Fig.S1

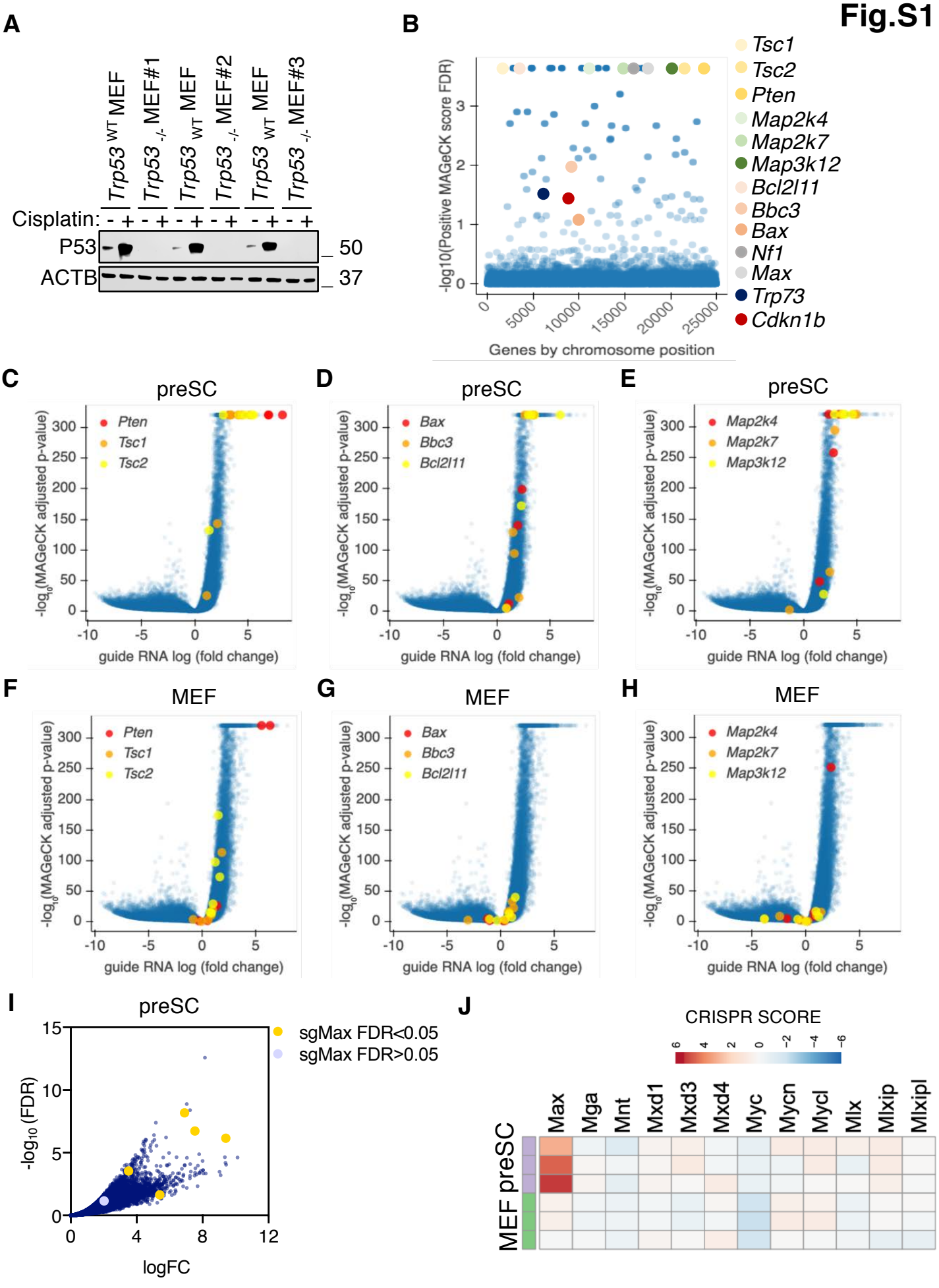
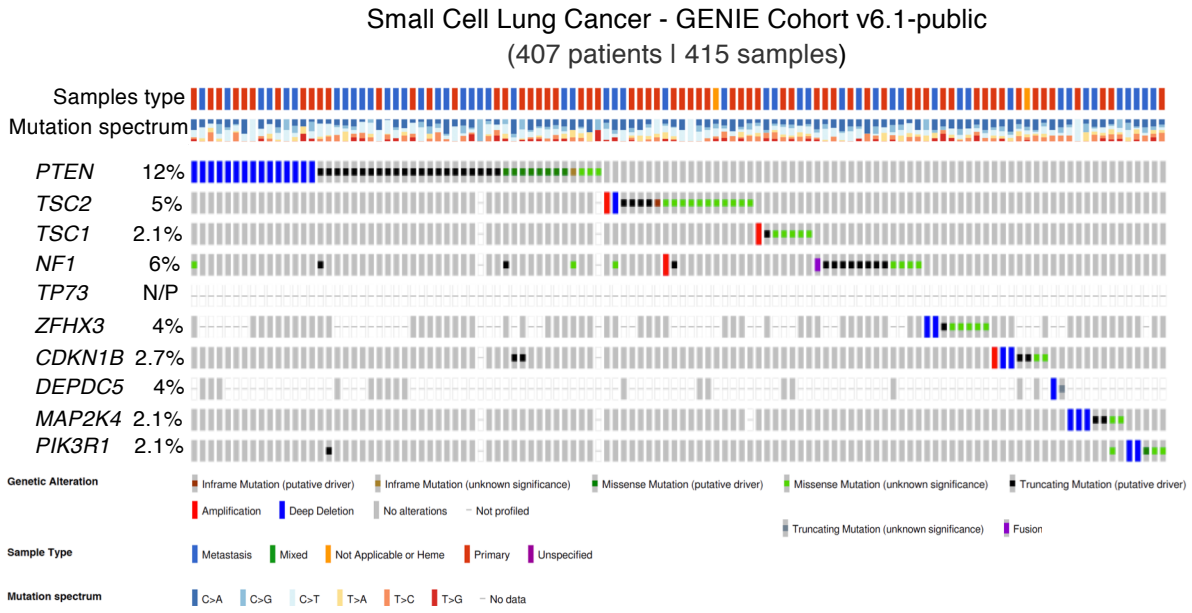

