Supporting information:

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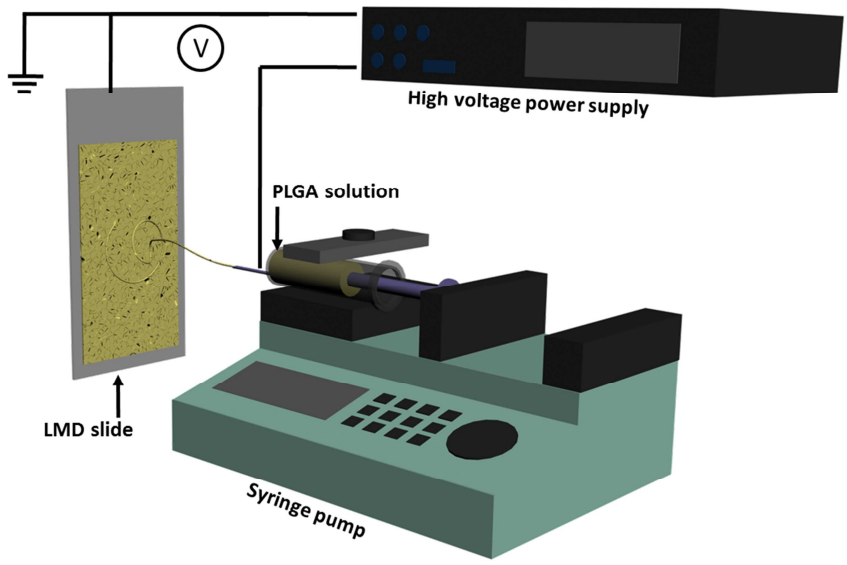
# 1. Experimental Section

## 1.1. Preparation of PLGA nanofiber (PN)-NanoVelcro substrate

We employed a previously described electrospinning method[1] for the deposition of PLGA nanofibers onto a commercial available laser micro-dissection (LMD) membrane slide. The PLGA working solution (10% w/v) was prepared by dissolving 1 g of PLGA pellets (DURECT Corporation) into 10 mL hexafluoroisopropanol (Sigma-Aldrich). Then the working solution was loaded into a glass syringe (Hamilton) and continuously ejected through a nozzle at a constant flow rate (0.2 µL $h^{-1}$) using an infusion pump (TS2-60, Baoding Longer). Meanwhile, an electric field of 15 kv/cm was applied between the nozzle and the ground collector by a high voltage power supply (Tianjin Dongwen high-voltage power supply co., ltd, China) **(Scheme S1)**. LMD membrane slides were placed in the middle of grounded collector for given durations to generate randomly orientated PLGA nanofibers with desired density and thickness. The resulting PN-NanoVelcro substrates were dried overnight in a vacuum chamber at room temperature.




**Scheme S1.**Experimental apparatus for deposition of electrospun PLGA nanofibers.

## 1.2. Fabrication of PDMS chaotic mixer

The PDMS chaotic mixer was fabricated based on a "soft-lithography" approach[2], which utilized a silicon mold with designed structures to replicate PDMS components of identical structure. In brief, a 3-inch wafer was spin-coated with a 100 µm layer of photoresist (SU8-2100, MicroChem Corp.), baked on a hot plate, and then exposed to UV light through a high-resolution mask using a top and bottom side aligner (Karl Suss MA6). Exposed wafer was developed and post-baked to obtain the first layer of photoresist structure, a serpentine

channel with a rectangular cross section (220 mm (L) x 1 mm (W) x 0.1 mm (H)). Then, the same wafer was spin-coated with another layer of photoresist, baked and precisely aligned to the second high-resolution mask. Similar procedures including UV exposure, development and post-bake was applied for the construction of photoresist herringbone structures on top of the serpentine channel. After these steps, the silicon mold for PDMS casting was produced.

