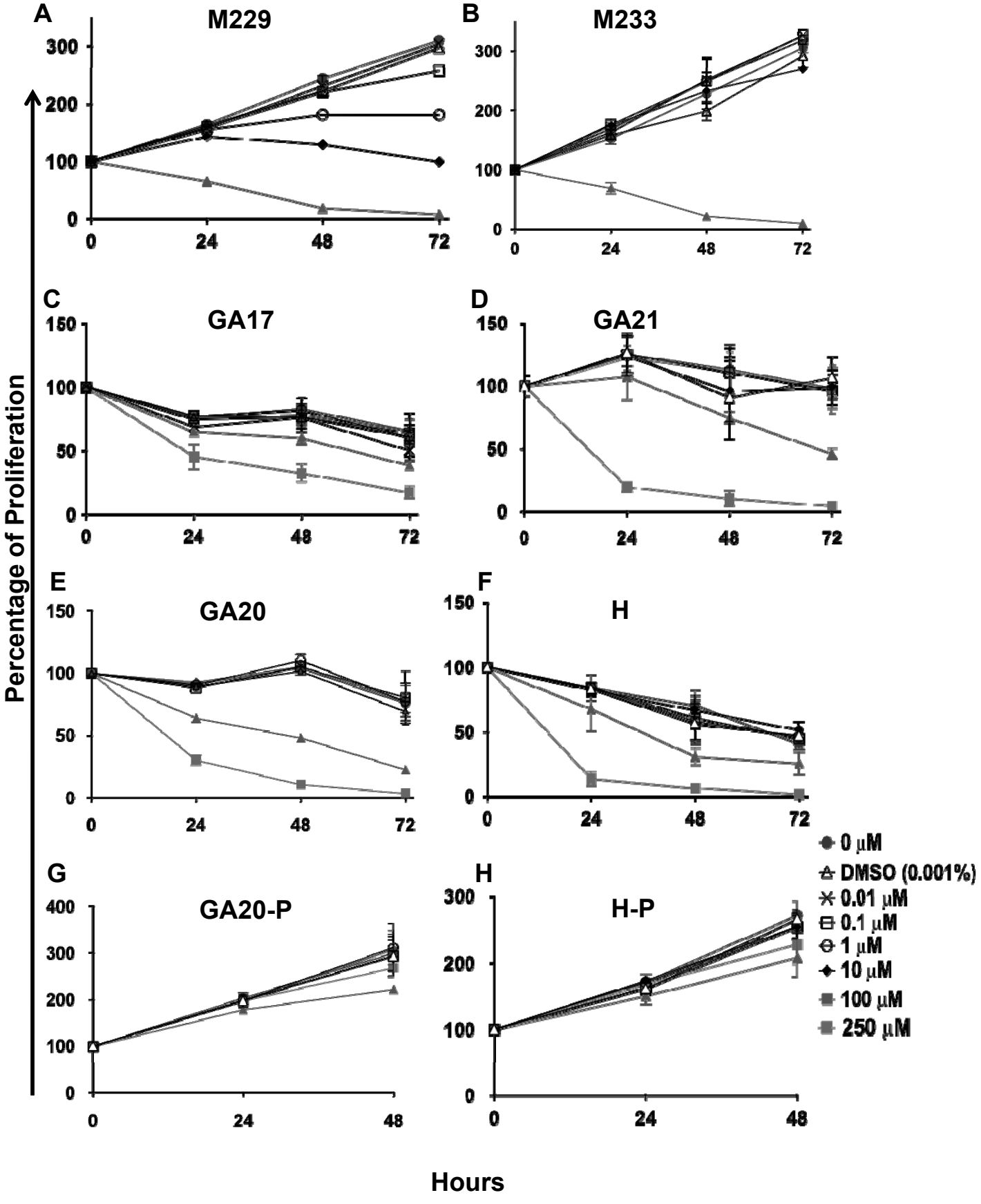
Supplemental Fig. 1.



