**Supplemental table 1:** Characteristics of treatment-naive CLL patients included in this study

| ID     | Sex | Age | Rai Stage | Mutation Status | Leukocyte count (×10^9/L) | % CD5+ CD19+ | % CD3+ | CMV status |
|--------|-----|-----|-----------|-----------------|---------------------------|--------------|---------|------------|
| CLL 1  | Male| 77  | 0         | M               | 71,4                      | 96,7         | 1,7     | +          |
| CLL 2  | Female| 62  | 0         | M               | 46,1                      | 91,1         | 6,7     | +          |
| CLL 3  | Female| 74  | 0         | M               | 39,6                      | 96,9         | 7,1     | +          |
| CLL 4  | Male| 59  | 0         | M               | 63,4                      | 83,5         | 10,8    | +          |
| CLL 5  | Female| 65  | 0         | UM              | 51,4                      | 64,9         | 5,3     | ?          |
| CLL 6  | Male| 74  | II        | M               | 68,9                      | 88,4         | 1,8     | +          |
| CLL 7  | Female| 67  | 0         | M               | 45,7                      | 85,4         | 9,6     | +          |
| CLL 8  | Male| 66  | I         | ?               | 32,1                      | 78,1         | 11,7    | +          |
| CLL 9  | Male| 55  | 0         | M               | 85,6                      | 84           | 9,6     | ?          |
| CLL 10 | Male| 61  | I         | ?               | 43,5                      | 90,5         | 7,4     | +          |
| CLL 11 | Male| 56  | 0         | M               | 93,9                      | 84,3         | 3,5     | +          |
| CLL 12 | Male| 85  | 0         | M               | 36,5                      | 86,1         | 10,5    | +          |
| CLL 13 | Female| 59  | 0         | UM              | 45,8                      | 82           | 11,6    | +          |
| CLL 14 | Female| 57  | 0         | ?               | 52,89                     | 80,9         | 11,6    | +          |
| CLL 15 | Male| 61  | 0         | UM              | 60,2                      | 92,8         | 4       | +          |
| CLL 16 | Male| 75  | NA        | ?               | 33,74                     | 10,6^1       | 8,3     | +          |
| CLL 17 | Female| 75  | 0         | UM              | 50                        | 78,3         | 3,6     | -          |
| CLL 18 | Female| 72  | I         | M               | 30,1                      | 85,7         | 6,9     | ?          |
| CLL 19 | Male| 63  | 0         | M               | 72,1                      | 94,7         | 4       | +          |
| CLL 20 | Male| 55  | I         | UM              | 51,7                      | 65,2         | 8,5     | -          |
| CLL 21 | Male| 67  | 0         | ?               | 127                       | 55,7         | 1,6     | ?          |
| CLL 22 | Male| 51  | 0         | UM              | 51,3                      | 85           | 3,5     | +          |
| CLL 23 | Male| 71  | I         | M               | 39                        | 19,5         | 0,4     | ?          |
| CLL 24 | Male| 30  | NA        | UM              | 286                       | 65,4         | 0,6     | +          |
| CLL 25 | Female| 80  | 0         | ?               | 142,23                    | 95,1         | 1,4     | -          |
| CLL 26 | Female| 55  | NA        | M               | 92,6                      | 91,9         | 5,4     | ?          |
| CLL 27 | Male| 73  | 0         | ?               | 129,7                     | 91,7         | 0,3     | +          |
| CLL 28 | Male| 57  | II        | UM              | 84,7                      | 90,7         | 3,6     | +          |
| CLL 29 | Male| 74  | 0         | M               | 61,3                      | 87,7         | 5,2     | +          |
| CLL 30 | Male| 81  | II        | ?               | 88,7                      | 95,5         | 2,5     | +          |
| CLL 31 | Female| 63  | 0         | M               | 43,9                      | 94,3         | 4,8     | +          |
| CLL 32 | Male| 66  | II        | M               | 23,07                     | 90,5         | 7,9     | +          |
| CLL 33 | Male| 69  | 0         | ?               | 61                        | 88,9         | 6       | ?          |
| CLL 34 | Male| 79  | 0         | ?               | 39                        | 92           | 3,4     | -          |
| CLL 35 | Male| 66  | 0         | ?               | 74,6                      | 93,1         | 8       | -          |
| CLL 36 | Male| 65  | I         | ?               | 132,7                     | 96,9         | 1,2     | ?          |
CLL 37  Male  79  0  M  127,8  97,1  2,1  +  
CLL 38  Female  72  NA  ?  745  79,5  4,4  +  
CLL 39  Male  72  0  M  202  95  2  -  
CLL 40  Male  68  NA  UM  268,4  97,2  1,5  +  
CLL 41  Female  80  I  M  65,31  93,7  4,9  +  
CLL 42  Female  77  0  M  59,6  91  3,1  +  
CLL 43  Female  85  0  UM  300,23  97,7  1  ?  
CLL 44  Female  62  0  M  72,2  98,6  1,2  ?  
CLL 45  Female  77  ?  UM  58.83  91.96  6.76  ?  
CLL 46  Male  84  0  M  49.27  92.58  6.13  ?  
CLL 47  Male  66  I  M  59.71  92.89  6.28  ?  
CLL 48  Male  70  0  M  70.24  93.56  5.86  ?  

