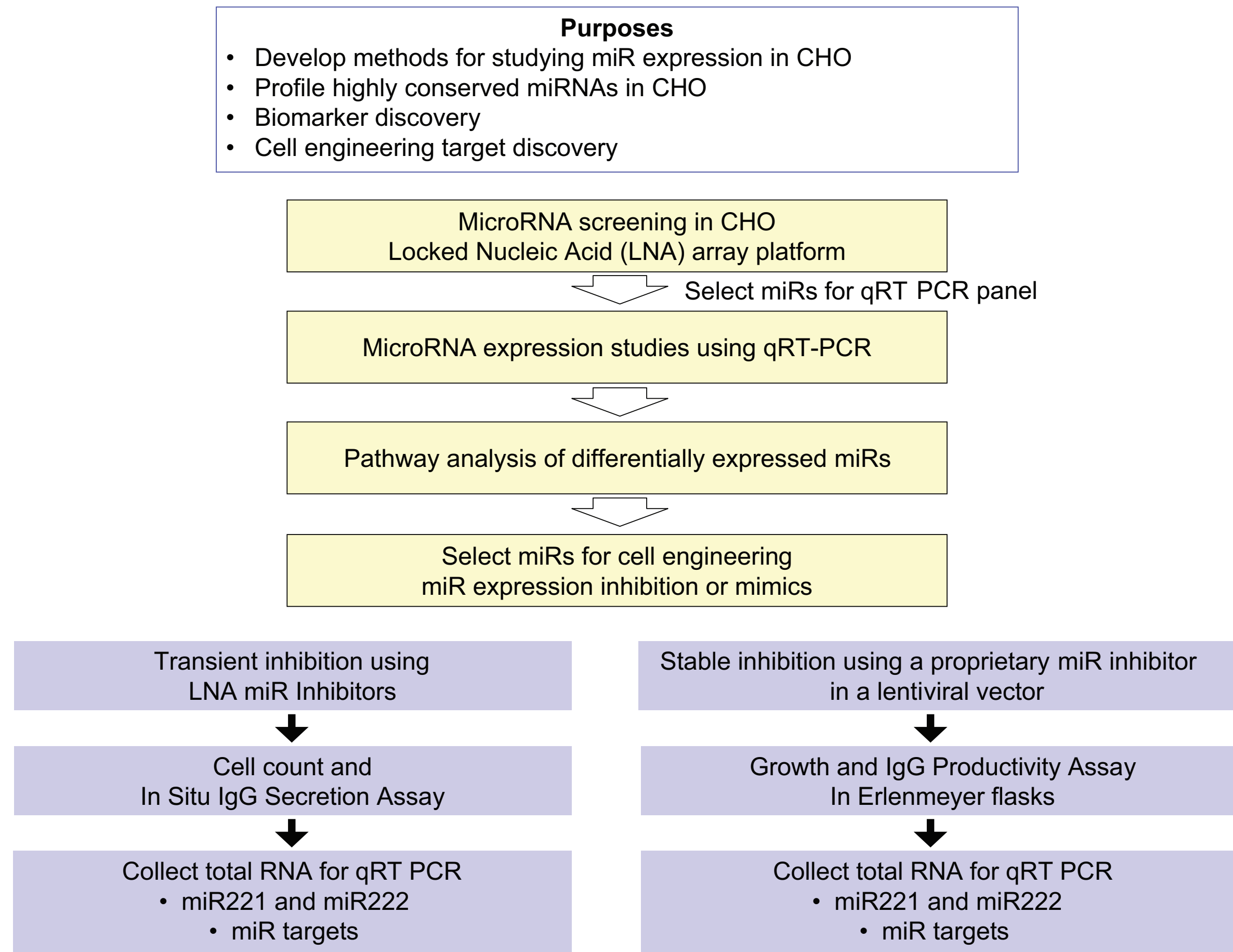


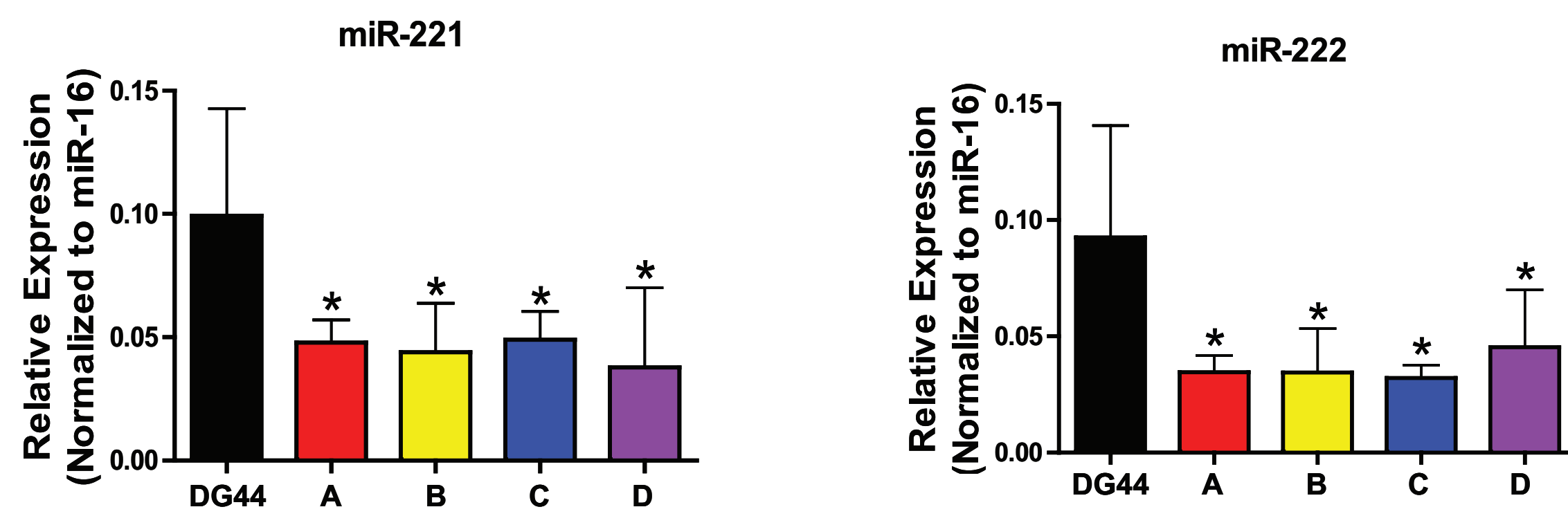
## Project Overview

Figure 1. Background and Experimental Design



