

Trojan, a possible regulator of apoptosis, belongs to a novel protein family

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Introduction

Aiming to identify novel proteins related to the immune system, we cloned a previously unknown antigen from chicken (*Gallus gallus*). The molecule is a leukocyte-specific, cell surface protein that we named "Trojan".

Trojan belongs to a novel gene family, that in chicken includes two more members, called "Mystran" and "Thracian". Here, we characterise Trojan, investigate its function and perform evolutionary analyses of its family.

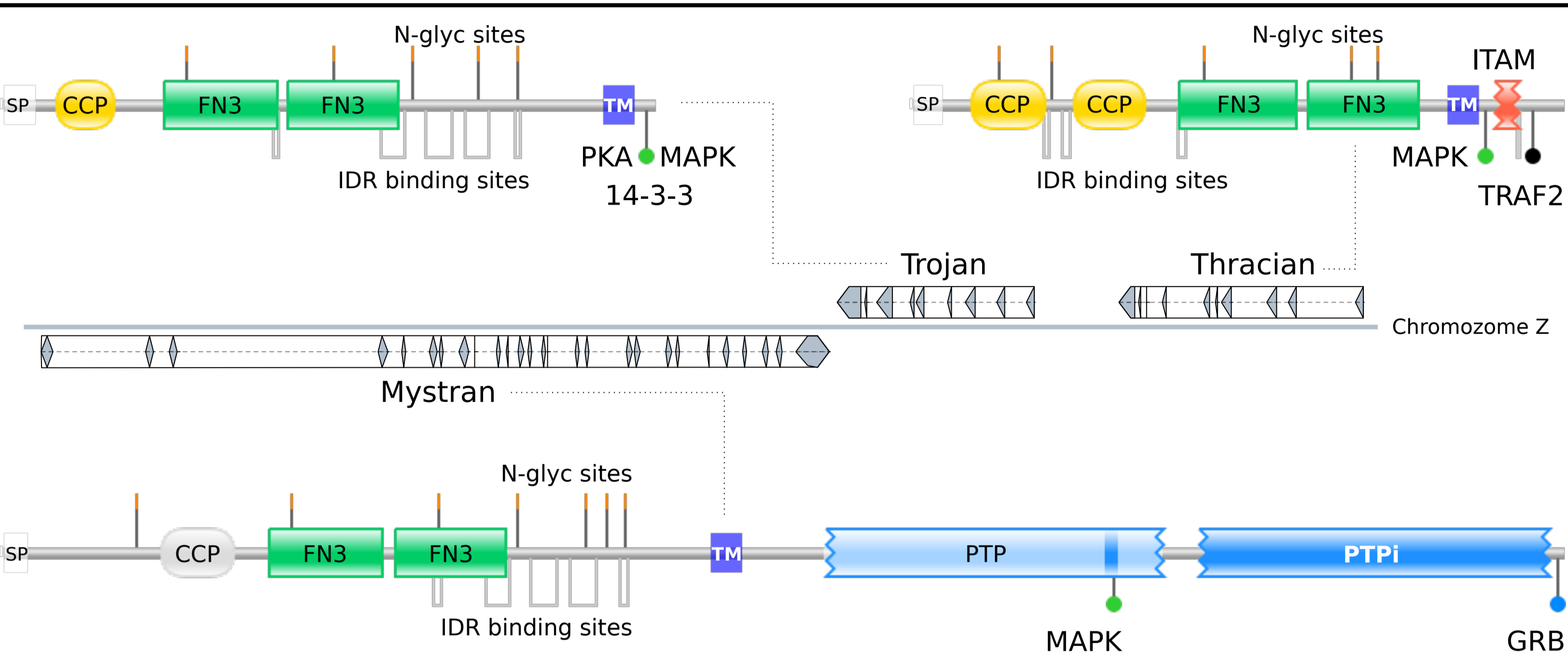


Figure 1. Trojan family in chicken is found on sex chromosome Z. Trojan is situated between Mystran (coding for a receptor type protein tyrosine phosphatase) and Thracian (coding for another transmembrane protein, containing an ITAM). Protein topology organisation is indicated, also showing target sites for PKA, MAPK, 14-3-3, ITAM, TRAF2 and GRB.

