

(A) Detection or recombinant (r)LILRA6 (A6) and rLILRB3 (B3) by rabbit anti-LILRB3 pAb. rLILR were seperated via SDS-PAGE, blotted onto membranes and detected using rabbit anti-LILRB3 pAb or rabbit IgG and goat anti-rabbit-IgG-HRP. (B) Illustrative HCD spectra of unique peptides of LILRB3: QRPAGAAETEPKDRGLL and RRSSPAADVQEENLY. The insets depict the amino acid sequence coverage obtained by fragmentation, the *b*-, *y*- fragment ions are red and blue colored separately. Precursor ions are in green color. Lower case "s" in the peptide sequence indicates a phosphorylated serine.



Differentiation of PLB-985 like cells after culture with 1.25% DMSO. **(A)** Binding of anti-LILRB3 or IgG2a mAb to undifferentiated PLB-985 cells, n = 4, Students t test, where * = p < 0.05, ** = p < 0.01. **(B)** PLB-985 cells were cultured in RPMI1640 + 10% FCS supplemented with or without 1.25% DMSO for 5 days. Cells were stained with monoclonal antibodies. Representative flow cytometry plots show binding of mAb to the surface of PLB-985 cells. Anti-IgG-PE used as a secondary mAb for anti-LILRB3. **(C)** Immunoprecipitation of LILRA6 and/or LILRB3 from the surface of resting neutrophils. Neutrophil lysates were incubated with 5 µg/ml anti-LILRA6 (aA6), anti-LILRB3 (aB3), IgG1 or IgG2a (i) and DB protein G. Immunoprecipitated proteins were eluted from beads, seperated via SDS-PAGE, blotted onto membranes and detected using rabbit anti-LILRB3 pAb and goat anti-rabbit-IgG-HRP.



LILRA6CD3ζ- and LILRB3CD3ζ- reporter cell lines. (A) A schematic of the LILRCD3z reporter cell lines. 2B4T cells express surface proteins composed of extracellular and transmembrane LILR domains fused to the CD3⁴ cytoplasmic domain. Cross-linking of fusion receptors induces ITAM (immunoreceptor tyrosine-based activation motif) phosphorylation by Src kinases, and signal transduction that culminates in activation of the NFAT (nuclear factor of activated T-cells) transcription factor. The cells contain an insert containing a gene encoding for GFP (green fluorescent protein) under the control of the NFAT promoter. (B) 2B4T cell lines were tested for binding of anti-LILRA6, anti-LILRB3 or isotype control mAbs. Goat anti-mouse-IgG-PE was used to detect mAb. Cell lines were transduced with lentiviral vectors encoding an LILRA6CD35 fusion protein, a lentiviral vector encoding an LILRB3CD3^{\(\zeta\)} fusion protein, or empty vector (EV) control. Representative flow cytometry plots are shown. (C) GFP-expression in 2B4T cells cultured in the presence of anti-CD3 or isotype control mAb. Representative flow cytometry plots are shown. (D) anti-CD3 mAb induced a significant increase in GFP-positive 2B4T cells in comparison to isotype control. A paired Student t-test was used to compare % of GFPpositive cells between anti-CD3 or isotype control mAb (n = 3), where * = p < 0.05, ** =p < 0.01.



ROS production by neutrophils. Relative luminescence units (RLU) was measured over 60 minutes as an indicator of ROS production. **(A)** Neutrophils were incubated on IgG1 or IgG2a coated plates in the presence of absence of 5 µg/ml FLIPr-like. **(B** and **C)** Neutrophils were incubated on anti-LILRA6 or IgG1 mAb coated plates. Representative plots (B) and integrated results (C) from different neutrophil populations were compared using area undercurve (AUC) values by Students t test, where * = p < 0.05, ** = p < 0.01. **(D)** Neutrophils were incubated on anti-CD89 or IgG1 coated plates in the presence of absence of 5 µg/ml FLIPr-like. **(F)** and **(G)** Mid-logarithmic cultures of *S. capitis* strains were incubated in the presence of indicated concentrations of anti-WTA-4497-IgA1 or anti-HAS-IgA1 as an isotype control. Surface bound IgA1 was detected with anti-hIgA-AF647 and flow cytometry. Strain ATCC 27840 is a wildtype strain, and ATCC 27840-H strain carries a vector for expression of a poly-RboP WTA backbone and β -GlcNAC modifications. Representative flow cytometry plots of n = 3 experiments are shown.