Supplemental information
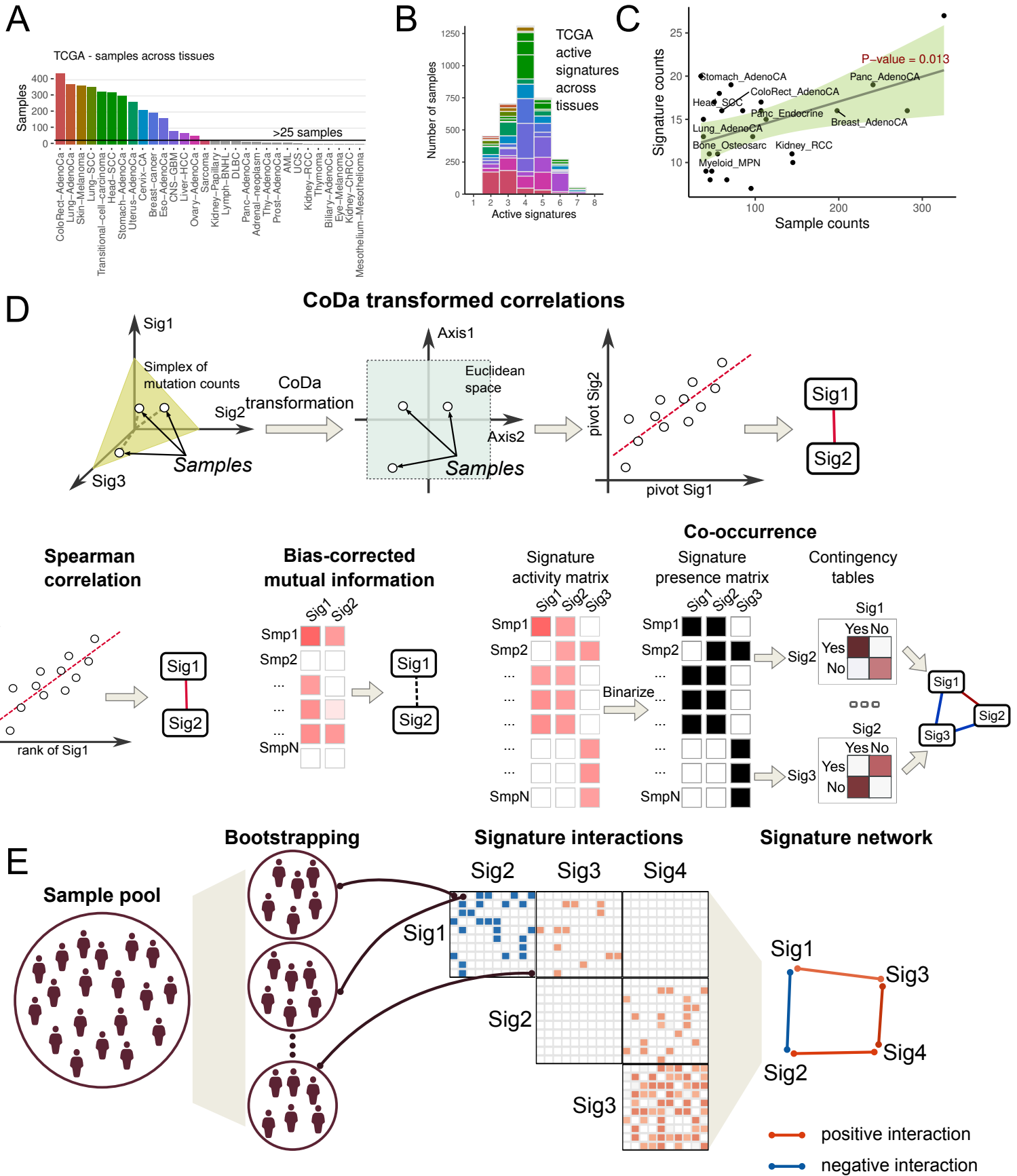
Pan-cancer analysis of the interplay between

# mutational signatures and cellular signaling

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Supplementary Figure 1

Supp. Fig. 1 Overview of the TCGA data and procedures for signature-signature metric calculation, related to Figure 1. A. The number of samples in TCGA across cancer types. **B.** Number of active mutational signatures in TCGA across cancer types. **C.** The relationship between the number of identified signatures and samples across tissues. **D.** Schematic diagram of used metrics. **E.** Bootstrapping procedure used for signature-signature interactions.

