Additional File 2

Figure S1. Relative gene expression of CYP epoxygenase (CYP2J2 and CYP2C19) and epoxide hydrolase (sEH) in paired normal mammary and tumor tissue specimen. Gene expression profile of **a** CYP2J2, **b** CYP2C19, and **c** seH in paired normal and tumor specimens with corresponding metabolomics data were measured by qRT-PCR analyses. All experiments were performed in triplicate. GADPH expression for each sample was used as control.

Figure S2. Representative strong positive, positive and weak positive/negative immunostaining and corresponding quantitative scoring analyses results of IHC analyses for tumor sections (*N* = 55) obtained against CYP3A4 antibodies. DAB-staining was used to visualize immunoreactive regions and cellular nuclei (blue) were counterstained with hematoxylin. Images were taken at 40x magnification. Quantitative scoring analyses of the immunostained tissues using IHC profiler shows the percentage contribution of DAB-stained cells with strong positive, positive and weak positive/negative immunoreactivity for CYP3A4 antibodies.

Figure S3. Comparison of CYP epoxygenase gene and protein expressions in tissues with corresponding oxylipin metabolome data. Oxylipin metabolomics, gene expression and immunohistochemical analyses show a direct relationships among total EET metabolite concentrations, CYP2C19 and CYP2J2 gene expression and IHC scores of **a** TNBC (*N* = 4), **b** ER+/PR+/HER2– (*N* = 6), **c** ER–/PR–/HER2+ (*N* = 5) and **d** TPBC (*N* = 5) tumor specimen.

Figure S4. Effects of CYP epoxygenase expression and EET supplementation on mesencymal-like TNBC cells. Metastatic phenotype of mesencymal-like TNBC B cells (MDA-MB-231) in 3D culture following treatment with the four EET isomers (2.5 nM each isomer, total EET concentration of 10 nM) in combination.

Figure S5. CYP epoxygenase overexpression predicts survival outcomes in breast cancer patients. Survival curves using a multigene classifier for CYP epoxygenases and hydroxylases in **a** TNBC (*N* = 178), **b** ER+/PR+/HER2– (*N* = 84) and **c** ER–/PR–/HER2+ tissues (*N* = 138) from a publicly available curated database of gene expressions, and overall survival information from GEO, EGA and TCGA (kmplot.com).

Figure S6. Biological processes and molecular functions enriched in CYP epoxygenase overexpressing specimens. GO enrichment analysis for **a** TNBC, **b** ER+/PR+/HER2–, **c** ER–/PR–/HER2+ and **d** TPBC specimens in the discovery set with mRNA expression *z* score of ≥ 2.0 for CYP2J2 and CYP2C9).

Figure S7. Pathway deregulation scoring (PDS) differentiates pathways regulated by CYP upregulation in breast cancer tumor subtypes. PDS for FA metabolism-related pathways were calculated for all samples downloaded from TCGA. Breast tissue specimens (*N* = 120) with high PDS scores for CYP450-related pathways (KEGG xenobiotic metabolism, KEGG arachidonic acid metabolism and REACTOME fatty acid metabolism) were selected for further analysis.

Figure S8. Key targetable molecular pathways altered in CYP epoxygenase overexpressing tumor specimens. Network of potential protein targets in **a** HER2+, **b** luminal (ER+/PR+/HER2- or TPBC), and **c** TNBC tumors are determined by Over Representation Analysis (ORA) and Network Topology-based Analysis (NTA) of gene enriched in the specimens examined.

Figure S1

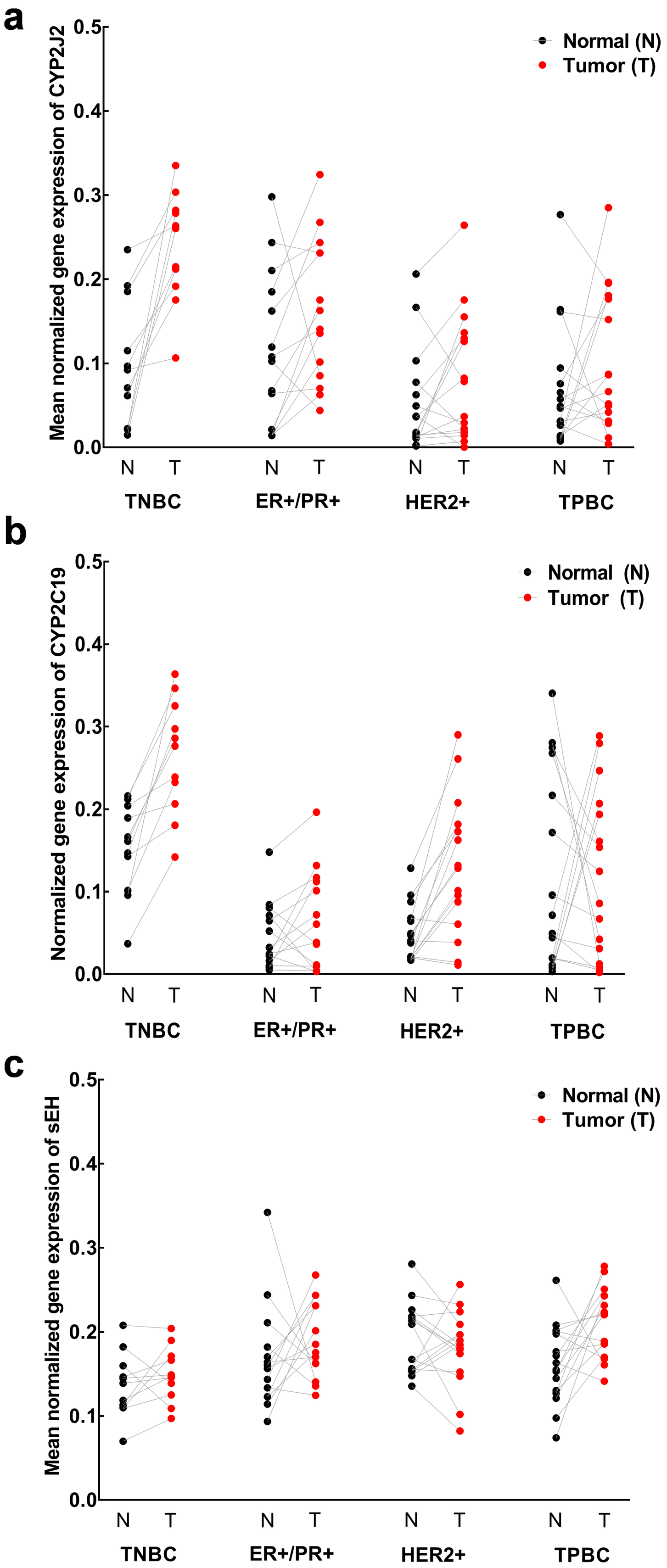
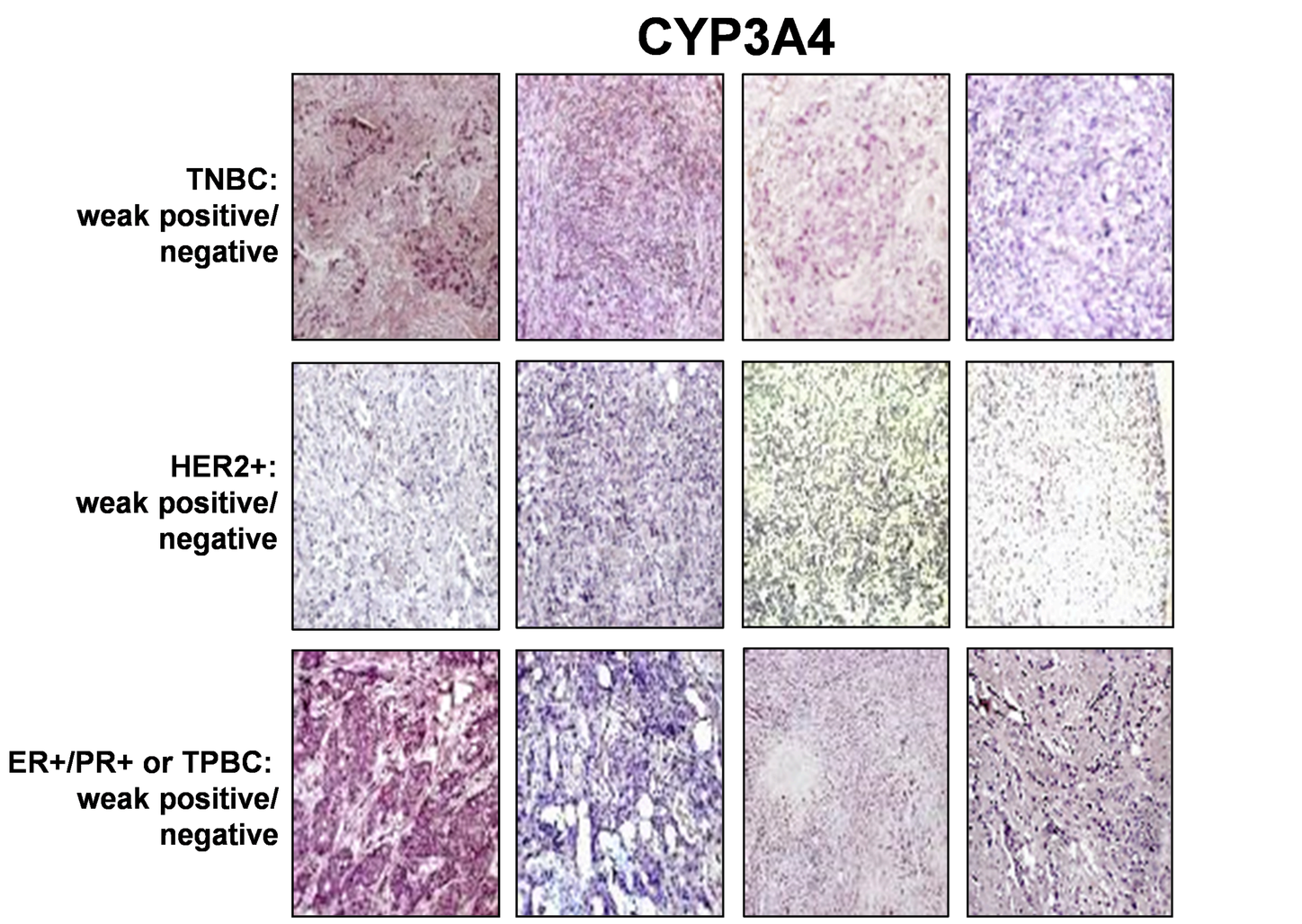