\(^1\)CLL cells in this patient had low CD5 expression. CD19\(^+\) fraction in this patient is 84.7%. A question mark indicates that the data is unavailable or could not be determined.

**Supplemental table 2:** Characteristics of age-matched healthy donors

| ID  | Sex  | Age |  | ID  | Sex  | Age |
|-----|------|-----|---|-----|------|-----|
| HD1 | Male | 73  | | HD20| Male | 68  |
| HD2 | Male | 73  | | HD21| Female | 67 |
| HD3 | Male | 71  | | HD22| Female | 67 |
| HD4 | Male | 68  | | HD23| Female | 70 |
| HD5 | Male | 67  | | HD24| Female | 69 |
| HD6 | Male | 69  | | HD25| Female | 61 |
| HD7 | Male | 66  | | HD26| ?  | 66 |
| HD8 | Male | 70  | | HD27| Female | 69 |
| HD9 | Male | 65  | | HD28| Female | 64 |
| HD10| Male | 68  | | HD29| Female | 64 |
| HD11| Male | 74  | | HD30| Male | 63 |
| HD12| Female | 73  | | HD31| Male | 66 |
| HD13| Male | 72  | | HD32| Male | 65 |
| HD14| Female | 73  | | HD33| Male | 60 |
| HD15| Male | 67  | | HD34| Male | 68 |
| HD16| Female | 68  | | HD35| Male | 61 |
| HD17| Male | 68  | | HD36| ?  | ? |
| HD18| Female | 65  | | HD37| ?  | ? |
| HD19| Male | 72  | |     |      |     |

**Supplemental table 3:** Patient characteristics of the HOVON139 study
| ID    | Hovon139 | Sex   | Age | Rai Stage | Mutation Status | Leukocyte count (x10^9/L) | % CD5+ | % CD19+ | % CD3+ | % CD4+ | % CD8+ | CMV status |
|-------|----------|-------|-----|-----------|-----------------|----------------------------|--------|---------|--------|--------|--------|------------|
| CLL 49 | Baseline | Female | 70  | I         | UM              | 25.5                       | 90.22  | 6.68    | 4.72   | 1.48   |        | +          |
| CLL 50 | Baseline | Male   | 75  | III       | ?               | 56.35                      | 94.04  | 4.64    | 1.22   | 3.07   |        | +          |
| CLL 51 | Baseline | Male   | 73  | IV        | ?               | 96.41                      | 95.83  | 3.18    | 1.1    | 1.35   |        | -          |
| CLL 52 | Baseline | Male   | 75  | IV        | M               | 93.58                      | 96.58  | 1.91    | 0.91   | 0.71   |        | -          |
| CLL 53 | Baseline | Female | 83  | III       | M               | 68.8                       | 45.89  | 5.26    | 4.06   | 0.88   |        | -          |
| CLL 54 | Baseline | Female | 57  | IV        | M               | 236.06                     | 96.59  | 2.16    | 1.41   | 0.56   |        | +          |
| CLL 49-C12 | Cycle 12 | Female | 71  | I         | UM              | 4.6                         | 0      | 93.81   | 49.31  | 43.72  |        | +          |
| CLL 50-C12 | Cycle 12 | Male   | 76  | III       | ?               | 4.51                       | 0      | 88.51   | 31.77  | 53.87  |        | +          |
| CLL 51-C12 | Cycle 12 | Male   | 74  | IV        | ?               | 5.93                       | 0      | 93.05   | 28.82  | 40.82  |        | -          |
| CLL 52-C12 | Cycle 12 | Male   | 76  | IV        | M               | 2.77                       | 0      | 72.3    | 39.16  | 30.68  |        | -          |
| CLL 53-C12 | Cycle 12 | Female | 85  | III       | M               | 4.65                       | 0      | 90.42   | 84.36  | 4.79   |        | -          |
| CLL 54-C12 | Cycle 12 | Female | 58  | IV        | M               | 5.16                       | 0      | 92.42   | 66.6   | 23.29  |        | +          |

