Supporting Information

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SI Methods

Subcloning and Mutagenesis. Genes for human rab9, rab7, rilp, and skip, as well as *Salmonella* sifA, were subcloned into pGex-5X-1 for protein expression in *E. coli*, or into pEGFP or mRFP mammalian expression vectors. Both rab7 and rab9 were subcloned into the BamHI and XhoI sites of pGex-5X-1. The sifA, rilp (amino acid 241 to 320) and the PH domain of skip (amino acid 762 to 885) were subcloned into the BamH1 and EcoR1 sites of pGex-5X-1. To construct the mRFP vector, the plasmid pEGFP was digested with Age1 and BspE1 to liberate GFP. The mRFP was PCR amplified, digested with Age1 and BspE1, and ligated into the vector to replace GFP. Mutants were generated by site directed mutagenesis using a Quickchange kit (Stratagene). Primers are listed in supporting information (SI) Table S2 and Table S3.

Protein Expression and Purification. Recombinant GST fusion proteins were produced from pGex-5X-1 fusion plasmids for Rab9, Q66LRab9, Rab7, Q67LRab7, RILP (amino acid 241 to 320) (1), SifA, and the SKIP PH domain (amino acid 762 to 885) (2). The proteins were expressed in *E. coli* BL21-CodonPlus (DE3) (Stratagene) and purified using glutathione-superflow resin (Clontech) according to the manufacturer's protocol. Proteins were dialyzed into Binding Buffer: 150 mM NaCl, 20 mM Tris pH 7.5, 2 mM DTT, and 0.1% Triton X-100. When appropriate, the GST tag was cleaved from SifA and SKIP PH proteins, using Factor Xa (Merck), and GST was removed by passage over glutathione resin. Protein concentrations were determined using Bio-Rad Protein Assay reagents (Bio-Rad).

Nucleotide Loading of Recombinant Rab Protein Pulldowns. GST-Rab proteins that had been loaded onto glutathione agarose overnight were washed once in Binding Buffer and three more times in Nucleotide Loading Buffer (3): 20 mM Tris pH 8.0, 200 mM $(NH_4)_2SO_4$, 0.4 mM EDTA, 2 mM DTT, 0.1% Triton X-100, 1 mM PMSF, and additional protease inhibitors (Complete mini-EDTA-free, Roche). The washed Rab-agarose was resuspended in Nucleotide Loading Buffer that had GTP, GDP, or GTPγS added to a final concentration of 5 mM. Following a 2 h room temperature incubation (rocking), MgAc was added to 10 mM for an additional 10 min incubation, and then the agarose was washed with binding buffer in preparation for binding assays. GST controls to be used in GST-Rab experiments were similarly treated as although "loading" with nucleotide.

Generation of HeLa Lysates. HeLa cells, grown in Minimum Essential Medium (Invitrogen) with 10% Fetal Bovine Serum (ATCC), were transfected with gfp-rab9 using FuGENE 6 Transfection Reagent (Roche). Forty-eight hours later, 2×10^7 HeLa cells were scraped and lysed in Triton Lysis Buffer: 1% Triton X-100, 150 mM NaCl, 20 mM Tris pH 7.5, 1 mM PMSF, and Protease inhibitors (Complete mini, Roche), nutated at 4 °C for 20 min and cleared by centrifugation at top speed in a chilled table top microcentrifuge (20,817 rcf). For binding assays, the supernatant was precleared with 100 μl clean glutathione agarose for 1 h at 4 °C, and precleared lysate was divided equally between test and control samples.

IFAs and Cell Imaging. Fixed cells were washed in PBS and incubated for 10 min in PBS with 50 mM NH_4Cl. Cells were permeabilized with Blocking Reagent: PBS with 10% horse serum and 1 μg/ml Saponin (SIGMA). Mouse anti-human LAMP1 (H4A3-c, Developmental Studies Hybridoma Bank, University of Iowa) primary antibody was diluted 1:500 and incubated with cells for 1 h at 37 °C. This was followed by a Cy5-conjugated AffiniPure Donkey Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc.) secondary used at 1:1000 dilution. Coverslips were mounted and imaged as described (4).

