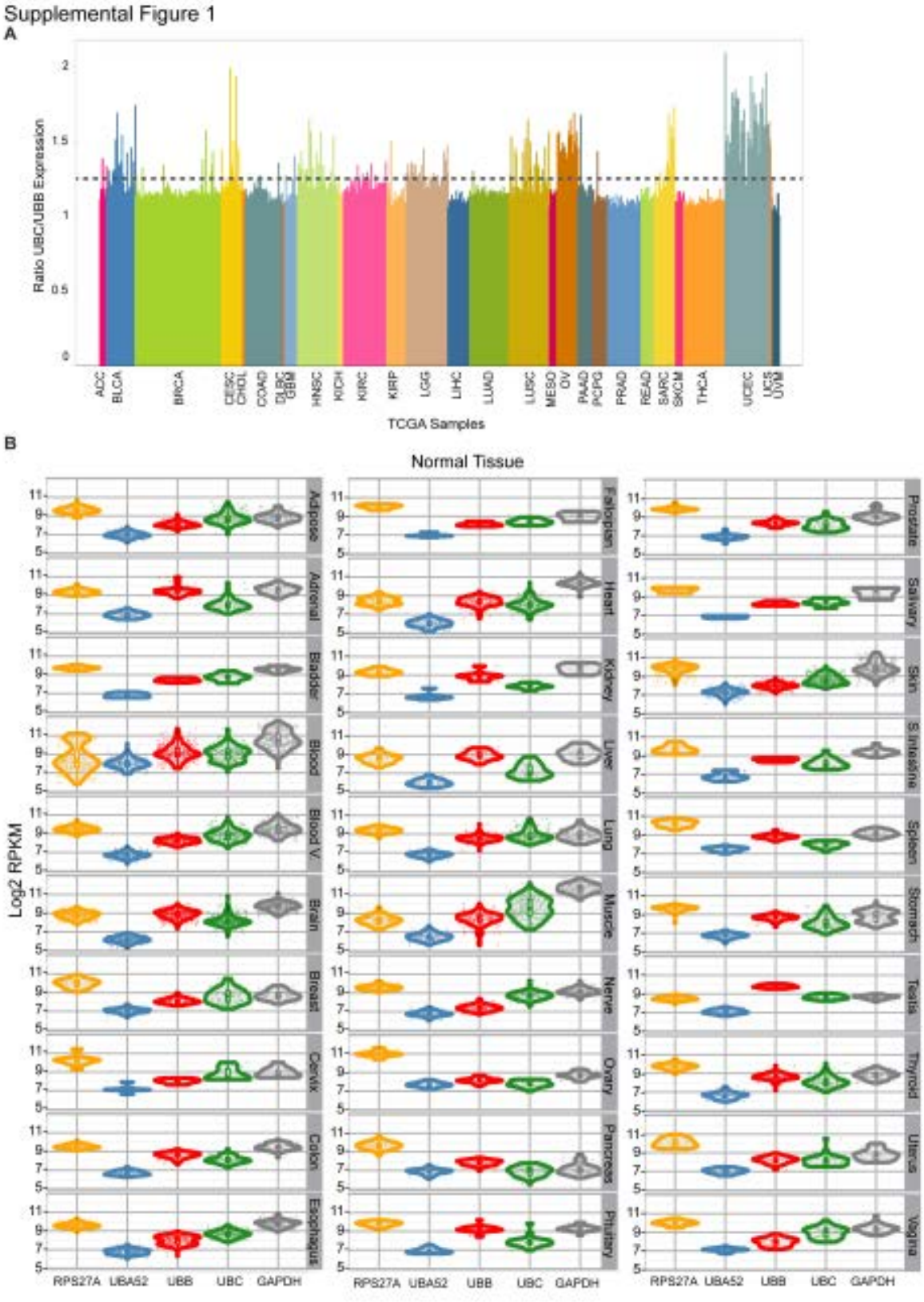
