Tissue-resident memory CD8$^+$ T cells amplify anti-tumor immunity by triggering antigen spreading through dendritic cells

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Tissue-resident memory CD8$^+$ T (Trm) cells mediate potent local innate and adaptive immune responses and play a central role against solid tumors. However, whether Trm cells cross-talk with dendritic cells (DCs) to support anti-tumor immunity remains unclear. Here we show that antigen-specific activation of skin Trm cells leads to maturation and migration to draining lymph nodes of cross-presenting dermal DCs. Tumor rejection mediated by Trm cells triggers the spread of cytotoxic CD8$^+$ T cell responses against tumor-derived neo- and self-antigens via dermal DCs. These responses suppress the growth of intradermal tumors and disseminated melanoma lacking the Trm cell-targeted epitope. Moreover, analysis of RNA sequencing data from human melanoma tumors reveals that enrichment of a Trm cell gene signature associates with DC activation and improved survival. This work unveils the ability of Trm cells to amplify the breadth of cytotoxic CD8$^+$ T cell responses through DCs, thereby strengthening anti-tumor immunity.
A characteristic of Trm cells is the expression of the homing receptor XCR1. This allows them to efficiently home to the draining lymph nodes (DLNs) of the skin after a priming event, such as the injection of the immunodominant OVA(257-264) peptide, readily infiltrating lung and breast tumors. Conversely, conventional type 1 migratory DCs in the skin. To this end, we generated ovalbumin (OVA)-specific skin Trm cells in mice using intradermal (i.d.) vaccination. This was followed by administration of an anti-CD8 antibody during the memory phase of the response (>4 weeks post vaccination), which efficiently deplete circulating CD8+ T cells (Supplementary Fig. 1a, b), including circulating memory and effector OVA-specific CD8+ T cells in lymphoid and non-lymphoid tissues, as previously shown. Then, depletion-resistant Trm cells (Fig. 1a) were specifically activated by i.d. injection of the immunodominant OVA125-264 peptide, readily producing IFN-γ and TNF-α within the first 6 h (Fig. 1a, b). Interestingly, we observed that skin XCR1+ conventional type 1 DCs (cDC1), also known as dermal DCs or DC DGs (Fig. 1c) upregulated CD80, CD86, MHC class II and IL-12 molecules 24 h after Trm cell activation (Fig. 1d–g). These data indicate that Trm cells induce maturation of dermal DCs, which are specialized in antigen cross-presentation and priming of CD8+ T cells. Then, we analyzed the presence of skin migratory DCs in draining lymph nodes based on the expression of high levels of MHC class II, CD207 (langerin) and CCR7 (Fig. 2a). We observed a marked accumulation of different migratory DC subsets at 24 and 48 h after Trm cell activation, including dermal DCs, Langerhans cells (LCs) and CD11b+ DCs (Fig. 2b). Among these subsets, dermal DCs displayed upregulated expression of the maturation marker CD86 (Fig. 2c). These results indicate that antigen-specific activation of Trm cells triggers maturation and migration to draining lymph nodes of skin-derived dermal DCs, revealing a cross-talk among these cells.

