ADVANCED MATERIALS

Supporting Information

for *Adv. Mater.*, DOI: 10.1002/adma.201905577

Hierarchical Ordered Assembly of Genetically Modifiable Viruses into Nanoridge-in-Microridge Structures

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Experiment section

Phage amplification and purification: The *E. coli* K12 ER2738 (NEB) strain and its engineered strain were used to produce both WT-phage and RGD-phage, respectively. The bacteria were incubated at 37°C with overnight shaking. The M13KO7 helper phage (NEB) was added into the overnight bacteria solution and incubated for 1 h. Then the mixture of phage and bacteria was transferred into a small flask and shaken for 3 h to allow phage to infect bacteria. Chloramphenicol antibiotics (50 μg ml^{-1}) was used to select the RGD-phage in this step. The product was subsequently transferred into 1 L LB broth and shaken for 24 h at 37°C. Kanamycin (70 μg ml^{-1}) was used to select the phage infected bacteria and IPTG was used to induce RGD phagemid expression. The resultant RGD-phage or WT-phage was separated from bacteria by centrifugation. The PEG-NaCl solution (16.7% PEG/3.3 M NaCl) was added to the supernatant to induce phage precipitation at 4°C overnight. Water was added to resuspend the precipitated phages. The above precipitation and suspension steps were repeated one more time for phage purification. The final phage solution was dialyzed against ddH_2O overnight for further use. A nanophotometer was used to detect the phage concentration.

Phage film fabrication by dip-pulling: The polylysine coated glass slides or coverslips were dipped into dialyzed phage solution and pulled from the solution at different speeds, which was controlled by a syringe pump (Stoelting). In evaporation change experiment, the normal

evaporation rate was under room temperature and the fast evaporation rate was applied in the chemical fume hood. Salt concentrations of the phage solutions were adjusted by adding NaCl solution. The pH values of the phage solutions were controlled by using buffers of different pH values (Table S1). The surface structures of the resultant phage films were confirmed by an Atomic Force Microscope (Bruker) and Optical Microscope (Nikon).

Polylysine treatment on different substrates: Flat substrates of gold, titanium and stainless steel were dipped in polylysine solution (0.01%) for 10 min at room temperature (RT), whereas polycarbonate plastic slide and silicon were incubated in 0.01% polylysine solution overnight at RT to form polylysine coatings. Phage film fabrication steps were the same as glass slides.

Cell culture: hiPSCs (ALSTEM, Inc.) were first cultured in mTeSR (Stemcell Tech.) to maintain their pluripotency. After 5-7 days, they were split into single cells and seeded on Matrigel (Corning)-coated plates for NPC differentiation. They were differentiated into NPCs according to the company's (Stemcell Tech) monolayer culture protocol. The STEMdiff™ Neural Induction Medium (Stemcell Tech) was used to differentiate hiPSCs into NPCs, which were then maintained in the STEMdiff™ Neural Progenitor Medium (Stemcell Tech).

Cell culture on phage films: Phage films were exposed to UV light for 4-6 h for sterilization. The sterilized phage films were pre-treated with laminin at 37°C for 1 h to form laminin coating. The hiPSCs-derived NPCs were seeded on the laminin coated-phage films and cultured in the STEMdiff™ Neural Progenitor medium. The medium was changed on the daily basis.

RT-qPCR: The NPCs on the phage films were harvested at different time points (10 coverslips for each group). They were digested by accutase and then resuspended in cold PBS. Cells (10^5 cells) were counted as a separate bio-repeat. The Power SYBR Green Cells-to-Ct Kit (Thermofisher) was used to perform the RT-qPCR. 3-4 bio-repeats were used at different time points for cells cultured on the WT-phage films, RGD-phage films and control substrate (no phage).

