Supporting Information

High-Resolution Visualisation of Antisense Oligonucleotide Release from Polymers in Cells

Jessica J. King[‡a,b], Kai Chen[‡a,b], Cameron W. Evans[a,b], Marck Norret[a,b], Ruba Almasri[a,b,c] Nathan J. Pavlos[d], Henry Y.L. Hui[e], Qiongxiang Lin[a,b], Uditi Bhatt[a,b], Stephen G. Young[f], Nicole M. Smith[a,b], Mehran Nikan[g], Clive A. Prestidge[c], Haibo Jiang[*a,b,h], K. Swaminathan Iyer[*a,b]

[a] School of Molecular Sciences, The University of Western Australia, Perth WA 6009, Australia.

[b] ARC Training Centre for Next-Generation in Biomedical Analysis, The University of Western Australia, Perth WA 6009, Australia

[c] UniSA Clinical & Health Sciences, University of South Australia, Adelaide SA, Australia

[d]School of Biomedical Sciences, The University of Western Australia, Perth WA 6009, Australia.

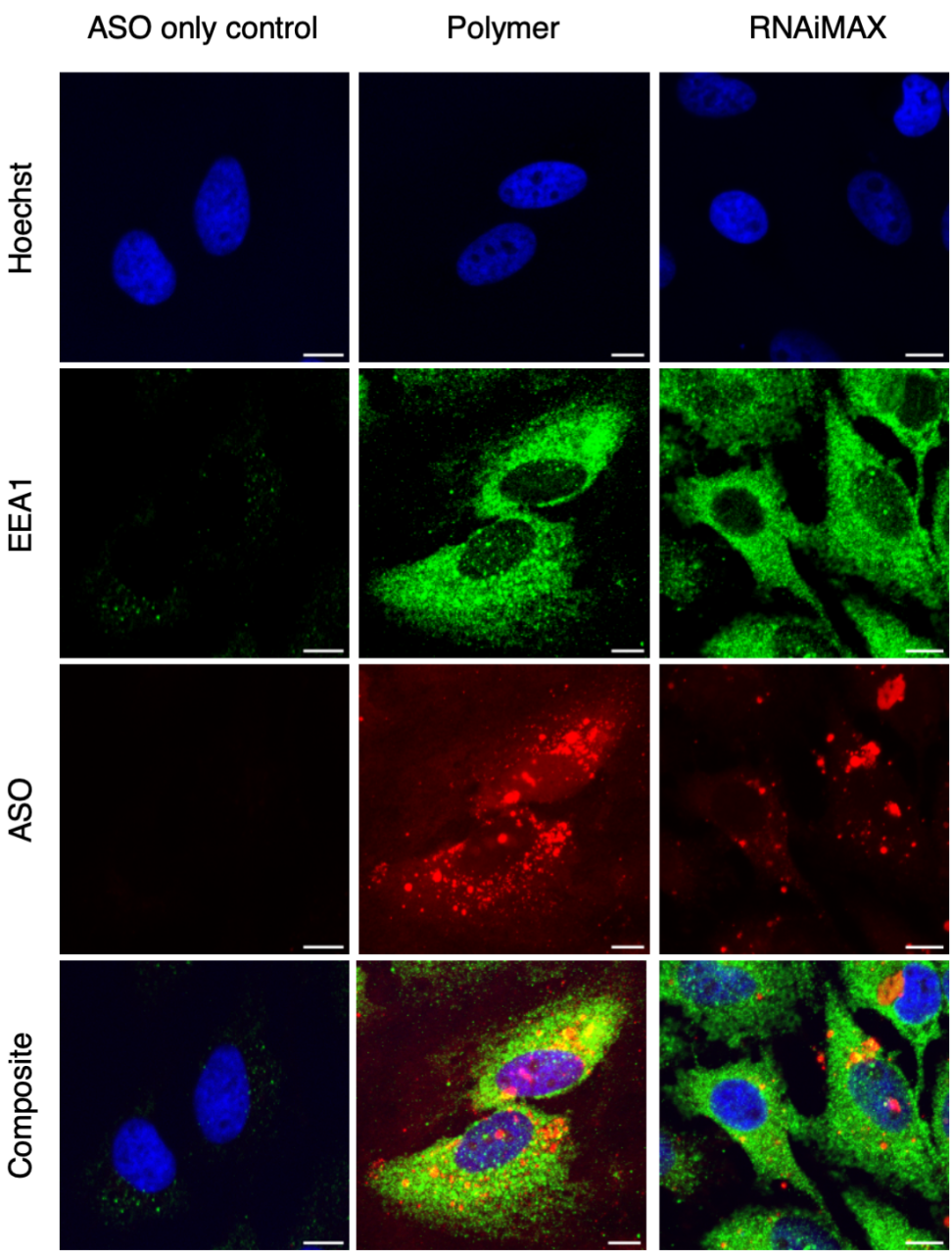
[e]Translational Cancer Pathology Laboratory, School of Biomedical Sciences, The University of Western Australia, Perth WA 6009, Australia