Trm cells spread CTL responses against tumor neo-antigens. Given the ability of Trm cells to activate migratory skin DCs and also mediate tumor-cell killing11,36, we hypothesized that
antigen-specific Trm cell-mediated tumor rejection would lead to the generation of secondary responses against other tumor-derived antigens, a phenomenon known as antigen spreading. To test this, mice bearing OVA-specific Trm cells were depleted from circulating CD8+ T cells and left during 8 weeks to replenish this compartment (Supplementary Fig. 1c). This allows resetting the endogenous repertoire in terms of specificity while maintaining OVA-specific Trm cells in the skin. Then, mice were challenged i.d. with MC38 cells expressing the OVA(257-264) peptide (MC38-OTI), which were rejected by OVA-specific Trm cells. As controls, unvaccinated mice (no Trm) challenged with MC38-OTI and Trm cell-bearing mice challenged with parental MC38 were used. Secondary CD8+ T-cell responses raised against highly relevant neo-epitopes present in MC38 cell line were analyzed 12 days later in tumor-draining lymph nodes (Fig. 3a). To this end, lymph node cells were ex vivo stimulated with neo-epitopes
Fig. 1 Skin Trm cell activation induces maturation of dermal DCs. C57BL/6 mice bearing OVA-specific skin Trm cells and depleted of circulating CD8+ T cells by administration of an anti-CD8 antibody were intradermally inoculated with control (CTRL) or OVA257-264 (OVA) peptides to activate Trm cells. a, b OVA-specific CD45+ CD8+ T cells in the skin were analyzed 6 h later by intracellular cytokine staining and flow cytometry. a Representative pseudocolor dot-plot showing CD69 and CD103 expression. b Representative pseudocolor dot-plots and graph showing IFN-γ and TNF-α production by skin Trm cells. c-g DCs in the skin were analyzed 24 h after peptide stimulation. c Gating strategy used to identify skin DC subpopulations, including CD11b+ DCs, Langerhans cells (LC) and dermal DCs (DDC) and representative histograms showing XCR1 expression in each subset. d-g Representative histograms (black: CTRL; red: OVA) and graphs showing the expression of CD80, CD86, MHCII and IL-12 of each skin DC subset. For quantification, the geometric mean fluorescence intensity (MFI) was normalized relative to the average of the control group. Pooled data from two independent experiments, n = 7 mice in the group treated with control peptide and n = 8 mice in the group stimulated with OVA257-264 peptide. Bars are the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 by Mann-Whitney unpaired test.

carrying missense mutations MUT 1 (SIIVFNLL from Dpgt1 gene), MUT 2 (AQLANDVVL from Reps1 gene) and MUT 3 (ASMTNMEML from Adpgk gene) and the production of effector molecules was analyzed by intracellular staining and flow cytometry. In contrast to control groups, rejection of MC38-OTI mediated by OVA-specific Trm cells resulted in the expansion of CD8+ T cells specific to all neo-epitopes tested (Fig. 3b, c), detected as IFN-γ-producing CD8+ T cells, which also expressed high levels of CD44 (Fig. 3d). These results indicate that spreading of CD8+ T-cell responses to multiple antigens is triggered by Trm cell-mediated tumor rejection. Neo-epitope-specific CD8+ T cells displayed high expression of other effector molecules, such as TNF-α, granzyme B and IL-2 (Fig. 3e, g), which is consistent with anti-tumor cytotoxic activity. Indeed, these mice were able to reject a re-challenge with MC38 cells, which express the neo-epitopes but cannot be recognized by OVA-specific Trm cells (Fig. 3h-k).

Trm cells promote melanoma-antigen spreading through DCs. To confirm these results in a relevant metastatic melanoma model, we used B16F10 cells, which are less immunogenic and express melanocyte-associated self-antigens, such as gp100. Favorably, responses against H-2 Kb-restricted gp100(25-33) peptide can be tracked by transferring congenic TCR-transgenic + CD8 T cells from pmel-1 mice without the need to wait for the replenishment of the endogenous repertoire. Mice bearing OVA-specific Trm cells and devoid of circulating CD8 + T cells received i.v. transfer of pmel-1 CD8+ T cells (Fig. 4a). The following day, mice were challenged i.d. with B16F10 cells expressing the OVA257-264 peptide (B16F10-OTI), which are rejected by OVA-specific Trm cells, as previously shown by us11. After 12 days, the generation of gp100-specific CTL responses was analyzed in the draining lymph nodes. Control groups were either left unchallenged (CTRL) or challenged with B16F10 parenteral cell line that do not activate OVA-specific Trm cells. As compared to control groups, only mice challenged with B16F10-OTI presented a significant expansion of gp100-specific CD8+ T cells (Fig. 4b, c), which produced IFN-γ after ex vivo peptide stimulation and displayed high expression of CD44 (Fig. 4d), indicating that they were efficiently primed. Since both B16F10-OTI and B16F10 cells express gp100 but only B16F10-OTI can be recognized by OVA-specific Trm cells, these results suggest that melanoma recognition by Trm cells triggers the spreading of CD8+ T-cell responses to tumor-derived antigens.

