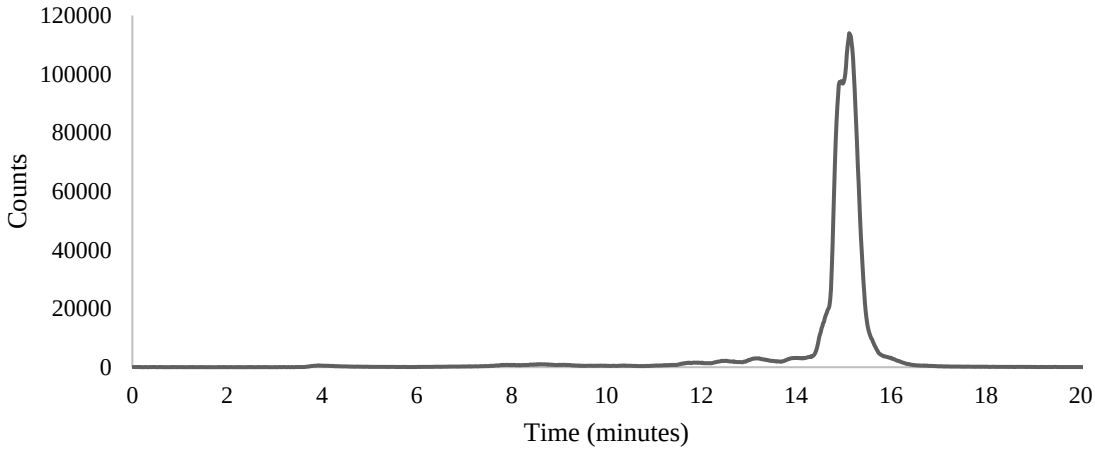
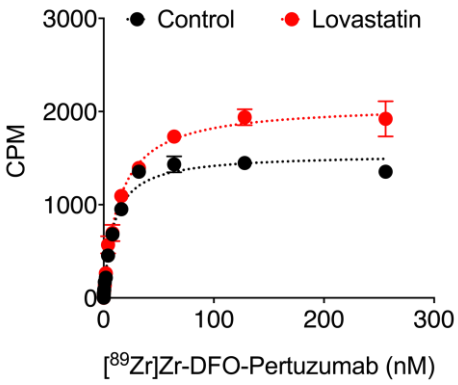
**Supplemental Data for Pereira et al: Temporal modulation of HER2 endocytosis increases pertuzumab tumor uptake and allows for pretargeted molecular imaging**

