Lysophosphatidic acid activated Calcium signaling is elevated in red cells from sickle cell disease patients

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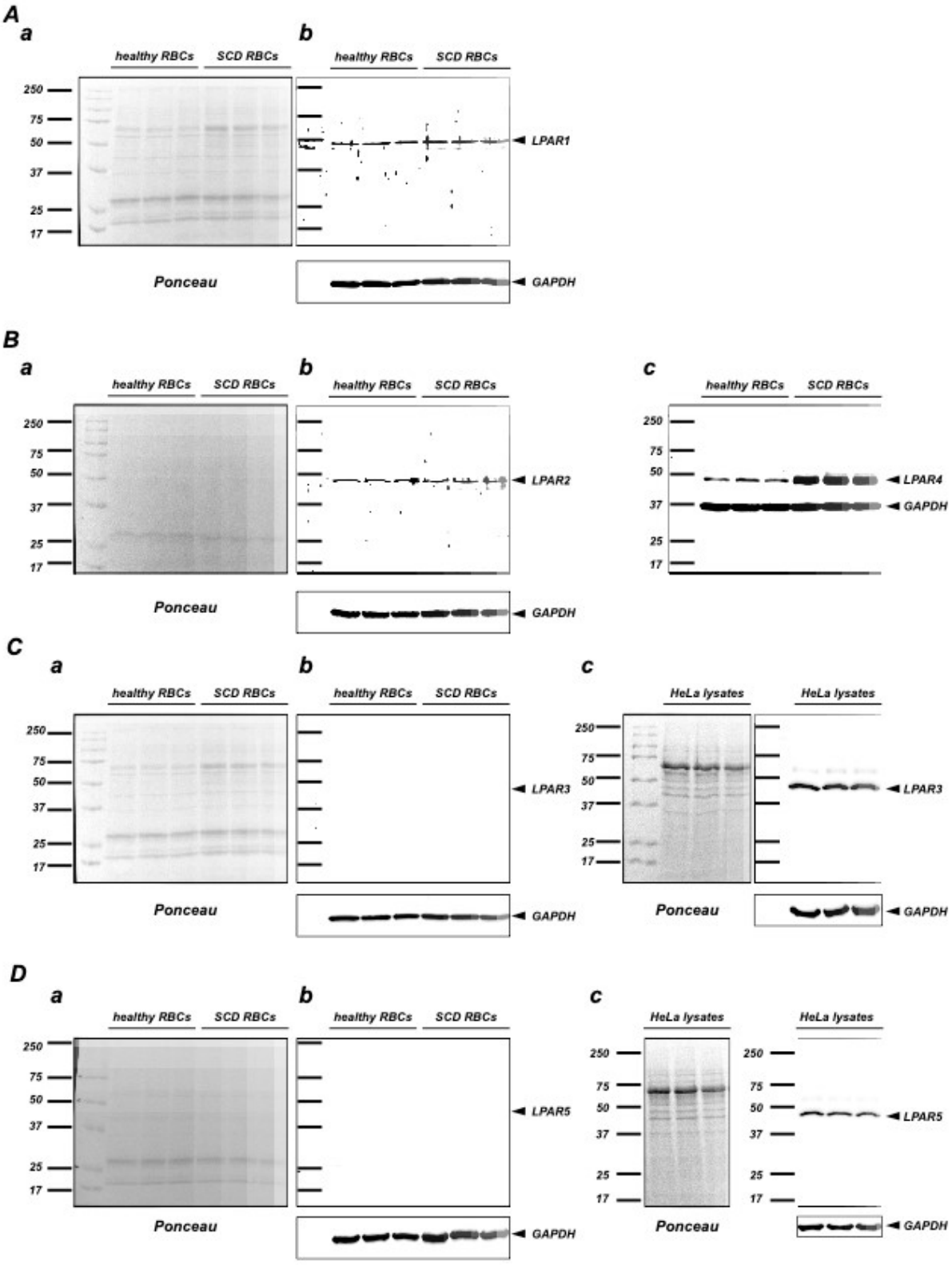
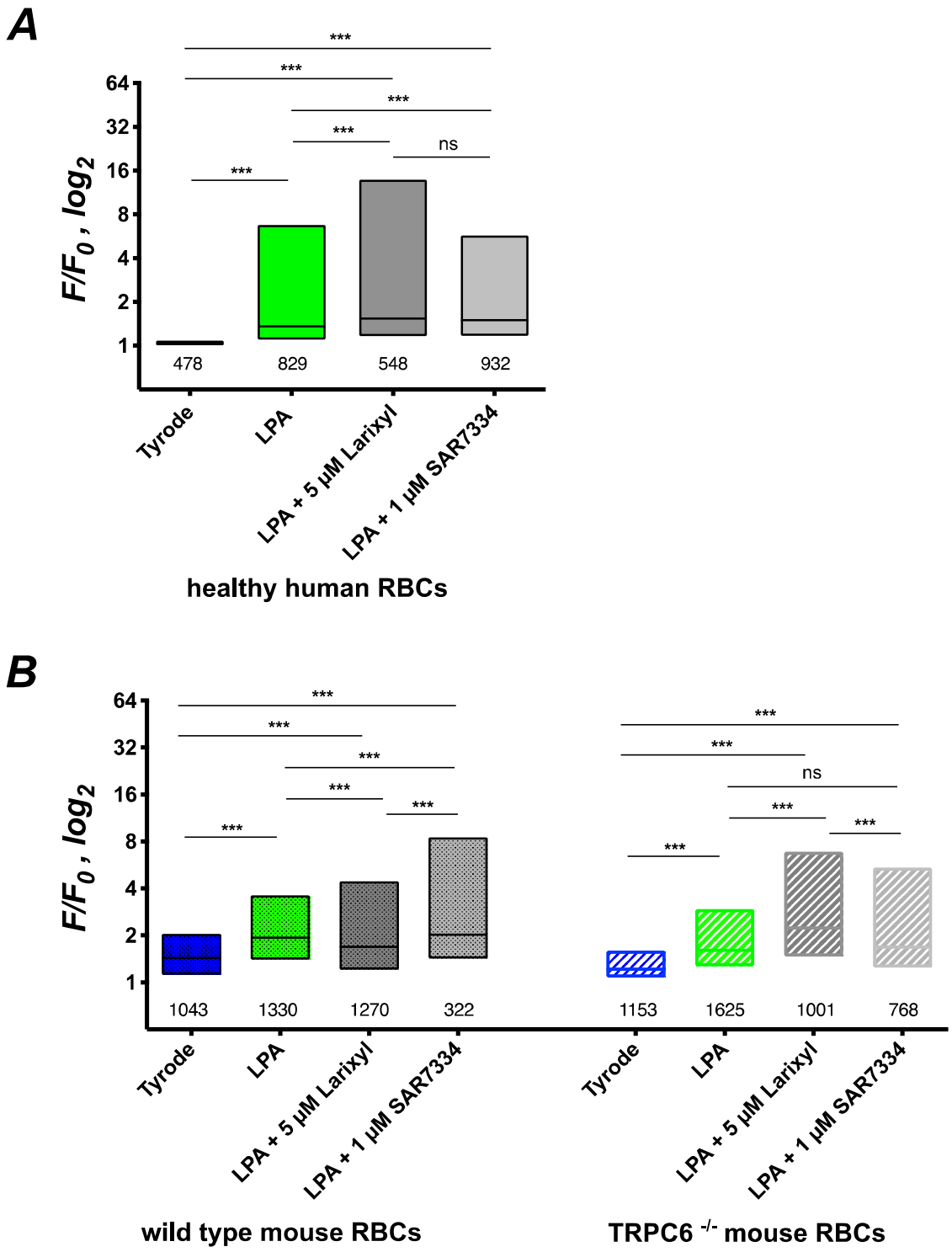
Supplemental Material



