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Supplemental Information

**TNF Induces Pathogenic Programmed Macrophage
Necrosis in Tuberculosis through a Mitochondrial-
Lysosomal-Endoplasmic Reticulum Circuit**

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SUPPLEMENTAL DATA TITLES AND LEGENDS

Table S1 (related to Figure 4 and Figure 7). Expression of ryanodine receptor isoforms in human macrophages and zebrafish blood monocytes.

| HUMAN | | ZEBRAFISH | |
|---------|--|-----------|--|
| Homolog | Expression | Homolog | Expression in blood mfap4+ cells (Athanasiadis et al., 2017) |
| RyR1 | Macrophages: (EMBL-EBI expression atlas) THP1: Protein Atlas (https://www.proteinatlas.org) | RyR1a | Yes (high) |
| | | RyR1b | nd |
| RyR2 | Lung macrophages (www.proteinatlas.org) | RyR2a | nd |
| | | RyR2b | Yes (high) |
| RyR3 | No data available | RyR3 | Yes (low) |

Table S2 (related to Figure 7). THP-1 macrophage death (%) in all experiments.

| <u>Experiment Code</u> | <u>UNINFECTED WELL, NO TNF</u> | <u>UNINFECTED WELL, +TNF</u> | <u>UNINFECTED CELLS, NO TNF</u> | <u>INFECTED CELLS, NO TNF</u> | <u>UNINFECTED CELLS, +TNF</u> | <u>INFECTED CELLS, +TNF</u> | <u>INF+TNF/INF-TNF</u> | <u>INF+TNF/Uninf+TNF within well ratio</u> | <u>UNINF+TNF/UNINF-TNF (ALL UNINFECTED WELLS)</u> | <u>INF-TNF/UNINF-TNF within well ratio</u> | <u>UNINF+TNF/UNINF-TNF</u> | <u>J/L</u> | <u>I/M</u> |
|------------------------|--------------------------------|------------------------------|---------------------------------|-------------------------------|-------------------------------|-----------------------------|------------------------|--|---|--|----------------------------|-------------|-------------|
| UWS003 | 0.70 | 1.40 | 16.00 | 11.10 | 14.00 | 47.60 | | 3.40 | | 0.69 | | | |
| | 0.60 | 0.60 | 12.70 | 4.70 | 15.60 | 30.70 | | 1.97 | | 0.37 | | | |
| <i>mean</i> | <i>0.65</i> | <i>1.00</i> | <i>14.35</i> | <i>7.90</i> | <i>14.80</i> | <i>39.15</i> | <i>4.96</i> | 2.68 | <i>1.54</i> | <i>0.53</i> | <i>1.03</i> | <i>5.05</i> | <i>4.81</i> |
| UWS004 | 7.71 | - | 12.90 | 13.73 | 13.04 | 24.60 | | 1.89 | | 1.06 | | | |
| | 6.46 | - | 9.68 | 11.85 | 4.00 | 22.54 | | 5.64 | | 1.22 | | | |