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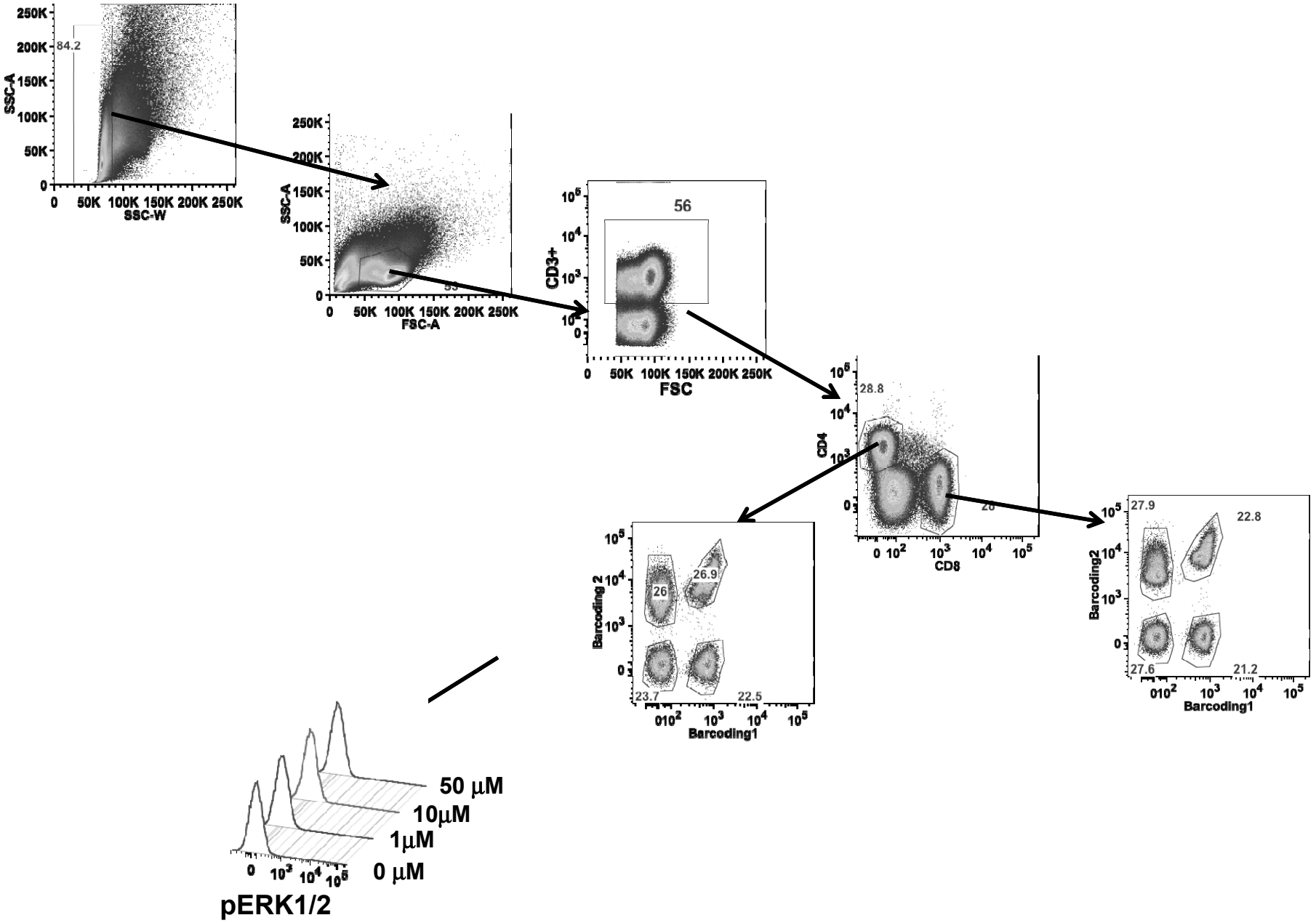

