

## Supplementary Information

### Folate Receptor Alpha Expression is Associated with Improved Disease Free Survival in Triple Negative Breast Cancer

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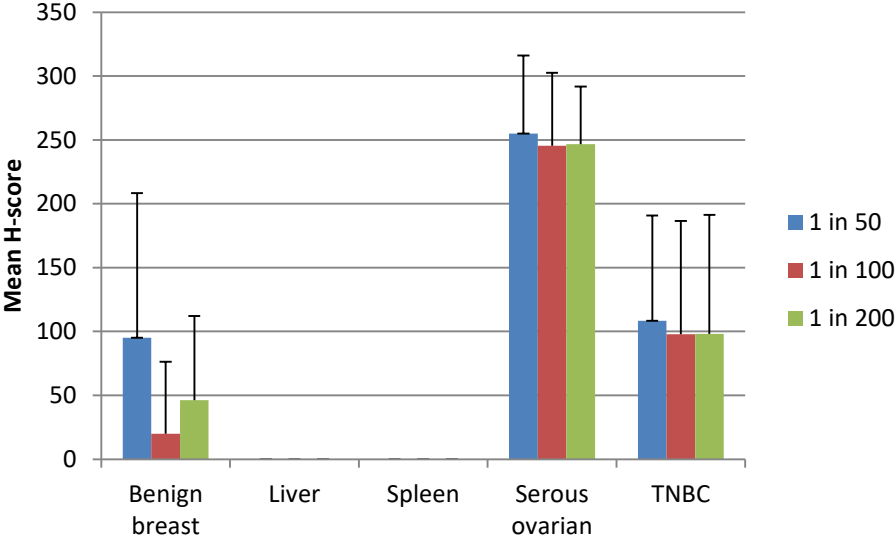
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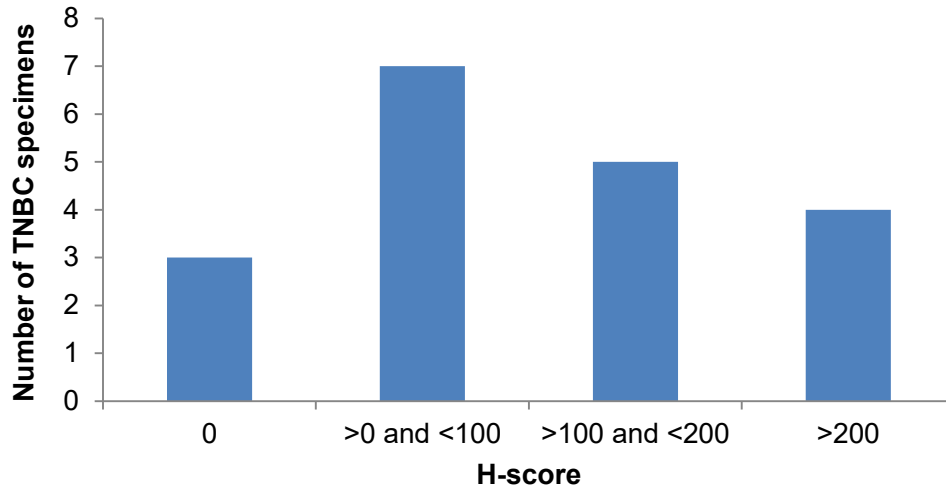
SUPPLEMENTARY FIGURES AND LEGENDS

