Table W1. Proteins Analyzed and Antibodies Used in This Study.

| | Antibody | Distributor | Dilution |
|----------------------------|----------|----------------------------------|----------|
| p ^{S118} ERα | No. 2511 | Cell Signaling, Danvers, MA | 1:2000 |
| FAK | No. 3285 | Cell Signaling, Danvers, MA | 1:2000 |
| GSK3-β | No. 9315 | Cell Signaling, Danvers, MA | 1:1000 |
| p ^{S9} GSK3-β | No. 9336 | Cell Signaling, Danvers, MA | 1:1000 |
| HSP27 | No. 2402 | Cell Signaling, Danvers, MA | 1:1000 |
| ILK | No. 3856 | Cell Signaling, Danvers, MA | 1:2000 |
| MET | No. 3127 | Cell Signaling, Danvers, MA | 1:1000 |
| PAI-1 | AHP1100 | Serotec, Oxford, United Kingdom | 1:5000 |
| PI3K | No. 4292 | Cell Signaling, Danvers, MA | 1:1000 |
| p38 | No. 9212 | Cell Signaling, Danvers, MA | 1:1000 |
| p ^{T180/Y182} p38 | No. 4631 | Cell Signaling, Danvers, MA | 1:1000 |
| ROCK | No. 4035 | Cell Signaling, Danvers, MA | 1:1000 |
| p ^{S727} STAT3 | No. 9136 | Cell Signaling, Danvers, MA | 1:1000 |
| uPA | Ab19893 | Abcam, Cambridge, United Kingdom | 1:500 |

Table W2. Patient and Disease Characteristics Related to the Collective of 52 Node-Positive Patients Analyzed to Study Protein Expression Correlations between Primary Tumor and Lymph Node Metastases.

| Factor | Patient Collective $(n = 52)$ | |
|-------------------------|-------------------------------|------|
| | No. Patients | % |
| Age | | |
| <50 | 6 | 11.5 |
| ≥50 | 46 | 88.5 |
| Tumor state | | |
| T1 | 11 | 21.2 |
| T2 | 31 | 59.6 |
| T3 | 7 | 13.5 |
| T4 | 3 | 5.8 |
| Nodal status | | |
| N1 | 22 | 42.3 |
| N2 | 16 | 30.8 |
| N3 | 14 | 26.9 |
| Subtype | | |
| Ductal | 38 | 73.1 |
| Lobular | 10 | 19.2 |
| Ductal-lobular | 2 | 3.8 |
| Other | 2 | 3.8 |
| Distant metastasis | | |
| Positive | 0 | 0.0 |
| Negative | 50 | 96.2 |
| Unknown | 2 | 3.8 |
| Grading | | |
| 1 | 1 | 1.9 |
| 2 | 24 | 46.1 |
| 3 | 27 | 51.9 |
| Hormone receptor status | | |
| Positive | 41 | 78.9 |
| Negative | 10 | 19.2 |
| Unknown | 1 | 1.9 |

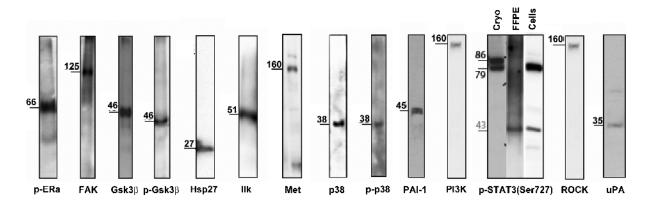


Figure W1. Validation of antibody specificity by Western blot analysis. All antibodies were validated using breast cancer FFPE tissue samples unless stated otherwise.

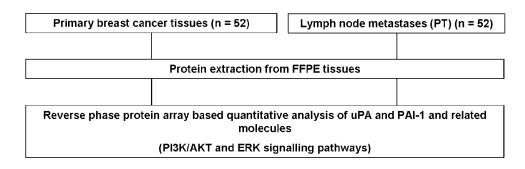


Figure W2. Strategy for the analysis of the collective used in this study.

Table W3. Correlation of the Expression of the 14 Analyzed Signaling Molecules between Primary Tumors and Lymph Node Metastases.

| | $r_{\rm s}$ | P (two-sided) |
|---------|-------------|---------------|
| FAK | 0.597 | .000 |
| GSK3-β | 0.270 | >.999 |
| pGSK3-β | 0.646 | .000 |
| ILK | 0.697 | .000 |
| MET | 0.603 | .001 |
| PI3K | 0.655 | .000 |
| ROCK | 0.571 | .000 |
| pp38 | 0.576 | .000 |
| p38 | 0.651 | .000 |
| PAI-1 | 0.173 | >.999 |
| uPA | 0.566 | .008 |
| pSTAT3 | 0.453 | .000 |
| pER | 0.446 | .000 |

The Spearman correlation coefficient r_s and P values are shown. All P values are adjusted for multiple testing as described in the Materials and Methods section. Statistically significant correlations are in bold face emphases.

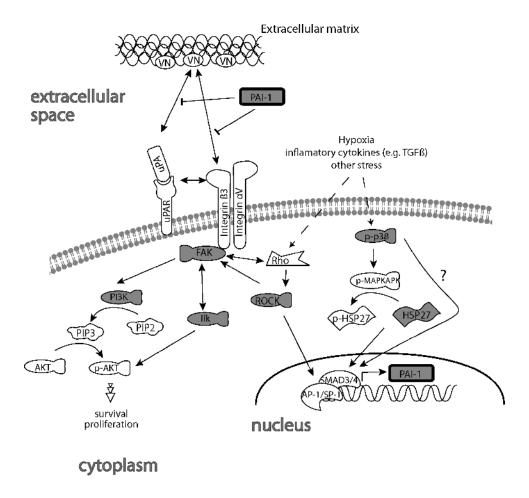


Figure W3. Overview of pathways correlating with high PAI-1 expression (gray). PAI-1 expression was found to be correlated with several proteins involved in AKT signaling (FAK, ILK, ROCK, and PI3K), supporting the assumption that PAI-1 is involved in the activation of AKT through integrin $\alpha_V \beta_3$ -induced PI3K activation. Furthermore, PAI-1 correlates with HSP27 and pp38, which are known to increase PAI-1 expression through transcription factor activation.