Supplementary Information

Extended Experimental Procedures

Induced macrophage functional assays

Phagocytosis assays were performed with the Vybrant Phagocytosis Assay Kit (Life Technologies, V6694). For cell migration assays, timelapse imaging was performed on a Nikon T1000 live imaging system. Images were captured every 2-5 minutes. Cell migration was measured with mTrackJ software (ImageJ: http://rsb.info.nih.gov/ij/). Formation of filopodia and lamellipodia were assessed by monitoring changes in cell shape, by ImageJ analysis.

Flow cytometry

For B cell to macrophage conversion, flow cytometry was performed as in Bussmann et al., 2009. Conjugated antibodies were used against CD19, Mac-1 and CD302 (BD Biosciences). For engraftment of iHeps in the colon, cells were sorted based on GFP expression, with autofluorescent resident colon macrophages gated out: conjugated antibodies were used against CD45 (BD Biosciences) and CX3CR1 (R&D Systems). Cells were analyzed and sorted directly into RLT lysis buffer (Qiagen) with an LSRII flow cytometer (BD Biosciences, San Diego, CA), and analysis performed with FlowJo software (Tree Star, Ashland, OR).

Constructs

Cdx2 and *Klf5* open reading frames were cloned from mouse jejunum cDNA into the lentiviral vector pSMAL or retroviral vector pGCDNsam. Other constructs used were: pMx-Klf4, pGCDNsam-Foxa1-IRES-GFP (Addgene plasmid 33007) 33006 pGCDNsam-Hnf4a-IRES-GFP (Addgene plasmid 33006), pGCDNsam-Foxa1 (Addgene plasmid 33003), pGCDNsam-Hnf4a (Addgene plasmid 33002). We also generated a construct in which the self-cleaving peptide sequence, T2A, was positioned in between Hnf4a and Foxa1 (pGCDNsam-Hnf4α-t2a-Foxa1). pMSCV-EGFP and pMSCV-EGFP-Cre (Addgene plasmid 24064) were used to excise *Cdx2* from $Cdx2^{Flox/Flox}$ fibroblasts.

Knockdown of gene expression

Short hairpins in the pLKO.1 and pLKO.5 vectors were obtained from the Broad Institute RNAi consortium: http://www.broadinstitute.org/rnai/trc. Knockdown phenotypes were confirmed with two independent shRNAs, sequences are as follows: Scramble: CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG, Ebf1: CCGGGCGCGACTGTGATCATCATAGCTCGAGCTATGATGATCACAGTCGCGCTTTTTG and CCGGCCCTGAAATGTGCCGAGTATTCTCGAGAATACTCGGCACATTTCAGGGTTTTTG, Pou2af1:

CCGGTCACTAATGTCACGCCAAGAACTCGAGTTCTTGGCGTGACATTAGTGATTTTTG and CCGGCCAGTCCTTCAGGACATGGATCTCGAGATCCATGTCCTGAAGGACTGGTTTTTG, Cdx2: CCGGGTTTCACTTTAGTCGATACATCTCGAGATGTATCGACTAAAGTGAAACTTTTTG, CCGGGCTCTCCGAGAGGCAGGTTAACTCGAGTTAACCTGCCTCTCGGAGAGCTTTTTG. C10 cells and iHeps were transduced with lentivirus in the presence of protamine sulfate (Sigma) for two days and then puromycin selected (3μg/ul, InvivoGen)(Barneda-Zahonero et al., 2013).

