Overexpression of the key metabolic protein CPT1A defines mantle cell lymphoma patients with poor response to standard high-dose chemotherapy independent of MIPI and complement established high-risk factors

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Supplementary Tables

Table S1. Multivariate Cox regression for CPT1A protein expression in the N-MCL2/3 cohort considering *TP53*.

Variable	MR cut-off	HR (95% CI)	Outcome	n
CTP1A	≥ 15%	11.43 (2.10 – 62.17)**	TTP	25
MIPI	Intermediate[1]	1.44 (0.39 – 5.31)		
	High[1]	6.44 (0.93 – 44.71)		
Morphology	Blastoid/pleomorphic	1.88 (0.07 – 3.84)		
Ki-67	≥ 30%	1.23 (0.19 - 3.40)		
TP53	Mutated	0.4 (0.38 – 15.79)		
CTP1A	≥ 69%	15.61 (1.20 – 203.70)*	OS	25
MIPI	Intermediate[1]	5.15 (0.74 – 35.83)		
	High[1]	8.19 (0.99 – 67.46)		
Morphology	Blastoid/pleomorphic	5.00 (0.63 – 40.07)		
Ki-67	≥ 30%	0.28 (0.03 – 2.43)		
TP53	Mutated	0.72 (0.09 – 5.54)		

[1]MIPI low risk was used as reference category.

* p-value < 0.05, ** p-value < 0.01

CI: Confidence Interval; HR: Hazard Ratio; MIPI: Mantle cell lymphoma International Prognostic Index; MR cut-off, maximally selected ranked statistics cut-off values; OS: Overall Survival; TTP: Time to progression

Supplementary Figure legends

Figure S1. Kaplan-Meier estimate and log-rank test for OS in the N-MCL2/3 cohort stratified by 15% of CPT1A positive cells evaluated by IHC.

Figure S2. IHC analysis demonstrated significant association of dichotomized CPT1A protein expression in relation to proliferation (Ki-67%) and morphology in both clinical trial N-MCL2/3 and the population-based BLISS cohorts, but not in relation to SOX11 expression. Top row: N-MCL3/3 cohort; Bottom row: BLISS cohort.

Figure S3. Differential expression analysis of A) cases with high and low proliferation, B) cases with non-classic vs classic MCL, and C) cases carrying *TP53* mutations vs wt. Top panel shows p-value distribution, bottom panel volcano plot.

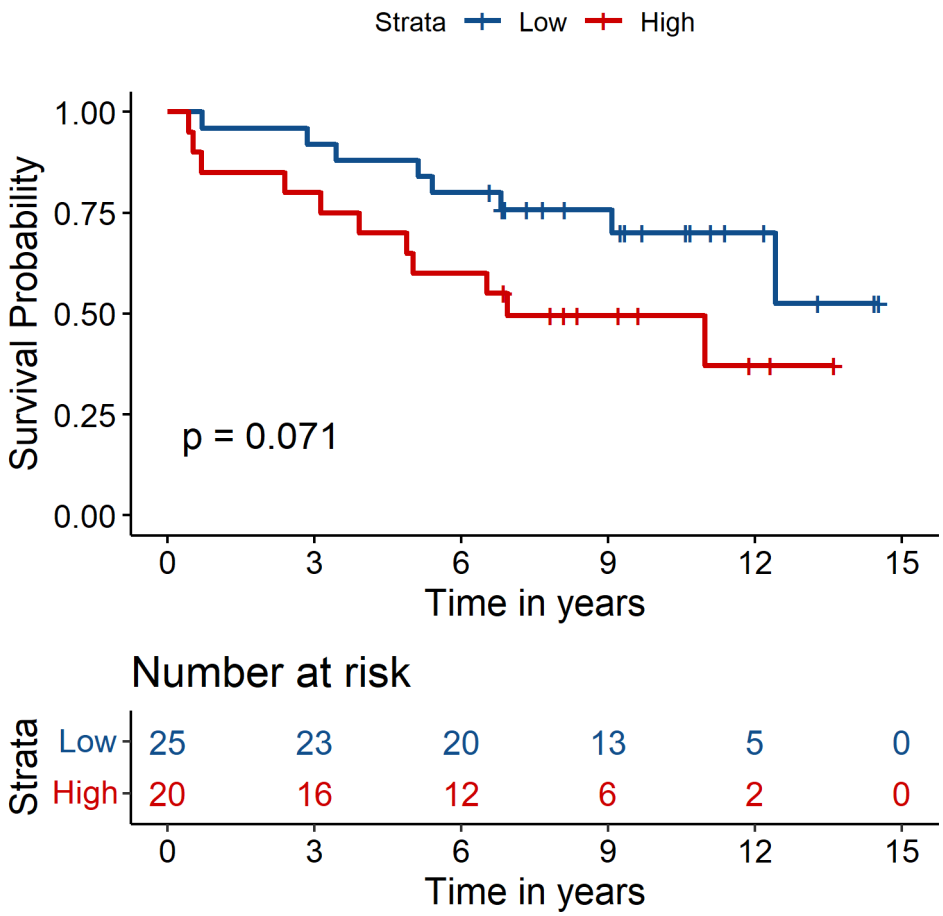
Figure S4. Pathway overview showing cell cycle regulatory genes with genes upregulated in cases with high proliferation shown in red and down-regulated genes in green.