a
Mean normalized gene expression of CYP2J2
0.5
0.4
0.3
0.2
0.1
0.0
Normal (N)
Tumor (T)
N T
N T
N T
N T
TNBC
ER+/PR+
HER2+
TPBC
b
Normalized gene expression of CYP2C19
0.5
0.4
0.3
0.2
0.1
0.0
Normal (N)
Tumor (T)
N T
N T
N T
N T
TNBC
ER+/PR+
HER2+
TPBC
c
Mean normalized gene expression of sEH
0.5
0.4
0.3
0.2
0.1
0.0
Normal (N)
Tumor (T)
N T
N T
N T
N T
TNBC
ER+/PR+
HER2+
TPBC

Figure S2


CYP3A4
TNBC:
weak positive/
negative
HER2+:
weak positive/
negative
ER+/PR+ or TPBC:
weak positive/
negative

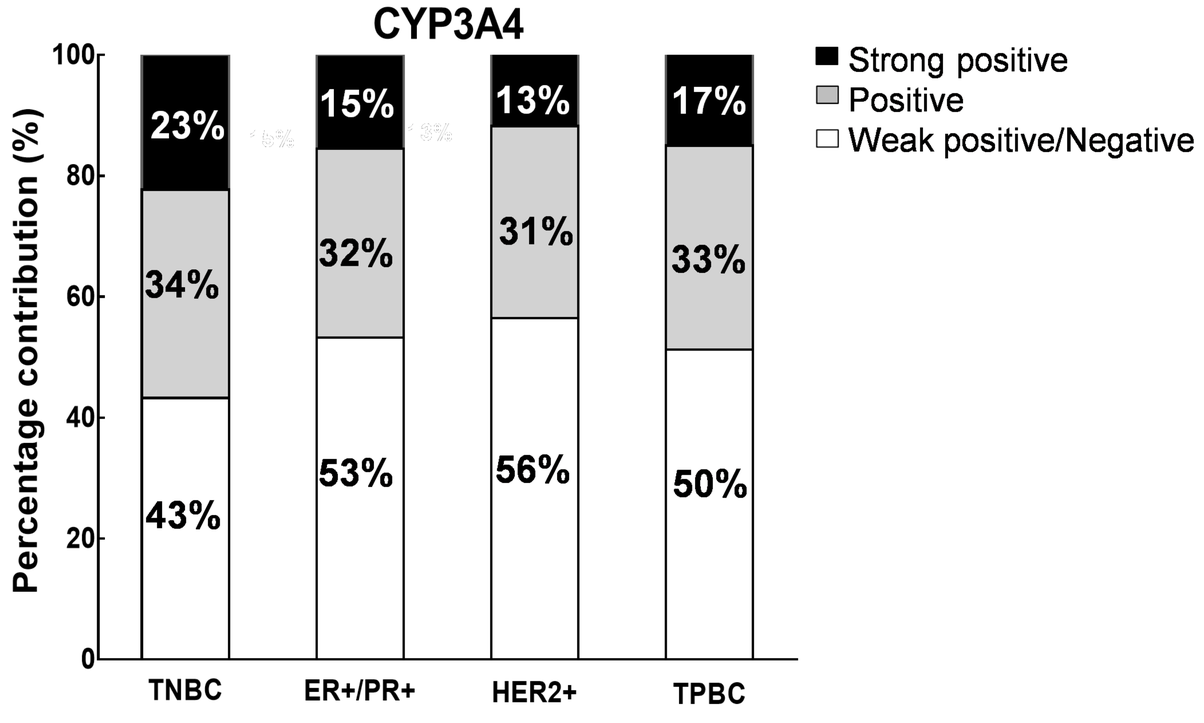
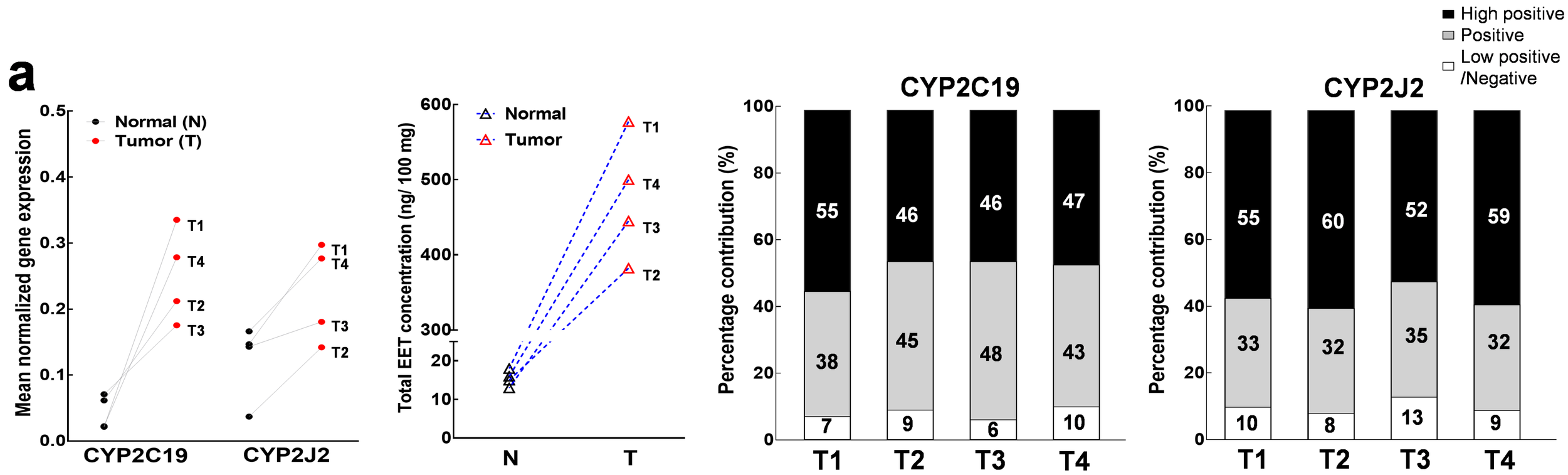
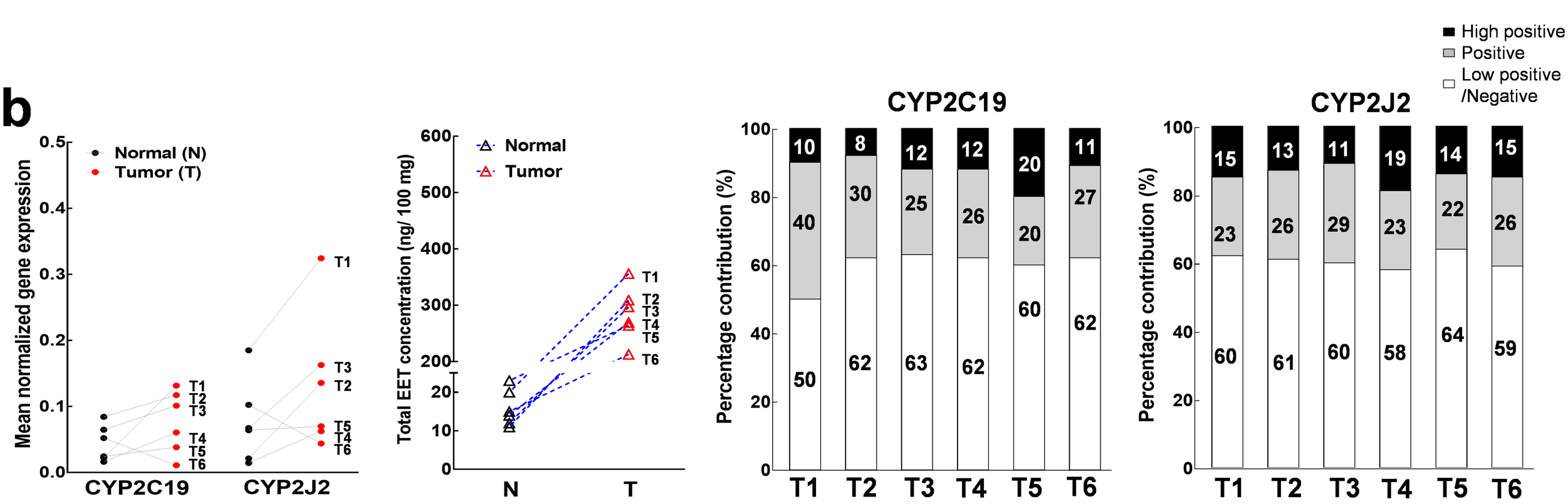
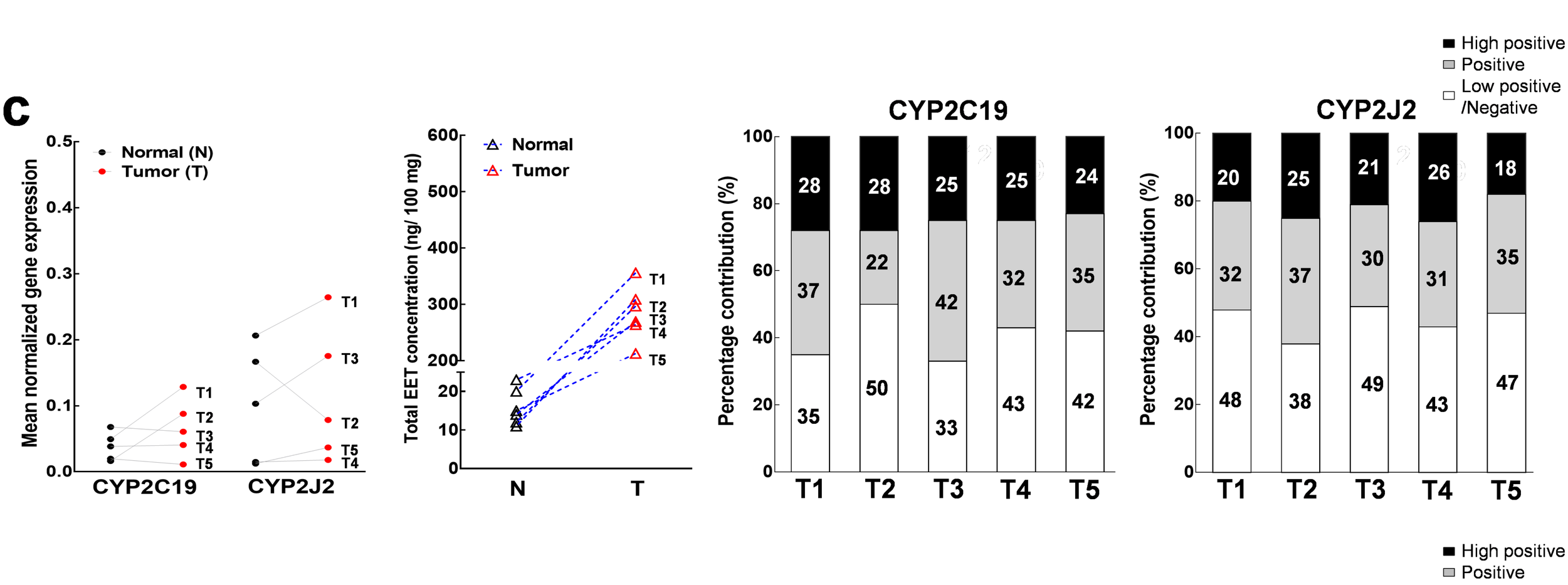

CYP3A4
Percentage contribution (%)
100
80
60
40
20
0
23%
34%
43%
15%
32%
53%
13%
31%
56%
17%
33%
50%
TNBC
ER+/PR+
HER2+
TPBC
Strong positive
Positive
Weak positive/Negative

Figure S3


a
Normal (N)
Tumor (T)
Mean normalized gene expression
CYP2C19
CYP2J2
T1
T4
T2
T3
Normal
Tumor
Total EET concentration (ng/ 100 mg)
N
T
High positive
Positive
Low positive /Negative
Percentage contribution (%)
55
38
7
46
45
9
46
48
6
47
43
10
33
10
60
32
8
52
35
13
59
32
9


b
Normal (N)
Tumor (T)
Mean normalized gene expression
CYP2C19
CYP2J2
T1
T2
T3
T4
T5
T6
Normal
Tumor
Total EET concentration (ng/ 100 mg)
N
T
High positive
Positive
Low positive /Negative
Percentage contribution (%)
10
40
50
8
30
62
12
25
63
12
26
62
20
20
60
11
27
62
15
23
60
13
26
61
11
29
60
19
23
58
14
22
64
15
26
59


c
Normal (N)
Tumor (T)
Mean normalized gene expression
CYP2C19
CYP2J2
T1
T2
T3
T4
T5
Normal
Tumor
Total EET concentration (ng/ 100 mg)
N
T
High positive
Positive
Low positive /Negative
Percentage contribution (%)
28
37
35
28
22
50
25
42
33
25
32
43
24
35
42
20
32
48
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31
43
18
35
47

