Supplemental data : Lacroix, El Messaoudi et al.

Supplemental Material and Methods

Antibody generation

Anti-COPR5 polyclonal antibody (EF1) was generated (Eurogentec) in Rabbits against a peptide corresponding to the last 18 amino-acids of human COPR5 (-PYYSKMVFETGQFDDAED) (Fig. S3). Specific IgGs were further purified on NHS-bound peptide columns and concentrated at 0.2 µg/µl. This antibody was used at dilutions of 1:250 for western blots and 1:50 for immunofluorescence staining of fixed cells.

Glycerol gradient analyis. This analysis was performed as described in Le Cam et al., 1999.

Overlay/Far Western

Overlay was performed essentially as previously described (Mateescu et al., 2004). Briefly, H4 and H3 N-terminal synthetic peptides were spotted onto nitrocellulose membrane and incubated with either purified GST-COPR5 or GST proteins. After washings, retained GST-COPR5 was detected by immunoblotting with an anti-GST Ab. .

PRMT's Methylation assay

Methylation assay was performed for 1h at 30°C in 20 µl using either GST-COPR5 beads that have been incubated with U2Os nuclear extracts, or Flag immunoprecipitates (performed on nuclear extracts prepared from Flag-PRMT5 transfected cells), in the presence of different substrates (20µg of histones, 1µg of H4r or H3r, 5 µg MBP), 1µCi of S[methyl-3H]-Adenosyl-L-methionine (SAM) (10Ci/mmol, Perkin Elmer # Net 155250UC) and 10 µl of 2xMAB (100 mM Tris 8.5, 40 mM KCl, 20mM MgCl2, 500mM sucrose and 20mM β-SH added extemporanously). Histone methylation was analyzed by fluorography after separation of substrats on a 15 % SDS-PAGE, stained by coomassie blue. Signal was amplified for 30 min in enhancer (Amersham), dried and exposed between 1 and 3 days at –80°C.

Nucleosome reconstitution

Mono-Nucleosome particles were reconstituted *in vitro* around a DNA fragment of the *CCNE1* promoter encompassing the nu12 DNA region. DNA fragment was amplified by PCR from CCNE1-Luc reporter construct using the GC genomic amplification PCR kit (BD) and the following primers : nuCE f biot-GTAAAAGAACACGCCCCCCG and nuCEr TGTCGAGCCGGCTGCTCCTG. DNA fragment was purified on agarose gel and then bound to streptavidin beads M280 (Dynal). Oligo-bound beads were first incubated 15 min à 37°C in 10µl of 2M NaCl, 0,1mg/mL BSA and 5µg of histones prepared from HeLa cell nuclei using a DNA/histone molar ratio of1/1. Nucleosomes were then formed by salt jump dilution method

according to Owen-Hughes et al., 1999. Histones were prepared from Hela nuclei recovered in 100μl of 7M guanidinium, 20mM tris 7.5, 10mM DTT, and then dialyzed overnight at 4° C in storage buffer (10mM tris 7.5, EDTA 1 mM, βmercaptoethanol 5 mM, 2M NaCl).

Supplemental references

El Messaoudi, S., Fabbrizio, E., Rodriguez, C., Chuchana, P., Fauquier, L., Cheng, D., Theillet, C., Vandel, L., Bedford, M. and Sardet, C. (2006) PRMT4 / CARM1 is a cyclin E1 gene regulator. *Proc Natl Acad Sci USA,* **36** : 13351-13356

Herbst, A. and Tansey, W.P. (2000) HAM: a new epitope-tag for in vivo protein labeling. *Mol Biol Rep*, **27** : 203-208

Le Cam, L.., Polanowska,, J. Geng ,Y., Fabbrizio, E., Olivier, M., Philips, A., Ng Eaton, E. Classon, M. and Sardet, C. (1999) timimg of cyclin E gene expression depends on the regulated association of a bipartite repressor element with a novel E2F complex. *EMBO. J.* **18**: 1878-1885,

Mateescu, B., England, P., Halgand, F., Yaniv, M. and Muchardt, C. (2004) Tethering of HP1 proteins to chromatin is relieved by phosphoacetylation of histone H3. *EMBO Rep*, **5** : 490-496