Supplementary Figure 2

A
Signature-signature interactions
across tissues in TCGA

B
Common signature-signature interactions
across tissues in TCGA
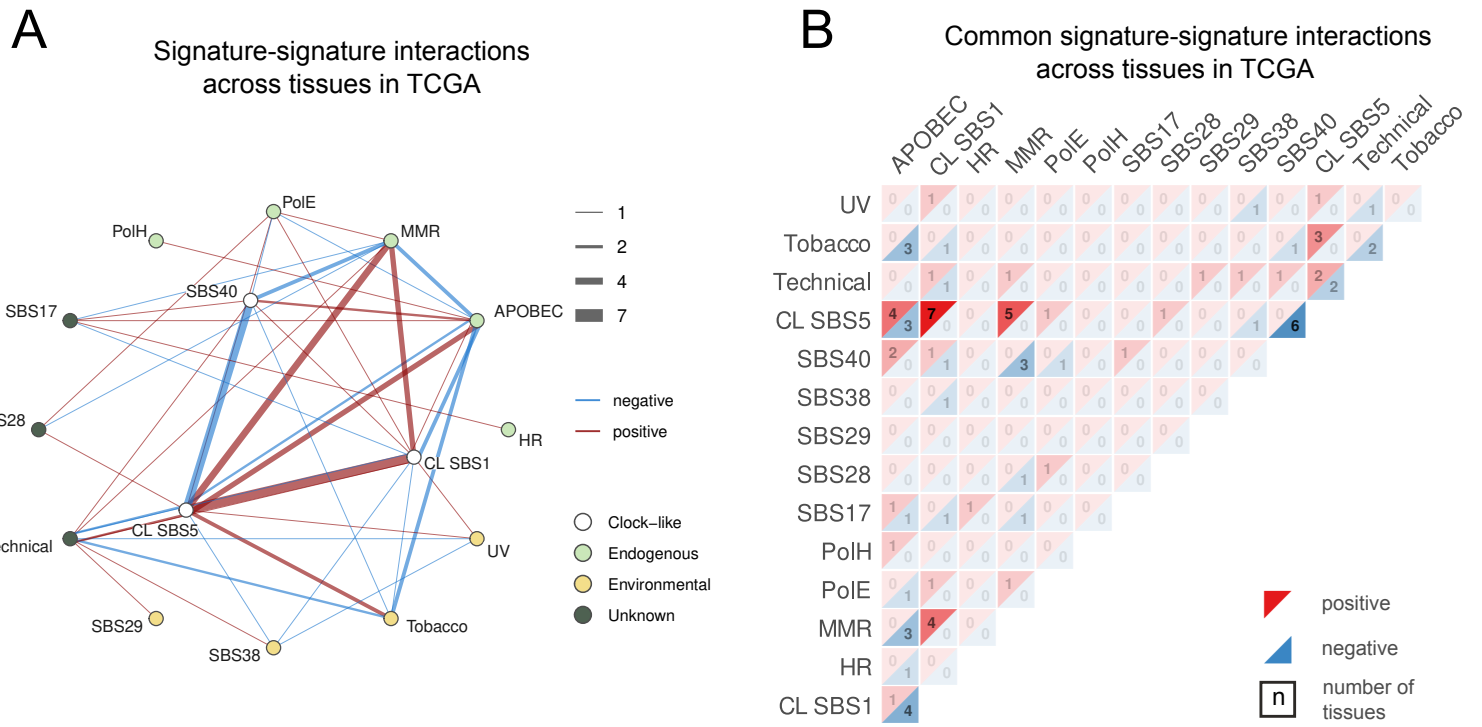
Supp. Fig. 2. Signature-signature interactions in TCGA, related to Figure 2. A. Network summary of all detected signature-signature interactions in TCGA. **B.** Summary of common interactions in TCGA across tissues. Positive (red) and negative (blue) interactions are summarized separately and the numbers indicate the number of tissues in which this interaction was detected.
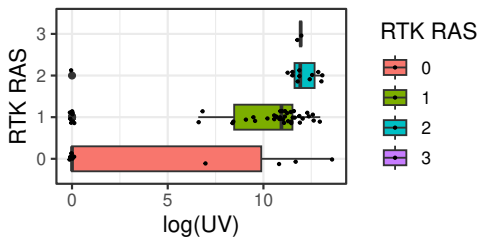
Supplementary Figure 3

