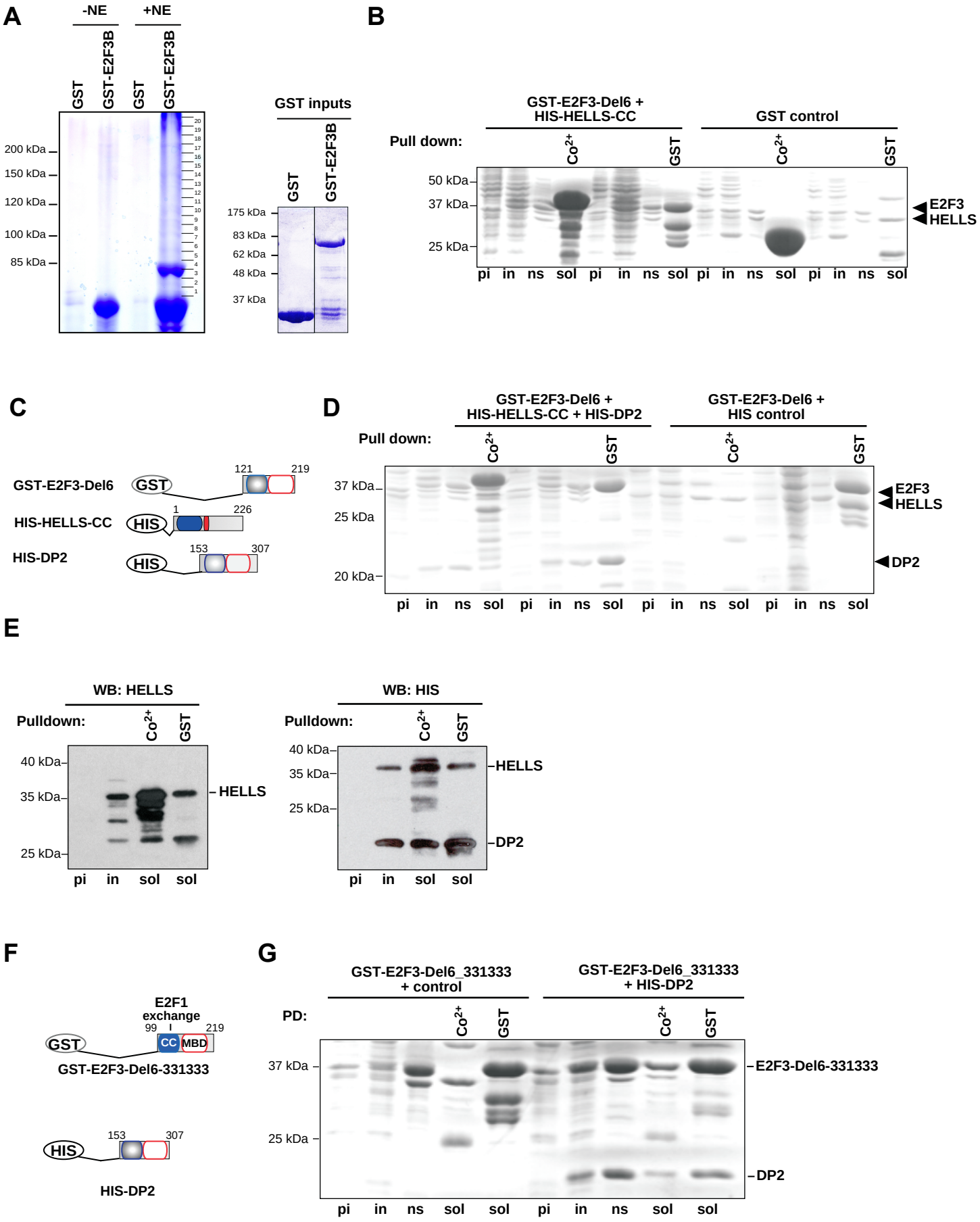
von Eyss *et al*, Supplementary Figure S1



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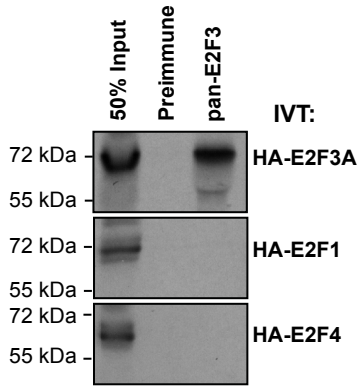
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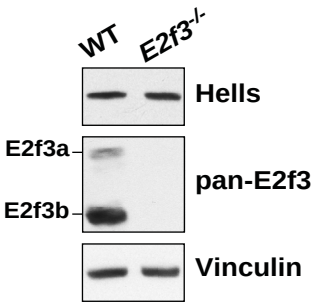
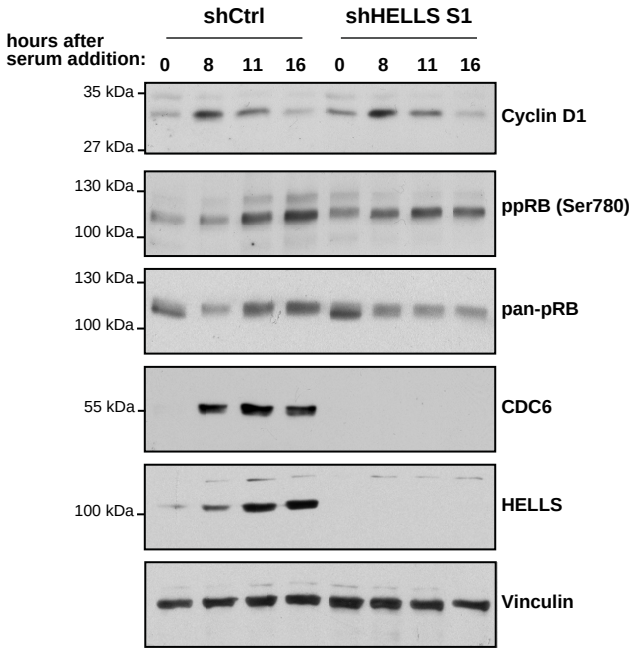
IVT:
72 kDa
55 kDa
HA-E2F3A
72 kDa
55 kDa
HA-E2F1
72 kDa
55 kDa
HA-E2F4

