Supplemental Information

Mitochondrial metabolism contributes to oxidative stress and reveals therapeutic targets in chronic lymphocytic leukemia

Jitschin et al.

Supplemental Figures

Figure 1: Follow-up time of CLL patients. Follow-up observational time (in months) is shown for ROS\textsuperscript{int/low} and ROS\textsuperscript{high} CLL patients.

Figure 2: Expression of activation markers in CD8\textsuperscript{+} T-cells of CLL patients with different ROS levels. The mean fluorescence intensity (MFI) of CD69, HLA-DR, and CD137 (activation markers) in CD8\textsuperscript{+} T-cells was assessed in ROS\textsuperscript{int/low} and ROS\textsuperscript{high} patients using flow cytometry (FACS). Bars indicate the standard error mean. Abbreviations: *, p<0.05.
Figure 3: CD8^+ T-cell maturation status in CLL patients with different ROS levels. A representative FACS analysis of T-cells is shown (left panel), which was used for determining their maturation status based on the CD45RA and CD27 expression: CD45RA^+CD27^neg terminally differentiated effector memory cells (EMRA), CD45RA^+CD27^- naïve (NA), CD45RA^negCD27^+ central memory (CM), and CD45RA^negCD27^neg effector memory (EM) cells. The right panel displays the maturation status of CD8^+ T-cells in ROS^int/low and ROS^high CLL patients.

Figure 4: Number of T-cells in CLL patients with different ROS levels. The number of T-cells per μl peripheral blood is shown for the ROS^int/low and ROS^high CLL patient groups as assessed by FACS.
Figure 5: **CD4⁺/CD8⁺ T-cell ratio in CLL patients with different ROS levels.** The ratio of CD4⁺/CD8⁺ T-cells was calculated based on FACS-analyses of CLL patient samples. Since CD4⁺ and CD8⁺ T-cells can display different sensitivities towards ROS-induced toxicities we compared their ratio in ROS\textsuperscript{int/low} and ROS\textsuperscript{high} CLL patients as shown.

Figure 6: **Proliferative status of CD4⁺ and CD8⁺ T-cells in CLL patients with different ROS levels.** It is well established that ROS impact T-cell proliferation. Therefore, we compared as shown here CD4⁺ and CD8⁺ T-cells positive for Ki67 (that is strictly associated with cell proliferation) as detected by FACS in ROS\textsuperscript{int/low} and ROS\textsuperscript{high} CLL patients.
Figure 7: Number of circulating CD56+ NK-cells in CLL patients with different ROS levels. The number of NK-cells per 1 peripheral blood is shown for the ROS_{med-low} and ROS_{high} CLL patient groups as assessed by FACS.

A.  

B.  

Figure 8: Coupling efficacy and uncoupling proteins in B- and CLL-cells. (A) Coupling efficacy in CLL- and B-cells. The coupling efficacy of the mitochondria in CLL-cells (n=4) and healthy donor derived B-cells (n=4) was measured using a cellular flux analyzer (Seahorse XFe96). The value is calculated based on the following formula: 1-(OCR upon oligomycin treatment/basal OCR). (B) The expression levels of uncoupling proteins (UCPs) in CLL- and B-cells. The relative gene expression of UCP1, 2, and 4 was determined by quantitative real time PCR in purified healthy donor-derived B-cells (n=5) and CLL-cells (n=5) respectively. Abbreviations: A.U., arbitrary units; N.D., not detected; OCR, oxygen consumption rate.
### Supplemental Tables

