Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information to

Reciprocal Upregulation of Scavenger Receptors Complicates Interpretation of Nanoparticle Uptake in Non-Phagocytic Cells

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Table S1. siRNAs used for silencing scavenger receptors in A549 cells. Two siRNAs were used to silenced each scavenger receptor in order to compare the efficiency of gene silencing.

Target	siRNA ID	Sense	Antisense
SR-A	S8987 (siRNA-1)	GGAGCGUGUUUACAAUFUAtt	UACAUUGUAAACACGCUCCtc
	S8988 (siRNA-2)	GGGACAUGGGAAUGCAAUAtt	UAUUGCAUUCCCAUCGUCCCtg
MARCO	S16549 (siRNA-1)	GCUUUUCACCAAAUUGCAAtt	UUGCAAUUUGGUGAAAAGCag
	S16551 (siRNA-2)	CAACUUCACUCAGAACCCAtt	UGGGUUCUGAGUGAAGUUGtc
SR-BI	S2648 (siRNA-1)	CAUGAUCAAUGGAACUUCUtt	AGAAGUUCCAUUGAUCAUGtt
	S2649 (siRNA-2)	GCCUCUACAUGAAAUCUGUtt	ACAGAUUUCAUGUAGAGGCtc
LOX-1	S9842 (siRNA-1)	CCAGCAAGCAAUUUCCUAUtt	AUAGGAAAUUGCUUGCUGGat
	S9843 (siRNA-2)	CAAGAGCAAGCAAACCUAAtt	UUAGGUUUGCUUGCUCUUGtg

Table S2. mRNA expression of scavenger receptors in A549 cells. The results are expressed as the difference in C_T obtained by qPCR between the scavenger receptor and GAPDH. A higher value indicates a lower mRNA expression level (n=4, mean ± S.D.). The results show a higher expression of SR-BI than SR-A, MARCO, LOX-1 and LDLR in A549 cells.

Receptor	$C_{T(SR)} - C_{T(GAPDH)}$
SR-A	18.9 ± 0.2
MARCO	19.5 ± 0.3
SR-BI	7.2 ± 0.1
LOX-1	19.1 ± 0.1
LDLR	9.2 ± 0.1

Table S3. Size characterization of the silica nanoparticles in relevant dispersions by dynamic light scattering.

	hydrodynamic diameter[*] (nm)			
foetal bovine serum conc.	0%	10%	30%	50%
10 nm	18[†]	171	106	119[‡]
50 nm	59	159	171	113
200 nm	241	297	253	252

[*] z-average diameter extracted by cumulant analysis, unless otherwise indicated

[†] Measured at 400 µg/ml; all other results at 100 µg/ml.

[‡] Main peak extracted by CONTIN size analysis; in addition a second peak at 3800 nm was observed.

