Supplemental Information: Mechanical activation and expression of HSP27 in epithelial ovarian cancer

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Supplemental Figures.

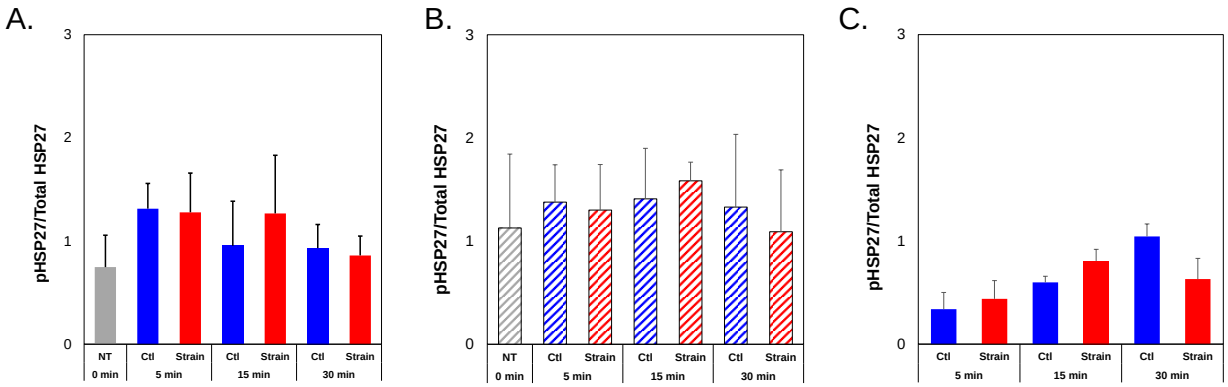

Figure S1. Changes in phosphorylated HSP27 relative to total HSP27 protein levels. Data is re-plotted from Figures 3 and 7 for (A) SKOV-3, (B), SKOV-3.tr, and (C) OVCAR-8 cell line exposed to varying time courses of strain.