A Pathway alterations across cancer types in TCGA

B RTK-RAS and UV mutations in melanoma
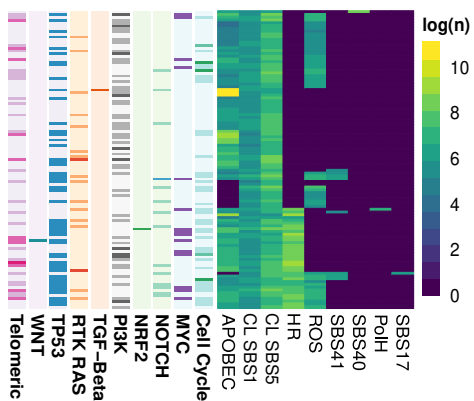
C Signature-pathway co-occurrence in TCGA
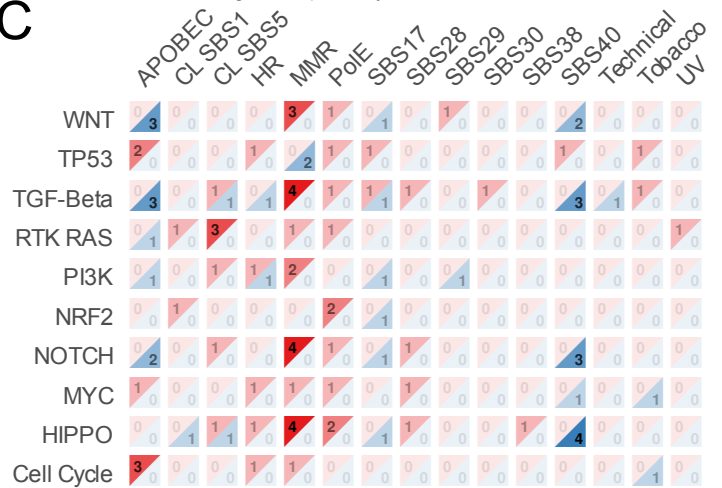
D BIpartite network of all signature-pathway interactions in TCGA

E Breast_AdenoCA - PCAWG
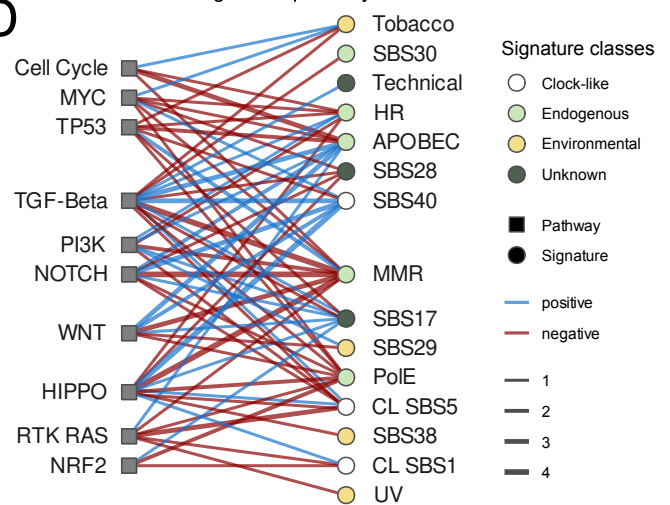
F Breast_cancer - TCGA

