Tissue imaging by 3D super-resolution fluorescence microscopy reveals molecular interactions between epithelial cells and tissue-resident T cells

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Epithelial tissues, such as skin, gut or lung, are the first line of animal defence against external factors. Mouse epidermis is populated by immune-monitoring γδ T cells, known as dendritic epidermal T cells (DETC), which almost entirely display a $V\gamma5^+$ T cell receptor (TCR) repertoire. A characteristic feature of DETC is the presence at steady-state of synaptic-like, TCR-enriched structures at the tips of dendrites which seemingly respond to changes in surrounding keratinocytes. A transmembrane-protein, Skint1, expressed by thymic epithelial cells is critical for DETC development, although its mechanism of action is unknown. We hypothesised that its sustained expression by keratinocytes may play a pivotal role in mediating the steady-state molecular interactions with DETC, implicating Skint1 in directly regulating the γδ TCR *in situ*.

We applied super-resolution fluorescence microscopy to investigate the role of Skint1 in defining tissue homeostasis and cell functionality. We used Structured Illumination Microscopy (SIM) to investigate protein clustering in epidermal tissue at 120nm resolution. Additionally, we applied two-colour Single Molecule Localization Microscopy (SMLM) approach revealing Skint1-TCR interactions in tissue at 15nm isotropic resolution.

By use of SIM we could visualize and quantitate Skint1 protein localization in the vicinity of γδ TCR clusters at the dendrite contact points between DETC and keratinocytes. The clustering of Skint1 on epithelial cell surface was further characterized in Skint1 mutant, Skint1 knockout, Skint1 transgenic and γδ TCR knockout mouse lines. Intriguingly, Skint clustering on epithelial cell surfaces was enriched wherever the TCR was present on apposing cells. Indeed, using two-colour SMLM, we showed that Skint1 molecules accumulated *in situ* at the TCR-rich tips of dendrites of $V\gamma5^+$ DETC. Additionally, we obtained evidence that the steady-state interactions also involve a related protein, Skint2. Thus, super-resolution fluorescence imaging supports the prospect that epithelial expression of Skint1 determines steady-state immunosurveillance by local γδ T cells *via* direct interactions with the γδ TCR.