Supporting Information

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SI Materials and Methods

ESC Transfections. ESCs maintained on a feeder layer in 12-well plates were transfected with 2 μg of circular BAC DNA or p53 shRNA lentiviral plasmids (Open Biosystems) using Lipofectamine 2000 Reagent (Invitrogen), according to the manufacturer's protocol. Selection began after 48 h, with the following concentration of drugs maintained for at least 5 d: 1.5 μg/mL puromycin (Sigma), 150 μg/mL hygromycin (Roche), 15 μg/mL blasticidin (Sigma), or 30 μg/mL zeocin (Invitrogen). Genomic DNA from BAC-transfected clones was isolated using DNAzol (Invitrogen), according to the manufacturer's instructions. Southern blot detection of transfected BAC DNA was performed using EcoRV digestion and a probe specific to the neomycin gene, as described (1). Total cellular RNA was isolated from BAC-transfected clones (undifferentiated ESCs or EBs) with TRIzol (Invitrogen), according to the manufacturer's instructions.

EB Formation. ESCs were cultured for at least one passage on gelatin-coated dishes before being seeded at a concentration of 1.25×10^3 cells/mL in bacterial-grade 60-mm dishes without LIF [Iscove's Modified Dulbecco's Medium; Sigma), 15% heat-inactivated FCS (Invitrogen), 5% serum-free and protein-free medium for hybridoma culture (Invitrogen), 2×10^{-3} M L-glutamine (Invitrogen), 50 μg/mL ascorbic acid (Sigma), and 3×10^{-4} M α-monothioglycerol]. EBs were counted at day 8 after LIF removal. For most experiments, EB numbers are expressed as relative values based on primary clone numbers.

Ribosome Profiling. Ten minutes before collection, 100 μg/mL cycloheximide was added to the culture medium. Cells were washed in ice-cold PBS supplemented with 100 μg/mL cycloheximide and were collected in polysome lysis buffer [15 mM Tris (pH 7.4), 250 mM NaCl, 15 mM $MgCl_2$, 1% Triton X-100, 100 μg/mL cycloheximide, 1 mM DTT, 400 U/mL RNaseOut (Invitrogen), and protease inhibitors]. Samples were centrifuged at 10,000 × *g* for 10 min at 4 °C. The resulting supernatant was layered on a 20–50% linear sucrose gradient and centrifuged in a Beckman SW41Ti rotor at 35,000 rpm for 3 h at 4 °C. After centrifugation, the A_{254} was monitored continuously and recorded using a Gradient Station IP instrument (Biocomp) attached to a UV-MII spectrophotometer (GE Healthcare).

GO Analysis. Each clone was annotated with a set of GO terms representing the union of the set of GO terms ascribed to all of the genes present in the deleted region of the clone. For each GO term assigned to at least one clone, a two-sided *t*-test was performed comparing the log EB density (normalized EB numbers) for clones annotated with that GO term against the log EB density for all other clones. GO terms then were ranked based on the significance of their effect on EB formation. The GOrilla application (2) also was used to determine GO enrichments from ranked gene lists.

Assessment of p53 Expression Levels. After shRNA selection, gene expression was assessed by qRT-PCR using a Roche Light Cycler 480 with Roche Universal ProbeLibrary (UPL) assays or TaqMan assays (endogenous control genes). For Trp53 (NM_011640) detection, UPL probe no. 25 and the primer pair acgcttctccgaagactgg + agggagctcgaggctgata were used. Reference gene assays (Gapdh and β-actin) were purchased from ABI (20× primer-probe mix, VIC-labeled). Protein levels were detected from whole-cell extracts (Laemmli buffer) by Western blotting using p53 (Cell Signaling Technology, catalog no. 2524) and α-tubulin (Cell Signaling Technology, catalog no. 2144) antibodies.

BrdU Pulse-Chase Assay. Cells were labeled with BrdU for 15 min and then were collected at various time points before being analyzed for fluorescence by flow cytometry using a BD Canto II cytometer (BD Bioscience) and BD FACSDiva 4.1 software.

