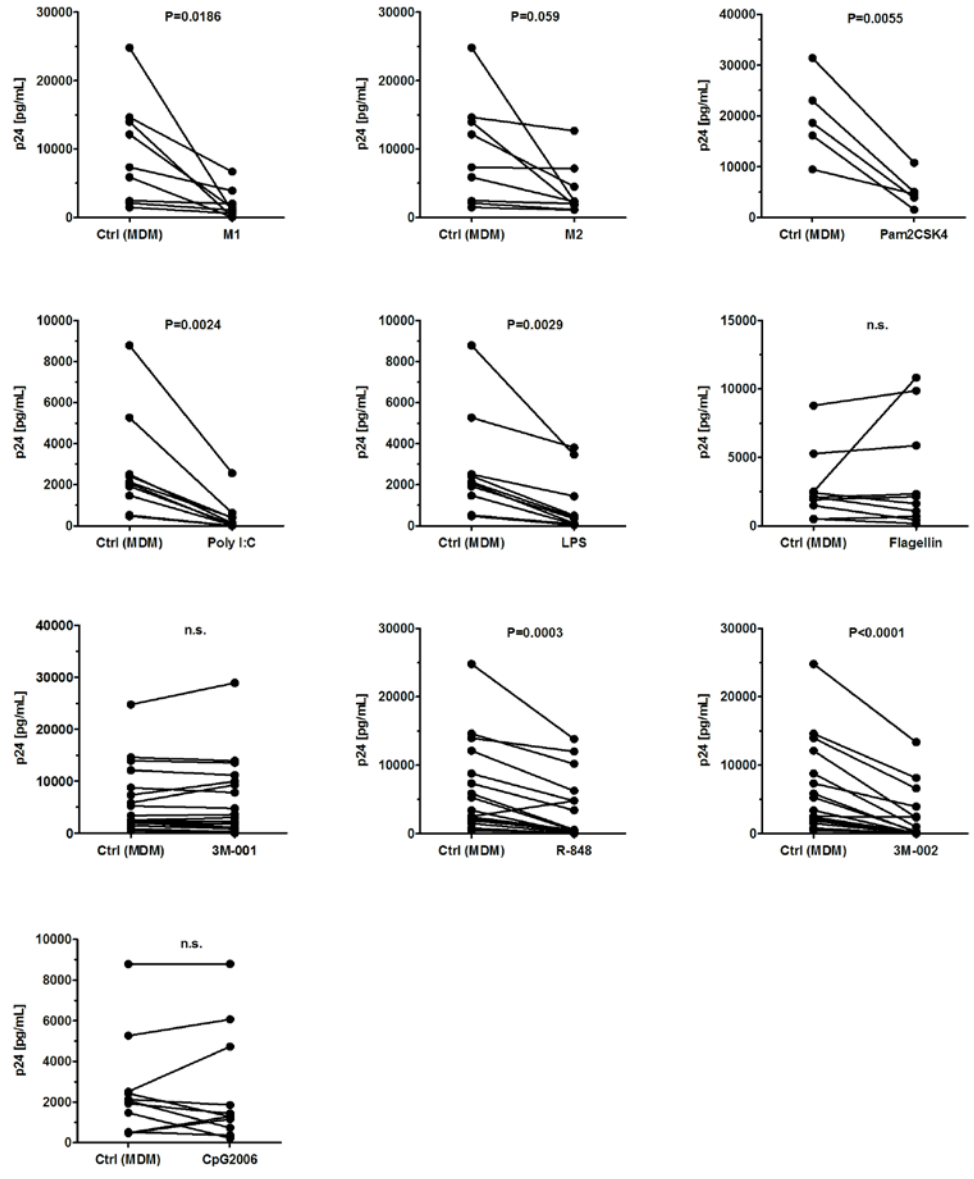
Supplementary Table

Suppl. Table 1: Polarization and priming of macrophages with the various TLR agonists did not affect the cell-surface expression of CD4 and CCR5 (mean fluorescence intensity, n=6)

