

Supplementary information to:

Letter to the editor:

ANTI-INFLAMMATORY AND ANTI-FIBROTIC EFFECTS OF BERBERINE-LOADED LIQUID CRYSTALLINE NANOPARTICLES

Amlan Chakraborty^{1,2,#,*} , Keshav Raj Paudel^{3,#} , Chao Wang² , Gabriele De Rubis^{4,5} ,
Dinesh Kumar Chellappan⁶ , Philip Michael Hansbro³ , Chrisan S. Samuel² ,
Kamal Dua^{4,5,*} 

¹ Faculty of Biology, Medicine and Health, The University of Manchester, Oxford Road, Manchester M13 9PL, U.K.

² Cardiovascular Disease Program, Biomedicine Discovery Institute and Department of Pharmacology, Monash University, Clayton, VIC 3800, Australia

³ Centre for Inflammation, Centenary Institute & University of Technology Sydney, School of Life Sciences, Faculty of Science, Sydney, New South Wales 2050 & 2007, Australia

⁴ Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Ultimo, NSW 2007, Australia

⁵ Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, University of Technology Sydney, Ultimo, NSW 2007, Australia

⁶ Department of Life Sciences, School of Pharmacy, International Medical University (IMU), Bukit Jalil, Kuala Lumpur, 57000, Malaysia

Equal contribution

* **Corresponding authors:** Dr. Amlan Chakraborty, Faculty of Biology, Medicine and Health, The University of Manchester, Oxford Road, Manchester M13 9PL, U.K.

E-mail: amlan.chakraborty@manchester.ac.uk

Dr. Kamal Dua, Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Ultimo, NSW 2007, Australia. E-mail: Kamal.Dua@uts.edu.au

<https://dx.doi.org/10.17179/excli2023-6467>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>).

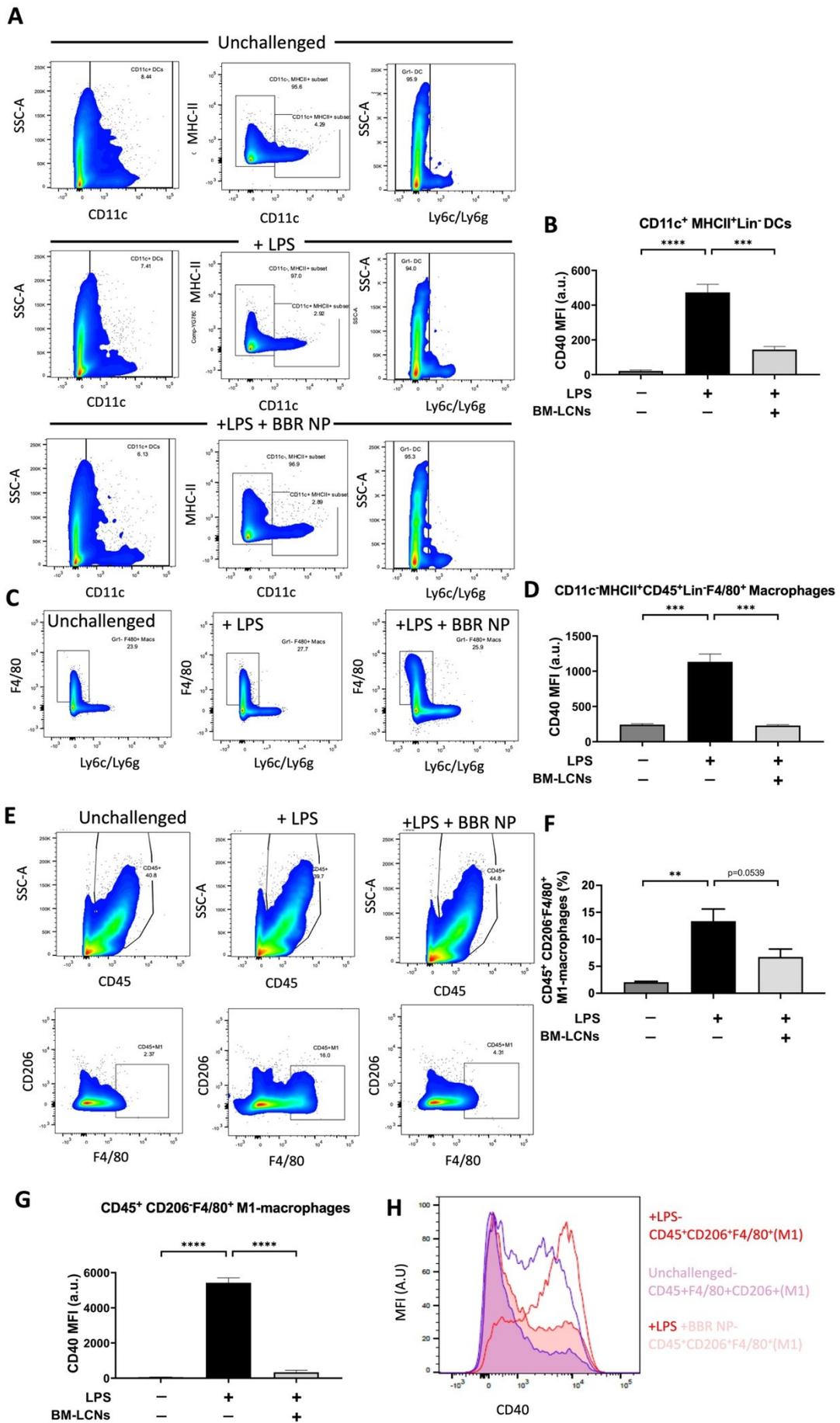
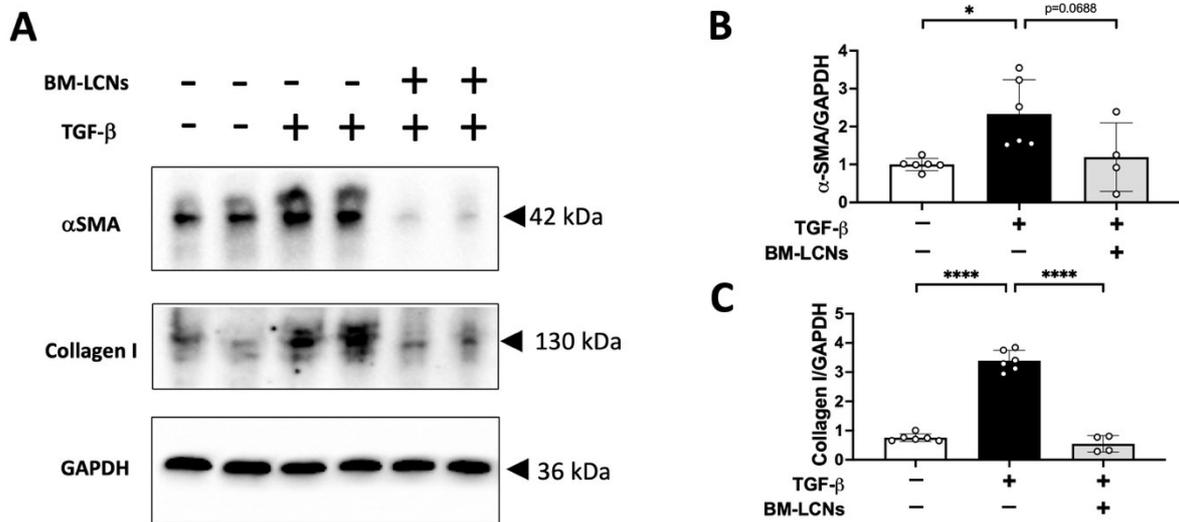


