Supplementary Information

Tracing the *in vivo* fate of nanoparticles with a "non-self" biological identity

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Supplementary Table 1

Characterization summary.

Nominal size	Surface	Fluorescent dye	Protein corona	Characterization data	
				hydrodynamic size [a] (nm [PdI])	ζ potential [b] (mV)
70 nm	Plain	FITC	–	74.0 ± 7.6 [0.042]	–20 ± 2
			FBS	103.4 ± 13.6 [0.069]	–19 ± 4
70 nm	Plain	Pacific Blue	–	62.4 ± 13.3 [0.182]	–21 ± 3
			FBS	109.1 ± 23.2 [0.181]	–18 ± 2
70 nm	NH_2	FITC	–	74.3 ± 8.5 [0.052]	–15 ± 2
			FBS	126.1 ± 17.5 [0.077]	–17 ± 3
70 nm	COOH	FITC	–	78.6 ± 5.7 [0.021]	–30 ± 6
			FBS	128.8 ± 23.6 [0.135]	–20 ± 3

[a] Dynamic light scattering; values are z-average ± PdI width obtained by the cumulant analysis.
[b] 10 mM sodium phosphate buffer (pH 7.4) as the dispersion medium. Values are mean ± s.d. of three measurements.

Supplementary Table 2

List of proteins identified in LC-MS/MS analysis of excised bands.

Band # (Mw)	Protein name (Gene name)	Symbol	Accession #	Mw (kDa)	Species	Protein score	Significant matches	Significant sequences[a]	Coverage %
1 (72 kDa)	Ahsg protein (*alpha-2-HS-glycoprotein 1*)	Ahsg1 (Fetua)	Q5U3D8	50.1	*Danio rerio*	3127	113	5 (5)	13
	Zgc:153748 (*3'-phosphoadenosine 5'-phosphosulfate synthase 2a*)	Papss2a	A0JPF3	69.2	*Danio rerio*	2275	79	17 (15)	34
	Polyadenylate-binding protein 1A (*poly(A) binding protein, cytoplasmic 1a*)	Pabpc1a	F1QB54	71.0	*Danio rerio*	1521	51	19 (7)	30
2 (15 kDa)	Haemoglobin subunit beta-2 (*haemoglobin, beta adult 2*)	Hbba2	Q90485	16.4	*Danio rerio*	12571	359	10 (2)	69
	Haemoglobin, subunit beta-1 (*haemoglobin, beta adult 1*)	Hbba1	Q90486	16.4	*Danio rerio*	12387	347	10 (2)	69
	Haemoglobin subunit alpha (*haemoglobin, alpha adult 1*)	Hbaa1	Q90487	15.5	*Danio rerio*	4082	197	8 (4)	52
	Si:xx-by187g17.5 (*haemoglobin, alpha adult 2*)	Hbaa2	Q5BJC7	15.4	*Danio rerio*	3634	130	4 (3)	37
	Apolipoprotein A-II (*apolipoprotein A-II*)	Apoa2	B3DFP9	15.5	*Danio rerio*	3235	86	9 (9)	43
3 (59 kDa)	ALB protein (*albumin*)	ALB	B0JYQ0	69.3	*Bos taurus*	9664	346	24 (2)	29
	Alpha-2-HS-glycoprotein (*alpha-2-HS-glycoprotein*)	AHSG (FETUA)	B0JYN6	38.4	*Bos taurus*	5009	123	9 (9)	24
	Alpha-1-antiproteinase (*alpha-1-antitrypsin*)	SERPINA1	P34955	46.1	*Bos taurus*	4128	165	20 (20)	38
4 (15 kDa)	Apolipoprotein A-II (*apolipoprotein A-II*)	APOA2	P81644	11.2	*Bos taurus*	3446	166	4 (4)	22

[a] Parentheses indicate the number of unique peptides.

List of proteins identified in LC-MS/MS analysis of excised bands (continued).

Band # (Mw)	Protein name (Gene name)	Symbol	Accession #	Mw (kDa)	Species	Protein score	Significant matches	Significant sequences[a]	Coverage %
5 (72 kDa)	Ahsg protein (*alpha-2-HS-glycoprotein 1*)	Ahsg1 (Fetua)	Q5U3D8	50.1	*Danio rerio*	3799	132	8 (8)	19
	Alpha-2-HS-glycoprotein (*alpha-2-HS-glycoprotein*)	AHSG (FETUA)	B0JYN6	38.4	*Bos taurus*	621[b]	16	5 (5)	18
6 (23 kDa)	Apolipoprotein A-I (*apolipoprotein A-I*)	APOA1	P15497	30.3	*Bos taurus*	32078	1086	33 (33)	83
	Apolipoprotein A-Ib (*apolipoprotein A-Ib*)	Apoa1b	A0A0R4 IKF0	30.1	*Danio rerio*	4029	149	19 (19)	57
	Apolipoprotein A-I (*apolipoprotein A-I*)	Apoa1	O42363	30.3	*Danio rerio*	2709	93	22 (22)	62
7 (23 kDa)	Apolipoprotein A-I (*apolipoprotein A-I*)	APOA1	P15497	30.3	*Bos taurus*	12244	496	28 (28)	62
	Apolipoprotein A-Ib (*apolipoprotein A-Ib*)	Apoa1b	A0A0R4 IKF0	30.1	*Danio rerio*	12222	468	16 (16)	47
	Apolipoprotein A-I (*apolipoprotein A-I*)	Apoa1	O42363	30.3	*Danio rerio*	2221	80	23 (23)	65

[a] Parentheses indicate the number of unique peptides.
[b] This protein may not represent the band excised but the score information is included for a comparison to the zebrafish orthologue.

Supplementary Table 3

Kinetic models used and fitted parameters (Exponential decay).

Model	Figure	RSE [a] (d.f.)	Fitted parameters		
			λ	y_0	y_f
Exponential decay $y = y_f + (y_0 - y_f)e^{-\lambda t}$	Fig. 2d (Unmodified)	3710 (28)	0.007	39010	0
	Fig. 2d (FBS-PC)	667 (47)	0.200	12360	1670
	Fig. 4f (Water)	668 (222)	0.020	9768	0
	Fig. 4f (Unmodified)	705 (222)	0.020	10260	0
	Fig. 4f (FBS-PC)	846 (222)	0.099	6957	0
	Fig. 4g (FBS-PC)	403 (129)	0.166	3990	0
	S. Fig. 10d (Unmodified)	1060 (222)	0.171	10820	1091
	S. Fig. 10f (FBS-PC)	911 (129)	0.173	8617	0
	Fig. 5b (FBS)	238 (201)	0.136	1317	687
	Fig. 5b (FBS-PC)	414 (201)	0.552	2206	1437
	Fig. 5b (LPS)	333 (405)	0.309	1888	1005
	Fig. 5c (inset)	11470 (201)	0.053	33100	0

