Supporting Information

YAP/TAZ cytoskeletal remodelling is driven by mechanotactic and electrotactic cues

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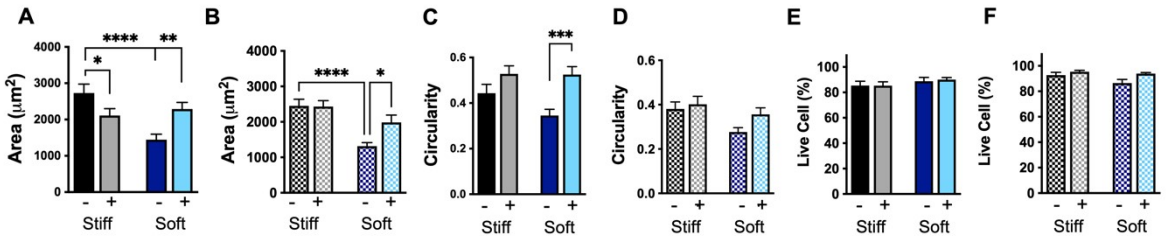

Figure S1. The EF affects cell morphology but not vitality. (A-D) Effect of the EF on the whole cell area of (A) U251-MG cells and (B) GL15 and the circularity of (C) U251-MG (n=32 for Stiff, n=37 for Soft substrates) and (D) GL15 cells (n=45 for Stiff, n=44 for Soft substrates). Note that cells exposed to an EF assumed a rounder morphology. (E-F) Cell viability upon EF exposure was evaluated by staining (E) U251-MG cells and (F) GL15 with calcein and propidium iodide to visualize live cells. For each condition, 3 different technical replicates were carried out and cell viability was calculated as the percentage of live cells on the total number of cells. (G-H). Significance was determined using two-way ANOVA followed by Tukey's multiple comparisons test with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$.

