Supplementary methods

Plasmids: roGFP (Addgene plasmid #49435) was PCR amplified and cloned into the pTR-GNP AAV backbone (pTR-GNP-roGFP). Human coding sequences for H2AX (NM_002105.3), TRF2 (NM_005652.5), LaminB1 (NM_005573.4) and HP1a (NM_001127322.1) were PCR amplified and cloned into the pTR-GNP AAV backbone using the appropriate restriction enzymes. Plasmids were amplified in SURE2 Competent Cells (Agilent, CA) at 30ºC to avoid loss of ITR sequences. Presence of ITR sequences was confirmed by SmaI digestion of the final plasmid preparations.
Cell culture: HL1 cell line was maintained, and FA treated as previously described (12). NRVMs were isolated and cultured as described previously (13).
AAV production and infection: The plasmids pTR-GNP for generating AAV expression cassette, pDP6rs for packaging AAV6 are gifts from Dr. Roger Hajjar (Mount Sinai School of Medicine, New York, NY) (14). We used helper virus-free, two-plasmid-based AAV packaging system for viral production. The viruses were produced by polyethylenimine (Polysciences, Warrington, PA)-mediated transfection in HEK293T cells (ATCC, #CRL-11268, Manassas, VA). After transfection for 72 hrs, the cells were harvested by centrifugation. After three rounds of freeze and thaw, the cell lysates were treated with Pierce Universal Nuclease for Cell Lysis (Pierce, Waltham, MA) to obtain the crude lysate. 20-100µL of crude virus lysate was added to 0.5 million NRVMs and the mixture was placed on a laminin-coated MakTek glass-bottom dish and incubated for 48-72h before imaging.
Microscopy and image analysis: Cells were imaged using a LSM510 Meta (Zeiss) microscope equipped with CO_2 and temperature-controlled cell culture chambers. After basal acquisition, equal volume 2X treatment medium was added. Excitation was performed using 405 and 488nm laser lines and fluorescence was collected using a filter BP505/550. A minimum of 20 sensor-expressing cells were imaged per condition by z-stack acquisition. Same cells were followed over time after treatment for a period of 10min. Intensity of maximum intensity projection images was quantified using ImageJ for 405 and 488 channels. For staining intensity quantification (γH2AX and H3K27me3), nuclei were segmented and per cell intensity in the green channel was measured using CellProfiler software. Individual values of at least 20 cells per experiment were averaged.