Figures and figure supplements

The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes

Paul Capewell *et al*

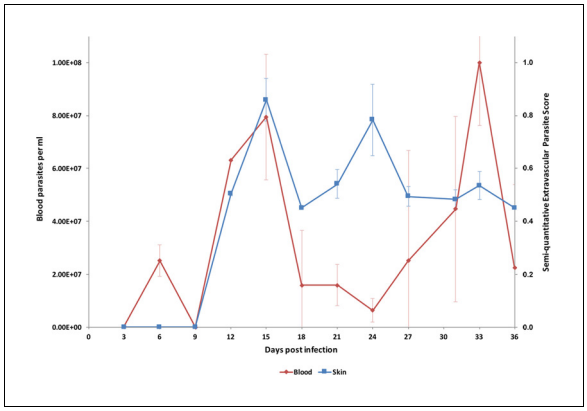

Figure 1. Parasite densities in the blood and in the extravascular tissue of the skin over a time-course. The blood parasitaemia of *T.b. brucei* strain STIB247 (red) and the semi-quantitative score of extravascular parasites in the skin (blue) are shown over a 36-day time-course following infection in Balb/C mice. Blood parasitaemia was measured using phase microscopy using methodology outlined in (***Lumsden, 1963***). Skin parasite burden is an average of five high-power fields scored by histological analysis (0 = no parasites detectable; 1 = low numbers of parasites; 2 = moderate numbers of parasites; 3 = large numbers of parasites). Standard error shown (n = 3).

DOI: 10.7554/eLife.17716.002

The following source data is available for figure 1:

Source data 1. Semi-quantitative evaluation of the parasite burden in skin sections (STIB247) Every three days for 36 days of a STIB247 T.b.

DOI: 10.7554/eLife.17716.003

Source data 2. Daily parasitaemia during STIB247 infection in Balb/C mice The daily parasitaemia during a 36-day STIB247 T.b.

