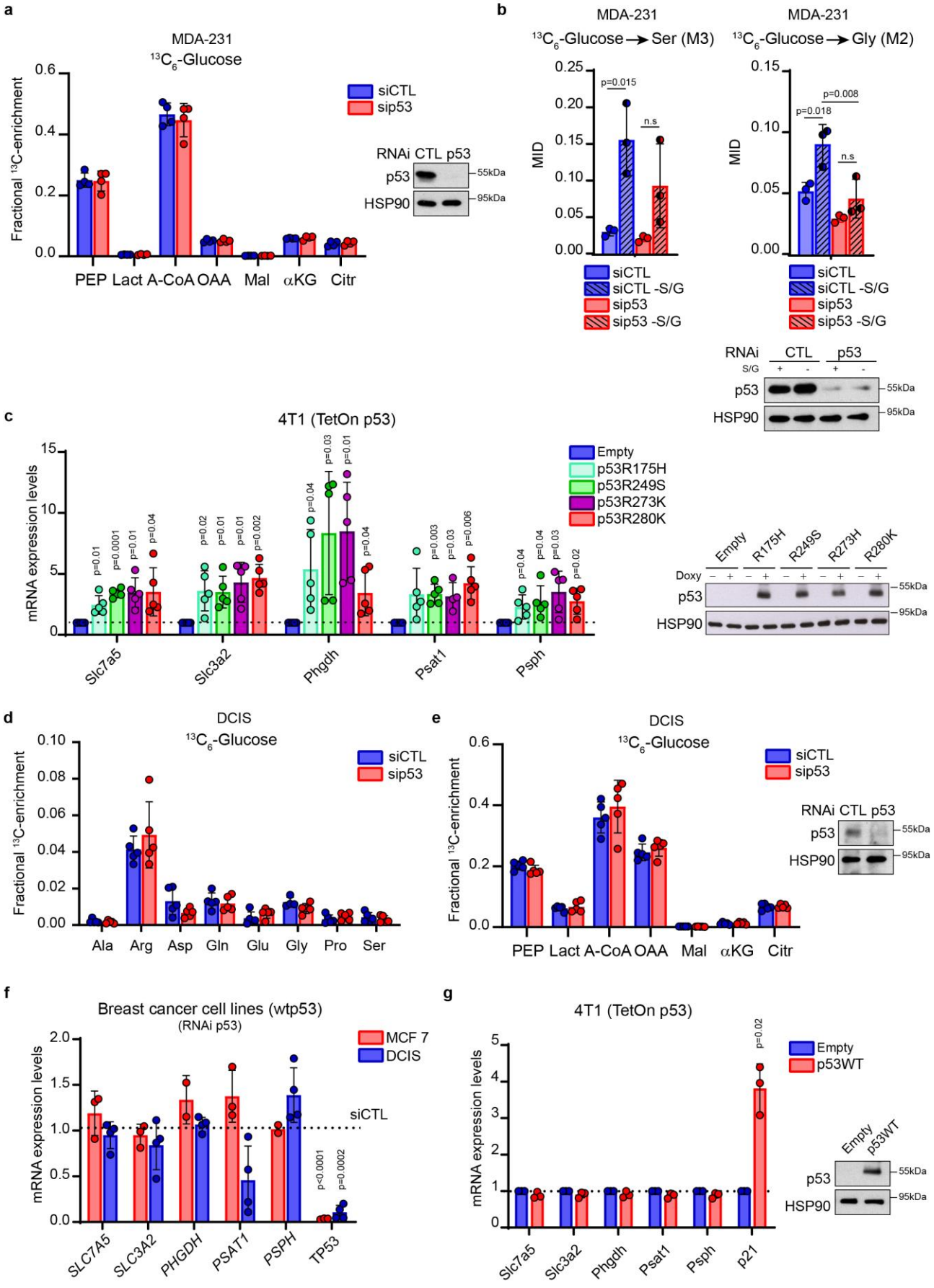
SUPPLEMENTARY INFORMATION Tombari et al.

Mutant p53 sustains serine-glycine synthesis and essential amino acids intake promoting breast cancer growth

SUPPLEMENTARY FIGURE 1



Supplementary Figure 1

a. Left panel: Mass isotopomer distribution (MID) of glycolysis and TCA cycle metabolites derived from [U-$^{13}C_6$]-Glucose in MDA-MB 231 cells upon control (siCTL) or p53 (sip53) silencing (PEP M3, Lact M3, A-CoA M2, OAA M4, Mal M2, αKG M5, Citr M6). For labeling experiments, cells were transfected with control or p53 siRNAs on the day of seeding and cultivated for 24h followed by incubation with [U-$^{13}C_6$]-Glucose in the medium for additional 24h. Right panel: western blot analysis of p53 levels in the above-described condition. HSP90 was used as loading control; n=4 biological replicates.

b. Upper panels: mass isotopomer distribution (MID) of serine M3 (left) and glycine M2 (right) from labeled [U-$^{13}C_6$]-Glucose in MDA-MB 231 cells upon control (siCTL) or p53 (sip53) silencing in complete medium or medium without serine and glycine (-S/G). For labeling experiments, cells were transfected with control or p53 siRNAs the day of seeding followed by incubation with [U-$^{13}C_6$]-Glucose in complete medium or medium without serine and glycine (-S/G), both with dialyzed serum, for additional 24h. Lower panel: western blot analysis of p53 levels in the above-described condition. HSP90 was used as loading control; n=3 biological replicates.

c. Left panel: qRT-PCR analysis of expression of indicated genes in 4T1 TetOn inducible clones in presence of doxycycline 1μg/mL for 24h. mRNA levels relative to those measured in 4T1 clone expressing empty vector (dotted line) are shown. Right panel: western blot analysis of p53 levels in 4T1 TetOn inducible clones in presence (+) or absence (-) of doxycycline 1μg/mL for 24h; n=5 biological replicates.

d. Mass isotopomer distribution (MID) of AAs from [U-$^{13}C_6$]-Glucose in MCF10DCIS.com cells upon control (siCTL) or p53 (sip53) silencing (isotopomers displayed in Fig. 1a are those reported in the graph). For labeling experiments, cells were transfected with control or p53 siRNAs on the day of seeding and cultivated for 24h followed by incubation with [U-$^{13}C_6$]-Glucose in the medium for additional 24h; n=5 biological replicates.

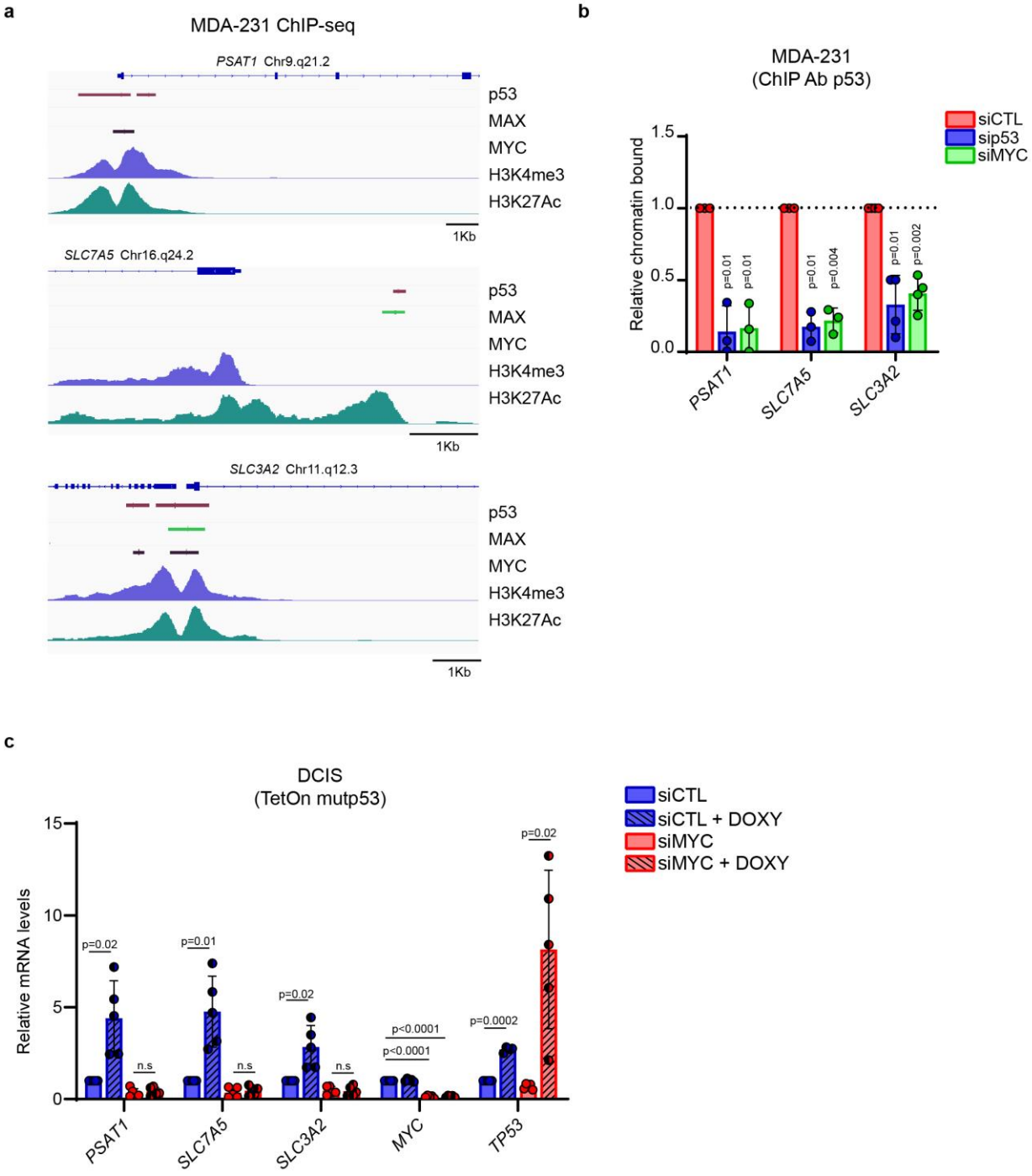
e. Left panel: Mass isotopomer distribution (MID) of glycolysis and TCA cycle metabolites derived from [U-$^{13}C_6$]-glucose in MCF10DCIS.com cells upon the above-described conditions (PEP M3, Lact M3, A-CoA M2, OAA M4, Mal M2 αKG M5, Citr M6). Right panel: western blot analysis of p53 levels in the above-described condition. HSP90 was used as loading control; n=5 biological replicates.