## Results

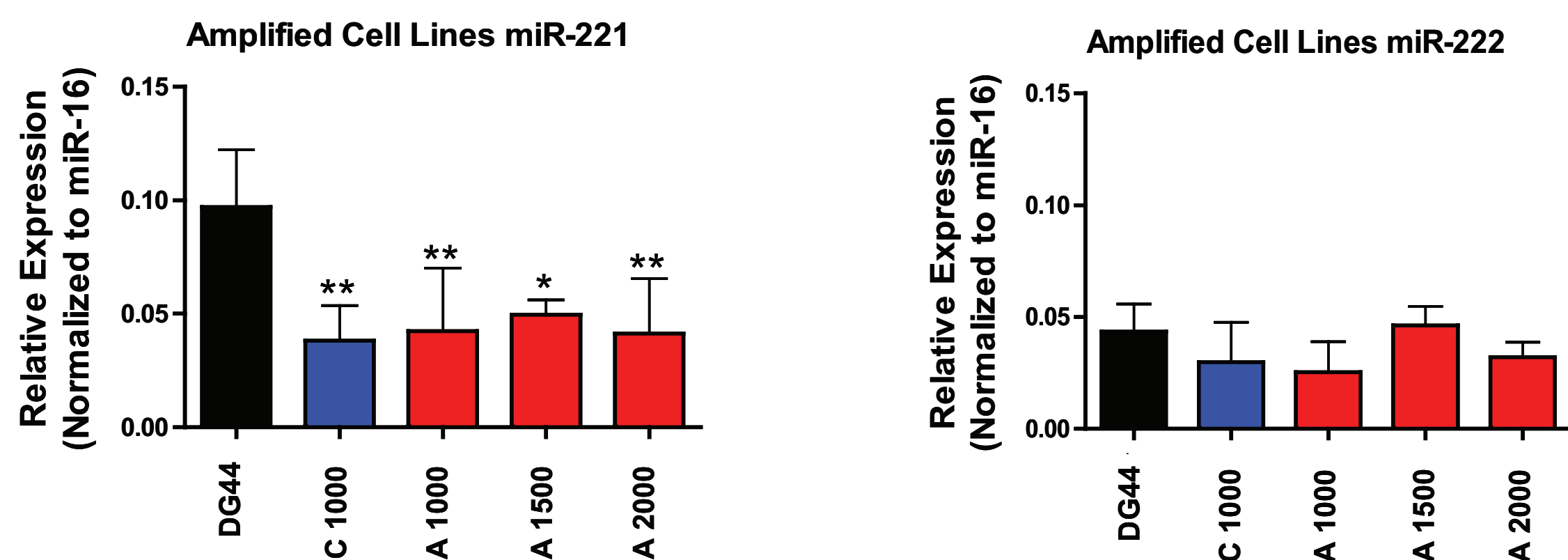
Figure 2. Differential Expression of miR-221 and miR-222 in IgG-Producing DG44 Lines by qRT-PCR



