Figure S1

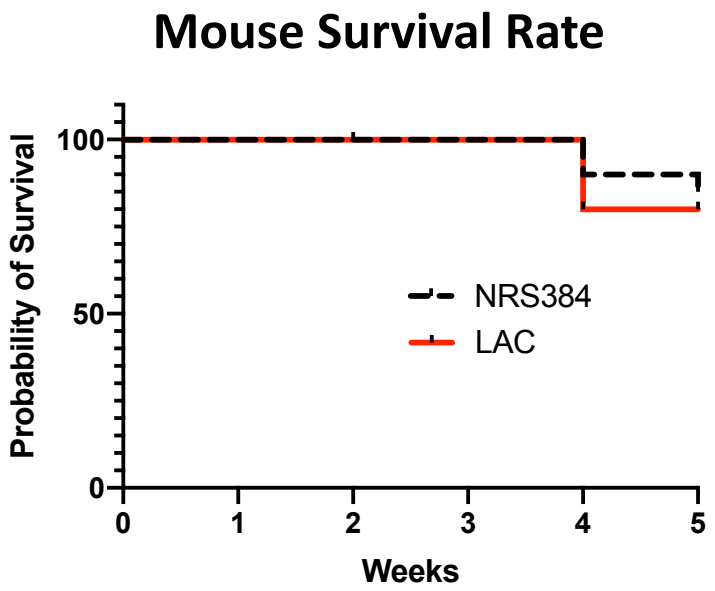

Mouse Survival Rate
Probability of Survival
100
50
0
0
1
2
3
4
5
Weeks
NRS384
LAC

Figure S2

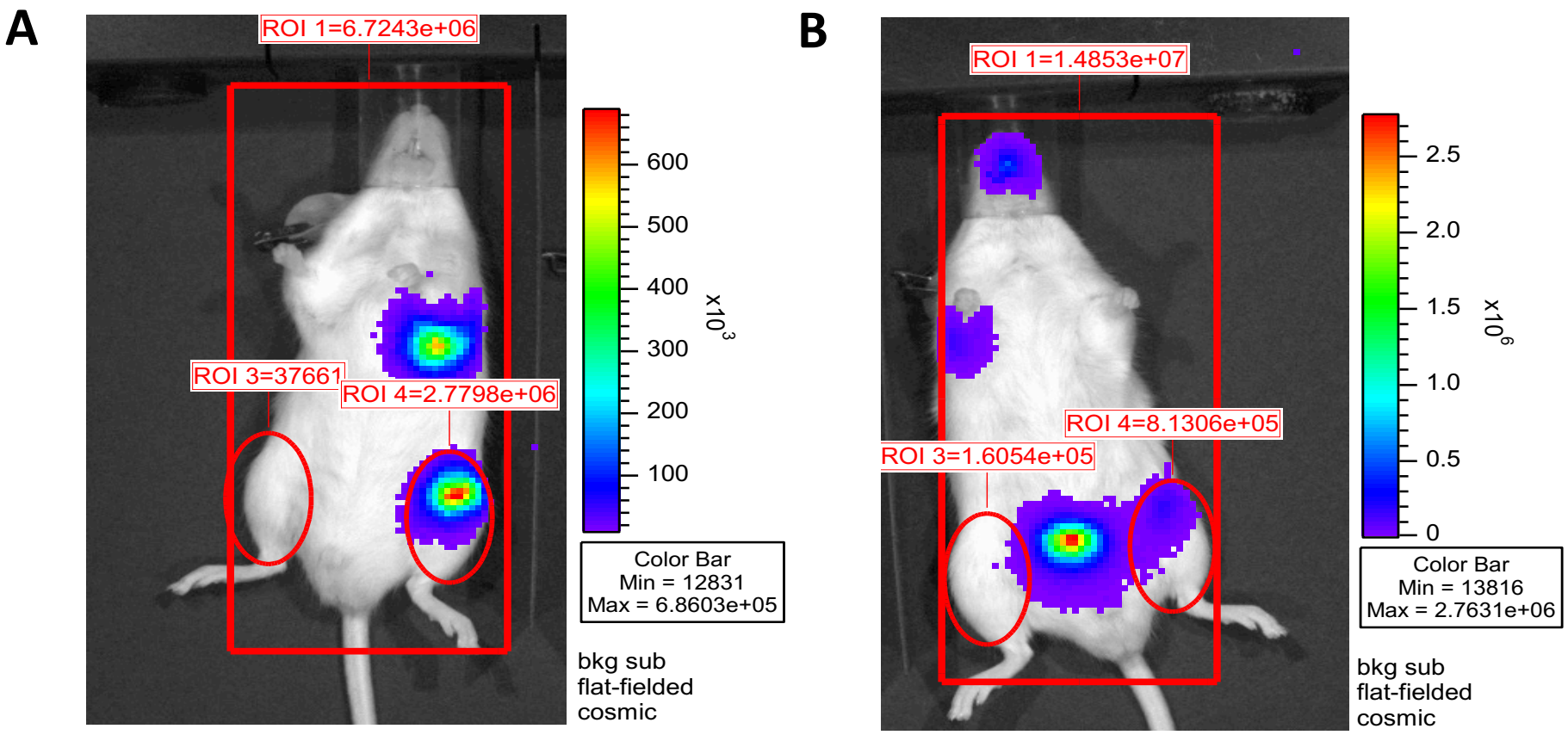
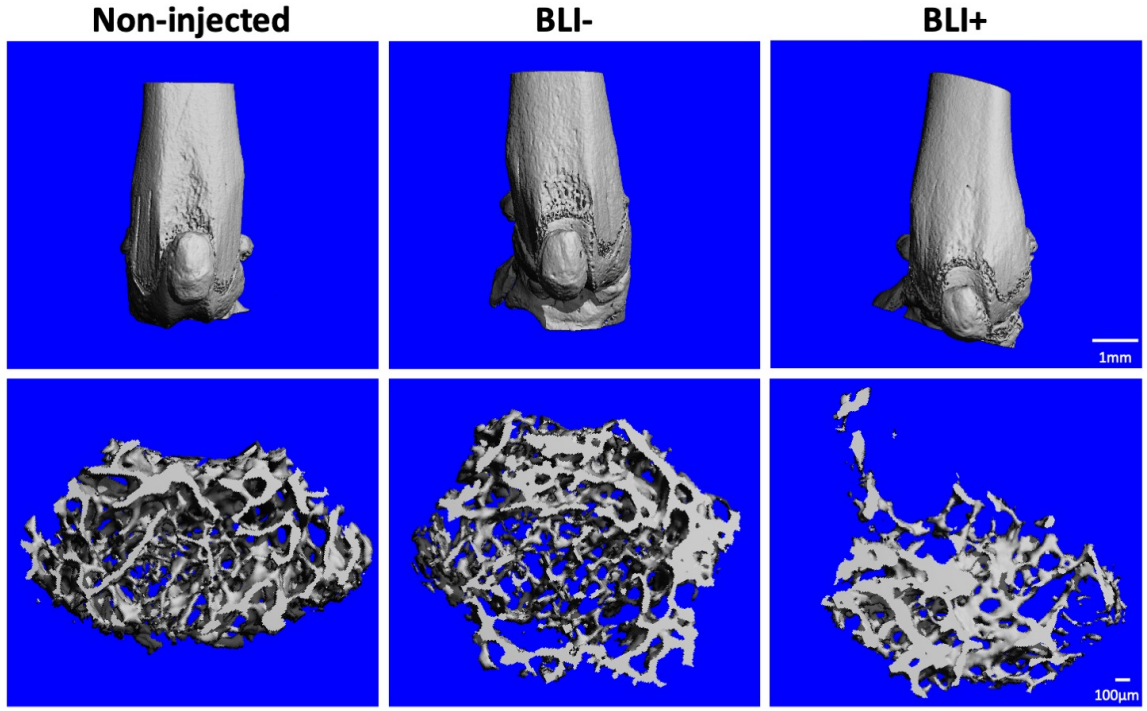

