**Manuscript Title:** Chronic reduction of store operated Ca2+ entry is viable therapeutically but is associated with cardiovascular complications

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**Animal model used, if applicable:** Mouse – STIM1 ConKI (Control), STIM1 KI, and STIM1 T cell cKO all on C57Bl6 background.

**Underlying hypothesis:** This study tests the viability and potential complications at the whole animal level of lowering SOCE levels which is associated with immunodeficiency.

**Definitions of ‘n’:**

Question 1: n = number of dishes imaged

Question 2: n = number of flow cytometry experiments

Question 3: n = number of mice

Question 4: n = number of mice

Question 5: n = number of dishes imaged

Question 6: n = number of mice

Question 7: n = number of Western Blots

Question 8: n = number of dishes imaged

Question 9: n = number of cells for density; number of puncta for intensity and area

Question 10: n = number of cells

Question 11: n = number of mice

Question 12: n = number of mice

Question 13: n = number of mice

Question 14: n = number of mice

Question 15: n = number of mice

Question 16: n = number of mice

Question 17: n = number of mice

Question 18: n = number of mice

**Statistical summary table:**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Experimental question number\* | Finding/ conclusion | Experimental location/ variable | Mean value | SD | n val. | P\*\* | Units | Data comparisons | Statistical test | Any other variable | Figure/ table in which data are presented | Comments |
| 1. SOCE levels in T cells | SOCE decreased in KI strain | T cells ConKI | 1.196 | 0.106 | 5 |  | Ratio | ConKI vs KI  ConKI vs cKO  KI vs cKO | Unpaired t-test |  | 1F |  |
| T cells KI | 0.442 | 0.067 | 5 | **<0.0001** |
| T cells cKO | 0.131 | 0.03 | 5 | **<0.0001** |
| 2. T cells STIM1 protein | No change | T cells ConKI | 1 | 0 | 6 | 0.979 | Fluorescence intensity | ConKI vs KI | Unpaired t-test |  | 2A |  |
| T cells KI | 1.002 | 0.172 | 6 |
| 3. Monocyte %  Monocyte # | Higher in KI | ConKI | 2.413 | 1.159 | 8 | **0.0365** | Percent | ConKI vs KI | Mann-Whitney test |  | 2C |  |
| KI | 7.08 | 5.227 | 10 |
| No difference | ConKI | 0.124 | 0.064 | 8 | 0.165 | number | ConKI vs KI | Mann-Whitney test |  |  |
| KI | 0.285 | 0.25 | 10 |
| 4. Lymphocyte subsets | No difference | CD4+ CD8-ConKI | 20.84 | 2.514 | 5 | 0.9384 | Percent | ConKI vs KI | Unpaired t-test |  | 2D |  |
| CD4+ CD8-KI | 20.94 | 1.246 | 5 |  |  |
| No difference | CD4-CD8+ ConKI | 14.84 | 2.415 | 5 | 0.771 | Percent | ConKI vs KI | Unpaired t-test |  |  |
| CD4-CD8+ KI | 15.22 | 1.46 | 5 |  |  |
| Higher in KI | CD4+ CD25+ ConKI | 9.138 | 0.602 | 5 | **0.0305** | Percent | ConKI vs KI | Unpaired t-test |  |  |
| CD4+ CD25+ KI | 11.35 | 1.788 | 5 |  |  |