[f] Department of Medicine, University of California, Los Angeles, CA 90095, USA.

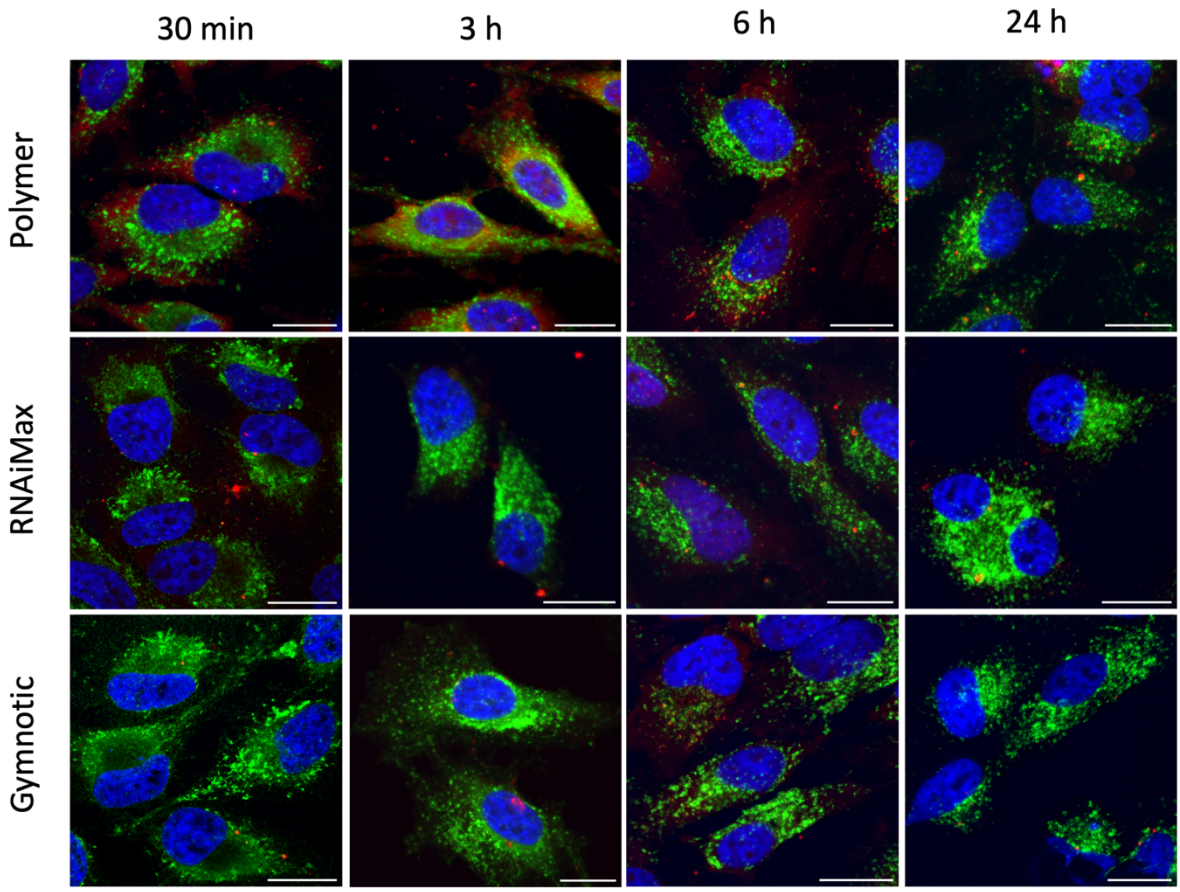
[g] Ionis Pharmaceuticals, Inc., Carlsbad, CA 92010, USA.

