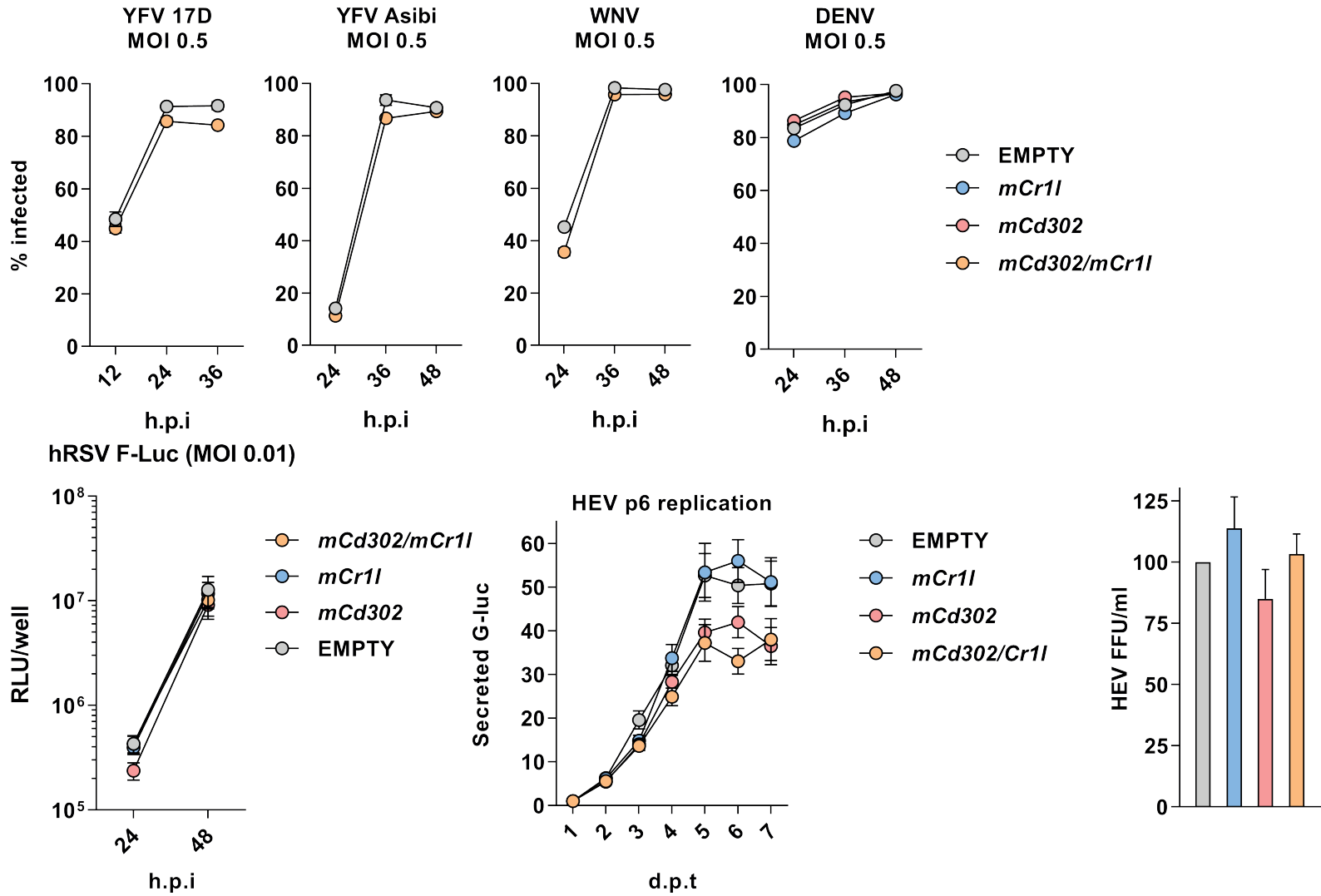

Fig. S2. No restriction of Flaviviruses, hRSV or HEV. Top panels: Infection time-course of indicated cell-lines with flaviviruses. Left to right: Yellow Fever virus (YFV) 17D strain, YFV Asibi strain, West-Nile virus (WNV) and Dengue virus (DENV). Bottom left panel. Infection time course with reporter human respiratory syncytial virus (hRSV). Bottom middle panel. G-luc secretion time-course of indicated cell-lines transfected with HEV p6 subgenomes. Bottom right panel. Infection of indicated cell-lines with hepatitis E virus (HEV).

