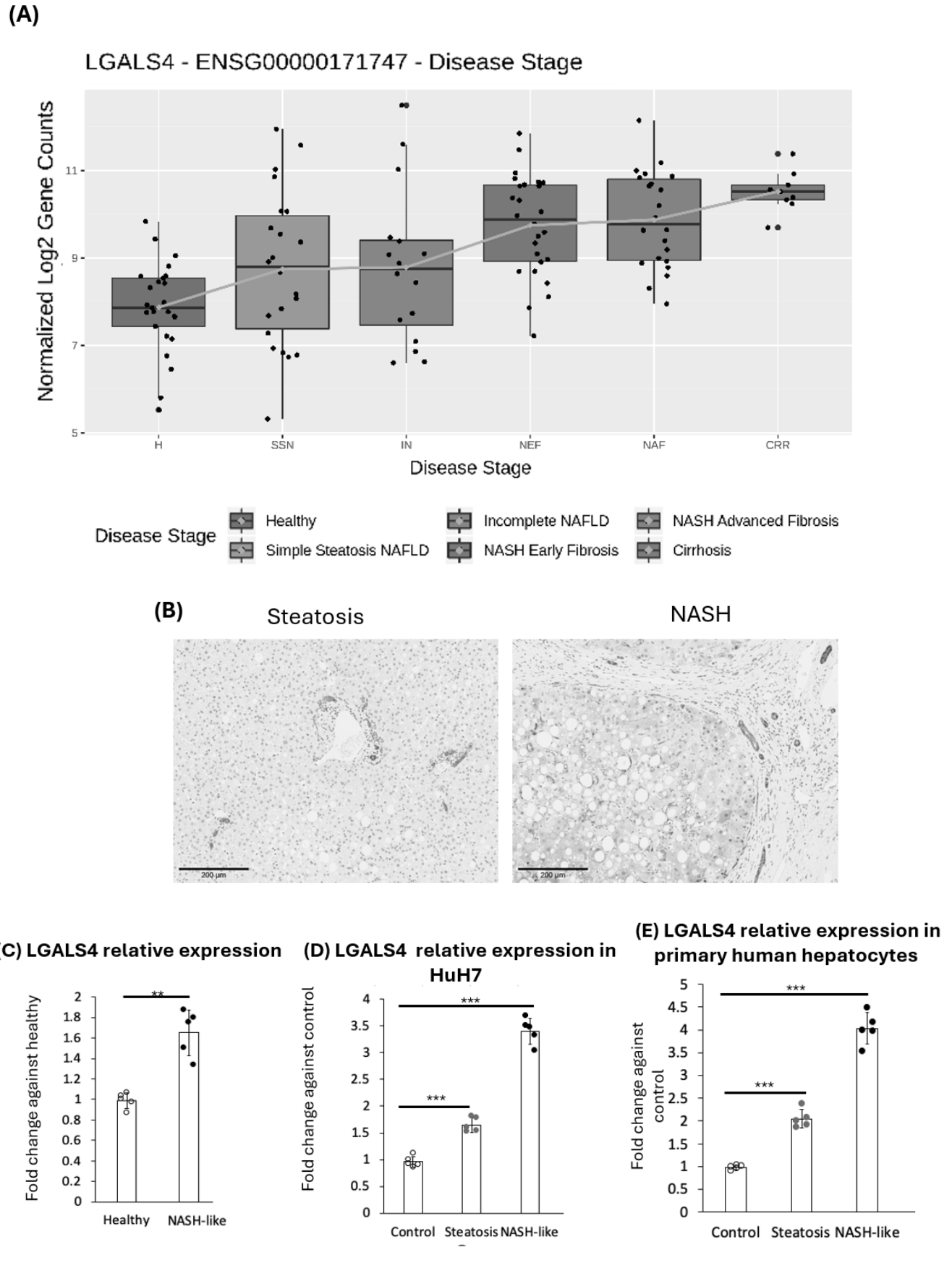
(A)
LGALS4 - ENSG00000171747 - Disease Stage
Normalized Log2 Gene Counts
11
9
7
5
H
SSN
IN
NEF
NAF
CRR
Disease Stage
Disease Stage
Healthy
Simple Steatosis NAFLD
Incomplete NAFLD
NASH Early Fibrosis
NASH Advanced Fibrosis
Cirrhosis
(B)
Steatosis
NASH
200 µm
200 µm
(C) LGALS4 relative expression
Fold change against healthy
**
Healthy
NASH-like
(D) LGALS4 relative expression in HuH7
Fold change against control
***
***
Control
Steatosis
NASH-like
(E) LGALS4 relative expression in primary human hepatocytes
Fold change against control
***
***
Control
Steatosis
NASH-like