f. qRT-PCR analysis of the indicated genes in MCF7 and MCF10DCIS.com cell lines upon silencing of p53. mRNA expression levels relative to those measured in control condition (dotted line) are shown; n=3 biological replicates for MCF7 and n=4 biological replicates for MMCF10DCIS.com.

g. Left panel: qRT-PCR analysis of expression of indicated genes in 4T1 TetOn inducible clones in presence of doxycycline 1µg/mL for 24h. mRNA levels relative to those measured in 4T1 clone expressing empty vector (dotted line) are shown. Right panel: western blot analysis of p53 levels in 4T1 TetOn inducible clones in presence of doxycycline 1µg/mL for 24h; n=5 biological replicates.

Graph bars represent mean ± s.d. Two-tailed Student's t-test or one-way ANOVA (Fisher's LSD).

SUPPLEMENTARY FIGURE 2



Supplementary Figure 2

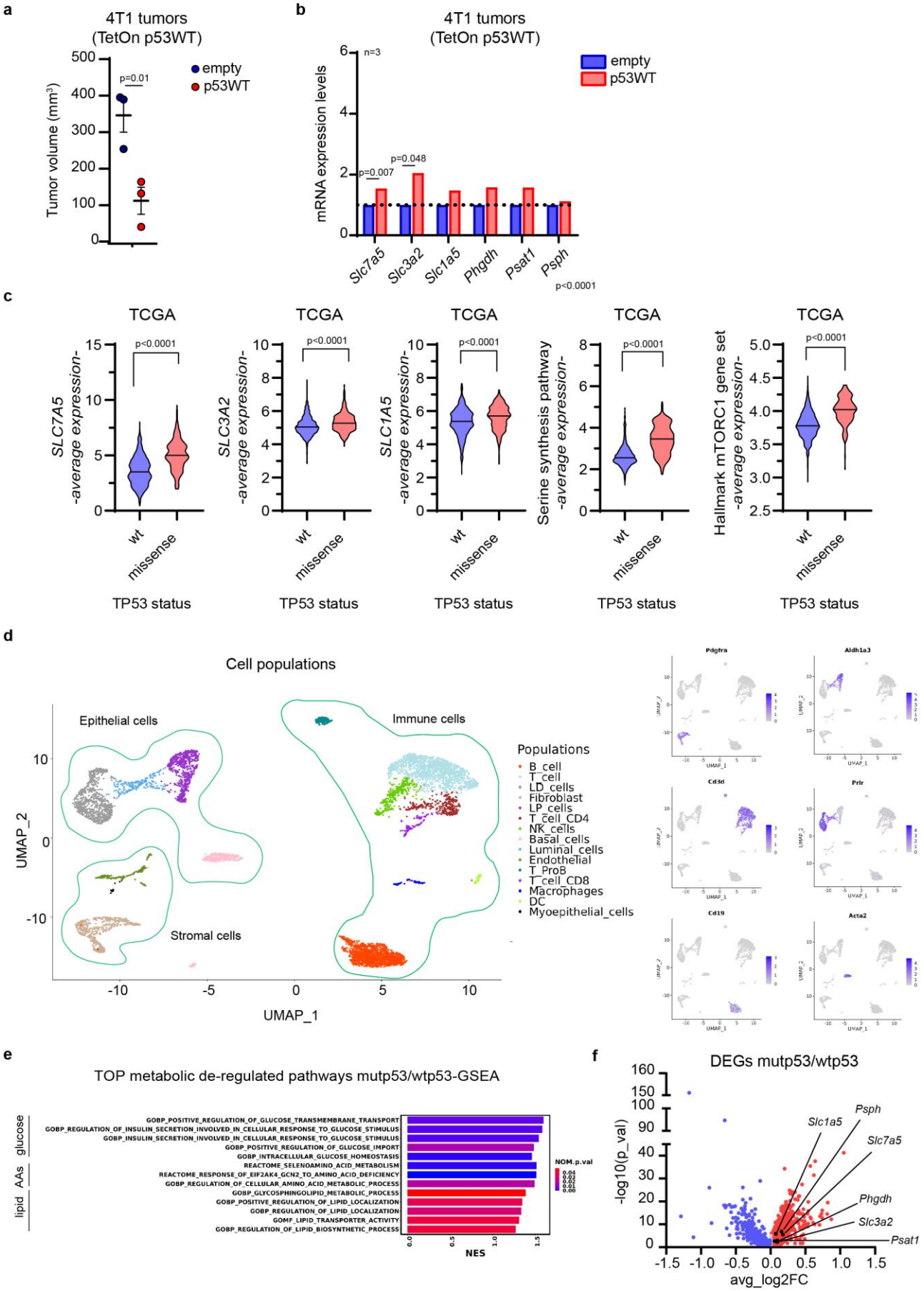
a. Scheme of *PSAT1*, *SLC7A5*, and *SLC3A2* loci, indicating promoter marks (H3K4me3 and H3K27Ac), putative TSS, and regions bound by mutp53, MAX and MYC.

b. Chromatin immunoprecipitation (ChIP) analysis of MDA-MB-231 cells upon control (siCTL), p53 (sip53) or MYC (siMYC) silencing with anti-p53 DO-1 antibody. Specific p53 binding to the indicated promoters was calculated as the ratio of fraction of input chromatin bound (2-ΔCT method) in p53-silenced (sip53) and MYC-silenced (siMYC) vs control-silenced (siCTL) cells. n=3 biological replicates for *PSAT1*, n=3 biological replicates for *SLC7A5*, n=4 biological replicates for *SLC3A2*.

c. qRT-PCR analysis of indicated genes in MCF10DCIS.COM TetOn inducible clones cultured upon control (siCTL) or MYC (siMYC) silencing in presence of doxycycline 1μg/mL for 3 days; n=5 biological replicates.

Graph bars represent mean ± s.d. Two-tailed Student's t-test.

SUPPLEMENTARY FIGURE 3



Supplementary Figure 3

a. Quantification of tumor volume in mice injected with indicated 4T1 TetOn inducible clones at day 24; n=3/condition.

b. qRT-PCR analysis of the indicated genes in 4T1 TetOn clones used in (**a)** n=3/condition.

c. Average expression levels of *SLC7A5*, *SLC3A2*, *SLC1A5*, serine synthesis pathway genes (i.e., *PHGDH*, *PSAT1,* and *PSPH*), and a gene set of mTORC1 activation in human breast cancer samples of the TCGA dataset (n=701) classified according to p53 status (wt and missense TP53 mutations).

d. Left panel: Uniform Manifold Approximation and Projection (UMAP) representation of scRNA-seq from wtp53 and mutp53 mice samples (wtp53: 3169 cells; mutp53: 3382 cells) colored according to cell type. Clusters were grouped into epithelial, stromal and immune populations. Right panels: FeaturePlots of known specific marker genes of the six more represented cell populations.

