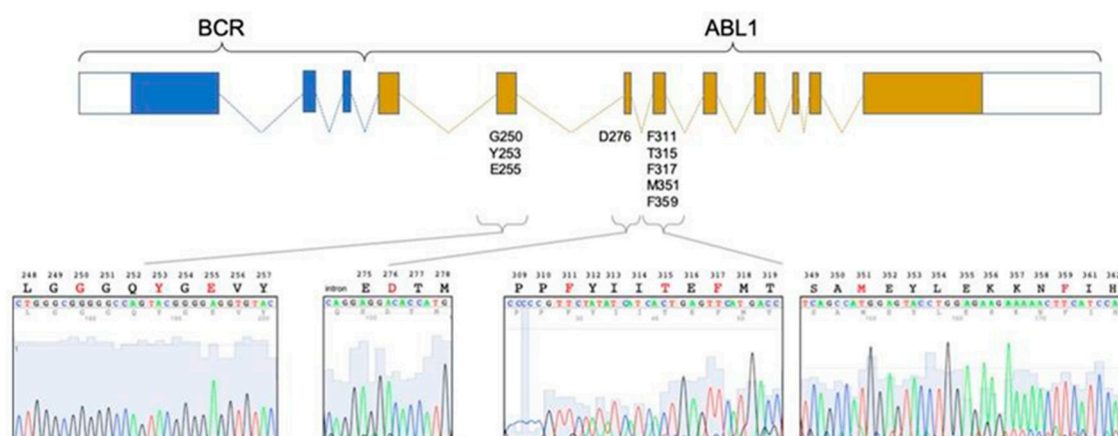


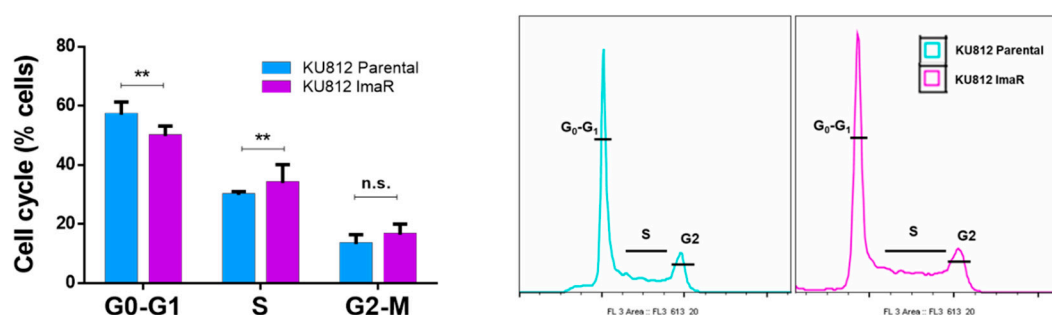
## Supplementary Materials:

# Metabolic Plasticity Is An Essential Requirement of Acquired Tyrosine Kinase Inhibitor Resistance in Chronic Myeloid Leukemia

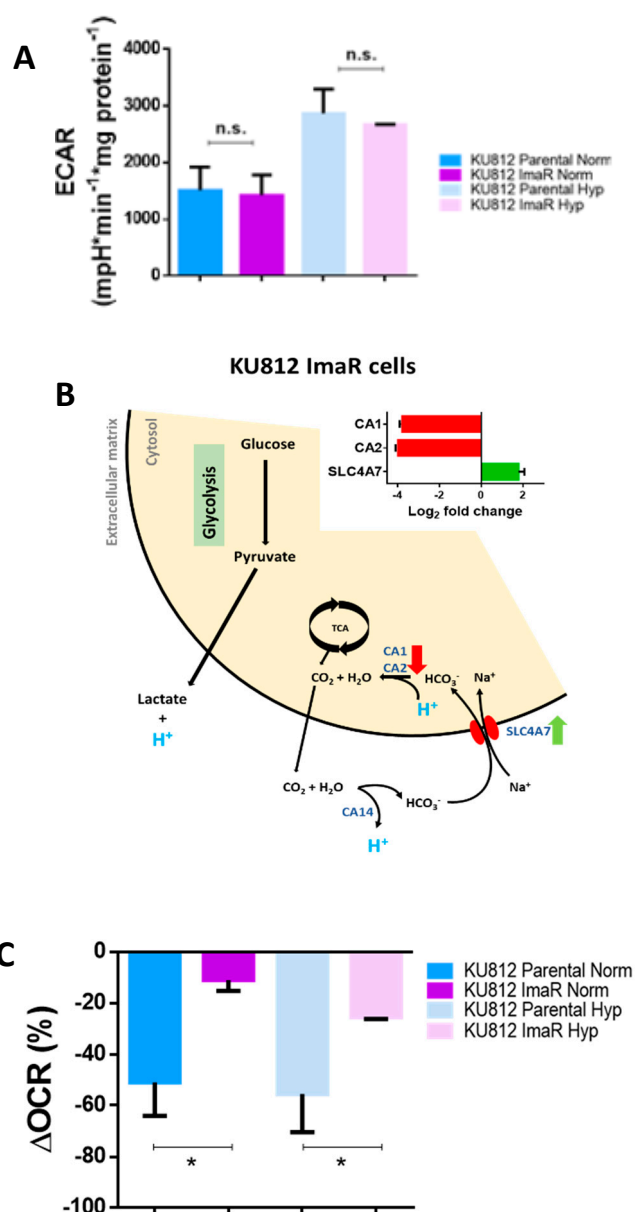
Miriam G. Contreras Mostazo, Nina Kurrle, Marta Casado, Dominik Fuhrmann, Islam Alshamleh, Björn Häupl, Paloma Martín-Sanz, Bernhard Brüne, Hubert Serve, Harald Schwalbe, Frank Schnuetgen, Silvia Marin and Marta Cascante