Figure 1

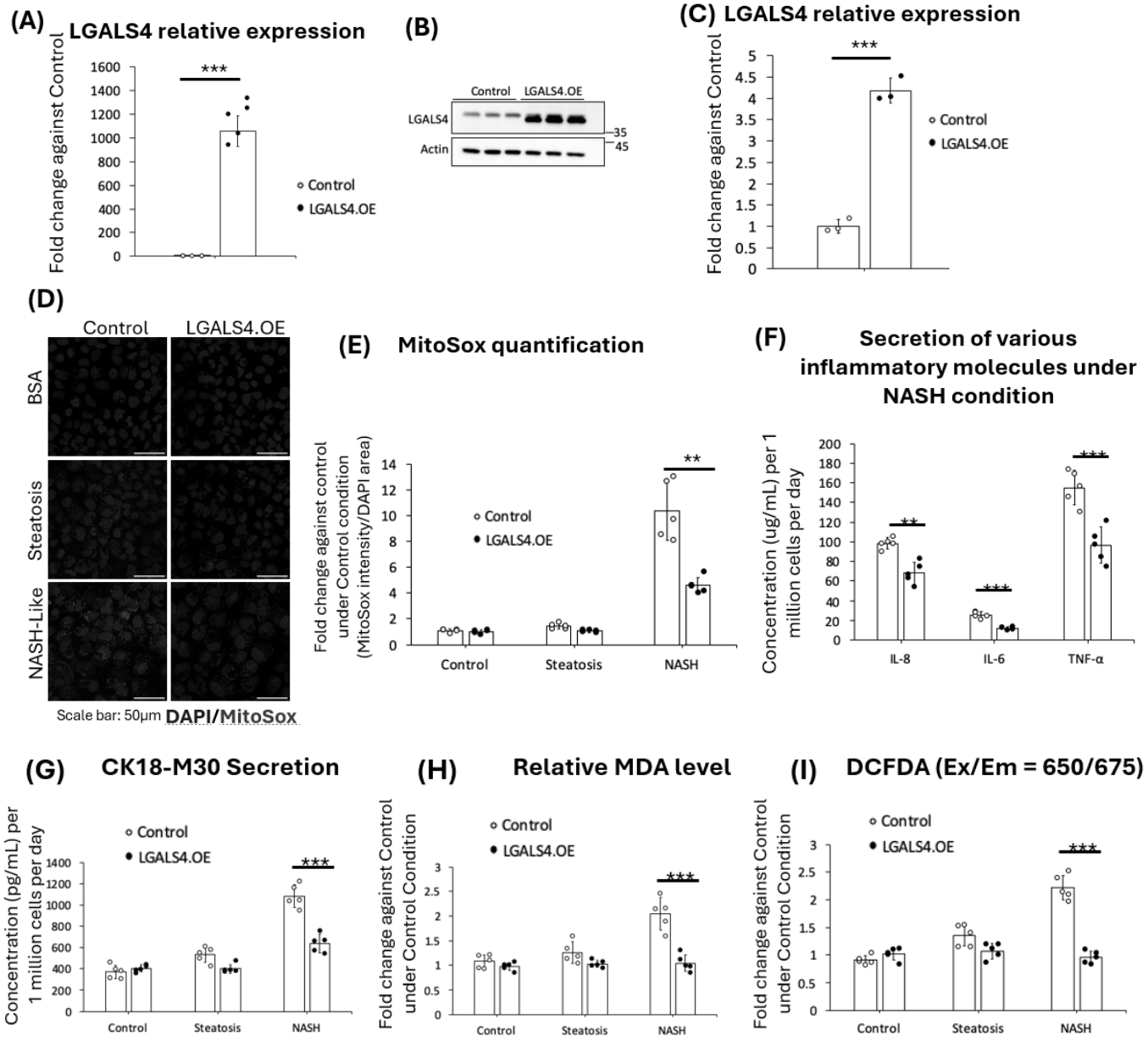
(A)
LGALS4 relative expression
Fold change against Control
***
Control
LGALS4.OE
(B)
Control
LGALS4.OE
LGALS4
Actin
35
45
(C) LGALS4 relative expression
Fold change against Control
***
Control
LGALS4.OE
(D)
Control
LGALS4.OE
BSA
Steatosis
NASH-Like
Scale bar: 50μm DAPI/MitoSox
(E) MitoSox quantification
Fold change against control under Control condition (MitoSox intensity/DAPI area)
**
Control
LGALS4.OE
Control
Steatosis
NASH
(F) Secretion of various inflammatory molecules under NASH condition
Concentration (ug/mL) per 1 million cells per day
**
***
***
IL-8
IL-6
TNF-α
(G) CK18-M30 Secretion
Concentration (pg/mL) per 1 million cells per day
Control
LGALS4.OE
***
Control
Steatosis
NASH
(H) Relative MDA level
Fold change against Control under Control Condition
Control
LGALS4.OE
***
Control
Steatosis
NASH
(I) DCFDA (Ex/Em = 650/675)
Fold change against Control under Control Condition
Control
LGALS4.OE
***
Control
Steatosis
NASH

Figure 2

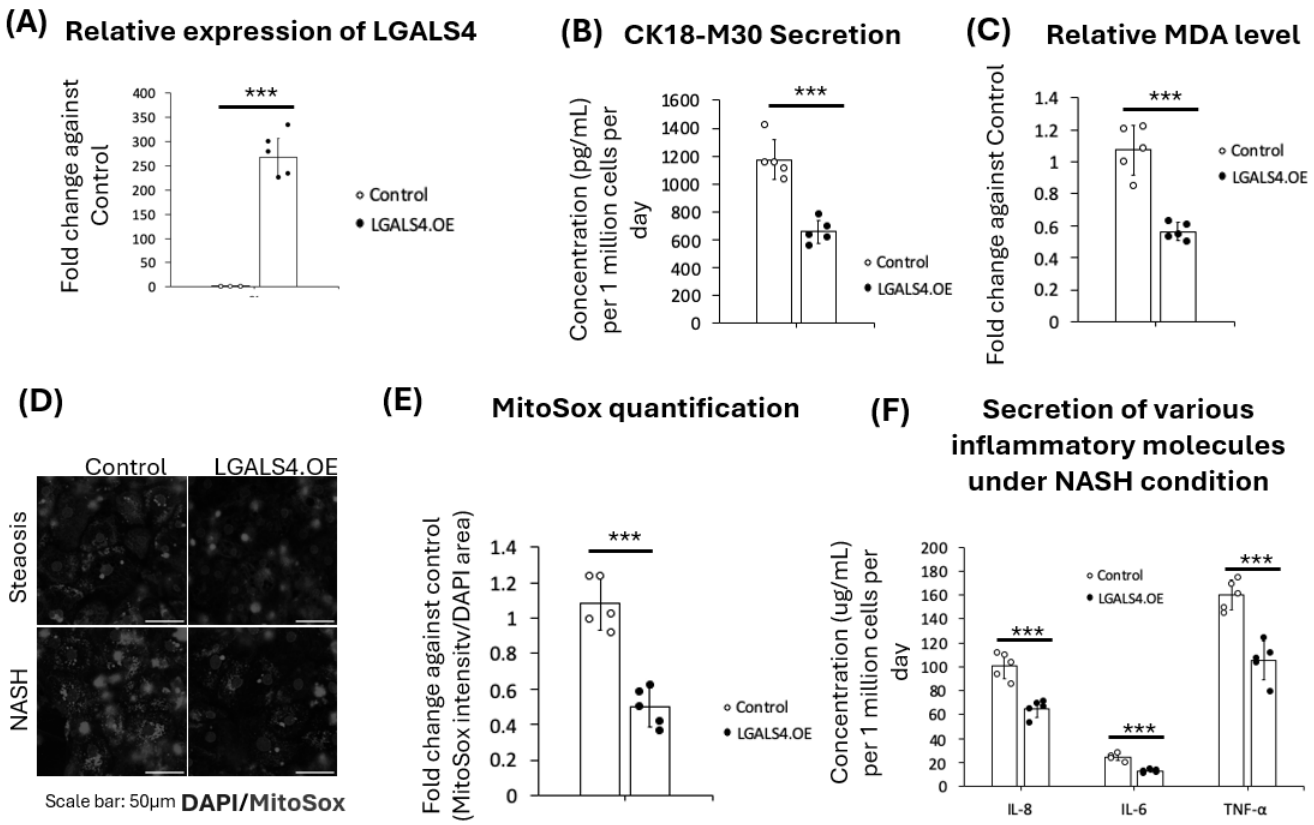
(A)
Relative expression of LGALS4
Fold change against Control
***
Control
LGALS4.OE
(B)
CK18-M30 Secretion
Concentration (pg/mL) per 1 million cells per day
***
Control
LGALS4.OE
(C)
Relative MDA level
Fold change against Control
***
Control
LGALS4.OE
(D)
Control
LGALS4.OE
Steaosis
NASH
Scale bar: 50μm DAPI/MitoSox
(E)
MitoSox quantification
Fold change against control (MitoSox intensity/DAPI area)
***
Control
LGALS4.OE
(F)
Secretion of various inflammatory molecules under NASH condition
Concentration (ug/mL) per 1 million cells per day
Control
LGALS4.OE
***
***
***
IL-8
IL-6
TNF-α

