Supplementary figures

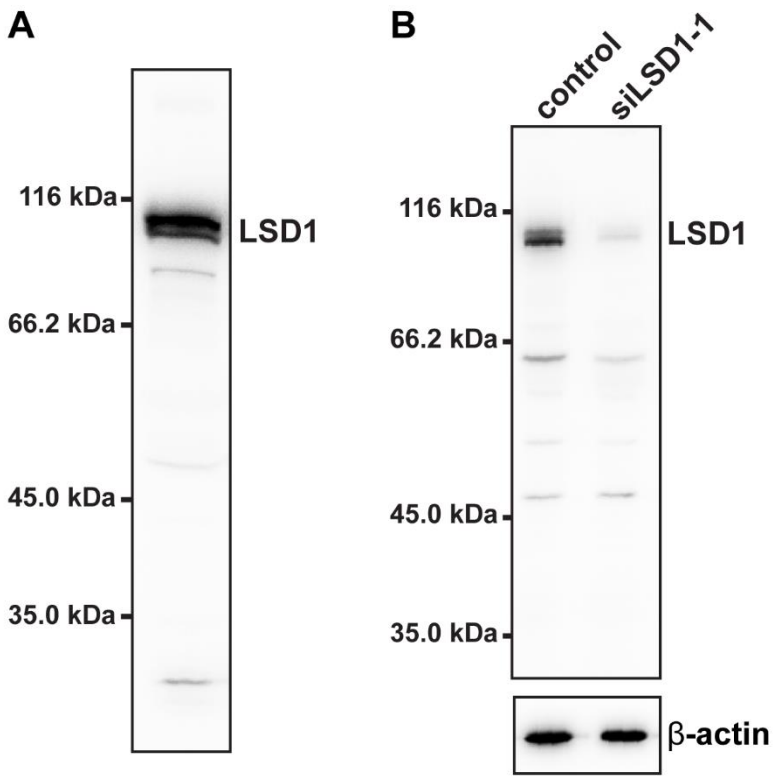

Fig. S1. Characterisation of a new *Xenopus laevis* LSD1 antibody

Antiserum generated in rabbits against the full length *Xenopus laevis* LSD1 protein was tested for specificity in immunoblots of lysates from *Xenopus* egg extracts (A) or HeLa cells (B) subjected to SDS-PAGE. Note the strong reduction of the human LSD1 signal upon RNAi mediated LSD1 downregulation.

