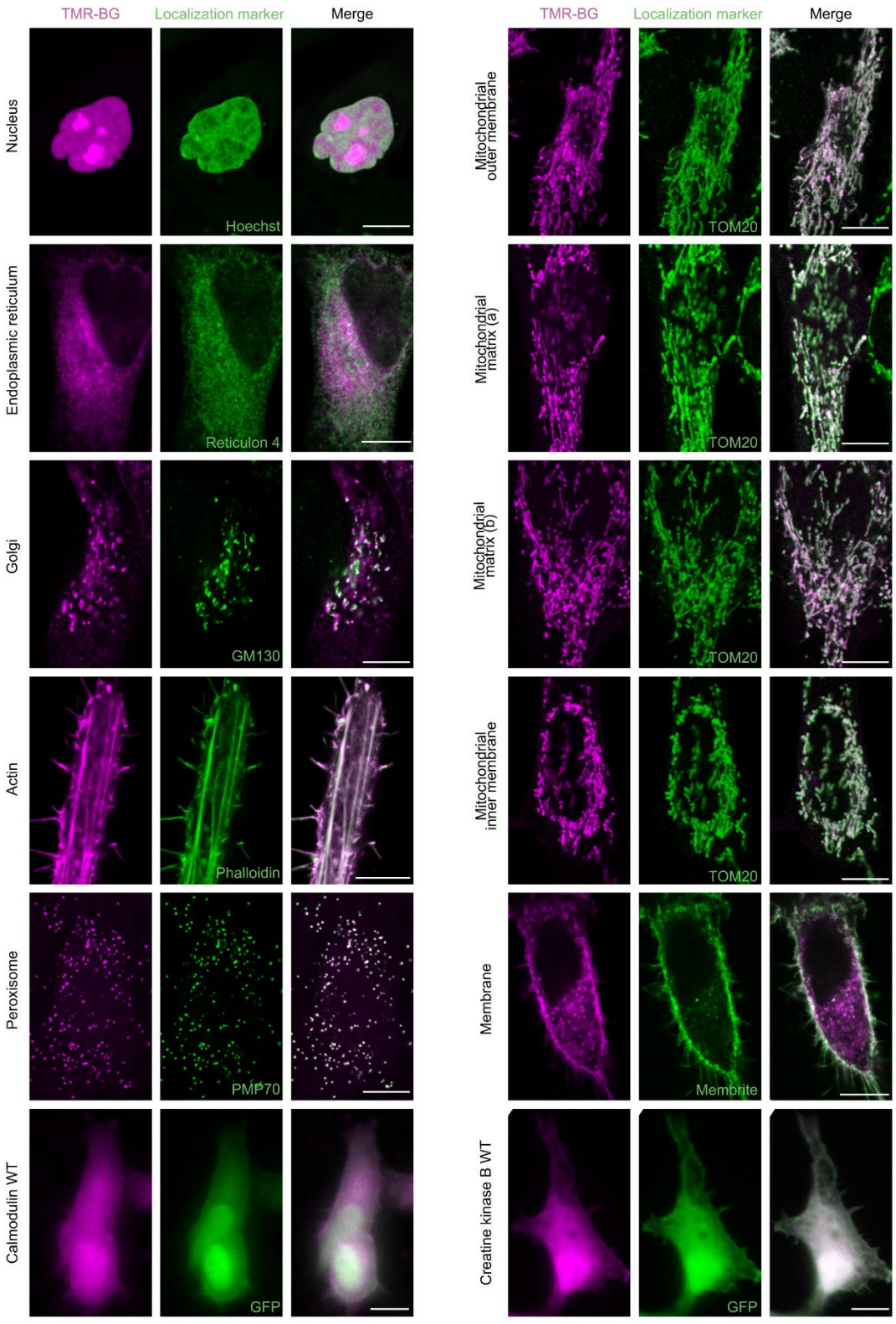
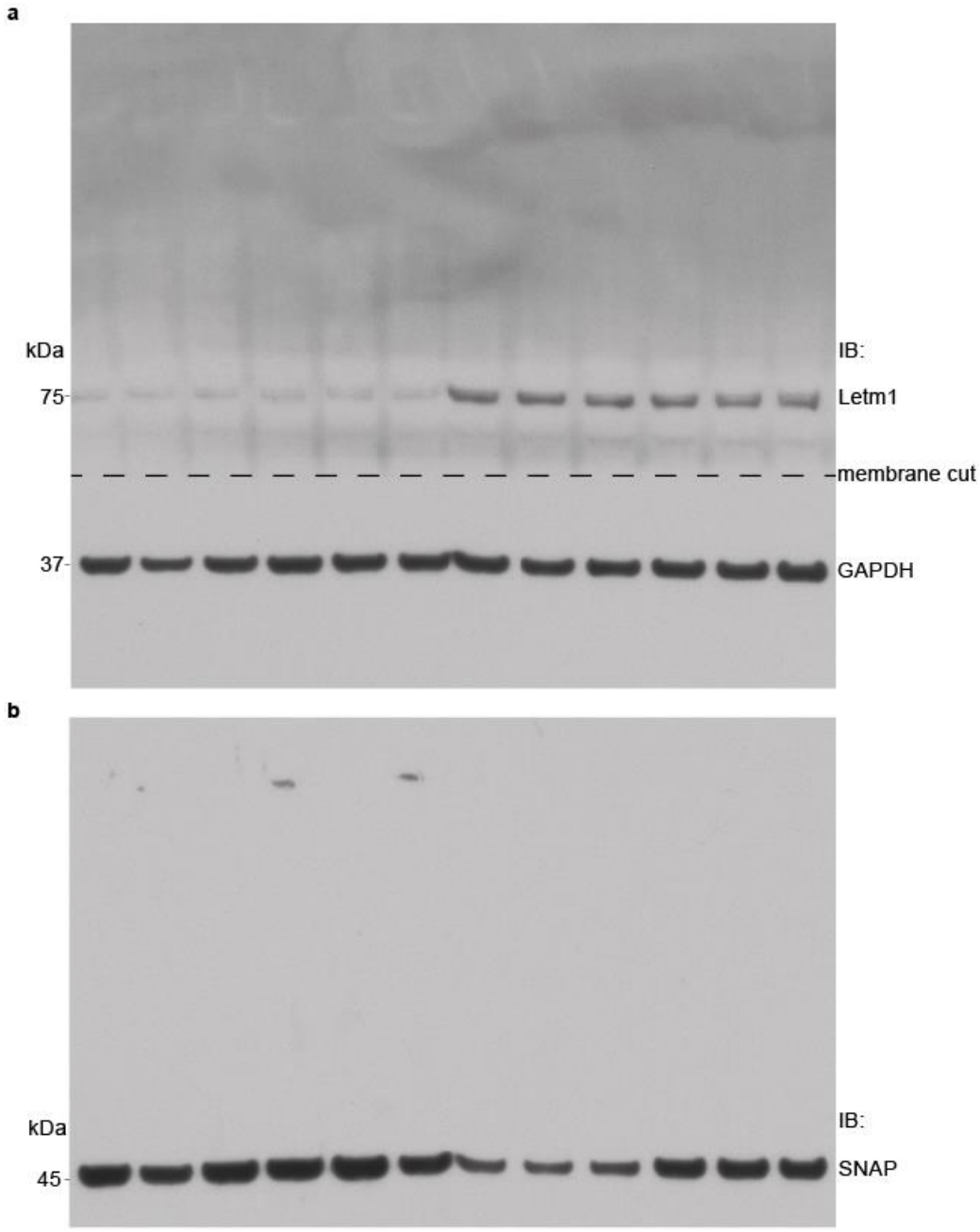
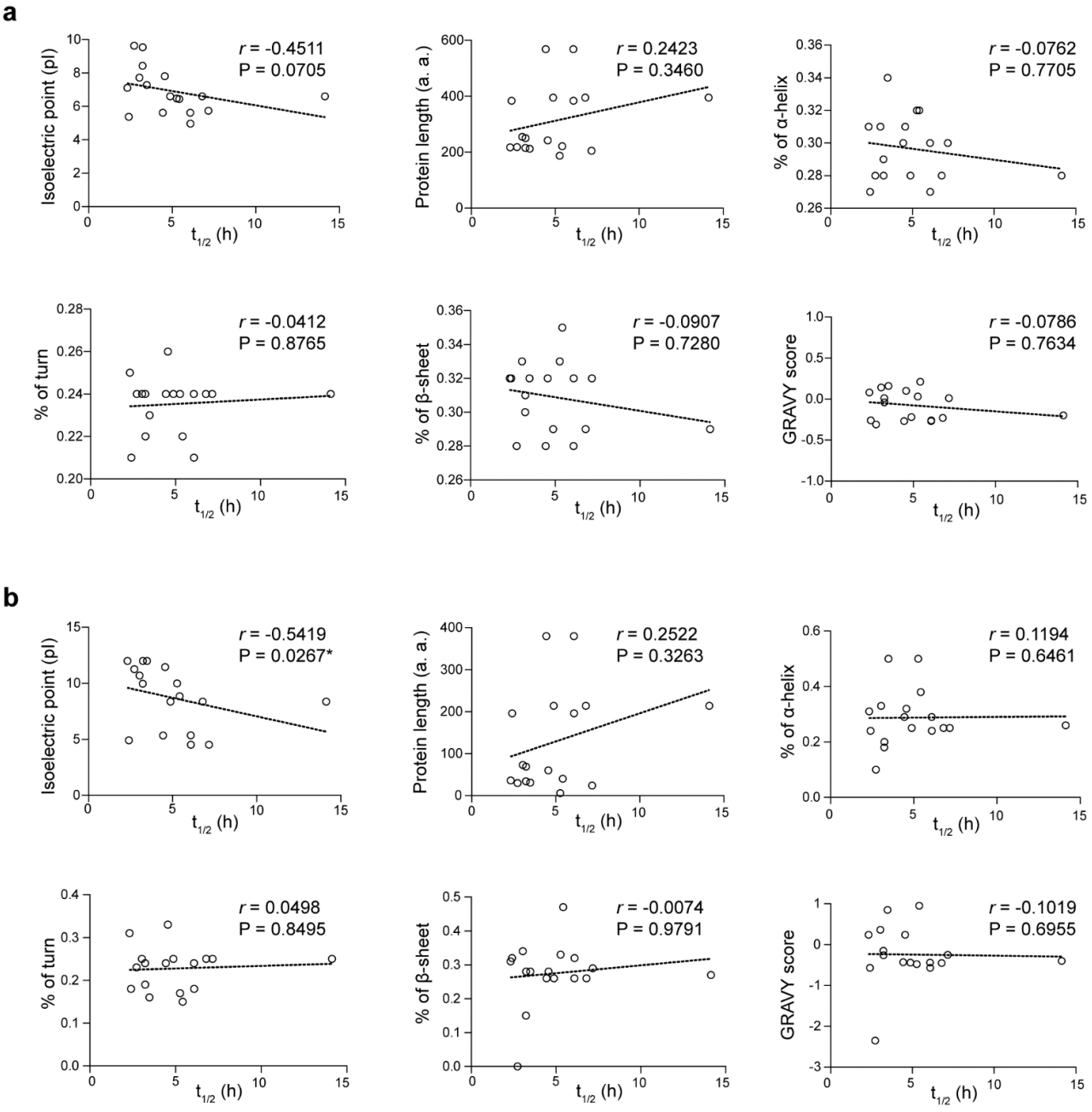


Supplementary Figure 1. Localization of SNAP-tag fusion constructs. The panels show the different SNAP sensors (summarized in Table 1), labelled with TMR-BG (magenta) and the respective colocalization marker (green). Note that NLS in cells is known to be enriched in nucleoli [87], while Hoechst, especially in human cells, is more evenly distributed. Along similar lines, Membrite labelling is a more reliable marker of the plasma membrane, while the palmitoylated-

