Extended Data Figure 3 | Screen for L1 regulators in HeLa cells and and L1- sequence-dependent L1 regulators. a. CasTLE analysis of two independent genome-wide screens in HeLa cells, with 20,514 genes represented as individual points. Genes at 10% FDR cutoff colored in red, CasTLE likelihood ratio test[11]. b. The maximum effect size (center value) estimated by CasTLE from two independent HeLa secondary screens with 10 different sgRNAs per gene. Bars, 95% credible interval (CI). L1 activators, red; L1 suppressors, blue. Genes whose CI include zero are colored in gray and are considered non-effective against L1. c. Scatter plots showing the secondary screen hits identified in K562 cells and HeLa cells (252 genes from two independent screens in each cell line), with Venn diagram comparing hits in the two cell lines is shown on the right. d. The maximum effect size (center value) of indicated heterochromatin regulators, estimated by CasTLE from two independent HeLa secondary screens with 10 different sgRNAs per gene. Error bars, 95% credible intervals of the estimated effect size. e. The maximum effect size (center value) of indicated DNA repair genes, estimated by CasTLE from two independent HeLa secondary screens with 10 different sgRNAs per gene. Error bars, 95% credible intervals of the estimated effect size. f. The (opt)-L1-GFP reporter retrotransposed more frequently than L1-GFP did in K562. The GFP(+) fraction of cells with the indicated L1 reporter after 15 days of dox induction was normalization to the L1-GFP sample. Box plots show median and interquartile range (IQR), whiskers are 1.5× IQR. n = 6 biologically independent replicates. g. The GFP(+) fraction of dox-induced Ctrl and mutant cell pools with the L1-GFP reporter or (opt)-L1-GFP reporter. Experiments were performed as Fig. 1e. Chromatin regulators (e.g. TASOR, MORC2, MPP8, SAFB) did not suppress the (opt)-L1-GFP reporter, in which 24% of the L1 ORF nucleotide sequence is altered, without changes in the encoded amino acid sequence[19,20], indicating their L1 regulation depends on the native nucleotide-sequence of L1Hs. h. K562 secondary screen with the (opt)-L1-G418^R reporter (252 genes from n = 2 independent screens) revealed genes that regulate retrotransposition dependent or nondependent on the native L1 nucleotide sequence. The K562 secondary screen candidates identified with L1-G418^R (252 genes from n = 2 independent screens) were labeled in blue. A Venn diagram comparing hits identified from the two L1-reporters is also shown.