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Differential requirements for HIV-1 Vif-mediated APOBEC3G degradation and RUNX1-mediated transcription by CBFβ

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Abstract

Core binding factor beta, a transcription regulator through RUNX binding, was recently reported critical for Vif function. Here, we mapped the primary functional domain important for Vif function to amino acids 15 to 126 of CBFβ. We also revealed that different lengths and regions were required for CBFβ assisting Vif or RUNX. The important interaction domains that are uniquely required for Vif but not RUNX functions represent novel targets for the development of HIV inhibitors.

Core binding factor β (CBFβ) regulates host genes specific to hematopoiesis and osteogenesis (1, 6, 31) by forming heterodimers with CBFα (i.e., RUNX). CBFβ does not function by binding DNA directly (1); instead, it greatly enhances the affinity of RUNX for DNA, specifically with the core binding sites of various promoters and enhancers (35) such as the MCSFR promoter (25). This enhancement would affect the transcription of various genes in human cells, resulting in the regulation of cell differentiation and proliferation (22, 31).

It has recently been shown by several research groups (10, 11, 13, 38, 39) that CBFβ is also a crucial determinant of the proper functioning of the viral infectivity factor (Vif) of HIV-1. HIV-1 Vif is necessary for viral survival in the host, since it serves to inactivate the host restriction factors (APOBEC3 proteins) present in HIV-1's natural target cells, including macrophages and CD4+ T cells (2, 3, 5, 8, 9, 14, 18, 23, 24, 26-29, 34). Vif hijacks the cellular Cullin5-ElonginB-ElonginC proteins to form a virus-specific E3 ubiquitin ligase complex (36) that targets APOBEC proteins (such as APOBEC3G [A3G]) for proteasomal degradation (4, 16, 17, 19-21, 30, 32, 36, 37). However, Vif's contribution to A3G degradation is almost completely abolished when the expression of CBFβ is silenced (13, 38). Furthermore, mutations in Vif that disrupt CBFβ binding prevent Vif from suppressing the antiviral activity of A3G. CBFβ interacts specifically with HIV-1 Vif to uniquely control its interaction with Culllin5, but not with ElonginB/ElonginC or A3G. Both wild-type CBFβ (either isoform 1-182 or isoform 1-187) and C-terminal truncated CBFβ (residues 1-140) interact with Vif in *vitro*, and Vif's solubility is greatly improved when it is co-expressed with CBFβ (39). CBFβ has been found to influence HIV-1 Vif's stability (13), Cul5 binding (38), and mediation of A3G degradation (10, 11, 13, 38, 39).

In the current study, we have further examined several internal regions of CBFβ that are crucial either for assisting Vif or regulating RUNX activity. By testing both N- and C-terminal truncation mutants of CBFβ, we have also identified a minimally functional fragment of CBFβ that can mediate the Vif-induced degradation of A3G. Overall, we have demonstrated that different domains of CBFβ are required for the protein's Vif- and RUNX-related functions, indicating that the CBFβ-Vif interaction and CBFβ-RUNX binding require different domains of CBFβ and suggesting new possibilities for anti-HIV-1 drug design.

CBFβ1-126 is fully functional in the Vif-induced depletion of A3G

The interaction between C-terminal truncated CBFβ and the Vif protein *in vitro* (39) triggered our interest in examining these CBFβ variants for their potency in contributing to Vif function. PCR was performed with pCBFβ-myc (38) as the template. The purified product was inserted into pcDNA3.1(-)(Invitrogen)to generate pCBFβ-HA. pCBFβ1-141-HA, pCBFβ1-135-HA, pCBFβ1-130-HA, pCBFβ1-126-HA, and pCBFβ1-120-HA were then constructed from pCBFβ-HA by site-directed mutagenesis and confirmed by DNA sequencing (Fig. 1A).

To determine the relative potency of CBFβ mutants with regard to their effects on Vif-mediated A3G degradation, we co-transfected HEK293T cells with the C-terminal truncated CBFβ constructs and Vif- and A3G-expressing vectors; the expression of endogenous CBFβ had been depleted in these cells by the introduction of shRNA (CloneID TRCN0000016644, TRCN0000016645, obtained from Open Biosystems). In brief, CBFβ-knockdown HEK293T cells at 80% confluency were co-transfected with 300ng of A3G-HA plasmid, 900ng of Vif-myc plasmid (both gifts of Dr. K. Strebel), and 1μg of one of the CBFβ-HA variant constructs. Cells were harvested at 48h post-transfection. Western blotting was carried out

with anti-HA antibody (Invitrogen, 715500) to detect A3G and CBFβ and anti-MYC antibody (Covance, MMS-101P) to detect Vif.

The interactions between CBFβ variants and Vif were then characterized by co-immunoprecipitation (Co-IP). Cells were harvested at 48h post-transfection, washed with 1×PBS, and suspended in lysis buffer (50mM Tris-HCl, pH 7.5, 150mM NaCl, and 0.5%-1.5% NP40, supplemented with Roche protease inhibitor cocktail). Samples were sonicated at 15% power for 60s with a 3-s break every 3s, then centrifuged to obtain a clear supernatant. Input samples were incubated with HA-labeled beads (Roche) for 3h, then washed several times with Wash Buffer (20mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1mM EDTA, and 0.05% Tween-20). The samples were then eluted with 100mM glycine-HCl, pH 2.5.

