The renal cancer risk allele at 14q24.2 activates a novel hypoxia-inducible transcription factor-binding enhancer of DPF3 expression

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Supplemental Information

Supplemental table and figure legends

Supplemental table 1

SNPs in high LD with rs4903064.

Supplemental table 2

Oligonucleotides used in this study.

Supplemental figure 1

DPF3 mRNA expression (TPM) in the TCGA normal tissue (green) and cancer (red) data sets. The box indicates kidney tumors. ccRCC tumors are highlighted in yellow (KIRC). Bars are mean values and numbers of samples per analysis are indicated below the graph. Data is derived from the GEPIA TCGA analysis tool (http://gepia.cancer-pku.cn).

Supplemental figure 2

A) Distribution of C and T alleles of rs4903064 in the 1000Genome project EUR cohort (blue), the CCC Erlangen-EMN ccRCC cohort (red) and non-ccRCC renal cancer patients from that cohort (grey). B) Relative expression levels of GLUT3 in the CCC Erlangen-EMN ccRCC cohort stratified according to the rs4903064 genotype. C) DPF3 expression in the cohort of patients with a non-ccRCC renal cancer according to the rs4903064 genotype. Bars indicate mean ±SD.

Supplemental figure 3

A) Luciferase Reporter assay performed in MCF-7 cells. Cells were transfected with plasmids and treated with 1mM DMOG or left untreated as described in main figure 2G. T test, ***, $p<0.001$. B) MCF-7 cells were depleted for the indicated HIF-α isoforms using siRNA and transfected with the reporter plasmid including the risk allele. Cells were exposed to 1mM DMOG for 16h. Reporter activity was normalized to the activity of co-transfected β-galactosidase. Bars indicate mean values of one experiment performed in duplicates. C) Western blot for HIF-1α and HIF-2α from lysates of MCF-7 cells treated with the indicated siRNA and exposed to 1mM of DMOG or left untreated. Cells were cultured in parallel to cells used for the reporter assay shown in B. Arrow indicates the HIF-2α band. D) Western blot for HIF-1α and HIF-2α from lysates of Hela cells treated with the indicated siRNA and exposed to 1mM of DMOG or left untreated. Cells were cultured in parallel to cells used for the reporter assay shown in main figure 2 H.

Supplemental figure 4

HIF-ChIP at the *DPF3* enhancer locus in two primary cell cultures of ccRCC cells isolated from individuals with the rs4903064 TT genotype or the CT genotype. Results are from one experiment and representative for three experiments per genotype.

Supplemental figure 5

A) Using available RNA-seq data from primary renal tubular cells (PTC) (Naas S., manuscript in preparation) genes within 500kb upstream or downstream of the DPF3 TSS were tested for expression (base mean >100) and regulation by the HIF-stabilizer DMOG. Of the expressed genes, three genes (ZFYVE1, PSEN1 and NUMB) were significantly induced by DMOG treatment ($p<0.05$). Independent HIF-binding signals could be detected at the ZFYVE1 and the NUMB locus indicating that expression of these genes is regulated by separate regulatory elements. B) Expression of PSEN1 was examined in isolated primary tubular cells exposed to 1mM DMOG for 16h or left untreated (control) by qPCR. The weak induction observed in RNA-seq experiments could not be reproduced in independent samples by qPCR. In addition, stratifying expression of PSEN1 under DMOG conditions according to the rs4903064 genotype revealed no genotype-specific effects.

Supplemental figure 6

A) FAIRE-seq tracks at the *DPF3* enhancer including the SNP rs4903064 (highlighted in yellow). The top three tracks (red) are FAIRE-seq tracks from primary renal tubular cells published in Grampp et al. 2017. The other tracks were generated by the ENCODE consortium. Data is in hg19. B) Relative DPF3 mRNA expression in different cell lines exposed to 1mM DMOG for 16h compared to untreated controls. The genotype of the cells for rs4903064 is indicated above the values. Data is from 1-3 experiments per cell line. Values are mean and error bars indicate standard deviation as applicable.

Supplemental figure 7

TCGA copy number variation data in the KIRC ccRCC cohort. Samples were stratified according to alterations at the HIF1A locus (x-axis) and numbers of mutations of DPF3 are shown in different colours. Data is from https://www.cbioportal.org/.

