Supplementary Figure 1 (Related to figure 1)

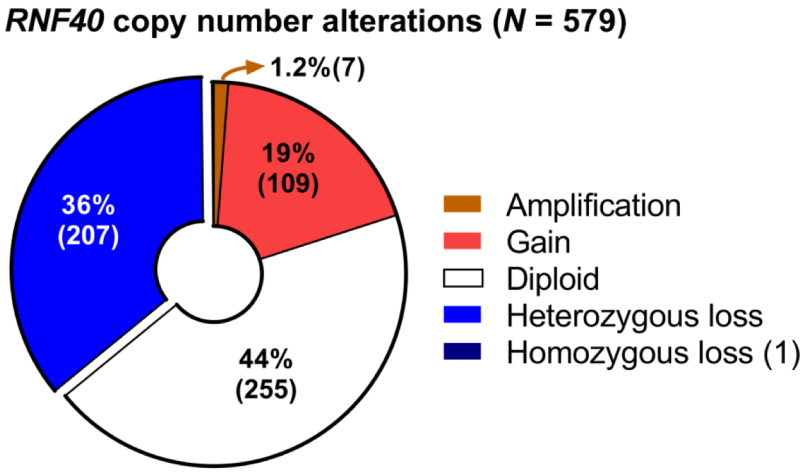
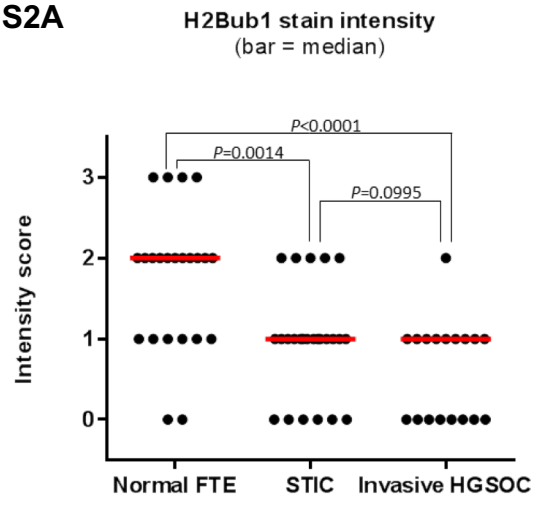


Figure S1. *RNF40* somatic copy number alterations in a cohort of 579 HGSOC cases from The Cancer Genome Atlas (TCGA). Heterozygous loss of *RNF40* is present in 36% of HGSOCs.

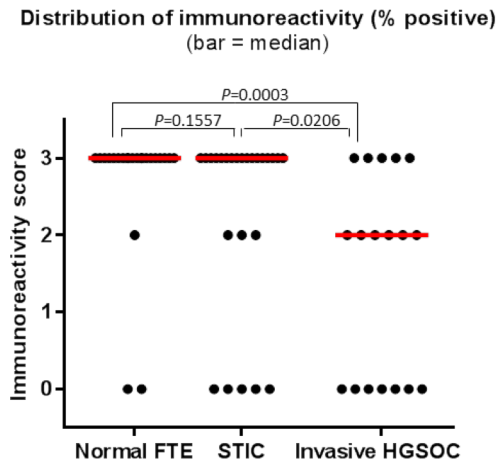
Supplementary Figure 2 (Related to figure 2)

S2A



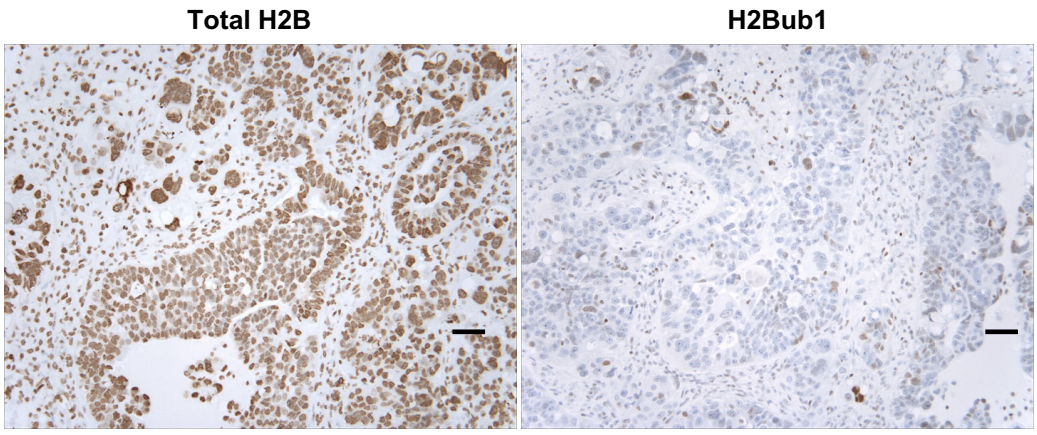
Supplementary Figure S2A. H2Bub1 stain intensity dot plot. Intensity of H2Bub1 stain in STIC and invasive HGSOC was lower (median score = 1+; i.e. weak) relative to normal FTE (median score = 2+; i.e. moderate) indicating decreased H2Bub1 expression in early and late forms of HGSOC.

S2B



Supplementary Figure S2B. Distribution of H2Bub1 immunoreactivity (% positive cells). Similar distribution of H2Bub1 positive nuclei (with any signal intensity above 0+) was observed in normal FTE and in STIC (median score = 3+; i.e. 75%-100% positive), but the distribution was lower in invasive HGSOC (median score = 2+; i.e. 10%-75% positive).