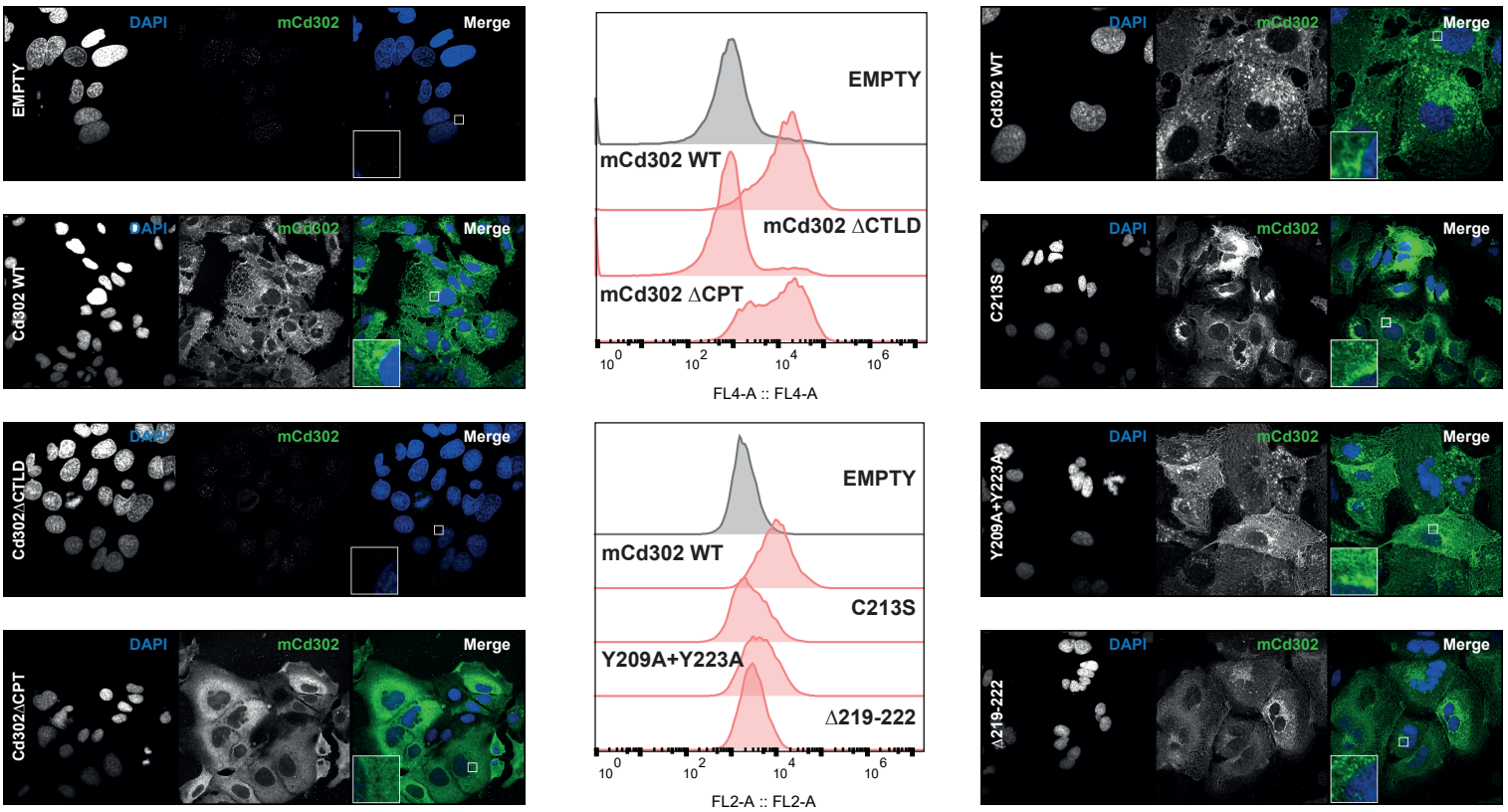

Fig. S3. Cellular localization of domain deletion and mutant Cd302. FACS and immunofluorescence staining of Cd302 domain deletion and cytoplasmic tail mutants. FACS plots of α-mCd302 staining (red) were performed on non-permeablized Huh-7.5 cell-lines ectopically expressing EMPTY, mCd302 or indicated mCd302 mutants. Immunofluorescence staining of mCd302 in the indicated cell-lines was determined by confocal microscopy. Inset panels magnify perinuclear concentration of Cd302.