To explore whether cross-presenting dermal DCs mediate antigen spreading, we carried out similar experiments using Langerin-DTR mice, which allow the selective depletion of CD207+ dermal DCs and LCs from the skin after diaphtheria toxin (DTx) administration40-42. Taking advantage of the relatively faster repopulation of dermal DCs (~2 weeks) derived from bone marrow precursors, in comparison to LCs (~4 weeks) that arise from slowly proliferating skin precursors, we performed the B16F10-OTI challenge in mice devoid of only LCs (single DTx administration two weeks before challenge) or depleted of both LCs and dermal DCs (continuous DTx administration starting one day before challenge) (Supplementary Fig. 2)43,44. Similar to wild-type mice, pmel-1 CD8+ T cells were clonally expanded following B16F10-OTI challenge in Langerin-DTR mice that were not treated with DTx (No DTx; DDC+/LC+) or received a single DTx dose (Single DTx; DDC+/LC-). Interestingly, the expansion of pmel-1 CD8+ T cells was severely reduced in the case of mice depleted of both dermal DCs and LCs by continuous DTx administration (Continuous DTx; DDC- LC-), indicating that dermal DCs are necessary for antigen spreading induced by Trm cells. If dermal DCs directly present tumor-derived antigens to naïve CD8 + T cells in the lymph nodes remain to be determined.

Trm cell-induced CTL spreading suppresses melanoma growth. We next determined whether secondary CD8+ T-cell responses triggered by Trm cells were able to protect against B16F10 melanoma cells lacking OVA antigen and cannot be recognized by vaccination-induced Trm cells45. To this end, mice that rejected B16F10-OTI cells were injected 2–3 weeks later in the opposite flank with B16F10 melanoma cells (re-challenge) (Fig. 5a). Interestingly, these mice suppressed the growth of cutaneous tumors as compared to control mice that did not receive initial B16F10-OTI challenge (Fig. 5b,c), and therefore did not prime gp100-specific pmel-1 CD8+ T cells. In addition, no protection against B16F10 re-challenge was observed in mice that rejected B16F10-OTI melanoma but that did not receive transfer of pmel-1 CD8+ T cells (Fig. 5b, c), directly implicating the participation of primed pmel-1 CD8+ T cells in the anti-tumor effects observed. These results imply that Trm cell-mediated melanoma rejection triggers the spreading of CD8+ T-cell responses against melanoma-associated antigens, providing cross-protection against melanoma lacking Trm cell-targeted antigen. This can potentially be important to control highly heterogeneous tumors containing antigen-loss escape mutants.

To address the potential of Trm cell-induced gp100-specific CTL responses to protect against tumors disseminated in distant tissues, we repeated the previous experiment but substituted i.d. for i.v. B16F10 re-challenge to form disseminated pulmonary melanoma foci. Similar to i.d. re-challenge experiments, we observed that gp100-specific CTLs educated upon Trm cell-mediated melanoma rejection suppressed the formation of melanoma foci, as compared to unchallenged or non-transferred controls (Fig. 5d). These data suggest that Trm cells can orchestrate the generation of systemic CTL responses, which have the potential to protect against metastatic tumors.

Trm cell-DC cross-talk in human melanoma. Finally, we set out to determine whether there is evidence of cross-talk between Trm cells and DCs in human cancer. Using previously described gene signatures for Trm cells, activated and immature DCs35,45, we
analyzed tumor transcriptomic data of patients with cutaneous melanoma available within The Cancer Genome Atlas. We found a striking correlation between Trm cell and activated DC signatures ($r = 0.862$), with a weaker correlation between Trm cells and immature DCs ($r = 0.446$; Fig. 6a). This is in line with our finding that Trm cells promote DC maturation in mice and suggests a similar process may occur in human melanoma. Whilst both Trm and activated DC enrichment correlated with better overall survival, this association was weaker for immature DCs (Fig. 6b). As these signatures are correlated, we carried out multivariable Cox regression analysis to estimate their individual contributions to patient survival, additionally correcting for total T-cell infiltrate using a previously published signature and stage, showing Trm cell enrichment to remain a strong predictor of survival (Fig. 6c). These results suggest that a similar Trm cell-DC cross-talk may occur in human melanoma.

