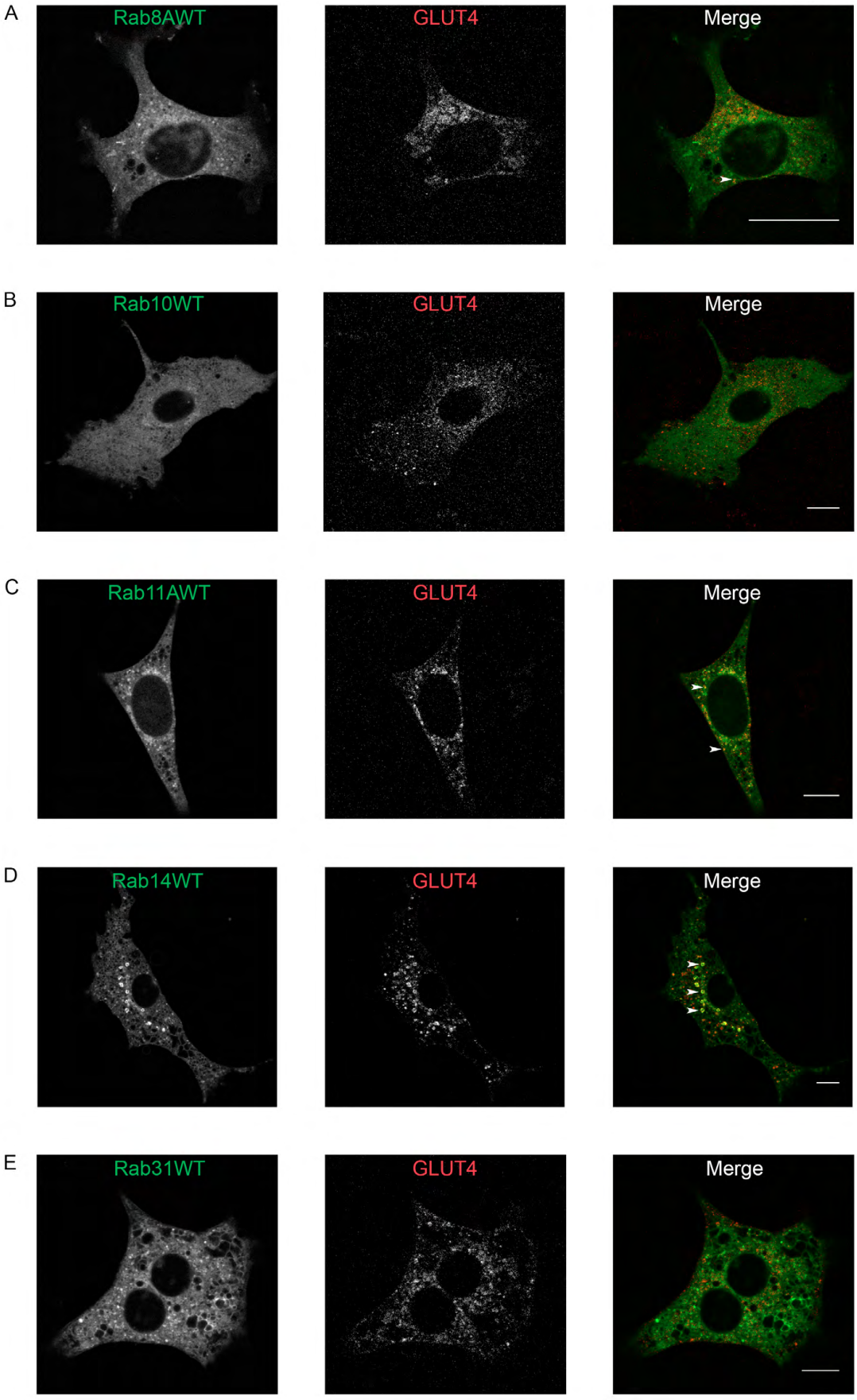

Fig. S1. Only Rab14 displays extensive colocalisation with GLUT4. 3T3-L1 adipocytes were electroporated with plasmids encoding HA-GLUT4-mCherry and either GFP-Rab8AWT (**A**), GFP-Rab10WT (**B**), GFP-Rab11AWT (**C**), GFP-Rab14WT (**D**) or GFP-Rab31WT (**E**). 24 hours later, cells were serum starved for 3 hours, fixed and imaged by confocal microscopy. White arrowheads indicate punctate/enlarged vesicular structures positive for Rab and GLUT4. Scale bars = 10 μm.