Gene expression analysis

RNA was extracted with the RNeasy Minikit (QIAGEN) and reverse transcribed with SuperScript III First-Strand Synthesis System (Life Technologies). TaqMan Universal PCR Master Mix (Life Technologies) was used for amplification and detection of cDNAs, and qPCR reactions were carried out with the Mx3005P qPCR System (Agilent). Ct values were calculated and normalized to housekeeping genes (*Gapdh* for B cell to macrophage conversion experiments and *Hprt* for fibroblast to iHep conversion experiments), and the relative expression ratio was calculated using the Pfaffl method (Pfaffl, 2001). Taqman primer sequences are as follows: Housekeeping genes: Hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) (Mm01545399_m1) and Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*)(Mm99999915_g1). Intestinal genes: Caudal type homeobox 2 (*Cdx2*) (Mm01212280_m1), Omega-3 Fatty Acid Receptor 1 (*O3far1*) (Mm00725193_m1), Forkhead box F2 (*Foxf2*) (Mm00515793_m1), Membrane bound O-acyltransferase domain containing 1 (*Mboat1*) (Mm00463555_m1), Tetraspanin-8 (*Tspan8*) (Mm00524563_m1), Phospholipase A2, group X (*Pla2g10*) (Mm00449532_m1), Transmembrane and immunoglobulin domain containing 1 (*Tmigd1*) (Mm00481764_m1), Amphiregulin (*Areg*) (Mm00437583_m1). Epithelial gene: E-cadherin (*Cdh1*) (Mm00486909_g1). Hepatic genes: Albumin (*Alb*) (Mm00802090_m1), Alpha fetoprotein (*Afp*) (Mm00431715_m1), Alpha-1 antitrypsin (*Serpina1*) (Mm02748447_g1), Sulfotransferase 1A1 (*Sult1a1*) (Mm01132072_m1), Glutathione S-transferase, alpha 4 (*Gsta4*) (Mm00494803_m1), Microsomal glutathione S-transferase 1 (*Mgst1*) (Mm00498294_m1). Fibroblast genes: Msh homeobox 2 (*Msx2*) (Mm00442992_m1), Snail family zinc finger 1 (*Snai1*)(Mm00441533_g1), Chemokine (C-X-C motif) ligand 5 (*Cxcl5*) (Mm00436451_g1), Paired related homeobox 2 (*Prrx2*) (Mm00436428_m1), Arylsulfatase Family, Member J (*Arsj*)(Mm00557970_m1). B cell genes: Immunoglobulin heavy gene (*Ighg*)(Mm01703611_g1), protein phosphatase, Mg2+/Mn2+ dependent, 1E (*Ppm1e*) (Mm00622953_s1), Cat eye syndrome chromosome region, candidate 2 (*Cecr2*)(Mm01183239_m1), Recombination activating gene 1 (*Rag1*) (Mm01270936_m1), Pre-B Lymphocyte 1 (*Vpreb*)(Mm00785614_sH), Early B-cell factor 1 (*Ebf1*)(Mm00432948_m1), POU class 2 associating factor 1 (*Pou2af1*)(Mm00448326_m1).

Macrophage genes: Integrin, alpha M (*Itgam*)(Mm00434455_m1), Insulin-like growth factor 1 (*Igf1*)(Mm00439560_m1), V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (*MafB*)(Mm00627481_s1), Ring finger protein 128 (*Rnf128*)(Mm00480990_m1), Peripheral myelin protein 22 (*Pmp22*)(Mm00476979_m1), Fc receptor, IgG, high affinity I (*Fcgr1*)(Mm00438874_m1), Macrophage scavenger receptor 1 (*Msr1*)(Mm00446214_m1).

Hepatic function assays

Primary hepatocytes were isolated from 10-week-old adult mouse livers by two-step collagenase digestion. Albumin and urea secretion, LDL uptake and PAS staining was assessed using a mouse Albumin ELISA kit (Abcam), QuantiChrom Urea Assay Kit (BioAssay Systems), incubation of iHeps with 10μg/ml DiI-labeled acetylated LDL (Biomedical Technologies) and PAS staining (Sigma) as in Sekiya and Suzuki, 2011.

iHep spheroid culture

To form iHep spheroids , iHeps were seeded in 2% Matrigel (BD Biosciences) in each well of a four-well plates precoated with 150μL Matrigel. Medium was changed every 2 d for iHep spheroids to develop within 3–5 d. To quantify lumen formation, iHep spheroids with a single central lumen were counted manually using an inverted microscope.

Supplementary Figure Legends

Figure S1, related to **Figures 1 and 2.** (**A**) B cell and macrophage GRN status in conversion of primary pre-B cells to macrophages (Di Tullio et al., 2011). (**B**) C10 B cell and macrophage GRN establishment does not significantly change following 48 and 96 hours of C/EBPα expression. n.s.: not significant. (**C**) FACS plots of Mac1 and CD19 expression over four days of conversion demonstrating similar kinetics as previously published (Bussmann et al., 2009). (**D**) Expression of surface antigen expression in induced macrophages: CD302 is not expressed at levels comparable to native macrophages. Expression data from Bussmann et al., 2009.

