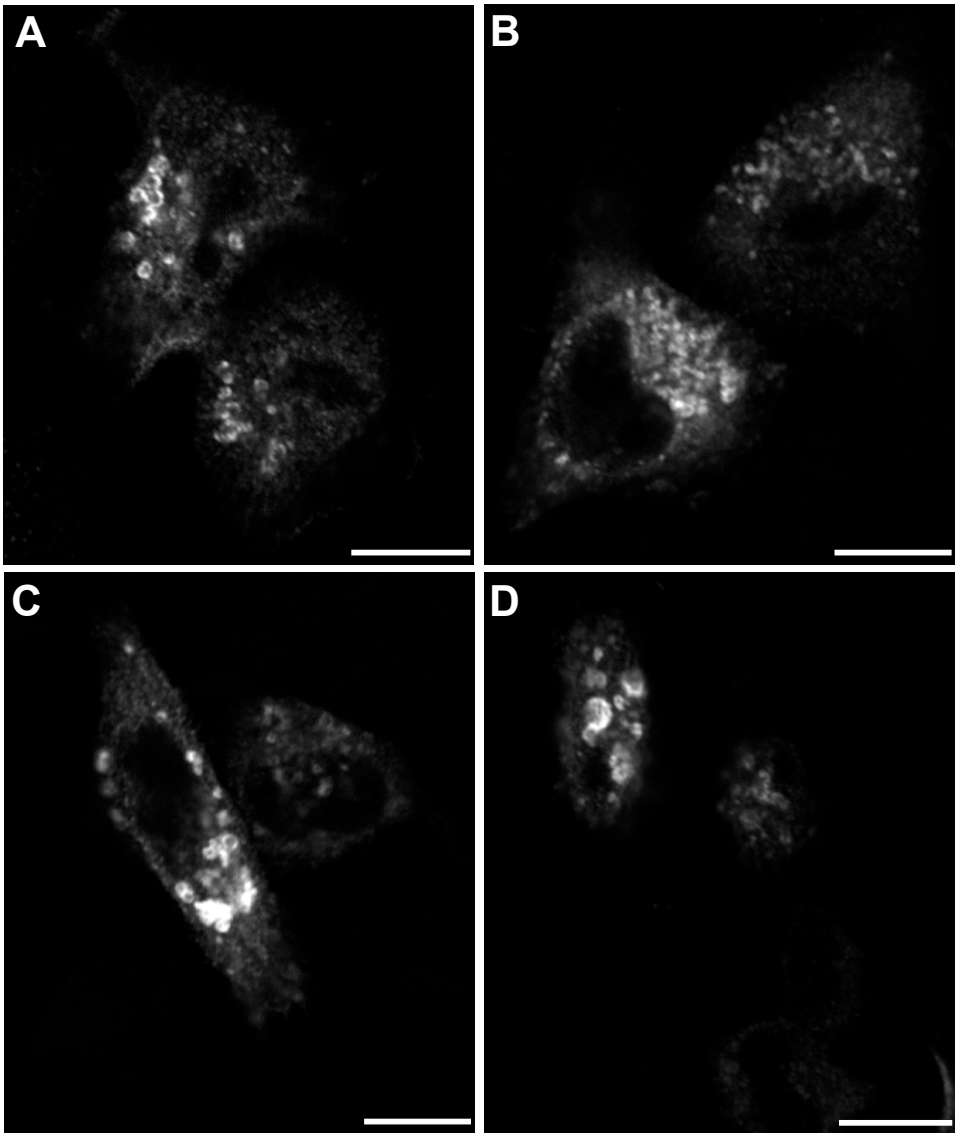
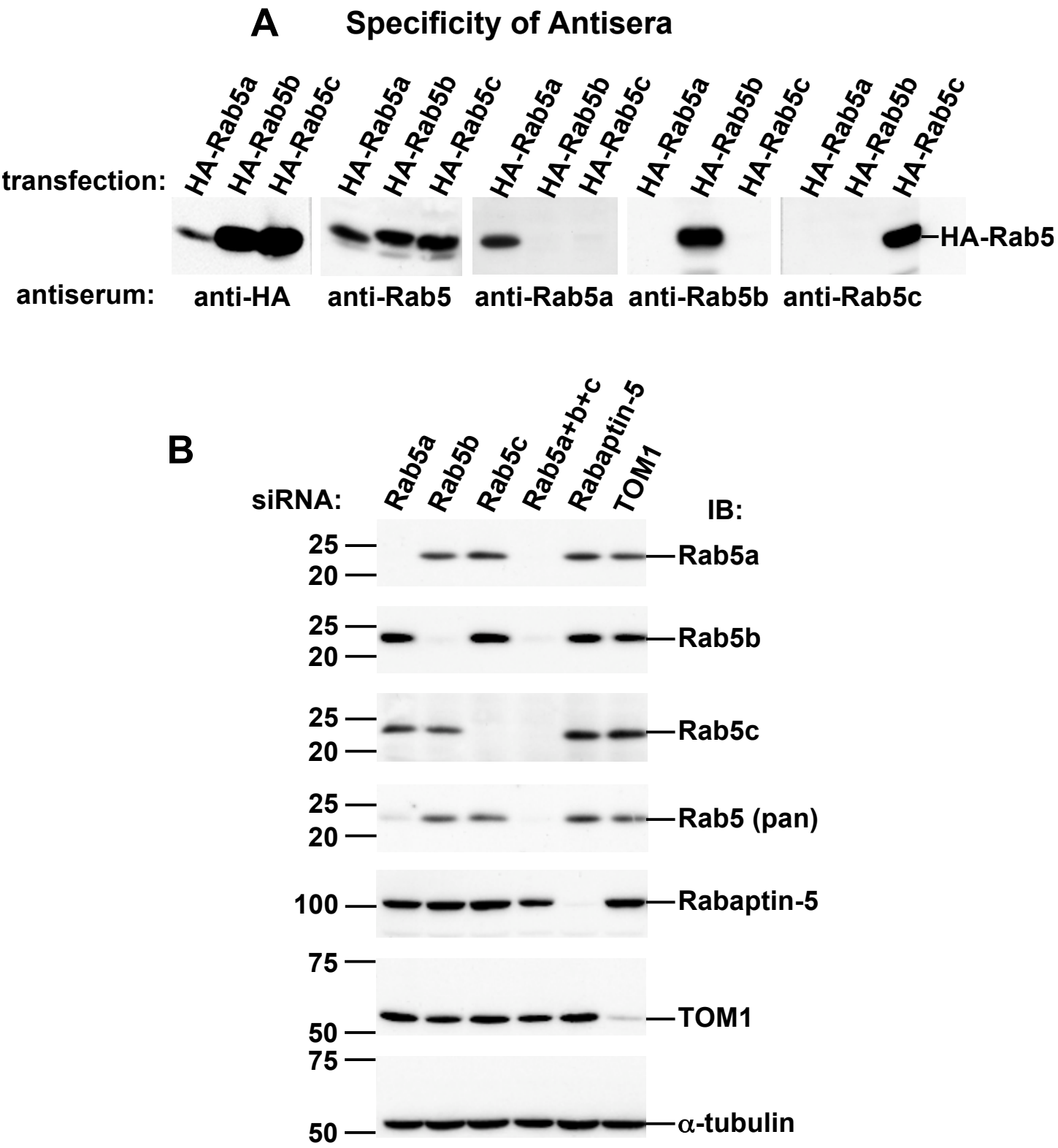
Supplementary Fig. S1



