Extracellular vesicles have an important function in cellular communication. Here, we show that human and mouse monocytes release TGF-β1-transporting vesicles in response to the pathogenic fungus *Candida albicans*. Soluble β-glucan from *C. albicans* binds to complement receptor 3 (CR3, also known as CD11b/CD18) on monocytes and induces the release of TGF-β1-transporting vesicles. CR3-dependence is demonstrated using CR3-deficient (CD11b knockout) monocytes generated by CRISPR-CAS9 genome editing and isolated from CR3-deficient (CD11b knockout) mice. These vesicles reduce the pro-inflammatory response in human M1-macrophages as well as in whole blood. Binding of the vesicle-transported TGF-β1 to the TGF-β receptor inhibits IL1B transcription via the SMAD7 pathway in whole blood and induces TGFB1 transcription in endothelial cells, which is resolved upon TGF-β1 inhibition. Notably, human complement-opsonized apoptotic bodies induce production of similar TGF-β1-transporting vesicles in monocytes, suggesting that the early immune response might be suppressed through this CR3-dependent anti-inflammatory vesicle pathway.
Extracellular vesicles (EVs) are released by a variety of human cells, and are important mediators in the coordination of the immune response in order to maintain host homeostasis. Depending on the stimulus, EVs transport a combination of nucleic acids, proteins, and lipids to other cells and have been implicated in certain pathological conditions, particularly microbial infections and cancer. C. albicans infections are a severe threat to life for immunocompromised persons, including patients who received an organ transplant, who are undergoing antitumor therapy, or who are infected with human immunodeficiency virus (HIV), as well as patients who have experienced major trauma or have extended stays in the intensive care unit. The pathogenicity of C. albicans depends on a broad range of virulence factors, and the fungus has developed evasion mechanisms to survive in the human host. In the process of systemic infection, C. albicans is recognized by immune cells due to the presentation of pathogen-associated molecular patterns (PAMPs), resulting in the initiation of a series of immune response mechanisms. β-glucan, which has been described as a major recognition molecule of C. albicans, is mostly detected via the dectin-1 receptor. Following recognition, innate immune responses against C. albicans include complement activation, phagocytosis, reactive oxygen species generation, pro-inflammatory cytokine release, and extracellular trap formation, but whether immune cells respond to in vivo fungal infection by generation of human EVs is yet unknown.

This study provides insight into the immunomodulatory properties of TGF-β1-transporting EVs that are generated by monocytes in response to the human pathogenic fungus C. albicans, as well as human apoptotic cells.

Results

C. albicans induces vesicle release from human blood monocytes. Human monocytes directly recognize C. albicans and react in multiple ways to the fungus. They take up fungal cells by phagocytosis; release DNA traps, similar to neutrophils, to immobilize the fungus; and secrete toxic reactive oxygen species. As monocytes also produce vesicles to communicate with other cells, we addressed the question whether C. albicans induces vesicle release in monocytes. Human blood monocytes were isolated fromuffy coats by magnetic sorting of CD14-positive cells (~95% purity), and incubated with complement-pre-opsonized C. albicans on a coverslip. After 1 h of incubation, the cells were fixed onto a microscopy slide, and the monocytes were monitored for the presence of vesicles using the previously described vesicle marker tetraspanin (CD63). Monocytes alone without C. albicans showed several vesicles, which predominantly surrounded the nucleus (Fig. 1a). When monocytes were incubated with C. albicans, vesicle formation substantially increased. Again, vesicles surrounded the nucleus, but were also found extracellularly, indicating vesicle release. Vesicles formed in response to C. albicans are referred to from here on as opsonized Candida-induced monotypic extracellular vesicles (MEVsCa) to distinguish them from the monotypic extracellular vesicles (MEVs) that are spontaneously produced in the absence of C. albicans.

To follow vesicle formation in real time, CD14+ monocytes in the presence of opsonized C. albicans were tracked by live cell imaging in culture dishes using nucleic acid staining—Sytos Orange, which does not penetrate living cells but can penetrate extracellular vesicles. Live cell imaging revealed phagocytosis of C. albicans by monocytes within minutes and generation of nucleic acid-containing vesicles. Release of vesicles was observed after ~20–40 min (Fig. 1b). Vesicle generation and release from monocytes in presence of C. albicans was captured in real time using dynamic light-scattering microscopy (DLSM) (Supplementary Video 1), confirming fast release of generated vesicle. To track vesicle generation by monocytes under more physiologic conditions, live cell imaging of monocytes was performed in an ex vivo whole-blood model system. Whole blood was infected with C. albicans, and monocytes were stained with anti-CD14 and vesicles were stained with anti-CD63. Vesicle generation was seen within 10 min after infection, and increased after 20 min (Fig. 1c). Released vesicles were also confirmed by scanning electron microscopy (SEM) (Fig. 1d). In summary, monocytes treated with opsonized C. albicans released EVs within 1 h after infection. In all subsequent experiments, C. albicans infection was performed for 1 h, unless otherwise indicated.

MEVsCa are double-layered vesicles. For detailed characterization, MEVsCa generated by isolated human blood monocytes in response to opsonized C. albicans were isolated using a polymer precipitation method. These vesicles were analyzed for their number and size by measuring the Brownian movement of vesicles in suspension using DLSM (Fig. 2a). The number of MEVsCa harvested from C. albicans-infected monocytes (5 × 10⁵) was about ten times higher than the number of MEVs harvested from the same number of uninfected monocytes (5 × 10⁵) (Fig. 2b). Thus, monocytes release substantially more vesicles upon infection with the fungus. Five major populations of MEVs were identified, with sizes ranging from 50 to 450 nm, and three major populations of MEVsCa were observed, with sizes ranging from 50 to 300 nm (Fig. 2c). For further characterization, cryogenic electron microscopy (Cryo-EM) and freeze-fracture electron microscopy (FFEM) were performed on MEVsCa. Cryo-EM verified the presence of 200 nm vesicles, and showed a double-layered membrane (Fig. 2d). Under FFEM, which revealed fractured concave and convex vesicles, the inner and outer membranes became visible (Fig. 2e).

In addition, the composition of MEVs and MEVsCa was determined using label-free liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics. Proteins were analyzed from vesicles derived from the same number of infected and control monocytes (1 × 10⁷) after tryptic digest. MEVsCa showed a significant increased level of 361 proteins compared with MEVs, while 29 proteins were significantly decreased in abundance (Fig. 2f). The significantly increased proteins were further categorized using Gene Ontology enrichment analysis for biological process, molecular function, and cellular compartment. Most (209) of the proteins with higher abundance in MEVsCa were extracellular exosome-related proteins (Fig. 2g). Heat shock proteins, along with histone fragments commonly found in vesicles, were found in MEVs and MEVsCa. The presence of CD14 in both MEVs and MEVsCa confirmed their monocytic origin. In addition, myeloid lineage marker such as CD11b, complement receptor type I (CR1), and Toll-like receptor 2 (TLR2) were increased in MEVsCa by 2+, 8+, and 11-fold, respectively (Fig. 2j). In addition, the tetraspanin CD9 was also identified on MEVsCa, together with commonly found annexins. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed a high abundance of physiologically important complement pathway proteins (Fig. 2h, i, j). As cytokines are important regulators of the immune system and are difficult to detect by mass spectroscopy, cytokine levels in MEVs and MEVsCa were determined by sandwich ELISA. Substantial amounts of transforming growth factor-β (TGF-β1) were detected in MEVsCa derived from infected monocytes but not in MEVs derived from the same number of uninfected monocytes (Fig. 2k). By contrast, levels of IL-6 (Fig. 2l), IL-10, and IL-1β (not shown) were very low or were not detected. To
exclude extracellular proteins in vesicle polymer precipitates, the results were confirmed also with EVs isolated by ultracentrifugation and size-exclusion chromatography (Supplementary Fig. 1). Furthermore, vesicle markers CD9 as well as HSP90 were identified on TGF-β1-transporting vesicles (70–130 nm) by size-exclusion chromatography, which in addition, characterized these vesicles as exosomes. Presence of few TGF-β1-transporting vesicles without vesicle markers is explained by simultaneous use of different antibodies.

MEVsCa transport TGF-β1. To verify the presence of TGF-β1 in MEVsCa, monocytes were incubated with C. albicans for 1 h on cover slips, and cells were fixed and stained with an antibody against TGF-β1. C. albicans-infected monocytes but not uninfected monocytes showed significant production of MEVsCa of 100–200 nm in size by confocal laser-scanning microscopy (CLSM) (Fig. 3a, b; Supplementary Fig. 2a). To visualize MEVsCa in more detail, TGF-β1 was labeled with immunogold, and the vesicles were analyzed by SEM. MEVsCa but not MEVs showed gold labeling on the surface, demonstrating the presence of TGF-β1 on the outer membrane. The size of MEVs and MEVsCa was ~100–300 nm (Fig. 3c), which was in the same range as the size measured by DLSM (Fig. 2c).

