Supporting information

Hierarchical thermoplastic rippled nanostructures regulate Schwann Cell adhesion, morphology and spatial organization

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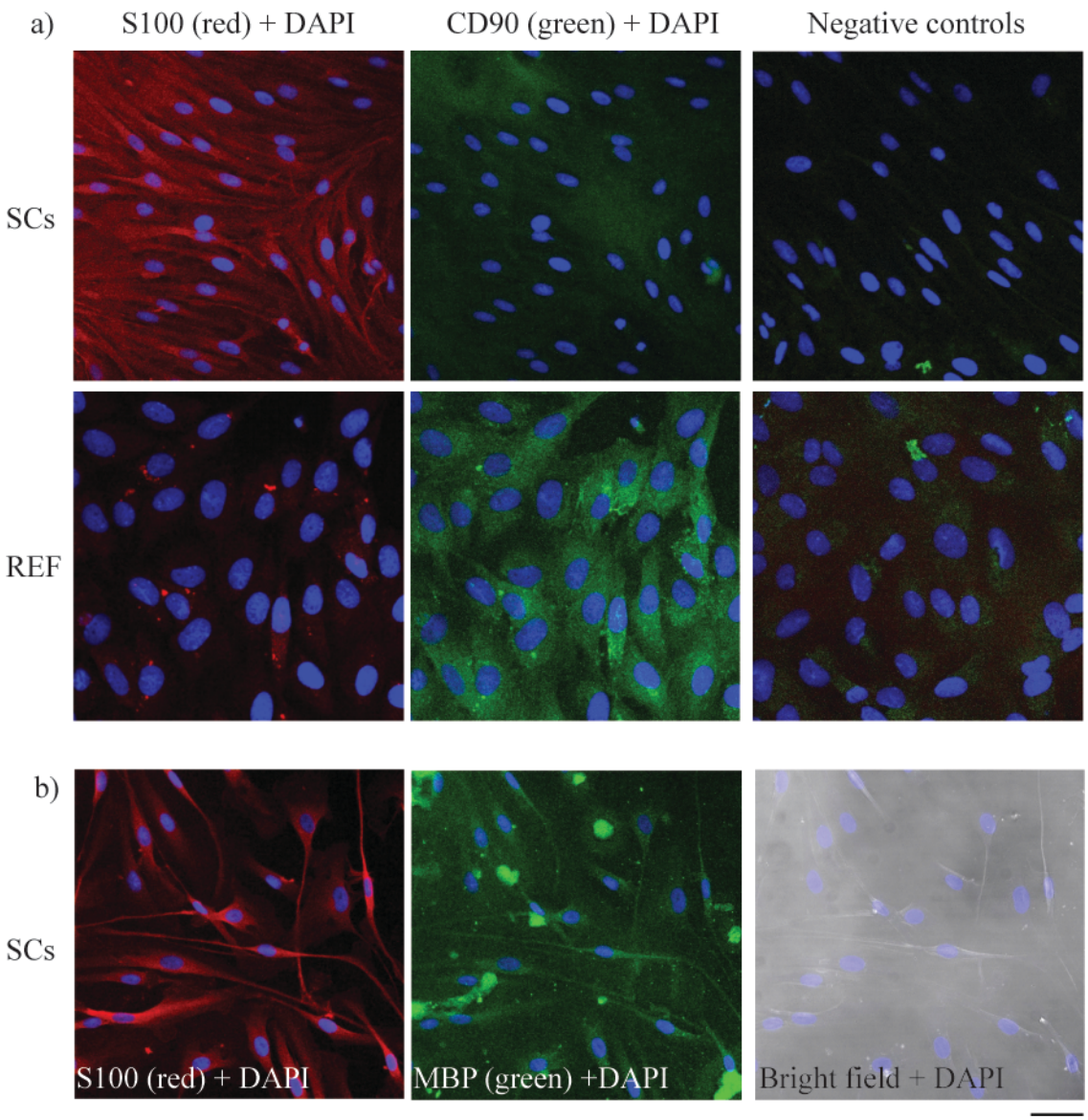

Figure S1. **a)** Confocal images of SCs (**first row**) and rat embryonic fibroblasts REF (**second row**) cultured for 4 days and immuno-stained for S100 (red, *first column*; specific SC marker) or CD90 (green, *second column*; specific fibroblast marker); all the samples (run and processed in parallel) were also stained for nuclei (blue; by DAPI). SC cultures showed specific S100 signal and no fibroblast contamination (CD90 negative); as positive control, REF fibroblasts showed no S100 staining and specific fibroblast CD90 signal.
Negative control experiments were also performed on SCs (*first row, last column*) and REF fibroblasts (*second row, last column*), incubating cells without the primary antibodies (but in presence of DAPI): staining was absent on sections that were incubated in such a solution (images show merged green and red channels). **b**) Confocal representative images of SCs immuno-stained for MBP (Abcam ab62631, 1:500; green) and S100 (red), with DAPI. Scale bar: 50 μm.

