ChIP-Seq	Total Tags	Binding Sites	Target Genes	3'UTR (%)	TSS (%)	Exon (%)	Intron (%)	Intergenic (%)	Promoter (%)	5'UTR (%)
Foxa1 in ♀control	26,733,886	9,337	5,598	0.58	1.32	2.43	44.91	42.18	8.43	0.16
Foxa2 in ♀control	26,932,294	17,531	8,253	0.68	1.82	2.06	53.25	37.03	5.01	0.14
Foxa1 in ♀control+DEN	26,395,516	14,939	7,185	0.61	1.45	1.93	49.12	39.69	6.65	0.17
Foxa2 in ♀control+DEN	28,225,206	38,309	11,657	0.63	1.56	1.92	50.06	39.98	5.70	0.13
Foxa1 in ♂control	29,685,552	17,796	7,960	0.60	1.35	1.74	46.76	43.64	5.78	0.12
Foxa2 in ♂control	30,325,531	11,613	6,170	0.74	1.73	2.31	51.27	38.95	4.89	0.11
Foxa1 in ♂control+DEN	26,465,443	28,449	10,225	0.66	1.49	1.87	49.49	40.66	5.68	0.13
Foxa2 in ♂control+DEN	30,923,817	18,835	8,524	0.56	1.31	1.88	47.42	42.13	6.54	0.14
ERα in ♀control	29,374,596	11,225	7,132	0.62	2.05	5.71	36.78	35.86	18.11	0.83
ERα in ♀control+DEN	25,931,991	13,033	6,010	0.68	1.71	3.06	45.62	38.59	10.02	0.31
ERα in ♀mutant+DEN	29,903,431	2,777	2,391	0.43	1.40	14.40	42.38	38.10	3.02	0.25
AR in ♂control	33,437,805	5,807	4,154	0.64	1.77	3.70	38.87	42.72	12.02	0.22
AR in ♂control+DEN	29,890,415	15,998	7,648	0.63	1.80	1.89	49.58	39.73	6.29	0.08
AR in ♂mutant+DEN	26,931,940	1,915	1,588	0.42	0.84	5.54	36.14	55.98	0.99	0.10

Table S1. Related to Figure 2

Genomic analysis from all ChIP-Seq datasets. ChIP was performed on liver chromatin of gender, genotype, and treatment as indicated in the first column. Binding sites and their nearest targeted genes were determined using the HOMER algorithm with motif filtering (Heinz et al., 2011). TSS, 100 bp surrounding transcription start sites; promoter, 1 kb surrounding transcription start sites.

Dual Binding Sites	3'UTR (%)	TSS (%)	Exon (%)	Intron (%)	Intergenic (%)	Promoter (%)	5'UTR (%)
Foxa1/ERα in ♀control	0.944811	1.619676	2.684463	39.75705	41.82663	11.71266	1.544691
Foxa2/ERα in ♀control	0.955171	1.6811	3.222109	39.7351	39.63321	13.20683	1.642894
Foxa1/ERα in ♀control+DEN	1.091582	1.415356	1.72988	47.05828	40.47179	7.779833	0.508788
Foxa2/ERα in ♀control+DEN	1.16327	1.534527	1.938784	46.34106	39.57594	8.844155	0.651761
Foxa1/AR in ♂control	0.97629	1.67364	2.161785	40.07438	45.74616	8.879591	0.627615
Foxa2/AR in ♂control	1.142573	1.564829	2.334824	42.5236	42.67263	9.289617	0.620964
Foxa1/AR in ♂control+DEN	1.163115	1.622789	0.968101	50.73826	40.41649	4.847472	0.285555
Foxa2/AR in ♂control+DEN	1.014607	1.434444	0.839675	50.75658	41.80005	3.988454	0.218665

Table S2. Related to Figure 2
Genomic distribution of Foxa/ERα or Foxa/AR dual binding sites. Dual binding sites were defined by close consensus binding elements within 250 bp.

