Mevalonate pathway-mediated ER homeostasis is required for haploid stability in human somatic cells

Supplemental materials and methods

Cholesterol measurement

For total cellular cholesterol measurement, cells were once washed with DPBS, resuspended in 850 µL DPBS, and lysed by sonication. Fifty µL cell lysis was mixed with 50 µL 0.1 M NaOH, incubated at 60°C for 2 h, and subjected to total protein measurement using Protein Assay Bicinchoninate kit (06385-00, nacalai tesque). For cholesterol extraction, the remaining cell lysis was mixed with 1 mL chloroform and 2 mL methanol, and incubated at 37°C for 2 h with vigorous agitation. After centrifugation at 1000 × g for 5 min, cholesterol extract was collected from the bottom layer. Cholesterol was further extracted from the remaining lysis by repeating the addition of 2 mL chloroform and centrifugation 3 times. The cholesterol extract was evaporated under a stream of N₂ and dissolved in 100 µL DPBS. The extracted total cholesterol was measured using LabAssay Cholesterol kit (294-65801, Wako) according to the manufacture’s instruction. The oxidized and condensed N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, sodium salt and 4-Aminoantipyrin were measured with 590 nm wavelength absorbance using iMark microplate reader (BIO-RAD). Cholesterol amount is then normalized to total cellular protein amount for comparison.

For visualization of intracellular cholesterol, the Cholesterol Cell-Based Detection Assay Kit (10009779, Cayman Chemical) was used according to the manufacture’s instruction.