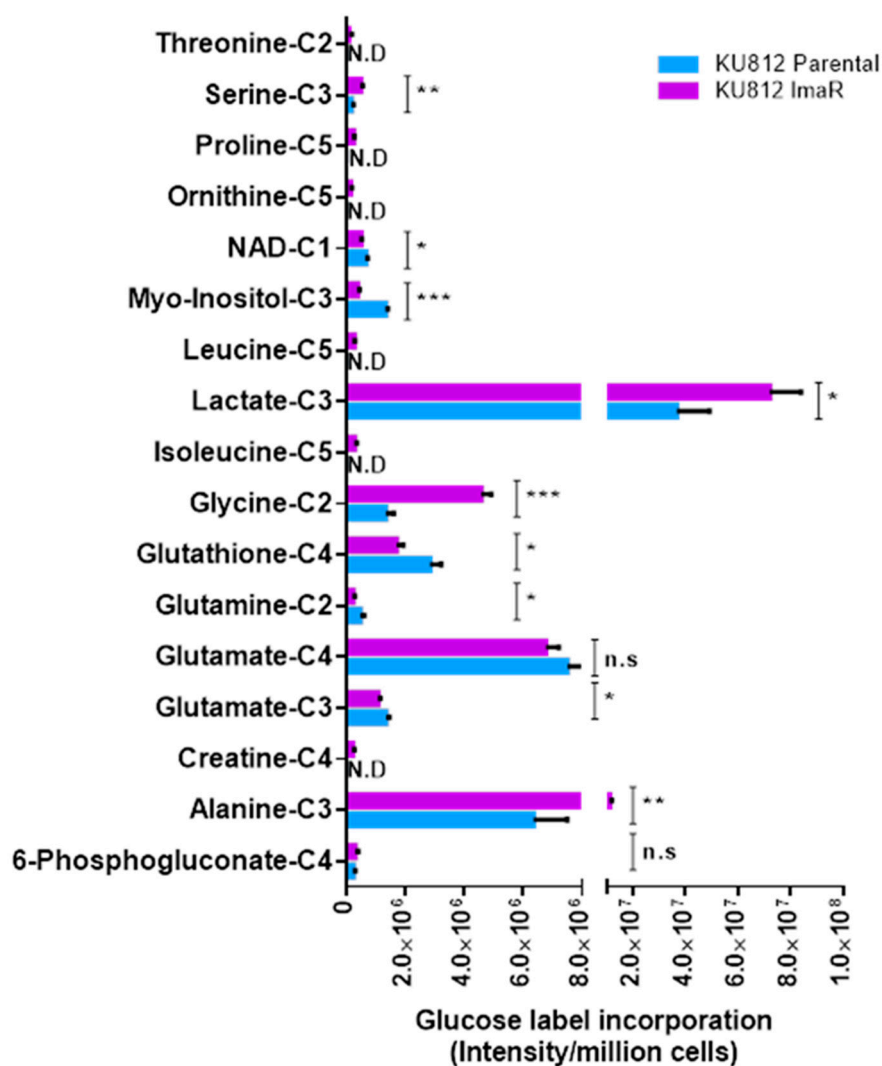
**Figure S1.** BCR-ABL1 mutation analysis. The nine indicated mutations account for 60% to 70% of all imatinib-resistant mutations. Exon 4,5 and 6 of ABL1 were amplified as explained in Materials and Methods and Sanger sequenced.



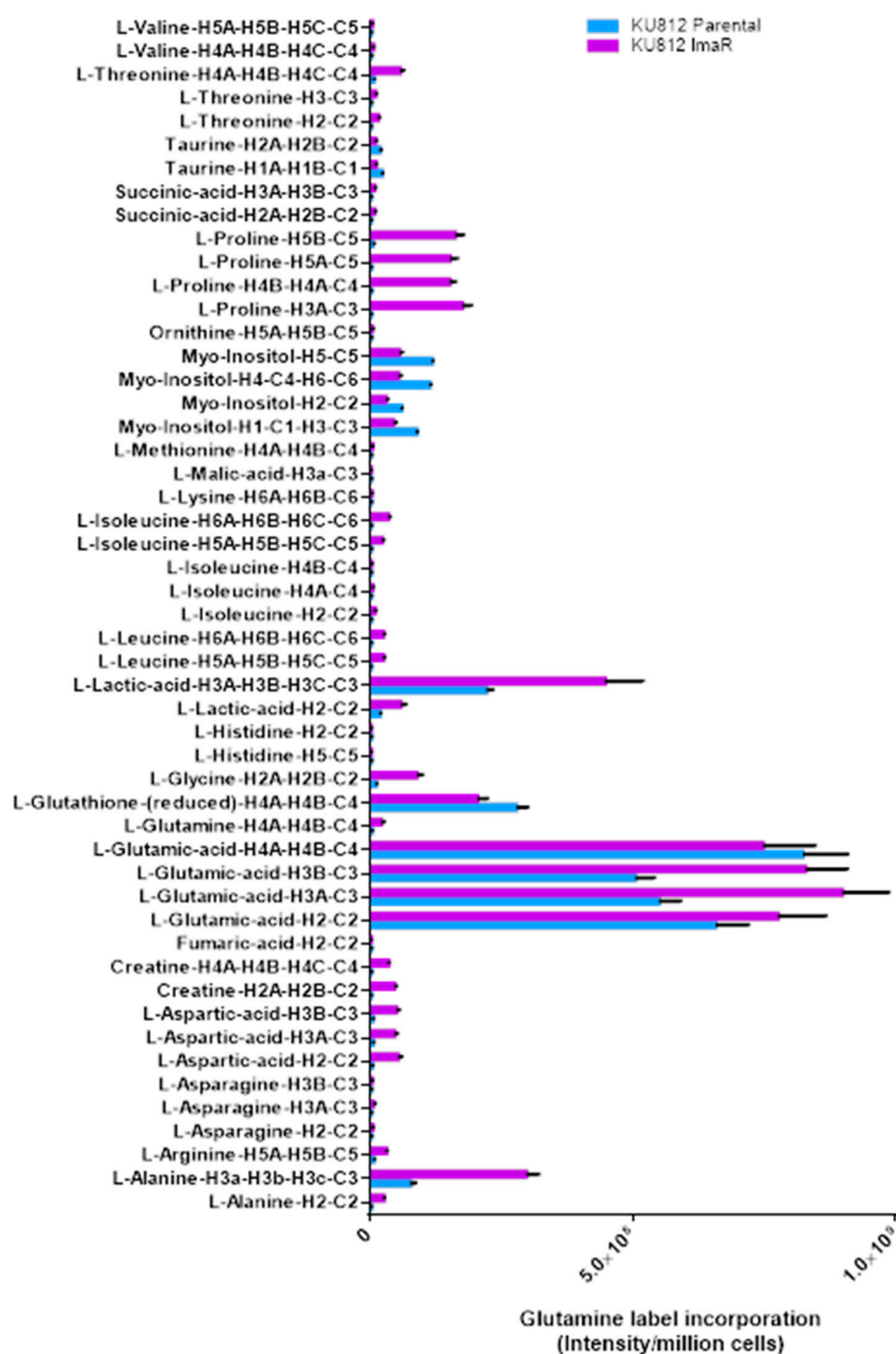
**Figure S2.** Cell cycle distribution of KU812 Parental and KU812 ImaR cells determined by flow cytometry. Figure shows one representative experiment (mean  $\pm$  SD for  $n = 3$  of  $n = 2$  independent experiments). In addition, significance was determined by two-tailed independent sample Welch's t-tests. Statistically significant differences between KU812 Parental and ImaR cells were indicated as  $p < 0.01$  (\*\*) and n.s. = non-significant differences.