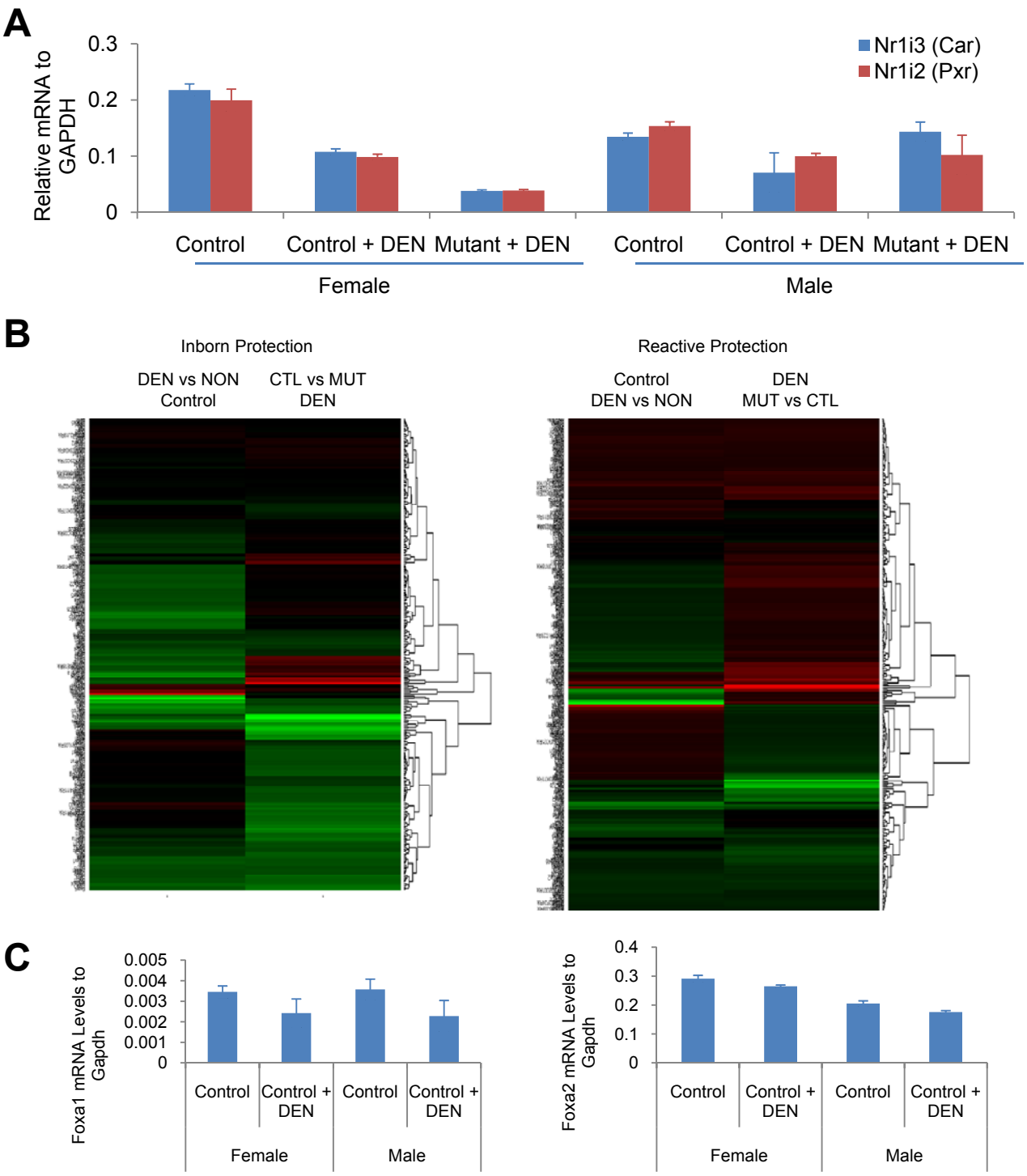
