Supplementary Information

for

Cell surface remodeling caused by the loss of TMEM30A in immune cells

Cenk O. Gurdap[1,#], Franziska Ragaller[1,#], Marion Muller[2,3,#], Ellen Sjule[1], Taras Sych[1], Linnea Blomén[4,5], Fredrik Thoren[4,5], Ilya Levental[6], Kandice Levental[6], Quentin Sattentau[2,7,*], Erdinc Sezgin[1,*]

[1] Science for Life Laboratory, Department of Women's and Children's Health, Karolinska Institutet, Tomtebodavägen 23, 17165 Solna, Sweden

[2] The Max Delbrück Centre for Molecular Medicine, Campus Berlin-Buch, 13125 Berlin, Germany.

[3] The Kennedy Institute of Rheumatology, University of Oxford, Roosevelt Drive, Oxford OX3 7FY, UK.

[4] Tumor Immunology (TIMM) Laboratory at Sahlgrenska Center for Cancer Research, University of Gothenburg, Gothenburg 413 90, Sweden.

[5] Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg 413 90, Sweden.

[6] Molecular Physiology and Biological Physics, Center for Membrane and Cell Physiology, School of Medicine, University of Virginia, Charlottesville, VA 22903

[7] Sir William Dunn School of Pathology, University of Oxford, Oxford, OX1 3RE, UK.

