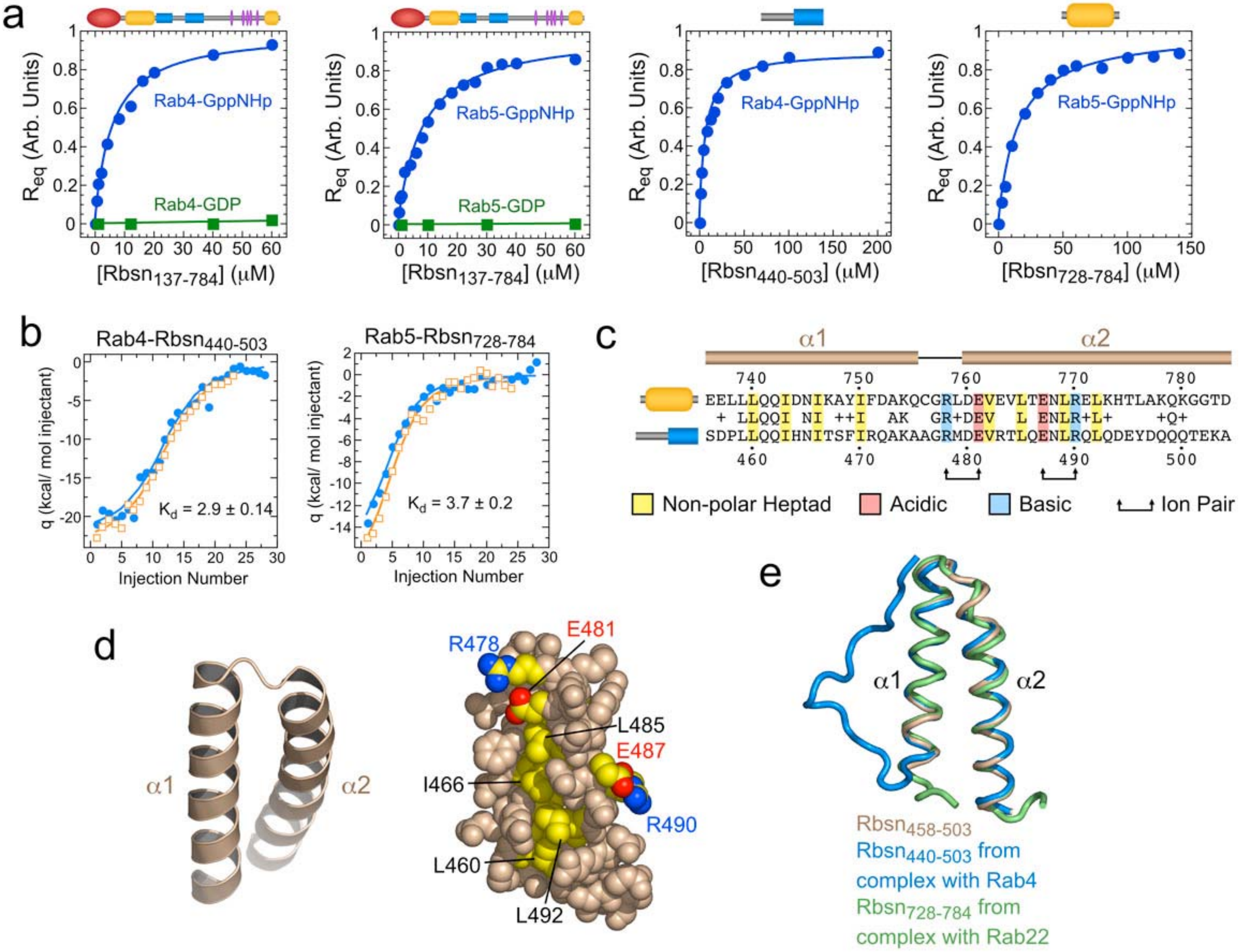

Supplementary Figure 4. Mapping and structure of the Rabenosyn-5 Rab binding domains

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 $Rbsn_{458-503}$ structure. **e,** Superposition of $Rbsn_{458-503}$ with $Rbsn_{440-503}$ from the complex with Rab4 and $Rbsn_{728-784}$ from the complex with Rab22.