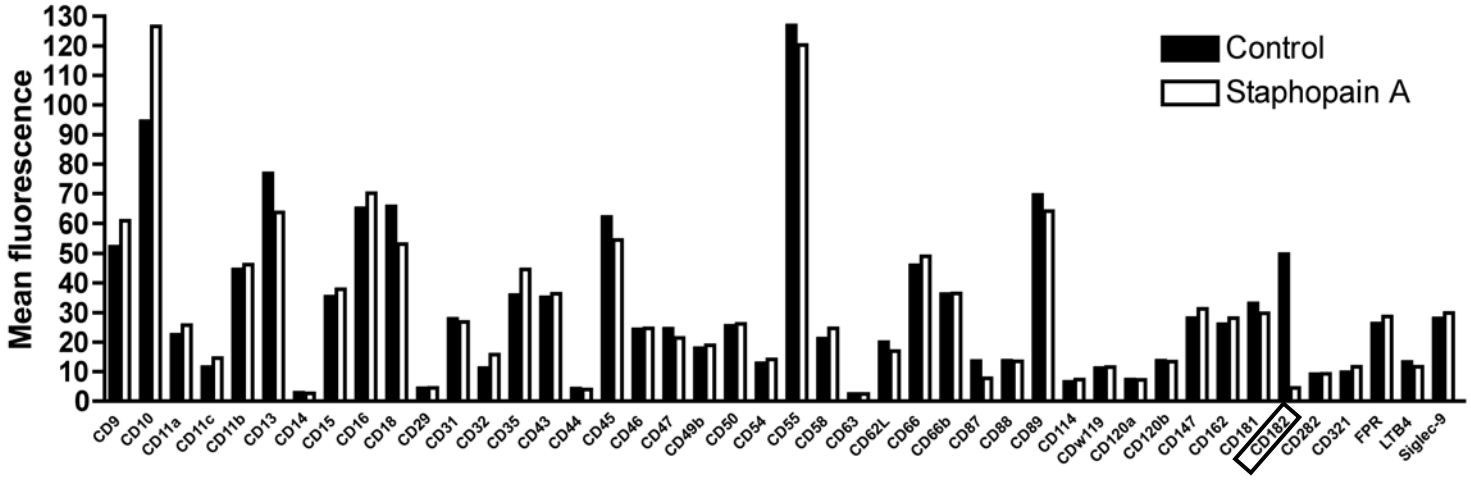
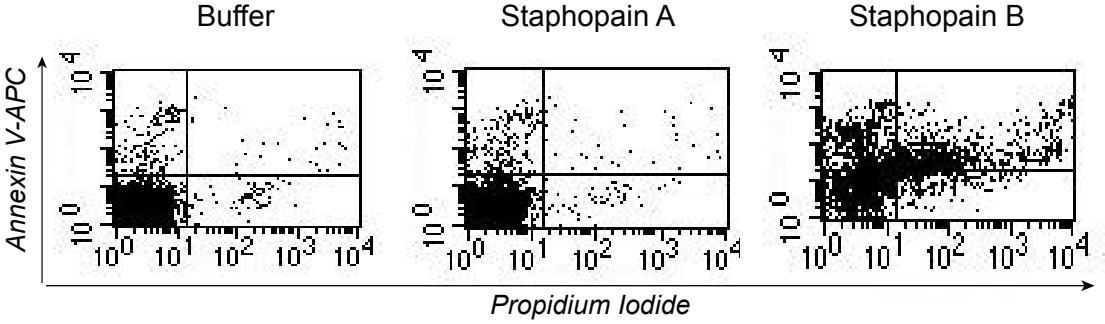
Supplemental Figure 1

