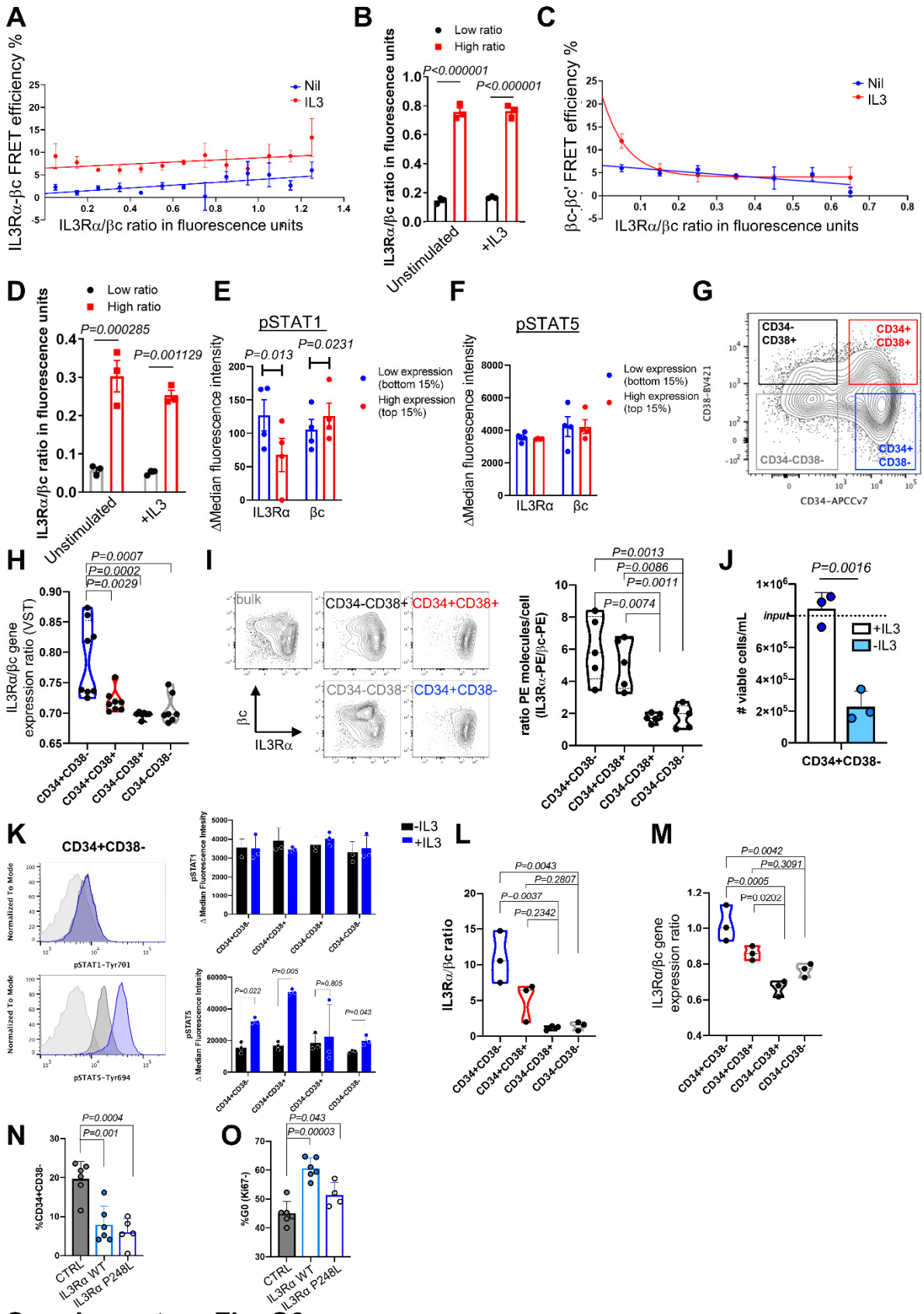

Supplementary Fig. S6

Supplementary Figure S6. Increasing IL3Rα/βc ratios lead to hexameric receptor assembly and augmented quiescence. A, %IL3Rα-βc FRET efficiency shown based on binned IL3Rα/βc expression ratios in fluorescence units. Each point is the mean of n=3 independent experiments. **B,** IL3Rα/βc ratio in fluorescence units in high and low ratio groups for IL3Rα-βc FRET experiments. Each point is the mean of n=3 independent experiments. **C,** %βc-βc' FRET efficiency shown based on binned IL3Rα/βc expression ratios in fluorescence units. Each point is the mean of n=3 independent experiments. **D,** IL3Rα/βc ratio in fluorescence units in high and low ratio groups for βc-βc' FRET experiments. Each point is the mean of n=3 independent experiments. **E, F,** Flow cytometric analysis of phosphorylated STAT1 **(E)** and STAT5 **(F)** signaling in FDH cells expressing βc and IL3Rα WT stratified on high vs. low IL3Rα and βc cell surface expression levels upon IL3 stimulation. ΔMFI: stimulated minus unstimulated median fluorescence intensity. **G-I,** The CD34 vs. CD38 defined subpopulations of OCI-AML22 **(G)** were assessed for IL3Rα/βc ratio (by RNA-seq, n=8 combined freshly thawed and various culture time points) **(H)** and IL3Rα/βc ratio by flow cytometric analysis of IL3Rα and βc expression on the cell surface of the CD34/CD38 defined subfractions using Quantibrite assay **(I)**. **J,** IL3 dependency of the sorted CD34+CD38- LSC fraction from OCI-AML22 according to cell counts after 7 days of culture +/- IL3. **K**, Phosphorylation of STAT-Tyr701 and STAT5-Tyr694 in the four sorted OCI-AML22 fractions delineated by CD34 and CD38 expression was assessed by Phosflow analysis upon 30 minutes of IL3 restimulation after 40 hours IL3 withdrawal (n=3). **L**, OCI-AML8227 cells were subjected to CD34, CD38 staining and via Quantibrite assay, surface abundance of either IL3Rα (CD123) or βc (CD131) was quantified by flow cytometry to determine IL3Rα/βc protein surface ratio of the individual fractions delineated by CD34 and CD38 expression (n=3). **M**, IL3Rα and βc transcript expression across the CD34/CD38 hierarchy of OCI-AML8227 according to RNA-seq. **N**, and **O**, Purified CD34+CD38- OCI-AML8227 were

transduced with control, IL3Rα WT and IL3Rα P248L vectors and after 2 weeks %CD34+CD38- was assessed (**N**) and BFP+CD34+CD38- cells were sorted and subjected to cell cycle analysis (**O**; 5-6 individual transductions, 2 independent experiments).