A
Trp53 WT MEF
Trp53 -/- MEF#1
Trp53 WT MEF
Trp53 -/- MEF#2
Trp53 WT MEF
Trp53 -/- MEF#3
Cisplatin: - + - + - + - + - + - +
P53
ACTB
50
37
B
-log10(Positive MAGeCK score FDR)
Genes by chromosome position
Tsc1
Tsc2
Pten
Map2k4
Map2k7
Map3k12
Bcl2l11
Bbc3
Bax
Nf1
Max
Trp73
Cdkn1b
C
preSC
Pten
Tsc1
Tsc2
-log10(MAGeCK adjusted p-value)
guide RNA log (fold change)
D
preSC
Bax
Bbc3
Bcl2l11
E
preSC
Map2k4
Map2k7
Map3k12
F
MEF
Pten
Tsc1
Tsc2
G
MEF
Bax
Bbc3
Bcl2l11
H
MEF
Map2k4
Map2k7
Map3k12
I
preSC
sgMax FDR<0.05
sgMax FDR>0.05
-log10 (FDR)
logFC
J
CRISPR SCORE
MEF preSC
Max
Mga
Mnt
Mxd1
Mxd3
Mxd4
Myc
Mycn
Mycl
Mlx
Mlxip
Mlxipl

**Figure S1, related to Figure 1. A genome scale CRISPR inactivation screen identifies candidate SCLC tumor suppressor genes and pathways**

**A**, Immunoblot for P53 in *Trp53* wild type or deleted MEFs treated with cisplatin at 10 μM for 48 hours. ACTB was used as loading control. **B**, Genomic position of top hits in preSCs. **C-E**, Volcano plot for selected genes in the PI3K-AKT pathway (**C**), the apoptotic pathway (**D**) and the SAPK pathway (**E**). Individual sgRNAs are depicted with adjusted p value from MAGeCK analysis plotted against the log2 fold change. **F-H**, As in **C-E** but for *Trp53*$^{-/-}$ MEFs. **I**, Scatter plot using EdgeR analysis showing each of the sgRNAs targeting *Max*, with 5/6 guides showing significant enrichment. **J**, Heat map for the MYC transcription factor network members in preSCs and MEFs. CRISPR scores are shown.

