Supplementary Material

Toll-like receptor 2 release by macrophages: an anti-inflammatory program induced by glucocorticoids and lipopolysaccharide

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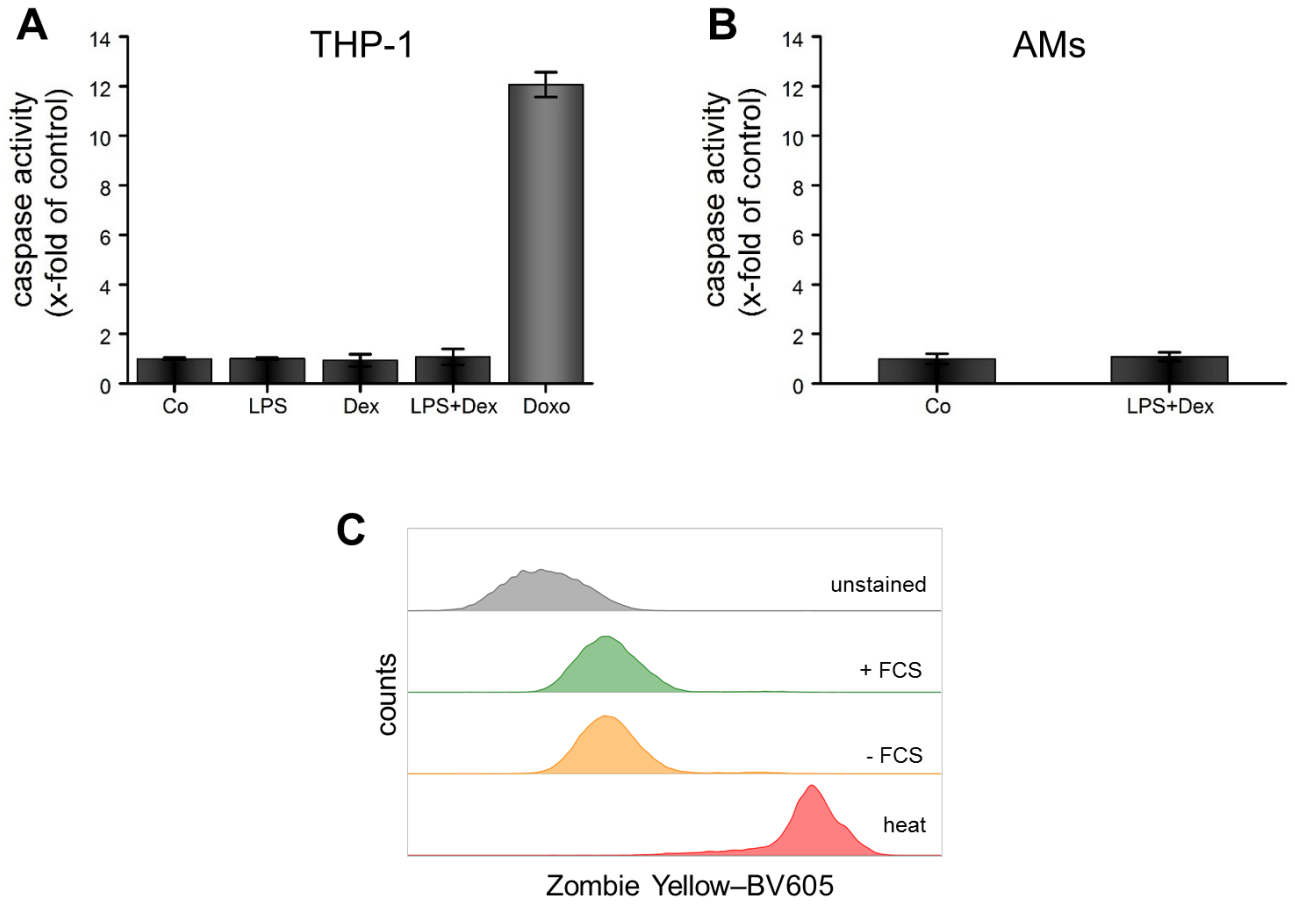
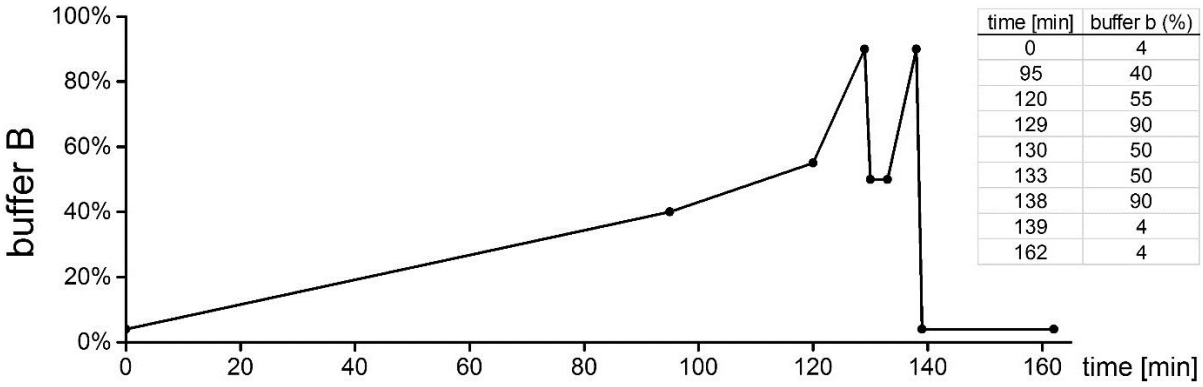
Supplementary Figures

