Identifying tmem127-deficient pheochromocytomas/paragangliomas via RET overexpression by immunohistochemistry

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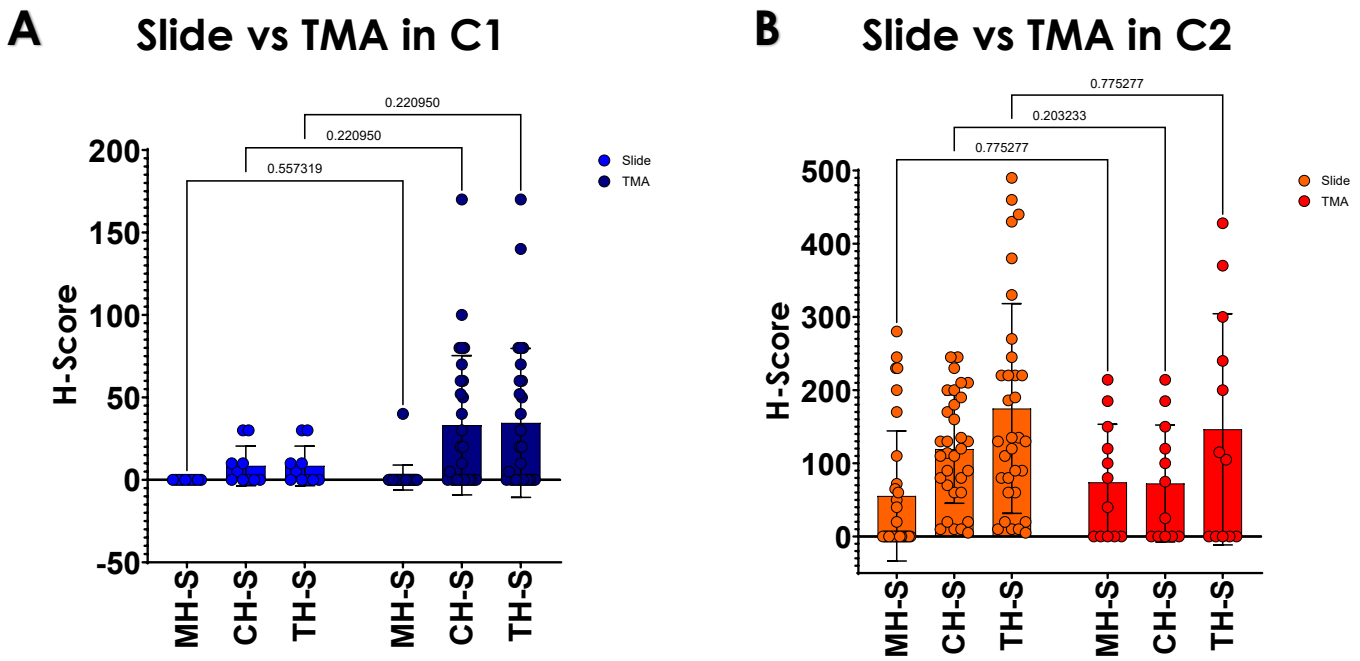
Supplementary Figures

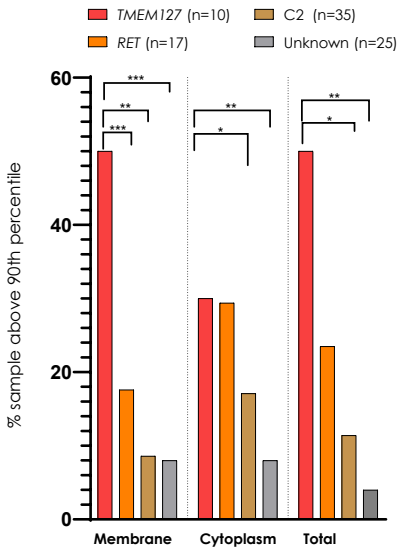


	Slide (n=10)	TMA (n=28)	Adjusted p
MH-S	0±0	1.42±7.55	0.5573
CH-S	8.5±12.03	33.10±42.22	0.2209
TH-S	8.5±12.03	34.53±45.14	0.2209

	Slide (n=32)	TMA (n=12)	Adjusted p
MH-S	55.37±88.82	74.08±79.23	0.7752
CH-S	119.50±74.03	72.41±79.91	0.2032
TH-S	174.87±143.25	146.50±157.93	0.7752

Supplementary Figure 1. Comparison between RET H-scores in individual slides versus TMA sections. **A)** Samples belonging to molecular cluster 1 (C1); **B)** Samples belonging to molecular cluster 2 (C2). Membrane, cytoplasm, and total (MH-S, CH-S, and T-HS) scores are as indicated. Statistical significance was assessed using one-way ANOVA.