Figure 1: BM-LCNs counteract LPS-induced Dendritic cell activation and macrophage. (A, B): BMDMs were treated with 100 ng/ml LPS for 24 hours and then exposed to BM-LCNs (100 ml formulating with 1 ml formulation containing 0.20 g Monoolein, 0.02 g Poloxamer 407, 5.00 mg Berberine Hydrochloride in sterile water) for 24 h. (A): representative dot plots showing the gating strategy employed for the analysis of CD40 expression on CD11c⁺MHCII⁺Lin⁻ BMDMs; (B): analysis of CD40 MFI on CD11c⁺MHCII⁺Lin⁻ BMDMs. (C, D): BMDMs were treated with 100 ng/ml LPS for 24 hours and then exposed to BM-LCNs (same concentration as stated before) for 24 hours. (C): representative dot plots showing the gating strategy employed for the analysis of CD40 expression on CD11c⁻MHCII⁺CD45⁺Lin⁻F4/80⁺ BMDMs; (D): analysis of CD40 MFI on CD11c⁻MHCII⁺CD45⁺Lin⁻F4/80⁺ BMDMs. (E-H): BMDMs were treated with 100 ng/ml LPS for 24 hours and then exposed to BM-LCNs for 24 hours. (E): representative dot plots showing the gating strategy employed for the analysis of the percentage of M1-skewed macrophages, defined as the CD45⁺CD206⁻F4/80⁺ subset of BMDMs; (F): analysis of the percentage of CD45⁺CD206⁻F4/80⁺ M1-skewed macrophages; (G): analysis of CD40 MFI on CD45⁺CD206⁻F4/80⁺ M1-skewed macrophages; (H): representative histogram showing the relative CD40 MFI in CD45⁺CD206⁻F4/80⁺ M1-skewed macrophages. Statistics: One-Way ANOVA (n = 3). **: p<0.01; ***: p<0.001; ****: p<0.0001.



Supplementary Figure 2: BM-LCNs counteract TGF-β-induced expression of α-SMA and Collagen I in BJ3 Human Dermal Fibroblasts. BJ3 HDFs were treated with 5 ng/ml TGF-β1 for 72 hours and exposed to BM-LCNs (100 ml formulating with 1 ml formulation containing 0.20 g Monoolein, 0.02 g Poloxamer 407, 5.00 mg Berberine Hydrochloride in sterile water). (A): representative western blots showing the effect of treatments on the expression of α-SMA, Collagen I, and GAPDH. (B): analysis of the relative expression of α-SMA; (C): analysis of the relative expression of Collagen I. Values in (B) and (C) are normalized by GAPDH expression. Statistics: One-Way ANOVA (n = 4-6). **: p<0.01; ***: p<0.001; ****: p<0.0001.