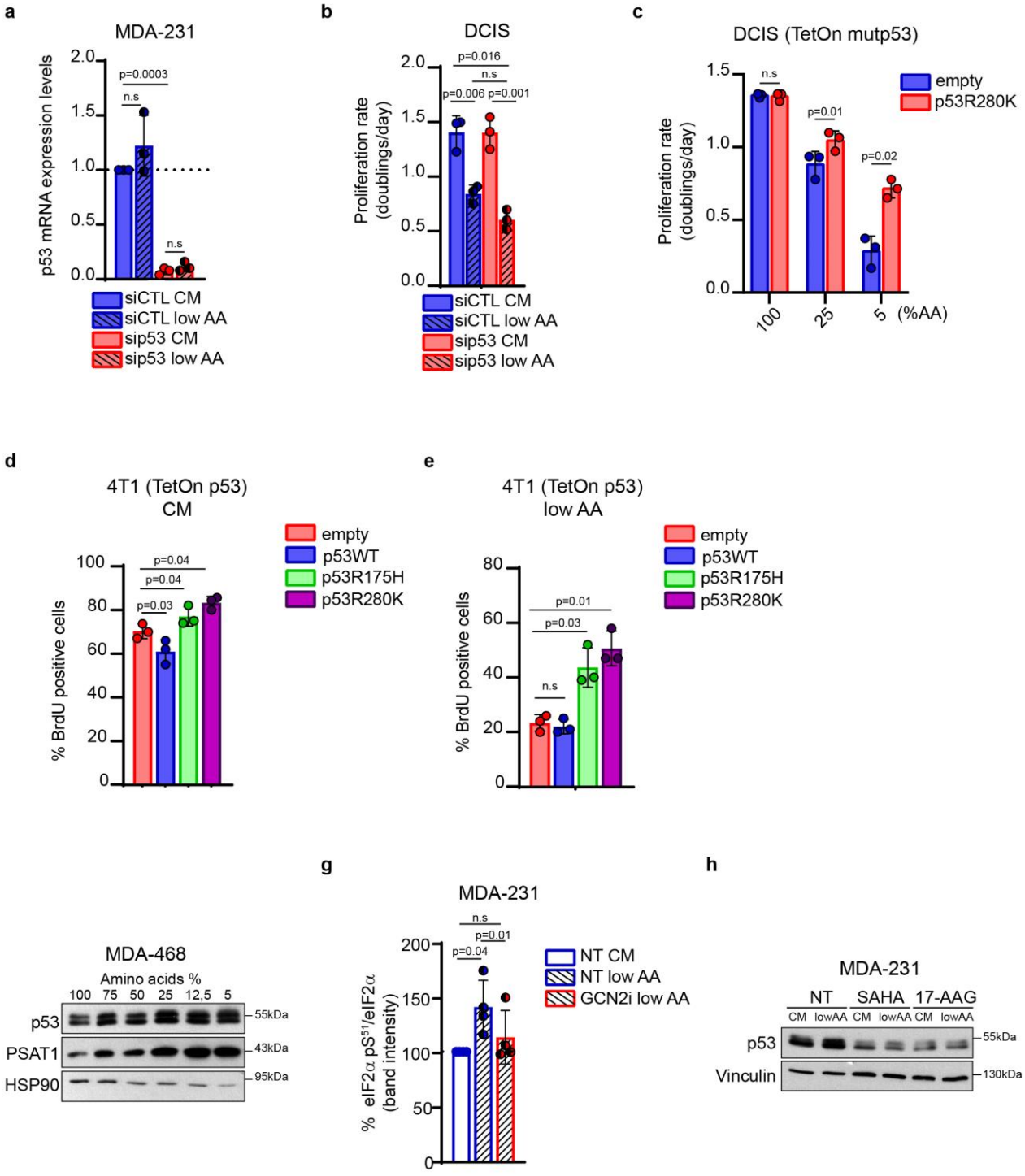
Abbreviations: LP, luminal progenitors; LD, luminal differentiated; DC, dendritic cells; NK, natural killer cells.

e. Gene sets enrichment analysis (GSEA) of glucose, AAs and lipid metabolism significantly enriched (NOM.p.val < 0.05) in mammary epithelial cells from $p53^{R172H/R172H}$ vs $p53^{+/+}$ mouse. The positive normalized enrichment score (NES) indicates the degree to which gene sets are overrepresented in the above-described conditions.

f. Volcano plot of genes significantly upregulated (red dots) and downregulated (blue dots) (padj<0.05) in mammary epithelial cells from $p53^{R172H/R172H}$ vs $p53^{+/+}$ mouse.

Two-tailed Student's t-test or Weighted Kolmogorov–Smirnov-like statistic or Wilcoxon Rank Sum test (pval) and bonferroni correction (padj).

SUPPLEMENTARY FIGURE 4



Supplementary Figure 4

a. qRT-PCR analysis of p53 mRNA level in MDA-MB 231cells grown for 72h in complete medium (CM) or medium containing 25% of AAs (low AA) upon silencing of p53. mRNA expression levels relative to those measured in control condition (dotted line) are shown; n=3 biological replicates.

b. Proliferation rate (doublings /day) of MCF10DCIS.COM cells cultured in complete medium (CM) or medium containing 25% of AAs (low AA) upon silencing of p53 for 3 days; n=3 biological replicates.

c. Proliferation rate (doublings/day) of MCF10DCIS.COM TetOn inducible clones cultured in medium with the indicated percentage of AAs for 3 days in presence of doxycycline 1μg/mL; n=3 biological replicates.

d-e. BrdU incorporation analysis in indicated 4T1 TetOn inducible clones grown in complete medium (CM) (**d**) or medium containing 25% of AAs (low AA) (**e**), in presence of doxycycline 1μg/mL. BrdU was added 3h (**d**) or 12h (**e**) before the end of the experiment. The percentage of BrdU positive cells out of 100-150 nuclei counted for each condition is shown; n=3 biological replicates.

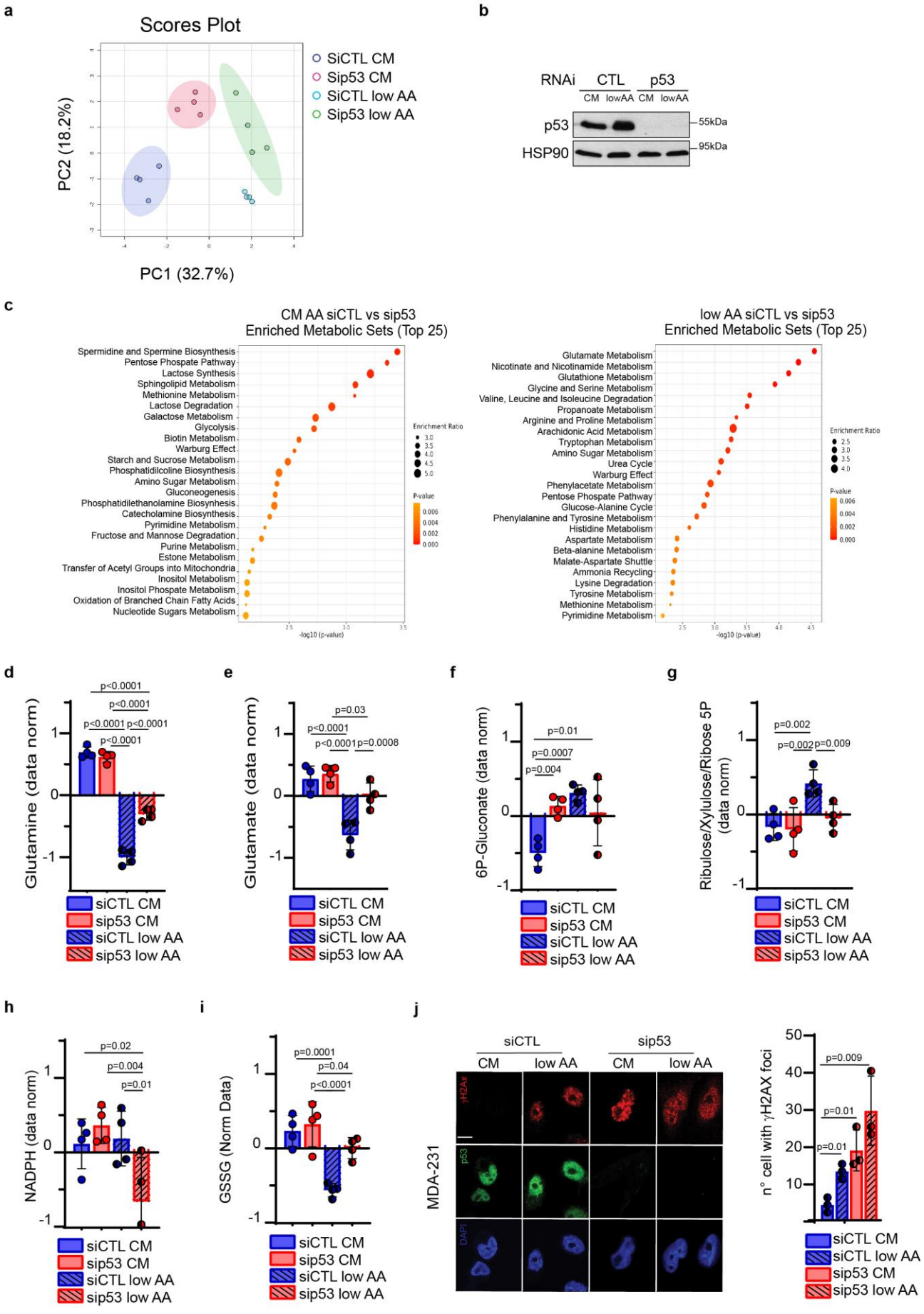
f. Western blot analysis of the indicated proteins in MDA-MB 468 cells grown for 72h in the indicated percentage of AAs; n=3.

g. Quantification of western blot shown in Fig. 3f. The percentage of eIF2α pS51 relative to eIF2α is shown; n=4 biological replicates.

h. Western blot analysis of the indicated proteins in MDA-MB 231 cells grown for 72h in complete medium (CM) or medium containing 25% of AAs (low AA), upon treatment with DMSO (NT), SAHA 1 μM, and 17-AAG 5 μM for 48h. Vinculin was used as loading control; n=3.

Graph bars represent mean ± s.d.. Two-tailed Student's t-test.

SUPPLEMENTARY FIGURE 5



Supplementary Figure 5

a. Principal component analysis (PCA) plot based on the metabolomic profile of MDA-MB 231 cells cultured for 3 days in complete medium (CM) or medium containing 25% of AAs (low AA) upon control (siCTL) or p53 (sip53) silencing. n=4 independent replicates (one single experiment).

b. Western blot analysis of p53 levels in the above-described conditions. HSP90 was used as loading control.

