

Extended Figure Legends

Figure 1. (A) NPs loaded with miR-23a and miR-150 protect the colonic epithelial cell barrier from colitogenic toxins. Caco-2/TC-7 cells plated for 21 days in transwell plates were treated with NPs loaded with miR-23a and miR-150 mimics or a non-targeting miRNA mimic (miR-Control) at a final concentration of 20nM for 48h. Transepithelial electrical resistance (TEER) was measured 24h after exposure to TcdA+B (50nM), or Lipopolysaccharides (LPS, 0.1ug/ml). **(B)** NPs significantly upregulate the intracellular levels of miR-23a and miR-150 in NCM356 IECs. NCM356 cells were treated with NPs and miRNA levels were assessed in cell extracts 24h later by RT-qPCR. Data normalised against RNU1A1 and 5S rRNA are expressed in comparison to vehicle (NP buffer)-treated cells.

Figure 2. (A) NPs significantly upregulate the intracellular levels of miR-23a and miR-150 in neuronal cells. SH-SY5Y cells were treated with NPs loaded with miR-23a and miR-150 mimics or a non-targeting miRNA mimic (miR-Control) at final concentrations of 2.5 and 5nM for 24h. miRNA levels were assessed in cell extracts 24h later by RT-qPCR. Data normalised against RNU1A1 and 5S rRNA are expressed in comparison to miR-Control -treated cells. **(B)** NPs protect brain-derived cells from LPS. SH-SY5Y cells treated with NPs loaded with miR-23a and miR-150 mimics or a non-targeting miRNA mimic (miR-Control) at final concentration of 10nM were treated with LPS (50ug/ml), and cell survival was assessed 48h later by calcein AM assay. A172 cells were treated with NPs at final concentration of 10nM, treated with LPS (50ug/ml), and cell survival was assessed 16 h later by CellTiter Glo assay, validated by IncuCyte live imaging. **(C)** Obefazimod (ABX464) decreases miR-124 levels in neuronal cells. Differentiating LUHMES cells were treated with increasing concentrations of ABX464 or vehicle (DMSO). miRNA levels were assessed in cell extracts 24h later by RT-qPCR. Data normalised against RNU1A1 and 5S rRNA are expressed in comparison to DMSO-treated cells. **(D)** Obefazimod (ABX464) protects brain-derived cells from LPS. SH-SY5Y cells pre-treated with 10uM ABX464 or DMSO, was treated with LPS (50ug/ml) or vehicle, and cell survival was assessed 48h later by calcein AM assay.

Experimental Procedures

Cell Culture and treatments

Human NCM356, NCM460 colonic epithelial cell lines (Incell, San Antonio, USA) were cultured in M3Base medium (M300F) supplemented with 10% foetal bovine serum (FBS) (10270-106; Life Technologies, Carlsbad, California, USA) and 1% Penicillin-Streptomycin (Monaghan et al., 2021).

Caco-2/TC-7 human colon adenocarcinoma cell line (Sigma-Aldrich) was cultured in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 20% FBS and 1% Penicillin-Streptomycin. For TEER analysis cells were grown in collagen-coated 12mm Transwells with 0.4µm pore membrane insert (Corning, UK) for 21 days. Transepithelial electrical resistance (TEER) was measured at 24h using EVOM3.

Human SH-SY5Y neuroblastoma cells obtained from the European Collection of Animal Cell Cultures (Salisbury, UK) and A-172 glioblastoma cells obtained from the American Type Culture Collection (Virginia, US) were grown in DMEM/Ham's F-12 (DMEM/F12) containing 10% foetal bovine serum, 2mM L-glutamine, 1% non-essential amino acids, penicillin, and streptomycin. (Ugun-Klusek et al., 2019). For SH-SY5Y growth assays, DMEM supplemented with Glutamax without antibiotics was used.

Human mesencephalic cell line (LUHMES, Lund human mesencephalic) purchased from American Type Culture Collection (Virginia, US) were cultured and differentiated following the established protocol (Scholz et al., 2011). Culture dishes were pre-coated with 50µg/mL Poly-L-ornithine (Sigma, USA) and 1µg/mL human plasma fibronectin (Gibco, USA). Cells were cultured in Advanced Dulbecco's modified Eagle's medium/F12, 1×N-2 supplement (Invitrogen, Karlsruhe, Germany), 2mM L-glutamine (Gibco, Rockville, MD, USA) and 40ng/mL recombinant basic fibroblast growth factor (R&D Systems, Minneapolis, MN, USA). To induce differentiation, cells were treated with Advanced Dulbecco's modified Eagle's medium/F12, 1×N-2 supplement, 2mM L-glutamine, 1mM dibutyryl cAMP (Sigma-Aldrich), 1µg/mL tetracycline (Sigma-Aldrich) and 20ng/mL recombinant human GDNF (R&D Systems). Cells were maintained in differentiation medium and treated after 3 days of differentiation.

Preparation of miRNA polyplex nanoparticles

miRNA polyplex nanoparticles were prepared using the following general procedure and was adapted to prepare nanoparticles with hsa-miR-23a-3p miRNA Agomir (MAH01609) and hsa-miR-150-5p miRNA Agomir (MAH01347) or miRNA Agomir Negative Control (MAH00000, ABM, Canada). 750 µL of miRNA (or miRNA mixtures) were prepared at 400 nM (~12 µg/mL) in sodium acetate buffer (25 mM, pH 5.5) and mixed via rapid pipetting with 750 µL of poly(β amino ester) cationic polymer at 1.54 mg/mL also dissolved in sodium acetate buffer (25 mM, pH 5.5) to form miRNA polyplex nanoparticles at a 128 polymer/miRNA w/w ratio with a final miRNA concentration of 200 nM (~6 µg/mL). The mixture was allowed to stand for 10 minutes to allow the polyplex nanoparticles to form and were used directly as described below.

