SUMOylated Non-canonical Polycomb PRC1.6 Complex as a Prerequisite for Recruitment of Transcription Factor RBPJ

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Research

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Abstract

Background

Notch signaling controls cell fate decisions in many contexts during development and adult stem cell homeostasis and, when dysregulated, leads to carcinogenesis. The central transcription factor RBPJ assembles the Notch coactivator complex in the presence of Notch signalling, and represses Notch target gene expression in its absence.

Results

We identified L3MBTL2 and additional members of the non-canonical polycomb repressive PRC1.6 complex in DNA-bound RBPJ associated complexes and demonstrate that L3MBTL2 directly interacts with RBPJ. Depletion of RBPJ does not affect occupancy of PRC1.6 components at Notch target genes. Conversely, absence of L3MBTL2 reduces RBPJ occupancy at enhancers of Notch target genes. Since L3MBTL2 and additional members of the PRC1.6 are known to be SUMOylated, we investigated whether RBPJ uses SUMO-moieties as contact points. Indeed, we found that RBPJ binds to SUMO2/3 and that this interaction depends on a defined SUMO-interaction motif. Furthermore, we show that pharmacological inhibition of SUMOylation reduces RBPJ occupancy at Notch target genes.

Conclusions

We propose that the PRC1.6 complex and its conjugated SUMO-modifications provide a scaffold that is recognized by RBPJ and promotes its recruitment to Notch target genes.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