Annexin V/Propidium Iodide Staining. ESCs or EBs were dissociated and counted before staining with Alexa350-annexin V (Invitrogen Molecular Probes) and propidium iodide (50 μg/mL) in accordance with the manufacturer's instructions. Fluorescence was evaluated by flow cytometry using a BD Canto II cytometer (BD Bioscience), and data were analyzed with BD FACSDiva 4.1 software.

qRT-PCR. Relative quantification of the target genes was calculated as described previously. Primers used for PCR reactions are provided in Table S2.

1. Kroon E, et al. (1998) Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. *EMBO J* 17(13):3714–3725.

2. Eden E, Navon R, Steinfeld I, Lipson D, Yakhini Z (2009) GOrilla: A tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics* 10:48.

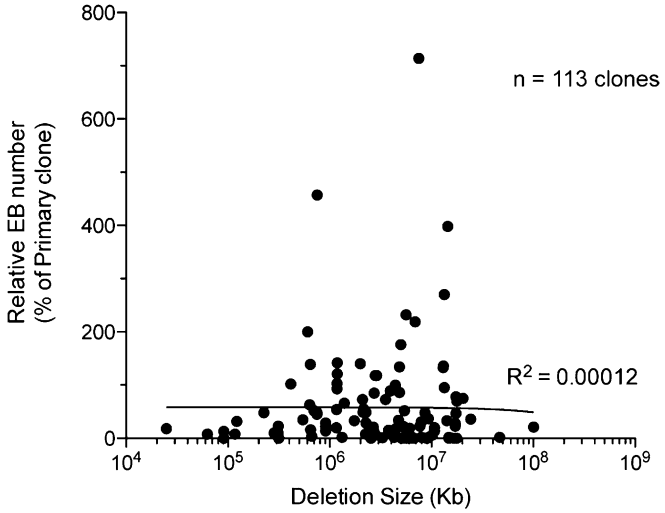

Fig. S1. Correlation between deletion size and EB numbers. Graphical representation of EB numbers compared with deletion size. The correlation coefficient shows there is no correlation between the two factors.

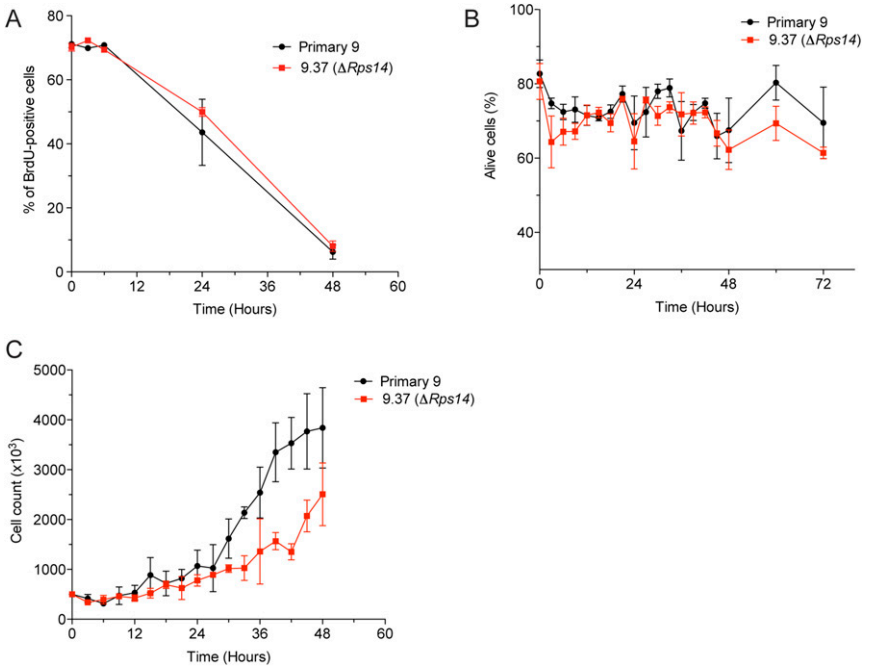

Fig. S2. Increased cell death upon EB formation. (*A*) BrdU pulse-chase experiments on self-renewing wild-type and *Rps14*$^{\Delta/wt}$ clones. The percentage of BrdU-positive cells is an indication of cell-cycle progression and was assessed 0, 3, 6, 24, and 48 h after the initial BrdU pulse. Error bars show SEM values of two independent experiments. (*B*) Cell viability experiments. Annexin V and propidium iodide staining was performed on differentiating (EB) wild-type and *Rps14*$^{\Delta/wt}$ clones. Viability was assessed every 3 h for the first 72 h of differentiation. Error bars show SD values of three biological replicates. (*C*) Total cell counts obtained before cell viability staining for the first 48 h of differentiation. Error bars show SD values of three biological replicates.