Figure S5. FEN1 and WEE1 expression levels are associated with high-risk MCL phenotypes, as assessed in the population-based BLISS cohort. IHC analysis demonstrated significant association of dichotomized A) WEE1 in relation to Ki-67 level, and B) WEE1 in relation to histological groups. C) WEE1 protein levels were not correlated to OS (same for *WEE1* gene). D) Dichotomized FEN1 expression was associated to Ki-67 high and E) non-classic morphology. F) FEN1 expression was significantly correlated to shorter OS, using a threshold of 39% as determined by maximally selected ranked statistics.

Figure S6. Deregulated genes in *TP53* and *ATM* mutated MCL. With a threshold of p<0.05, 23 enriched pathways were identified for TP53. A) UpSet plot of the top 10 significantly (p<6E-3)

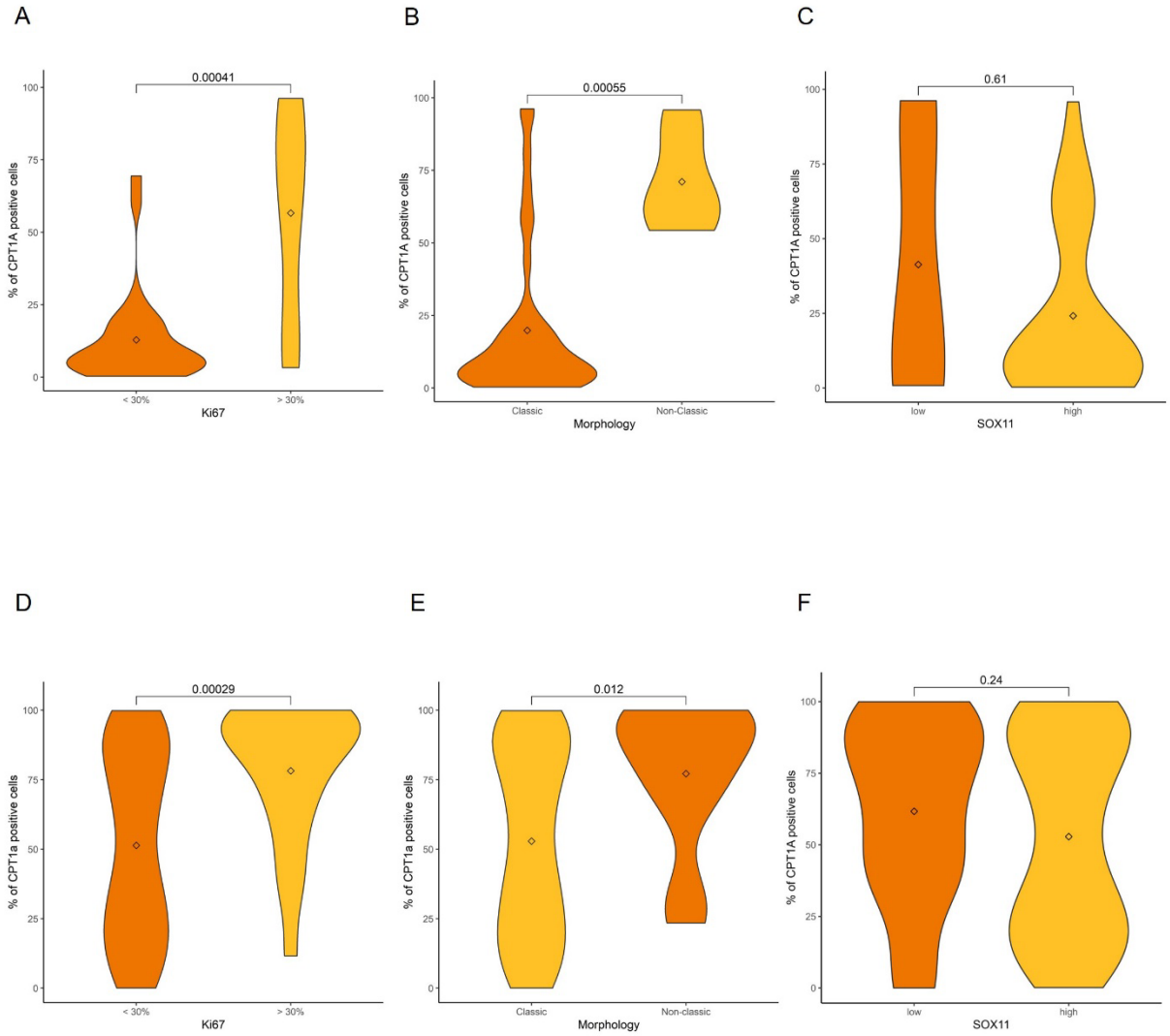
enriched pathways for *TP53* mutated vs wt, colored by relative expression with red and green denoting up- and downregulation in *TP53* mutated MCL, respectively. Heatmap with log FCs for *TP53* mutated vs wt, with log FCs for Ki-67 high vs low, and non-classic (blastoid) vs classic MCL added for comparison. B) Top eight clusters of significantly enriched pathways for *TP53* mutated vs wt. C) *TP53* and *ATM* mutations were mutually exclusive in the selected MCL cohort. Each column represents one sample, with black=mutated, white=wt, and grey=unknown. D) Top 20 significantly enriched pathways for *ATM* mutated vs wt, clustered by involved genes. E) Deregulated genes in steroid biosynthesis, and pathway cluster 2, including e.g., *TP53, PTEN*, and genes related to apoptosis (*CASP3, TUBA1C, MAPK8, MAPK9,* and *FASLG*)

