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Supplemental Information

FOXA1 Mutations Reveal Distinct Chromatin Profiles and Influence Therapeutic Response in Breast Cancer

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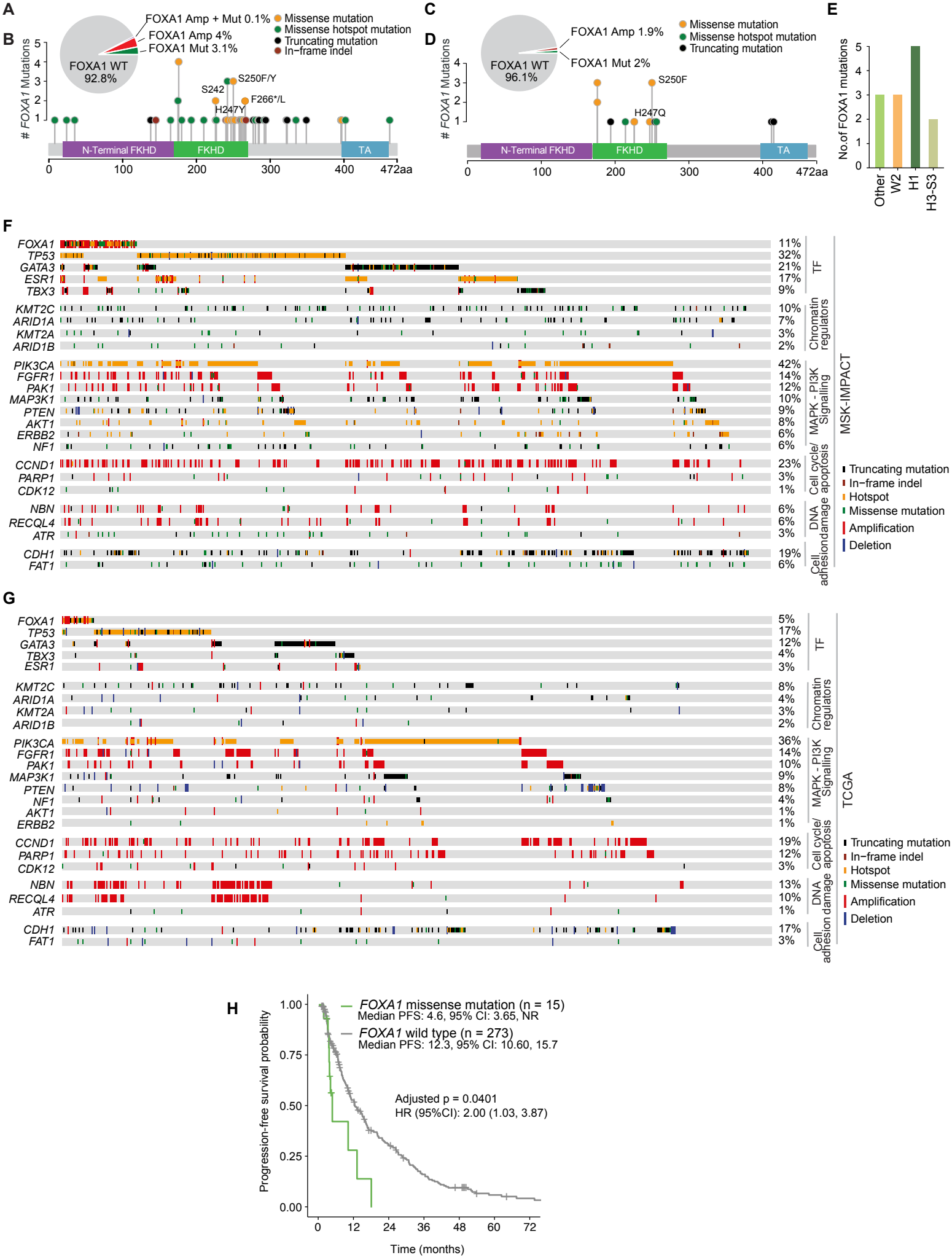

A
B
FOXA1 Amp + Mut 0.1%
FOXA1 Amp 4%
FOXA1 Mut 3.1%
FOXA1 WT 92.8%
Missense mutation
Missense hotspot mutation
Truncating mutation
In−frame indel
FOXA1 Mutations
S250F/Y
S242
F266*/L
H247Y
N-Terminal FKHD
FKHD
TA
0
100
200
300
400
472aa
C
D
FOXA1 Amp 1.9%
FOXA1 Mut 2%
FOXA1 WT 96.1%
S250F
H247Q
E
No.of FOXA1 mutations
Other
W2
H1
H3-S3
F
FOXA1 11%
TP53 32%
GATA3 21%
ESR1 17%
TBX3 9%
KMT2C 10%
ARID1A 7%
KMT2A 3%
ARID1B 2%
PIK3CA 42%
FGFR1 14%
PAK1 12%
MAP3K1 10%
PTEN 9%
AKT1 8%
ERBB2 6%
NF1 6%
CCND1 23%
PARP1 3%
CDK12 1%
NBN 6%
RECQL4 6%
ATR 3%
CDH1 19%
FAT1 6%
TF
Chromatin regulators
MAPK - PI3K Signalling
Cell cycle/ apoptosis
DNA damage
Cell adhesion
MSK-IMPACT
Truncating mutation
In−frame indel
Hotspot
Missense mutation
Amplification
Deletion
G
FOXA1 5%
TP53 17%
GATA3 12%
TBX3 4%
ESR1 3%
KMT2C 8%
ARID1A 4%
KMT2A 3%
ARID1B 2%
PIK3CA 36%
FGFR1 14%
PAK1 10%
MAP3K1 9%
PTEN 8%
NF1 4%
AKT1 1%
ERBB2 1%
CCND1 19%
PARP1 12%
CDK12 3%
NBN 13%
RECQL4 10%
ATR 1%
CDH1 17%
FAT1 3%
TCGA
H
FOXA1 missense mutation (n = 15)
Median PFS: 4.6, 95% CI: 3.65, NR
FOXA1 wild type (n = 273)
Median PFS: 12.3, 95% CI: 10.60, 15.7
Adjusted p = 0.0401
HR (95%CI): 2.00 (1.03, 3.87)
Progression-free survival probability
Time (months)

Figure S1. Related to Figure 1. *FOXA1* hotspot mutations cluster at C-terminal FKH domain and are mutually exclusive with *ESR1* mutations.

(A, C) Pie charts representing frequency of *FOXA1* alteration types (wild-type (WT), amplification (Amp), mutation (Mut) or both (Amp + Mut)) among breast cancer patients from the curated list utilized for survival analysis in Figure 1G and TCGA respectively.