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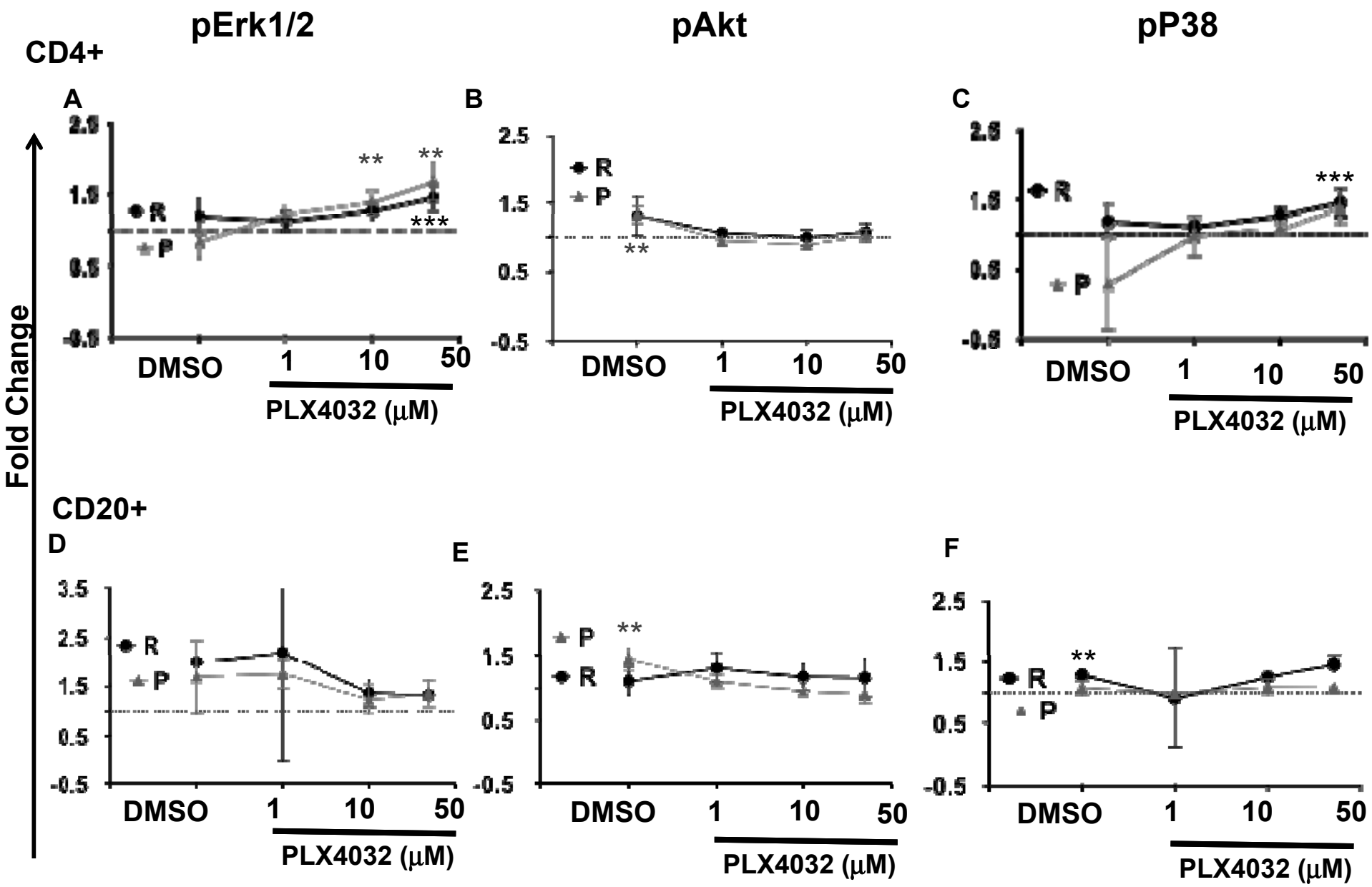
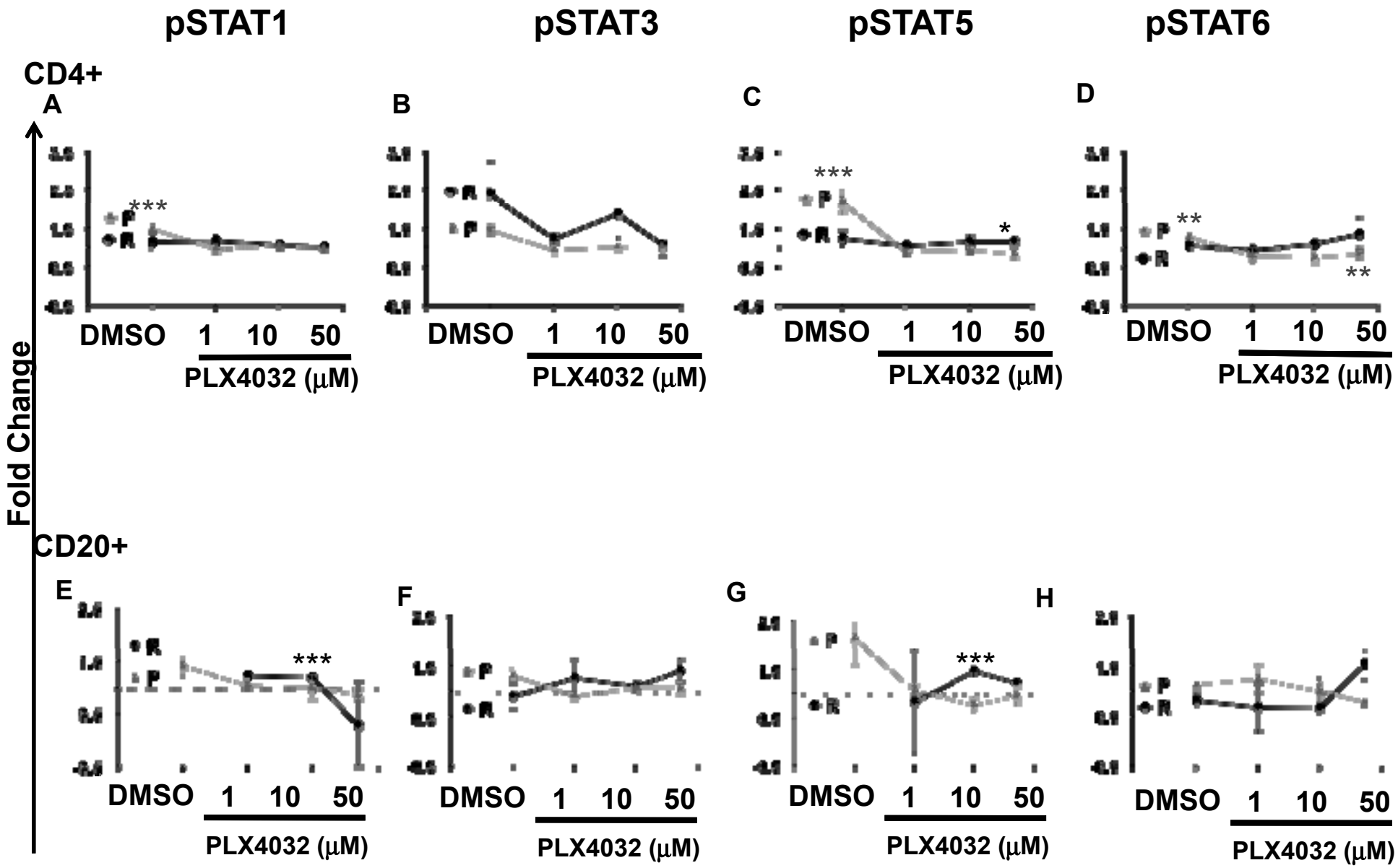