Figure S2, related to **Figure 2.** (**A**) qPCR confirmation of *Ebf1* and *Pou2af1* knockdown in induced macrophages. (**B**) Formation of lamellipodia and filopodia as measured by shape change and (**C**) phagocytic function of the induced macrophages following *Ebf1* and *Pou2af1* knockdown, relative to scramble control and the RAW264.7 macrophage cell line. (**D**) qPCR analysis of B-cell (left panel) and macrophage (right panel) marker expression following knockdowns and conversion to induced macrophages. (**E**) Example of a regulatory loop identified from CellNet GRNs, in which loss of

Pou2af1 is predicted to increase B-cell factor (*Pax5* and *Ebf1*) expression. (**F**) Repression of the macrophage GRN by Pou2af1, explaining the upregulation of macrophage fate and function following *Pou2af1* knockdown. For example, *Tfec*, a gene repressed by Ebf1 and Pou2af1 in the engineered macrophages, is linked to upregulation of *Ifi204*, a macrophage transcription factor whose expression favors macrophage differentiation (Dauffy et al., 2006).

Figure S3, related to **Figure 3.** (**A**) Efficiency of conversion to iHeps: Alkaline phosphatase staining of E-cadherin expressing colonies. (**B**) Immunofluorescence staining of E-cadherin and Albumin in iHeps and MEFs. (**C**) Visualization of glycogen storage via Periodic Acid-Schiff (PAS) staining in iHeps. (**D**) Low density lipoprotein (LDL) uptake in iHeps and MEFs. Scale bars as indicated. (**E**) Relative albumin expression in MEFs, iHeps and primary hepatocytes. (**F**) Relative urea production in MEFs, iHeps and primary hepatocytes. (**G**) Relative liver marker expression in adult hepatocytes, dedifferentiated iHeps, fetal liver and iHeps.

Figure S4, related to **Figure 4.** (**A**) Knockdown efficiency of *Cdx2.* (**B**) Intestinal gene expression in Sekiya iHeps. (**C**) Intestinal gene expression in two independent replicate iHep lines. (**D**) Heatmaps of gene expression associated with liver function and Hox patterning following Cdx2 knockdown. (**E**) Cell and tissue type classification heatmap following *Cdx2*-knockdown engineered iHeps and the corresponding establishment of GRNs.

Figure S5, related to **Figures 4 and 5.** (**A**) Schematic and confirmation of conditional Cdx2 knockout following the introduction of Cre recombinase. (**B**) Timecourse of low MOI iHep behavior and relative expression of *Cdx2* and liver markers in low and high MOI iHeps. (**C**) Characterization of low MOI iHeps augmented with Cdx2.

Figure S6, related to **Figure 6.** (**A**) iHep spheroid formation in Matrigel following *Cdx2* knockdown and in the presence of Wnt3a and R-spondin. (**B**) Lgr5 expression in 2D-cultured iHeps and spheroids, from MEFs derived from B6.129P2-*Lgr5*$^{tm1(cre/ERT2)Cle}$/J embryos. Lgr5 expression is detected in <1% of iHep spheroids. (**C**) Klf4-iEPs do not engraft mouse colon: whole-mount image. Scale bar, 0.5cm. (**D**) Patch of GFP+ long-term engrafted iHeps/iEPs in mouse colon. Scale bar, 100μm. (**E**) Flow cytometry analysis of iHep engraftment and resident colon macrophage autofluorescence. (**F**) Engraftment of Cre-EGFP transduced iHeps into *Rosa26-Lox-STOP-Lox-Tomato* transgenic mouse colon. Stop codon excision is not detected from the absence of Tomato expression, ruling out fusion of iHeps with resident colon cells. *Rosa26-Lox-STOP-Lox-Tomato*

(B6;129S6-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$/J) colon was directly transduced with Cre-EGFP, resulting in stop codon excision and tomato expression as a positive control.

Table 1, related to **Figure 2.** The Pou2af1 network that influences the set of macrophage and B cell genes that are up-regulated upon *Pou2af1* knockdown. There are 2 types of rows indicated by the TG (Targeted Gene) type column. 'Macrophage' means that the target gene in the row is one of the macrophage genes upregulated following *Pou2af1* knockdown. These rows list the TF (Transcription Factor) that is proposed to repress (or in some cases, promote) the target gene. These TFs can be Pou2af1 itself, or TFs that Pou2af1 is predicted to regulate. If Pou2af1 represses a TF, then that TF must promote the expression of the macrophage target gene in order to be listed in the table. The other type of row describes the relationship between Pou2af1 and these intermediate TFs.

