YOUNG SCIENCE FORUM:
IL FUTURO NASCE IN LABORATORIO

IL-12 Family Cytokines Shape Metabolic Adaptations in Chronic Lymphocytic Leukemia

Nadia Bertola

IRCCS AOM Ospedale Policlinico San Martino

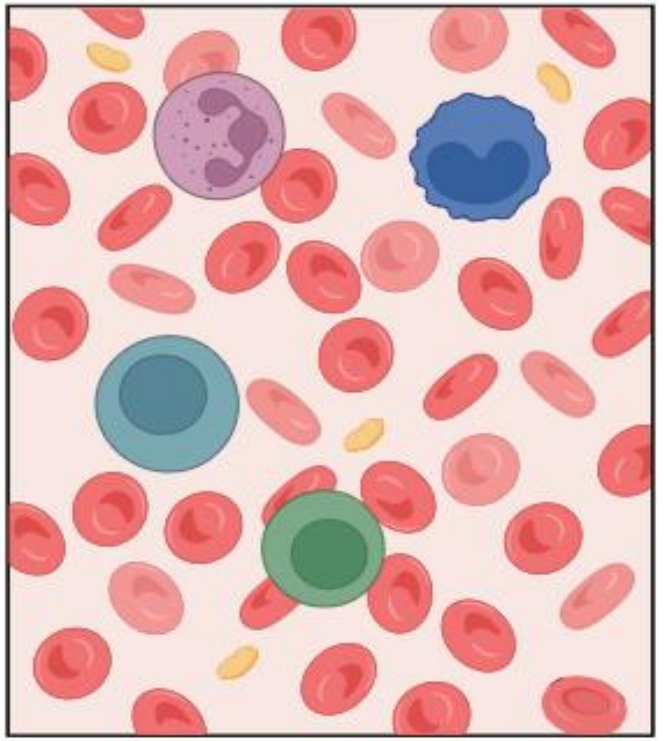
Conflict of interest disclosure

I have no, real or perceived, direct or indirect conflicts of interest that relate to this presentation.

Leucemia Linfatica Cronica

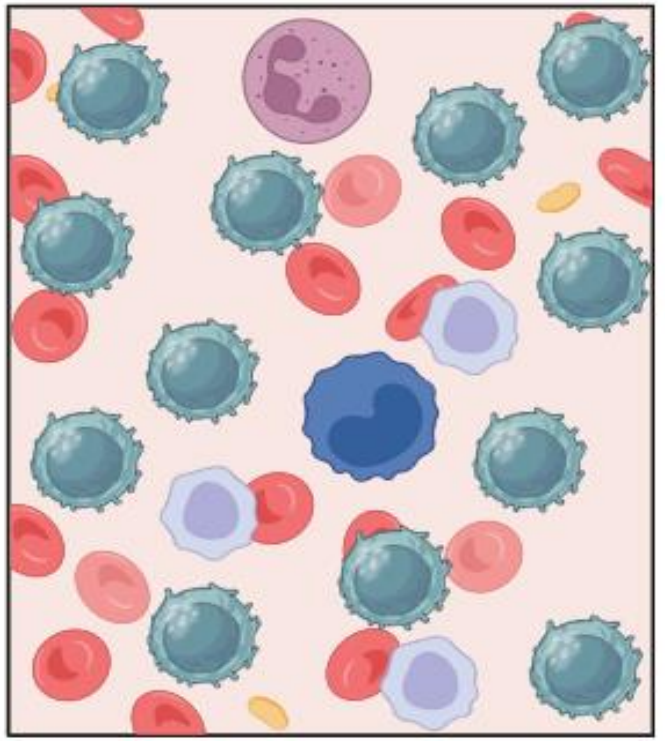
- Leucemia più frequente dell'adulto nei Paesi occidentali
- Accumulo progressivo di linfociti B monoclonali maturi ($CD19^+/CD5^+$) nel sangue periferico, midollo osseo e tessuti linfoidi
- Decorso clinico eterogeneo, da forme indolenti a forme rapidamente progressive
- Sopravvivenza, proliferazione e resistenza terapeutica del clone leucemico dipendono dalle interazioni con il microambiente tumorale
- Presenza nel microambiente di segnali di attivazione che sostengono la crescita del clone leucemico

Donatore sano



CD4+ T cells	30–60%
CD8+ T cells	15–40%
B cells	**5–10%**
NK cells	5–30%
Monocytes	5–10%
Dendritic cells	1–2%

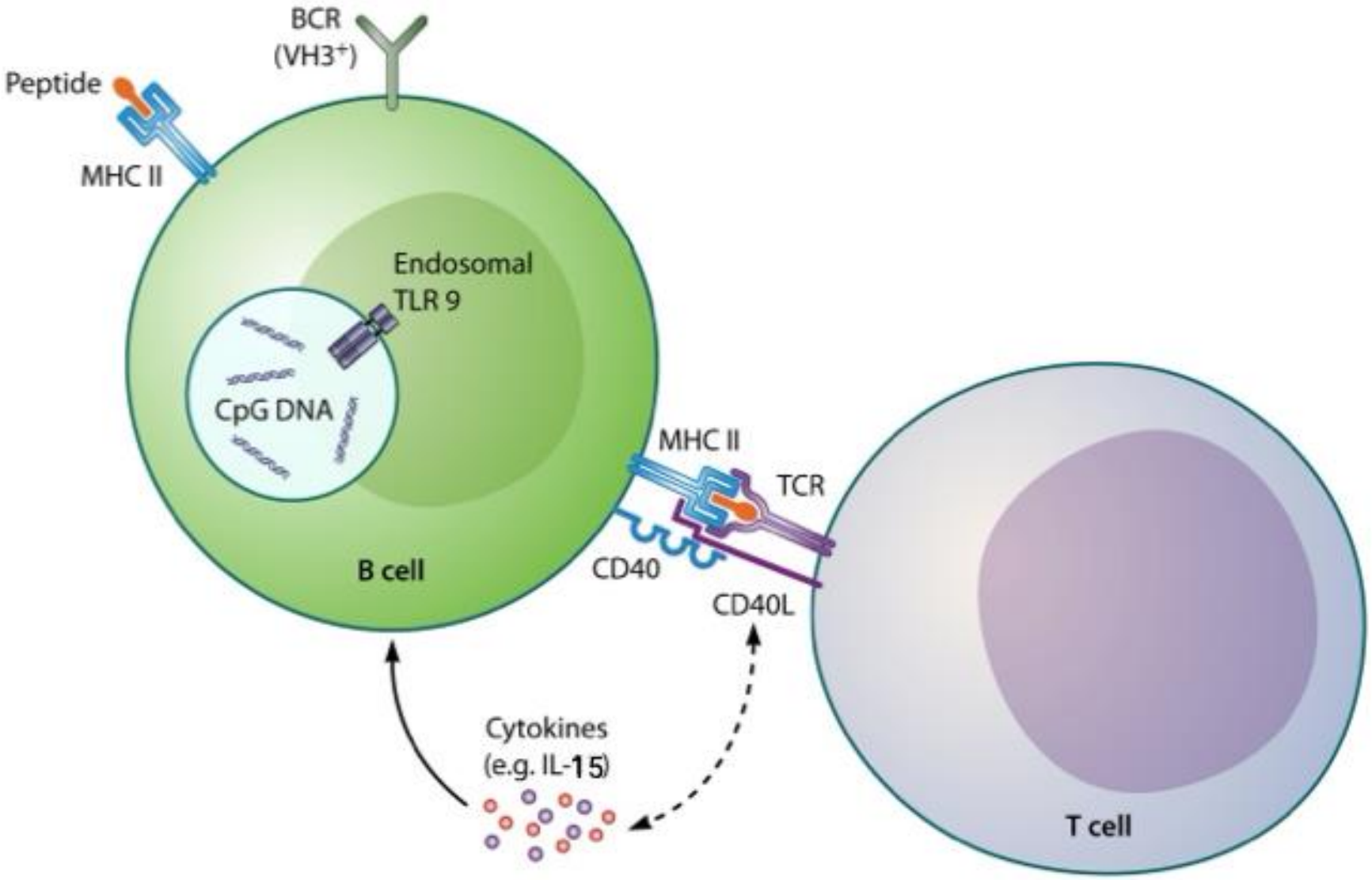
Paziente LLC



CD4+ T cells	2-15%
CD8+ T cells	1–8%
CLL B cells	**30–98%**
NK cells	1–10%
Monocytes	5–10%
Dendritic cells	1–2%