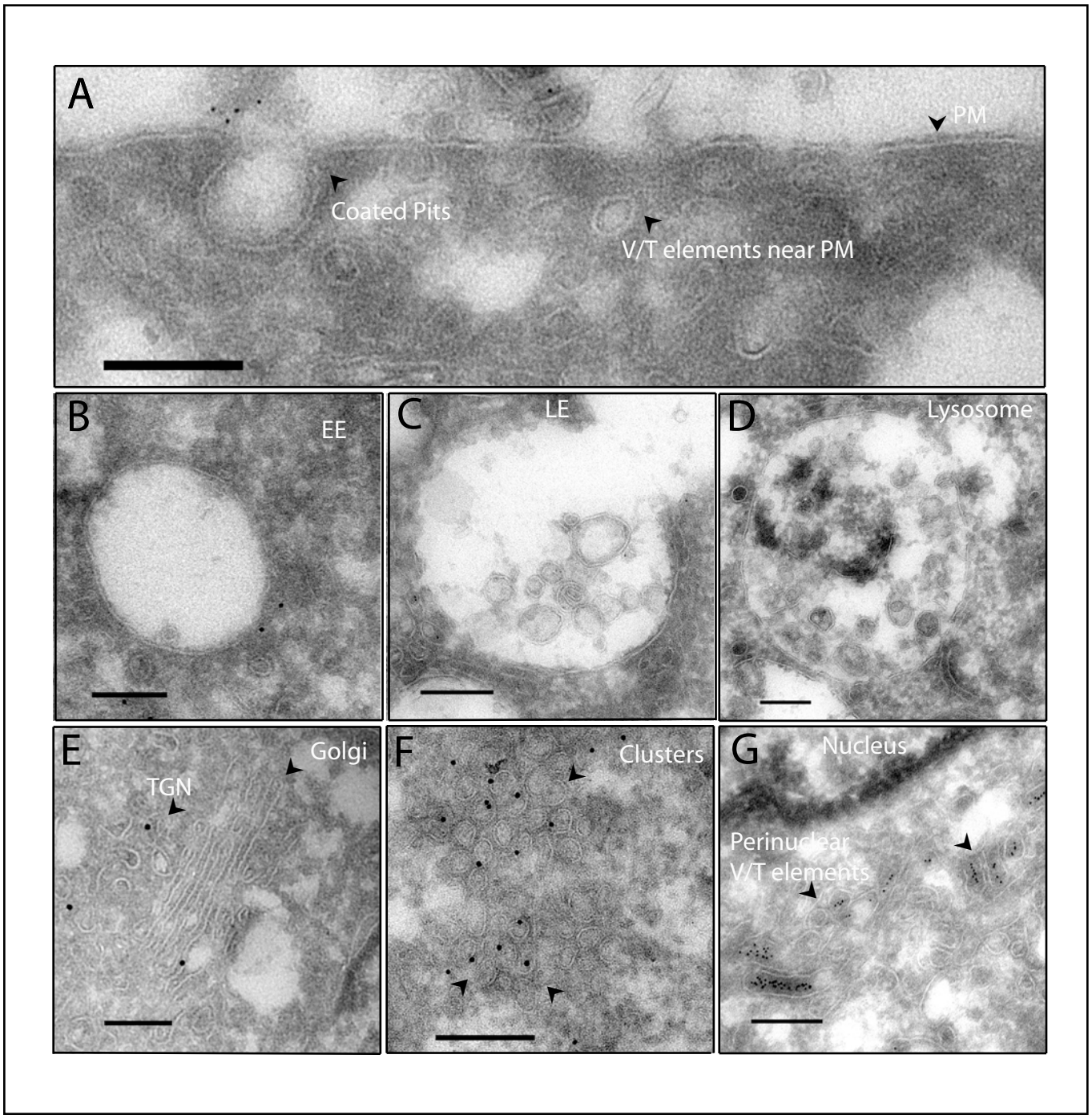

Fig. S2. Compartments labelled with gold particles during internalisation of HA-antibodies by HA-tagged GLUT4. Representative transmission electron microscopy images of intracellular compartments in 3T3-L1 adipocytes. The following compartments were identified based on organelle morphology: plasma membrane, coated pits/vesicles at and vesicles/tubules near to the plasma membrane (**A**), early endosomes (**B**), late endosomes (**C**), lysosomes (**D**), Golgi and TGN (**E**), clusters of vesicles (**F**), and V/T elements in the perinuclear region (**G**). Scale bars = 200 nm.

