Supplementary figure legends

Figure S1. Tumor targeting ability of ppGpp-defective *S. typhimurium*. C57BL/6 or BALB/c athymic nu^-/nu^- mice (n = 5 per group) were injected subcutaneously with each tumor cell line. After tumors reached 100 mm^3, the mice were intravenously injected with bioluminescent ΔppGpp. (A) Non-invasive *in vivo* imaging of bacterial bioluminescence. (B) Signal intensity was assessed by measuring the total flux from the region of interest, which was selected manually for each group at Day 4 post-injection. In the box and whisker plot, the lines at the top and bottom of the boxes represent the upper and lower quartiles, respectively. The line within the box represents the median value. The whiskers mark the 10^{th} and 90^{th} percentiles. **P* = 0.001.

Figure S2. Adhesion of bacteria to several different tumor types. $\Delta ppGpp^{RGD}$ was induced to express $OmpA^{RGD}$ by addition of $_L$-arabinose. ΔppGpp and $\Delta ppGpp^{AAA}$ (instead of RGD) were also induced to express $OmpA^{AAA}$ and used as controls. Infection of tumor cells with ΔppGpp, $\Delta ppGpp^{AAA}$, or $\Delta ppGpp^{RGD}$ was carried out at a MOI of 100:1. For the competition assay, cells were pre-incubated for 2 h with 1 μM synthetic RGD peptide (ACDCRGDCFCG) and then infected with $\Delta ppGpp^{RGD}$ (MOI, 1:100). For visualization by confocal microscopy, cell nuclei (blue), the cytoskeleton (red), and *Salmonella* (green) were stained with DAPI and antibodies against F-actin and *Salmonella*, respectively. Incubation of tumor cells with (A) low level and (B) high level expression of αvβ3 integrin with bacteria. Scale bar in (A) = 40 μm; scale bar in (B) = 20 μm. Results are representative of at least three independent experiments.

Figure S3. The binding of $\Delta ppGpp^{RGD}$ to tumor cells. $\Delta ppGpp^{RGD}$ induced to express $OmpA^{RGD}$ by addition of $_L$-arabinose. Infection of MDA-MB-231 with $\Delta ppGpp^{RGD}$ was carried out for 2 h at a MOI of 1:100. For confocal microscopy, cell nuclei (blue), the cytoskeleton (red), and *Salmonella* (green) were stained with DAPI and antibodies specific for F-actin and *Salmonella*, respectively. Representative confocal Z-slices are shown. Three-

dimensional reconstruction sections are shown below (X-Z section) and to the right (Y-Z section) of the merged panel. Images show adhesive bacteria (green) in the cytoplasm surrounded by the cytoskeleton (red). Results are representative of at least three independent experiments. Scale bar = 10 μm.

