Supplemental Information

Supplemental Figures

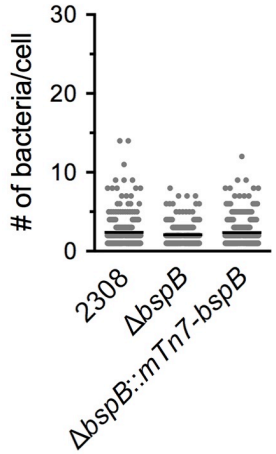

Figure S1. Comparable uptake of *Brucella* strains in BMMs. Related to Figure 1. BMMs were infected with various $DsRed_m$-expressing *B. abortus* strains for 4 h and numbers of bacteria/cell were counted via fluorescence microscopy. Lines indicate means from 3 independent experiments.

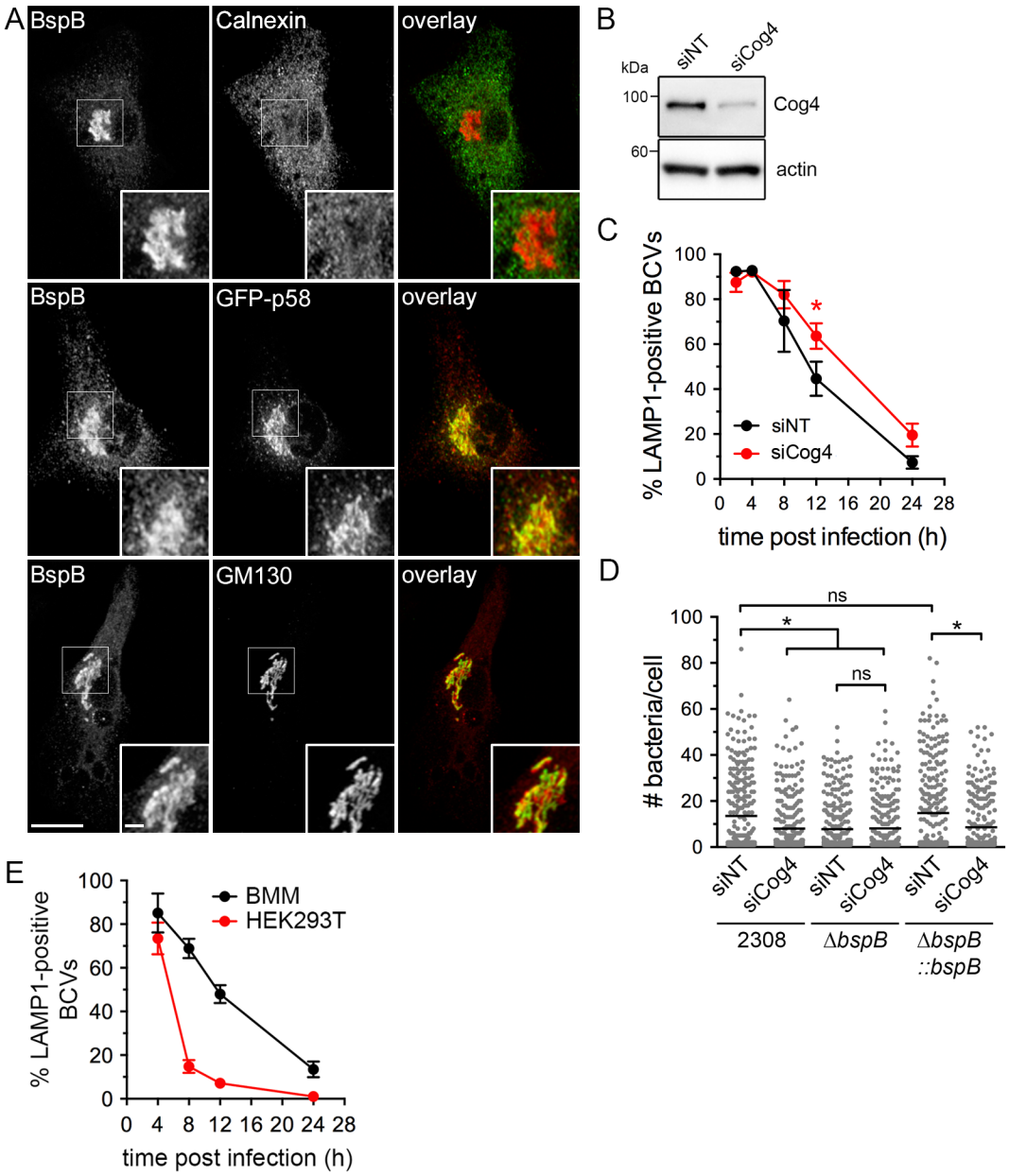

Figure S2. Golgi targeting of BspB in BMMs, role of Cog4 in rBCV biogenesis and bacterial replication and accelerated rBCV biogenesis in HEK293T cells. Related to Figure 3.