Table 2, related to **Figure 2.** Macrophage genes that are significantly de-repressed following *Ebf1*- and *Pou2af1*-knockdown.

References

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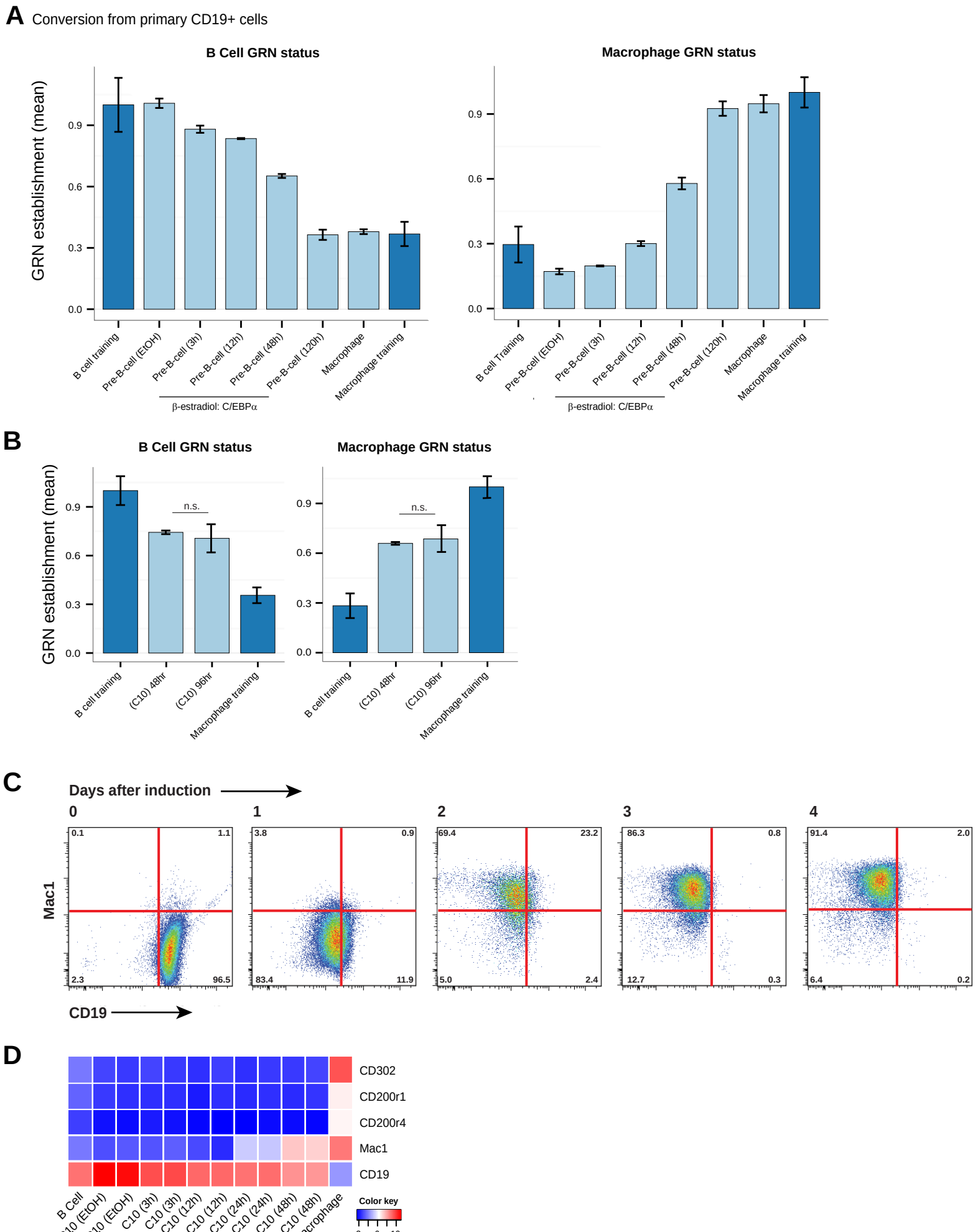
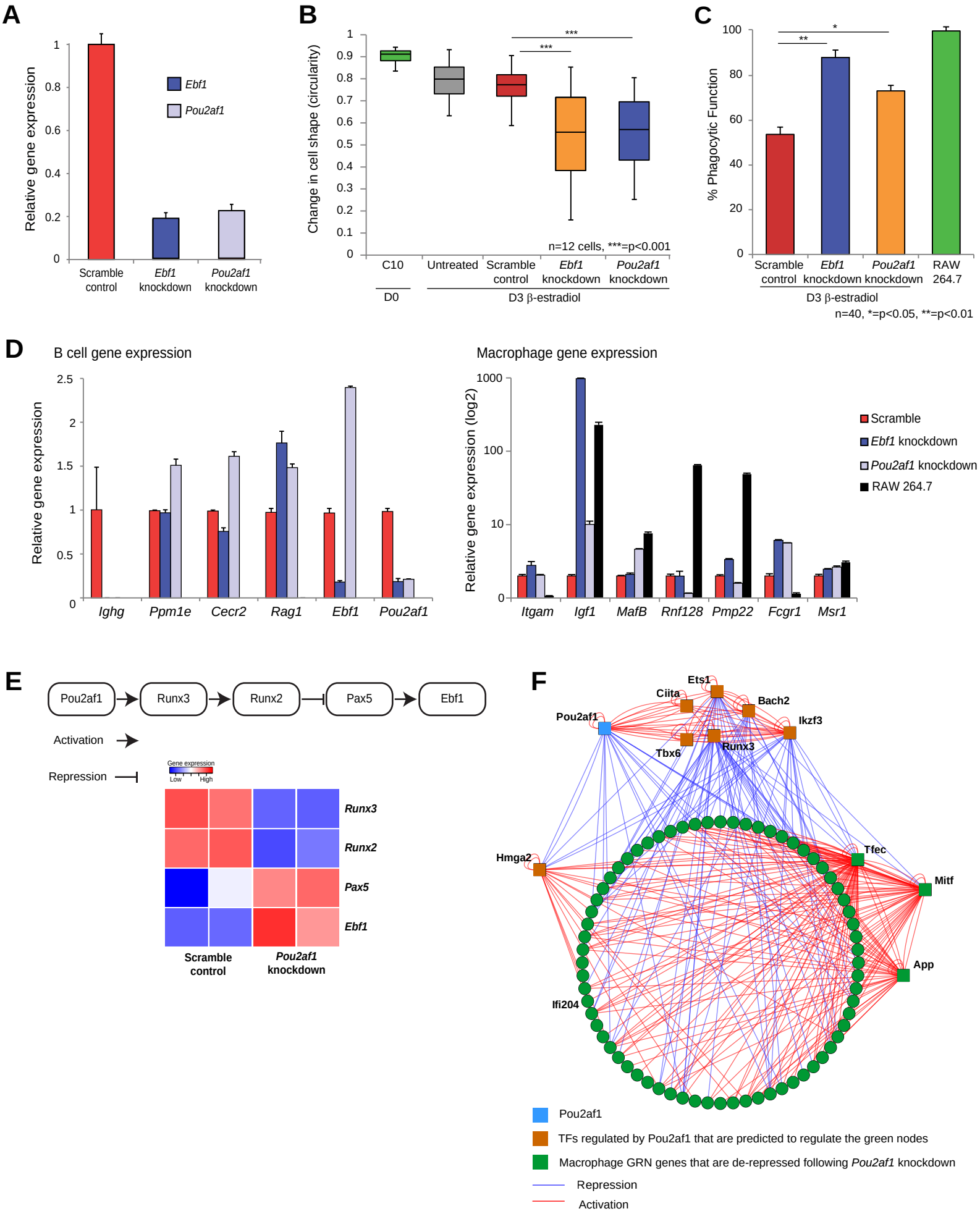
Supplemental Figure 1



