

## Supplementary Materials for

### **Integrated molecular imaging reveals tissue heterogeneity driving host-pathogen interactions**

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#### **The PDF file includes:**

Fig. S1. Iron- and manganese-responsive fluorescent bacterial reporters coupled with LA-ICP-MS reveal lesional dynamics during invasive infection.

Fig. S2. MALDI IMS reveals the bacterial niche and lesional dynamics in abscessed tissues during invasive *S. aureus* infection.

Legends for movies S1 to S10

#### **Other Supplementary Material for this manuscript includes the following:**

(available at

[www.sciencetranslationalmedicine.org/cgi/content/full/10/432/eaan6361/DC1](http://www.sciencetranslationalmedicine.org/cgi/content/full/10/432/eaan6361/DC1))

Movie S1 (.mp4 format). Full MRI image volume.

Movie S2 (.mp4 format). Co-registration of MRI to 3D BLI.

Movie S3 (.mp4 format). Co-registration of blockface digital imaging to 3D BLI.

Movie S4 (.mov format). Co-registration of blockface digital imaging to LA-ICP-MS imaging of calcium, copper, iron, magnesium, manganese, phosphorous, and zinc.

Movie S5 (.mp4 format). Co-registration of blockface digital imaging to 3D BLI and LA-ICP-MS imaging of iron.

Movie S6 (.mp4 format). Co-registration of MRI to MALDI IMS signal for the S100A8 component of calprotectin ( $m/z = 10,164$ ).

