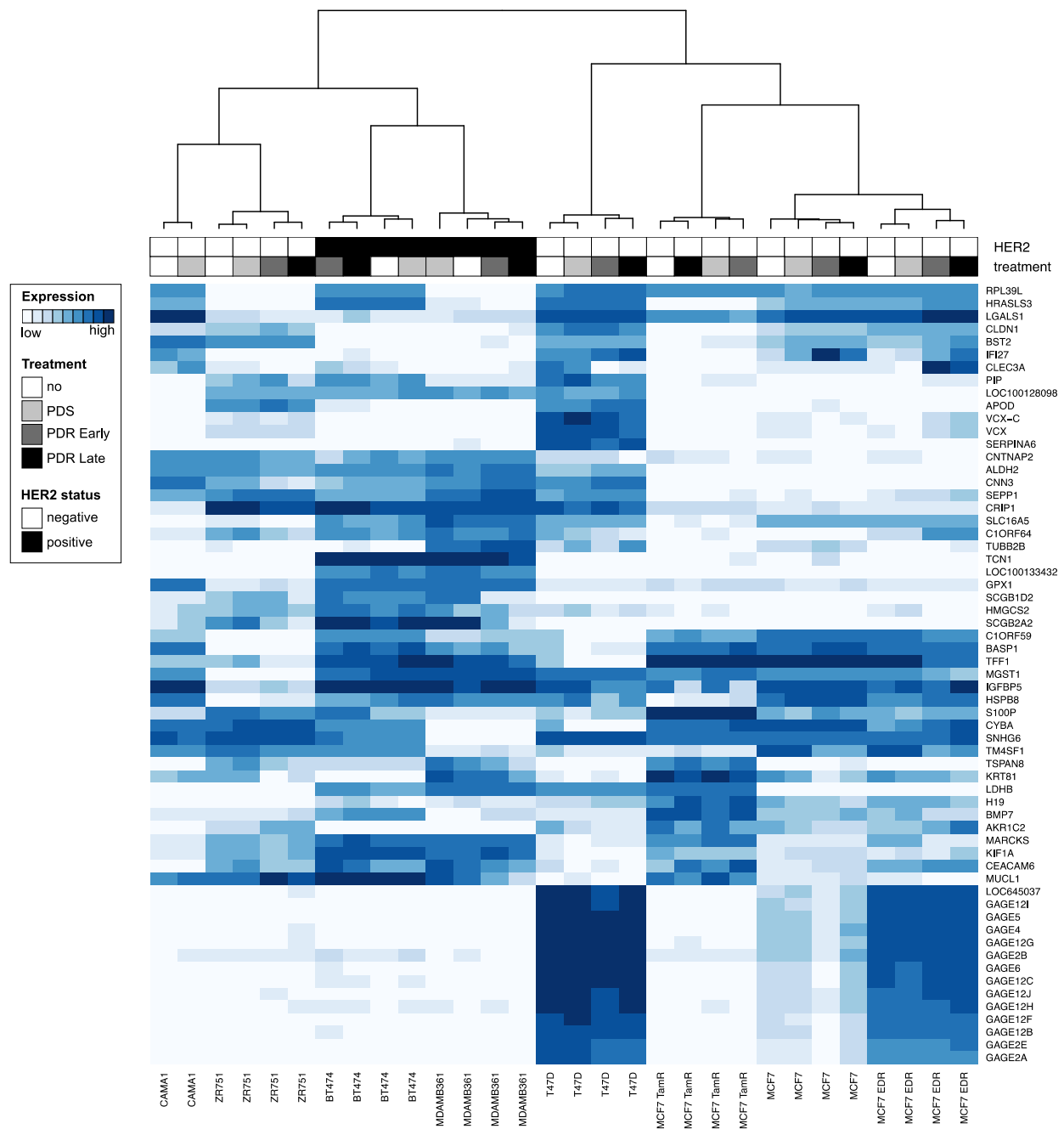
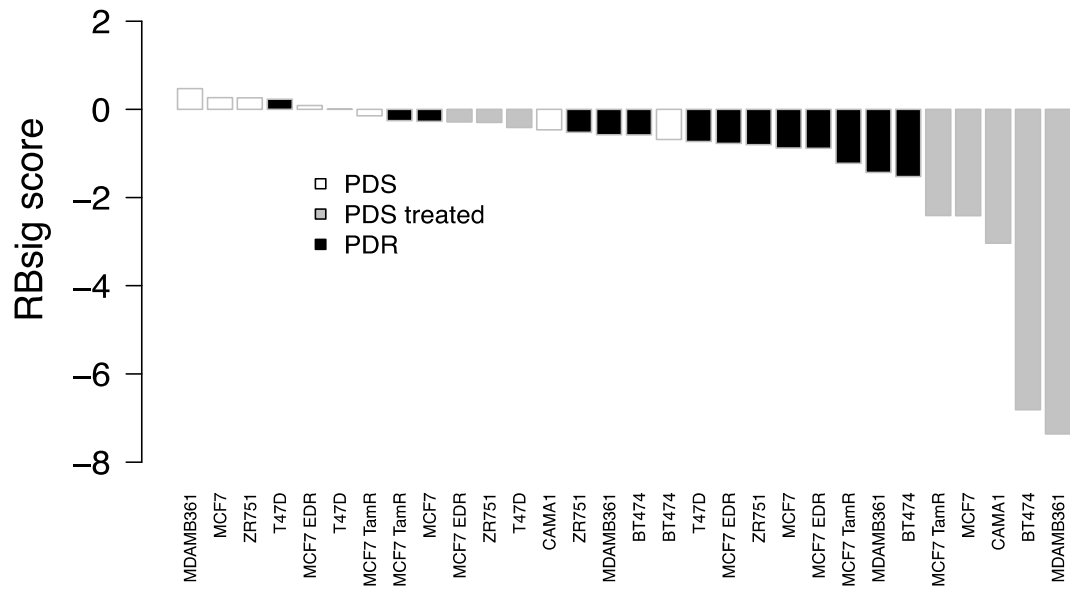