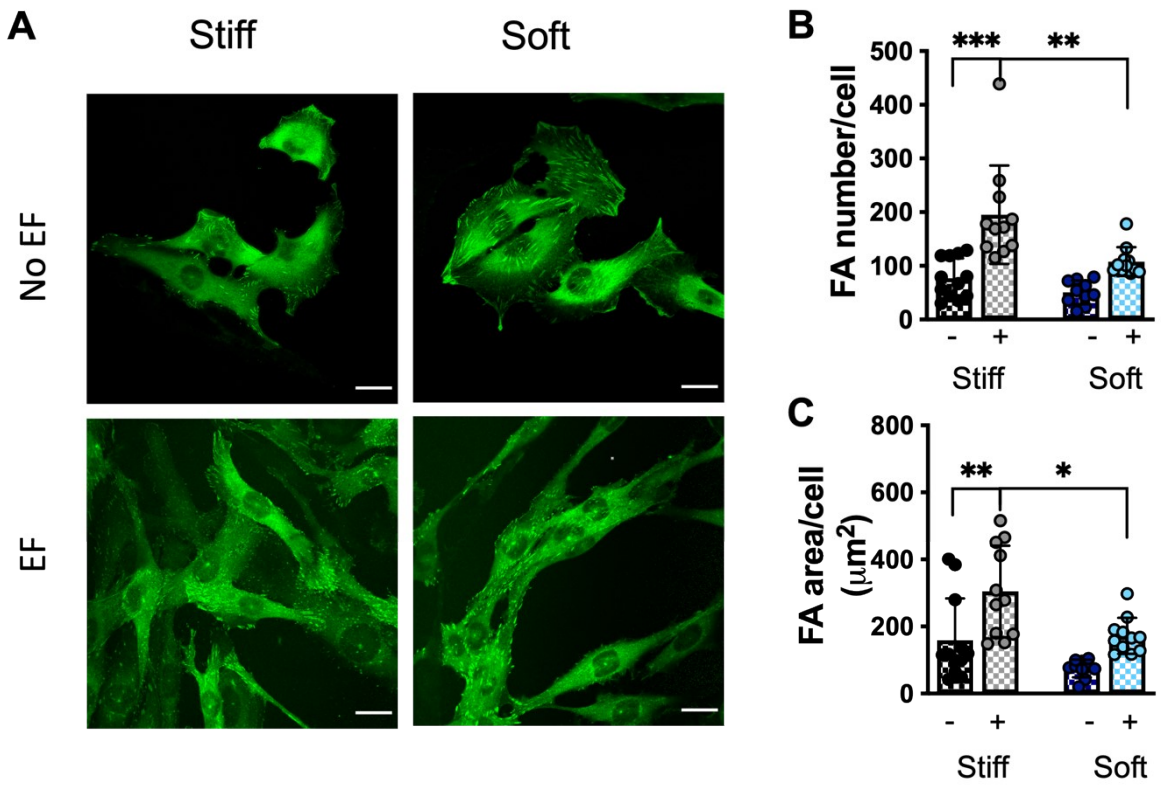

Figure S2. FA distribution in GL15 cells is affected by the EF. (A) Representative confocal images of GL15 cells stably expressing PXN-GFP (green) plated onto substrates of different stiffness with and without the effect of a 3 h EF treatment. Scale bar 25 μm. (B-C) Bar graphs showing quantification of the number of FAs per cell (FA number) (B) and total FA area (C). (Untreated: n= 12 for Stiff, n=10 for Soft substrates, EF: n=11 for Stiff, n=11 for Soft substrates for 3 different technical replicates). Significance was determined using two-way ANOVA followed by Tukey's multiple comparisons test with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$.

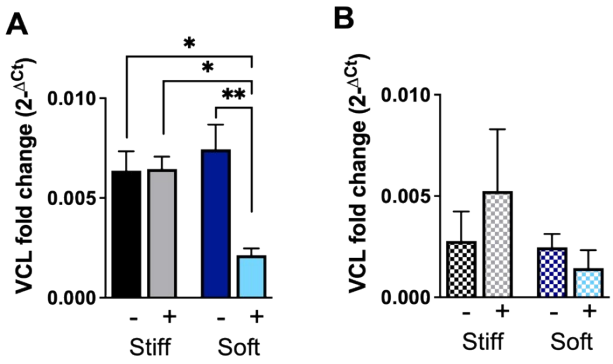

Figure S3. EF regulates VCL expression. *VCL* expression in U251-MG cells (A) and GL15 (B) were validated by qPCR. Values are expressed as means ± S.E.M., for at least 3 different technical replicates. Significance was determined using two-way ANOVA followed by Tukey's multiple comparisons test with * $p < 0.05$, ** $p < 0.01$.

