# Supplementary information

## Engineering of a graphene oxide-based 2D platform for immune activation and modulation

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# TABLE S1

**Table S1. Main physicochemical properties of GO nanosheets used in this study.**

Properties including lateral dimensions, thickness, optical properties, degree of defects, interlayer distance, surface charge, functionalization degree, chemical composition, and purity are indicated below.

| Material property | Technique | Result |
|---|---|---|
| Lateral dimensions | SEM | 50 - 950 nm [n = 946] |
| | | 95% < 450 nm |
| | | Mean 144 nm |
| | AFM | 10 - 890 nm [n = 2890] |
| | | 95% < 350 nm |
| | | Mean 72 nm |
| Thickness | AFM | 1 nm (1 layer) |
| Optical properties | Absorption spectroscopy | $\varepsilon_{232}$ (mL $\mu g^{-1}$ $cm^{-1}$)= 0.050 |
| Degree of defects ($I_D/I_G$) | Raman spectroscopy | 1.12 ± 0.02 [n=3] |
| Peak (2θ) | XRD | 12.36 ° [n=1] |
| Interlayer distance (nm) | | 0.71 |
| Surface charge (ζ-Potential) | Electrophoretic mobility | - 47.2 ± 0.7 mV |
| Functional groups | FTIR | v(C-H): 2838 $cm^{-1}$ |
| | | v(C=O): 1735 $cm^{-1}$ |
| | | v(C=C): 1645 $cm^{-1}$ |
| | | v(O-H): 1423 $cm^{-1}$ |
| | | v(C-O): 1062 $cm^{-1}$ |
| Functionalization degree | TGA | 30-75°C: 7% (water) |
| | | 200-250°C: 30% |
| | | 250-950°C: 20% |
| | | TOTAL 50% |
| Chemical composition (%) | XPS | O: 29.6, C: 68.5, N: 1.1, S: 0.9 |
| Purity % (C+O) | | 98,0 |
| C:O ratio | | 2,3 |

