

Supplementary Information

The proteolysis of methylated SOX2 protein is regulated by L3MBTL3 and CRL4^{DCAF5} ubiquitin ligase

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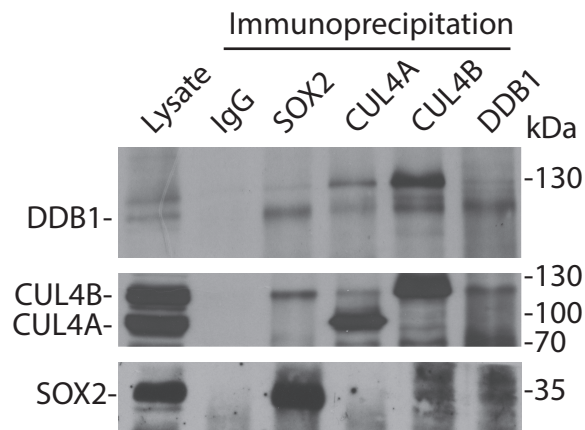
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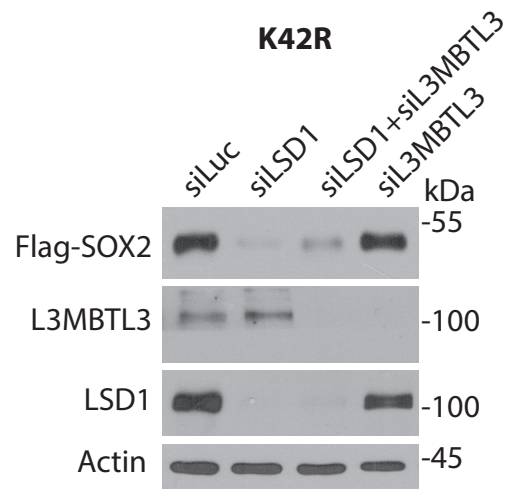
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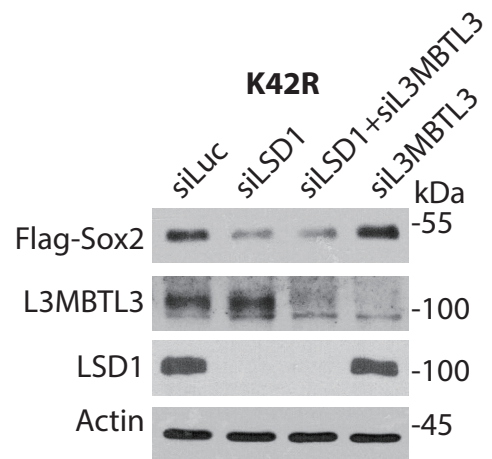
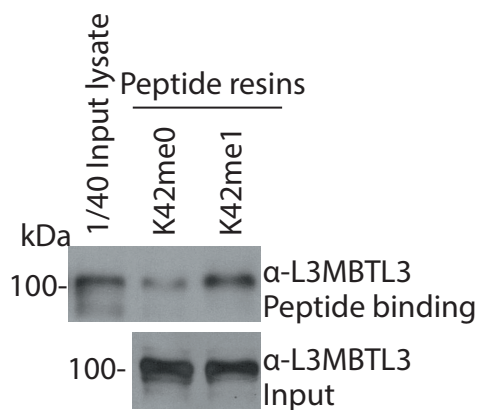
A.



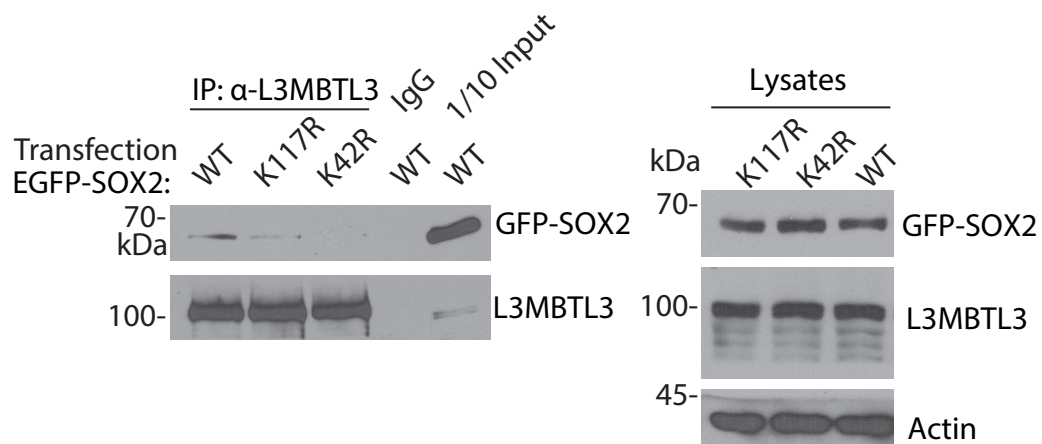
B.



C.



D.



Supplementary Figure 1. SOX2 interacts with CRL4 and L3MBTL3.

A. Endogenous CUL4A, CUL4B, and DDB1 interact with SOX2. SOX2, CUL4A, CUL4B, and DDB1 were immunoprecipitated from mouse embryonic stem cells and their interactions were examined by Western blot analyses as indicated.

B. PA-1 cells stably expressing the Flag-SOX2 K42R mutant were transfected with 50 nM siRNAs for luciferase (control), LSD1, L3MBTL3+LSD1, and L3MBTL3 for 48 hours. The Flag-SOX2 K42R mutant and indicated proteins were analyzed by Western blotting. Two independent experiments were shown to indicate that loss of L3MBTL3 either partial or fail to rescue the protein level of Flag-SOX2 in LSD1 knockdown cells.

C. The K42me0 and K42me1 peptide resins were incubated with the GST-L3MBTL3-3MBT recombinant protein as in Figure 4D. The proteins associated with the washed peptide resins were blotted with anti-L3MBTL3 antibodies.

D. GFP-tagged SOX2 (wildtype), K42R, and K117R mutant expression constructs were transfected into 293 cells. The L3MBTL3 complexes were immunoprecipitated by anti-L3MBTL3 antibodies and Western blotted by anti-SOX2 antibodies as in Figure 4G.