SUPPORTING INFORMATION

Exposure to graphene oxide sheets alters the expression of reference genes used for real-time RT-qPCR normalization

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Supporting Tables

Table S1. Physicochemical characterization of GO material used in this study.

	Technique	***Results***
Lateral dimensions*	*AFM*	0.050 – 0.5 µm
	TEM	0.1 – 2 µm
Thickness*	*AFM*	1.4 ± 0.5 nm (1-2 layers)
Optical Properties	*Absorbance (λ = 230 nm)*	$A = 0.050 * C_{GO}$ (µg/mL)
	Fluorescence (λ_{exc} = 525 nm)	$F_{600} = 0.823 * C_{GO}$ (µg/mL)
Degree of defects (I_D/I_G)	*Raman spectroscopy*	1.36 ± 0.03
Surface charge	*ζ-potential*	-55.9 ± 1.4 mV
Functionalisation degree	*TGA*	41%
Chemical composition (Purity)	*XPS*	C: 67.6%, O: 32.2%, (99.8%) S: 0.2%
C:O ratio	*XPS*	2.1
π-π, O=C-O, C=O, C-O-C, C-C & C=C	*XPS*	3.0%, 15.3%, 31.2%, 11.3%, 39.2%

*Lateral dimensions and thickness are reported as a range between the minimum and maximum sizes detected. Full characterization of the material is provided in Mukherjee et al[15].

Table S2. MIQE checklist.

See separate XLS file

Table S3. RNA yield and integrity assessment, MCF7 samples.

Sample	[RNA] µg/ml	$A_{260/280}$	$A_{260/230}$	28S/18S	RIN
Untreated 1	554	2.01	2.1	3.0	9.2
Untreated 2	583	2.06	2.02	2.4	9.2
Untreated 3	535	1.87	1.93	3.2	9.5
5 µg/ml 1	559	2.02	2.08	3.8	9.3
5 µg/ml 2	639	2.04	2.18	2.5	9.6
5 µg/ml 3	563	2.02	1.82	2.8	9.3
10 µg/ml 1	636	2.02	1.99	3.7	9.4
10 µg/ml 2	597	2.01	2.11	3.3	9.4
10 µg/ml 3	723	2.03	2.19	3.9	9.4
50 µg/ml 1	523	1.79	1.70	3.0	9.6
50 µg/ml 2	594	2.02	1.75	3.0	9.8
50 µg/ml 3	529	1.99	2.09	2.7	9.8

[RNA] µg/ml, $A_{260/A280}$ and $A_{260/230}$ are calculated by spectroscopy; 28S/18S ratios and RIN numbers are calculated upon RNA denaturation and electrophoretic separation **(Figure S1, a)**. The table shows the results corresponding to all four experimental groups, n=3.

Table S4. RNA yield and integrity assessment, MEF samples.

Sample	[RNA] µg/ml	A260/280	A260/230	28S/18S	RIN
Untreated 1	358	1.89	1.88	3.1	9.1
Untreated 2	333	1.96	1.93	2.8	9.3
Untreated 3	324	2.01	2.19	2.7	9.3
5 µg/ml 1	266	2.09	1.71	2.2	9.6
5 µg/ml 2	223	2.03	2.1	2.5	8.9
5 µg/ml 3	246	1.99	2.14	2.4	9.1
10 µg/ml 1	371	1.82	1.95	2.5	9.3
10 µg/ml 2	263	1.77	1.77	2.1	9.3
10 µg/ml 3	252	1.92	1.77	2.3	9.3
50 µg/ml 1	96	1.96	2.04	2.0	9.4
50 µg/ml 2	176	1.99	2.09	2.4	9.2
50 µg/ml 3	110	1.71	1.75	2.6	9.3

[RNA] µg/ml, $A_{260/A280}$ and $A_{260/230}$ are calculated by spectroscopy; 28S/18S ratios and RIN numbers are calculated upon RNA denaturation and electrophoretic separation **(Figure S1, b)**. The table shows the results corresponding to all four experimental groups, n=3.

Table S5. Details of primer pairs used in MCF7 study (human).