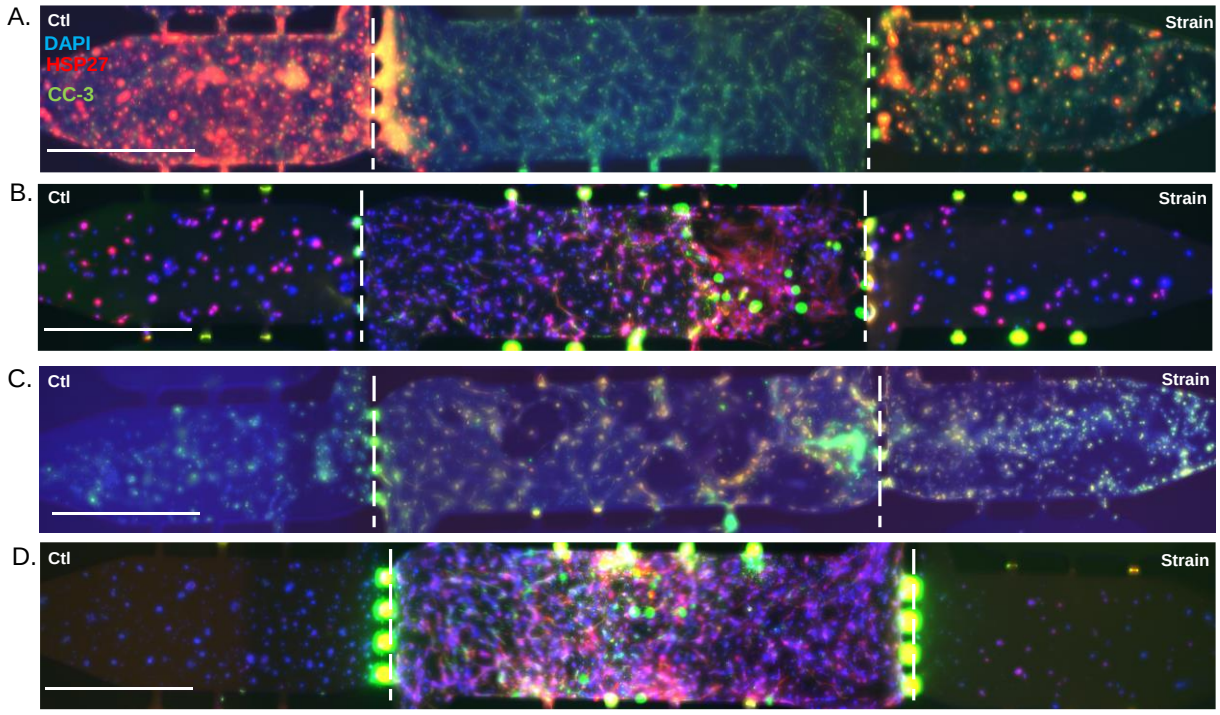

Figure S2. Representative full-length fluorescence images of 3D microtissues models. Systems are loaded with either SKOV-3 cells or SKOV-3.tr cells and treated with Paclitaxel. Systems are stained for HSP27 (TXRED), CC-3 (GFP), and DAPI. (A,B) Devices with SKOV-3 cells placed under oscillatory tensile strain for 24h (A) or 72h (B) vs control (non-strained cells) in the side chambers. (C,D) Devices with SKOV-3.tr cells placed under oscillatory tensile strain for 24h (C) or 72h (D) vs control (non-strained cells) in the side chambers. Scale bar = 200µm. White dashed lines show interfaces between chamber regions. These images contain all chambers and all fluorescence channels from Fig. 5.

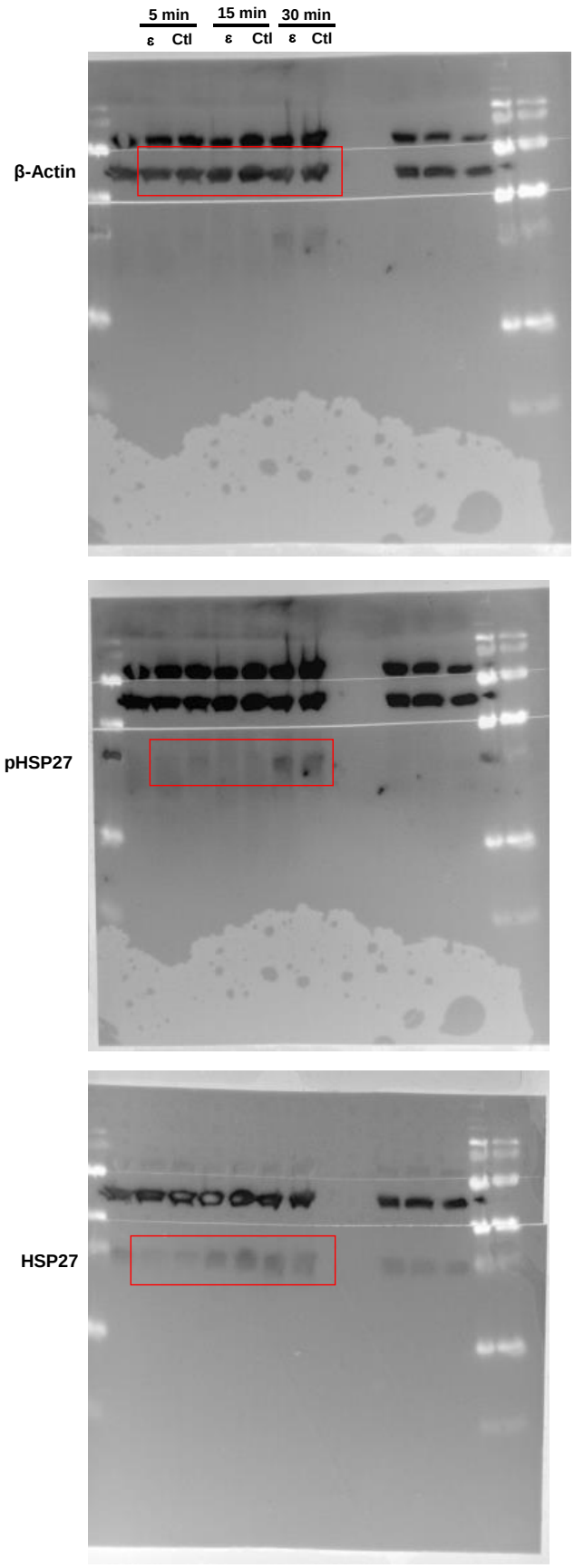
5 min
15 min
30 min
ε
Ctl
ε
Ctl
ε
Ctl
β-Actin
pHSP27
HSP27

Figure S3. Uncropped Western blot images for SKOV-3 cells treated with strain. Data shown in Fig. 3, including both pHSP27, total HSP27, and β-actin loading controls, is highlighted with red boxes. All samples originated in the same experiment, and membranes were processed in parallel. Additional bands are internal controls or different experiments not included in this manuscript. Different exposure times are shown for the different proteins, due to inconsistencies with binding affinities and resulting intensities for the different primary antibodies. Total HSP27 was measured on the same membrane after processing with stripping solution; this is the reason why the image is not perfectly aligned with the β-actin and pHSP27 images. White bands are ladder (PageRuler Plus, ThermoFisher #26619). All images have been post-processed with the same techniques to ensure accurate quantification and comparisons.

