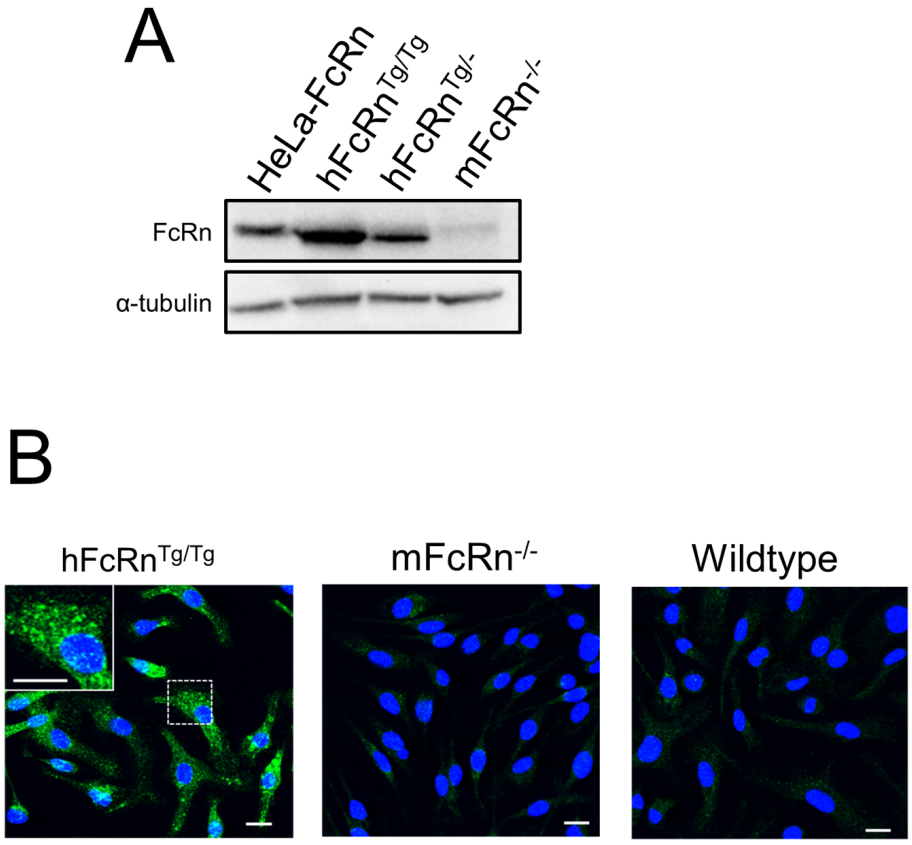


Figure S1 Analysis of BMDM from mFcRn$^{-/-}$ and hFcRn$^{Tg/Tg}$ mice

(A) *Immunoblotting*: BMDMs from either mFcRn-/-, hFcRn$^{Tg/Tg}$ or hFcRn$^{Tg/-}$ mice, as indicated, and HeLa cells stabling expressing hFcRn were lysed in reducing buffer and extracts resolved on 10 % PAGE gels. Proteins were then transferred onto PVDF membranes and probed with antibodies to human FcRn (rabbit polyclonal antibody (HPA012122), and mouse anti-α-tubulin antibodies, using a chemiluminescence detection system.

(B) *Detection of hFcRn in BMDMs by immunofluorescence:* BMDMs derived from mFcRn-/-, hFcRn$^{Tg/Tg}$ and wild type mice were fixed, stained for human FcRn with rabbit polyclonal antibody (HPA012122), followed by Alexa Fluor 488-conjugated anti-rabbit IgG. Nuclei were stained with DAPI (blue). Bars represent 5µm.

(C) *Alexa488-HSA internalisation in BMDMs:* FACS analysis to quantify the internalisation of Alexa488-HSA(HSA-AF488) in BMDMs derived from hFcRn$^{Tg/Tg}$ or mFcRn-/- mice. BMDM were incubated with HSA-AF488 for 15 min. Note the similar level of HSA-AF488 uptake by both hFcRn$^{Tg/Tg}$ and mFcRn-/- BMDMs.

