Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Enhanced Enzymatic Degradation Resistance of Plasmid DNA in Ionic Liquids

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Experimental Procedures

Materials: VR1020 vector containing the YFP gene was kindly provided by Prof Ross Coppel's group from the Department of Biochemistry, Monash University. Phosphate buffered saline (PBS) tablets, polyethylenimine (PEI), tris base, Ethylenediaminetetraacetic acid (EDTA), glacial acetic acid and agarose from Sigma Aldrich. Dulbecco Modified Eagle medium (DMEM), Fetal Calf Serum (FCS), Optimem, SYBR Safe, and were purchased from Life Technologies. The GeneRuler 1kB plus ladder and 6x loading dye were purchased from Thermo Fisher Scientific. QIAGEN Plasmid Giga Kit was purchased from QIAGEN. bCDHP is prepared as described previously [4] by partial neutralisation of locally prepared CDHP with choline hydroxide solution (20 wt% in water). The target proton activity (pH = 7.2) of the mixture is initially determined and subsequently confirmed by determination of the pH titration curve of the CDHP diluted in water at 0.1 M concentration. Final water- bCDHP mixtures are made by dilution with deionisied water. Human Embryonic Kidney (HEK) 293T cells were purchased from CellBiolabs.

Bacterial Cell Culturing to isolate pDNA: The yellow fluorescent protein (YFP) inserted into the mammalian expression vector pVR1020 was streaked on to a LB agar plate containing kanamycin (50 μg/ml) using the four quadrant streak method.

A single colony of *E. coli* DH5 α containing pDNA–YFP was lifted from a streaked plate and inoculated in LB medium (2.4 L) containing kanamycin (50 μg/ml). The growth culture was incubated at 37°C and agitated at 200 rpm overnight. The plasmids were purified from cells using the QIAfilter plasmid giga kit according to the manufacturer's instructions.

The purity of the extracted pDNA was assessed using a Nanodrop 2000c, and stock solutions of concentrations (1000 ng/µL and 2000 ng/µL) were prepared using ultrapure water and placed into cold storage (-80°C) for later use.

Agarose Gel Electrophoresis: Agarose gels (0.5% (w/v)) were prepared in 1 X TAE solution and heated in a microwave for 1 minute to dissolve the agarose. Upon cooling to room temperature 1x SYBR safe added to the solution before pouring into a casting tray secures in place with a casting gate and inserting a 10 well comb.

The gels were used to run the YFP-pDNA samples in a Mini-Sub Cell GT Cell using a PowerPac. All gels were run at 90V for 55 minutes or as the loading dye approached the end of the gel. Gels were visualised using a Molecular Imager Gel Doc XR System with Quantity One software.

Mammalian Cell Culturing: The gene expression of YFP-pDNA after storing it in 20% (w/) bCDHP and 50% (w/w) bCDHP was examined. HEK 293T cells were plated in a 6 well plate with 200000 cells/well in DMEM with 10% FCS. The cells were incubated at 37^{o}C with 5% CO_2 and grown for 24 hours. 293T cells were then transfected with YFP-pDNA (2.5 µg YFP-pDNA per well) at N/P ratio of 20 with PEI and incubated for 48 hours.

Flow Cytometry: YFP-pDNA expression in HEK 293T cells was determined by flow cytometry. Following a 48 hour transfection of HEK 293T cells with YFP-pDNA, 293T cells were trypsinized, washed three times in PBS before resuspending in PBS with 2% FCS. The samples were kept on ice and analysed by flow cytometry on a CyAn Flow Cytometer. Data was analysed using Flow Jo V10. Just prior to analysis by flow cytometry, propidium iodide was added to stain dead cells.

Transfection for Confocal Microscopy: The samples were prepared as for flow cytometry, but were then stained with Hoechst Dye for 15 to 20 minutes to stain the nuclei. This dye stains the nuclei of the cells blue whilst those cells expressing successfully transfected expressed yellow fluorescent protein (green). A 40X objective lens was used to obtain the images.