a



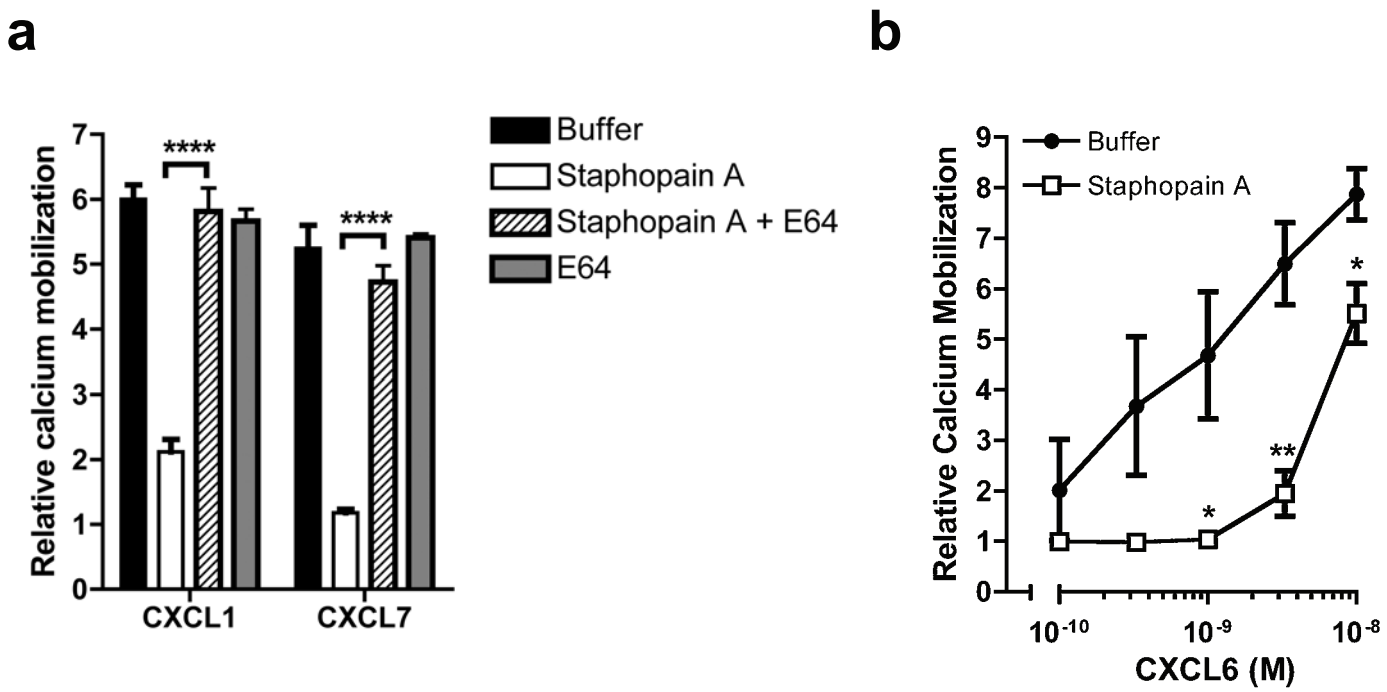
b



Supplemental figure 1. Staphopain A inhibits antibody binding to CXCR2 on neutrophils

(A) Mean fluorescence data of figure 1a. Neutrophils were incubated with 0.5 μM Staphopain or buffer for 15 min at 37^{o}C. After washing, cells were stained with a panel of blocking antibodies against surface-expressed receptors. Figure is a representative of three separate experiments using different donors. (B) Staphopain A does not induce apoptosis in neutrophils. Neutrophils were incubated with Buffer, 0.5 μM Staphopain A or Staphopain B for 75 min at 37^{o}C. After washing, cells were stained with APC-labeled Annexin V and Propidium Iodide. Figure represents two separate experiments using different donors.