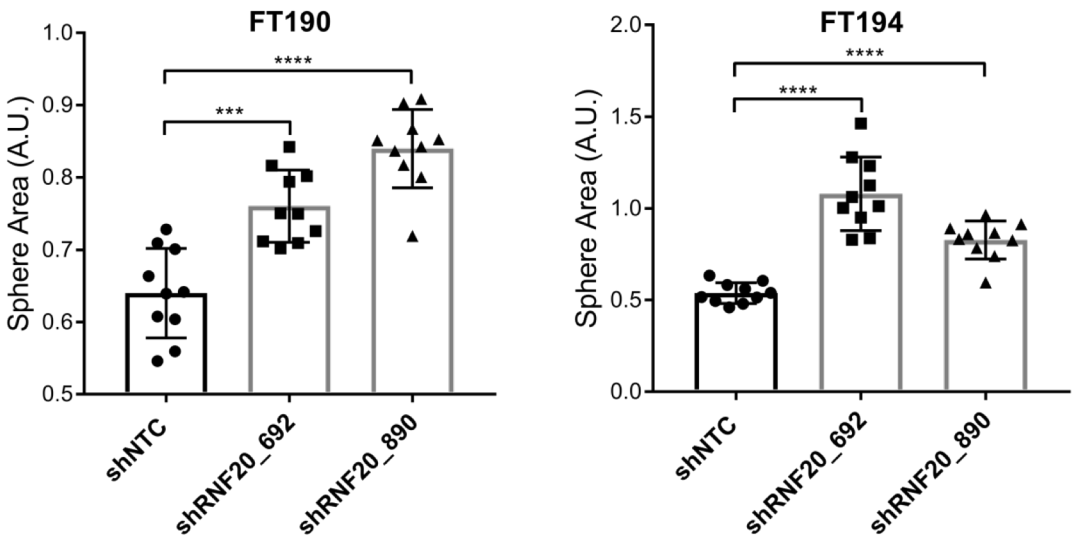
S2C



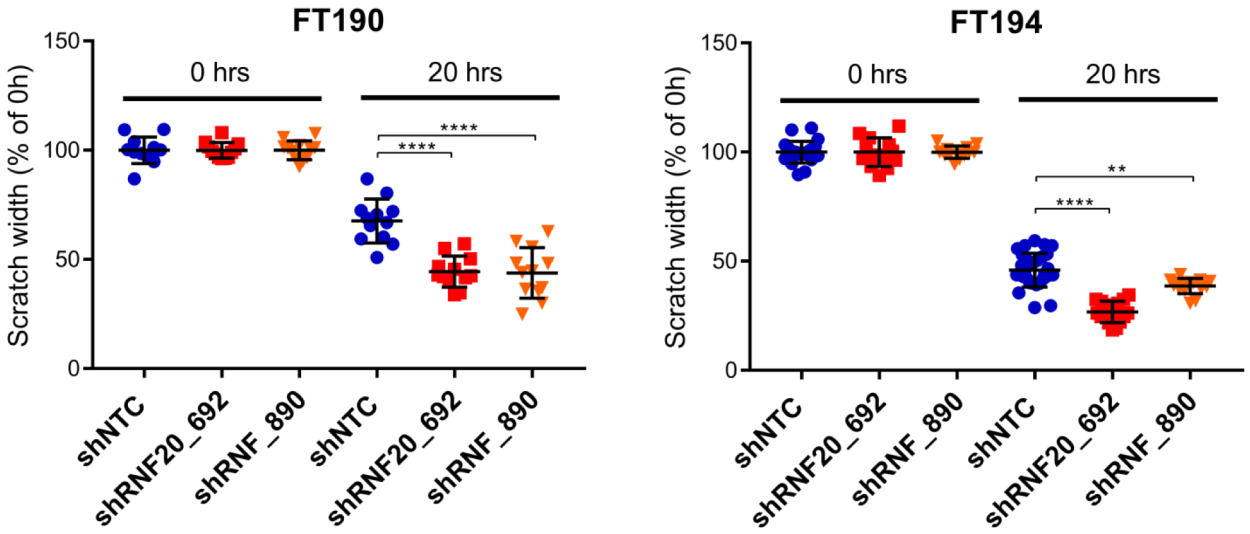
Supplementary Figure S2C. Immunohistochemical analysis of Total H2B and H2Bub1 levels in invasive high-grade serious ovarian cancer in the tissue samples from the same patient. Total H2B is ubiquitously expressed in cancer and normal cells. H2Bub1 is either absent or present at low levels in cancer cells, while scattered normal cells stained positive (all micrographs were imaged at 20X, scale bar = 20 μm).

Supplementary Figure 3 (Related to figure 3)