| <i>mean</i> | <i>7.09</i> | <i>-</i> | <i>11.29</i> | <i>12.79</i> | <i>8.52</i> | <i>23.57</i> | <i>1.84</i> | 3.76 | <i>n/a</i> | <i>1.14</i> | <i>0.75</i> | <i>3.29</i> | <i>2.44</i> |
| CMB008 | 14.25 | 12.00 | 9.26 | 13.53 | 27.76 | 35.67 | | 1.28 | | 1.46 | | | |
| | - | - | - | - | 12.09 | 31.29 | | 2.59 | | | | | |
| <i>mean</i> | <i>-</i> | <i>-</i> | <i>-</i> | <i>-</i> | <i>19.93</i> | <i>33.48</i> | <i>2.47</i> | 1.94 | <i>0.84</i> | | <i>2.15</i> | <i>1.33</i> | <i>1.15</i> |
| CMB010 | 6.96 | 7.03 | 11.11 | 16.67 | 11.20 | 46.81 | | 4.18 | | 1.50 | | | |
| | 11.62 | 12.23 | 9.20 | 18.46 | 14.34 | 39.33 | | 2.74 | | 2.01 | | | |
| | 7.00 | 16.14 | 17.48 | 18.42 | 27.96 | 57.53 | | 2.06 | | 1.05 | | | |
| <i>mean</i> | <i>8.53</i> | <i>11.80</i> | <i>12.60</i> | <i>17.85</i> | <i>17.83</i> | <i>47.89</i> | <i>2.68</i> | 2.99 | <i>1.38</i> | <i>1.52</i> | <i>1.42</i> | <i>1.97</i> | <i>1.90</i> |
| CMB018 | 5.90 | 11.31 | 7.97 | 7.88 | 30.47 | 48.84 | | 1.60 | | 0.99 | | | |
| | 9.67 | 9.61 | 9.41 | 8.64 | 11.23 | 27.91 | | 2.49 | | 0.92 | | | |
| <i>mean</i> | <i>7.79</i> | <i>10.46</i> | <i>8.69</i> | <i>8.26</i> | <i>20.85</i> | <i>38.38</i> | <i>4.65</i> | 2.04 | <i>1.34</i> | <i>0.95</i> | <i>2.40</i> | <i>2.14</i> | <i>1.94</i> |
| CMB019 | 6.58 | 7.46 | 8.04 | 5.10 | 13.27 | 34.31 | | 2.59 | | 0.63 | | | |
| | 8.41 | 19.75 | 13.81 | 16.20 | 15.70 | 30.41 | | 1.94 | | 1.17 | | | |
| <i>mean</i> | <i>7.50</i> | <i>13.61</i> | <i>10.93</i> | <i>10.65</i> | <i>14.49</i> | <i>32.36</i> | <i>3.04</i> | 2.26 | <i>1.82</i> | <i>0.90</i> | <i>1.33</i> | <i>2.50</i> | <i>2.29</i> |
| CMB021 | 13.16 | 8.13 | 9.33 | 3.73 | 14.38 | 10.81 | | 0.75 | | 0.40 | | | |
| | 11.05 | 8.98 | 20.73 | 5.26 | 20.78 | 13.98 | | 0.67 | | 0.25 | | | |
| | 7.82 | 13.15 | 13.38 | 7.69 | 24.62 | 13.43 | | 0.55 | | 0.57 | | | |
| <i>mean</i> | <i>10.68</i> | <i>10.09</i> | <i>14.48</i> | <i>5.56</i> | <i>19.93</i> | <i>12.74</i> | <i>2.29</i> | 0.66 | <i>0.94</i> | <i>0.41</i> | <i>1.38</i> | <i>1.60</i> | <i>1.67</i> |
| CMB029 | 25.58 | 28.79 | 19.80 | 25.00 | 30.25 | 32.26 | | 1.07 | | 1.26 | | | |
| | 16.46 | 25.93 | 19.93 | 33.60 | 22.90 | 30.44 | | 1.33 | | 1.69 | | | |
| <i>mean</i> | <i>21.02</i> | <i>27.36</i> | <i>19.86</i> | <i>29.30</i> | <i>26.57</i> | <i>31.35</i> | <i>1.07</i> | 1.20 | <i>1.30</i> | <i>1.47</i> | <i>1.34</i> | <i>0.81</i> | <i>0.80</i> |

