# Supporting Information

# for

# Early breast cancer screening using iron/iron oxide-based nanoplatforms with sub-femtomolar limits of detection

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**Determination of matrix effects on the observed fluorescence intensities of the nanoplatforms, relative error from 10 repetitive protease measurements, and comparison of cancer stages and boxplots for each of the investigated proteases**

## 1. Determination of Matrix Effects on the Observed Fluorescence Intensities of the Nanoplatforms

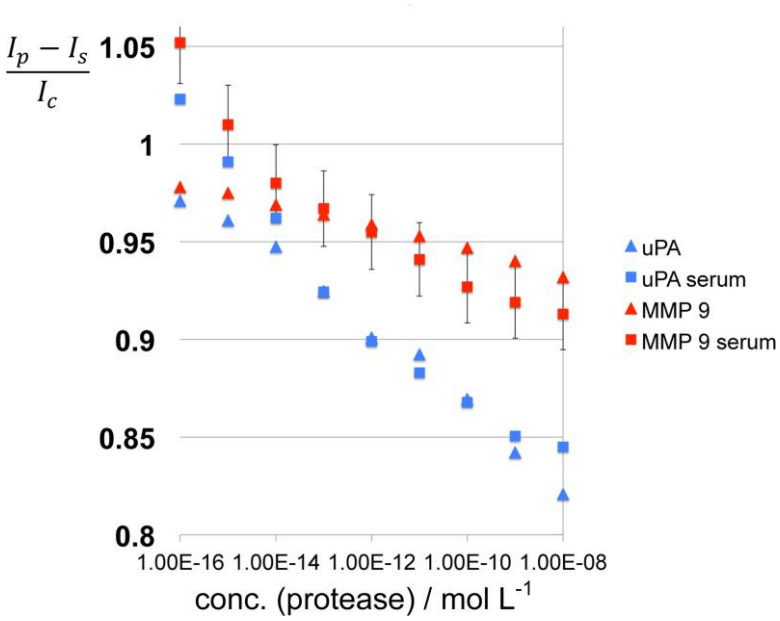


**Figure S1:** Matrix effects for MMP9 and uPA after 60 min of incubation at 25°C under standard conditions. Triangles: fluorescence readings in PBS; Squares: fluorescence readings in PBS containing inactivated serum. $I_p$: fluorescence signal after 60 min. of incubation; $I_c$: fluorescence signal in the absence of protease after 60 min. incubation; $I_s$: fluorescence signal of serum/PBS-dextran alone. Experimental errors are indicated. A detailed mechanistic discussion of the reasons why uPA and MMP9 are defying the "light switch paradigm" is provided in reference S1.

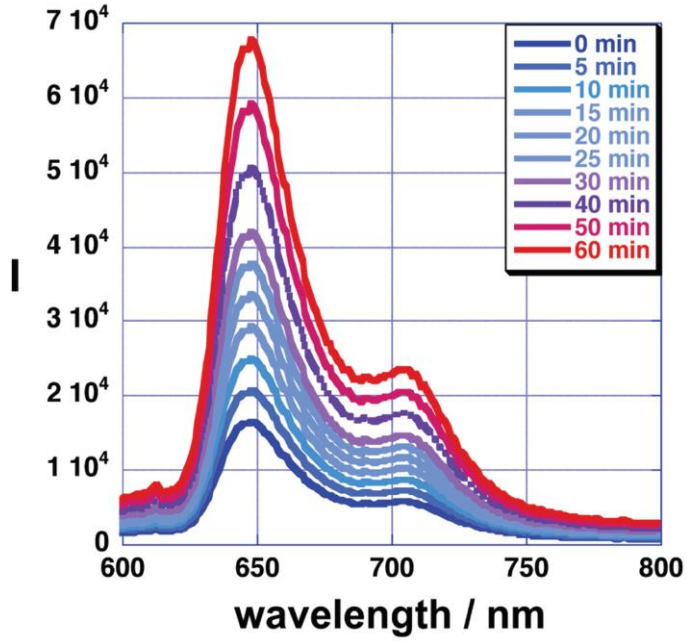


**Figure S2:** "Light-Switch Effect" of the Fe/$Fe_3O_4$-nanoplatform for detecting MMP13: fluorescence increase as a function of reaction time under standard conditions at 25°C after addition of 1.0 x $10^{-12}$ mol $l^{-1}$ of MMP13, with permission from Royal Society of Chemistry (reference S1).

