Supplementary Information

RNA sequences

1. Homo sapiens keratin 18, type I (KRT18), transcript variant 2, mRNA.

ATGAGCTTCACCACTCGCTCCACCTTCTCCACCAACTACCGGTCCCTGGG

CTCTGTCCAGGCGCCCAGCTACGGCGCCCGGCCGGTCAGCAGCGCGGCCA

GCGTCTATGCAGGCGCTGGGGGCTCTGGTTCCCGGATCTCCGTGTCCCGC

TCCACCAGCTTCAGGGGCGGCATGGGGTCCGGGGGCCTGGCCACCGGGAT

AGCCGGGGGTCTGGCAGGAATGGGAGGCATCCAGAACGAGAAGGAGACCA

TGCAAAGCCTGAACGACCGCCTGGCCTCTTACCTGGACAGAGTGAGGAGC

CTGGAGACCGAGAACCGGAGGCTGGAGAGCAAAATCCGGGAGCACTTGGA

GAAGAAGGGACCCCAGGTCAGAGACTGGAGCCATTACTTCAAGATCATCG

AGGACCTGAGGGCTCAGATCTTCGCAAATACTGTGGACAATGCCCGCATC

GTTCTGCAGATTGACAATGCCCGTCTTGCTGCTGATGACTTTAGAGTCAA

GTATGAGACAGAGCTGGCCATGCGCCAGTCTGTGGAGAACGACATCCATG

GGCTCCGCAAGGTCATTGATGACACCAATATCACACGACTGCAGCTGGAG

ACAGAGATCGAGGCTCTCAAGGAGGAGCTGCTCTTCATGAAGAAGAACCA

CGAAGAGGAAGTAAAAGGCCTACAAGCCCAGATTGCCAGCTCTGGGTTGA

CCGTGGAGGTAGATGCCCCCAAATCTCAGGACCTCGCCAAGATCATGGCA

GACATCCGGGCCCAATATGACGAGCTGGCTCGGAAGAACCGAGAGGAGCT

AGACAAGTACTGGTCTCAGCAGATTGAGGAGAGCACCACAGTGGTCACCA

CACAGTCTGCTGAGGTTGGAGCTGCTGAGACGACGCTCACAGAGCTGAGA

CGTACAGTCCAGTCCTTGGAGATCGACCTGGACTCCATGAGAAATCTGAA

GGCCAGCTTGGAGAACAGCCTGAGGGAGGTGGAGGCCCGCTACGCCCTAC

AGATGGAGCAGCTCAACGGGATCCTGCTGCACCTTGAGTCAGAGCTGGCA

CAGACCCGGGCAGAGGGACAGCGCCAGGCCCAGGAGTATGAGGCCCTGCT

GAACATCAAGGTCAAGCTGGAGGCTGAGATCGCCACCTACCGCCGCCTGC

TGGAAGATGGCGAGGACTTTAATCTTGGTGATGCCTTGGACAGCAGCAAC

TCCATGCAAACCATCCAAAAGACCACCACCCGCCGGATAGTGGATGGCAA

AGTGGTGTCTGAGACCAATGACACCAAAGTTCTGAGGCATTAA

2. Homo sapiens keratin 19, type I (KRT19), mRNA.

ATGACTTCCTACAGCTATCGCCAGTCGTCGGCCACGTCGTCCTTCGGAGG

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CCAGCATTCACGGGGGCTCCGGCGGCCGCGGCGTATCCGTGTCCTCCGCC

CGCTTTGTGTCCTCGTCCTCCTCGGGGGCCTACGGCGGCGGCTACGGCGG

CGTCCTGACCGCGTCCGACGGGCTGCTGGCGGGCAACGAGAAGCTAACCA

TGCAGAACCTCAACGACCGCCTGGCCTCCTACCTGGACAAGGTGCGCGCC

CTGGAGGCGGCCAACGGCGAGCTAGAGGTGAAGATCCGCGACTGGTACCA