#### Supplemental Table 1: Patients’ characteristics.

<table>
<thead>
<tr>
<th>UPN</th>
<th>Sex</th>
<th>Age</th>
<th>Binet</th>
<th>CTx</th>
<th>UPN</th>
<th>Sex</th>
<th>Age</th>
<th>Binet</th>
<th>CTx</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL1</td>
<td>M</td>
<td>60</td>
<td>A</td>
<td>No</td>
<td>CL39</td>
<td>M</td>
<td>78</td>
<td>C</td>
<td>No</td>
</tr>
<tr>
<td>CL2</td>
<td>M</td>
<td>46</td>
<td>B</td>
<td>No</td>
<td>CL40</td>
<td>M</td>
<td>82</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL3</td>
<td>M</td>
<td>55</td>
<td>B</td>
<td>No</td>
<td>CL41</td>
<td>F</td>
<td>71</td>
<td>B</td>
<td>No</td>
</tr>
<tr>
<td>CL4</td>
<td>M</td>
<td>78</td>
<td>A</td>
<td>No</td>
<td>CL42</td>
<td>M</td>
<td>65</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL5</td>
<td>F</td>
<td>80</td>
<td>ND</td>
<td>&gt;2 yrs. ago</td>
<td>CL43</td>
<td>M</td>
<td>69</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL6</td>
<td>M</td>
<td>72</td>
<td>B</td>
<td>No</td>
<td>CL44</td>
<td>M</td>
<td>71</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL7</td>
<td>M</td>
<td>75</td>
<td>C</td>
<td>No</td>
<td>CL45</td>
<td>M</td>
<td>72</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL8</td>
<td>M</td>
<td>73</td>
<td>C</td>
<td>No</td>
<td>CL46</td>
<td>M</td>
<td>60</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL9</td>
<td>M</td>
<td>74</td>
<td>A</td>
<td>No</td>
<td>CL47</td>
<td>M</td>
<td>70</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL10</td>
<td>M</td>
<td>80</td>
<td>A</td>
<td>No</td>
<td>CL48</td>
<td>M</td>
<td>58</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL11</td>
<td>F</td>
<td>77</td>
<td>C</td>
<td>No</td>
<td>CL49</td>
<td>M</td>
<td>82</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL12</td>
<td>M</td>
<td>63</td>
<td>A</td>
<td>No</td>
<td>CL50</td>
<td>M</td>
<td>72</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL13</td>
<td>M</td>
<td>65</td>
<td>A</td>
<td>No</td>
<td>CL51</td>
<td>M</td>
<td>76</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL14</td>
<td>F</td>
<td>69</td>
<td>A</td>
<td>No</td>
<td>CL52</td>
<td>M</td>
<td>58</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL15</td>
<td>M</td>
<td>70</td>
<td>B</td>
<td>No</td>
<td>CL53</td>
<td>F</td>
<td>78</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL16</td>
<td>M</td>
<td>71</td>
<td>C</td>
<td>No</td>
<td>CL54</td>
<td>M</td>
<td>80</td>
<td>C</td>
<td>No</td>
</tr>
<tr>
<td>CL17</td>
<td>M</td>
<td>69</td>
<td>A</td>
<td>No</td>
<td>CL55</td>
<td>M</td>
<td>73</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL18</td>
<td>M</td>
<td>53</td>
<td>A</td>
<td>No</td>
<td>CL56</td>
<td>F</td>
<td>72</td>
<td>C</td>
<td>No</td>
</tr>
<tr>
<td>CL19</td>
<td>M</td>
<td>74</td>
<td>A</td>
<td>No</td>
<td>CL57</td>
<td>M</td>
<td>66</td>
<td>ND</td>
<td>No</td>
</tr>
<tr>
<td>CL20</td>
<td>F</td>
<td>62</td>
<td>A</td>
<td>No</td>
<td>CL58</td>
<td>F</td>
<td>71</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL21</td>
<td>F</td>
<td>68</td>
<td>A</td>
<td>No</td>
<td>CL59</td>
<td>M</td>
<td>73</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL22</td>
<td>M</td>
<td>68</td>
<td>A</td>
<td>No</td>
<td>CL60</td>
<td>F</td>
<td>57</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL23</td>
<td>M</td>
<td>56</td>
<td>A</td>
<td>No</td>