G PCAWG - Skin_Melanoma
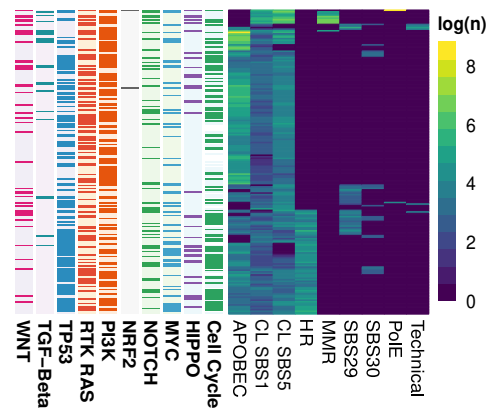
H PCAWG - Skin_Melanoma Signature-pathway interactions
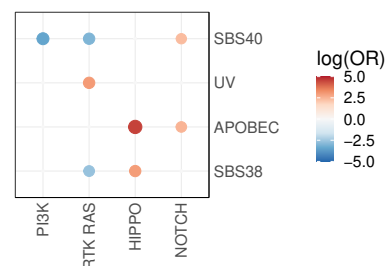
Supp. Fig. 3. Signature-pathway interactions in TCGA and case-inspections for selected tissues, related to Figure 3. A. Numeric summary of pathway alterations in TCGA. **B.** Boxplot demonstrating the interaction between UV signature and resulting RTK-RAS mutation counts in melanoma. The boxes indicate 25th and 75th percentile, whiskers extend no longer than 1.5 * IQR (inter-quartile range). **C.** Summary of signature-pathway co-occurrence across tissues in TCGA. Positive (red) and negative(blue) interactions are summarized separately and the numbers indicate the number of tissues in which this interaction has been detected. **D.** Bipartite graph of signature-pathway co-occurrence in TCGA. **E-G.** Signature activities along with annotations of pathway alterations in selected tissues. **H.** Significant signature-pathway interactions in melanoma in PCAWG dataset.