**Fig. 2** Trm cell activation promotes migration of dermal DCs. C57BL/6 mice bearing OVA-specific skin Trm cells and depleted of circulating CD8$^+$ T cells by administration of an anti-CD8 antibody were intradermally inoculated with control (CTRL) or OVA(257-264) peptides followed by analysis of inguinal draining lymph nodes after 24 h (OVA 24 h) and 48 h (OVA 48 h). a Gating strategy used to identify the different DC subsets in skin draining lymph nodes, including: lymph node-resident DCs (ResDC), migratory DCs (MigDC), CD11b$^+$ DCs, LCs and DDCs. Representative histograms showing CCR7 expression on relevant subpopulations. b Quantification of total numbers of the different DC subsets. c Representative histograms (black: CTRL; red: OVA 24 h; blue: OVA 48 h) and graphs showing CD86 expression of each DC population. For quantification, the geometric mean fluorescence intensity (MFI) was normalized relative to the average of the control group. Pooled data from two independent experiments $n = 9$ mice per group. Bars are the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ by Mann-Whitney unpaired test.
**Discussion**

There is compelling evidence supporting a central role for Trm cells in anti-tumor immunity. However, the precise mechanisms by which Trm cells can control solid tumors are just starting to be deciphered. Here we show that, in parallel to their well-documented direct tumor killing capability,

Trm cells can activate cross-presenting dermal DCs, resulting in the subsequent priming and expansion of new CD8+ T cells specific to tumor-derived neo- and self-antigens. Importantly, this secondary response confers protection against re-challenge with tumor cells.
lacking the antigen initially recognized by Trm cells. To our knowledge, this is the first study to show that Trm cells can orchestrate the broadening of circulating CTL responses via DCs to strengthen protective immunity. Interestingly, these secondary CTL responses were able to suppress the growth of cutaneous tumors and also disseminated melanoma in the lungs. The ability of Trm cells to trigger the generation of broader and systemic CTL responses to control disseminated tumors implies that they can overcome their tissue-restricted nature, spreading their protective potential to other organs. This notion is supported by our

Fig. 4 Trm cells promote melanoma-antigen spreading through dermal DCs. C57BL/6 and Langerin-DTR mice bearing OVA-specific skin Trm cells and depleted of circulating CD8⁺ T cells were intravenously transferred with gp100-specific CD90.1⁺ pmel-1 CD8⁺ T cells and one day later challenged intradermally with B16F10-OTI melanoma cells. Control groups were either unchallenged (CTRL) or challenged with or B16F10 melanoma cells that do not activate Trm cells. Analysis of gp100-specific pmel-1 CD8⁺ T cells was performed 12 days after tumor challenge in inguinal draining lymph nodes stimulated ex vivo with cognate gp100(25-33) peptide to analyze IFN-γ production by intracellular cytokine staining. a Experimental scheme. b Representative pseudocolor dot-plots showing the frequency of gp100-specific pmel-1 CD8⁺ T cells and IFN-γ production. c Graph showing frequencies of gp100-specific pmel-1 CD8⁺ T cells. d Representative histograms (CTRL: black histogram, upper panel; B16F10: red histogram, middle panel; B16F10-OTI: blue histogram, lower panel) and geometric mean fluorescence intensity values of CD44 expression in CD90.1⁺ pmel-1 CD8⁺ T cells. e, f Representative pseudocolor dot-plots (e) and graph (f) showing the frequency of gp100-specific pmel-1 CD8⁺ T cells and IFN-γ production in Langerin-DTR mice that were either: non-depleted of LCs and DDCs (No DTx; DDC⁺/LC⁺); depleted of only LCs by administrating a single dose of diphtheria toxin (DTx) 14 days before the tumor challenge (Single DTx; DDC⁺/LC⁻); or depleted of both DDCs and LCs through the continuous administration of DTx (Continuous DTx; DDC⁻/LC⁻). Pooled data from three independent experiments in b–d, n = 13 mice per group, and two independent experiments in e–f, n = 7–8 mice per group. Bars are the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, by Mann-Whitney unpaired test.
findings in human data indicating that a Trm cell gene signature positively correlates with overall survival.

