Science Advances
AAAS

Supplementary Materials for

Epidermal mutation accumulation in photodamaged skin is associated with skin cancer burden and can be targeted through ablative therapy

Ho Yi Wong *et al.*

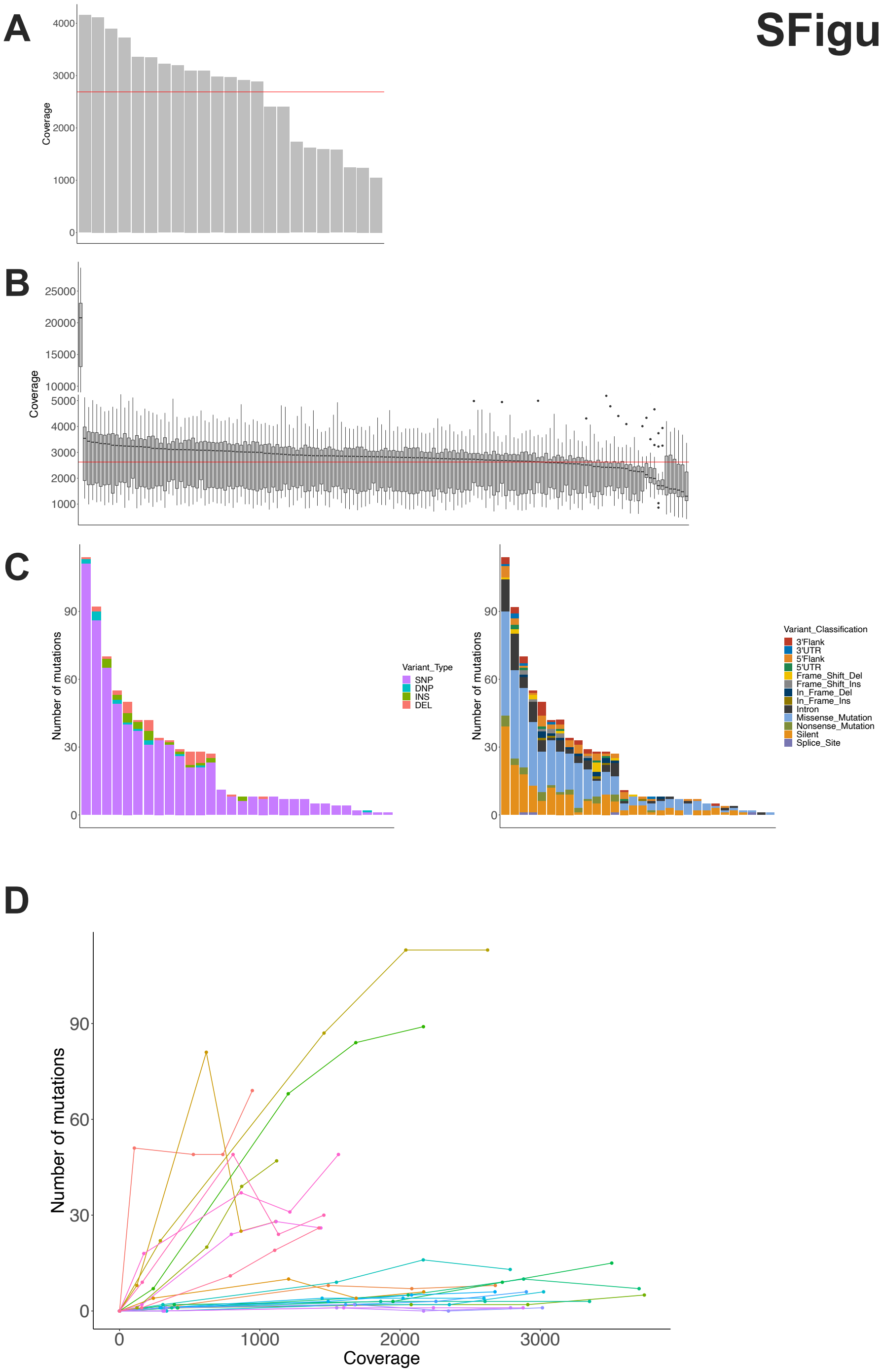
Corresponding author: Kiarash Khosrotehrani, k.khosrotehrani@uq.edu.au

Sci. Adv. **9**, eadf2384 (2023)
DOI: 10.1126/sciadv.adf2384

This PDF file includes:

Figs. S1 to S14
Tables S1 to S3

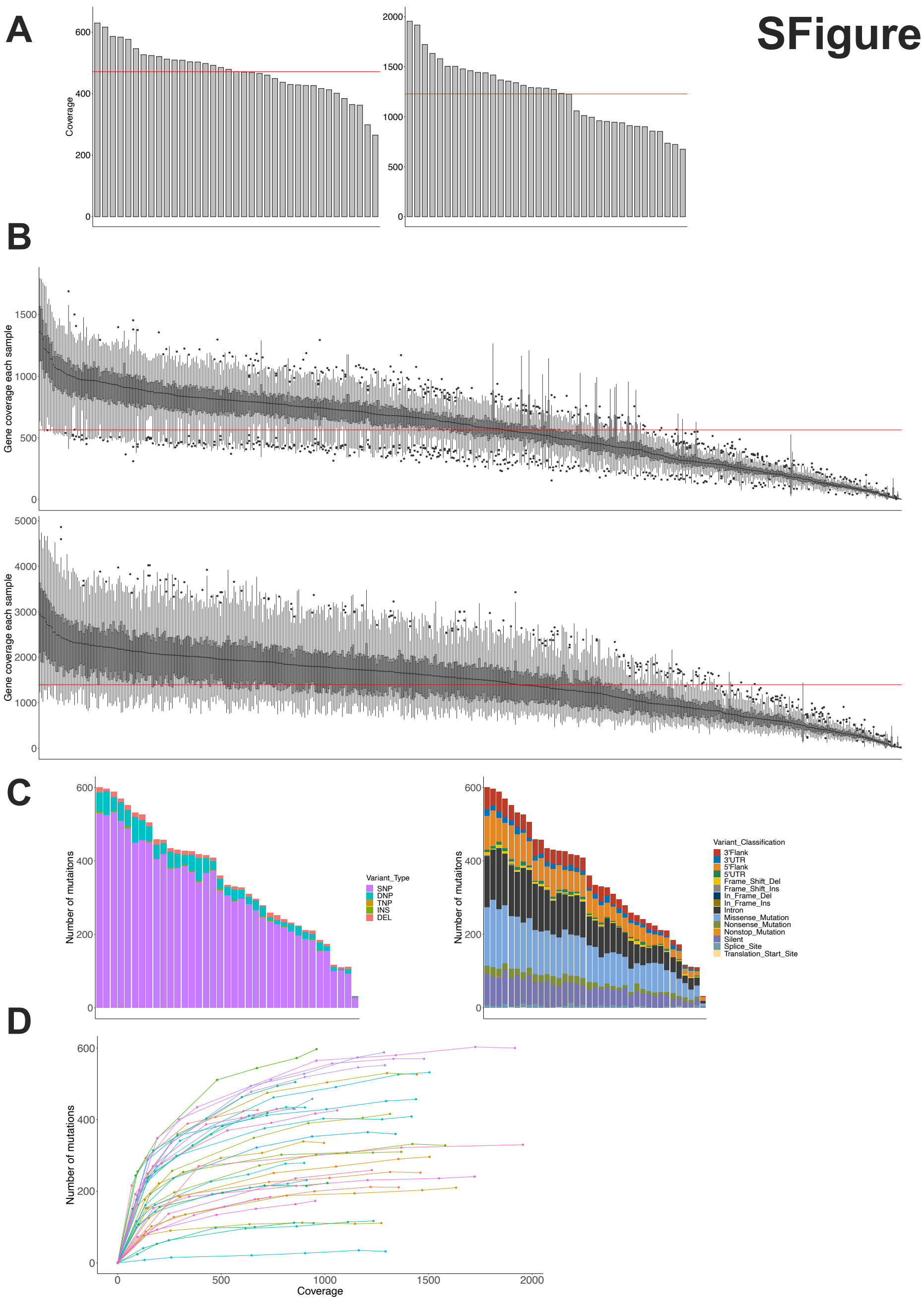
SFigure 1



SFigure 1. Sequencing depth and summary of murine samples.

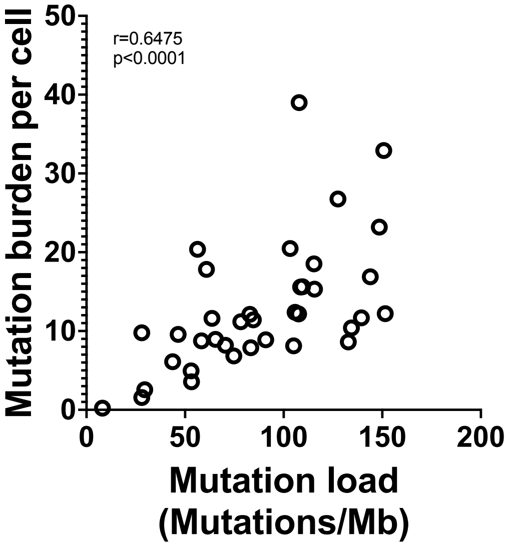
(A) Sequencing coverage for each sample. Red line indicates average coverage of all samples. **(B)** Average coverage of each panel gene. Red line indicates average coverage of all panel genes. **(C)** Distribution of variant types (left) and classifications (right) in each sample. **(D)** Downsampling analysis of murine samples. SNP: single nucleotide polymorphism. DNP: double nucleotide polymorphism. INS: insertion. DEL: deletion. UTR: untranslated region.

SFigure 2

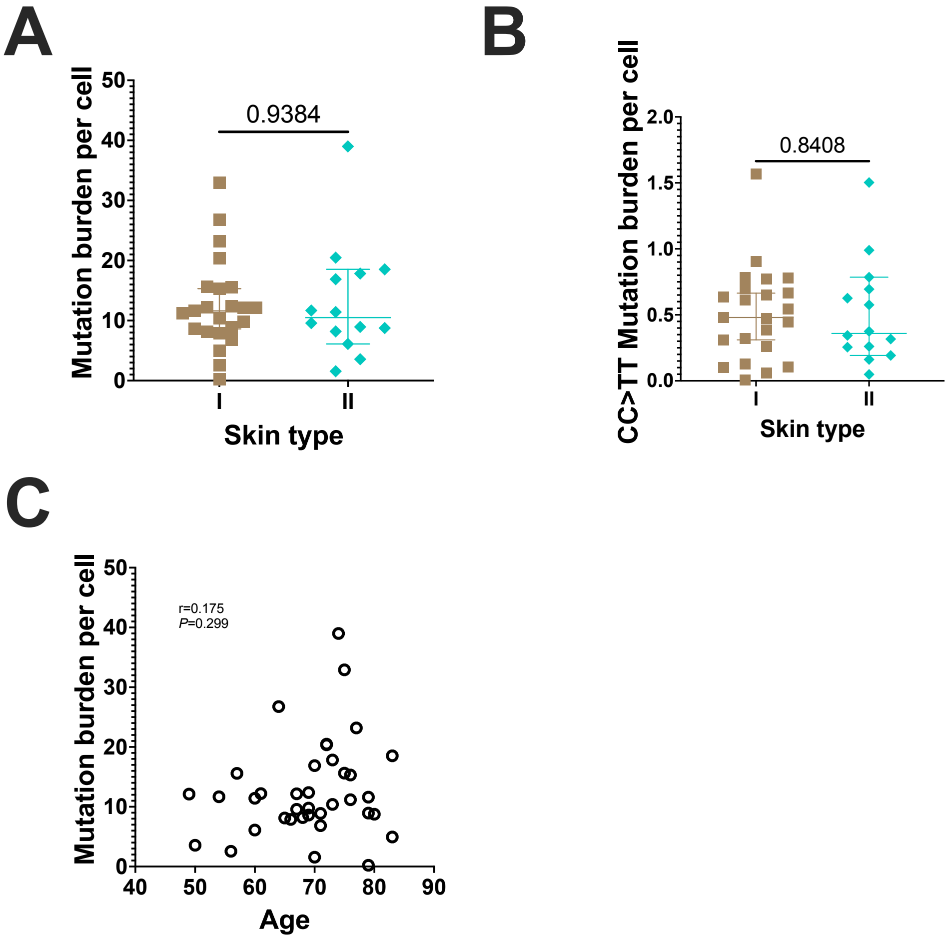


SFigure 2. Sequencing depth and summary of patient cohort.