| <u>Experiment Code</u> | <u>UNINFECTED WELL, NO TNF</u> | <u>UNINFECTED WELL, +TNF</u> | <u>UNINFECTED CELLS, NO TNF</u> | <u>INFECTED CELLS, NO TNF</u> | <u>UNINFECTED CELLS, +TNF</u> | <u>INFECTED CELLS, +TNF</u> | <u>INF+TNF/INF-TNF</u> | <u>INF+TNF/Uninf+TNF within well ratio</u> | <u>UNINF+TNF/UNINF-TNF (ALL UNINFECTED WELLS)</u> | <u>INF-TNF/UNINF-TNF within well ratio</u> | <u>UNINF+TNF/UNINF-TNF</u> | <u>J/L</u> | <u>I/M</u> |
|------------------------|--------------------------------|------------------------------|---------------------------------|-------------------------------|-------------------------------|-----------------------------|------------------------|--|---|--|----------------------------|------------|------------|
| CMB031 | 2.42 | 13.54 | 6.59 | 15.17 | 8.64 | 20.78 | | 2.41 | | 2.30 | | | |
| | 4.22 | 7.20 | - | - | 13.13 | 23.34 | | 1.78 | | | | | |
| <i>mean</i> | 3.32 | 10.37 | - | - | 10.89 | 22.06 | 1.45 | 2.09 | 3.12 | | 1.65 | 0.91 | 0.88 |
| CMB040 | 2.49 | 3.44 | 4.17 | 7.69 | 5.42 | 5.95 | | 1.10 | | 1.84 | | | |
| | 7.62 | 2.09 | 2.94 | 11.54 | 10.81 | 15.46 | | 1.43 | | 3.93 | | | |
| <i>mean</i> | 5.06 | 2.77 | 3.56 | 9.62 | 8.12 | 10.71 | 1.11 | 1.26 | 0.55 | 2.88 | 2.28 | 0.44 | 0.49 |
| CMB075 | 11.44 | 10.61 | 13.15 | 24.27 | 15.71 | 49.40 | | 3.14 | | 1.85 | | | |
| | 11.49 | 9.26 | 12.86 | 34.88 | 7.36 | 25.71 | | 3.49 | | 2.71 | | | |
| | 10.48 | 18.13 | 9.21 | 15.69 | 13.91 | 32.50 | | 2.34 | | 1.70 | | | |
| <i>mean</i> | 11.14 | 12.67 | 11.74 | 24.95 | 12.33 | 35.87 | 1.44 | 2.99 | 1.14 | 2.09 | 1.05 | 1.43 | 1.37 |
| CMB076 | 11.89 | 7.25 | 10.20 | 22.90 | 3.40 | 14.90 | | 4.38 | | 2.25 | | | |
| | 11.00 | 8.05 | 11.60 | 16.10 | 7.90 | 31.40 | | 3.97 | | 1.39 | | | |
| | 12.43 | 14.12 | 17.16 | 28.40 | 12.10 | 43.10 | | 3.56 | | 1.66 | | | |
| <i>mean</i> | 11.77 | 9.81 | 12.99 | 22.47 | 7.80 | 29.80 | 1.33 | 3.97 | 0.83 | 1.76 | 0.60 | 2.25 | 2.21 |
| CMB077 | 5.13 | 4.56 | 4.79 | 7.09 | 5.08 | 13.60 | | 2.68 | | 1.48 | | | |
| (Mtb infection) | 4.38 | 5.28 | 5.36 | 8.47 | 6.47 | 18.90 | | 2.92 | | 1.58 | | | |
| | 2.65 | 7.81 | 3.94 | 6.03 | 3.70 | 12.90 | | 3.49 | | 1.53 | | | |
| | 5.01 | 6.74 | 3.08 | 5.68 | 5.01 | 9.59 | | 1.91 | | 1.84 | | | |
| | 4.25 | 6.62 | 5.05 | 2.99 | 7.66 | 17.82 | | 2.33 | | 0.59 | | | |
| | 5.59 | 6.54 | 4.23 | 5.30 | 5.12 | 9.00 | | 1.76 | | 1.25 | | | |
| <i>mean</i> | 4.50 | 6.26 | 4.41 | 5.93 | 5.51 | 13.64 | 2.30 | 2.51 | 1.39 | 1.38 | 1.25 | 1.82 | 1.84 |