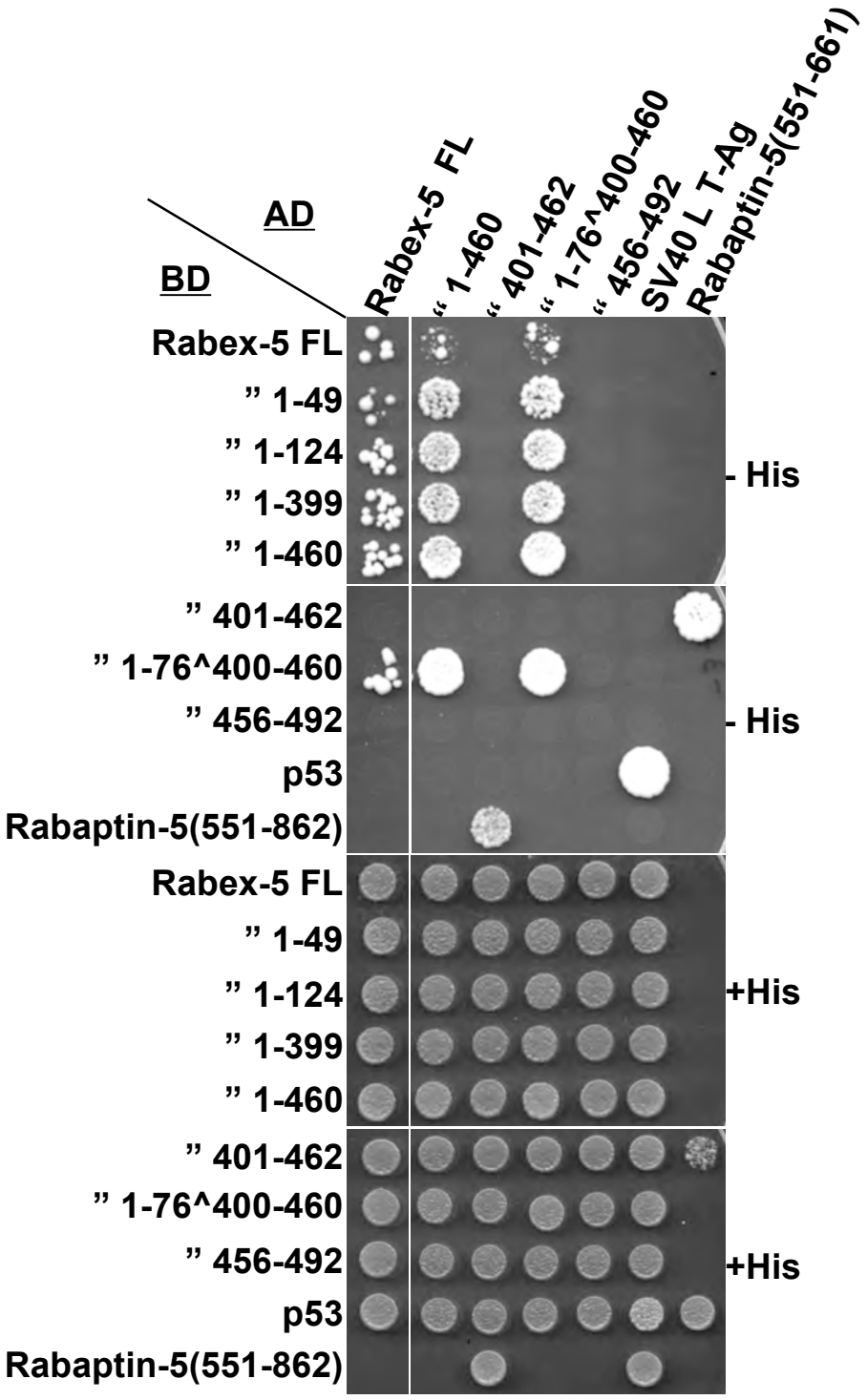
Supplementary Figure S1: **Distribution of *myc*-Rabex-5 wild-type in transiently transfected HeLa cells.** Cells were transfected, fixed and incubated as described in the legend to Figure 2. Four examples are shown in panels A-D. The size of vesicles containing myc-Rabex-5 appears proportional to the expression levels of the recombinant protein. For quantitative analysis see legend to Figure 2. Scale bars = 10 μm