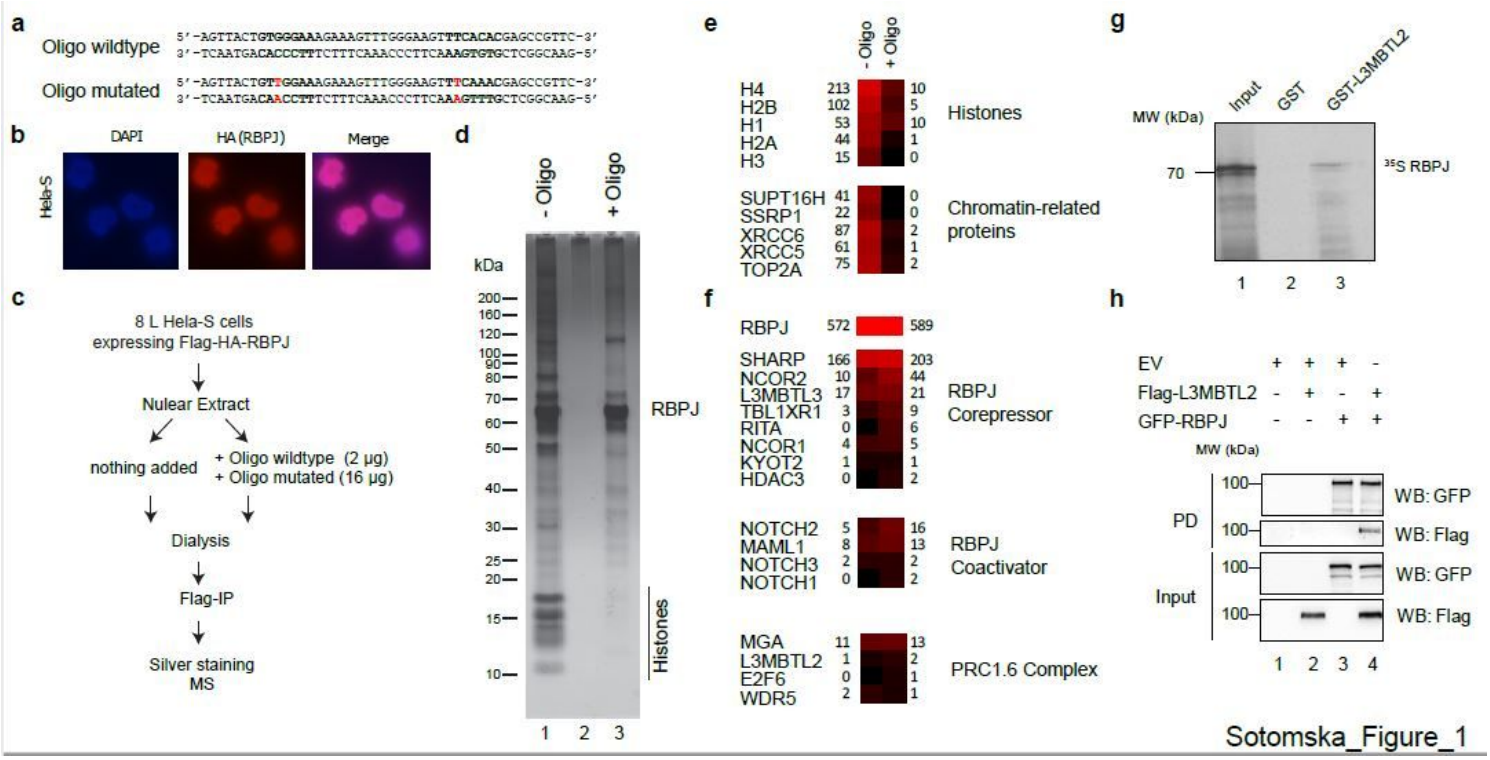

Figure 1

Oligonucleotide-assisted complex purification of RBPJ and validation of a direct RBPJ-L3MBTL2 interaction. (a) Oligonucleotides used to stabilize RBPJ complexes during purification. The sequence of the double-stranded oligo is based on the Hes1 promoter as described in (27). (b) Immunofluorescence of Flag-HA-tagged RBPJ expressed in HeLa-S cells. (c) Experimental outline of oligonucleotide-assisted complex purification of Flag-HA-RBPJ from HeLa-S cells. (d) Silver staining of purified RBPJ complexes, obtained in the presence and absence of the oligonucleotides. (e) Example proteins, that are strongly reduced in the RBPJ complex 550 purified in presence of the oligonucleotides. (f) Proteins associated with the RBPJ coactivator or corepressor complexes. Components of the PRC1.6 are putative novel RBPJ associated proteins. The numbers in (e) and (f) indicate the total peptide numbers identified by mass-spectrometry (see also Additional file 1: Table S1). (g) GST pulldown assays were performed with GST-L3MBTL2 or GST only and [35S] methionine-labelled RBPJ. Bound proteins were separated in SDS-PAGE and visualized by autoradiography. (h) Co-Immunoprecipitation experiments were performed using Flag-L3MBTL2 and GFP RBPJ overexpressed in HEK293T cells. GFP-RBPJ and Flag-L3MBTL2 were expressed in 293T cells. Lysates were subjected for GFP immunoprecipitation, followed by Western blotting. Control cells were transfected with pcDNA GFP plasmid.

