



Supplemental Figure 1. Screen of provirus silencing factor by lentiviral CRISPR-gRNA library.

(a) Selection of reporter cell line for the screen. GFP expressions were analyzed by flow cytometry at day 5 post transfection of *Setdb1* gRNA. #7 cell line (Clone 7) showed strong enhancement of proviral GFP expression by *Setdb1* gRNA plasmid transduction. (b) FACS analysis of proviral GFP expression in clone 7 grown in serum/LIF or 2i/LIF medium at 4 days after lentiviral infection. Clone 7 grown in 2i/LIF showed reactivation of proviral GFP (left). In addition, any lentiviral infection further enhanced proviral GFP expression in clone 7 cultured with 2i (right), but not with serum (center). (c) Bisulfite sequencing analysis of MSCV promoter in clone 7 grown in serum/LIF (top) or 2i/LIF (bottom) medium. (d) Validation of several gRNAs by western blotting at day 5 after transfection of gRNA plasmid. (e) Validation of top 47-95 genes by gRNA plasmid transfection. GFP expressions were analyzed by flow cytometry at day 5 after the gRNA transfection. Y-axis represents fraction of GFP positive cells. Red bar represents the fraction of GFP positive cells in Clone 7 transfected with empty vector. (f) GO term enrichment analysis for cellular component of top 100 genes. The analysis was performed by DAVID. (g) List of the protein complex components associated with chromatin found in top 100 genes. (h) Transfection of gRNA specific to HUSH components followed by FACS analysis at day 5 after gRNA plasmid transfection. Both *Mphosph8* and *Pphln1* gRNAs enhanced proviral GFP expression.