Supplementary Information

Absence of systemic inflammation in nebulized LPS-induced ALI mice

To confirm the absence of systemic inflammation in nebulized LPS-induced ALI animal model (C57BL/6 male mice), the liver tissue from each mouse was extracted and analyzed for MPO activity as a marker of systemic inflammation. Our data revealed similar MPO activity in the liver tissue of all the ALI mice relative to baseline controls (each, $p>0.05$, n=6/group; Supplementary Fig. S1).

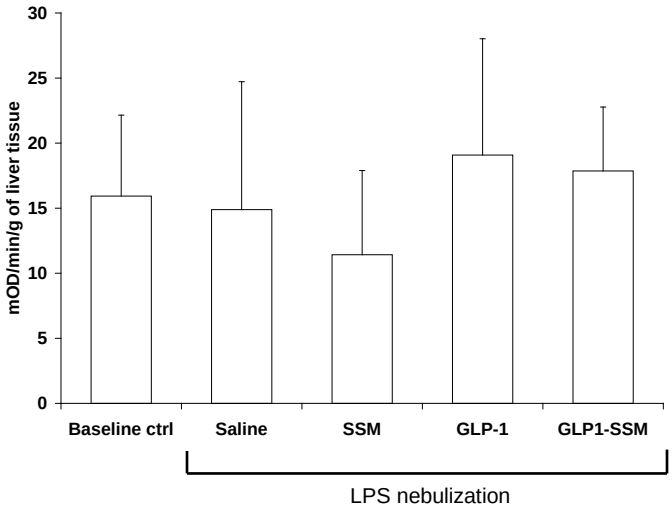

Figure S1. Myeloperoxidase activity (mOD/min/g of tissue) of the liver tissue of ALI mice. No statistical significance was observed between the different treatment groups of ALI mice and baseline control mice (n=6/group). P>0.05 among all groups.

Sterile filtration of GLP1-SSM through 0.2 µm membrane filter

The rationale of this experiment was to determine the presence of any significant changes in the physicochemical characteristics and yield of GLP1-SSM after filtration through 0.2 µm membrane filter. GLP1-SSM was prepared at the

peptide and lipid concentrations of 33.3 μM and 1 mM respectively. After equilibration, 1.0 ml each of the preparation was filtered through Durapore® membrane filter composed of tortuous pores or Nuclepore™ membrane filter composed of straight through pores, both with 0.2 μm pores. The non-filtered control and filtrates collected downstream of either membrane filters were assessed for the following properties: particle sizes (Agilent 7030 Nicomp DLS), peptide α-helicity (Jasco J-710 Spectropolarimeter) and peak fluorescence emission (SLM Aminco Spectrofluorimeter) under the same experimental conditions as described under methods.

The phospholipid and peptide yields of filtered GLP1-SSM dispersion were determined using Bartlett's assay and GLP-1 ELISA respectively. To measure the phosphate content (which would correlate to phospholipid quantity) using the modified Bartlett's assay, GLP1-SSM were first subjected to heat-induced digestion at 150 ^{o}C using 70 % perchloric acid. Thereafter, ammonium molybdate, sodium bisulfate and amidol were added to the samples to induce formation of colored complex with the free phosphate group. Absorbance of the complex was measured at 830 nm using DU 800 UV/Visible Spectrophotometer (Beckman Coulter, Fullerton, CA). The phosphate content of the samples was determined from a standard curve of absorbance versus phosphate concentration using inorganic phosphate as standard. To quantitate the amount of GLP-1 in samples, commercially available GLP-1 ELISA kit (Bachem, Torrance, CA) was used according to manufacturer instructions. The test samples were diluted with saline in 2 serial dilutions (1000x dilution followed by

500x dilution) so that the theoretical peptide concentration in samples would be within the optimal detection range of the ELISA kit.

