

## Supporting Information

### High-content and high-throughput identification of macrophage polarization phenotypes

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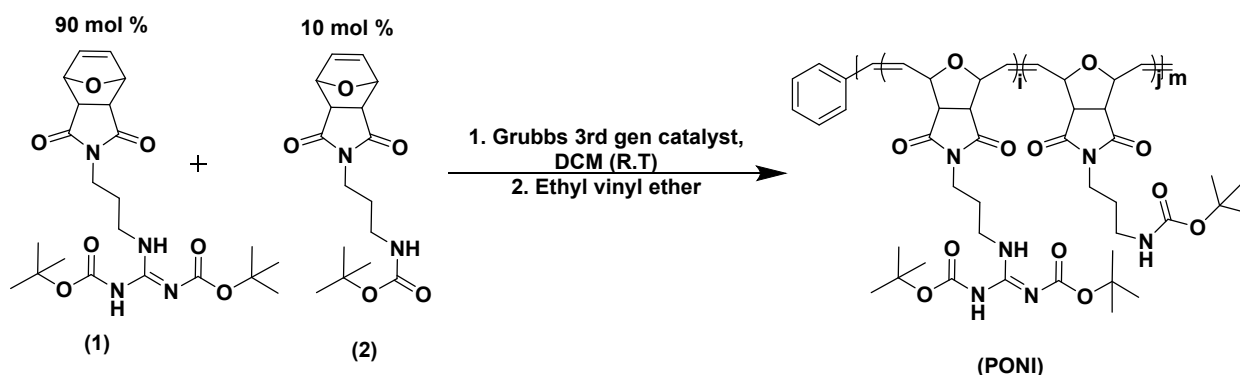
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## Table of contents

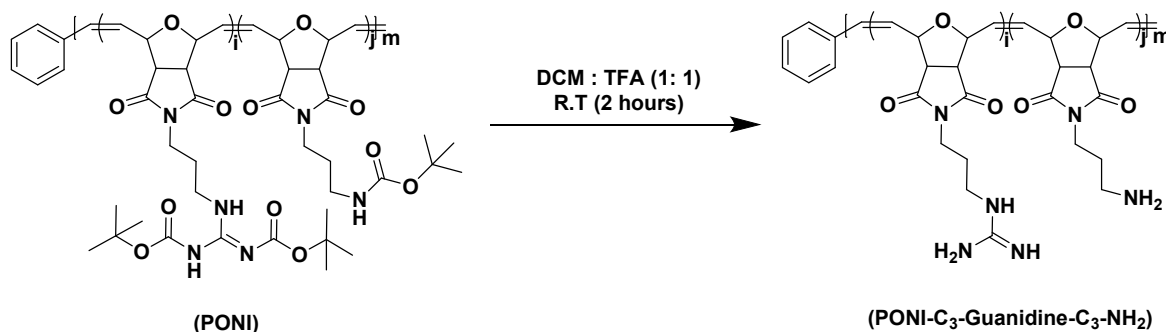
1.	Synthesis of PONI polymer	S3-4
2.	Characterization of PONI polymer and polymer-GFP assembly	S5-7
3.	RT-PCR of M1 and M2 activation markers	S8-9
4.	Sensing data	
	4.1 for M1 and M2 subtypes in RAW 264.7 cells	S10-12
	4.2 for M1 and M2 subtypes in bone marrow-derived macrophages	S13-15
	4.3 for RAW 264.7 cells exposed to conditioned media	S16-17

## 1. Synthesis of PONI polymer

The monomers **1** & **2** were made according to a previous report.<sup>1</sup>

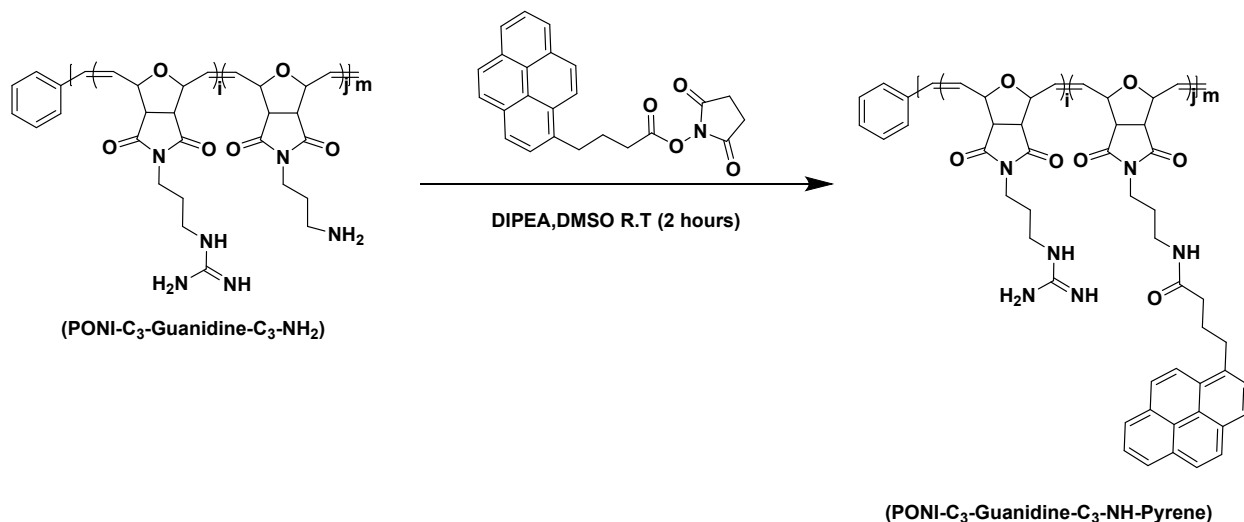


To a 15 mL pear-shaped air-free flask equipped with a stir bar was added **1** (0.5 g, 1.076 mmol, 0.9eq), **2** (39 mg, 0.1196 mmol, 0.1eq) and 5 mL of DCM. In a separate 10ml pear-shaped air-free flask was added Grubbs 3<sup>rd</sup> generation catalyst (13.6 mg, 0.015 mmol) and 1 mL DCM. Both flasks were sealed with septa and attached to a schlenk nitrogen/vacuum line. Both flasks were freeze-pump-thawed three times. After thawing, Grubbs 3<sup>rd</sup> generation catalyst was removed via syringe and quickly added to the flask containing **1** & **2** and allowed to react for 15 min. After the allotted time, ethyl vinyl ether (300  $\mu$ L) was added and allowed to stir for 20 mins. Afterwards, the reaction was diluted to two times the volume and precipitated into a heavily stirred solution of hexane. The precipitated polymer was filtered and dissolved into tetrahydrofuran (THF). The polymer was precipitated again into hexane and filtered to yield **PONI**. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) 11.49 (s, 1H), 8.48 (m, 1H), 6.53 (m, 1H), 6.1 (br, 1H), 5.79 (br 1H), 5.3 (m, 1H), 5.1 (br 1H), 4.5 (br, 1H), 4.5 (br, 1H), 3.58 (t, 3H), 3.41 (m, 4H), 2.88 (m, 1H), 1.9 (m, 2H), 1.59 (s, 9H), 1.51 (m, 24 H), 1.45 (m, 2H). The polymer was also characterized by GPC (gel permeation chromatography) in tetrahydrofuran. The M<sub>w</sub> was 50,209 and the PDI (Poly-dispersity index) was 1.04.



