# Association of the Structure Specific Recognition Protein 1 with the Lens Epithelium-Derived Growth Factor/p75 modulates HIV-1 replication

## SUPPLEMENTARY DATA

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## Supplementary Legends

**Supplementary Figure S1: Protein domains in LEDGF/p75, LEDGF/p52, and SSRP1.** (a) Two domains, the PWWP and the integrase binding domain (IBD), two AT hook motifs, and six charged and conserved regions (CR) have been described in LEDGF/p75. (b) The splicing variant LEDGF/p52 has a small and unique eight amino acids-long C-terminal region and shares the entire N-terminal region of LEDGF/p75 that includes the PWWP domain, the AT hook motifs, and CR1-3. (c) SSRP1 contains two structural domains, a structure-specific recognition (SSRC) motif and an HMG-box domain, and four evolutionarily conserved regions called N- terminal domain (NTD), middle domain (MD), intrinsically disordered domain (IDD), and C-terminal domain (CID).

**Supplementary Figure S2: Subcellular distribution of SSRP1 mutants.** LEDGF/p75-deficient HEK 293T cells were transiently co-transfected with FLAG-tagged LEDGF/p75 wild type (WT) and Myc-tagged SSRP1 WT or mutants lacking the N-terminal (NTD) or the HMG domains. Thirty-six hours after transfection total cell lysates (T), chromatin non-bound (S1), and chromatin bound (P1) fractions were obtained from these cells and analyzed by immunoblot with anti-FLAG or anti-Myc antibodies.

**Supplementary Figure S3: Evaluating each region of LEDGF/p75 for its interaction with SSRP1.** Densitometry analysis of the interaction of SSRP1 with LEDGF/p75 wild type (WT) or (a) mutants lacking C-terminus protein regions including the IBD domain and different charged regions (CR) or (b) mutants lacking the N-terminus protein regions, including the PWWP domain or different charged regions (CR). Bars represent the densitometry quantification of the levels of SSRP1 detected by immunoblot analysis in the different immunoprecipitation reactions.

Immunoprecipitations were performed as described in 1a using S2 fractions obtained from si1340/1428 cells expressing Myc-SSRP1 WT alone (none) or together with different FLAG-tagged LEDGF/p75 proteins.

**Supplementary Figure S4: Defining the role of the PWWP subdomains in the SSRP1 interaction.** (a-c) Analysis of the binding of LEDGF/p75 PWWP mutants to SSRP1 by immunoprecipitation. si1340/1428 cells were co-transfected with plasmids expressing Myc-SSRP1 WT and FLAG-tagged LEDGF/p75 WT (lanes 1) or PWWP mutants (lanes 2), or an empty plasmid (lanes 3). In these experiments the following mutants were analyzed: (a) LEDGF/p75ΔPWWPβ-barrel, (b) FLAG-LEDGF/p75ΔPWWPα-helix, and (c) LEDGF/p75W21A. Immunoprecipitations were performed as described in figure legend 1a.

**Supplementary Figure S5: Analyzing the function of SSRP1 regions in the interaction with LEDGF/p75**

HEK293T cells were co-transfected as described in figure legend 3. The Myc-tagged SSRP1 mutants analyzed were: (a) SSRP1ΔSSRC, (b) SSRP1ΔMD, (c) SSRP1ΔIDD, and (d) SSRP1ΔCID. S2 fractions were obtained from transfected cells and subjected to immunoprecipitation as described in figure legend 1a.