Circular Dichroism Spectroscopy: The YFP-pDNA samples were prepared at stock concentrations (500 ng/μL) and degraded at 37^{o}C for 10 minutes before diluting the solution (50 ng/μL) to measure on the circular dichrosim (CD) spectrometer. The samples were run from 200 nm to 350 nm and an accumulation of three measurements, per sample was obtained. The resulting data was smoothed by a 25 pt. algorithm using OriginPro 8.

UV-Vis Spectroscopy*:* The melting temperature curves for YFP-pDNA were measured using a Cary UV-VIS, controlled by a thermostat and using a thermal package. YFP-pDNA was prepared (131 μM, 50 ng/μL) in its respective buffer and heated to 95^{o}C for three minutes before slowly cooling to room temperature and conducting the measurements. The absorbance was measured using a wavelength of 264 nm, with a slit average of 2 nm, at an average time of 0.2 s and ramping at 1^{o}C/min. The thermal package was then used to derive the 1st derivative, and conduct the two state process for van't Hoff's model. The raw data measured was smoothed using a 25 pt. algorithm using OriginPro 8.

VR1020 YFP

Sequence

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGA
CGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCT
ACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCA
CCCTCGTGACCACCTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATGA
AGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCT
TCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACC
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GCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGA
AGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAG
CTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGA

CAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATC

ACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGT

ACAAGTAA

Construct

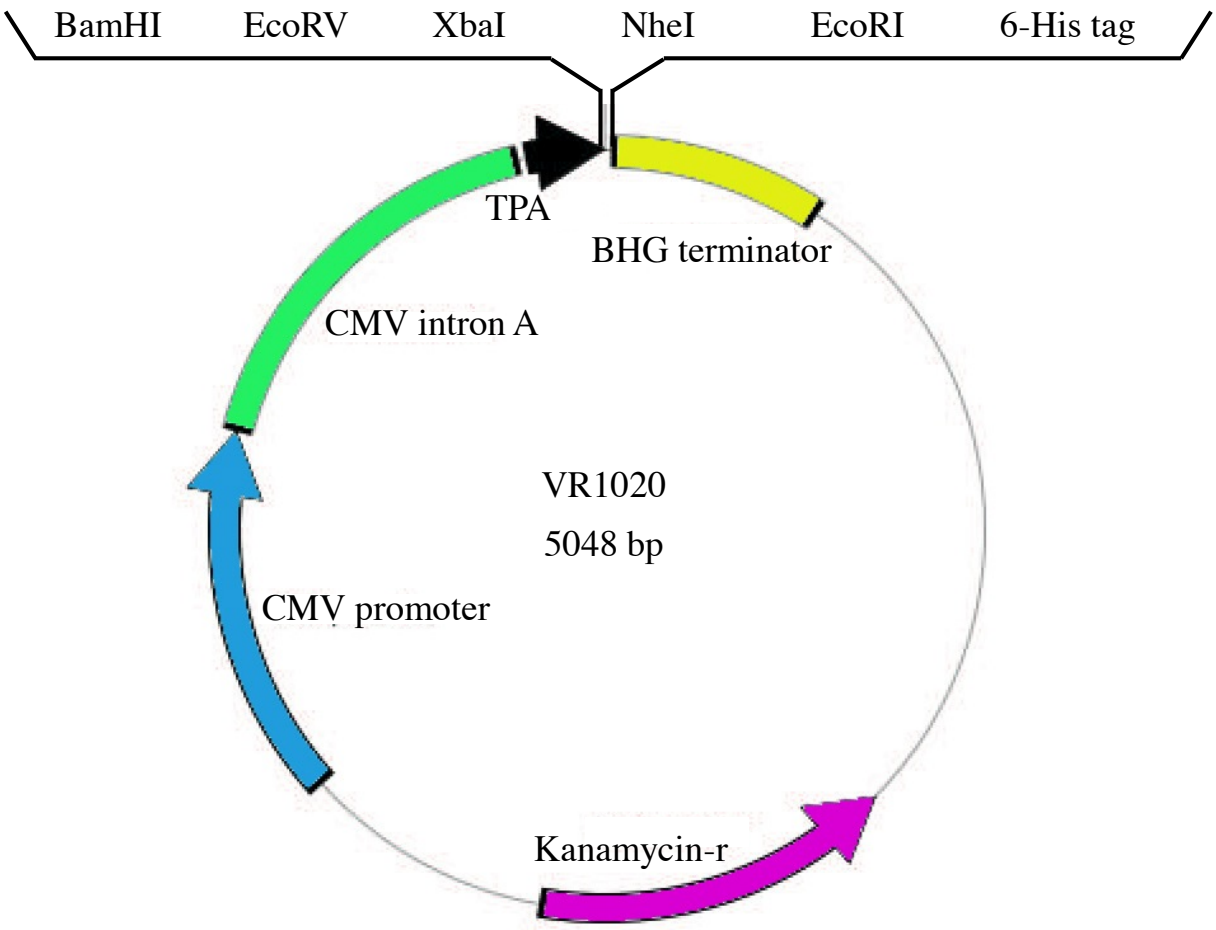

Figure S1: Schematic showing the modified VR1020 vector. Cloning sites for BamHI, EcoRV, XbaI, NheI and EcoRI and a hexahistidine tag were inserted between BamHI and BglII sites of the original VR1020 vector and YFP gene was inserted between BamI and EcoRI sites.

Melting Curves

Untreated YFP-pDNA

Melting temperature curves were run on a Cary UV-Vis with a 6 cell Peltier. The peak of the pDNA in each buffer was first determined before the melting temperature was run at a slit of 2 nm and average time of 0.2 s. Each sample was run in triplicates before conducting thermodynamic analysis.

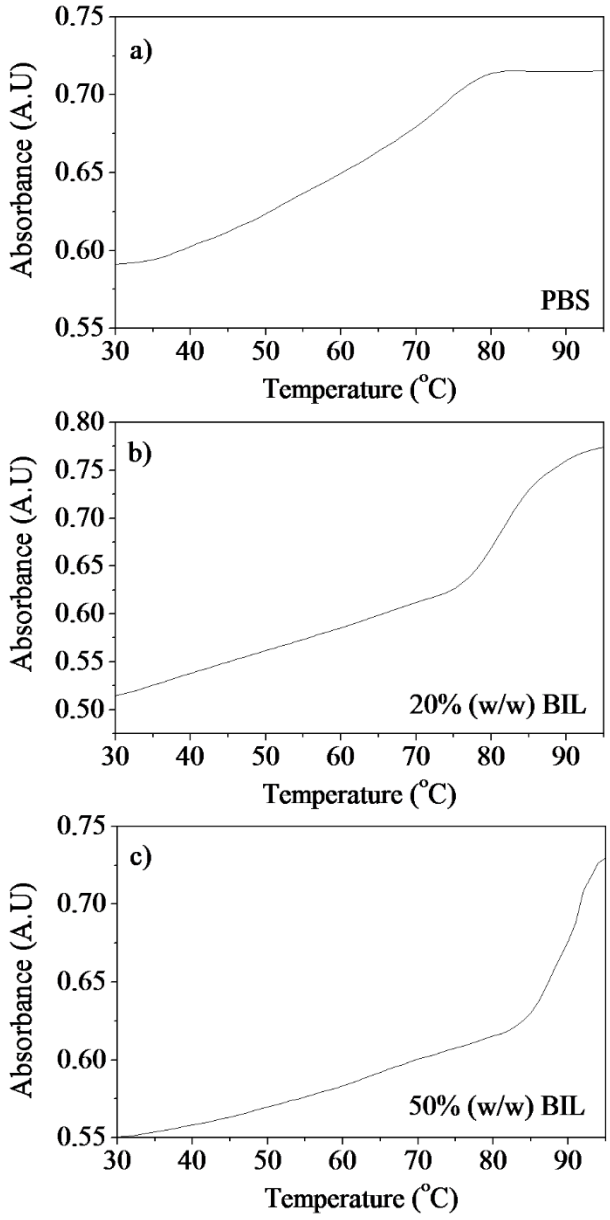

Figure S2: Melting Temperature profile of YFP-pDNA in (a) PBS, (b) 20% (w/w) bCDHP and (c) 50% (w/w) bCDHP.

