SUPPLEMENTARY MATERIAL

Glucose metabolic reprogramming of ER+ breast cancer in the acquired resistance to the CDK4/6 inhibitor palbociclib

Nicla Lorito[1,5] , Marina Bacci[1,5] , Alfredo Smiriglia[1] , Michele Mannelli[1], Matteo Parri[1], Giuseppina Comito[1], Luigi Ippolito[1], Elisa Giannoni[1], Martina Bonechi[2], Matteo Benelli[3], Ilenia Migliaccio[2], Luca Malorni[2,4], Paola Chiarugi[1] and Andrea Morandi[1*]

[1]Department of Experimental and Clinical Biomedical Sciences, Viale Morgagni 50, I-50134 Florence, University of Florence, Italy.
[2] Translational Research Unit, Azienda USL Toscana Centro, Via Suor Niccolina Infermiera 20, I-59100 Prato, Hospital of Prato, Italy.
[3] Bioinformatics Unit, Azienda USL Toscana Centro, Via Suor Niccolina Infermiera 20, I-59100 Prato, Hospital of Prato, Italy.
[4] “Sandro Pitigliani” Oncology Department, Azienda USL Toscana Centro, Via Suor Niccolina Infermiera 20, I-59100 Prato, Hospital of Prato, Italy.

[5]these authors contributed equally to the study

The Supplementary data file contains:

Supplementary Figures 1-6
1 Supplementary Table (.xls file)

*Materials request should be addressed to Andrea Morandi (andrea.morandi@unifi.it)