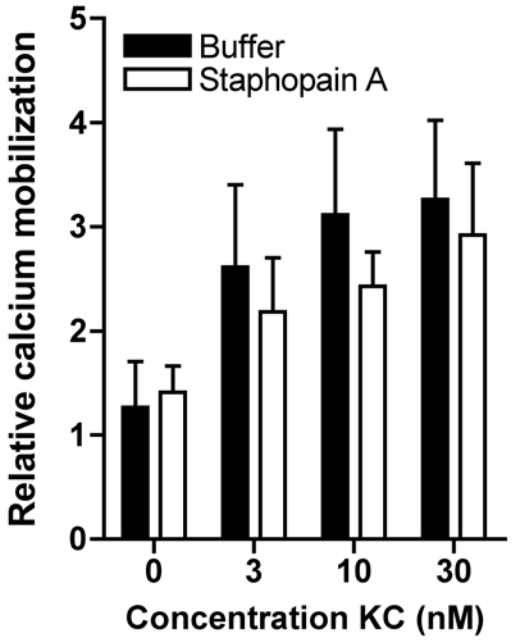
Supplemental Figure 2



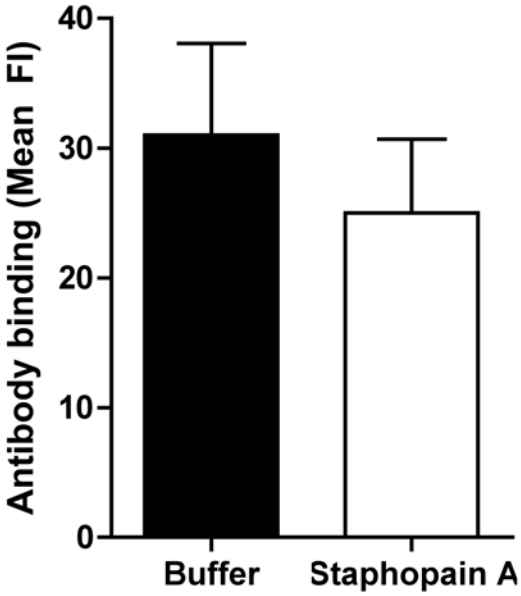
Supplemental figure 2. Staphopain A blocks calcium mobilization of neutrophils. Fluo-3 labelled human neutrophils were pre-incubated with buffer or 0.5 μM Staphopain A for 15 min at 37^{o}C. After washing, cells were stimulated with different concentrations of chemokines. (A) The cysteine protease inhibitor E-64 (10 μM) reverses the inhibitory action of Staphopain A (0.5 μM). CXCL1 and CXCL7 at $1x10^{-8}$ M. (B) Staphopain A blocks CXCL6 dependent calcium mobilization of neutrophils. Figures represent the mean ± SE of three separate experiments. The relative calcium mobilization was calculated by dividing the fluorescence after stimulation by the baseline fluorescence. *, $P<0.05$; **,$P<0.01$; ***,$P<0.005$ Staphopain A versus buffer (2-tailed Student's *t* test).