Immunofluorescent staining and intensity analysis: Paraformaldehyde (4%) was used to fix the cells for 20 min at RT after PBS washing (3 times) steps. The cells were treated with Triton X-100 (0.3%) at RT for 5 min to allow for the penetration of the cell membrane. After penetration, BSA (10%) was used for blocking for 1 h at RT. Then, the cells were incubated with the primary antibody specific for nestin (1:200), MAP2 (1:500), GFAP (1:5000) (Abcam) and βIII-Tubulin (1:500) (Thermofisher) overnight in BSA (3%) solution. The secondary antibodies (Goat Anti-Mouse IgG H&L, Goat Anti-Chicken IgY H&L, Goat Anti-Rabbit IgG H&L*)* were labeled with Alexa Fluor® 488 and Alexa Fluor® 555 (Abcam). DAPI was incubated with the cells to stain the nucleus. The stained cells were fluorescently imaged. The average immunofluorescent intensity of the cells was analyzed by Image J, which was based on randomly selected ten cells in about 5 images.

Statistical analysis: One-way ANOVA was adopted to perform statistical analysis. Different substrate groups were compared with a p value less than 0.05, 0.01, 0.001, or 0.0001 considered significantly different.

Table S1. Buffers used for achieving different pH values.

pH value	Buffer
5.0	HAc-NaAc
6.0	Na_2HPO_3-NaH_2PO_3
9.0	Na_2CO_3-$NaHCO_3$
10.0	Na_2CO_3-$NaHCO_3$
11.0	Na_2CO_3

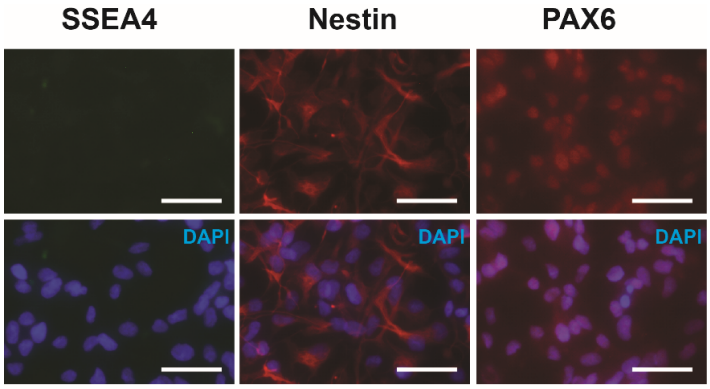

Figure S1. Confirmation of conversion of hiPSCs into NPCs. After 21 days of culture in Neural induction medium (Stemcell Tech.), hiPSCs were successfully differentiated into NPCs. SSEA4 is a pluripotent marker, and Nestin and PAX6 are NPC markers. Scale bar: 50 µm.

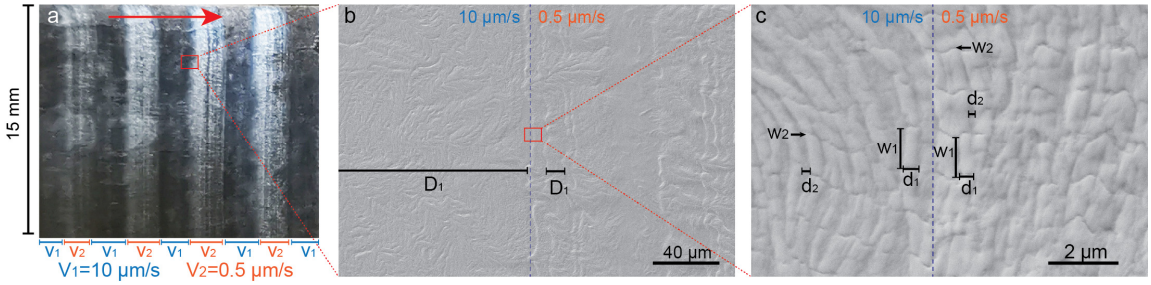

Figure S2. Optical and SEM image of RGD-phage film formation under periodic pulling speeds. (a) Optical image of the film formed when a polylysine treated glass slide was vertically pulled out of the phage solution under periodic pulling speeds (10 µm/s and 0.5 µm/s). (b) SEM image of the highlighted area in the rectangle in (a). (c) SEM image of the highlighted area in the rectangle in (b). Both (a) and (b) showed that the width of the microridges (D_1) and nanogrooves (d_2) increased with the speed increase while other size parameters were nearly unaffected by the speed changes. It should be noted that in some areas of the low speed side, d_2 (the nanogroove size) became very small, making two neighboring nanoridges seem to be nearly linked. Blue lines indicated the speed boundaries. Red arrow indicated the pulling direction. (Phage concentration, 1 x 10^{14} pfu/ml)