Supplementary Figure 4

A TCGA signature-pathway analysis summaries
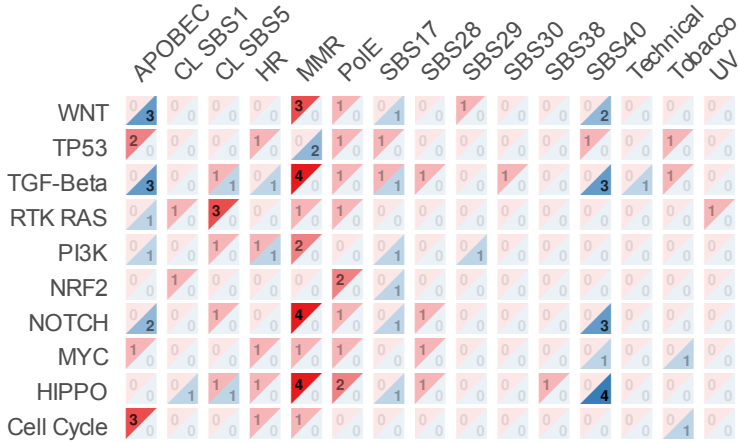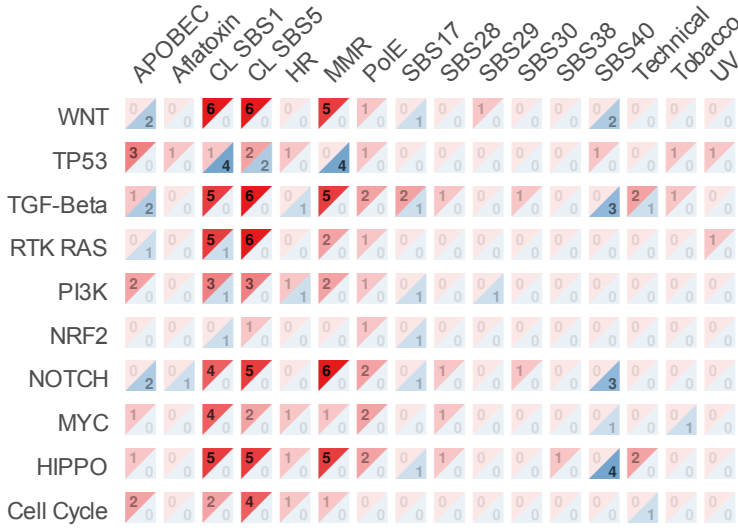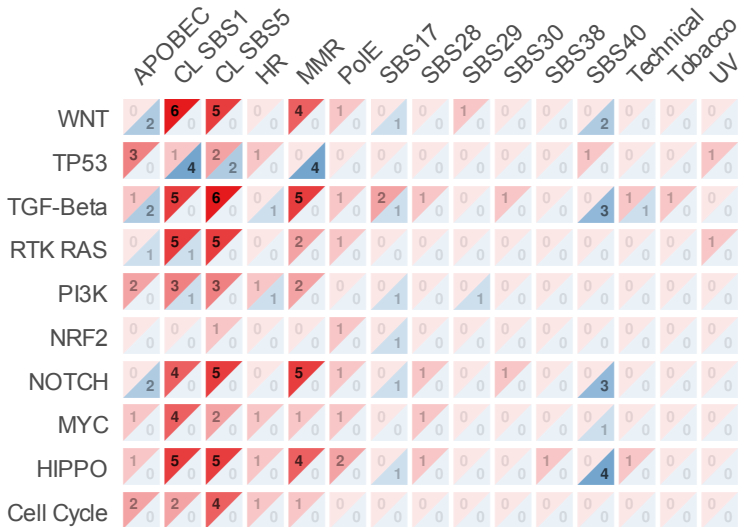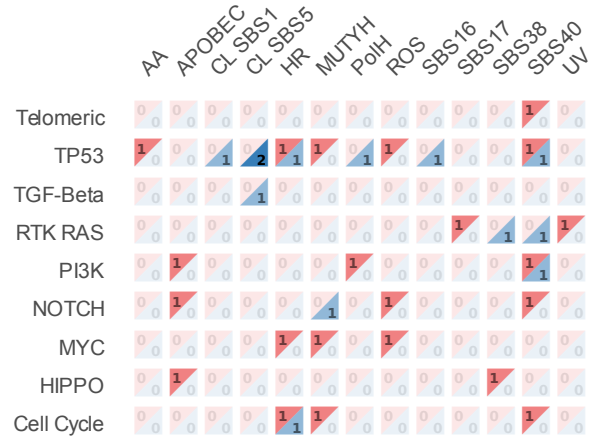
B PCAWG signature-pathway analysis summaries
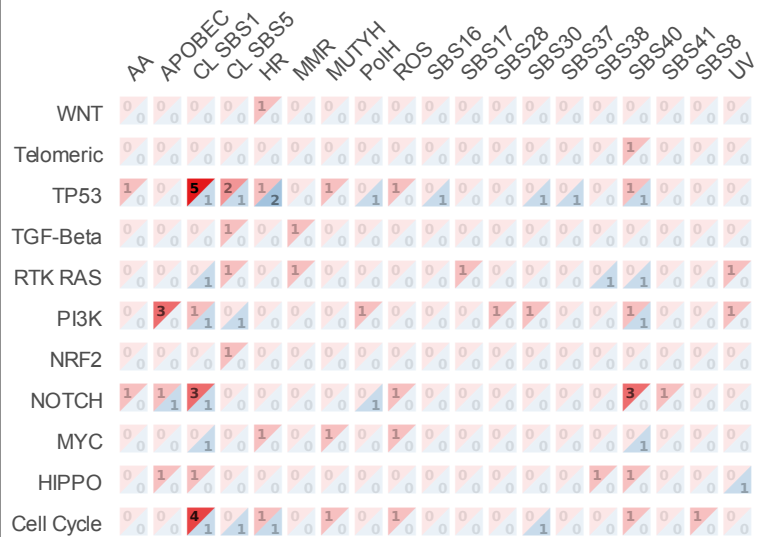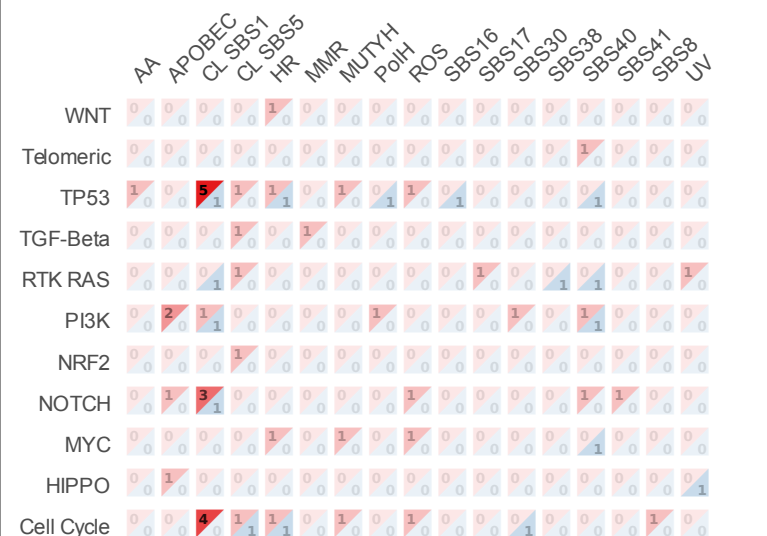
Supp. Fig. 4. Summary of signature-pathway interactions across tissues in TCGA and PCAWG, related to Figure 4. Summaries of interaction metrics across PCAWG and TCGA.

