Supplementary materials

Cytosolic HMGB1 mediates LPS-induced autophagy in microglia by interacting with NOD2 and suppresses its proinflammatory function

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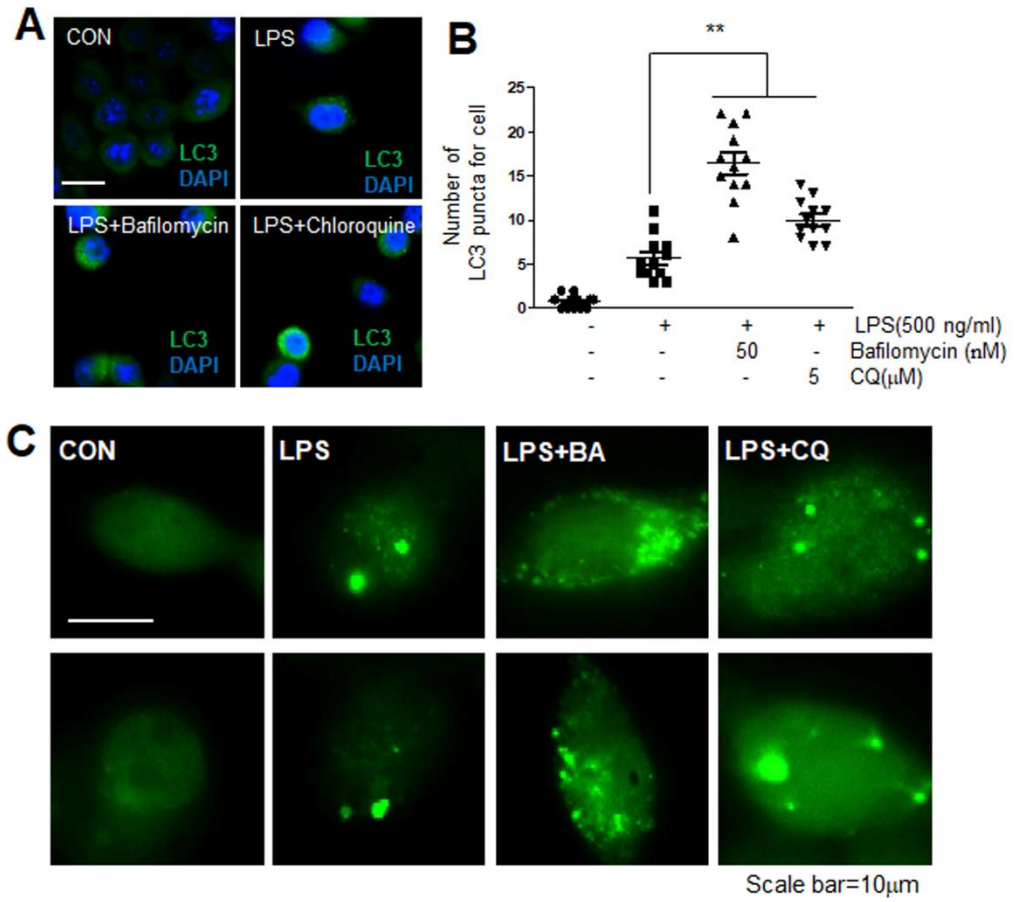
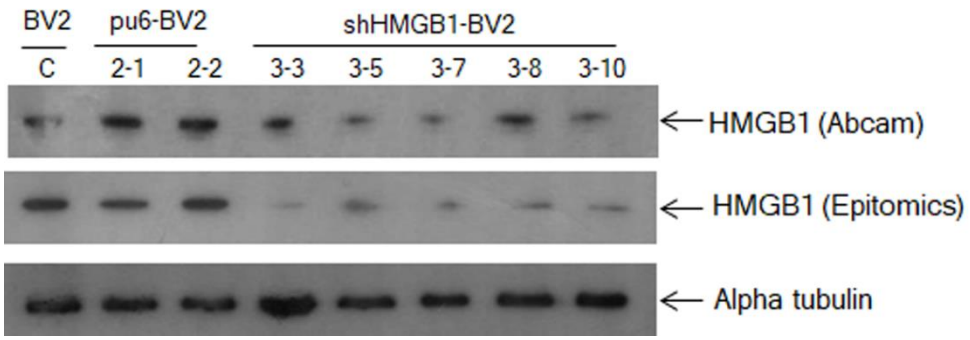


Supplementary Figure S1. Suppression of LPS-mediated autophagy flux in BV2 cell by Chloroquine and Bafilomycin A1

BV2 cells were pretreated with Bafilomycin A1 (BA, 50 nM) or chloroquine (CQ 5 mM) for 2 h and then treated with LPS (500 ng/ml) for 24 h. Immunofluorescence staining was conducted at 12 and 24 h after LPS treatment with anti-LC3 antibody (green) and DAPI (blue). Representative images are shown in A, and quantification of the dot- or ring-shaped LC3 signals (representing autophagosomes) are shown in B (n=12). Images in C are high magnification pictures. Scale bar, 20 μm for A and 10 μm for C.



Supplementary Figure S2. Generation of HMGB1-deficient stable BV2 cell lines

BV2 cells were transfected with pU6 plasmid expressing HMGB1 shRNA (shHMGB1-BV2) or empty pU6 plasmid (pU6-BV2). At 24 h post transfection, HMGB1 expression levels were examined in five cell lines after clonal selection using two different antibodies. We selected one cell line, namely, shHMGB1-BV2 (3-5), was selected and used in the subsequent experiments.