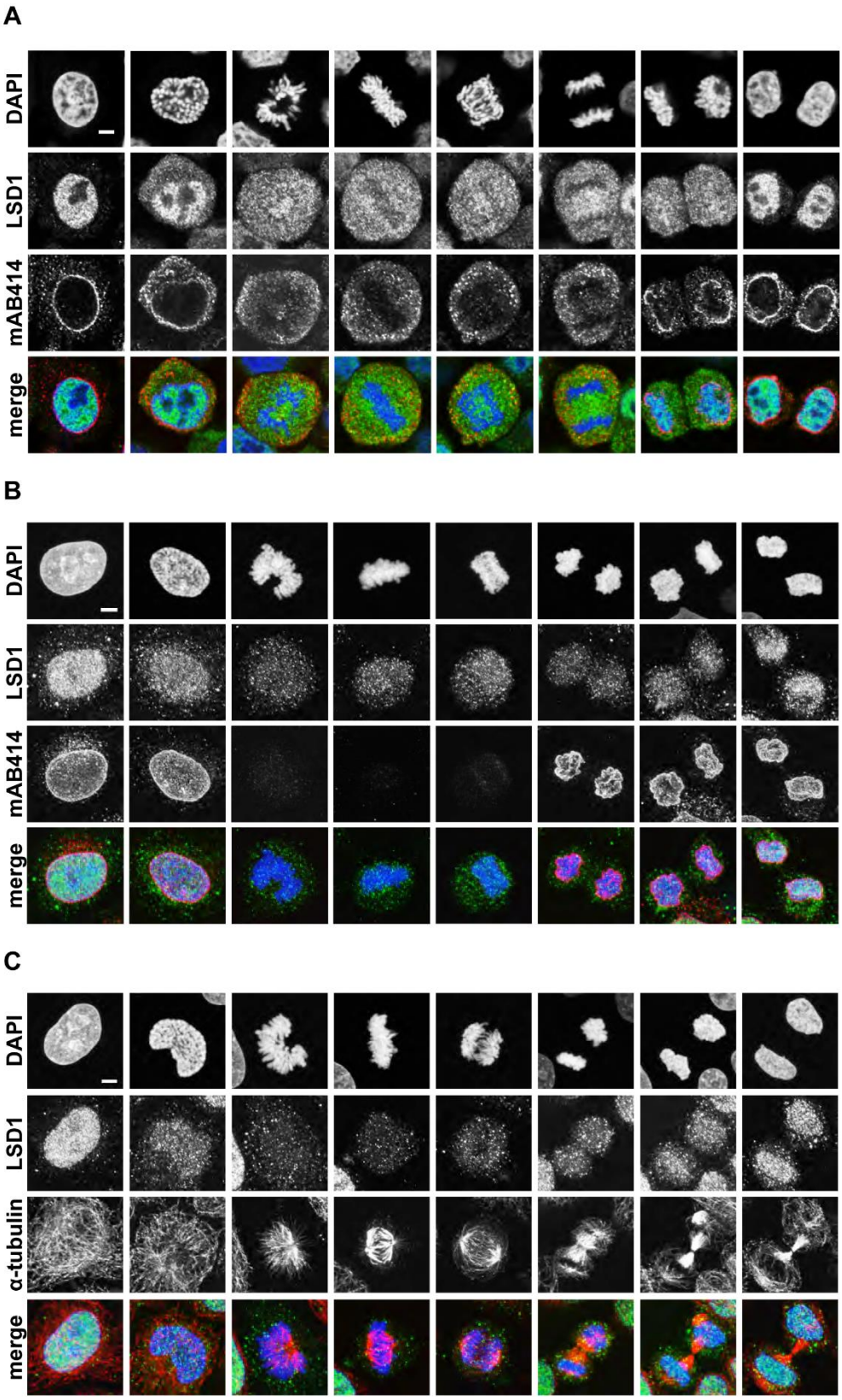

Fig. S2. LSD1 localisation in HeLa cells

(A) The cell cycle-dependent localization of LSD1 in unsynchronised HeLa cells was analyzed by immunofluorescence and confocal microscopy. LSD1 (green in overlay) and NPCs (mAB414, red) were immunolabeled and chromatin was stained with DAPI (blue).

(B) LSD1 (green) and NPCs (mAB414, red) immunolabeled in cells permeabilized with 0.1% Triton-X 100 prior to fixation. Chromatin was stained with DAPI (blue). Maximum intensity projections are shown.

(C) LSD1 (green) and α-tubulin (red) were immunolabeled in cells permeabilized with 0.1% Triton-X 100 prior to fixation. Chromatin was stained with DAPI (blue). Maximum intensity projections are shown.

