



Article

# In Vitro and In Vivo Assessment of PEGylated PEI for Anti-IL-8/CxCL-1 siRNA Delivery to the Lungs

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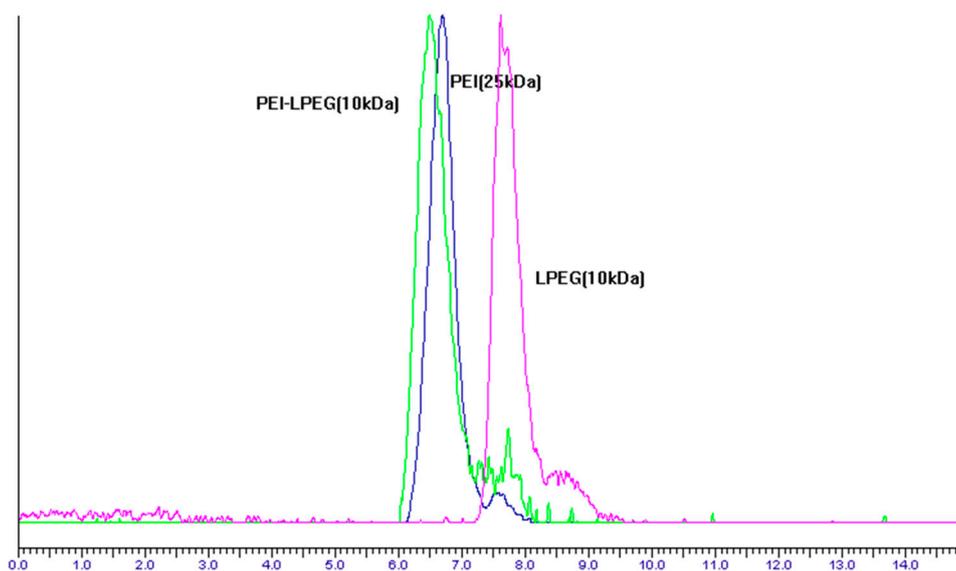
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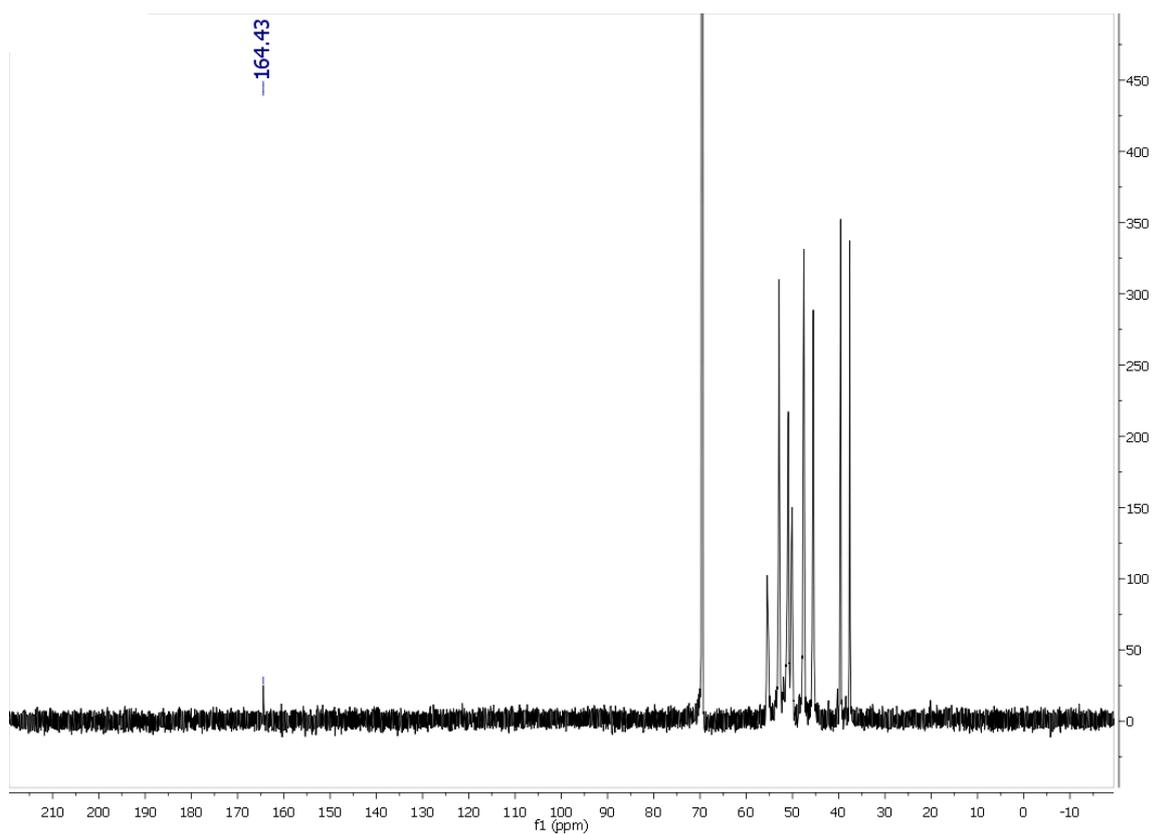
<sup>5</sup> Aerogen Ltd. Galway Business Park, Galway, H91 HE94, Ireland

