Supplemental Figure S1

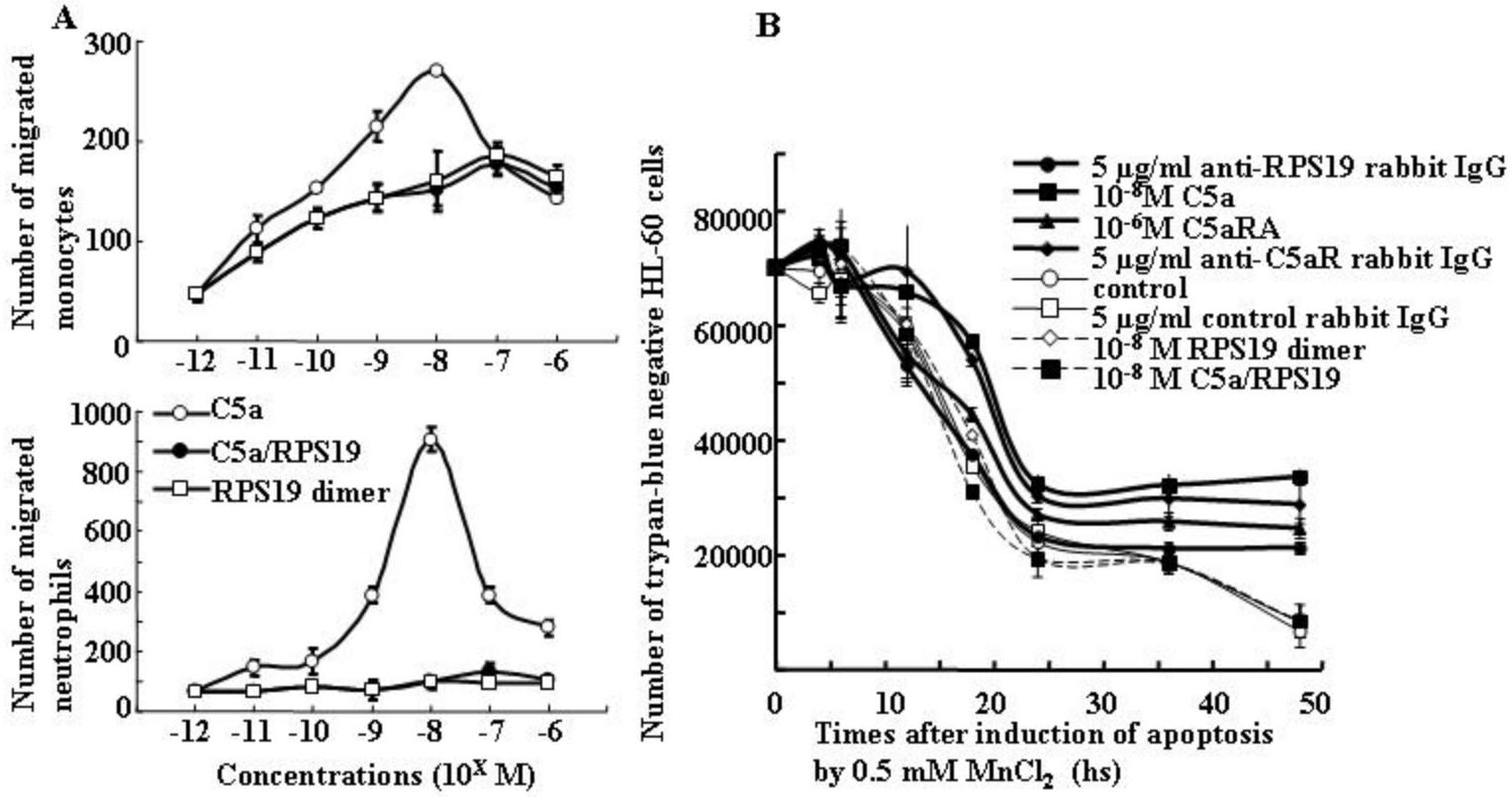

Figure S1. C5a/RPS19 as a substitute for the RP S19 dimer. The recombinant proteins were prepared using an *E. coli* expression system with pET32a as the vector and Rosetta-gami B (DE3) Lys-S as the host bacteria. We initially prepared C27R-C5a cDNA and C27R-G73D-C5a/RPS19 chimera by PCR. The cDNAs were subcloned into pET32a and transfected into the cells. Trx-tag-His-tag-S-tag-C5a (C5a) or Trx-tag-His-tag-S-tag-C5a/RPS19 (C5a/RPS19) was purified using a Hi-TrapTM Chelating HP column and a Hi-TrapTM Heparin HP column [Reference 24]. (A) The monocyte and neutrophil chemotactic potencies of C5a/RPS19 (white square) were compared with that of C5a (white circle) and the RP S19 dimer (black circle) in chemotaxis chamber, respectively. (B) Viable cells in 96 well tissue culture plates were counted at several time points after 0.5 mM manganese (II)-loading by trypan-blue exclusion in the presence of control PBS (thick line with white circle), 5 μg/ml of control rabbit IgG (thick line with white square), 10^{-8}M the RP S19 dimer (thick dot line with white diamond), 10^{-8}M C5a/RPS19 (thick dot line with black square), 5 μg/ml of anti-RP S19 rabbit IgG (thin line with black circle), 10^{-8}M C5a (thin line with black square), 10^{-6}M C5aRA (thin line with black triangle), and 5 μg/ml of anti-C5aR rabbit IgG (thin line with black diamond), respectively. Data are mean values ± SD.

