



Cell synchronization as a tool to optimize expression of metabotropic glutamate receptors in stable, inducible mammalian expression system.



Chruścicka B., Burnat G., Brański P., Pilc A.
Neurobiology Department Institute of Pharmacology PAS, 12 Smetna St., 31-343 Krakow, Poland

Introduction

The metabotropic glutamate receptors play important neuromodulatory role throughout the brain. Intervention in glutamatergic neurotransmission via mGluRs has been investigated in the treatment of many psychiatric and neurological disorders. Expression of mGluRs in heterologous mammalian cells is a method for their functional characterization and tool to study the agonist, antagonist and allosteric effects.

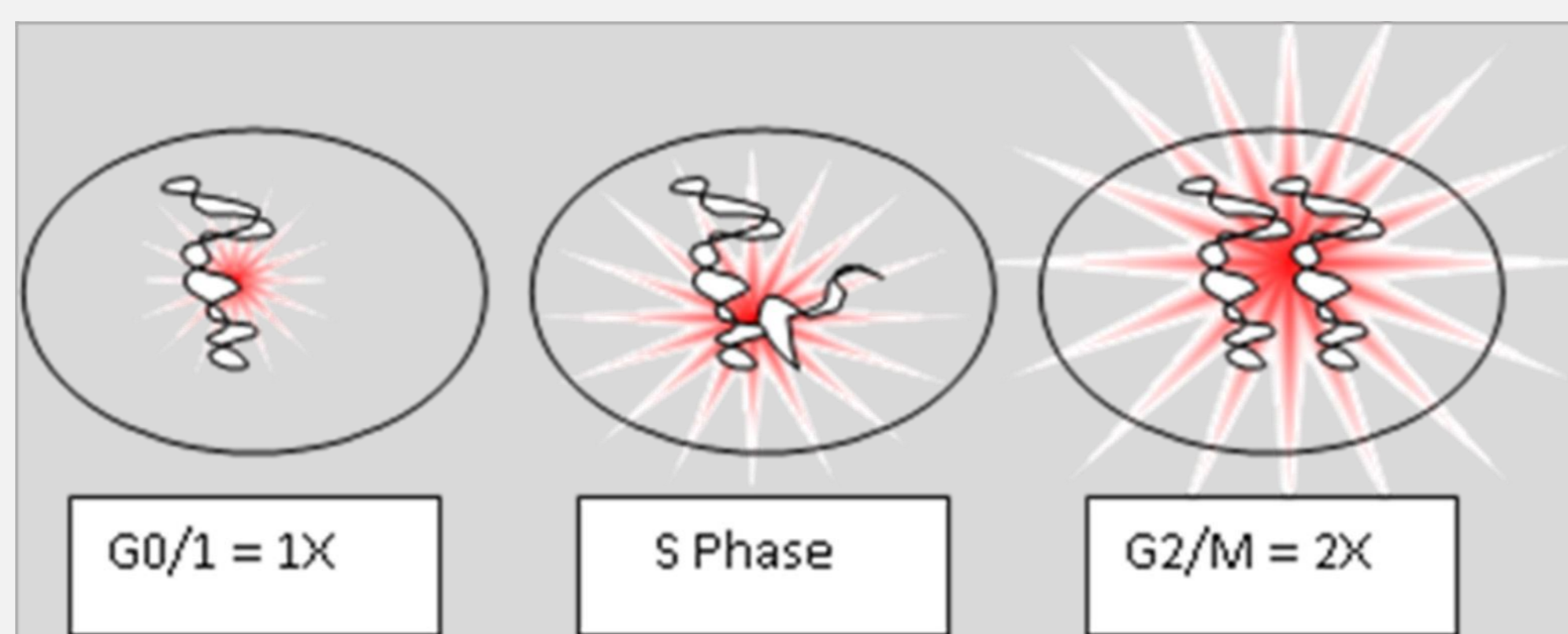
Methods and results

1. mGluR2 cell line

To obtain cell line with mGlu2 receptor expression, cDNA encoding mGluR2 was cloned into pcDNA5/FRT/TO vector under the control of a tetracycline regulated promoter. HEK293 cells containing inducible expression system were transfected and then antibiotic selection was performed. Expression of mGluR2 is activated in the presence of tetracycline.