Supplemental figure 8

Relative expression values of DPF3 in primary renal tubular cells. Values were stratified according to the rs4903064 genotype and the gender of the donors indicated with symbols.

Supplemental figure 9

A) Relative expression values of DPF3 in isolated tumor cells carrying the risk allele C compared to corresponding primary tubular cells and stratified according to the PBRM1 mutation status (as defined in B). T test: not significant (ns). B) Western blots for PBRM1 using lysates of isolated renal tumor cells. Only tumor cells carrying a risk allele were used for analysis in A. N: normal, T: tumor.

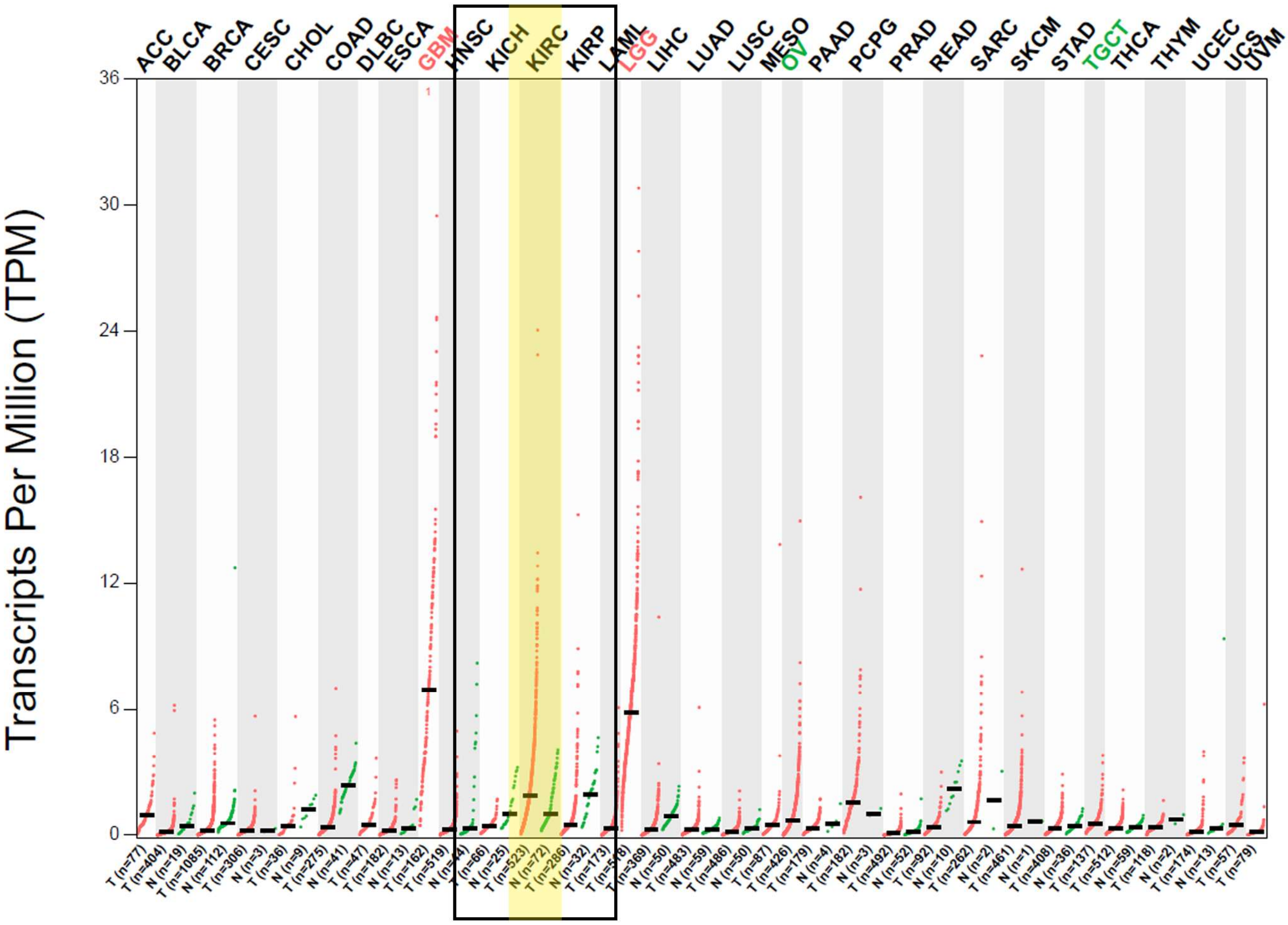
Supplemental table 1

QRSID	RSID	RSALIAS	CHR	POS1	POS2	DIST	R2	D	DPRIME	MAJOR	MINOR	MAF
rs4903064	rs11620775	rs60339375	14	72812462	72808736	-3975	0.852665	0.167232	0.943894	C	T	0.233598
rs4903064	rs12050132	NA	14	72812462	72809542	-3170	0.946882	0.179261	0.983637	A	T	0.245527
rs4903064	rs4903064	NA	14	72812462	72812462	0	1	1	1	T	C	0.241551
rs4903064	rs7152005	rs61351179	14	72812462	72813710	998	0.801788	0.160207	0.936109	C	T	0.225646
rs4903064	rs28840762	rs58119506	14	72812462	72819416	6704	0.852665	0.167232	0.943894	T	C	0.233598
rs4903064	rs2332920	rs59774530	14	72812462	72819750	7038	0.852665	0.167232	0.943894	A	G	0.233598
rs4903064	rs8015900	rs56991741	14	72812462	72821222	8510	0.821913	0.162709	0.942424	T	A	0.227634
rs4903064	rs2109794	rs61636270	14	72812462	72822147	9435	0.824029	0.166305	0.907761	G	A	0.241551

