Supplementary information:-

1. Preparation of substrates:-

SEBS(KRATON 1726-G)was solvent processed with chloroform as solvent to form thin stretchable films of SEBS. Briefly, SEBS was dissolved in chloroform by sonication and stirring for around 4-5 hours till a translucent solution was obtained. This solution was carefully poured in a flat bottom glass petri-dish to allow the solvent to evaporate which left behind a thin film of SEBS. Subsequently, the film was peeled off and 1 ×1.2 cm rectangular substrates were cut and plasma treated for 2 minutes, at 0.5 bar pressure and 0.08 A current. This reduced the hydrophobicity of the substrates which prevented the delamination of PEDOT:PSS films when incubated in the cell culture media.

The aqueous dispersion of PEDOT:PSS (Agfa, Orgacon Printing Ink EL-P3040) was spin coated on SEBS films at 2500 RPM, 60 s to obtain the films of thickness ~ 90 nm and they were post annealed at 65 ^{0}C for 12 hrs.. These substrates were further heat treated in vacuum at around 60^{o} c for 2 hours to prepare PEDOT:PSS coated substrates (CP substrates.

The setup for straining the PEDOT:PSS coated SEBS substrates was a homebuilt setup with a calibrated screw gauge of least count (L.C.) ~ 32μm (fig. 2.6). Substrates were strained by clamping at the two ends and were uniaxially strained to different strain regimes of 10%, 20%, 30% and 5 cycles of 30% strain. The strained conducting substrates were maintained in stretched condition by wedging them cleanly to a glass slide of the same dimension using araldite. The glass slide was previously cleaned to remove any impurity by sonicating in 1:1:1 (volume ratio) solution of isopropyl alcohol (IPA), acetone, and chloroform for 10 min. Glass slides were then

boiled for 3 min in trichloroethylene (TCE) and IPA separately, followed by rinsing in deionized water and blow drying.

The pristine SEBS substrates were prepared in the similar manner following the similar procedure barring the coating with conducting polymer PEDOT:PSS. The stretched cycles substrate was left with a residual strain without wedging it to a glass slide.

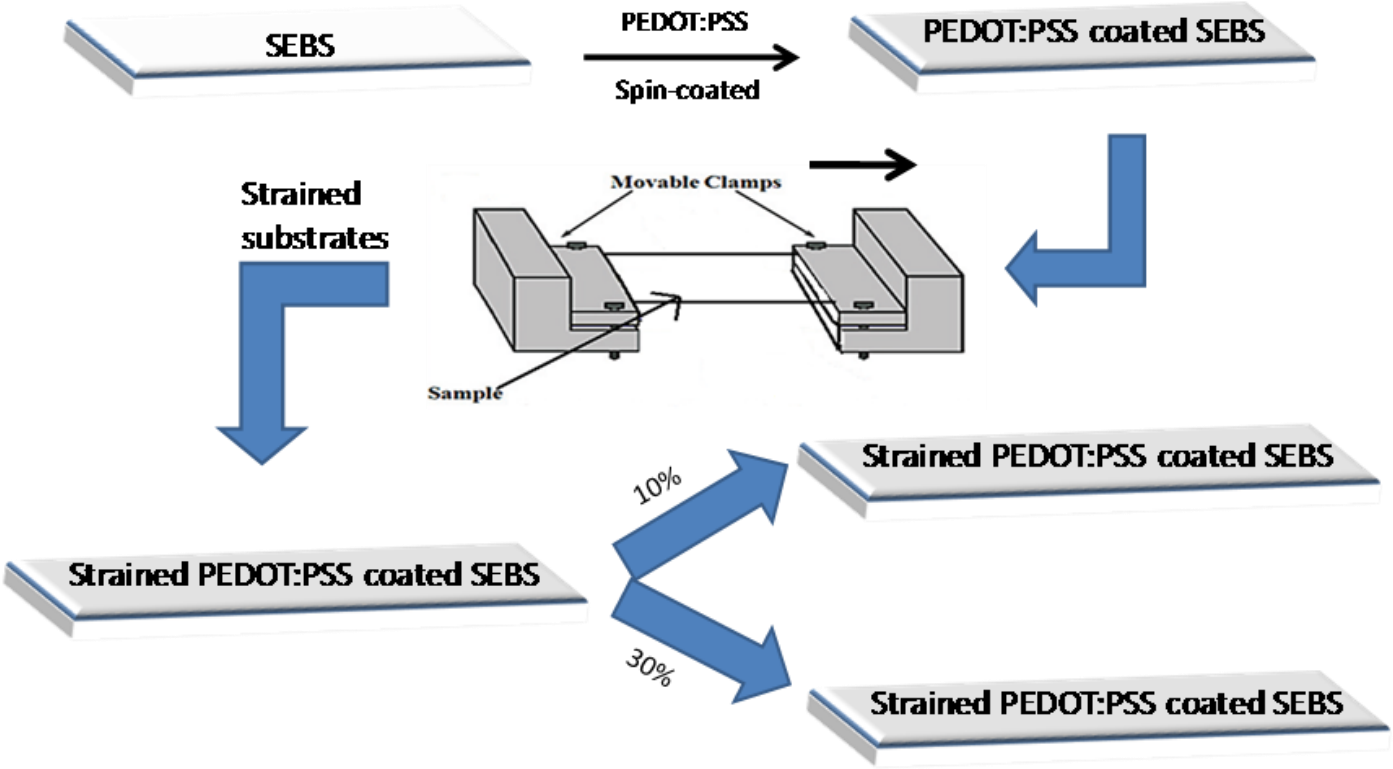


Fig. S1. Schematic of substrate architecture and preparation. Pristine SEBS and PEDOT:PSS coated SEBS substrate were stretched using the straining set-up to obtain different substrates.

2. Embryonic stem cell culture:-

Mouse D3 ES cells (ES-D3; ATCC#CRL-11632) were grown on Mitomycin-C treated primary mouse embryonic fibroblast (MEF) feeder layer on 0.1% gelatin substrate in embryonic stem cell growth medium [ESGM - consisting of DMEM, 10% defined FBS, 10% NCS, 2mM L-Glutamine, 1X Nucleoside, 0.1mM β-mercaptoethanol and 1000U/ml LIF]. Briefly, culture flasks were incubated with 0.1% gelatin solution for 30 minutes at room temperature and washed with 1X PBS. Frozen vials containing ES cells were thawed rapidly in 37^0C water bath and washed twice with 1X PBS. Finally, cells were re-suspended in ESGM and incubated at 37^0C in 5% CO_2 incubator for 1-2 days. ES cells grown on MEF feeder layer were used for making stocks while for regular experiments, feeder free ES culture protocol was used. Proliferating ES cells appeared as spherical colonies with intact boundary and individual cells were seen separately. ES cells generally do not grow well in low numbers hence appropriate number of cells should be cultured in culture dishes and should not be splitted more than 1:10 ratio.

2.1. Preparation of MEF feeder layer :-

ES cells are usually grown on a layer of mitotically inactivated primary mouse embryonic fibroblasts (MEF) to promote growth and prevent differentiation. Since these cells stop dividing after a couple of passages, embryonic fibroblasts need to be isolated freshly, each time from E13 embryos. For MEF isolation, pregnant mouse was sacrificed by cervical dislocation and E13.5 embryos were transferred into 1X PBS in a Petri-dish. Embryos were washed in 1X PBS and their head, heart, viscera and liver were removed. Decapitated embryos were transferred to 0.25% Trypsin/EDTA solution and minced finely with curved scissors. The minced tissue was incubated at 37^0C for 10-15 minutes and the trypsin was inactivated by adding 100-200 µl FBS.

Subsequently, it was transferred to a conical tube and allowed to settle down and the supernatant was removed and washed with DMEM. The tube was centrifuged for 10 minutes at 1000 rpm and the pellet was re-suspended in the MEF medium. Cells were allowed to grow for 24-48 hours and were subsequently frozen. For Mitomycin-C treatment, confluent primary MEF cells were plated on gelatin coated culture flask and incubated at 37^0C overnight in MEF medium.10 µg/ml Mitomycin(dissolved in MEF medium) was added to recover the cells and incubated for 3 hours, the cells were then washed with MEF medium thrice after removing the Mitomycin containing medium completely. Finally, MEF medium was replaced with ESGM and the plate was incubated until ES cells were seeded.

2.2. ES cell passaging:-

~70-80% confluent ES cells were washed with 1X PBS and trypsinized using 1ml of 0.05% Trypsin-EDTA for 3-5 minutes at 37^0C. Trypsin was inactivated by adding 100µl of serum and the cells were washed twice with 2ml 1X PBS and pelleted down by centrifugation at 1800 rpm for 5 minutes. Pelleted cells were re-suspended in 4ml fresh ESGM and ~$1x10^6$ cells were plated on inactivated MEF/gelatin coated T-25 flask.