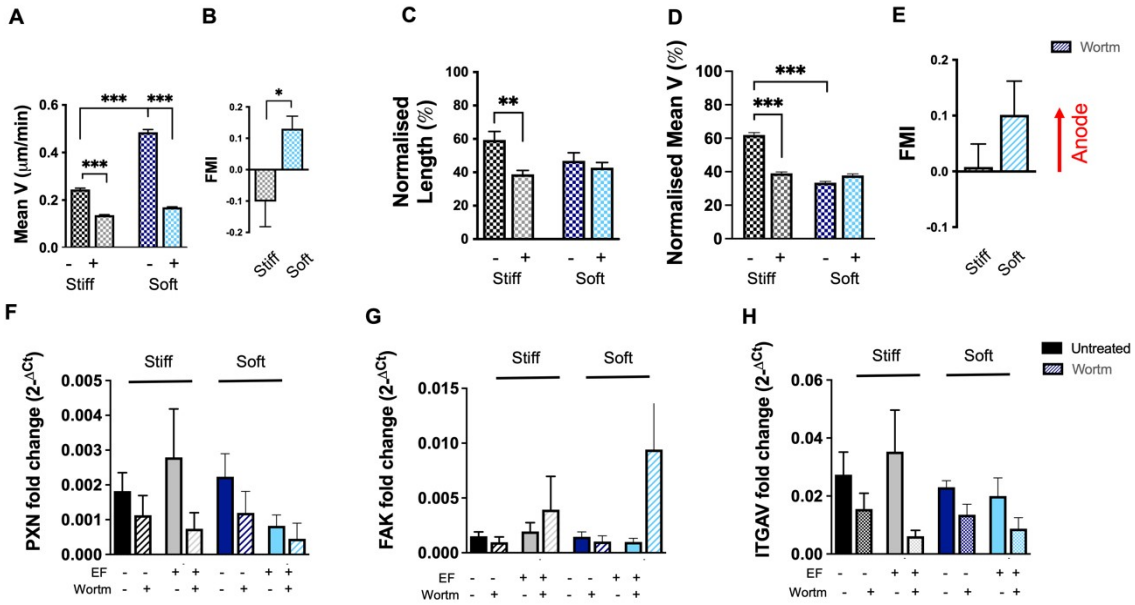

Figure S4. The EF regulates cell migration overriding mechanotactic cues of GL15 cells. (A-B) Motility parameters of PI3K-inhibited GL15 cells normalised to untreated GL15 cells on their respective substrates, showing path length (A) and velocity (B) without (-) and with (+) an EF, (Untreated: n=61 for Stiff, n=46 for Soft substrates, EF: n=46 for Stiff, n=71 for Soft substrates). (C-E) Normalised motility values of GL15- PI3K-inhibited cells to untreated

cells on their respective substrates, showing path length (C) and motility (D), with (+) and without (-) an EF, and their FMI distribution under an EF (E) showing that PI3K-inhibited cells reverted their directionality on Stiff substrates from cathodic to anodic (PI3K treated cells with EF: n=45 for Stiff, n=93 for Soft substrates). (F-H) Gene expression levels of *PXN* (F), *FAK* (G) and *ITGAV* (H) were determined using qPCR. Data are from at least three independent experiments, two-way ANOVA followed by Tukey's multiple comparisons test, ***$p < 0.001$, and unpaired two-tailed Mann-Whitney U test was run between each pair of groups, *$p<0.05$. Values are expressed as means ± S.E.M.