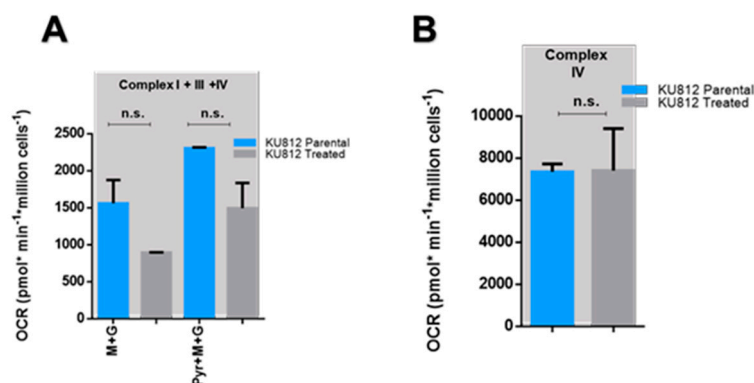
**Figure S3.** Glycolytic profile, protein profile differences of protein associated with H<sup>+</sup> balance and Crabtree effect of KU812 Imatinib resistant compared to KU812 Parental cells. A) ECAR after glucose addition calculated using ECAR values from Figure 3C. B) The protein expression profiling of the carbonic anhydrase (CA) 1 and 2, and the sodium bicarbonate cotransporter (SLC4A7) obtained under normoxia using SILAC proteomic experiments. Log<sub>2</sub> fold change values were calculated and represented by green colour = protein upregulation; and red = protein downregulation. Data are provided as mean ± SD of n=2 of one representative experiment C) Crabtree effect determined by adding 10 mM glucose to glucose-deprived media. Values of ΔOCR are expressed as % of the basal OCR values in absence of glucose. Data are provided as mean ± SD of n=3. Significance was determined by two-tailed independent sample Student's t-test. Statistical differences between KU812 P vs. ImaR cells under normoxic or hypoxic incubation conditions are indicated as p<0.05 (\*) and p≥0.05 (n.s.=non-significant differences).



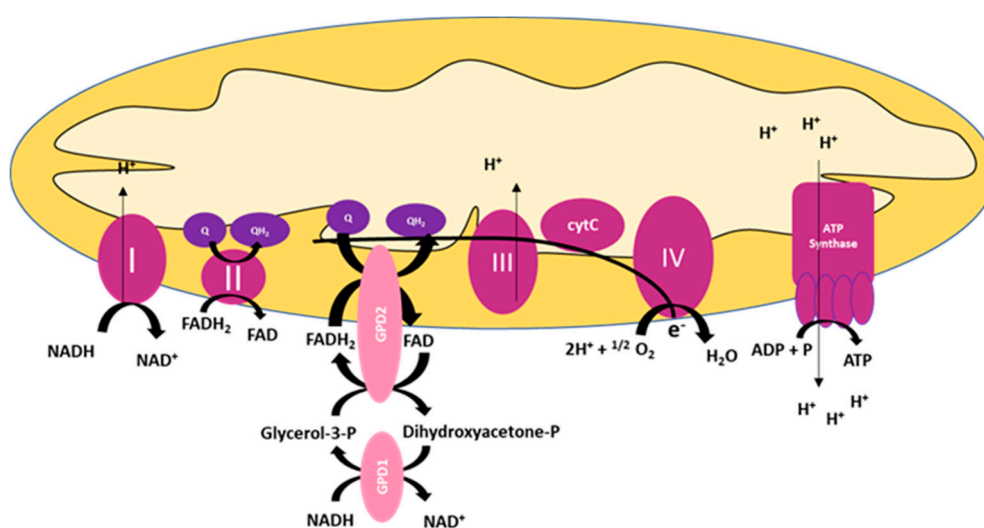
**Figure S4.**  $^{13}\text{C}$  Glucose label incorporation in KU812 Parental and ImaR cells under normoxia measured by NMR. Intensity values were normalized by million cells. Figure shows one representative experiment (mean  $\pm$  SD for  $n = 3$  replicates). Significance was determined by two-tailed independent sample Student's  $t$ -tests. Statistically significant differences between KU812 Parental and KU812 ImaR cells were indicated as  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*), and  $p \geq 0.05$  (n.s. = non-significant differences). Abbreviations: ImaR, imatinib resistant; N.D., non-detected metabolites.



