Disorders of the eye leading to visual impairment are a major issue that affects millions of people. On the other side ocular toxicities were described for e.g. molecularly targeted therapies in oncology and may hamper their development. Current ocular model systems feature a number of limitations affecting human-relevance and availability. To find new options for pharmacological treatment and assess mechanisms of toxicity, hence, novel complex model systems that are human-relevant and readily available are urgently required. Here, we report the development of a human immunocompetent Choroid-on-Chip (CoC), a human cell-based in vitro model of the choroid layer of the eye integrating melanocytes and microvascular endothelial cells, covered by a layer of retinal pigmented epithelial cells. Immunocompetence is achieved by perfusion of peripheral immune cells. We demonstrate controlled immune cell recruitment into the stromal compartments through a vascular monolayer and in vivo-like cytokine release profiles. To investigate applicability for both efficacy testing of immunosuppressive compounds as well as safety profiling of immunoactivating antibodies, we exposed the CoCs to cyclosporine and tested CD3 bispecific antibodies.
Organ-on-chip (OoC) technology allows building complex in vitro models tailored specifically to the tissue/organ needs. OoC models mimic the microphysiological environment cells experience in a tissue including the vasculature-like perfusion. They are developed to potentiate several functional readouts using very low cell numbers. Over the past years, the technology has emerged as a powerful tool to support drug discovery and development with a potential for pharmaceutical R&D cost reduction. The rise of complex treatment modalities, increasing attrition rates and low of predictivity of current model systems created a need for human-relevant and well-characterized in vitro models to support drug development (i) in efficacy testing by building in vitro disease models, and (ii) in toxicity testing by providing a unique tool for mechanistic studies. Of particular interest for both efficacy and toxicity testing, is the human eye. Millions of people worldwide are affected by ocular disorders leading to visual impairment. Moreover, ocular toxicities have emerged as an issue of targeted therapies and have been reported for immunotherapies. Hence, to develop ophthalmic drugs and study ocular toxicity, novel human-relevant ocular tissue models are urgently needed.

One such ocular tissue is the choroid of the eye which belongs to the uveal tract. It is a thin, highly vascularized and pigmented tissue positioned between the neurosensory retina and the sclera, which takes care of the outer retinal demands for nutrition supply and removal of toxic metabolic products. The human choroidal tissue is underlined by the retinal pigmented epithelium (RPE) and its main cell types are melanocytes, choriocapillar microvascular endothelial cells (MVEC), fibrocytes and immune cells. Uveitis refers to a group of ocular disorders characterized by inflammation of the uveal tract, which encompasses the iris, ciliary body and choroid. The inflammatory state is characterized by cytokine release and substantial T cell recruitment and infiltration. Uveitis is the 4th leading cause of blindness, responsible for 10% of blindness in the US. Its origin can be infectious (20%) or noninfectious (immune-mediated, drug induced or idiopathic). Uveitis is categorized based on the primary anatomical location of inflammation. Posterior uveitis involves inflammation of the retina, choroid and sometimes the optic nerve and in spite of not being the most common type of uveitis it is indeed the most sight-threatening kind, with potential for causing severe structural damage and visual impairment.

Immunotherapies have been responsible for an increase of drug-induced uveitis and ocular side effects are observed in up to 70% of patients under immunoncological treatments including inhibitors of VEGFR, EGFR, tumor specific proteins, as well as estrogen receptor modulators, interferons and checkpoint inhibitors. All types of checkpoint inhibitors have shown ocular side effects. Uveal effusion was reported for anti-CD137 and anti-PDL1 checkpoint inhibitor agents, both small molecules and monoclonal antibodies. Ipilimumab, a monoclonal antibody targeting cytotoxic T lymphocyte antigen-4 (CTLA-4) has been associated with choroidal neovascularization that leads to the disruption of the choroid-retina barrier causing meaningful visual impairment. Interestingly, uveitis treatment covers immunosuppression with small molecules (e.g., corticosteroids, cyclosporine, tacrolimus, or methotrexate) and more recently biologic therapies targeting the inhibition of activated T cells VEGF (e.g., adalimumab, infliximab) as well as pro-inflammatory cytokines such as TNF-α and IL-6 (e.g., tocilizumab and sarilumab in non-infectious intermediate, posterior, and panuveitis).

The lack of cross-reactive, pharmacologically relevant animal species poses a challenge in nonclinical safety assessment in the development of novel complex treatment modalities as in cancer immunotherapy. Translation of findings e.g., in non-human primates to patients can be hampered due to species differences in their immune systems. Two remarkable differences between humans and rhesus and cynomolgus macaques are that CD4 + CD8 + double-positive T cells are more abundant in macaques and display unique responses to different type I interferons and that cynomolgus plasmacytoid dendritic cells (pDCs) have a unique pSTAT5 response to IL-6. This is particularly interesting for posterior uveitis since IL-6 is a therapeutic target under phase I/II clinical investigation and pDCs have been reported to be decreased relative to monocyte derived DCs possibly leading to biased CD4+ T cell immune responses (increased Th1 and decreased Treg cells) in non-infectious uveitis in humans.

Hence, advanced human-based and immunocompetent in vitro models of the choroid of the eye are urgently required to study the interplay between the vascular network and the human immune system. A model could support research on early detection of drug-induced uveitis and on the discovery of new therapeutic strategies for diseases such as posterior uveitis, regardless of its etiology. Expended testing with several donors would further help to inform about the mechanistic insights of idiosyncratic effects in a patient-specific manner.