Differential expression of miR-221 (left panel), miR-222 (right panel) in four IgG-producing DG44-derived cell lines (500 nM MTX cultures). Relative expression levels were normalized to miR-16.

\* $p < 0.05$ , ANOVA and Dunnett's Multiple Comparison Test.

Figure 3. Post-Amplification Expression of miR-221 and miR-222 in the MTX Responsive and Non-Responsive Cell Lines



Differential expression of miR-221 (left panel), miR-222 (right panel) in cell line C and A (Fig.2) Amplified by sequential increase of MTX concentration. Cell line A demonstrated increased IgG productivity but not cell line C (data not shown here).

Relative expression levels were normalized to miR-16.

\* $p < 0.05$ , ANOVA and Dunnett's Multiple Comparison Test.

Figure 4. Modulating miR Expression to Control Cell Cycle for Increased IgG Secretion

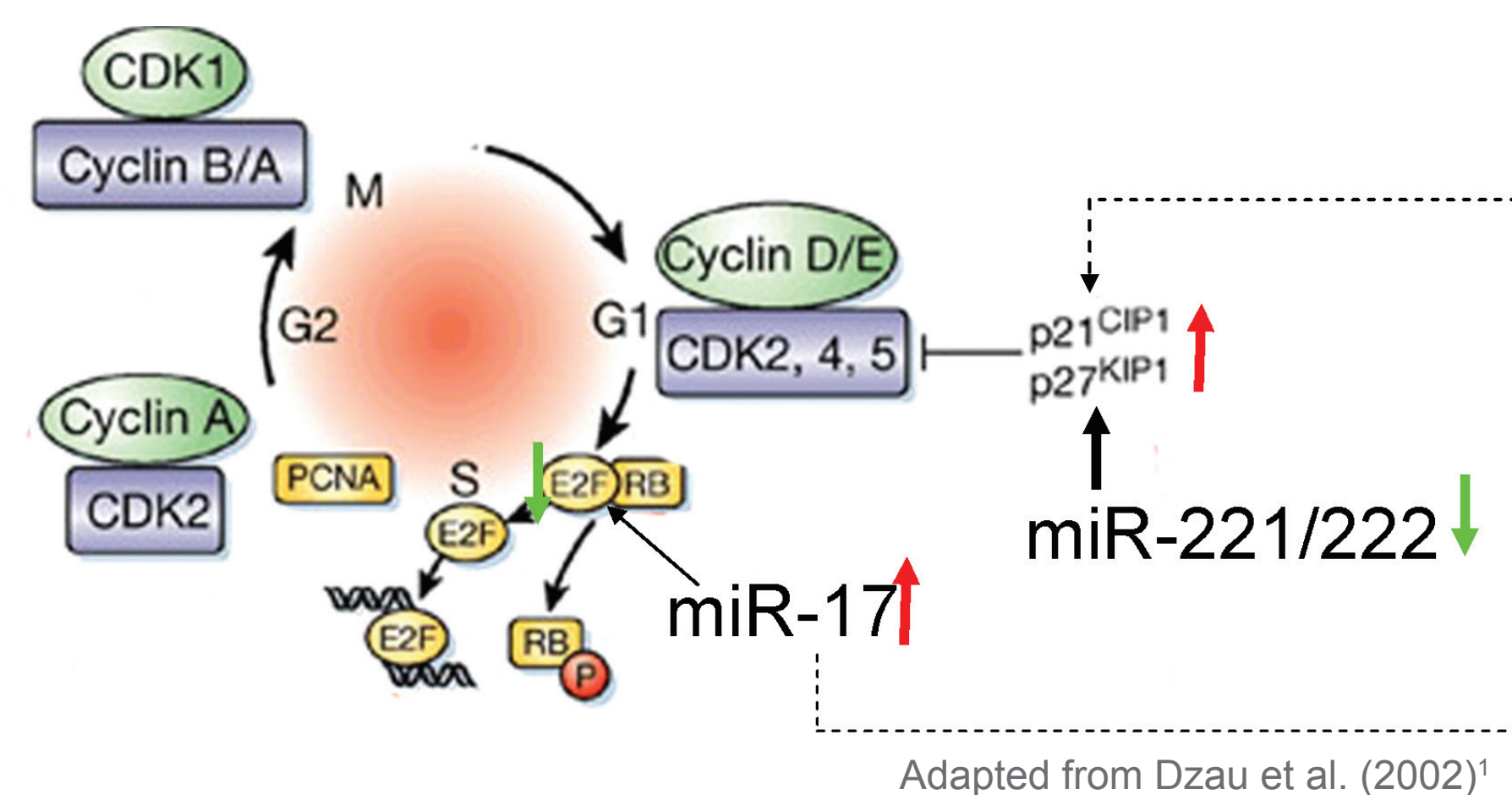
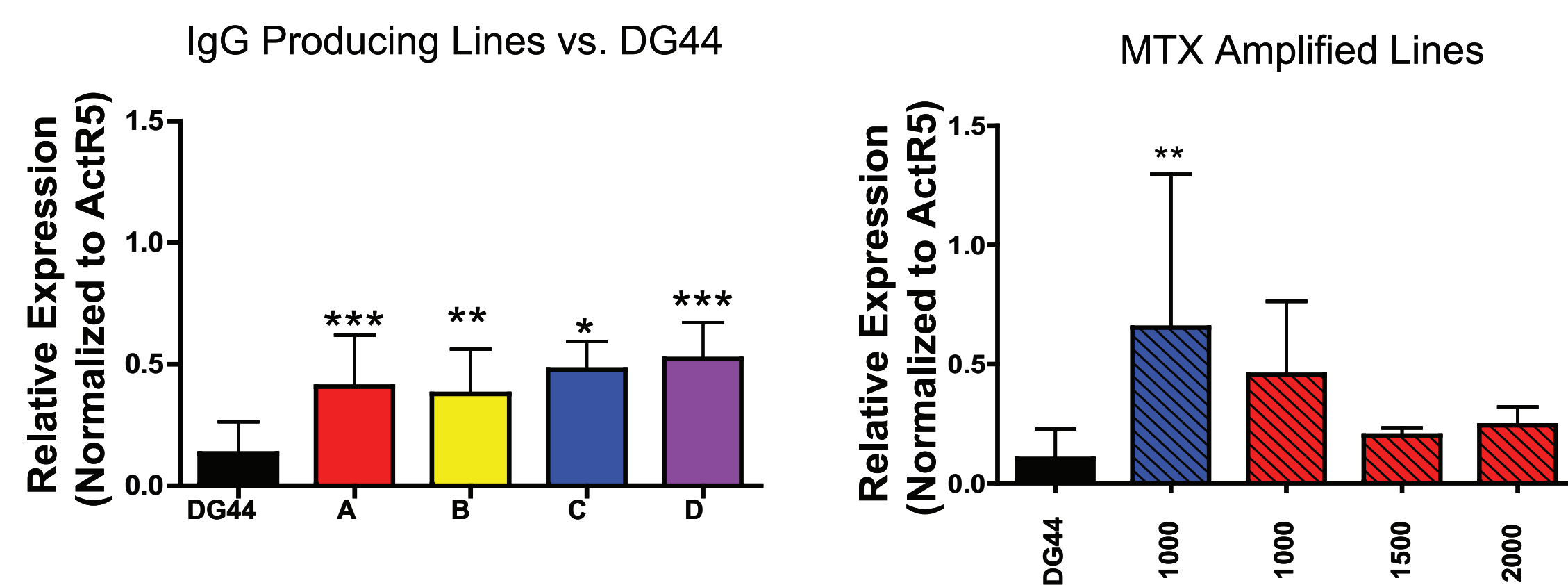