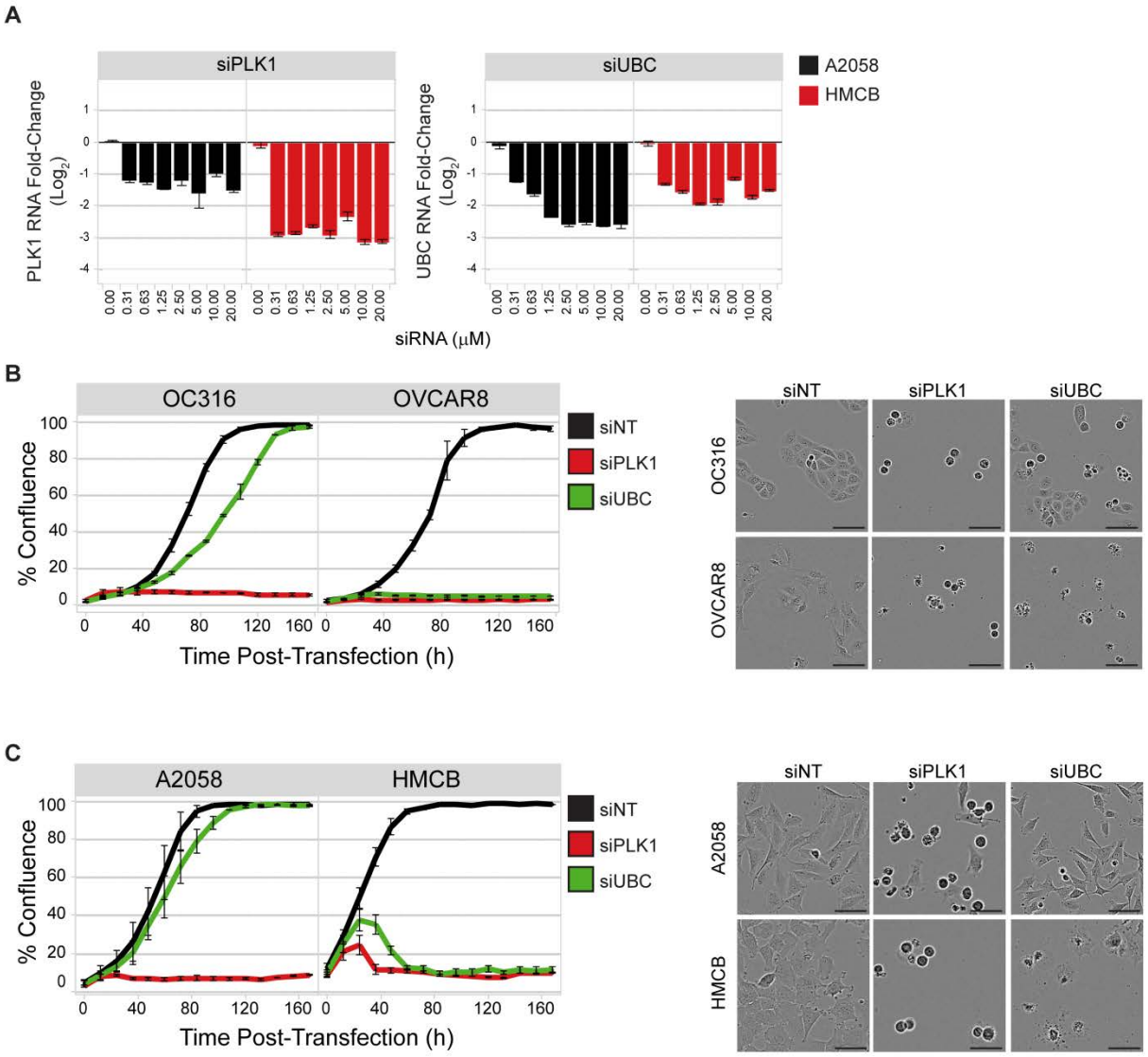


