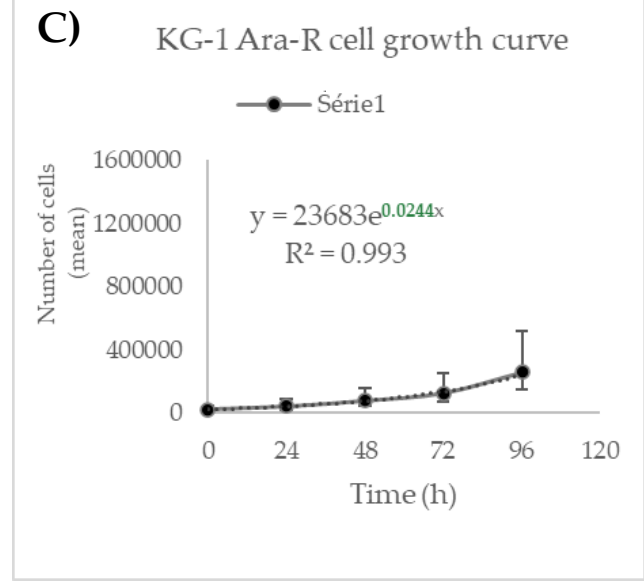
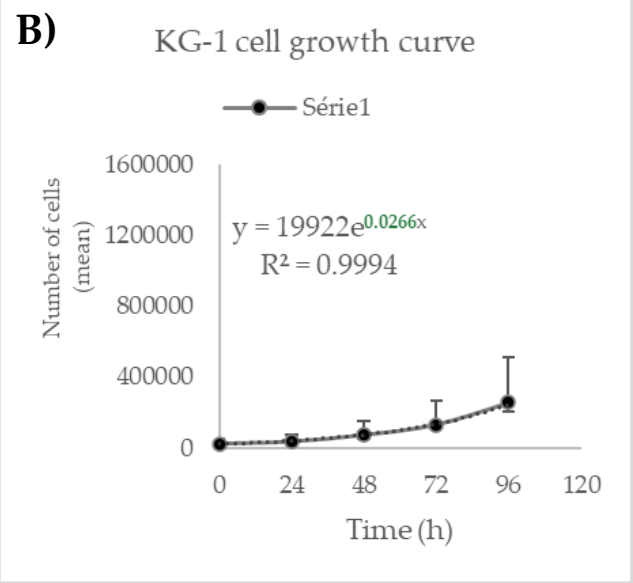


**Figure S1. Induction of Ara-C resistance in AML cell lines.** (A) KG-1 and (B) MOLM13 cells were exposed to increasing concentrations of cytarabine (Ara-C). Resistance to Ara-C was induced in KG-1 and MOLM13 cells for 3 and 6 months, respectively.

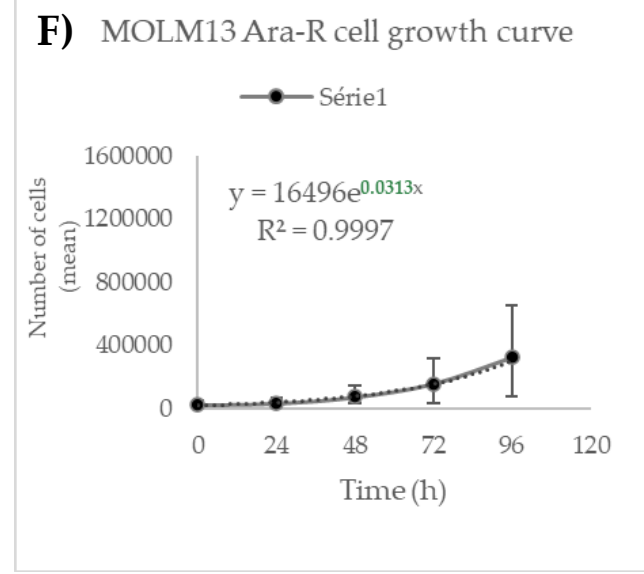
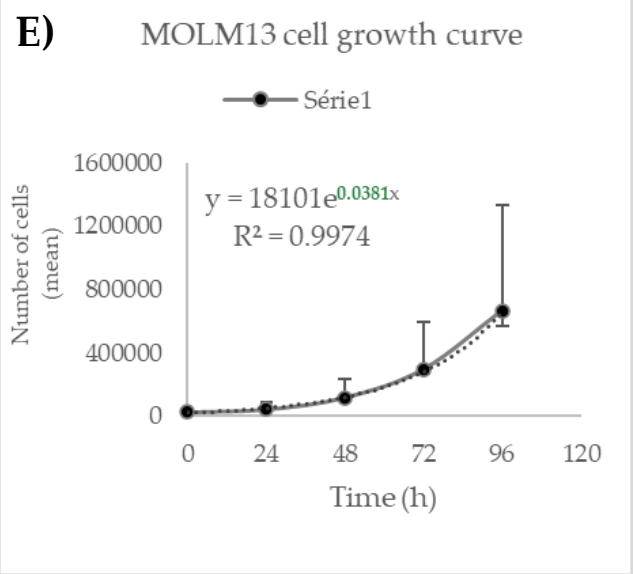
A)

