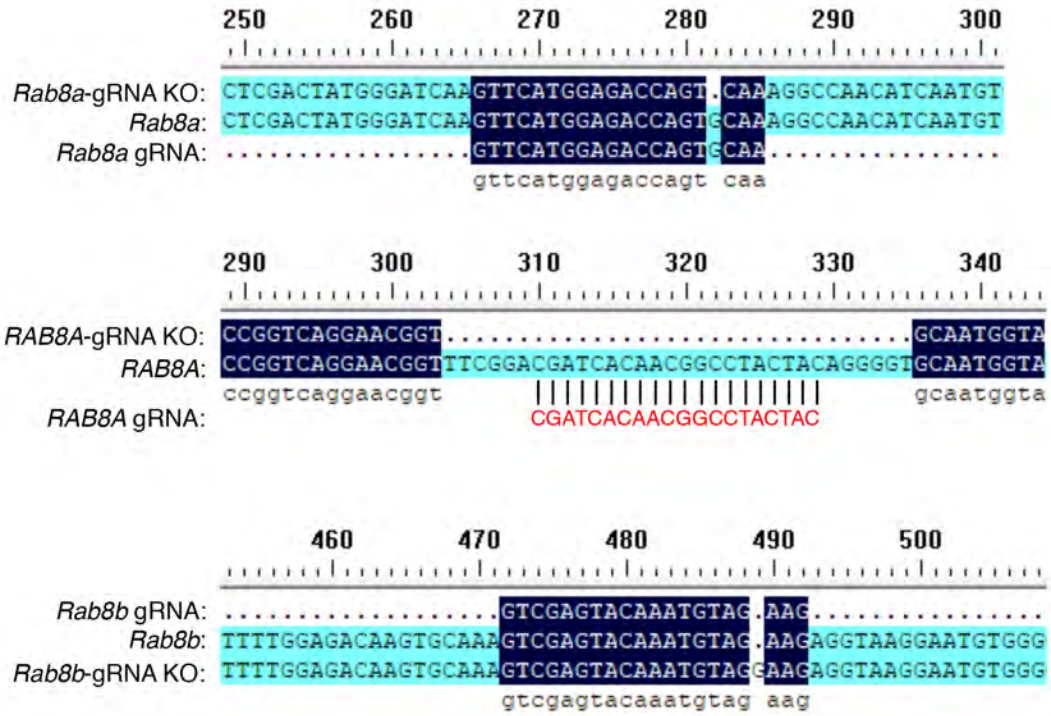
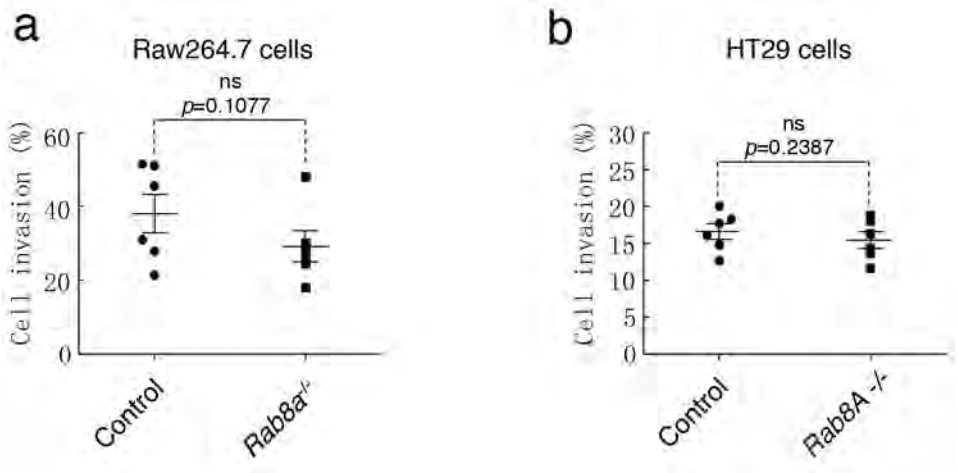
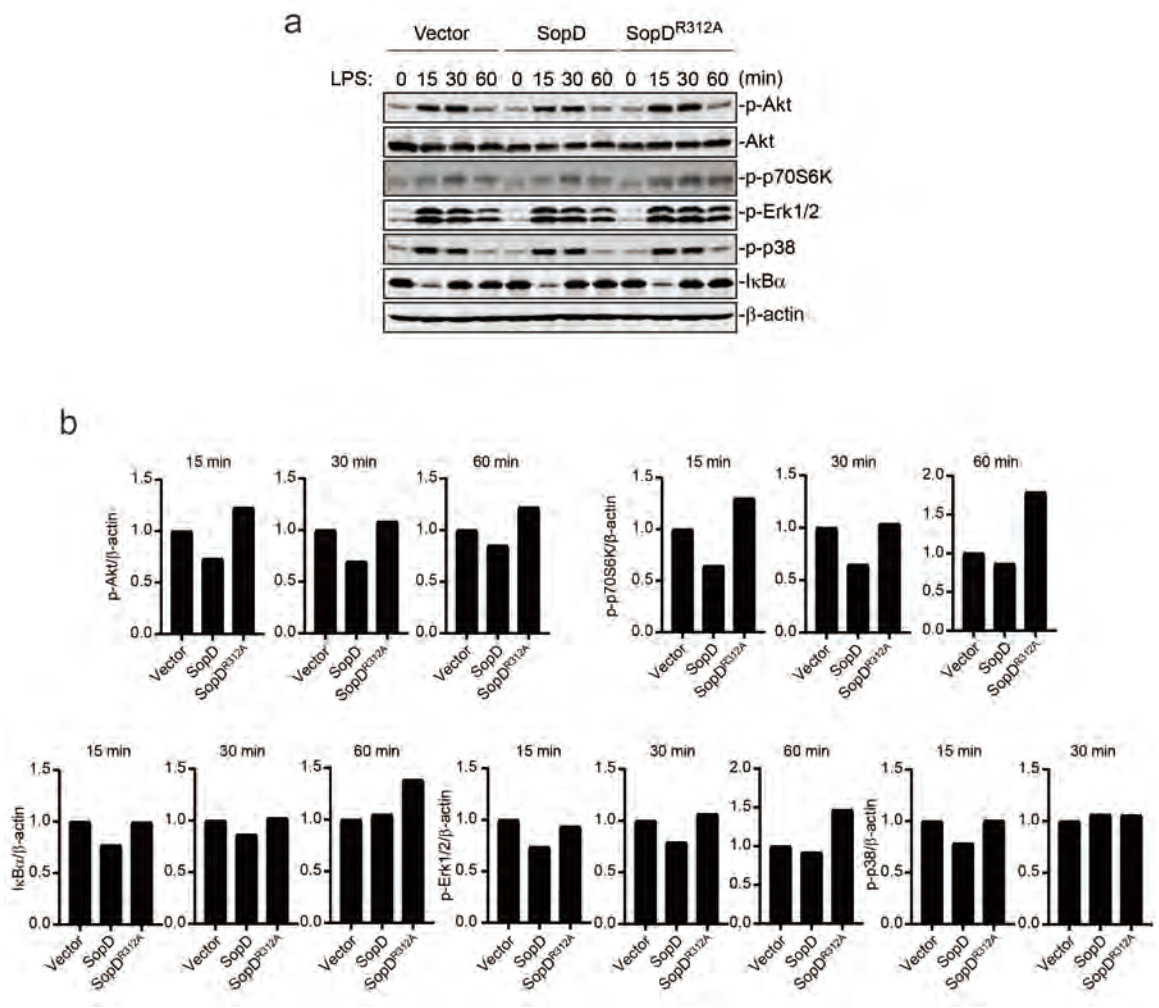
Supplementary Fig. 1. Sequence alignment of SopD and SopD2. The key GAP catalytic residue for their GAP activity, Arg315 in SopD2 and Arg312 in SopD, are denoted in red. A residue in SopD (Glu293), which is essential for its ability to form a complex with Rab8, and the residue in the equivalent position in SopD2, are denoted in orange. Identical (*) and similar (:) residues are also indicated.