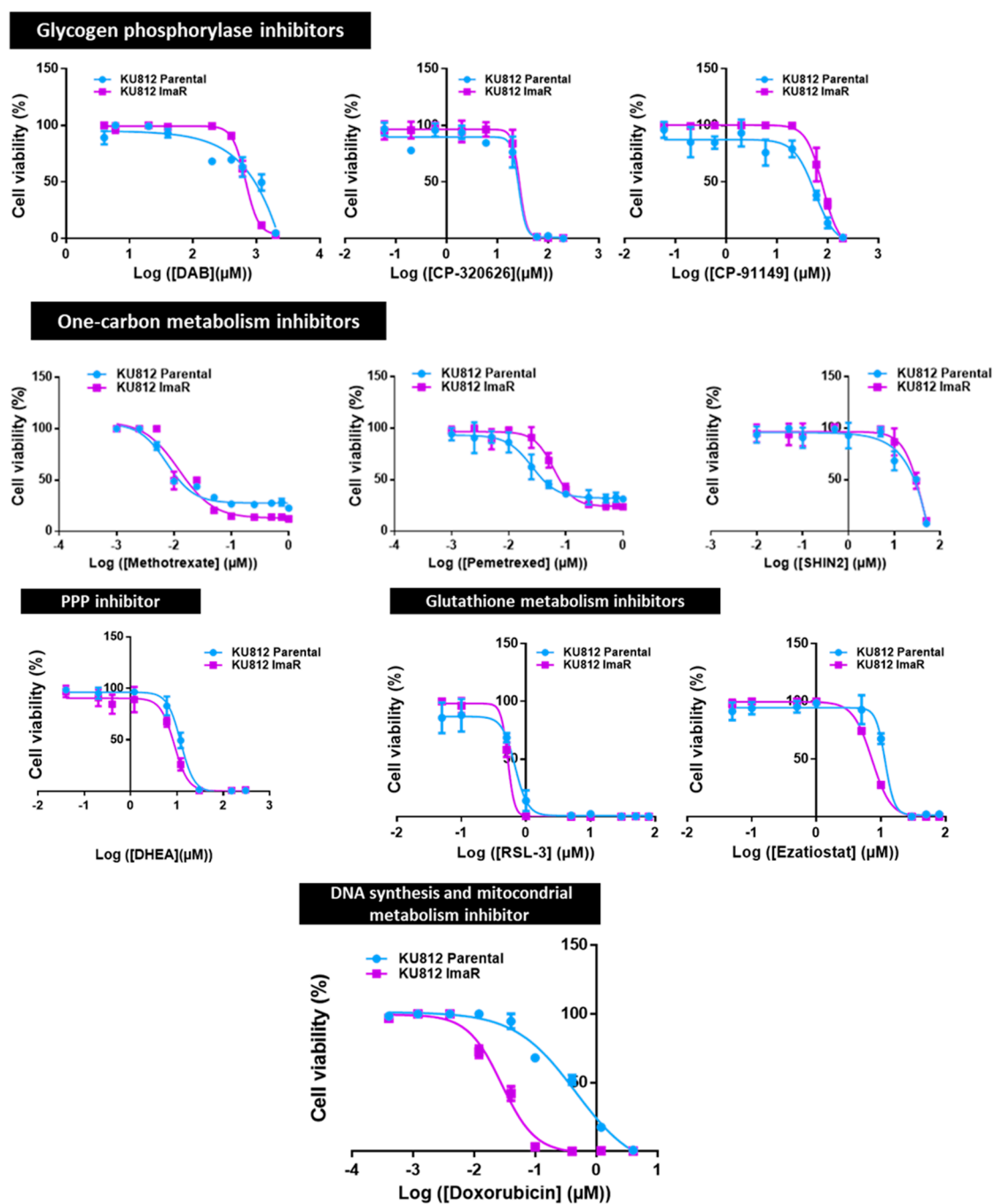
**Figure S5.** <sup>13</sup>C Glutamine label incorporation in KU812 Parental and ImaR cells under normoxia measured by NMR. Intensity values were normalized by million cells. Figure shows one representative experiment (mean±SD for n=3 replicates). Statistically significant differences between KU812 Parental and KU812 ImaR cells are not shown in this Figure; they are represented in Table S1. Abbreviations: ImaR, imatinib resistant; and N.D., non-detected metabolites.



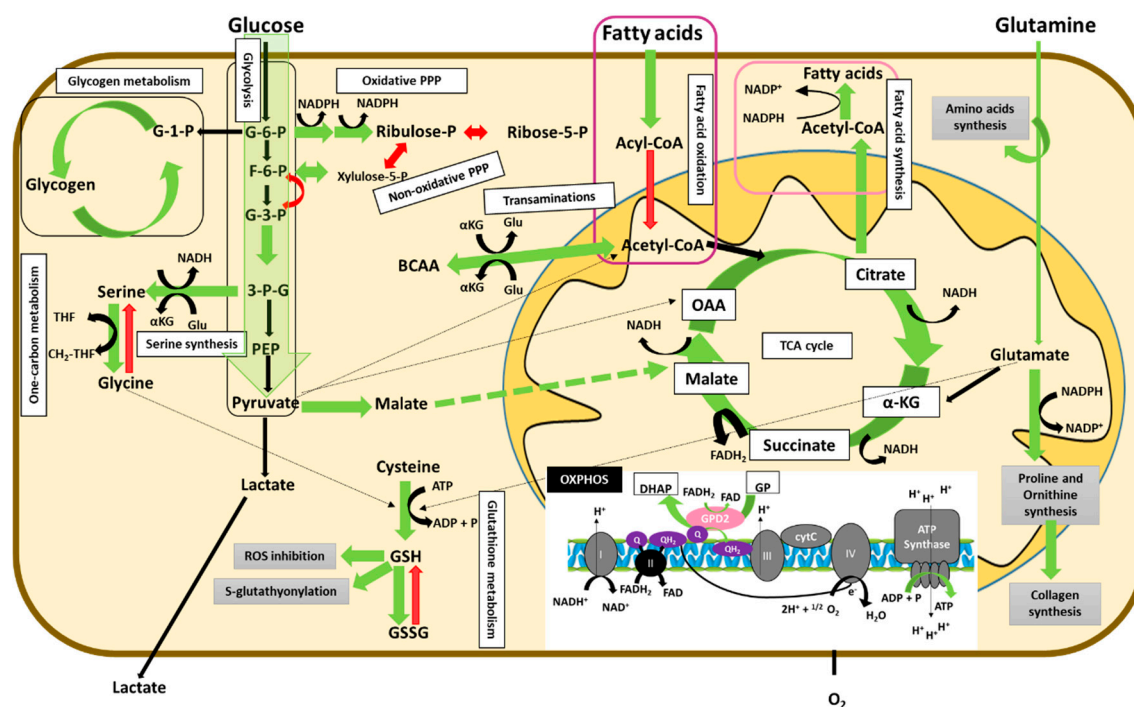
**Figure S6.** Activity of the different electron transport chain complexes of KU812 Imatinib-treated vs. KU812 Parental cells. OCR measurements of KU812 Parental and Treated (KU812 Parental cells treated with 80nM imatinib for 72 h) were determined by Oroboros Oxygraph-2k respirometer under normoxia. OCR were measured after sequential injections of substrates and inhibitors. For **A** (approach to study complex I activity): 2 mM malate + 5 mM ADP + 10 mM glutamate (M+G), and 5mM pyruvate (P). For **B** (complex IV activity): 5 mM Ascorbate + 5 mM ADP + 0.5 mM tetramethyl-p-phenyldiamine dihydrochloride (TMPD). Data were normalised by cell number. Data are provided as mean  $\pm$  SD of  $n = 2$  and significance was determined by two-tailed independent sample Welch's t-test. Statistically significant differences between KU812 P and Treated cells are indicated as  $p \geq 0.05$  (n.s. = non-significant differences).