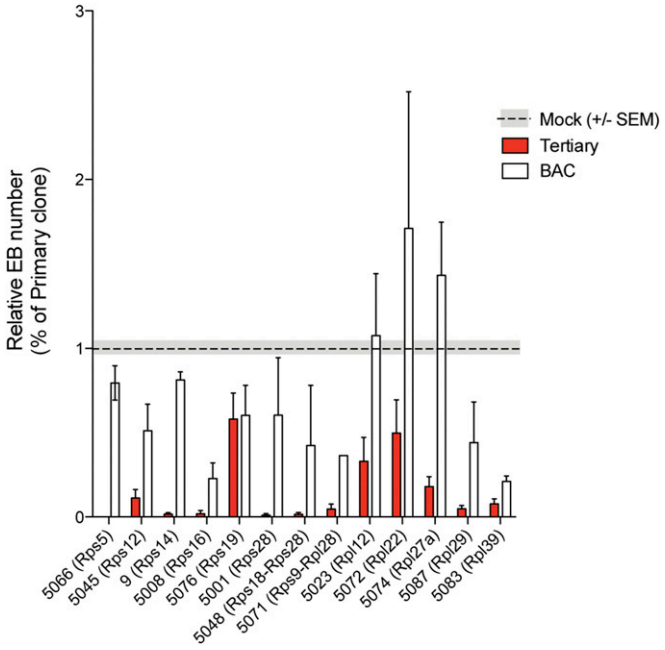

Fig. S3. BAC complementation. Complementation experiments using ΔRP-ESCs. Relative EB numbers (based on primary clone values) obtained from mock-transfected (red bars) and BAC-transfected (white bars) clones are represented. Error bars show the SEM of at least two independent experiments.

