

## Supplementary Information

### RNA sequences

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

ATGAGCTTCACCACTCGCTCCACCTTCTCCACCAACTACCGGTCCCTGGG  
CTCTGTCCAGGCGCCCAGCTACGGCGCCCGGCCGGTCAGCAGCGCGGCCA  
GCGTCTATGCAGGCGCTGGGGGCTCTGGTTCCCGGATCTCCGTGTCCCGC  
TCCACCAGCTTCAGGGGCGGCATGGGGTCCGGGGGCCTGGCCACCGGGAT  
AGCCGGGGGTCTGGCAGGAATGGGAGGCATCCAGAACGAGAAGGAGACCA  
TGCAAAGCCTGAACGACCGCCTGGCCTCTTACCTGGACAGAGTGAGGAGC  
CTGGAGACCGAGAACCGGAGGCTGGAGAGCAAAATCCGGGAGCACTTGGA  
GAAGAAGGGACCCCAGGTCAGAGACTGGAGCCATTACTTCAAGATCATCG  
AGGACCTGAGGGCTCAGATCTTCGCAAATACTGTGGACAATGCCCCGCATC  
GTTCTGCAGATTGACAATGCCCGTCTTGCTGCTGATGACTTTAGAGTCAA  
GTATGAGACAGAGCTGGCCATGCGCCAGTCTGTGGAGAACGACATCCATG  
GGCTCCGCAAGGTCATTGATGACACCAATATCACACGACTGCAGCTGGAG  
ACAGAGATCGAGGCTCTCAAGGAGGAGCTGCTCTTCATGAAGAAGAACCA  
CGAAGAGGAAGTAAAAGGCCTACAAGCCCAGATTGCCAGCTCTGGGTTGA  
CCGTGGAGGTAGATGCCCCCAAATCTCAGGACCTCGCCAAGATCATGGCA  
GACATCCGGGGCCCAATATGACGAGCTGGCTCGGAAGAACCGAGAGGAGCT  
AGACAAGTACTGGTCTCAGCAGATTGAGGAGAGCACCCACAGTGGTCACCA

CACAGTCTGCTGAGGTTGGAGCTGCTGAGACGACGCTCACAGAGCTGAGA  
CGTACAGTCCAGTCCTTGGAGATCGACCTGGACTCCATGAGAAATCTGAA  
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AGATGGAGCAGCTCAACGGGATCCTGCTGCACCTTGAGTCAGAGCTGGCA  
CAGACCCGGGCAGAGGGACAGCGCCAGGCCAGGAGTATGAGGCCCTGCT  
GAACATCAAGGTCAAGCTGGAGGCTGAGATCGCCACCTACCGCCGCCTGC  
TGGAAGATGGCGAGGACTTTAATCTTGGTGATGCCTTGGACAGCAGCAAC  
TCCATGCAAACCATCCAAAAGACCACCACCCGCCGGATAGTGGATGGCAA  
AGTGGTGTCTGAGACCAATGACACCAAAGTTCTGAGGCATTAA

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

ATGACTTCCTACAGCTATCGCCAGTCGTCGGCCACGTCGTCCTTCGGAGG  
CCTGGGCGGCGGCTCCGTGCGTTTTGGGCCGGGGGTCGCCTTTCGCGCGC  
CCAGCATTACGGGGGCTCCGGCGGCGCGGCGTATCCGTGTCCTCCGCC  
CGCTTTGTGTCCTCGTCCTCCTCGGGGGCCTACGGCGGCGGCTACGGCGG  
CGTCCTGACCGCGTCCGACGGGCTGCTGGCGGGCAACGAGAAGCTAACCA  
TGCAGAACCTCAACGACCGCCTGGCCTCCTACCTGGACAAGGTGCGCGCC  
CTGGAGGCGGCCAACGGCGAGCTAGAGGTGAAGATCCGCGACTGGTACCA  
GAAGCAGGGGGCCTGGGCCCTCCCGCGACTACAGCCACTACTACACGACCA  
TCCAGGACCTGCGGGACAAGATTCTTGGTGCCACCATTGAGAACTCCAGG

