

SUPPLEMENTARY INFORMATION

FOR

Molecular characterization of a marine turtle tumor epizootic, profiling external, internal and post-surgical regrowth tumors

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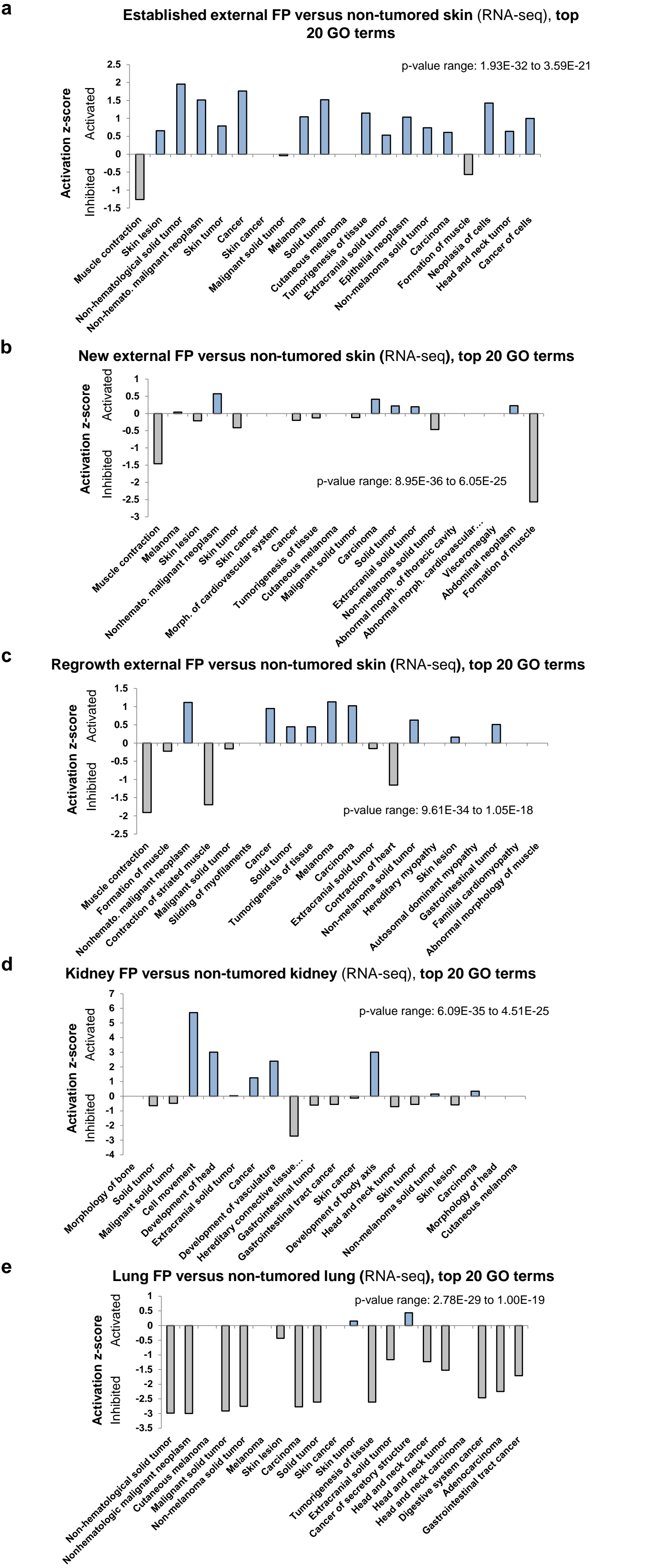