Number of cells (mean of four independent experiments)				
Cell line	KG-1		KG-1 Ara-R	
Time (h)	MEAN	SD ±	MEAN	SD ±
24	36979	10225	42813	19153
48	74583	18887	78958	35929
72	133125	18576	126979	56648
96	256979	47888	258021	111830
Td = ln(2)/B (h)	26.058		28.408	
μ = [ln(N(96)/N(24))]/t(72) (h <sup>-1</sup> )	0.027		0.025	

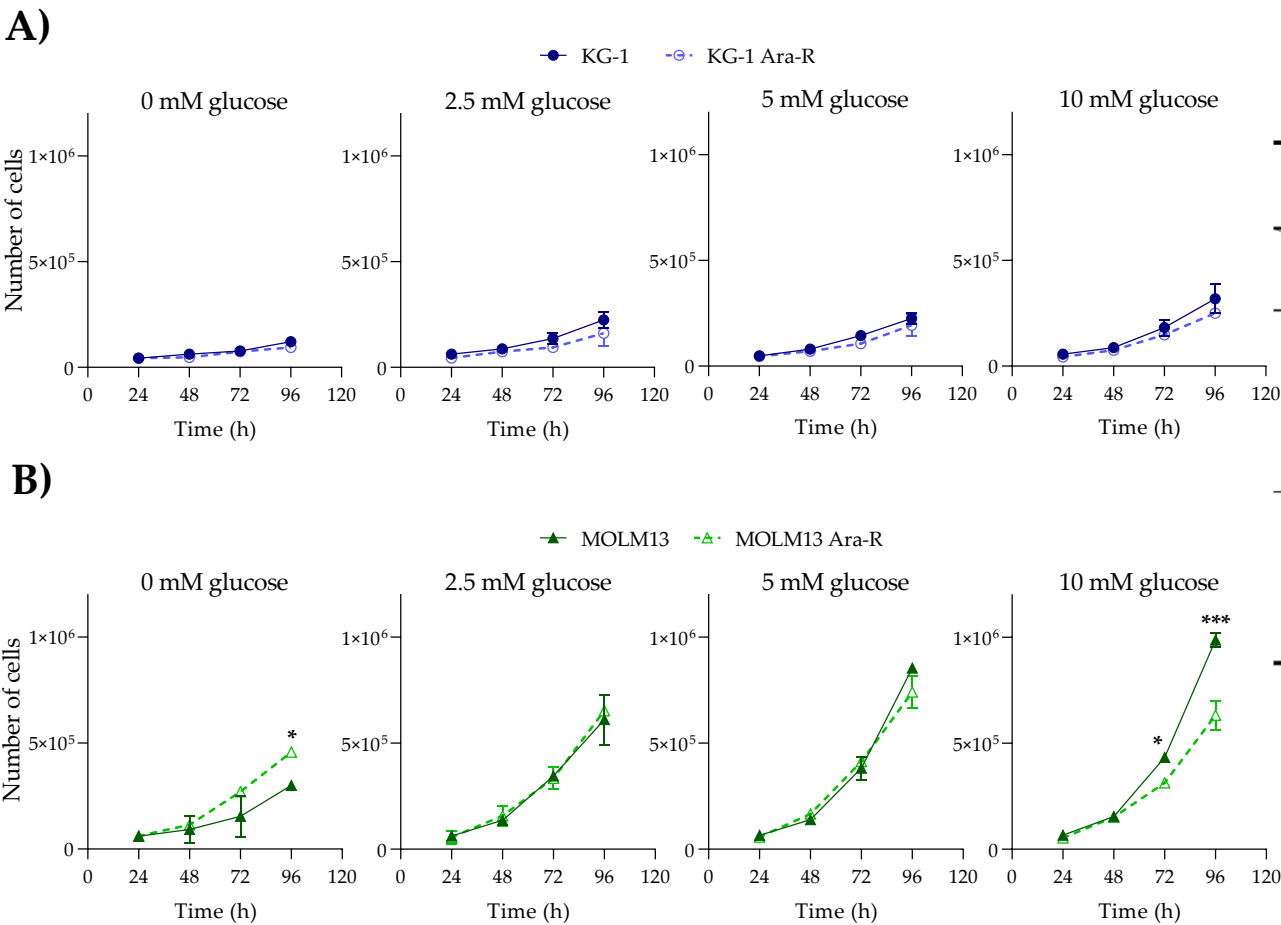


D)

