

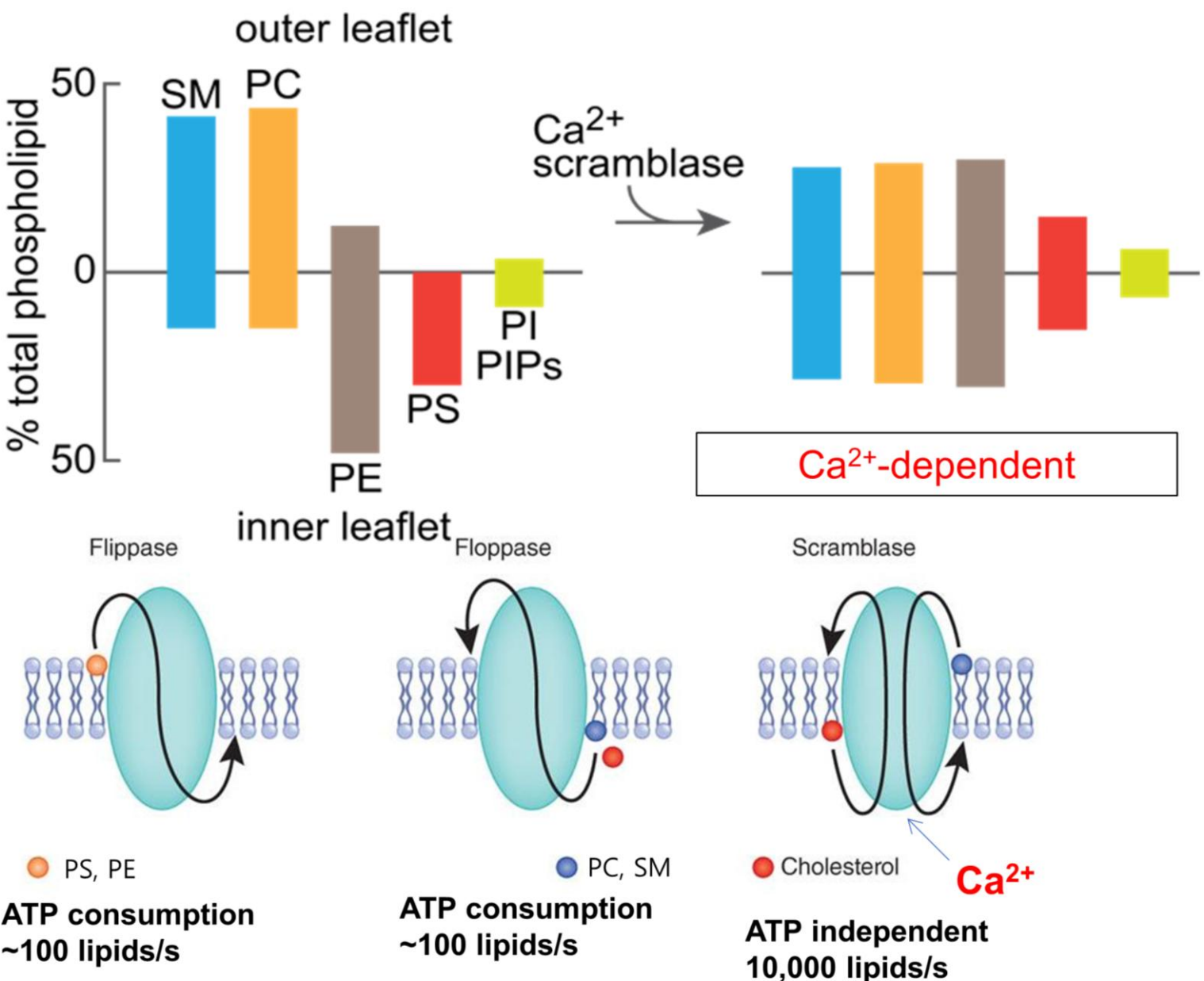
Characterization of TMEM16F-Specific Affibodies and Their Cellular Effects

