



Supplementary Figure 3. **A** Cell lysates of SW480, DLD-1 and HCT116 cells were prepared by adding 20mM N-ethylmaleimide (NEM) or not. The lysates were blotted by anti-ELOC antibody. **B** The SUMO modification sites of ELOC was predicted using online SUMOylation prediction tools JASSA. **C** Either UBC9, SEN1, SEN2, or SEN3 was stably knocked down in HCT116 cells. Cell lysates were prepared for immunoprecipitation with anti-SUMO1 antibody, and blotted by anti-ELOC antibody. **D, E, F** Either Flag-SEN1 (**D**), Flag-SEN2 (**E**) or Flag-SEN3 (**F**) was overexpressed in HEK293T cells stably expressed Myc-ELOC, HA-SUMO1 and GFP-UBC9. Cell lysates were prepared for immunoprecipitation with anti-Myc antibody, and blotted by anti-HA antibody. **G** Either Myc-ELOC WT or Myc-ELOC (K32R) mutant form was expressed in SW480 cells. Cell lysates were prepared for immunoprecipitation with anti-Myc antibody, and blotted by the indicated antibodies. **H** Either UBC9 or SEN1 was knocked down in SW480 cells with stable USP51 knockdown. The relative mRNA level of HIF1A was measured under normoxia and hypoxia. **I** Either UBC9 or SEN1 was knocked down in HCT116 cells with stable USP51 overexpression. The relative mRNA level of HIF1A was measured under normoxia and hypoxia. Data are expressed as mean \pm SD, n=3 biological replicates. ns: not significant.