

Figure S1: FACS analysis for TLR localization in HUVEC. Exemplary data from non-permeabilized (surface) and permeabilized (intracellular) cells stained with secondary antibody only (sec. Ab., grey area) or with antibodies against TLR3&4 as indicated.

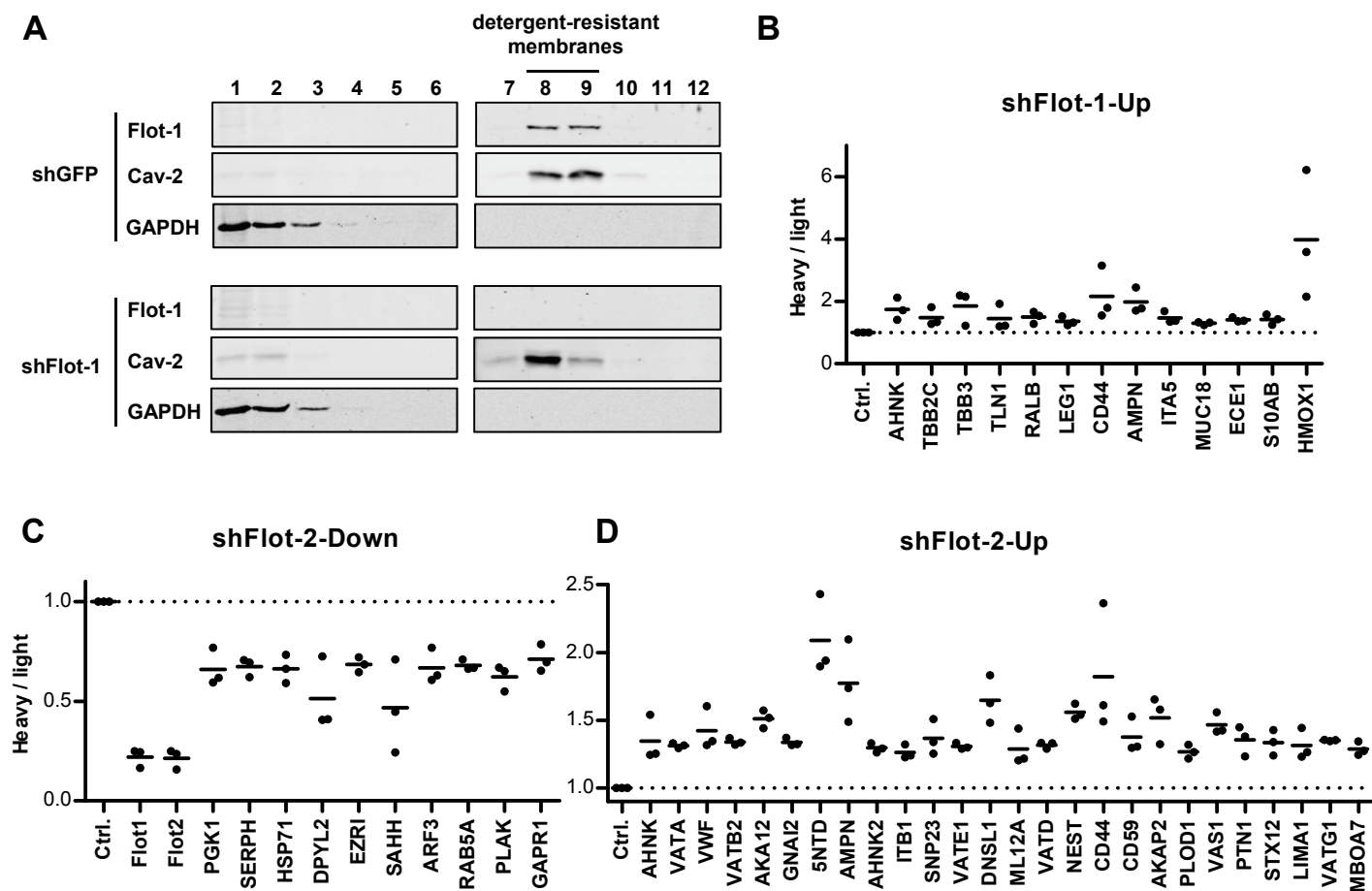


Figure S2: Impact of down-regulation of flotillins on the detergent resistant membrane fraction of HUVECs (A) Exemplary Western blot of density fractionation for detergent-resistant membranes probed for the proteins indicated in HUVECs transduced with shGFP and shFlot-1. Numbers above the blots indicate the fraction collected after sucrose gradient ultra-centrifugation where 1 illustrates 40% and 12 reflects the 5% sucrose fraction. Fractions 8 and 9 were pooled and subjected to proteome analysis. **(B-D)** Subset of SILAC-mass spectrometric analyses on the effect of shFlot-1 and shFlot-2 on protein abundance in the detergent-resistant membrane fraction of HUVECs. Depicted are proteins increased >20% **(B&D)** or decreased <20% **(C)** by the shRNA indicated. N=3.