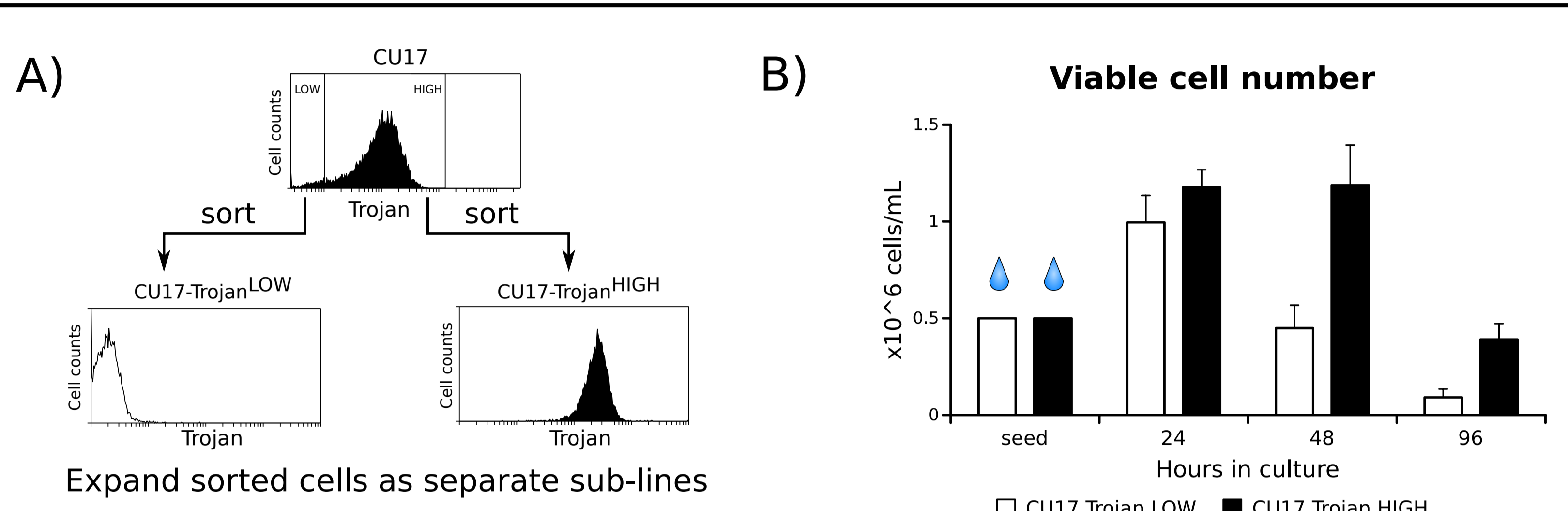


Figure 4. Trojan expressing cells are less susceptible to 5-FU induced apoptosis.

A) CU17 cells with lowest and highest expression levels of Trojan were sorted and expanded as separate sub-lines, termed CU17-Trojan^{LOW} and CU17-Trojan^{HIGH}, respectively. B) After 5-FU treatment, CU17-Trojan^{HIGH} (black bars) showed a higher count of viable cells than CU17-Trojan^{LOW} (white bars).

