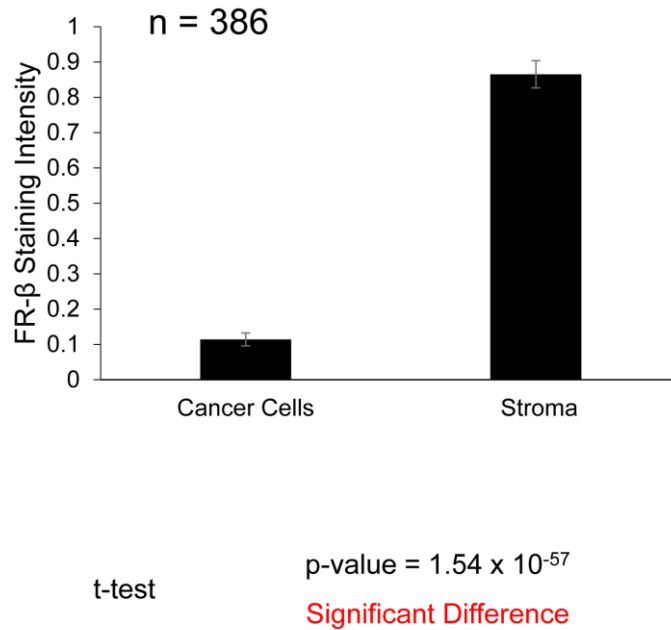
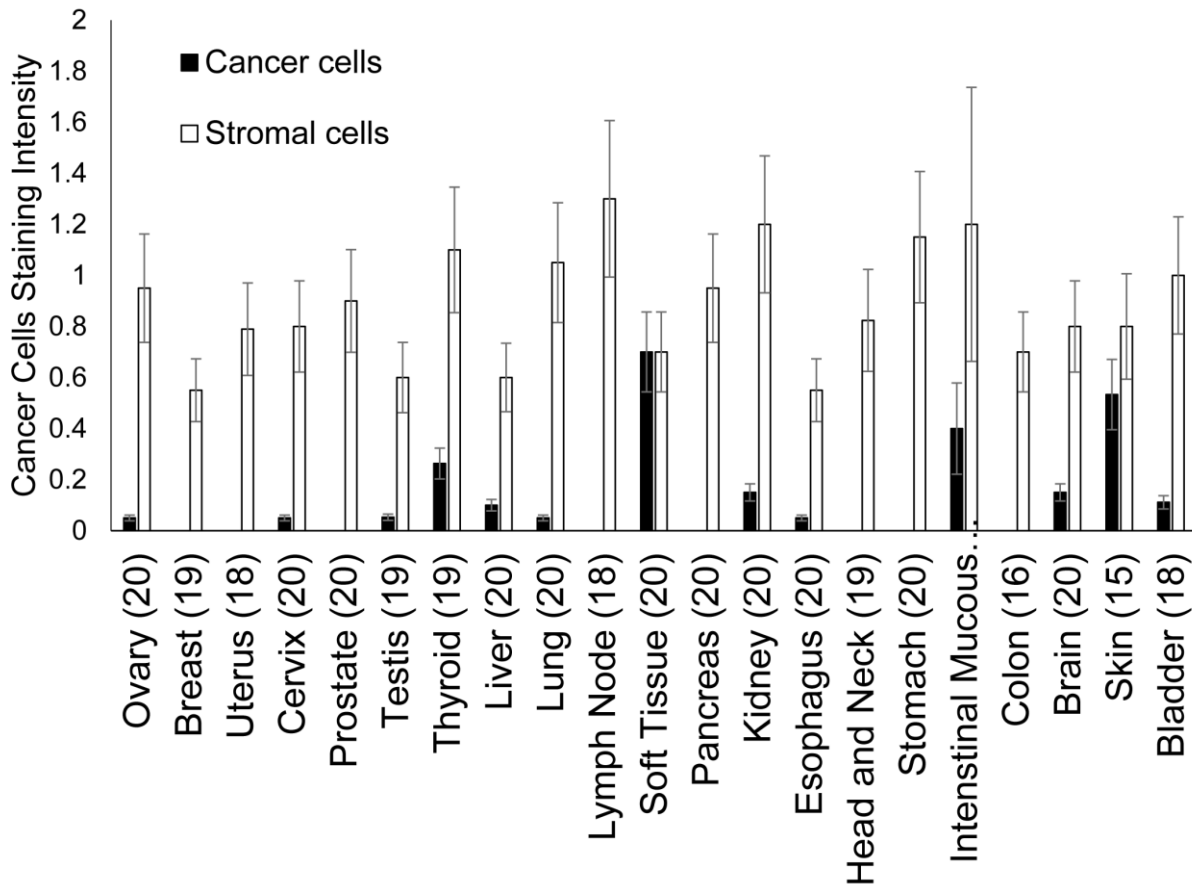


