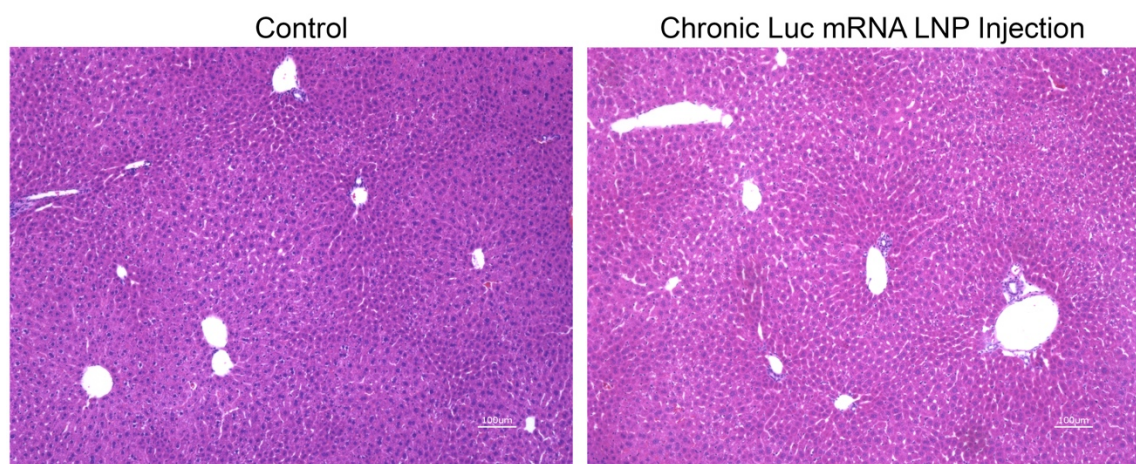


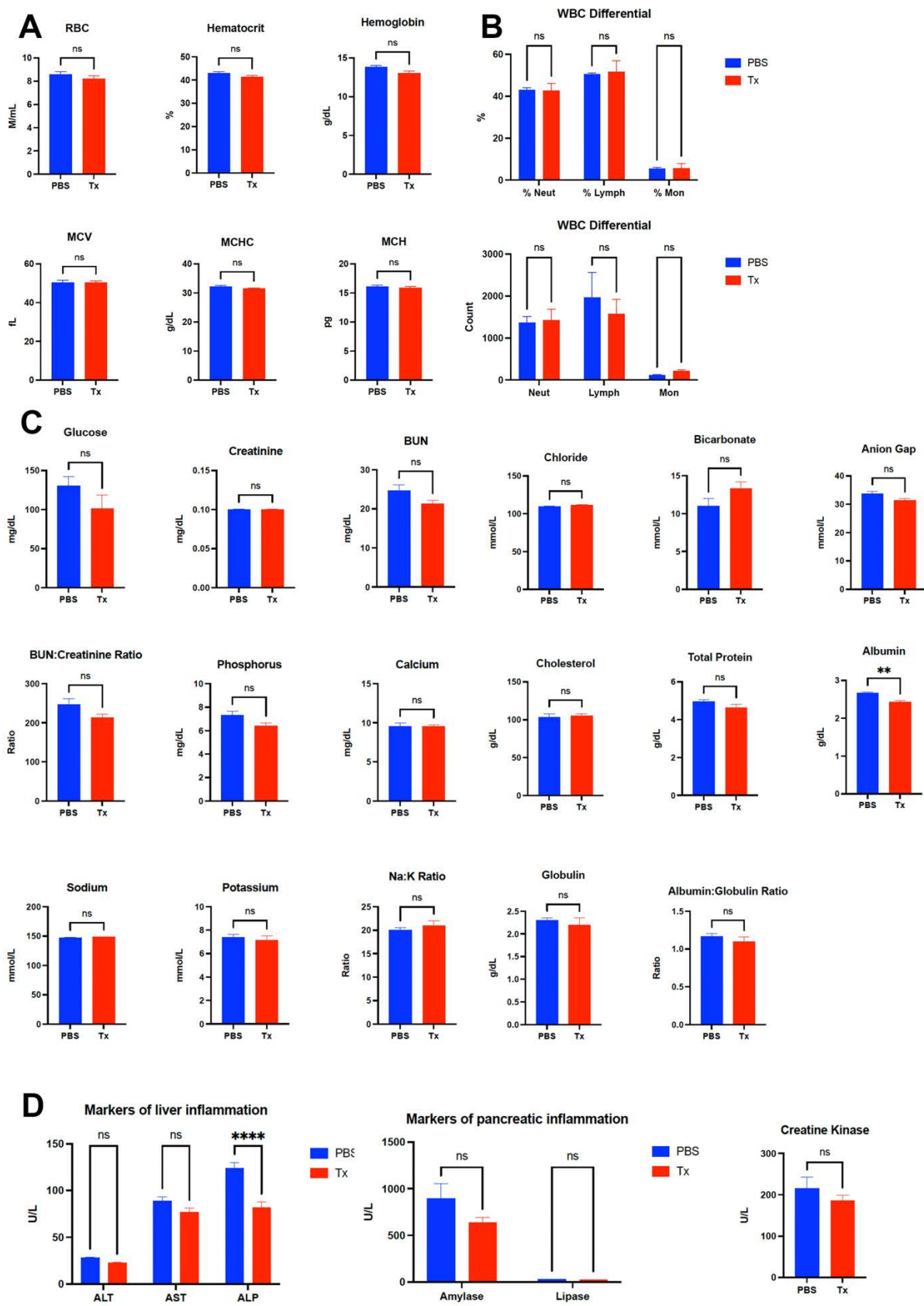
## Supporting Information

**Nanoparticle-Based Follistatin Messenger RNA Therapy for Reprogramming Metastatic Ovarian Cancer and Ameliorating Cancer-Associated Cachexia**

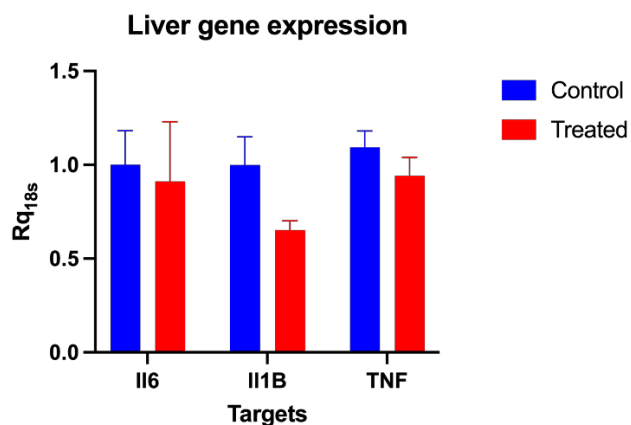
Tetiana Korzun, Abraham S. Moses, Jeonghwan Kim, Siddharth Patel, Canan Schumann, Peter R. Levasseur, Parham Diba, Brennan Olson, Katia Graziella De Oliveira Rebola, Mason Norgard, Youngrong Park, Ananiya A. Demessie, Yulia Eygeris, Subisha Sundaram, Vladislav Grigoriev, Tanja Pejovic, Jonathan R. Brody, Olena R. Taratula, Xinxia Zhu, Gaurav Sahay, Daniel L. Marks\*, Oleh Taratula\*