ATTGTCCTGCAGATCGACAATGCCCCGTCTGGCTGCAGATGACTTCCGAAC  
CAAGTTTGAGACGGAACAGGCTCTGCGCATGAGCGTGGAGGCCGACATCA  
ACGGCCTGCGCAGGGTGCTGGATGAGCTGACCCTGGCCAGGACCGACCTG  
GAGATGCAGATCGAAGGCCTGAAGGAAGAGCTGGCCTACCTGAAGAAGAA  
CCATGAGGAGGAAATCAGTACGCTGAGGGGCCAAGTGGGAGGCCAGGTCA  
GTGTGGAGGTGGATTCCGCTCCGGGCACCGATCTCGCCAAGATCCTGAGT  
GACATGCGAAGCCAATATGAGGTCATGGCCGAGCAGAACCGGAAGGATGC  
TGAAGCCTGGTTCACCAGCCGACTGAAGAATTGAACCGGGAGGTGCTG  
GCCACACGGAGCAGCTCCAGATGAGCAGGTCCGAGGTTACTGACCTGCGG  
CGCACCCCTTCAGGGTCTTGAGATTGAGCTGCAGTCACAGCTGAGCATGAA  
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AGCTGGCGCATATCCAGGCGCTGATCAGCGGTATTGAAGCCCAGCTGGGC  
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GGACATCAAGTCGCGGCTGGAGCAGGAGATTGCCACCTACCGCAGCCTGC  
TCGAGGGACAGGAAGATCACTACAACAATTTGTCTGCCTCCAAGGTCCTC  
TGA

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

ATGACCATGTATTTGTGGCTTAACTCTTGGCATTTGGCTTTGCCTTTCT  
GGACACAGAAGTATTTGTGACAGGGCAAAGCCCAACACCTTCCCCCACTG

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GCTGTCATTTCAACCACAACAATAGCTACTACTCCATCTAAGCCAACATG  
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CTAAATTATTTACAGCAAAGCTAAATGTTAATGAGAATGTGGAATGTGGA  
AACAACTTGCACAAACAATGAGGTGCATAACCTTACAGAATGTAAAAA  
TGCGTCTGTTTCCATATCTCATAATTCATGTACTGCTCCTGATAAGACAT  
TAATATTAGATGTGCCACCAGGGGTTGAAAAGTTTCAGTTACATGATTGT  
ACACAAGTTGAAAAAGCAGATACTACTATTTGTTTAAAATGGAAAAATAT  
TGAAACCTTTACTTGTGATACACAGAATATTACCTACAGATTTTCAGTGTG  
GTAATATGATATTTGATAATAAAGAAATTAAATTAGAAAACCTTGAACCC  
GAACATGAGTATAAGTGTGACTCAGAAATACTCTATAATAACCACAAGTT  
TACTAACGCAAGTAAAATTATTAACAGATTTTGGGAGTCCAGGAGAGC  
CTCAGATTATTTTTGTAGAAGTGAAGCTGCACATCAAGGAGTAATTACC  
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TACAATCAGTTTGGAGAAACAGAAGTGAATTTGTCTGAATTACATCCATA  
TCTACATAACATGAAGAAAAGGGATCCACCCAGTGAGCCGTCTCCACTAG  
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CCCATATGACTATAACAGAGTGCCACTTAAACATGAGCTGGAAATGAGTA  
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TGAAGGTTCTGAACCCACGAGTGGCACTGAGGGGCCAGAACATTCTGTCA  
ATGGTCCTGCAAGTCCAGCTTTAAATCAAGGTTCATAG

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

ATGCAGGAGCCGGGGCAGATTGTGGAGACCTACACGGAGGAGGATCCTGA  
GGGAGCCATGTCTGTAGTCTCTGTGGAGACCTCAGATGATGGGACCACTC  
GGCGCACAGAGACCACGGTCAAGAAAGTAGTGAAGACTGTGACAACACGG  
ACAGTACAGCCAGTCGCTATGGGACCAGACGGGTTGCCTGTGGATGCTTC  
ATCAGTTTCTAACAACCTATATCCAGACTTTGGGTCGTGATTTCCGCAAGA  
ATGGCAATGGGGGACCTGGTCCCTATGTGGGGCAAGCTGGCACTGCTACC  
CTTCCTAGGAACTTCCACTACCCTCCTGATGGTTATAGTCGCCACTATGA  
AGATGGTTATCCAGGTGGCAGTGATAACTATGGCAGTCTGTCCCGGGTGA  
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AGTAGACAGGATGTGTATGGGCCCCCAACCCAGGTTCTGGGTAGGTGGGAG  
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TCAAATGGAGATTGTGGACCATGCACTGCATGCCTTGACAGATGAAGTG  
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TGTGTGCTGGGCGCTGGACGTATGGTCGATACATCCGCTCTGCTCTGCGT  
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CTCGCAACAAAGAATTAATTGGTAAACATGCTATTCCTAACTTGGTAAAG  
AATCTGCCAGGAGGACAGCAGAACTCCTCTTGGAATTTCTCTGAGGACAC  
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CCGGAACCAAAAATCAGATAAGAAACCTGATCGGGAAGAAATTCAGATGA  
GCAATATGGGATCAAACACAAAATCACTAGATAACAACTATTCCACACCA  
AATGAGAGAGGAGACCACAATAGAACACTGGATCGATCGGGGGATCTAGG  
CGACATGGAGCCATTGAAGGGAACAACACCCTTGATGCAGAAGATTTAG