GAAGCAGGGGCCTGGGCCCTCCCGCGACTACAGCCACTACTACACGACCA

TCCAGGACCTGCGGGACAAGATTCTTGGTGCCACCATTGAGAACTCCAGG

ATTGTCCTGCAGATCGACAATGCCCGTCTGGCTGCAGATGACTTCCGAAC

CAAGTTTGAGACGGAACAGGCTCTGCGCATGAGCGTGGAGGCCGACATCA

ACGGCCTGCGCAGGGTGCTGGATGAGCTGACCCTGGCCAGGACCGACCTG

GAGATGCAGATCGAAGGCCTGAAGGAAGAGCTGGCCTACCTGAAGAAGAA

CCATGAGGAGGAAATCAGTACGCTGAGGGGCCAAGTGGGAGGCCAGGTCA

GTGTGGAGGTGGATTCCGCTCCGGGCACCGATCTCGCCAAGATCCTGAGT

GACATGCGAAGCCAATATGAGGTCATGGCCGAGCAGAACCGGAAGGATGC

TGAAGCCTGGTTCACCAGCCGGACTGAAGAATTGAACCGGGAGGTCGCTG

GCCACACGGAGCAGCTCCAGATGAGCAGGTCCGAGGTTACTGACCTGCGG

CGCACCCTTCAGGGTCTTGAGATTGAGCTGCAGTCACAGCTGAGCATGAA

AGCTGCCTTGGAAGACACACTGGCAGAAACGGAGGCGCGCTTTGGAGCCC

AGCTGGCGCATATCCAGGCGCTGATCAGCGGTATTGAAGCCCAGCTGGGC

GATGTGCGAGCTGATAGTGAGCGGCAGAATCAGGAGTACCAGCGGCTCAT

GGACATCAAGTCGCGGCTGGAGCAGGAGATTGCCACCTACCGCAGCCTGC

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TGA

3. Homo sapiens protein tyrosine phosphatase, receptor type, C (PTPRC), transcript variant 2, mRNA.

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GGACACAGAAGTATTTGTGACAGGGCAAAGCCCAACACCTTCCCCCACTG

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GCTGTCATTTCAACCACAACAATAGCTACTACTCCATCTAAGCCAACATG

TGATGAAAAATATGCAAACATCACTGTGGATTACTTATATAACAAGGAAA

CTAAATTATTTACAGCAAAGCTAAATGTTAATGAGAATGTGGAATGTGGA

AACAATACTTGCACAAACAATGAGGTGCATAACCTTACAGAATGTAAAAA

TGCGTCTGTTTCCATATCTCATAATTCATGTACTGCTCCTGATAAGACAT

TAATATTAGATGTGCCACCAGGGGTTGAAAAGTTTCAGTTACATGATTGT

ACACAAGTTGAAAAAGCAGATACTACTATTTGTTTAAAATGGAAAAATAT

TGAAACCTTTACTTGTGATACACAGAATATTACCTACAGATTTCAGTGTG

GTAATATGATATTTGATAATAAAGAAATTAAATTAGAAAACCTTGAACCC

GAACATGAGTATAAGTGTGACTCAGAAATACTCTATAATAACCACAAGTT

TACTAACGCAAGTAAAATTATTAAAACAGATTTTGGGAGTCCAGGAGAGC

CTCAGATTATTTTTTGTAGAAGTGAAGCTGCACATCAAGGAGTAATTACC

TGGAATCCCCCTCAAAGATCATTTCATAATTTTACCCTCTGTTATATAAA