To observe MEVsCa under physiological conditions, MEVsCa were also tracked ex vivo in C. albicans-infected whole blood using live cell imaging and CLSM. Monocytes were tracked over time with anti-CD14 labeling, and vesicles were tracked with anti-TGF-β1 labeling. Shortly after infection (15 min), the monocytes in the blood began to form TGF-β1-transporting vesicles intracellularly, which can be seen as red dots within the monocytes (Fig. 3d). When C. albicans started forming hyphae (45 min later), TGF-β1-transporting vesicles from the same
**Fig. 2 MEV_{Ca} are double-layered vesicles.** a Tracking of EVs by dynamic light-scattering microscopy (DLSM). EVs were isolated from 5 × 10^{5} uninfected or opsonized C. alibans-infected monocytes (MEVs or MEV_{Ca}, respectively) by polymer precipitation. Representative data of three independent experiments (three donors) are shown. b MEV_{Ca} are significantly increased compared with MEVs (data are presented as mean values ± SD, p = 0.0027, unpaired two-tailed t test, n = 3 different donors). EVs isolated from same number of infected or uninfected monocytes were counted by DLSM. c Size distribution of MEVs and MEVs_{Ca} as determined by DLSM using NanoSight NTA 3.2 software. Graphs were generated by overlaying the size distribution of MEVs and MEVs_{Ca} from n = 3 donors. d The double-membrane structure and round shape of the MEVs_{Ca} are visible by cryogenic electron microscopy (Cryo-EM). Bars: 100 nm. e MEVs_{Ca} structure and shape is confirmed by freeze-fracture electron microscopy (FFEM). Bars: 100 nm. f MEVs_{Ca} show significantly higher protein content compared with MEVs (p < 0.05, unpaired two-tailed t test, n = 3 different donors). Proteins from MEVs and MEVs_{Ca} (each from 1 × 10^{7} monocytes) were detected by label-free LC-MS/MS-based proteomics. g Most of the proteins enriched in MEVs_{Ca} compared with MEVs are extracellular exosome-related proteins (Gene Ontology (GO) enrichment analysis). h Heatmap of complement and coagulation proteins upregulated in MEVs_{Ca} (Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis). i Complement pathway proteins were significantly increased in MEVs_{Ca} (Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis). j Heatmap of complement and coagulation proteins upregulated in MEVs_{Ca}. Comparisons in g-i rely on protein content of MEVs_{Ca} from three different donors. k Diagram of an MEVs_{Ca} showing the presence of receptors, marker proteins, and cytokines detected in the current study. l TGF-β1 (data are presented as mean values ± SD, p = 0.0013, unpaired two-tailed t test, n = 3 donors), but not IL-6, is significantly increased in MEVs_{Ca} compared with MEVs (data are presented as mean values ± SD, p = 0.2432, unpaired two-tailed t test, n = 3 different donors). Cytokines from MEVs and MEVs_{Ca} (each isolated from monocytes (5 × 10^{5})) were determined by ELISA.
monocytes were detected extracellularly (Fig. 3d). After 1 h of infection, significantly increased numbers of TGF-β1-transporting vesicles (BEVsCa) were detected in the blood, in contrast to uninfected blood (Fig. 3e, f).

To confirm the formation of TGF-β1-transporting vesicles in vivo, mice were infected with C. albicans and killed 1 day later, and extensively perfused liver tissue was stained for TGF-β1. TGF-β1-transporting vesicles were abundant in the liver tissue of infected, but not control, mice (Fig. 3g, h). Similarly, the vesicle marker RhoA was abundant (Supplementary Fig. 2b; Fig. 3i). To further confirm the generation of TGF-β1-transporting vesicles, blood was collected from the mice, vesicles were isolated, and the TGF-β1 content of the vesicles was analyzed by ELISA. The results confirmed a significant ($p = 0.0083$, unpaired two-tailed
The text reads as follows:

**Fungal β-glucan interaction with CR3 induces MEVs<sub>Ca</sub> release.** To determine how TGF-β1-transporting MEVs<sub>Ca</sub> are generated, we focused on CR3, as CR3 has been described as a key recognition receptor for pathogens<sup>14</sup>. Therefore, CR3 was blocked with the anti-cholesterol drug simvastatin<sup>15</sup>. When CR3-blocked monocytes were infected with opsonized C. albicans<sup>–/–</sup> (CD11b KO) mice (Supplementary Fig. 3a, b). Peripheral blood donors were selected and assayed for their TGF-β1 content by ELISA. No significant difference in TGF-β1 content was observed between MEVs and MEVs<sub>Ca</sub> derived from CD11b KO mouse monocytes, but MEVs<sub>Ca</sub> generated from wild-type monocytes showed a significant 4–6-fold increase in TGF-β1 levels compared with MEVs (p = 0.0021, unpaired two-tailed t test, n = 5) (Fig. 4e).

Having shown the central role of CR3 in the production of TGF-β1-transporting vesicles by C. albicans-infected monocytes, we were interested in identifying the ligand of CR3 responsible for this effect. As iC3b is deposited onto C. albicans due to opsonization, iC3b was used to induce TGF-β1 vesicle release by binding to the so-called I-domain of CR3 (Fig. 4f). Human blood monocytes were stimulated for 1 h with iC3b, and vesicles were isolated and assayed for their TGF-β1 content by ELISA. No significant difference was observed between MEVs and vesicles induced with iC3b, indicating that iC3b is not relevant in TGF-β1-containing vesicle release. As soluble β-glucan is commonly expressed on the fungus<sup>16,17</sup> and has been previously described to bind to the lectin-like site (LLS) of CR3<sup>18</sup>, purified soluble β-glucan from Saccharomyces cerevisiae was used to induce vesicles (MEVs<sub>Ca</sub>-βG) in human monocytes. Similarly, soluble β-glucan was extracted/enriched from C. albicans and used to induce vesicles (MEVs<sub>Ca</sub>-βG). Both types of monocyte-derived vesicle showed significant TGF-β1 content (p = 0.0027, p = 0.0077, unpaired two-tailed t test, n = 3) (Fig. 4g). Similar to induction with whole C. albicans cells, the number of vesicles released from monocytes increased by about tenfold upon stimulation with soluble β-glucan from C. albicans (Fig. 4i). MEVs<sub>Ca</sub>-βG were composed of six major vesicle populations, with sizes ranging from 50 nm to 650 nm (Fig. 4j). To determine whether β-glucan interacted directly with CR3, a proximity ligation assay (PLA) was performed. In the presence of C. albicans, soluble β-glucan on the C. albicans surface interacted with CD11b on the monocyte surface (Fig. 4k). When enriched soluble β-glucan was used instead of whole C. albicans cells in the same PLA, the interaction between soluble β-glucan and CD11b was confirmed. No fluorescence was detected from CR3-expressing monocytes alone in the absence of soluble β-glucan (Fig. 4k). iC3b also bound to CD11b on the monocyte (Supplementary Fig. 3e), and both iC3b and soluble β-glucan-induced reactive oxygen species (ROS) formation in monocytes upon binding (Supplementary Fig. 3f).

Label-free LC-MS/MS-based proteomics was also used to determine the composition of β-glucan-induced vesicles. MEVs<sub>Ca</sub>-βG showed a significant increase of 420 proteins compared with MEVs, while the level of 13 proteins were significantly decreased (Fig. 5a). According to a Gene Ontology enrichment analysis for cellular compartments, again most of the identified proteins (238) were extracellular exosome-related proteins, similarly to the proteins identified in whole C.
albicans-induced vesicles (Fig. 5b). KEGG pathway enrichment analysis also showed a high abundance of complement pathway proteins (Supplementary Fig. 4a). In addition, out of 100 commonly reported vesicle markers (ExoCarta)19, 68 were upregulated in both types of vesicles (MEVsCa and MEVsCa-sβG; Fig. 4c). These data confirm that soluble β-glucan is a component of C. albicans that induces TGF-β1-transporting vesicles.