Abstract

The TMEM16 (Anoctamin) family comprises a group of transmembrane proteins involved in diverse physiological processes, including ion transport and phospholipid scrambling. TMEM16F (Anoctamin 6), a phospholipid scramblase and nonselective ion channel, plays a central role in membrane remodeling, blood coagulation, immune responses, and cell death pathways through its ability to externalize phosphatidylserine in response to elevated intracellular calcium levels. Consequently, modulating TMEM16F activity has emerged as a promising strategy for the development of new therapeutic applications. Despite the functional importance of TMEM16F, TMEM16F modulators have received little study. In a previous study, we generated TMEM16F-specific affibodies by biopanning a phage display library for affibodies that bind to brain-specific TMEM16F (hTMEM16F) variant 1. In this study, we selected six other affibodies from among the 38 previously sequenced affibody candidates and characterized them. After purification, we confirmed that two of these affibodies bound to human TMEM16F with high affinity. To provide functional insights into how these affibodies modulate TMEM16F activity, we tested whether they could exert functional effects at the cellular level. Finally, we show that TMEM16F affibody attenuated the neuronal cell death induced by glutamate and microglial phagocytosis, suggesting that these affibodies might have potential therapeutic and diagnostic applications.

Introduction

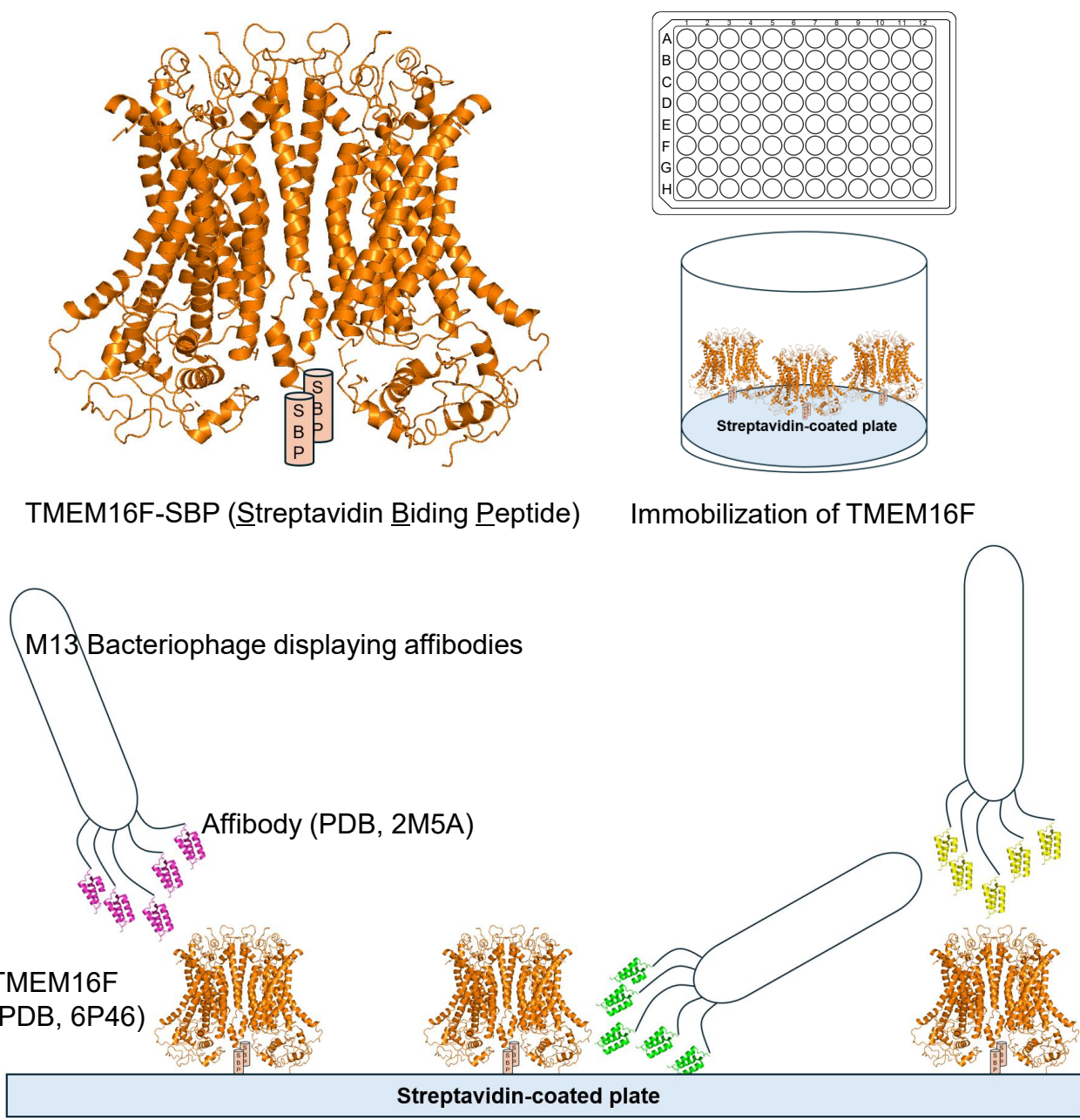
Asymmetry of plasma membrane & lipid scrambling