S3A



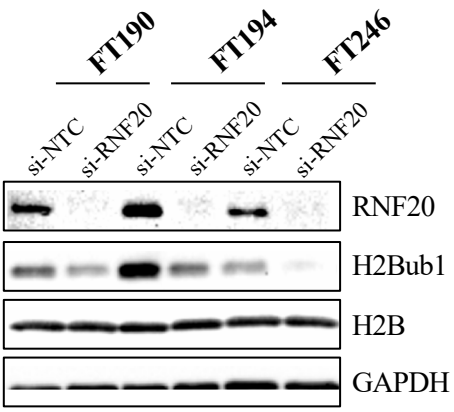
S3B



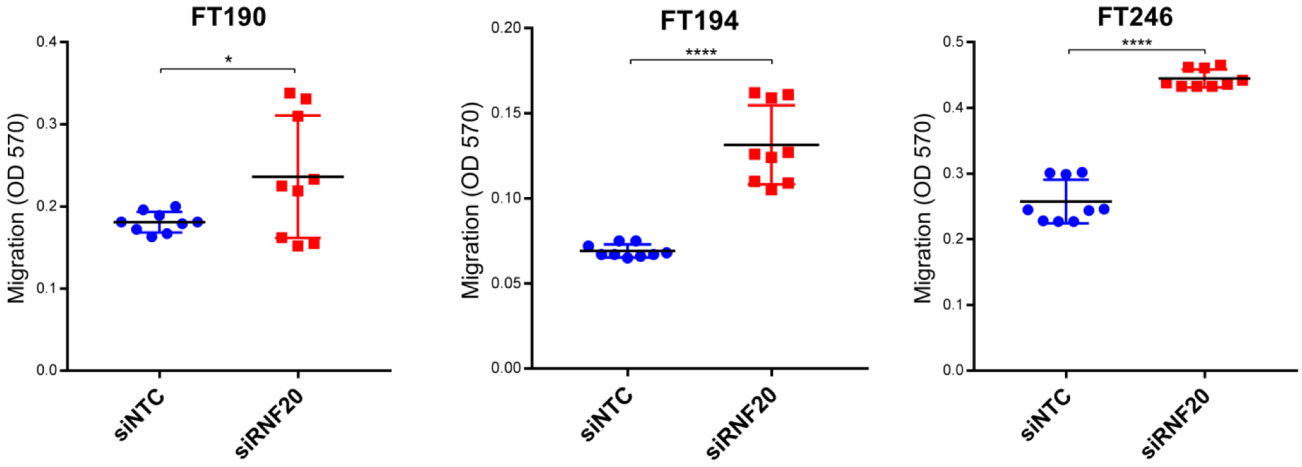
Supplementary Figure S3. RNF20 and H2Bub1 depletion escalates oncogenic behavior in FTSEC cell lines. (**S3A)** Hanging drop sphere formation assay showing modest increase in sphere forming ability of RNF20-depleted cells. (**S3B)** Scratch assay showing that RNF20 deficient cells have significantly higher rates of gap closing ability. A.U., Arbitrary unit. **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.

Supplementary Figure 4 (Related to figure 3 and figure 4)

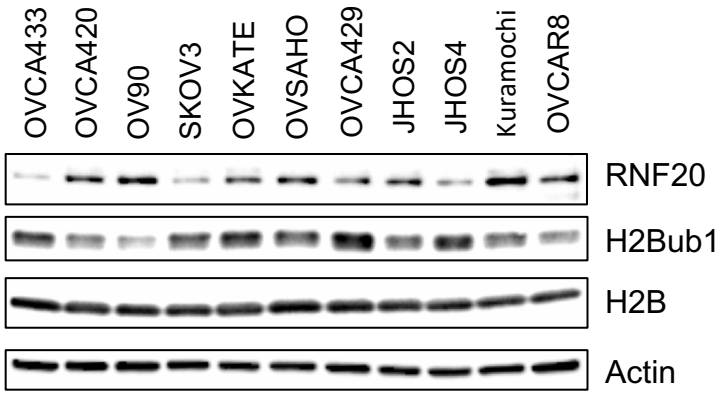
S4A



S4B



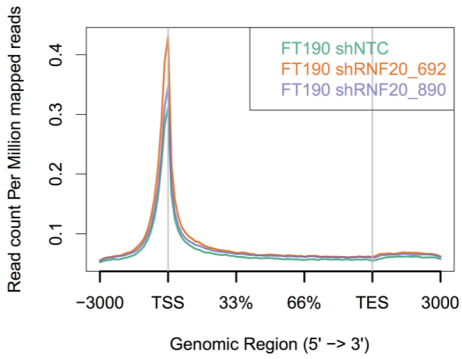
S4C



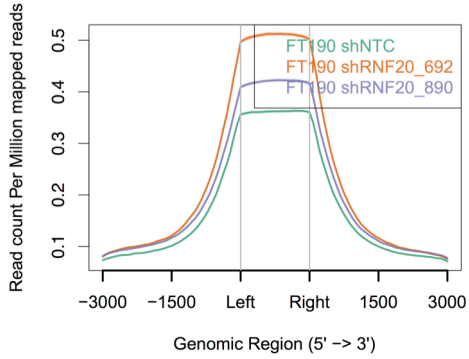
Supplementary Figure S4. RNF20 and H2Bub1 depletion escalates oncogenic behavior in FTSECs. RNF20 was silenced by pooled siRNAs in three FTSEC cell lines. (**S4A)** Western blot was used to validate RNF20 knockdown and the resulting H2Bub1 downregulation. (**S4B)** RNF20/H2Bub1 downregulation by using siRNA promoted trans-well migration in FTSEC cell lines. This data further confirms the observation that RNF20 knockdown promotes migration as observed in figure 3D by using stable hairpins to knockdown RNF20. **(S4C)** Levels of RNF20 and H2Bub1 in a panel of HGSOC cell lines. *, $p \leq 0.05$; ****, $p \leq 0.0001$.

Supplementary Figure 5 (Related to figure 5)