# Fig.S2

**A**




**B**

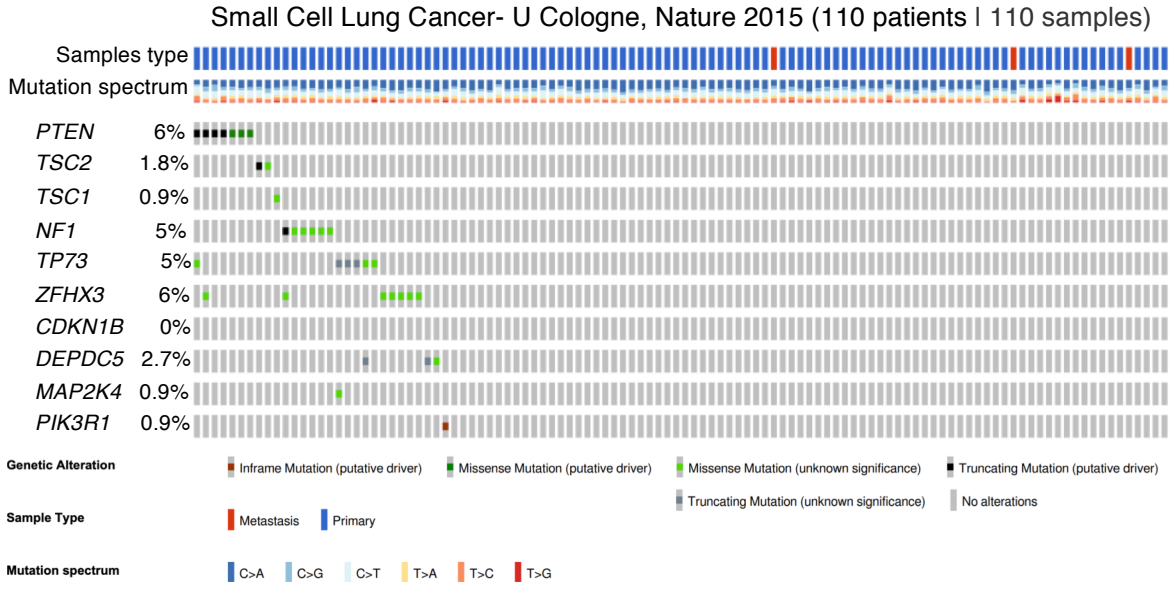



**Figure S2, related to Figure 1. Genomic analyses for the selected CRISPR-Cas9 screen hits**

**A,** Oncoprint, for the selected genes, using the GENIE Cohort v6.1-public SCLC sample set, which includes targeted sequencing data for a subset of cancer-mutated genes and includes many metastatic SCLC samples (407 patients, 415 samples). **B,** Oncoprint, for the selected genes, using the George et al SCLC dataset (110 patients, 110 samples), which is mainly composed of primary SCLC samples.

# Fig.S3

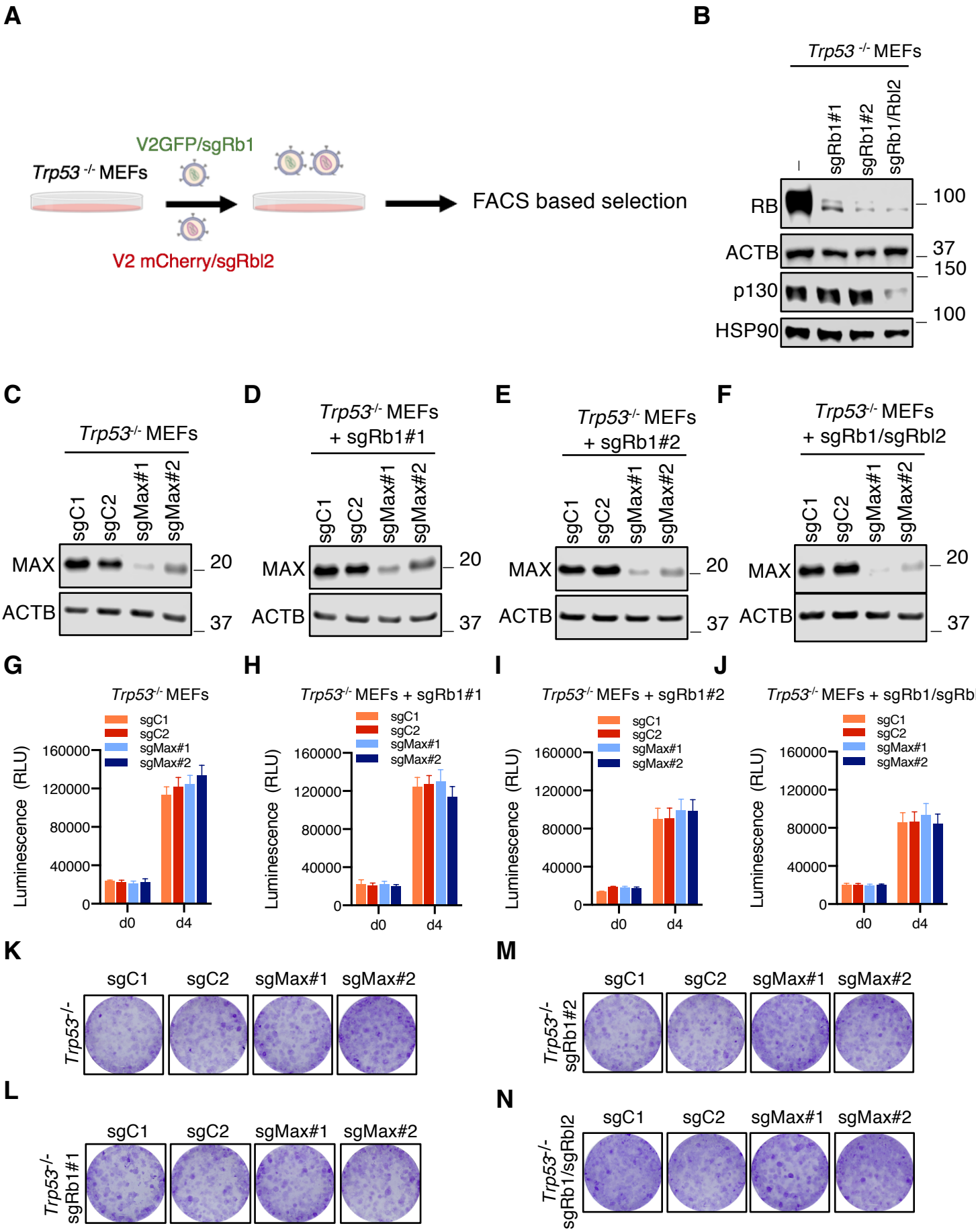

A
V2GFP/sgRb1
Trp53 -/- MEFs
V2 mCherry/sgRbl2
FACS based selection
B
Trp53 -/- MEFs
sgRb1#1
sgRb1#2
sgRb1/Rbl2
RB
ACTB
p130
HSP90
100
37
150
100
C
Trp53-/- MEFs
sgC1
sgC2
sgMax#1
sgMax#2
MAX
ACTB
20
37
D
Trp53-/- MEFs + sgRb1#1
E
Trp53-/- MEFs + sgRb1#2
F
Trp53-/- MEFs + sgRb1/sgRbl2
G
Trp53-/- MEFs
H
Trp53-/- MEFs + sgRb1#1
I
Trp53-/- MEFs + sgRb1#2
J
Trp53-/- MEFs + sgRb1/sgRbl2
Luminescence (RLU)
160000
120000
80000
40000
0
d0
d4
K
Trp53-/-
M
Trp53-/- sgRb1#2
L
Trp53-/- sgRb1#1
N
Trp53-/- sgRb1/sgRbl2

