Supplementary Material:

Supplementary Figure 1: Correlation of estrogen receptor status and Plexin B1 defines a threshold for Plexin B1 Affymetrix expression values

Scatter plot of Affymetrix expression values of Plexin B1 (probe set ID 215807_s_at) vs. the rank of expression among 119 breast cancer samples. Estrogen receptor positive samples are represented by blue dots while ER negative samples shown as red dots. The horizontal line and shaded region represents the Affymetrix expression value of 500 which was adopted as a biological threshold based on Plexin B1 expression in ER negative samples. The absolute numbers of ER positive and negative samples above and below this threshold is given.

Supplementary Figure 4: Detection of Plexin B1 protein on tumor cells

Plexin B1 protein was detected in 5 µm sections of tumor tissue from a breast cancer patient using a monoclonal antibody directed against Plexin B1 and visualized using a Cy3 labeled secondary antibody (red staining). DAPI was used to counterstain the nuclei of cells (blue staining). **A:** normal mammary epithelial cells, **B:** tumor cells of an invasive ductal mammary carcinoma. No staining was seen when mammary carcinoma without Plexin B1 mRNA expression were analyzed (data not shown).

Supplementary Figure 5: Plexin B1 gene expression among different normal tissues

Affymetrix microarray data of Plexin B1 (probe set ID 215807_s_at) expression in 79 human tissues were retrieved from the SymGene database (<u>http://symatlas.gnf.org/SymAtlas/</u>) and represented as bar chart. Median, threefold and tenfold median values are represented by black, blue and red lines, respectively.

Supplementary Figure 6: Plexin B1 is dysregulated among "uncoupled" breast cancers

Box plot of Affymetrix expression values of Plexin B1 (probe set ID 215807_s_at) in different sample groups. Samples are stratified as stem cell like (SCL) and Non-SCL breast cancers and by their estrogen receptor status. Among ER positive Non-SCL tumors those with a normal coupling between receptor status and proliferation are separated from the "uncoupled" tumors ("ER⁺ unc.").

Supplementary Figure 7: Validation of Plexin B1 expression by quantitative Real-Time PCR

Reverse transcription based Real Time PCR was performed on 29 independent tissue samples from tumors which were profiled before on Affymetrix microarrays. Box plots of Δc_t values (Plexin B1 vs. GPDH) are given and samples stratified by the Plexin B1 expression value on the Affymetrix platform.

Supplementary Figure 8: Multidimensional scaling of Plexin B1 vs. ESR1 and proliferation

All 119 breast cancer samples were ranked according to their Affymetrix expression values of Plexin B1 (215807_s_at), ESR1 (205225_at), and their proliferative activity based on the cluster of 136 proliferation associated genes. Tumor samples were then placed in a three dimensional scatter plot according to these ranks and a surface visualisation was obtained by using local linear regression as a smoother with a normal kernel and a bandwith of 3.0.

Supplementary Figure 9: Analysis of the proliferation state of breast cancer samples

A cluster of 136 highly correlated genes well known for their association with proliferation was used to obtain a robust quantitative metric of the proliferation. Each marker was median centered over all samples, subsequently the median expression of all marker determined for each sample and finally as an ordinal variable all samples ranked according this proliferation metric from left to right. The 136 applied probe sets are listed in Suppl. Table 4.

Supplementary Figure 10: Detection of Plexin B1 protein in normal human skin tissue

Plexin B1 protein was detected in 15 μ m sections of normal skin tissue using a monoclonal antibody directed against Plexin B1 and visualized using a Cy3 labeled secondary antibody (red staining). IHC was performed as described for the detection of Plexin B1 in breast cancer samples. The epidermis including str. corneum, str. spinosum and str. basale are positive for Plexin B1 protein expression. No staining was observed in the dermis.

Supplementary Table 3: Differential expression of Plexin B1 according to published microarray datasets

Supplementary Table 4: Genes applied for proliferation metric

List of the 136 highly correlated proliferation associated probe sets used for the analysis of proliferative state of samples in Supplementary Figure 9.

Figure 1: Correlation of estrogen receptor status and Plexin B1 defines a threshold for Plexin B1 Affymetrix expression values



Supplementary Figure 4: Detection of Plexin B1 protein on tumor cells









Supplementary Figure 5: Plexin B1 gene expression among different normal tissues



Supplementary Figure 6: Plexin B1 is dysregulated among "uncoupled" breast cancers









Supplementary Figure 8: Scatter plot of Plexin B1 vs. ESR1 and proliferation

Supplementary Figure 9: Analysis of the proliferation state of breast cancer samples

