Supporting Information for:

Changes in the Morphology and Proliferation of Astrocytes Induced by Two Modalities of Chemically Functionalized Single-Walled Carbon Nanotubes are Differentially Mediated by Glial Fibrillary Acidic Protein

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This file includes:

Supporting Figures S1-S4

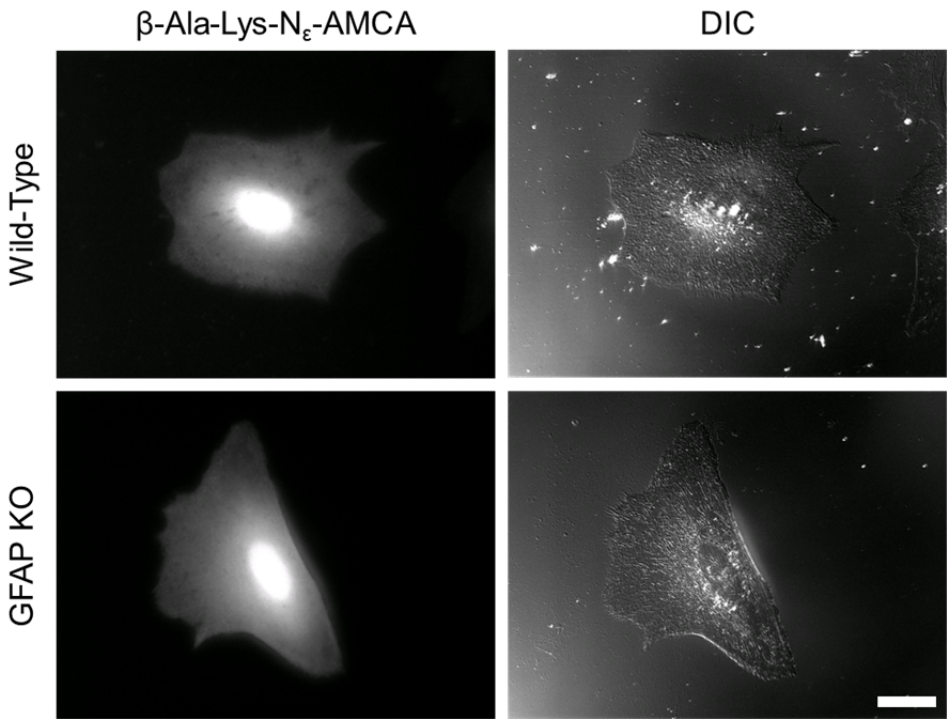

Figure S1. GFAP KO cells accumulate β-Ala-Lys-$N_ε$-AMCA through the astrocyte specific PepT2 peptide transporters similar to wild-type cells confirming their astrocytic identity. Images of live wild-type and GFAP KO astrocytes loaded with β-Ala-Lys-Nε-AMCA (left column) and their corresponding differential interference contrast (DIC) images (right column). We examined 23 wild-type and 22 GFAP KO live cells. Scale bar, 20 μm.