Apoptotic bodies induce release of TGF-β1-transporting MEVs. TGF-β1 has been described primarily as an anti-
Fig. 4 Fungal soluble β-glucan interaction with CR3 induces MEV<sub>Ca</sub> release. a TGF-β1 is significantly upregulated in MEVs<sub>Ca</sub> compared with MEVs, but not when CR3 was blocked (data presented as mean values +/− SD, p = 0.0019, unpaired two-tailed t test, n = 3 donors). MEVs were isolated from 1-h-infected monocytes (5 × 10<sup>5</sup>), and TGF-β1 was detected by ELISA. b CD11b knockout (KO) THP-1 cells generated via CRISPR-CAS9 lacks CD11b expression by western blot and flow cytometry (see uncropped WB and gating strategy in Supplementary Fig. 7a, c). c TGF-β1 significantly increases in wild-type THP-1-derived TVS<sub>Ca</sub>, but not EVs from CD11b KO THP-1 cells. EVs were isolated from uninfected or C. albicans-infected (1 h) wild-type or CD11b KO THP-1 cells (TVs or TVS<sub>Ca</sub> respectively) (each 5 × 10<sup>5</sup>), data are presented as mean values +/− SD, p = 0.0064, unpaired two-tailed t test, p = 0.0014, one-way ANOVA, n = 3 donors). d TGF-β1 significantly increases in ex vivo blood cell infection-derived vesicles (BCEVs<sub>Ca</sub>) compared with control blood cell-derived vesicles (BCEVs) in wild-type mice (C57BL/6), but not CR3-deficient (CD11b KO) mice (B6.129S4-Lt[tg]flm1Myj/J). In all cases, vesicles were isolated from 1-h-infected blood cells (1 × 10<sup>7</sup> EVs) (data are presented as mean values +/− SD, p = 0.0293, unpaired two-tailed t test, p = 0.0110, one-way ANOVA, n = 3 donors). e MEVs<sub>Ca</sub> from wild-type but not from CR3-deficient mice show increased TGF-β1 concentration compared to MEVs (data are presented as mean values +/− SD, p = 0.0082, unpaired two-tailed t test, p = 0.0021, one-way ANOVA, n = 5 wild-type mice and n = 3 or CR3-deficient mice). Monocytes were generated from mouse bone marrow-derived stem cells. MEVs and MEVs<sub>Ca</sub> (1 h infection) were each isolated from 5 × 10<sup>5</sup> monocytes. FCR3 (CD11b/CD18) harbours two binding domains: the I-domain (binds iC3b) and the lectin-like site (LLS) domain (binds soluble β-glucan (sβG)) (modified from O’Brien et al. 18). g MEVs<sub>sβG</sub> and MEVs<sub>Ca-sβG</sub> (induced for 1 h by sβG from S. cerevisiae and C. albicans, respectively) but not by iC3bVEs significantly increase compared to MEVs. EVs were isolated from activated monocytes (5 × 10<sup>5</sup>) (data presented as mean values +/− SD, p = 0.0053, p = 0.0227, p = 0.0077, unpaired two-tailed t test, n = 3 donors). h MEVs or MEVs<sub>sβG</sub> tracking by DSLM. Data are representative of four independent experiments. I MEVs<sub>sβG</sub> significantly increase compared with MEVs (data are presented as mean values +/− SD, p = 0.0023, unpaired two-tailed t test, n = 5 donors). j Size distributions of EVs determined with NanoSight NTA 3.2 software. EVs were isolated from 5 × 10<sup>5</sup> induced or control monocytes. Graphs were generated by overlaying the size distribution of MEVs and MEVs<sub>sβG</sub> derived from five donors. k sβG from C. albicans binds to CR3 on monocytes as seen by proximity ligation assay (PLA). PLA staining- red: sβG/CD11b complexes, blue: DNA. Bars: 10 μm. Representative data of n = 5 experiments (five donors).

MEVs<sub>Ca</sub> downregulate IL-6 expression in human macrophages. Detection of increased abundance of endocytosis proteins in MEVs<sub>Ca</sub> and MEVs<sub>sβG</sub> compared with MEVs (Supplementary Fig. 4b, c) suggested uptake by and interaction of these EVs with other cells. As discussed above, TGF-β1 acts predominantly as an anti-inflammatory cytokine. To assess the functional effects of TGF-β1-transporting vesicles, human blood monocytes were differentiated into macrophages and subsequently incubated with TGF-β1-transporting vesicles. To inhibit inflammation, TGF-β1 on the vesicles interacts with TGF-βRII on macrophages. Therefore, binding of TGF-β1-transporting vesicles to this receptor was evaluated by PLA. Co-incubation revealed complex formation between TGF-β1 on the vesicle and TGF-βRII on the macrophage, in contrast to MEVs, which did not show any interaction with TGF-βRII in the PLA (Fig. 5g).

As TGF-β1-transporting vesicles were expected to dampen the inflammatory response after binding to the TGF-βRII, macrophages were first incubated with lipopolysaccharide (LPS) to induce production of the inflammatory cytokine IL-6<sup>22</sup>. TGF-β1-transporting vesicles significantly reduced IL-6 production by macrophages (p = 0.0339, unpaired two-tailed t test, n = 3), as measured by ELISA (Fig. 5h). When TGF-β1 on vesicles was blocked with TGF-β1-neutralizing antibodies, LPS-induced IL-6 was not reduced (Fig. 5h). This result confirmed the anti-inflammatory role of TGF-β1 vesicles.

MEVs<sub>Ca</sub> reduce inflammation via the SMAD7 pathway. To determine whether TGF-β1-transporting vesicles regulate IL-6 also in systemic C. albicans infection, human whole blood was infected with C. albicans for 4 h, and IL-6 transcription was assayed in whole blood cells by qPCR. No changes of IL-6 transcription was detected after infection and with TGF-β1-neutralizing antibodies (Fig. 6a).

To further follow the function of TGF-β1-transporting vesicles in systemic C. albicans infection, interaction of these vesicles with human blood monocytes was assessed by PLA. Incubation of monocytes with TGF-β1-transporting vesicles for 15 min revealed the formation of complexes between TGF-β1 on vesicles and TGF-βRII on monocytes. Vesicles from uninfected monocytes did not show any fluorescent signal (Fig. 6b). This result demonstrates that TGF-β1-transporting vesicles generated in response to C. albicans bind to monocytes via TGF-βRII. Tracking these vesicles with SEM confirmed binding of multiple vesicles also to the C. albicans surface inflammatory cytokine<sup>20</sup>, and targeted disruption of the mouse Tgfr1 gene results in several inflammatory diseases<sup>21</sup>. To determine whether C. albicans exploits a physiological regulatory mechanism to dampen the immune response to the fungus, we aimed to identify a physiological condition where TGF-β1-transporting vesicles are released by monocytes in the absence of an infection. As apoptosis is characterized as a process of cell clearance without inflammation, we wondered whether human apoptotic bodies induce similar TGF-β1-transporting vesicles in monocytes. Apoptotic bodies were generated from human umbilical vein endothelial cells (HUVECs), isolated, opsonized in complement-active human serum, and incubated for 1 h with human blood monocytes. The vesicles induced in response to opsonized apoptotic bodies (MEVs<sub>AB</sub>) were isolated and assayed for TGF-β1 by ELISA. Apoptotic body-induced significantly higher amounts of TGF-β1-transporting vesicles (MEVs<sub>AB</sub>) compared with MEVs (p = 0.0008, unpaired two-tailed t test, n = 3) (Fig. 5d). This effect was also analyzed in the ex vivo blood system. Apoptotic body-induced blood cell vesicles (BCEVs<sub>AB</sub>) carried about twice as much TGF-β1 compared with blood cell vesicles (BCEVs) induced in the absence of apoptotic bodies (Fig. 5e). To investigate whether CR3 is involved in this process, apoptotic cells were incubated in whole blood in the presence of simvastatin, which blocks CR3 activation. Under these conditions, the amount of TGF-β1 in BCEVs<sub>AB</sub> was significantly reduced (p = 0.0133, unpaired two-tailed t test, n = 3) and comparable with the levels in BCEVs derived in the absence of apoptotic cells (Fig. 5e).