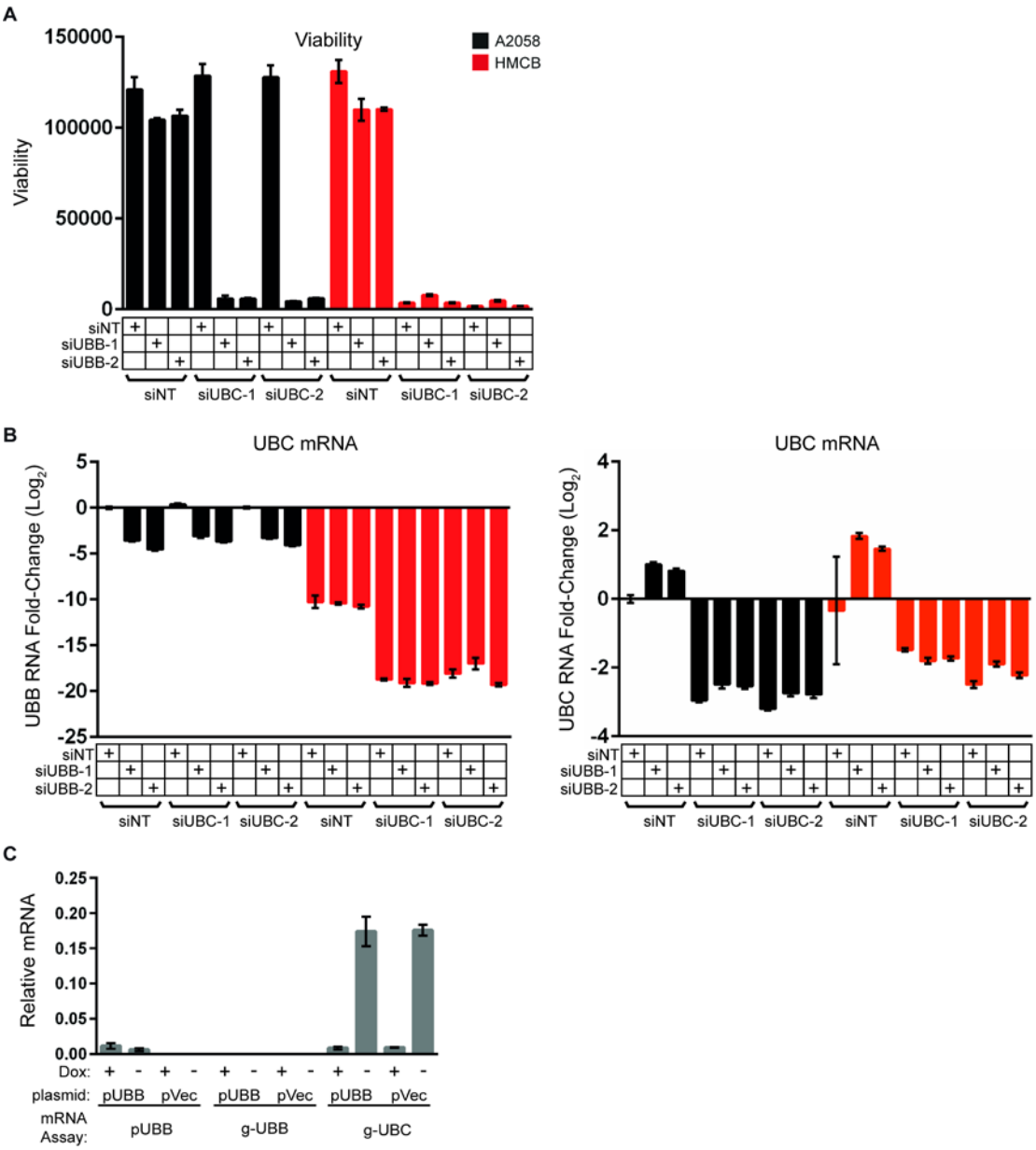
Supplementary Figure 1. Expression of ubiquitin-encoding genes across TCGA.
(a) Expression levels of *UBB* within each patient-derived tumor across TCGA is shown as the ratio of *UBC*:*UBB*. Dashed line shows upper inner fence cutoff (see Methods) and distinguishes outlier patients for low *UBB* expression (samples the cross dashed line). **(b)** Extended survey of ubiquitin gene expression across tissues from healthy donors with *GAPDH* as an internal comparator.