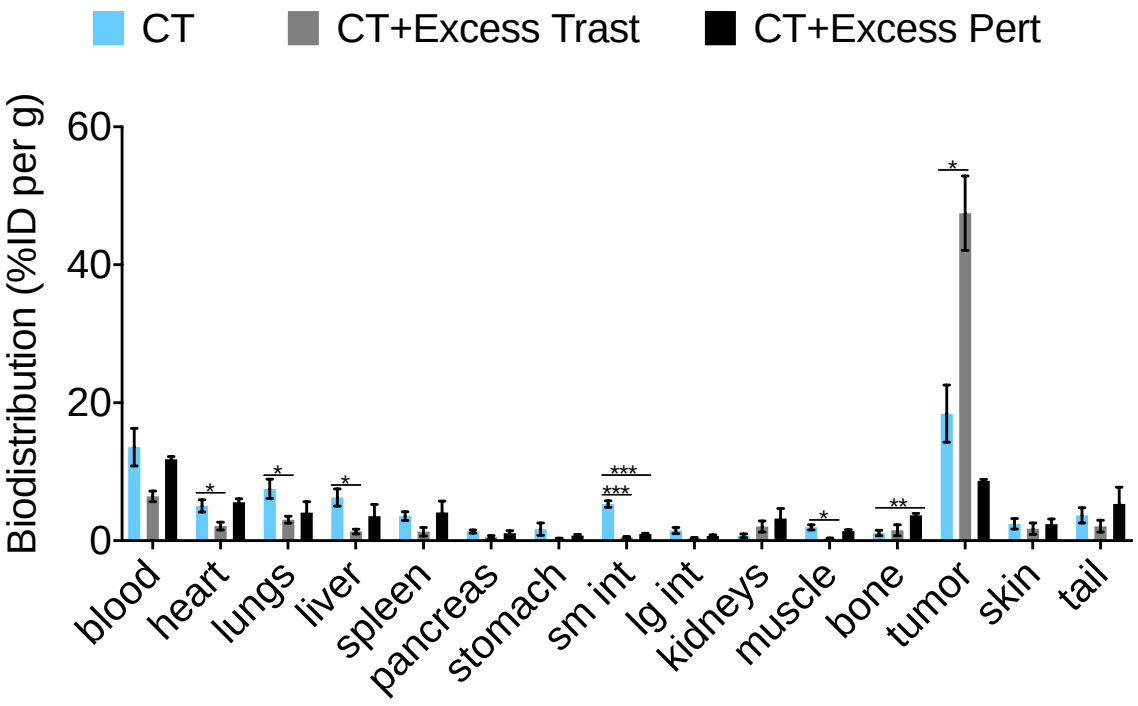



**Supplemental Figure 1.** Representative quality control (QC) radio-HPLC trace for $^{18}$F-labeled Tz, which was obtained with high purity (>98%) and high molar activity (55.5 GBq/µmol).

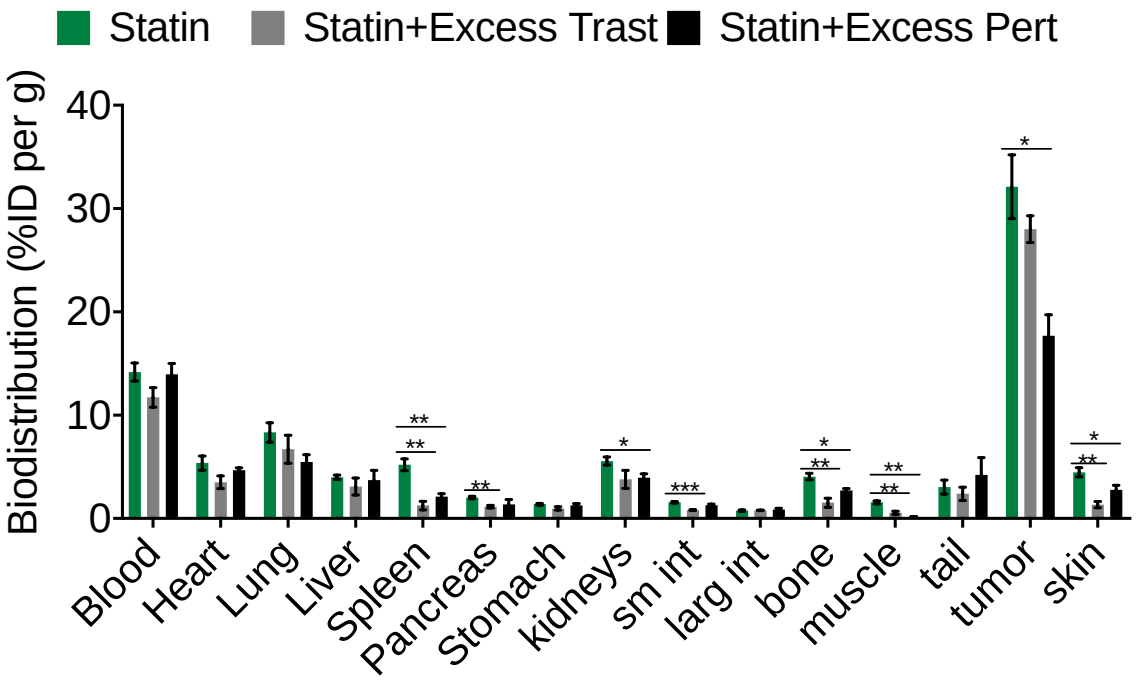



| Condition | Bmax (HERs/cell x $10^5$) |
|---|---|
| Control | 3.9 |
| Lovastatin | 5.4* |

