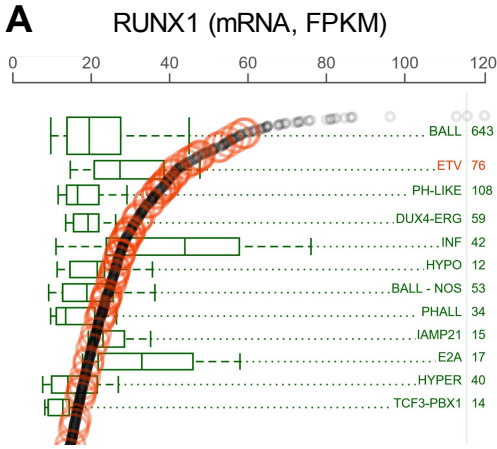
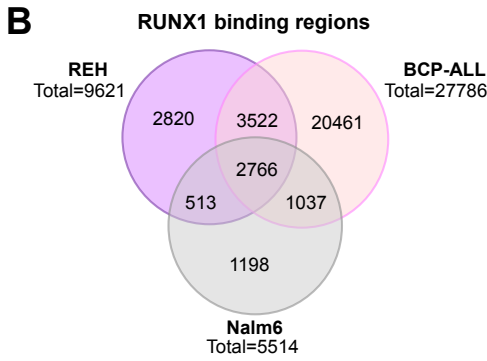
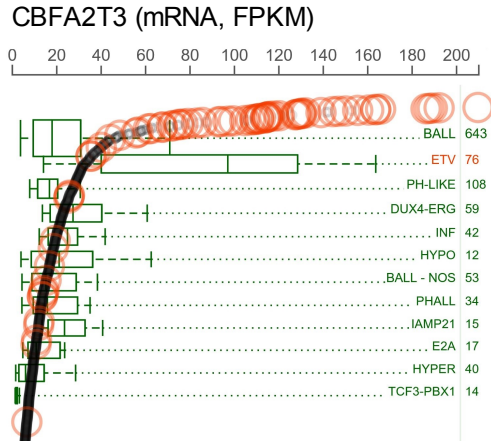


