Figure captions and legends

Figure 1. Schematic structure of Affitoxin.

Figure 2. Physicochemical characterization of purified Affitoxin.
A. 50 μg of purified Affitoxin was resolved in 4-12% Bis-Tris polyacrylamide gel along with pre-stained protein weight standards. **B**. MALDI-TOF MS of purified Affitoxin.

Figure 3. Kinetic analysis of $Z_{HER2:342}$ Affibody molecule (A) or Affitoxin (B) binding to HER2/Fc-covered surface.
Binding of 0.32, 0.63, 1.25, 2.5, 5 nM wild type $Z_{HER2:342}$ Affibody molecule (**A**) and 6.17, 18.5, 55.5, 166.6, 500 nM of Affitoxin (**B**) to HER2/Fc on the sensor chip was tested using SPR-based binding assay. The red lines represent a global analysis of data using a Langmuir binding model. The Affibody molecule-HER2/Fc complex was allowed to dissociate for 2000 seconds in order to observe a measurable decayKinetic analysis of $Z_{HER2:342}$ Affibody molecule (A) or Affitoxin (B) binding to HER2/Fc-covered surface. Kinetic data are summarized in **C.**

Figure 4. Specificity of Affitoxin binding confirmed by confocal imaging and flow cytometry.
A. Cells were incubated with 0.1 μM of Alexa Fluor® 488-labeled Affitoxin for 6 hours with and without Affibody molecules. B. HER2 expression level in five different breast cancer cell lines was determined by ELISA and is expressed in nanograms of HER2 per milligram of protein lysate. Cells were incubated with 5 μg/ml of Alexa Fluor® 488-labeled Affitoxin. Binding efficacy was determined by flow cytometry. C. BT474 cells were incubated with 5 μg/ml of Alexa Fluor® 488-labeled Affitoxin and increasing concentrations of Affibody. Binding efficacy was determined by flow cytometry and quantified by GraphPad software.

Figure 5. Efficacy of Affitoxin measured by inhibition of protein synthesis (A) and intracellular ATP level (B).
Five different breast cancer cells lines were transfected with pCMV-GLuc plasmid containing secreted *Gaussia* luciferase gene under control of strong CMV promoter. *Gaussia* luciferase activity was measured in the medium 24 hours after treatment with Affitoxin. The same cell lines were assessed for intracellular ATP level 72 hours following treatment (CellTiter-Glo, Promega).

Figure 6. Effect of exposure time on Affitoxin efficacy.
BT474 cells were treated with 43.5 pM Affitoxin for indicated time periods. After that medium containing Affitoxin was changed and incubation was continued up to 72 hours and cell viability was measured using CellTiter-Glo.

Figure 7. Affitoxin efficacy in the presence of Affibody excess
BT474 cells were treated with 2pM Affioxin in presence of 100nM Affibody and 100nM BSA. Cells were incubated for 72 hours and cell viability was measured using CellTiter-Glo.

Supplementary Figure 1. HER2 Affitoxin sequence.

Supplementary Figure 2. Affitoxin binding to cells expressing different number of HER2 receptors.
Five different breast cancer cell lines were incubated with 5 μg/ml of Alexa Fluor® 488-labeled Affitoxin at 37°C followed by flow cytometry analysis. Red curves show background of non-stained cells, green fluorescence of cells probed with Affitoxin