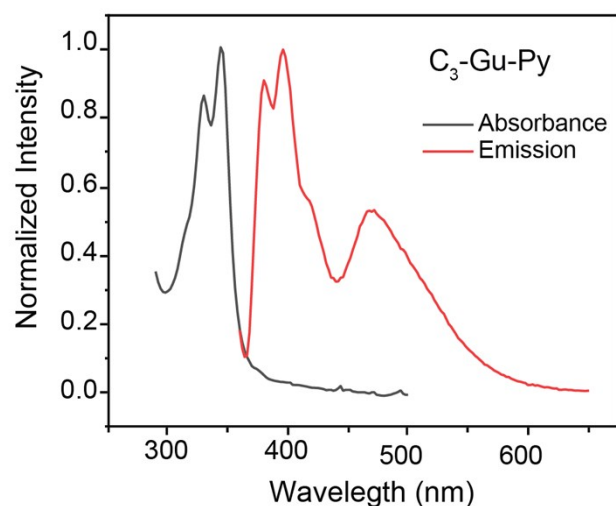
To a 20 mL scintillation vial equipped with a magnetic stir bar was added polymer **PONI** (0.4 g). 4 mL of trifluoroacetic acid was added to dissolve the polymer; the mixture was transferred to a 50 mL round bottom flask equipped with a stir bar and 4 mL of dichloromethane. This mixture was allowed to react at room temperature for two hours, followed by solvent removal via rotary

evaporation with dichloromethane 3 times (5 mL each time). The material was dried under vacuum overnight to yield **PONI-C<sub>3</sub>-Guanidine-C<sub>3</sub>-NH<sub>2</sub>**.

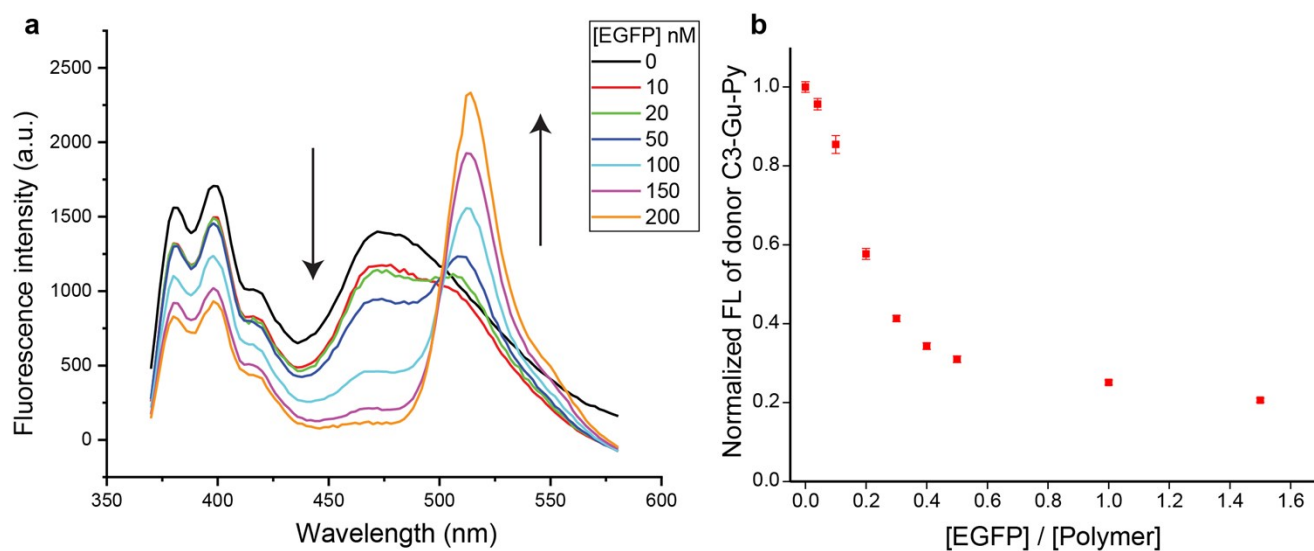


To a 20 mL scintillation vial equipped with a stir bar was added Polymer **PONI-C<sub>3</sub>-Guanidine-C<sub>3</sub>-NH<sub>2</sub>** (approximately 100 mg), which was dissolved in 2 mL of DMSO followed by the addition of ~100  $\mu$ L DIPEA. Separately, pyrene-NHS (3 mg) was dissolved in DMSO, added to the stirred reaction mixture, and allowed to react for two hours at room temperature. The progress of the reaction was monitored with TLC (9.5/0.5 – ethyl acetate/methanol) to ensure the dye was conjugated (free dye moves while polymer remains on the baseline). After the completion of the reaction, the product **PONI-C<sub>3</sub>-Guanidine-C<sub>3</sub>-NH-Pyrene** was precipitated in diethyl ether and centrifuged (repeated twice more to improve the purity). It was then dried under vacuum and used for the experiments.

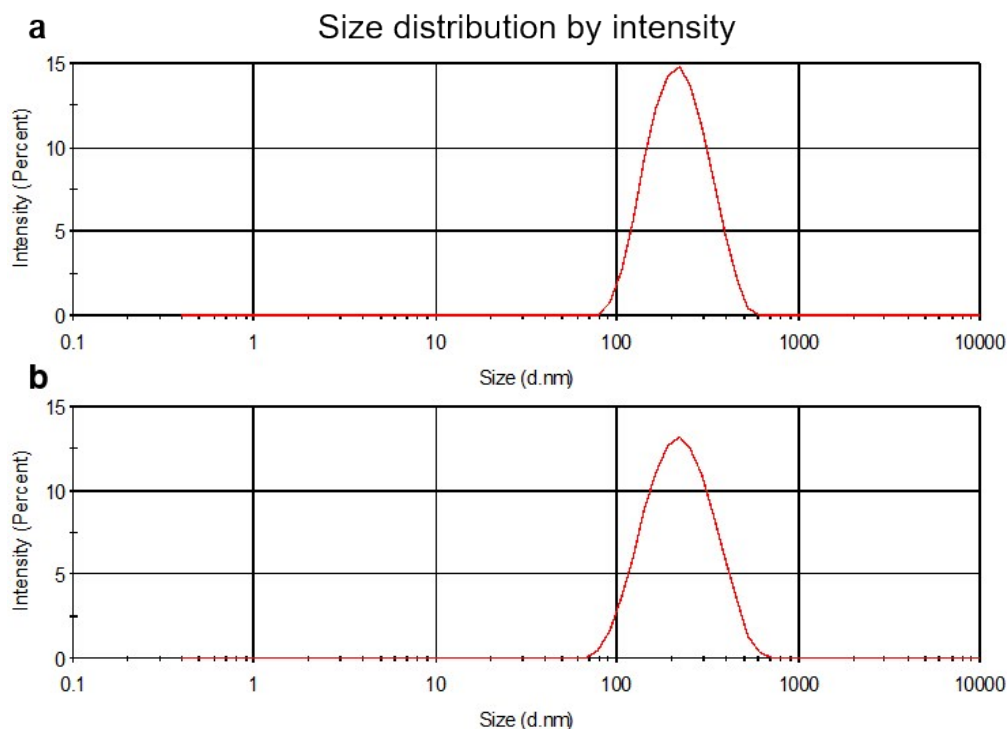
## 2. Characterization of PONI polymer and polymer-GFP assembly