Clark *Nat Immunol.* 2011 (5):373-5

Materials & Methods

Biopanning of TMEM16F-specific affibody using phage display



To screen for affibodies specific for TMEM16F, purified human TMEM16F protein was used for biopanning. Seven rounds of biopanning were performed to isolate highly specific affibodies to hTMEM16F. To increase the selection stringency, the number of washes was increased for each round. Significant enrichment of hTMEM16F-binding peptides (160-fold increase) was obtained after seven rounds of biopanning. Affibody phages from the last round of panning were randomly selected to examine their binding to hTMEM16F using ELISA. Streptavidin was used as a negative control because it was used to attach TMEM16F protein to the plate via its SBP tag. The individual affibody phages were then incubated on hTMEM16F- and streptavidin-coated plates, and the streptavidin-only-coated plate was used as a background control.

Results

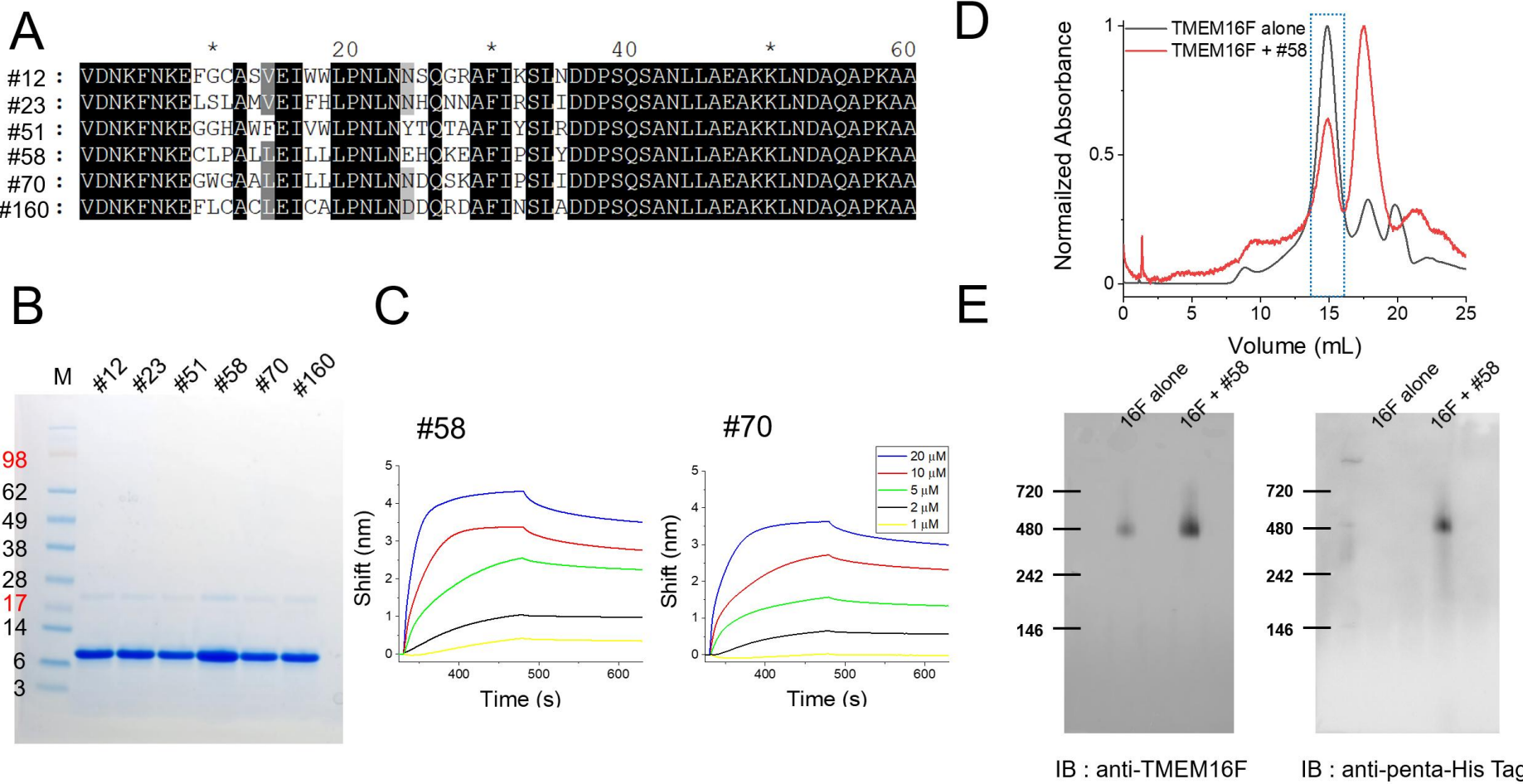


Fig 1. Screening for TMEM16F-specific affibodies using phage display

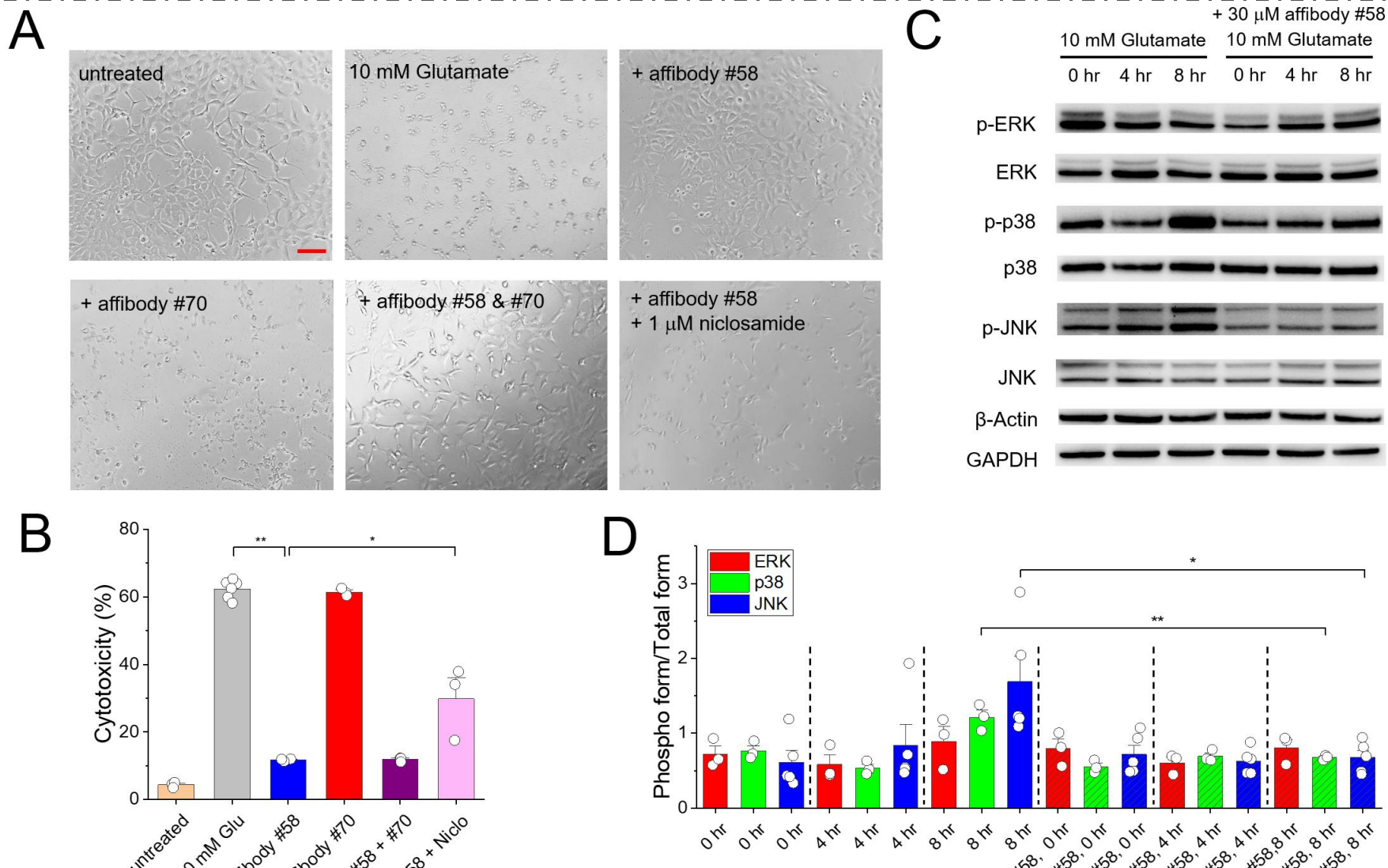


Fig 2. Effect of candidate TMEM16F affibodies on glutamate-induced neuronal cell death.

