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Supplemental information

Discovery of a novel SHIP1 agonist that promotes degradation of lipid-laden phagocytic cargo by microglia

Chiara Pedicone, Sandra Fernandes, Alessandro Matera, Shea T. Meyer, Stewart Loh, Jeung-Hoi Ha, Denzil Bernard, John D. Chisholm, Rosa Chiara Paolicelli, and William G. Kerr

Compound	MW	CLogP[a]	CLogS[b]	Calculated Water Solubility
Pelorol	372.50	6.31	-6.16	0.000259 mg/mL
AQX-MN100	312.49	7.18	-6.49	0.000100 mg/mL
K306	493.62	1.88	-3.83	0.0733 mg/mL

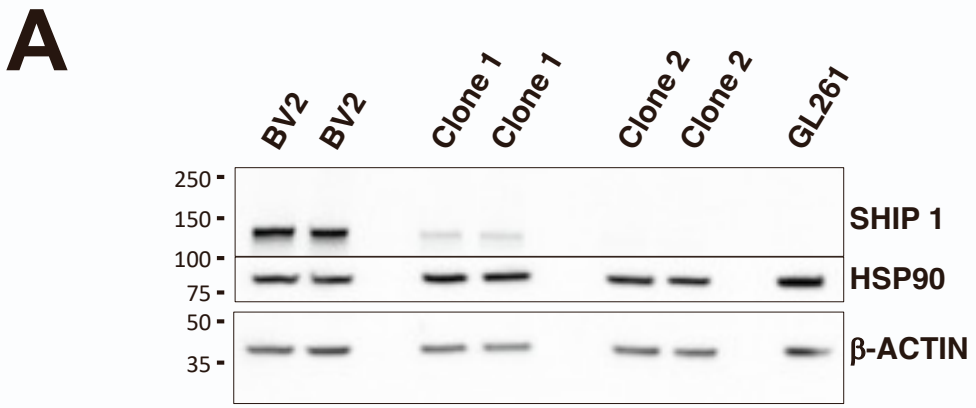
Supplemental Table1. K306 and AQX-MN100 Water solubility.-Related to Star Methods

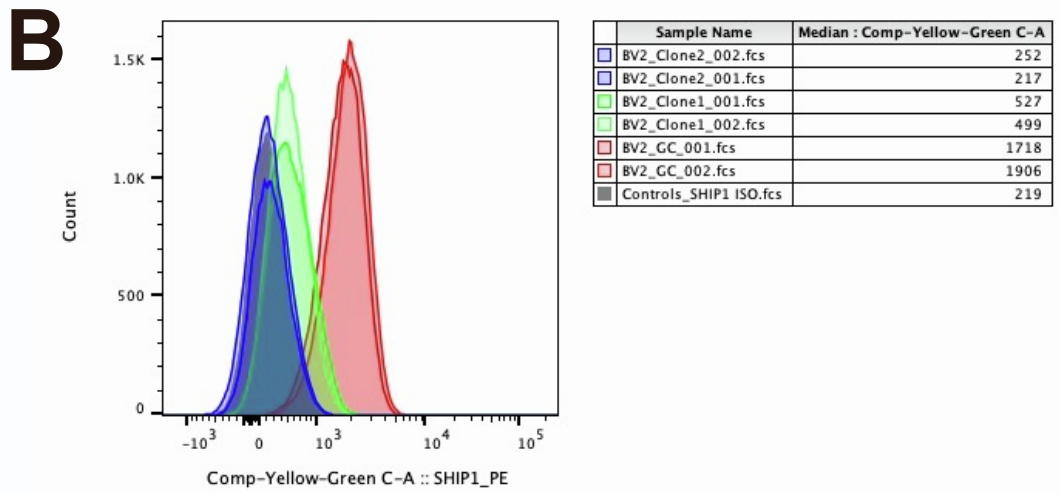
[a]CLogP was calculated using the XLOGP3 atomistic and knowledge-based method (Wang et al., 1997). XLOGP version 3.2.2 was used.