# Assessment of folate receptor- $\beta$ expression in human neoplastic tissues

## Supplementary Material



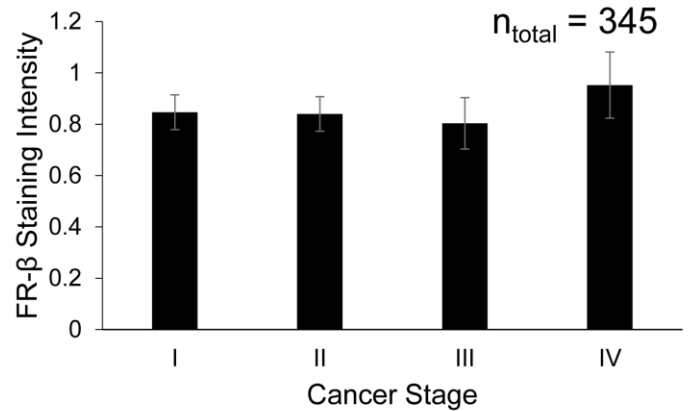
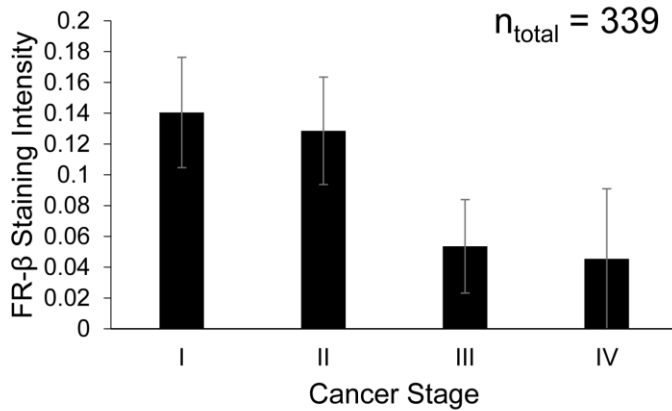
**SI Figure 1: FR- $\beta$  staining intensity of cancer and stromal cells.** IHC was performed on a tissue microarray using the antibody m909. The staining intensity within the tumor and stroma were graded on a scale of 0 to 3 and the average value is plotted (error bars represent SEM). A t-test (assuming equal variance and 2-tailed) was used to determine if there were any statistically significant differences between cancer and stromal cells.



**SI Figure 2: FR- $\beta$  staining intensity in cancer and stromal cells.** IHC was performed on a tissue microarray using the antibody m909. The staining intensity within the tumor and stroma were graded on a scale of 0 to 3. Average staining intensity is plotted for each cancer tissue type (error bars represent SEM) and the number of samples is shown in parenthesis.

## Cancer Cells

## Stromal Cells



1-Sided  
ANOVA

$$F(3,335) = 1.023$$

$$p\text{-value} = 0.383$$

No Significant Difference

$$F(3,341) = 0.193$$

$$p\text{-value} = 0.901$$

No Significant Difference

Spearman  
Correlation  
Analysis

$$\rho = -0.0884$$

$$p\text{-value} = 0.1041$$

No Significant Correlation

$$\rho = 0.0041$$

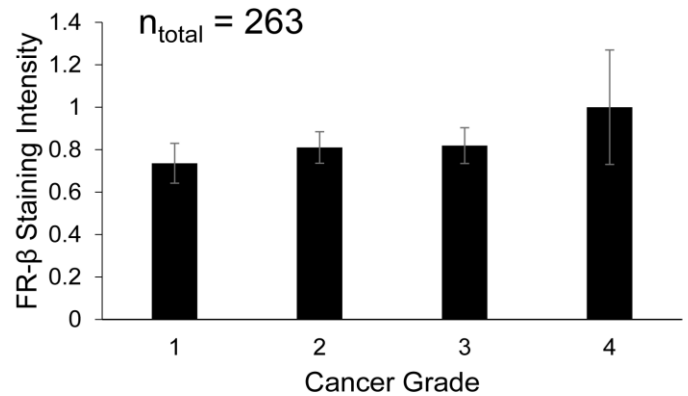
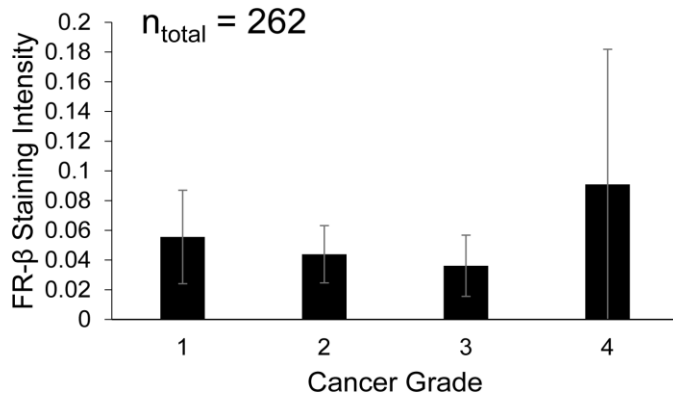
$$p\text{-value} = 0.9767$$

