Supporting information for

Exploitable length correlations in peptide nanofibres

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Abbreviations: Fmoc: 9-fluorenylmethoxycarbonyl; HBTU: *O*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate; RP-HPLC: reversed phase high pressure liquid chromatography; MALDI-ToF: matrix-assisted laser desorption/ionization time of flight; MOPS: 3-(*N*-morpholino)-propanesulfonic acid; TIS: tri-isopropyl silane; TFA: trifluoroacetic acid.

Peptide synthesis: peptides were assembled on an automatic microwave peptide synthesizer, Liberty-1 (CEM Corp.), using Fmoc-Gln(Trt)-Wang resin and standard solid phase Fmoc/tBu protocols and HBTU/DIPEA as coupling reagents. Following synthesis completion the peptides were cleaved from the resin by TFA:TIS:H_2O – 95:2.5:2.5%. Peptides were purified by semi-preparative RP-HPLC on a JASCO HPLC system (model PU-980; Tokyo, Japan) and their purity and identity were confirmed by analytical HPLC and MALDI-ToF mass spectrometry (Bruker Daltonics) with 2,5-dihydroxybenzoic acid as a matrix. MS $[M+H]^+$: T2 – m/z 3067.7 (calc.), 3069.6 (found); T3 – m/z 4574.7 (calc.), 4573.3 (found); T4 – m/z 6084.2 (calc.), 6083.9 (found); T3(-) – m/z 4575.6 (calc.), 4575.7 (found).

High performance liquid chromatography: analytical and semi-preparative gradient HPLC was performed on a Jasco HPLC system using a Grace C8 analytical (5 μm, 4.6 mm i.d. x 250 mm) and semi-preparative (5 μm, 10 mm i.d. x 250 mm) columns with 10-70% gradient of B (A: 95% CH_3CN, 5% H_2O, 0.1% TFA; B: 95% CH_3CN, 5% H_2O, 0.1% TFA) over 30 minutes at 1 mL/min and 4.7 mL/min flow rates, respectively, with detection at 214, 240 and 280 nm.

Fibre assembly and visualisation by TEM: 300 µL samples (100 µM in each peptide) were incubated overnight in filtered (0.22 µm) 10 mM MOPS, pH 7.4, at 20°C. After incubation, a peptide aliquot (25 µL) was applied to a formvar/carbon coated copper/palladium support grids subjected to plasma glow discharge. After 5 minutes excess solution was blotted away, the sample was stained with 2% phosphotungstic acid and then examined using a Phillips BioTwin transmission electron microscope at the accelerating voltage of 80 kV.

Circular dichroism (CD) spectroscopy: CD spectra were obtained using a Chirascan Plus spectropolarimeter (Applied Photophysics Ltd.) equipped with a Peltier temperature controller. All measurements were taken in millidegrees for 100-µm peptide in a 260-190 nm wavelength range using 1 nm step, 1 nm bandwidth, 1 second time/point and 4 acquisitions in a quartz cuvette with 0.05 cm pathlength. After baseline subtraction and normalisation for the concentration of peptide bonds the spectra were converted to mean residue delta epsilon $\Delta\varepsilon$ (M^{-1} cm^{-1} res^{-1}). CD spectra at variable temperature were recorded every 1°C from 20 to 90°C with 180 seconds equilibration time for each spectrum. Thermal denaturation curves were obtained by plotting the $\Delta\varepsilon$ at 222 nm as a function of temperature, the first derivative of which provided *Tm* values.

Fourier-transform infrared (FT-IR) spectroscopy: All FTIR spectra were collected using a Tensor-37 series FTIR spectrophotometer with a BioATR II unit (Bruker Optics, UK) as the sampling platform with a photovoltaic MCT detector and a Bruker Optics workstation, which was equipped with OPUS software. Aqueous samples of very low volume (15 µl, 100 µM) were placed in a circular sampling area of radius 2 mm with a path length of 6 µm. This multi-reflection ATR accessory is based on a dual crystal technology, which has an upper silicon crystal and a hemispherical zinc selenide (ZnSe) lower crystal that does not come into contact with the sample. The temperature of the sample was maintained at 20°C by means of flow connectors to a circulating water bath. This accessory was purged continuously throughout the experiment with dry nitrogen via telescopic inserts that seals the optical path inside the spectrometer sample compartment. All FTIR spectra were collected between 4000 and 850 cm^{-1} with resolution 4 cm^{-1}, scanner velocity 20 kHz, 128 scans, phase resolution 32 and zero filling factor 4.