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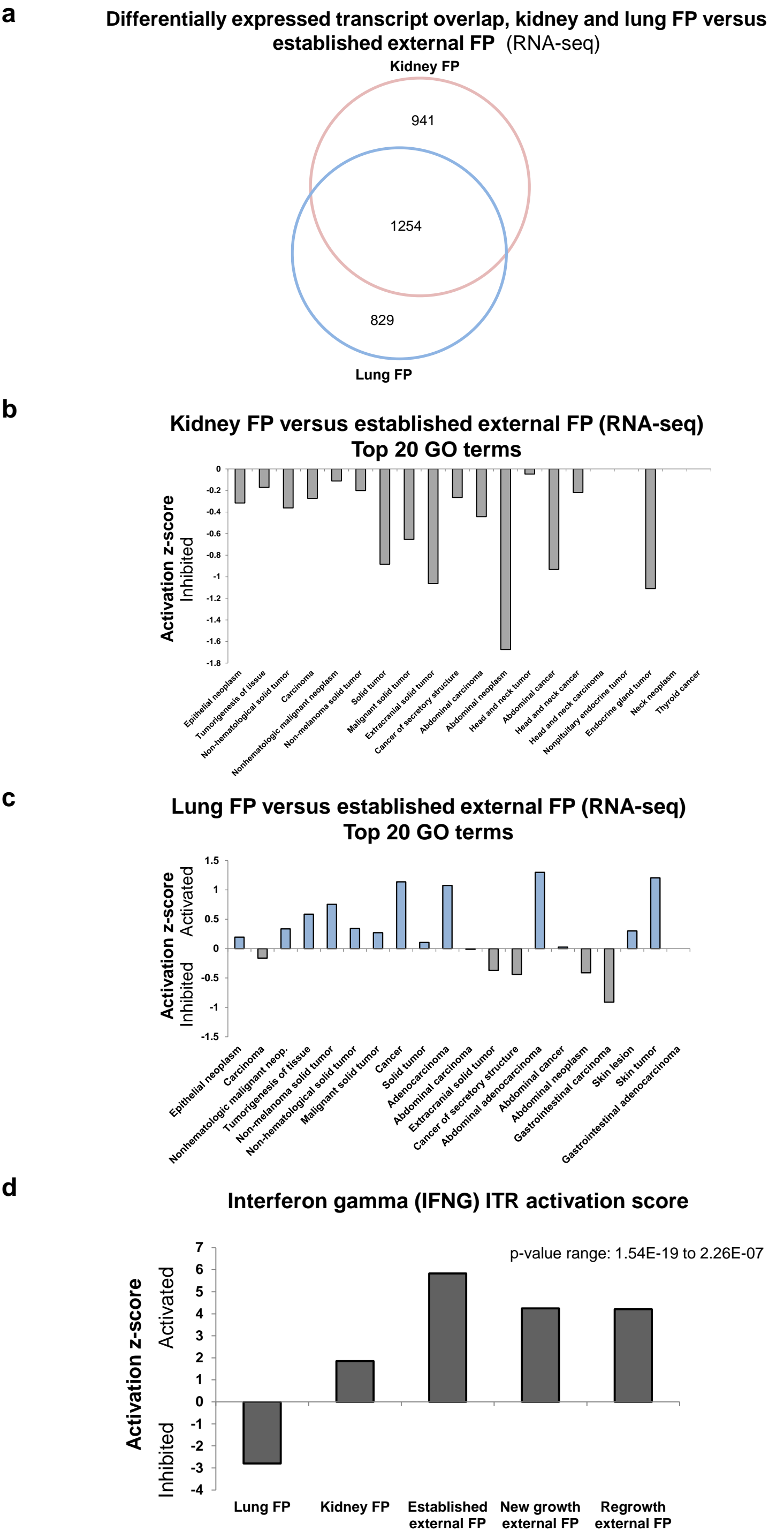
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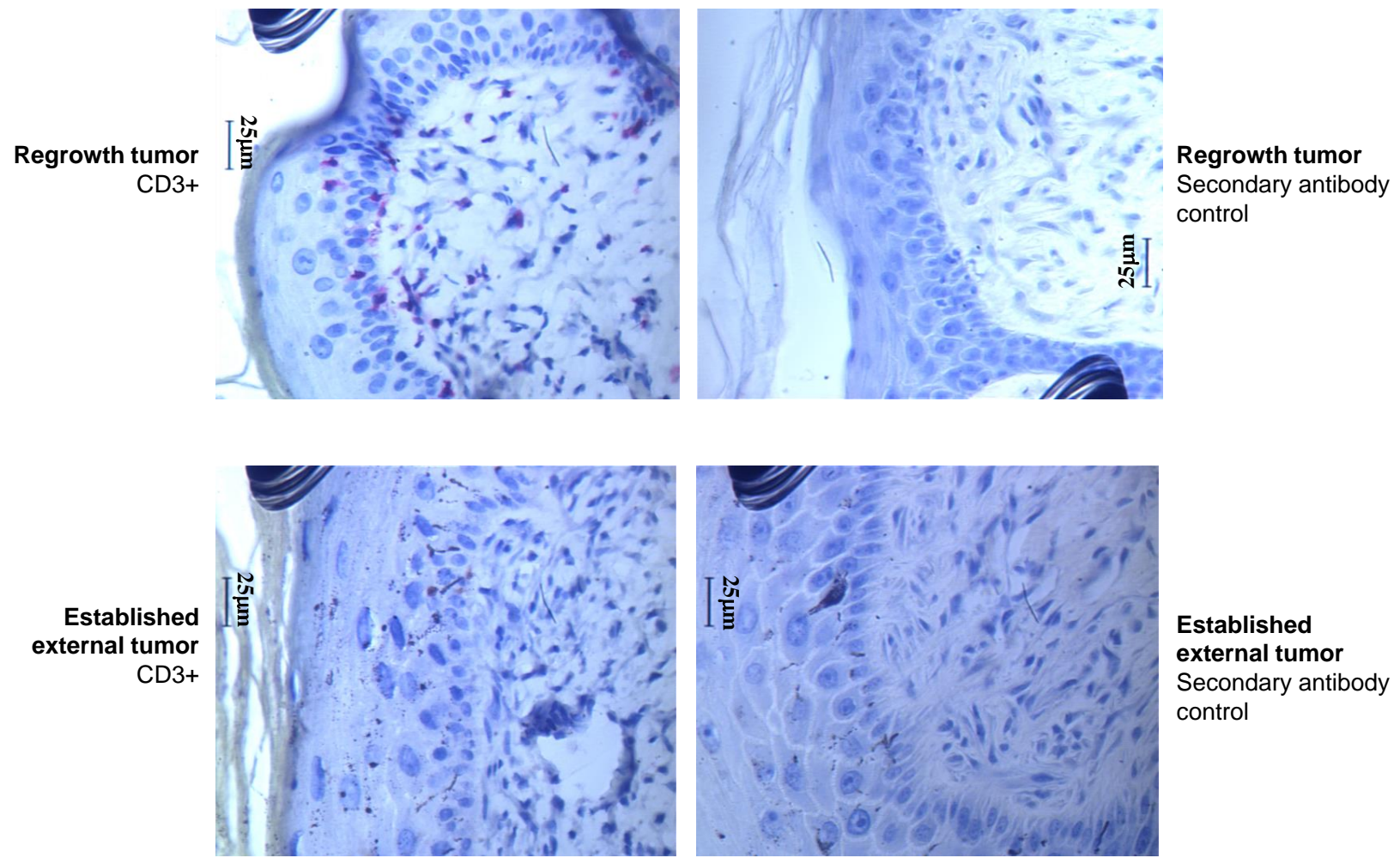