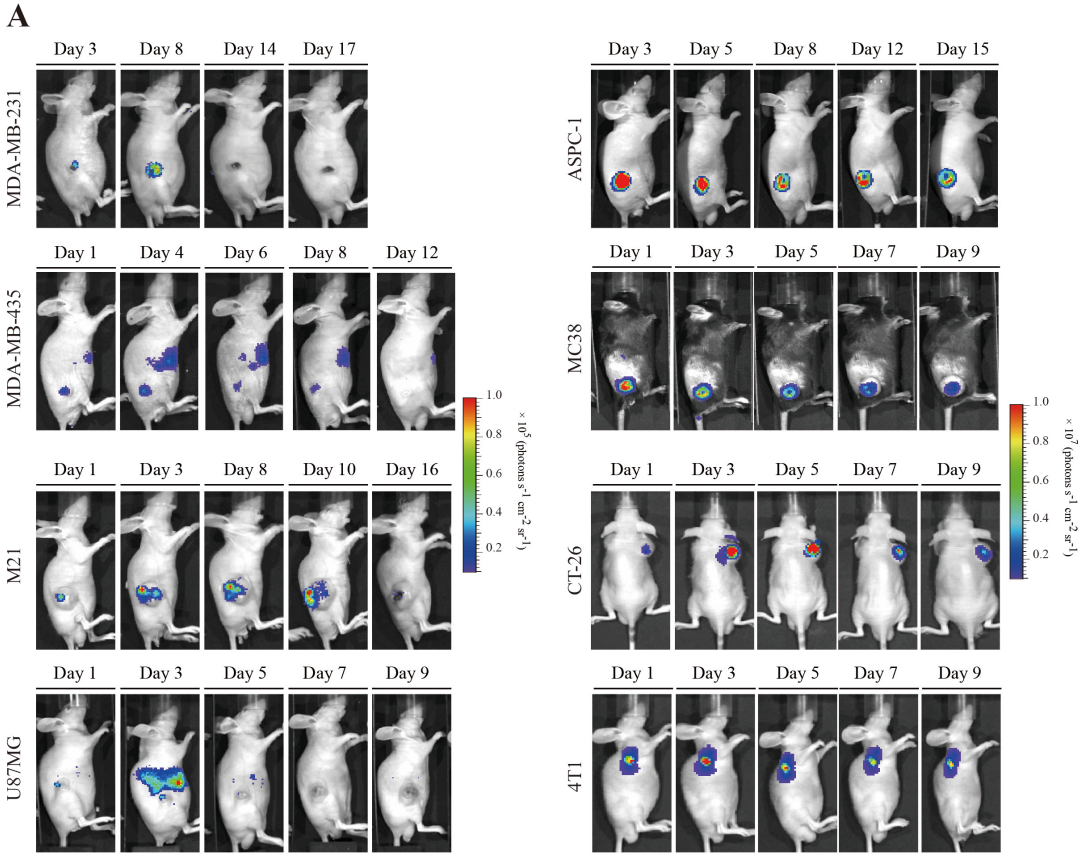
Figure S4. The invasion of bacteria to tumor cells. $\Delta ppGpp^{RGD}$ was induced to express $OmpA^{RGD}$ by addition of $_L$-arabinose. ΔppGpp and $\Delta ppGpp^{AAA}$ (instead of RGD) were also induced to express $OmpA^{AAA}$ and used as controls. The actual number of intracellular gentamycin-resistant bacteria after the incubation of human cancer cells (MCF7, M21L, U87MG, M21, MDA-MB-231, and MDA-MB-435). *Salmonella* WT used as positive controls. The results are representative of at least three independent experiments.

Figure S5. *In vivo* imaging of RGD-displaying *S. typhimurium* in the M21 or M21L xenograft models. BALB/c athymic nu^-/nu^- mice (n = 7 per group) were injected subcutaneously with M21 or M21L (1×10^7) cells. When the tumors reached approximately 100 mm^3, mice were intravenously injected with bioluminescent bacteria (ΔppGpp, $\Delta ppGpp^{AAA}$, or $\Delta ppGpp^{RGD}$). (A) Non-invasive *in vivo* imaging of bacterial bioluminescence in representative mice. (B) Signal intensity in tumor regions of interest was assessed by measuring the toral flux. Regions of interest were selected manually within each tumor and results are shown as a bar graph after bacterial injection. Left panel, M21; Right panel, M21L. (C) The bacterial viable counting of tumor in M21 or M21L mouse models after bacteria injection.