AGAGACAGAAAAAGATTGCCTCAATCTGGATAAAAACCTGATCAAATATG

ATTTGCAAAATTTAAAACCTTATACGAAATATGTTTTATCATTACATGCC

TACATCATTGCAAAAGTGCAACGTAATGGAAGTGCTGCAATGTGTCATTT

CACAACTAAAAGTGCTCCTCCAAGCCAGGTCTGGAACATGACTGTCTCCA

TGACATCAGATAATAGTATGCATGTCAAGTGTAGGCCTCCCAGGGACCGT

AATGGCCCCCATGAACGTTACCATTTGGAAGTTGAAGCTGGAAATACTCT

GGTTAGAAATGAGTCGCATAAGAATTGCGATTTCCGTGTAAAAGATCTTC

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CCTGGAGAACCCTTTATTTTACATCATTCAACATCTTATAATTCTAAGGC

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CAGGGATGAAACTGTTGATGATTTCTGGAGGATGATTTGGGAACAGAAAG

CCACAGTTATTGTCATGGTCACTCGATGTGAAGAAGGAAACAGGAACAAG

TGTGCAGAATACTGGCCGTCAATGGAAGAGGGCACTCGGGCTTTTGGAGA

TGTTGTTGTAAAGATCAACCAGCACAAAAGATGTCCAGATTACATCATTC

AGAAATTGAACATTGTAAATAAAAAAGAAAAAGCAACTGGAAGAGAGGTG

ACTCACATTCAGTTCACCAGCTGGCCAGACCACGGGGTGCCTGAGGATCC

TCACTTGCTCCTCAAACTGAGAAGGAGAGTGAATGCCTTCAGCAATTTCT

TCAGTGGTCCCATTGTGGTGCACTGCAGTGCTGGTGTTGGGCGCACAGGA

ACCTATATCGGAATTGATGCCATGCTAGAAGGCCTGGAAGCCGAGAACAA

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TACAATCAGTTTGGAGAAACAGAAGTGAATTTGTCTGAATTACATCCATA

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AGGCTGAATTCCAGAGACTTCCTTCATATAGGAGCTGGAGGACACAGCAC

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CCCATATGACTATAACAGAGTGCCACTTAAACATGAGCTGGAAATGAGTA

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GAGGAACCAAGCAAATACATCAATGCATCTTTTATAATGAGCTACTGGAA

ACCTGAAGTGATGATTGCTGCTCAGGGACCACTGAAGGAGACCATTGGTG

ACTTTTGGCAGATGATCTTCCAAAGAAAAGTCAAAGTTATTGTTATGCTG

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AAATTGAATTTGATAATGAAGTGGACAAAGTAAAGCAGGATGCTAATTGT

GTTAATCCACTTGGTGCCCCAGAAAAGCTCCCTGAAGCAAAGGAACAGGC

TGAAGGTTCTGAACCCACGAGTGGCACTGAGGGGCCAGAACATTCTGTCA

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4. Homo sapiens catenin (cadherin-associated protein), delta 1 (CTNND1), transcript variant 21, mRNA.

