

## Expanded View Figures

**Figure EV1. Mis18BP1 amino acid conservation and Mis18 complex formation with the well conserved Mis18BP1 N-terminal region (amino acids 20–130) *in vivo*.**

- A Alignments include *Homo sapiens* (*hs*), *Mus musculus* (*mm*), *Bos taurus* (*bt*), *Gallus gallus* (*gg*), and *Xenopus tropicalis* (*xt*). Black boxes indicate SANTA and SANT domains, respectively. Multiple sequence alignment (conservation score is mapped from red to cyan, where red corresponds to highly conserved and cyan to poorly conserved) was performed with MUSCLE (MUltiple Sequence Comparison by Log-Expectation, EMBL-EBI) [31] and edited with Aline (sequence alignment editor) [32].
- B Representative images (left) and quantification (right) for the recruitment of mCherry-Mis18BP1<sub>20–130</sub> and mCerulean-Mis18 $\beta$  to the alphoid<sup>tetO</sup> array in HeLa 3-8 cells expressing TetR-eYFP-Mis18 $\alpha_{wt}$ . Middle bars show median whilst error bars show SEM. Mann–Whitney test vs. TetR-eYFP;  $P \leq 0.0001$ ,  $n \geq 63$ . Scale bar, 5  $\mu$ m.