Supplementary Figure 5

A
PCAWG - the survival effect of carrying
a combination of interacting signatures over
a reference of non-carriers

negative ●
positive ▲

APOBEC+HR
SBS29+SBS40
ROS+SBS40

log(HR)

Tissue
● Eso_AdenoCA
● Liver_HCC
● Panc_AdenoCA

B
PCAWG - Cox regression coefficients
for ROS and SBS40 interaction
in medulloblastoma, pancreatic,
prostate and ovarian adenocarcinomas

|	coef	exp(coef)	se(coef)	z	Pr(>|z|)	
age_at_diagnosis	0.01	1.01	0.01	2	0.05	
Cancer.TypesOvary_AdenoCA	1.25	3.5	0.41	3.05	0	***
Cancer.TypesPanc_AdenoCA	1.14	3.12	0.43	2.66	0.01	*
Cancer.TypesProst_AdenoCA	−1.4	0.25	0.51	−2.73	0.01	*
ROS	0.46	1.59	0.16	2.82	0	***
SBS40	0.02	1.02	0.16	0.14	0.89	
ROS*SBS40	0.96	2.62	0.36	2.68	0.01	*

C
TCGA - the survival effect of carrying
a combination of interacting signatures over
a reference of non-carriers

HR+SBS17
APOBEC+SBS17
CL_SBS5+Tobacco
CL_SBS5+UV
CL_SBS1+SBS5
CL_SBS1+UV
SBS38+Technical
APOBEC+SBS40
MMR+SBS17

log(HR)