## 2. Relative Error from 10 Independently Performed Protease Measurements

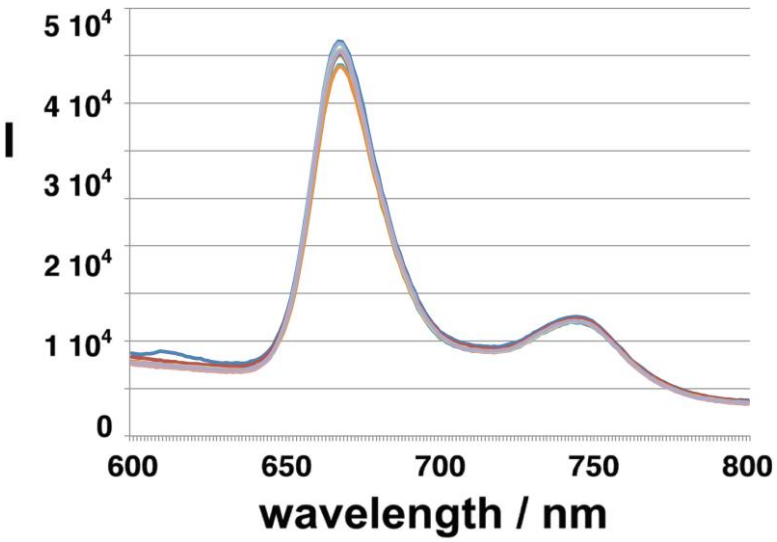


**Figure S3:** 10 independent repetitions of measuring the activity of the $Fe/Fe_3O_4$-nanoplatform for detecting MMP13 under standard conditions at 25°C after addition of $1.0 \times 10^{-13}$ mol $l^{-1}$ of MMP13. The relative error was determined to be 3 percent.

### 3. Boxplots and Bar-Graphs for Nine Proteases (Cathepsin L is also shown in the main text)

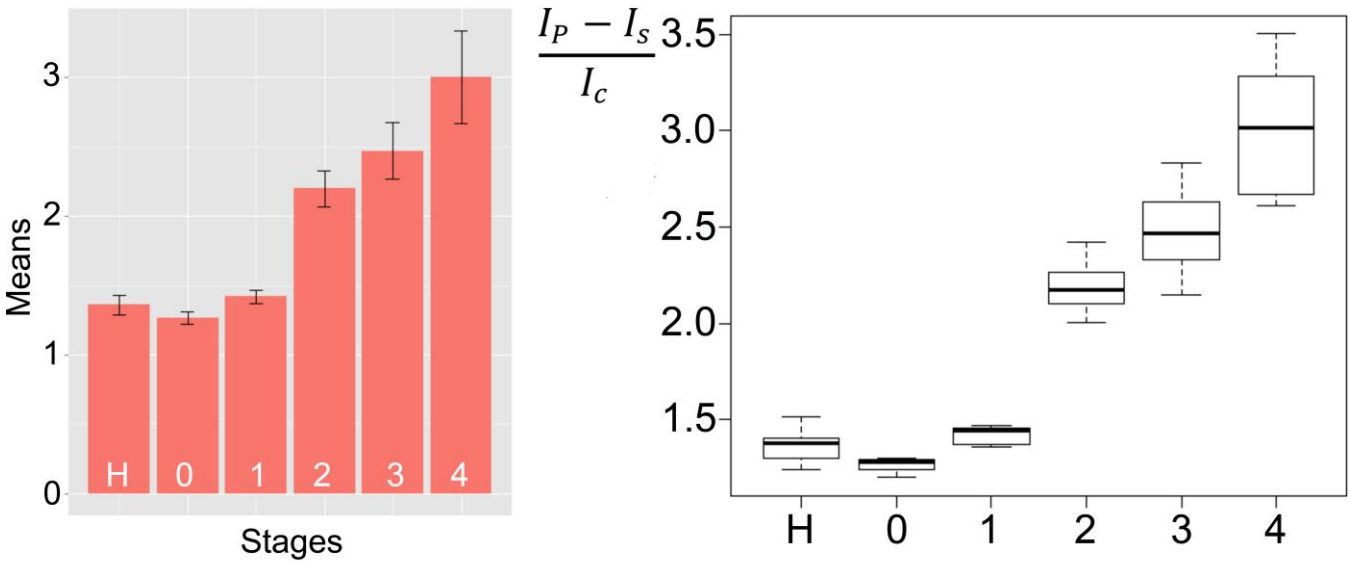


**Figure S4:** Bar graph (left, showing means and standard deviations) and box plot (right, indicating the observed data range) for cathepsin B. The group sizes are H (apparently healthy control group, n=20), 0: breast cancer stage 0 (n=4), 1: breast cancer stage 1 (n=9), 2: breast cancer stage 2 (n=9), 3: breast cancer stage 3 (n=12); 4: breast cancer stage 4 (n=12). All biospecimens were obtained from the Southeastern Nebraska Cancer Center (SNCC). Breast cancer has been staged according to the TNM staging system.[S1]

**Table S1:** Means, Standard Deviations, and Average Protease Activities in Serum for Cathepsin B (CTS B).

| Stages | Means | Standard Deviation | Average Protease Activity in Serum (mol $L^{-1}$) |
|---|---|---|---|
| H | 1.360853 | 0.07226045 | $2.4 \times 10^{-16}$ |
| 0 | 1.264777 | 0.04452960 | $1.4 \times 10^{-16}$ |
| 1 | 1.418908 | 0.04501105 | $3.3 \times 10^{-16}$ |
| 2 | 2.198153 | 0.12710588 | $2.6 \times 10^{-14}$ |
| 3 | 2.469330 | 0.20346940 | $1.1 \times 10^{-13}$ |
| 4 | 3.002602 | 0.33220471 | $1.8 \times 10^{-12}$ |