ATGCAGGAGCCGGGGCAGATTGTGGAGACCTACACGGAGGAGGATCCTGA

GGGAGCCATGTCTGTAGTCTCTGTGGAGACCTCAGATGATGGGACCACTC

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ACAGTACAGCCAGTCGCTATGGGACCAGACGGGTTGCCTGTGGATGCTTC

ATCAGTTTCTAACAACTATATCCAGACTTTGGGTCGTGATTTCCGCAAGA

ATGGCAATGGGGGACCTGGTCCCTATGTGGGGCAAGCTGGCACTGCTACC

CTTCCTAGGAACTTCCACTACCCTCCTGATGGTTATAGTCGCCACTATGA

AGATGGTTATCCAGGTGGCAGTGATAACTATGGCAGTCTGTCCCGGGTGA

CCCGCATTGAGGAGCGGTATAGGCCCAGCATGGAAGGCTACCGGGCACCT

AGTAGACAGGATGTGTATGGGCCCCAACCCCAGGTTCGGGTAGGTGGGAG

CAGCGTGGATCTGCATCGCTTTCATCCAGAGCCTTATGGGCTAGAGGATG

ACCAGCGTAGTATGGGCTATGATGACCTGGATTATGGTATGATGTCTGAT

TATGGCACTGCCCGTCGGACTGGGACACCCTCTGACCCTCGTCGGCGCCT

CAGGAGCTATGAAGACATGATTGGTGAGGAGGTGCCATCGGATCAATACT

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AGCCTGCGCAAAGGAGGGCCTCCACCTCCTAATTGGAGACAGCCAGAGCT

GCCAGAGGTGATCGCCATGCTTGGATTCCGCTTGGATGCTGTCAAGTCCA

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CAAGAGAAGGCTCTTTCTGCCATAGCTGACCTCCTGACTAATGAACATGA

ACGGGTGGTGAAAGCTGCATCTGGAGCACTGAGAAACCTGGCTGTGGATG

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CGAAGCCAGAGCAGTCATTCATATGATGATAGTACTCTCCCTCTCATTGA

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GCAATATGGGATCAAACACAAAATCACTAGATAACAACTATTCCACACCA

AATGAGAGAGGAGACCACAATAGAACACTGGATCGATCGGGGGATCTAGG

CGACATGGAGCCATTGAAGGGAACAACACCCTTGATGCAGAAGATTTAG

5. d2egfp

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AAACTCAATGTATCTTATCATACGAATTCCTACACATTATCCTAGCAGAAGCACATGCAGGGT

GACGGTCCACGCTCTCCTGGGCACAAATGGGCAGCGTGCCATCCTGCTCCTCCACCTCCG

GAAGCCATGGCTAAGCTTGTACAGCTCGTCCATGAGAGTGATCCCGGCGGTCACGAACTCC

AGCAGGATGTGATCGCGCTTCTCGGGGTCTTTGCTCAGGGACTGGGTGCTCAGGTAGTTG

TCGGGCAGCAGCACGCCGTCGCCGATGGGGGTCTGCTGGTAGTGGTCGAGCTGCACGCT
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CGTTGTGGCTGTAGTTGTACTCCAGCTTGCCCAGGATGTTGCCGTCCTTGAAGTCGATGCC
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GCACGGGCAGGCCGGTGGTGCAGATGATCAGGGTCAGCTTGCCGGTGGCATCGCCCTCG
CCGCCGGACACGCTGAACTGGCCGTTTACGTCGCCGAGCTCGACCAGGATGGGCACCCC
GGTGAACAGCTCGCCCTTGCTCACCATC

6. Mouse ECad

GTGTAGACCTCGGAACTGAATGCATCCTTCAAATCTCACTGAATCGGGAGTCTTCCGAAAAG
CTTGTGGAGCTTTAGATGGGTACACGCTGGGAAACATGGGATTTGATCTGAACCAGGTCTG
TGATCACTATCTCCATGCTGTTGTCATTCTGATCTGTTCTGCAACGAATCCCTCAAATTGTAG
GTGTTGACGTCATCTGTCCCTATTGACAGTGAACAGAGGTGAGCACACTGATGACCAGAGT
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AGTCTCTGCATATGTGCTGTTCTTCACAATCATCTGTGGCGATGATGAACAGGACCAGGAGA
AGAGTGAAGGTCTGGATCCAAGATGGTCTTGAGCTGCGTCATTATAGTCCTTTCTTGGTTGC
AAAATTTTGTATTCGCCAATCTCTTGGTCTTTGTTCTGGTTATCGATGGCAGGAACTTGCAAT
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GAGATAATCGTAGTCCTGGTC

7. human ECad

TCAAAATTCACTCTGCCCAGACTTTGAATCGGGTGTCGAGGCCTTTTGACTGTAATCACATG
TATTCAGCGTGACTTTGGATGTGAGCAATTCTGCTTGGGCAGCTGATGGGAGGAATAAAGG