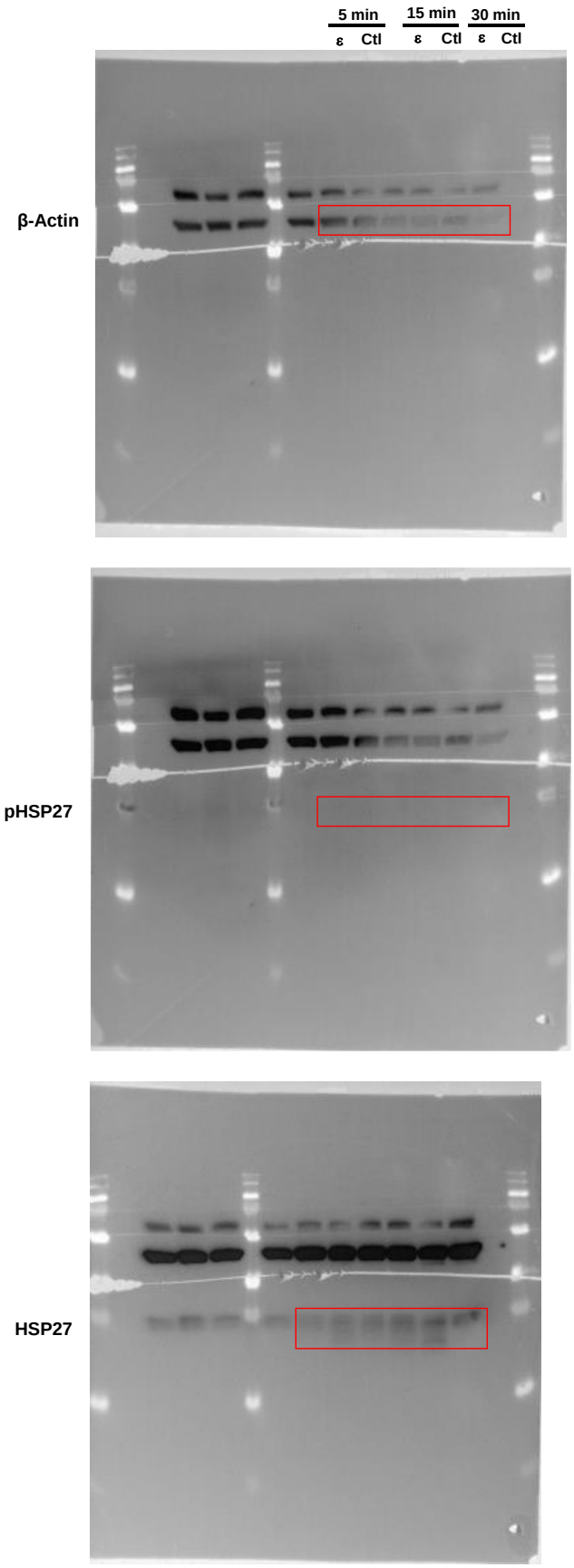
5 min
15 min
30 min
ε
Ctl
ε
Ctl
ε
Ctl
β-Actin
pHSP27
HSP27

Figure S4. Uncropped Western blot images for SKOV-3.tr cells treated with strain. Data shown in Fig. 3, including both pHSP27, total HSP27, and β-actin loading controls, is highlighted with red boxes. All samples originated in the same experiment, and membranes were processed in parallel. Additional bands are internal controls or different experiments not included in this manuscript. Different exposure times are shown for the different proteins, due to inconsistencies with binding affinities and resulting intensities for the different primary antibodies. Total HSP27 was measured on the same membrane after processing with stripping solution; this is the reason why the image is not perfectly aligned with the β-actin and pHSP27 images. White bands are ladder (PageRuler Plus, ThermoFisher #26619). All images have been post-processed with the same techniques to ensure accurate quantification and comparisons.