	MDM	M1	M2	polyI:C	LPS	flagellin	3M-002
CD4	4.8±0.9	4.2±0.9	4.8±0.9	4.5±0.9	4.5±1.2	4.6±1.2	4.5±0.8
CCR5	22.2±9.	23.6±6.6	22.2±10.3	20.4±8.2	17.2±5.8	24.5±13.4	19.6±9.8

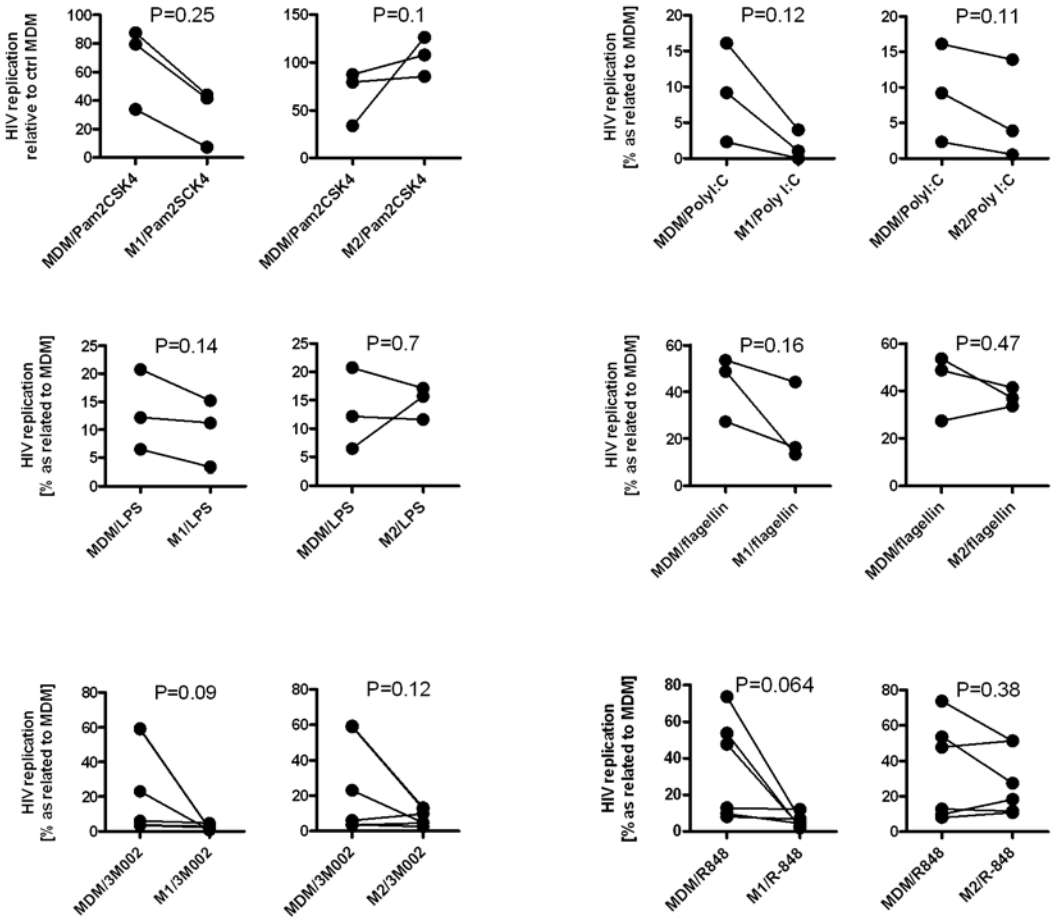
Supplementary Figures

Suppl. Fig. 1:



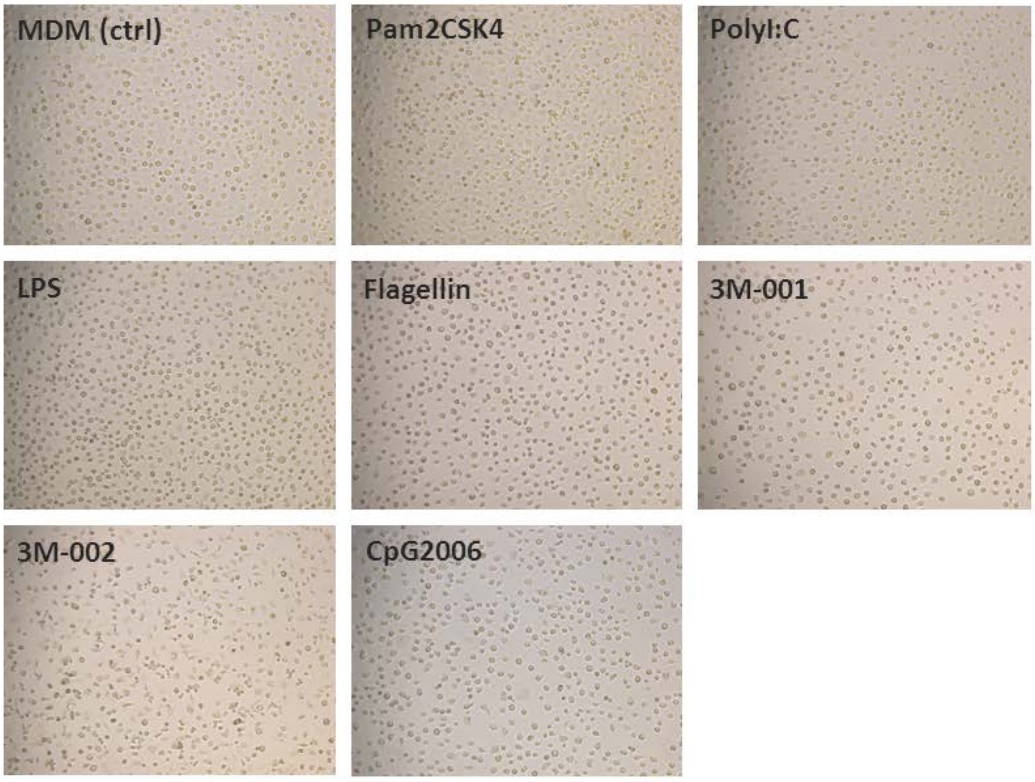
Suppl. Fig. 1: M1 polarization and triggering TLR2, 3, 4 and 8 rendered MDMs poorly permissive to HIV infection. Here we present the p24 Ag levels from day 7 after HIV infection of the experiment in Fig. 1B. In Fig. 1B, we presented the AUC of the p24 Ag over time of the individual experiment normalized to the untreated HVI-infected control. For the details of the experimental design, see legend to Fig. 1. Statistical analysis was done using paired T-test with two-tailed p-value. Pam2CSK4 triggers TLR2, PolyI:C TLR3, LPS TLR4 , flagellin TLR5, 3M-001 TLR7, 3M-002 TLR8, ккR-848 TLR7/8 and CpG2006 TLR9.

Suppl. Fig. 2:



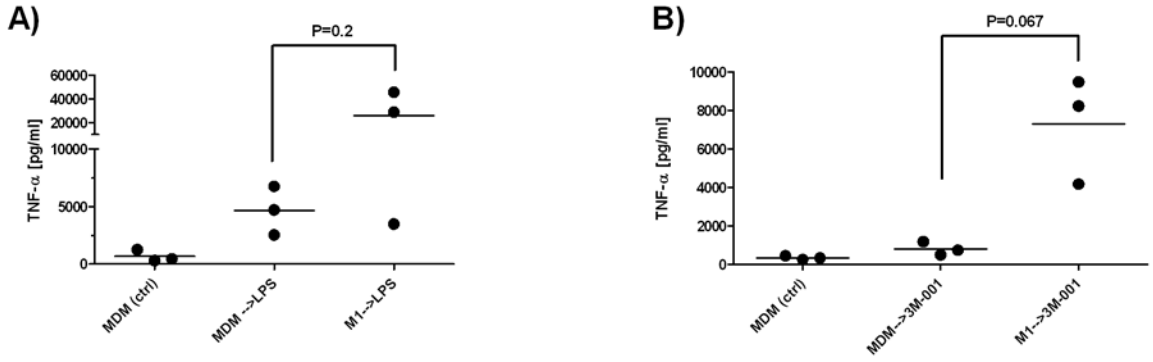
Suppl. Fig. 2: TLR triggering reinforces the low permissiveness of polarized macrophages. The effects of TLR agonists on HIV inhibition were compared in matched specimens with and without polarization. Macrophages were treated as described in Fig. 1 with the indicated TLR agonists (for acronyms, see Suppl. Fig. 1) and infected with YU-2. Statistics used paired T-test with two-tailed p-value.