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GACACAGCGTGAGAGGTTGTCATTCTGATCGGTTATTAAAGACCTCCTGGGTGAATGTAGGT

GTTCACATCATCGTTGGCTGAGGATGGTGTAAGTGTGTTCCTGTTAATGGTGACCAGGGTAT

ACGTAGGGAAATTATCGTTGGTGTCAGTGACTGGTGGGATTGAAGATCGGAAATTGTCCAC

CATCATCATTTGTTCACTGGATTTGTGGTGCAGTGTAGGATGTGATTTCCTTCCATAAATGTG

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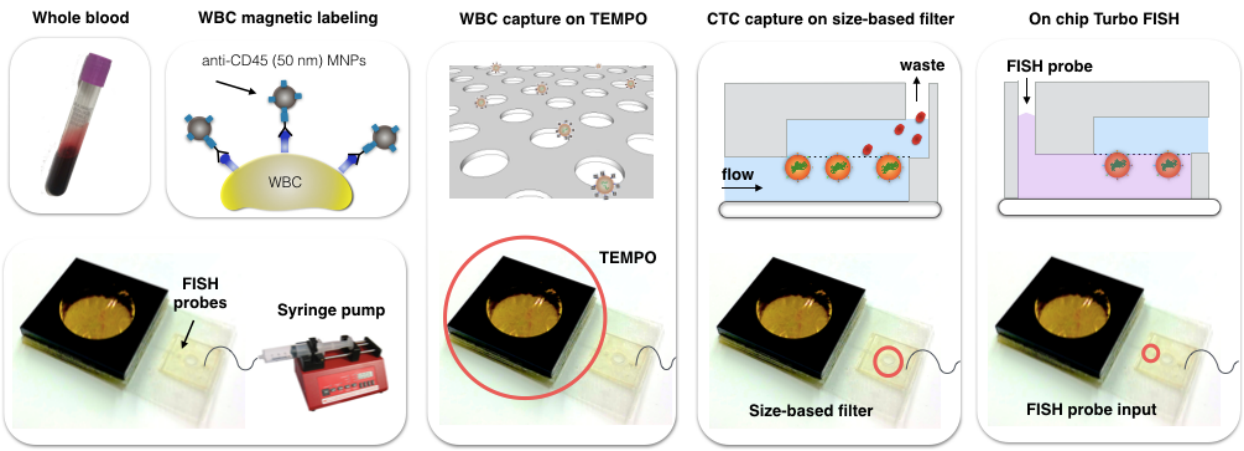

Fig. S1. A Schematic of the step-by-step operation of our CaTCh FISH Chip. From whole blood, WBCs are magnetically labeled with anti-CD45 MNPs for negative depletion. Labeled WBCs are captured on TEMPO at the upstream. Then, WBC-depleted whole blood travels through the device and potential CTCs are captured on the size-based filter where RBCs continuously move through the filter with the flow and are removed through the waste outlet. Finally, FISH probes are introduced and incubated on chip for 5 mins. After wash, we directly image single RNA molecules of CTCs captured in the device.