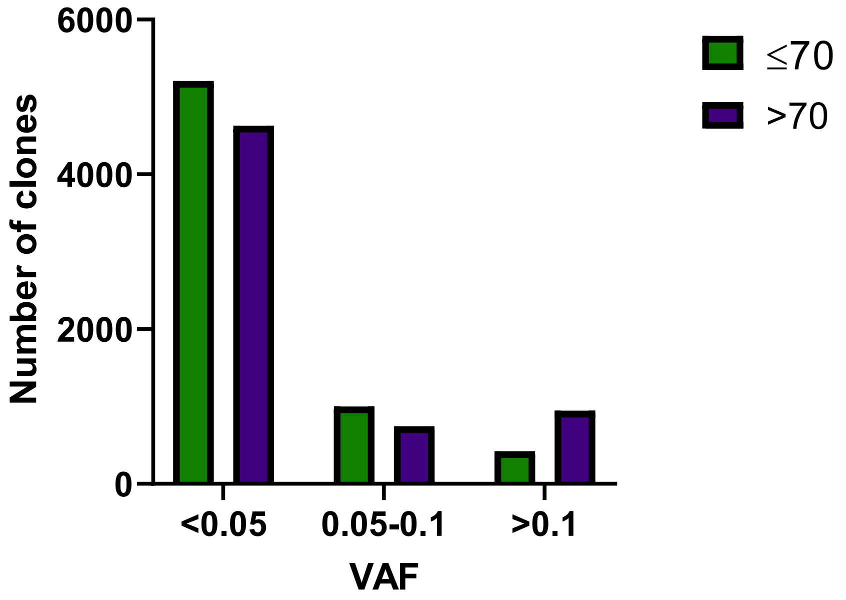
(A) Sequencing coverage of saliva (left) and skin (right) samples. Red line indicates average coverage of all samples. **(B)** Average coverage of each gene in the saliva (top) and skin (bottom) samples. Red line indicates average coverage of all panel genes. **(C)** Distribution of variant types (left) and classifications (right) of each sample. **(D)** Downsampling analysis and saturation plot of each sample. SNP: single nucleotide polymorphism. DNP: double nucleotide polymorphism. TNP: triple nucleotide polymorphism. INS: insertion. DEL: deletion. UTR: untranslated region.



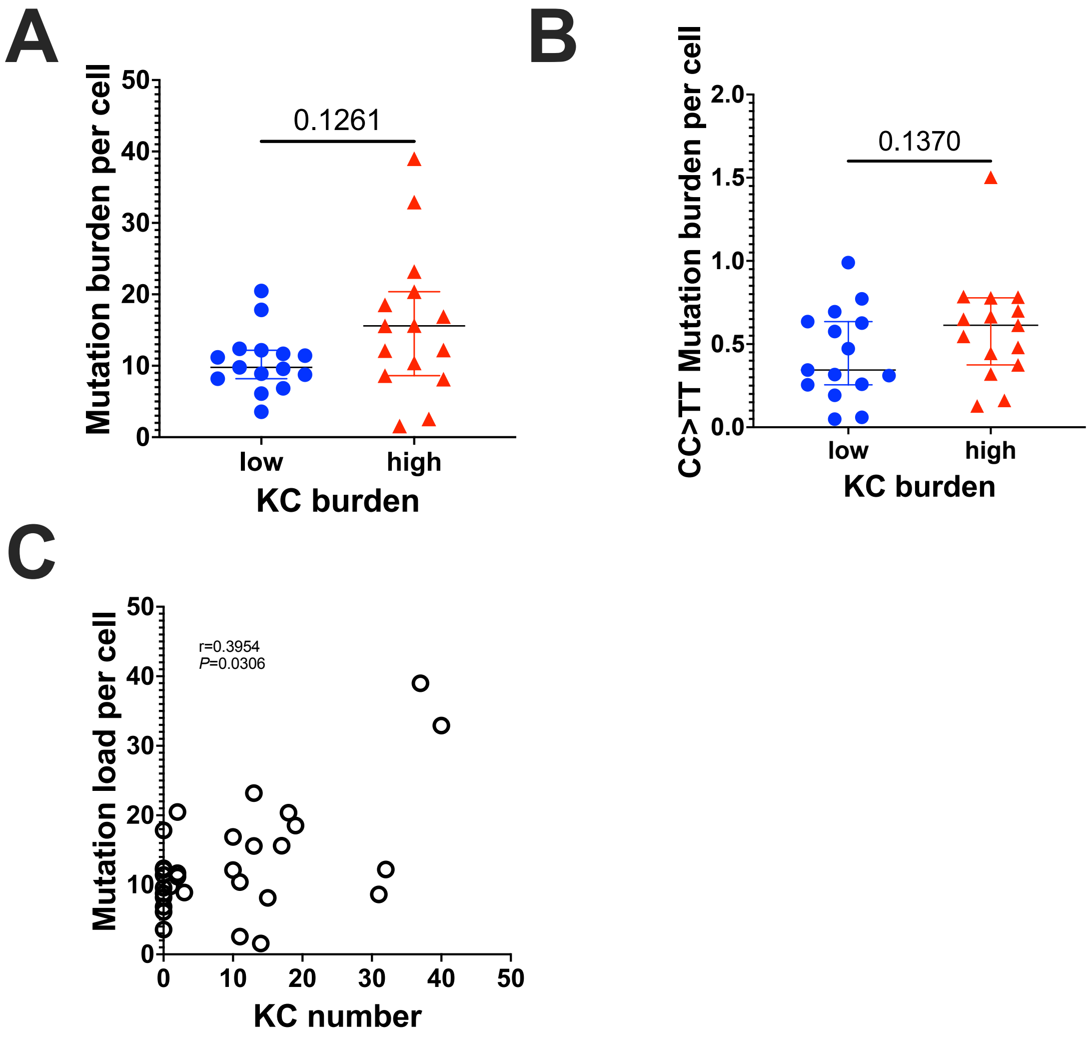
SFigure 3. Correlation of mutation load and mutation burden per cell of 37 KC patients (Spearman correlation, r=0.6475, *p*<0.0001).



SFigure 4. Mutation burden per cell analysis according to skin type and age. (A) Mutation burden per cell in patients with Fitzpatrick skin phototype I and II (Mann-Whitney test, *p*=0.93). **(B)** CC>TT mutation burden per cell in patients with Fitzpatrick skin phototype I and II (Mann-Whitney test, *p*=0.84). **(C)** Spearman correlation between mutation burden per cell and age of patients (r=0.175, *p*=0.299).