Supplementary Figure S1

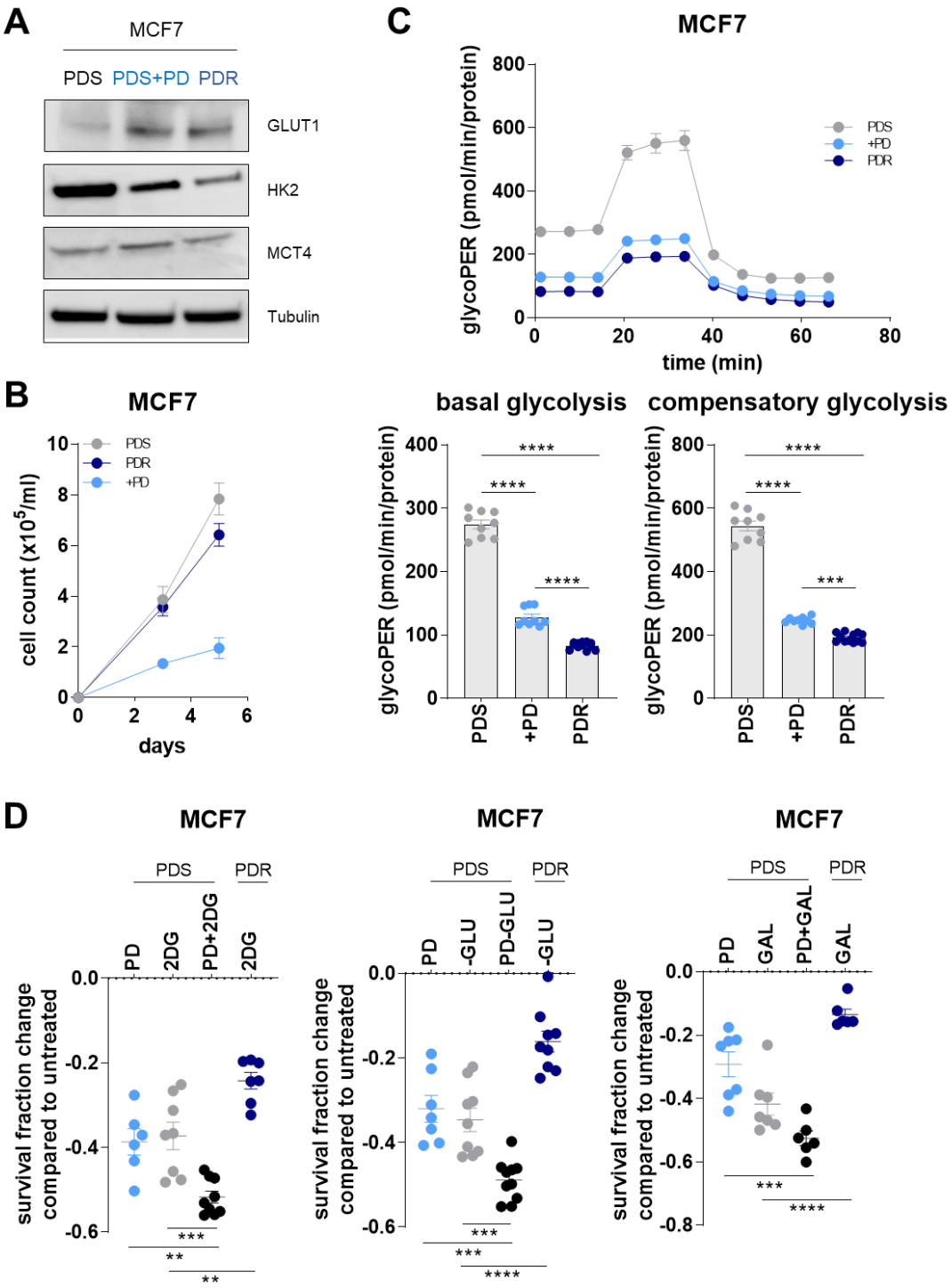

Figure S1. ER+/HER2- MCF7 cells recapitulate the metabolic behaviour of the ER+/HER2- breast cancer cells. (**A**) Total protein lysates from PDS, PDS+3 days PD and PDR were subjected to Western blot analysis as indicated. (**B**) No significant reduction in cell survival between PDS and PDR derived cells was observed by cell counting within the 5-day time range. Significant differences were observed between PDS and PDS treated with PD. Data represent means ± SEMs. Two-way ANOVA; Bonferroni corrected. (**C**) PDS (either in presence or absence of 1μM palbociclib, PD) and PDR cells were subjected to Seahorse XFe96 Glycolytic Rate analysis. PD treated PDS and PDR cells showed reduced basal and compensatory glycolysis when compared to untreated PDS. (**D**) Impairment of glycolysis (e.g. 2-DG, glucose deprivation and galactose containing medium) increased palbociclib antiproliferative effect in MCF7 PDS while had minimum effects in PDR counterpart. One-way ANOVA; Dunnett's corrected; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

