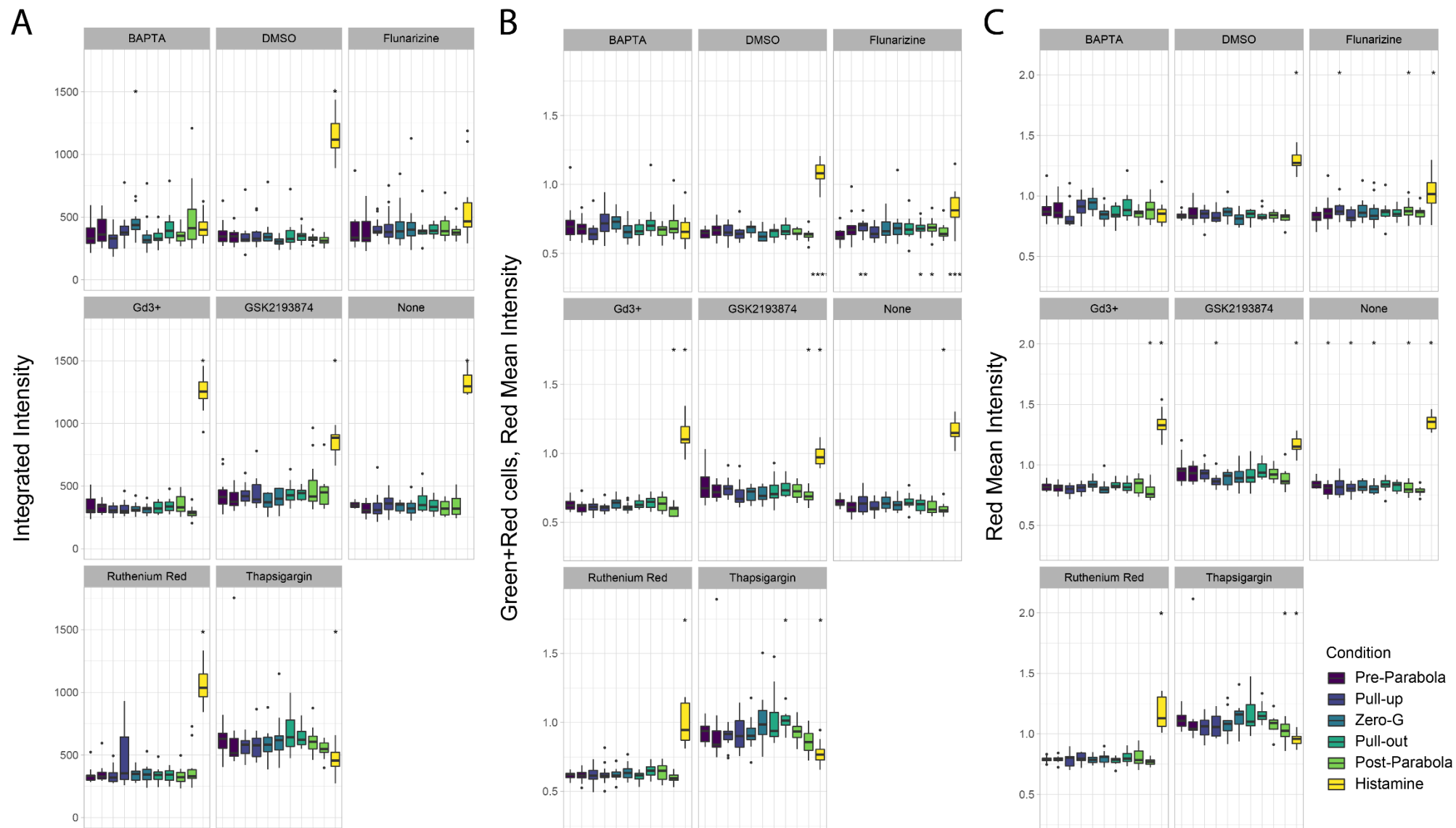


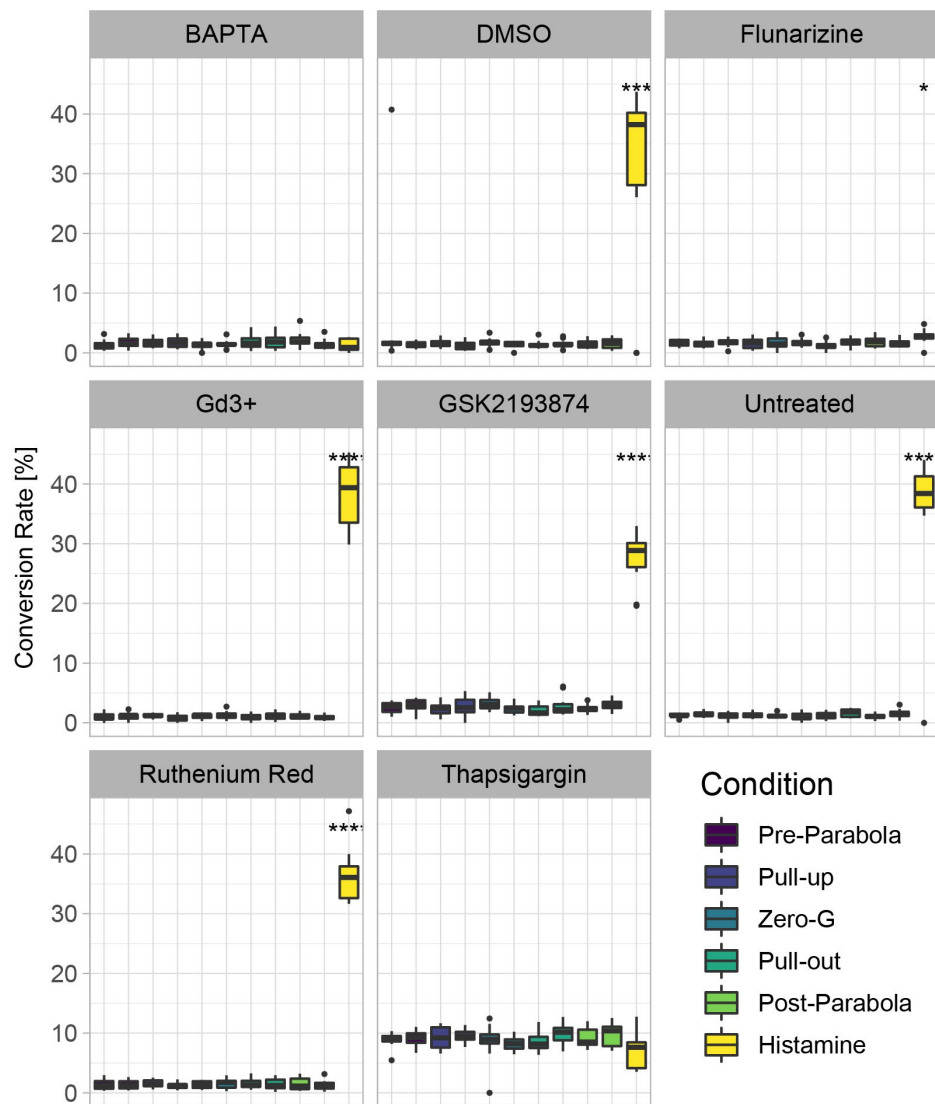
Retrograde Analysis of Calcium Signaling by CaMPARI2 Shows Cytosolic Calcium in Chondrocytes Is Unaffected by Parabolic Flights

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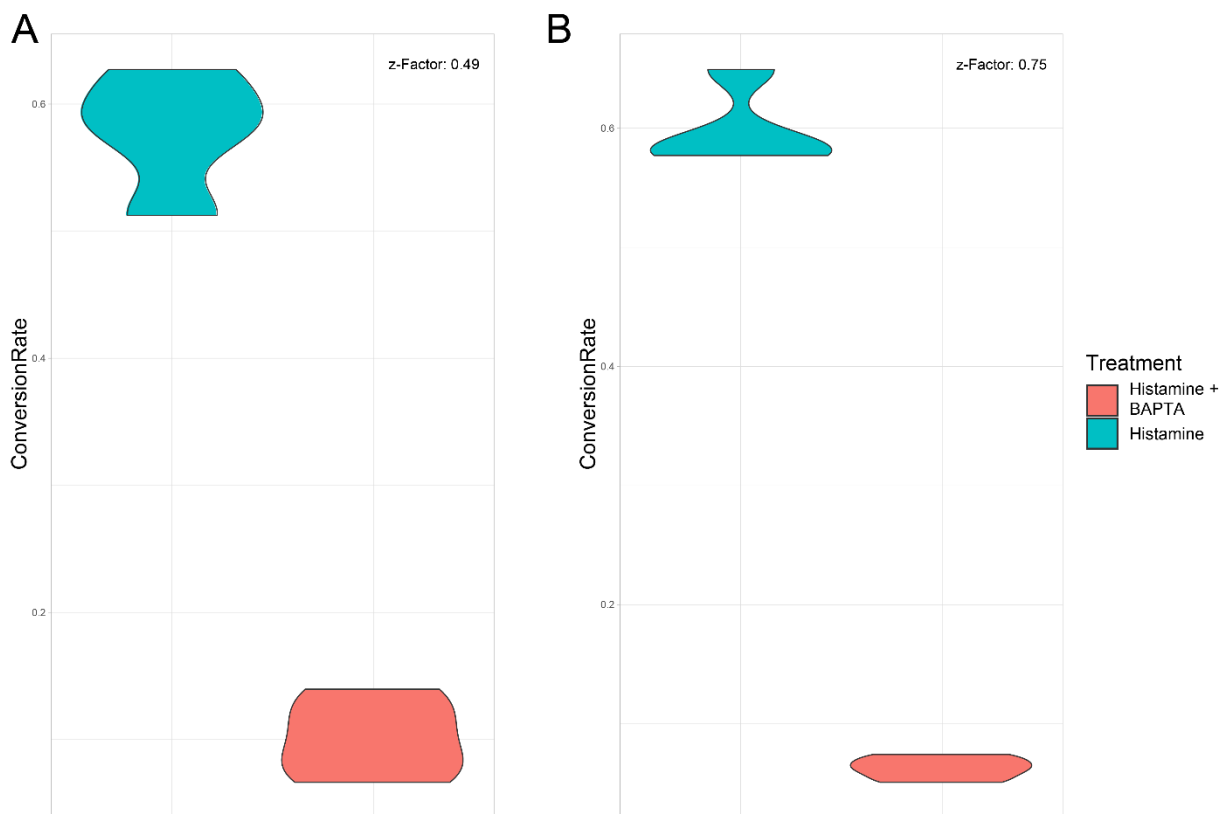
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Supplementary Figure S1: Different metrics used for the analysis of high throughput microscopy image data after the parabolic flight. Eight 96-well plates were used for each of the three parabolic flights. Each plate was treated with one of the displayed chemicals 5 hours before photoconversion. The cells were illuminated over four parabolas (Parabola 0, 10, 20, and 30), each well for 8 seconds during the corresponding flight