[b]CLogS was calculated using the topological method (Delaney, 2004).

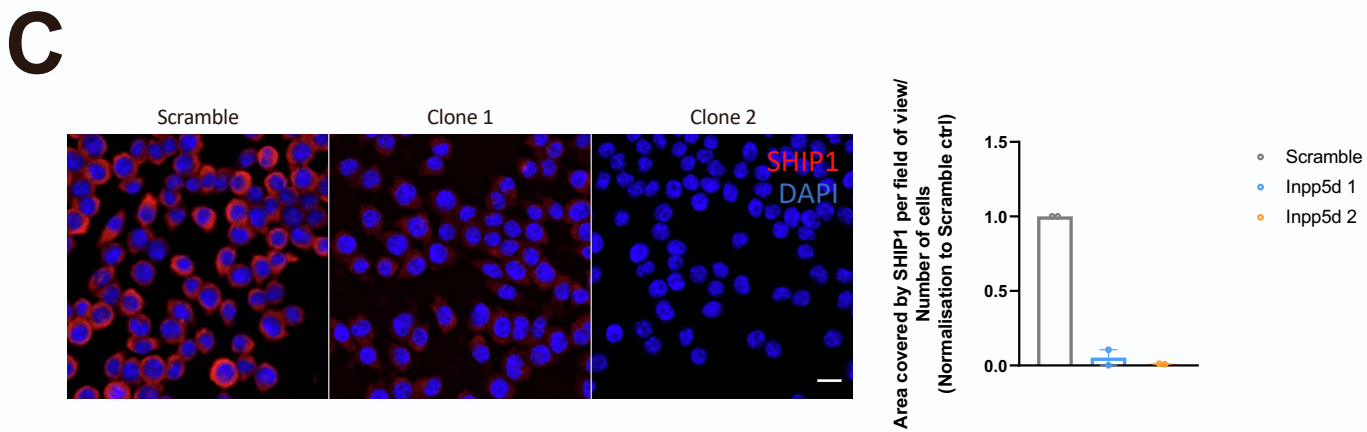
Plasmid	Forward	Reverse
SHIP1 cloning primers (pS97splitRBP_S1-Enz)	5’-GTCGCGGCCGCGAACAAGCACTCAGAGCAGC-3’	5’-CGGCGGATCCGAGCTTCTCCCTCGTCTTGC-3’
SHIP1deltaC2 cloning primers (pS97splitRBP_S1ΔC2)	5’-GTCGCGGCCGCGAACAAGCACTCAGAGCAGC-3’	5’-TTAGGATCCCTGTCCTTGGCTGTCAAC-3’
SHIP2 cloning primers (pS97splitRBP_S2-Enz)	5’-CGTGCGGCCGCCAAGCACTCCAAGCAGGACGAG-3’	5’-GCGCGGATCCCACCTTCATGGAGCCTCTG-3’