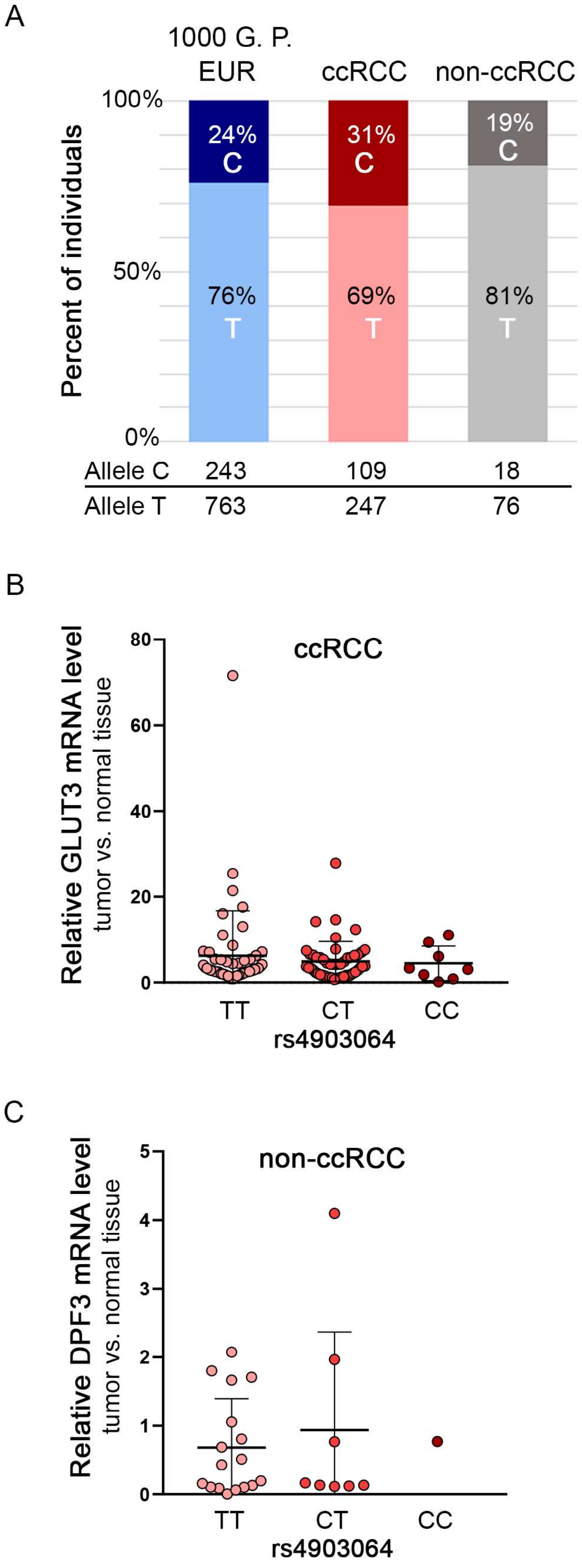
Supplemental table 2

CRISPR/Cas9 guides	
DPF3 exon2 guide1 fw	CACCGACAGCCGTGAGTTGTAACTC
DPF3 exon2 guide1 rev	AAACGAGTTACAACTCACGGCTGTC
DPF3 exon2 guide2 fw	CACCGCTCGGGGACCAGTTCTACA
DPF3 exon2 guide2 rev	AAACTGTAGAACTGGTCCCCGAGC
PAGE primers	
DPF3 CRISP ex2 Cloning KPNI fw	GCATGGTACCCTCGGTGCCTCTTCTCCATC
DPF3 CRISP ex2 Cloning NHEI rev	GCTAGCTAGCTCATTGGCAAGGCTCTTGGT
ICE primers	
ICE DPF3 Primer fw	AATGCCCCACACCATCCTTG
ICE DPF3 Primer rev	TGGGACCTCATGCTTCCTAGA
ICE sequencing	
ICE sequencing	TATCTCAGAGGCCCATGTGC
Expression primer	
HPRT fw	GACCAGTCAACAGGGGACAT
HPRT rev	CTGAGCCTTCCTCAGCGATT
CA9 fw	GAGGCCTGGCCGTGTTG
CA9 rev	CTGAGCCTTCCTCAGCGATT
DPF3 fw	AGCGTGCGTCTTCCCTT
DPF3 rev	GCTCCACTTCAGGTTTTATCTCC
EGLN3 fw	GGCCATCAGCTTCCTCCTG
EGLN3 rev	GGTGATGCAGCGACCATCA
DPF3 ex3_4 fw	CGGCTGCTGGAGATAAAACC
DPF3 ex3_4 rev	TCCACCTTCTTCTCAACCCC
PSEN1 fw	TATCAAGTACCTCCCTGAAT
PSEN1 rev	ACCATTGTTGAGGAGTAAAT
ChIP	
DPF3 hif bind(14q24.2 HIF ChIP fw)	TGGAGGCTTTGTAGCTAGG
14q24.2 HIF ChIP rev	CACACTGCTCTGTGTCAGCA
EGLN3 hif bind fw	AGTGTCCGTTCCCAGCTCAG
EGLN3 hif bind rev	TAGGCACAGTAAACAGGCC
CCND1 neg3 fw	GGATCACCAGGTGTATTCGG
CCND1 neg3 rev	CATCCAAGGGGGATACACAC
Reporter cloning	
14q24.2 reportert fw KPNI	GCATGGTACCAGGAGGCAGGGTTGGTATTT
14q24.2 reportert REV NHEI	GCTAGCTAGCTTCCCGTCTTTTGTTTGGTC