***Salmonella* Infection of HeLa Cells.** Infections of HeLa cells were performed as follows: *Salmonella typhimurium* bacteria were inoculated from a stationary phase culture into LB Amp and after 5–10 h, bacteria were diluted 1:10 into 5 ml of LB containing Amp and 0.3 N NaCl, in a screw cap 15 ml conical tube, and incubated overnight at 37 °C (without shaking) to induce SPI-1. The bacterial OD at 600 nm was multiplied by 1.4×10^9 to calculate number of bacteria per milliliter. Bacteria were diluted into serum free media (SFM) to achieve a MOI of 100 bacteria per cell. Media covering the cells was replaced with Serum Free Media (SFM) containing bacteria, plates were centrifuged at 1000 RPM in a Beckman Coulter Allegra 6R centrifuge for 5 min and incubated at 37 °C and 5% CO_2 for 1 h. Following the incubation, cells were washed and incubated for an hour in MEM + 10% FBS with 100 μg/ml Gentamycin, followed by incubation in MEM + 10% FBS with 10 μg/ml Gentamycin.

Generation of mCherry *Salmonella*. To generate monomeric Cherry fluorescent bacteria, mCherry was cloned into the FPV25.1 GFP vector. GFP was digested out of FPV25.1 with XbaI and HindIII and the mCherry sequence was PCR amplified using the 5′primer GCACtctagaatggtgagcaagggcgaggagg and the 3′ primer GCACaagctt TTACTTGTACAGCTCGTCCATGC-CGCCGG. The mCherry PCR product was digested with XbaI and HindIII and cloned into the similarly digested FPV25.1. FPV25.1-mCherry was electroporated into SL 1344 *Salmonella* and used for subsequent infections. The mCherry plasmid was kindly provided by from Roger Y. Tsien (University of California, San Diego, LaJolla, CA (5).

Dynamic Light Scattering. A Zetasizer Nano ZEN1600 DLS photometer using Dispersion Technology Software 4.10 (Malvern Instruments Ltd.) was used to collect and analyze data for the dynamic light scattering experiment. Molecular weights of solution species were estimated using the Protein Utility component of Malvern Software through MHKS relations for globular proteins (6).

Sif quantitation. We counted the number of cells with Sifs using minimum length limits of 3, 5, 10, 15, and 20 microns and expressed this number as "percentage of infected cells" with Sifs per experiment. Sifs were defined as largely continuous, long, tubular membranes. Discontinuous structures that appeared as beads-on-a-string were not counted as Sifs in these experiments. We required that experiments have a minimum of 30% Sifs in the GFP control to be included in this dataset (for ≥ 3 μm length). Total cells imaged over all five experiments: GFP [180], GFP-Rab9 [252], GFP-DNRab9 [163]. Average number of infected, transfected cells imaged per experiment, per transfection type is 40 cells.

1. Wu M, Wang T, Loh E, Hong W, Song H (2005) Structural basis for recruitment of RILP by small GTPase Rab7. *EMBO J* 24:1491–1501.
2. Boucrot E, Henry T, Borg J-P, Gorvel J-P, Meresse S (2005) The intracellular fate of *Salmonella* depends on the recruitment of kinesin. *Science* 308:1174–1178.
3. Hoshino M, *et al.* (1987) Interaction of. *ras* oncogene product p21 with guanine nucleotides. *J Biochem* (*Tokyo*) 102:503–511.
4. Bahattacharjee S, van Ooij C, Balu B, Adams JH, Haldar K (2007) Maurer's clefts of *P. falciparum* are secretory organelles that concentrate virulence protein reporters for delivery to the host erythrocyte. *Blood*, in press.
5. Shaner NC, *et al.* (2004) Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma sp* red fluorescent protein. *Nat Biotechnol* 22:1567–1572.
6. Harding SE (1997) The intrinsic viscosity of biological macromolecules. Progress in measurement, interpretation and application to structure in dilute solution. *Prog Biophys Mol Biol* 68:207–262.

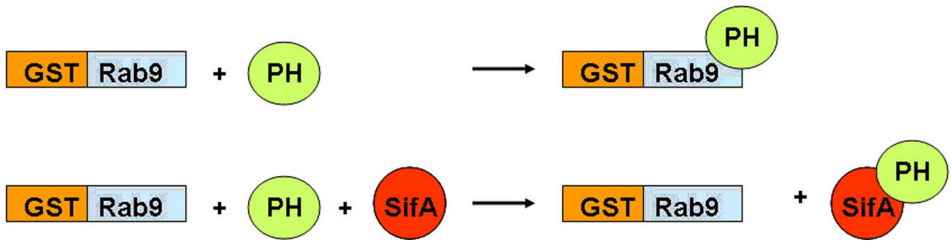

Fig. S1. Model of competition pulldown results. Cartoon illustrates results presented in Fig. 2 *B* and *C*. GST-Q66L-Rab9 or GFP-Rab9 bind to SKIP PH, but when SifA is added, SifA binds and sequesters the SKIP PH domain protein, making it unavailable to Rab9.