DOI: 10.7554/eLife.17716.004

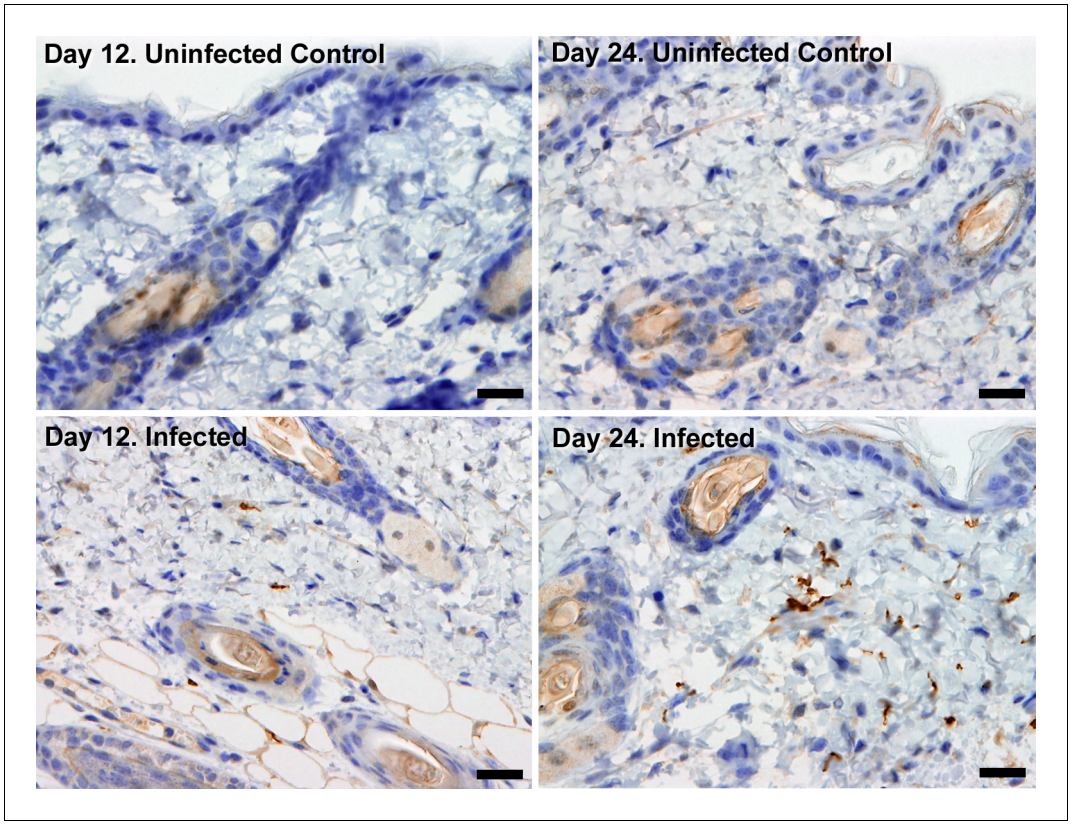

Figure 2. Extravascular localisation of trypanosomes during an infection. Histological sections of dorsal skin from uninfected and infected Balb/C mice stained with trypanosome-specific anti-ISG65 antibody (brown), counterstained with Gill's Haematoxylin stain (blue) at 12 days and 24 days post-inoculation with T.b. *brucei* strain STIB247. Parasites are visible in extravascular locations of the skin including the deep dermis and subcutaneous adipose tissue from day 12. The scale bar represents 20 μm.

DOI: 10.7554/eLife.17716.005

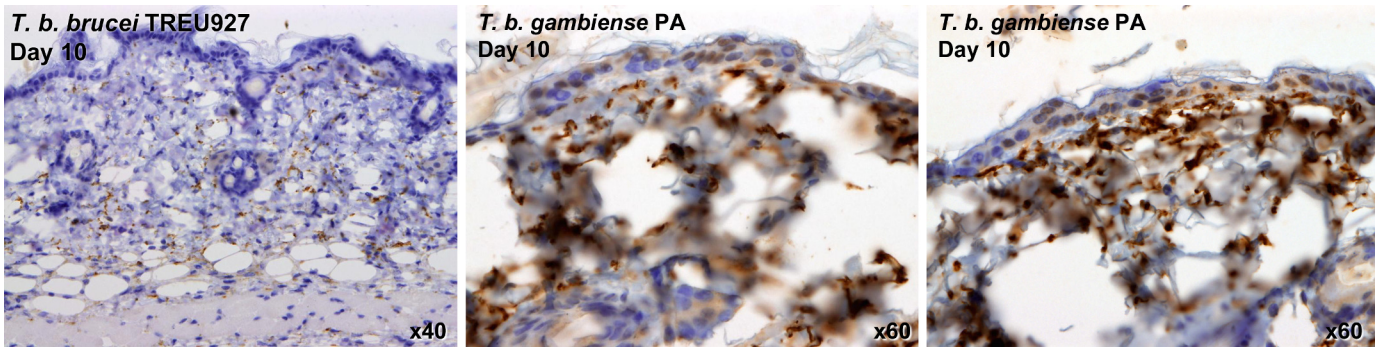

Figure 2—figure supplement 1. Skin invasion by T.b. brucei strain TREU927 and T.b. gambiense strain PA. Histological sections of dorsal skin from a mouse infected with *T.b. brucei* strain TREU927 at 20x magnification and two mice infected with *T.b. gambiense* strain PA at 40x magnification 10-days post-inoculation. Trypanosome-specific anti-ISG65 antibody reveals the presence of extravascular parasites (brown) and the slides were counterstained with Gill's Haematoxylin stain (blue) to reveal host skin structure.

