

Supplementary file

Tethering Interleukin-22 to Apolipoprotein A-I Ameliorates Mice from Acetaminophen-induced Liver Injury

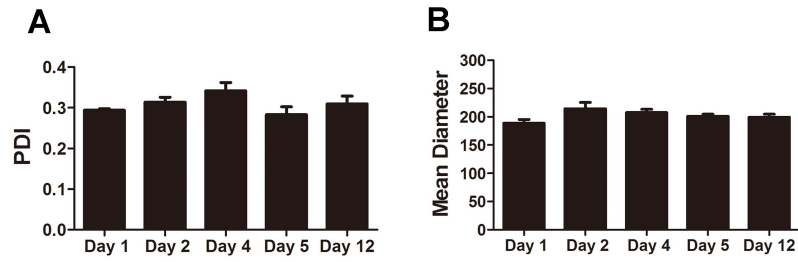
Wei Chen^{†,1}, Xuyao Zhang^{†,1}, Jiajun Fan^{†,1}, Wenjing Zai¹, Jingyun Luan¹, Yubin Li¹,
Shaofei Wang¹, Qicheng Chen¹, Yichen Wang¹, Yanxu Liang¹, Dianwen Ju^{*,1}

¹Department of Microbiological and Biochemical Pharmacy & The Key Laboratory of
Smart Drug Delivery, Ministry of Education, School of Pharmacy, Fudan University,
826 Zhangheng Road, Shanghai, 201203, P. R. China.

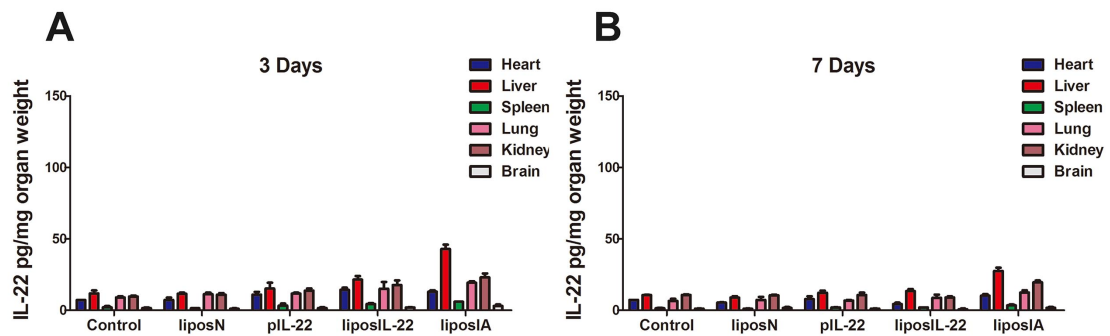
[†]These authors contributed equally to this work.

*Correspondence to: Dianwen Ju, Tel: +86 21 5198 0037; Fax: +86 21 5198 0036;