Scale bars: 5 μm

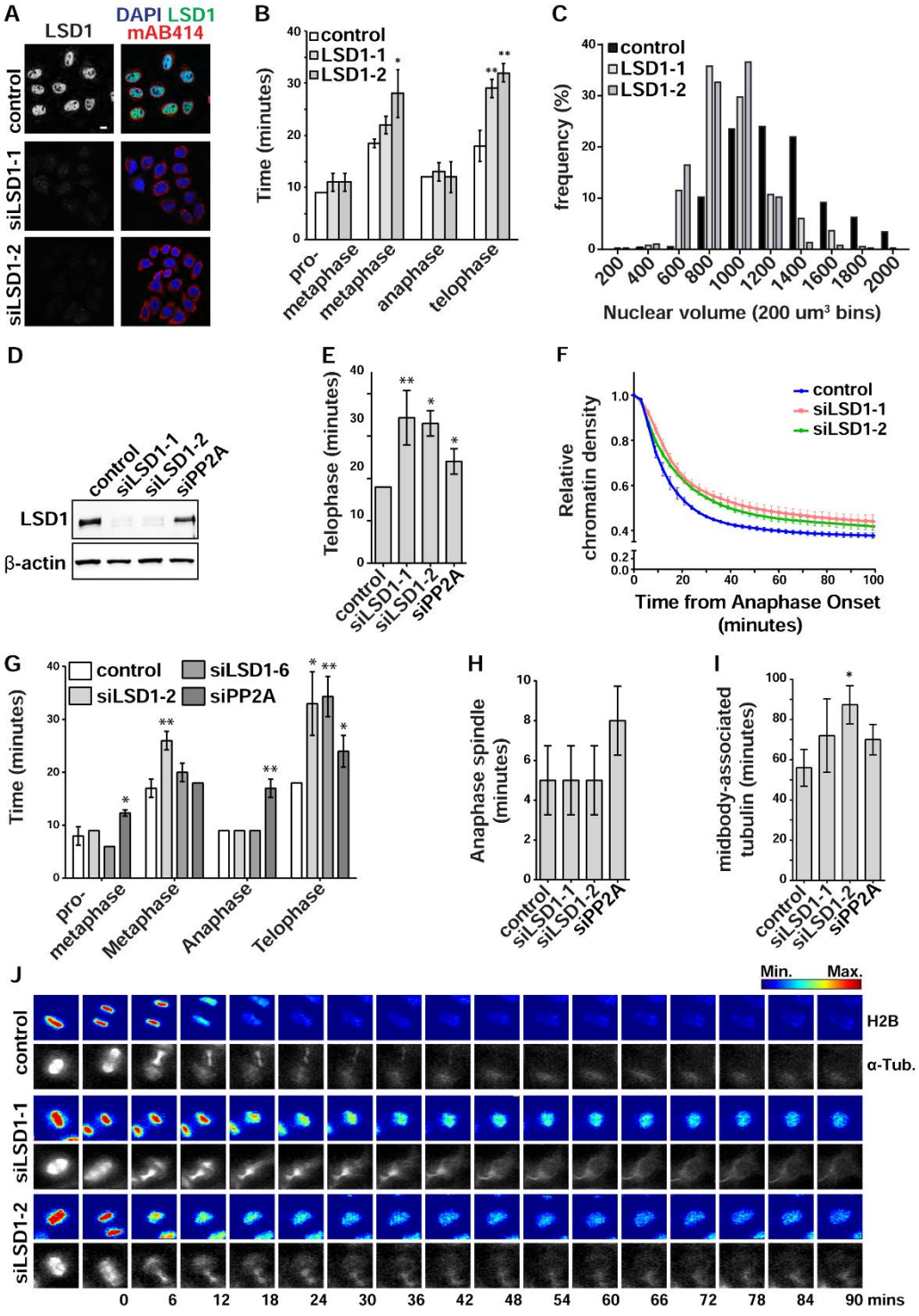

Fig. S3. Further characterization of siRNA mediated downregulation of LSD1 in HeLa cells

(A) HeLa cells transfected with 20 nM control, LSD1-1 or LSD1-2 siRNA oligos were fixed 48h post-transfection and processed for immunofluorescence. LSD1 (top panel, green in overlay) and NPCs (mAB414, red in overlay) were immune-labeled and chromatin was stained with DAPI (blue in overlay). Scale bar: 10 µm

(B) HeLa cells stably expressing H2B-mCherry and transfected with 20 nM siRNA as indicated were subjected to time-lapse microscopy starting 30h post-transfection. Mitotic events were analyzed with CellCognition. The mean of the median time spent in each cell cycle stage indicated is plotted for more than 100 mitotic events per condition in 3 independent experiments. Error bars represent s.d. *P<0.05, Student's t-test.