B


WT
E2f3-/-
Hells
E2f3a
E2f3b
pan-E2f3
Vinculin

von Eyss *et al*, Supplementary Figure S3

A

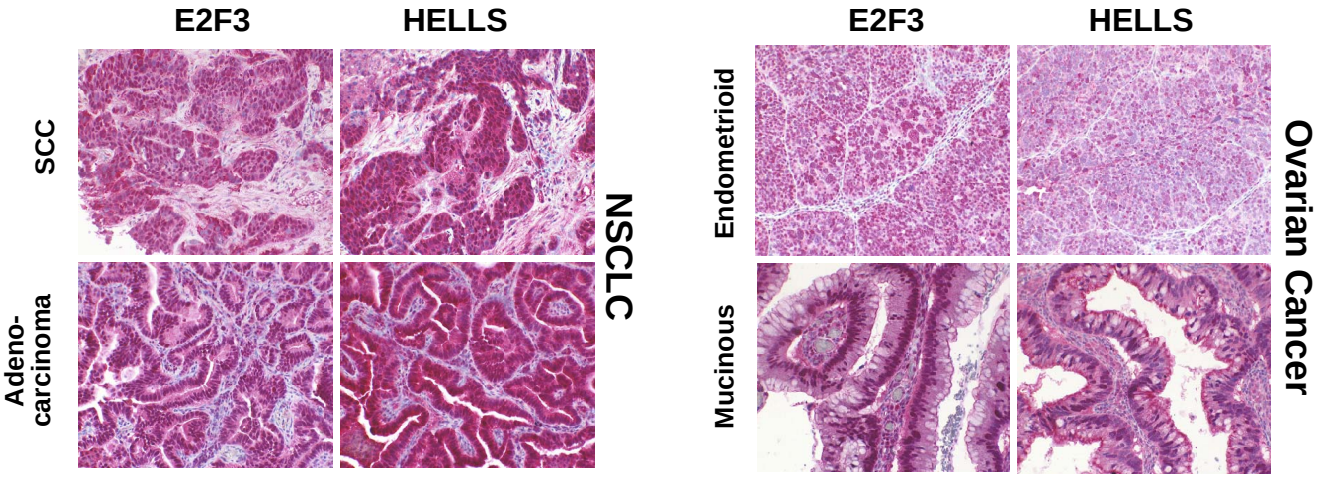


von Eyss *et al*, Supplementary Figure S4

A

Tumor Subtype:	Tumor staining positive for E2F3		Tumor staining positive for HELLS	
Non-small cell lung carcinoma	13 of 15	86,67%	13 of 15	86,67%
Small cell lung carcinoma	12 of 12	100%	12 of 12	100%
Retinoblastoma	6 of 6	100%	4 of 6	67%
Transitional cell carcinoma (bladder)	10 of 10	100%	10 of 10	100%
Ovarian Cancer	12 of 17	70,59%	13 of 17	76,47%

B



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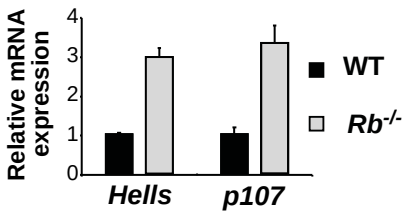

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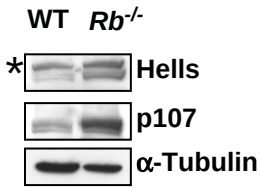

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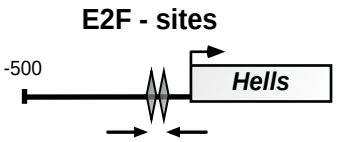

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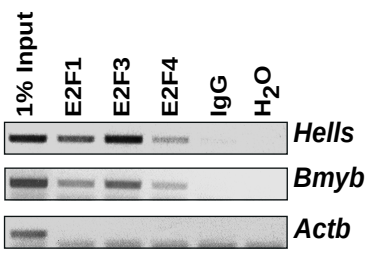

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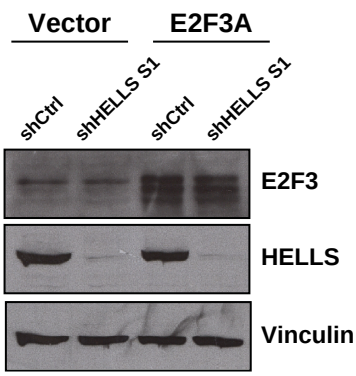
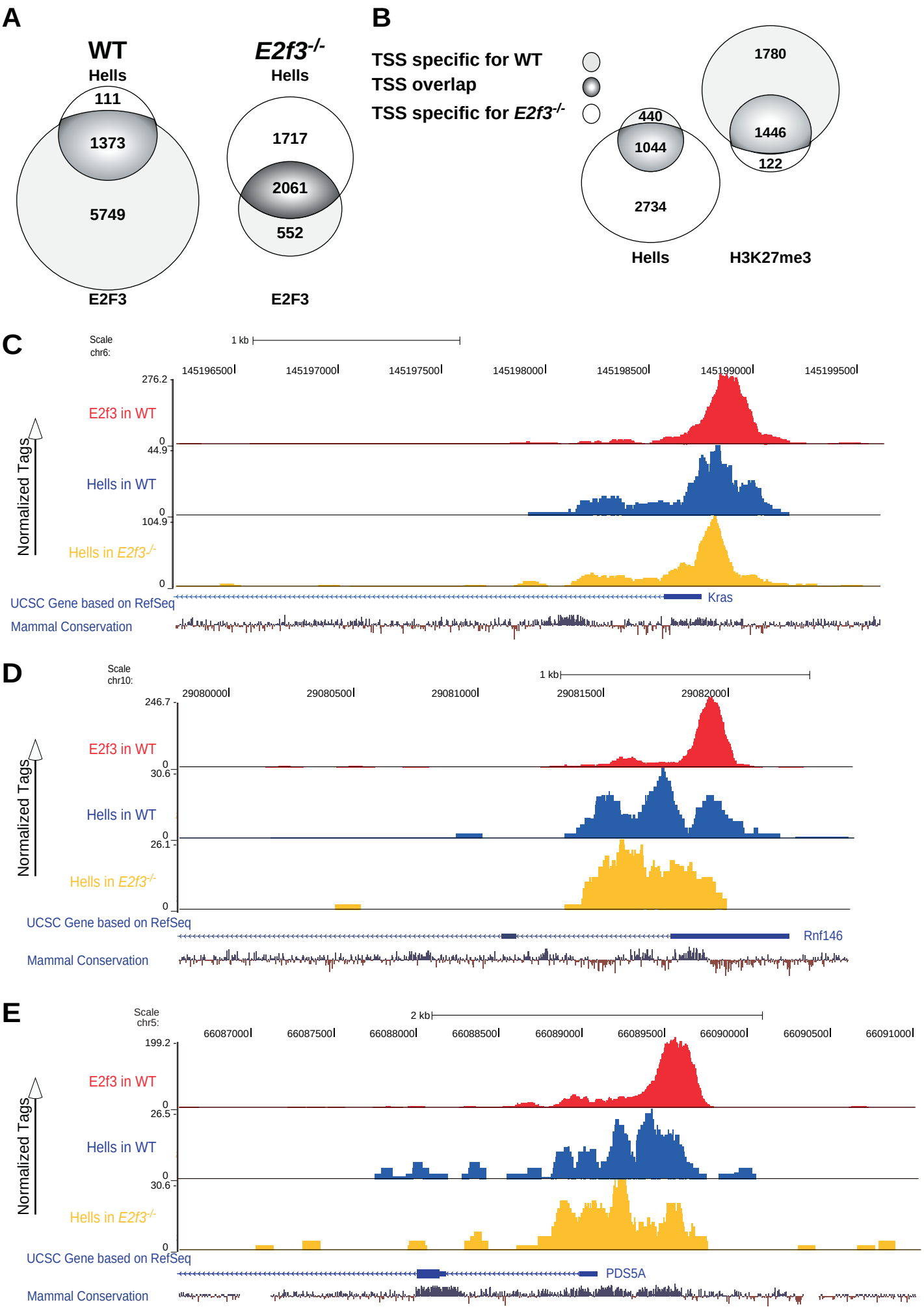
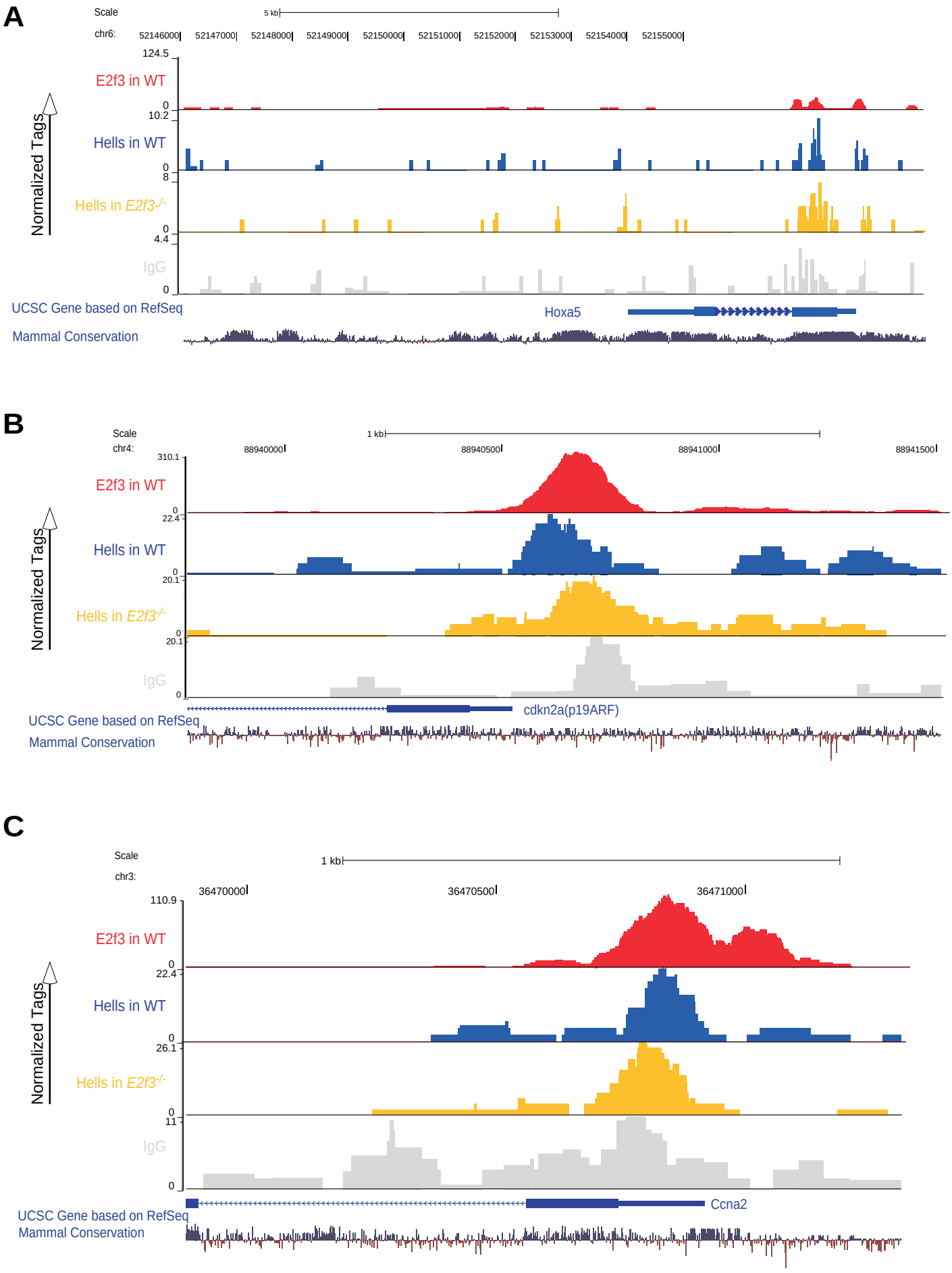


