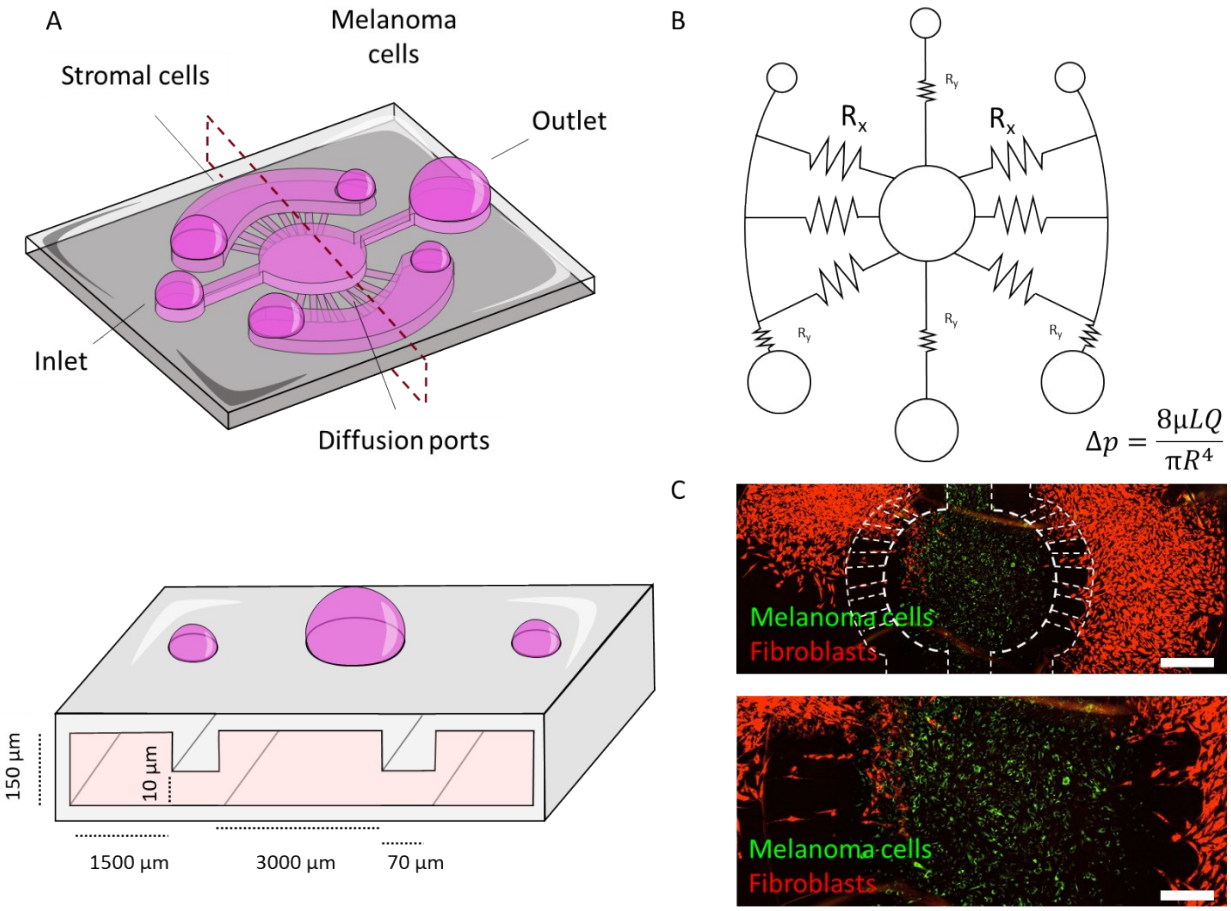
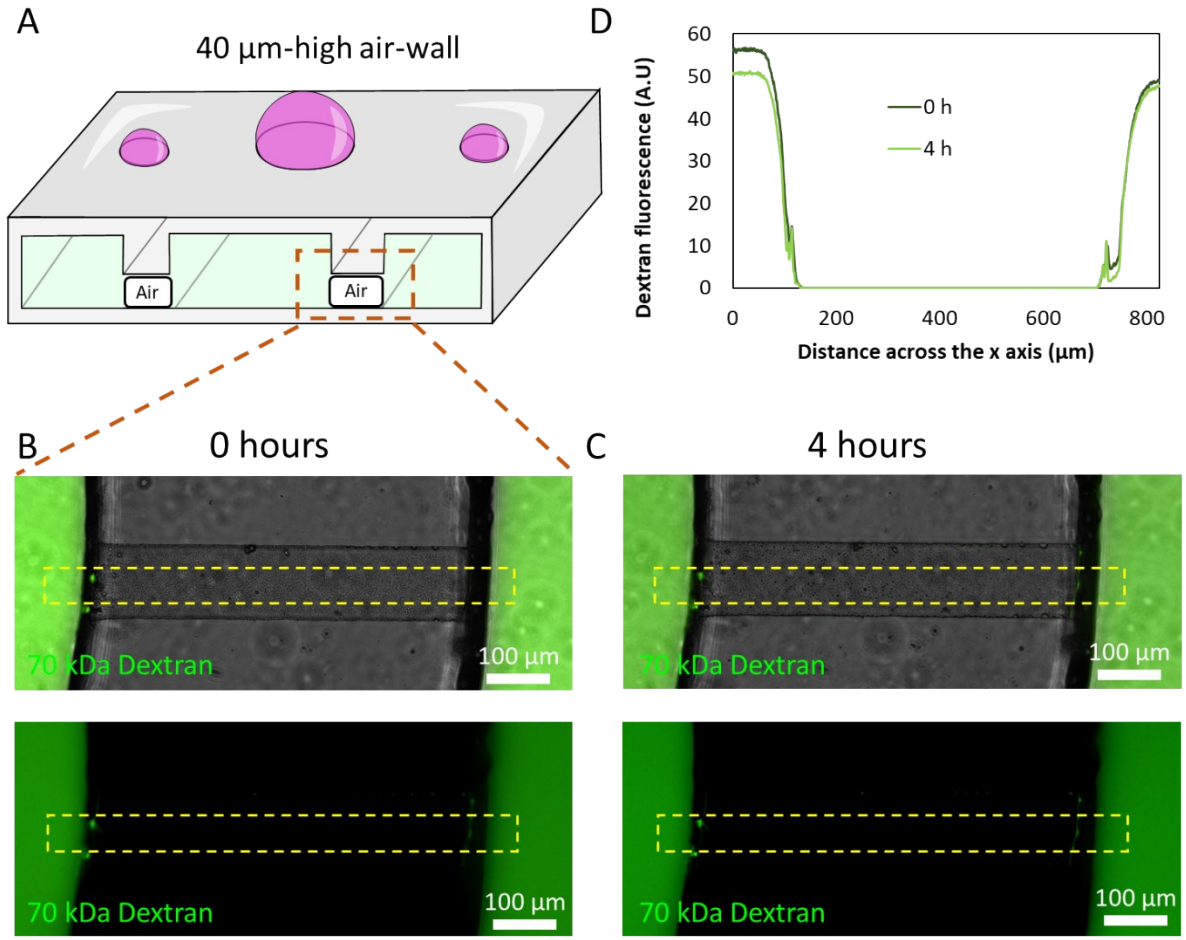
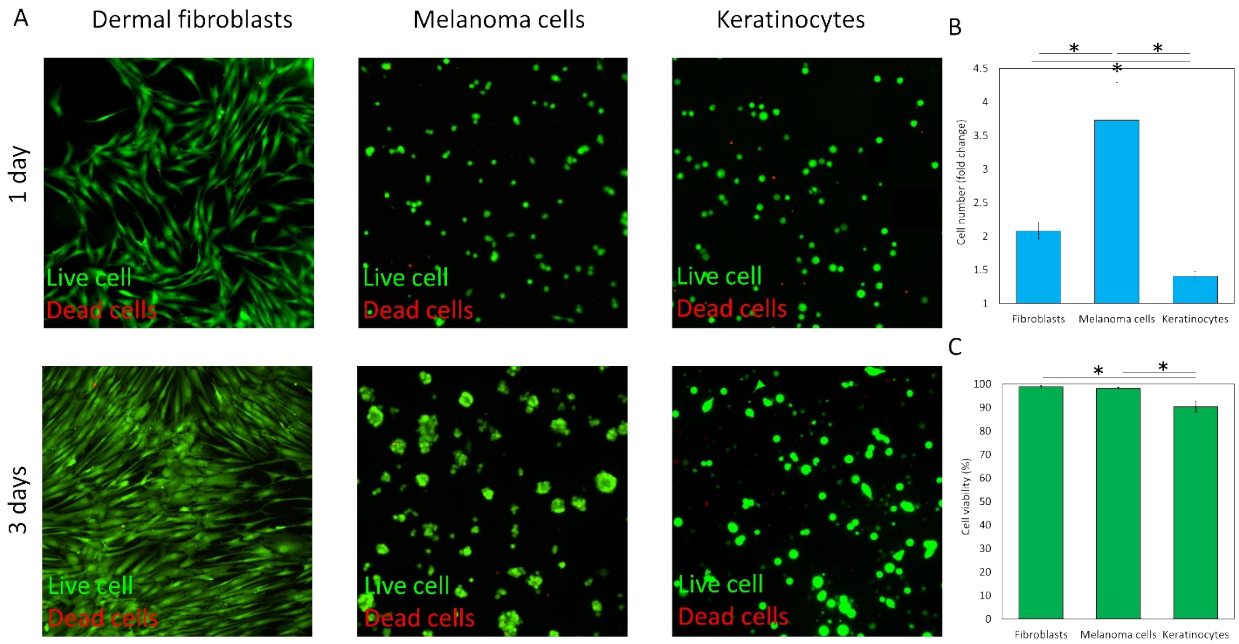
Electronic Supplementary Material (ESI) for Lab on a Chip.



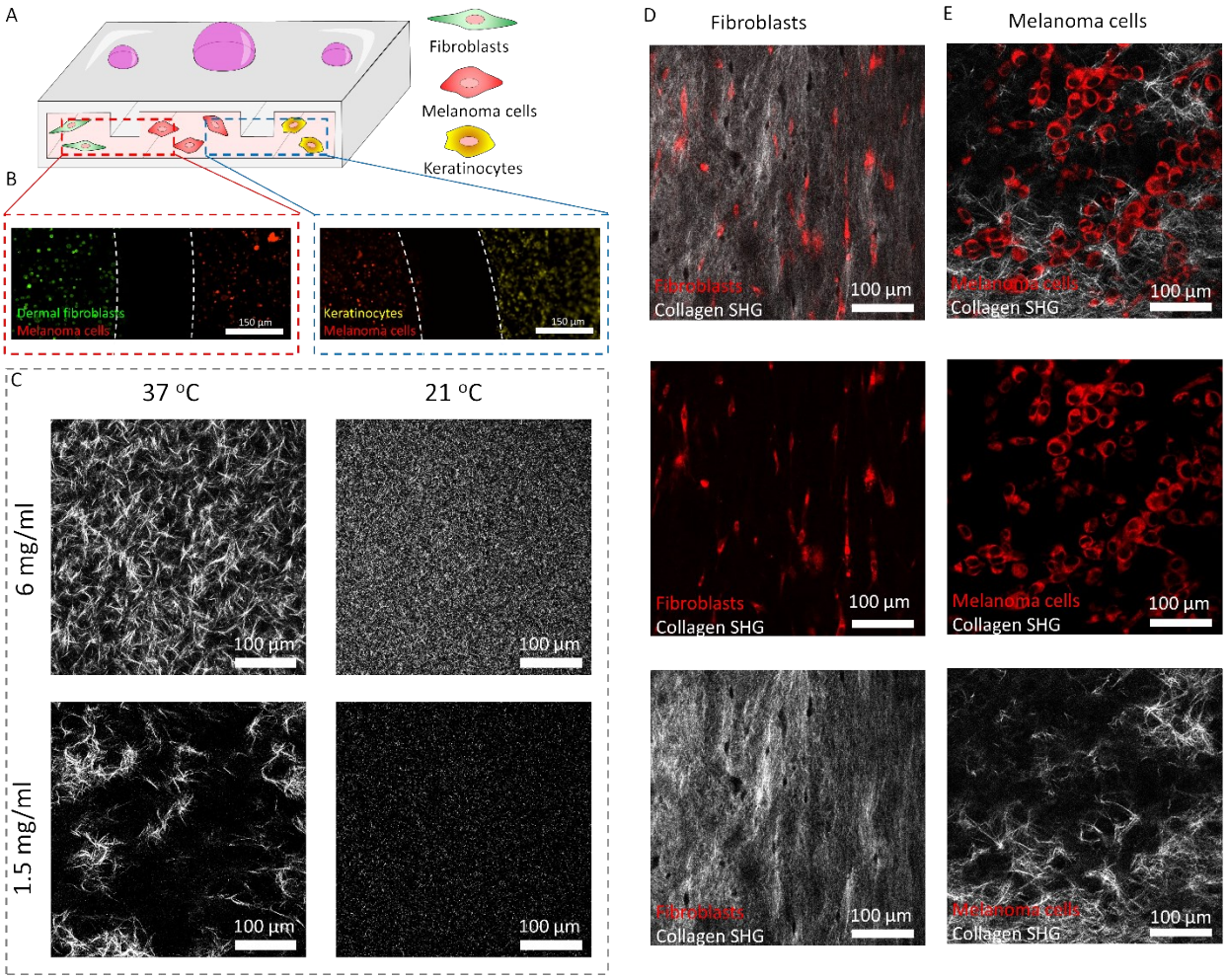
Supporting Figure 1. Cell confinement based on fluidic resistance. A) Microdevice scheme. The connection microchannels present a smaller cross-section compared with central channel connecting with the central microchamber. B) Schematic representation of the microdevice demonstrating the difference in fluidic resistance generated by the connection microchannels compared with the path defined by the central chamber and central channel. Bottom right shows the Hagen–Poiseuille equation where Δp is the pressure difference between the two ends, μ is the dynamic viscosity, L is the length of the channel, Q is volumetric flow rate, and R is the radius of the channel. C) Confocal image showing the confinement achieved by fluidic resistance using melanoma cells (in green) and dermal fibroblasts (in red). Fibroblasts can be observed inside the central chamber, demonstrating the presence of crossflow from the lateral chambers towards the central chamber.



