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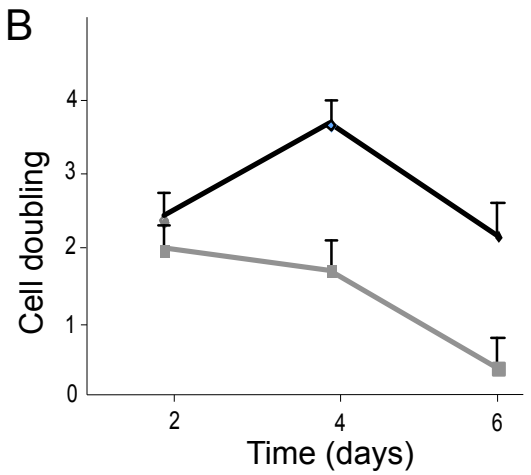
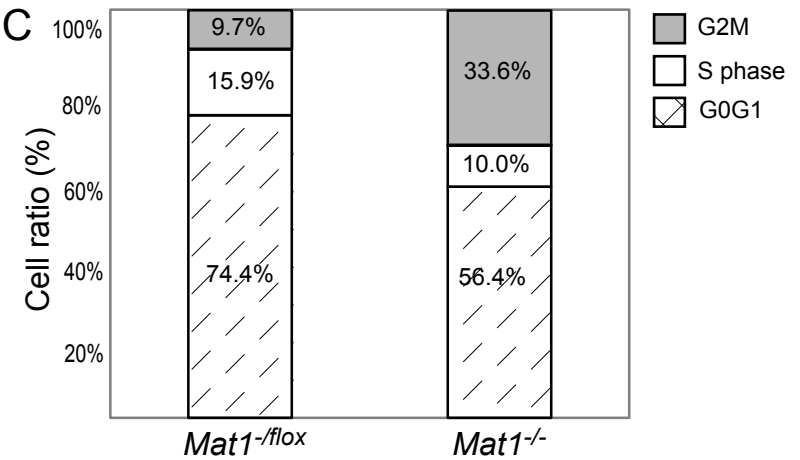
GO term	P-value	Sample Freq	Background Freq
GO:0000278 **cell cycle**	4.07E-26	40.2%	3.6%
GO:00000087 **cell division**	4.51E-23	30.8%	1.6%
GO:0000775 **cell cycle process**	2.00E-24	23.9%	2.2%
GO:0000910|GO:00077104|GO:0016288 **M phase**	1.69E-22	23.9%	1.5%
GO:0006259 **cell cycle phase**	3.65E-22	23.9%	1.8%
GO:0044430 **DNA metabolic process**	8.19E-11	17.9%	2.2%
GO:0034728 **DNA replication**	4.61E-13	16.2%	0.8%





Supplementary Figure 1. **Decreased proliferation of *Mat1*$^{-/-}$ MEFs concomitant with an increase in cells in G2/M during prolonged culture. (A)** Gene ontology (GO) category enrichment analysis among upregulated genes (fold change >2, p-value <0.05) in *Mat1*$^{-/-}$ MEFs 96h after Mat1 deletion sorted by P-value demonstrates significant increases in cell cycle and M-phase genes. **(B)** Analysis of cell population doubling times in *Mat1*$^{-/flox}$ and *Mat1*$^{-/-}$ MEFs at indicated times following AdCre (grey line) or AdLacZ (black line) demonstrates comparable doubling times at 2 days, and significantly decreased values from 4 days onwards. Values represent averages of three independent experiments with error bars showing standard deviations between experiments. **(C)** Analysis of cell cycle stage distribution in *Mat1*$^{-/-}$ MEFs and *Mat1*$^{-/flox}$ MEFs performed 96h after infection by analyzing fixed, propidium iodide stained cells by flourescence activated cell sorting (LSR, Bekton Dickinson) and analysis using Cell Quest Software. Distribution in G0/G1, S and G2/M phases as indicated demonstrates a significant increase in G2/M phase cells in *Mat1*$^{-/-}$ MEFs.

