Movie 1 - High laser power induces severe tissue photodamage. Anesthetized rats were injected with Texas red-dextran (red) and the salivary glands were exposed and imaged in time-lapse by two-photon microscopy. A wavelength of 750 nm was used to reveal the parenchyma (cyan). The power measured at the objective was 30 mW. After 30 seconds the parenchyma shows signs of photodamage that results in its complete disruption.

Movie 2– High laser power stops intracellular movements. Anesthetized rats were injected with Texas red-dextran (red) and Alexa 488 dextran (green) and the salivary glands were exposed and imaged in time-lapse by two-photon microscopy. A wavelength of 930 nm was used to reveal the elastic fiber (cyan). The power measured at the objective was 15 mW. Endosomal structures in superficial stromal cells are initially very motile, but after 1 min from the beginning of the imaging session suddenly halt.

Movie 3- Systemically injected Hoechst rapidly label the nuclei. The salivary glands of an anesthetized rat were exposed and 70 kDa Texas Red-dextran was injected systemically to label the vasculature (red). The glands were imaged in time lapse by two-photon microscopy (excitation 800 nm) using a 60X water immersion lens (NA 1.2, Olympus) while Hoechst was injected. The dye rapidly diffuses from the vasculature (red) and labels the nuclei (green)

Movie 4- Dynamics of mitochondria in the liver of a live rat. The liver of an anesthetized rat was exposed, bathed with Rhodamine 123, a probe to measure mitochondrial potential and imaged by two-photon microscopy (excitation 750 nm) using a 60X water immersion lens (NA 1.2, Olympus). In hepatocytes, metabolically active mitochondria are densely packed around the nucleus and very dynamic. Bar 5 μm

Movie 5- Actin-rich protrusions in metastatic cells implanted in the tongue of an immunocompromised mouse. Human oral squamous carcinoma cells (HN12) expressing GFP-lifeact were injected in the tongue of immunocompromised mice and imaged by two-photon microscopy (excitation 930 nm) using a 25X water immersion lens (NA 1.05, Olympus). Cells are located approximately 300 μm below the surface of

the tongue. F-actin is enriched in actin-rich protrusion at the cell surface of the tumor cells

Movie 6- Membrane trafficking in the kidney of a live mouse. The kidney of a mouse expressing mGFP was exposed and a proximal tubule was imaged by confocal microscopy (excitation 488 nm). mGFP is localized both at the plasma membrane and in intracellular structures. Note small intracellular vesicles trafficking within the tubule. In the center a blood vessel (note the streaks due to the fast movement of red blood cells). Bar 20 μm.

Movie 7- Membrane trafficking in the small intestine of a live mouse. The small intestine of a mouse expressing mGFP was exposed and enterocytes were imaged by confocal microscopy (excitation 488 nm). mGFP is localized primarily at the plasma membrane and in some intracellular structures.

Movie 8- GLUT4 vesicles in the soleus muscle of a live mouse. The soleus muscle of a mouse expressing GFP-GLUT4 was exposed and imaged by two-photon microscopy (excitation 930 nm). GFP-GLUT4 is localized in small vesicles that in resting conditions exhibited low mobility.

Movie 9- Volume rendering of salivary glands expressing GFP-lifeact. The salivary glands of a mouse expressing GFP-lifeact were exposed and imaged by confocal microscopy (excitation 488 nm). A z-scan up to 60 μm was performed to capture a single acinus and the volume rendering was realized using Imaris (bitplane). The myoepithelial cells enclose the acinus. Note the apical canaliculi within the acinus.

Movie 10- Neutrophil migration in mammary glands. The mammary glands of a mouse expressing RFP-lifeact and GFP myosin IIb were exposed and imaged by confocal microscopy (excitation 488 nm and 561 nm). A neutrophils was caught while sampling the tissue. Note the enrichment of myosin IIb in the protrusions that form both in the front and in the rear of the cells. Bar, 20 μm

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Supplemental Material to:

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Everything you need to know about intravital microscopy
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