Supplemental data

Figure S1. Bioenergetic profile: a schematic overview of the effects of mitochondrial inhibitors on the electron transport chain (A) Oligomycin is an ATP synthase inhibitor, Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) is a protonophore that uncouples ATP synthesis from the electron transport chain (ETC), rotenone is a Complex I inhibitor, and antimycin A is a Complex III inhibitor. (B) Using the XF-24 Extracellular Flux Analyzer, O_2 consumption rate (OCR) was measured prior to the addition of drugs (basal OCR) and then following the addition of the indicated drugs. Reduction in OCR after oligomycin indicates the amount of O_2 consumed for mitochondrial ATP generation. FCCP allows protons to flow back into the matrix independent of the ATP synthase; cells attempt to maintain the chemiosmotic gradient after FCCP by moving protons back out to the intermembrane space, which requires the use of the ETC and the consumption of O_2 as the final electron acceptor. After FCCP the maximum capacity of the mitochondria to use OXPHOS is revealed. Spare respiratory capacity (SRC) is the difference between maximal OCR and basal OCR and as such is an indicator of how close to its bioenergetic limit the cell is functioning.

Figure S2. Activation of DCs by a range of TLR agonists results in impairment of mitochondrial respiration, in iNOS expression and in NO production (A) DCs were seeded in a Seahorse XF-96 analyser, stimulated with indicated TLR ligands or medium for 24 h, and real time basal OCR was determined as well as in response to sequential treatments with oligomycin (ATP-synthase inhibitor), FCCP (uncoupler of OXPHOS), and antimycin-A/rotenone (electron transport chain inhibitors). Data represent mean ± SD of triplicates. One of three experiments is shown. (B) Intracellular iNOS expression in 24 h TLR-activated DCs was determined by FACS. One experiment of three is shown.

(C) Nitrite levels were determined in culture supernatants using the Griess assay 24 h after stimulation with indicated TLR agonists. Data represent mean ± SD of three experiments.

Figure S3. Neither TLR agonists nor cytokines induce iNOS expression in conventional splenic DCs Conventional splenic DCs were stimulated with TLR ligands or cytokines and 24 h later analysed for (A) iNOS expression and (B) surface expression of activation markers. While the stimulations variously resulted in upregulation of activation marker expression, none resulted in iNOS expression (B). Data represent mean ± SD of duplicates. One of two experiments is shown.