SFig1.

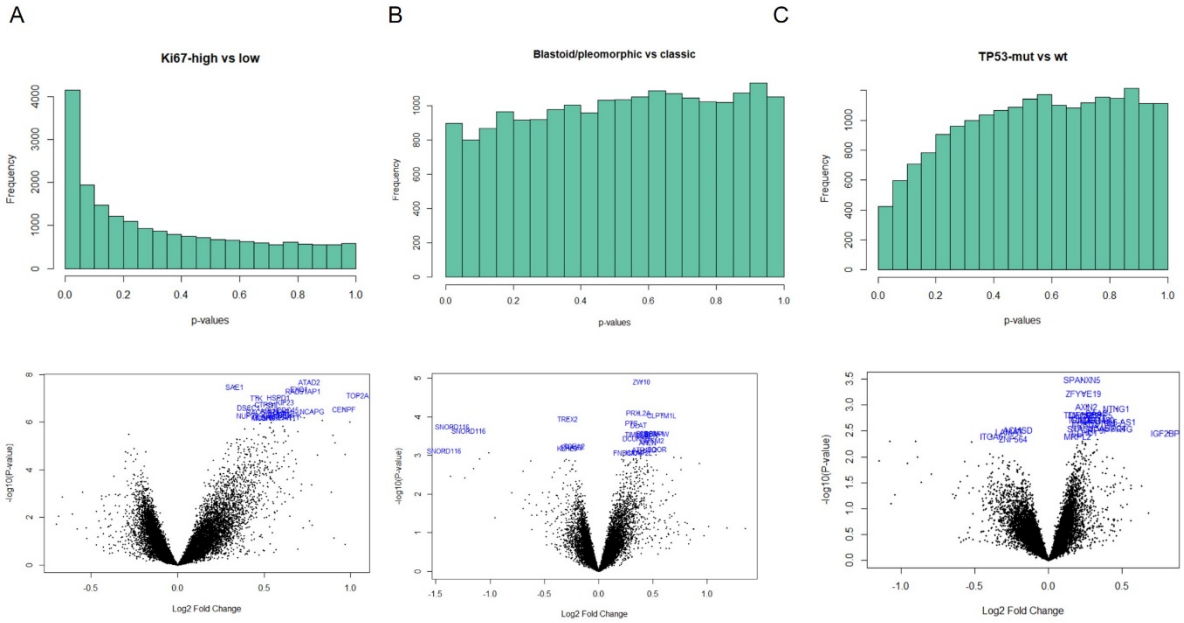

Strata
Low
High
1.00
0.75
0.50
0.25
0.00
Survival Probability
p = 0.071
0
3
6
9
12
15
Time in years

Number at risk
Strata
Low
High
25
23
20
13
5
0
20
16
12
6
2
0
0
3
6
9
12
15
Time in years

SFig2.

