Enhancer landscape of lung carcinoids reveals sensitivity to FGF signaling inhibition

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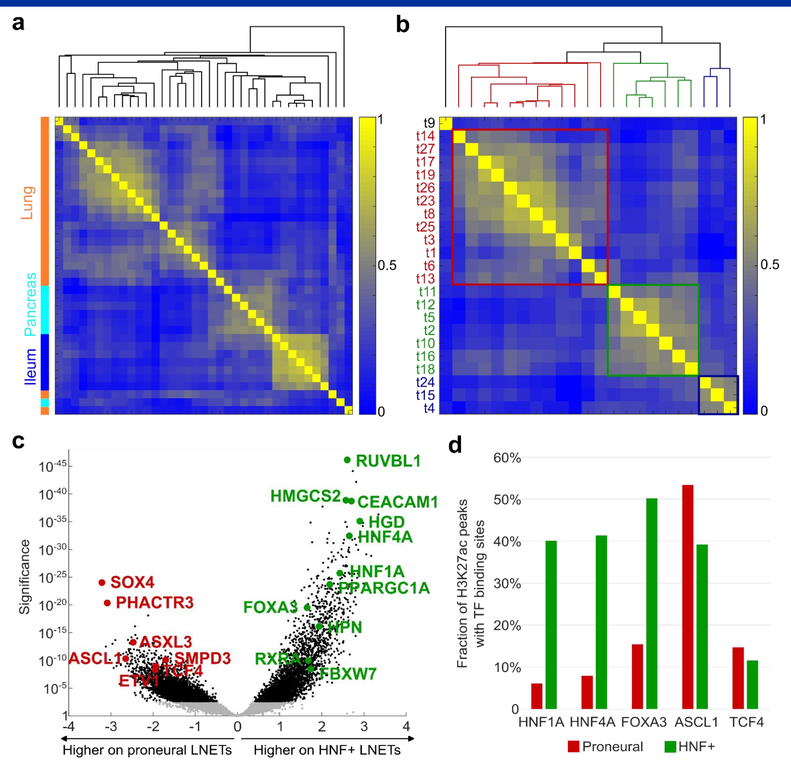
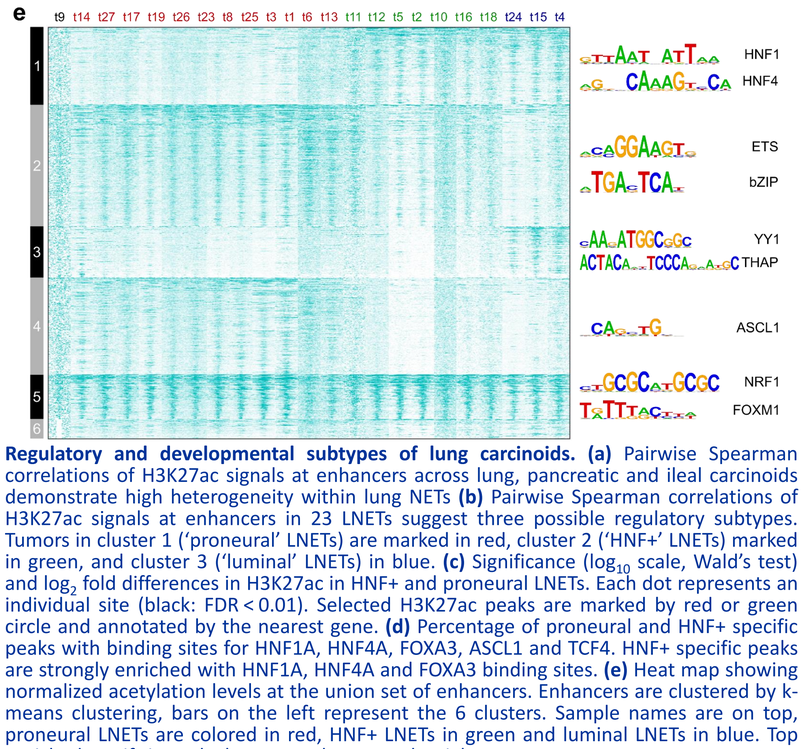
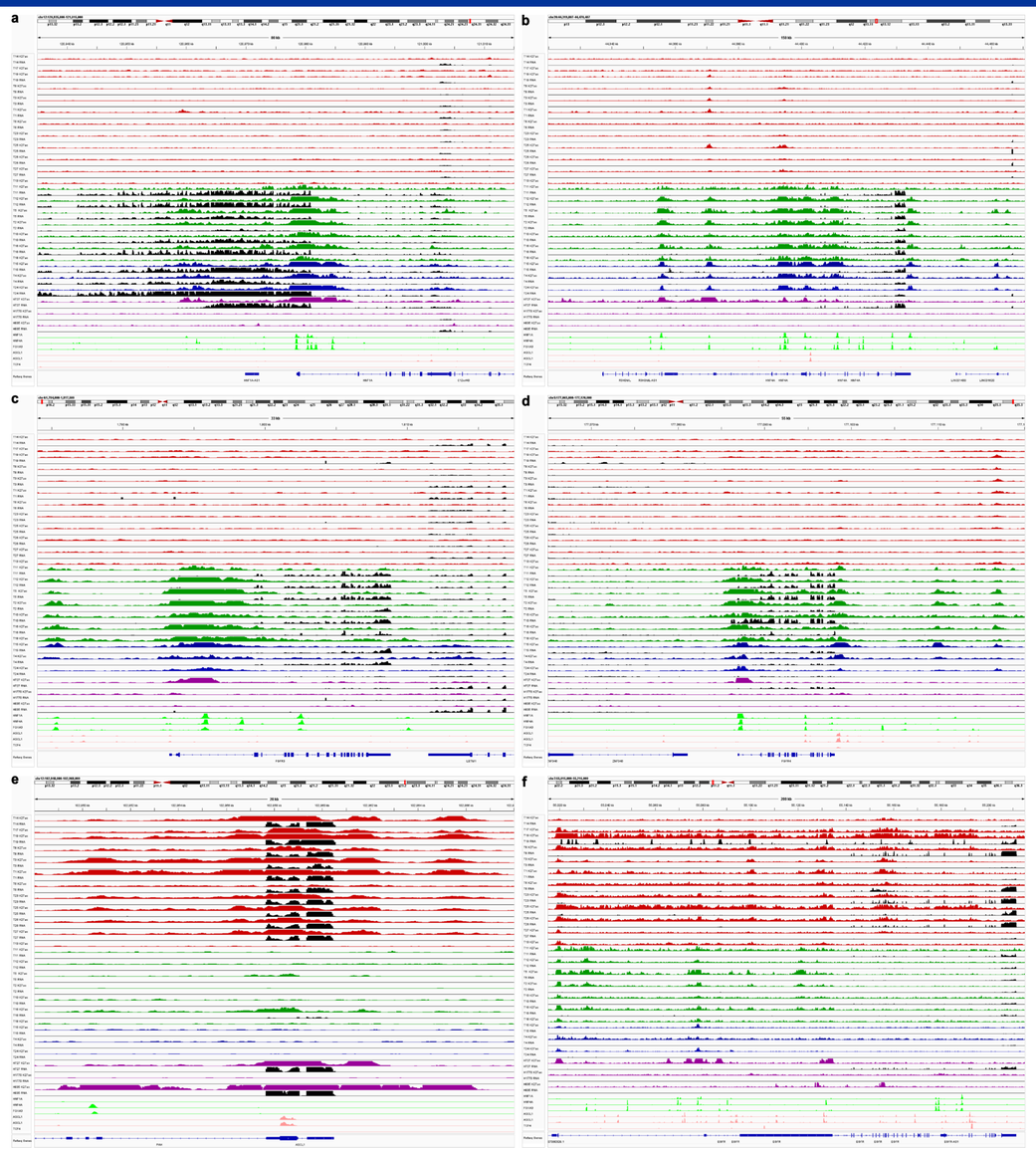
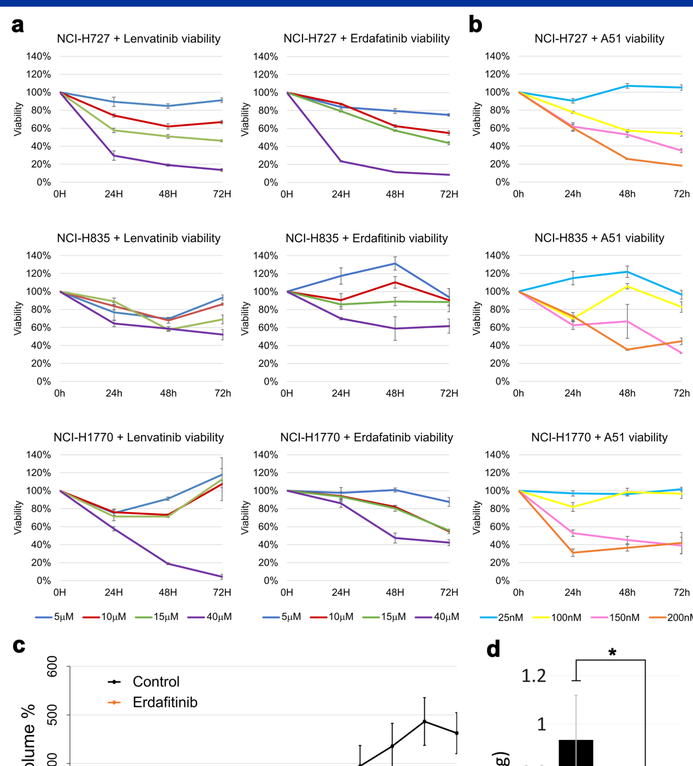