We first discovered that CBFβ1-141 could still inactivate A3G in the presence of Vif (Fig. 1B); this inhibition was later determined to be due to a potent interaction between CBFβ1-141 and Vif (Fig. 1C) that resembled our previously reported finding in *E. coli* (39). Further study revealed that a deletion of amino acid residues 127-182 or fewer residues of (CBFβ1-135, 1-130, and 1-126) had very little impact on CBFβ's ability to assist Vif, suggesting that the long C-terminal tail of CBFβ is not required for the Vif-induced degradation of A3G or even for binding to Vif (Fig. 1B-D). However, a further deletion of six more residues at the C-terminus (CBFβ1-120) almost completely abolished the ability of CBFβ to contribute to Vif-induced A3G degradation (Fig. 1B). Our co-IP results indicated that these six residues (121-126) were critical for the interaction of CBFβ with Vif (Fig. 1D). Therefore, binding to Vif is critical for CBFβ enhancement of Vif function. Vif expression appears quite variable, depending on the co-transfected CBFβ variant. However, there is no direct correlation between Vif function and the ability of CBFβ truncations to enhance Vif expression. For

example, CBF-beta 1-120 showed increased Vif expression but did not support Vif-mediated A3G degradation (Fig. 1B).

CBFβ1-130, but not 1-126, can fully support RUNX1-mediated gene transcription

Inside eukaryotic cells, CBFβ has a natural binding factor named RUNX, and CBFβ-RUNX binding has been shown to be essential for regulation of host gene expression (7, 12). It would therefore be interesting to determine whether a CBFβ variant that is able to assist Vif could still regulate a RUNX1-mediated promoter. Therefore, we transfected CBFβ-knockdown HEK293T cells with 500ng of a firefly luciferase MCSFR promoter construct (pMCSFR-luc) (15), 500ng of the RUNX1c-myc plasmid (a gift of Dr. A. Friedman), and 1ug of each CBFβ-HA variant construct in triplicate. Forty eight hours post transfection, the cells were lysed, and the luciferase activity was quantified with the Promega Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol.

Surprisingly, although CBFβ1-126 was fully functional in terms of the Vif-induced degradation of A3G, it lost the ability to regulate RUNX1 (a >2-fold decrease); in contrast, all other longer variants, including CBFβ1-130, maintained their ability to regulate RUNX1 (Fig. 2A). Further investigation suggested that although binding to RUNX was detected for all the other functional CBFβ variants (Fig. 2B), CBFβ1-126 and 1-120 were unable to bind RUNX1 efficiently (Fig. 2B). Therefore, we conclude that different lengths of CBFβ are required for its role in Vif function and for its role in RUNX function, with residues 127-130 being essential for RUNX1-mediated gene transcription.

CBFβ acts through different domains in its interactions with Vif and RUNX1

The differing potencies of CBFβ1-126 and CBFβ1-130 with regard to RUNX1 led us to hypothesize that CBFβ may function through different domains in assisting Vif and RUNX1. We therefore tested this hypothesis using CBFβd2-14 and d69-90 (Fig. 3A), which were previously reported to have differing abilities to bind Vif (38). Consistently, CBFβ variant d2-14, which could still bind Vif, was fully functional in assisting with the degradation of A3G (Fig. 3B and 3C), indicating that the first 14 residues of CBFβ are also dispensable for Vif assistance. In contrast, CBFβ variant d69-90 failed to bind Vif and assist in degrading A3G (Fig. 3B and 3C), confirming that the CBFβ-Vif interaction is essential for the downstream degradation of A3G. The RUNX1-mediated promoter test presented a different story, however, with d2-14, but not d69-90, failing to bind RUNX and support RUNX function (Fig. 3D and 3E). Therefore, CBFβ indeed interacts with RUNX and Vif through different binding regions.

Loop3, but not other loops of CBFβ, is important for the inactivation of A3G

CBFβd69-90, whose deletion covers the majority of the longest loop (Loop3, residue 68-85) in the published CBFβ structure (PDB: 1H9D) (33), is deficient for Vif binding (38), indicating the importance of this loop for Vif binding. We therefore introduced several mutations into Loop3: pCBFβLoop3.1-HA, pCBFβLoop3.2-HA, and pCBFβLoop3.3-HA were synthesized and sequenced by Shanghai Generay Biotech Co., Ltd (Shanghai, China)(Fig. 4A). Further tests indicated that only the first six amino acids of Loop3 were important for the degradation of A3G, since mutations in this area led to a partial rescue of A3G in the presence of Vif (Fig. 4B and 4D). We did notice that the expression of Loop3.1 in Fig. 4B was less than wild type CBFβ, so a dose manner was applied to wild type CBFβ and we again observed the inability of Loop3.1 in assisting Vif (Fig. 4C). Consisting of CBFβd69-90, this mutation did not alter CBFβ's capacity to assist RUNX1 (Fig. 4E and 4F).

Similar tests were also performed with other CBFβ loops including Loop1, Loop2, and Loop4 to identify other potential Vif binding domains (Fig. 5A). Loop5 was excluded because of its possible interaction with RUNX according to the reported crystal structure (33). Analysis of Loop1, Loop2, and Loop4 mutants indicated that these areas were not important for either Vif-mediated degradation of A3G or for RUNX1-mediated regulation of gene expression (Fig. 5B-E). It is possible that these loop mutants may have an increased affinity for Vif (Fig. 5C).