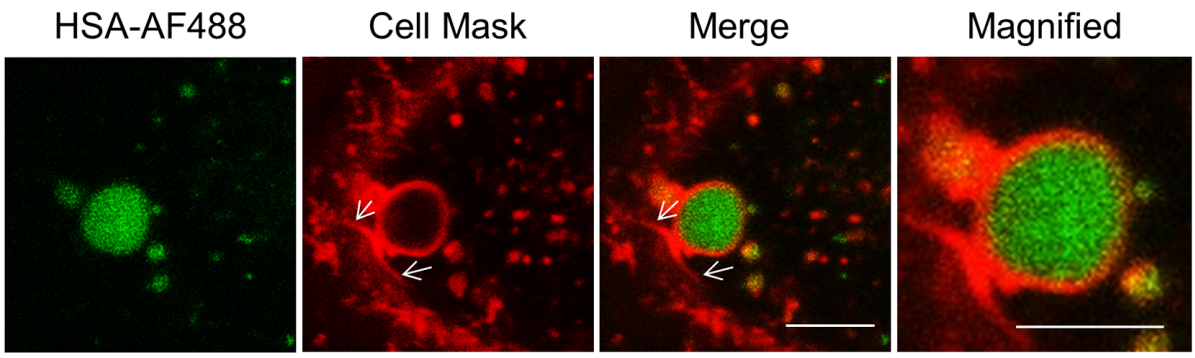
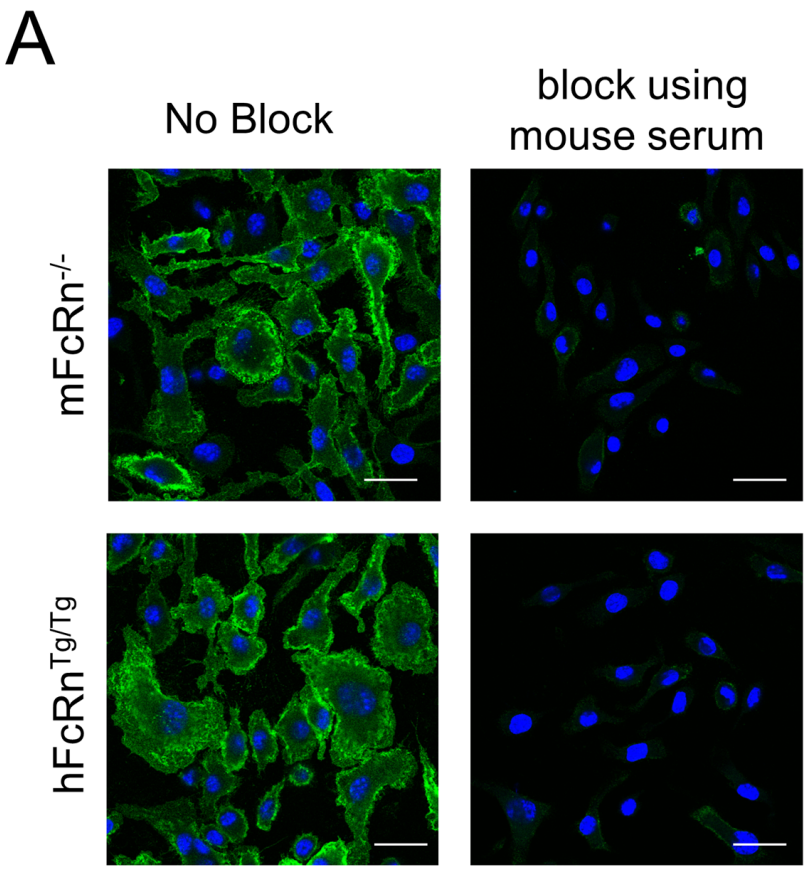