SNAP sensor is present both on plasma membrane as well as on other membranes within cells, as also previously observed for palmitoylated proteins [88]. Calmodulin WT and Creatine kinase B WT expressing cells were co-transfected with GFP, which is soluble and serves here as a control for soluble protein localization (reproduced from Figure 1). For the localization of the Rab5 sensors, please refer to main Figure 5**.** Scale bars 10 μm.



Supplementary Figure 2. Western blot to study Rab5a-WT and Rab5a-Q79L association with the membrane. **a**. Immunoblot for Letm1 (75 kDa) and GAPDH (37 kDa). The position where the membrane was cut (but exposed on the same film) is indicated by a dashed line. **b**. Immunoblot for SNAP-tag (45 kDa).



Supplementary Figure 3. Correlations of protein half-lives with biochemical properties including (a) or excluding (b) SNAP-tag. a-b. Correlations between protein half-lives with various characteristics of proteins considering (**a**) or not considering (**b**) the SNAP-tag sequence in the calculation. These measures include the isoelectric point, the protein length, the percentage of alpha-helix, turn or beta-sheet and grand average of hydropathy (GRAVY), a measure of hydrophobicity. See methods for details. All the data are presented as scatter plots with linear fits represented by a segmented line. *r* – Spearman's rank correlation coefficient (N = 17, data does not follow Gaussian distribution).