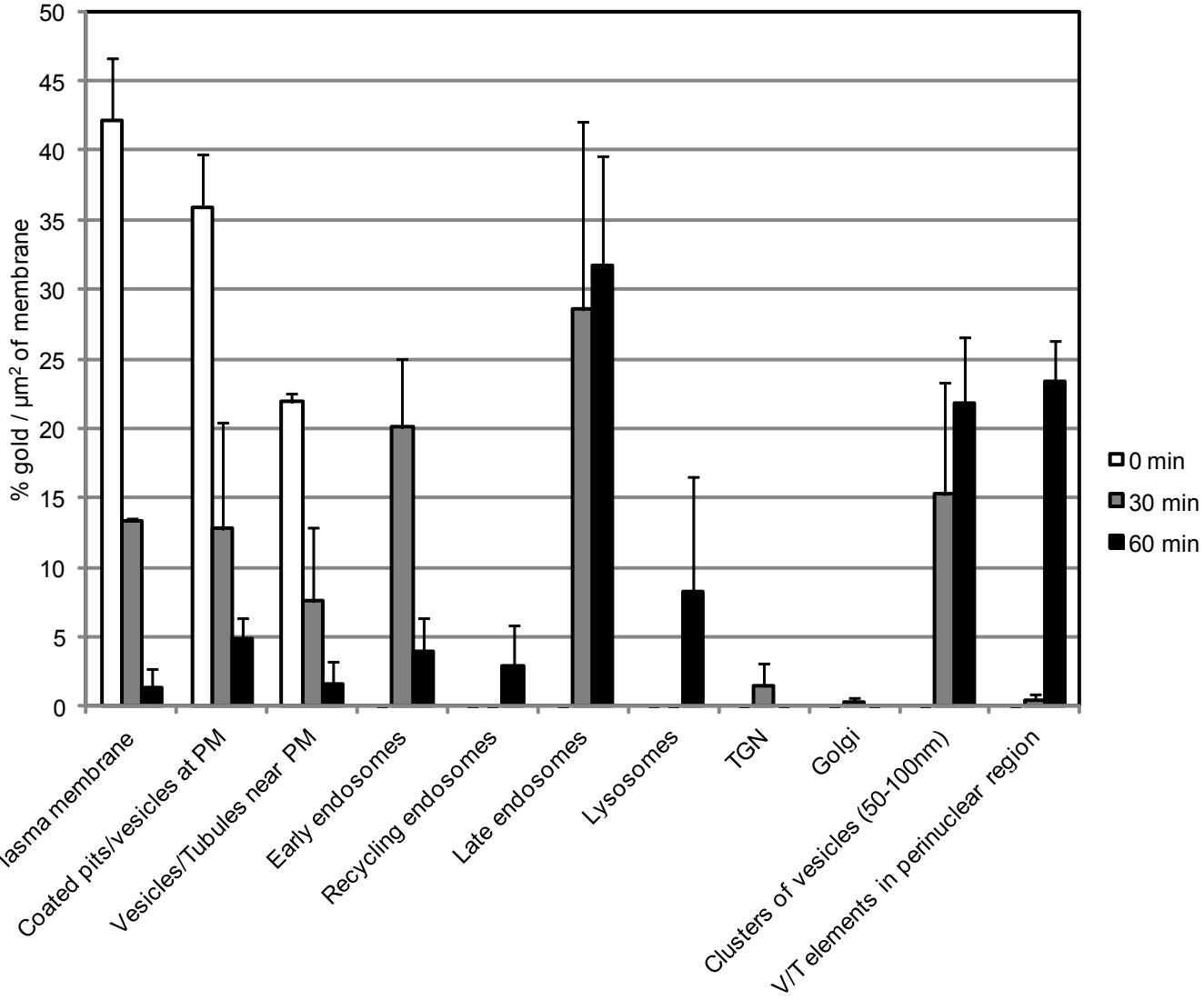

Fig. S3. The internalisation of GLUT4 from the plasma membrane to perinuclear compartments can be followed using electron microscopy. Scrambled 3T3-L1 adipocytes expressing HA-GLUT4-GFP were serum starved for 3 hours and stimulated for 20 minutes with 87 nM insulin. Cells were incubated with anti-HA antibody, rabbit anti-mouse bridging antibody and 5 nm Protein A gold for 20 minutes at 15°C. Cells were subsequently warmed to 37°C for the indicated times, before fixation, processing for transmission electron microscopy and quantification, as described in Materials and Methods. The graph represents the amount of gold in each compartment as a percentage of total gold. Data are from a single experiment with 3 cells analysed per time point.

