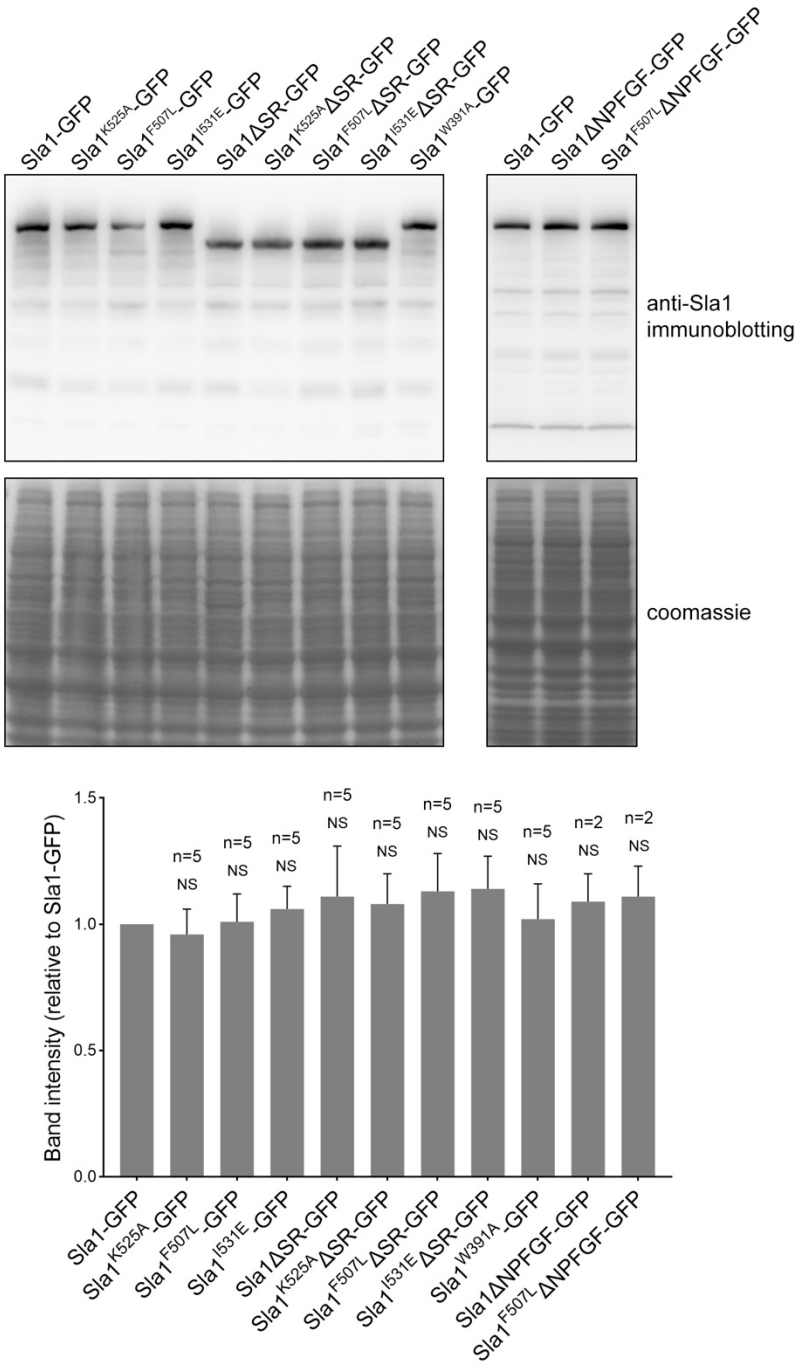

Figure S1. Immunoblotting analysis of strains expressing wild type and mutant forms of Sla1-GFP. Upper panels, total cell extracts were generated from strains expressing Sla1-GFP (SDY1422), Sla1^{K525A}-GFP (SDY603), Sla1^{F507L}-GFP (SDY601), Sla1^{I531E}-GFP (SDY599), Sla1ΔSR-GFP (SDY712), Sla1^{K525A}ΔSR-GFP (SDY738), Sla1^{F507L}ΔSR-GFP (SDY736), Sla1^{I531E}ΔSR-GFP (SDY718), Sla1^{W391A}-GFP (SDY832), Sla1ΔNPFGF-GFP (SDY1486), or Sla1^{F507L}ΔNPFGF-GFP (SDY1488) from the *SLA1* endogenous locus and analyzed by immunoblotting with anti-Sla1 antibodies. Middle panels, loading control. Lower panel, quantification of the band intensity for Sla1-GFP mutants relative to wild type Sla1-GFP (5 biological replicates were analyzed for strains shown in the left panels and 2 biological replicates for strains shown in the right panels). Differences in band intensities relative to wild type Sla1-GFP were not significant (NS).