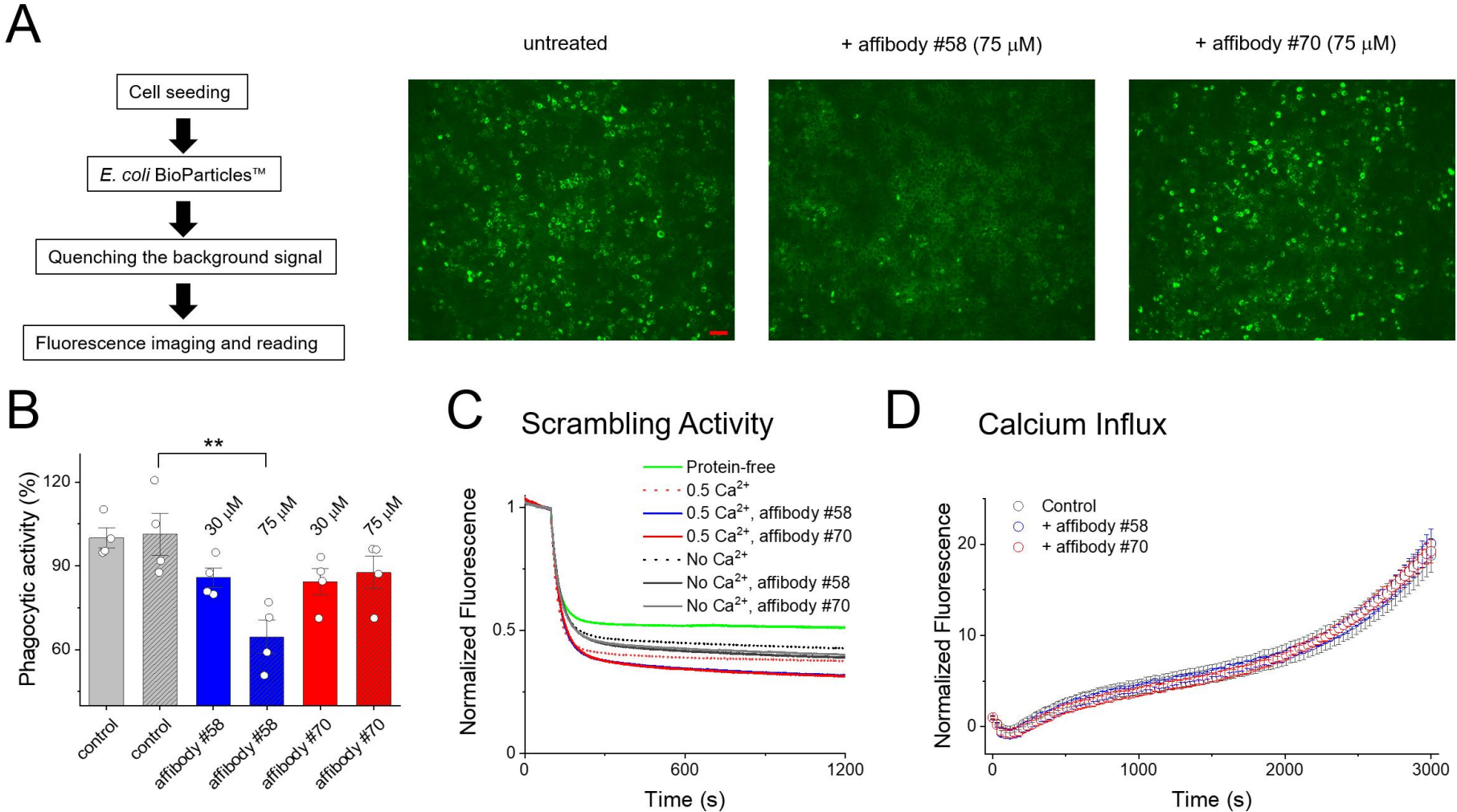


Fig 3. Effect of candidate TMEM16F affibodies on phagocytic activity of microglial cells

Summary

1. TMEM16F-specific affibody (#58) could affect the neuronal cell death and phagocytic activity of microglial cells
2. #58 affibody could potentiate the lipid scrambling activity of TMEM16F

Acknowledgement

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