Evidences of a transcriptional co-activator function of cohesin STAG/SA/Scc3

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Running title: Cohesin STAG/SA/Scc3 is a transcriptional co-activator
SUMMARY

Cohesins hold sister chromatids together from DNA replication until they are segregated. Although cohesins Smc1, Smc3 and Scc1/Rad21 are involved in chromatid cohesion and other cellular processes, little is known about the other mitotic cohesin subunit Scc3/STAG. Here we describe that STAG/Scc3 may act as a transcriptional co-activator. STAG2 is able to enhance the activity of the TNFα, the CD69 and the human immunodeficiency virus (HIV) LTR promoters in a NF-κB-dependent manner. In addition, STAG2 interacts with the viral transactivator Tat and enhances the Tat-mediated activation of the HIV-LTR promoter. Moreover, STAG2 co-activates a multimeric NF-κB reporter construct and enhances the activity of the transactivation domain of p65/RelA in a Gal4 system. This function is dependent on one of the LXXLL co-activation motives present in this cohesin and is substantiated by the interaction of STAG2 with the p65 subunit of NF-κB. These results describe a novel activity for cohesins, suggesting a role for STAG/Scc3 in transcriptional regulation.
INTRODUCTION

During cell division, chromosomes are duplicated and segregated into daughter cells. After DNA replication sister chromatids are held together by protein complexes known as cohesins. This cohesion allows chromatids to be aligned on the mitotic spindle in metaphase until they are pulled to opposite spindle poles by microtubules at the onset of anaphase. In budding yeast, cohesin complexes are formed by four core subunits: two structural maintenance of chromosome proteins (Smc1 and Smc3), and two sister chromatid cohesion proteins (Scc1 and Scc3) (1). In vertebrates, two Scc3 homologues have been described (STAG1 and STAG2), which integrate independent cohesin complexes (2). Cohesins form a ring around the sister DNA molecules, with Smc1 and Smc3 forming a V-shaped heterodimer bridged by Scc1 (3,4). Scc3 binds to the complex by associating with the C-terminal domain of Scc1 (3). At the metaphase-anaphase transition, the anaphase promoting complex (APC) triggers the ubiquitin-dependent degradation of securin, activating the endopeptidase separase, which cleaves Scc1 leading to chromatid segregation (1,5). Scc1 cleavage is enhanced after its phosphorylation by Polo-like kinases (PLKs) (6). In vertebrates, removal of cohesins from the chromosomes is achieved through two steps. The bulk of cohesin is released from chromosomes at prophase by a mechanism involving cohesin phosphorylation by PLKs without cleavage of Scc1/Rad21 (7). At the onset of anaphase, the residual amount of cohesins, mainly located at the centromeric heterochromatin, are removed
from chromosomes by Scc1/Rad21 cleavage and sister chromatids are then separated (8). The persistent localization of cohesins at the centromeric region prevents precocious chromatid separation and is mediated by Swi6, a conserved heterochromatin protein (9). Swi6 is able to interact with Scc3, suggesting that recruitment of the cohesin complex is mediated through this interaction (10). In addition, cohesin is also recruited to other DNA regions to which Swi6 binds.

Although previous work on cohesins has been mainly focused on chromatid cohesion and segregation, certain cohesins have been implicated in different cellular processes (11). In this regard, caspase proteolysis of Rad21 can be observed in cells undergoing apoptosis in response to diverse stimuli, preceding chromatin condensation and amplifying the death signal (12,13). On the other hand, a post-translationally modified form of Smc3 carrying chondroitin sulphate chains is over-expressed and secreted in colon carcinomas (14). Cohesin function is also essential for DNA repair (15). In this context, Smc1 is an effector of the ATM/NBS pathway and forms part of the DNA damage response network (16,17) and Rad21 depletion is associated with increased radiation sensitivity (18). The possible function of Scc3/STAG, however, is not completely understood. Whereas depletion of Rad21 in Drosophila melanogaster cells results in premature sister chromatid separation and mitosis delay, much milder effects are observed in STAG-depleted cells (19). These results suggest that Scc3/STAG may be involved in other processes besides chromatid cohesion. Herein, we provide evidences suggesting that Scc3/STAG functions as a
transcriptional co-activator by a mechanism involving protein-protein interactions with transcription factors.