No Significant Correlation

**SI Figure 3: Correlation analysis of FR-β staining intensity and cancer stage.** IHC was performed on a tissue microarray using the antibody m909. The staining intensity within the tumor and stroma were graded on a scale of 0 to 3 and the average is plotted (error bars represent SEM). A 1-sided ANOVA and Spearman correlation analysis were used to determine if there were any statistically significant differences between the individual groups and/or if there was a significant correlation between the intensity staining and the cancer stage.

## Cancer Cells

## Stromal Cells



1-Sided ANOVA  
 $F(3,258) = 0.269$   
 $p\text{-value} = 0.848$   
No Significant Difference

1-Sided ANOVA  
 $F(3,259) = 0.387$   
 $p\text{-value} = 0.763$   
No Significant Difference

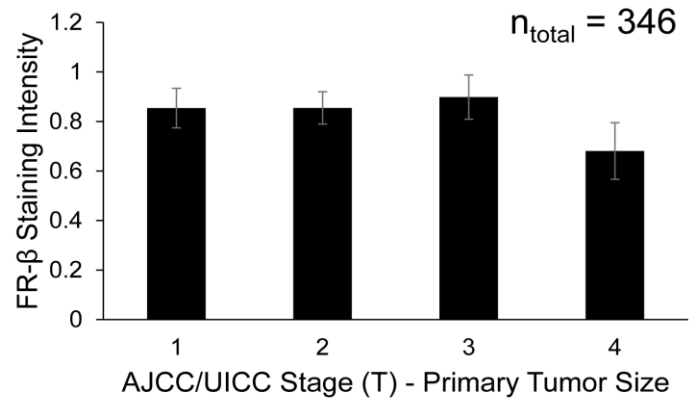
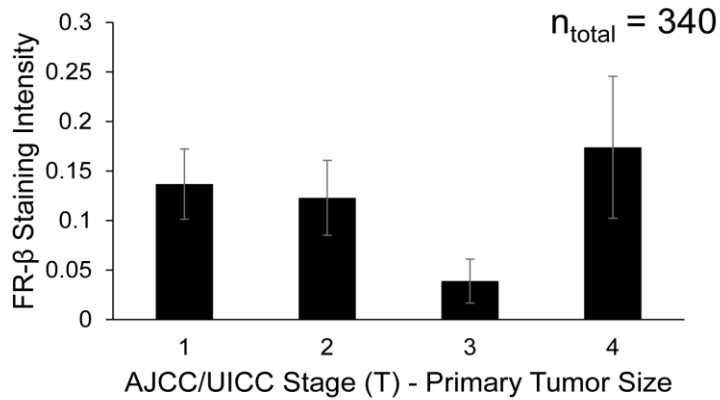
Spearman Correlation Analysis  
 $\rho = -0.0131$   
 $p\text{-value} = 0.8322$   
No Significant Correlation

Spearman Correlation Analysis  
 $\rho = 0.0454$   
 $p\text{-value} = 0.4629$   
No Significant Correlation

**SI Figure 4: Correlation analysis of FR-β staining intensity and cancer grade.** IHC was performed on a tissue microarray using the antibody m909. The staining intensity within the tumor and stroma were graded on a scale of 0 to 3 and the average is plotted (error bars represent SEM). A 1-sided ANOVA and Spearman correlation analysis were used to determine if there were any statistically significant differences between the individual groups and/or if there was a significant correlation between the intensity staining and the cancer grade.

## Cancer Cells

## Stromal Cells



1-Sided ANOVA  
 $F(3,336) = 1.606$   
 $p\text{-value} = 0.188$   
No Significant Difference