(B, D) Lollipop plots depicting distribution of FOXA1 mutations (truncating, missense and in-frame indels) found among breast cancer patients from the curated list utilized for survival analysis in Figure 1G and TCGA respectively, along the protein sequence.

(E) Bar-plot showing number of mutations per subdomains indicated, for patients in TCGA cohort. W2, Wing2; H1, Helix1; H3-S3, from helix3 to third beta strand.

(F, G) Association of full cohort of samples, including *FOXA1* mutant and wild-type (WT) samples, with pattern, frequency and type of genomic alterations of the key breast cancer genes indicated among breast cancer patients from www.cbioportal.org (F) and TCGA (G). Genes with alteration frequency > 5% were represented.

(H) Kaplan-Meier curves displaying progression-free survival of patients harboring either WT or mutant *FOXA1* under aromatase inhibitor treatment, excluding patients harboring concomitant *ESR1* mutations. P value as indicated, log rank test.

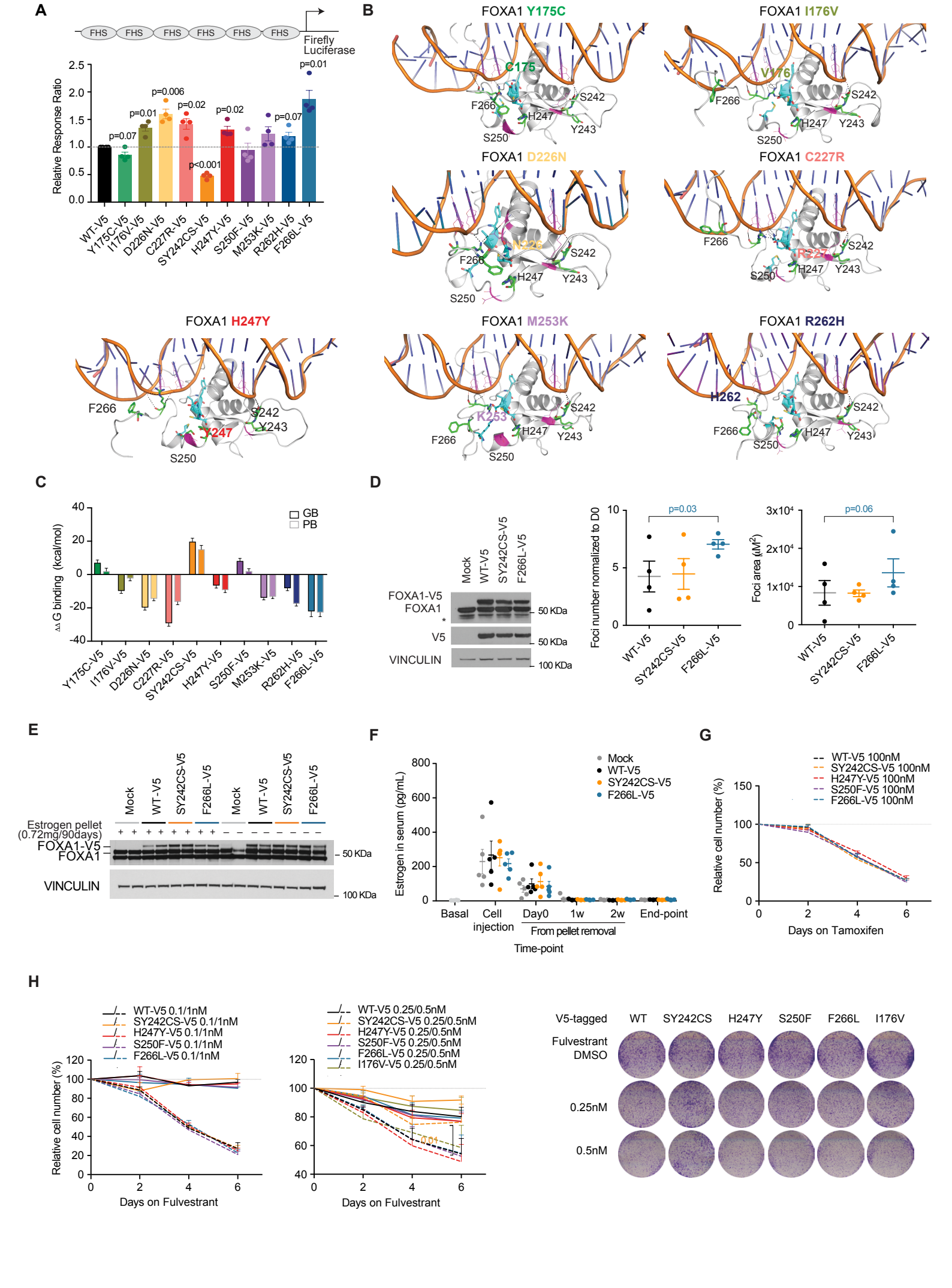

A
FHS FHS FHS FHS FHS FHS
Firefly Luciferase
Relative Response Ratio
p=0.07
p=0.01
p=0.006
p=0.02
p<0.001
p=0.02
p=0.07
p=0.01
WT-V5
Y175C-V5
I176V-V5
D226N-V5
C227R-V5
SY242CS-V5
H247Y-V5
S250F-V5
M253K-V5
R262H-V5
F266L-V5
B
FOXA1 Y175C
FOXA1 I176V
FOXA1 D226N
FOXA1 C227R
FOXA1 H247Y
FOXA1 M253K
FOXA1 R262H
C
ΔΔG binding (kcal/mol)
GB
PB
D
Mock
WT-V5
SY242CS-V5
F266L-V5
FOXA1-V5
FOXA1
V5
VINCULIN
50 KDa
100 KDa
Foci number normalized to D0
p=0.03
Foci area (µM²)
p=0.06
E
Estrogen pellet (0.72mg/90days)
FOXA1-V5
FOXA1
VINCULIN
50 KDa
100 KDa
F
Estrogen in serum (pg/mL)
Mock
WT-V5
SY242CS-V5
F266L-V5
Basal
Cell injection
Day0
1w
2w
End-point
From pellet removal
Time-point
G
WT-V5 100nM
SY242CS-V5 100nM
H247Y-V5 100nM
S250F-V5 100nM
F266L-V5 100nM
Relative cell number (%)
Days on Tamoxifen
H
WT-V5 0.1/1nM
SY242CS-V5 0.1/1nM
H247Y-V5 0.1/1nM
S250F-V5 0.1/1nM
F266L-V5 0.1/1nM
Relative cell number (%)
Days on Fulvestrant
WT-V5 0.25/0.5nM
SY242CS-V5 0.25/0.5nM
H247Y-V5 0.25/0.5nM
S250F-V5 0.25/0.5nM
F266L-V5 0.25/0.5nM
I176V-V5 0.25/0.5nM
0.01
Days on Fulvestrant
V5-tagged
WT
SY242CS
H247Y
S250F
F266L
I176V
Fulvestrant
DMSO
0.25nM
0.5nM

Figure S2. Related to Figure 2. *FOXA1* forkhead mutations show enhanced transcriptional activity, and structural alterations.