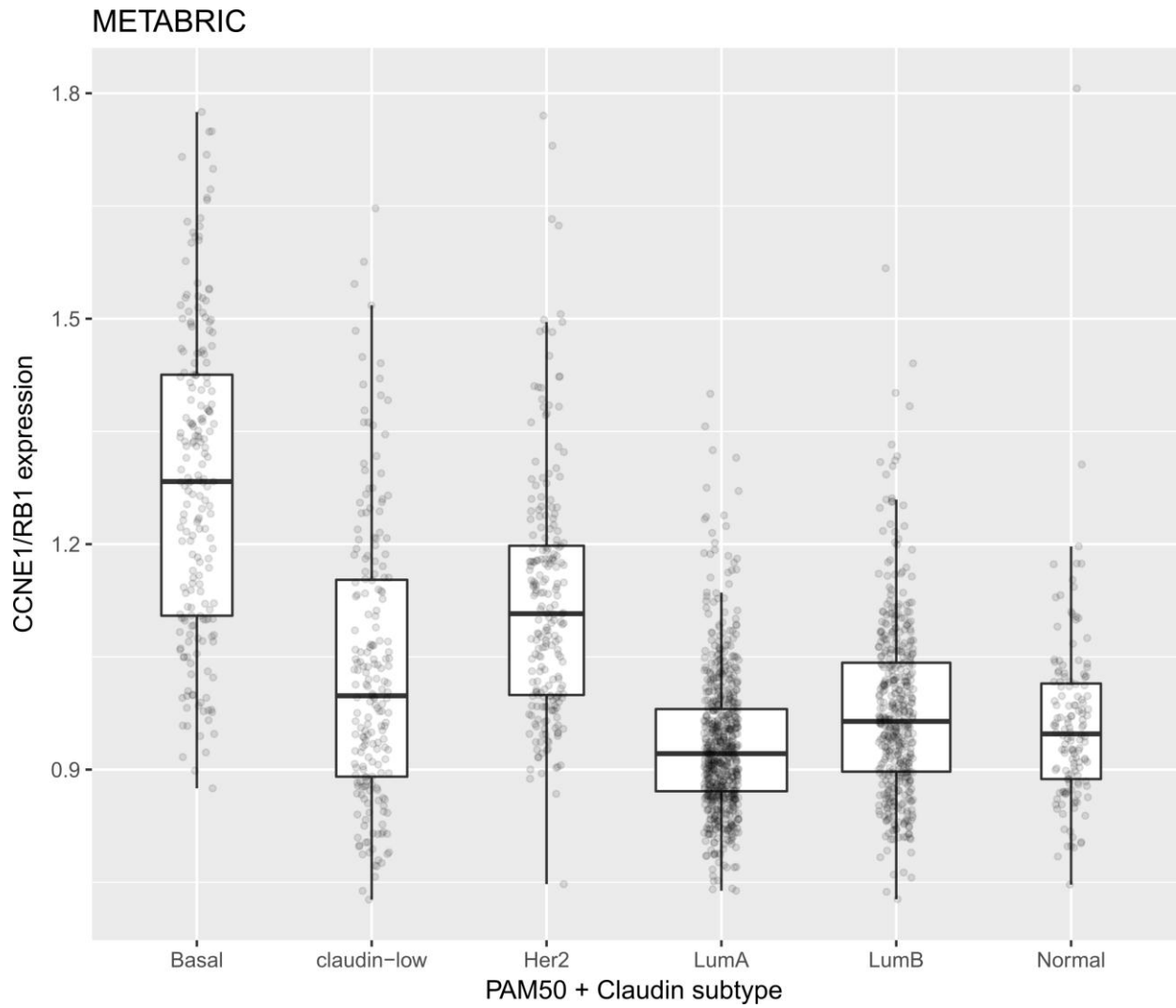
Supplementary Figure s1. Representative images of β -galactosidase senescence assay.