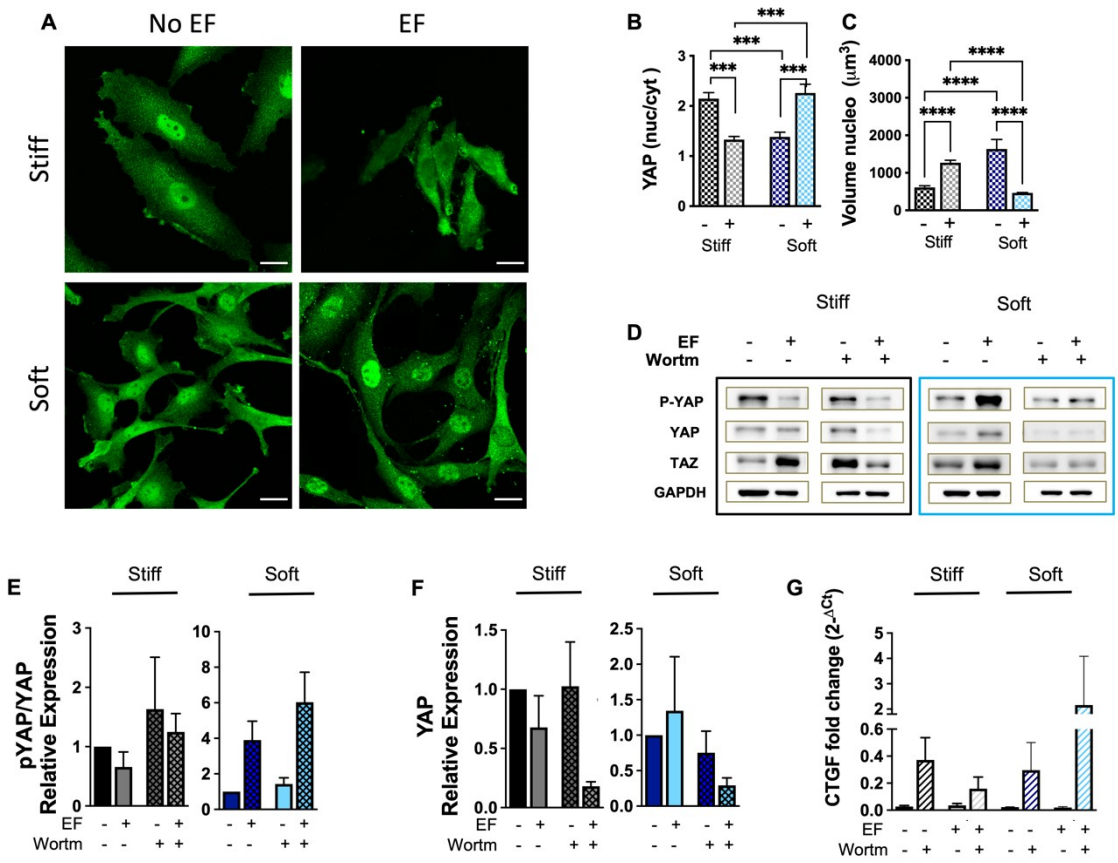

Figure S5. Effect of the EF on YAP expression in GL15 cells. (A) Representative confocal images of GL15 cells cultured on substrates of different rigidities depicting YAP localization with/without exposure to an EF of 2V/cm. Cells were stained with anti-YAP (green). Scale bar 25 µm. (B) Quantification of the YAP nuclear/cytoplasmic ratio in response to substrate

rigidity and exposure to an EF. (Untreated: n=39 for Stiff, n=33 for Soft substrates, EF: n=35 for Stiff, n=29 for Soft substrates, from at least 3 technical replicates) (C) Quantification of nuclear volume of GL15 cells on substrates of different stiffness and w/wo EF (Untreated: n=64 for Stiff, n=48 for Soft substrates, EF: n=57 for Stiff, n=100 for Soft substrates, from at least 3 technical replicates). (D) Representative western blot membranes of YAP, TAZ, p-YAP ^{Ser127} and GAPDH protein expression of GL15 cells with (+) and without (-) an EF and with (+) and without (-) treatment with the PI3K inhibitor (Wortm = wortmannin), cultured on substrates of different rigidity. (E-F) Bar graphs showing the protein quantification of p-YAP^{Ser127}(E) and YAP (F) in wortmannin-treated GL15 cells. Protein expression is normalized to untreated cells not exposed to EFs. Data are normalised to GAPDH expression from at least 3 technical replicates, p-values calculated with the unpaired two-tailed Mann-Whitney U test, *$p<0.05$; **$p<0.01$. (G) RT-qPCR quantification of the canonical YAP target genes *CTGF* in U251-MG cells with and without PI3K-inhibition cultured on substrates of different rigidity and w/wo exposure to an EF of 2 V/cm. Gene expression levels were normalized to GAPDH in all RT-qPCR experiments from at least 3 technical replicates. Three-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Data are expressed as means ± S.E.M.