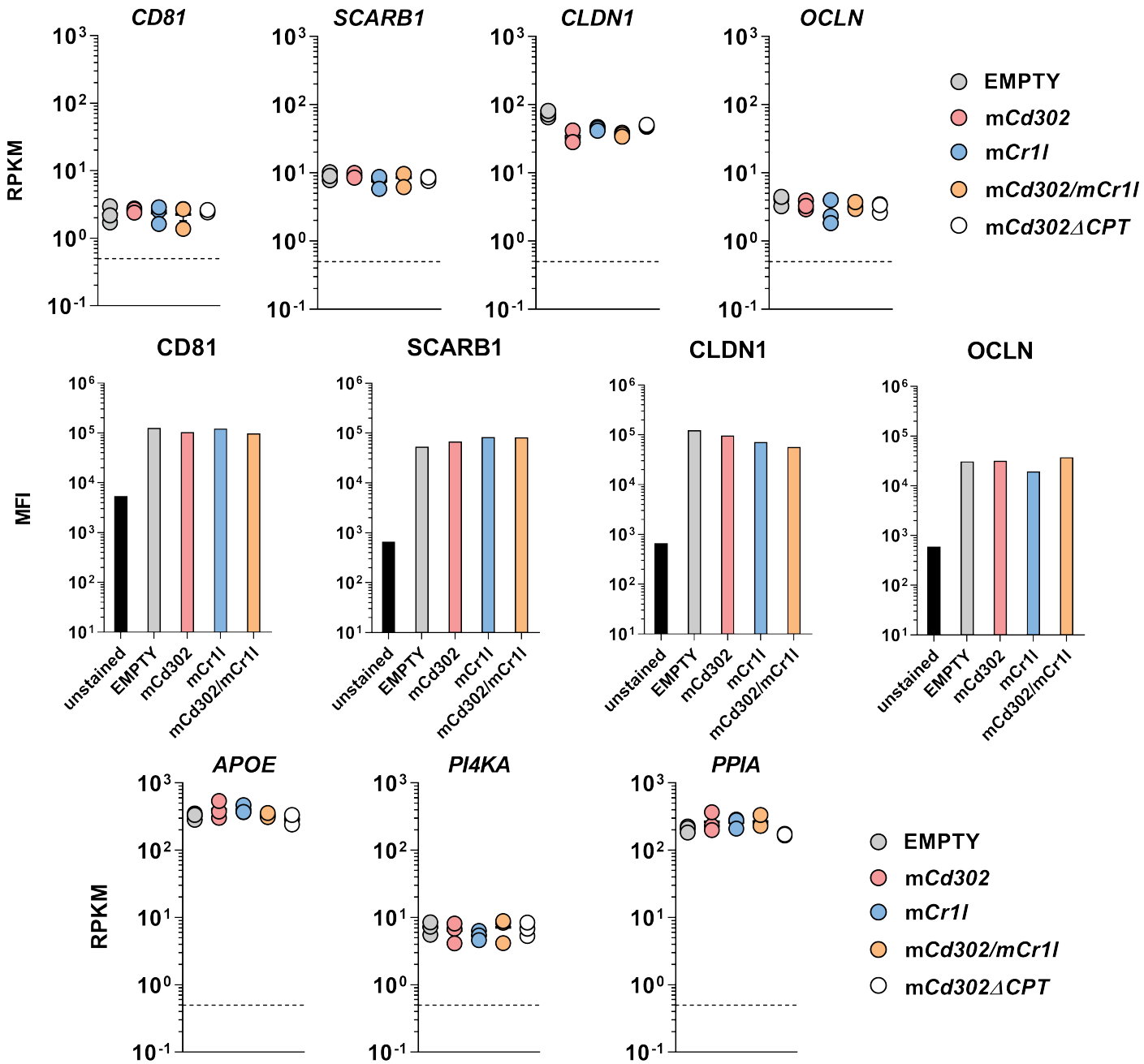

Fig. S4. Ectopically expressing Huh-7.5 cells show no depletion of HCV cofactors. Top panels. Relative mRNA expression (RPKM) of HCV entry factors in Huh-7.5 cells ectopically expressing the indicated genes, as determined by RNA-seq. For each cell-line, n=3 non-identical replicate passages were sequenced. Dashed lines represent a tissue specific gene expression RPKM threshold of 0.5. Middle panels. Flow cytometric analysis of HCV entry factors in non-permeablized Huh-7.5 cells ectopically expressing the indicated factors. MFI: mean fluorescence intensity. Bottom panels. Relative mRNA expression (RPKM) of HCV replication cofactors in Huh-7.5 cells ectopically expressing the indicated genes, as determined by RNA-seq. For each cell-line, n=3 non-identical replicate passages were sequenced. Dashed lines represent a tissue specific gene expression RPKM threshold of 0.5.