A
ROI 1=6.7243e+06
ROI 3=37661
ROI 4=2.7798e+06
600
500
400
300
200
100
x10^3
Color Bar
Min = 12831
Max = 6.8603e+05
bkg sub
flat-fielded
cosmic
B
ROI 1=1.4853e+07
ROI 4=8.1306e+05
ROI 3=1.6054e+05
2.5
2.0
1.5
1.0
0.5
0
x10^6
Color Bar
Min = 13816
Max = 2.7631e+06
bkg sub
flat-fielded
cosmic

Figure S3


A
Non-injected
BLI-
BLI+
1mm
100µm

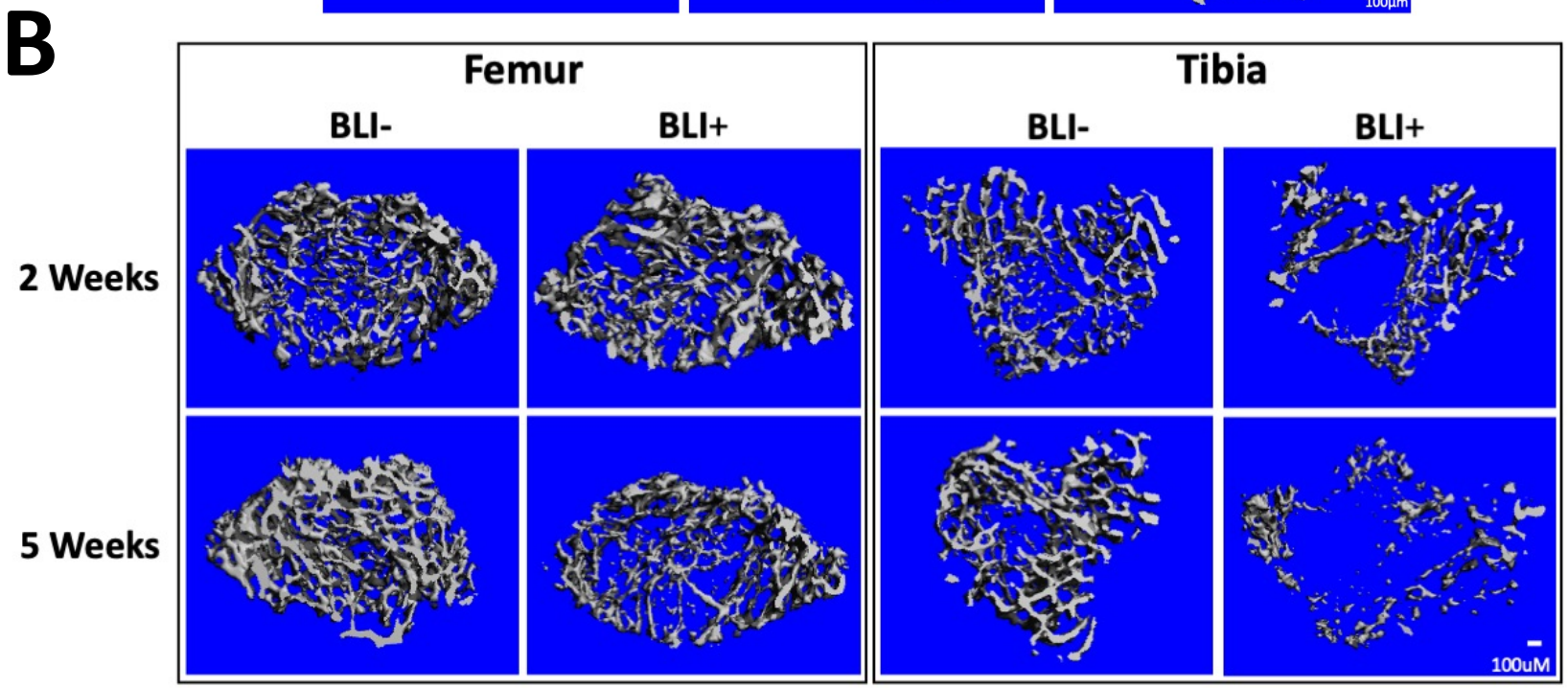
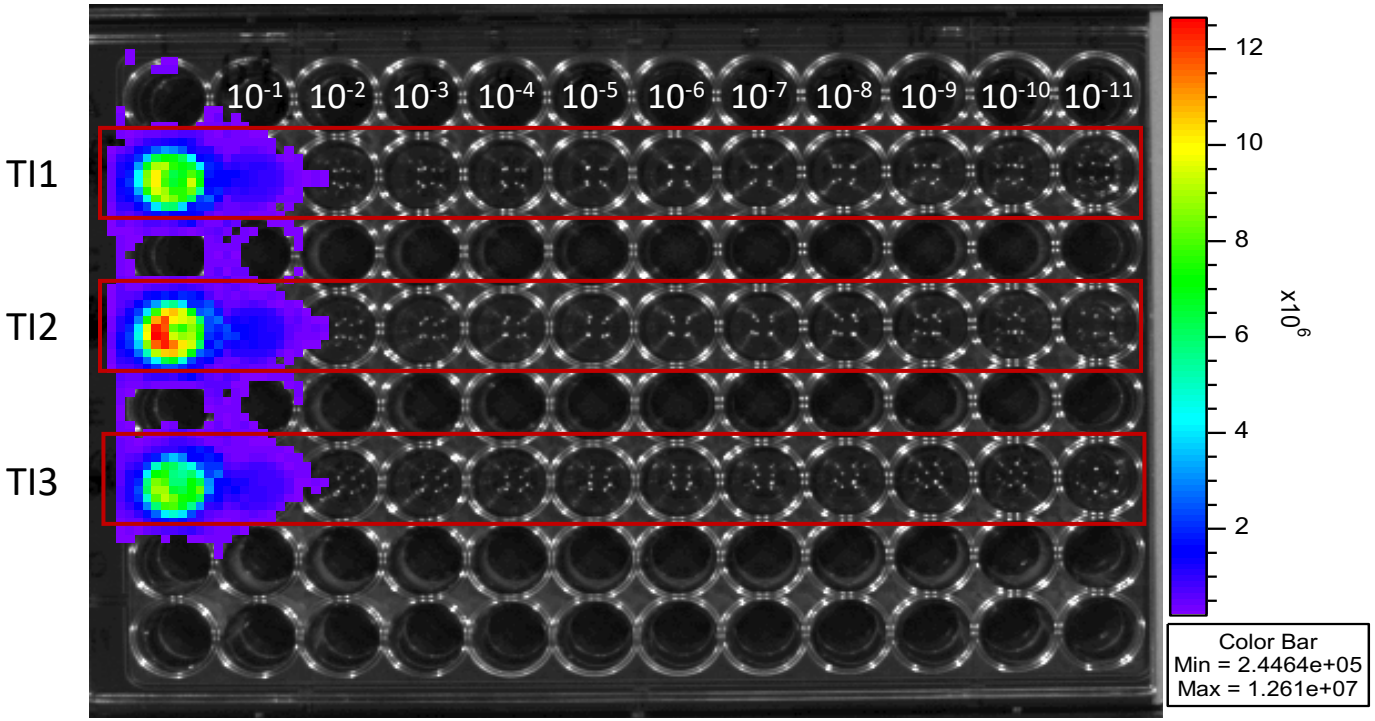