Figure 3

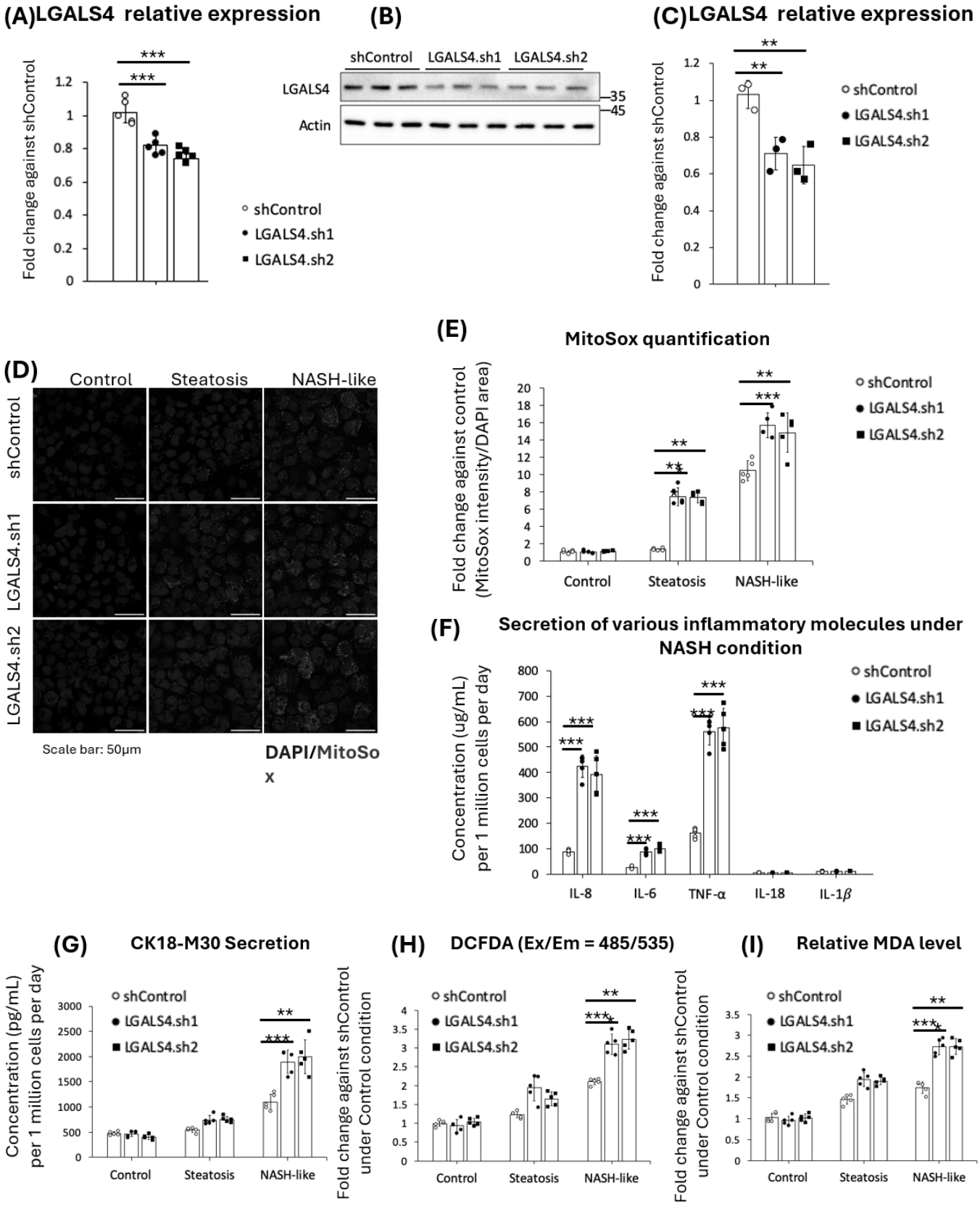
(A) LGALS4 relative expression
Fold change against shControl
shControl
LGALS4.sh1
LGALS4.sh2
(B)
shControl
LGALS4.sh1
LGALS4.sh2
LGALS4
Actin
35
45
(C) LGALS4 relative expression
Fold change against shControl
(D)
Control
Steatosis
NASH-like
shControl
LGALS4.sh1
LGALS4.sh2
Scale bar: 50μm
DAPI/MitoSox
(E)
MitoSox quantification
Fold change against control (MitoSox intensity/DAPI area)
Control
Steatosis
NASH-like
(F)
Secretion of various inflammatory molecules under NASH condition
Concentration (ug/mL) per 1 million cells per day
IL-8
IL-6
TNF-α
IL-18
IL-1β
(G)
CK18-M30 Secretion
Concentration (pg/mL) per 1 million cells per day
Fold change against shControl under Control condition
(H)
DCFDA (Ex/Em = 485/535)
(I)
Relative MDA level
Fold change against shControl under Control condition