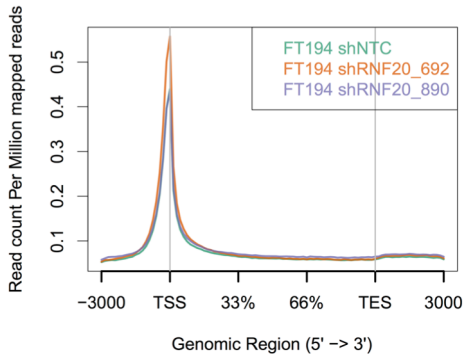
S5A



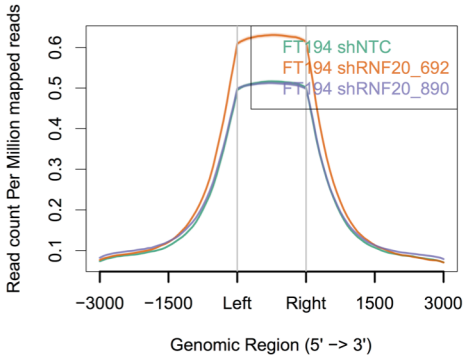
S5B



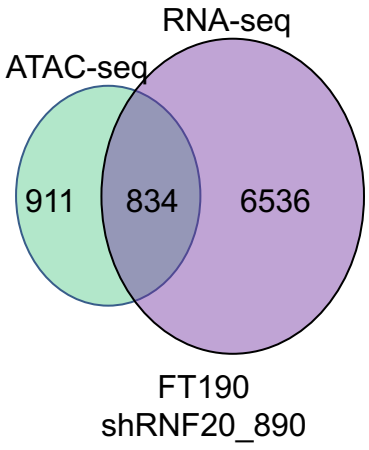
S5C



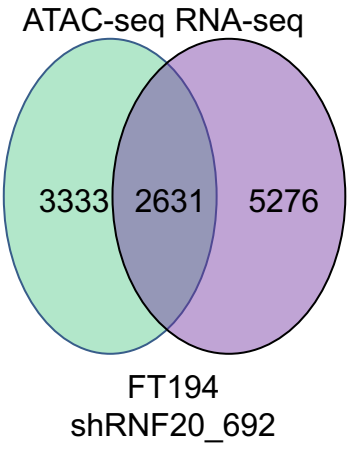
S5D



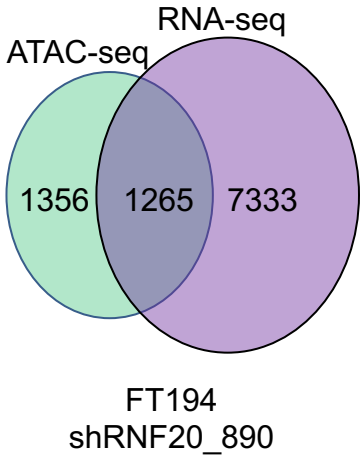
S5E



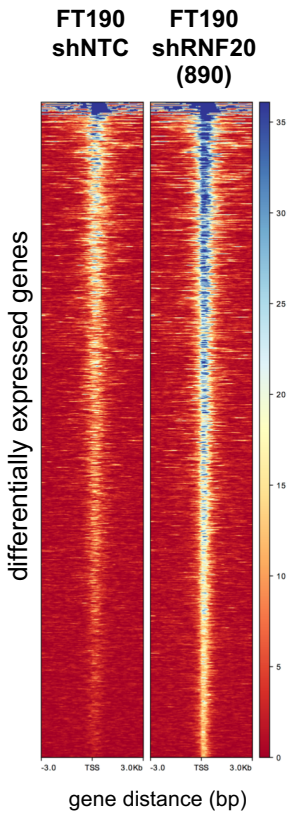
S5F



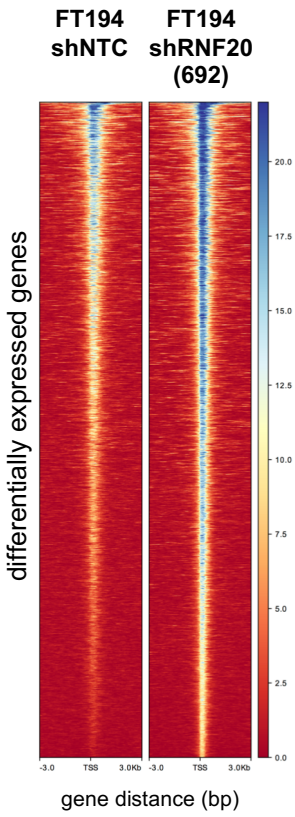
S5G



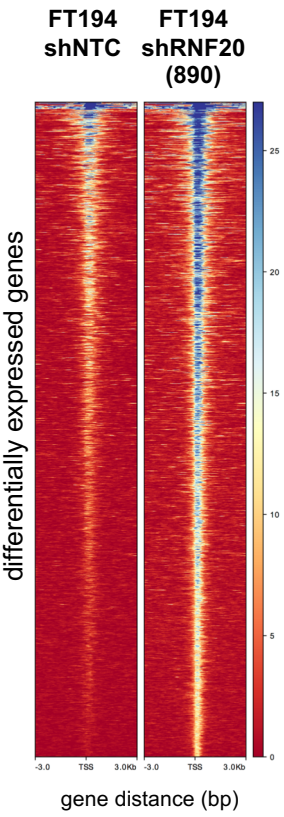
S5H



S5I



S5J

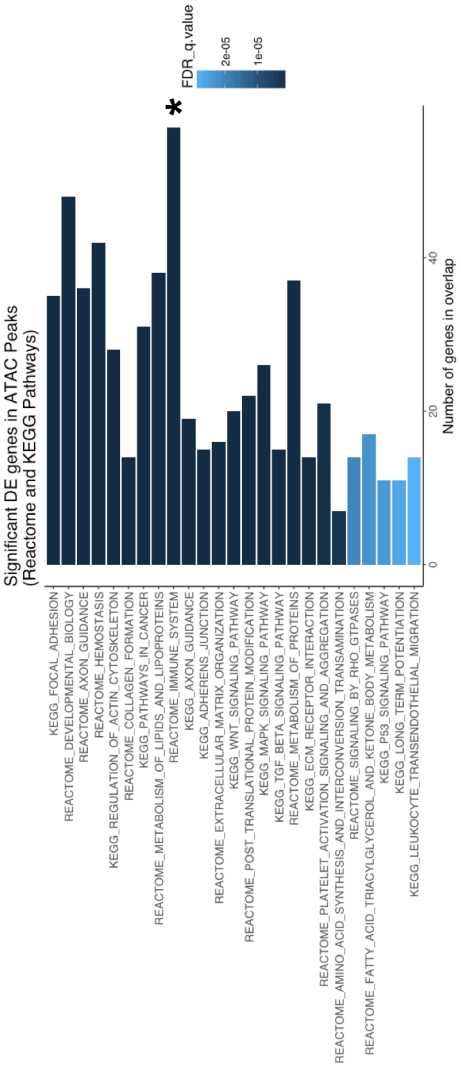


Supplementary Figure 5 (Related to figure 5)

