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Abstract

Since targeting c-Myc-Max interaction is considered to have broad therapeutic applications in cancers, several technologies have been developed to analyze the interaction for drug screening purpose. These technologies, however, rely on either biochemical assay disregarding innate complexity of living cells or transient expression in a cell line making such an assay complicated for high throughput screening. In this study, without changing the sequence coding the binding domain between c-Myc and Max, we genetically modified the c-Myc and the Max gene in order to reduce the toxicity in cells when over-expressed. By combining these two modified genes with our recently developed Fluoppi, a protein-protein interaction technology, we successfully established a stable cell line that visualizes c-Myc-Max interaction. Furthermore, the disruption of the interaction was also visualized by expressing a competitive poly peptide real time. Taken together, the cell line we established provides a simple method that can be used in a high-throughput format for c-Myc-Max inhibitor drug screening.

Method

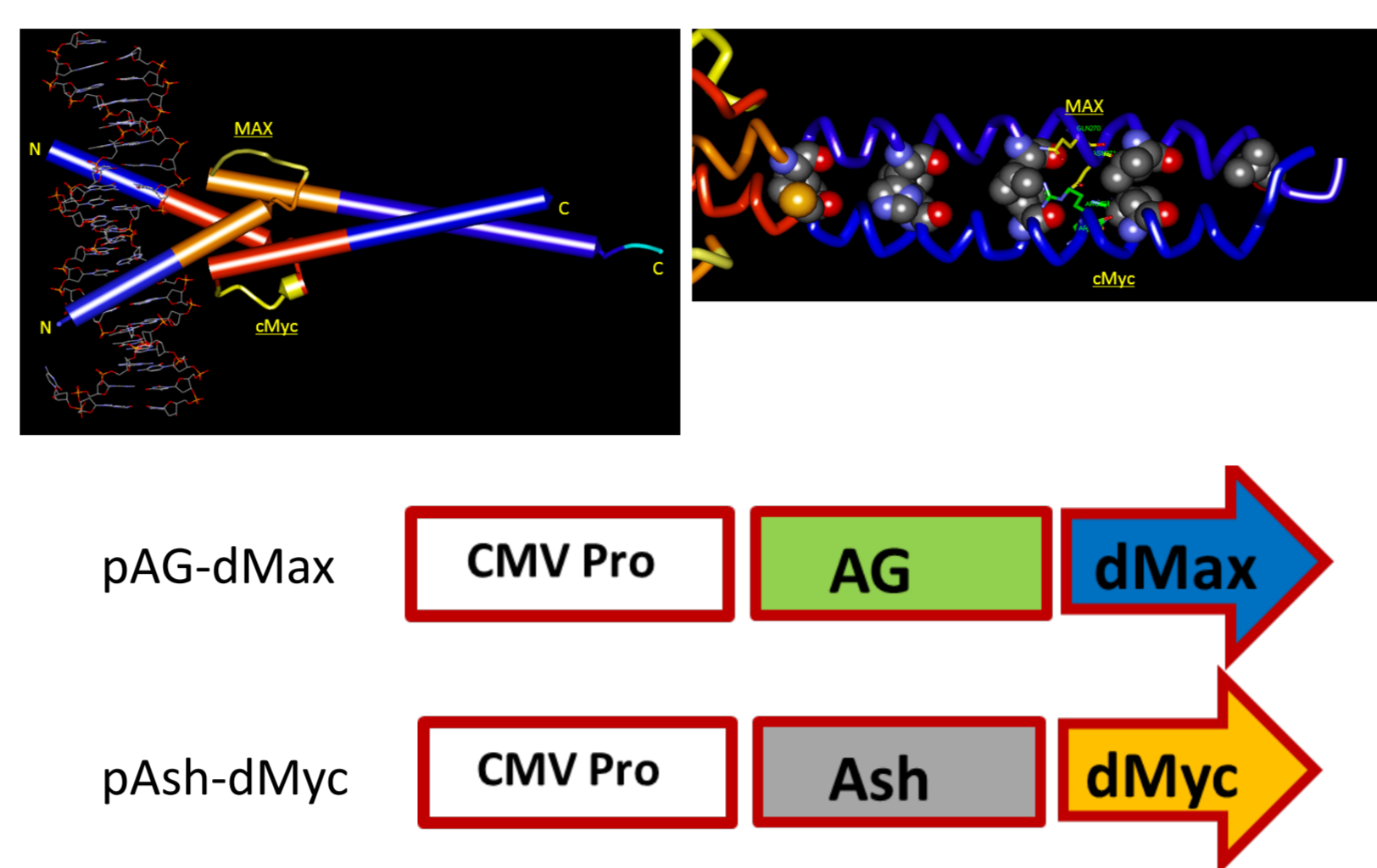


Fig. 2 Construction of Fluoppi plasmid pair for cMyc-Max PPI assay.