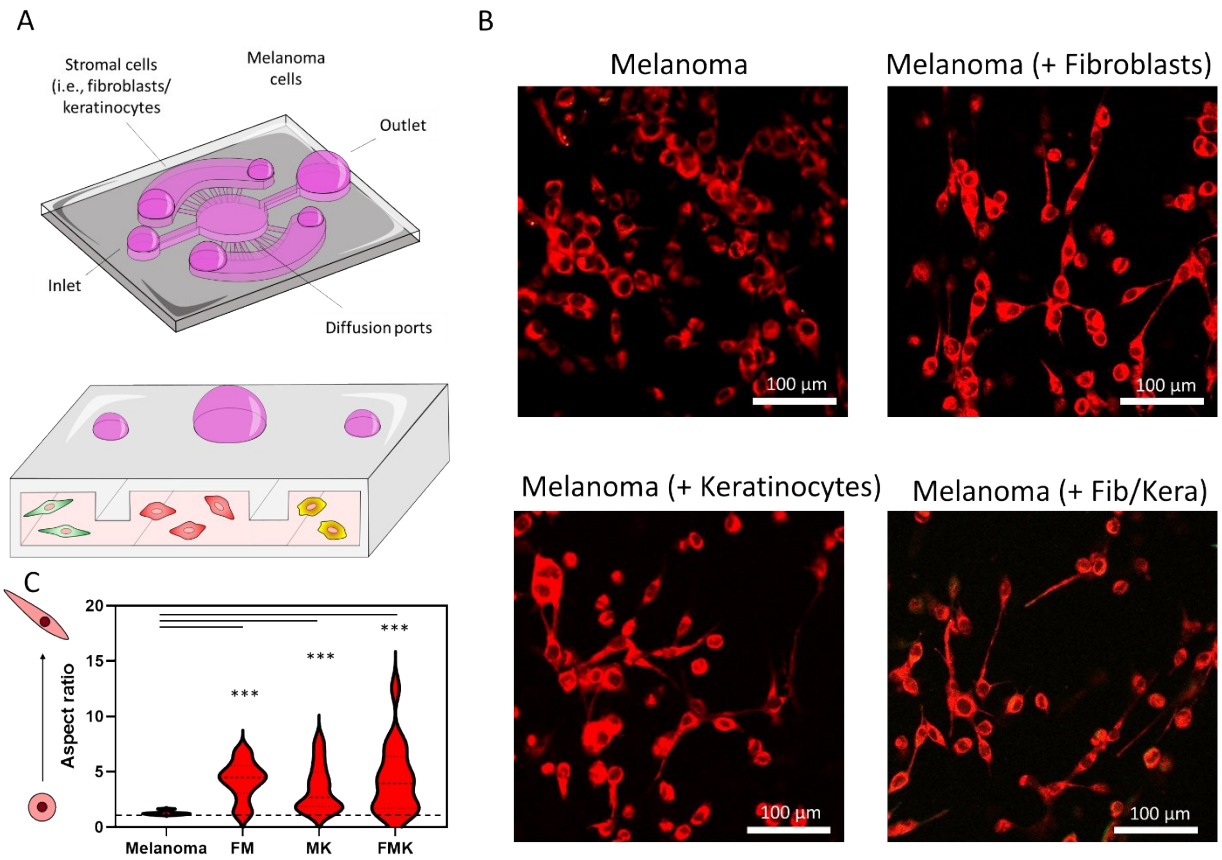
Supporting Figure 2. Air-walls prevent molecular diffusion. A) Schematic showing a 40µm-high air-wall separating a 10µM fluorescent 70kDa-dextran solution. The dextran solution was perfused in the central and lateral chambers, generating an air-wall in the connection channel. B-C) Fluorescence microscopy images showing dextran fluorescence at 0 and 4 hours post-dextran injection. D) Graph analyzed the fluorescent profile across the delimited region in B and C (i.e., yellow rectangle). The profile showed no dextran diffusion across the connection channel after 4 hours in culture. Experiments were repeated at least three independent times with replicates showing showed similar trends.