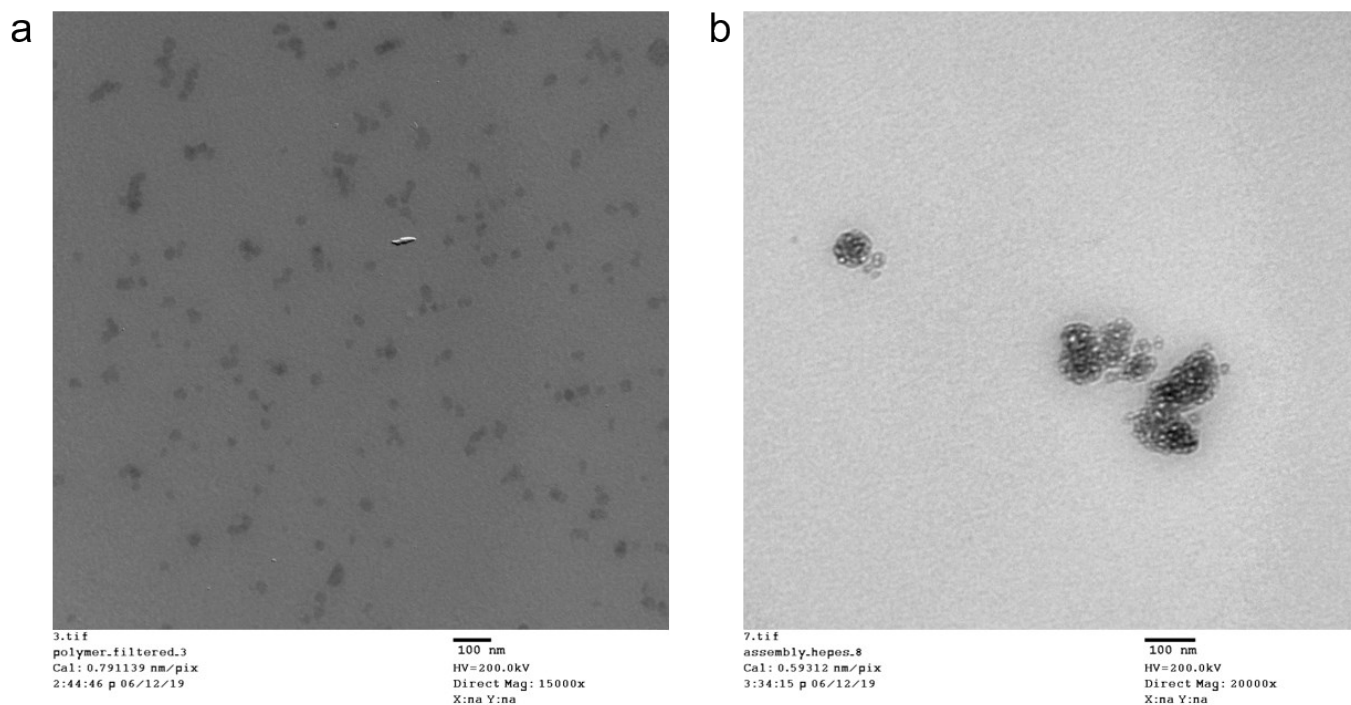
**Figure S1. Optical property of PONI-C<sub>3</sub>-Gu-Py.** Absorbance and emission spectra of PONI-C<sub>3</sub>-Gu-Py polymer was measured using Molecular Devices Spectramax M2 plate reader.



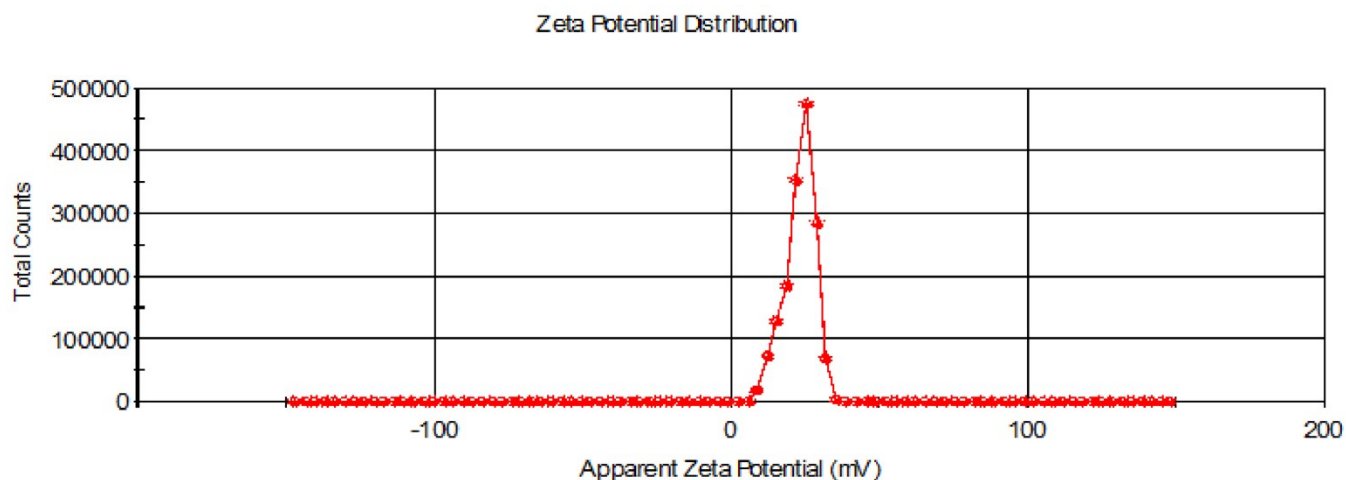
**Figure S2. Fluorescence titrations (a) and quenching (b).** 0.5  $\mu$ M C<sub>3</sub>-Gu-Py was titrated with varying concentrations of EGFP in 10 mM HEPES buffer. Fluorescence spectrum was recorded at pyrene excitation of 344 nm. A decrease in pyrene emission at 470 nm and increase of EGFP emission at 510 nm was observed. Each value is the average of three independent measurements.



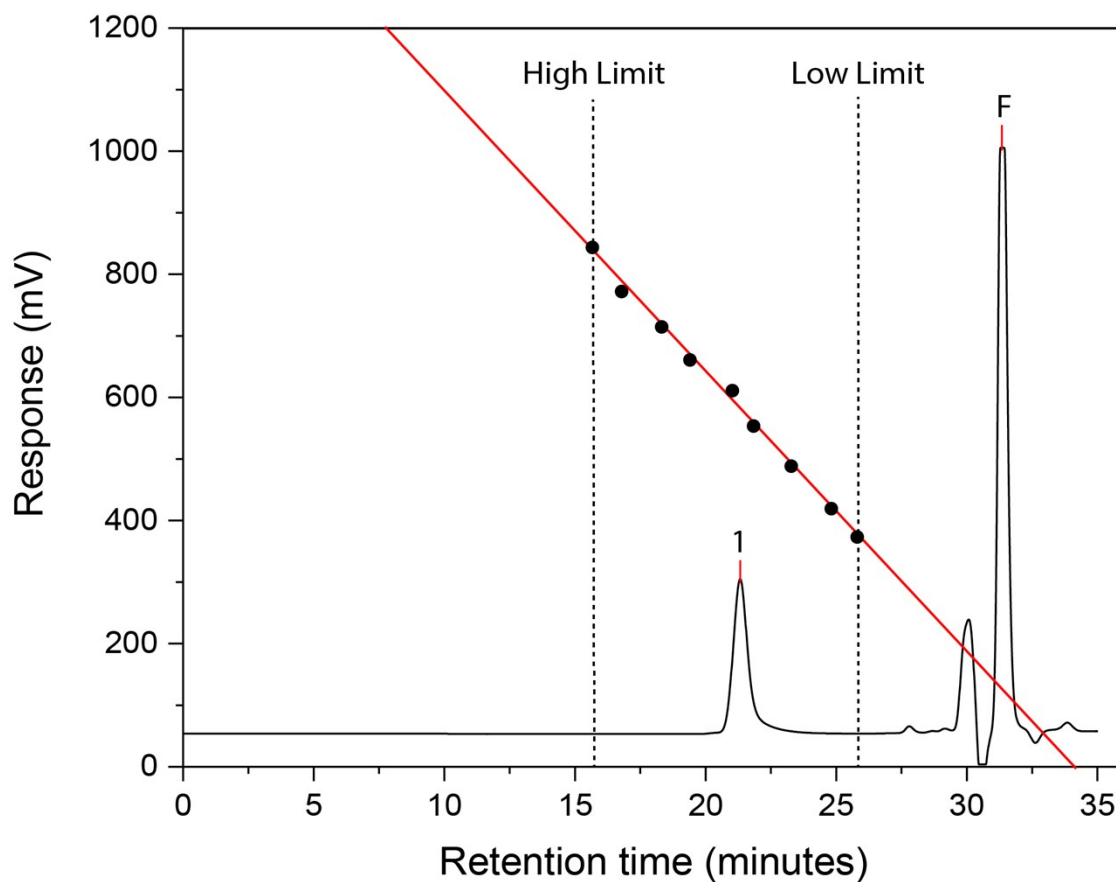
**Figure S3. Hydrodynamic size of PONI-C<sub>3</sub>-Gu-Py polymer (a) and polymer-GFP assembly (b) in 10mM HEPES buffer.** C<sub>3</sub>-Gu-Py polymer formed a complex with an average diameter of  $230 \pm 84.4$  nm. With the addition of EGFP, the size of polymer-EGFP assembly is approximately  $237 \pm 97.7$  nm in diameter.