Chiorazzi et al. 2005, NEJM

B cells activation



Adapted from Haas et al. 2011

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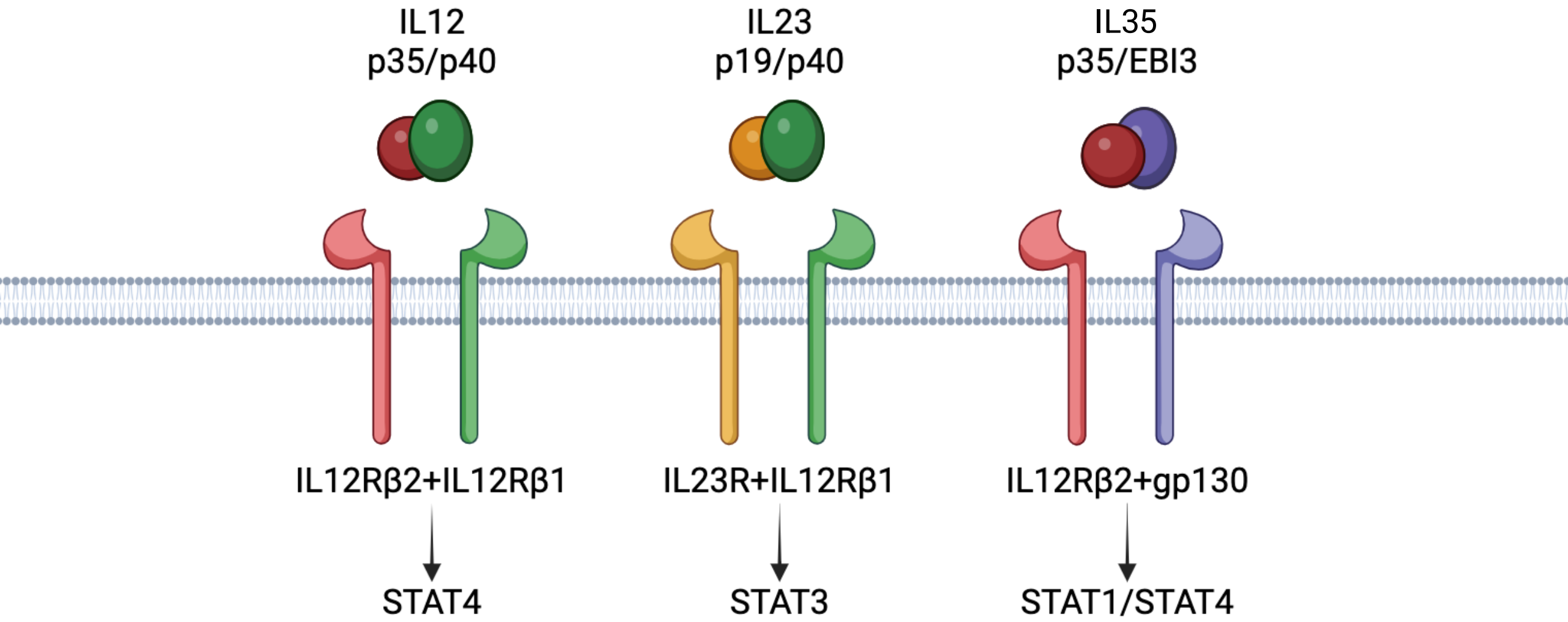
CANCER

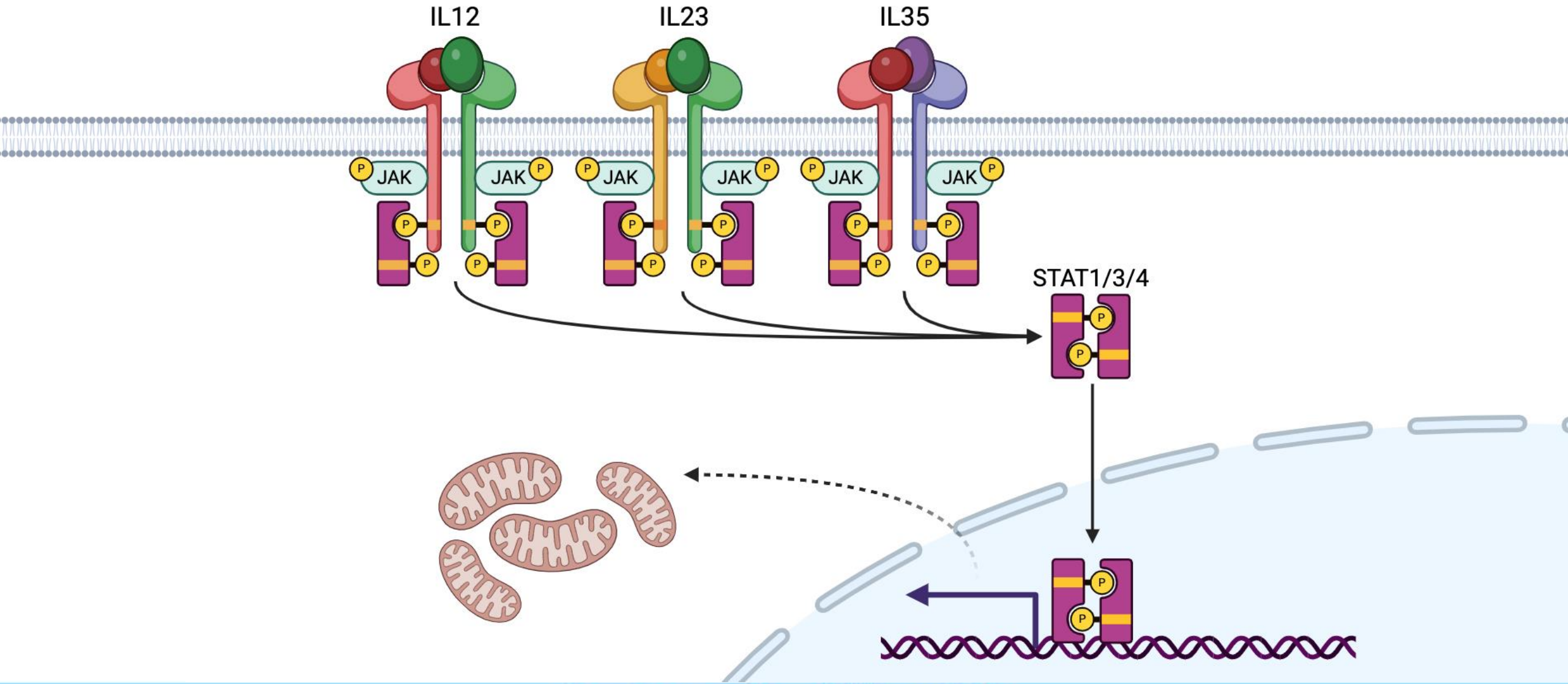
Microenvironmental regulation of the IL-23R/IL-23 axis overrides chronic lymphocytic leukemia indolence

Giovanna Cutrona,[1*†] Claudio Tripodo,[2*] Serena Matis,[1] Anna Grazia Recchia,[3,4] Carlotta Massucco,[1] Marina Fabbi,[5] Monica Colombo,[1] Laura Emionite,[6] Sabina Sangaletti,[7] Alessandro Gulino,[2] Daniele Reverberi,[1] Rosanna Massara,[1] Simona Boccardo,[8] Daniela de Totero,[1] Sandra Salvi,[8] Michele Cilli,[6] Mariavaleria Pellicanò,[3,4] Martina Manzoni,[9,10] Sonia Fabris,[10] Irma Airoldi,[11] Francesca Valdora,[1,12] Silvano Ferrini,[5] Massimo Gentile,[3,4] Ernesto Vigna,[3,4] Sabrina Bossio,[4] Laura De Stefano,[4] Angela Palummo,[4] Giovanni Iaquinta,[4] Martina Cardillo,[12] Simonetta Zupo,[13] Giannamaria Cerruti,[13] Adalberto Ibatici,[14] Antonino Neri,[9,10] Franco Fais,[1,12] Manlio Ferrarini,[15] Fortunato Morabito[3,4]