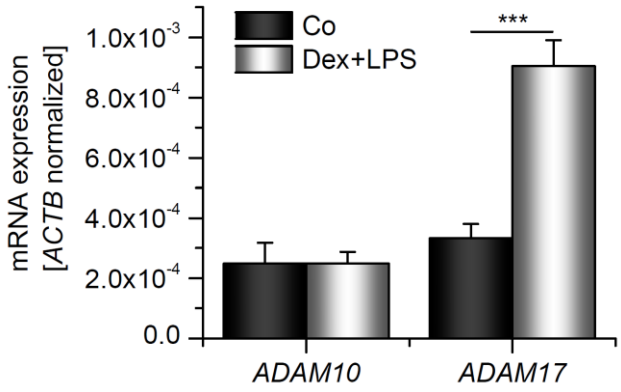


Supplementary Figure 1. *Treatment schemes and serum deprivation do not impair cell viability.* A, B: Caspase-3-like activity assay. Cells were treated with the vehicle control (0.1% DMSO, Co), LPS (100 ng/mL), Dex (1 µM), or LPS + Dex for 3 d in serum-free medium. Treatment with 10 µM doxorubicin (Doxo) for 24 h served as a positive control. An increase in apoptosis-asscociated caspase-3-like activity was neither detected in THP-1 cells (A) nor in AMs (B) (n = 3). C: Zombie yellow viability staining. Differentiated THP-1 cells were cultured in in serum-containing (+ FCS) or serum-free (- FCS) medium for 3 d. Cells incubated at 60°C for 1 h (heat) served as a positive control. Cell viability was not affected by serum-free medium (n = 2, triplicates).

