Supplementary Materials

Methods

Overexpression of HNF4α and knockdown of HB-EGF in UMSCs

HNF4α cDNA was cloned from human umbilical cord MSCs into pHelper 1.0 plasmid. The UMSCs were then infected with lentiviral particles of HNF4Sα-GFP or GFP (used as a control). Overexpression of HNF4α was confirmed by confocal laser-scanning microscope and Western blotting. The sh-RNA for HB-EGF was constructed with reference from published literatures, then infected with HNF4α-overexpressed UMSCs. Knockdown of HB-EGF was confirmed by qRT-PCR and Western blotting

Western blot

Western blotting was performed to analyze the protein expression levels. The cell extracts were isolated from cultured hepatocytes using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher scientific, Waltham, MA, USA). The primary antibodies against HNF4α (Abcam), HB-EGF (Abcam) and β-actin as control (Sigma-Aldrich) were utilized.

Harvest of conditioned medium

Microcapsules of human hepatocytes, with or without HNF4α-UMSCs or UMSCs were cultured for four days, and the conditioned medium (CM) was harvested and stored at -80 °C.

RNA extraction and real-time PCR

RNA extraction and real-time PCR were performed as described previously.[18] The PCR primers are listed in Table S1. Total liver RNA was extracted using TRIzol (Takara, Tokyo, Japan) reagent according to the manufacturer's instructions. The cDNA was synthesized with PrimeScript RT reagent Kit (Takara). q-PCR was performed using CFX 96 q-PCR system (BIO-RAD, Hercules, CA, USA). A SYBR RT-PCR kit (Takara) was used for quantitative real-time PCR analysis. The relative expression levels for target gene

were normalized by β-actin or GAPDH.

Supplementary figure and table legends

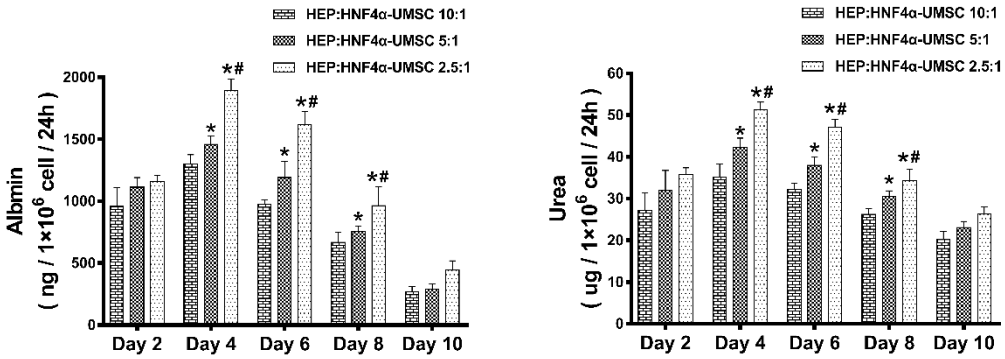

Figure S1: Primary hepatocytes were co-encapsulated with HNF4α-UMSCs at a ratio of 10:1, 5:1, and 2.5:1. Measurement of albumin secretion and urea synthesis in the supernatant of microcapsules in different groups at varied time points.

