

MAIT cell visualization in human skin: comparative staining analysis with two differentially labeled MR1-tetramers

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Mucosal-associated invariant T (MAIT) cells are a subset of unconventional T cells known to play a central role in immune surveillance at mucosal and epithelial surfaces. Canonical human MAIT lymphocytes use the semi-invariant TCRV α 7.2 receptor microbial antigens in an MHC related class I (MR1)-restricted manner. The development of MR1 5-OP-RU tetramers enabled the identification of classical MAIT cells based on T-cell receptor specificity for ribityl antigens, but the integration of MR1-tetramers into routine tissue staining protocols remains limited. Therefore, the aim of this study was to optimize visualization of MAIT cells in skin samples with MR1-tetramers conjugated to either phycoerythrin (PE) or Alexa Fluor 488 fluorescent dye.

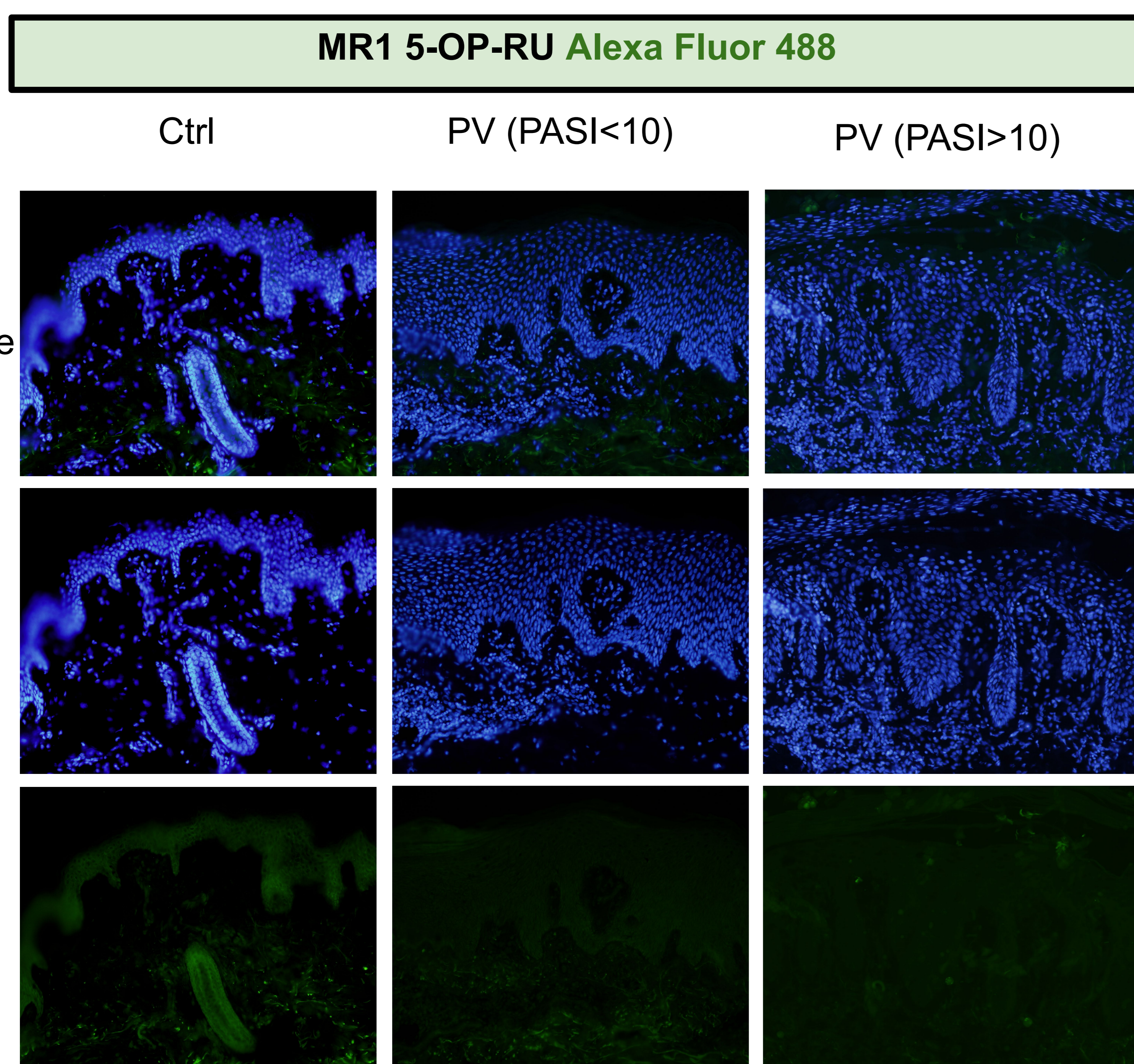


Figure 2. Immunofluorescent staining with MR1 5-OP-RU Alexa Fluor 488 of healthy control (Ctrl) skin and psoriatic skin (PV PASI < 10 and PASI > 10).

RESULTS:

A total of 18 skin images were analyzed. MAIT cells were rare in both healthy and psoriatic skin, and were found along the basal membrane of the epidermis, with a more prominent distribution within the papillary dermis, especially in the papillary loops. The average number of MR1 5-OP-RU PE+ cells per image was 1.75, and 1.8 after MR1 5-OP-RU Alexa Fluor 488 staining. The maximum number of PE- or AlexaFluor-488-positively labeled cells per image was 4 and 3, respectively.