Number of cells (mean of four independent experiments)				
Cell line	MOLM13		MOLM13 Ara-R	
Time (h)	MEAN	SD ±	MEAN	SD ±
24	43125	8344	34444	14181
48	117813	12348	74861	35607
72	298229	32103	159167	123627
96	668750	100606	326389	248965
Td = ln(2)/B (h)	18.193		22.145	
μ = [ln(N(96)/N(24))]/t(72) (h <sup>-1</sup> )	0.038		0.031	



**Figure S2. Calculation of doubling time (Td) and growth rates (μ) in cytarabine (Ara-C)-resistant cell lines.** A-C) KG-1, KG-1 Ara-R and D-F) MOLM13, MOLM13 Ara-R cell lines were seeded into 24-well plates in complete RPMI medium. B-C, E-F) Growth curves were generated from mean of cell numbers of four independent experiments shown in A-C) for KG-1 and KG-1 Ara-R cells, and in D-E) for MOLM13 and MOLM13 Ara-R cells, using manual cell counting in the Neubauer chamber every 24h for 4 days. Td was calculated from line equation were the green values corresponded to the B value.

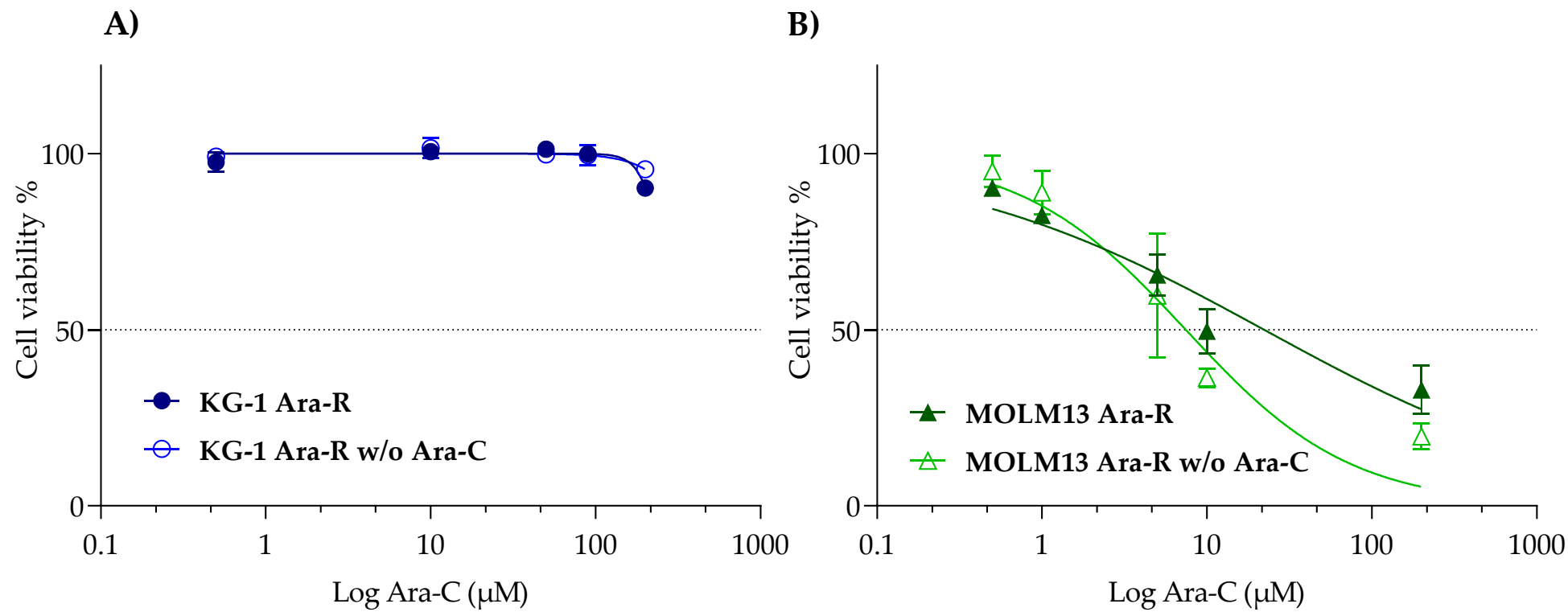


**C)**

DOUBLING TIME (Td) ± SD (h)					GROWTH RATE (μ) ± SD (h <sup>-1</sup> )			
Glucose (mM)	0	2.5	5	10	0	2.5	5	10
Cell line								
KG-1	44.68 ± 3.05	32.61 ± 4.93*	30.44 ± 1.51*	26.67 ± 0.58*	0.014 ± 0.001	0.018 ± 0.006	0.021 ± 0.004	0.024 ± 0.002
KG-1 Ara-R	52.41 ± 5.57	38.40 ± 6.96	34.26 ± 1.51	28.40 ± 2.46*	0.011 ± 0.001	0.018 ± 0.002	0.020 ± 0.004	0.025 ± 0.007
MOLM13	25.15 ± 3.33	20.30 ± 1.63	18.54 ± 0.56	17.96 ± 0.53*	0.023 ± 0.005	0.032 ± 0.004	0.036 ± 0.003	0.038 ± 0.004*
MOLM13 Ara-R	26.34 ± 5.00	20.16 ± 0.58	19.33 ± 0.84	20.21 ± 0.42	0.028 ± 0.001	0.035 ± 0.008	0.036 ± 0.006	0.034 ± 0.001