<td>CL61</td>
<td>F</td>
<td>75</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL24</td>
<td>F</td>
<td>79</td>
<td>A</td>
<td>ND</td>
<td>CL62</td>
<td>F</td>
<td>78</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL25</td>
<td>F</td>
<td>69</td>
<td>A</td>
<td>No</td>
<td>CL63</td>
<td>F</td>
<td>72</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL26</td>
<td>F</td>
<td>74</td>
<td>A</td>
<td>No</td>
<td>CL64</td>
<td>M</td>
<td>70</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL27</td>
<td>M</td>
<td>73</td>
<td>A</td>
<td>No</td>
<td>CL65</td>
<td>F</td>
<td>76</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL28</td>
<td>M</td>
<td>71</td>
<td>A</td>
<td>No</td>
<td>CL66</td>
<td>F</td>
<td>68</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL29</td>
<td>F</td>
<td>64</td>
<td>A</td>
<td>No</td>
<td>CL67</td>
<td>M</td>
<td>77</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL30</td>
<td>M</td>
<td>91</td>
<td>C</td>
<td>No</td>
<td>CL68</td>
<td>M</td>
<td>69</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL31</td>
<td>F</td>
<td>73</td>
<td>A</td>
<td>No</td>
<td>CL69</td>
<td>F</td>
<td>71</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL32</td>
<td>F</td>
<td>81</td>
<td>A</td>
<td>No</td>
<td>CL70</td>
<td>F</td>
<td>71</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL33</td>
<td>M</td>
<td>58</td>
<td>A</td>
<td>No</td>
<td>CL71</td>
<td>F</td>
<td>74</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL34</td>
<td>F</td>
<td>73</td>
<td>A</td>
<td>No</td>
<td>CL72</td>
<td>F</td>
<td>56</td>
<td>C</td>
<td>No</td>
</tr>
<tr>
<td>CL35</td>
<td>F</td>
<td>78</td>
<td>A</td>
<td>No</td>
<td>CL73</td>
<td>M</td>
<td>62</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL36</td>
<td>F</td>
<td>73</td>
<td>A</td>
<td>No</td>
<td>CL74</td>
<td>F</td>
<td>48</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL37</td>
<td>M</td>
<td>53</td>
<td>A</td>
<td>No</td>
<td>CL75</td>
<td>M</td>
<td>68</td>
<td>A</td>
<td>No</td>
</tr>
<tr>
<td>CL38</td>
<td>M</td>
<td>69</td>
<td>B</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: UPN, unique patient number; M, male; F, female; ND, not determined; CTx, chemotherapy
## Supplemental Table 2: Antibody list.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Fluorochrome</th>
<th>Clone</th>
<th>Isotype</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 AAD</td>
<td>FITC</td>
<td></td>
<td></td>
<td>Biolegend</td>
</tr>
<tr>
<td>Annexin V</td>
<td></td>
<td></td>
<td></td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD11b</td>
<td>PE Cy7</td>
<td></td>
<td>ICRF44 Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD137</td>
<td>PE Cy7</td>
<td>4B4-1</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD14</td>
<td>PerCP Cy5.5</td>
<td>M5E2</td>
<td>Mouse IgG2a, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD19</td>
<td>V500</td>
<td>HIB19</td>
<td>Mouse IgG1, κ</td>
<td>BD Biosciences</td>
</tr>
<tr>
<td>CD19</td>
<td>PE Cy7</td>
<td>HIB19</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD19</td>
<td>APC</td>
<td>HIB19</td>
<td>Mouse IgG1, κ</td>
<td>BD Biosciences</td>
</tr>
<tr>
<td>CD23</td>
<td>PerCP Cy5.5</td>
<td>M-L 233</td>
<td>Mouse IgG1, κ</td>
<td>BD Biosciences</td>
</tr>
<tr>
<td>CD27</td>
<td>APC Cy7</td>
<td>O323</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD3</td>