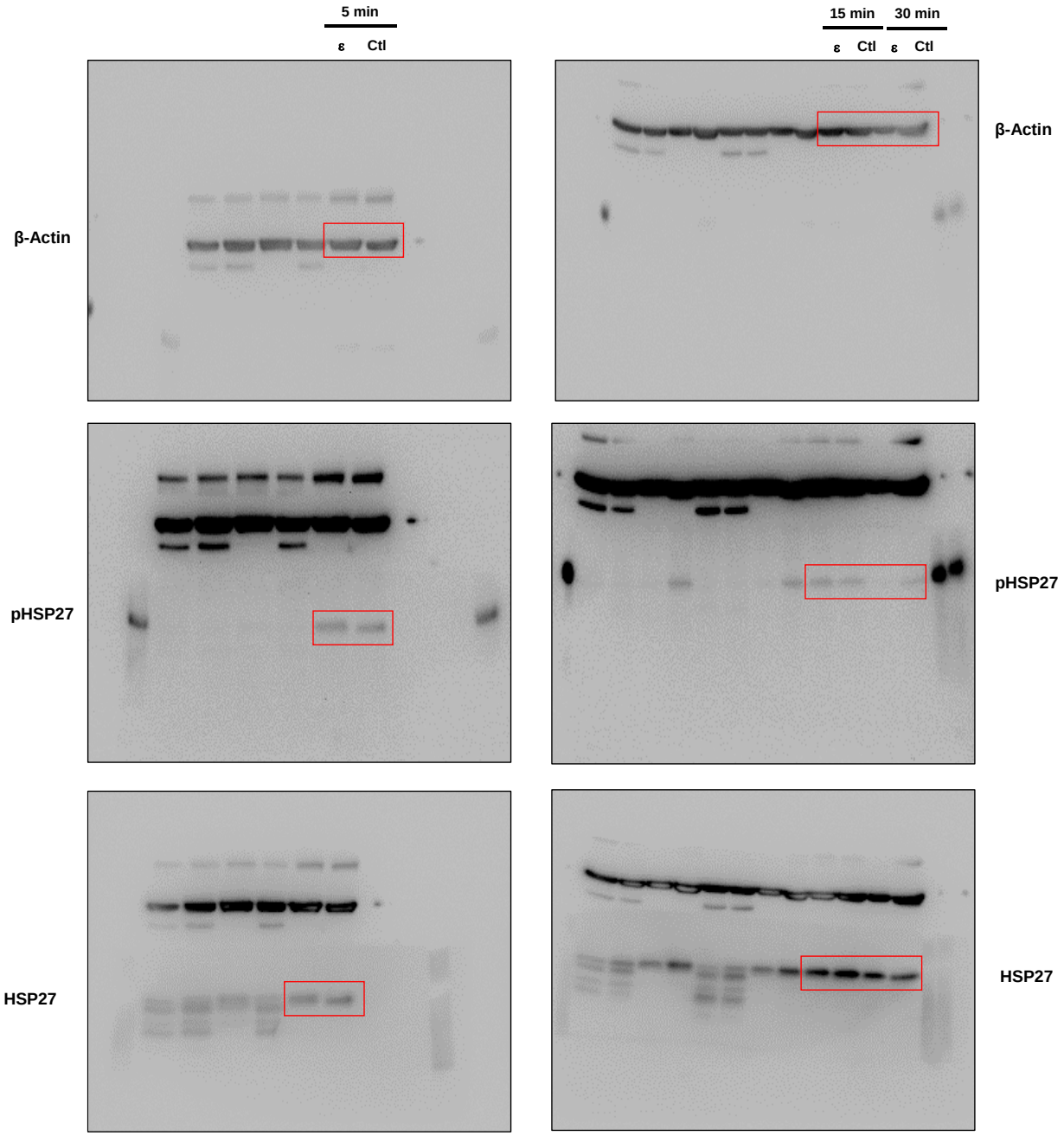

Figure S5. Uncropped Western blot images for OVCAR-8 cells treated with strain. Data shown in Fig. 7, including both pHSP27, total HSP27, and β-actin loading controls, is highlighted with red boxes. All samples originated in the same experiment, and membranes were

processed in parallel. Different exposure times are shown for the different proteins, due to inconsistencies with binding affinities for the different primary antibodies. Additional bands are internal controls or different experiments not included in this manuscript. Total HSP27 was measured on the same membrane after processing with stripping solution; this is the reason why the image is not perfectly aligned with the β-actin and pHSP27 images. All images have been post-processed with the same techniques to ensure accurate quantification and comparisons.