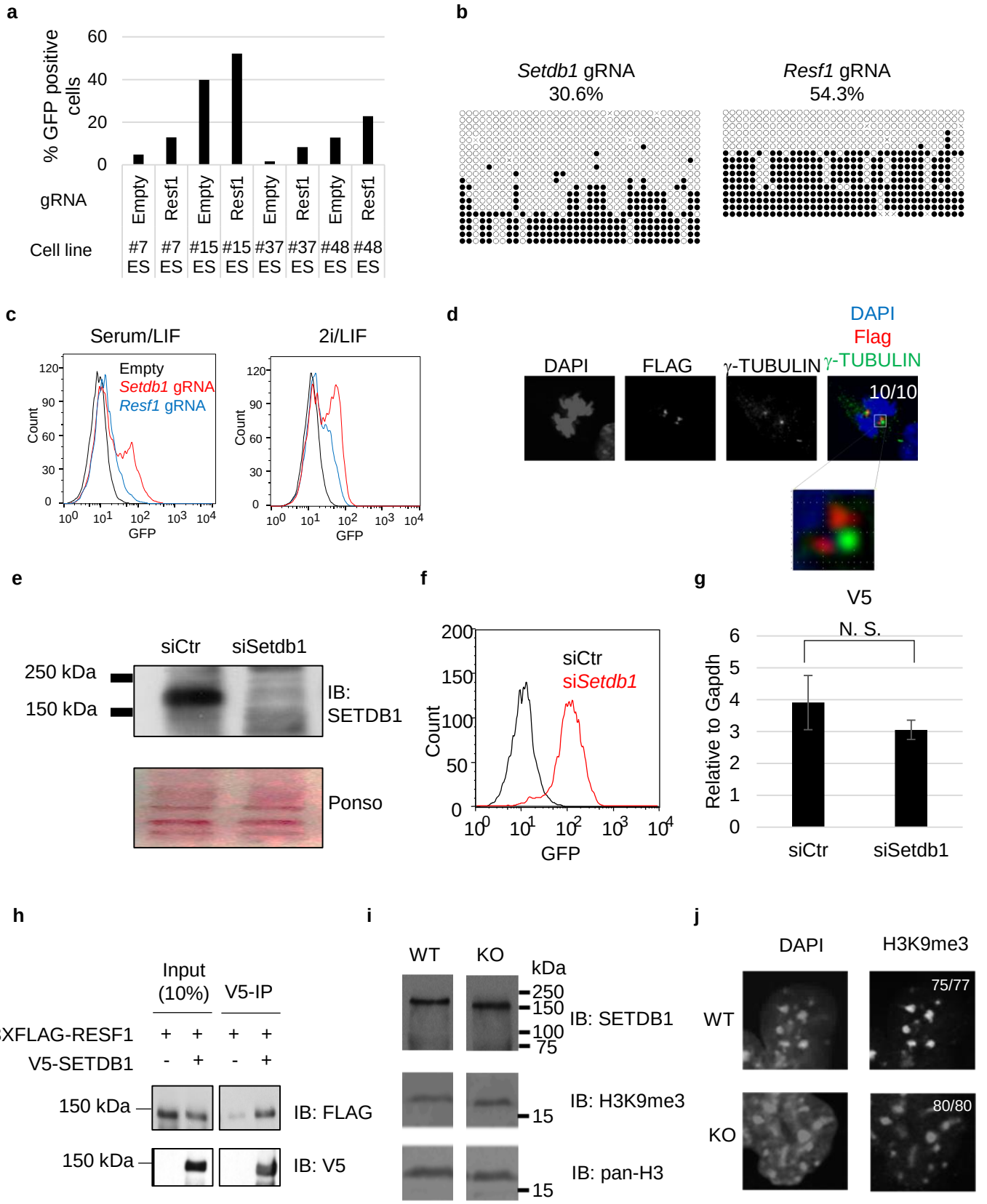
Fukuda_Fig_S5



Supplemental Figure 5. Characterization of *Resf1* KO mESCs containing MSCV-GFP reporter provirus. (a) Enhancement of proviral GFP expression by the transfection of *Resf1* gRNA plasmid in different MSCV-GFP reporter mES cell lines. Proviral GFP expression was analyzed by flow cytometry at 5 days after gRNA transfection. (b) Effect of *Setdb1* and *Resf1* gRNA transfection on DNA methylation of MSCV promoter. DNA methylation of MSCV promoter in GFP positive cells at 5 days after *Setdb1* (left) or *Resf1* (right) gRNA plasmids was analyzed by bisulfite sequencing. (c) FACS analysis of proviral GFP expression at 5 days after gRNA plasmid transfection in clone 7 grown in serum/LIF (left) or 2i/LIF (right). (d) Cellular localization of FLAG-tagged RESF1 in M phase analyzed by immunofluorescence staining. FLAG signals were surrounded around g-Tubulin in all mitotic cells analyzed (10 of 10 mitotic cells). (e-g) Effect of SETDB1 knockown on RESF1 enrichment in MSCV promoter. siSetdb1 and siCtr were treated to V5-RESF1 expressing mESCs for 6 days, and then, SETDB1 protein level, GFP expression level and RESF1 enrichment in MSCV promoter were analyzed by western blotting (e), FACS (f) and ChIP-qPCR (g), respectively. (h) Coimmunoprecipitation of V5-SETDB1 with 3XFLAG-RESF1 from transfected cells. 293FT cells were transfected with plasmids expressing 3XFLAG-RESF1 and V5-SETDB1. 48 hours after the transfection, the cell lysates were immunoprecipitated with anti-V5 antibody, and then the immunoprecipitated complexes were subjected to Western blot analysis with an anti-V5 antibody or anti-FLAG antibody. (i) Western blotting analysis of SETDB1 and H3K9me3 in WT and *Resf1* KO mESCs. (j) Distribution of H3K9me3 in nucleus from WT and *Resf1* KO interphase cells analyzed by immunofluorescence. H3K9me3 foci was found in DAPI dense region in the majority of analyzed cells (75/77 cells in WT and 80/80 cells in *Resf1* KO cells).