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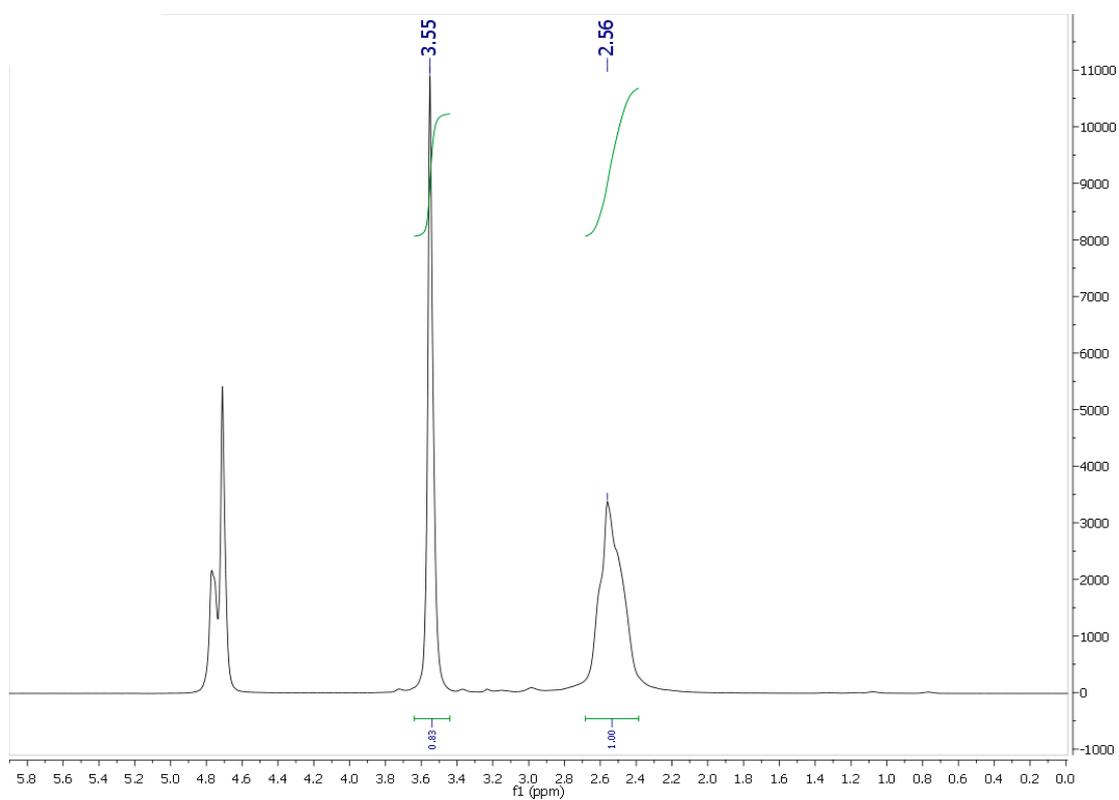
## Supplementary Materials:



