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SUPPLEMENTARY MATERIAL

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Optimization of the autophagy measurement in a human cell line and primary cells by flow cytometry

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Key words: Autophagy; tuberculosis; BCG; flow cytometry; LC3; PBMC; THP-1.

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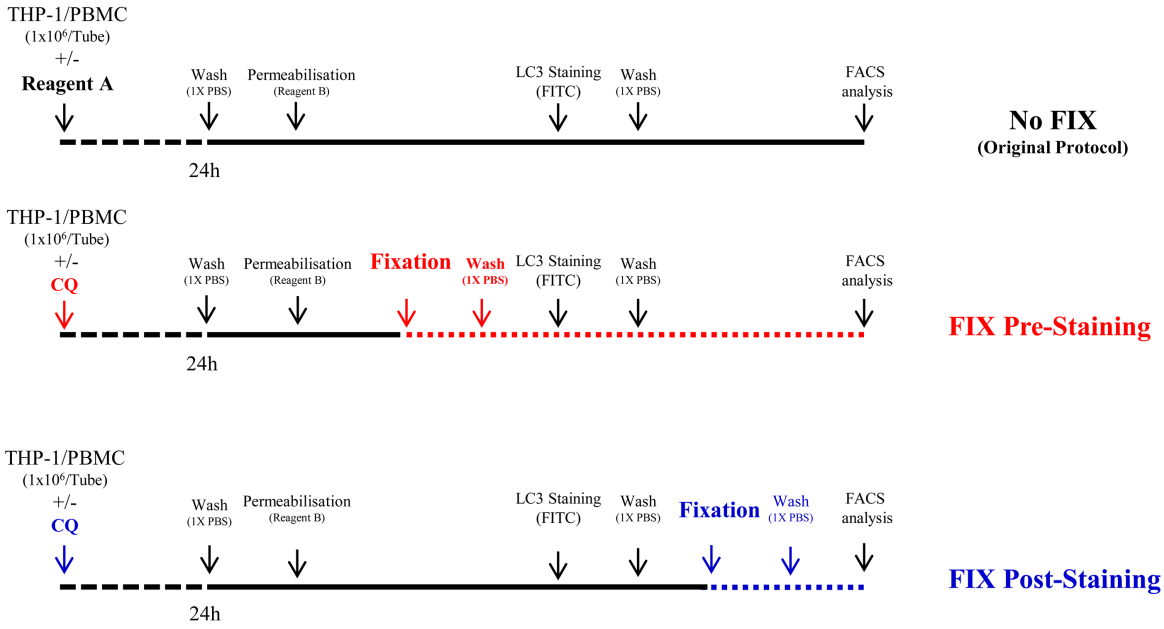
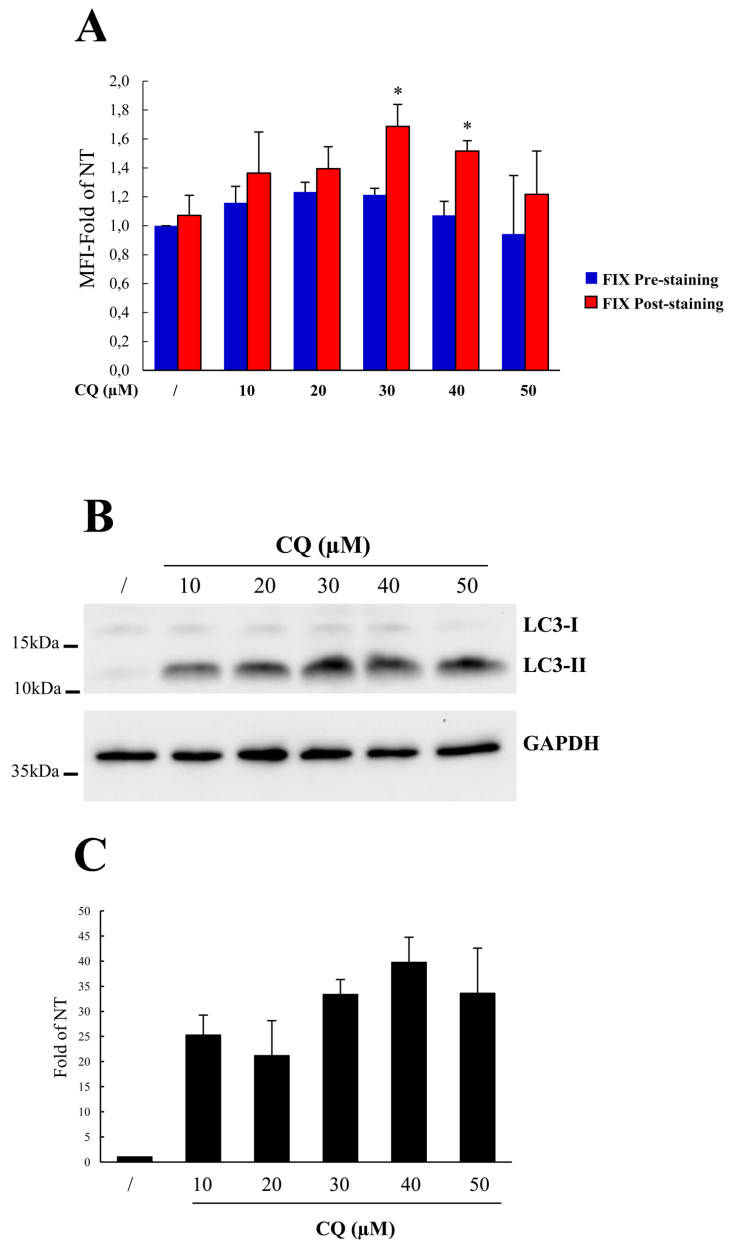


Supplementary Figure 1.

Schematic representation of the experimental procedures used for LC3 evaluation. See details in the text. In red and blue are highlighted the differences from the manufacture's protocol.



Supplementary Figure 2.

LC3 accumulation induced by different CQ treatments in human PBMC. A) Human PBMC were isolated from healthy donors and either stimulated with different doses of CQ, as indicated, or left untreated (NT) for 4 h; LC3 levels were measured by flow cytometry using the FlowCellect Kit in which a fixation step was introduced either before (Fix Pre-staining; blue columns) or after LC3 labelling (Fix Post-staining; red columns); data represent MFI of LC3 normalized to MFI of untreated cells analysed using the pre-staining fixation protocol. Data are expressed as average ±SD (n=4); *P<0.05 Fix-Pre-staining *vs* Fix-Post-staining. B) A representative immunoblotting analysis of LC3 in PBMC cells treated as in A; GAPDH levels were used as loading control. C) Quantification of the ratio of LC3-II/GAPDH band intensities obtained by immunoblotting analysis, as in B, and expressed as average ±SD (n=4).