Owen-Hughes, T., Utley, R.T., Steger, D.J., West, J.M., John, S., Cote, J., Havas, K.M. and Workman, J.L. (1999) Analysis of nucleosome disruption by ATP-driven chromatin remodeling complexes. *Methods Mol Biol*, 119 : 319-331

Supplemental Figures :

S1A

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ClustalW format http://www.bioinformatics.nl/tools/muscle.html
WUR MUSCLE (3.51) multiple sequence alignment


Homo            MDLQAAGAQAQGAAEPSRGPPLPSARGAPPSPEAGFATADHSGQERETEKAMDRLARGTQ
Macaca          MDPQAARAQARRAAEPSRGPPLPSAQEAPPSPEAGFATADHSSQERETEKAMDRLARGAQ
Bos             MDPPTAGAQSLGAAEQPRGLQLPSGREAPPSPGTAFAPADHSSQEKATENATDRLANGAQ
Mus             MDPQAA---------TGRGPGERSSQEA-PSAEAGFATADLSGRETETELAVDRLASGAQ
Rattus          MDPQAA---------TGPGPGEPSAWEA-PSAEAGLATADLSGGETETELDVDRLASGAQ
                **  :*            *    *.. * **. :.:*.** *. *  **   **** *:*

Homo            SIPNDSPARGEGTHSEEEGFAMDEEDSDGELNTWELSEG-TNCPPKEQPGDLFNEDWDSE
Macaca          SVPNDSPAQGEGTHSEEEGFAMDEEDSDGELNTWELSEG-TNCPPKEQPGDIFNEDWDLE
Bos             SIPHDSPAHGEGTHCEEEGFAEDDEDSDGEPSPWELSEGMSGCLPKEQAGDLFHEDWDLE
Mus             SIPADIPAHAEGPSSEEEGFAVEKE-ADGELYAWELSEG-PSCPPMEQAADLFNEDWDLE
Rattus          SIPTDVPTHAEGPSSEEEGFAMEKE-ADGELYAWELSEG-PACPPMEQASGLFNEDWDLE
                *:* * *:..**. .****** :.* :***  .****** . * * **...:*:**** *

Homo            LKADQGNPYDADDIQESISQELKPWVCCAPQGDMIYDPSWHHPPPLIPYYSKMVFETGQF
Macaca          LKADQGNPYDADDIQESISQELKPWVCCAPQGDMIYDPSWHHPPPLIPHYSKMVFETGQF
Bos             LKADQGNPYDADDIQGCLSQEVRPWVCCAPQGDMIYDPSWHHPPPLIPHYSKMVFETGQF
Mus             LKADQGNPYDADDIQGSISQEIKPWVCCAPQGDMIYDPSWHHPPPLIPHYSKMVFETGQF
Rattus          LKADQGNPYDADDVQGSISQEIKPWVCCAPQGDMMYDPSWHHPPPLIPHYSKMVFETGQF
                *************:* .:***:.***********:*************:***********

Homo            DDAED
Macaca          DDAED
Bos             DDAED
Mus             DDAED
Rattus          DDAED
                *****
```

S1B

```
MUSCLE (3.51) multiple sequence alignment


Homo            MDLQAAGAQAQGAAEPSRGPPLPSARGAPPSPEAGFATADHSGQERETEKAMDRLARGTQ
Gallus          ----MAATLEHTSFEEEQPPNKKETMTWKPTE----VKPKILGQTKADECLLKNILCVLD
                     *.:  : : * .. *   .:    *:     ....  ** .  *  :..:    :

Homo            SIPNDSPARGEGTHSEEEGFAMDEEDSD--GELNTWELSEGTNCPPKEQPGDLFNEDWDS
Gallus          SDSEGSEFSDISACEDDVSLHTDTDMEDLCGELS--RMPEDVTFPQQQTASTYEVEDWDK
                * .:.*   . .: .:: .:  * : .*  ***.   :.*... * :: ..    ****.

