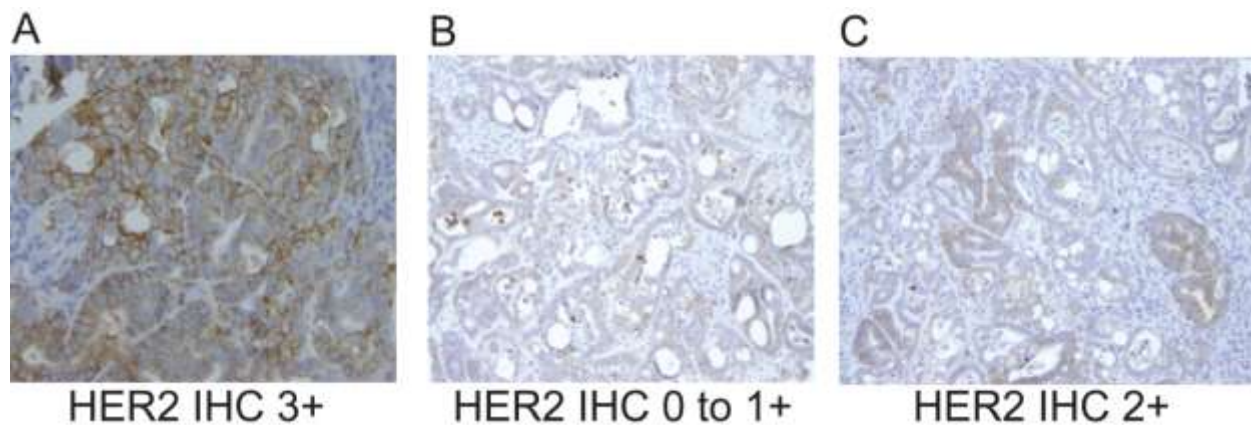


Gastric NCI-N87 HER2/Cep17

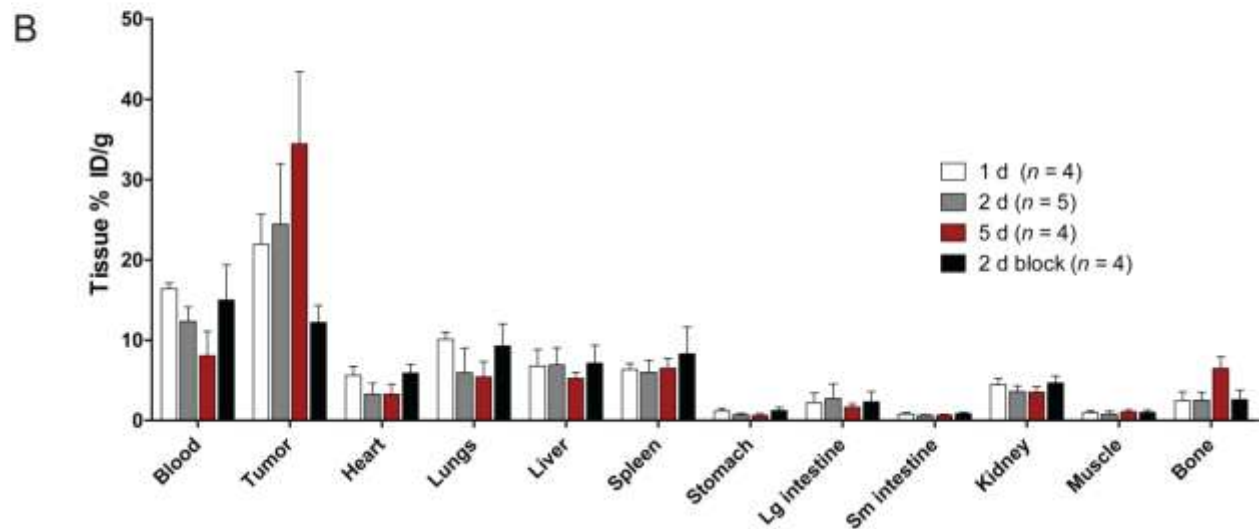
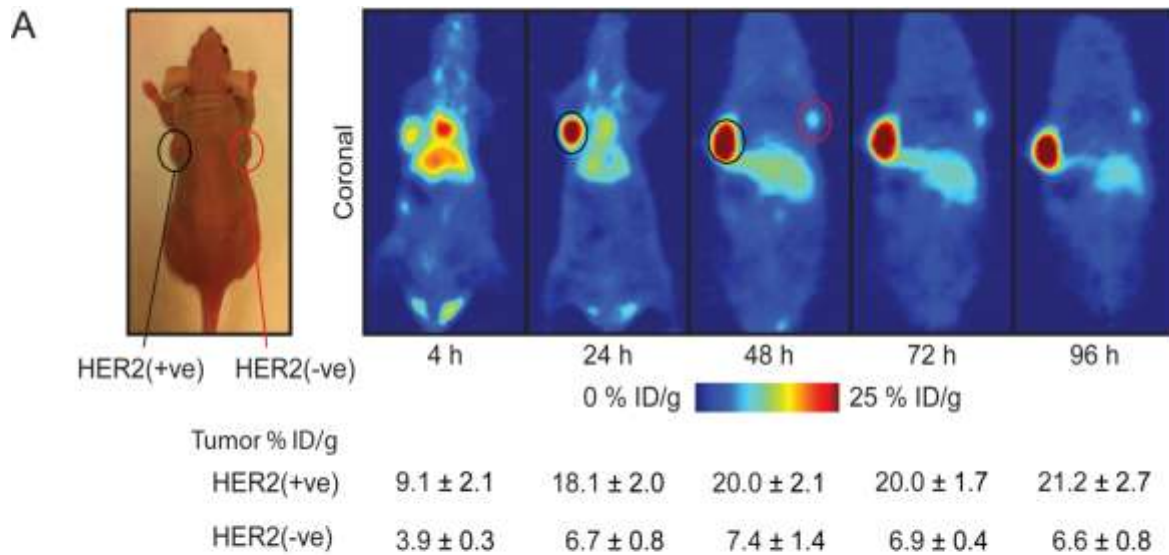
HER2 = red, Cep17 = green