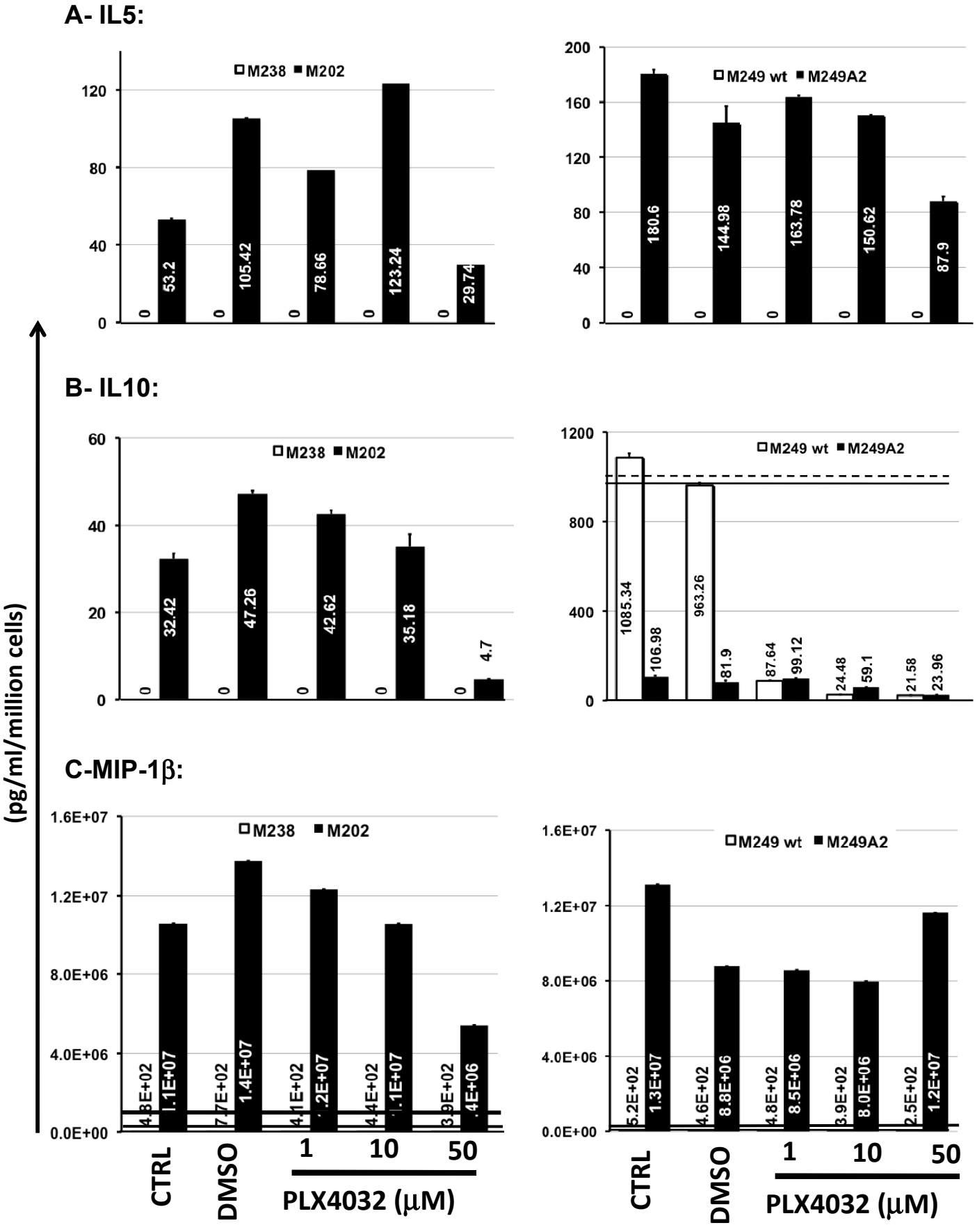