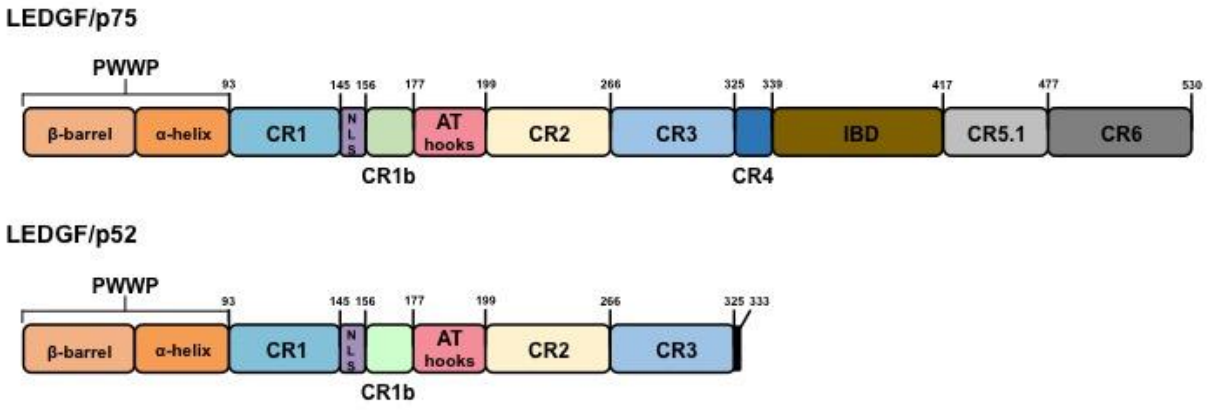
**Supplementary Figure S6: Viability of SSRP1 knockdown cells.** (a) Cells were infected as described for figure legend 4a. ATP content (viability) was measured along with luciferase. (b) Cells were infected as described for figure legend 5c. ATP content (viability) was measured along with luciferase.

**Supplementary Figure S7: Analysis of the chromatin binding strength of SSRP1 and LEDGF/p75 in LEDGF/p75 and SSRP1-deficient cells by immunoblotting.** Chromatin-bound

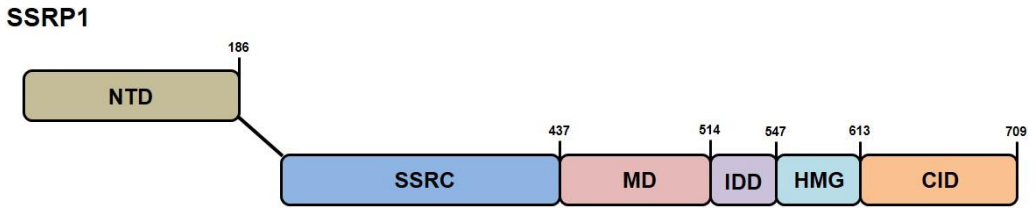
(P1) and non-bound fractions (S1) were obtained from SupT1 cells expressing control (Scr) or SSRP1- (a) or LEDGF/p75- (b) specific shRNAs. A total cell lysate (T) was obtained by lysing the aforementioned cells in 2X Laemmli sample buffer. The presence of SSRP1 and LEDGF/p75 proteins was detected in these fractions by immunoblot analysis. β-actin was detected as a loading control. To avoid saturation of the immunoblot signals, the amount of P1 fractions loaded was 1/5 of the total and S1 fractions.