The few available in vitro models of the human choroid are mostly 2D, integrate few cell types and lack vascularization or immune components; aspects that are all crucial for mechanisms of toxicity or disease triggering events. In general, in vitro models that study immune cell infiltration are still limited and encompass mostly three-dimensional (3D) skin and tumor models.

To address the key physiological characteristics related to choroidal drug reactions and considering immunology, we developed a human immunocompetent choroidal in vitro model that mimics the tissue vascularization, pigmentation and immune response in the presence of circulating immune cells. Here we provide a comprehensive characterization of the novel model and describe how the immunocompetent chip responded to immunosuppressive treatment with cyclosporine upon T cell activation and to T cell bispecific antibody (TCB) containing the T cell receptor binding domain.

Results and discussion

Design, concept and characterization of the Choroid-on-Chip Platform concept. To mimic the tissue complexity of the human choroid, we designed a 3-channel microfluidic platform that allows for the perfusion of circulating immune cells. The Choroid-on-Chip (CoC) comprises three main cellular components (Fig. 1a–c). The epithelium consists of a monolayer of human induced pluripotent stem cell (iPSC)-derived RPE in the top channel. The endothelium consists of two confluent monolayers of human primary MVECs seeded in the central channel facing on the upper side the RPE and on the lower side the melanocyte compartment. This channel is perfused using a syringe pump at a flow rate of 40 µL/h. The stromal component in the bottom channel features a cell laden hydrogel incorporating melanocytes at defined cell densities in a 3D arrangement (Fig. 1d, orthogonal view). All the epithelial, endothelial and stromal components together form a robust, viable 3D tissue (Fig. 1d). The cells are homogenously seeded and distributed in a pigmented tissue throughout the entire length of the chip (Fig. 1e). The dimensions of the chip were chosen to match in vivo tissue dimensions: The total height of the 3D tissue on chip is 300 µm; the human choroidal thickness was estimated to be 266.8 ± 78.0 µm by Yiu et al.

Outer blood-retina barrier characterization. To mimic the outer blood-retina barrier (oBBR), RPE and MVECs were cultured on
the two sides of the upper membrane, top and bottom respectively (Fig. 1a). The membranes were coated with Laminin, Collagen and Fibronectin, as described in detail in the Methods section, to mimic the Bruchs’ Membrane composition. The RPE formed a pigmented monolayer throughout the entire chip expressing tight junction protein zonula occludens 1 (ZO-1) and tyrosinase-related protein 1 (TYRP1), involved in the generation of melanin. MVEC s formed confluent and CD31 positive monolayers throughout the entire length of the endothelial channel. To further assess how our model mimics the oBRB, we evaluated the permeability of the endothelial layer towards the RPE side (oBRB) and towards the stromal side of the tissue (Fig. 2c). Our data showed retention of both Carboxyfluorescein (0.377 kDa) and Dextran Texas Red (70 kDa) in the presence of the cell monolayers in all conditions. The retention was higher for the oBRB than for the endothelial-stromal barrier (twofold) and for the larger molecular weight molecule compared to the lower one. A comprehensive quantitative analysis of the absolute difference between the permeability of the endothelial-stromal barrier and the oBRB is hampered by the differences in tissue composition of RPE and stromal channel: the hydrogel in the stromal channel leads to a slower intra-channel diffusion in contrast to the medium in the RPE compartment. However, it is worth mentioning that the relative differences are indeed in alignment with the in vivo situation and ex-vivo data showing a tighter barrier where the RPE is located. The permeability of the choriocapillaris is expected to be 1–2 orders of magnitude higher than the oBRB. The endothelial-RPE barrier is reported to be 30 times less permeable to the 70 kDa dextran than to the carboxyfluorescein. We did not observe the same magnitude of differences in the permeability as described in ex-vivo and other in vitro studies. However, the chip was not specifically designed to quantify barrier properties but to study effects of drugs and/or immune response in the stromal and epithelial compartments.

**Choroidal stroma and melanocyte characterization.** The melanocyte seeding process in the CoC was designed to achieve two different melanocyte densities, 5 and 50% (Fig. 3a, b). Morphology, pigmentation, proliferation (Ki-67) and viability were characterized for high and low melanocyte cell densities after 2 weeks in culture. Interestingly, the main functional difference between the chips cultured with high and low melanocyte density was in IL-6, secreted proportionally to the melanocyte cell density, conversely to secretion of the pro-inflammatory cytokine TNF-α (Fig. 3d).
In vivo, uveal melanocytes are located in the choroidal stroma and are essential for the normal ocular homeostasis and function, namely for light absorption, regulation of oxidative stress, immune regulation, angiogenesis and inflammation\textsuperscript{33}. Uveal melanocyte size and pigmentation varies within ethnicities and species. Rhesus macaques feature 50% of the choroidal tissue filled with melanocytes in opposition to 5% observed for white humans, corresponding to a density of 34.2 uveal melanocytes per 10,000 \mu m\textsuperscript{2} with an average size of 28.5 \mu m\textsuperscript{2}\textsuperscript{2,28}. The CoC aimed at recapitulating those cell densities with the low and high melanocyte densities, respectively, being able to address possible species differences for non-clinical drug development. Different melanocyte densities are accompanied by differences in melanin content with pharmacological implications such as binding of small molecule drugs to melanin\textsuperscript{34} and by different IL-6 levels. IL-6 is secreted by both melanocytes and RPE and is a key cytokine for the immunological state of the eye involved in maintaining uveal tissue homeostasis\textsuperscript{35}. IL-6 also plays a central role in ocular disease: intraocular IL-6 levels have been found elevated in a plethora of retinal diseases including uveitis, AMD and diabetic eye disease\textsuperscript{36,37}. Interestingly, IL-6 and TNF-\alpha were the only cytokines detected in human vitreous of controls\textsuperscript{37}, which corresponds to the cytokine profile of the low melanocyte density condition of our model (Fig. 3c). This condition was, hence, used for the further studies.