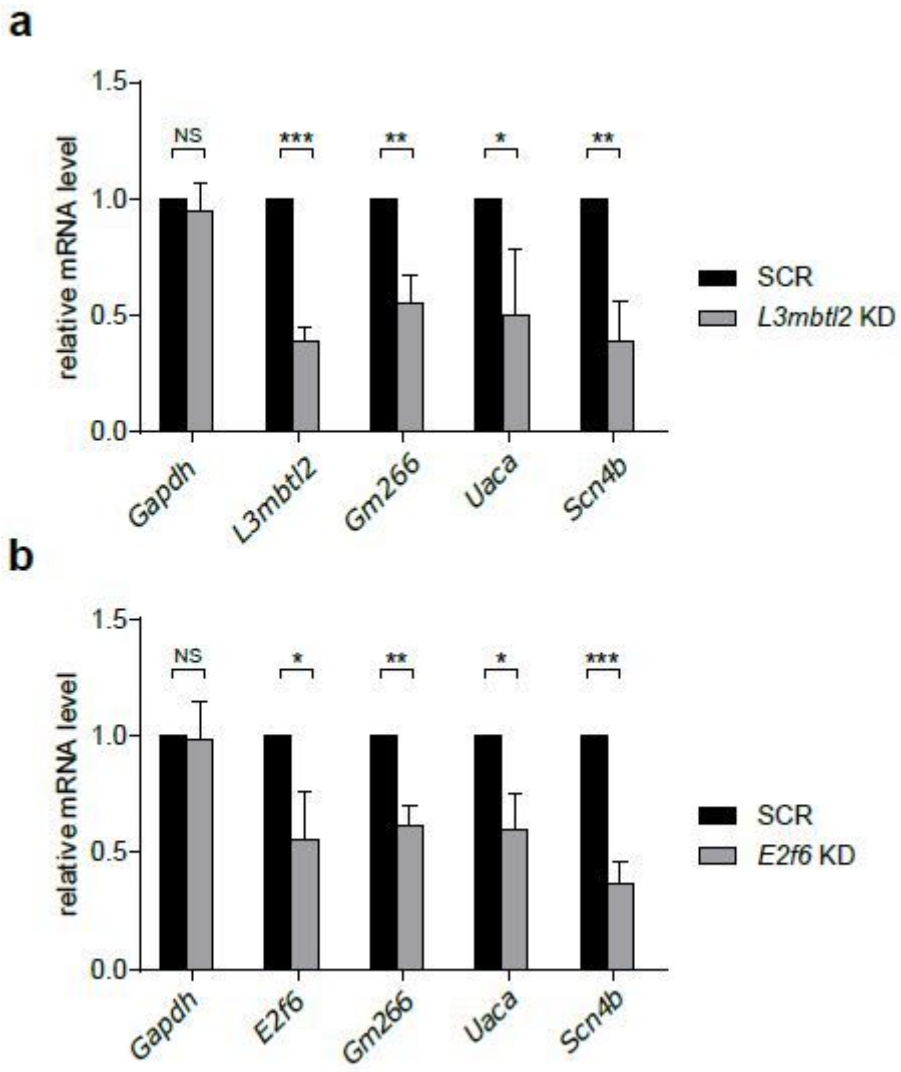

Figure 2

L3mbtl2 and E2f6 silencing functionally affects Notch target genes in mouse leukemia pre-T cells. (a) Beko cells were lentivirally infected with indicated shRNAs targeting L3mbtl2 or (b) E2f6 gene. 48 hours after the last infection, cells were selected with puromycin. Indicated mRNA levels were measured by quantitative real time PCR. Data was normalised to Hprt, Gapdh served as a control. (*P < 0.05, **P < 0.01, ***P < 0.001, [NS] not significant, unpaired Student's t-test)