| Lower in KI | CD19+B220+ConKI | 85.04 | 1.549 | 5 | **0.0158** | Percent | ConKI vs KI | Unpaired t-test |  |  |
| CD19+B220+KI | 81.16 | 2.384 | 5 |  |  |
| 5. BTP2 Inhibition | Effective | CD4 T cells | 2.486 | 0.214 | 4 |  | Fluorescence Ratio | Dose dependent inhibition | ANOVA |  | 2G |  |
| 100 nM | 1.254 | 0.252 | 4 | **<0.0001** |  |  |
| 1 µM | 0.260 | 0.036 | 4 | **<0.0001** |  |  |
| Effective | Jurkat con | 2.429 | 0.124 | 3 |  | ANOVA |  |  |
| 100 nM | 1.371 | 0.402 | 3 | **0.0018** |  |  |
| 1 µM | 0.158 | 0.032 | 3 | **<0.0001** |  |  |
| 6. Lung inflammation | Lower in KI | ConKI PBS | 0.111 | 0.192 | 3 |  |  | Levels of inflammation | ANOVA |  | 2I |  |
| ConKI Papain | 2.556 | 0.193 | 3 | **0.0001** | ConKI PBS vs Papain |  |  |
| KI PBS | 0.222 | 0.192 | 3 | **0.0023** | KI PBS vs Papain |  |  |
| KI Papain | 1.667 | 0.333 | 3 | **0.0249** | Papain ConKI vs KI |  |  |
| 7. MEFs STIM1 protein | Similar | ConKI | 1.066 | 0.334 | 5 | 0.47 | ConKI vs KI | Protein level | Unpaired t test |  | 3A |  |
|  | KI | 1.194 | 0.178 | 5 |  |  |
| 8. MEF SOCE and store content | Lower in KI | SOCE ConKI | 0.894 | 0.309 | 21 | **<0.0001** | ConKI vs KI | SOCE levels | Unpaired t test |  | 3B |  |
| SOCE KI | 0.307 | 0.109 | 24 |  |  |
| Similar | Store ConKI | 27x104 | 2.1x 104 | 4 | 0.11 | ConKI vs KI | Ca2+ store content | Unpaired t test |  | 3C |  |
| Store KI | 29.7x 104 | 1.8x 104 | 4 |  |  |
| 9. STIM1 puncta | Higher in TG  Lower in KI after TG | TIRF Con density | 0.247 | 0.465 | 34 |  |  | STIM1 puncta density | ANOVA |  | 3E |  |
| TIRF | TIRF Con-TG density | 3.86 | 1.815 | 24 | **<0.0001** | Con vs Con-TG |  |  |
| TIRF KI density | 0.053 | 0.13 | 40 | **<0.0001** | KI vs KI-TG |  |  |
| TIRF KI-TG density | 0.843 | 0.748 | 26 | **<0.0001** | Con-TG vs KI-TG |  |  |
| Higher in TG | TIRF Con intensity | 327.4 | 16.38 | 33 |  |  | STIM1 puncta density | ANOVA |  | 3F |  |
| TIRF Con-TG intensity | 420.5 | 52.73 | 232 | **<0.0001** | Con vs Con-TG |  |  |
| TIRF KI intensity | 324.5 | 14.99 | 9 | **<0.0001** | KI vs KI-TG |  |  |
| TIRF KI-TG intensity | 431.4 | 42.48 | 97 | **0.06** | Con-TG vs KI-TG |  |  |
| Higher in TG  Higher in Con after TG | TIRF Con area | 0.1837 | 0.0878 | 33 |  |  |  |  |  | 3G |  |
| TIRF Con-TG area | 0.264 | 0.2066 | 232 | **0.02** | Con vs Con-TG |  |  |
| TIRF KI area | 0.132 | 0.0375 | 9 | 0.23 | KI vs KI-TG |  |  |
| TIRF KI-TG area | 0.209 | 0.1381 | 97 | **0.01** | Con-TG vs KI-TG |  |  |
| STIM1 puncta AiryScan | Higher in TG  Lower in KI after TG | Airyscan Con density | 0.1282 | 0.1909 | 17 |  |  | STIM1 puncta density | ANOVA |  | 3I |  |
| Airyscan Con-TG density | 1.844 | 0.7275 | 20 | **<0.0001** | Con vs Con-TG |  |  |  |
| Airyscan KI density | 0.2773 | 0.1530 | 12 | 0.4222 | KI vs KI-TG |  |  |  |
| Airyscan KI-TG density | 0.4116 | 0.2693 | 17 | **<0.0001** | Con-TG vs KI-TG |  |  |  |