**Figure S7.** Diagram of the oxidative phosphorylation and glycerol-3-Phosphate shuttle.



**Figure S8.** Effects of different metabolic inhibitors and AML conventional drugs in KU812 Parental and ImaR cell viability in normoxia. KU812 parental and ImaR cells were incubated 72 h with DMSO (vehicle control) or the log concentrations of the inhibitors shown. IC<sub>50</sub>s shown in Table 1. Figure shows one representative experiment (mean  $\pm$  SD for  $n = 3$ ) of  $n = 3$  independent experiments.



**Figure S9.** Schematic diagram of the metabolic reprogramming upon TKI resistance acquisition. Upregulated pathways are highlighted in green and downregulated pathways are highlighted in red.

**Table S1.** Exchange flux values of KU812 Parental and ImaR in normoxia after specific incubation times (Parental Norm = 96 h, ImaR Norm = 48 h, Parental Hyp = 72 h and ImaR Hyp = 24 h). Exchange flux values were obtained using Biocrates kit. Table shows the values of one representative experiment (mean  $\pm$  SD for  $n = 3$  replicates). Cysteine was not shown due to the inability of Biocrates kit to measure it.

| EXTRACELLULAR FLUXES NORMOXIA |                |       |            |       |        |
|-------------------------------|----------------|-------|------------|-------|--------|
| Kpc (nmol*millioncell-1*h-1)  |                |       |            |       |        |
|                               | KU812 Parental |       | KU812 ImaR |       |        |
|                               | Amino acids    |       |            |       |        |
|                               | Mean           | SD    | Mean       | SD    | pvalue |
| Ala                           | 5.02           | 0.92  | 3.72       | 0.52  | 0.101  |
| Arg                           | -0.65          | 0.25  | -3.87      | 4.05  | 0.365  |
| Asn                           | -0.26          | 0.02  | -0.61      | 0.49  | 0.394  |
| Asp                           | 0.02           | 0.12  | -0.41      | 0.13  | 0.013  |
| Cit                           | 0.01           | 0.00  | -0.10      | 0.04  | 0.006  |
| Gln                           | -9.12          | 0.24  | -22.44     | 4.79  | 0.034  |
| Glu                           | 2.63           | 0.65  | 3.97       | 0.38  | 0.036  |
| Gly                           | 0.25           | 0.19  | 0.39       | 0.28  | 0.492  |
| His                           | -0.21          | 0.10  | -0.46      | 0.20  | 0.128  |
| Ile                           | -1.43          | 0.09  | -2.86      | 0.39  | 0.003  |
| Leu                           | -1.93          | 0.13  | -4.13      | 0.68  | 0.005  |
| Lys                           | -1.25          | 0.12  | -2.52      | 0.58  | 0.020  |
| Met                           | -0.32          | 0.01  | -0.92      | 0.17  | 0.004  |
| Orn                           | 0.61           | 0.09  | 1.80       | 0.60  | 0.028  |
| Phe                           | -0.45          | 0.04  | -1.11      | 0.23  | 0.008  |
| Pro                           | 0.20           | 0.15  | 2.47       | 0.27  | 0.000  |
| Ser                           | -1.79          | 0.11  | -5.05      | 0.89  | 0.003  |
| Thr                           | -0.52          | 0.02  | -1.91      | 0.68  | 0.025  |
| Trp                           | -0.12          | 0.01  | -0.25      | 0.09  | 0.062  |
| Tyr                           | -0.38          | 0.06  | -1.01      | 0.09  | 0.001  |
| Val                           | -1.17          | 0.11  | -2.56      | 0.47  | 0.007  |
| Polyamines                    |                |       |            |       |        |
| Putrescine                    | -4E-05         | 4E-05 | -5E-04     | 2E-04 | 0.010  |
| Spermidine                    | -7E-06         | 2E-05 | 2E-05      | 4E-05 | 0.362  |
| Spermine                      | -7E-05         | 2E-05 | -8E-04     | 1E-03 | 0.409  |

**Table S2.** Exchange flux values of KU812 Parental and ImaR in hypoxia (1% O<sub>2</sub>) after specific incubation times (Parental Norm = 96 h, ImaR Norm = 48 h, Parental Hyp = 72 h and ImaR Hyp = 24 h). Exchange flux values were obtained using Biocrates kit. Table shows the values of one representative experiment (mean ± SD for *n* = 3 replicates). Cysteine was not shown due to the inability of Biocrates kit to measure it.