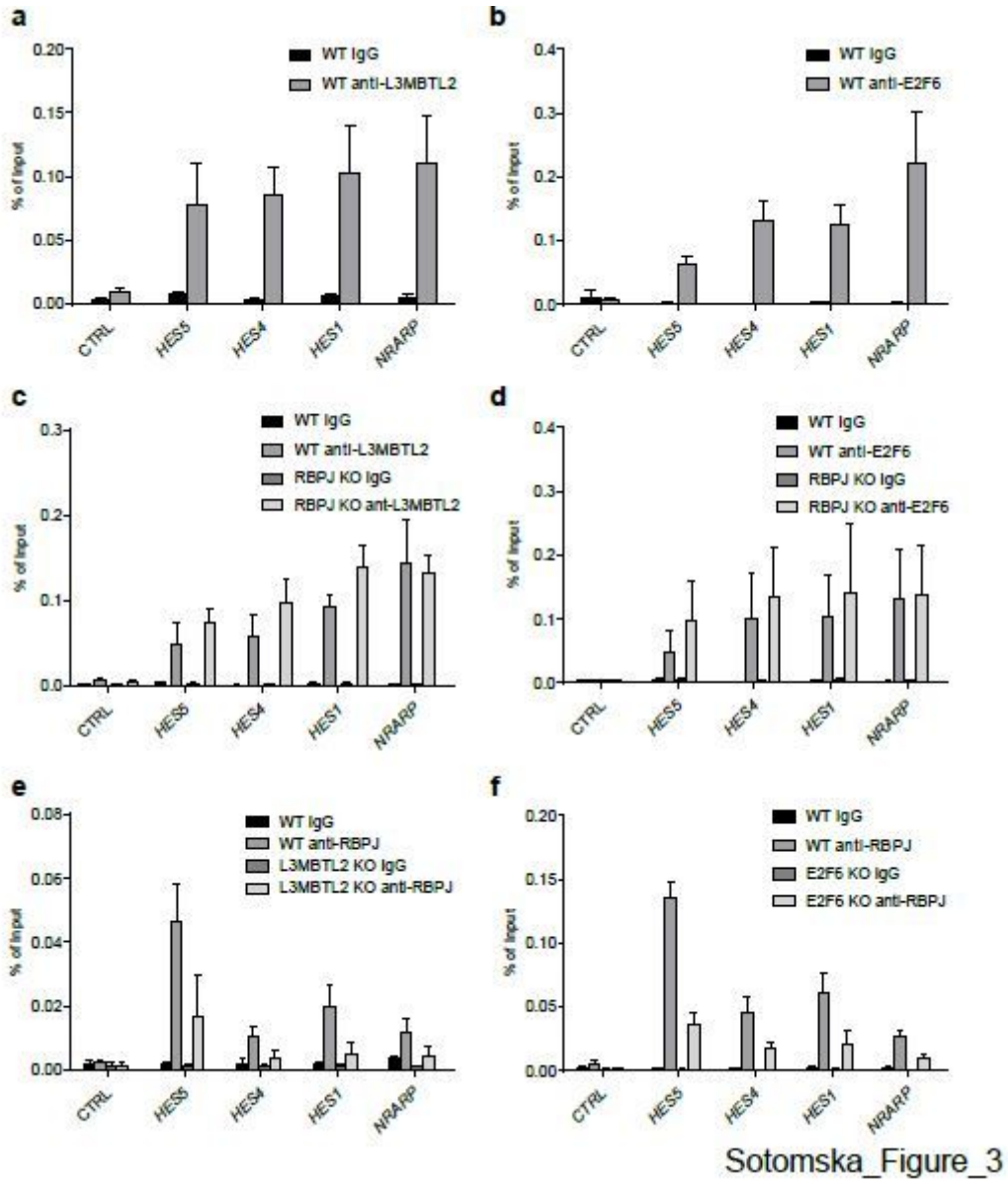

Figure 3

Changes in RBPJ binding depend on the presence of L3MBTL2 and E2F6. (a) ChIP-qPCR experiments showing binding of L3MBTL2 to regulatory elements of Notch target genes in HEK 293 cells. CDC7-2Kb served as a negative control (CTRL). (b) ChIP-qPCR experiments showing binding of E2F6 to regulatory elements of Notch target genes in HEK 293 cells. Gene Desert served as a negative control (CTRL). The mean of at least three technical replicates +/- SD. (c) ChIP-qPCR analysis of L3MBTL2 binding at regulatory elements of Notch target genes in CRISPR/Cas9 mediated RBPJ depleted cells. (d) ChIP-qPCR analysis 575 of E2F6 binding in CRISPR/Cas9 mediated RBPJ depleted cells in comparison with control cells. CDC7-2Kb served as a negative control (CTRL) (e) ChIP-qPCR analysis of RBPJ binding at regulatory elements of Notch target genes in L3MBTL2 KO and (f) E2F6 KO cells in comparison with the control cells. Gene Desert served as a negative control (CTRL). The mean of at least three independent biological replicates +/- SD.