Supplemental Figure S1: Gel electrophoresis analysis of LPA-receptors 1-5 in human red blood cells (RBCs) of healthy donors and sickle cell disease (SCD) patients. (**A**) Analysis of LPA-receptor 1 (LPAR1). (**Aa**) Ponceau stained gel to see the total protein distribution. (**Ab**) Western blot of the LPAR1 (for methodological details see main document). The GAPDH was used for normalization of the quantitative analysis (compare Figure 2A of the main document). GAPDH is plotted separately because it required a different exposure time than LPAR1. (**B**) Analysis of LPA-receptor 2 (LPAR2) and LPA-receptor 4 (LPAR4). (**Ba**) Ponceau stained gel to see the total protein distribution. The same gel was used for both Western blots (LPAR2 and LPAR4). (**Bb**) Western blot of the LPAR2 (for methodological details

see main document). The GAPDH was used for normalization of the quantitative analysis (compare Figure 2A of the main document). GAPDH is plotted separately because it required a different exposure time than LPAR2. (**Bc**) Western blot of the LPAR4. Here GAPDH could be visualized in the same blot. (**C**) Analysis of LPA-receptor 3 (LPAR3). (**Ca**) Ponceau stained gel to see the total protein distribution. (**Cb**) Western blot of the LPAR3 (for methodological details see main document). The GAPDH was used for normalization of the quantitative analysis (compare Figure 2A of the main document). GAPDH is plotted separately because it was recorded at a different exposure time than LPAR3. (**Cc**) Since the Western blot of LPAR3 did not show detectable bands for RBCs, a positive control was performed with HeLa cells. The Poncaeu staining as well as the Western blot of LPAR3 are depicted. GAPDH is plotted separately because it required a different exposure time than LPAR3. (**D**) Analysis of LPA-receptor 5 (LPAR5). (**Da**) Ponceau stained gel to see the total protein distribution. (**Db**) Western blot of the LPAR5 (for methodological details see main document). The GAPDH was used for normalization of the quantitative analysis (compare Figure 2A of the main document). GAPDH is plotted separately because it was recorded at a different exposure time than LPAR5. (**Dc**) Since the Western blot of LPAR5 did not show detectable bands for RBCs, a positive control was performed with HeLa cells. The Poncaeu staining as well as the Western blot of LPAR5 are depicted. GAPDH is plotted separately because it required a different exposure time than LPAR5.



Supplemental Figure S2: Test of the reported TRPC6 inhibitors larixyl acetate and SAR7334. (**A**) Statistical evaluation of human RBCs pretreated with the TRPC6 inhibitors larixyl acetate (Larixyl) and SAR7334 followed by LPA stimulation. (**B**) Statistical evaluation of wild type mouse RBCs in comparison to RBC from TRPC6$^{-/-}$ mice for perfusion with Tyrode (control), LPA stimulation and LPA stimulation with pretreatment of 5 µM Laryxil or 1 µM SAR7334. The numbers below the boxes indicate the number of cells measured in at least 3 different experiments. ns stands for not significant ($p>0.05$) and *** for $p<0.001$. All measurements were performed at room temperature.