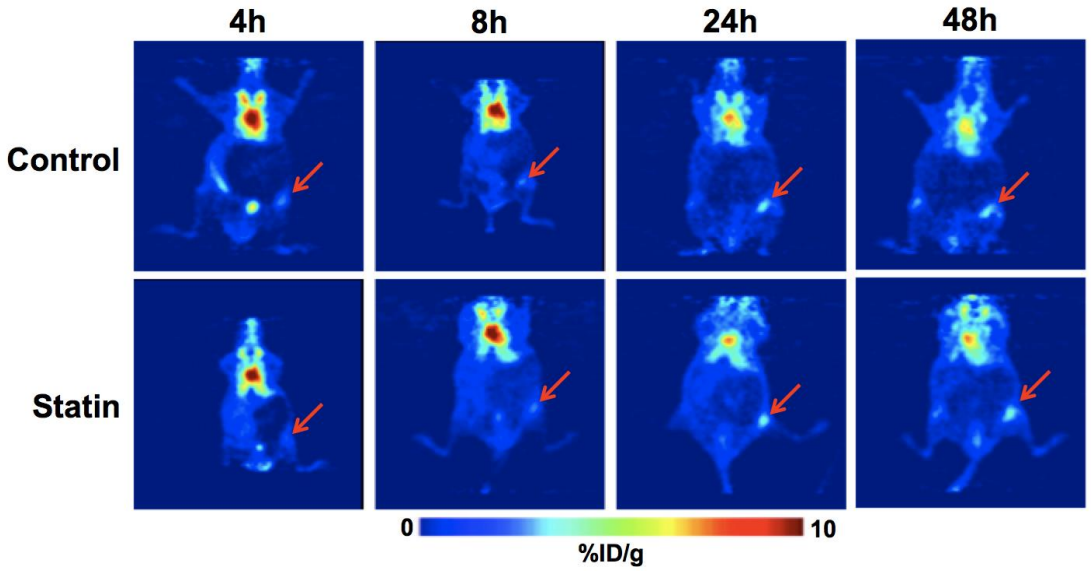
**Supplemental Figure 2.** Binding kinetics of $^{89}$Zr-labeled pertuzumab in control and lovastatin-treated NCI-N87 cells. NCI-N87 control and lovastatin-treated cells were incubated with $^{89}$Zr-labeled pertuzumab (0 to 256 nM) for 2 h at 4 °C (upper panel). Specific binding of $^{89}$Zr-labeled pertuzumab (red or black circles) and non-linear regression curve fit (dotted lines). Data are presented as mean ± S.E.M, n = 3. Binding parameters (lower panel) of $^{89}$Zr-labeled pertuzumab to NCI-N87 cells control or treated with lovastatin (*$P$ < 0.05 based on a Student's t-test).



