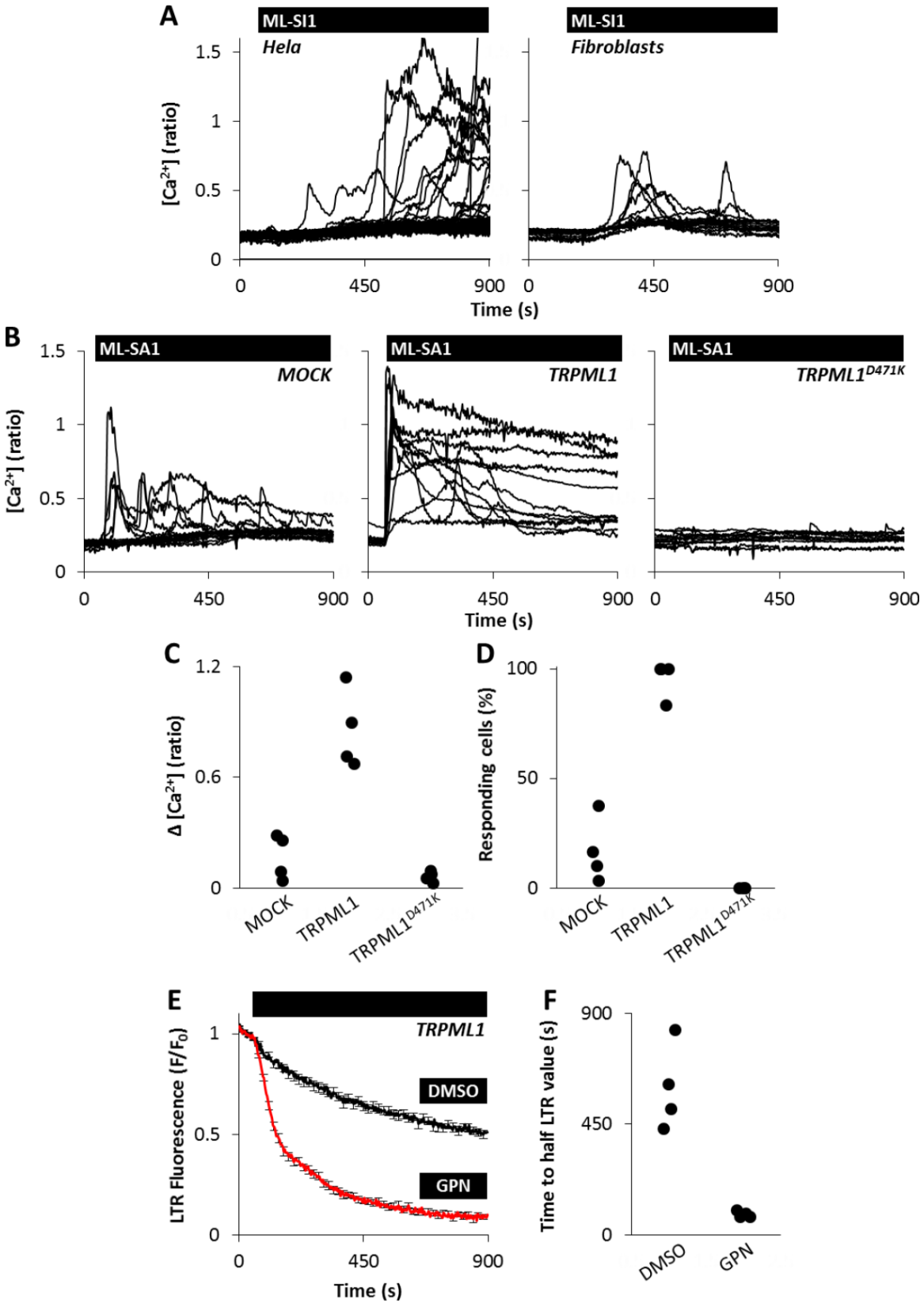


Figure S1. (A) The mucolipin inhibitor, ML-SI1 elevates cytosolic Ca^{2+} levels. Cytosolic Ca^{2+} levels of Hela cells and fibroblasts stimulated with 20 µM ML-SI1. (B-D) Agonist-evoked Ca^{2+} signals require TRPML1. (C) Cytosolic Ca^{2+} levels of individual mock transfected fibroblasts or fibroblasts expressing TRPML1 or TRPML1^{D471K} stimulated with 20 µM ML-SA1. (C-D) Summary data (12-97 cells). (E-F) GPN compromises lysosome integrity. (E) Lysotracker® red fluorescence levels of TRPML1 expressing Hela cells stimulated with vehicle (0.1 % DMSO) or 200µM GPN. (F) Summary data quantifying the time taken to achieve half-maximal loss of fluorescence (87-108 cells).

