Supporting Information MATERIALS AND METHODS

siRNA transfections

siRNA transfections of HMG cells with silencer select siRNA (Thermo Fisher Scientific Cat# 4392420) for *TLR7* (Assay ID s27842), *TLR8* (Assay ID s27920) or negative control siRNA (Thermo Fisher Scientific Cat# 4390843) were performed according to manufacturer's protocol. Two days later cells were analyzed for target gene silencing by qPCR analysis using TaqMan Gene expression assay (Applied Biosystems Cat# 4331182) for *TLR7* (Hs01933259_s1) and *TLR8* (Hs00152972_m1) and used in experiments.

Cycloheximide chase assay

HMG cells were seeded at a density of $2.5x10^5$ cells /well in a 48 well plate. At day 6, cells were transfected with ssRNA40/lyovec for 24h and 48h. For inhibition of protein synthesis, cycloheximide (1μM, Cell Signaling Technology Cat# 2112S) was added to the culture medium in the presence of ssRNA40 at different time points (0h, 6h, 12h, 24h) post ssRNA40 treatment. For 0h and 6h cycloheximide co-treatment, HMG cells were chased for 24h, and for 12h and 24h cycloheximdie co-treatment, cells were chased for 48h post ssRNA40 treatment. At 24h and 48h cells were harvested and lysates were assessed for OPTN, SQSTM1 and NDP52 expression and turnover by immunoblotting.

Supporting Information FIGURE LEGENDS

Supporting Information Figure S1. HIV ssRNA mediated inflammasome activation results in reduced autophagy/mitophagy in microglia cells. Relative fold change (densitometric analysis) in autophagy receptors NDP52, OPTN, SQSTM1 (a), core autophagy proteins LC3B, BECN1 (b) and mitophagy associated proteins pTBK1, and Parkin (c) in HMG cells following incubation with ssRNA40 for 24h and 48h. Results are presented as mean ± SD; n=3 *P<0.05.

Supporting Information Figure S2. HIV ssRNA mediated inflammasome activation results in reduced turnover of autophagy receptors in microglia cells. Protein synthesis is inhibited in ssRNA40 (5μg/mL) treated HMG cells by adding cycloheximide (CHX, 1μM) at different time points (0h, 6h, 12h and 24h) post ssRNA40 treatment. Expression of OPTN, SQSTM1 and NDP52 is compared to untreated ssRNA40 exposed HMG cells and cycloheximide (0h, 6h) co-treated HMG cells at 24h, and untreated ssRNA40 exposed HMG cells and cycloheximide (12h, 24h) co-treated cells at 48h by immunoblotting. *Top*, representative immunoblots showing expression of autophagy receptors OPTN, p62/SQSTM1 and NDP52 in untreated and ssRNA40 treated HMG cells at 24h and 48h with or without cycloheximide. *Bottom*, relative fold change (densitometric analysis) in expression of OPTN, p62/SQSTM1 and NDP52 in untreated and ssRNA40 treated HMG cells at 24h and 48h with or without cycloheximide. Results are presented as mean ± SD; n=3, *P<0.05.

Supporting Information Figure S3. **ssRNA40 activates NLRP3 inflammasome activation via TLR7 and TLR8.** HMG cells transfected with non-specific control siRNA (siControl), *TLR7* siRNA (si*TLR7*), *TLR*8 siRNA (si*TLR8*) or both si*TLR7* and si*TLR8* (si*TLR7/8*) were treated for 24h with vehicle or ssRNA40 (5μg/mL). (a) Cells were lysed and qPCR analysis was performed to quantitate the relative expression of *TLR7* and *TLR8* mRNA. (b) Culture supernatants were analyzed for IL-1β release by ELISA. (c) Cells were lysed and immunoblotting was performed with antibodies to LC3B-II, OPTN, SQSTM1 and β-Actin. *Top*, representative immunoblots of LC3B, OPTN, SQSTM1and β-Actin. *Bottom*, relative fold change (densitometric analysis) in expression of LC3B-II, OPTN and p62/SQSTM1. Results are presented as mean ± SD; n=3, *P<0.05.