2.3. ES cell freezing:-

One confluent T-25 flask was trypsinized and washed in 1X PBS. After washing, ~$2x10^6$cells /vial were re-suspended in freezing medium (ESGM with 10% DMSO). The tubes were then transferred to cryo-baby and placed at -70^0C for overnight (for cooling the cells at the rate of 1^0C/min) and the cells were transferred to liquid nitrogen next day.

2.4. Embryoid body (EB) generation and RA induction:-

Proliferating ES cells were trypsinized and plated on uncoated plates in EB medium (ESGM without LIF and β-mercaptoethanol) for four days followed by 0.5μM RAfor an additional 4 days to promote the neuronal induction in the EB.RA acts as a morphogen and induces neuronal lineage since activation of RA receptors in EB leads to the development of neuronal subtypes.

2.5. ES cell derived neural progenitors (ES-NP) generation:-

ES-NPs were generated from ES cells by modifying previously described protocol [199]. RA-induced EBs was partially differentiated on poly-D-Lysine (150μg/ml) and laminin (1μg/ml) substrates for two days in neuron differentiation medium (DMEM/F12 supplemented with 1% N2 supplement, 0.5% FBS, heparin (2μg/ml) and FGF2 (10ng/ml). Partially differentiated EBs were further trypsinized and plated on uncoated 6-well plates (~$1.5x10^6$ cells/well) in ES-NP proliferation medium consisting of DMEM/F12 supplemented with 1% N2 supplement, Heparin (2μg/ml) and combinations of FGF2 (20ng/ml) or EGF (10ng/ml).

3. Substrate preparation for cell culture:-

Polymeric substrates (conducting and non-conducting) were prepared as described earlier. In addition to these substrates, controls were used in the experiments which consisted of glass coverslips and PEDOT:PSS coated glass coverslips. All the substrates were first washed with ethanol and then irradiated with UV for 2 hours. These substrates were dried and incubated overnight with penstrep solution to prevent any contamination. Substrates were re-washed with 1x PBS next day and subsequently coated with poly-D-lysine (150μg/ml) for 4-6 hrs. The substrates were again washed with 1x PBS and coated with laminin (1μg/ml) which was diluted

in DMEM/F12 medium for 1 hr at 37^{o}c. Substrates were washed twice with 1x PBS before seeding ES-NPs on them.

4. ES-NP differentiation on polymeric substrates

ES-NPs were differentiated onpoly-D-lysine (150µg/ml) and laminin (1µg/ml) coated polymeric substrates.The differentiation medium consisted of DMEM/F12 supplemented with 1% N2 supplement, FGF2 (10ng/ml) or EGF (10ng/ml), Heparin (2µg/ml) and 1.0% FBS. Heparin was added in medium in combination with FGF2 only. Differentiation procedure was carried out for 6-8 days post which cells were fixed for immunofluorescence analysis and processed with DAPI, β-III tubulin, GFAP or phalloidin.

5. Immunofluorescence analysis

Immunoflourescence analysis was carried out for detection of cell specific markers. Briefly, 4% paraformaldehyde-fixed cells were blocked in 5% NGS (Sigma-Aldrich) and permeabilized with 0.2–0.4% Triton-X 100 followed by an overnight incubation with primary antibodies at 4°C (β-III tubulin, 1:200, GFAP 1:400).For visualizing actin cytoskeleton, cells were incubated with FITC conjugated phalloidin(1:750).Cells were examined for fluorescence following incubation with appropriate secondary antibody conjugated to FITC (1:400), cy3 (1:400) and DAPI (1:50000) in an upright fluorescent microscope (Olympus BX-61) and images were captured using a cooled CCD camera with a 20x objective. Confocal imaging was done using Nikon A1 and Zeiss LSM 510 Meta confocal microscope.

6. Quantitative analysis

Cell counting was done from the acquired immunostained images of neuronal and glial cells. Briefly, 7–8 fields of each polymeric substrate were imaged, and the number of β-III tubulin positiveand GFAP positive cells were obtained by manual counting of the merged images. Cells stained with nuclear stain DAPI were used for counting total number of cells. One-way ANOVA analysis was performed in all the statistical analysis and significance is depicted at $p < 0.5$.

Neurite length was measured by Simple Neurite Tracer plug-in of the Image J software. The neurite length of the neurons well-spread on the substrate was sampled and those within the aggregates were excluded and it was measured from the point of attachment of the neurite to the cell body along its entire length till its terminal end. The branched terminals of the neurites were considered individually and their length was measured as described above. The area of the aggregates was measured by binarizing the DAPI images of the differentiated cells on the substrates by Image J software. The aggregate was chosen with the help of Wand tool and the area was measured and saved with ROI manager. The aggregate alignment was considered by measuring the angle between the longer axis of the ellipsoidal aggregate with the direction of the local defect, formed orthogonal to strain direction. The aggregates were positively scored for the alignment if they made an angle of 20^{o} or less with the local defects.

All the experiments were performed in duplicates (n=2) and repeated three times (N=3) for performing the quantitative analysis thus negating the chances of sudden variations in the data.

The immunostained images shown in paper are the average representative of all the experiments performed on the particular substrate.

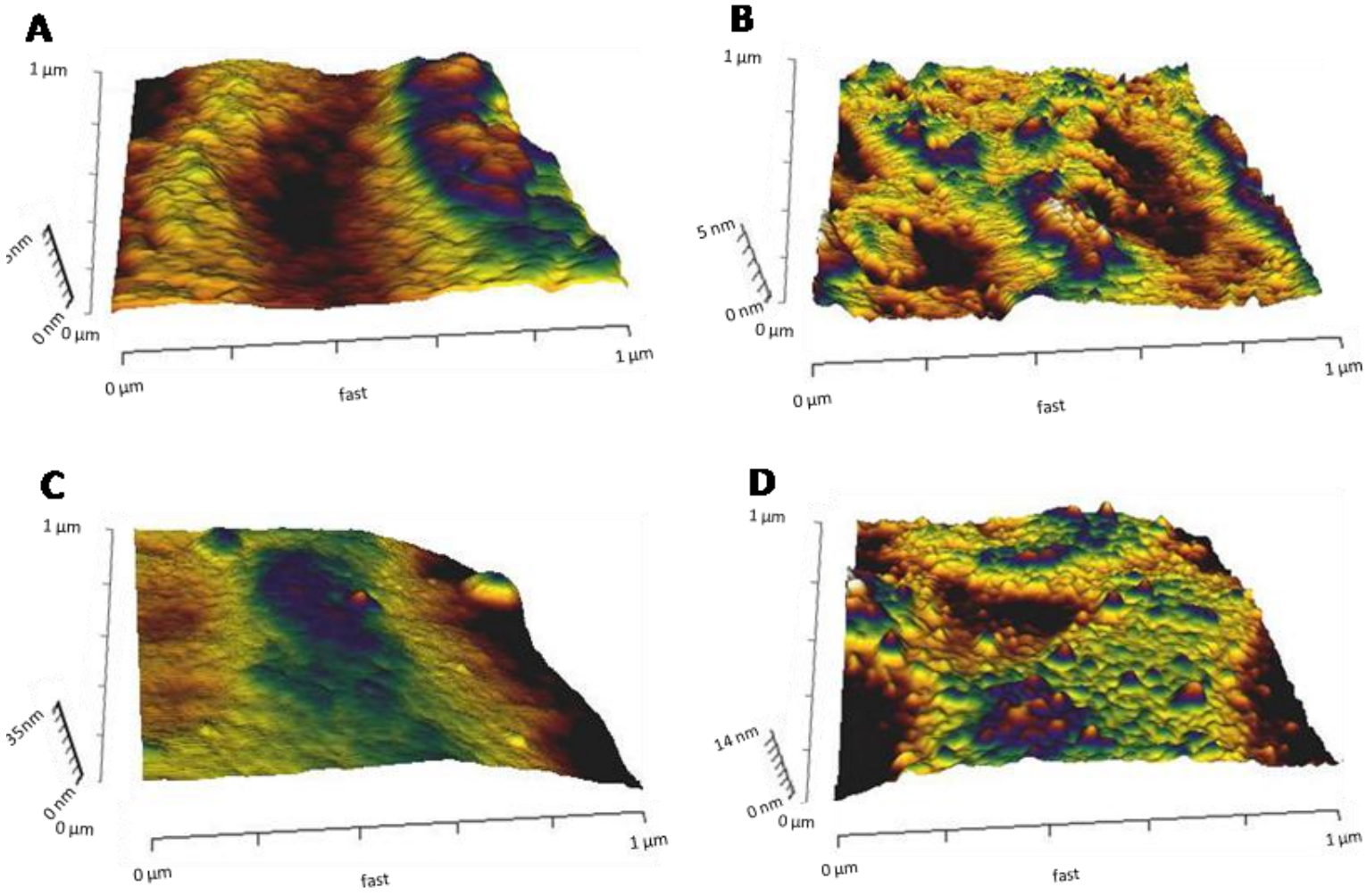
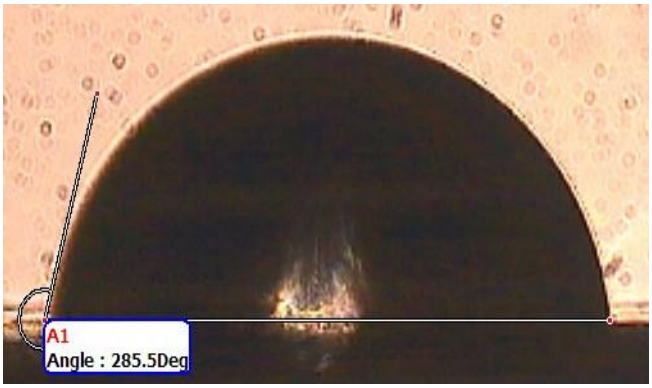