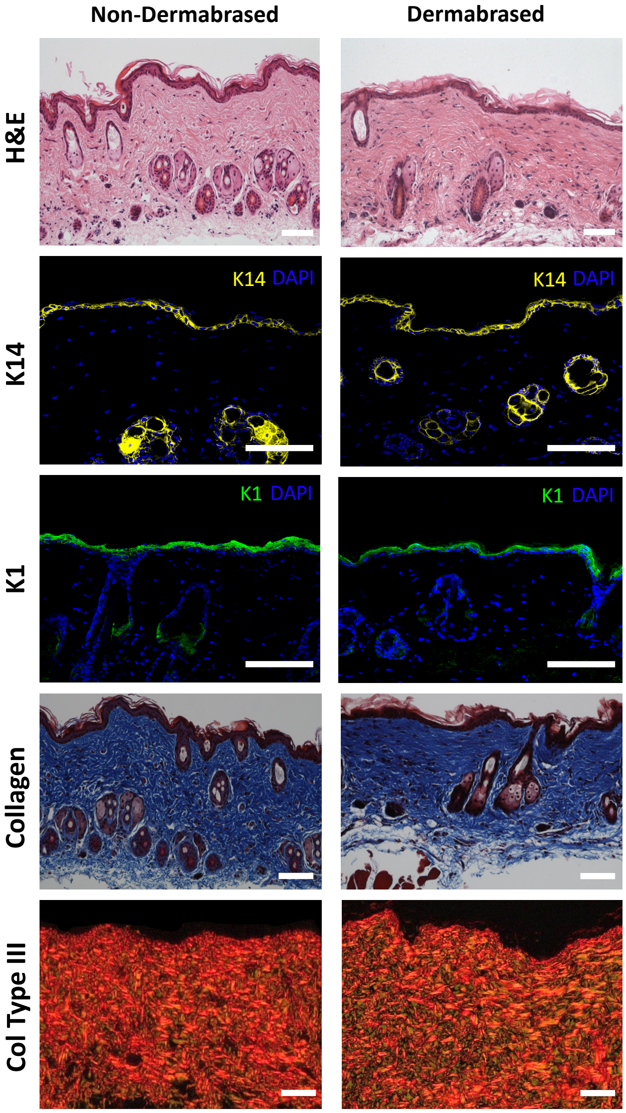


SFigure 5. Quantification of clone size distribution in ≤70 and >70-year-old groups.
The number of clones with VAF <0.05 ,0.05-0.1 and >0.1 were quantified in patients who were 70-year-old or younger and those who were more than 70 years old (Chi2 test, *p*<0.0001).



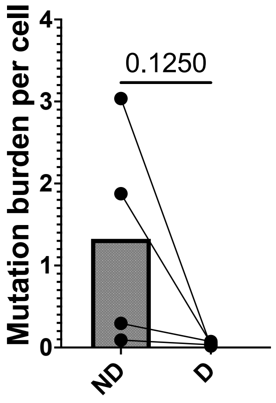
SFigure 6. Estimation of mutation burden per cell for 30 (age and sex-matched) low and high-risk KC patients.

(A) Mutation burden per cell in low and high-risk KC patients (Mann-Whitney test, *p*=0.1261). **(B)** CC>TT mutation burden per cell in low and high-risk KC patients (Mann-Whitney test, *p*=0.1370). **(C)** Spearman correlation between mutation burden per cell and the number of KC (r=0.3954, *p*=0.0306).

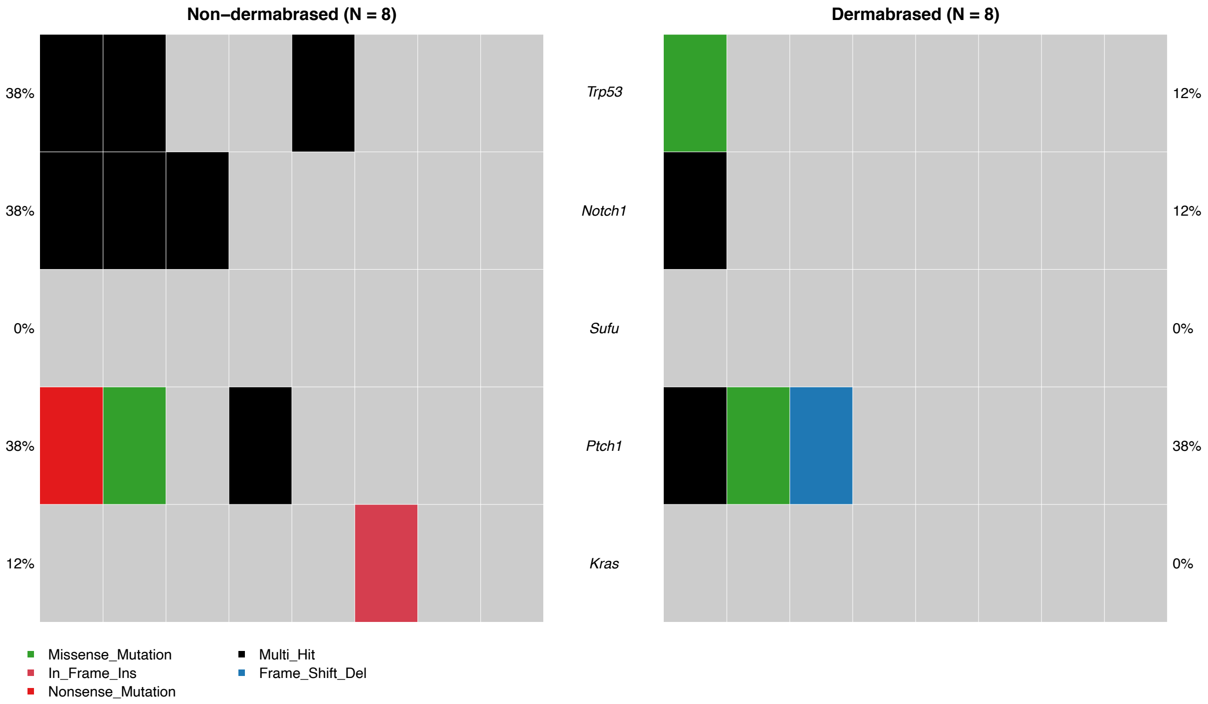


SFigure 7. Dermabrasion does not cause damage to hair follicle or fibrosis.