Linear dichroism (LD) spectroscopy: Solution-phase flow linear dichroism spectroscopy was performed on a Jasco-810 spectropolarimeter using a photo elastic modulator 1/2 wave plate. A micro-volume quartz Couette flow cell with 0.5 mm annular gap and quartz capillaries were used (all from Kromatec Ltd, UK). Molecular alignment was achieved by applying the constant laminar flow of the sample solution between two coaxial cylinders—a stationary quartz rod and a rotating cylindrical capillary which was obtained by maintaining the rotation speed at 3000 rpm. All the spectra were recorded in a 260-190 nm wavelength range using 1 nm step, 1 nm bandwidth, 50 nm/min scan speed, 4 second response and 3 acquisitions. The spectra were recorded for 100-µM peptide in 10 mM filtered (0.22 µm) MOPS buffer, pH 7.4 and processed by subtracting non-rotating

baseline spectra. The resulting spectra were normalised for the concentration of peptide bonds and converted to mean residue delta epsilon $\Delta\varepsilon$ (M^{-1} cm^{-1} res^{-1}).

Photon Correlation Spectroscopy: Assembled templates (100 μM) were analysed using a Zetasizer Nano (ZEN3600, Malvern Instruments Ltd, Worcestershire, UK). ζ-potential measurements were carried out in folded capillary cells at 25 °C, and ζ-potential values were obtained through the fitting of autocorrelation data using the manufacture's software, Zetasizer Software (version 7.03).

Gel electrophoresis: all experiments were performed using proprietary native Tris-Glycine gel (10%), running buffers and MW protein markers of 20, 66, 146, 242, 480, 720, 1048 and 1236 kDa (Invitrogen). Gels were run at 125 V constant.

Cell culture: Human dermal fibroblasts (Invitrogen, UK) were maintained in Medium 106 supplemented with low serum growth supplement (2% v/v) and antibiotics (10 μg/mL gentamicin; 0.25 μg/mL amphotericin B) in 25 cm^3 culture flasks. The cells were incubated at 37 °C, 5% CO_2 and 95% air humidity. At 70-80% confluency cells were washed with PBS to remove the unattached cells and then adhered cells were trypsinized (trypsin/EDTA 0.025:0.01%) followed by trypsin neutralizer (all from Invitrogen, UK). The harvested cells (of passages 3 to 5) were seeded for subsequent cellular analysis. For cytoskeletal visualization Nunc LabTek chambered cover glass slides were used as substrates. For cell viability and proliferation assays, cells were seeded at a given density (10 or 15 x 10^3) in the serum-free medium on substrates (sterile 96 well plates) coated with 50 μL of template or collagen (500 μg/mL). The medium was then switched to the medium (Medium 106) supplemented with low serum growth supplement (2% v/v) for proliferation after 24-h incubations.

Quantitative cell viability and proliferation assays: cell proliferation rates and viability were determined by PrestoBlue® and CyQUANT® assays on day 1 and 4 (all from Life Technologies, UK).

PrestoBlue® assay. PrestoBlue® reagent is supplied as a 10x solution and added to each well by diluting (1x) in the serum-free culture medium. The cells were incubated for 30 minutes at 37 °C in 200 μL of the reagent. The fluorescence of each well was measured with a microplate reader (BMG Labtech, Germany), with 544 nm excitation and 590 nm emission filters. A standard calibration curves were generated by plotting measured fluorescence values versus cell numbers. A cell dilution series (500-50000 cells) was done by seeding cells on sterile 96-well plates with overnight incubation before each given time point.