This reverse flow of information from adaptive to innate immune responses was initially described during anti-viral immune responses. A few reports have demonstrated that following Trm cell activation, a strong innate-like alarm state is induced in the tissue through the production of a plethora of effector molecules. Among these, TNF-α has been shown to promote maturation of DCs. However, whether Trm cell-induced DC maturation results in the generation of new CD8+ T-cell responses had not previously been addressed. The present study reveals that such cross-talk between Trm cells and DCs occurs in the context of anti-tumor immunity and, more importantly, that it results in the propagation of circulating anti-tumor CTL responses.

The results obtained in mouse models are supported by human data showing a strong correlation between Trm cell and activated DC gene signatures in tumors from melanoma patients. The broader anti-tumor CTL responses triggered by Trm cells can eventually underlie the association between Trm cell infiltration and higher density of CTLs observed in some human solid tumors, as well as the superior predictive potential and better response to immunotherapy that Trm cells have in comparison to total CD8+ T-cell infiltration. This mechanism may have broader implications because Trm cell-infiltration has been shown to predict better clinical outcome in other types of solid tumors.

On the other hand, cross-presenting migratory DCs are key players in the generation of anti-tumor T-cell immunity and their absence abolishes the rejection of immunogenic tumors and decreases the response to immune checkpoint blockade and adoptive T-cell therapy.

Emerging evidence indicates that effective anti-tumor immunity requires the coordinated action of tissue-resident and circulating T-cell compartments. However, how these two compartments team-up to control tumors is poorly understood. It has been previously demonstrated that virus-specific Trm cells can recruit circulating bystander memory CD8+ T cells to the infection site after antigen recognition through the production of IFN-γ. In tumor models, circulating Tcm cells have in comparison to total CD8+ T cells (no challenge + pmel-1) or challenged but did not receive pmel-1 CD8+ T cells (no challenge + pmel-1) challenged but did not received pmel-1 CD8+ T cells (B16F10-OTI + pmel-1). Two to three weeks later, mice were re-challenged intradermally in the contralateral flank (b-c) or intravenously (d) with B16F10 melanoma cells. a Experimental scheme. b Individual curves showing tumor growth. c Graph showing the mean of tumor volume in all groups. d Representative picture of lungs and graph showing the number of melanoma foci. Pooled data from two independent experiments in b, c, n = 10, and three independent experiments in d n = 22 mice. Bars are the mean ± SEM. *p < 0.05, **p < 0.01, ***p ≤ 0.001, and ****p ≤ 0.0001 by two-way ANOVA Bonferroni post-hoc test for c, and Mann-Whitney unpaired test for d.
Our findings suggest a novel mechanism by which resident and circulating T cells can collaborate to fight tumors. The mechanism described in this manuscript could be of particular relevance to control highly heterogeneous tumors, which represents a major challenge to oncological treatments and, in particular, immunotherapies. Indeed, tumor-cell exon sequencing has revealed that multiple regions inside the same tumor or different lesions in the same patient have divergent mutation patterns and probably a differential expression of antigens. Consequently, Trm cells derived from different metastasis of the same patient have a high interlesional TCR diversity.