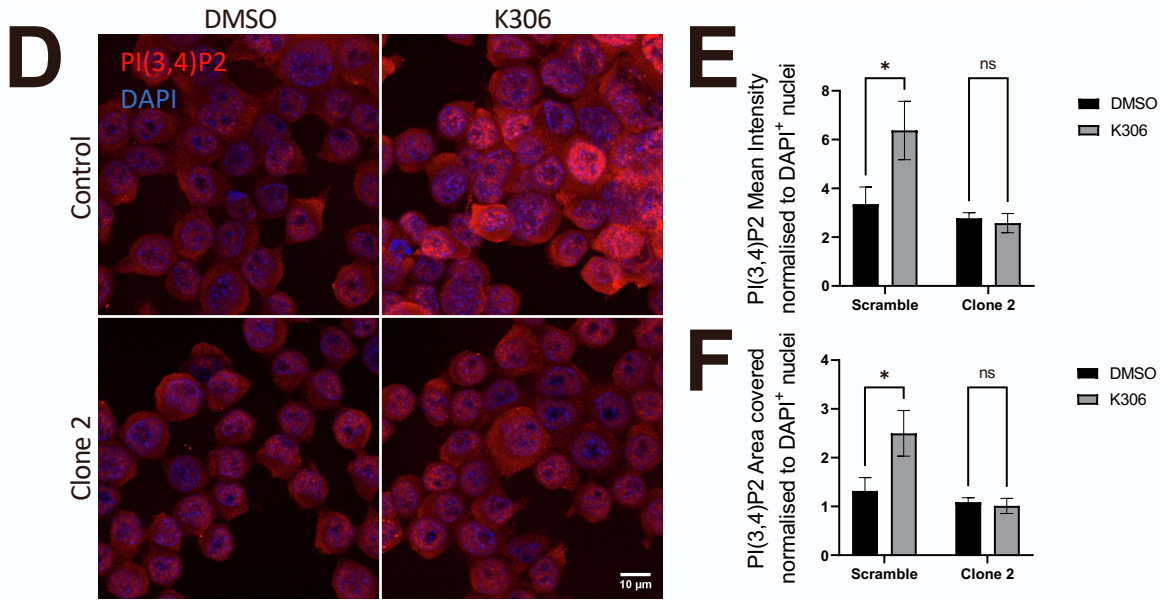
Supplemental Table2. Related to Star Methods Cloning Primers for SHIP1-Enzyme, SHIP2ΔC2 and SHIP2-Enzyme.