B
Femur
Tibia
BLI-
BLI+
BLI-
BLI+
2 Weeks
5 Weeks
100uM

Figure S4

A


10-1 10-2 10-3 10-4 10-5 10-6 10-7 10-8 10-9 10-10 10-11
TI1
TI2
TI3
12
10
8
6
4
2
x10^6
Color Bar
Min = 2.4464e+05
Max = 1.261e+07

B

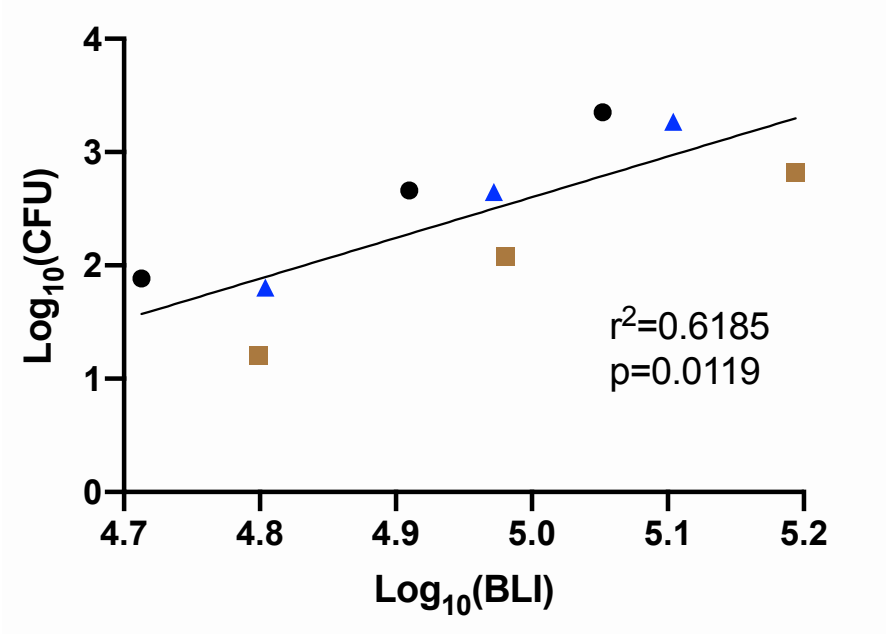
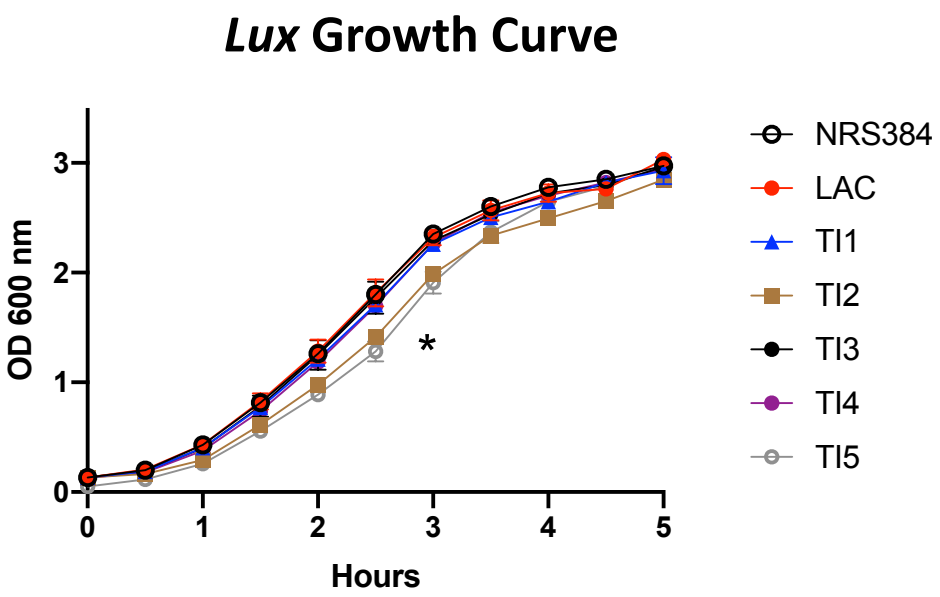