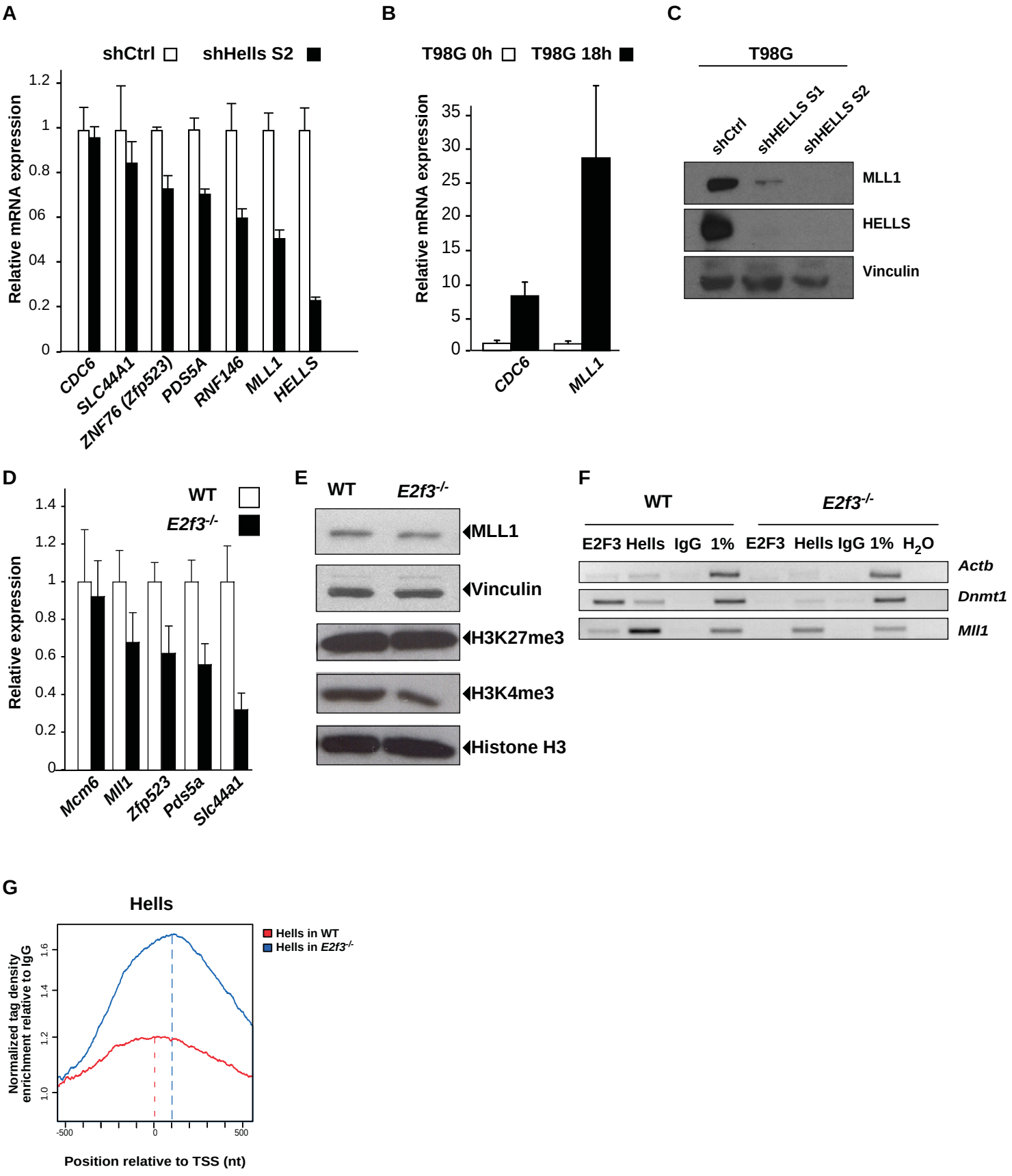
von Eyss *et al*, Supplementary Figure S5