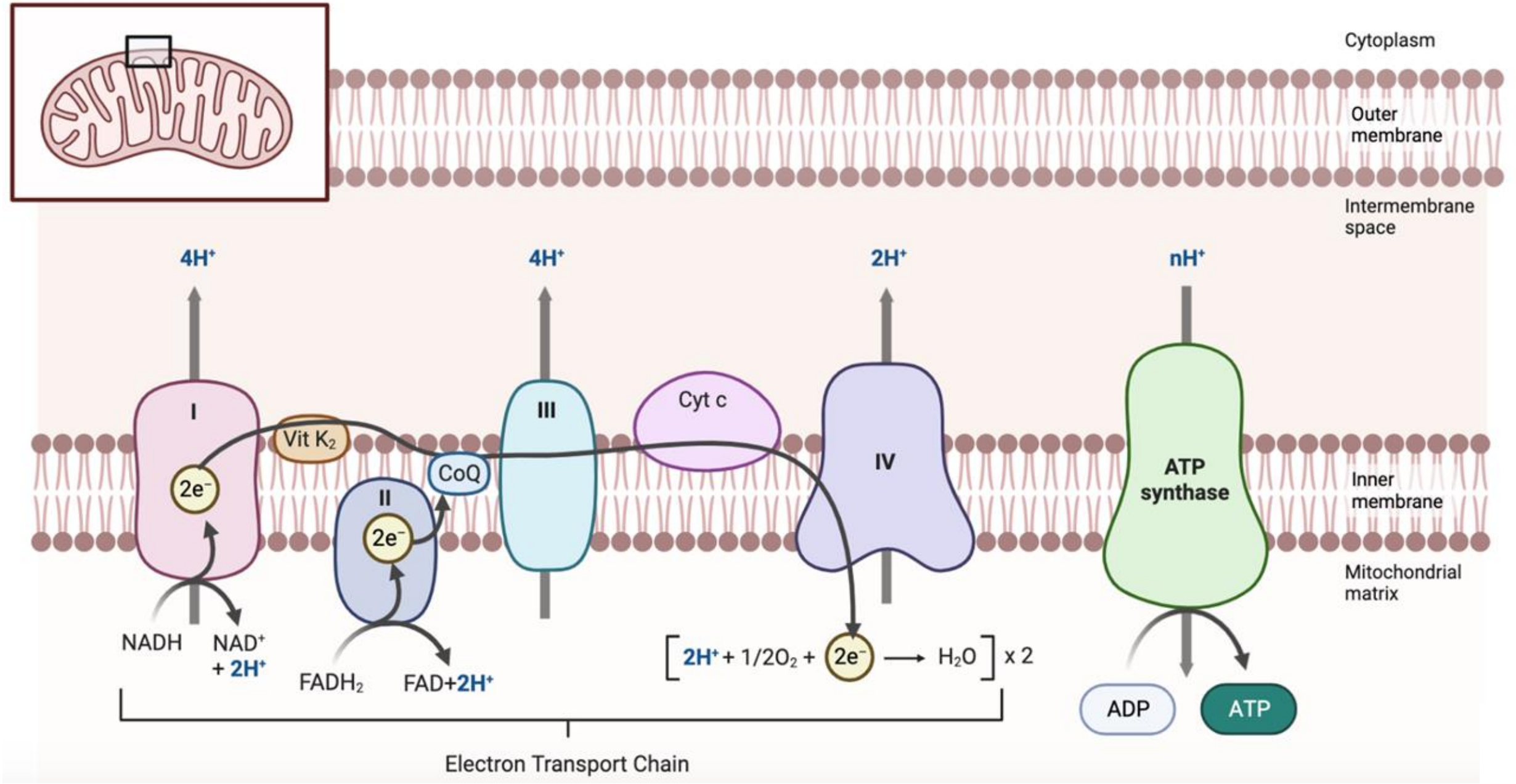
IL12 cytokine family



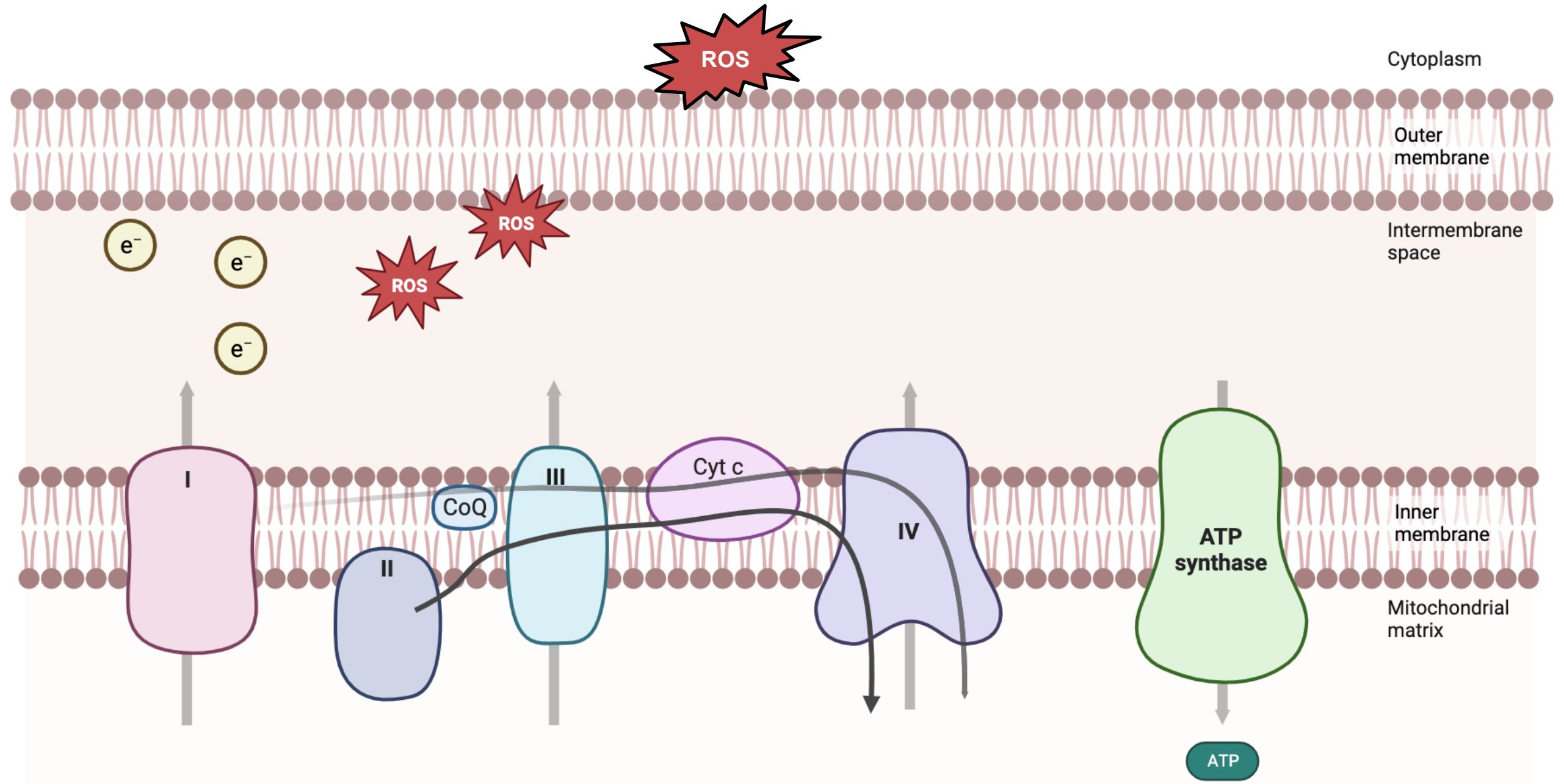
IL12 cytokine family signaling



Oxidative Phosphorylation Machinery



Defective OxPhos



AIMS OF THE STUDY

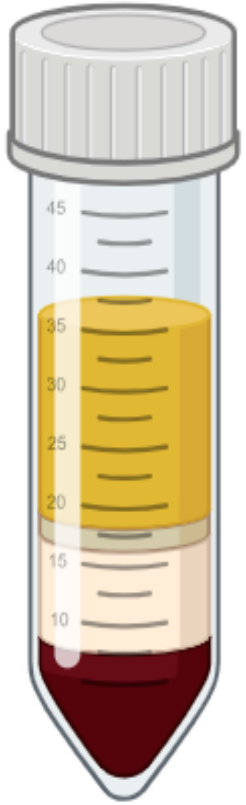
Role of IL12 family in regulating CLL cells' clonal expansion

Following B-cell activation ± treatment with IL12 cytokines family:

1. Expression of IL12 family receptor chains
2. Analysis of receptor heterodimer composition
3. Analysis of secreted cytokines
4. Assessment of STAT protein activation
5. Metabolic analysis