**Figure S3, related to Figure 3. MAX depletion in *Rb1* and/or *Rbl2* depleted *Trp53*$^{-/-}$ MEFs**

**A**, Schematic of the strategy to generate *Rb1* and/or *Rbl2* depleted MEFs. **B**, Immunoblotting results following lentiviral sgRNA expression for the indicated proteins in *Trp53*$^{-/-}$ MEFs. ACTB or HSP90 were used as a loading control. **C-F**, Immunoblotting results following lentiviral transduction of sgRNA(s) targeting *Max* in *Trp53*$^{-/-}$ MEFs **(C)**, *Trp53*$^{-/-}$; sgRb1#1 MEFs **(D)**, *Trp53*$^{-/-}$; sgRb1#2 MEFs **(E)** and *Trp53*$^{-/-}$; sgRb1; sgRbl2 MEFs **(F)**. ACTB was used as a loading control. **(G-J)**, CellTiter-Glo viability assay for the indicated MEFs are shown. Cells were seeded at $3x10^3$ cells/well (96 well plate). Representative experiments from 4 independent experiments are shown. Error bars represent mean ±SD. **(K-N)**, Colony formation assays for the indicated MEFs are shown. Cells were seeded at $5x10^3$ cells/well (6 well plate), expanded for 9 days after which crystal violet was used to stain colonies (n=3 independent experiments).

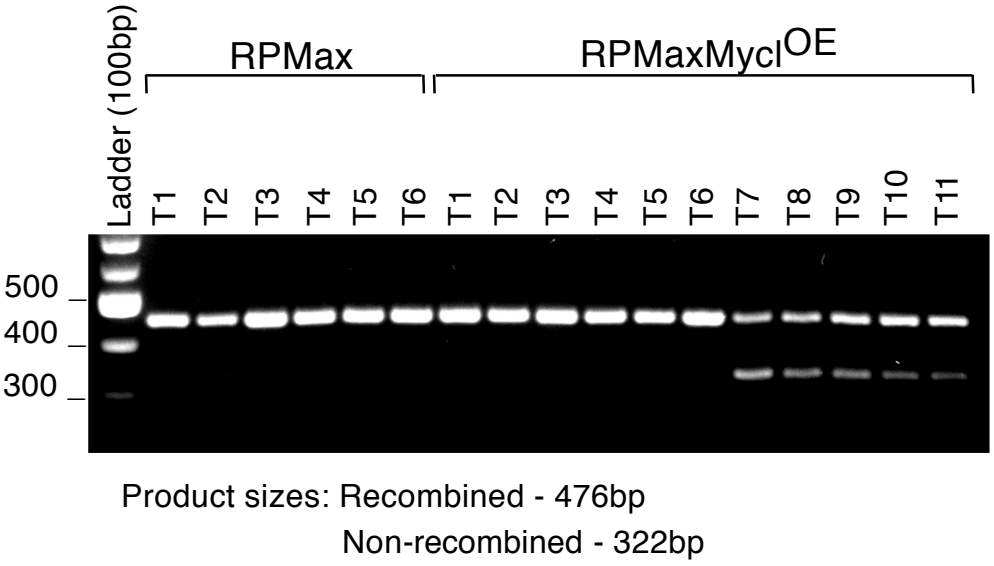



**Figure S4, related to Figure 4. *Max* recombination in RPMaxMycl$^{OE}$ mice**

Genomic DNA was extracted for the indicated tumors and PCR performed with three primers to distinguish the recombined (476bp) and non-recombined *Max* allele (322bp). RPMax tumors were used as positive controls for *Max* recombination (both alleles, T1-T6). Eleven RPMaxMycl$^{OE}$ tumors are shown and grouped into *Max*-deleted (both copies, T1-T6) and *Max*-retained (heterozygous, T7-T11).