Supplemental Figure 1. Additional activation/inhibition z-scores of the top 20 disease-associated GO terms associated with transcripts differentially expressed in different types of fibropapillomatosis tumors (RNA-seq), as detected by IPA, ranked by *P*-value (calculated by right-tailed Fisher's Exact Test, with Benjamini-Hochberg correction). **a)** Established external FP versus non-tumored skin. A total of 689 of the significant DEs were recognized by IPA and used in the analysis. **b)** New external FP versus non-tumored skin. A total of 698 of the significant DEs were recognized by IPA and used in the analysis. **c)** Regrowth external FP versus non-tumored skin. A total of 507 of the significant DEs were recognized by IPA and used in the analysis. **d)** Kidney FP versus non-tumored kidney tissue. A total of 618 of the significant DEs were recognized by IPA and used in the analysis. **e)** Lung FP versus non-tumored lung tissue. A total of 653 of the significant DEs were recognized by IPA and used in the analysis.



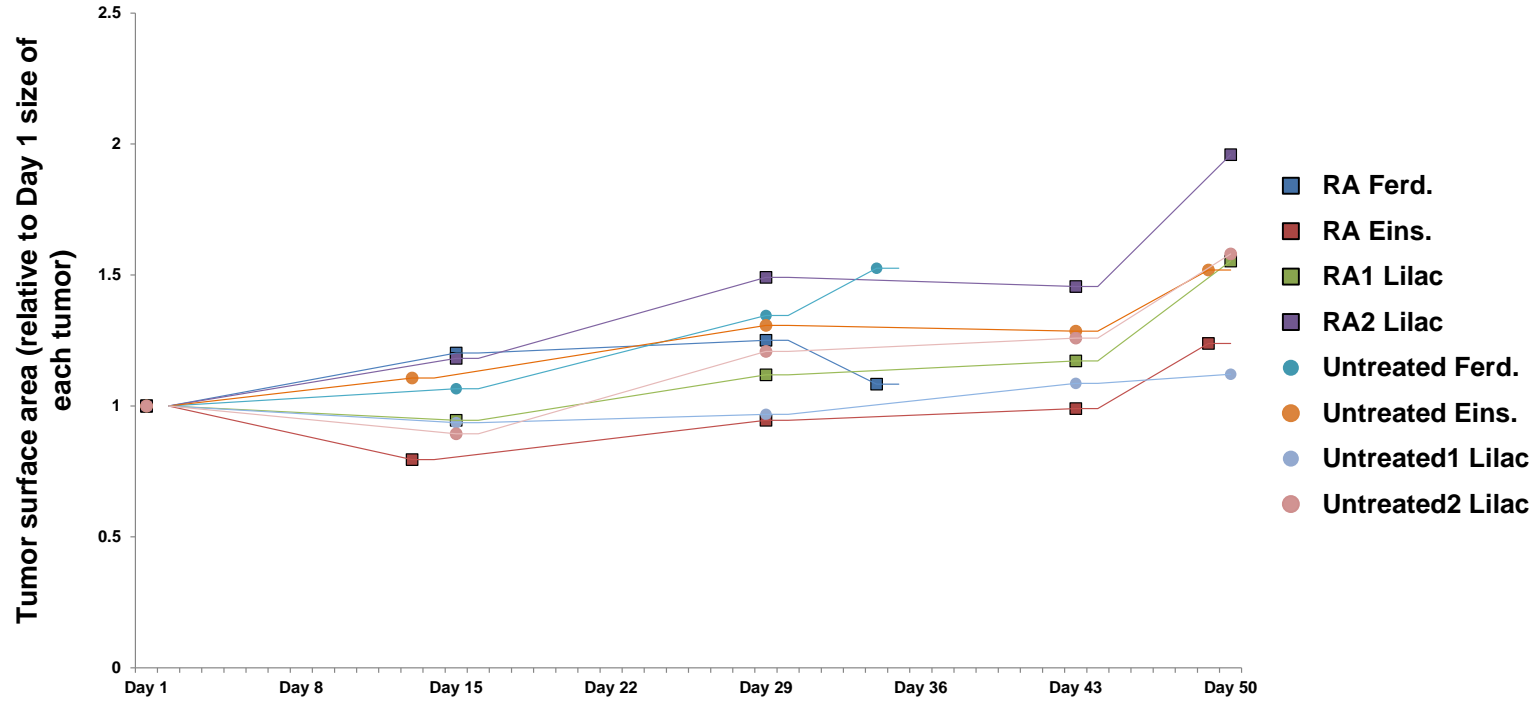
Supplemental Figure 2. Additional fibropapillomatosis transcriptomic comparisons. **a)** Overlap of transcripts from RNA-seq data significantly differentially expressed (DE) (as called by DESeq2) in fibropapillomatosis between either kidney FP compared to established external FP, or lung FP compared to established external FP. Transcripts were considered significant if passing the following cut-offs: adjusted *P*-value of < 0.05 and log₂ fold change of > 2 and < -2. **b, c)** Activation/inhibition z-scores of the top 20 disease-associated gene ontology (GO) terms associated with transcripts differentially expressed in different types of fibropapillomatosis tumors (RNA-seq), as detected by IPA, ranked by *P*-value (calculated by right-tailed Fisher's Exact Test, with Benjamini-Hochberg correction). **b)** Kidney FP versus established external FP tumors. **c)** Lung FP versus established external FP tumors. **d)** Activation z-scores of the Interferon gamma (IFNG) inferred transcriptional regulator (ITR) associated with transcripts differentially expressed across the five different types of fibropapillomatosis tumors, when compared to their respective non-tumor tissue sources.