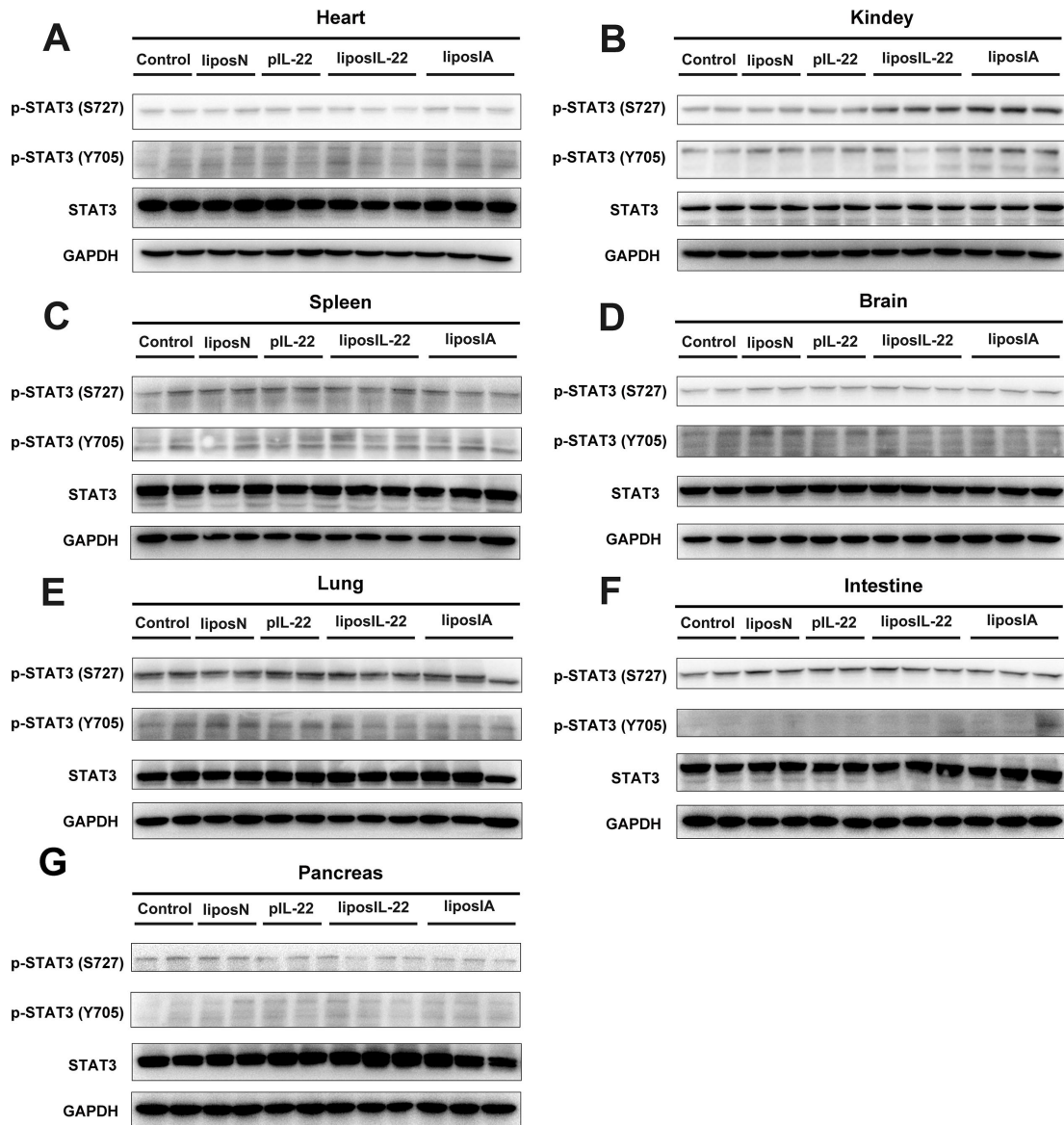
E-mail: dianwenju@fudan.edu.cn



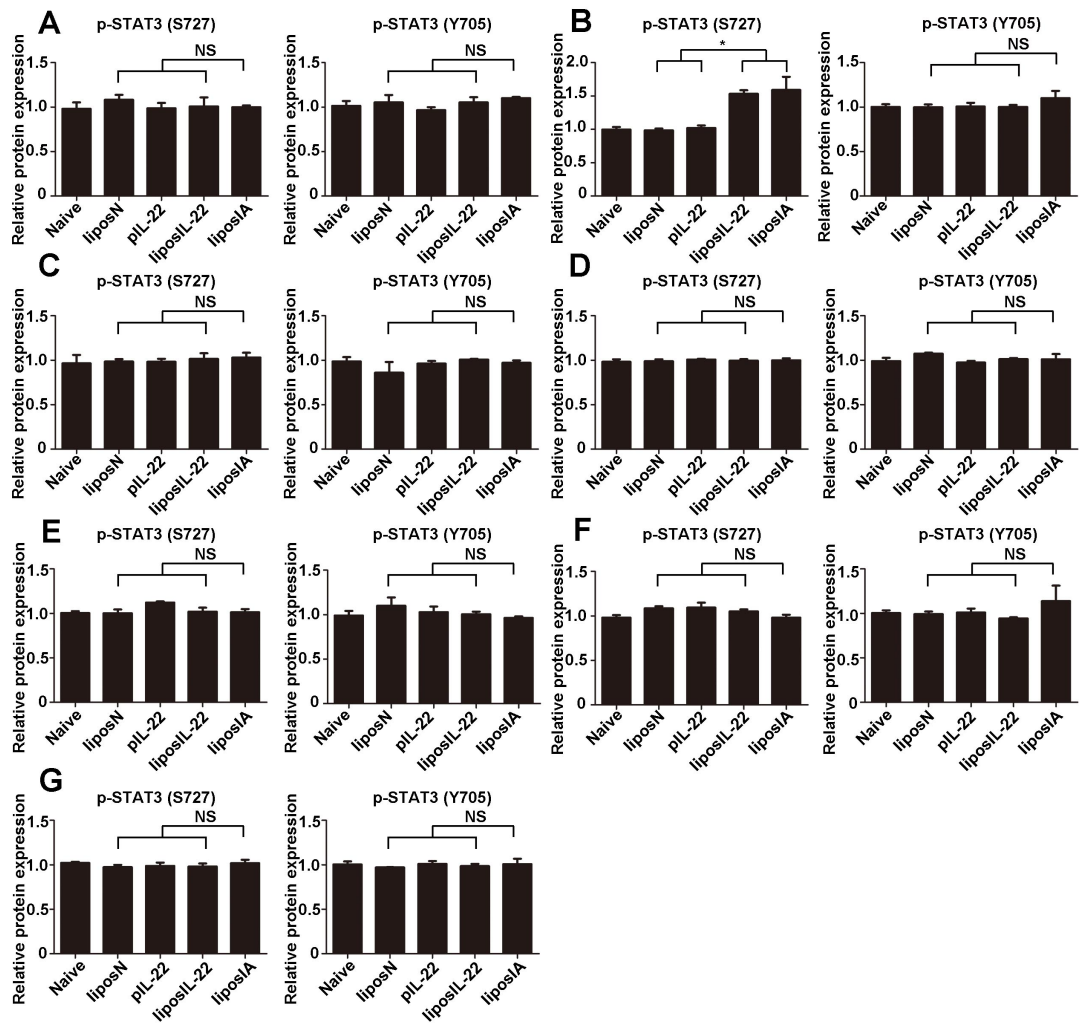
Supplementary figure S1. LiposIA were stored at 4°C for extended periods of time. After a minimum of 12 d of storage, no statistically significant changes in polydisperse index value (**A**) and particle size (**B**) were calculated using a t-test compared with the initial particle size, indicating the particles remained stable. Error bars \pm SD and $N = 3$.