TD treated YFP-pDNA

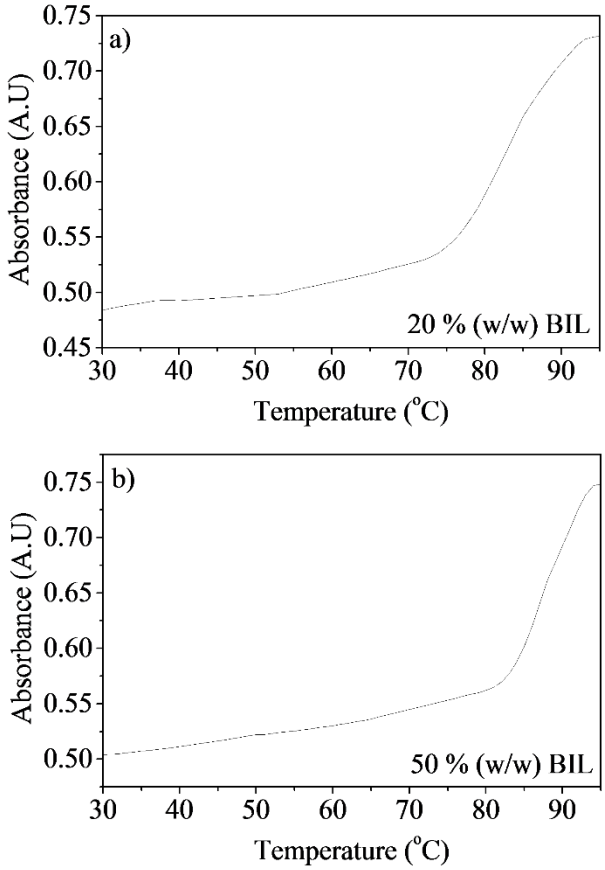

Figure S3: Melting Temperature profile of YFP-pDNA in (a) 20% (w/w) bCDHP and (b) 50% (w/w) bCDHP.

YFP Expression over 28 days

The YFP-DNA was treated for 10 minutes at 37°C and subsequently stored at room temperature for which the results can be seen below.