a

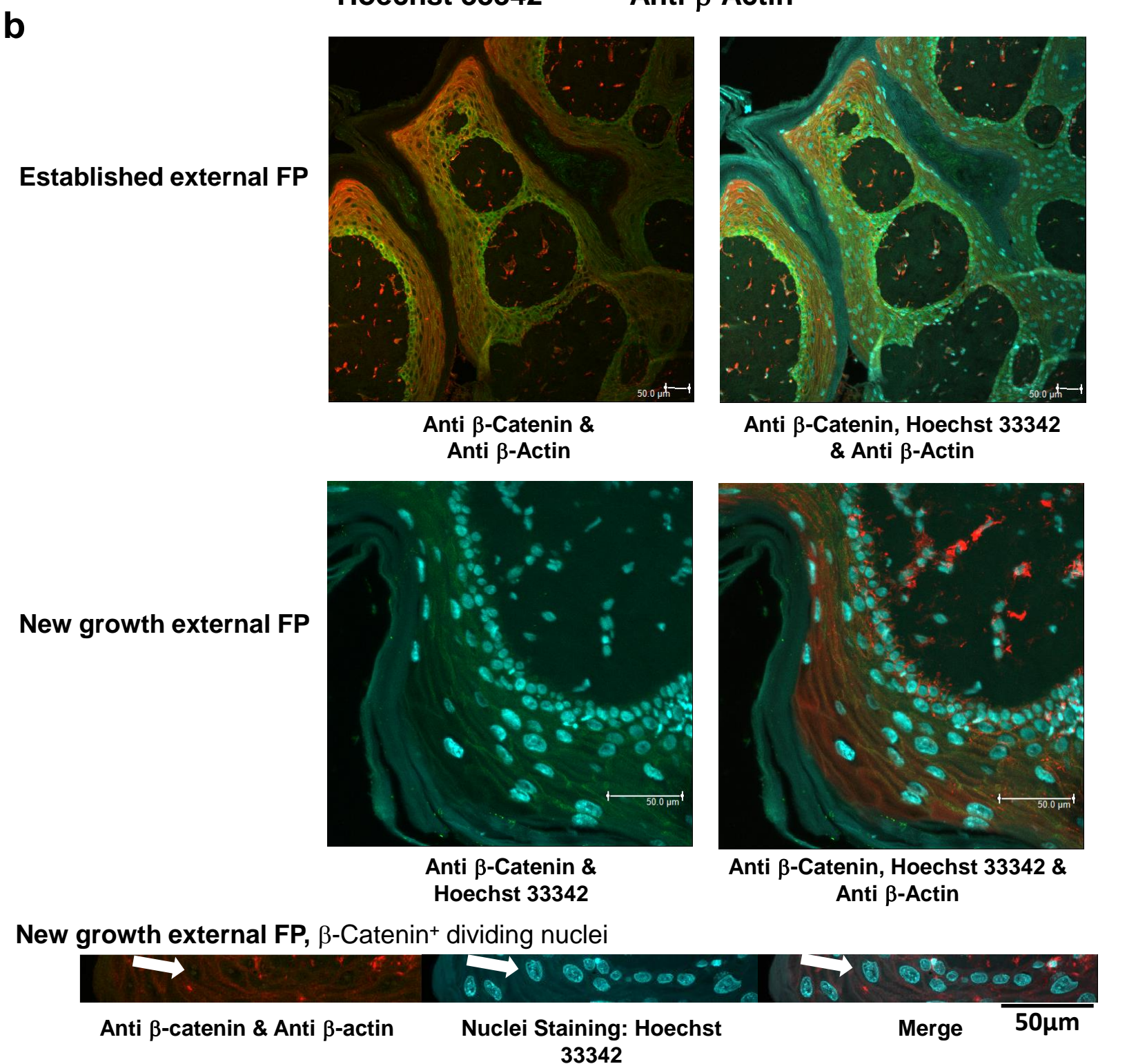