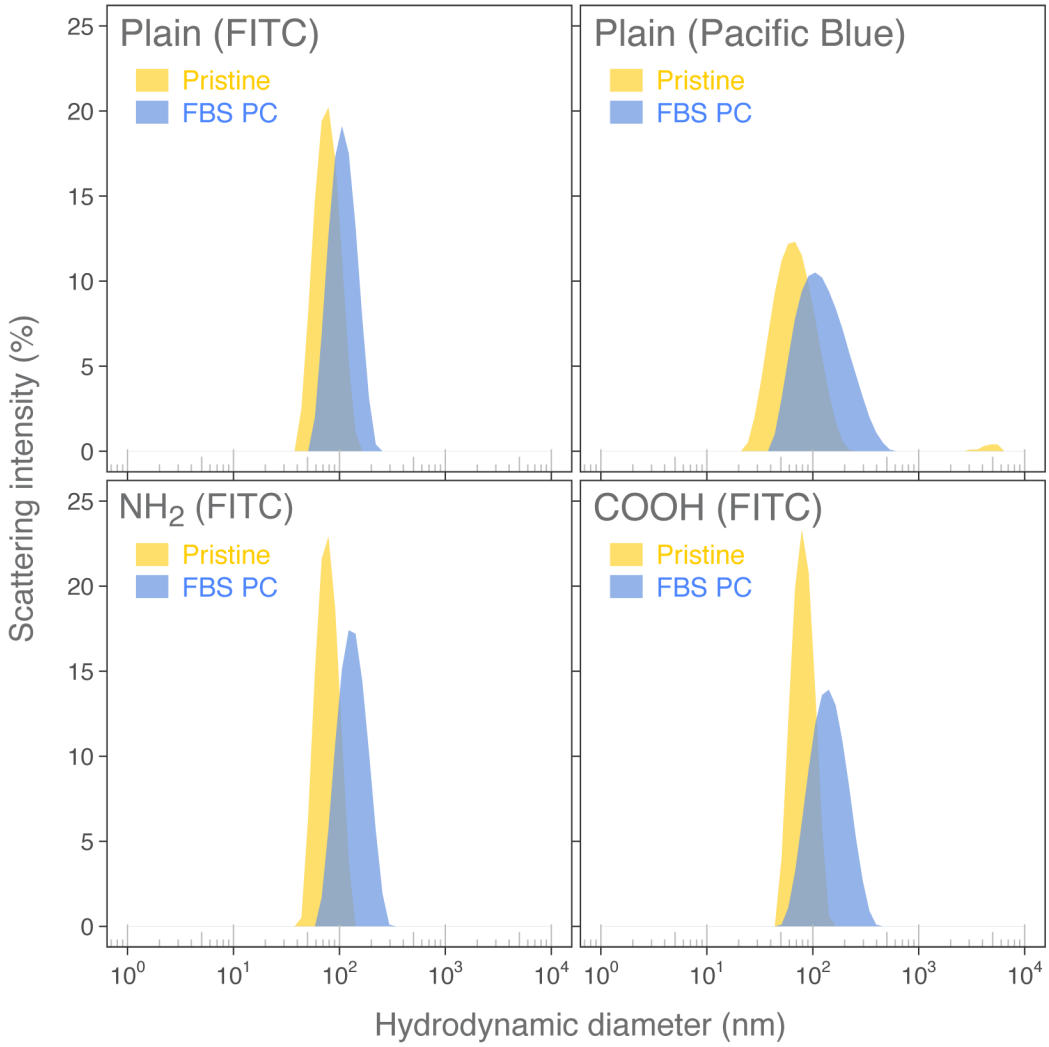
[a] Residual standard error on (degrees of freedom); the square root of the residual sum of squares divided by the residual degrees of freedom.

Kinetic models used and fitted parameters (Logistic/Gompertz growth).

Model	Figure	RSE [a] (d.f.)	Fitted parameters		
			λ	y_0	y_f
Gompertz growth $y = y_f \times \left(\frac{y_0}{y_f}\right) e^{-\lambda t}$	Fig. 2e (Unmodified)	508 (27)	0.037	1273	4000 [b]
	Fig. 2f (FBS-PC)	574 (47)	0.053	303	10970
	Fig. 3c (Ratio)	0.18 (21)	0.299	0.46	2.58
	S. Fig. 10e (Unmodified)	806 (222)	0.826	290	1855
Logistic growth $y = y_f \times \frac{y_0}{(y_f - y_0)e^{-\lambda t} + y_0}$	Fig. 5c (All MΦ)	3.48 (201)	0.162	1.70	100 [b]
	Fig. 5c (M1-like MΦ)	1.30 (201)	0.207	0.48	20.6
	Fig. 5d (FBS-PC)	15.83 (201)	0.752	5.33	55.7
	Fig. 5d (LPS)	10.15 (405)	1.138	0.90	46.6

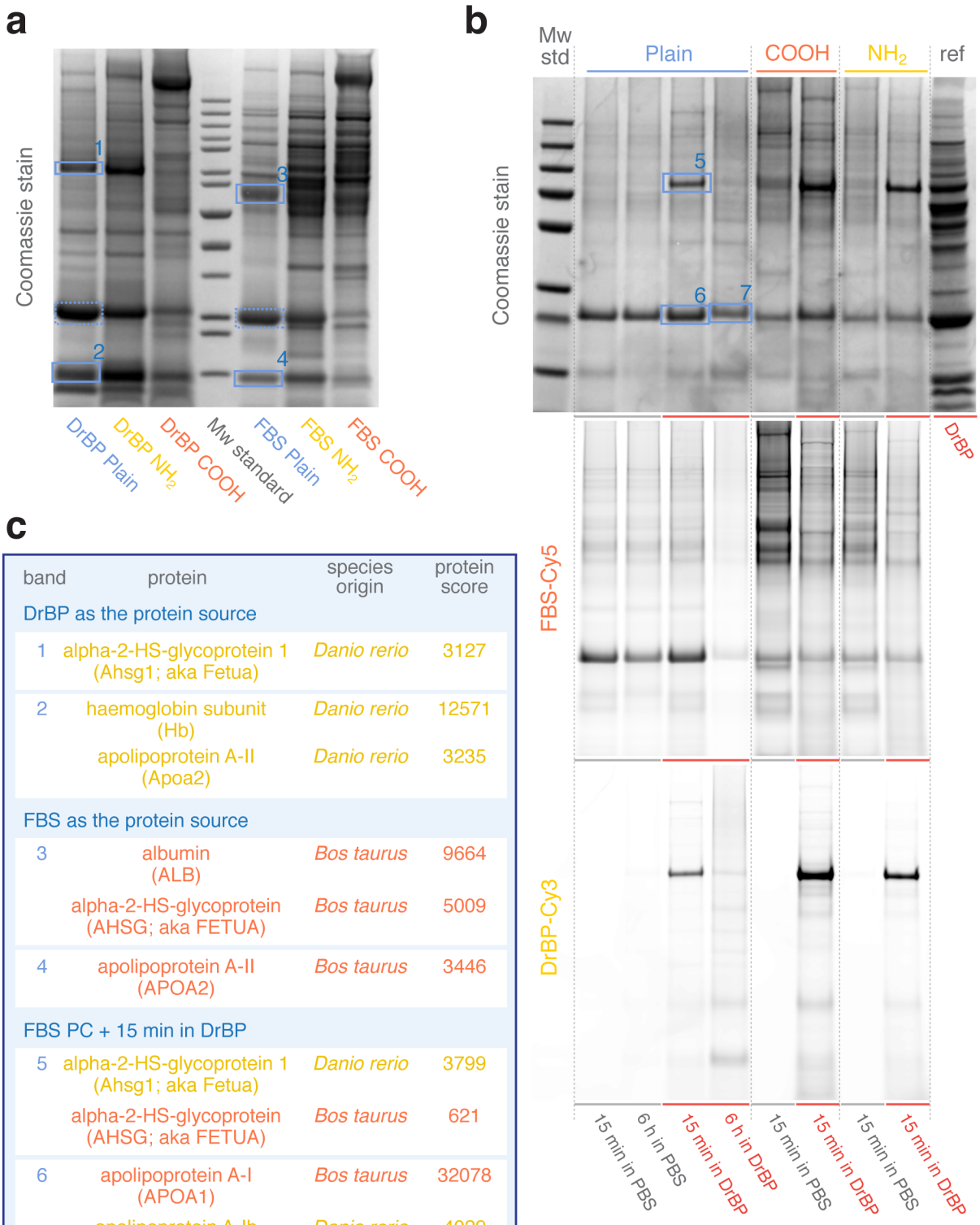
[a] Residual standard error on (degrees of freedom); the square root of the residual sum of squares divided by the residual degrees of freedom.
[b] The fitted value corresponds to the upper constraint manually defined in the port algorithm.