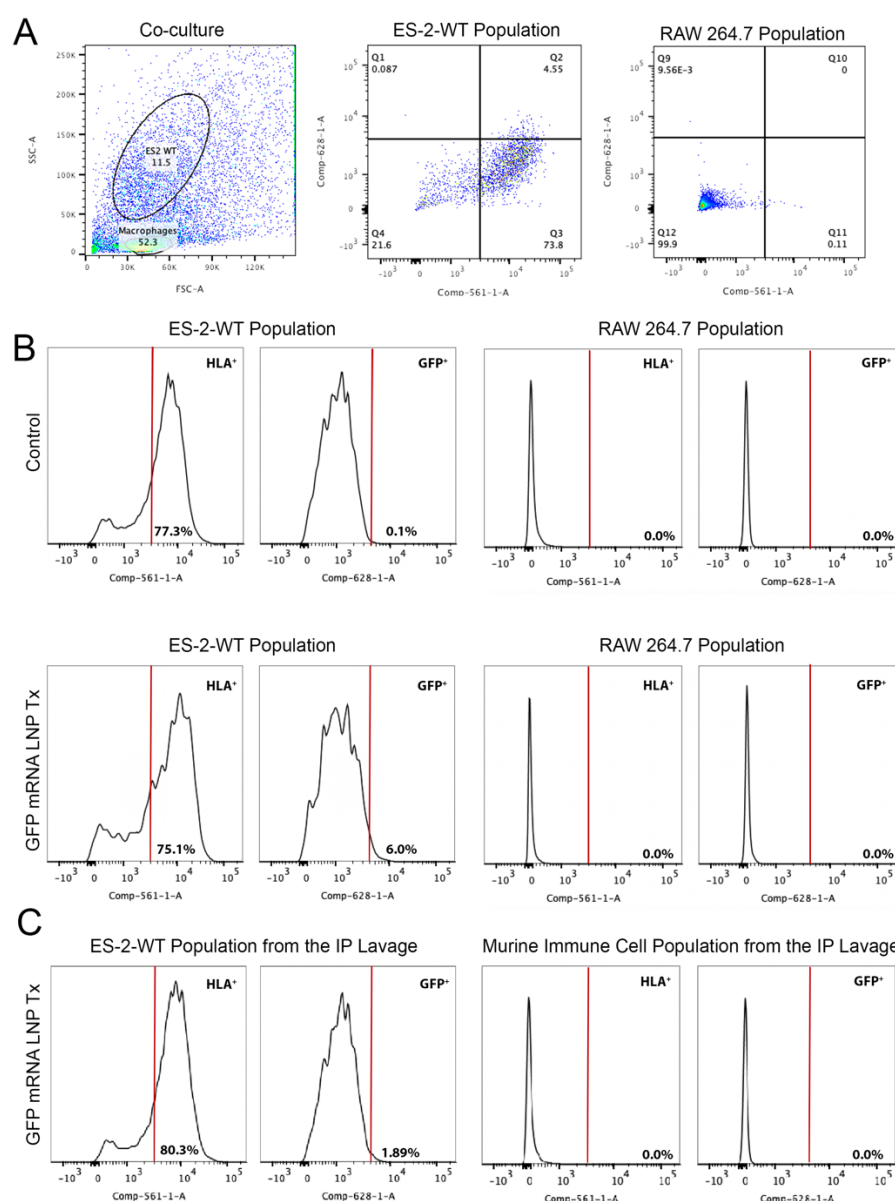
**Figure S1:** Representative histopathological examination of murine liver sections after chronic injection of Luc mRNA LNPs.



**Figure S2:** Blood analysis for evaluation of systemic toxicity of chronically injected Luc mRNA LNPs. (A) erythrocytes and (B) leukocytes evaluation; (C) blood chemistry; (D) markers of liver and pancreatic inflammation.

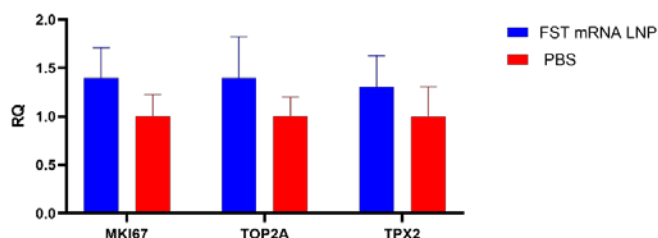


**Figure S3:** Expression of hepatic inflammatory phase reactants in mice receiving 3 daily injections of *Luc* mRNA LNPs.