In this study, we have now for the first time screened both N- and C-termini of CBFβ for a primary functional domain that is important for Vif function. Our results with C-terminal truncations of different lengths suggested that the entire C-terminal tail, together with the last helix (H4), is not required for assisting Vif; our examination of CBFβd2-14, on the other hand, revealed that residues 2-14 of CBFβ are also dispensable. Therefore, we conclude that CBFβ 15-126 contains the minimum fragment required for CBFβ to maintain the ability to assist in the Vif-induced degradation of A3G.

CBFβ is essential for mouse embryo development. It was surprising that we connected functional CBFβ truncations such as CBFβ1-126 and CBFβ1-130 to Vif and RUNX, respectively, since it has been reported that CBFβ155 isoform composed with CBFβ1-133 plus 161-182 cannot restore the development of murine embryos (35). Since we have determined that the C-terminal tail of CBFβ (131-182) is dispensable for both Vif-induced A3G degradation and RUNX1-mediated transcription of the MCSFR promoter, it is possible that the C-terminal tail of CBFβ has a novel function in embryo development. It is also possible that different domains on CBFβ are required for the regulation of different host genes.

We have previously reported that loop3 of CBFβ may be important for Vif binding (38). In the current study, we have determined that the first six amino acids (68-73) in loop3 of CBFβ are important for Vif binding and function. During the preparation of this paper, Hultquist et al. reported their findings (11), which shared some similarities with ours. For example, they observed that CBFβF68D was defective for Vif binding and function. It is not clear whether only F68 in loop3 is important for Vif binding and function. We observed that CBFβF68S could still support Vif function (data not shown). Further study will be required to determine the importance of amino acids 69-73 of CBFβ in Vif binding/function.

Various distinct regions of CBFβ were shown in this study to be important either for Vif's ability to degrade A3G or for RUNX-mediated regulation of host genes. However, no regions yet have been discovered that have an impact on both functions of CBFβ. Hultquist et al. recently screened a series of CBFβ mutants and also found no mutants that affected both the Vif-related and RUNX-related activities (11). In addition, according to the known structure of the binding between CBFβ and RUNX, the region (loop3) of CBFβ that was found to be critical for Vif binding has no contact with RUNX1. Therefore, the one protein, CBFβ, must fulfill different requirements for HIV-1 Vif-mediated A3G degradation and RUNX1-mediated transcription. A more detailed understanding of the CBFβ-Vif binding interface should provide crucial information for designing anti-HIV-1 drugs that will not interrupt the normal CBFβ-RUNX function in human cells.

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References

1. **Adya, N., L. H. Castilla, and P. P. Liu.** 2000. Function of CBFbeta/Bro proteins. Semin Cell Dev Biol **11:**361-8.
2. **Bieniasz, P. D.** 2004. Intrinsic immunity: a front-line defense against viral attack. Nat Immunol **5:**1109-15.
3. **Chiu, Y. L., and W. C. Greene.** 2008. The APOBEC3 cytidine deaminases: an innate defensive network opposing exogenous retroviruses and endogenous retroelements. Annu Rev Immunol **26:**317-53.
4. **Conticello, S. G., R. S. Harris, and M. S. Neuberger.** 2003. The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. Curr Biol **13:**2009-13.
5. **Cullen, B. R.** 2006. Role and mechanism of action of the APOBEC3 family of antiretroviral resistance factors. J Virol **80:**1067-76.
6. **de Bruijn, M. F., and N. A. Speck.** 2004. Core-binding factors in hematopoiesis and immune function. Oncogene **23:**4238-48.
7. **Durst, K. L., and S. W. Hiebert.** 2004. Role of RUNX family members in transcriptional repression and gene silencing. Oncogene **23:**4220-4.
8. **Goff, S. P.** 2004. Retrovirus restriction factors. Mol Cell **16:**849-59.
9. **Harris, R. S., and M. T. Liddament.** 2004. Retroviral restriction by APOBEC proteins. Nat Rev Immunol **4:**868-77.
10. **Hultquist, J. F., M. Binka, R. S. LaRue, V. Simon, and R. S. Harris.** 2012. Vif proteins of human and simian immunodeficiency viruses require cellular CBFbeta to degrade APOBEC3 restriction factors. J Virol **86:**2874-7.
11. **Hultquist, J. F., R. M. McDougle, B. D. Anderson, and R. Harris.** 2012. HIV-1 Vif and the RUNX Transcription Factors Interact with CBFbeta on Genetically Distinct Surfaces. AIDS Res Hum Retroviruses.
12. **Ito, Y.** 2004. Oncogenic potential of the RUNX gene family: 'overview'. Oncogene **23:**4198-208.
13. **Jager, S., D. Y. Kim, J. F. Hultquist, K. Shindo, R. S. LaRue, E. Kwon, M. Li, B. D. Anderson, L. Yen, D. Stanley, C. Mahon, J. Kane, K. Franks-Skiba, P. Cimermancic, A. Burlingame, A. Sali, C. S. Craik, R. S. Harris, J. D. Gross, and N. J. Krogan.** 2012. Vif hijacks CBF-beta to degrade APOBEC3G and promote HIV-1 infection. Nature **481:**371-5.
14. **Jarmuz, A., A. Chester, J. Bayliss, J. Gisbourne, I. Dunham, J. Scott, and N. Navaratnam.** 2002. An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics **79:**285-96.
15. **Kamikubo, Y., L. Zhao, M. Wunderlich, T. Corpora, R. K. Hyde, T. A. Paul, M. Kundu, L. Garrett, S. Compton, G. Huang, L. Wolff, Y. Ito, J. Bushweller, J. C. Mulloy, and P. P. Liu.** 2010. Accelerated leukemogenesis by truncated CBF beta-SMMHC defective in high-affinity binding with RUNX1. Cancer Cell **17:**455-68.