Supplemental Figure S2

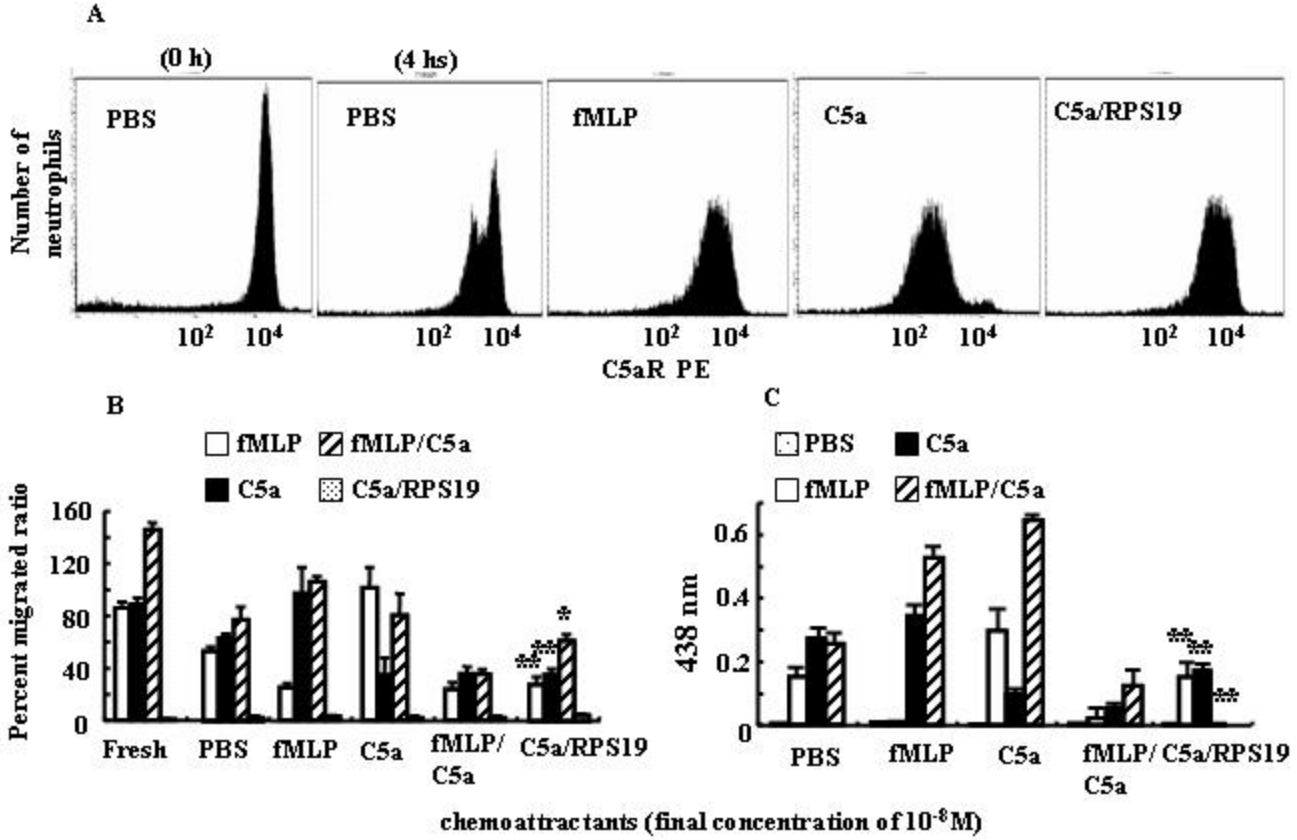

Figure S2. Antagonistic effect of C5a/RPS19 on C5aR of neutrophils. (A) The expression of C5aR was observed by FACS with PE-conjugated anti-C5aR monoclonal antibody (red). Chemotaxis (B) and respiratory burst reaction (C) assays were performed in the presence of fMLP (white column), C5a (black column), a mixture of C5a and fMLP (hatched column) and C5a/RPS19 (dotted column). Results are expressed as the percent ratio of number of migrated 4 hs-culture neutrophils by a sample to number of migrated fresh neutrophils by the same sample (B) and the row data (C). Data are mean values ± SD. (P<0.05: *, P<0.01: **).

Supplemental Figure S3

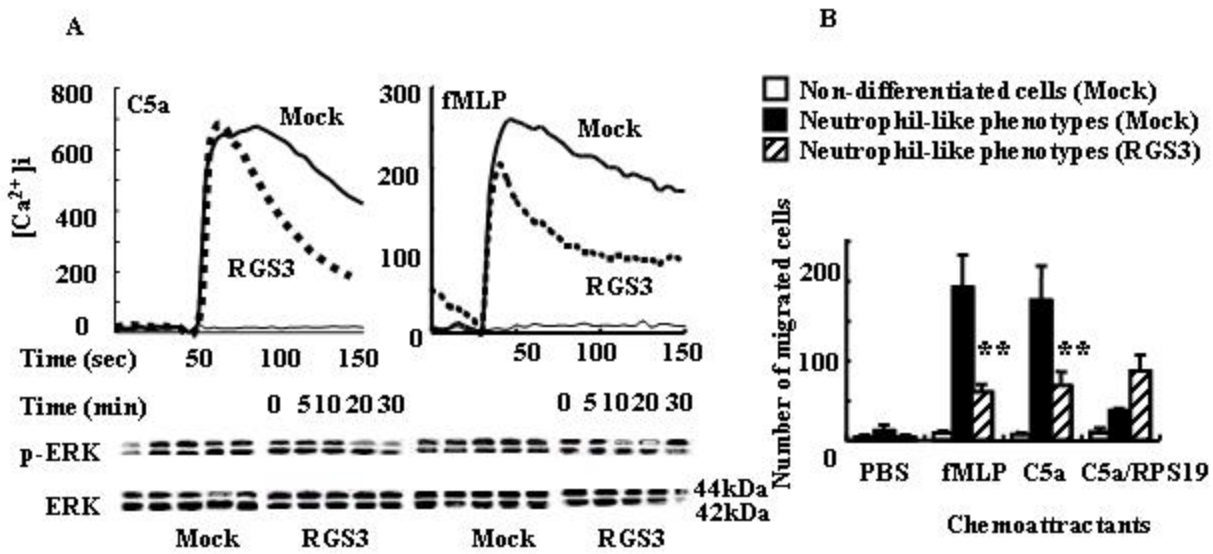

Figure S3. Cross-inhibition of C5aR- and FPR-mediated signals in the neutrophil-phenotypes derived from RGS3 over-expressing HL-60 cells. RGS3-HL-60 were differentiated by 1.25% dimethyl sulfoxide for 7 days to the neutrophil-phenotypes. (A) Cytoplasmic Ca^{2+} influx was measured using Fra-2 AM, and phosphorylated ERKs were detected by Western blot with anti-p-ERK and anti-ERK rabbit IgGs in Mock- and RGS3-HL-60-derived neutrophil-phenotypes at each time point after stimulation with 10^{-8}M C5a and fMLP. (B) The chemotactic responses of non-differentiated Mock- (white columns) or differentiated Mock- (black columns) and RGS3-HL-60 (hatched columns) were measured in a multi-well chamber by the stimulation with 10^{-8}M C5a, fMLP, and C5a/RPS19. Data are mean values ± SD. (P<0.05: *, P<0.01: **).