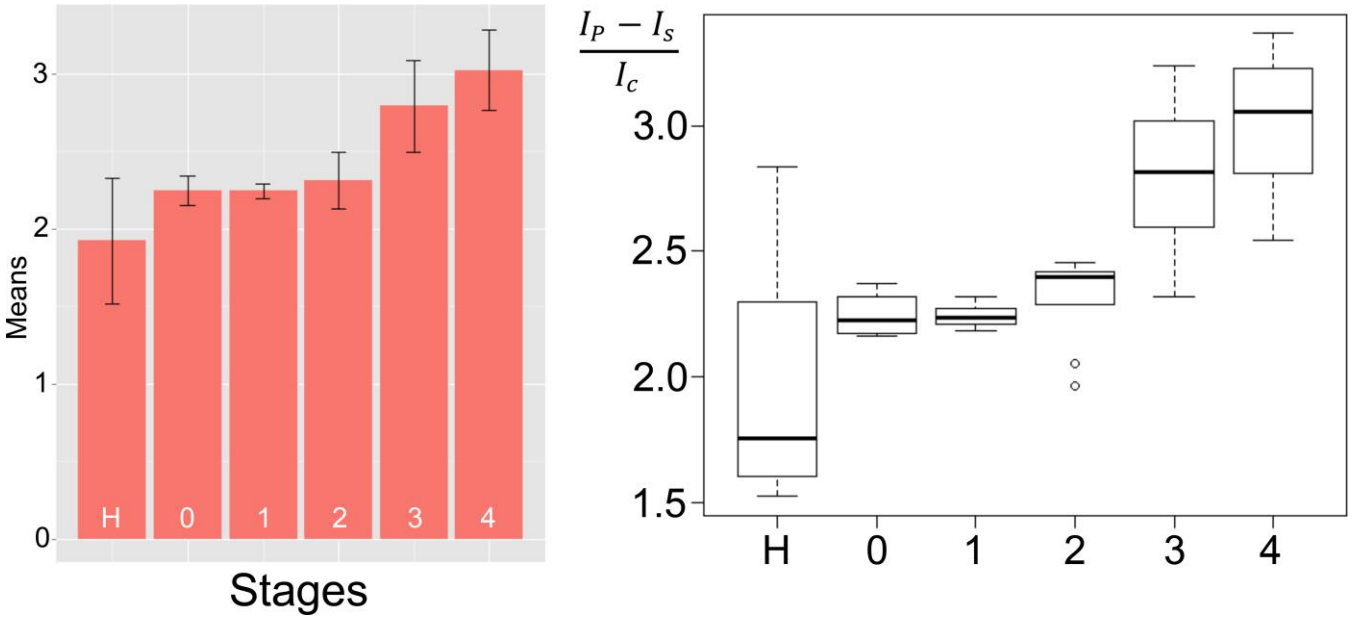


**Figure S5:** Bar graph (left, showing means and standard deviations) and box plot (right, indicating the observed data range) for cathepsin L. The group sizes are H (apparently healthy control group, n=20), 0: breast cancer stage 0 (n=4), 1: breast cancer stage 1 (n=9), 2: breast cancer stage 2 (n=9), 3: breast cancer stage 3 (n=12); 4: breast cancer stage 4 (n=12). All biospecimens were obtained from the South Eastern Nebraska Cancer Center (SNCC). Breast cancer has been staged according to the TNM staging system.[S1]

**Table S2:** Means, Standard Deviations, and Average Protease Activities in Serum for Cathepsin L (CTS L).

| Stages | Means | Standard Deviation | Average Protease Activity in Serum (mol $L^{-1}$) |
|---|---|---|---|
| H | 1.923536 | 0.40659660 | $1.8 \times 10^{-15}$ |
| 0 | 2.245194 | 0.09365240 | $8.5 \times 10^{-15}$ |
| 1 | 2.243285 | 0.04673341 | $8.4 \times 10^{-15}$ |
| 2 | 2.312233 | 0.18165558 | $1.1 \times 10^{-14}$ |
| 3 | 2.790727 | 0.29286377 | $5.4 \times 10^{-14}$ |
| 4 | 3.021944 | 0.25981286 | $1.2 \times 10^{-13}$ |