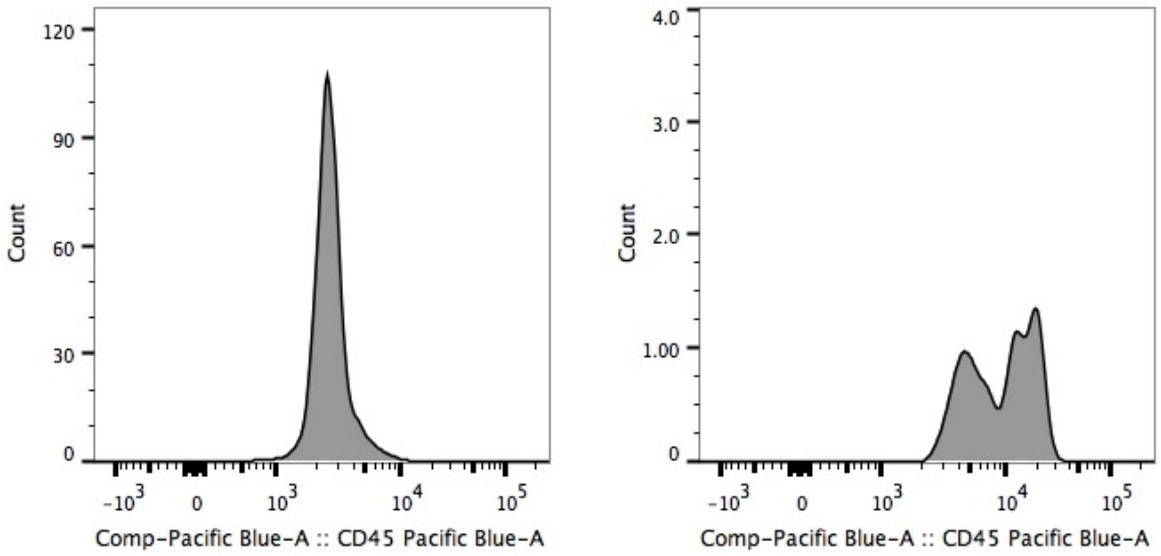

Fig. S2. Flow Cytometry Profiling of CD45 expression of leukocytes in the input **(a)** and the output **(b)** of the CaTCh FISH platform. The cells from the output do not have low CD45 expression, leading to the conclusion that heterogeneity in CD45 expression did not play a major role in the performance of our device. All cells were stained with Cell Tracker (Fisher) to enable all cells to be gated and their CD45 profiled, even those potentially negative for CD45.

