Supplementary Materials.

Supplementary Table 1. Proteins with plasma case/control ratios up- or down-regulated >1.5-fold ($p<0.05$) or "cancer only" at the preclinical time point.

Supplementary Table 2. All plasma proteins identified by LC-MS/MS from all four tumor stages. For each protein, case/control plasma ratios in the HER2/neu and confounder mouse models and subcellular location are shown. MS events in breast cancer cell lines are also indicated. Case/control expression ratios of the genes in breast, liver, buffy coat, spleen, and thymus are also shown. Red indicates increased case/control ratios (>1.5-fold), and green indicates decrease in case/control ratios (>1.5 fold).

Supplementary Table 3. Case/control ratios for plasma proteins measured by Rules Based Medicine Rodent Multi-Analyte Profile version 2.0. The same plasma samples were used as for IPAS analysis. Red indicates plasma proteins that increased > 1.5-fold in case vs. control mice. Plasma samples from 5 to 12 mice were analyzed for each tumor stage.

Supplementary Table 4. Detailed protein identification information. The relationship between ProteinProphet score and false discovery rate is shown. Detailed information is shown for each protein identified at each time point including IPI accession, description, molecular weight, SwissProt accesscion, ProteinProphet score, total peptides identified, and peptide sequences identified.

Supplementary Figure 1. Pathway analyses of dysregulated proteins at the pre-clinical tumor stage. A) Gene Ontology (GO) Process (Metacore) and B) canonical pathway (Ingenuity) analyses of dysregulated proteins at the pre-clinical tumor stage divided by subcellular location.

Supplementary Figure 2. Pathway analyses of dysregulated proteins at the pre-clinical, 0.5 cm, and 1.0 cm tumor stages. A) Gene Ontology (GO) Process analysis (Metacore). B) GeneGo pathway maps. C-D) Selected signaling pathways from B are shown. Thermometers 1, 2, and 3 indicate levels of proteins at the pre-clinical, 0.5 cm, and 1.0 cm time points respectively. Red is increased and green is decreased in case vs. control mice.

Supplementary Figure 3. Correlation of case/control ratios of breast specific plasma proteins with case/control ratios of breast cancer gene expression. (A) Case/control ratios for proteins that were increased in plasma from 1.0 cm tumor bearing mice (>1.5-fold, $p<0.05$) and also identified in breast cancer cells by proteomic profiling. (B) Case/control gene expression ratios for the same genes from laser capture microdissected breast cancer cells (LCM GE) and non-dissected breast tissue (Breast GE).

Supplementary Figure 4. Correlation of case/control ratios of immune cell specific plasma proteins and case control ratios of immunologic tissue gene expression. (A) Case/control ratios for proteins that were increased in plasma from 1.0 cm tumor bearing mice (>1.5-fold, $p<0.05$) and that were known to be expressed in tumor infiltrating cells. (B) Case/control gene expression ratios for the same genes in buffy coat, spleen, and thymus.

Supplementary Figure 5. Correlation between mass spectrometry and Rules Based Medicine measurements. Shown are case/control ratios for eight plasma proteins measured by both methods at four different tumor stages. The Pearson r for the correlation = 0.8362 (p-value<0.0001) indicating the measurements are highly correlated.

Supplementary Figure 6. Histograms showing distribution of peptide case/control ratios for the four tumor stages. Numbers in parentheses indicate total number of quantified peptides at each stage. Histograms were centered to give a median of 0 for the peptide ratios.