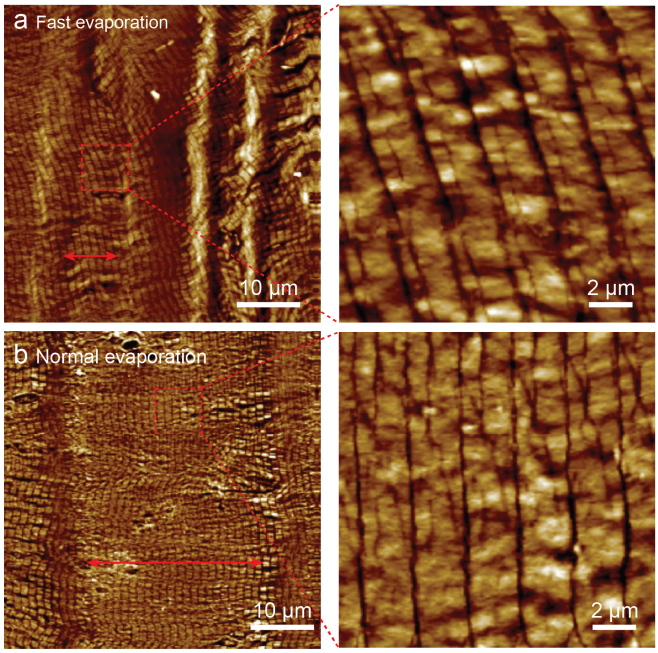

Figure S3. Surface morphologies of phage films assembled form wild-type phages at different evaporation rates by the dip-pulling method. (a) Phage film structure formed under the fast evaporation rate in a chemical fume hood. (b) Phage film structure formed under the normal room condition. Red arrows indicated the microridge width D_1. (Phage concentration, 1 x 10^{14} pfu/ml; pulling speed, 1.5 μm/s).