DOI: 10.7554/eLife.17716.006

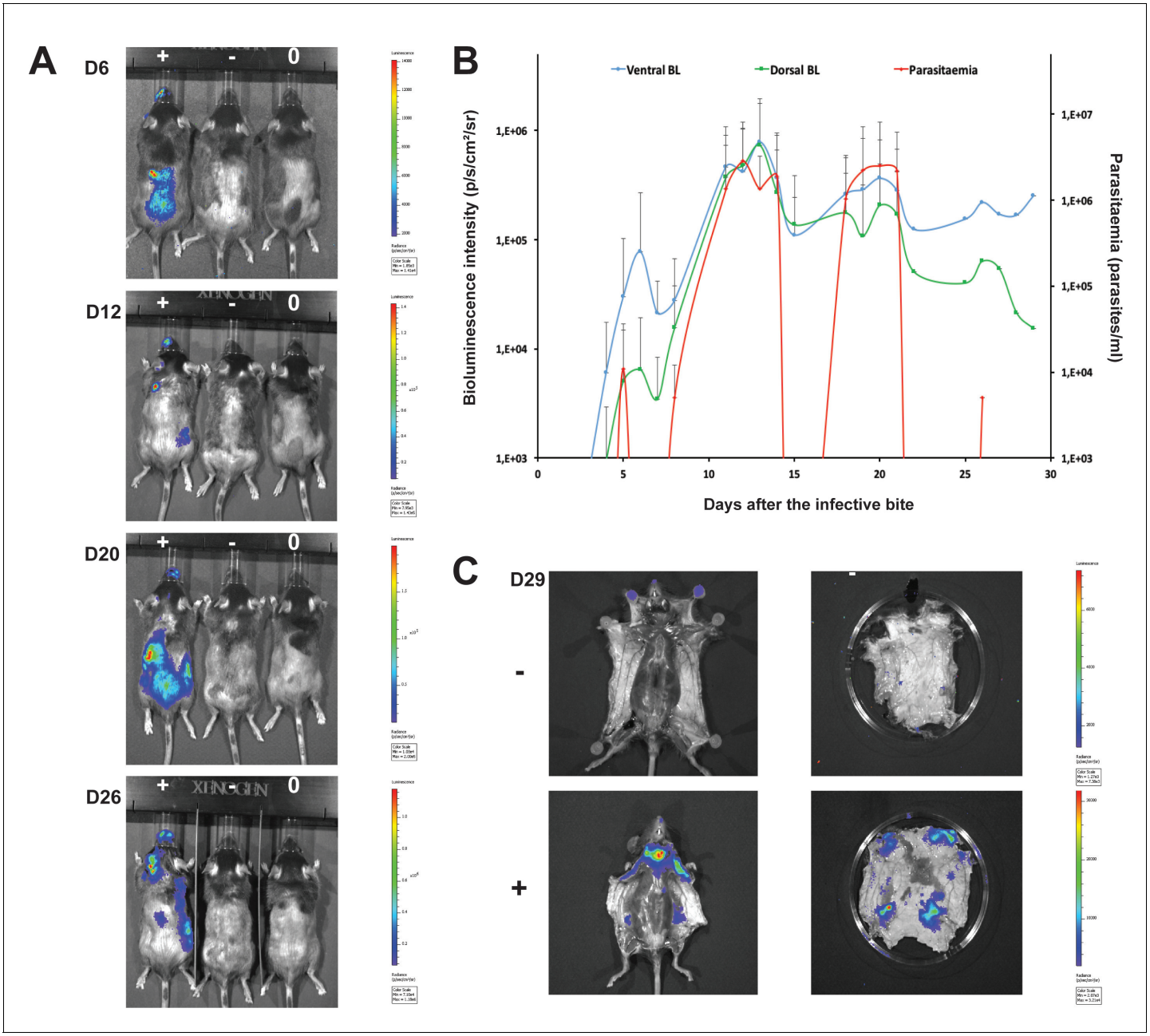

Figure 3. Dynamics of parasite distribution in the extravascular tissue of the skin and in the blood during a representative course of infection following natural transmission. A total of seven mice were infected by the single infective bite of an individual G.m. *morsitans* on the belly with the *T.b. brucei* AnTat1.1E AMLuc/tdTomato strain. Panels **A** and **C** depict representative patterns. (**A**) Examples of bioluminescence profiles of 3 mice (+ bitten by an infected fly, - bitten by an uninfected fly and 0 not bitten) 6, 12, 20 and 26 days after the bite are shown. (**B**) Ventral (blue) and dorsal (green) bioluminescence (BL) intensities (in p/s/cm^2/sr on the left Y-axis) and parasitaemia (in parasites/ml in red on the right Y-axis) were measured daily for 29 days and plotted as mean ± SD (n = 7 mice). (**C**) The entire skins of mice (+) and (-) were dissected for bioluminescence imaging 29 days after the bite. For the mouse (+), ***Figure 3—figure supplement 1*** shows the bioluminescence profile of dissected organs, ***Figure 3—figure supplement 2*** presents the skin inflammation, and ***Figure 3—figure supplement 3*** shows labelled parasites in skin sections.

