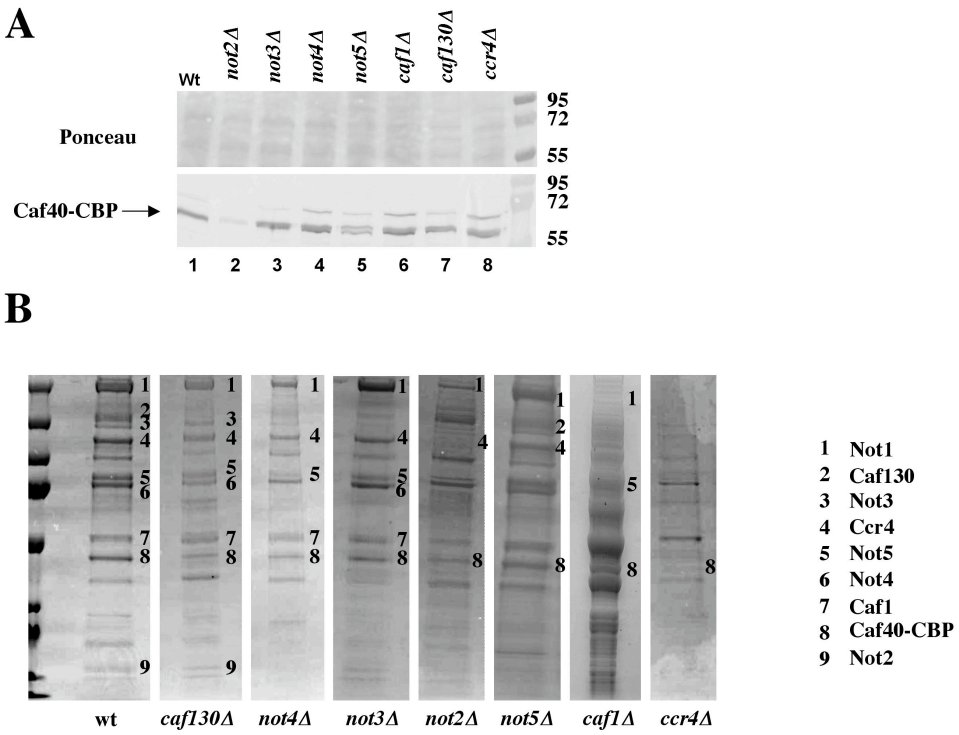
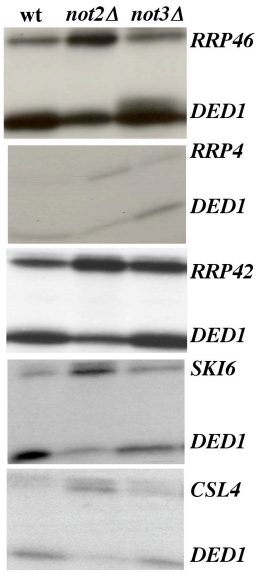


Supplementary Figure 1

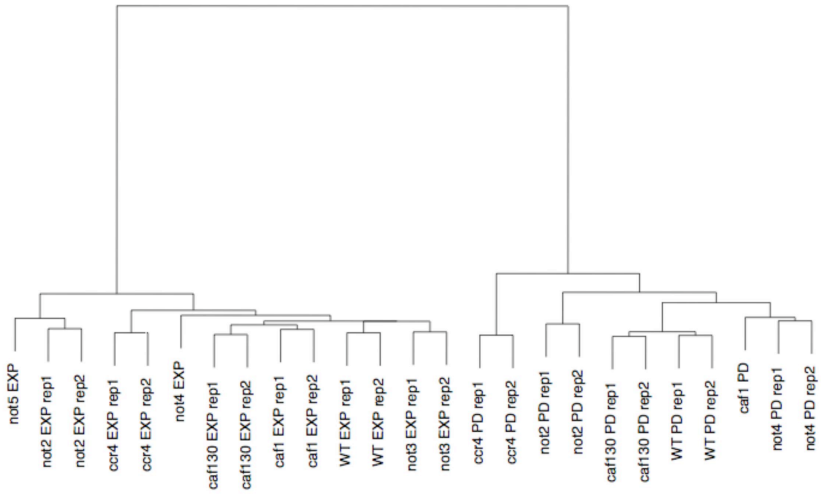
Supplementary Fig.1. Tandem affinity purification of Caf40p from wild-type and mutant strains reveals distinct roles of the other subunits for Ccr4-Not complex integrity. 20 l of wild-type and mutant cells expressing Tap-tagged Caf40p were grown exponentially for total protein extract preparation. (**A**) 50 µg of total extract were separated by SDS-PAGE. Equal loading was confirmed by ponceau staining of the membrane (upper panel) followed by western blot analysis with antibodies against CBP (lower panel) to evaluate the expression of tagged Caf40p in the different strains. (**B**) Equivalent amounts of total protein extract (2 g) from all strains were bound to an IgG column. After cleavage by TEV protease, the eluted proteins were bound to a calmodulin column. Bound proteins were finally eluted by EGTA and an equivalent proportion of each eluate was analyzed by PAGE on a 4-12% gradient gel stained with coomassie The proteins identified by mass

spectrometry in the gel at the indicated positions, and/or by western blot in a separate analysis of eluted material, are indicated. The data for purification from the wild-type and *not4Δ* has already been published (Lenssen et al. 2007), and is added here for comparative purposes.



Supplementary Figure 2

Supplementary Fig.2. S1 analysis confirms an increase of the various exosome mRNAs specifically in *not2Δ*. Total cellular RNA was extracted from the indicated strains grown exponentially, and 30 µg were analyzed by S1 digestion for the levels of *RRP46*, *RRP4*, *RRP42*, *SKI6* and *CSL4* mRNAs as indicated. *DED1* was measured as an internal control for each hybridization reaction.



Supplementary Figure 3

Supplemenatary Fig.3. Cluster dendogram of the correlation between samples. Distance was defined as 1 – Pearson's correlation coefficient. Agglomeration was made using Ward's minimum variance algorithm.

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