Supplemental figure 1



Supplemental figure 2



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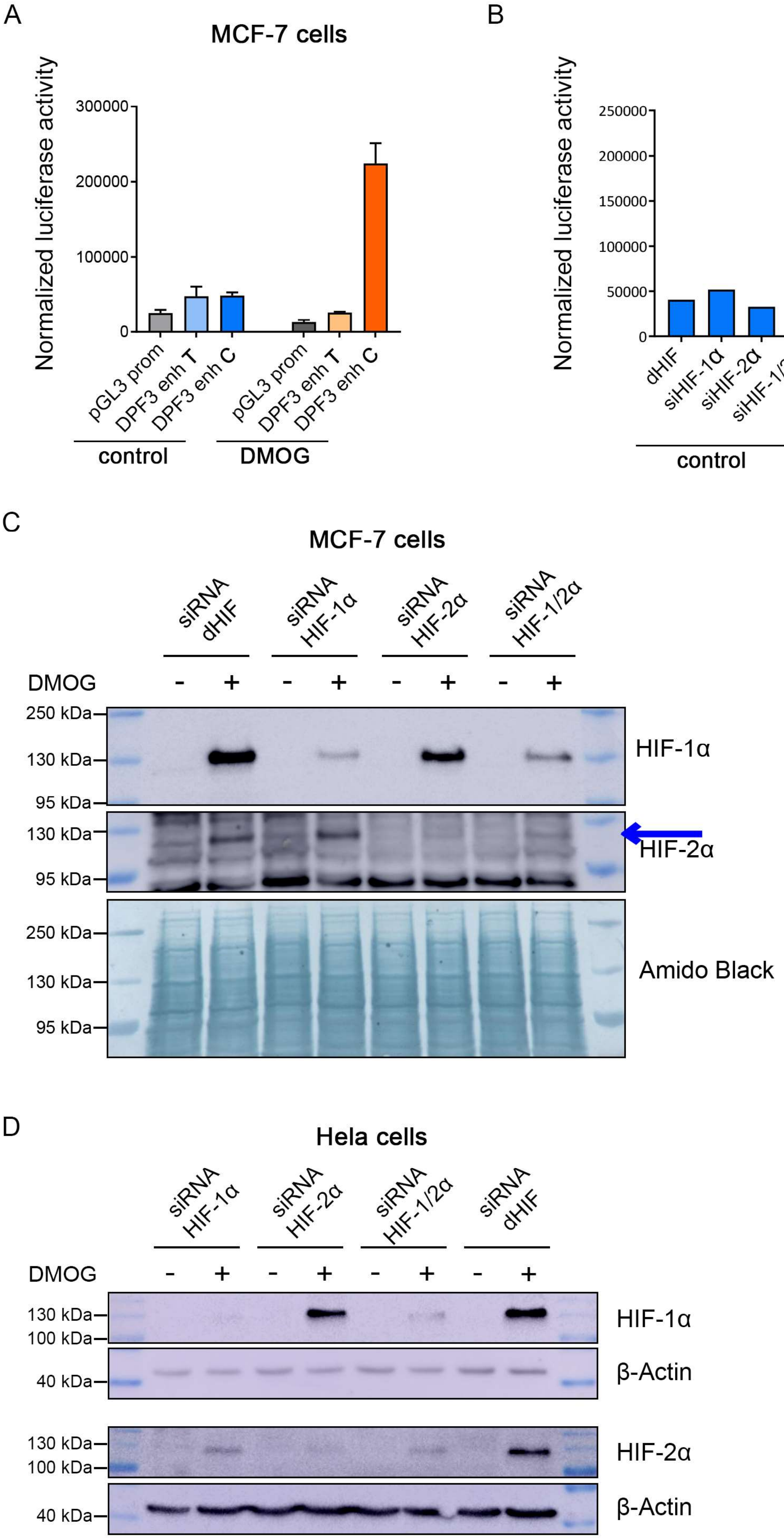