On the other hand, the adaptive immune system exerts a selective pressure on tumor cells, driving the survival of more resistant cancer cell subpopulations, a phenomenon known as immune editing. As a result, cancer cell clones that do not express immune targeted antigens can escape immune control and form new tumors. In this regard, Trm cell responses can drive the control of resistant clones, such as antigen-loss escape mutants, by broadening anti-tumor CTL responses against multiple tumor-derived antigens, as shown here.

In summary, we propose that Trm cells represent a new orchestrator of anti-tumor immunity. Interestingly, it has been suggested that Trm cells are major targets of checkpoint blockade and that checkpoint blockade promotes Trm cell formation in tumors. Hence, we envision that the ability of Trm cells to increase the breath of anti-tumor T-cell immunity via DCs may play an important role in cancer immunotherapy. Accordingly, recent studies have evidenced the importance of the cross-talk between DCs and CD8+ T cells for effective cancer immunotherapy. Moreover, a recent study has revealed that PD-1 blockade leads to the expansion of new tumor-reactive T-cell clones in patients with advanced skin cancer. In consequence, the development of therapeutic approaches, such as vaccines, T-cell-based therapies and monoclonal antibodies, that boost the

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**Figure 6** Trm cell signature correlates with DC maturation and improved survival. The relationship between previously described gene transcription signatures of Trm cells, DCs and survival was investigated in the TCGA melanoma cohort. 

- **a** Correlation between Trm cell gene signature and either mature or immature DC gene signatures, summarized as enrichment z-scores (n = 468). Pearson correlation coefficients and associated p-values are shown.
- **b** Kaplan-Meier plots showing the overall survival of patients (n = 407 with available data) grouped according the median value of signatures (light blue curves: upper half; red curves: lower half) corresponding to Trm cells, mature DC and immature DCs. Log-rank p-values are shown.
- **c** Multivariable Cox survival regression carried out on the same cases represented in **b**. The forest plot shows the hazard ratio, 95% confidence interval and associated p-values for each variable in the model.

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ability of Trm cells to broaden anti-tumor CTL immunity are expected to have a greater protective potential in cancer, particularly to control highly heterogeneous tumors and metastatic disease.

**Methods**

**Animals.** C57BL/6J wild-type (CD45.2), B6.Cg-Thy1.2/Cy Tg(TcraTcrb)18Tgl/J (clone 53-19C2), CD4-APC (clone 17A2), CD3-APC (clone 17A2), CD3-PerCp/Cy5.5 (clone 17A2), CD8-Brillant Violet 421 (clone 53-6-7), CD4-PE (clone 30-F11), CD45-PerCP (clone 30-F11), CD45.1-PE/Cy7 (clone 42A1), CD45.2-PE/Cy7 (clone 42A1), CD68-PE/Cy7 (clone 203.124), CD69-APC/Cy7 (clone H1.2F3), CD69-APC (clone H1.2F3), CD44-PerCP (clone IM7), IFN-γ-PE (clone XMG1.2), IFN-γ-APC (clone XMG1.2), TNF-α-APC/Cy7 (clone MP6X-XT22), CD11b-PerCP (clone M1/70), CD207-PE (clone 4C7), XCR1-APC (clone ZET), XCR1-PerCP/Cy5.5 (clone ZET), CD11c-PE/Cy7 (clone N418), MHCII-APC/Cy7 (clone MI114.15.2), CD24-PerCP/Cy5.5 (clone MI169), CD80-APC (clone 16-10A1), CD80-PerCP/Cy7 (clone 16-10A1), CD86 Brilliant Violet 421 (clone GL-1), CCR7-PE/Cy7 (clone 4B12), IL-2-PE/Cy7 (clone JES6-5H4) IL-12-23-APC (clone 15C6.15), granzyme B-APC (clone GB1) and viability dye Zombie Aqua (ref 423101). Samples were acquired in a FACSCanto II cytometer (BD Biosciences) and data were analyzed using FlowJo version X.7.7 (Tree Star, Inc.). Gating strategies for all flow cytometry experiments are shown in Supplementary Fig. 4.