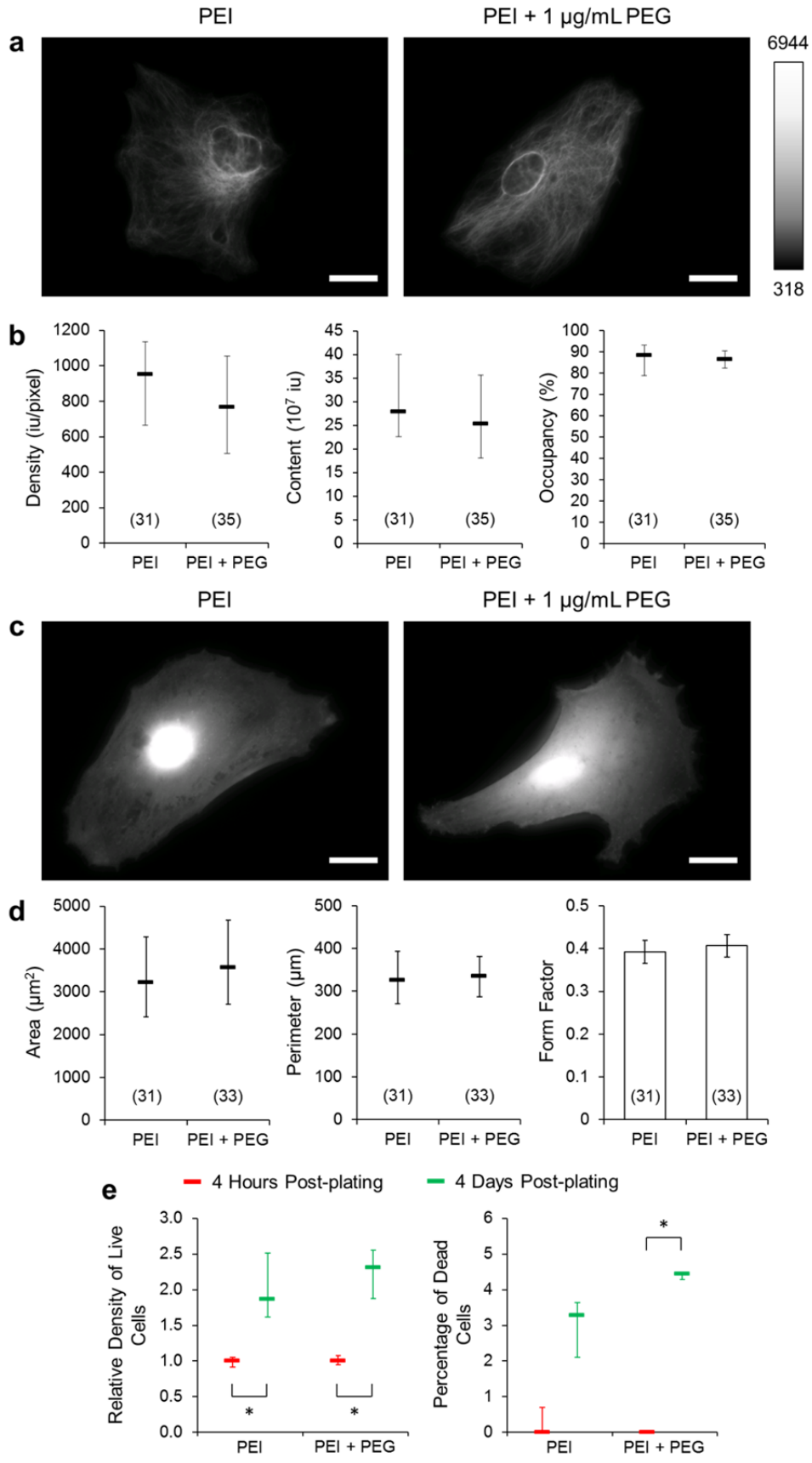
a
PEI
PEI + 1 µg/mL PEG
6944
318
b
Density (iu/pixel)
Content (10^7 iu)
Occupancy (%)
(31)
(35)
PEI
PEI + PEG
c
PEI
PEI + 1 µg/mL PEG
d
Area (µm²)
Perimeter (µm)
Form Factor
(31)
(33)
PEI
PEI + PEG
e
4 Hours Post-plating
4 Days Post-plating
Relative Density of Live Cells
Percentage of Dead Cells
*
PEI
PEI + PEG