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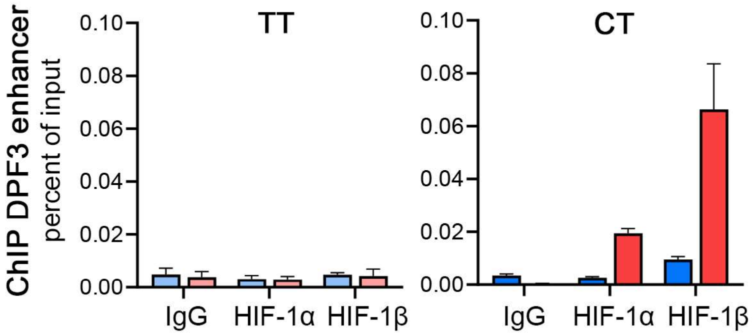
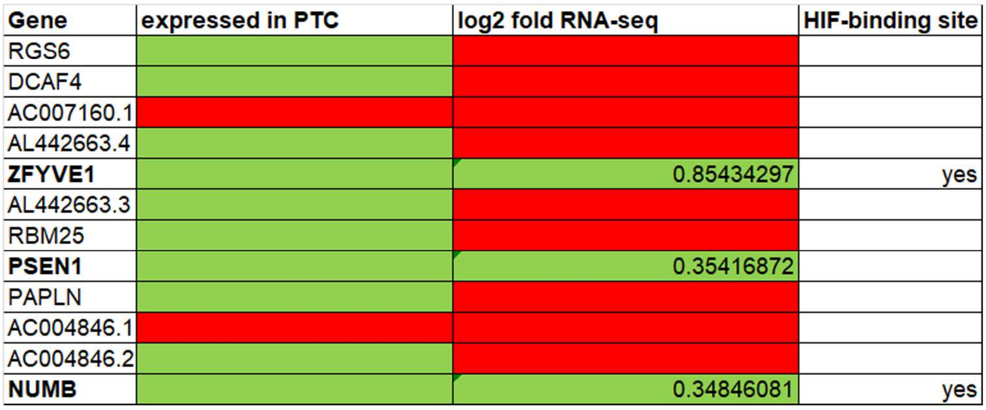


