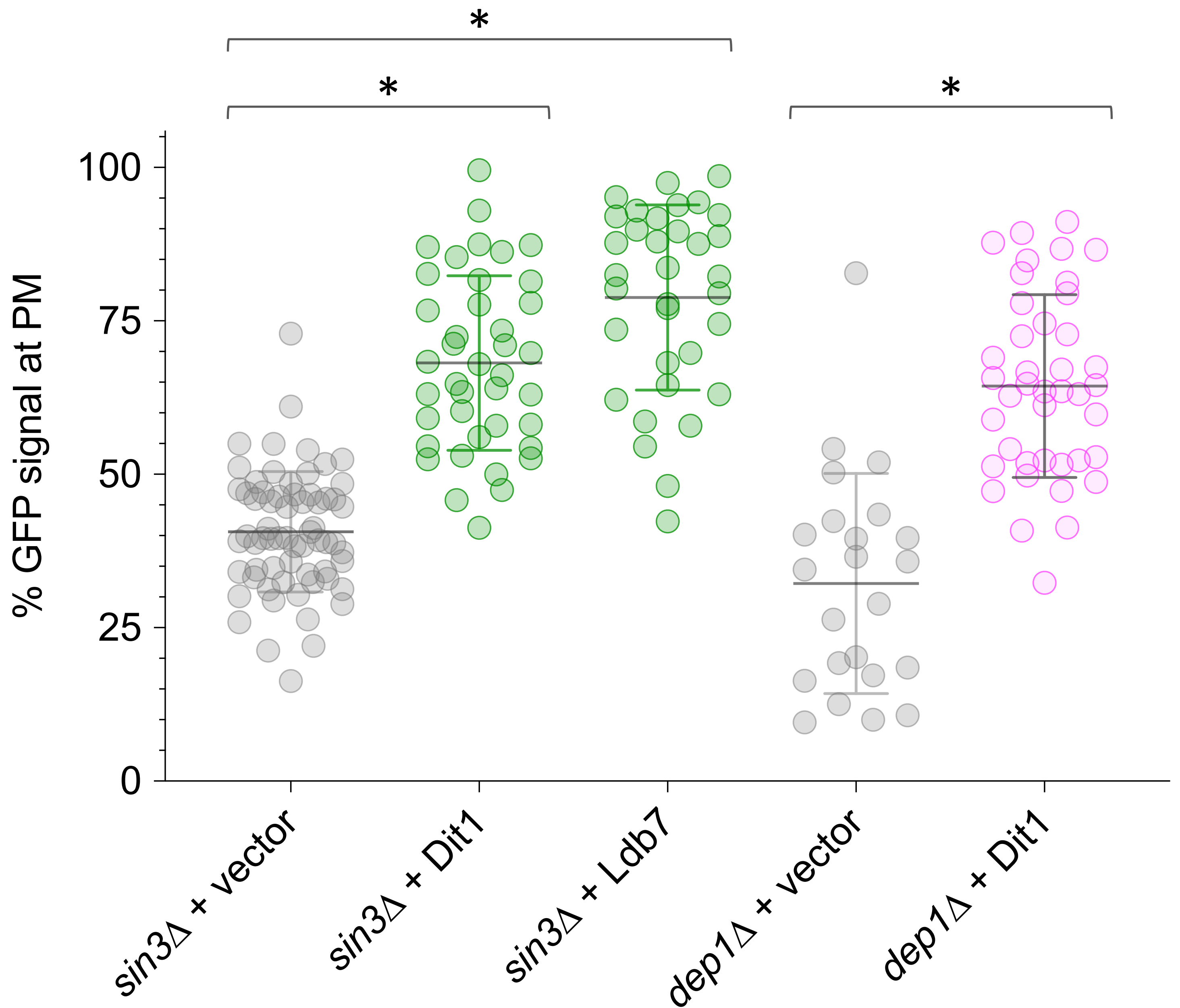


	<i>transformant</i>				<i>transformant</i>		
	1	2	3		1	2	3
Vector				<i>FIS1</i>			
<i>YAR029W</i>				<i>VNX1</i>			
<i>TPS1</i>				<i>NHX1</i>			
<i>VPS51</i>				<i>VPS55</i>			
<i>DUR3</i>				<i>TLG2</i>			
<i>PPA1</i>				<i>LTV1</i>			
<i>EMP46</i>				<i>YPT11</i>			
<i>PDA1</i>				<i>YPT31</i>			
<i>VAM6</i>				<i>NEM1</i>			
<i>ERG2</i>				<i>ERG24</i>			
<i>VPS30</i>				<i>GTR1</i>			

