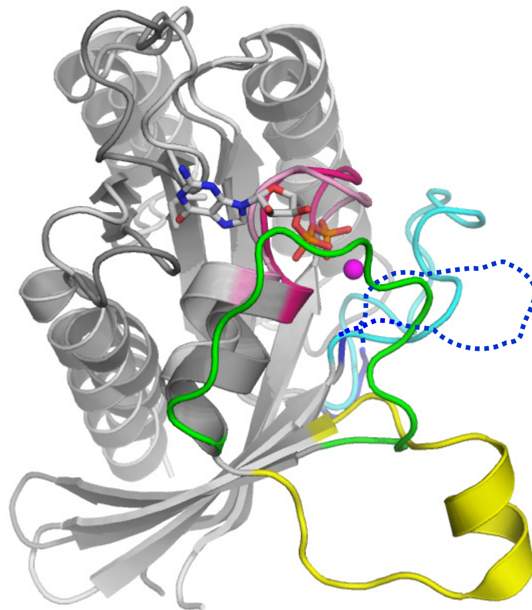
Supplementary Table 1. Data collection, phasing, and structure refinement statistics.

	SidMcd−Rab1(1-175;N121I)	
	Native	Se-Met
Data Collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions,		
a, b, c (Å)	68.8, 73.8, 73.6	69.0, 73.7, 73.7
Wavelength (Å)	1.0000	0.97945 (*peak*)
Resolution (Å)	30 – 1.5	30 – 1.87
R_{sym}[a]	6.1 (12.4)[b]	14.6 (40.9)
$I/\sigma(I)$	59.7 (13.9)	16.3 (2.9)
Completeness (%, > 0 σ)	98.3 (96.7)	95.8 (79.5)
Redundancy	10.6	7.7
Phasing		
Phasing power[c]		0.68 / 0.82
R_{Cullis} (iso)[d], centric / acentric		0.81 / 0.89
R_{Cullis} (ano)[e]		0.84
Figure of merit[f], centric / acentric		0.42 / 0.35
Refinement		
Resolution (Å)	30 – 1.5	
No. of reflections (> 0 σ)	59667	
R_{work}[g] / R_{free}	22.12 / 22.81	
No. atoms, proteins / water	2921 / 117	
R.m.s.d., bond lengths (Å) / angles (°)	0.00501 / 1.17206	
Average B-factor (Å^2),		
Rab1(1-175;N121I)	18.00	
SidMcd	14.41	
Ramachandran plot (%),		
most favoured region	92.9	
additional allowed region	7.1	

[a]$R_{sym} = \Sigma |I_{obs} - I_{avg}| / I_{obs}$, where I_{obs} is the observed intensity of individual reflection and I_{avg} is average over symmetry equivalents. [b]The numbers in parentheses are statistics from the highest resolution shell. [c]Phasing power is the mean amplitude calculated from the heavy-atom model divided by the lack of cloure error. [d]R_{cullis} (iso) is the mean residual lack of closure error divided by the isomorphous difference. [e]R_{cullis} (ano) is the mean residual lack of closure error divided by the anomalous difference. [f]Figure of merit = $\langle| \Sigma P(\alpha) e^{i\alpha} / \Sigma P(\alpha) |\rangle$, where α is the phase and P(α) is the phase probability distribution. [g]$R_{work} = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively. R_{free} was calculated with 5% of the data.

