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Supplementary data for:

C2ORF29/CNOT11 and CNOT10 form a new module of the CCR4-NOT complex

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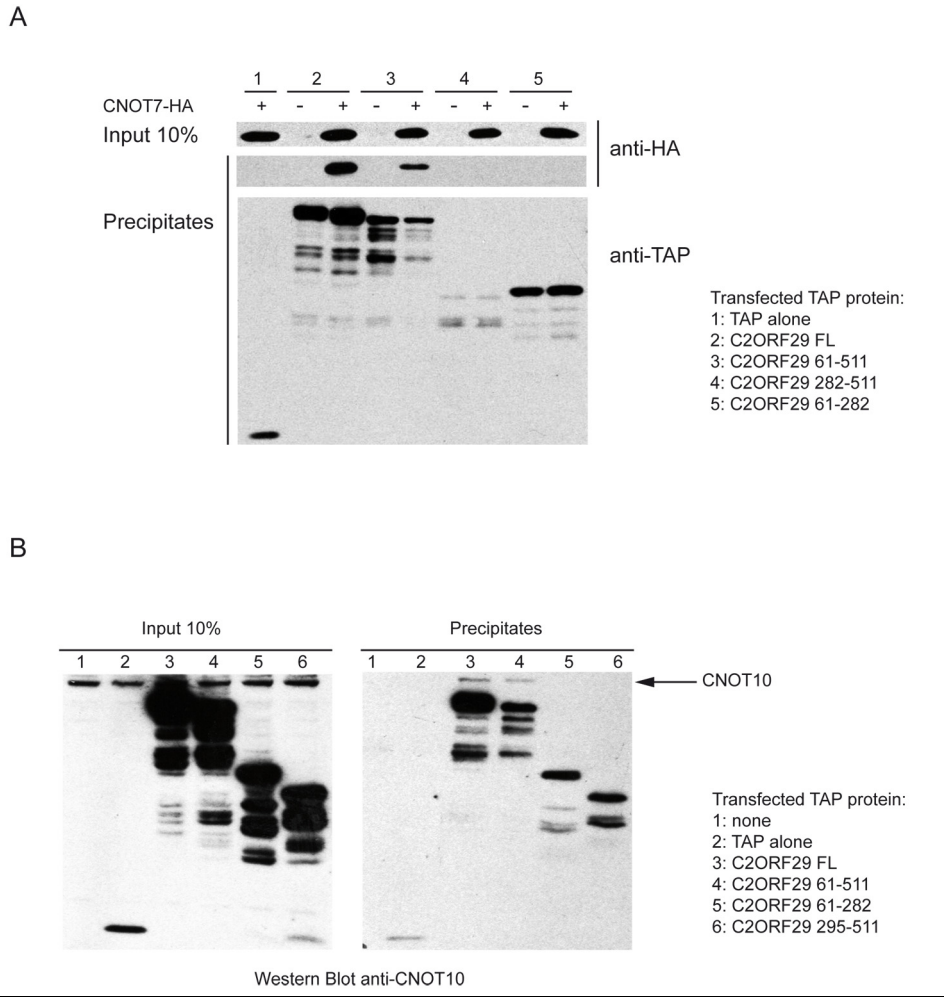
Supplementary Table 1: Identification of the proteins associated with CNOT7-TAP by mass spectrometry.

For each bands visible in Fig.1, the proteins identified with more than 2 unique peptides are indicated. The numbers of total peptides and of unique peptides obtained in the mass spectrometric analysis are indicated as well as the name of the yeast CCR4-NOT orthologue. NR: not relevant; X: no homologue in the yeast genome.

Band number	Protein	Total peptides	Unique peptides	Yeast orthologue
1	CNOT1	74	37	Not1
2	CNOT1	54	31	Not1
3	CNOT1	59	33	Not1
4	CNOT3	7	5	Not3/Not5
5	CNOT10	21	11	X
6	CNOT2 CNOT6L CNOT6	26 15 4	9 8 3	Not2 Ccr4 Ccr4
7	CNOT2 CNOT6 CNOT6L	12 9 6	6 6 4	Not2 Ccr4 Ccr4
8	β tubulin	7	6	NR
9	CNOT2 C2ORF29	10 4	5 3	Not2 X
10	CNOT7 CAPZA1	26 7	7 4	Caf1 NR
11	CNOT9/RQCD1	30	13	Caf40



Supplementary Figure 1: The C-terminal conserved region of C2ORF29 is not sufficient for its association with the CCR4-NOT complex

(A) HEK293 cells were co-transfected with plasmids expressing the TAP tag alone, the full-length C2ORF29-TAP protein, or deletion derivatives thereof,, and a plasmid expressing CNOT7-HA or the empty pCIneo vector. Cells were lysed and the TAP-tagged proteins were precipitated with IgG Sepharose beads. Co-precipitation of CNOT7-HA was analyzed by western blotting. Note that the C2ORF29-TAP protein corresponding to amino acids 282 to 511, which corresponds in sequence alignment to the length of the *Dm* C2ORF29 protein, is not well expressed in human cells.