In addition, CR3 dependence was investigated in an ex vivo system using whole blood from wild-type and CD11b KO mice. Peripheral blood cells (1 × 10<sup>7</sup>) from each of these mice were incubated with opsonized apoptotic bodies generated from whole blood of respective mouse for 1 h, and vesicles were subsequently isolated and assayed for TGF-β1. No significant difference in TGF-β1 content was observed between BCEVs and BCEVs<sub>AB</sub> derived from CD11b KO mouse monocytes, but BCEVs<sub>AB</sub> generated from wild-type monocytes revealed a significant increase in TGF-β1 levels compared with BCEVs (p = 0.0163, unpaired two-tailed t test, n = 3) (Fig. 5f). Thus, TGF-β1-containing vesicles are released by monocytes in a CR3-dependent manner in response to apoptotic bodies, and this pathway is also used by C. albicans.
When TGF-β1 was labeled with immunogold, TGF-β1-transporting vesicles were observed, particularly on C. albicans hyphae (Fig. 6d). To determine the effect of TGF-β1-containing vesicles in C. albicans infection, human whole blood was infected with C. albicans for 4 h, and then blood cells were lysed, and proteins were separated by SDS-PAGE and immunoblotted for markers of TGF-β1 pathway activation, including phosphorylated SMAD2/3 and SMAD7. C. albicans infection resulted in strong induction of SMAD2/3 phosphorylation (Fig. 6e). However, when TGF-β1 was blocked during C. albicans whole blood infection with
a neutralizing antibody, SMAD2/3 phosphorylation was inhibited (Fig. 6e). As SMAD2/3 phosphorylation leads to the upregulation of SMAD7, which subsequently signals through NF-κB, we also determined the expression of SMAD7 by western blot analysis. SMAD7 was substantially upregulated during *C. albicans* infection, and was reduced in the presence of an anti-TGF-β1 antibody (Fig. 6e).

RNA-seq of infected and control monocytes confirmed these results, as infected monocytes showed significant upregulation of SMAD7 and NFKBIA as well as SMAD2 (Fig. 6f, g). Induction of SMAD7 and IκBα is also reported to inhibit IL-1β and TNF-α in renal inflammation by blocking NF-κB-mediated transcription (Fig. 6h)24. A low amount of IL1B production was detected early during whole blood infection with *C. albicans* (4 h), and blocking
Fig. 6 MEVsCa reduce inflammation via the SMAD7 pathway. a No change in IL6 transcription occurs in whole-blood ex vivo infection with C. albicans, also after blocking TGF-β1 (data are presented as mean values ± SD, p = 0.9947, unpaired two-tailed t test, n = 3 donors). b TGF-β1 on MEVsCa binds to TGF-βRII on monocytes. No such interaction was observed with MEVs. Cells were incubated with EVs for 30 min, and TGF-β/βRII complexes detected by PLA using CLSM. PLA staining: red: TGF-β1/βRII complexes, blue: DNA. Bars: 10 μm. c MEVsCa and d TGF-β1 adhere to C. albicans and its hyphae. Monocytes were infected with opsonized C. albicans for 1 h, and immunogold-labeled TGF-β1 visualized by SEM. e Phosphorylated SMAD2/3 and SMAD7 in whole blood infected with C. albicans ex vivo (1 h), but not when TGF-β1 was neutralized. Cells were lysed and intracellular proteins were separated and immunoblotted using an anti-TGF-βRII antibody together with the nucleic acid dye Sytox Orange. f Little upregulation of IL1β expression is observed in whole blood infected with C. albicans ex vivo, but strong upregulation of IL1β occurs when TGF-β1 or TGF-βRII was blocked during ex vivo infection (data are presented as mean values ± SD, p = 0.0222, p = 0.0005, unpaired two-tailed t test, n = 3 donors). j IL1β expression is not upregulated in whole blood from wild-type mice (C57BL/6) infected with C. albicans, but in blood from CR3-deficient (CD11b KO) mice (B6.129S4-Itgamtm1Myd/J) (data are presented as mean values ± SD, p = 0.3785, p = 0.0015, unpaired two-tailed t test, n = 4 donors). After 4 h whole blood infection, cells were lysed, and RNA was isolated and subjected to comparative qPCR.

MEVsCa act anti-inflammatory on endothelial cells. To verify the presence of TGF-β1-transporting vesicles in vivo, liver tissue sections from C. albicans-infected mice (24 h) were screened for TGF-β1 expression by CLSM. The sections were stained with an anti-TGF-β1 primary antibody and a fluorescently labeled secondary antibody together with the nucleic acid dye Sytox Orange. Strong TGF-β1 staining was identified along, and in endothelial cells of blood vessels of infected mice, but little or no staining was observed in tissues from uninfected mice (Fig. 7a). As blood vessels easily come in contact with endothelial cells, these cells were subjected to further studies. First, BCEVs and BCEVsCa were isolated by polymer precipitation. The total amount of BCEVsCa was significantly higher (about 4 fold) compared with BCEV (Fig. 7c). BCEVsCa size ranged from 40 to 400 nm (Fig. 7d). To confirm the presence of TGF-β1-transporting vesicles during whole-blood infection, the membrane and cytosol fractions of the vesicles were separated by lysis of the vesicles and subsequent centrifugation. Supernatants and membrane fractions were separated and immunoblotted using an anti-TGF-β1 antibody. BCEVsCa, but not BCEVs showed the presence of TGF-β1 in the membrane fraction (Fig. 7e). To understand the effect of TGF-β1-transporting vesicles on the blood vessel endothelium in systemic candidiasis, the interaction of the vesicles with human endothelial cells was assessed. First, the interaction of BCEVsCa with HUVECs was assayed by PLA. After 30 min of incubation, multiple complexes between TGF-β1-βRII on isolated vesicles and TGF-βRII on the HUVEC surface were detected. Vesicles from uninfected blood cells, however, did only generate low or no signals (Fig. 7f, g). Following incubation of vesicles with HUVECs for 6 h, the effect of TGF-β1-transporting vesicles on the HUVEC phenotype was determined by qPCR. HUVECs treated with TGF-β1-transporting vesicles revealed an upregulation of several anti-inflammatory cytokines, including TGFB1 and IL4 (Fig. 7h; Supplementary Fig. 6). Increased production of TGF-β1 in endothelial cells was confirmed by immunofluorescence using LSM and measuring intracellular TGF-β1 by ELISA. HUVECs showed significantly more intracellular TGF-β1 when they were incubated with TGF-β1-containing blood vesicles from C. albicans-infected blood cells than when they were incubated with vesicles from uninfected blood (Fig. 7i–k). Treatment of the HUVECs with vesicles did not affect the cell viability (Supplementary Fig. 6). To confirm that this effect is mediated by TGF-β1 on vesicles, HUVECs were then incubated with reconstitent TGF-β1, which also resulted in upregulation of TGFB1 (Fig. 7l). To exclude an effect by the pathogen itself, HUVECs were incubated with C. albicans for 6 h. C. albicans alone failed to induce anti-inflammatory cytokines in HUVECs (Fig. 7m). These results suggest that TGF-β1-transporting vesicles are induced by C. albicans in blood cells, which subsequently bind to endothelial cells to amplify the TGF-β1 signal. Altogether these results demonstrate that TGF-β1-transporting vesicles are released in response to C. albicans, attach to endothelial cells, and upregulate the expression of TGF-β1 in vitro and in vivo.

Discussion EVs are central regulators of the immune response and are used as a form of cellular communication, particularly during bacterial or viral infections. However, the generation, composition, and action of vesicles released by immune cells during in vivo fungal infections are so far not described. We show here that the human pathogenic fungus C. albicans mimics apoptotic cells in inducing vesicle release from human blood monocytes that transport TGF-β1 to other cells (Fig. 8). TGF-β1 significantly contributes to the development of immune tolerance on mucosal surfaces and supports C. albicans commensalism. However, in early systemic infection, TGF-β1-transporting vesicles can reduce the host immune response against the fungus, which favors survival of the fungal cells. About tenfold more vesicles were released per monocyte when the cells were incubated with C. albicans cells. Release of vesicles from monocytes was observed in real time within 20 min upon infection with C. albicans and thus constitutes an immediate innate immune response. A similar early vesicle production was reported from neutrophils exposed to Staphylococcus aureus. The vesicles released in response to C. albicans showed a spherical-to-round morphology, as previously described for other
vesicles, and ranged from 80 to 500 nm in size, which is characteristic for exosomes and microvesicles. Intracellularly, the vesicles formed within monocytes stained positive for CD63 and tetraspanins, as previously reported for vesicles generated by monocytes in response to bacterial infection. However, CD63 was lost on released EVs, and thus represents a less consistent marker for monocyte-derived vesicles. By contrast, the vesicle marker CD9 was consistently detected on MEVs generated, both with and without C. albicans infection. C. albicans-induced vesicles were found to contain complement proteins (C3, factor H (FH)), which are often transported in vesicles. Complement plays an important role in C. albicans infection, and the fungus has developed several evasion mechanisms. Finally, like monocytes, the vesicles also expressed CD11b and CD14. EV formation in response to C. albicans and Aspergillus fumigatus was recently also reported. However, experimental conditions were substantially different in these studies regarding cell type and incubation time.