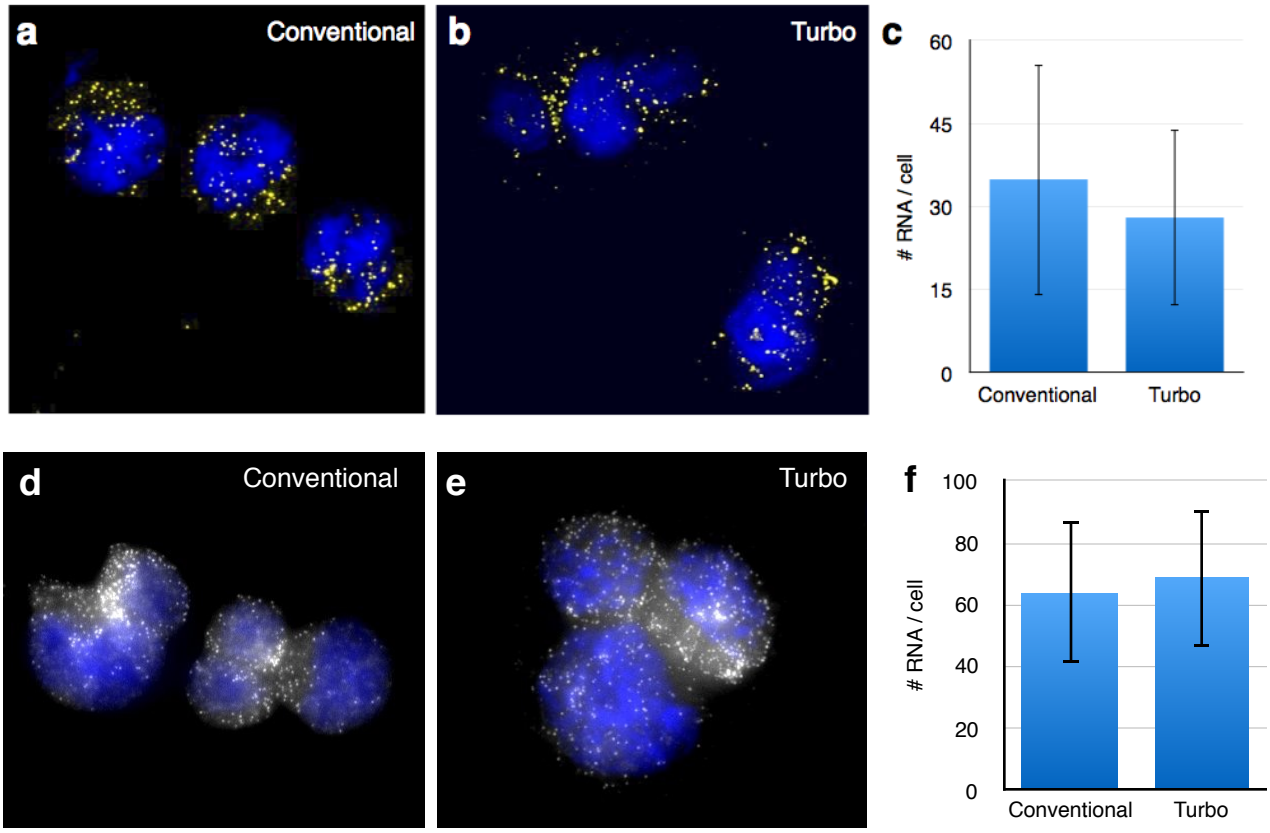

Fig. S3. Comparison of conventional and Turbo RNA FISH. We directly compare the number of CD45 RNA measured per cell (Jurkat) using conventional single molecule RNA FISH (**a**) and our Turbo RNA FISH protocol (**b**), and showed that there was not a significant difference in the number of (**c**) punctates.(P = 0.32) Each image is a representative image of $N = 20$ cells. Error bars represent standard deviation. We also compare the number of CK19 RNA measured per cell (PD7591) using conventional single molecule RNA FISH (**d**) and our Turbo RNA FISH protocol (**e**), and showed that there was not a significant difference in the number of (**f**) punctates.(P = 0.29) Each image is a representative image of $N = 50$ cells. Error bars represent standard deviation.