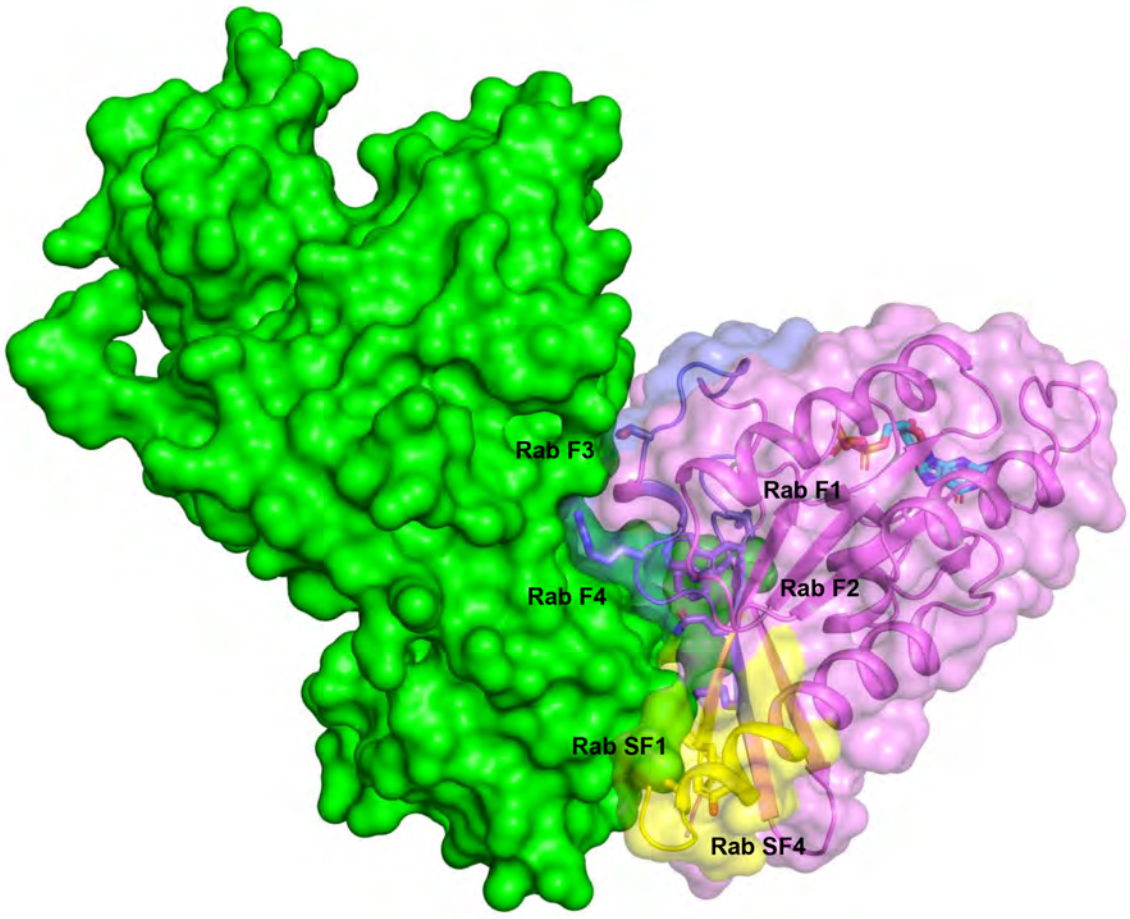
Supplementary Fig. 2. Nucleotide sequence of the CRISPR/Cas9-generated Rab8a- and Rab8b-deficient cells. The relevant sequences of the wild type and inactivated loci of the indicated genes are shown. Genomic DNA was obtained from the indicated cell lines and analyzed by PCR with specific primers as indicated in Methods. The light blue and blue represent the genomic DNA sequence of Rab8a, RAB8A and Rab8b wildtype and knockout cells. Blue also represents the gRNA sequence to target Rab8a and Rab8b. Red represents the gRNA sequence to target RAB8A