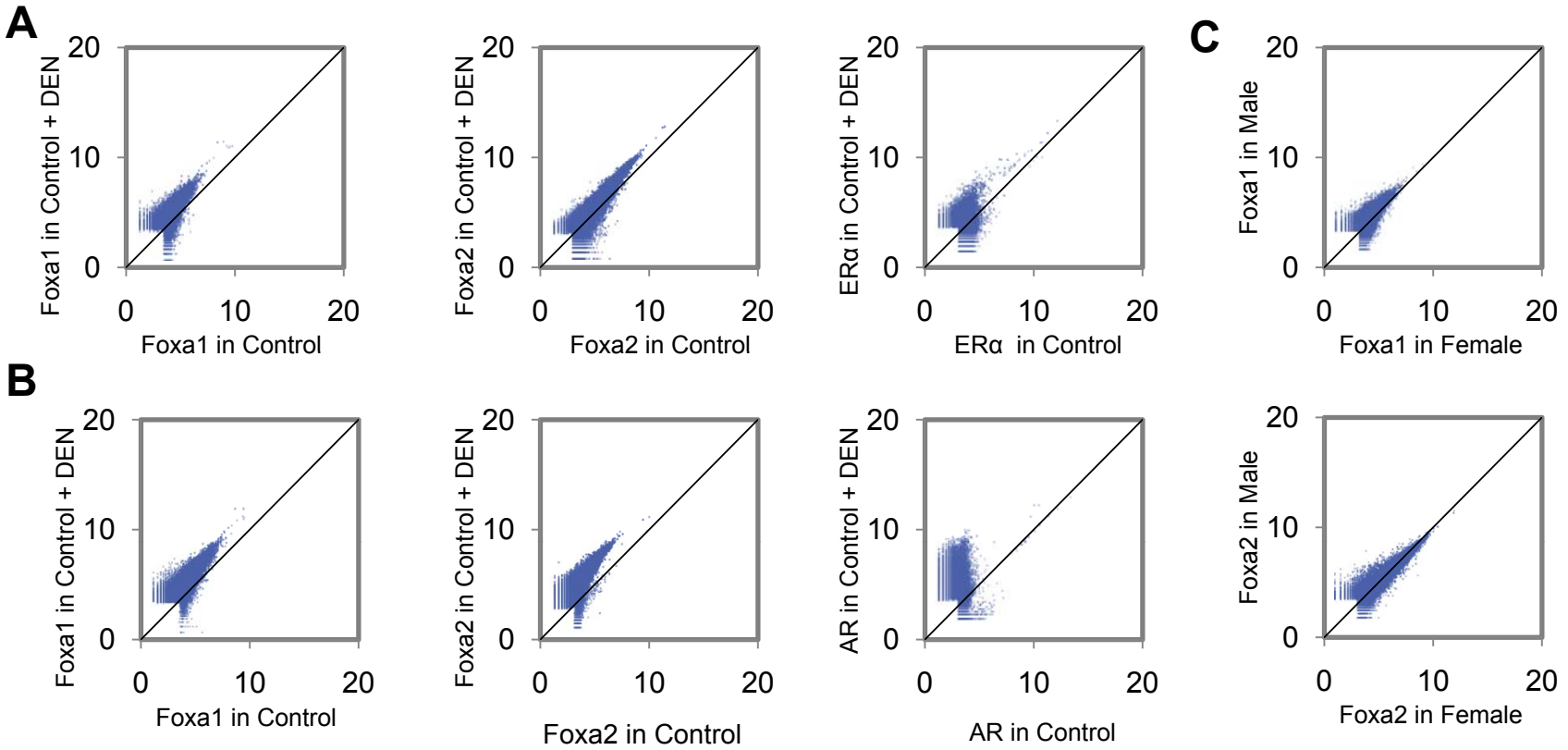