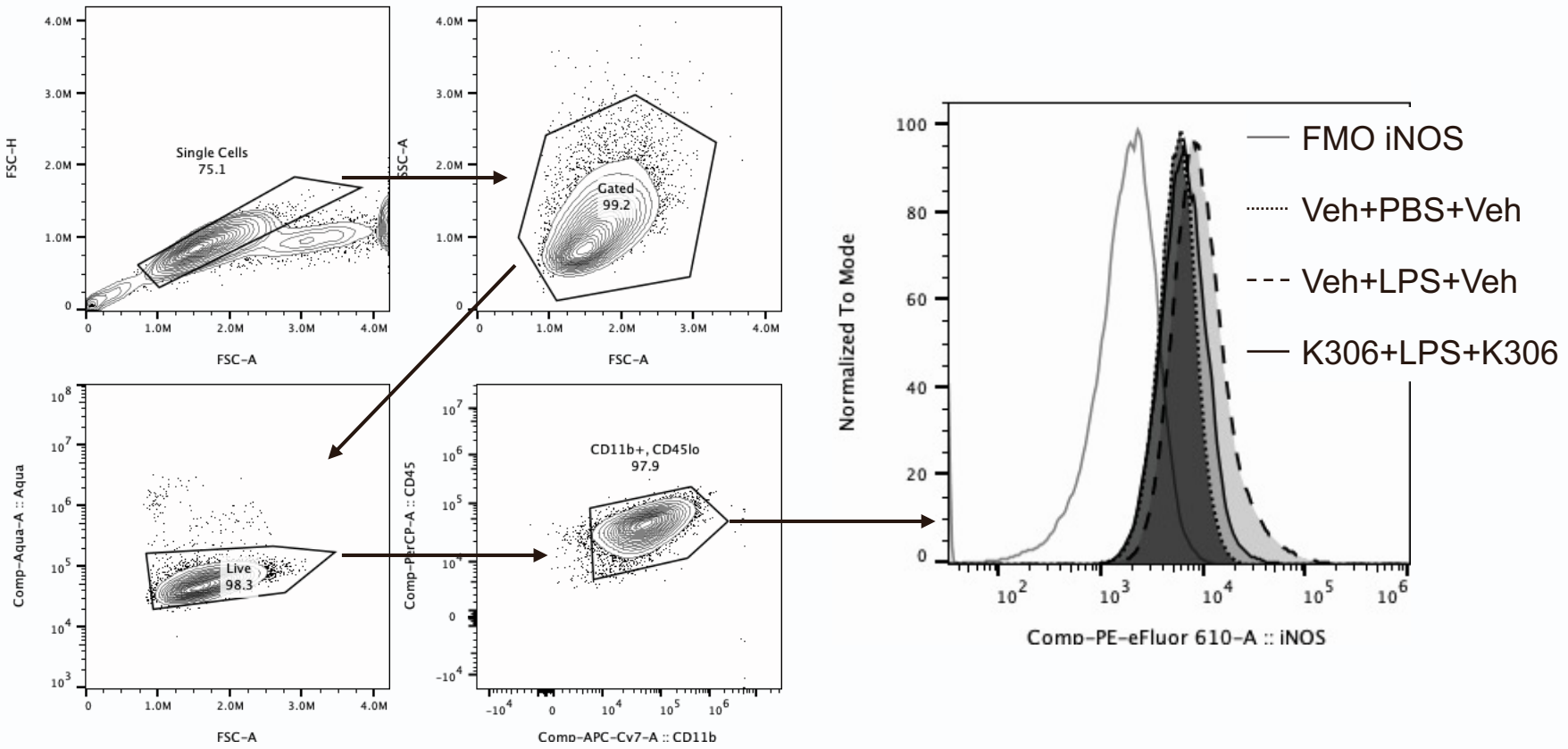


	Sample Name	Median : Comp-Yellow-Green C-A
	BV2_Clone2_002.fcs	252
	BV2_Clone2_001.fcs	217
	BV2_Clone1_001.fcs	527
	BV2_Clone1_002.fcs	499
	BV2_GC_001.fcs	1718
	BV2_GC_002.fcs	1906
	Controls_SHIP1 ISO.fcs	219