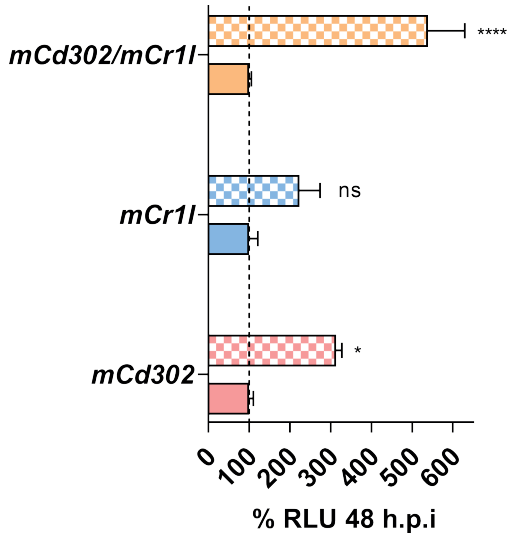

Fig. S5. Silencing of ectopic *Cd302/Cr1l* expression enhances HCV infection rates. Partial ablation of reporter HCVcc restriction via siRNA silencing in Huh-7.5 cells ectopically expressing the indicated murine factors. Cells were treated with either scrambled control siRNAs (color-filled bars) or siRNAs targeted to over-expressed murine factors (checked bars) 24 hours prior to infection. Dashed line represents normalised infection rate observed at 48 h.p.i in cells treated with control siRNAs (100%). Data represents means of n=3 experiments + SEM.

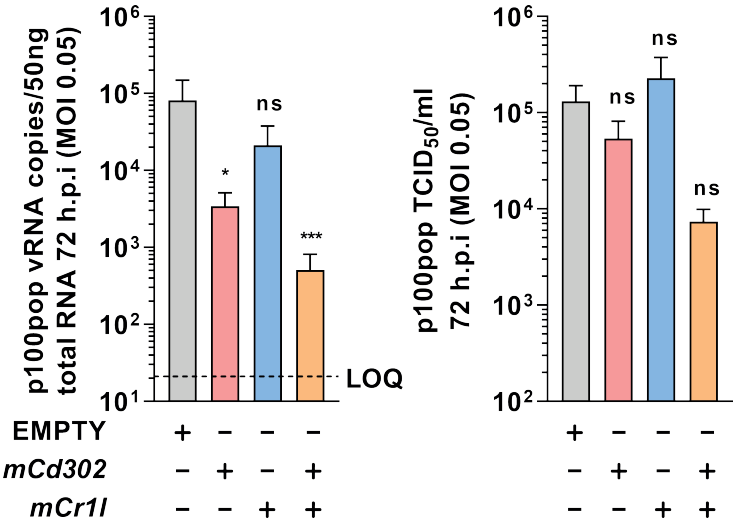

Fig. S6. Murine *Cd302* and *Cr1l* restrict HCV strain p100pop. Infection of the indicated cells with HCV p100pop results in reduced vRNA and virus production at 72 h.p.i. Data represents mean values for n=5 experiments + SEM.

