Supporting information

Regulation of collectins by uPARAP is governed by unique structural elements in the receptor FNII domain

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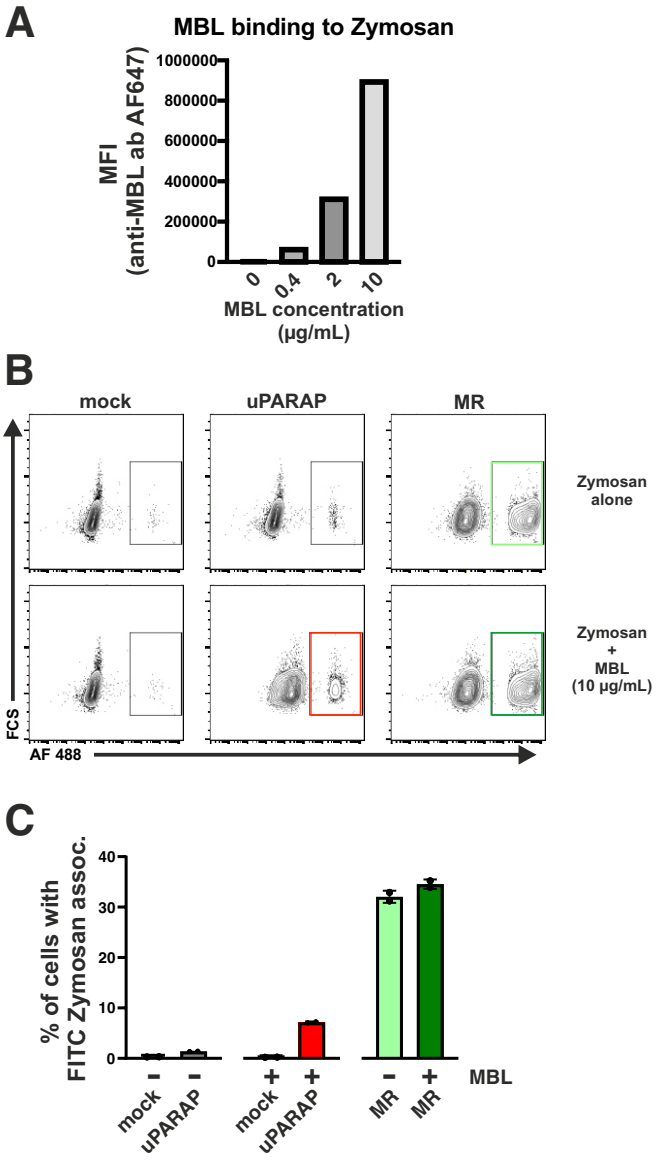
Material included:

Figure S-1:

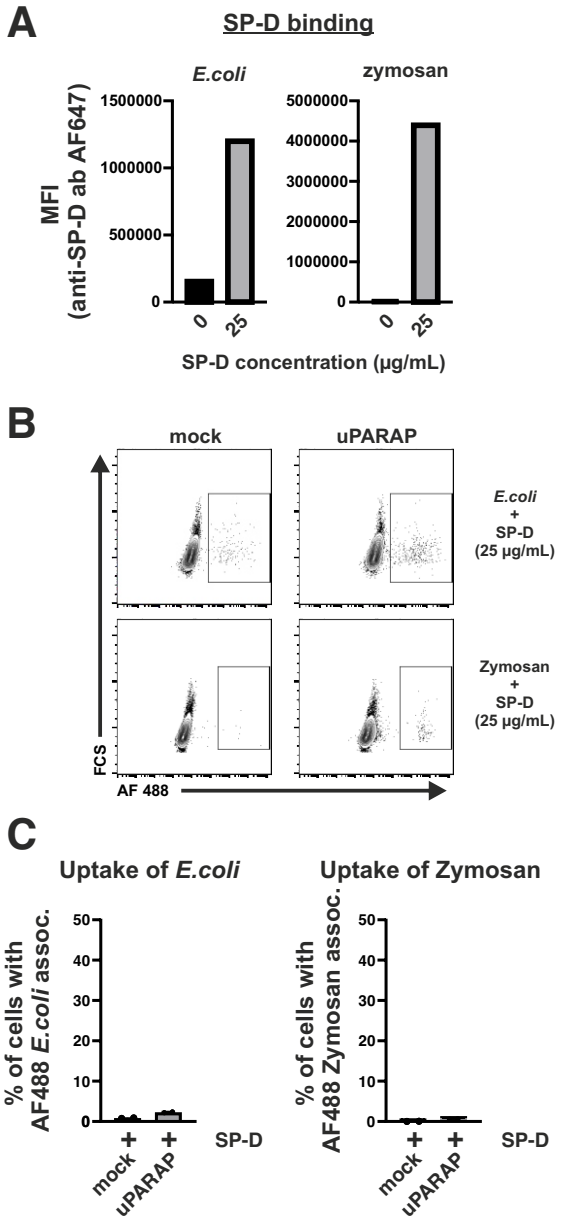


Supporting figure 1. uPARAP interacts with MBL immobilized on the surface of zymosan bioparticles

A, Quantification of MBL binding to the surface of zymosan bioparticles. Immobilized MBL was detected using a primary anti-MBL antibody followed by a AF 647 conjugated secondary antibody. The AF 647 signal associated with the bioparticles was quantified using flow cytometry. MFI, mean fluorescence intensity. In this experiment, a potent concentration-dependent immobilization of MBL on the surface of zymosan bioparticles was detected.

B and C, Assay for the association between bioparticles and mock CHO-K1 cells or cells expressing uPARAP or MR. Examples of flow charts showing fluorescence associated with cells exposed to AF 488 conjugated zymosan bioparticles that were pre-incubated with MBL (B, bottom panels) or incubated without collectin (B, top panels). The percentages of cells scoring positive for an association with bioparticles are presented in bar charts (C). Analysis was performed in duplicate. Data is presented as mean (SD). Expression of uPARAP in the CHO-K1 cells induced an association with the zymosan particles pre-incubated with MBL only, thereby indicating a direct interaction between uPARAP and immobilized MBL. The expression of MR induced a strong association between cells and zymosan particles, but this effect was independent of MBL. This observation is in line with MR's well-known ability to interact directly with carbohydrate moieties present on the surface of zymosan and related pathogens.

Figure S-2:



Supporting figure 2. SP-D does not induce an association between uPARAP expressing cells and pathogens

A, Quantification of SP-D binding to the surface of E.coli and zymosan bioparticles. Immobilized SP-D was detected using a primary anti-SP-D antibody followed by a AF 647 conjugated secondary antibody. The AF 647 signal associated with the bioparticles was quantified using flow cytometry. SP-D was efficiently immobilized on the surface of both types of bioparticles.

B and C, Assay for the association between bioparticles and mock CHO-K1 cells or cells expressing uPARAP. Examples of flow charts showing fluorescence associated with cells exposed to AF 488 conjugated E.coli (B, top panels) or zymosan (B, bottom panels) bioparticles that were pre-incubated with SP-D. The percentages of cells scoring positive for an association with bioparticles are presented in bar charts (C). Analysis was performed in duplicate. Data is presented as mean (SD). SP-D failed to induce an association between uPARAP-expressing cells and the two types of bioparticles.