Project funded by 5x1000 IRCCS AOM Ospedale Policlinico San Martino

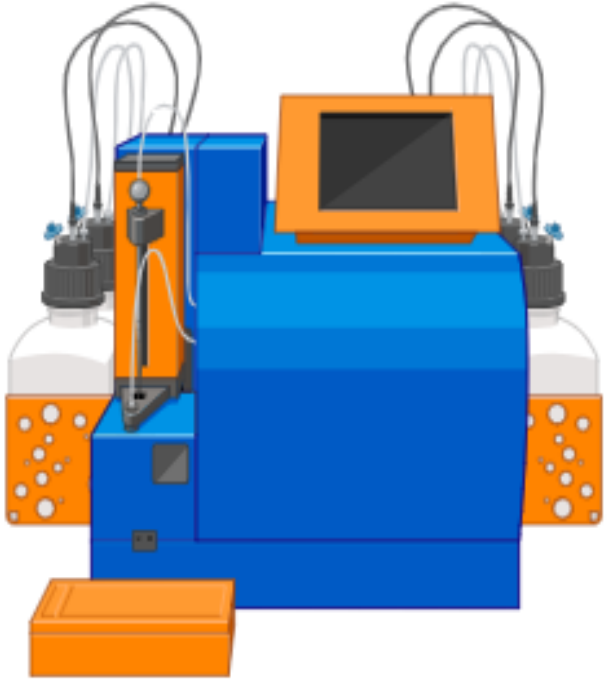
B cells' purification

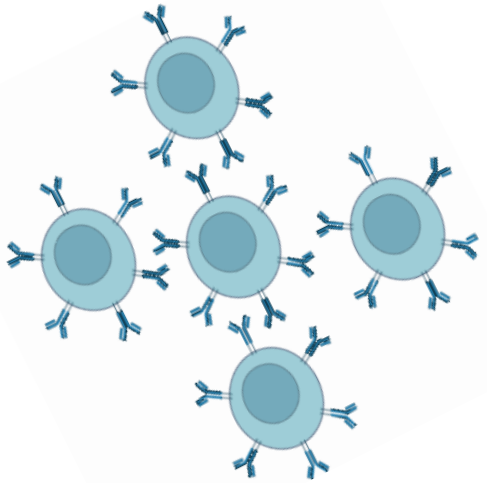


Plasma

PBMCs

RBCs



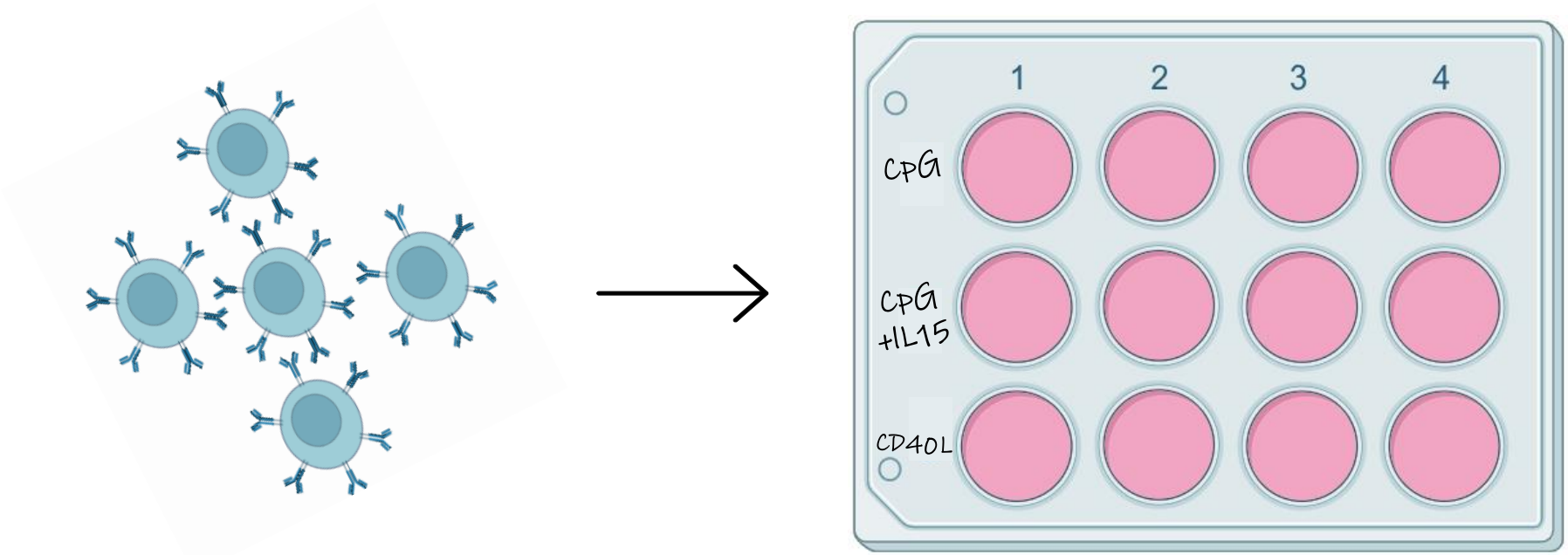


PBMCs isolation

AutoMACS® Neo Separator (Miltenyi)

Purified B cells

Activation

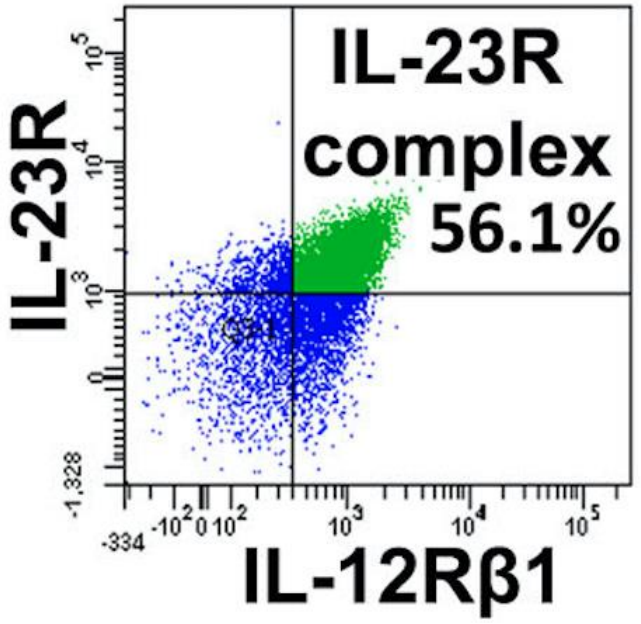


Purified B cells

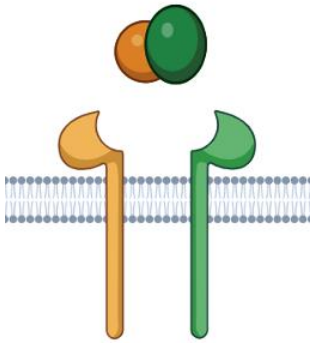
Activation (96h)