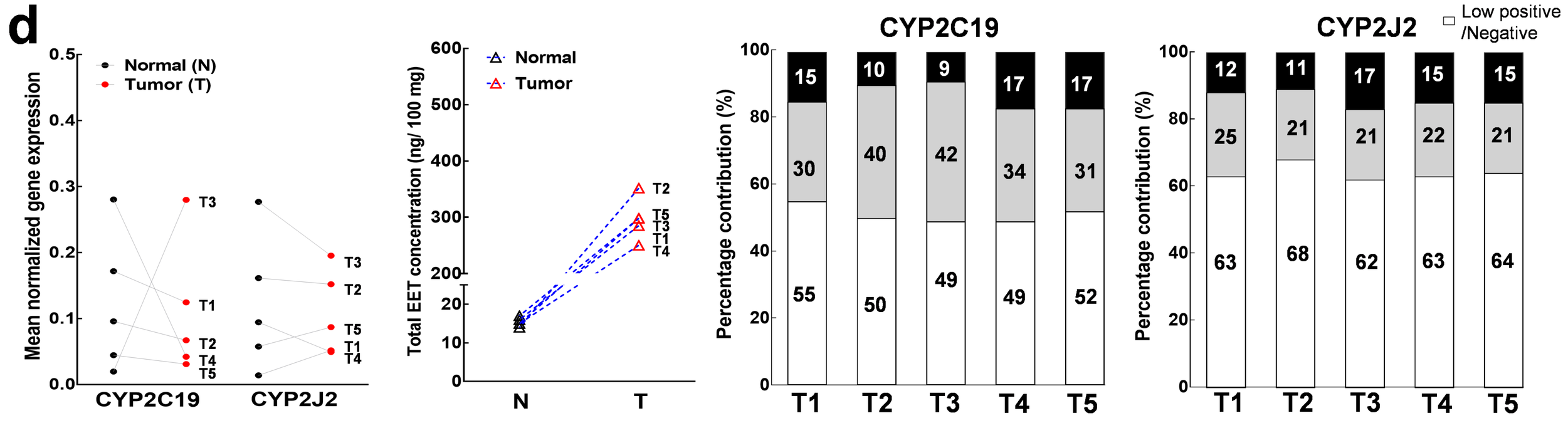

d
Normal (N)
Tumor (T)
Mean normalized gene expression
CYP2C19
CYP2J2
T1
T2
T3
T4
T5
Normal
Tumor
Total EET concentration (ng/ 100 mg)
N
T
High positive
Positive
Low positive /Negative
Percentage contribution (%)
15
30
55
10
40
50
9
42
49
17
34
49
17
31
52
12
25
63
11
21
68
17
21
62
15
22
63
15
21
64

Figure S4

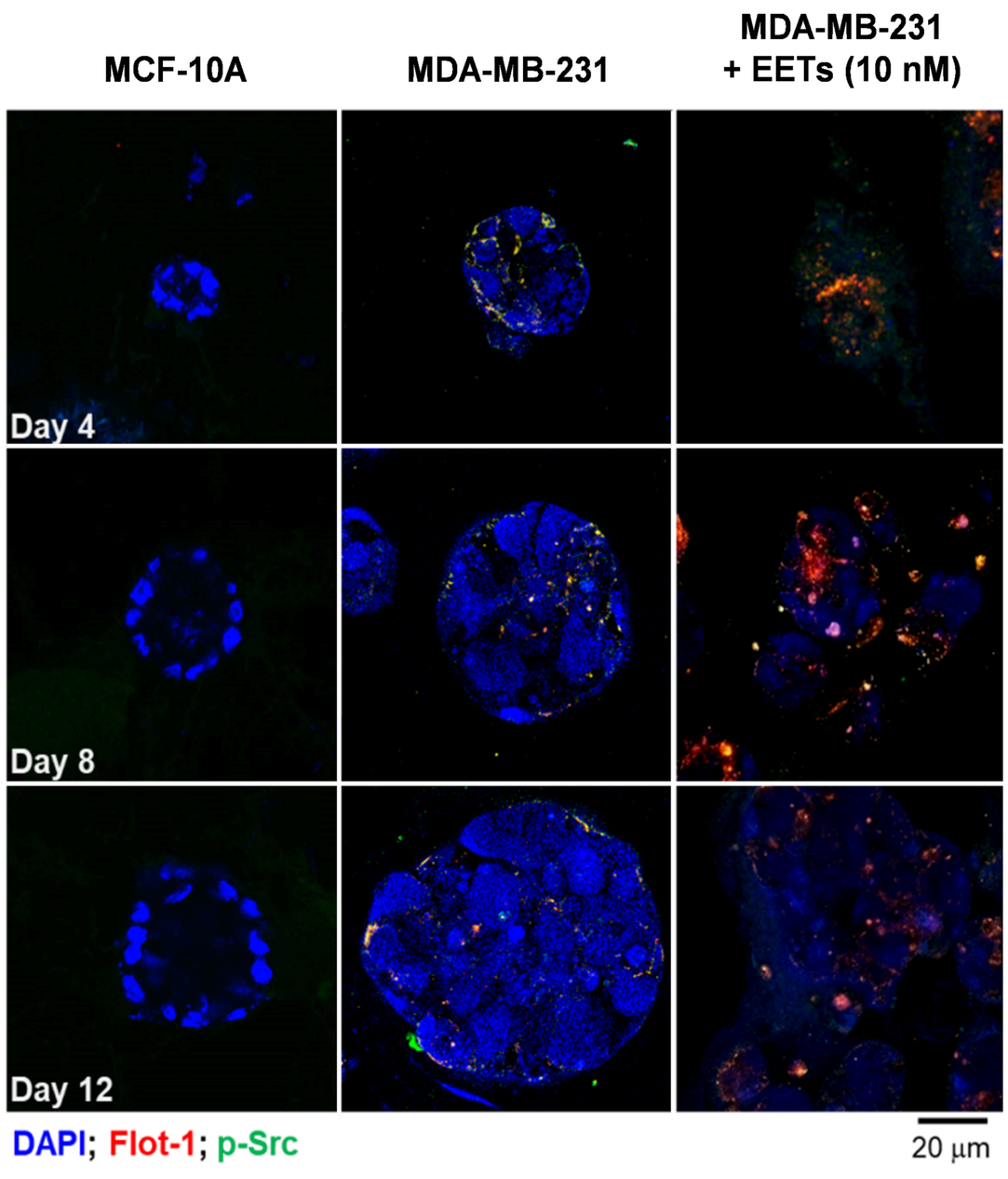

MCF-10A
MDA-MB-231
MDA-MB-231
+ EETs (10 nM)
Day 4
Day 8
Day 12
DAPI; Flot-1; p-Src
20 μm