Before usage, the silicon mold was treated with trimethylchlorosilane (TMSC) vapor. Well-mixed PDMS pre-polymer (RTV 615, GE Silicones; A and B in 10 to 1 ratio) was poured onto the silicon mold, degassed and baked in an oven at 80 $^{o}$C for 48 h. After polymerization, the PDMS sheet was carefully peeled off from the mold, cut into desired pieces and punched with two through holes for tubing connection.

Assembly and surface modification of PN-NanoVelcro chip: to assemble the PN-NanoVelcro chip, PDMS chaotic mixer chip was first placed on top of the PN-NanoVelcro substrate ensuring overlapping of the mixer channel and PLGA nanofiber. The two components were then sandwiched by a home-machined holder set in precise alignment. Afterwards, the whole device was secured by screws at four different corners, and therefore forms a sealed channel allowing the sample injection.

To achieve specific immobilization of CTCs, capture agent, biotiynlated anti-EpCAM, was conjugated on the PLGA nanofibers. Briefly, 0.5 mL EDC (8 mg mL$^{-1}$) and sulfo-NHS (2 mg mL$^{-1}$) prepared by 1 x PBS was slowly loaded into the channel to convert the carboxyl group on the terminal of PLGA molecule to an amine-reactive sulfo-NHS ester. Then, the channel was rinsed with multiple PBS solution to eliminate the free EDC and sulfo-NHS. Streptavidin (250 μg mL$^{-1}$) was then introduced into the channel and reacted with the sulfo-NHS ester. After removing free streptavidin molecules, biotinylated anti-EpCAM (8 μg mL$^{-1}$, in 500-μL PBS with 1% (w/v) BSA) was injected into the channel and incubated at room temperature for 1 h. PN-NanoVelcro chip coated with anti-EpCAM was then rinsed with multiple PBS and ready for subsequent sample processing.

## 1.3. Cell

While PC3 and LNCaP cells were obtained from ATCC, C4-2 cells, an androgen-independent derivative of LNCaP was developed by Dr. Chung's laboratory [3]. Prostate cancer cell lines were cultured in humidified incubator with 5% $CO_2$ using RPMI medium with 10% fetal bovine serum (Omega Scientific), 1% glutamine, and 1% Penicillin-Streptomycin (Invitrogen).Before use, cells were detached with sodium citrate (0.015 M sodium

citrate with 0.135 M KCl), washed and then resuspended in $Ca^{2+}$ and $Mg^{2+}$ free PBS.

## 1.4. Blood

Blood samples were obtained from prostate cancer patients at UCLA and CSMC under Institutional Review Board approved protocols at each site. Normal blood used for cell line spiking studies was obtained from the healthy volunteer donors from the age of 20 to 40. All blood specimens were collected into EDTA-containing vacutainer tubes (BD bioscience) according to the standard protocol and processed within 24 h.

## 1.5. Capture efficiency assessment

To validate and optimize experiment conditions, CellSearch™ CTC control kit containing 200/mL of pre-stained EpCAM-positive SK-BR-3 breast cancer cells, and pre-stained prostate cancer cells was spiked into PBS and normal blood as a model system for parameter optimization. For determining the exact cell number in the experiment, the suspended prostate cancer cells were stained with a lipophilic tracer DiO (Invitrogen) and then counted by a hemocytometer. The CellSearch™ CTC control kit was commercially fluorescently labeled and could then be counted in the hemocytometer . The preliminary number given by the hemocytometer was then used in a series of serial dilutions to achieve a goal concentration. Using that concentration, we plated the cells on a 48 well plate. The plate was then placed under a fluorescence microscope and an exact cell concentration was determined by averaging three wells. Based on the exact cell concentration, the specific number of cells was added to the blood or PBS in an eppendorf tube and then loaded into a 1 mL disposable syringe and introduced into the PN-NanoVelcro chip at constant flow rate by a syringe pump (KDS200, KD-Scientific). After processing, captured cell were fixed by 2% paraformaldehyde (PFA), stained with DAPI and enumerated under an automatic imaging system. The imaging system was composed of an upright fluorescent microscope (Eclipse 90i, Nikon) with the NIS-Element imaging software (Nikon), a precision motorized stage (ProScan II system, Prior Scientific), and a fluorescent light source (SPECTRAX, Lumencor). After automatically scanning through the entire substrate under a 4X objective, all the DAPI+/green fluorescence+ (for CellSearch CTC control kit) or DAPI+/DiO+(for pre-stained prostate cancer cells) events were automatically selected, and a manual confirmation followed to determine the exact number of cancer cells on the substrate. The capture efficiencies were calculated as the number of cancer cells captured on the substrate divided by the total number of cells loaded into the chip. In **Figure 2c**, when we were assessed the distribution of our CTCs in each channel, we plotted our figure according to the assigned channel numbers as illustrated in **Figure S1**.