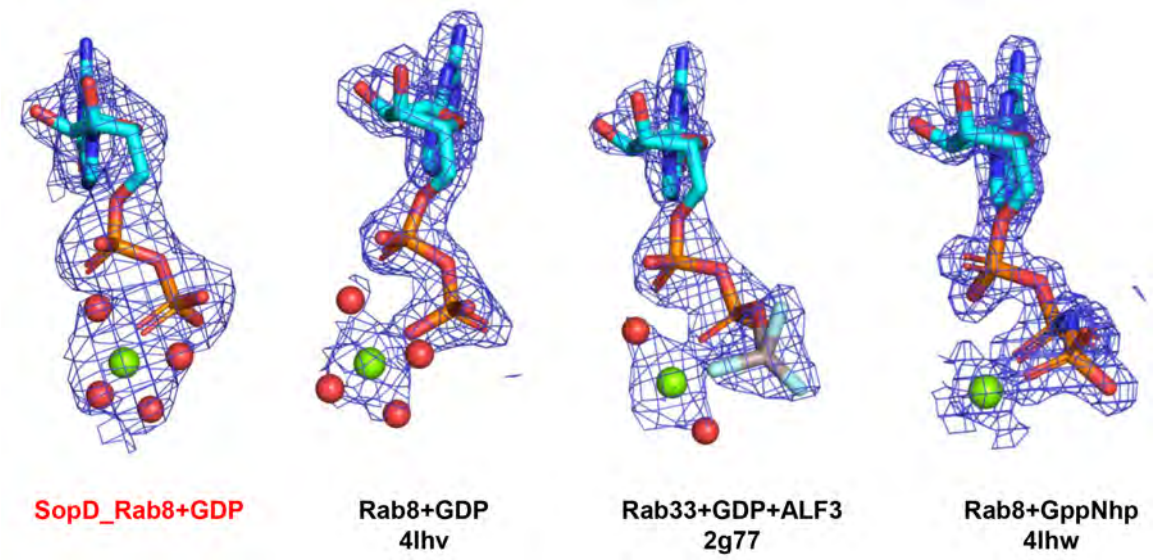
Supplementary Fig. 3. *S.* Typhimurium invasion of Rab8a-deficient cells. Raw264.7 (MOI=5) (**a**), HT29 (MOI=20) (**b**), and their Rab8a-deficient derivative cells (**a** and **b**) were infected with wild-type *S*. Typhimurium and the levels of internalized bacteria were determined by the gentamicin protection assay. Data represent the percentage of inoculum (mean ± SD) that survived the gentamicin treatment due to bacterial internalization. n.s.: differences not statistically significant (unpaired two-sided *t* test). Each circle or square represents the data of a single independent invasion assay conducted as indicated in Materials and Methods [in all cases, each invasion assay was carried out 6 times (n=6)].