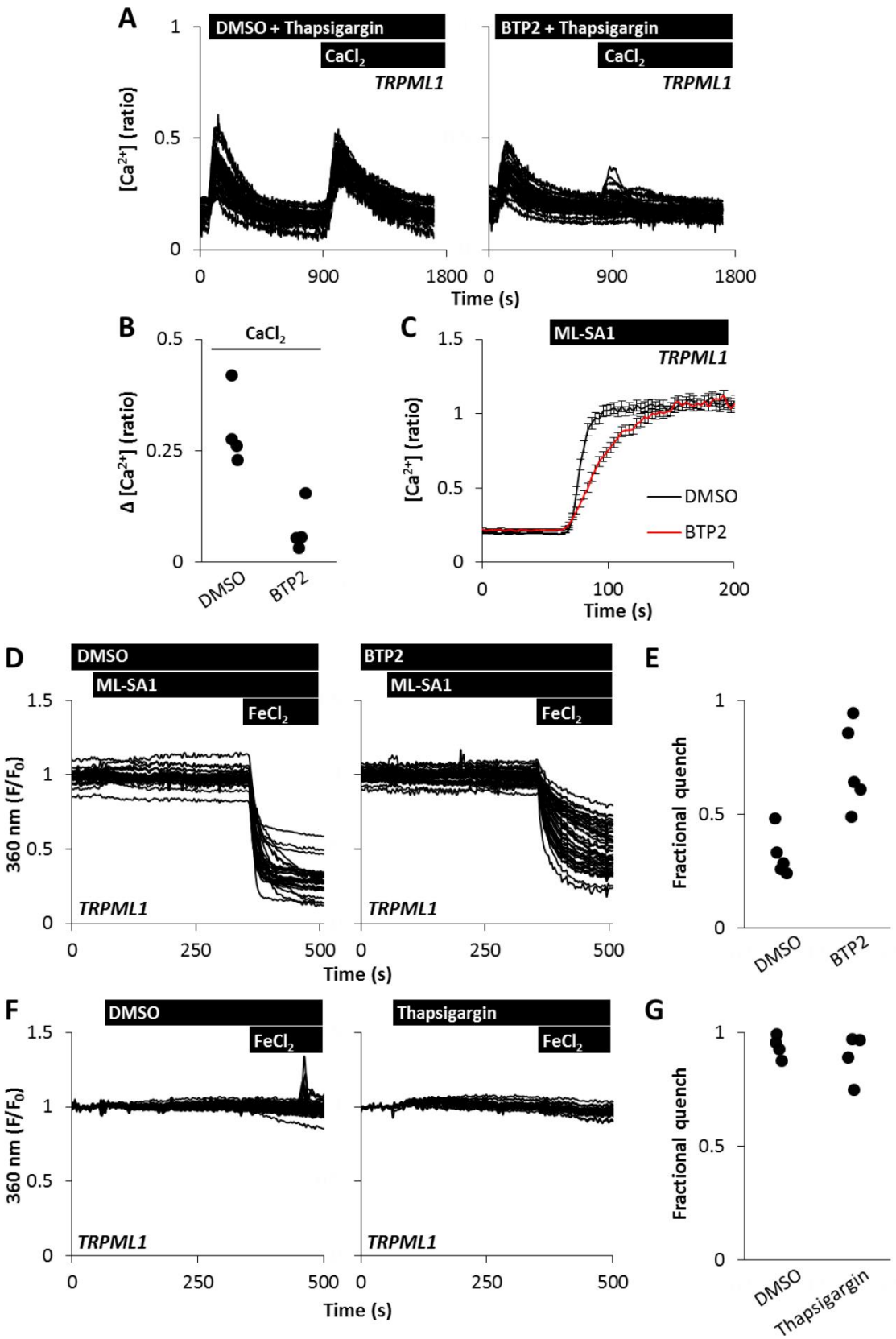
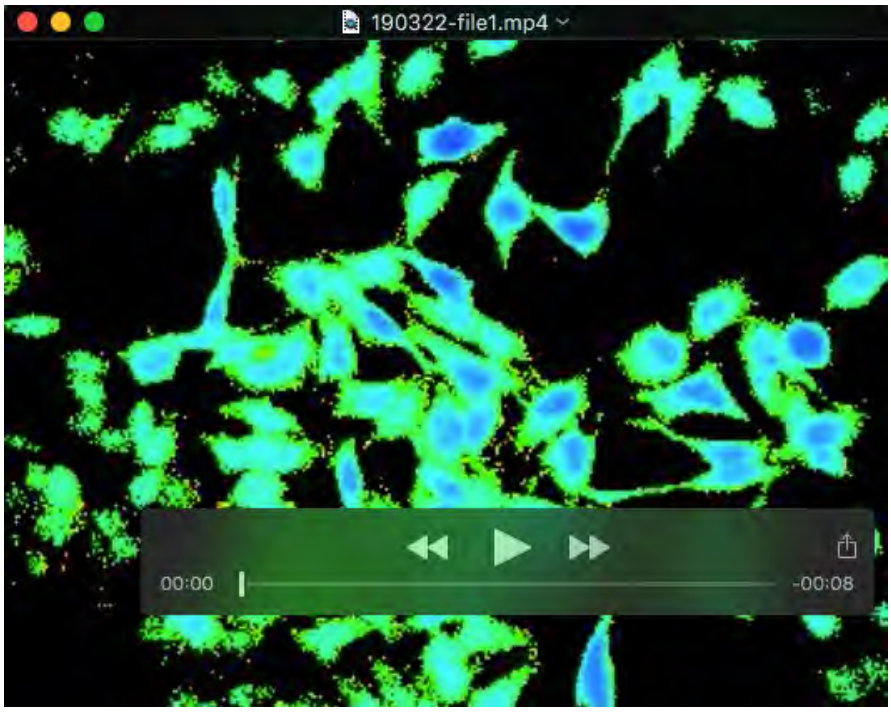
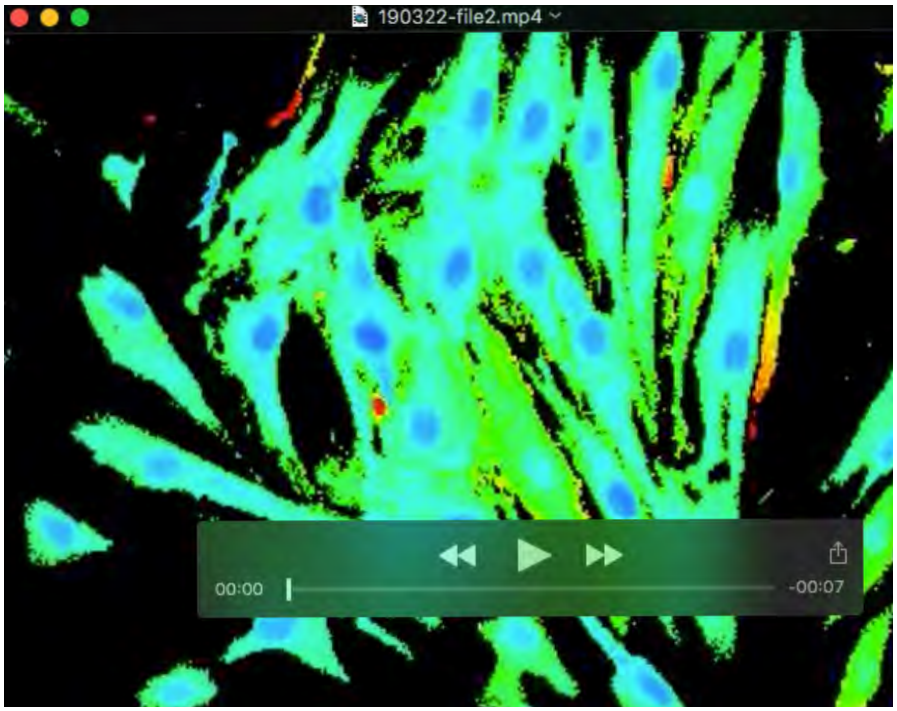