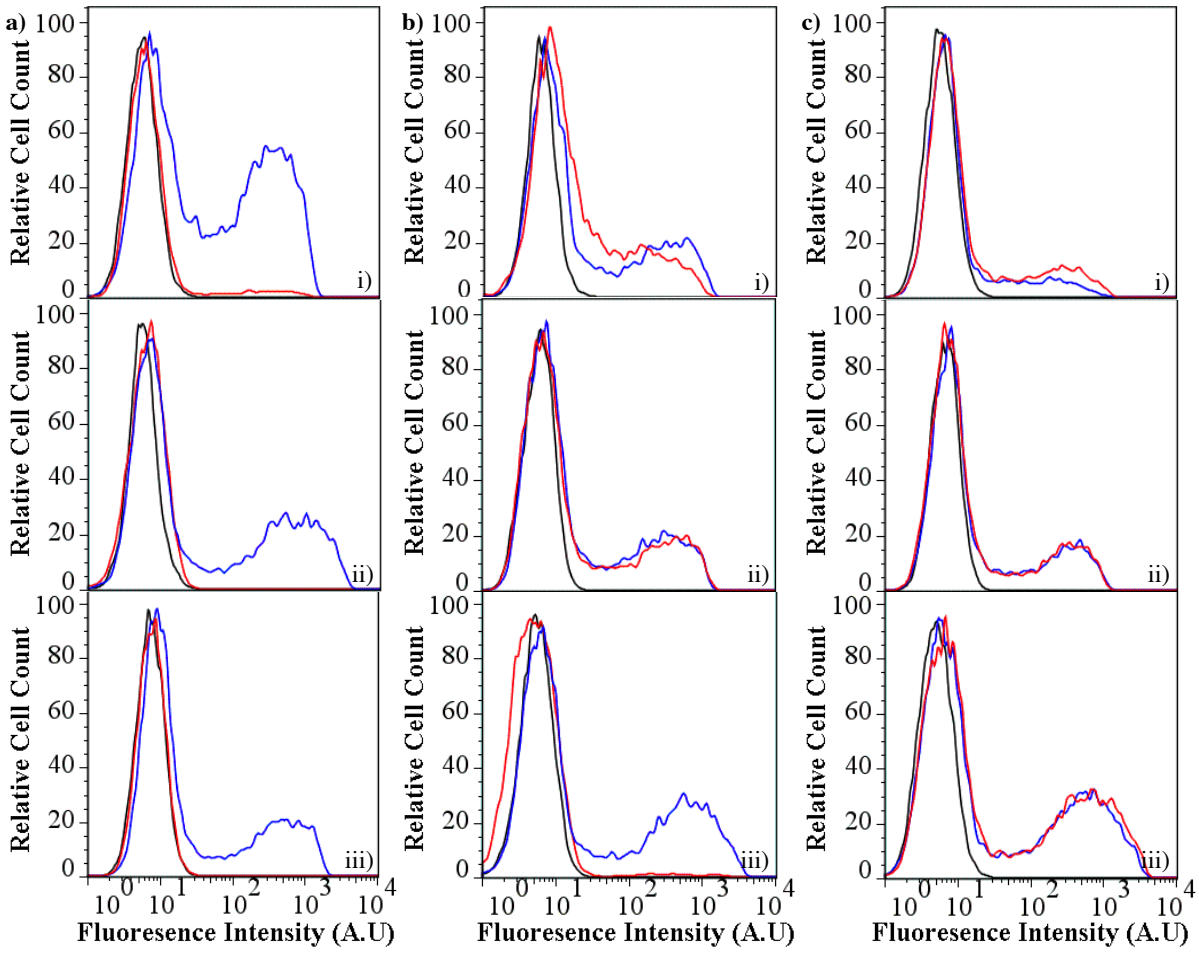

Figure S4: FACS analysis for transfections of aged YFP-pDNA in (a) PBS, (b) 20% (w/w) bCDHP and (c) 50% (w/w) bCDHP for i. day 0, ii. day 14 and iii. Day 28. The black line in the histogram represents the buffer, blue line represents YFP-pDNA and the red line represents YFP-pDNA treated with TD.

Flow cytometry transfection efficiency

This is a quantitative analysis of Figure 4. It can be seen that only untreated PBS can remain biologically active whereas YFP-pDNA in bCDHP remains active at 37°C for more than 7 days.