**EXPERIMENTAL PROCEDURES**

*Antibodies, reagents and plasmids*

Antibody against human STAG2 has been previously described (20). Anti-p300 was purchased from Oncogene (Boston, MA) and anti-p65 were from Santa Cruz (Santa Cruz, CA). PMA and the calcium ionophore A23187 were obtained from Sigma (St. Louis, MO). The pSTAG2 vectors derive from pCDNA3.1 and express the full length (1-1162) and mutant STAG2 proteins under the control of the CMV promoter. pSTAG2-ΔE (127-1162), pSTAG2-EEV (127-815) and pSTAG2-XX (415-1162) bear different deletions of the STAG2 protein. pSTAG2-m1, pSTAG2-m2 and pSTAG2-m12 were generated by directed mutagenesis of the LXXLL sites at aa 502 (m1), 540 (m2) or both (m12) in which the last two Leu were replaced by two Ala. pGFP-STAG2, pGFP-STAGm1 and pGFP-STAGm2 carry the same constructs linked to the C-terminal domain of the GFP. pCMV-Tat, pSTAG1 and pCMV2N3T-CBP are expression vectors for Tat, STAG1 and CBP, respectively. The reporter constructs pTNFα-Luc, pAIM-Luc and pHIV-LTR-Luc bear the TNFα promoter (~600), the CD69 promoter (~1400) and the HIV-LTR promoter, respectively upstream of the firefly luciferase gene. pLTR-ΔκB-Luc carries the HIV-LTR with a deletion of the NF-κB site. The reporter plasmids KBF-Luc and Gal4-Luc carry multimeric copies of responsive sites for the transcription factors NF-κB and Gal4, respectively. pGal4-p65, pGal4-p65mut, pGal4-cRel and pGal4-Sp1 express chimerical constructs bearing the
DNA-binding domain of Gal4 linked to the transcription activation domains of p65, p65mut (carrying a double mutation Ser’Ala at 529/536), cRel and Sp1, respectively. The reporter plasmid pRL-null bears the Renilla luciferase gene preceded by no promoter and was co-transfected in all points (0.5 µg) and used to normalize all the firefly luciferase values obtained. pGST-Tat, pGST-p65 and pGST-STAG2 express the glutathion-S-transferase linked to Tat, p65 or STAG2-ΔE, respectively.

**Cells and transfections**

The lymphoblastoid T cell line Jurkat, the erythroleukemic K562, the hepatoblastoma cell line HepG2 and the kidney embryonic 293 cells were maintained in RPMI (Jurkat and K562) and DMEM (HepG2 and 293), supplemented with 10% FCS, 2 mM L-Glutamine, 100 U/ml Penicillin and 100 µg/ml Streptomycin. The Stag2 KO cells were obtained from 129 Sv mouse embryonic stem cells in order to generate Stag2 KO mice (our unpublished results). Loss of STAG2 expression in KO cells was confirmed by Western blot (inset, Fig. 3D). Jurkat cells (2 × 10^6) were transfected with 0.2 µg of the reporter plasmid, 1 µg of pCDNA3.1 and the indicated amount of pSTAG2 using 4.5 µg of lipofectin (Invitrogen, Carlsbad, CA). HepG2, 129Sv wild type and Stag2 KO cells were transfected with the same amount of DNA using DOSPER (Roche, Mannheim, Germany) according to the manufacturer’s instructions. After 6 h, the medium was replaced, the cells were incubated for additional 18 h, lysed and luciferase activity was
measured.