**Perfusion of circulating immune cells.** To integrate an immune component into the CoC, freshly isolated human peripheral blood mononuclear cells (PBMCs) were perfused through the endothelial channel. The perfusion process and parameters were carefully chosen to assure cell viability while avoiding immune cell activation that would lead to an uncontrolled and undesired immune cell response (Fig. 4). The capacity of the immune cells to adhere to the membrane and migrate towards the melanocyte compartment was initially assessed in acellular chips. We perfused PBMCs and activated T cells ("Act", treated with anti CD3/CD28 antigens) through chips containing either bare hydrogel or hydrogel supplemented with CCL19, a chemokine responsible for directing T cells from the blood stream into lymph nodes\textsuperscript{38}. In the absence of T cell activation and CCL19, almost no immune cells were present in the melanocyte compartment whereas cell recruitment was clearly observable in the presence of CCL19 and T cell activation (Fig. 4a).

In the second step, we perfused PBMCs and activated T cells through the fully assembled CoC to better understand the immune cell recruitment into the melanocyte compartment and evaluate if T cell activation would mimic the disease phenotype of
uveitis. After 24 h of linear perfusion, more than 50% of the perfused immune cells remained in the chip (Fig. 4b); moreover, cells in the effluent were not proliferative (<5% of Ki-67 positive cells, Supplementary Fig. 1). The cell viability remained above 75% for both PBMCs and activated T cells. Fraction of T cells positive for activation marker CD69 was twofold lower in the chip effluent than for plate controls (Fig. 4c). In contrast to this, CD25, a later marker of T cell activation was not changed by activation, probably due to the short period of activation of 24 h. This suggests that activated cells stay in the chip, adhering to the endothelium and/or migrating towards the other compartments as also observed by quantitative image analysis of the entire chip and measurement of cytokines in the chip effluent. Treatment with CD3/CD28 antigens led to an increase in PBMC and T cell migration particularly towards the lower half of the melanocyte compartment (Fig. 4d, e). T cell activation resulted in consistently higher cytokine levels for all tested cytokines. IL-10, particularly was not detected in PBMCs and detected at a low concentration in activated cells, consistent with the early time point of 24 h (Fig. 4f). IL-6 mediates T cell migration in the presence of extracellular matrix, requiring integrin signal transduction pathways and a gradient for chemotactic migration. Therefore, IL-6 increase could explain the increased recruitment of immune cells. This is reinforced by the higher proportion of immune cells that migrated into the melanocyte compartment in high melanocyte density chips: 3-fold increase for all immune cells and 7-fold for T cells (Supplementary Fig. 2), when perfused with PBMCs. In high melanocyte density chips, T cell activation did not result in an increase of immune cell recruitment (Supplementary Fig. 3).

Exposure of the immunocompetent CoC to immunomodulatory drugs

Cyclosporine A. The capacity of the CoC to respond specifically to immunomodulatory stimuli was evaluated using the well-known immunosuppressor Cyclosporine A (CsA). The CoCs were exposed to different doses of CsA while perfused with PBMCs activated via anti CD3/CD28 antigens. CsA treatment did not significantly affect immune cell viability (Fig. 5A). However, the cell recovery in the effluents was significantly higher for CsA treated CoCs than for controls with activated T cells or PBMCs (Fig. 5b). The immunosuppressing effect of CsA was observed in
a reduction on CD69 expression in CD8a positive cells, statistically significant for both concentrations (Fig. 5c), a reduction of all measured cytokines in the chip (Fig. 5d) and of all except for IL-2 in the plate controls (Fig. 5e). The IL-2 increase in chips caused by T cell activation is slightly reverted in the high CsA condition on the chip but not on the plate controls. Most importantly, these effects correlate with CsA concentration dependent reduction in immune cell recruitment. Treatment with high concentrations of CsA reversed the infiltration of immune cells by 2-fold relative to activated T cells and 1.8-fold relative to low CsA condition (Fig. 5f, g).

