Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Supplementary Tables

Supplementary Table 1. Physicochemical and biological properties of sGO and LGO flakes.

Physicochemical properties	Technique	sGO	LGO
	Optical microscopy	Non-detectable (< 2 μm)	up to 30 μm
Lateral dimension	AFM	< 1 μm	5-20 μm
	TEM	50 nm-3 μm	0.5-15 μm
Thickness	AFM	1-3 nm	1-2 nm
Defects Density	Raman $I_{(D)}/I_{(G)}$ ratio	1.32 ± 0.05	1.30 ± 0.05
Surface Charge	ζ-potential	-55.0 ± 5 mV	-53 ± 5 mV
Functionalisation degree	TGA	43%	44%
Chemical composition	XPS (at. %)	C: 70.3 ± 1.0; O: 29.4 ± 1.0; S: 0.2 ± 0.1; Si: 0.1 ± 0.1	C: 69.1 ± 1.2; O: 30.5 ± 1.2; S: 0.4 ± 0.1
Purity	XPS (C at. % + O at. %)	99.7	99.6
C:O ratio	XPS (C at. % / O at. %)	2.4 ± 0.1	2.3 ± 0.1
π-π*, O=C-O, C=O, C-O, C-C and C=C	XPS (C1s spectra, C at. %)	1.9 ± 0.6, 5.0 ± 0.9, 3.2 ± 0.5, 45.4 ± 3.0, 44.5 ± 3.0	1.2 ± 0.2, 3.8 ± 0.3, 4.0 ± 0.4, 44.0 ± 2.0, 47.0 ± 2.0
Biological properties			
Endotoxin content	TNF-α ELISA measurement	n/a*	n/a*

* **See Supplementary Figure 1**

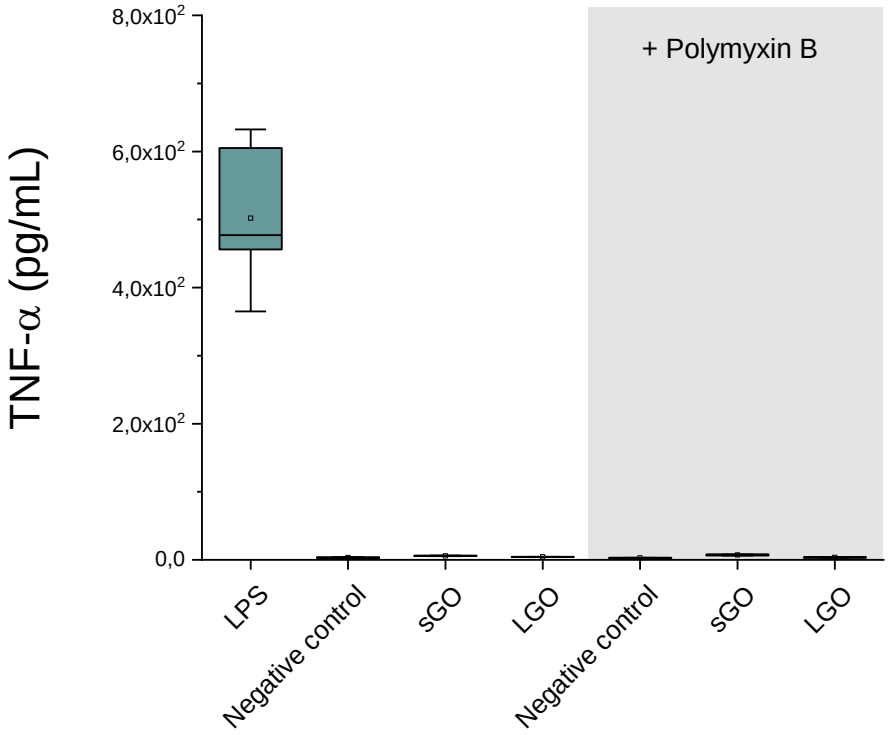
Supplementary Table 2. Antibodies used to assess DC activation and antigen presentation