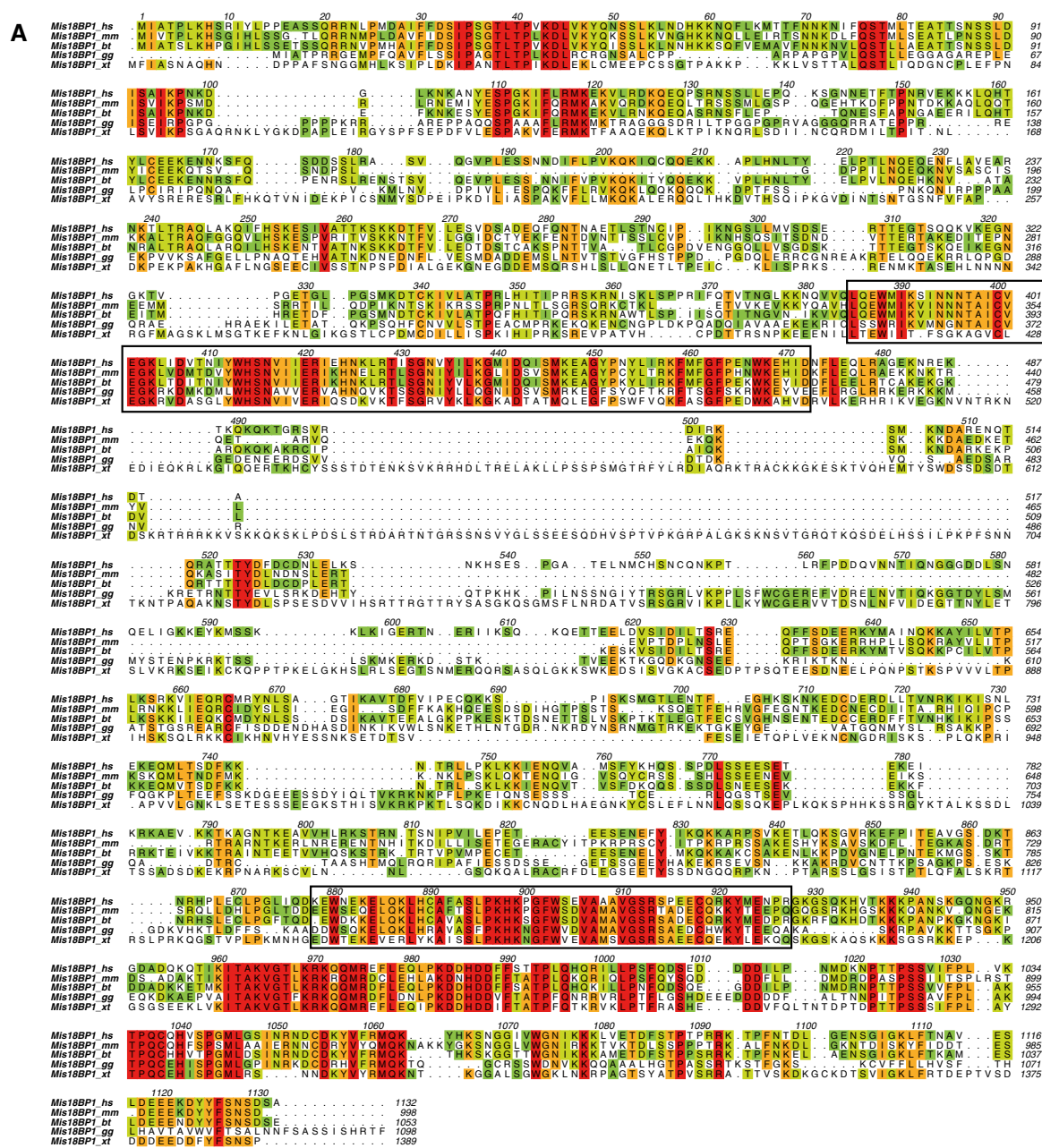
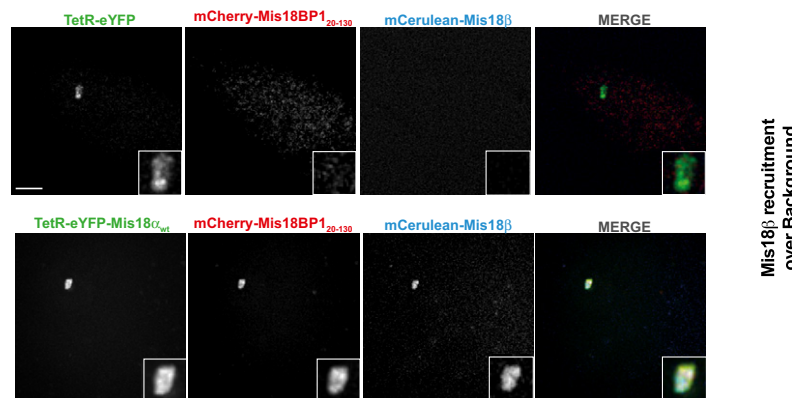
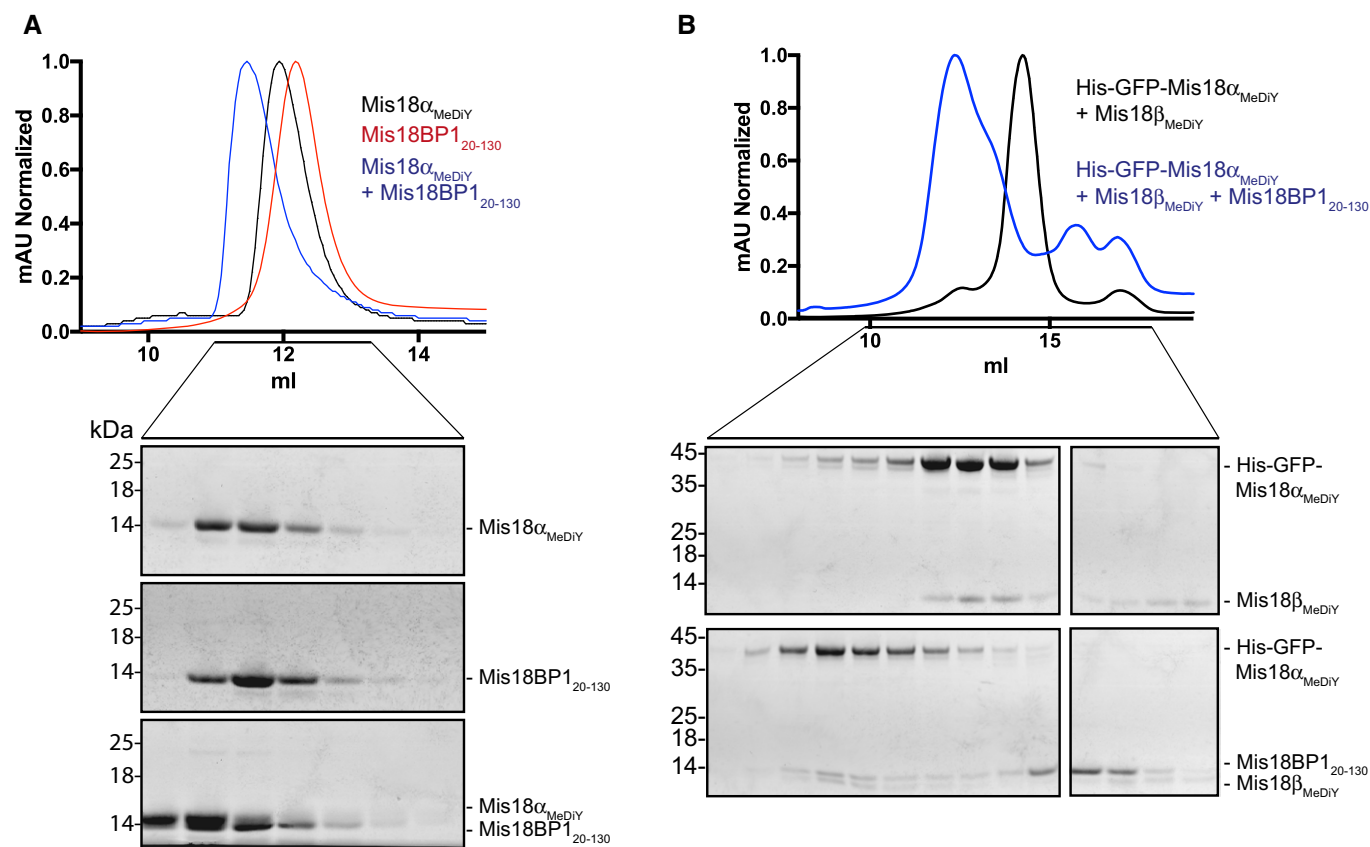
**B**