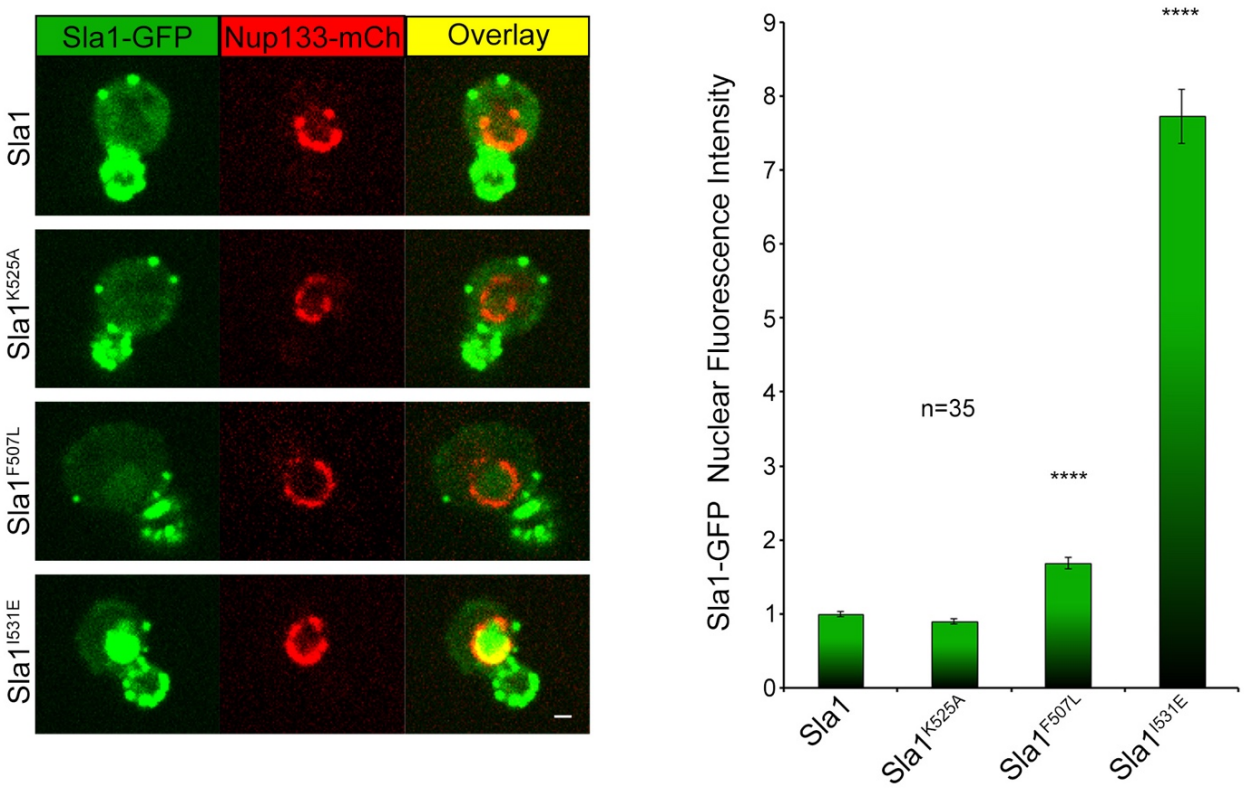

Figure S2. Sla1 localizes to the nucleus when NPFxD binding is disrupted. Live-cell confocal fluorescence microscopy imaging of cells expressing Sla1-GFP wild type, or the indicated SHD1 mutant from the endogenous locus and Nup133-3xmCherry expressed from a plasmid. Left, representative frames of Sla1-GFP and Nup133-3xmCherry in wild type and Sla1 mutant cells. Right, quantification of the Sla1-GFP nuclear/cytoplasm fluorescence intensity ratio showing a significant increase in Sla1-GFP nuclear localization was determined for F507L and I531E mutants (n=35 cells, P=0.0566 for $Sla1^{K525A}$, P<0.0001 for $Sla1^{F507L}$ and $Sla1^{I531E}$). Scale bar = 1 µm.