Figure 4

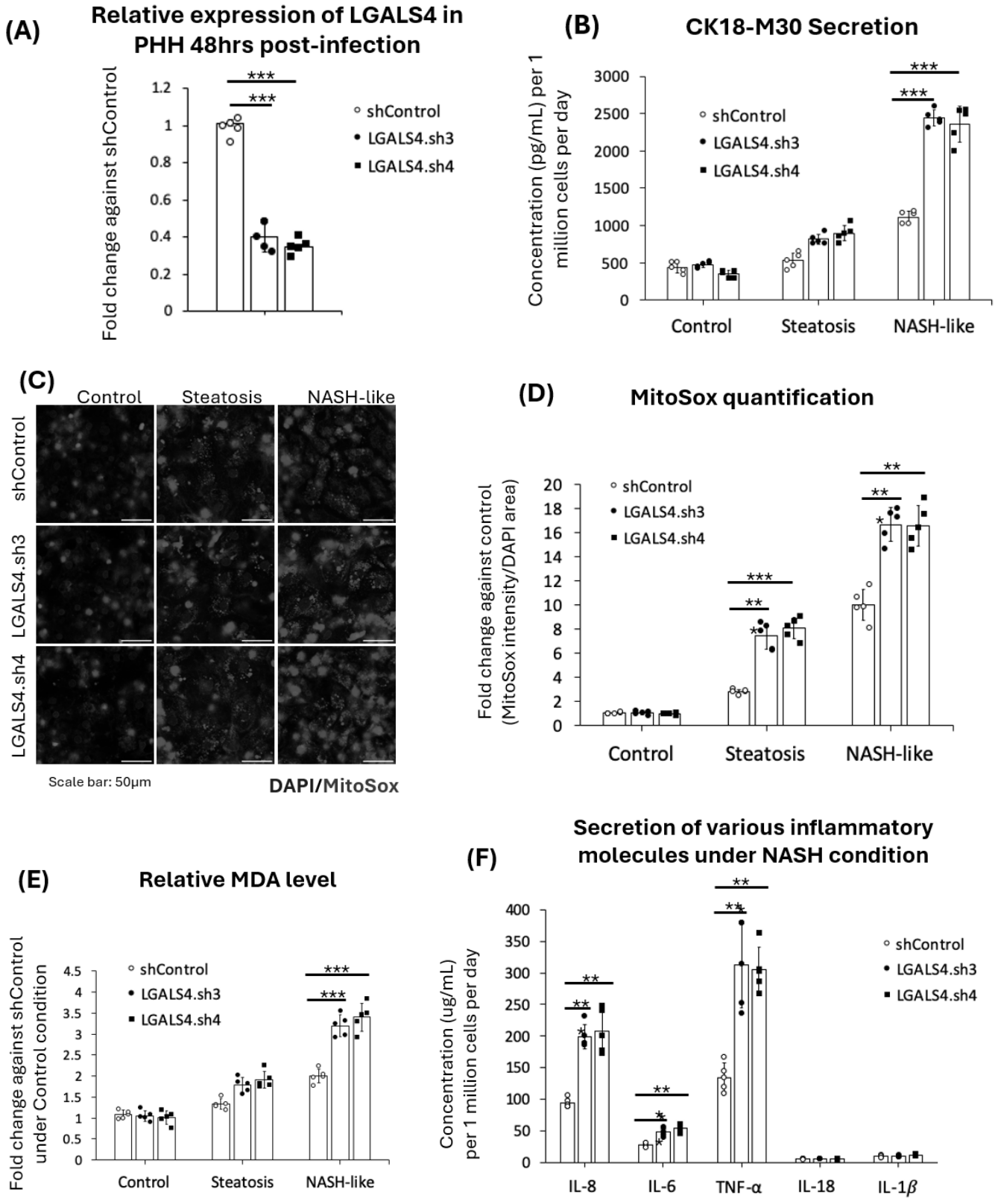
(A)
Relative expression of LGALS4 in PHH 48hrs post-infection
Fold change against shControl
shControl
LGALS4.sh3
LGALS4.sh4
(B)
CK18-M30 Secretion
Concentration (pg/mL) per 1 million cells per day
Control
Steatosis
NASH-like
(C)
Control
Steatosis
NASH-like
shControl
LGALS4.sh3
LGALS4.sh4
Scale bar: 50μm
DAPI/MitoSox
(D)
MitoSox quantification
Fold change against control (MitoSox intensity/DAPI area)
(E)
Relative MDA level
Fold change against shControl under Control condition
(F)
Secretion of various inflammatory molecules under NASH condition
Concentration (ug/mL) per 1 million cells per day
IL-8
IL-6
TNF-α
IL-18
IL-1β