von Eyss *et al*, Supplementary Figure S6

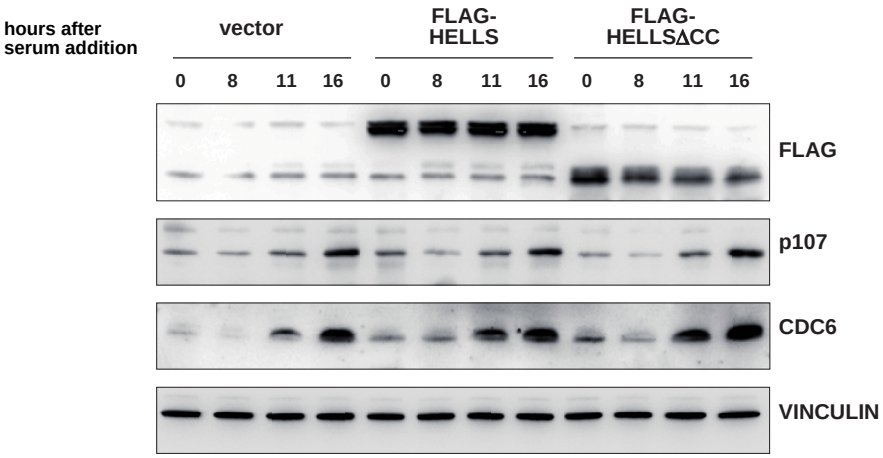


von Eyss *et al*, Supplementary Figure S7



von Eyss *et al*, Supplementary Figure S8

A



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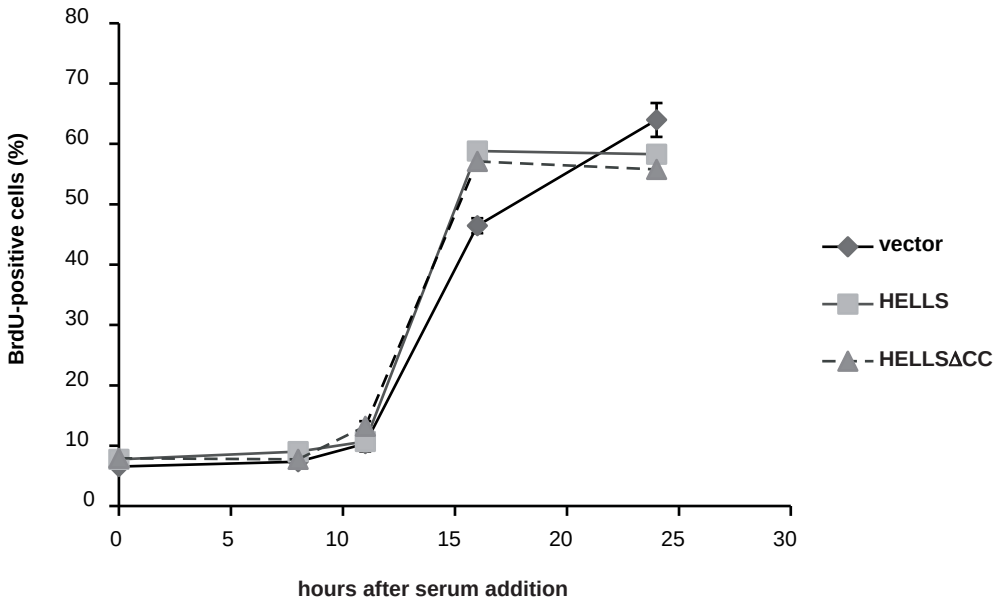

Supplemental Figure Legends

Supplemental Figure S1.

The interaction of HELLS with E2F3 was identified via mass spectrometry and it is specified via the coiled-coil domains of each protein. (A) GST-E2F3B or GST control extract was incubated without (-NE) or with (+NE) nuclear extracts prepared from HCT116 cells. Protein complexes were separated by SDS-PAGE and analyzed by Coomassie staining. The GST-E2F3B lane was sliced into 20 pieces and subjected to mass spectrometry. The GST-E2F3B Protein is marked with an asterisk (left). The equal loading of GST-inputs is depicted by a Coomassie staining (right). (**B)** E2F3-Del6 and HELLS-CC or GST-control only, were co-expressed from a singular vector. E2F3:HELLS are capable to interact *in vitro*. Various bacterial extracts containing GST-E2F3-Del6 and HIS-HELSS-CC were separated via SDS-PAGE and stained with Coomassie before induction (pi), after IPTG induction (in), the non-soluble protein fraction (ns) as well as the soluble fractions (sol). The latter fractions were subjected to pull downs using either metal affinity chromatography resin (Co^{2+}) or GST-beads (GST). No specific bands are visible in the GST control precipitates. **(C**-**E**) E2F3-Del6, HELLS-CC and DP2 co-expressed from a singular vector are capable to interact *in vitro*. **(C**) Schematic drawing of the constructs used. (**D**) Various bacterial extracts containing GST-E2F3-Del6, HIS-HELLS-CC and HIS-DP2 or alternatively GST-E2F3-Del6 alone were separated via SDS-PAGE and stained with Coomassie before induction (pi), after IPTG induction (in), the non-soluble protein fraction (ns) as well as the soluble fractions (sol). The latter fractions were subjected to pull downs using either metal affinity chromatography resin (Co^{2+}) or GST-

beads (GST). The individual proteins are marked with an arrow head. (**E**) The presence of HIS-HELLS and HI-DP2 in the direct (Co^{2+}) or the indirect (GST) pulldowns was identified using antibodies that are either HELLS-specific (left) or HIS-specific (right).

Supplemental Figure S2.

The HELLS:E2F3 interaction is specific (**A)** IVT assays were used to verify that the pan-E2F3 antibody that was raised against the full-length E2F3B in rabbits was specific to E2F3A/B and did not recognize E2F1 or E2F4. The IVT-HA-E2F1, -E2F3A or -E2F4 proteins were ^{35}S-Methionine labeled and immunoprecipitated using preimmune serum or pan-E2F3 antibody. The precipitates were separated via SDS-PAGE with subsequent autoradiography. (**B**) The protein expression of *Hells* is not changed in *E2f3*$^{-/-}$ as compared to wildtype (WT) mouse embryonic fibroblasts, as analyzed by western blots. The expression of both E2f3-isoforms is undetectable in *E2f3*$^{-/-}$ compared to WT MEFs. Vinculin served as a loading control.

Supplemental Figure S3.