Fig. S2. AFM images of (A) SEBS PEDOT:PSS 0% Stretched (B) SEBS 0% Stretched (C) SEBS PEDOT:PSS 30% Stretched and (D) SEBS 30% Stretched substrates. Variations in the nano-topographical features are clearly evident on the substrates.

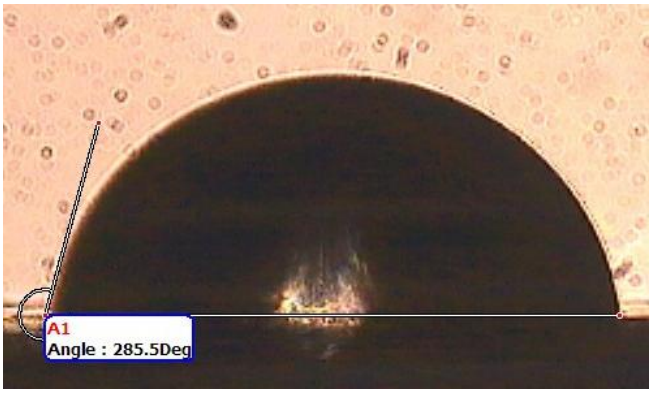
A



74.5± 1.7^{O}

SEBS PEDOT:PSS 0%
Stretched

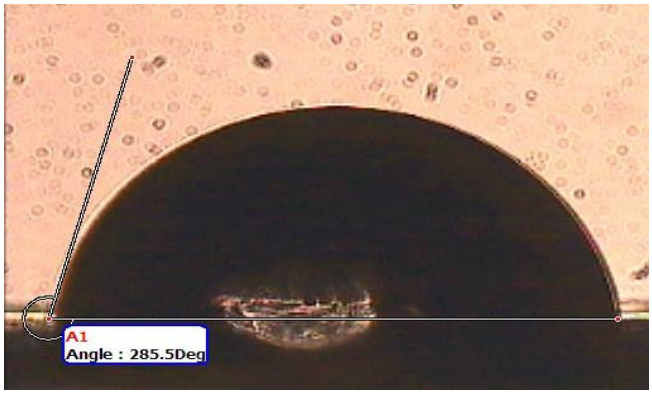
B



74.5± 1.6^{O}

SEBS PEDOT:PSS 10%
Stretched

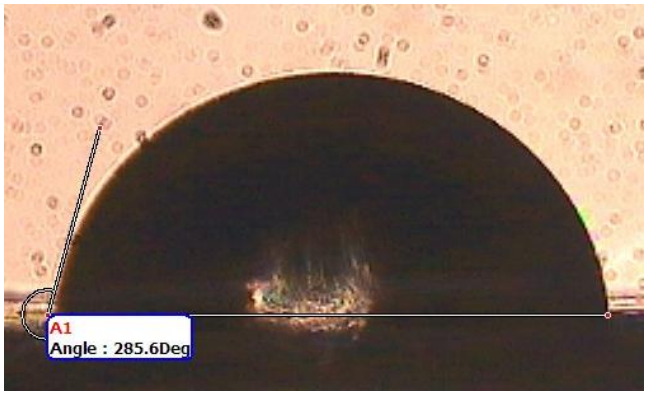
C



74.5± 1.6^{O}

SEBS PEDOT:PSS 20%
Stretched

D



74.4± 1.6^{O}

SEBS PEDOT:PSS 30%
Stretched

Fig. S3. Contact angle measurement of PEDOT:PSS coated SEBS substrates (A) Unstrained CP substrate (B)-(D) Strained substrates. Hydrophobicity of the substrate does not change upon the application of strain.

7. Maintenance of embryonic stem cells on polymeric substrates

Mouse Embryonic Stem cells (ES cells) are commonly cultured on glass coverslips coated with Poly-D-lysine and laminin. ES cells cultured on glass coverslips in N2 differentiation medium differentiated uniformly into neuronal cells. Conducting polymer PEDOT:PSS is biologically compatible and has been widely used for neuronal culture thereby showing its biocompatible nature while SEBS has not yet been used for biological applications. So, green Fluorescent protein (GFP) expressing plasmid transfected ES cells were used for culturing on SEBS and PEDOT:PSS coated SEBS substrates (Fig. S5). GFP tagged ES cells were then observed throughout the period of differentiation for 8-10 days and it was seen that the cells adhered and differentiated into neuronal cells extending out neurites on all the substrates. Cells were indicated as neuronal cells on the basis of morphology since immunocytochemical analysis was not performed.

