Supporting Information

Distinct macrophage uptake of engineered and biological particles driven by host age and sex

Riki Toita[a, 1, *], Yuki Shimizu[b, 1], Jeong-Hun Kang[c]

[a] Molecular Biosystems Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Ikeda, Osaka, Japan; [b] Molecular Biosystems Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan; [c] National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka, Japan; [1] These authors contributed equally to this study.

*Corresponding author: Riki Toita, Ph.D.

Molecular Biosystems Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Osaka, Japan.

E-mail: toita-r@aist.go.jp

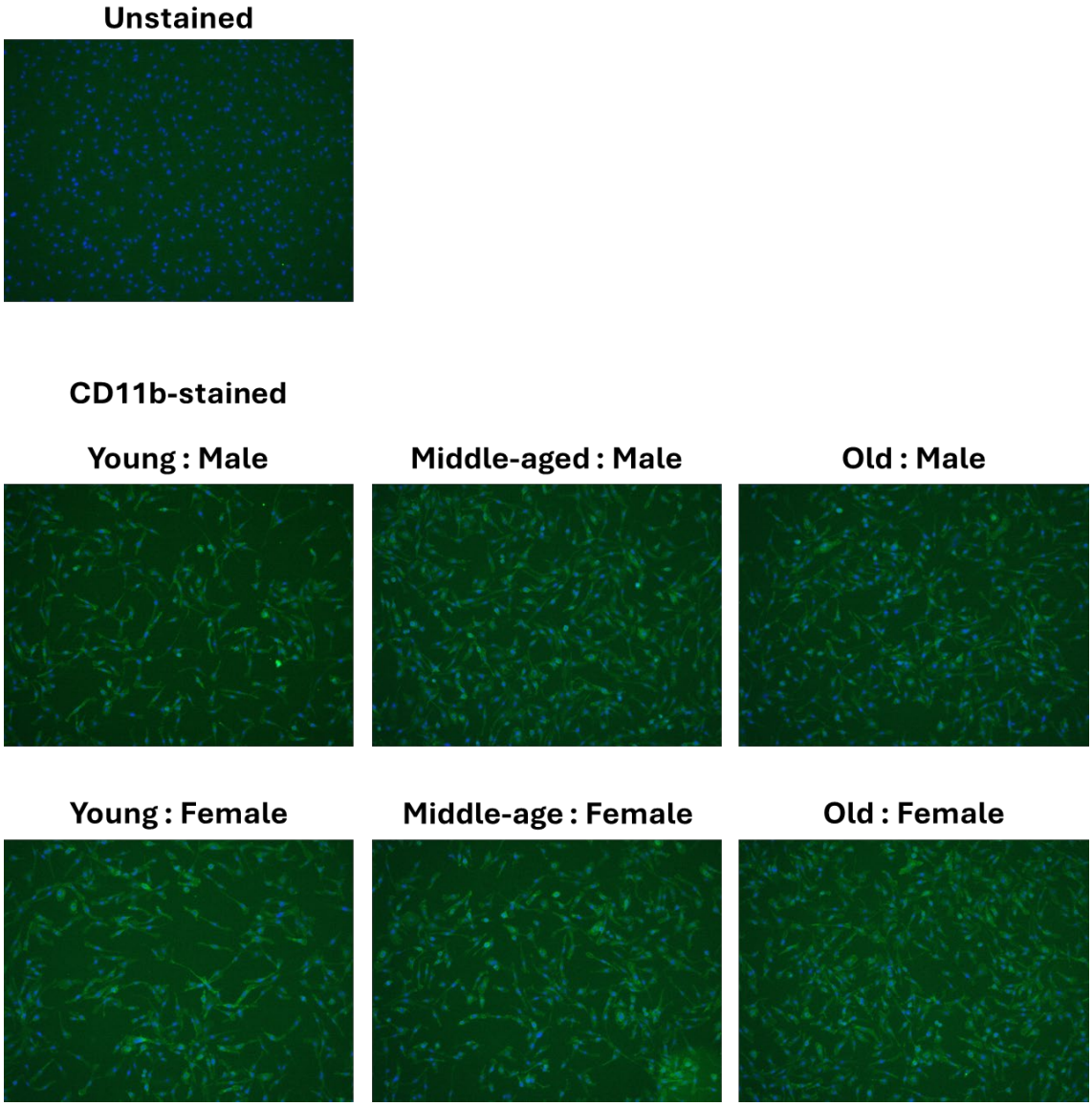

Figure S1. CD11b immunostaining of bone marrow-derived macrophages. After fixation with 4% paraformaldehyde in PBS, cells were stained with an Alexa Fluor 488–conjugated anti-mouse/human CD11b antibody (clone M1/70; 1:100; BioLegend, San Diego, CA, USA), and nuclei were counterstained with Hoechst 33342 (1:500; Dojindo, Kumamoto, Japan). Images were acquired using a ZOE Fluorescence Cell Imager (Bio-Rad Laboratories, Hercules, CA, USA). Brightness was adjusted uniformly across all images using PowerPoint.