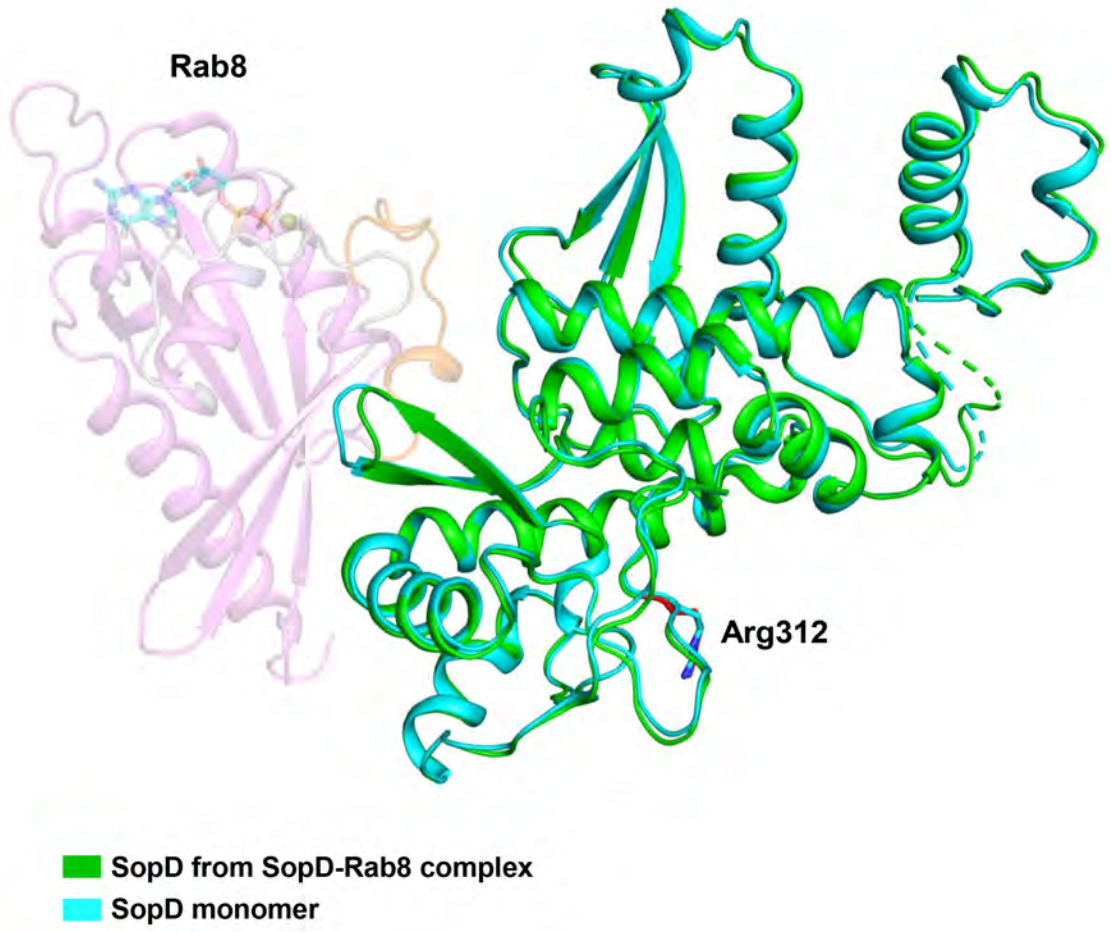
Supplementary Fig. 4. Effect of the expression of SopD or its catalytic mutant SopD^{R312A} on LPS-induced Akt, p70S6K, Erk1/2, p38 MAP, and NF-κB signaling. (**a** and **b**). Raw264.7 cells stably expressing HA-tagged SopD or its GAP-deficient mutant SopD^{R312A} were treated with LPS (100 ng/ml) for the indicated times, lysed, and analyzed by immunoblotting with antibodies specific for the phosphorylated state of Akt, p70S6K, p38, and Erk1/2, as well as an antibody to I-κBα and β-actin (as a loading control). The quantification of the intensity of the western blot bands is shown. The quantification of the Western blots is shown in (**b**). This experiment is an independent repetition of the experiment shown in Fig. 2e.



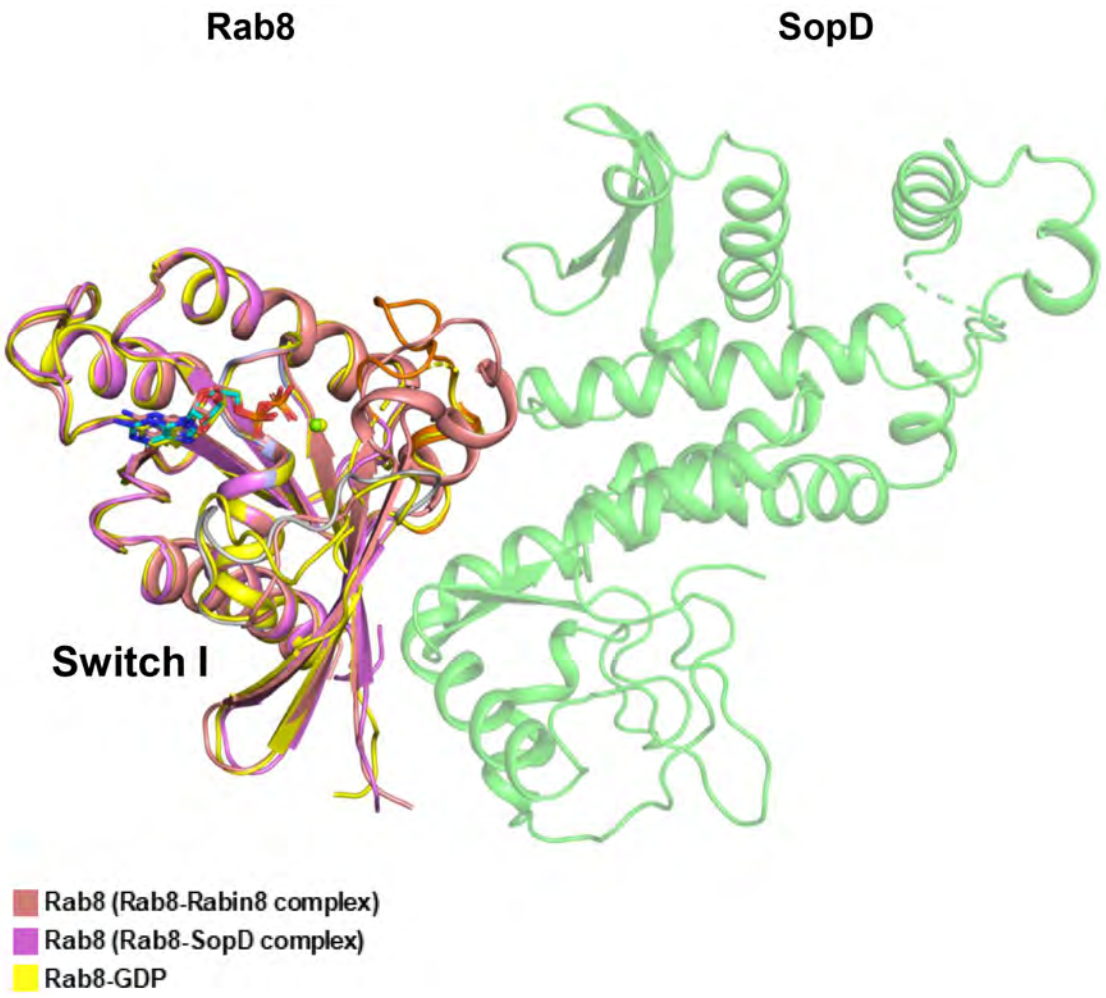
Supplementary Fig. 5. Surface representation of the overall structure of the SopD/Rab8 complex. The position of the structural motif of Rab8 involved in its interaction with SopD as well as the location of the RabF (blue) and RabSF (yellow) motifs of Rab8 are indicated. The amino acid residues from Rab8 RabF and RabSF motifs involved in the interaction with SopD are shown as sticks.