Supplemental Figure 3

a



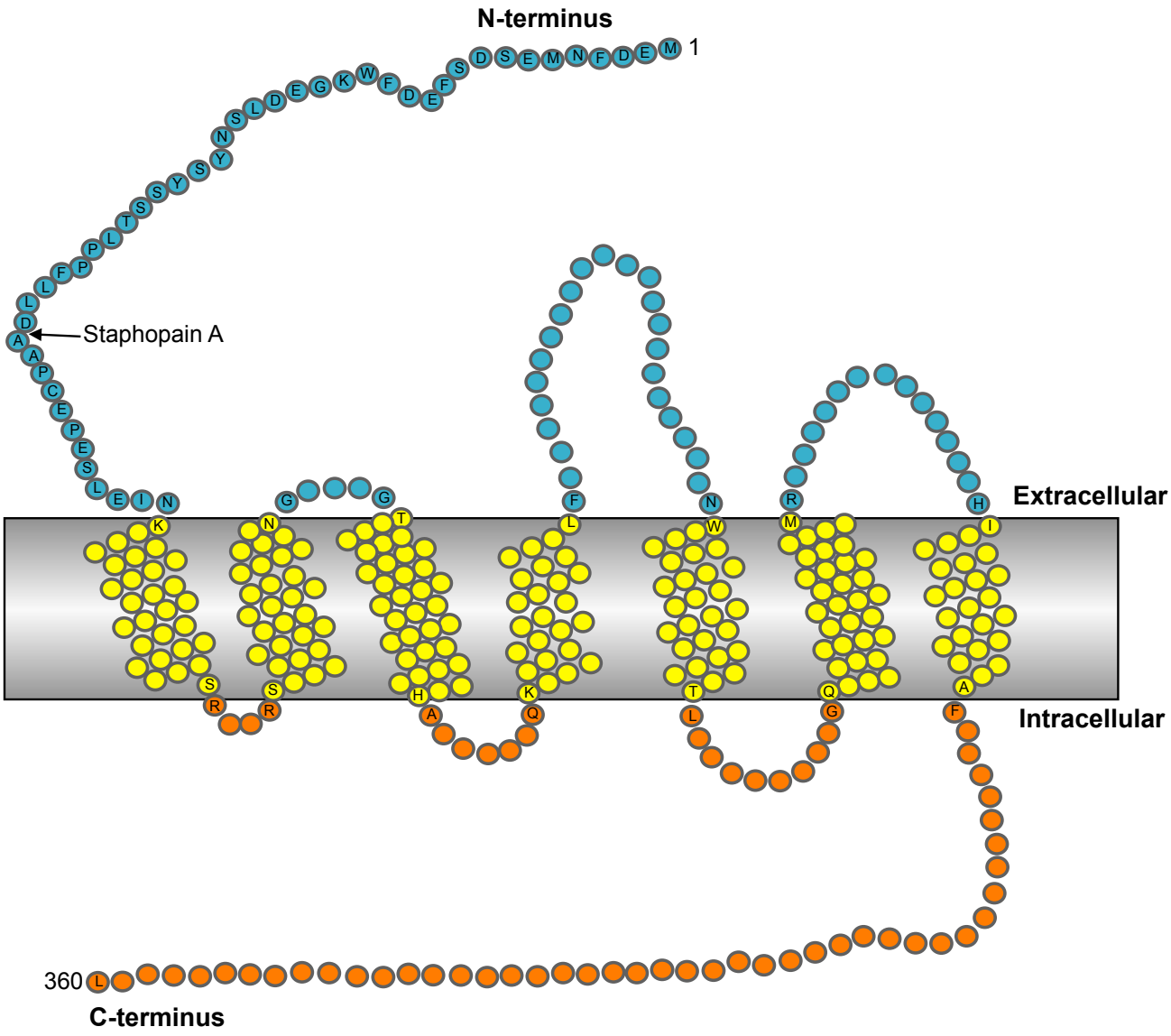
b



Supplemental figure 3. Staphopain A does not inactivate murine CXCR2.

Murine neutrophils were isolated from the bone marrow of C57Bl/6 mice. (A) Fluo-3 labelled murine neutrophils were pre-incubated with buffer or 0.5 μM Staphopain A for 15 min at 37^{o}C. After washing, cells were stimulated with KC (murine CXCL1). The relative calcium mobilization was calculated by dividing the fluorescence after stimulation by the baseline fluorescence. (B) Murine neutrophils were pre-incubated with buffer or 0.5 μM Staphopain A for 15 min at 37^{o}C. Cells were subsequently stained with a monoclonal antibody specific for the N-terminus of murine CXCR2. Both figures represent the mean ± SD of two separate experiments using different mice.