(A) Impact of ectopic expression of V5-tagged *FOXA1* variants (Y175C, I176V, D226N, C227R, SY242CS, H247Y, S250F, M253K, R262H, F266L) on luciferase reporter activity (n=4 independent experiments as indicated by dots). Two-tailed Student's t-test with Welch's T test for different variance correction, p-values as indicated. Error bars, mean ± SEM.

(B) Impact of ectopic expression of V5-tagged *FOXA1* variants (Y175C, I176V, D226N, C227R, H247Y, M253K, R262H) 3-D structural interaction of the forkhead domain (FKHD) with DNA.

(C) Impact of ectopic expression of V5-tagged *FOXA1* variants (Y175C, I176V, D226N, C227R, SY242CS, H247Y, S250F, M253K, R262H, F266L) on binding free energy ($\Delta\Delta G$) calculated using the Generalized Born (GB) and Poisson-Boltzmann (PB) formalisms averaged over the conformations of the complexes sampled during atomistic Molecular Dynamics (MD) simulations.

(D) Effect of ectopic expression of *FOXA1* WT-V5, SY242CS-V5, and F266L-V5 on protein abundance (left, representative experiment is shown) and foci number and area under estrogen deprivation in T47D cells (right, n=4 independent experiments as indicated by dots). *, unspecific band.

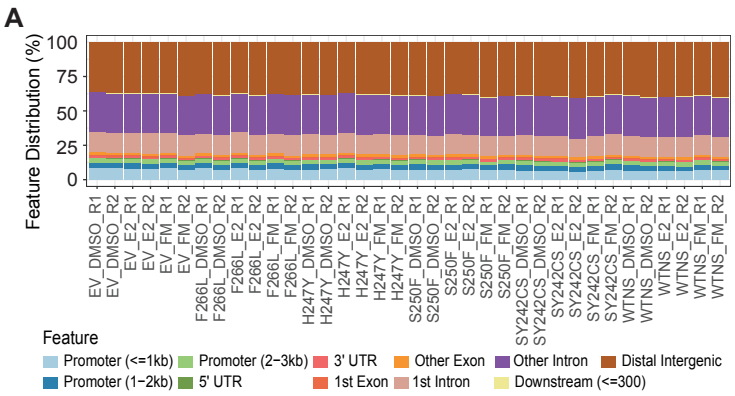
One-tailed Student's t-test, p-values as indicated.

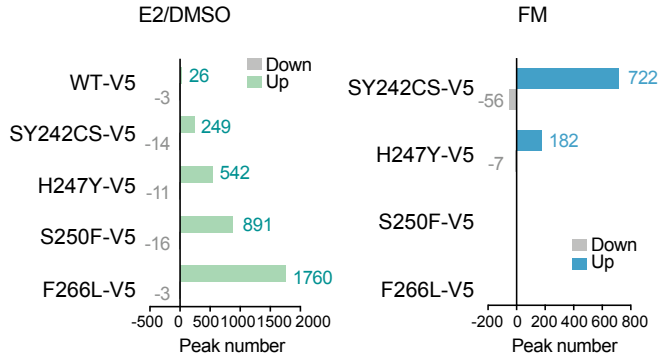
(E) Immunoblot of depicted proteins from representative xenograft tumors of Mock or V5-tagged *FOXA1* variant (WT, SY242CS and F266L) expressing MCF7 cells.

(F) Estrogen levels (pg/mL) in mice sera during indicated time-points of the *in vivo* experiment (n=4 mice for the basal group and n=5 mice for the rest).

(G) Impact of ectopic expression of V5-tagged *FOXA1* variants (Y175C, I176V, D226N, C227R, SY242CS, H247Y, S250F, M253K, R262H, F266L) on cell-number upon tamoxifen treatment (100nM) *in vitro* (n=3 independent experiments). Two-tailed Student's t-test.

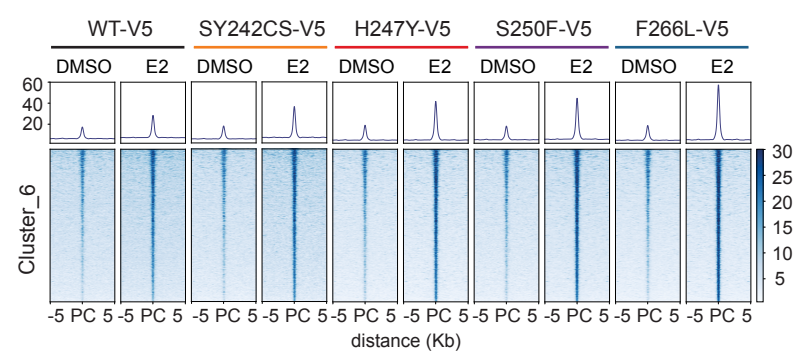
(H) Impact of ectopic expression of V5-tagged *FOXA1* variants (Y175C, I176V, D226N, C227R, SY242CS, H247Y, S250F, M253K, R262H, F266L) on cell-number upon fulvestrant treatment (0.1 and 1nM (n=3 independent experiments), left; 0.25 and 0.5nM (n=3 independent experiments), middle; representative images of crystal violet staining upon 0.25 and 0.5nM fulvestrant treatment, right) *in vitro.* Two-tailed Student's t-test, p-values as indicated.