CsA was selected not only for its immunosuppressive properties, but also for its effects on improving uveitis symptoms. CsA has a paradoxical effect on T cells since it is able to suppress T cell activation and proliferation at high doses but, at lower doses, it is reported to have an immunomodulatory effect with potential application for immuno-oncology therapies. The mechanism that supports the effect of this drug for treating uveitis is that...
uveitis is characterized by T cell activation and infiltration into the choroidal tissue, as well as other inflammatory cells including B cells and macrophages. As mentioned above, IL-6 is increased in posterior uveitis and identified as a potential therapeutic target. Our data show a similar biological response when the chip is treated with activated cells covered by cytokine production (IL-6 increase) and immune cell recruitment to the choroid. Taking a closer look into the correlation of cytokine secretion in the CoCs and the immune cell recruitment, CsA effectively inhibits IL-2 production in vivo and in vitro, as observed for the high CsA concentration in the chip but not in the plate control. IL-2 is required to maintain the activation state of T cells. Activation of T cells only led to an increase of IL-6 in the CoCs, but not in the plate control. In addition to melanocytes and RPE, monocytes can also produce IL-6, promoting the proliferation and migration of T cells in a concerted way. CsA is able to directly reduce IL-6 production by monocytes, in agreement with the 10-fold reduction of IL-6 levels in CsA treated chips (Fig. 5d). The monocyte role was not explored in depth in the present work. However, monocyte migration towards uveal...
melanocytes after being in contact with conditioned medium from activated T cells that contains increased IFN-γ and TNF-α has been described in vitro. T cells produce these cytokines after T cell receptor stimulation and induce monocyte production of GM-CSF. Consequent monocyte activation promotes monocyte adherence, migration, chemotaxis. Our data also show elevated IFN-γ and TNF-α in the CoCs perfused with activated T cells, which might suggest a T cell dependent monocyte activation and migration, represented by the CD3 negative PBMCs population, which was reduced by more than 2-fold with high CsA concentrations (Fig. 5i). Interestingly, in the chips perfused with activated T cells, only 50% of the migrated cells were CD3 positive, independently of their depth of penetration into the melanocyte compartment. For chips perfused with PBMCs, most of the cells were in the upper half of the compartment, near the endothelial cells (Fig. 5f). This was not reverted by CsA treatment suggesting that CsA treatment affected the number of cells that infiltrate the melanocyte compartment, but not the spatial distribution of immune cell types. To observe the effect of activation on T cell proliferation and possibly a difference in the spatial distribution of immune cell types, the evaluation period would need to be increased because 24 h might be insufficient. CsA was also reported to reduce T-cell trans-endothelial migration. That effect is reflected by the CsA-induced reduction of the number of T cells by 1.2- and 1.8-fold for low and high CsA, respectively, relative to the activated T cells.

**Bi-specific T cell engagers (TCBs).** Bi-specific T cell engagers (TCBs) have been investigated for immuno-oncology therapies for more than 30 years. However, there are still unresolved potential safety liabilities for this mode of action revealed in toxicity studies with non-human primates and in clinical studies. TCBs are antibodies that target a constant-component of the T cell/CD3 complex and a tumor-associated antigen (TAA). The relative abundance of T cells in blood, and their cytotoxicity and proliferation capacity upon activation make T cells an effector cell of choice. To test the capacity of the CoC for immune safety assessment, we tested two antibodies, labeled as A and B. Both contain the T cell receptor binding domain but lack the TAA. Both TCBs were compared with a commercially available reagent developed to activate and expand human T cells via exposure to beads containing anti CD3/CD28 antigens. The PBMC suspension was supplemented with the different T cell activators and then perfused through the CoC for 24 h as described for the CsA study. The PBMCs recovered in all effluents showed good viability (Fig. 6a). The treatment with TCB B led to a higher cell recovery than TCB A or with the activated condition after chip perfusion and in the plate controls (Fig. 6b).

Both TCBs led to a similar low increase in IL-2 as for the commercial T cell activator (Act). TCB B elicited minimal cytokine release, with some increase mainly evident for granzyme B. TCB A led to significantly higher levels of TNF-α and IFN-γ when compared to the commercial activator, while this latter showed higher levels for IL-10, IL-6 and granocyte B. This is particularly interesting since T cell secretion of TNF-α and IFN-γ leads to monocyte migration towards uveal melanocytes, as discussed above. This could lead to an untargeted, undesired and possibly detrimental immune response. Our data show that TCB B is leading to similar T cell recruitment to the melanocyte compartment as TCB A and the activated condition but to less recruitment of CD3 negative cells. These observations and the higher presence of cells in the chip effluent in TCB B treated chips support its higher efficiency on T cell-specific recruitment while producing lower levels of pro-inflammatory cytokines.

**Conclusion**

Altogether, this microphysiological CoC model recapitulates key features of the human choroid such as cellular composition, pigmentation, tissue-specific endothelial barriers as well as cytokine secretion and responds to immunomodulatory strategies, both to immunosuppressors and immunoactivators. Within this study we built a tool (i) for probing mechanisms of uveitic side effects of immune-related therapeutics for cancer treatment as demonstrated with the TCB data (Fig. 6) as well as (ii) for screening of uveitis treatments in a model of the inflammatory choroid, artificially obtained by systemic T cell activation, displaying the main uveitis features on the chip. Thereby, although not capturing the entire universe of events, the main molecular events of drug induced uveitis (i) inflammation and (ii) immune cell infiltration into the choroid could be mimicked; similarly, their reduction upon CsA treatment, a classical immunosuppressant used for uveitis treatment, could be recapitulated. Since drug-induced uveitis is idiosyncratic, a drug, e.g., pilimimumab, would not necessarily trigger such effects in a model. The CoC was also sensitive to detect differences in the immunological responses to two TCBs providing hints on their potential safety profile. The data presented here sustain the relevance of using an advanced cell culture system integrating several cell types involved in the immune response of a specific tissue to support pharmaceutical compound ranking and decision making. The immune response in the CoC was shown to be sensitive to the mechanisms of T cell activation and suppression, key aspects for a human-relevant healthy and diseased in vitro model. Furthermore, the processes established for building the CoC model can potentially be translated to other tissues where the stromal,
endothelial and epithelial cells are key elements of mechanisms of disease and/or toxicity and/or expanded to test multiple donors for exploratory mechanistic studies on patient-specific idiosyncrasies. This could help building a toolbox of in vitro models recapitulating aspects of the immune system to study immune-oncology therapies such as checkpoint inhibitors, TCBs and CAR-T cells.