HELLS depletion does not alter the pRB G0/G1 checkpoint. (**A**) T98G cells were treated as detailed (Figure 3) and the induction of Cyclin D1 and the Cyclin D1-dependent phosphorylation site Serine 780 in pRB was assayed by western blot in addition to pan-pRB, CDC6 and HELLS. Vinculin served as a loading control.

Supplemental Figure S4.

The impact of elevated HELLS and E2F3 expression on the development of human tumours. In many specific human cancer types a strong correlation between HELLS and E2F3 expression can be found. (**A-B**) The expression of E2F3 or HELLS was assessed in tumours using human tumour biopsies cut to semi-thin paraffin sections and specifically stained using antibodies against pan-E2F3 or HELLS. The analyzed subgroups of cancers are: non-small cell lung cancer, small cell lung cancer, retinoblastoma, transitional cell carcinoma and ovarian cancer. (**A**) Combined results for positive staining for E2F3 and HELLS in all cancers that were not further subclassed, due to the fact that fewer specimen were available. (**B**) Examples of immunhistochemistry (IHC) using specific antibodies against either E2F3 or HELLS of human tumour paraffin sections (secondary antibody coupled to peroxidase, developed with Fast Red; nuclear hematoxylin stained in blue). On the left side examples of non-small cell lung cancer (NSCLC) IHCs are shown. Two types of NSCLC are presented: the squamous cell carcinoma (SCC) and the adenocarcinoma with strong expression of either protein in epithelia structures. On the right side, examples of ovarian cancer IHCs for HELLS and E2F3 are shown. Two specific cancer types are presented: the endometriod and the mucinous ovarian cancer. The endometriod showed a less strong expression of either protein compared to mucinous in epithelia structures. (**C**) The RNA expression of murine HELLS (*Hells)* is elevated in pRB-deficient ($Rb^{-/-}$) MEFs, as examined by quantitative real time PCR. The *Hells* upregulation in $Rb^{-/-}$ MEFs is comparable to the known E2f-target *p107*. The expression of the RNA was normalized to β-actin (*Actb)* and the RNA in WT-cells was set to 1. (**D**) The protein expression of *Hells* is elevated in in $Rb^{-/-}$ MEFs, as analyzed by western blots. Again, the upregulation of Hells was compared to p107. The star denotes a non-specific band. α-tubulin served as a loading

control. (**E**) Schematic drawing of the *Hells* promoter, including the transcriptional start site (arrow) and *in silico* mapped E2F-sites (rectangles). (**F**) Verification of *Hells* as an E2F-target using chromatin immunoprecipitation analysis in WT MEFs using antibodies for E2F1, E2F3, E2F4 or control serum (IgG). The *Bmyb* promoter served as a positive E2F-target and the *β-Actin* (Actnb) promoter as a negative control. The water (H_2O) control did not contain DNA. (**G**) The tumour propelling E2F3-activity depends on HELLS. The DU145 PCA line was infected with a retrovirus containing E2F3A or with a control virus (Vector) and assessed for soft-agar growth (Figure 5E-F). Both ensuing lines were infected with lentiviral shHELLS S1 or shCtrl. The E2F3 over-expression and HELLS knockdown was verified by a confirmatory western blot with antibodies against E2F3 and HELLS. Vinculin served as a loading control.

Supplemental Figure S5.

Overlap between Hells and E2f3 bound genes on the genome-wide level. (**A**) Venn diagram showing vast overlap of Hells bound promoters with the promoters bound by E2f3 identified by ChIP-Seq using anti-Hells or anti-E2f3 and IgG in wildtype and *E2f3*$^{-/-}$ mouse embryonic fibroblasts; MEFs (see Experimental Procedures). Promoters are counted as targets if their TSS is within 1kb distance of enriched regions' summits in the ChIP-Seq libraries. There is a significant reduction of E2f3-bound targets and there appears to be a slight increase of HELLS-bound targets *E2f3*$^{-/-}$ MEFs. The data sets are included in Supplementary Tables S2 and S3. (**B**) Venn diagram showing overlap of Hells bound promoters in the absence or presence of E2f3. The number of H3K27me3 enriched TSS are reduced in the absence of E2f3. (**C-E**) Hells and E2f3 are highly enriched at TSS of many

putative target genes. Chromatin enrichment of E2f3 and Hells shown in screenshots from the UCSC genome browser displaying number of ChIP-Seq normalized tags (per 10 million mapped tags) mapping to the genomic contexts of (**C**) *Kras*, (**D**) *RNF146* and (**E**) *PDS5A*. These three genes are among the significantly enriched targets of HELLS according to the MACS $10\log_{10}$(p-value) score. Depicted are E2f3 or Hells enrichment in Wild-type (WT) MEFs in addition to Hells in *E2f3*$^{-/-}$ MEFs. All views show the genomic region of the genes to be enriched for E2F3 and Hells predominantly in the regions close to their TSS.

Supplemental Figure S6.

Hells and E2f3 are also found at other target genes by conventional ChIP and also in the tag density representation. Chromatin enrichment of E2f3, Hells and IgG in wildytpe MEFs shown in screenshots from the UCSC genome browser displaying number of tags mapping to the genomic contexts of (**A**) *Hoxa5*, (**B**) *cdkn2a/p19ARF* and (**C**) *Ccna2*. Depicted are E2f3 or Hells enriched promoters in Wild-type (WT) MEFs. In addition Hells enriched targets are also shown in *E2f3*$^{-/-}$ MEFs. The IgG shows also binding to the regions surrounding the start sites, creating "background". Such promoters are likely to be called insignificant by MACS. All views show the genomic region of the genes to be enriched for Hells predominantly in the regions close to their TSS. The Hells positioning in the mutant condition is visibly shifted as compared to the one in WT cells.

Supplemental Figure S7.

MLL1 is a cell-cycle regulated gene which needs E2F3 (and Hells) for proper activation. (**A**) Quantitative real time PCR (qRT-PCR) analysis depicting the relative expression of representative HELLS-target genes (Supplementary Tables S2-S3) in human T98G glioblastoma cells after lentiviral depletion of HELLS. *CDC6* served as control. Note that the human homolog of murine *Zfp523* is termed *ZNF76*. (**B**) *MLL1* is induced in the S-phase. *MLL1* was analyzed by qRT-PCR in synchronized S-phase T98G cells, which were serum-arrested and entered the S-phase at 18 hours after serum addition. *CDC6* is a S-phase specific control. (**C**) MLL1 expression depends on HELLS. Effects of depleting HELLS determined by western blot analysis for MLL1, HELLS and Vinculin, using two independent hairpins (shHELLS S1 or S2) or control infections (shCtrl). (**D**) Relative expression of putative Hells target genes such as *Mll1, Zfp523, Pds5a* and *Slc44a1* in wildtype (WT) and *E2f3*$^{-/-}$ MEFs using qRT-PCR. The expression of *Mcm6* serves a proliferation control (**E**) Western blot analysis using whole cell extracts of WT and *E2f3*$^{-/-}$ MEFs showing downregulation for Mll1, but not the loading control Vinculin. Histone extracts show a decline of H3K4me3 but not H3K27me3 or total Histone H3 in *E2f3*$^{-/-}$ compared to WT MEFs. (**F**) Confirmatory chromatin immunoprecipitation analyses of Hells-target genes in WT and *E2f3*$^{-/-}$ MEFs. The β-actin (Actb) promoter served as a control. The enrichment of E2F3 and Hells was assessed using *Dnmt1* and *Mll1* primers spanning the genomic regions around the TSS. IgG served as a control. (**G**) Ratio of normalized Hells tag density over normalized IgG tag density around TSS in WT (red) and *E2f3*$^{-/-}$ (blue) MEFs . Vertical axis gives ratios of Hells-target TSS over Hells non-target TSS.