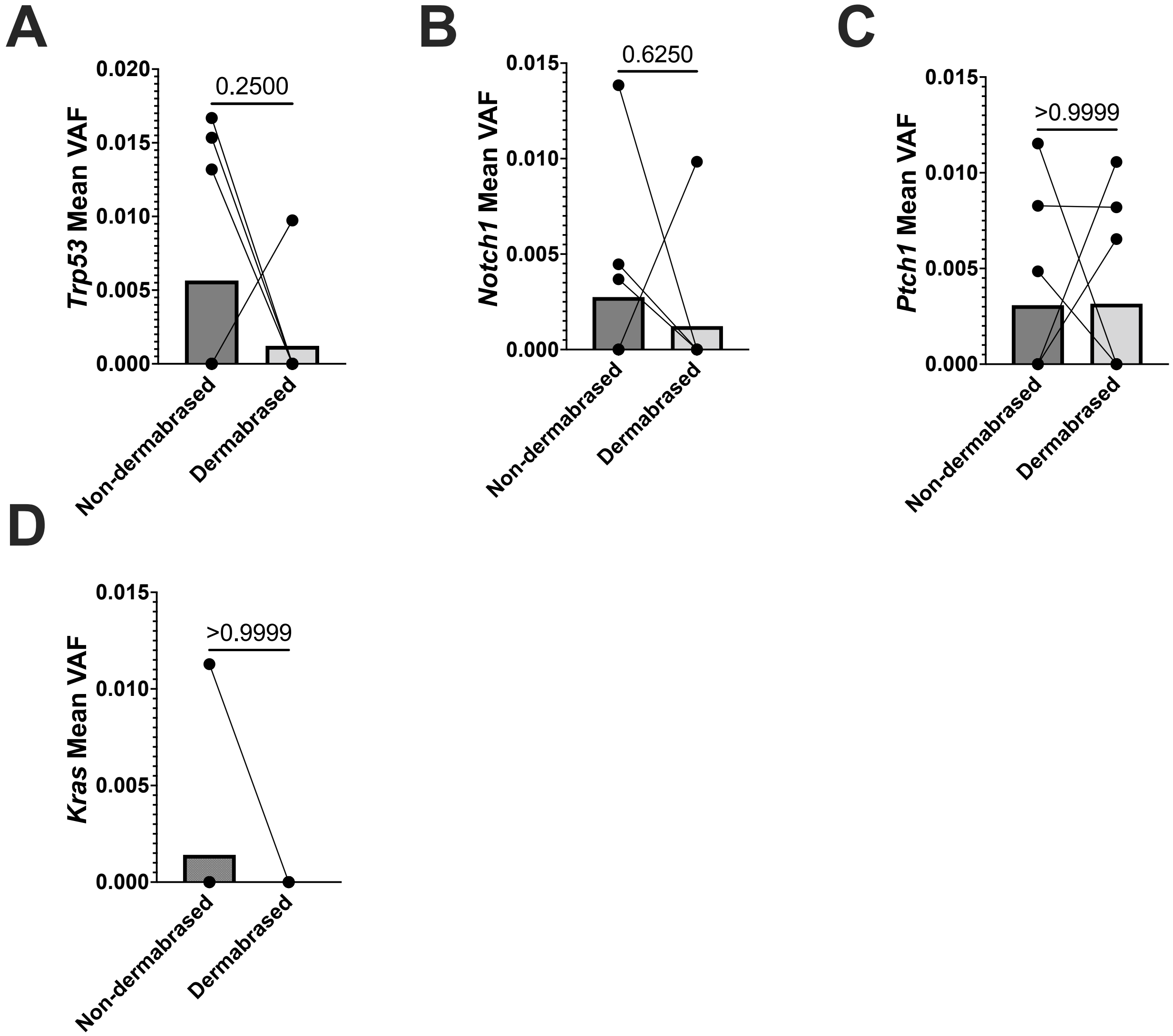
From top to bottom: Representative images of hematoxylin and eosin, K14, K1, Trichrome (Collagen) and Sirius red (Collagen Type III) staining in non-dermabrased and dermabrased skin. Scale bar=50um.



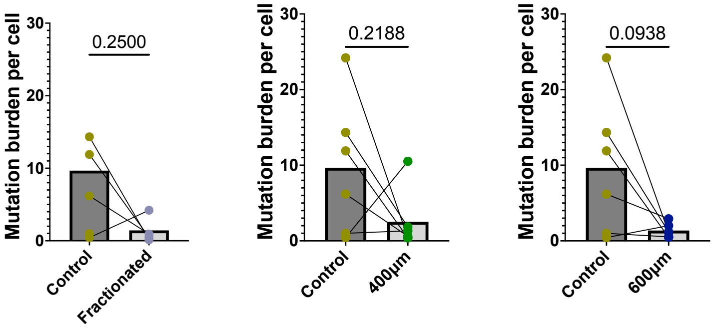
SFigure 8. Estimation of mutation burden per cell upon dermabrasion.
The estimation of mutation burden per cell in the non-dermabrased (ND) and dermabrased (D) murine epidermis (Wilcoxon test, *p*=0.125). Bar plot represents mean.



