Supplementary Figures

The SUMO isopeptidase activity of SENP7 can sustain the expression of developmental genes but not a repressive state on chromosome X

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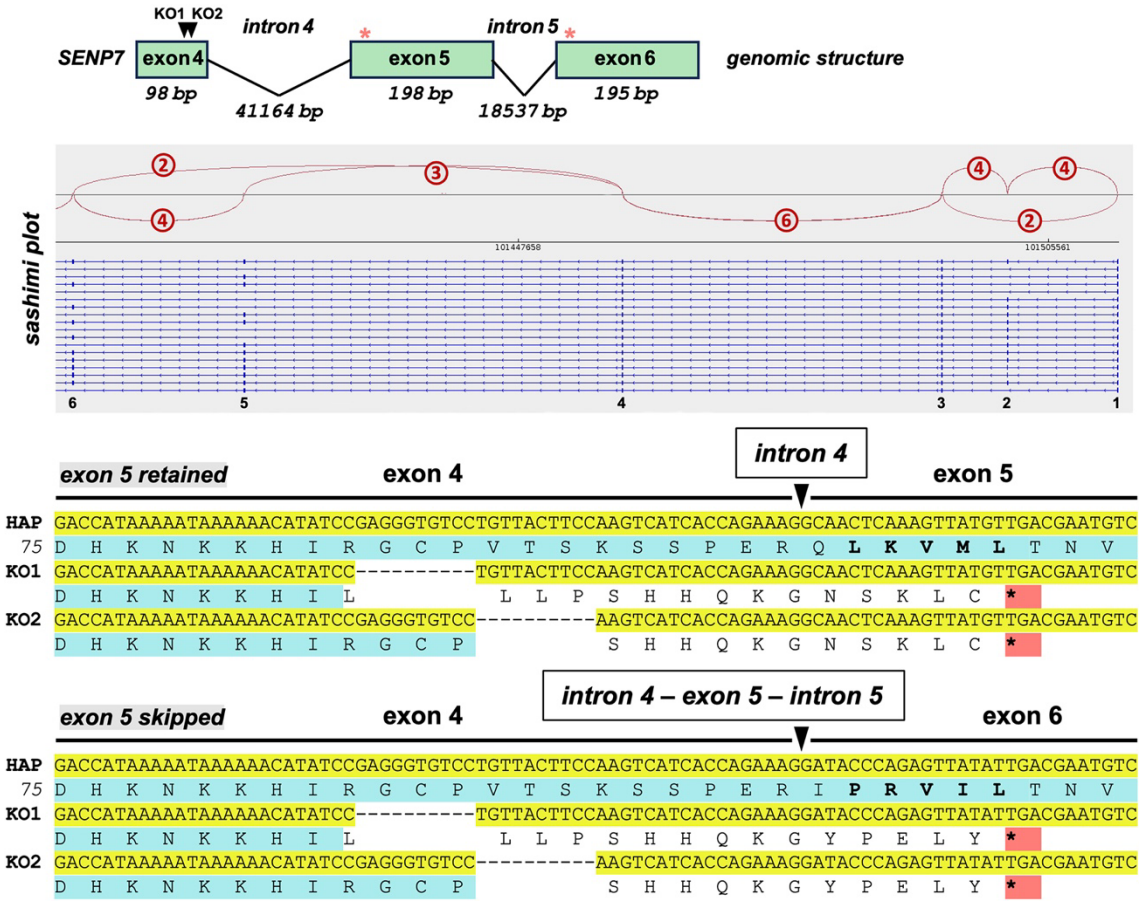
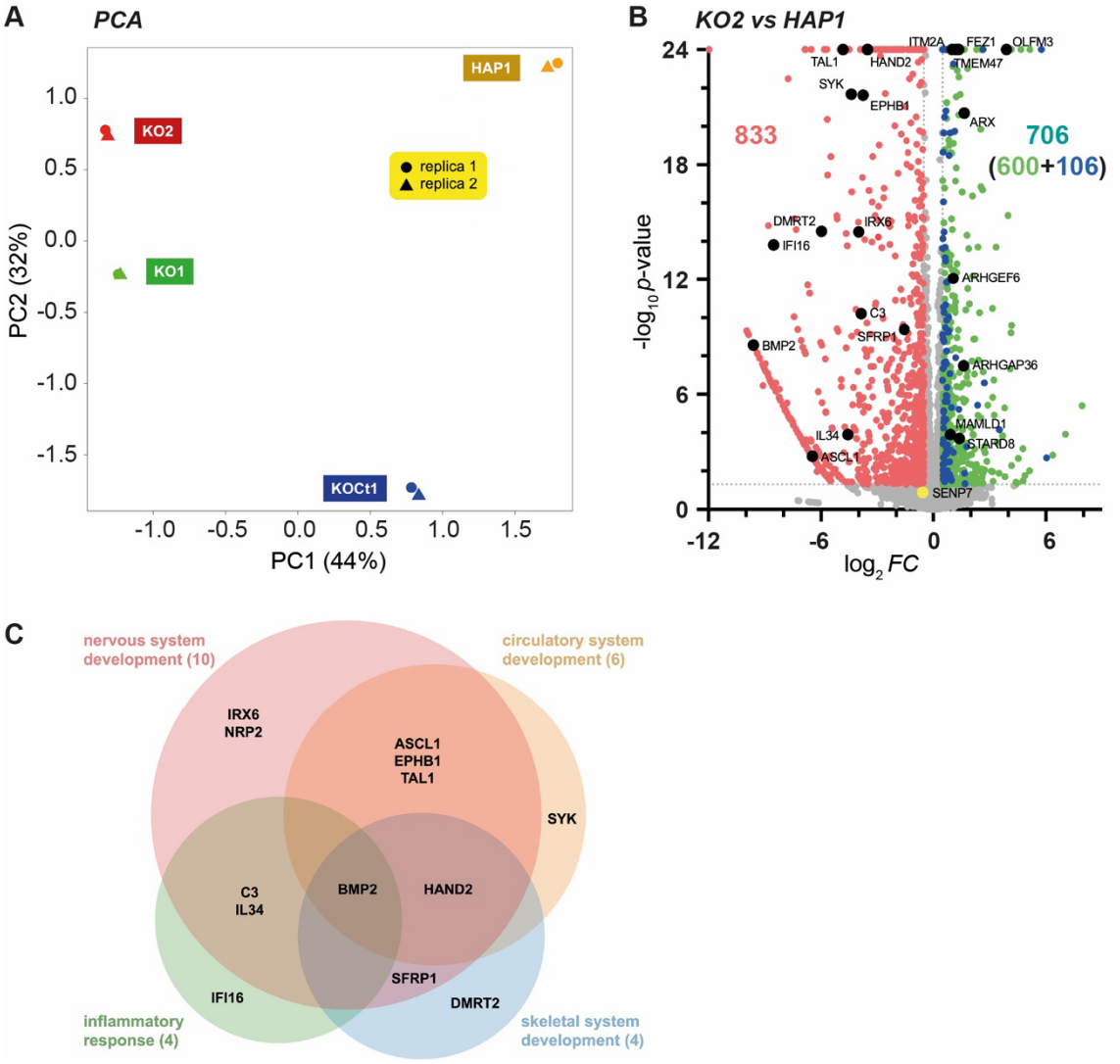
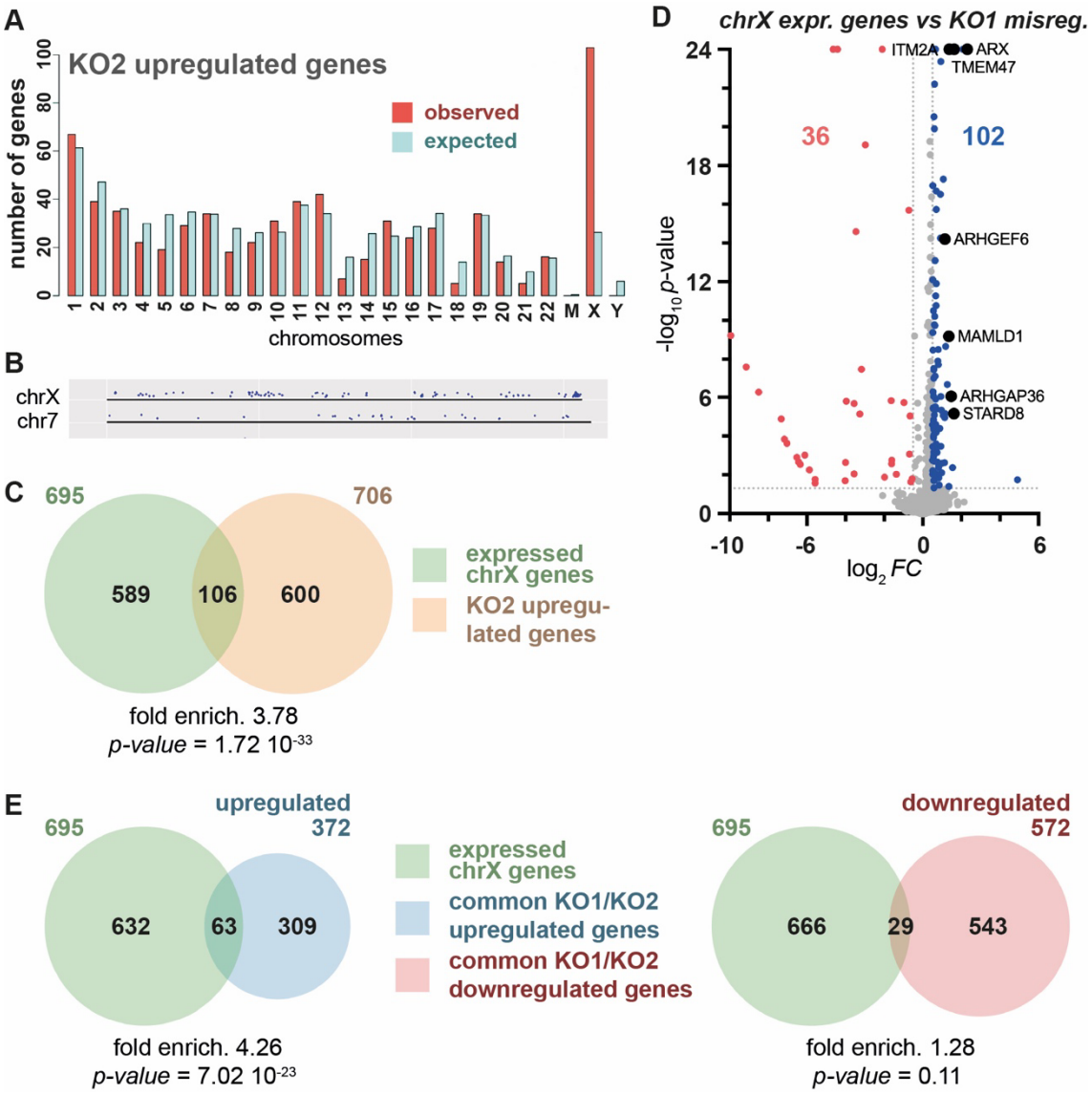


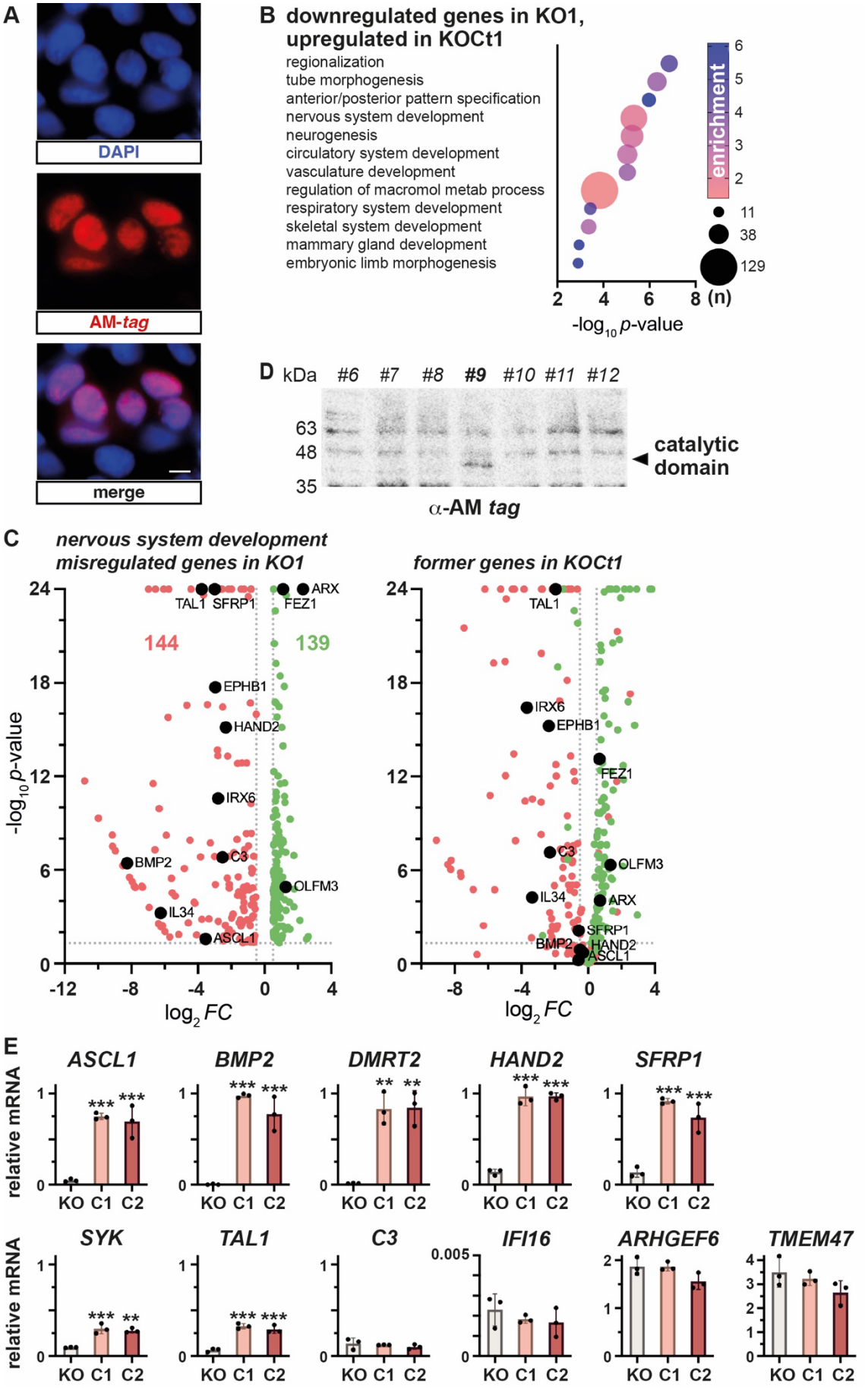
Supplementary Fig. S1 Location of mutations in *SENP7* KO cells. Upper panel: schematic representation of the *SENP7* genomic locus corresponding to exons 4 to 6 and introns 4 and 5. Arrowheads point to the positions of the 10-bp