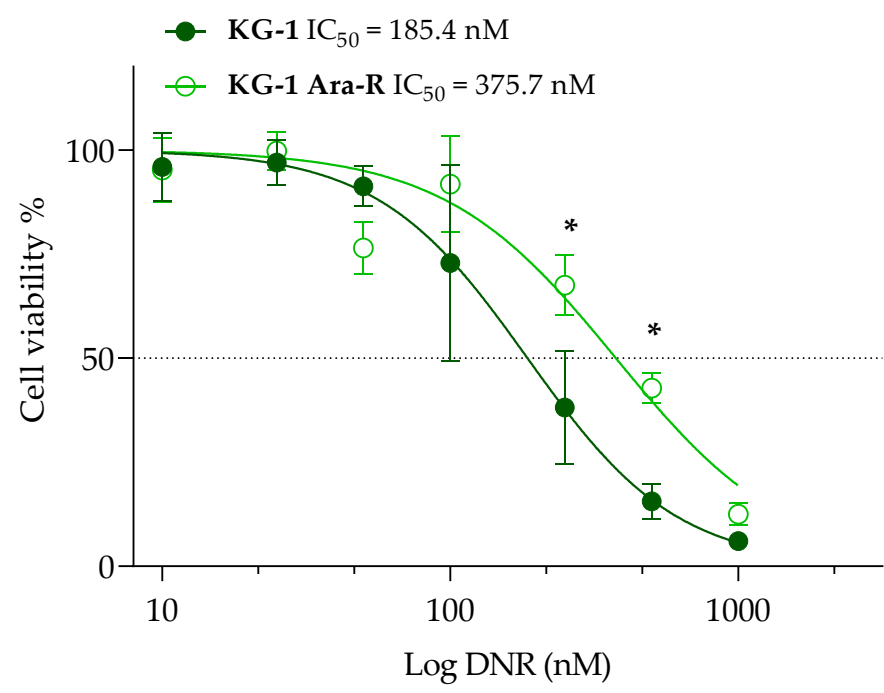
\* Significantly different comparing the different glucose concentrations with absence of glucose (p ≤ 0.05).

**Figure S3. Effect of different glucose concentrations on cell growth rates and doubling times of AML Ara-C resistant and parental cell lines.** Cell growth curves of A) KG-1 and KG-1 Ara-R, and B) MOLM13 and MOLM13 Ara-R were analyzed over multiple population doublings.  $2.5 \times 10^4$  cells were plated with 0, 2.5, 5 and 10mM glucose. Cells were counted every 24h using Trypan blue dye. C) Values of growth rates ( $\mu$ ) and doubling times (Td) were calculated from the respective line equations. Statistical significance was determined by two-way ANOVA followed by Sidak's Multiple Comparison Test. \*  $p \leq 0.05$ ; \*\*\*  $p \leq 0.001$ . At least three independent experiments with three replicates were performed.