CyQUANT® assay. CyQUANT® GR dye was prepared in a cell-lysis buffer (according to the manufacturer's protocols) prior to each experiment by diluting this stock solution (400x) into the buffer. After days 1 and 4, the cells grown on the various substrates were washed gently in PBS and stored in a -70 °C freezer. At the same time, to generate calibration data, a cell pellet ($1x10^6$) prepared to provide a cell dilution series was also frozen. Cells grown on the substrates along with the cell pellet were thawed at 37 °C. CyQUANT GR dye in the lysis buffer was added (1 mL) to the pellet

and the lysate was re-suspended by brief vortexing. A cell dilution series (500-50000 cells) was created with the CyQUANT GR in cell lysis buffer in a final volume of 200 µL. A standard calibration curve was generated by plotting measured fluorescence values versus cell numbers. To quantify cell numbers grown on the substrates, the same volume of the reagent was added to each well. All of the samples were dark-incubated for 10 minutes at room temperature. The fluorescence of each well was measured with the microplate reader, with 485 nm excitation and 520 nm emission filters.

Cytoskeletal visualization: actin staining was performed using *Alexa-Fluor 488*® conjugated to phalloidin (Life Technologies, UK), following 1 and 4-day incubations, cells were rinsed with warm PBS (pH 7.4), fixed in 10% neutral buffered formalin solution (Sigma Aldrich, UK) for 15 min at room temperature, washed with PBS and permeabilised using 0.1% Triton- X 100 in PBS. Cells were then extensively washed in PBS and incubated for 30 min at room temperature with 10 µg/mL phalloidin in PBS. After post-stain washing with PBS, cells were mounted in ProLong Gold with 4',6-diamidino-2-henylindole (DAPI) (Life Technologies, UK) and imaged using an inverted confocal laser scanning microscope (CLSM) (FV-1000, Olympus).

Statistical analysis: statistical analysis for all the analytical data was performed by OriginPro 8.5 using ANOVA followed by a Fisher post-test for three independent experiments each done in triplicate for each test. Other multiple-means comparison tests with p values <0.05 considered significant, were also performed to allow comparison. The results are expressed as an average ± standard deviation.

Tables and Figures

Table S1. $\Delta\varepsilon_{222}/\Delta\varepsilon_{208}$ values, % helix and ζ-potential for the assembled templates used in the study.

Peptide	MOPS		50% TFE		ζ-potential, mV
	$\Delta\varepsilon_{222}/\Delta\varepsilon_{208}$	% α-helix*	$\Delta\varepsilon_{222}/\Delta\varepsilon_{208}$	% α-helix*	
T2	1.01 ± 0.004	96.8	0.86 ± 0.004	87.8	14.1 ± 0.4
T3	1.12 ± 0.004	92.1	0.92 ± 0.002	96.2	12.3 ± 0.5
T4	1.30 ± 0.002	88.7	0.94 ± 0.002	96.6	14.5 ± 0.7
T3(-)	1.07 ± 0.001	78.9	0.94 ± 0.002	93.8	-17.2 ± 1.5

*% α-helix calculated using the CDNN software from Applied Photophysics (*Böhm G, Muhr R, Jaenicke R. Protein Eng. 1992;5:191–195*).

Table S2 Position of Amide I and II bands by FT-IR spectroscopy.

Peptide	Amide I	Amide II (cm^{-1})
T2	1646 cm^{-1}, 1630 cm^{-1}*	1548 cm^{-1}, 1515 cm^{-1}*
T3	1647 cm^{-1}, 1630 cm^{-1}*	1548 cm^{-1}, 1515 cm^{-1}*
T4	1648 cm^{-1}, 1630 cm^{-1}*	1548 cm^{-1}, 1515 cm^{-1}*
T3(-)	1649 cm^{-1}	1548 cm^{-1}