Figure S1. Related to Figure 1

(A) Gene expression levels of constitutive androstane receptor (Car, or Nr1i3) and pregnane X receptor (Pxr, or Nr1i2) in liver of female and male control and Foxa1/2 mutant mice treated or not treated with DEN.

(B) The change of gene expression of Foxa/ERα dual targets in female control mice was mostly reversed in mutant mice during hepatocarcinogenesis in both groups of inborn and reactive protection. DEN, mice with carcinogen; NON, mice without carcinogen. MUT, mutant mice; CTL, control mice.

(C) Hepatic Foxa1 and Foxa2 expression in female and male control mice with or without carcinogen (DEN) treatment.



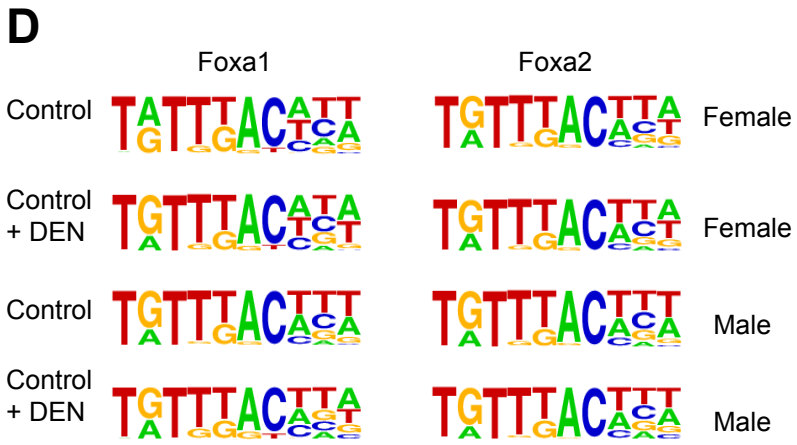


Figure S2. Related to Figure 2
Genome-wide Occupancy of Foxa1, Foxa2, and ERα/AR in control and carcinogen-treated liver