$F(3,342) = 0.857$   
 $p\text{-value} = 0.494$   
No Significant Difference

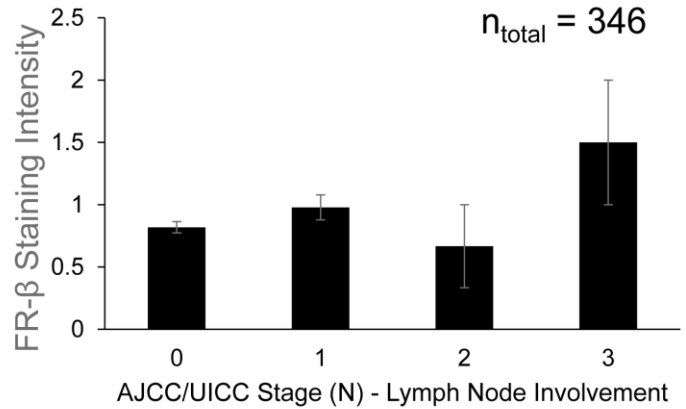
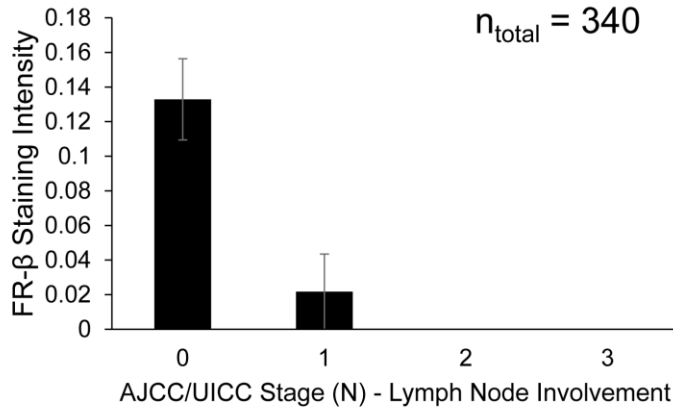
Spearman Correlation Analysis  
 $\rho = -0.0606$   
 $p\text{-value} = 0.2650$   
No Significant Correlation

$\rho = 0.0389$   
 $p\text{-value} = 0.4709$   
No Significant Correlation

**SI Figure 5: Correlation analysis of FR-β staining intensity and AJCC/UICC T value (size of primary tumor).** IHC was performed on a tissue microarray using the antibody m909. The staining intensity within the tumor and stroma were graded on a scale of 0 to 3 and the average is plotted (error bars represent SEM). A 1-sided ANOVA and Spearman correlation analysis were used to determine if there were any statistically significant differences between the individual groups and/or if there was a significant correlation between the intensity staining and the primary tumor size.

## Cancer Cells

## Stromal Cells



1-Sided ANOVA  
 $F(3,336) = 1.456$   
 $p\text{-value} = 0.226$   
No Significant Difference

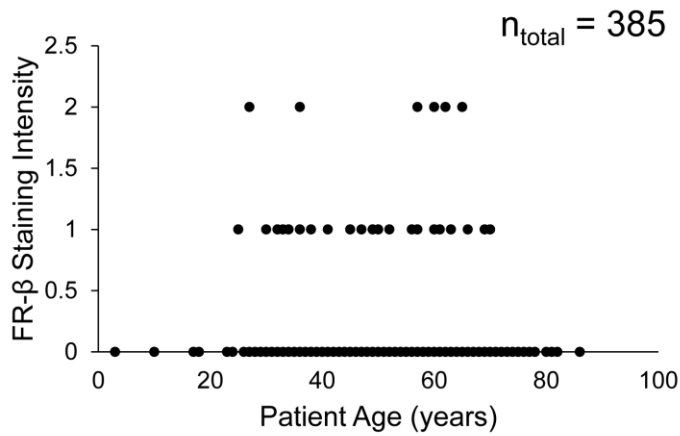
$F(3,342) = 1.176$   
 $p\text{-value} = 0.319$   
No Significant Difference

Spearman Correlation Analysis  
 $\rho = -0.1161$   
 $p\text{-value} = 0.0323$   
**Significant Correlation**

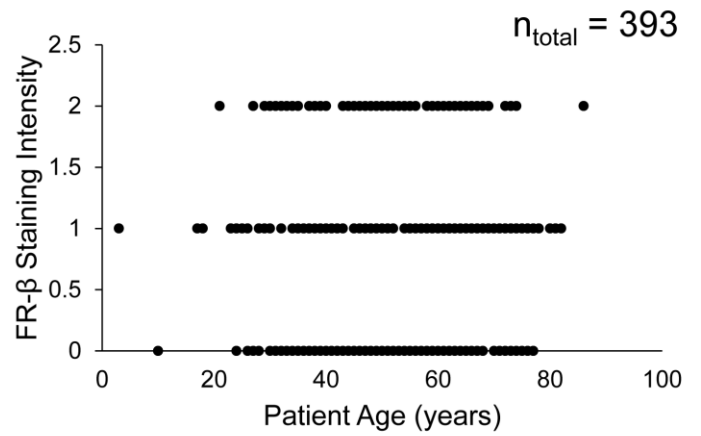
$\rho = 0.0745$   
 $p\text{-value} = 0.9406$   
No Significant Correlation

**SI Figure 6: Correlation analysis of FR-β staining intensity and AJCC/UICC N value (level of lymph node involvement).** IHC was performed on a tissue microarray using the antibody m909. The staining intensity within the tumor and stroma were graded on a scale of 0 to 3 and the average is plotted (error bars represent SEM). A 1-sided ANOVA and Spearman correlation analysis were used to determine if there were any statistically significant differences between the individual groups and/or if there was a significant correlation between the intensity staining and the lymph node involvement.