(B) HEK293 cells were transfected with plasmids expressing the TAP tag alone, the full-length C2ORF29-TAP protein, or deletion derivatives thereof. After cell lysis, the TAP-tagged proteins were precipitated with IgG sepharose. Co-precipitation of CNOT10 was visualized by western blotting with a rabbit antiserum directed against endogenous CNOT10. Note that the rabbit antiserum recognizes also the TAP-tagged proteins due to the presence of a protein A domain in the tag.

Supplementary Table 2: Plasmids

Plasmid	Vector	Insert	Comments
GST-CAF1	pGEX-2T	Encodes a GST-murine CNOT7 fusion protein	Kind gift from Dr. L. Corbo (Centre Léon Bérard, Lyon, France), our stock pBS2190
pBS2567	pCIneo (Promega) cut XhoI-XmaI	Encodes a murine CNOT7-TAP fusion protein	PCR cut XhoI-NcoI amplified with OBS800 and OBS801 from pBS2190
			and NcoI-XmaI fragment from plasmid pBS1479 [1]
pBS3620	pCIneo (Promega) cut XhoI-XmaI	Encodes the TAP tag	PCR cut XhoI-XmaI amplified with OBS3715 and OBS3716 from pBS1479 [1]
MGC5752134	pCMV-SPORT6	Human C2ORF29 cDNA	From the Mammalian Gene Collection [2], our stock pBS3424
pBS3554	pCIneo (Promega) cut XhoI-XmaI	Encodes a human C2ORF29-TAP fusion protein	PCR cut XhoI-NcoI amplified with OBS3521 and OBS3522 from pBS3424
			and NcoI-XmaI fragment from pBS1479
pBS2594	pCIneo cut XhoI-XmaI	Encodes a murine CNOT7-HA protein	XhoI-NcoI fragment from pBS2567
			and AflIII-XmaI HA tag
pBS2560	pEGFP-C1 (CLONTECH)	Encodes a GFP-human CNOT6 fusion protein	described in [3]
pB27		Encodes a LexA Binding Domain	From Hybrigenics, our stock pBS4411
pP6		Encodes a Gal4 Activating Domain	From Hybrigenics, our stock pBS4403
MGC5266600	pBluescriptR	Human CNOT1 cDNA	From the Mammalian Gene Collection [2], our stock pBS3422
MGC3955575	pOTB7	Human CNOT10 cDNA	From the Mammalian Gene Collection [2], our stock pBS3430
pBS4568	pB27 (Hybrigenics) cut SfiI	Encodes a LexA Binding Domain - human C2ORF29 (amino acids 61 to 511) fusion protein	PCR cut SfiI amplified with OBS5380 and OBS5381 from pBS3424
pBS4569	pB27 (Hybrigenics) cut SfiI	Encodes a LexA Binding Domain - human CNOT10 protein	PCR cut SfiI amplified with OBS5382 and OBS5383 from pBS3430
pBS4625	pB27 (Hybrigenics) cut SfiI	Encodes a LexA Binding Domain – human CNOT1 (amino acids 1 to 331) protein	PCR cut SfiI amplified with OBS5376 and OBS5604 from pBS3422
pBS4570	pP6 (Hybrigenics) cut NotI-BamHI	Encodes a Gal4 Activating Domain – human C2ORF29 (amino acids 61 to 511) fusion protein	PCR cut NotI-BamHI amplified with OBS5386 and OBS5387 from pBS3424
pBS4571	pP6 (Hybrigenics) cut NotI-BamHI	Encodes a Gal4 Activating Domain – human CNOT10 fusion protein	PCR cut NotI-BamHI amplified with OBS5384 and OBS5385 from pBS3430

pBS4626	pP6 (Hybrigenics) cut NotI-BamHI	Encodes a Gal4 Activating Domain – human CNOT1 (amino acids 1 to 240) fusion protein	PCR cut NotI-BamHI amplified with OBS5607 and OBS5608 from pBS3422
pBS4409	pP6 (Hybrigenics) cut SfiI	Encodes a Gal4 Activating Domain – human CNOT7 fusion protein	From Hybrigenics
pTet-BBB		Transcribes a β-globin mRNA reporter	Kindly provided by Dr. Ann-Bin Shyu (The University of Texas Medical School, Houston, Texas, USA), [4], our stock pBS2894
pBS3809	pCIneo (Promega) cut NheI-XmaI	Encodes a human C2ORF29 amino acids 61 to 511-TAP fusion protein	PCR cut NheI-NcoI amplified with OBS4004 and OBS3522 from pBS3424 and NcoI-XmaI fragment from pBS1479
pBS3811	pCIneo (Promega) cut NheI-XmaI	Encodes a human C2ORF29 amino acids 61 to 282-TAP fusion protein	PCR cut NheI-NcoI amplified with OBS4004 and OBS4006 from pBS3424 and NcoI-XmaI fragment from pBS1479
pBS3810	pCIneo (Promega) cut NheI-XmaI	Encodes a human C2ORF29 amino acids 282 to 511-TAP fusion protein	PCR cut NheI-NcoI amplified with OBS4005 and OBS3522 from pBS3424 and NcoI-XmaI fragment from pBS1479
pBS4134	pCIneo (Promega) cut NheI-XmaI	Encodes a human C2ORF29 amino acids 295 to 511-TAP fusion protein	PCR cut NheI-ClaI amplified with OBS4670 and OBS3522 from pBS3424 and ClaI-XmaI fragment from pBS3554