Figure S1

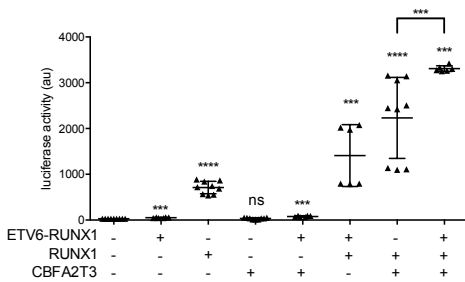
patients' cells



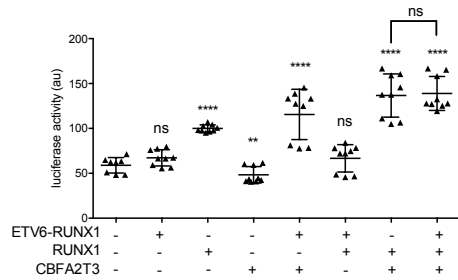
patients' cells



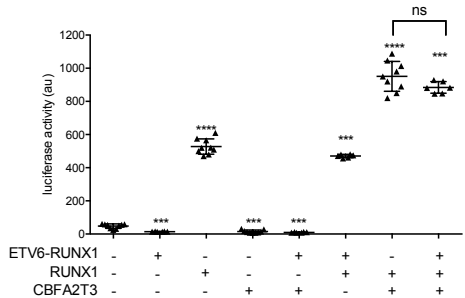
C RUNX1 promoter



D CBFA2T3 enhancer



E RUNX1-consensus motif repetition



F

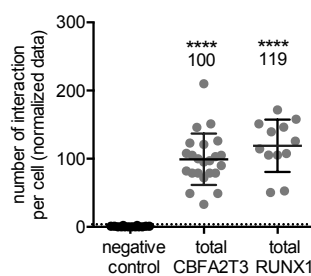


Figure S2

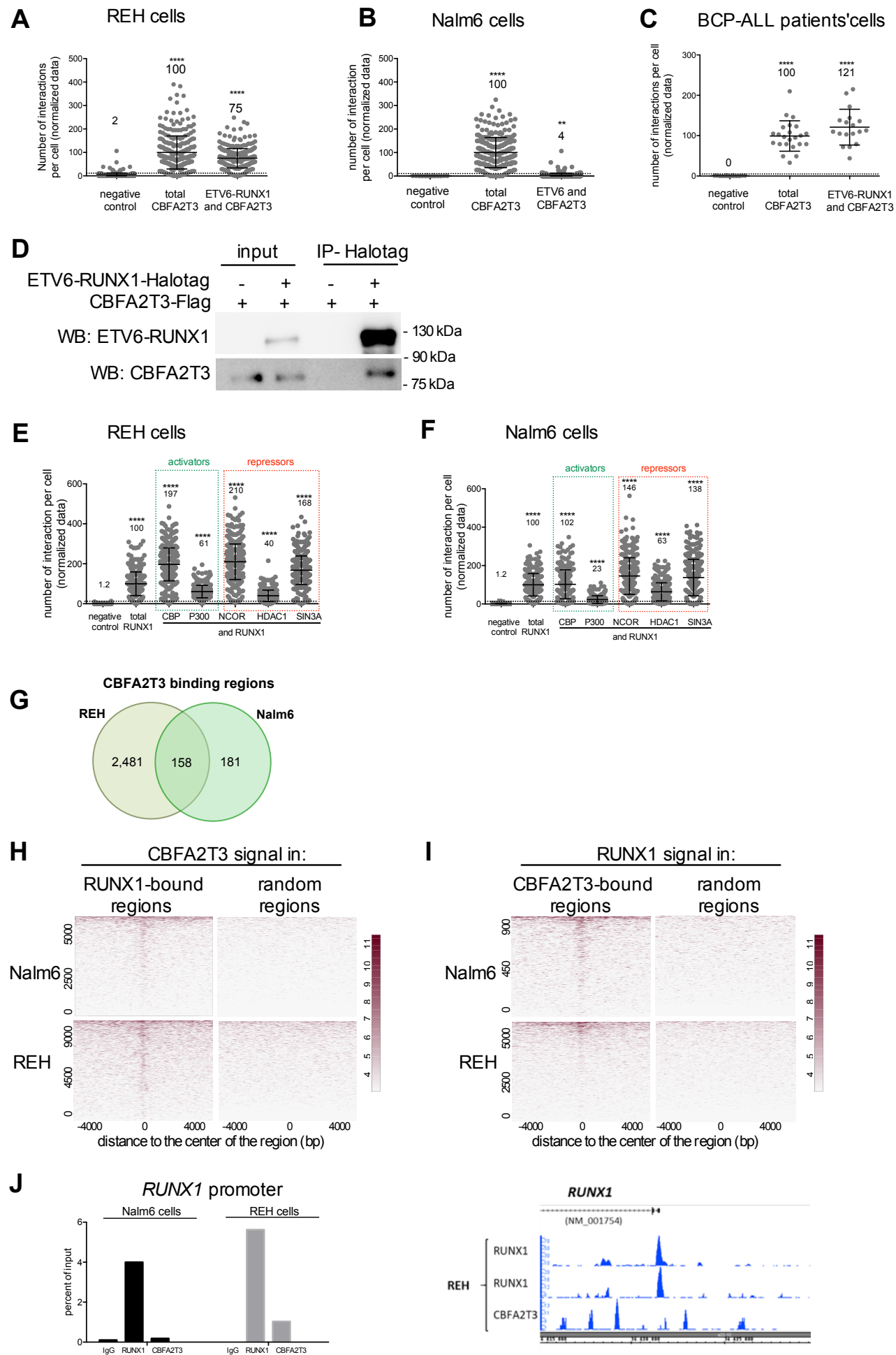
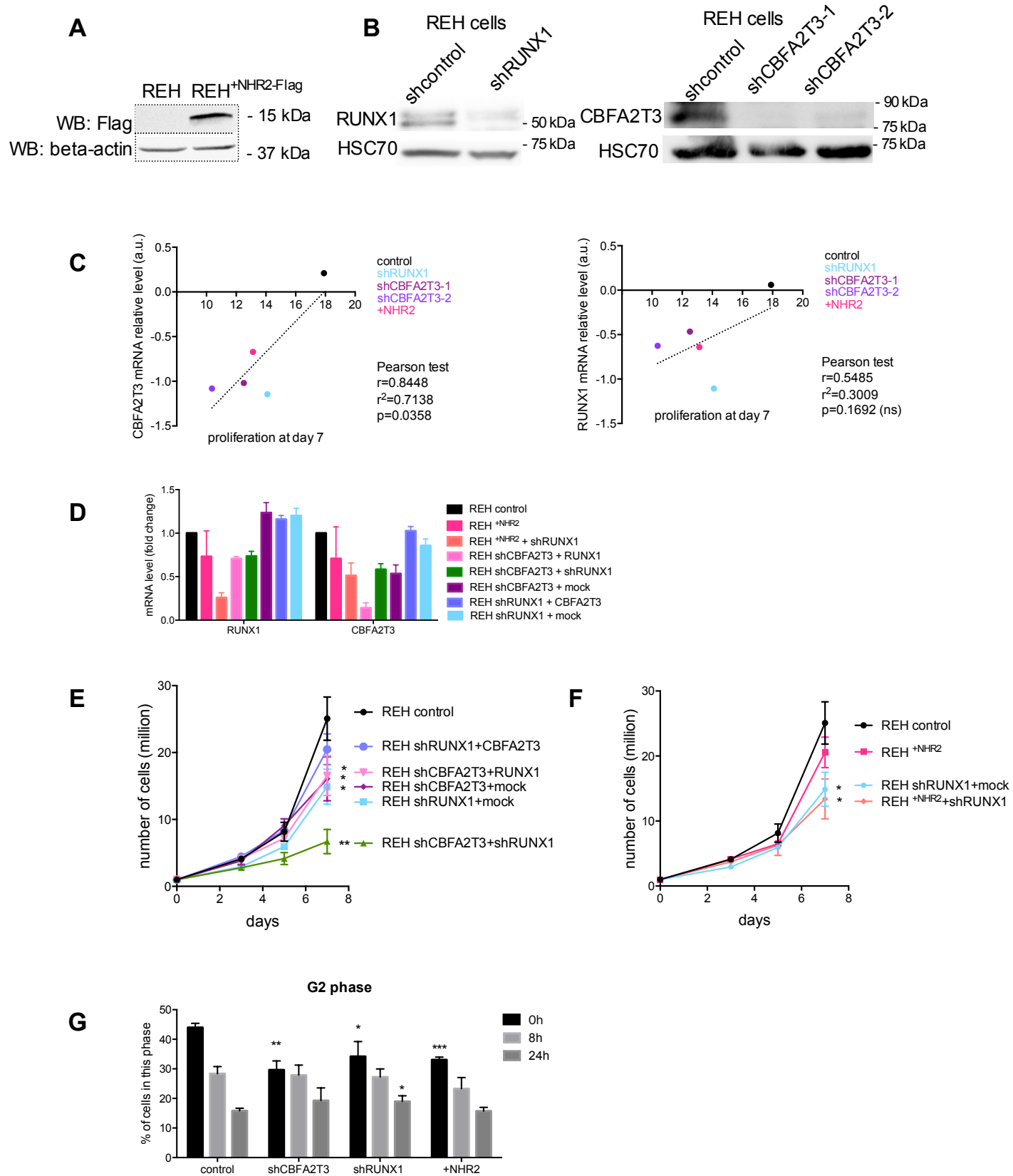