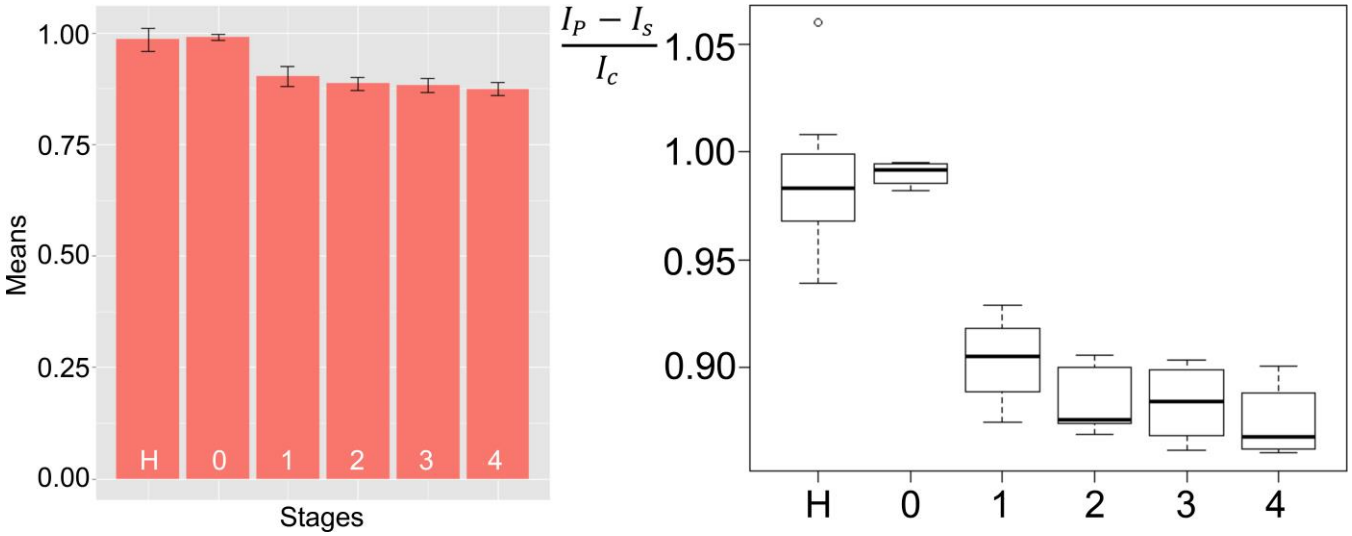


**Figure S6:** Bar graph (left, showing means and standard deviations) and box plot (right, indicating the observed data range) for urokinase plasminogen activator (uPA). The group sizes are H (apparently healthy control group, n=20), 0: breast cancer stage 0 (n=4), 1: breast cancer stage 1 (n=9), 2: breast cancer stage 2 (n=9), 3: breast cancer stage 3 (n=12); 4: breast cancer stage 4 (n=12). All biospecimens were obtained from the South Eastern Nebraska Cancer Center (SNCC). Breast cancer has been staged according to the TNM staging system.[S1]

**Table S3:** Means, Standard Deviations, and Average Protease Activities in Serum for Urokinase Plasminogen Activator (uPA).

| Stages | Means | Standard Deviation | Average Protease Activity in Serum (mol $L^{-1}$) |
|---|---|---|---|
| H | 0.9848335 | 0.025264087 | $1.3 \times 10^{-15}$ |
| 0 | 0.9900917 | 0.005991484 | $7.8 \times 10^{-16}$ |
| 1 | 0.9034983 | 0.022280082 | $2.1 \times 10^{-12}$ |
| 2 | 0.8860111 | 0.015128888 | $1.0 \times 10^{-11}$ |
| 3 | 0.8832422 | 0.015965042 | $1.3 \times 10^{-11}$ |
| 4 | 0.8741700 | 0.014332318 | $2.0 \times 10^{-11}$ |

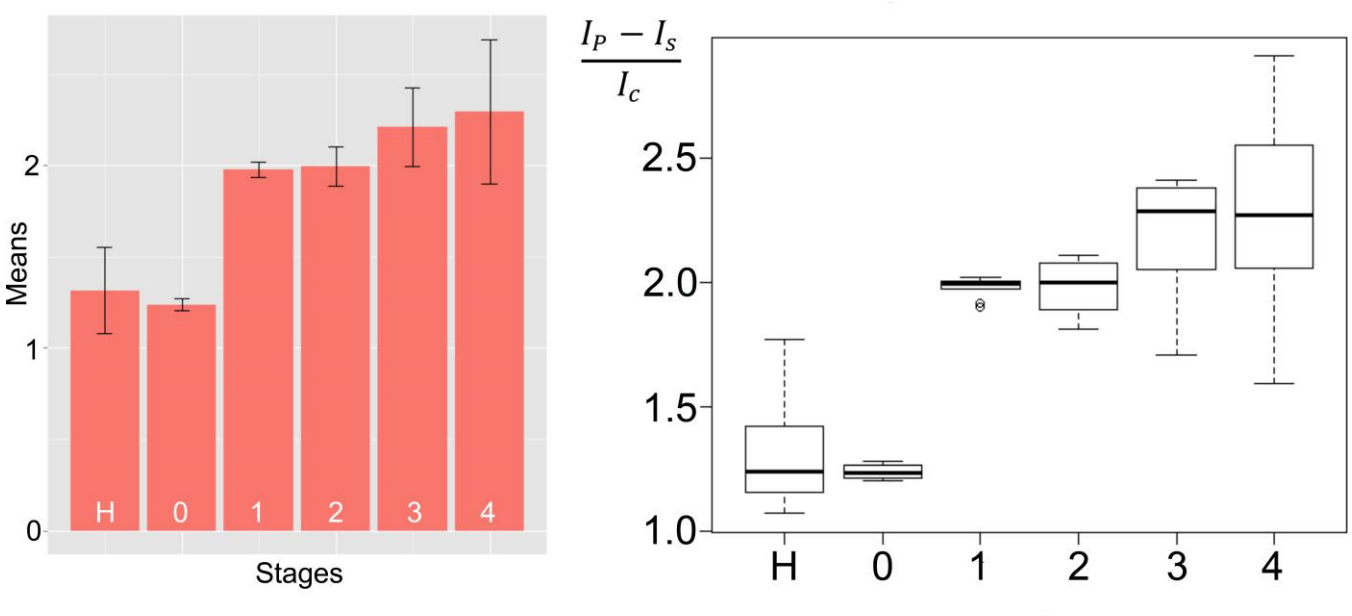


**Figure S7:** Bar graph (left, showing means and standard deviations) and box plot (right, indicating the observed data range) for matrix metalloproteinase 1 (MMP 1). The group sizes are H (apparently healthy control group, n=20), 0: breast cancer stage 0 (n=4), 1: breast cancer stage 1 (n=9), 2: breast cancer stage 2 (n=9), 3: breast cancer stage 3 (n=12); 4: breast cancer stage 4 (n=12). All biospecimens were obtained from the South Eastern Nebraska Cancer Center (SNCC). Breast cancer has been staged according to the TNM staging system.[S1]