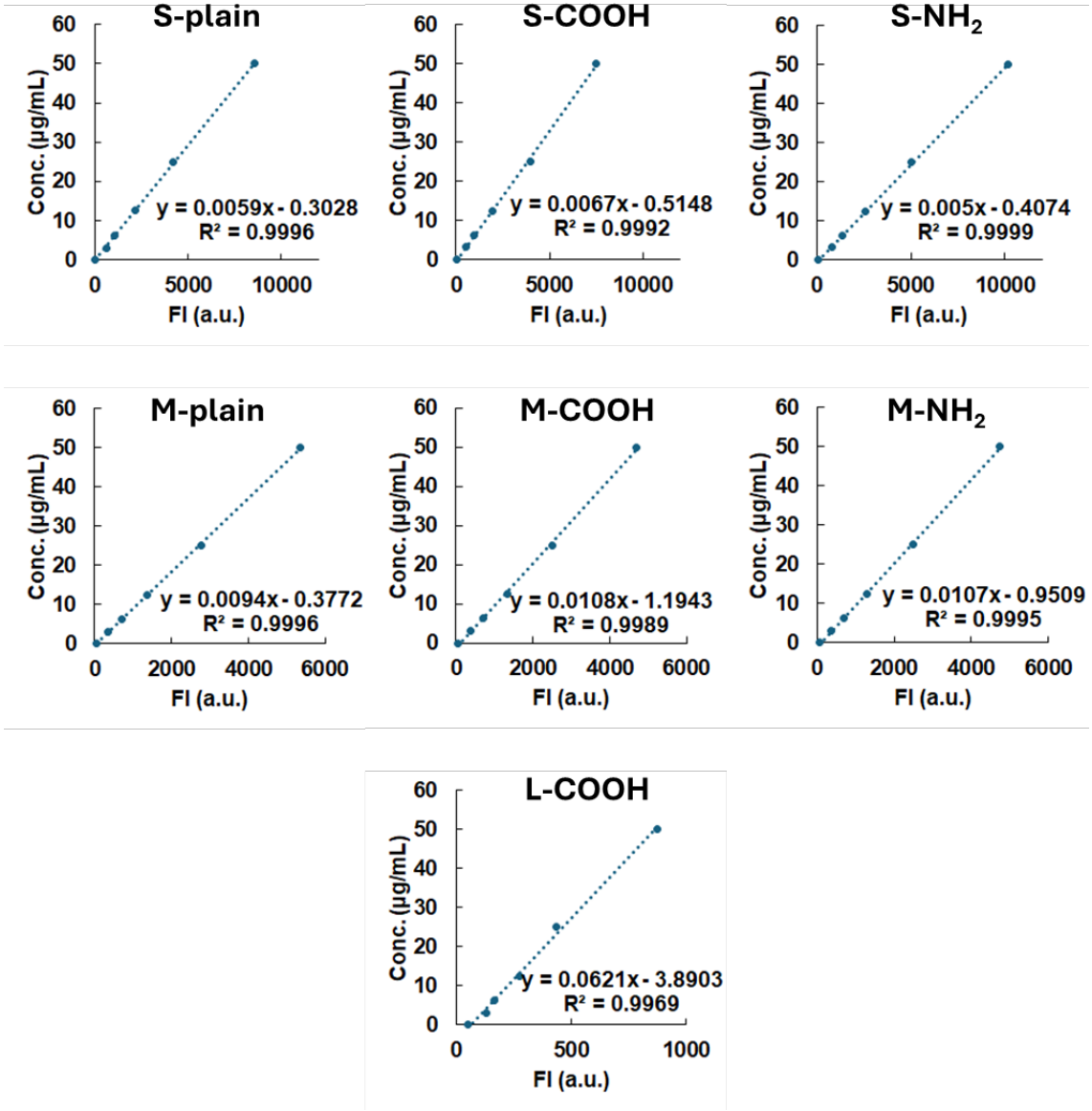

Figure S2. Standard slopes of fluorophore-modified polymeric particles.

