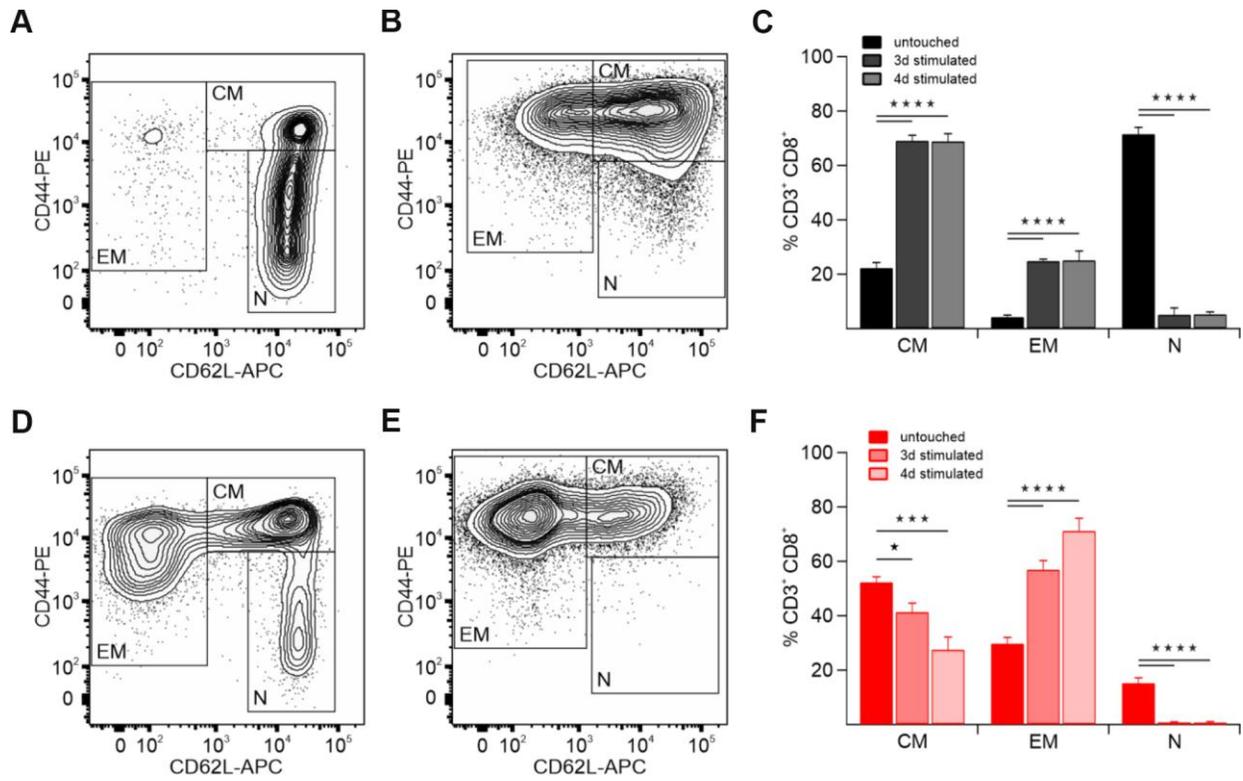
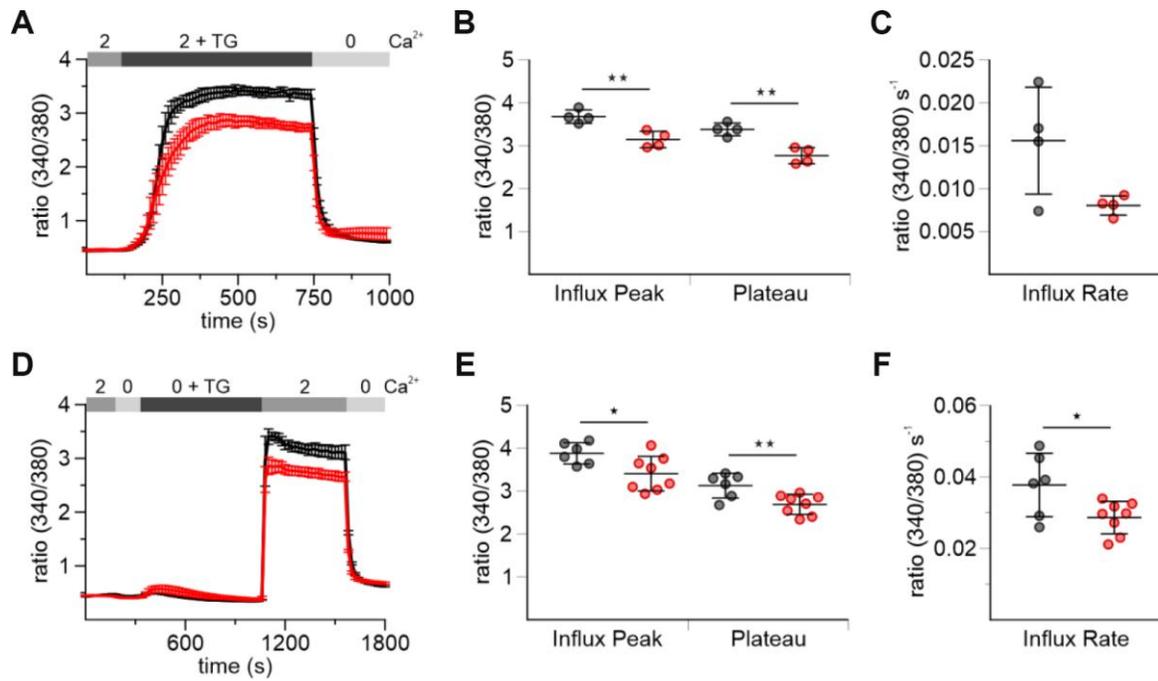