Table S1: Flotillin-1 SILAC LTQ-Orbitrap-MS: List of proteins significantly up-regulated (>20%) by shFlot-1 relative to shGFP in the detergent-resistant membrane of HUVECs (n=3).

Protein		Accession	Score	Mean heavy / light	SEM
AHNK	Neuroblast differentiation-associated protein AHNAK	Q09666	3698	1.75	0.21
TBB2C	Tubulin beta-2C chain	P68371	1364	1.48	0.17
TBB3	Tubulin beta-3 chain	Q13509	1343	1.85	0.32
TLN1	Talin-1	Q9Y490	789	1.45	0.24
RALB	Ras-related protein Ral-B	P11234	604	1.49	0.11
LEG1	Galectin-1	P09382	602	1.36	0.08
CD44	Isoform Reticulocyte of CD44 antigen	P16070-12	520	2.16	0.50
AMPN	Aminopeptidase N	P15144	451	1.98	0.24
ITA5	Integrin alpha-5	P08648	404	1.47	0.11
MUC18	Cell surface glycoprotein MUC18	P43121	288	1.30	0.03
ECE1	Isoform C of Endothelin-converting enzyme 1	P42892-3	193	1.41	0.04
S10AB	Protein S100-A11	P31949	168	1.42	0.09
HMOX1	Heme oxygenase 1	P09601	151	3.98	1.19

Table S2: Flotillin-2 SILAC LTQ-Orbitrap-MS: List of proteins significantly down-regulated (>20%) by shFlot-2 relative to shGFP in the detergent-resistant membrane of HUVECs (n=3).

Protein		Accession	Score	Mean Heavy / light	SEM
Flot1	Flotillin-1	O75955	1469	0.22	0.03
Flot2	Flotillin-2	Q14254	1260	0.21	0.03
PGK1	Phosphoglycerate kinase 1	P00558	759	0.66	0.05
SERPH	Serpin H1	P50454	650	0.67	0.03
HSP71	Heat shock 70	P08107	592	0.66	0.04
DPYL2	Dihydropyrimidinase-related protein 2	Q16555	370	0.51	0.11
EZR1	Ezrin	P15311	363	0.68	0.03
SAHH	Adenosylhomocysteinase	P23526	237	0.47	0.14
ARF3	ADP-ribosylation factor 3	P61204	213	0.67	0.05
RAB5A	Ras-related protein Rab-5A	P20339	204	0.68	0.02
PLAK	Junction plakoglobin	P14923	182	0.62	0.04
GAPR1	Golgi-associated plant pathogenesis-related protein 1	Q9H4G4	164	0.63	0.03

Table S3: Flotillin-2 SILAC LTQ-Orbitrap-MS: List of proteins significantly up-regulated (>20%) by shFlot-2 relative to shGFP in the detergent-resistant membrane of HUVECs (n=3).