Antibody	Fluorophore	Clone	Dilution	Supplier	Cat No.
CD86	Alexa Fluor 488	GL-1	1:800	BioLegend	105018
CD80	PerCP/Cy5.5	16-10A1	1:600	BioLegend	104722
MHCI (H-2Kb)	APC	AF6-88.5.5.3	1:500	eBioscience	17-5958-80
MHCII (I-A/I-E)	Alexa Fluor 700	M5/114.15.2	1:1000	BioLegend	107622
CD11c	APC/eFluor 780	N418	1:200	eBioscience	47-0114-82
Cellular Amines (Viability Dye)	Zombie UV	-	1:2000	BioLegend	423108
CD40	PE	3/23	1:200	BioLegend	124610
H-2Kb bound SIINFEKL	PE	25-D1.16	1:200	BioLegend	141603
CD83	PE/Cy7	Michel-19	1:300	BioLegend	121518

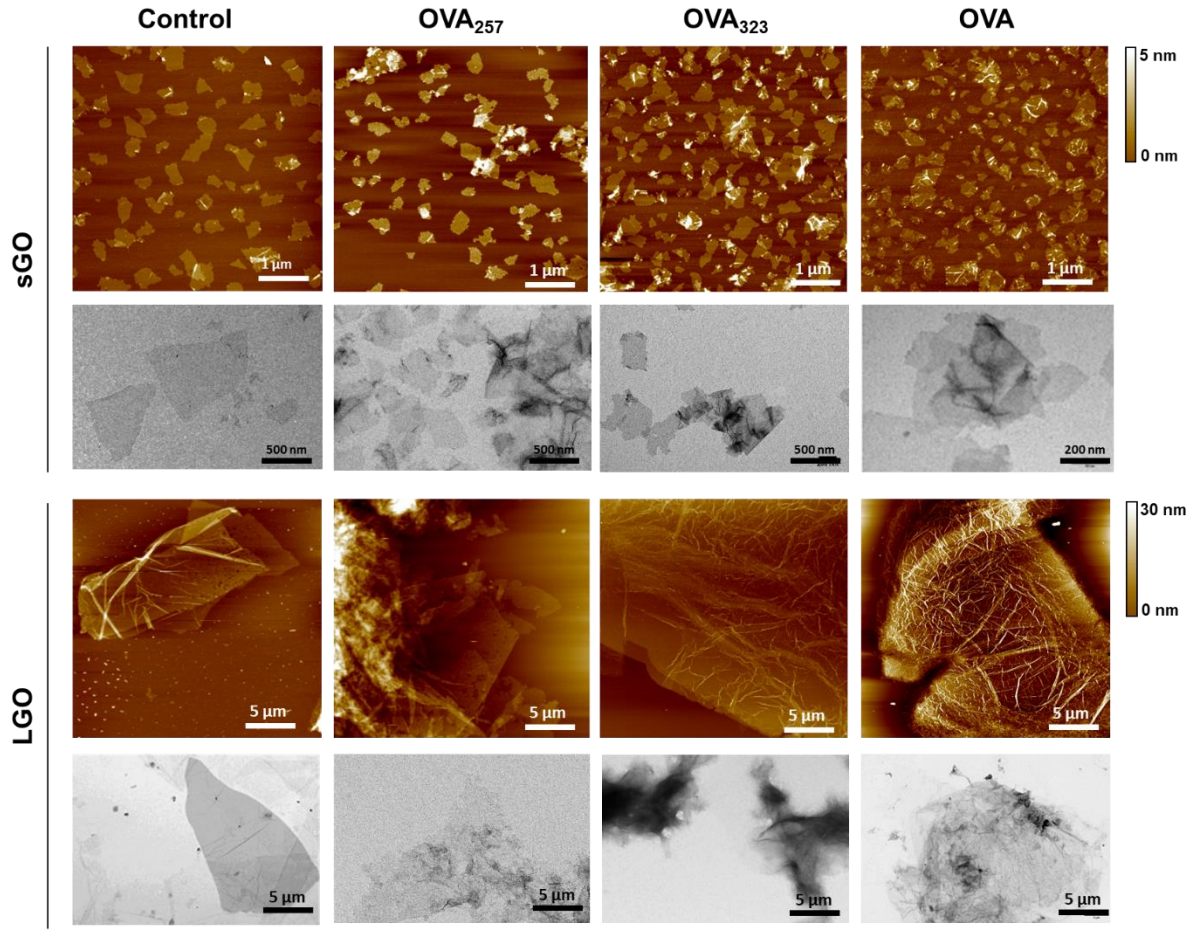
Supplementary Table 3. Antibodies used to assess T cell activation