# FIGURE S1

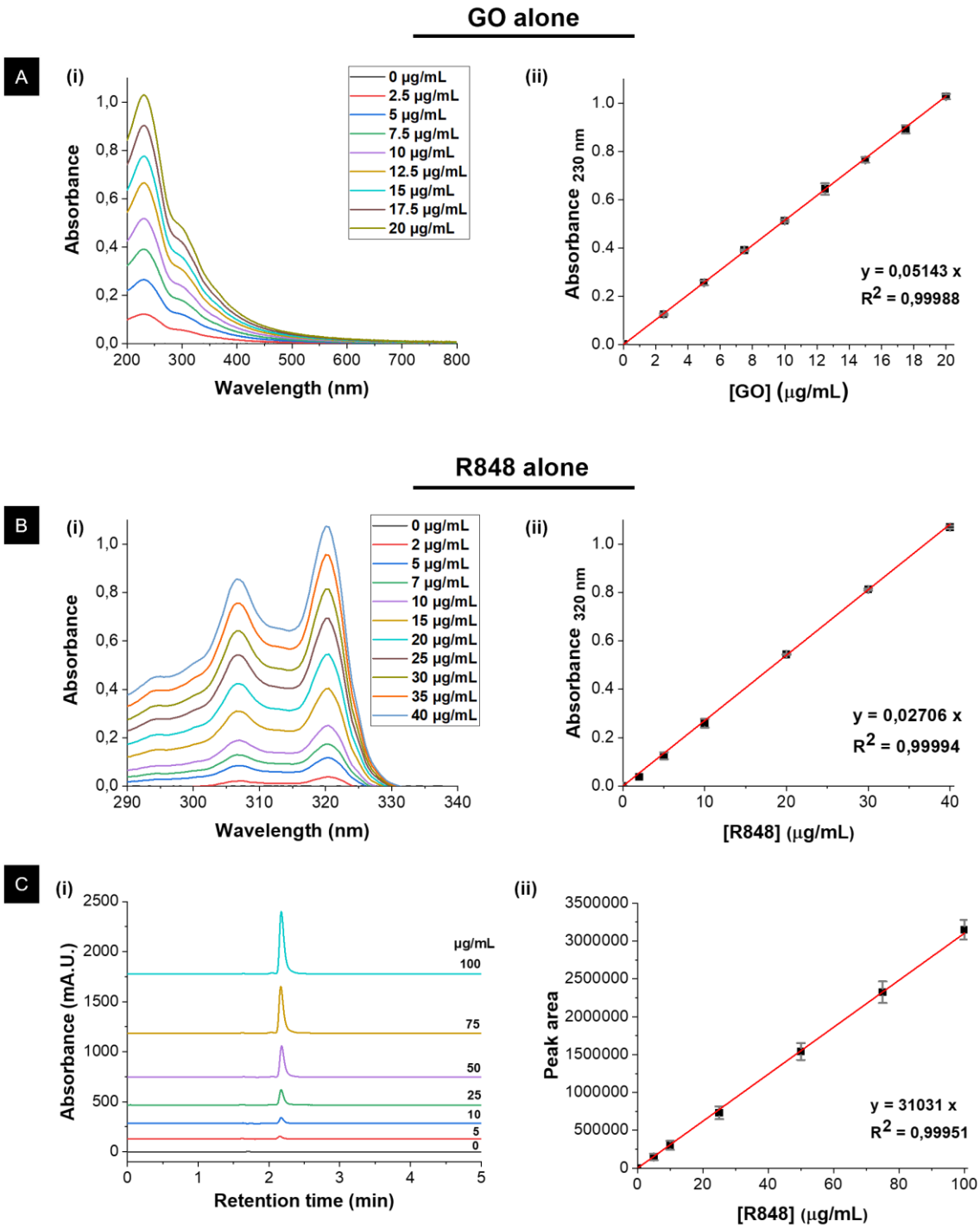


**Figure S1. Calibration data of GO and R848 alone.**

**(A)** UV-Vis calibration data of GO at concentrations ranging between 0 and 20 µg/mL at the wavelength of 230 nm. **(B)** UV-Vis calibration curve of R848 at concentrations ranging between 0 and 40 µg/mL at the wavelength of 320 nm. **(C)** HPLC calibration curve of R848 at concentrations ranging between 0 and 100 µg/mL at the wavelength of 254 nm. In each case, the following is presented: **(i)** raw data of one indicative experimental run; **(ii)** calibration curves from data expressed as mean ± SD (n=3).