Suppl. Fig.3:



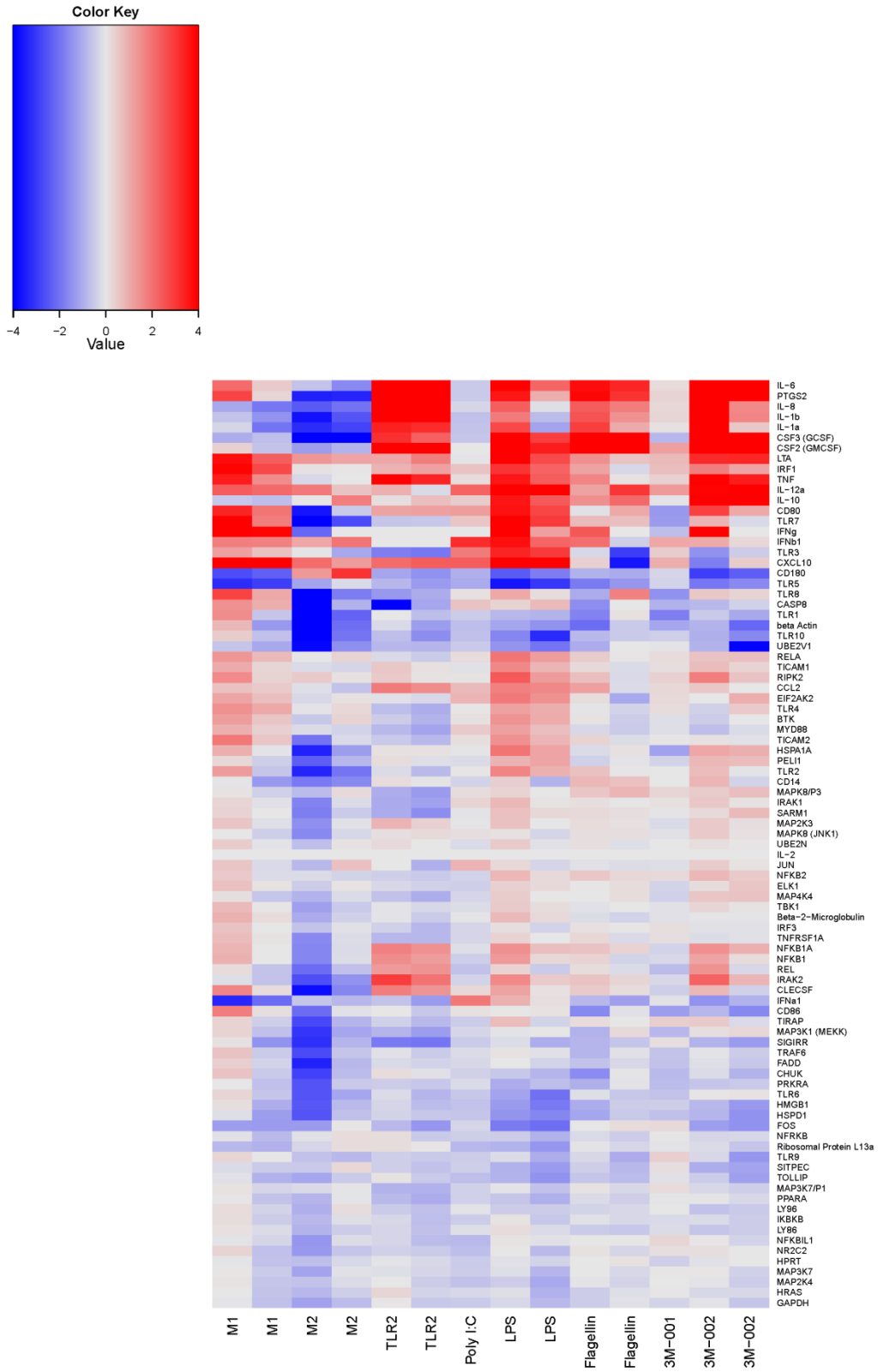
Suppl. Fig. 3: Macrophages lost no viability for 7 days when cultured with the distinct TLR agonists as indicated.