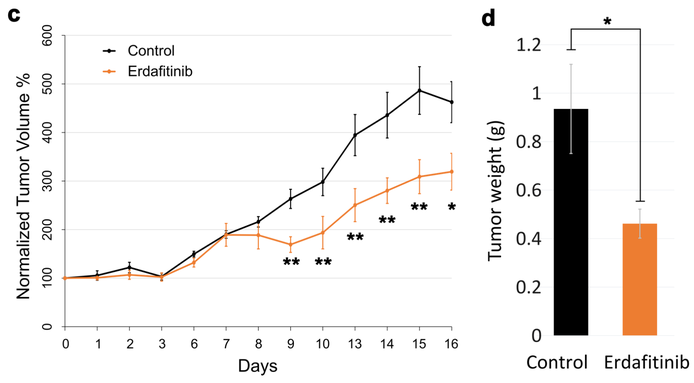


Regulatory and developmental subtypes of lung carcinoids. (a) Pairwise Spearman correlations of H3K27ac signals at enhancers across lung, pancreatic and ileal carcinoids demonstrate high heterogeneity within lung NETs **(b)** Pairwise Spearman correlations of H3K27ac signals at enhancers in 23 LNETs suggest three possible regulatory subtypes. Tumors in cluster 1 ('proneural' LNETs) are marked in red, cluster 2 ('HNF+' LNETs) marked in green, and cluster 3 ('luminal' LNETs) in blue. **(c)** Significance (log_{10} scale, Wald's test) and log_2 fold differences in H3K27ac in HNF+ and proneural LNETs. Each dot represents an individual site (black: FDR < 0.01). Selected H3K27ac peaks are marked by red or green circle and annotated by the nearest gene. **(d)** Percentage of proneural and HNF+ specific peaks with binding sites for HNF1A, HNF4A, FOXA3, ASCL1 and TCF4. HNF+ specific peaks are strongly enriched with HNF1A, HNF4A and FOXA3 binding sites. **(e)** Heat map showing normalized acetylation levels at the union set of enhancers. Enhancers are clustered by k-means clustering, bars on the left represent the 6 clusters. Sample names are on top, proneural LNETs are colored in red, HNF+ LNETs in green and luminal LNETs in blue. Top enriched motifs in each cluster are shown on the right.



HNF1A, HNF4A, FGFR3 and FGFR4 demonstrate strong enhancer acetylation and expression specifically at HNF+ LNETs, while the ASCL1 and EGFR locus is preferentially acetylated in proneural LNETs. (a-e) Genomic view of the **(a)** HNF1A, **(b)** HNF4A, **(c)** FGFR3, **(d)** FGFR4, **(e)** ASCL1, and **(e)** EGFR loci, showing RNA signal (black) and H3K27ac signals of proneural (red), HNF+ (green) and luminal (blue) lung neuroendocrine tumors, as well as the NCI-H727, NCI-H1770 and NCI-H835 lung neuroendocrine neoplasms cell lines (purple). Hepatic factors (HNF1A, HNF4A and FOXA3) ChIP-seq tracks in HepG2 cells are shown in light green and proneural transcription factors (ASCL1 and TCF4) ChIP-seq tracks in neuroblastoma cells in light red. RNA-seq signals are normalized to 5 bins per million mapped reads (BPM). H3K27ac signals are scaled by promoter-based DESeq2 normalization. Multiple HNF1, HNF4 and FOXA3 binding sites are found at FGFR3 and FGFR4 enhancers and promoters, suggesting FGFR expression is downstream of hepatic factors activation.





Erdafitinib significantly slows tumor growth. (a) Relative cell line viability by WST-1 assay of H727, H835 and H1770 lines during treatment with Lenvatinib (left) or Erdafitinib (right). Values represent ratio between viability of treated and control cells (DMSO). Error bars represent SEM. **(b)** Relative cell line viability during treatment with A51. Values represent viability ratio to DMSO control. Error bars represent SEM. **(c)** Normalized tumor volume of H727 mouse xenograft treated with Erdafitinib (12.5mg/kg, orange) or PBS 1% tween control (black). Error bars represent SEM. P-values are calculated by one tail t-test. **(d)** Average tumor weight of the PBS control group (black) and Erdafitinib treated group (orange) at the end of the experiment after mice were sacrificed. Error bars represent SEM. P-values are calculated by one tail t-test. * $p < 0.05$. ** $p < 0.01$

For more details see our preprint at https://doi.org/10.1101/2023.11.17.566871 and our website at http://yotamdrier.ekmd.huji.ac.il/ or contact yotam.drier@mail.huji.ac.il

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