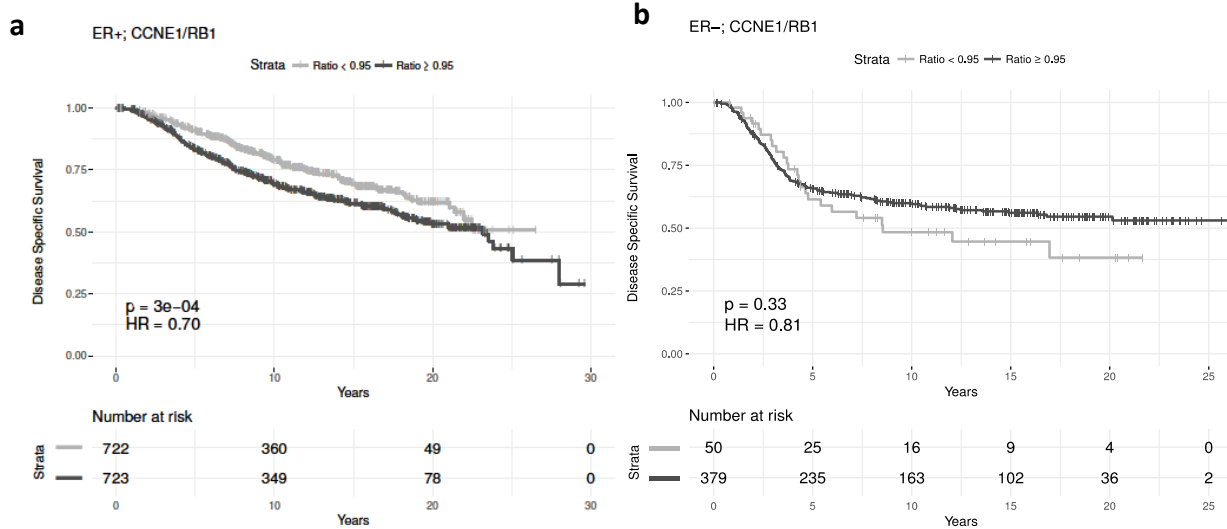
Supplementary Figure s2. Hierarchical clustering of PDS, PDS treated and PDR cell line models (n=30) using ‘1 – Pearson’s correlation’ as distance measure on the expression values of the top 2.5%o variable genes (n=61). Details about the different treatments and HER2 status are reported in the annotation track above the heat map.