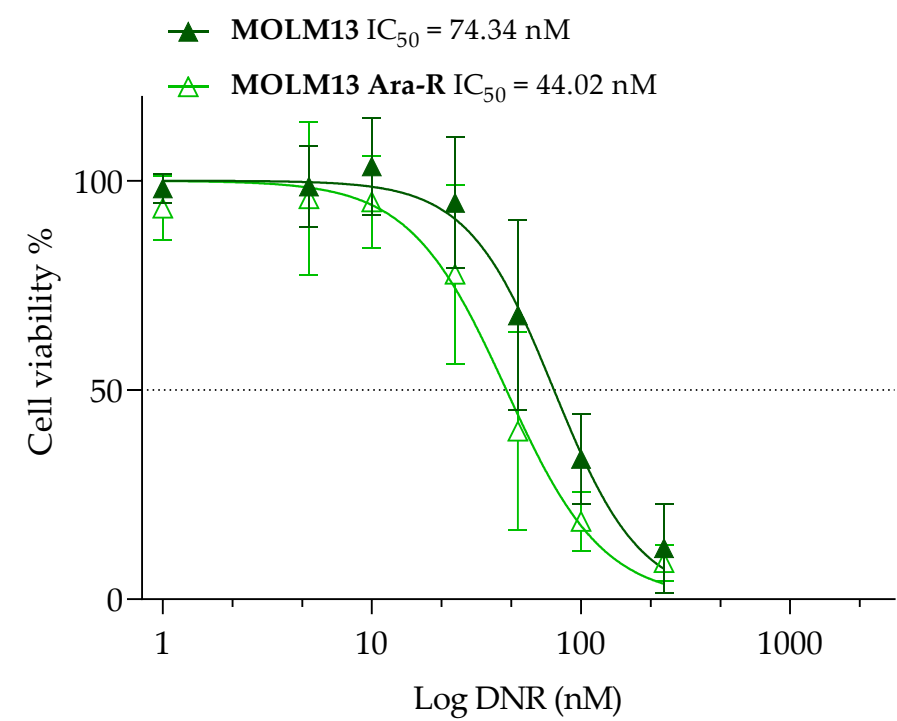


**Figure S4. Sensitivity of cytarabine (Ara-C)-resistant cells in absence of Ara-C.** Ara-C-resistant (A) KG-1 and (B) MOLM13 cells were cultured for three weeks with and without Ara-C. Cells were plated and tested with a range of Ara-C concentrations for 48h. Values are expressed as percentage of cell viability relative to vehicle-treated cells normalized to 100%. Values are given as mean  $\pm$  SD. Two-way ANOVA followed by Sidak's Multiple Comparison Test. At least three independent experiments with two replicates were performed.

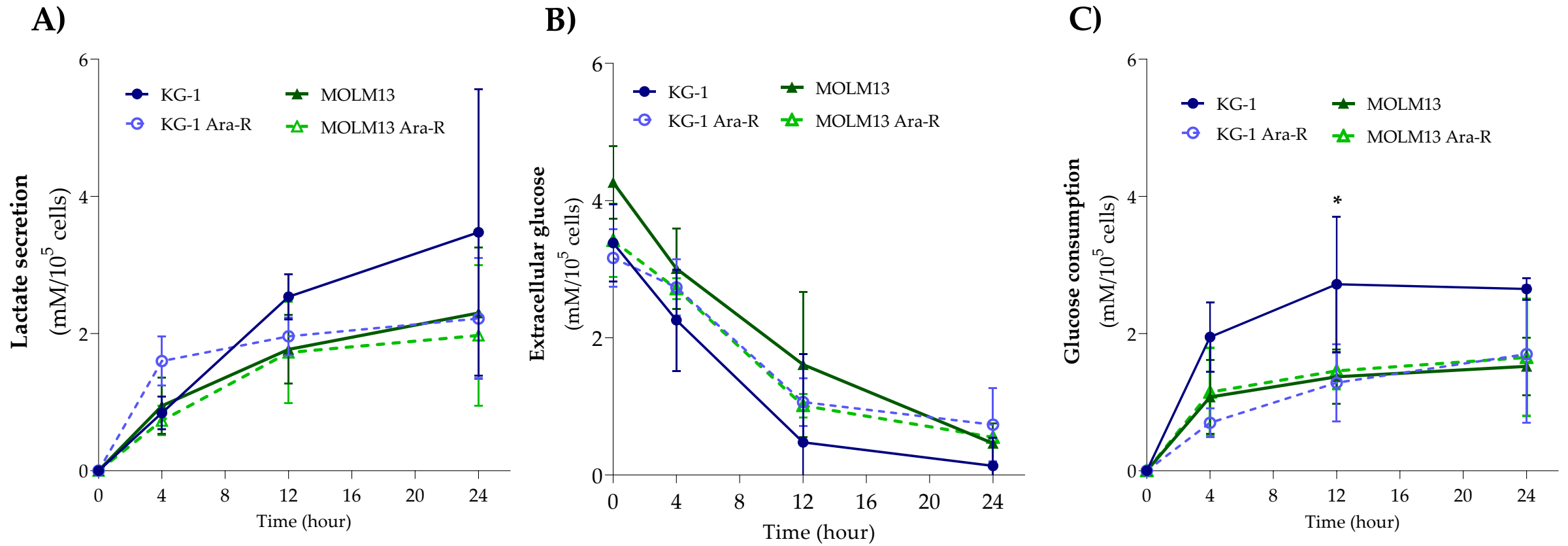
A)