Interaction type
● negative
▲ positive

Tissue
● ColoRect_AdenoCa
● Lung_AdenoCa
● Skin_Melanoma
● Stomach_AdenoCa

D
PCAWG - the survival effect of carrying
a combination of interacting signature-pathway pair
over a reference of non-carriers

MYC+ROS
ROS+TP53

log(HR)

● CNS_Medullo
● Prost_AdenoCA

Interaction type
▲ positive

E
TCGA - the survival effect of carrying
a combination of interacting signature-pathway pair over
a reference of non-carriers

APOBEC+TGF.Beta
APOBEC+NOTCH
NOTCH+SBS40

log(HR)

negative ●

Tissue
● Cervix_CA
● Uterus_AdenoCa

Supp. Fig. 5. Survival analysis results for survival models with non-epistatic interaction models, related to Figure 5. A. Survival effect of signature-signature interactions obtained from survival models that compare samples of carriers of both interactors to non-carriers. The error bars in A, C, D, E correspond to the 95% confidence interval of the HR estimate. **B.** Regression coefficients of SBS40 and ROS interactions in specified tissues. The asterisk flags following p-value thresholds - P < 0.05 (*), P < 0.01 (**), P < 0.001 (***). **C-E**. Similar to panel **A**, interactions in TCGA for signature-signature interactions and signature-pathway interactions in PCAWG and TCGA.

Supplementary Figure 6

A
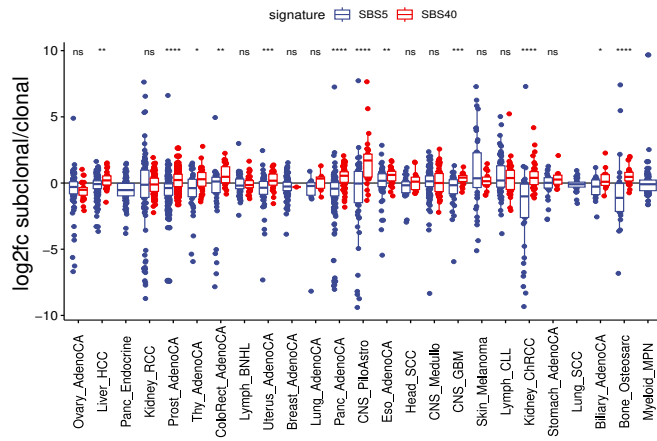
Supp. Fig. 6. The logFC fraction of subclonal vs clonal mutations attributed to SBS5 and SBS40 signatures across cancer types in PCAWG dataset based on data analyzed by Gerstung et al.[s1]. Related to Figure 5.