Materials and methods
Microfluidic chip fabrication by UV-lithography and replica molding. The CoC is based on a tailored five-layered microfluidic platform comprising three microstructured polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning, USA) layers, which are separated by two isoporous, semipermeable PET membranes (Fig. 1a). 100 µm high channel structures in the PDMS pieces were generated using two differently patterned master wafers fabricated via photolithographic processes53. The PDMS elastomer base and curing agent were mixed in a 10:1 (w/w) ratio and desiccated to remove air bubbles. Two different replica molding approaches were conducted as described previously53. Standard molding was used to obtain 3 mm and 0.5 mm thick PDMS pieces with channel structures for the RPE and Melanocyte layers, respectively. Curing was achieved at 80 °C for 4 h. The endothelial layer was fabricated using exclusion molding to achieve a 0.1 mm thin layer with through hole channel structures. Layers were cured at 80 °C for 1 h. Commercially available semipermeable polyethylene terephthalate (PET) membranes (ηp = 3 µm; ρp = 8 × 10^5 pores per cm²; TRAKETCH® PET 3.0 p S210 × 300, SABEU GmbH & Co. KG, Northeim, Germany) were functionalized by a plasma-enhanced, chemical vapor deposition (PECVD) process as described previously53. Microfluidic chips

Fig. 6 Effect of the TCBs on the Immuno-competent Choroid-chip with low melanocyte density after 24 h of perfusion or cell culture (plate controls) respectively, in PBMCs treated and non-treated with TCB A or TCB B (5 µg/mL). a Viability of the immune cells collected in the chip effluents and plate control supernatants (n = 3–10, the dots represent each data point). b Fraction of cells recovered in the chip effluents and plate control supernatants relative to the number of recovered cells in the PBMC control (n = 3–10). c Cytokine profile in the TCB A and TCB B treated choroid chips relative to PBMCs (n = 6–12). d Quantification of immune cell recruitment by image analysis of the whole chip, distinguishing PBMCs (cell tracker labeled prior perfusion) and T cells (CD3 post fixation staining) in the top (dark red and green) and bottom (light red and green) halves of the melanocyte compartment chips with low melanocyte density (n = 3–9). e Representative images of the melanocyte compartment containing labeled PBMCs (Cell tracker, red) and T cells (CD3, green) represented as maximum intensity projections for each of the analyzed condition. Scale bar = 500 µm. Bars represent Averages ± SEM. Statistical analysis represents the comparison of the TCBs treatment in PBMCs with cells activated with antiCD3/CD28 beads (*P < 0.1, **P < 0.05, ***P < 0.01), unless labeled otherwise, and the comparison of the total and bottom compartments of the TCBs treatment in PBMCs with non-treated PBMCs (*P < 0.1). Statistical analysis is detailed in Supplementary data 1, including detailed information of the number of biological replicates per group, exact p values and individual data points.
were cultured with gentamicin for the first 15 min. To enhance bonding strength, assembled parts were placed at 80 °C for at least 10 min and after assembly of the entire chip.

Prior to cell injection, all chips were O2: plasma sterilized (50 W, 0.2 cm2·min−1 O2) for 5 min. Afterwards, channels were filled with Dulbecco’s phosphate-buffered saline with MgCl2/ CaCl2 (PBS; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and centrifuged under sterile conditions for 3 min at 200 × g to remove residual air from the systems.

Cell isolation and culture. All research was carried out in accordance with the rules for investigation of human subjects as defined in the Declaration of Helsinki. Patients gave a written agreement according to the permission of the Landesärztekammer Baden-Württemberg and the IRB (IRB: F-2012-078 & F-2020-166-1) that all primary melanocytes and microvascular endothelial cells with and without cell concentration and viability were monitored using the Guava Reagent (Luminex) by the manufacturer instructions. Blood was collected up to 1 h before isolation. After centrifugation at 200 × g for 5 min, cells were resuspended in BRDM supplemented with 10% FCS, 20 ng/mL of EGF (Cell Guidance Systems), depending on the adherence and passage. Cells were seeded at 75,000 cells per cm2 and kept in culture in parallel with the chip culture until the cell size and viability were monitored using the Guava Reagent (Luminex) by the manufacturer instructions. 