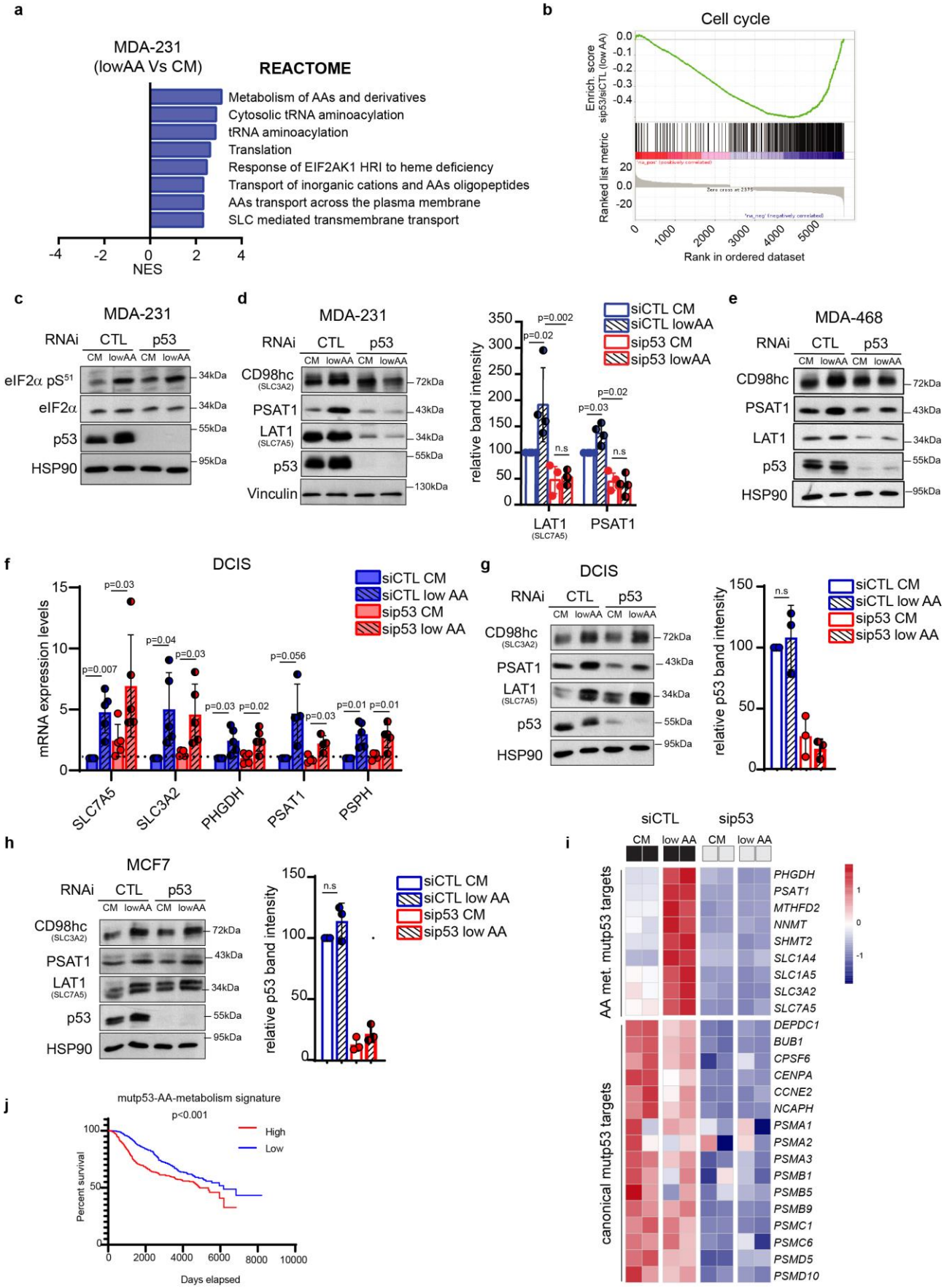
c. Left panel: dot Plot of the top 25 Enriched Metabolites Sets - quantitative enrichment analysis (Pathway based – SMPDB) on the results of metabolomic analysis (FDR < 0.05) in MDA-MB-231 cells cultured in control silencing (siCTL) vs p53 silencing (sip53) in complete medium (CM). Right panel: dot Plot of the top 25 Enriched Metabolites Sets - quantitative enrichment analysis (Pathway based – SMPDB) on the results of metabolomic analysis (FDR < 0.05) in MDA-MB-231 cells cultured in control silencing (siCTL) vs p53 silencing (sip53) in medium containing 25% of AAs (low AA). n=4 independent replicates (one single experiment).

d-i. Histograms showing the normalized abundance of glutamine (**d**), glutamate (**e**), 6-phosphogluconate (**f**), ribose-5-phosphate/xylulose-5-phosphate/ribulose-5-phosphate (three different isomers here measured as sum) (**g**), NADPH (**h**) and oxidized glutathione (i) from LC-MS analysis. n=4 independent replicates (one single experiment).

j. Left panel: representative immunofluorescence images of γH2AX positive MDA-MB 231 cells, cultured for 3 days in complete medium (CM) or in medium containing 25% of AAs (low AA) upon control (siCTL) or p53 (sip53) silencing. Right panel: quantification of γH2AX positive MDA-MB 231 cells. Scale bar 20 μm. The number of γH2AX positive cells out of 100 cells/condition is shown; n=3 biological replicates.

Two-tailed Student's t-test or Ordinary one-way ANOVA test (Fisher's LSD).

SUPPLEMENTARY FIGURE 6



Supplementary Figure 6

a. Reactome gene sets significantly enriched (padj value < 0.05) in MDA-MB-231 cells cultured in low AA vs complete medium (CM) in control silencing. The positive normalized enrichment score (NES) indicates the degree to which Reactome gene sets are overrepresented in the conditions described above. Gene expression data were obtained from n=2 biological replicates for each condition (GSE214494).

b. Gene set enrichment analysis (GSEA) of "Cell cycle" in MDA-MB-231 cells cultured in low AA upon p53 silencing vs control silencing (sip53/siCTL). n=2 biological replicates for each condition (GSE214494).

c, d. Western blot analyses of the indicated proteins in MDA-MB 231 cells cultivated for 72h in complete medium (CM) and in medium containing 25% of AAs (low AA) upon silencing of p53 (**c**, **d** left panel). Quantification of LAT1 and PSAT1 western blot bands expressed as percentage relative to Vinculin. HSP90 and Vinculin were shown as loading controls (**d**, right panel); n=4 biological replicates.

e. Western blot analysis of the indicated proteins in MDA-MB 468 cells cultivated for 72h in complete medium (CM) and in medium containing 25% of AAs (low AA) upon silencing of p53. HSP90 was shown as loading control; n=3.

f. qRT-PCR of indicated genes in DCIS cells cultivated for 72h in complete medium (CM) or medium containing 25% of AAs (low AA) upon silencing of p53. mRNA levels relative to control condition (dotted line) are shown; n=5 biological replicates.

g, h. Left panels: western blot analysis of the indicated proteins in DCIS (**g**) and MCF7 (**h**) cells cultivated for 72h in complete medium (CM) and in medium containing 25% of AAs (low AA) upon silencing of p53. HSP90 was shown as loading control. Right panels: quantification of p53 levels relative to HSP90 in western blots; n=3.

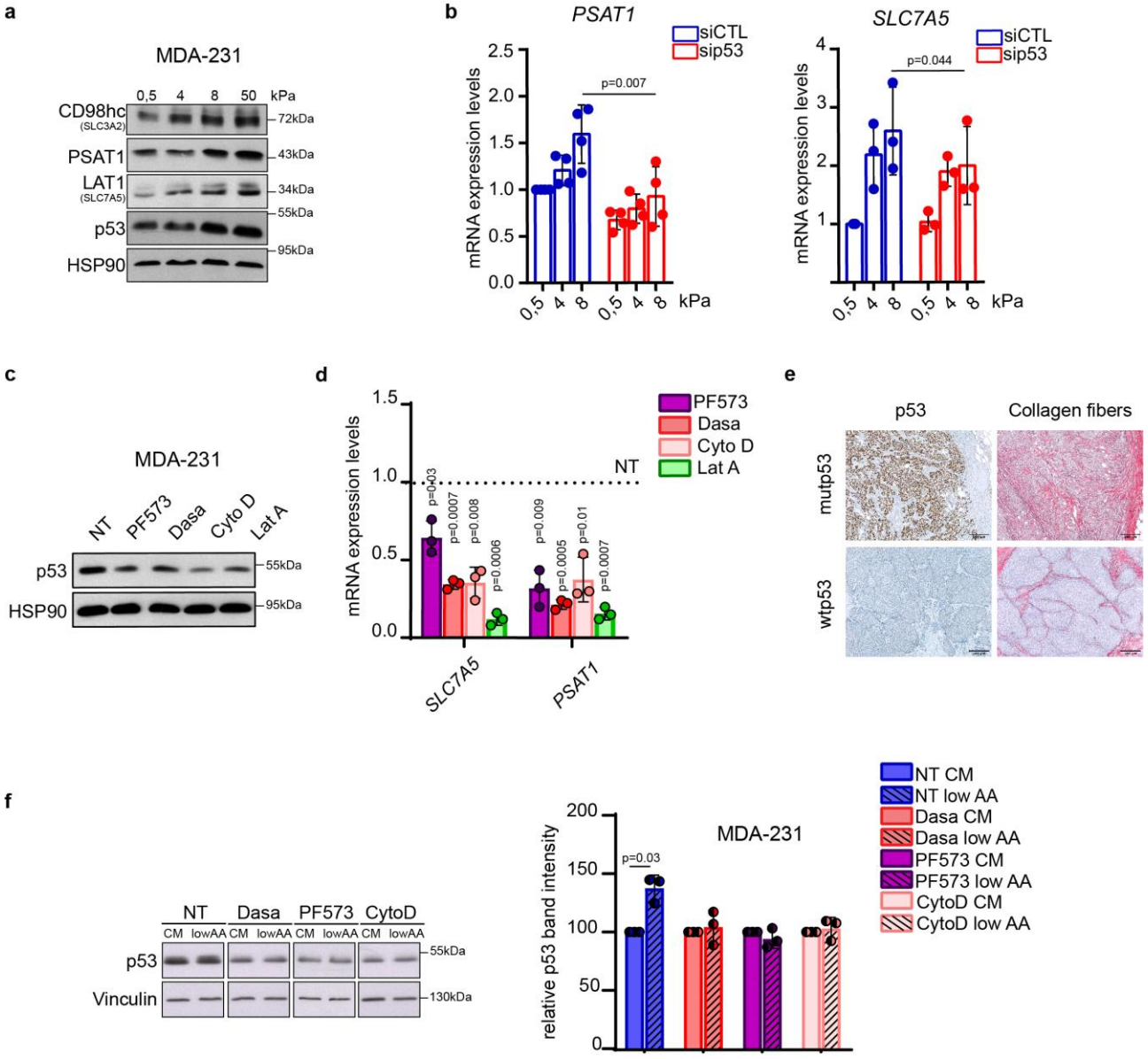
i. Heatmap of RNA-seq data of genes related to AA biosynthesis, intake, and metabolism in MDA-MB 231 cells in complete medium (CM) and in medium containing 25% of AAs (low AA) upon