| Higher in TG  Lower in KI after TG | Airyscan Con intensity | 480.2 | 54.04 | 470 |  |  | STIM1 puncta density | ANOVA |  | 3J |  |
| Airyscan Con-TG intensity | 606.4 | 169.1 | 4142 | **<0.0001** | Con vs Con-TG |  |  |  |
| Airyscan KI intensity | 469.6 | 44.2 | 363 | **0.0170** | KI vs KI-TG |  |  |  |
| Airyscan KI-TG intensity | 492.9 | 67.56 | 626 | **<0.0001** | Con-TG vs KI-TG |  |  |  |
| Higher in TG  Lower in KI after TG | Airyscan Con area | 0.0368 | 0.0257 | 470 |  |  |  | ANOVA |  | 3K |  |
| Airyscan Con-TG area | 0.0687 | 0.0569 | 4142 | **<0.0001** | Con vs Con-TG |  |  |  |
| Airyscan KI area | 0.035 | 0.0236 | 363 | **0.02** | KI vs KI-TG |  |  |  |
| Airyscan KI-TG area | 0.0427 | 0.035 | 626 | **<0.0001** | Con-TG vs KI-TG |  |  |  |
| 10. NFAT translocation | Lower in KI | ConKI | 0.4916 | 0.2952 | 50 |  |  |  | Unpaired t test |  | 3N |  |
| KI | 0.1409 | 0.1340 | 53 | **<0.0001** | ConKI vs KI |  |  |  |
| 11. Litter size | No difference | C57Bl6 (WT) | 5.526 | 2.687 | 152 |  |  |  | ANOVA |  | 4A |  |
| ConKI | 5.727 | 2.556 | 121 | 0.518 | WT vs ConKI |  |  |  |
| KI | 5.764 | 2.285 | 203 | 0.3862 | WT vs KI |  |  |  |
| 12. Rotarod assay | No difference | |  | | --- | | Con Trial 1 | | Trial 2 | | Trial 3 | |  | | Trial 4 | | Trial 5 | | |  | | --- | | 156.24 | | 222.52 | | 221.33 | |  | | 261.33 | | 279.00 | | |  | | --- | | 73.978 | | 74.465 | | 71.131 | |  | | 48.635 | | 41.652 | | 21 |  |  |  |  |  | 4B | See Tukey multiple comparison test table below |
| |  | | --- | | Con KI T1 | | Trial 2 | | Trial 3 | |  | | Trial 4 | | Trial 5 | | |  | | --- | | 151.47 | | 195.61 | | 242.53 | |  | | 235.28 | | 260.78 | | |  | | --- | | 80.947 | | 89.606 | | 70.536 | |  | | 64.994 | | 65.820 | | 36 |  |  |  |  |  |  |
| |  | | --- | | KI Trial1 | | Trial 2 | | Trial 3 | |  | | Trial 4 | | Trial 5 | | |  | | --- | | 147.00 | | 206.09 | | 236.36 | |  | | 250.36 | | 272.59 | | |  | | --- | | 63.275 | | 77.897 | | 72.923 | |  | | 49.2110267984968 | | 50.0879775677632 | | 44 |  |  |  |  |  |  |
| 13. Sweat Assay | Higher after ACh | ConKI | 474.5 | 291.8 | 8 | **<0.0001** | ConKI vs ConKI ACh |  | Paired t test |  | 4C |  |
| KI | 277.6 | 192.9 | 7 | **0.01** | KI vs KI Ach |  |  |  |
| ConKI+ ACh | 897 | 386.3 | 8 | 0.1535 | ConKI vs KI |  | Unpaired t test |  |  |
| KI+ ACh | 637.3 | 422.4 | 7 | 0.2354 | Ach ConKI vs KI |  |  |  |
| 14. Glycemia | No difference between ConKI and KI | GTT Chow ConKI | 37.97 | 7.586 | 16 |  |  |  | Unpaired t test |  | 4D |  |
| KI | 39.66 | 7.357 | 16 | 0.5287 | ConKI vs KI |  |  |  |
| Body weight ConKI T0 | 31.12 | 2.367 | 16 | **<0.0001** | ConKI T0 vs T5 |  | ANOVA |  | 4E |  |
| Body weight ConKI T5 wks | 29.2 | 3.912 | **<0.0001** | KI T0 vs T5 |  |  |  |
| Body weight KI T0 | 44.61 | 3.666 | 0.23 | T0 ConKI vs KI |  |  |  |
| Body weight KI T5 wks | 43.31 | 6.644 | 0.41 | T5 ConKI vs KI |  |  |  |