Figure EV1.



**Figure EV2. Mis18 $\alpha_{MeDIY}$  can directly interact with Mis18BP1 $_{20-130}$ .**

A, B SEC profiles and respective SDS-PAGE analyses of (A) Mis18 $\alpha_{MeDIY}$ , Mis18BP1 $_{20-130}$ , and Mis18 $\alpha_{MeDIY}$  mixed with two times molar excess of Mis18BP1 $_{20-130}$ . Mis18 $\alpha_{MeDIY}$  elutes at 11.9 ml while Mis18 $\alpha_{MeDIY}$ /Mis18BP1 $_{20-130}$  elutes at 11.5 ml. Proteins were separated using a Superdex 75 10/300 column and (B) His-GFP-Mis18 $\alpha_{MeDIY}$ /Mis18 $\beta_{MeDIY}$  and His-GFP-Mis18 $\alpha_{MeDIY}$ /Mis18 $\beta_{MeDIY}$  mixed with two times molar excess of Mis18BP1 $_{20-130}$  elutes at 14.3 ml and 12.4 ml, respectively. Proteins were analyzed using a Superdex 200 increase 10/300 column.

Mis18 $\alpha/\beta$	
Molecules	calculated MW in kDa
5	126.5
6	151.8
7	177.1

His-GFP-Mis18 $\alpha$ His-Mis18 $\beta$	
Molecules	calculated MW in kDa
3 $\alpha$ 3 $\beta$	247.5
4 $\alpha$ 2 $\beta$	276.0
5 $\alpha$ 1 $\beta$	304.5

His-Mis18 $\alpha$ / His-GFP-Mis18 $\beta$	
Molecules	calculated MW in kDa
3 $\alpha$ 3 $\beta$	247.5
4 $\alpha$ 2 $\beta$	221.4
5 $\alpha$ 1 $\beta$	195.3

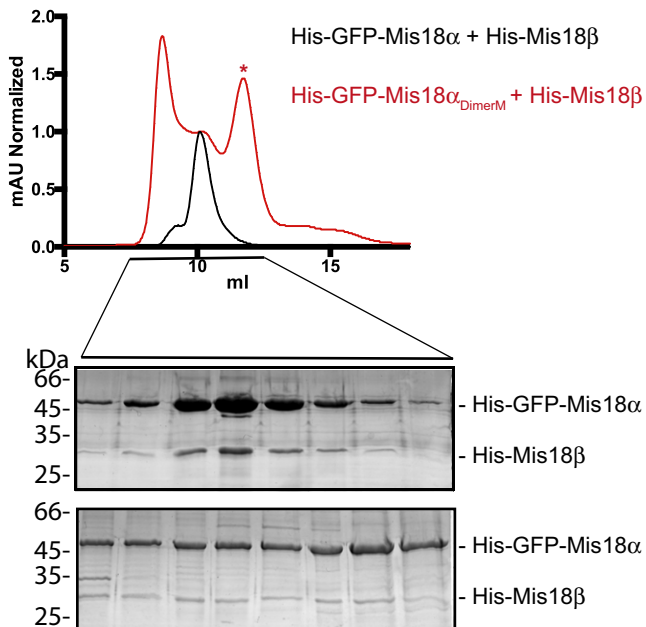
His-GFP-Mis18 $\alpha_{C-term}$ / His-MBP-Mis18 $\beta_{C-term}$	
Molecules	calculated MW in kDa
1 $\alpha$ 1 $\beta$	84.8
2 $\alpha$ 1 $\beta$	119.6
1 $\alpha$ 2 $\beta$	134.8