Supplementary Figure 10: Detection of Plexin B1 protein in human skin tissue



Epidermis

Dermis

Supplementary Table 3: Differential expression of Plexin B1 according to published microarray datasets

| Study | Platform | Reporter | Test | p-value |
|----------------------|--|---|---|--|
| Wang et al. | Affymetrix U133A | 215807_s_at | T-Test | 0.00005 |
| van de Vijver et al. | Inkjet spotted oligos | AB007867 | T-Test | 0.003 |
| Zhao et al. | spotted cDNA | IMAGE:2541458 | T-Test | 0.017 |
| Gruvberger et al. | spotted cDNA | IMAGE:755952 | T-Test | 0.035 |
| Sotiriou et al. 2003 | spotted cDNA | IMAGE:755952 | T-Test | 0.110 |
| Sorlie et al. 2003 | spotted cDNA | IMAGE:755952 | T-Test | 0.800 |
| West et al. | Affymetrix HuGeFL | X87904_at | T-Test | 0.310 |
| Perou et al. | spotted cDNA | IMAGE:755952 | T-Test | 0.018 |
| Sotiriou et al. 2003 | spotted cDNA | IMAGE:755952 | T-Test | 0.032 |
| Sorlie et al. 2003 | spotted cDNA | IMAGE:755952 | T-Test | 0.082 |
| van t'Veer et al. | Inkjet spotted oligos | AB007867 | T-Test | 0.011 |
| van de Vijver et al. | Inkjet spotted oligos | AB007867 | T-Test | 0.035 |
| van de Vijver et al. | Inkjet spotted oligos | NM_002673 | T-Test | 0.144 |
| van t'Veer et al. | Inkjet spotted oligos | AB007867 | Cox-Regr. | 0.006 |
| van de Vijver et al. | Inkjet spotted oligos | AB007867 | Cox-Regr. | 0.024 |
| | StudyWang et al.van de Vijver et al.Zhao et al.Gruvberger et al.Sotiriou et al. 2003Sorlie et al. 2003West et al.Perou et al.Sotiriou et al. 2003Sorlie et al. 2003Sorlie et al. 2003van t'Veer et al.van de Vijver et al. | StudyPlatformWang et al.Affymetrix U133Avan de Vijver et al.Inkjet spotted oligosZhao et al.spotted cDNAGruvberger et al.spotted cDNASotiriou et al. 2003spotted cDNASorlie et al. 2003spotted cDNAWest et al.Affymetrix HuGeFLPerou et al.spotted cDNASotiriou et al. 2003spotted cDNAVest et al.Affymetrix HuGeFLPerou et al.spotted cDNASorlie et al. 2003spotted cDNAVan t'Veer et al.Inkjet spotted oligosvan de Vijver et al.Inkjet spotted oligosvan t'Veer et al.Inkjet spotted oligosvan de Vijver et al.Inkjet spotted oligos | StudyPlatformReporterWang et al.Affymetrix U133A215807_s_atvan de Vijver et al.Inkjet spotted oligosAB007867Zhao et al.spotted cDNAIMAGE:2541458Gruvberger et al.spotted cDNAIMAGE:755952Sotiriou et al. 2003spotted cDNAIMAGE:755952Sorlie et al. 2003spotted cDNAIMAGE:755952West et al.Affymetrix HuGeFLX87904_atPerou et al.spotted cDNAIMAGE:755952Sotiriou et al. 2003spotted cDNAIMAGE:755952Sotiriou et al. 2003spotted cDNAIMAGE:755952Sotiriou et al. 2003spotted cDNAIMAGE:755952Sotiriou et al. 2003spotted cDNAIMAGE:755952Van t'Veer et al.Inkjet spotted oligosAB007867van de Vijver et al.Inkjet spotted oligosAB007867 | StudyPlatformReporterTestWang et al.Affymetrix U133A215807_s_atT-Testvan de Vijver et al.Inkjet spotted oligosAB007867T-TestZhao et al.spotted cDNAIMAGE:2541458T-TestGruvberger et al.spotted cDNAIMAGE:755952T-TestSotiriou et al. 2003spotted cDNAIMAGE:755952T-TestSorlie et al. 2003spotted cDNAIMAGE:755952T-TestWest et al.Affymetrix HuGeFLX87904_atT-TestPerou et al.spotted cDNAIMAGE:755952T-TestSotiriou et al. 2003spotted cDNAIMAGE:755952T-TestVest et al.Affymetrix HuGeFLX87904_atT-TestSotiriou et al. 2003spotted cDNAIMAGE:755952T-TestVan t'Veer et al.Inkjet spotted oligosAB007867T-Testvan de Vijver et al.Inkjet spotted oligosAB007867Cox-Regr.van de Vijver et al.Inkjet spotted oligosAB007867Cox-Regr.van de Vijver et al.Inkjet spotted oligosAB007867Cox-Regr. |