# Fig.S5

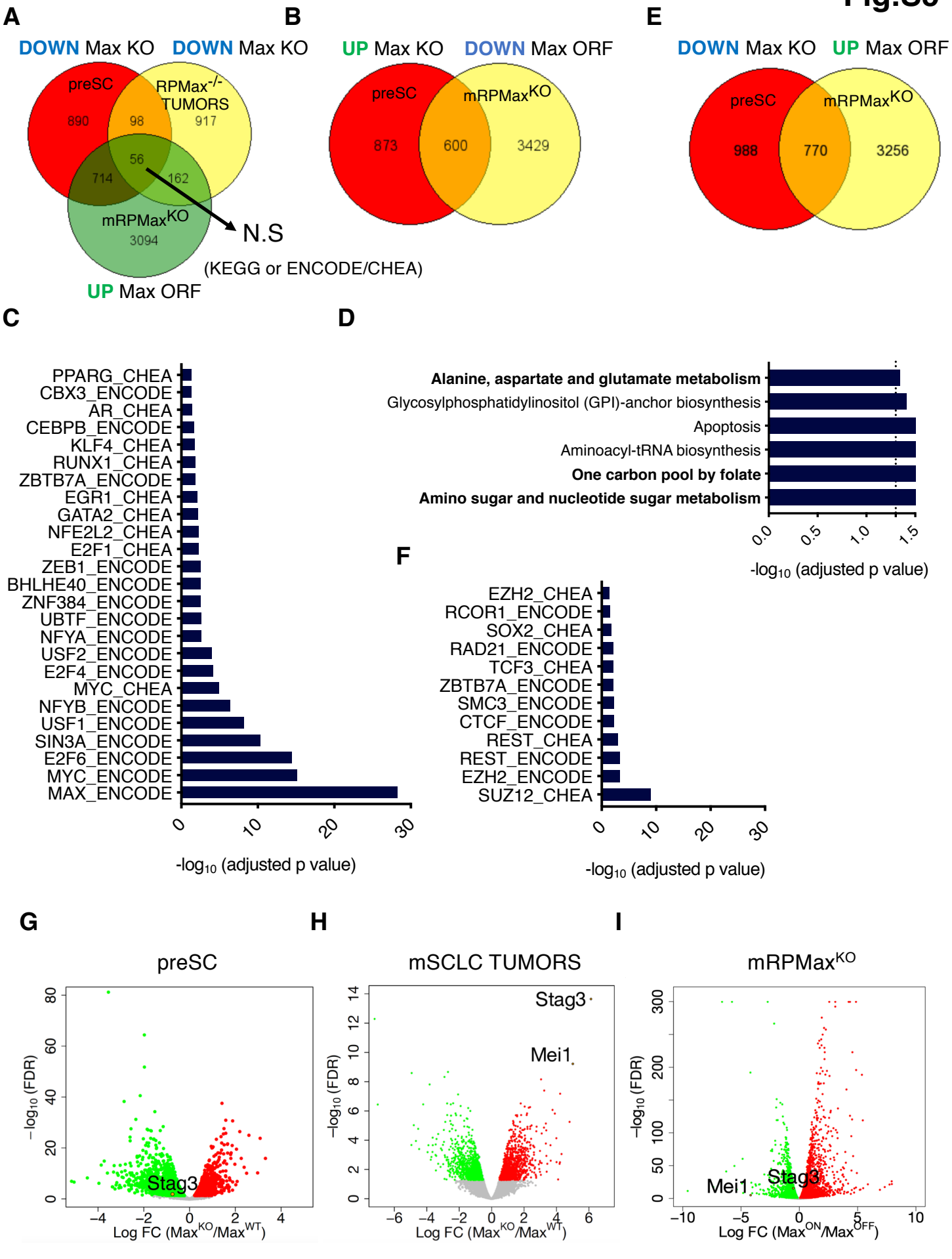
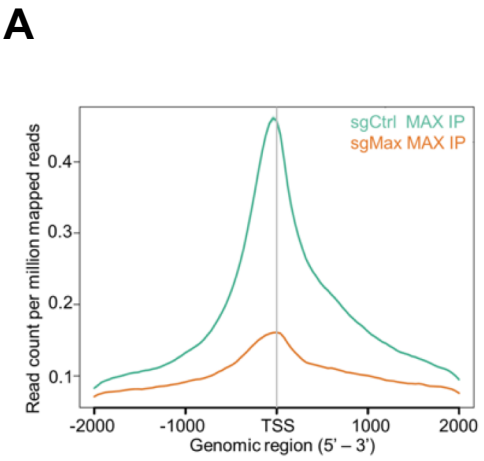
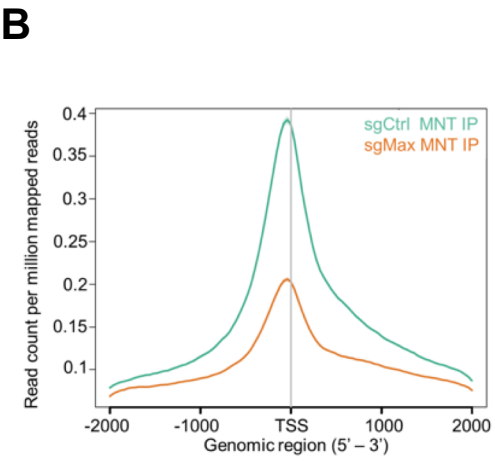