The identification of heat shock proteins like HSP90 in C. albicans-induced vesicles suggested a mechanism of cytokine packaging into vesicles. EVs often transport cytokines to protect these digestion-sensitive proteins in the blood. As cytokines play a major role in the immune response to C. albicans, vesicles were screened for the presence of cytokines. Surprisingly, low amounts of the pro-inflammatory cytokines IL-1β and IL-6 were detected in C. albicans-induced vesicles from isolated monocytes or infected whole-blood cells, but high concentrations of the anti-inflammatory cytokine TGF-β1 were detected. So far,
TGF-β1-containing vesicles have been observed in a parasitic infection model and were shown to be released by cancer cells, but the mechanism of their induction and their role in the immune response remain unclear. Generation of TGF-β1-transporting vesicles by *C. albicans* is shown here using an ex vivo whole-blood infection model, and in vivo in blood and liver tissue from *C. albicans*-infected mice.

Notably, the induction of TGF-β1-transporting vesicles from monocytes depends on the presence of CR3 on the monocytes. Previous studies indicated that CR3 is associated with TGF-β1 generation in dendritic cells, but did not report the significant link between CR3 and TGF-β1-transporting vesicle formation. In addition, opsonization with C3b, which binds to CR3, was previously considered to be important in bacteria-induced immune cell vesicle production, but was not reported for *C. albicans*. We identified soluble β-glucan of *C. albicans* as a ligand of CR3 and a trigger for the release of TGF-β1-transporting vesicles. This is in agreement with previous studies showing that CD11b, which forms CR3 together with CD18, harbors a carbohydrate-binding site LLS that binds fungal-derived soluble, but not insoluble, β-glucan. In addition, CD11b and CD18 together form the so-called I-domain, which then binds the complement protein iC3b. iC3b is generated from the cleavage of C3b deposited onto *C. albicans*; however, in our study, iC3b did not induce any TGF-β1-transporting vesicles.

Apoptotic cells and apoptotic bodies have been reported to induce TGF-β1 in phagocytic cells. We hypothesized that production of TGF-β1-transporting vesicles might be a conserved mechanism in human physiology used by *C. albicans* and evaluated vesicle induction from monocytes incubated with apoptotic cells. Indeed, incubation of monocytes with apoptotic cells resulted in the release of TGF-β1-transporting vesicles, as...
observed with *C. albicans*. Such vesicle generation was also very fast (within minutes), and again the presence of CR3 was a prerequisite for vesicle release. As TGF-β1-transporting vesicles were generated by monocytes and whole-blood cells in response to apoptotic bodies, we concluded that *C. albicans* likely exploits this pathway for immune suppression.

TGF-β1 is a pleiotropic cytokine produced by many cell types, including immune and nonimmune cells, and it regulates multiple cellular functions. TGF-β1 primarily inhibits the function of inflammatory immune cells. Human macrophages induce CD4+Foxp3+–regulatory T cells via binding and release of TGF-β1. High concentrations of TGF-β1 promote this Foxp3 induction and consequently formation of immune suppressive iTreg cells. We show that TGF-β1 vesicles are released by monocytes in response to apoptotic cells and can reduce the very early induction of pro-inflammatory immune cells. Human macrophages induce CD4+Foxp3+–regulatory T cells via binding and release of TGF-β1.

**Methods**

**Human cells.** Human monocytes were isolated from sterile buffy coats (JENA University Hospital, Germany) or from fresh blood collected in Na-heparin tubes (BD Biosciences) from healthy volunteers after informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated from blood or buffy coats using Biocoll (Density 1.077 g/ml) (Biochrom) gradient centrifugation. Contaminant platelets were removed by low speed centrifugation of 160×g. The lymphocyte population from PBMCs was decreased by using 46% Percoll (density 1.133 g/ml) (GE healthcare) gradient centrifugation. Monocytes were then isolated by negative selection procedure using the pan monocyte isolation kit, (Miltenyi Biotec, cat no. 130-096-357) according to the manufacturer’s protocol. Purity of the monocytes was assessed by detecting the presence of CD14 surface marker using flow cytometer.

Human monocharyotypic THP-1 cell line (ACC 16, DMSZ) was maintained in M medium (RPMI 1640 medium (Lonza) supplemented with 10% FCS (Thermofischer), 2 mM ultra glutamine (Lonza), 25 µg/mL of gentamicin sulphate (Lonza)) at 37 °C, 95% humidity, and 5% CO2. Human M1 macrophages were generated from lymphocyte-depleted PBMCs. Monocytes from lymphocyte-depleted PBMCs were isolated by adherence after 2 h incubation. Peripheral blood mononuclear cells (PBMCs) were maintained in M medium for 2 h. M1 macrophages were generated by differentiation of adherent monocytes in M medium supplemented with 50 ng/mL of recombinant human GM-CSF (Peprotech) for 7 days at 37 °C, 95% humidity, and 5% CO2. During differentiation procedure, fresh M medium supplemented with GM-CSF was introduced after 4 days. After 7 days, macrophages were harvested using accutase (Capricorn Scientific).

Human fresh blood was collected in Na-heparin tubes from healthy volunteer by venipuncture. Whole blood cells were isolated by centrifugation at 2000×g, 15 min, 4 °C. Human umbilical vein endothelial cells (HUVECs) were maintained in H medium (DMEM medium (Lonza) supplemented with 10% FCS (Thermofischer), 2 mM ultra glutamine (Lonza), 25 µg/ml of gentamicin sulphate (Lonza)) at 37 °C, 95% humidity, and 5% CO2.

**Mouse cells.** Experiments were conducted in compliance with European and German regulations. Protocols were approved by the responsible Federal State authority and ethics committee (Zuchtrahmenantrag 02-05/16 and Tötunganzeige FSU-02-2017). C57BL/6 mice and B6.129S4-Itgamtm1Myd/J mice (CD11b knocked out) (Jackson Laboratory) were housed in ventilated cages with free access to water and food. C57BL/6 mice were maintained with heterozygous breeding, and B6.129S4-Itgamtm1Myd/J mice were maintained with KO breeding. Male and female mice with the age between 9 and 22 weeks were used for the experiments. Mice were euthanized with CO2. Mouse fresh blood was collected from euthanized (CO2) mice immediately by allowing blood to flow into heparin containing medium and was kept in ice. Whole blood cells were isolated by centrifugation at 2000×g, 15 min, 4 °C. Serum from the supernatant was used for *C. albicans* opsonization. Femur and tibia bones were isolated from euthanized mouse. Bones were sterilized with ethanol, bone orifice was cut open, and flushed with M medium using a 27-g needle (B. Braun) to harvest the cells of marrow. Collected bone marrow suspension was strained through a 70-µm nylon web. Cells were transferred to ultra-low attachment T25 cell culture flasks (Corning). Bone marrow stem cell differentiation was carried out in M medium supplemented with 30 ng/mL of recombinant M-CSF (BioLegend) at 37 °C, 95% humidity, and 5% CO2. After 5 days of differentiation, mouse bone marrow-derived macrophages were harvested from the supernatant. Purity of the macrophages was analyzed by detecting the presence of CD115 using flow cytometer (Supplementary Figs. 3 and 7) using Alexa Fluor 488 anti-mouse CD115 antibody (BioLegend, cat no. 135512) (1:200). Presence of CD11b was also detected on monocytes using flow cytometer using Alexa Fluor 647 anti-mouse CD11b antibody (BioLegend, cat no. 101218) (1:200). Presence of the ITGAM gene was analyzed in all mice. Isolated genomic DNA from C57BL/6 or B6.129S4-Itgamtm1Myd/J mice was subjected to PCR reaction using common forward primer, reverse primer for C57BL/6, and reverse primer for B6.129S4-Itgamtm1Myd/J (Supplementary Table 1). This yields 264 and 166-bp long PCR products, respectively (Supplementary Fig. 3a). PCR products were resolved on 2% agarose gel visualized with ethidiun bromide stain (uncropped gel picture in Supplementary Fig. 7).

**Microbial strain and culture.** *C. albicans* wild-type (SC5314) was maintained on yeast extract–peptone–dextrose (YPD) agar (2% d-glucose, 1% peptone, 5% yeast.
extract in agar) for no more than 1 month. After C. albicans was grown overnight in YPD medium at 30 °C with shaking, fungal cells were resuspended in fresh YPD medium at the same temperature. After 3 h of growth by light in 10% N2H, cells were taken into the culture, and further tested for CD11b using mouse monoclonal antibody (R&D Systems, cat no. 393102) (1:200) and secondary goat anti-Mouse Alexa Fluor 647 (Thermo Fisher). After fixation, cells were immobilized with TGF-β1 (R&D Systems) (2000 pg/ml) was dissolved in Minduction medium. TGF-β1-transporting vesicles was performed by isolating these vesicles from the total vesicle population from whole-blood cells (1 × 10^9) treated with C. albicans using M-PluriSelect (pluriSelect, cat no. 19-00900-20). Therefore, the final vesicles obtained were both CDF and TGF-β1-transporting vesicles. After removing cell debris, cells, and C. albicans, MEVs were in parallel also isolated by ultracentrifugation at 120,000 × g for 1 h at 4 °C. Whole-blood cells were plated on a 100 mm (Quantifoil Micro Tools), and excess of vitrified, aliquoted, and stored at −80 °C for further use.