# FIGURE S2

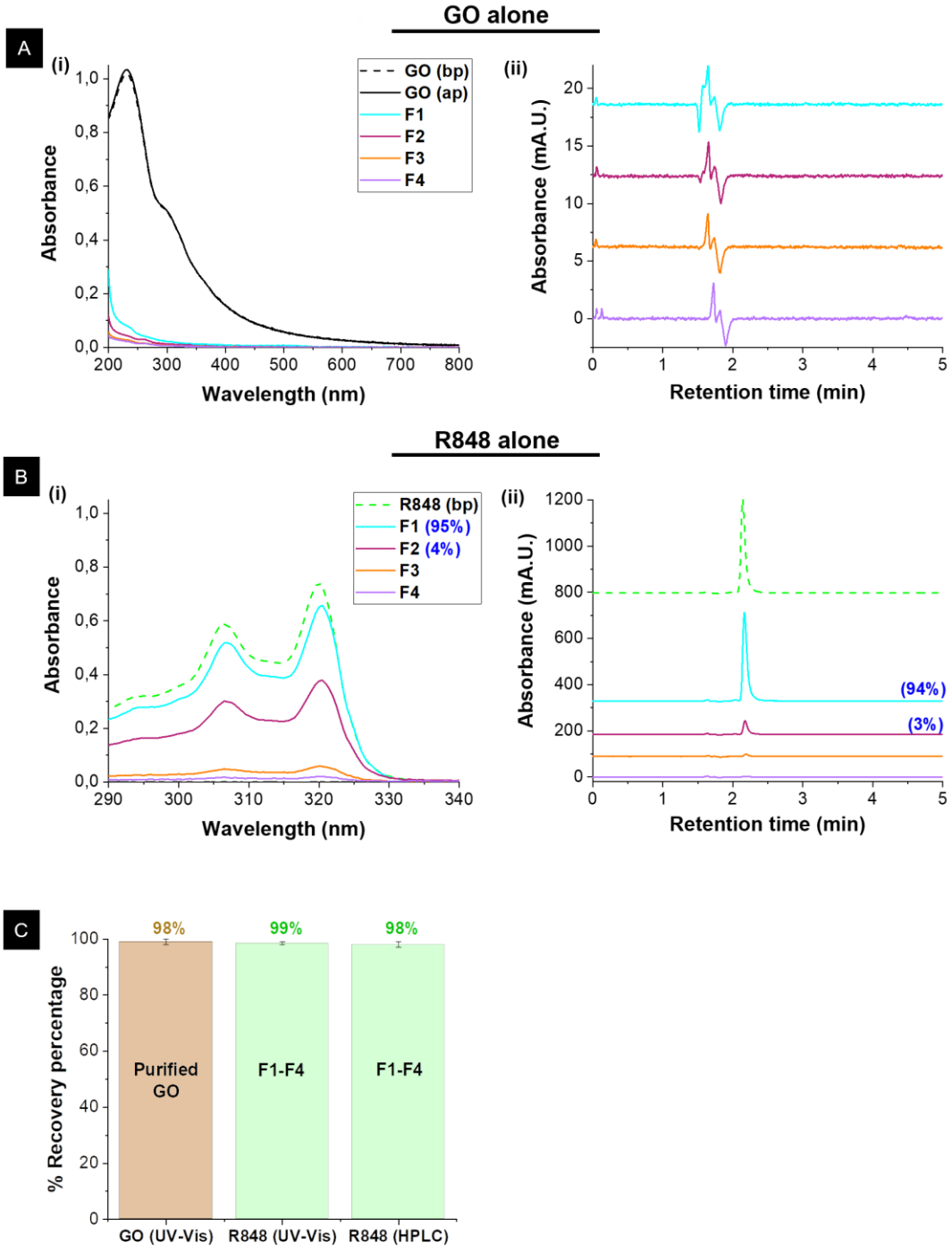


**Figure S2. Validation of purification method for GO and R848 controls.**

**(A)** GO control purification. **(i)** UV-Vis spectrum before (bp) (dashed line) and after (ap) purification (solid line) (GO (20 µg/mL)), and signal of the corresponding filtrates (F1-F4) in the range 200-800 nm; **(ii)** HPLC chromatograms of GO filtrates (F1-F4). **(B)** R848 control purification. **(i)** UV-Vis spectrum before purification (bp) (dashed green line) and signal of the corresponding filtrates (F1-F4) in the range of 290-340 nm; **(ii)** HPLC chromatograms of R848 before purification (bp) and of the corresponding filtrates (F1-F4). All R848 quantity at the end of the procedure is present only in the filtrates. **(C)** Percentage (%) of GO recovery in the purified sample fraction on the filter (brown bar); and percentage (%) of R848 recovery washed in the filtrates (green bars) quantified by the two independent techniques and measurements: UV-Vis spectroscopy and HPLC. The results (generated by applying the formula (1)) are expressed as a mean ± SD (n=3).

