Supporting Information for

Supramolecular Peptide Nanofibers Engage Mechanisms of Autophagy in Antigen-presenting Cells

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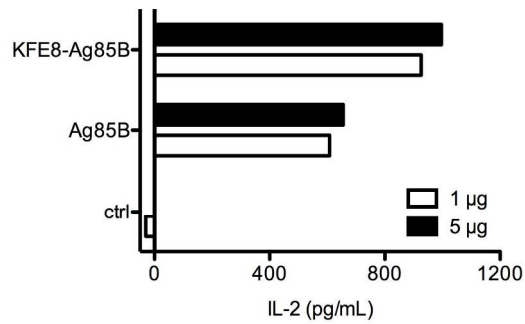E-mail address: jarudra@utmb.edu

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Figure S1. Peptide antigens linked to self-assembling domains are more effectively presented by DCs compared to soluble antigen. IL-2 production by BB7 cells (T cells recognizing the Ag85B epitope) presented on BCG-infected DCs treated with KFE8-Ag85B is significantly higher compared to soluble Ag85B at two different doses tested (n=2 replicates/group).
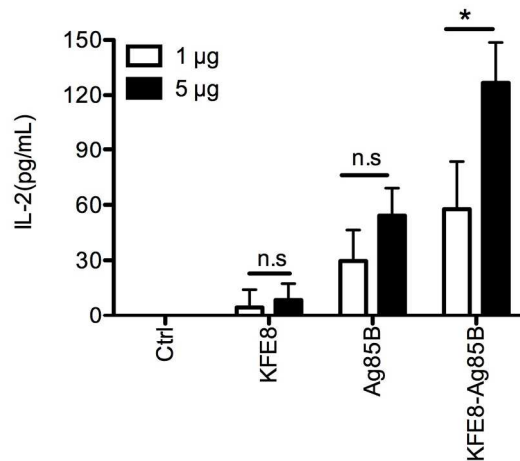

Figure S2. Enhanced antigen-presentation and higher IL-2 production is observed in macrophages treated with KFE8-Ag85B compared to macrophages treated with soluble Ag85B peptide even without BCG infection (n=4 replicates/group). . *p<0.05 by students t-test (n=3 replicates/group).
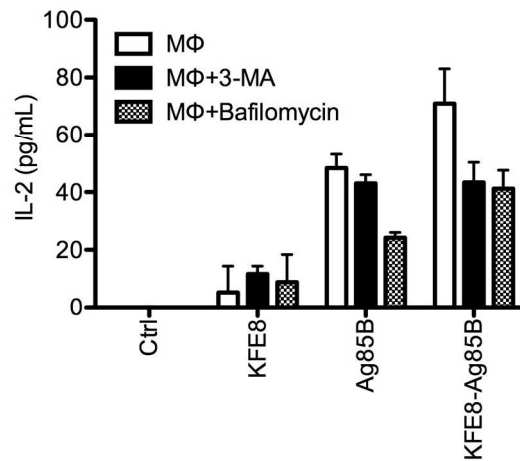
Figure S3. Addition of autophagy inhibitors 3-MA and bafilomycin leads to reduced IL-2 production by macrophages treated with KFE8-Ag85B. A reduction in IL-2 levels was also observed in macrophages treated with soluble Ag85B peptide in the presence of bafilomycin (n=2 replicates/group).
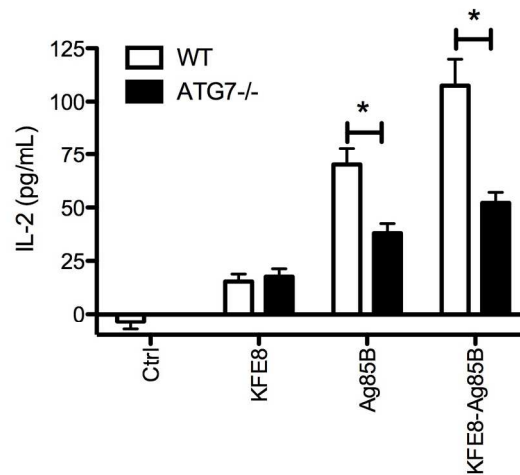