ELISA. Cytokines were measured in EVs isolated from 5 × 10^5 human monocytes, 5 × 10^6 human M1 macrophages, 1 × 10^8 mouse monocytes, or 1 × 10^7 human/ mouse whole-blood cells. TGF-β1 was measured with Human TGF-β1 DuoSet ELISA kits (R&D Systems, cat no. DY679) or Human/Mouse TGF-β1 ELISA Ready-SET-Go! Kit (Bio-吃惊, cat no. 88-8350-88). IL-10, IL-6, and IL-1β were measured using the human IL-10 ELISA Ready-SET-Go! Kit (Bio-吃惊, cat no. 88-7106-22), human IL-6 ELISA Ready-SET-Go! Kit (Bio-吃惊, cat no. 88-7066-22), or human IL-1β ELISA Ready-SET-Go! Kit (Bio-吃惊, cat no. 88-7251-86). In all cases, the ELISA was performed according to the manufacturer’s protocol. CD9 and HSP90 were measured with ELISA on immobilized vesicle using anti-CD9 antibody (Novus Biologicals, cat no. NB500-327) (2 µg/mL) and anti-HSP90 antibody (Abcam, cat no ab79848) (2 µg/mL), respectively. Vesicles in different fractions of size-exclusion chromatography was immobilized with TGF-β1 capture antibody from Human TGF-β1 DuoSet ELISA, and CD9 or HSP90 was detected on vesicles by biotinylated CD9 and HSP90 antibody and streptavidin-conjugated HRP.

Vesicle counting. Isolated vesicles were counted using NS300 dynamic light-scattering microscope (Malvern) fitted with NanoSight NTA 3.2 software. Vesicles isolated from 5 × 10^5 monocytes or 5 × 10^7 whole-blood cells were dispersed in 1 mL of DPBS and injected though the microscope at 100 (AU) pump flow rate. Videos were captured at 24 fps, three times 60 s for each sample, and analyzed using NanoSight NTA 3.2. Fractions of 1 mL obtained from size-exclusion chromatography were visually screened.

Cryo-TEM. EVs isolated from 4 × 10^4 infected monocytes (MOI 1:1) were collected in 4-mL suspension, and were applied onto copper EM-grids covered by a QUANTICHOL Multi A holey carbon film (Quantifoil Micro Tools), and excess of liquid was blotted automatically between two strips of filter paper. Subsequently, the samples were rapidly plunge-frozen in liquid ethane (cooled by liquid nitrogen at about −180 °C) in a cryobox (Carl Zeiss). Excess of ethane was removed with a Leica EM CPD300 Automated Critical Point Dryer (Leica) and finally coated with carbon (10 nm) in a BAL-TEC MED 020 Sputter Coating System (BAL-TEC). SEM images were acquired at different magnifications in a Zeiss-LEO 1530 Gemini field-emission scanning electron microscope (Carl Zeiss) at 8-kV acceleration voltage and a working distance of 7 mm using an intense secondary electron detector for secondary electron imaging and a scintillation type backscatter electron detector (Centaurus Detector, K.E. Developments) for antibody-gold detection.

**In vitro and ex vivo vesicle generation and isolation.** Vesicles were generated from human monocytes, mouse bone marrow-derived monocytes, or THP-1 cells or M1 macrophages or whole-blood cells (human/mouse) after 1 h of induction or from untreated control cells in M_induction medium at 37 °C, 95% humidity, and 5% CO2. Cells were incubated with osazonized C. albicans (MOI 1:1) to isolate infection-derived vesicles. For measuring CR3 dependency, cells were treated with 30 µg/mL of solubilized CR3 (Sigma-Aldrich) prior to incubation to inhibit CR3. In the absence of C. albicans, cells were induced with 50 µg/mL of ICB (CompTech), or 10 µg/mL of α-scerine-derived sβIg (InVivoGen), or 10 µL/10^6 cells of C. albicans-derived sβG. In case of apoptotic body-mediated induction, cells were incubated with osazonized apoptotic bodies which were isolated from double the number of MOI (1:2) of induced cells. For measuring CR3 dependency, cells were treated with 30 µM simvastatin (Sigma-Aldrich) prior to incubation with apoptotic bodies. Cells and C. albicans were removed by centrifugation at 3000 × g for 15 min at 4 °C from the supernatant and accumulated EVs collected isolated using ExoQuick-TC (System Biosciences) according to the manufacturer’s protocol.

**Distribution of CDF-β1-transporting vesicles was performed by isolating these vesicles from the total vesicle population from whole-blood cells (1 × 10^9) treated with C. albicans using M-PluriSelect (pluriSelect) according to the manufacturer’s protocol. TGF-β1-transporting vesicles were isolated by positive selection with mouse anti-TGF-β1 antibody (R&D Systems, cat no. MAB240).**

**Apoptotic body generation.** Apoptotic bodies were generated from HUVEC cell line and whole-blood cells using UV irradiation. After harvesting, HUVEC cells and whole-blood cells were washed with and suspended in DPBS. Cells were then transferred into six-wells plates and exposed to UV light for 4 h for whole-blood cells and 6 h for HUVEC cells. Dead cells were removed by centrifugation at 300 × g for 10 min, and apoptotic bodies were harvested by centrifugation at 3000 × g for 20 min. Apoptotic bodies were opsonized by incubation in 10% NHS at 37 °C for 30 min.

**ΔCR3 THP-1 cell.** CRISPR/Cas9 method was used to knockout the CD11b from THP-1 cell line and generate ΔCR3 THP-1 cells. Adjacent to protoplascter adjacent motif (PAM), 20 base-guide oligo sequences were designed to target the ITGAM gene. The guide oligo sequence was designed to be inserted upstream the single-guide RNA scaffold into the plasmid pPSpCas9(2B)-2A-GFP (PSG485) (which was a gift from Feng Zhang, Addgene plasmid # 48138;http://n2t.net/addgene:48138;RRID:Addgene_48138)) into the BbsI site in the 5′ end of the U6 promoter using the golden gate assembly protocol. The guide oligos were as follows (Supplementary Table 1). Guide oligos were annealed and phosphorylated in a thermal cycler with 100 µM of each sense- and anti-sense- guide oligo, 1 µL of 10X T4 ligation buffer, 1 µL T4-polynukleotidase. Annealing and phosphorylation was performed at 37 °C for 30 min, 95 °C for 5 min, and ramped down to 25 °C at 5 °C min−1. For the insertion of annealed guide oligos, 1 µg of pPSpCas9(2B)-2A-GFP was digested with 1 U BbsI (New England Biolabs) at 37 °C for 30 min. Digested plasmid was separated by agarose gel electrophoresis, and the insert was purified using QIAquick gel extraction kit (Qiagen) according to the manufacturer’s protocol. In all, 1 µL of (1:200) annealed guide oligo was ligated to 50 ng of digested pPSpCas9(2B)-2A-GFP using 1 U quick ligase enzyme (New England Biolabs) at room temperature for 10 min. Guide oligo-pPSpCas9(2B)-2A-GFP was then transformed into B. subtilis. Successful transformants were selected on ampicillin on LB agar plates. Transformation colonies were again cultured under selection pressure, and guide oligo-pPSpCas9(2B)-2A-GFP was isolated from E. coli. Guide oligo-pPSpCas9(2B)-2A-GFP was then transfected into THP-1 cells by Amaxa® Human Monocyte Nucleofector® Kit according to the protocol. Transfected cells were maintained in similar conditions as THP-1 cells, and after 3 days, cells were stained for CD11b with mouse anti-human CD11b (BioLegend, cat no. 393102) (1:200) and secondary goat anti-Mouse Alexa Fluor 647 (Thermo Fisher, cat no. A-21235) (1:1000). Stained cells were then sorted for CD11b-negative and GFP-positive signals using BD FACSaria Cell sorter. Sorted cells were taken into the culture, and further tested for CD11b using flow cytometry and western blotting.
Freeze-fracture TEM. Vesicles isolated from 5 × 10⁵ infected monocytes (MOI 1:1) were collected in 5-μL suspension and enclosed between two 0.1-mm-thick copper grids for freeze-fracture technique. The specimens were negatively stained with 0.1% saponin, and imaged under low-dose conditions. The replicas were cut and examined with a JEM-2100F electron microscope (JEOL Ltd) at 80 kV. The images were recorded with a 9k charge-coupled-device (CCD-camera and acquisition software EMAN v 4.009.1.7, TVIPS).

