1 <p align="center">**Supplementary Materials**</p>

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3 **MASLD-mimicking microenvironment drives an aggressive phenotype and**

4 **represses IDH2 expression in hepatocellular carcinoma**

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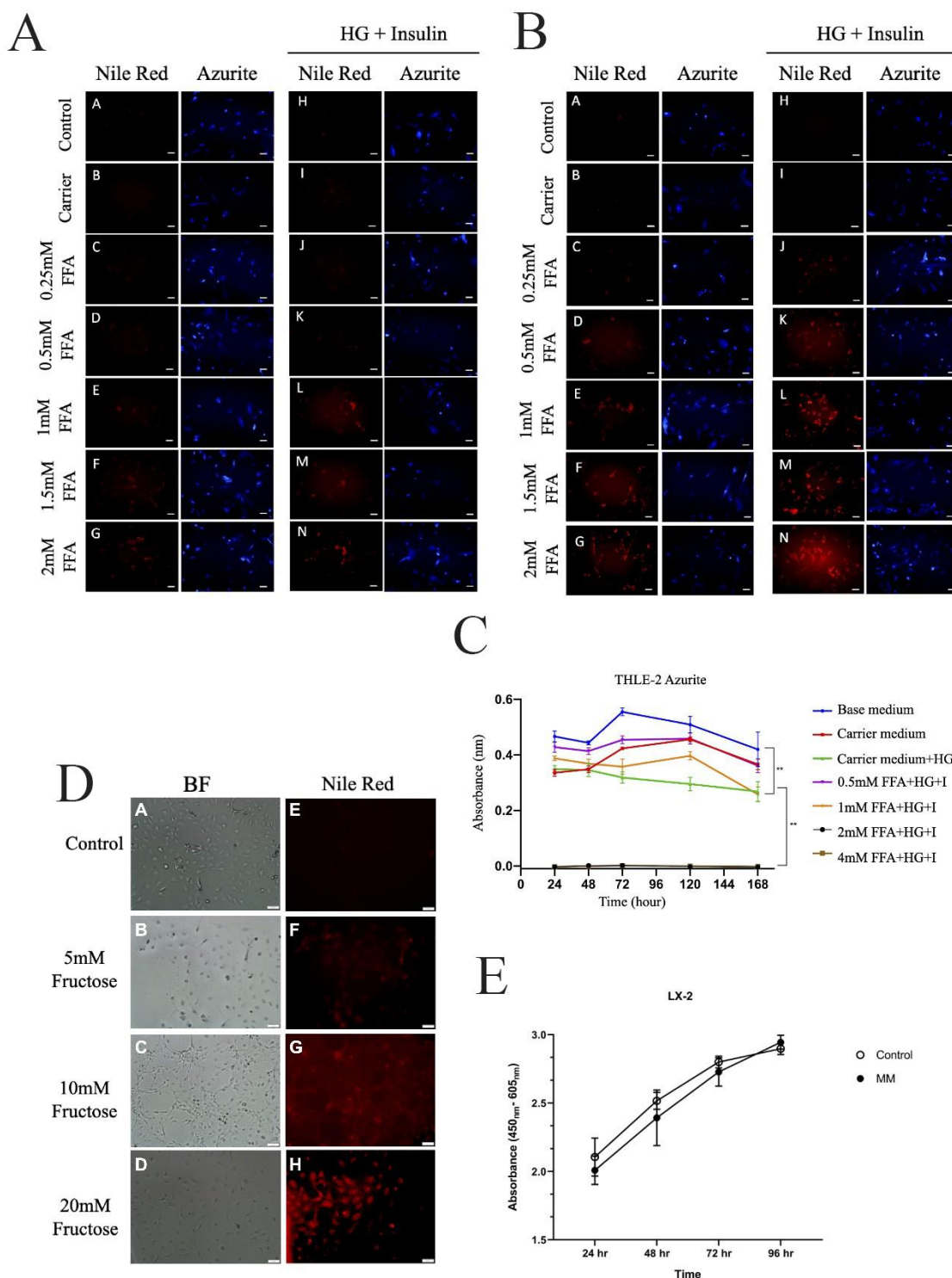
18 35100, Turkey.