The c-Myc and Max genes were genetically modified without changing the sequence coding the binding domain between them. The modified genes, dMax and dMyc, were cloned into Fluoppi plasmid vector pair. The resulting plasmid pairs are co-transfected into the cells, and followed by fluorescent image assay after 24 hours. In order to establish a stable cell line, cells were treated with two appropriate antibiotics for double selection.

Results (Continued)

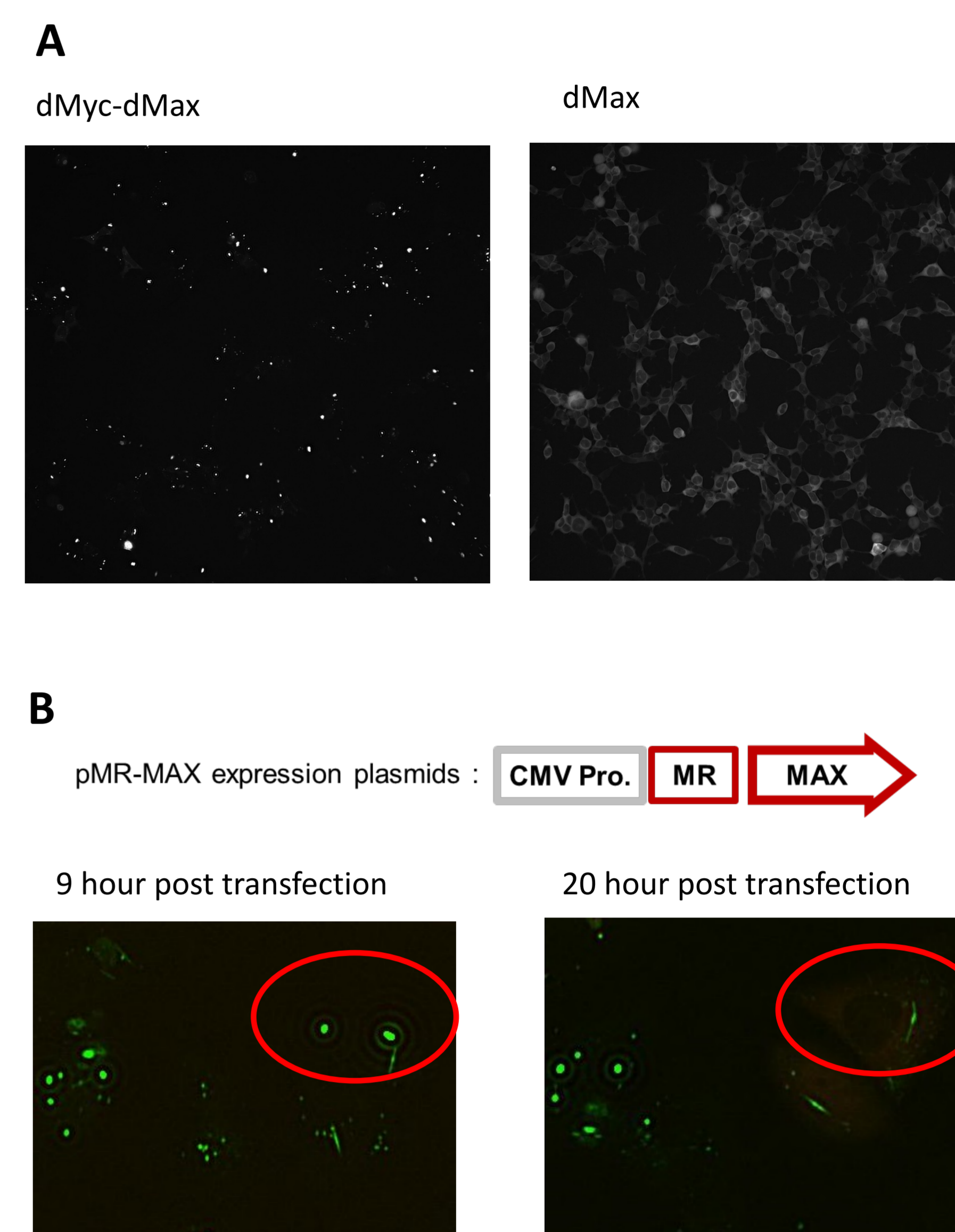


Fig. 4 Stable cell line visualizing PPI between c-Myc and Max and disruption of the PPI by a competitive binding of a poly-peptide.