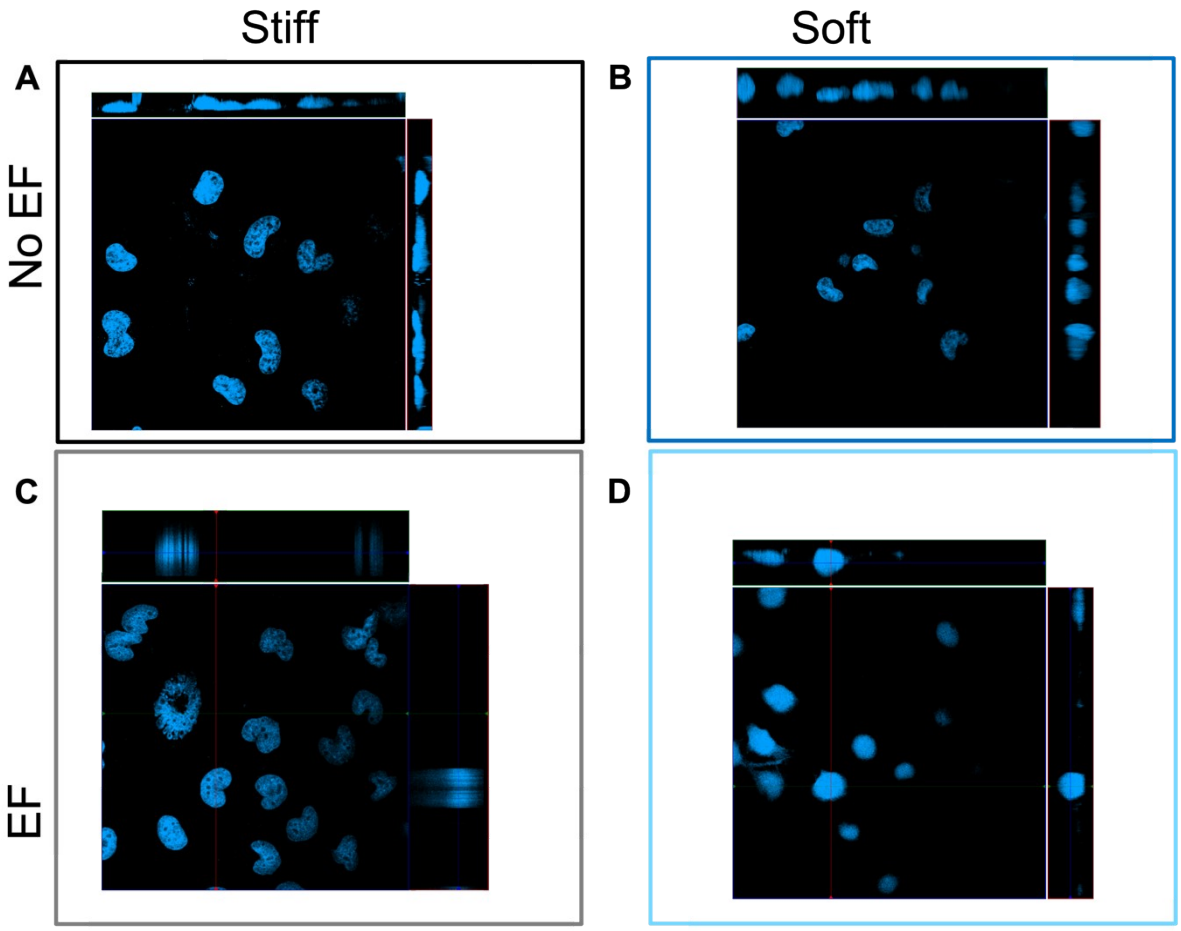

Figure S6. Representative confocal laser scanning microscopy ortho-images of the nuclear volume of U251-MG cells. The x/y planes correspond to the top views layers and the marginal images correspond to the cross-sections for nuclear volumes of cells without exposure to an EF on (A) Stiff and (B) Soft and after exposure to an EF on (C) Stiff and (D) substrates. The images are representative of at least three independent experiments.

