



**Supplementary Figure 4. Mapping and structure of the Rabenosyn-5 Rab binding domains**

**a**, Concentration dependence of the equilibrium SPR signal ( $R_{eq}$ ) for the interaction of Rab4 and Rab5 with Rabenosyn-5 constructs. **b**, Validation of SPR dissociation constants by isothermal titration microcalorimetry (ITC). Data were analyzed as described in Cronin, T. C., DiNitto, J. P., Czech, M. P., & Lambright, D. G. Structural determinants of phosphoinositide selectivity in splice variants of Grp1 family PH domains. *Embo J* **23**, 3711-3720 (2004). **c**, Annotated BLAST alignment of the C-terminal Rab5 binding domain of Rabenosyn-5 with the homologous core of the central Rab4 binding domain. The observed secondary structure applicable to both sequences is shown above the alignment. **d**, Ribbon and space filling representations of the Rbsn<sub>458-503</sub> structure. **e**, Superposition of Rbsn<sub>458-503</sub> with Rbsn<sub>440-503</sub> from the complex with Rab4 and Rbsn<sub>728-784</sub> from the complex with Rab22.