Figure S5

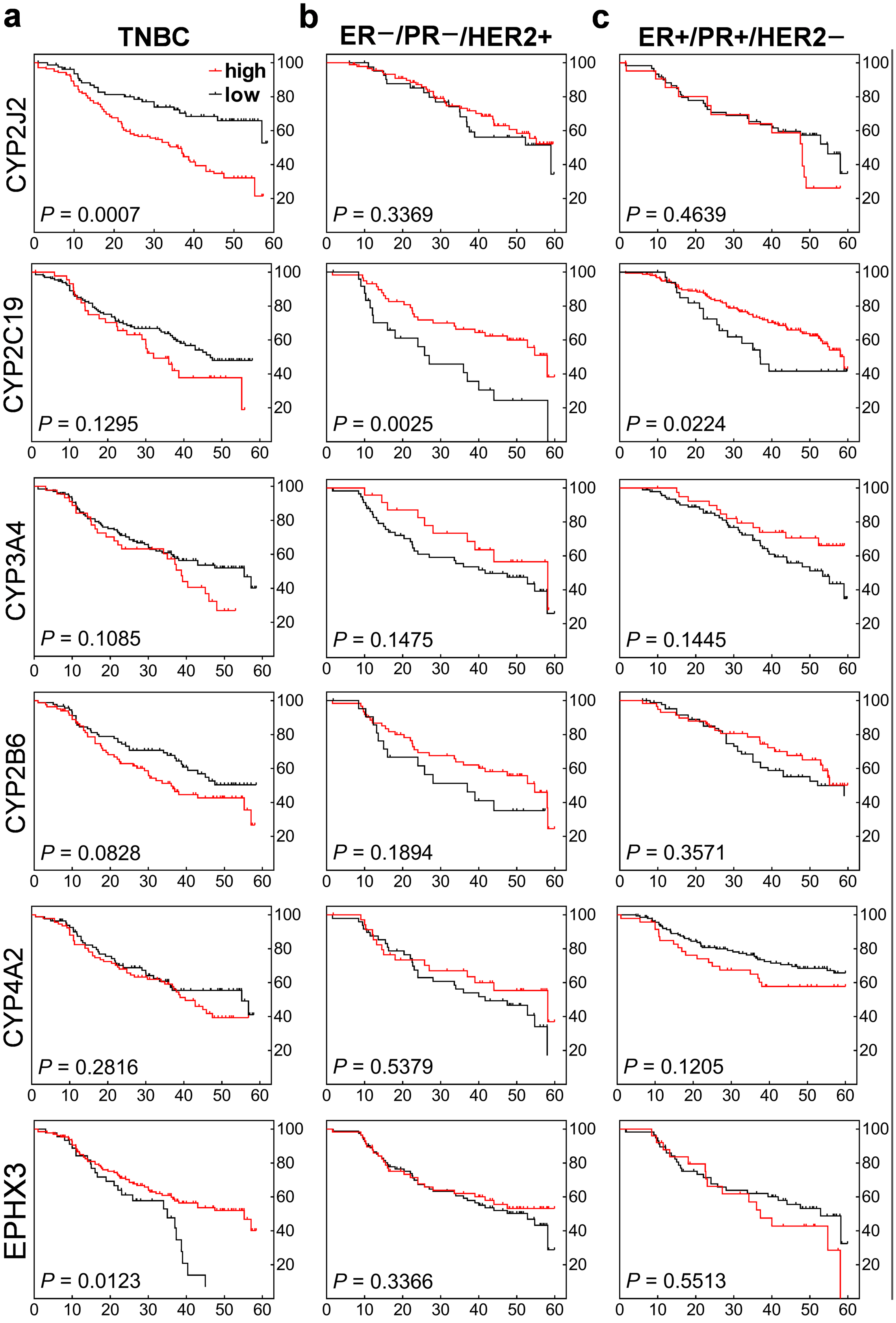
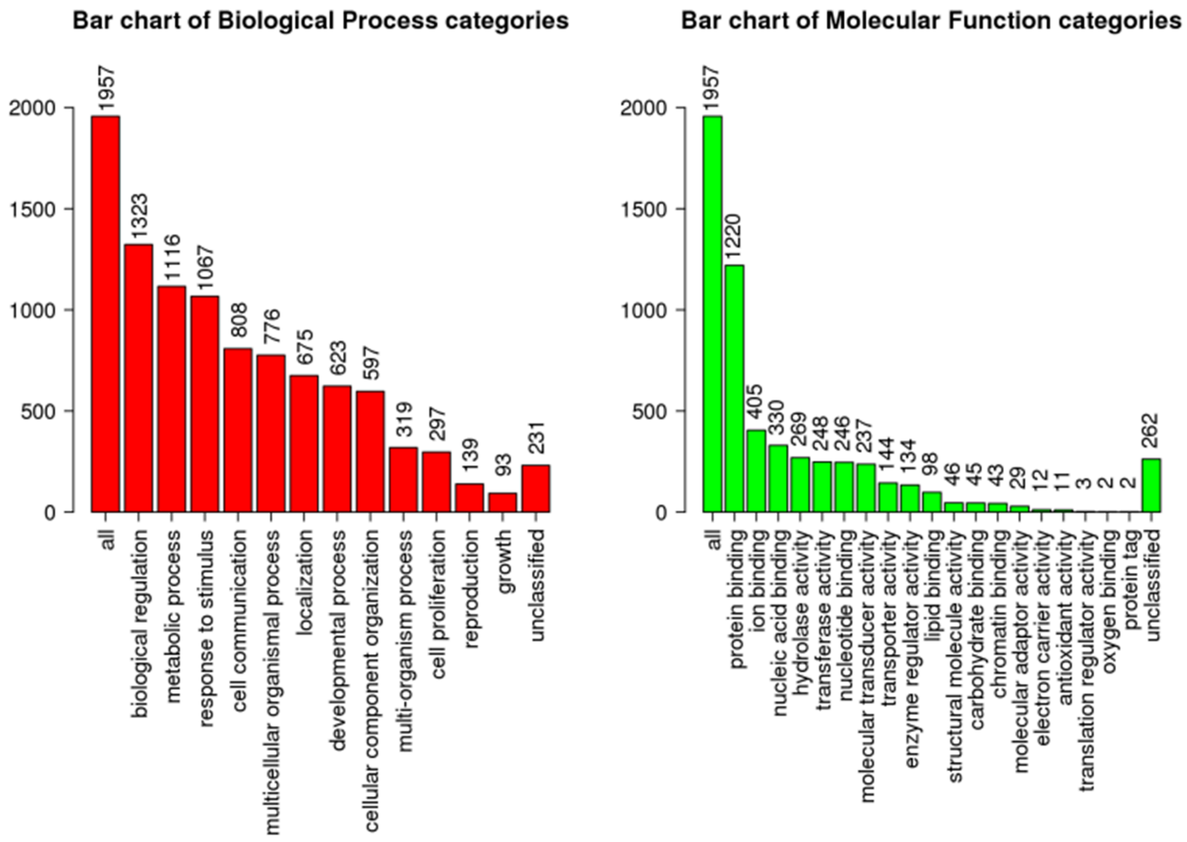
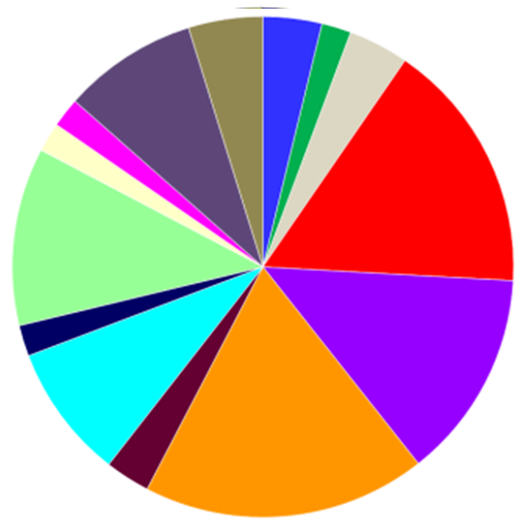
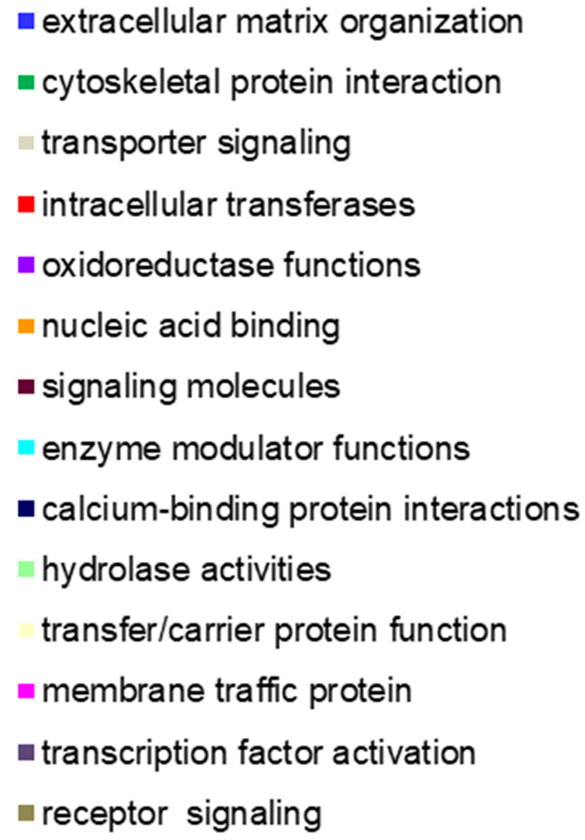

a
TNBC
b
ER−/PR−/HER2+
c
ER+/PR+/HER2−
high
low
CYP2J2
CYP2C19
CYP3A4
CYP2B6
CYP4A2
EPHX3
P = 0.0007
P = 0.3369
P = 0.4639
P = 0.1295
P = 0.0025
P = 0.0224
P = 0.1085
P = 0.1475
P = 0.1445
P = 0.0828
P = 0.1894
P = 0.3571
P = 0.2816
P = 0.5379
P = 0.1205
P = 0.0123
P = 0.3366
P = 0.5513
0 10 20 30 40 50 60
100 80 60 40 20
Percent Survival
Time (months)