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GO term	# of probes	Enrichment	P-value
mRNA metabolic process GO: 0016070	25	5.3	1.82E-06
RNA binding GO: 0003723	39	3.1	6.79E-04
RNA processing GO: 0006396	29	4.2	3.56E-06
mRNA processing GO: 0006397	25	6.1	3.85E-07
RNA splicing GO: 0008380	22	7.1	1.03E-07

Supplementary Figure 2. Gene ontology analysis of TFIIH kinase-dependent mRNAs. (A) Gene ontology (GO) enrichment in mRNA processing-related genes among downregulated mRNAs in *Mat1*$^{-/-}$ MEFs. Over-represented Biological Function GO groups in the 365 downregulated probe set after filtering (minimum 10 probes) and ranking according to p-value. Enrichment is calculated as Sample Frequency/Background Frequency for each GO term. There is significant overlap in the GO classes so that 52 probes (45 genes) cover all classes.

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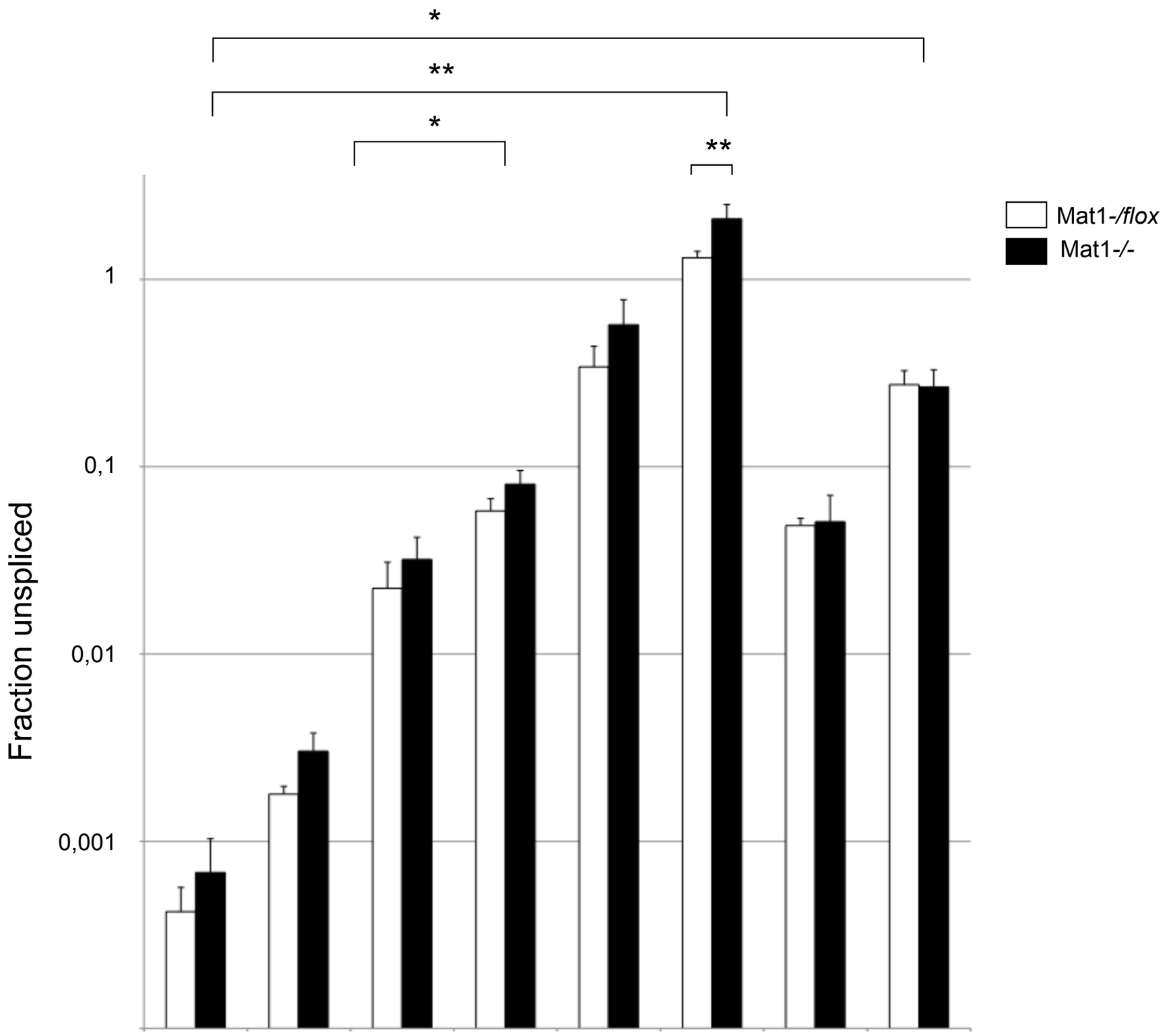

