Electronic Supplementary Material (ESI) for RSC Advances.


Supporting information

# Substrate porosity induces phenotypic alterations in retinal cells cultured on silicon nanowires

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| Antibody - Antigen | Retinal Distribution | Dilution | Type - Host | Source - Code |
|---|---|---|---|---|
| β-tubulin Isotype III | *Inner retinal neurons* | *1:1,500* | *MAb Mouse* | Sigma-Aldrich, St. Louis, MO, USA T8660 |
| Rho 1D4 | *Rods* | *1:150* | *MAb Mouse* | Robert S Molday, Univ of British Columbia, Vancouver, Canada |
| Cone arrestin | *Cones* | *1:500* | *PAb Rabbit* | Cheryl Craft, Univ Southern California, Los Angeles, CA, USA |
| Recoverin | *Rods, Cones, Cone bipolar cells* | *1:10,000* | *PAb Rabbit* | *Chemicon Intl, Temecula, CA, USA AB5585* |
| PKC (protein kinase C) | *Bipolar cells* | *1:800* | *MAb Mouse* | *Meridian Life Science Inc, Memphis, TN, USA K01107M* |
| Chx10 | *Bipolar cells, Progenitor cells* | *1:300* | *PAb Sheep* | Exalpha Biologicals, Inc, Shirley, MA, USA X1179P |
| Brn-3a | *Retinal ganglion cells* | *1:50* | *PAb Goat* | Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA sc-31984 |
| *TRPV4 (transient receptor potential cation channel, subfamily V, member 4)* | *Retinal ganglion cells* | *1:600* | *PAb Rabbit* | LifeSpan BioSciences, Inc, Seattle, WA, USA LS-C94498 |
| GAFP (glial fibrillary acidic protein) | *Glial cells* | *1:700* | *PAb Rabbit* | *DAKO A/S, Glostrup, Denmark* Z0334 |

**Table S1.** Antibodies used in the analysis. For more details, see Piret et al, 2013. (PAb, polyclonal antibody; MAb, monoclonal antibody)