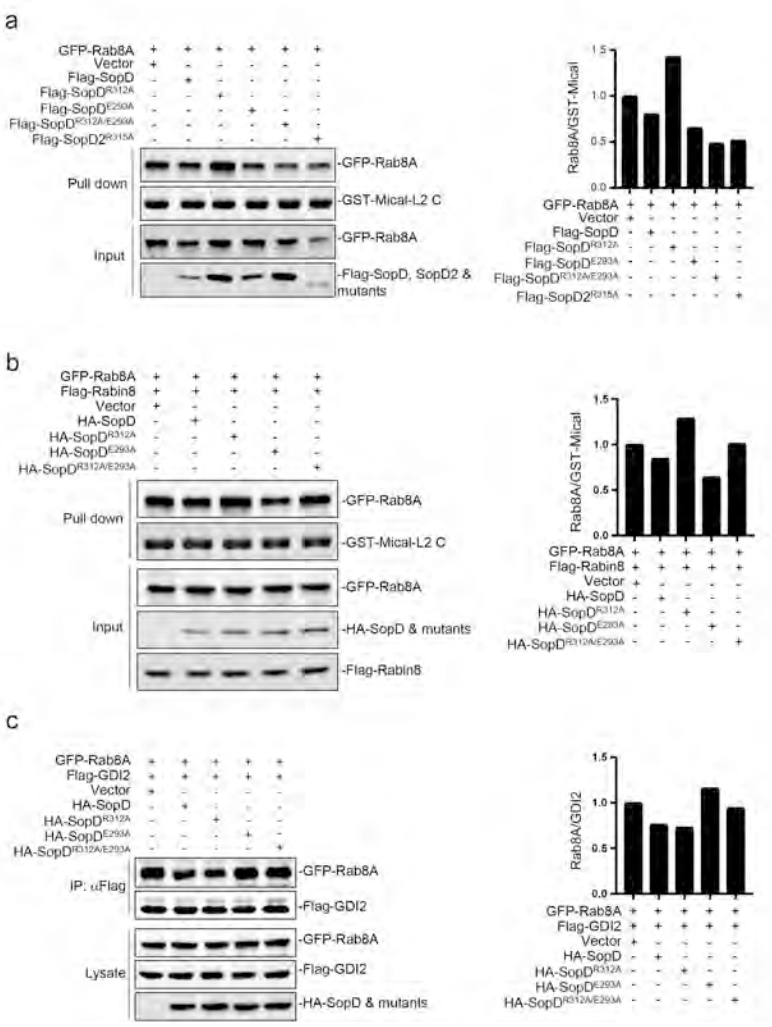
Supplementary Fig. 6. Simulated annealing omit maps for different nucleotides bound to different Rab GTPases. Nucleotides are shown in stick model. Carbon atoms are colored in cyan, oxygen in red, nitrogen in blue, phosphorus in orange, aluminum in light gray, and fluorine in light blue, aluminum trifluoride is shown in stick, water as a red sphere and magnesium as a green sphere. Blue mesh represents the difference density map of the molecules contoured at 1.5 σ.