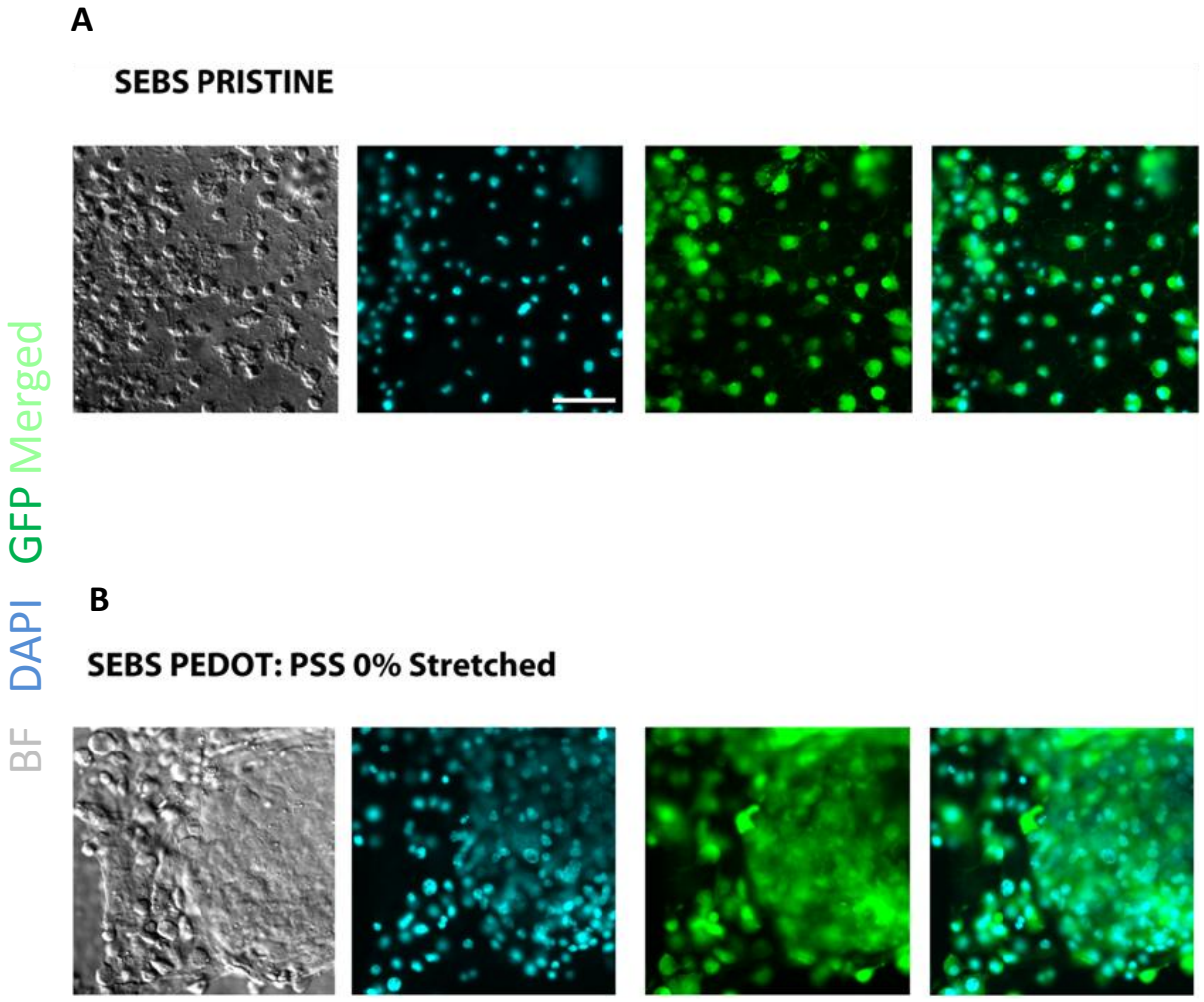


Fig. S4. Differentiation and Proliferation of GFP tagged ES-NP cells on (A) SEBS pristine (B) SEBS PEDOT:PSS 0% Stretched. ES cells were transfected with pce-3 plasmid and GFP was constitutively expressed under CAG promoter. Heterogeneous population of cells differentiated from ES-NPs express GFP. Scale bar 50 µm.

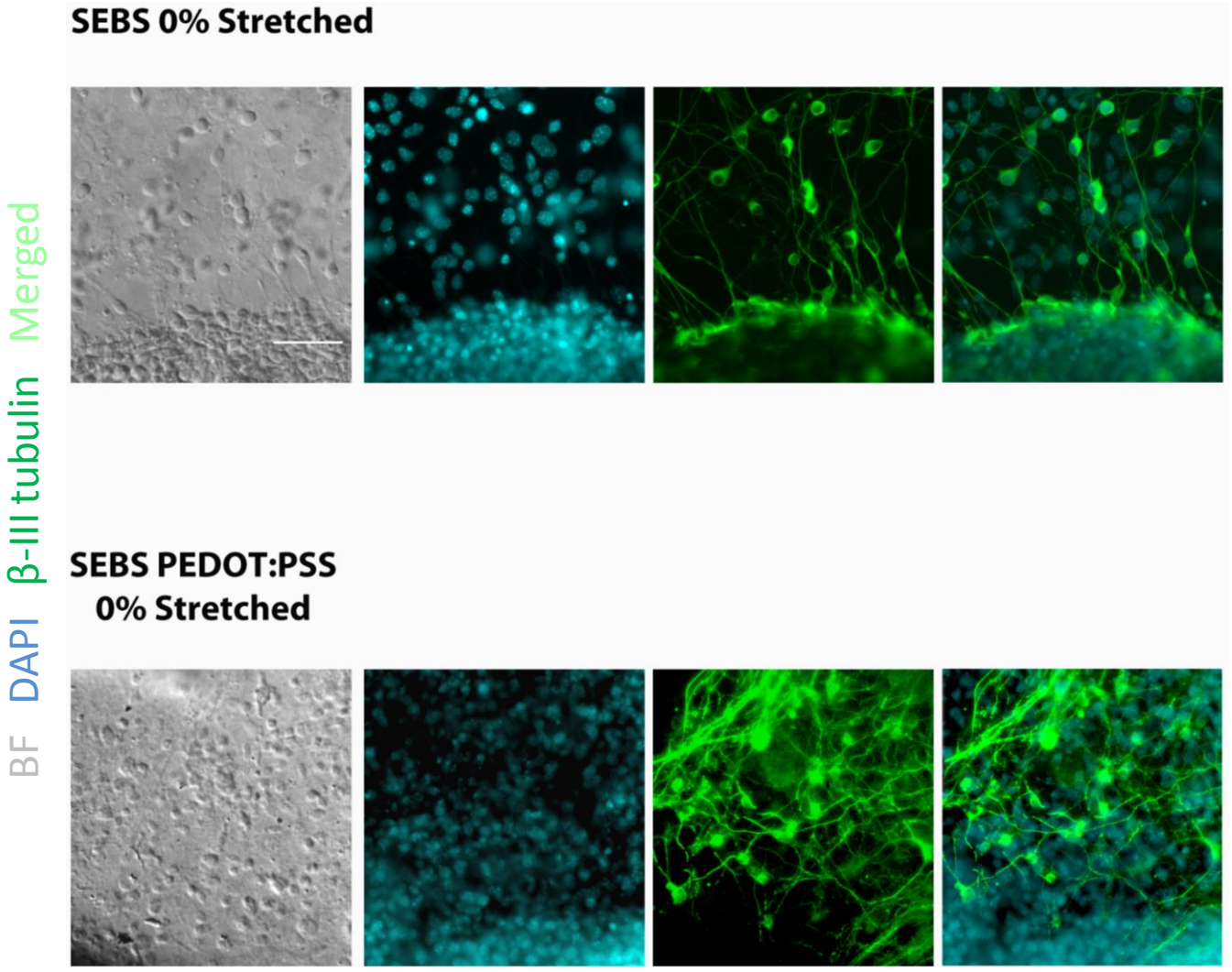


Fig. S5 - Differentiation of ES-NPs on unstrained conducting CP-coated SEBS and non-conducting pristine SEBS substrates. Scale bar 50 µm.

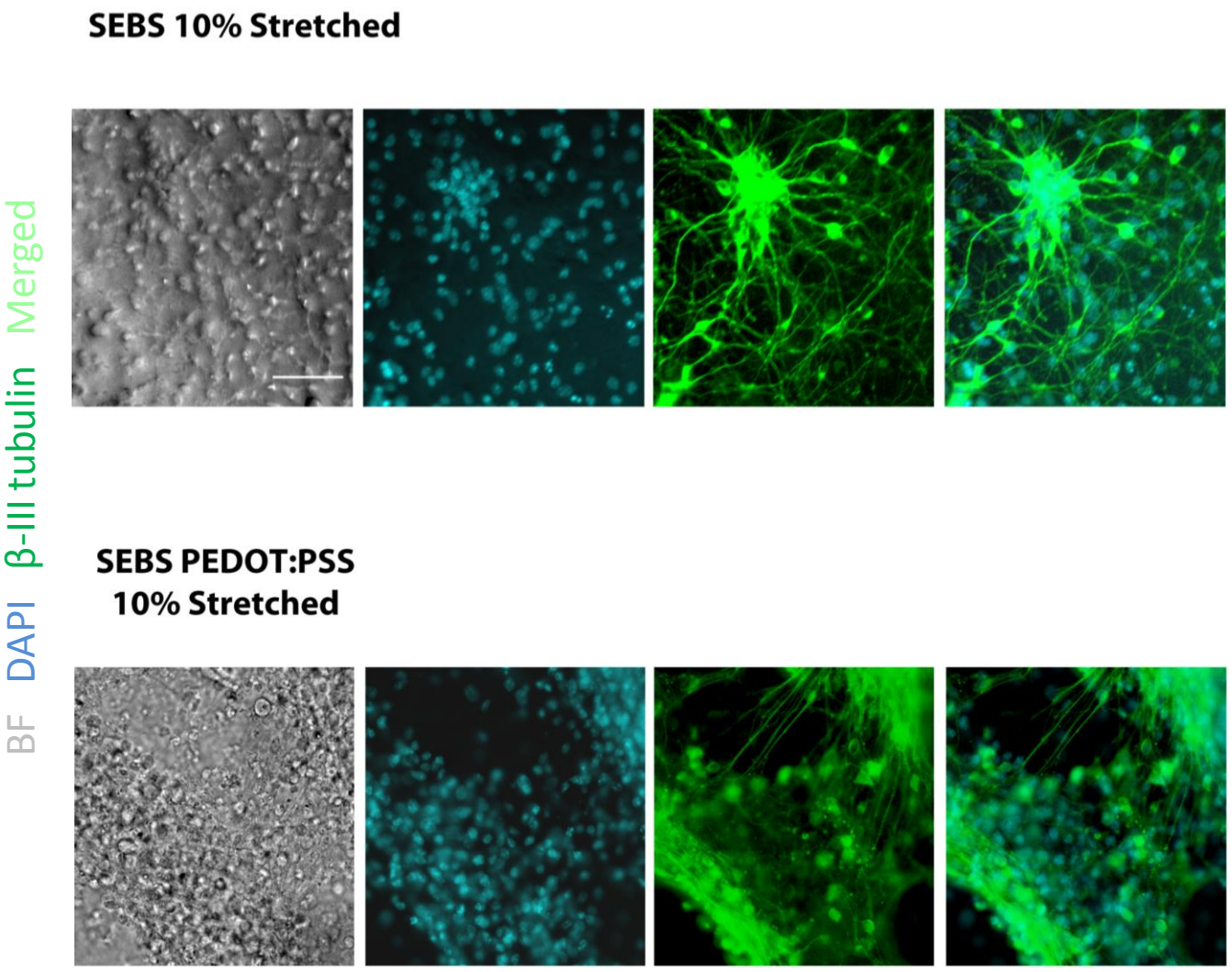


Fig. S6. Differentiation of ES-NPs on strained (10%) CP coated SEBS and non-conducting pristine SEBS substrates. Neuronal differentiation decreases on straining CP coated substrates and tendency to remain within the aggregates increases. Scale bar 50µm.