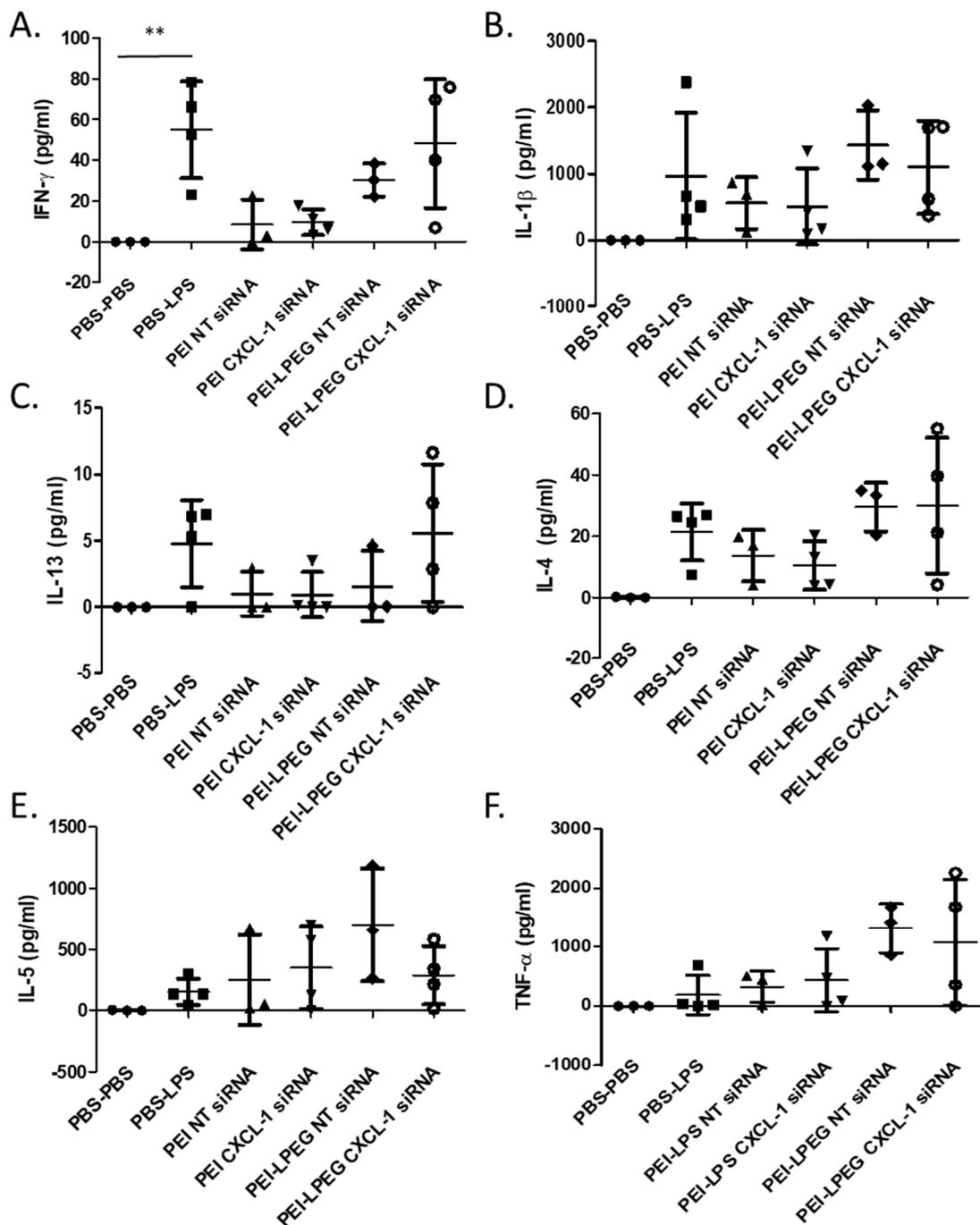
**Figure S1.** GPC size and purity analysis of synthesised PEI-LPEG overlaid with its respective starting materials.



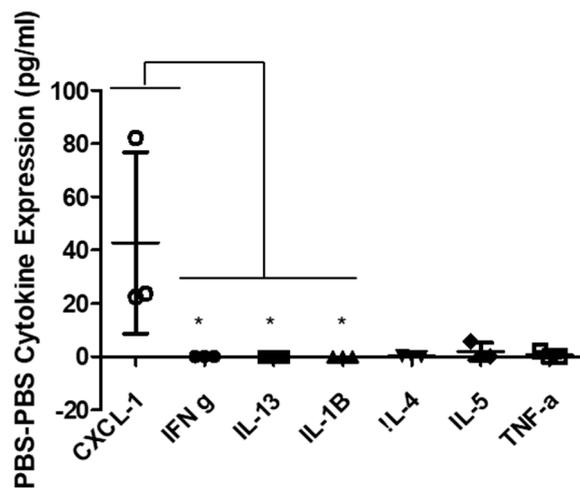
**Figure S2.** PEI-LPEG carboxylic bonding presence was shown in  $^{13}\text{C}$  NMR at 164 ppm.