(A) Representative confocal micrographs of BMMs transduced to express HA-BspB for 24 h. HA-BspB (red) localization to the ER (calnexin), the ERGIC (GFP-p58) and the Golgi apparatus (GM130) are shown. Scale bars, 10 μm and 2 μm (insets).

(B) Representative Western blot analysis of Cog4 depletion in BMMs upon siRNA nucleofection, compared to non-targeting (siNT) siRNA treatment. β-actin was used as loading control.

(C) BCV remodeling in BMMs treated with either siNT or siCog4 siRNAs for 72 h prior to infection with *B. abortus* 2308::mTn*7*-*DsRed*$_m$. Data are means ± SD from 3 independent experiments. Asterisks indicate statistically significant differences compared to siNT control as determined by a two-way ANOVA followed by Bonferroni's multiple comparisons test (P<0.05).

(D) *Brucella* replication in BMMs treated with either siNT or siCog4 siRNAs for 72 h prior to infection with DsRed$_m$-expressing *B. abortus* wild type (2308), Δ*bspB*, or complemented Δ*bspB B. abortus* strains for 24 h. Lines indicate from 3 independent experiments. Asterisks indicate statistically significant differences compared to untreated control determined by a Kruskal-Wallis test with Dunn's multiple comparisons test (P<0.05).. ns, not significant.

(E) BMMs and HEK293T cells were infected with *B. abortus* 2308::mTn*7*-*DsRed*$_m$, processed at various times post infection for immunostaining of LAMP1, and LAMP1-positive BCVs were scored by fluorescence microscopy. Data are means ± SD from 3 independent experiments.