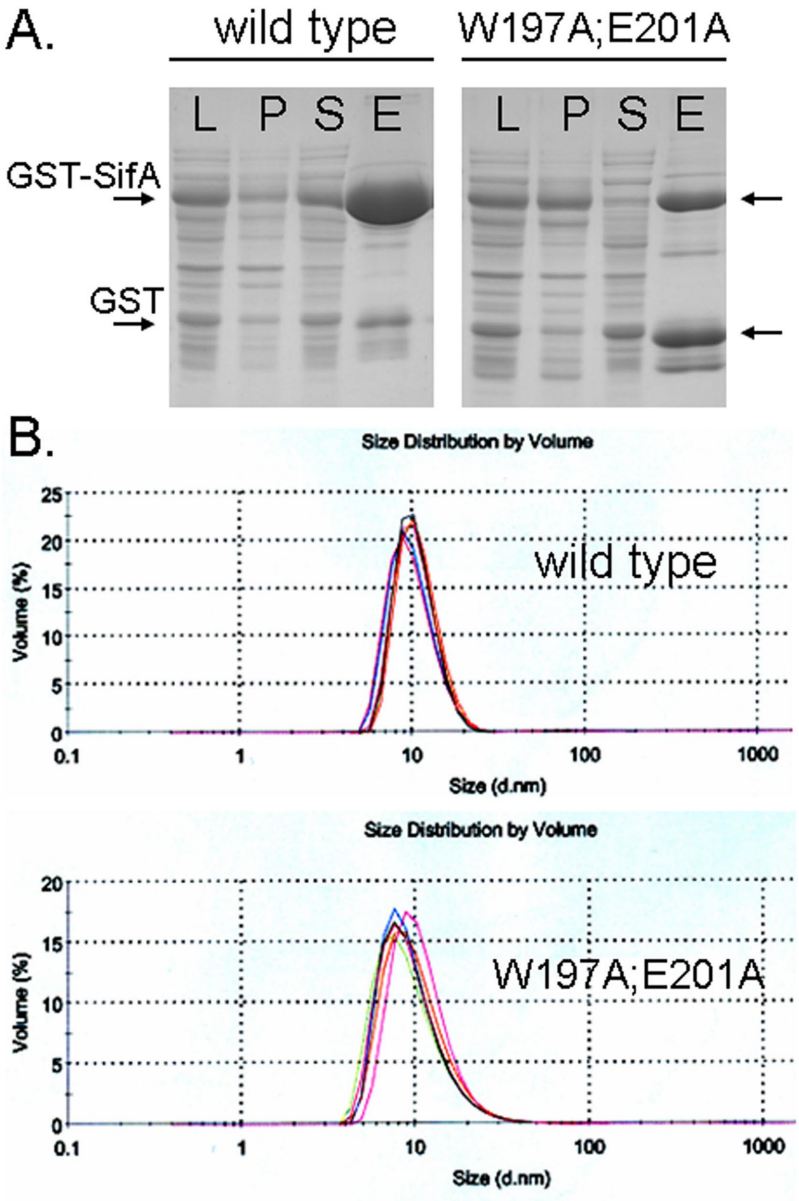

Fig. S2. Comparison of wild-type GST-SifA and GST-(W197A;E201A)SifA protein behavior during expression/purification and in solution. (*A*) Purification of wild type and a W197A, E201A mutant form of GST-SifA. SDS/PAGE gel of equivalent amounts of cell lysate (L), cell pellet (P), supernatant (S), or eluate (E) for wild-type GST-SifA and GST-(W197A,E201A)SifA. Mutant SifA was eluted in half the volume compared with wild type. (*B*) A dynamic light scattering (DLS) experiment indicates that wild-type and mutant proteins both have stable biological assemblies in solution. DLS analysis revealed single peaks in particle size volume distributions for both wild-type GST-SifA and for GST-(W197A;E201A)SifA, yielding an average hydrodynamic diameter of 10.4 $\pm$ 0.4 nm with width of 3.0 $\pm$ 0.1 nm for wild type, and a 10.2 $\pm$ 0.4 nm diameter with width of 8.0 $\pm$ 0.3 nm for mutant protein. Diameters of 10.4 and 10.2 nm correspond to theoretical molecular weights of 159.4 and 152.3 kDa, respectively, for globular proteins, estimated by sing Mark–Houwink–Kuhn–Sakurada (MHKS) relations for globular proteins. Taking