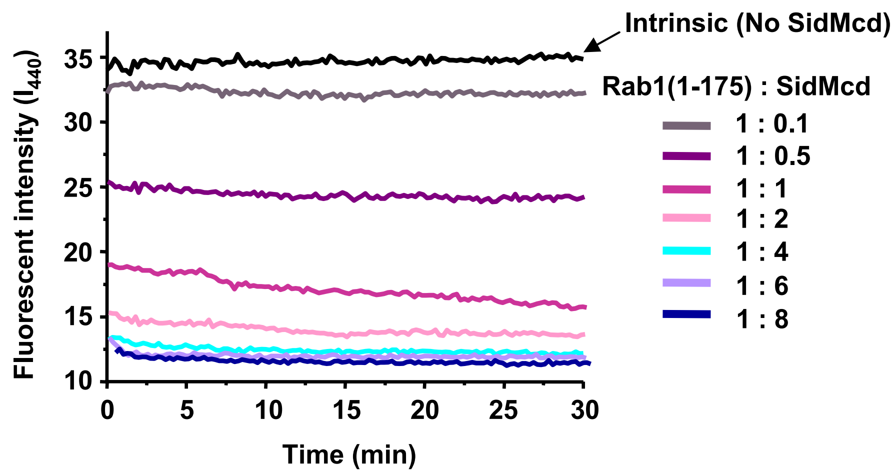


Rab1	Switch I	Switch II	P-loop	Others
GDP-bound				
SidMcd-bound				

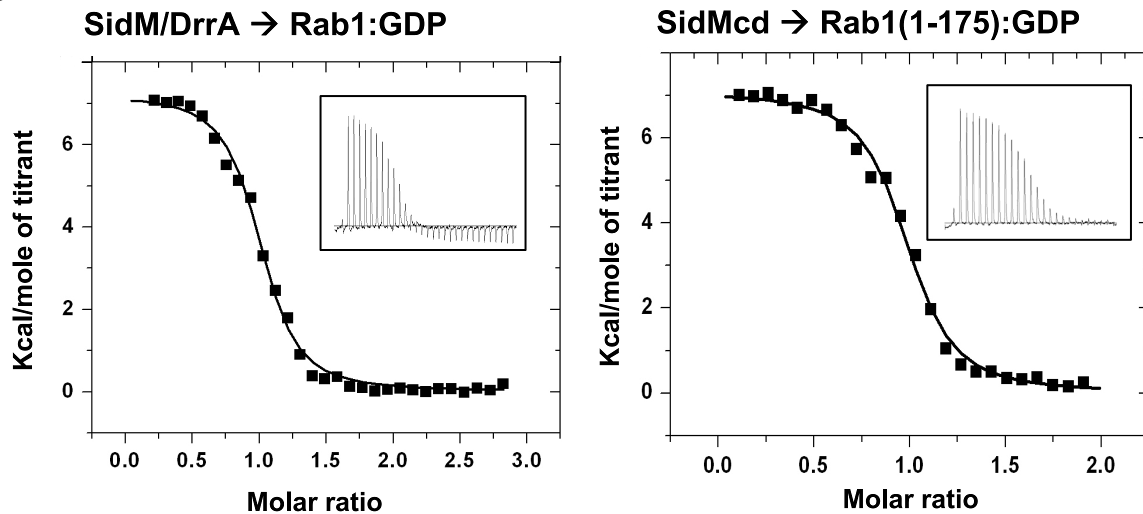
Supplementary Figure 1. Conformational changes of Switch I, Switch II and P-loop

The structures of isolated Rab1:GDP (PDB code: 2FOL) and Rab1 in complex with SidMcd are superposed. The orientation of the SidMcd-bound Rab1 is the same as that shown in Figure 1A. These three loops are highlighted in different colors indicated in the table to contrast their conformations in the two structures. Bound Mg^{2+}-GDP is shown in ball-and-sticks. The dotted line represents a disordered region.

A



B



In the syringe	In the cell	K_D (nM)
SidM/DrrA	Rab1:GDP	84.0 ± 10.7
SidMcd	Rab1(1-175):GDP	78.8 ± 10.8

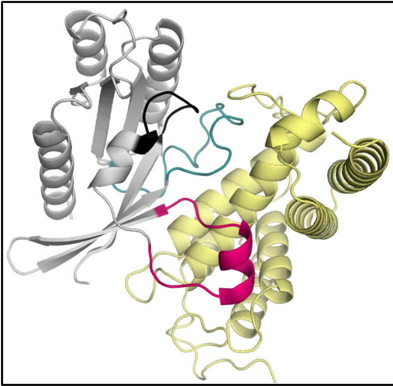
Supplementary Figure 2. Interaction between SidM/DrrA and Rab1

(A) SidMcd binding results in the eviction of GDP. Increasing amount of SidMcd was added to the Rab1:mant-GDP complex in a Tris-HCl buffer (pH 8.0) containing 150 mM NaCl and 0.5 mM MgCl2, and the fluorescence from bound mant-GDP was monitored at 440 nm. The fluorescence intensity decreased as the molar ratio of SidMcd to Rab1:mant-GDP increased. Although fluorescence was recorded immediately after mixing the two reactants, gradual decrease in the fluorescence intensity was not observed, indicating that the eviction of mant-GDP was completed within the dead time.