# FIGURE S3

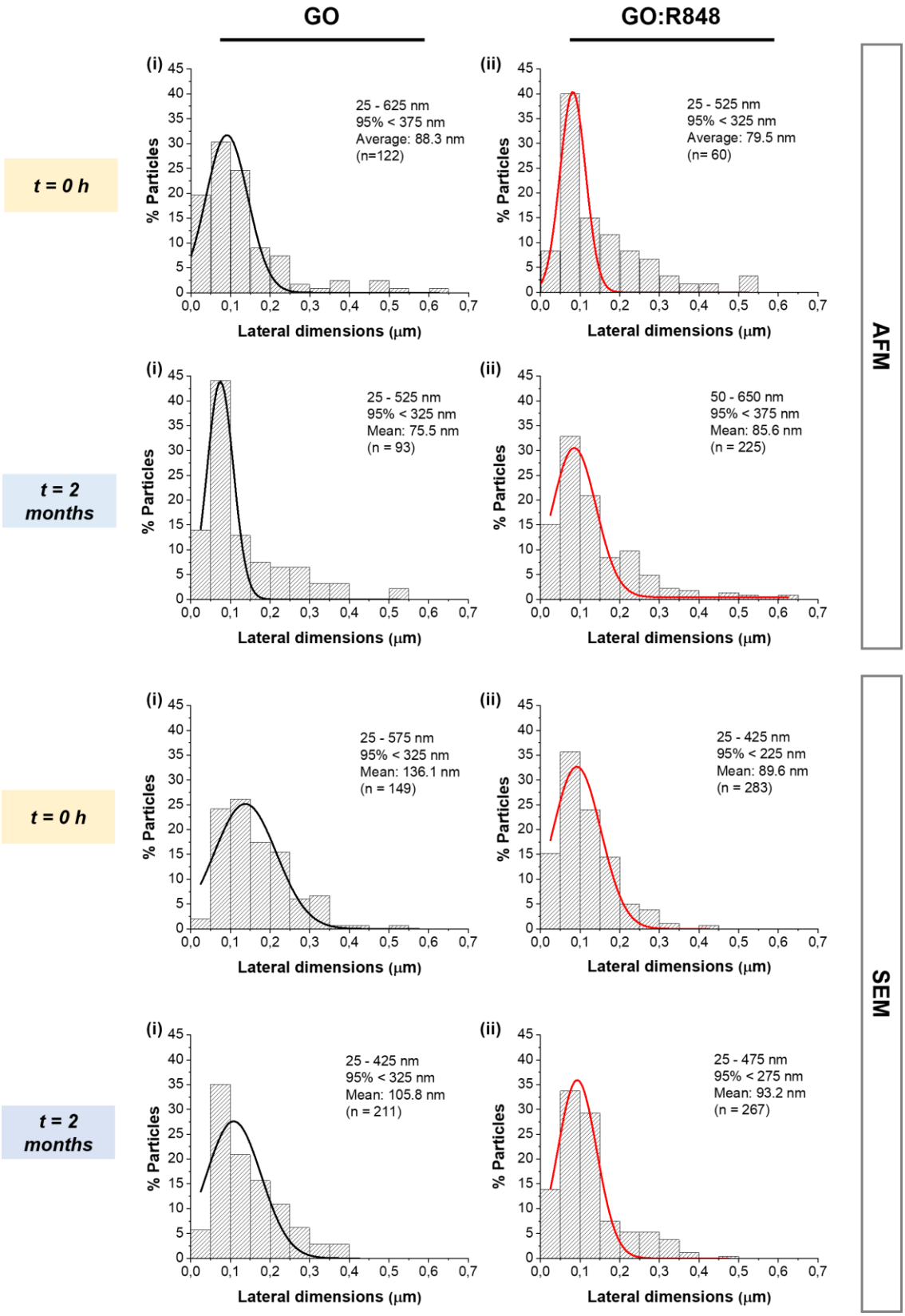


**Figure S3. Size distribution of GO nanosheets alone and in complex by AFM and SEM.**

The data were obtained from AFM and SEM images at t=0 h (yellow) and t=2 months (blue) for **(i)** GO control; **(ii)** GO:R848 complex after purification (ap) complex.