Figure 5

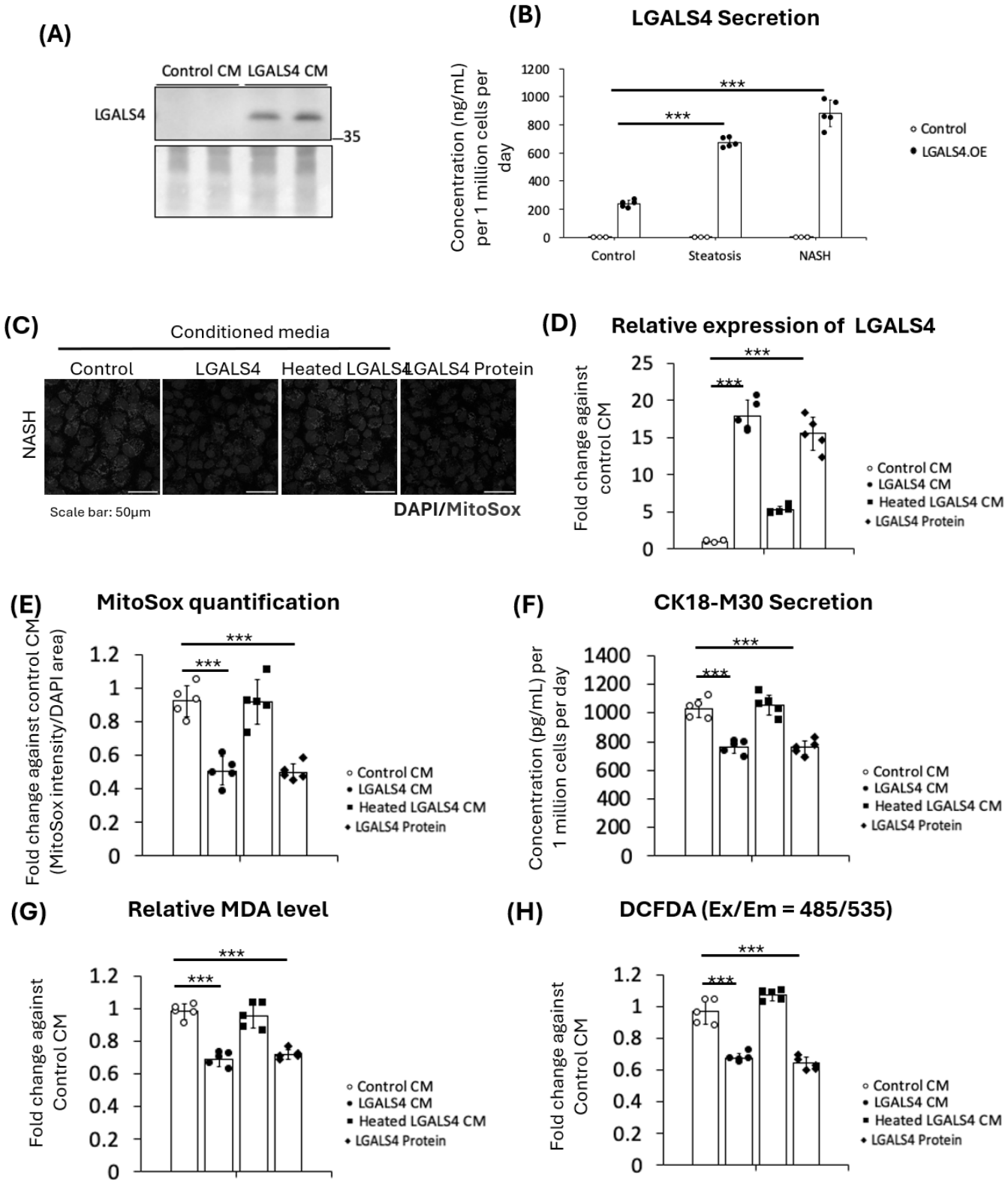
(A)
Control CM
LGALS4 CM
LGALS4
35
(B)
LGALS4 Secretion
Concentration (ng/mL) per 1 million cells per day
1200
1000
800
600
400
200
0
***
***
Control
Steatosis
NASH
Control
LGALS4.OE
(C)
Conditioned media
Control
LGALS4
Heated LGALS4
LGALS4 Protein
NASH
Scale bar: 50μm
DAPI/MitoSox
(D)
Relative expression of LGALS4
Fold change against control CM
25
20
15
10
5
0
***
***
Control CM
LGALS4 CM
Heated LGALS4 CM
LGALS4 Protein
(E)
MitoSox quantification
Fold change against control CM (MitoSox intensity/DAPI area)
1.2
1
0.8
0.6
0.4
0.2
0
***
***
Control CM
LGALS4 CM
Heated LGALS4 CM
LGALS4 Protein
(F)
CK18-M30 Secretion
Concentration (pg/mL) per 1 million cells per day
1400
1200
1000
800
600
400
200
0
***
***
Control CM
LGALS4 CM
Heated LGALS4 CM
LGALS4 Protein
(G)
Relative MDA level
Fold change against Control CM
1.2
1
0.8
0.6
0.4
0.2
0
***
***
Control CM
LGALS4 CM
Heated LGALS4 CM
LGALS4 Protein
(H)
DCFDA (Ex/Em = 485/535)
Fold change against Control CM
1.2
1
0.8
0.6
0.4
0.2
0
***
***
Control CM
LGALS4 CM
Heated LGALS4 CM
LGALS4 Protein