16. **Liu, B., X. Yu, K. Luo, Y. Yu, and X. F. Yu.** 2004. Influence of primate lentiviral Vif and proteasome inhibitors on human immunodeficiency virus type 1 virion packaging of APOBEC3G. J Virol **78:**2072-81.
17. **Luo, K., Z. Xiao, E. Ehrlich, Y. Yu, B. Liu, S. Zheng, and X. F. Yu.** 2005. Primate lentiviral virion infectivity factors are substrate receptors that assemble with cullin 5-E3 ligase through a HCCH motif to suppress APOBEC3G. Proc Natl Acad Sci U S A **102:**11444-9.
18. **Malim, M. H., and M. Emerman.** 2008. HIV-1 accessory proteins--ensuring viral survival in a hostile environment. Cell Host Microbe **3:**388-98.
19. **Marin, M., K. M. Rose, S. L. Kozak, and D. Kabat.** 2003. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat Med **9:**1398-403.
20. **Mehle, A., J. Goncalves, M. Santa-Marta, M. McPike, and D. Gabuzda.** 2004. Phosphorylation of a novel SOCS-box regulates assembly of the HIV-1 Vif-Cul5 complex that promotes APOBEC3G degradation. Genes Dev **18:**2861-6.
21. **Mehle, A., B. Strack, P. Ancuta, C. Zhang, M. McPike, and D. Gabuzda.** 2004. Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. J Biol Chem **279:**7792-8.
22. **Miyazono, K., S. Maeda, and T. Imamura.** 2004. Coordinate regulation of cell growth and differentiation by TGF-beta superfamily and Runx proteins. Oncogene **23:**4232-7.
23. **Navarro, F., and N. R. Landau.** 2004. Recent insights into HIV-1 Vif. Curr Opin Immunol **16:**477-82.
24. **Niewiadomska, A. M., and X. F. Yu.** 2009. Host restriction of HIV-1 by APOBEC3 and viral evasion through Vif. Curr Top Microbiol Immunol **339:**1-25.
25. **Rhoades, K. L., C. J. Hetherington, J. D. Rowley, S. W. Hiebert, G. Nucifora, D. G. Tenen, and D. E. Zhang.** 1996. Synergistic up-regulation of the myeloid-specific promoter for the macrophage colony-stimulating factor receptor by AML1 and the t(8;21) fusion protein may contribute to leukemogenesis. Proc Natl Acad Sci U S A **93:**11895-900.
26. **Rogozin, I. B., M. K. Basu, I. K. Jordan, Y. I. Pavlov, and E. V. Koonin.** 2005. APOBEC4, a new member of the AID/APOBEC family of polynucleotide (deoxy)cytidine deaminases predicted by computational analysis. Cell Cycle **4:**1281-5.
27. **Rose, K. M., M. Marin, S. L. Kozak, and D. Kabat.** 2004. Transcriptional regulation of APOBEC3G, a cytidine deaminase that hypermutates human immunodeficiency virus. J Biol Chem **279:**41744-9.
28. **Rose, K. M., M. Marin, S. L. Kozak, and D. Kabat.** 2004. The viral infectivity factor (Vif) of HIV-1 unveiled. Trends Mol Med **10:**291-7.
29. **Sheehy, A. M., N. C. Gaddis, J. D. Choi, and M. H. Malim.** 2002. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature **418:**646-50.
30. **Sheehy, A. M., N. C. Gaddis, and M. H. Malim.** 2003. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat Med **9:**1404-7.
31. **Speck, N. A.** 2001. Core binding factor and its role in normal hematopoietic development. Curr Opin Hematol **8:**192-6.
32. **Stopak, K., C. de Noronha, W. Yonemoto, and W. C. Greene.** 2003. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol Cell **12:**591-601.
33. **Tahirov, T. H., T. Inoue-Bungo, H. Morii, A. Fujikawa, M. Sasaki, K. Kimura, M. Shiina, K. Sato, T. Kumasaka, M. Yamamoto, S. Ishii, and K. Ogata.** 2001.

Structural analyses of DNA recognition by the AML1/Runx-1 Runt domain and its allosteric control by CBFbeta. Cell **104:**755-67.

34. **Turelli, P., and D. Trono.** 2005. Editing at the crossroad of innate and adaptive immunity. Science **307:**1061-5.