(C) Quantification of nuclear volume based on DAPI staining in HeLa cells transfected with 20 nM control, LSD1-1 or LSD1-2 siRNA oligos and fixed 48h post-transfection. Nuclear volume measurements from 383 nuclei in 3 independent experiments were pooled for each condition and assigned to 200 μm^3 bins. The nuclear volume frequency for each bin is plotted and the center of each bin is indicated on the x-axis.

(D) Western blot analysis of whole cell lysates from HeLa cells stably expressing H2B-mCherry/α-tubulin-EGFP and transfected with 20 nM control, LSD1-1, LSD1-2, or PP2A siRNA oligos as indicated. Lysates were harvested 48h post-transfection.

(E) HeLa cells stably expressing H2B-mCherry/ α-tubulin-EGFP and transfected with 20 nM siRNA were subjected to time-lapse microscopy starting 30h after transfection. Mitotic events were analyzed with CellCognition. The mean of the median time spent in telophase based on chromatin features is plotted for more than 100 mitotic events per condition in 3 independent experiments. Error bars represent s.d. *P<0.05, **P<0.01, Student's t-test.

(F) The average fluorescence intensity of the H2B-mCherry signal was extracted from the CellCognition data acquired in (B) as an indication of chromatin density. The density was normalized to the first anaphase frame in individual mitotic tracks and the mean relative density for more than 100 mitotic events from three independent experiments is plotted over time. Error bars represent s.d.

(G) HeLa cells stably expressing H2B-mCherry/ α-tubulin-EGFP and transfected with 20 nM siRNA were subjected to time-lapse microscopy starting 30h post-transfection. Mitotic events were analyzed with CellCognition. The mean of the median time spent in each cell cycle stage based on chromatin features is plotted for more than 100 mitotic events per condition in 3 independent experiments. Error bars represent s.d. *P<0.05, **P<0.01, Student's t-test.

(H) HeLa cells stably expressing H2B-mCherry/ α-tubulin-EGFP and transfected with 20 nM siRNA were subjected to time-lapse microscopy starting 30h post-transfection. Mitotic events were analyzed with CellCognition. The mean of the median duration of the anaphase spindle, based on the α-tubulin signal, is plotted for more than 100 mitotic events per condition in 3 independent experiments. Error bars represent s.d.

(I) HeLa cells stably expressing H2B-mCherry/ α-tubulin-EGFP and transfected with 20 nM siRNA were subjected to time-lapse microscopy starting 30h post-transfection. Mitotic events were analyzed with CellCognition. The mean of the median duration of detectable midbody-associated α-tubulin is plotted for more than 100 mitotic events per condition in 3 independent experiments. Error bars represent s.d. *P<0.05, Student's t-test.

(J) HeLa cells stably expressing H2B-mCherry/ α-tubulin-EGFP and transfected with 20 nM siRNA were subjected to time-lapse microscopy starting 30h after transfection. Maximum intensity projections of the mCherry and EGFP signals from five optical z sections are shown. Mitotic tracks are normalized to the first anaphase frame.