| EXTRACELLULAR FLUXES HYPOXIA |                |       |            |       |        |
|------------------------------|----------------|-------|------------|-------|--------|
| Kpc (nmol*millioncell-1*h-1) |                |       |            |       |        |
|                              | KU812 Parental |       | KU812 ImaR |       |        |
|                              | Amino acids    |       |            |       |        |
|                              | Mean           | SD    | Mean       | SD    | pvalue |
| Ala                          | 3.19           | 0.51  | 1.85       | 0.63  | 0.045  |
| Arg                          | -1.95          | 0.03  | 10.68      | 6.07  | 0.099  |
| Asn                          | -0.86          | 0.88  | 0.32       | 0.57  | 0.308  |
| Asp                          | -0.36          | 0.30  | 1.06       | 1.07  | 0.091  |
| Cit                          | -0.02          | 0.00  | 0.09       | 0.18  | 0.356  |
| Gln                          | -6.39          | 1.10  | 6.54       | 15.92 | 0.358  |
| Glu                          | 0.90           | 0.71  | 7.01       | 3.50  | 0.041  |
| Gly                          | 1.32           | 0.31  | 3.44       | 1.29  | 0.359  |
| His                          | -0.02          | 0.09  | 0.41       | 0.68  | 0.336  |
| Ile                          | -0.42          | 0.33  | -1.00      | 1.10  | 0.433  |
| Leu                          | -0.83          | 0.22  | -1.97      | 1.02  | 0.130  |
| Lys                          | -0.24          | 0.64  | -1.49      | 0.87  | 0.115  |
| Met                          | 0.08           | 0.01  | -0.01      | 0.73  | 0.871  |
| Orn                          | 2.15           | 0.51  | 1.90       | 0.66  | 0.639  |
| Phe                          | 0.09           | 0.25  | -0.54      | 0.30  | 0.049  |
| Pro                          | 0.46           | 0.22  | 1.47       | 0.44  | 0.023  |
| Ser                          | -1.58          | 0.26  | -8.33      | 0.95  | 0.000  |
| Thr                          | -0.25          | 0.37  | 0.13       | 1.51  | 0.695  |
| Trp                          | 0.02           | 0.02  | -0.25      | 0.18  | 0.054  |
| Tyr                          | 0.13           | 0.29  | -0.93      | 0.31  | 0.012  |
| Val                          | 0.03           | 0.53  | -1.51      | 0.81  | 0.051  |
| Polyamines                   |                |       |            |       |        |
| Putrescine                   | 4E-04          | 2E-04 | -2E-03     | 3E-04 | 0.001  |
| Spermidine                   | -1E-06         | 2E-05 | 5E-05      | 2E-04 | 0.739  |
| Spermine                     | -3E-05         | 3E-05 | 1E-04      | 2E-04 | 0.315  |

**Table S3.** Intracellular content of KU812 Parental and ImaR in normoxia after 24 h incubation time. Intracellular contents were obtained using Biocrates kit. Table shows the values of one representative experiment (mean  $\pm$  SD for  $n = 3$  replicates). Cysteine was not shown due to the inability of Biocrates kit to measure it. Abbreviations: D, discarded.

| INTRACELLULAR CONTENT NORMOXIA |                |        |            |        |        |
|--------------------------------|----------------|--------|------------|--------|--------|
| nmol/mg protein                |                |        |            |        |        |
|                                | KU812 Parental |        | KU812 ImaR |        |        |
|                                | Amino acids    |        |            |        |        |
|                                | Mean           | SD     | Mean       | SD     | pvalue |
| Ala                            | 271.86         | 130.35 | 448.71     | 198.69 | 0.267  |
| Arg                            | 89.71          | 14.48  | 96.74      | 48.08  | 0.820  |
| Asn                            | 253.43         | 14.36  | 500.37     | 178.44 | 0.075  |
| Asp                            | 180.60         | 72.28  | 222.23     | 39.51  | 0.523  |
| Cit                            | D              | D      | D          | D      | D      |
| Gln                            | 1162.32        | 500.57 | 2141.94    | 847.04 | 0.160  |
| Glu                            | D              | D      | D          | D      | D      |
| Gly                            | 275.13         | 142.35 | 768.70     | 235.22 | 0.057  |
| His                            | 26.28          | 3.18   | 47.00      | 28.66  | 0.281  |
| Ile                            | 67.55          | 13.16  | 138.49     | 32.39  | 0.025  |
| Leu                            | 55.69          | 8.21   | 170.55     | 50.71  | 0.018  |
| Lys                            | 23.24          | 4.06   | 20.24      | 1.44   | 0.295  |
| Met                            | 19.15          | 3.70   | 52.75      | 28.28  | 0.111  |
| Orn                            | 6.84           | 0.59   | 38.37      | 11.96  | 0.010  |
| Phe                            | 18.59          | 1.46   | 34.06      | 6.78   | 0.018  |
| Pro                            | 43.54          | 1.65   | D          | D      | D      |
| Ser                            | 39.94          | 14.85  | 81.03      | 69.67  | 0.374  |
| Thr                            | 65.16          | 10.89  | 134.42     | 19.08  | 0.005  |
| Trp                            | 3.42           | 1.48   | 8.04       | 1.58   | 0.021  |
| Tyr                            | 30.27          | 3.48   | 60.51      | 21.60  | 0.075  |
| Val                            | 22.63          | 5.55   | 21.91      | 10.02  | 0.918  |
| Polyamines                     |                |        |            |        |        |
| Putrescine                     | 23.63          | 7.46   | 4.22       | 0.90   | 0.011  |
| Spermidine                     | 31.66          | 2.06   | 26.32      | 7.98   | 0.324  |
| Spermine                       | 15.49          | 0.66   | 9.63       | 2.82   | 0.025  |

**Table S4.** Intracellular content of KU812 Parental and ImaR in hypoxia (1% O<sub>2</sub>) after 24 h incubation time. Intracellular contents were obtained using Biocrates kit. Table shows the values of one representative experiment (mean  $\pm$  SD for  $n = 3$  replicates). Cysteine was not shown due to the inability of Biocrates kit to measure it. Abbreviations: D, discarded.