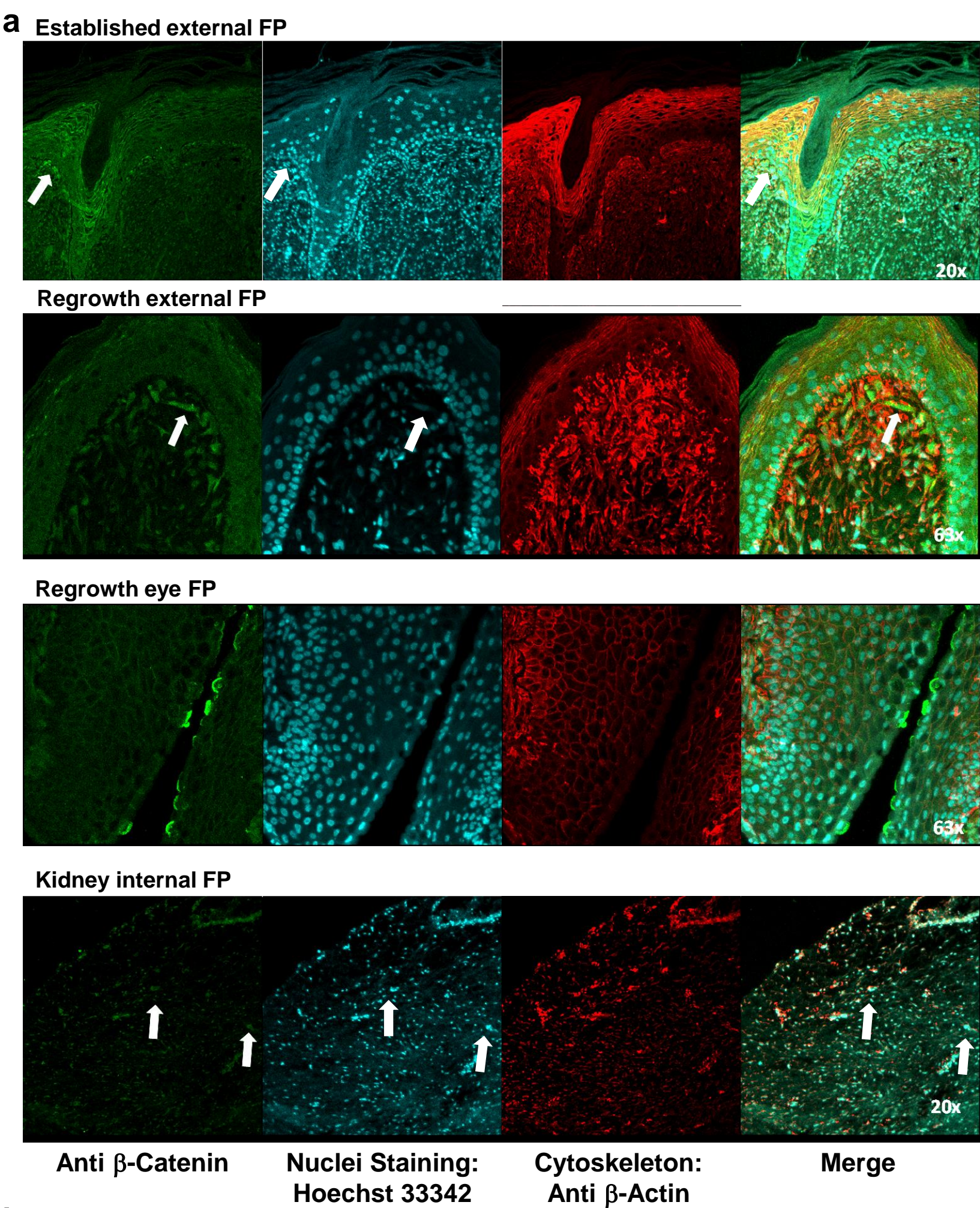