control (siCTL) or p53 (sip53) silencing. Two columns for each condition represent n=2 biological replicates.

j. Cumulative incidence of overall survival calculated using Kaplan-Meier survival analysis in human breast cancer samples of the Metabric dataset (n=997) stratified based on high or low mutp53 AA metabolism signature.

Graph bars represent mean ± s.d. Two-tailed Student's t-test or log-rank test or Wilcoxon Rank Sum test (pval) and bonferroni correction (padj).

SUPPLEMENTARY FIGURE 7



Supplementary Figure 7.

a. Western blot analysis of the indicated proteins in MDA-MB 231 cells cultured on fibronectin-coated hydrogels with 0,5, 4, 8, and 50 kPa elastic moduli for 48h. HSP90 was used as loading control; n=3.

b. qRT-PCR of indicated genes in MDA-MB 231 cells cultured on fibronectin-coated hydrogels with 0,5, 4 and 8 kPa elastic moduli for 48h upon control (siCTL) or p53 (sip53) silencing. n=4 biological replicates for *PSAT1* and n=3 biological replicates for *SLC7A5*.

c. Western blot analysis of p53 protein in MDA-MB 231 treated with DMSO (NT), PF573228 (PF573) 10 µM, Dasatinib (Dasa) 0,5 µM, Cytochalasin D (Cyto D) 1 µM, Latrunculin A (Lat A) 0,5 µM, for 48h. HSP90 was shown as loading control; n=3.

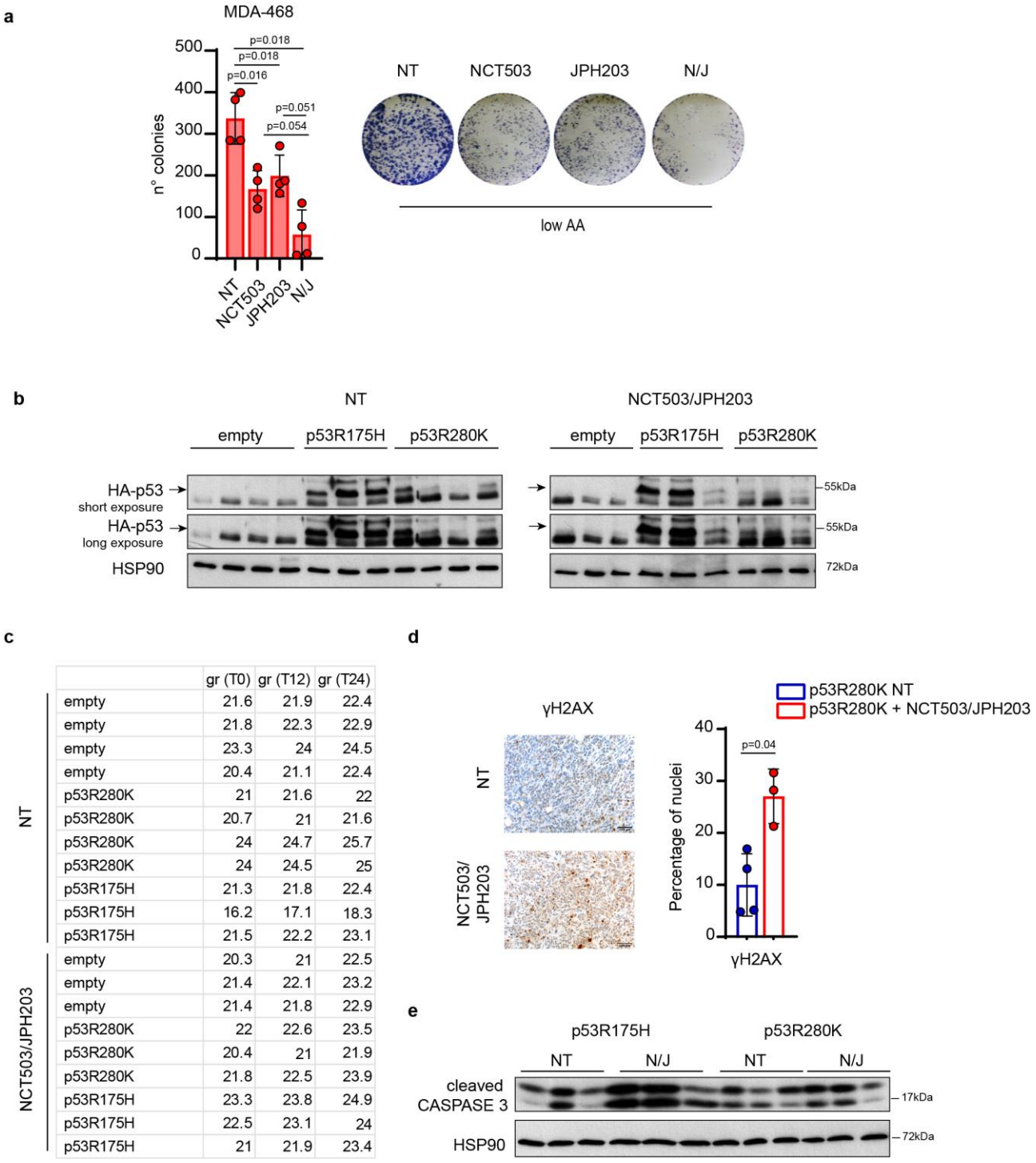
d. qRT-PCR of indicated genes in MDA-MB 231 cells treated with DMSO (NT), PF573228 (PF573) 10 µM, Dasatinib (Dasa) 0,5 µM, Cytochalasin D (Cyto D) 1 µM, Latrunculin A (Lat A) 0,5 µM, for 48h; n=3 biological replicates.

e. Representative images of p53 immunohistochemical staining and Picrosirius Red of breast cancer samples of Fig. 2d. Original magnification, x50. Scale bar, 500 µm.

f. Left panel: western blot analysis of p53 protein in MDA-MB 231 cultivated for 72h in complete medium (CM) and in medium containing 25% of AAs (low AA) and treated with DMSO (NT), Dasatinib (Dasa) 0,5µM, PF573228 (PF573) 10µM, and Cytochalasin D (Cyto D) 1µM. Vinculin was shown as loading control. Right panel: quantification of p53 levels relative to vinculin in western blot; n=3 biological replicates.

Graph bars represent mean ± s.d. Two-tailed Student's t-test.

SUPPLEMENTARY FIGURE 8



		gr (T0)	gr (T12)	gr (T24)
NT	empty	21.6	21.9	22.4
	empty	21.8	22.3	22.9
	empty	23.3	24	24.5
	empty	20.4	21.1	22.4
	p53R280K	21	21.6	22
	p53R280K	20.7	21	21.6
	p53R280K	24	24.7	25.7
	p53R280K	24	24.5	25
	p53R175H	21.3	21.8	22.4
	p53R175H	16.2	17.1	18.3
	p53R175H	21.5	22.2	23.1
NCT503/JPH203	empty	20.3	21	22.5
	empty	21.4	22.1	23.2
	empty	21.4	21.8	22.9
	p53R280K	22	22.6	23.5
	p53R280K	20.4	21	21.9
	p53R280K	21.8	22.5	23.9
	p53R175H	23.3	23.8	24.9
	p53R175H	22.5	23.1	24
	p53R175H	21	21.9	23.4

Supplementary Figure 8

a. Left panel: quantification of colonies formed by MDA-MB 468 cells grown in medium containing 25 % AAs (low AAs), treated with DMSO (NT), NCT-503 10μM, JPH203 10μM, or combination of NCT503 10μM - JPH203 10μM (N/J). Right panel: representative images of colonies described above; n=4 biological replicates.

b. Western blot analysis of HA-p53 protein in lysates of tumors formed by indicated 4T1 TetOn inducible clones treated with placebo (NT) or combination of NCT503/JPH203. HSP90 was shown as loading control.