### Cancer Cells



### Stromal Cells

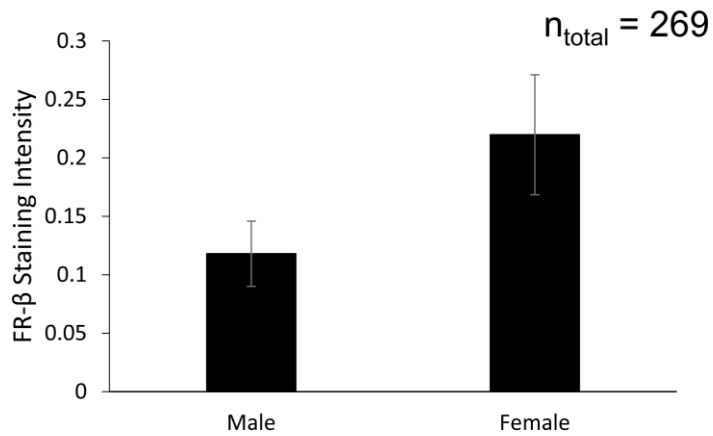


1-Sided ANOVA -  
-  
-  
Spearman Correlation Analysis  $\rho = -0.0608$   
p-value = 0.2339  
No Significant Correlation

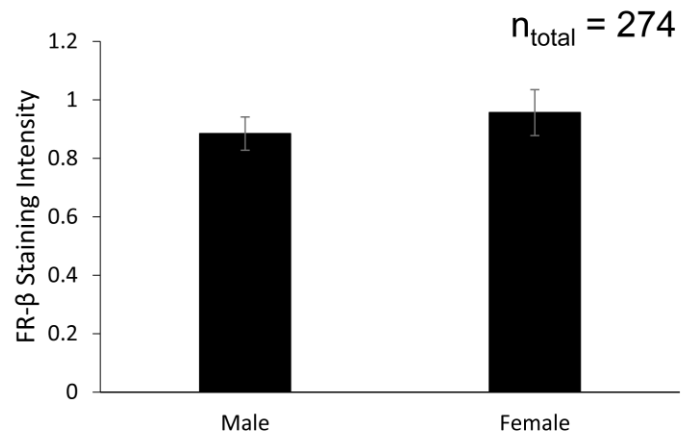
-  
-  
-  
 $\rho = 0.0371$   
p-value = 0.4630  
No Significant Correlation

**SI Figure 7: Correlation analysis of FR- $\beta$  staining intensity and patient age at cancer excision.** IHC was performed on a tissue microarray using the antibody m909. A Spearman correlation analysis were used to determine if there was a significant correlation between the intensity staining and the patient's age.