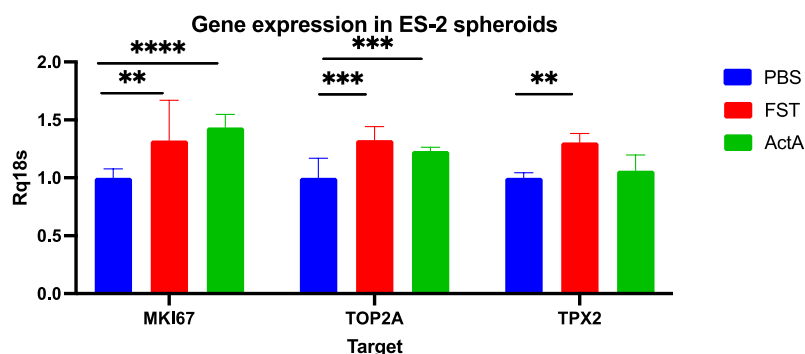


**Figure S4:** Proportion and type of cells internalizing GFP mRNA LNPs and expressing GFP. (A) Flow cytometry analysis and gating approach in evaluating in vitro and ex vivo samples, Comp – 561- 1 - A – anti-HLA-ABC antibody; Comp -628 – 1 - A – anti-GFP antibody. (B)

Analysis of in vitro subculture of ES-2-WT and Raw 264.7 cells (3:1), where controls were treated with PBS and GFP mRNA LNP Tx samples were treated with GFP mRNA LNP formulation. (C) ES-2-WT-bearing mice were treated with GFP mRNA LNPs (2ug mRNA per mouse), control mice were injected with PBS. Collected IP lavage fluid containing mixture of ES-2-WT cells and murine immune cells was subjected to flow cytometry analysis.

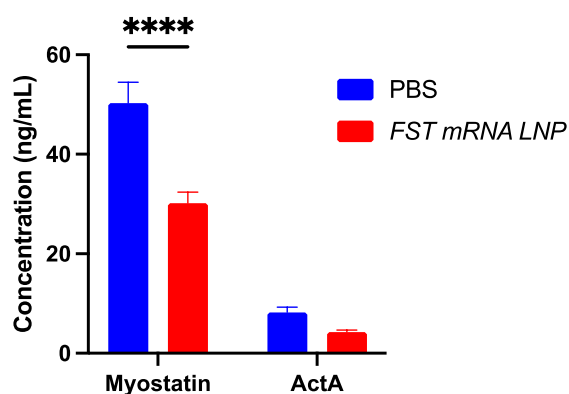


**Figure S5:** Proliferation markers in ES-2 cell clusters from treatment and control groups.

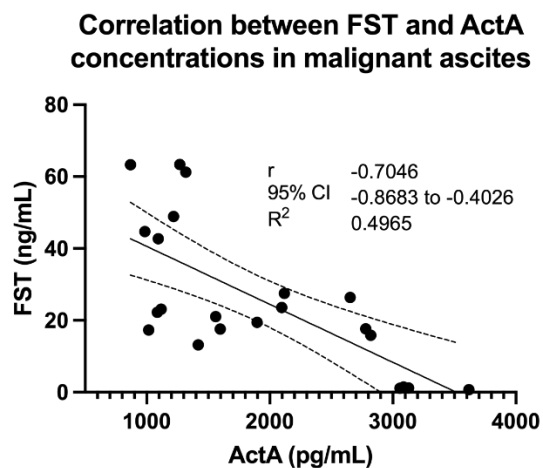


**Figure S6:** In vitro evaluation of gene expression from ES-2-WT spheroids.

**TGF-B family proteins concentration in IP lavage**



**Figure S7:** Myostatin and ActA concentration in ES-2-WT malignant ascites from peritoneal cavity collected from control and FST mRNA LNP-treated mice.



**Figure S8:** Correlation between FST and ActA concentrations from pooled samples of healthy and ES-2-WT-bearing mice, as well as mice treated with FST mRNA LNP formulation.