Supplemental Figure 2



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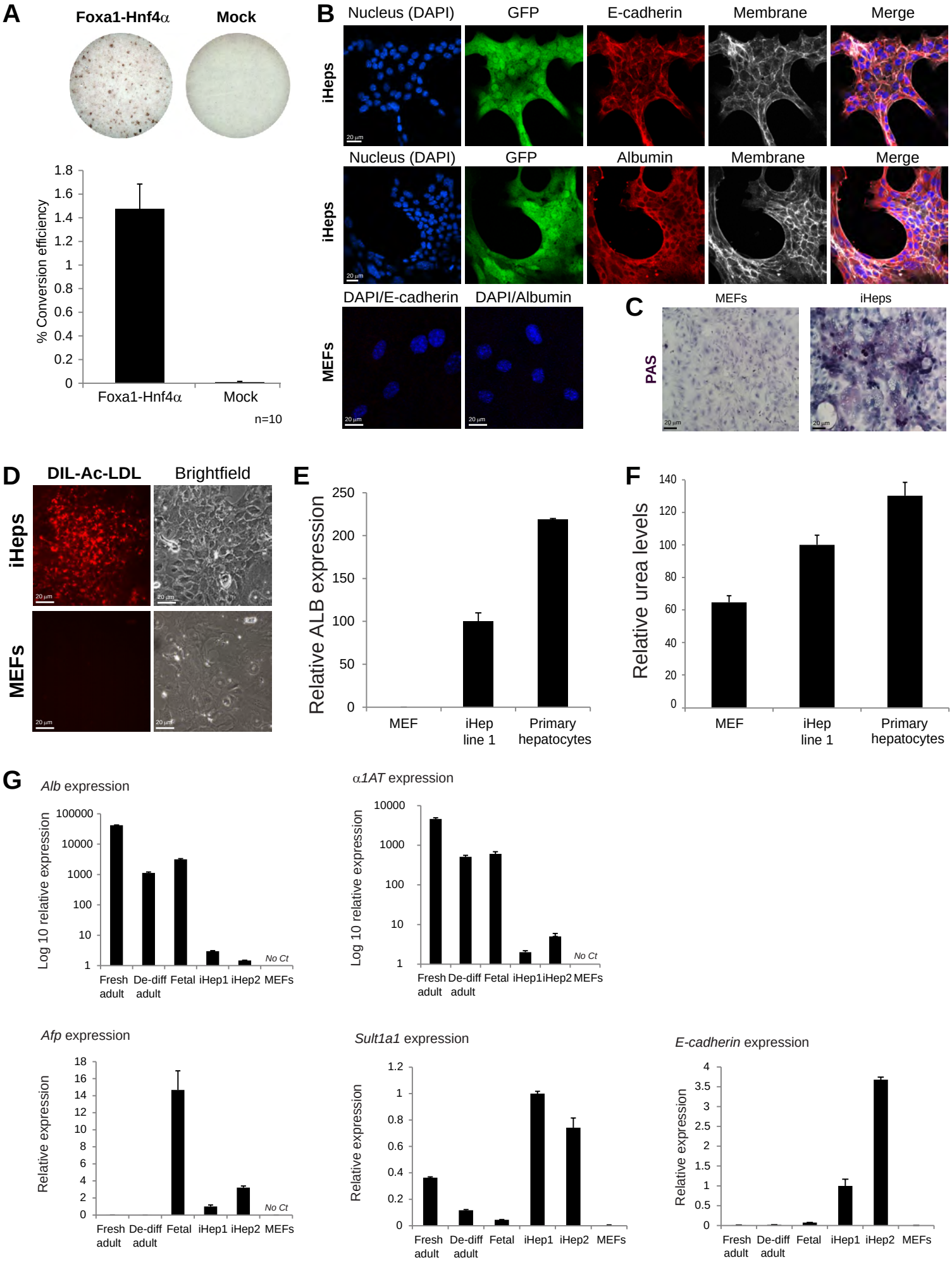