Figure S4. Antigenic-processing and presentation of self-assembling peptide nanofibers by DCs involves autophagy. Data demonstrating that IL-2 production is significantly reduced in Atg7-/- DCs treated with KFE8-Ag85B nanofibers and overlaid with BB7 cells. Interestingly, Atg7-/- DCs treated with soluble Ag85B also exhibited similar behavior. *p<0.05 by students t-test (n=3 replicates/group).
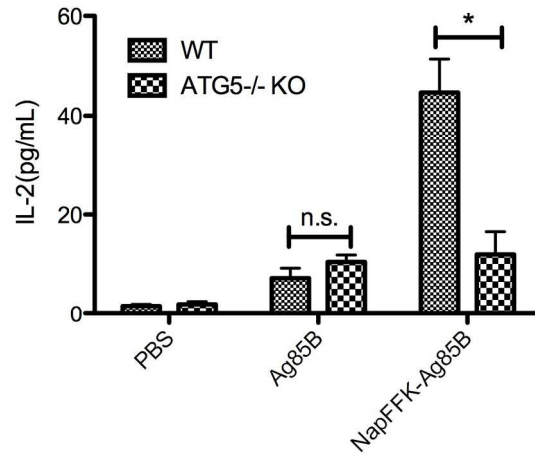
Figure S5. Data demonstrating higher IL-2 production in MΦs treated with NapFFK-Ag85B nanofibers compared to soluble antigen Ag85B and significant reduction of IL-2 in Atg5-/- MΦs treated with NapFFK-Ag85B nanofibers. *p<0.05 by students t-test (n=3 replicates/group).
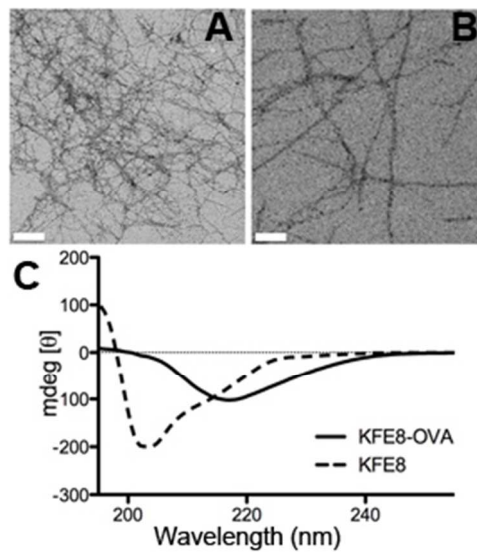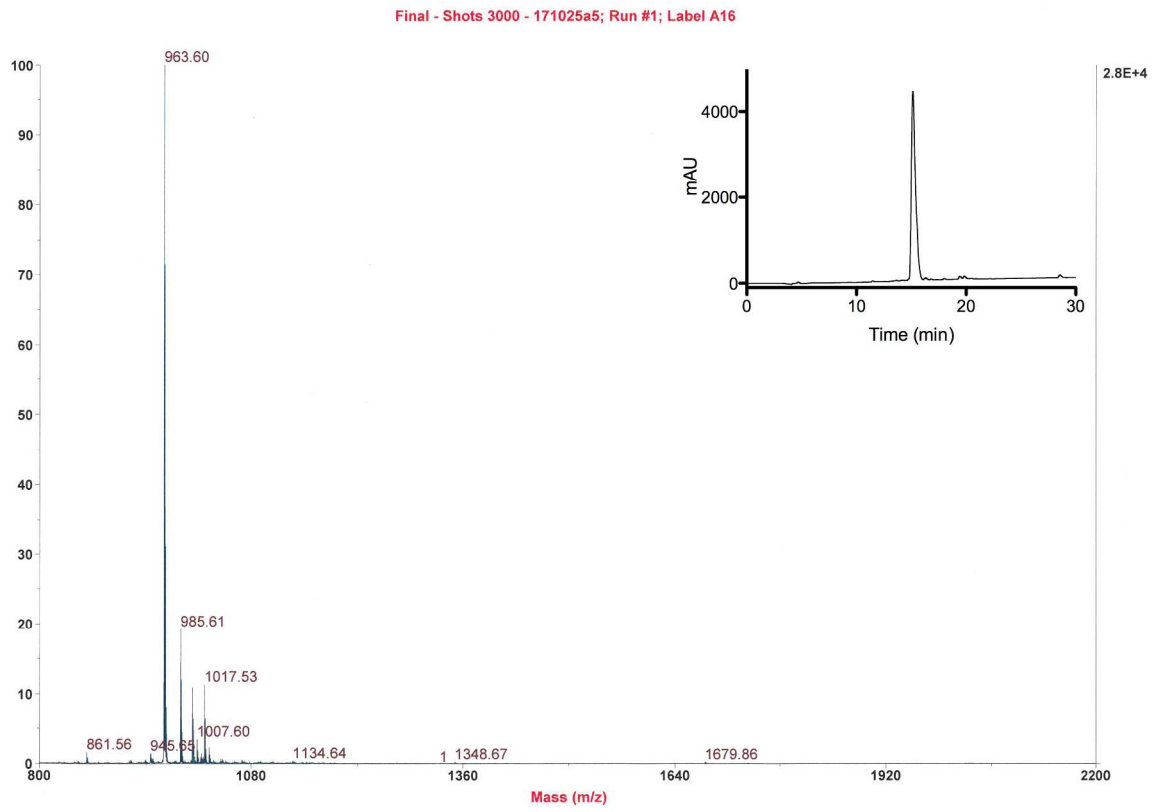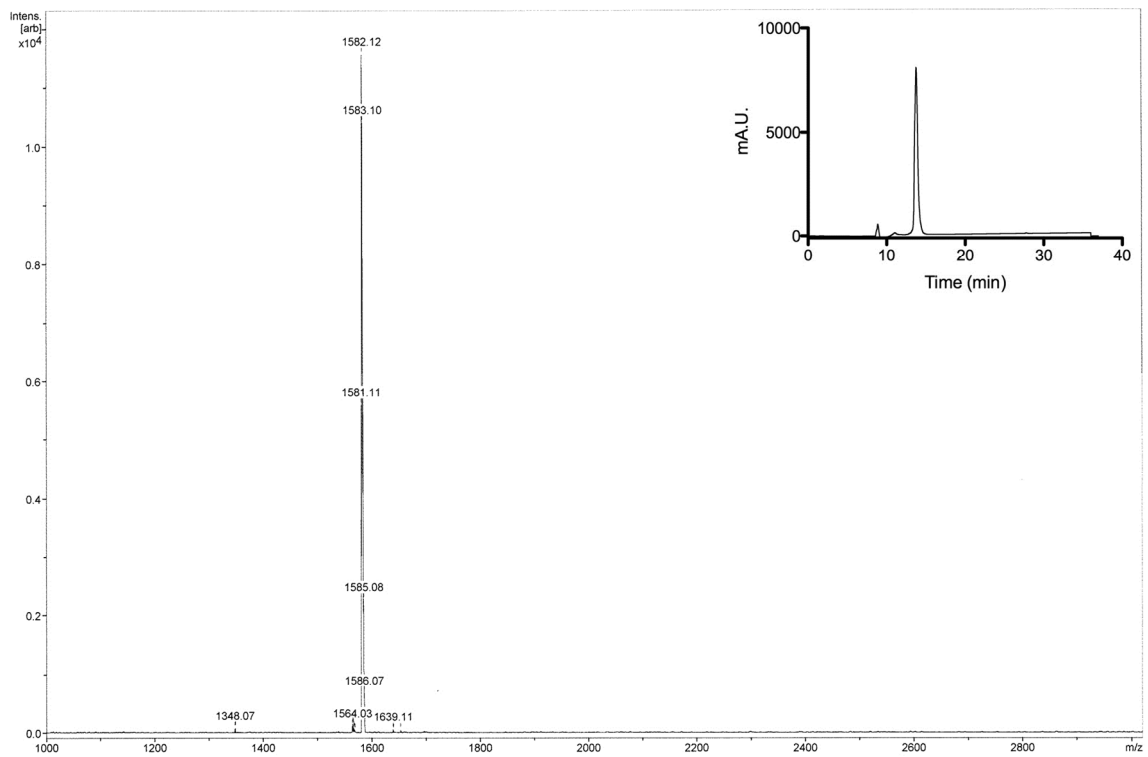