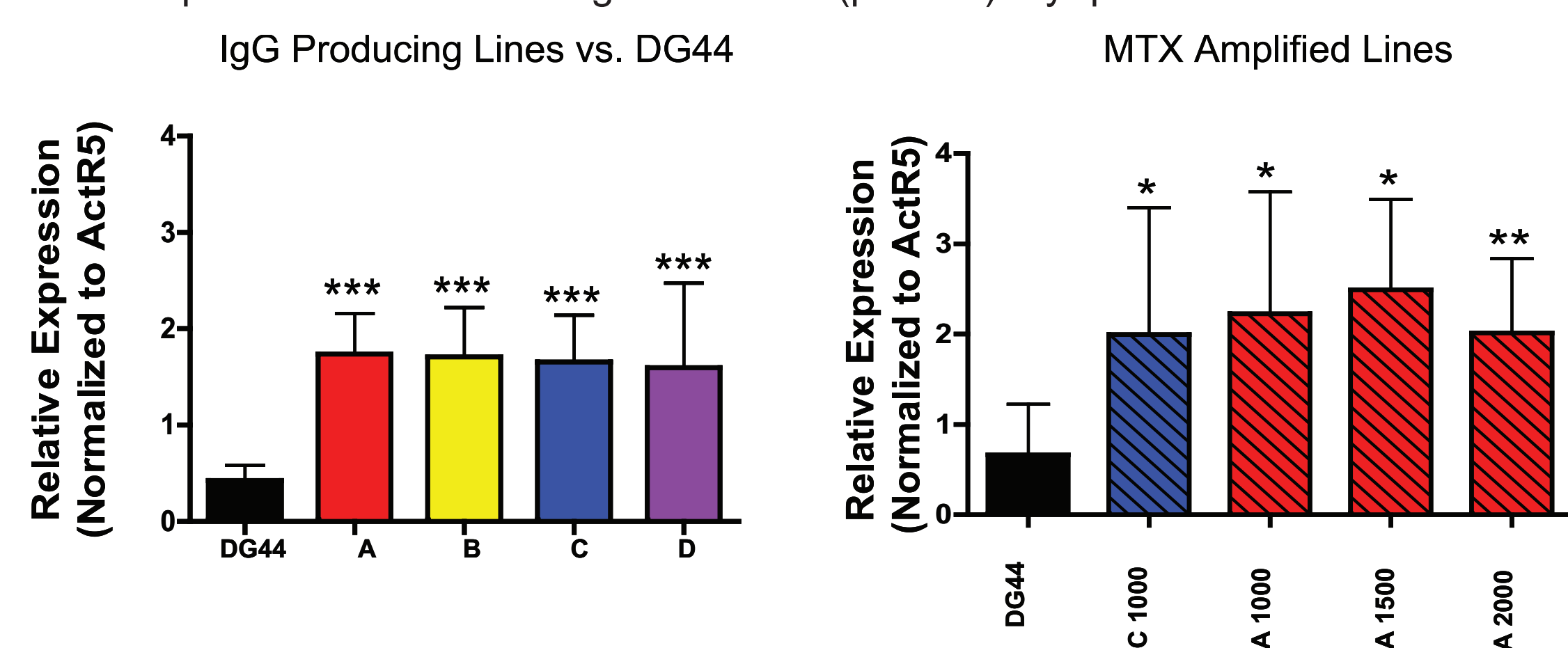
Figure 5. Relative Expression of miR-221/222 Target - Cdkn1b (p27Kip1)<sup>2</sup> by qRT-PCR



Differential expression of Cdkn1b in IgG producing cell lines A – D (left panel), and amplified cell line C and A (right panel). Relative expression levels were normalized to ActR5 (multiplexed qRT-PCR).

\* $p < 0.05$ , ANOVA and Dunnett's Multiple Comparison Test.