Figure S2: HSA-AF488 does not colocalise with tubules emanating from macropinosomes in BMDM from mFcRn$^{-/-}$ mice

mFcRn$^{-/-}$ BMDMs were plated in 8 well chamber slides and rendered quiescent by culturing overnight in C-RPMI in the absence of mCSF-1. BMDM were then incubated in Cell Mask Deep Red (1/1000 dilution), to stain the PM, and 100 µg/mL HSA-AF488 and in Leibovitz's media supplemented with mCSF-1 for 10 min. BMDM were washed in PBS and cells were imaged live in Leibovitz's media using an SP8 confocal microscope over 20 min. Images shown were snapshots of HSA-AF488 and Cell Mask Deep Red in mFcRn$^{-/-}$ BMDMs. Bar represents 10 µm.



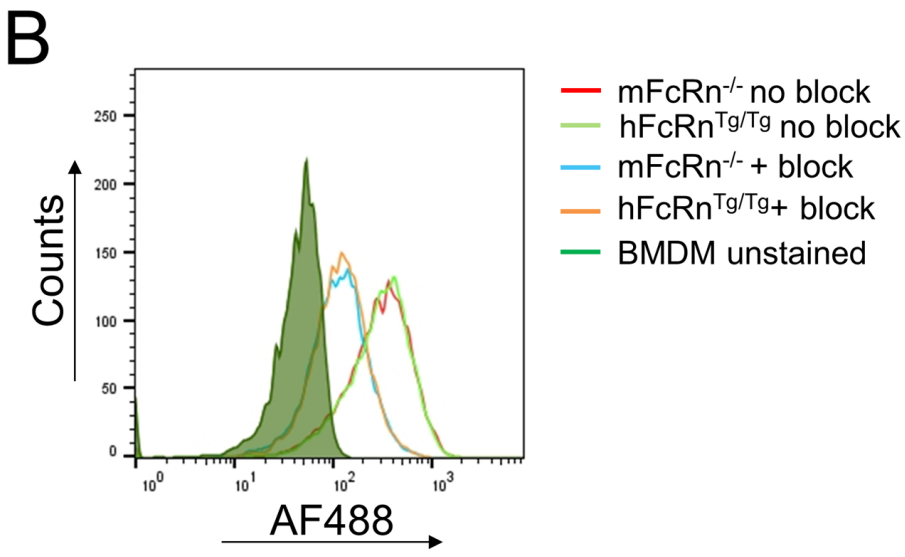
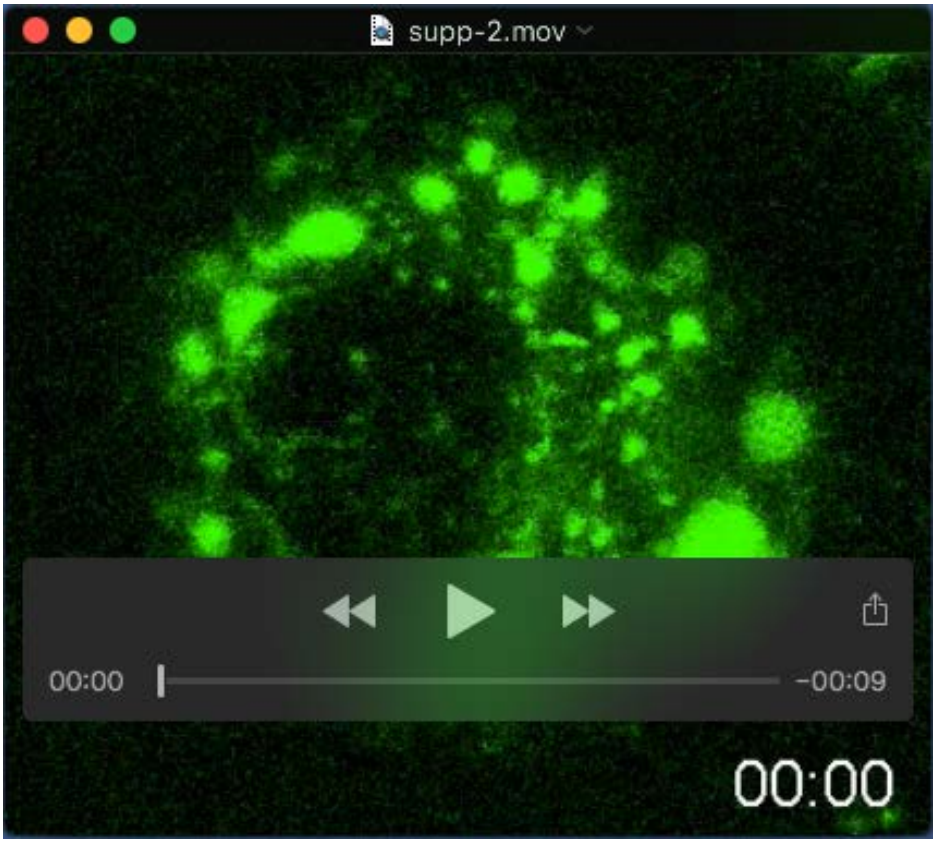