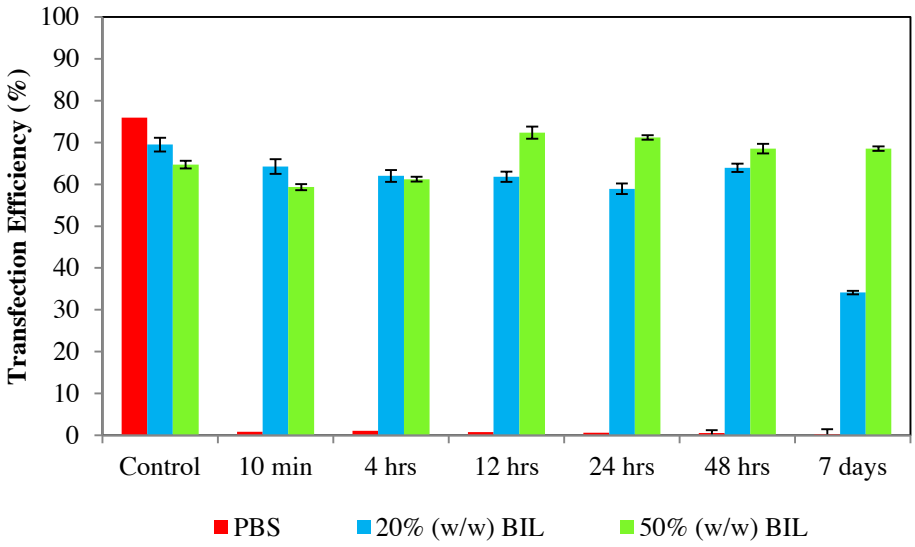

Figure S5: Transfection efficiencies calculated using the geometric mean of flow cytometry data. The times specified above are those for which the YFP-pDNA was incubated with TD at 37°C.

Cell Viability

The cell viability was measured using propidium iodide to distinguish dead cells from live cells and normalized against PBS treated cells.

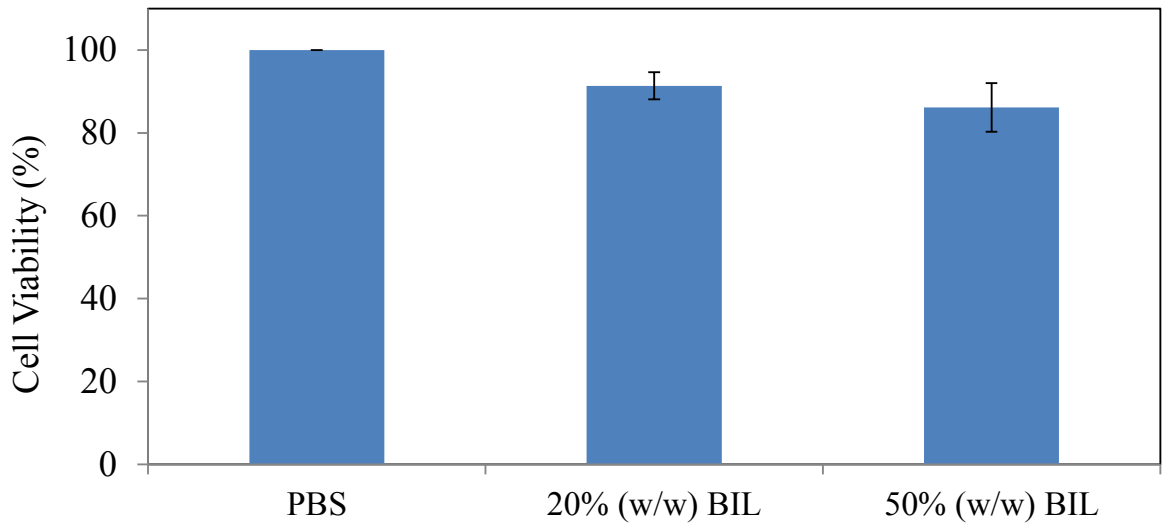

Figure S6: Effect of BILs media on the viability of cells, HEK 293T. Flow cytometry was used and the cells were stained with propidium iodide to identify dead cells from live cell populations for the cell viability quantification of cells treated 20% (w/w) bCDHP and 50% (w/w) bCDHP compared against PBS.