Supplemental Figure 1. NCI-N87 gastric cancer cell line is HER2 amplified.

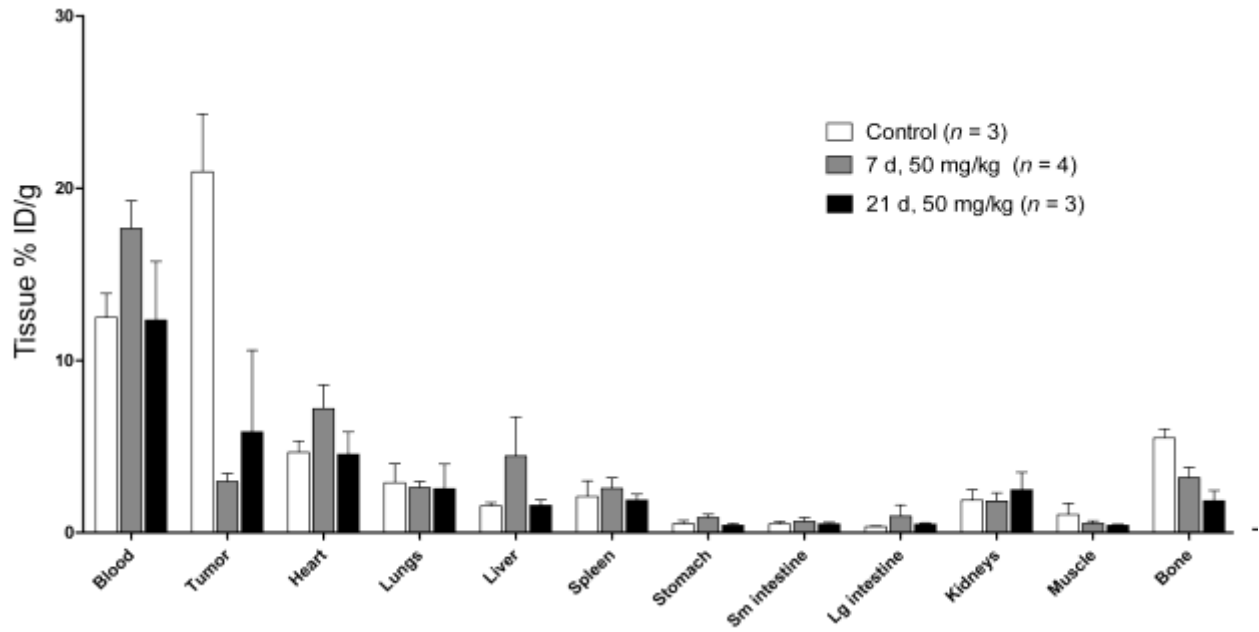
Fluorescence in-situ hybridization (FISH) analysis of NCI-N87 cell line confirms HER2 amplification, HER2/Cep17 >20. HER2=red Cep 17=green.