Supplementary Table 4: Genes applied for proliferation metric

| Affymetrix probe set | Gene Symbol |
|----------------------|----------------|
| 219918 s at | ASPM |
| 209464 at | AURKB |
| 210334 x at | BIRC5 |
| 202094 at | BIRC5 |
| 202095 s at | BIRC5 |
| 205733 at | BLM |
| 219555 s at | BM039 |
| 222118 at | BM039 |
| 212949 at | BRRN1 |
| 209642 at | BUB1 |
| 215509 s at | BUB1 |
| 203755 at | BUB1B |
| 218542 at | C10orf3 |
| 217835 x at | C20orf24 |
| 213226 at | CCNA2 |
| 203418 at | CCNA2 |
| 214710 s at | CCNB1 |
| 202705 at | CCNB2 |
| 205034 at | CCNE2 |
| 211814 s at | CCNE2 |
| 208696_at | CCT5 |
| 210559 s at | CDC2 |
| 203214 x at | CDC2 |
| 203213_at | CDC2 |
| 202870 s at | CDC20 |
| 204695 at | CDC25A |
| 204696 s at | CDC25A |
| 221436 s at | CDCA3 |
| 221520 s at | CDCA8 |
| 204252 at | CDK2 |
| 211804 s at | CDK2 |
| 209714 s at | CDKN3 |
| 204962 s at | CENPA |
| 210821 x at | CENPA |
| 205046 at | CENPE |
| 207828 s at | CENPE |
| 209172 s at | CENPE |
| 218447 at | DC13 |
| 201584 s at | DDX39 |
| 218726 at | DKFZn762F1312 |
| 203764 at | DI G7 |
| 213092 x at | DNAIC9 |
| 213092_A_at | DNAIC9 |
| 213000_5_at | DONSON |
| 221077_5_dt | ESPI 1 |
| 38158 at | EST L1 |
| 204603 at | ESI EI EVOI |
| 204005_at | EAUI |
| 205558_8_at | ELEZ |
| 2108/3_8_at | FDAU3 |
| 204/68_s_at | FENI |

| Affymetrix probe set | Gene Symbol |
|----------------------|-------------|
| 204767 s at | FEN1 |
| 221591 s at | FLJ10156 |
| 220060 s at | FLJ20641 |
| 202580 x at | FOXM1 |
| 207590 s at | FSHPRH1 |
| 214804 at | FSHPRH1 |
| 214431 at | GMPS |
| 204317 at | GTSE1 |
| 215942 s at | GTSE1 |
| 204318 s at | GTSE1 |
| 204315 s at | GTSE1 |
| 200853 at | H2AFZ |
| 213911 s at | H2AFZ |
| 218663 at | HCAP-G |
| 218663 at | HCAP-G |
| 218662 s at | HCAP-G |
| 218662 s at | HCAP-G |
| 203744 at | HMGB3 |
| 207165 at | HMMR |
| 209709 s at | HMMR |
| 217755 at | HN1 |
| 206102 at | KIAA0186 |
| 204444 at | KIF11 |
| 206364 at | KIF14 |
| 218755 at | KIF20A |
| 211519 s at | KIF2C |
| 209408 at | KIF2C |
| 218355 at | KIF4A |
| 209680 s at | KIFC1 |
| 219306 at | KNSL7 |
| 204162 at | KNTC2 |
| 201088 at | KPNA2 |
| 211762 s at | KPNA2 |
| 203276 at | LMNB1 |
| 222039 at | LOC146909 |
| 203362 s at | MAD2L1 |
| 201475 x at | MARS |
| 220651 s at | MCM10 |
| 202107 s at | MCM2 |
| 212142 at | MCM4 |
| 212141 at | MCM4 |
| 222036 s at | MCM4 |
| 222037 at | MCM4 |
| 201930 at | MCM6 |
| 204825 at | MELK |
| 212023_s_at | MKI67 |
| 212020_s_at | MKI67 |
| 212021_s_at | MKI67 |
| 212022_s_at | MKI67 |
| 218982 s at | MRPS17 |
| | |

| Affymetrix probe set | Gene Symbol |
|----------------------|-------------|
| 201710_at | MYBL2 |
| 211080_s_at | NEK2 |
| 204641_at | NEK2 |
| 218039_at | NUSAP1 |
| 219978_s_at | NUSAP1 |
| 213599_at | OIP5 |
| 204267_x_at | PKMYT1 |
| 202240_at | PLK1 |
| 219510_at | POLQ |
| 218009_s_at | PRC1 |
| 203554_x_at | PTTG1 |
| 222077_s_at | RACGAP1 |
| 205024_s_at | RAD51 |
| 203022_at | RNASEH2A |
| 201890_at | RRM2 |
| 209773_s_at | RRM2 |
| 214096_s_at | SHMT2 |
| 214437_s_at | SHMT2 |
| 205339_at | SIL |
| 201195_s_at | SLC7A5 |
| 201342_at | SNRPC |
| 203145_at | SPAG5 |
| 208079_s_at | STK6 |
| 204092_s_at | STK6 |
| 203046_s_at | TIMELESS |
| 209753_s_at | TMPO |
| 209754_s_at | TMPO |
| 203432_at | TMPO |
| 220865_s_at | TPRT |
| 210052_s_at | TPX2 |
| 204033_at | TRIP13 |
| 204649_at | TROAP |
| 204822_at | TTK |
| 202954_at | UBE2C |
| 202779_s_at | UBE2S |
| 204026_s_at | ZWINT |