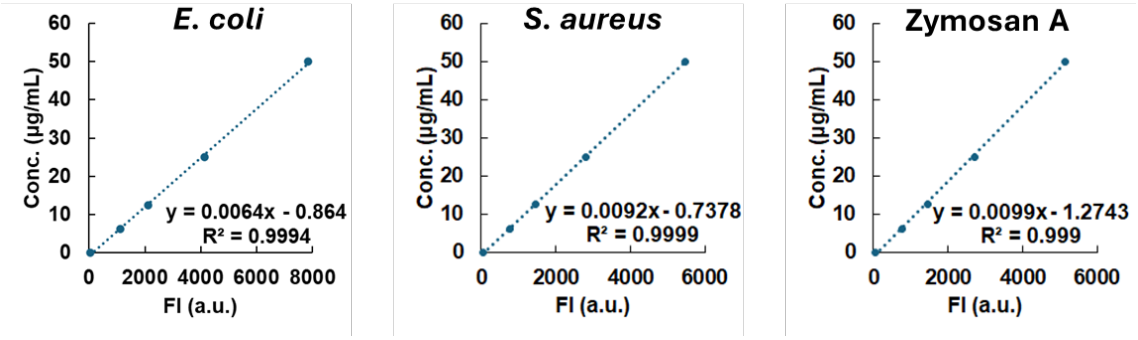

Figure S3. Standard slopes of fluorescein-modified bioparticles.