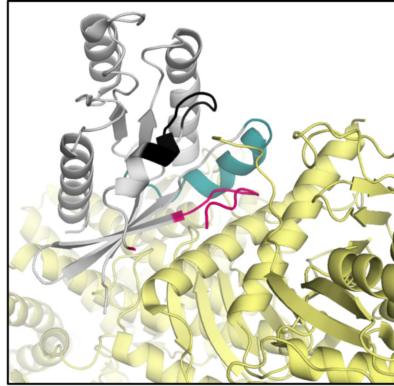
(B) Quantitative analysis. Two ITC runs are shown: titration of full-length SidM/DrrA into full-length, non-prenylated Rab1 (*left*) and titration of SidMcd into Rab1(1-175) (*right*). The both titrations were performed in the absence of added Mg^{2+} and GDP. The deduced K_D values, shown in the table, are similar to each other. Of note, the both interactions are endothermic.

A

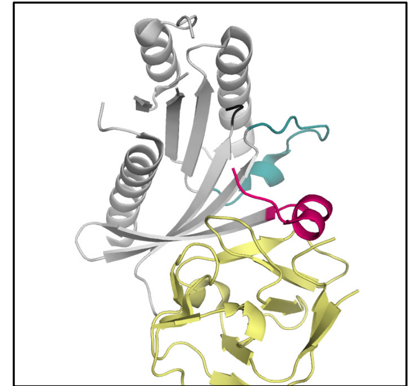
SidMcd-bound Rab1



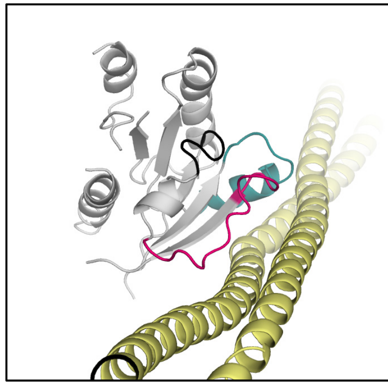
TRAPP I-bound Ypt1



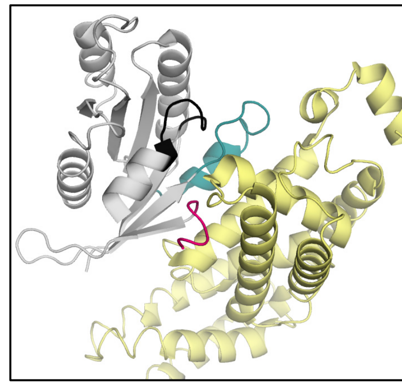
Mss4-bound Rab8



Sec2p-bound Sec4p

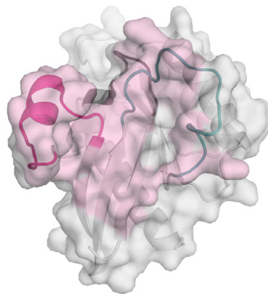


Rabex5-bound Rab21

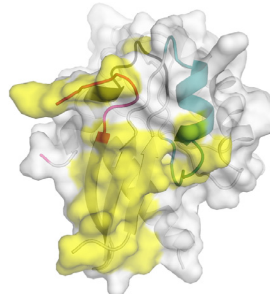


(Switch I Switch II P-loop)