DOI: 10.7554/eLife.17716.007

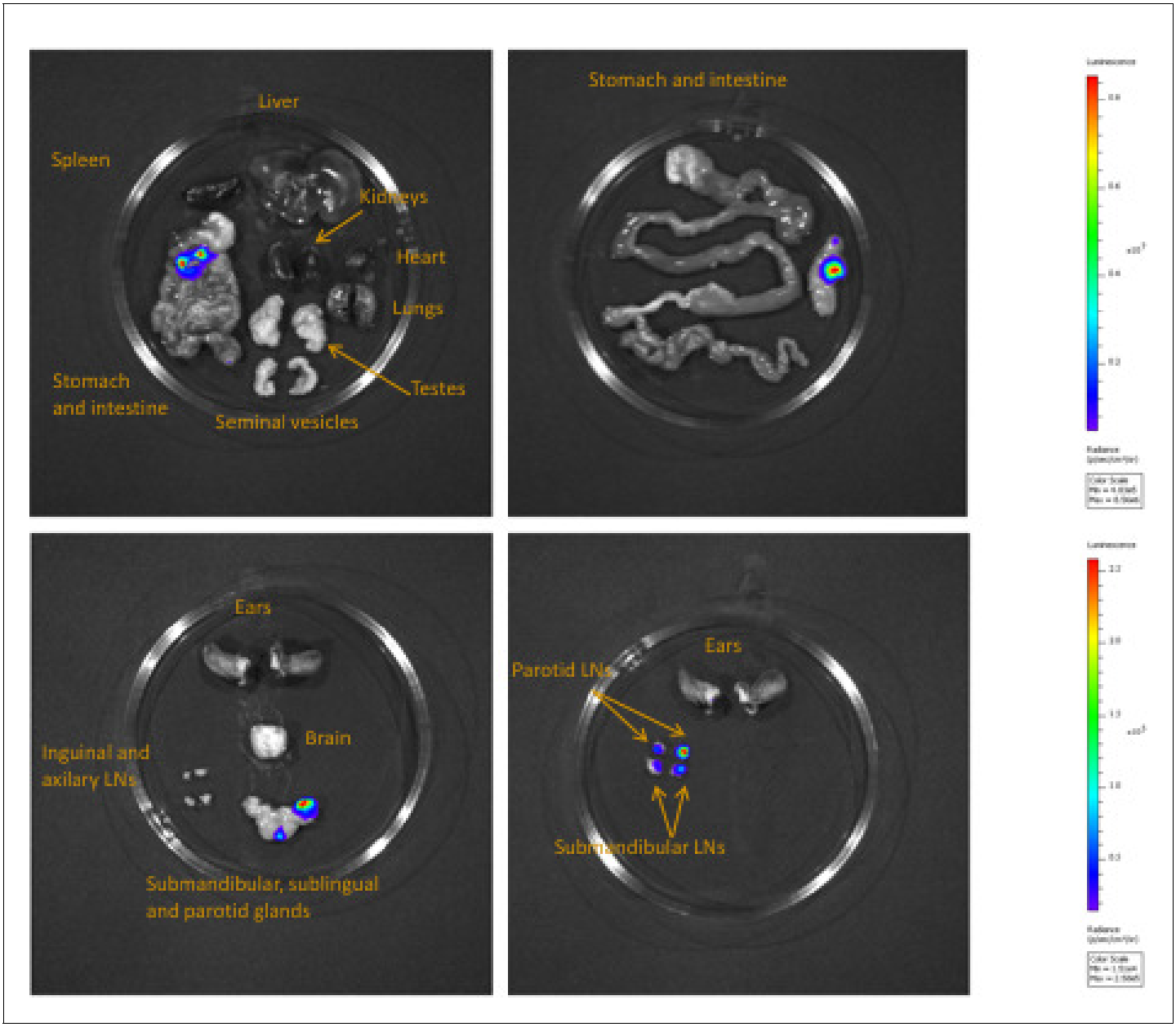

Figure 3—figure supplement 1. Bioluminescence mostly originates from parasites in the skin. Mouse (+) was sacrificed and dissected for bioluminescence imaging 29 days after the infective bite. ***Figure 3C*** shows the bioluminescence profile of its entire skin and dissected organs are shown here.

