

GR-independent down-modulation on GM-CSF bone marrow-derived dendritic cells by the selective glucocorticoid receptor modulator Compound A

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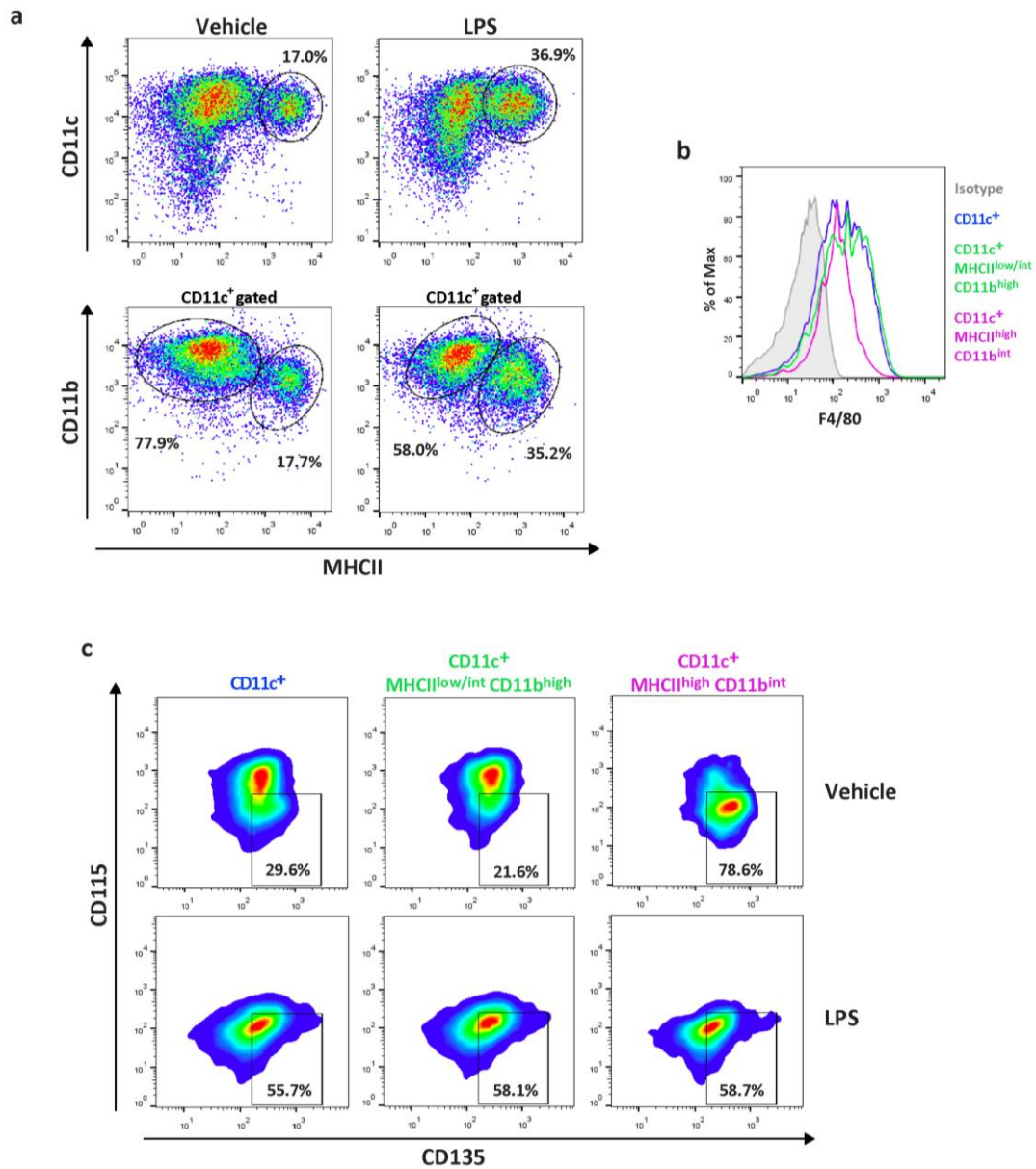
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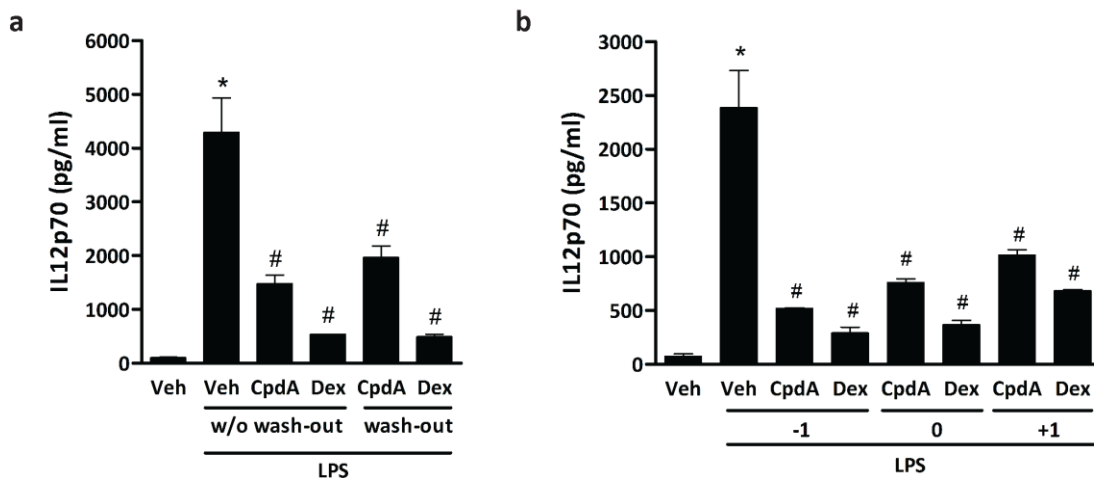
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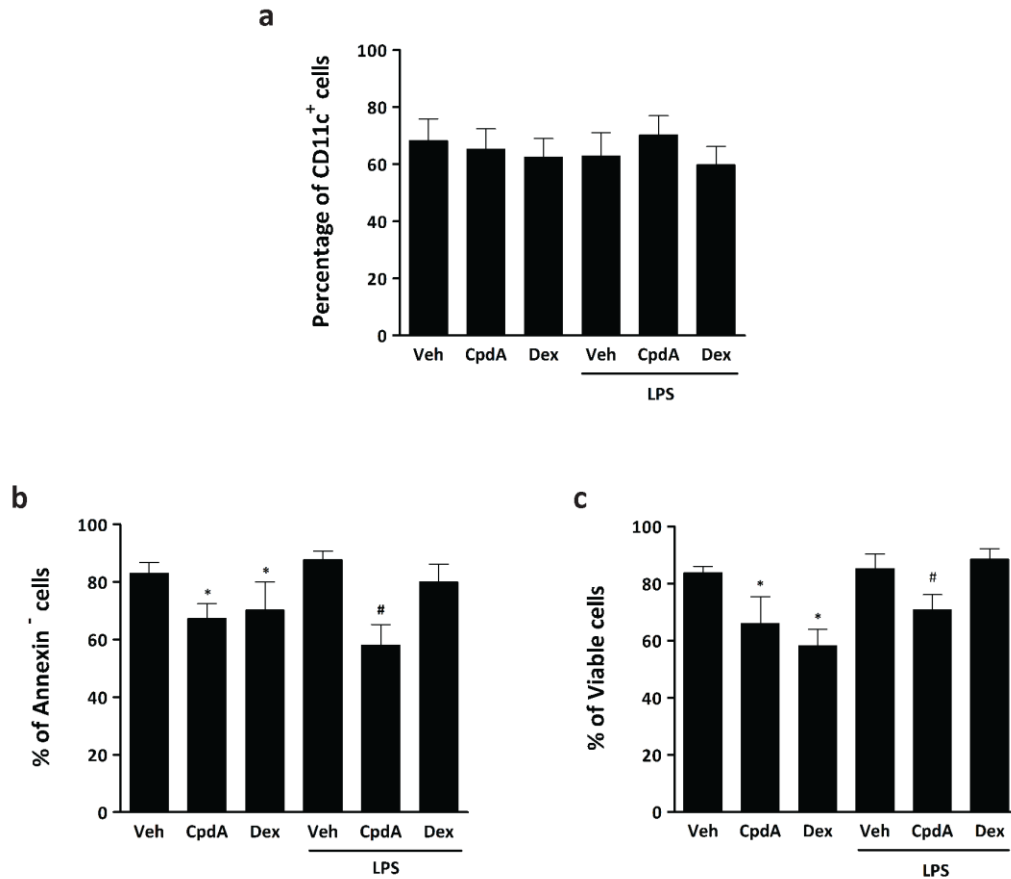
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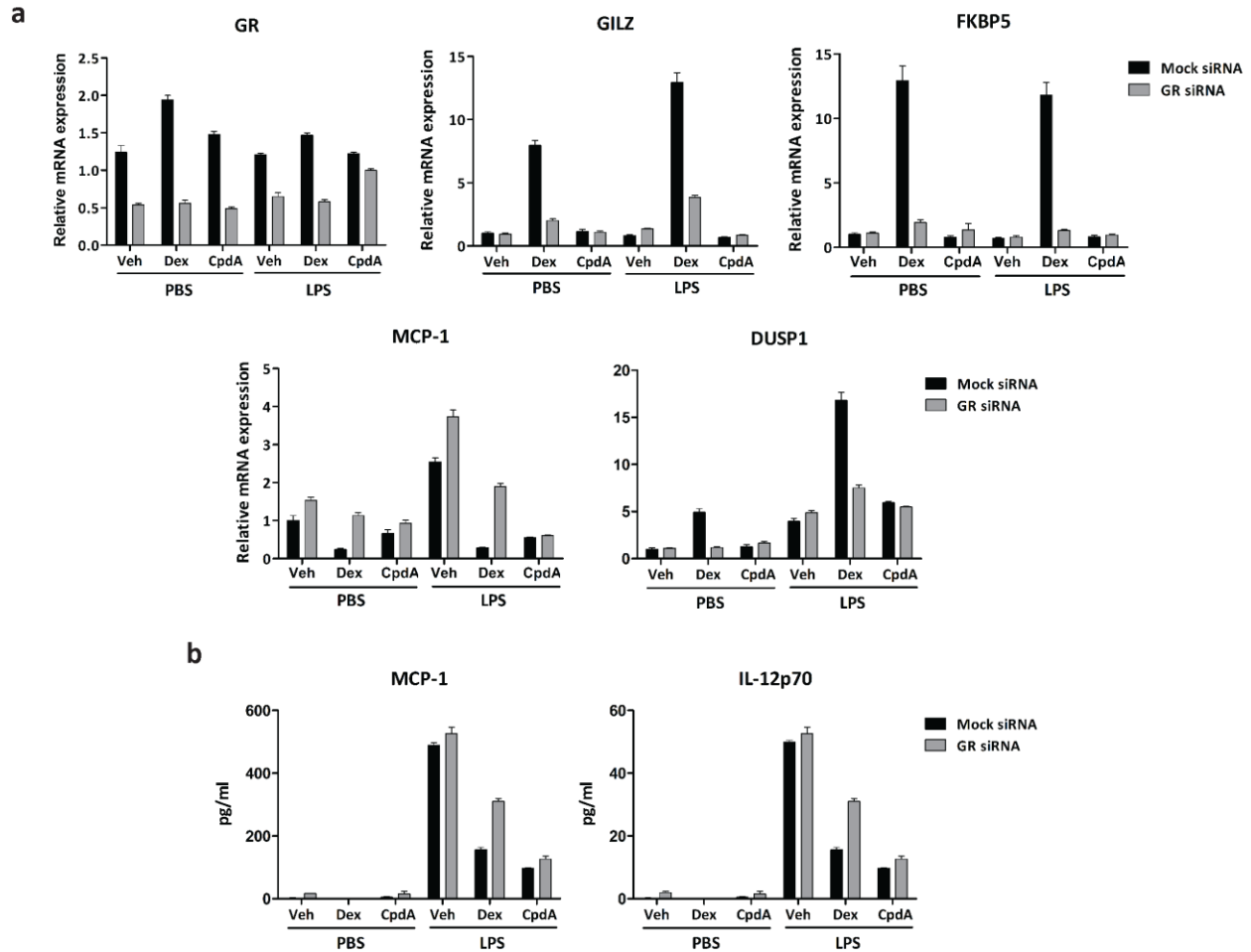
Supplementary Figure S1: Phenotype characterization of GM-CSF-conditioned bone marrow derived dendritic cells (BMDC). Representative flow cytometry analysis of GM-CSF+IL4-stimulated BM cultures harvested after 7 days in culture. **a)** CD11c⁺ cells were analyzed on the basis of CD11b and MHCII expression under basal and LPS-stimulated conditions. Circles enfold cell sub-populations and numbers