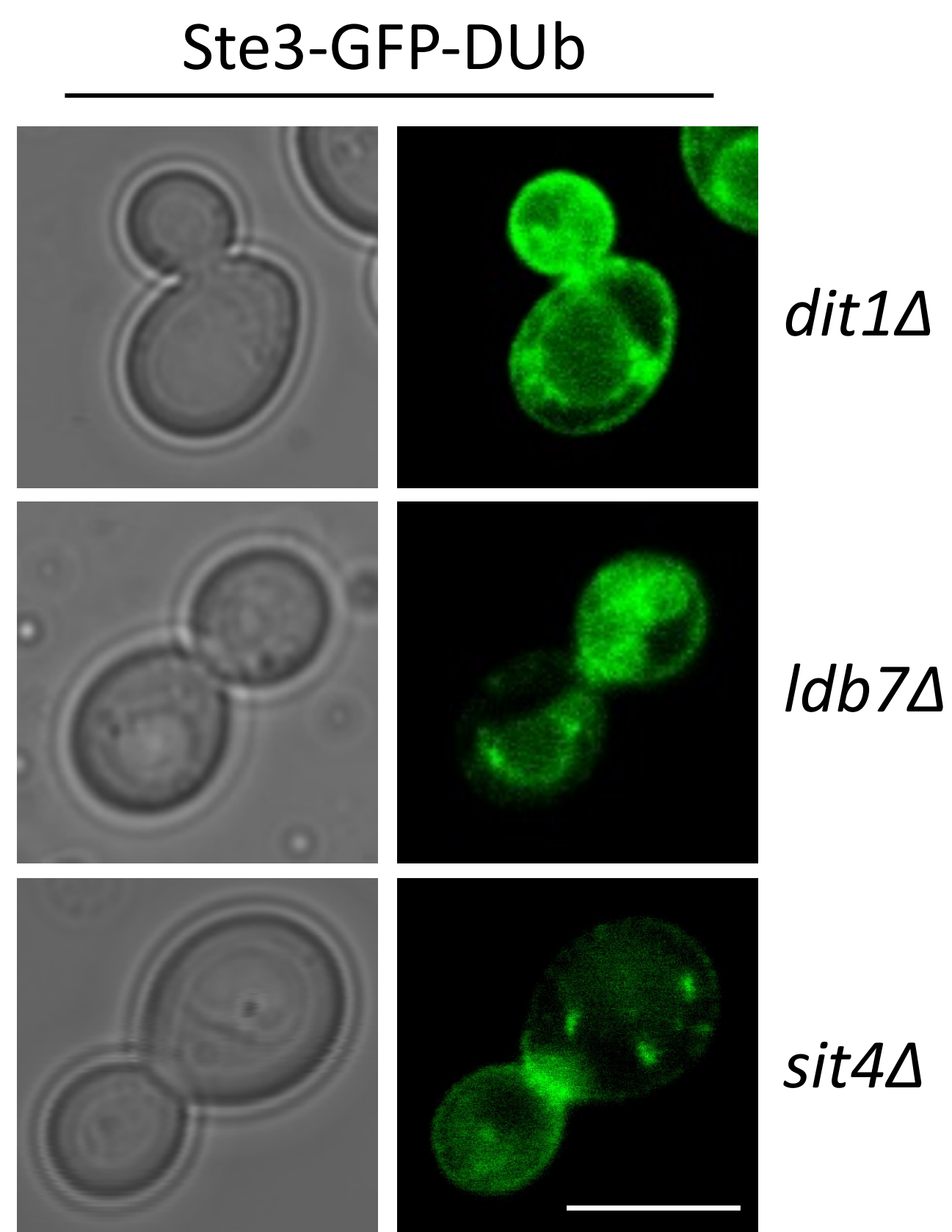
Supplemental Figure S1: over-expressor screen in wild-type background

Wild-type cells stably expressing Ste3-GFP-DUb were transformed with over-expression plasmids for listed genes before growth in selective media to mid-log phase and confocal microscopy. Three individual clones of each transformation was visualised but none of these over-expression plasmids induced a recycling defect of Ste3-GFP-DUb.



Supplemental Figure S2: Additional recycling complementation experiments

sin3Δ and *dep1Δ* mutant cells expressing Ste3-GFP-DUb from a chromosomal copy and transformed with indicated plasmids were grown to mid-log phase before confocal microscopy to determine localisation. The surface levels of Ste3-GFP-DUb in each condition was quantified as a percentage and asterisks (*) used to indicate significant difference of $p < 0.0001$ from *t*-test comparisons.



Supplemental Figure S3: Recycling defects in mutants from screen

Indicated mutants expressing Ste3-GFP-DUb were grown to mid-log phase before preparation for confocal microscopy and visualisation of the reporter. Scale bar, 5 μm .