Confocal Microscopy

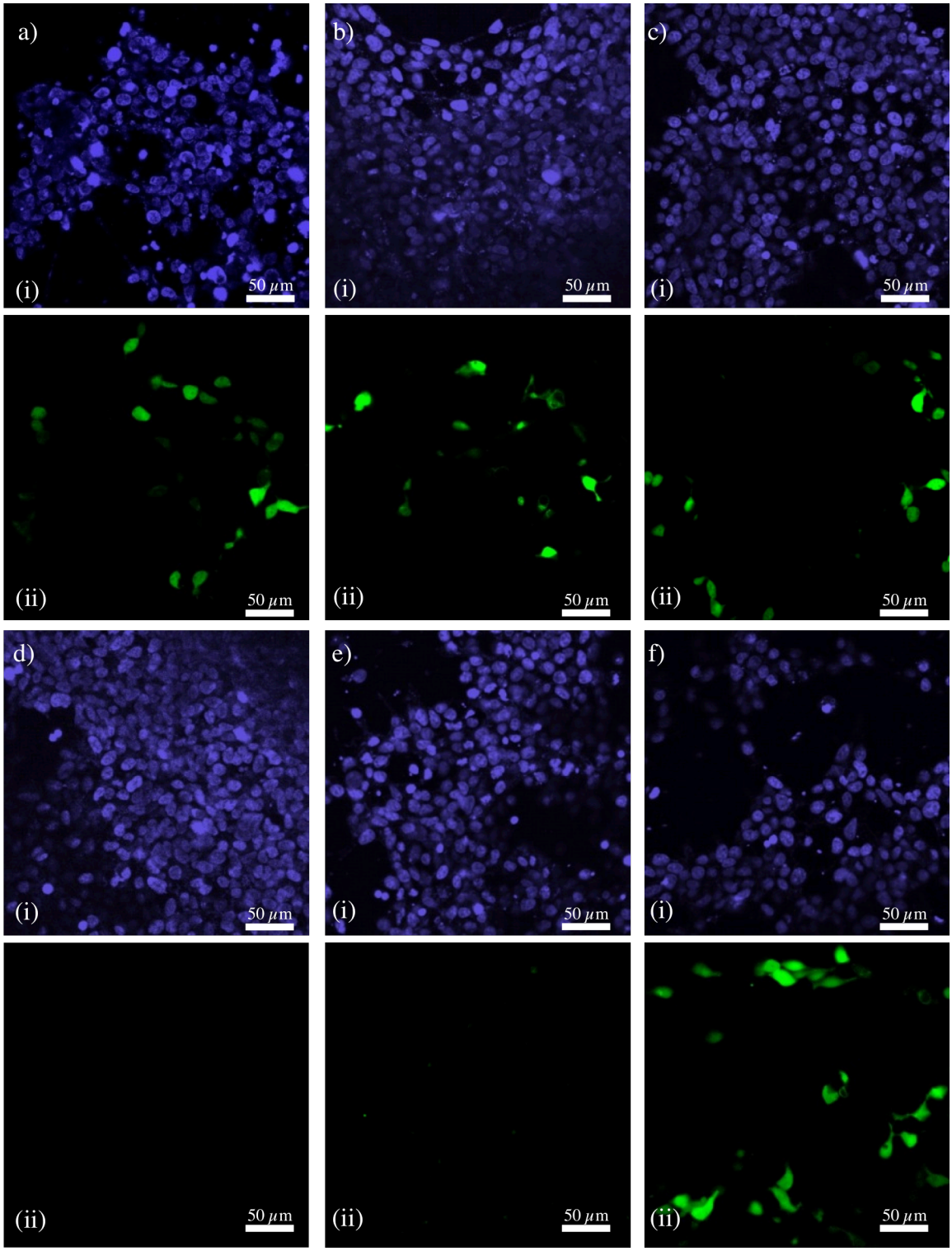

Figure S7: Confocal microscopy images for transfection at day 28: (a) YFP-pDNA in PBS (b) YFP-pDNA in 20% (w/w) bCDHP (c) YFP-pDNA in 50% (w/w) bCDHP (d) YFP-pDNA in PBS + DNase (e) YFP-pDNA in 20% (w/w) bCDHP + DNase (f) YFP-pDNA in 50% (w/w) bCDHP + DNase. For each image (i) Blue stain of nucleic of the HEK 293T cells present and (ii) Green represents YFP that have been successfully transfected and expressed in HEK 293T cells.