

Appendix

Structure of the RZZ complex and molecular basis of Spindly-driven corona assembly at human kinetochores

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Appendix Figure S1 *Comparison of structural homologs of RZZ subunits*

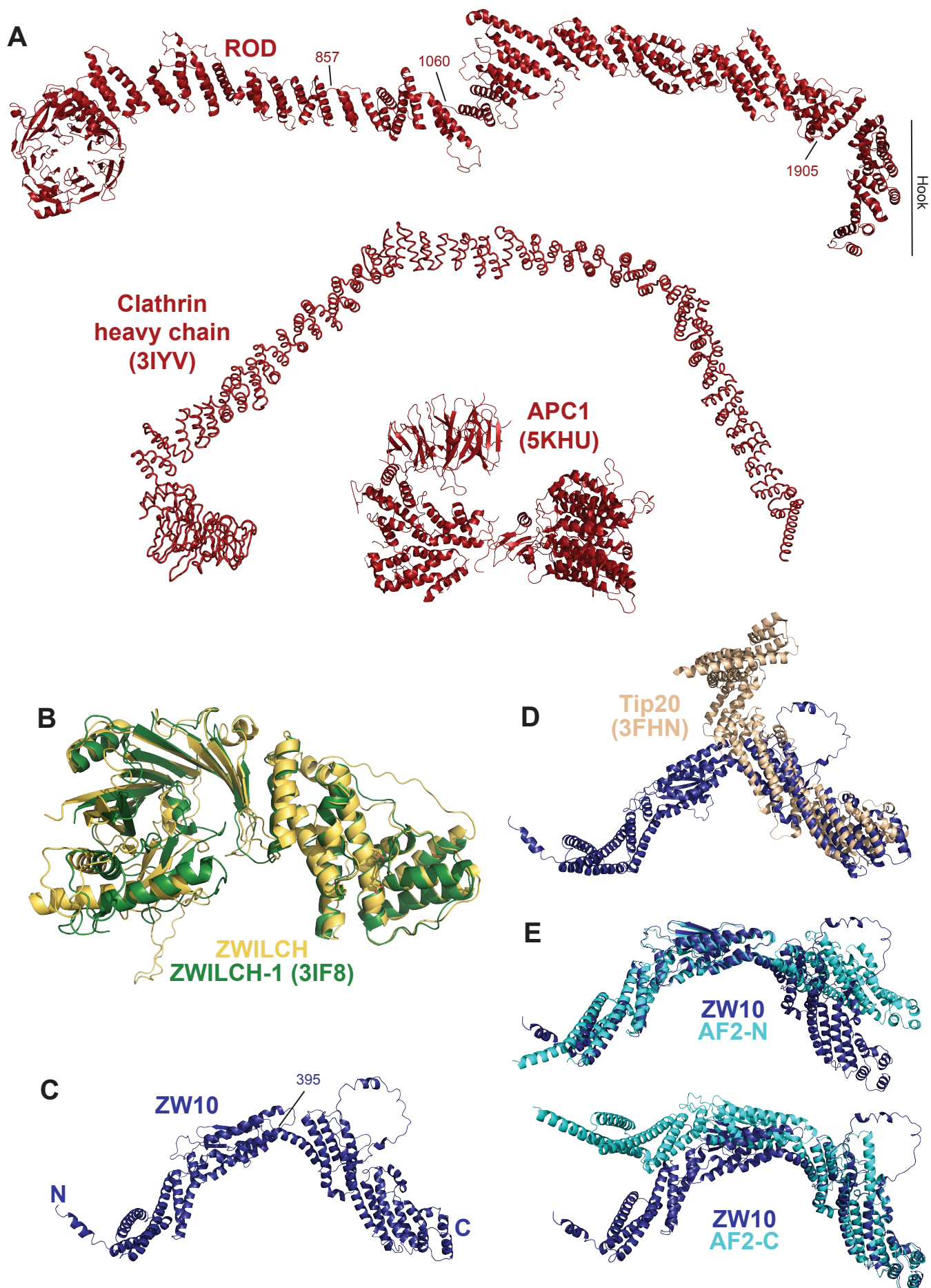
(A) Cartoon model of ROD and of the indicated proteins that share the same structural organization of ROD. PDB ID codes are included. (B) Structural superposition of Zwi1 in our cryo-EM reconstruction (yellow) and of the previously published crystal structure of Zwi1 (green) (Civril et al., 2010). (C) Cartoon model of ZW10. (D) Cartoon model of the superposition of ZW10 with Tip20, a related *S. cerevisiae*'s homolog. The interdomain angle of the N- and C-terminal domains is very different in the two structures. (E) AF2 prediction of ZW10 (cyan) superposed to the experimental model of ZW10. The N- and C-terminal domains, independently superposed, are predicted almost perfectly. In the absence of other restraints (as they may exist in the RZZ complex), only the hinge angle is different.