the expected molecular weights of these fusion proteins into account and the fact that GST is known to dimerize in solution, we interpret that this peak, representing the major species in solution, corresponds to a GST-SifA:GST-SifA dimer species. Six replicate scans were taken for each protein. The scatter and thus readout diameter will be dominated by the larger molecular weight species in a mixture. However, we see a tendency of the peak to shift to the left and a wider peak width for the mutant protein compared to wild type. This is likely because of contribution from smaller molecular weight species in the purified mutant protein mixture (see *A*). Importantly, large molecular weight aggregates are not seen to a significant extent with either wild-type or mutant GST-SifA proteins.

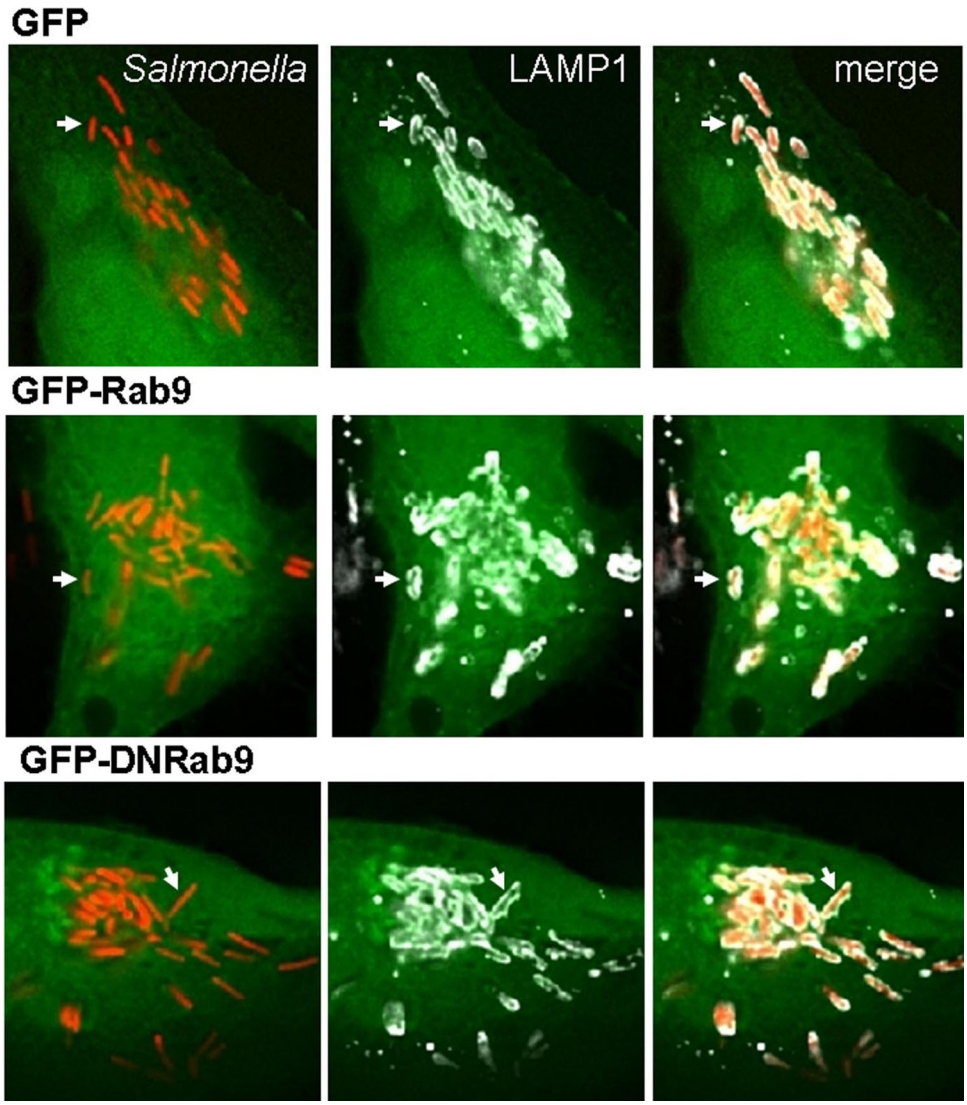

Fig. S3. LAMP1-stained SCV detected in infected HeLa cells expressing GFP, GFP-Rab9, or GFP-DNRab9. Monolayers expressing GFP, GFP-Rab9, or GFP-DNRab9 (green) were infected with mCherry expressing *S. typhimurium* (red) for 12 h and then were fixed and stained for LAMP1 (white). Arrows point out individual bacteria for comparison.

