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Supplemental Information

Membrane Potential Distinctly Modulates Mobility and Signaling of IL-2 and IL-15 Receptors in T Cells

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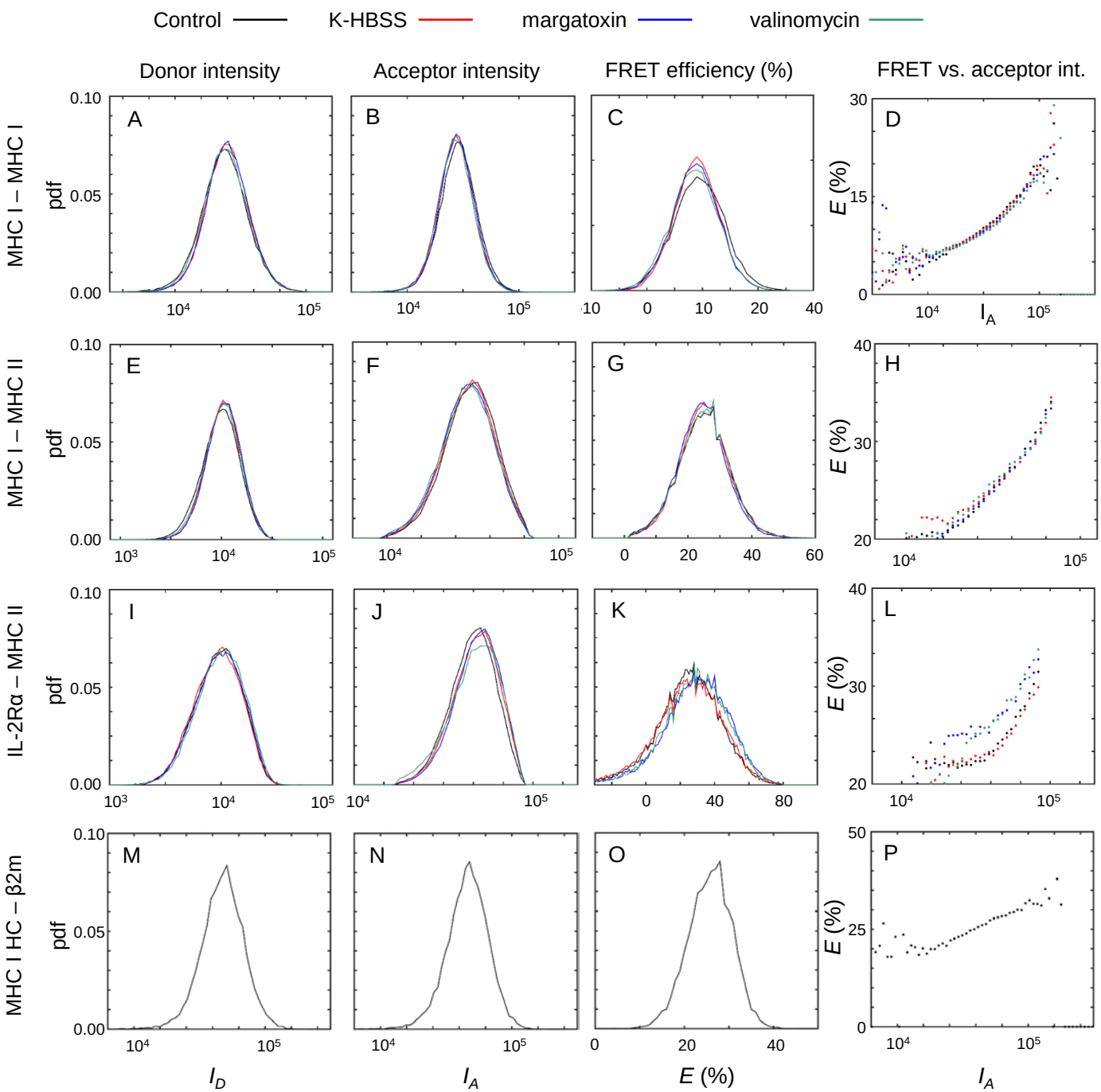