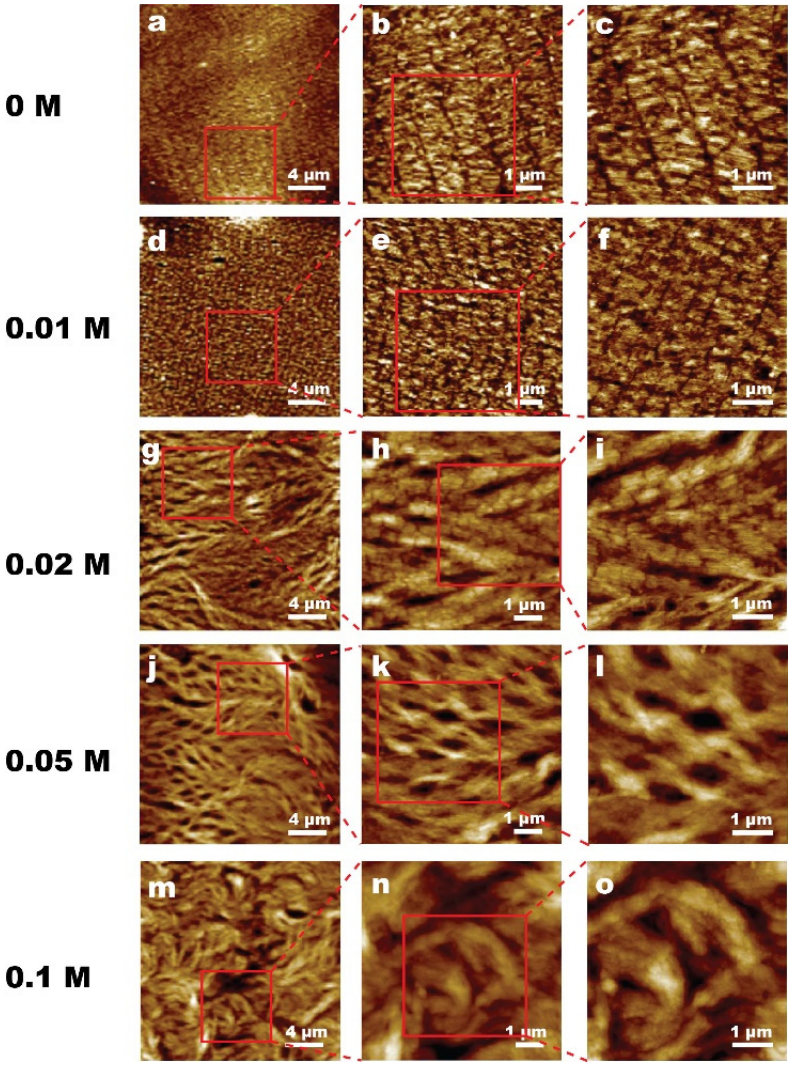

Figure S4. Self-assembly of wild-type phages at different salt (NaCl) concentrations into films by the dip-pulling method. The salt concentrations were found to tremendously influence the structure of the phage assemblies. When the salt concentration was higher than 0.01 M, the phages were assembled into a cross-linking pattern instead of the parallel-aligned nanoridge pattern. (Phage concentration, $7x10^{13}$ pfu/ml; pulling speed, 1.5 μm/s).

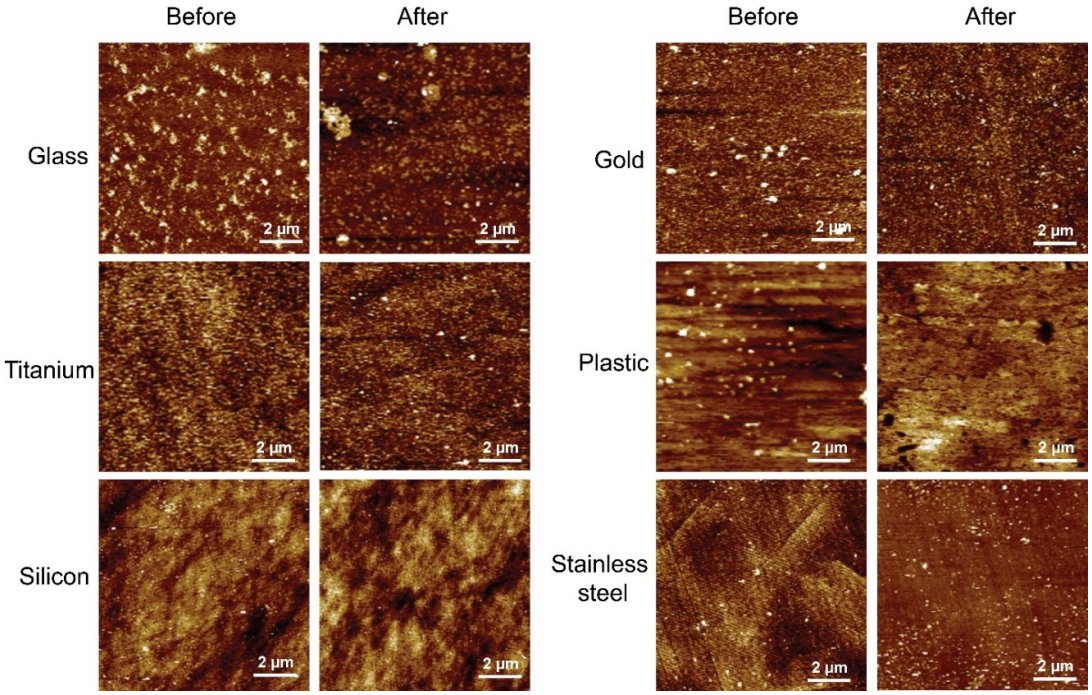

Figure S5. AFM imaging of a variety of substrates before and after polylysine treatment. Different substrates were coated with 0.01% polylysine before the formation of the phage films. None of the pre-treated substrates showed the NiM structure.