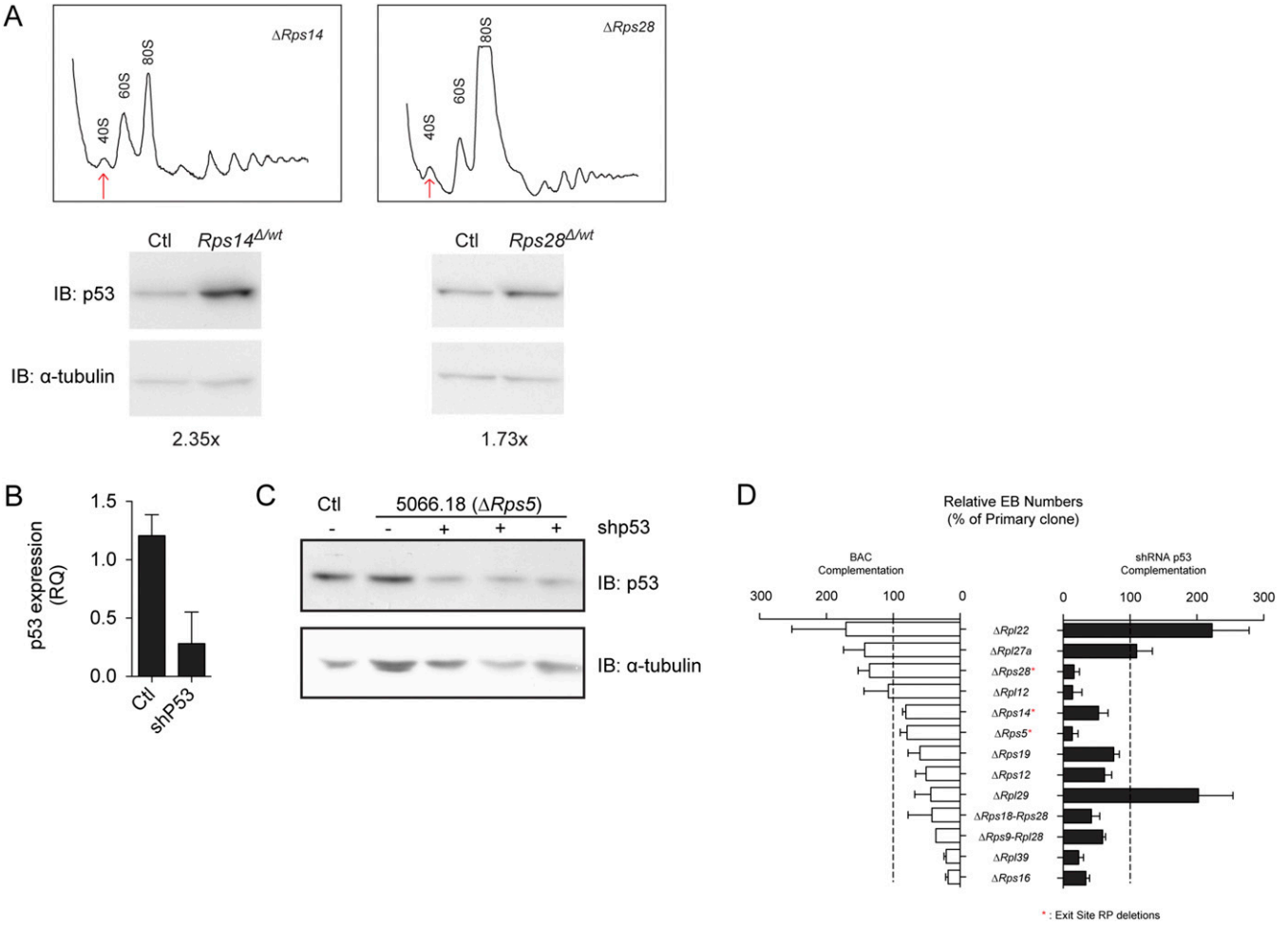

Fig. S4. p53 status and evaluation of knockdown efficiency. (*A*) Polysome profiles and p53 expression levels of *Rps14*$^{\Delta/wt}$ and *Rps28*$^{\Delta/wt}$ clones. The reduced 40S peak is indicated by red arrows. Western blot quantifications were made by normalizing p53 levels on α-tubulin with ImageJ software. (*B*) qRT-PCR assessment of relative p53 expression (RQ) of mock-transfected (Ctl, $n = 3$) and sh-p53–transfected ($n = 10$) cells from family 5066. The ΔCt values range from 5.88 (Ctl) to 9.87 (sh-p53 with ∼92% knockdown). (*C*) Western blot analysis of p53 protein levels in mock- and sh-p53–transfected clones ($n = 3$ independent transfections) from family 5066. α-Tubulin is used as loading control. IB, immunoblot. (*D*) Relative EB numbers (expressed as a percentage of primary clone numbers) following BAC or shRNA p53 transfection. Error bars show the SEM of at least two (BAC transfections) or four (shp53 transfections) independent experiments performed in duplicate.