Supplemental Figure 4



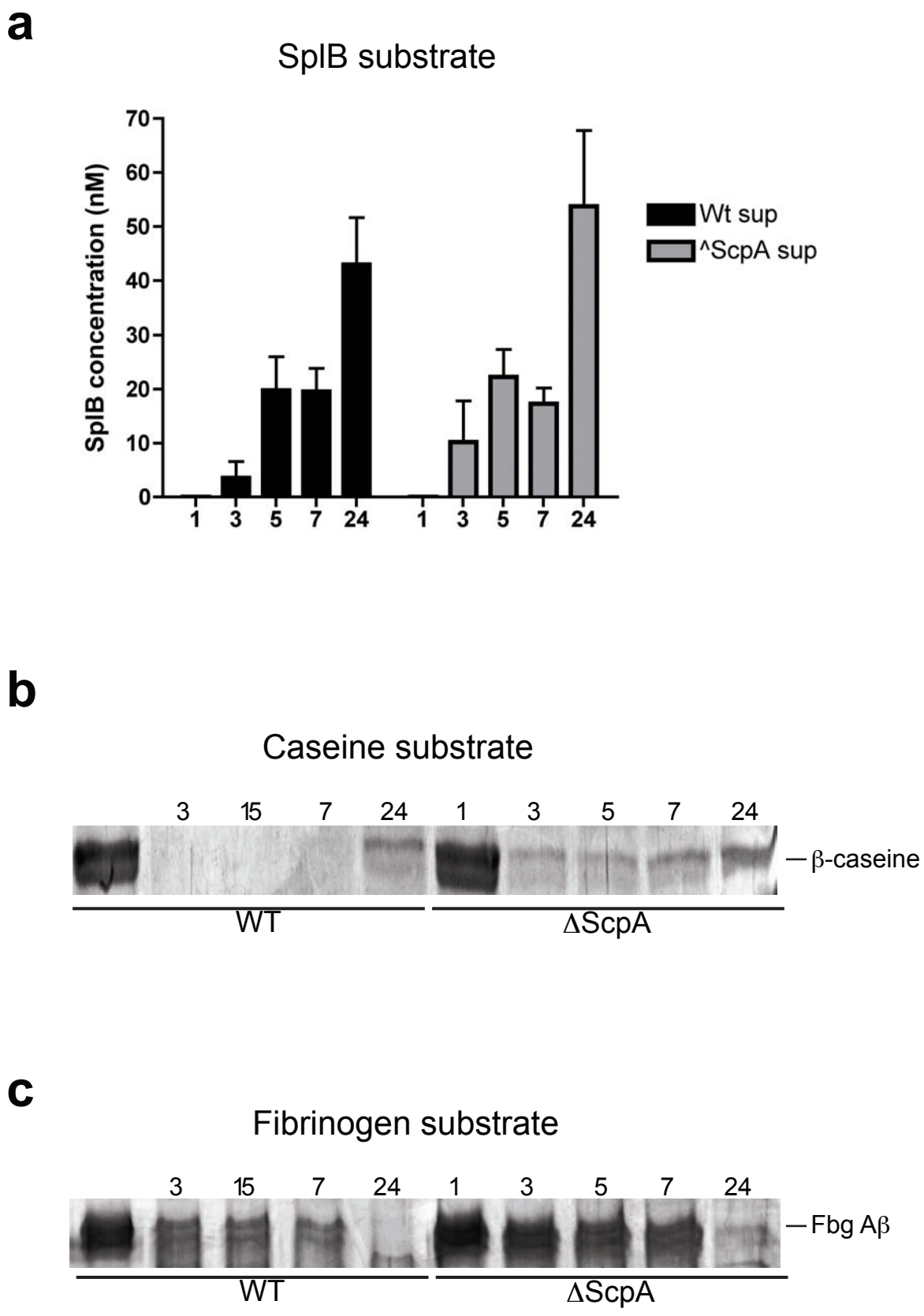
Human CXCR2

MEDFNMESDSFEDFWKGEDLSNYSYSSTLPPFLLDAAPCEPESLEINKYFVVIIYALVFLLSLLGNSLVMLVILYSRVG
RSVTDVYLLNLALADLLFALTLPIWAASKVNGWIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKR
YLVKFICLSIWGLSLLLALPVLLFRRTVYSSNVSPACYEDMGNNTANWRMLLRILPQSFGFIVPLLIMLFCYGFTLRTL
FKAHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTQVIQETCERRNHIDRALDATEILGILHSCLNPLIYAFIG
QKFRHGLLKILAIHGLISKDSLPKDSRPSFVGSSSGHTSTTL

Supplemental figure 4. Predicted topology plot for human CXCR2

Above: Predicted topology plot of human CXCR2 in the context of a membrane. Transmembrane helices are predicted by snake plot designer from the GPCR-SSFE database (www.ssfa-7tmr.de). The folding of extracellular and cytoplasmic domains is hypothetical. Below: Amino acid sequence of human CXCR2 (Uniprot #P25025), colour coding according to topology plot. The arrow indicates the cleavage site of Staphopain A.

Supplemental Figure 5



Supplemental figure 5. Protease expression in bacterial supernatants.

Supernatants of *S. aureus* strain USA300 (WT) and its isogenic mutant (*ΔscpA*) (described in figure 7A) were tested for the presence of other proteases than Staphopain A. (A) Supernatants (undiluted) were incubated with a SplB-specific fluorescent substrate (WELQ-AMC, 0.1 mM (Dubin *et al,* 2008)) for 15 minutes at 37 ^{o}C and fluorescence was detected using a fluorometer (Ex355/Em460). Figure represents mean± SE of two separate experiments. B) Supernatants (undiluted) were incubated with β-caseine (10 μM) for three hours at 37 ^{o}C. (C) Fibrinogen is cleaved by Staphopain A and B (Ohbayashi *et al*.,2010). Supernatants (undiluted) were incubated with human fibrinogen (1 μM) for 30 minutes at 37 ^{o}C. (B-C) Cleavage was analysed by SDS-PAGE and Instant Blue staining. Representatives of two separate experiments.