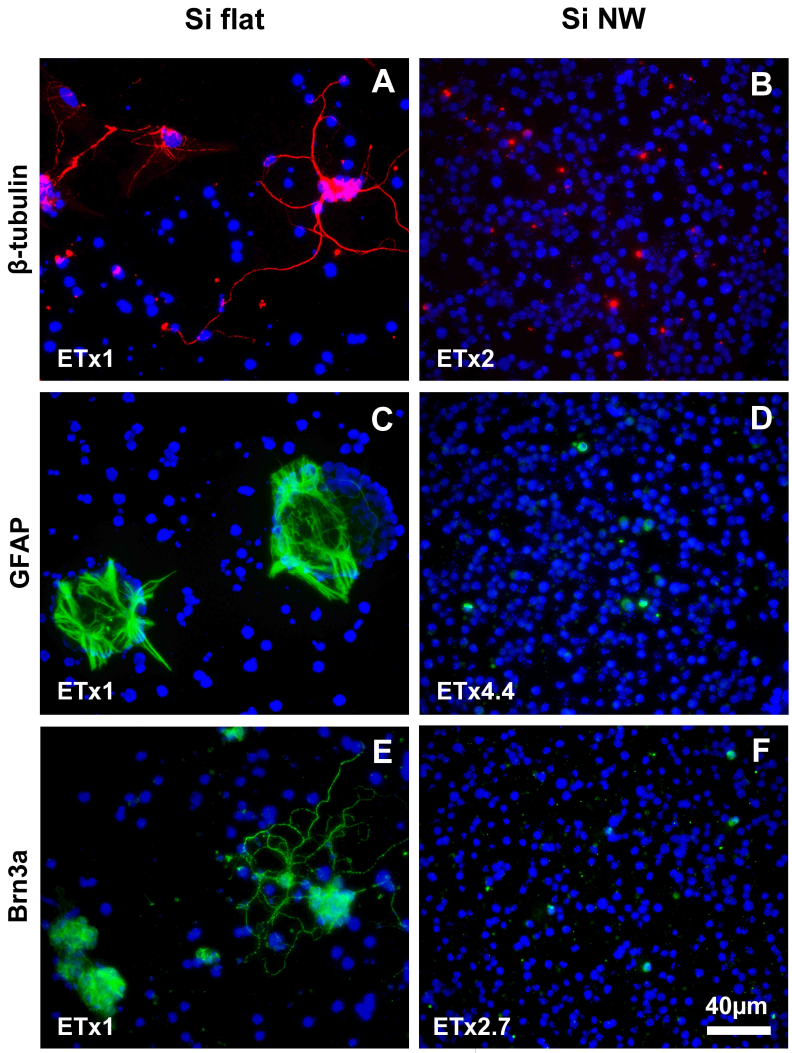


**Fig. S1.** Fluorescence images of retinal cell cultures after 18DIV showing β-tubulin III positive cells (red) and cell nuclei (DAPI, blue) on flat Si **(A)** and Si NW **(B)** substrates, GFAP positive cells (green) and cell nuclei (DAPI, blue) on flat Si **(C)** and Si NW **(D)** substrates, and Brn3a positive cells (green) and cell nuclei (DAPI, blue) on flat Si **(E)** and Si NW **(F)** substrates. The Exposure Time (ET) required to image the cells cultured on Si NW substrates was increased by a factor that is indicated at the bottom left of each panel. Scale bar, 40 µm for all panels.

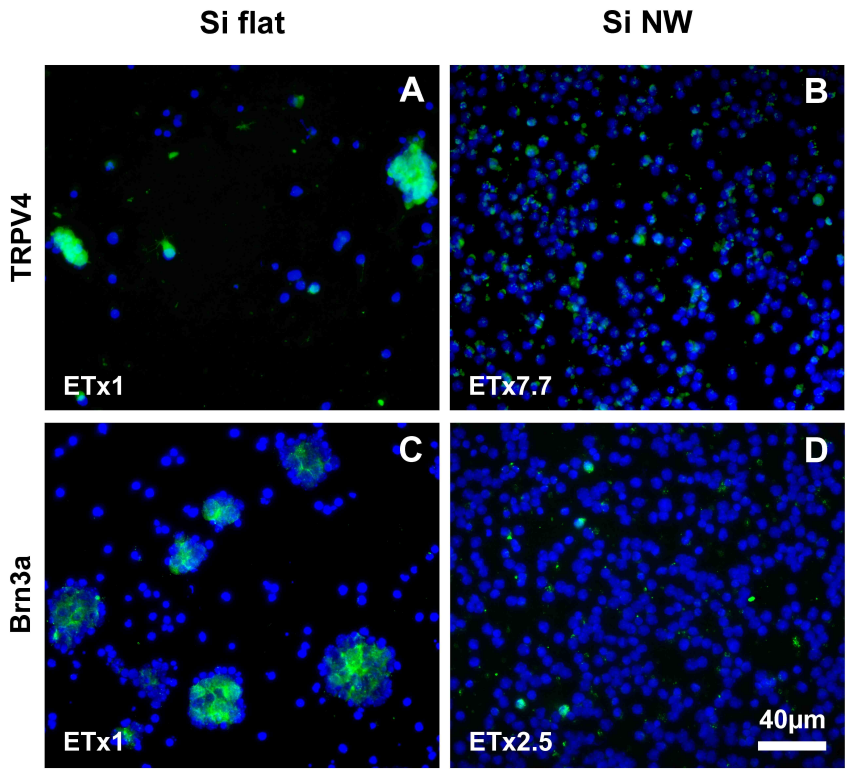


**Fig. S2**. Fluorescence images of retinal cell cultures after 3DIV showing TRPV4 positive cells (green) and cell nuclei (DAPI, blue) on flat Si **(A)** and Si NW **(B)** substrates, as well as Brn3a positive cells (green) and cell nuclei (DAPI, blue) on flat Si **(C)** and Si NW **(D)**. The Exposure Time (ET) required to image the cells cultured on Si NW substrates was increased by a factor that is indicated at the bottom left of each panel. Scale bar, 40 µm for all panels.