Supplementary Table 3: Sequences of DNA and RNA oligonucleotides

Name	Sequence
R2 RNA	5'P-CCUAUAGUGAGUCGUAUUAA-ddC3'
OBS800	CCGCTCGAGTATGCCAGCAGCAACCGTAGATC
OBS801	TTGCCCATGGCTGACTGCTTGCTGGCTTCCT
OBS3715	CTGCTCGAGACCATGGAAAAGAGAAGATGGAAA
OBS3716	CTGCCCGGGTCAGGTTGACTTCCCCGCGGA
OBS3521	ATTGCTAGCGGATCCAGGTTGATGCCCGGCGGAGG
OBS3522	TTGTCTAGAGCCATGGCTTTTGACATTTCGGTCTCAG
OBS4004	ATTGCTAGCGGATCCAGGTTGATGAGCTTGACCCCGAAGG
OBS4005	ATTGCTAGCGGATCCAGGTTGATGTTTATTCGTCCACCGCCTC
OBS4006	TTGTCTAGAGCCATGGCTGGTCGAAAATGGCTTTCAAT
OBS4069	AATTCACTCCTCAGGTGCAG
OBS4420	CTGGAATTCGCGGTTAAATTAATACGACTCACTATAGGT
OBS4437	CTGGAATTCGCGGTTAAA
OBS4670	ATTGCTAGCGGATCCAGGTGGATGGAGGATGAACTTGCTTGGCTAA
OBS5376	ATCGGCCGGACGGGCCATGAATCTTGACTCGCTCTCG
OBS5380	ATCGGCCGGACGGGCCATGAGCTTGACCCCGAAGGAG
OBS5381	ATCGGCCCCAGTGGCCCTTATTTTGACATTTCGGTCTCAGAAG
OBS5382	ATCGGCCGGACGGGCCATGGCTGCAGACAAGCCT
OBS5383	ATCGGCCCCAGTGGCCCTTACTTTCTCTGCACAGTGGTGA
OBS5384	ATCGCGGCCGCCATGGCTGCAGACAAGCCT
OBS5385	ATCGGATCCTTACTTTCTCTGCACAGTGGTGA
OBS5386	ATCGCGGCCGCCATGAGCTTGACCCCGAAGGAG

OBS5387	ATCGGATCCTTATTTTGACATTTCGGTCTCAGAAG
OBS5604	ATCGGCCCCAGTGGCCCTTATGCTCCATCACTTTTATCTTTCC
OBS5607	ATCGCGGCCGCCATGAATCTTGACTCGCTCTCG
OBS5608	ATCGGATCCTTATGCTCCATCACTTTTATCTTTCC

Supplementary Table 4: Sequences of siRNAs

siRNA target	siRNA Sequences
CNOT1	CUAUAAAGAGGGAACGAGA
	GGCCAAAUUGUCUCGAAUA
	CCAGAAACUUUGGCGACAA
	CAAGUUAGCACUAUGGUAA
CNOT2	CAUGAGCAGUUCAGGGUUA
	GAUUGGACCUUUCAGAUUU
	UAACCCAACUCCAUUAAUA
	GAAGAACGAGUAUGGAUUA
CNOT7	CAGCUAGGACUGACAUUUA
	GGAGAAUUCAGGAGCAAUG
	UCAUAGCGGUUACGACUUU
	GUUAGAGCUGGAACGGAUA
CNOT8	CCAUAGAUCUCCUUGCUAA
	AAUAUCAGCUUCUGCGGUG
	GAGAAUAGCCAGGUUAUCU
	UUUCGUAGUUCCAUAGAUU
CNOT9	CACCAGCCUUGGAGUUAUU
	UGAAGCAUGUAGUGAGAUG
	UGACAGCACACCAGUCUAA
	CCUUUAUGUUUGCGAAUUA
CNOT10	UACGAGCUUAUAUCCAAAUGA
	CAGGGAAGAACCCGAGTCGAA
	GUGUACGUUACUAACCAAUAA
	UUGGUCAAGGCUAUCAUCGUA
C2ORF29	GGAUGAACUUGCUUGGCUA
	GUGUGGAGAUCAAACGAAU
	GGAAGUUGUAAAUCGACUA
	AUUCAGUAGGAUACGAGAA

SUPPLEMENTARY REFERENCES

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