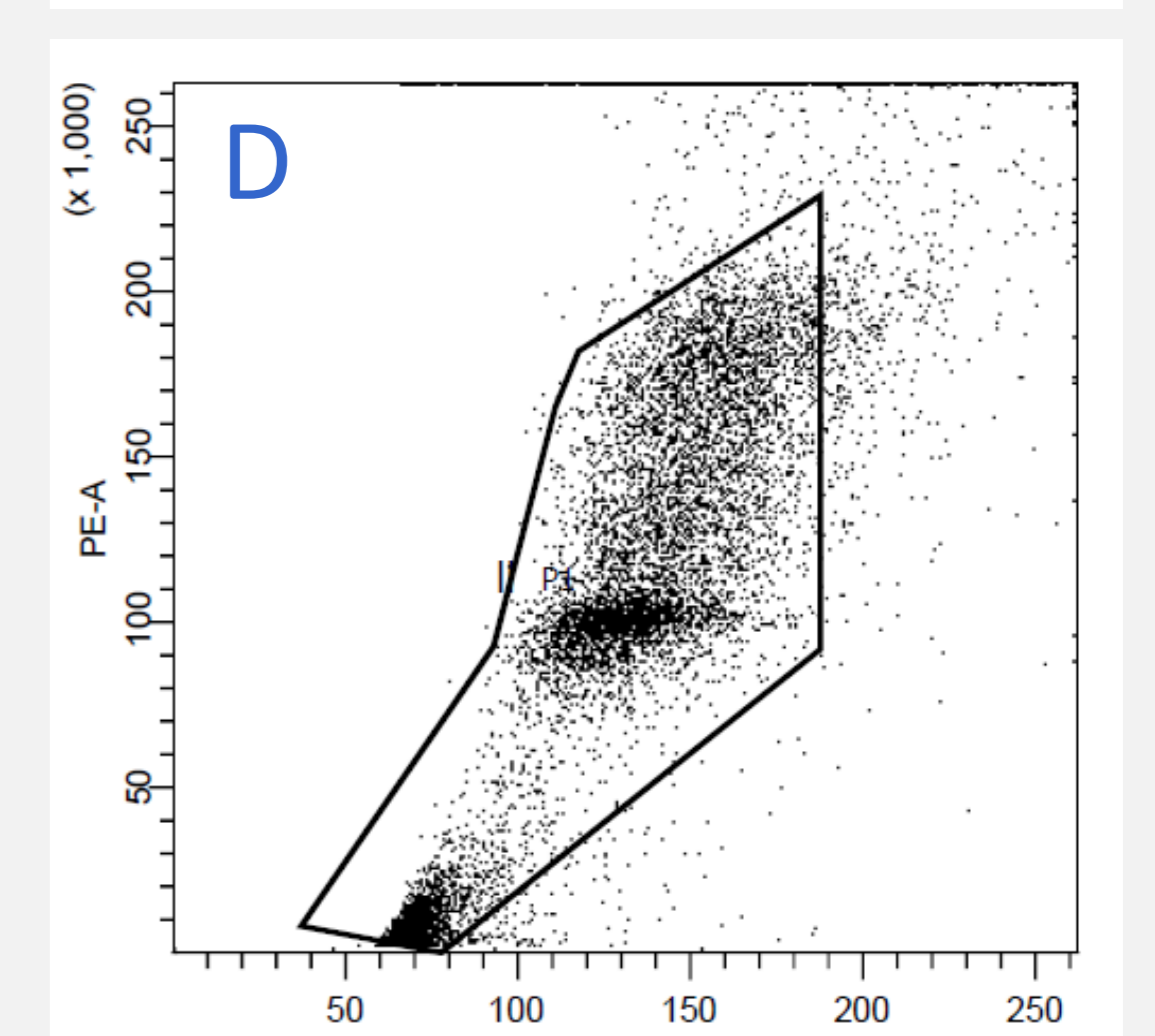