Log10(CFU)
Log10(BLI)
4
3
2
1
0
4.7
4.8
4.9
5.0
5.1
5.2
r2=0.6185
p=0.0119

Figure S5

A


Lux Growth Curve
OD 600 nm
Hours
*
NRS384
LAC
TI1
TI2
TI3
TI4
TI5

B

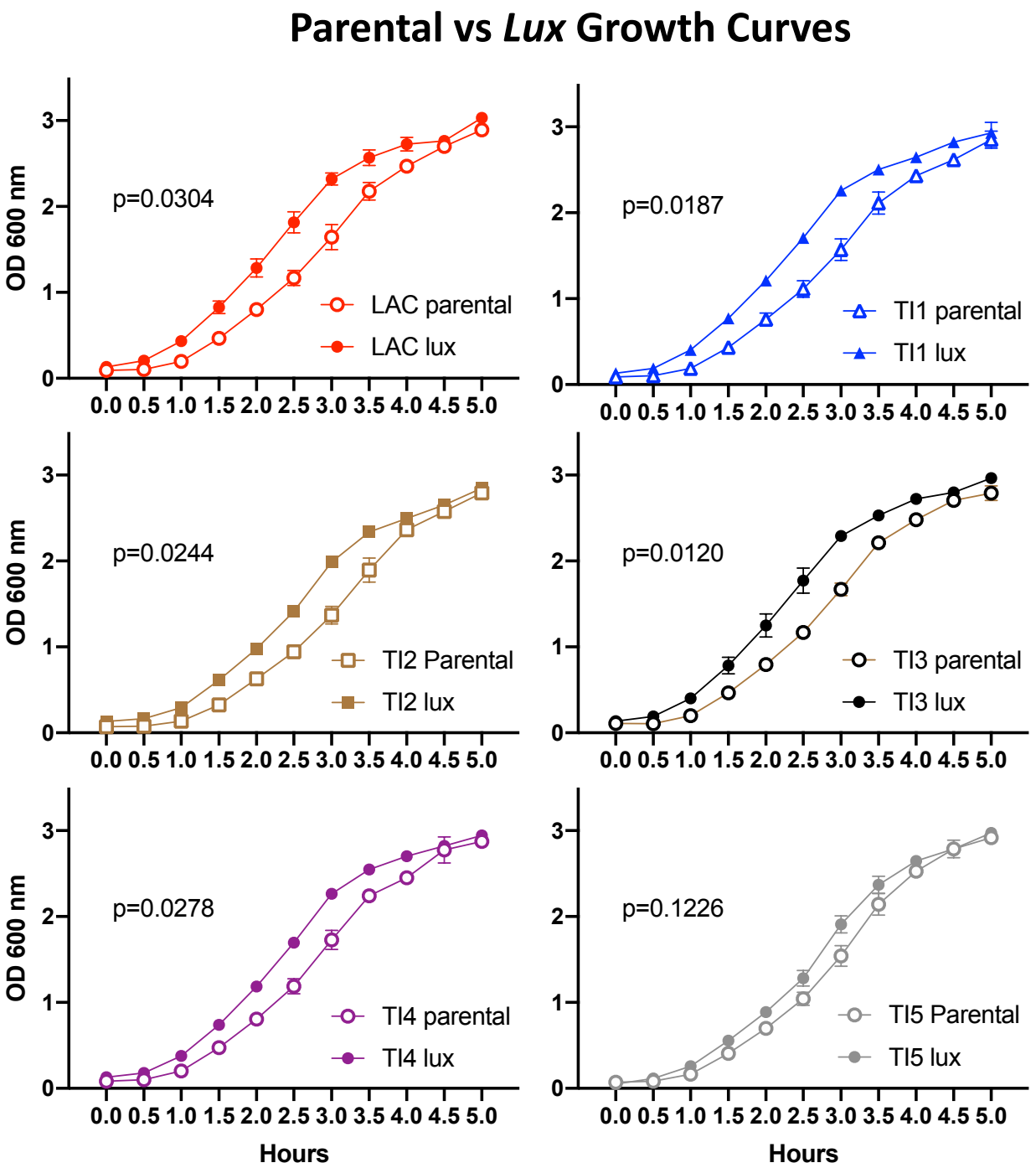
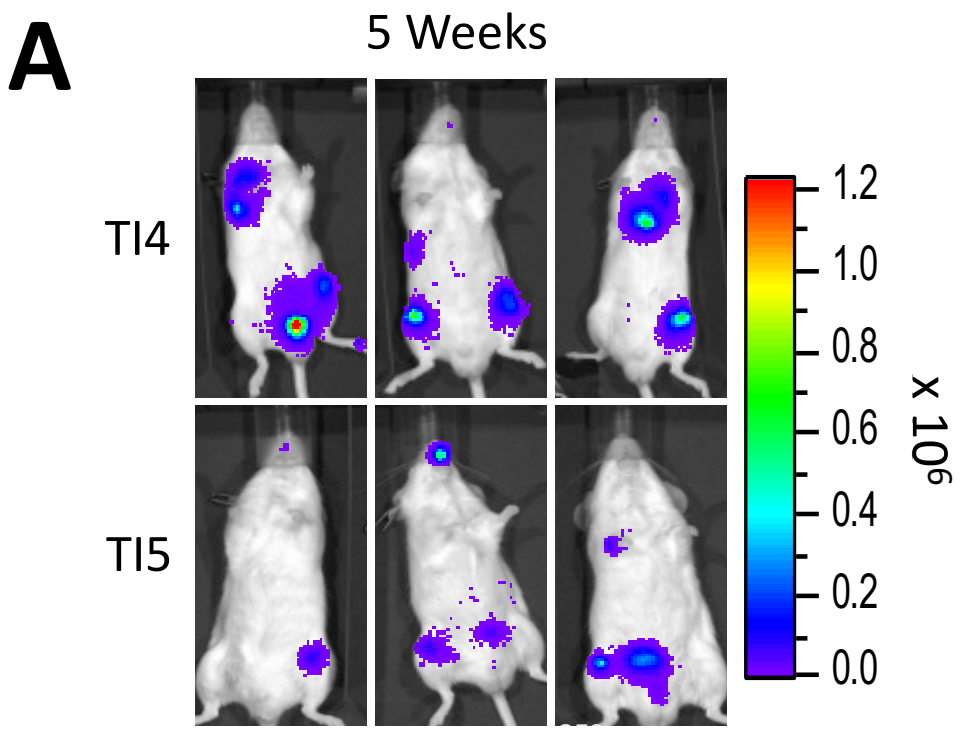
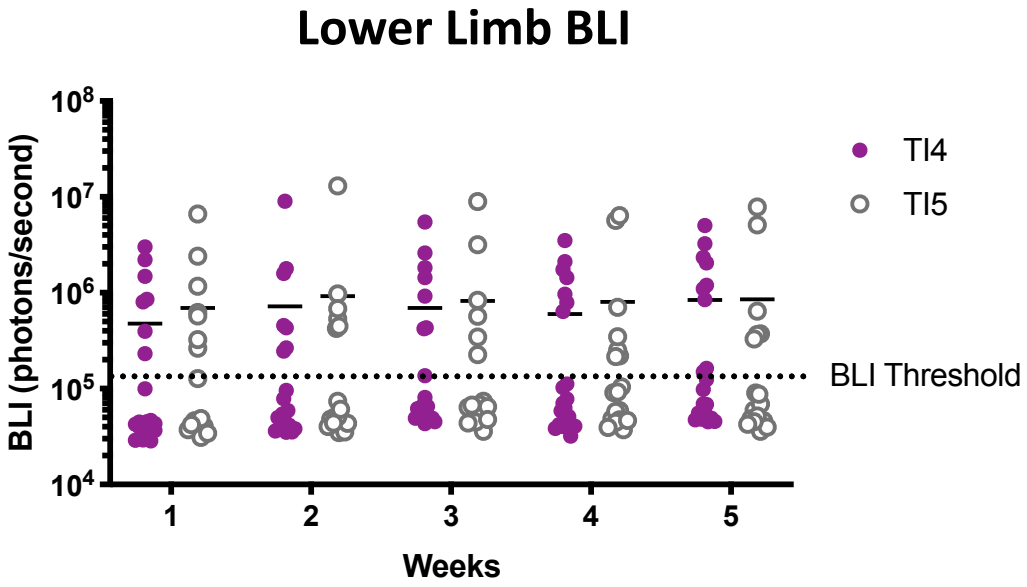