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Supplementary Figure 1. Designing and optimization of the composition of a
metabolic medium. (A and B) Accumulation of fat droplets within the cells with
different concentrations of FFA, and BSA. Represent images taken from conditions
with increasing ratio of FFA prepared in 1.25% BSA (A), represent images taken from
conditions with increasing ratio of FFA prepared in 2.5% BSA (B); (C) Testing
cytotoxic effects of FFA and auxiliary components (BSA, ethanol, insulin, glucose)

33   using MTT assay in THLE2 normal hepatocyte cell line; (D) Effect of various fructose

34   concentrations on lipid droplet biogenesis in LX-2 cells; (E) Proliferation and cell

35   viability assay of LX-2 cells treated with control medium or MM. Scale bar: 50um**.**

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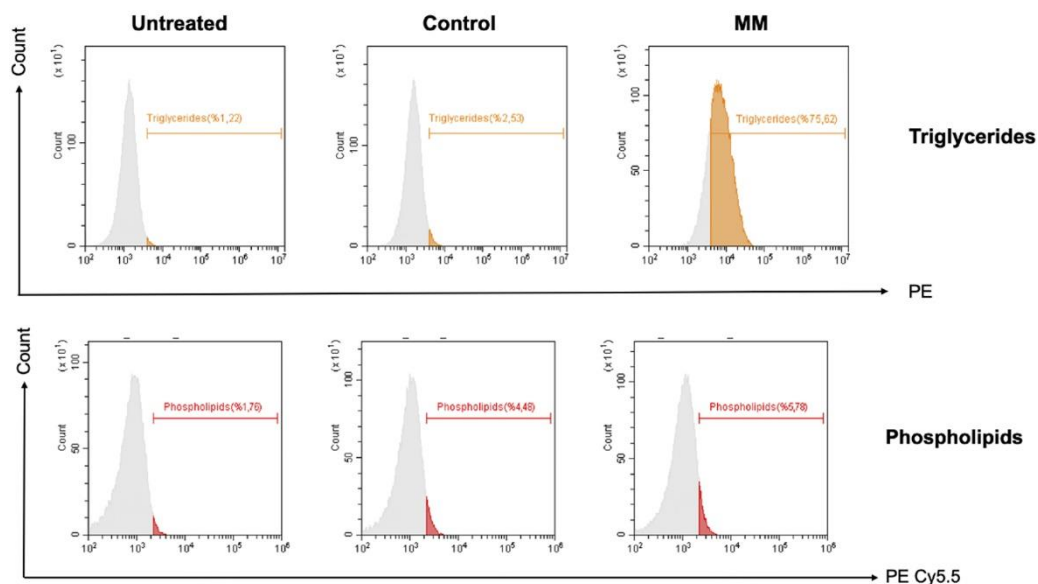
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38   **Supplementary Figure 2.** Nile Red staining in LX-2 cells treated with control

39   condition or MM for 24 h assessed by flow cytometry. At 24 h post MM or control

40   treatment, cells were collected, fixed in 2% PFA for 20 min at room temperature and

41   stained with Nile Red (1:200) for 10 min at 37°C (dark). Cells were acquired on

42   CytoFlex equipped with a blue laser and 4 channels (Beckman-Coulter). Nile Red

43   staining on the cells was detected using PE (585/42 BP) channel for triglycerides and

44   PE Cy5.5 (690/50 BP) channel for phospholipids ($n = 3$).

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46   **Supplementary Table 1. Primer sequences used in RT-qPCR experiments**

Gene	F/R	5' > 3'
RPL41	F	GAAACCTCTGCGCCATGA
	R	TCTTTCTTCTTTTGCGCTTCA
IDH2	F	TCGTGCGCTCGCTCTG
	R	TCCTTTTGTCGGCATAGTGG
aSMA	F	AAAGCAAGTCCTCCAGCGTT
	R	TTAGTCCCGGGGATAGGCAA
CXCR 4	F	GGTGGTCTATGTTGGCGTCT
	R	ACACAACCACCCACAAGTCA

FAS	F	CAGGCACACACGATGGAC
	R	CGGAGTGAATCTGGGTTGAT
IL1B	F	GGCCACATTTGGTTCTAAGAAA
	R	TAAATAGGGAAGCGGTTGCTC
TGFB1		QuantiTect Primer Assay (Qiagen) for TGFβ (QT00000728)

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