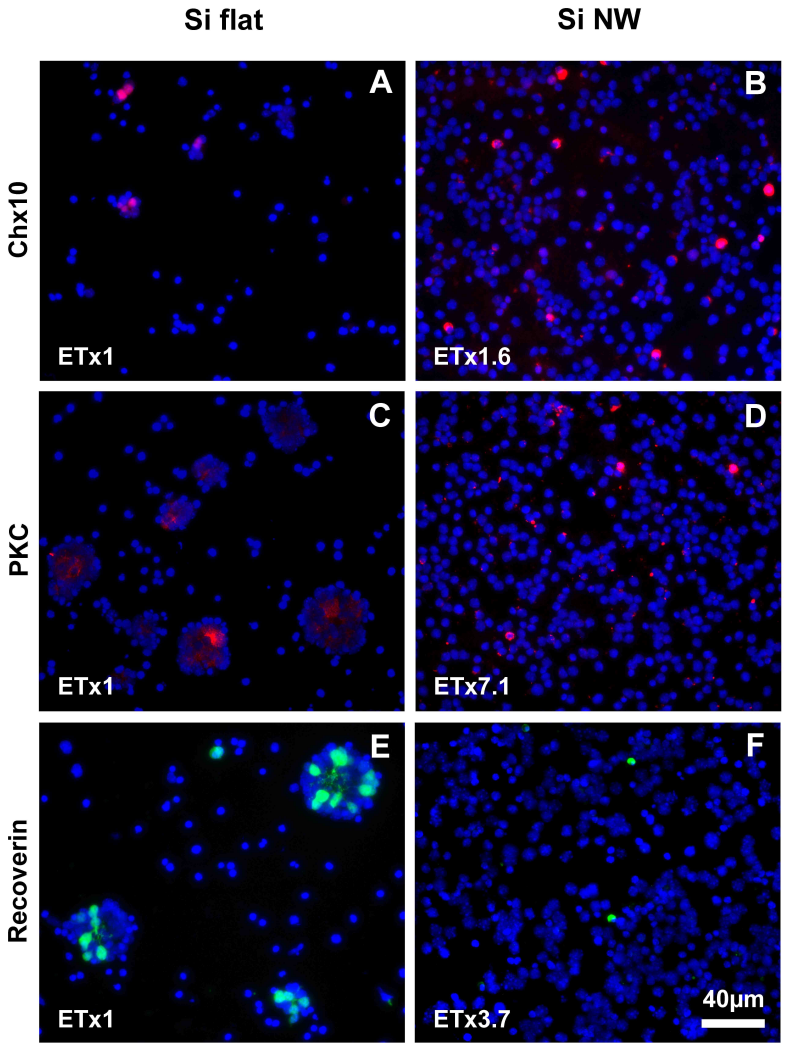


**Fig S3.** Fluorescence images of retinal cell cultures after 3DIV showing Chx10 positive cells (red) and cell nuclei (DAPI, blue) on flat Si **(A)** and Si NW **(B)** substrates, PKC positive cells (red) and cell nuclei (DAPI, blue) on flat Si **(C)** and Si NW **(D)** substrates, and recoverin positive cells (green) and cell nuclei (DAPI, blue) on flat Si **(E)** and Si NW **(F)** substrates. The Exposure Time (ET) required to image the cells cultured on Si NW substrates was increased by a factor that is indicated at the bottom left of each panel. Scale bar, 40 µm for all panels.