Label-free LC-MS/MS. EVs were isolated from 1 × 10⁶ human monocytes—control (MEVs), C. albicans-infected (MEVs_C2), C. albicans-derived sG-induced (MEVs_C2-sG), and collected in a final volume of 100 μL of DPBS and stored until analysis. For analysis, the samples were thawed with 400 μL chilled MeOH. Forced precipitation and phase separation was performed with 100 μL chilled 20% (v/v) isopropanol to precipitate and separate the lipids. After centrifugation, MeOH was removed, and samples were almost dried. Samples were resolubilized in 100 μL denaturation buffer (50 mM triethylammonium bicarbonate (TEAB) in 50% trifluoroethanol (TFE)/H2O (ν/ν)) by pipetting and ultrasonication. After solubilization, samples were incubated at 55 °C for 1 h with 2 μL of reduction buffer (500 mM TCEP (tris (2-carboxyethyl)phosphine) in 100 mM TEAB) followed by incubation at RT for 30 min with 2 μL alklylation buffer (625 mM CAA (2-chloroacetamide) in 100 mM TEAB). After alkylation, the precipitation, phase separation, and drying steps were repeated as mentioned before. Samples were then resolubilized in 100 μL 100 mM TEAB by sonication. C-terminus of lysine of sample proteins was cleaved with 2 μL of lysC (1 μg/μL) (Promega) by mixing and incubating for 16 h at 37 °C. The reaction was stopped with 10 μL 10% HCOOH. Samples were dried and solubilized in 0.05% trifluoroacetic acid and 2% acetonitrile in water by pipetting, vortexing, and ultrasonication. Samples were filtered through 0.2-μm spin filter, transferred to HPLC vials, and stored at −80 °C until LC-MS/MS analysis.

LC-MS/MS analysis was performed on an Ultimate 3000 nano RSLC system connected to a Qeaxtive Plus mass spectrometer (both Thermo Fisher Scientific, Waltham, MA, USA). Peptide trapping for 5 min on an Acclaim Pep Map 100 column (2 cm × 75 μm, 3 μm) at 5 μL/min was followed by separation on an analytical column—Qexactive Plus RSLC nano column (2 cm × 75 μm, 3 μm) by using a gradient phase elution of eluent A (0.1% (v/v) formic acid in water) with mobile B (0.1% (v/v) formic acid in 90% acetonitrile/water) as performed: 0–5 min at 4% B, 50 min at 7% B, 100 min at 15% B, 140 min at 25% B, 180 min at 45% B, 200 min at 65% B, 210–215 min at 90% B, 215.1–240 min at 4% B. Positively charged ions were generated at spray voltage, for examination in a Zeiss EM902A electron microscope (Carl Zeiss) operated at 80 kV. Images were recorded with a 1 k (1024 × 1024) FastScan-CCD-camera (CCD-camera and acquisition software EMAN v 4.009.1.7, TVIPS).

Immunofluorescence. Monocytes were seeded on poly-L-Lysine-coated 12-mm cover slip and infected with opsonized C. albicans (MOI 1:1) for 30 min or 1 h in Mdmnimum medium at 37 °C. 95% humidity, and 5% CO₂. Control monocytes were prepared in a similar manner without infection. After co-incubation cells were fixed with 4% formaldehyde.

For detection of intracellular vesicle or TGF-β1, cells were permeabilized with 0.1% saponin, blocked with blocking solution (10% FCS, 1% BSA, and 0.1% Tween 20 in DPBS). CD63 was stained with Alexa Fluor 647 anti-human CD63 antibody (BioLegend, cat. no. 353015) (1:100), CD14 with Alexa Fluor 488 anti-human CD14 antibody (BioLegend, cat. no. 367130) (1:200), and sytox blue (5 μM) were used for quantification. The reactions were also incubated with 50 μg/mL of iC3b, and sytox blue (5 μM) were added for 30 min at 45 °C. Then, the replica was washed four times in HBSS + Ca++ and Mg++ before being used for examination in a Zeiss EM902A electron microscope (Carl Zeiss) operated at 80 kV. Images were recorded with a 1 k (1024 × 1024) FastScan-CCD-camera (CCD-camera and acquisition software EMAN v 4.009.1.7, TVIPS).

PLA assay was used to describe the interaction between soluble βG or iC3b and CD11b. Monocytes were seeded onto 12-mm glass cover slip. Seeded cells were incubated with BCEV or BCEV_sG isolated from equal amount of blood cells after 6 h. After fixation and blocking the cells, intracellular TGF-β1 was stained with mouse anti-TGF-β1 antibody (R&D Systems, cat. no. MA5240) (3 μg/mL) and Alexa Fluor 647 goat anti-mouse IgG (H+L) secondary antibody (Thermo Fischer, cat. no. A21235) (1:500). Nucleic acid was stained with sytox orange (Thermo Fischer) (5 μM). Coverslips were fixed on a glass slide, and images were capture with LSM 710 fitted with ZEN 2011 software. Intracellular TGF-β1 or CD63 was measured with ZEN 2011 by measuring the fluorescence intensity inside the cell.

For detection of intracellular vesicle or TGF-β1 in HUVEC cells, HUVEC cells were seeded onto 12-mm glass cover slip and infected with opsonized C. albicans (MOI of 1:1) or 10 μL/10⁶ cells of C. albicans-derived sG or 50 μg/mL of iC3b for 15 min. Cells were fixed with 4% formaldehyde, permeabilized with 0.1% saponin, blocked with Duolink blocking solution (Duolink Aldrich). Cells were incubated with mouse anti–TGF-β1 antibody (Biosupplies, cat no. 400-2) (1:200) for (for opsonized C. albicans and sG) or mouse anti-iC3b antibody (Calbiochem; cat no. MAB9892) (1:200) and rabbit anti-CD11b antibody (Abcam, cat. no. 133357) (1:200). PLA assay was performed using the Duolink In Situ Red Starter Kit Mouse/Rabbit (Sigma-Aldrich, cat. no. DUO92101) according to the manufacturer's protocol.

The PLA assay was also used to describe the interaction between TGF-β1 and TGFβRII. Monocytes or M1 macrophages or HUVECs were seeded onto 6.7-mm poly-L-Lysine-coated diagnostic slides. Monocytes and macrophages were treated with MEV or MEV_sG—isolated from equal amounts of cells—for 15 min, whereas HUVECs were treated with BEV or BEV_sG—isolated from equal amounts of blood cells—for 30 min. Cells were fixed with 4% formaldehyde, permeabilized with 0.1% saponin, and blocked with Duolink blocking solution (Sigma-Aldrich). Cells were treated with mouse anti-TGF-β1 antibody (R&D Systems, cat. no. MA5240) (3 μg/mL) and rabbit anti-TGFβRII antibodies (Sigma-Aldrich, cat. no. A/V47433) (1:200). PLA assay was performed using the Duolink In Situ Red Starter Kit Mouse/Rabbit (Sigma-Aldrich, cat. no. DUO92101) according to the manufacturer's protocol.

For detection of ROS, human monocytes (1 × 10⁶) were seeded into 96-well black flat bottom plates (Greiner). Cells were also treated with 50 μg/mL of iC3b, 10 μL/10⁶ cells of C. albicans-derived sG. The reactions were also incubated with CellROX deep red reagent (Thermo Fischer) (5 μM). After 30 min, ROS was detected by measuring the fluorescence intensity of CellROX using a Safire plate reader (Tecan).