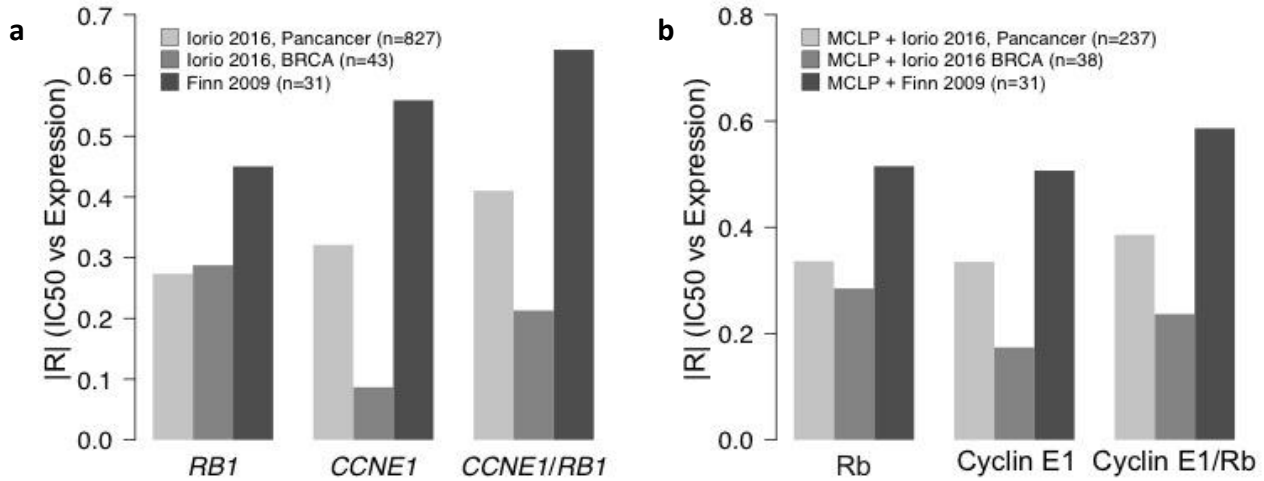
Supplementary Figure s3. Bar plot of RBsig score across PDS, PDS treated and PDR cell line models.



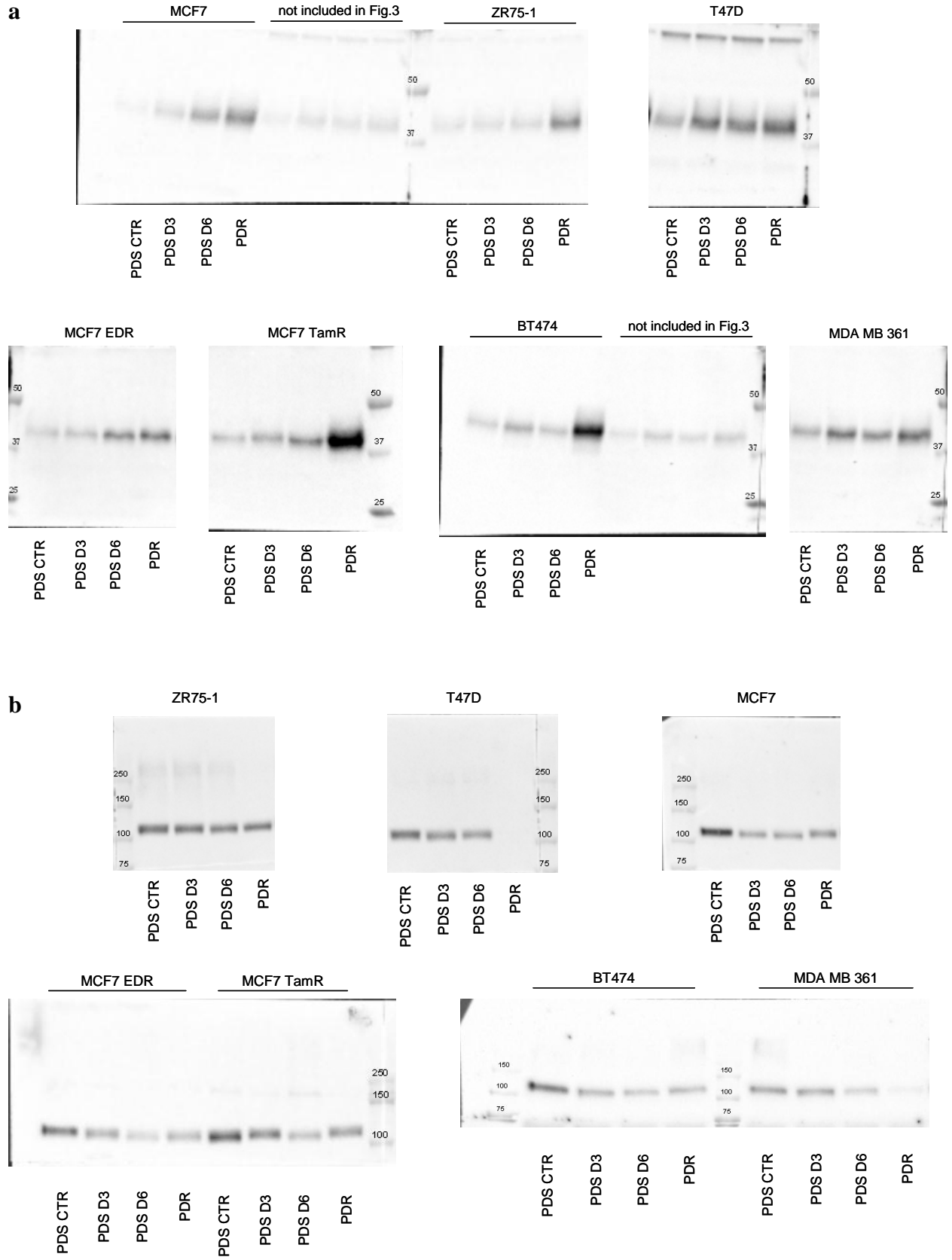
Supplementary Figure s4. Box plots of the distribution of the ratio between the expression values of *CCNE1* to *RB1* across the different PAM50 subtypes in the METABRIC dataset¹⁵.