35. **Wang, Q., T. Stacy, J. D. Miller, A. F. Lewis, T. L. Gu, X. Huang, J. H. Bushweller, J. C. Bories, F. W. Alt, G. Ryan, P. P. Liu, A. Wynshaw-Boris, M. Binder, M. Marin-Padilla, A. H. Sharpe, and N. A. Speck.** 1996. The CBFbeta subunit is essential for CBFalpha2 (AML1) function in vivo. Cell **87:**697-708.

36. **Yu, X., Y. Yu, B. Liu, K. Luo, W. Kong, P. Mao, and X. F. Yu.** 2003. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science **302:**1056-60.

37. **Yu, Y., Z. Xiao, E. S. Ehrlich, X. Yu, and X. F. Yu.** 2004. Selective assembly of HIV-1 Vif-Cul5-ElonginB-ElonginC E3 ubiquitin ligase complex through a novel SOCS box and upstream cysteines. Genes Dev **18:**2867-72.

38. **Zhang, W., J. Du, S. L. Evans, Y. Yu, and X. F. Yu.** 2012. T-cell differentiation factor CBF-beta regulates HIV-1 Vif-mediated evasion of host restriction. Nature **481:**376-9.

39. **Zhou, X., S. L. Evans, X. Han, Y. Liu, and X. F. Yu.** 2012. Characterization of the interaction of full-length HIV-1 Vif protein with its key regulator CBFbeta and CRL5 E3 ubiquitin ligase components. PLoS One **7:**e33495.

Figure legends

Figure 1. CBFβ N-terminal residues1-126 are sufficient to support Vif in inducing A3G degradation. A. Cartoon of CBFβ C-terminal truncation. B. Effect of C-terminal CBFβ deletions on Vif-mediated A3G degradation. A3G-HA, HXB2-Vif, and CBFβ variants were co-transfected into CBFβ-knockdown-HEK293T cells, and Western blotting was carried out to detect A3G, Vif, and CBFβ expression. Results are representative of three independent experiments. C,D. Co-IP to detect interactions between HIV-Vif and the CBFβ variants in the absence of A3G. Results are representative of three independent experiments.

Figure 2. CBFβ1-130, but not 1-126 or 1-120, can interact with RUNX1c and regulate gene transcription mediated by RUNX1c. A. Luciferase assays confirmed that CBFβ1-130 is functional in terms of RUNX1c regulation of the MCSFR promoter. It has been reported that endogenous RUNX1 expression is very low in HEK293 cells. For the MCSFR promoter assay, exogenous RUNX1 is introduced into HEK293 cells as previously described (15). For all luciferase tests, the ratio of the luciferase count of the MCSFR promoter-Luc plus Runx1 and a control vector was used as the reference and was set to 1. Results are representative of four independent experiments. Each bar is the average of three replicates from the same experiment (error bars indicate mean±SD). B. CBFβ1-130 can still interact with RUNX1c, while CBFβ1-126 and 1-120 lost such capability. Results are representative of three independent experiments.

Figure 3. CBFβ acts through different domains to assist Vif and RUNX1. A. Cartoon of CBFβ domain truncations. B. Functional comparison of wild type and mutant CBFβ, N-terminal CBFβ truncation d2-14 could support Vif function in degrading A3G, while d69-90 was defective for Vif-induced A3G degradation. Results are representative of five independent experiments. C. Co-IP showed that CBFβd2-14 could bind Vif, but d69-90 could not. Results are representative of three independent experiments. D. CBFβd69-90 could bind RUNX1c, but d2-14 could not. Results are representative of three independent experiments. E. Luciferase assays confirmed that CBFβd69-90 was fully functional in terms of RUNX1c regulation of the MCSFR promoter, but d2-14 was not. Results are representative of three independent experiments. Each bar is the average of three replicates from the same experiment (error bars indicate mean±SD).

Figure 4. Mutations occurring on Loop3 of CBFβ have no impact on RUNX1-mediated transcription, while the first six residues of Loop3 are important for Vif-induced degradation of A3G. A. Mutations designed for Loop3 of CBFβ. In general, Ala scanning mutagenesis was performed. However, to achieve the maximum effect, small amino acids such as Gly and Ala were changed to charged amino acids. Hydrophobic amino acids with aromatic long side chain such as Phe and Try were changed to hydrophilic Ser residues. A similar strategy was also applied to the other loop mutations. B. The first 6 aa in Loop3 are important for Vif-induced A3G degradation. Results are representative of four independent experiments. C. Functional comparison of wild type CBFβ and mutant CBFβ (Loop3.1 and F68D). Both mutant CBFβ Loop3.1 and F68D lost the capability of assisting Vif to degrade A3G when compared to wild type CBFβ. Results are representative of three independent experiments. D. Co-IP indicated that Loop3.1 malfunctioned because of compromised interaction with Vif. Results are representative of three independent experiments. E. Co-IP confirmed that the mutations on Loop3 did not affect the CBFβ-RUNX1c interaction. Results are representative of three independent experiments. F. Luciferase assays confirmed that all Loop3 mutants were fully functional with regard to Runx1c-mediated regulation of the MCSFR promoter. Results are representative of three independent experiments. Each bar is the average of three replicates from the same experiment (error bars indicate mean±SD).

