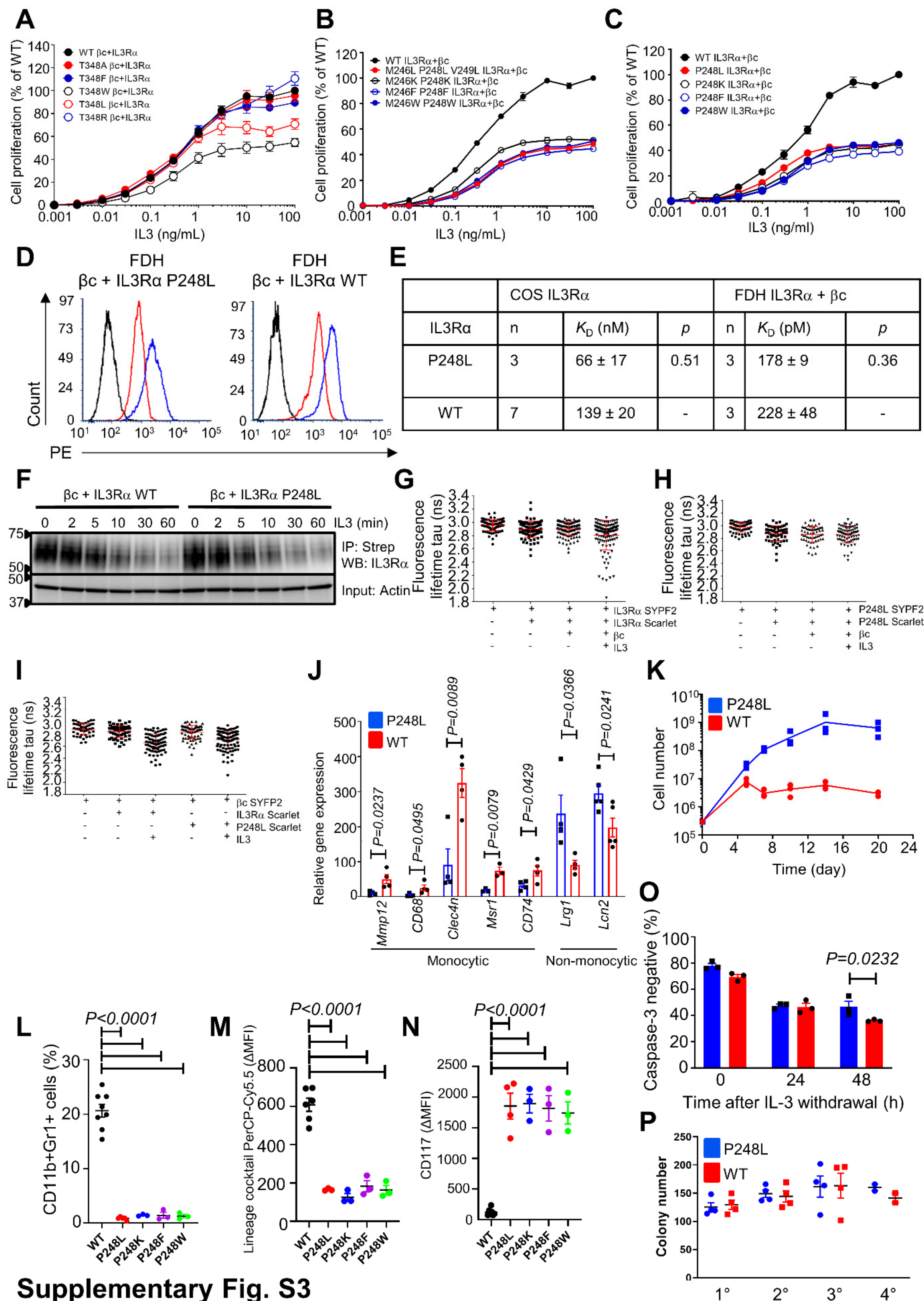

Supplementary Fig. S3

Supplementary Figure S3. IL3Rα P248 at the IL3R assembly interface is critical for cell differentiation. A, B, C, IL3 induced proliferation of FDH cells expressing: **A,** IL3Rα and βc WT (black filled circle) or βc mutants of the "assembly interface", T348A (red filled circle), T348F (blue filled circle), T348W (black open circle), T348L (red open circle) and T348R (blue open circle); **B,** βc and IL3Rα WT (black filled circle) or IL3Rα mutants of the "assembly interface", M246K/P248K (black open circle), M246W/P248W (blue filled circle), M246F/P248F (blue open circle) or M246L/P248L/V249L (red filled circle); **C,** βc and IL3Rα WT (black filled circle) or IL3Rα P248 mutants, P248K (black open circle), P248W (blue filled circle), P248F (blue open circle) or P248L (red filled circle). Proliferation of FDH cells expressing IL3Rα WT or mutant is normalized to the maximum proliferation of FDH cells expressing WT IL3Rα and βc in 100 ng/mL IL3 for 48 hours. Data are the mean of triplicate determinations from a representative experiment ± standard deviation of the mean (SD). Similar results were obtained in 3 independent experiments. **D,** Flow cytometric analysis of cell surface βc and IL3Rα in FDH cells expressing βc and IL3Rα P248L or WT. Representative histograms are shown with IL3Rα (blue), βc (red) and isotype control (black). **E,** The binding affinity of IL3 for IL3Rα WT or IL3Rα P248L in the absence or presence of βc was measured in saturation binding assays using radio-iodinated IL3 and indicated as K_D ± standard error of the mean (SEM). *P* values are expressed relative to IL3Rα WT in respective cell lines. **F,** Stability of IL3Rα P248L compared to IL3Rα WT at the cell surface as assessed by biotinylation of all cell surface proteins followed by streptavidin (strep) pulldown enrichment and immunoblotting of pulldowns for IL3Rα. **G,** Raw IL3Rα-IL3Rα' FLIM data from a representative experiment (of n=4 independent experiments) with cells expressing IL3Rα-mScarlet-I or IL3Rα-SYPF2 fusion proteins and βc. FLIM donor lifetimes were converted into %FRET efficiencies (shown in Fig. 2D, 2E) as indicated in Methods. **H,** as for **(G)** but for IL3Rα P248L. **I,** Raw IL3Rα-βc FLIM data from a representative experiment (of n=3 independent experiments) with cells expressing

IL3Rα-mScarlet-I or IL3Rα P248L-mScarlet-I and βc-SYFP2 fusion proteins. **J,** Expression of monocytic differentiation genes in FDH cells after 5 days in IL3 normalized to RPLP0 expression. n=4 independent experiments. Blue: IL3Rα P248L (hexamer); red: IL3Rα WT (dodecamer). **K,** Cumulative number of FDH cells cultured with IL3 quantified at specific time points up to 20 days. The cell number from n=3 independent experiments are shown at each time point and the connecting line indicates the mean from independent experiments. **L, M, N,** Flow cytometric analysis of %CD11b+Gr1+ **(L)**, mature lineage cocktail **(M)** and CD117 expression **(N)** in FDH cells expressing βc and various IL3Rα P248 mutants (n=3). **O, P**, Fetal liver cells from $\beta c^{-/-}\beta_{IL3}^{-/-}$ mice were transduced to express βc and IL3Rα P248L (hexamer, blue) or WT (dodecamer, red) and assessed for % caspase-3 negative cells after IL3 withdrawal (n=3) **(O)** and serial replating colony numbers in cytokine cocktail (n=3) **(P).**