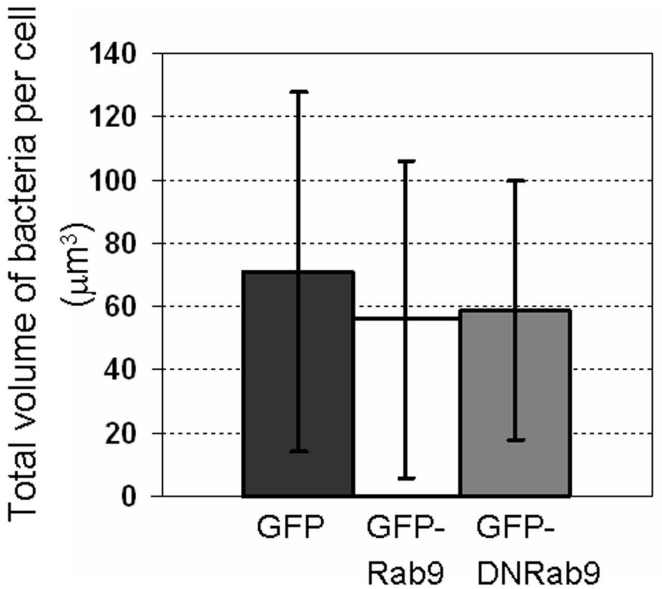

Fig. S4. Quantification of effects of Rab9 overexpression on bacterial proliferation. *Salmonella* infection levels in HeLa cells expressing GFP, GFP-Rab9, or GFP-DNRab9 were scored based on total bacterial volume per cell, calculated by using Volocity version 3.5.1 (Improvision). Results from five separate experiments were combined and averaged. More than 160 cells were counted for each category. Bar plot shows average over all data. Error bars denote standard deviation.

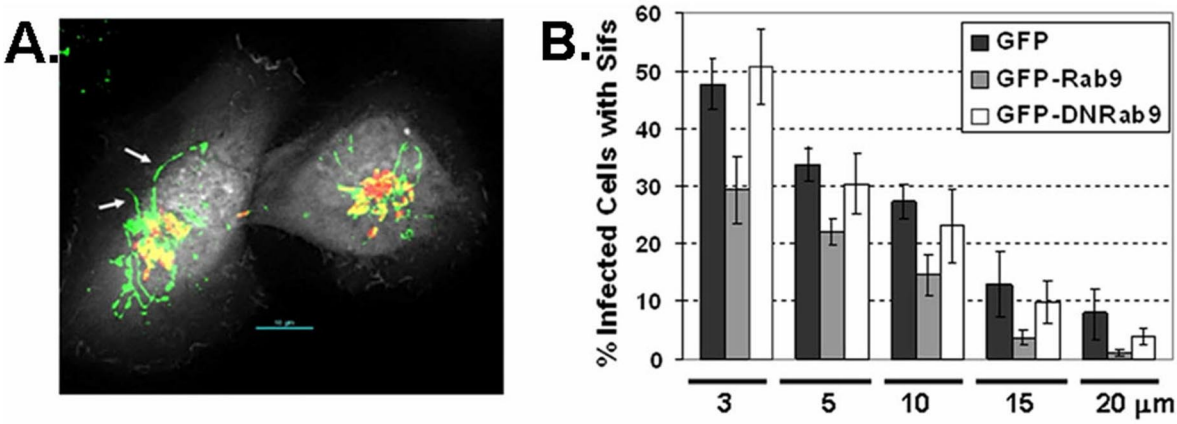

Fig. S5. Rab9 overexpression reduces Sif size in *Salmonella* infected HeLa cells. (*A*) Examples of Sifs. HeLa cells expressing GFP (white) and infected with mCherry *Salmonella* (red) are stained for LAMP1 (green). Arrows point at selected Sifs. (*B*) Bar plot showing average percentage of infected cells with Sifs for HeLa cells overexpressing GFP (black), GFP-Rab9 (gray), or GFP-DNRab9 (white bars) ($n = 5$ experiments). Error bars are represented as $\pm$ SEM. Data are plotted using 3, 5, 10, 15, or 20 μm as the minimum size for Sifs counted in each analysis, indicated below plots. [Scale bar, 10 mm (cyan).]