DOI: 10.7554/eLife.17716.008

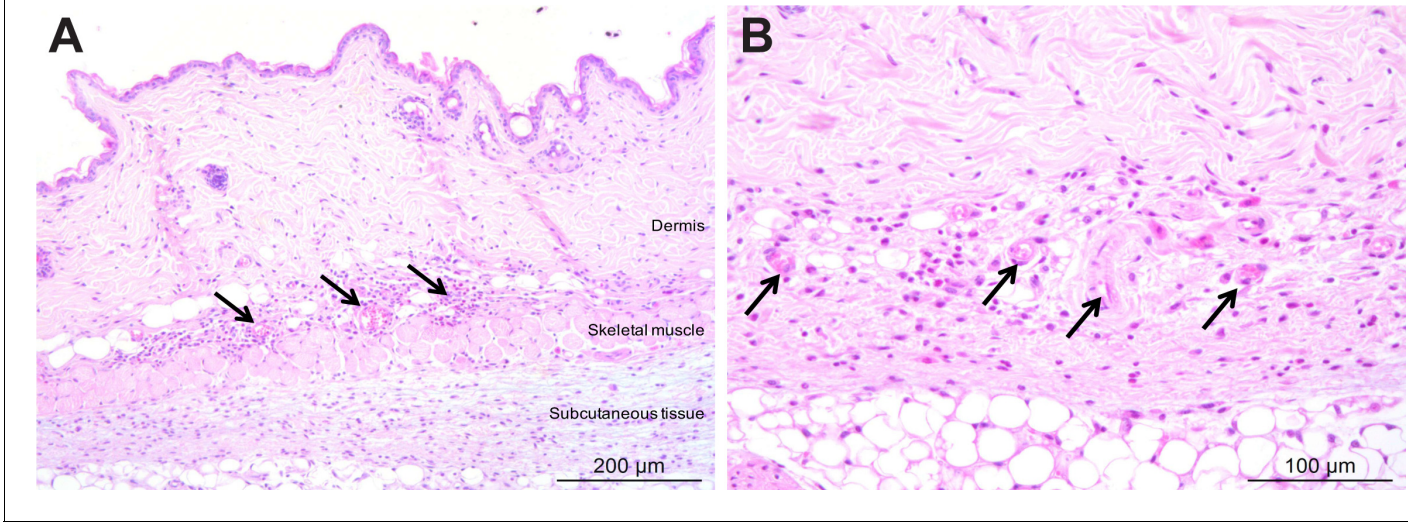

Figure 3—figure supplement 2. Mild inflammation of skin tissues one month after an infection by natural transmission. After 29 days, the most bioluminescent skin region of mouse (+) was dissected, fixed in paraformaldehyde, embedded in paraffin and stained with HE. Multifocal inflammatory infiltrates containing neutrophils were located in the dermis and subcutaneous tissue and associated with oedema (arrows in **A**). Inflammatory foci were generally centred on blood vessels (arrows in **B**).