A stable cell line that visualizes PPI between c-Myc and Max was established (A). To investigate whether the PPI is reversible, the established cell line was further transfected by a plasmid, pMR-Max, expressing a poly-peptide derived from Max and fused with a red fluorescent protein (MR). Cells were tracked for 20 hours, by taking a series of time laps images. The punctum disappeared after 20 hours of transfection only in the cells expressing the red fluorescence, indicating that the PPI was disrupted by a competitive binding of MR-Max to c-Myc (B).

Results

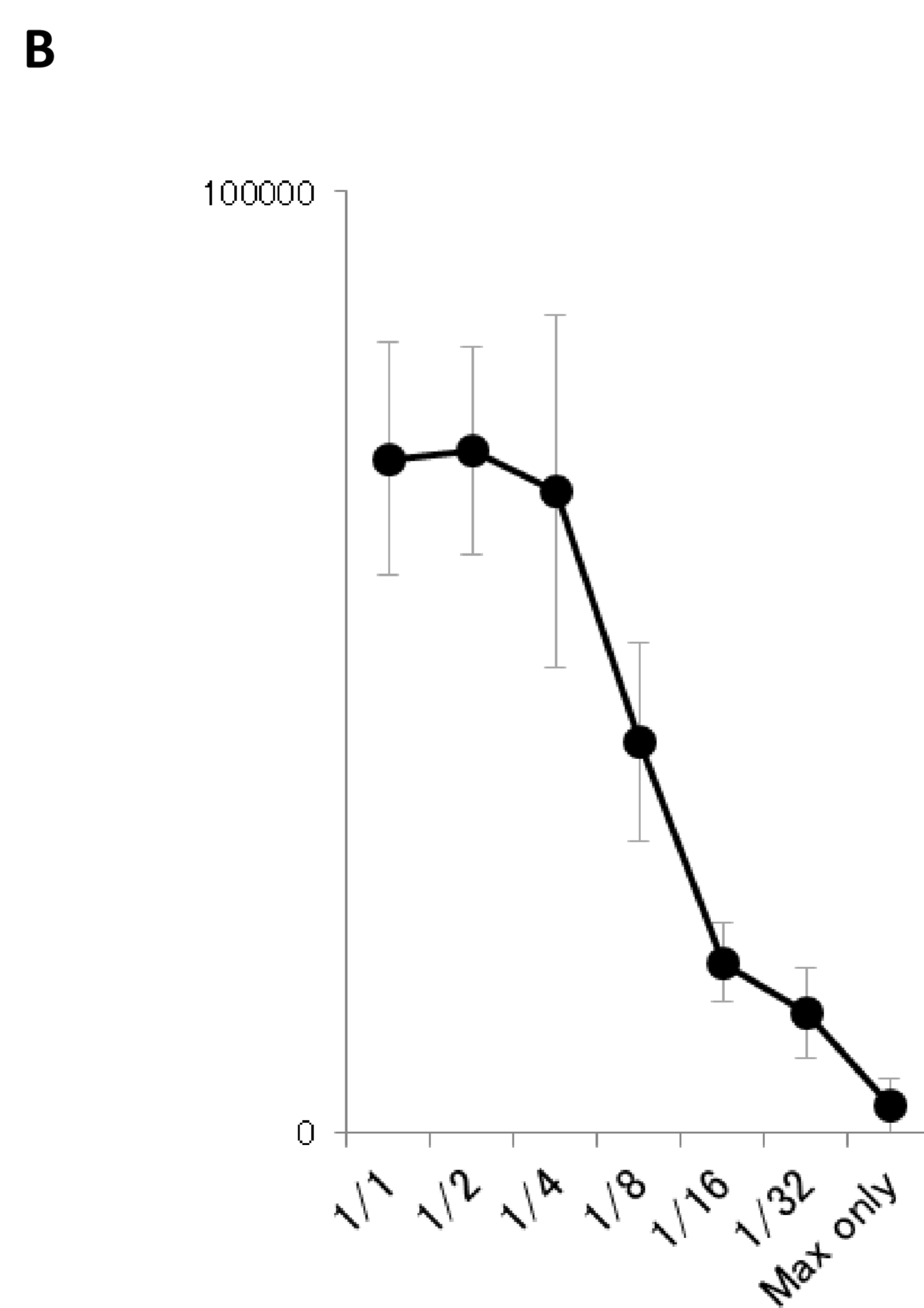
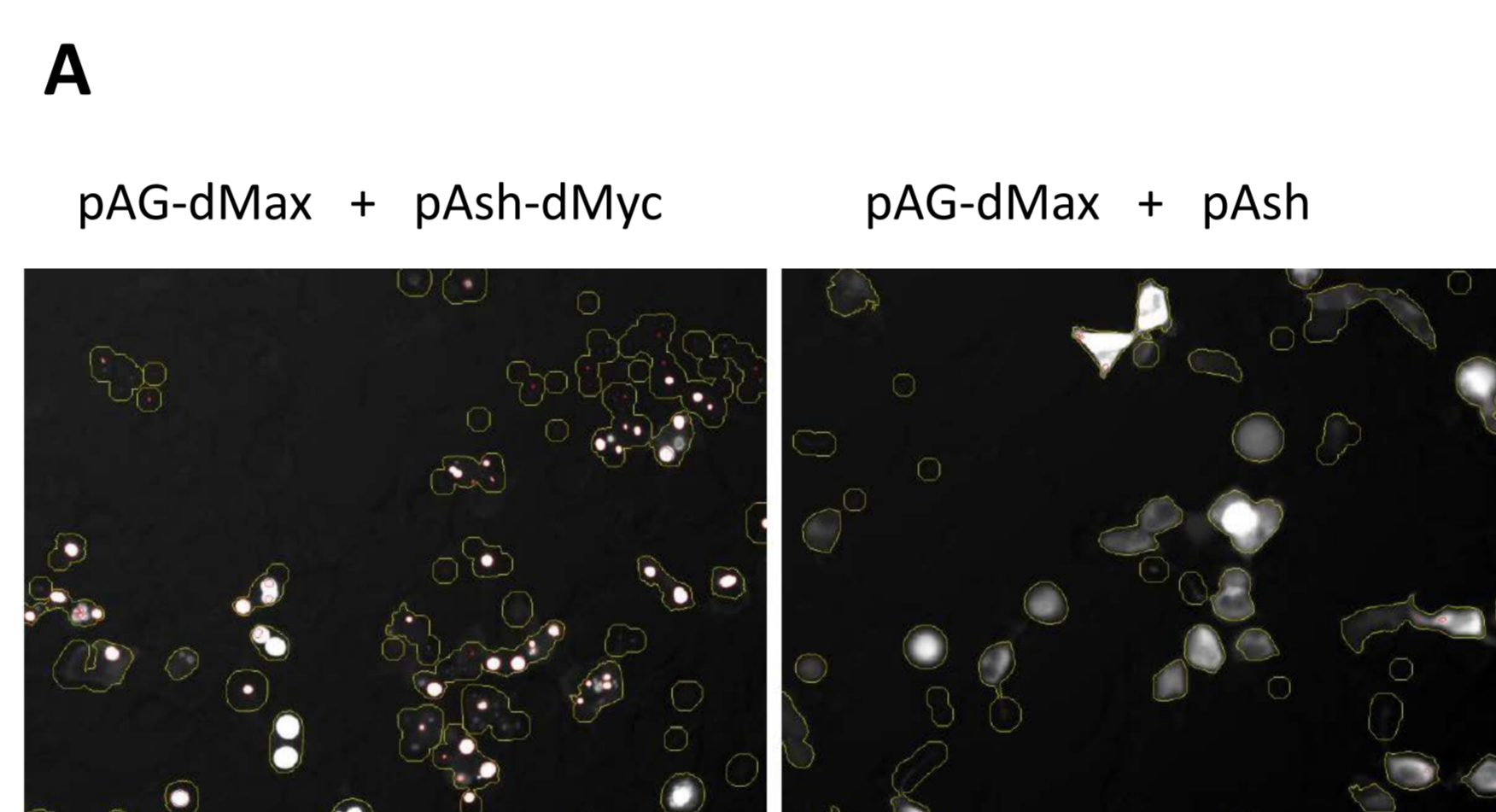


Fig. 3 Confirmation of PPI-specific puncta

Cells were co-transfected with Fluoppi plasmid pair, pAsh-dMyc and pAG-dMax at different ratio, and were analyzed by using a high content fluorescent image analyzer. Fluorescent punctum were detected 24 hours after transfection (A). Fluoppi signals appeared in a pAsh-dMyc/pAG-dMax ratio dependent manner, indicating the signal is c-Myc-Max interaction specific (B).

