

Recombinant Human Activated Protein C Regulates Integrin-Mediated Neutrophil Migration

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INTRODUCTION

During sepsis, tissue injury results from excessive infiltration and sequestration of activated neutrophils caused by dysregulation of cell surface adhesion molecules such as the integrins. Recombinant human activated protein C (rhAPC) has been shown to protect patients with severe sepsis, although the mechanism underlying this protective effect remains unclear. Here, we show that rhAPC directly binds to β_1 and β_3 integrins and inhibits neutrophil migration, both in vitro and in vivo. In contrast with a previous study, blocking of endothelial protein C receptor (EPCR) had little effect on human neutrophil migration. Furthermore, Gla-less APC, a mutant form of APC that lacks the EPCR binding motif, could inhibit neutrophil adhesion to fibronectin (FN). We found that human APC possesses a RGD sequence, which is critical for the inhibition. Mutation of this sequence abolished both integrin binding and inhibition of neutrophil migration. Thus, we conclude that leukocyte integrins are novel cellular receptors for rhAPC, and the interaction decreases neutrophil recruitment into tissues, providing a potential mechanism by which rhAPC may protect from sepsis.

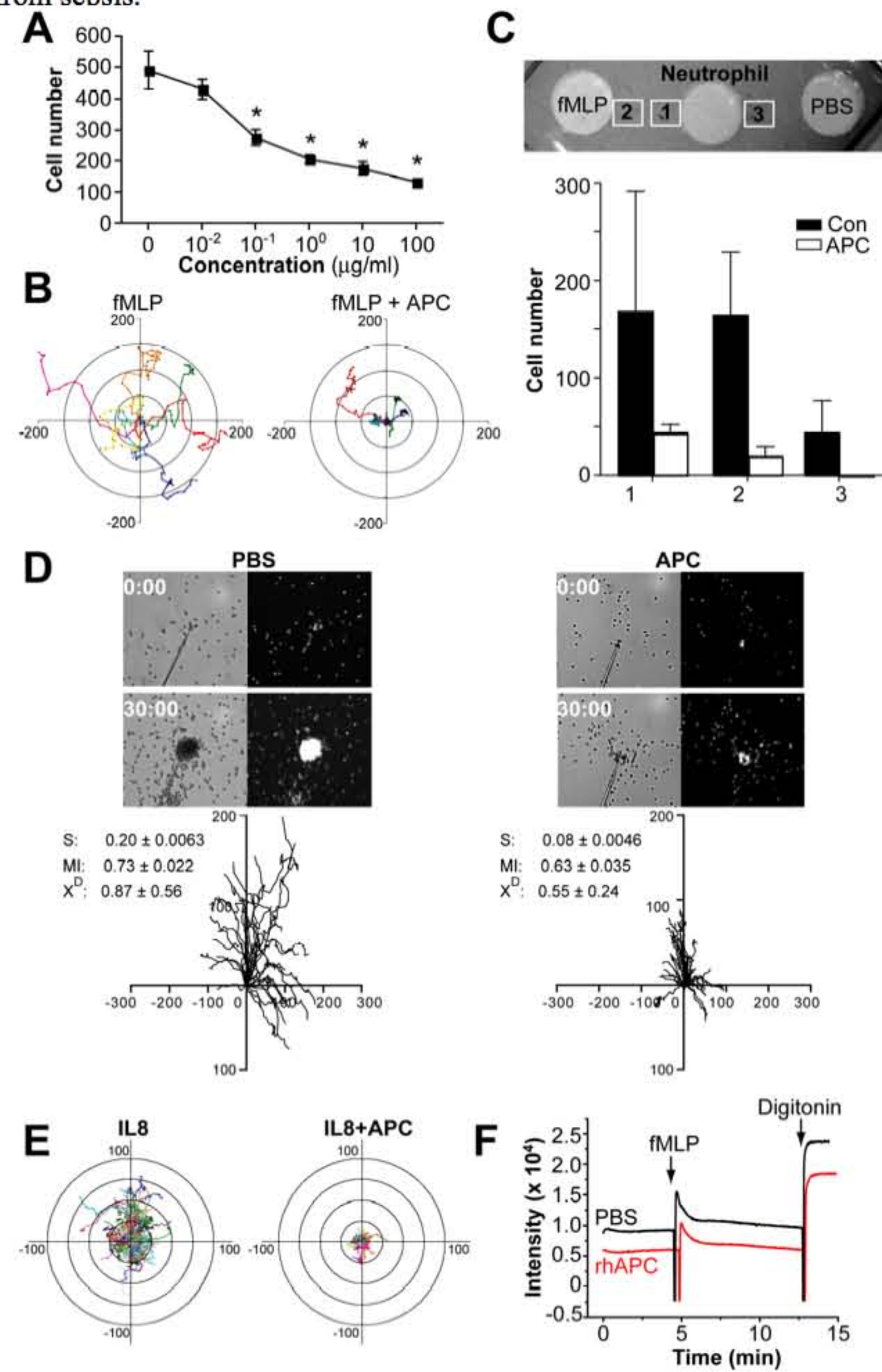


Figure 1. rhAPC inhibits neutrophil migration.

