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Supplemental Material to:

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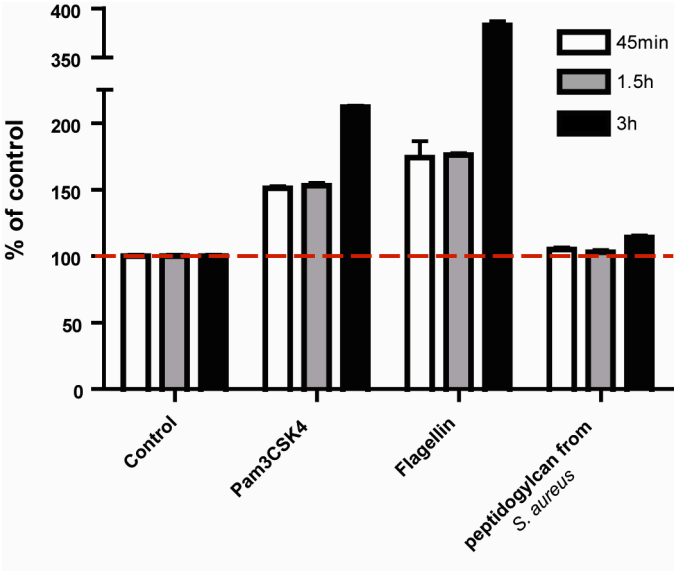
Macrophages mediate colon carcinoma cell adhesion in the rat liver after exposure to lipopolysaccharide

Oncoimmunology 2012; 1(9)
http://dx.doi.org/10.4161/onci.22303

http://www.landesbioscience.com/journals/oncoimmunology/article/22303/



Supplemental figure 1: Intracellular ROS levels in macrophages after treatment with control medium, 50 μg/ml Pam3CSK4 (TLR1/2 ligand), 5μg/ml flagellin (TLR5 ligand), or 50 μg/ml peptidoglycan from *S. aureus* (TLR2 ligand).

Supplemental material and methods

Bone marrow -derived macrophages

Bone marrow was harvested from freshly isolated femur, tibia and humerus from Wag/Rij rats. After removal of connective tissues and muscles, bone marrow was flushed and single cell suspensions were made by passing bone marrow through sterile 70 μm filters (BD Falcon, Bedford, MA). Macrophages differentiation was induced by incubating bone marrow cells for 7 days with complete DMEM supplemented with 15% L929 conditioned medium (containing macrophage-colony stimulating factor). Macrophages were harvested by 15 minutes incubation with trypsin-EDTA and subsequent scraping with a cell scraper. Macrophages were seeded in 96-well plates ($2x10^5$/ well; Corning Incorporated, NY) for experiments.

ROS measurement

Macrophages were incubated with control medium HBSS or ligands for Toll-like receptors (50 μg/ml Pam3CSK4, 5μg/ml flagellin, and 50 μg/ml peptidoglycan from *S. Aureus*) for 45 minutes, 1.5 hour or 3 hours. After washing 10 μM CM-H_2DCFDA (Molecular Probes, Breda, The Netherlands), reflecting intracellular ROS levels, was added and cell were incubated for 1 hour in the incubator whereafter fluorescence was measured (485 nm excitation/520 nm emission filters; Fluostar Galaxy, BMG Labtechnologies, Offenburg, Germany).