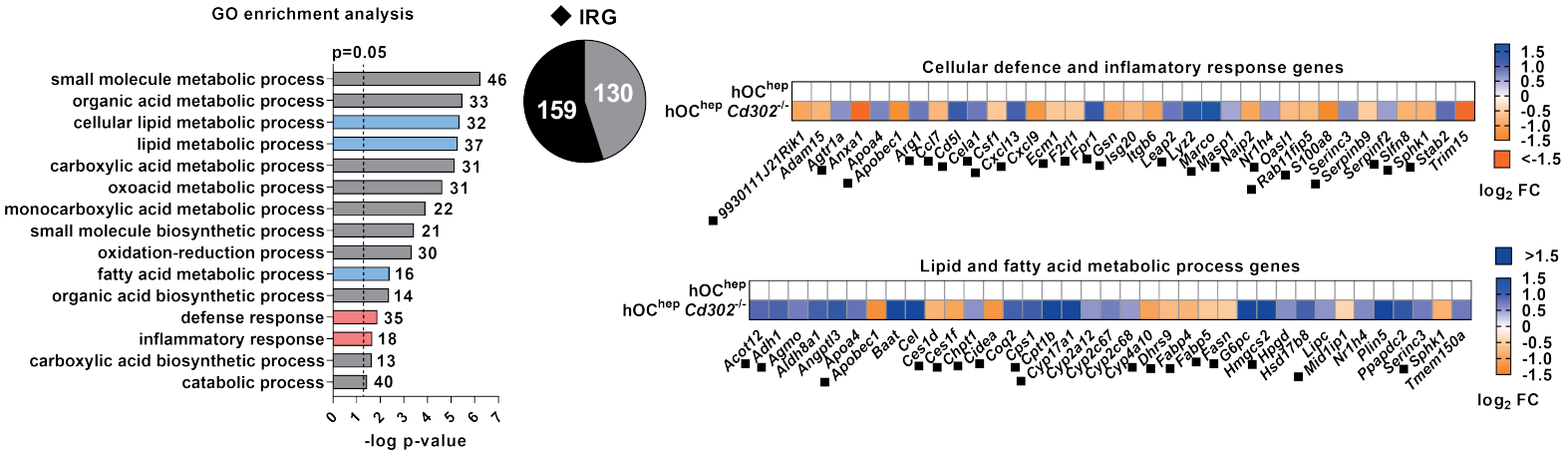

Fig. S7. Ablation of *Cd302* expression in humanized murine hepatocytes modulates the intrinsic transcriptional landscape. Left panel. Gene ontology (GO) analyses of significant biological process gene sets, determined when comparing global transcriptomic data from plated hepatocytes of hOC^{hep} *Cd302*$^{-/-}$ mice (n=3) to parental hOC^{hep} mice (n=3). Numbers associated with biological process categories (y-axis) indicate the number of significant DEGs in each GO category. Right panels. *Cd302* ablation in murine hepatocytes dysregulates expression of genes associated with cellular defense and metabolic processing. Pie chart displays the proportion of IRGs in DEGs associated with hOC^{hep} *Cd302*$^{-/-}$ hepatocytes. Black = IRGs; grey = non-IRGs. Top right. Heat map represents significantly dysregulated genes associated with cellular defense and the inflammatory response in hOC^{hep} *Cd302*$^{-/-}$ hepatocytes when compared to hOC^{hep} hepatocytes. Bottom right. Heat map represents significantly dysregulated genes associated with lipid and fatty acid metabolic processing. Both heat maps represent combinations of the GO categories presented to the left and individual heat map cells represent the mean fold-change in RPKM of n=3 replicates.

