**Supplemental Figure 1. Quantitative standardization of metal-conjugated antibodies.** Specially designed TMAs including positive and negative controls, cell lines and routine cancer tissues were used to determine the titer with highest signal-to-noise ratio and specificity of the staining pattern.

**Supplemental Figure 2.** **Comparison of conjugated and unconjugated antibodies.** The performance of metal-conjugated antibodies was analyzed against the average of multiple staining runs with the same unconjugated immunoglobulin. R2: linear regression coefficient.

**Supplemental Figure 3. Methods for noise determination using IMC data. (A)** shows an adaptive strategy based on intensity and density of pixels for a given markers. A bimodal distribution is identified and the pixels in the first peak are given value zero using automated analysis. **(B)** depicts a method based on top percentile selection, used to maximize the corresponding signal of each marker. PRE: before noise determination; POST: after noise determination.

**Supplemental Table 1. Metal-tagged antibody panel used in the study.** Protein targets have been validated and standardized using QIF and conjugated antibodies.

**Supplemental Table 2. Clinico-pathological characteristics of the Yale case-control series.** Tissues from trastuzumab-treated patients were matched by age and stage. Histospots were stained and acquired alongside.