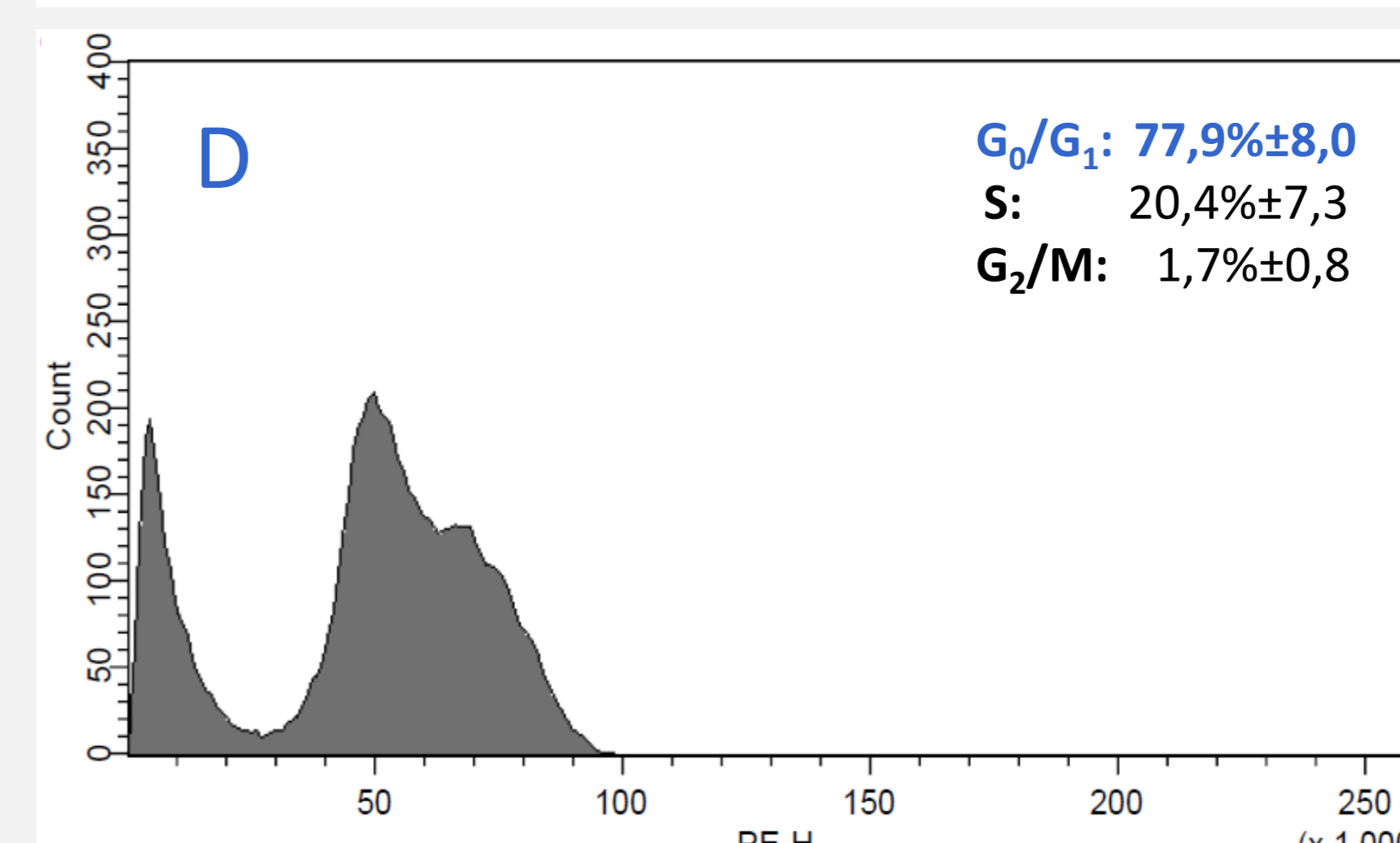
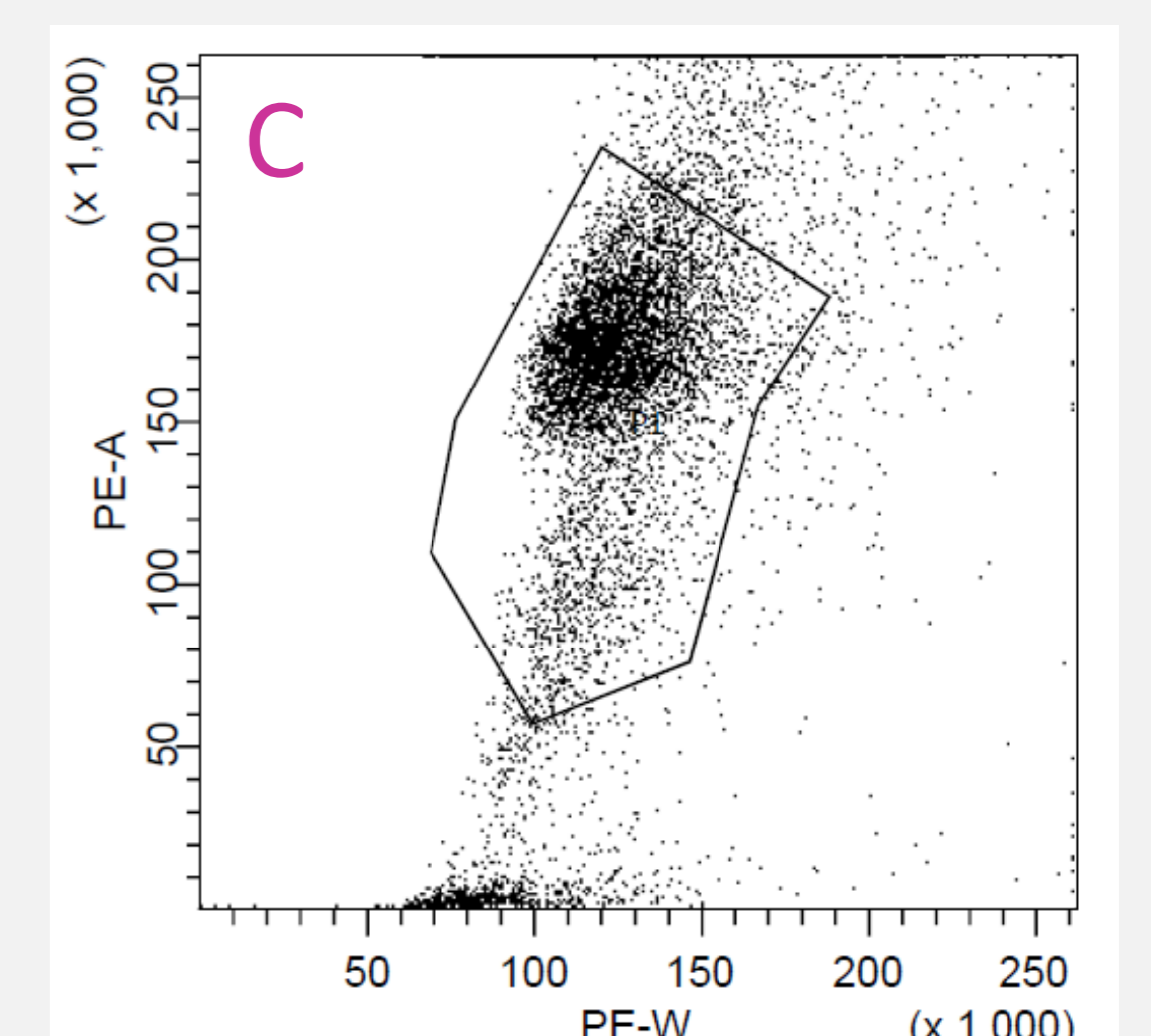