Co-expression of IL12 family receptor chains in CLL cells

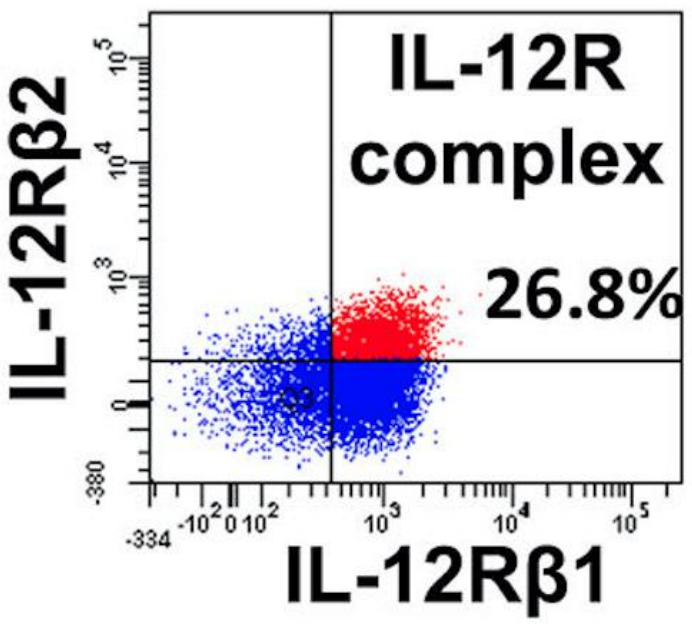
CpG 96h



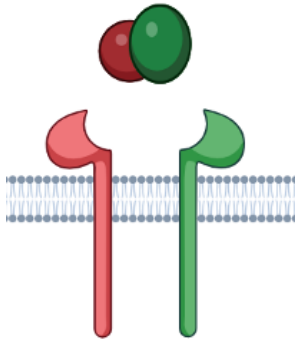
IL23
p19/p40



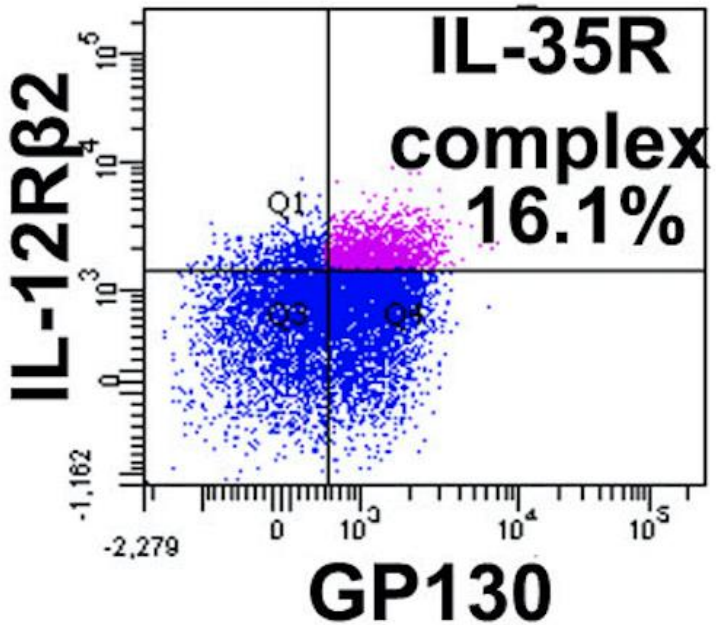
IL23R+IL12Rβ1



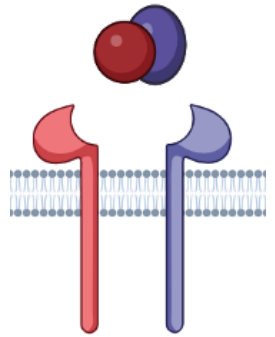
IL12
p35/p40



IL12Rβ2+IL12Rβ1

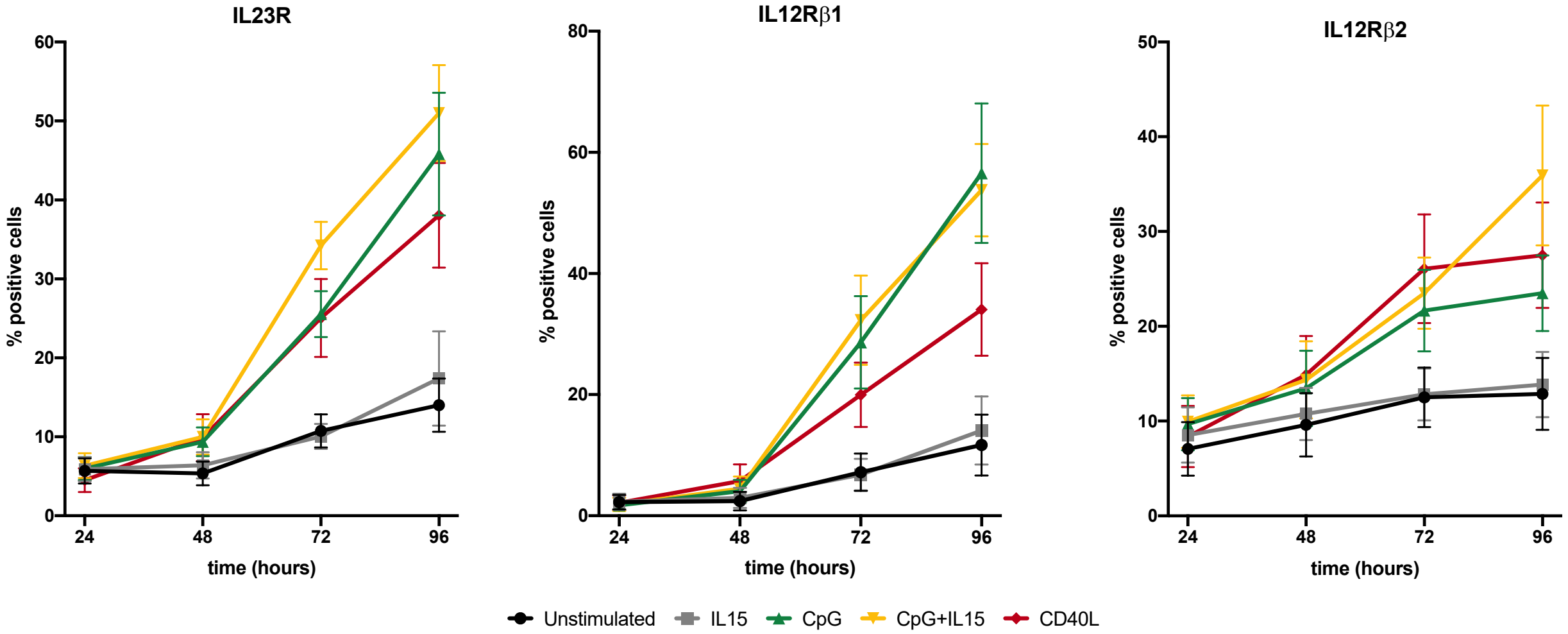


IL35
p35/EBI3



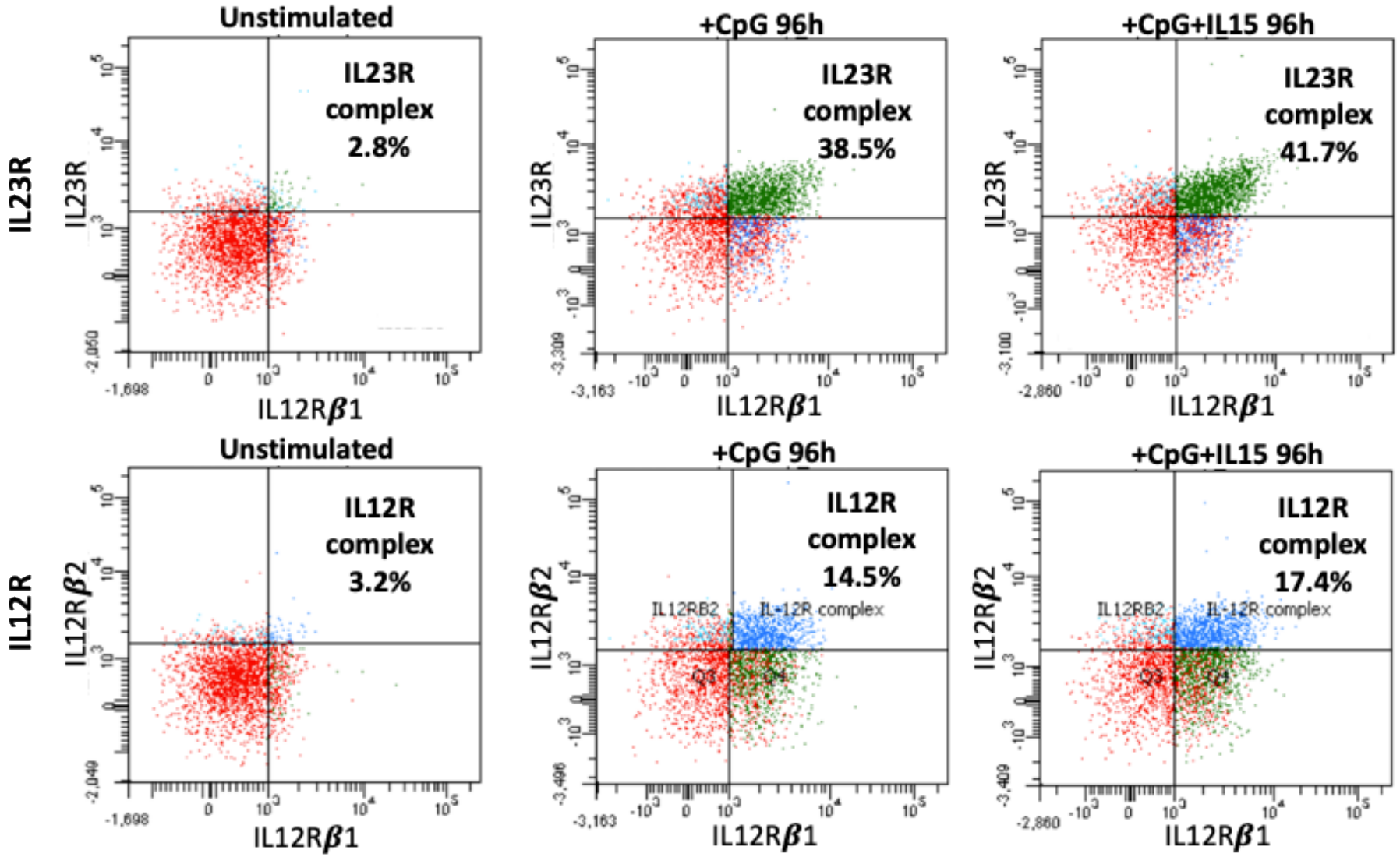
IL12Rβ2+gp130