Figure S6. Nanofibers of KFE8 (A) and KFE8-OVA (B) as observed using TEM. Scale bar = 100 nm. Circular dichrosim spectra of KFE8 and OVA-KFE8 indicating transition from a cross-beta structure to pure beta-sheet (C). Peptide concentration was 0.75 mM in ultrapure water.
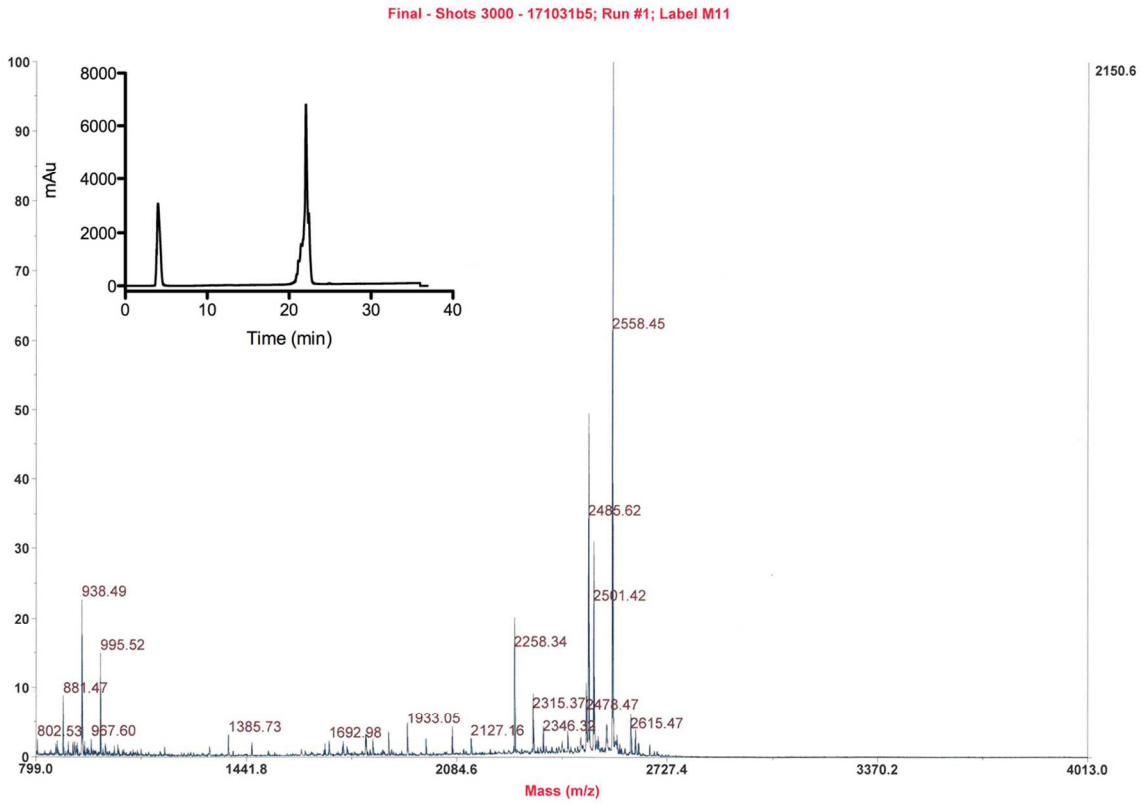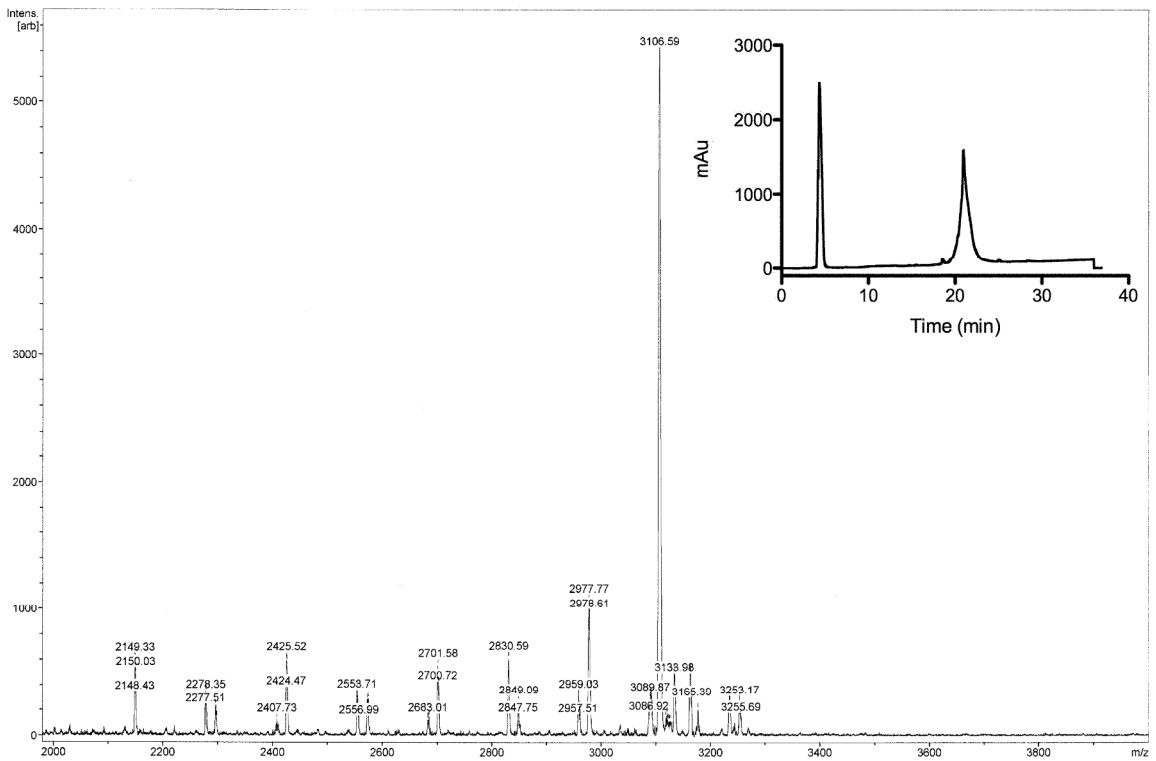
(i)