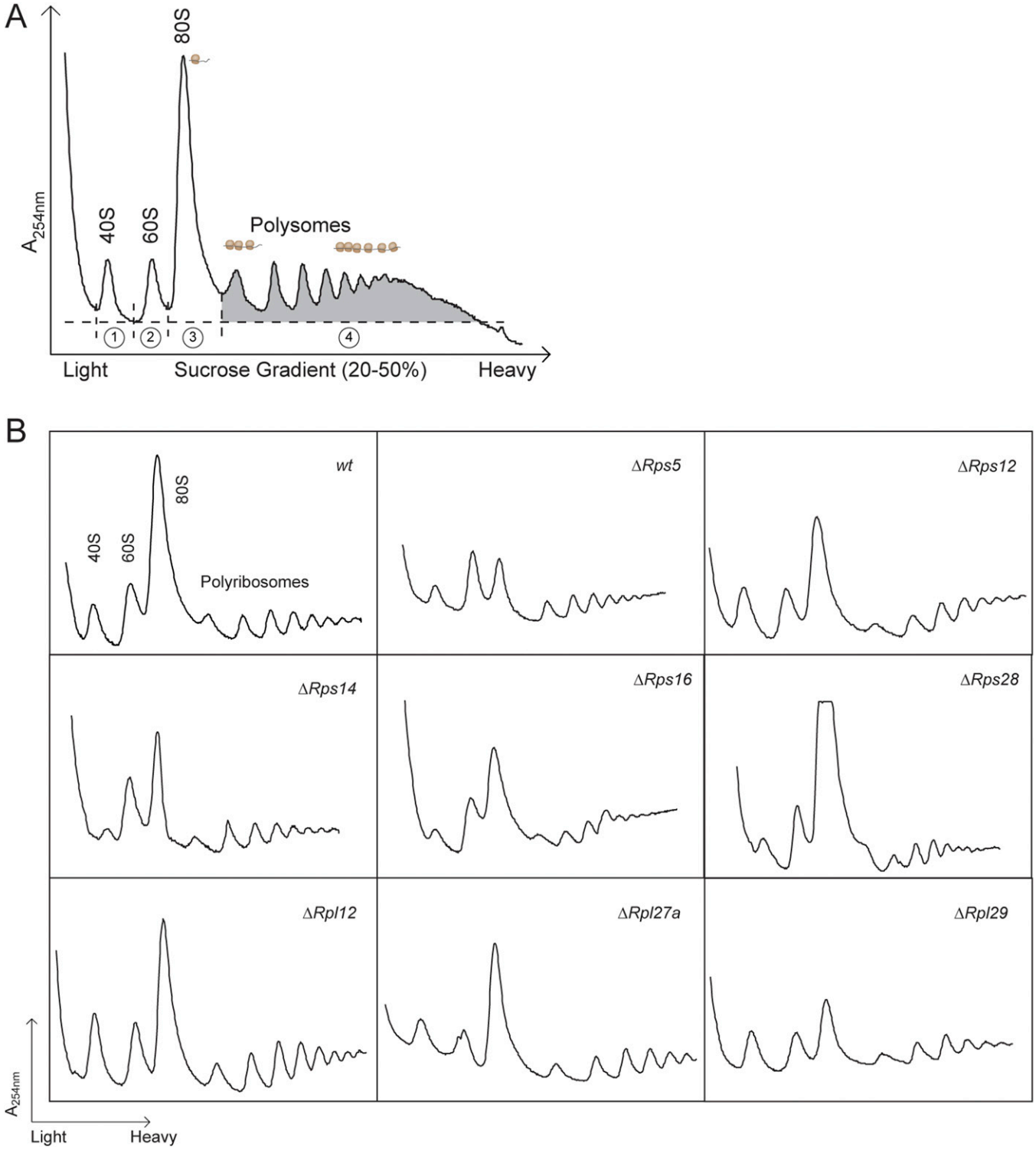

Fig. S5. Polyribosome profiles of undifferentiated ΔRP-ESCs. (*A*) AUC evaluation. A horizontal line has been drawn from baseline levels, and peaks were delimited manually (dashed lines). For example, the highlighted gray region represents the polysomal AUC to be calculated. The 40S, 60S, 80S, and polysomal regions are labeled 1 to 4, respectively. Quantitation was done using ImageJ image analysis software. (*B*) Ribosomal profiles of all tested Rp-deleted clones (*Rps* and *Rpl* genes) in self-renewal conditions (undifferentiated). Profiles shown are representative of at least two independent experiments. For a full description of the experimental setup, see *SI Materials and Methods*.