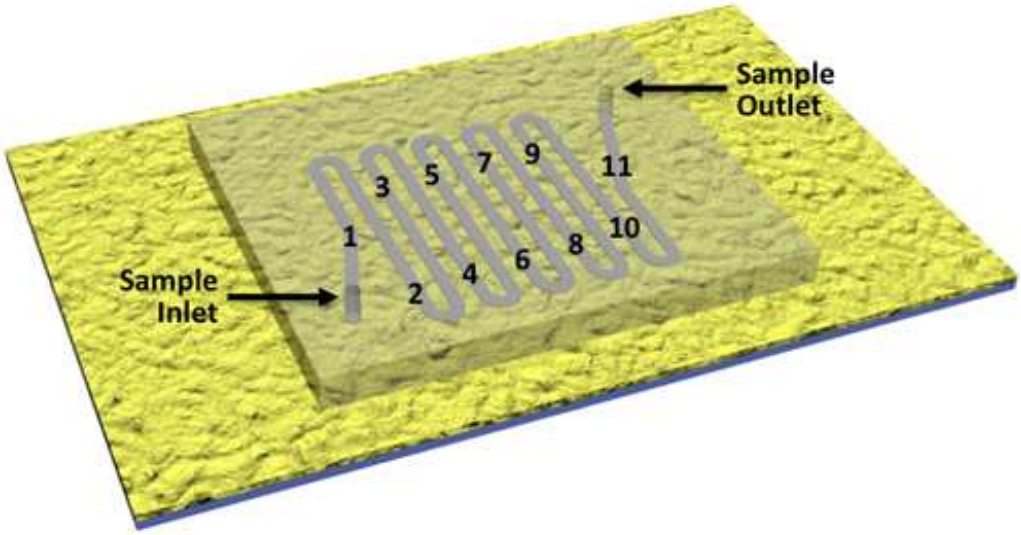


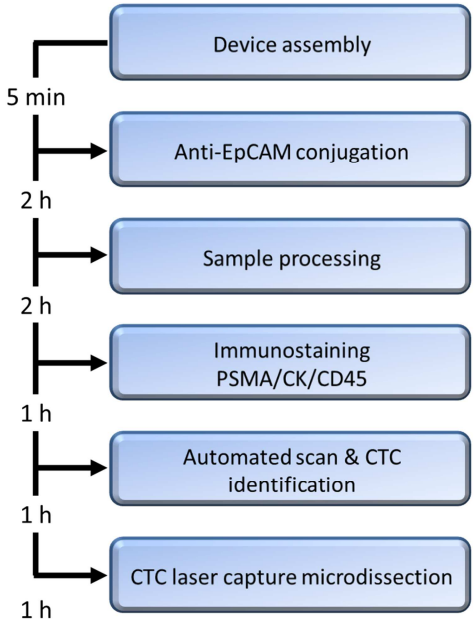

**Figure S1** Chip layout and the spatial locations of assigned channel numbers (indicated in **Figure 2c**)

## 1.6. CTC identification and registration under a fluorescent microscope

A modified protocol was employed for the cell line validation and patient sample study. Briefly, 1 mL patient sample and cancer cell spiked artificial sample were loaded in a syringe and run through the PN-NanoVelcro chip. Captured cells were fixed by methanol for 10 minutes at -20 $^{o}$C and then blocked with 2% BSA at room temperature for 30 minutes. An antibody cocktail consisting of FITC-conjugated anti-PSMA, PE-conjugated anti-CK and Cy5-conjugated anti-CD45 in 2% BSA was used for immunocytochemistry staining. For the validation studies, the sample was then mounted with DAPI mounting solution and then subject to the imaging. To identify CTCs, the PN-NanoVelcro substrate was scanned by above mentioned automatic imaging system. We first scanned through the substrate under 4X objective, creating a mosaic micrograph for the determination of fluorescence intensity of all automatic counted events. X-Y scatter plots summarizing the CK and CD45 expressions of individual cells (including CTCs and WBCs) on a NanoVelcro substrate were exported and plotted in Microsoft Excel. After identifying potential cancer cells populations in the Excel plot, we set the limitations in the NIS-Element review and previewed the 4X micrographs. The micrographs in **Figure 4** were taken under a 10X objective after manually confirming and enumerating the cancer cells by their morphology.

For the validation of our staining and CTC characterization protocol, we performed a control study using five samples of healthy donors' blood. There were no CTCs fitting our criteria in the study. For real patient samples for sequencing, the substrate were not stained with DAPI. After staining, the PN-NanoVelcro substrate was first washed by PBS and then treated with ethanol and frozen dry. After identifying the

PSMA+/CK+/CD45- cells in the abovementioned microscope, CTCs' locations were recorded for subsequent laser capture dissection (LCM) **(Scheme S2)**.