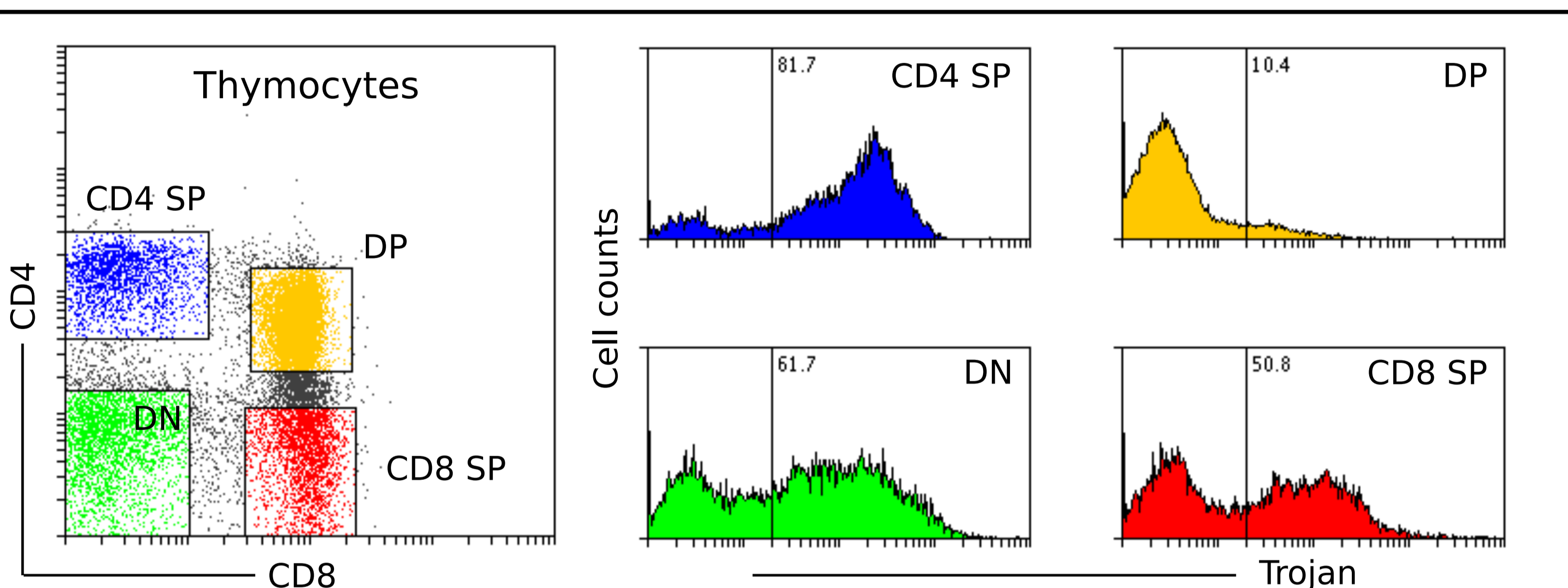


Figure 2. Trojan diminishes from the surface of DP thymocytes. Thymocyte sub-populations were defined by the expression of CD4 and CD8 co-receptors. Populations of DN, DP, CD4 SP and CD8 SP cells were gated (left) and the expression of Trojan was analysed for each (right). The observed specific expression was similar to that of the anti-apoptotic BCL-2 and the pro-survival IL-7R. By drawing a parallel based on the expression patterns, we hypothesised an anti-apoptotic or proliferative role for Trojan.