Supplementary Table 1 related to Figures 2-5: Signature annotations

Signature	Class	Description	Annotation	Origin
SBS1	Ageing	Ageing	CL SBS1	Clock-like
SBS2	APOBEC/AID	APOBEC	APOBEC	Endogenous
SBS3	BER/HR	HR	HR	Endogenous
SBS4	Environ	Tobacco-smoking	Tobacco	Environmental
SBS5	Unknown	Unknown	CL SBS5	Clock-like
SBS6	MMR	MMR	MMR	Endogenous
SBS7a	UV	UV	UV	Environmental
SBS7b	UV	UV	UV	Environmental
SBS7c	UV	UV	UV	Environmental
SBS7d	UV	UV	UV	Environmental
SBS8	Unknown	Unknown	SBS8	Unknown
SBS9	Polymerase	PolH	PolH	Endogenous
SBS10a	Polymerase	PolE	PolE	Endogenous
SBS10b	Polymerase	PolE	PolE	Endogenous
SBS11	Chemo	Temozolomide treatment	Chemo	Environmental
SBS12	Unknown	Unknown	SBS12	Unknown
SBS13	APOBEC/AID	APOBEC	APOBEC	Endogenous
SBS14	MMR	PolE + MMR	MMR	Endogenous
SBS15	MMR	MMR	MMR	Endogenous
SBS16	Unknown	Unknown	SBS16	Unknown
SBS17a	Unknown	Unknown	SBS17	Unknown
SBS17b	Unknown	Unknown	SBS17	Unknown
SBS18	Environ	ROS	ROS	Endogenous
SBS19	Unknown	Unknown	SBS19	Unknown
SBS20	MMR	POLD1 + MMR	MMR	Endogenous
SBS21	MMR	MMR	MMR	Endogenous
SBS22	Environ	Aristolochic acid	AA	Environmental
SBS23	Unknown	Unknown	SBS23	Unknown
SBS24	Environ	Aflatoxin	Aflatoxin	Environmental
SBS25	Chemo	Chemotherapy	Chemo	Environmental
SBS26	MMR	MMR	MMR	Endogenous
SBS27	Technical	Technical	Technical	Unknown
SBS28	Unknown	Unknown	SBS28	Unknown
SBS29	Environ	Tobacco chewing	SBS29	Environmental
SBS30	BER/HR	NTHL1	SBS30	Endogenous
SBS31	Chemo	Platinium chemotherapy	Chemo	Environmental
SBS32	Chemo	Azathioprine	Chemo	Environmental
SBS33	Unknown	Unknown	SBS33	Unknown
SBS34	Unknown	Unknown	SBS34	Unknown
SBS35	Chemo	Platinium chemotherapy	Chemo	Environmental

SBS36	BER/HR	MUTYH	MUTYH	Endogenous
SBS37	Unknown	Unknown	SBS37	Unknown
SBS38	UV	Indirect effect of UV	SBS38	Environmental
SBS39	Unknown	Unknown	SBS39	Unknown
SBS40	Unknown	Unknown	SBS40	Clock-like
SBS41	Unknown	Unknown	SBS41	Unknown
SBS42	Environ	Haloalkane exposure	Haloalkane	Environmental
SBS43	Technical	Technical	Technical	Unknown
SBS44	MMR	MMR	MMR	Endogenous
SBS45	Technical	Technical	Technical	Unknown
SBS46	Technical	Technical	Technical	Unknown
SBS47	Technical	Technical	Technical	Unknown
SBS48	Technical	Technical	Technical	Unknown
SBS49	Technical	Technical	Technical	Unknown
SBS50	Technical	Technical	Technical	Unknown
SBS51	Technical	Technical	Technical	Unknown
SBS52	Technical	Technical	Technical	Unknown
SBS53	Technical	Technical	Technical	Unknown
SBS54	Technical	Technical	Technical	Unknown
SBS55	Technical	Technical	Technical	Unknown
SBS56	Technical	Technical	Technical	Unknown
SBS57	Technical	Technical	Technical	Unknown
SBS58	Technical	Technical	Technical	Unknown
SBS59	Technical	Technical	Technical	Unknown
SBS60	Technical	Technical	Technical	Unknown
SBS84	APOBEC/AID	AID	AID	Endogenous
SBS85	APOBEC/AID	Indirect effect of AID	AID	Endogenous
SBS86	Chemo	Unknown chemotherapy	Chemo	Environmental
SBS87	Chemo	Thiopurine chemotherapy	Chemo	Environmental
SBS88	Environ	Colibactin exposure	Colibactin	Environmental
SBS89	Unknown	Unknown	SBS89	Unknown
SBS90	Chemo	Duocarmycin exposure	Chemo	Environmental

Supplementary References

S1. Gerstung, M., Jolly, C., Leshchiner, I., Dentro, S.C., Gonzalez, S., Rosebrock, D., Mitchell, T.J., Rubanova, Y., Anur, P., Yu, K., et al. (2020). The evolutionary history of 2,658 cancers. Nature 578, 122–128. 10.1038/s41586-019-1907-7.