[h] Department of Chemistry, Faculty of Science, University of Hong Kong, Pok Fu Lam, Hong Kong.

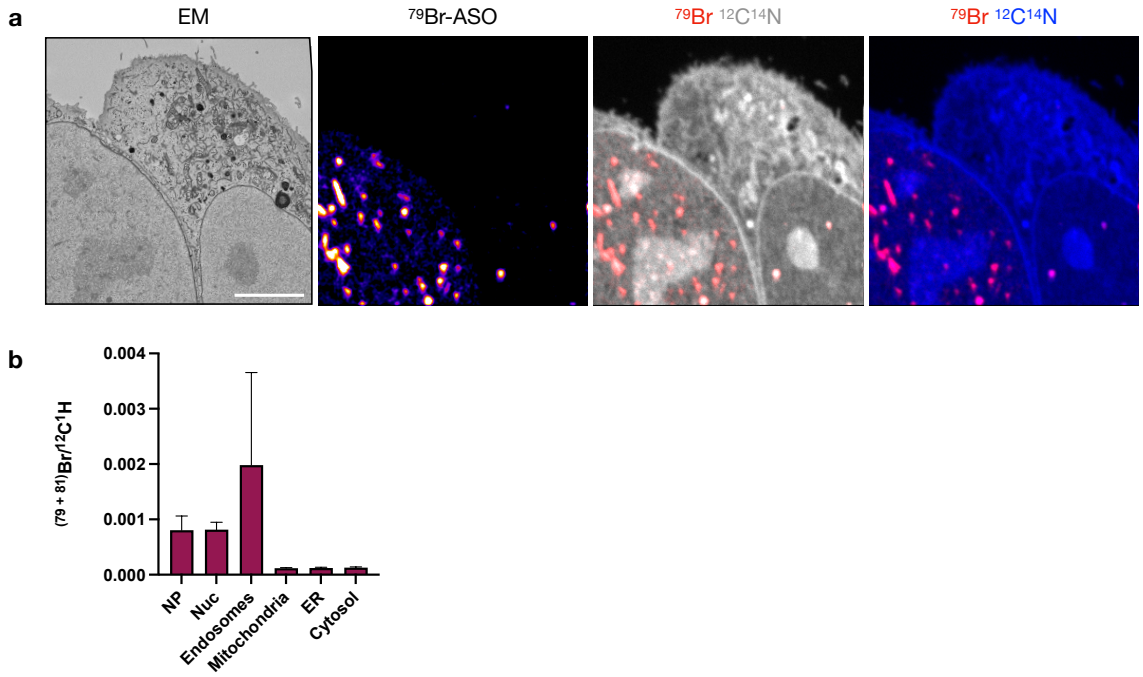
Contents



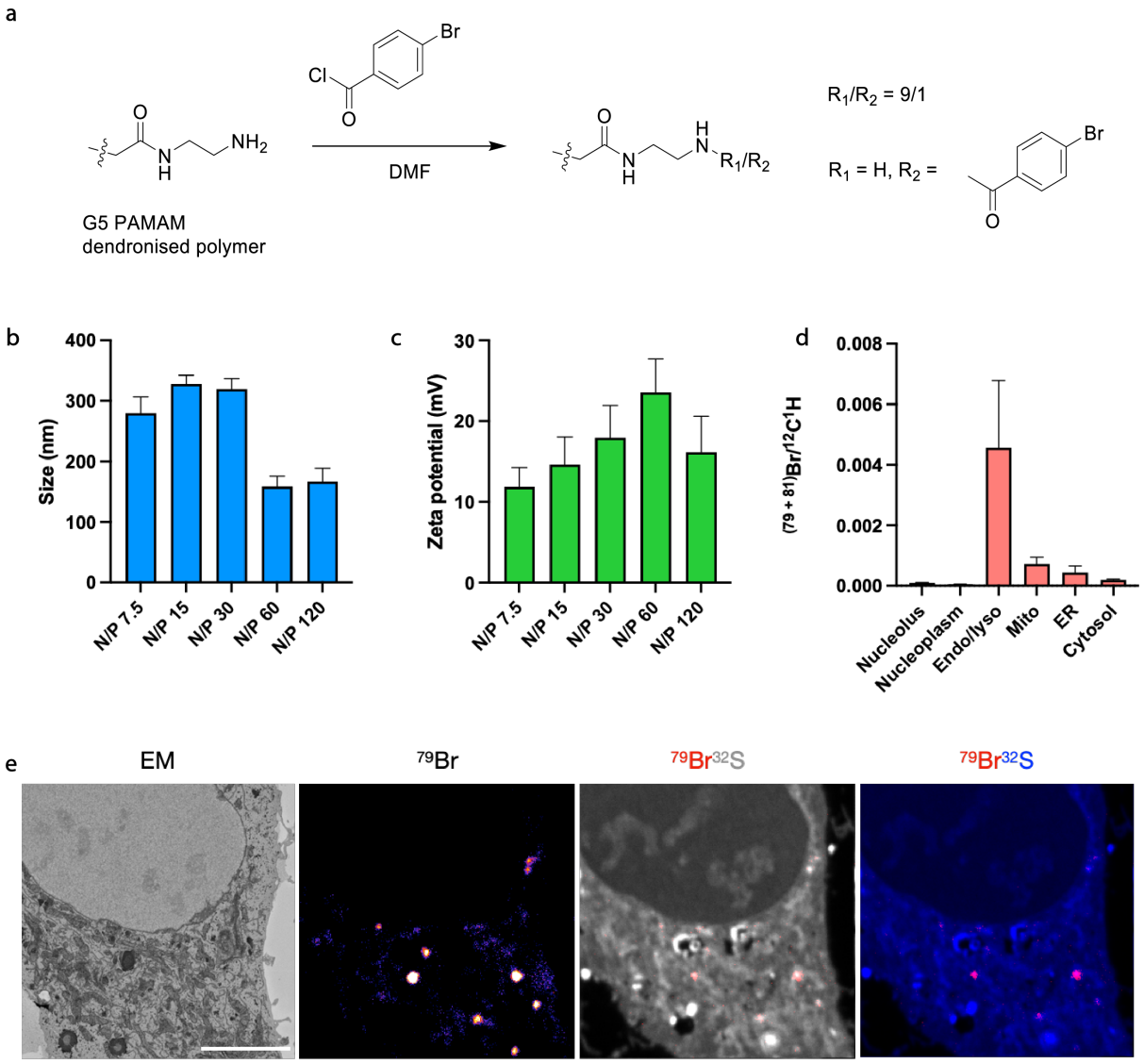
Supplementary Figure S1. Diffuse distribution of ASO after 24 h results in efficient knockdown activity. Representative confocal images of Cy3-ASO (100 nM) delivery into cells after 24 h incubation. ASO (Cy3, red), EEA1 (Alexa Fluor 488, green) and nuclei (Hoechst 34580, blue). Scale bar 10 μm.