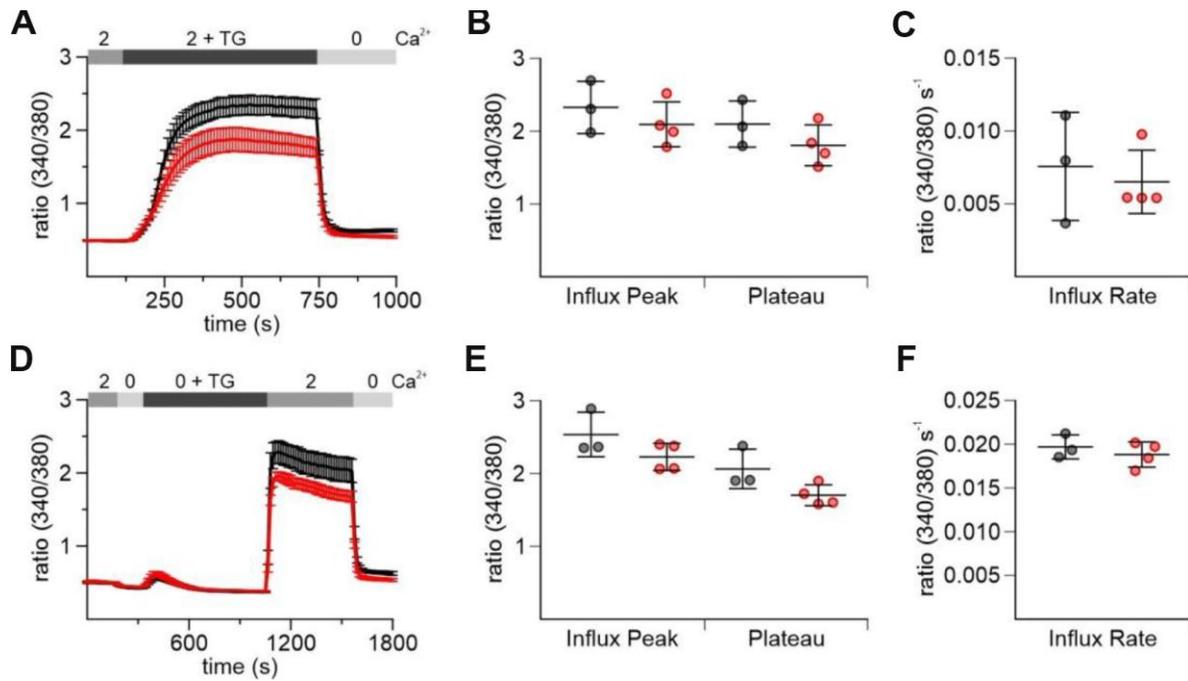
SUPPLEMENTARY FIGURES



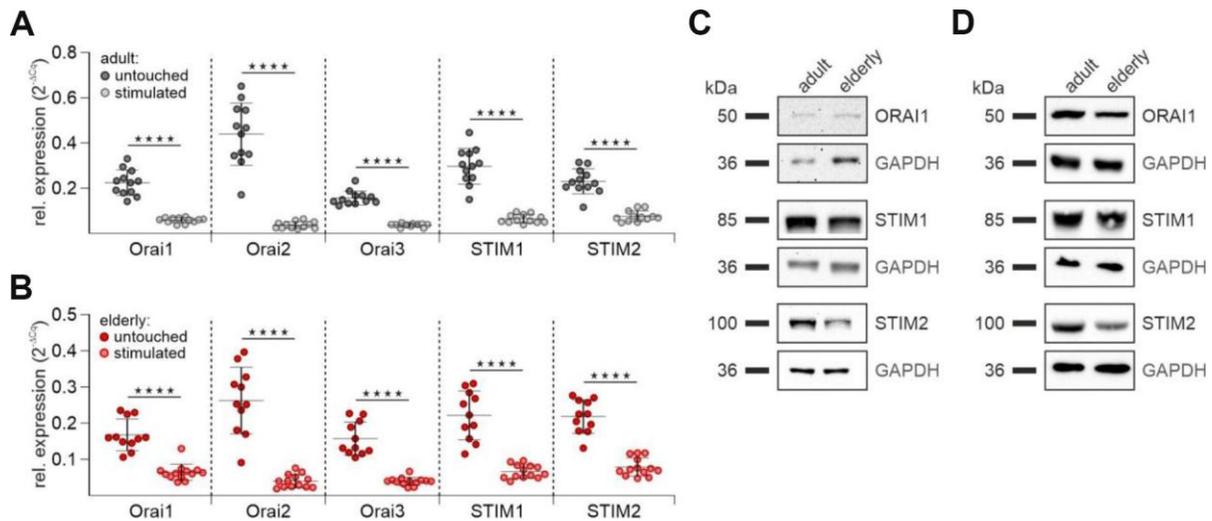
Supplementary Figure 1. CD8⁺ T cell subtype distribution shifts from more naïve to more memory cells in elderly mice. Exemplary contour plots of untouched CD8⁺ T cells from adult (A) and elderly (B) and stimulated CD8⁺ T cells from adult (D) and elderly mice (E). (C) Subtype distribution from untouched (n = 35), three (n = 20) and four days stimulated (n = 10) CD8⁺ T cells from adult mice. (F) Subtype distribution from untouched (n = 31), three (n = 20) and four days stimulated (n = 8) CD8⁺ T cells from elderly mice. Data obtained are presented as mean ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.



Supplementary Figure 2. Untouched CD8⁺ T cells from elderly mice exhibit reduced thapsigargin (TG)-induced Ca²⁺ signals. (A) Fura2-AM based Ca²⁺ Imaging with 1 μM TG as stimulus applied in the presence of 2 mM [Ca²⁺]_{ext} (combined Ca²⁺ protocol) of CD8⁺ T cells from adult (black, n = 4) and elderly (red, n = 4) mice. Scatter dot plot in (B) displays the corresponding statistic of Ca²⁺ influx peak and Ca²⁺ plateau and in (C) the corresponding influx rates. (D) Ca²⁺ Imaging with 1 μM TG applied in the absence of [Ca²⁺]_{ext} before re-addition of 2 mM Ca²⁺ (re-addition protocol) of CD8⁺ T cells from adult (black, n = 6) and elderly (red, n = 8) mice. The scatter dot plot in (E) displays the corresponding statistic of Ca²⁺ influx peak and Ca²⁺ plateau and (F) the corresponding influx rates. Ca²⁺ data are presented as mean ± SEM. Scatter dot plots are presented as mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.