A
DOWN Max KO
DOWN Max KO
preSC
RPMax-/- TUMORS
890
98
917
56
714
162
mRPMaxKO
3094
N.S
(KEGG or ENCODE/CHEA)
UP Max ORF
B
UP Max KO
DOWN Max ORF
preSC
mRPMaxKO
873
600
3429
E
DOWN Max KO
UP Max ORF
preSC
mRPMaxKO
988
770
3256
C
PPARG_CHEA
CBX3_ENCODE
AR_CHEA
CEBPB_ENCODE
KLF4_CHEA
RUNX1_CHEA
ZBTB7A_ENCODE
EGR1_CHEA
GATA2_CHEA
NFE2L2_CHEA
E2F1_CHEA
ZEB1_ENCODE
BHLHE40_ENCODE
ZNF384_ENCODE
UBTF_ENCODE
NFYA_ENCODE
USF2_ENCODE
E2F4_ENCODE
MYC_CHEA
NFYB_ENCODE
USF1_ENCODE
SIN3A_ENCODE
E2F6_ENCODE
MYC_ENCODE
MAX_ENCODE
0
10
20
30
-log10 (adjusted p value)
D
Alanine, aspartate and glutamate metabolism
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis
Apoptosis
Aminoacyl-tRNA biosynthesis
One carbon pool by folate
Amino sugar and nucleotide sugar metabolism
0.0
0.5
1.0
1.5
-log10 (adjusted p value)
F
EZH2_CHEA
RCOR1_ENCODE
SOX2_CHEA
RAD21_ENCODE
TCF3_CHEA
ZBTB7A_ENCODE
SMC3_ENCODE
CTCF_ENCODE
REST_CHEA
REST_ENCODE
EZH2_ENCODE
SUZ12_CHEA
0
10
20
30
-log10 (adjusted p value)
G
preSC
−log10 (FDR)
Stag3
Log FC (MaxKO/MaxWT)
H
mSCLC TUMORS
Stag3
Mei1
−log10 (FDR)
Log FC (MaxKO/MaxWT)
I
mRPMaxKO
−log10 (FDR)
Mei1
Stag3
Log FC (MaxON/MaxOFF)

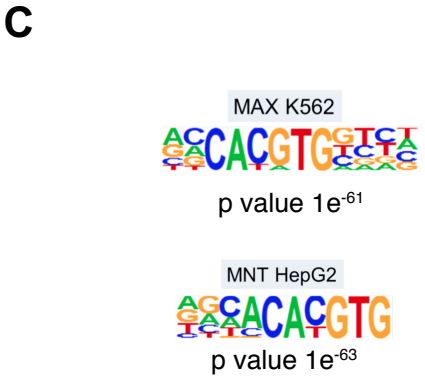
**Figure S5, related to Figure 5. Transcriptional analyses of MAX altered SCLCs**

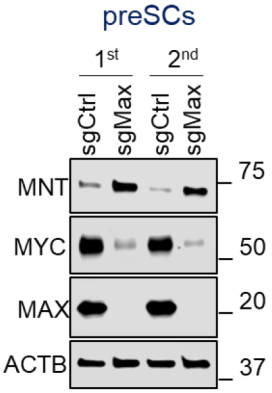
**A**, Venn diagram of the genes downregulated upon *Max* loss in preSCs and mSCLCs tumors and upregulated upon MAX restoration in mRPMaxKO mSCLC line. N.S: not significant. **B**, Venn diagram of the upregulated genes upon *Max* loss in preSCs and downregulated upon MAX restoration in mRPMaxKO mSCLC line. The list of genes used to generate the Venn diagrams in **A** and **B** were selected from EdgeR analysis with a cut off of FDR<0.05. **C**, ENCODE or CHEA binding analysis for the 600 genes commonly shared across the two matched cell based models. An adjusted p value of p<0.05 was considered significant. **D**, KEGG pathway analysis for the 600 genes commonly shared (see **S5B**) across the two matched cell-based models. An adjusted p value of p<0.05 was considered significant. **E**, Venn diagram of the downregulated genes upon *Max* loss in preSCs and upregulated upon MAX restoration in mRPMaxKO mSCLC line. The list of genes used to generate the Venn diagram were selected from EdgeR analysis with a cut off of FDR<0.05. **F**, ENCODE or CHEA binding analysis for the 770 genes commonly shared in the 2 matched cell based models. An adjusted p value of p<0.05 was considered significant. **G-I**, Volcano plot from the preSC *Max*-KO vs *Max*-WT (**G**), mSCLC tumors *Max* deleted vs *Max* control (**H**) and mRPMaxKO, a MAX null cell line inducible for MAX restoration (**I**) is shown. Significant genes upregulated upon *Max* perturbation (red) or downregulated (green). An FDR<0.05 was considered significant. Individual genes of interest are depicted. *Stag3* and *Mei1* are shown, when detected, for each model.

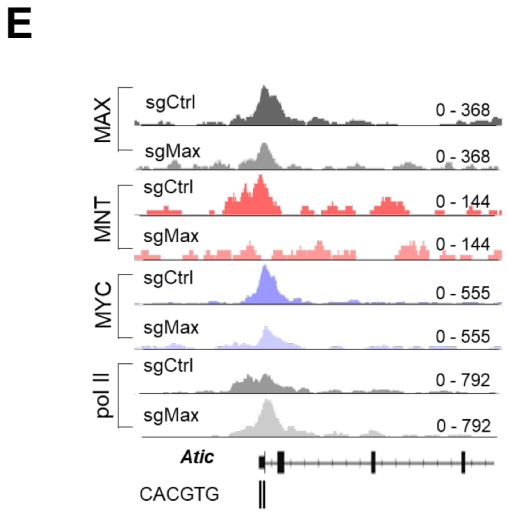
# Fig.S6

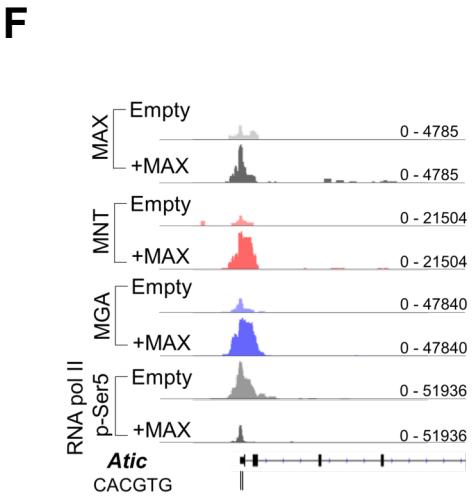

A
sgCtrl MAX IP
sgMax MAX IP
Read count per million mapped reads
Genomic region (5' – 3')
-2000
-1000
TSS
1000
2000