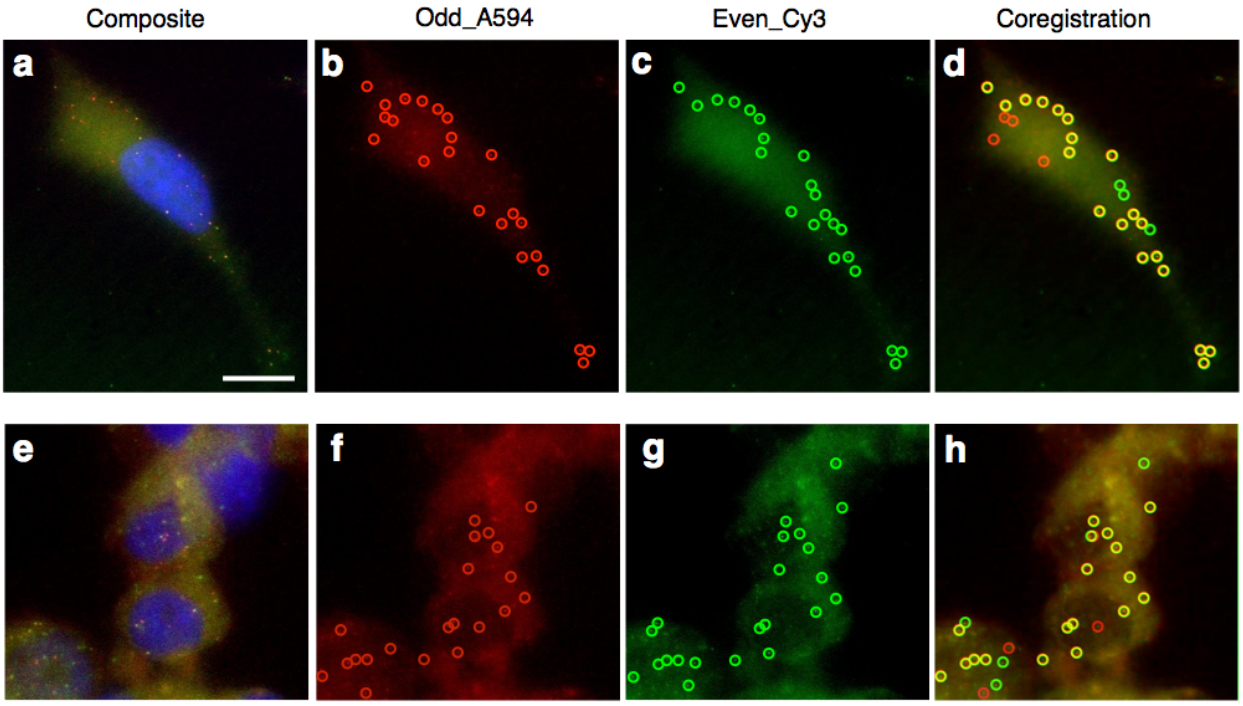

Fig. S4. Quantification of sensitivity and specificity of RNA FISH using odds and evens co-localization. We quantify the rate of falsely positive and falsely negative punctates using our Turbo FISH method by labeling the same strand of RNA with two differently colored probes, in both TurboFISH on a glass slide (**a-d**) and on our chip (**e-h**). We partitioned the probe set (32 oligos) to the even and odd numbered oligonucleotides and coupled each subset with a different fluorophore (odds: Alexa 594 (**b**), evens: Cy3 (**c**)). **a.** Composite image of DAPI and RNA punctates using TurboFISH on a glass slide.(scale bar = 10 μm) **d.** Co-localization between **b** and **c** is shown, wherein yellow circles represent co-localization, red indicate only an odd punctate and green only an even punctate. For TurboFISH on a glass slide, 76% of our punctates co-registered,(N = 20 cells) consistent with previously published results using conventional single molecule RNA FISH.[48] For on-chip Turbo FISH, we found 79% co-registration (N=15 cells), also consistent with previously published results.

	CK18	CK19	Ctnnd1	Ecad	Cocktail	CD45
Jurkat	0	0	0	0	0	57 ± 4.24
MiaPaCa2	8.67 ± 0.47	2.65 ± 0.56	18 ± 2.83	0	29.31 ±2.81	0
AsPC-1	10.9 ± 0.79	16.5 ± 2.12	15.8 ± 1.13	56.6 ± 0.96	99.7 ± 3.68	3.56 ± 0.02
Capan2	23.1 ± 1.03	29 ± 1.41	34 ± 0	28 ± 4.24	114.1 ± 2.2	0.83 ± 0.24

Mean ± Standard deviation

Fig. S5. Raw counts of RNA punctates for RNA marker validation. We counted individual RNA molecules from RNA FISH images for CK18, CK19, Ctnnd1, ECad, CD45 from Jurkat, MiaPaCa2, AsPC-1, and Capan2 pancreatic human cell lines. Reported numbers are the average of RNA punctates per cell from two independent experiments, with standard deviation.

	PDAC patients (n=12)
Median age (range)	66 (51-83)
Sex	
Male	6 (50%)
Female	6 (50%)
Metastatic Disease	
M0 (No metastases)	3 (25%)
M1 (Metastases)	9 (75%)
ECOG Status	
0	6 (50%)
1	6 (50%)
2+	-
Treatment status at draw [1] (n=14)	
Pre-Treatment	8 (57%)
Post- Resection	2 (14%)
On Chemotherapy	4 (29%)

[1] 14 total samples were tested, two patients had a second draw after medical intervention

Fig. S6. Patient demographic information. Patient demographic information includes median age, sex, the presence of metastasis, Eastern Cooperative Oncology Group (ECOG) status, and treatment status.