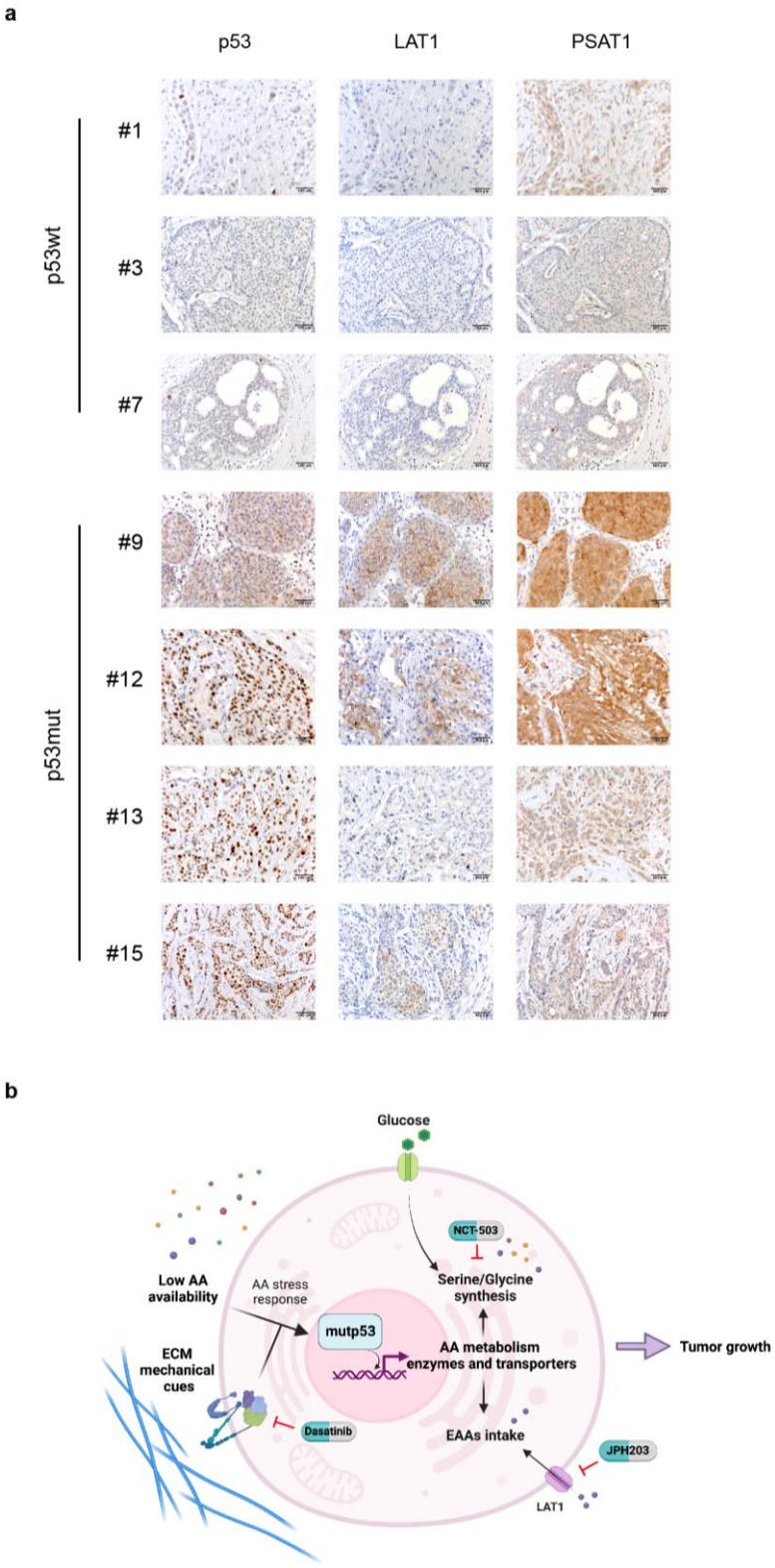
c. Table indicating the body weight of mice at the day of injection (T0) and after 12 (T12) and 24 (T24) days.

d. Representative images and quantitative analyses of γH2AX immunohistochemical staining in mouse tumor samples of Fig. 6d and e. Original magnification, x200. Scale bar, 100 μm.

e. Western blot analysis of cleaved CASPASE 3 in lysates of tumors in **b**. HSP90 was shown as loading control.

Two-tailed Student's t-test.

SUPPLEMENTARY FIGURE 9



Supplementary Figure 9

a. Representative images of immunohistochemical staining of p53, PSAT1 and LAT1 in seven BCs cases from which PDOs were derived. Original magnification, x200. Scale bar, 100 μm.

b. Schematic summary model. Mutp53 potentiates two branches of amino acid metabolism (i.e., serine/glycine synthesis and EAAs intake) promoting the expression of SSP enzymes and EAAs transporter in response to environmental stresses to sustain tumor growth. Treating patient-derived tumor organoids bearing mutp53 with SSP and LAT1 inhibitors or with mechanosignaling inhibitor (e.g., Dasatinib) restricts their viability. Created with BioRender.com.

Supplementary Table 1: List of AA metabolism-related genes presenting at least one p53 peak in their promoter region in ChIP-seq of MDA-MB-231 cells (GEO GSE95303). The columns show gene name, peak number, peak score, peak genomic coordinates, histone marks (GEO GSE49651) and TFs bound.

Gene Symbols	mutp53 Peak	Score	Chromosome	Peak Start	Peak End	Peak H3K27Ac Score	Peak H3K27Ac Start	Peak H3K27Ac End	Peak H3K4me3 Score	Peak H3K4me3 Start	Peak H3K4me3 End	TFs bound
AHCY	Peak1	98	chr20	34312651	34313044	660,22	34311907	34317582	-	-	-	
	Peak2	37	chr20	34314324	34314819	660,22	34311907	34317582	-	-	-	
	Peak3	19	chr20	34316205	34316664	660,22	34311907	34317582	-	-	-	
ASL	Peak1	3	chr7	66075633	66075816	447,09	66074860	66077308	3100	66074899	66077182	
	Peak2	3	chr7	66075997	66076291	447,09	66074860	66077308	3100	66074899	66077182	
ASS1	Peak1	3	chr9	130445623	130445792	-	-	-	2535,7	130444012	130446241	
BCAT2	Peak1	7	chr19	48812180	48812653	414,47	48809919	48813326	3100	48809825	48813288	
	Peak2	21	chr19	48810720	48811297	414,47	48809919	48813326	3100	48809825	48813288	
CTH	Peak1	11	chr1	70410503	70411604	618,26	70409582	70412708	3109,63	70409883	70413167	
DHFR	Peak1	10	chr5	80655042	80655377	1920,97	80652883	80656825	3100	80652808	80656357	
	Peak2	4	chr5	80654707	80654865	1920,97	80652883	80656825	3100	80652808	80656357	
	Peak3	4	chr5	80654054	80654504	1920,97	80652883	80656825	3100	80652808	80656357	
MAT2B	Peak1	11	chr5	163503160	163503490	3100	163502173	163507918	3100	163502616	163507814	
	Peak2	6	chr5	163504630	163504939	3100	163502173	163507918	3100	163502616	163507814	
	Peak3	4	chr5	163505343	163505693	3100	163502173	163507918	3100	163502616	163507814	
	Peak4	5	chr5	163505819	163506017	3100	163502173	163507918	3100	163502616	163507814	
MTHFD1	Peak1	20	chr14	64387787	64388716	2313,71	64386818	64390404	3100	64386741	64390029	
MTHFD1L	Peak1	9	chr6	150857628	150857864	713,62	150856301	150858872	-	-	-	
	Peak2	10	chr6	150865420	150865695	3100	150864600	150868032	3100	150864705	150867797	
	Peak3	3	chr6	150866826	150867053	3100	150864600	150868032	3100	150864705	150867797	
MTHFD2	Peak1	9	chr2	74198239	74198814	884,91	74197934	74200401	3100	74197995	74200656	
	Peak2	9	chr2	74199081	74199427	884,91	74197934	74200401	3100	74197995	74200656	
MTHFR	Peak1	27	chr1	11805395	11806282	2793,98	11800238	11808449	1105,45	11804400	11807850	
MTR	Peak1	31	chr1	236794891	236795587	1498,38	236794048	236797389	3100	236794407	236797390	
	Peak2	5	chr1	236795660	236795860	1498,38	236794048	236797389	3100	236794407	236797390	
MTRR	Peak1	15	chr5	7868884	7869249	1144,86	7867900	7870481	3100	7867745	7870454	
PHGDH	Peak1	5	chr1	119711853	119712095	-	-	-	659,24	119711509	119713484	
PSAT1	Peak1	26	chr9	78296255	78297415	3100	78295123	78299134	3100	78295572	78299291	ZMYND8;BRD9;JUN;CDKN1B;BRD2;BRD4;SOX4;BRD2;ZMYND8;FOXM1;CDKN1B;ESR1;BRD9;JUN;FOSL1;MYC
	Peak2	7	chr9	78297548	78297969	3100	78295123	78299134	3100	78295572	78299291	no TFs
PSPH	Peak1	5	chr7	56051790	56051948	2803,93	56049793	56054340	3100	56049924	56053245	
	Peak2	16	chr7	56051180	56051631	2803,93	56049793	56054340	3100	56049924	56053245	
SHMT1	Peak1	7	chr17	18363438	18363605	444,51	18362019	18364398	3100	18361996	18364364	
	Peak2	6	chr17	18362713	18363247	444,51	18362019	18364398	3100	18361996	18364364	
SLC1A5	Peak1	43	chr19	46787916	46789019	3100	46782271	46789346	3100	46783564	46789115	
	Peak2	16	chr19	46787125	46787503	3100	46782271	46789346	3100	46783564	46789115	
SLC1A7	Peak1	65	chr1	53146101	53146747	669,13	53145095	53148852	-	-	-	
SLC25A15	Peak1	4	chr13	40789811	40790206	957,39	40788391	40790805	3100	40788560	40790909	
SLC38A2	Peak1	9	chr12	46373568	46374156	1581,38	46363889	46375053	3100	46366612	46374297	
	Peak1	4	chr12	46372993	46373342	1581,38	46363889	46375053	3100	46366612	46374297	
	Peak1	17	chr12	46372537	46372821	1581,38	46363889	46375053	3100	46366612	46374297	
SLC3A2	Peak1	6	chr11	62854838	62855296	1633,76	62852395	62858111	3100	62852787	62858710	BRD2, TP53, BRD4, MYC
	Peak2	70	chr11	62855445	62856510	1633,76	62852395	62858111	3100	62852787	62858710	BRD4;BRD2;MYC;BRD9;FOXM1;CDKN1B;SOX4;MAX;ZMYND8;TP53;YAP1;ESR1;TEAD4;JUN;FOSL1;STAT3
SLC6A9	Peak1	10	chr1	44036619	44036876	-	-	-	-	-	-	
	Peak2	9	chr1	44031110	44031861	3162,8	44027570	44032401	3100	44028932	44032464	
SLC7A1	Peak1	13	chr13	29594285	29594798	1929,22	29593831	29596880	3100	29593645	29596725	
SLC7A5	Peak1	14	chr16	87871659	87871843	1126,78	87866285	87872163	3100	87866415	87869952	JUN;YAP1;BRD4;TEAD4;E2F1;ZMYND8;CDKN1B;BRD2;BRD9;SOX4;STAT3;MAX;FOXM1
SLC7A6	Peak1	8	chr16	68263906	68264329	3100	68263057	68267225	3100	68263308	68266840	
	Peak2	13	chr16	68265149	68265771	3100	68263057	68267225	3100	68263308	68266840	

