Revised Supplementary Figure Legends

Supplementary Figure 1 (**related to Figure 1**): **BCL11A and BCL11B interact with MTA1 and MTA3. (A)** Endogenous BCL11B proteins interact with endogenous MTA1 proteins in Jurkat cells. Jurkat whole cells extracts were prepared in IPH buffer, incubated with total IgG and with anti-MTA1 antibodies and immunoblotted with polyclonal antibodies against BCL11B to detect co-immunoprecipitation (left panels, **IP**). The membrane was then stripped and re-probed with anti-MTA1 antibodies to verify the presence of MTA1 proteins. 1% of each lysate were directly resolved by SDS-PAGE and immunoblotted with anti-BCL11B antibodies (Left panel, **INPUT**). **(B)** BCL11B interacts with MTA3. A similar experiment was conducted in Jurkat cells with anti-MTA3 antibodies. **(C, D, E, F)** Expression levels of the two *BCL11* paralogs, *BCL11A* and *BCL11B* and of the *MTA1* and *MTA3* corepressors in MOLT4 and Jurkat cells. Total RNAs were extracted and RT-qPCR experiments were performed to analyse the expression levels of *BCL11A* (C), *BCL11B* (D), *MTA1* (E) and *MTA3* (F) in MOLT and Jurkat cells (G) BCL11A interacts with MTA1. HEK293T were transiently transfected for 48h with the indicated plasmids and the empty pcDNA3-FLAG vector used as a control. Whole cells extracts were prepared in IPH buffer, incubated with anti-MTA1 antibodies (Top panels, **IP MTA1**) and immunoblotted with polyclonal antibodies against BCL11A to detect co-immunoprecipitation. The membrane was then re-probed with anti-MTA1 antibodies to verify the presence of MTA1 proteins in the immunoprecipitates. 1% of each lysate were directly resolved by SDS-PAGE and immunoblotted with the indicated antibodies (Lower panels, **INPUT**). (H) BCL11A interacts with MTA3. A similar experiment was conducted in HEK293T cells with BCL11A and MTA3 expression vectors.

Supplemental Figure 2 (related to Figure 2): **The various 1-20 S2X BCL11B-Gal4-NLS-HA constructs display a diffuse nuclear localization whereas the various FLAG-BCL11B mutants display a punctuated nuclear localization. (A-B)** Immunofluorescence analyses of the 1-20 S2X BCL11B-Gal4-NLS-HA constructs. HEK293T were mock-transfected or transfected with the indicated expression vectors for the various BCL11B N-terminal fragments cloned in frame with the Gal4 binding domain, a Nuclear localization signal (NLS) and an epitope tag (HA) in the Gal4-NLS-HA vectors. These expression vectors have been used in the co-immunoprecipitation experiments and in the luciferase reporter assays. 48 hours after transfection, cells were fixed with paraformadehyde and analysed by conventional immunofluorescence microscopy using anti-HA monoclonal antibodies. Nuclei are seen as Hoechst-positive staining. The merging of the two images is shown in the right-hand panels.

(C, D, E and F) Western blot analyses of the HEK293T cell extracts used in the luciferase transactivation assays. HEK293T were transfected with the empty pGL3 or with the PGL3Luc vectors together with the indicated empty Gal-NLSHA or 1-20 S2X BCL11B Gal4-NLS-HA expression vectors. 48 hours after transfection cells were lysed in Luc assay buffer. These total cell extracts were used to measure Luciferase activity and aliquots were also analyzed by SDS/PAGE and immunoblotting analyses with HA antibodies. Panel C corresponds to extracts used in Figure 2D; Panel D to extracts used in Figure 2E, Panel E to extracts used in Figure 2F and Panel F to extracts used in Figure 2G.

(G) Immunofluorescence analyses of wt BCL11B and of the point (S2A, S2A) or deletion (ΔMSRRKQ) mutants. HEK293T cells were transiently transfected with expression vectors for wt FLAG-tagged-BCL11B or for the various BCL11B mutants (S2D, S2A and ΔMSRRKQ) used in the co-immunoprecipitation experiments. Immunofluorescence

microscopy analyses using the anti-FLAG monoclonal antibodies were performed as described in panels A and B.

Supplemental Figure 3 (related to Figure 4): **Time course of BCL11B Serine 2 phosphorylation and SUMOylation in HEK293T cells activated with PMA. (A)** Western blot analyses. 48h after transfection with wt FLAG-BCL11B, HEK293T cells were treated with DMSO (vehicle) or PMA (1μM in DMSO) as indicated before being harvested and analysed by Western blotting with anti Phospho-Ser2 BCL11B, with anti BCL11B antibodies which detect SUMOylated forms of BCL11B (indicated by an arrowhead) and with the FLAG antibodies (which for unknown reasons do not detect SUMOylation of BCL11B). The SUMOylation site is perfectly conserved between the murine and human BCL11B proteins. Phospho-erk1/2 and actin were used as controls for PKC activation and for equal loading respectively. Then, quantification of phosphorylated and SUMOylated BCL11B were performed with the Fujifilm MultiGauge software. **(B)** Quantification of FLAG-BCL11B in DMSO and in activated conditions for each time point. The levels of BCL11B compared to actin were quantified with the Fujifilm MultiGauge software.