Supplemental Figure S8

Overexpressed HELLS does not hinder cell cycle entry, as would be expected for a repressor. (**A**) For the cell cycle analysis, FLAG-tagged HELLS was overexpressed in T98G cells using the pLEGO lentiviral vector. The infected cells were serum starved for 72 hours and re-stimulated with 20% FCS. For western blot analysis cells were harvested at 0, 8, 11, 16 hours. An antibody against the Flag tag was deployed to detect the exogenous full length HELLS and HELLS without the N-terminal CC-domain that is the domain required for E2F3-interaction. The expression of cell cycle-related proteins were analyzed using antibodies recognizing p107 and CDC6. VICULIN served as a loading control. (**B**) For BrdU-FACS, cells were treated as above and pulse-labeled with BrdU for 1h and subsequently fixed. Cells were stained with a FITC-labeled anti-BrdU antibody according to the manufacturer's protocol and counterstained with propidium iodide.

Supplemental Materials

8 Supplemental Figures
4 Supplemental Tables

Supplemental Materials and Methods

GST-proteins

GST-proteins were produced in BL21(DE3) and purified according to standard protocols using CL4B Sepharose (GE Health Care). We used bacterial expressed E2F3b as bait since it is identical to E2F3a apart from its N-terminus and displays improved solubility.

Mass spectrometry analysis

GST-bound proteins were separated on an 8 % SDS-gel. The SDS-gel lane was cut into 20 pieces. Peptides were generated by in-gel digestion as described (Shevchenko et al., 2006). The extracted peptides were separated on a 15 cm pepMap column (Dionex) and sprayed into a Q-TRAP 4000 hybrid mass spectrometer (ABSciex). Peptide spectra were assigned using the MASCOT software (Matrix Science) with mass tolerances of 0.4 Da for precursor and fragment masses.

Pull-down experiments with proteins expressed in bacteria

For bacterial pull-downs single and coexpression plasmids were generated (Scheich et al. 2007). The CC and MBD sequence of human E2F3A was cloned into the pQLinkG vector,

resulting in the expression of a GST-fusion protein. Both Hells and DP2 were expressed as His-tagged fusion proteins using the pQLinkH vector. Coexpression plasmids were expressed upon induction with 0.5 mM isopropyl-beta-D-thiogalactopyranoside in E. coli Rosetta (DE3) at 17 °C overnight. Cells were suspended in lysis buffer (50 mM sodium/potassium phosphate buffer pH 7.5, 0.5 M NaCl, 5% glycerol, 0.2 % CHAPS, 25 U/mL benzonase, and 0.1 mg/mL lysozyme) and disrupted by repeated freeze-thaw cycles. After centrifugation affinity beads, either Co^{2+}-Talon Superflow, Clontech or Glutathione Sepharose 4B (GE Healthcare) were added to the supernatant to purify specifically tagged proteins and their associated complexes. The beads were washed twice with 20 mM Tris-HCl pH 8.0, 0.2 M NaCl, and 20 mM imidazole (Co^{2+}) or 20 mM Tris pH 8.0, 0.2 M NaC (GST-beads) and purified proteins/complexes are eluted with 20 mM Tris-HCl pH 8.0, 0.2 M NaCl, and 250 mM imidazole (Co^{2+}-beads) or 15 mM GSH (GST-beads), respectively. Pull-down experiments are assessed by SDS-PAGE and western blotting. Usually samples pre-induction (pi), the cell suspension before harvest (in), the pellet after cell disruption ns), and affinity beads-purified samples are analyzed.

qRT-PCR and qCHIP

All experiments were performed at least three times. RNA was prepared using Trizol (Invitrogen) according to the manufacturers' protocol. For qRT-PCR and qChIP analysis the PCR reaction was performed using SybrGreen (Abgene) according to the supplied protocol. The calculation for the qChIPs was performed as described previously (Osterloh et al., 2004). For qRT-PCRs the expression was normalized to GAPDH and the shControl or WT sample was set as one, respectively. In synchronization experiments 0h was set as

one and the other values are given as fold induction. The PCR reaction was performed in triplicates and the values represent average ± SD.

Lentiviral infections

For lentiviral infections, 293TN cells were transfected with the packaging plasmids pLP1 and pLP2 (Invitrogen) and the VSV-G envelope as well as pSicoR (Ventura et al., 2004) using calcium phosphate. Viral supernatants were harvested after 48h and 72h of transfection, pooled and filtered through a 0.44µm filter. Viral supernatants were diluted 1:1 with fresh medium and supplemented with 6µg/ml polybrene. To infect target cells, they were incubated with this supernatant for 24h. All experiments were performed at maximum two days after infection to prevent secondary effects. The HA-ER-E2F3A retrovirus (Muller et al., 2001) was infected using standard procedures and induced for 1h using 300nM 4-Hydroxytamoxifen (OHT) or with Ethanol (Taubert et al., 2004)

Lentiviral HELLS overexpression

C-terminally Flag-tagged full length HELLS was subcloned into the lentiviral pLEGO vector. For the lentiviral HELLS DelCC construct, the region was amplified from a pQCXIP-HELLS constuct by PCR at the same time introducing an SV40 NLS (MPKKKRKV) at the N-terminus and subsequently cloned into the pLEGO vector. The primers used for amplification of HELLS DelCC construct: forward 5`AGTCGCGGCCGCATGCCTAAAAAAAAACGTAAAGTTCAAGTAGAAGGCATG GAATGGC´3. Reverse: 5`CGTTAATTAAGCGTACGAGGCCTACC´3

Nuclear extracts and whole cell extracts

Cells were washed twice with ice-cold PBS and scraped into PBS. Cells were swollen in hypotonic buffer (25mM Hepes pH 7.8, 1.5mM MgCl2, 10mM KCl, 0.1% NP-40, 1 mM DTT, 0.25mM PMSF) for 10 min and then sheared twelve times using a tight pestel (Dounce homogenizer, Sigma). Nuclei were spun down, resuspended in HEGN450 buffer (450mM KCl, 20mM HEPES pH 7.9, 0.2mM EDTA, 10% glycerol, 0.1% NP-40) and rocked for 45min on ice. The lysates were cleared by centrifugation and diluted 1:2 with HEGN0 (HEGN450 without KCl) to obtain a KCl concentration of 150mM KCl. Whole cell extracts were prepared using TNN buffer (50 mM Tris, pH 7.5, 120 mM NaCl, 5 mM EDTA, 0.5% IGEPAL CA-630, 10 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 100 mM NaF, 0.1% Protease Inhibitor Cocktail Sigma; P8340). Histone extracts were made according to publicly available protocols (Abcam).