*shoulder to the main band

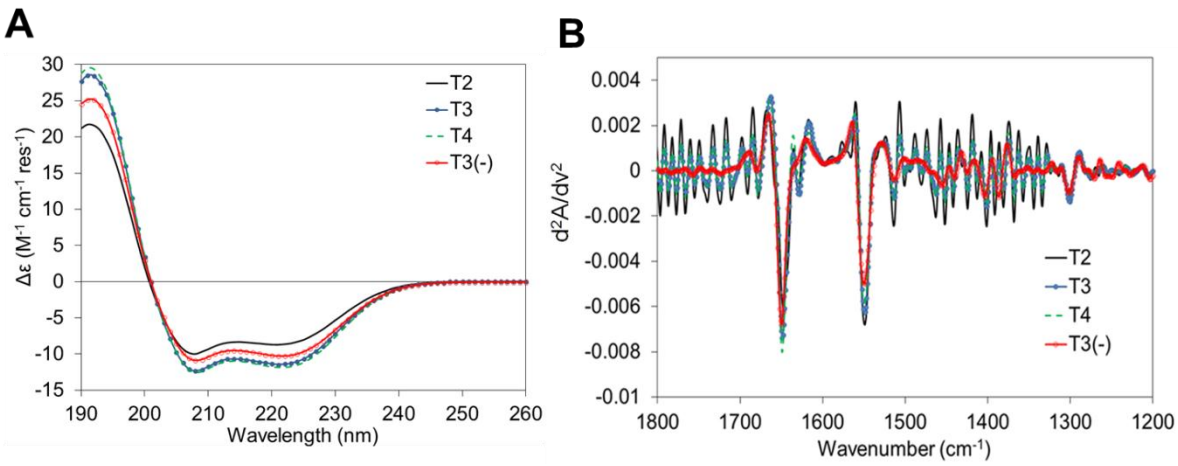

Figure S1. Template folding. (A) CD spectra in 50% TFE and (B) second derivative of FTIR spectra in 10 mM MOPS buffer at pH 7.4 for T2 (solid black line), T3 (closed circles blue line), T4 (dashed green line) and T3(-) (open circles red line).

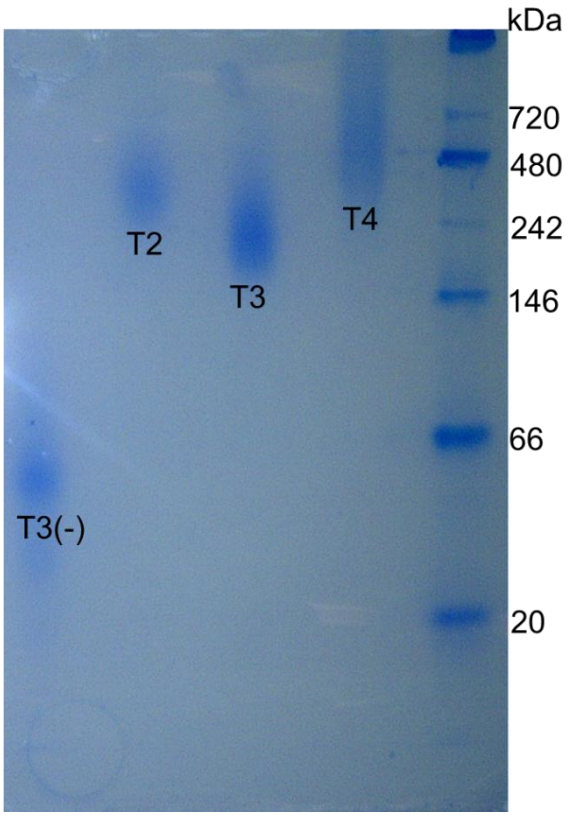

Figure S2. Native gel electrophoresis (Tris-Glycine 10%) of the templates as marked in the gel.