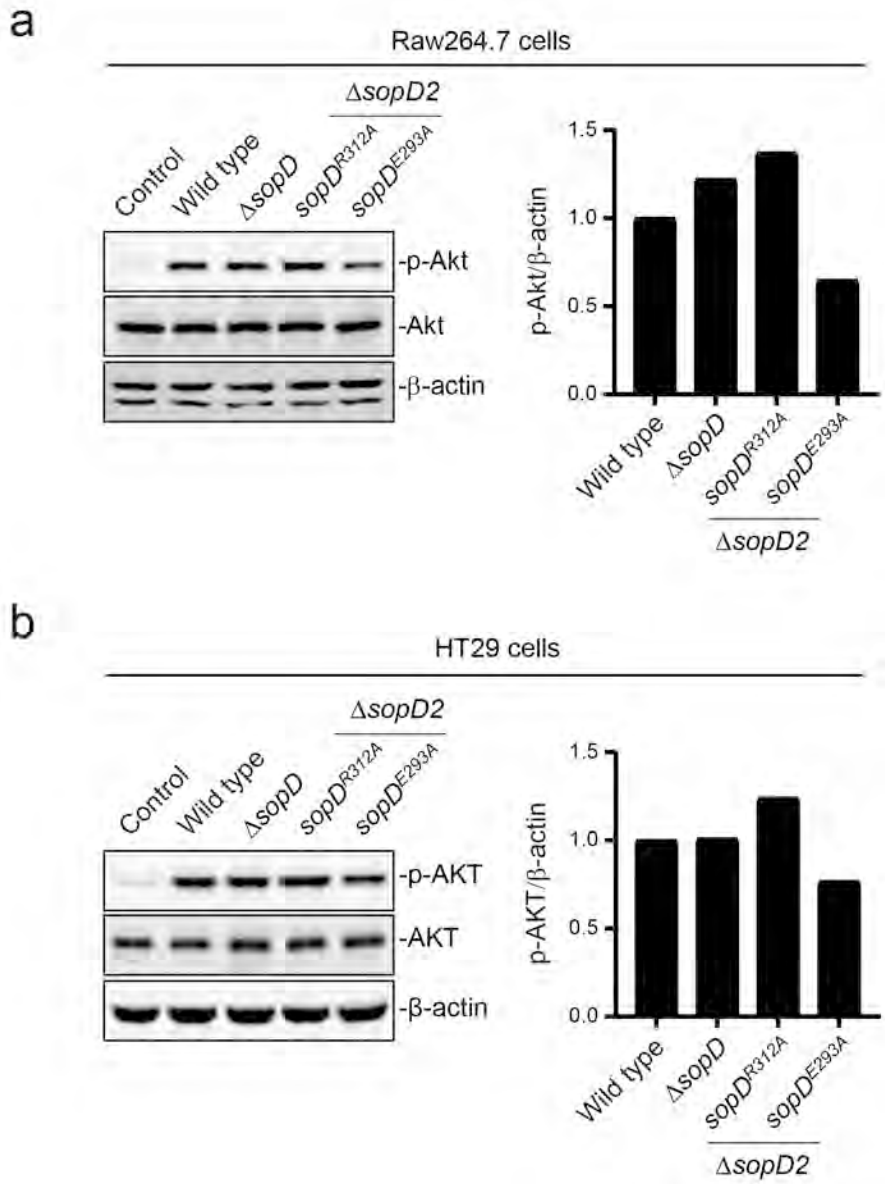
Supplementary Fig. 7. Structural alignment between free SopD and SopD bound to Rab8. The structure superimposition of the SopD monomer (cyan, RCSB ID 5CPC) and SopD extracted from the SopD/Rab8a complex (green) is shown as a ribbon. The GAP catalytic residue (Arg312) is shown as stick.



Supplementary Fig. 8. Structure superimposition of Rab8 as it appears in the Rab8/SopD complex, with Rab8 as it appears in the Rab8/Rabin8 complex or bound to GDP. The switch I region of Rab8 from Rab8-SopD complex is colored in gray.



Supplementary Fig. 9. SopD activates Rab8 through a functional interface unrelated to its GAP activity. (**a** and **b**) Effect of the transient expression of SopD^{R312A} (a GAP-defective mutant) or SopD^{E293A} (a mutant unable to form a complex with Rab8) on the levels of Rab8 activation. HEK-293T cells were transiently co-transfected with plasmids expressing wild-type SopD or the indicated mutants along with plasmids expressing GFP-tagged Rab8A (**a**) and, when indicated (**b**), Flag-tagged Rabin8, an exchange factor for Rab8a. Twenty hours after transfection, cells were lysed and the levels of GTP-loaded Rab8A were determined with the GST-Mical-L2 affinity probe as described in Methods. The quantifications of the intensity of the western blot bands is show. This experiment is an independent repetition of the experiment shown in Fig. 4a and 4b). (**c**) HEK-293T cells were transiently co-transfected with plasmids expressing GFP-tagged Rab8A and Flag-tagged GDI2, along with plasmids expressing the indicated HA-tagged SopD constructs. Twenty hours after transfection, cells were lysed and the levels of Rab8a/GDI2 complex were determined by immunoprecipitation with an anti-Flag antibody (directed to the tag in GDI2) and immunoblotting with an anti-GFP antibody (directed to the tag in Rab8A). The quantification of the intensity of the Western blot bands is shown. The quantification of the Western blots is shown in the bar graphs. This experiment is an independent repetition of the experiments shown in Fig. 4a, 4b, and 4e.



Supplementary Fig. 10. SopD positively and negatively modulates *S.* Typhimurium-induced inflammatory signaling through its independent Rab8-modulating activities. Akt activation in cells infected with *S.* Typhimurium strains expressing different *sopD* mutants. Raw264.7 (MOI=2) or HT29 (MOI=10) cells were infected with wild-type *S.* Typhimurium, the Δ*sopD* isogenic mutant, or the Δ*sopD2* mutant expressing the indicated mutant alleles of *sopD* for 60 minutes and Akt activation was analyzed by immunoblotting with antibodies specific for the phosphorylated state of Akt and β-actin (as a loading control). The quantification of the intensity of the bands of the western blots is shown in the bar graphs. This experiment is an independent repetition of the experiment shown in Fig. 6a.