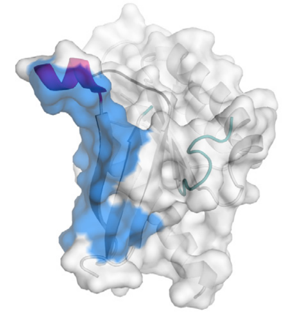
B



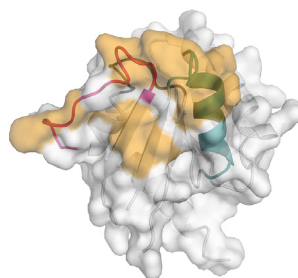
SidMcd-bound Rab1
(SidMcd-interacting residues)



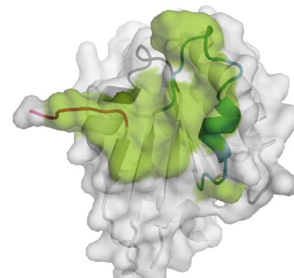
TRAPP I-bound Ypt1
(TRAPP I-interacting residues)



Mss4-bound Rab8
(Mss4-interacting residues)

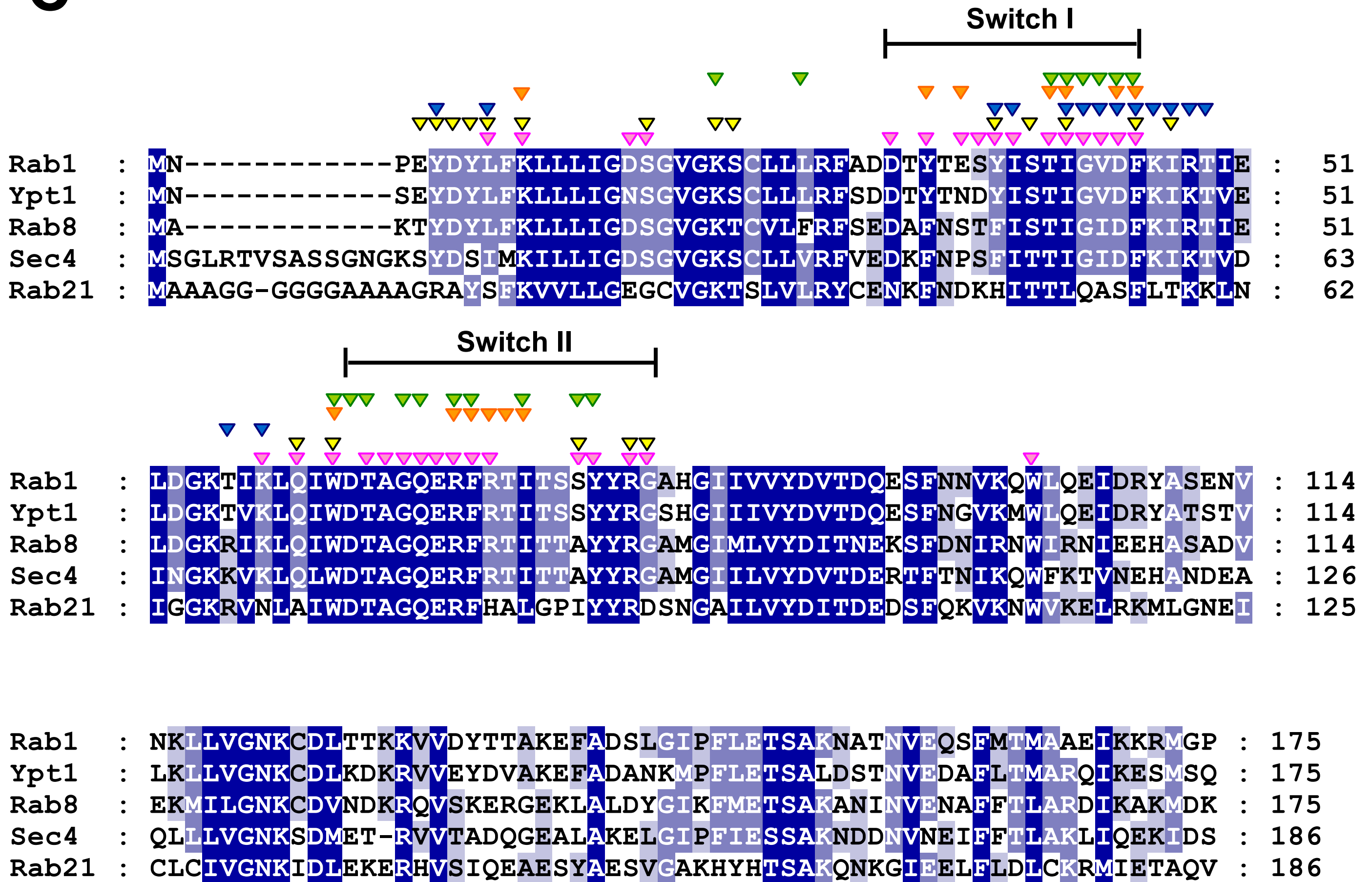


Sec2p-bound Sec4p
(Sec2p-interacting residues)



Rabex5-bound Rab21
(Rabex5-interacting residues)