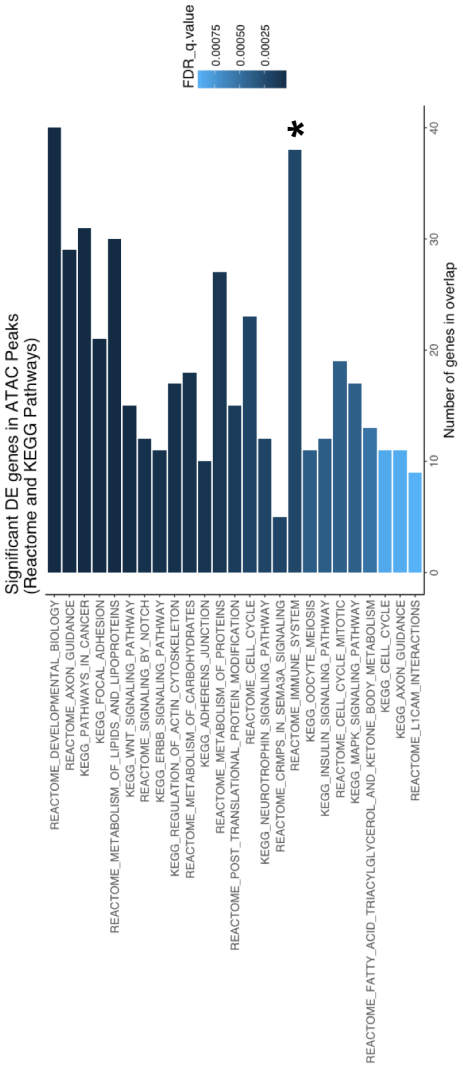
Supplementary Figure S5. RNF20-depletion changes chromatin accessibility and re-distributes open chromatin. **(S5A)** The distribution of ATAC-seq reads in FT190 shRNF20-depleted cells show enrichment around transcription start site and **(S5B)** known DNase I hypersensitivity sites as compared to control cells. **(S5C)** The distribution of ATAC-seq reads in FT194 shRNF20-depleted cells show enrichment around transcription start site and **(S5D)** known DNase I hypersensitivity sites as compared to control cells. **(S5E-S5G)** Overlap between ATAC-seq peaks and RNA-seq significantly differentially expressed genes. All overlap genes were upregulated in RNA-seq. **(S5H-S5J)** Heatmaps for the set of significantly differentially expressed genes within ATAC-seq peaks for control and RNF20-depleted FT190 and FT194 cells. ATAC-seq reads are centered on transcription start sites (TSS) with 3KB of flanking sequence. The color scale indicates an increase in read counts from red to blue, with darker blue representing a more open chromatin state.

Supplementary Figure 6 (Related to figure 5)

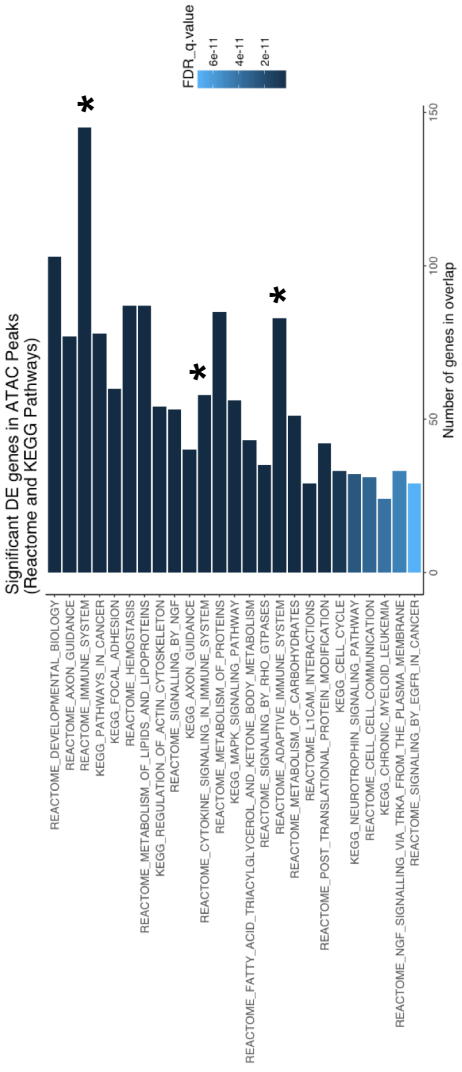
S6A (FT190 shRNF20_692)