Figure S6

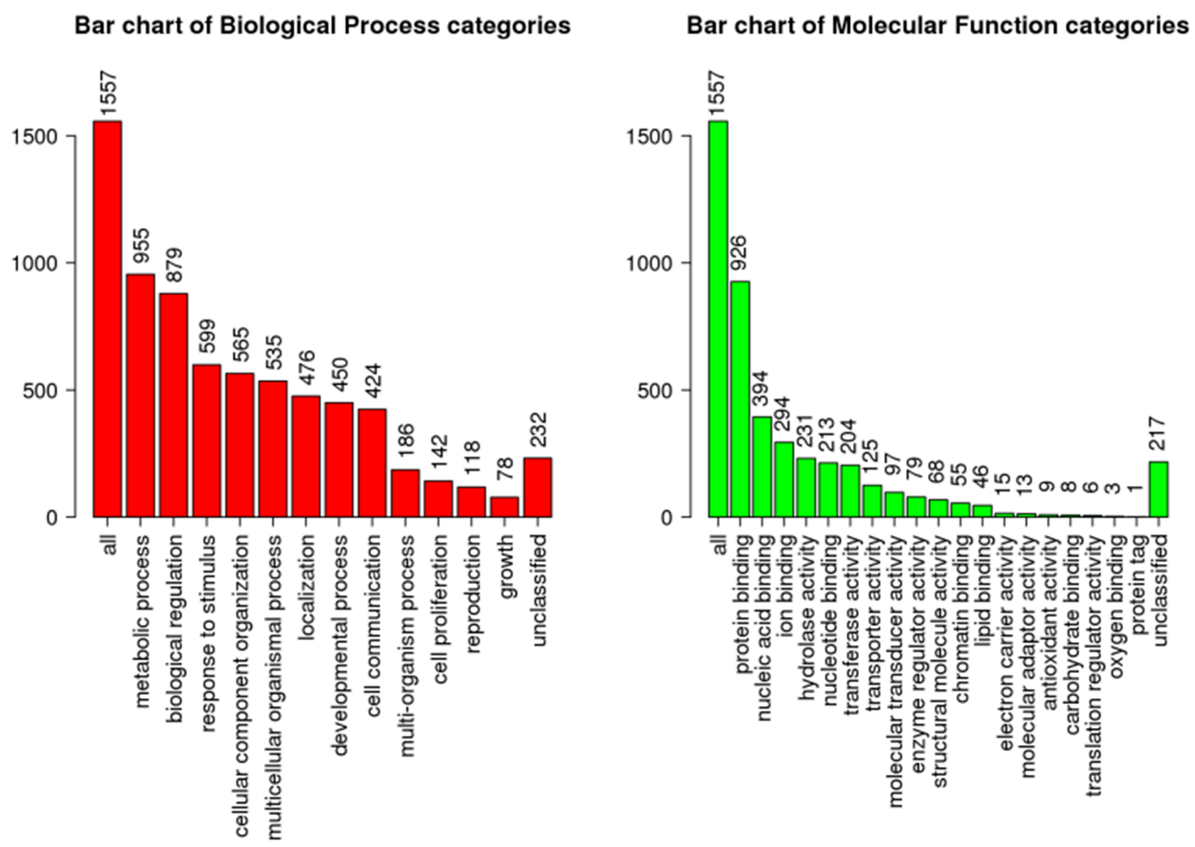
a

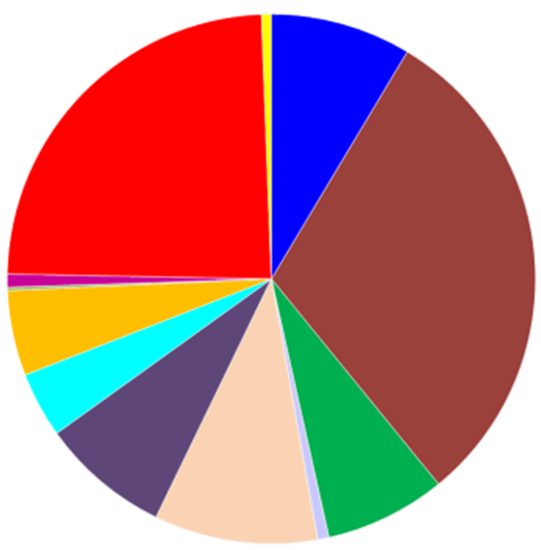


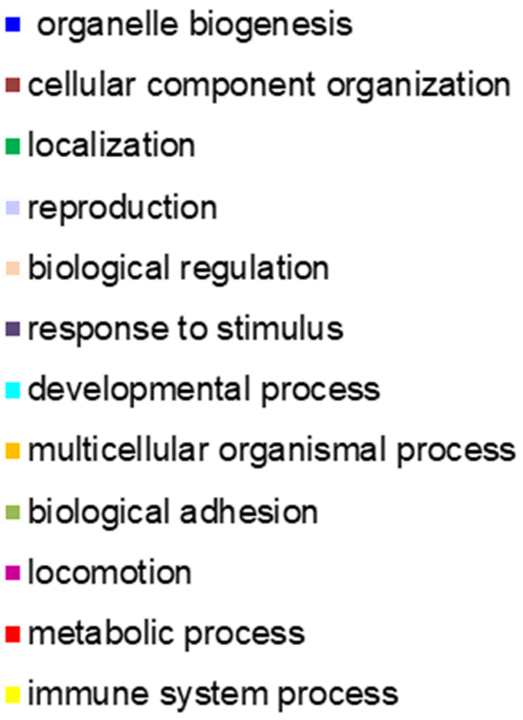




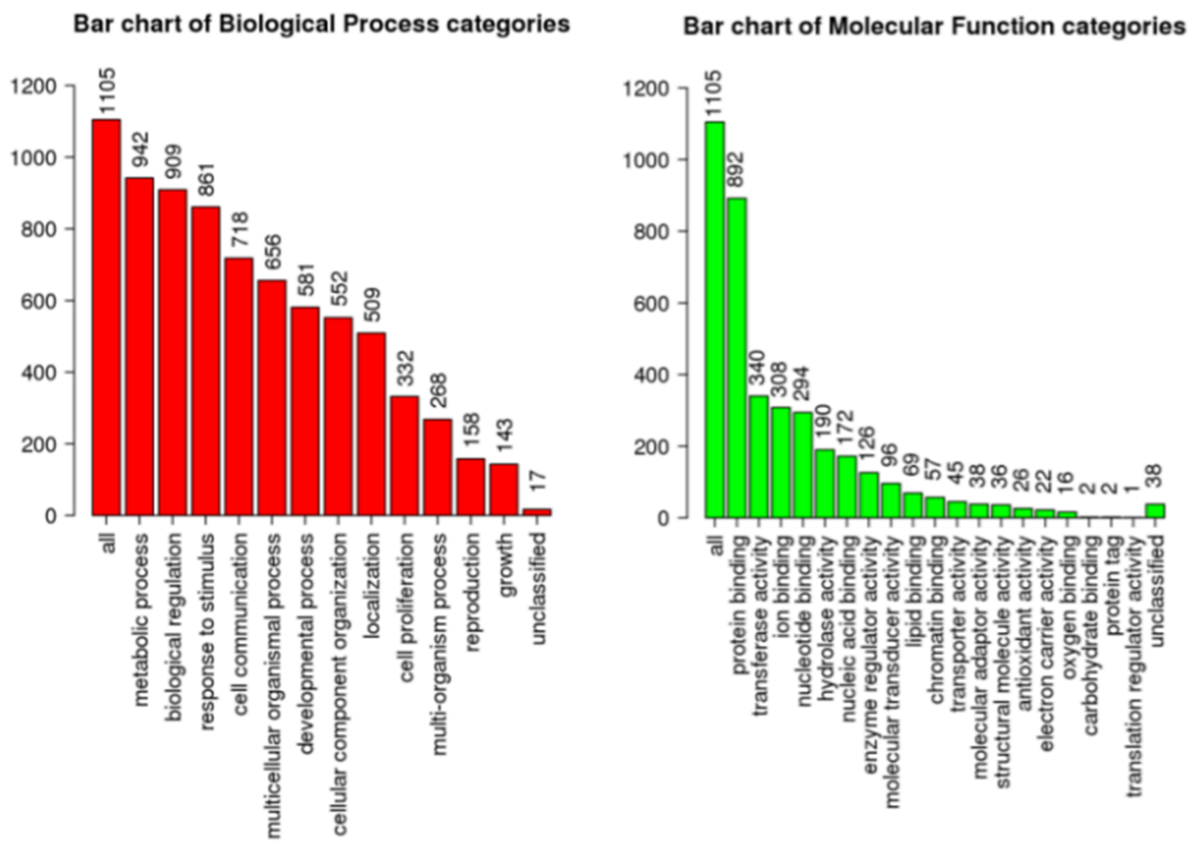
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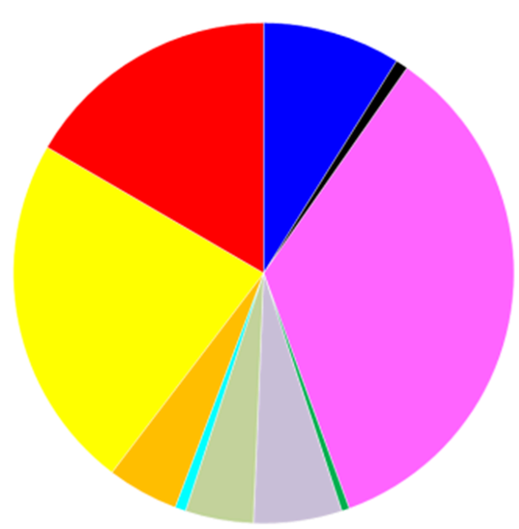