Expression of IL12 family receptor chains in CLL cells

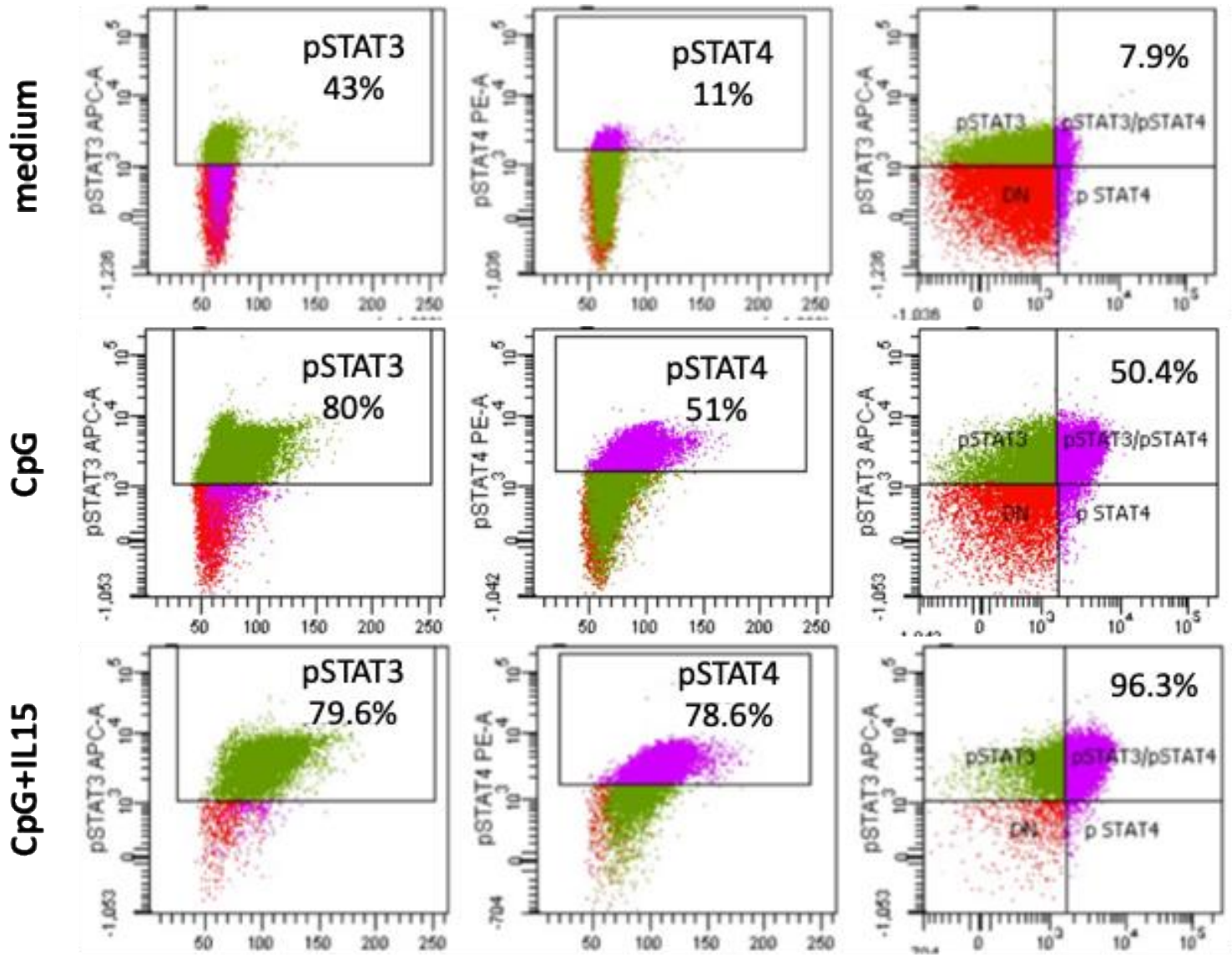


n=10

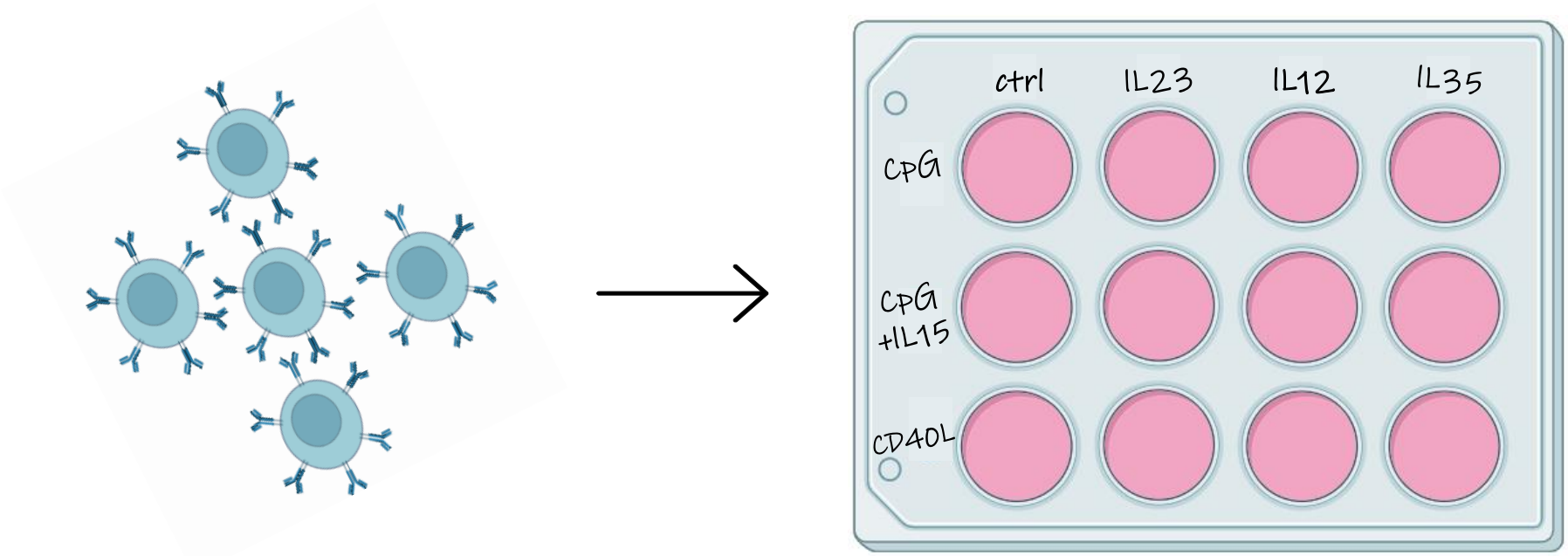
Co-expression of IL12 family receptor chains in normal B cells



STAT protein activation in CLL cells



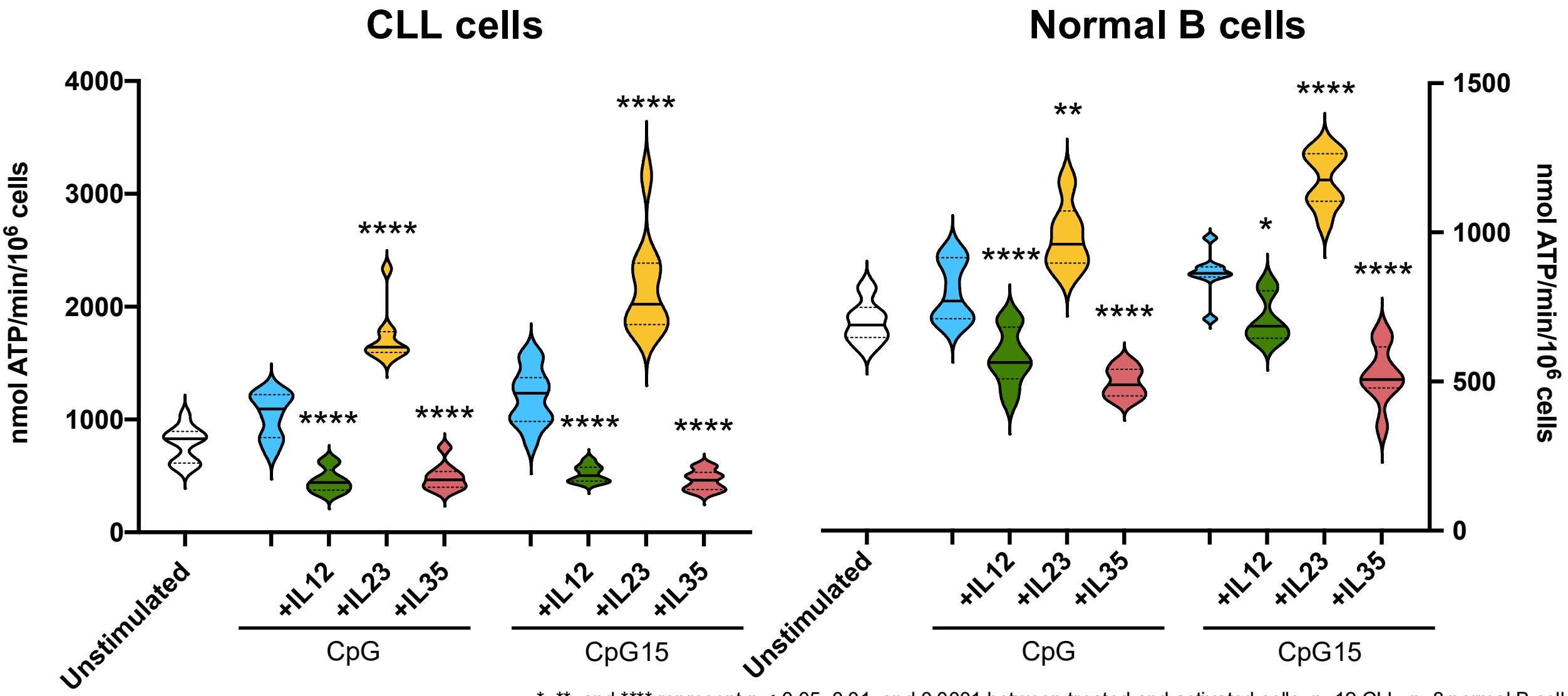
Cytokines treatments



Purified B cells

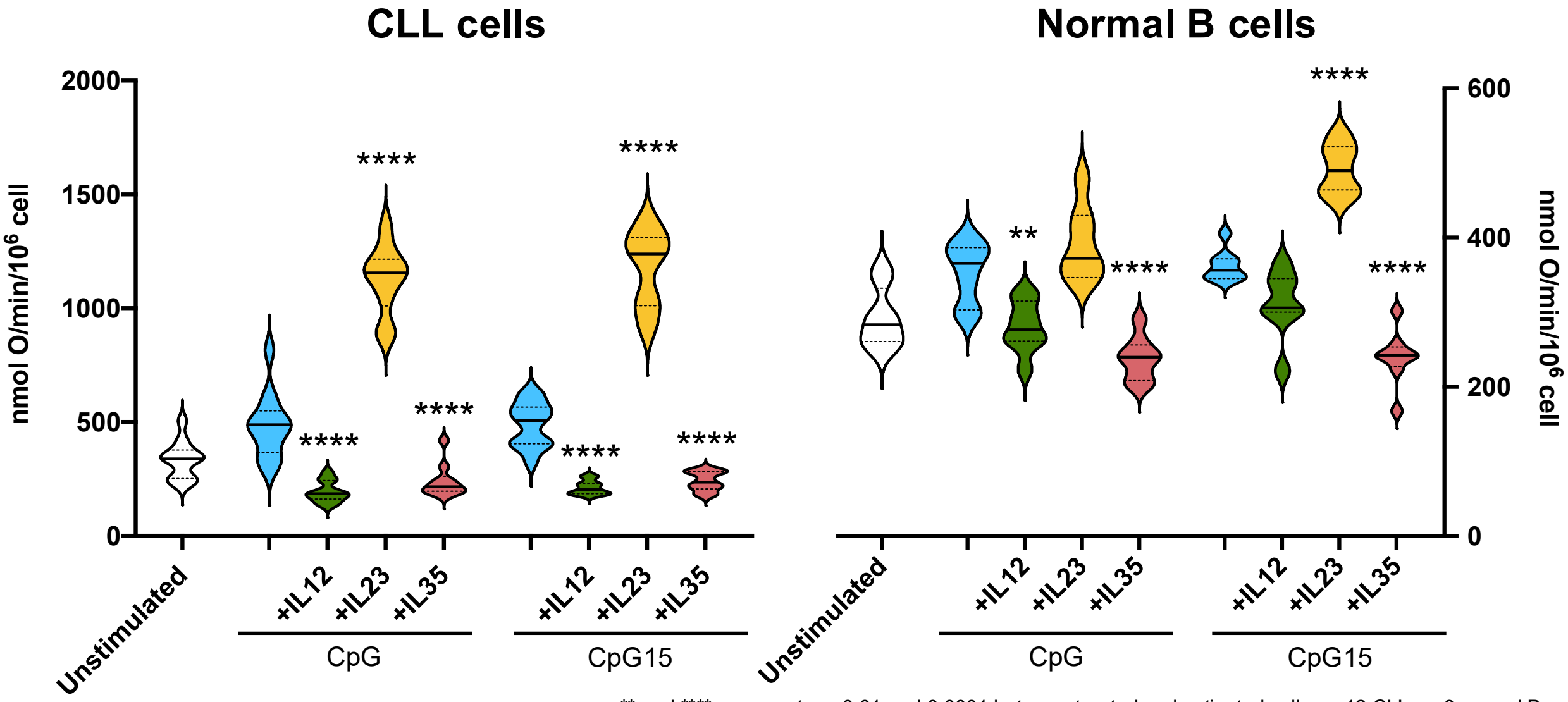
Activation (72h)
+ treatment (24h)