Figure 6

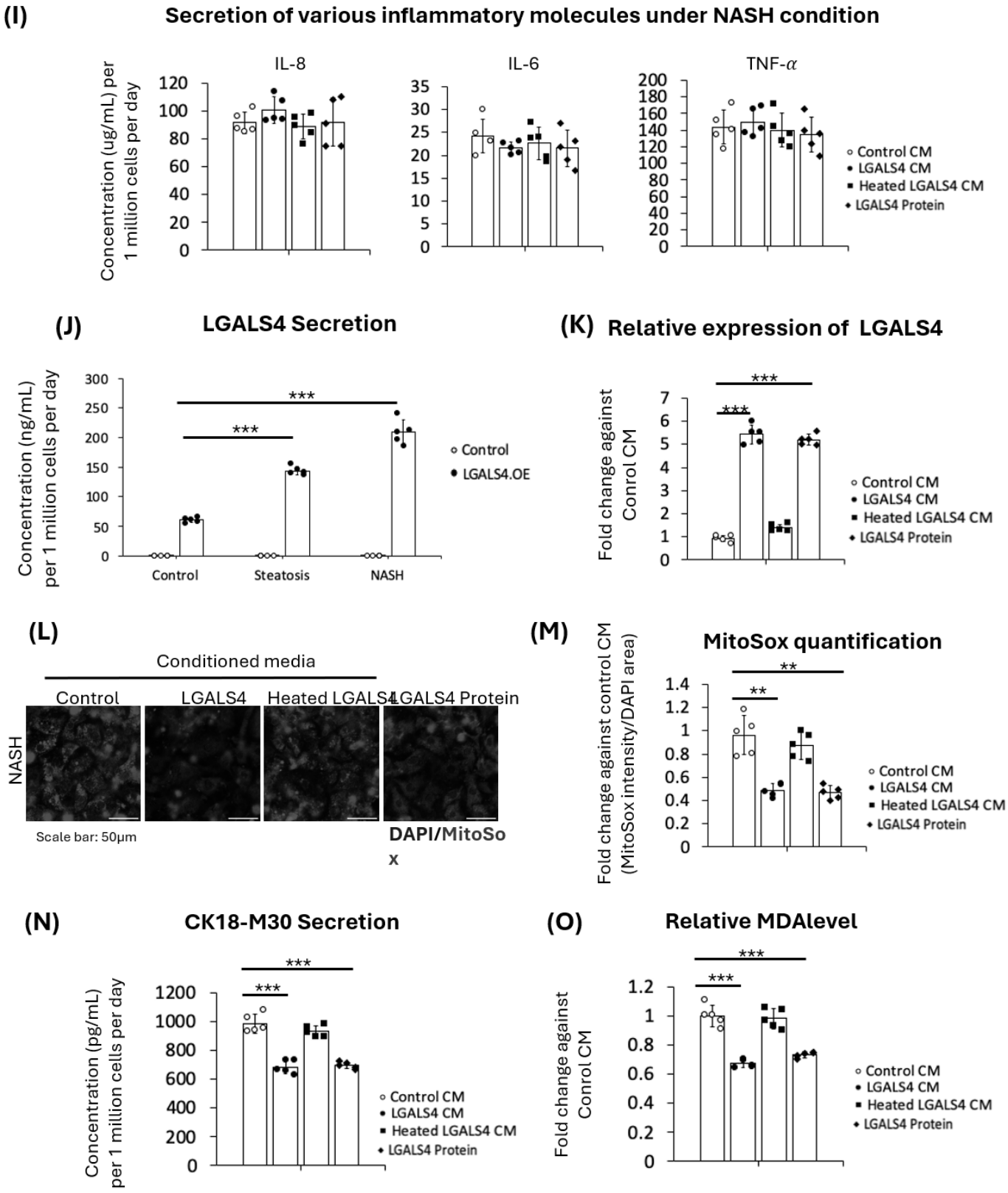

Figure 6 (continued)

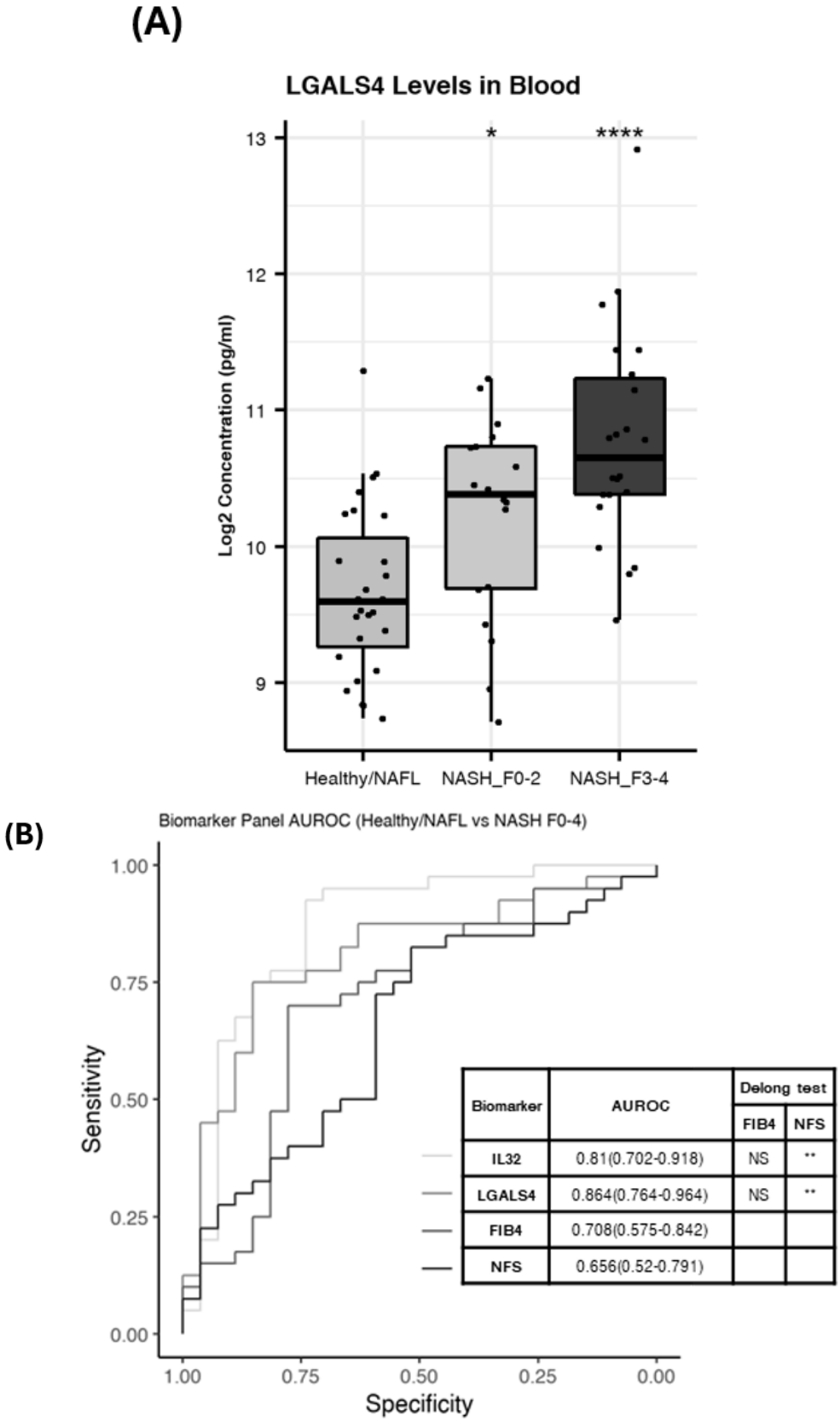
(A)
LGALS4 Levels in Blood
Log2 Concentration (pg/ml)
13
12
11
10
9
*
****
Healthy/NAFL
NASH_F0-2
NASH_F3-4
(B)
Biomarker Panel AUROC (Healthy/NAFL vs NASH F0-4)
Sensitivity
Specificity
1.00
0.75
0.50
0.25
0.00
Biomarker
AUROC
Delong test
FIB4
NFS
IL32
0.81(0.702-0.918)
NS
**
LGALS4
0.864(0.764-0.964)
NS
**
FIB4
0.708(0.575-0.842)
NFS
0.656(0.52-0.791)