Supplementary Figure S2

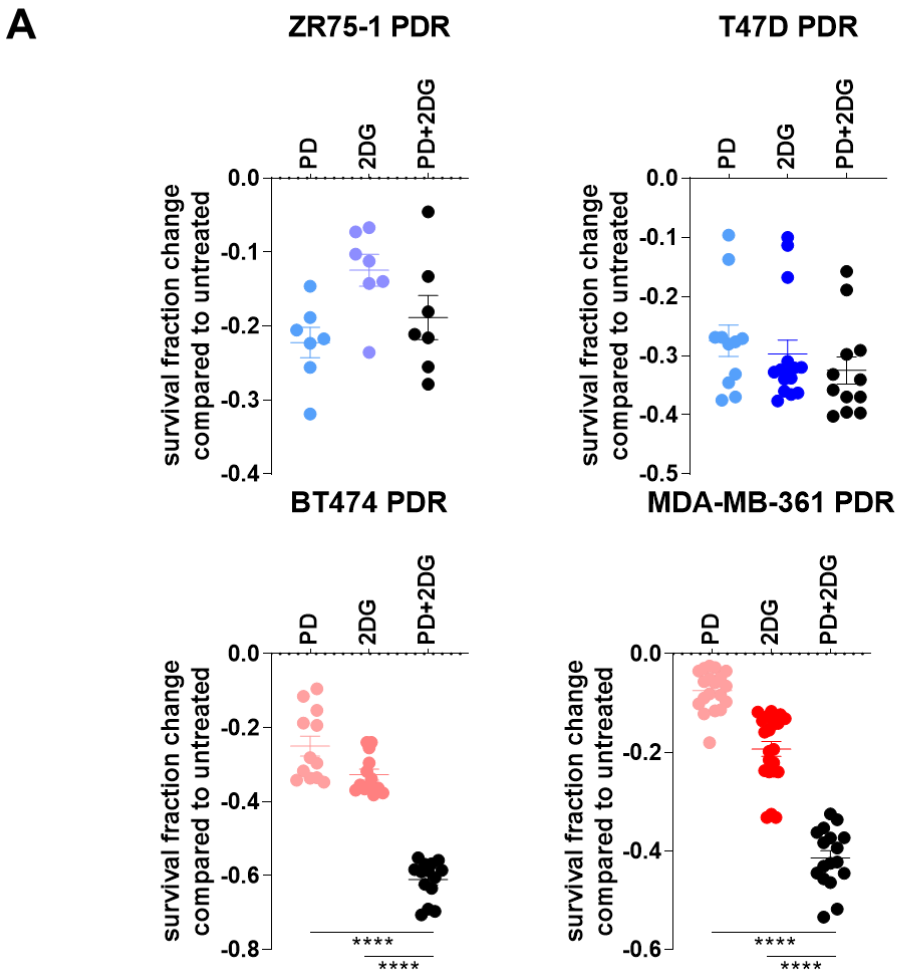

Figure S2. PDR cells are re-sensitised to palbociclib when glycolysis is targeted.
(**A**) Impairment of glycolysis using 2-DG has stronger anti-survival effects in HER2+/ER+ cells (red shades) while has no effects on the HER2-/ER+ cells. One-way ANOVA; Dunnett's corrected; **** $P < 0.0001$.