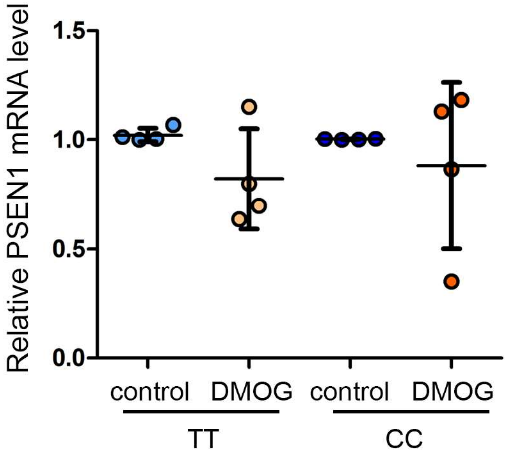
Supplemental figure 5

A

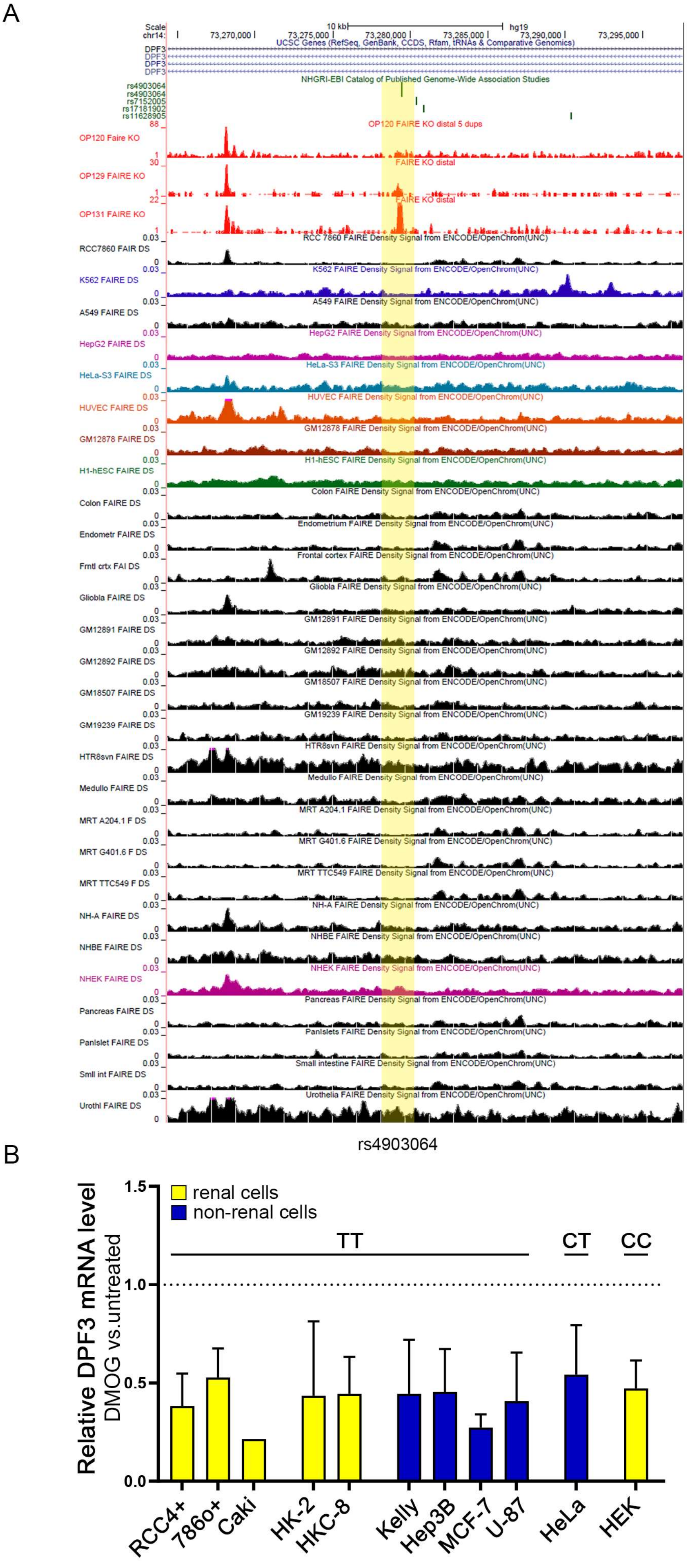


Gene	**expressed in PTC**	**log2 fold RNA-seq**	**HIF-binding site**
RGS6	🟩	🟥	
DCAF4	🟩	🟥	
AC007160.1	🟥	🟥	
AL442663.4	🟩	🟥	
ZFYVE1	🟩	🟩 0.85434297	yes
AL442663.3	🟩	🟥	