**Figure S4. TEM image of PONI-C<sub>3</sub>-Gu-Py polymer (a) and polymer-EGFP assembly (b) in 10 mM HEPES buffer.** C<sub>3</sub>-Gu-Py polymer formed a complex of size  $\sim 25$  nm. The difference in sizes measured by DLS and TEM can be attributed to the drying process during TEM sample preparation as well as the high vacuum conditions in the TEM chamber. Upon the addition of EGFP, larger complexes were observed.

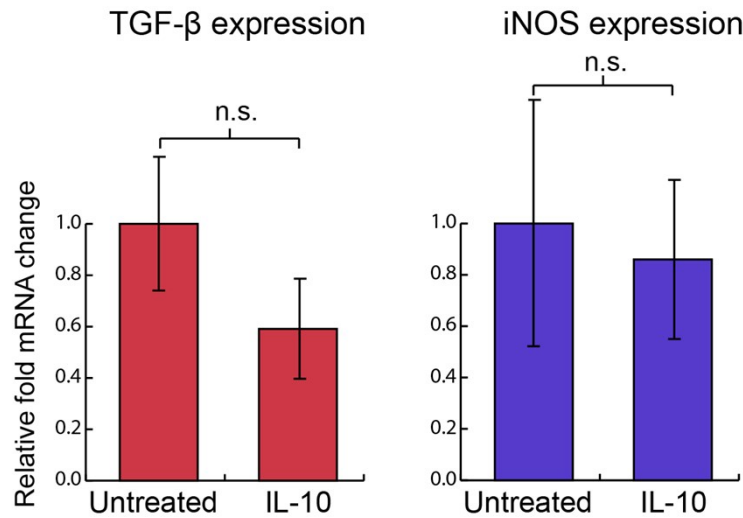


**Figure S5.** Zeta potential of PONI-C<sub>3</sub>-Gu-Py polymer in water. The value is  $23.2 \pm 5.1$  mV.

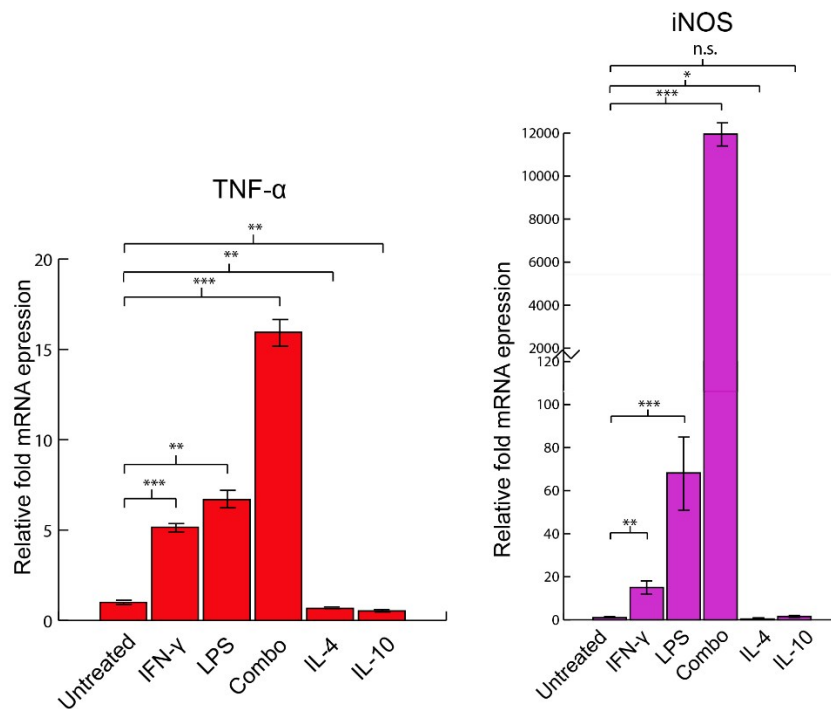


**Figure S6. Characterization of Boc-protected polymer via gel permeation chromatography.** GPC trace shows that PONI-C<sub>3</sub>-Guanidine-C<sub>3</sub>-NHBOC has  $M_w = 26$  kDa,  $M_n = 24.5$  kDa and a polydispersity index of 1.06, determined by GPC using polystyrene standards, THF as the eluent, and toluene as the flow marker.

### 3. RT-PCR of M1 and M2 activation markers

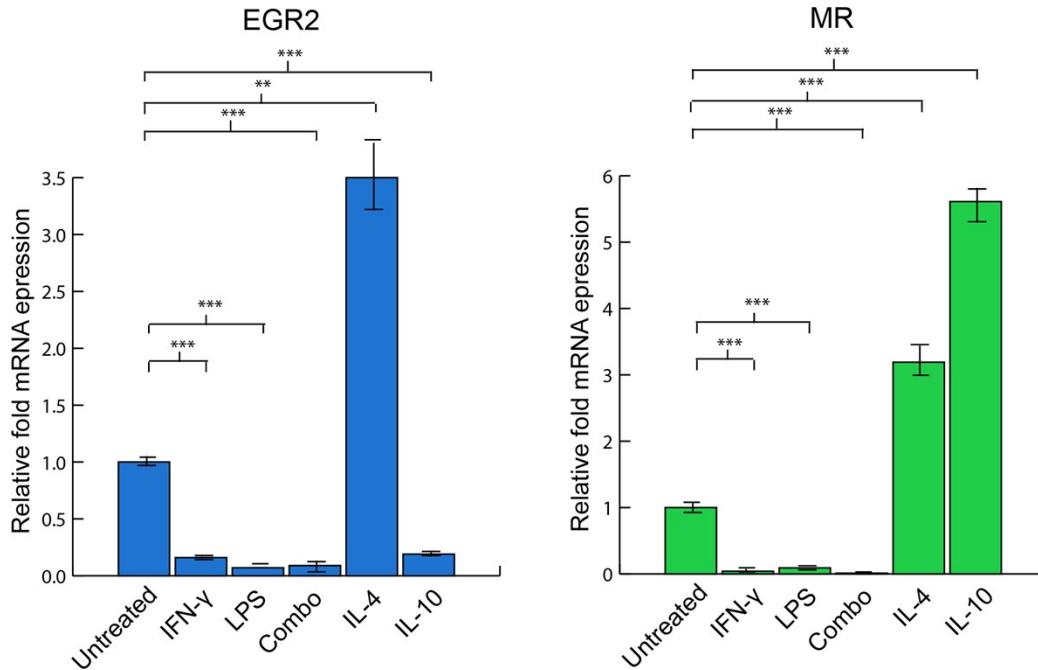


**Figure S7. RT-PCR quantification of M2 state in IL-10 activated RAW 264.7 macrophages.** TGF- $\beta$  is M2-associated gene. Reduction of M1-associated gene iNOS is used to evaluate M2 state. Control = non-treated cells. Fold changes in mRNA level were normalized to  $\beta$ -actin. Statistical significance was determined by two-tailed student t-test. \* =  $p < 0.1$ , \*\* =  $p < 0.05$ , \*\*\* =  $p < 0.005$ ,  $n = 3$  biological replicates (3 technical replicates were used each). n.s. = not significant.