Based on our data, no significant changes in the physicochemical properties of GLP1-SSM were seen after the liquid preparation was filtered through either membrane filter. GLP-1 association to SSM persisted post filtration as indicated by the fluorescence and CD results (Supplementary Fig. S2a and b). In addition, no significant retention of phospholipid and peptide by the membrane filters was detected. The measured phosphate (which relates to phospholipid) and peptide concentrations in filtered GLP1-SSM were comparable to pre-filtered controls (Supplementary Fig. S2c and d). Therefore, it could be concluded that GLP1-SSM dispersion was robust to the process of sterile filtration through 0.2 μm membrane filter and this process could be used to sterilize the liquid formulation without adversely affecting its physicochemical properties and yield.

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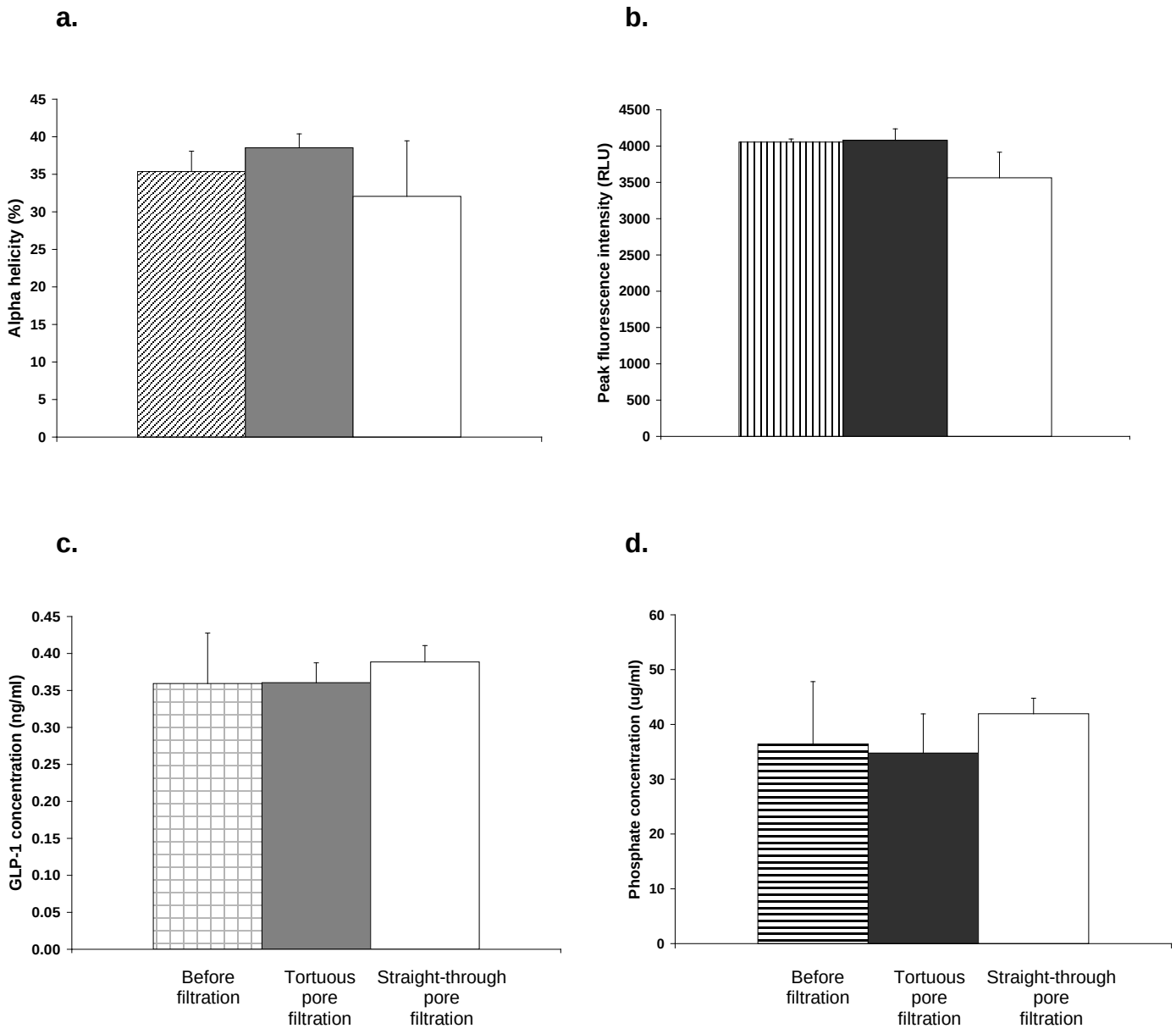