Parental vs *Lux* Growth Curves
OD 600 nm
Hours
p=0.0304
LAC parental
LAC lux
p=0.0187
TI1 parental
TI1 lux
p=0.0244
TI2 Parental
TI2 lux
p=0.0120
TI3 parental
TI3 lux
p=0.0278
TI4 parental
TI4 lux
p=0.1226
TI5 Parental
TI5 lux

Figure S6


A
5 Weeks
TI4
TI5
1.2
1.0
0.8
0.6
0.4
0.2
0.0
x 10⁶


B
Lower Limb BLI
BLI (photons/second)
10⁸
10⁷
10⁶
10⁵
10⁴
1
2
3
4
5
Weeks
TI4
TI5
BLI Threshold

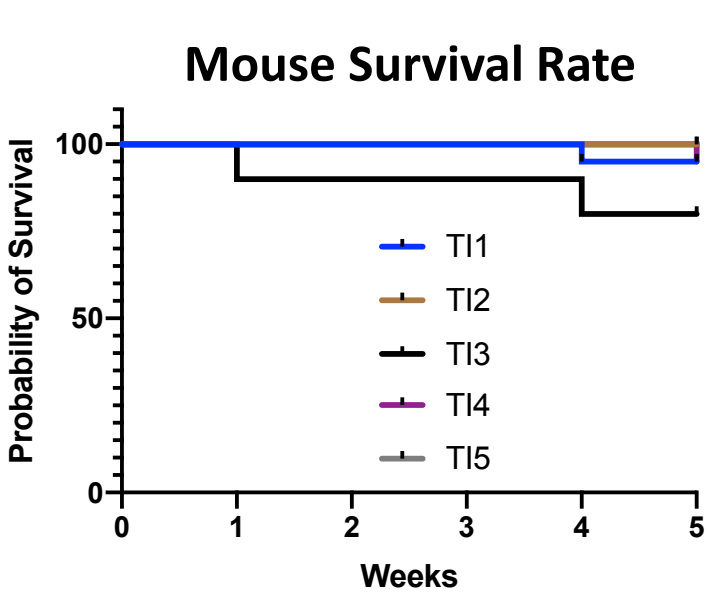

C
Mouse Survival Rate
Probability of Survival
100
50
0
0
1
2
3
4
5
Weeks
TI1
TI2
TI3
TI4
TI5