Supplementary Table 1: List of bacterial strains used in this study

Strain	Relevant genotype	Reference
SB300	Mouse-passed *S*. Typhimurium SL1344	PMID7015147
SB0923	*ΔsopB*	PMID11136447
SB3334	*ΔsopD*	This study
SB3339	*sopDR312A ΔsopD2*	This study
SB3896	*sopDE293A ΔsopD2*	This study

Supplementary Table 2: Data Collection and Refinement Statistics

Data Collection	
Space group	P 31 2 1
Cell dimensions	
a,b,c (Å)	114.66 , 114.66 , 107.07
α,β,γ(°)	90 , 90 , 120
Resolution (Å)	47.12-2.301 (2.383-2.301)
Rmerge	0.063(1.604)
Mean I/σ(I)	19.32(1.24)
Completeness (%)	99.0(92.0)
Redundancy	9.0(4.86)
$CC_{1/2}$	0.999(0.566)
Refinement	
Resolution (Å)	47.12-2.301 (2.383-2.301)
Number of reflections	36,321
R_{work}/R_{free}	0.188/0.215
Number of Atoms	
Protein	3,715
Ligand	97
Solvent	120
Average B Factor (Å^2)	
Wilson	61.0
Protein	85.28
Ligand/ion	100.63
Solvent	78.82
RMSDs	
Bond lengths (Å)	0.009
Bond angles (°)	0.69
Ramachandran Plot Statistics (%)	
Favored regions	96.71
Allowed regions	3.29
Disallowed regions	0.00

*Numbers in parenthesis represent values in the highest resolution shell.

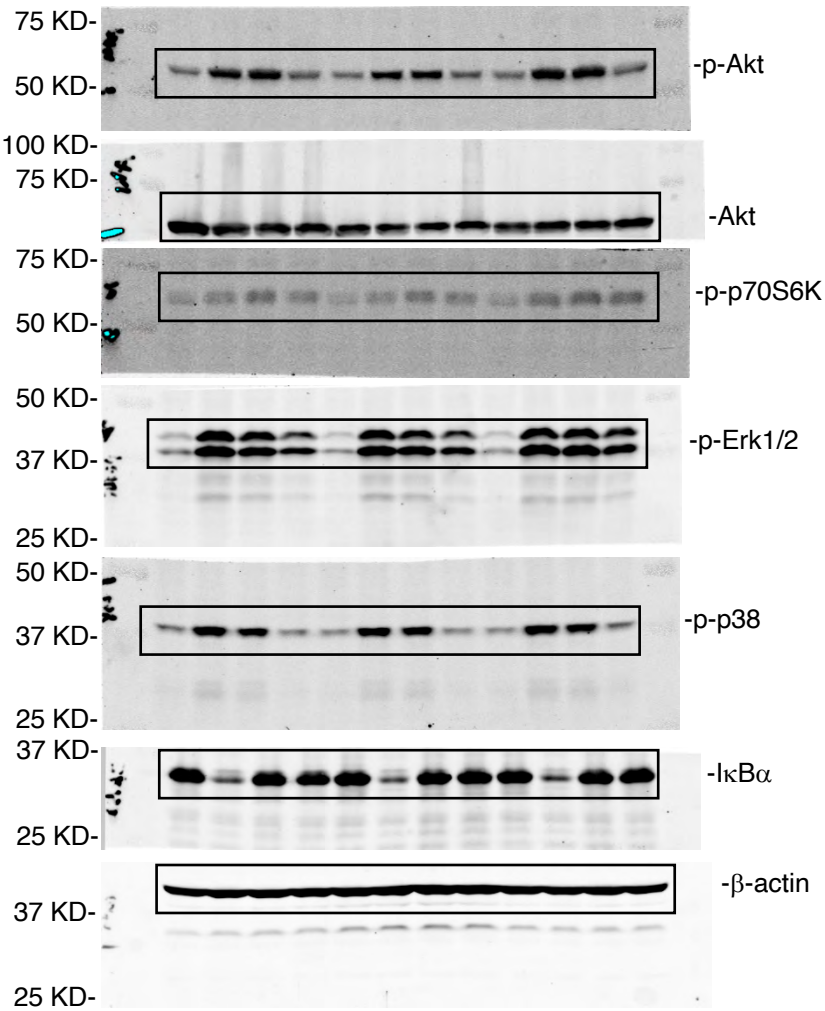
Supplementary Table 3: Primers used in the construction and screening of CRISPR/Cas9 genome edited cell lines

Primer Name	Nucleotide Sequence
sg-Rab8a	5’-GTTCATGGAGACCAGTGCAA-3’
sg-Rab8b	5’-GTCGAGTACAAATGTAGAAG-3’
sg-Rab8A	5’-CGATCACAACGGCCTACTAC-3’
Rab8a-identification-Fw	5’-CTTAGGCTATGCCACATTCCTC-3’
Rab8a-identification-Rv	5’-TGGGTGTAGGTAGTGGGTACTAGG-3’
Rab8b-identification-Fw	5’-ATGAGGGTGGTTACCTTGGAGAGC-3’
Rab8b-identification-Rv	5’-GCAGGGTGATGACAAATGACAGAG-3’
Rab8A-identification-Fw	5’-AGGGAGAATTCCCTGGAAGCAAAG-3’
Rab8A-identification-Rv	5’-TTAAACCTTGCATTGTCTCTGC-3’