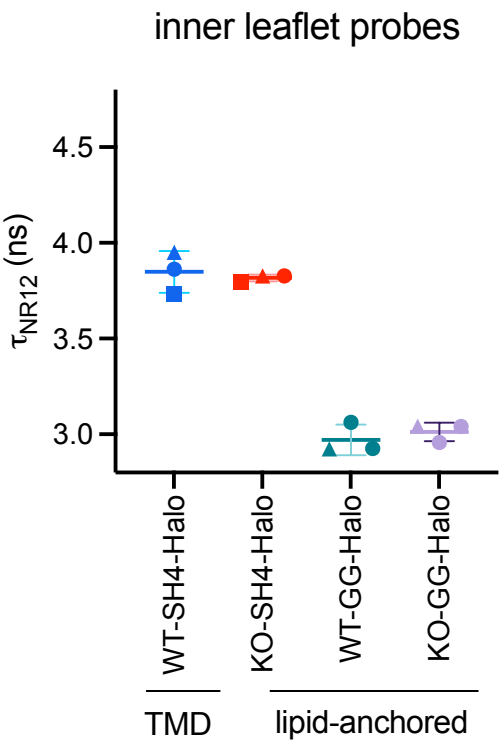
 # These authors contributed equally

*** Correspondence:**

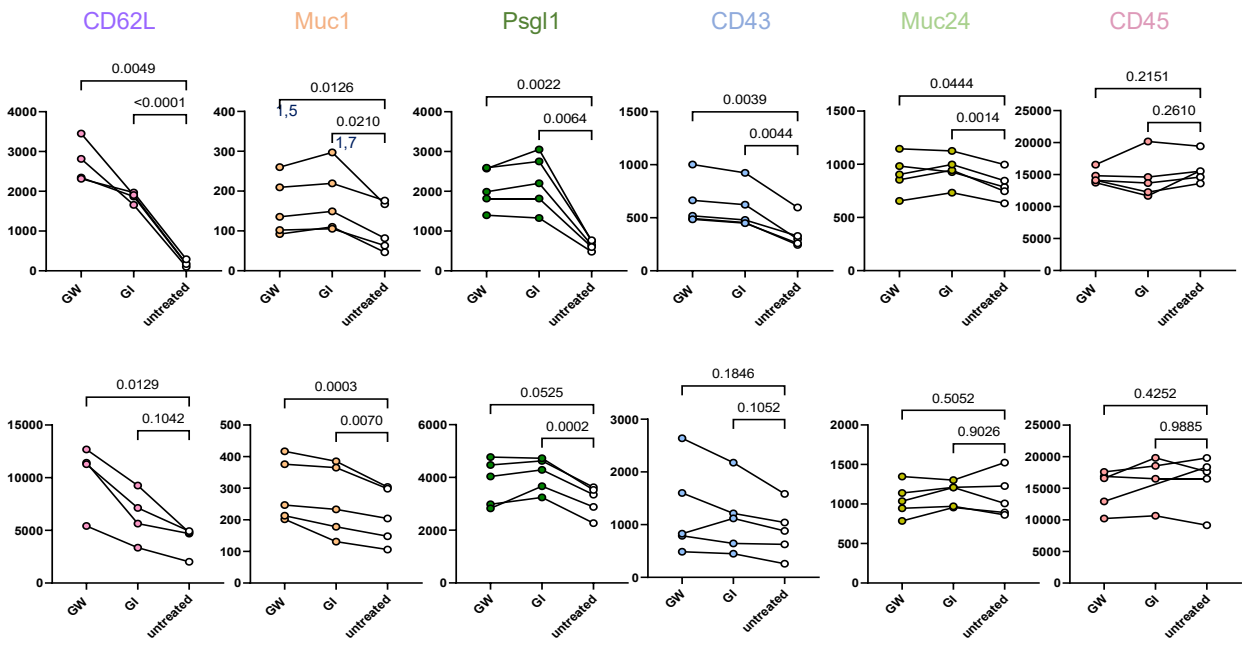
erdinc.sezgin@ki.se

quentin.sattentau@path.ox.ac.uk

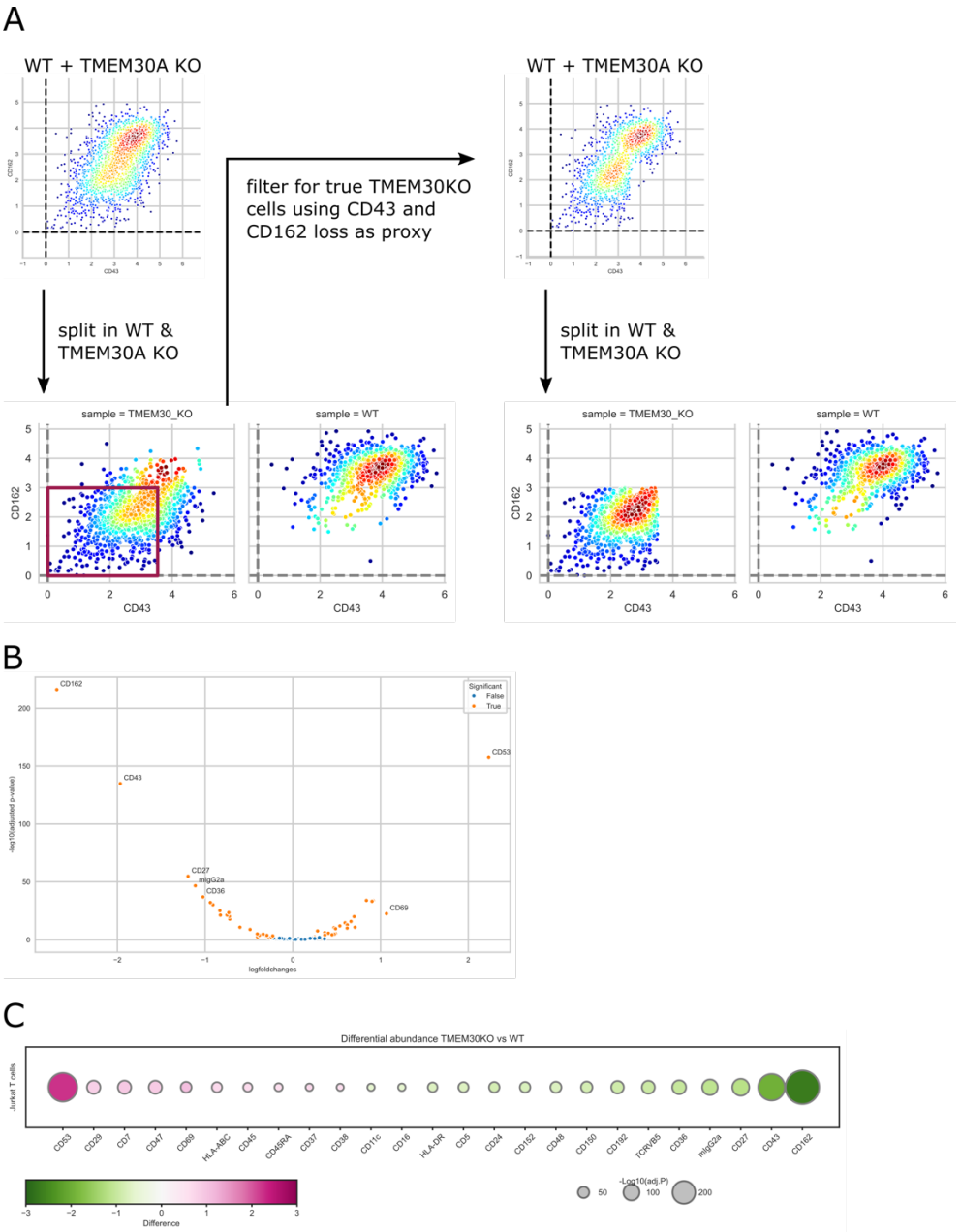
Supplementary Figures:



Supplement Figure S1. Lipid order in the inner leaflet does not change notably. HaloTag-Nile Red was used to measure local lipid environment of three reporter proteins: one ordered domain (SH4) and one disordered domain (GG) preferring. Lifetime of Nile Red is shown for three peptides. Each point is a replicate.



Supplementary Figure S2. The protein shedding by ADAM10 in Jurlat WT and TMEM30A-KO cells. Top panel shows the TMEM30A-KO cells and bottom panel is WT cells. ADAM10 inhibitor GI254023X (GI) and ADAM family inhibitor GW280264X (GW) reduces the shedding significantly in TMEM30A-KO cells and notably in WT cells.



Supplement Figure S3: Surface protein abundance after filtering for true TMEM30A KO cells. A) TMEM30A KO cells are a cell pool and not all cells have the KO as indicated by cells expressing CD43 and CD162. TMEM30A KO cells are filtered for cells with a shifted clr value of CD162<3 and CD43>3.5. After filtering two distinct cell populations are visible. B) Volcano plot for the abundance of proteins differentially regulated in WT vs. true KO Jurkat cells. Orange dots indicate significantly up- or downregulated proteins (adjusted p-value < 0.01). Labels are added for proteins with a log fold change > 1. C) Proteins of differential abundance (log fold change >0.6) in WT vs. true KO Jurkat cells. Color corresponds log fold change and size of the bubbles indicates the adjusted p-value.