Supplemental Figure 2



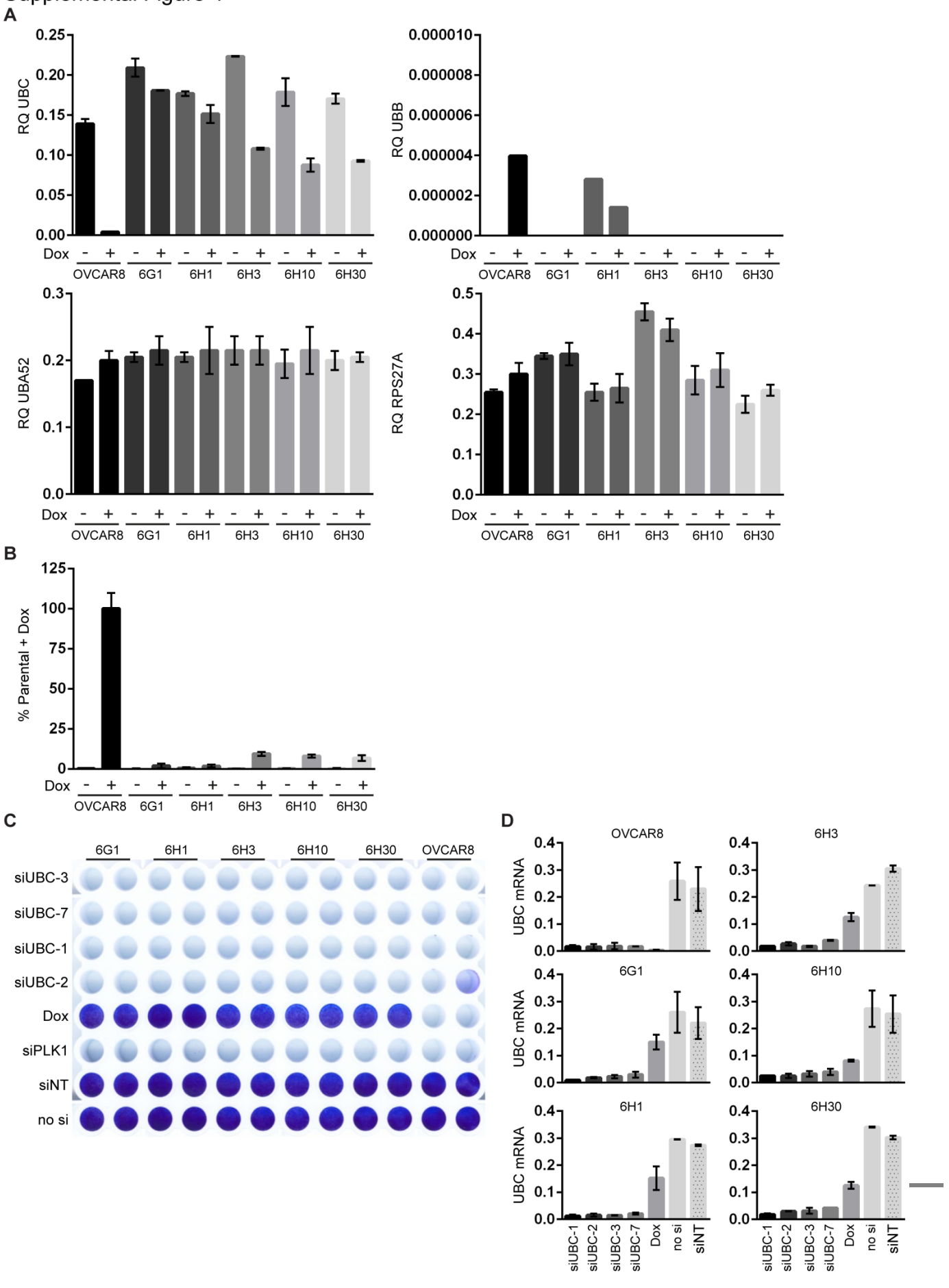
Supplementary Figure 2. Target mRNA knockdown and cell viability by live cell imaging.
(a) Effects of siRNA on target mRNA knockdown for *PLK1* and *UBC* in OC316 (black) and OVCAR8 (red) cells. The concentration of gene-specific siRNA used in the transfection is shown on the X-axis, mRNA knockdown effects of si*PLK1 and siUBC* is shown on left and right, respectively. The total siRNA amount was 20 nM; adjusted with non-targeting control siRNA. Technical quadruplicates with mean -/+ SEM were performed twice. **(b,c)** Growth of two lineage-matched cell lines was continuously monitored using Incucyte (Essen) real-time microscopy following transfection of siRNAs. A2058 and HMCB are skin cancer cell lines, harboring UBB^{WT} and UBB^{LO}, respectively. While OC316 and OVCAR8 are HGSOC lines as previously described. siRNAs targeting *PLK1* are lethal to all four cell lines, whereas knockdown of *UBC* was lethal selectively in the UBB^{LO} lines, OVCAR8 and HMCB. 5 nM siRNA was used in quadruplicates to assess confluence with mean -/+ SEM. **(Right)** Representative phase contrast images of cells taken thirty-six hours post-transfection. Scale bar in each image is 50 μm. Magnification is 10X. This study was performed twice.