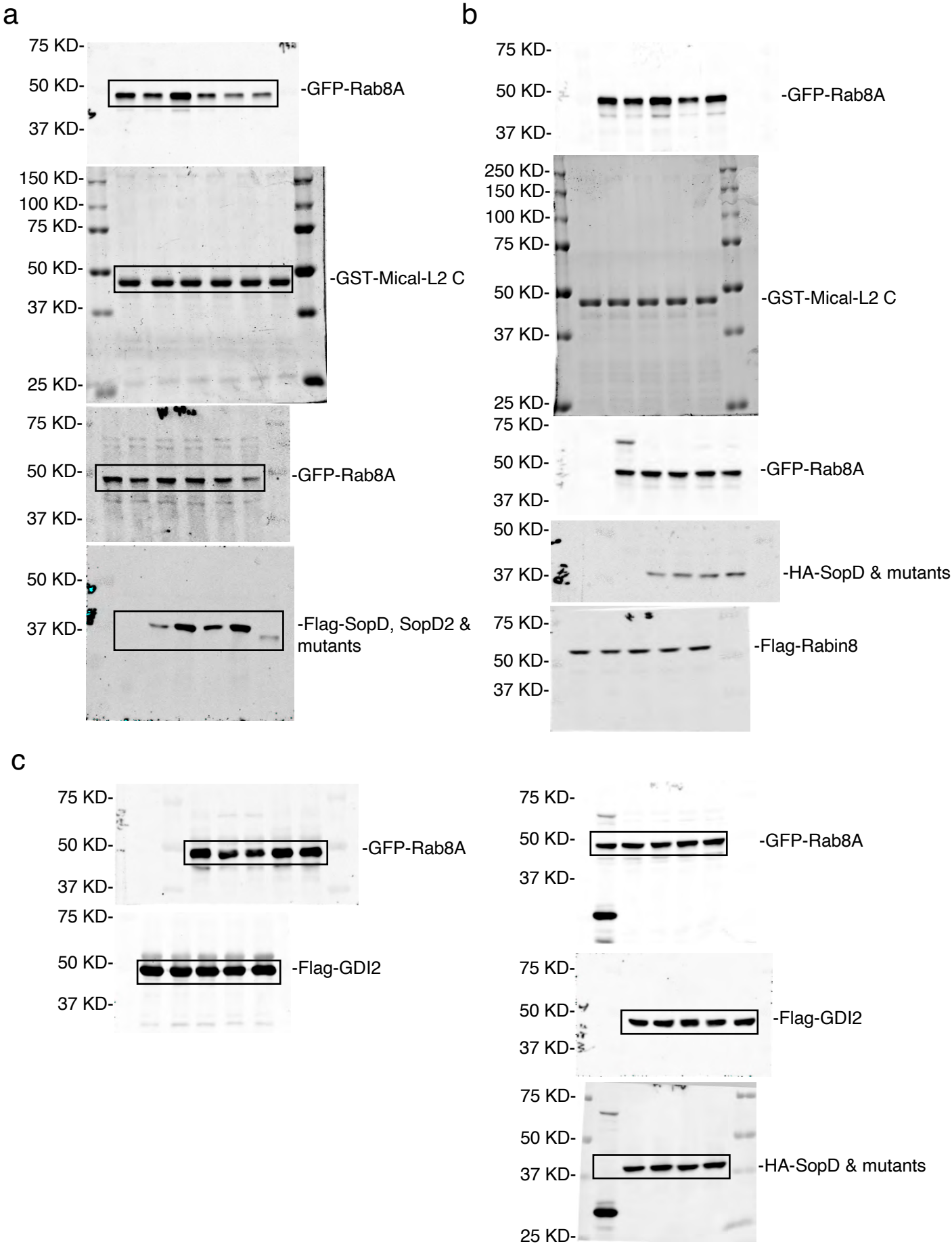
Supplementary Table 4: Specific sequences of primers used in the RT-PCR experiments

Gene	Forward	Reverse
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG
IL10	GGCACCCAGTCTGAGAACAG	ACTCTGCTGAAGGCATCTCG
IL-1β	GCAGGCCGCGTCAGTTGTTG	CCCGGAGCGTGCAGTTCAGT
TNFα	GCCGCATCGCCGTCTCCTAC	CCTCAGCCCCCTCTGGGGTC
Gapdh	ACGGCCGCATCTTCTTGTGCA	ACGGCCAAATCCGTTCACACC
Il10	CGGGAAGACAATAACTGCACCC	CGGTTAGCAGTATGTTGTCCAGC
Il-1β	AAAGCCTCGTGCTGTCGGACC	CAGGGTGGGTGTGCCGTCTT
Tnfα	GGTGATCGGTCCCCAAAGGGATGA	TGGTTTGCTACGACGTGGGCT

Source Data Supplementary Fig. 4. Uncropped blots corresponding to Supplementary Fig. 4a.



Source Data Supplementary Fig. 9. Uncropped blots corresponding to Supplementary Fig. 9a, 9b and 9c.



Source Data Supplementary Fig. 10. Uncropped blots corresponding to Supplementary Fig. 10a and 10b.