**Table S4:** Means, Standard Deviations, and Average Protease Activities in Serum for Matrix Metalloproteinase 1 (MMP 1).

| Stages | Means | Standard Deviation | Average Protease Activity in Serum (mol $L^{-1}$) |
|---|---|---|---|
| H | 1.314616 | 0.23507424 | $1.6 \times 10^{-15}$ |
| 0 | 1.236894 | 0.03339194 | 5.9 x 10-$^{16}$ |
| 1 | 1.978331 | 0.04220099 | $7.8 \times 10^{-12}$ |
| 2 | 1.995024 | 0.10799028 | $9.6 \times 10^{-12}$ |
| 3 | 2.210357 | 0.21628141 | $1.5 \times 10^{-10}$ |
| 4 | 2.292405 | 0.39496657 | $4.3 \times 10^{-10}$ |

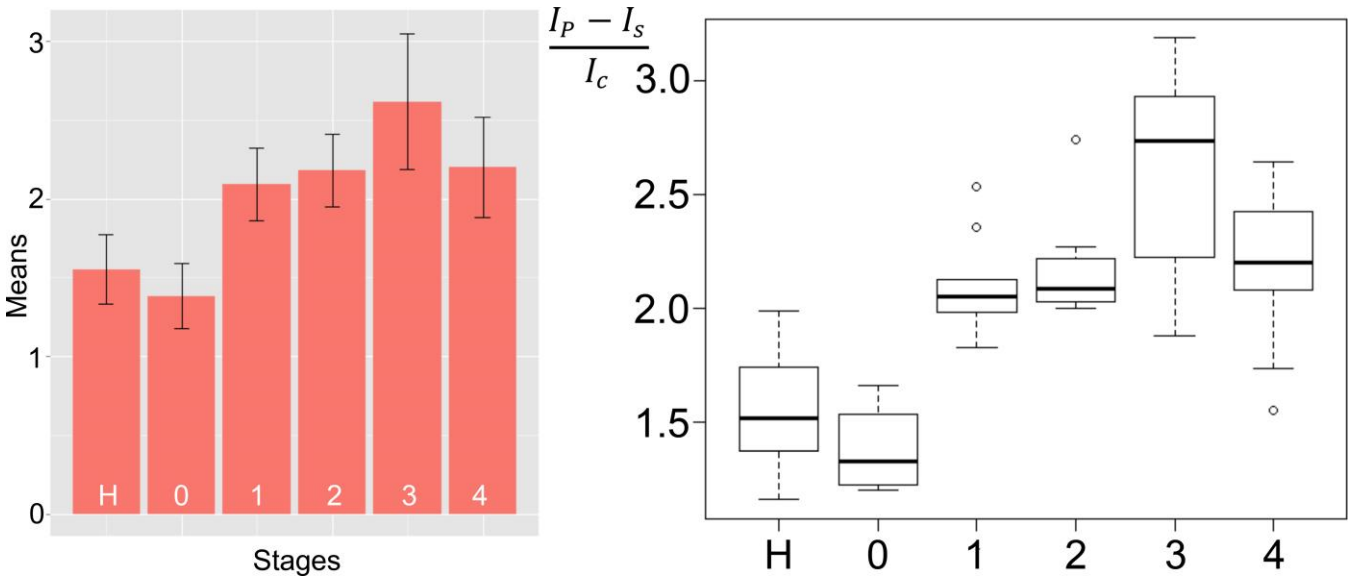


**Figure S8:** Bar graph (left, showing means and standard deviations) and box plot (right, indicating the observed data range) for matrix metalloproteinase 2 (MMP 2). The group sizes are H (apparently healthy control group, n=20), 0: breast cancer stage 0 (n=4), 1: breast cancer stage 1 (n=9), 2: breast cancer stage 2 (n=9), 3: breast cancer stage 3 (n=12); 4: breast cancer stage 4 (n=12). All biospecimens were obtained from the South Eastern Nebraska Cancer Center (SNCC). Breast cancer has been staged according to the TNM staging system.[S1]

**Table S5:** Means, Standard Deviations, and Average Protease Activities in Serum for Matrix Metalloproteinase 2 (MMP 2)

| Stages | Means | Standard Deviation | Average Protease Activity in Serum (mol $L^{-1}$) |
|---|---|---|---|
| H | 1.551050 | 0.2210302 | $4.0 \times 10^{-15}$ |
| 0 | 1.381780 | 0.2078706 | $6.4 \times 10^{-16}$ |
| 1 | 2.093351 | 0.2287855 | $1.4 \times 10^{-12}$ |
| 2 | 2.181590 | 0.2301482 | $3.6 \times 10^{-12}$ |
| 3 | 2.616193 | 0.4300199 | $4.0 \times 10^{-10}$ |
| 4 | 2.200369 | 0.3192078 | $4.4 \times 10^{-12}$ |