# FIGURE S4

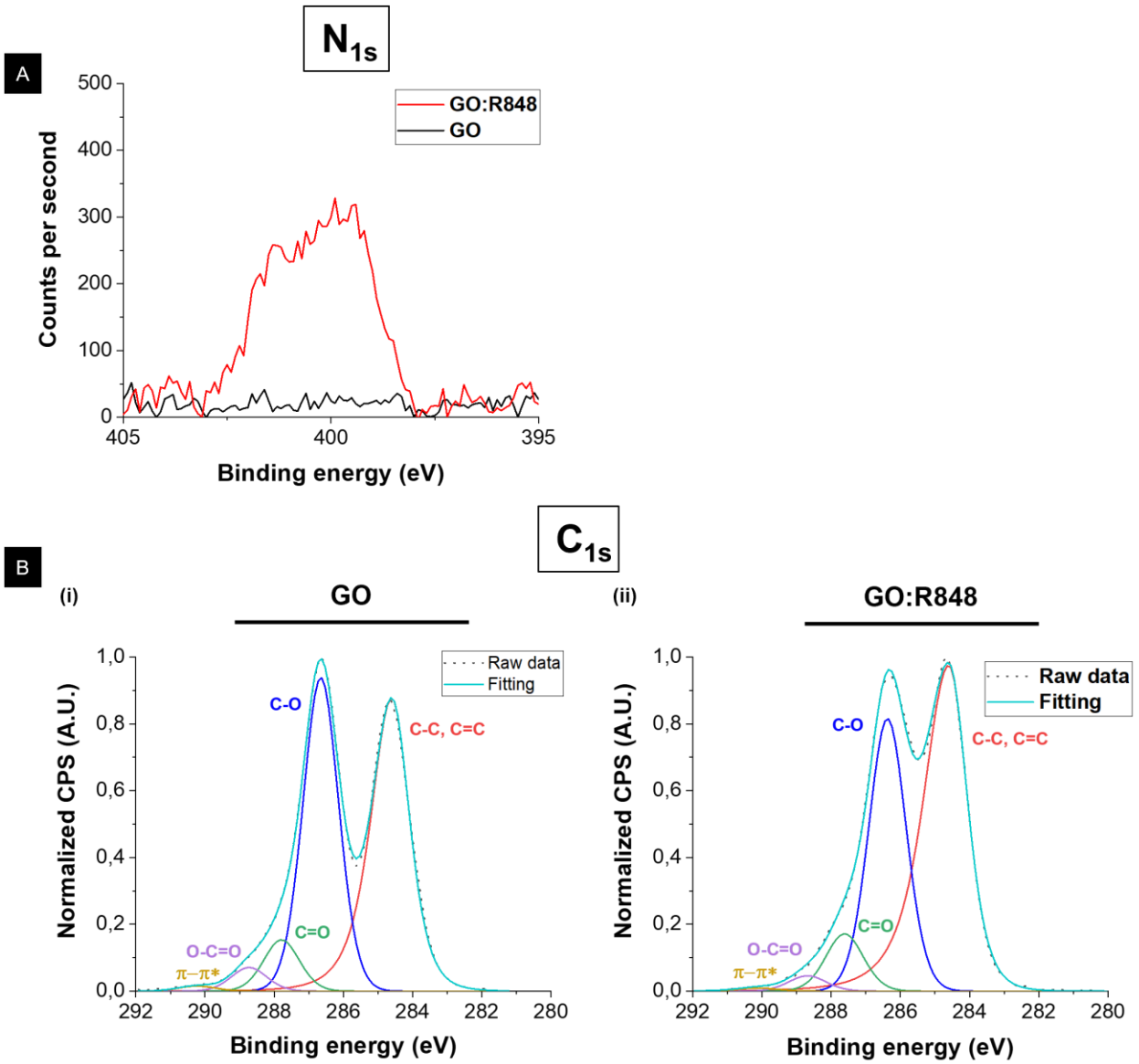


**Figure S4. $N_{1s}$ and $C_{1s}$ high-resolution XPS spectra**.

**(A)** $N_{1s}$ high resolution spectra for GO and GO:R848 complex after purification (ap) displayed in the binding energy range of 405-395 eV. **(B)** Deconvolution of $C_{1s}$ high-resolution spectrum in the binding energy range of 292-280 eV highlighting the corresponding functional groups for: **(i)** GO control; **(ii)** GO:R848 complex.