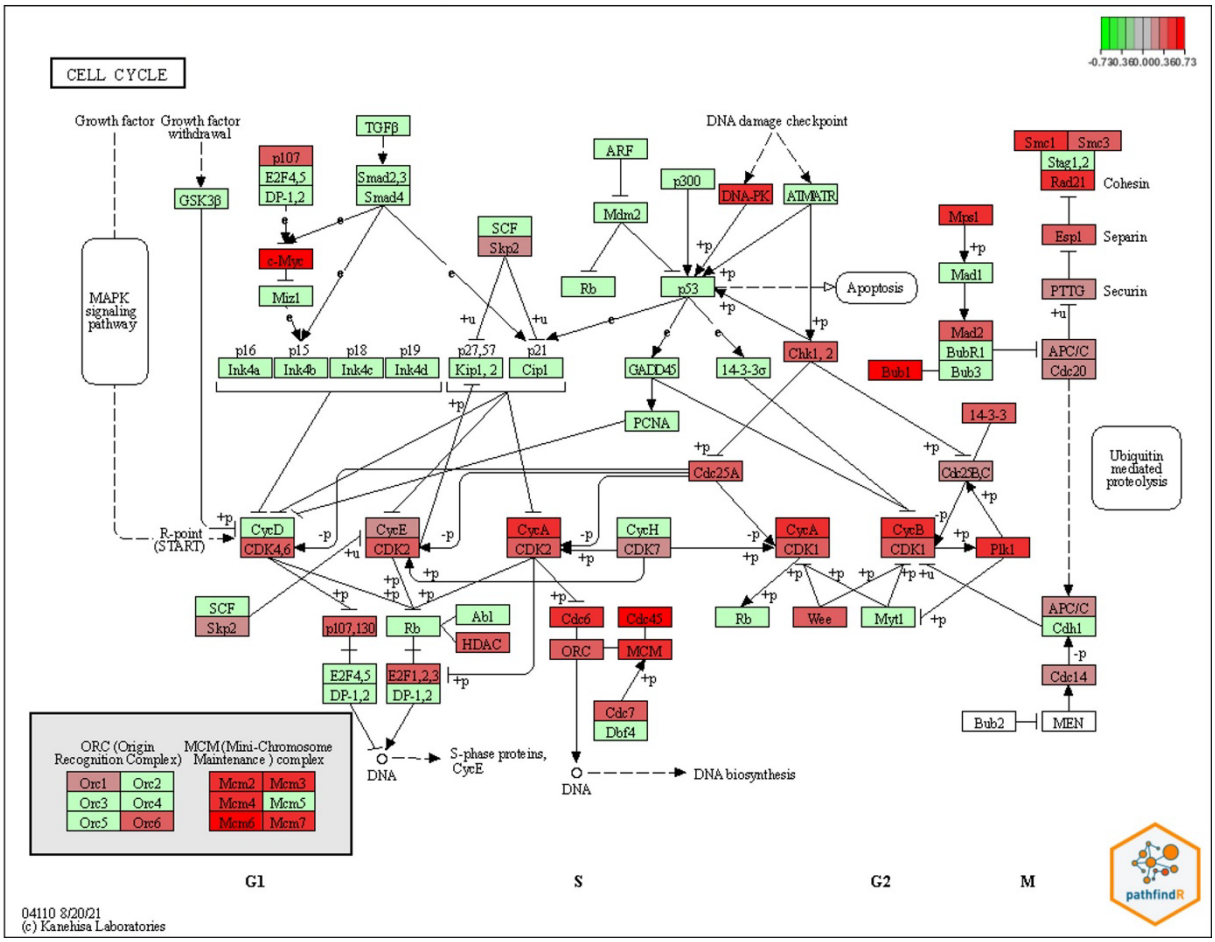

A
B
C
0.00041
0.00055
0.61
% of CPT1A positive cells
100
75
50
25
0
< 30%
> 30%
Ki67
Classic
Non-Classic
Morphology
low
high
SOX11
D
E
F
0.00029
0.012
0.24
% of CPT1a positive cells
Ki67
Morphology
SOX11

SFig3.