Mitochondrial Metabolism: ATP synthesis



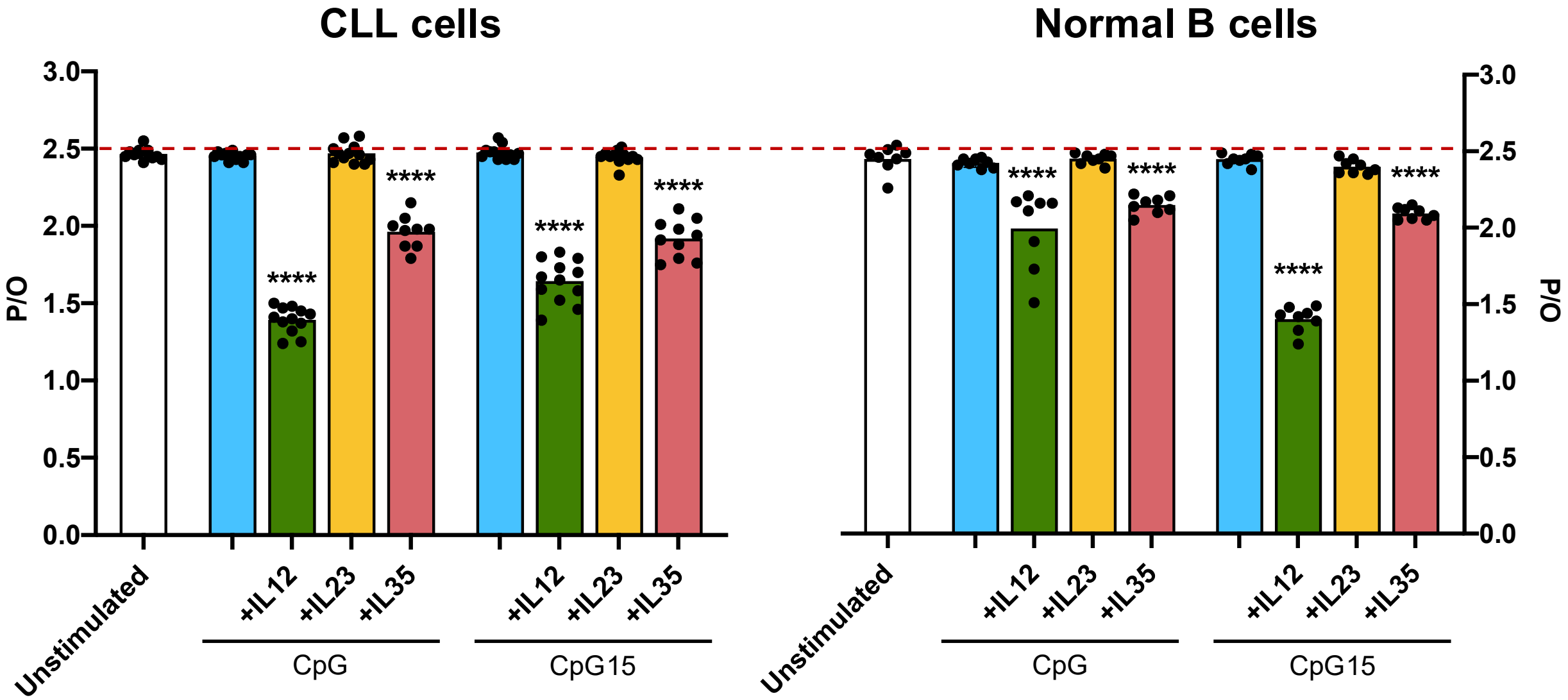
*, **, and **** represent p < 0.05, 0.01, and 0.0001 between treated and activated cells; n=12 CLL, n=8 normal B cells

Mitochondrial Metabolism: Oxygen Consumption Rate



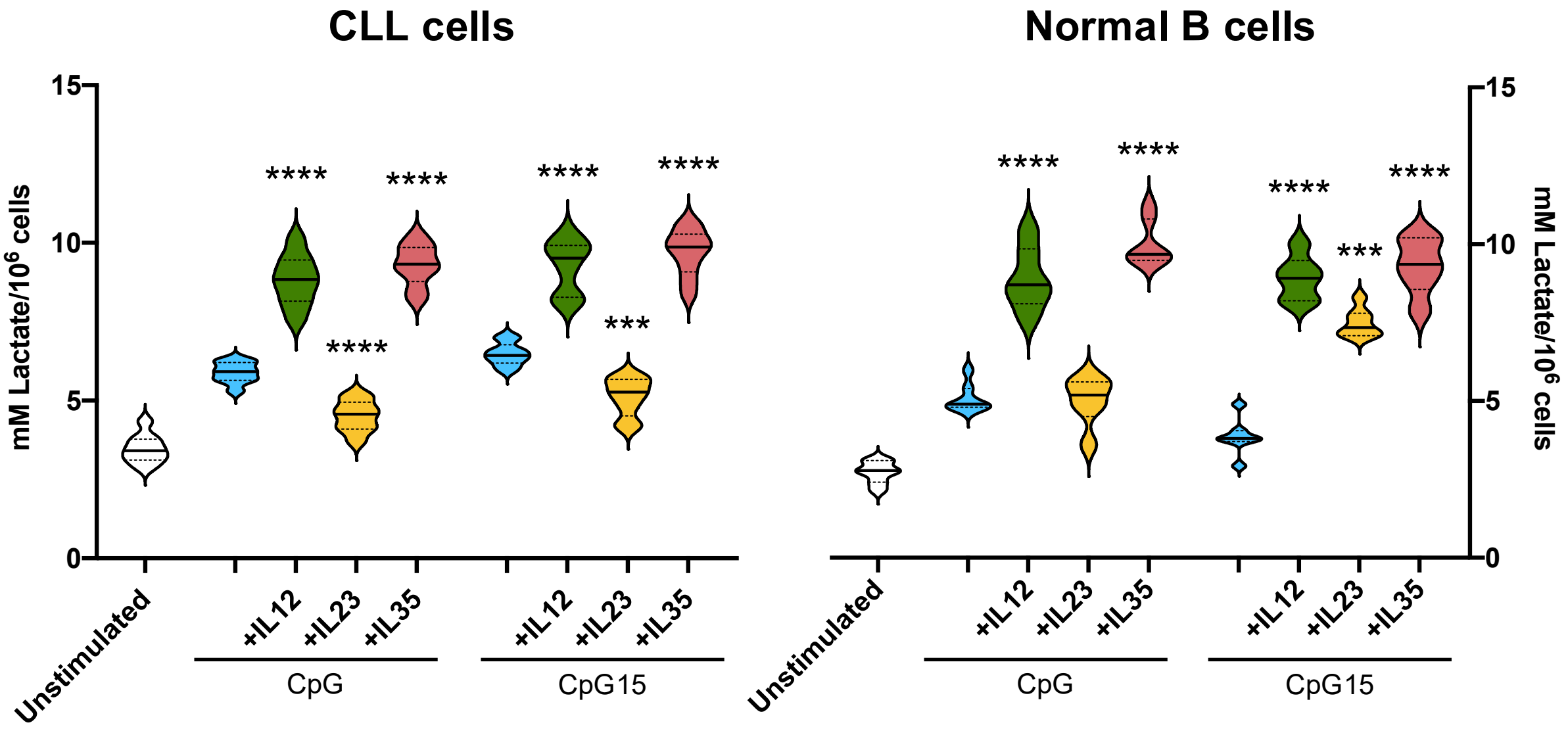
** and **** represent p < 0.01 and 0.0001 between treated and activated cells; n=12 CLL, n=8 normal B cells

Mitochondrial efficiency



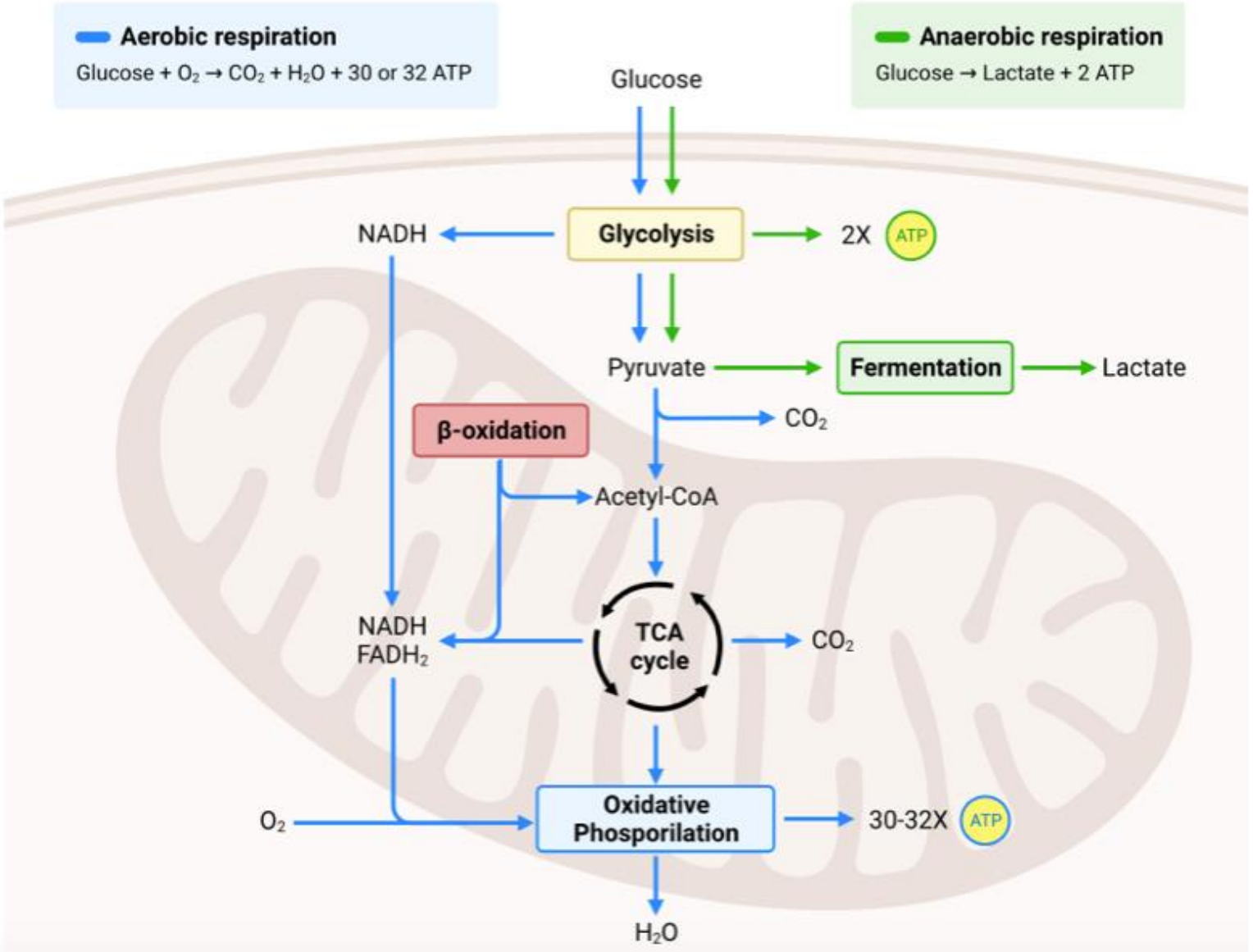
**** represents p < 0.0001 between treated and activated cells; n=6 CLL, n=4 normal B cells