Supplementary Figure 3. Analysis of spliced and unspliced RNA levels in *Mat1*$^{-/-}$ MEFs. (A) Analysis of splicing of endogenous U2- and U12-type introns in *Mat1*$^{-/-}$ and *Mat1*$^{-/flox}$ MEFs in genes with variable levels of unspliced RNA. RNA of *Dock1, Pex16, Tcea2, and Pcyt2* was analyzed using primers spanning an unspliced exon-intron boundary and is displayed as a fraction of the corresponding spliced RNA on a logarithmic scale (primers indicated in Supplemental Materials and Methods). Error bars indicate standard deviation between four biological experiments. Significant (*; P < 0,05) or highly significant (**; P < 0,01) increases in unspliced U2 and U12 RNA in *Mat1*$^{-/-}$ cells are shown, where brackets indicate samples used in paired t tests: *Tcea2* U12 (P=0.0095); *Pex16* U2 and U12 (P=0.0282); *Dock1, Pex16*, and *Tcea2* U2 and U12 (P=0.0058). Levels of spliced U2 RNA in in *Mat1*$^{-/-}$ cells were not significantly changed (*Pcyt2, Tcea2*) or decreased (*Dock1,* 0.56; *Pex16*, 0.83) and no significant alteration was noted in ratio of spliced U2 and U12-type RNAs.

Supplementary Materials & methods

Generation of immortal Mat1$^{-/flox}$ MEFs

MEFs were isolated from E12.5 *Mat1$^{-/flox}$* embryos and cultured in DMEM (Gibco) supplemented with 10% fetal calf serum and antibiotics. Immortal *Mat1$^{-/flox}$* MEFs were generated as previously described (10). For deletion of Mat1, MEFs were infected with adenoviruses encoding CRE-recombinase (AdCre) or LacZ (AdLacZ) using multiplicity of infection of 1500 MOI overnight at 37 °C.

Western blotting

Western blotting and kinase assays were as described (10). Briefly, MEFs were lysed in Laemlli sample buffer and centrifuged at 13 000 rpm 15 min. Antibodies: α-Mat1 (Santa Cruz FL-309), α-Cdk7 (Santa Cruz sc-7273), α-p62 (Genway), α-p44 (Genway), α-actin (Sigma AC-40), α-ser5 (Nordic Biosite H14), α-ser2 (Nordic Biosite H5), α-Pol II (Santa Cruz N20 and Nordic Biosite 8WG16).

Immunofluorescence staining

Immunostaining of cells was done as previously described (10). Antibodies used: α-ser5 (Nordic Biosite H14), α-ser2 (Nordic Biosite H5), α-Pol II (Santa Cruz N20).

Affymetrix data analysis

Data analysis and comparison of the data set to other published microarray studies was performed using GeneSpring GX (Agilent Technologies), Microsoft Excel, and

Filemaker Pro software. For standard comparison of differentially regulated genes at a given timepoint samples arranged by genotype were filtered for expression (at least 1/6 raw values between 20-100% percentile for inclusion), for significance (paired T-test, $p<0,05$) and fold change (>1,5). For analysis of general changes in signal intensities, a normalization between genotypes at the 72h time point was performed using a normalization factor generated through pairwise comparisons of triplicate averages of 45 Probe Sets: AFFX labeling controls (28 probes), AFFX prelabeled hybridization controls (excluding AFFX-Cre due to the Adeno-CRE infection; 14 probes) and AFFX mouse non-PolII/PolI controls (3 probes) leading to a normalization factor of 1,0374 for *Mat1*$^{-/-}$ samples.