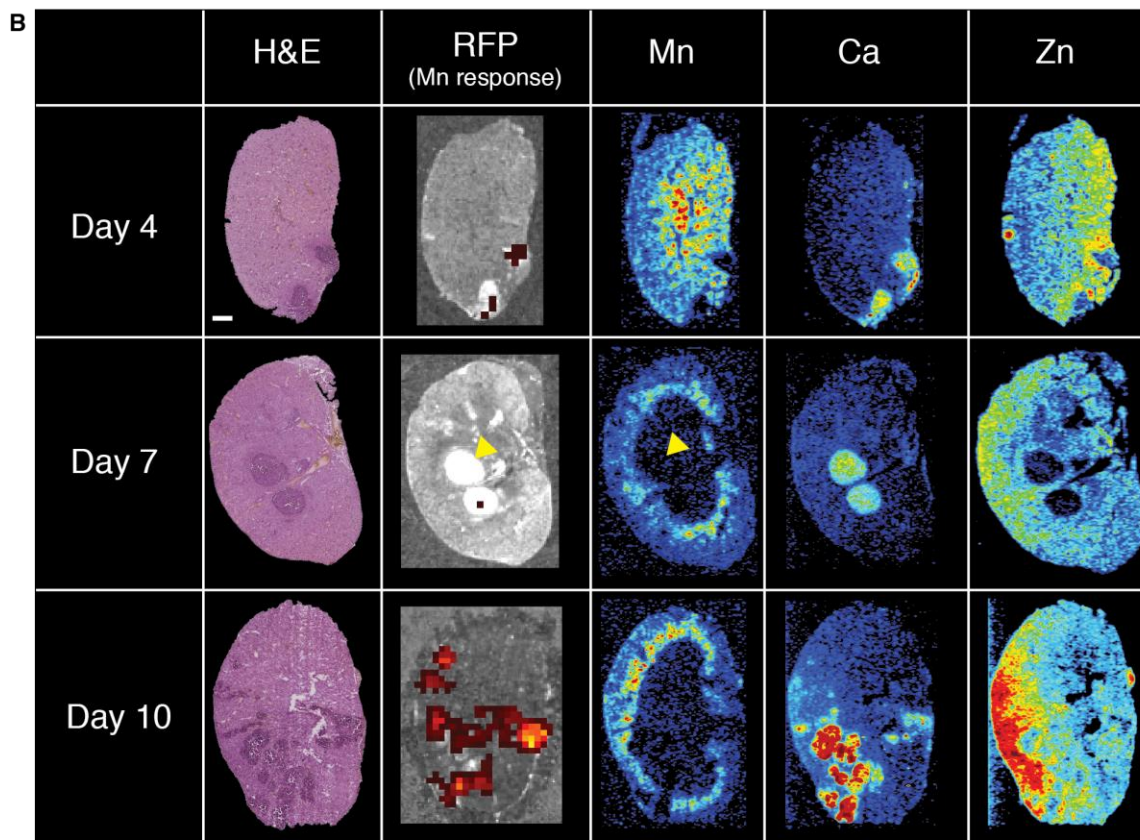
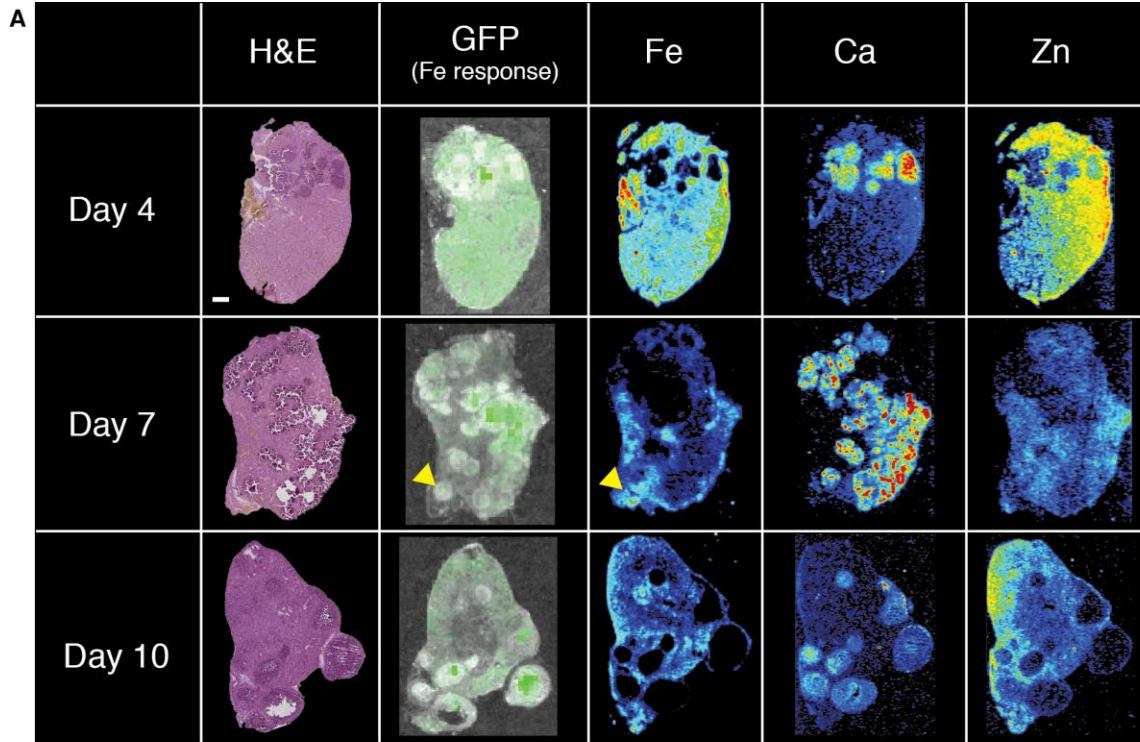
Movie S7 (.mp4 format). Co-registration of blockface digital imaging to MALDI IMS signal for the S100A8 component of calprotectin ( $m/z = 10,164$ ).

Movie S8 (.mp4 format). Co-registration of blockface digital imaging to MALDI IMS signal for the S100A8 component of calprotectin ( $m/z = 10,164$ ).