| Glucose ConKI T0 | 177.1 | 35.79 | 16 | **0.002** | ConKI T0 vs T5 |  | ANOVA |  | 4F |  |
| Glucose ConKI T5 wks | 178.4 | 38.56 |  | **<0.0001** | KI T0 vs T5 |  |  |  |
| Glucose KI T0 | 255.9 | 56.87 | 16 | 0.99 | T0 ConKI vs KI |  |  |  |
| Glucose KI T5 wks | 279.9 | 87.55 | 0.65 | T5 ConKI vs KI |  |  |  |
| HFD GTT ConKI | 48.03 | 8.797 | 16 | 0.2796 | ConKI vs KI |  | Unpaired t test |  | 4I |  |
| HFD GTT KI | 51.5 | 9.018 |  |  |
| 15. Cardiovascular phenotype | Higher BP in KI | Mean BP Con night | 101.1 | 10.22 | 4 | **0.0134** | Con D vs N |  | ANOVA |  | 5A |  |
| Con Morn | 87.51 | 12.36 | 4 | **0.0004** | KI D vs N |  |  |  |
| KI Night | 113.6 | 3.01 | 7 | **0.0110** | N Con vs KI |  |  |  |
| KI Morn | 97.12 | 3.188 | 7 | **0.0424** | D Con vs KI |  |  |  |
| Diastolic BP Con night | 86.93 | 7.254 | 4 | **0.0038** | Con D vs N |  |  | 5B |  |
| Con Morn | 73.58 | 7.652 | 4 | **0.0001** | KI D vs N |  |  |  |
| KI Night | 98.13 | 3.98 | 7 | **0.0056** | N Con vs KI |  |  |  |
| KI Morn | 83.19 | 5.038 | 7 | **0.0147** | D Con vs KI |  |  |  |
| Systolic BP Con night | 114.8 | 13.8 | 4 | 0.1009 | Con D vs N |  |  | 5C |  |
| Con Morn | 102.4 | 18.58 | 4 | **0.0037** | KI D vs N |  |  |  |
| KI Night | 129.5 | 5.618 | 7 | **0.0330** | N Con vs KI |  |  |  |
| KI Morn | 111.4 | 3.09 | 7 | 0.1734 | D Con vs KI |  |  |  |
| Higher HR in KI | HR Con night | 488.1 | 33.09 | 4 | **0.0008** | Con D vs N |  |  | 5D |  |
| Con Morn | 424.4 | 27.23 | 4 | **<0.0001** | KI D vs N |  |  |  |
| KI Night | 541.7 | 18.72 | 7 | **0.0013** | N Con vs KI |  |  |  |
| KI Morn | 477.9 | 16.01 | 7 | **0.0014** | D Con vs KI |  |  |  |
| No difference | Activity Con night | 0.1213 | 0.0520 | 4 | **0.013** | Con D vs N |  |  | 5E |  |
| Con Morn | 0.0433 | 0.0172 | 4 | **<0.0001** | KI D vs N |  |  |  |
| KI Night | 0.1489 | 0.0529 | 7 | 0.2862 | N Con vs KI |  |  |  |
| KI Morn | 0.0298 | 0.0224 | 7 | 0.5970 | D Con vs KI |  |  |  |
| No difference | Temp Con Morn | 33.67 | 0.471 | 4 | **0.0113** | Con D vs N |  |  | 5F |  |
| Con Night | 34.54 | 0.5062 | 4 | **0.0162** | KI D vs N |  |  |  |
| KI Morn | 33.77 | 0.4428 | 7 | 0.563 | N Con vs KI |  |  |  |
| KI Night | 34.38 | 0.3581 | 7 | 0.743 | D Con vs KI |  |  |  |
| Higher in KI | AngII BP 1 week ConKI | 115.6 | 5.386 | 6 |  |  |  |  |  |  |  |
| KI | 154.6 | 17.34 | 6 | **0.0004** | ConKI vs KI |  | Unpaired t test |  | 5G |  |
| No difference between ConKI and KI | KCl contraction N ConKI | 0.01676 | 0.008134 | 9 | **0.0069** | ConKI N vs H |  | ANOVA |  | 5H |  |
| H ConKI | 0.03036 | 0.008733 | 6 | 0.4817 | N ConKI vs KI |  |  |  |
| N KI | 0.0138 | 0.01025 | 9 | 0.1932 | H ConKI vs KI |  |  |  |
| H KI | 0.02358 | 0.007165 | 6 | **0.0446** | KI N vs H |  |  |  |
| No difference between ConKI and KI | Aorta thickness ConKI | 91.11 | 12.81 | 13 |  |  |  | Unpaired t test |  | 6D |  |