Quantitative RT-PCR analysis

RNA was isolated using RNEasy isolation kit (Qiagen) according to manufacturer's protocol. dscDNA was amplified using Power SYBR Green PCR Master Mix (Applied Biostystems). Relative mRNA amounts were assayed by normalizing PCR cycles to GAPDH for each sample unless otherwise indicated using 7500 Fast Real-Time PCR System Software. *Mat1*$^{-/-}$ samples were compared to *Mat1*$^{-/flox}$ samples. Technical triplicates were run from 3-5 independent experiments in all cases.

Transfections and cell treatments

For siRNA knockdown experiments, *Mat1*$^{-/flox}$ MEFs were transfected with 100 nmol siRNA diluted in OptiMEM (Gibco) and incubated with Lipofectamine 2000 (Invitrogen) following manufacturer's instructions. Transfections were repeated 24h later. RNA was

collected 72h from first transfection. siRNA used: Mat1: ON-TARGETplus SMARTpool (Dharmacon catalog number L-047470-00), control: non-targeting siRNAPOOL (Dharmacon catalog number D-001810-10-20).

Splicing analysis

RNA from four parallel samples of *Mat1*$^{-/-}$ and *Mat1*$^{-/flox}$ MEFs were DNAse-treated with DNA free kit (Ambion) followed by cDNA synthesis was done with Superscript III reverse transcriptase (Invitrogen) according to manufacturer's instructions using 2 μg RNA and 500 ng random primers (Promega). Control reactions were assembled without reverse transcriptase. QRT-PCR was done using Lightcycler 480 SYBR Green I Master mix according to manufacturer's protocol (Applied Biosystems). Primers were designed similarly as described earlier (45). In each run, samples were included in triplicate and concentration standards in duplicate. Results were analyzed with Lightcycler 480 software release 1.2.

Primers used in qRT-PCR analysis

b-actin

F: 5'- GGCTGTATTCCCCTCCATCG-3'

R: 5'- CCAGTTGGTAACAATGCCATGT-3'

Crabp2

F: 5'- ATGCCTAACTTTTCTGGCAACT-3'

R: 5'- GCACAGTGGTGGAGGTTTTGA-3'

Dnajc3

F: 5’-GGCGCTGAGTGTGGAGTAAAT-3’

R: 5’-GCGTGAAACTGTGATAAGGCG-3’

Gapdh

F: 5’-AAGGTCGGAGTCAACGGATT-3’

R: 5’-TTGATGACAAGCTTCCCGTT-3’

Gjb2

F: 5’- ATCCTCGGGGGTGTCAACAA-3’

R: 5’- AGACAAAATCGGCTTGCTCATC-3’

Mat1

F: 5’-AAGCCAGTGACGTTTTCCAC-3’

R: 5’-AAGAAGTATAGCCGCCAGCA-3’

MMP3

F: 5’- ACATGGAGACTTTGTCCCTTTTG-3’

R: 5’- TTGGCTGAGTGGTAGAGTCCC-3’

Ppih

F: 5’-TTGGACCCATTCATTAACCTGC-3’

R: 5’-CAGTGGGCATCAGGATCAGAG-3’

Pnrc2

F: 5’- GTAGAGGGAAGGAGGCTTGG-3’

R: 5’-ATGAGCAATCTTCGTCTGGGA-3’

Rab2b

F: 5’-GGTGTGGGGAAGTCATGTCTC-3’

R: 5’-GTTGACCATACGTGCTCCAAA-3’

Rpl30

F: 5’- AGTCGATCAACTCTAGGCTCC-3’