(A, B) Genome-wide occupancy of Foxa1, Foxa2, and ERα/AR in female (A) and male (B) control livers with and without carcinogen (DEN) administration. Values are log2 of total tag number at each peak region from pooled peaks of two comparisons after normalization to 10 million ChIP-Seq tags each.

(C) Genome-wide occupancy of Foxa1 and Foxa2 between female and male control livers without carcinogen (DEN) administration. Values are from log2 of total tags number from pooled peaks after normalization to 10 million ChIP-Seq tags each.

(D) Motif analysis using HOMER for Foxa1 or Foxa2 binding sites in female and male control liver with or without carcinogen treatment.

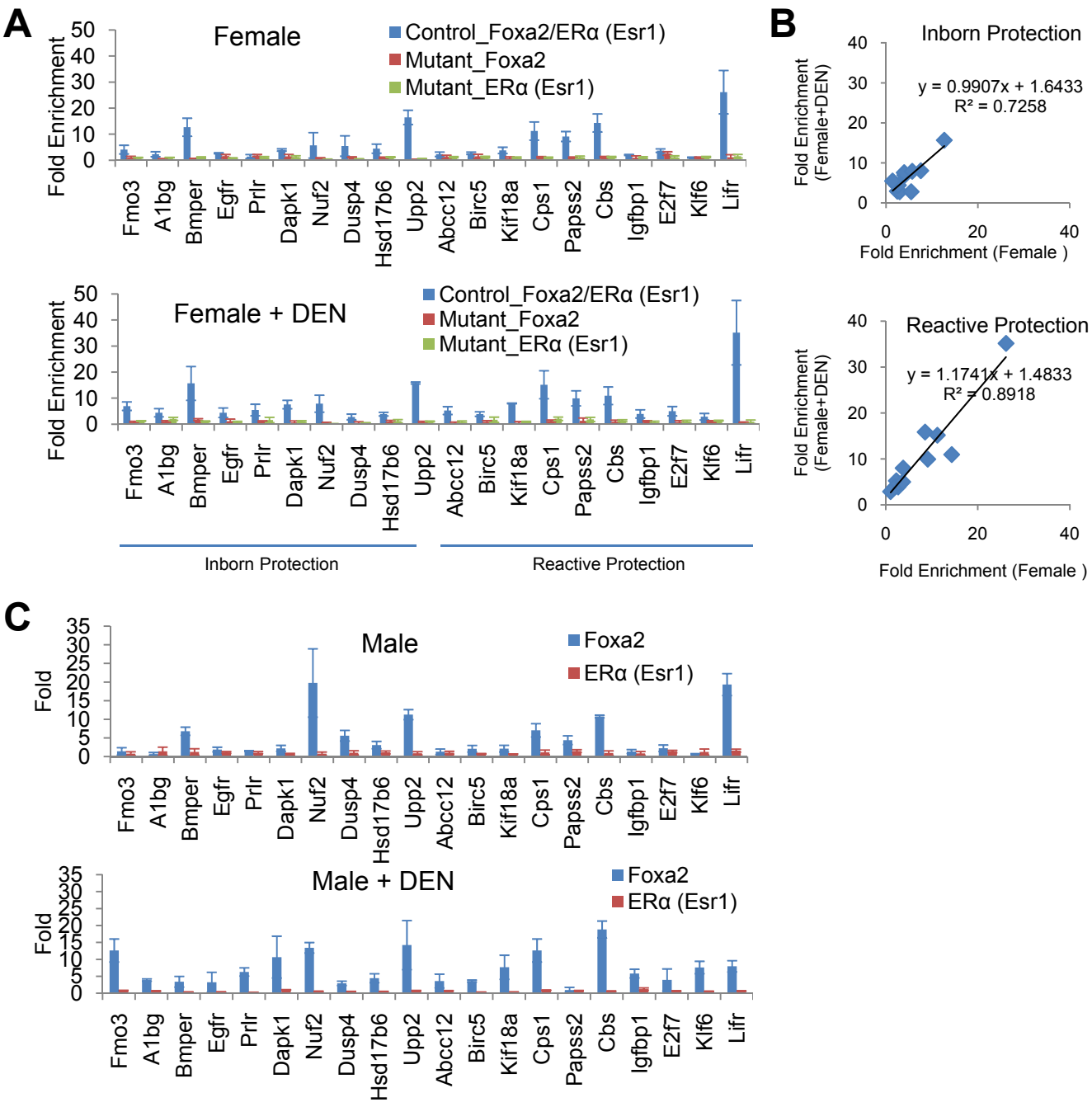


Figure S3. Related to Figure 3

Occupancy of Foxa2 and ERα in control and carcinogen-treated liver

(A, C) Occupancy of Foxa2 and ERα in female (A) and male (C) control livers with and without carcinogen (DEN) administration by ChIP-qPCR. Ers1, gene name for ERα.

(B) Correlation analysis of ChIP enrichment of Foxa2/ERα dual targets between female mice with and without DEN administration for genes classified to potentially function in inborn or reactive protection.