Figure S1

Homo- and heteroassociations of membrane proteins detected by flow cytometric FRET measurements on FT7.10 cells. Proteins were labeled with donor- (Alexa 546) and acceptor-tagged (Alexa 647) mAbs. The top row (panels A-D) displays the homoassociation of MHC I (labeled with A546-W6/32 and A647-W6/32 mAbs). A) Cell-by-cell intensity distributions I_D of donor-tagged MHC I (corrected for FRET quenching); B) Intensity distribution I_A of acceptor-tagged proteins; C) Cell-by-cell average FRET efficiency histograms; D) Dependence of the FRET efficiency E on the acceptor intensity I_A; here, E values were binned within short intervals of I_A. The second and third rows (E-H, I-L) show data for the heteroassociation of MHC I with MHC II (labeled with A546-W6/32 + A647-L243) and of IL-2Rα with MHC II (labeled with A546-anti-Tac + A647-L243) in a similar fashion. The fourth row (M-P) displays FRET measured between the heavy and light chains of MHC I (labeled with A546-W6/32 + A647-L368). This is a mixture of intra- and intermolecular FRET processes occurring between the two chains within the same complex or between chains of distinct, homoassociated MHC I complexes; hence the increase of E upon increasing acceptor intensity (and total MHC I expression). Control samples were incubated in HBSS, depolarization was achieved either by K-HBSS buffer or by margatoxin; hyperpolarization was induced by valinomycin. N~55000, 75000, 55000 and 17000 cells were measured for the different D-A pairs.

