A βc + IL3Rα M246L P248L V249L   βc + IL3Rα WT

Time (min): 0 2 5 15 30 60   0 2 5 15 30 60

Src Family_P
p21 CIP/WAF1
p70 S6 Kinase_P
mTOR_P
YAP_P
Src_P
Cyclin D1
Cyclin E1
FAK_P
CDK2E
CDL6E
IKK a/b_P
CK2-alpha
Lck_P
p27 Kip1
Shc_P (Tyr239,240) ←
Shc_P (Tyr317)
Jak2_P ←
NF-kB p65_P
IkBa_P
Smad2_P; Smad3_P
SHP-2_P
Akt_P ←
p44/42 MAPK (Erk1/2)_P ←
Mcl-1
c-Myc
Stat1_P (Tyr701) ←
S6 Ribosomal Protein_P
p53_P
Stat5_P ←
Beta Catenin
ATM
Aurora A_P; Aurora B_P; Aurora C_P
Bad_P (Ser112)
Bad_P (Ser136)
Bax
Beta Catenin_P (Ser33, Ser37, Thr41)
Beta Catenin_P (Thr41, Ser45)
Caspase 3 cleaved
cdc2_P (Tyr15)
cdc2_P (Tyr15)
cdc25c_P
c-Jun_P (Ser73)
c-Jun_P (Ser73)
c-Met_P
Cyclin D1_P
Bcl-xL
Stat3_P
XIAP
Chk2

B

Untransduced, shCont, shSTAT1_1, shSTAT1_2, shSTAT1_3, shSTAT1_4

100 / 75 — STAT1
100 / 75 — STAT5
50 / 37 — Actin

C

Relative gene expression (shControl / shSTAT1_3)
P=0.0471 (Kit), *P=0.0496* (Mpl)
Kit, Flt3, Mpl

Relative gene expression (shControl / shSTAT1_4)
P=0.0391 (Kit), *P=0.0154* (Flt3), *P=0.0372* (Mpl)
Kit, Flt3, Mpl

D

Ann V-negative (%)
P=0.0173 *P=0.0380*
shCont, shSTAT1_3, shCont, shSTAT1_4

E

Day 2 — Sig DE: 743 (q<0.05, FC>2)
568 down reg   175 up reg
-log10(PValue) vs logFC

Day 5 — Sig DE: 1567 (q<0.05, FC>2)
764 down reg   803 up reg
-log10(PValue) vs logFC

F HALLMARK UNFOLDED PROTEIN RESPONSE
NES:1.89
q-value: 0.001
Hex up / Dod down   Hex down / Dod up

G NELSON EOSINOPHIL IL3 UP
NES: -1.818
q-value: 0.000
Hex up / Dod down   Hex down / Dod up

H HALLMARK INTERFERON ALPHA RESPONSE
NES: -2.389
q-value: 0.000
Hex up / Dod down   Hex down / Dod up

I HALLMARK INTERFERON GAMMA RESPONSE
NES: -2.268
q-value: 0.000
Hex up / Dod down   Hex down / Dod up

J STAT1 TARGET GENES UP
NES: -2.030
q-value: 0.000
Hex up / Dod down   Hex down / Dod up

Supplementary Fig. S4

Supplementary Figure S4. The IL3R dodecamer activates STAT1 to induce cell differentiation. A, Heat map representation of protein levels in lysates from FDH cells expressing βc and IL3Rα M246L/P248L/V249L or WT, after stimulation with 100 ng/mL IL3 for 0 to 60 minutes, as determined by RPPA. The levels of each protein are normalized to Time 0 for the respective cell line and presented using a red to blue (highest to lowest) color gradient. Targets indicated by arrows were validated subsequently with immunoblotting and shown in Fig. 4A and 4E. **B,** Representative immunoblot of STAT1 and STAT5 protein expression after independent STAT1 shRNA knockdown in FDH cells expressing βc and IL3Rα WT. **C,** Expression of genes associated with stem and progenitor cells in FDH cells expressing βc and IL3Rα WT transduced with lentivirus encoding Control (shCont) or STAT1 (shSTAT1_3 or shSTAT1_4) shRNA after treatment with IL3 for 2 days normalized to *RPLP0* expression (n=3-4). **D,** Survival of FDH cells expressing βc and IL3Rα WT assessed by %Annexin V (Ann V) negative staining after STAT1 shRNA knockdown and following IL3 withdrawal (n=3). **E,** Number of differentially expressed genes between cells expressing βc and IL3Rα P248L (IL3R hexamer) vs. βc and IL3Rα WT (IL3R dodecamer) after 2 and 5 days of IL3 treatment. q<0.05, fold change >2. **F,** GSEA showing gene sets associated with unfolded protein response are enriched in genes upregulated in cells expressing IL3R hexamer after 5 days of IL3 treatment. **G,** GSEA showing that expression of genes associated with IL3 responses in differentiated human eosinophils is downregulated in cells expressing IL3R hexamer. **H, I,** GSEA showing gene sets associated with the IFNα **(H)** and IFNγ response **(I)** are downregulated in FDH cells expressing IL3R hexamer after 5 days of IL3 treatment. **J,** GSEA plot showing negative enrichment of STAT1 target genes (55) in FDH cells expressing IL3R hexamer.