Gene symbol	Accession number (mRNA)	Primer sequences	Amplicon size (bp)	E	R^2
RPS13	NM_001017.2	**Fwd** CGCTCTCCTTTCGTTGCCT **Rv** CGCTGCGTCGATAGGGTAAA	96	2.11	0.9956
RPL27	NM_000988.3	**Fwd** ATCGCCAAGAGATCAAAGATAA **Rv** TCTGAAGACATCCTTATTGACG	123	2.02	0.9993
RPL30	NM_000989.3	**Fwd** ACAGCATGCGGAAAATACTAC Rv AAAGGAAAATTTTGCAGGTTT	158	1.99	0.9987
OAZ1	NM_004152.3 NM_001301020.1	**Fwd** CTCCACTGCTGTAGTAACCCG Rv GATCCCTCTGACTATTCCCTCG	104	1.75	0.9998
ACTB	NM_001101.3	**Fwd** AGCACAGAGCCTCGCCTTT **Rv** GAGCGCGGCGATATCATCA	82	2.0	0.9961
GAPDH	NM_001289746.1 NM_001289745.1 NM_001256799.2 NM_002046.5	**Fwd** CCACATGGCCTCCAAGGAGTAAGAC **Rv** AGGAGGGGAGATTCAGTGTGGTGGG	131	2.0	0.9984
MAPK1	NM_002745.4 NM_138957.3	**Fwd** TCCCAAATGCTGACTCCAAAG **Rv** CATGTCGAACTTGAATGGTGC	164	2.08	0.9993
UBC	NM_021009.6	**Fwd** GCCTTAGAACCCCAGTATCAG **Rv** AAGAAAACCAGTGCCCTAGAG	74	1.98	0.9907
HMBS	NM_000190.3 NM_001024382.1 NM_001258208.1 NM_001258209.1 XM_017017629.1 XM_005271531.1 XM_005271532.1 XM_005271533.3 XM_011542796.1	**Fwd** AGCTTGCTCGCATACAGACG **Rv** AGCTCCTTGGTAAACAGGCTT	157	2.03	0.9990
TBP	NM_003194.4 NM_001172085.1	**Fwd** CCACTCACAGACTCTCACAAC **Rv** CTGCGGTACAATCCCAGAACT	127	2.00	0.9968

E, efficiency of qPCR reaction; R^2, coefficient of determination from linear regression of Cq values (cDNA serial dilution). Primer pairs amplify all transcription variants with equal amplicon length.

Table S6. Details of primer pairs used in MEF study (mouse).

Gene symbol	Accession number (mRNA)	Primer sequences	Amplicon size (bp)	E	R^2
Rps13	NM_026533.3	**Fwd** CCCAGGTCCGTTTTGTGACT **Rv** TCCTCTCAAGGTGCTTTCGG	132	2.01	0.9972
Rpl27	NM_011289.3	**Fwd** CAAAAACGCAGTGCCCGA **Rv** CTTACGGAGAGGTGGCTTCA	120	2.27	0.9907
Rpl30	NM_009083.4 NM_001163485.1	**Fwd** GAAGAGCTTTGCATTGTGGGAG **Rv** CCATCTTCCTGCCTTAGGTGC	102	1.95	0.9948
OAZ1	NM_008753.4 NM_001301034.1	**Fwd** GGGTTGCCCTTAATTGCTGT **Rv** TCTTGTCGTTAGACGTCGGC	187	1.99	0.9985
Actb	NM_007393.5	**Fwd** CTGAGCTGCGTTTTACACCC **Rv** CGCCTTCACCGTTCCAGTTT	200	1.93	0.9995
Gapdh	NM_001289726.1 NM_008084.3 XM_017321385.1	**Fwd** AGGTCGGTGTGAACGGATTTG **Rv** TGTAGACCATGTAGTTGAGGTCA	123	2.00	0.9988
Mapk1	NM_011949.3 NM_001038663.1 XM_006522147.3	**Fwd** GGTTGTTCCCAAATGCTGACT **Rv** CAACTTCAATCCTCTTGTGAGGG	84	1.99	0.9979
Ubc	NM_019639.4	**Fwd** CAAACAGGAAGACAGACGTACC **Rv** CCCATCACACCCAAGAACAAG	80	2.04	0.9986
Hmbs	NM_013551.2 NM_001110251.1	**Fwd** ATCTTGGACCTAGTGAGTGTGT **Rv** GTACAGTTGCCCATCTTTCATCA	141	2.01	0.9985
Tbp	NM_013684.3	**Fwd** TTTGGCTAGGTTTCTGCGGT **Rv** GCCCTGAGCATAAGGTGGAA	195	2.04	0.9984

E, efficiency of qPCR reaction; R^2, coefficient of determination from linear regression of Cq values (cDNA serial dilution). Primer pairs amplify all transcription variants with equal amplicon length.