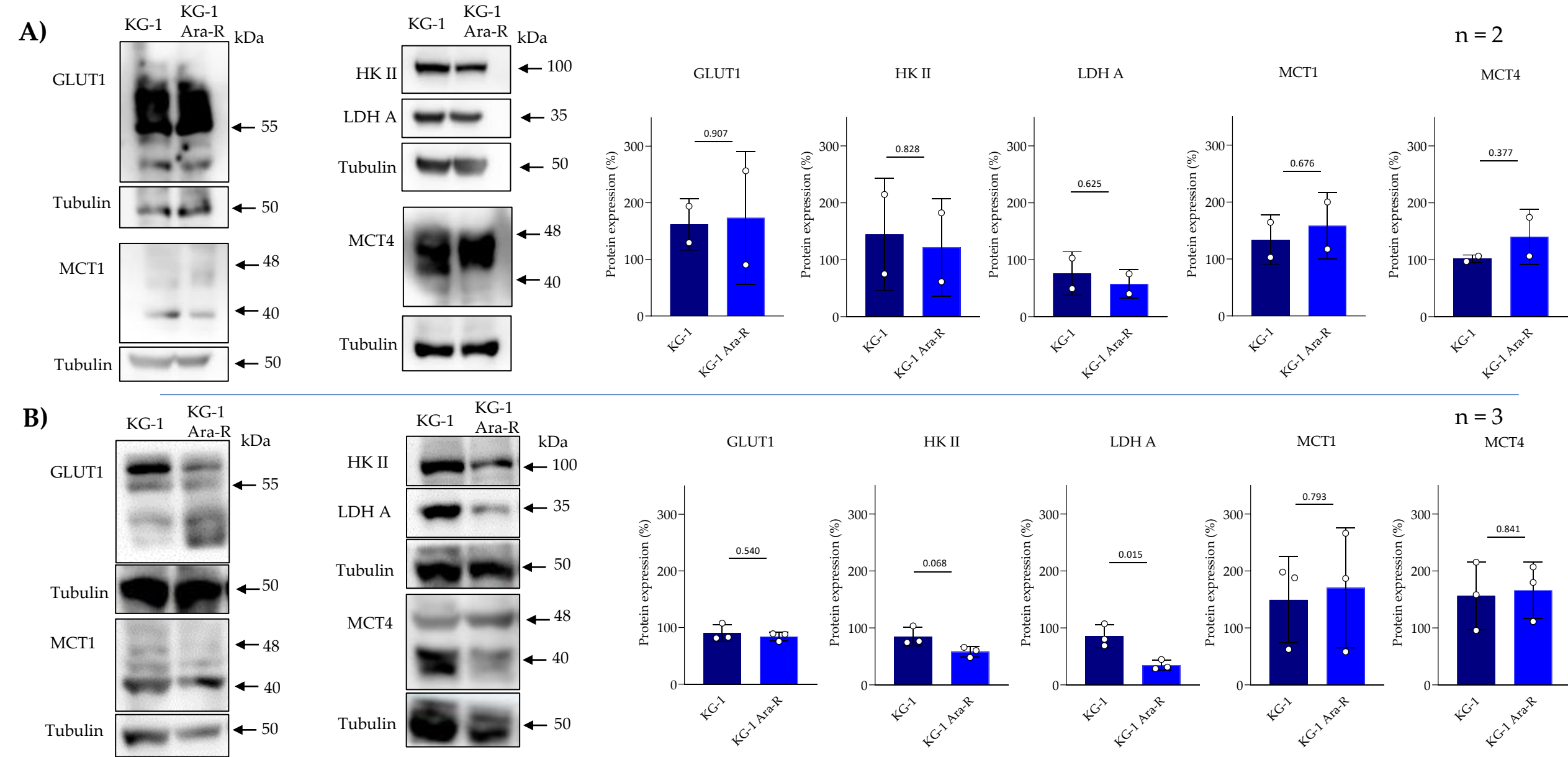
B)