Figure S2. (A-B) BTP2 inhibits store-operated Ca^{2+} entry. (A) Cytosolic Ca^{2+} levels of TRPML1-expressing Hela cells in nominally Ca^{2+}-free medium sequentially stimulated with 1 µM thapsigargin and 2mM $CaCl_2$ in the presence of vehicle (0.1 % DMSO) or 20 µM BTP2. (B) Summary data (111-117 cells). (C). BTP2 slows ML-SA1–evoked Ca^{2+} signals. Cytosolic Ca^{2+} levels of TRPML1-expressing Hela cells (presented as mean +/- s.e.m. of 120-121 cells, n=3) stimulated with 20 µM ML-SA1 in the presence of vehicle (0.1 % DMSO) or 20 µM BTP2. (D-E) BTP2 slows ML-SA1–evoked Fe^{2+}-entry. (D) Quench of Fura-2 fluorescence in Hela cells expressing TRPML1 stimulated with 20 µM MLSA1 and then 1 mM $FeCl_2$. Experiments were performed in nominally Ca^{2+}-free medium either in the presence of vehicle (0.1 % % DMSO) or 20 µM BTP2. (E) Summary data (124-144 cells). (F-G) Depletion of ER Ca^{2+} stores does not evoke Fe^{2+} entry. (F) Quench of Fura-2 fluorescence in Hela cells expressing TRPML1 stimulated with 1 mM $FeCl_2$ in the presence of vehicle (0.1 % DMSO) or 1 µM thapsigargin. (G) Summary data (84-130 cells).



Movie S1. Activation of TRPML evokes global Ca^{2+} signals in Hela cells. Effect of 20 µM ML-SA1 on cytosolic Ca^{2+} levels of Fura-2 loaded Hela cells. Images were acquired every 3 s and played back at a rate of 40 frames per second. Warmer colours represent an increase in Fura-2 fluorescence ratio which is proportional to Ca^{2+} concentration.



Movie S2. Activation of TRPML evokes global Ca^{2+} signals in fibroblasts. Effect of 20 µM ML-SA1 on cytosolic Ca^{2+} levels of Fura-2 loaded fibroblasts. Images were acquired every 3 s and played back at a rate of 40 frames per second. Warmer colours represent an increase in Fura-2 fluorescence ratio which is proportional to Ca^{2+} concentration.