b

Time-course of retinoic acid (RA) treated and untreated FP tumors



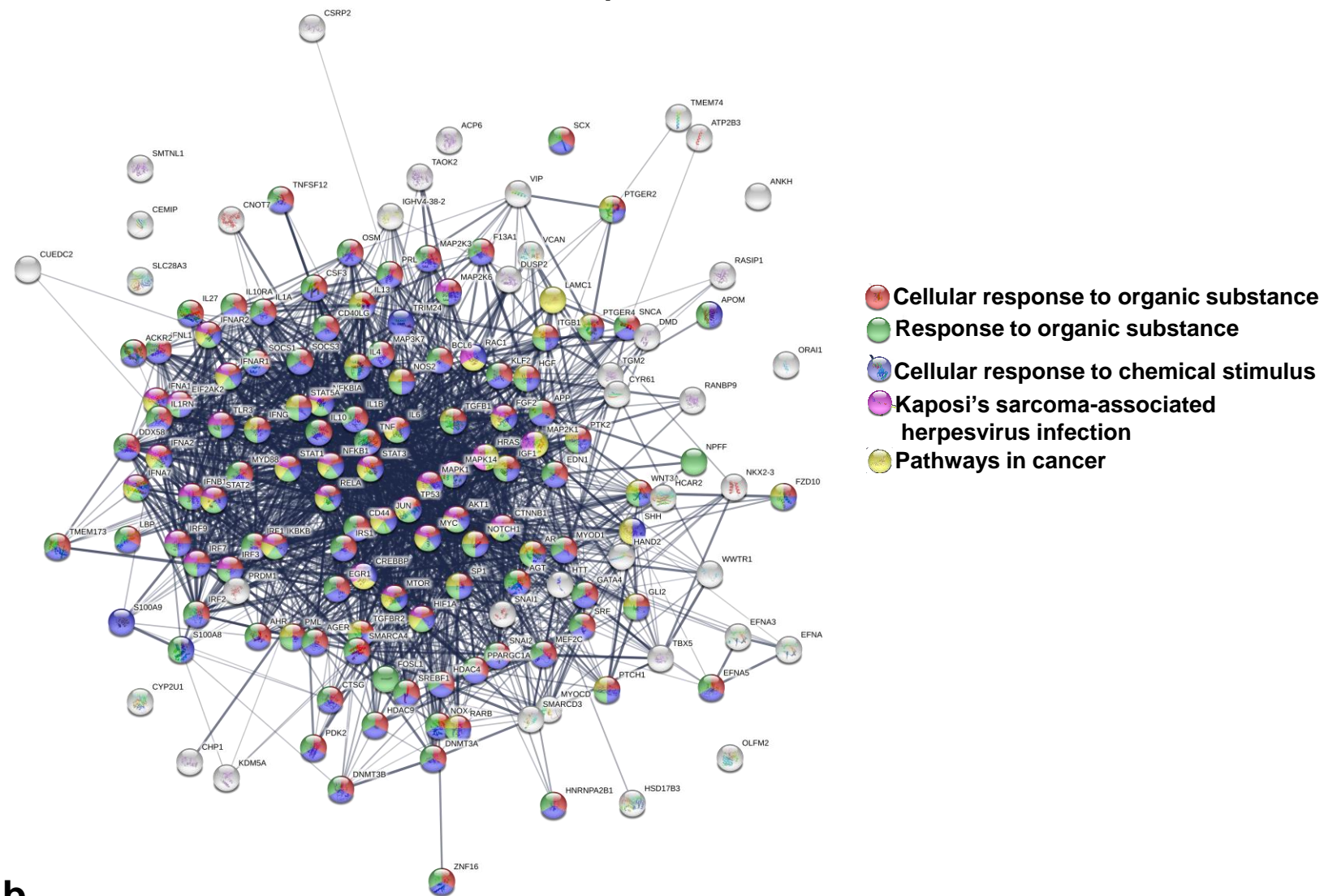
Supplemental Figure 3. Additional CD3 staining and RA treatment tumor growth profiling. **a)** CD3 antibody-based staining (red/purple) of T lymphocyte infiltration in an established external tumor and regrowth external tumor, nuclei are counterstained with Hematoxylin (blue staining). **b)** Time-course of relative tumor growth profiles of retinoic acid (RA, Tretinoin cream 0.1%) treated and untreated fibropapillomatosis tumors. Profiles for eight tumors across three individual *C. mydas* patients are shown. Duration of treatment was under veterinary determination. Patient ‘name’ abbreviations, Ferd., ‘Ferdinand’ (07-2018-Cm), Eins., ‘Einstein’ (28-2018-Cm) and ‘Lilac’ (25-2018-Cm). Tumor growth is relative to the size of each individual tumor before treatment, i.e. Day 1.