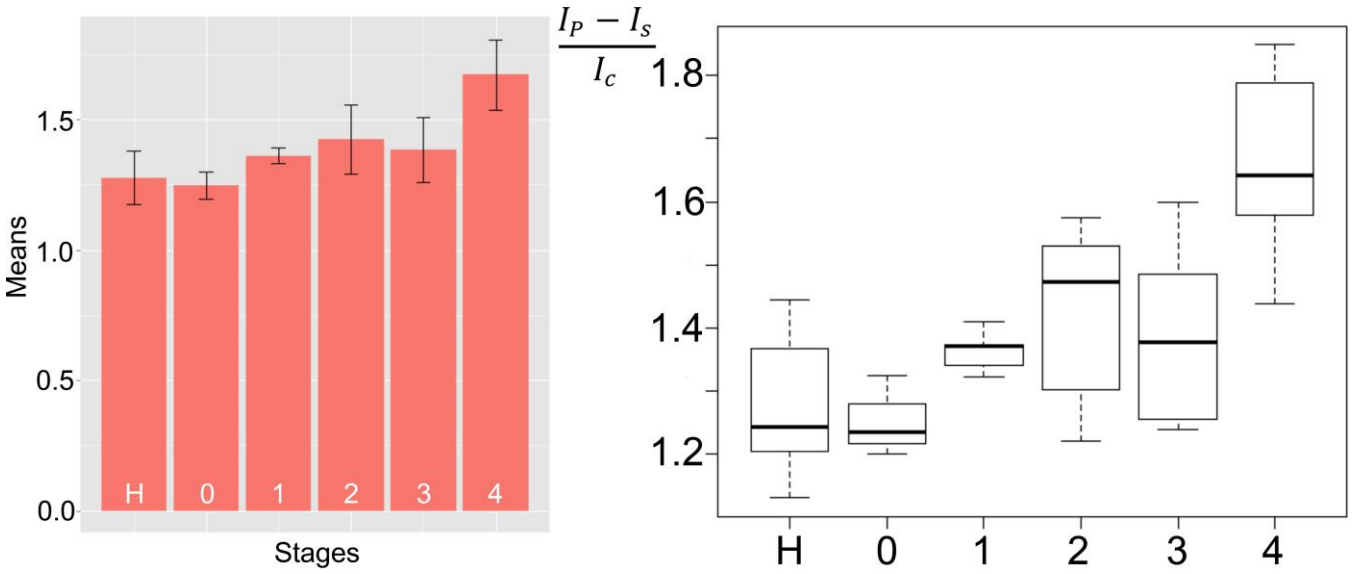


**Figure S9:** Bar graph (left, showing means and standard deviations) and box plot (right, indicating the observed data range) for matrix metalloproteinase 3 (MMP 3). The group sizes are H (apparently healthy control group, n=20), 0: breast cancer stage 0 (n=4), 1: breast cancer stage 1 (n=9), 2: breast cancer stage 2 (n=9), 3: breast cancer stage 3 (n=12); 4: breast cancer stage 4 (n=12). All biospecimens were obtained from the South Eastern Nebraska Cancer Center (SNCC). Breast cancer has been staged according to the TNM staging system.[S1]

**Table S6:** Means, Standard Deviations, and Average Protease Activities in Serum for Matrix Metalloproteinase 3 (MMP 3)

| Stages | Means | Standard Deviation | Average Protease Activity in Serum (mol $L^{-1}$) |
|---|---|---|---|
| H | 1.276774 | 0.10293781 | $3.4 \times 10^{-16}$ |
| 0 | 1.248445 | 0.05253079 | $2.2 \times 10^{-16}$ |
| 1 | 1.361347 | 0.02882905 | $1.2 \times 10^{-15}$ |
| 2 | 1.423178 | 0.13355403 | $2.9 \times 10^{-15}$ |
| 3 | 1.383381 | 0.12401589 | $1.6 \times 10^{-15}$ |
| 4 | 1.670680 | 0.13369459 | $1.2 \times 10^{-13}$ |