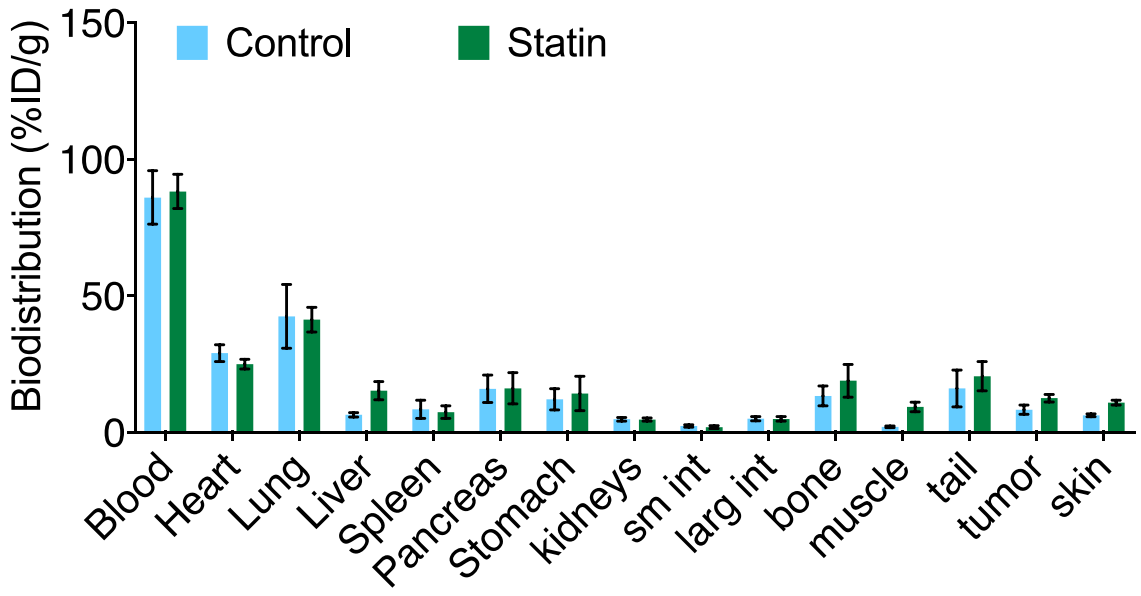

**Supplemental Figure 3.** Biodistribution in control (CT) athymic nude mice bearing subcutaneous NCI-N87 gastric tumors with and without blocking with unlabeled pertuzumab or trastuzumab. [$^{89}$Zr]Zr-DFO-pertuzumab (4.44-5.18 MBq, 42-49 μg protein) was administered by tail vein injection. Blocking experiments were performed by administration of $^{89}$Zr-labeled pertuzumab in the presence of a 40-fold molar excess of pertuzumab or trastuzumab. Biodistribution studies were performed at 48 h post-injection of $^{89}$Zr-labeled pertuzumab. Data represent the mean ± SEM ($n$ = 5 mice per group, * $P$ < 0.05, **$P$ < 0.01, and **$P$ < 0.01 based on a Student's $t$ test). Tumor-to-organ ratios radioactivity are shown in Supplementary Table 2.



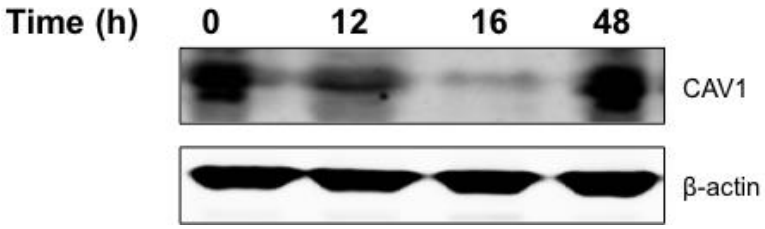

**Supplemental Figure 4.** Biodistribution in lovastatin-treated athymic nude mice bearing subcutaneous NCI-N87 gastric tumors with and without blocking with unlabeled trastuzumab or pertuzumab. Lovastatin (8.3 mg/kg) was orally administered 12 h prior to and at the same time as the tail vein injection of [$^{89}$Zr]Zr-DFO-pertuzumab (4.44-5.18 MBq, 42-49 μg protein). Blocking experiments were performed by administration of $^{89}$Zr-labeled pertuzumab in the presence of a 40-fold molar excess of trastuzumab or pertuzumab. Biodistribution studies were performed at 48 h post-injection of $^{89}$Zr-labeled pertuzumab. Data represent the mean ± SEM, ($n$ = 5 mice per group, *$P$ < 0.05, **$P$ < 0.01, and ***$P$ < 0.001 based on a Student's $t$ test). Tumor-to-organ ratios radioactivity are shown in Supplemental Table 3.