DOI: 10.7554/eLife.17716.009

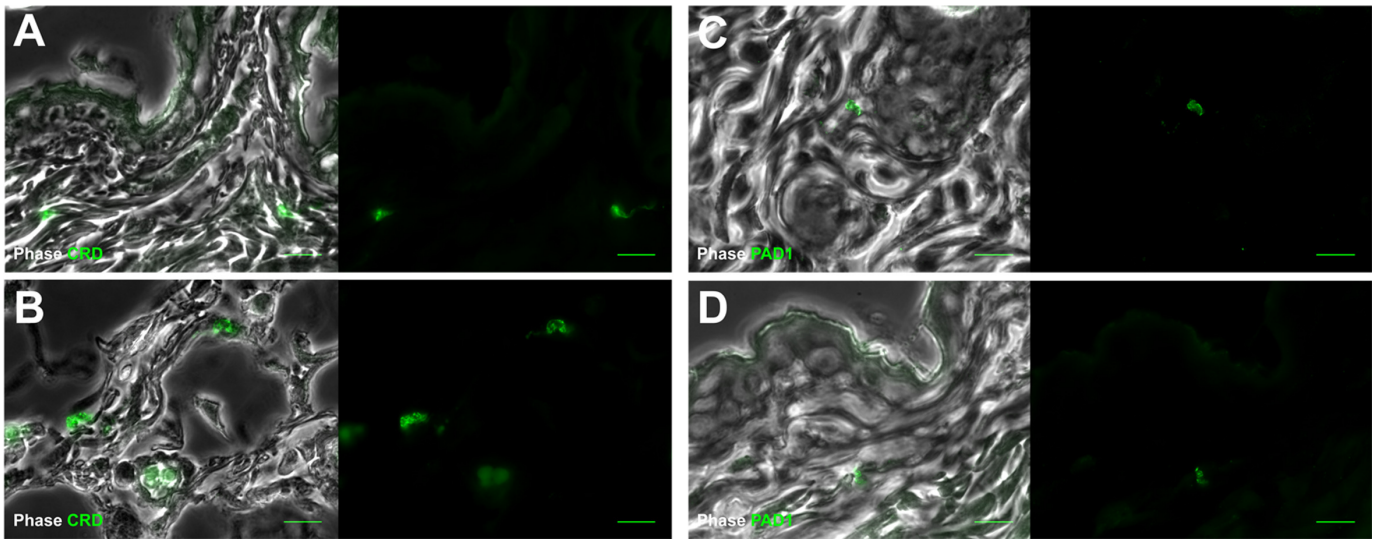

Figure 3—figure supplement 3. Extravascular parasites in the skin express both VSGs and PAD1 surface markers. After 29 days, the most bioluminescent skin region of mouse (+) was dissected, fixed in paraformaldehyde, embedded in paraffin and treated for IFA with the anti-CRD antibody that predominately labels parasites expressing VSGs (**A**–**B**), or the anti-PAD1 antibody specific to transmission form 'stumpy' cells (**C**–**D**).
DOI: 10.7554/eLife.17716.010

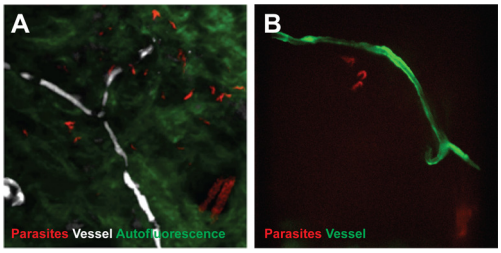

Figure 4. Extravascular localisation of trypanosomes during an infection visualised using multi-photon microscopy (**A**) and spinning-disk confocal microscopy (**B**). (**A**) Still-image extracted from video (***Video 1***) of multi-photon live imaging of dorsal skin during a trypanosome infection. Intravenous non-targeted quantum dots (white) highlight blood vessels. *T.b. brucei* STIB 247 parasites transfected with mCherry to aid visualisation (red) are clearly visible and motile outside the vasculature and within the extravascular skin matrix (green). (**B**) Still-image extracted from (***Video 3***) of spinning-disk confocal live imaging of the ear of an *Kdr (Flk1)* C57BL/6J Rj mouse during a trypanosome infection. *T.b. brucei* AnTat1.1E AMLuc/tdTomato parasites expressing tdTomato (red) are moving in the extravascular region surrounding a vessel of the dermis (green).
DOI: 10.7554/eLife.17716.011

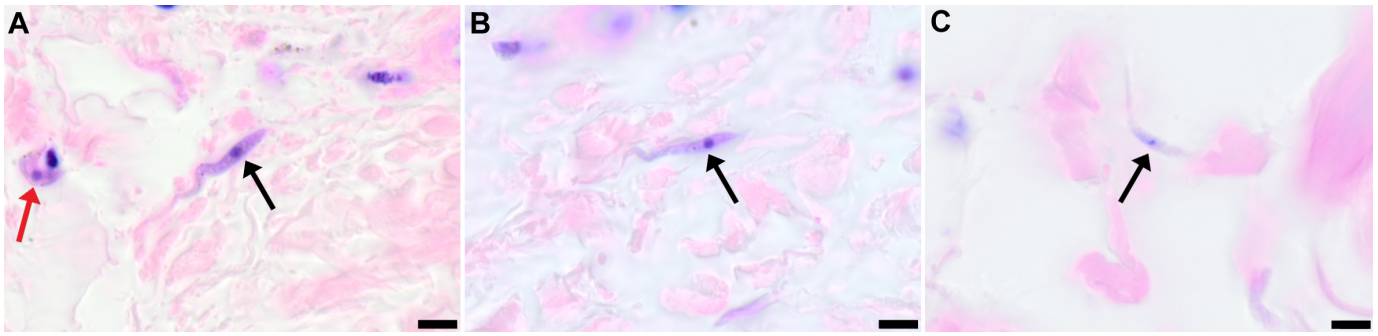

Figure 5. Extravascular localisation of trypanosomes in previously unidentified human cases of trypanosomiasis Histological sections of skin collected from previously unidentified cases of human trypanosomiasis from the Democratic Republic of Congo, showing the presence of extravascular parasites in biopsies from three individuals (**A**, **B** and **C**). Skin biopsies were collected as part of a national onchocerciasis screening programme that took place in the same geographic region as an active trypanosomiasis focus. Slides were stained with Giemsa and examined under oil immersion at 100x magnification. In addition to visible slender forms (black arrows) in the extravascular tissue of the skin, a clearly identifiable stumpy transmission form with typical morphology and an unattached undulating membrane is also present in the skin of one individual (red arrow in **A**). The scale bar represents 5 μm.

DOI: 10.7554/eLife.17716.021