Figure S3



Supplemental Figure 1

Figure S1

A. Data of mRNA levels for *RUNX1* and *CBFA2T3* (expressed in Fragments Per Kilobase Million – FPKM) have been extracted from the St. Jude Children's Research Hospital RNA-Seq Pediatric Cancer Data Portal(8), and different subtypes of childhood BCP-ALL are shown. Data have been updated on June, 16th, 2020. BALL: B-cell Acute Lymphoblastic Leukemia, ETV: ETV6-RUNX1 BCP-ALL, PH-LIKE: Philadelphia chromosome-like BCP-ALL, INF: Infant BCP-ALL; HYPO: hypodiploid BCP-ALL, NOS: not otherwise specified, E2A: E2A-PBX1 BCP- ALL subtype, PHALL: BCR-ABL1 BCP-ALL, HYPER: Hyperdiploid BCP-ALL. Subtypes with less than 5 samples are not included.

B. Venn-diagrams presenting the intersection between RUNX1 ChIP-Seq binding regions in REH cells (common regions in both replicates), in Nalm6 cells (common regions in both replicates), and in bone marrow mononuclear cells isolated from 3 BCP-ALL patients. For the BCP-ALL patients, we considered only the common regions observed in 2 out of the 3 patients.

C-E. Luciferase assays with a plasmid containing the *RUNX1* promoter (C), *CBFA2T3* enhancer (D) and a repetition of RUNX1-consensus motif (E) upstream a minimal promoter and a luciferase ORF, in presence of ETV6-RUNX1, RUNX1 and CBFA2T3 expressing plasmids in HEK293T cells. Luciferase levels (Firefly luciferase/Renilla luciferase) are represented using a scatter dot plot indicating the means and S.D. ns: non-significant, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ in Mann-Whitney tests compared to the control situation (first left condition).