Antibody/Cell Tracer	Fluorophore	Clone	Dilution	Supplier	Cat No.
CFSE	FITC	-	-	Sigma	21888
T-bet	PerCP/Cy5.5	4B10	1:100	BioLegend	644806
Foxp3	APC	FJK-16s	1:200	eBioscience	17-5773-82
TCR beta	APC/eFluor 780	H57-597	1:200	eBioscience	47-5961-82
CD11c	BV421	N418	1:200	BioLegend	117330
CD62L	BV605	MEL-14	1:200	BioLegend	104437
CD25	BV650	PC61	1:100	BioLegend	102038
CD69	BV711	H1.2F3	1:200	BioLegend	104537
CD44	BV785	IM7	1:400	BioLegend	103059
Cellular Amines (Viability Dye)	Zombie UV	-	1:2000	BioLegend	423108
CD4	PE	RM 4.5	1:400	eBioscience	12-0042-83
CD8	PE/Cy7	53-6.7	1:400	BioLegend	100722

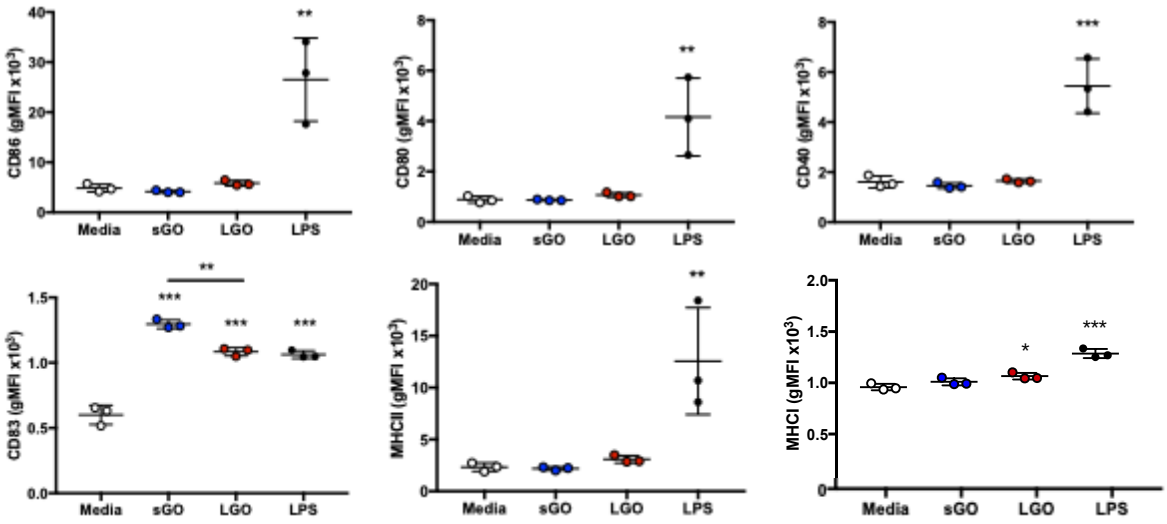
Supplementary Figures



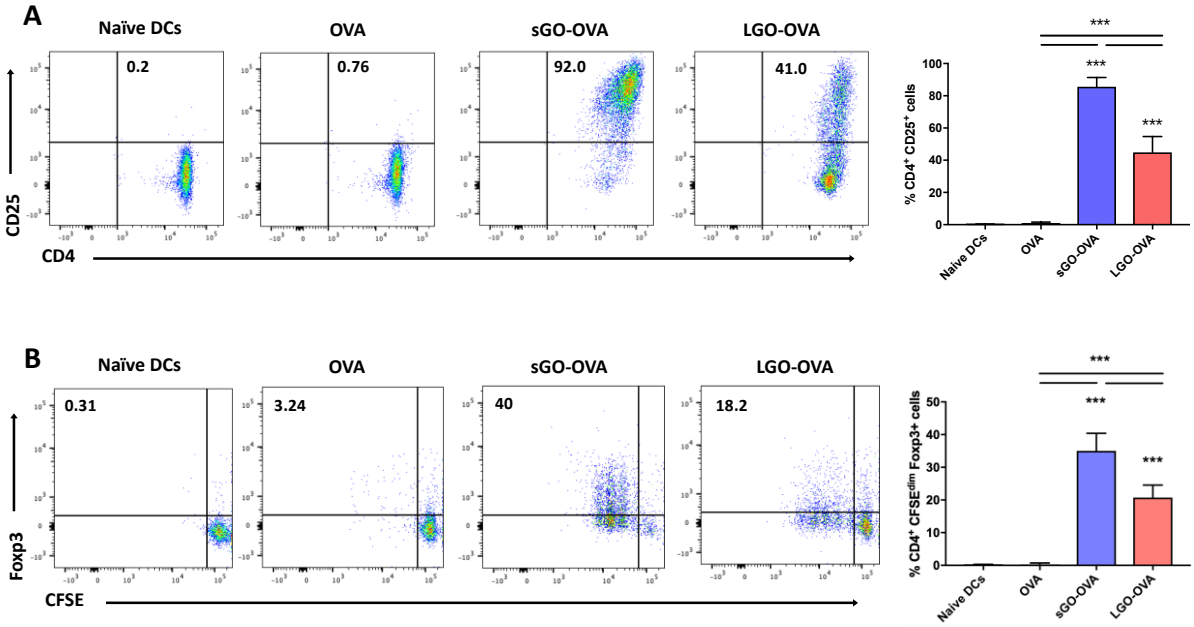