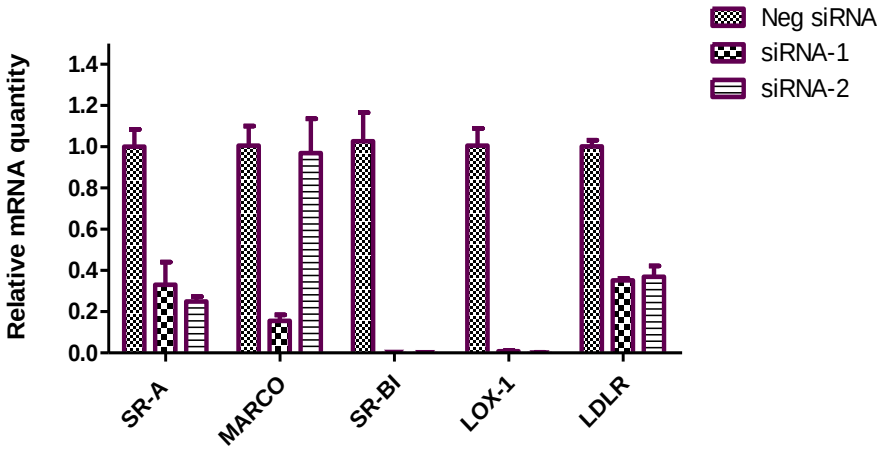

Fig. S1. Efficiency of gene silencing of scavenger receptors in A549 cells. Two separate duplexes (Table S1) were used and the efficiency of gene silencing was determined by measuring the relative reduction of scavenger receptor expression using quantitative real time PCR. In the case of SR-BI and LOX-1, both siRNA-1 and siRNA-2 can effectively reduce the mRNA expression (relative mRNA amount less than 0.02). siRNA-2, siRNA-1, siRNA-2, siRNA-2, siRNA1 were selected to silence SR-A, MARCO, SR-BI, LOX-1, and LDLR respectively, in subsequent experiments due to better silencing efficiency.

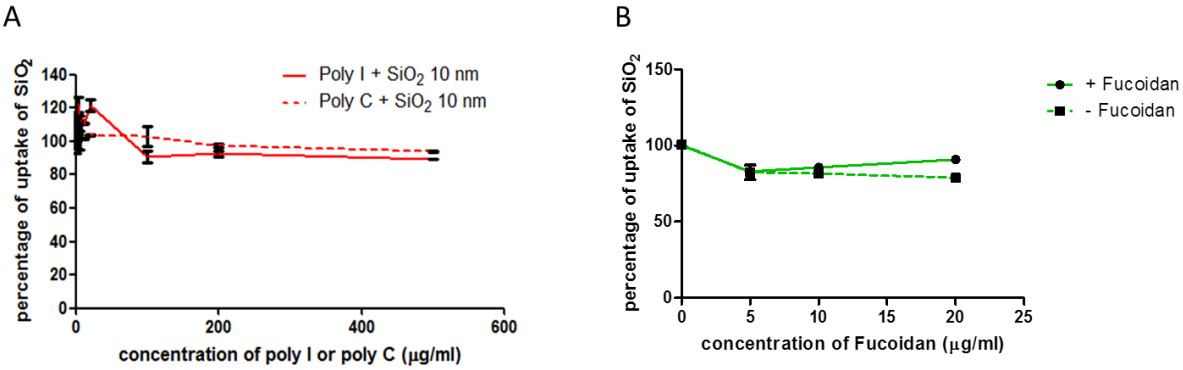

Fig. S2. Lack of inhibition of 10 nm silica nanoparticle uptake by poly I and fucoidan. A549 cells were pre-incubated with either (A) poly I or poly C or (B) fucoidan at different concentrations for 30 min before exposure to 100 µg/mL of 10 nm silica nanoparticles in 10% foetal bovine serum for 3 h. Results are presented as the median cell fluorescence intensity due to nanoparticle uptake relative to cells without pre-incubation with inhibitor. The results show that (A) even when the concentration of poly I is increased up to 500 µg/mL, or (B) after pre-incubation with fucoidan, the uptake of the 10 nm silica nanoparticles is unchanged with respect to control, suggesting that the nanoparticles are not taken up by scavenger receptors.