1Baseline: treatment-naive CLL patients. 2Cycle 12: twelve cycles of Venetoclax in combination with Obinutuzumab for the first six cycles

**Supplemental table 4: Differentially expressed genes within CD4 T cells stimulated in presence or absence of CLL cells**

| Gene   | log2 FC | Adj. p-value | Gene   | log2 FC | Adj. p-value | Gene   | log2 FC | Adj. p-value |
|--------|---------|--------------|--------|---------|--------------|--------|---------|--------------|
| SCARB1 | -1.240301741 | 2.10E-09     | PROX2 | -0.646061593 | 5.31E-10   | GALK1 | 1.111583261 | 1.50E-07     |
| GALK1  | -1.111583261 | 1.50E-07     | ERCC2 | -0.630300323 | 0.001262578 | GPC1  | 0.72503437  | 0.01198916   |
| GAA    | -0.003277955 | 0.000302359  | G6PD  | -0.400351214 | 0.004869829 | STKMN1| 0.69417378  | 1.88E-09     |
| S100A4 | -0.75524433  | 0.00420516   | GPX4  | -0.384450784 | 0.004492655 | KIF20A| 0.59483884  | 0.001748579  |
| SLC25A1| -0.74511113  | 1.25E-05     | JUNB  | 0.316265588  | 0.018820984 | AURKA | 0.575201443 | 7.32E-05     |
| GP1C   | -0.72503437  | 0.01199816   | LSP1  | 0.305435845  | 0.009500884 | HMMR  | 0.569200161 | 7.58E-07     |
| FOSL2  | -0.605212511 | 1.44E-05     | TXNRD1| 0.457463709  | 5.08E-05   | B3GAT3| 0.556618355 | 0.003383917  |
| NFI3L3 | -0.515484795 | 0.01748579   | GCLC  | 0.493887316  | 0.019271186 | SLC37A4| 0.473354028 | 0.020772596  |
| SLC37A4| -0.473354028 | 0.020772596  | SRXN1 | 0.60120192   | 0.000377942 | PGLS  | 0.434548386 | 0.03757863  |
| MIF    | -0.425059695 | 0.000727116  | NQO1  | 0.676169852  | 0.004885086 | MIF   | 0.425059695 | 0.000727116  |
| PFKL   | -0.410566578 | 0.001690407  | MBP   | 0.847645234  | 3.49E-05   | PFKL  | 0.410565678 | 0.00169047   |
| HK1    | -0.391635219 | 2.75E-05     | IPCEF1 | 1.523738984 | 5.97E-09   | GNE   | 0.410222078 | 0.030820935  |
| DDT4   | -0.383315724 | 0.023263142  |        |         |              | KIF2A | 0.40394636  | 0.000162101  |
| ALDOA  | -0.306238294 | 0.01086485   |        |         |              | G6PD  | 0.600351214 | 0.00468929   |
| ENO2   | -0.303246207 | 0.03964087   |        |         |              | HK1   | 0.391635219 | 2.75E-05     |
| GPI    | -0.286169911 | 0.017969678  |        |         |              | MGST3 | -0.70936292 | 0.043380845  |