Figure 7

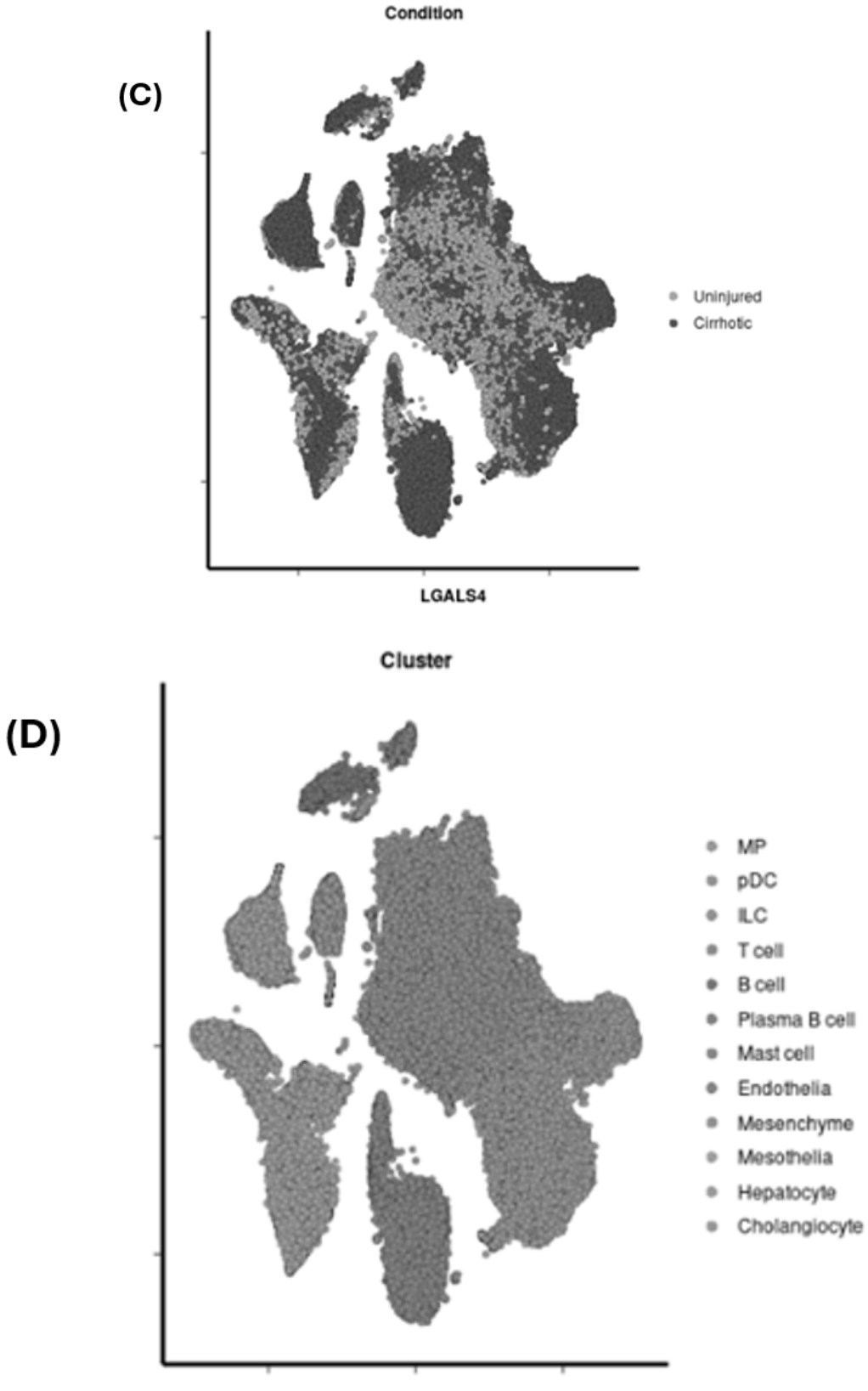

Figure 7 (continued)