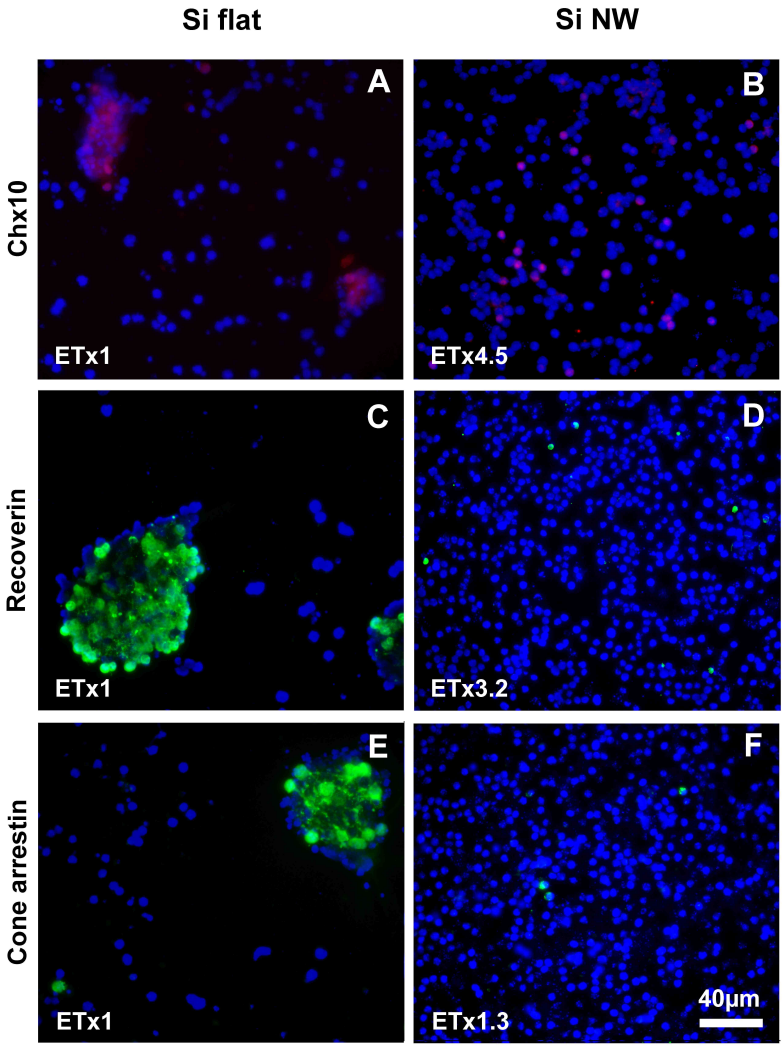


**Fig. S4.** Fluorescence images of retinal cell cultures after 18DIV showing Chx10 positive cells (green) and cell nuclei (DAPI, blue) on flat Si **(A)** and Si NW **(B)** substrates, recoverin positive cells (red) and cell nuclei (DAPI, blue) on flat Si **(C)** and Si NW **(D)** substrates, and cone arrestin positive cells (green) and cell nuclei (DAPI, blue) on flat Si **(E)** and Si NW **(F)** substrates. The Exposure Time (ET) required to image the cells cultured on Si NW substrates was increased by a factor that is indicated at the bottom left of each panel. Scale bar, 40 µm for all panels.

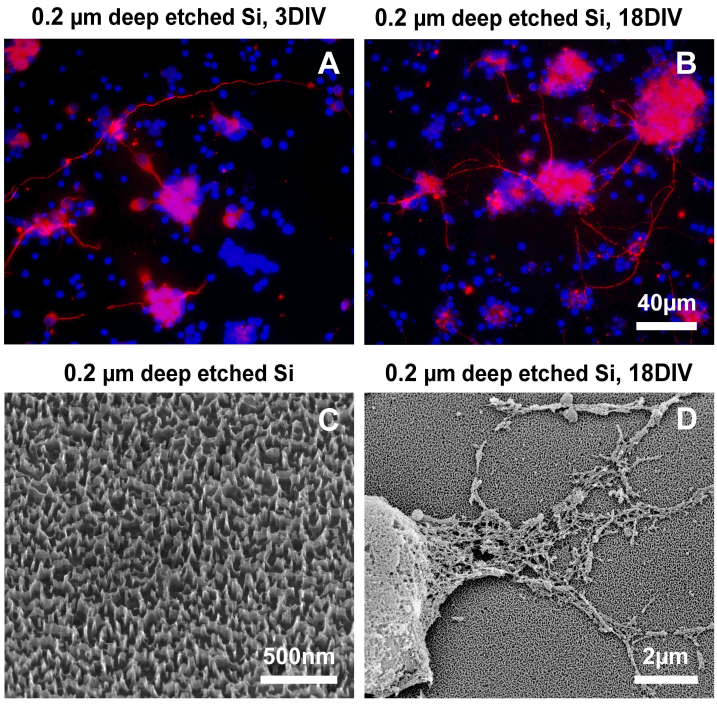


**Fig. S5.** Fluorescence images of retinal cell cultures showing cell nuclei (DAPI, blue) and β-tubulin III positive cells (red) on the 0.2 µm deep etched Si after 3DIV **(A)** and after 18DIV **(B)**. SEM images of the 0.2 µm deep etched Si before retinal cell culture **(C)**, and after 18DIV of culture **(D)**. Scale bar, 40 µm for fluorescence images. Scale bars, 500 nm **(C)** and 2 µm **(D)** for SEM images (tilt 30°).