**Pull down assays**

Pull down experiments were carried out as previously described (21). Briefly, GST fusion proteins were expressed in *E. coli* by induction with 0.1 mM IPTG at 30°C for 30 min. The cells were lysed in EBC buffer and the GST fusion proteins were purified using glutathione-sepharose 4B (Amersham Pharmacia Biotech, Uppsala, Sweden). Nuclear extracts of Jurkat cells stimulated for 60 min with 10 ng/ml PMA and 0.5 µM calcium ionophore were incubated overnight at 4°C with 10 µg of purified GST fusion protein immobilized on glutathione-sepharose. After washing, bound proteins were fractionated by SDS-PAGE and analyzed by western blot.

**Immunoprecipitation**

Nuclear pellet of Jurkat cells was extracted 1h on ice in buffer containing: 400mM NaCl, 20 mM Hepes pH 7.9, 1 mM EDTA, 1 mM EGTA, 1mM PMSF and protease inhibitors. For IP experiments, 100 µl of nuclear extract were mixed with 100 µl of nuclear extraction buffer and Nonidet NP 1/1000.
RESULTS

STAG2 co-activates transcription

To unveil the possible role of STAG2, the main Scc3 isoform in human tissue culture cells (2), we searched for short sequence homology with other proteins. Analysis of the STAG2 sequence revealed a glutamine-rich region in its C-terminal domain which is also present in transcriptional co-activators of the p160 family (SRC1, TIF-2 and RAC3) (Fig. 1). In addition, STAG2 presented three LXXLL motives, which are found in transcriptional co-activators, including CBP, p300 and the p160 family, and are necessary for their co-activation function (22).

To study whether STAG2 could act as a transcriptional co-activator, we analyzed the effect of STAG2 on the activity of three different reporter constructs bearing the proximal regions of the CD69 and TNFα promoters and the HIV long terminal repeat (LTR), which acts as a viral promoter driving transcription of the HIV RNA. When the constructs containing the HIV-LTR or the CD69 promoter were co-transfected into the T-cell line Jurkat along with the expression vector pSTAG2, a STAG2-mediated up-regulation of the transcriptional activity of HIV-LTR, but not of CD69 promoter, was observed (Fig. 2A). In contrast, when co-transfections were performed in the erythroleukemia cell line K562, over-expression of STAG2 resulted in the activation of both CD69 and TNFα promoters (fig.2B). Given that the activity of
CD69 and TNFα promoters is regulated by members of the NF-κB family in myeloid but not in lymphoid cells, these results suggested that the co-activation function of STAG2 was achieved through NF-κB elements of these promoters. To rule out the possibility that an increase in STAG2 expression may result in reduced cell viability, we included in the co-transfection experiments the non-inducible reporter plasmid pRL-null. Analysis of the Renilla luciferase activity showed similar results in the presence of different amounts of pSTAG2 up to 1 µg, indicating that the increased STAG2 expression was not interfering with cell viability (data not shown). We performed similar experiments transfecting expression vectors for the cohesin STAG1 and the co-activator CBP. Both STAG1 and CBP showed similar co-activation activity in Jurkat cells as STAG2 (Fig. 2C), suggesting that the ability of the latter to enhance the activity of the HIV-LTR transcription was conserved in its homologue STAG1 and was quantitatively similar to that exhibited by other co-activators like CBP.

The activity of the HIV-LTR promoter is dependent on the presence of both the NF-κB responsive element and the TAR site, which is recognized by the viral transactivator Tat to induce HIV transcription (23). To determine whether Tat activity was necessary for the enhancement of the HIV-LTR activity by STAG2, we transfected Jurkat cells with the HIV-LTR reporter plasmid and pSTAG2 in the absence (Fig. 3A) or presence (Fig. 3B) of a Tat expression vector. As shown in Fig. 3A and 3B, induction of the HIV-LTR activity by STAG2 was increased in the presence of Tat. Deletion of the NF-κB responsive element in the viral LTR (HIV-LTR-ΔκB) resulted in a
decrease in the ability of STAG2 to enhance the promoter activity, suggesting that STAG2 could act as a co-activator for this transcription factor. To study whether STAG2 could contribute to the induction of the HIV-LTR by Tat, we transfected Jurkat cells with the HIV-LTR reporter plasmid, along with Tat and/or STAG2 expression vectors. We observed that the ability of Tat to activate the HIV-LTR was increased in the presence of STAG2 (Fig. 3C). In addition, the activation of the HIV-LTR by Tat was higher in the wild type Stag-expressing cells than in the Stag2 knockout cells (Fig. 3D). To further investigate the involvement of STAG2 in HIV transcription, we performed pull-down assays with GST-Tat. As shown in Fig. 3E, STAG2 interacted with Tat in Jurkat cell extracts. All these data strongly suggest that STAG2 enhances the ability of Tat to induce the HIV promoter.