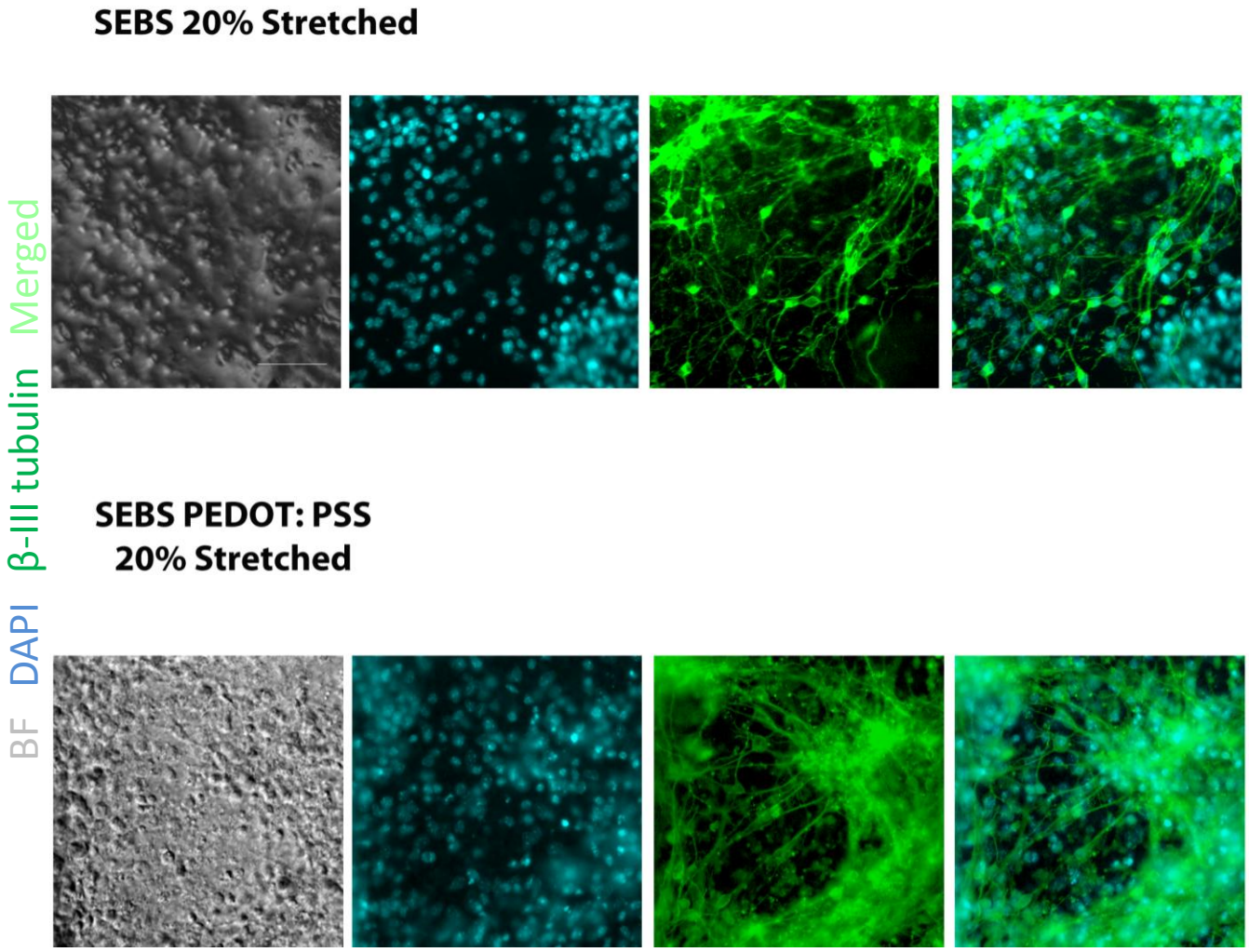


Fig. S7. Differentiation of ES-NPs on strained (20%) CP coated SEBS and non-conducting pristine SEBS substrates. Neuronal differentiation decreases further on straining CP coated substrates and tendency to remain within the aggregates markedly increases. Scale bar 50µm.

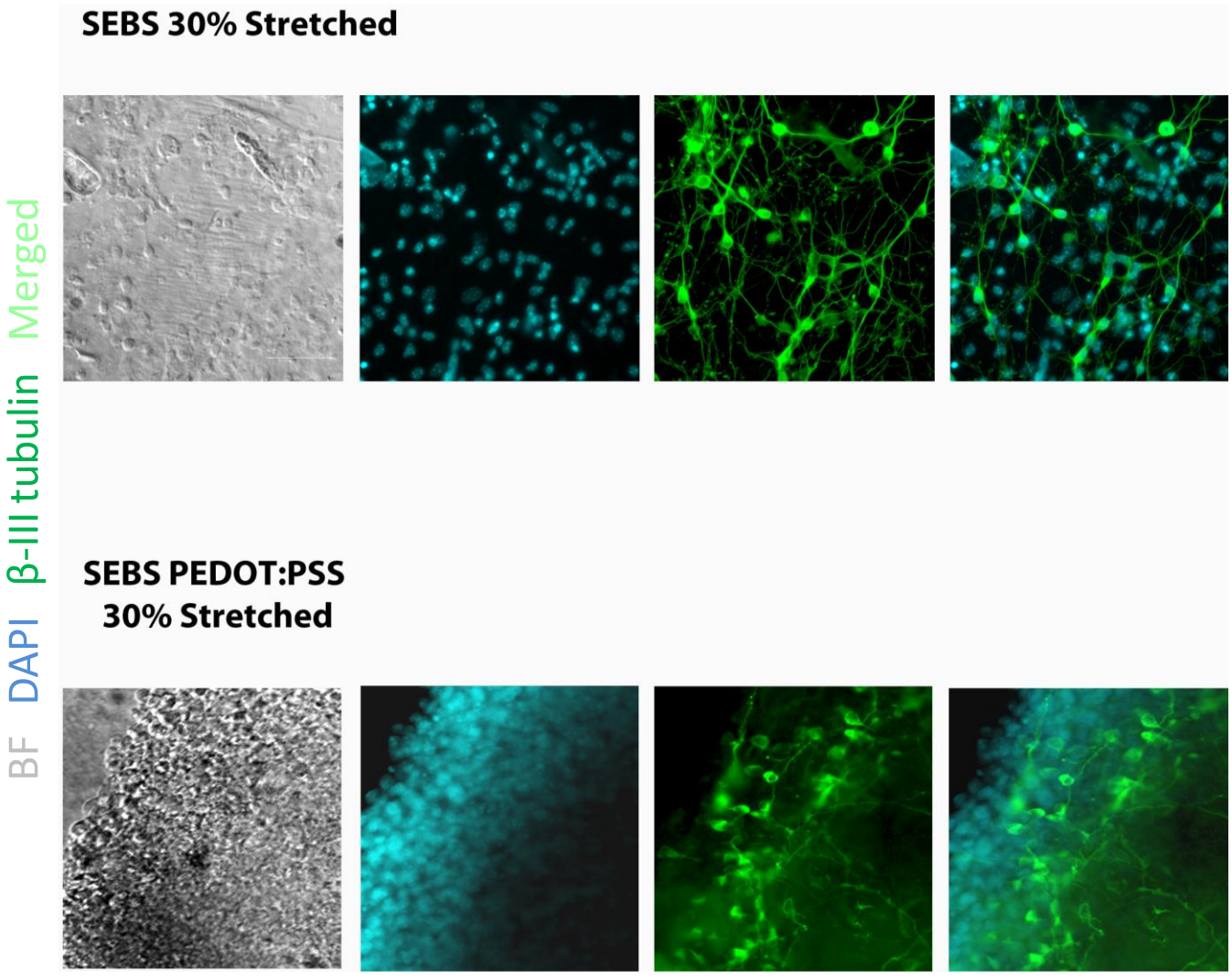


Fig. S8. Differentiation of ES-NPs on strained (30%) CP coated SEBS and non-conducting pristine SEBS substrates. Neuronal differentiation decreases further on straining CP coated substrates and tendency to remain within the aggregates increases significantly on these substrates. Scale bar 50µm.