Figure S6. Systemic toxicity of RGD-displaying Salmonellae. BALB/c athymic nu^-/nu^- mice (n = 5 per group) were injected subcutaneously with MDA-MB-231 cells. After tumors reached 100 mm^3, the mice were intravenously injected with PBS, ΔppGpp, or $\Delta ppGpp^{RGD}$, followed by intraperitoneal administration of $_L$-arabinose at 0 day post-injection (dpi) or 3 dpi. The level of serum aspartate aminotransferase, alanine aminotransferase, blood urea

nitrogen, creatinine, plasma C-reactive protein, and procalcitonin was measured at 5 dpi. In the box and whisker plot, the lines at the top and bottom of the boxes represent the upper and lower quartiles, respectively. The line within the box represents the median value. The whiskers mark the 10^{th} and 90^{th} percentiles. *$P < 0.01$. Normal values: ALT, 17–77 IU/L; AST, 54–298 IU/L; blood urea nitrogen, 8–33 mg/dL; creatinine, 0.2–0.9 mg/dL; CRP, < 0.5 mg/dL; and procalcitonin, < 0.5 ng/mL. Yellow-shaded areas = normal range for the measured parameter.

Figure S1


A
MDA-MB-231
Day 3
Day 8
Day 14
Day 17
MDA-MB-435
Day 1
Day 4
Day 6
Day 8
Day 12
M21
Day 1
Day 3
Day 8
Day 10
Day 16
U87MG
Day 1
Day 3
Day 5
Day 7
Day 9
ASPC-1
Day 3
Day 5
Day 8
Day 12
Day 15
MC38
Day 1
Day 3
Day 5
Day 7
Day 9
CT-26
Day 1
Day 3
Day 5
Day 7
Day 9
4T1
Day 1
Day 3
Day 5
Day 7
Day 9
1.0
0.8
0.6
0.4
0.2
× 10^5 (photons s^-1 cm^-2 sr^-1)
× 10^7 (photons s^-1 cm^-2 sr^-1)

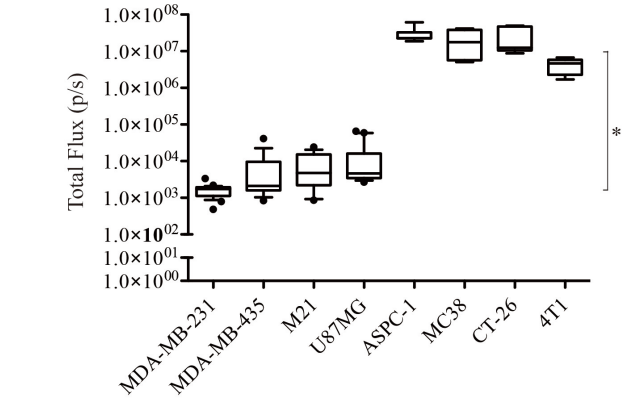

B
Total Flux (p/s)
1.0×10^08
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
1.0×10^01
1.0×10^00
*
MDA-MB-231
MDA-MB-435
M21
U87MG
ASPC-1
MC38
CT-26
4T1