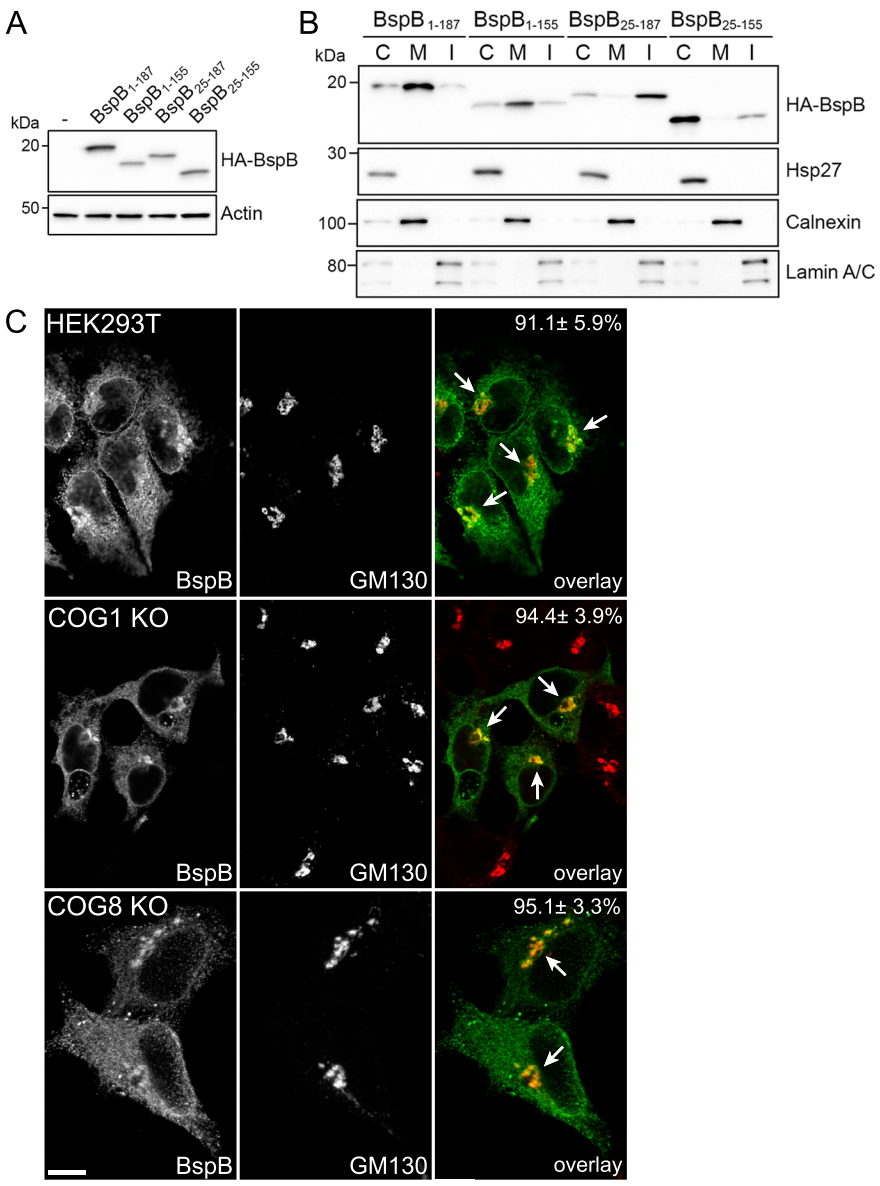

Figure S3. BspB subcellular fractionation and Golgi targeting in wild type, COG1 and COG8 KO HEK293T cells. Related to Figure 4

(A) Representative Western blot analysis of HA-BspB truncations production in HeLa cells after 18h of transfection. (-) refers to cells transfected with empty vector pCMV-HA. β-actin was used as a loading control.

(B) Detergent-based subcellular fractionation of HeLa cells expressing either HA-BspB$_{1-187}$, HA-BspB$_{1-155}$, HA-BspB$_{25-187}$, or HA-BspB$_{25-155}$ into saponin-soluble cytosolic (C),

Triton X-100-soluble membrane (M) and insoluble (I) fractions. Western blots were probed with either anti-BspB, anti-Hsp27 (cytosolic marker), anti-calnexin (membrane marker) and anti-Lamin A/C (nuclear insoluble marker) antibodies.

(C) Representative confocal micrographs of wild type, COG1 and COG8 KO HEK293T cells transfected with pCMV-HA-*bspB* for 24 h and immunostained for GM130 (red) and HA-BspB (green). Arrows indicate Golgi targeting of BspB in all transfected cells. Numbers indicate the percentage of cells where BspB localized to GM130-positive structures (mean ± SD). Scale bar, 10 μm.