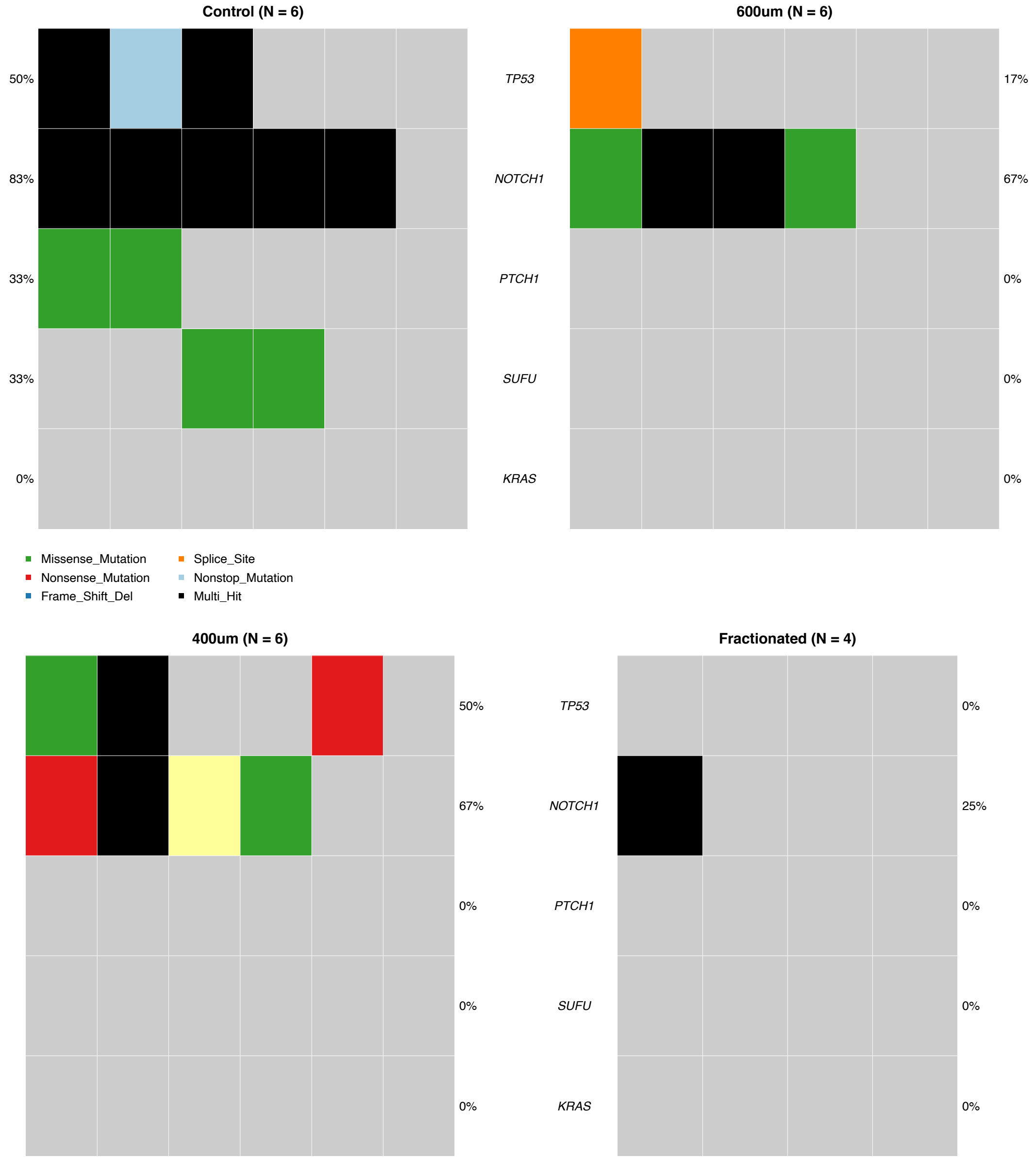
SFigure 9. Waterfall plot of KC driver genes with or without dermabrasion. Waterfall plots showing mutations in KC genes *(Trp53*, *Notch1*, *Ptch1*, *Sufu* and *Kras*) in each sample from the non-dermabrased and dermabrased areas.



SFigure 10. Mean VAF of KC driver genes with or without dermabrasion.
Mean VAF of (A) *Trp53*, (B) *Notch1*, (C) *Ptch1* and (D) *Kras* in the epidermis from the non-dermabrased and dermabrased areas (Wilcoxon test). Bar plots represent mean.

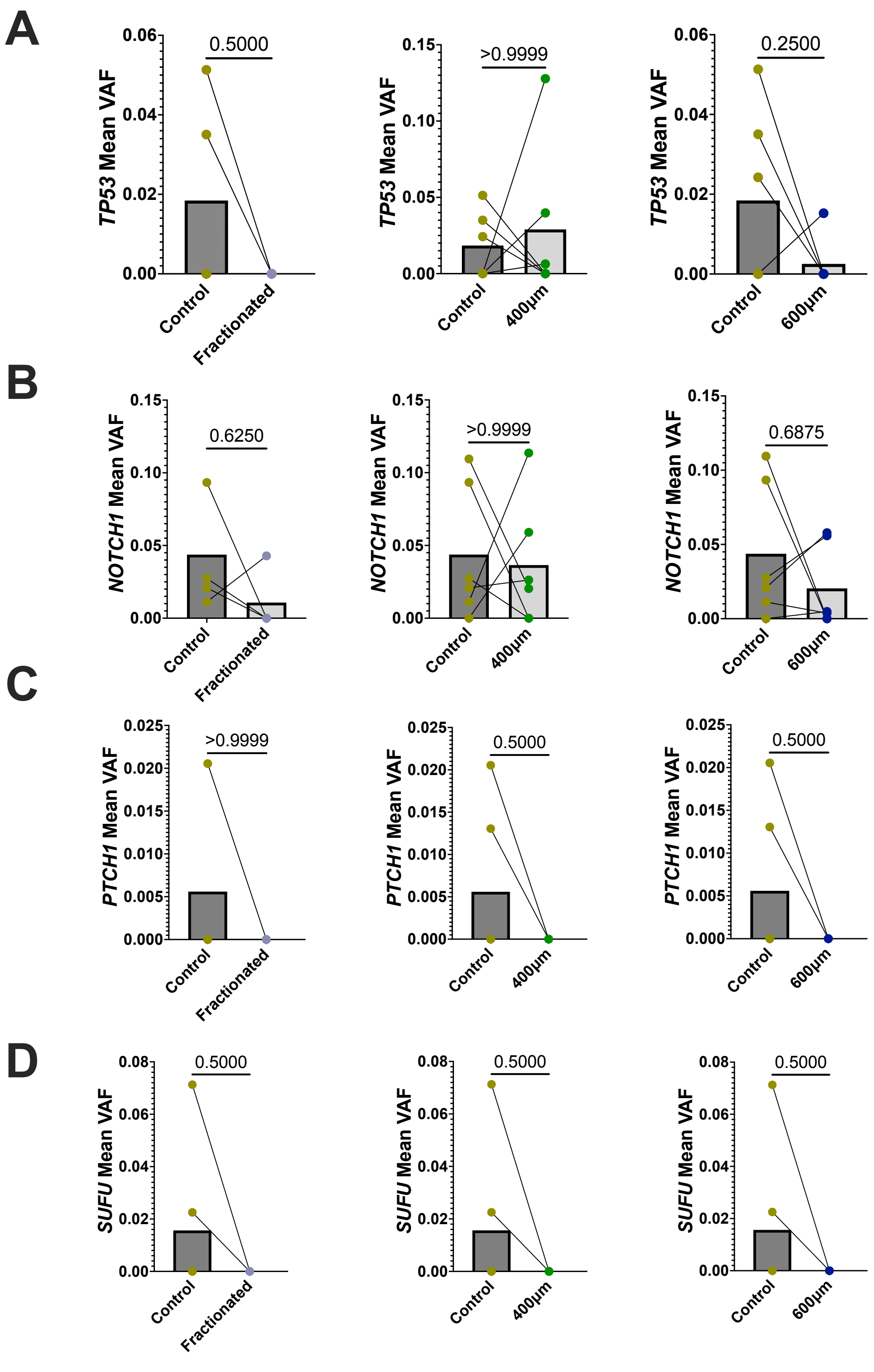


SFigure 11. Estimation of mutation burden per cell upon laser ablation.
The estimation of mutation burden per cell in the epidermis from KC patients who were treated with 600µm (right), 400µm (middle) and fractional laser (left) (Wilcoxon test). Bar plots represent mean.