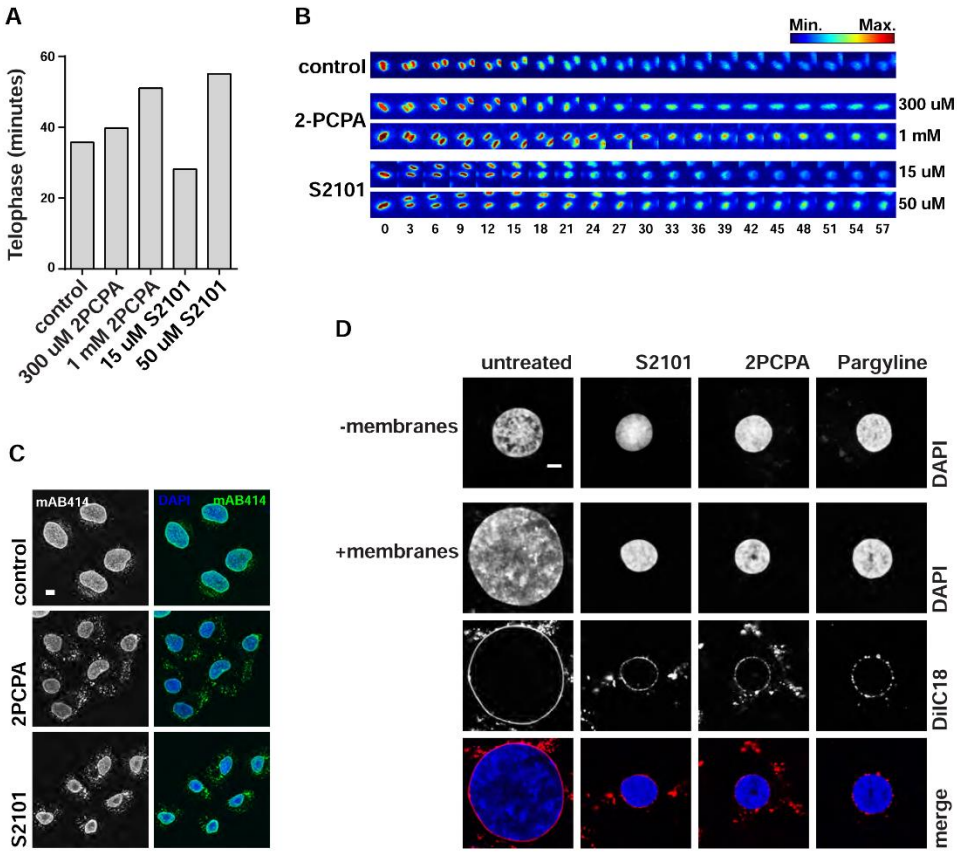

Fig. S4. Chemical inhibition of LSD1 extends the duration of telophase and promotes the formation of annulate lamellae in HeLa cells but does not block membrane-independent chromatin decondensation *in vitro*

(A) HeLa cells stably expressing H2B-mCherry were treated with different concentrations of the LSD1 inhibitors 2-PCPA and S2101 as indicated and immediately subjected to time-lapse microscopy. Mitotic events were analyzed with CellCognition (control: 73 events, 300 µM 2-PCPA: 35 events, 1mM 2-PCPA: 10 events, 15 µM S2101: 62 events, and 50 µM S2101: 22 events). The number of mitotic events analysed was drastically reduced in the presence of LSD1 inhibitors due to cell lethality at the effective concentrations. The median time spent in telophase is plotted and individual data are shown.

(B) HeLa cells stably expressing H2B-mCherry were treated with different concentrations of the LSD1 inhibitors 2-PCPA and S2101 as indicated and immediately subjected to time-lapse microscopy. Representative maximum intensity projections of the mCherry signal from five optical z sections are represented as a heat maps. Mitotic tracks are normalized to the first anaphase frame.

(C) HeLa cells treated with 300 µM 2-PCPA or 15 µM S2101 as indicated for 24h, fixed, and processed for immunofluorescence. NPCs were immuno-labelled using mAB414 (green in overlay) and DNA was stained with DAPI (blue). Maximum intensity projections are shown.

(D) Mitotic chromatin clusters from HeLa cells were incubated with Xenopus egg extracts in the presence or absence of added membranes. Where indicated 5 mM pargyline, 2.5 mM 2PCPA or 0.25 mM S2101 was added to decondensation reactions at t = 0. After 120 min samples were fixed and analyzed by confocal microscopy. Added membranes were pre-labeled with DiIC18 (red in overlay) and chromatin was stained with DAPI (blue in overlay). Scale bar: 5 µm.