Appendix Figure S2 *Multiple sequence alignment of ROD and ZW10*

ROD and ZW10 sequences from *Caenorhabditis elegans* (Ce), *Drosophila melanogaster* (Dm), *Xenopus tropicalis* (Xt), *Danio rerio* (Dr), *Bos taurus* (Bt), *Mus musculus* (Mm), and *Homo sapiens* (Hs) were aligned with MAFFT (Katoh et al., 2002) and visualized with software developed in house. The secondary structure of the two RZZ subunits (straight black lines on green, β -strands; loopy black lines on red, helices; grey, coils) is displayed above the aligned sequences. For ROD, note in the second row a helical hairpin discussed in the text that lines the farnesyl-binding cavity, and the corresponding short deletions in species (Ce and Dm) where Spindly is not farnesylated.

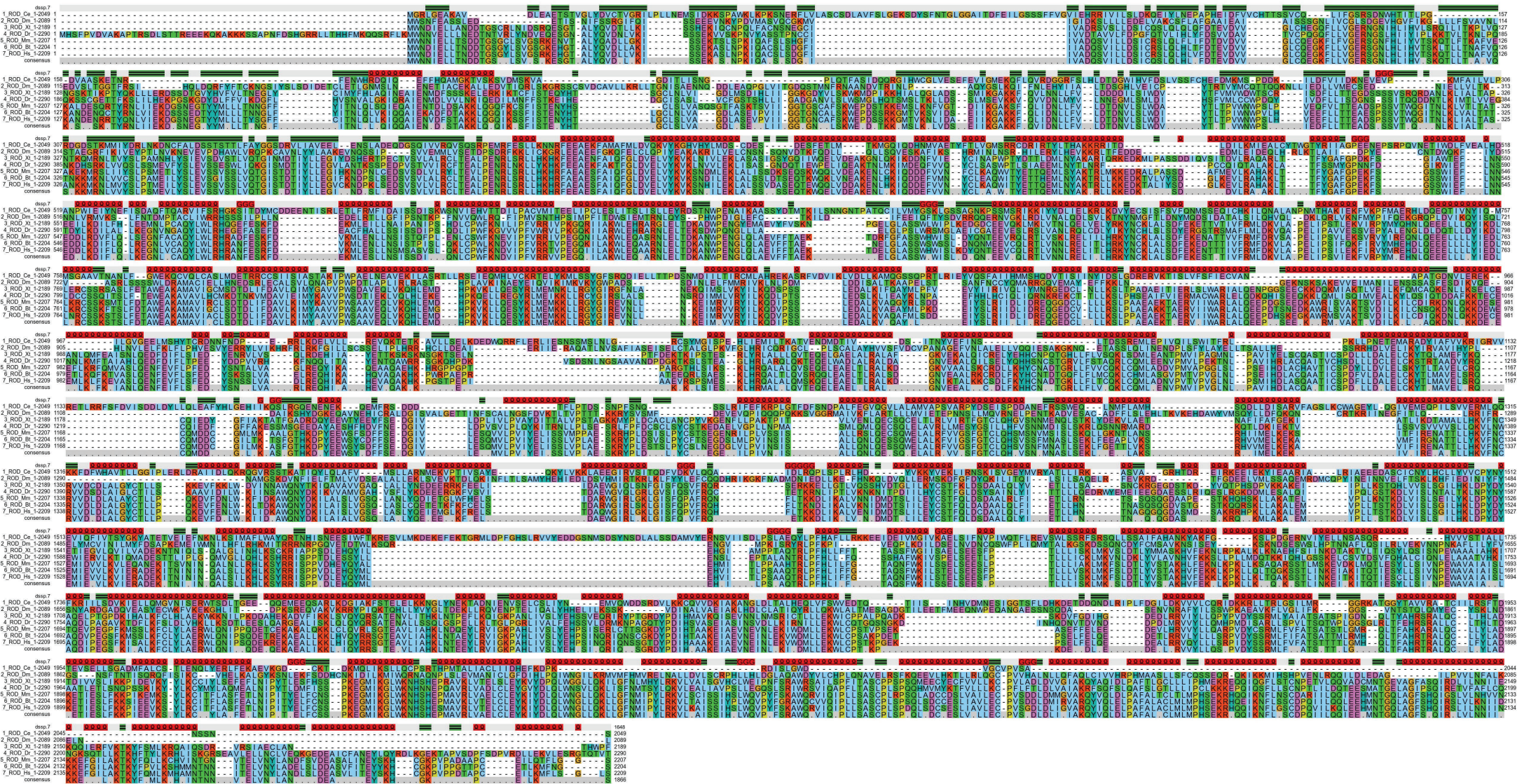
Appendix Figure S3 *Additional biochemical experiments*

(A) Heat-induced filaments of the indicated species. Note that Spindly^{F(250-605)} and Spindly^{F(Δ 276-306)} may show a combination of slight precipitation and filamentation when filamentation is induced with heat. The same positive control is also shown in [Figure EV3D](#). (B) Size-exclusion chromatography of Spindly^{F(Δ 276-306)}, RZZ, and their complex. (C) Titration of ^{mCh}RZZ and Spindly^F at the indicated concentrations.