Supplementary Figure 3. Stimulated CD8⁺ T cells from elderly mice exhibit reduced thapsigargin (TG)-induced Ca²⁺ signals. (A) Fura2-AM based Ca²⁺ Imaging with 1 μM TG as stimulus applied in the presence of 2 mM [Ca²⁺]_{ext} (combined Ca²⁺ protocol) of CD8⁺ T cells from adult (black, n = 3) and elderly (red, n = 4) mice. The scatter dot plot in (B) displays the corresponding statistic of Ca²⁺ influx peak and Ca²⁺ plateau and in (C) the corresponding influx rates. (D) Ca²⁺ Imaging with 1 μM TG applied in the absence of [Ca²⁺]_{ext} before re-addition of 2 mM Ca²⁺ (re-addition protocol) of CD8⁺ T cells from adult (black, n = 3) and elderly (red, n = 4) mice. The scatter dot plot in (E) displays the corresponding statistic of Ca²⁺ influx peak and Ca²⁺ plateau and (F) the corresponding influx rates. Ca²⁺ signalling curves are presented as mean ± SEM. Scatter dot plots are presented as mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.



Supplementary Figure 4. mRNA expression of SOCE components declines significantly with stimulation/activation of CD8⁺ T cells from both age groups. (A) Relative mRNA expressions of Orai1, 2 and 3 and STIM1 and 2 of untouched (dark grey, n = 12) and stimulated (light grey, n = 12) CD8⁺ T cells from adult mice. (B) Relative mRNA expressions of Orai1, 2 and 3 and STIM1 and 2 of untouched (dark red, n = 11) and stimulated (light red, n = 13) CD8⁺ T cells from elderly mice. Representative Western blots of SOCE components from untouched (C) and stimulated (D) CD8⁺ T cells. Scatter dot plots are presented as mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.