<td>PE</td>
<td>UCHT1</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD3</td>
<td>PE Cy7</td>
<td>HIT3a</td>
<td>Mouse IgG2a, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD33</td>
<td>APC</td>
<td>WM53</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD3ζ-chain</td>
<td>PE</td>
<td>6B10.2</td>
<td>Mouse IgG1</td>
<td>eBioscience</td>
</tr>
<tr>
<td>CD4</td>
<td>FITC</td>
<td>RPA-T4</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD4</td>
<td>PE Cy7</td>
<td>Sk3</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD45RA</td>
<td>PE Cy7</td>
<td>H1100</td>
<td>Mouse IgG2b, κ</td>
<td>BD Biosciences</td>
</tr>
<tr>
<td>CD45RO</td>
<td>PE</td>
<td>UCHL1</td>
<td>Mouse IgG2a, κ</td>
<td>BD Biosciences</td>
</tr>
<tr>
<td>CD5</td>
<td>PE</td>
<td>UCHT-2</td>
<td>Mouse IgG1, κ</td>
<td>BD Biosciences</td>
</tr>
<tr>
<td>CD69</td>
<td>AF 647</td>
<td>FN50</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD8</td>
<td>PerCP Cy5.5</td>
<td>Sk1</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>CD95</td>
<td>PE Cy7</td>
<td>DX2</td>
<td>Mouse IgG1, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>gp91phox</td>
<td>PE</td>
<td>NL7</td>
<td>Mouse IgG1</td>
<td>Santa Cruz Biotech</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>APC Cy7</td>
<td>L243</td>
<td>Mouse IgG2a, κ</td>
<td>Biolegend</td>
</tr>
<tr>
<td>Ki67</td>
<td>FITC</td>
<td>20Raj1</td>
<td>Mouse IgG1, κ</td>
<td>eBioscience</td>
</tr>
</tbody>
</table>
# Supplemental Table 3: Primer list.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>p22</td>
<td>QT00082481/Qiagen</td>
<td></td>
</tr>
<tr>
<td>rac1</td>
<td>QT00065856/Qiagen</td>
<td></td>
</tr>
<tr>
<td>g6pd</td>
<td>QT00071596/Qiagen</td>
<td></td>
</tr>
<tr>
<td>glut1</td>
<td>QT00068957/Qiagen</td>
<td></td>
</tr>
<tr>
<td>glut3</td>
<td>QT00047124/Qiagen</td>
<td></td>
</tr>
<tr>
<td>hk2</td>
<td>QT00013209/Qiagen</td>
<td></td>
</tr>
<tr>
<td>ppargc1b</td>
<td>QT00081865/Qiagen</td>
<td></td>
</tr>
<tr>
<td>ldha</td>
<td>QT00001687/Qiagen</td>
<td></td>
</tr>
<tr>
<td>pdk1</td>
<td>QT00069636/Qiagen</td>
<td></td>
</tr>
<tr>
<td>cox2</td>
<td>QT00040586/Qiagen</td>
<td></td>
</tr>
<tr>
<td>p67phox</td>
<td>QT00089341/Qiagen</td>
<td></td>
</tr>
<tr>
<td>p40phox</td>
<td>QT00077847/Qiagen</td>
<td></td>
</tr>
<tr>
<td>erra</td>
<td>QT00018536/Qiagen</td>
<td></td>
</tr>
<tr>
<td>nrf1</td>
<td>QT01154076/Qiagen</td>
<td></td>
</tr>
<tr>
<td>nrf2</td>
<td>QT02451519/Qiagen</td>
<td></td>
</tr>
<tr>
<td>tfam</td>
<td>QT00012782/Qiagen</td>
<td></td>
</tr>
<tr>
<td>cclc</td>
<td>ATG-GAG-GTG-CAA-TTA-ACA-GAC</td>
<td>ACT-GCA-TTG-CCA-CCT-TTG-CA</td>
</tr>
<tr>
<td>cclm</td>
<td>GCT-GTA-TCA-GTG-GGC-ACA-G</td>
<td>CGC-TTG-AAT-GTC-AGG-AAT-AC</td>
</tr>
<tr>
<td>mnsod</td>
<td>CTT-CAG-CTG-GCA-CTG-AAG-TTC-AAT</td>
<td>CTG-AAG-GTA-AGC-GTG-GTC-CT-CC</td>
</tr>
<tr>
<td>ho1</td>
<td>CTT-CTT-CAC-CTT-CCC-CAA-CA</td>
<td>AGC-TCC-TGC-ACC-TCC-TCA-AC</td>
</tr>
<tr>
<td>trx1</td>
<td>ACG-CTG-CAG-GTG-ATA-AAC</td>
<td>CTG-ACA-GTC-ATC-CAC-ATC-TAC</td>
</tr>
<tr>
<td>tRNA Leu(UUC)</td>
<td>CAC-CCA-AGA-ACA-GGG-TTT-GT</td>
<td>TGG-CCA-TGG-GTA-TGT-TGT-TA</td>
</tr>
<tr>
<td>beta2mg</td>
<td>TGC-TGT-CTG-CAT-CTT-TGA-TGT-ATC-T</td>
<td>TCT-CTG-CTC-CCC-ACC-TCT-AGT-T</td>
</tr>
<tr>
<td>actin</td>
<td>CGA-TCC-ACA-CGG-AGT-CTC-CTG</td>
<td>GGA-TGC-AGA-AGG-AGA-TCA-CTG</td>
</tr>
</tbody>
</table>