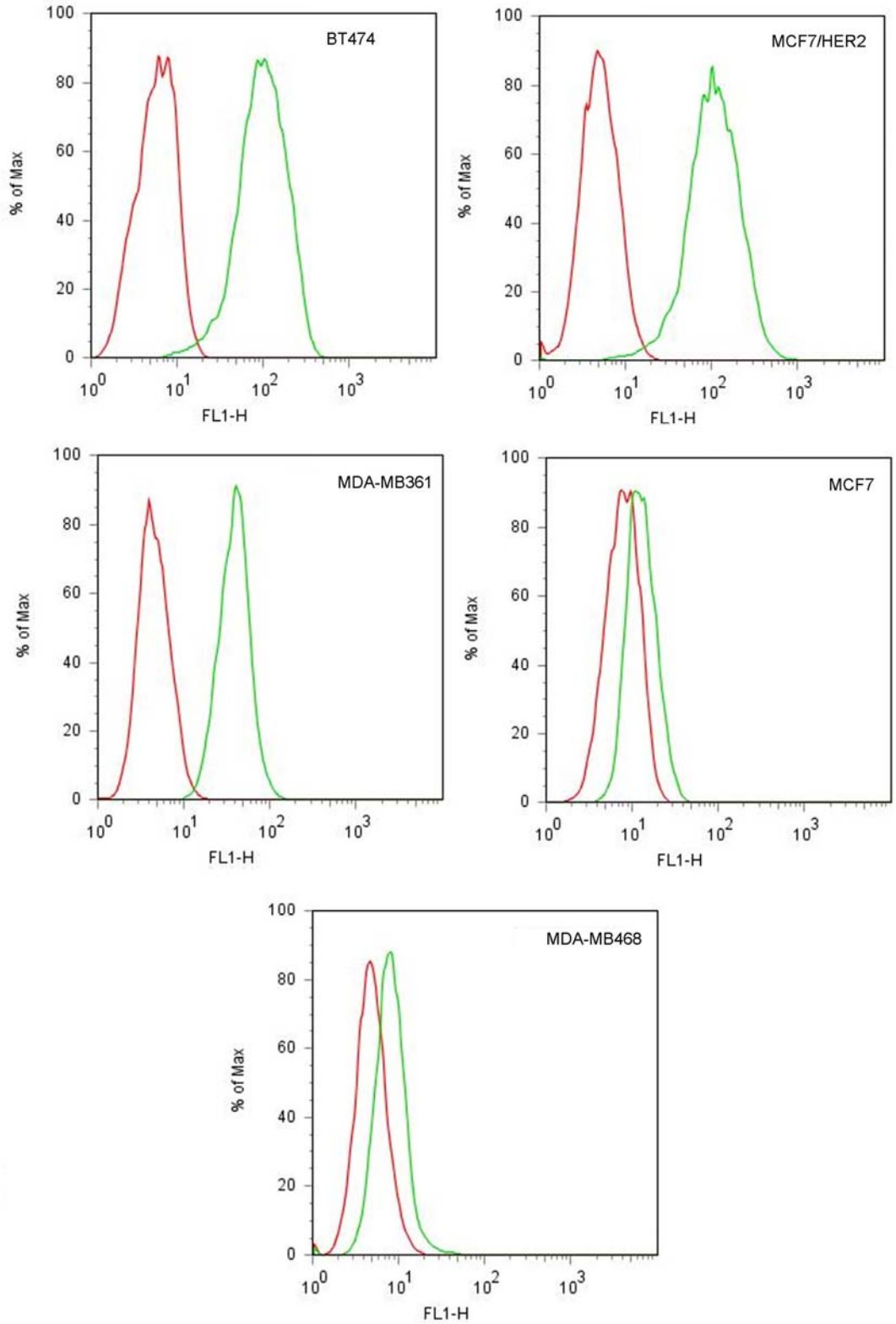
Zielinski et al. Supplementary Table 1. List of primers used for Affitoxin cloning.

Primer name	Sequence
P1	CACCATGGTAGACGCTTCCGGAGGTCCCGAGGGCGGC
P2	GCTCAGCGCGGCCGCTTACTTCAGGTCCTCGCGCGGCGG
P3	CACCATGGGCCATCACCACCATCACCATCTGCAGGTAGATAACAAATTCAACAAAGAAATG
P4	TCCACCTGATCCTCCACCTCCGTCTACTTTCGGCGCCTGAGC
P5	GGAGGTGGAGGATCAGGTGGACCCGAGGGCGGCAG
P6	TTACAGTTCGTCTTTCGGCGGTTTGCCGG

MGSSHHHHHHLQVDNKFNKEMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQS
ANLLAEAKKLNDAQAPKVDGGGGSGGPEGGSLAALTAHQACHLPLETFTRHRQ
PR↓GWEQLEQCGYPVQRLVALYLAARLSWNQVDQVIRNALASPGSGGDLGEAI
REQPEQARLALTLAAAESERFVRQGTGNDEAGAANGPADSGDALLERNYPTGA
EFLGDGGDVSFSTRGTQNWTVERLLQAHRQLEERGYVFVGYHGTFLEAAQSIV
FGGVRARSQDLDAIWRGFYIAGDPALAYGYAQDQEPDARGRIRNGALLRVYVP
RSSLPGFYRTSLTLAAPEAAGEVERLIGHPLPLRLDAITGPEEEGGRLETILG
WPLAERTVVIPSAIPTDPRNVGGDLDPSSIPDKEQAISALPDYASQPGKPPKD
EL

Legend

HER2-Affibody
Flexible spacer
Pseudomonas Exotoxin PE38 Translocation Domain
↓-endosomal cleavage site
Pseudomonas Exotoxin PE38 Activity Domain
KDEL sorting signal
Hexahistidin tag
Cysteins used for labeling by Alexa dyes


BT474
MCF7/HER2
MDA-MB361
MCF7
MDA-MB468
% of Max
FL1-H
0
20
40
60
80
100
10^0
10^1
10^2
10^3