# FIGURE S5

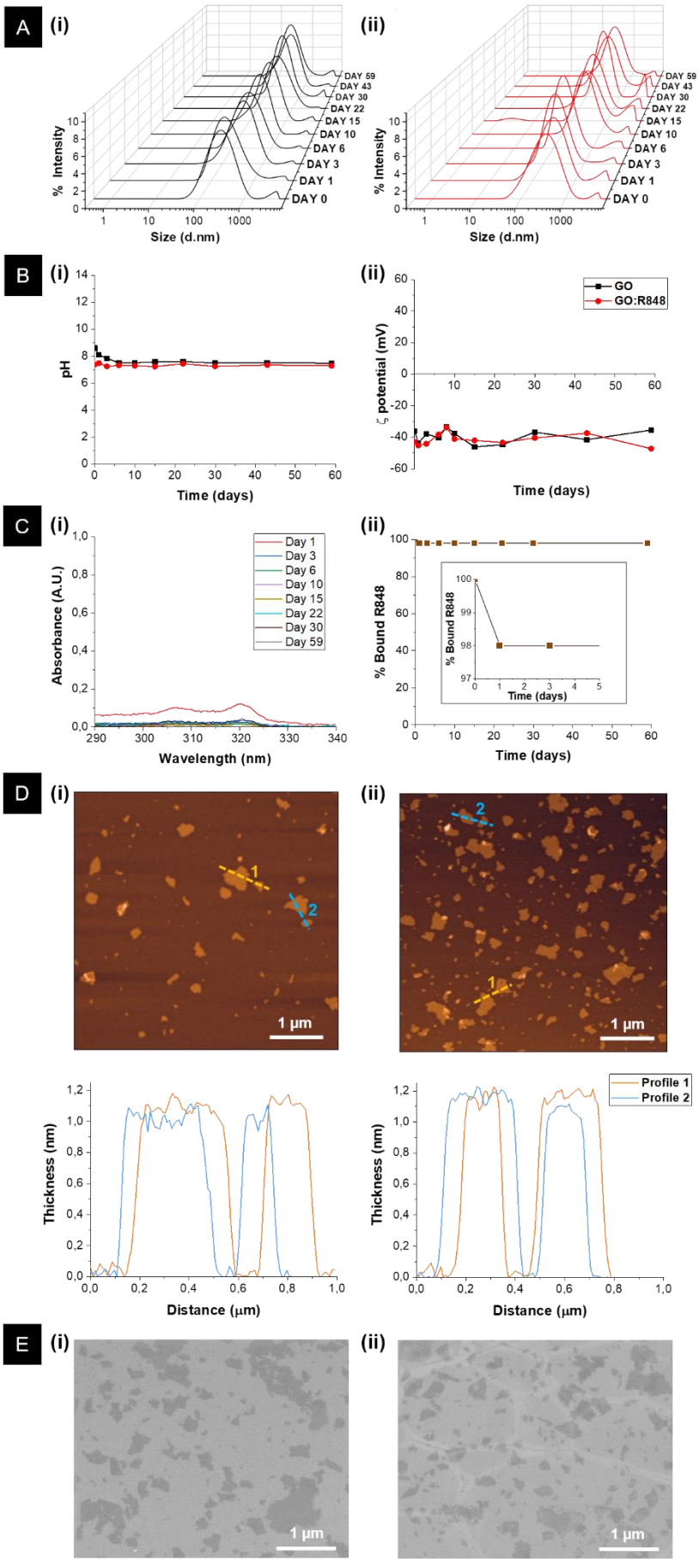


**Figure S5. Colloidal stability of GO:R848 complex after purification (ap) over 2 months.**

**(A)** Dynamic light scattering (DLS) plots showing the evolution of mean particle size over time. **(i)** GO control; **(ii)** GO:R848 complex. **(B)** Measurements over 2 months. **(i)** pH monitoring; **(ii)** Average particle surface charge (ζ-potential) for GO control (black) and GO:R848 complex (red). **(C) (i)** UV-Vis spectra of GO:R848 filtrates (unbound R848) in the range of 290-340 nm over 2 months; Note that the spectrum shown in each time point is the sum of absorbance from all individual filtrates (F1-F4); **(ii)** Percentage (%) of R848 that remains bound on the GO surface over 2 months; Inset: Magnified plot showing a 2 % R848 release from the GO surface in the first day. **(D)** AFM height images after 2 months from corresponding nanosheets cross section and thickness graphs (shown underneath) for **(i)** GO control; **(ii)** GO:R848 complex. **(E)** SEM micrographs after 2 months for **(i)** GO control; **(ii)** GO:R848 complex. In all images, scale bars are 1 µm.