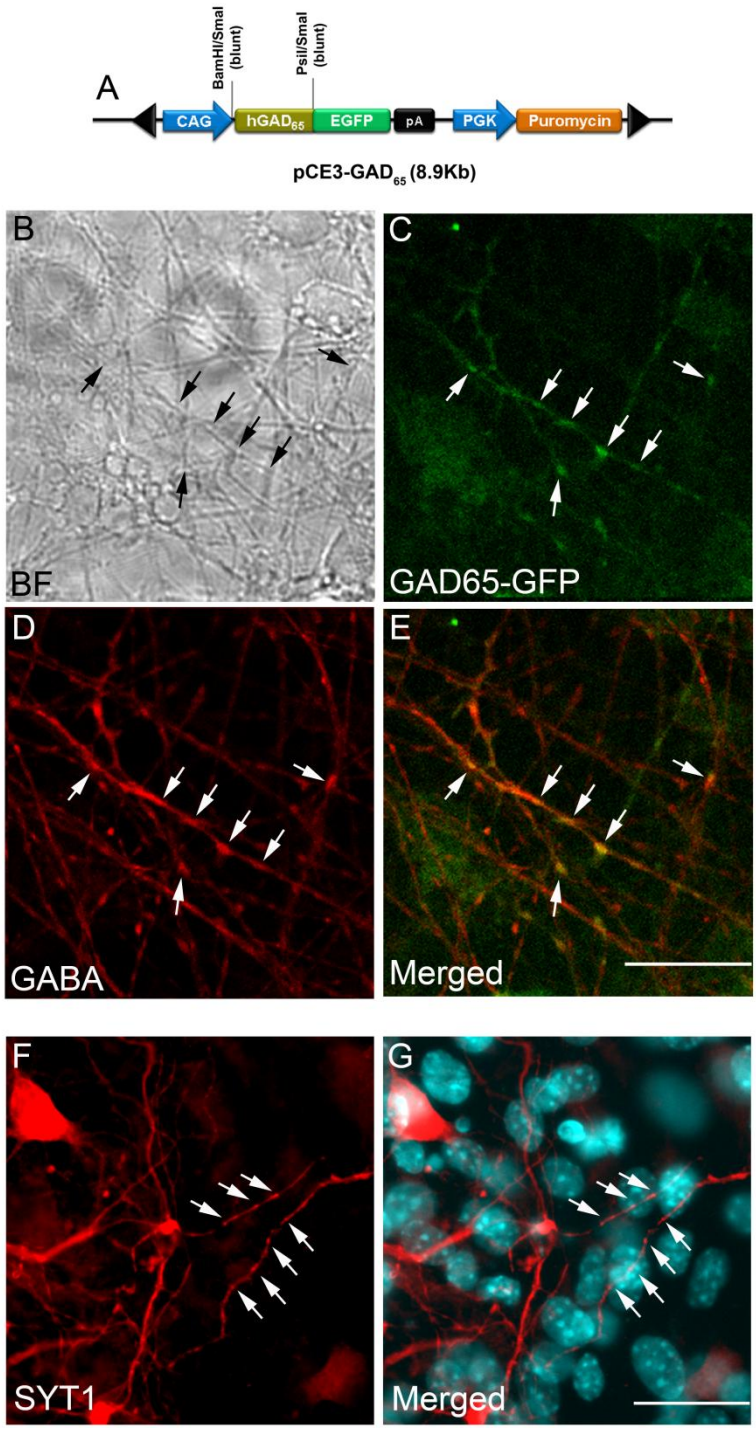


Fig. S9. A) Schematic representation of pCE3-GAD65 construct used for the generation of GAD65-ES cells. (B-C) GAD65-ES cell-derived neurons, shows the presence of localized GAD65-EGFP spots in their dendritic membranes. (D-E) These GAD65-EGFP spots co-localized with GABA, confirming the dendritic localization and GABA synthesis by GAD6-EGFP fusion protein in differentiated neurons. (F-G) Synaptotagmin-1 localizes to the synaptic terminals of the ES-NP differentiated neurons. Scale bar 25 µm.

7. Conducting polymer does not significantly influence the glial differentiation potential of embryonic stem cell derived neural progenitors

We also compared the glial differentiation potential of ES-NPs on CP and SEBS substrates. Upon exposure to differentiation conditions, the ES-NPs also differentiated into glial fate which was indicated by $GFAP^{+ve}$ (Glial fibrillary acidic protein) staining (Fig. 7A-H). The number of $GFAP^{+ve}$ cells was observed to be less influenced by the strain on the PEDOT:PSS coated substrates. Differentiation on unstrained (0%) PEDOT:PSS substrates resulted in 26.2±1.5% glial cells while that on strained (10%) PEDOT:PSS substrates generated 22.3±2.3% glial cells (Fig. 7I). Glial differentiation was also less dependent on the conducting nature of the polymer surface. On 20% strained CP-coated substrates, 22.9±2.3% of glial cells were found to be differentiated from ES-NPs which does not vary much from 25.7±3.2% glial cell differentiation on 20% strained pristine SEBS control substrates.

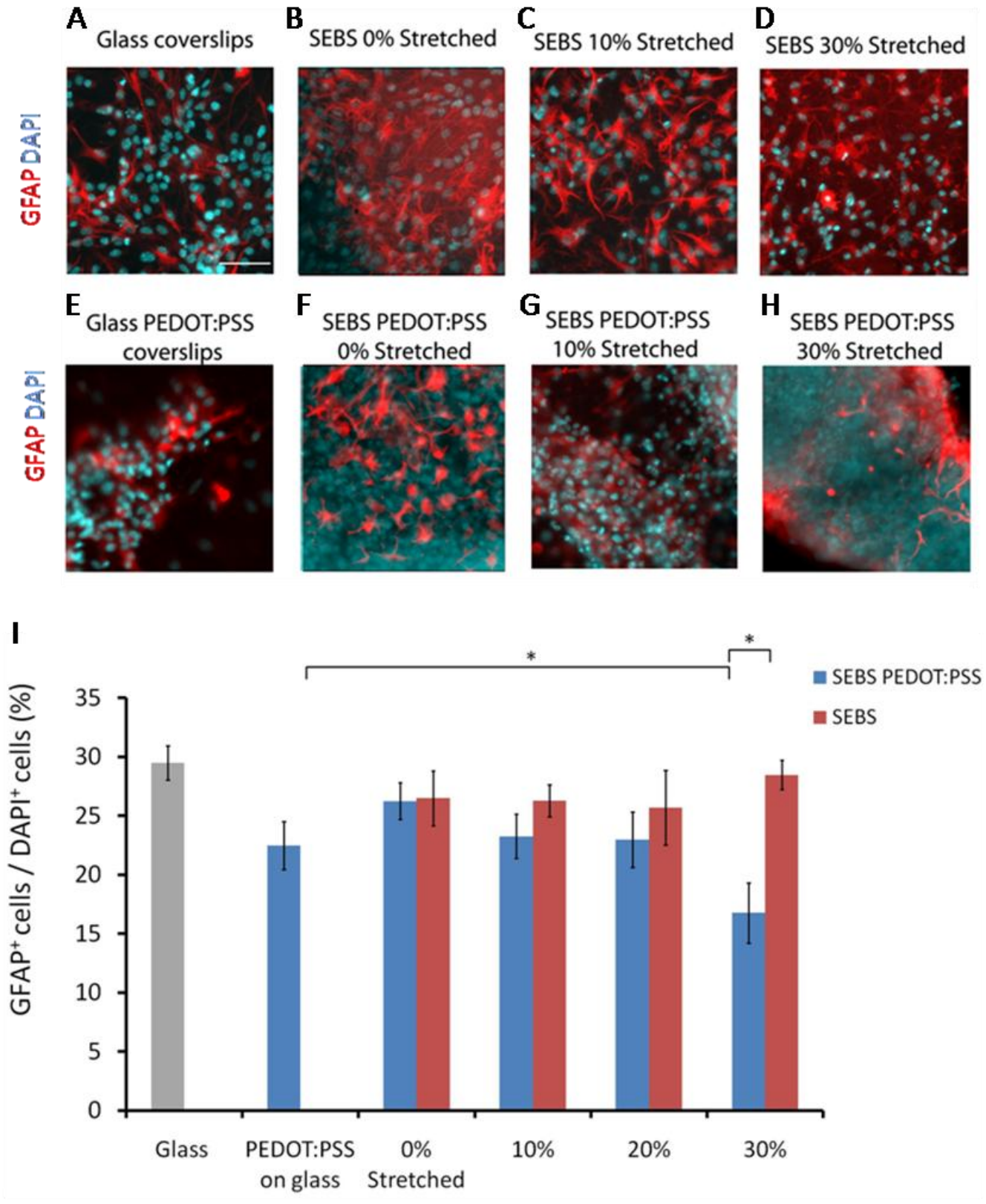


Fig. S10. Immunocytochemistry for differentiation of ES-NPs into glia (GFAP) on (B-D) SEBS and (F-H) PEDOT:PSS coated SEBS substrates, (A,E) on glass-coverslips and PEDOT:PSS coated glass-coverslips (controls). (I) Distribution of the glial differentiation on each substrate (*$p<0.05$, One-way ANOVA). Data are represented as mean ± SD, n=3. (A-H): Scale bar 50µm.