Supplementary Figure 1



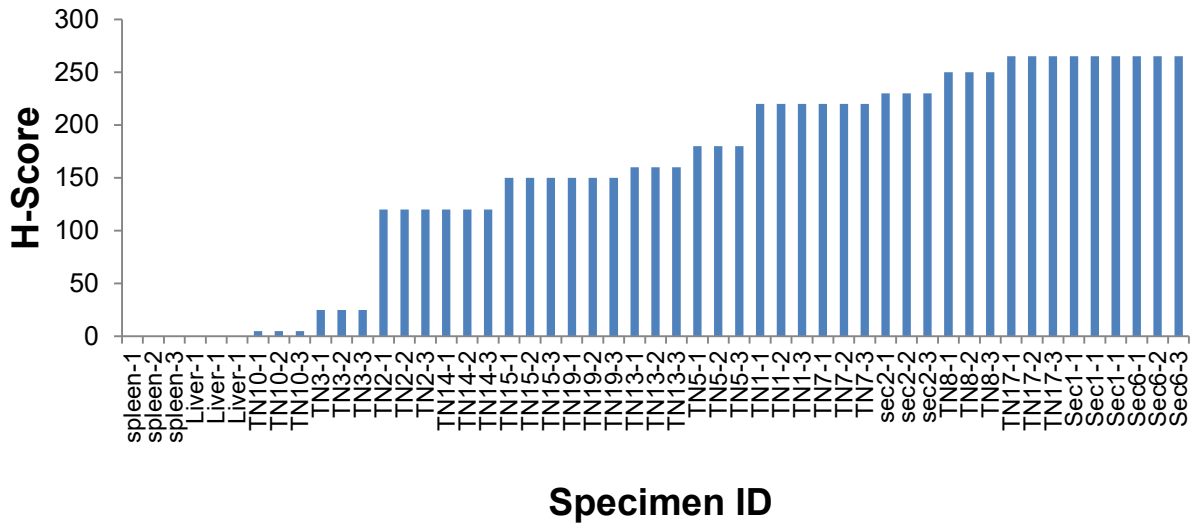
**Fig. S1: The antibody (lot # 13J40007) was tested at 1:50, 1:100, 1:200 dilutions on an optimization TMA. H-score distributions were similar for each concentration. Error bars are standard deviation.**

## Supplementary Figure 2



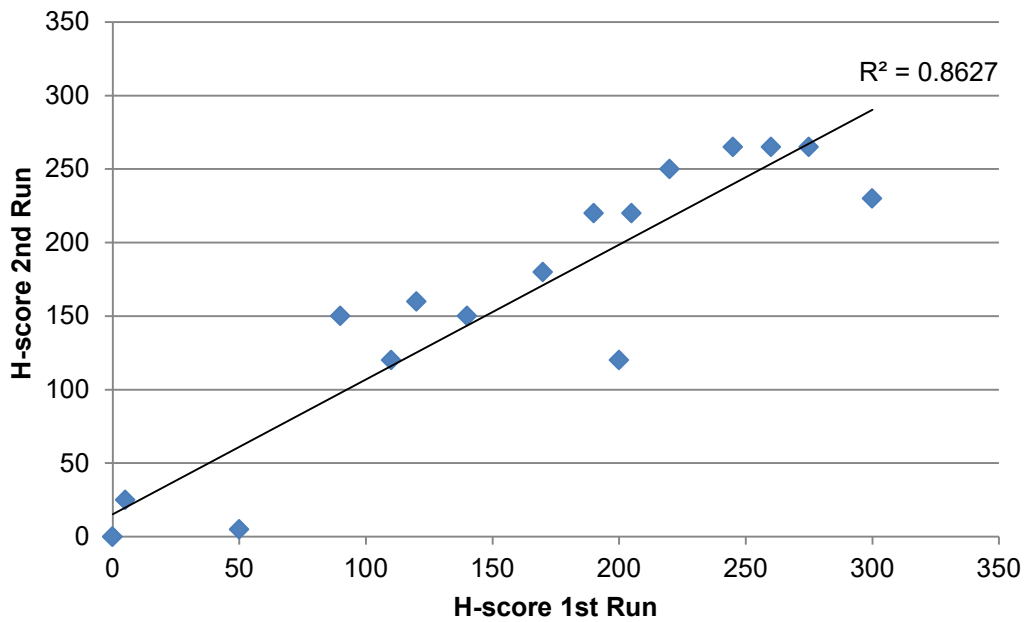
**Fig. S2: FR $\alpha$  expression is heterogeneous in TNBC.** Distribution of H-scores in 19 TNBC specimens at an antibody dilution of 1:100 using antibody lot # 13J40007. At 1:100, H-scores for 19 TN tumors ranged from 0 to 300. 3 /19 (16%) tumors showed zero staining (H-score of 0 for all three replicate punches) and 16 of 19 (84%) had a mean H-score >zero.