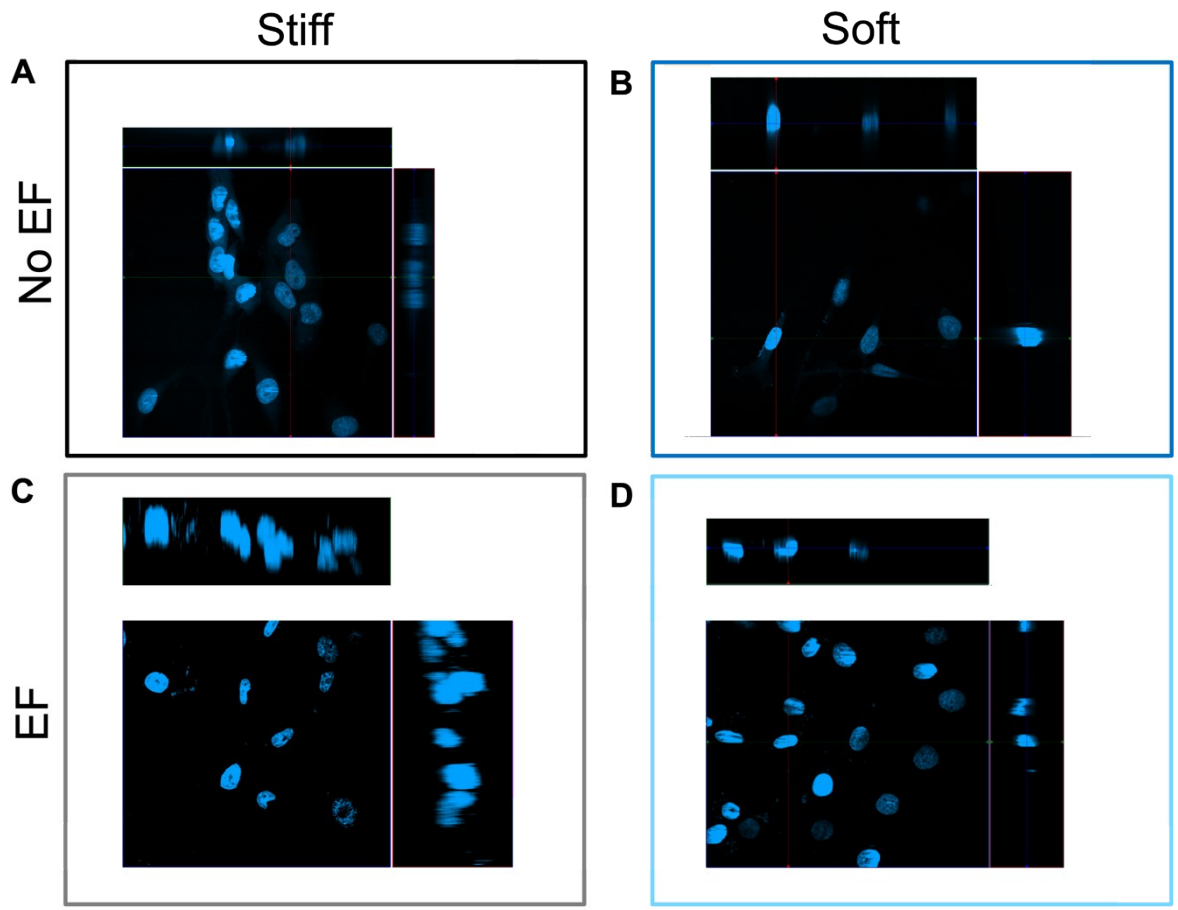

Figure S7. Representative confocal laser scanning microscopy ortho-images of the nuclear volume of GL15 cells. The x/y planes correspond to the top views layers and the marginal images correspond to the cross-sections for nuclear volumes of cells without exposure to an EF on (A) Stiff and (B) Soft and after exposure to an EF on (C) Stiff and (D) substrates. The images are representative of at least three independent experiments.