Supplementary Figure s5. a) Kaplan-Meier curves according to high (above the median, dark grey) or low (below the median, light grey) levels of *CCNE1/RB1* in the METABRIC dataset¹⁵ (n=1904) for ER+ (n=1445) samples. b) Kaplan-Meier curves according to high (above the median, dark grey) or low (below the median, light grey) levels of *CCNE1/RB1* in the METABRIC dataset¹⁵ (n=1904) for ER- samples (n=429).



Supplementary Figure s6. a) Bar plot of the absolute value of Pearson's correlation coefficient of IC50 values versus protein expression of *RB1*, *CCNE1* and *CCNE1/RB1* by considering both IC50 values and expression data for 827 cell lines of different tumors from Iorio *et al.*, 2016¹² (light grey); both IC50 values and expression data for 43 breast cancer cell lines from Iorio *et al.*, 2016¹² (middle grey); IC50 values and expression data for 31 breast cancer cell lines from Iorio *et al.*, 2016¹² and Finn *et al.*, 2009¹⁴, respectively (dark grey). b) Bar plot of the absolute value of Pearson's correlation coefficient of IC50 values versus expression of Rb, Cyclin E1 and Cyclin E1/Rb by considering IC50 values and protein expression data for 237 cell lines of different tumors from Iorio *et al.*, 2016¹² and MCLP¹³, respectively (light grey); IC50 values and protein expression data for 38 breast cancer cell lines from Iorio *et al.*, 2016¹² and MCLP¹³, respectively (middle grey); IC50 values and protein expression data for 31 breast cancer cell lines from Finn *et al.*, 2009¹⁴ and MCLP¹³, respectively (dark grey).



Supplementary Figure s7. Uncropped scans of cyclin E1 (a) and Rb (b) blots

Supplementary Table s1: IC50 values of PDS and PDR models. IC50 values were assessed after 9 days by methylene blue assay. Mean value of IC50 of three independent experiments +/- standard error (SE) was calculated (P-values <0.05, by Student's t test, were considered significant).

| | IC50 PDS (SE) [nM] | IC50 PDR (SE) [nM] | IC50 PDR/IC50 PDS | IC50 PDR/IC50 PDS P-value |
|---------------|--------------------|--------------------|-------------------|---------------------------|
| T47D | 180.6 (13.5) | 5766.2 (30.6) | 31.9 | 0.000 |
| ZR75-1 | 78.3 (10.1) | 1429.0 (131.1) | 18.3 | 0.009 |
| MCF7 | 670.2 (79.7) | 4112.8 (784.3) | 6.1 | 0.048 |
| MCF7 EDR | 18.1 (3.7) | 580.4 (102.0) | 32.1 | 0.031 |
| MCF7 TamR | 309.5 (44.6) | 5001.2 (112.5) | 16.2 | 0.001 |
| BT474 | 174.7 (26.3) | 3073.2 (242.5) | 17.6 | 0.007 |
| MDA MB 361 | 216.2 (31.2) | 2653.2 (293.0) | 12.3 | 0.014 |
| CAMA1 | 101.0 (28.5) | NA | NA | NA |

Supplementary Table s2: Normalized expression (FC, see Methods) of the top 100 differentially expressed genes for PDS treated and PDR cell lines. Columns 2 and 3 report averaged (i.e., by mean) FC values. Differential expression between PDS treated and PDR cell lines is calculated by Wilcoxon-Mann-Whitney test (column 4). Resulting p-values are adjusted by Benjamini Hochberg correction (column 5).