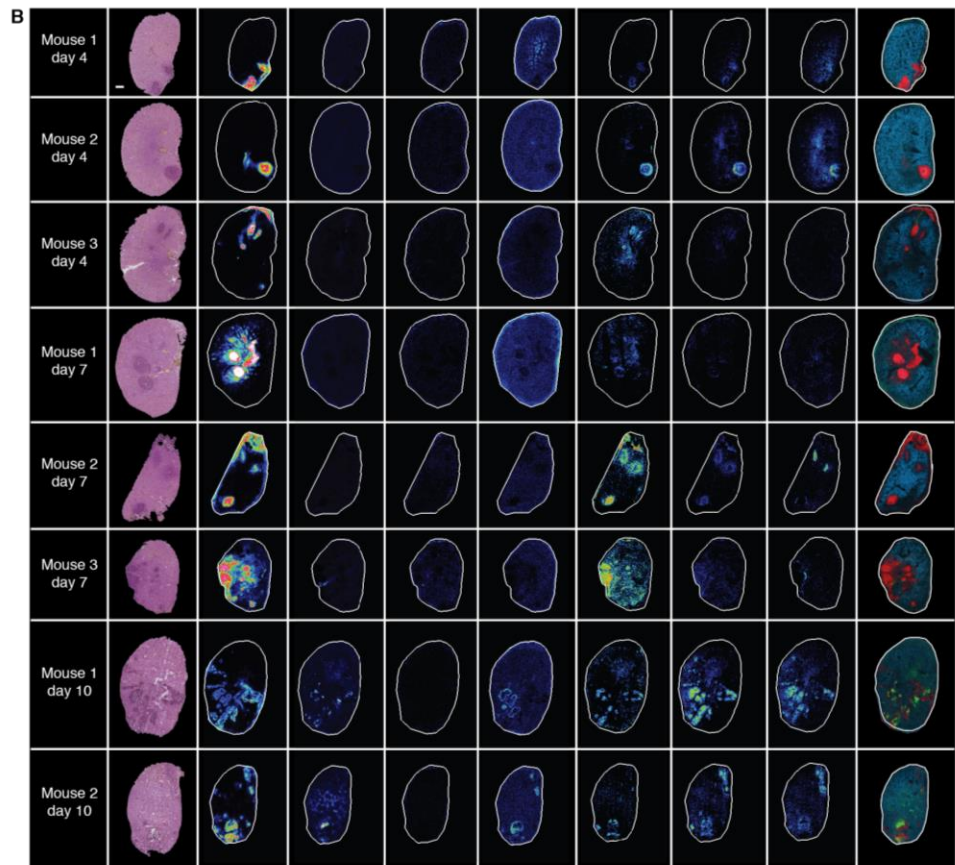
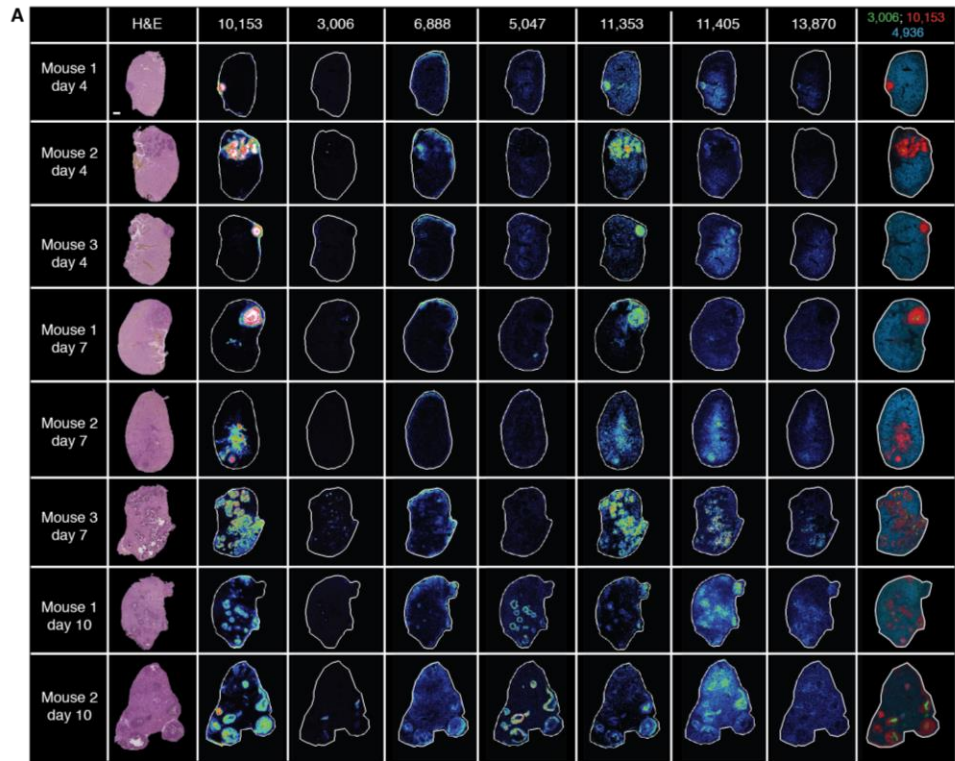
Movie S9 (.mov format). Co-registration of blockface digital imaging to MALDI IMS signal for the S100A8 component of calprotectin ( $m/z = 10,164$ ) and LA-ICP-MS imaging of calcium.