Protein		Accession	Score	Mean Heavy / light	SEM
AHNK	Neuroblast differentiation-associated protein AHNAK	Q09666	4382	1.35	0.10
VATA	V-type proton ATPase catalytic subunit A	P38606	2326	1.31	0.01
VWF	von Willebrand factor	P04275	1636	1.42	0.09
VATB2	V-type proton ATPase subunit B, brain isoform	P21281	1502	1.34	0.01
AKA12	Isoform Gamma of A-kinase anchor protein 12	Q02952-3	1454	1.51	0.04
GNAI2	Guanine nucleotide-binding protein G(i) subunit alpha-2	P04899	1247	1.31	0.03
5NTD	5'-nucleotidase	P21589	740	2.09	0.17
AMPN	Aminopeptidase N	P15144	708	1.77	0.18
AHNK2	Isoform 3 of Protein AHNAK2	Q8IVF2-3	655	1.30	0.02
ITB1	Isoform Beta-1B of Integrin beta-1	P05556-2	638	1.26	0.03
SNP23	Synaptosomal-associated protein 23	O00161	782	1.37	0.08
VATE1	V-type proton ATPase subunit E 1	P36543	504	1.31	0.01
DNSL1	Deoxyribonuclease-1-like 1	P49184	488	1.65	0.10
ML12A	Myosin regulatory light chain 12A	P19105	430	1.29	0.08
VATD	V-type proton ATPase subunit D	Q9Y5K8	408	1.32	0.01
NEST	Nestin	P48681	349	1.62	0.06
CD44	Isoform Reticulocyte of CD44 antigen	P16070-12	331	1.82	0.27
CD59	CD59 glycoprotein	P13987	314	1.38	0.08
AKAP2	A-kinase anchor protein 2	Q9Y2D5	307	1.52	0.10
PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	Q02809	287	1.27	0.03
VAS1	V-type proton ATPase subunit S1	Q15904	210	1.47	0.05
PTN1	Tyrosine-protein phosphatase non-receptor type 1	P18031	198	1.35	0.06
STX12	Syntaxin-12	Q86Y82	185	1.34	0.05
LIMA1	Isoform 3 of LIM domain and actin-binding protein 1	Q9UHB6-3	183	1.31	0.07
VATG1	V-type proton ATPase subunit G 1	O75348	180	1.35	0.00
MBOA7	Isoform 3 of Lysophospholipid acyltransferase 7	Q96N66-3	156	1.29	0.03