Figure S7

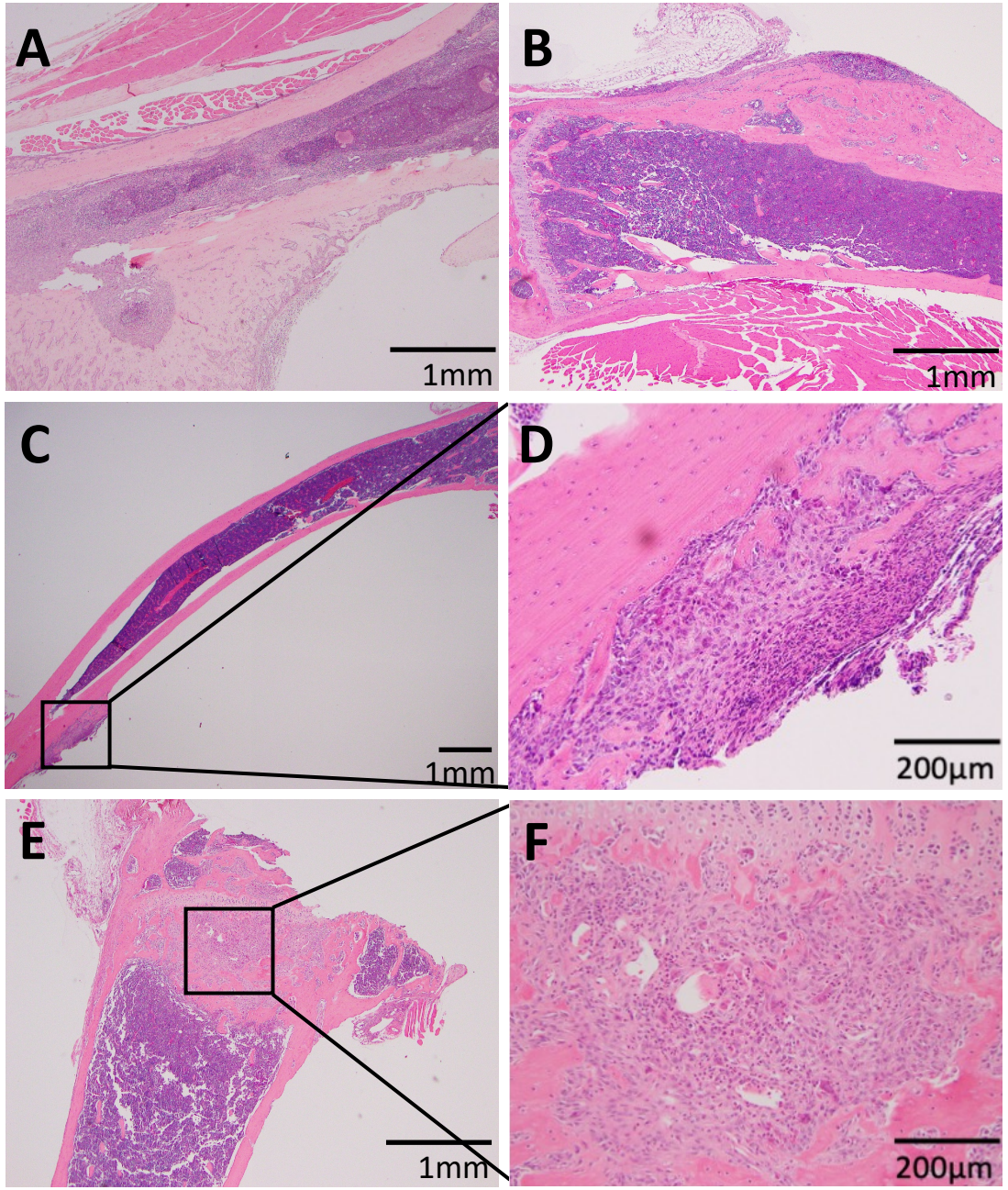


Figure S8

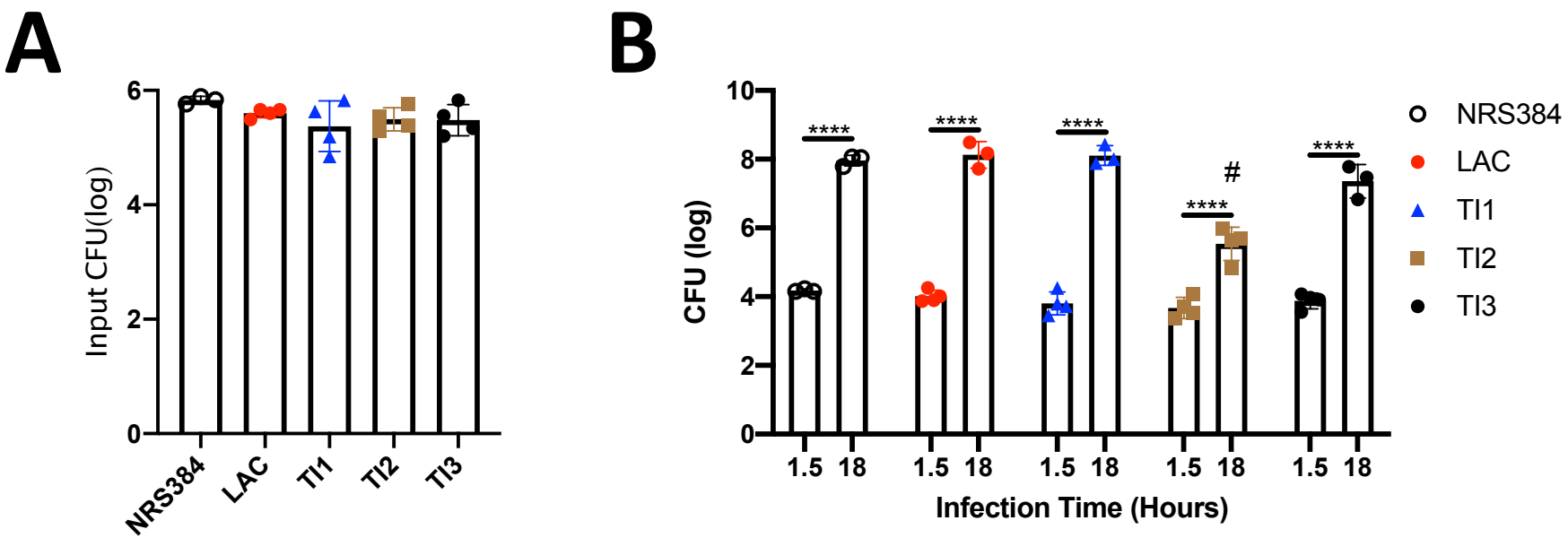
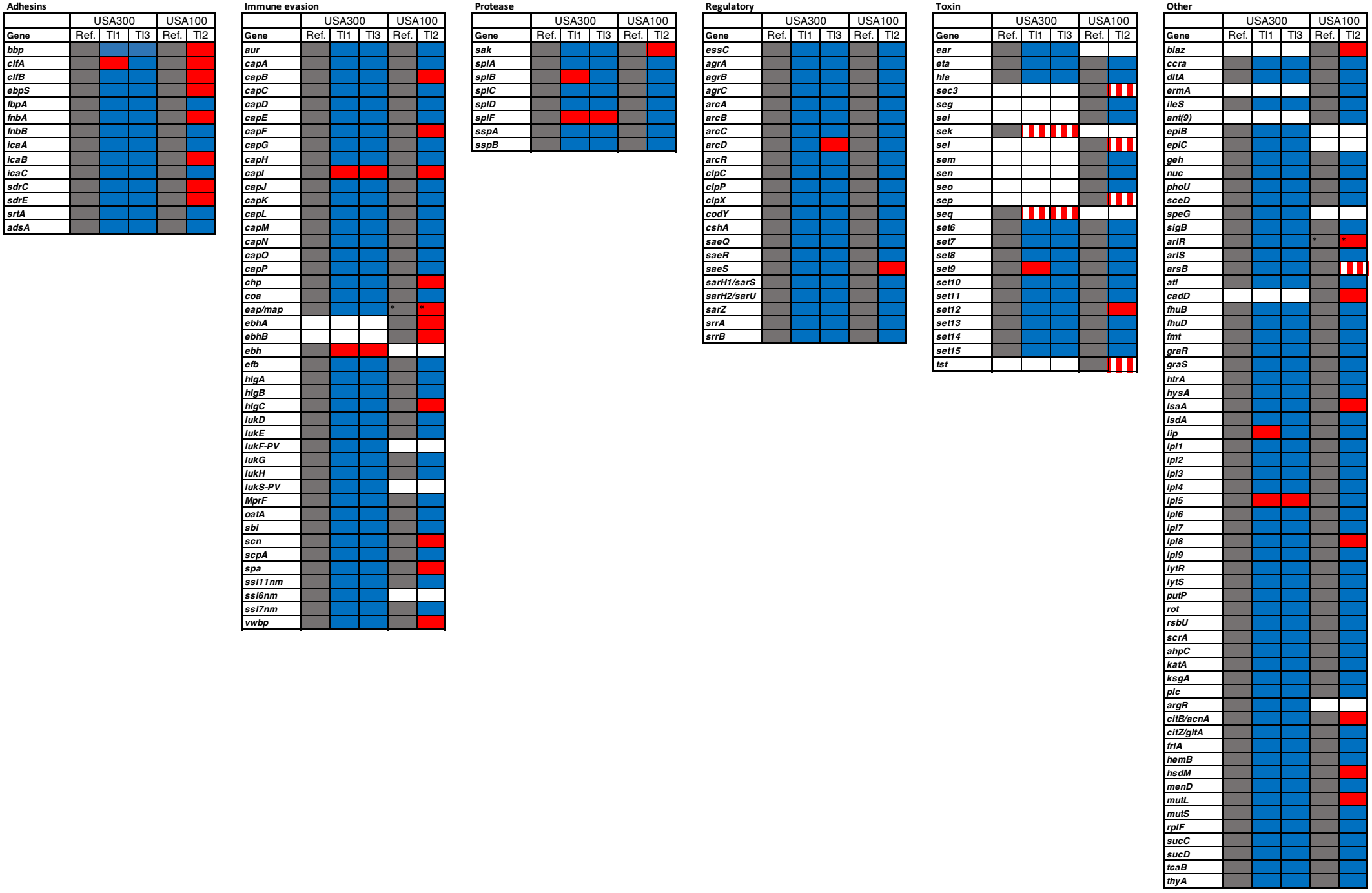

A
Input CFU(log)
6
4
2
0
NRS384
LAC
TI1
TI2
TI3
B
CFU (log)
10
8
6
4
2
0
****
****
****
#
****
****
1.5
18
Infection Time (Hours)
NRS384
LAC
TI1
TI2
TI3