Homo            ELKADQGNPYDADDIQESISQELKPWVCCAPQGDMIYDPSWHHPPPLIPYYSKMVFETGQ
Gallus          ELEESECSPYDAGDLYCGSFQENNLLASYSFREDSLYNPCCHHAACLAFTLFVRMTEVGQ
                **: .: .****.*:  .  ** :  .. : . * :*:*. **.. *    .  : *.**

Homo            FDDAED
Gallus          FDDADE
                ****::
```

Figure S1: (A) alignment of mammalian COPR5 protein sequences.
(B) alignment of human and chicken COPR5 protein sequences

S1C

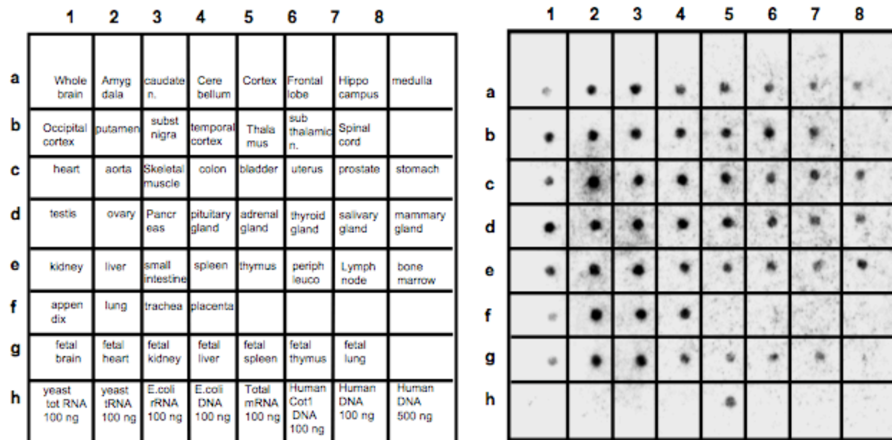

Figure S1C : COPR5, an ubiquitously expressed gene. Northern blot analysis of human *COPR5* mRNA expression. RNA Master Blot (Clontech) of different tissue RNAs, spotted as indicated in the left panel, was probed with a [^{32}P]labelled *COPR5* cDNA (right panel). Lanes a-f correspond to adult tissues. Lane g correspond to fetal tissues. Lane h corresponds to various controls.

S2

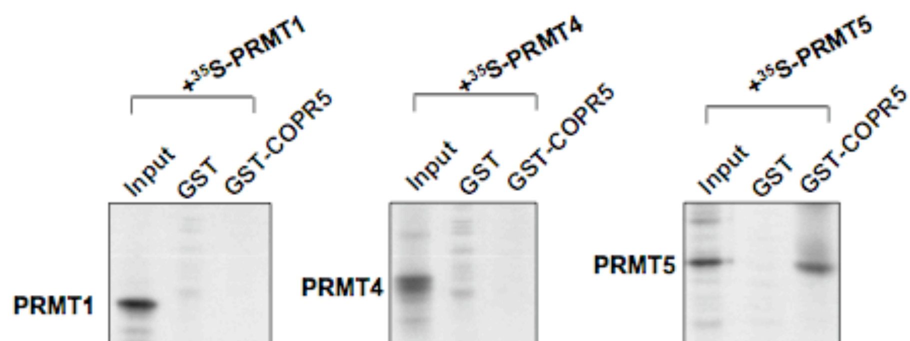

Figure S2: COPR5 is a PRMT5 binding protein. Similar amounts of GST and GST-COPR5 were bound to glutathione beads and then incubated in the presence of *in vitro* translated and [^{35}S]-radiolabed PRMT1, PRMT4 and PRMT5 proteins. The proteins bound by these beads, and a fraction of the PRMTs used as input were analyzed by fluorography.

S3

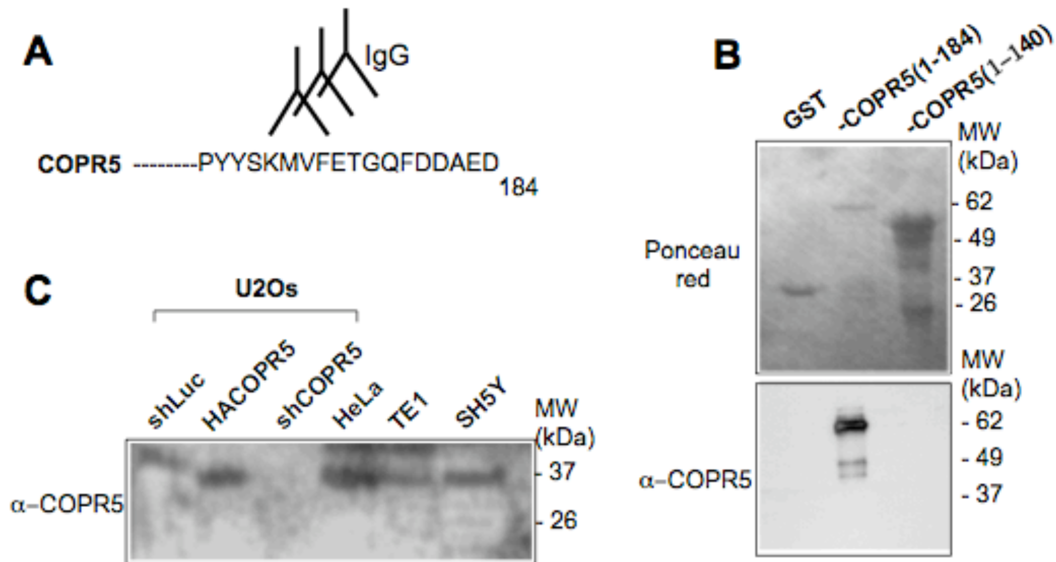

Figure S3: (A) Development of an a rabbit polyclonal antibody directed against the last 18 C-terminal of the Human COPR5 protein.