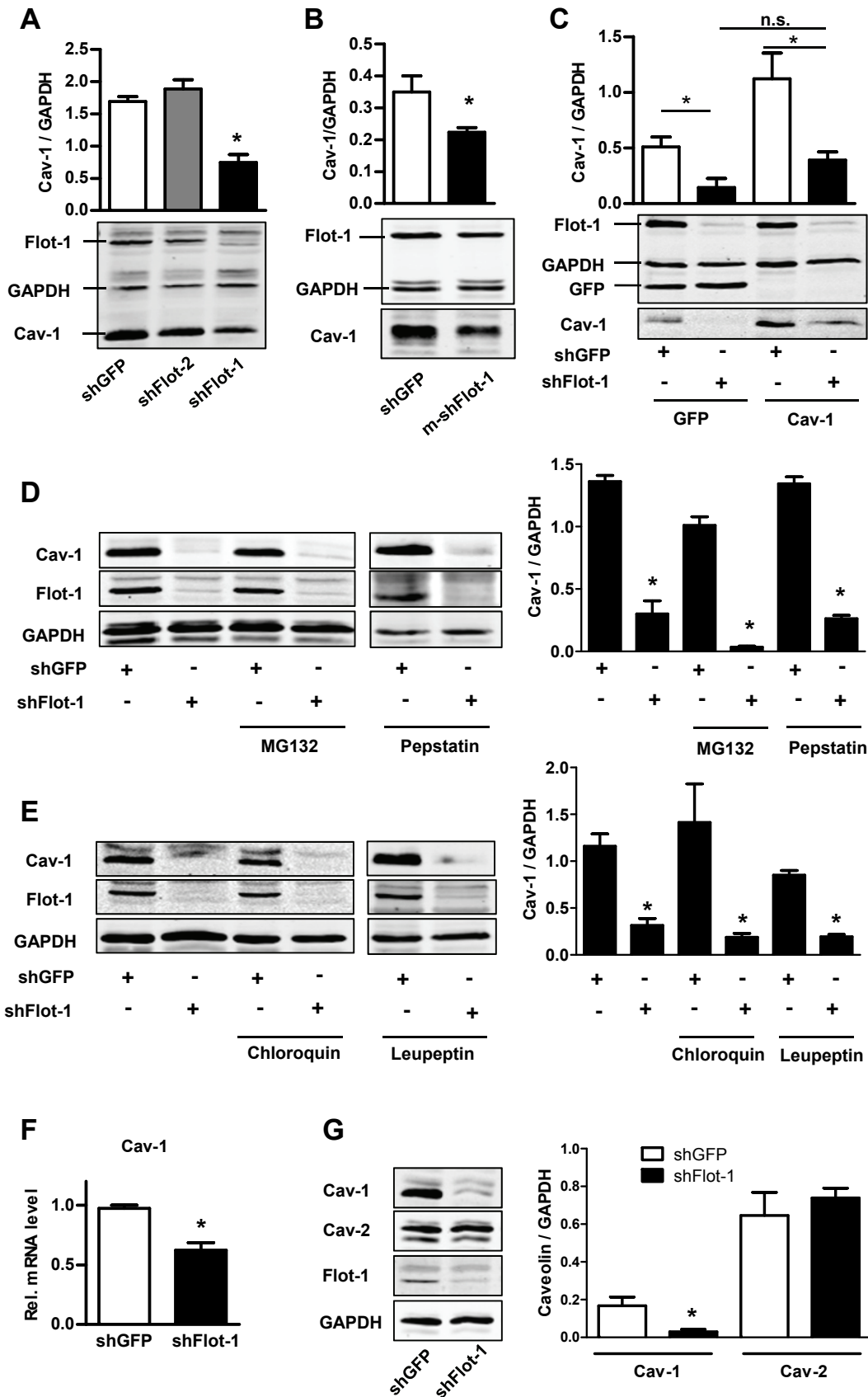


Figure S3: Analysis of the interaction of flotillin-1 and caveolin-1. (A-E, G) Western blot and densitometry for the proteins indicated in HUVECs (A,C-G) and in mouse lung endothelial cells (B) transduced with shGFP, shFlot-2, shFlot-1 or mouse(m)-shFlot-1 and in (C) electroporated with plasmids coding for GFP (pcDNA3) or cav-1 (pCMV6-XL5). Cav-1: Caveolin-1, Flot-1: flotillin-1 in the presence or absence of MG132 (10 $\mu\text{mol/l}$, 5.5 h), pepstatin (2 $\mu\text{mol/l}$, 22 h), chloroquin (100 $\mu\text{mol/l}$, 25 h), leupeptin (30 $\mu\text{mol/l}$, 24 h). N=3, * $p < 0.05$, n.s.=not significant. (F) qRT-PCR for caveolin-1 relative to RNA polymerase II in HUVECs transduced with shGFP or shFlot-1. N=3, * $p < 0.05$ n.s.=not significant.

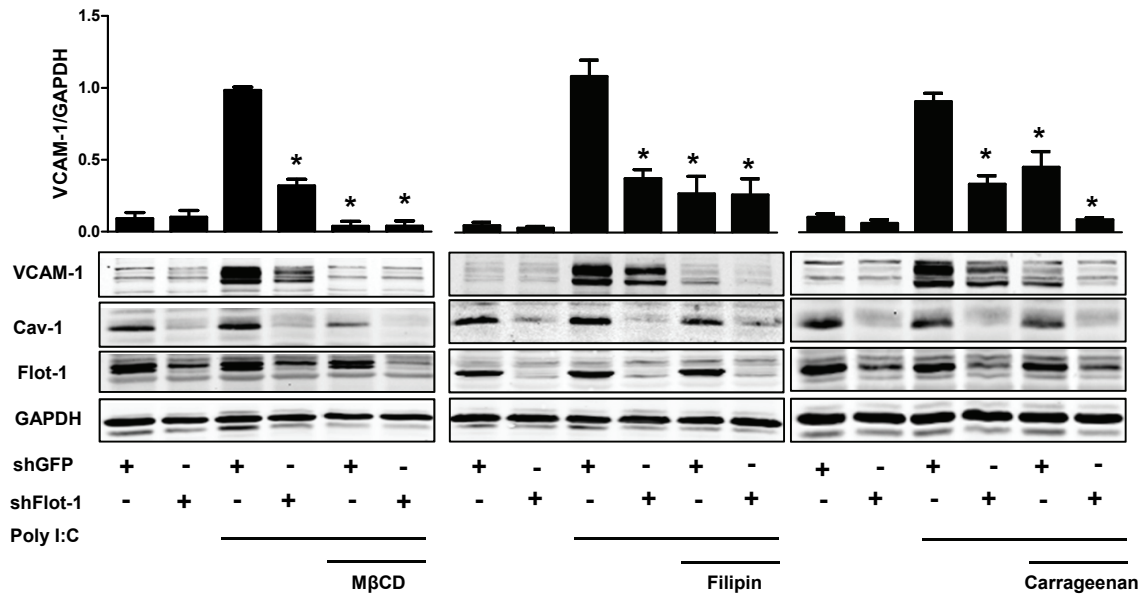


Figure S4: Effect of cholesterol-modulating compounds on poly-I:C-induced signaling. Exemplary Western blots and densitometry for the proteins indicated in HUVECs transduced with shGFP or shFlot-1 with and without stimulation with poly-I:C (10 μ g/ml, 180 min) in the presence or absence of methyl- β -cyclodextrin (M β CD, 5 mmol/l, 60 min pretreatment), filipin (5 μ g/ml, 30 min pretreatment) and Carrageenan (250 μ g/ml, 60 min pretreatment). N=3, *p<0.05.