**Supplementary Figure S1**

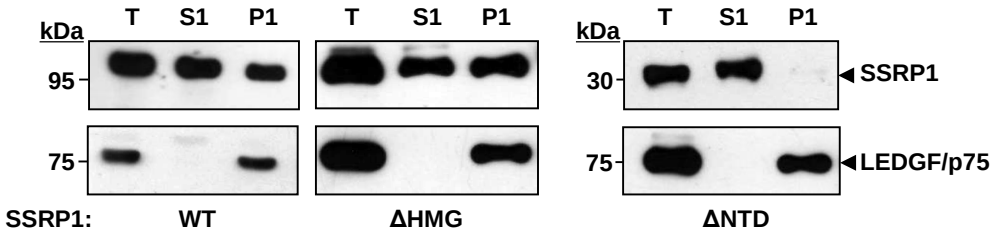
**a**



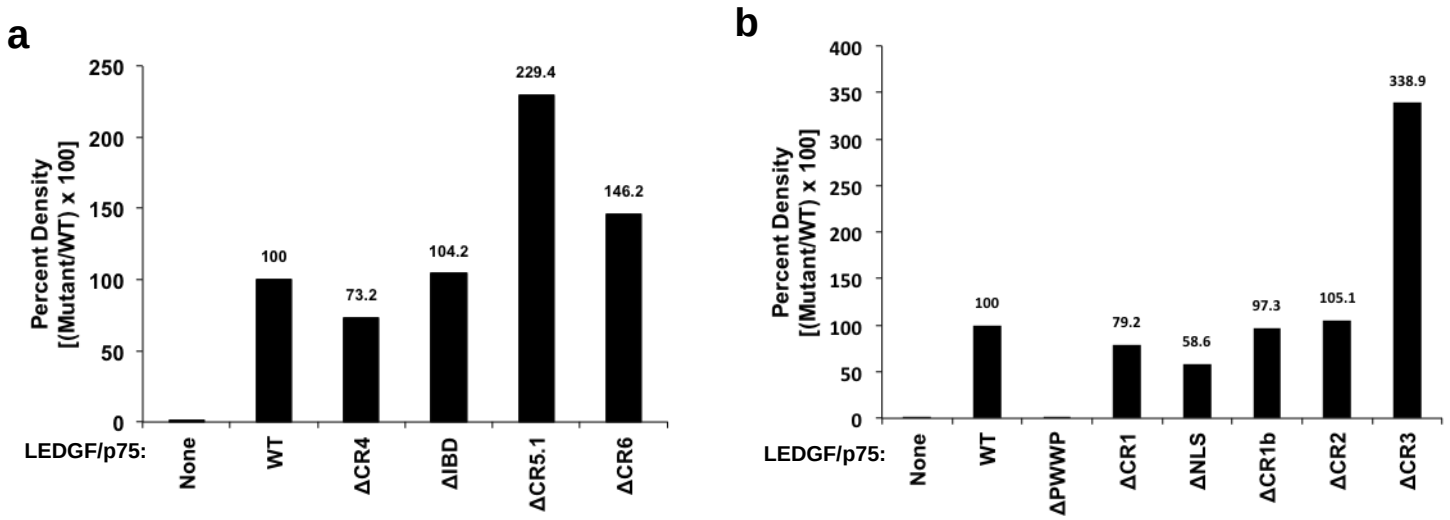

**b**