Figure S2

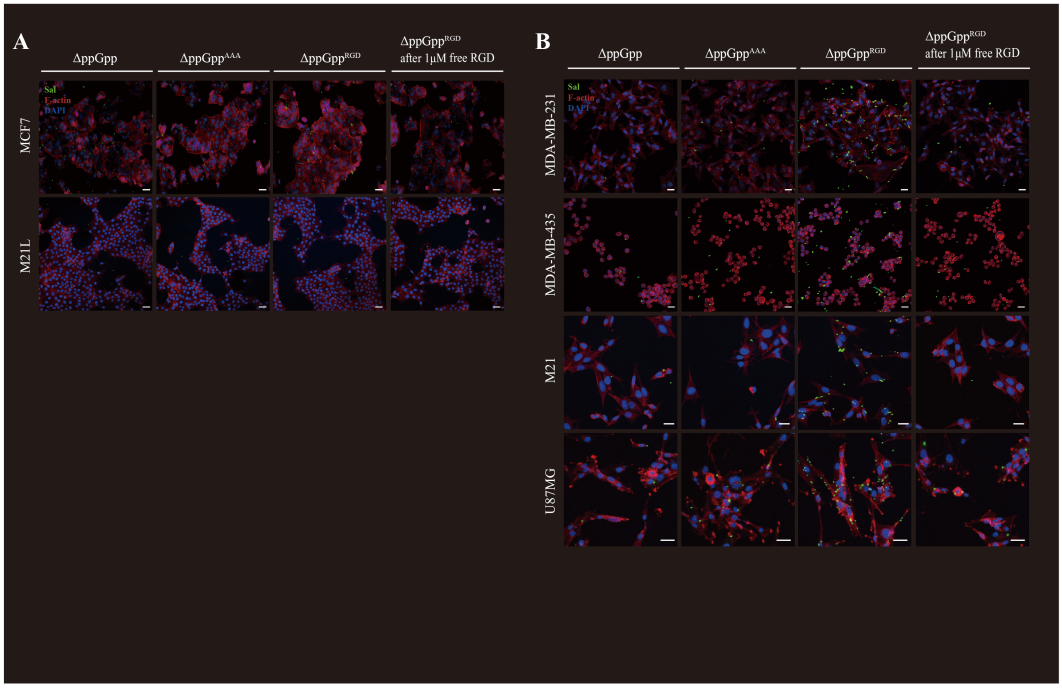

A
ΔppGpp
ΔppGpp^AAA
ΔppGpp^RGD
ΔppGpp^RGD after 1μM free RGD
Sal
F-actin
DAPI
MCF7
M21L
B
ΔppGpp
ΔppGpp^AAA
ΔppGpp^RGD
ΔppGpp^RGD after 1μM free RGD
Sal
F-actin
DAPI
MDA-MB-231
MDA-MB-435
M21
U87MG

Figure S3

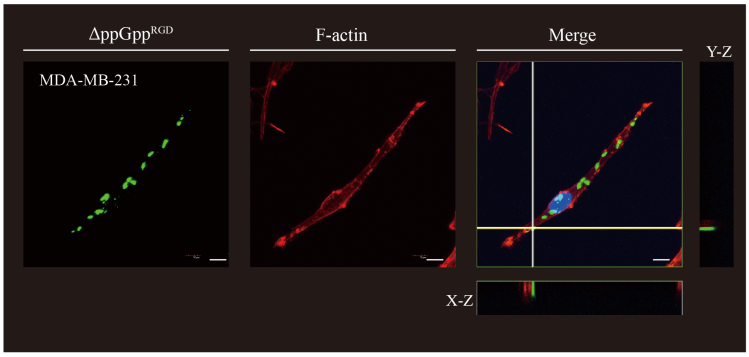


Figure S4

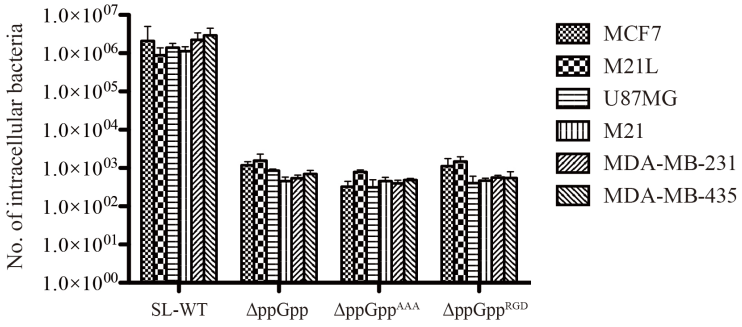

No. of intracellular bacteria
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
1.0×10^01
1.0×10^00
SL-WT
ΔppGpp
ΔppGpp^AAA
ΔppGpp^RGD
MCF7
M21L
U87MG
M21
MDA-MB-231
MDA-MB-435