| INTRACELLULAR CONTENT HYPOXIA |                |        |            |        |        |
|-------------------------------|----------------|--------|------------|--------|--------|
| nmol/mg protein               |                |        |            |        |        |
|                               | KU812 Parental |        | KU812 ImaR |        |        |
|                               | Amino acids    |        |            |        |        |
|                               | Mean           | SD     | Mean       | SD     | pvalue |
| Ala                           | 196.81         | 70.77  | 555.71     | 205.39 | 0.267  |
| Arg                           | 124.89         | 82.72  | 168.62     | 8.77   | 0.820  |
| Asn                           | 374.27         | 155.35 | 584.22     | 159.18 | 0.075  |
| Asp                           | 41.06          | 15.07  | 176.95     | 111.59 | 0.523  |
| Cit                           | D              | D      | D          | D      | D      |
| Gln                           | 2102.55        | 122.34 | 2939.16    | 475.32 | 0.160  |
| Glu                           | D              | D      | D          | D      | D      |
| Gly                           | 341.37         | 25.92  | 1847.53    | 898.01 | 0.057  |
| His                           | 50.56          | 17.24  | 85.98      | 22.96  | 0.281  |
| Ile                           | 124.73         | 37.62  | 306.43     | 65.23  | 0.025  |
| Leu                           | 138.61         | 18.59  | 352.46     | 29.64  | 0.018  |
| Lys                           | 68.98          | 24.70  | 89.94      | 44.29  | 0.295  |
| Met                           | 36.54          | 14.07  | 84.08      | 35.11  | 0.111  |
| Orn                           | 60.62          | 32.88  | 52.32      | 16.46  | 0.010  |
| Phe                           | 33.73          | 3.72   | 75.25      | 11.72  | 0.018  |
| Pro                           | 223.72         | 47.62  | D          | D      | D      |
| Ser                           | 99.18          | 67.01  | 107.43     | 8.54   | 0.374  |
| Thr                           | 98.19          | 10.44  | 247.54     | 98.36  | 0.005  |
| Trp                           | 8.06           | 1.54   | 14.28      | 5.59   | 0.021  |
| Tyr                           | 55.90          | 13.18  | 113.93     | 32.12  | 0.075  |
| Val                           | 66.16          | 32.79  | 104.60     | 37.65  | 0.918  |
| Polyamines                    |                |        |            |        |        |
| Putrescine                    | 22.53          | 9.31   | 1.05       | 0.19   | 0.011  |
| Spermidine                    | 41.91          | 0.44   | 59.18      | 36.97  | 0.324  |
| Spermine                      | 19.49          | 6.13   | 25.64      | 13.60  | 0.025  |

Original Western Blot:

Figure 3.E

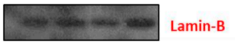
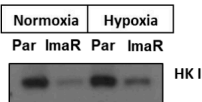


Figure 4.B

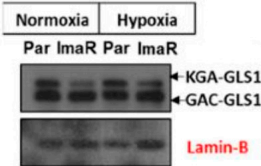
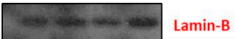
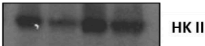
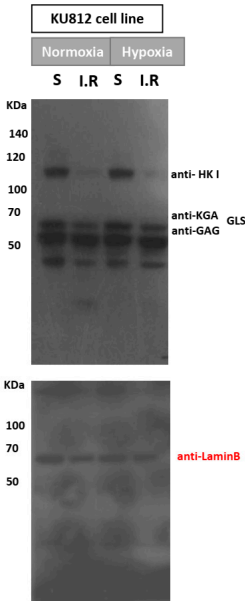


Figure 3.E

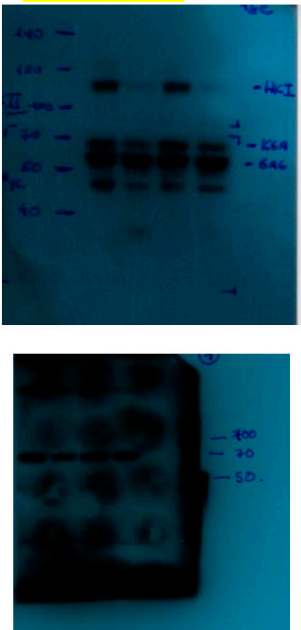


Membrane 1:

HKI and the two isoforms of GLS were performed in the same membrane.

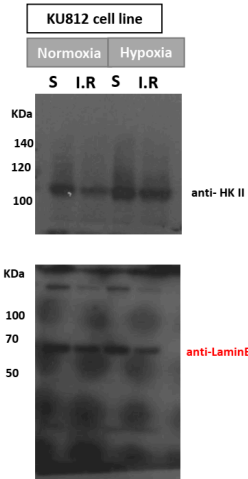


ORIGINAL SCANS



Membrane 2:

HKII membrane.



ORIGINAL SCANS

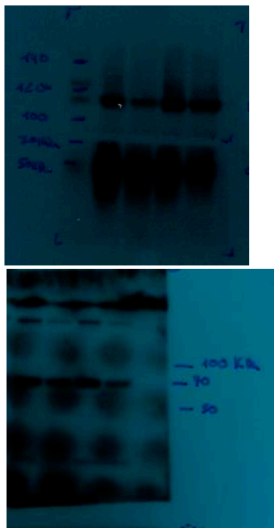
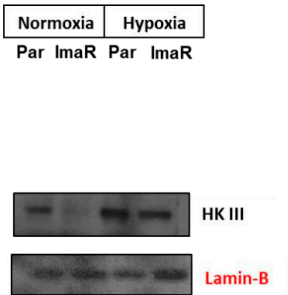
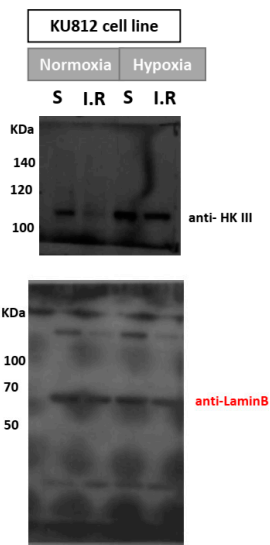


Figure 3.E



Membrane 3:

HKIII membrane.