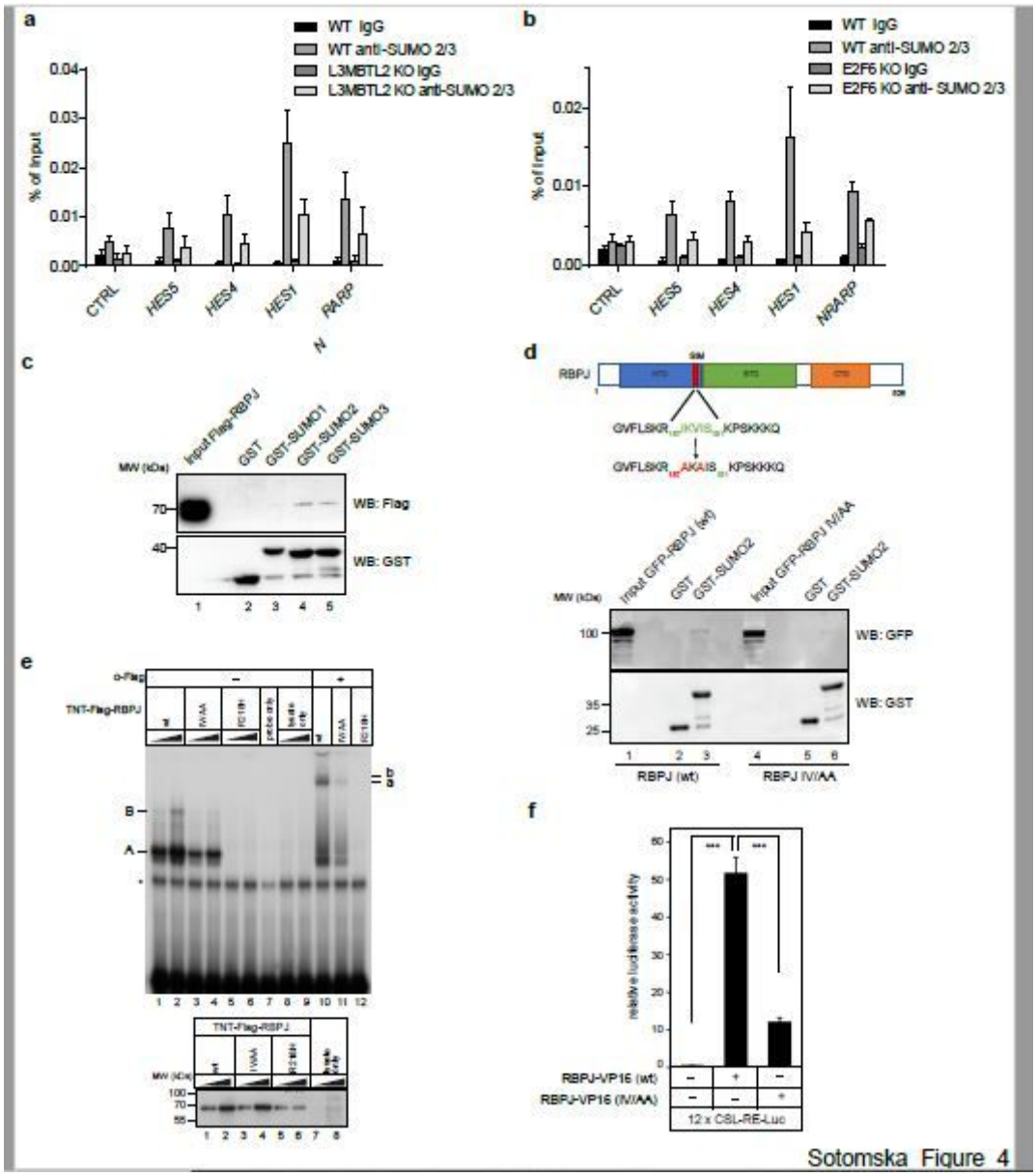

Figure 4

SUMO moieties are found at Notch target genes and are bound non covalently by RBPJ. (a) ChIP qPCR analysis of SUMO2/3 enrichment at regulatory elements of Notch target genes in HEK293 L3MBTL2 KO or (b) E2F6 KO cells. Gene Desert served as a negative control (CTRL). The mean of at least three independent biological replicates ±SD. (c) GST-SUMO1, GST-SUMO2 and GST-SUMO3 fusion proteins were expressed in bacteria and purified. HEK 293T cells were transiently transfected with Flag-RBPJ and whole cell extract was incubated with GST fusion proteins immobilized on sepharose beads. Flag-RBPJ binds non-covalently to GST SUMO2 and GST-SUMO3 but not to GST-only. (d, upper panel) Schematic representation of the wild type and the mutated SIM of RBPJ. (d, lower panel) GST SUMO2 fusion protein was expressed in bacteria and purified. HEK 293T cells were transiently transfected with GFP-RBPJ wild type or GFP-RBPJ IV/AA mutant and whole cell extracts were incubated with GST fusion protein immobilized on sepharose beads. (e, upper panel) Electrophoretic Mobility Shift Assay (EMSA) analysis of RBPJ wt and RBPJ IV/AA mutant binding to DNA. Oligomeric duplex DNA probe with RBPJ binding