B
sgCtrl MNT IP
sgMax MNT IP
Read count per million mapped reads
Genomic region (5' – 3')
-2000
-1000
TSS
1000
2000


C
MAX K562
p value 1e-61
MNT HepG2
p value 1e-63


D
preSCs
1st
2nd
sgCtrl
sgMax
sgCtrl
sgMax
MNT
MYC
MAX
ACTB
75
50
20
37


E
MAX
MNT
MYC
pol II
sgCtrl
sgMax
0 - 368
0 - 144
0 - 555
0 - 792
Atic
CACGTG


F
MAX
MNT
MGA
RNA pol II p-Ser5
Empty
+MAX
0 - 4785
0 - 21504
0 - 47840
0 - 51936
Atic
CACGTG

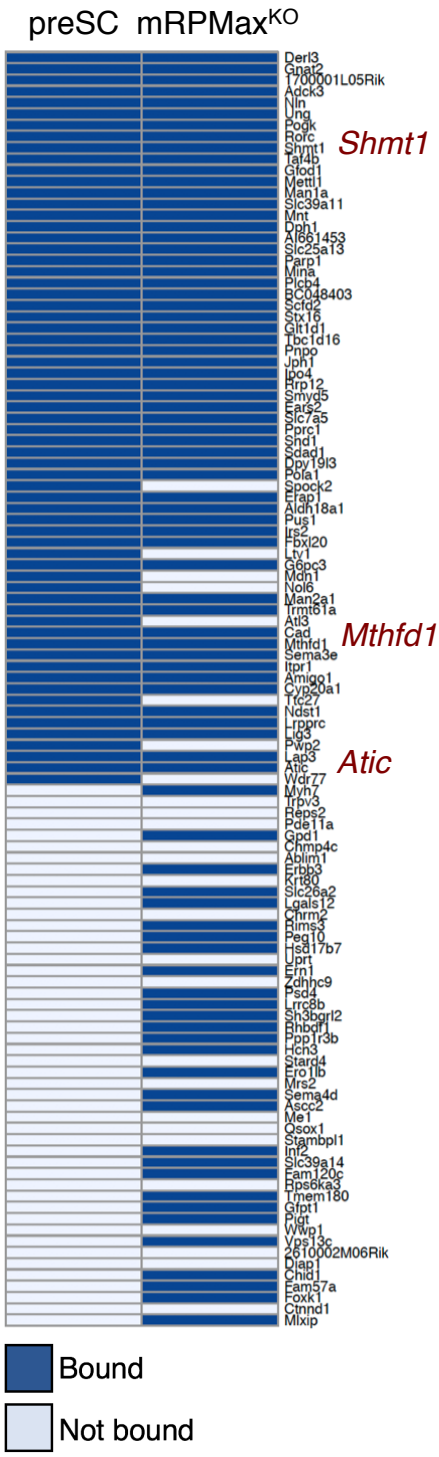

G
preSC
mRPMaxKO
Shmt1
Mthfd1
Atic
Bound
Not bound

**Figure S6, related to Figure 6. Genomic occupancy of MAX altered SCLCs**

Metaplots depicting MAX (**A**) and MNT (**B**) occupancy in control and *Max*-null preSCs. **C**, HOMER analysis for known motifs enriched for in MAX-bound sequences in sgCtrl preSCs **D**, Western blots showing MYC, MAX and MNT levels in WT and *Max*-null preSCs. **E**, Representative tracks for MAX, MNT, MYC and pol II enrichment at the Atic promoter in preSCs. **F**, Representative tracks depicting MAX, MNT MGA and RNA pol II phospho-Ser5 at the Atic promoter in empty or mRPMaxKO MAX-restored SCLC cell line. **G**, Heatmap of MAX bound genes in both preSCs and mRPMaxKO models. Highlighted in red are selected one carbon genes.