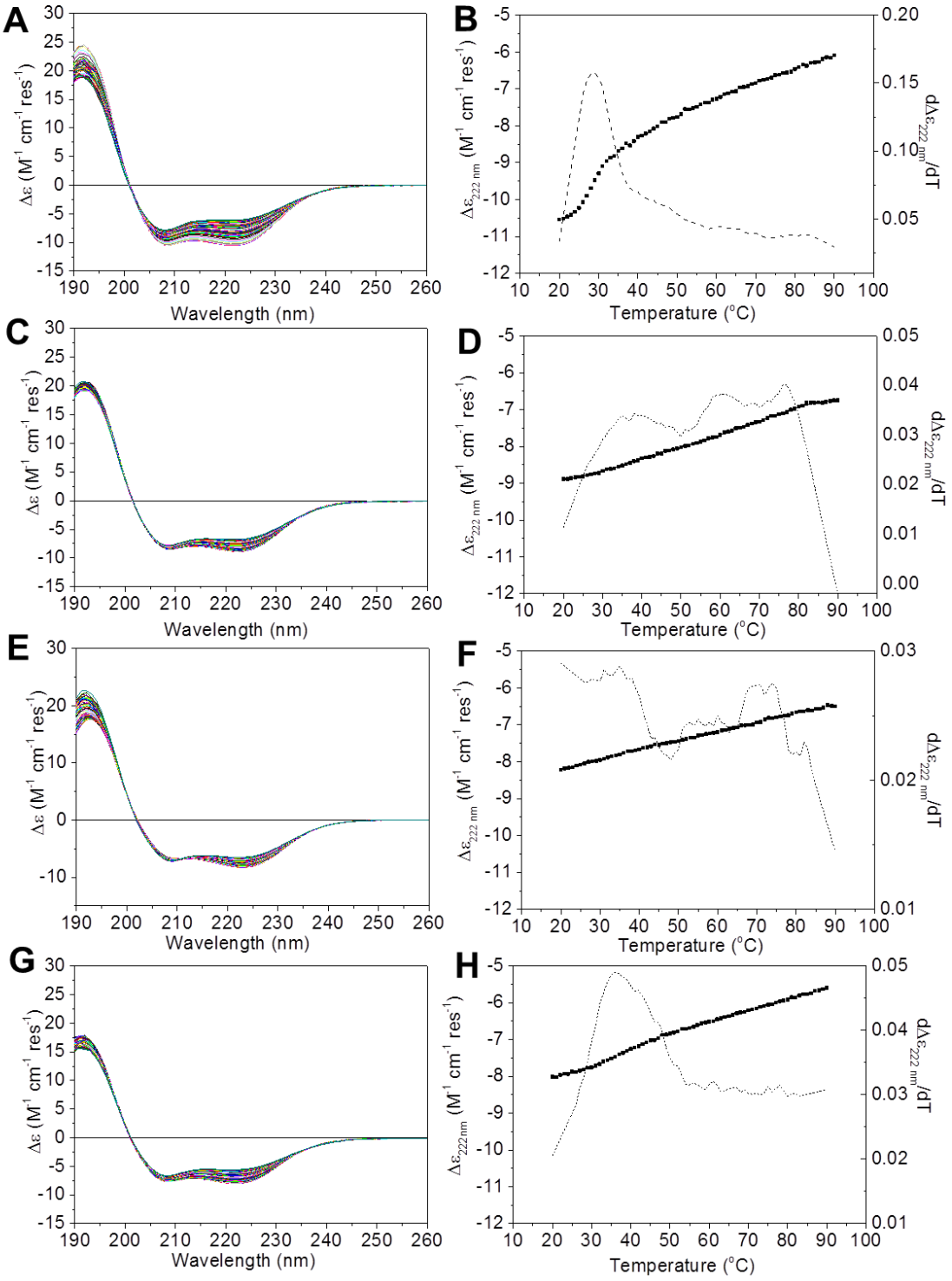

Figure S3. Template folding as a function of temperature. CD spectra (left) and thermal unfolding curves with their first derivatives (right) for (A, B) T2, (C, D) T3, (E, F) T4, (G, H) T3(-). Folding conditions: 10 mM MOPS, pH7.4, 100 µM peptide.