Supplementary Figure 1



Supplementary Figure 1. DLS analysis on the hydrodynamic size distributions. Size distributions of 70 nm SiO_2 nanoparticles with or without pre-formed FBS PC, fitted by the CONTIN algorithm. Three different surfaces (Plain, NH_2 and COOH) and two different dyes (FITC and Pacific Blue) used in this study were analyzed.

Supplementary Figure 2

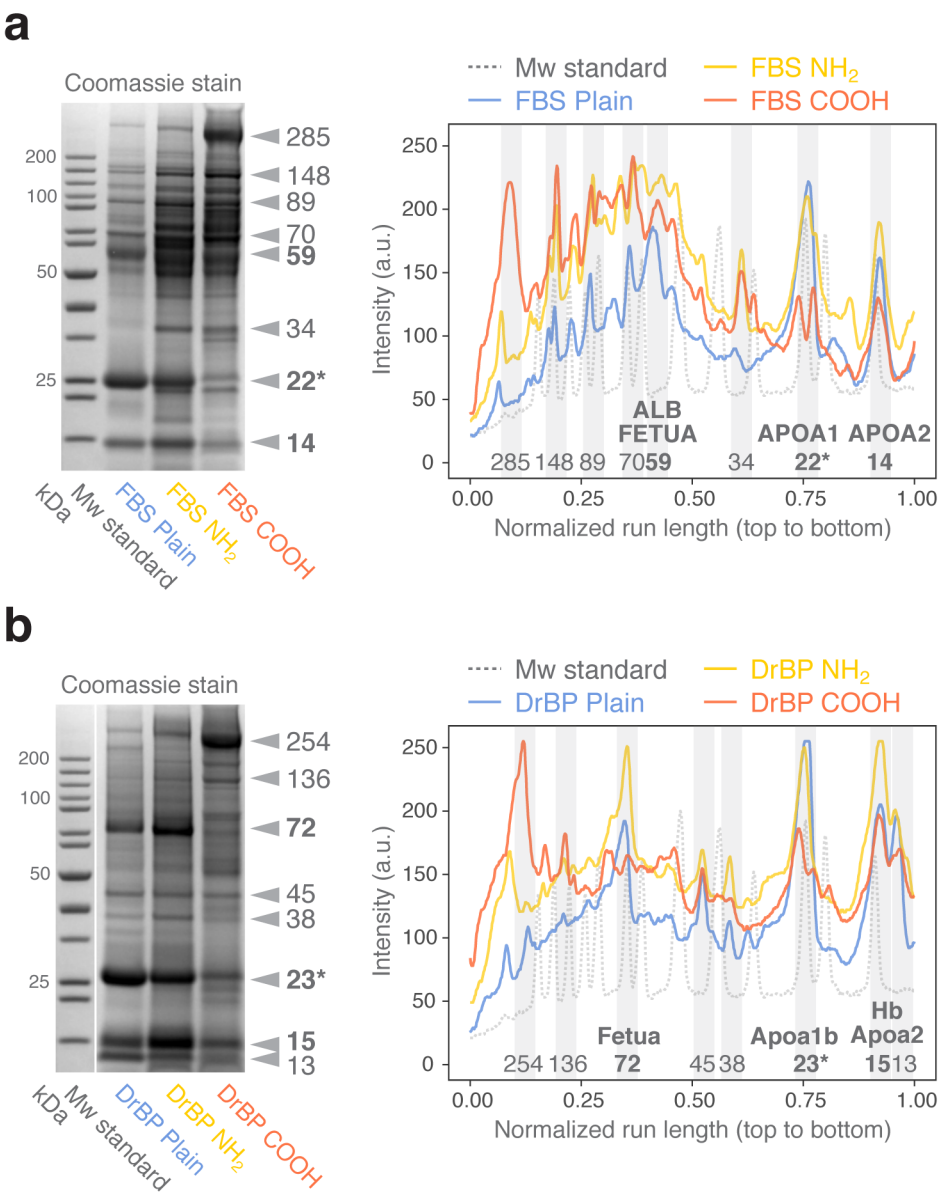


c

band	protein	species origin	protein score
DrBP as the protein source			
1	alpha-2-HS-glycoprotein 1 (Ahsg1; aka Fetua)	*Danio rerio*	3127
2	haemoglobin subunit (Hb)	*Danio rerio*	12571
	apolipoprotein A-II (Apoa2)	*Danio rerio*	3235
FBS as the protein source			
3	albumin (ALB)	*Bos taurus*	9664
	alpha-2-HS-glycoprotein (AHSG; aka FETUA)	*Bos taurus*	5009
4	apolipoprotein A-II (APOA2)	*Bos taurus*	3446
FBS PC + 15 min in DrBP			
5	alpha-2-HS-glycoprotein 1 (Ahsg1; aka Fetua)	*Danio rerio*	3799
	alpha-2-HS-glycoprotein (AHSG; aka FETUA)	*Bos taurus*	621
6	apolipoprotein A-I (APOA1)	*Bos taurus*	32078
	apolipoprotein A-Ib (Apoa1b)	*Danio rerio*	4029
FBS PC + 6 h in DrBP			
7	apolipoprotein A-I (APOA1)	*Bos taurus*	12244
	apolipoprotein A-Ib (Apoa1b)	*Danio rerio*	12222