**Scheme S2** Workflow and respective operation time in each steps of PN-NanoVelcro Chip for real patient CTC isolation

## 1.7. CTC laser capture microdissection (LCM) isolation:

Before LCM-based single-CTC isolation, the CTC immobilized on the PN-NanoVelcro Chips are first identified and registered using the first fluorescent microscope (Nikon 90i) in conjunction with an auto-scan imaging software (Nikon, Element), please see more details in Experimental section 1.6. In order to achieve the isolation of high purity CTCs, a second microscope, the ArcturusXT$^{TM}$ LCM System (Applied Biosystems$^{TM}$) was utilized to eliminate WBC contamination by selective dissecting identified CTCs. Briefly, CTCs identified with the imaging system were further confirmed by the ArcturusXT$^{TM}$ LCM system at three different fluorescent wavelengths (488nm, 546nm and 674nm). Afterwards, a CapSure$^{TM}$ HS Cap was placed on top of the region of identified CTCs. Then, an 810 nm IR laser beam was applied to melt the polymer membrane on the cap. The resultant conical polymer pillar, so called sticky finger, dropped down and adhered onto the PN-NanoVelcro substrate. In the following, a 355 nm UV laser beam was utilized to cut through the PN-NanoVelcro substrate in a designed route around CTCs excluding surrounding WBCs. Finally, the HS Caps with dissected CTCs were stored in -20°C until next step analysis was performed.

## 1.8. Viability test

To test the viability of captured cancer cells, prostate cancer cell lines were spiked into RPMI culture medium and processed through the PN-NanoVelcro chips. Meanwhile, the same batch of harvested cells was loaded to a 48 well plate and maintained in the incubator. Two hour after the sample processing, the viability of captured cells and the control groups were evaluated using a Calcein AM/Ethidium bromide fluorescent assay (Invitrogen). The live cells are defined as any cells stained by Calcein AM but not Ethidium bromide. The viability was obtained by dividing the live cells counts by the total cell counts **(Figure S2)**. As there was clear overlap of error bars between the groups, there should not be any significant difference in the study.