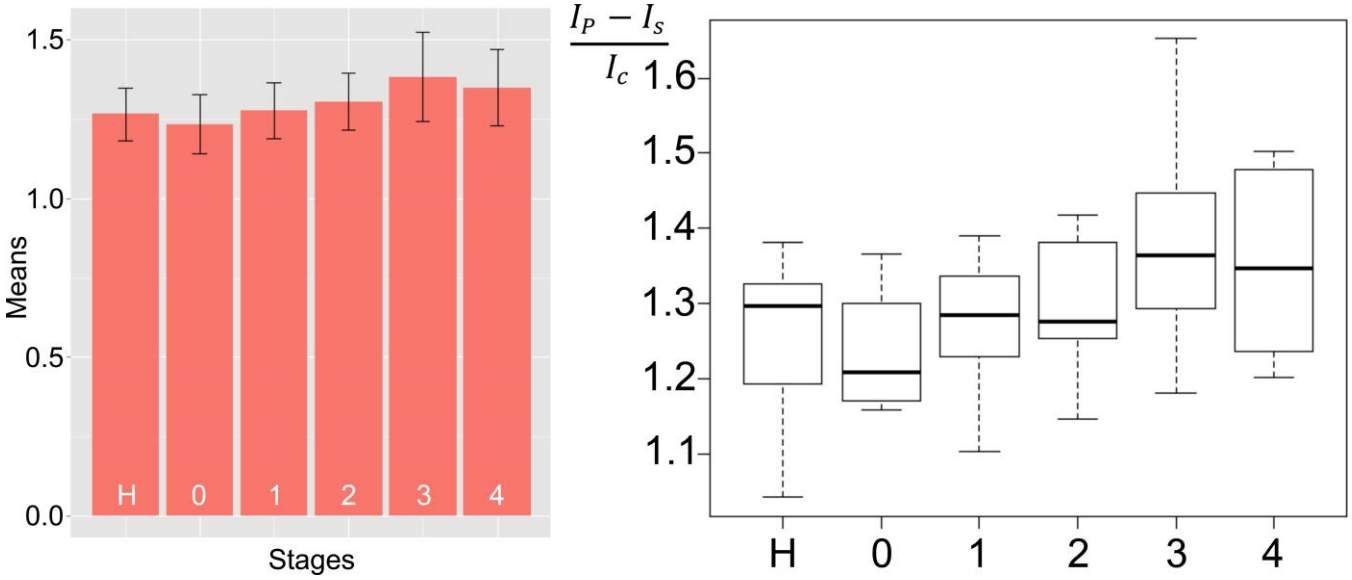


**Figure S10:** Bar graph (left, showing means and standard deviations) and box plot (right, indicating the observed data range) for matrix metalloproteinase 7 (MMP 7). The group sizes are H (apparently healthy control group, n=20), 0: breast cancer stage 0 (n=4), 1: breast cancer stage 1 (n=9), 2: breast cancer stage 2 (n=9), 3: breast cancer stage 3 (n=12); 4: breast cancer stage 4 (n=12). All biospecimens were obtained from the South Eastern Nebraska Cancer Center (SNCC). Breast cancer has been staged according to the TNM staging system.[S1]

**Table S7:** Means, Standard Deviations, and Average Protease Activities in Serum for Matrix Metalloproteinase 7 (MMP 7).

| Stages | Means | Standard Deviation | Average Protease Activity in Serum (mol $L^{-1}$) |
|---|---|---|---|
| H | 1.265858 | 0.08413963 | $6.4 \times 10^{-16}$ |
| 0 | 1.235173 | 0.09279042 | $4.5 \times 10^{-16}$ |
| 1 | 1.276960 | 0.08834555 | $7.2 \times 10^{-16}$ |
| 2 | 1.306292 | 0.09032285 | $1.0 \times 10^{-15}$ |
| 3 | 1.384025 | 0.14060255 | $2.5 \times 10^{-15}$ |
| 4 | 1.349619 | 0.12004177 | $1.7 \times 10^{-15}$ |

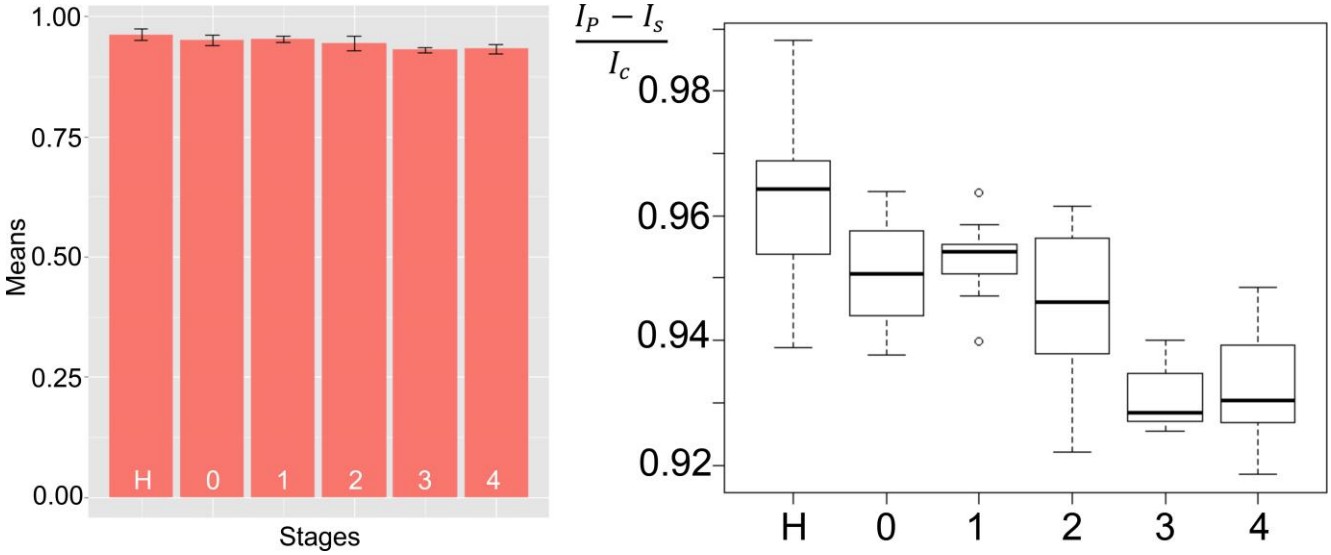


**Figure S11:** Bar graph (left, showing means and standard deviations) and box plot (right, indicating the observed data range) for matrix metalloproteinase 9 (MMP 9). The group sizes are H (apparently healthy control group, n=20), 0: breast cancer stage 0 (n=4), 1: breast cancer stage 1 (n=9), 2: breast cancer stage 2 (n=9), 3: breast cancer stage 3 (n=12); 4: breast cancer stage 4 (n=12). All biospecimens were obtained from the South Eastern Nebraska Cancer Center (SNCC). Breast cancer has been staged according to the TNM staging system.[S1]