c





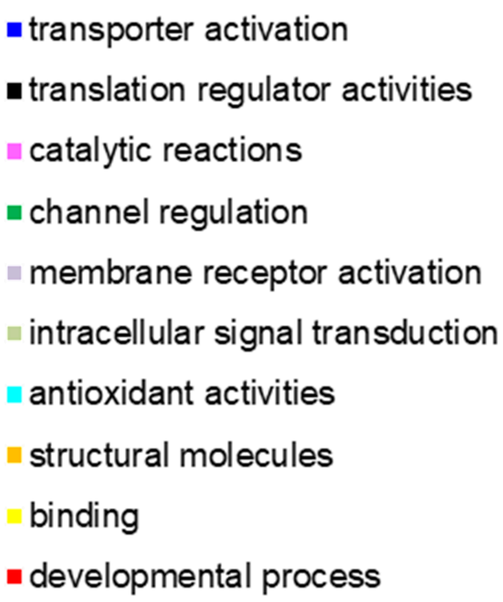


Figure S7

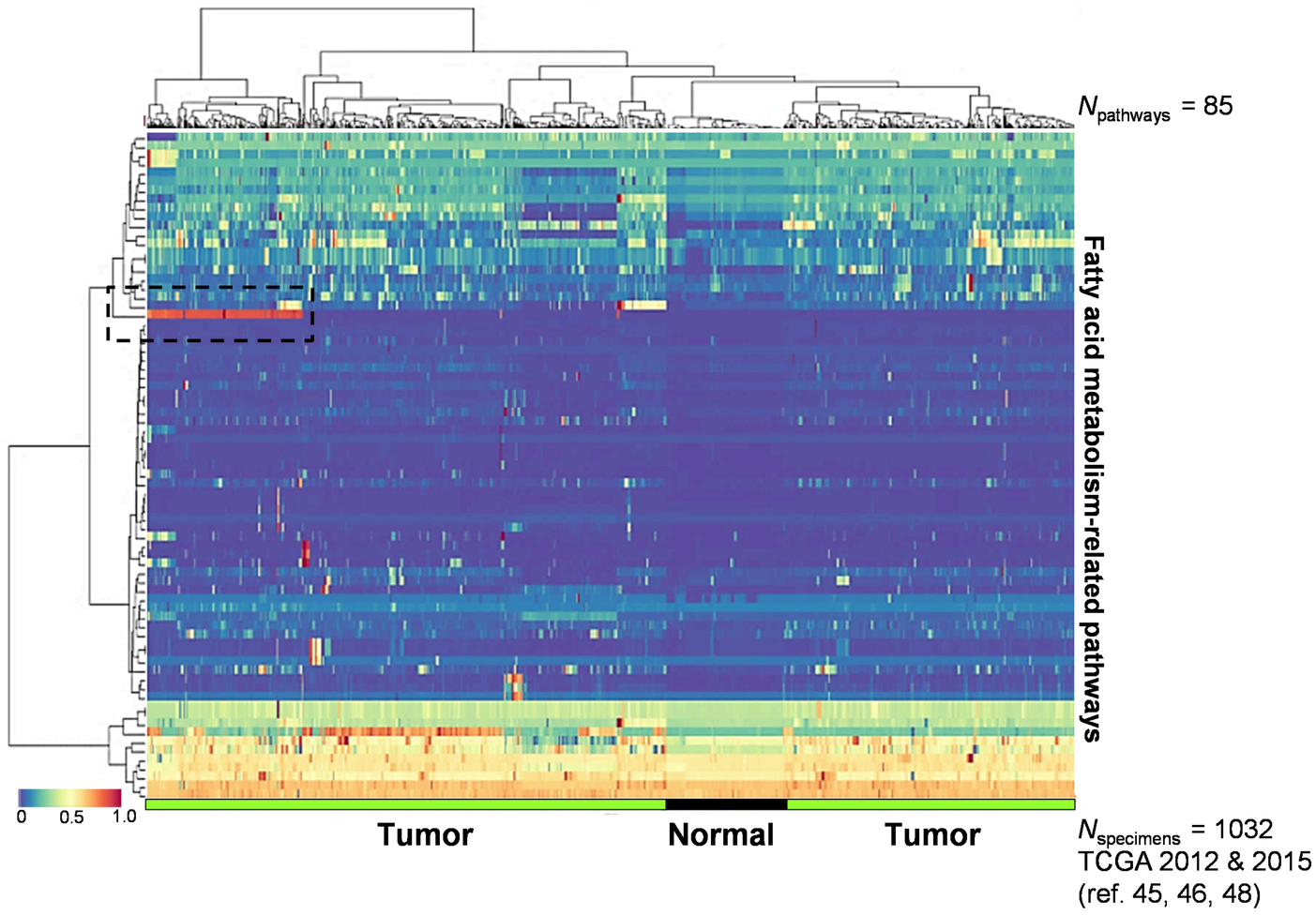
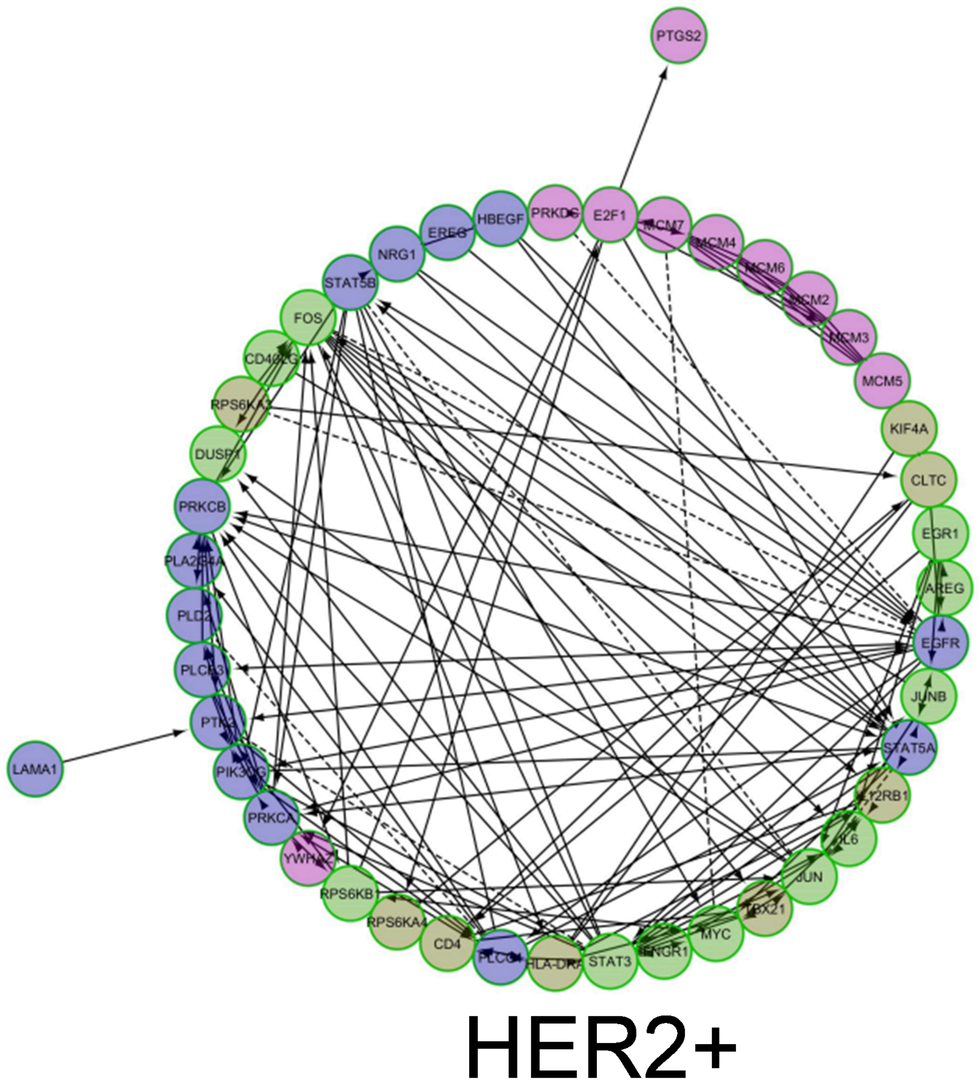