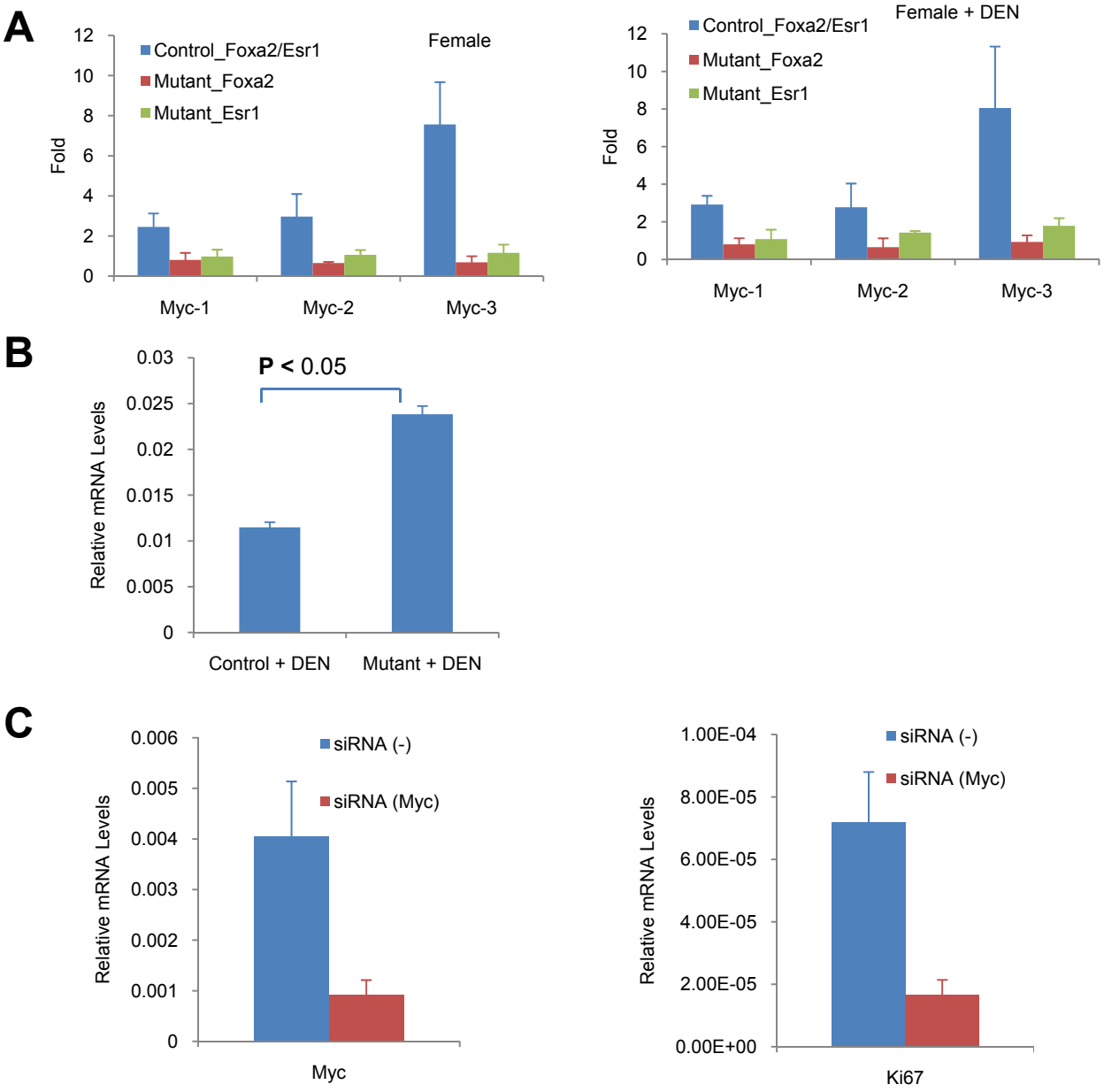


Figure S4. Related to Figure 4
Comprehensive protection to hepatocarcinogenesis by the co-regulation of Foxa1/2 and ERα

(A) Chromatin immunoprecipitation (ChIP) assays for Foxa2/ERα dual targets in female mice with/without carcinogen (DEN) administration. Ers1, gene name for ERα.

(B) Myc expression in control and Foxa1/2 mutant female liver after DEN treatment. n=4 in each group.

(C) Suppression of cell proliferation, as measured by Ki67 expression, by Myc siRNA in primary hepatocytes isolated from female DEN-treated mutant mice.

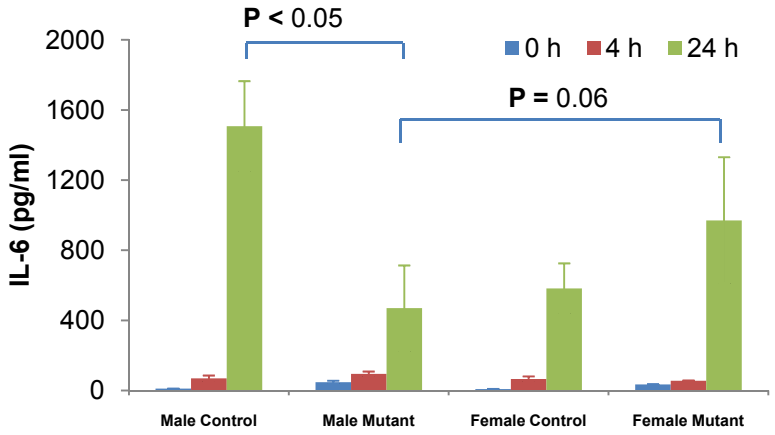


Figure S5. Related to Figure 5
Serum interleukin-6 (IL-6) levels in mice at 0, 4 and 24 hours after carcinogen administration
8-week old control and Foxa1/2 mutant mice of both genders were injected Intraperitoneally with DEN (100 µg/g body weight) before the analysis of serum IL-6 levels.