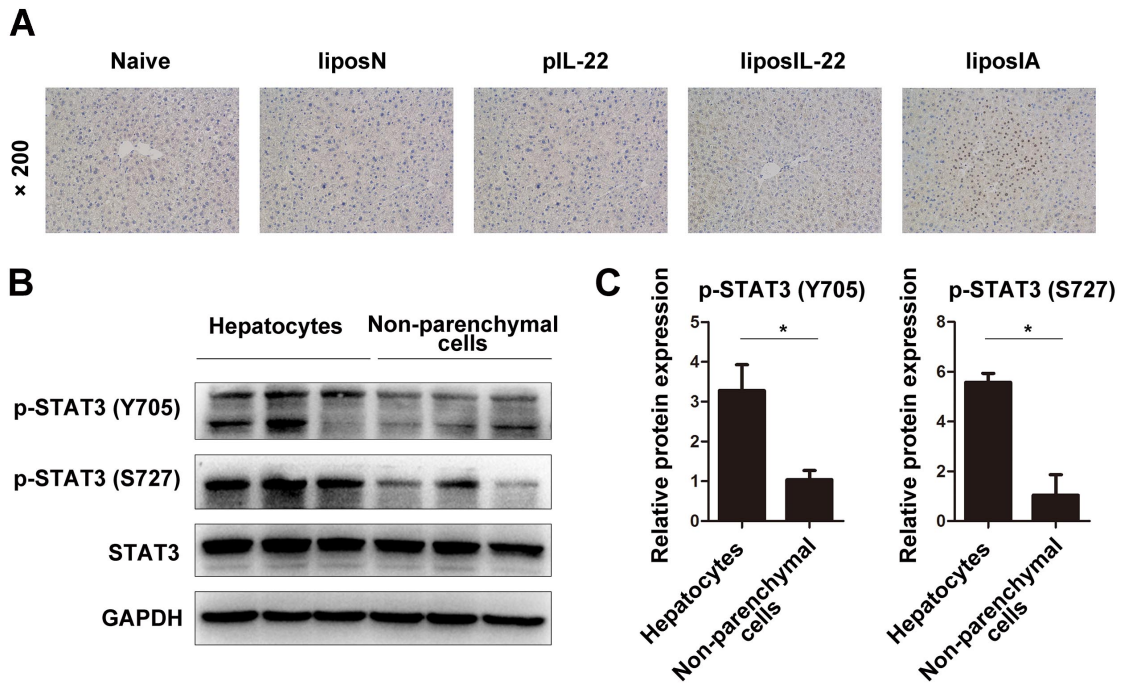
Supplementary figure S2. (**A**, **B**) IL-22 concentrations measured by ELISA in selected organs (heart, liver, lungs, spleen, kidney and brain) 3 or 7 days after a single dose injection with 0.5 mg of liposIA, liposIL-22, liposN or PBS (Control) ($N = 5$; mean \pm SD).