## Cancer Cells



## Stromal Cells

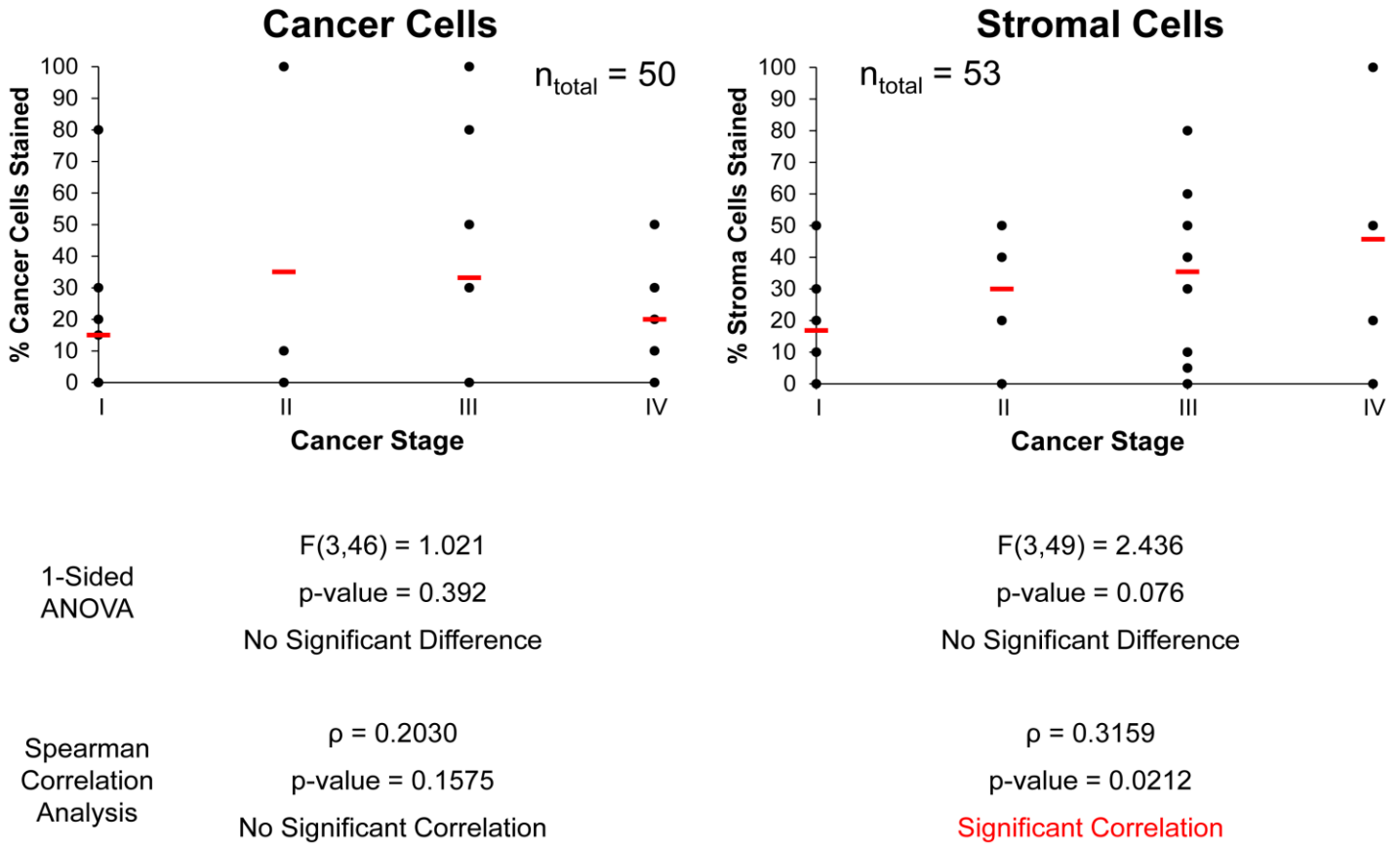


t-test  
p-value = 0.0834  
No Significant Difference

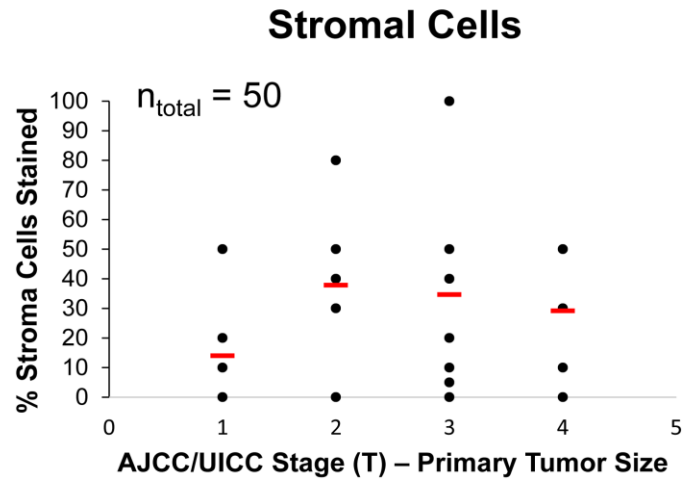
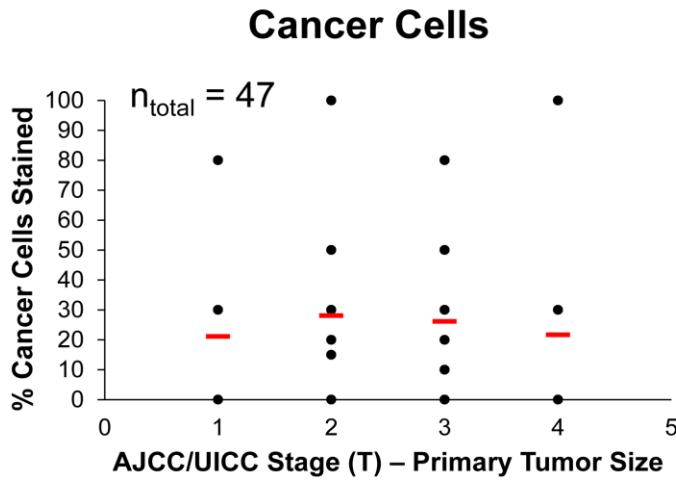
p-value = 0.4621  
No Significant Difference

**SI Figure 8: Correlation analysis of FR- $\beta$  staining intensity and patient sex.** IHC was performed on a tissue microarray using the antibody m909. The staining intensity within the tumor and stroma were graded on a scale of 0 to 3 and the average is plotted (error bars represent SEM). A t-test (assuming equal variance and 2-tailed) was used to determine if there were any statistically significant differences in staining intensity between males and females.