Live cell imaging. Monocytes were incubated with opsonized C. albicans (MOI 1:1) cells in a 30-mm culture dish in Mdmnimum medium, placed in the incubation chamber of LSM710 at 37 °C and 5% CO₂. Alexa Fluor 488 anti-human CD14 antibody (BioLegend, cat. no. 676730) (1:200), sytox blue (5 μM) were added for quantification. Data normalization was deliberately not used in order to display differences on the protein level in the course of the host–pathogen interaction. P-values were calculated based on the 2 s t test using the abundance values of all biological replicates. P-values < 0.05 were considered significant.
Monocytes with opsonized C. albicans cells (MOI 1:1) in M\textsubscript{induction} medium were also injected into the flow cell of NanoSight NS300. After monocytes attached to the flow cells, video of scattered light was recorded in real time for 10 min with NTA 3.2 and subsequently analyzed. Human blood was diluted with M\textsubscript{induction} medium and incubated with C. albicans (MOI 1:1) cells in a 30-mm culture dish placed into the incubation chamber of LSM710 at 37 °C and 5% CO\textsubscript{2}. To observe vesicle formation from monocytes in whole blood, the reaction was treated with Alexa Fluor 488 anti-human CD14 antibody (BioLegend, cat no. 367130) (1:200), Alexa Fluor 647 anti-human CD63 antibody (BioLegend, cat no. 353015) (1:200), and sytox orange (5 µM). To observe TGF-β1 vesicle formation in monocytes, the reaction was treated with Alexa Fluor 488 anti-human CD14 antibody (BioLegend, cat no. 367130) (1:200), Brilliant Violet 421 anti-human LAP (TGF-β1) antibody (BioLegend, cat no. 349613) (1:200), and sytox orange (5 µM). To observe vesicle generation, pictures were taken every 5 min for 1 h using ZEN 2011. After 1 h, images of TGF-β1 vesicles were taken from different places both from control and infected blood. TGF-β1 vesicles were counted from an area of 212 µm\textsuperscript{2} by subjecting the images to following Image J (v.1.52) algorithm:

```
run("8-bit");
setAutoThreshold("Yen dark");
run("Threshold...");
setThreshold(60, 255);
setOption("BlackBackground", false);
run("Convert To Mask");
run("Watershed");
run("Analyze Particles...");
show-Outlines display clear summarize;
close();
```

### Mouse model of disseminated candidiasis for immunohistochemistry

Protocols were approved by the responsible Federal State authority and ethics committees (Thüringer Landesamt für Verbraucherschutz, permit number: 03-00668/09). For mouse experiments in whole blood in vitro, mice were housed in ventilated cages with free access to water and food. Male and female mice with the age between 9 and 22 weeks were used for the experiments. For infection, C. albicans was grown for 12 h at 30 °C in YPD medium, washed three times in sterile PBS, and diluted to the desired concentration. The mice were infected via the tail vein tail with 2.5 × 10\textsuperscript{9} C. albicans cfu/g body weight. Liver was collected 24 h post infection, fixed in 10% neutral buffered formalin (Histofix, Carl Roth, Karlsruhe, Germany), embedded in paraffin, and sectioned at 4-µm thickness. For staining the tissues, paraffin-embedded liver sections were deparaffinized with treatments with RotiClear (Carl Roth), 100% ethanol, 90% ethanol subsequently. Sectioned tissues were boiled in 10 mM Na\textsubscript{2}citrate buffer (pH 6.5) for antigen retrieval, and permeabilized with 0.1% saponin (Sigma-Aldrich). Tissues were stained for CD9 and perilipin using rabbit anti-mouse/human CD9 antibody (R&D Systems) or rabbit anti-human perilipin antibody (x Cell Biologics, cat no. A-12144) (1:500) with antibodies directed against CD9 and perilipin. Tissue sections were stained for TGF-β1 using rabbit antihuman TGF-β1 antibody (Invivogen) (1 µg/mL) and a goat anti-rabbit immunoglobulin-conjugated HRP secondary antibody (Dako, cat no. P0481-1) (1:1000) in reducing conditions. Phosphorylated Smad2/3 proteins were detected using rabbit anti-human phosphorylated Smad2/3 antibody (R&D Systems) or rabbit anti-smad2/3 antibody (BioLegend, cat no. 10-6801-02) (1:1000). For detection of TGF-β1, HUVECs were treated with BCEO\textsubscript{4+} for 30 min, unbound vesicles were removed, and after 6 h cells were lysed, and TGF-β1 was measured with human TGF-beta 1 DuoSet ELISA.

### Ex vivo C. albicans infection

Fresh blood was collected from healthy human volunteers in Na-heparin tubes (BD Biosciences) after informed consent. In total, 1 ml of blood was diluted with 4 ml M\textsubscript{induction} medium and infected with 1 × 10\textsuperscript{8} C. albicans and incubated for 1 h (for western blot) or 4 h (for qPCR) at 37 °C. 95% humidity, and 5% CO\textsubscript{2}. To block TGF-β1, blood was treated with 10 µg of human anti-TGF-β1-neutralizing antibody (Invivogen, cat no. maba-htgβ3-3), or 300 mM SB 431542 (R&D Systems) before infecting with C. albicans.

For mouse experiments, fresh blood was collected from euthanized mouse immediately by cardiac puncture in Na-heparin tubes (BD Biosciences). In total, 100 µl of blood was diluted with 400 µl M\textsubscript{induction} medium and infected with 1 × 10\textsuperscript{7} C. albicans for 4 h at 37 °C, 95% humidity, and 5% CO\textsubscript{2}.

### EV interaction with HUEVC cells

HUEVC cells were treated with EVEs isolated from equal numbers of infected (BCEO\textsubscript{4+}) and control blood cells (BCEV) in the M\textsubscript{induction} medium for 6 h at 37 °C, 95% humidity, and 5% CO\textsubscript{2}. In absence of EVEs, HUEVCs were treated with 0.5 ng/mL or 0.8 ng/mL human recombinant TGF-β1 (R&D Systems) or C. albicans (MOI 1:1). For detection of TGF-β1, HUVECs were treated with BCEV or BCEO\textsubscript{4+} for 30 min, unbound vesicles were removed, and after 6 h cells were lysed, and TGF-β1 was measured with human TGF-beta 1 DuoSet ELISA.

### Western blot

Cells or vesicles were lysed in lysis buffer (25 mM Tris HCl (pH 7.6) + 150 mM NaCl, 1% NP-40 1% sodium deoxycholate, 1% SDS) in presence of 1 mM PMSF (phenylmethylsulfonyl fluoride), and centrifuged at 14,000 x g for 10 min. Supernatant protein was subjected to isolate Poly (A) mRNA with poly-T oligoattached magnetic beads (Invitrogen). Following purification, the poly(A)- or poly(A)+ RNA fractions were fragmented into small pieces using divalent cations under elevated temperature. Then the cleaved RNA fragments were reverse-transcribed to create the final cDNA library in accordance with the protocol for the mRNA-Seq sample preparation kit (Illumina). The average insert size for the paired-end libraries was 300 bp ±50 bp. Paired-end sequencing was performed on an Illumina Hiseq 4000 (I-bio) following the vendor’s recommended protocol. First, Concatenate all reads in files were used to remove the reads that contained adaptor contamination, low quality bases, and undetermined bases. Then sequence quality was verified using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). HISAT2 was used to map reads to the genome of Homo sapiens. The mapped reads of each sample were assembled using StringTie. Then, the transcripts from different samples were merged to reconstruct a comprehensive transcriptive sequence using perl scripts and gffcompare. After the final transcriptome was generated, StringTie and Ballgown was used to estimate the expression levels of all transcripts. StringTie was used to calculate expression levels for mRNAs by calculating FPKM. The differentially expressed mRNAs were selected with log2 fold change >1 and log2 fold change >1.5.

### RNA-Seq

RNA from monocytes infected with opsonized C. albicans for 1 h and from untreated monocytes from four different donors was isolated using a total RNA purification kit (Norgen Biotechn). Probes from different donors were pooled, and RNA was polyA sequenced using the Illumina method (by LC Sciences). The transcripts from different samples were merged to reconstruct a comprehensive transcriptive sequence using perl scripts and gffcompare. After the final transcriptome was generated, StringTie and Ballgown was used to estimate the expression levels of all transcripts. StringTie was used to calculate expression levels for mRNAs by calculating FPKM. The differentially expressed mRNAs were selected with log2 fold change >1 and log2 fold change >1.5.

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were captured and analyzed using StepOne v2.3 qPCR. Primers used for the study are listed in Supplementary Table 1. qPCR data was used for HUVEC qPCR, and murine

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Conceptualization: C.S. and L.D.H.; methodology: L.D.H., C.S., E.A.H.J., M.W., and T.K.; analysis: L.D.H. and T.K.; investigation: L.D.H., E.A.H.J., M.Z.H., M.F.G., T.K., M.W., D.I.P., G.B., L.J.D., N.B., and C.S.p; writing—original draft: C.S. and L.D.H.; review & editing: C.S., L.D.H., and P.F.Z.; visualization: C.S. and L.D.H.; funding acquisition: C.S.; resources: C.S., F.F.Z., B.J., O.K., L.J.D., M.W., C.S.p., and N.B.; project administration: C.S. and L.D.H.; supervision: C.S. and L.D.H.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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