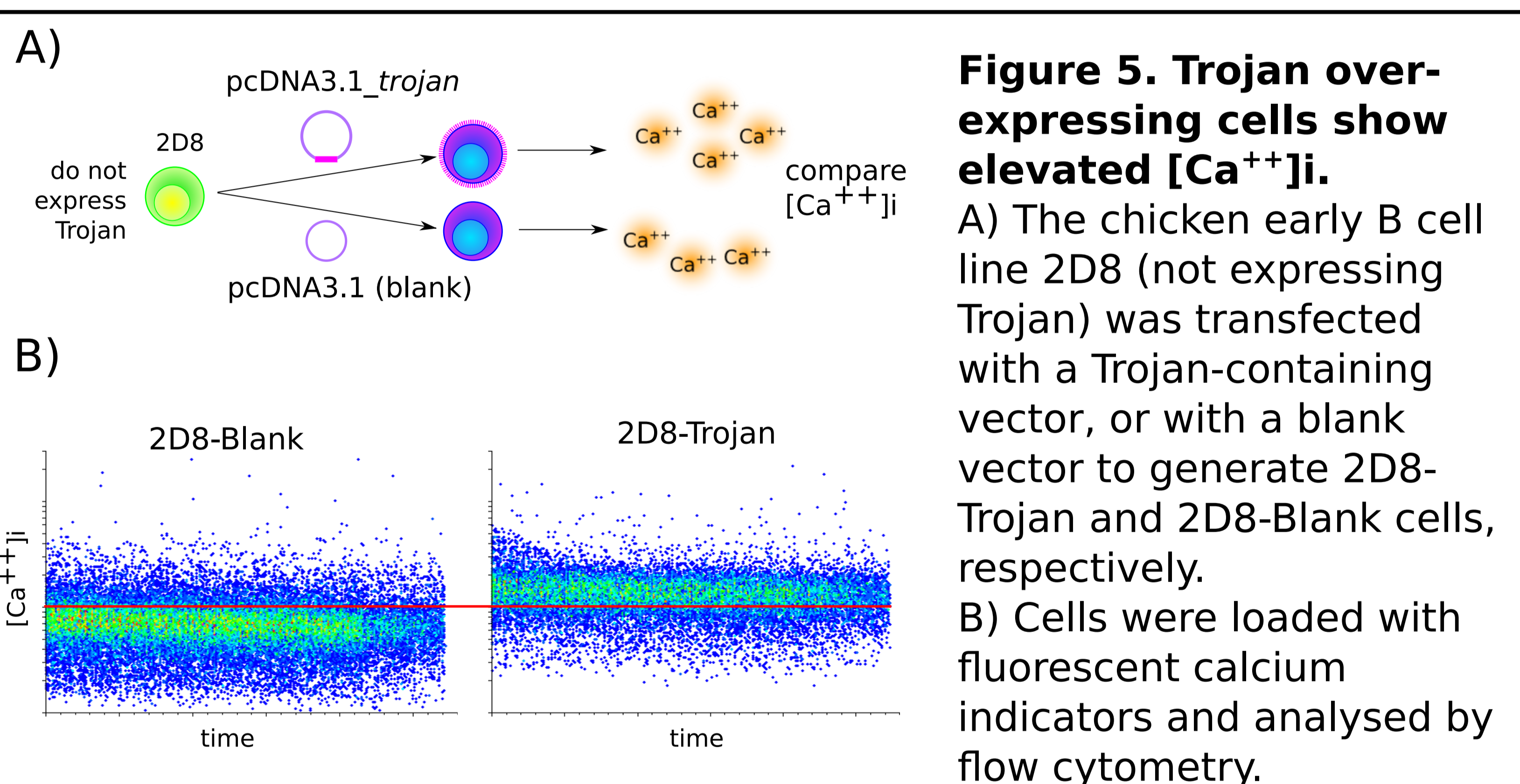


Figure 5. Trojan over-expressing cells show elevated [Ca⁺⁺]. A) The chicken early B cell line 2D8 (not expressing Trojan) was transfected with a Trojan-containing vector, or with a blank vector to generate 2D8-Trojan and 2D8-Blank cells, respectively. B) Cells were loaded with fluorescent calcium indicators and analysed by flow cytometry. The 2D8-Trojan cells were found to have a higher baseline of intracellular calcium concentration [Ca⁺⁺]_i, compared to 2D8-Blank cells. Hence, we expect that Trojan has a signalling potential, linked to intracellular calcium mobilisation.