Primary microvascular human endothelial cells (MVECs) and melanocytes were isolated from adult human skin tissue of plastic surgeries received from Dr. Ulrich E. Ziegler (Klinik Charlottenhaus, Stuttgart, Germany). Visible blood vessels and connective tissue were removed and the skin biopsies were cut into 8 cm2 pieces and further cut into strips. Tissues were incubated with a 2 U/ml disperse solution (Sera Electrophoresis, Heidelberg, Germany) in PBS overnight at 4 °C. Samples were then washed in PBS and epidermis and dermis were separated for further melanocyte and microvascular endothelial cell isolation, respectively. Human MVECs were isolated using the literature28. The total volume of the stromal channel corresponds to 1.5 µL and a 10 000 cells/cm2 and kept in culture in parallel with the chip culture until the cell concentration and viability were monitored using the Guava Reagent (Luminex) by the manufacturer instructions. Blood was collected from elective surgeries for the collection of primary melanocytes and microvascular endothelial cells and with the ethical Committee of the Eberhard Karls University Tübingen (Nr. 678/2017BO2, for iPSC derived retinal pigmented epithelial cells and Nr. 495/2018-B02 for the isolation of PBMCS from whole blood). Human melanocytes and microvascular endothelial cells were isolated from biopsies that were taken from female, pre-obese donors (BMI 25.0–29.9, as per the WHO classification), aged 25–65. Human PBMCS were isolated from healthy donors (BMI <25.0, as per the WHO classification), aged 25–35. Retinal pigmented epithelial (RPE) cells were obtained from human induced pluripotent stem cells as described previously42. RPE cells were maintained in B27-, retinal differentiation medium (BRDM) consisting of DMEM/F12 and DMEM, high glucose (1:1) with 2% B27 without vitamin A, 1% non-essential aminos acids and 1% antibiotic antimycotic solution (all from ThermoFisher Scientific, USA). Cell culture media was changed daily and cells were used up to passage three. Passage 1 of cells was performed using Accumax at 37 °C and 5% CO2 for 10–30 min, depending on the adherence and passage. Cells were seeded at 75,000 cells per cm2 in BRDM supplemented with 10% FCS, 20 mg/mL of EGF (Cell Guidance Systems), 20 ng/mL of FGf2 (Cell Guidance Systems), 10 µM of ROCK-inhibitor Y-27632 (Selleck Chem). After 24 h the medium was changed to unsupplemented BRDM and changed daily thereafter. Pasaging was never performed earlier than every 4 weeks and never used preconfluent phenotype.

Cell seeding on the choroid-chip. Prior to cell injection, the RPE channel was coated with 50 µl of 50 µg/ml laminin in DMEM/F12 and DMEM, high glucose (1:1) for 2 h at 37 °C, 95% Humidity, 5% CO2 (will be referred to as “incubation conditions”). Afterwards, RPE cells were added with mechanical and chemical collection of RPE cells using a cell scraper. Cells were stripped through a 100 µm mesh and then centrifuged at 200 × g for 5 min. Cells were cultured in endothelial cell growth media in a 75 cm2 cell culture flasks. Both melanocytes and endothelial cells were cultured with gentamicin for the first 10 days of culture and used in passage 2 or 3 for this study. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using the MACS Prep PBMC Isolation kit (Milteny Biotec), according to the manufacturer instructions. Blood was collected up to 1 h before isolation. After isolation, PBMCs were labeled with CellTracker™ Deep Red Dye (Thermofisher Scientific) at 1 µM in X-vivo medium (Bioyim) for 45 min at 37 °C and protected from light. The cell concentration and viability were monitored using the Guava ViaCount™ Reagent (Luminex) by flow cytometry using a Guava EasyCyte HT (Guava, Luminox) according to the manufacturer instructions. To evaluate the proportion of the cell volume to the total stromal channel volume, the total volume of the stromal channel corresponds to 1.5 µL and a high and low melanocyte concentration were achieved corresponding to the chorioid of a white human (5%) and of a rhesus macaques (50%) as described in the literature28. The total volume of the stromal channel corresponds to 1.5 µL and a volume of 5 µL was used for cell seeding and an expansion factor of 3.68 was used to account for the melanocyte morphology change while spreading. The calculation of the melanocyte morphology change was based on passing a line across the cell on size. Prior to melanocyte seeding, the chip was connected to a syringe pump to apply a media perfusion through the endothelial channel at a flow rate of 5 µL/h with positive pressure. The cell-hydrogel suspension was injected into the stromal channel, which was subsequently closed. Chips were then placed in an incubator, allowing cells to attach to the top membrane. Afterwards, the chip was flipped back to the upright position and placed for an additional hour in the incubator. Filter pipet tips filled with 100 µL ECGM media each were added to the in- and outlet of the endothelial channel for 24 h of static culture. RPE and stromal channels were completely closed off.

Melanocytes, embedded into a dextran-CD hydrogel, supplemented with RGD peptide (Celllendes; Catalog no. G93-1), were injected into the stromal channel of the chip, 24 h after MVEC seeding. The hydrogel was prepared according to manufacturer instructions. The melanocytes were passaged as described above and the average cell diameter assessed using ImageJ (Supplementary material 2). By calculating the proportion of the cell volume to the total stromal channel volume, high and low melanocyte concentration were achieved corresponding to the chorioid of a white human (5%) and of a rhesus macaques (50%) as described in the literature28. The total volume of the stromal channel corresponds to 1.5 µL and a volume of 5 µL was used for cell seeding and an expansion factor of 3.68 was used to account for the melanocyte morphology change while spreading. The calculation of the melanocyte morphology change was based on passing a line across the cell on size. Prior to melanocyte seeding, the chip was connected to a syringe pump to apply a media perfusion through the endothelial channel at a flow rate of 5 µL/h with positive pressure. The cell-hydrogel suspension was injected into the stromal channel, which was subsequently closed. Chips were then placed in an incubator, allowing cells to attach to the top membrane. Afterwards, the chip was flipped back to the upright position and placed for an additional hour in the incubator. Filter pipet tips filled with 100 µL ECGM media each were added to the in- and outlet of the endothelial channel for 24 h of static culture. RPE and stromal channels were completely closed off.