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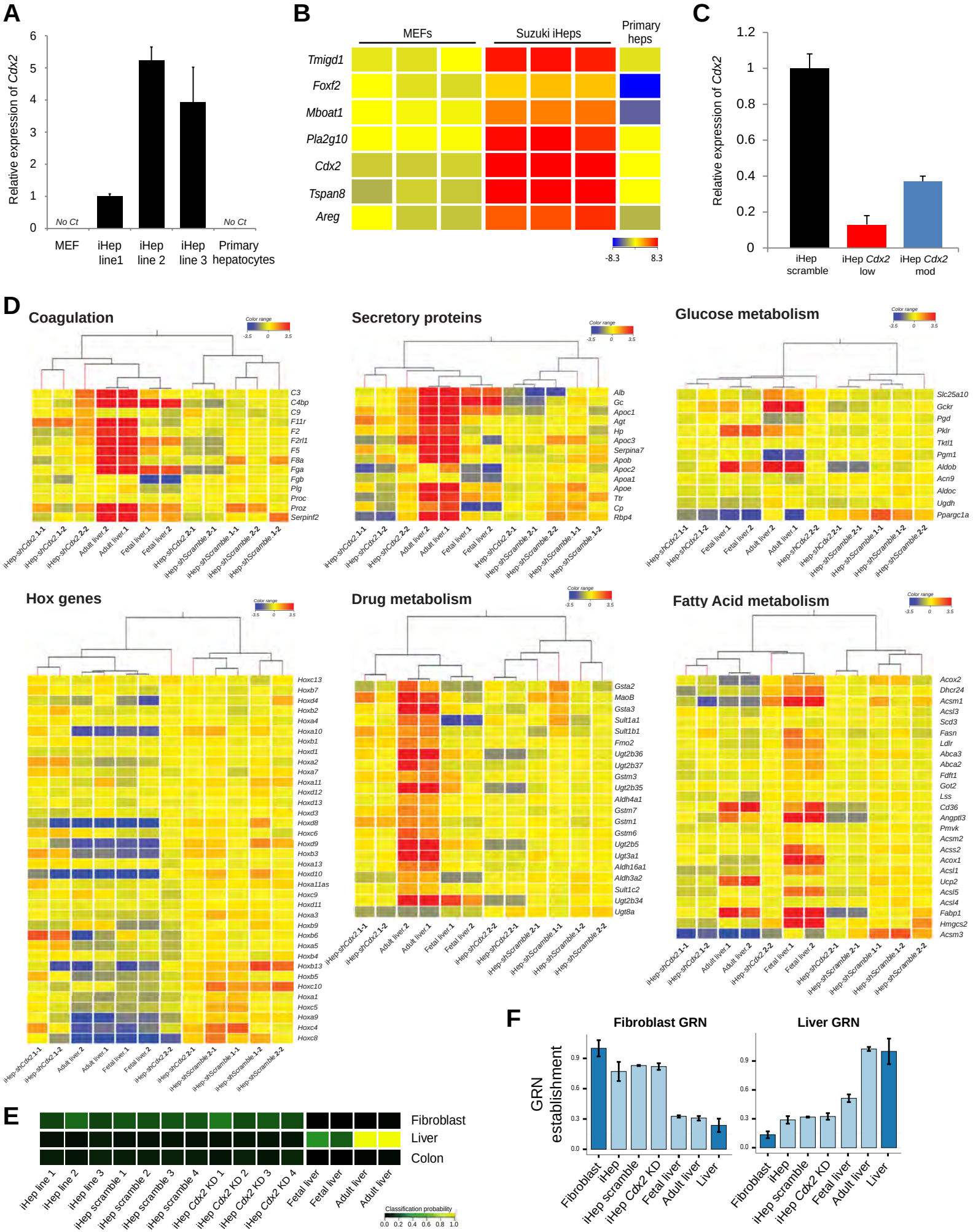

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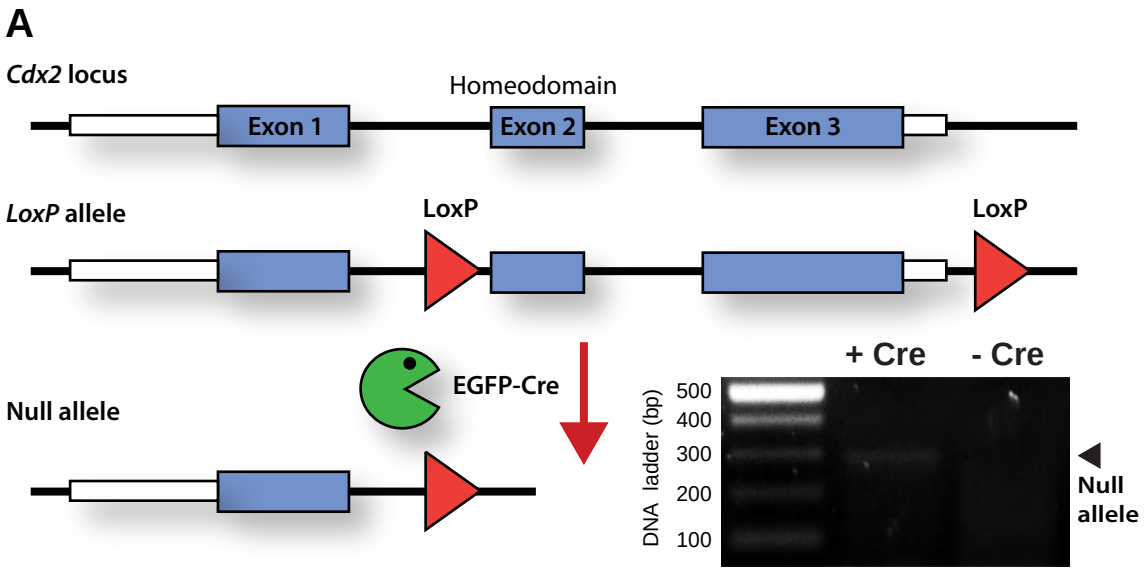