Figure S2. PEG does not induce any changes in the morpho-functional properties of cultured wild-type astrocytes. (a) Images of wild-type astrocytes plated onto the PEI-coated coverslips, in the absence (untreated control) and presence of the PEG solute (1 μg/mL), and labeled for GFAP using indirect immunocytochemistry. Scale bar, 20 μm. Gray scale is a linear representation of the fluorescence intensities of the pixels in the images, expressed in fluorescence intensity units (iu). (b) Summary graphs showing the median effects of PEG solute on GFAP-ir parameters. Density is shown in fluorescence intensity units (iu) per area (pixel). Number of astrocytes studied in each condition is given in parentheses. The dashes represent medians with interquartile range (IQR). Lack of statistical significance was established by Mann-Whitney U-tests. (c) Images of live wild-type astrocytes plated in matching conditions as above, and loaded with the vital fluorescent dye, calcein. Scale bar, 20 μm. (b) Summary graphs showing the effects of PEG solute on the morphology of astrocytes. The open bars represent mean ± standard error of mean. Lack of statistical significance for form factor was established by Student's t-test. Other annotations as in (b). (e) Summary graphs showing the median relative density of live cells (left), normalized to the median number of live astrocytes plated onto PEI at 4 h post-plating, and median percentage of dead cells (right) in matching setting as above. We used seven coverslips per data point. Mann-Whitney U- test was used for the comparison between the untreated and PEG treated groups and Wilcoxon Signed-Rank test was used for the comparison between the time points in each treatment. *$p < 0.05$. Other annotations as in (b). Note that there is a significantly higher proportion of dead astrocytes in the presence of PEG at 4 d vs. 4 h post-plating, albeit the death toll at either time point was not different from that of control astrocytes grown in the absence of PEG.

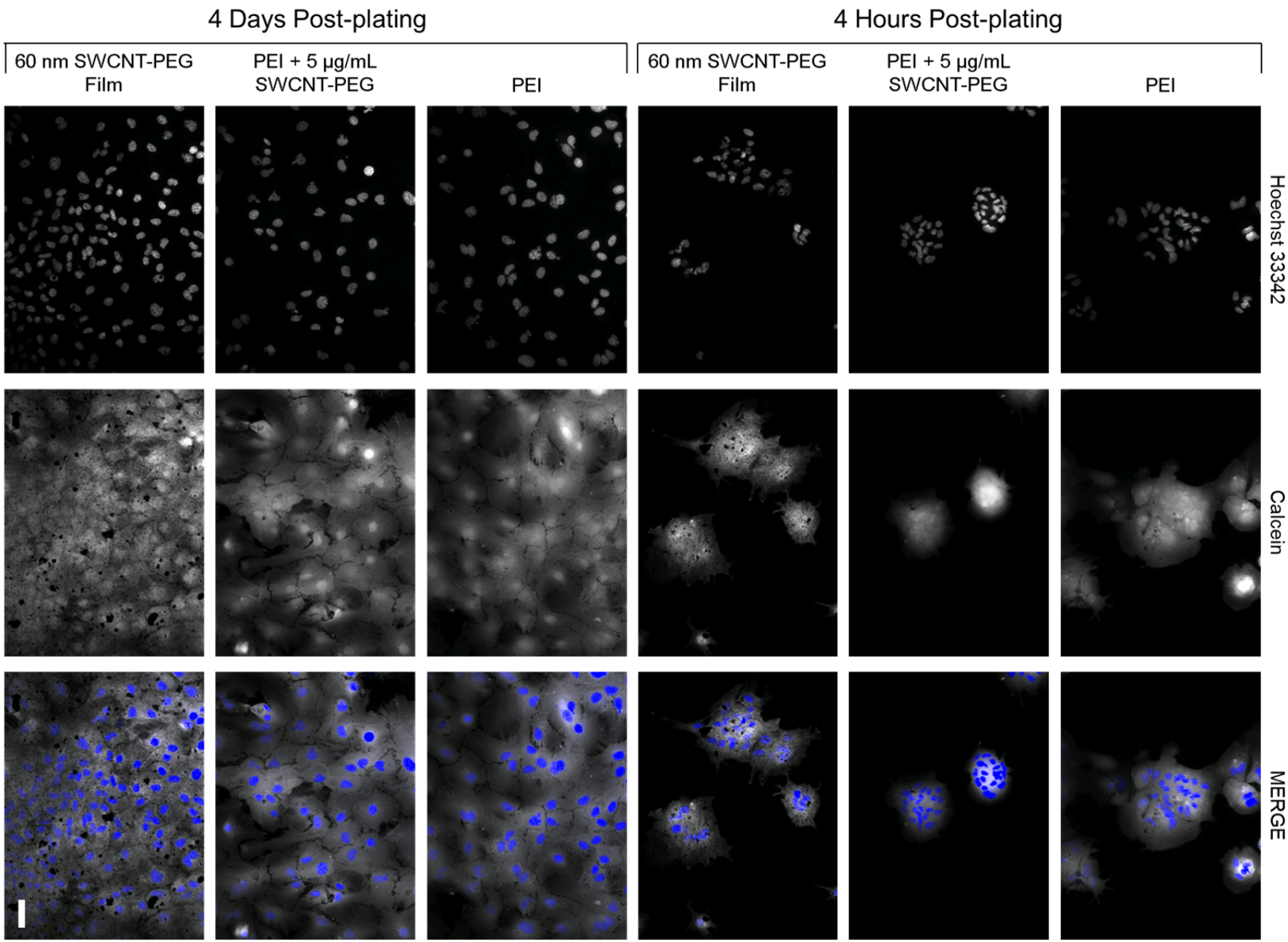
4 Days Post-plating
4 Hours Post-plating
60 nm SWCNT-PEG Film
PEI + 5 µg/mL SWCNT-PEG
PEI
60 nm SWCNT-PEG Film
PEI + 5 µg/mL SWCNT-PEG
PEI
Hoechst 33342
Calcein
MERGE