Supplemental Fig. 4.



Supplemental Fig. 5



Supplemental Figures

Supplemental Fig. 1. PBMC and melanoma cell line proliferation curves with increasing concentrations of PLX4032. A and B) $BRAF^{V600E}$ mutant melanoma cell lines M229 (A) and M233 (B); C to F) non-proliferating PBMC from three patients with metastatic melanoma GA17 (C), GA21 (D) and GA20 (E), and a healthy donor (F); G and H) proliferating PBMC from a patient with melanoma GA20-P (G) and a healthy donor H-P (H). Samples were treated with increasing concentrations of PLX4032 in a time course study up to 72 hours for non-proliferating PBMC and melanoma cells (x axis), and for up to 48 hours for proliferating PBMC. Data represent the mean ± s.e.m. of three independent experiments with triplicate samples for each condition.

Supplemental Fig. 2. Gating strategy and analysis of fluorescently barcoded cells used for phosphoflow analysis. Single cells were gated on lymphocytes by size criteria, CD3+ T cells, and then CD4+ and CD8+ cells. Four populations of cells, each one exposed to 0, 1, 10 or 50 μM of PLX4032 for PBMC, and 0, 0.1, 1, or 10 μM of PLX4032 for M233 and M229 for 24 hours, were analyzed simultaneously based on fluorescent dye barcoding with Ax350-NHS and Ax-750-NHS. Intracellular phosphoproteins were analyzed in each cell population by mean fluorescence intensity (MFI) after subtracting the background. DMSO-exposed samples (0.0005%) were analyzed in parallel.

Supplemental Fig. 3. Phosphorylation of cell signaling proteins in CD4+ and CD20+

cells in the presence of increasing concentrations of PLX4032 over 24 hour exposure. Fold change over control in phosphoprotein levels for p-ERK1/2, pAKT, and pP38 in CD4+ (top row) and CD20+ (bottom row). P= five day proliferating PBMC at day zero of the experiment. DMSO. Y-axis= percentage of fold change respect control. Points, means (n=3); bars, s.e.m.

Supplemental Fig. 4. Phosphorylation of STAT cell signaling proteins in CD4+ and CD20+ cells in the presence of increasing concentrations of PLX4032 over 24h exposure. Fold change over control in phosphoprotein levels for phosphorylated signal transducers and activators of transcription (STAT) proteins pSTAT1, pSTAT3, pSTAT5 and pSTAT6 in CD4+ (top row) and CD20+ (bottom row). X-axis: P (filled triangles) represent five day proliferating PBMC. R (filled circles) represent resting PBMC. DMSO (0.0005%). Y-axis= percentage of fold change compared to controls. Points, means (n=3); bars, s.e.m.

Supplemental Fig. 5. Antigen-specific IL-5, IL-10 and MIP-1β secretion by the MART-1 TCR transgenic T cells previously exposed to increasing concentrations of PLX4032 and stimulated with MHC and antigen-matched or -mismatched melanoma cells. The secretion of IL-5 (A), IL-10 (B) and MIP-1β (C) was quantitated in supernatants of co-cultures of MART-1-specific TCR transgenic lymphocytes and the MART-1+ human melanoma cell lines M202 (HLA-A*0210+, $BRAF^{V600E}$-, $NRAS^{Q61L}$+), M238 (HLA-A*0210-, $BRAF^{V600E}$+, $NRAS^{Q61L}$-), M249wt (HLA-A*0210-, $BRAF^{V600E}$+, $NRAS^{Q61L}$-), M249-A2.1 (HLA-A*0210+, $BRAF^{V600E}$+, $NRAS^{Q61L}$-). Columns represent the mean +/-

s.d. of duplicate samples. The horizontal red line in each plot represents the basal cytokine secretion of PBMC transduced cells; Y axis= pg/ml/million cells.