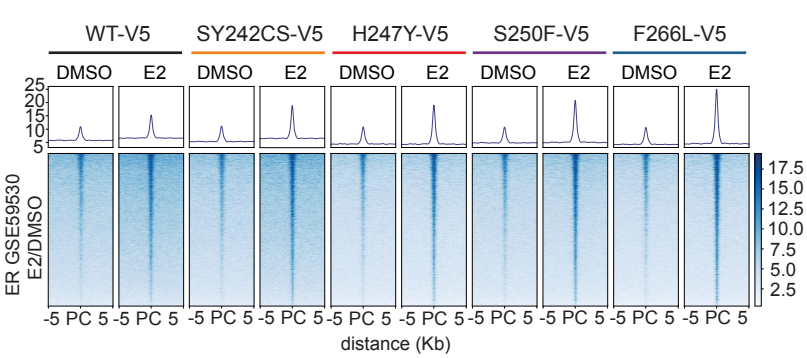




C

	Cl	Top enriched *de novo* motifs	T%	BG%	p-val
1		FORKHEAD	65.92	14.50	$1e^{-574}$
		YY2	7.06	0.37	$1e^{-122}$
		SMAD	10.34	1.46	$1e^{-100}$
2		FORKHEAD	45.99	17.96	$1e^{-161}$
		DEAF1	2.24	0.31	$1e^{-27}$
		MYB	3.14	0.71	$1e^{-18}$
3		FORKHEAD	72.40	29.44	$1e^{-470}$
		AP1	22.91	5.80	$1e^{-193}$
		GRHL1	27.02	15.96	$1e^{-48}$
4		Alternative motif	70.08	28.23	$1e^{-631}$
		FORKHEAD	19.36	9.24	$1e^{-81}$
		AP2gamma	20.68	12.34	$1e^{-47}$
5		FORKHEAD	64.54	20.31	$1e^{-427}$
		AP1	29.79	11.20	$1e^{-118}$
		GRHL1	24.68	14.10	$1e^{-37}$
6		NUCLEAR RECEPTOR	43.84	17.60	$1e^{-704}$
		FORKHEAD	54.28	27.38	$1e^{-154}$
		GATA	18.53	10.67	$1e^{-27}$
7		FORKHEAD	81.28	24.26	$1e^{-630}$
		ARID	29.77	9.16	$1e^{-150}$
		GATA	9.91	4.63	$1e^{-22}$





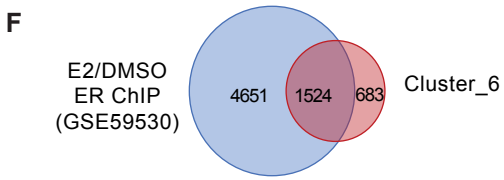


Figure S3. Related to Figure 3. *FOXA1* Wing2 mutations show enhanced affinity for ER loci and ER-mediated transcription

(A) Stacked bar plot depicting genomic feature distribution per ChIP-seq sample (n=2 biological replicates).

(B) Bar plots representing number of differential peaks for *FOXA1* mutant (SY242CS-V5, H247Y-V5, S250F-V5 and F266L-V5) and control (WT-V5, wild-type) MCF7 cells upon estrogen stimulation (left panel) and differential peaks for *FOXA1* mutant (SY242CS-V5, H247Y-V5, S250F-V5 and F266L-V5) as compared to *FOXA1* WT in full-media (FM, right panel).

(C) Table depicting top enriched Homer *de novo* motifs and the transcription factors associated with, per ChIP-seq cluster indicated. Fisher's exact test, p-value (p-val) as indicated. T%, percentage of target sites; BG%, background percentage; Cl, cluster.

(D) Tornado plot representing FOXA1 binding peaks at cluster 6 in the absence (DMSO) or presence of estrogen (E2) in *FOXA1* mutant (SY242CS-V5, H247Y-V5, S250F-V5 and F266L-V5) and WT-V5 MCF7 cells. PC, Peak center. Average of 2 biological replicates is represented.

(E) Tornado plot representing FOXA1 binding peaks at cluster 6 for *FOXA1* mutant (SY242CS-V5, H247Y-V5, S250F-V5 and F266L-V5) and WT-V5 in the absence (DMSO) or presence of estrogen (E2) at estrogen induced ER binding sites (from public dataset GSE59530 (Franco et al., 2015). PC, Peak center.

(F) Venn diagram illustrating the overlap between cluster 6 peaks and estrogen induced ER binding sites (from public dataset GSE59530 (Franco et al., 2015). DMSO, estrogen depletion; E2, upon estrogen stimulation.