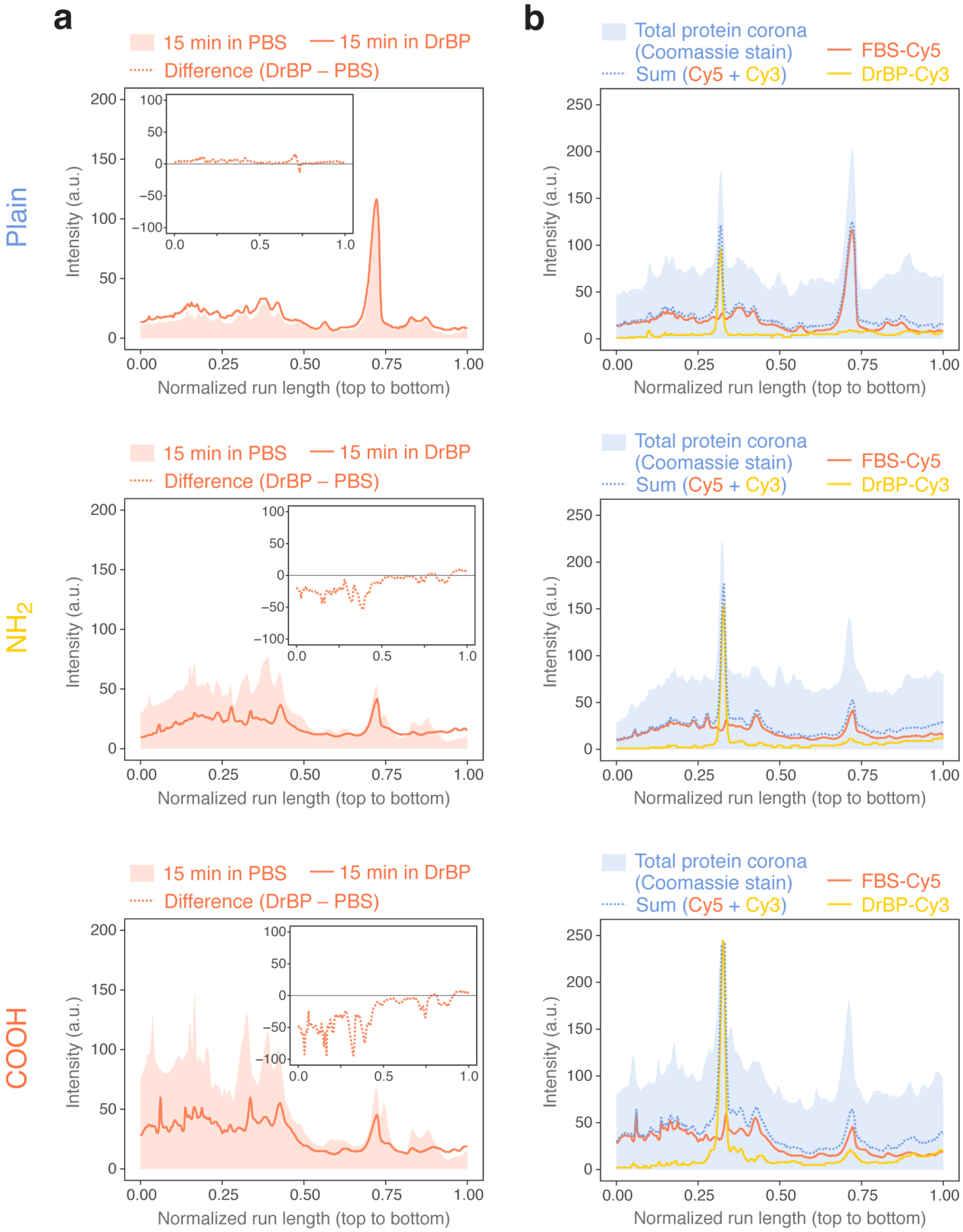
Supplementary Figure 2. Original gel images and protein identification. a,b, Original gel images (**a, b**) and fluorescence scans (**b**) used for **Fig. 1c-e** additionally showing the results from two different surface functionalizations (-NH_2 and -COOH) and at the 6 h time point for the Plain surface (**b**). Note that the nominal mass of nanoparticles from which the PC was stripped is different between the gels in **a** (150 μg) and **b** (30 μg). **c,** MS/MS protein identification of the selected bands designated in **a** and **b**. The bands designated with dashed line rectangles in **a** were excluded from analysis since we have previously identified them as Apoa1b/APOA1 in similar experiments[1] and in this study after 15 min/6 h incubation in DrBP. Protein entries in yellow and orange are from protein databases for *Danio rerio* and *Bos taurus*, respectively. See Supplementary Table 2 for details of the identified proteins.

Supplementary Figure 3



Supplementary Figure 3. Comparison of SDS-PAGE profiles for different surface functionalizations. a,b, SDS-PAGE profiles for FBS PC (**a**) and DrBP PC (**b**). Major protein bands common to all surface types are indicated as arrowheads (gel images) and as grey-shaded areas (profile plots) along with molecular weights estimated from the molecular weight standards and, where available, the protein names identified (see Supplementary Table 2). Asterisks denote the proteins identified in our previous report[1]. a.u., arbitrary unit.

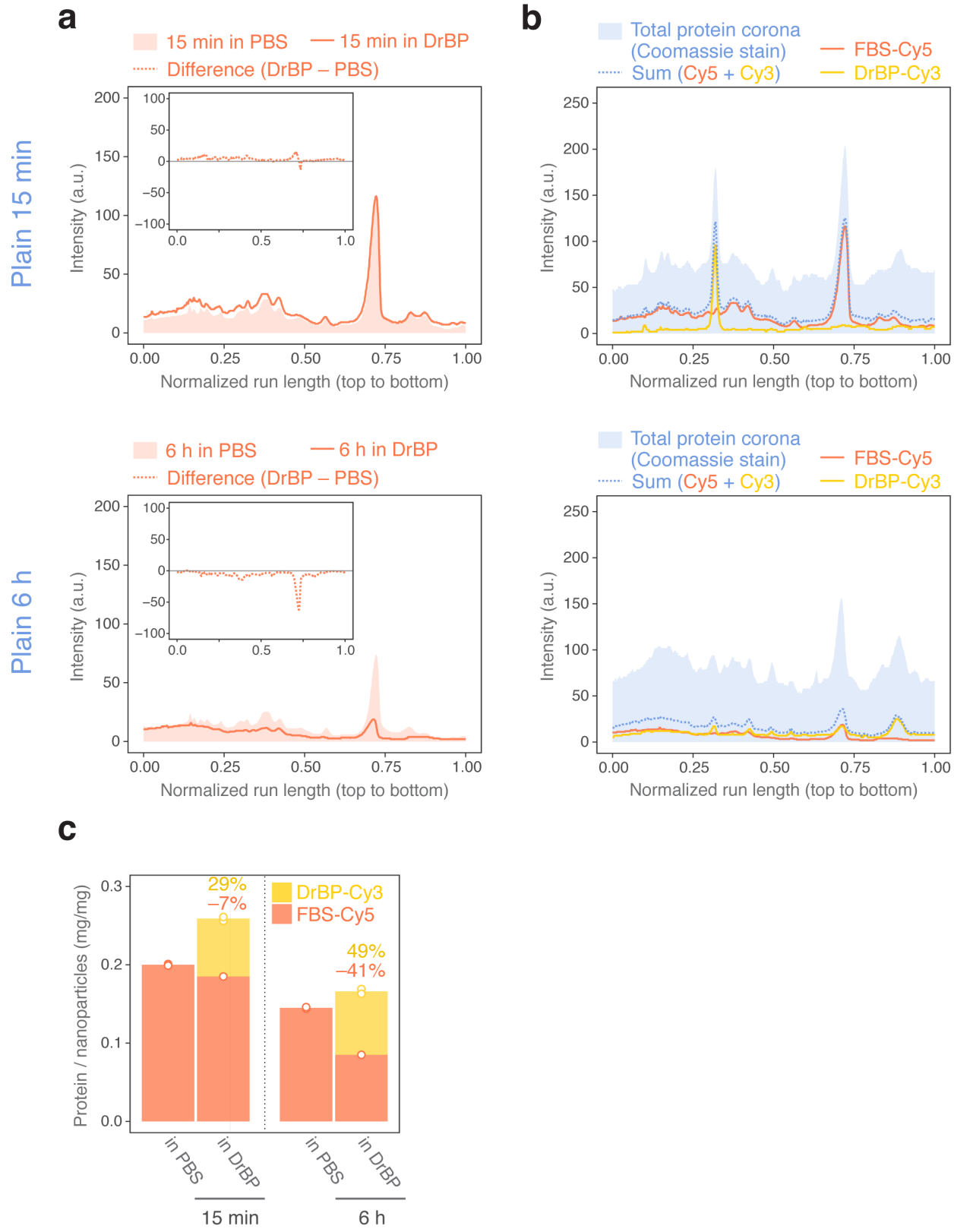
Supplementary Figure 4



Supplementary Figure 4. PC retention profiles for different surface functionalizations. a, PC retention profiles for FBS-Cy5 following 15 min incubation in PBS or DrBP. The difference is plotted in the inset, showing a major decrease of higher molecular weight proteins for NH_2 and COOH. **b,**

The modified PC compositions after 15 min incubation in DrBP. Dashed line is the sum of FBS-Cy5 and DrBP-Cy3 signals that generally follows the peak positions identified by Coomassie Brilliant Blue staining of the total PC. See Supplementary Fig. 2b for the fluorescence scans. a.u., arbitrary unit.