### Supplementary Figure 3



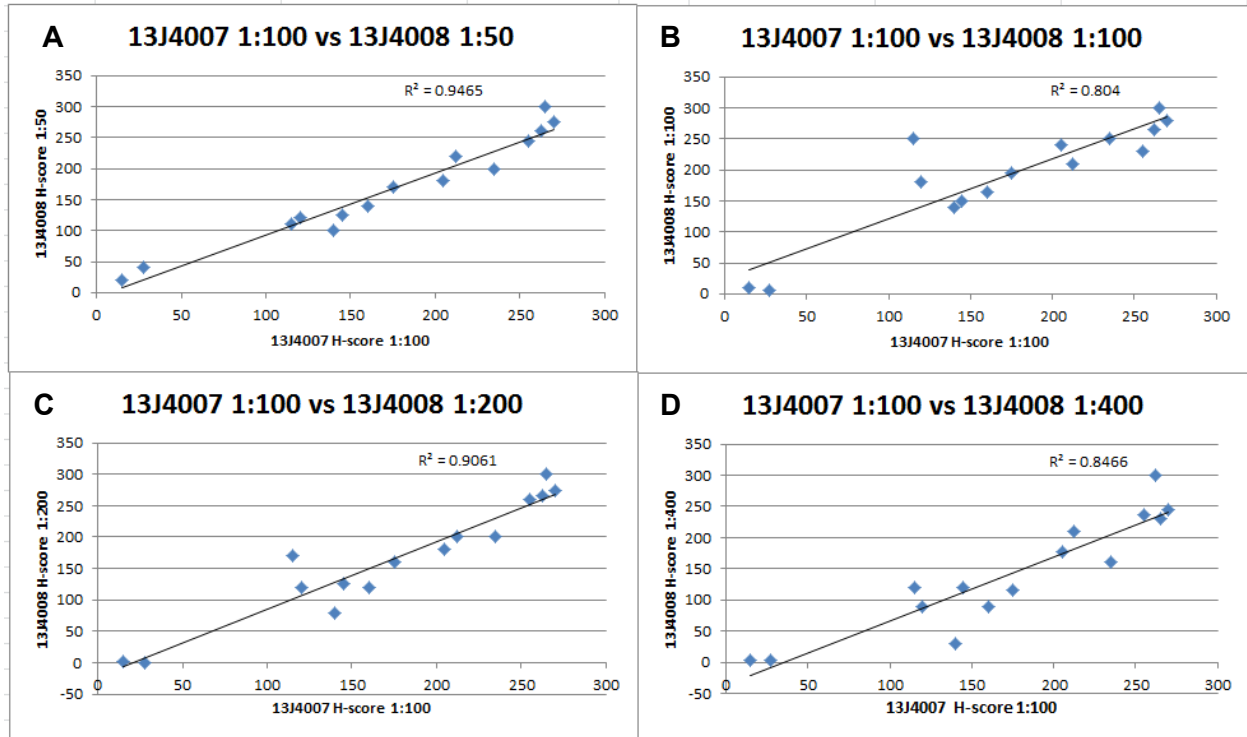
**Fig. S3. Intra-assay variation in FR $\alpha$  IHC staining of TNBC sections is negligible.** Shown are FR $\alpha$  H-scores of three 5  $\mu$ m sections from TNBC specimens, serous ovarian cancer (Sec) and liver and spleen specimens, stained using antibody lot # 13J40007. Adjacent 5  $\mu$ m were sections were taken from the blocks of TNBC specimens: TN1, TN2, TN3, TN5, TN7, TN8, TN10, TN13, TN14, TN 17 and TN19, serous ovarian (Sec) patients, Sec1, Sec2 and Sec6 and liver and spleen to represent a range of intensities.