Supplementary Table 2: List of known specific marker genes used to identify cell types of scRNAseq analysis of wtp53 mouse ($p53^{+/+}$) and mutp53 mouse ($p53^{R172H/R172H}$).

Basal_cells	Luminal_cells	LD_cells	LP_cells	Myoepithelial_cells	Endothelial	Fibroblast	T_cell	CD4	T_ProB	CD8	B_cell	NK	Macrophage	DC
Trp63	Epcam	Esr1	Elf5	Acta2	Mcam	Vim	Cd3d	Cd4	Il7r	Cd8a	Cd19	Gnly	Cd80	Cd1e
Snai2	Krt18	Pgr	Kit	Tagln	Vcam1	Pdgfra	Cd3e	Ptprc	Ptprc	Ptprc	Cd22	Nkg7	Cd68	Fcer1a
Krt14	Krt8	Ar	Cd14	Mylk	Vwf	Pdgfrb	Tnfrsf4				Ptprc	Ptprc	C1qa	Cd208
Krt5	Krt19	Foxa1	Aldh1a3	Myl9	Pecam1		Ptprc						C1qb	Cd265
Itga6	Gata3	Prlr	Hey2	Vim	Sele								Adgre1	Xcr1
Procr		Wnt4	Ehf		Cd93								Ptprc	Batf3
Zeb2		Tnfsf11	Tnfrsf11a		Nectin3									Fscn1
		Areg			Tek									
		Cited1												
		Batf												
		Tbx3												

Supplementary Table 3: Percentages of cell populations obtained from scRNAseq analysis of wtp53 mouse ($p53^{+/+}$) and mutp53 mouse ($p53^{R172H/R172H}$).

	wtp53	**mutp53**
B_cell	21,7	17,3
T_cell	25,2	20,8
LD_cells	13,4	10,5
Fibroblast	5,7	9,7
LP_cells	8,5	10,9
T_cell_CD4	4,9	5
NK_cells	4,7	5,1
Basal_cells	3,3	7,5
Luminal_cells	3	3,5
Endothelial	2,8	3,8
T_ProB	2	2
T_cell_CD8	1,8	1,5
Macrophages	1,2	1,2
DC	1,5	0,8
Myoepithelial_cells	0,3	0,4

Supplementary Table 4: List of significant indicated DEGs from scRNAseq data of epithelial population comparing mutp53 mouse ($p53^{R172H/R172H}$) with wtp53 mouse ($p53^{+/+}$). DEGs have been calculated using the R package Seurat and sorted in decreasing avg_log2FC order. Wilcoxon Rank Sum test (pval) and bonferroni correction (padj). Cutoff padj<0.05.

Gene	avg_log2FC	-log10(p_val)	p_val	pct.1	pct.2
Slc7a5	0,15	5,68	2,09E-06	0,491	0,369
Psph	0,12	6,84	1,45E-07	0,276	0,177
Slc3a2	0,07	2,44	0,003666308	0,892	0,835
Phgdh	0,07	2,49	0,003216926	0,312	0,248
Slc1a5	0,05	5,79	1,61E-06	0,298	0,201
Psat1	0,04	2,53	0,002981782	0,386	0,31

Supplementary Table 5: List of 51 significantly different metabolites identified in the experimental conditions (SiQ 100%, Sip53 100%, SiQ 25%, Sip53 25%). ANOVA p-value (FDR) cutoff 0.05, post-hoc analysis Fisher's LSD.