Supplementary Figure S3

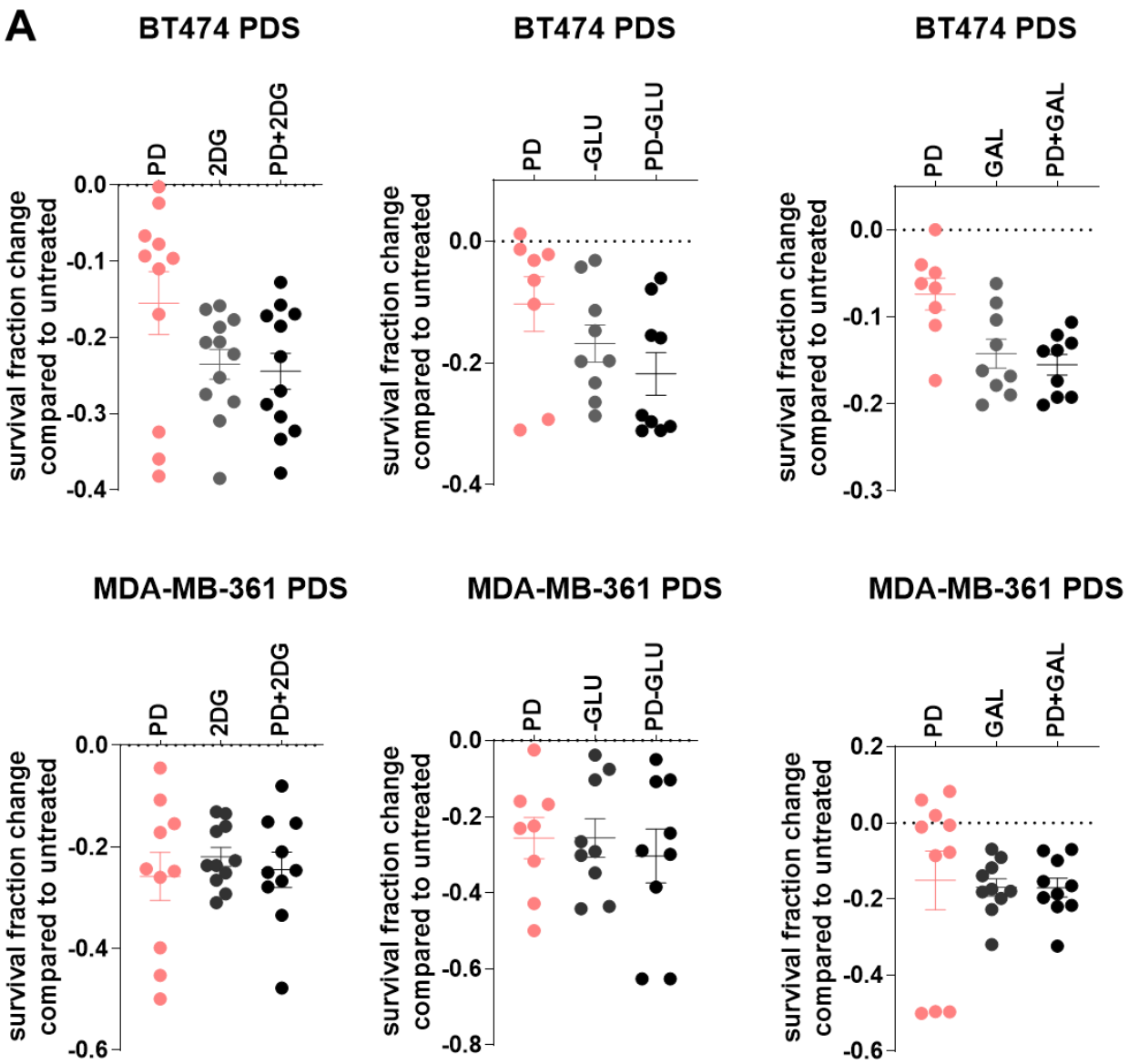

Figure S3. Targeting glycolysis alone or in combination with PD in ER+/HER2+ PDS cells has no major impact when compared to palbociclib alone.
(**A**) Impairment of glycolysis (using 2-DG, glucose deprivation or galactose-containing medium) has no stronger anti-survival effects when compared to vehicle-treated PDS cells. One-way ANOVA; Dunnett's corrected.