Chromatin-Immunoprecipitation (ChIP) and Re-ChIP

The ChIP assays were essentially performed as described (Osterloh et al., 2007). Briefly, 2-5 µg of antibody was incubated with 20µl Protein A or G Dynabeads (Invitrogen) in a volume of 500 µl PBS + 5 mg/ml BSA, at 4°C on a rotating wheel. The beads were collected on a magnetic device and resuspended in 100 µl PBS + 5 mg/ml BSA per ml chromatin. The antibody bound to the magnetic beads and the chromatin was incubated at 4°C on a rotating wheel. The beads were successively washed with 1 ml Sonication buffer (50 mM Hepes pH 7.9, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS, 0.25 mM PMSF, protease inhibitor cocktail (Sigma), with 1 ml

high salt buffer (sonication buffer including 500 mM NaCl) and with 1 ml LiCl wash buffer (20 mM Tris, pH 8.0, 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, 0.5% Na-deoxycholate, 0.25 mM PMSF and protease inhibitor cocktail, Sigma). Each washing step was repeated and included a 3 minute incubation on a rotating wheel at 4°C. Finally, the chromatin was eluted with 50 mM Tris, pH 8.0, 1 mM EDTA, 1% SDS, 50 mM $NaHCO_3$ at 65°C for 30 minutes. For Re-ChIP experiments, the beads were washed three times with sonication buffer and eluted with 50 µl of peptide (Sc-878p) for 15 minutes at room temperature. The eluted chromatin was diluted in 1 ml Sonication buffer supplemented with 1 mg/ml BSA and 100 µg sonicated salmon sperm. The immunoprecipitation and washing was subsequently performed as described elsewhere. For semiquantitive PCRs, different cycle numbers (29-32 cycles) were used to keep the PCR signal in a linear range.

ChIP-Seq

The ChIP for the ChIP-Seq analysis was performed as described above, but with minor modifications. 10^8 WT or *E2f3*$^{-/-}$ cells were lyzed and 10µg of antibody or µg IgG used (I5006; Sigma). The DNA was purified with a MinElute PCR Purification column (Qiagen) and eluted in 11 µl. All reagents used were supplied with the Genomic Sample Prep Kits (Illumina). Blunt ends were created to repair the ends of the ChIP-DNA and the DNA was cleaned with the aid of a PCR purification column (Qiagen) according to the manufacturers suggestion. Again, DNA was purified with the MinElute PCR Purification column (Qiagen). Next, adapters (Sample Prep Kit) were ligated to the ChIP-DNA. The adapter-ligated ChIP-DNA was amplified using Kit-derived oligonucleotides (Sample Prep Kit) with a 22 cycles PCR-run. The size selection of the chip library was attained by

separation on a 2%-Agarose gel. The PCR amplified DNA between 200-300 bp was cut out and cleaned with the help of a MinElute gel extraction kit (Qiagen). The size distribution and quantity of the DNA was analyzed through a Bioanalyzer chip (Agilent) before the DNA was used for cluster generation. Single-end sequencing of 36 nucleotides was performed using a Genome Analyzer IIx.

Solexa read processing and mapping

Solexa reads were base called using the manufacturer's software SCS 2.5 / RTA 1.5. Subsequently, 3' ligation adapter sequences were identified and removed from the reads. Reads were clipped to 25 bases, and reads shorter than 17 bases were discarded. The remaining reads were mapped against the mouse genome (NCBI37/mm9) allowing up to edit distance 1 per read using a custom read mapping pipeline. Only reads mapping uniquely to the genome were considered. The mapped reads were reduced to non-redundant tag position sets.

Adapter removal

To remove 3' ligation adapter sequences, all adapter prefix occurrences up to edit distance 4 were considered. The best matching adapter prefix occurrence of each read was identified using a binomial model with parameters p, n, and k, where p, the average sequencing accuracy, was set to 0.95, and n is the length of the prefix occurrence and k is the edit distance, i.e. the number of mismatches and indels (=insertions and deletions) in the alignment of the adapter sequence to the adapter prefix occurrence in the read. For each

read we removed the prefix occurrence that had the highest log-likelihood according to this model.

Tag density normalization

To compare distributions of aligned deep sequencing reads between the different libraries we applied the following normalization procedure. First aligned tag position sets were smoothed by applying a sliding window of size 100 bases, calculating for each position the relative frequency of positions with aligned tags in the window centered on that position. This relative frequency was divided by the number of aligned tag positions and multipled by 10 million to yield mapping density units of tags per 10 million aligned tags.

Tag density analysis around transcription start sites

A non-redundant set of RefSeq (Pruitt *et al,* 2005) transcription start sites (TSS) was extracted from the UCSC table browser (Karolchik *et al,* 2004). For each library, we determined the expected tag densities per 10 million aligned tags for each position in windows of size 1.5 around the TSS. From these densities we computed the relative enrichment of each library to the IgG library in the same genotype.

Identification of binding regions

The mapped reads were reduced to non-redundant tag position sets. Regions enriched for E2F3 and HELLS were determined using MACS (Zhang *et al,* 2008) version 1.4.0beta. The IgG mapped read sets were pooled and used as control in the analyses. Default parameter settings were used.

Determining target genes

Target genes were defined by finding transcription start sites situated closer than 1kb to summits of enriched regions according to the MACS analysis of the ChIP-Seq libraries.

Comparison with H3K4me3 data

MEF histone modification data of Mikkelsen *et al,* (2007), in particular H3K4me3, were retrieved from the supplement of Young *et al,* (2011). These data are given in terms of Ensembl gene IDs, that we mapped to RefSeq genes. We performed the analysis of overlapping target genes and H3K4me3 on the level of RefSeq genes. Note that our Hells-E2f3-H3K27me3 overlap analysis was performed in terms of shared TSS. As there may be multiple RefSeq genes that share a given TSS, the numbers reported in the H3K4me3 overlap analysis add to higher counts than in the H3K27me3 analysis.

Creating E2F3-swapping mutants

The marked box swapping mutant, HA-E2F333133, was a kind gift of J.Nevins (Halstrom and Nevins, 2003). To generate HA-E2F331333, we used pcDNA3-HA-E2F1 as a template to amplify its coiled coil domain with the aid of the following primers: 5'-gtcaaggcctgtcaaaagaagtgcgcgagctccaacaggaagagcagcaattagatgaactgatccaaagc-3' and 5'-gctttggatcagttcatctaattgctgctcttcctgttggagctcgcgcacttcttttgacaggccttgac-3'. Subsequently, the amplicon was used together with flanking primers and pcDNA3-HAE2F3A as a template.