**SI Figure 9: Correlation analysis of FR- $\beta$  % cells staining positive and cancer stage.** IHC was performed on a tissue microarray using the antibody m909. The approximate percentage of positively staining cells within the tumor and stroma were determined (red bars represent population mean). A 1-sided ANOVA and Spearman correlation analysis were used to determine if there were any statistically significant differences between the individual groups and/or if there was a significant correlation between the percentage of positive staining cells and the stage of the cancer.



1-Sided ANOVA  
 $F(3,43) = 0.111$   
 $P\text{-value} = 0.953$   
 No Significant Difference

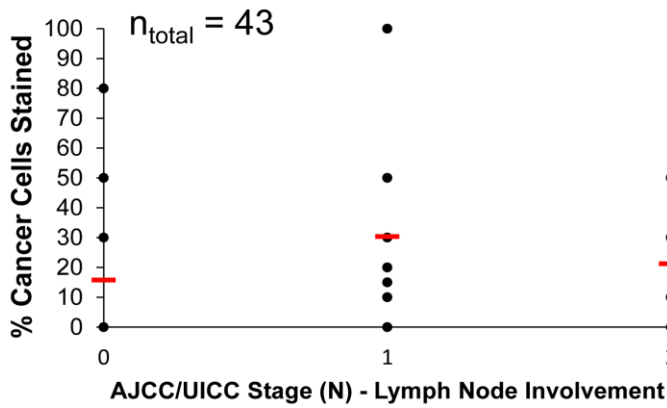
1-Sided ANOVA  
 $F(3,46) = 1.694$   
 $p\text{-value} = 0.181$   
 No Significant Difference

Spearman Correlation Analysis  
 $\rho = -0.0036$   
 $p\text{-value} = 0.9808$   
 No Significant Correlation

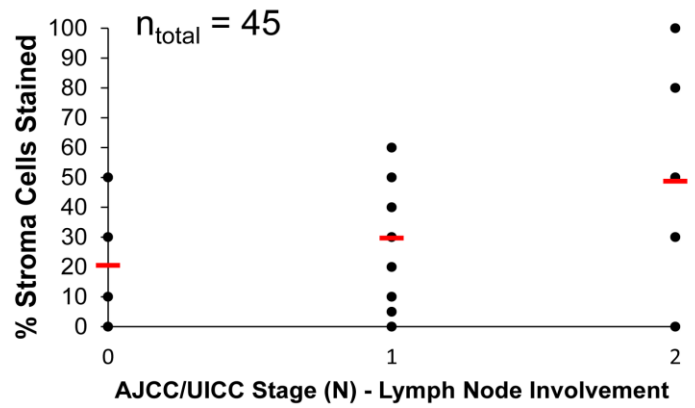
Spearman Correlation Analysis  
 $\rho = 0.1382$   
 $p\text{-value} = 0.3386$   
 No Significant Correlation

**SI Figure 10: Correlation analysis of FR-β % cells staining positive and AJCC/UICC T value (size of primary tumor).** IHC was performed on a tissue microarray using the antibody m909. The approximate percentage of positively staining cells within the tumor and stroma were determined (red bars represent population mean). A 1-sided ANOVA and Spearman correlation analysis were used to determine if there were any statistically significant differences between the individual groups and/or if there was a significant correlation between the percentage of positive staining cells and the size of the primary tumor.

### Cancer Cells



### Stromal Cells



1-Sided ANOVA  
 $F(2,38) = 0.862$   
 $P\text{-value} = 0.430$   
 No Significant Difference

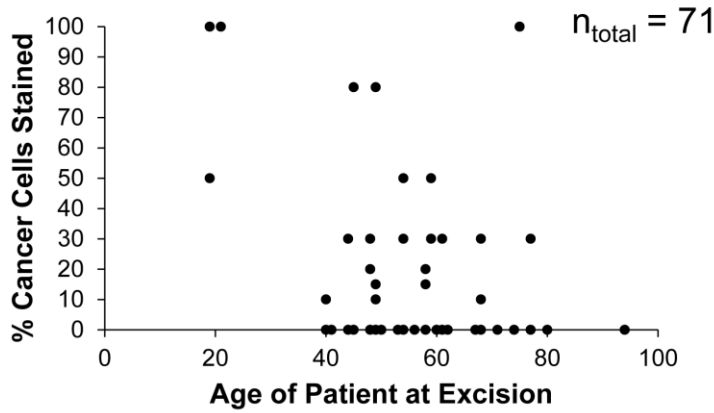
$F(2,40) = 3.508$   
 $p\text{-value} = 0.039$   
**Significant Difference**

Spearman Correlation Analysis  
 $\rho = 0.1905$   
 $p\text{-value} = 0.2212$   
 No Significant Correlation