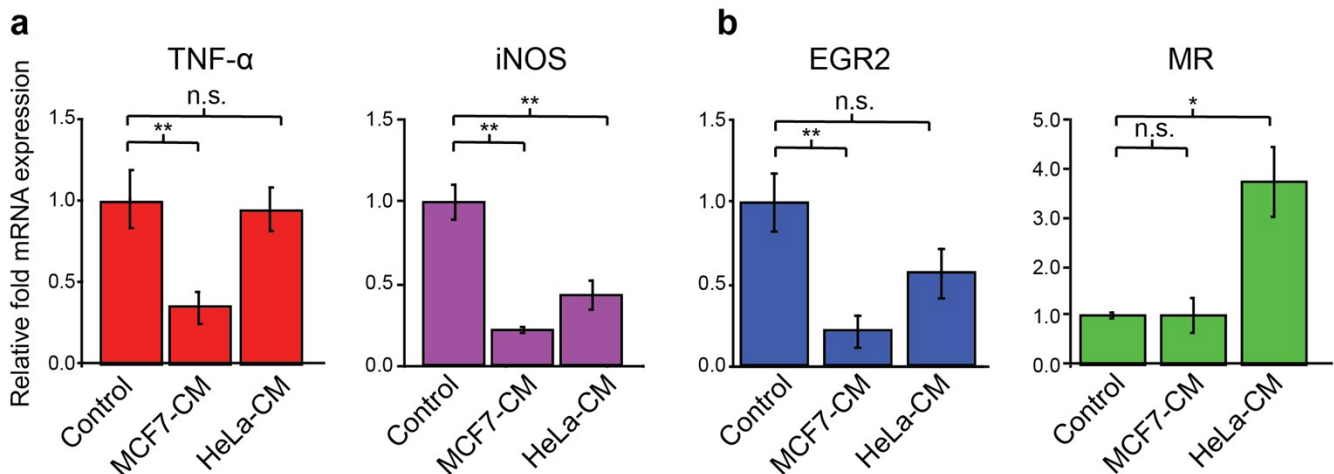


**Figure S8. RT-PCR quantification of M1-associated genes in primary bone marrow-derived macrophages, according to treatment group.** Control = non-treated cells; combo = cells treated with both IFN- $\gamma$  and LPS. Fold changes in mRNA level were normalized to  $\beta$ -actin. \* =  $p < 0.1$ , \*\* =  $p < 0.05$ , \*\*\* =  $p < 0.005$ ,  $n = 3$  biological replicates (3 technical replicates were used each). n.s. = not significant.





**Figure S9. RT-PCR quantification of key M2 genes in primary bone marrow-derived macrophages, according to treatment group.** Control = non-treated cells. Fold changes in mRNA level were normalized to  $\beta$ -actin. \* =  $p < 0.1$ , \*\* =  $p < 0.05$ , \*\*\* =  $p < 0.005$ ,  $n = 3$  biological replicates (3 technical replicates were used each). n.s. = not significant.



**Figure S10. RT-PCR quantification macrophages exposed to conditioned media from different cancer cells.** (a) mRNA quantification of M1-associated genes, TNF- $\alpha$  and iNOS. (b) mRNA quantification of M2-associated genes, EGR2 and MR. Control = non-treated cells. MCF7-M and HeLa-M = conditioned media from MCF7 and HeLa cells, respectively. Fold changes in mRNA level were normalized to  $\beta$ -actin. \* =  $p < 0.1$ , \*\* =  $p < 0.05$ , \*\*\* =  $p < 0.005$ ,  $n = 3$  biological replicates (3 technical replicates were used each). n.s. = not significant.

#### 4. 4.1 Sensing data for M1 and M2 subtypes in RAW 264.7 cells

**Table S1. Normalized fluorescence responses and LDA output for M1 and M2 activated RAW 264.7 cells.** Score (1) and score (2) correspond to Fig. 3 in the main text.

Sample name	I/I <sub>0</sub>					LDA output	
	Pyrene monomer	Pyrene excimer	EGFP	FRET	Pyrene minor peak	Score (1)	Score (2)
M1: LPS	1.389	1.233	1.787	0.801	1.257	-8.050	-2.673
M1: LPS	1.356	1.204	1.765	0.786	1.287	-10.093	-1.379
M1: LPS	1.351	1.196	1.739	0.819	1.285	-9.452	-1.357
M1: LPS	1.418	1.237	1.788	0.881	1.322	-8.018	-1.203
M1: LPS	1.494	1.344	1.815	0.975	1.447	-7.040	-2.114
M1: LPS	1.563	1.376	1.877	1.046	1.514	-6.671	-0.947
M1: LPS	1.506	1.315	1.885	0.966	1.451	-8.633	-0.495
M1: LPS	1.552	1.381	1.905	0.921	1.486	-8.158	-1.261
M1: Combo	1.522	1.049	1.452	1.505	1.259	7.912	6.055
M1: Combo	1.463	1.000	1.375	1.410	1.234	6.377	7.125
M1: Combo	1.471	1.022	1.380	1.425	1.297	5.229	7.595
M1: Combo	1.490	1.047	1.382	1.397	1.302	5.676	7.254
M1: Combo	1.580	1.109	1.458	1.497	1.399	6.359	8.043
M1: Combo	1.612	1.125	1.535	1.558	1.442	5.599	8.368
M1: Combo	1.652	1.185	1.556	1.630	1.457	7.789	6.385
M1: Combo	1.668	1.213	1.598	1.647	1.536	5.887	6.917
Control	1.378	1.257	1.714	1.351	1.235	0.642	-8.605
Control	1.441	1.274	1.728	1.413	1.285	1.862	-6.972
Control	1.457	1.253	1.673	1.494	1.293	3.820	-5.730
Control	1.455	1.270	1.701	1.503	1.336	2.359	-5.821
Control	1.502	1.293	1.741	1.488	1.364	2.404	-4.958
Control	1.561	1.350	1.829	1.592	1.430	2.772	-5.681
Control	1.759	1.570	1.995	1.414	1.591	2.259	-5.991
Control	1.739	1.620	2.043	1.395	1.613	0.668	-8.563
M1: IFN- $\gamma$	1.445	1.169	1.475	1.591	1.231	8.815	-3.380
M1: IFN- $\gamma$	1.472	1.184	1.492	1.588	1.289	7.867	-2.046
M1: IFN- $\gamma$	1.517	1.206	1.499	1.654	1.312	9.532	-1.809
M1: IFN- $\gamma$	1.537	1.242	1.563	1.777	1.368	9.394	-2.920
M1: IFN- $\gamma$	1.573	1.296	1.656	1.827	1.433	8.316	-3.769
M1: IFN- $\gamma$	1.586	1.286	1.669	1.784	1.443	7.494	-2.411
M1: IFN- $\gamma$	1.702	1.394	1.839	1.986	1.531	9.383	-4.483
M1: IFN- $\gamma$	1.625	1.350	1.738	1.880	1.483	8.380	-4.439
M2: IL-10	1.303	1.016	1.437	1.284	1.209	-0.207	1.766
M2: IL-10	1.319	1.028	1.481	1.243	1.252	-2.077	2.744