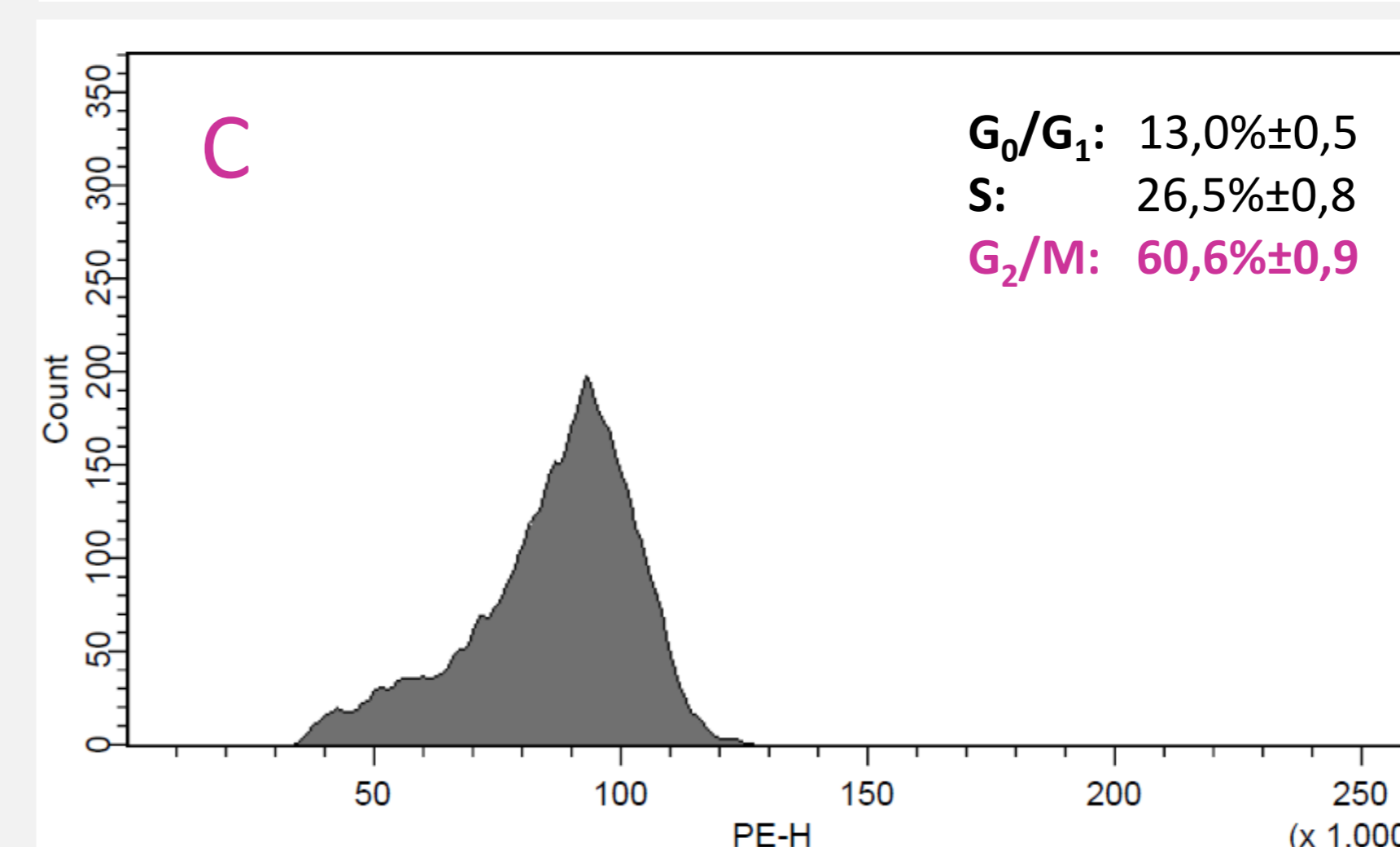