Movie S10 (.mp4 format). Co-registration of blockface digital imaging to MALDI IMS signals for the S100A8 component of calprotectin ( $m/z = 10,164$ ) and *S. aureus*  $\delta$ -hemolysin ( $m/z = 3006$ ).

## Supplementary Materials



**Fig. S1. Iron- and manganese-responsive fluorescent bacterial reporters coupled with LA-ICP-MS reveal lesional dynamics during invasive infection.** Groups of mice were infected with *S. aureus* containing iron ( $P_{isdAgfp}$ , **A**) or manganese ( $P_{mntArfp}$ , **B**) responsive fluorescent reporters. At 4, 7, or 10 days post-infection, infected kidneys were removed, flash-frozen, and sectioned prior to imaging for fluorescence (Fe=GFP; Mn=RFP) and LA-ICP-MS (for Ca, Zn, and either Fe or Mn). Serial sections were also imaged via MALDI IMS (see Figure S2), and then stained via H&E. Yellow arrowheads depict representative abscesses with lower levels of metal starvation signal via fluorescent imaging, and the corresponding lesion showing differential surrounding metal abundance via LA-ICP-MS for Fe or Mn. One representative mouse is shown for each time point (n=3 for days 4 and 7, n=2 for day 10) and each fluorescent reporter. White bar represents 1 mm scale.



**Fig. S2. MALDI IMS reveals the bacterial niche and lesional dynamics in abscessed tissues during invasive *S. aureus* infection.** Groups of mice were infected with *S. aureus* strain Newman  $P_{isdAgfp}$  (A) or Newman  $P_{mntArfp}$  (B) (see fig. S1) and infected kidneys were subsequently excised at days 4 (n=3 per strain), 7 (n=3 per strain), or 10 (n=2 per strain) post-infection. Whole kidneys were then flash-frozen, sectioned, and imaged via MALDI IMS. At the completion of MALDI IMS, sections were stained with H&E. The following analytes are depicted:  $m/z$  10,153=S100A8,  $m/z$  3006=delta toxin,  $m/z$  5047=CsbD-like,  $m/z$  11,353=unknown,  $m/z$  11,405=unknown, and  $m/z$  13,870=unknown. The rightmost panel is an overlay of delta toxin (green), S100A8 (red), and thymosin beta 10 ( $m/z$  4936; depicts background kidney anatomy). White bar represents 1 mm scale.

**Movie S1. Full MRI image volume.** Abdominal transverse MRI imaging of a mouse intravenously infected 4 days prior with *S. aureus* Newman  $pisdl.Xen1$ . Abscesses appear as spherical punctate lesions with loss of signal in the hepatic and renal parenchyma.

**Movie S2. Co-registration of MRI to 3D BLI.** Abdominal transverse MRI (see movie S1) and three-dimensional bioluminescent (BLI) imaging were performed on a mouse intravenously infected 4 days prior with *S. aureus* Newman  $pisdl.Xen1$ . Movie depicts the co-registration of MRI and BLI imaging volumes. Bioluminescent signal is depicted by yellow-orange spheres. Abscesses appear as spherical punctate lesions with loss of signal in the hepatic and renal parenchyma.

**Movie S3. Co-registration of blockface digital imaging to 3D BLI.** The abdominal portion of a mouse intravenously infected 4 days prior with *S. aureus* Newman *pisdl.Xen1* was transversely sectioned and each section was digitally imaged to create a serial blockface imaging volume. Movie depicts the blockface imaging volume co-registered to the BLI imaging volume (see movie S2). Bioluminescent signal is depicted by yellow-orange spheres. Abscesses appear as white spherical punctate lesions within the hepatic and renal parenchyma.