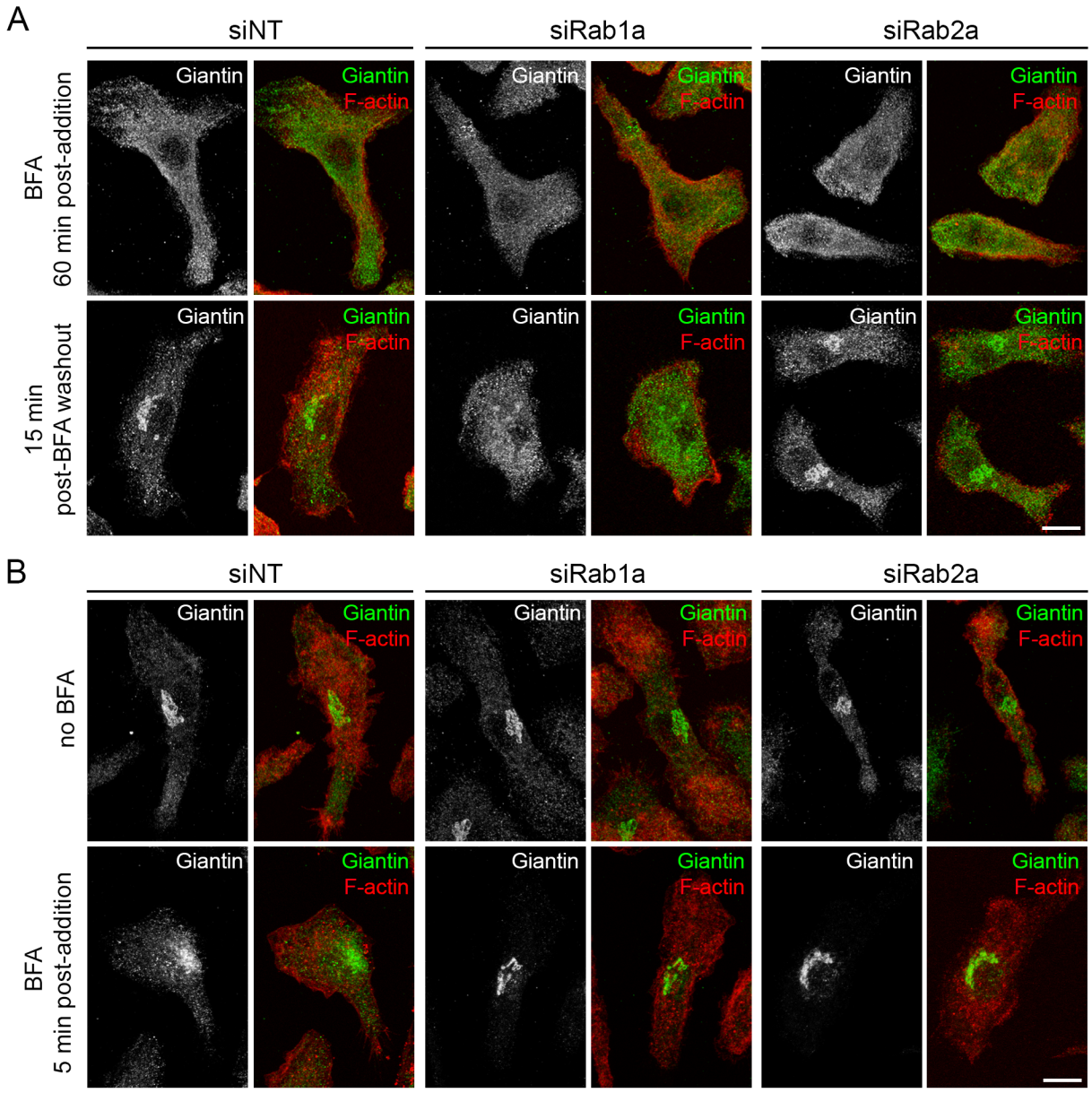

Figure S4. Effects of Rab1a and Rab2a siRNA-mediated depletions on anterograde and retrograde secretory transport in BMMs. Related to Figure 5.

(A) BMMs were either treated with siNT, siRab1a or siRab2a siRNAs for 72 h, then treated for 60 min with 5 µg/ml BFA to induce redistribution to the ER of the Golgi protein Giantin (upper panels). BFA was washed out and BMMs were processed after 15 min for Giantin immuno-staining (lower panels). F-actin staining using phalloidin was

used to delineate cell shapes. Rab1a depletion blocked anterograde transport of Giantin back to Golgi membranes after BFA washout. Scale bars, 10 μm

(B) BMMs were either treated with siNT, siRab1a or siRab2a siRNAs for 72 h (upper panels), then treated with 5 μg/ml BFA for 5 min to induce redistribution to the ER of the Golgi protein Giantin via retrograde transport. BMMs were processed for Giantin immuno-staining (lower panels). F-actin staining using phalloidin was used to delineate cell shapes. Rab1a and Rab2a depletions impaired retrograde transport of Giantin upon addition of BFA. Scale bars, 10 μm