Supplementary Figure 1. Presence of endotoxins evaluated in bone marrow-derived macrophages by TNF-α ELISA. Mouse bone marrow-derived macrophages were exposed to non-toxic concentrations of sGO and LGO (50 µg/mL) or to cell culture medium. LPS at 100 ng/mL was used as positive control of inflammation. Polymyxin B (10 µM) was used as control to inhibit TLR4 dependent secretion of TNF-α. TNF-α concentrations are expressed in pg/mL using mouse TNF-α standard curve.



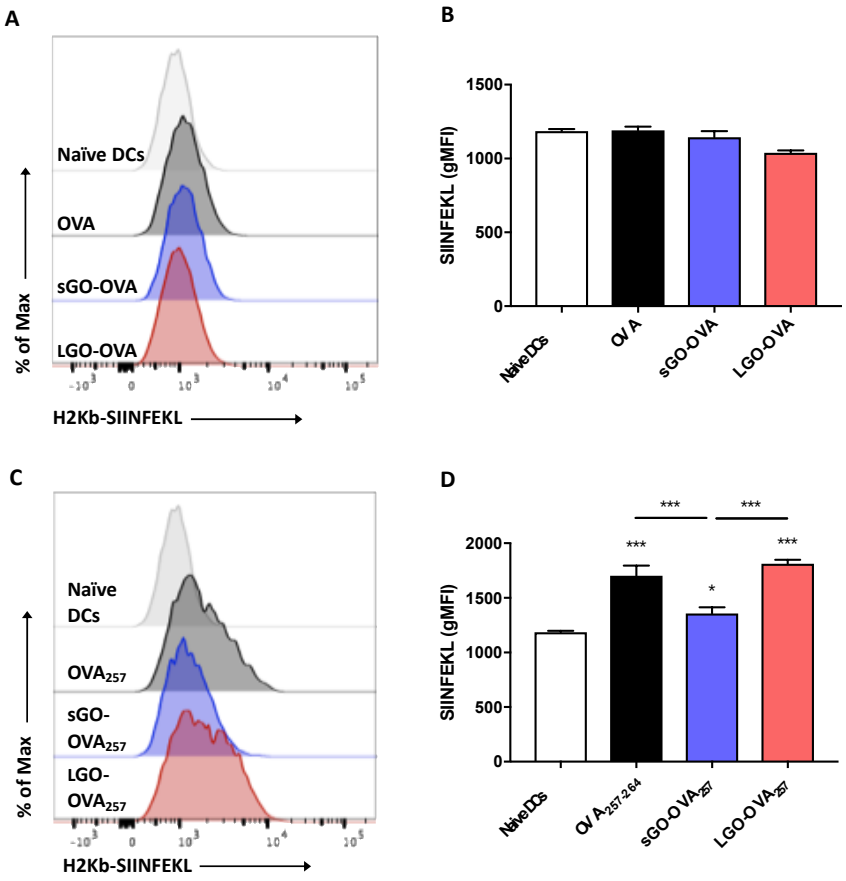
Supplementary Figure 2. Adsorption of OVA peptides (OVA_{257}, OVA_{323}) and OVA protein to sGO and LGO. Height AFM images and TEM micrographs are shown to evidence the formation of the complexes. Higher ratios were used to make the biomolecules visible in the AFM and TEM (1.5 µg of OVA peptides and 50 µg of OVA protein were adsorbed to 5 µg of sGO and LGO).