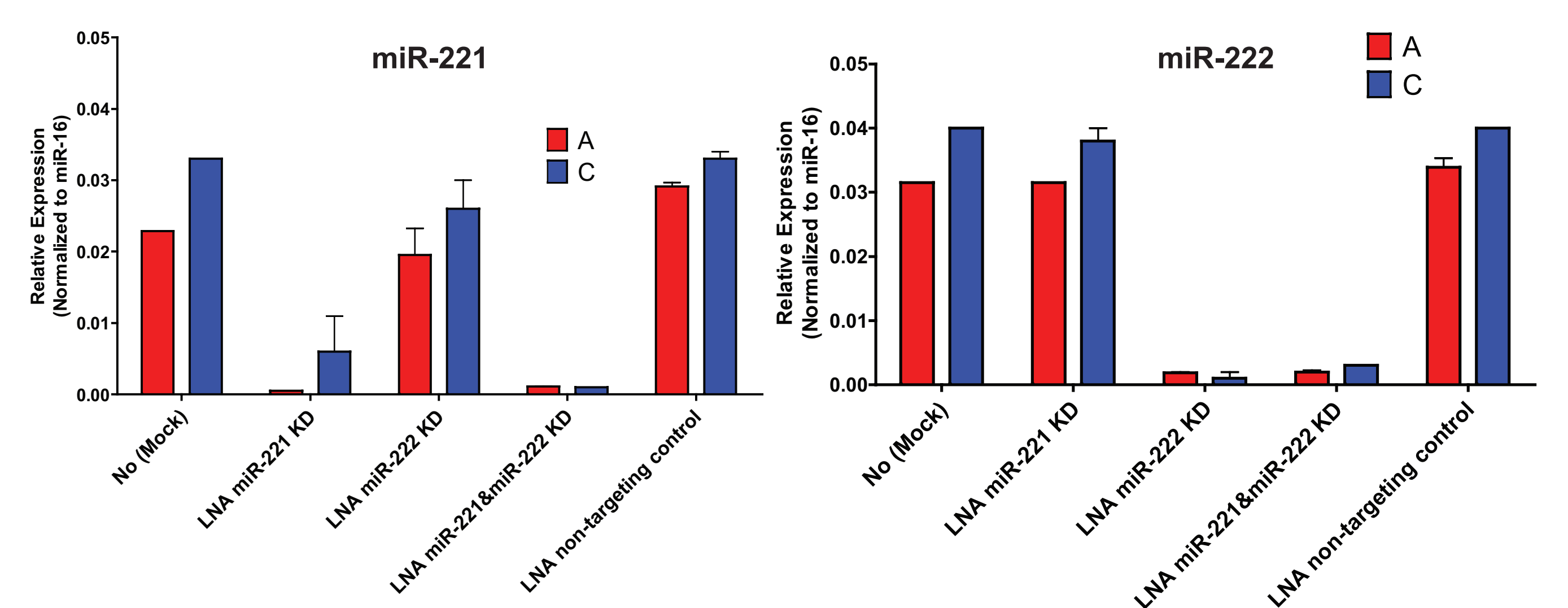
Figure 6. Relative Expression of miR-17 Target - Cdkn1a (p21<sup>WAF1</sup>)<sup>3</sup> by qRT-PCR



Differential expression of Cdkn1a in IgG producing cell lines A – D (left panel), and amplified cell line C and A (right panel). Relative expression levels were normalized to ActR5 (multiplexed qRT-PCR).

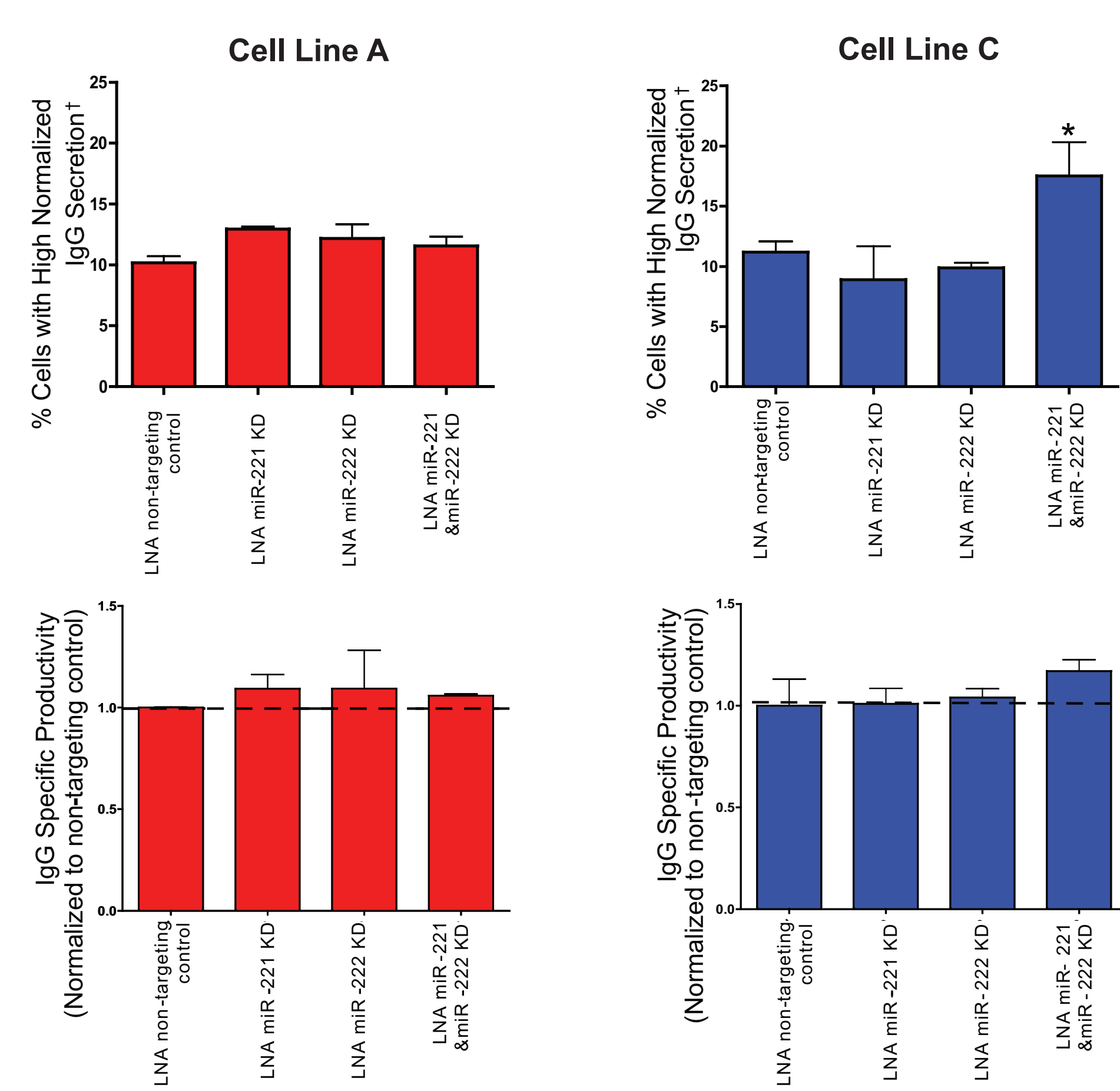
\* $p < 0.05$ , ANOVA and Dunnett's Multiple Comparison Test.