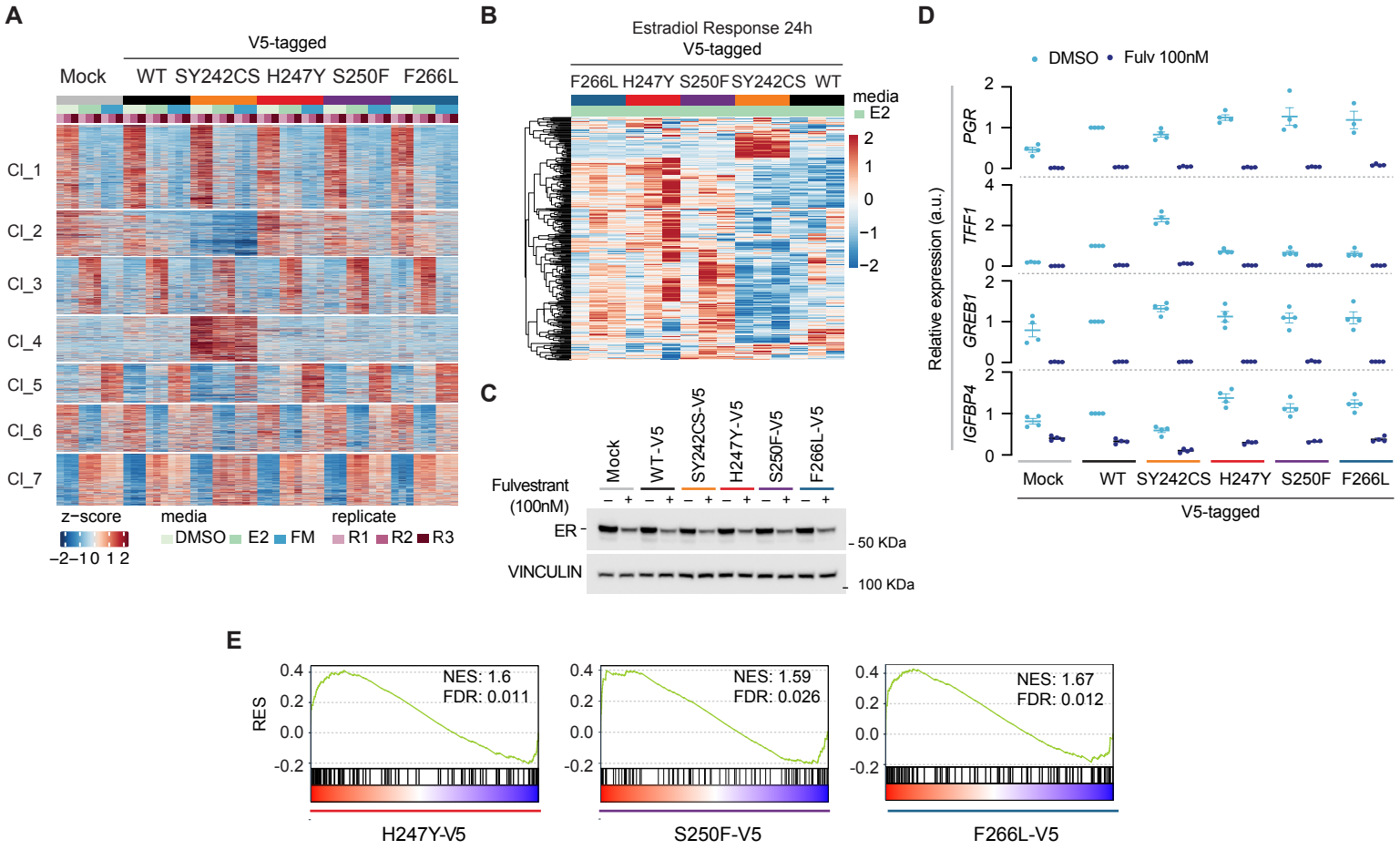

A
V5-tagged
Mock
WT SY242CS H247Y S250F F266L
Cl_1
Cl_2
Cl_3
Cl_4
Cl_5
Cl_6
Cl_7
z−score
−2−1 0 1 2
media
DMSO E2 FM
replicate
R1 R2 R3
B
Estradiol Response 24h
V5-tagged
F266L H247Y S250F SY242CS WT
media
E2
2
1
0
−1
−2
C
Mock
WT-V5
SY242CS-V5
H247Y-V5
S250F-V5
F266L-V5
Fulvestrant
(100nM)
ER
VINCULIN
50 KDa
100 KDa
D
DMSO
Fulv 100nM
Relative expression (a.u.)
PGR
TFF1
GREB1
IGFBP4
Mock
WT
SY242CS
H247Y
S250F
F266L
V5-tagged
E
RES
NES: 1.6
FDR: 0.011
NES: 1.59
FDR: 0.026
NES: 1.67
FDR: 0.012
H247Y-V5
S250F-V5
F266L-V5

Figure S4. Related to Figure 4. *FOXA1* mutants display enhanced ER-dependent estrogen response.

(A) Heatmap of k-means clustering of RNA-seq peaks from *FOXA1* mutant (SY242CS-V5, H247Y-V5, S250F-V5 and F266L-V5) and control (WT-V5, wild-type and Mock, empty vector) MCF7 cells, representing seven clusters (n=3 biological replicates).

(B) Heatmap showing expression level of genes from Gene Set Enrichment Analysis (GSEA) dataset 'Dutertre Estradiol Response 24h Up' in RNA-seq from *FOXA1* Wing2 mutant cells (H247Y-V5, S250F-V5, F266L-V5 and SY242CS-V5) as compared to *FOXA1* WT-V5 cells.

(C-D) Impact of fulvestrant treatment (100nM, 72h) on ER protein levels (C, representative experiment is shown) and shared FOXA1 and ER target gene expression (*IGFBP4, PGR, TFF1, GREB1*) in V5-tagged *FOXA1* variant (SY242CS, H247Y, S250F, F266L) expressing MCF7 cells (D) n=4 biological replicates.

(E) Gene Set Enrichment Analysis (GSEA) plot showing 'Y537S *ESR1* versus WT *ESR1* in HD' from Jeselsohn et al., 2018 enriched in RNA-seq from *FOXA1* Wing2 mutant cells (H247Y-V5, S250F-V5 and F266L-V5) as compared to *FOXA1* WT-V5 cells. RES, Running Enrichment Score, as indicated. FDR represents p adjusted value, calculated using the GSEA package. HD, Hormone depleted; RES, Running Enrichment Score.

DMSO, estrogen depletion; E2, upon estrogen stimulation; FM, in full media.