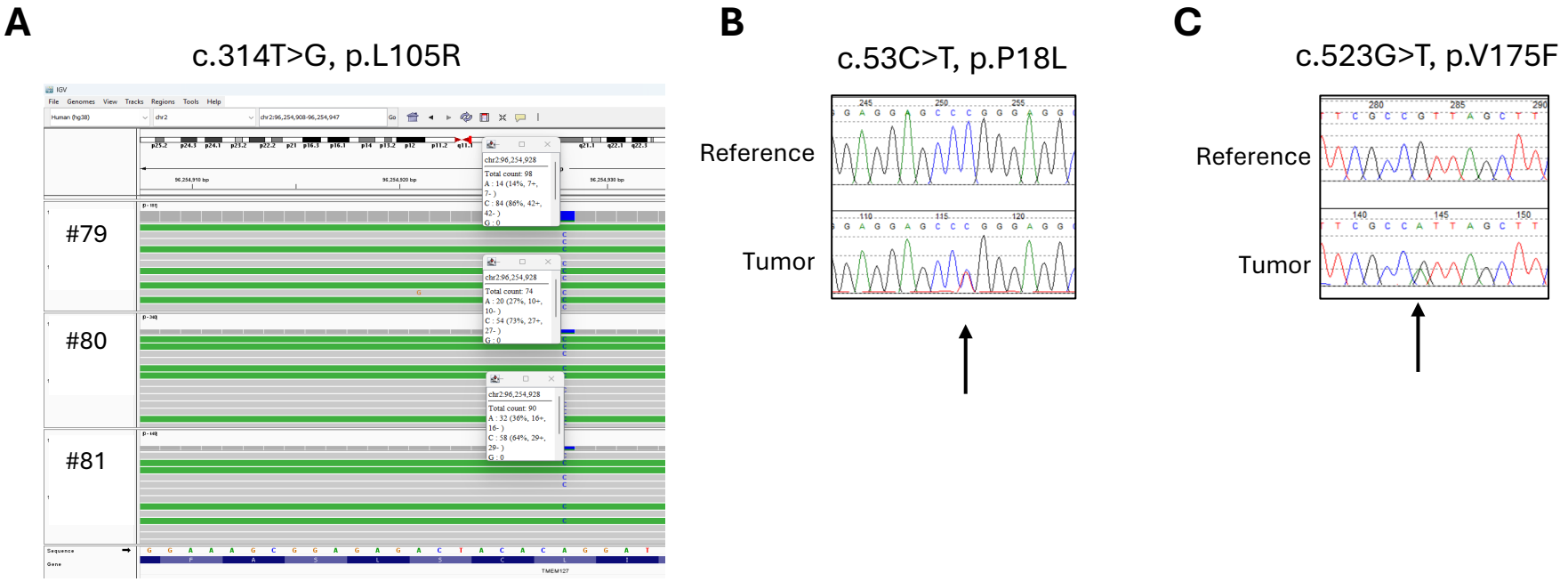
A



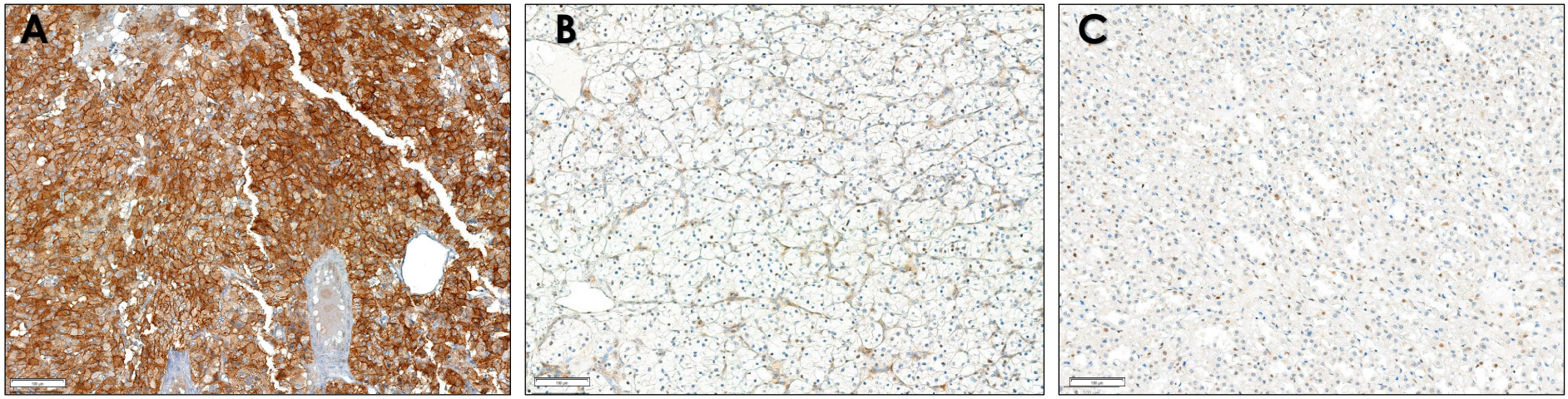
B

Comparison groups	Adjusted p value		
	MH-S	CH-S	TH-S
TMEM127 vs SDH (SDHA+SDHB+SDHD)	<0.0001	0.0309	<0.0001
TMEM127 vs. FH	<0.0001	0.0249	<0.0001
TMEM127 vs. EPAS1	<0.0001	0.0009	<0.0001
TMEM127 vs. RET, RET::GRB2	0.0312	>0.9999	0.7521
TMEM127 vs. HRAS	0.0393	0.1966	0.0194
TMEM127 vs. MAX	<0.0001	0.0506	<0.0001
TMEM127 vs. NF1	0.0003	0.1959	0.0013
TMEM127 vs. CSDE1 +PIK3CA	0.9477	0.9933	0.9329
TMEM127 vs. UBTF::MAML3	0.3234	0.5266	0.1882
TMEM127 vs. Unknown	0.0001	0.1346	0.0005