Supplementary Figure S4

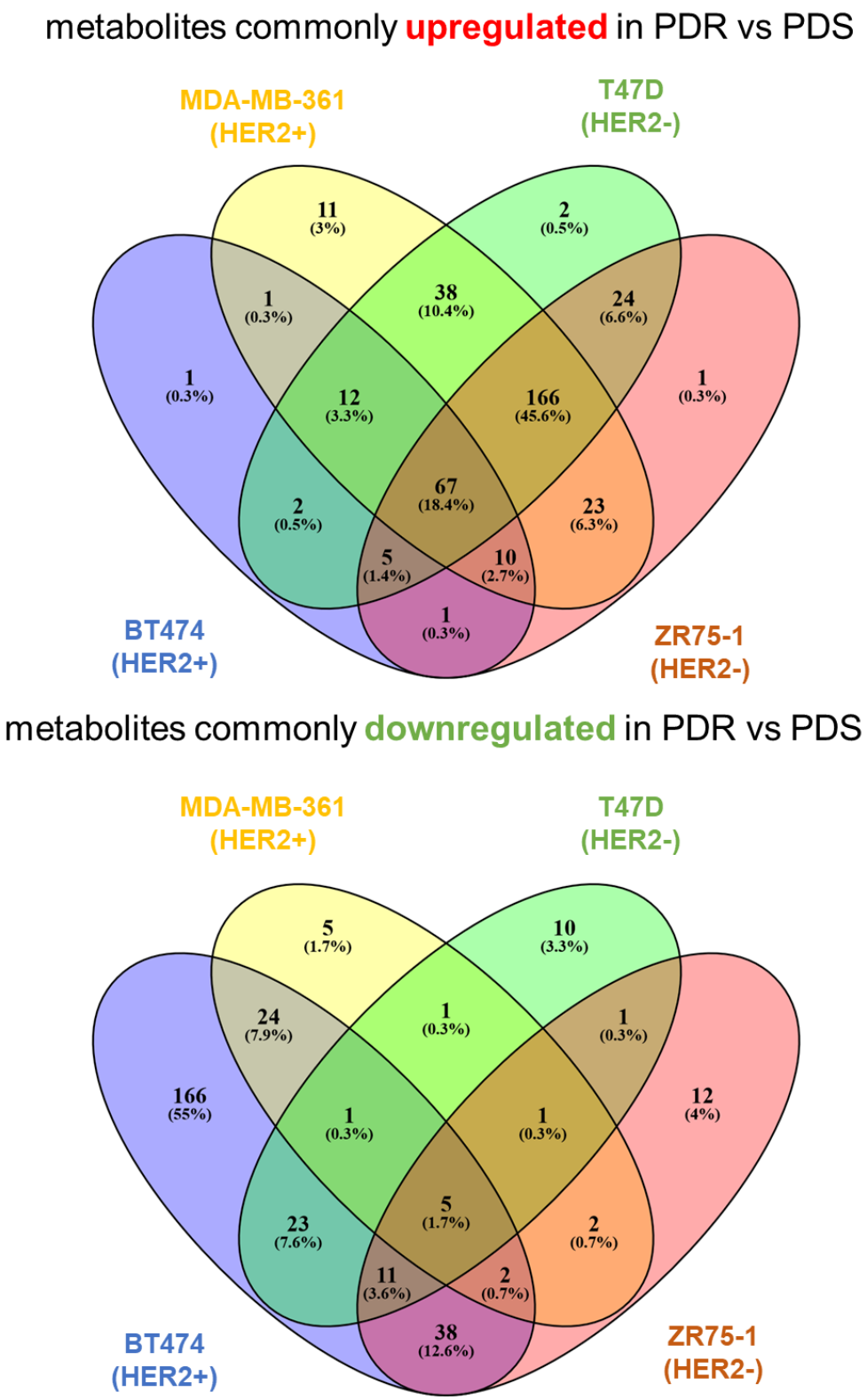

Figure S4. Commonly deregulated metabolites between the different PDR cell lines. Metabolites (either identified or left as retention time and m/z value – see Table S1 for details) were subjected to Venn diagram analysis (https://bioinfogp.cnb.csic.es/tools/venny/) to highlight potential commonly altered metabolites between the different cell lines, when PDR.

Supplementary Figure S5

A

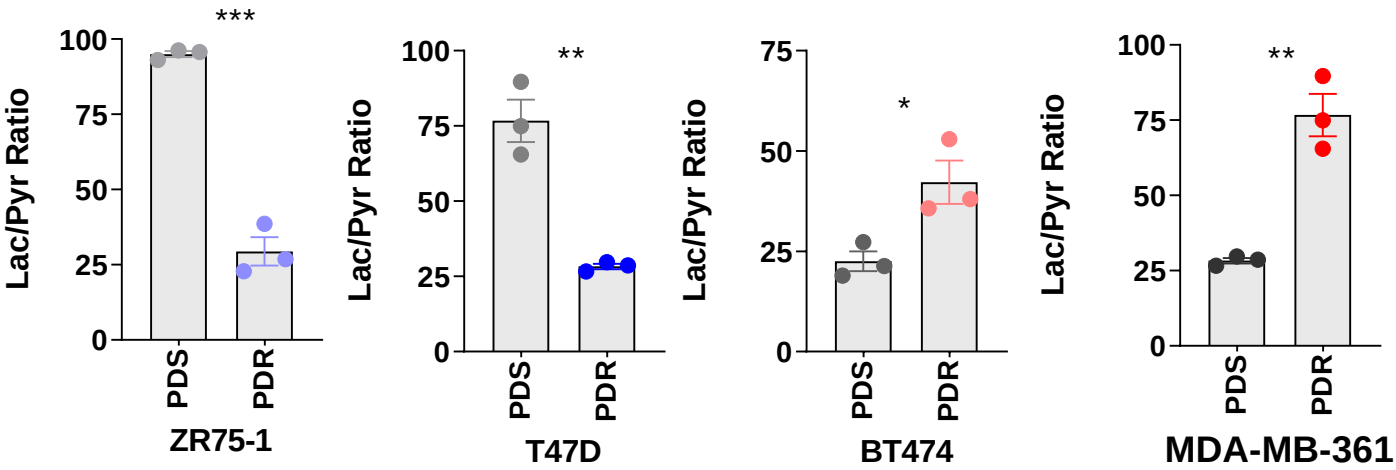

Figure S5. Aerobic glycolysis is enhanced in ER+/HER2+ and reduced in ER+/HER2- PDR cells when compared to PDS cells.
(**A**) Lactate to pyruvate ratio, as an indicator of fermentation propensity of the cell lines analyzed, showed that ER+/HER2- PDR cells are less reliant on aerobic glycolysis when compared to ER+/HER2+ PDR cells (both relative to the PDS counterparts).