Supplemental Figure S4

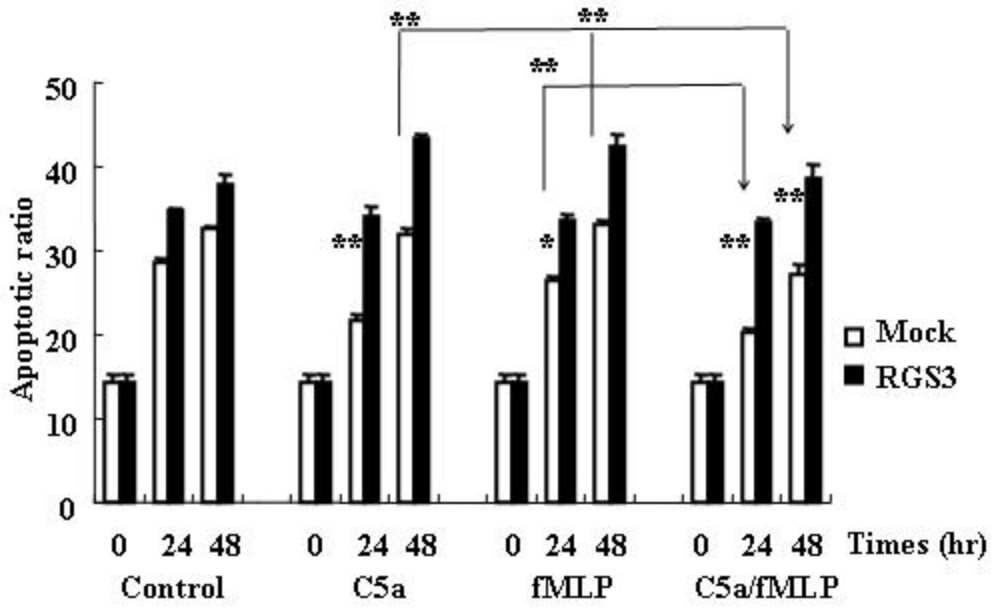

Figure S4. Anti-apoptosis by the fMLP-FPR system. Apoptotic ratios were calculated in cell cycle analysis using Mock-HL-60 (white column) or RGS3-HL-60 (black column) at each time point after a half-dose of manganese (II)-loading in the presence of control PBS, C5a, fMLP, and a mixture of C5a and fMLP, respectively. Data are mean values ± SD. (P<0.05: *, P<0.01: **).

Supplemental table S1

Lists of inhibitors		
pertussis toxin (PTX)	catalysis of the ADP-rybosylation of Gαi, Gαo, and Gαs protein	Sigma (Kyoto, Japan)
PD98059	ERK inhibitor	Sigma (Kyoto, Japan)
SB203580	p38MAPK inhibitor	Sigma (Kyoto, Japan)
C5aR antagonistic/partial agonistic peptide (C5aRA)	NMePhe-Lys-Pro-dCha-dCha-dArg	a kind gift of Prof. N. Nishino of Kyushu Institute of Technology, Kitakyushu, Japan [Reference 29]

Lists of antibodies		
RP S19	rabbit IgG (NY-1) recombinant human RP S19 protein rabbit IgG (NY-4) Ile-Ala-Gly-Gln-Val-Ala-Ala-Ala-Asn-Lys-Lys-His-Cys (RP S19 from Ile134 to His145 with a Cys at the C-terminal)	[Reference 11]
C5aR	rabbit IgG, mouse IgG (S5/1), or FITC-conjugated monoclonal antibody (P12/1)	Santa Cruz (California, USA)
p-ERK and ERK2	rabbit IgGs (Tyr204) or (C-14)	Santa Cruz (California, USA)
RGS3	rabbit IgG (H-300)	Santa Cruz (California, USA)
rabbit IgG	HRP- or FITC-conjugated goat IgG	Santa Cruz (California, USA)
mouse IgG	PE-conjugated goat IgG	Santa Cruz (California, USA)
	FITC- or PE-conjugated annexin V	MBL (Nagoya, Japan)
	Control mouse and rabbit IgG	Sigma (Kyoto, Japan