phase. For each flight phase and the histamine post-flight control, the average of different metrics of 12 wells (4 wells per flight) were calculated using the IncuCyte 2021A mask described in materials and methods 2.9. In this supplemental figure the integrated red intensity (A, Red integrated intensity per well/green+red object counts per well), the red mean intensity of objects showing green and red fluorescence (B, green+red objects red mean intensity object average) and (C) the red mean intensity of red objects. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Supplementary Figure S2: Conversion rates of cells transfected with CaMPARI2 and subjected to a parabolic flight. During each flight phase (Condition) 2 wells containing transfected C28/I2 chondrocyte cells were illuminated for 8 seconds with 405 nm photoconversion light. The histamine positive control was performed directly after the parabolic flight with a 100 μ M histamine treatment followed by 8 seconds of photoconversion. Each box in the figure displays the average of 12 wells. * $p < 0.05$, *** $p < 0.001$.



Supplementary Figure S3: Z'-Factor analysis of all four parabolas of the second (A) and third (B) parabolic flight, comparing the conversion rate of CaMPARI2-F391W transfected human C28/I2 chondrocytes. Both conditions are treated with 100 μ M histamine to induce photoconversion of CaMPARI2-F391W with 405 nm light, one condition was pretreated with 10 μ M of the calcium chelator BAPTA-AM as a negative control. The calculated Z'-factors are 0.49 for flight 2 (A) and 0.75 for flight 3 (B).