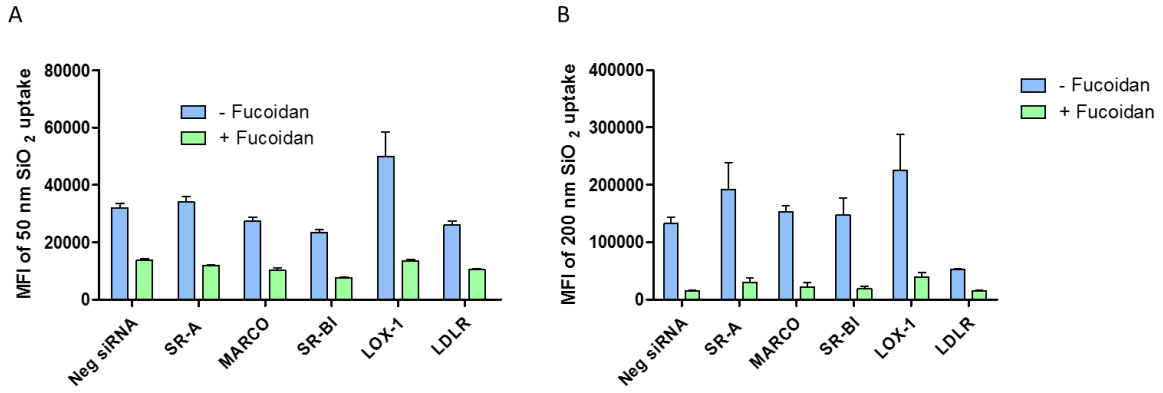

Fig. S3. 50 and 200 nm silica nanoparticle uptake in silenced cells pre-incubated with fucoidan inhibitor. The cells were pre-incubated with fucoidan at 10 µg/mL concentrations for 30 min before being exposed for 3 h to 100 µg/mL (A) 50 nm or (B) 200 nm silica nanoparticles dispersed in 10% foetal bovine serum. For both 50 and 200 nm silica nanoparticles uptake was decreased after pre-incubation with fucoidan, consistent with the results from pre-incubation with poly I (Fig. 1E-F).

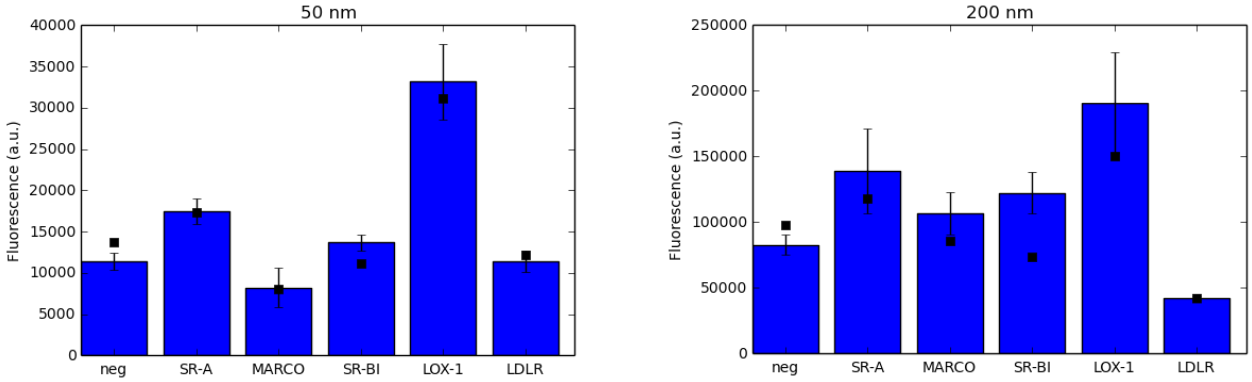

Fig. S4. Check of results from mathematical extraction of uptake fluxes into A549 cells. As a check on the results, the fluxes extracted from the experimental data using the mathematical model (Fig. 3) were used to predict (or, rather, postdict) the uptake that should be observed experimentally (Fig. 1) given how silencing one scavenger receptor affects the other receptors (Fig. 2). (A) 50 nm and (B) 200 nm silica nanoparticles. (Data points) Model prediction. (Bars) Experimental data from Fig. 1E-F, here shown as the part of the uptake that is mediated by scavenger receptors. This was calculated by subtracting the data which is not blocked by a scavenger inhibitor (polyI) from the average of the uptake without blocking agent and in the presence of negative inhibitor (polyC). The results show generally good agreement between the experimental data and the results expected based on the extracted fluxes, except for uptake into SR-BI silenced cells.