**STAG2 co-activates the NF-κB transcription factor**

Since the NF-κB responsive elements appeared to be necessary for the induction of HIV-LTR as well as the CD69 and TNFα promoters, we studied whether STAG2 could enhance NF-κB-driven transcription in an independent promoter context. We transfected Jurkat cells with a reporter construct bearing three tandem NF-κB responsive elements (pKBF-Luc) along with pSTAG2. Analysis of the reporter construct showed an induction of NF-κB-driven transcription in the presence of increased STAG2 expression (Fig. 4A). To find out whether the effect of STAG2 on NF-κB-mediated transcription was specific of the cell type employed, we transfected
the hepatic cell line HepG2 with pKBF-Luc in the presence or absence of pSTAG2, obtaining similar results.

We confirmed the co-activation of NF-κB by STAG2 using the Gal4 transcription system, which allows the study of transcription factor activities independently of endogenous factors. Jurkat cells were transfected with a reporter construct in which luciferase expression is driven by four tandem copies of the responsive element for the yeast transcription factor Gal4 (pGal4-Luc), along with expression vectors containing chimeras of the DNA binding domain of Gal4 linked to the transcription activation domain of the NF-κB subunits p65, cRel or the unrelated transcription factor Sp1. These constructs are constitutively active and are not regulated by the signaling pathways that govern the activation of the endogenous transcription factors. Co-transfection of the STAG2 expression vector increased the activation mediated by Gal4-p65, but not by a mutant Gal4-p65 form in which the transcriptional activity has been abolished (Fig. 4B). In addition, STAG2 co-activated the transcription mediated by Gal4-cRel but not by Gal4-Sp1. These results demonstrate that STAG2 selectively enhances NF-κB-mediated transcription independently of other stimuli.

To determine whether STAG2 could interact with the transcription machinery components, we performed pull down assays using nuclear extracts of Jurkat cells and a GST-p65 chimera in which the p65 transcription activation domain is linked to the glutathione S-transferase. As shown in Fig. 5A, STAG2 co-precipitated with GST-p65, but not with GST alone, suggesting that STAG2 and p65 could form part of the
same transcriptional complexes. The co-activator p300 was used as a positive control. Accordingly, when we analyzed the ability of the two proteins to interact with each other endogenously, we observed that STAG2 interacted with p65 as shown by immunoprecipitation experiments using an anti-p65 antibody (Fig. 5B).