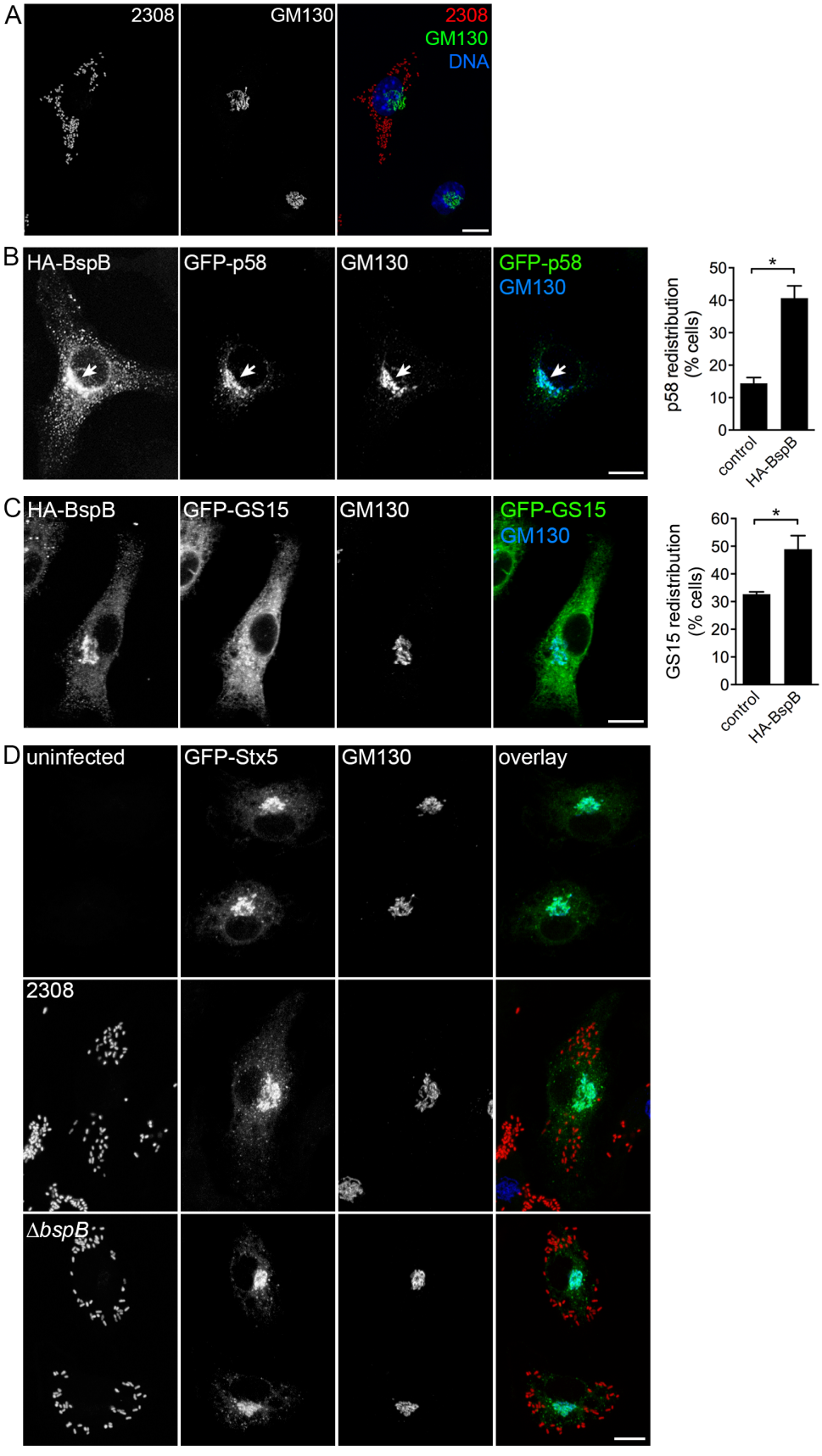
A
2308
GM130
2308
GM130
DNA
B
HA-BspB
GFP-p58
GM130
GFP-p58
GM130
p58 redistribution (% cells)
50
40
30
20
10
0
*
control
HA-BspB
C
HA-BspB
GFP-GS15
GM130
GFP-GS15
GM130
GS15 redistribution (% cells)
60
50
40
30
20
10
0
*
control
HA-BspB
D
uninfected
GFP-Stx5
GM130
overlay
2308
ΔbspB

Figure S5. Effect of *Brucella* infections or ectopically expressed BspB on the cellular distribution of the ERGIC and the Golgi SNAREs GS15 and Syntaxin5 in BMMs. Related to Figure 6

(A) Representative confocal micrographs of BMMs infected with wild type *B. abortus* 2308::mTn*7*-*DsRed*$_m$ (red) for 24 h, and immuno-stained for the Golgi marker GM130 (green). BMM DNA nuclei were labelled using Hoechst 33342 (Thermo Fisher Scientific). Scale bar, 10 µm

(B) Representative confocal micrographs of BMMs expressing GFP-p58 and HA-BspB via retroviral transduction for 24 h, and immuno-stained for the Golgi marker GM130. Scale bar, 10 µm. Quantification of GFP-p58 redistribution in BMMs expressing or not (control) HA-BspB. Data are means ± SD from 3 independent experiments. Asterisk indicates statistical significant differences compared to control BMMs, as determined by an unpaired two-tailed *t* test ($P < 0.05$).

(C) Representative confocal micrographs of BMMs expressing GFP-GS15 and HA-BspB via retroviral transduction for 24 h, and immuno-stained for the Golgi marker GM130. Scale bar, 10 µm. Quantification of GFP-GS15 redistribution in BMMs expressing or not (control) HA-BspB. Data are means ± SD from 3 independent experiments. Asterisk indicates statistical significant differences compared to control BMMs, as determined by an unpaired two-tailed *t* test ($P < 0.05$).