Figure S3: Mouse serum blocks IgG binding to FcγR on cell surface

(A-B) mFcRn$^{-/-}$ and hFcRn$^{Tg/Tg}$ BMDMs were plated in 8 well chamber slides and BMDMs were plated in 8 well chamber slides and rendered quiescent by culturing overnight in C-RPMI in the absence of mCSF-1. To block FcγR, BMDM were incubated with mouse serum (1/100 dilution in C-RPMI) for 30 min on ice and washed with PBS. BMDM were then incubated with 100 µg/mL IgG-AF488 in the presence or absence of mouse serum in serum-free media supplemented with mCSF-1 for 15 min. BMDM were then washed and fixed in 4% PFA.

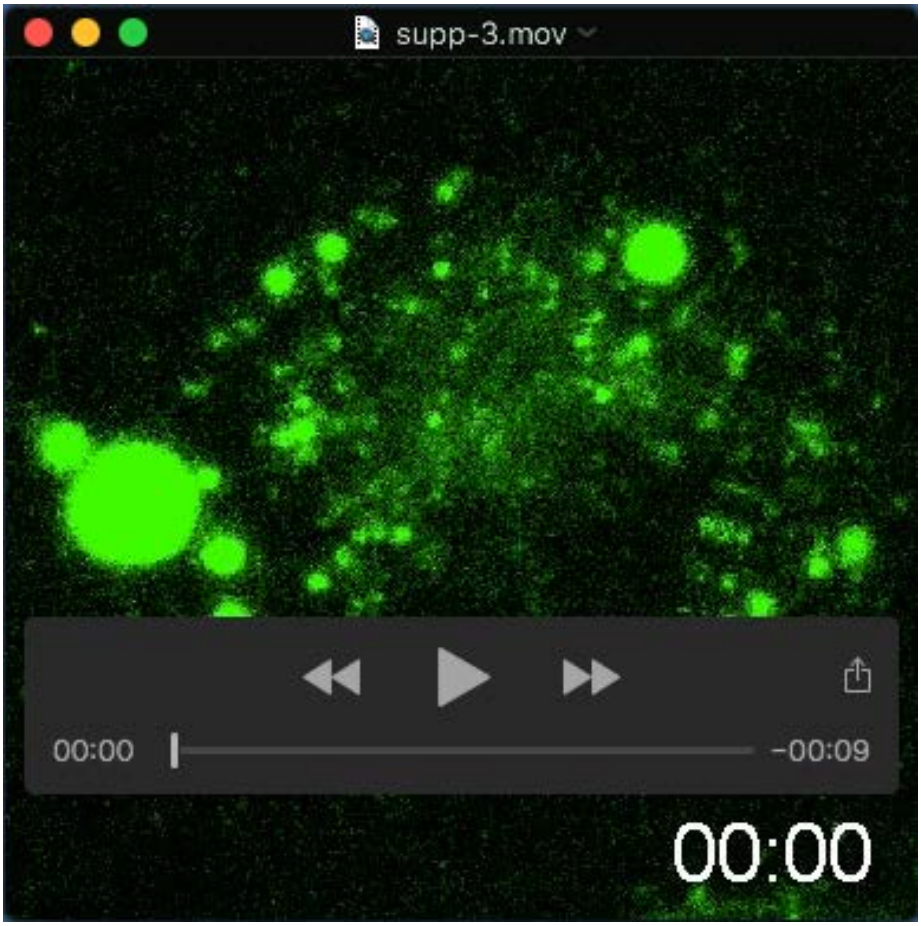
(A) Confocal images of IgG-AF488 (green) and DAPI (blue) in mFcRn$^{-/-}$ and hFcRn$^{Tg/Tg}$ BMDMs in control conditions and FcγR block conditions. Bars represent 10 µm.