Mechanism of the assay

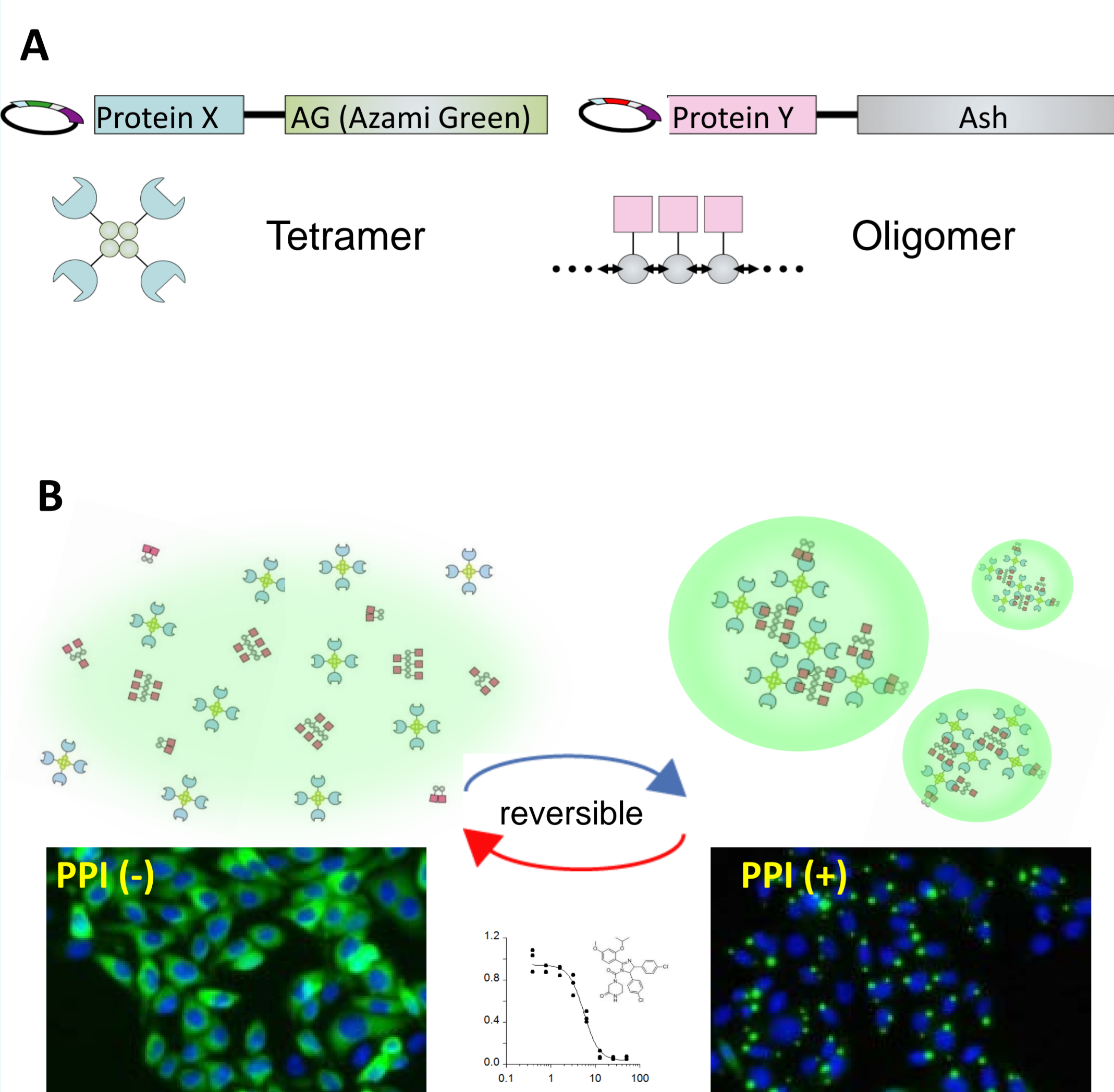


Fig. 1 Mechanism of the assay

A AG fluorescent proteins form a tetramer, and Ash-tags form a linear oligomer spontaneously when expressed in a cell. Fusion of protein X or Y to each tags results in two multimeric building blocks of proteins carrying multivalent Xs or Ys.