One of the LXXLL motives of STAG2 is necessary for its co-activation activity

To study the contribution of the different domains of STAG2 to its co-activation activities, we transfected Jurkat cells with the reporter construct pGal4-Luc, pGal4-p65 and expression vectors bearing different deletions or point mutations of the STAG2 cDNA. As shown in Fig. 6A, deletion of the first N-terminal 127 aa (pSTAG2-ΔE) and the last 348 aa (pSTAG2-EEV) had no effect on the co-activation of p65 by STAG2. In addition, a construct lacking the first 415 aa of STAG2 (pSTAG2-XX) showed the same co-activation capacity as the wild type form. The region spanning aa 415-815 carries two LXXLL motifs (aa 502-506 and 540-544), which are essential for the activity of other co-activators, including SRC1, TIF-2 or p300 (22). To determine the relative contribution of these LXXLL motives to the co-activation activity of STAG2, we mutated these motives separately (pSTAG2-m1 and pSTAG2-m2) or together (pSTAG2-m12). Whereas mutation of the LPQLL motif (m1, aa 502-506) showed no effect, mutation of the LDALL motif (m2, aa 540-544), either alone or together with LPQLL (m12), resulted in a strong decrease in the co-activation activity of STAG2 (Fig. 6A). To rule out the possibility that the STAG2-m2 construct could be deficiently
expressed or have decreased stability, we transfected Jurkat cells with pGal4-Luc, pGal4-p65 and expression vectors bearing the STAG2 cDNA linked to the GFP. Again pGFP-STAG2-m2 showed less co-activation efficiency than the wild type and the pGFP-STAG2-m1 constructs (Fig. 6B). Western blot analysis of 293 cells transfected with the different constructs showed similar expression levels of all the GFP-STAG2 fusion proteins (Fig. 6C), indicating that the decrease of transcription enhancement observed in the presence of the mutated LDALL motif was due to a loss of the co-activation function rather than to a loss of expression of STAG2.
DISCUSSION

Transcriptional co-activators are multifunctional complexes that are recruited by sequence-specific transcription factors and enhance transcriptional activation of target genes (24). The p160 family of co-activators (SRC1, TIF-2 and RAC3) was initially identified as nuclear receptor-interacting proteins that were able to enhance receptor-mediated transcriptional activation (25). p160 co-activators potentiate transcription of the HIV-LTR promoter stimulated by the viral transactivator Tat (26). The ability of p160 proteins to enhance the HIV-LTR activity is dependent on the presence of the transcription factor NF-κB and is mediated by some of the LXXLL motives of the co-activator (26). Interestingly, STAG2 is able to enhance the Tat-stimulated activity of the HIV-LTR promoter in a NF-κB-dependent manner and to interact with the viral transactivator. This cohesin also enhanced the activity of two other promoters, CD69 and TNFα, in K562 cells, where their activity is dependent on NF-κB (27).

Interestingly, STAG2 did not enhance the CD69 promoter activity in Jurkat cells, in which the promoter is rather dependent on Egr, ATF/CREB and AP-1 transcription factors (28), supporting the idea of specific co-activation of NF-κB by STAG2. In addition, STAG2 co-activates NF-κB-mediated transcription through one of its LXXLLL motives and is able to interact with this transcription factor.

LXXLL co-activation motives are also present in the other two human homologues of Scc3 (STAG1 and STAG3), but not in other mitotic cohesins Rad21,
SMC1 and SMC3 (Fig. 7A). In addition, the positions of the three LXXLL co-activation motives found in human STAG2 are conserved in other vertebrate STAG2 proteins and LXXLL motives can be found in the Scc3/STAG isoforms of different organisms, ranging from yeast to plants or insects (Fig. 7B). Taken together, our results demonstrate that the 540 LXXLL co-activation motif is essential for the enhancement of transcription mediated by STAG2 and suggests that this conserved motif may have a key role in other Scc3/STAG2 activities.

This is the first description of a role of a cohesin in transcriptional regulation. Although alternative activities had been reported for cohesins Smc1, Smc3 and Rad21 besides from chromatid cohesion (12-17), the possible function of Scc3/STAG remained elusive. Here we propose a possible function for Scc3/STAG as a transcriptional co-activator. It has recently been suggested that the cohesin regulatory factor Scc2 might facilitate communication between enhancer and promoters (11). Our results support this idea and propose a possible mechanism for the regulation of gene transcription by cohesins. The interaction between STAG2 and the NF-κB subunit p65 may help to recruit other components of the transcriptional co-activator complexes. In this regard, although STAG2 did not exhibit histone acetyl transferase (HAT) activity (data not shown), it has been described to interact with other acetyl transferases like Eco1, a conserved protein necessary for the establishment of sister chromatid cohesion during S phase in budding yeast (29). Since acetylation of p65 controls its transcriptional activity (30) it is tempting to speculate that STAG2 might regulate NF-
κB-driven transcription by recruiting acetyl transferases.