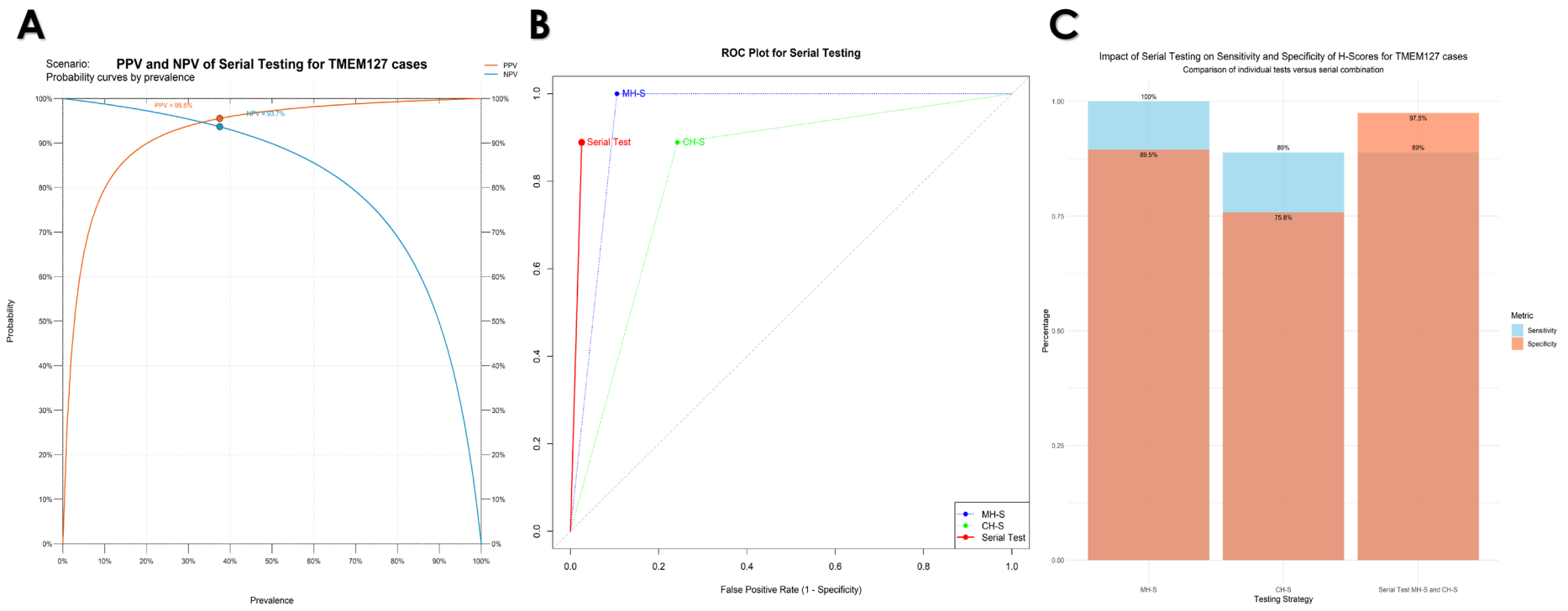
Supplementary Figure 2. A) Percentage of samples displaying RET IHC H-Scores above the 90th percentile in each of the indicated groups: tumors with TMEM127 pathogenic variants (n=10, including a sample previously reported in ref[23]); the remaining groups are the same as shown in Figure 2D: tumors with RET pathogenic variants, tumors belonging to Cluster 2 (C2, kinase signaling, except for TMEM127-mutant tumors, and samples with unknown genotype (including RET VUS and TMEM127 VUS), distribution of membrane, cytoplasm and total staining. P values were calculated by one-way ANOVA. Comparisons with TMEM127 group are indicated by (*). Other comparisons were not statistically significant; **B)** Statistical analysis of pairwise genotype comparisons of RET immunohistochemistry scores for membrane (MH-S), cytoplasm (CH-S) or total (TH-S) H-scores using Tukey's multiple comparisons test (related to Figure 2C and Table 3);



Supplementary Figure 3. Loss of heterozygosity analysis of tumors carrying the indicated *TMEM127* variants. **A)** Variant classified as VUS, Next-generation sequencing (IGV) of three tumors from the same patient (#79=left pheochromocytoma, #80 and #81 are two separate tissue blocks from the right pheochromocytoma). The variant nucleotide is detected at a frequency of 86%, 73%, 64%, in these three samples, respectively. **B,** Variant classified as benign, **C)** Variant classified as VUS. Sanger sequencing of the B and C tumors relative to the reference sequence, arrow indicates the variant region showing heterozygosity.



Supplementary Figure 4. A, B) RET immunohistochemistry of a pheochromocytoma (A) and renal cell carcinoma (B) from the same individual carrying a *TMEM127* pathogenic variant; **C)** Renal cell carcinoma from an unrelated patient (carrying a pathogenic *SDHB* variant). Scale bars are 100µm



Supplementary Figure 5: A) Serial testing using MH-S (71) and CH-S (97) to identify *TMEM127* cases (only known pathogenic variants included in TMEM127 group). Sensitivity= Sensitivity A X Sensitivity B=.890X1= 1 X 0.890= 89%, Specificity= Spec A + Spec B – (Spec A X Spec B)= 0.758 + 0.895 – (0.758 X 0.895) =0.975 X 100 = 97.5%; **B)** Post Test Prob=0.375, PPV Serial= .890 X 0.375/ (.890 X 0.375) + [(1-.975) X (1- 0.375)]= 0.955x100=95.5%, NPV Serial= .975 X (1-0.375) (.975 X (1 - .375) + [(1-.890) X .375]= 0.936X100=93.6%; **C)** comparison plots of MH-S, CH-S, and Serial test