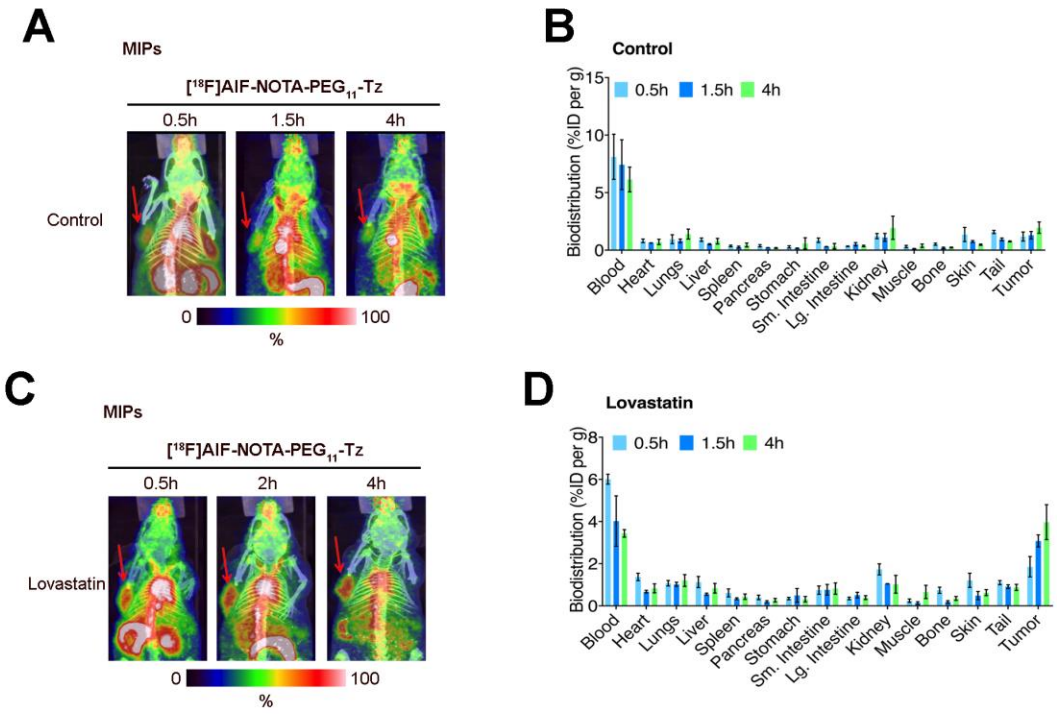

**Supplemental Figure 5.** Representative coronal PET images of [$^{89}$Zr]Zr-DFO-pertuzumab in athymic nude mice bearing orthotopic MDA-MB-231 mammary fat pad tumors treated with lovastatin. Lovastatin (8.3 mg/kg of mice) was orally administered 12 h prior and at the same time as the tail vein injection of [$^{89}$Zr]Zr-DFO-pertuzumab (4.44-5.18 MBq, 42-49 μg protein). Control mice received oral saline instead of lovastatin.




**Supplemental Figure 6.** Biodistribution of $^{89}$Zr-labeled pertuzumab in lovastatin-treated athymic nude mice bearing orthotopic MDA-MB-231 mammary fat pad tumors. Lovastatin (8.3 mg/kg) was orally administered 12 h prior to and at the same time as the tail vein injection of [$^{89}$Zr]Zr-DFO-pertuzumab (4.44-5.18 MBq, 42-49 μg protein). Biodistribution studies were performed at 48 h post-injection of $^{89}$Zr-labeled pertuzumab. Data represent the mean ± SEM, (*n* = 3 mice per group).




**Supplemental Figure 7.** Western blot of CAV1 in the total cell lysate of NCI-N87 subcutaneous tumors from athymic nude mice. Lovastatin (8.3 mg/kg) was orally administered twice, with an interval of 12 h between each administration. Tumor lysates were prepared at 0, 12, 16, and 48 h after lovastatin administration and analyzed by Western blot.




**Supplemental Figure 8. (A, C)** Representative maximum intensity projection PET images (MIPs) and (**B, D)** biodistribution data at 0.5, 1.5, and 4 h post-injection of [$^{18}$F]AlF-NOTA-PEG$_{11}$-Tz in athymic nude mice bearing subcutaneous gastric tumors. Lovastatin (8.3 mg/kg) was orally administered 12 h prior to and at the same time as the tail vein injection of trastuzumab-TCO, and then at the same time as the tail vein injection of [$^{18}$F]AlF-NOTA-PEG$_{11}$-Tz. Mice were given trastuzumab-TCO (0.42 nmol) 24 h prior to injection of the $^{18}$F-labeled tracer (10.36-11.1 MBq, 0.83 nmol) via the tail vein. Control (CT) mice received with PBS instead of lovastatin. Biodistribution data represent the mean ± SEM for five mice.