**Flow cytometry staining.** Cells were incubated 10 min with the TruStain scX (clone 1F9) washed and incubated with the antibodies for 20 minutes followed by two washes with PBS. Monoclonal antibodies specific for mouse molecules were purchased from Biolegend: CD3-FTC (clone 17A2), CD3-APC (clone 17A2), CD3-PerCp/Cy5.5 (clone 17A2), CD8-Brillant Violet 421 (clone 53-6-7), CD4-PE (clone 30-F11), CD45-PerCP (clone 30-F11), CD45.1-PE/Cy7 (clone 42A1), CD45.2-PE/Cy7 (clone 42A1), CD68-PE/Cy7 (clone 203.124), CD69-APC/Cy7 (clone H1.2F3), CD69-APC (clone H1.2F3), CD44-PerCP (clone IM7), IFN-γ-PE (clone XMG1.2), IFN-γ-APC (clone XMG1.2), TNF-α-APC/Cy7 (clone MP6X-XT22), CD11b-PerCP (clone M1/70), CD207-PE (clone 4C7), XCR1-APC (clone ZET), XCR1-PerCP/Cy5.5 (clone ZET), CD11c-PE/Cy7 (clone N418), MHCII-APC/Cy7 (clone MI114.15.2), CD24-PerCP/Cy5.5 (clone MI169), CD80-APC (clone 16-10A1), CD80-PerCP/Cy7 (clone 16-10A1), CD86 Brilliant Violet 421 (clone GL-1), CCR7-PE/Cy7 (clone 4B12), IL-2-PE/Cy7 (clone JES6-5H4) IL-12-23-APC (clone 15C6.15), granzyme B-APC (clone GB1) and viability dye Zombie Aqua (ref 423101). Samples were acquired in a FACSCanto II cytometer (BD Biosciences) and data were analyzed using FlowJo version X.7.7 (Tree Star, Inc.). Gating strategies for all flow cytometry experiments are shown in Supplementary Fig. 4.

**Ex vivo intracellular cytokine staining.** Inguinal lymph nodes were obtained 12 days after the tumor challenge and CD8+ T cells were stimulated ex vivo with the gp100(25-33) peptide (KYPQRNQDWL, synthesized at GenScript) for 8 h. Brefeldin A (1 µg/mL, Sigma–Aldrich, ref B6542) was added during the last 6 h. In the case of neo-epitopes MUT1 (SIIVFNLL), MUT2 (AQLANDYVL) and MUT3 (ASMTNELM), stimulation was carried out during 20 h. Brefeldin A (1 µg/mL, Sigma–Aldrich, ref B6542) was added during the last 4 h. Intracellular staining was performed using the Cytofix/Cytoperm Fixation/Permeabilization solution set (BD Biosciences, ref 554774) according to the manufacturer’s instructions.

**In vivo Trm cell staining and intracellular cytokine staining.** OVA-specific Trm cells were stained by intradermal injection with 20 µg of OVA 257–264 peptide (SIINFEKL) or control SURV 20–24 peptide (ATFKNWFFL) diluted in PBS near to the vaccination site. Mice were sacrificed 6, 24 or 48 h later and lymph nodes were harvested as described above. In the case of intracellular cytokine staining of skin Trm cells, 20 µg of brefeldin A was co-injected with the peptides. In the case of intracellular IL-12 staining of skin DCs, brefeldin A was injected after 24 h and analysis was performed 4 h later.

**Diphtheria toxin administration.** To deplete langerin-expressing DCs, Langerin-DTR mice received 1 µg of diphtheria toxin (Sigma–Aldrich, ref D0564 1MG) by intravenous injection in the tail vein. In the experiments where DC depletion was continuously maintained, mice received 0.35 µg of diphtheria toxin intraperitoneally every 3 days.