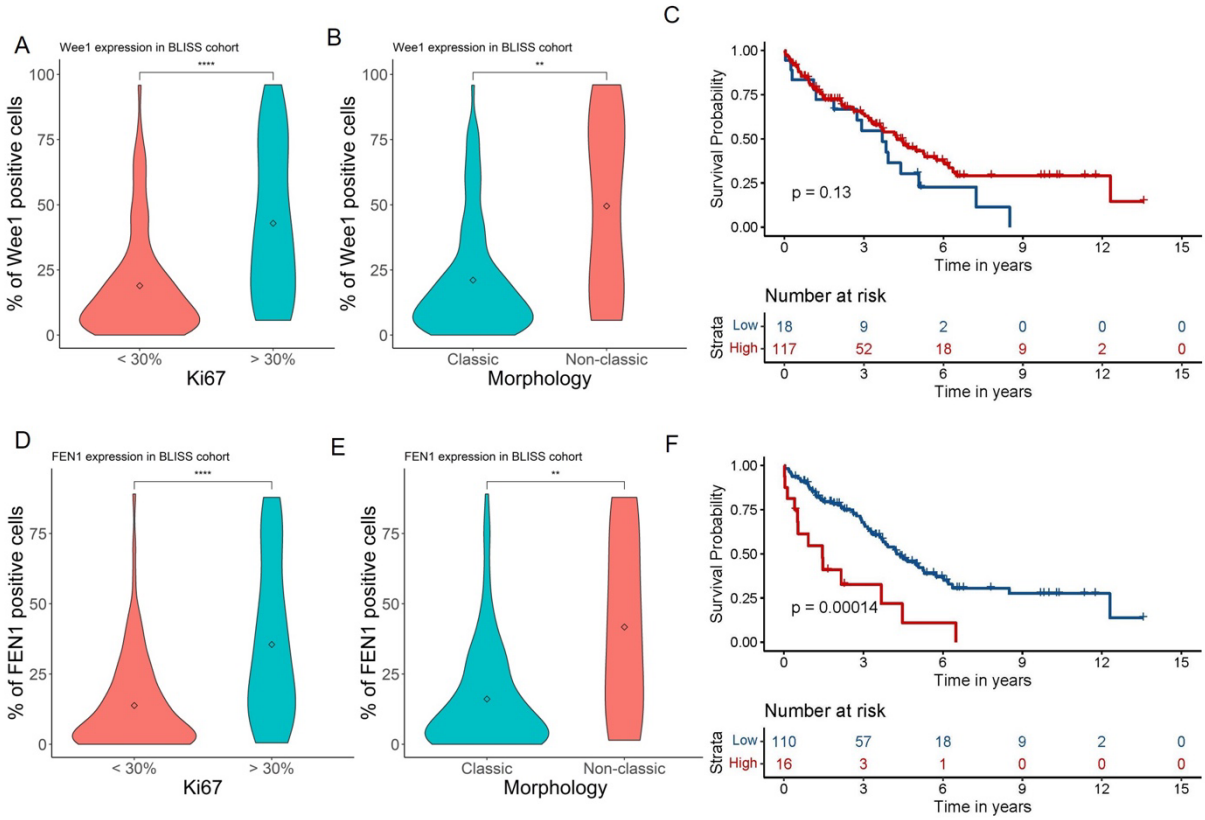

A
B
C
Ki67-high vs low
Blastoid/pleomorphic vs classic
TP53-mut vs wt
Frequency
p-values
-log10(P-value)
Log2 Fold Change
SAE1
ATAD2
TOP2A
CENPF
ZWINT
SNORD116
TREX2
SPAN0N5
ZFYVE19
AXIN2
MTMG1
IGF2BP

SFig4.