(B) mFcRn$^{-/-}$ and hFcRn$^{Tg/Tg}$ BMDMs containing IgG-AF488 under control condition and FcγR block condition. Total fluorescence was analysed by FACS and histograms were overlaid in Flowjo.



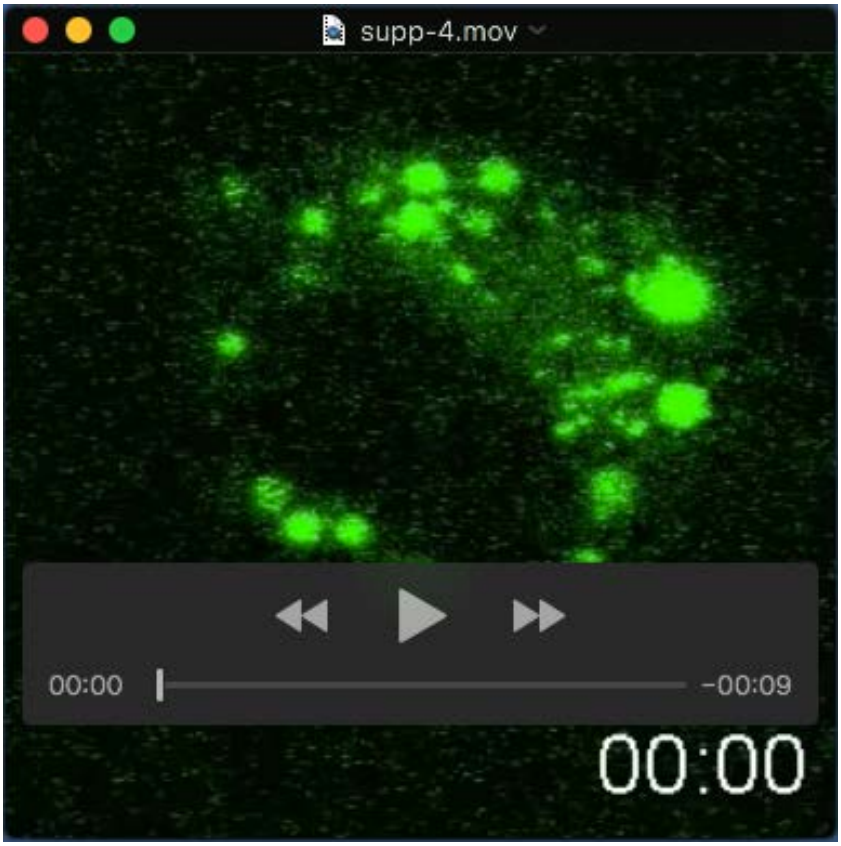
Movie 1: HSA-AF488 in hFcRn$^{Tg/Tg}$ BMDM