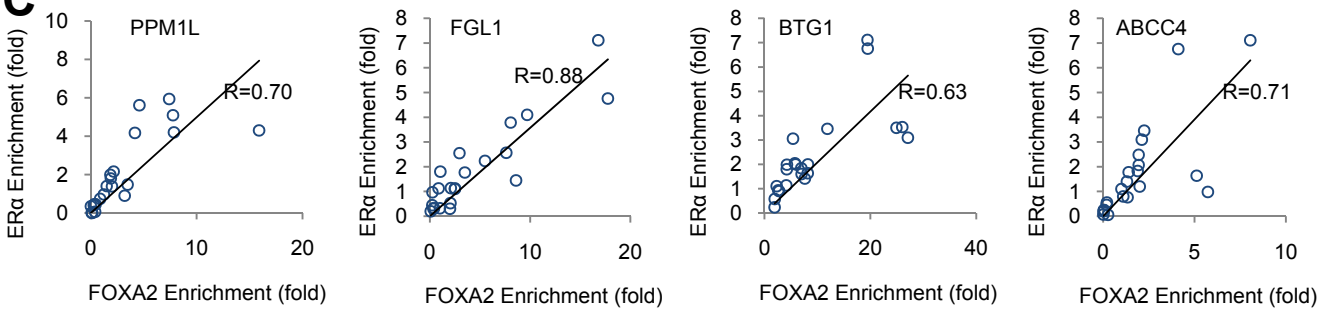
A

	FOXA2					ERα					Mutation				
ChIP/SNP	Normal	HCC-1	HCC-2	HCC-3	HCC-4	Normal	HCC-1	HCC-2	HCC-3	HCC-4	HCC-1	HCC-2	HCC-3	HCC-4	SNP Description
KLF6	6.1	2.9	2.2	5.5	27.5	2.4	2.1	1.8	5.9	22.9	NO	YES	NO	NO	AAACA > AAAA(14)CA
MYC (1)	9.9	4.1	12.3	2.6	7	4.5	2.8	1.8	6.1	3.3	YES	NO	NO	YES	TTTTT > TT(16)TTTT
PLXNA2	3.9	1.9	3.7	3.2	4.5	3	1.6	1.4	2.2	2.7	YES	NO	NO	NO	AAATA > AAGTA
ACMSD	5	2.1	18.4	3.4	16.1	3.5	1.3	4.4	3.4	6.2	YES	NO	NO	NO	TGTTT > T--TT
PTP1B	17.5	0.6	8.6	2.1	24.1	4.3	0.6	1.6	2.2	6.5	NO	NO	NO	NO	TCTTT > TCTTC
NIPAL1	13.9	2.6	4.1	3.6	13.5	4.2	2.8	1.3	7.6	5.4	NO	AAACA > AAAGT*	AAACA > AAACG*	NO	AAACA > AACCA
ANKRD50	3.6	3.3	4.8	4	2.6	3.7	1.8	0.8	2.8	0.9	NO	AAACA > AAAGT*	AAACA > AAACC*	NO	AAACA > AAAGA
GCLC	5.1	1.9	6.5	4.5	6.2	4.5	2.5	2.2	4.1	2.8	NO	NO	NO	NO	TGTTT > CGTTT
ASL	4.7	2.8	3.2	11.8	2.5	3.8	3.4	1.9	5.2	1.6	NO	NO	NO	NO	AAACA > GAACA
IPMK	7.9	1.6	6.8	1.4	14.2	4.9	1	1.4	1	5.4	YES	NO	YES	NO	AAACA > AAGCA
PIK3AP1	5.3	2.6	1.1	4	8.4	2.5	2.1	0.6	2.1	3.1	NO	NO	NO	NO	AAATA > AAATG
SERPINA5	8.8	1.1	5.5	1.1	2.6	3.4	0.8	2.2	2	2.1	NO	NO	NO	YES(homo)	TGTTT > TGCTT
CYP2A13	7.2	1.4	10.1	1	2.8	3.2	1.6	0.8	1.1	1.4	NO	NO	NO	NO	AAACA > AAATA
MYC (2)	5.7	5.9	7.6	2.6	5.4	5.1	2	1.4	0.9	3	NO	NO	YES	NO	TATTT > ---- T

B

	FOXA2					ERα					Mutation				
ChIP/SNP	Normal	HCC-1	HCC-2	HCC-3	HCC-4	Normal	HCC-1	HCC-2	HCC-3	HCC-4	HCC-1	HCC-2	HCC-3	HCC-4	SNP Description
PPM1L	7.4	1	15.9	1.5	3.2	4.2	0.9	4.3	1.8	1.4	YES	NO	YES	YES	AAATA > AGATA
FGL1	10	0.4	1	2.5	2	4.1	0.3	0.3	1.8	1.1	YES (homo)	YES (homo)	YES (homo)	YES (homo)	TATTT > TATTC
BTG1	19.5	4.2	2.3	8.2	7	3.5	2	1.1	1.6	1.8	YES	YES(homo)	YES	YES	TATTT > TACTT
ABCC4	7.0	1.3	1.1	1	2.0	3.8	1.4	0.8	1.1	1.2	YES	YES	YES*	YES	AAACA > CAACA

C



FigureS6. Related to Figure 7

The relationship between hepatocellular carcinoma (HCC) and single nucleotide polymorphisms (SNPs) at FOXA2 binding sites in women

(A,B) DNA sequencing and ChIP assays with anti-FOXA2 or anti-ERα revealed that mutations at the core binding element of FOXA2 binding were associated with impaired binding of both FOXA2 and ERα in four women livers with HCC. The ChIP enrichment of the normal liver was assayed from normal subjects without mutations in both alleles. YES, mutation presented in one allele; YES(homo), mutations in both alleles; NO, no mutation in either alleles. *, a novel mutation/SNP. The first column contains gene names for those genes associated with SNPs at the core binding element of FOXA2 binding sites.

(C) Correlation analysis of co-binding of FOXA2 and ERα from all 22 normal and HCC samples (n=11 each).