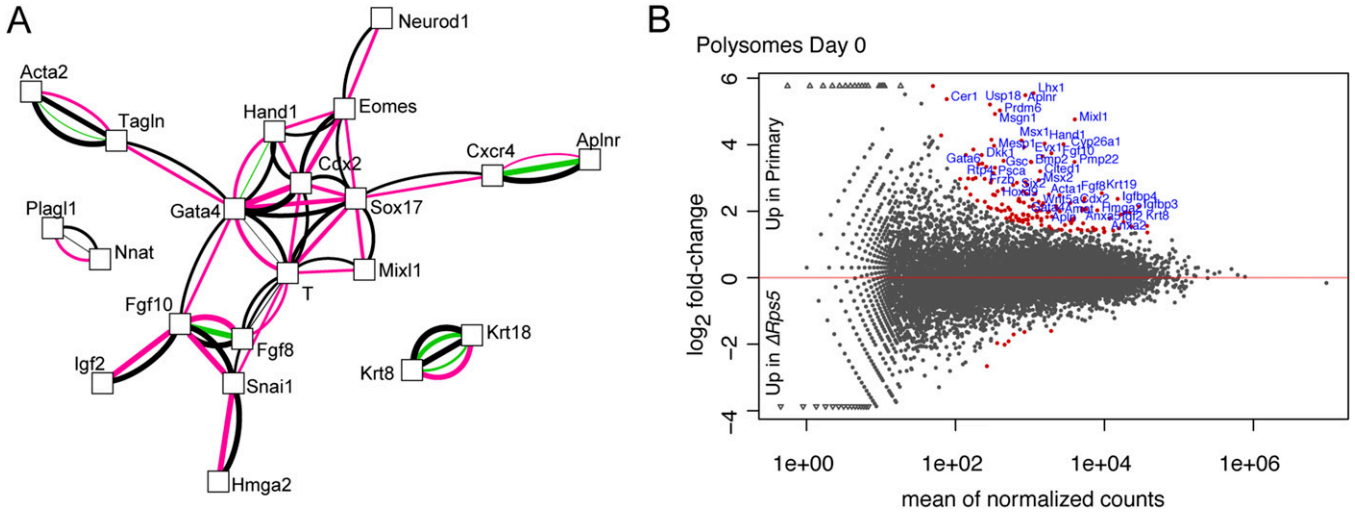

Fig. S6. Differential expression analysis of wild-type vs. *Rps5*$^{\Delta/wt}$ deleted clones. (*A*) Molecular network, built in String (1), of genes identified from DESeq analysis of EB polysomal fractions. Genes with an adjusted *P* value (P_{adj}) < 0.1 have been used, and unlinked nodes have been removed. Edges (colored connections) represent predicted functional links, as established by String. (*B*) DESeq analysis obtained from ESC polysomal fractions. Genes that are differentially expressed (P_{adj} < 0.1) are shown in red. Genes with a P_{adj} < 0.01 are labeled and are enriched for mesodermal differentiation-related genes.

1. Franceschini A, et al. (2013) STRING v9.1: Protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res* 41(Database issue):D808–D815.