Suppl. Fig. 4:



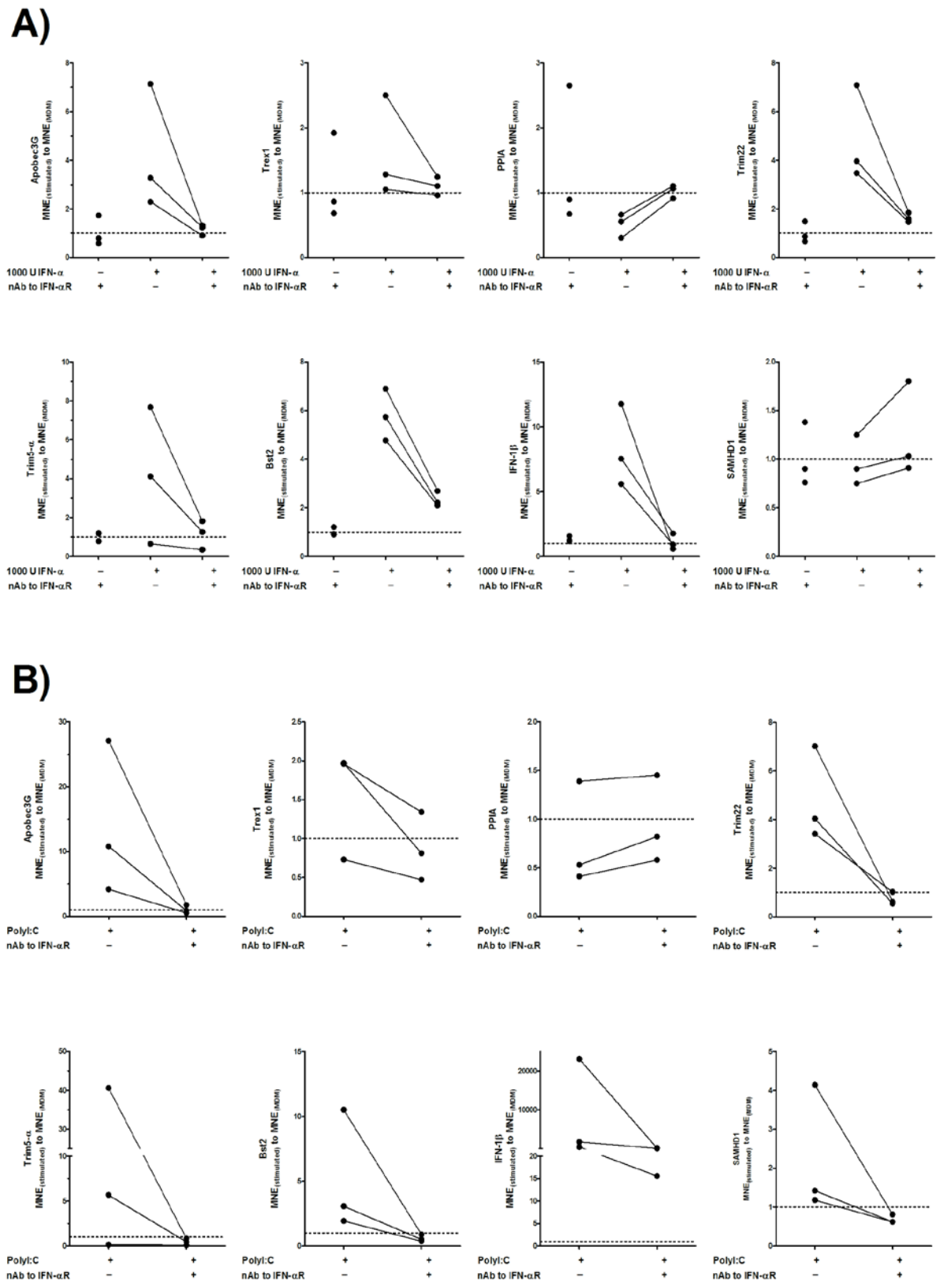
Suppl. Fig. 4: M1-polarized macrophages had increased reactivity in response to LPS or 3M-001 (TLR7 agonist). Polarized macrophages were exposed to either LPS (A) or 3M-001 (B) for 24 hours. Supernatants were then harvested and analyzed for TNF-α; (for abbreviations, see Fig. 1). Statistics done using paired T-test with two-tailed p-value.