Supplemental tables S2

Lists of plasmid vectors		
pCAIN	pCAGGS-internal ribosome entry site and neomycin-resistant vectors	[Reference 30] [Reference 31]
pCAIN-wild type RP S19 or 137N RP S19	pCAIN bearing wild type RP S19 cDNA or Gln137Asn mutant RP S19 cDNA	[Reference 11]
pCAIN- RGS3	pCAIN bearing RGS3 cDNA	Template; pRCCMV-RGS3 primer pair; forward -CCGGAATTCATGTTTGAGACGGAG- reverse -CCGCTCGAGCGGTCCCCCGGGCTAAAGCGG-
CS-RfA-EG	 pENTR4-H1 pCAG-HIVgp pCMV-VSV-G-RSV-Rev	[Reference 32] Entry plasmid Packaging plasmid VSV-G- and Rev-expression plasmids
CS-control shRNA-EG	sense only control RGS3 RNA (Control shRNA)	5'-GATCTGGATGTTTGAGACGGAGGCATTCAAGAGATTTTTTT-3' 5'-CTAGAAAAAAATCTCTTGAATGCCTCCGTCTCAAACATCCA-3'
CS-RGS3 shRNA-EG	hairpin RGS3 shRNA (RGS3 shRNA)	5'- GATCTGATGTTTGAGACGGAGGCATTCAAGAGATGCCTCCGTCTCAAA CATCTTTTTTT-3' 5'- CTAGAAAAAAAGATGTTTGAGACGGAGGCATCTCTTGAATGCCTCCGT CTCAAACATCA-3'

To establish HL-60 transformations that had CS-RfA-EG vector plasmids with control shRNA (RGS3^{cont}-HL-60) or RGS3 shRNA (RGS3^{low}-HL-60), 293T cells (5X10^6 cells/ml) were maintained in DMEM medium containing 10 % FBS in poly-lysine coated 10-cm-diameter tissue culture plates for 24 hours, then changed to fresh medium. 450 μl of plasmid DNA solution containing 17 μg of the packaging plasmid and 10 μg of the VSV-G- and Rev-expressing plasmids were mixed with 50 μl of 2.5 M $CaCl_2$ and 500 μl of 2X HEBS (1.5 mM Na_2HPO_4, 280 mM NaCl, and 50 mM HEPES). HIV-1-based lentivirus was recovered by ultracentrifugation and resuspended in 50 μl of HBSS medium [Reference 33]. We mixed lentivirus in 100 μl of HL-60 cells (1 X 10^6 cells /ml) at multiplicity of infection 10 and established 3 RGS3^{cont}- and RGS3^{low}-HL-60 in a selection medium with 1 mM Zeosin.

Supplemental tables S3

List of transcripts	Primers
C5aR; 810 bp	forward-GAGAGCAAGTCATTCACGCG- reverse- AGTGCAGTGGTGCGATCA-
HA-RP S19; 477 bp Wild type RP S19; 450 bp	Forward-GGAATTCCATATGCCTGGATACCCATAC- reverse–GTAGAACCAGTTCTCATCGTAG- forward–TACCCATACGATGTTCCAGA- reverse–GTAGAACCAGTTCTCATCGTAG-
RGS1: 193 bp	forward-ACTGGGAAGGCCAGGTAACT- reverse–ACACCGGACAAGGAATAGTAGC-
RGS3: 214 bp	forward-CACAGGCCTTTTATTTGCTCC- reverse-CACCATTCTCCAGCTTCAGG-
RGS14: 80 bp	forward-CATACATCAAGGACAAACCCAG- reverse–GTACCCCACCCACATAGAC-
RGS18: 708 bp	forward-ATGGAAACAACATTGCTTTTCT- reverse–TTATAACCAAATGGCAACATCTGA-