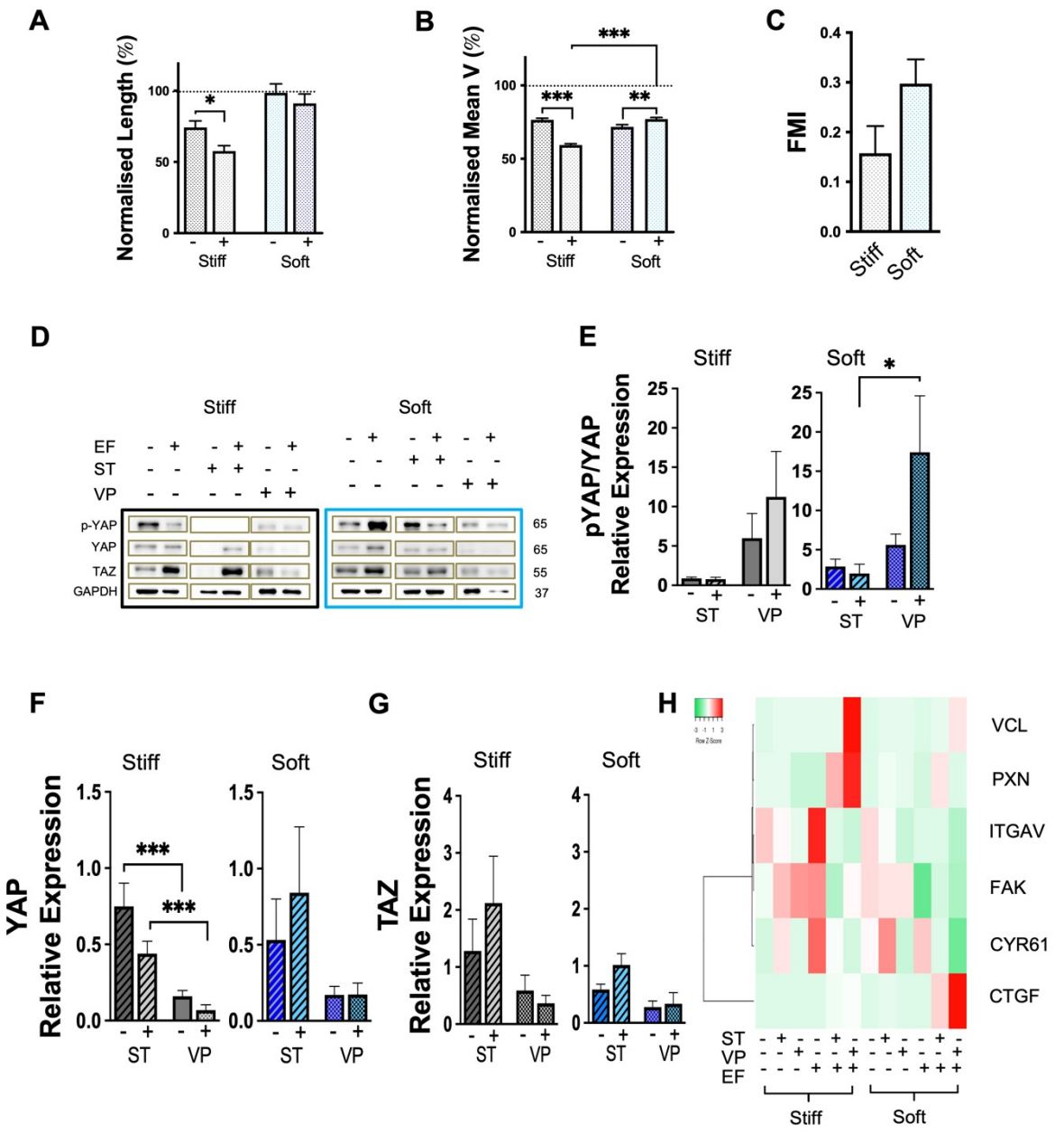

Figure S8. Effects of YAP-TEAD interaction inhibition on motility and YAP expression in GL15 cells. (A-C) Motility parameters of GL15 cells treated with ST. Values are normalised to their respective untreated cells, seeded on substrates of different rigidity, showing the effects of inhibition w/wo exposure to an EF of 2 V/cm. Bar graphs quantifying (A) path length, (B) instantaneous speed and (C) directionality of migration. ST-treated cells without EF: n=88 for Stiff, n=76 for Soft substrates; EF: n=67 for Stiff, n=51 for Soft substrates; from at least 3 different technical replicates, two-way ANOVA followed by Tukey's multiple comparisons test, * $p < 0.05$, ** $p < 0.01$, ***$p<0.001$, and unpaired two-tailed Mann-Whitney U test,