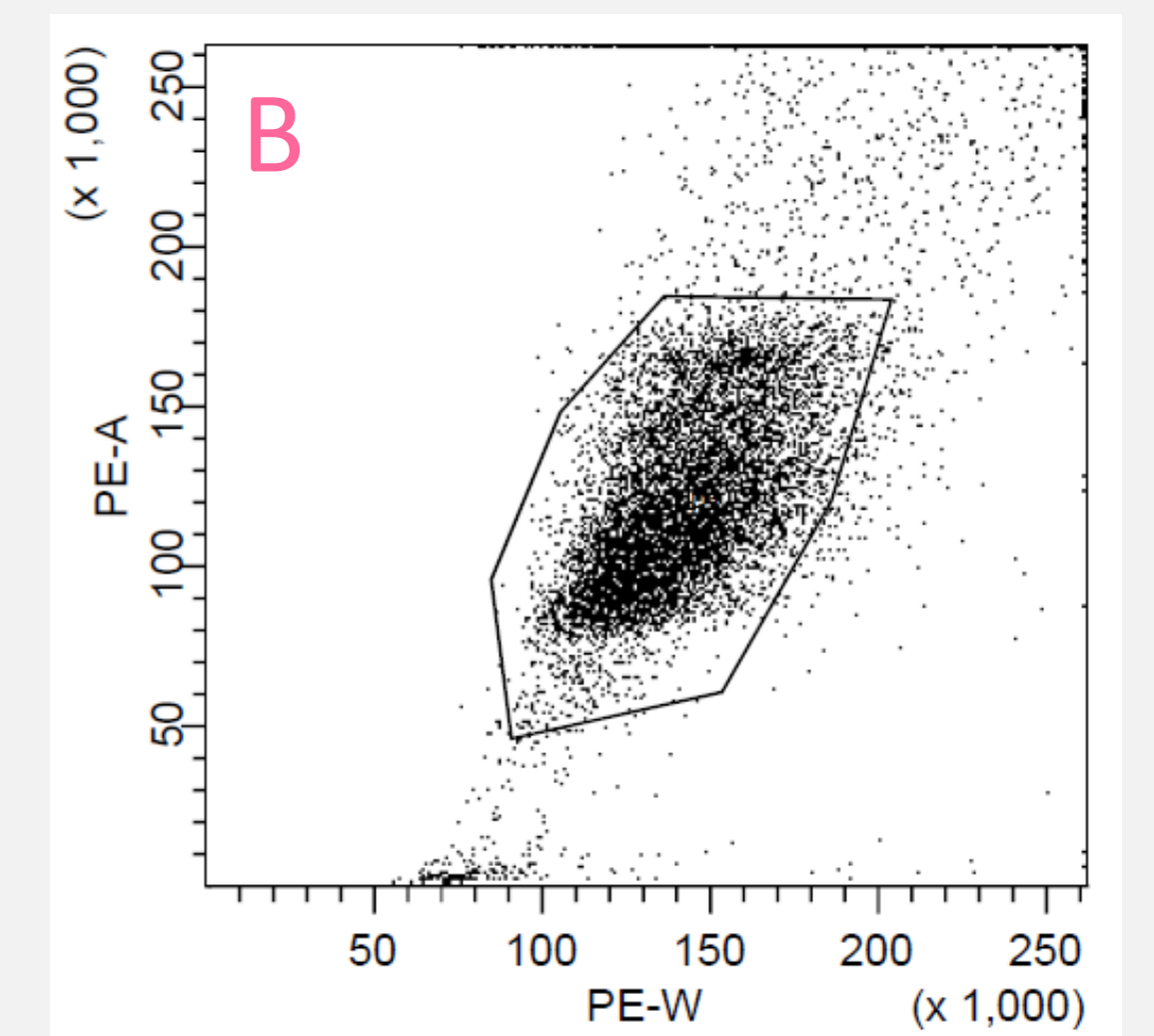
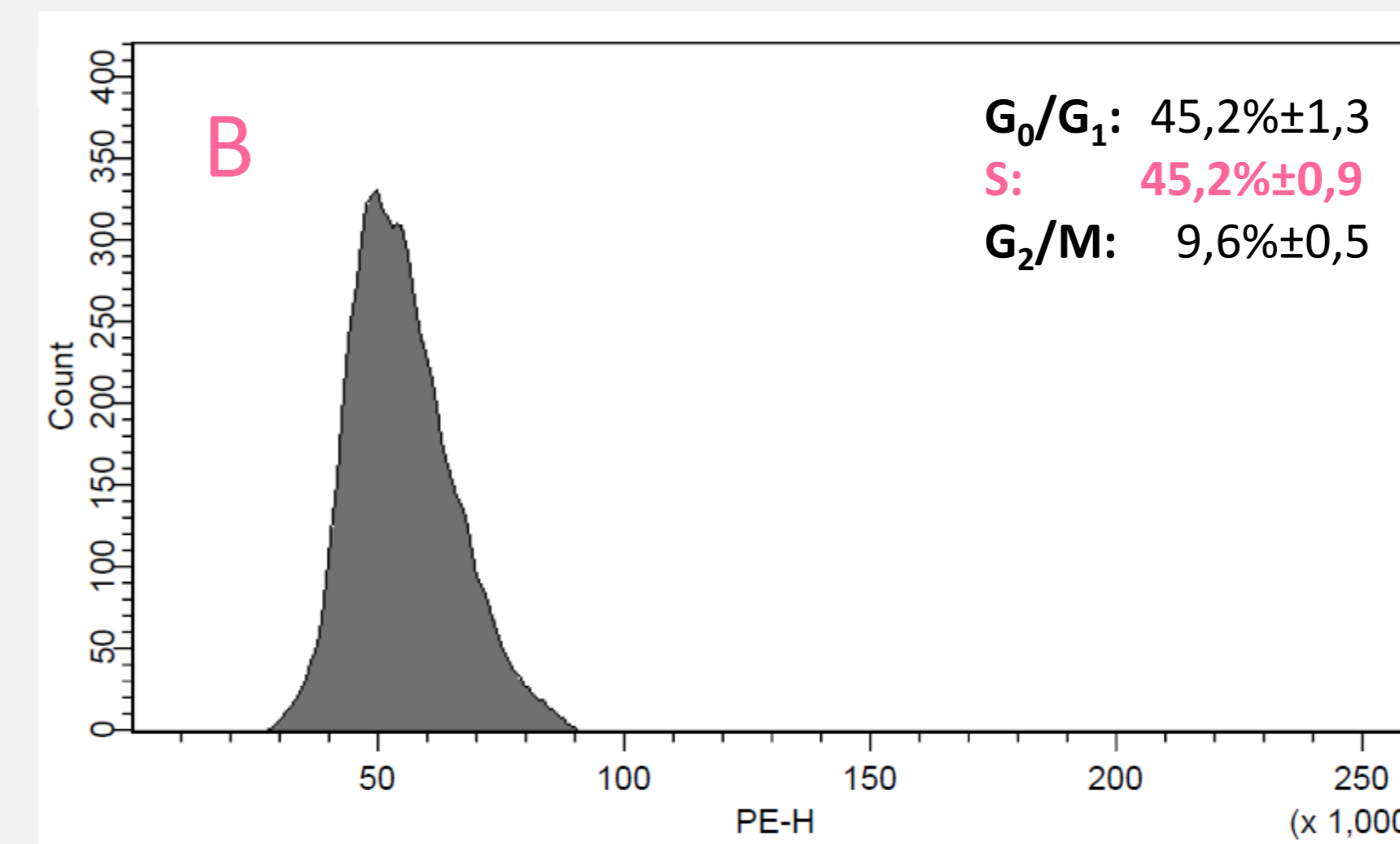