#### 5. d2egfp

CAAATAAAGCAATAGCACAAATTTACAAATAAATTTTTTTCACTGCATTCTTGTGGTTTGTCC  
AAACTCAATGTATCTTATCATACGAATTCCTACACATTATCCTAGCAGAAGCACATGCAGGGT  
GACGGTCCACGCTCTCCTGGGCACAAATGGGCAGCGTGCCATCCTGCTCCTCCACCTCCG  
GAAGCCATGGCTAAGCTTGTACAGCTCGTCCATGAGAGTGATCCCGGCGGTACGAACTCC  
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TCGGGCAGCAGCACGCCGTCGCCGATGGGGGTCTGCTGGTAGTGGTCGAGCTGCACGCT  
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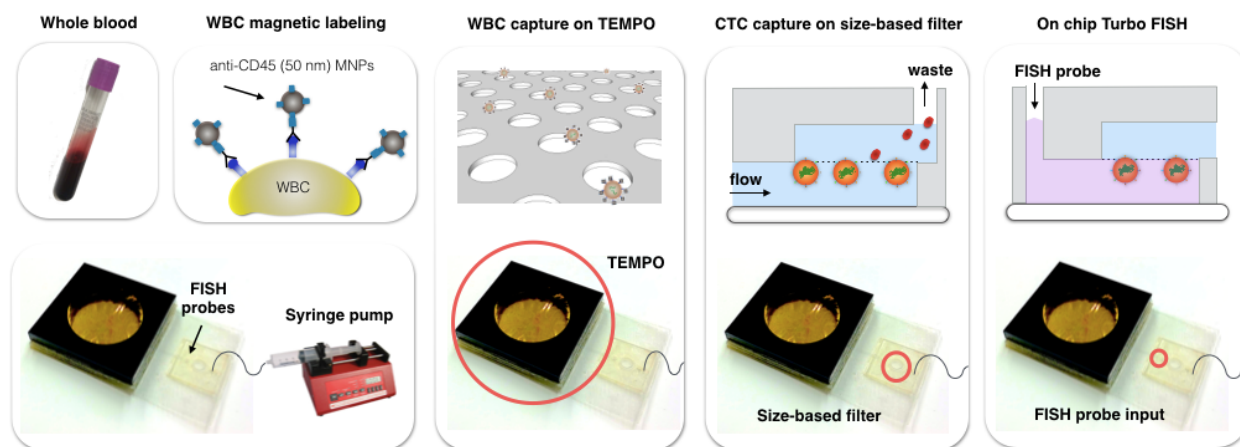
#### 6. Mouse ECad