ORIGINAL SCANS

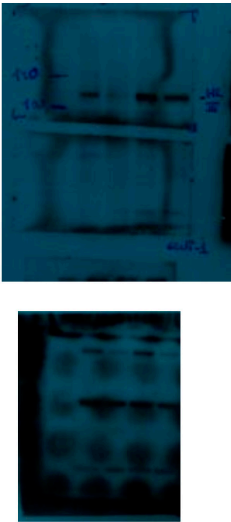
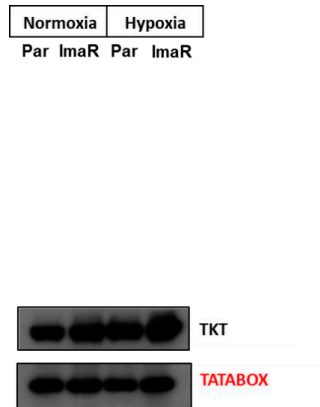
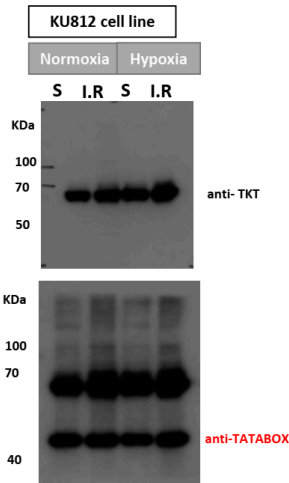


Figure 3.E



Membrane 4:

TKT membrane.



ORIGINAL SCANS

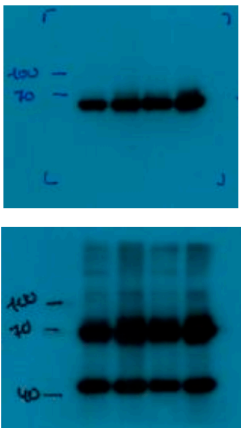
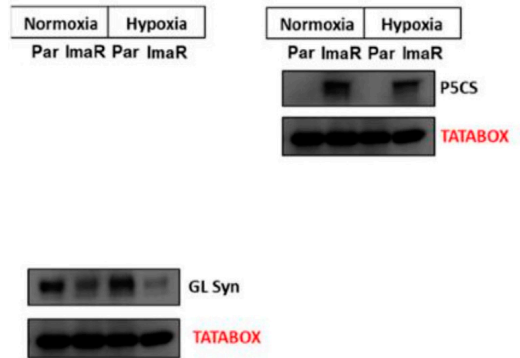
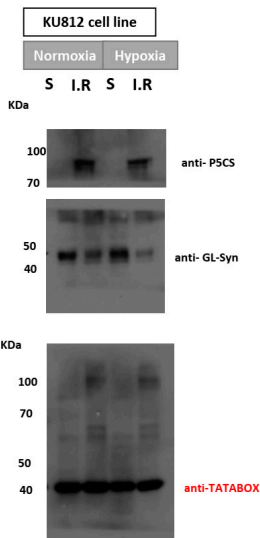


Figure 4.B



Membrane 5:

P5CS and GL-Syn proteins were performed in the same membrane.



ORIGINAL SCANS

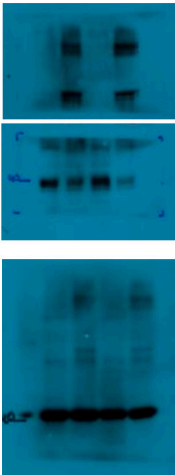
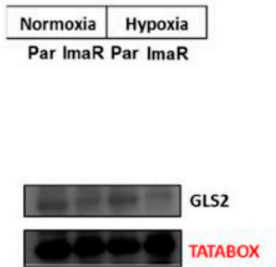


Figure 4.B



Membrane 6:

GLS2 protein

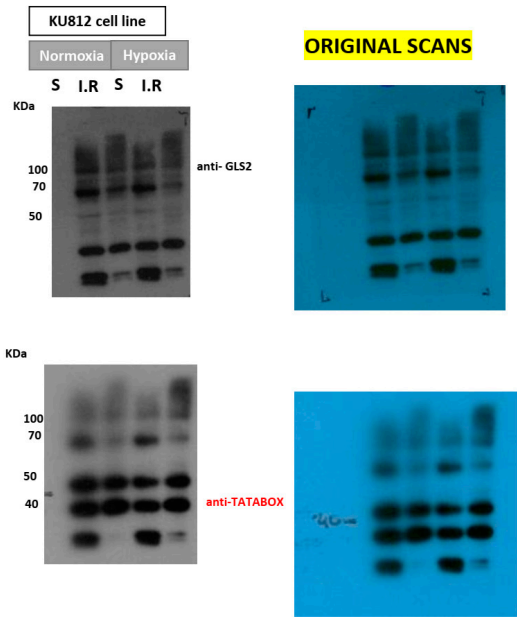
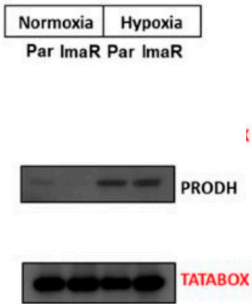


Figure 4.B



Membrane 7:

PRODH protein

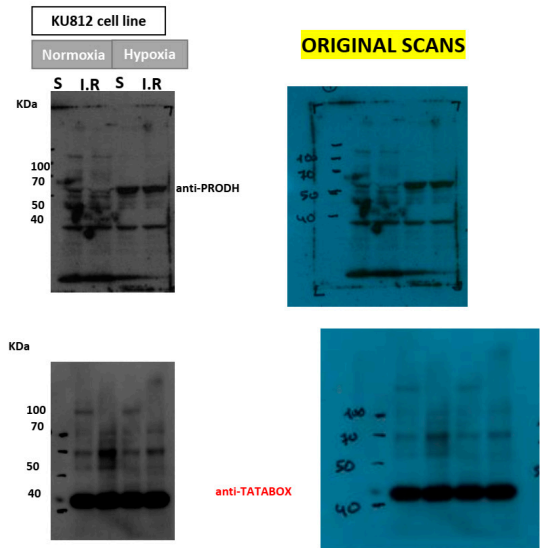
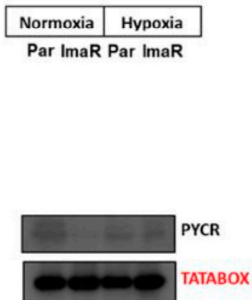


Figure 4.B



Membrane 8:

PYCR protein