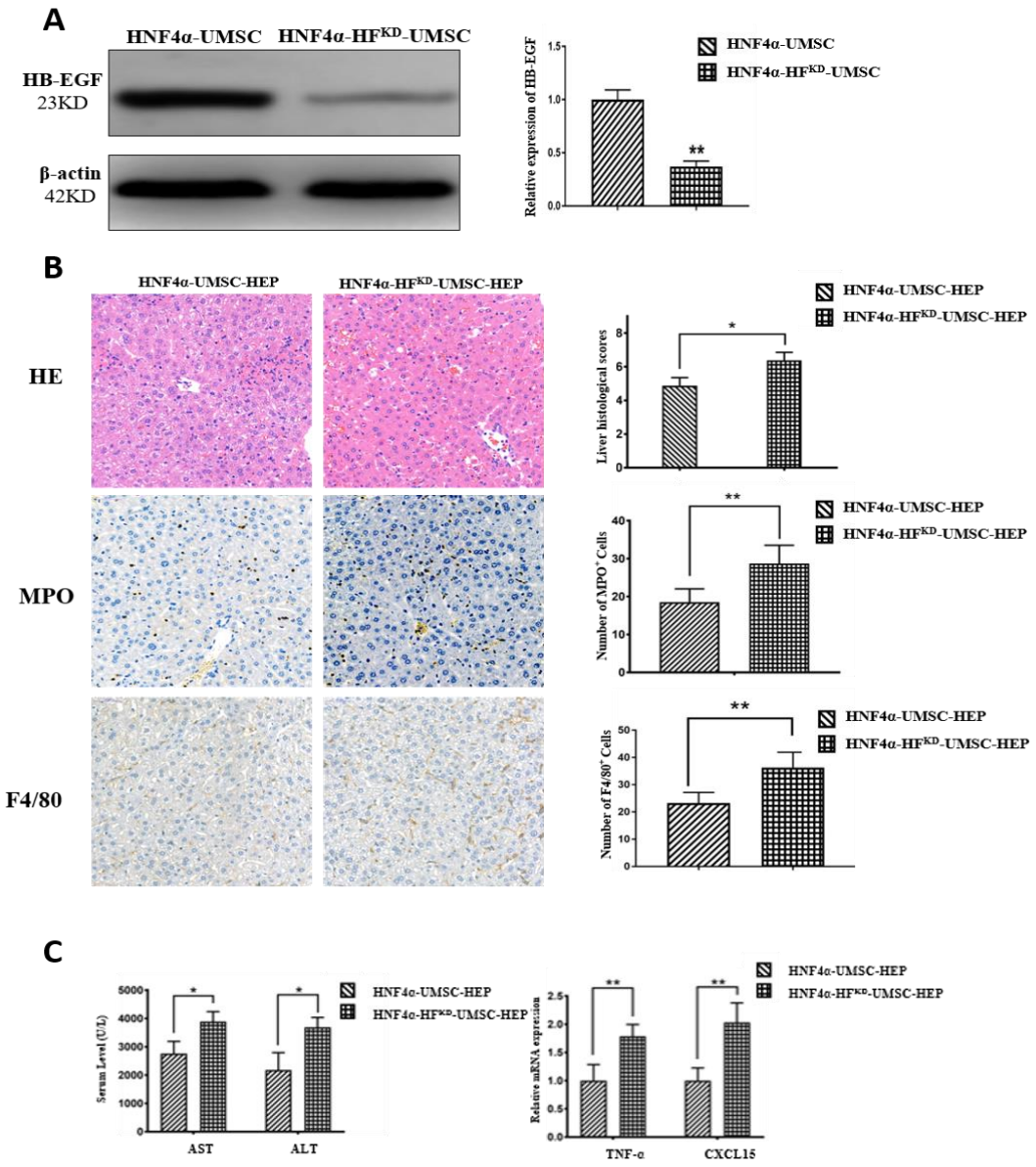

Figure S2: Assess the essential influence of HB-EGF in UMSCs on inflammation resolution effect on ALF mice. **(A)** Confirmation of knockdown of HB-EGF in HNF4α-UMSCs. **(B)** HE staining and immunochemistry images of liver sections with MPO and F4/80 antibodies (original magnification, × 200). Quantification of liver histological scores, MPO and F4/80 positive cells in sights. **(C)** ELISA analysis on ALT and AST concentrations in plasma of ALF mice treated with HNF4-α-UMSC-HEP and HNF4α-HFKD-UMSC-HEP. The mRNA levels of TNF-α and IL-8 levels in the liver tiusses of ALF mice treated with HNF4-α-UMSC-HEP and HNF4α-HFKD-UMSC-HEP.