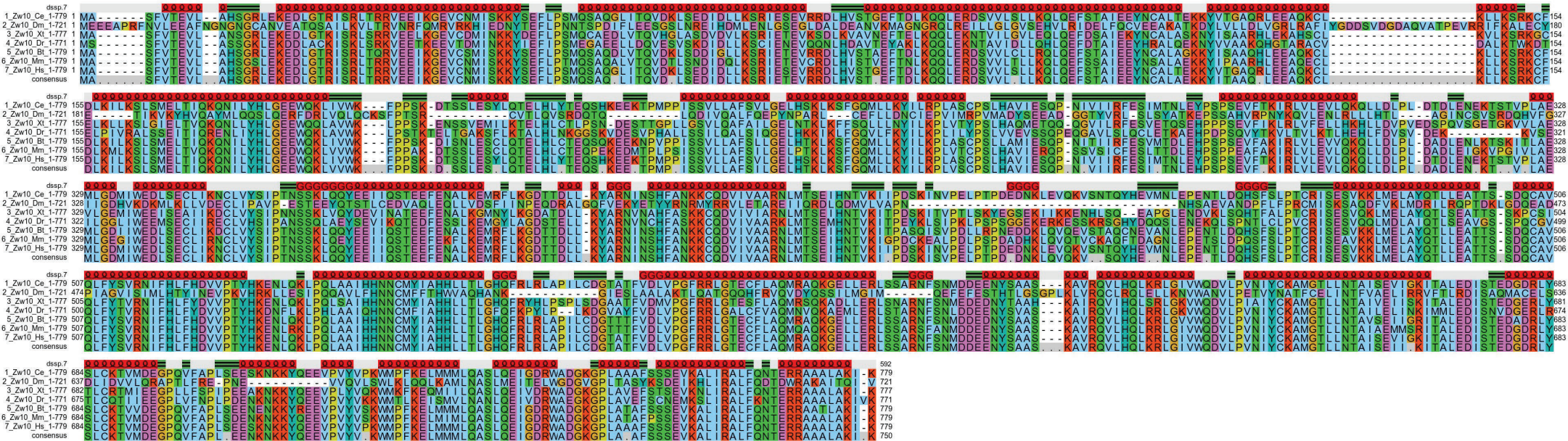
Appendix Figure S1

Multiple sequence alignment of ROD

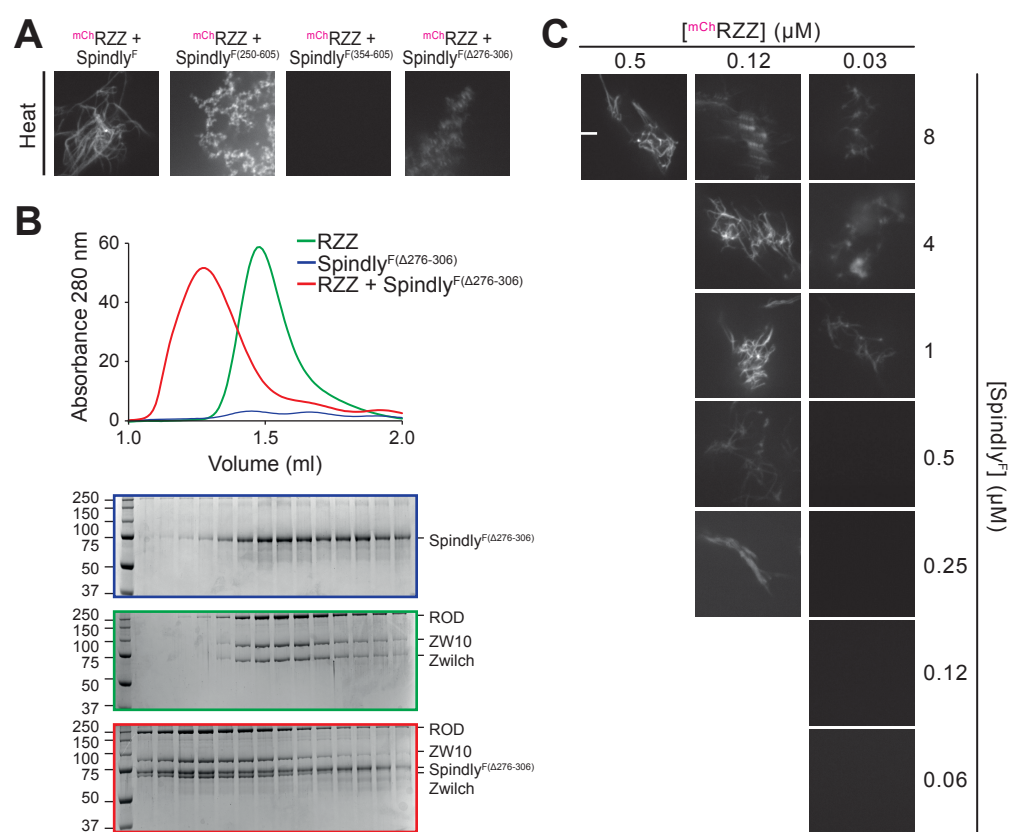


Appendix Figure S2

Multiple sequence alignment of ZW10



Appendix Figure S2



Appendix Figure S3