hFcRn$^{Tg/Tg}$ BMDMs were plated in 35mm Ibidi dishes and rendered quiescent by culturing overnight in C-RPMI in the absence of mCSF-1. BMDM were then incubated in 100 µg/mL HSA-AF488 in Leibovitz's media supplemented with 50 ng/mL mCSF-1 for 10 min. BMDM were washed in PBS and cells were imaged live in Leibovitz's media using an SP8 confocal microscope over 20 min. The delay between image capture was 12 sec.



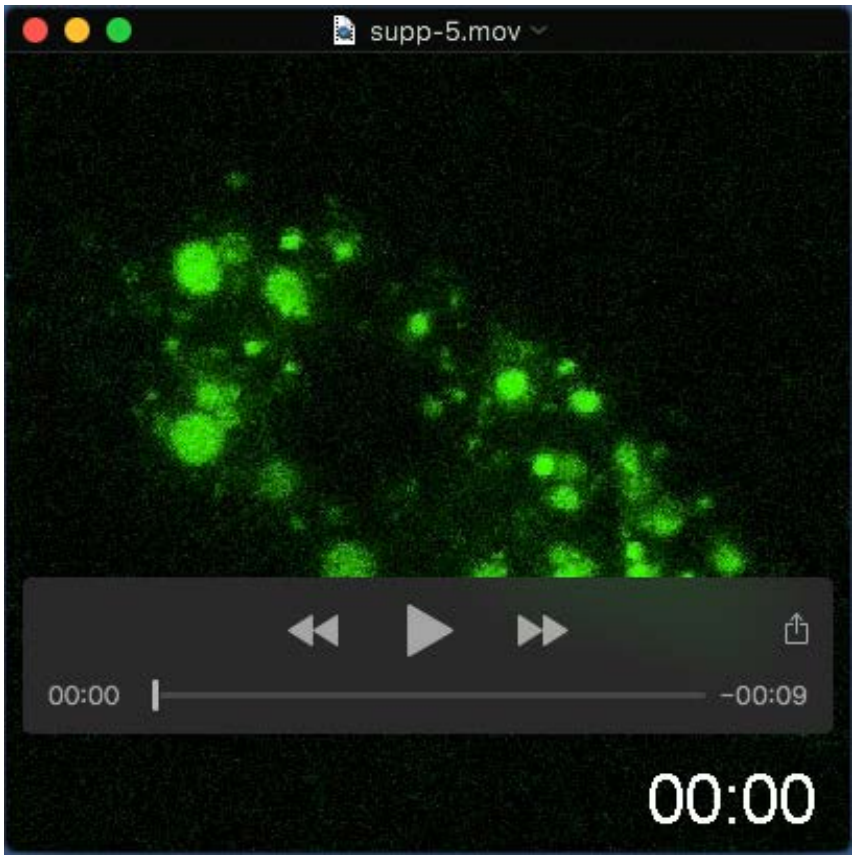
Movie 2: HSA-AF488 in mFcRn$^{-/-}$ BMDM

mFcRn$^{-/-}$ BMDMs were plated in 35mm Ibidi dishes and rendered quiescent by culturing overnight in C-RPMI in the absence of mCSF-1. BMDM were then incubated in 100 µg/mL HSA-AF488 in Leibovitz's media supplemented with 50 ng/mL mCSF-1 for 10 min. BMDM were washed in PBS and cells were imaged live in Leibovitz's media using an SP8 confocal microscope over 20 min. The delay between image capture was 12 sec.



