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**Supplementary Figure 1: Amplification plot of preliminary MYB primer screening on primary ACC tumor tissue.** Quant Studio 7 amplification plot output of 10 MYB primers with cDNA from ACC tumor tissue.

**Supplementary Figure 2: Primer screen on blood samples for MYB amplification in metastatic samples.** Six primer pairs spanning exons 2-8 run on control and ACC patient blood to identify the difference between metastatic samples compared to disease free and control patients.

**Supplementary Figure 3: Relative expression of individual patients for MYB primer pair amplification of exons 2-3.** Relative expression levels of control, NED, local, and metastatic MYB whole blood levels.

**Supplementary Figure 4: Relative expression of MYB exons 14-15 in patient whole blood.** Endogenous levels of MYB in whole blood measured by the amplification of MYB exons typically lost in translocation events.

Supplementary Figure 5: Violin plots of 30 genes overexpressed in spatial transcriptomic primary ACC tumor and circulating tumor cell cluster from blood scRNA-seq. Violin plots of genes that are differentially expressed in both primary tumor and CTCs in blood.

**Supplementary Figure 6: Progression of MYB level in sample T5 overtime.** Relative expression of MYB in whole blood for sample T5 (February 2020), T5B (April 2021), and T5C (December 2021).

**Supplementary Figure 7: Keratin expression in different clusters from blood single-cell sequencing on metastatic ACC patient.** RNA expression for Keratins 1-30 were examined and detection of RNA for keratin 1, 2, 5, 7, 8, 10, 17, 18, and 23 was observed.

**Supplementary Figure 8: Examining Sox4 expression in whole blood via RT-qPCR.** Relative expression of Sox4 for 4 different primer pairs amplifying varying regions of the gene for control, NED, local, and metastatic disease patients. medRxiv preprint doi: https://doi.org/10.1101/2024.10.15.24315549; this version posted October 17, 2024. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY-NC-ND 4.0 International license.

## Supplementary Table 1: Table of primers used in RT-qPCR experiments.

Name	Notes	5'	3'
MYB-8	Spanning exons 2-3	CCCAAGTCTGGAAAGCGTCA	TTCATCCTCTTCCCGGGTCC
MYB-12	Spanning exons 14-15	ATGGCACCAGCATCAGAAGA	AGGATGCAGGTTCCCAGGTA
MYB-1	Spanning exons 3-4	AGCTGGTGGAACAGAATGGA	GTACTTTCTGCCATCGGTGC
MYB-2	Spanning exons 3-4	AGCTGGTGGAACAGAATGGAA	ACTTTCTGCCATCGGTGCTG
MYB-3	Spanning exons 4-5	CCTGAGCTCATCAAGGGTCC	ACCAACGTTTCGGACCGTATT
MYB-4	Spanning exons 4-5	CCCTGAGCTCATCAAGGGTC	AACAGACCAACGTTTCGGAC
MYB-6	Spanning exons 5-6	ATGGGCAGAAATCGCAAAGC	CCTTCCTGTTCGACCTTCCG
MYB-7	Spanning exons 6-7	CAGCCCACTGTTAACAACGAC	CAGCTGGCTGAGGGACATTG
MYB-9	Spanning exons 7-8	TGCTCCTAATGTCAACCGAG	CATGTGTGGTTCTGTGTTGGTA
MYB-10	Spanning exons 5-6	ATGGGCAGAAATCGCAAAGC	CGACCTTCCGACGCATTGTA
MYB-11	Spanning exons 7-8	AGTCAATGTCCCTCAGCCAG	CGCTTTTCCTTCTCAGGGTCT
UBE2D2	Normalizing gene	GTACTCTTGTCCATCTGTTCTCTG	CCATTCCCGAGCTATTCTGTT
RPS18	Normalizing gene	GTTCCAGCATATTTTGCGAGT	GTCAATGTCTGCTTTCCTCAAC