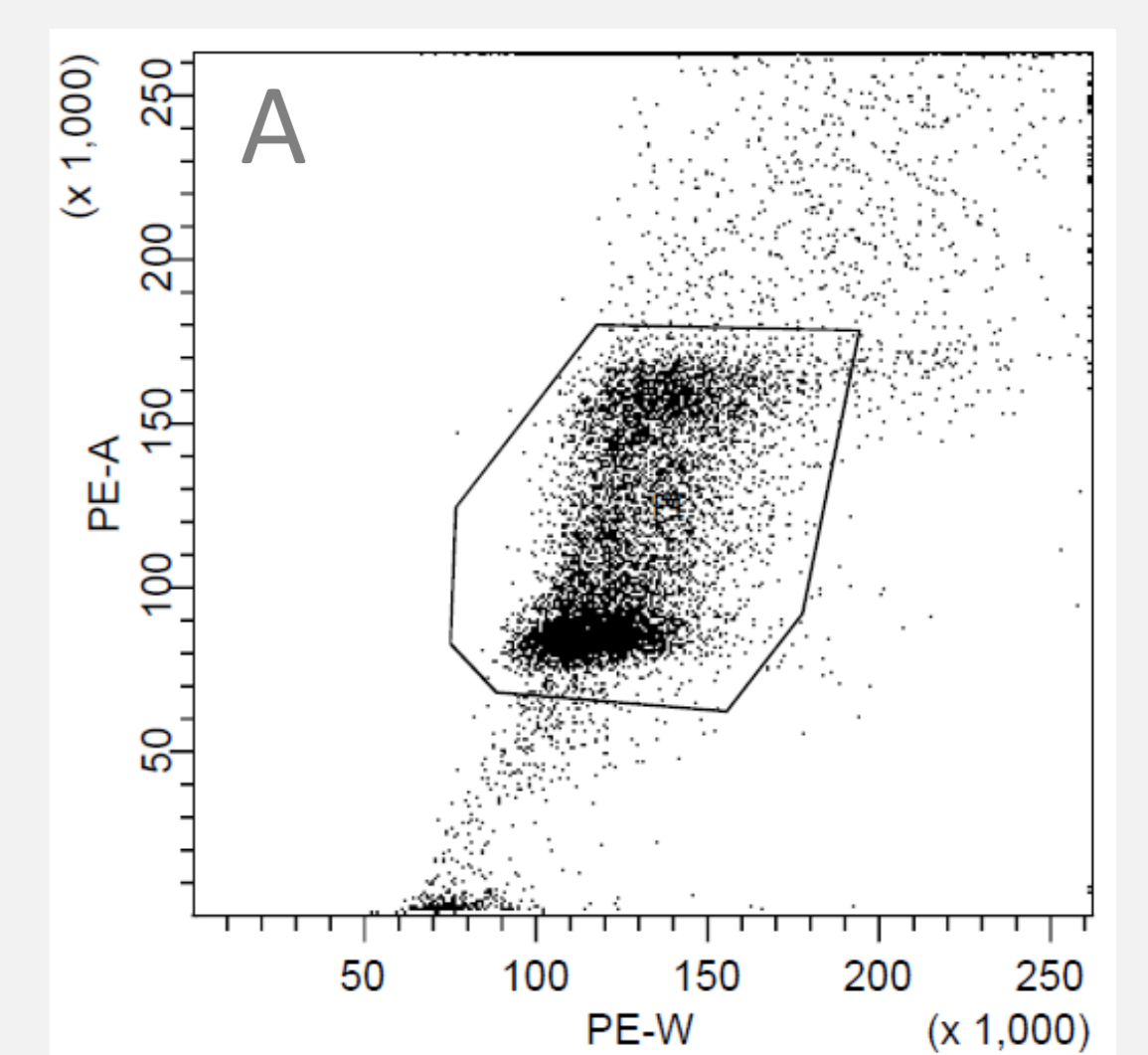