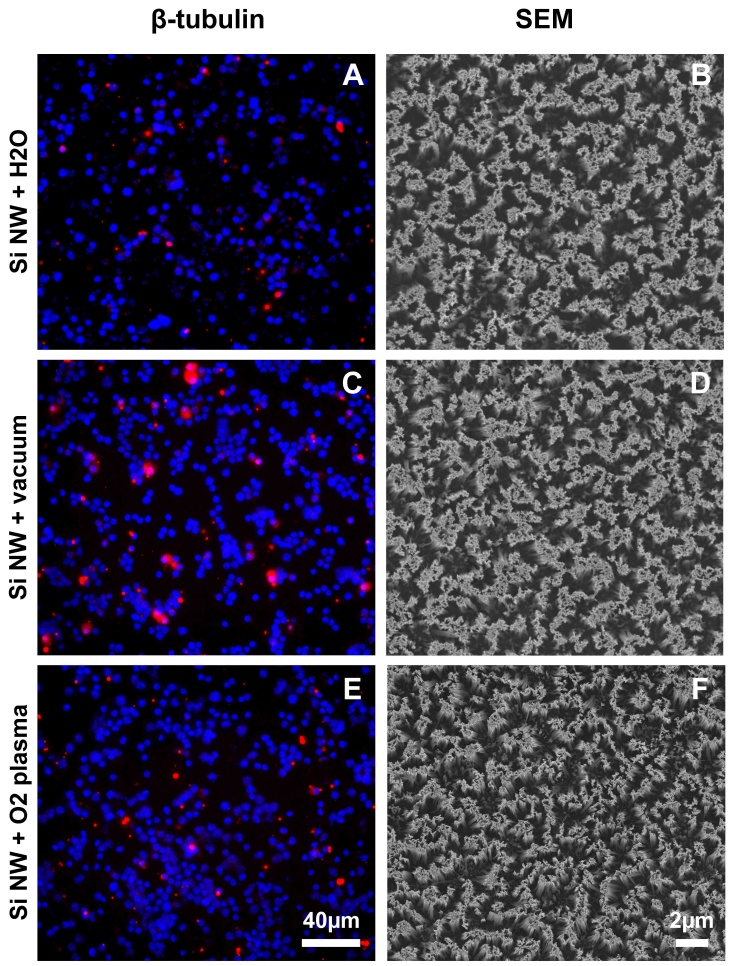


**Fig. S6.** Fluorescence images showing cell nuclei (DAPI, blue) and β-tubulin III positive cells (red) among cells cultured for 3DIV on Si NW substrates that were put through one of following three extra cleaning step: rinsed in water ($H_2O$) overnight **(A)**, exposed to high vacuum **(C)**, and exposed to an oxygen plasma ($O_2$; **E**). To the right (**B**, **D**, and **F)**, SEM images (top views) showing the Si NW substrates after each of the cleaning steps. Scale bar, 40 µm for fluorescence images. Scale bar, 2 µm for SEM images.