F. Quantitation of endogenous total RUNX1 and total CBFA2T3 proteins in BCP-ALL patient cells measured by PLA presented with the mean values $\pm$ S.D. A negative control is also included. The data are normalized against total CBFA2T3. The mean value is indicated above each plot. The positive threshold value is represented by the dotted line as described in (Debaize et al, molecular cytogenetics 2017). **** $p < 0.0001$ in Fisher's exact test compared to the negative control condition.

Supplemental Figure 2

Figure S2.

A-C: Quantitation of ETV6-RUNX1, ETV6 and CBFA2T3 protein co-localization per nucleus–visualized by PLA dots in REH cells (A), Nalm6 cells (B), and BCP-ALL patient cells (C) presented with the mean values $\pm$ S.D. Positive controls (total CBFA2T3) and negative controls were included. The data are normalized against total CBFA2T3. The mean value is indicated above each plot. The positive threshold value is represented by the dotted line as described in (Debaize et al, molecular cytogenetics 2017). ** $p < 0.01$, **** $p < 0.0001$ in Fisher's exact test compared to the negative control condition.

D: Co-immunoprecipitation (IP) using anti-HaloTag antibody in HEK293T cells expressing ETV6-RUNX1-HaloTag and CBFA2T3-Flag plasmids. Western blots were performed with RUNX1 and CBFA2T3 antibodies.

E-F: Quantitation of RUNX1 and various activators or repressor protein co-localization per nucleus—visualized by PLA dots in REH cells (A), and Nalm6 cells (B) presented with the mean values $\pm$ S.D. Positive controls (total RUNX1) and negative controls were included. The data are normalized against total RUNX1. The mean value is indicated above each plot. The positive threshold value is represented by the dotted line as described in (Debaize et al, molecular cytogenetics 2017).

G. Venn-diagrams presenting the intersection between CBFA2T3 ChIP-Seq binding regions in REH cells and in Nalm6 cells.

H,I. (H) heatmap of Chip-Seq CBFA2T3 signals into Chip-Seq RUNX1-bound regions or random regions in Nalm6 cells (top line) and in REH cells (bottom line). (I) heatmap of Chip-Seq RUNX1 signals into Chip-Seq CBFA2T3-bound regions or random regions in Nalm6 cells (top line) and in REH cells (bottom line).

J. left panel: Chromatin Immunoprecipitation-qPCR with IgG, RUNX1, CBFA2T3 and Histone H3 (positive control) antibodies in Nalm6 and REH cells on the *RUNX1* promoter. The results are expressed as percentage of the input. Right panel: ChIP-Seq profiles across the human *RUNX1* gene. Genomic tracks display ChIP-Seq profiles for RUNX1 and CBFA2T3 from REH cells. ChIP-Seq reads were aligned to the reference human genome version GRCh37 (hg19). The figure is centered on the *RUNX1* promoter.

Supplemental Figure 3

Figure S3.

A, B. Western blots of total protein extracts from REH control cells and REH expressing CBFA2T3^{NHR2}-Flag cells (REH^{+NHR2}) (A), REH shRUNX1 (B), REH shCBFA2T3-1 (B), REH shCBFA2T3-2 (B). Western blots were performed with Flag, RUNX1 and CBFA2T3 antibodies, and beta-actin or HSC70 for the loading control.

C. XY plots showing the relative mRNA level of *CBFA2T3* and *RUNX1* according to the proliferation rate expressed as the number of cells (expressed in million) at day 7 from the experiment of Figure 7 A and B. Each dot corresponds to the mean on the mRNA level and of the proliferation rate for each condition. A Pearson correlation test has been performed.

D. Quantification of *CBFA2T3* and *RUNX1* mRNA level in the different cell lines of (E) and (F).

E, F. Proliferation curves for REH^{shcontrol}, REH^{shRUNX1+mock}, REH^{shCBFA2T3+mock}, REH^{shCBFA2T3+shRUNX1}, REH^{shCBFA2T3+RUNX1}, REH^{shRUNX1+CBFA2T3} (E), and for REH^{shRUNX1+mock}, REH^{+NHR2} cells, REH^{shRUNX1+NHR2} cells (F). Three experiments are represented for each condition. For more readability, statistical analyses have been run only for the last day (day 7) and compared to the condition REH^{shcontrol}. * $p < 0.05$; ** $p < 0.005$.

G. Progression in G2 phase after 0, 8 and 24hours after nocodazole-block release in REH^{shcontrol}, REH^{shRUNX1} REH^{shCBFA2T3} REH^{+NHR2} cells. The results are expressed as the percentage of cells in G2-phase. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$.