Extended Data Figure 4 | MORC2, MPP8 and TASOR silence L1 transcription. a. Relative genomic copy number of newly integrated L1-GFP reporters in the indicated mutant K562 pools after dox-induction. PspGI-assisted qPCR assay used here was designed to selectively detect spliced GFP rather than the unspliced version (see Methods section). The L1-GFP copies were normalized to beta-actin DNAs; data then normalized to Ctrl. As a putative L1 activator, SLTM shows an opposite effect on the DNA copy number, compared with L1 suppressors. Center value as median. n = 3 technical replicates per gene. b. RNA-seq data in Ctrl K562 cells showing that most heterochromatin regulators in Fig. 2a are expressed, supporting the selective effect of HUSH and MORC2 in L1 regulation. c. Western blots validating the knockout (KO) effects in independent KO K562 cell clones. Ctrl samples were loaded at 4 different amounts (200%, 100%, 50%, 25% of KO clones). Three experiments repeated independently with similar results. To obtain KO clones, we sorted mutant K562 pools (cells used in Fig. 1e,f) into 96-well plates, expanded cells and screened for KO clones through western blotting. Of note, all K562 KO clones were derived from the same starting L1-GFP reporter line, and thus do not differ in reporter transgene integrations among the clones. d. Representative images of single molecule FISH (smFISH) assays targeting ACTB mRNAs and RNA transcripts from L1-GFP reporters in Ctrl and KO K562 clones after 5 days of dox-induction. No signal was observed from L1-GFP reporters without dox-induction (data not shown). Two experiments repeated independently with similar results. See also panel e and Fig. 2b (showing L1-GFP mRNA only). e. Quantitation of the L1-GFP transcription level from the indicated number of K562 cells, determined by smFISH assays (panel d and Fig. 2b). The number of L1-GFP mRNA transcripts is normalized to the number of beta-actin mRNAs within each K562 cell. Box plots show median and interquartile range (IQR), whiskers are 1.5× IQR. P-value, two-sided Wilcoxon test. 95% CI for median from 1,000x bootstrap: Control: 0.059-0.082; MORC2: 0.106-0.123; MPP8: 0.264-0.410; TASOR: 0.514-0.671. f. MORC2, MPP8, and TASOR KOs increase the genomic copy number of newly integrated L1-GFP reporters. PspGI-assisted qPCR assays were performed as in panel a), but using clonal KO K562 clones instead of mutant cell pools. Data normalized to Ctrl. n = 3 technical replicates, center value as median. g. MORC2 KO, MPP8 KO, and TASOR KO increase the expression of endogenous L1s. RT-qPCR experiments were performed as in (Fig. 1f), but using clonal KO K562 clones instead of mutant cell pools. n = 2 biological replicates x 3 technical replicates (center value as median). The primers do not target the L1-GFP reporter and the cell lines were not dox-induced, so these RT-qPCR assays will not detect L1-GFP transcripts. h. Western blots showing depletion effects of MORC2, MPP8 and TASOR in the mutant pools of K562 cells (left) and in the mutant pools of H9 hESCs without transgenic L1 reporters (right). Two experiments repeated independently with similar results. i. Northern blots showing increased transcription from the L1-GFP reporter in KO K562 clones (same cell lines as in panel c) after 5 days' dox-induction. Two experiments repeated independently with similar results. As observed in Fig. 2b, while HUSH KO significantly increases L1-GFP transcription, MORC2 KO leads to only a modest increase. This is probably because the L1-GFP reporter does not contain the native L1 5' UTR sequence, where MORC2 intensively binds (See Extended Data Fig. 7f,g). The 5 kb and 1.9 kb marks on the membrane refer to the 28S rRNA and 18S rRNA bands respectively. j. Northern blots showing that disruption of MORC2, MPP8 and TASOR increases the expression level of endogenous L1Hs in hESCs, same cell lines as in panel h). Size marker indicated as in panel i). Two experiments repeated independently with similar results. k. Western blots showing protein abundance of L1_ORF1p and HSP90 in the mutant pools of K562 cells and hESCs (same cell line as shown in panel h). Two experiments repeated independently with similar results. Experiments were performed without dox-induction of the transgenic L1 reporter. Due to the strong signal of bands from the KO samples, the blots were exposed for a very short time and the band signal in the Ctrl samples were relatively very weak compared to the KO samples; same case for panels i, j).