| GeneID (HGNC) | PDS treated (FC) | PDR (FC) | P-value PDR vs PDS treated (WMW) | Adj P-value (FDR) |
|---------------|------------------|--------------|----------------------------------|-------------------|
| LOC645411 | -0,110083652 | 0,21532616 | 1,25E-05 | 0,302242237 |
| FPR1 | -0,23060604 | 0,10827435 | 7,51E-05 | 0,90672671 |
| CNTNAP2 | -0,19271208 | -1,073305596 | 0,000118835 | 0,957100416 |
| FOS | 0,243863753 | -0,597847134 | 0,000187635 | 1 |
| CCNE1 | -0,206864622 | 1,073067251 | 0,000281452 | 1 |
| SYCE2 | -0,439702695 | -0,007074492 | 0,000281452 | 1 |
| EFNB2 | 0,216479809 | -0,714866509 | 0,000419051 | 1 |
| ELF1 | 0,1996513 | -0,303259103 | 0,000419051 | 1 |
| HS.335413 | 0,028845452 | 0,369625895 | 0,000600432 | 1 |
| JAG2 | -0,132061152 | 0,288982933 | 0,000600432 | 1 |
| TOB2 | 0,087238226 | 0,389363543 | 0,000600432 | 1 |
| LOC401074 | 0,035871249 | -0,403548612 | 0,000850611 | 1 |
| UTP14C | 0,146617934 | -0,27379496 | 0,000850611 | 1 |
| MRPL37 | -0,257050105 | -0,094243669 | 0,001175845 | 1 |
| MTIF3 | 0,148457856 | -0,179777014 | 0,001175845 | 1 |
| MUTED | 0,145058856 | -0,125525875 | 0,00161366 | 1 |
| PAM | 0,426600634 | -0,152727975 | 0,00161366 | 1 |
| SLC44A3 | 0,354837442 | -0,011074872 | 0,00161366 | 1 |
| WBP11P1 | 0,159173042 | -0,060948698 | 0,00161366 | 1 |
| CD58 | 0,101009028 | -0,34012774 | 0,00217031 | 1 |
| DHRS7 | 0,286372012 | -0,06211949 | 0,00217031 | 1 |
| GLT25D1 | -0,150749421 | 0,182148104 | 0,00217031 | 1 |
| LOC646223 | 0,02217994 | -0,321545645 | 0,00217031 | 1 |
| LOC646358 | 0,086064327 | -0,229496171 | 0,00217031 | 1 |
| RPAIN | -0,166256313 | 0,028027778 | 0,00217031 | 1 |
| TBL1X | -0,03114284 | -0,290368787 | 0,00217031 | 1 |
| ZNF274 | -0,137263148 | 0,117572506 | 0,00217031 | 1 |
| ATP7B | 0,209383044 | -0,348126158 | 0,002895831 | 1 |
| C10RF27 | 0,200062836 | -0,085729713 | 0,002895831 | 1 |
| COQ10A | -0,155120037 | 0,278661382 | 0,002895831 | 1 |
| N6AMT2 | 0,145846053 | -0,17789196 | 0,002895831 | 1 |
| NDRG4 | 0,068042824 | 0,324150284 | 0,002895831 | 1 |
| DHODH | -0,183774849 | 0,081362269 | 0,003713278 | 1 |
| BAZ2B | 0,178258073 | -0,065866732 | 0,003802733 | 1 |
| C16ORF38 | 0,290284736 | -0,016237359 | 0,003802733 | 1 |
| CAP2 | 0,272511767 | -0,222774758 | 0,003802733 | 1 |
| CCNA1 | 0,181125585 | -0,198687909 | 0,003802733 | 1 |
| CCNDBP1 | 0,33262544 | -0,069143591 | 0,003802733 | 1 |
| DHDH | -0,196398612 | 0,134514237 | 0,003802733 | 1 |
| DNAJC12 | 0,427118157 | -0,000914239 | 0,003802733 | 1 |