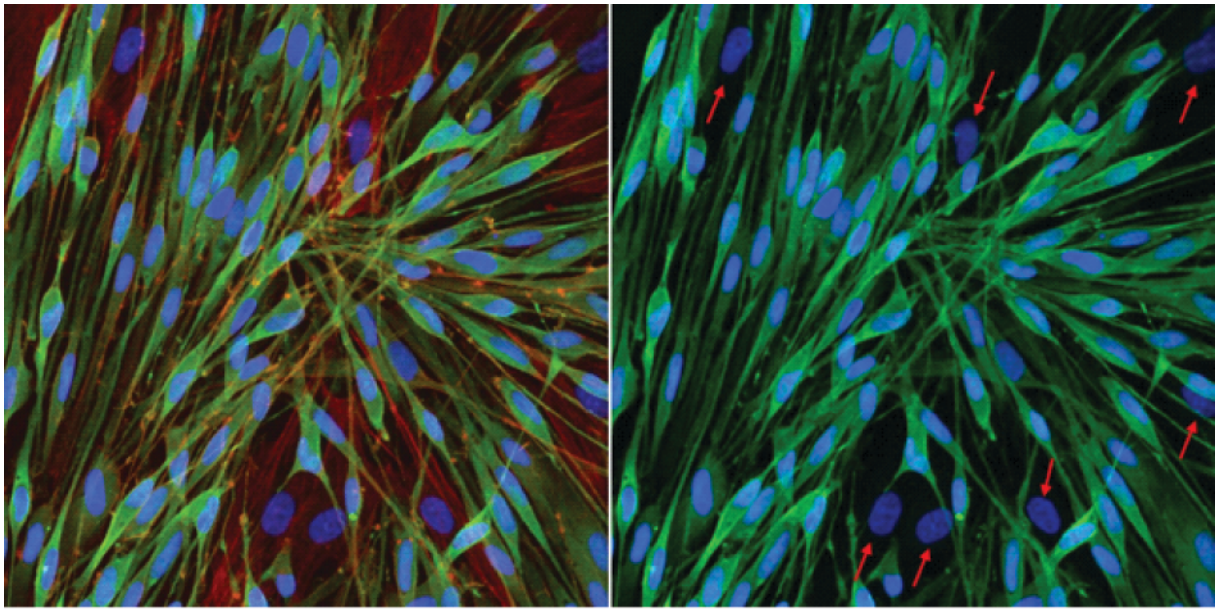

Figure S2. Representative confocal image of SCs before immunodepletion on Flat Pet substrate; SCs were immuno-stained for S100 (green; specific SC marker) and actin fibers (red), along with DAPI nuclear stining (blue). Fibroblasts are visible (*red large cells*) thanks to their large morphology and high actin fiber content; they (indicated by red arrows in the right panel) are also S100 negative (no green staining); SCs show instead S100 staining and lower actin fiber content. Scale bar: 50 μm.