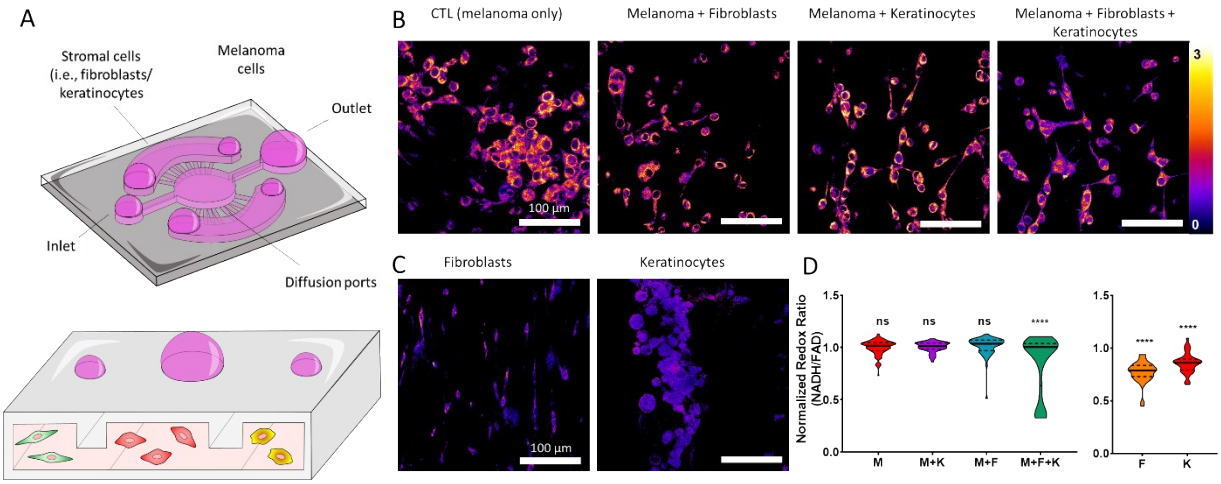
Supporting Figure 3. Cell viability and proliferation in the microdevice. A) Dermal fibroblasts, melanoma cells, and keratinocytes were individually cultured in different microdevices and cell viability was analyzed after 1 and 3 days. Images show the three cell types proliferated inside of the microdevice and retained high cell viability (>90%). B) Graph bar shows cell proliferation analysis. Cell number was quantified after 1 and 3 days in the microdevice and used to calculate the cell number fold change. C) Graph shows cell viability analysis after 3 days in the microdevice. Asterisk denotes p-value <0.05Experiments were repeated at least three independent times with replicates showing showed similar trends.



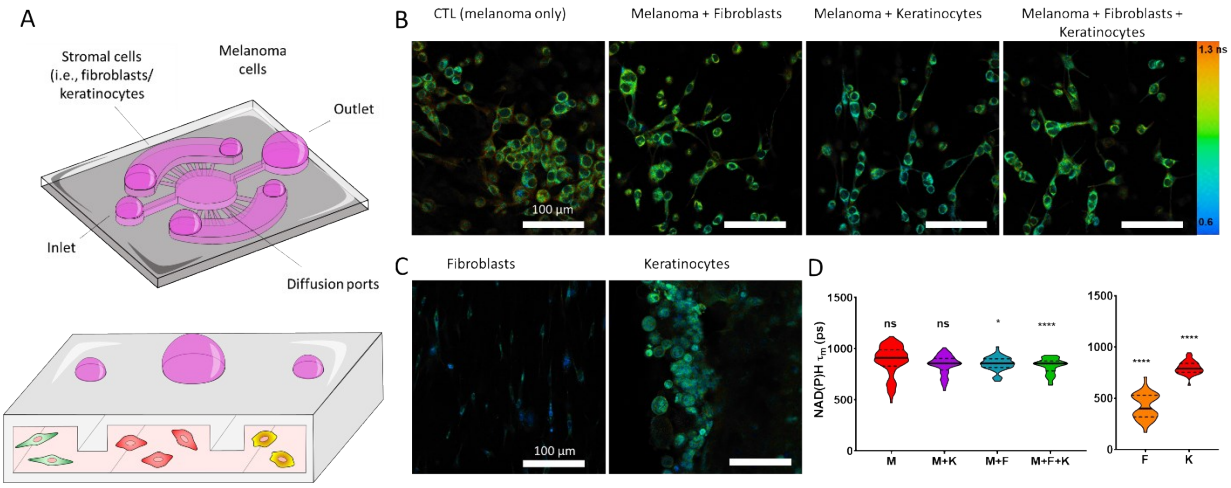
Supporting Figure 4. Co-culture in 3D ECM. A) Scheme showing the microdevice with the three cell types embedded in a 3D collagen hydrogel. B)Fluorescence microscopy images show fibroblasts (in green), melanoma cells (in red), and keratinocytes (in yellow) after injecting them in their respective microchamber. C) Collagen second harmonic generation (SHG) showing the effect of collagen density and polymerization temperature on collagen fiber structure. D) Images show fibroblasts (in red) and collagen fibers (in gray). Fibroblasts contracted the collagen hydrogel and caused directional orientation of the fibers. E) Similar images showing melanoma cells. No significant change in collagen structure was observed.