Supplemental Figure 3



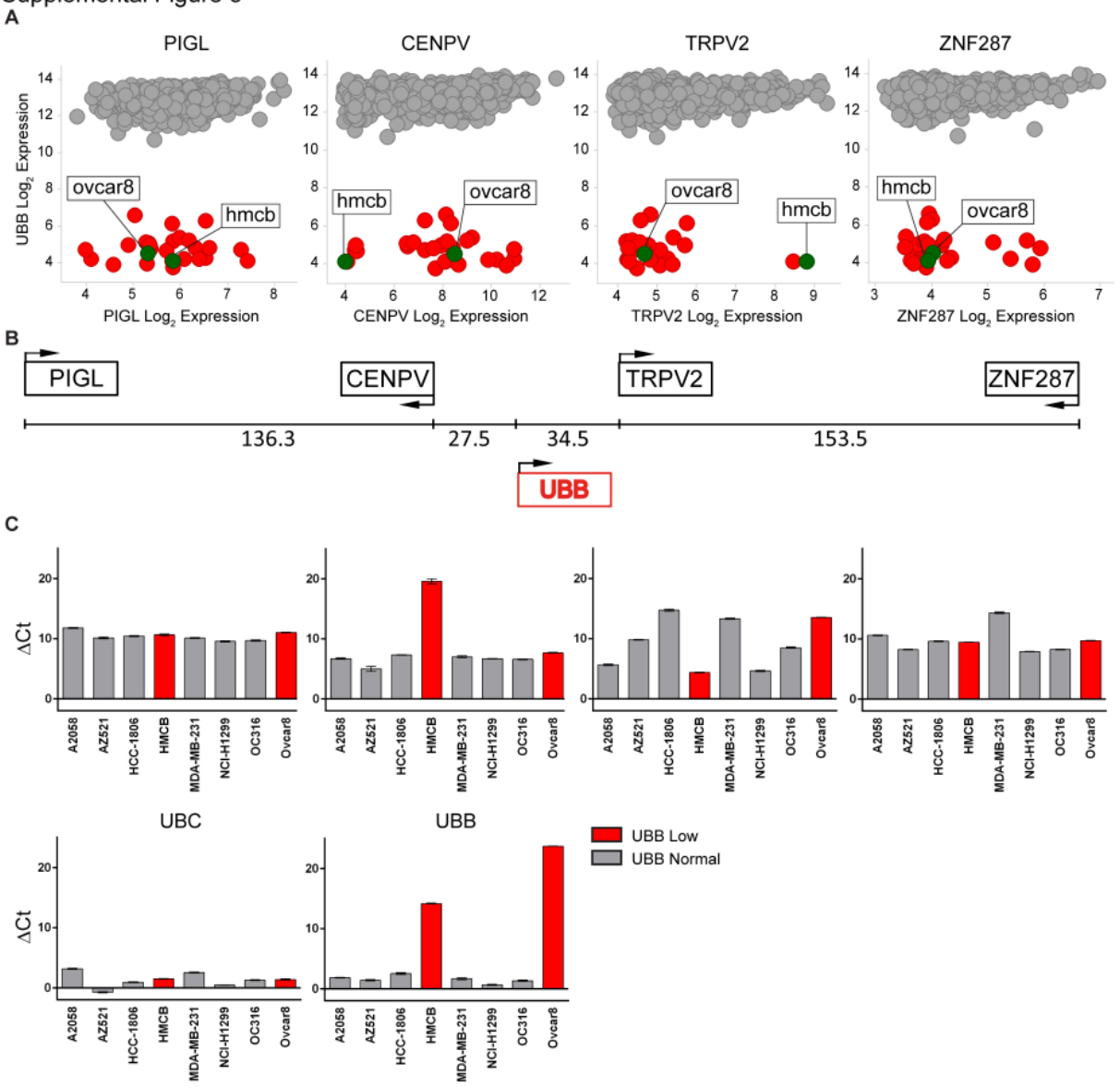
Supplementary Figure 3. *UBB* and *UBC* are a synthetic lethal gene pair.
(a) Skin cancer cell lines A2058 (*UBB*^{WT}, black) and HMCB (*UBB*^{LO}, red) cells were transfected with a combination of two siRNAs for each of *UBB* and *UBC* at 5 nM each as shown in the matrix below the figure. Every pairwise combination of two siRNAs for *UBC*, *UBB* and NT were used to transfect these cells, and viability was measured by CTG seventy-two hours later. Study was performed in biological quadruplicate with mean -/+ SEM, repeated twice. **(b)** Twenty-four hours after transfection target mRNA knockdown was measured as (a). Values are shown as fold-change (log_2) relative to siNT for A2058. **(c)** OVCAR8 cells containing a Dox-inducible hairpin for *UBC* were transfected with plasmid encoding *UBB* (pUBB). Expression of the plasmid-borne *UBB* (p*UBB*), endogenous, genomic, *UBB* (g-*UBB*) and *UBC* (g-*UBC*) is shown in RNA isolated twenty-four hours post-transfection. This study reflects analysis of technical replicates with standard error of the mean and was performed twice.