Cells were treated with miRNA polyplex nanoparticles or ABX464 (Cambridge Bioscience, Cat. No. HY-100870) in serum free medium for 2 hrs. Poly (beta amino esters) derived from an 'alkyl' diacrylate and amino alcohol monomer high molar mass linear were used to produce miRNA polyplex nanoparticles.

RNA isolation and RT-qPCR for miRNAs

RNA was extracted using the miRNeasy Tissue/Cells Advanced Mini Kit (217604). For RT-qPCR miRNA analysis, reverse transcription was performed using miRCURY LNA RT Kit (339340) and quantitative polymerase chain reaction (qPCR) hsa-miR-23a-3p (YP00204772), hsa-miR-150-5p (YP00204772), hsa-miR-124-3p (YP00206026), using miRCURY LNA SYBR Green PCR Kit (339346, Qiagen, Hilden, Germany) on CFX384 real-time PCR detection system (Bio-Rad, Hercules, California, USA). The qPCR conditions applied were 95°C for 10 min, and 40 cycles of 95°C for 10 s and 60°C for 1 min, followed by melting curve analysis. qPCR reactions were performed in quadruplicates and RNUA1 and 5S rRNA were used as reference genes.

Cell survival analysis

SH-SY5Y cells were plated on 96-well black clear-bottom plates. After 2 days of treatment with either vehicle or NPs/ABX164 and with 2 extra days with either vehicle or LPS, cell survival was assessed using calcein AM (ThermoFisher) staining at a final concentration of 3µM. Cells were incubated at 37°C in the dark for 30min and fluorescence was measured using a fluorescence plate reader (FLUOstar BMG LabTech) at ex/em wavelengths of 485/535. Fluorescence intensity of samples were compared to fluorescence intensity of cells treated with NP-Controls or Vehicle and without LPS-treatment to determine the % survival as compared to controls.

Following A172 treatments in 96 well plates, cell survival was assessed at 16h using the CellTiter Glo Luminescence Cell Viability Assay (G7573, Promega) according to the

manufacturer's instructions. Luminescence readings were acquired with CLARIOstar plate reader (BMG Labtech). Data were expressed as mean luminescence (arbitrary units) \pm s.e.m. (control cells were set as 100%).

References

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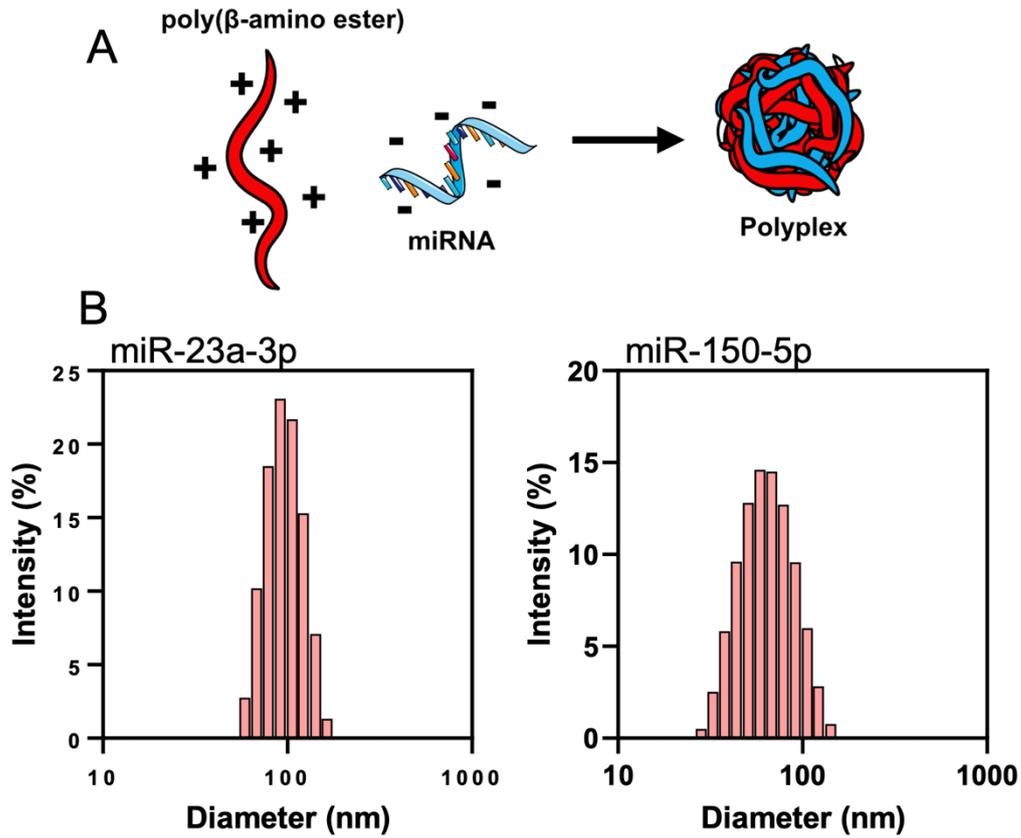


Figure S1: (A) Cartoon depiction of poly(β -amino ester) (PBAE) and miRNA formulation to prepare polyplex nanoparticles. (B) Particle size distribution of representative miR-23a-3p and miR-150-5p PBAE formulations as measured by dynamic light scattering.