Figure 7. Transient Knockdown of miR-221/222 Using LNA miR Inhibitors



Cells were collected 96 hours after electroporation of LNA miR Inhibitors for miR-221 and/or miR-222 in cell lines A and C. miR-221 (left panel) and miR-222 (right panel) relative expression levels were normalized to ActR5 (multiplexed qRT-PCR).

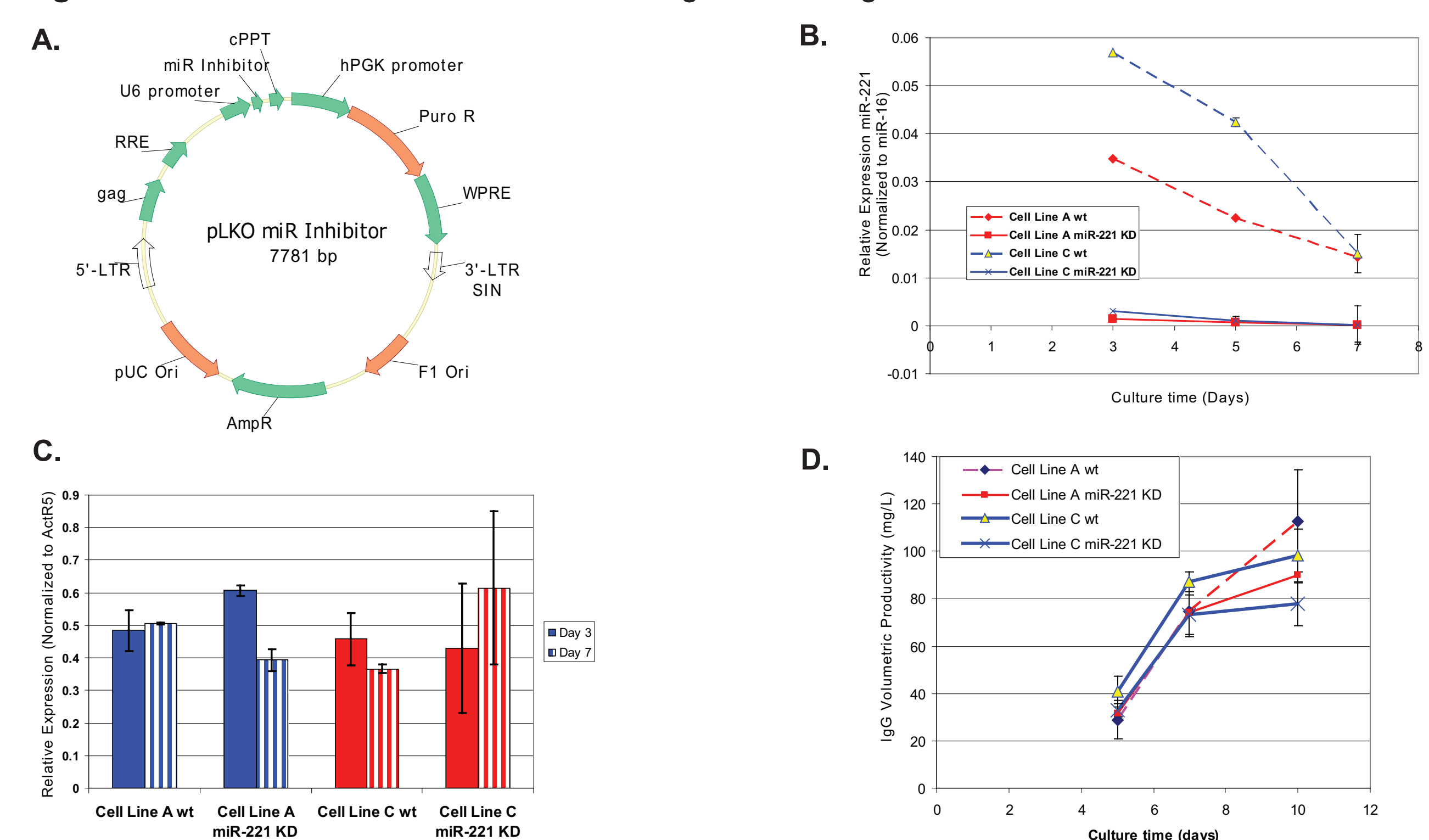
Figure 8. IgG Secretion 96-Hour Post Transient Knockdown of miR-221/222