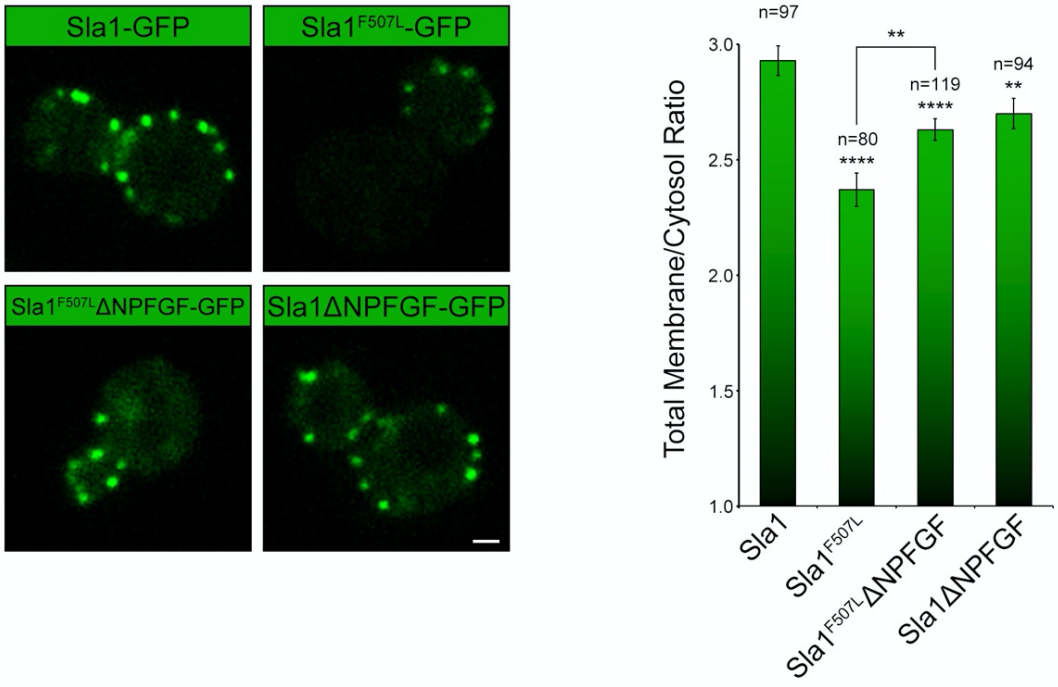

Figure S3. The NPFGF-COOH sequence at the Sla1 C-terminus affects Sla1 recruitment to the plasma membrane. Live-cell confocal fluorescence microscopy analysis of yeast cells expressing either Sla1-GFP (SDY1422), Sla1^{F507L}-GFP (SDY601), Sla1^{F507L}ΔNPFGF-GFP (SDY1488) or Sla1ΔNPFGF-GFP (SDY1486) from the endogenous *SLA1* locus. Left, panels show representative frames from each strain. Right, quantification represented as Total Membrane/Cytosol Ratio of fluorescence intensity across the entire cell plasma membrane was performed as described under Materials and Methods and shows a recruitment defect for Sla1^{F507L}-GFP, Sla1^{F507L}ΔNPFGF-GFP and Sla1ΔNPFGF-GFP relative to Sla1-GFP (n=97 cells for Sla1; n=80 cells, P<0.0001 for Sla1^{F507L}-GFP; n=119, P<0.0001 for Sla1^{F507L}ΔNPFGF-GFP; n=94, P<0.01 for Sla1ΔNPFGF-GFP). Importantly, the plasma membrane levels of Sla1^{F507L}ΔNPFGF-GFP were higher than Sla1^{F507L}-GFP (P<0.01). Scale bar = 1 μm.