Supporting Figures

Supporting Figure 1

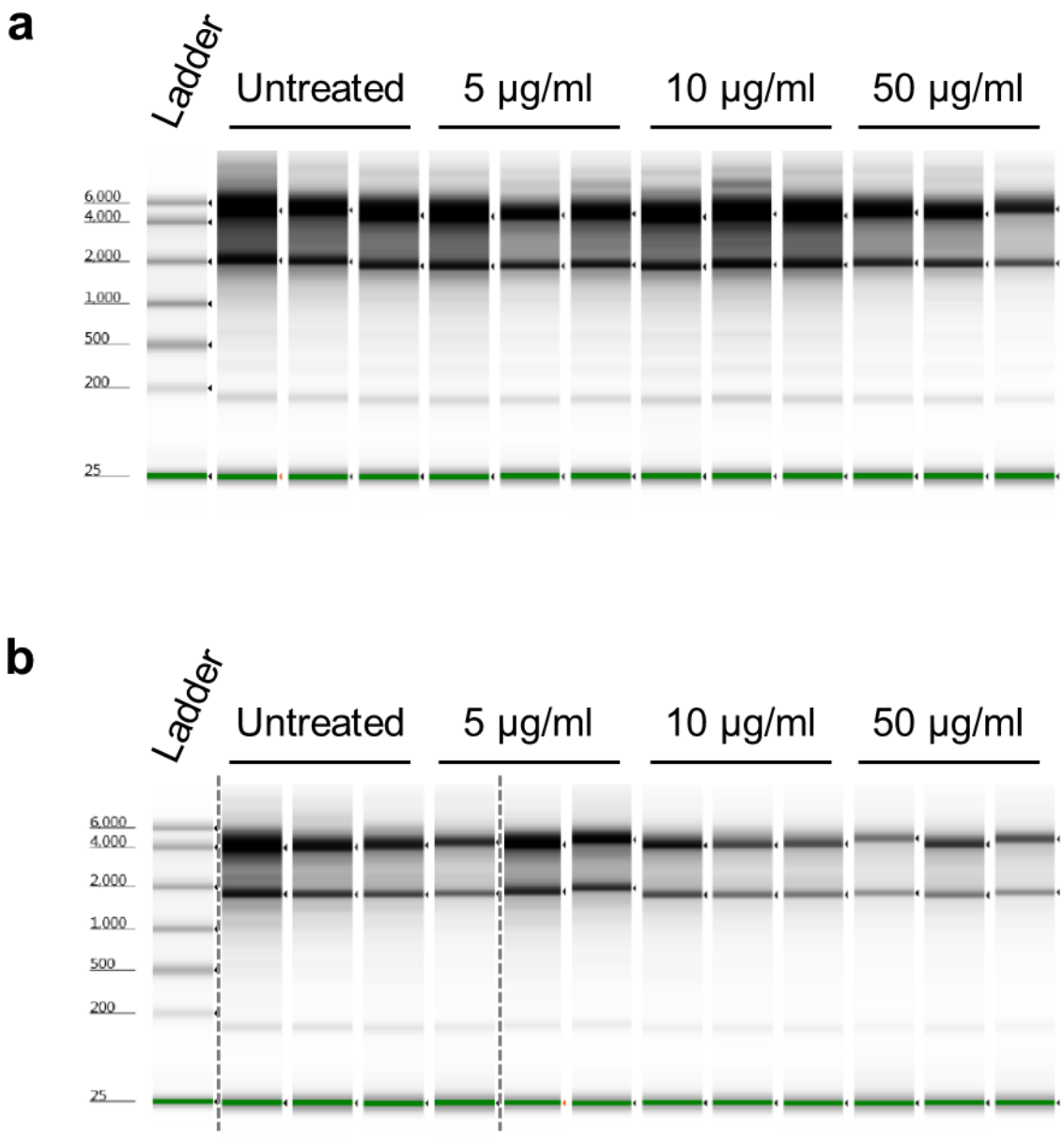

Figure S1. Analysis of RNA integrity on the 2200 TapeStation system. Gel electrophoresis shows the separation profile of individual **(a)** MCF7 and **(b)** MEF samples, including 28S, 18S, small rRNAs and lower marker. RIN numbers and 28S/18S ratios are provided in **Tables S3 and S4.** When gels have been cropped to organize the samples in consecutive order, cropping sites are delineated by grey dashed lines.