Supporting Figure 5 Morphological analysis in 3D collagen hydrogel. A) Scheme illustrating the experimental protocol: melanoma cells were confined in the central chamber using the air-wall method with/without dermal fibroblasts/keratinocytes in the lateral chambers. Cells were embedded in a 3D collagen hydrogel to study the effect of a 3D environment. After 3 days in culture, the microdevices were imaged to visualize cell morphology. B) Fluorescence microscopy images showed melanoma cells stained in red after 3 days in culture in monoculture (control condition) and in the presence of fibroblasts, or keratinocytes, or both in the lateral chambers. When cultured alone, melanoma cells displayed a rounded morphology. When cultured in the presence of fibroblasts, keratinocytes, or both, melanoma cells spread and displayed a more elongated morphology C) Violin graph showed the melanoma cell aspect ratio in monoculture and in the presence of dermal fibroblasts and keratinocytes. * and *** denote p-value<0.05 and <0.005. Experiments were repeated at least three independent times with replicates showing showed similar trends.



Supporting Figure 6 Optical metabolic imaging in 3D. A) Schematic representation of the triple co-culture inside the microdevice, including melanoma cells, dermal fibroblasts, and keratinocytes. B) Melanoma cells were imaged by multi-photon microscopy. Images show ratio of NAD(P)H autofluorescence divided by FAD autofluorescence, defined as the optical redox ratio. Melanoma cells were imaged alone and in the presence of dermal fibroblasts and/or keratinocytes. C) Images of fibroblasts and keratinocytes alone are also shown. D) Left graph shows the quantification of the optical redox ratio of melanoma cells alone and in the co-culture conditions. Right graph shows the optical redox ratio of dermal fibroblasts and keratinocytes alone. Melanoma cells alone (M), dermal fibroblasts alone (F), keratinocytes alone (K), melanoma with dermal fibroblasts (M + F), melanoma cells and keratinocytes (M + K), and the triple co-culture (M + F + K). Asterisk denotes a p-value<0.05. Experiments were repeated at least three independent times with replicates showing showed similar trends.



Supporting Figure 7 NAD(P)H Fluorescence Lifetime Microscopy in 3D. A) Schematic representation of the triple co-culture inside the microdevice, including melanoma cells, dermal fibroblasts, and keratinocytes. B) Melanoma cells were imaged by multi-photon fluorescence lifetime microscopy and images show NAD(P)H mean fluorescence lifetimes (τm). Melanoma cells were imaged alone and in the presence of dermal fibroblasts and/or keratinocytes. C) Images of NAD(P)H tm for fibroblasts and keratinocytes alone are also shown. D) Left graph shows the quantification of melanoma cell NAD(P)H τm alone and in the co-culture conditions. Right graph shows keratinocyte and dermal fibroblast NAD(PH) τm. Melanoma cells alone (M), dermal fibroblasts alone (F), keratinocytes alone (K), melanoma with dermal fibroblasts (M + F), melanoma cells and keratinocytes (M + K), and the triple co-culture (M + F + K). Asterisk denotes a p-value<0.05. Experiments were repeated at least three independent times with replicates showing showed similar trends.