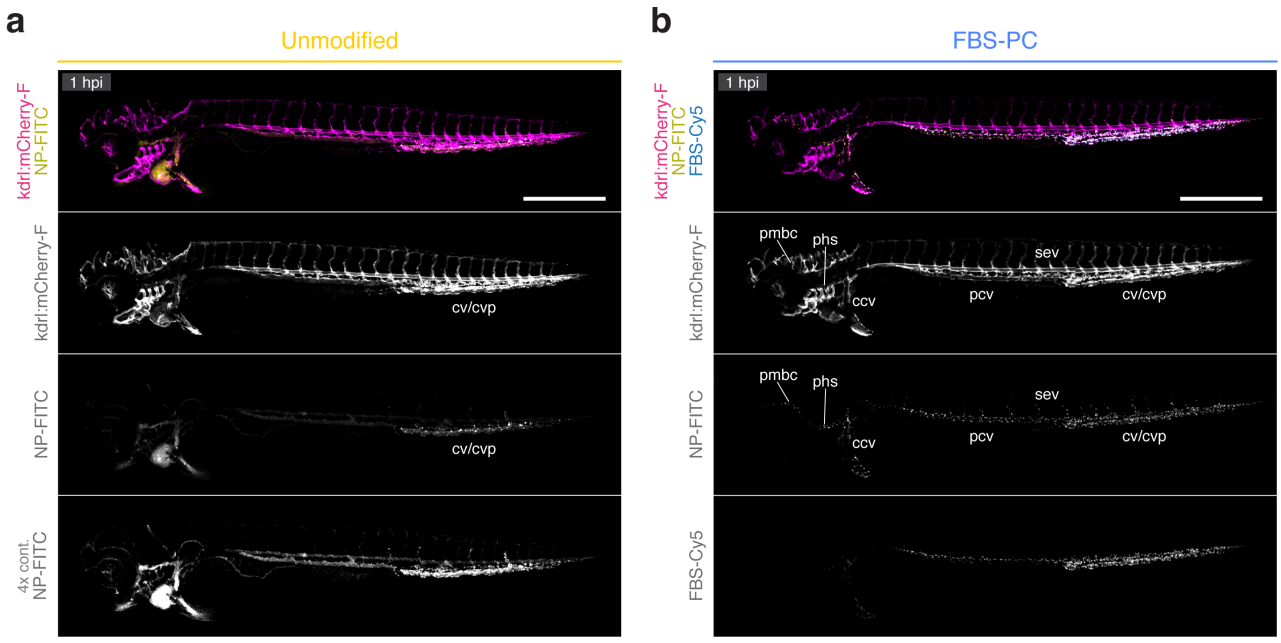
Supplementary Figure 5



Supplementary Figure 5. PC retention profiles at two time points. a, PC retention profiles for FBS-Cy5 following 15 min and 6 h incubation in PBS or DrBP. The difference is plotted in the inset, showing a major decrease of the band representing APOA1 at 6 h. **b,** The modified PC compositions

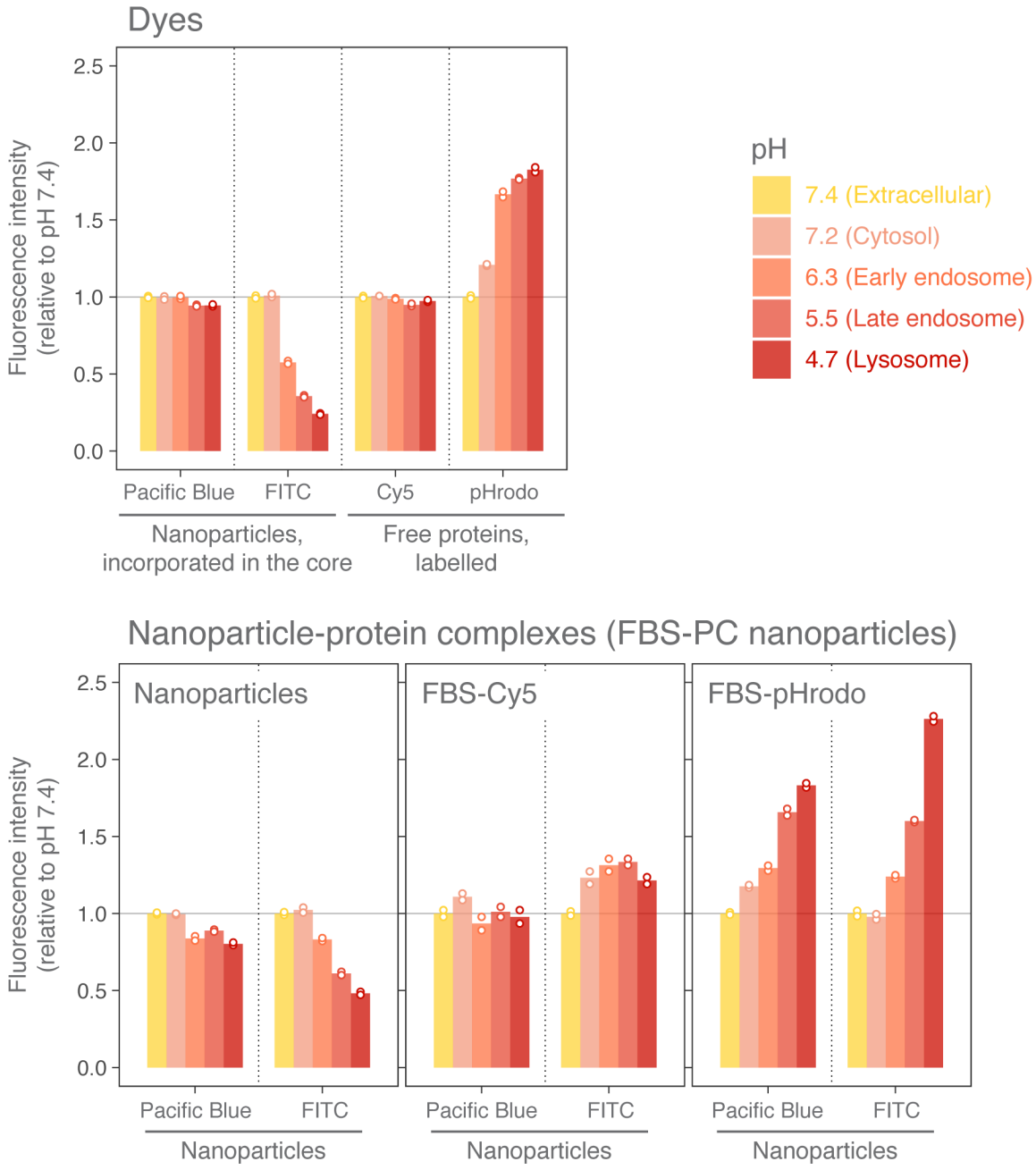
after 15 min and 6 h incubation in DrBP. Dashed line is the sum of FBS-Cy5 and DrBP-Cy3 signals that generally follows the peak positions identified by Coomassie Brilliant Blue staining of the total PC. Note the decrease of the band representing Fetua (DrBP-Cy3) and nearly equal contribution of APOA1 (FBS-Cy5) and Apoa1b (DrBP-Cy3) for the band corresponding to Apoa1b indicating competitive replacement of FBS by DrBP at 6 h. See Supplementary Fig. 2b,c for the fluorescence scans and protein scores for APOA1 *versus* Apoa1b. a.u., arbitrary unit. **c,** Fluorimetry-based quantification of FBS-Cy5 and DrBP-Cy3 in the PC. The values shown above the columns are the percentage decrease of FBS as compared to "in PBS" and the fraction of DrBP in the total PC. Columns represent the mean of two measurements (empty points).