Supporting Figure 2

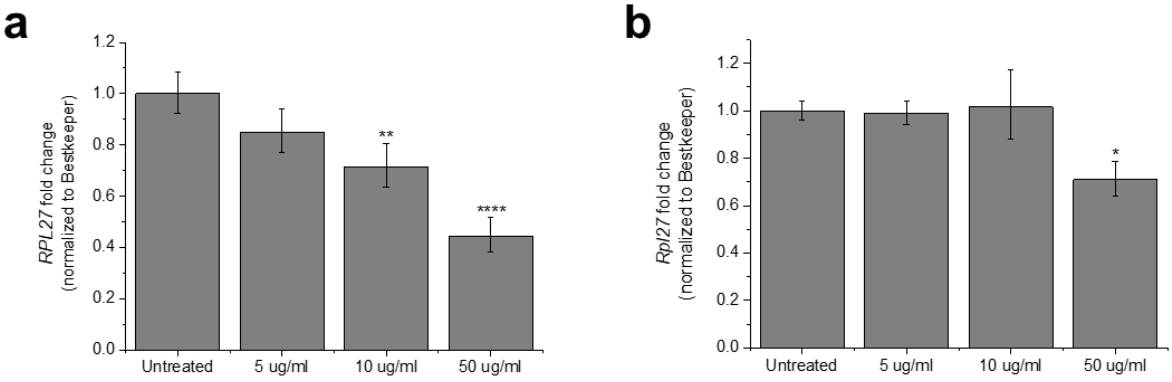

Figure S2. *RPL27* mRNA levels normalized to Bestkeeper index. (a) *RPL27* expression in MCF7 cells exposed to GO, normalized to Bestkeeper index. **(b)** *Rpl27* expression in MEFs exposed to GO, normalized to Bestkeeper index. Bars represent fold change; error bars represent propagation of standard error (SE). *p<0.05, **p<0.01 and ****p<0.0001, assessed by one-way ANOVA and Tukey's test, n=3.

Supporting Figure 3

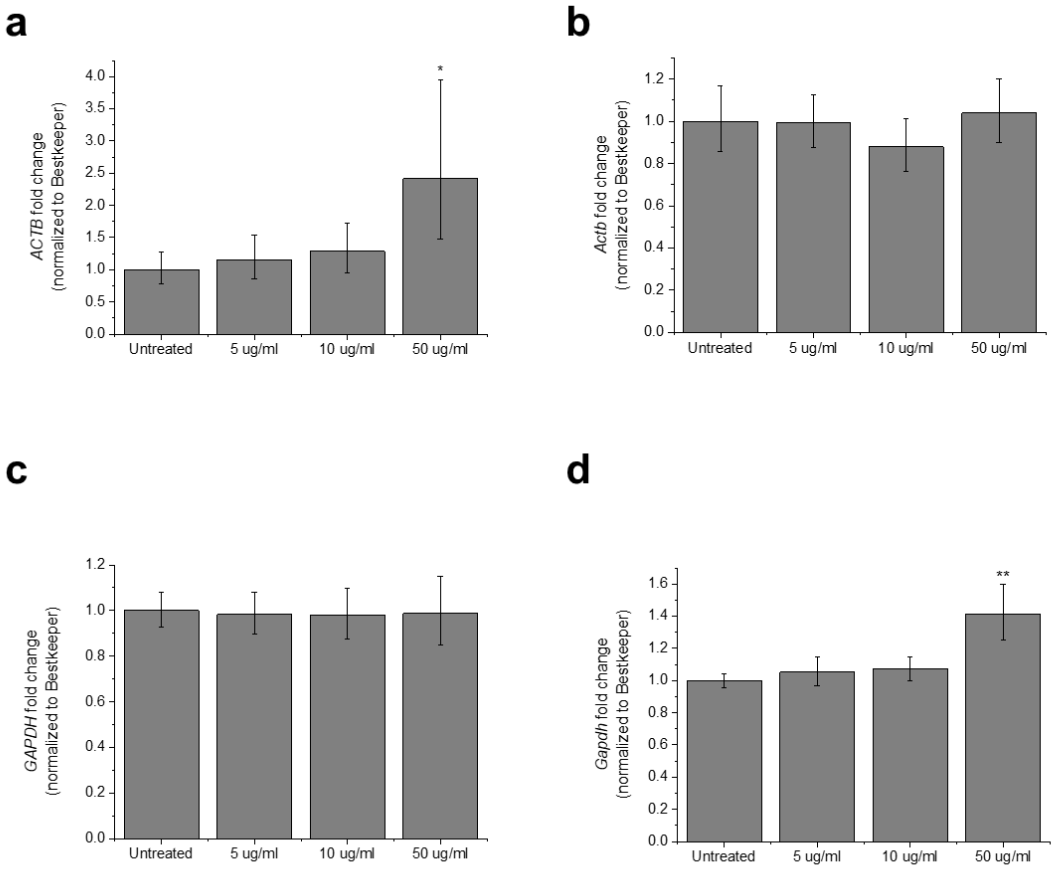

Figure S3. *ACTB* and *Gapdh* mRNA levels normalized to Bestkeeper index. (a) *ACTB* and **(b)** *GAPDH* expression in MCF7 cells exposed to GO, normalized to Bestkeeper index. **(c)** *Actb* and **(d)** *Gapdh* expression in MEFs exposed to GO, normalized to Bestkeeper index. Bars represent fold change; error bars represent propagation of standard error (SE). *p<0.05 and **p<0.01, assessed by one-way ANOVA and Tukey's test, n=3.