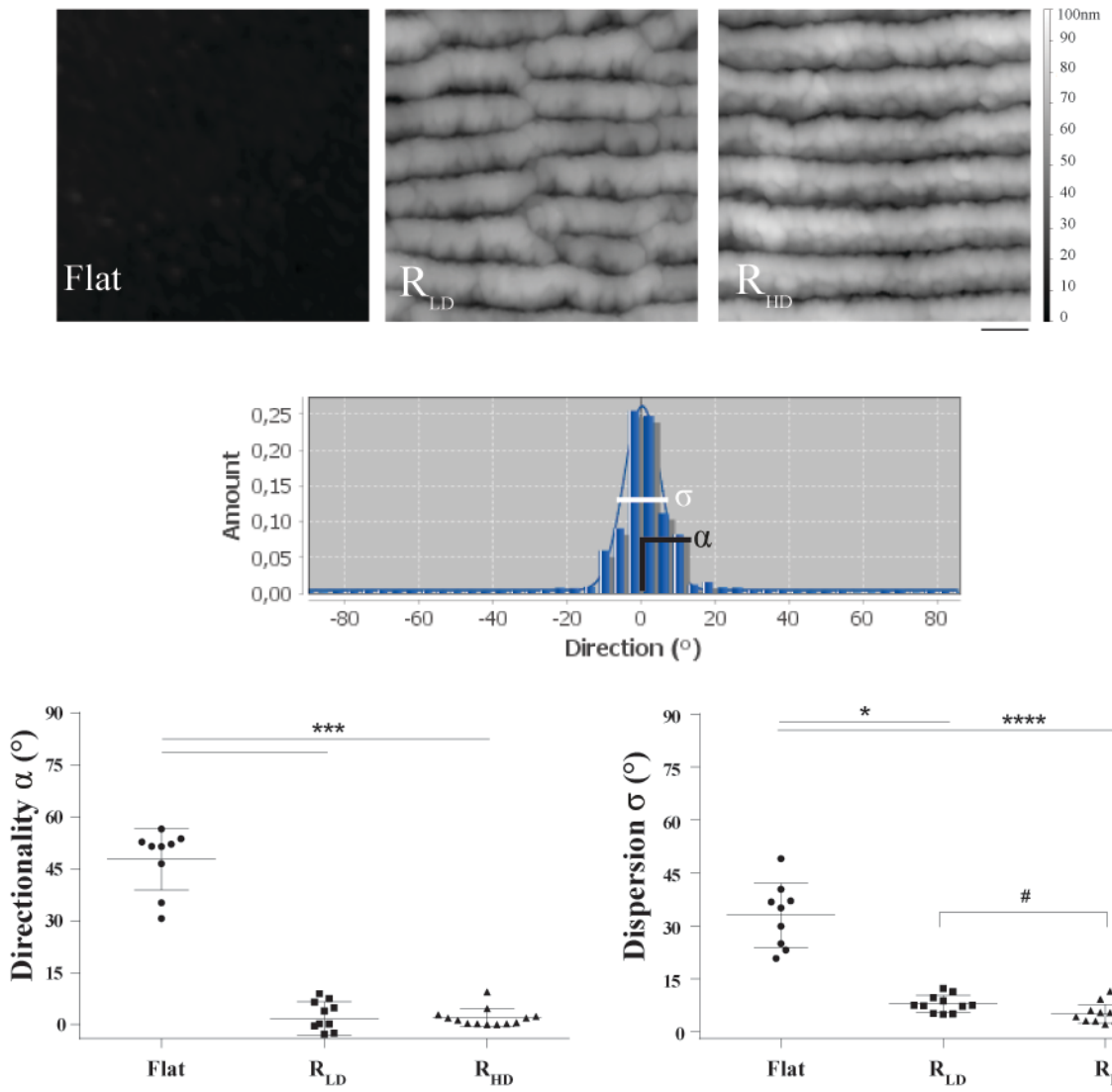

Figure S3. Nano-ripples directionality analysis. a) AFM images of Flat, R_{LD} and R_{HD} periodic nano-rippled PET surfaces; scale bar = 300nm. b) Representative image of fast Fourier transform (FFT) of R_{HD} topography. c) Directionality (α) and dispersion (σ) parameters calculated for the different substrates: */***/**** P<0.05/0.001/0.0001, One-Way ANOVA Kruskal-Wallis test; # P<0.05 R_{LD} vs. R_{HD}, Mann-Whitney test. Data= mean ± SD.

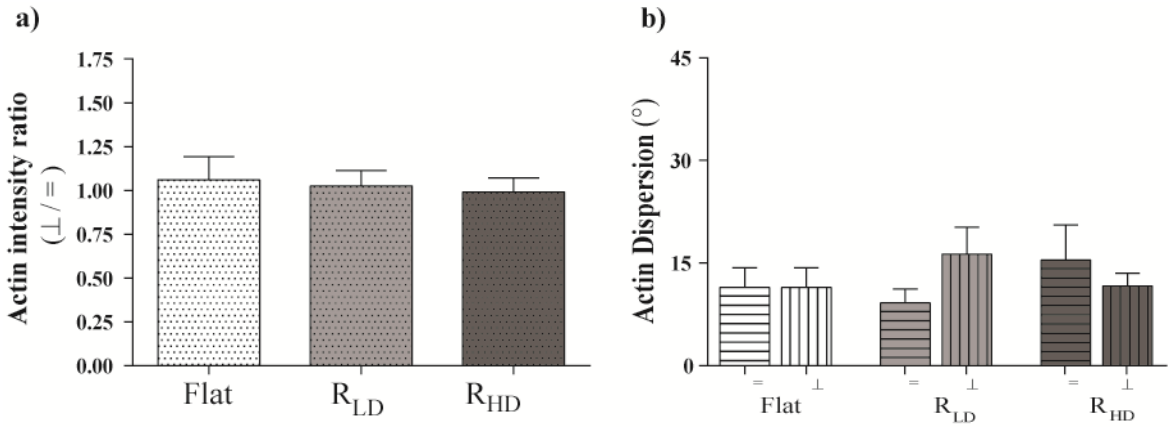

Figure S4. a) Intensity ratio between the actin signal of perpendicularly-oriented SCs protrusion tips and that of protrusion tips with parallel alignment, for each substrate. b) FFT actin dispersion (°) for perpendicularly-oriented SCs (⊥; i.e. with alignment angle ≥ 60° vs. pattern direction) and SCs with parallel orientation (=; i.e. with alignment angle ≤ 30° vs. pattern direction). n ≥ 3. Data = mean ± SEM.