M2: IL-10	1.295	1.019	1.453	1.239	1.238	-2.078	2.291
M2: IL-10	1.336	1.058	1.484	1.272	1.304	-2.280	2.739
M2: IL-10	1.338	1.070	1.477	1.270	1.276	-1.147	1.634
M2: IL-10	1.366	1.089	1.506	1.274	1.339	-2.315	2.800
M2: IL-10	1.367	1.112	1.536	1.320	1.351	-2.289	1.528
M2: IL-10	1.380	1.132	1.513	1.341	1.360	-1.140	1.043
M2: IL-4	1.316	1.070	1.641	0.984	1.257	-7.816	2.129
M2: IL-4	1.312	1.106	1.591	1.121	1.292	-5.778	0.185
M2: IL-4	1.340	1.108	1.628	0.949	1.316	-8.366	2.657
M2: IL-4	1.308	1.104	1.574	1.058	1.294	-6.447	0.769
M2: IL-4	1.393	1.126	1.586	1.118	1.374	-5.318	3.495
M2: IL-4	1.365	1.178	1.666	0.988	1.377	-8.520	0.928
M2: IL-4	1.403	1.156	1.662	1.027	1.398	-7.761	3.288
M2: IL-4	1.414	1.210	1.671	1.039	1.416	-7.145	1.265

**Table S2. Percentage of accurate classification of M1 and M2 activated RAW 264.7 cells from Jackknifed analysis.** The results show an overall 100% correct classification.

	M1: Combo	M1: IFN- $\gamma$	M2: IL-10	M2: IL-4	M1: LPS	Control	% correct
M1: Combo	8	0	0	0	0	0	100
M1: IFN- $\gamma$	0	8	0	0	0	0	100
M2: IL-10	0	0	8	0	0	0	100
M2: IL-4	0	0	0	8	0	0	100
M1: LPS	0	0	0	0	8	0	100
Control	0	0	0	0	0	8	100
<b>Total</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>100</b>

**Table S3. Prediction of RAW 264.7 cell polarization state using training set from Figure 3 and Table S1.** The results show an overall 91% correct unknown identification.

Unknown sample #	I/I <sub>0</sub>					True ID	Identified as	Correct prediction
	Pyrene monomer	Pyrene excimer	EGFP	FRET	Pyrene minor peak			
1	1.405	1.167	1.682	1.112	1.256	LPS	LPS	Yes
2	1.346	1.081	1.680	0.983	1.275	LPS	IL-4	No
3	1.457	1.259	1.798	1.071	1.421	LPS	LPS	Yes
4	1.375	1.129	1.648	1.094	1.298	LPS	IL-4	No
5	1.415	1.270	1.808	0.842	1.357	LPS	LPS	Yes
6	1.476	1.274	1.820	1.013	1.422	LPS	LPS	Yes
7	1.453	1.329	1.861	0.828	1.428	LPS	LPS	Yes
8	1.419	1.223	1.737	0.937	1.328	LPS	LPS	Yes
9	1.496	1.032	1.520	1.455	1.265	Combo	Combo	Yes
10	1.423	0.970	1.317	1.359	1.223	Combo	Combo	Yes
11	1.479	1.033	1.438	1.459	1.293	Combo	Combo	Yes

12	1.398	0.996	1.407	1.367	1.300	Combo	Combo	Yes
13	1.533	1.060	1.460	1.490	1.332	Combo	Combo	Yes
14	1.456	1.265	1.694	1.464	1.285	M0	M0	Yes
15	1.408	1.217	1.715	1.444	1.273	M0	M0	Yes
16	1.459	1.270	1.710	1.533	1.309	M0	M0	Yes
17	1.494	1.303	1.730	1.580	1.356	M0	M0	Yes
18	1.526	1.311	1.752	1.640	1.397	M0	M0	Yes
19	1.593	1.369	1.793	1.744	1.471	M0	M0	Yes
20	1.653	1.430	1.867	1.675	1.524	M0	M0	Yes
21	1.687	1.442	1.906	1.563	1.581	M0	M0	Yes
22	1.462	1.206	1.456	1.610	1.259	IFN-γ	IFN-γ	Yes
23	1.455	1.161	1.526	1.621	1.252	IFN-γ	IFN-γ	Yes
24	1.429	1.174	1.512	1.598	1.260	IFN-γ	IFN-γ	Yes
25	1.468	1.192	1.490	1.650	1.295	IFN-γ	IFN-γ	Yes
26	1.479	1.248	1.502	1.687	1.351	IFN-γ	IFN-γ	Yes
27	1.519	1.278	1.591	1.809	1.371	IFN-γ	IFN-γ	Yes
28	1.557	1.301	1.592	1.776	1.447	IFN-γ	IFN-γ	Yes
29	1.606	1.292	1.564	1.713	1.474	IFN-γ	IFN-γ	Yes
30	1.314	1.076	1.603	1.102	1.237	IL-4	IL-4	Yes
31	1.298	1.069	1.595	1.145	1.242	IL-4	IL-4	Yes
32	1.323	1.082	1.645	1.160	1.246	IL-4	IL-4	Yes
33	1.337	1.134	1.657	1.121	1.296	IL-4	IL-4	Yes
34	1.326	1.116	1.624	1.142	1.288	IL-4	IL-4	Yes
35	1.366	1.169	1.656	1.232	1.356	IL-4	IL-4	Yes
36	1.403	1.171	1.624	1.277	1.391	IL-4	IL-10	No
37	1.382	1.141	1.602	1.231	1.372	IL-4	IL-10	No
38	1.355	1.032	1.525	1.270	1.266	IL-10	IL-10	Yes
39	1.301	1.049	1.509	1.262	1.249	IL-10	IL-10	Yes
40	1.377	1.090	1.615	1.276	1.314	IL-10	IL-10	Yes
41	1.343	1.097	1.526	1.312	1.307	IL-10	IL-10	Yes
42	1.389	1.102	1.571	1.247	1.338	IL-10	IL-10	Yes
43	1.359	1.125	1.524	1.277	1.342	IL-10	IL-10	Yes
44	1.441	1.143	1.613	1.368	1.387	IL-10	IL-10	Yes
45	1.393	1.148	1.574	1.319	1.398	IL-10	IL-10	Yes

## 4.2 Sensing data for M1 and M2 subtypes in bone marrow-derived macrophages

**Table S4. Normalized fluorescence responses and LDA output for polarized primary bone marrow-derived macrophages.** Score (1) and score (2) correspond to Fig. 4 in the main text.