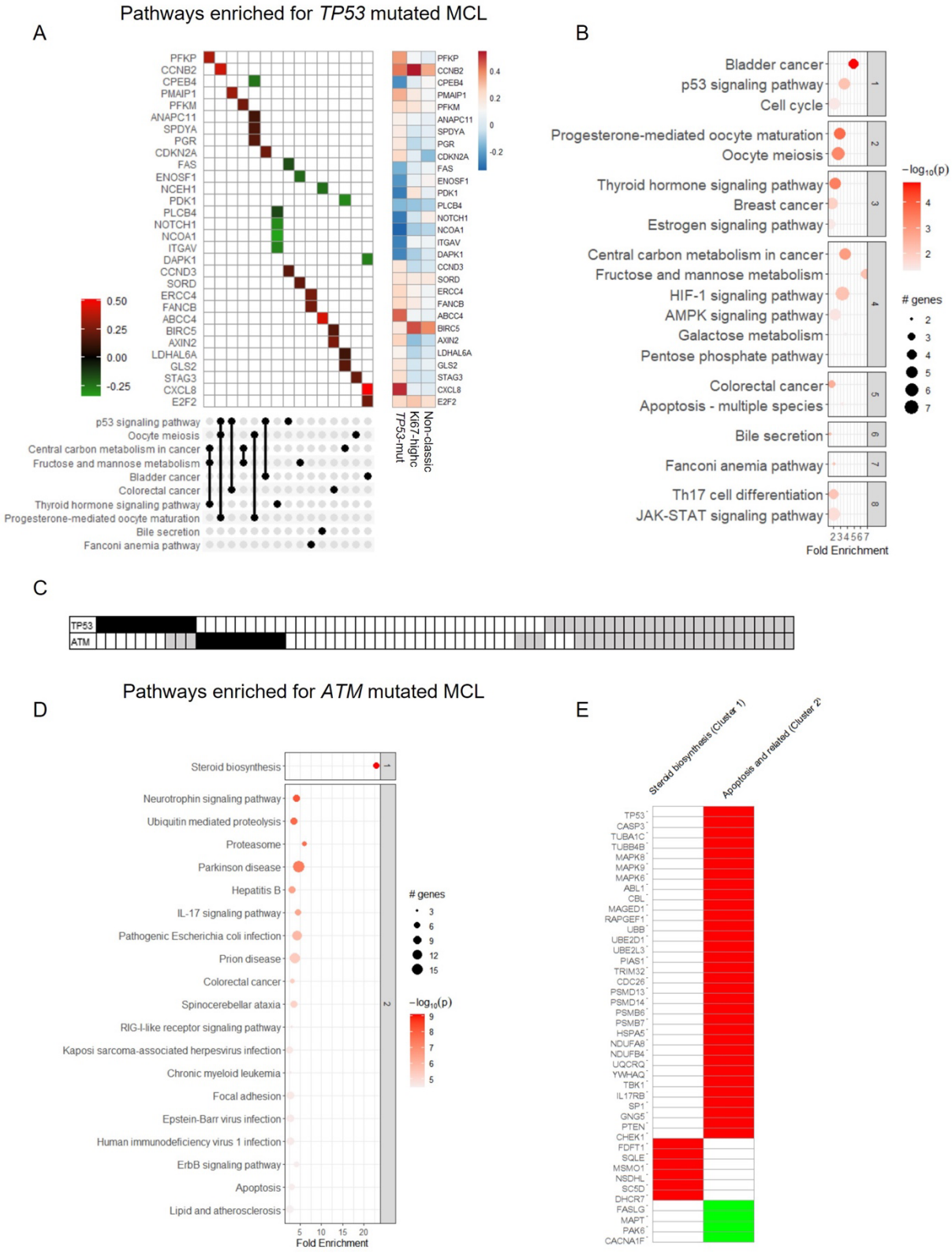
Cell cycle regulation in Ki67 high vs low MCL



SFig 5.


A
Wee1 expression in BLISS cohort
****
% of Wee1 positive cells
100
75
50
25
0
< 30%
> 30%
Ki67
B
Wee1 expression in BLISS cohort
**
% of Wee1 positive cells
Classic
Non-classic
Morphology
C
Survival Probability
1.00
0.75
0.50
0.25
0.00
p = 0.13
0
3
6
9
12
15
Time in years
Number at risk
Strata
Low
High
18
9
2
0
0
0
117
52
18
9
2
0
Time in years
D
FEN1 expression in BLISS cohort
****
% of FEN1 positive cells
75
50
25
0
< 30%
> 30%
Ki67
E
FEN1 expression in BLISS cohort
**
% of FEN1 positive cells
Classic
Non-classic
Morphology
F
Survival Probability
p = 0.00014
Time in years
Number at risk
Strata
Low
High
110
57
18
9
2
0
16
3
1
0
0
0
Time in years

SFig6.


Pathways enriched for *TP53* mutated MCL
A
B
C
Pathways enriched for *ATM* mutated MCL
D
E
Non-classic
Ki67-highc
TP53-mut
Fold Enrichment
genes
Steroid biosynthesis (Cluster 1)
Apoptosis and related (Cluster 2)
TP53
ATM