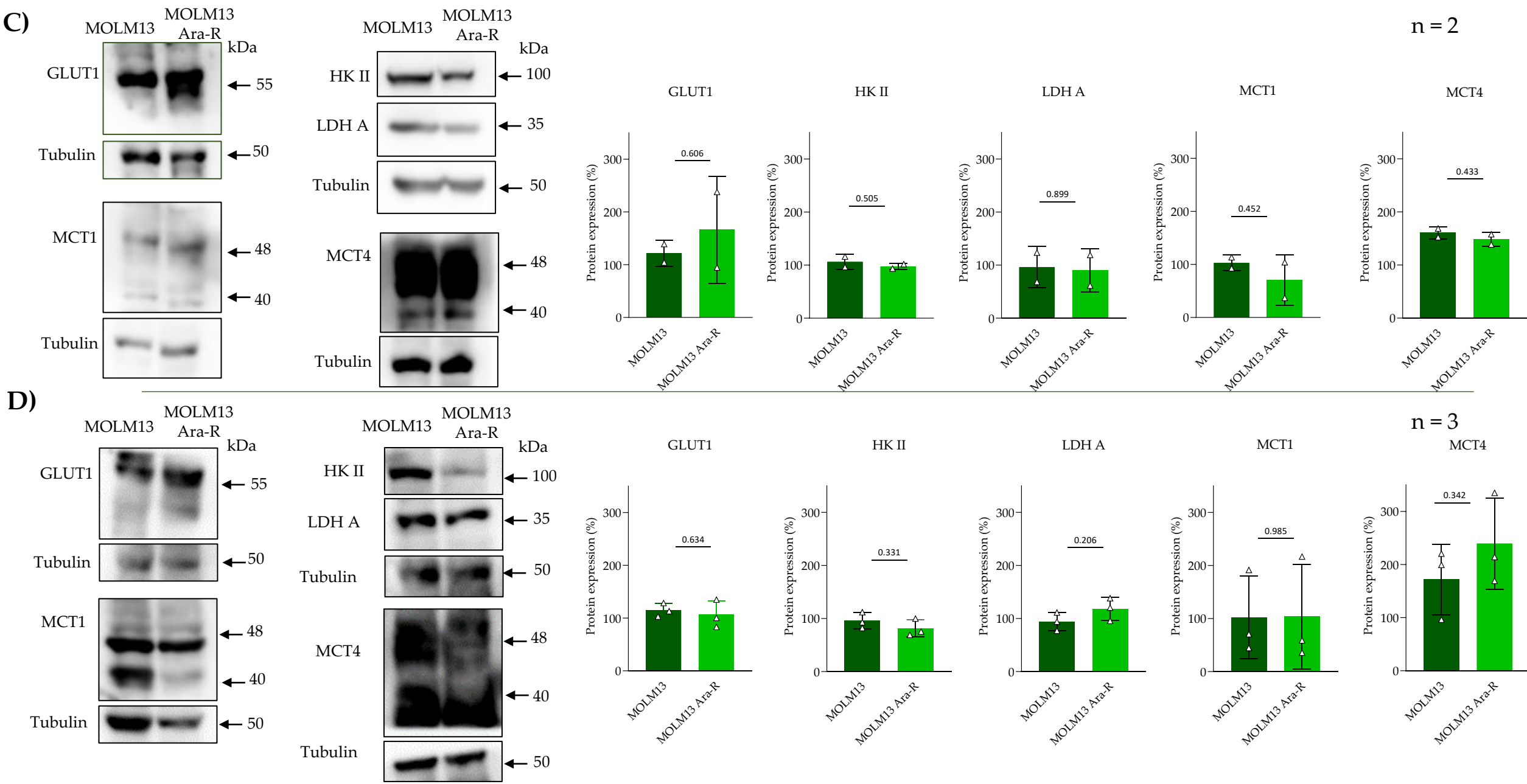
**Figure S5. Sensitivity of cytarabine (Ara-C)-resistant cells to daunorubicin (DNR).** Ara-C-resistant (A) KG-1 and (B) MOLM13 cells were plated and tested with a range of DNR concentrations. Values are expressed as percentage of cell viability relative to vehicle-treated cells normalized to 100%. Values are given as mean  $\pm$  SD. Two-way ANOVA followed by Sidak's Multiple Comparison Test. At least three independent experiments with two replicates were performed. \*  $p \leq 0.05$ .



**Figure S6. Extracellular lactate and glucose, glucose consumption in parental and Ara-C resistant cell lines.** (A) Extracellular lactate levels and (B) extracellular glucose levels and (C) glucose consumption were evaluated at 0, 4, 12, and 24h for KG-1 and KG-1 Ara-R, and MOLM13, and MOLM Ara-R cells. Glucose consumption corresponds to the difference between 0 and 4, 12 or 24h of extracellular glucose levels. Results are presented as mean  $\pm$  SD from three independent experiments. Statistical significance estimated by two-way ANOVA followed by Sidak's Multiple Comparison Test: \* $p < 0.05$  (C).