Our findings are consistent with recent results describing that chromatin-remodelling complexes can load cohesins onto DNA, correlating with histone modification (31). In addition, Scc3/STAG can be recruited by Swi6/HP1 (10) to centromeric regions. Heterochromatin protein 1 (HP1) is a conserved heterochromatic adaptor molecule involved in both gene silencing and supra-nucleosomal chromatin structure (32). However, HP1 has recently been involved in induced gene expression in euchromatin (33). Taken together, these results suggest that the association of STAG2 with Swi6/HP1 could enhance gene transcription. Swi6 forms part of transcriptional complexes that regulate many genes at the G1-S transition (34). Since cohesins are loaded onto DNA at the S phase, the co-activation activity of STAG2 could help to control the expression of genes involved in cell cycle progression. The ability to interact with transcription factors and enhance their transcriptional activity represents a new feature of cohesins and will help us to gain sight into the function of this intriguing complex.
ACKNOWLEDGMENTS

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FIGURE LEGENDS

Figure 1. STAG2 shows partial homology with transcriptional co-activators of the p160 family. STAG2 presents a glutamine-rich region (grey box), also present in p160 co-activators and three LXXLL co-activation motives (black bars). The position of the first leucine of each LXXLL motif is indicated.

Figure 2. STAG2 is a transcriptional co-activator. A, B Jurkat cells (A) and K562 cells (B) were transfected with 0.2 µg of reporter plasmids carrying the luciferase gene under the control of the CD69 promoter (pAIM1.4-Luc), the TNFα promoter (TNFα-615-Luc) and the HIV-LTR promoter (HIV-LTR-Luc), along with 2 ng of the STAG2 expression vector pSTAG2 and 1 µg of the empty vector pCDNA3 (for pAIM1.4-Luc and TNFα-615-Luc) or 1 µg of the Tat expression vector pCMV-Tat. The amount of luciferase was determined 18 h later and the results expressed as fold induction over the value without pSTAG2. C, Similar co-activation efficiency is displayed by STAG2, STAG1 and CBP. Jurkat cells were transfected with 0.2 µg of HIV-LTR-Luc, 2 ng of expression vectors for STAG2, STAG1 and CBP and 1 µg of pCMV-Tat. The results are expressed as in A.

Figure 3. STAG2 enhances Tat-induced HIV-LTR activation in a NF-κB-dependent manner. Jurkat cells were transfected with 0.2 µg of the wild type reporter plasmid
HIV-LTR-Luc or the mutant HIV-LTR-ΔκB-Luc, which carries a deletion of the NF-κB-responsive element, along with 2 ng of pSTAG2 and 1 µg of either pCDNA3 (A) or pCMV-Tat (B). The results are expressed as fold induction over the value without pSTAG2. C, Jurkat cells were transfected with 0.2 µg of HIV-LTR-Luc in the presence or absence of 2 ng of pSTAG2 and 1 µg of pCMV-Tat (Tat) or pCDNA3 (-). D, Wild type Stag2-expressing cells (wt) or Stag2-knockout cells (STAG2 KO) were transfected with 0.2 µg of HIV-LTR-Luc along with 1 µg of pCMV-Tat (Tat) or pCDNA3 (-). All the results are expressed as in A and are representative of three independent experiments. E, STAG2 interacts with Tat. Nuclear extracts of Jurkat cells were incubated with sepharose beads bound either to the glutathione-S-transferase (GST) or to GST-Tat. Sepharose-bound extracts were separated by SDS-PAGE and the presence of STAG2 was analyzed by western blot.