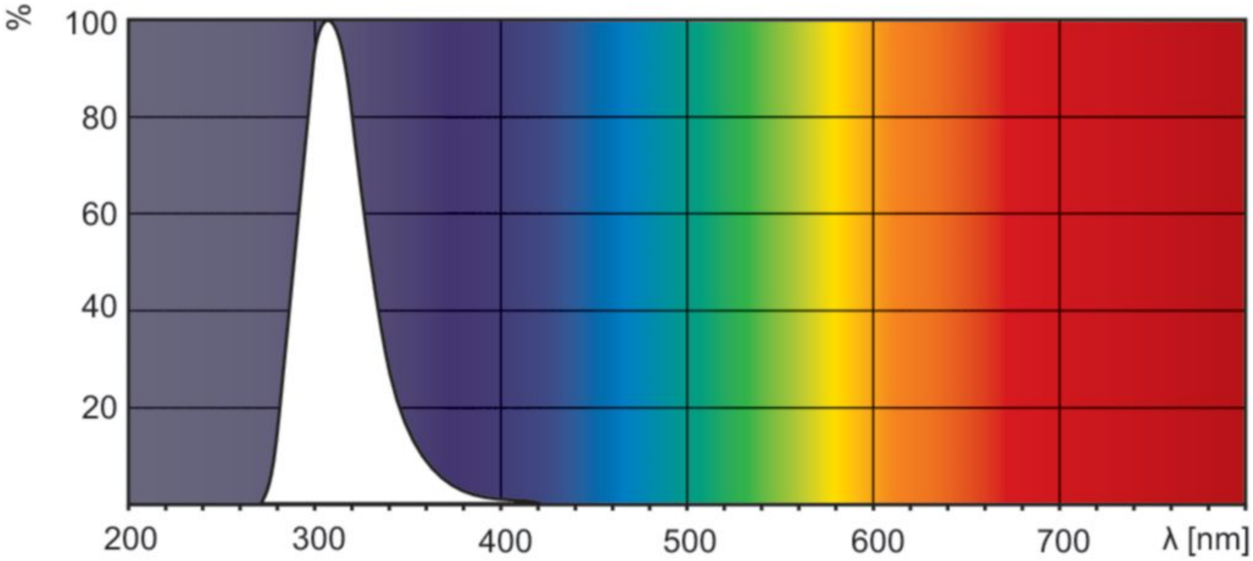


SFigure 12. Waterfall plots of KC driver genes with or without laser ablation

Waterfall plots showing mutations in KC genes *(TP53*, *NOTCH1*, *PTCH1*, *SUFU* and *KRAS*) in each sample from the control areas (top left), 600µm (top right), 400µm (bottom left) and fractionated (bottom right) laser treated areas.



SFigure 13 Mean VAF of KC driver genes with or without laser ablation.
Estimation of mean VAF of (A) *TP53*, (B) *NOTCH1*, (C) *PTCH1* and (D) *SUFU* in the epidermis from the control areas, 600µm, 400µm and fractionated laser treated areas (Wilcoxon test). Bar plots represent mean.



SFigure 14. The spectral emission of the UVB lamps (TL 40W/12 RS SLV, Philips).

STable 1. The size and the number of targeted genes in the sequencing panels.

	Samples	Panel	No. genes	Panel size
Mouse	Chronic UV	Sureselect XT HS custom	152*	0.67Mb
	Dermabrasion	Sureselect XT HS custom	152*	0.67Mb
Human	Risk prediction	Community Design Glasglow Cancer plus	352	3.96Mb
	Laser ablation	ClearSeq Cancer Comprehensive	152*	0.78Mb

*Same 152 genes targeted

STable 2. Targeted genes in Agilent comprehensive cancer panel.

ABCB1	*CYP2D6*	*IKZF1*	*NPM1*	*SLC34A2*
ABCC2	*DDR1*	*IL2RA*	*NRAS*	*SLC45A3*
ABL1	*DDR2*	*IL2RB*	*PDGFRA*	*SLCO1B1*
ABL2	*DDX3X*	*IL2RG*	*PDGFRB*	*SMAD4*
AKT1	*DNMT3A*	*INPP4B*	*PHF6*	*SMARCA4*
AKT2	*DPYD*	*JAK1*	*PIK3CA*	*SMARCB1*
AKT3	*EGFR*	*JAK2*	*PIK3R1*	*SMO*
ALK	*ERBB2*	*JAK3*	*PSMB1*	*SNCAIP*
APC	*ERBB3*	*KDM6A*	*PSMB2*	*SOS1*
ASXL1	*ERBB4*	*KDR*	*PSMB5*	*SPRED1*
ATM	*ERG*	*KIT*	*PSMD1*	*SRC*
ATRX	*ESR2*	*KRAS*	*PSMD2*	*STK11*
BRAF	*ESR1*	*LAMA2*	*PTCH1*	*SUFU*
BRCA1	*EZH2*	*LCK*	*PTEN*	*TAS2R38*
BRCA2	*FBXW7*	*LTK*	*PTPN11*	*TET2*
CBL	*FGFR1*	*MAP2K1*	*RAF1*	*TP53*
CDA	*FGFR2*	*MAP2K2*	*RARA*	*TRRAP*
CDH1	*FGFR3*	*MAP2K4*	*RARB*	*TYK2*
CDKN2A	*FGFR4*	*MAP3K1*	*RARG*	*UGT1A1*
CDKN2B	*FLT1*	*MAPK1*	*RB1*	*VHL*
CEBPA	*FLT3*	*MED13*	*RET*	*WT1*
CHD7	*FLT4*	*MET*	*ROS1*	*YES1*
CHIC2	*FSTL5*	*MLH1*	*RPS6KB1*	*ZMYM3*
CREBBP	*GNA11*	*MLL*	*RUNX1*	
CRLF2	*GNAQ*	*MPL*	*RXRA*	
CSF1R	*GNAS*	*MST1R*	*RXRB*	
CTNNB1	*GSTP1*	*MTOR*	*RXRG*	
CYP19A1	*H3F3A*	*MYC*	*SHH*	
CYP2A6	*HNF1A*	*MYD88*	*SHOC2*	
CYP2B6	*HRAS*	*NELL2*	*SLC22A1*	

CYP2C19	*IDH1*	*NF1*	*SLC22A2*	
CYP2C9	*IDH2*	*NOTCH1*	*SLC31A1*	

STable 3. Targeted Genes in Agilent Glasglow Cancer Plus panel.