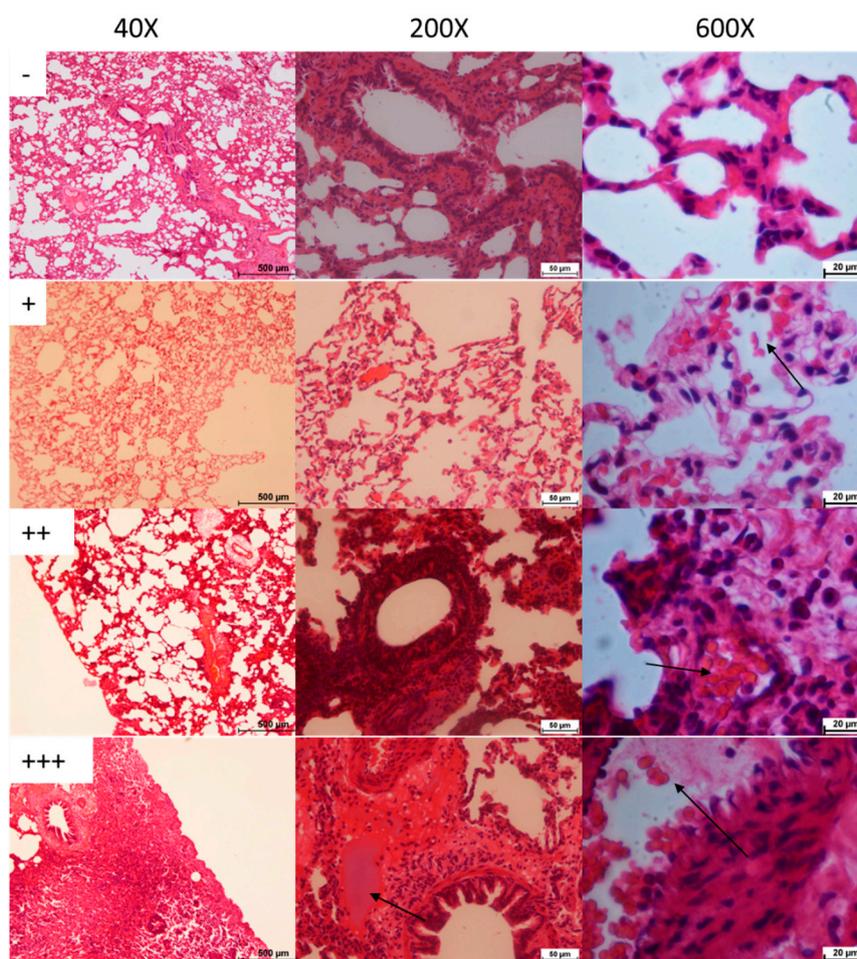
**Figure S3.**  $^1\text{H}$  NMR of synthesised PEI-LPEG polymer with PEI present at 2.5 ppm and PEG present at 3.5 ppm.



**Figure S4.** Rat Demonstration 7-Plex Ultra-Sensitive Kit analysis of inflammatory cytokine responses elicited by intratracheal instillation of PBS-PBS, PBS-LPS, non-targeting (NT) or anti-CXCL-1 siRNA nanoparticles in a rat model. (A) interferon- $\gamma$  (IFN- $\gamma$ ) (B) interleukin-1 $\beta$  (IL-1 $\beta$ ) (C) IL-13 (D) IL-4 (E) IL-5, and (F) tumour necrosis factor alpha (TNF- $\alpha$ ) (significance vs. PBS-LPS treated samples, Kruskal-Wallis test and Dunn's post-hoc test, min of  $n = 3 \pm SD$ , \*\*  $p < 0.01$ ).



**Figure S5.** BAL cytokine expression levels in PBS-PBS treated rats (minimum  $n = 3 \pm$  SD, Kruskal-Wallis test and Dunn's post-hoc test, \*  $p < 0.05$ ).



**Figure S6.** Pulmonary histopathology semi quantitative scoring of neutrophil-rich inflammation. Haematoxylin and eosin stained lung sections from top PBS, PBS-LPS and siRNA nanoparticle treated rats were scored based on the degree of neutrophil-rich inflammation observed (- absent, + mild, ++ moderate and +++ highly inflamed). Images were acquired at 40 $\times$ , 200 $\times$  and 600 $\times$  magnification with

arrows indicating evidence of inflammation and of blood and protein in the alveoli and loss of the alveolar lining at higher levels of severity.



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