Figure 5. Mutations occurring in other loops of CBFβ have no impact on either RUNX1-mediated transcription or Vif-induced A3G degradation. A. Mutations designed for Loops1, 2, and 4. B. No crucial residue was detected in these loops in terms of Vif-induced A3G degradation. Results are representative of four independent experiments. C. Co-IP confirmed that mutations in Loops1, 2, or 4 do not affect the CBFβ-Vif interaction. Results are representative of three independent experiments. D. Co-IP confirmed that mutations in

Loops1, 2, or 4 do not affect the CBFβ-RUNX1c interaction. Results are representative of three independent experiments. E. Luciferase assays confirmed that all these mutants were fully functional in terms of RUNX1c-mediated regulation of the MCSFR promoter. Results are representative of three independent experiments. Each bar is the average of three replicates from the same experiment (error bars indicate mean±SD).

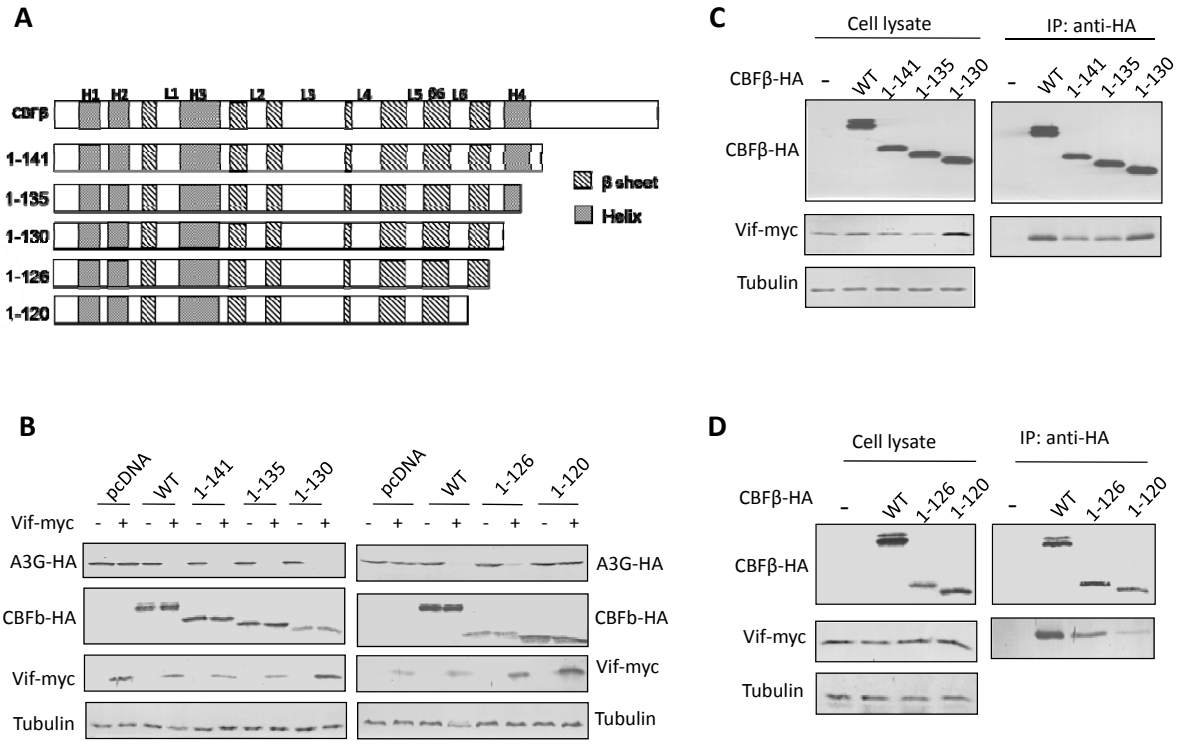
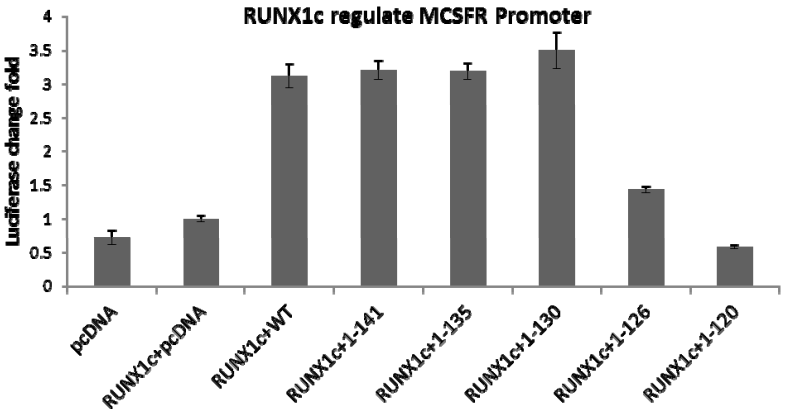


Fig.1

A



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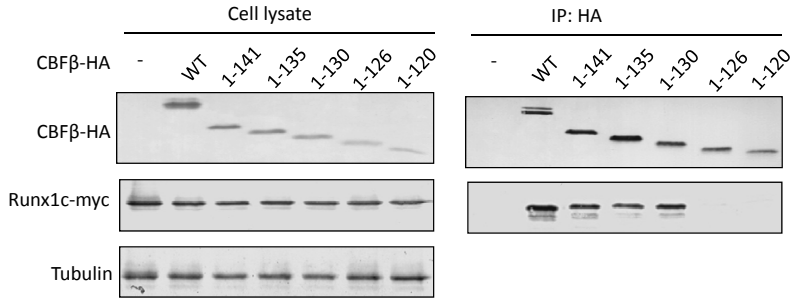