| | | | | |
|--------------|--------------|--------------|-------------|---|
| LOC645018 | -0,285789064 | 0,074474228 | 0,003802733 | 1 |
| MAST4 | -0,2606596 | 0,175404124 | 0,003802733 | 1 |
| NAT6 | -0,156234618 | 0,101242918 | 0,003802733 | 1 |
| NRCAM | 0,064529211 | -0,461146289 | 0,003802733 | 1 |
| CRELD1 | 0,40867371 | 0,14679551 | 0,00495356 | 1 |
| CXCL12 | 0,372685386 | -0,348401384 | 0,00495356 | 1 |
| CXORF26 | 0,1389814 | -0,087579432 | 0,00495356 | 1 |
| CYP2D7P1 | 0,075643719 | -0,080779408 | 0,00495356 | 1 |
| DSE | 0,131450304 | -0,273675209 | 0,00495356 | 1 |
| DYNLL2 | -0,191684851 | 0,071249835 | 0,00495356 | 1 |
| ERRFI1 | 0,296544557 | -0,050562421 | 0,00495356 | 1 |
| F8A2 | -0,060096157 | -0,368636667 | 0,00495356 | 1 |
| HMGCS2 | 0,898669527 | -0,200977899 | 0,00495356 | 1 |
| IKZF4 | -0,043543007 | 0,185814257 | 0,00495356 | 1 |
| LOC100133489 | -0,141261656 | 0,155419833 | 0,00495356 | 1 |
| MPPED2 | 0,126355766 | -0,368479988 | 0,00495356 | 1 |
| NFKB1 | 0,074306206 | -0,233144083 | 0,00495356 | 1 |
| POLR2F | -0,30245563 | 0,035829904 | 0,00495356 | 1 |
| RAB20 | 0,244550809 | -0,091427026 | 0,00495356 | 1 |
| RAB7A | 0,200049299 | -0,006506248 | 0,00495356 | 1 |
| RDBP | -0,22057093 | 0,137403184 | 0,00495356 | 1 |
| SAMD4B | -0,063063188 | 0,137098774 | 0,00495356 | 1 |
| UBA6 | 0,082164351 | -0,202367235 | 0,00495356 | 1 |
| ARPC4 | 0,067217198 | -0,090379758 | 0,006367076 | 1 |
| C6ORF173 | -0,760443889 | -0,034238186 | 0,006367076 | 1 |
| C8ORF51 | 0,030977767 | 0,283991824 | 0,006367076 | 1 |
| CLDN4 | -0,014521548 | 0,3306664 | 0,006367076 | 1 |
| CREB5 | 0,198764684 | -0,118410946 | 0,006367076 | 1 |
| DEPDC1B | -0,643640868 | -0,189350356 | 0,006367076 | 1 |
| GPR98 | -0,067179155 | -0,398581397 | 0,006367076 | 1 |
| LOC100131187 | 0,203005431 | -0,087315038 | 0,006367076 | 1 |
| LOC100134370 | 0,274472143 | -0,24252304 | 0,006367076 | 1 |
| LOC731049 | -0,905534399 | -0,14957954 | 0,006367076 | 1 |
| MAPK12 | -0,124432332 | 0,306733884 | 0,006367076 | 1 |
| POLR2H | -0,173800303 | 0,01368544 | 0,006367076 | 1 |
| RAD54B | -0,463291261 | -0,03537668 | 0,006367076 | 1 |
| RARRES3 | -0,094438764 | 0,558895626 | 0,006367076 | 1 |
| RDM1 | -0,058209154 | 0,158044825 | 0,006367076 | 1 |
| RPS6KA2 | 0,384357211 | -0,024420578 | 0,006367076 | 1 |
| SETBP1 | 0,053243649 | -0,257669907 | 0,006367076 | 1 |
| SPOCD1 | 0,38903287 | -0,048784508 | 0,006367076 | 1 |
| STAT6 | 0,261560997 | 0,071226029 | 0,006367076 | 1 |
| TTK | -0,837477834 | -0,075720263 | 0,006367076 | 1 |
| VARS | 0,147704938 | -0,042794633 | 0,006367076 | 1 |
| ZNF789 | -0,075540639 | 0,20506801 | 0,006367076 | 1 |
| ASAH1 | 0,105646451 | -0,083133756 | 0,00812459 | 1 |
| BMI1 | -0,087853771 | -0,360512386 | 0,00812459 | 1 |
| C12ORF24 | -0,179408237 | 0,232263162 | 0,00812459 | 1 |