Metabolites	f.value	p.value	-LOG10(p)	FDR	Fisher's LSD
Glutamine	260,14	3,51E-11	10,454	3,06E-09	SiP53 100% - SiP53 25%; SiP53 100% - SiQ 25%; SiQ 100% - SiP53 25%; SiP53 25% - SiQ 25%; SiQ 100% - SiQ 25%
L-Acetylcamitine	65,21	1,07E-07	6,9724	4,64E-06	SiP53 25% - SiP53 100%; SiQ 100% - SiP53 100%; SiQ 25% - SiP53 100%; SiP53 25% - SiQ 100%; SiQ 25% - SiQ 100%
Iso-Citrate	51,536	3,97E-07	6,4017	1,15E-05	SiP53 25% - SiP53 100%; SiQ 100% - SiP53 100%; SiQ 25% - SiP53 100%; SiQ 25% - SiQ 100%
pentanoylcarnitine	35,106	3,21E-06	5,4938	6,98E-05	SiP53 25% - SiP53 100%; SiP53 100% - SiQ 100%; SiP53 25% - SiQ 100%; SiP53 25% - SiQ 25%; SiQ 25% - SiQ 100%
Stearoylcamitine	33,497	4,12E-06	5,3852	7,17E-05	SiP53 25% - SiP53 100%; SiP53 100% - SiQ 100%; SiP53 25% - SiQ 100%; SiP53 25% - SiQ 25%; SiQ 25% - SiQ 100%
Oleoylcamitine	28,035	1,05E-05	4,9787	0,0001372	SiP53 100% - SiQ 100%; SiP53 100% - SiQ 25%; SiP53 25% - SiQ 100%; SiP53 25% - SiQ 25%
Malate	27,767	1,10E-05	4,9571	0,0001372	SiQ 100% - SiP53 100%; SiP53 100% - SiQ 25%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
Putrescine	24,799	1,98E-05	4,7042	0,00021492	SiP53 25% - SiP53 100%; SiQ 100% - SiP53 100%; SiQ 25% - SiP53 100%; SiQ 100% - SiP53 25%; SiQ 25% - SiP53 25%
Glucose	24,124	2,27E-05	4,6431	0,00021986	SiP53 100% - SiQ 100%; SiQ 25% - SiP53 100%; SiP53 25% - SiQ 100%; SiQ 25% - SiQ 100%
Glutathione	21,773	3,82E-05	4,4184	0,00033198	SiP53 100% - SiP53 25%; SiQ 100% - SiP53 100%; SiP53 100% - SiQ 25%; SiQ 100% - SiP53 25%; SiP53 25% - SiQ 25%; SiQ 100% - SiQ 25%
Propionylcarnitine	20,262	5,46E-05	4,263	0,00039695	SiP53 25% - SiP53 100%; SiP53 100% - SiQ 100%; SiQ 25% - SiP53 100%; SiP53 25% - SiQ 100%; SiQ 25% - SiQ 100%
Asp	19,995	5,83E-05	4,2347	0,00039695	SiP53 100% - SiP53 25%; SiQ 100% - SiP53 100%; SiQ 100% - SiP53 25%; SiQ 25% - SiP53 25%; SiQ 100% - SiQ 25%
Glu	19,922	5,93E-05	4,2268	0,00039695	SiP53 100% - SiP53 25%; SiP53 100% - SiQ 25%; SiP53 25% - SiQ 25%; SiQ 100% - SiQ 25%
Fructose 1,6-bisphosphate	18,428	8,69E-05	4,0612	0,00051977	SiP53 25% - SiP53 100%; SiQ 100% - SiP53 100%; SiQ 25% - SiP53 100%; SiQ 100% - SiP53 25%
PEP	18,16	9,33E-05	4,0303	0,00051977	SiP53 25% - SiP53 100%; SiQ 25% - SiP53 100%; SiP53 25% - SiQ 100%; SiQ 25% - SiQ 100%
Fumarate	18,067	9,56E-05	4,0196	0,00051977	SiQ 100% - SiP53 100%; SiP53 100% - SiQ 25%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
O-palmitoleoylcarnitine	17,166	0,00012227	3,9127	0,00062573	SiP53 100% - SiQ 100%; SiP53 100% - SiQ 25%; SiP53 25% - SiQ 100%; SiP53 25% - SiQ 25%
Thr	16,385	0,00015267	3,8162	0,00073792	SiP53 100% - SiP53 25%; SiP53 100% - SiQ 25%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
GSSG	15,475	0,00019984	3,6993	0,00091504	SiP53 100% - SiP53 25%; SiP53 100% - SiQ 25%; SiP53 25% - SiQ 25%; SiQ 100% - SiQ 25%
L-Palmitoylcarnitine	15,153	0,00022047	3,6567	0,00095903	SiP53 25% - SiP53 100%; SiP53 100% - SiQ 100%; SiP53 25% - SiQ 100%; SiP53 25% - SiQ 25%; SiQ 25% - SiQ 100%
Ser	13,935	0,00032453	3,4887	0,0013445	SiP53 100% - SiP53 25%; SiP53 100% - SiQ 25%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
Ile	13,439	0,00038273	3,4171	0,0015135	SiP53 100% - SiP53 25%; SiQ 100% - SiP53 25%; SiQ 25% - SiP53 25%; SiQ 100% - SiQ 25%
Phe	12,98	0,00044771	3,349	0,0016935	SiP53 100% - SiP53 25%; SiQ 100% - SiP53 25%; SiQ 25% - SiP53 25%
Met	11,209	0,0008539	3,0686	0,0030012	SiP53 100% - SiP53 25%; SiQ 100% - SiP53 25%; SiQ 25% - SiP53 25%
Citrate	11,183	0,00086243	3,0643	0,0030012	SiP53 25% - SiP53 100%; SiQ 100% - SiP53 100%; SiQ 25% - SiP53 100%
SAM	10,806	0,00099927	3,0003	0,0033437	SiQ 100% - SiP53 100%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
UDP	10,141	0,0013073	2,8836	0,0042124	SiQ 100% - SiP53 100%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
Lactate	9,6655	0,0015959	2,797	0,0049588	SiP53 25% - SiP53 100%; SiQ 100% - SiP53 100%; SiQ 25% - SiP53 100%
CTP	9,5599	0,0016698	2,7773	0,0050094	SiQ 100% - SiP53 100%; SiQ 100% - SiP53 25%; SiP53 25% - SiQ 25%; SiQ 100% - SiQ 25%
Asn	9,4634	0,0017407	2,7593	0,0050482	SiP53 100% - SiP53 25%; SiQ 100% - SiP53 25%; SiQ 25% - SiP53 25%
SAH	9,2128	0,0019421	2,7117	0,0054505	SiQ 100% - SiP53 100%; SiQ 25% - SiP53 100%; SiQ 100% - SiP53 25%
UTP	8,8338	0,0023006	2,6382	0,0062548	SiQ 100% - SiP53 100%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
ATP	8,5123	0,0026662	2,5741	0,0070292	SiQ 100% - SiP53 100%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
His	8,3239	0,0029118	2,5358	0,0073529	SiP53 100% - SiP53 25%; SiP53 100% - SiQ 25%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
Val	8,2905	0,0029581	2,529	0,0073529	SiP53 100% - SiP53 25%; SiQ 100% - SiP53 25%; SiQ 25% - SiP53 25%
Dihydroxyacetone phosphate	7,9143	0,0035428	2,4507	0,0085617	SiQ 100% - SiP53 100%; SiQ 25% - SiP53 100%; SiQ 25% - SiP53 25%
Gly	7,5897	0,0041575	2,3812	0,0097757	SiP53 100% - SiP53 25%; SiP53 100% - SiQ 25%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
Gluconate 6P	7,4726	0,004409	2,3557	0,010094	SiP53 100% - SiQ 100%; SiP53 25% - SiQ 100%; SiQ 25% - SiQ 100%
Glucose 6-Phosphate	7,148	0,0052048	2,2836	0,011611	SiQ 25% - SiP53 100%; SiP53 25% - SiQ 100%; SiQ 25% - SiQ 100%
Ribu-5P	7,0681	0,0054257	2,2655	0,011801	SiQ 25% - SiP53 100%; SiQ 25% - SiP53 25%; SiQ 25% - SiQ 100%
GMP	6,8058	0,006231	2,2054	0,012983	SiP53 25% - SiP53 100%; SiQ 100% - SiP53 100%; SiQ 25% - SiP53 100%
Isobutyryl-L-carnitine	6,7947	0,0062679	2,2029	0,012983	SiP53 100% - SiQ 25%; SiP53 25% - SiQ 25%; SiQ 100% - SiQ 25%
aKG	5,6482	0,011947	1,9227	0,024172	SiP53 25% - SiP53 100%; SiP53 25% - SiQ 100%; SiP53 25% - SiQ 25%
dGTP	5,562	0,012577	1,9004	0,024868	SiQ 100% - SiP53 100%; SiQ 100% - SiP53 25%; SiQ 100% - SiQ 25%
Sarcosine	5,1917	0,015757	1,8025	0,029916	SiP53 100% - SiP53 25%; SiQ 25% - SiP53 25%; SiQ 25% - SiQ 100%
Taurine	5,1856	0,015818	1,8009	0,029916	SiP53 100% - SiQ 100%; SiP53 25% - SiQ 100%; SiP53 25% - SiQ 25%
NADPH	4,9985	0,017783	1,75	0,032918	SiP53 100% - SiP53 25%; SiQ 100% - SiP53 25%; SiQ 25% - SiP53 25%
NADH	4,9105	0,018806	1,7257	0,034086	SiP53 25% - SiQ 100%; SiQ 25% - SiQ 100%
Ala	4,8034	0,020144	1,6959	0,035766	SiQ 25% - SiP53 25%; SiQ 25% - SiQ 100%
Ac-CoA	4,6856	0,021743	1,6627	0,037833	SiQ 100% - SiP53 25%; SiQ 25% - SiP53 25%
Arg	4,3725	0,026765	1,5724	0,045658	SiQ 25% - SiP53 25%; SiQ 25% - SiQ 100%

Supplementary Table 6: Mutp53 AA metabolism signature. List of 213 genes found significantly upregulated in siCTL MDA-MB-231 cells grown in low AA vs CM condition, and downregulated in sip53 vs siCTL MDA-MB-231 cells grown in low AA condition.

mutp53 AA metabolism signature					
TNFSF18	EIF4EBP1	GYPC	FADS3	SLC43A1	LDLRAP1
PSAT1	HIF1A-AS3	SLFN12	PSPH	LEPR	WWC3
NXPH4	PEAR1	SLC1A3	CHML	ROBO3	FBXO25
NNMT	ADM2	LGALS8	PDGFA	CCN2	VLDLR
LINC02331	ANOS1	BEX2	EPRS1	RBCK1	TRIB3
ST6GALNAC3	SHMT2	AARS1	SLC8B1	TARS1	LONP1
SLC16A1	ANKRD1	PID1	PHLDB3	PYCR1	RSL24D1
JDP2	NXN	NLRP1	LINC00662	ZNF598	FLNB
APOL6	ANKFN1	CT83	PCID2	NARS1	CHIC2
GPR17	SLC7A5	VSIR	ACSS3	RBMS2	PRPF39
PHGDH	CHAC1	NCOA7	CDC42EP1	LMO4	DDIT4
PCK2	NIBAN1	OGFRL1	NANOS1	HSPA13	LITAF
ASNS	OVCH2	RPS6KA2	LINC02693	SPIRE1	CALCOCO2
SFTA1P	GPT2	LINC02783	TTC9	UHRF1BP1	SNTB1
KISS1	TLCD5	RASGRF1	TGFB2	FAT3	NTN4
ANGPT4	TPM1	LIMCH1	TIMM44	NFE2L2	CEP120
TUBE1	SARS1	ATF3	TMC8	BCAT1	MIR4435-2HG
SLC1A4	MTHFD1L	SYTL1	ANXA3	PAWR	CEBPB
SHOC1	SLC3A2	WARS1	SLC39A14	DDX60L	ATF4
FSTL1	YARS1	SLC7A1	XPOT	GFPT1	PPP1R15B
ASS1	SLC22A15	ADARB1	MILR1	UBE2J1	FAM241A
VLDLR-AS1	CASP4	AXL	CARS1	KLF13	ADCY6
C6orf132	ELF3	DOCK11	CNGA1	FMNL1-DT	TACC2
FAM167A	TMEM156	PDZD2	LPAL2	DYSF	ARHGEF17
SLC1A5	GARS1	UGCG	EIF2S2	PPARD	SLC38A1
MTHFD2	PABPC1L	SERTAD4	HAX1	PDLIM5	KLHL42
CSF2RB	TSEN15	RPL13AP20	SLC20A2	SESN2	RIOK3
SORBS2	PLXNA2	PPL	CRIM1	PRNP	PARP6
MAP3K20	PRKCE	MIR325HG	KLHL5	IGDCC4	WWC2
CREB3L1	AJUBA	MARS1	ARHGEF2	PXK	KIRREL1
GABRG1	CCND2	CEBPG	ZNF419	DNASE2	DPY19L3
PM20D2	PPME1	COL8A1	ARHGAP23	ECHDC2	PARVA
CTH	PEA15	MOCOS	LARS1	LOC100128398	SMIM13
STC2	PNMA2	LSM8	CLIC4	TMEM268	
MKX	SHANK2	TOM1L1	PDCD4	CD55	
INSYN2B	SLC7A11	IARS1	CHRM3-AS2	ATP13A3	