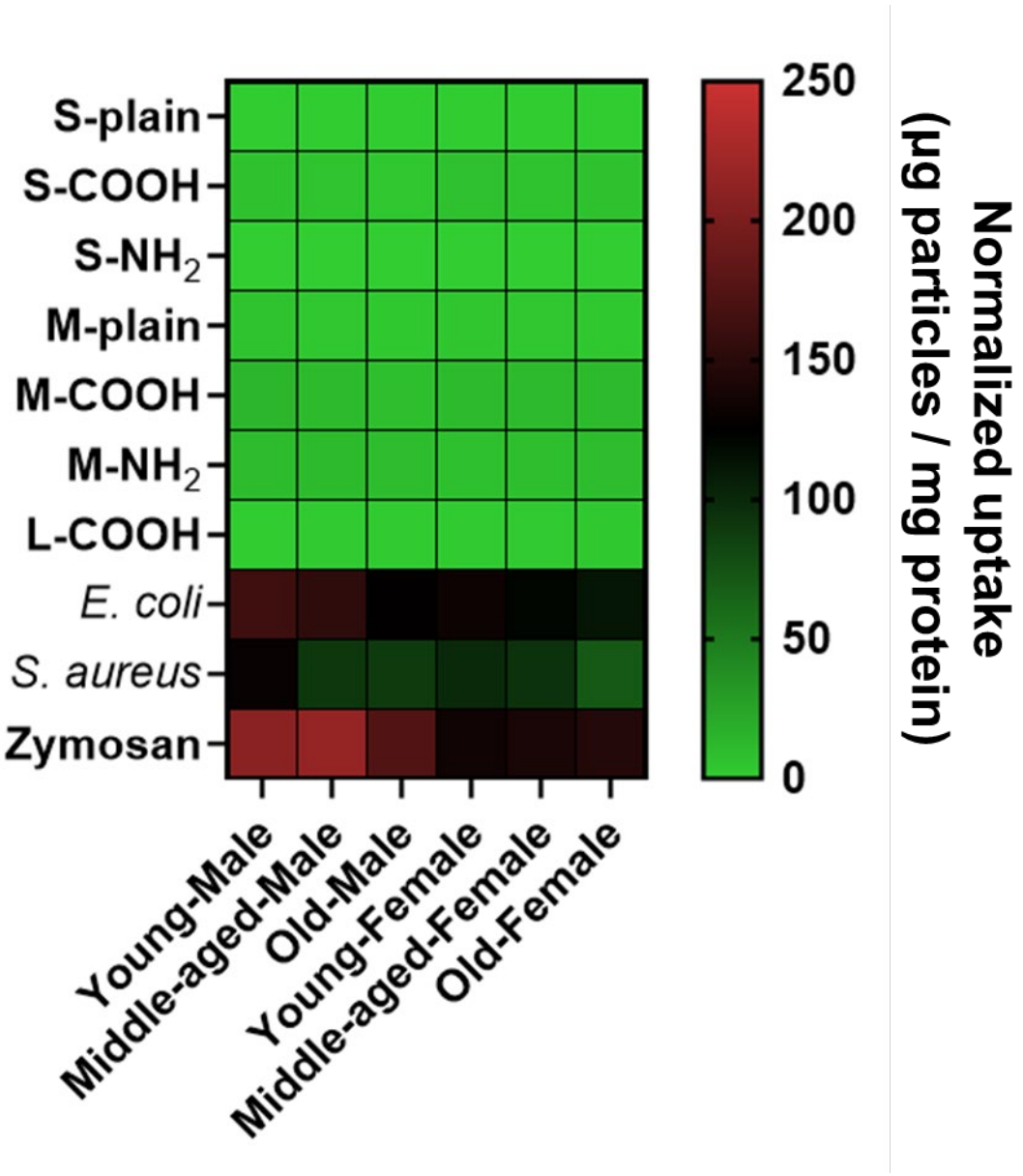

Figure S4. Amount of particle uptake 4 h after exposure of macrophages to particles.

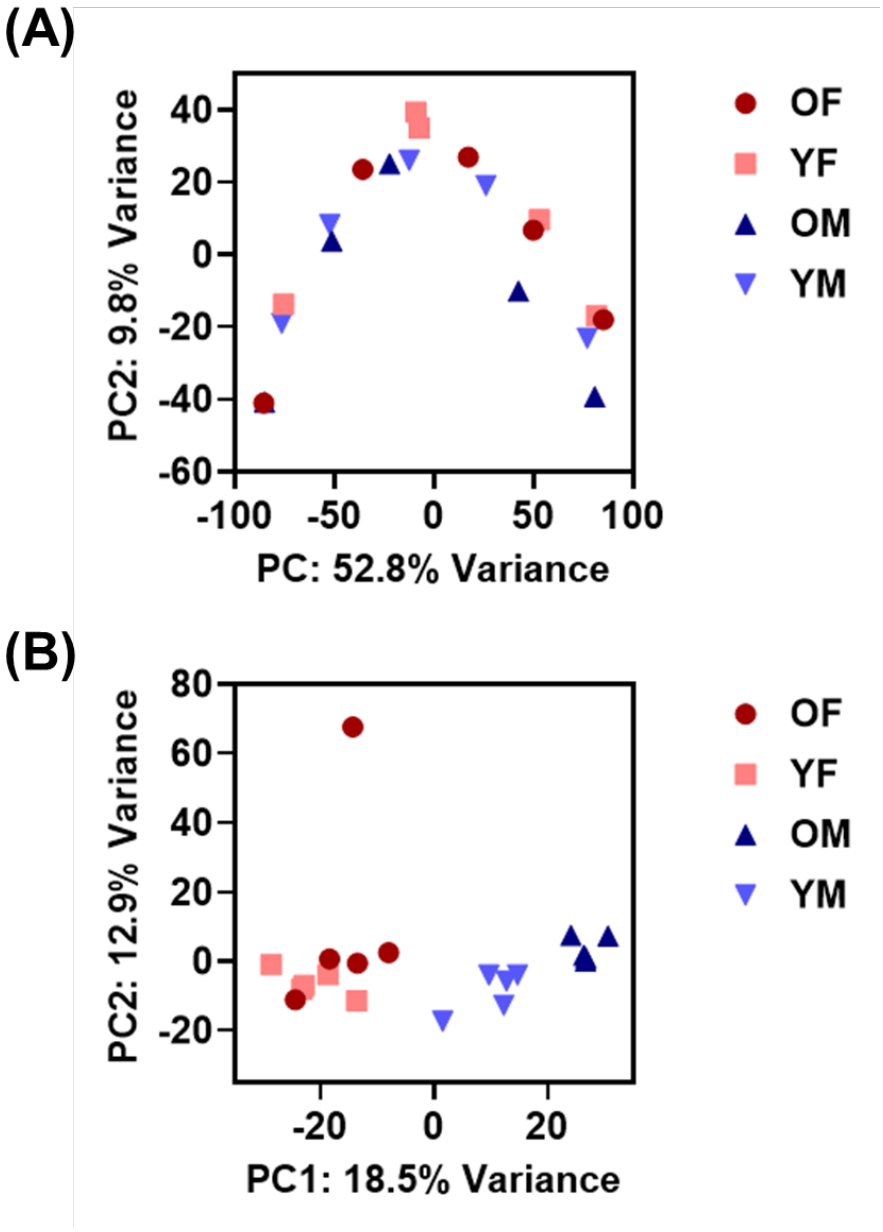

Figure S5. Principal component analysis (PCA). (A) PCA of raw count data before batch correction. (B) PCA after batch correction using ComBat-seq, showing mitigation of batch effects across samples and identification of a potential outlier sample.