Supplemental Figure 4



Supplementary Figure 4. Ex vivo analysis of shUBC-resistant tumor cells.
Dox-resistant tumor nodules recovered from five mice in the H8 (*shUBC* + *Dox*) study arm were re-established for growth in cell culture. **(a)** Expression levels of each of the ubiquitin-encoding genes in these lines (6G1, 6H1, 6H3, 6H10, 6H30) are shown. Dox treatment produces a strong reduction of *UBC* mRNA in parental cells (upper left, labeled OVCAR8) which is attenuated to various extents in each of the resistant lines. Note that the Y-axis values are specific for each gene. Relative Quantitation (RQ) for each mRNA was determined in technical replicates with standard error of the mean and performed three times. **(b)** Expression of the short hairpin RNA that targets *UBC* is induced by Dox treatment in parental cells but is blunted in each of the Dox-resistant clones. **(c)** Growth phenotypes following transfection of siRNAs that target *UBC*, *PLK1*, or NT in parental OVCAR8-*shUBC* cells and in the five Dox-resistant lines indicates that the latter retain sensitivity to *UBC* knockdown. Resistance to Dox (100 ng/ml) is retained in these ex vivo cultures. Four different siRNAs that target *UBC* were transfected into these cells as biological replicates. Cells were fixed and stained with crystal violet six days after transfection. **(d)** *UBC* expression in each of the ex vivo established lines following transfection of *UBC* siRNAs. The effect of Dox treatment on *UBC* levels in the ex vivo cell lines is also shown and Dox-insensitive lines can be compared to parental OVCAR8 (top left). Error is shown as standard deviation between biological replicates.

Supplemental Figure 5



Supplementary Figure 5. Transcriptional silencing of *UBB* is regulated locally at a gene-proximal level. **(a)** Expression of the four nearest-neighbor genes to *UBB* shows that their transcription regulation is independent of *UBB* expression status across the CCLE. Expression of *UBB* neighbor genes on X-axis is shown with *UBB* expression levels on the Y-axis. OVCAR8 and HMCB are highlighted among all UBB^{LO} cell lines (red) to illustrate divergent expression patterns of neighboring genes, *CENPV* and *TRPV2*. **(b)** Linear map of *UBB* region from chromosome 17p12 showing distances and orientation of transcription start sites in kilobases. **(c)** Relative mRNA expression levels of the four *UBB*-flanking genes shown above (see a, b) were measured in eight cell lines. Increasing ΔCt values indicates decreased expression and is consistent with expression data in the CCLE expression profile database. Expression of *UBC* and *UBB* is shown in these cell lines at bottom. Technical quadruplicates were analyzed by RT-qPCR shown as mean -/+ SEM and was performed twice.