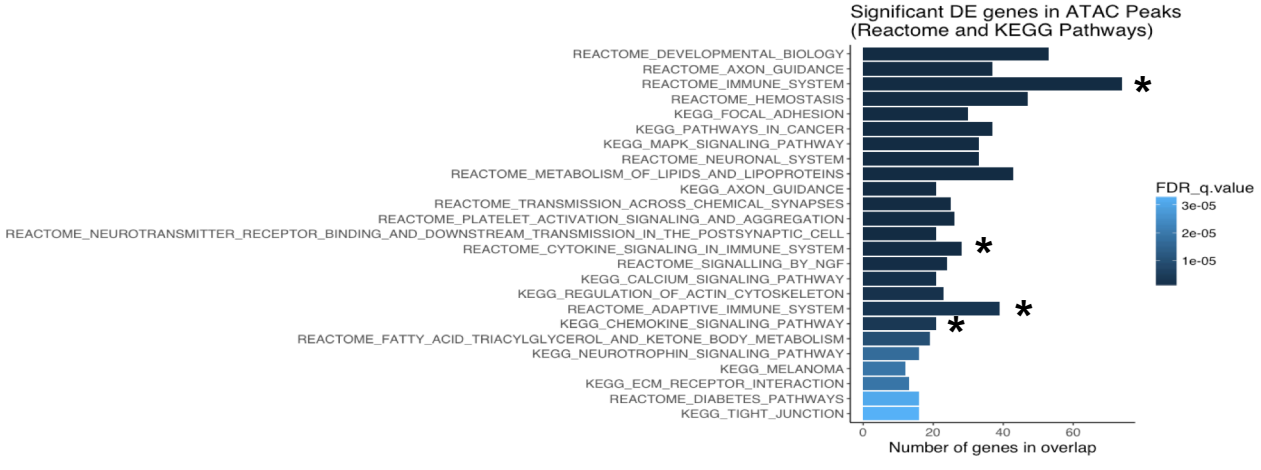
S6B (FT190 shRNF20_890)



S6C (FT194 shRNF20_692)



S6D (FT194 shRNF20_890)

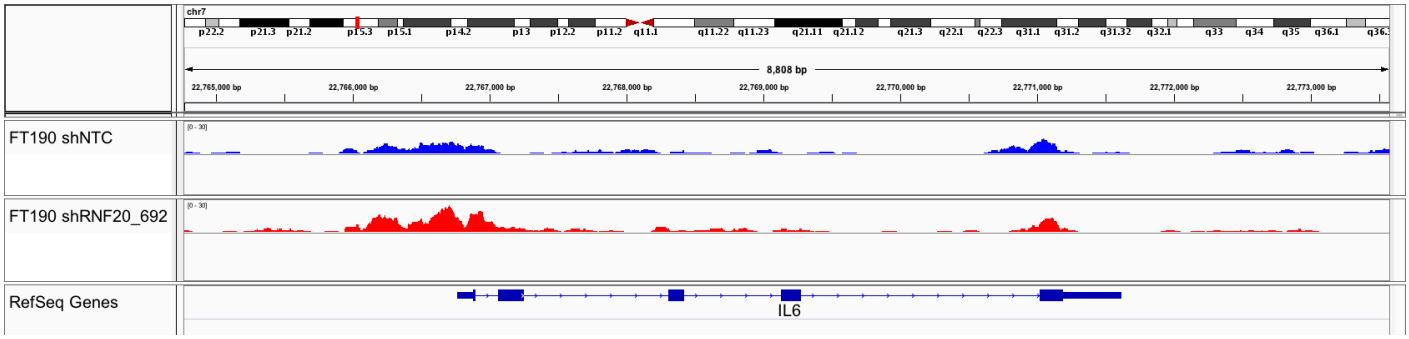


Supplementary Figure 6 (Related to figure 5)

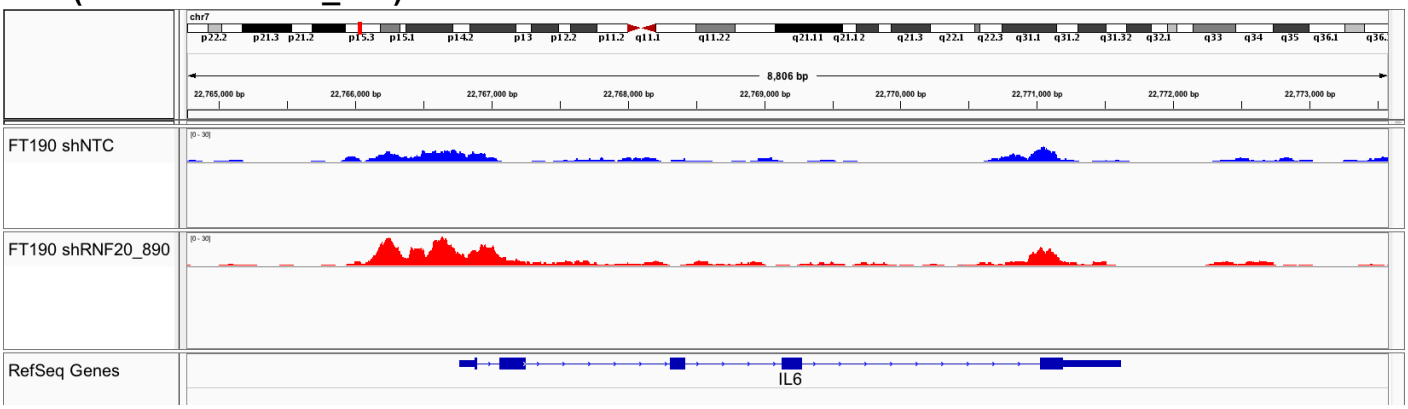
Supplementary Figure S6. RNF20-depletion changes chromatin accessibility and alters gene expression of immune signaling pathways in FTSEC cells. (**S6A-S6D)** MSigDB enrichment analysis of the set of significantly differentiated expressed RNA-seq genes within ATAC-seq peaks indicates significant enrichment for pathways related to immune system function. Reactome and KEGG pathways were tested and the top 25 most significant pathways are shown.