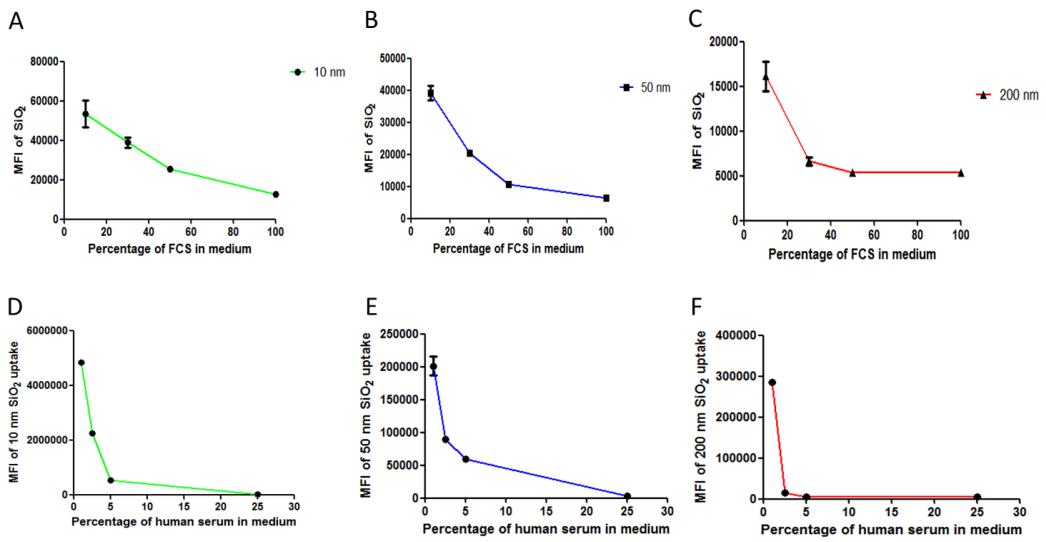

Fig. S5. Silica nanoparticle uptake in the presence of different concentrations of foetal bovine and human serum in A549 cells. Cells were exposed to (A) 10 (B) 50 and (C) 200 nm silica nanoparticles in different concentration of foetal bovine serum. The same experiments was performed with human serum (D, E, F respectively).

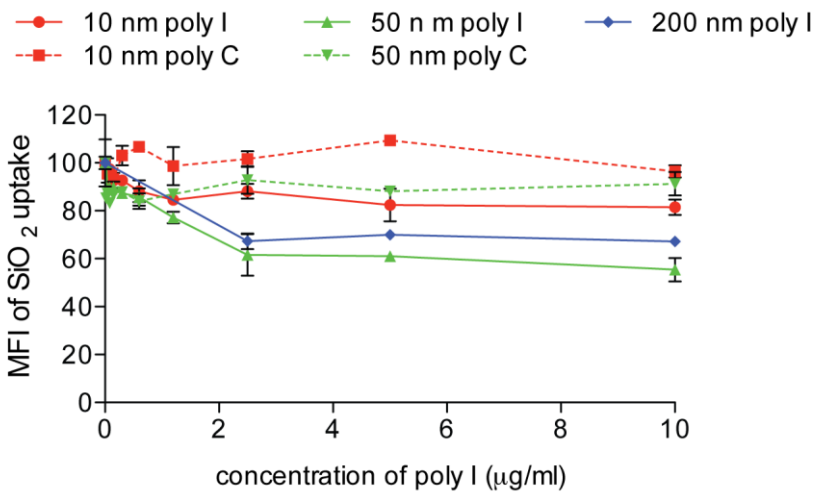

Fig. S6. Uptake of 10, 50 and 200 nm silica nanoparticles in the presence of inhibitors of scavenger receptors in 30% foetal bovine serum. Cells were pre-incubated with either poly I (inhibitor) or poly C (negative control) at different concentrations for 30 min before being exposed for 3 h to 100 µg/mL of 10, 50 and 200 nm silica nanoparticles dispersed in 30% foetal bovine serum. Results are presented as the median cell fluorescence intensity due to nanoparticle uptake relative to cells without pre-incubation with inhibitor.