Supplementary Figure 6



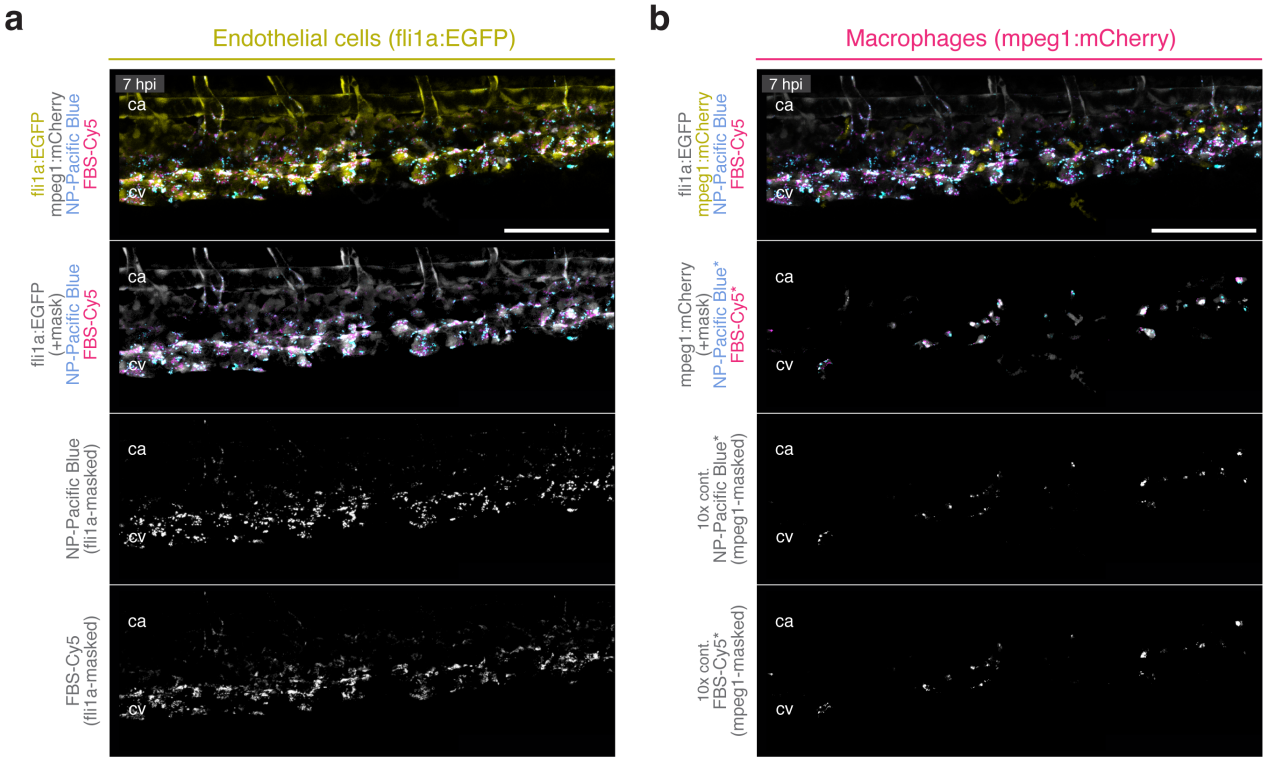
Supplementary Figure 6. Biodistribution mapping of IV injected nanoparticles. a,b, *Tg(kdrl:mCherry-F)* embryos at 3 dpf were injected with FITC-labelled nanoparticles (NP-FITC) with or without pre-formed FBS PC (FBS-Cy5) and imaged at 1 hpi. Representative tiled images depict the blood vessel networks (*kdrl:mCherry-F*) and biodistribution of unmodified (**a**) and FBS-PC nanoparticles (**b**). The NP-FITC signals of the bottom panel in **a** are multiplied by 4-fold to aid visualization of blood-borne nanoparticles that remain circulating in the bloodstream. Anatomical annotations are indicated where the nanoparticles are sequestered. Anterior left, dorsal top. Scale bars, 500 μm. pmbc, primordial midbrain channel. phs, primary head sinus. ccv, common cardinal vein. pcv, posterior caudal vein. cv, caudal vein. cvp, caudal vein plexus. sev, intersegmental veins.

Supplementary Figure 7



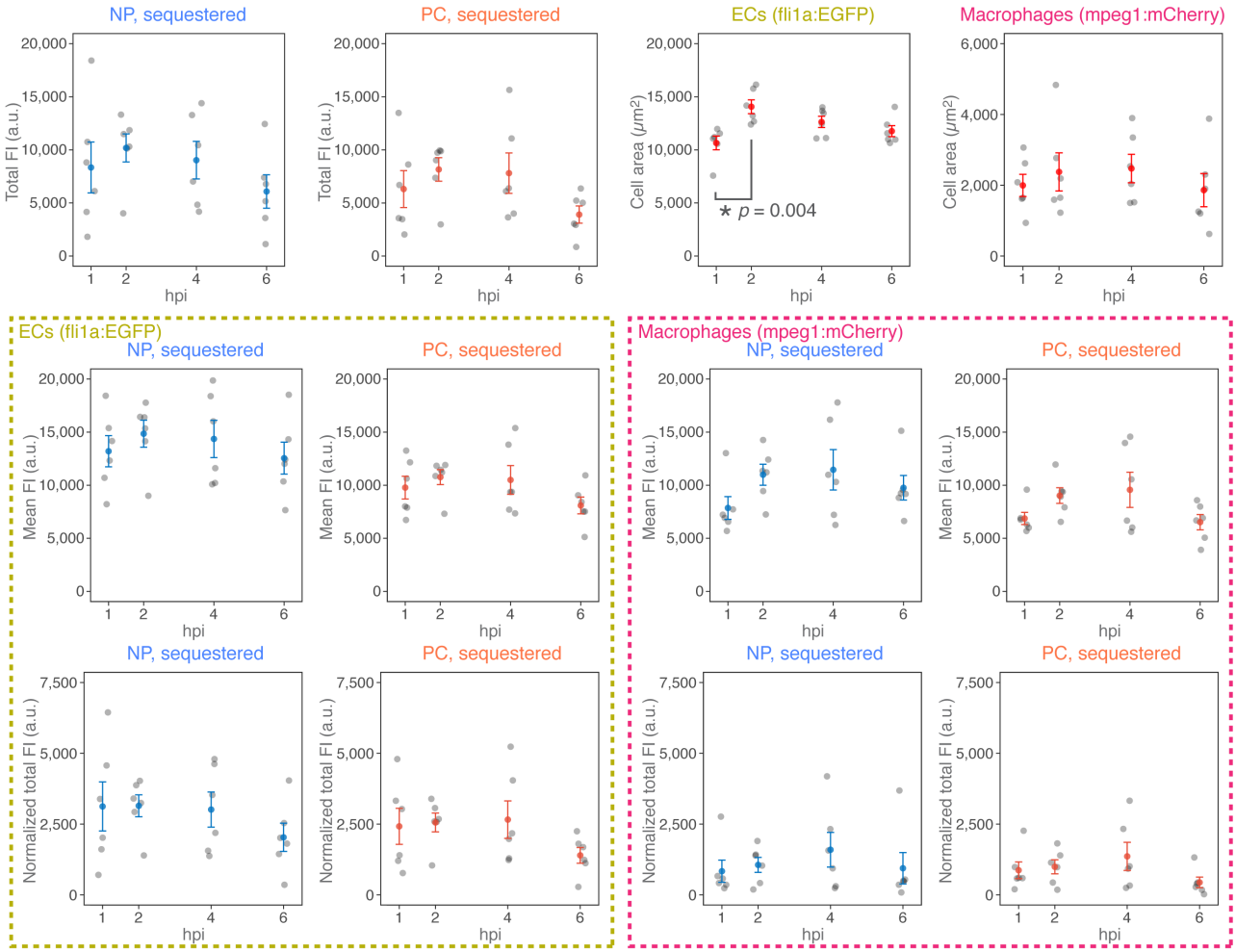
Supplementary Figure 7. Effects of pH on the fluorescence intensity of FBS-PC nanoparticles. Fluorescence intensities of Pacific Blue and FITC nanoparticles, free proteins labelled with Cy5 or pHrodo, and the nanoparticle-protein complexes (FBS-PC nanoparticles) labelled with the mixture of Cy5 and pHrodo were measured by fluorimetry at varying pH relevant for intracellular environments. Columns represent the mean of two measurements (empty points). Phosphate-citrate buffer was used to achieve pH of choice while retaining the buffering capacity. DLS was performed on the nanoparticles at pH ranges between 4 to 7.4 and confirmed no pH-induced agglomeration.