Fig S1

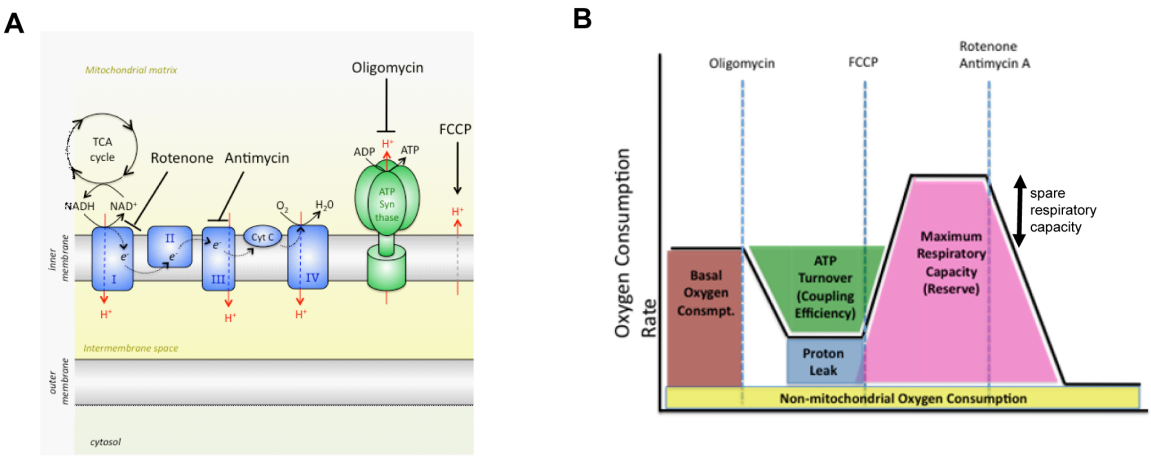
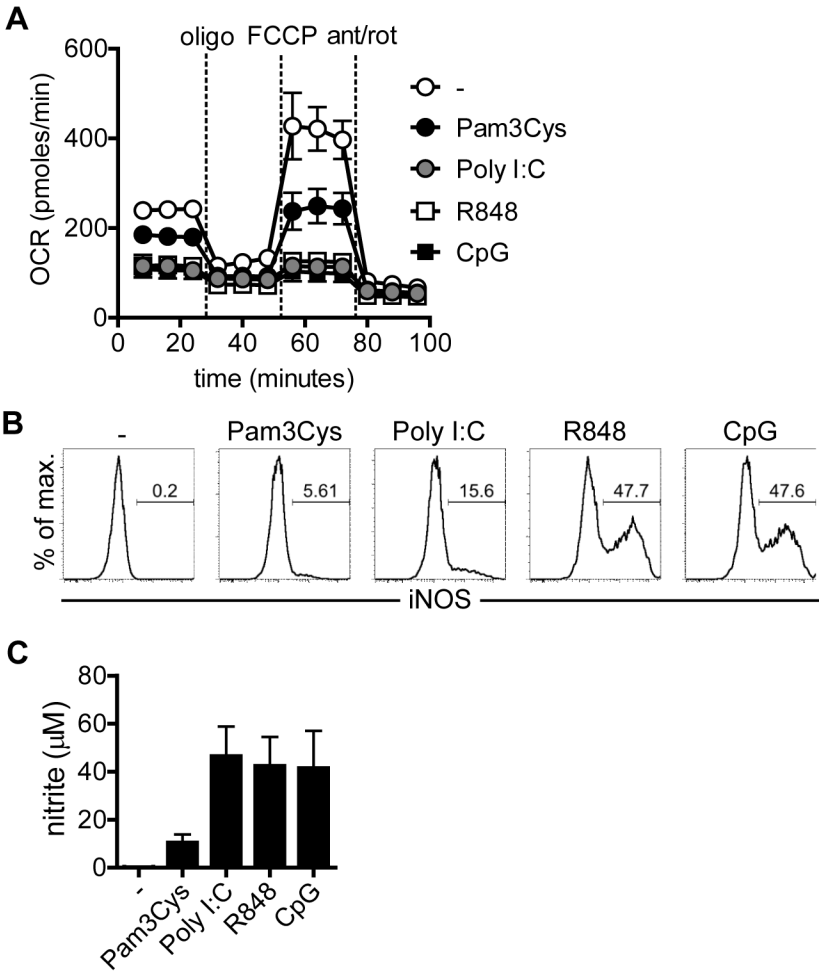

A
Mitochondrial matrix
Oligomycin
TCA cycle
Rotenone
Antimycin
FCCP
ADP
ATP
NADH
NAD+
ATP Syn thase
Cyt C
inner membrane
Intermembrane space
outer membrane
cytosol
B
Oligomycin
FCCP
Rotenone Antimycin A
Oxygen Consumption Rate
Basal Oxygen Consmpt.
ATP Turnover (Coupling Efficiency)
Proton Leak
Maximum Respiratory Capacity (Reserve)
spare respiratory capacity
Non-mitochondrial Oxygen Consumption