| KI | 92.06 | 14.51 | 0.8593 | ConKI vs KI |  |  |  |
| 16. αMHC-cre hypomorph | Similar HR in αMHC and ConKI | HR ConKI | 711.6 | 16.46 | 12 |  |  |  | ANOVA |  | 7B |  |
| HR KI | 744.6 | 22.95 | 14 | **0.0001** | ConKI vs KI |  |  |  |
| αMHC | 715 | 26.26 | 10 | 0.7077 | ConKI vs αMHC |  |  |  |
| 17. HRV analyses | Higher in KI | LF ConKI | 2.885 | 1.044 | 4 |  |  |  | Unpaired t test |  | 7C |  |
|  | LF KI | 4.856 | 1.147 | 7 | **0.0199** | ConKI vs KI |  |  |  |
|  | No difference | HF ConKI | 3.29 | 1.74 | 4 |  |  |  |  |  |
|  | HF ConKI | 2.71 | 1.891 | 7 | 0.6275 | ConKI vs KI |  |  |  |
|  | Higher in KI | Ratio ConKI | 1.963 | 0.561 | 4 |  |  |  |  |  |
|  | KI | 4.186 | 1.737 | 7 | **0.0375** | ConKI vs KI |  |  |  |
|  | No difference | pNN6 ConKI | 22.67 | 9.924 | 4 |  |  |  |  | 7D |  |
|  | KI | 15.61 | 6.872 | 7 | 0.1941 | ConKI vs KI |  |  |  |
|  | SDNN ConKI | 11.92 | 3.552 | 4 |  |  |  |  |  |
|  | KI | 15.23 | 2.393 | 7 | 0.0953 | ConKI vs KI |  |  |  |
|  | RMSSD ConKI | 6.445 | 2.232 | 4 |  |  |  |  |  |
|  | KI | 5.369 | 1.539 | 7 | 0.3651 | ConKI vs KI |  |  |  |
| 18. Catecholamines | Higher in KI | Epi ConKI | 5.124 | 3.427 | 15 |  |  |  | Unpaired t test |  | 7E |  |
|  | KI | 12.47 | 6.694 | 16 | **0.0007** | ConKI vs KI |  |  |  |
|  | Norepi ConKI | 2.862 | 0.4514 | 8 | **0.0286** |  |  | Unpaired t test |  |  |
|  | KI | 3.906 | 1.123 | 8 | ConKI vs KI |  |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **Rotarod Assay Tukey's multiple comparisons test** | Mean Diff. | 95.00% CI of diff. | Adjusted P Value |
|  |  |  |  |
| Trial 1 |  |  |  |
| Con/Con vs. Con/KI | 4.766 | -48.30 to 57.83 | 0.9720 |
| Con/Con vs. KI/KI | 9.238 | -43.96 to 62.44 | 0.8996 |
| Con/KI vs. KI/KI | 4.472 | -34.93 to 43.87 | 0.9584 |
|  |  |  |  |
| Trial 2 |  |  |  |
| Con/Con vs. Con/KI | 26.91 | -27.50 to 81.33 | 0.4381 |
| Con/Con vs. KI/KI | 16.43 | -38.52 to 71.39 | 0.7333 |
| Con/KI vs. KI/KI | -10.48 | -52.39 to 31.43 | 0.8146 |
|  |  |  |  |
| Trial 3 |  |  |  |
| Con/Con vs. Con/KI | -21.19 | -60.78 to 18.39 | 0.3828 |
| Con/Con vs. KI/KI | -15.03 | -71.52 to 41.46 | 0.7815 |
| Con/KI vs. KI/KI | 6.164 | -36.52 to 48.85 | 0.9336 |
|  |  |  |  |
| Trial 4 |  |  |  |
| Con/Con vs. Con/KI | 26.06 | -11.09 to 63.20 | 0.2035 |
| Con/Con vs. KI/KI | 10.97 | -23.74 to 45.68 | 0.7075 |
| Con/KI vs. KI/KI | -15.09 | -45.85 to 15.68 | 0.4612 |
|  |  |  |  |
| Trial 5 |  |  |  |
| Con/Con vs. Con/KI | 18.22 | -16.64 to 53.09 | 0.3997 |
| Con/Con vs. KI/KI | 6.409 | -27.56 to 40.37 | 0.8827 |
| Con/KI vs. KI/KI | -11.81 | -42.57 to 18.94 | 0.6190 |

\*You may use multiple lines for the same question to indicate multiple comparisons

\*\* Authors may wish to make the text bold where p is considered significant against a stated confidence limit.