Mis18 $\alpha/\beta$ / Mis18BP1 <sub>20-130</sub>	
Molecules (4 $\alpha$ 2 $\beta$ + ?)	calculated MW in kDa
1 Mis18BP1	165.6
2 Mis18BP1	178.2
3 Mis18BP1	190.8

Mis18 $\alpha/\beta$ / His-Sumo-Mis18BP1 <sub>20-130</sub>	
Molecules (4 $\alpha$ 2 $\beta$ + ?)	calculated MW in kDa
1 Mis18BP1	179.8
2 Mis18BP1	206.6
3 Mis18BP1	233.4

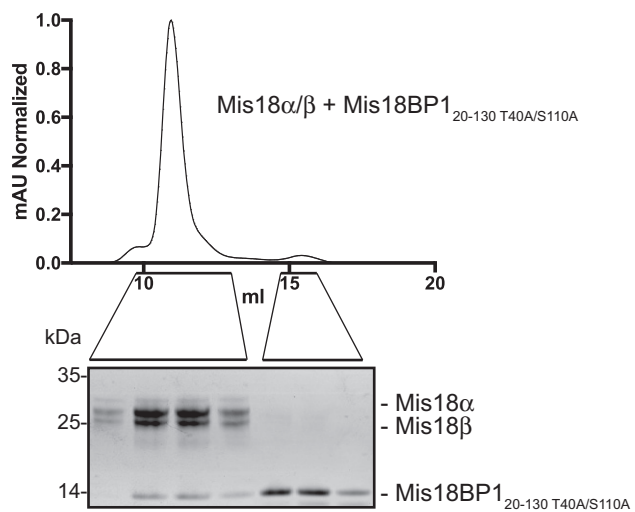
**Figure EV3. Predicted molecular weights for Mis18 complexes with different subunit stoichiometry.**

A–F Predicted MWs of (A) Mis18 $\alpha/\beta$ , (B) His-GFP-Mis18 $\alpha$ /His-Mis18 $\beta$ , (C) His-Mis18 $\alpha$ /His-GFP-Mis18 $\beta$ , (D) His-GFP-Mis18 $\alpha_{C-term}$ /His-MBP-Mis18 $\beta_{C-term}$ , (E) Mis18 $\alpha/\beta$ /Mis18BP1<sub>20-130</sub>, (F) Mis18 $\alpha/\beta$ /His-Sumo-Mis18BP1<sub>20-130</sub> complexes with different subunit compositions. These values together with the measured MWs from SEC-MALS analysis shown in Fig 3 were used to deduce the correct subunit composition.



**Figure EV4. Characterization of Mis18 $\alpha_{DimerM}$ /Mis18 $\beta$ .**

SEC profiles and SDS-PAGE analyses of His-GFP-Mis18 $\alpha$ /His-Mis18 $\beta$  and His-GFP-Mis18 $\alpha_{DimerM}$ /His-Mis18 $\beta$  complexes. SEC analyses were carried out using a Superdex 200 increase 10/300 column. Sample used for SEC-MALS is indicated with an asterisk (\*).



**Figure EV5. Mis18BP1<sub>20-130 T40A/S110A</sub> binds Mis18α/β *in vitro*.**  
 SEC profile and respective SDS-PAGE analysis of Mis18α/β mixed with two times molar excess of Cdk1 non-phosphorylatable mutant, Mis18BP1<sub>20-130 T40A/S110A</sub>. SEC run was carried out using a Superdex 200 increase 10/300 column.