GRANTS:

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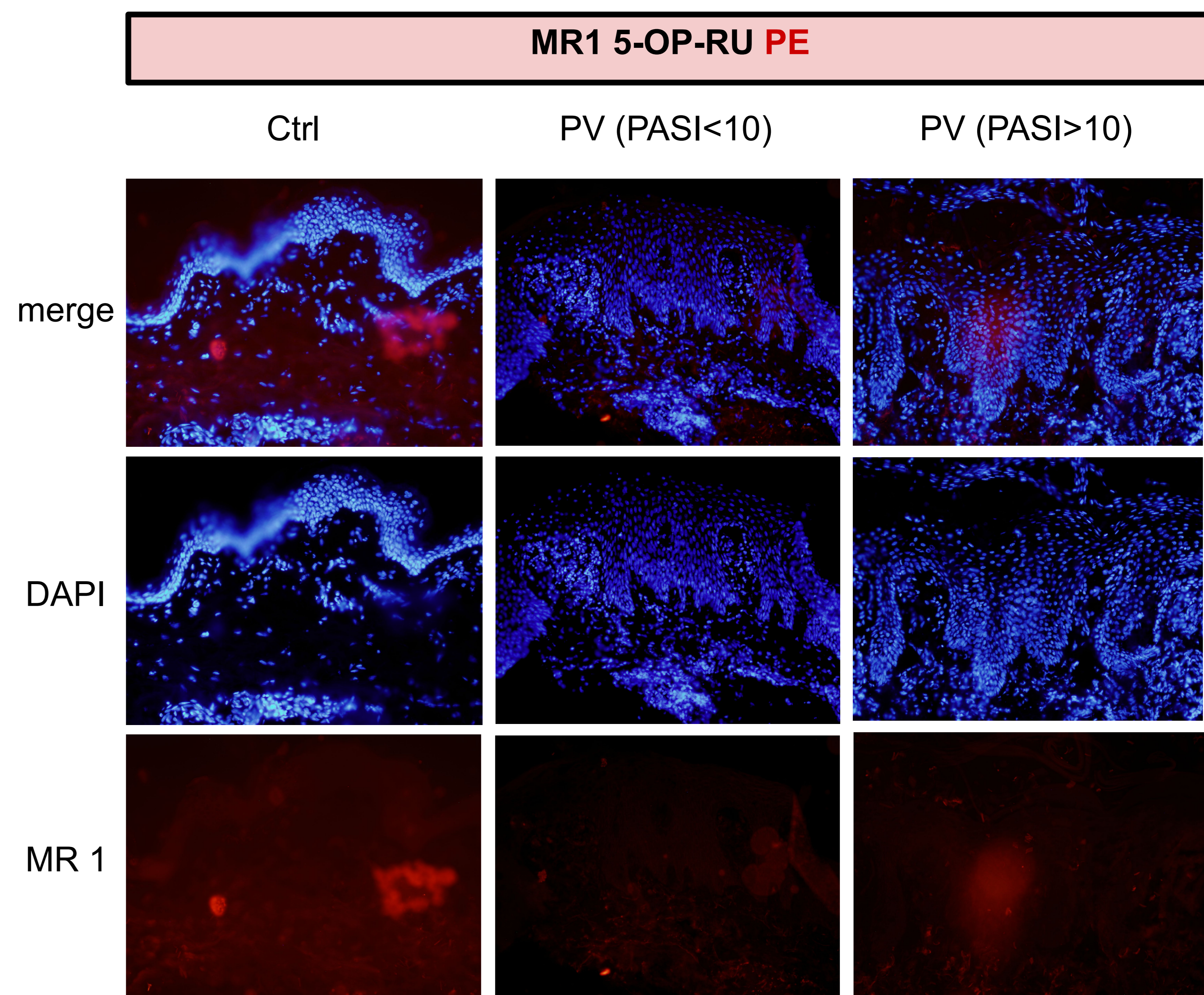


Figure 1. Immunofluorescent staining with MR1 5-OP-RU PE of healthy control (Ctrl) skin and psoriatic skin (PV PASI < 10 and PASI > 10).

MATERIALS AND METHODS:

Two psoriatic (PASI<10 and PASI>10) and one healthy skin samples were fixed in paraformaldehyde, cryoprotected in a sucrose gradient, and snap frozen, before sectioning on the cryostat. Each sample was stained with MR1 5-OP-RU PE and Alexa Fluor 488-conjugated tetramer, and nuclei were stained with DAPI. Slides were imaged under a fluorescence microscope and images were processed using ZEN lite software.

CONCLUSION:

MR1-Alexa Fluor 488 skin labeling provides better stability and reliable visualization over a longer period of time, while PE-labeled tetramers show better performance due to lower background noise. Skin autofluorescence and photoconversion of DAPI hampered visualization of MAIT cells within the epidermis. Further improvements of our protocol are required for comprehensive MAIT cell staining in psoriasis-afflicted and healthy skin samples.