$N_{pathways}$ = 85
Fatty acid metabolism-related pathways
0
0.5
1.0
Tumor
Normal
Tumor
$N_{specimens}$ = 1032
TCGA 2012 & 2015
(ref. 45, 46, 48)

Figure S8

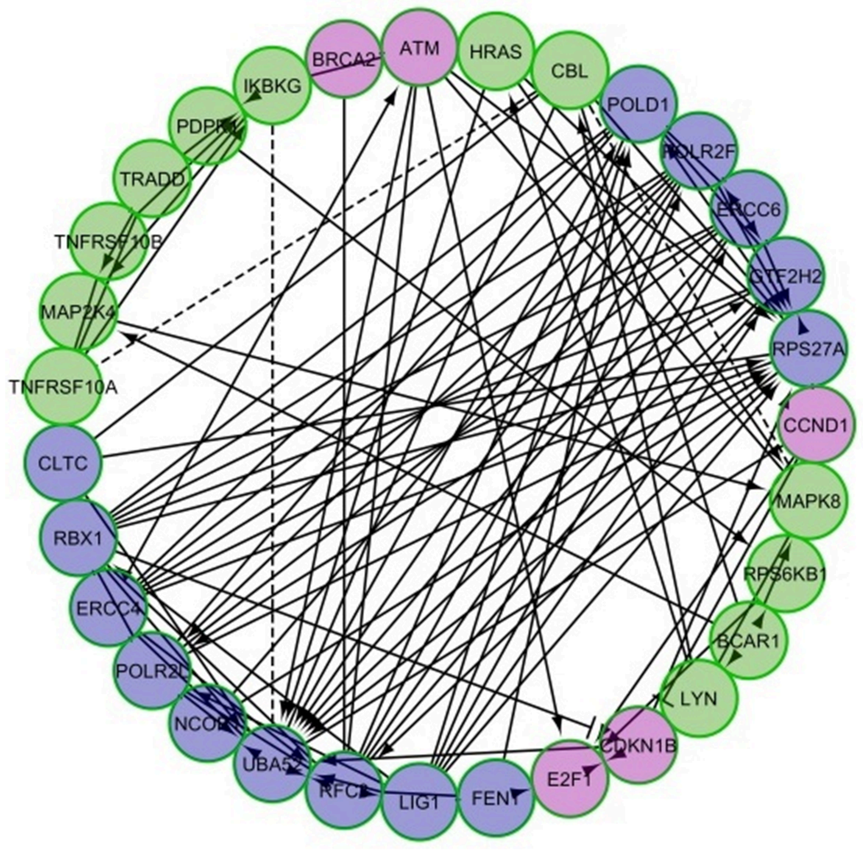
a



HER2+

Top 10 GO processes	Target
G1/S transition of mitotic cell cycle	STAT5B
MAPK cascade	AREG
mitotic cell cycle	IFNGR1
cell activation	LAMA1
regulation of protein phosphorylation	NRG1
positive regulation of protein phosphorylation	MCM2
immune system process	EREG
leukocyte differentiation	MAP3K5
regulation of immune system process	S1PR3
positive regulation of immune system process	EGR1

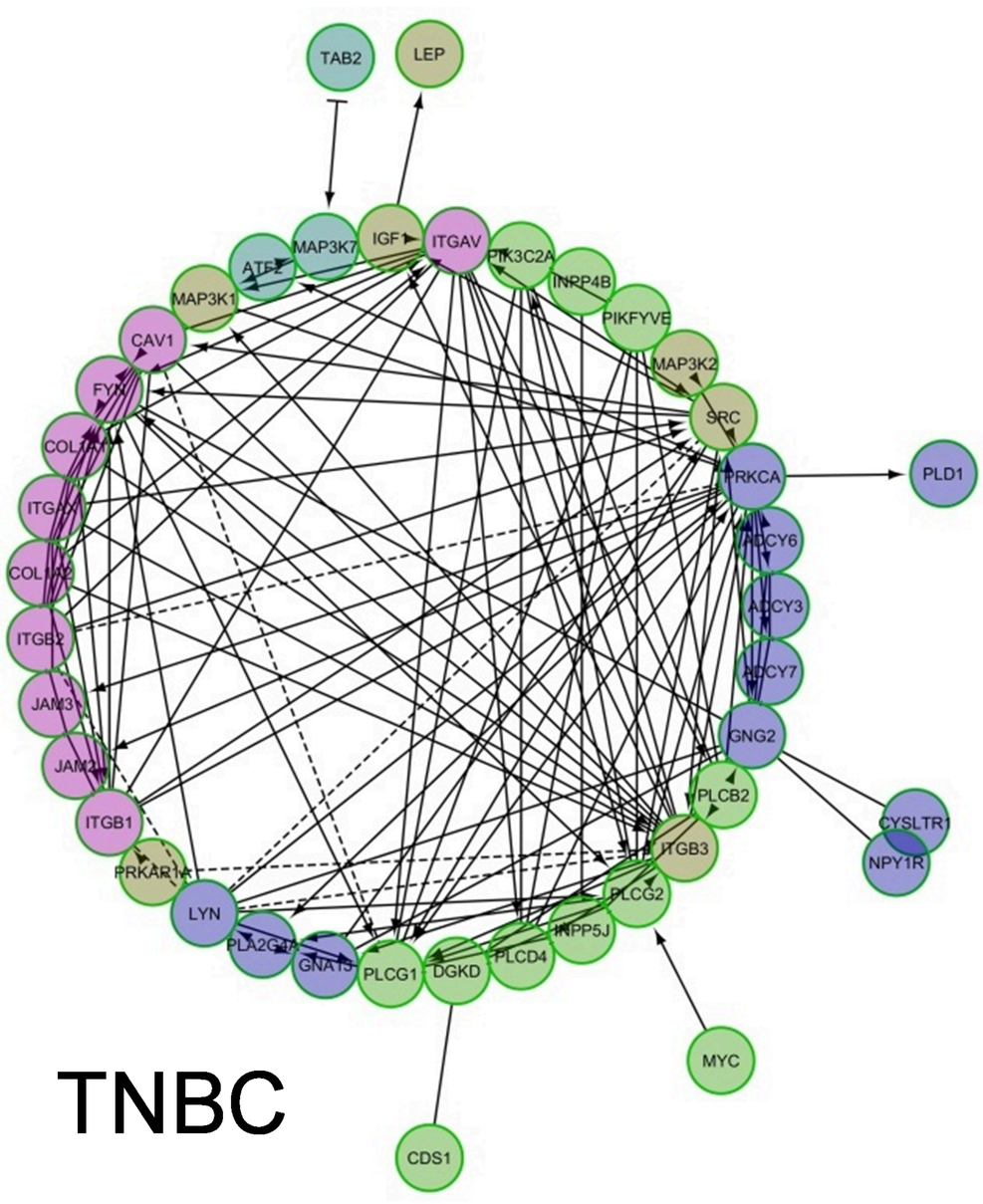
b



TPBC or ER+/PR+/HER2–

Top 10 GO processes	Target
establishment of planar polarity	GTF2H2B
cellular response to DNA damage stimulus	FEN1
mitotic cell cycle	MAPK8
transcription-coupled nucleotide-excision repair	TNFRSF10B
regulation of protein phosphorylation	GTF2H2
generation of precursor metabolites and energy	CDKN1B
oxidative phosphorylation	BRCA2
mitochondrial electron transport, NADH to ubiquinone	CASP7
purine nucleotide metabolic process	KAT2B
protein complex assembly	ERCC6

c



TNBC

Top 10 GO processes	Target
angiogenesis	GSTM4
blood vessel development	SRC
cell activation	ITGAV
cellular lipid metabolic process	LPCAT3
positive regulation of protein phosphorylation	CYSLTR1
vasculature development	DGKG
system process	ITGAM
circulatory system process	ITPKA
phosphorus metabolic process	SLC2A1
angiogenesis	GSTM1