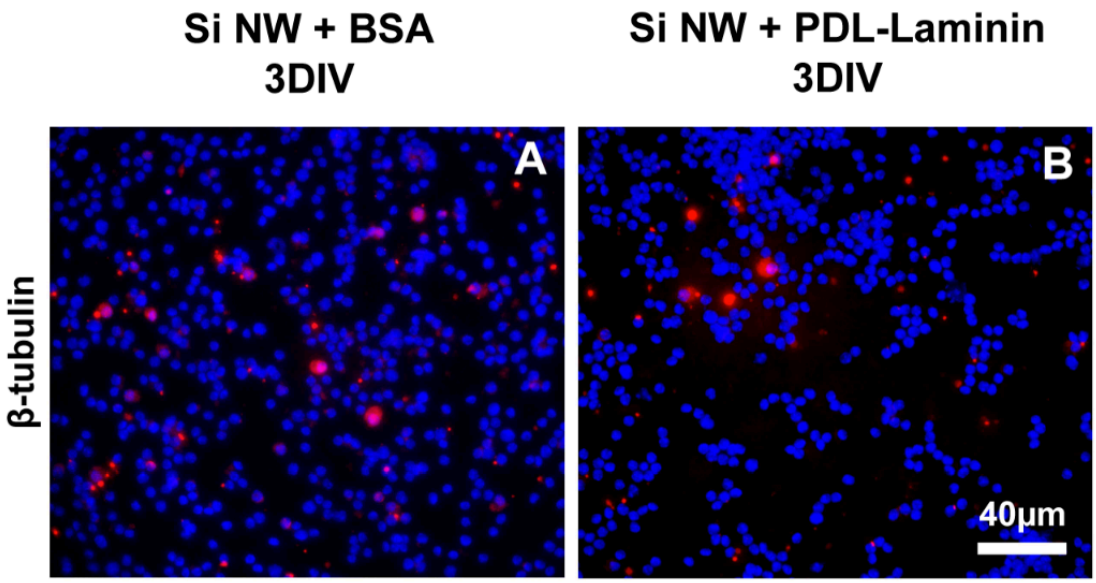


**Fig. S7.** Fluorescence images of retinal cell cultures showing cell nuclei (DAPI, blue) and β-tubulin III positive cells (red) after 3DIV on the Si substrates previously coated with BSA **(A)**, and on the Si substrates previously coated with PDL-Laminin **(B)**. Scale bar, 40 µm for both panels.

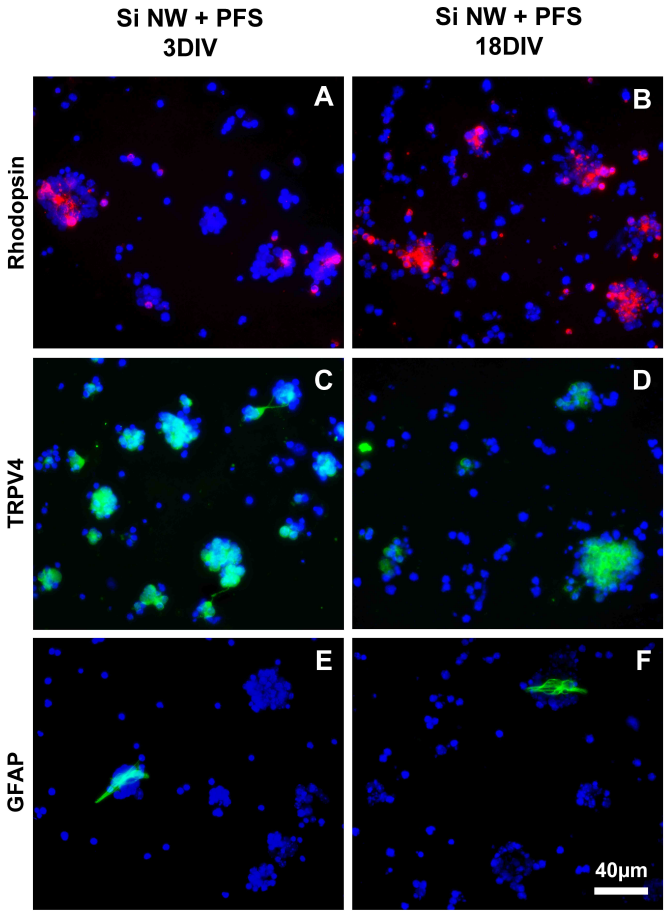


**Fig. S8.** Fluorescence images of retinal cells cultured on Si NW substrates functionalized with perfluorosilanes (PFS) showing rhodopsin positive cells (red) and cell nuclei (DAPI, blue) after 3DIV **(A)** and 18DIV **(B)**, TRPV4 positive cells (green) and cell nuclei (DAPI, blue) after 3DIV **(C)** and 18DIV **(D)** and GFAP positive cells after 3DIV **(E)** and 18DIV **(F)**. Scale bar, 40 µm for all panels.