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GTGTTGACGTCATCTGTCCCTATTGACAGTGAACAGAGGTGAGCACACTGATGACCAGAGT  
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CAAAATGCCAAAGGATGTATTGCTGCTTGGATCCGATACGTGATCTTCTGGAAAATGGCACC  
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AGAGTGAAGGTCTGGATCCAAGATGGTCTTGAGCTGCGTCATTATAGTCCTTTCTTGGTTGC  
AAAATTTTGTATTCGCCAATCTCTTGGTCTTTGTTCTGGTTATCGATGGCAGGAACTTGCAAT  
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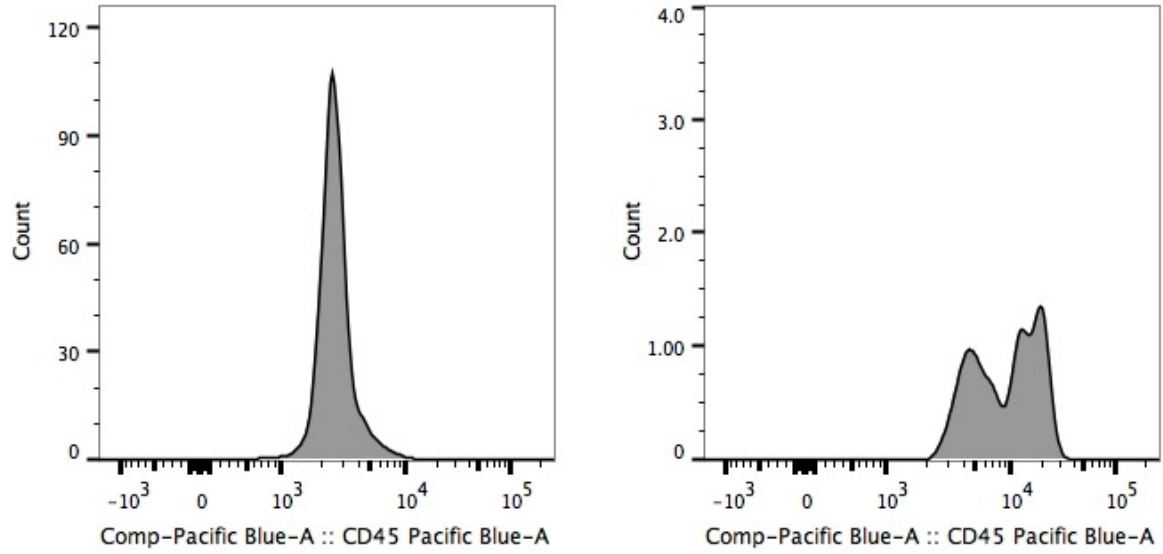
#### 7. human ECad