Supplementary Figure 8



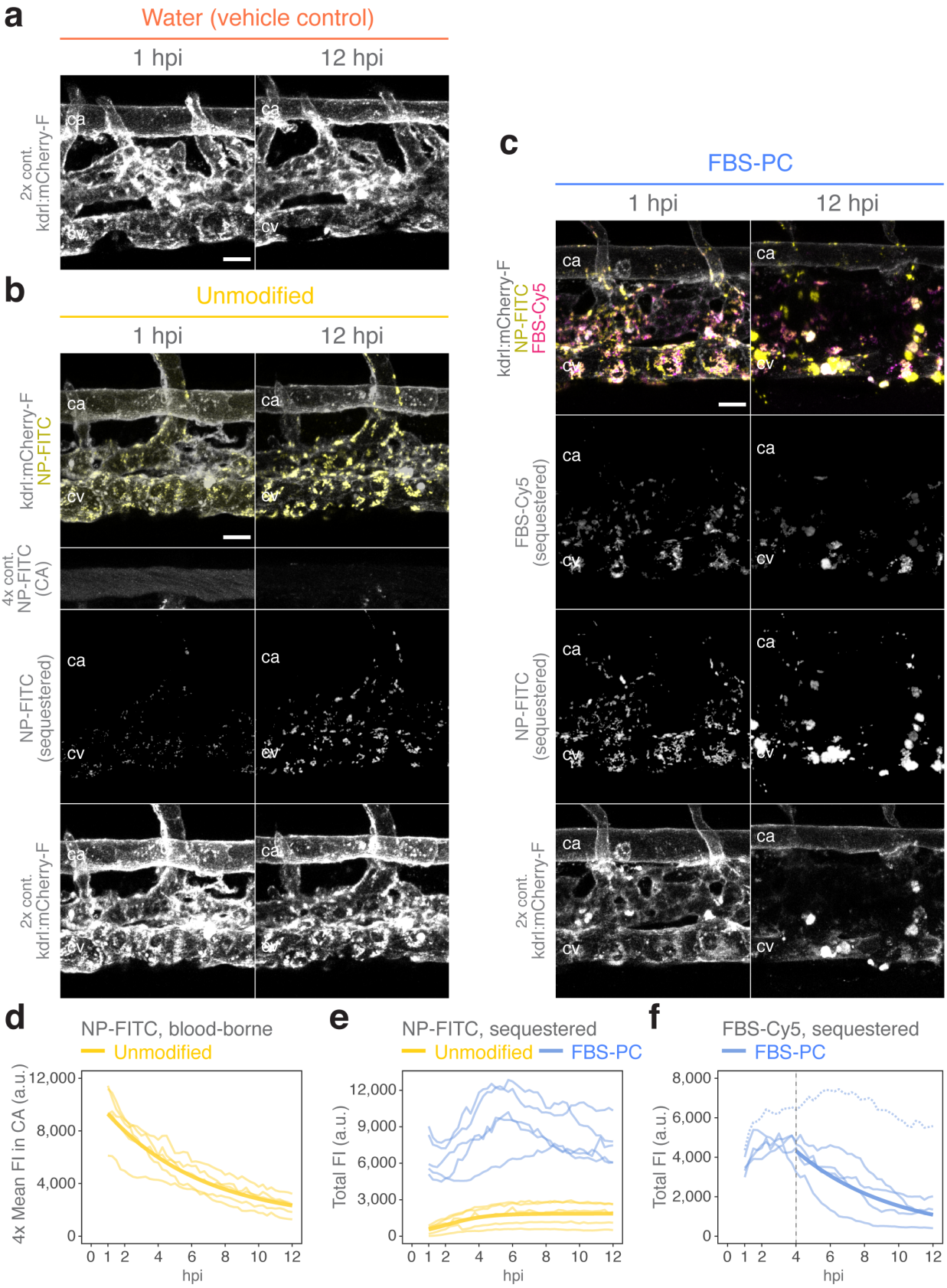
Supplementary Figure 8. The 3D mask approach to analyze cell type-specific signals. a,b, *Tg(fli1a:EGFP); Tg(mpeg1:mCherry)* embryos at 3 dpf were injected with Pacific Blue-labelled nanoparticles (NP-Pacific Blue) with pre-formed FBS PC (FBS-Cy5) and imaged at 7 hpi. Representative tiled images showing EC- (**a**) and macrophage-specific (**c**) signals for NP-Pacific Blue and FBS-Cy5. Asterisks in **b** indicate enhanced contrast (10-fold) applied to nanoparticle and FBS-Cy5 signals to aid visualization. Anterior left, dorsal top. Scale bars, 250 μm.

Supplementary Figure 9



Supplementary Figure 9. Supplementary image analysis results for Fig. 4a-d. *Tg(fli1a:EGFP); Tg(mpeg1:mCherry)* embryos at 3 dpf were injected with Pacific Blue-labelled nanoparticles with pre-formed FBS PC for each time point of 1, 2, 4 and 6 hpi and imaged independently. Values are plotted as individual embryos (grey points, $n = 6$) and the mean ± s.e.m. (blue/orange/red-coloured). Significant differences were tested by one-way ANOVA with Tukey's HSD post-hoc comparisons (degrees of freedom = 20). "Normalized total FI" is total FI divided by the cell area (ECs or macrophages). a.u., arbitrary unit. FI, fluorescence intensity.

Supplementary Figure 10



Supplementary Figure 10. Supplementary image analysis results for Fig. 4e-g. a-f, *Tg(kdrl:mCherry-F)* embryos at 3 dpf were injected with FITC-labelled nanoparticles (NP-FITC)

with or without pre-formed FBS PC (FBS-Cy5) and imaged every 15 min starting at 1 hpi. Representative images showing the intact blood vessels (**a, b**), blood clearance of unmodified nanoparticles (**b**), and loss of fluorescence signals from scavenger ECs and sequestered FBS-PC nanoparticles both as NP-FITC and FBS-Cy5 (**c**). The mCherry and NP-FITC (in CA) signals are multiplied by 2-fold and 4-fold, respectively, to aid visualization. Anterior left, dorsal top. Scale bars, 20 μm. Kinetics for clearance of blood-borne nanoparticles (**d**) and sequestration of nanoparticles (**e**) and FBS PC (**f**) are plotted as individual embryos (thin lines, $n = 5$) and, where possible, curve-fitted (thick lines). Note that the NP-FITC fluorescence is quenched after endolysosomal sequestration, resulting in the signal reduction over time even though the blood vessels are intact. The curve fitting in **f** was performed after excluding an outlier (the dashed line) and between 4-12 hpi where steady decreases are observed. a.u., arbitrary unit. FI, fluorescence intensity. See Movies 4-6 for the time-lapse sequences.

Descriptions of movie files

Movie 1. Time-lapse imaging of nanoparticle sequestration at 3-30 mpi. *Tg(kdrl:mCherry-F)* embryos at 3 dpf were injected with FITC-labelled nanoparticles (NP-FITC) without pre-formed FBS PC and imaged every 3 min. Representative movies showing sequestration of unmodified nanoparticles. The huge clusters of nanoparticles that move around are likely those associated with macrophages[2]. Left panel, merged channel for total signals: *kdrl:mCherry* in grey and NP-FITC in yellow. Right panel, single channel for sequestered nanoparticles: NP-FITC in grey. Anterior left, dorsal top.