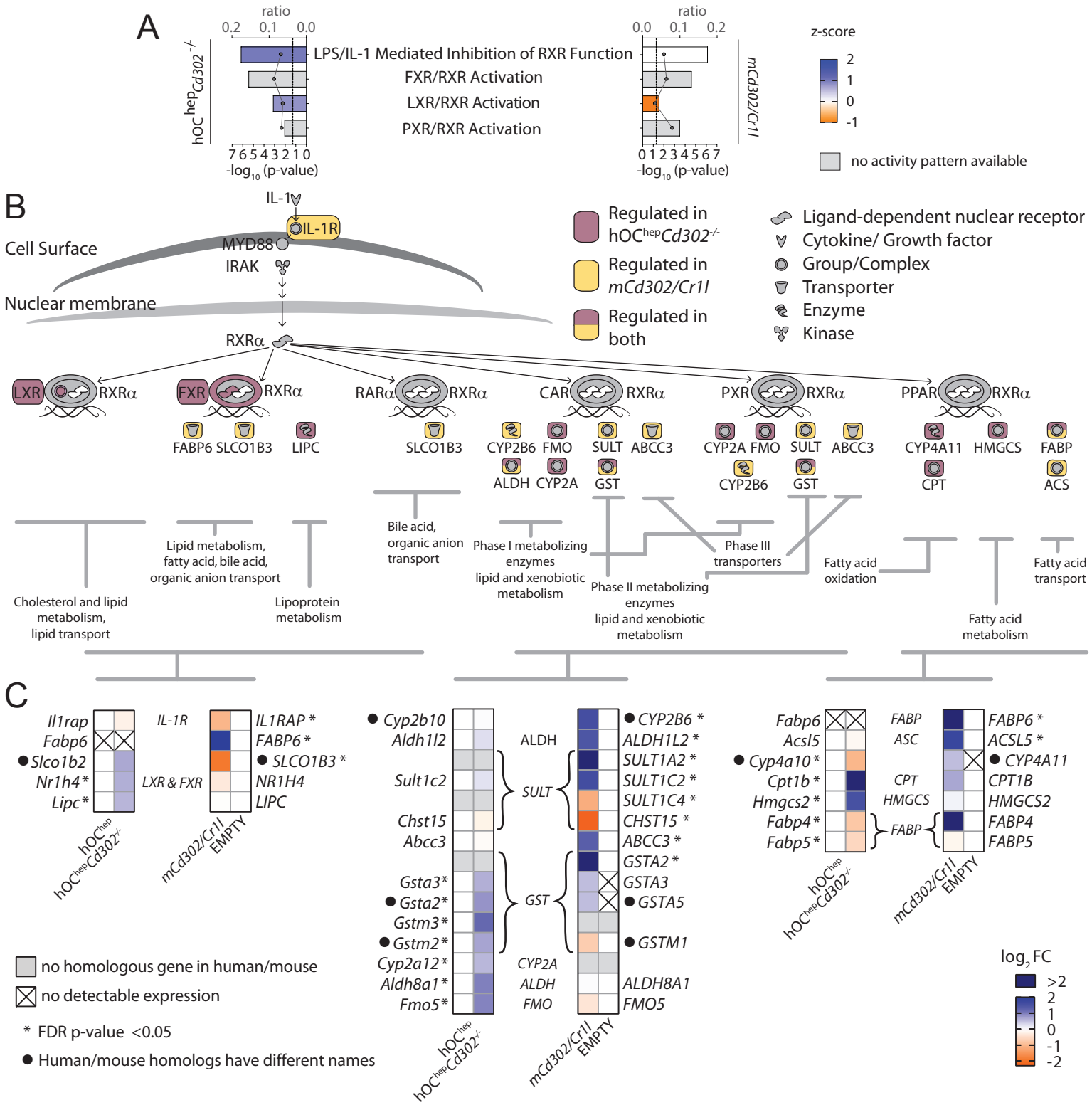

Fig. S8. Overlap between canonical pathways dysregulated in Huh-7.5 cells overexpressing m*Cd302/Cr1l* and hOC^{hep} *Cd302*$^{-/-}$ hepatocytes. (**A**) Shared pathways which were significantly targeted in both systems and include nuclear receptors. The ratio between dysregulated molecules to total molecules belonging to the pathway are presented as grey dots linked by a line and plotted on the upper x-axis. Bars representing p-value (-log$_{10}$) for each pathway are plotted on the lower x-axis. Bar color illustrates the z-score, which indicates the predicted directionality for each pathway with blue indicating activation, orange indicating inhibition and white suggesting no predicted change. Z-scores represent a statistical measure based on the observed gene expression. Only genes with an FDR-p-value of <0.05 were included in the analysis. Pathways highlighted with grey bars indicate no activity pattern is available based on current knowledge. (**B**) Cartoon representation of the targeted canonical pathway "LPS/IL-1 Mediated Inhibition of RXR Function", which possesses the highest –log$_{10}$ (p-value) in both systems. Significantly dysregulated molecules are color-coded according to the system in which they are dysregulated, with the metabolic processes which are influenced by the associated molecules depicted below. Only targeted molecules downstream of nuclear receptors are presented. (**C**) DEGs involved in these pathways and their RPKM fold-change relative to controls are presented as heatmaps. If the gene is a member of a group of genes presented in (B), the group name is designated adjacent to the heatmap.