Supplementary Figure S6

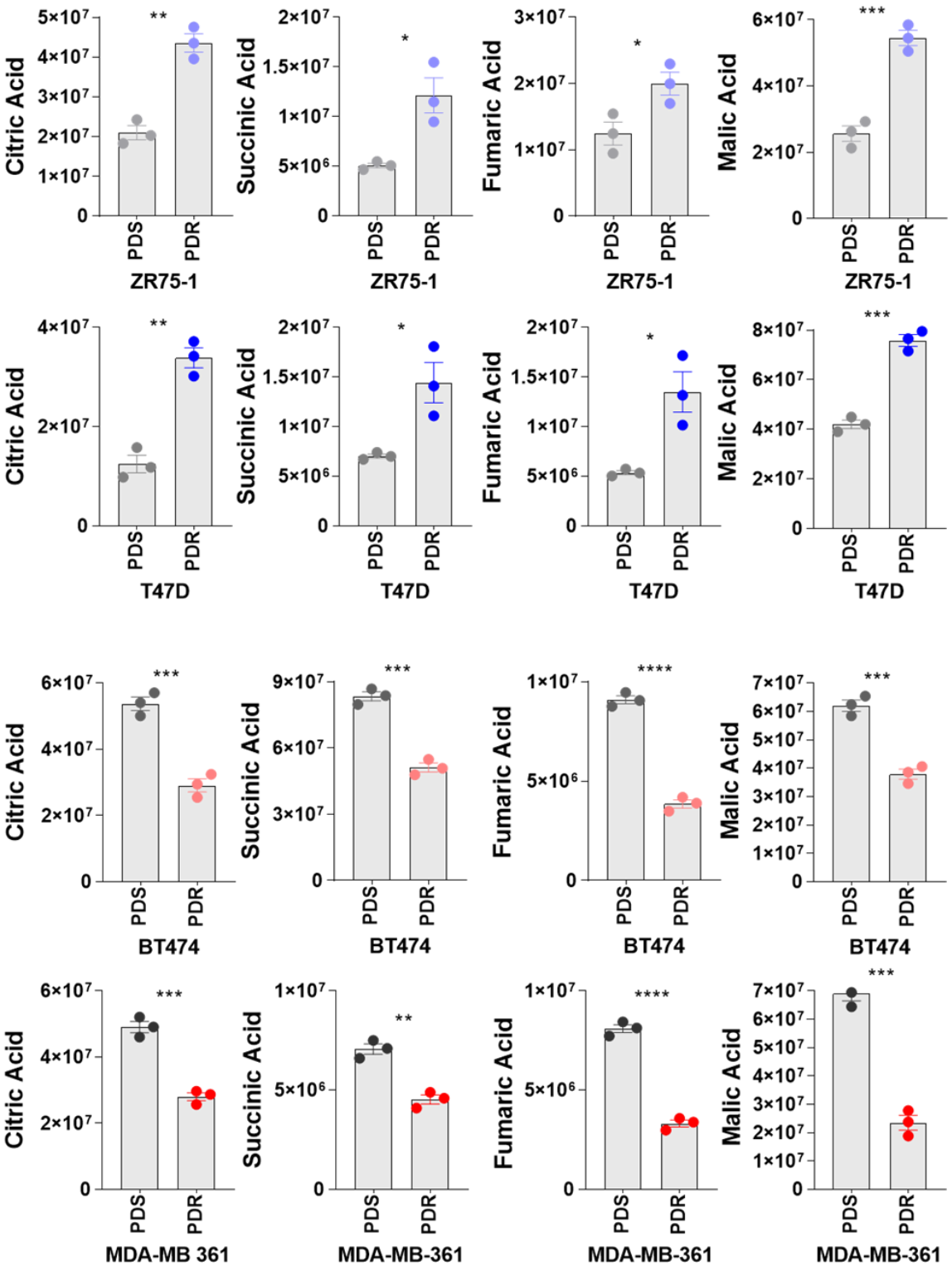

Figure S6. TCA intermediates are enhanced in ER+/HER2- and reduced in ER+/HER2+ PDR cells when compared to PDS cells.

(**A**) Intracellular relative abundance of the TCA cycle intermediates citrate, succinate, fumarate and malate as indication of oxidative phosphorylation reliance of the cell lines analyzed. Student *t*-test, ; * P < 0.05, ** P < 0.01; *** P < 0.001; **** P < 0.0001