### Supplementary Figure 4



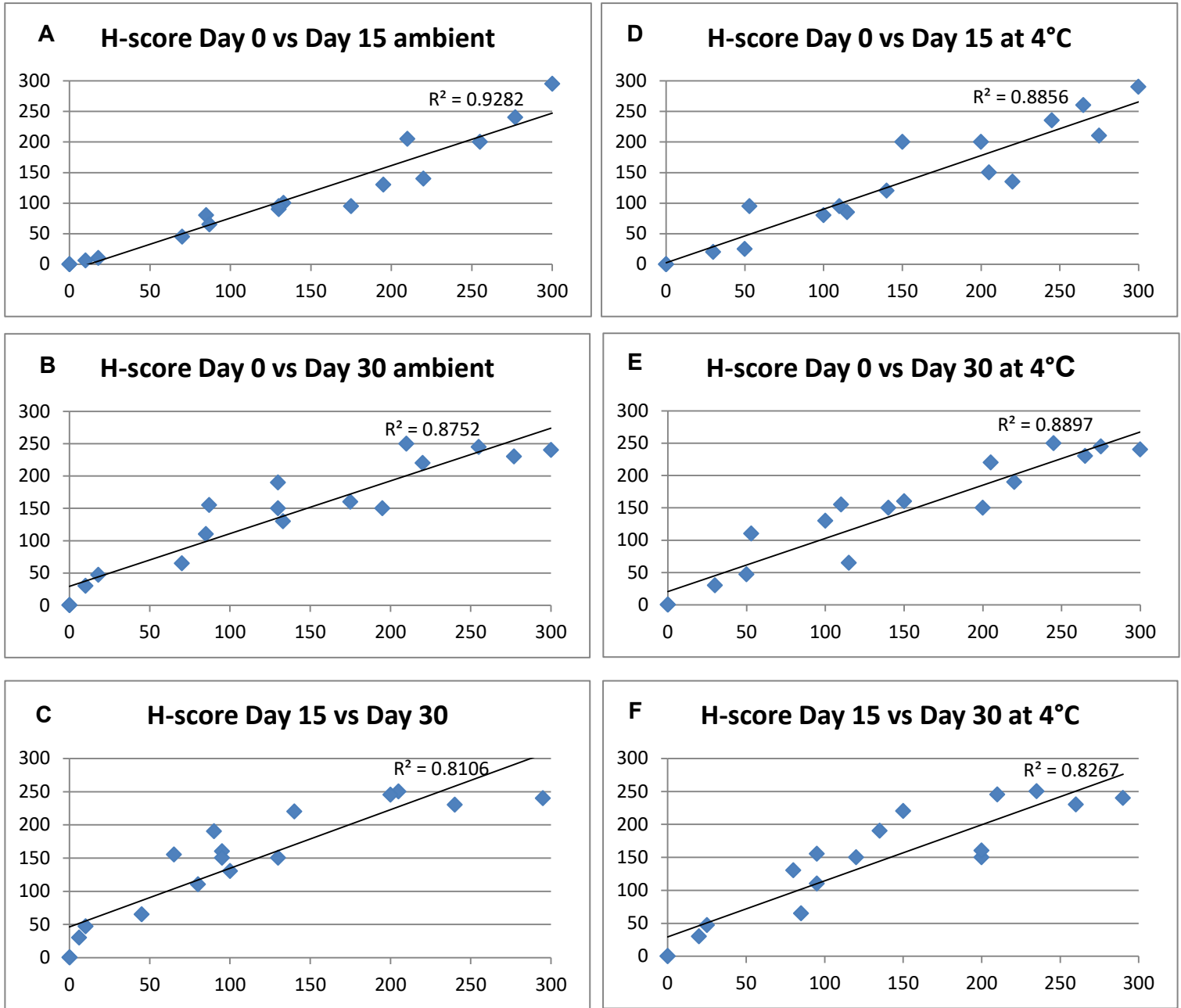
**Fig. S4: Inter-assay variation in FR $\alpha$  IHC staining of TNBC sections is negligible**  
Shown are coordinated FR $\alpha$  H-scores of two 5  $\mu$ m sections from TNBC specimens, serous ovarian cancer (Sec) as well as liver and spleen specimens, stained using antibody lot # 13J40007. Adjacent 5  $\mu$ m section were taken from the blocks of 11 TNBC specimens, three serous ovarian tumors, as well as liver and spleen to represent a range of intensities and stained on a separate day as those in Fig. S2. Sections were processed at antibody dilution of 1:100 in the same batch with the same reagents, same technician and scored by the same pathologist. Linear regression to assess correlation of H-scores on same samples processed at two different times showed a correlation of  $R^2=0.86$ .

### Supplementary Figure 5



**Fig. S5. FR $\alpha$  antibody lots showed high reproducibility.** Adjacent 5  $\mu$ m were section was taken from the blocks of TN patients: TN1, TN2, TN3, TN5, TN7, TN8, TN10, TN13, TN14, TN 17 and TN19, Serous Ovarian patients Sec 1, Sec2 and Sec6 and liver and spleen to represent a range of intensities (based on observed H-scores from the TMA in section 1). Sections were processed with a new lot of antibody (13J4008) at dilutions of 1:50 (A), 1:100 (B), 1:200 (C) and 1:400 (D) in the same batch with the same reagents, same technician and scored by the same pathologist. Linear regression was performed to assess correlation of H-scores on each dilution of the new antibody lot (13J4008) and compared against the optimized 1:100 dilution of the original antibody lot (13J4007). We observed correlations of R<sup>2</sup> = 0.95, 0.80, 0.91 and 0.85 respectively.

### Supplementary Figure 6



**Fig. S6. FR $\alpha$  antibody was stable up to 30 days at room temperature and 4°C.** For sections stored at room temperature and 4°C, we observed good correlation of H-scores between samples stored for 15 and 30 days compared to those that were stained immediately after the slides was cut. Correlation of H-score between samples stored for 30 days versus those immediately stained was highly similar to correlation observed for inter-assay and different antibody lot experiments described above.