RBM25	🟩	🟥	
PSEN1	🟩	🟩 0.35416872	
PAPLN	🟩	🟥	
AC004846.1	🟥	🟥	
AC004846.2	🟩	🟥	
NUMB	🟩	🟩 0.34846081	yes

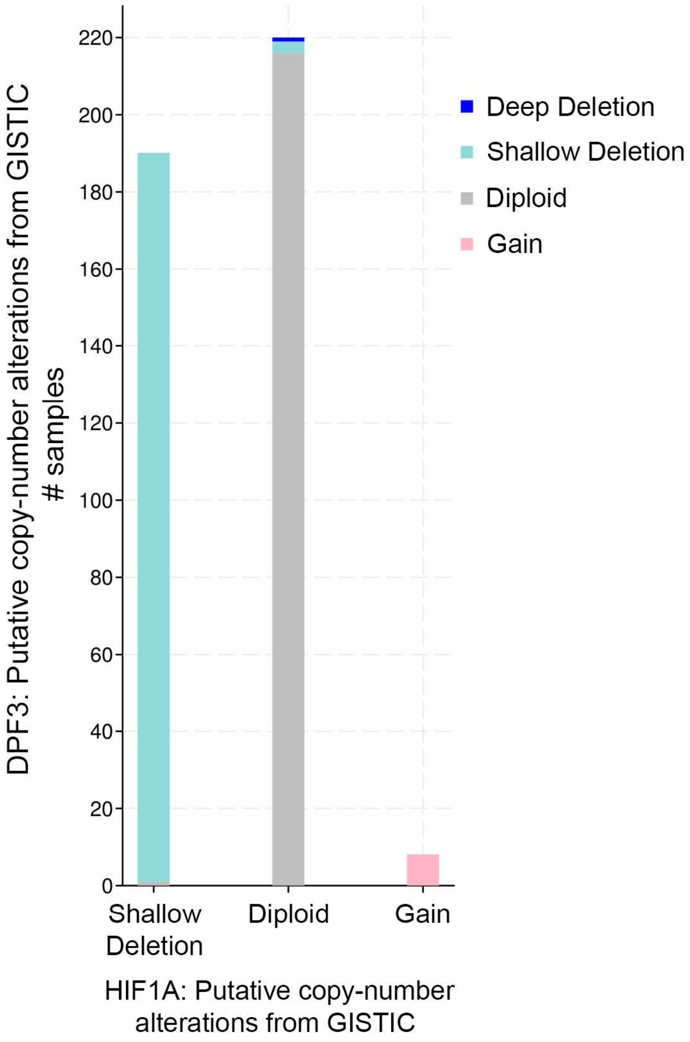
B



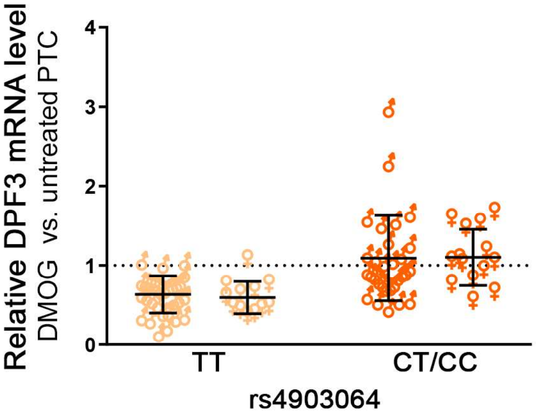
Supplemental figure 6



Supplemental figure 7



Supplemental figure 8



Supplemental figure 9