In vivo 2-color multiphoton imaging of genetic reporter fibroblasts in the skin

Fibroblasts are cells that synthesize collagen and support the maintenance of connective tissue in the skin, tendons, and joints. They maintain a critical role in the immune system's response to tissue injury by promoting wound healing at sites of injury and inflammation. Our lab is interested in the role of fibroblasts in the development of both acute and chronic pain and leukocyte infiltration. Therefore, we designed a novel multidisciplinary technique to study the activation of these cells in vivo. We created a genetic reporter animal that fluorescently tags fibroblasts bright red (tdTomato) and utilized next generation imaging, multiphoton microscopy. This technique allows users to create time-lapse videos and/or pictures of protein interacting with live cells from whole imaged tissues and provides an accurate model of the microenvironment in tissues.

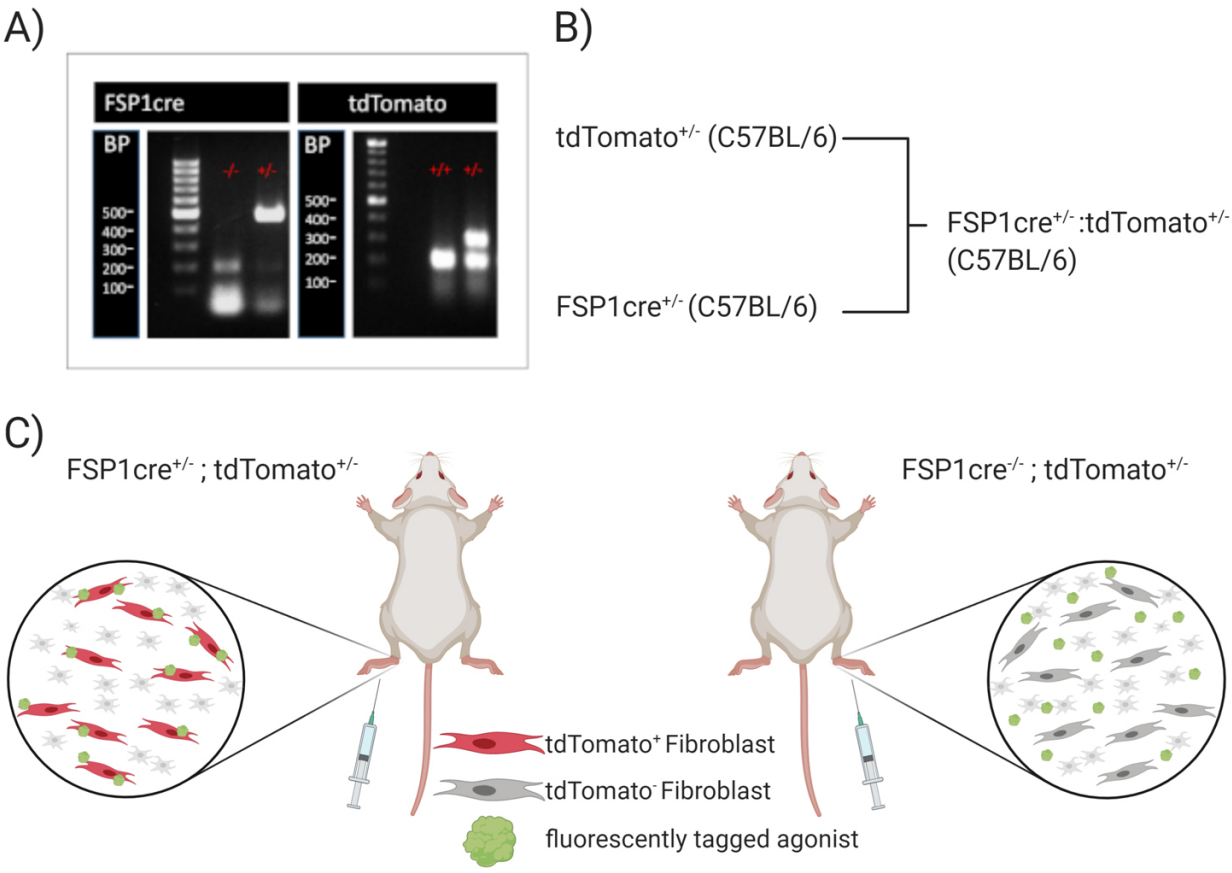

Fig. 1. tdTomato expressed only in FSP1+ Fibroblast in a Cre – Dependent manner. A) Representative PCR results depicting mice both positive and negative for Fibroblast-specific protein 1 cre (FSP1cre) expressing tdTomato. B) FSP1cre transgenic mice bred on a C57BL/6 background are crossed with tdTomato mice bred on a C56BL/6 background to generate mice that expressed tdTomato in FSP1+ fibroblast in a cre-dependent fashion. C) Representative pictograph

showing uptake of the fluorescently tagged agonist by TLR4 expressed on tdTomato positive fibroblast and the lack of uptake in the mice without TLR4 in the entire body (TLR4KO) after agonist injection.

The immune system serves as the body's first line of defense against foreign invaders. Bacterial components are recognized by receptors on the surface of fibroblasts called toll-like receptor 4 (TLR4). We utilized an agonist that mimics bacteria to bind to TLR4. When this receptor is activated, it causes the production of inflammatory mediators and changes to the composition of the extracellular matrix. We and others believe this activated phenotype can promote recruitment of leukocytes to sites of inflammation.

By utilizing a fluorescently tagged version of this TLR4 agonist (green fluorescent protein) and our reporter mice, we were able to visualize the interactions between fibroblasts and the agonist in real-time. We found that following administration, fibroblasts expressing TLR4 bind the agonist, with high levels of colocalization occurring. In contrast, mice that have no TLR4 in the entire body ($TLR4^{KO}$) do not bind the agonist.

Utilizing the methods described provide benefit over existing methods to visualize activation of cells in real-time. *In vivo* experiments provide a more accurate insight to the functional state of cells and their interactions in tissue. Moreover, *in vivo* imaging decreases the likelihood of confounding results stemming from the trauma of tissue dissociation. In addition, in an experimental setting, which requires continuous and extended imaging sessions, multiphoton microscopy has a decreased rate of damaging light-sensitive fluorophores.

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