Supplementary Table 1: Cell lines with *UBB* in OFF state.

Cell Line	Lineage
nb1	autonomic ganglia
ks1	central nervous system
efe184	endometrium
hec1a	endometrium
mfe280	endometrium
ten	endometrium
em2	CML
st486	Burkitt's lymphoma
snu283	large intestine
jhh1	liver
ncih82	Lung-small cell
ncih322	Lung-adenocarcinoma
kyse150	esophagus
caov4	ovary
jhoc5	ovary
jhom1	ovary
ovk18	ovary
ovcar8	ovary
rmugs	ovary
snu8	ovary
colo818	skin
hmcb	skin
k029ax	skin
skut1	leiomyosarcoma
cal33	upper aerodigestive
yd38	upper aero-digestive

Supplementary Table 1: Cell lines with *UBB* in OFF state.
The 26 cell lines from the CCLE that express low levels of UBB are listed along with their corresponding tumor lineage.

Supplementary Table 2: Lineage frequency of *UBB*^{OFF} in CCLE.

Lineage	UBB "off"	Total	Frequency
autonomic ganglia	1	16	0.063
biliary tract	0	8	na
bone	0	26	na
breast	0	71	na
central nervous system	1	55	0.018
endometrium	4	28	0.143
eye	0	7	na
haem and lymph	2	191	0.011
kidney	0	23	na
large_intestine	1	68	0.015
liver	1	28	0.036
lung	3	185	0.016
esophagus	1	26	0.038
ovary	7	52	0.135
pancreas	0	47	na
pleura	0	14	na
prostate	0	7	na
salivary gland	0	2	na
skin	4	79	0.05
soft tissue	1	23	0.043
stomach	0	39	na
thyroid	0	12	na
upper aero-digestive track	2	33	0.061
urinary track	0	27	na

Supplementary Table 2: Lineage frequency of *UBB*^{OFF} in CCLE.
Ovarian and endometrial cancers most frequently inactivate *UBB* expression among cell lines in the CCLE. Total number of cell lines from corresponding lineages is shown along with the number of lines with low *UBB* expression

Supplementary Table 3: Mouse Survival Study

Day	A6 shControl (Dox)		A6 shControl (Non-Dox)		H8 shUBC (Dox)		H8 shUBC (Non-Dox)	
	per cent	no. mice	per cent	no. mice	per cent	no. mice	per cent	no. mice
0	100	7	100	10	100	10	100	8
1	100	7	100	10	100	10	100	8
37	100	7	100	10	100	10	100	8
40	100	7	90	9	100	10	100	8
41	100	7	80	8	100	10	100	8
44	100	7	70	7	100	10	100	8
46	86	6	70	7	100	10	100	8
49	86	6	50	5	100	10	100	8
50	57	4	50	5	100	10	100	8
51	43	3	50	5	100	10	100	8
55	14	3	40	4	100	10	100	8
56	14	3	20	4	100	10	100	8
58	14	3	10	1	100	10	100	8
62	14	1	0	0	100	10	100	8
64	0	0	0	0	100	10	88	7
66	0	0	0	0	100	10	50	4
70	0	0	0	0	100	10	38	3
71	0	0	0	0	100	10	13	1
92	0	0	0	0	100	10	0	0
93	0	0	0	0	90	9	0	0
98	0	0	0	0	60	6	0	0
99	0	0	0	0	30	3	0	0
105	0	0	0	0	20	2	0	0
106	0	0	0	0	10	1	0	0

Supplementary Table 3: Mouse survival study

OVARC8 cells with shControl (A6) or shUBC (H8) were implanted into mice and treated with and without Dox. Survival was monitored as described in Methods. The number of mice in each arm alive at each time point is shown as the per cent of starting population and number of animals. Study was continued until all mice were euthanized.