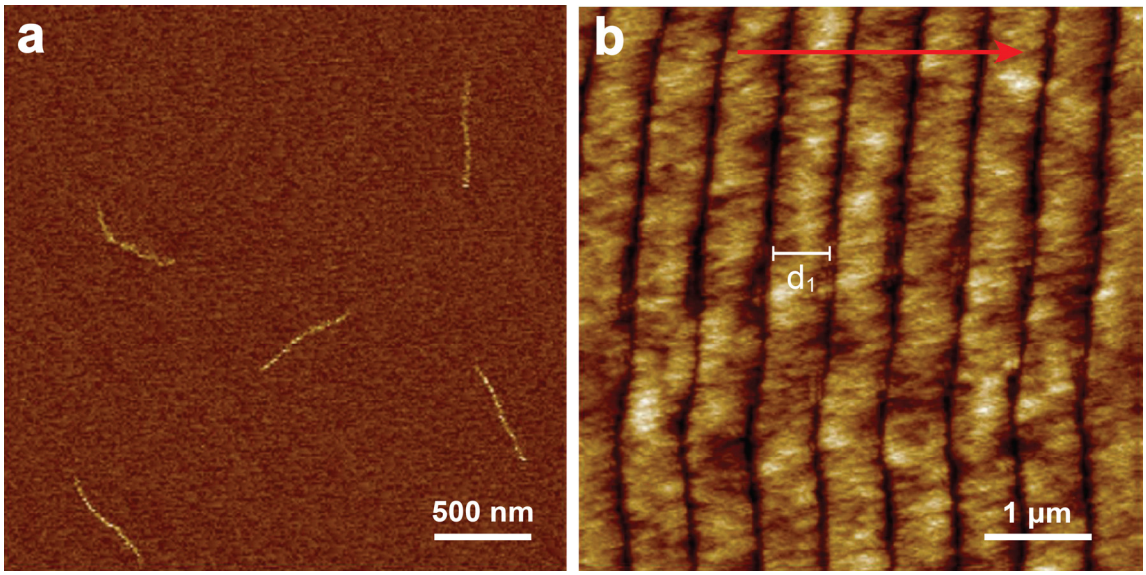

Figure S6. AFM imaging of individual RGD-phage nanofibers and the films assembled from them by the dip-pulling method (Phage concentration, 1 x 10^{14} pfu/ml; pulling speed, 1.5 µm/s.). An RGD-engineered phage is about 550 nm in length. The size d_1 (along the pulling direction, highlighted as a red arrow) of the parallel-aligned nanoridges of the RGD-phage film is equal to the length of the RGD-phage.