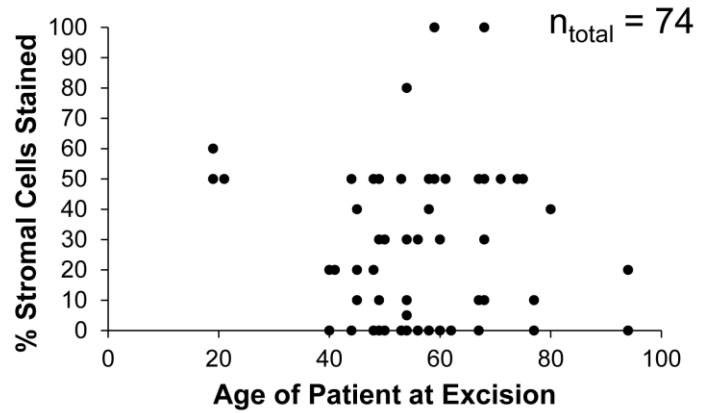
$\rho = 0.3250$   
 $p\text{-value} = 0.0293$   
**Significant Correlation**

**SI Figure 11: Correlation analysis of FR- $\beta$  % cells staining positive and AJCC/UICC N value (level of lymph node involvement).** IHC was performed on a tissue microarray using the antibody m909. The approximate percentage of positively staining cells within the tumor and stroma were determined (red bars represent population mean). A 1-sided ANOVA and Spearman correlation analysis were used to determine if there were any statistically significant differences between the individual groups and/or if there was a significant correlation between the percentage of positive staining cells and the level of lymph node involvement.

### Cancer Cells



### Stromal Cells



1-Sided ANOVA -  
-  
-

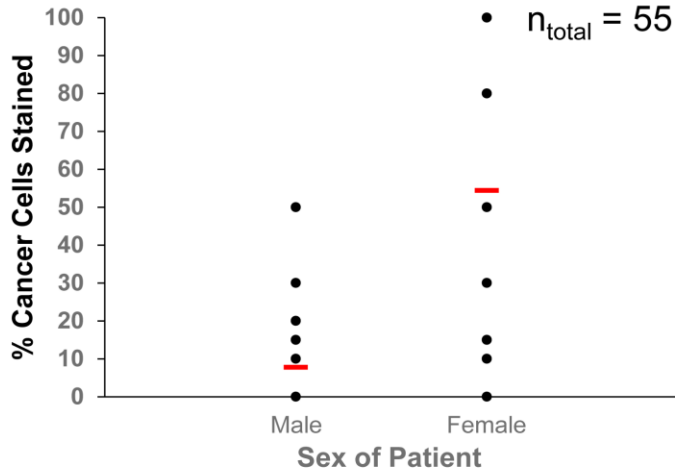
Spearman Correlation Analysis  
 $\rho = -0.2337$   
p-value = 0.0501  
No Significant Correlation

1-Sided ANOVA -  
-  
-

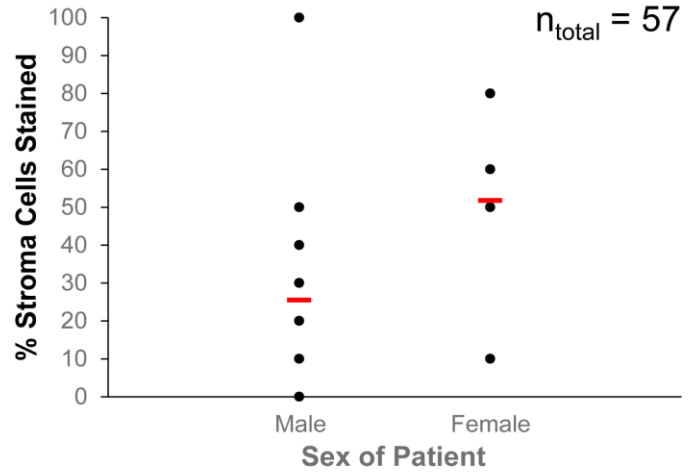
Spearman Correlation Analysis  
 $\rho = -0.0285$   
p-value = 0.8096  
No Significant Correlation

**SI Figure 12: Correlation analysis of FR- $\beta$  % cells staining positive and patient age at tumor excision.** IHC was performed on a tissue microarray using the antibody m909. The approximate percentage of positively staining cells within the tumor and stroma were determined. A Spearman correlation analysis was used to determine if there was a significant correlation between the percentage of positive staining cells and the patient age at tumor excision.

### Cancer Cells



### Stromal Cells



t-test

p-value = 0.0001

Significant Difference

p-value = 0.00002

Significant Difference

**SI Figure 13: Correlation analysis of FR-β % cells staining positive and patient sex.** IHC was performed on a tissue microarray using the antibody m909. The approximate percentage of positively staining cells within the tumor and stroma were determined (red bars represent population mean). A t-test (assuming equal variance and 2-tailed) was used to determine if there were any statistically significant differences between males and females.