**Movie S4. Co-registration of blockface digital imaging to LA-ICP-MS imaging of calcium, copper, iron, magnesium, manganese, phosphorous, and zinc.** The blockface imaging volume encompassing the abdominal portion of a mouse intravenously infected 4 days prior with *S. aureus* (see movie S3) was co-registered to LA-ICP-MS imaging of calcium, copper, iron, magnesium, manganese, phosphorous, and zinc concentrations. The final sequence of the movie contains all elements co-registered together. LA-ICP-MS analysis is limited to the right kidney only. Heat maps depict metal concentrations in arbitrary units. Each imaging volume is oriented obliquely to demonstrate the signal in three dimensions.

**Movie S5. Co-registration of blockface digital imaging to 3D BLI and LA-ICP-MS imaging of iron.** The blockface imaging volume encompassing the abdominal portion of a mouse intravenously infected 4 days prior with *S. aureus* (see movie S3) was co-registered to both the BLI imaging volume (red spheres) and the LA-ICP-MS imaging volume for iron (yellow). LA-ICP-MS analysis is limited to the right kidney only.



**Movie S6. Co-registration of MRI to MALDI IMS signal for the S100A8 component of calprotectin ( $m/z = 10,164$ ).** Abdominal transverse MRI (see movie S1) and MALDI IMS were performed on a mouse intravenously infected with *S. aureus* 4 days prior. The resulting imaging volumes were co-registered. MALDI IMS of the S100A8 component of calprotectin ( $m/z=10,164$ ) is depicted in yellow. MALDI IMS analysis was limited to the right kidney.

**Movie S7. Co-registration of blockface digital imaging to MALDI IMS signal for the S100A8 component of calprotectin ( $m/z = 10,164$ ).** The blockface imaging volume encompassing the abdominal portion of a mouse intravenously infected 4 days prior with *S. aureus* (see movie S3) was co-registered to the MALDI IMS signal for the S100A8 component of calprotectin ( $m/z=10,164$ ), depicted in blue. MALDI IMS analysis was limited to the right kidney. Abscesses appear as white spherical punctate lesions within the hepatic and renal parenchyma.

**Movie S8. Co-registration of blockface digital imaging to MALDI IMS signal for the S100A8 component of calprotectin ( $m/z = 10,164$ ).** The blockface imaging volume encompassing the abdominal portion of a mouse intravenously infected 4 days prior with *S. aureus* (see movie S3) was co-registered to the MALDI IMS signal for the S100A8 component of calprotectin ( $m/z=10,164$ ), depicted in blue. MALDI IMS analysis was limited to the right kidney. Abscesses appear as white spherical punctate lesions within the hepatic and renal parenchyma. Each imaging volume is oriented obliquely to demonstrate the signal in three dimensions.



**Movie S9. Co-registration of blockface digital imaging to MALDI IMS signal for the S100A8 component of calprotectin ( $m/z = 10,164$ ) and LA-ICP-MS imaging of calcium.** The blockface imaging volume encompassing the abdominal portion of a mouse intravenously infected 4 days prior with *S. aureus* (see movie S3) was co-registered to both the MALDI IMS signal for the S100A8 component of calprotectin ( $m/z=10,164$ ; depicted in blue) and LA-ICP-MS imaging of calcium (depicted in orange). Each imaging volume is oriented obliquely to demonstrate the signal in three dimensions. MALDI IMS and LA-ICP-MS analyses were limited to the right kidney. The first sequence shows co-registration of blockface and MALDI IMS, the second sequence shows co-registration of blockface and LA-ICP-MS, and the final sequence shows all three imaging volumes together. Heat maps depict analyte concentrations in arbitrary units.

**Movie S10. Co-registration of blockface digital imaging to MALDI IMS signals for the S100A8 component of calprotectin ( $m/z = 10,164$ ) and *S. aureus*  $\delta$ -hemolysin ( $m/z = 3006$ ).** The blockface imaging volume encompassing the abdominal portion of a mouse intravenously infected 4 days prior with *S. aureus* (see movie S3) was co-registered to both the MALDI IMS signal for the S100A8 component of calprotectin ( $m/z=10,164$ ; depicted in blue) and the MALDI IMS signal for *S. aureus* delta toxin ( $m/z=3006$ ; depicted in red). MALDI IMS analysis was limited to the right kidney. Each imaging volume is oriented obliquely to demonstrate the signal in three dimensions.