# FIGURE S6

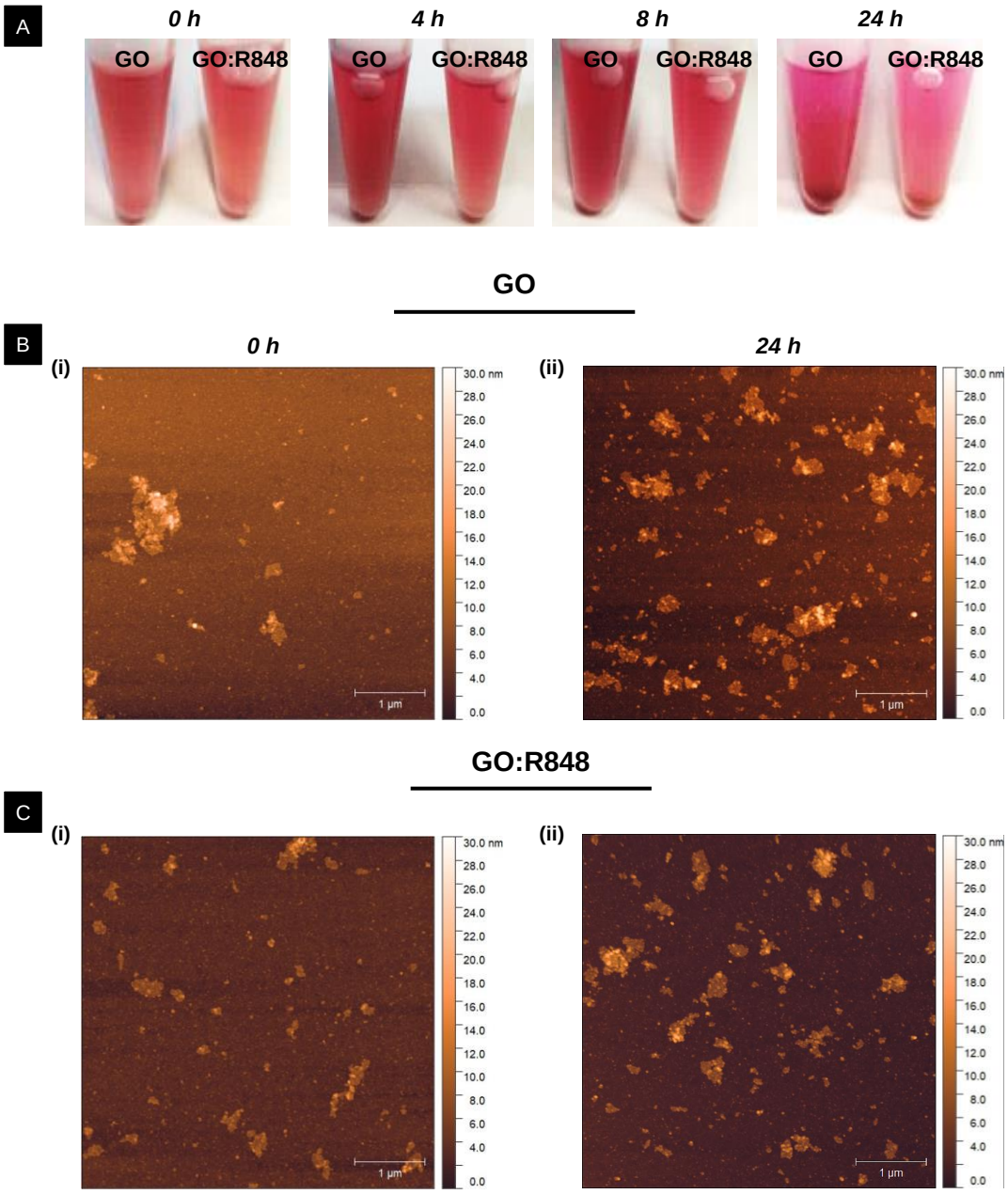


**Figure S6. Colloidal stability of GO:R848 complexes after purification (ap) in cell culture media (DMEM + 10% FBS) for 24 h.**

**(A)** Visual aspect of GO control and GO:R848 complex in media at various time points: 0, 4, 8 and 24 h. **(B)** AFM height images of GO control for **(i)** 0 h and **(ii)** 24 h. **(C)** AFM height images of GO:R848 complex for **(i)** 0 h and **(ii)** 24 h. Note that all samples were measured at a final GO concentration of 0.1 mg/mL after dilution in media.

# FIGURE S7

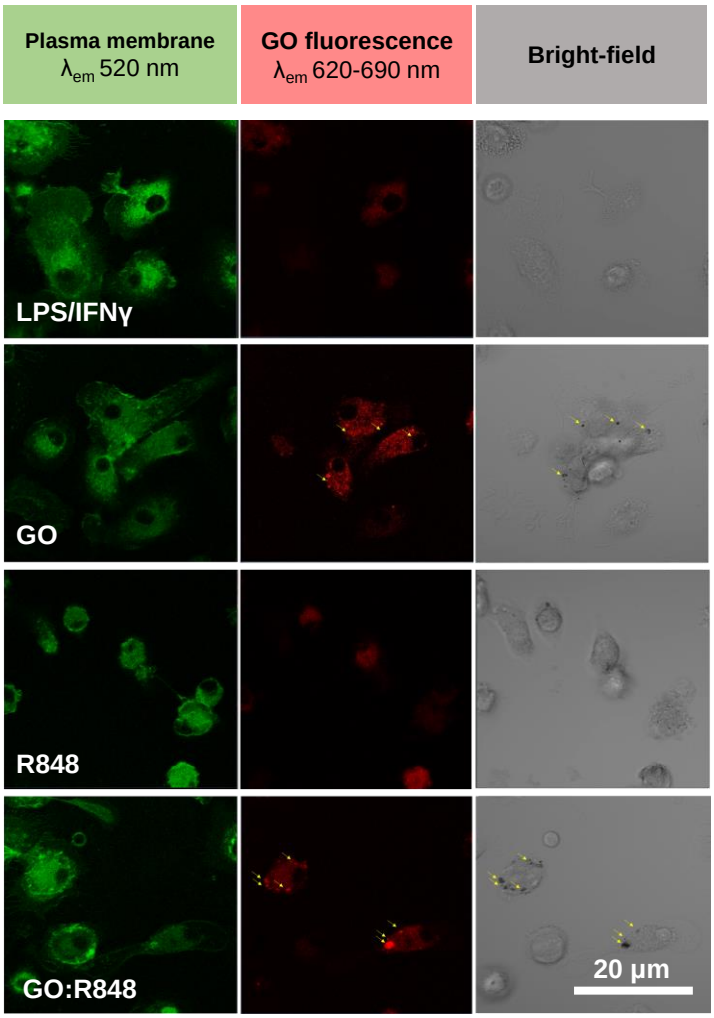


**Figure S7. Confocal images taken with zoom factor 1, showing the co-localization of GO and GO:R848 (10:4) with BMDM membrane dye (green), 2 h post-treatment.**

Red fluorescence field showing the autofluorescence of GO. Bright field confirmed the presence of GO black particles. Images were taken via confocal microscope at 40× magnification. Representative images from n=6/condition, Scale bar 20 µm.