Sample name	I/I <sub>0</sub>					LDA output	
	Pyrene monomer	Pyrene excimer	EGFP	FRET	Pyrene minor peak	Score (1)	Score (2)
Control	1.239	1.042	1.317	1.187	1.182	-9.640	-0.413
Control	1.264	1.069	1.345	1.205	1.189	-9.779	0.842
Control	1.334	1.112	1.407	1.259	1.266	-7.781	2.211
Control	1.349	1.135	1.475	1.279	1.270	-7.137	2.528
Control	1.296	1.135	1.440	1.266	1.237	-9.886	1.782
Control	1.317	1.136	1.444	1.271	1.244	-9.452	2.313
Control	1.313	1.113	1.455	1.269	1.250	-8.166	1.001
Control	1.303	1.119	1.488	1.295	1.249	-9.420	-0.145
M1: IFN- $\gamma$	1.227	0.997	1.272	1.103	1.168	-4.761	0.108
M1: IFN- $\gamma$	1.235	1.004	1.266	1.112	1.155	-6.250	0.666
M1: IFN- $\gamma$	1.259	1.045	1.307	1.148	1.200	-5.874	1.425
M1: IFN- $\gamma$	1.254	1.013	1.301	1.162	1.225	-5.328	-0.866
M1: IFN- $\gamma$	1.255	1.024	1.353	1.157	1.197	-4.886	-0.330
M1: IFN- $\gamma$	1.247	1.063	1.381	1.179	1.217	-5.687	0.023
M1: IFN- $\gamma$	1.306	1.085	1.454	1.238	1.256	-5.219	0.241
M1: IFN- $\gamma$	1.304	1.090	1.424	1.242	1.244	-7.133	0.760
M1: LPS	1.242	0.880	1.259	0.957	1.204	9.808	-0.936
M1: LPS	1.247	0.908	1.256	0.977	1.245	9.695	-0.518
M1: LPS	1.303	0.923	1.237	0.991	1.279	10.436	1.540
M1: LPS	1.298	0.960	1.350	1.033	1.291	10.526	0.594
M1: LPS	1.305	0.968	1.395	1.034	1.269	10.848	1.037
M1: LPS	1.359	0.961	1.409	1.069	1.282	10.509	1.383
M1: LPS	1.375	0.975	1.446	1.117	1.319	9.840	0.320
M1: LPS	1.342	0.979	1.473	1.113	1.303	9.615	-0.601
M1: Combo	1.187	0.908	1.213	0.967	1.205	6.085	-1.459
M1: Combo	1.213	0.917	1.225	0.962	1.196	6.723	0.043
M1: Combo	1.220	0.942	1.250	1.008	1.219	4.855	-0.387
M1: Combo	1.291	0.979	1.296	1.054	1.256	5.165	1.596
M1: Combo	1.279	0.979	1.334	1.067	1.250	4.925	0.514
M1: Combo	1.289	0.988	1.362	1.079	1.259	5.326	0.573
M1: Combo	1.273	0.979	1.340	1.073	1.246	4.415	0.106
M1: Combo	1.376	1.029	1.450	1.159	1.301	4.980	1.873
M2: IL-4	1.268	0.927	1.257	1.058	1.233	3.275	-1.086
M2: IL-4	1.258	0.953	1.251	1.050	1.211	1.883	0.353

M2: IL-4	1.256	0.949	1.221	1.064	1.215	0.247	-0.078
M2: IL-4	1.253	0.960	1.237	1.071	1.235	0.807	-0.282
M2: IL-4	1.283	0.983	1.309	1.104	1.241	1.096	0.129
M2: IL-4	1.325	1.019	1.358	1.135	1.294	2.740	1.261
M2: IL-4	1.306	1.010	1.384	1.128	1.247	1.786	0.721
M2: IL-4	1.337	1.052	1.412	1.169	1.293	1.475	1.739
M2: IL-10	1.173	0.915	1.232	1.031	1.167	-0.168	-3.173
M2: IL-10	1.079	0.885	1.207	0.979	1.083	-2.150	-5.076
M2: IL-10	1.153	0.919	1.187	1.018	1.142	-2.227	-2.581
M2: IL-10	1.168	0.912	1.200	1.027	1.129	-2.665	-2.570
M2: IL-10	1.215	0.989	1.277	1.112	1.215	-3.139	-1.565
M2: IL-10	1.168	0.974	1.274	1.095	1.146	-5.825	-2.543
M2: IL-10	1.234	1.003	1.325	1.134	1.227	-2.697	-1.539
M2: IL-10	1.252	0.993	1.376	1.134	1.200	-1.791	-1.534

**Table S5. Percentage of accurate classification of polarized primary bone marrow-derived macrophages from Jackknifed analysis.** The results show an overall 96% correct classification.

	M1: Combo	M1: IFN- $\gamma$	M2: IL-10	M2: IL-4	M1: LPS	Control	% correct
M1: Combo	8	0	0	0	0	0	100
M1: IFN- $\gamma$	0	7	0	0	0	1	88
M2: IL-10	0	1	7	0	0	0	88
M2: IL-4	0	0	0	8	0	0	100
M1: LPS	0	0	0	0	8	0	100
Control	0	0	0	0	0	8	100
<b>Total</b>	8	8	7	8	8	9	<b>96</b>

**Table S6. Prediction of primary bone marrow-derived macrophages polarization state using training set from Figure 4 and Table S4.** The results show an overall 92% correct unknown identification.

Unknown sample #	I/I <sub>0</sub>					True ID	Identified as	Correct prediction
	Pyrene monomer	Pyrene excimer	EGFP	FRET	Pyrene minor peak			
1	1.246	1.051	1.346	1.178	1.174	M0	M0	Yes
2	1.230	1.026	1.289	1.163	1.183	M0	M0	Yes
3	1.274	1.077	1.375	1.196	1.212	M0	IFN- $\gamma$	No
4	1.266	1.092	1.364	1.252	1.224	M0	M0	Yes
5	1.289	1.106	1.422	1.270	1.224	M0	M0	Yes
6	1.306	1.064	1.397	1.219	1.237	M0	IFN- $\gamma$	No
7	1.349	1.143	1.438	1.298	1.281	M0	M0	Yes
8	1.353	1.131	1.468	1.274	1.240	M0	M0	Yes
9	1.247	1.042	1.340	1.153	1.206	IFN- $\gamma$	IFN- $\gamma$	Yes
10	1.223	1.014	1.299	1.134	1.177	IFN- $\gamma$	IFN- $\gamma$	Yes
11	1.234	1.037	1.319	1.145	1.182	IFN- $\gamma$	IFN- $\gamma$	Yes