Immune cell perfusion and treatment. Isolated and pre-labeled PBMCS were cultured at a concentration of 1.0 × 106 cells/ml. Plate controls were performed for the flow cytometry and for the cytokine measurements in all treated- and non-treated conditions in static 24-well cell culture plates. The total volume for each chip perfused and the plate controls was kept equally at 960 µL, assuring the same cell concentration and treatment concentration. Chips were cultured in an incubator that allowed for chip handling under sterile conditions (Incubator FlowBox™, A. Gilgen, Germany) to mimic the incubator. The outlet of the channel was equipped with a reservoir holding the PBMC suspension, while the pump was changed to the withdraw mode at 40 µL/h, maintaining the flow direction. After 24 h, the chip effluents and plate control supernatants were collected for further analysis and the chips were fixed for immunofluorescent labeling.

T cell activation was achieved by adding 5 µL of anti-CD3/CD28 antibodies (TransAct, Milteny Biotec) to the 1.0 × 106 cell suspension, being present for the 24 h of perfusion in the chip or static in the plate controls.

Cyclosporin (CsA) treatment was performed for the 24 h of perfusion using two concentrations, which were selected to mimic low and high plasma concentrations of 100 and 500 ng/mL52,56 and to evaluate a dose response of its effects on chips perfused with activated T cells. Therefore, CsA treatment on chip was performed only in combination with the T cell activation. PBMCs were also treated with CsA in the plate controls to confirm that CsA treatment is not affecting the immune cell phenotype and cytokine profile.

T cell specific antibody (TCB) treatment was performed for the 24 h of perfusion. Both TCB A and TCB B were added at the concentration of 5 µg/ml to PBMCs.

Evaluation of immune cells in chip effluents and plate control supernatants. The effluents of the immune cell perfusion were collected and centrifuged at 200 × g for 5 min. The cell pellet was re-suspended in 950 µL of PBS for evaluation of cell number and viability using the Guava ViaCount™ Reagent (Luminex) by flow cytometry (Guava, Luminox), according to the manufacturer instructions, and for flow cytometry analysis. The supernatant was centrifuged for 10 min at 10,000 × g at 4 °C to remove cell debris and stored at −80 °C for further analysis of the cytokine profile.

For flow cytometry analysis cells were washed and labeled with anti-CD98-PerPC, anti-CD25-APC and anti-CD69-FITC (Biolegend) for 20 min at 4 °C. Antibodies were used at concentrations recommended by the manufacturer. As...
buffer solution and for the washing step PBS containing 2 mM of EDTA and 0.1% of BSA was used. Autofluorescence and isotype controls were performed to confirm similar levels of unspecific signal in every staining. Table 1 specifies the used antibodies and isotype controls. The analysis was performed using the Guava Incyte software. The PBMCs were gated on the FCS/SSC plot before gating for CD8α. The quantification of the fraction of CD25 and CD69 positive cells was performed for the CD8α positive population (Supplementary Fig. 4).

For the cytokine measurement, the samples were thawed and analyzed using the LEGENDPlex Human CD8/NK Panel (Biolegend), according with the manufacturer instructions, using a Guava EasyCyte HT (Guava, Lumine). The samples were diluted 1:100 to assure that the analyses are within the calibration curves. The analysis was performed on the LEGENDplex™ v.8.0 software.

**Immunofluorescence staining.** Chips were washed twice with 100 µL of PBS by gravity flow through the endothelial and RPE channel and then fixed with 4% ROTrH®/Histofix solution (Roth) for 10 min at room temperature (RT) using the same method. The chips were washed with PBS after fixation and stored until further processing in PBS at 4°C. Blocking of unspecific binding and permeabilization was achieved by incubation with a 3% BSA and 0.1% Triton X-100 solution in PBS for 30 min at RT. Chips stained for CD3 were not permeabilized to avoid losing the cell tracker label in the PBMCs. All primary and secondary antibodies, specified in Table 2, were used at dilutions of 1:50 and 1:100, respectively. Positive and negative controls were used for all antibodies as well as secondary antibody controls where applicable. The highest concentrations were selected considering the background/signal ratio and the high tissue to media ratio in the chip that is significantly lower than in tissue slices or cells cultured as monolayers in well plates. High antibody concentrations were tested and were required due to the high tissue to media ratio. Using 30 µL of antibody solution per chip, primary antibodies were incubated overnight at 4°C and secondary antibodies for 1 h at RT. Chips were washed three times with PBS after each of the antibody incubation steps using gravity flow. Following the incubation with the secondary antibodies, chips were incubated with DAPI in 0.2% saponin solution in PBS for 45 min at RT. Chips were washed three times each with 0.2% saponin solution in PBS and PBS only, and then stored at 4°C until imaged.

**Permeability assays.** For the evaluation of the endothelial and outer blood-retina barrier, chips were perfused with Carboxyfluorescein (0.377 kDa, Sigma, 21877) and Dextran Texas Red (70 kDa, Thermo Scientific, D1830) added to the EGC media at concentrations of 100 µM and 14.3 µM, respectively. Compounds and concentrations were based on previous studies31. Image acquisition was performed for the CD8α positive population (Supplementary Fig. 4). The cytokine measurement, the samples were thawed and analyzed using the LEGENDPlex Human CD8/NK Panel (Biolegend), according with the manufacturer instructions, using a Guava EasyCyte HT (Guava, Lumine). The samples were diluted 1:100 to assure that the analyses are within the calibration curves. The analysis was performed on the LEGENDplex™ v.8.0 software.