Supplementary Figure 3. Graphene oxide has minimal effect on expression of costimulatory markers in BMDCs. gMFI of CD86, CD80, CD40, CD83, MHCII and MHCI as assessed by flow cytometry. Data are presented as mean ± STDEV (data is representative of 1 of 3 independent experiments each with 3 intra experimental replicates). Statistical analysis was performed by one-way ANOVA with Bonferroni's *post hoc* test, *p<0.05, **p<0.01, ***p<0.001 (above bars *versus* control).



Supplementary Figure 4. OT-II CD25 and Foxp3 expression flow cytometry. BMDCs were pulsed for 2h with 10 µg OVA protein or GO-OVA complexes (5µg:10µg). BMDCs were then washed and co-cultured with CFSE labelled OT-II CD4+ T cells for 72h. (A) The % of $CD4^+$ T cells expressing CD25 was analysed via flow cytometry. (B) The % of $CD4^+$ $CFSE^{dim}$ dividing T cells expressing Foxp3 was analysed via flow cytometry. Data are presented as mean ± STDEV (data is representative of 1 of 3 independent experiments each with 3 intra experimental replicates) . Statistical analysis was performed by one-way ANOVA with Bonferroni's *post hoc* test. ***p<0.001 (above bars *versus* OVA alone control).



Supplementary Figure 5. OT-I antigen presentation on MHCI. (A, B) BMDCs were pulsed for 18 hours with 10 µg OVA protein or GO-OVA complexes (5µg:10µg) and the presentation of MHCI-bound SIINFEKL was assessed by flow cytometry. (C, D) BMDCs were pulsed for 18 hours with 5 ng OVA peptide (257-264) or GO-OVA peptide complexes (5µg:5ng) and the presentation of MHCI-bound SIINFEKL was assessed by flow cytometry. Data are presented as mean + STDEV (data is representative of 1 of 3 independent experiments each with 3 intra experimental replicates). Statistical analysis was performed by one-way ANOVA with Bonferroni's *post hoc* test. *P<0.05, ***p<0.001 (above bars *versus* naïve DCs).

Supplementary Videos

Video S1. Naïve DCs were imaged using a confocal microscope. DC plasma membranes were stained with CellMask Red plasma membrane stain (excitation at 633 nm/ emission max at 665 nm).

Video S2. Processing of OVA_{DQ} (488/520 nm, green) was imaged using a confocal microscope. DC plasma membranes were stained with CellMask Red plasma membrane stain (excitation at 633 nm/ emission max at 665 nm).

Video S3. Processing of sGO-OVA_{DQ} (488/520 nm, green) was imaged using a confocal microscope. DC plasma membranes were stained with CellMask Red plasma membrane stain (excitation at 633 nm/ emission max at 665 nm).

Video S4. Processing of LGO-OVA_{DQ} (488/520 nm, green) was imaged using a confocal microscope. DC plasma membranes were stained with CellMask Red plasma membrane stain (excitation at 633 nm/ emission max at 665 nm).