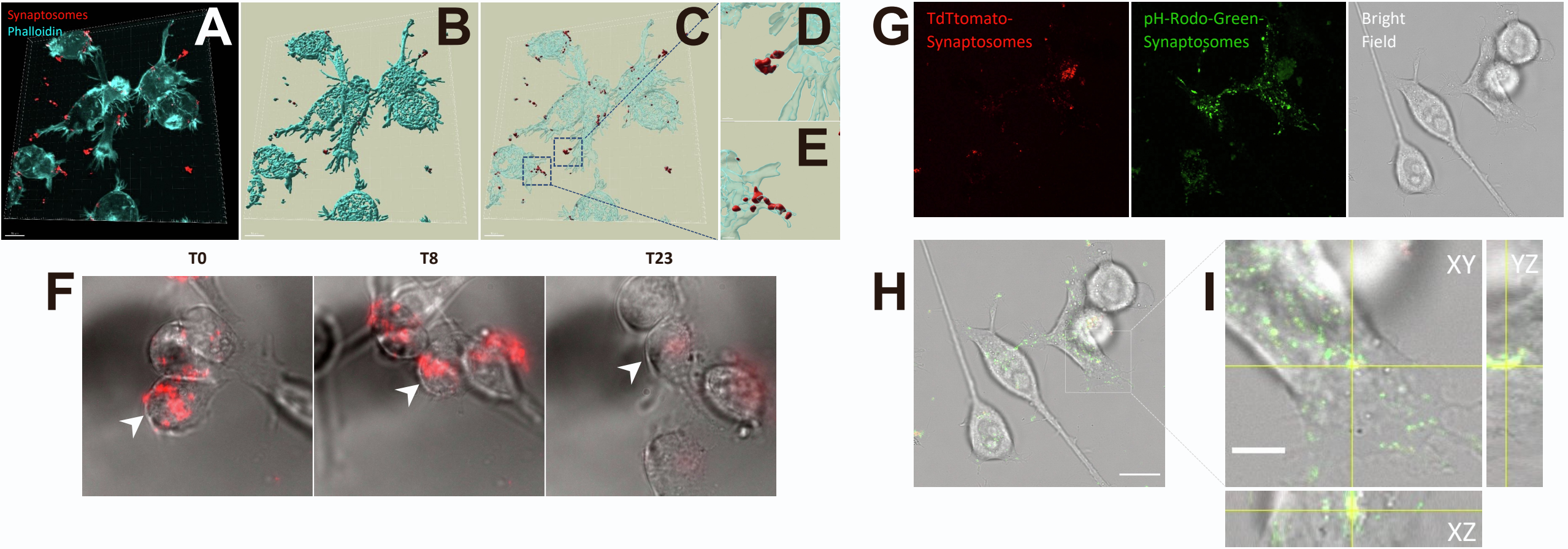




Supplemental Figure 1. Validation of BV2 gene edited clones and characterization. - Related to Star Methods: Validation of SHIP1 expression loss in gene-edited BV2 cell clones (Clone 1, Clone 2) vs. a scrambled control guide RNA clone (BV2). **(A)** Western Blot (WB) for SHIP1 protein expression and blotted for β-actin and HSP90 as loading controls, showing two independent lysate samples for each clone. **(B)** Intracellular Flow Cytometry (IFC) for the clones and scrambled control BV2 cells and relative quantitation. **(C)** Immuno-Fluorescence (IF) confocal microscopy imaging for SHIP1 of the three BV2 clones described above and relative quantitation of SHIP1 staining in each clone is shown at right. Scale bar 20 µm **(D)** Max-projection of 4.16 µm z-stack confocal acquisition of PI(3,4)P2 in parental BV2 cells and clone 2, treated with vehicle or K306 10 µM for 1h. Relative quantifications of PI(3,4)P2 mean intensity and area covered normalised to DAPI$^+$ nuclei are shown in **(E)** and **(F)** respectively. Two-way ANOVA with Šidák correction, *p<0.05 in **(E)** and **(F).**



Supplemental Figure 2. Gating strategy for BV2 cells treated with K306 or control and LPS.- **Related to Star Methods.** Singlets were gated by FSC-H vs FSC-A, debris were removed in SSC-A vs FSC-A, dead cells were excluded in Zombie Aqua vs FSC-A with viable BV2 microglia cells identified as CD45-PerCP+CD11b-APC-Cyanine7+ cells. The right panel shows histograms for iNOS-PE_efluor610 staining and an FMO stain (gray line), BV2 treated with Veh+PBS+Veh (dotted line), Veh+LPS+Veh (dashed line) or K306+LPS+K306 (solid black line). Data were acquired on Cytek Aurora, unmixed on SpectroFlow and further analyzed in FlowJo version 10.8.1.



Supplemental Figure 3. Synaptosome uptake and degradation.- Related to Star Methods (A) Representative Z-stack acquired by confocal microscopy and **(B)** relative three-dimensional reconstruction of BV2 microglial cells labelled by Phalloidin-647, and TdTomato-labelled synaptosomes. **(C)** Volume rendering of microglial cells in transparency confirms internalization of synaptosomes; scale bar: 10µm. **(D,E)** inset of synaptosomal cargoes engulfed by BV2 cells; scalebar: 2µm. **(F)** Time-lapse micrographs of BV2 microglia cells after 1 hour-incubation with TdTomato-positive synaptosomes (T0) and after 120 mins (T8) and 345 mins (T23), acquired by light sheet fluorescent microscopy every 15 minutes, showing progressive degradation of the cargo inside the cell (white arrowhead). **(G)** Max-projection of 5µm z-stack confocal acquisition of TdTomato-labelled synaptosomes, conjugated to pHrodo-Green pH-sensitive dye, 3 hours after incubation with BV2 cells. **(H)** Single focal plane of merged channels representing pHrodo-Green-positive synaptosomes internalized by microglial cells co-labelled with TdTomato; scale bar: 15µm. **(I)** Orthogonal view of the inset in B, pointing to co-localization across the 5µm Z-stack between TdTomato and pHrodo-Green signals; scale bar 5µm.