**Table 1 Flow cytometry antibodies and isotype controls.**

| Fluorophore | Reactivity | Antigen | Clone | Lot number | Product reference |
|------------|------------|---------|-------|------------|------------------|
| APC        | Anti-human | CD25    | B9C6  | B258747    | 302610           |
| FITC       | Anti-human | CD69    | FNS50 | B224681    | 310904           |
| PerCP      | Anti-human | CD8a    | HIT8a | B241517    | 300922           |
| FITC       | Mouse      | IgG1,k  | MOPC-21 | B199152 | 400107           |
| APC        | Mouse      | IgG1,k  | MOPC-21 | B257953 | 400119           |
| PerCP      | Mouse      | IgG1,k  | MOPC-21 | B226117 | 400147           |

**Table 2 Immunofluorescence antibodies.**

| AB            | Supplier, Reference | Host | Reactivity |
|---------------|---------------------|------|------------|
| CD1-PECAM-1   | Dako, M0823         | Mouse| Human      |
| ZO-1          | ThermoFisher, 40-2200| Rabbit| Human      |
| TYPRI         | Abcam, 3312         | Mouse| Human      |
| Ki-67         | R&D Systems, AF7617 | Sheep| Human      |
| CD3-FITC      | Biocell, 300406     | Mouse| Human      |
| Alexa Fluor 488 | ThermoFisher, A11008| Goat | Rabbit     |
| Alexa Fluor 464 | ThermoFisher, A11003| Goat | Mouse      |
| Alexa Fluor 464 | ThermoFisher, A21098| Donkey| Sheep      |

**Confocal image acquisition and analysis.** Whole chips were imaged using a Zeiss LSM 710 version 3.4c Quasar NLO equipped with the inverted microscope platform Axio Observer.Z1. All images were acquired with an EC Plan-Neofluor 10x/0.30 M27 air objective. The sample was excited with three different lasers. A HeNe laser was used to excite the red cell tracker at 633 nm and emission was measured at 638–755 nm. FITC and DAPI were excited by an argon laser at 488 nm and Diode 405–30 at 405 nm, respectively. Emissions of these fluorochromes were measured at 495–630 nm and 410–501 nm, respectively. The pinhole of the red, green and blue channel was set to 90 µm, 23 µm and 17 µm, respectively. Digital gain was constantly kept at a 1.0 for all channels. The red master gain was increased to enhance the signal in respect to the background. The Hybrid 3D Median filter plugin was used to reduce noise in the red and green channel. Finally, closing was performed on the green channel by using the Morphological Filters (3D) function implemented in the MorphoLib plugin59 with a ball of radius = 2, to fill circles resulting from surface staining of T cells.

**Statistics and reproducibility study design.** Every chip is considered an independent biological experiment. For each run of chips, the same condition was performed at least twice. For all graphs with a statistical analysis, the minimum sample size is 3. A minimum of 2 runs and a maximum of 12 runs was performed. The sample size criteria depended on the logistical complexity of the whole...
experiment, primary cell availability, distribution of donors to assure paired conditions, availability of pumping systems and technical issues. The criteria was the following: for proof of concept data, the sample size is at least of 2; for the validation data (PBMCs vs Act) and for the proof of concept study a minimum of 4 (CoA study and TCB data). Every test was performed in parallel with a respective control (PBMC and Act). Chips were excluded and not analyzed if there would be technical issues with the connection and pumping system. Only chips with the correct efficient volume were considered. Replication was successful. Variability is within biological variability for a complex system with 4 different cell types cultured simultaneously. Chips were randomly allocated into experimental groups using a numbering system for distinguishing them. The numbering system was kept until analysis.

Bar graphs are represented as average values ± SEM. The number of independent experiments, biological replicates, is detailed in each figure caption. Statistically significant differences among two groups were analyzed using a One-way ANOVA for cell viability, cell fractions, CD69 expression and cytokine analysis and using mixed-effects model (Restricted Maximum Likelihood (REML)) for the migration analysis were PBMCs and CD3+ cells - considered dependent variables. This model was chosen instead of Two-way ANOVA due of the different number of biological experiments per condition and the results can be interpreted like repeated measures ANOVA. Graphpad Prism software (version 8.2.0), with the threshold for significance set at p < 0.1. Those are referred in the text and exhaustively described in Supplementary Data 1.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The source data for the graphs and charts are available as Supplementary Data 1 and any remaining information can be obtained from the corresponding author upon reasonable request.

Code availability
The code used for image analysis is fully uploaded in the Supplementary material 4.

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12 COMMUNICATIONS BIOLOGY | https://doi.org/10.1038/s42003-021-02977-3 | www.nature.com/commsbio
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**Author contributions**

P.L., S.K., M.C., A.S. designed the study; P.L. and C.P. designed and developed the chip production protocols, K.S. performed the COMSOL simulation; M.C., K.L. and K.S. performed experiments; M.C. collected and analyzed data; M.J.F. performed the image analysis; L.M. contributed to the RPE differentiation; M.W. contributed to the collection of immune cells. S.L., K.A., M.M., V.N., A.M.G., and A.S. provided conceptual advice.

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**Competing interests**

M.M., V.N., A.S., A.M.G., and S.K. are employees of F. Hoffmann-La Roche Ltd. M.C., C.P., and P.L. hold a patent related to the technology presented in the manuscript (WO2020120466). The remaining authors declare no competing interests.

**Additional information**

**Supplementary information**

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