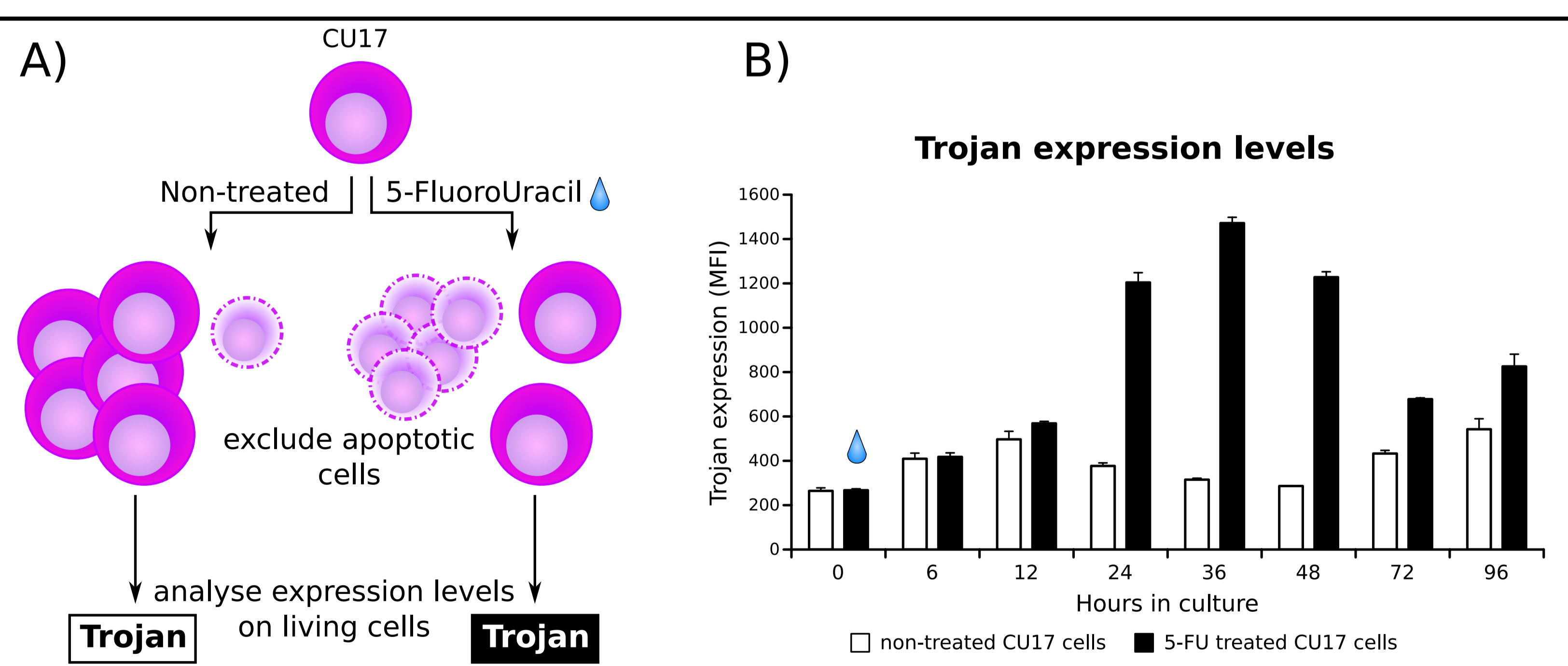


Figure 3. Upon apoptosis induction, surviving cells increase the Trojan level on their surface.

A) The chicken CD4⁺ T cell line CU17 was treated with 5-Fluorouracil (5-FU) or appropriate control buffer. Changes in the expression of Trojan on living cells was analysed by flow cytometry at different time points. B) Defined by its mean fluorescence intensity (MFI), Trojan was found to rise on the surface of 5-FU treated CU17 cells (black bars), as compared to control cells (white bars).