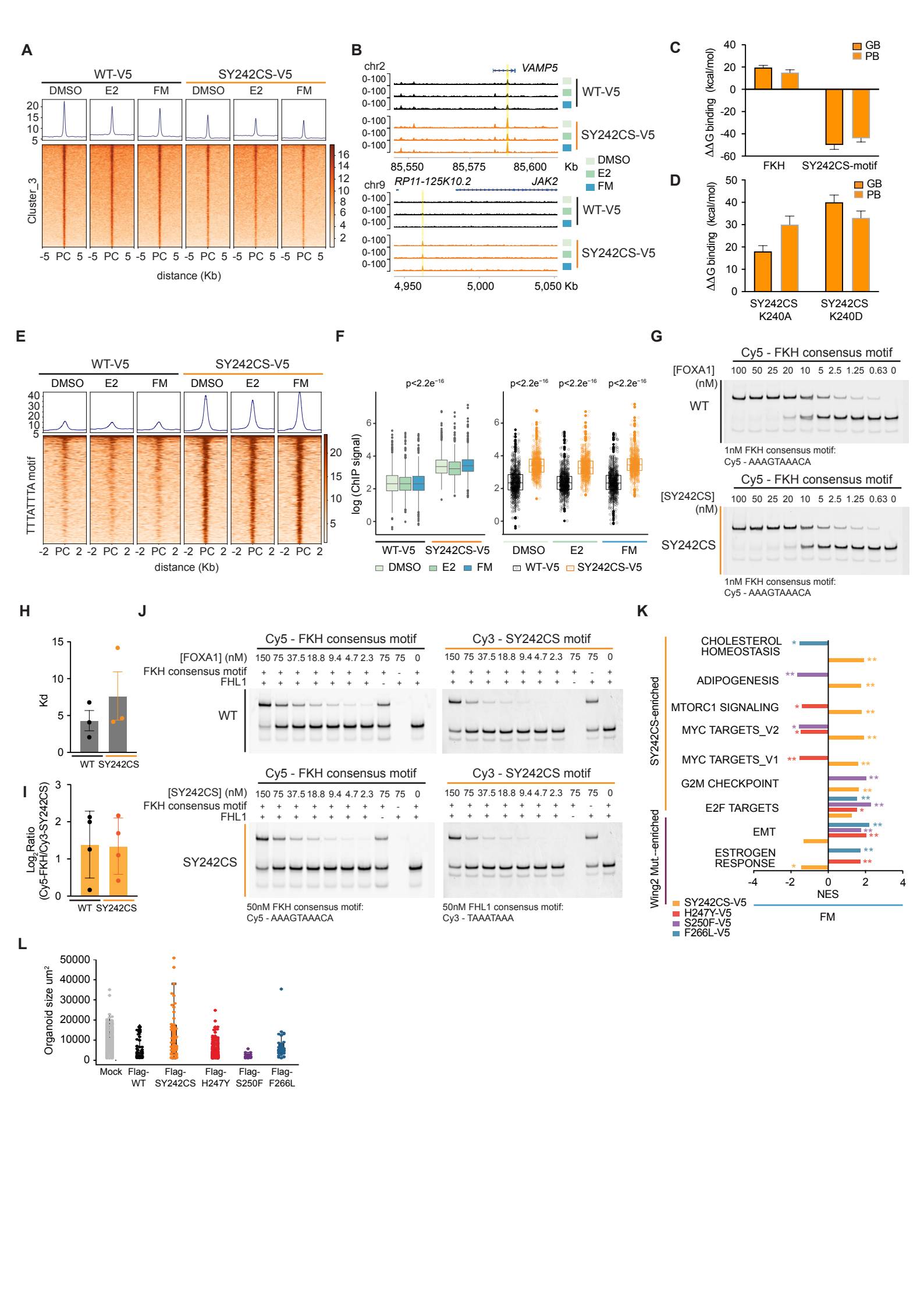

A
WT-V5
SY242CS-V5
DMSO
E2
FM
Cluster_3
distance (Kb)
B
chr2
VAMP5
WT-V5
SY242CS-V5
85,550
85,575
85,600
Kb
chr9
RP11-125K10.2
JAK2
4,950
5,000
5,050 Kb
C
GB
PB
ΔΔG binding (kcal/mol)
FKH
SY242CS-motif
D
SY242CS K240A
SY242CS K240D
E
TTTATTTA motif
F
p<2.2e-16
log (ChIP signal)
G
Cy5 - FKH consensus motif
[FOXA1] (nM)
WT
1nM FKH consensus motif:
Cy5 - AAAGTAAACA
[SY242CS] (nM)
SY242CS
H
Kd
J
Cy5 - FKH consensus motif
Cy3 - SY242CS motif
FKH consensus motif
FHL1
50nM FKH consensus motif:
Cy5 - AAAGTAAACA
50nM FHL1 consensus motif:
Cy3 - TAAATAAA
I
Log2 Ratio (Cy5-FKH/Cy3-SY242CS)
K
CHOLESTEROL HOMEOSTASIS
ADIPOGENESIS
MTORC1 SIGNALING
MYC TARGETS_V2
MYC TARGETS_V1
G2M CHECKPOINT
E2F TARGETS
EMT
ESTROGEN RESPONSE
SY242CS-enriched
Wing2 Mut.-enriched
NES
SY242CS-V5
H247Y-V5
S250F-V5
F266L-V5
L
Organoid size um2
Mock
Flag-WT
Flag-SY242CS
Flag-H247Y
Flag-S250F
Flag-F266L

Figure S5. Related to Figure 5. *FOXA1* SY242CS shows enrichment for alternative transcriptomic pathways.

(A) Tornado plot showing ChIP-seq peaks of FOXA1 cluster 3 in Figure 3B from MCF7 cells bearing either *FOXA1* WT or SY242CS under the conditions indicated. PC, Peak center. Average of 2 biological replicates is represented. Average of 2 biological replicates is represented.
(B) ChIP-seq tracks of *FOXA1* in either SY242CS-V5 or WT *FOXA1* MCF7 cells at *EFEMP1* and *FST* gene loci at chromosome 2 and 5 respectively, under media condition indicated.
(C-D) Quantification of binding free energy ($\Delta\Delta G$) of SY242CS to either FKH or SY242CS-specific motifs (C) and of SY242CS K240A or K240D mutants to SY242CS-specific motif, calculated using the Generalized Born (GB) and Poisson-Boltzmann (PB) formalisms averaged over the conformations of the complexes sampled during atomistic Molecular Dynamics (MD) simulations. Error bars equal SEM.
(E-F) Tornado plot (E, average of 2 biological replicates is represented) and quantification (F) of peaks that contain the SY242CS motif TTTATTTA in both WT or *FOXA1* SY242CS mutant sites in all three medias. Anova test, p<2.2*10-16.
(G-H) Representative gel image (G) and quantification (H) of EMSAs where FOXA1 WT and SY242CS peptides were titrated using a fluorescently labeled probe of the *FOXA1* consensus forkhead (FKH) DNA element. The dissociation constant (Kd) was estimated by non-linear curve-fitting from three experiments. Error bars equal SEM.
(I-J) Representative gel image (I) and quantification (J) of competition ('picking') EMSA of FOXA1 WT and FOXA1 SY242CS peptides simultaneously incubated with the FKH consensus motif (AAAGTAAACA, Cy5) or the SY242CS-specifc motif (TAAATAAA, Cy3). Relative proportions of protein-bound Cy3 and Cy5 probes in the same gel are determined using successive scans with Cy5 or Cy3 emission filters, respectively. Error bars equal STD.
(K) Bar plot representing selected Hallmark datasets in *FOXA1* SY242CS-V5 or Wing2 mutant MCF7 cells as compared to the *FOXA1* WT-V5 control cells under the culture media condition indicated. NES, Normalized Enrichment Score. *, padj<0.05; **, padj<0.01; padj<0.001.
(L) Quantification of organoid size (μm_2) measured seven days after seeding, from representative experiment. Values for sample size (indicated as dots) (n=1) Error bars equal STD.
DMSO, estrogen depletion; E2, upon estrogen stimulation; FM, in full media.