Figure S9


Adhesins
Gene | USA300 Ref. | USA300 TI1 | USA300 TI3 | USA100 Ref. | USA100 TI2
bbp
clfA
clfB
ebpS
fbpA
fnbA
fnbB
icaA
icaB
icaC
sdrC
sdrE
srtA
adsA
Immune evasion
Gene | USA300 Ref. | USA300 TI1 | USA300 TI3 | USA100 Ref. | USA100 TI2
aur
capA
capB
capC
capD
capE
capF
capG
capH
capI
capJ
capK
capL
capM
capN
capO
capP
chp
coa
eap/map
ebhA
ebhB
ebh
efb
hlgA
hlgB
hlgC
lukD
lukE
lukF-PV
lukG
lukH
lukS-PV
MprF
oatA
sbi
scn
scpA
spa
ssl11nm
ssl6nm
ssl7nm
vwbp
Protease
Gene | USA300 Ref. | USA300 TI1 | USA300 TI3 | USA100 Ref. | USA100 TI2
sak
splA
splB
splC
splD
splF
sspA
sspB
Regulatory
Gene | USA300 Ref. | USA300 TI1 | USA300 TI3 | USA100 Ref. | USA100 TI2
essC
agrA
agrB
agrC
arcA
arcB
arcC
arcD
arcR
clpC
clpP
clpX
codY
cshA
saeQ
saeR
saeS
sarH1/sarS
sarH2/sarU
sarZ
srrA
srrB
Toxin
Gene | USA300 Ref. | USA300 TI1 | USA300 TI3 | USA100 Ref. | USA100 TI2
ear
eta
hla
sec3
seg
sei
sek
sel
sem
sen
seo
sep
seq
set6
set7
set8
set9
set10
set11
set12
set13
set14
set15
tst
Other
Gene | USA300 Ref. | USA300 TI1 | USA300 TI3 | USA100 Ref. | USA100 TI2
blaz
ccra
dltA
ermA
ileS
ant(9)
epiB
epiC
geh
nuc
phoU
sceD
speG
sigB
arlR
arlS
arsB
atl
cadD
fhuB
fhuD
fmt
graR
graS
htrA
hysA
IsaA
IsdA
lip
lpl1
lpl2
lpl3
lpl4
lpl5
lpl6
lpl7
lpl8
lpl9
lytR
lytS
putP
rot
rsbU
scrA
ahpC
katA
ksgA
plc
argR
citB/acnA
citZ/gltA
frlA
hemB
hsdM
menD
mutL
mutS
rplF
sucC
sucD
tcaB
thyA

Supplemental Figure Legends

Figure S1

Percentage of mouse survival by week after injection with either NRS384 or LAC. Mice were sacrificed early (3 or 4 weeks post-injection) if lameness or hind-limb paralysis was observed.

Figure S2

Explanation of BLI signal measurements. Regions of interest (ROIs) used to measure the BLI signal from each hind limb are shown. (A) An example of a mouse with one BLI+ (left) leg and one BLI- (right) leg. BLI+ designation relies upon a discrete BLI signal focus originating from the left leg exceeding a signal of 70,000 photons/second. (B) An example of a mouse with one BLI+ (left) leg and one BLI- (right) leg. The right leg of this mouse is designated as BLI- even though it has a BLI signal of 1.6054×10^5 because there is no clear focus of signal originating from the leg and high BLI signal originating from the bladder at the edge of the leg ROI.

Figure S3

Effects of HOM on different bone compartments. (A) Representative microCT reconstructions of cortical (upper panels) and trabecular (lower panels) bone of femurs from control (Non-injected) mice or BLI- and BLI+ femurs after NRS384 injection. (B) Representative microCT reconstructions of trabecular bone of BLI- and BLI+ femurs and tibias from LAC injected mice measured at 2- or 5-weeks post injection. Scale bars 1 mm for cortical scans and 100 µm for trabecular scans.

Figure S4

In vitro CFU and BLI correlation with *lux* operon in clinical isolates. (A) BLI image of serially diluted TI1, TI2, and TI3. Bacteria were grown overnight in TSB at 1:200 shaking at 220 rpm overnight and subcultured 1:100 for 2 hours before dilutions. (B) Correlation of the BLI signal from 10^{-4}, 10^{-5}, and 10^{-6} dilutions (A and B) from each isolate and the CFU count for these dilutions. CFUs enumerated from sampling of each dilution grown overnight on TSA. Blue triangle, TI1; brown squares, TI2; black dots, TI3.

Figure S5

Bacterial growth assay. (A) The OD_{600} of each *lux* strain and isolate was measured every 30 minutes for 5 hours to determine growth curves. *p=0.0011, TI2 and TI5 compared to all other strains by repeated measures two-way ANOVA with Geisser-Greenhouse correction and Tukey's post-hoc test, n=2 repeats. (B) The OD_{600} of each parental strain or isolate was measured every 30 minutes for 5 hours and compared to its respective *lux* transformed strain or isolate by repeated measures ANOVA, n=2 repeats.

Figure S6

Characterization of additional clinical isolates of *S. aureus*. (A) *In vivo* bioluminescent imaging (BLI) of three representative mice for each isolate at 5 weeks post-injection. (B) The quantification of BLI signal measured in the hind limb of each injected mouse over the 5 weeks post-injection (n=20/strain; BLI Threshold 70,000 photons/second). (C) Percentage of mouse survival after injection with each clinical isolate. TI2, TI4, and TI5 injected mice all had 100% survival.

Figure S7

Unusual histological formations with HOM infections. (A) Abscess formed along the periosteum of the distal tibia with robust periosteal bone formation encompassing the abscess from a TI3-injected mouse. (B-D) Inflammation with reactive bone formation on the periosteal surface of two tibias of LAC-injected mice (D is higher magnification of black boxed area in C). (E,F) Fibrosis and new bone formation directly under the growth plate of a tibia from a TI2 injected mouse.

Figure S8

Osteoclast intracellular proliferation assay. (A) Confirmation of equivalent input CFU for each strain from the bacteria-containing media used to infect osteoclast cultures. (B) Intracellular CFU measured from lysed osteoclasts 1.5 or 18 hours post infection. Each dot represents a biological replicate (cells cultured from a different mouse on a different day). There is no difference in phagocytosis of bacteria (1.5 hour timepoint) in any strain. ****$p<0.0001$, 1.5 hour vs 18 hour timepoints, and #$p\leq0.0003$, 18 hour TI2 vs all other groups, by Two-way ANOVA with Tukey's post-hoc test.

Figure S9

Clinical isolate polymorphisms by gene. 173 virulence associated genes (modified from (31)) and their presence, absence, and sequence relative to the reference strain. USA300_FPR3757 is the USA300 reference genome, and N315 is the USA100 reference genome. Grey = present in reference genome, White = absent in reference genome, Blue = present and identical sequence to reference genome, Red = present with at least one SNP, and Red/White stripped = absent in isolate but present in reference genome. * denotes a truncated gene. Genes are organized by function, which is listed above each table.