Fig. 2

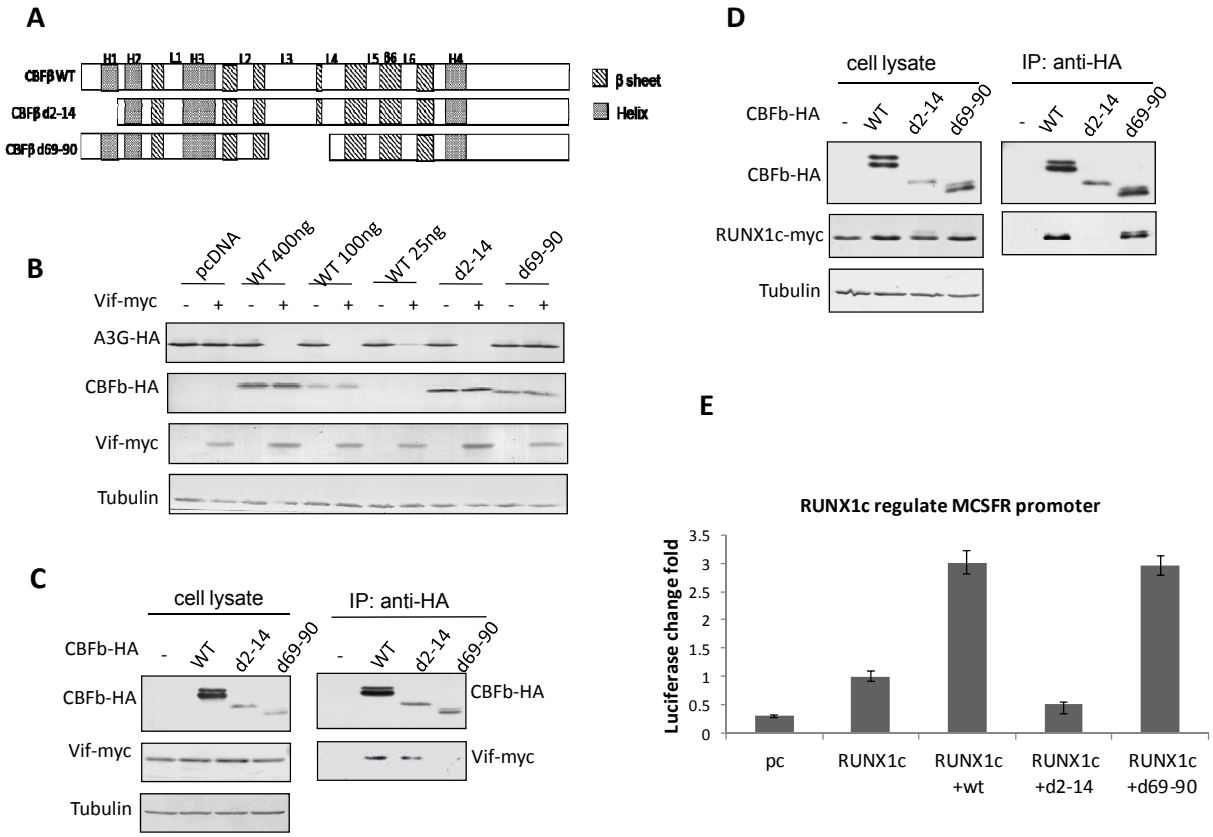

Fig. 3