Suppl. Fig. 5:


Color Key
−4
−2
0
2
4
Value
IL−6
PTGS2
IL−8
IL−1b
IL−1a
CSF3 (GCSF)
CSF2 (GMCSF)
LTA
IRF1
TNF
IL−12a
IL−10
CD80
TLR7
IFNg
IFNb1
TLR3
CXCL10
CD180
TLR5
TLR8
CASP8
TLR1
beta Actin
TLR10
UBE2V1
RELA
TICAM1
RIPK2
CCL2
EIF2AK2
TLR4
BTK
MYD88
TICAM2
HSPA1A
PELI1
TLR2
CD14
MAPK8/P3
IRAK1
SARM1
MAP2K3
MAPK8 (JNK1)
UBE2N
IL−2
JUN
NFKB2
ELK1
MAP4K4
TBK1
Beta−2−Microglobulin
IRF3
TNFRSF1A
NFKB1A
NFKB1
REL
IRAK2
CLECSF
IFNa1
CD86
TIRAP
MAP3K1 (MEKK)
SIGIRR
TRAF6
FADD
CHUK
PRKRA
TLR6
HMGB1
HSPD1
FOS
NFRKB
Ribosomal Protein L13a
TLR9
SITPEC
TOLLIP
MAP3K7/P1
PPARA
LY96
IKBKB
LY86
NFKBIL1
NR2C2
HPRT
MAP3K7
MAP2K4
HRAS
GAPDH
M1
M1
M2
M2
TLR2
TLR2
Poly I:C
LPS
LPS
Flagellin
Flagellin
3M−001
3M−002
3M−002

Suppl. Fig. 5: M1- and M2-polarized macrophages and MDMs treated for 10 hours with polyI:C, LPS, flagellin, 3M-001 or 3M-002 were subjected to gene profiling with the "Toll-Like Receptor Signaling Pathway PCR Array" from SABiosciences Qiagen. (For MDM primed by either Pam2CSK4, LPS, flagellin, 3M-002 and M1- and M2-polarized macrophages n=2 (D1=donor 1; D2=donor 2), for MDMs primed by either polyI:C or 3M-001 n=1. M1 and M2 indicate M1- and M2-polarized macrophages, respectively). Heat maps were done using R/Bioconductor package gplots. The values are given in log2-ratios (i.e., -1 corresponds to a twofold down-regulation, 0 means constant expression and 1 corresponds to twofold up-regulation) (for abbreviations, see Fig. 1).

Suppl. Fig. 6


A)
Apobec3G
Trex1
PPIA
Trim22
Trim5-α
Bst2
IFN-1β
SAMHD1
1000 U IFN-α
nAb to IFN-αR
B)
Poly I:C
nAb to IFN-αR

Suppl. Fig. 6: The antibody to IFN-αR (from Alexis #PBL21385) was highly efficient in blocking the IFN pathway. MDMs were pre-treated with the neutralizing antibody to IFN-αR at a concentration of 2 μg/ml for 2 hours followed by adding either 100 U/ml of IFN type 1 (Universal type 1 IFN, PBL, product number 11200-1, PBL Interferon Source, Piscataway, NJ 08854 USA) (A) or PolyI:C (B) and thus resulting in a final concentration of the neutralizing antibody to IFN-αR of 1 μg/ml. Controls were handled identically but without pretreatment with the neutralizing antibody to IFN-αR. 24 hours later MDMs were harvested for quantifying the ISGs IFIT1 and RSAD2 (A) or the various HIV restriction factors (B). All values were expressed as a ratio between stimulated and unstimulated MDMs.