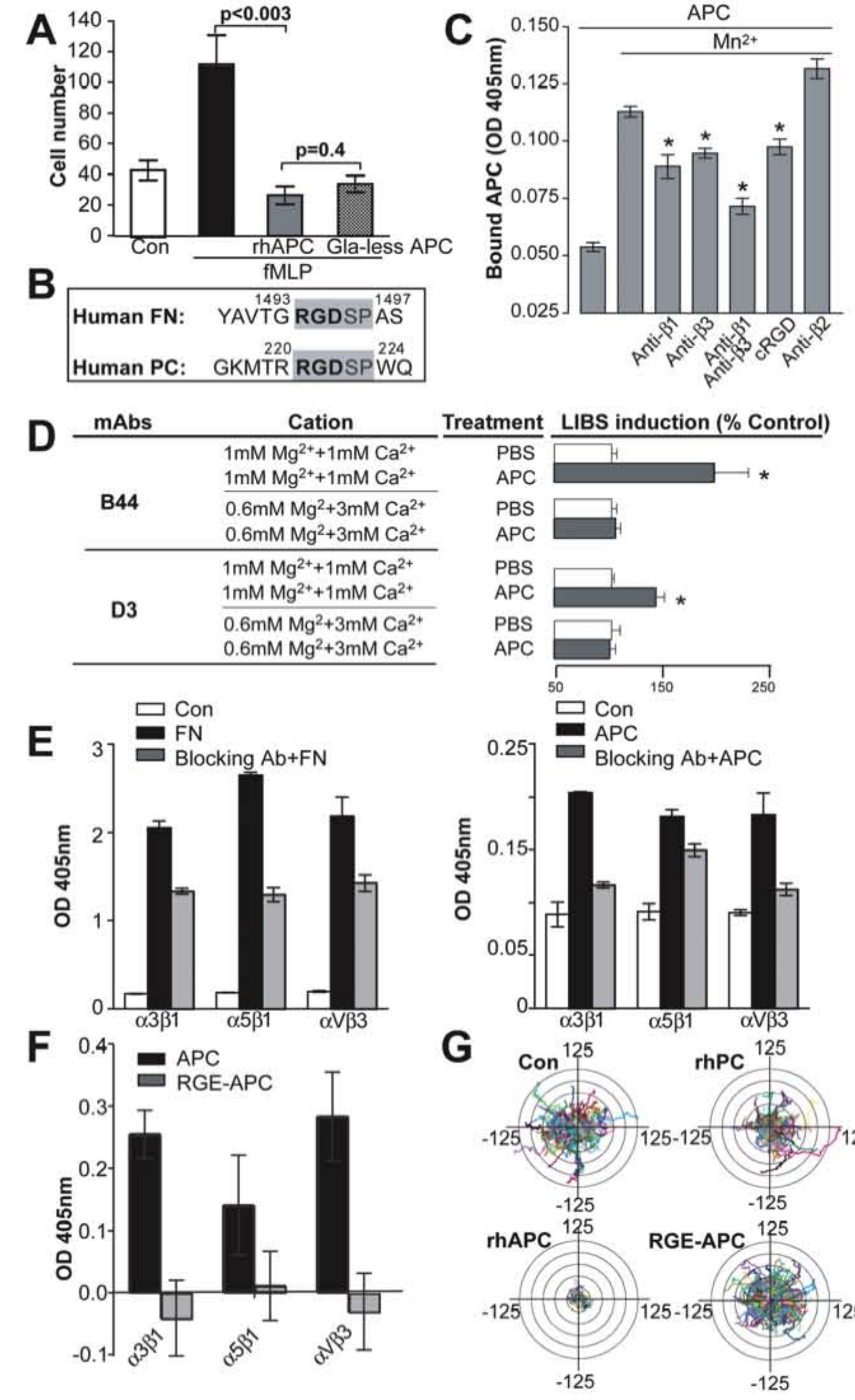


Figure 2. Direct binding of rhAPC to neutrophil integrins.

	rhAPC (WT)	RGE-APC
K_m	315.12 ± 44.43	278.20 ± 36.13
k_{cat}	164.35 ± 13.11	146.91 ± 15.37
K_{cat}/K_m	0.54 ± 0.035	0.54 ± 0.024

Table 1. Enzyme kinetic constants of wild type APC and RGE-APC.

CONCLUSION The results presented here demonstrate that rhAPC inhibits neutrophil adhesion and migration on extracellular matrix proteins by directly binding to integrins (β_1 and β_3 integrins) at the neutrophil surface. Therefore, we conclude that leukocyte integrins are novel cellular receptors for rhAPC and that specific APC-integrin interactions inhibit neutrophil migration.