# FIGURE S8

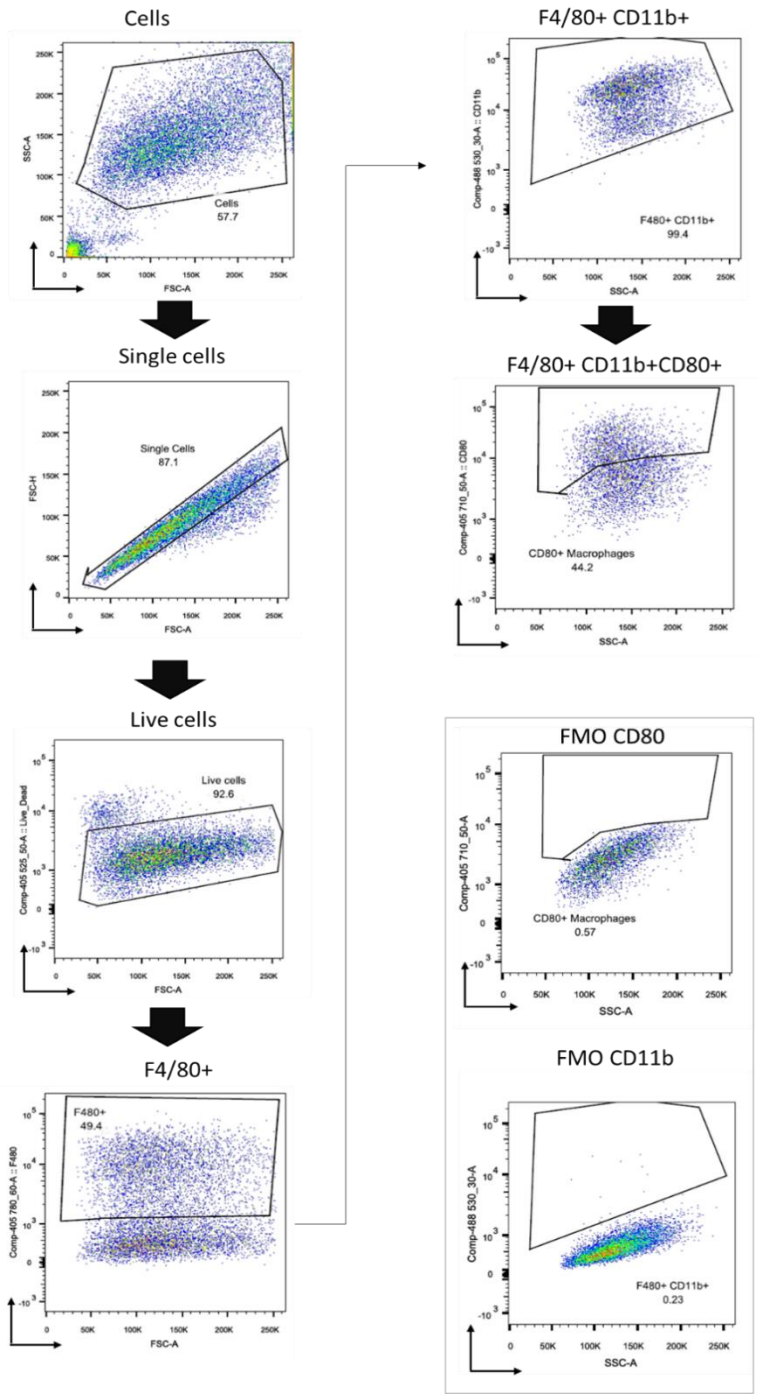


**Figure S8. Flow cytometry dot plots and gating strategy used for CD80+/F480+ CD11b+ cells.**

Indicative FACS data is shown of cells first isolated from debris; single cells gated to avoid clumps; live cells selected to avoid dead cells. F480+ cells were taken forward from live cells and based on the fluorescence minus one (FMO) controls of CD11b and CD80, CD11b+ and CD80+ cells were gated.