Supplementary Table 1

Gene	Forward Primer	Reverse Primer
Human ALB	TGCAACTCTTCGTGAAACCTATG	ACATCAACCTCTGGTCTCACC
Human CK18	GGCATCCAGAACGAGAAGGAG	ATTGTCCACAGTATTTGCGAAGA
Human CYP3A4	AAGTCGCCTCGAAGATACACA	AAGGAGAGAACACTGCTCGTG
Human GAPDH	GGCTGTTGTCATACTTCTCATGG	GGAGCGAGATCCCTCCAAAAT
Mouse INOS	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
Mouse CD86	TCAATGGGACTGCATATCTGCC	GCCAAAATACTACCAGCTCACT
Mouse TNFα	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
Mouse CXCL 15	TCGAGACCATTTACTGCAACAG	CATTGCCGGTGGAAATTCCTT
Mouse Arg-1	CTCCAAGCCAAAGTCCTTAGAG	GGAGCTGTCATTAGGGACATCA
Mouse Ppar-γ	GGAAGACCACTCGCATTCCTT	GTAATCAGCAACCATTGGGTCA
Mouse CD206	CTCTGTTCAGCTATTGGACGC	TGGCACTCCCAAACATAATTTGA
Mouse β-actin	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC

Table S1: The PCR primers used in the study.

Supplementary Table 2

Name	UMSC	HNF4α-UMSC	Ratio
Artemin	3.57	8.37	2.34
BDNF	6.14	7.67	1.25
beta-NGF	5.46	7.64	1.40
EGF	6.09	7.89	1.30
EGF R / ErbB1	6.17	7.90	1.28
EG-VEGF / PK1	5.67	7.94	1.40
FGF Basic	5.58	7.33	1.31
FGF-9	5.75	8.13	1.41
GCSF	7.22	8.21	1.14
GDF1	5.75	7.98	1.39
GDF3	7.64	8.60	1.13
GDF5	5.51	8.30	1.51
GDF8	3.80	8.37	2.20
GDF9	3.94	8.25	2.09
GDF11	4.58	8.24	1.80
GM-CSF	5.81	7.75	1.33
HB-EGF	5.30	8.24	1.55
HGF	4.62	8.23	1.78
ICAM-5	6.95	7.51	1.08
IGFBP-1	6.25	7.75	1.24
IGFBP-2	6.16	7.74	1.26
IL-1 ra	7.23	7.44	1.03
IL-4	6.32	7.93	1.25
IL-10	5.95	8.63	1.45
IL-11	5.71	8.79	1.54
IL-13	7.07	7.68	1.09
LIF	7.03	7.35	1.05
M-CSF	6.09	8.46	1.39
Neurturin	6.85	7.44	1.09
NT-3	6.74	7.62	1.13
PDGF-AA	4.69	8.44	1.80
PDGF-AB	3.83	8.47	2.21
PDGF-BB	2.83	8.31	2.94
Persephin	6.34	7.46	1.18
TGF-beta 1	0.00	7.70	14.74
TGF-beta 2	6.00	7.37	1.23
TGF-beta 5	6.77	7.22	1.07

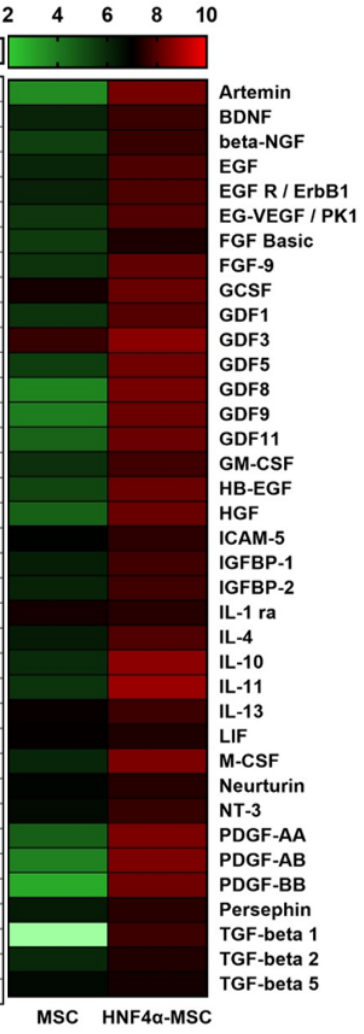

Table S2: The relative intensities of signals were listed in the below table and the list of relative intensities of signals of growth factors in the CMs of HNF4α-UMSCs and UMSCs which are significantly high in HNF4α-UMSCs groups. Heat map is shown in the right panel.