**Upper panels:** Cells were plated 24 hours after LNA electroporation for the in situ IgG Secretion Assay<sup>4</sup>. Detection was performed 48 hrs after electroporation. High IgG secretion cutoff is 90th percentile of the control population. LNA inhibition of miR-221 and miR-222 led to mild increase in high IgG secreting population in Cell Line C but not Cell Line A. ANOVA,  $p < 0.05$ . Dunnett's Multiple Comparison Test, LNA non-targeting as control, \* $p < 0.05$

**Lower panels:** Cells were plated in 6-well static cultures 24 hours after LNA electroporation, and harvested 5 days after plating for viable cell density and IgG productivity using ForteBio Octet system.

Figure 9. Stable Inhibition of miR-221 in Two IgG Producing DG44 Lines



**Panel A:** Lentiviral expression vector for proprietary miR inhibitors. Puromycin selection was performed after transduction to generate stable pools for miR-221 knockdown. Growth and productivity assays in Erlenmeyer flask cultures were performed using the miR-221 KD lines and the wildtype lines. **Panel B:** Relative expression of miR-221 by qRT-PCR. Expression of miR-222 showed no significant change (data not shown). **Panel C:** Relative expression of Cdkn1b on Day 3 and Day 7 by qRT-PCR. Numerical increase in Cell Line A-miR-221 KD on day 3 and in Cell Line C-miR-221 on day 7 was observed. **Panel D:** Volumetric IgG productivity of the miR-221 KD and wildtype cell lines.

## Conclusions

- In the IgG producing CHO DG44 cell lines, miR-221 and miR-222 are differentially expressed compared to the parental CHO DG44 cell line. In an MTX-amplified cell line, miR-221 is significantly down-regulated at increased MTX concentrations albeit without significant dose-response
- Cdkn1b and Cdkn1a, two of the validated target genes for cell cycle modulating miRs (miR-221, miR-222 and miR-17) are significantly up-regulated in the IgG-producing DG44 lines
- LNA miR Inhibitors effectively inhibited miR-221 and miR-222 with high specificity. Transient inhibition of both miR-221 and miR-222 expression led to mild increase in IgG secretion per cell in one of the two IgG producing lines (Line C)
- Stable miR inhibition was achieved by Lentiviral expression of a proprietary miR inhibitor with high specificity for miR-221. However, neither cell line A nor C demonstrated significant increase in IgG productivity after transduction. Both cell lines showed numerical increase in miR-221 target Cdkn1b expression
- Based on the transient inhibition data, ongoing work includes simultaneous stable inhibition of miR-221 and miR-222 in both cell lines A and C. Expression of miR-17 mimics will also be explored.

## Acknowledgements

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## References

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