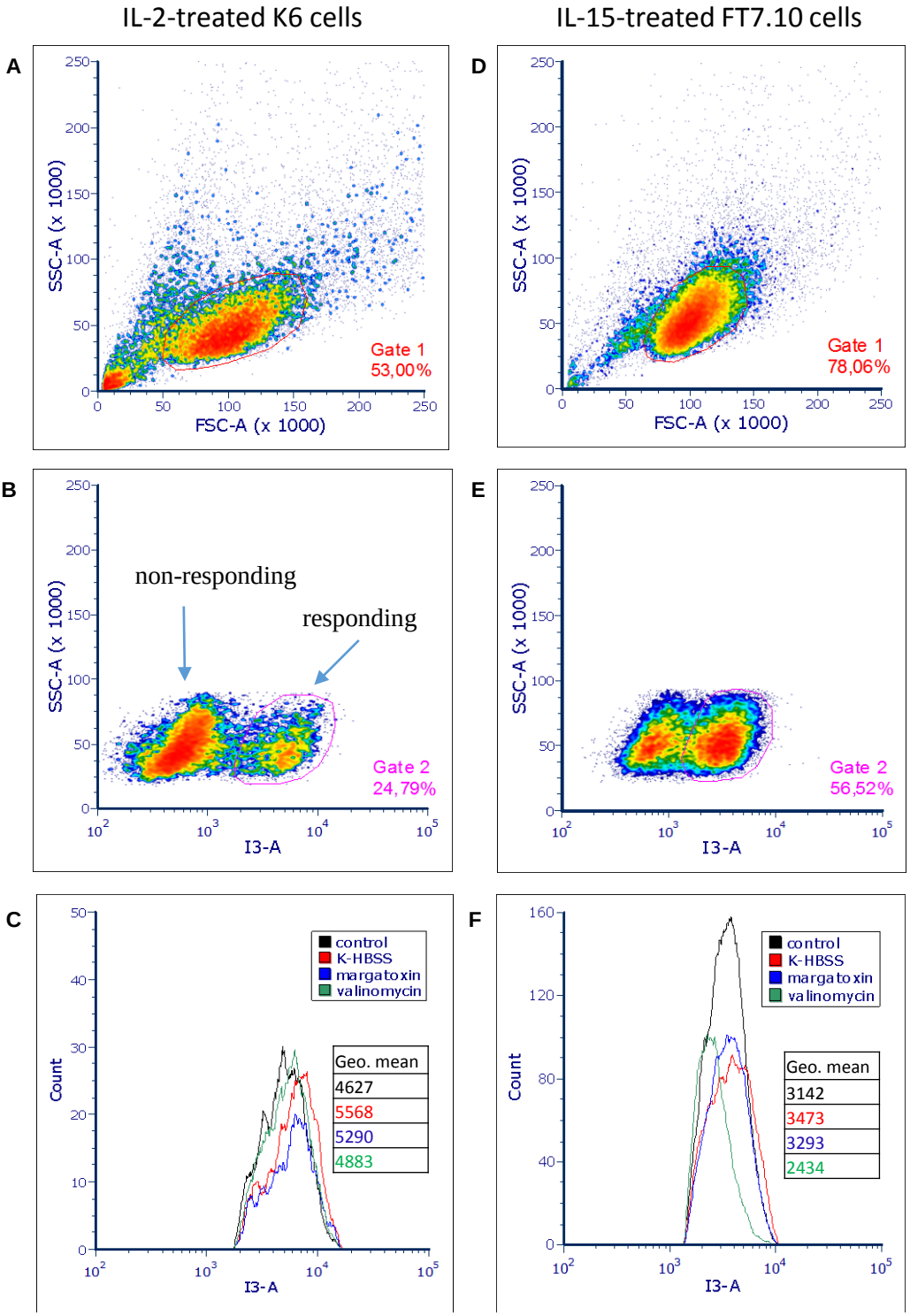

Figure S2

Flow cytometric analysis of IL-2/IL-15 induced STAT5 phosphorylation. Flow cytometry was used to measure STAT5 phosphorylation on a cell-by-cell basis using Alexa647-anti-PSTAT mAbs. Control samples were incubated in HBSS, depolarization was achieved either by K-HBSS buffer or by margatoxin (1.5 nM); hyperpolarization was induced by valinomycin (10 µM). A-C) K6 cells were stimulated with IL-2 (50 pM, 10 min, 37°C). D-F) FT7.10 cells were treated with IL-15 (50 pM, 5 min, 37°C). First, debris was excluded on the side scatter vs. forward scatter plot (panels A, D showing data of the control sample). Next, populations responding to the IL-2/IL-15 treatment were selected on a SSC vs. I3 (PSTAT5) plot (panels B, E showing the control sample); this plot allowed a better separation between responding and non-responding populations than the I3 histograms. Panels C and F display the I3 intensity histograms of the PSTAT5 signals of control, depolarized and hyperpolarized samples. Geometric means of I3 are also shown for the presented experiments.

Charge distribution in and near the transmembrane regions of IL-2/IL-15 receptor subunits, MHC glycoproteins and the transferrin receptor

The electric field across the plasma membrane can act on proteins having an uneven charge distribution in or near their transmembrane regions (TMRs). We identified TMRs of membrane receptors by using an online transmembrane protein topology prediction program (1) or the UniProt database. All four receptor subunits of IL-2/15R have single transmembrane helices. The TMR (bold letters) and 10 flanking amino acids for these proteins are shown below; negatively charged amino acids are marked red and are singly underlined, positively charged ones are blue and doubly underlined. Also shown are examples of one allele each of MHC I and II chains: the HLA-A-68 allele and HLA-DR A α chain, and the transferrin receptor.

IL-2Rα: 231-ETSIFTTEYQ**VAVAGCVFLLISVLLLSGLTW**QRRQRKSRRTI

IL-15Rα: 196-VYPQGHSDTT**VAISTSTVLLCGLSAVSLLACYL**KSRQTPPLAS

IL-2/15Rβ: 233-PAALGKDTIP**WLGHLLVGLSGAFGFIILVYLL**INCRNTGPWL

γ_c: 253-ENPFLFALEA**VVISVGSMGLIISLLCVYFWL**EQTMPRIPTL

HLA-A-68: 296-LRWEPSSQPT**IPIVGIIAGLVLFGAVITGAVVAAV**MWRRKSSDRK

HLA-DR-A α chain: 209-SPLPETTENV**VCALGLTVGLVGIIIGTIFII**KGVRKSNAAE

Transferrin receptor: 58-KPKRCSGSIC**YGTIAVIVFFLIGFMIGYLGY**CKGVEPKTECE

IL-2Rα contains two negatively charged glutamic acids (E-231, E-238) near the extracellular side of the TMR and six positively charged amino acids, five arginines (R) and a lysine (K) near the intracellular side of the TMR. The dipole moment points inward, toward the intracellular side. IL-15Rα contains a potentially positively charged histidine (H-201) and a negatively charged aspartate (D-203) near the extracellular side of the TMR, and two positively charged amino acids near the intracellular side of the TMR (K-229, R-231). Histidine has a pK of 6.0, so H-201 may be positive at physiological pH. The dipole moment may point inward, but it is probably smaller than that of IL-2Rα because there are fewer positive charges near the intracellular side of the TMR and because H-201 may partially cancels the effect of D-203 at the extracellular side. The shared β chain of IL-2R and IL-15R contains a positive and a negative charge (K-233, D-234) next near to each other near the ec. side of the TMR, and a positive charge (R-268) near the intracellular side of the TMR; it also possesses a histidine inside the TMR. The shared γ_c chain has two negative charges (E-253, E-261) near the ec. side, and a negative (E-284) and a positive (R-289) charge near the ic. side of the TMR. The MHC I allele shown has a positively and a negatively charged amino acid in the vicinity of the ec. side and five positive residues near the ec. side of the TMR. HLA-DR A α chain has two negatively charged residues (E-2013, E-216) near the ec. side of the TMR, and three positively charged residues and a negative one at the ic. side. The transferrin receptor differs from the other studied

proteins in that it has three positive charges at the extracellular flank of the TMR; it also has two positive charges at the cytoplasmic flank. From the studied proteins IL-2Rα and HLA-A-68 stand out with their largest positive net charges near the ic. side of the TMR (6 and 5 positive amino acids, respectively).

Supporting reference

1. Kahsay, R. Y., G. Gao, and L. Liao. 2005. An improved hidden Markov model for transmembrane protein detection and topology prediction and its applications to complete genomes. Bioinformatics 21:1853-1858.