(ii)

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(iii)

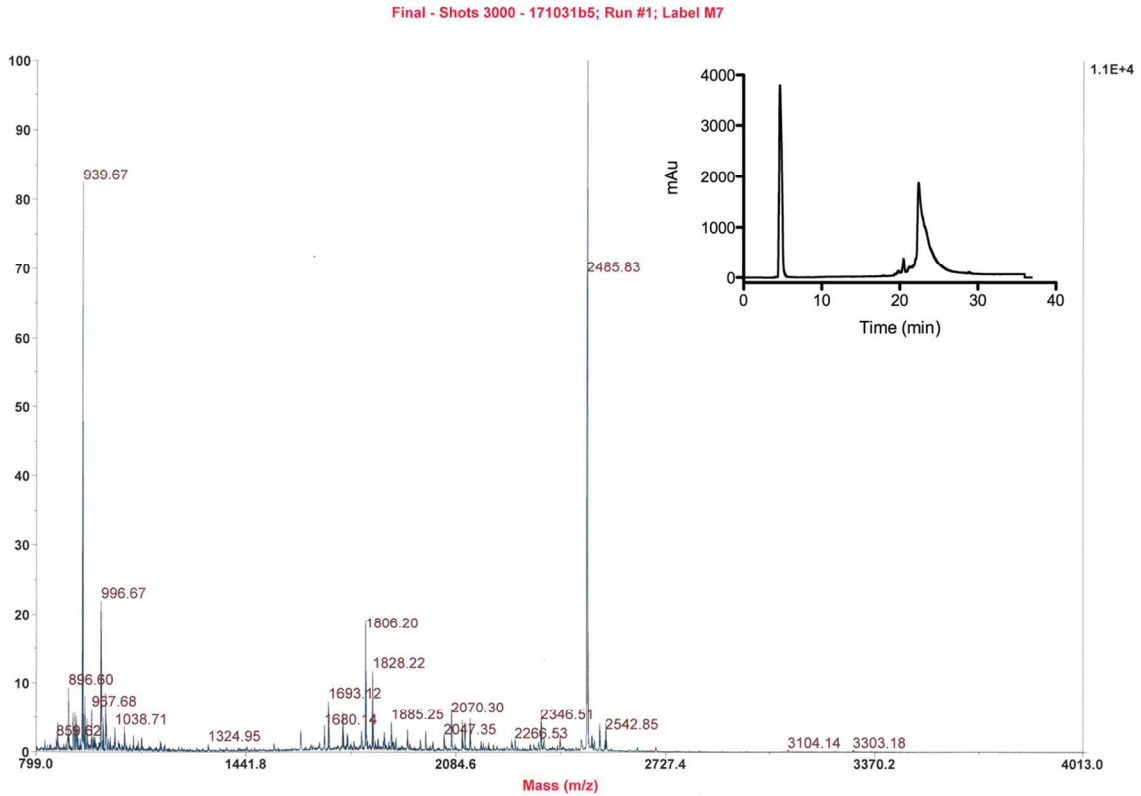Final - Shots 3000 - 171031b5; Run #1; Label M11

(iv)

S7

(v)

Figure S7. HPLC and mass spectra for peptides used in the study. (i) OVA peptide, Cal'd 963.1 Da, Obs'd 963.3 Da, purity >90%. (ii) Ag85B peptide, Cal'd 1581.66 Da, Obs'd 15812.12 Da, purity >90%. (iii) Nap-Ag85B peptide, Cal'd 2558.7 Da, Obs'd 2558.45 Da, purity >80%. (iv) KFE8-Ag85B peptide, Cal'd 3104.4 Da, Obs'd 3103.87 Da, purity >80%. (v) OVA-KFE8 peptide, Cal'd 2485.87 Da, Obs'd 2485.83, purity >80%.