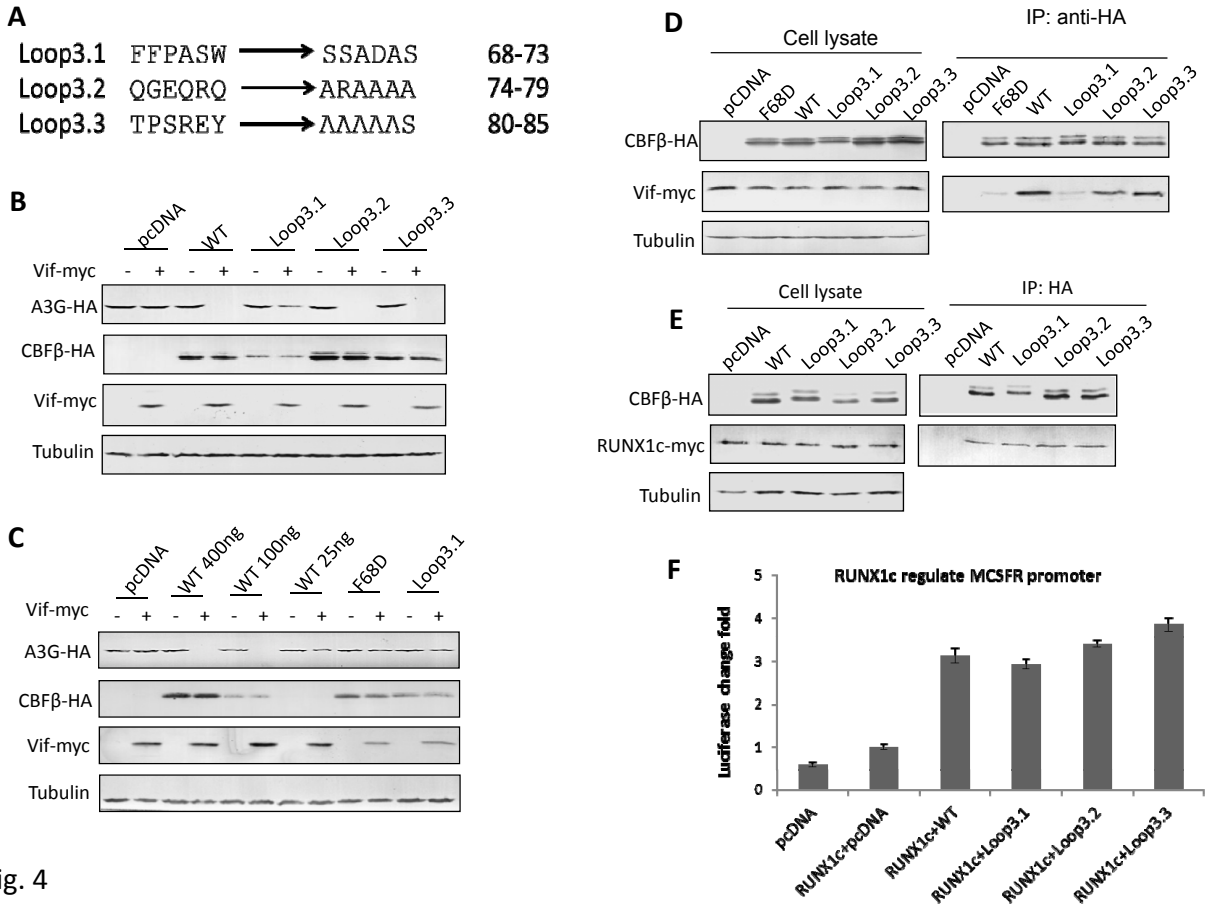

Fig. 4

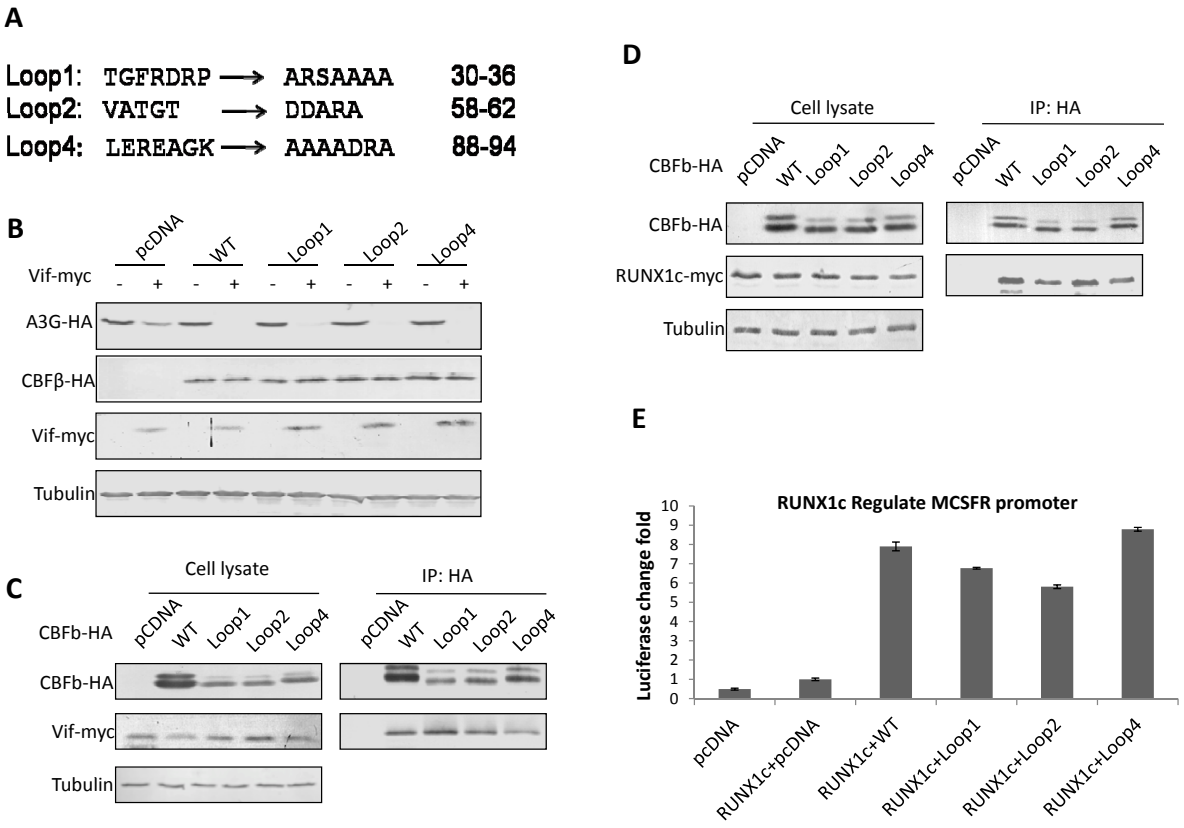

Fig. 5