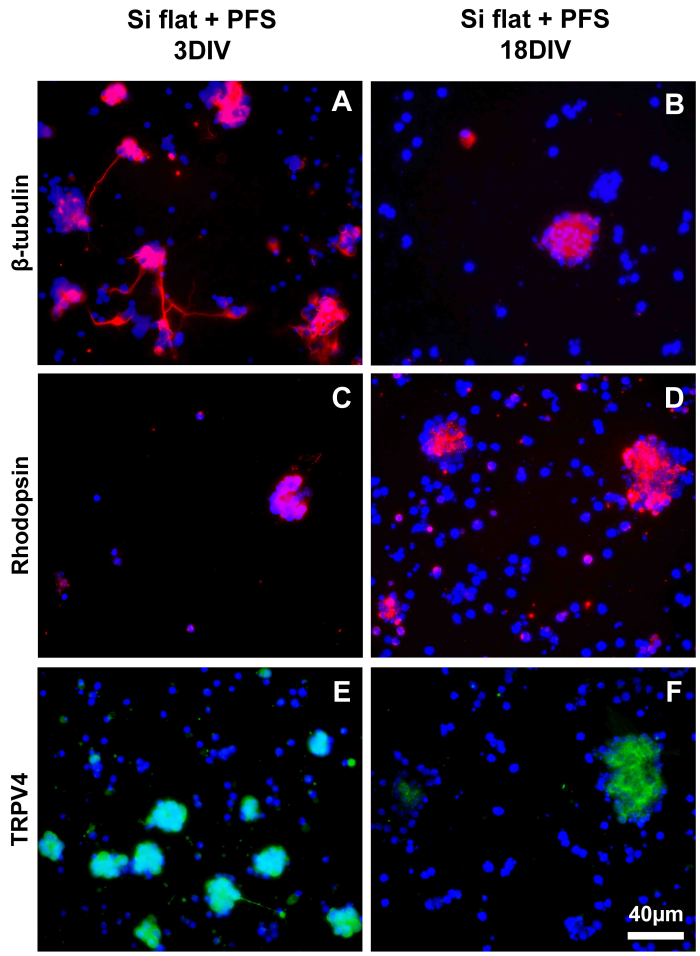


**Fig. S9.** Fluorescence images of retinal cells cultured on flat Si substrates functionalized with perfluorosilane (PFS) showing β-tubulin III positive cells (red) and cell nuclei (DAPI, blue) after 3DIV **(A)** and 18DIV **(B)**, rhodopsin positive cells (red) and cell nuclei (DAPI, blue) after 3DIV **(C)** and 18DIV **(D)**, and TRPV4 positive cells (green) and cell nuclei (DAPI, blue) after 3DIV **(E)** and 18DIV **(F)**. Scale bar, 40 μm for all panels.

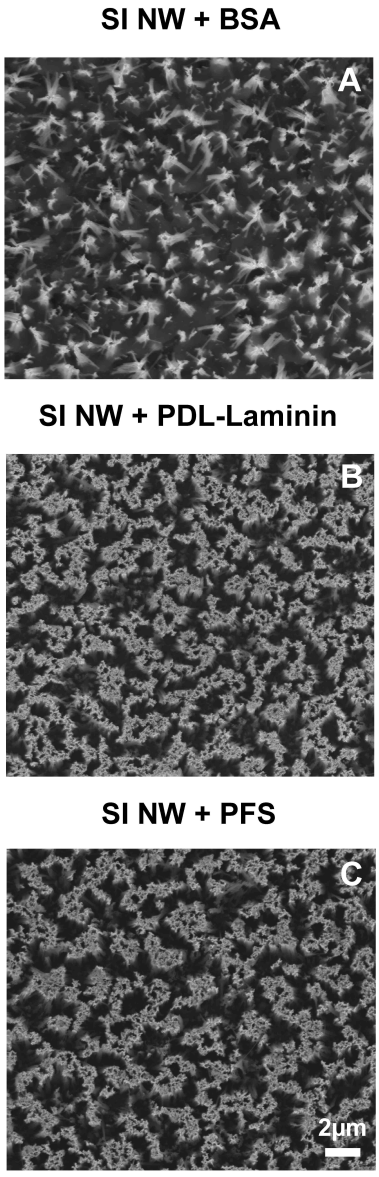


**Fig. S10.** SEM images (top views) of Si NW substrates after coating with BSA **(A)**, with PDL-Laminin **(B)**, and after functionalization with perfluorosilanes (PFS) **(C)**. Scale bar, 2 µm for all panels.