**Supplemental Table 1.** NCIN87 tumor-to-organ ratios at 48 h after injection of $^{89}$Zr-pertuzumab in control and lovastatin-administrated mice.

| | Control | Lovastatin |
|---|---|---|
| Blood | 1.36 | 2.27 |
| Heart | 3.64 | 5.98 |
| Lung | 2.45 | 3.85 |
| Liver | 2.94 | 8.05 |
| Spleen | 5.16 | 6.17 |
| Pancreas | 13.75 | 15.84 |
| Stomach | 10.81 | 23.25 |
| Kidneys | 3.46 | 5.77 |
| Small intestine | 12.41 | 20.48 |
| Large intestine | 25.39 | 41.55 |
| Bone | 9.49 | 7.92 |
| Muscle | 16.37 | 20.48 |
| Tail | 7.50 | 10.55 |
| Tumor | 1.00 | 1.00 |
| Skin | 5.01 | 7.17 |

**Supplemental Table 2.** NCIN87 tumor-to-organ ratios at 48 h after injection of $^{89}$Zr-pertuzumab in control mice after blocking with unlabeled trastuzumab or pertuzumab.

| | Control | Control + Blocking Trastuzumab | Control + Blocking Pertuzumab |
|---|---|---|---|
| Blood | 1.36 | 7.39 | 0.73 |
| Heart | 3.64 | 22.39 | 1.55 |
| Lungs | 2.45 | 15.55 | 2.14 |
| Liver | 2.94 | 35.41 | 2.45 |
| Spleen | 5.16 | 36.05 | 2.11 |
| Pancreas | 13.75 | 90.26 | 8.06 |
| Stomach | 10.81 | 168.69 | 11.29 |
| Small Intestine | 3.46 | 91.56 | 9.05 |
| Large Intestine | 12.41 | 132.13 | 12.40 |
| Kidneys | 25.39 | 22.87 | 2.71 |
| Muscle | 9.49 | 155.00 | 6.28 |
| Bone | 16.37 | 31.23 | 2.36 |
| Tumor | 1.00 | 1.00 | 1.00 |
| Skin | 7.50 | 26.96 | 3.61 |
| Tail | 5.01 | 22.50 | 1.63 |

**Supplemental Table 3.** NCIN87 tumor-to-organ ratios at 48 h post-injection of $^{89}$Zr-pertuzumab in statin-treated mice after blocking with unlabeled trastuzumab or pertuzumab.

| | Statin | Statin + Blocking Trastuzumab | Statin + Blocking Pertuzumab |
|---|---|---|---|
| Blood | 2.27 | 2.39 | 1.27 |
| Heart | 5.98 | 7.99 | 3.79 |
| Lung | 3.85 | 4.17 | 3.23 |
| Liver | 8.05 | 9.04 | 4.76 |
| Spleen | 6.17 | 22.21 | 8.35 |
| Pancreas | 15.84 | 24.40 | 13.05 |
| Stomach | 23.25 | 28.72 | 13.89 |
| Kidneys | 5.77 | 7.39 | 4.48 |
| Small Intestine | 20.48 | 34.20 | 13.73 |
| Large Intestine | 41.55 | 35.51 | 20.68 |
| Bone | 7.92 | 18.31 | 6.52 |
| Muscle | 20.48 | 50.29 | 109.99 |
| Tail | 10.55 | 11.74 | 4.19 |
| Tumor | 1.00 | 1.00 | 1.00 |
| Skin | 7.17 | 20.91 | 6.42 |

**Supplemental Table 4.** NCIN87 tumor-to-organ ratios at 4 h post-injection of [$^{18}$F]AlF-NOTA-PEG$_{11}$-Tz in control and statin-treated mice administrated with pertuzumab-TCO.

| | Control | Lovastatin |
|---|---|---|
| Blood | 0.13 | 0.48 |
| Heart | 0.41 | 1.32 |
| Lung | 0.31 | 0.97 |
| Liver | 0.57 | 1.74 |
| Spleen | 0.82 | 2.73 |
| Pancreas | 1.08 | 3.81 |
| Stomach | 1.33 | 4.14 |
| Kidneys | 0.28 | 0.88 |
| Small Intestine | 0.53 | 2.57 |
| Large Intestine | 4.68 | 4.97 |
| Bone | 0.70 | 2.71 |
| Muscle | 2.51 | 4.75 |
| Tail | 0.72 | 2.09 |
| Tumor | 1.00 | 1.00 |
| Skin | 1.23 | 3.87 |