Table S1. Comparison of percentages of infected cells with Sifs, for cells expressing GFP-Rab9 or GFP-DNRab9, vs. GFP controls

μm	% Sifs	% Sifs	P	% Sifs	P
	GFP ± SEM	GFP-Rab9 ± SEM		GFP-DNRab9 ± SEM	
3	48 ± 4	29 ± 6	0.047	51 ± 7	0.754
5	34 ± 3	22 ± 2	0.028	30 ± 5	0.465
10	27 ± 3	14 ± 3	0.016	23 ± 6	0.753
15	13 ± 6	3.7 ± 1.3	0.142	10 ± 4	0.754
20	8.0 ± 4.1	1.1 ± 0.5	0.332	3.8 ± 1	0.753

Wilcoxon-Mann-Whitney tests comparing results for GFP with GFP-Rab9 or comparing GFP with GFP-DNRab9, show that the difference between GFP and GFP-Rab9 is significant ($P < 0.05$) when using a 3, 5 or 10 micron cutoff size for Sifs in the analysis. Values provided are the average over five experiments. SEM is standard error of the mean.

Table S2. Constructs were cloned into pGex5X-1, or mutagenized, using the following primers (F = forward, R = Reverse)

Construct	Primer sequence 5′ to 3′
SUBCLONING	
GST-Rab9 F	GCA CGG ATC CCC GCAGGAAAATCTTCACTTTTTAAAG
GST-Rab9 R	GCA CCT CGA GTC AACAGCAAGATGAGCTAGG
GST-Rab7 F	GCA CGG ATC CCC ACCTCTAGGAAGAAAGTG
GST-Rab7 R	GCA CCT CGA GTC AGCAACTGCAGCTTTCTGC
GST-SifA F	GCA CGA ATT CCC GATTACTATAGGGAATGG
GST-SifA R	GCA CCT CGA GTT AGCCGCTTTGTTCTGAGCGAACG
GST-RILP(aa241–320) F	GCA GAT CTC CTG CCGCTTCAGTCGGGAGG
GST-RILP(aa241–320) R	GCG AAT TCT CAG CCAGCCTCATCCTCACTGC
GST-PH(aa762–885) F	GCA GAT CTC ATG CCACTGCTCACCCCCCG
GST-PH(aa762–885) R	GGG AAT TCT CAG CAGGGGCTGGGAGCTACG
MUTAGENESIS	
Rab9 Q66L F	GAC ACG GCA GGT CTGGAGCGATTCCGAAG
Rab9 Q66L R	CTT CGG AAT CGC TCCAGACCTGCCGTGTC
Rab7 Q67L F	GAC ACA GCAGGA CTGGAACGGTTCCAGTCTC
Rab7 Q67L R	GAG ACTGGA ACC GTTCCA GTCCTGCTGTGTC
SifA W197A;E201A F	GGA CAT TTA GATGGGGCG AAAGCGCAAGCAAAGGCAACCTACC
SifA W197A;E201A R	GGTAGG TTG CCT TTGCTT GCGCTTTCGCCCCATCTAAATGTCC

Table S3. Constructs were cloned into RFP, or mutagenized, using the following primers (F = forward, R = Reverse)

Construct	Primer sequence 5′ to 3′
SUBCLONING	
mRFP F	GCACACCGGTCGCCACCATGGCCTCCTCCGAGG
mRFP R	GCACTCCGGAGGCGCCGGTGGAGTGGCGGCC
mRFP-SKIP F	GCACAGATCTGAGCCGGGGGAGGTGAAGGACCGG
mRFP-SKIP R	GCAC GAATTCTCAGCACCAGGGGTCTCGGGAGGCTCG
mRFP-SKIP(ΔRUN) F	GCAGATCTTACATGCCCGACTACTACAAAC
mRFP-SKIP(ΔRUN) R	GCGAATTCTCAGCACCAGGGGTCTCGGGAG
MUTAGENESIS	
SKIP(1–760(gap)877-end) F	G TCCCTGGGCCCCACGCCCCAGGGCGTAGCTC
SKIP(1–760(gap)877-end) R	GAGCTACGCCCTGGGGCGTGGGGCCCAGGGAC