**p<0.01. (D) Representative western blot membranes of YAP, TAZ, p-YAP ^{Ser127} and GAPDH protein expression of GL15 cells with (+) and without (-) an EF and with (+) and without (-) treatment with the ST and VP inhibitors. (E-G) Quantification of p-YAP^{Ser127} (E) YAP (F) and TAZ (G) protein levels in GL15 cells after treatment with ST and VP and w/wo an EF. Values are normalised to untreated cells on their respective substrates. Data are normalised to GAPDH expression from at least 3 technical replicates, two-way ANOVA followed by Tukey's post hoc test for multiple comparisons, * $p < 0.05$, ***p<0.001. Values are expressed as means ± S.E.M. (H) Heatmap visualisation of RT-qPCR analysis of mRNA levels of *CYR61*, *CTGF*, *PXN*, *FAK*, *VCL* and *ITGAV* expression in TEAD-inhibited GL15 cells with (+) and without (-) EF. Relative quantification of each gene expression level was normalised according to the GAPDH gene expression. Columns represent treatments with (+) and without (-) EF and, of untreated (-), ST-inhibited (+) and VP-inhibited (+) GL15 cells. Green represents downregulation, and red represents upregulation. Colour intensity represents the mRNA expression values. Rows, mRNA of the genes.

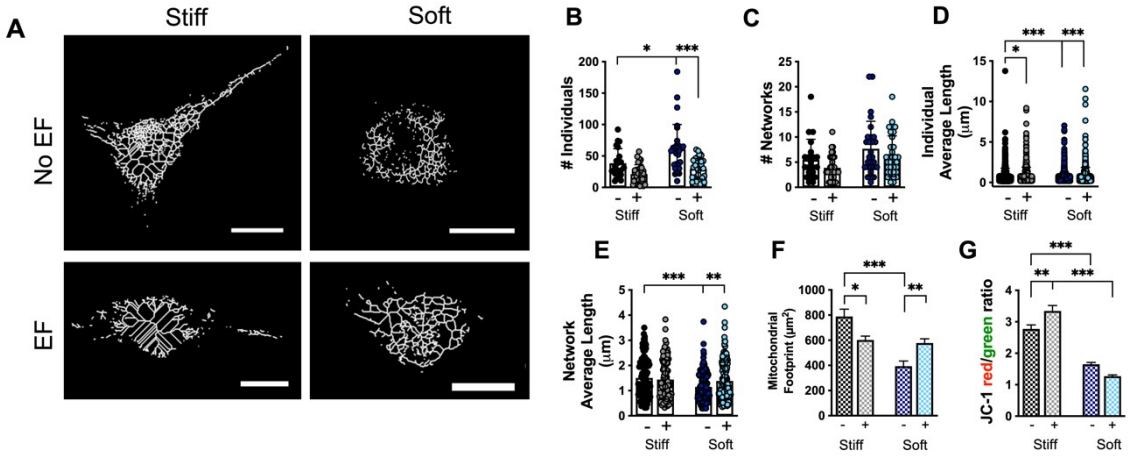

Figure S9. Mitochondria of GL15 cells are affected by mechanotactic and electrotactic cues. (A) Representative mitochondrial skeletonised structures obtained from GL15 cells cultured on substrates of different rigidity w/wo 3 h of EF (2 V/cm), scale bar 25 μm. Bar graphs quantifying (B) number of individuals, (C) number of networks, (D) mean summed branch lengths, (E) mean network branches and (F) mitochondrial footprint (Untreated: n=21 for Stiff, n=28 for Soft substrates, EF: n=33 for Stiff, n=34 for Soft substrates for at least 10 different confocal acquisitions). (G) Analysis of mitochondrial membrane potential using JC-1 staining. Cells were exposed to EF on substrates of different rigidity and fluorescence transition from red to green was evaluated. (Untreated: n=166 for Stiff, n=127 for Soft substrates, EF: n=125 for Stiff, n=88 for Soft substrates. Data are from at least three independent experiments, two-way ANOVA followed by Tukey's multiple comparisons test, * $p < 0.05$, ** $p < 0.01$, ***$p<0.001$. Values are expressed as means ± S.E.M.