A fluorescent timer reporter enables sorting of insulin secretory granules by age

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Running title: *Sorting out young and old insulin granules*

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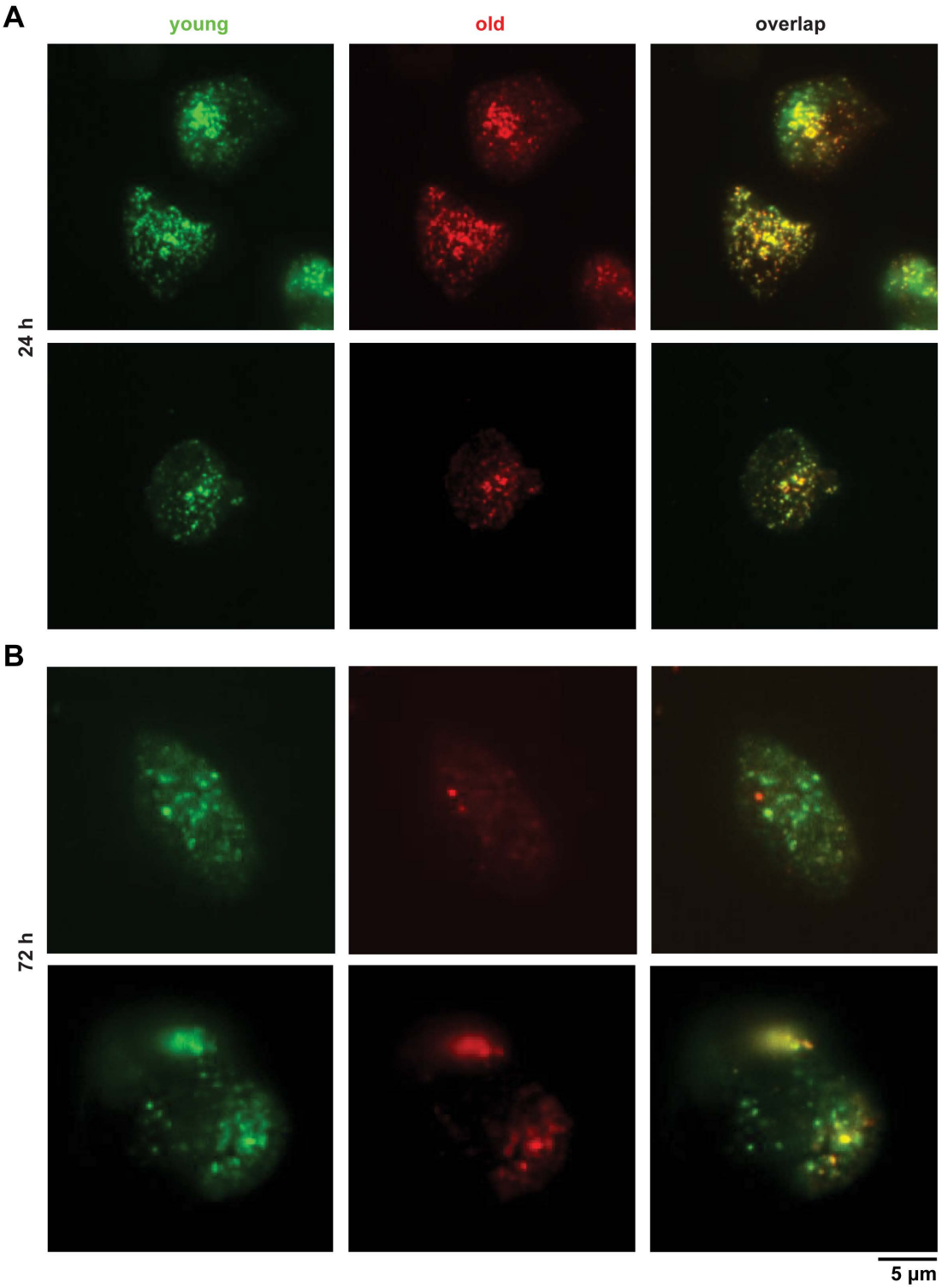
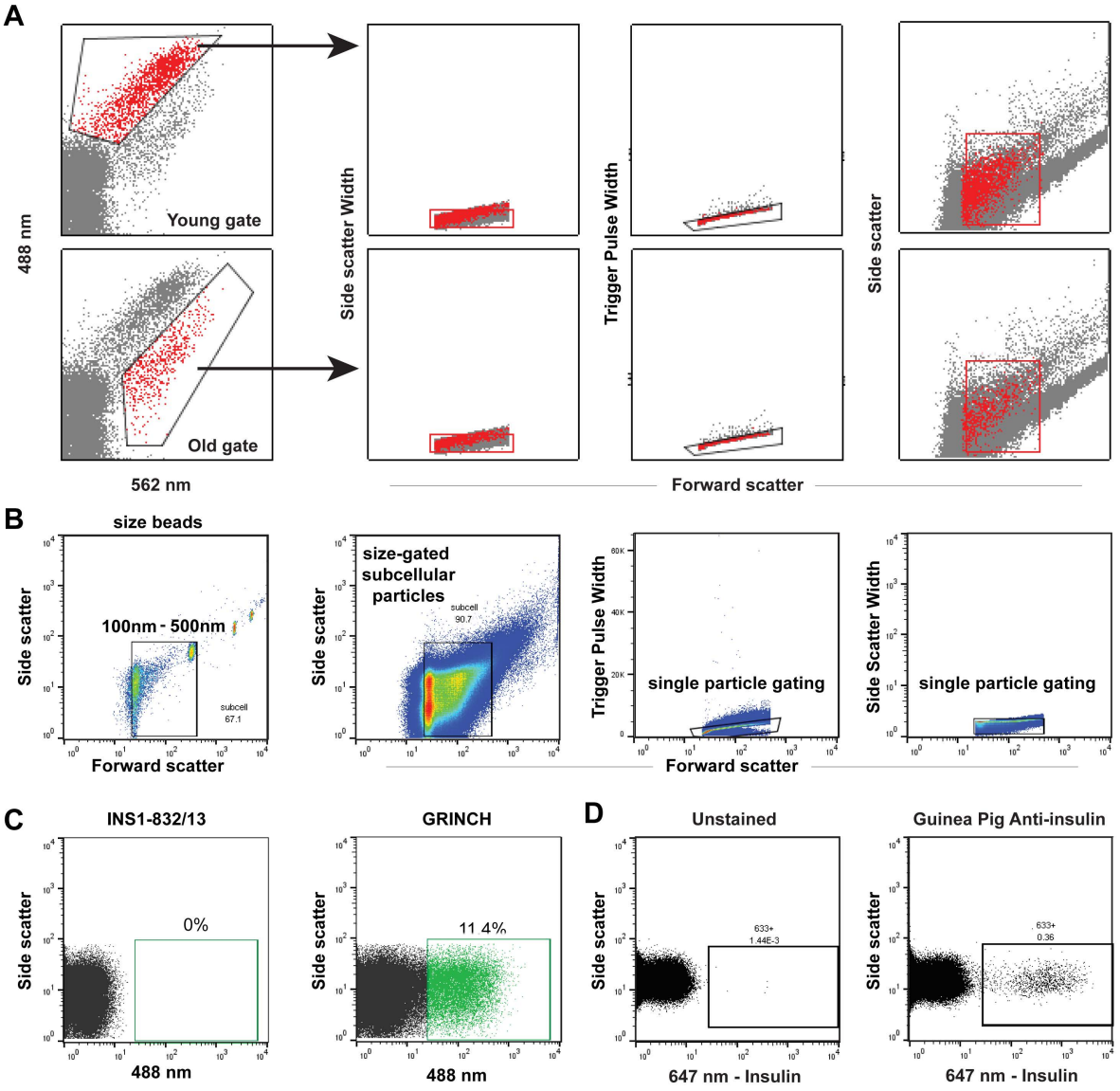
Supplementary Figure 1



Supplementary Figure 1. Young and old insulin granules labelled with syncollin-dsRedE5TIMER differentially localise at the plasma membrane. TIRF microscopy at 488 nm and 561 nm at an illumination angle of ~110 nm at the plasma membrane of INS1 rat beta cell line fixed at **(A)** 24 h or **(B)** 72 h post-transduction with syncollin-dsRedE5TIMER.

Supplementary Figure 2



Supplementary Figure 2. Syncollin-dsRedE5TIMER-expressing granules are genuine granules in INS1 cells. (A) Backgating analysis of young and old granule populations. **(B)** FAOS gating strategy for single subcellular particles between 100 nm and 500 nm, as described in Figure 2B, can be applied unchanged to the INS1 rat beta cell line. **(C)** FAOS analysis of INS1 832/13 cells and hPro-CpepsfGFP-expressing INS1 832/13 GRINCH cells, gated for GFP fluorescence at 488 nm. **(D)** FAOS analysis of unstained Min6 and Min6 stained with guinea pig anti-insulin and Alexa Fluor 647-conjugated anti-guinea pig secondary fluorophore, gated for fluorescence at 633 nm.