Supplemental Figure 4. Additional fibropapillomatosis tumor immunohistochemistry. **a)** Antibody-based immunohistochemistry of established external, regrowth external, regrowth eye external and kidney internal tumor tissue. Tissue sections are stained for β -catenin (anti- β -catenin antibody) and counter stained with Hoechst 33342 to visualize nuclei and Anti β -actin. Selected cells with nuclear (activated) β -catenin staining are indicated by white arrows. **b)** Established external and new growth tumor sections, stained for β -catenin (anti- β -catenin antibody) and counter stained for Hoechst 33342 and Anti β -actin, highlighting the membrane localization of β -catenin in epidermal tumor cells, top, and dividing nuclei localization, bottom.

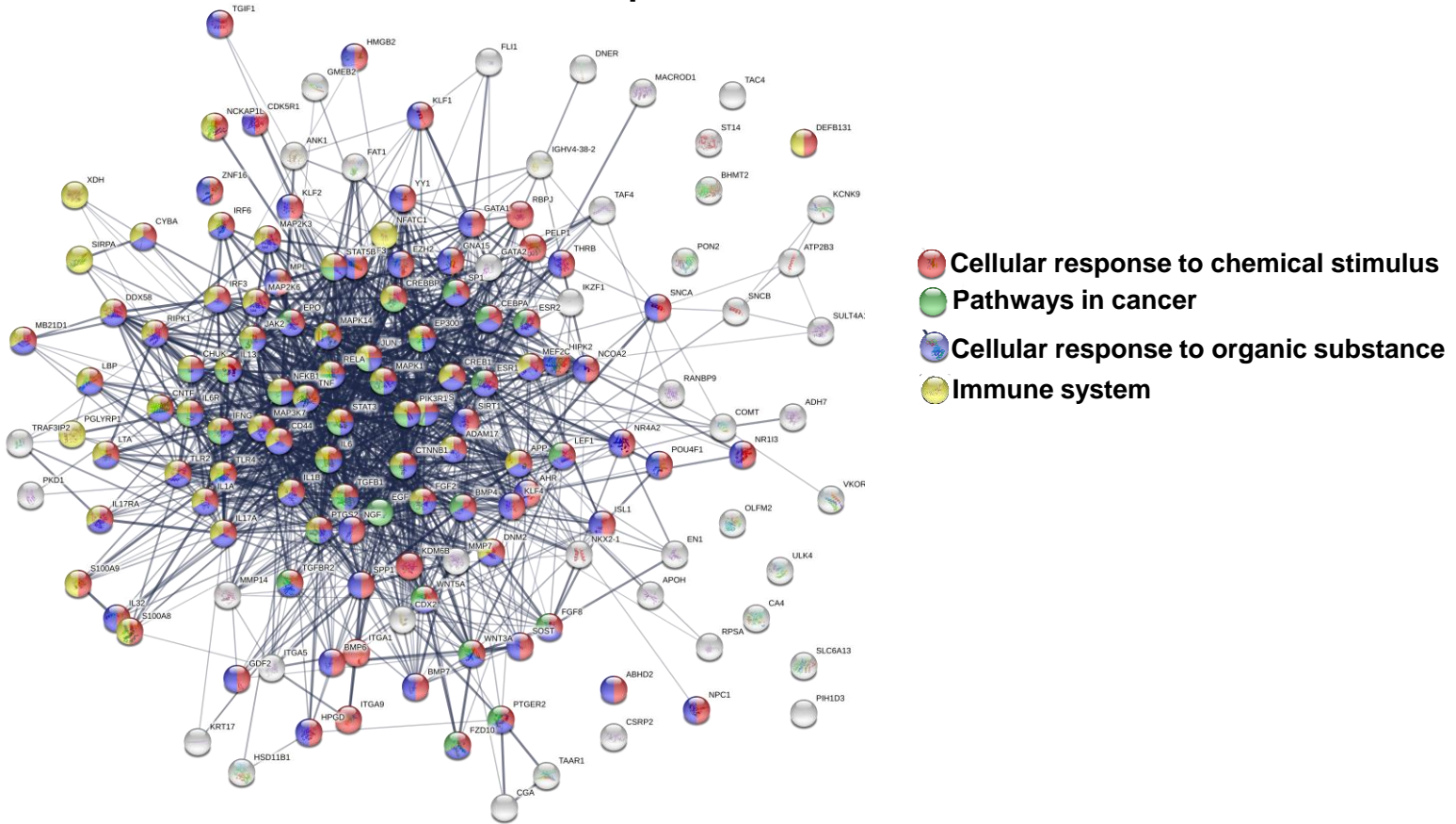
a

**Established external FP versus non-tumored skin (RNA-seq)
Top 200 ITRs**



b

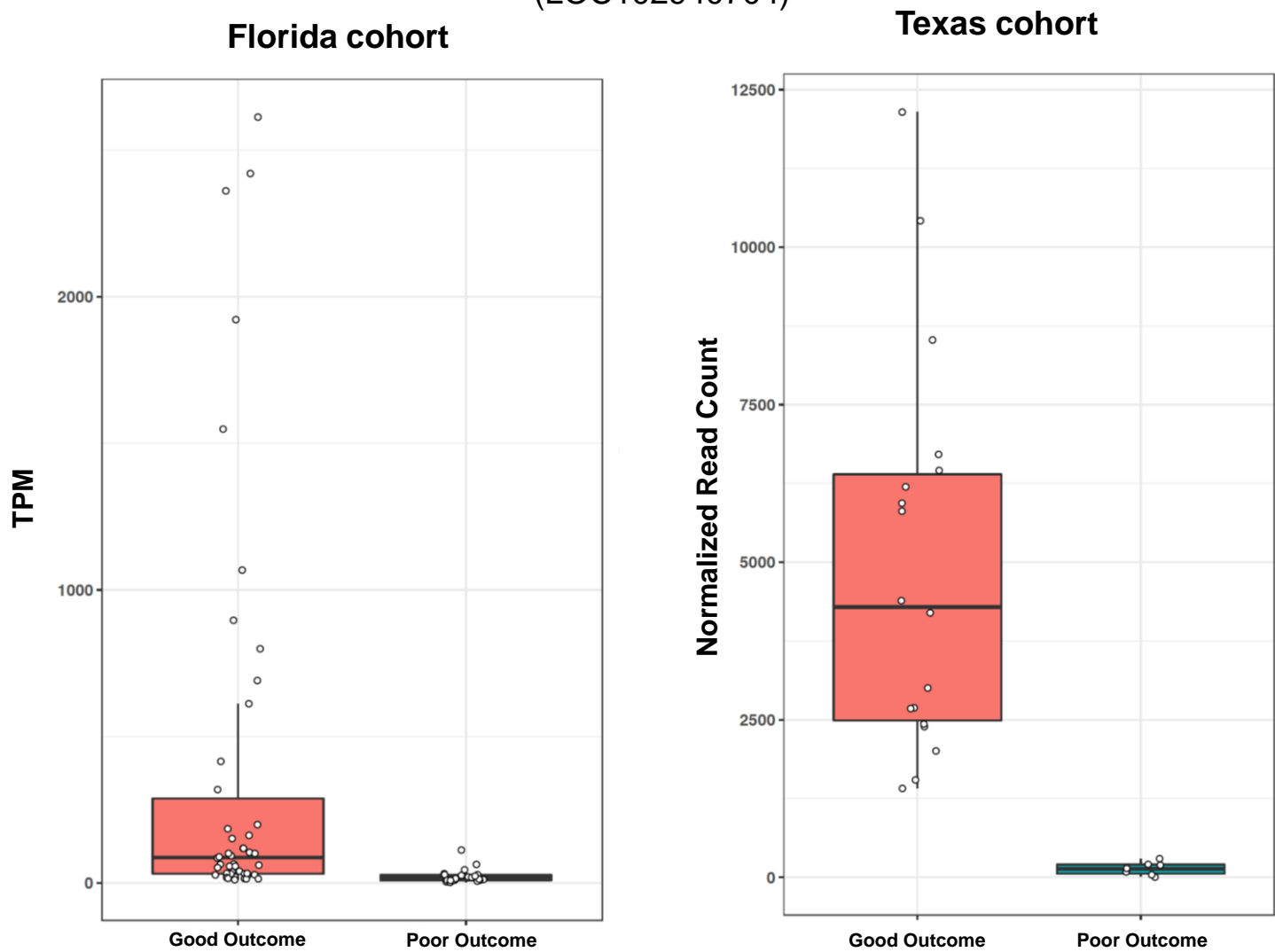
**Lung FP versus non-tumored skin (RNA-seq)
Top 200 ITRs**



Supplemental Figure 5. Additional fibropapillomatosis inferred transcriptional regulator (ITR) interaction networks. **a, b)** Interaction networks of the top 200 ITRs of established **a)** external tumors and of **b)** lung tumors.

a

Interferon alpha-inducible protein 27 protein 2B
(LOC102940704)



Supplemental Figure 7. Additional outcome expression data. **a)** Expression levels of Interferon alpha-inducible protein 27 protein 2B between tumors of good outcome and poor outcome patients, as detected by RNA-seq. Top: Florida cohort. Bottom: Texas cohort. Florida cohort; N = 69 samples. Per outcome: released (good outcome) = 7 turtles; died/euthanized (poor outcome) = 5 turtles. Texas cohort; N = 25 samples. Per outcome: prolonged rehabilitation (poor outcome) = 2 turtles; short rehabilitation (good outcome) = 1 turtle.