**Table S8:** Means, Standard Deviations, and Average Protease Activities in Serum for Matrix Metalloproteinase 9 (MMP 9).

| Stages | Means | Standard Deviation | Average Protease Activity in Serum (mol $L^{-1}$) |
|---|---|---|---|
| H | 0.9622952 | 0.011980785 | $8.6 \times 10^{-13}$ |
| 0 | 0.9507300 | 0.010709674 | $5.9 \times 10^{-12}$ |
| 1 | 0.9530756 | 0.006783337 | $4.0 \times 10^{-12}$ |
| 2 | 0.9439189 | 0.014798504 | $1.8 \times 10^{-11}$ |
| 3 | 0.9308592 | 0.005204805 | $1.6 \times 10^{-10}$ |
| 4 | 0.9327775 | 0.009121815 | $1.2 \times 10^{-10}$ |

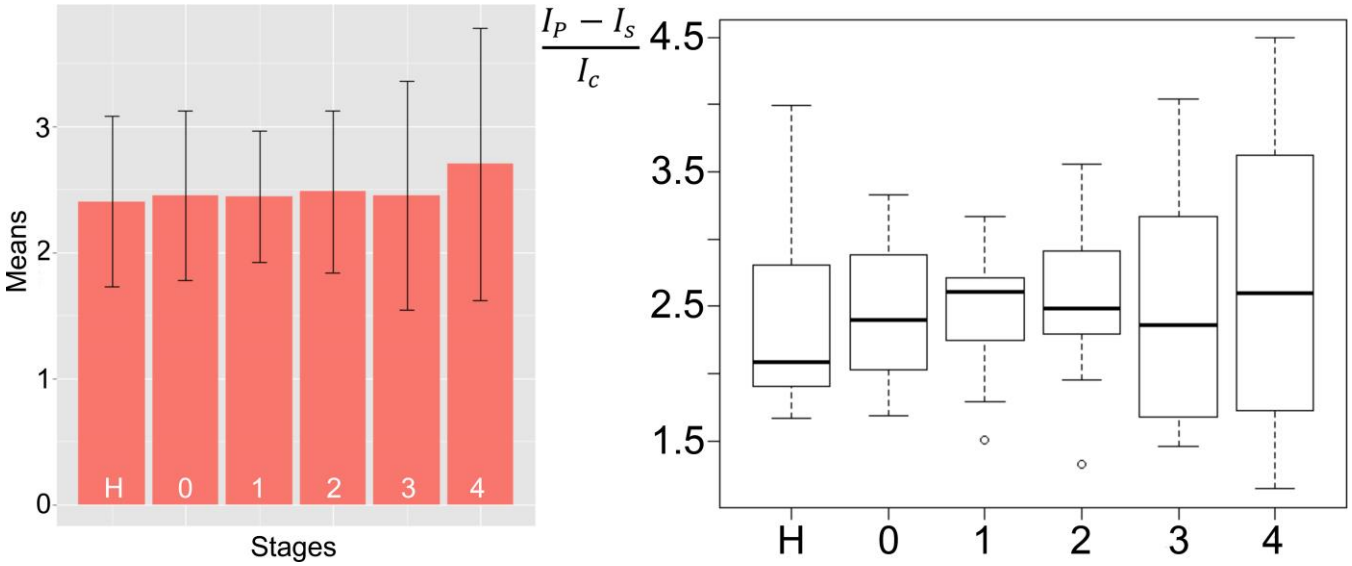


**Figure S12:** Bar graph (left, showing means and standard deviations) and box plot (right, indicating the observed data range) for matrix metalloproteinase 13 (MMP 13). The group sizes are H (apparently healthy control group, n=20), 0: breast cancer stage 0 (n=4), 1: breast cancer stage 1 (n=9), 2: breast cancer stage 2 (n=9), 3: breast cancer stage 3 (n=12); 4: breast cancer stage 4 (n=12). All biospecimens were obtained from the South Eastern Nebraska Cancer Center (SNCC). Breast cancer has been staged according to the TNM staging system.[S1]

**Table S9:** Means, Standard Deviations, and Average Protease Activities in Serum for Matrix Metalloproteinase 13 (MMP 13).

| Stages | Means | Standard Deviation | Average Protease Activity in Serum (mol $L^{-1}$) |
|---|---|---|---|
| H | 2.402871 | 0.6767673 | $3.2 \times 10^{-15}$ |
| 0 | 2.454853 | 0.6726423 | $3.7 \times 10^{-15}$ |
| 1 | 2.447914 | 0.5209525 | $3.6 \times 10^{-15}$ |
| 2 | 2.483194 | 0.6442519 | $4.0 \times 10^{-15}$ |
| 3 | 2.453076 | 0.9055961 | $3.7 \times 10^{-15}$ |
| 4 | 2.701158 | 1.0781773 | $7.5 \times 10^{-15}$ |

S1: Singletary, S.E., Connolly, J.L., 2006. Breast cancer staging: working with the sixth edition of the AJCC Cancer Staging Manual. CA Cancer J Clin 56(1), 37-47; quiz 50-31.