(B) Recombinant GST proteins (GST alone or GST fused to full length COPR5 (-COPR5(1-184)) or to a truncated version of COPR5 (-COPR5(1−140) deleted from the C-ter epitope recognized by the antibody) were probed by immunoblotting with the α−COPR5. **(C)** Imunoblotting analysis of various cellular extracts probed with α−COPR5. Cellular extracts were prepared from i/ U20s (osteosarcoma cell line) transduced with retroviral vectors coding for either control shRNA (shLuc), shRNA directed against COPR5 (shCOPR5), or for Ha-tagged COPR5; ii/ other cancer cell lines, Hela (Cervix carcinoma), SH5Y (Neuroblastoma) and TE1 (oesophageal carcinoma).

S4

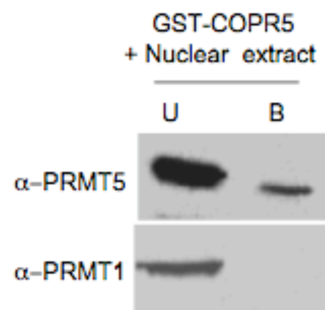

Figure S4 : PRMT5, but not the other H4R3 methyl-transferase PRMT1, is present on GST-COPR5 beads used in the methylation assay shown figure 2A. U2Os nuclear extracts were added to GST-COPR5 beads as in Fig.2A and after extensive washing, the bound (B) and unbound (U) fractions were probed for the presence of PRM5 and PRMT1 by immunoblotting using polyclonal α-PRMT5 and α-PRMT1 antibodies, respectively.

S5

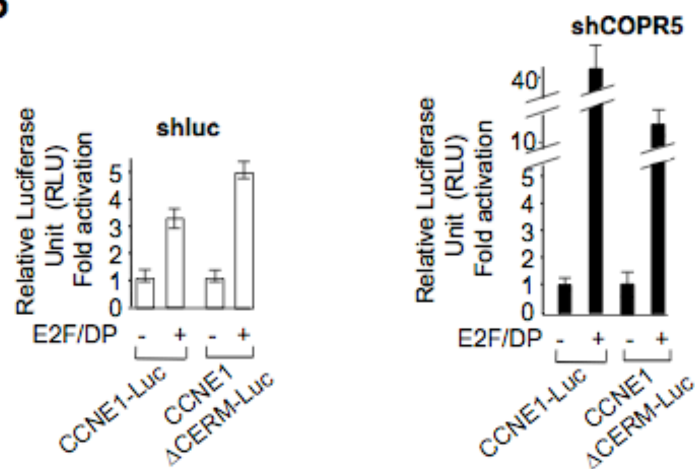

Figure S5 : shCOPR5-mediated depletion of COPR5 in U2Os cells potentiates the transactivation of the *CCNE1* promoter by E2F/DP. Cells infected either with shCOPR5 or shluc retroviral particles were transfected with the CCNE1ELuc or CCNE1ΔCERMLuc reporter constructs together with plasmids encoding E2F, DP and CMV-βgal. Results normalized to β-galactosidase activity are expressed as fold activation and are representative of two independent experiments performed in duplicates.