## Development of a high-throughput platform for screening lipid nanoparticles for mRNA delivery

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Supplementary Figures



**Figure S1.** Stability of automated MC3 LNPs encapsulating eGFP mRNA stored in 4°C for two weeks. (a) hydrodynamic size and (b) mRNA expression in H358 cells.

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**Figure S2.** Automated DLS measurements. a. comparison of DLS measurements carried out using the manual (cuvette) and automated (384-well plate) platforms. b. Size (Z-ave) and polydispersity index (PDI) for 3 LNPs obtained with the manual and automated DLS platforms.



**Figure S3.** Automated Ribogreen assay validation. a. mRNA standard curves obtained by manual pipetting (manual platform) vs Hamilton-assisted pipetting (automated platform). b. calculated encapsulation efficiency for 4 different LNPs using the manual and automated platforms.



**Figure S4.** Automated TNS assay (pKa) measurements. a. comparison of the TNS assay carried out using the manual and automated platforms. b. normalised TNS fluorescence of MC3 LNPs fitted into sigmoidal curves.



**Figure S5.** Diagram illustrating an example of an automated workflow of LNP functional delivery. The steps involved in the quantification of eGFP fluorescence following successful LNP delivery of eGFP mRNA include: 1) LNPs are produced with the automated platform and dosed into a 384-well plate containing cells using a common liquid handler (*e.g.*, ECHO, Hamilton), 2) The cells are imaged using an automated live-cell imaging system (*e.g.* Incucyte) equipped with fluorescence detectors over an appropriate time-course, 4) Fluorescence and phase images are acquired at

predefined time points over the course of the experiment, 5) kinetics data can be easily plotted and analysed using the incorporated software or another graphing software.



**Figure S6.** Characterisation of ten novel LNPs-mRNA regarding mRNA loading efficiency as measured by Ribogreen fluorescence assay (n=3).



**Figure S7.** *In vivo* functional delivery of automated LNPs compared with standard LNPs encapsulating pDNA encoding luciferase. a, whole body luminescence imaging and quantification. P>0.05, unpaired t-test. b, *ex vivo* organ luminescence imaging and quantification. Mice (n=3) were dosed at 0.25 mg/kg via tail vein i.v. injection and sacrificed at 48 h. P > 0.05. Two-way ANOVA followed by Tukey's multiple comparisons.