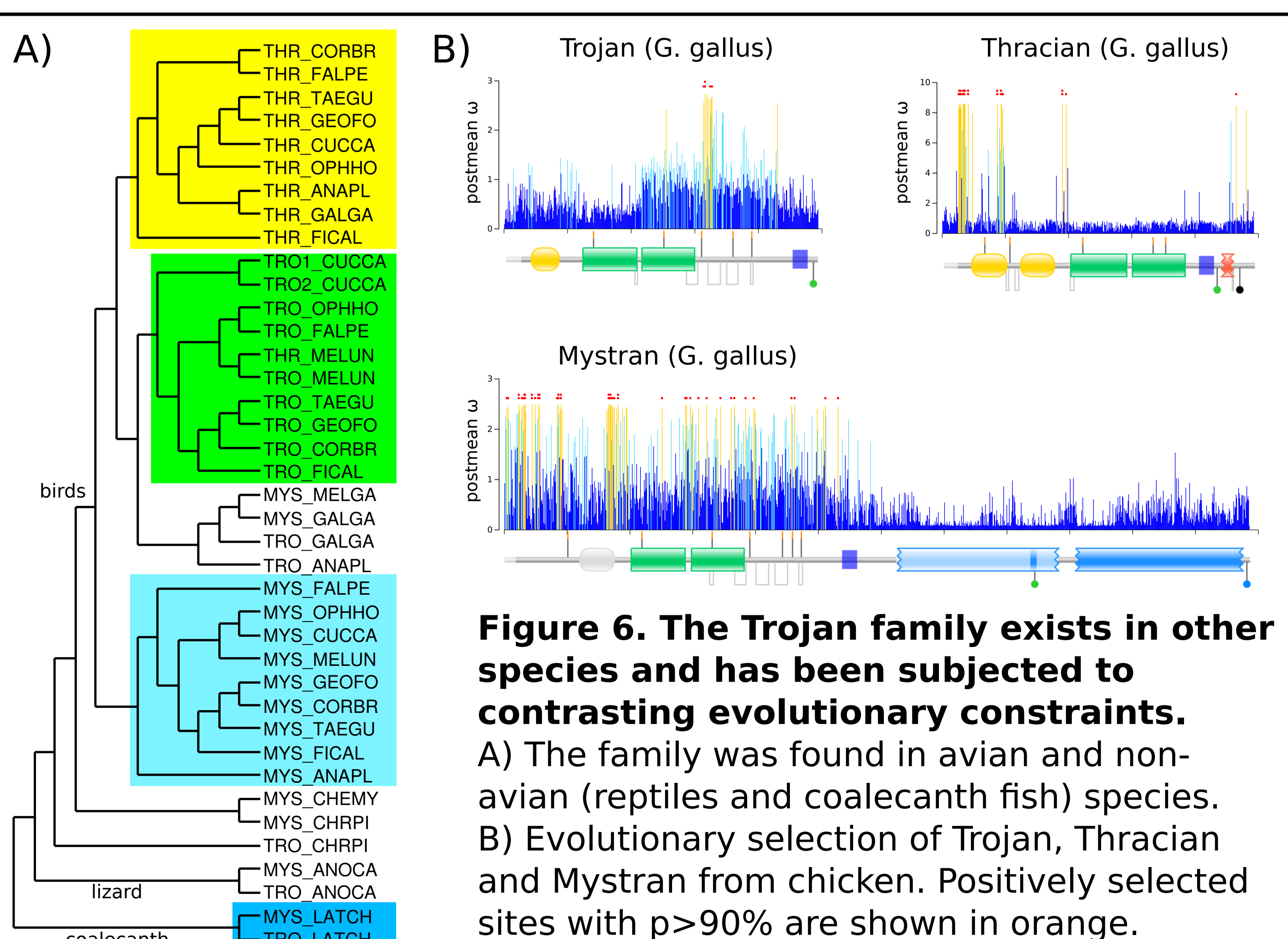


Figure 6. The Trojan family exists in other species and has been subjected to contrasting evolutionary constraints.

A) The family was found in avian and non-avian (reptiles and coelacanth fish) species. B) Evolutionary selection of Trojan, Thracian and Mystran from chicken. Positively selected sites with $p > 90\%$ are shown in orange.