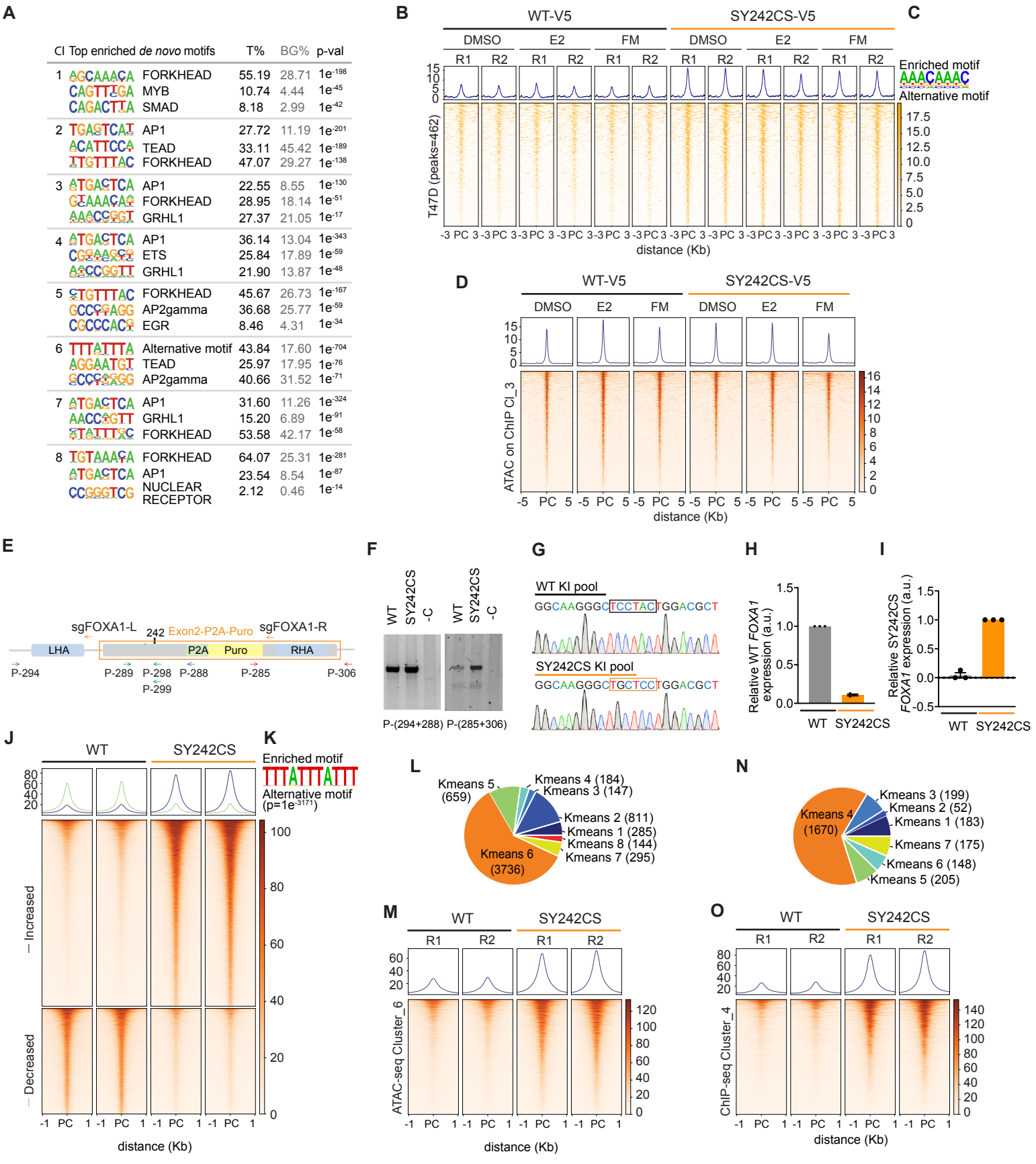

A
Cl Top enriched de novo motifs T% BG% p-val
1 FORKHEAD 55.19 28.71 1e-198
MYB 10.74 4.44 1e-45
SMAD 8.18 2.99 1e-42
2 AP1 27.72 11.19 1e-201
TEAD 33.11 45.42 1e-189
FORKHEAD 47.07 29.27 1e-138
3 AP1 22.55 8.55 1e-130
FORKHEAD 28.95 18.14 1e-51
GRHL1 27.37 21.05 1e-17
4 AP1 36.14 13.04 1e-343
ETS 25.84 17.89 1e-59
GRHL1 21.90 13.87 1e-48
5 FORKHEAD 45.67 26.73 1e-167
AP2gamma 36.68 25.77 1e-59
EGR 8.46 4.31 1e-34
6 Alternative motif 43.84 17.60 1e-704
TEAD 25.97 17.95 1e-76
AP2gamma 40.66 31.52 1e-71
7 AP1 31.60 11.26 1e-324
GRHL1 15.20 6.89 1e-91
FORKHEAD 53.58 42.17 1e-58
8 FORKHEAD 64.07 25.31 1e-281
AP1 23.54 8.54 1e-87
NUCLEAR RECEPTOR 2.12 0.46 1e-14
B
WT-V5
SY242CS-V5
DMSO
E2
FM
R1
R2
T47D (peaks=462)
distance (Kb)
C
Enriched motif
Alternative motif
D
WT-V5
SY242CS-V5
ATAC on ChIP Cl_3
distance (Kb)
E
sgFOXA1-L
242
Exon2-P2A-Puro
sgFOXA1-R
LHA
P2A
Puro
RHA
P-294
P-289
P-298
P-299
P-288
P-285
P-306
F
WT
SY242CS
-C
P-(294+288)
P-(285+306)
G
WT KI pool
GGCAAGGGCTCCTACTGGACGCT
SY242CS KI pool
GGCAAGGGCTGCTCCTGGACGCT
H
Relative WT FOXA1 expression (a.u.)
WT
SY242CS
I
Relative SY242CS FOXA1 expression (a.u.)
WT
SY242CS
J
WT
SY242CS
Increased
Decreased
distance (Kb)
K
Enriched motif
Alternative motif
(p=1e-3171)
L
Kmeans 5 (659)
Kmeans 4 (184)
Kmeans 3 (147)
Kmeans 2 (811)
Kmeans 1 (285)
Kmeans 8 (144)
Kmeans 7 (295)
Kmeans 6 (3736)
N
Kmeans 4 (1670)
Kmeans 3 (199)
Kmeans 2 (52)
Kmeans 1 (183)
Kmeans 7 (175)
Kmeans 6 (148)
Kmeans 5 (205)
M
WT
SY242CS
R1
R2
ATAC-seq Cluster_6
distance (Kb)
O
WT
SY242CS
R1
R2
ChIP-seq Cluster_4
distance (Kb)

Figure S6. Related to Figure 6. *FOXA1* SY242CS gained binding sites correlate with increased accessibility and mRNA upregulation.

(A) Table depicting top enriched Homer *de novo* motifs and the transcription factors associated with, per ATAC-seq cluster indicated. Fisher's exact test, p-value (p-val) as indicated. T%, percentage of target sites; BG%, background percentage; Cl, cluster.
(B-C) Tornado plot (F) representing ATAC-seq peaks for *FOXA1* SY242CS and WT T47D cells under the conditions indicated, and top enriched homer *de novo* motif for the represented peaks (G). PC, Peak center; R1, biological replicate one; R2, biological replicate two.
(D) Tornado plot representing chromatin accessibility of peaks in ChIP-seq cluster 3 for *FOXA1* SY242CS and WT MCF7 cells under the conditions indicated. PC, Peak center.
(E) Schematic depicting the KI detection strategy of FOXA1 WT and SY242CS. Orange arrows: sgRNAs. Blue and red arrows: primers detecting left and right on-target insertion in genome. Green arrows: Primers detecting WT (P-289+P-299) or SY242CS FOXA1 (P-289+P298) cDNAs.
(F) Agarose gel showing positive bands for left and right insertion in the genome for WT and SY242CS pooled MCF7 cells.
(G) Sanger sequencing of the positive WT and SY242CS pooled MCF7 cells.
(H-I) Relative WT (H) or SY242CS (I) expression. Error bars equal SEM.
(J-K) Tornado plot showing increased and decreased differential chromatin accessibility peaks in WT or SY242CS pooled KI MCF7 cells. Also shown is the enriched motif for the SY242CS alternative motif TTTATTATTT ($p=1e^{-3171}$) by Fisher's Exact test (n=2 biological replicate).
(L-M) Pie plot (L) and tornado plot (M) showing the distribution of overlapping peaks between the KI ATAC-seq peaks (SY242CS KI vs. WT KI) and the ATAC-seq cluster 6 obtained by overexpressing FOXA1 variants in MCF7 cells.
(N-O) Pie plot (N) and tornado plot (O) showing the distribution of overlapping peaks between the KI ATAC-seq peaks (SY242CS KI vs. WT KI) and the ChIP-seq cluster 4 obtained by overexpressing FOXA1 variants in MCF7 cells.