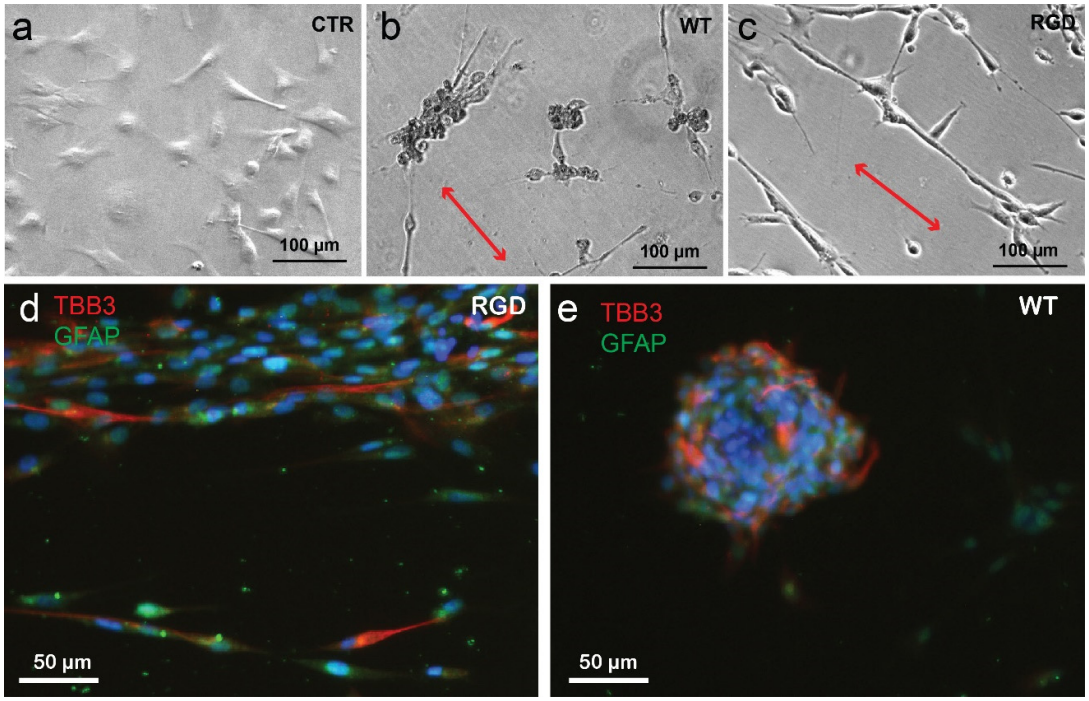

Figure S7. Morphologies and bidirectional differentiation of the cells on the different phage substrates formed under the same conditions (Phage concentration, 1 x 10^{14} pfu/ml; pulling speed, 1.5 μm/s.). HiPSCs-derived NPCs were seeded onto different substrates, including WT-phage films, RGD-phage films and bare control substrate (CTR, the polylysine-coated glass slides) without phages. (a-c) Optical images of the cells on different substrates on Day 5. Compared to the control group (a), the cells grew on the phage films (b-c) showed different morphologies on Day 5. The NPCs were grown into clusters on WT-phage films (b), but were aligned parallel to the microridge length direction on the RGD-phage films (c). Red arrows denoted the microridge length direction. (d-e) The NPCs on RGD-phage film (d) and WT-phage film (e) were bidirectionally differentiated into neurons and astrocytes by phage films only on Day 8. TBB3 is a neuron marker (red) and GFAP is an astrocyte marker (green).

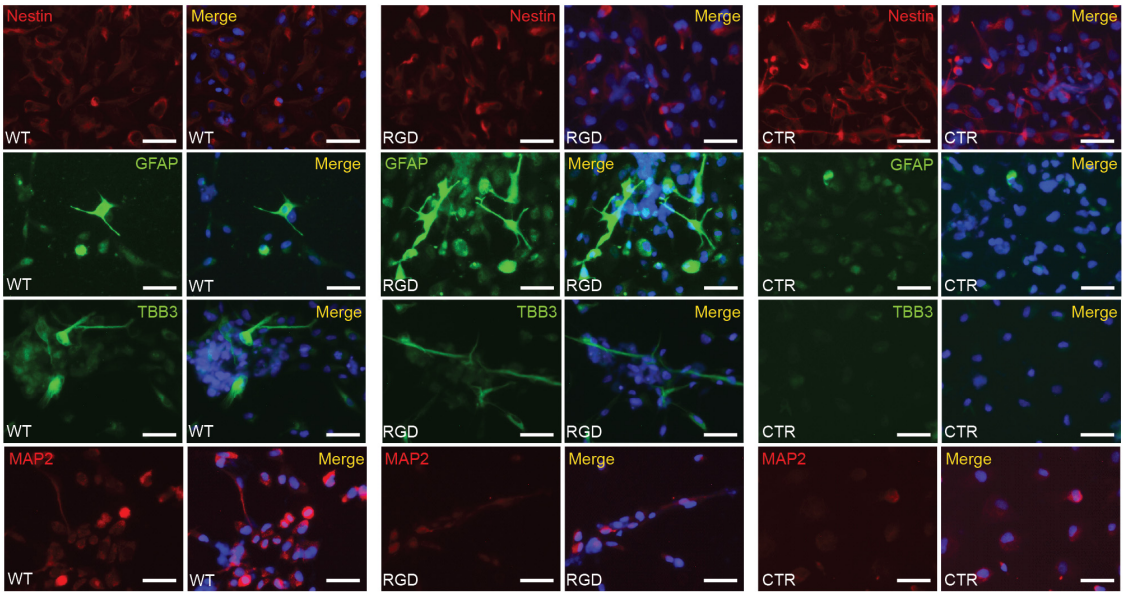

Figure S8. Immunofluorescent images of hiPSC-derived NPCs on different substrates on Day 8. Cells on the WT-phage films had a higher expression level of TBB3 and MAP2 marker, while those on the RGD-phage films showed a higher expression level of GFAP marker. The CTR showed a higher expression level of Nestin marker. Compared to the CTR (without phage) group, the NPCs showed a neural differentiation tendency on the phage films with the WT-phage and RGD-phage film presenting a better induction capability towards neurons and astrocytes, respectively. Scale bar, 50 μm.