Figure S5

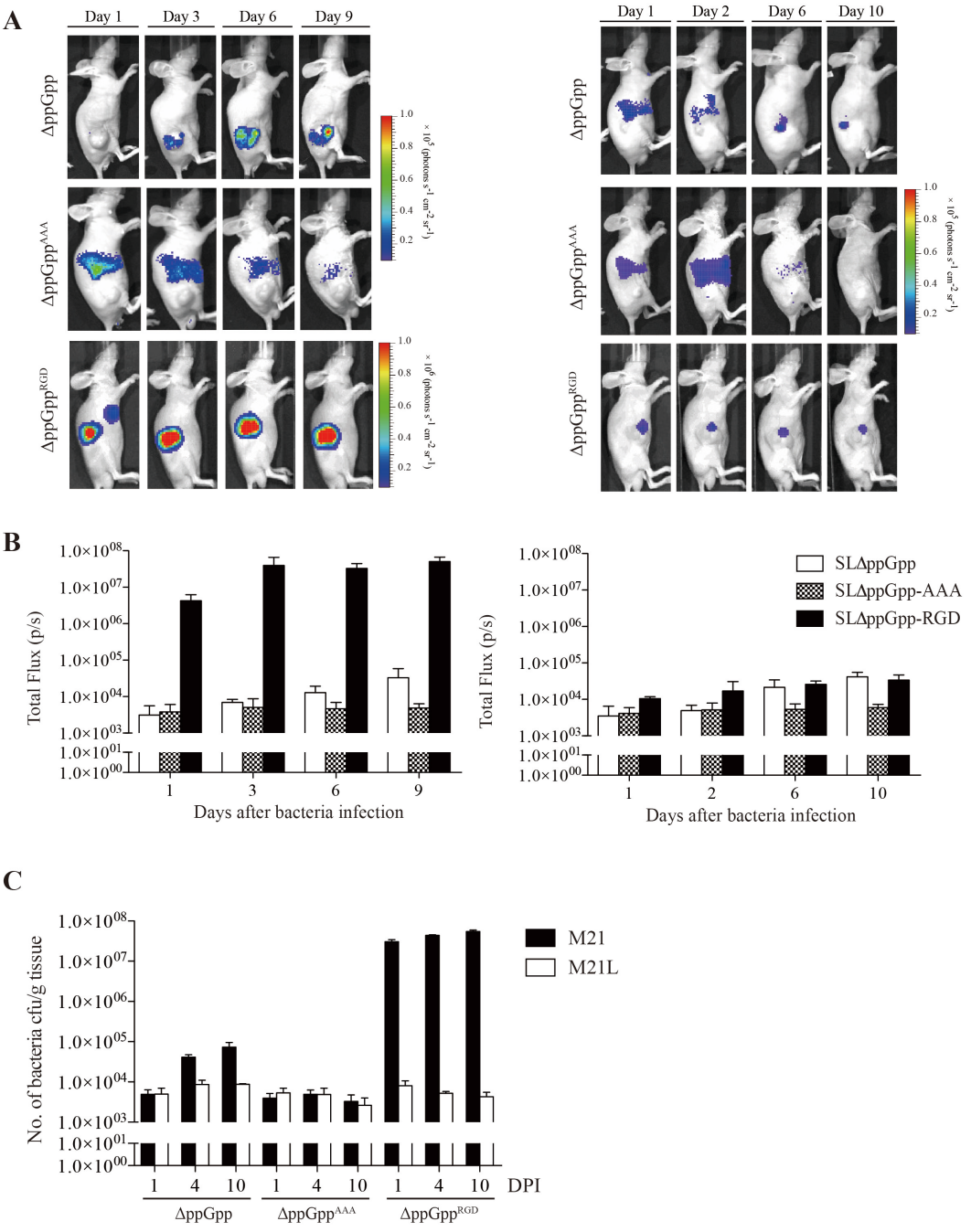
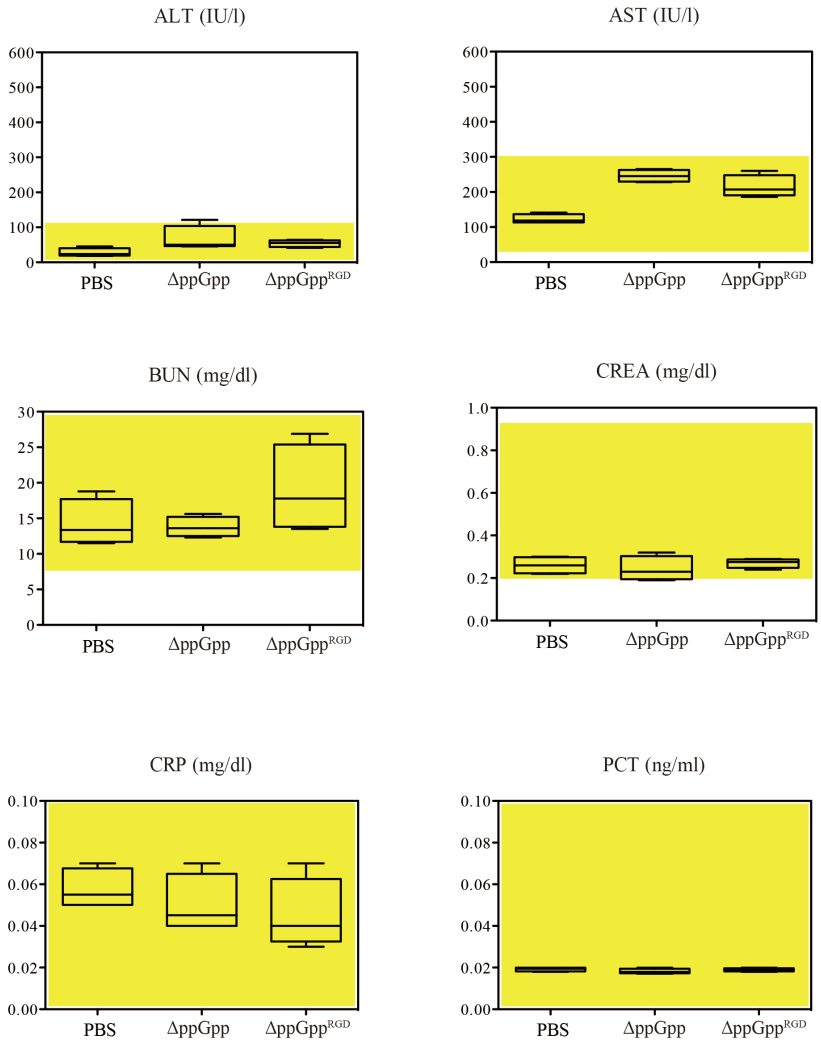

A
Day 1
Day 3
Day 6
Day 9
ΔppGpp
ΔppGpp^AAA
ΔppGpp^RGD
Day 1
Day 2
Day 6
Day 10
B
Total Flux (p/s)
Days after bacteria infection
SLΔppGpp
SLΔppGpp-AAA
SLΔppGpp-RGD
C
No. of bacteria cfu/g tissue
M21
M21L
DPI

Figure S6


ALT (IU/l)
600
500
400
300
200
100
0
PBS
ΔppGpp
ΔppGpp^RGD
AST (IU/l)
600
500
400
300
200
100
0
PBS
ΔppGpp
ΔppGpp^RGD
BUN (mg/dl)
30
25
20
15
10
5
0
PBS
ΔppGpp
ΔppGpp^RGD
CREA (mg/dl)
1.0
0.8
0.6
0.4
0.2
0.0
PBS
ΔppGpp
ΔppGpp^RGD
CRP (mg/dl)
0.10
0.08
0.06
0.04
0.02
0.00
PBS
ΔppGpp
ΔppGpp^RGD
PCT (ng/ml)
0.10
0.08
0.06
0.04
0.02
0.00
PBS
ΔppGpp
ΔppGpp^RGD

Graphical Abstract