**OXPHOS**
| Gene       | Value 1          | Value 2          | Gene       | Value 1          | Value 2          |
|------------|------------------|------------------|------------|------------------|------------------|
| TPI1       | 0.255670194      | 0.016363494      | CYB5R3     | -0.488712828     | 0.001520846      |
| ENO1       | 0.301249359      | 0.255670194      | GOT2       | 0.367672651      | 0.00499025       |
| F3         | 0.079575531      | 0.021937668      | ALDOA      | 0.303246207      | 0.03696087       |
| BCL2       | 0.305289719      | 0.021937668      | EC1        | 0.3028287271     | 0.02168434       |
| CDKN1B     | 0.375404873      | 0.021937668      | ATP6V0C    | 0.3028287271     | 0.02168434       |
| BHLHE40    | 0.388939038      | 0.021937668      | ECH1       | 0.3028287271     | 0.02168434       |
| NAGK       | 0.409895785      | 0.021937668      | TIMM13     | 0.3028287271     | 0.02168434       |
| ANXA2      | 0.425758513      | 0.021937668      | ATP5F1D    | 0.3028287271     | 0.02168434       |
| ZNF292     | 0.495211345      | 0.021937668      | MRPL34     | 0.3028287271     | 0.02168434       |
| PNRC1      | 0.556316974      | 0.021937668      | MRPL34     | 0.3028287271     | 0.02168434       |
| MX1        | 0.592970233      | 0.021937668      | UQCRCP1    | 0.3028287271     | 0.02168434       |
| TIPARP     | 0.625814109      | 0.021937668      | GPX4       | 0.3028287271     | 0.02168434       |
| ALDH7A1    | 0.659258573      | 0.021937668      | GPD1       | 0.3028287271     | 0.02168434       |
| MAP3K1     | 0.71239225       | 0.021937668      | IDH2       | 0.3028287271     | 0.02168434       |
| IER3       | 0.749211345      | 0.021937668      | CD44       | 0.3028287271     | 0.02168434       |
| RORA       | 0.804630444      | 0.021937668      | UQCRFS1    | 0.3028287271     | 0.02168434       |
| KLHL24     | 0.854949481      | 0.021937668      | SLC25A3    | 0.3028287271     | 0.02168434       |
| CDKN1A     | 0.903134961      | 0.021937668      | PROK1      | 0.3028287271     | 0.02168434       |
| ZFP36      | 0.929197334      | 0.021937668      | CYC1       | 0.3028287271     | 0.02168434       |
| RBP9       | 0.990507781      | 0.021937668      | GPA        | 0.3028287271     | 0.02168434       |
| NR3C1      | 1.100082169      | 0.021937668      | OXA1L      | 0.3028287271     | 0.02168434       |
| IGFBP3     | 1.116996358      | 0.021937668      | IMMT       | 0.3028287271     | 0.02168434       |
| BTG1       | 1.150588097      | 0.021937668      | CS         | 0.3028287271     | 0.02168434       |
| SDC4       | 1.27492178       | 0.021937668      | ATP5F1A    | 0.3028287271     | 0.02168434       |
| PAM        | 1.293135558      | 0.021937668      | MDH1       | 0.3028287271     | 0.02168434       |
| KIF7       | 1.455100232      | 0.021937668      | ATP5F1B    | 0.3028287271     | 0.02168434       |
| IL6        | 1.72141499       | 0.021937668      | HSPA9      | 0.3028287271     | 0.02168434       |
| HMOX1      | 2.06290102       | 0.021937668      | CASP7      | 0.3028287271     | 0.02168434       |
| TGFBI      | 4.544926526      | 0.021937668      | ATP1B1     | 4.544926526      | 0.02168434       |
Supplemental figure 1

A

Unstimulated  aCD3  aCD3/aCD28

HD

CD4

CD25  2-NBDG

CD4

GLUT-1  4-1BB

CD4

PD-1

CLL

CD4

CD25  2-NBDG

CD4

GLUT-1  4-1BB

CD4

PD-1

B

ECAR (%) vs. Time (minutes)

aCD3/aCD28

HD

CLL

MitoSOX

Normalized To Mode

Unstim: 244  Stim: 429

MitoSOX

Normalized To Mode

Unstim: 737  Stim: 1118

MitoTracker Orange

Normalized To Mode

Unstim: 7417  Stim: 9780

MitoTracker Orange

Normalized To Mode

Unstim: 24912  Stim: 31690

C

OCR (%) vs. Time (minutes)
Supplemental figure 1

PBMCs from CLL and HD were thawed and T cells were stimulated for 2 days using either CD3, or CD3+CD28 antibodies. Subsequently, CD4 T cells were analyzed by flow cytometry. (A) Representative flow cytometry plots are shown. Gating strategy of CD4 T cells stimulated with or without CD3, or CD3+CD28 antibodies and subsequently analyzed for expression of GLUT-1, 4-1BB, CD25, CD71, PD-1. (B) CD4 T cells derived from CLL or HD PBMCs were cell sorted and immediately analyzed for extracellular flux. 1 CLL patient and 1 HD were stimulated with CD3 and CD28 antibodies. (C) Gating strategy of CD4 T cells derived from CLL patients and HD and analyzed for mitochondrial potential and ROS.

Supplemental figure 2

PBMCs from CLL and HD were thawed and CD4 T cells were directly analyzed by flow cytometry. Representative flow cytometry plots are shown. (A) Gating strategy of naive (Tn), effector (Te), and memory (Tm) CD4 T cells based on expression of CD27 and CD45RA. (B) CD4 T cells were cell sorted and immediately analyzed for extracellular flux during a mitochondrial stress test.

Supplemental figure 3

CLL patients who underwent 12 cycles of venetoclax had donated PBMCs prior and post Ven +Obi treatment. These PBMCs as well as PBMCs from HD were thawed after which T cells were stimulated for 2 days using either CD3, or CD3+CD28 antibodies. Representative flow cytometry plots are shown. Gating strategy of Tregs from patients included in the HOVON139 cohort.