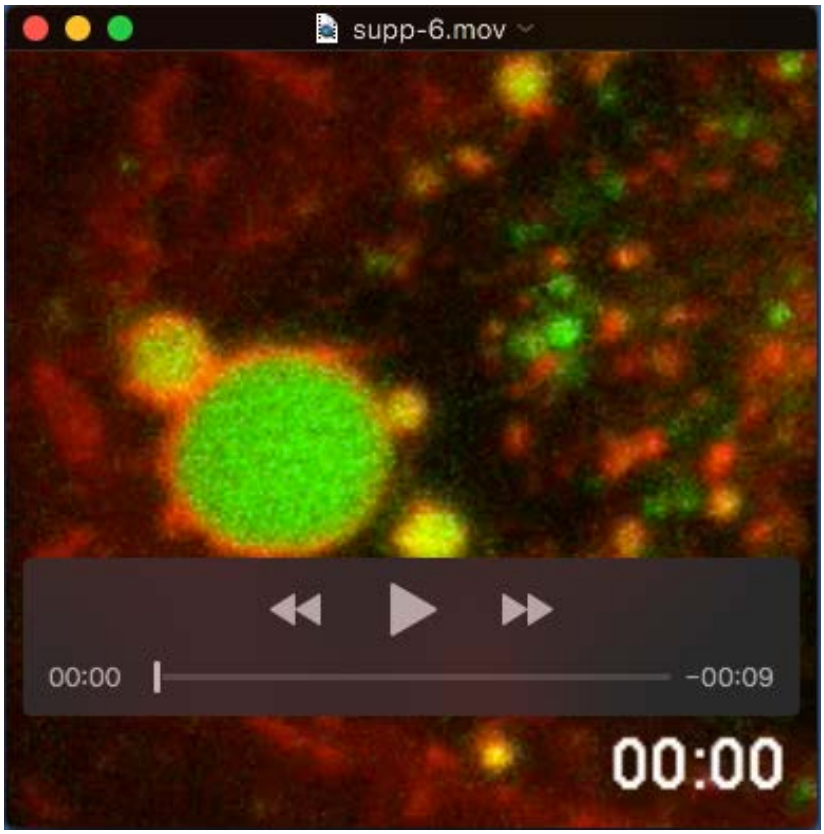
Movie 3: rHSA^{H464Q}-AF488 in hFcRn$^{Tg/Tg}$ BMDM

hFcRn$^{Tg/Tg}$ BMDMs were plated in 35mm Ibidi dishes and rendered quiescent by culturing overnight in C-RPMI in the absence of mCSF-1. BMDM were then incubated in 100 µg/mL rHSA^{H464Q}-AF488 in Leibovitz's media supplemented with 50 ng/mL mCSF-1 for 10 min. BMDM were washed in PBS and cells were imaged live in Leibovitz's media using an SP8 confocal microscope over 20 min. The delay between image capture was 12 sec.



Movie 4: rHSA^{H464Q}-AF488 in mFcRn$^{-/-}$ BMDM

mFcRn$^{-/-}$ BMDMs were plated in 35mm Ibidi dishes and rendered quiescent by culturing overnight in C-RPMI in the absence of mCSF-1. BMDM were then incubated in 100 µg/mL HSA^{H464Q}-AF488 in Leibovitz's media supplemented with 50 ng/mL mCSF-1 for 10 min. BMDM were washed in PBS and cells were imaged live in Leibovitz's media using an SP8 confocal microscope over 20 min. The delay between image capture was 12 sec.



Movie 5: HSA-AF488 in mFcRn$^{-/-}$ BMDM labelled with CellMask Deep Red

mFcRn$^{-/-}$ BMDMs were plated in 35mm Ibidi dishes and rendered quiescent by culturing overnight in C-RPMI in the absence of mCSF-1. BMDM were then incubated in 100 µg/mL HSA-AF488 and cell mask deep red (1/1000 dilution) in Leibovitz's media supplemented with mCSF-1 for 10 min. BMDM were washed in PBS and cells were imaged live in Leibovitz's media using an SP8 confocal microscope over 20 min. The delay between image capture was 12 sec.