



**FIGURE S1.** Additional measures of fluorescence. The relative green fluorescence of three tested GFPs expressed from the intermediate *TEF2p* promoter was assessed by (a) microscopy and (b) flow cytometry.  $n = 1$ ; 10,000 cells/strain. (c) Representative histograms of green fluorescence intensity measured by flow cytometry of wild type cells and a strain with *TDH3p-GFP* integrated at Site 1. These data correspond with a subset of Figure 2f Fluorescence microscopy of yeast with RFP-tagged markers (d) RFP-Vps1, (e) Snf7-RFP, (f) RFP-Vam3 and (g) Pex25-GFP displaying changes in localization as promoter strength increases  $RPL18Bp < TEF2p < TDH3p$ .  $n = 2$ . Scale bars indicate 2  $\mu$ m.