**Figure 4. STAG2 enhances NF-κB-driven transcription.** A, The T lymphoblastic Jurkat cells and the hepatoblastoma HepG2 cells were transfected with 0.2 µg of the reporter plasmid pKBF-Luc, which carries three tandem copies of a NF-κB-responsive element, with or without 2 ng of pSTAG2 and 1 µg of pCDNA3. Luciferase activity was determined 18 h later and the results expressed as fold induction over the value without pSTAG2. The results are representative of three independent experiments. B, STAG2 co-activates NF-κB-mediated transcription in a Gal4 system. Jurkat cells were transfected with 0.2 µg of the reporter plasmid pGal-Luc, carrying multimeric copies of
a Gal4-responsive element, along with 1 µg of pCDNA3 in the presence or absence of 2 ng of pSTAG2. In addition, 2 ng of the expression vectors for the Gal4 chimeras pGal4-p65, pGal4-p65mut, pGal4cRel and pGal4-Sp1, carrying the DNA-binding domain of the yeast transcription factor Gal4 linked to the transcription activation domains of the transcription factors p65, mutated p65, cRel and Sp1, respectively, were added. Luciferase activity was determined as in A.

**Figure 5. STAG2 interacts with the p65 subunit of NF-κB.** A, Nuclear extracts of Jurkat cells were incubated with sepharose beads bound either to the glutathione-S-transferase (GST) or to GST-p65, in which GST is linked to the transcription activation domain of the NF-κB subunit p65. sepharose-bound extracts were separated by SDS-PAGE and the presence of STAG2 and p300 was analyzed by western blot. B, Jurkat nuclear extracts were subjected to immunoprecipitation with anti-p65 and anti-STAG2, using anti-STAG3 and anti-CCR6 as negative controls. The resulting complexes were resolved by SDS-PAGE and the presence of STAG2 was analyzed by western blot. The arrow indicates the STAG2 band.

**Figure 6. Analysis of the contribution of the different domains of STAG2 to its co-activation capacity.** A, Jurkat cells were transfected with 0.2 µg of pGal4-Luc, along with 2 ng of pGal4-p65, 1 µg of pCDNA3 and 2 ng of expression vectors bearing different deletions or point mutations of the LXXLL motives as depicted in the figure.
The amount of luciferase was determined 18 h later and the results expressed as fold induction over the value without pSTAG2. The results are representative of three independent experiments. B, Jurkat cells transfected with 0.2 µg of pGal4-Luc, 2 ng of pGal4-p65, 1 µg of pCDNA3 and 2 ng of expression vectors, carrying the wild type mutant1 and mutant2 STAG2 constructs linked to GFP. Luciferase activity was analyzed as in A. C, 293 cells were transfected with 1 µg of each GFP construct and the relative expression of each GFP-STAG2 protein was analyzed by western blot using an anti-GFP.

Figure 7. Comparison of the presence of the LXXLL co-activation motives in different cohesins. A, LXXLL co-activation motives (black bars) are present in the three human Scc3 homologues STAG2, STAG1 and STAG3, but not in other mitotic cohesins. B, The position of the three LXXLL co-activation motives of STAG2 is conserved in Homo sapiens (hSTAG2), Mus musculus (mSTAG2) and Xenopus laevis STAG2 (xSTAG2). LXXLL co-activation motives can also be found in the Scc3 homologues of different organisms, including Drosophilamelanogaster (dSTAG), Arabidopsis thaliana (aSTAG), Saccharomyces pombe (Psc3) and Saccharomyces cerevisiae (Scc3). The position of the first leucine of each LXXLL motif is indicated.
Figure 3: 

(A) Luciferase activity (Fold induction) for different STAG2 conditions and reporters: HIV-LTR-Luc and HIV-LTR-ΔκB-Luc. 

(B) Luciferase activity (Fold induction) with Tat treatment for HIV-LTR-Luc and HIV-LTR-ΔκB-Luc reporters. 

(C) Luciferase activity (Fold induction) with and without STAG2 treatment. 

(D) Western Blot (WB) of STAG2 in wild type (wt) and knockout (KO) conditions. 

(E) Western Blot (WB) of GST and GST-Tat proteins. 

Lara-Pezzi et al. Fig. 3
Lara-Pezzi et al. Fig. 5
A

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C

WB: GFP

Lara-Pezzi et al. Fig. 6
Evidences of a transcriptional co-activator function of cohesin STAG/SA/Sec3
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