12	1.282	1.067	1.386	1.216	1.256	IFN-γ	IFN-γ	Yes
13	1.280	1.090	1.448	1.245	1.240	IFN-γ	IFN-γ	Yes
14	1.212	1.037	1.325	1.149	1.197	IFN-γ	IFN-γ	Yes
15	1.247	1.047	1.345	1.184	1.221	IFN-γ	IFN-γ	Yes
16	1.279	1.072	1.427	1.216	1.257	IFN-γ	IFN-γ	Yes
17	1.279	0.970	1.414	1.045	1.285	LPS	LPS	Yes
18	1.336	0.993	1.431	1.078	1.293	LPS	LPS	Yes
19	1.391	0.985	1.428	1.074	1.340	LPS	LPS	Yes
20	1.377	1.000	1.484	1.108	1.315	LPS	LPS	Yes
21	1.346	0.979	1.446	1.053	1.299	LPS	LPS	Yes
22	1.352	0.960	1.461	1.088	1.294	LPS	LPS	Yes
23	1.227	0.913	1.247	0.983	1.234	LPS	Combo	No
24	1.270	0.932	1.250	0.982	1.234	LPS	LPS	Yes
25	1.205	0.934	1.219	0.981	1.231	Combo	Combo	Yes
26	1.248	0.956	1.281	1.041	1.236	Combo	Combo	Yes
27	1.189	0.954	1.289	1.011	1.232	Combo	Combo	Yes
28	1.201	0.942	1.249	1.036	1.248	Combo	Combo	Yes
29	1.304	1.042	1.461	1.149	1.272	Combo	Combo	Yes
30	1.181	0.926	1.217	1.014	1.229	Combo	Combo	Yes
31	1.251	0.989	1.342	1.083	1.284	Combo	Combo	Yes
32	1.288	0.965	1.321	1.096	1.274	Combo	IL-4	No
33	1.305	1.016	1.405	1.129	1.228	IL-4	IL-4	Yes
34	1.319	1.007	1.413	1.147	1.269	IL-4	IL-4	Yes
35	1.326	1.038	1.432	1.155	1.283	IL-4	IL-4	Yes
36	1.268	0.927	1.257	1.058	1.233	IL-4	IL-4	Yes
37	1.258	0.953	1.251	1.050	1.211	IL-4	IL-4	Yes
38	1.256	0.949	1.221	1.064	1.215	IL-4	IL-4	Yes
39	1.253	0.960	1.237	1.071	1.235	IL-4	IL-4	Yes
40	1.283	0.983	1.309	1.104	1.241	IL-4	IL-4	Yes
41	1.185	0.955	1.232	1.093	1.173	IL-10	IL-10	Yes
42	1.170	0.922	1.188	1.029	1.159	IL-10	IL-10	Yes
43	1.117	0.921	1.210	1.056	1.172	IL-10	IL-10	Yes
44	1.211	0.985	1.264	1.094	1.231	IL-10	IL-10	Yes
45	1.206	0.975	1.264	1.117	1.197	IL-10	IL-10	Yes
46	1.286	1.040	1.453	1.165	1.237	IL-10	IL-10	Yes
47	1.213	0.983	1.256	1.103	1.194	IL-10	IL-10	Yes
48	1.218	0.979	1.280	1.103	1.200	IL-10	IL-10	Yes

### 4.3 Sensing data for RAW 264.7 cells exposed to conditioned media

**Table S7. Normalized fluorescence responses and LDA output of RAW 264.7 cells under cancer cell conditioned media stimulation.** Score (1) and score (2) correspond to Fig. 5 in the main text.

Sample name	I/I <sub>0</sub>					LDA output	
	Pyrene monomer	Pyrene excimer	EGFP	FRET	Pyrene minor peak	Score (1)	Score (2)
HeLa-CM	1.080	0.752	1.206	0.876	1.018	6.314	2.256
HeLa-CM	1.099	0.787	1.184	0.877	1.047	5.478	0.342
HeLa-CM	1.124	0.791	1.211	0.893	1.077	6.063	0.278
HeLa-CM	1.109	0.801	1.189	0.908	1.048	6.881	0.706
HeLa-CM	1.174	0.832	1.225	0.924	1.129	7.694	-0.819
HeLa-CM	1.223	0.843	1.387	1.039	1.188	7.215	2.446
HeLa-CM	1.096	0.759	1.232	0.833	1.035	6.799	2.325
HeLa-CM	1.132	0.780	1.201	0.895	1.097	5.402	-0.672
Control	1.052	0.777	1.233	0.841	1.051	-4.339	0.176
Control	1.078	0.782	1.280	0.869	1.079	-3.590	0.889
Control	1.087	0.810	1.315	0.883	1.102	-6.042	0.775
Control	1.099	0.821	1.398	0.870	1.117	-7.009	2.537
Control	1.120	0.837	1.362	0.896	1.157	-8.006	0.025
Control	1.131	0.830	1.382	0.879	1.159	-6.211	0.787
Control	1.126	0.842	1.386	0.854	1.145	-5.772	1.240
Control	1.142	0.858	1.475	0.720	1.158	-5.935	2.856
MCF7-CM	1.072	0.769	1.139	0.874	1.068	-1.381	-2.570
MCF7-CM	1.072	0.764	1.152	0.856	1.062	-0.526	-1.922
MCF7-CM	1.102	0.810	1.176	0.915	1.091	-0.059	-2.066
MCF7-CM	1.088	0.808	1.201	0.905	1.080	-1.796	-1.256
MCF7-CM	1.110	0.789	1.201	0.895	1.106	-0.152	-1.782
MCF7-CM	1.128	0.819	1.219	0.922	1.128	-0.533	-2.065
MCF7-CM	1.146	0.841	1.242	0.928	1.145	-0.210	-1.975
MCF7-CM	1.139	0.836	1.218	0.923	1.139	-0.284	-2.511

**Table S8. Percentage of accurate classification of RAW 264.7 cells exposed to cancer cell conditioned media using Jackknifed analysis.** The results show an overall 100% correct classification.

	Control	HeLa-CM	MCF7-CM	% correct
Control	8	0	0	100
Hela-CM	0	8	0	100
MCF7-CM	0	0	8	100
<b>Total</b>	8	8	8	<b>100</b>



**Table S9. Prediction of macrophage polarization state of RAW 264.7 cells cultured in cancer cell conditioned media.** Training set is from Fig. 5 and Table S7. The results show an overall 96% correct unknown identification.

Unknown sample #	I/I <sub>0</sub>					True ID	Identified as	Correct prediction
	Pyrene monomer	Pyrene excimer	EGFP	FRET	Pyrene minor peak			
1	1.077	0.763	1.121	0.847	1.039	Hela-CM	Hela-CM	Yes
2	1.104	0.736	1.203	0.822	1.048	Hela-CM	Hela-CM	Yes
3	1.136	0.818	1.247	0.920	1.099	Hela-CM	Hela-CM	Yes
4	1.172	0.805	1.333	0.938	1.148	Hela-CM	Hela-CM	Yes
5	0.992	0.735	1.155	0.787	0.985	Control	Control	Yes
6	1.013	0.733	1.184	0.755	1.020	Control	Control	Yes
7	1.038	0.767	1.194	0.797	1.055	Control	Control	Yes
8	1.043	0.765	1.249	0.783	1.054	Control	Control	Yes
9	1.059	0.802	1.243	0.812	1.071	Control	Control	Yes
10	1.070	0.791	1.275	0.802	1.094	Control	Control	Yes
11	1.090	0.843	1.361	0.841	1.136	Control	Control	Yes
12	1.114	0.819	1.316	0.889	1.146	Control	Control	Yes
13	1.102	0.839	1.299	0.886	1.140	Control	Control	Yes
14	1.122	0.834	1.351	0.891	1.155	Control	Control	Yes
15	1.053	0.763	1.138	0.842	1.041	MCF7-CM	MCF7-CM	Yes
16	1.072	0.780	1.144	0.845	1.069	MCF7-CM	MCF7-CM	Yes
17	1.078	0.780	1.167	0.852	1.060	MCF7-CM	MCF7-CM	Yes
18	1.125	0.807	1.219	0.920	1.120	MCF7-CM	MCF7-CM	Yes
19	1.097	0.798	1.217	0.858	1.105	MCF7-CM	MCF7-CM	Yes
20	1.105	0.827	1.227	0.908	1.122	MCF7-CM	Control	No
21	1.146	0.846	1.242	0.942	1.154	MCF7-CM	MCF7-CM	Yes
22	1.145	0.860	1.247	0.949	1.149	MCF7-CM	MCF7-CM	Yes
23	1.157	0.885	1.237	0.993	1.181	MCF7-CM	MCF7-CM	Yes
24	1.166	0.899	1.250	1.002	1.162	MCF7-CM	MCF7-CM	Yes

## References

(1) R. F. Landis, A. Gupta, Y. W. Lee, L. S. Wang, B. Golba, B. Couillaud, R. Ridolfo, R. Das and V. M. Rotello, *ACS Nano*, 2017, **11**, 946–952.