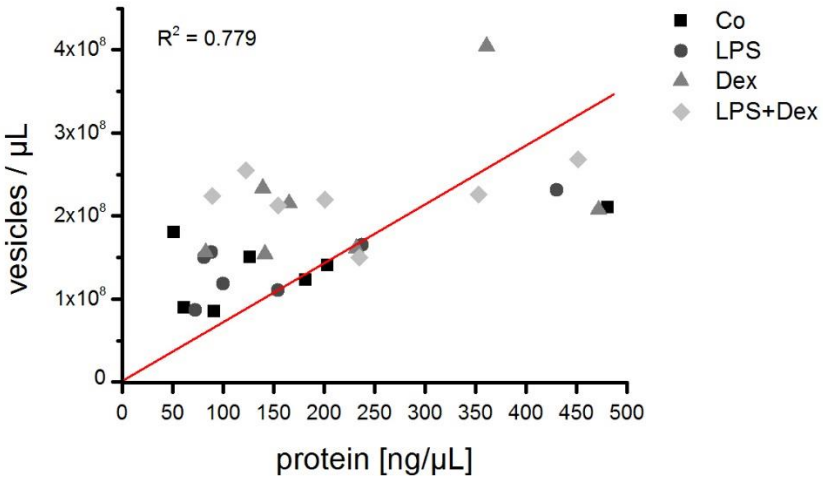


time [min]	buffer b (%)
0	4
95	40
120	55
129	90
130	50
133	50
138	90
139	4
162	4

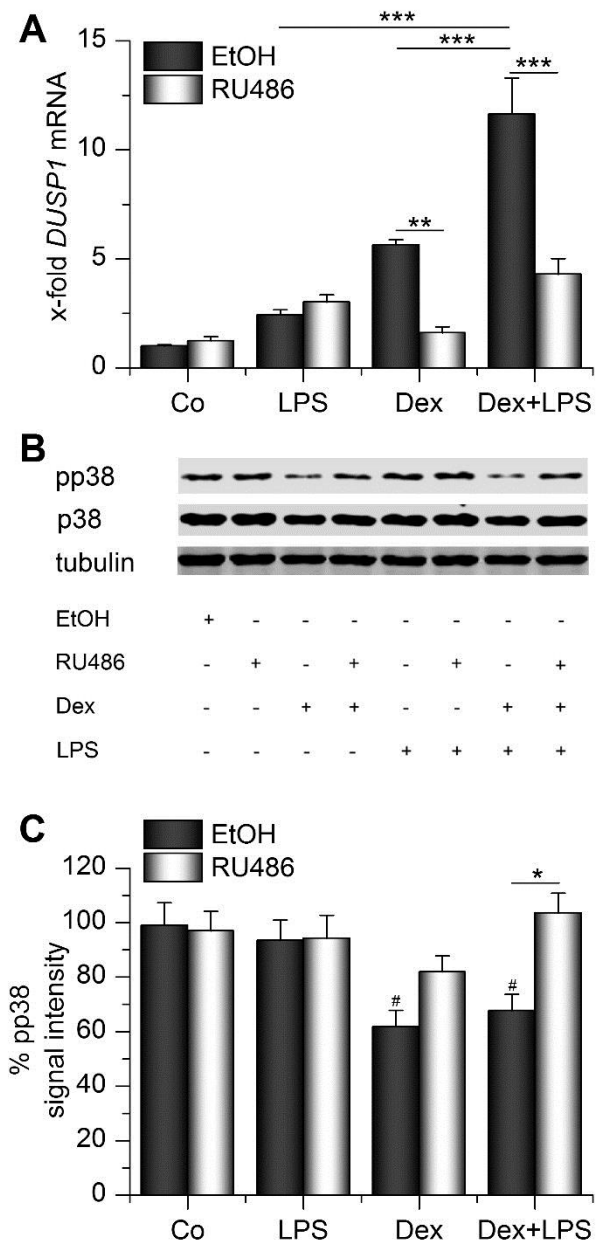
Supplementary Figure 2. *Gradient for nano-liquid chromatography.* Time points for changes in buffer B concentrations are given in the table.



Supplementary Figure 3: ADAM10 *and* ADAM17 *expression in AMs*. AMs were treated with vehicle (Co) or LPS (100 ng/ml) + Dex (1 µM) for 24 h. *ADAM10* and *ADAM17* expression was determined by qPCR, and values were normalized to the housekeeping gene *ACTB*. The means of 3 experiments performed in triplicate + SEM are shown. ***$p < 0.001$. p-values were generated by ANOVA with Bonferroni's post-hoc test.



Supplementary Figure 4: *Correlation between EV concentration and protein amount.* EVs were isolated from THP-1 macrophages treated with the vehicle control (0.1% DMSO, Co), LPS (100 ng/mL), Dex (1 µM) or LPS + Dex for 3 d in serum-free medium. Values for seven individual isolations are given. ■ = Co, ● = LPS, ▲ = Dex and ♦ = LPS+Dex.



Supplementary Figure 5: *Influence of dexamethasone and LPS on* DUSP1 *expression and p38 MAPK activation.* AMs were preincubated with the GR inhibitor RU486 (10 µM) or solvent control (0.1% EtOH) and treated with LPS (100 ng/mL), Dex (1 µM), or both for 24 h. A: *DUSP1* expression was measured by qPCR. B, C: Phospho-p38 and total p38 were analyzed by Western Blot. Tubulin served as a loading control. Data from at least three independent experiments performed in duplicate with cells from different donors are presented as means + SEM and A, C: *$p < 0.05$, **$p < 0.01$. ***$p < 0.001$. C: #$p < 0.05$ vs. vehicle-treated Co. p-values were generated by ANOVA with Bonferroni's post-hoc test.