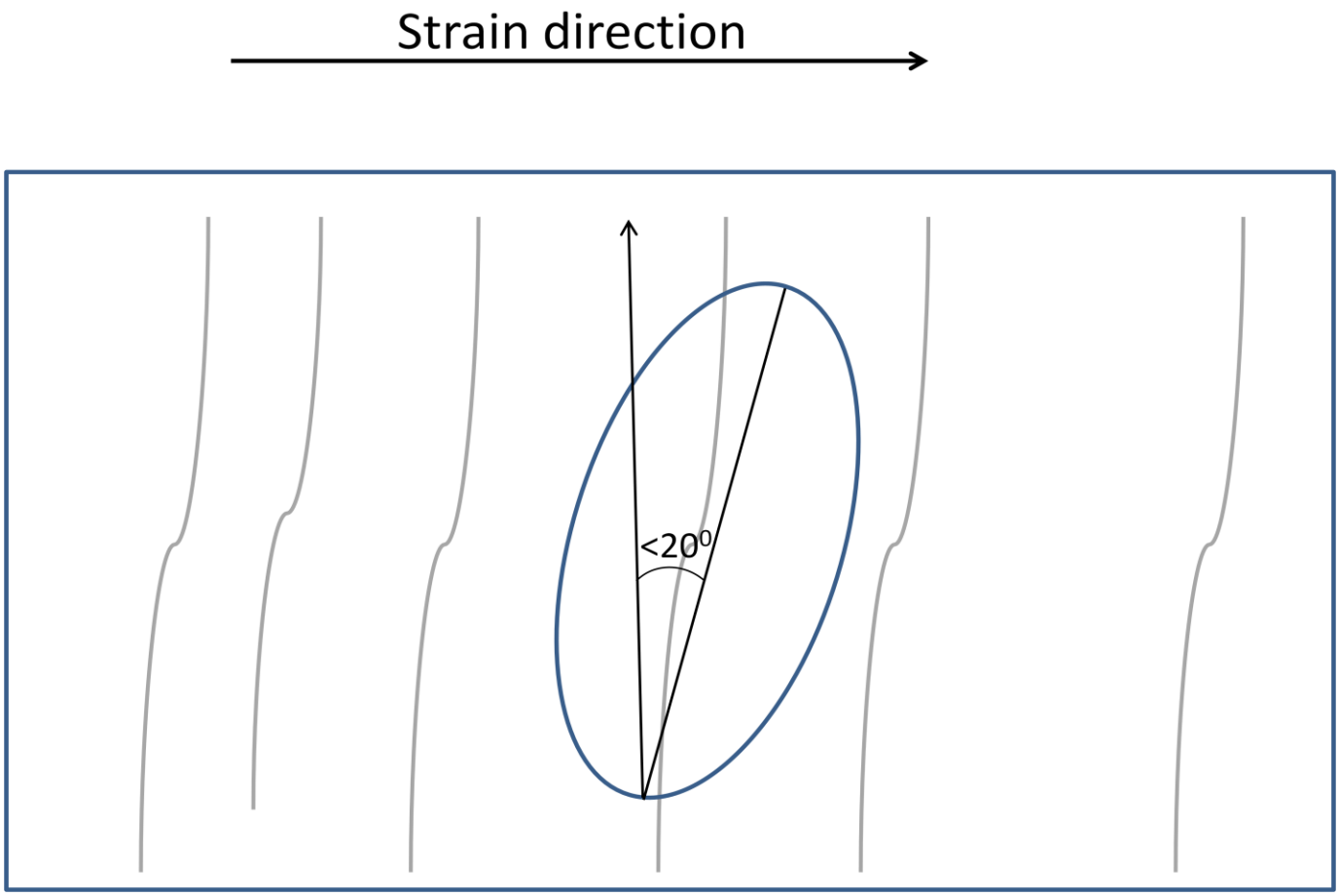
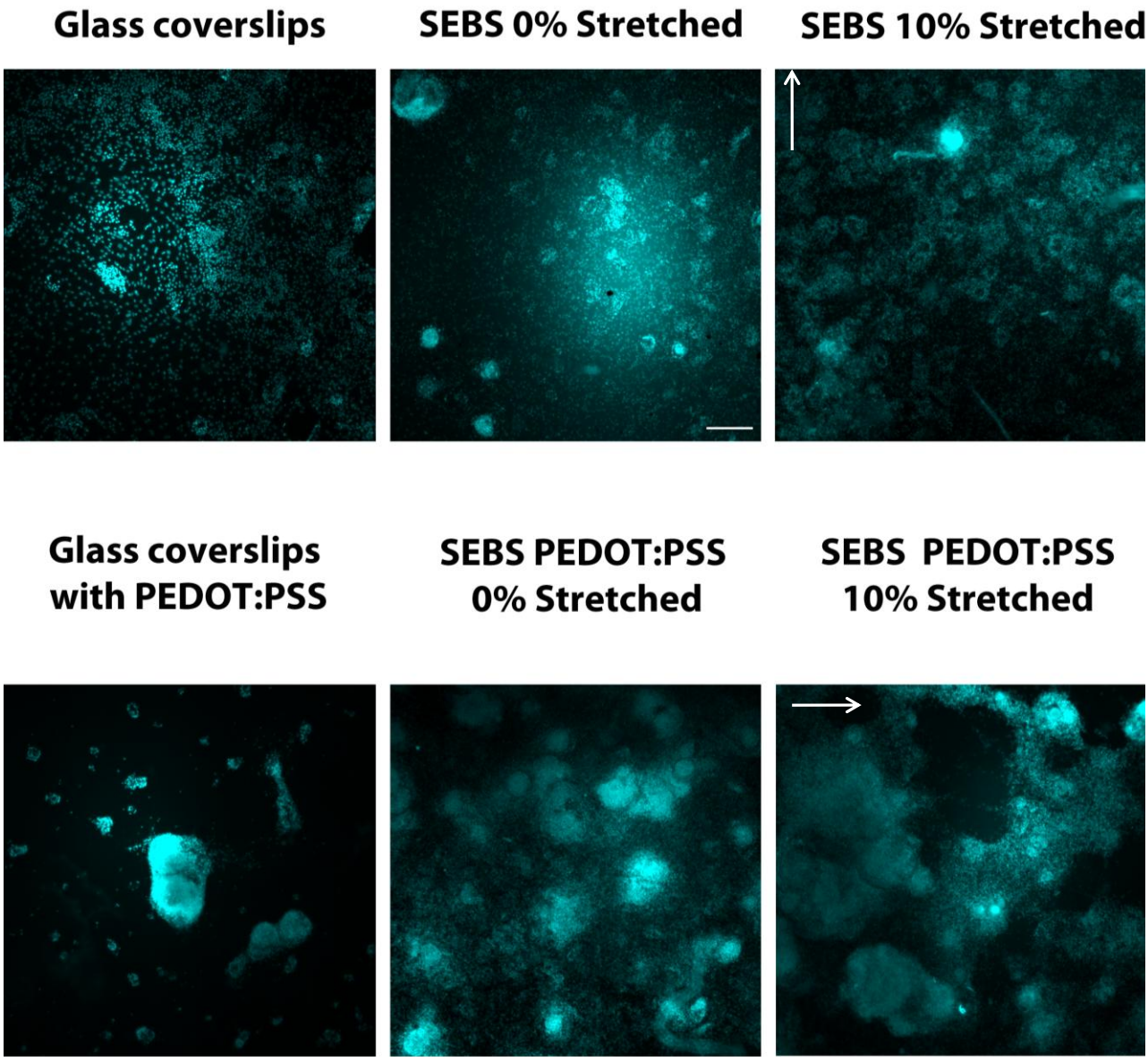


Fig. S11. Pictorial representation of the aggregate aligned along the crack patterns on the strained CP substrates.