indicate the percentage of cells in each population. **b)** Histograms showing the expression of F4/80 by CD11c⁺ (blue line), CD11c⁺MHCII^{low/int}CD11b^{high} (green line) and CD11c⁺MHCII^{high}CD11b^{int} (purple line) subpopulations. Filled histogram depicts isotype-irrelevant specificity control. **c)** CD115 and CD135 expression on CD11c⁺, CD11c⁺MHCII^{low/int}CD11b^{high} and CD11c⁺MHCII^{high}CD11b^{int} BMDC subpopulations. Boxes represent CD115⁺CD135⁺ subpopulations with corresponding percentage of cells.



Supplementary Figure S2: CpdA impairs IL12p70 secretion by BMDC. Evaluation of different treatment schemes. **a)** The transient presence of CpdA impairs IL12p70 by LPS-challenged BMDC. Cells were pretreated with vehicle, CpdA 10 μ M or Dex 0.1 μ M for 1h and then, washed exhaustively before they were challenged with LPS. After 24h, secreted IL12p70 was determined by ELISA. w/o wash-out (without wash-out). **b)** CpdA impairs IL12p70 secretion by BMDC when it was applied 1h before, simultaneously or 1h after LPS 1 μ g/ml. After 24h, secretion levels of IL12p70 were determined by ELISA. Data are shown as mean $\pm$ SD of triplicate determinations from one representative out of two independent experiments. (*) $p < 0.05$ vs. vehicle, (#) $p < 0.05$ vs. vehicle + LPS.



Supplementary Figure S3: Effect of CpdA on the recovery and viability of BMDC in culture. BMDC were pretreated with vehicle, CpdA 10 μ M or Dex 0.1 μ M for 1h and stimulated with 1 μ g/ml of LPS or left unstimulated. After 24h, **a)** the percentage of CD11c⁺ cells was analyzed by flow cytometry, **b)** the percentage of Annexin-V⁻ BMDC was analyzed by flow cytometry, **c)** the percentage of viable cells was determined by Trypan Blue exclusion. Data are shown as mean $\pm$ SD of at least four independent experiments. (*) $p < 0.05$ vs. vehicle, (#) $p < 0.05$ vs. vehicle + LPS.



Supplementary Figure S4: The effects of CpdA on BMDC are not affected by GR knockdown. For efficient RNA interference, 3×10^5 cells were transfected with 150 nM mouse GR siRNA (Dharmacon) or 150 nM non-silencing control scrambled (mock) siRNA (RL, Dharmacon) by using the Amaxa® Mouse Dendritic Cell Nucleofector® Kit (Lonza). Sixteen hours after transfection, cells were pre-treated with vehicle, CpdA 10 μ M or Dex 0.1 μ M for 1h before they were stimulated with LPS or PBS. **a)** RNA was isolated and levels of GR, GILZ, FKBP5, MCP-1 and DUSP1 mRNA were measured by qRT-PCR. **b)** supernatants were collected and secretion of MCP-1 and IL-12p70 was determined by ELISA. Data is presented as mean $\pm$ SD of different determinations from one representative (different than the presented in Fig. 6) out of three independent experiments.