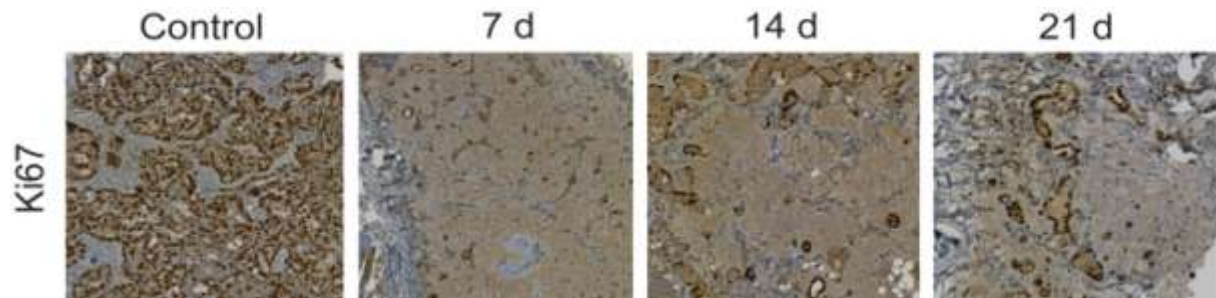
Supplemental Figure 2. Single-dose afatinib inhibits downregulation of total HER2 in HER2-positive NCI-N87 xenografts. Immunohistochemistry (IHC) analysis at baseline (panel A, untreated), 24h (panel B) and 48h (panel C) after a single dose of afatinib 25 mg/kg p.o.



Supplemental Figure 3. ^{89}Zr -trastuzumab shows uptake in NCI-N87 tumors for up to 96 hours. **A.** Coronal ^{89}Zr -trastuzumab PET images of mice bearing s.c. NCI-N87 and MK-74 tumors imaged from 4h to 96 h p.i. The regions-of-interest (%ID/g) for ^{89}Zr -trastuzumab indicated. **B.** Tissue biodistribution of ^{89}Zr -trastuzumab in NCI-N87 xenografts untreated.



Supplemental Figure 4. Tissue biodistribution of ^{89}Zr -trastuzumab in NCI-N87 xenografts treated with afatinib. Tissue biodistribution of ^{89}Zr -trastuzumab in NCI-N87 xenografts at baseline, 7 and 21 days post afatinib 50mg/kg daily treatment.



Supplemental Figure 5. Ki67 staining of tumors harvested from NCI-N87 xenografts after afatinib therapy. IHC staining of Ki67 in tumors treated with vehicle-only (control) or for 7, 14 and 21 days with afatinib. Positive staining (brown) for Ki67 is seen in the control tumors, which is abolished after 7 days of treatment, and is seen to slowly recover after 21 days. Bar = 100mm.

Supplemental Table 1. Full biodistribution of mice bearing NCI-N87 tumors*

Tissue	Control n=3	7 days n=4	21 days n=3
Tumor	21.0 ± 3.4	3.0 ± 0.5	5.9 ± 4.7
Blood	12.5 ± 1.4	17.7 ± 1.6	12.3 ± 3.4
Heart	4.7 ± 0.7	7.2 ± 1.4	4.5 ± 1.3
Lungs	2.9 ± 1.1	2.6 ± 0.4	2.5 ± 1.4
Liver	1.6 ± 0.2	4.4 ± 2.3	1.6 ± 0.3
Spleen	2.1 ± 0.9	2.6 ± 0.6	1.9 ± 0.3
Stomach	0.5 ± 0.2	0.9 ± 0.2	0.4 ± 0.1
Small intestines	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
Large intestines	0.3 ± 0.04	1.0 ± 0.6	0.5 ± 0.1
Kidneys	1.9 ± 0.6	1.8 ± 0.5	2.5 ± 1.0
Bone	5.5 ± 0.5	3.2 ± 0.6	1.8 ± 0.6
Muscle	1.0 ± 0.7	0.5 ± 0.1	0.4 ± 0.1

*Different cohorts of mice were treated with afatinib (50 mg/kg) p.o. daily for 1-3 weeks. A separate group of mice was dosed with the drug vehicle (0.9% saline).