Fig.S7

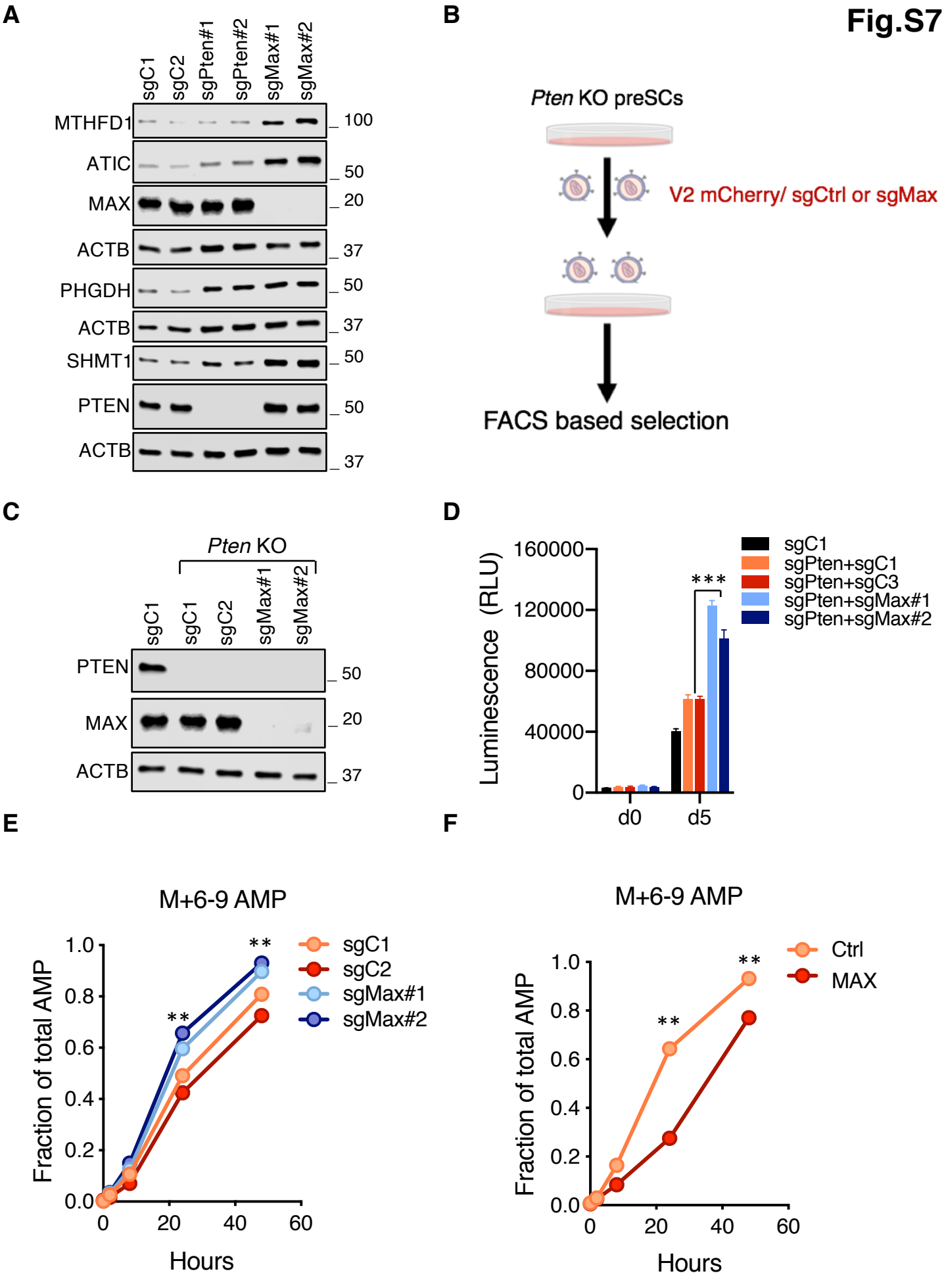

A
sgC1
sgC2
sgPten#1
sgPten#2
sgMax#1
sgMax#2
MTHFD1
ATIC
MAX
ACTB
PHGDH
ACTB
SHMT1
PTEN
ACTB
100
50
20
37
50
37
50
50
37
B
Pten KO preSCs
V2 mCherry/ sgCtrl or sgMax
FACS based selection
C
Pten KO
sgC1
sgC1
sgC2
sgMax#1
sgMax#2
PTEN
MAX
ACTB
50
20
37
D
Luminescence (RLU)
160000
120000
80000
40000
0
d0
d5
***
sgC1
sgPten+sgC1
sgPten+sgC3
sgPten+sgMax#1
sgPten+sgMax#2
E
M+6-9 AMP
Fraction of total AMP
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
Hours
**
**
sgC1
sgC2
sgMax#1
sgMax#2
F
M+6-9 AMP
Fraction of total AMP
Hours
**
**
Ctrl
MAX

**Figure S7, related to Figure 7. PTEN and MAX regulation of one carbon metabolism**

**A**, Immunoblotting for the indicated one carbon proteins following lentiviral sgRNA expression for the indicated genes in preSCs. ACTB was used as a loading control. **B**, Schematic of the strategy to generate *Pten* KO preSCs depleted for MAX. **C**, Immunoblotting following lentiviral sgRNA expression for the indicated genes in preSCs. ACTB was used as a loading control. **D**, CellTiter-Glo viability assay for the indicated preSCs are shown. Cells were seeded at $7.5x10^3$ cells/well (96 well plate). Representative experiments from 4 independent experiments are shown. *** $p<0.0001$, unpaired student's t-test. **E**, Fraction carbon labelled for AMP (M+ 6-9) are shown upon *Max* loss in preSCs after incubation with U-$^{13}$C glucose. ** $p<0.01$, unpaired student's t-test. **F**, Fraction carbon labelled for AMP (M+ 6-9) are shown upon MAX restoration in mRPMax$^{KO}$ cells after incubation with U-$^{13}$C glucose. ** $p<0.01$, unpaired student's t-test.