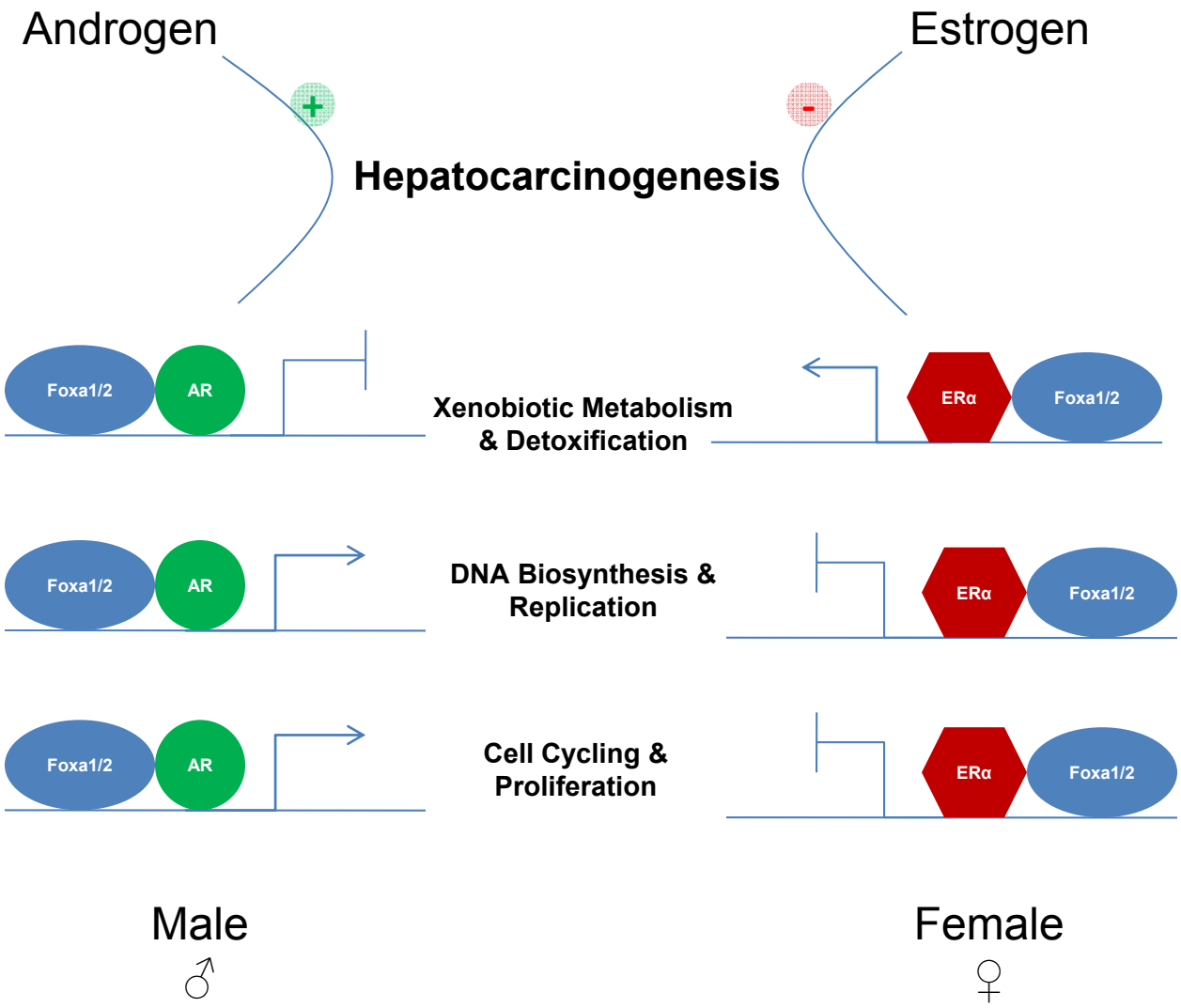


Figure S7. Related to Figure 4
Foxa1/2 as the central regulator of gender dimorphism in liver cancer
Estrogen/androgen signaling prevents/promotes liver cancer in females/males, respectively. ERα-dependent prevention and AR-mediated liver cancer promotion depend on Foxa1/2. Foxa1/2 and ERα/AR co-regulate multiple pathways of hepatocellular carcinogenesis, including xenobiotic metabolism and carcinogen detoxification, DNA biosynthesis and replication, and cell cycling and proliferation.