C

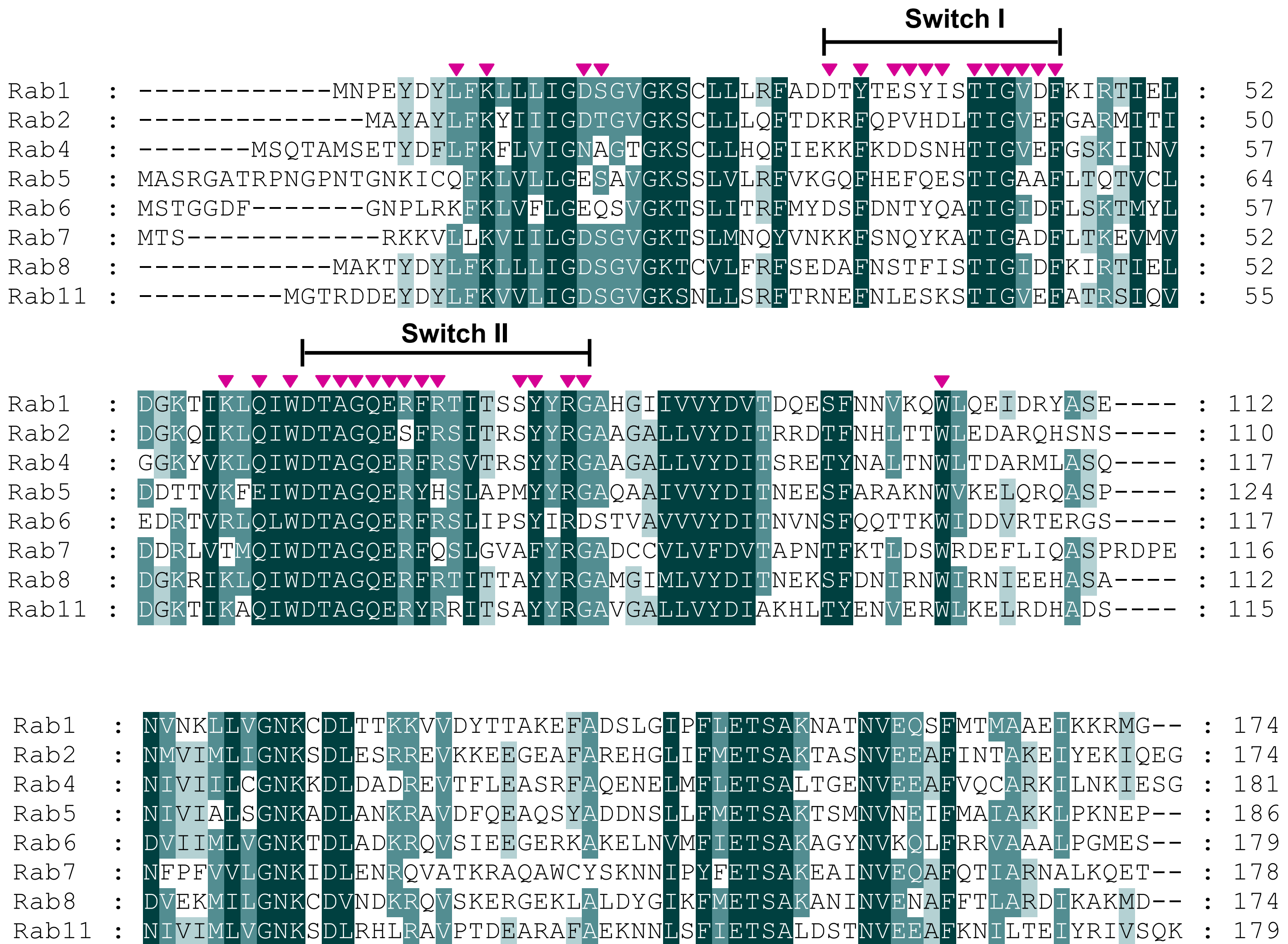


Supplementary Figure 3. Comparison of the five available structures of RabGEF-Rab

(A) Rabs (gray) bound to RabGEF (yellow) are shown in the same orientation. Switch I, Switch II and P-loop of Rabs are in red, cyan and black, respectively. The PDB codes are; TRAPP I–Ypt1 (3CUE), Mss4–Rab8 (2FU5), Sec2p–Sec4p (2OCY), and Rabex5–Rab21 (2OT3).

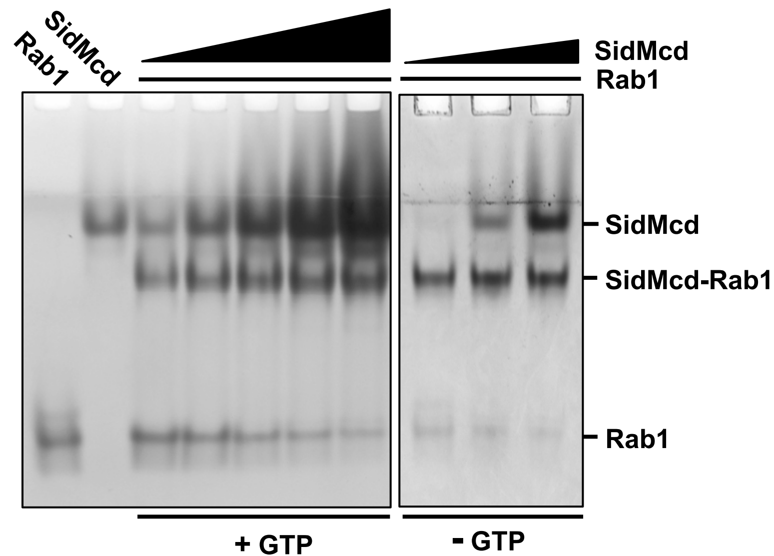
(B) Representation of the RabGEF-interacting residues. The residues of the Rabs involved in the intermolecular interaction (< 4.0 Å) with the RabGEFs were identified and mapped on the structures of Rabs shown in the same orientation. Switch I, Switch II and P-loop are indicated by the same colors used in **A**.

(C) The RabGEF-interacting residues are indicated by the triangles at the top of the sequence alignment of the five Rabs. The triangles are color-coded according to the coloring scheme used in **B**. The two switch regions of Rab1 are indicated by black bars. Switch I and II in Rab1 provides most of the residues involved in the intermolecular interaction (24 out of 32 residues).



Supplementary Figure 4. Sequence alignment of eight different Rabs

The SidMcd-interacting residues are indicated by triangles. Among these residues, those on the Switch I are least conserved. The two switch regions are indicated by black bars.



Supplementary Figure 5. Weak binding affinity between SidMcd and Rab1 in the presence of GTP

In the presence of 1 mM GTP, Rab1:GTP (10 μM) was incubated with SidMcd at 10, 20, 40, 160, and 320 μM for 20 min in a buffer solution containing 20 mM Tris-HCl (pH 7.5), 100 mM NaCl and 1 mM $MgCl_2$. On the native gel, free Rab1 is observable even at the highest molar ratio (*left*). In contrast, most of Rab1 was in complex with SidMcd at 10, 20, 40 μM in the absence of GTP (*right*).