**Table S1:** Dynamic Light Scattering Properties of Assembled mRNA LNP platforms

LNP	Dynamic Light Scattering		
	$d$ (nm)	$PDI$	$\zeta$ (mV)
<i>Luc</i> mRNA LNP	84.15	0.148	9.07
<i>EGFP</i> mRNA LNP	102.00	0.207	-2.38
<i>FST</i> mRNA LNP	84.57	0.065	-7.78
<i>EGFP/FST</i> mRNA LNP	86.55	0.217	3.04
Empty LNP	72.22	0.116	1.55

**Table S2:** Estimated mean survival for evaluation of combinatorial treatment with CDDP and FST or *FST* mRNA LNPs

Treatment Group	Mean Survival	Std. Error	95% LCL	95% UCL
PBS	12.2	1.16	9.93	14.47
CDDP	21.2	1.66	17.96	24.44
FST <sub>H</sub>	17.0	1.34	14.37	19.63
CDDP+FST <sub>H</sub>	23.8	1.96	19.96	27.64
<i>FST</i> mRNA LNP	25.8	2.80	20.31	31.29
CDDP+ <i>FST</i> mRNA LNP	33.8	2.35	29.19	38.41

**Table S3: mRNA ORF sequences**

<i>FST</i> mRNA ORF (CleanCap®)
ATGGTCCGCGCGAGGCACCAGCCGGGTGGGCTTTGCCTCCTGCTGCTGCTGCTCT GCCAGTTCATGGAGGACCGCAGTGCCAGGCTGGGAACTGCTGGCTCCGTCAAGC GAAGAACGGCCGCTGCCAGGTCCTGTACAAGACCGAACTGAGCAAGGAGGAGTG CTGCAGCACCGCCGGCTGAGCACCTCGTGGACCGAGGAGGACGTGAATGACAA CACTCTTCAAGTGGATGATTTTCAACGGGGGCGCCCCAACTGCATCCCCTGT AAAGAAACGTGTGAGAACGTGGACTGTGGACCTGGGAAAAAATGCCGAATGAAC AAGAAGAACAAACCCCGCTGCGTCTGCGCCCCGGATTGTTCCAACATCACCTGGA AGGGTCCAGTCTGCGGGCTGGATGGGAAAACCTACCGCAATGAATGTGCACTCCT AAAGGCAAGATGTAAAGAGCAGCCAGAACTGGAAGTCCAGTACCAAGGCAGATG TAAAAGACTTGTGCGGGATGTTTTCTGTCCAGGCAGCTCCACATGTGTGGTGGAC CAGACCAATAATGCCTACTGTGTGACCTGTAATCGGATTTGCCAGAGCCTGCTT CCTCTGAGCAATATCTCTGTGGGAATGATGGAGTCACCTACTCCAGTGCCTGCCA CCTGAGAAAGGCTACCTGCCTGCTGGGCAGATCTATTGGATTAGCCTATGAGGGA AAGTGTATCAAAGCAAAGTCCTGTGAAGATATCCAGTGCCTGGTGGGAAAAAA TGTTTATGGGATTTCAAGGTTGGGAGAGGCCGGTGTTCCTCTGTGATGAGCTGT GCCCTGACAGTAAGTCGGATGAGCCTGTCTGTGCCAGTGACAATGCCACTTATGC CAGCGAGTGTGCCATGAAGGAAGCTGCCTGCTCCTCAGGTGTGCTACTGGAAGTA AAGCACTCCGGATCTTGCAACTCCATTTGGAAGACACCGAGGAAGAGGAGGAA GATGAAGACCAGGACTACAGCTTTCCTATATCTTCTATTCTAGAGTGGTAA
<i>FLuc</i> mRNA ORF Sequence (CleanCap® FLuc mRNA, L-7202)
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GA

*EGFP* mRNA ORF Sequence (CleanCap® EGFP mRNA, L-7601)

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