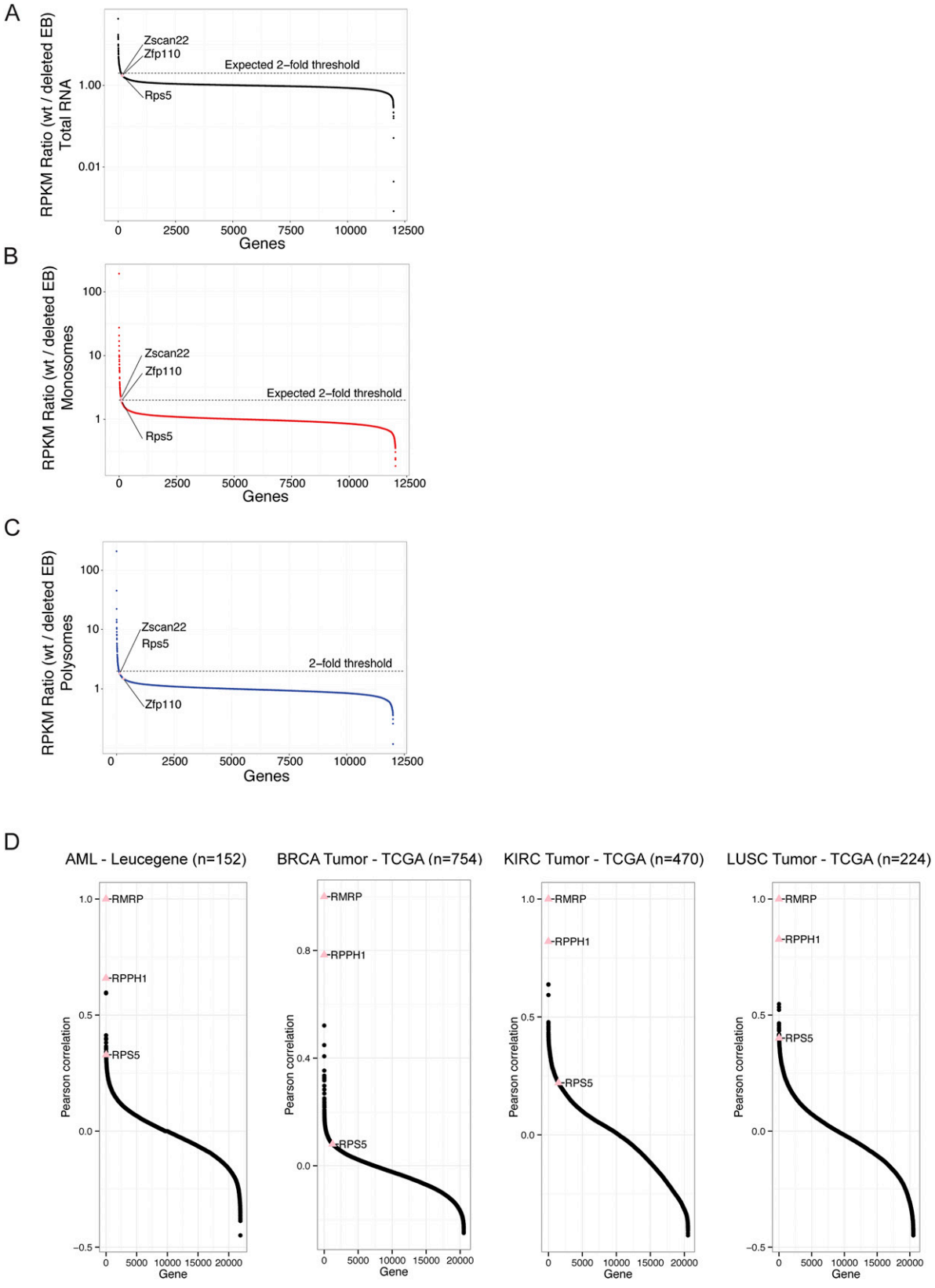

Fig. S7. Enrichment graphs of next-generation sequencing experiments. (*A*) Enrichment curve showing RPKM ratios (wild-type/*Rps5*$^{\Delta/wt}$ EBs) of genes found in total RNA extracts. (*B*) Enrichment curve showing RPKM ratios (wild-type/*Rps5*$^{\Delta/wt}$ EBs) of genes found in monosomal RNA extracts. (*C*) Enrichment curve showing RPKM ratios (wild-type/*Rps5*$^{\Delta/wt}$ EBs) of genes found in polysomal RNA extracts. Genes with RPKM ≥1 are shown (n = 12,009 genes). Genes included in the deleted interval are identified on each graph (*Zfp128* RPKM <1). The dashed lines represent the theoretical twofold threshold expected for hemizygous

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deletions. (*D*) Pearson's correlation coefficient curve of $\log_2$-transformed expression values for *RMRP* in human cancer samples obtained from the The Cancer Genome Atlas (1) collection and the Institute for Research in Immunology and Cancer Leucégène Project.

1. Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455(7216):1061–1068.