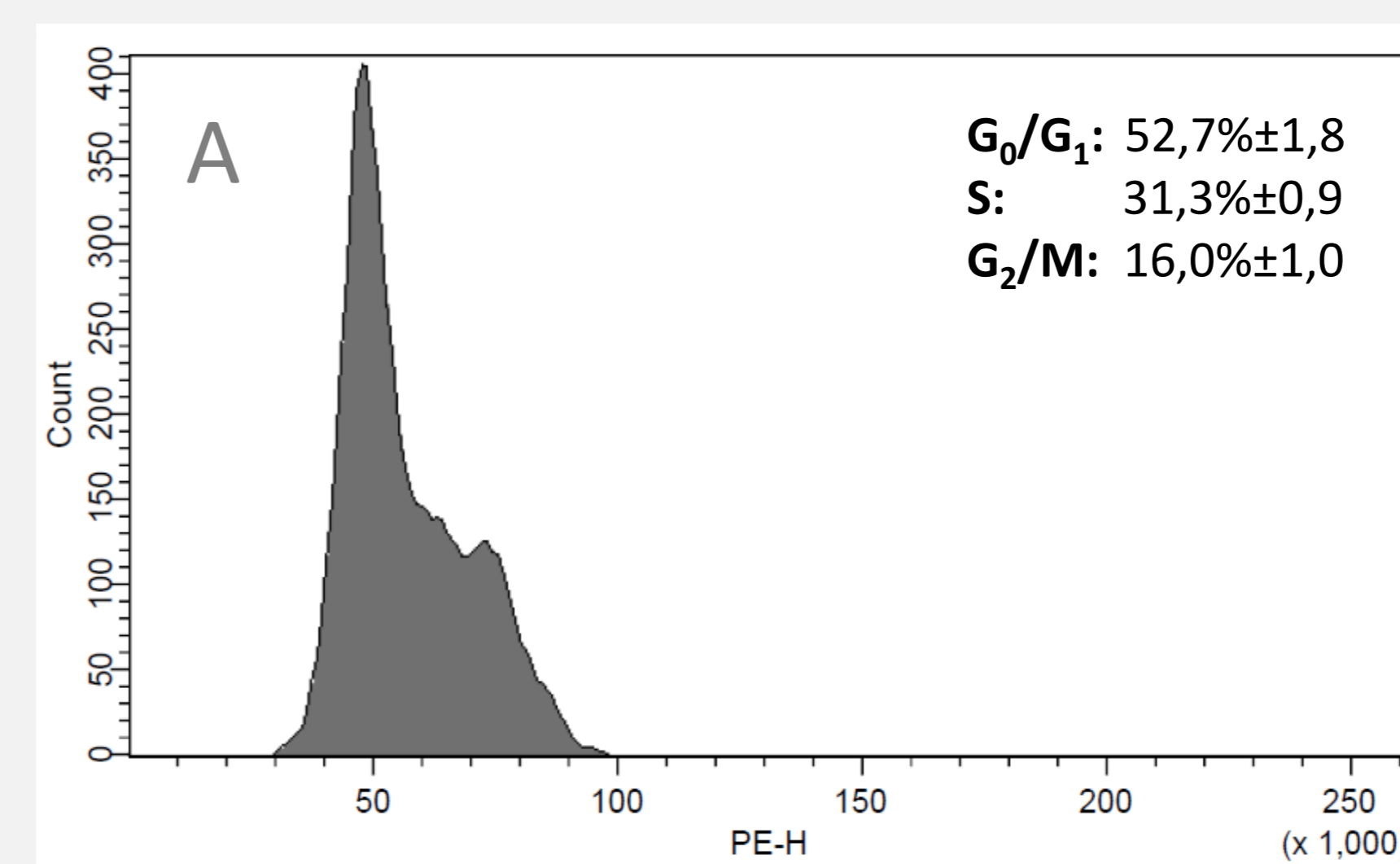
2. Cell synchronization