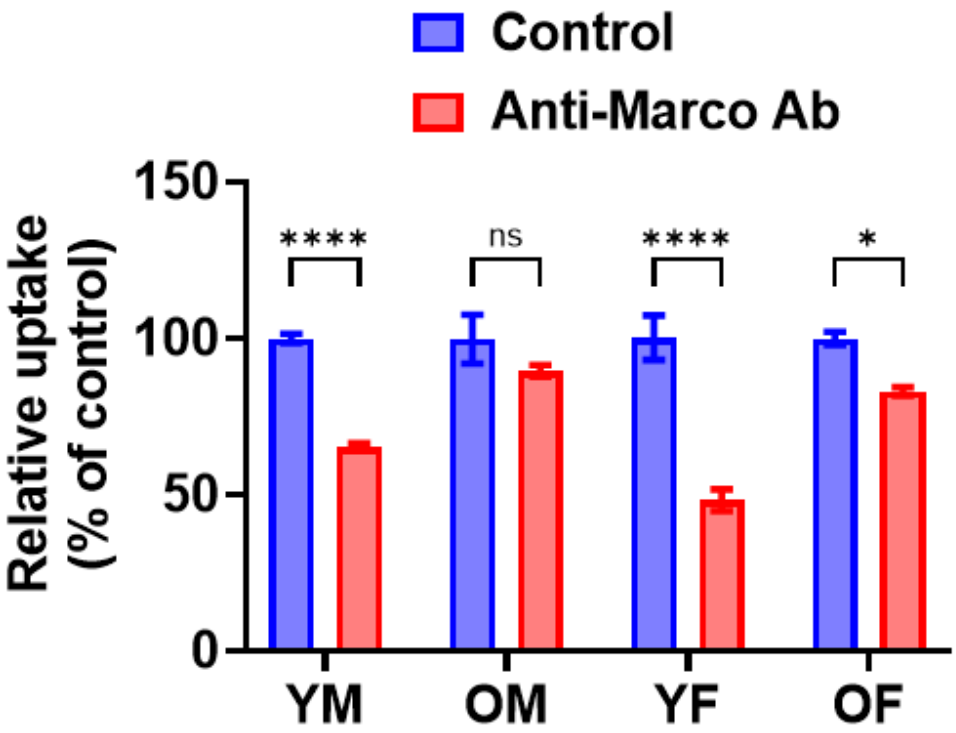

Figure S6. Blocking the MARCO receptor with an anti-MARCO antibody reduces the uptake of L-COOH particles by bone marrow-derived macrophages. Y, young; O, old; M, male; F, female. Data are means ± SE (n = 5). Statistical significance was determined by paired t-test. *$p < 0.05$; ****$p < 0.0001$; ns, not significant.

Table S1. List of common DEGs showing sex-dependent differences in macrophages.

High in female (8 genes)	**Low in female (83 genes)**		
C1qtnf6	*Eif2s3y*	*Col5a1*	*Cnn3*
Pde1c	*Ddx3y*	*Col16a1*	*Thbs2*
Eif2s3x	*Kdm5d*	*Sema7a*	*Col8a1*
Padi4	*Uty*	*Map1b*	*Amotl2*
Odf3l1	*Gm29650*	*Phldb2*	*Col12a1*
Cpne8	*ENSMUSG00000121547*	*Col3a1*	*Fn1*
Gdf3	*Grem2*	*Cyp1b1*	*Timp3*
Xist	*Cd248*	*Sparc*	*Fermt2*
	Vgll3	*Col5a2*	*Ccn2*
	Ccn5	*Cxcl12*	*Grb10*
	Eml1	*Acta2*	*Dpysl3*
	Loxl1	*Fstl1*	*Lamb1*
	Nid1	*Col6a1*	*Tgfb1i1*
	Tjp1	*Col5a3*	*Mxra8*
	Itga11	*Loxl2*	*Parva*
	Col1a1	*Fat1*	*Cemip*
	Actg2	*Fkbp10*	*Fscn1*
	Hspg2	*Fmod*	*Msrb3*
	Postn	*Mrc2*	*Aebp1*
	Ddr2	*Bgn*	*Bmp1*
	Col6a3	*Serpinh1*	*Tead1*
	Igfbp7	*Adam12*	*Ccdc80*
	Pxdn	*Fbn1*	*Enah*
	Crlf1	*Cald1*	*Timp1*
	Col1a2	*Myl9*	*Tnc*
	Podnl1	*Ccn4*	*Ltbp1*
	Fbln2	*Cdh11*	*Inhba*
	Cdh2	*Adamts2*	