TCAAATTCACCTCTGCCCAGACTTTGAATCGGGTGTGAGGCCTTTTGACTGTAATCACATG  
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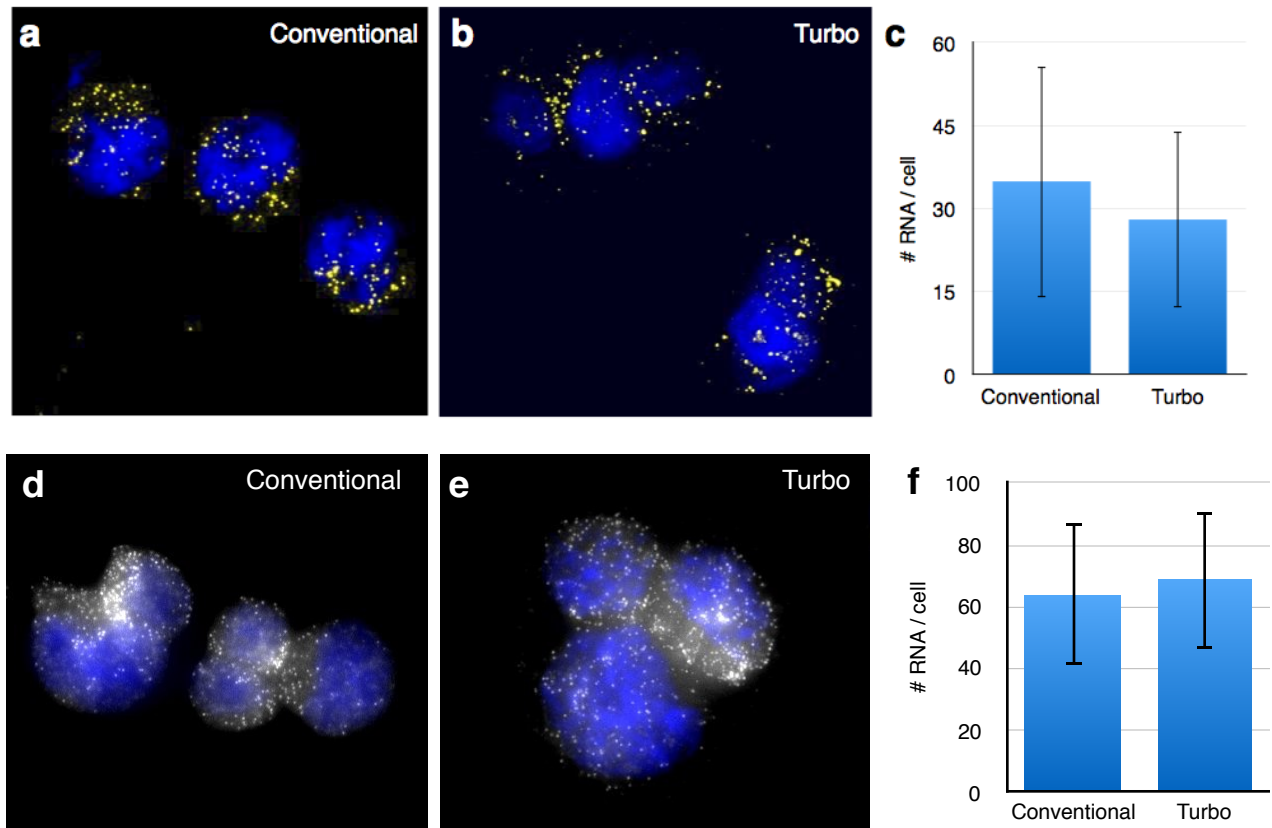
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CATCATCATTTGTTCACTGGATTTGTGGTGCAGTGTAGGATGTGATTTCTTCCATAAATGTG  
TCTGGCTTCTCTCCAAATCCGATATGTTTCACGTGCTCAAAATCCTCCTGGAGAACCATTGT  
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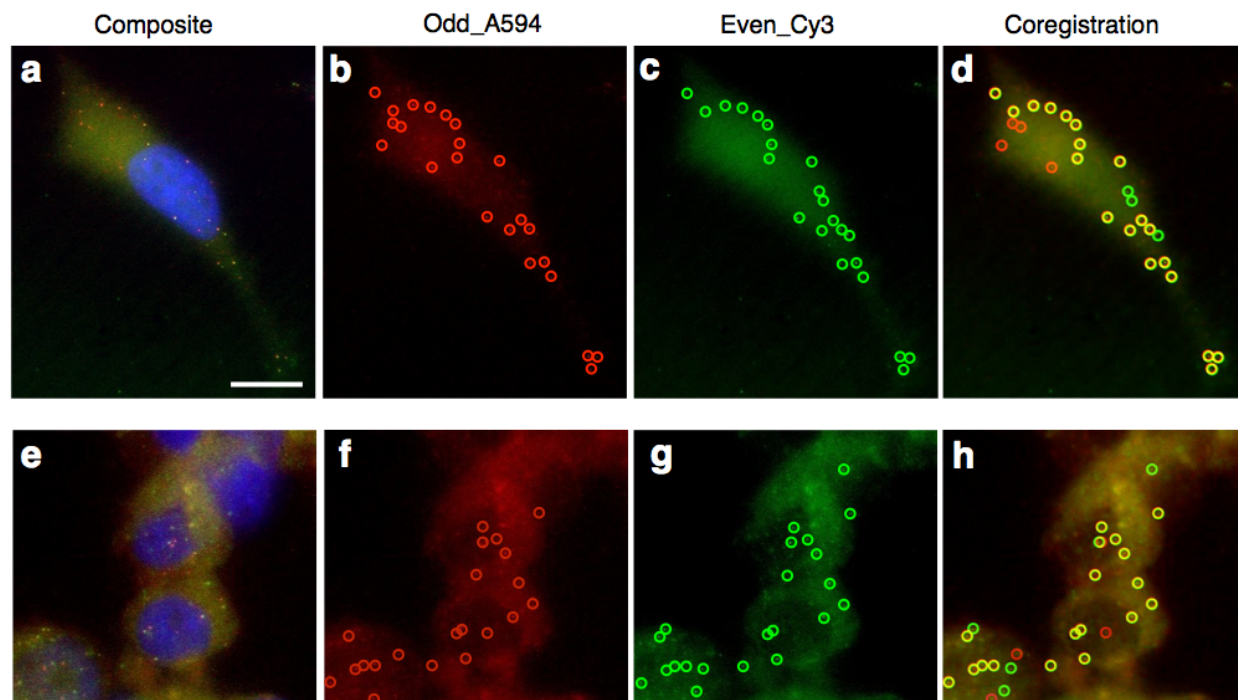
**Fig. S1. A Schematic of the step-by-step operation of our CaTCh FISH Chip.** From whole blood, WBCs are magnetically labeled with anti-CD45 MNPs for negative depletion. Labeled WBCs are captured on TEMPO at the upstream. Then, WBC-depleted whole blood travels through the device and potential CTCs are 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  $\mu$ m) **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.<sup>48</sup> 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 <sub>1</sub> (n=14) |                      |
| Pre-Treatment                                | 8 (57%)              |
| Post- Resection                              | 2 (14%)              |
| On Chemotherapy                              | 4 (29%)              |

<sup>1</sup> 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.