Figure S2. Characterization of GLP1-SSM before and after filtration through 0.2 µm membrane filters of tortuous or straight-through pores. a-b, Percent α-helicity (**a**) and peak fluorescence emission (**b**) of GLP-1 (33.3 µM) in association with SSM (1 mM) before and after filtration through 0.2 µm membrane filter (n=3; p>0.05). Deconvolution of CD spectra was done by fitting the data into simulations using SELCON® to calculate the percentage of α-helical

structures. **c-d,** Concentrations of GLP-1 (**c**) and phosphate (**d**) detected in GLP1-SSM dispersion before and after filtration (n=3; p>0.05).

Upregulation of BALF cAMP in GLP1-SSM treated LPS-induced ALI mice.

To determine the level of cAMP in collected murine BALF, commercially available ELISA kit (Cayman Chemical) was used as instructed in product manual. Using C57B6/DBA transgenic mice with NF-κB luciferase reporter gene (HIV-LTR/luciferase; HLL) developed by Yull et al (22), the mice were induced with ALI via nebulized LPS and injected with single dose of GLP1-SSM (15 nmol/mouse s.c.) immediately post LPS nebulization. The mice were sacrificed 4 h after completion of nebulization and BALF was collected as described in Method. The measured cAMP concentration in BALF was found to be significantly elevated in GLP1-SSM (15 nmol/mouse s.c.) treated mice (2.5 ± 1.1 pmol/ml) relative to those given saline (0.6 ± 0.1 pmol/ml), SSM (0.3 ± 0.2 pmol/ml) or GLP-1 in saline (0.8 ± 0.6 pmol/ml) (each, $p<0.05$ compared to GLP1-SSM treatment group, n=5/group; Supplementary Fig S3).

Supplementary Information

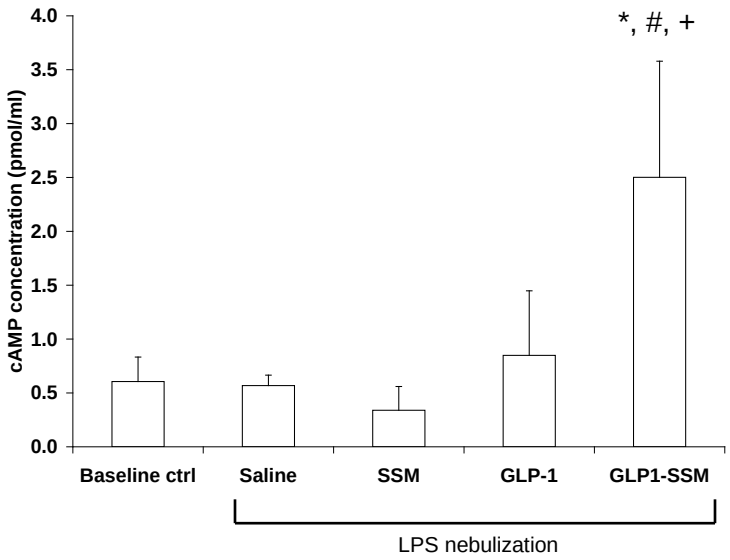

Figure S3. Cyclic AMP concentration in BALF of ALI mice. HLL mice induced with ALI were treated with saline, SSM, GLP-1 in saline (GLP-1; 15 nmol/mouse) or GLP1-SSM. *, # and + indicate $p<0.05$ compared to saline, SSM and GLP-1 treatments respectively (n=5/group except for baseline ctrl, n=3).