**Cell lines.** Mouse melanoma cell line B16F10 (ATCC CL8-6475) was obtained from American Type Culture Collection. MC38 tumor cells were kindly provided by Dr. Burkhard Becher (University of Zurich, Switzerland) to Dr. Sergio A. Quezada. B16F10-OTL5X-ZsGreen (B16F10-OTLI) and MC38-OTL5X-ZsGreen (MC38-OTL) cells were generated by lentiviral transduction of B16F10 cell line with the pLVX-OTL5X-ZsGreen vector encoding the OTI epitope minigene fused to ZsGreen11. B16F10 and MC-38 cell lines were cultured in complete RPMI 1640 (ThermoFisher Scientific, ref 61870-036) and DMEM (HyClone, ref SH30081.02) media, respectively, supplemented with penicillin, streptomycin (ThermoFisher Scientific, ref 11140052), sodium pyruvate (ThermoFisher Scientific, ref 11360070) and 10% of heat-inactivated fetal bovine serum (ThermoFisher Scientific, ref 10437010) in a humidified incubator at 37 °C with 5% CO2. All cell lines were routinely tested for mycoplasma contamination.

**Tumor challenge.** Mice were injected intradermally in the lower back skin close to the vaccination site with 50 µL of PBS containing 1 × 10^6 of tumor cells. Tumor growth was monitored by measuring perpendicular tumor diameters with calipers. Tumor volume was calculated using the following formula: \( V = \frac{D \times d^2}{2} \), where \( V \) is the volume (mm^3), \( D \) is larger diameter (mm) and \( d \) is smaller diameter (mm). Mice were sacrificed when moribund or when the mean tumor diameter was ≥15 mm, according to the approved ethical protocol. When indicated, mice were re-challenged with 1 × 10^5 B16F10 or MC38 cells in the contralateral site. For intravenous re-challenge 1 × 10^6 B16F10 melanoma cells in 200 µL of PBS were inoculated through the tail vein. Mice were sacrificed two weeks later and lungs were obtained, washed in PBS and stored in 3 mL of Fekete’s solution. Lung foci quantification was performed taking pictures of the lungs on both sides (Canon EOS rebel T5) followed by quantification of dark melanoma foci.

**RNA sequencing analysis.** Upper quartile normalized RSEM expected RNA transcript counts and clinical data47 from The Cancer Genome Atlas (TCGA) project were downloaded from the National Cancer Institute GDC PanCanAtlas project website (https://gdc.cancer.gov/about-data/publications/pancanatlas) and cutaneous melanoma cases (SKCM) filtered. Trm cell and DC gene signatures were previously described by Charoetntong et al. and Savas et al.35,45. A tumor-infiltrating T-cell signature was used as previously described by Danaher et al.48. Non-protein coding genes were removed from these signatures for consistency with TCGA data. For each signature, enrichment scores were calculated by taking the mean log2 [1 + normalized expression of each gene, followed by z-score transformation. The correlation between Trm cell and DC gene signatures was evaluated by Pearson correlation.

**Statistical analysis.** Statistical analysis was performed using Graphpad Prism software (Graphpad Software Inc.). RNA sequencing and survival analyses were carried out in the R statistical programming environment. Mann-Whitney or unpaired t-tests were performed to calculate p-values. p-values for tumor growth was performed using two-way ANOVA Bonferroni post-hoc test. Error bars in figures indicate the mean plus SEM. Survival analysis by Cox regression was carried out with the “survival” package and Kaplan-Meier survival curves were drawn using the “survminer” package with patients grouped on the median value of each variable tested and with log-rank p values reported. Overall p value ≤0.05 was considered statistically significant; *p ≤0.05, **p ≤0.01, ***p ≤0.001 and ****p ≤0.0001.
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**Author contributions**

A.L. conceived and designed the project. E.M., F.G.C. and P.C.M. developed methodology. E.M., F.G.C., P.C.M., E.L., X.D., I.S.A., D.A.F. and E.R. acquired the data. A.L., E.M., F.G.C., P.C.M. and E.G. analyzed and interpreted the data. S.A.Q. contributed with technical and material support. A.L. wrote and reviewed the manuscript. All the authors discussed the results and the manuscript.

**Additional information**

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