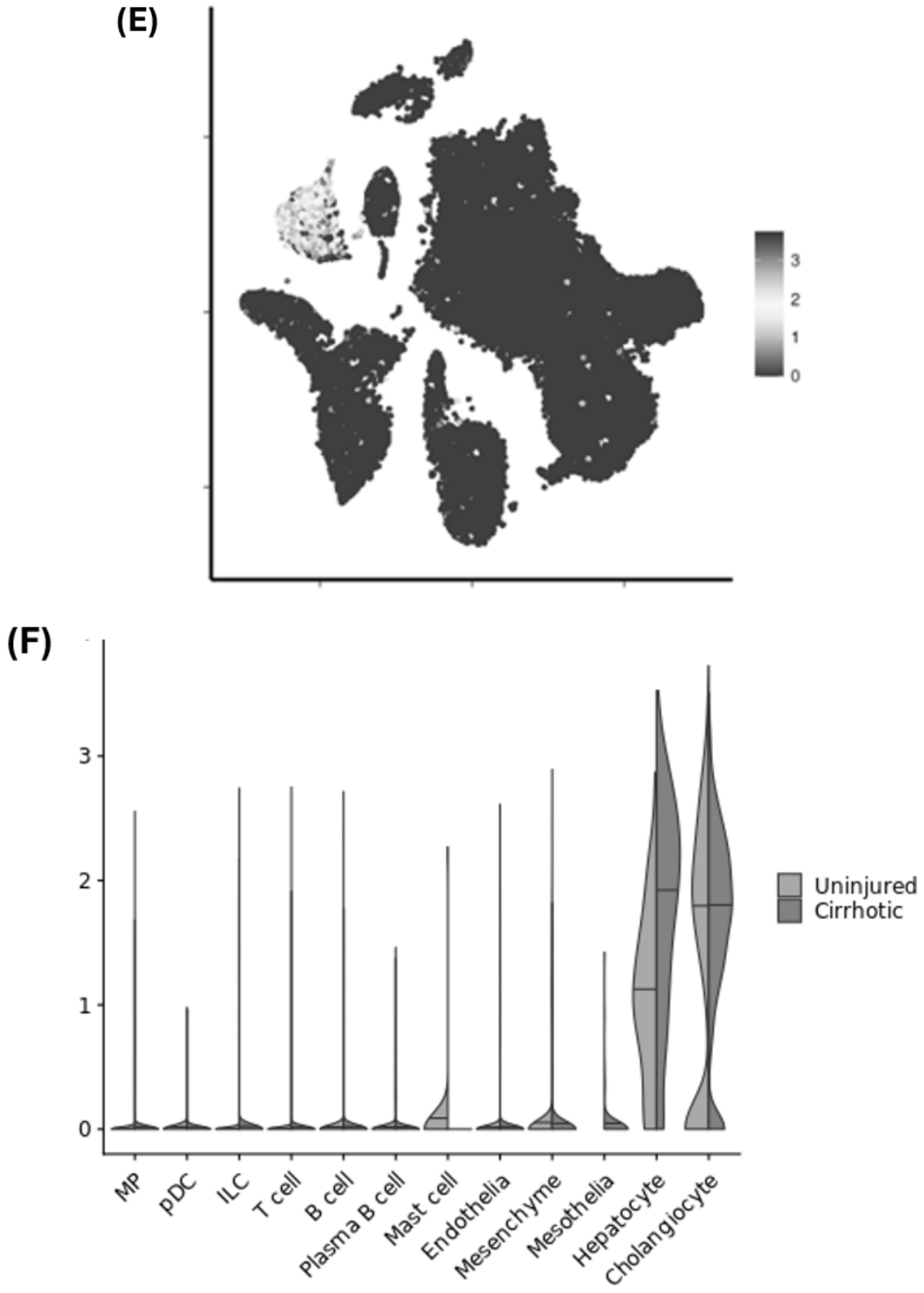

Figure 7 (continued)

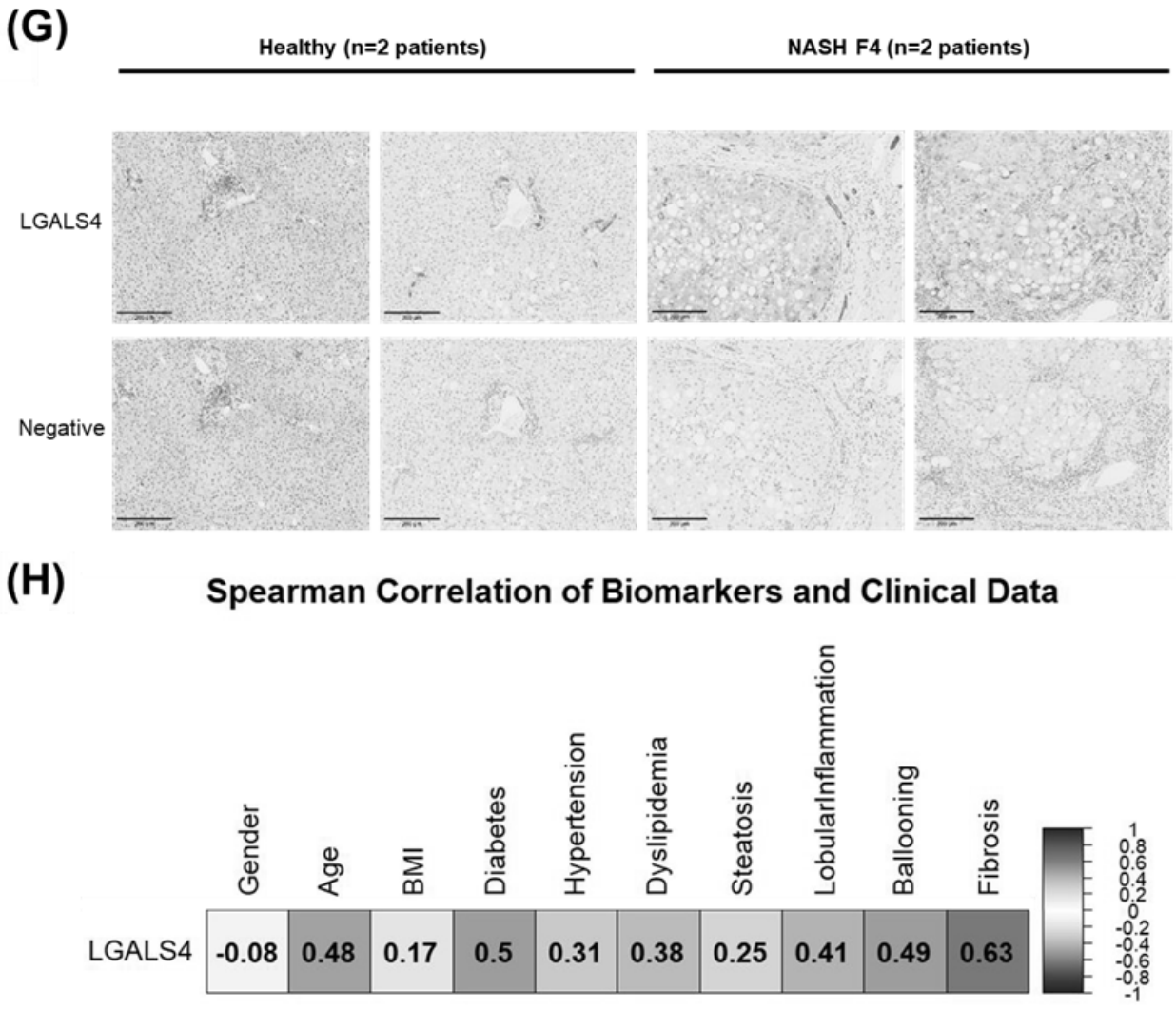

Figure 7 (continued)