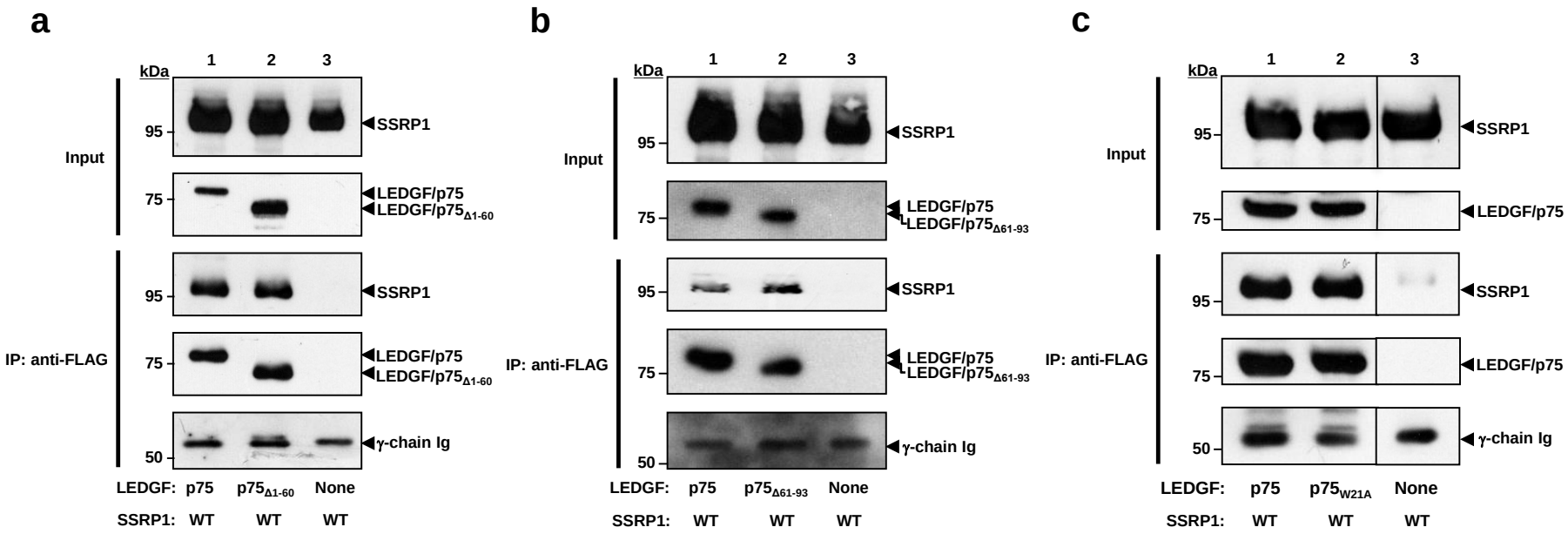
**Supplementary Figure S2**



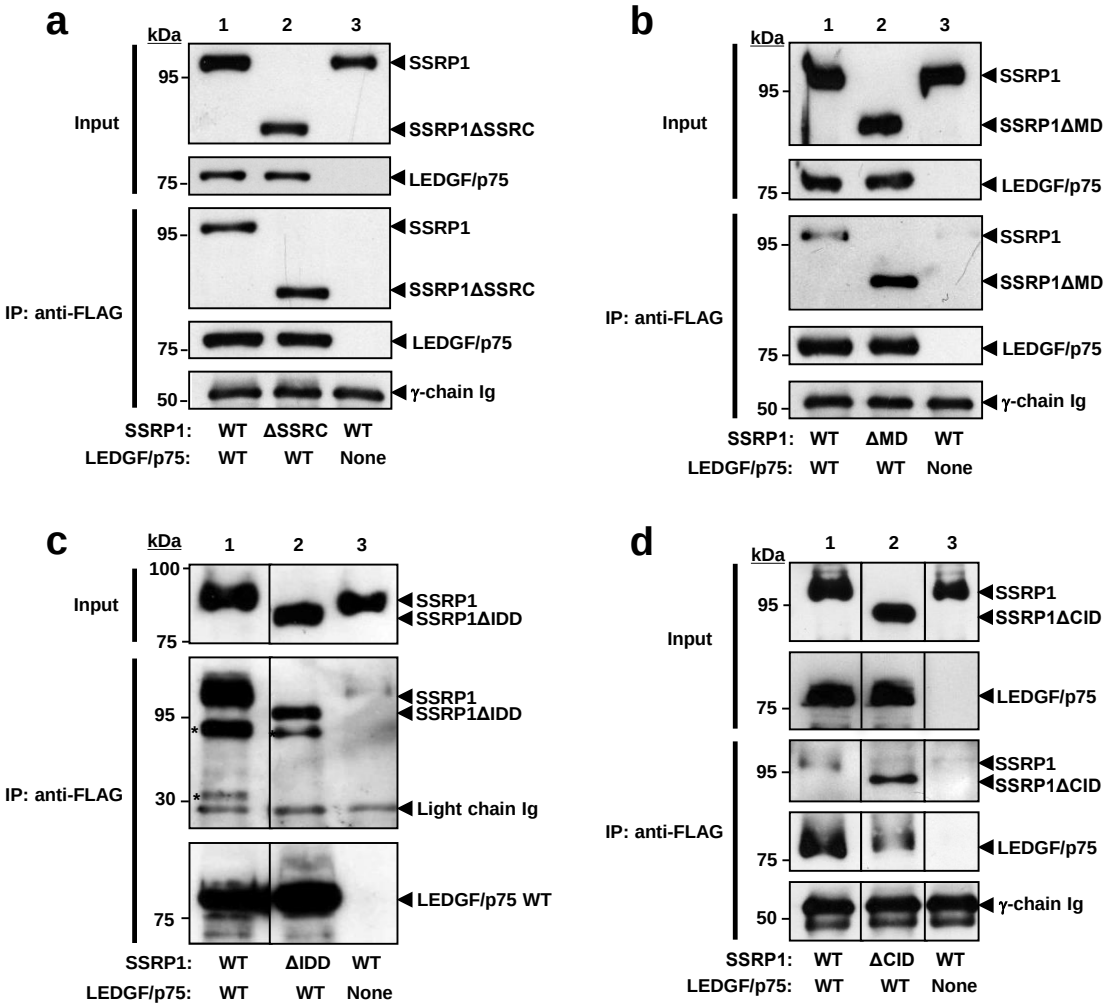
**Supplementary Figure S3**