Supplementary Tables

Supplementary Table 1: PCR conditions.

Gene	Sequence (5'→3') forward primer	Sequence (5'→3') reverse primer	Probe sequence (5'FAM→3'BHQ)	Probe [nM]	$MgCl_2$ [mM]	Annealing [°C]
ACTB	TGCGTGACATTAAGGAGAAG	GTCAGGCAGCTCGTAGCTCT	CACGGCTGCTTCCAGCTCCTC	60	4	60
ADAM10	TGCCCAGATATCCAGTCATGTT	TCACCATGAAACTGATGTTACGG	no probe	N/A	N/A	60
ADAM17	AGAGAACCACCTGAAGAGCTTG	TCCCCTCTGCCCATGTATCT	no probe	N/A	N/A	60
CCL2	TTGATGTTTTAAGTTTATCTTTCATGG	CAGGGGTAGAACTGTGGTTCA	no probe	N/A	N/A	60
CXCL10	GAGCCTACAGCAGAGGAACC	AAGGCAGCAAATCAGAATCG	TCCAGTCTCAGCACCATGAATCAAA	60	4	60
DUSP1	CAGCTGCTGCAGTTTGAGTC	AGGTAGCTCAGCGCACTGTT	no probe	N/A	N/A	64
FPR2	GCATCCTCAGGAAAATGCACC	GCATCCTCAGGAAAATGCACC	no probe	N/A	N/A	60
ICAM	GAAGTGGCCCTCCATAGACA	TCAAGGGTTGGGGTCAGTAG	no probe	N/A	N/A	60
IL10	CAACAGAAGCTTCCATTCCA	AGCAGTTAGGAAGCCCCAAG	AGCCTGACCACGCTTTCTAGCTGTTGAG	100	4	60

MMP9	CTTTGAGTCCGGTGGACGAT	TCGCCAGTACTTCCCATCCT	no probe	N/A	N/A	60
SELE	AGCCCAGAGCCTTCAGTGTA	CCCTGCATGTCACAGCTTTA	no probe	N/A	N/A	60
TNF	CTCCACCCATGTGCTCCTCA	CTCTGGCAGGGGCTCTTGAT	CACCATCAGCCGCATCGCCGTCTC	100	3	60
TLR1	AGCAAAGAAATAGATTACACATCA	TTACCTACATCATACACTCAAAT	ATTCCTCCTGTTGATATTGCTTTTG	60	5	57
TLR2	GCAAGCTGCGGAAGATAATG	CGCAGCTCTCAGATTTACCC	ATGGACGAGGCTCAGCGGGAAG	60	3	60
TLR4	ATGAAATGAGTTGCAGCAGA	AGCCATCTGTGTCTCCCTAA	AAGTGATGTTTGATGGACCTCTGAATCT	60	4	58
TLR6	TTTACTTGGATGATGGTGAATAGT	AGTTCCCCAGATGAAACATT	GTCGTAAGTAACTGTCTGGAGGTGC	100	5	57
VCAM	CGAGACCACCCCAGAATCTA	CTGTGGTGCTGCAAGTCAAT	no probe	N/A	N/A	60

Supplementary Table 2: Antibodies and TLR2 ligands for flow cytometry.

antibody / ligand	amount per sample	order no.	supplier
FITC anti-human CD9, Mouse IgG1, kappa [HI9a 25]	0.5 µg	BLD-312103	Biozol
FITC anti-human CD63, Mouse IgG1, [H5C6]	1 µg	BLD-353005	Biozol
FITC Mouse IgG1, kappa Isotype Ctrl (FC) [MOPC-21]	1 µg	BLD-400109	Biozol
APC anti-human TLR2 (CD282) Mouse IgG2a [TL2.1]	2 µg	17-9922-41	ThermoFisher
APC Mouse IgG2a kappa Isotype Control [eBM2a]	2 µg	17-4724-81	ThermoFisher
Rhodamine-conjugated Pam_3CSK_4	0.5 µg	tlrl-rpms	Invivogen