Supplementary Fig. S2



Supplementary Figure S2: **Specificity of antisera against different isoforms of Rab5 and effect of siRNA treatment on endogenous levels of Rab5 in HeLa cells** *A*. HeLa cells were transiently transfected with the indicated HA-Rab5 constructs. Cell lysates were blotted with mouse monoclonal anti-HA or the indicated anti-Rab5 polyclonal antisera. Notice that the rabbit polyclonal anti-Rab5 (pan anti-Rab5; second blot from left) reacts with all Rab5 isoforms but exhibits higher avidity for Rab5a (comparison of anti-HA and pan anti-Rab5 blots). *B*. HeLa cells were transfected with the indicated siRNAs (TOM1 siRNA was used as control) and lysed 72 h after post-transfection. Cell lysates were blotted with anti-Rab5, anti-Rabaptin-5, anti-TOM1 and anti-α-tubulin (to control for equivalence of protein content in samples).

Supplementary Fig. S3



Supplementary Figure S3: **Self-Association of Rabex-5**. Constructs representing the indicated fragments of Rabex-5 were subcloned in both Gal4 activation and binding domain vectors (AD and BD, respectively). Yeast co-transformants were plated on medium without histidine (-His) to detect HIS3 reporter gene activation due to interaction of constructs, and on medium with histidine (+His) as a control for loading and growth of the co-transformants. Controls for non-specific interactions included co-transformation of pGAD con *myc*-Rabex-5 WT structs with a Gal4 BD-p53 plasmid, as well as co-transformation of pGBT9 constructs with a Gal4 AD-SV40 large T-antigen plasmid (T-Ag). Co-transformation with vectors encoding Gal4 BD-p53 and Gal4 AD-SV40 large T-antigen provided a positive control for interactions. The AD-Rabaptin-5(551-661) positive control was only co-transformed with BD-Rabex-5(401-462) or BD-p53; similarly, the BD-Rabaptin-5 (551-862) positive control was only co-transformed with AD-Rabex-5(400-460) or AD-SV40 LT-Ag. Notice that the Rabex-5 C-terminal coiled-coil (BD-Rabex-5(401-462) or AD-Rabex-5(401-462) constructs) interacts with Rabaptin-5 (AD-Rabaptin-5(551-661) or BD-Rabaptin-5(551-862)) but does not self-associate with an affinity detectable by yeast-two hybrid assays.