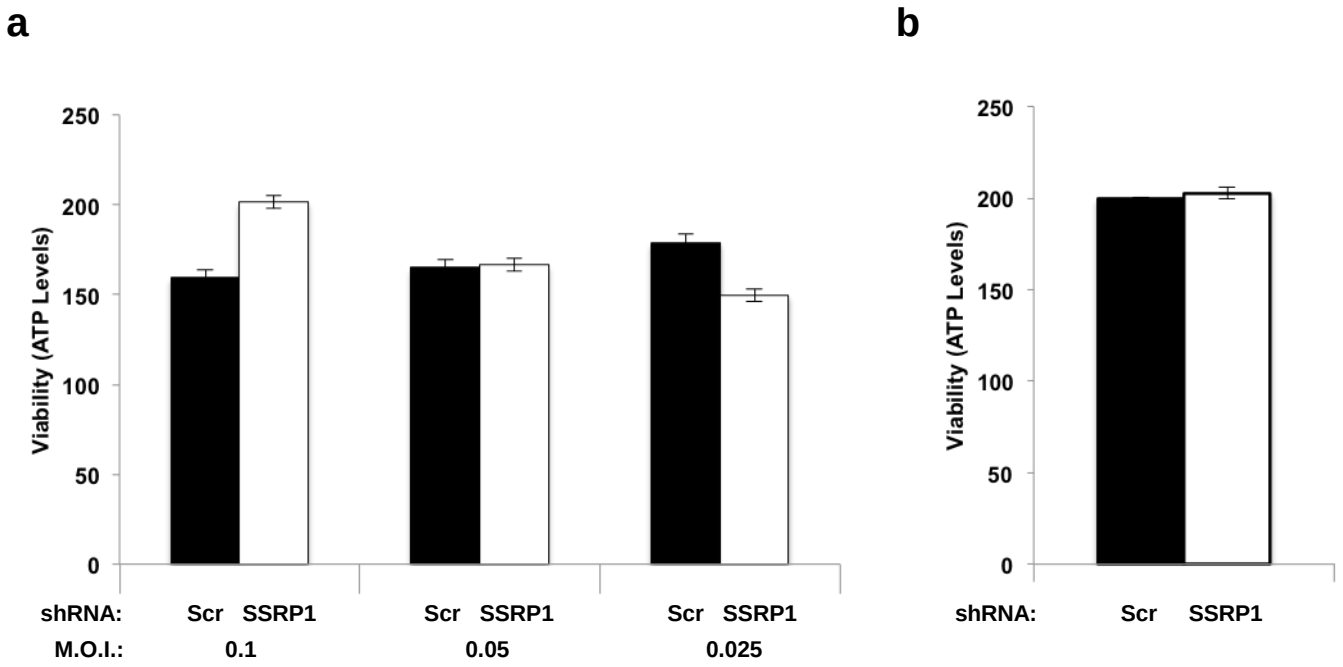
## Supplementary Figure S4



## Supplementary Figure S5



**Supplementary Figure S6**



**Supplementary Figure S7**