deletions in exon 4 in KO1 and KO2 cells, which, whether exon 5 is retained or skipped, lead to frameshifts and the appearance of premature STOP codons (red asterisks) as depicted in lower panels. Length of exons and introns (bp) is indicated. Middle panel: sashimi plot obtained from RNA-seq reads overlapping contiguous exons, indicating that in HAP1 cells exon 5, but not exon 4, can be skipped in a number of cases. Several *SENP7* variants are shown in blue, with the exon numbers below. Red numbers indicate relative quantity of the indicated splicing. Lower panels: DNA and amino acid sequences around exons 4-6 of *SENP7* showing the frameshift provoked by the 10-bp deletions in exon 4 of KO1 and KO2 cells, and the appearance of STOP codons (red boxed asterisks) if exon 5 is retained or skipped. The position of amino acid 75 (D) is indicated. PxVxL motifs previously described to interact with HP1 protein are highlighted in bold.



Supplementary Fig. S2 Transcriptomic analysis of *SENP7* KO cells. A Principal Component Analysis (PCA) representation of the RNA-seq experiments. Sample replicates (1 and 2) for HAP1, KO1, KO2, and KOCt1 cells are indicated in the same color, with different symbols, as indicated. **B** Volcano plot of DEGs in *SENP7* KO cells (KO2) in relation to HAP1 cells, obtained from RNA-seq analysis. DEGs out of *p*-value and fold change (FC) cutoffs are in grey. Downregulated genes are shown in red, and upregulated genes in blue (chrX genes) and green (rest of genes). Selected genes are marked in black. *SENP7* is marked in yellow. The number of downregulated and upregulated genes is indicated. **C** Overlapping GO categories for selected DEGs validated by qPCR in this study, related to *development* and *inflammatory response*. Numbers indicate the number of genes in each category.