Table S1. DelES families used in validation experiments

Family ID	Validated clone	RP gene deleted
9	9.18	*Rps14*
	9.37	*Rps14*
5001	5001.02	*Rps28, Rpl10a*
	5001.06	*Rps28*
	5001.18	*Rps28*
	5001.30	*Rps28*
5002	5002.25	—
	5002.31	—
	5002.37	—
5006	5006.03	—
	5006.13	—
	5006.28	—
5007	5007.04	—
	5007.06	—
	5007.09	—
5008	5008.31	*Rps16*
	5008.20	—
	5008.38	*Rps16*
	5008.42	*Rps16*
5010	5010.14	—
	5010.19	—
	5010.22	—
5016	5016.22	—
	5016.25	—
	5016.30	—
5017	5017.04	—
	5017.05	—
	5017.11	—
5023	5023.12	*Rpl12*
	5023.21	—
	5023.30	*Rpl12*
5026	5026.09	—
	5026.17	—
	5026.18	—
5029	5029.01	—
	5029.15	—
	5029.38	—
5030	5030.07	—
	5030.16	—
	5030.24	—
	5030.28	*Rps3, Rps11, Rps13, Rps16, Rps17, Rps19, Rpl13a, Rpl18, Rps27a*
5032	5032.09	—
	5032.16	—
	5032.23	—
5034	5034.02	*Rpl3*
	5034.11	*Rpl3*
5035	5035.01	—
	5035.10	—
	5035.30	—
5045	5045.10	—
	5045.16	*Rps12*
5048	5048.31	*Rps18, Rps28, Rpl7l1*
	5048.34	*Rps18, Rps28*
5056	5056.02	—
	5056.11	—
	5056.33	—
5066	5066.18	*Rps5*

Table S1. Cont.

Family ID	Validated clone	RP gene deleted
5067	5067.09	—
	5067.30	—
	5067.36	—
5068	5068.04	—
	5068.05	—
	5068.09	—
	5068.22	—
5071	5071.15	—
	5071.21	*Rps9, Rpl28*
5072	5072.15	*Rpl22*
	5072.19	—
5074	5074.18	*Rpl27a*
	5074.26	*Rpl27a*
	5074.31	*Rpl27a*
5076	5076.07	*Rps19*
	5076.39	*Rps19*
5078	5078.03	—
	5078.05	—
	5078.09	—
5079	5079.26	—
	5079.29	—
	5079.32	—
5081	5081.06	—
	5081.39	—
5082	5082.12	—
	5082.29	—
	5082.31	—
5083	5083.14	—
	5083.26	*Rpl39l*
5084	5084.28	—
	5084.33	—
5085	5085.15	—
	5085.16	—
5087	5087.27	—
	5087.42	—
	5087.44	*Rpl29*
5088	5088.10	—
	5088.14	—
	5088.32	—

Identification of all clones used in validation experiments are grouped by anchor points (families). The presence of a RP gene deletion is indicated in the last column.

Table S2. Primers and probes used for qRT-PCR studies

Primers	Sequence
Rmrp_fwd	gctctgaaggcctgtttcct
Rmrp_rev	cttcttggcgggctaacagt
Rmrp_HybProbe	cttatcctttcgcctagggg
Rpph1_fwd	ggtgagttcccagagagcag
Rpph1_rev	cagccattgaactcgcact
Rpph1_HybProbe	agcttggaacagactcacgg
Rps5_fwd	cactgcgtcgagtgaatcag
Rps5_rev	gctcatctgcaaggcactc
Tp53_fwd	acgcttctccgaagactgg
Tp53_rev	agggagctcgaggctgata

The table shows the sequences used for qRT-PCR studies. Unless otherwise mentioned, probes from Roche Universal Probe Library were used.

Dataset S1. Analysis of GO-term clustering from the EB-formation screen

Dataset S1

The dataset shows the complete GO term clustering analysis file performed on 320 deletion clones with available mapping information. See *SI Materials and Methods* for complete methodology used for clustering.

Dataset S2. List of significant ($P_{adj} < 0.1$) genes obtained from DESeq analysis

Dataset S2

The dataset shows the list of genes that are differentially expressed in wild-type and *Rps5*$^{\Delta/wt}$ clones in the EB polysomal fraction. Only significant ($P_{adj} < 0.1$) genes are shown.

Dataset S3. Analysis of GO-term clustering of polyribosomal fractions

Dataset S3

Complete GO term clustering analysis based on processes performed on 12,009 genes (RPKM ≥1) from next-generation sequencing of total, monosomal, and polysomal RNA extracted from sucrose gradient fractions. See *SI Materials and Methods* for complete methodology used for clustering.