Supplemental Figure 4 (related to Figure 6): **Effects of Anacardic acid on the SUMOylation of BCL111B**. Jurkat cells were treated with DMSO or activated for 60 minutes and pretreated or not with the SUMOylation inhibitor Anacardic Acid (AA) for the indicated times. Total cells extracts obtained in denaturing conditions were analyzed by immunoblotting with the indicated antibodies. The SUMOylation inhibitor Anacardic acid is also known to inhibit various histone acetylase and thus to have a negative effect of transcription. This could explain the decreased levels of BCL11B (top panels) and several SUMOylated proteins detected by the anti-SUMO2 antibodies (third panel). The same

extracts were also analyzed with anti-RanGAP1 antibodies. pErk1/2 was used as a control for activation and Erk2 as a loading control.

Supplemental Figure 5 (related to Figure 7): **PMA/Ionomycin activation of Jurkat cells does not induce detectable ubiquitination of BCL11B**. Jurkat cells were activated with PMA/Ionomycin for 5 hours and pretreated or not for 1 hour and for all the activation treatment (5 hours) with the proteasome inhibitor MG132. Total cells extracts were prepared in RIPA buffer and immunoprecipitated with anti-BCL11B antibodies or rabbit IgG as control. The immunoprecipitates (Top Panels: IP) and 1.5% of each lysate (Bottom Panels: Input) were resolved by SDS-PAGE and immunoblotted with the indicated antibodies.

Supplemental Figure 6 (related to Figure 7 for panels A and B, to Figure 8 for panels C to I and to Figure 9 for panels G and H): RT-qPCR analyses of gene expression levels in activated Jurkat cells. (A-B) *BCL11B* and *CXCR4* are repressed upon prolonged activation of Jurkat cells. Jurkat cells were treated with DMSO or activated with PMA/Ionomycin (P/I) for 5 hours. Total RNAs were extracted and RT-qPCR experiments were performed to analyse the expression levels of *BCL11B* and *CXCR4*. **(C)** Prolonged Activation of the human CD4+ T cell line Jurkat by PMA/Ionomycin induced a huge increase of *IL2* expression. Jurkat cells were treated with DMSO or activated with P/I for the indicated times. Total RNAs were extracted and RT-qPCR experiments were performed to analyse the increase of *IL2* expression levels after activation of Jurkat cells. These results correspond to the experiment shown partially (0, 5 and 30 minutes) as Figure 8A and presented here in totality with a different scale to visualize the huge expression of *IL2* mRNAs levels after 2 and 5 hours of activation. (**D, E**) MTA1 and RbAp48 RNAs levels during PMA/Ionomycin activation of Jurkat cells. The same mRNAs samples than those used here in panel C (and partially shown

in Figure 8A) were also analyzed by RT-qPCR for the expression of MTA1 (panel D) and RbAP48 (Panel E). (**G, H**) The PKC inhibitor Gö6983 does not significantly affect expression levels of *MTA1* and *RbAp48*. Jurkat cells were treated with DMSO or activated with P/I for 30 minutes without or with pre-incubation with the PKC inhibitor Gö6983. Total mRNAs were extracted and RT-qPCR experiments were performed to analyse the expression levels of *MTA1* and *RbAp48*. The same RNAs samples have been used for the quantification of *BCL11B* and *IL2* expression levels (Figure 9 A and 9B).

Supplementary Figure 7 (related to Figures 8 and 9): Pilot ChIP experiments on the IL2 and Id2 promoters in un-stimulated Jurkat cells.

(**A**) Schematic drawing of the human *IL2* proximal promoter region. The TATA box as well as the region between -243 and -201, named the upstream site 1 (US1) are represented as described in Cismaciu et al. (2006) (reference 19 in the main text). The BCL11B binding site contained in this region is shown with the **TGGGC** core sequence highlighted in bold and underlined. The oligonucleodites used in the ChIP experiments are schematically shown by arrows. (**B**) Pilot ChIP experiments on the *IL2* and *Id2* promoters. Pilot ChIP experiments were performed on chromatin prepared from Jurkat cells grown in standard conditions with antibody against BCL11B, MTA1 (sc-10813, used in the ChIP experiments presented as Figure 8) or rabbit IgG on chromatin prepared as described in the Materials and Methods section. The bound material was eluted and analysed by quantitative PCR using primers flanking the US1 BCL11B binding site identified in the human *IL2* promoter and a newly identified core TGGGC BCL11B binding site (–316 to -312) located upstream of the transcription start site (TSS) in the human *Id2* gene (see Figure 8D). *GAPDH* was used as an internal nonbinding control. (**C**) Validation of another MTA1 antibody in pilot ChIP experiments. During the revision of the manuscript, the anti-MTA1 sc-10813 was no more

available from the manufacturer. We therefore checked another anti-MTA1 antibody (ab50263) from Abcam on the same Jurkat chromatin. This antibody has been used in the experiments presented as Figure 9. (**D**) Validation of the MTA3 antibody in pilot ChIP experiments. Similar pilot ChIP experiments were performed to validate the anti MTA3 antibody ab87275 from Abcam.

Supplemental Figure 8 (related to Figure 10): RT-qPCR analyses of gene expression levels in activated primary human CD4$^+$ T cells.

(**A, B and C**) MTA1, MTA3 and RbAp48 RNAs levels during activation of primary human CD4$^+$ T cell by PMA/Ionomycin or by anti-CD3/CD28 antibodies. The same mRNAs samples than those used in Figure 10 (Panels A and C) were also analyzed by RT-qPCR for the expression of MTA1 (panel A), MTA3 (panel B) and RbAP48 (panel C).