Anaerobic Metabolism: Lactate release

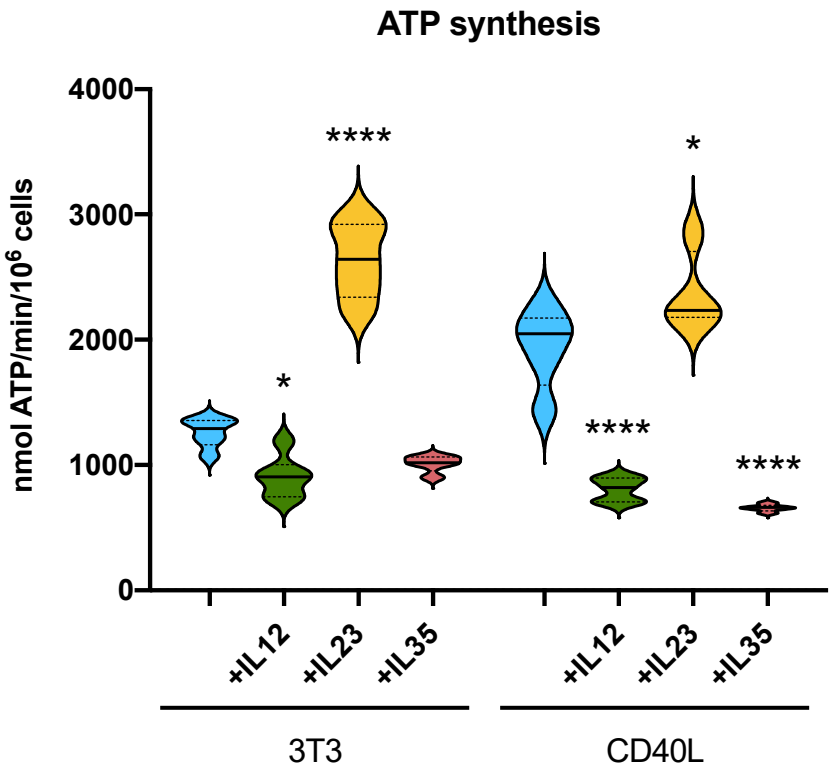


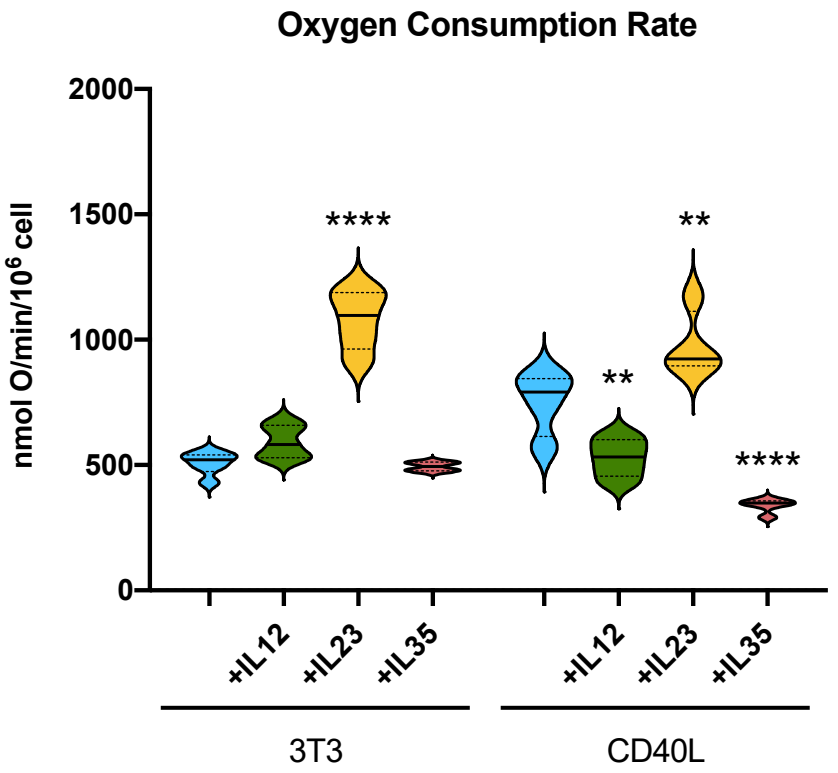
*** and **** represent p < 0.001 and 0.0001 between treated and activated cells; n=9 CLL, n=7 normal B cells

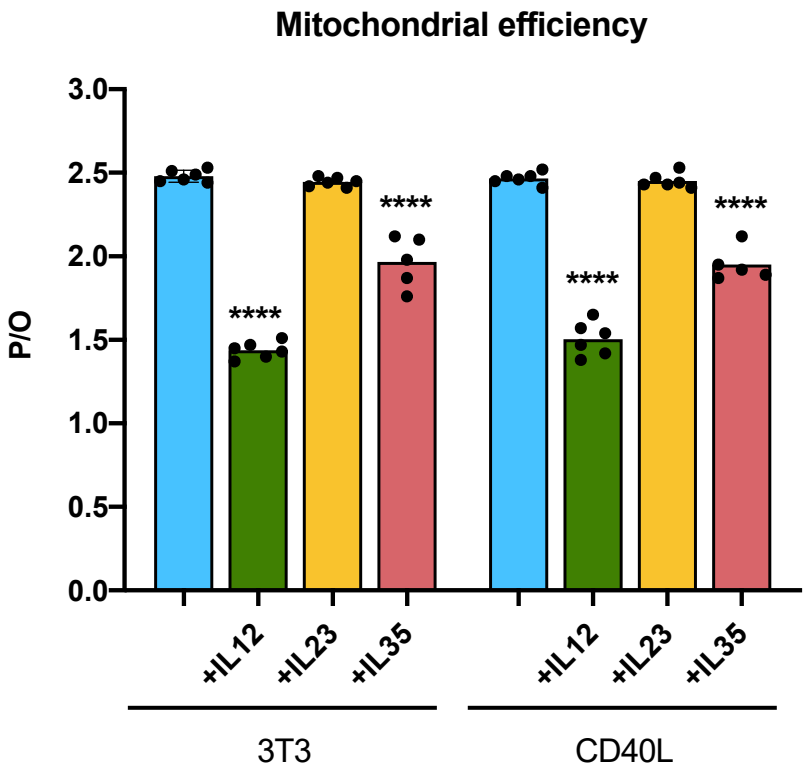
OxPhos vs glycolysis



CLL Mitochondrial Metabolism: 3T3 and CD40L







*, **, and **** represent $p < 0.05$, 0.01 and 0.0001 between treated and activated cells; n=6

CONCLUSIONS

- CpG±IL15 stimulation is highly effective in inducing the expression of individual receptor chains and complete IL12-family receptors, particularly the IL23R complex.
- IL23 was the sole cytokine in the family, promoting oxidative phosphorylation while maintaining mitochondrial efficiency.
- IL12 and IL35 increased lactate release and uncoupling between the electron transport chain and ATP synthase, indicating decreased metabolic efficiency.

CONCLUSIONS

Different cytokines within this family exert distinct effects on CLL metabolism

- CpG±IL15 stimulation is highly effective in inducing the expression of individual receptor chains and complete IL12-family receptors, particularly the IL23R complex.
- IL23 was the sole cytokine in the family, promoting oxidative phosphorylation while maintaining mitochondrial efficiency.
- IL12 and IL35 increased lactate release and uncoupling between the electron transport chain and ATP synthase, indicating decreased metabolic efficiency.

IL12-family cytokines may represent potential targets to improve CLL treatment and management

U.O. Patologia Molecolare
Fabiana Ferrero
Giovanna Cutrona
Monica Colombo
Rosanna Massara
Maria Cristina Capra
Ennio Nano
Franco Fais

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Thank you!

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CYMACS Lab
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