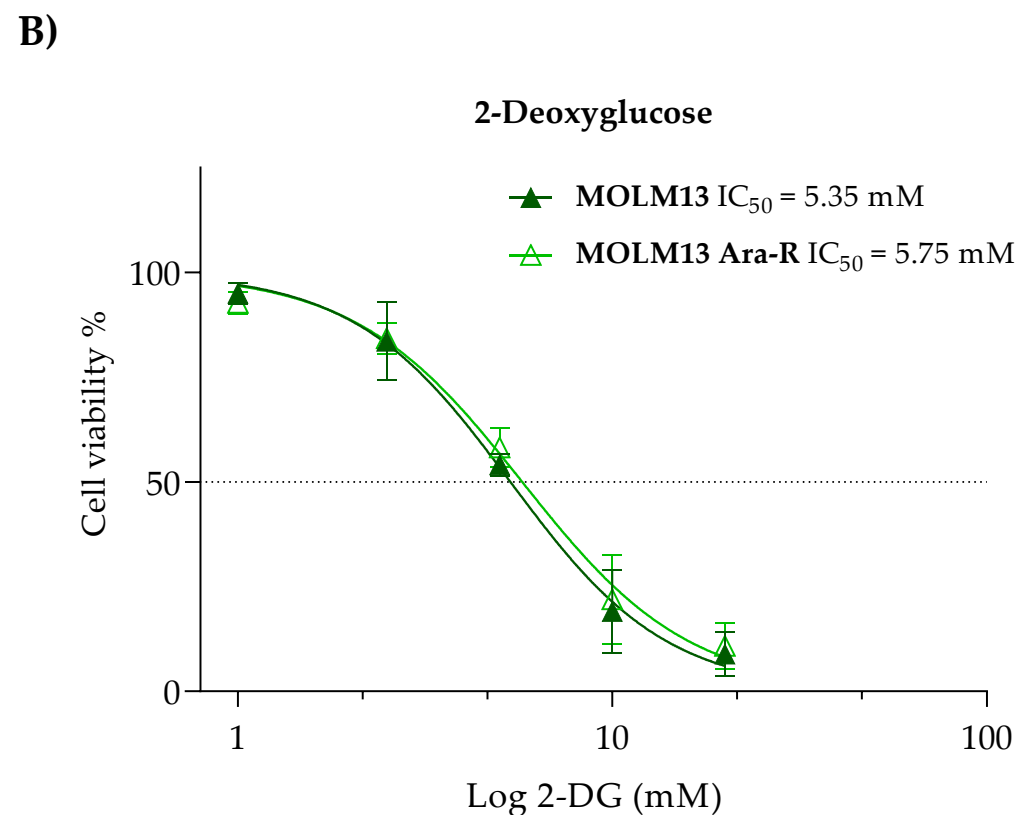
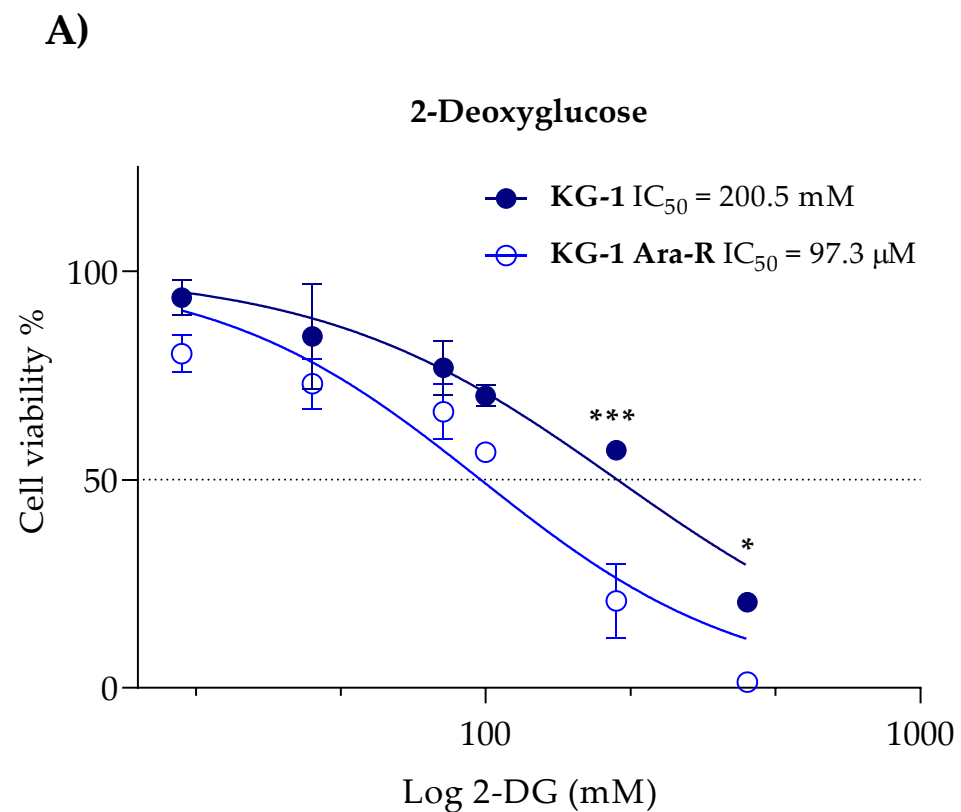


**Figure S7. Characterization of glycolytic phenotype-associated protein expression in cytarabine (Ara-C)-resistant cell lines.** Expression levels of GLUT1, HKII, LDHA, MCT1, and MCT4 proteins assessed by western blot in the cell pairs A,B) KG-1/KG-1 Ara-R and C,D) MOLM13/MOLM13 Ara-R cells at A,C) 6h and B,D) 24h.  $\alpha$ -Tubulin was used as a loading control. Levels of protein expression were normalized for tubulin. Results were quantified using the ImageJ software and results are presented as mean  $\pm$  SD of at least two independent experiments. Values were expressed as protein levels relative to parental cells normalized for 100%. Statistical significance was estimated by the unpaired two-tailed Student's t-test.



**Figure S7.** (continued).





**Figure S8. Effect of 2-deoxy-glucose (2-DG) on Ara-C-resistant and parental AML cell viability.** Dose-response curve to generate  $IC_{50}$  values of (A) KG-1 and KG-1 Ara-R cell lines and (B) MOLM13 and MOLM13 Ara-R cell lines in response to 2-DG. Values are expressed as cell viability relative to vehicle-treated cells normalized to 100%. Values are given as mean  $\pm$  SD. \* $p < 0.05$ ; \*\*\* $p < 0.001$  (Two-way ANOVA followed by Sidak's Multiple Comparison Test). Results are from at least three independent experiments with two replicates.