A


Glass coverslips
SEBS 0% Stretched
SEBS 10% Stretched
Glass coverslips with PEDOT:PSS
SEBS PEDOT:PSS 0% Stretched
SEBS PEDOT:PSS 10% Stretched

B

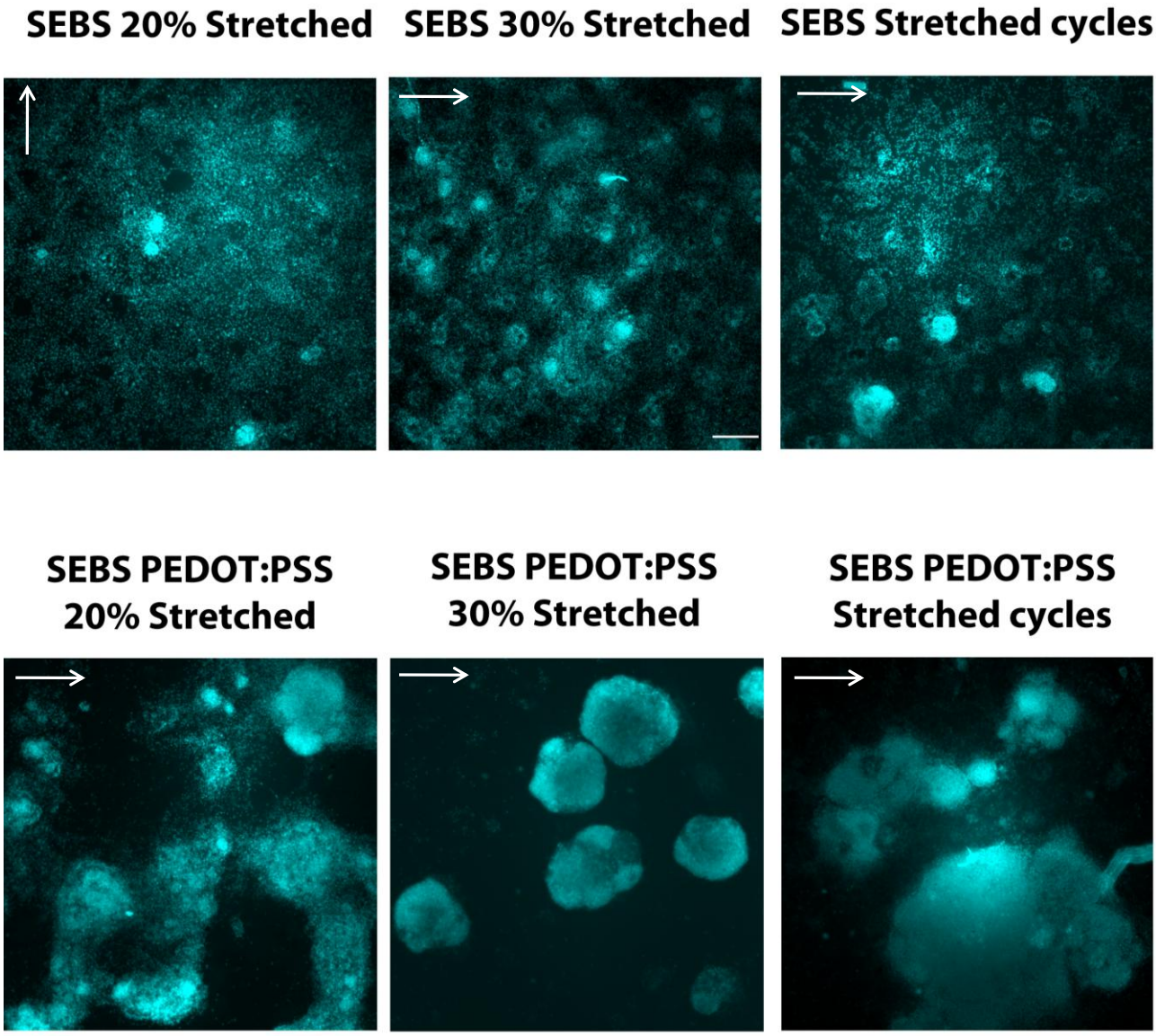


Fig. S12. (A,B). DAPI images of cells on polymeric substrates for analyzing the role of directionality provided by the defect patterns. The arrows indicate the strained direction. More number of aggregates was aligned along the direction of local defects on strained CP substrates. Scale bar 50µm.

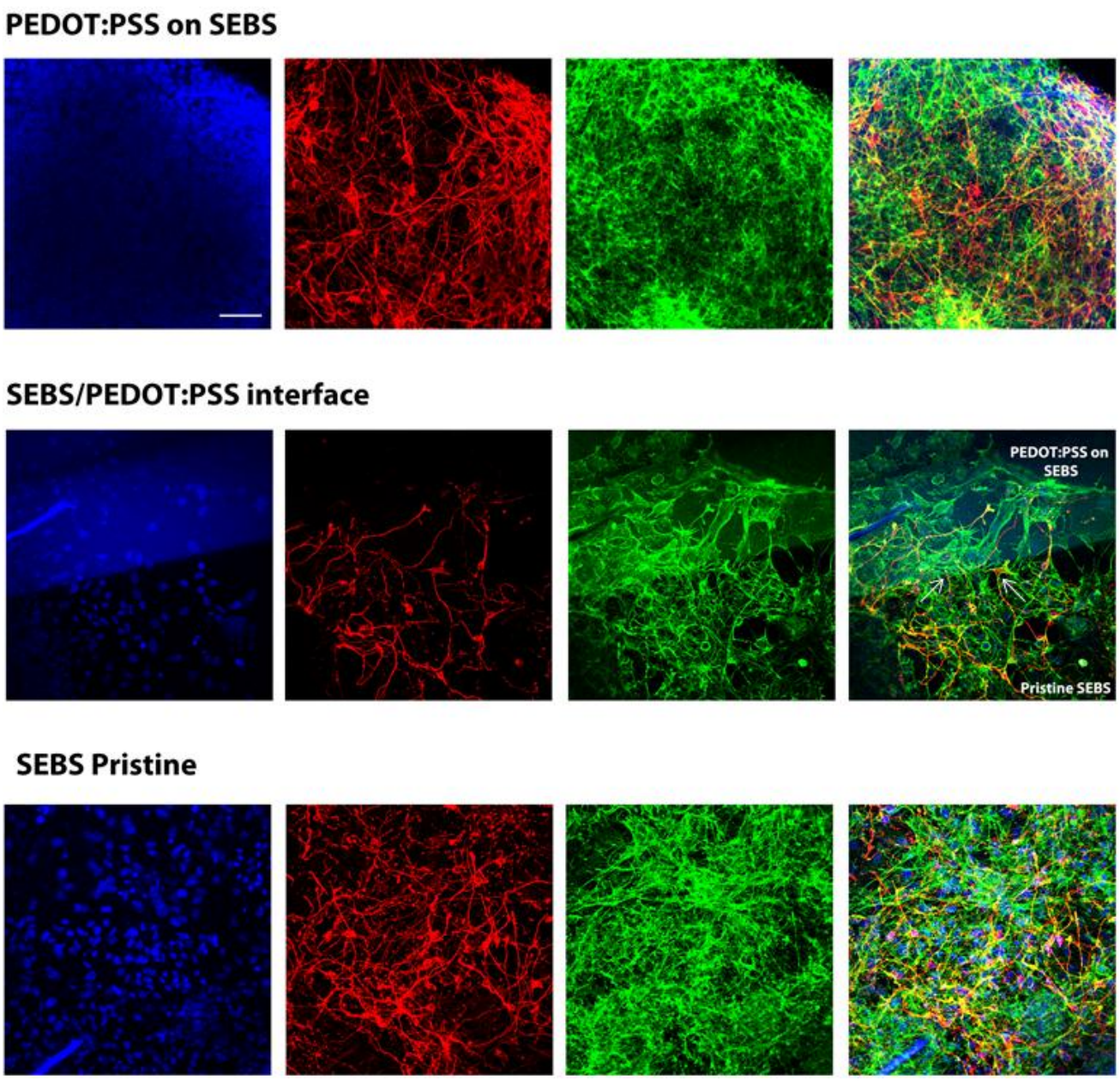


Fig. S13. Arrangement of actin fibers in neurons differentiated from ES-NPs on SEBS/PEDOT: PSS coated SEBS substrates. Scale bar, 50µm.