sites (bold): 5'-CCT GGA ACT ATT TTC CAC GGT GCC CTT CCG CCC ATT TTC CCA CGA GTC G-3'. DNA-protein complexes are indicated as A and B. Supershifted complexes after addition of Flag antibodies are indicated by a and b. The asterisk indicates a nonspecific background band. (e, lower) Western blot showing the in vitro translated Flag-RBPJ proteins used in the EMSA. (g) Transactivation capacities of RBPJ-VP16 fusion proteins. Hela cells were cotransfected with either RBPJ-VP16 wt or RBPJ-VP16 IV/AA mutant together with 12 x CSL-RE-Luc reporter construct containing 12 RBPJ DNA binding sites upstream of the luciferase gene. The mean of at least four independent biological replicates +/- SD is shown (***, p<0.0001, unpaired students T-test).

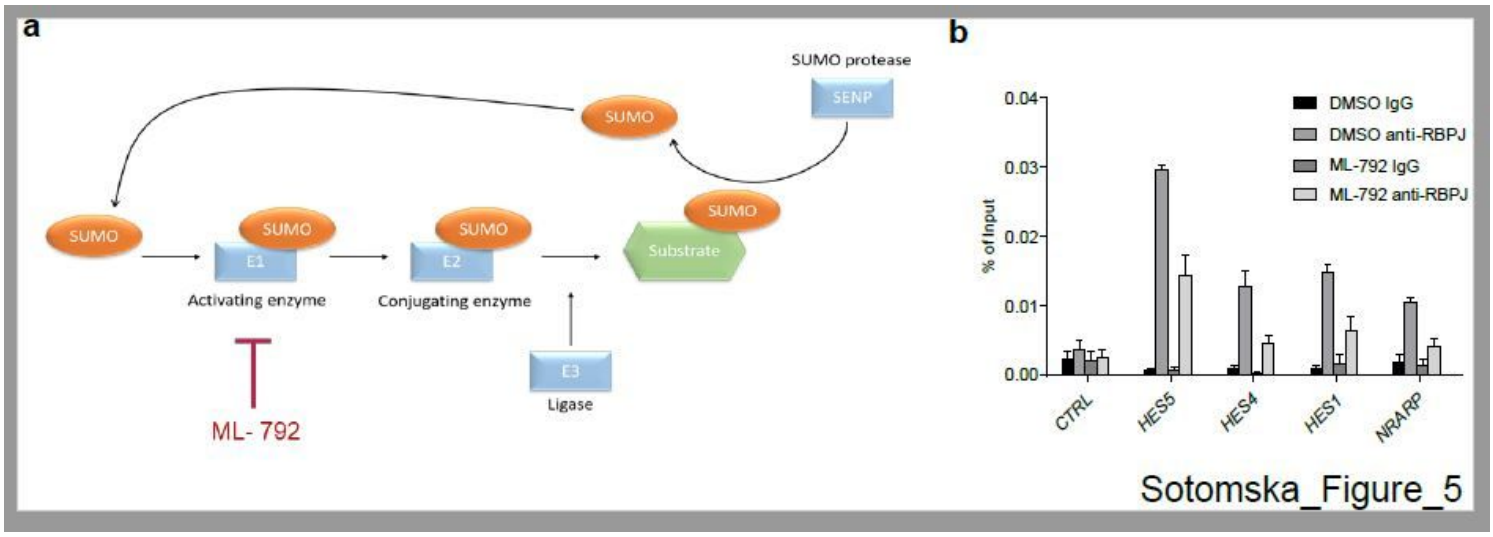

Figure 5

RBPJ recruitment is impaired upon SUMO inhibition. (a) Schematic representation of the SUMO pathway. SUMO after establishing ATP dependent thioester bond with heterodimeric Aos1/Uba2 SUMO activating E1 enzyme (SAE), is transferred to E2 enzyme (Ubc9) and subsequently bound to the substrate by E3 SUMO ligase. ML-792 selectively blocks E1 (SAE). (b) ChIP qPCR analysis of endogenous RBPJ enrichment at regulatory elements of Notch target genes in HEK293 upon 24h treatment with 10μM ML-792 or the vehicle. Gene Desert served as a negative control (CTRL). The mean of at least three independent biological replicates ±SD.

Supplementary Files

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- SotomskaAdditionalfile1TableS1.xlsx
- SotomskaAdditionalfile3TableS2.xlsx