Fig S2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400
200
0
0 20 40 60 80 100
time (minutes)
-
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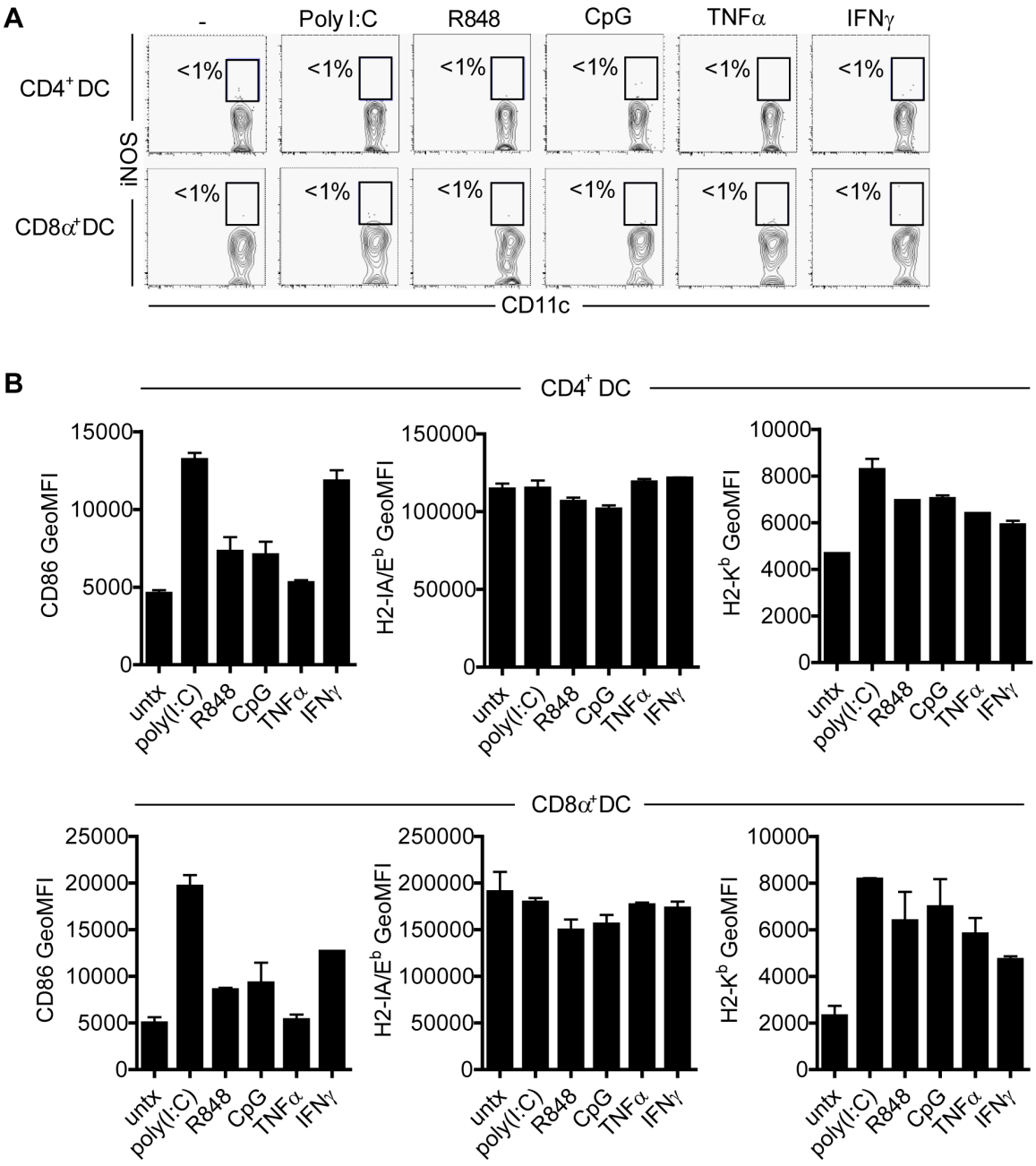
Poly I:C
R848
CpG
B
-
Pam3Cys
Poly I:C
R848
CpG
% of max.
0.2
5.61
15.6
47.7
47.6
iNOS
C
nitrite (μM)
80
60
40
20
0
-
Pam3Cys
Poly I:C
R848
CpG

Fig S3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R848
CpG
TNFα
IFNγ
CD4+ DC
CD8α+DC
iNOS
<1%
CD11c
B
CD4+ DC
CD8α+DC
CD86 GeoMFI
H2-IA/Eb GeoMFI
H2-Kb GeoMFI
untx
poly(I:C)
R848
CpG
TNFα
IFNγ