Supplementary Fig. S3 Chromosome X altered transcription in *SENP7* KO cells. A Bar diagram showing the expected number of upregulated DEGs per chromosome, and the real observed value for them in KO2 cells. **B** Linear distribution of upregulated DEGs along chrX in KO2 cells, together with upregulated DEGs in chr7 for comparison with a chromosome of similar size. **C** Representation with a Venn diagram of the overlap between the 695 genes from chrX, which are expressed in HAP1 cells, and the 706 genes upregulated in KO2 cells. The fold enrichment and its associated *p*-value of the overlap, based on the hypergeometric test, are also indicated. **D** Volcano plot of FC and *p*-value in KO1 cells of the 695 genes on chrX whose expression is detected in HAP1 cells. DEGs out of cutoffs are in grey, while downregulated and upregulated genes are in red and blue, respectively, shown with the number of misregulated genes. Selected genes are indicated in black. **E** Venn diagrams of the overlap between the 695 genes on chrX expressed in HAP1cells and the commonly upregulated (left) or downregulated (right) genes in KO1 and KO2 cells, associated with the hypergeometric test-determined fold enrichment and *p*-value.


A
DAPI
AM-tag
merge
B downregulated genes in KO1, upregulated in KOCt1
regionalization
tube morphogenesis
anterior/posterior pattern specification
nervous system development
neurogenesis
circulatory system development
vasculature development
regulation of macromol metab process
respiratory system development
skeletal system development
mammary gland development
embryonic limb morphogenesis
enrichment
6
5
4
3
2
11
38
129
(n)
2
4
6
8
-log10 p-value
D kDa #6 #7 #8 #9 #10 #11 #12
63
48
35
catalytic domain
α-AM tag
C nervous system development misregulated genes in KO1
former genes in KOCt1
144
139
TAL1
SFRP1
ARX
FEZ1
EPHB1
HAND2
IRX6
BMP2
C3
OLFM3
IL34
ASCL1
-log10 p-value
log2 FC
E
ASCL1
BMP2
DMRT2
HAND2
SFRP1
SYK
TAL1
C3
IFI16
ARHGEF6
TMEM47
relative mRNA
KO C1 C2

Supplementary Fig. S4 Stable cell line expressing the SENP7 catalytic domain. A The cellular localization of the expressed catalytic domain construct, N- and C-terminally tagged with NLS and AM epitope sequences, respectively, was analyzed by immunofluorescence with the anti-AM antibody (red). Nuclei were stained with DAPI (blue). Scale bar: 5 µm. **B** Bubble representation of GO analysis of genes downregulated in KO1 cells, which are upregulated in KOCt1 cells. Size and color correspond to the number of genes (n) and enrichment, respectively, as indicated in the legend. Top-12 non-redundant categories according to *p*-values (Benjamini) are shown. **C** Volcano plots of DEGs related to nervous system development in KO1 cells (left) and how they appear in KOCt1 cells (right). DEGs outside the cutoffs are in grey, while downregulated and upregulated genes are in red and green, respectively, together with the number of misregulated genes. Selected genes are shown in black. **D** Western blot analysis of additional puromycin-resistant clones putatively expressing the SENP7 catalytic domain (see Fig. 4B), tested with the anti-AM antibody. Clone #9 expresses the expected protein and was designated KOCt2. **E** Relative mRNA levels of the indicated genes in KO1 (KO), KOCt1 (C1) and KOCt2 (C2) cells, analyzed by qPCR. Note that values for KO1 and KOCt1 cells are the same as in Figs. 5D and 8C for the corresponding genes. Data are mean values ± s.d. from 3 independent experiments analyzed in duplicate. Statistical significance of the differences was determined through ANOVA ($p < 0.05$) followed by Tukey's post-test. Differences with KO1 cells are indicated on top of each bar. Differences between KOCt1 and KOCt2 cells were not significant. ** $p < 0.01$; *** $p < 0.001$.