(D) Representative confocal micrographs of BMMs expressing GFP-GS15 via retroviral transduction for 24 h, left uninfected or infected with either *B. abortus* 2308 or Δ*bspB* bacteria for 24 h, and immuno-stained for the Golgi marker GM130. Scale bar, 10 µm.

Table S1: Oligonucleotides used in this study. Related to the STAR Methods section

Oligonucleotides		
RC603-miniTn7K-dsRed Forward: 5'-ATCATCCTCATCACCGACAA-3'	Myeni et al., 2013	N/A
RC604-miniTn7K-dsRed Reverse: 5'-GCTATATTCTGGCGAGCGAT-3'	Myeni et al., 2013	N/A
WSU0003-*bspB* Forward *Eco*RI: 5'-CCGGAATTCGGCGCCCCGTTCTTTTCCTG-3'	This paper	N/A
WSU0004-$bspB_{25}$ Forward *Eco*RI: 5'-CCGGAATTCGGCACTTCACCGGAAGCGAGATCG-3'	This paper	N/A
WSU0005-*bspB* Reverse *Xho*I: 5'-CCGACTCGAGTTATGTTTGGGGGCGGCGAAGG-3'	This paper	N/A
WSU0006-$bspB_{155}$ Reverse *Xho*I: 5'-CCGACTCGAGTTACGCACCGCGGCGCAGCACAAG-3'	This paper	N/A
WSU0203-*bspB* Tn7K *Eco*RI Forward: 5'-AGCTCGAATTCGGATTGCTTCGTCACCGATTTG-3'	This paper	N/A
WSU0204-*bspB* Tn7K *Kpn*I Reverse: 5'-AAGGTACCTTATGTTTGGGGGCGGCGAAG-3')	This paper	N/A
WSU0247-EGFP Forward *Eco*RI: 5'-TAGCGGAATTCGTCGCCACCATGGTGAGC-3'	This paper	N/A
WSU0249-EGFP Forward *Bam*HI: 5'-CGGGATCCGTCGCCACCATGGTGAGC-3'	This paper	N/A
WSU0255-Rab2a Reverse *Bam*HI: 5'-CGGGATCCCGTTGGGCAGCTAGACAG-3'	This paper	N/A
WSU0262-Mutagenesis Rab1a Q70L Forward: 5'-TGGGACACAGCAGGCCTGGAAAGATTTCGAACAA TCAC-3'	This paper	N/A
WSU0263-Mutagenesis Rab1a Q70L Reverse: 5'-GTGATTGTTCGAAATCTTTCCAGGCCTGCTGTGT CCCA-3'	This paper	N/A
WSU0265-Rab1a Reverse *Bam*HI: 5'-CGGGATCCTTAGCAGCAACCTCCACC-3'	This paper	N/A
WSU0298-Rab1a Forward *Sac*I: 5'-ACCGAGCTCGCGATATGTCCAGCATGAATCCCG-3'	This paper	N/A
WSU0301-*bspB* Reverse *Bam*HI stop: 5'-CGGGATCCTTATGTTTGGGGGCGGCGAAGG-3'	This paper	N/A
WSU0302-$bspB_{155}$ Reverse *Bam*HI stop: 5'-CGGGATCCTTACGCACCGCGGCGCAGCACAAG-3'	This paper	N/A
WSU0303-*bspB* Forward Kozak *Eco*RI: 5'-CCGGAATTCAGCATGGGACGCCCCGTTCTTTTCCT G-3'	This paper	N/A
WSU0304-$bspB_{25}$ Forward Kozak *Eco*RI: 5'-CCGGAATTCAGCATGGGACACTTCACCGGAAGCGA GATC-3'	This paper	N/A
WSU0305-Rab2a Forward *Xho*I: 5'-GGCCTCGAGCCATGGCGTACGCCTATCTC-3'	This paper	N/A
WSU0350-pEGFP N1 Reverse *Bam*HI: 5'-CGCGGATCCGCTTTACTTGTACAGCTCGTCCATG-3'	This paper	N/A
WSU0353-pEGFP C1 Reverse: 5'-TGATCAGTTATCTAGATCCGGTGG-3'	This paper	N/A
WSU0354-pEGFP C1 Forward *Cla*I: 5'-TAATATCGATGCCACCATGGTG-3'	This paper	N/A