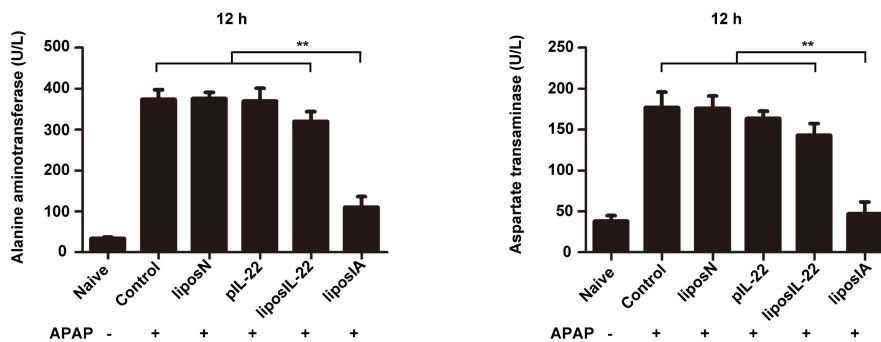
Supplementary figure S3. (A-G) Signaling transductions activated by liposIA were analyzed by western blot against phosphorylated and total protein levels of STAT3 in selected organs (heart, kidney, spleen, brain, lung, intestine and pancreas) 2 days after a single dose injection with 0.5 mg of liposIA, liposIL-22, pIL-22, liposN or PBS (Control).



Supplementary figure S4. Densitometric values of p-STAT3 (A, heart; B, kidney; C, spleen; D, brain; E, lung; F, intestine; G, pancreas) were quantified and normalized to control ($n = 3$; mean \pm SD; * $P < 0.05$, ** $P < 0.01$). The values of control were set to 1.

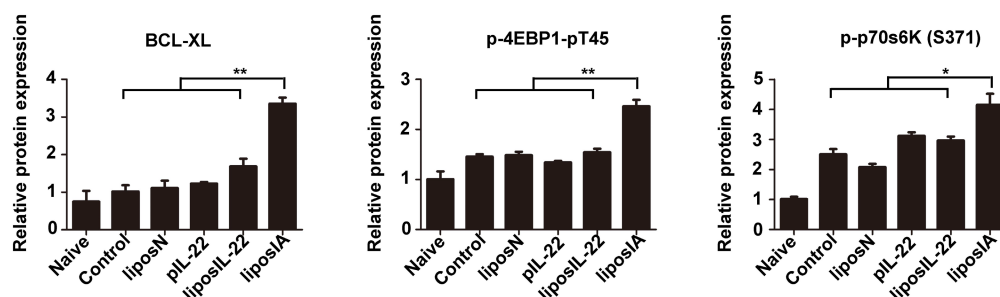


Supplementary figure S5. Hepatocytes, not non-parenchymal cells, are the major target cells. (A) Representative liver immunohistochemistry (p-STAT3) after a single dose injection with liposIA, liposIL-22, pIL-22 and liposN at 2 days. (B) Signaling transductions activated by liposIA were analyzed by western blot. (C) Densitometric values of p-STAT3 were quantified and normalized to control ($n = 3$; mean \pm SD; $*P < 0.05$). The values of non-parenchymal cells were set to 1.



Supplementary figure S6. C57BL/6 mice were injected with 0.5 mg of liposIA, lipoIL-22, pIL-22, liposN, PBS (control), followed 2 days later by intraperitoneal

injection 500 mg/kg of APAP. Serum was collected at 12 h for determination of ALT, AST levels (**A, B**) ($N = 5$; mean \pm SD; ** $p < 0.01$, *** $p < 0.001$).



Supplementary figure S7. C57BL/6 mice were intramuscularly injected with 0.5 mg of liposIA, lipoIL-22, pIL-22, liposN, PBS (control), and challenged 2 days later with an injury dose of acetaminophen (APAP). Naive mice as blank control group. Mice were then killed 24 h post APAP injection, total liver extracts were prepared and subjected to western blot analysis with various antibodies. Densitometric values were quantified and normalized to naive group. The values of naive were set to 1.