AKT1	*HIST2H3C*	*RUNX1*	*EPHA2*	*PSIP1*
AKT2	*HLA-A*	*SETBP1*	*ERCC2*	*PTK2*
AKT3	*HLA-B*	*SETD2*	*ERCC3*	*PTPRD*
ALK	*HLA-C*	*SF3B1*	*ERCC4*	*QSER1*
AMER1	*HNF1A*	*SMAD4*	*ERCC5*	*RAD51B*
APC	*HRAS*	*SMARCA4*	*ETV1*	*RAD51C*
APLNR	*IDH1*	*SMARCB1*	*ETV4*	*RAD51D*
AR	*IDH2*	*SMO*	*ETV5*	*RAD52*
ARAF	*IGF1R*	*SOCS1*	*FANCA*	*RAD54L*
ARID1A	*JAK1*	*SPOP*	*FANCC*	*RASA1*
ARID1B	*JAK2*	*SRC*	*FANCD2*	*RBM10*
ARID2	*JAK3*	*STAG1*	*FANCE*	*RFX5*
ASXL1	*JUN*	*STAG2*	*FANCF*	*RFXAP*
ATM	*KDR*	*STAT3*	*FANCG*	*RHEB*
ATR	*KIT*	*STAT5B*	*FANCL*	*RICTOR*
ATRX	*KLF4*	*STK11*	*FANCM*	*RIT1*
AURKA	*KMT2A*	*SYK*	*FAT1*	*RPL22*
AXL	*KRAS*	*TERT*	*FLT1*	*SERPINB3*
B2M	*MAP2K1*	*TGFBR2*	*FOXA1*	*SERPINB4*
BAP1	*MAP2K2*	*TP53*	*FOXA2*	*SLC34A2*
BCL2	*MAP2K4*	*TSC1*	*FOXL2*	*SMAD2*
BLM	*MAP3K1*	*TSC2*	*FOXO1*	*SMAD3*
BRAF	*MAPK1*	*U2AF1*	*FUBP1*	*SMC1A*
BRCA1	*MAX*	*VHL*	*GATA6*	*SMC3*
BRCA2	*MCL1*	*WT1*	*GNA13*	*SMG1*
CBL	*MDM2*	*YAP1*	*GPS2*	*SOS1*
CCND1	*MED12*	*ABL1*	*HIF1A*	*SOX17*
CCND2	*MEN1*	*ABL2*	*HIST1H1C*	*SOX9*
CCND3	*MET*	*ABR*	*IDO1*	*SPEN*
CCNE1	*MLH1*	*ABRAXAS1*	*IDO2*	*SRSF2*

CD274	*MSH2*	*ACVR1B*	*IFNGR1*	*STAT1*
CD58	*MSH6*	*ACVR2A*	*IFNGR2*	*TAF1*
CDK12	*MTOR*	*AJUBA*	*IL6ST*	*TAF3*
CDK2	*MUTYH*	*AKAP9*	*IRF1*	*TAP1*
CDK4	*MYB*	*ALOX12B*	*KDM5C*	*TAP2*
CDK6	*MYC*	*ALOX15B*	*KDM6A*	*TAPBP*
CDKN1A	*MYCN*	*ARHGAP35*	*KEAP1*	*TBL1XR1*
CDKN1B	*NBN*	*ARID5B*	*KMT2B*	*TBX3*
CDKN2A	*NF1*	*ASXL2*	*KMT2C*	*TCF12*
CDKN2B	*NF2*	*AURKB*	*KMT2D*	*TCF7L2*
CHEK2	*NFE2L2*	*AURKC*	*LZTR1*	*TET2*
CIITA	*NOTCH1*	*AXIN1*	*MDM4*	*TGFBRN*
CREBBP	*NOTCH2*	*AXIN2*	*MECOM*	*TMPRSS2*
CTCF	*NOTCH3*	*BARD1*	*MGA*	*TP53BP1*
CTNNB1	*NOTCH4*	*BCOR*	*MGMT*	*TP73*
DAXX	*NPM1*	*BIRC3*	*MRE11*	*TRAF7*
DICER1	*NRAS*	*BRIP1*	*MSH3*	*UVRAG*
DNMT3A	*NTRK1*	*CARD11*	*MYCL*	*WRN*
EGFR	*PALB2*	*CASP8*	*MYH9*	*XBP1*
EP300	*PBRM1*	*CBFB*	*NAB2*	*XPO1*
EPHA3	*PDCD1LG2*	*CD74*	*NCOA2*	*ZFHX3*
ERBB2	*PDGFRA*	*CDC73*	*NCOR1*	*ZFP36L1*
ERBB3	*PDGFRB*	*CDH1*	*NLRC5*	*ZMYM2*
ERBB4	*PHF6*	*CDK8*	*NRG1*	*ZMYM3*
ERG	*PIK3CA*	*CDKN1C*	*NSD1*	*ZNF703*
ESR1	*PIK3CB*	*CDKN2C*	*NSD3*	*ZNF750*
ETV6	*PIK3R1*	*CHD4*	*NTRK2*	
EZH2	*PMS2*	*CHD8*	*NTRK3*	
FAS	*POLE*	*CHEK1*	*PARP1*	
FBXW7	*POLQ*	*CIC*	*PAX5*	
FGF19	*PPP2R1A*	*CKS1B*	*PCBP1*	

FGFR1	*PTCH1*	*CTLA4*	*PIAS3*	
FGFR2	*PTEN*	*CUL3*	*PIAS4*	
FGFR3	*PTPN11*	*CUX1*	*PIK3CD*	
FGFR4	*PAC1*	*CYLD*	*PIK3R2*	
GATA3	*PAD21*	*DDR2*	*PIM1*	
GNA11	*PAD50*	*DDX3X*	*PLCG1*	
GNAQ	*PAF1*	*DDX5*	*PMS1*	
GNAS	*RB1*	*DEFB134*	*POLQ*	
H3F3A	*RET*	*DHX9*	*PPM1D*	
H3F3B	*RHOA*	*DNMT3A*	*PPP2R2A*	
HGF	*RNF43*	*EIF4A2*	*PPP4R2*	
HIST1H3B	*ROS1*	*ELF3*	*PPP6C*	
HIST1H3C	*RPL5*	*ELOC*	*PRKAR1A*	