B In case of no interaction, the fluorescent proteins distribute in a diffuse manner throughout the cytosol. If interact, they form clustered localization using their multivalent arms, appearing as a bright and quantifiable puncta in the cells under the exposure of an appropriate UV light.

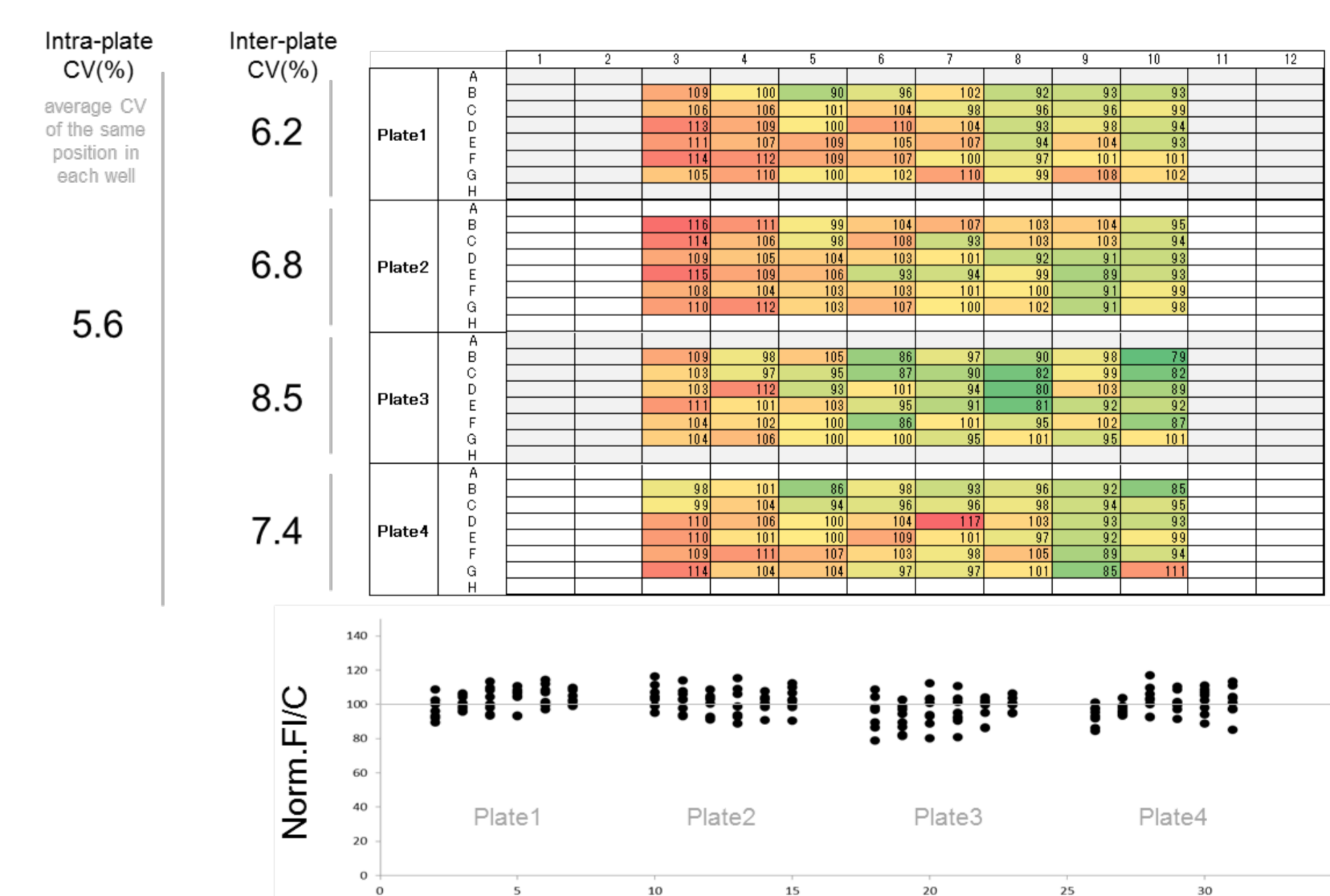


Fig. 5 The assay is stable intra- and inter- plate for high throughput format

Conclusion

We successfully established a stably transfected cell line that visualizes c-Myc-Max interaction. Disruption of the interaction was visualized by expressing a competitive poly peptide. The PPI assay by using the cell line is stable intra- and inter- plate in a high throughput format, indicating the potential application to be used for c-Myc-Max inhibitor drug screening in a simple way.

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