Supplementary Figure 7 (Related to figure 5)

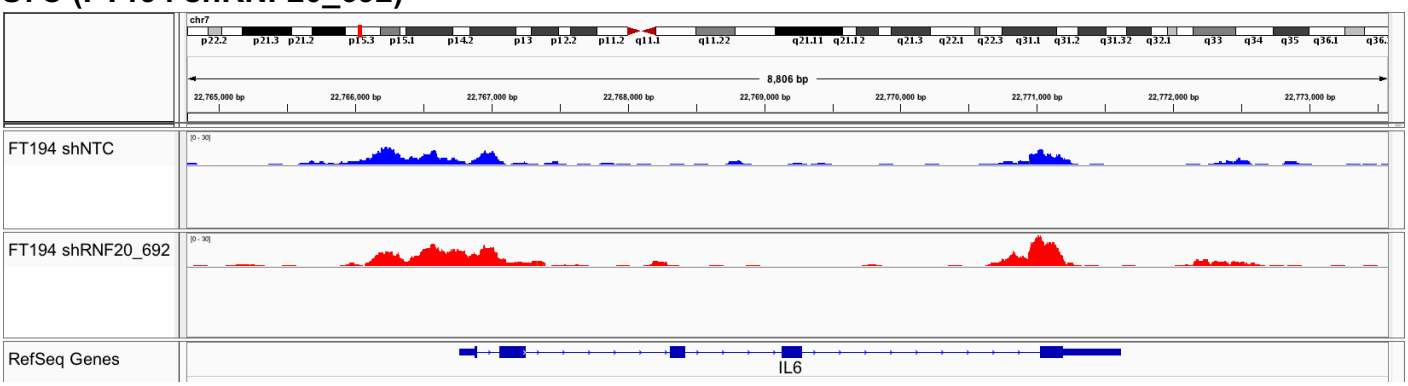
S7A (FT190 shRNF20_692)



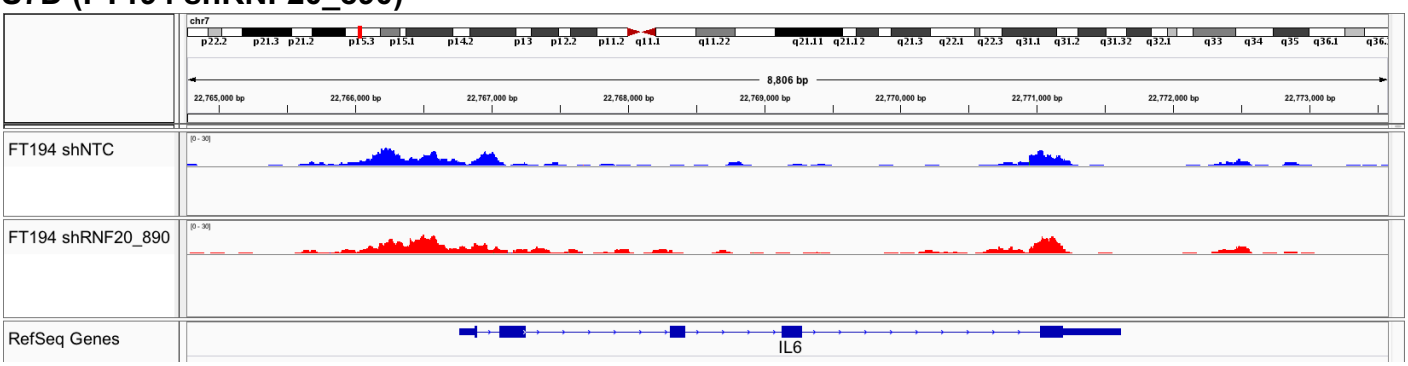
S7B (FT190 shRNF20_890)



S7C (FT194 shRNF20_692)



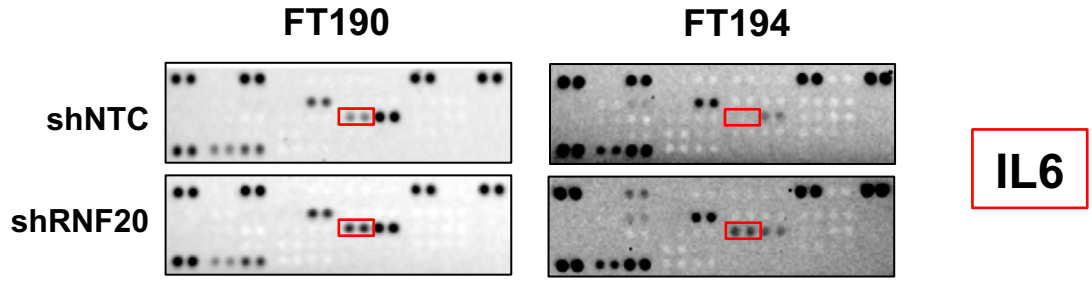
S7D (FT194 shRNF20_890)