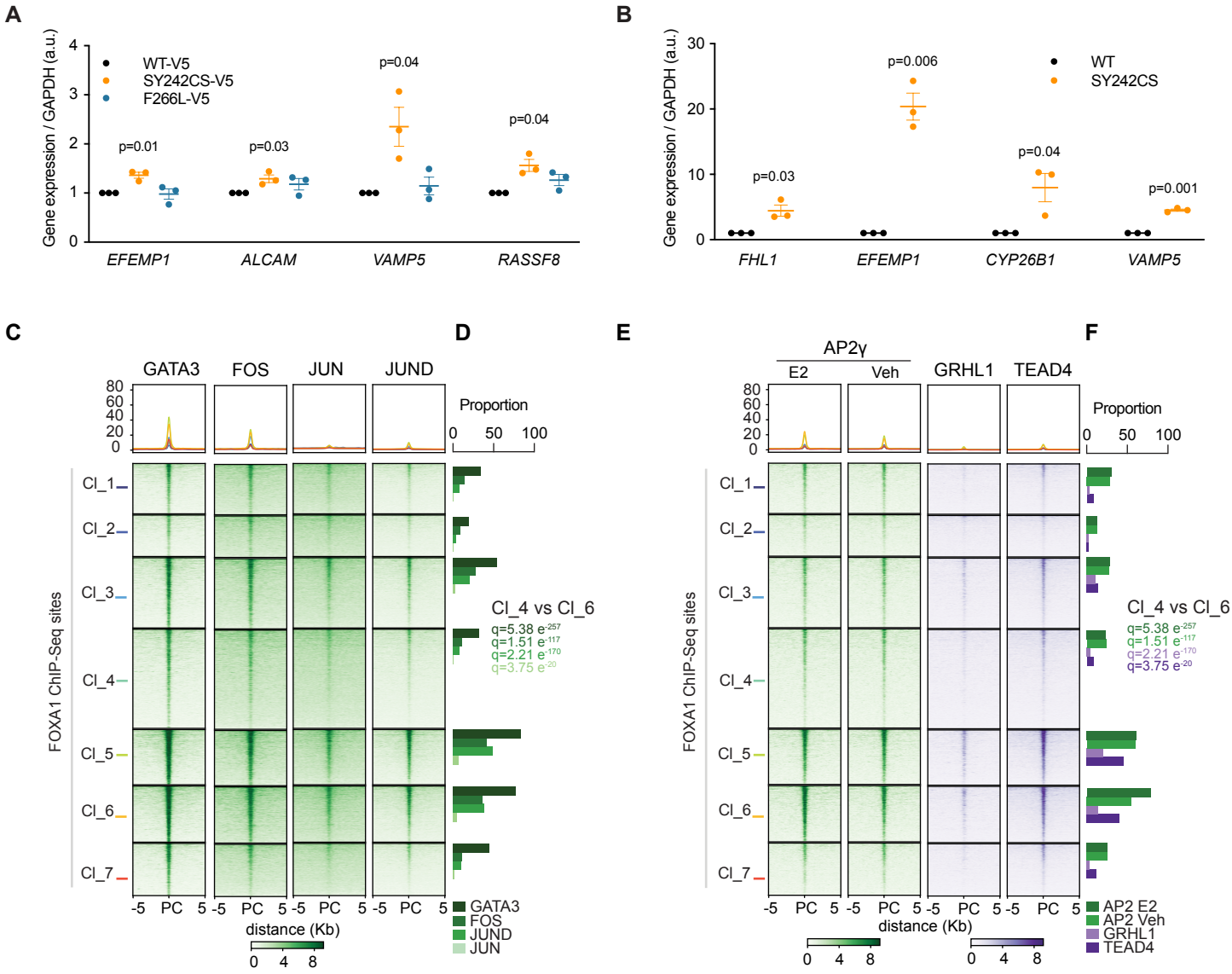

A
Gene expression / GAPDH (a.u.)
WT-V5
SY242CS-V5
F266L-V5
p=0.01
p=0.03
p=0.04
p=0.04
EFEMP1
ALCAM
VAMP5
RASSF8
B
Gene expression / GAPDH (a.u.)
WT
SY242CS
p=0.03
p=0.006
p=0.04
p=0.001
FHL1
EFEMP1
CYP26B1
VAMP5
C
GATA3
FOS
JUN
JUND
FOXA1 ChIP-Seq sites
Cl_1
Cl_2
Cl_3
Cl_4
Cl_5
Cl_6
Cl_7
distance (Kb)
D
Proportion
Cl_4 vs Cl_6
q=5.38 e-257
q=1.51 e-117
q=2.21 e-170
q=3.75 e-20
GATA3
FOS
JUND
JUN
E
AP2γ
E2
Veh
GRHL1
TEAD4
F
Proportion
Cl_4 vs Cl_6
AP2 E2
AP2 Veh
GRHL1
TEAD4

Figure S7. Related to Figure 7. FOXA1 cooperating factors show high chromatin occupancy at *FOXA1* sites

(A) mRNA expression quantified by RT-QPCR of genes indicated in T47D cells expressing *FOXA1* variants (SY242CS-V5 and F266L-V5) as compared to *FOXA1* WT-V5 cells in FM.
(B) mRNA expression quantified by RT-QPCR of genes indicated in pooled KI MCF7 cells (WT and SY242CS FOXA1) in FM.
(C-D) Tornado plots (C) and bar plot (D) showing ChIP-seq peaks of public ChIP-seq data for transcription factors with enriched occupancy at FOXA1 ATAC-seq clusters in Figure 6A (GATA3 (ENCSR000BST) and AP1 (FOS, ENCSR569XNP; JUN, ENCSR176EXN; JUND, ENCSR000BSU) at FOXA1 ChIP-seq sites per cluster in Figure 3B indicated. Statistical test of cluster 4 vs cluster 6 as indicated.
(E-F) Tornado plots (E) and bar plot (F) showing ChIP-seq peaks of public ChIP-seq data for transcription factors with enriched occupancy at FOXA1 ATAC-seq clusters in Figure 6A (AP2gamma (GSE26741), GRHL1 (GSE140185) and TEAD (ENCSR000BUO)) at FOXA1 ChIP-seq sites per cluster in Figure 3B indicated. Statistical test of cluster 4 vs cluster 6 as indicated.
PC, Peak center; DMSO, estrogen depletion; E2, upon estrogen stimulation; Veh, vehicle treated.