Movie 2. Time-lapse imaging of nanoparticle sequestration at 3-30 mpi. *Tg(kdrl:mCherry-F)* embryos at 3 dpf were injected with FITC-labelled nanoparticles (NP-FITC) with pre-formed FBS PC (FBS-Cy5) and imaged every 3 min. Representative movies showing sequestration of FBS-PC nanoparticles. Left panels, merged channels for total signals: *kdrl:mCherry* in grey, NP-FITC in yellow and FBS-Cy5 in magenta, included (top) or excluded (bottom). Right panels, single channels for sequestered nanoparticles: NP-FITC in grey (bottom) and FBS-Cy5 in grey (top). Anterior left, dorsal top.

Movie 3. Time-lapse imaging of nanoparticle acidification at 3-24 mpi. Wild-type embryos at 3 dpf were injected with FITC-labelled nanoparticles with pre-formed FBS PC (FBS-Cy5 and FBS-pHrodo) and imaged every 3 min. Representative movies showing sequestration of dual-labelled FBS PC and the fluorescence ratios, 0-2 scaled. Left panel, merged channel for total signals: FBS-Cy5 in magenta and FBS-pHrodo in cyan. Right panel, fluorescence ratios for sequestered FBS PC: 0-2 scaled from purple/dark to yellow/white. Anterior left, dorsal top.

Movie 4. Time-lapse imaging of blood vessel integrity at 1-12 hpi. *Tg(kdrl:mCherry-F)* embryos at 3 dpf were injected with a vehicle control (water) and imaged every 15 min starting at 1 hpi. Representative movies showing intact blood vessels over time. The mCherry signals are multiplied by 2-fold to aid visualization. Single channel images for total signals: *kdrl:mCherry* in grey with (right panel) and without (left panel) the 2-fold enhanced contrast. Anterior left, dorsal top.

Movie 5. Time-lapse imaging of nanoparticle sequestration and blood vessel integrity at 1-12 hpi. *Tg(kdrl:mCherry-F)* embryos at 3 dpf were injected FITC-labelled nanoparticles (NP-FITC) without pre-formed FBS PC and imaged every 15 min starting at 1 hpi. Representative movies showing sequestration of unmodified nanoparticles. Left/top panel, merged channel for total signals: *kdrl:mCherry* in grey and NP-FITC in yellow. Left/bottom panel, single channel for sequestered nanoparticles: NP-FITC in grey. Right/top panel, single channel for total signals: *kdrl:mCherry* in

grey (2-fold enhanced contrast). Right/bottom panel, single channel for all nanoparticles: NP-FITC in grey (4-fold enhanced contrast to aid visualization of blood-borne nanoparticles). Anterior left, dorsal top.

Movie 6. Time-lapse imaging of nanoparticle sequestration and loss of blood vessel integrity at 1-12 hpi. *Tg(kdrl:mCherry-F)* embryos at 3 dpf were injected with FITC-labelled nanoparticles (NP-FITC) with pre-formed FBS PC (FBS-Cy5) and imaged every 15 min starting at 1 hpi. Representative images showing loss of fluorescence signals from scavenger ECs and sequestered FBS-PC nanoparticles. Left/top panel, merged channel for total signals: *kdrl:mCherry* in grey, NP-FITC in yellow and FBS-Cy5 in magenta. Left/bottom panel, single channel for sequestered nanoparticles: NP-FITC in grey. Right/top panel, single channel for total signals: *kdrl:mCherry* in grey (2-fold enhanced contrast). Right/bottom panel, single channel for sequestered FBS PC: FBS-Cy5 in grey. Anterior left, dorsal top.

Movie 7. Time-lapse imaging of non-activated macrophages at 1-12 hpi. *Tg(mpeg1:mCherry); Tg(tnfa:GFP-F)* embryos at 3 dpf were injected with a vehicle control (water) and imaged every 20 min starting from 1 hpi. Representative movies showing no *tnfa* induction over time. Left panel, merged channel for total signals: *mpeg1:mCherry* in grey and *tnfa:GFP-F* in yellow. Right panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey and *tnfa:GFP-F* in yellow. Anterior left, dorsal top.

Movie 8. Time-lapse imaging of nanoparticle sequestration and non-activated macrophages at 1-12 hpi. *Tg(mpeg1:mCherry); Tg(tnfa:GFP-F)* embryos at 3 dpf were injected with Pacific Blue-labelled nanoparticles (NP-Pacific Blue) without pre-formed FBS PC and imaged every 20 min starting from 1 hpi. Representative movies showing sequestration of unmodified nanoparticles and no *tnfa* induction over time. Note that NP-Pacific Blue is prone to photobleaching and the overall fluorescence intensity decreases over time. Left/top panel, merged channel for total signals: *mpeg1:mCherry* in grey, *tnfa:GFP-F* in yellow and NP-Pacific Blue in cyan. Right/top panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey, *tnfa:GFP-F* in yellow and NP-Pacific Blue in cyan. Left/bottom panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey and *tnfa:GFP-F* in yellow. Right/bottom panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey and NP-Pacific Blue in cyan. Anterior left, dorsal top.

Movie 9. Time-lapse imaging of non-activated macrophages with a FBS protein control at 1-12 hpi. *Tg(mpeg1:mCherry); Tg(tnfa:GFP-F)* embryos at 3 dpf were injected with FBS proteins only (at the equivalent protein mass of FBS-PC nanoparticles) and imaged every 20 min starting from 1

hpi. Representative movies showing no sequestration of FBS proteins (FBS-Cy5) and no *tnfa* induction over time. Left/top panel, merged channel for total signals: *mpeg1:mCherry* in grey, *tnfa:GFP-F* in yellow and FBS-Cy5 in magenta. Right/top panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey, *tnfa:GFP-F* in yellow and FBS-Cy5 in magenta. Left/bottom panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey and *tnfa:GFP-F* in yellow. Right/bottom panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey and FBS-Cy5 in magenta. Anterior left, dorsal top.

Movie 10. Time-lapse imaging of nanoparticle sequestration and M1-like polarized macrophages at 1-12 hpi. *Tg(mpeg1:mCherry); Tg(tnfa:GFP-F)* embryos at 3 dpf were injected with Pacific Blue-labelled nanoparticles (NP-Pacific Blue) with pre-formed FBS PC (FBS-Cy5) and imaged every 20 min starting from 1 hpi. Representative movies showing sequestration of FBS-PC nanoparticles and *tnfa* induction over time. Note that NP-Pacific Blue is prone to photobleaching and thus FBS-Cy5 is shown instead as a proxy for both components of FBS-PC nanoparticles. Left/top panel, merged channel for total signals: *mpeg1:mCherry* in grey, *tnfa:GFP-F* in yellow and FBS-Cy5 in cyan. Right/top panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey, *tnfa:GFP-F* in yellow and FBS-Cy5 in cyan. Left/bottom panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey and *tnfa:GFP-F* in yellow. Right/bottom panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey and FBS-Cy5 in cyan. Anterior left, dorsal top.

Movie 11. Time-lapse imaging of M1-like polarized macrophages with a positive control at 1-12 hpi. *Tg(mpeg1:mCherry); Tg(tnfa:GFP-F)* embryos at 3 dpf were injected with a positive control (LPS) and imaged every 20 min starting from 1 hpi. Representative movies showing *tnfa* induction over time. Left panel, merged channel for total signals: *mpeg1:mCherry* in grey and *tnfa:GFP-F* in yellow. Right panel, merged channel for macrophage-masked signals: *mpeg1:mCherry* in grey and *tnfa:GFP-F* in yellow. Anterior left, dorsal top.

References

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