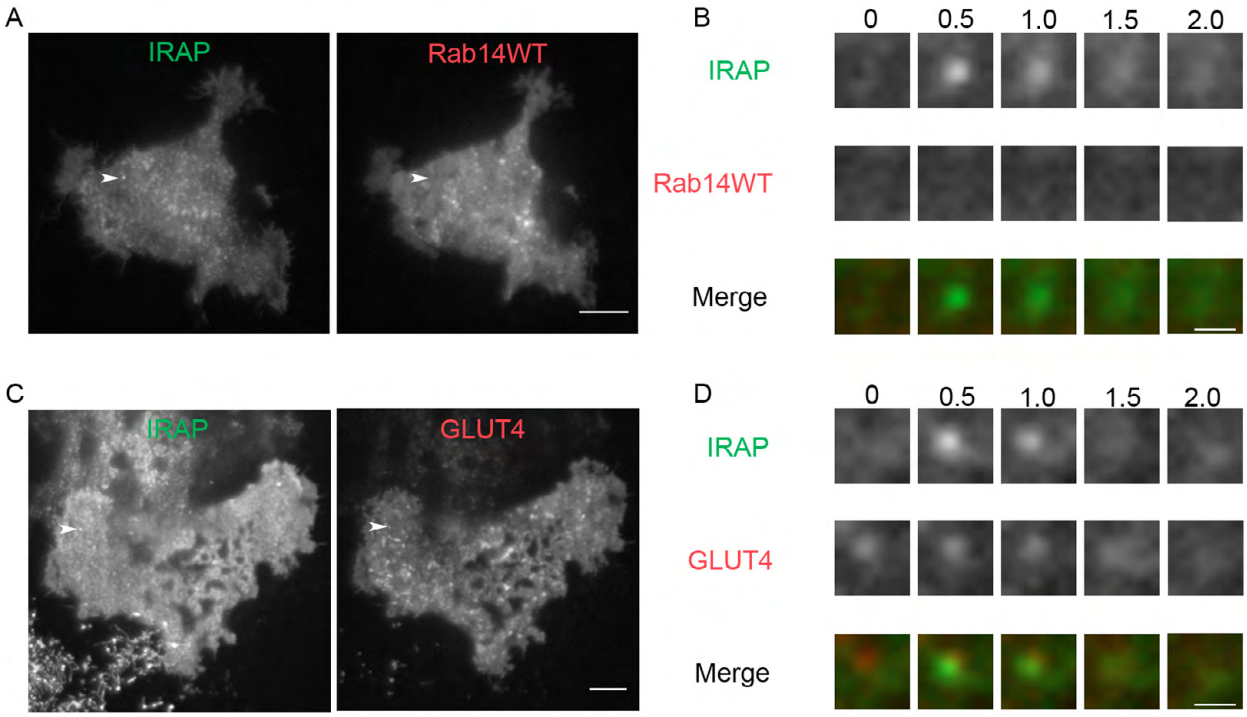

Fig. S4. The majority of IRAP-pHluorin vesicles fusing with the plasma membrane are devoid of Rab14WT. (**A**,**B**) 3T3-L1 adipocytes were electroporated with plasmids encoding mCherry-Rab14WT and IRAP-pHluorin. 24 hours later, cells were serum starved for 3 hours, and imaged by total internal reflection fluorescence (TIRF) microscopy using a penetration depth of 90 nm. Insulin was added at a concentration of 87 nM on the microscope. The entire cell is shown (A) in addition to an individual vesicle (B). Scale bar in A = 10 µm. Scale bar in B = 1 µm. (**C**,**D**) 3T3-L1 adipocytes were electroporated with plasmids encoding HA-GLUT4-mCherry and IRAP-pHluorin. 24 hours later, cells were serum starved for 3 hours, and imaged by TIRF microscopy using a penetration depth of 90 nm. Insulin was added at a concentration of 87 nM on the microscope. The entire cell is shown (C) in addition to an individual vesicle (D). Scale bar in C = 10 µm. Scale bar in D = 1 µm.

Table S1. Rab4 and Rab14 give rise to a similar degree of vesicle enlargement. 3T3-L1 adipocytes were electroporated with plasmids encoding either GFP-Rab4WT or GFP-Rab14WT. 24 hours later, cells were serum starved for 3 hours, fixed and imaged by confocal microscopy. Shown are the mean number of enlarged Rab positive vesicles per cell ± s.e.m., and the mean diameter of enlarged Rab positive vesicles ± s.e.m. Data are from 15 cells from 1 experiment.

	Rab4WT	**Rab14WT**
Mean number of Rab + enlarged vesicles/cell	2.93±0.60	3.00±0.87
Mean diameter of Rab + enlarged vesicles (µm)	1.15±0.06	1.02±0.04
Number of vesicles analysed	44	45