Figure S3. Examples of the images of wild-type astrocytes used in the proliferation study. Images of wild-type astrocytic nuclei labeled with the cell permeable nuclear dye, Hoechst 33342, (left column) and corresponding wild-type astrocytes loaded with the vital fluorescent dye, calcein (middle column). We used seven coverslips per condition. Right column shows the merge of the images. Top set) The first three rows show images of wild-type astrocytes plated onto the PEI-coated coverslips in the absence and presence of 5 μg/mL SWCNT-PEG and onto the 60 nm thick CNT film, respectively, 4 h post-plating. Bottom set) The next rows (fourth - sixth) show images of wild-type astrocytes plated in matching conditions as above, but imaged at the later time point, 4 days post-plating. Note that the images in the first and fourth rows are the same as in Figure 3a. Scale bar, 50 μm.

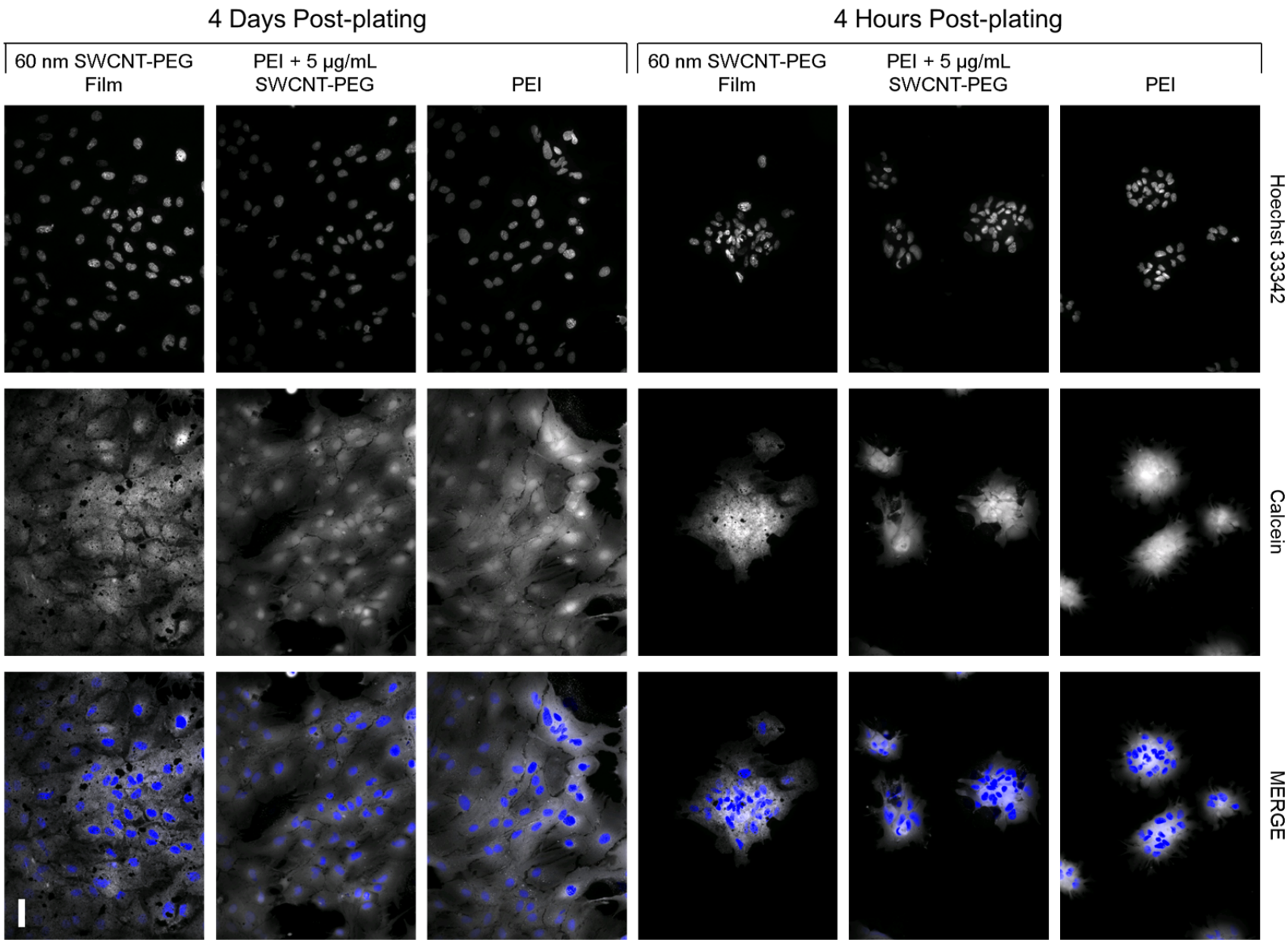
4 Days Post-plating
4 Hours Post-plating
60 nm SWCNT-PEG Film
PEI + 5 µg/mL SWCNT-PEG
PEI
60 nm SWCNT-PEG Film
PEI + 5 µg/mL SWCNT-PEG
PEI
Hoechst 33342
Calcein
MERGE

Figure S4. Examples of the images of GFAP KO astrocytes used in the proliferation study. Images of GFAP KO astrocytic nuclei labeled with the cell permeable nuclear dye, Hoechst 33342, (left column) and corresponding GFAP KO astrocytes loaded with the vital fluorescent dye, calcein (middle column). We used seven coverslips per condition. Right column shows the merge of the images. Top set) The first three rows show images of GFAP KO astrocytes plated onto the PEI-coated coverslips in the absence and presence of 5 μg/mL SWCNT-PEG and onto the 60 thick nm CNT film, respectively, 4 h post-plating. Bottom Set) The next rows (fourth - sixth) show images of GFAP KO astrocytes plated in matching conditions as above, but imaged at the later time point, 4 days post-plating. Note that the images in the first and fourth rows are the same as in Figure 3a. Scale bar, 50 μm.