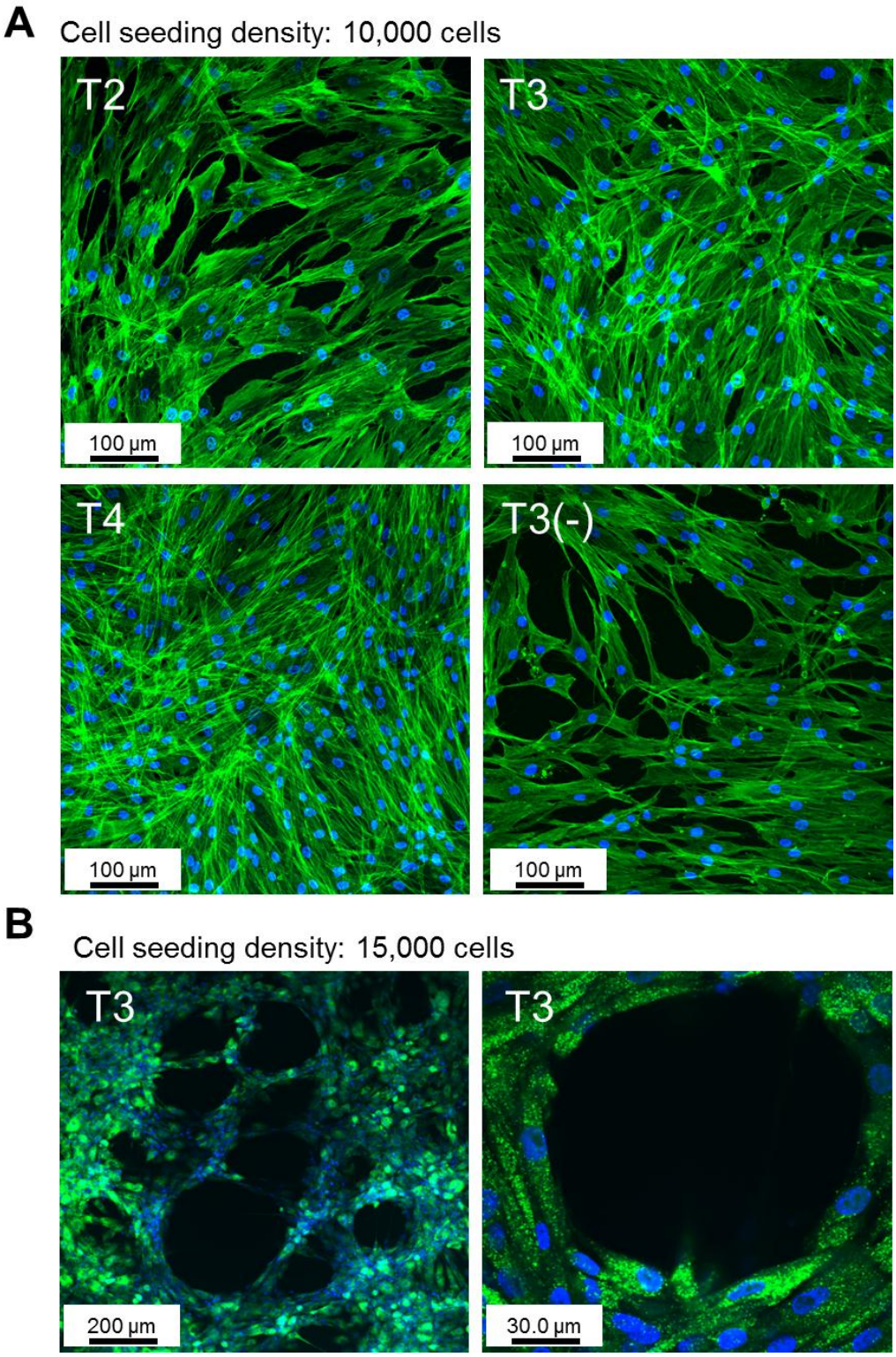

Figure S4. Fluorescence micrographs of human dermal fibroblasts after (A) 4-day and (B) 1-day incubations on the templates. Fluorescent stains Alexa-Fluor 488 phalloidin and 4′,6-diamidino-2-phenylindole highlight actin (green) and nuclear DNA (blue), respectively.