R: 5’- TAACCAACTTCGCTTTGCCTT-3’

Tuba4

F: 5’-ATGCGCGAGTGCATTTCAG-3’

R: 5’-CACCAATGGTCTTATCGCTGG-3’

Primers used in splicing analysis (intron/exon number)

Dock1 U12 intron (41)

5’- ACATTAAGACTTCTCCCGGCCA

3’- ACAACCAAGAGAAGCCCTGCTC

Dock1 U2 intron (34)

5’- ATCATGAAGTGGAAGGAGGCA

3’- AATCCCACCGTGAATCAACAC

Dock1 U12 exon (41-42)

5’- GCTGAAAAAATGAAGACGACGT

3’- TGAAGCACTGGATGTACTGGC

Dock1 U2 exon (34-35)

5’- GCTGGATCATGAAGTGGAAGGA

3’- GCAGTGCTCCAGGAGGATTTTAT

Pcyt2 U12 intron (8)

5’- TATGTGGCTGGTGCCTTTGA

3’- TGCCATTCTCCCTGGTGAA

Pcyt2 U2 intron (2)

5’- ATTATGGCCACTCCAACCAGC

3’- CATTGGACTGTCCGGCACA

Pcyt2 U12 exon (8-9)

5’- GTTTGCTTCTGGGAAGGAGC

3’- ACGTGCCCGATGTGGAA

Pcyt2 U2 exon (2-3)

5’- TTATGGCCACTCCAACCAGCT

3’- TGCTTGGCAATCTCCTCATCC

Pex16 U12 intron (7)

5’- GCAGAGTCTTTGTACATCGCCC

3’- CGCCACCAGCACAGCTAAG

Pex16 U2 intron (6)

5’- GAACAGTAAGTGGAAGATGTAGCTGG

3’- CGGGCTCACACTGACCTCA

Pex16 U12 exon (7-8)

5’- GCAGGAGACCATTGCAGAGTC

3’- CCAGGCTGAGCAAGTGCAG

Pex16 U2 exon (6-7)

5’- AACCCTCCAGAACAGCCCAT

3’- TCTGCCGAATTTCCCGC

Tcea2 U12 intron (6)

5’- ATTCCCCATATGACTCCCAGG

3’- CAGTATTTCCCACGTCCAGGA

Tcea2 U2 intron (2)

5’- AAGAGTCCTTGGTGAGGATCCG

3’- TTGACAGACATCCCAACACGG

Tcea2 U12 exon (6-7)

5’- CATCTCAGATCGAGGAGTGCAT

3’- TTAGAGATTCGGCTCCGCA

Tcea2 U2 exon (2-3)

5’- GCACTTGCTCCAGTCCACC

3’- GCAATGAGCTCCTCATCTGAAC

Primers used in ChIP experiment

Actb TSS (+1 - +167)

(F): ACTGTCGAGTCGCGTCCA

(R): CATGGTGTCCGTTCTGAGTG

Actb body (+1048 - +1218)

(F): GATATCGCTGCGCTGGTC

(R): CATCGATCCCCAAGAAAACC

Rpl30 TSS (-13 - +112)

(F): GTGGGAGCTCCTTCCTTTCT

(R): AATCCAGAGCGTCAAACACC

Rpl30 body(+1257 - +1360)

(F): AGCACTTTGGAGCACTGTTG

(R): CTTCAGTGCCAAGTTAAAGGA

Tuba4a TSS (-40 - +60)

(F): CGCCTCCTGCCTATAAGAGC

(R): TGATGAGTTTGGGCTGTACG

Tuba4a body (+1889 - +2035)

(F): TGTCTGGAACATGGGATTCA

(R): GTAGGCTCCAGGTCCACAAA

Rab2b TSS (+1 – 142)

(F): GGCAAATAAGGAGACCCGTA

(R): CTGTGTCTCCGATGATGATGT

Rab2b body (+961 - +1081)

(F): ACGGACATTGCAGTTCTTCC

(R): TCTCTGGGAGTTTGTGTCCA