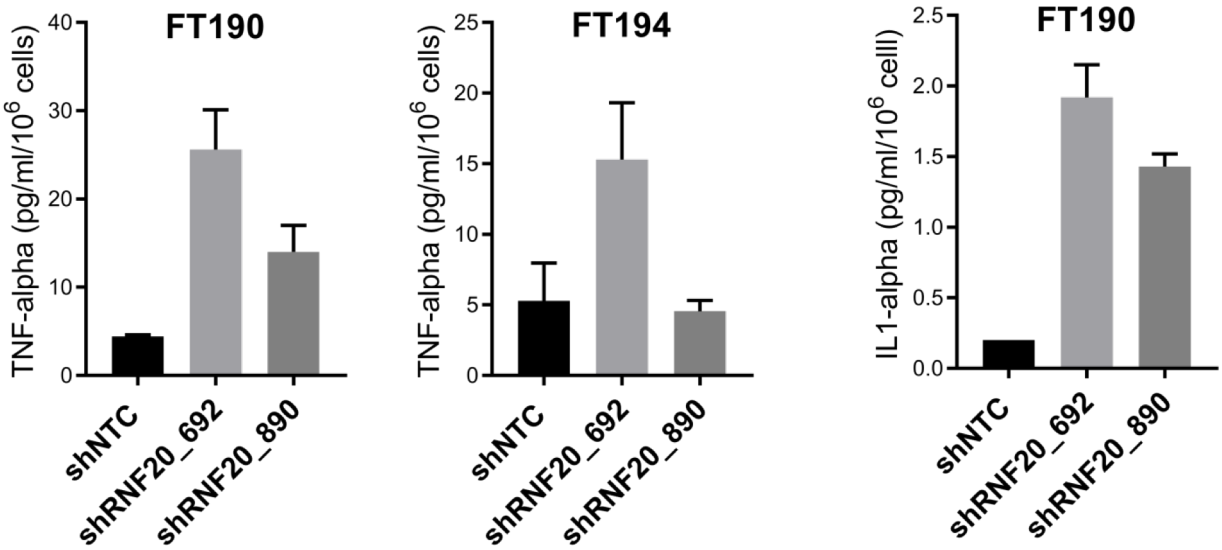
Supplementary Figure S7. RNF20-depletion changes chromatin accessibility and alters gene expression of cytokine IL6 in FTSEC cells. (**S7A-S7D)** IGV browser view of the IL6 gene region shows a greater number of ATAC-seq reads in the RNF20-depleted cells (red) as compared to control cells (blue). * pathways related to immune signaling.

Supplementary Figure 8 (Related to figure 6)

S8A



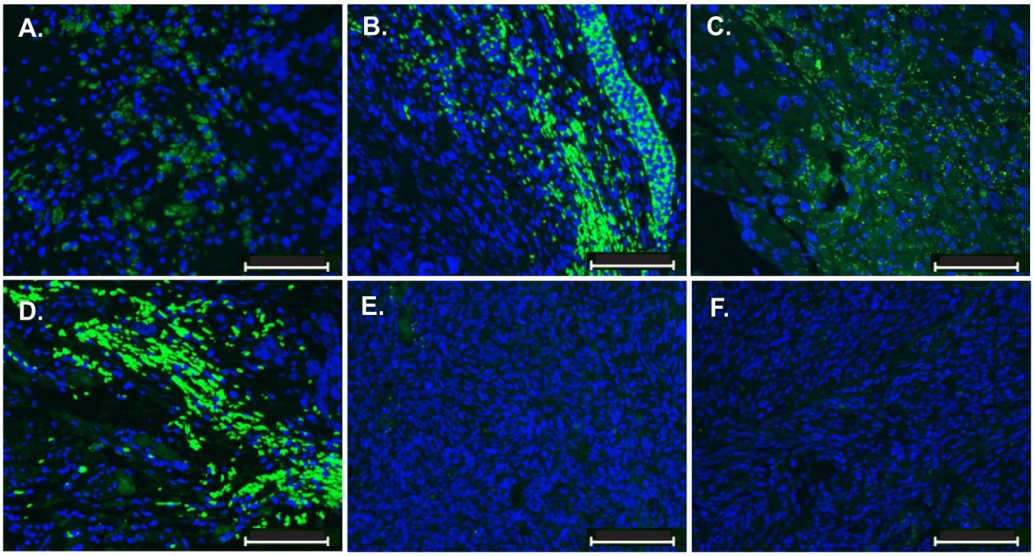
S8B



Supplementary Figure S8. RNF20 and H2Bub1 deficiency alters the levels of cytokines. (**S8A)** Images of the array related to figure 6A, identifies IL6 as the significantly altered cytokine. (**S8B)** ELISA assay shows an increase in the levels of TNFα and IL1α in RNF20 deficient cells.

Supplementary Figure 9

S9



Supplementary Figure S9. Representative images for RNA *in situ* hybridization of IL6 expression in FFPE tumor and normal tissue sections. Representative tissue sections of IL6 fluorescent FITC (green) probe were merged with respective DAPI (blue) staining. **(A)** Weak IL6 staining; weak H2Bub1 tumor lesion. **(B-D)** High IL6 staining; H2Bub1 negative tumor lesion. **(E-F)** Negative IL-6 staining; ovary control tissue and uterus control tissue, respectively. Magnification=20x; Scale bar=100μm.

Supplementary Table 1. Antibody information and staining conditions for immunohistochemistry (IHC)

Protein	Source	Catalog #	Clone	IHC Dilution (Incubation time)	Positive control (IHC)
p53 (TP53)	Santa Cruz Biotechnology	SC-126	DO-1 mouse monoclonal	1:1200 (40 min)	colon cancer
RNF20	Bethyl Laboratories	A300-715-M	Rabbit polyclonal		
RNF40	Cell Signaling Technologies	12187	D2R20 rabbit monoclonal		
H2Bub1	Millipore-Upstate	05-1312	Clone 56 mouse monoclonal	1:200 (40 min)	adjacent normal FTE
H2Bub1	Cell Signaling Technologies	5546	Rabbit monoclonal		
Total H2B	Cell Signaling Technologies	12364	Rabbit monoclonal		
GAPDH	Cell Signaling Technologies	5174	D16H11 Rabbit monoclonal		
Human/Primate IL6 Antibody Neutralizing Ab	R&D Systems	MAB206-500	6708 Monoclonal mouse IgG_1		
Mouse IgG_1 Isotype Control Antibody	R&D Systems	MAB002	11711 Monoclonal mouse IgG_1		