The efficacy of cell synchronization was verified by flow cytometric analysis of DNA content after propidium iodide staining:



Synchrony of stably transfected cells were performed by means of three different synchronization protocols:

- Usynchronous HEK293 cell culture (10% FBS, 2mM Glu) is characterised by a large number of cells in G_0/G_1 phase and distinct population of cells in G_2/M phase.
- To arrest cells at the beginning of S phase a double thymidine block (16h and 18h) was used.
- To obtain G_2/M phase block, cells were supplemented with nocodazole for 18h.
- G_0/G_1 population of cells was obtained by serum starvation for 48h.

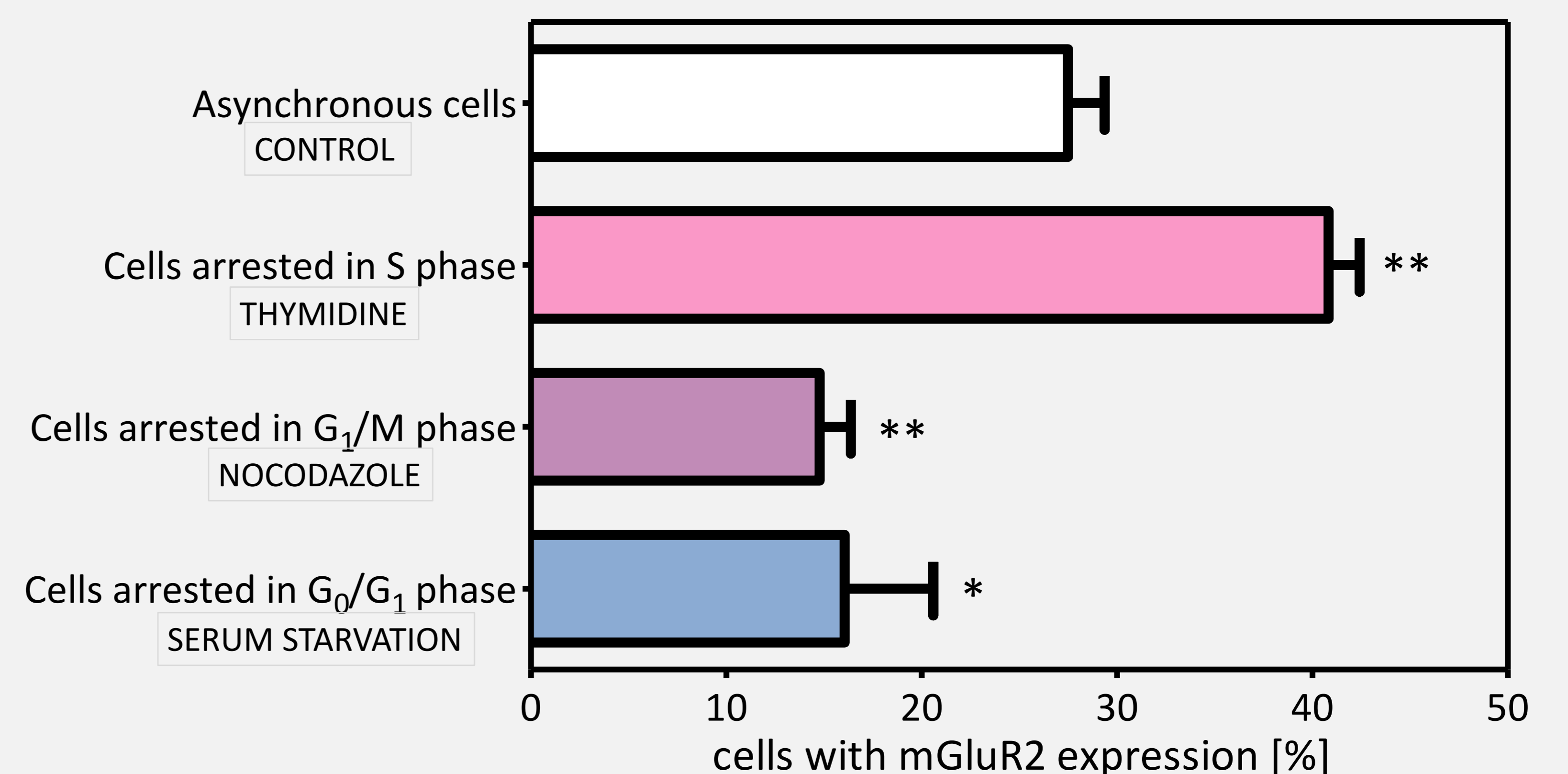


3. Comparison of mGlu2 receptor surface expression in HEK293 cells, depending on cell cycle phase:

After three different synchronization procedures and induction of mGluR2 expression by tetracycline, immunofluorescence staining was performed.

Evaluation of mGluR2 surface expression was verified by flow cytometric analysis.

Statistical analysis was performed with the use of One-way ANOVA and Dunnett's Multiple Comparison Test.



Conclusions

The results indicate that modification of inducible expression system by HEK293 cell synchronization seems to have significant effect on surface mGluR2 expression level ($F(3,41)=22.8, p<0.0001$). The expression of mGluR2 is significantly decreased in G_2/M phase blocked cells after nocodazole treatment and in G_0/G_1 blocked cells after serum starvation. In contrast, the level of mGluR2 surface expression is significantly higher after double thymidine block.

Acknowledgements

Barbara Chruścicka acknowledges the financial support from the project Interdisciplinary PhD Studies "Molecular sciences for medicine" (co-financed by the European Social Fund within the Human Capital Operational Programme).