Primers

ChIPs

hβ-Actin	CAAGGCGGCCAACGCCAAAACTCT	GCCATAAAAGGCAACTTTCGGAACG
hCCNB1	CGATCGCCCTGGAAACGCATTC	CCAGCAGAAACCAACAGCCGTTC
hCDC6	GTAGCAAAAAAAGAGGCGGTG	AGCCCGGCCTCGATTCT
hHOXA7	GGAGCAAAGGAGCAAGAAGTC	GCCGACTTCTTGCTCCTTTG
hLefty2	GAAGAGCTTGAATGTGGATTGGG	TTCCCTGTTCTTCAAACACCGTCC
hMCM4	CCGAGCGAGGCCTACTTCT	GGACAGTGCCGCTTCTTTCA
hp107	GGATGACAACACGTCCCG	CACGGCCCCCGACTTC
hp107 +2kb up	GATGTTCCTGCTTCAGCCTCC	TGGGCAACATAGTCAGATACTATCTCCC
hPCNA	TTTCAGTGTGCGATTAGC	CCATTCGACCTAAATAACG
hU2	TTTGCTCCCACTGCCGTC	CTGAGTCTTTCGGTGCCC
mp107	ATCTTCTTATCCCATTCCGGGAGACG	GGGCTCGTCCTCGAACATATCC
mHells	GCGTCCAAATCTCGTGAGC	GCGAGCTAAATCCTCACACC
mBmyb	CTCGTGTCTTGTACGCTTCGCC	CACGTTCCCAGGAACTGCAGCT
mβ-Actin:	GCTTCTTTGCAGCTCCTTCGTTG	TTTGCACATGCCGGAGCCGTTGT
mMll11	CCTGCAATCTGCATATGAGCC	CATGCACTTATCCAGGTTGCC
mDnmt1	CCTTGCTCCCTCAAGCTCC	GGCTGTGCTTCCTGTCGC
mZfp523	GCGAGAGGGAAGTGCGG	CGACAGACCACCATGCACC
hsMLL1	TCGGGCTAACCCATCTTGTA	GGGAGAGCAGCTTCCAGTAT
hsRNF146	GAGAAAAGACTGCGAGGTGG	GAGGATCCTAGAGTGGCCG
hsHSPD1_TSS	AAGACTCGGAGGCGGAAG	CTCAAGGGTCAAATCGCGTC
hsHSPD1_300bp	CTGACGGGAAGTGGGTGG	TGAGGGACAGAGTGCAGG
hsPDS5A	GAGACAGTGCCGCTCTCC	GTCCCGGACGGACACAAG
hsKRAS	GGAACGCATCGATAGCTCTG	GCCGCCTAGGAAAATCGAG
hsHsapds5a	AGAGAAATAGCCCGGAAACTTG	GTCCTGAACGGATAGCTGTATCT
hsaNek6fwd	GATAGGCCGAGGACAGTTCAG	TGGCGTCCATCATCTCAAAGA
hsaKrasfwd	GGACTGGGGAGGGCTTTCT	GCCTGTTTTGTGTCTACTGTTCT
hsaZnf76	CAACAGCCTACGTCCAGCAA	AATGTATGCGGTGGTCCCATC
hsaRalgps2	TGCAGGACCAAGACATCTGTT	GATAGCCATTTCGTGCCACTC
hsaXpo4	ACGCGGCTAAAGTTCTGATGG	TGGCGTTGTTCATTATTGACCA
hsaRnf146	CTACAAACAGGAAAGCGAACGA	CGTGCTTACAGGGCAGACT

qRT-PCR

hE2F1	GCAGATGGTTATGGTGATCAAAGCCC	ATGATGGTGGTGGTGACACTATGG
hE2F3b	CTTACAGCAGCAGGCAAAGCG	GGAGCTGAATGAACTTCTTGGTGAGC
hCCNA2	CGCTGGCGGTACTGAAGTC	AAGGAGGAACGGTGACATGC
hCDC6	ACAAATGTCCAAACCGTAACCT	GGTAAATGGGGAGTGTTGCATA
hHells	TCAAATGAATGCTTCAGGACC	GGACAATCAGTATTTCAAACCC
hMcm3	TCGGGCCTTGAAAACATTCGT	GATGCGAGTGAGCAAACCAAT

hMcm4	ACCTCTATACATGCAACGACTTG	GTCAGGGTAACGGTCAAAGAAG
hMcm6	TCGGGCCTTGAAAACATTCGT	GATGCGAGTGAGCAAACCAAT
hp107	ATCTTCTTATCCCATTCCGGGAGACG	GGGCTCGTGCTCGAACATATCC
hS18	TACTCAACACCAACATCGATGGGC	ATCTTGTACTGGCGTGGATTCTGC
hGAPDH	ACATCAAGAAGGTGGTGAAGCAGG	AGCTTGACAAAGTGGTCGTTGAGG
mβ-Actin	TCTACGAGGGCTATGCTCTC	GGATGCCACAGGATTCCATAC
mHells	TTCGGAAATGTAATGGACAGC	GGGCCACATACAAGAAAAGG
mHspd1	CTCGAGCCTTAATGCTTCAAGG	CAATTGACTTTGCAACAGTGACC
mKras	CAAGAGCGCCTTGACGAT	CCAAGAGACAGGTTTCTCCAT
mMcm3	GAATGAAAAGAGGGCTAACCGC	GCGAACCAGAGAGCATTTAGTAA
mMcm6	GAGTCTACCCTTACCTGTGTCG	GGAAAGTTCCACTCACAAGCTC
mMll1	GACACGGACTCCTAGAGCGAAC	TCATTGGCTGGATTCTGTGTTG
mNek6	AGAAGCAGAAGCGGCTTATCC	CGTAGCTGTGATGAACACGTTG
mPrdm10	TCAGTGGACGGTCCAGAGC	TGTTGAGCTGTGGCATCCTG
mRalgps2	GTGGTTTCTGGCTTACAGAGTC	GCAAGGAGTCATCTTTAAGCTGC
mSlc44a1	GCATTTGCTGCCTTTGGTG	TGCTGAGGTGCAGAAGTTGG
mZfp523	CAAACGGTGAGGCTCAGTGAT	CACCTGGTGAATGTACGCAGT
hsahspd1	CTTCGGTTACCCACAGTCTTTC	ACCTTGAAGCATTAAGGCTCG
hsaslc44a1	GGACCGTAGCTGCACAGAC	TGCTGCACCTGTTGCTATTGA

shRNA

hHells 1 fw: TGAAGGTCATTAGTGATAAATTCAAGAGATTTATCACTAATGACCTTCTTTTTTC
hHells 1 rev: TCGAGAAAAAAGAAGGTCATTAGTGATAAATCTCTTGAATTTATCACTAATGACCTTCA

hHells 2 fw: TCTATTGCATTGATGATTCATTCAAGAGATGAATCATCAATGCAATAGTTTTTTC
hHells 2 rev: TCGAGAAAAAACTATTGCATTGATGATTCATCTCTTGAATGAATCATCAATGCAATAGA

Antibodies

CDC6	Oncogene	ab-3
CYCLIN D1	Santa Cruz	sc-92
E2F1	sc-193	Santa Cruz
pan-E2F3	J.A. Lees	Parisi et al., 2007
pan-E2F3	Santa Cruz	sc-878
pan-E2F3	Ziebold-lab	full length E2F3B as an antigen
HA-Tag	Covance	MMS-101R
HELLS	Santa Cruz	sc-28202

HELLS	Novus	100-2784
HELLS	K. Muegge	Xi et al., 2007
HIS	Sigma	H1029
Histone H3	Santa Cruz	sc-10809
Histone H3K4me3	Cell Signaling	C42D8
Histone H3K27me3	Millipore	07-449
MLL1	Bethyl Laboratories Inc	A300-086A
p107	Santa Cruz	sc-318
pRB	Cell Signaling	9309
ppRB (Ser780)	Cell Signaling	9307
VINCULIN	Sigma	VD9131

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