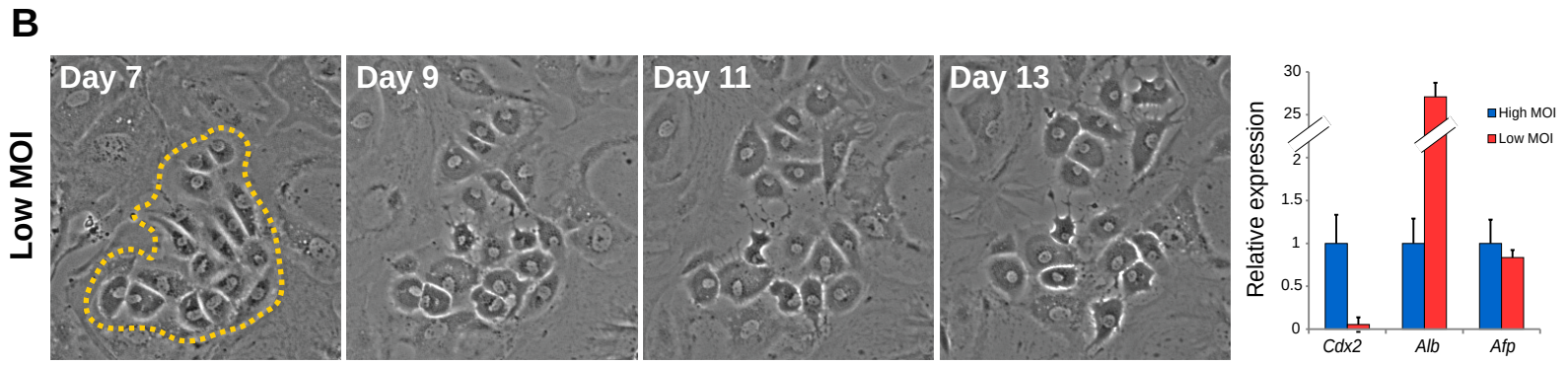

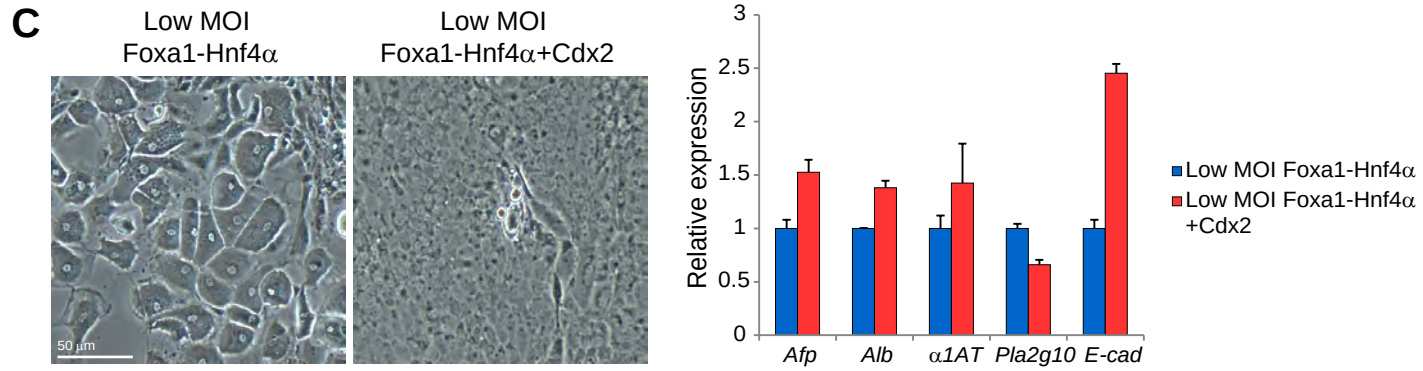

Supplemental Figure 6