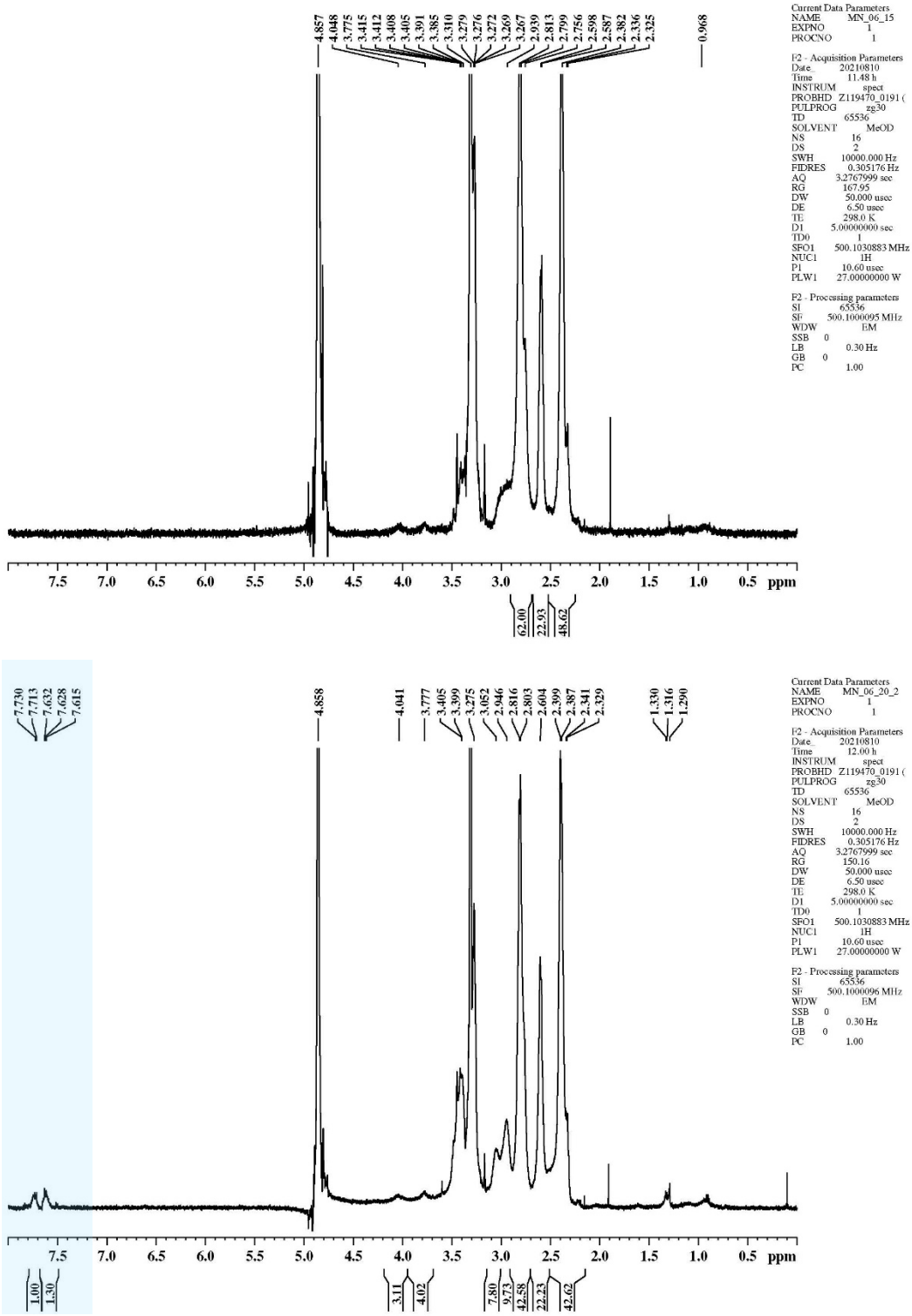
Supplementary Figure S2. Representative confocal images of Cy3-labelled *MALAT1* ASO (100 nM) delivery into cells after 30 min, 3 h, 6 h, and 24 h incubation. ASO-Cy3 (red), Rab7 (green) and nuclei (blue). Scale bar 20 μm.



Supplementary Figure S3. RNAiMAX delivers ASO into nucleus of HeLa cells. **(a)** Scanning electron microscopy (SEM) and NanoSIMS images showing the delivery of ASO into the nucleus of HeLa cells by RNAiMAX. SEM image, bromine labelled ASO (^{79}Br NanoSIMS image), composite ^{79}Br (red), $^{12}C^{14}N$ (grey). **(b)** Quantification of ASO (^{79}Br signal) in subcellular compartments.



Supplementary Figure S4. Bromine labelled polymer facilitated polymer tracking via NanoSIMS. **(a)** Bromine labelling of generation 5 (G5) dendronised polymer. **(b)** Size of polyplex complexes at various N/P ratios. Data shown as mean ± S.E.M. **(c)** Surface charge (zeta potential) of polyplexes at different N/P ratios. Data shown as mean ± S.E.M. **(d)** Quantification of bromine (*i.e.,* polymer) signal, collected from 10 cells. **(e)** Representative images of the localisation of bromine labelled polymer (unbound to ASO) in cells. SEM image, bromine labelled polymer (^{79}Br NanoSIMS image), composite ^{79}Br (red), ^{32}S (grey), composite ^{79}Br (green), ^{32}S (blue).



Supplementary Figure S5. ^{1}H NMR spectra (500 MHz, MeOD, 25 °C) of G5 PAMAM dendronised polymer (top), and bromobenzyl modified polymer used for NanoSIMS (bottom). The appearance of signals at δ_H 7.73–7.61 ppm (blue shaded region) confirms polymer labelling with Br.