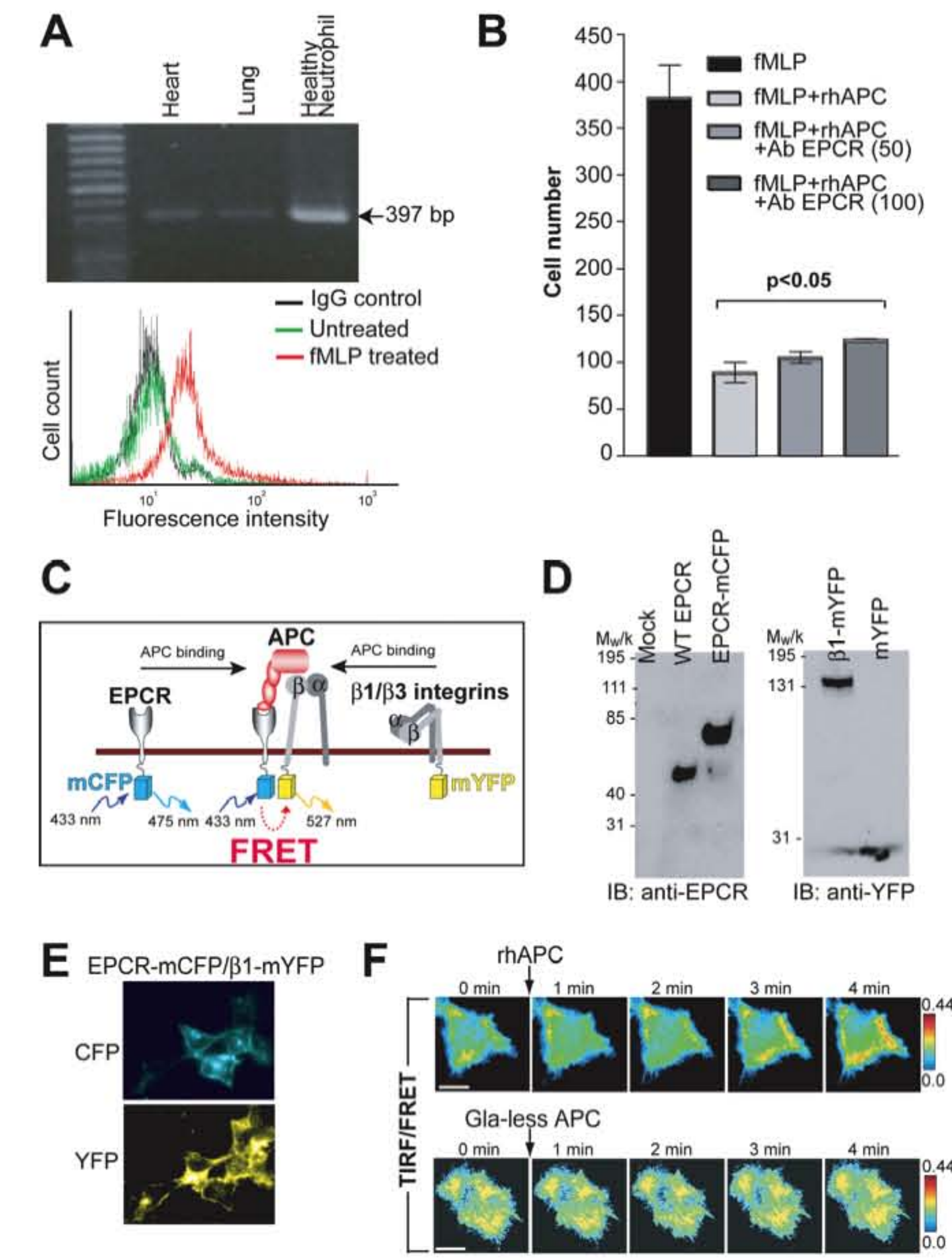


Figure 3. Simultaneous binding of rhAPC to neutrophil integrins and EPCR.

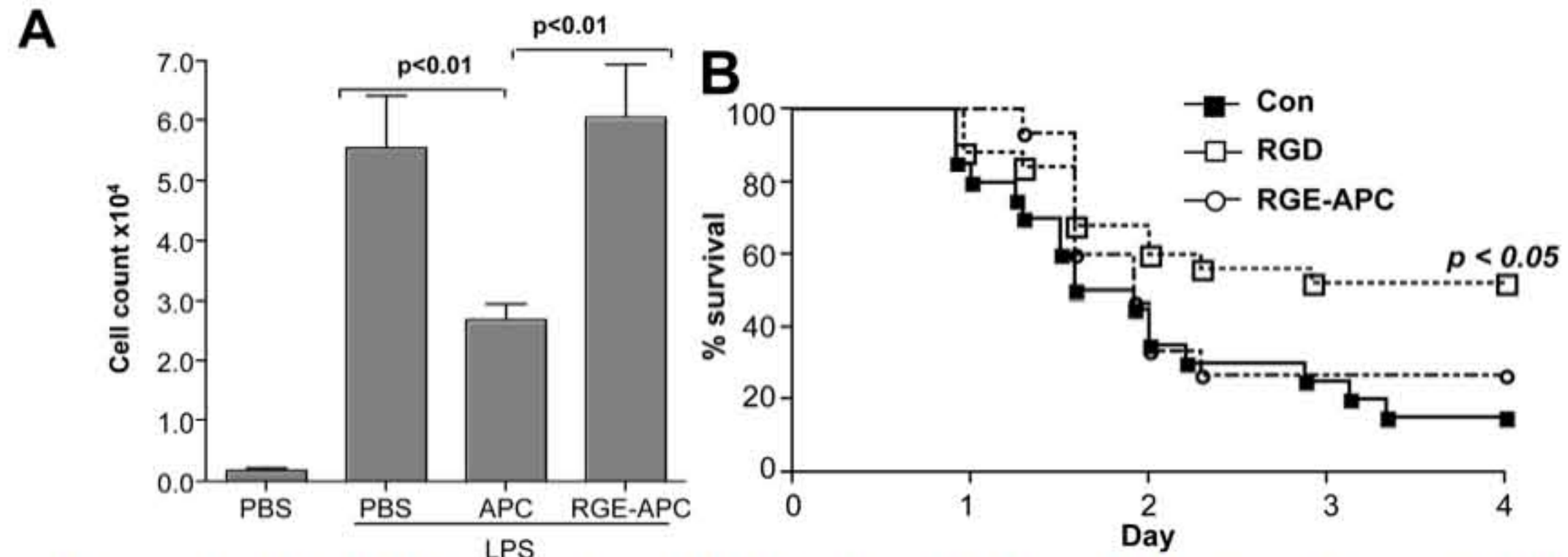


Figure 4. The RGD sequence of rhAPC is critical for inhibition of neutrophil migration in vivo.

SIGNIFICANCE

- 1) Novel cellular receptors for rhAPC.
- 2) Mechanisms for rhAPC in inhibition of neutrophil migration.
- 3) New therapeutic opportunity for severe sepsis.