| | | | | |
|--------------|--------------|--------------|------------|---|
| C6ORF1 | 0,179589247 | 0,040074171 | 0,00812459 | 1 |
| CCNL1 | 0,153333764 | -0,089016073 | 0,00812459 | 1 |
| CGGBP1 | -0,014048652 | -0,318930478 | 0,00812459 | 1 |
| FAHD2B | 0,176107377 | 0,437570788 | 0,00812459 | 1 |
| FAM5C | 0,167786417 | -0,489243505 | 0,00812459 | 1 |
| FMO5 | 0,349333081 | 0,013545757 | 0,00812459 | 1 |
| HS.127242 | -0,019305103 | 0,170883787 | 0,00812459 | 1 |
| HS.290694 | -0,086962278 | 0,136011899 | 0,00812459 | 1 |
| HS.541226 | -0,317506153 | 0,076922138 | 0,00812459 | 1 |
| ITM2B | 0,189482934 | -0,191279326 | 0,00812459 | 1 |
| LOC100129268 | -0,135632017 | 0,042684121 | 0,00812459 | 1 |

Supplementary Table s3: Quantification of western blot bands for cyclin E1 and Rb normalized against total proteins

| | Cyclin E1 | | | | | Rb | | | |
|------------|-----------|------|------|-------|--|------|------|------|------|
| | CTR | D3 | D6 | PDR | | CTR | D3 | D6 | PDR |
| T47D | 1,00 | 1,46 | 1,62 | 2,47 | | 1,00 | 0,69 | 0,63 | 0,00 |
| ZR75-1 | 1,00 | 1,24 | 1,37 | 6,63 | | 1,00 | 0,88 | 0,75 | 0,59 |
| MCF7 | 1,00 | 4,91 | 8,14 | 16,23 | | 1,00 | 0,30 | 0,29 | 0,36 |
| MCF7 EDR | 1,00 | 1,62 | 3,55 | 5,96 | | 1,00 | 0,40 | 0,19 | 0,34 |
| MCF7 TamR | 1,00 | 2,03 | 5,38 | 34,87 | | 1,00 | 0,76 | 0,25 | 0,68 |
| BT474 | 1,00 | 1,37 | 1,23 | 7,83 | | 1,00 | 0,38 | 0,26 | 0,38 |
| MDA MB 361 | 1,00 | 1,88 | 1,74 | 3,90 | | 1,00 | 0,90 | 0,36 | 0,13 |

Supplementary Table s4: Primary and secondary antibodies working conditions.

| Antibody | Clone | Company | Dilution | Secondary Antibody |
|------------------------------|--------------|---------------------------|-----------------|---------------------------|
| CDK2 | 78B2 | Cell Signaling Technology | 1/500 o.n. | anti-R 1/2000 1h |
| CDK4 | D9G3E | Cell Signaling Technology | 1/1000 o.n. | anti-R 1/2000 1h |
| Cyclin A1 | BF683 | Merk Millipore | 1/1000 o.n. | anti-M 1/2000 1h |
| Cyclin D1 | 92G2 | Cell Signaling Technology | 1/1000 o.n. | anti-R 1/2000 1h |
| Cyclin E1 | HE12 | Cell Signaling Technology | 1/1000 o.n. | anti-M 1/2000 1h |
| Cyclin E2 | | Cell Signaling Technology | 1/500 o.n. | anti-R 1/2000 1h |
| E2F1 | | Cell Signaling Technology | 1/1000 o.n. | anti-R 1/2000 1h |
| E2F2 | EPR8622 | Abcam | 1/2000 o.n. | anti-R 1/2000 1h |
| GAPDH | D16H11 | Cell Signaling Technology | 1/10000 1h | anti-R 1/2000 1h |
| p107 | C-18 | Santa Cruz Biotechnology | 1/1000 1h | anti-R 1/2000 1h |
| p130 | EP2141Y | Abcam | 1/1000 1h | anti-R 1/2000 1h |
| p16 | EP435Y-129R | Abcam | 1/250 o.n. | anti-R 1/2000 1h |
| p21 | 12D1 | Cell Signaling Technology | 1/1000 o.n. | anti-R 1/2000 1h |
| p27 | D69C12 | Cell Signaling Technology | 1/1000 o.n. | anti-R 1/2000 1h |
| pRb S807/811 | D20B12 | Cell Signaling Technology | 1/2000 o.n. | anti-R 1/2000 1h |
| Rb | 4H1 | Cell Signaling Technology | 1/8000 o.n. | anti-M 1/5000 1h |
| | | | | |
| o.n. = 4°C overnight | | | | |
| 1h = 1 hour room temperature | | | | |
| anti-R = anti-rabbit | | | | |
| anti-M = anti-mouse | | | | |
| | | | | |