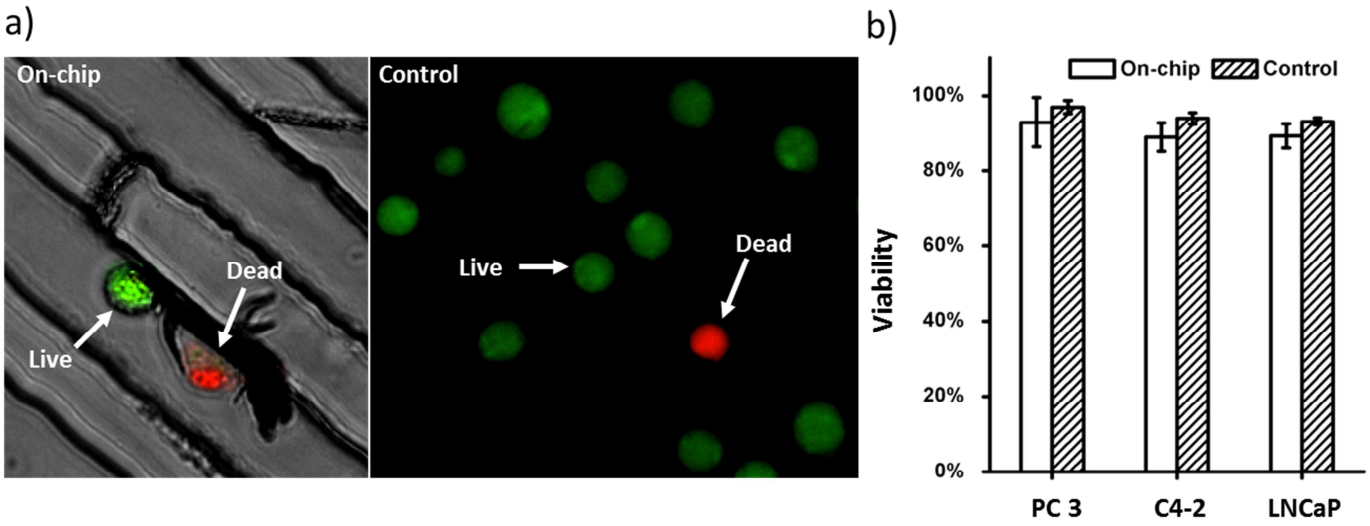
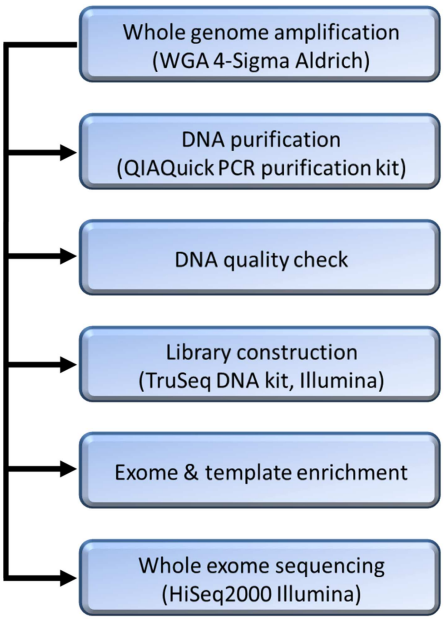



**Figure S2** Viability of captured cell lines was evaluated using a fluorescent assay LIVE (green) /DEAD (red). a) fluorescent image of live cells (green) and dead cell (red); b) Viability test were conducted 2 h after sample processing, and the same batch of cell lines suspended in 24 well plates with culture medium were tested at the same incubation time. Error bars show standard deviations (n=3-4).

## 1.9. General procedure for CTC whole exome sequencing

Isolated CTC first went through whole genome amplification. Successfully amplified samples were selected for PCR purification. The purified products were then subject to DNA quality check before library construction. Standard exome capture and template enrichment were conducted afterwards. Finally, HiSeq 2000 whole exome sequencing was performed. The workflow was summarized in **Scheme S3**.




**Scheme S3** Workflow of WGA and Exome-Seq

### 1.9.1. Whole genome amplification

Genomic DNA from LCM captured CTCs was subjected to WGA using the GenomePlex Single Cell WGA4 Kit (Sigma-Aldrich) following manufacturer's protocol. First, the Capsure™ HS Caps were placed into the alignment tray. The extraction buffer containing 1 µL lysis and proteinase K digestion solution in 10 µL of deionized water was added to the file ports of the caps. 0.5-mL microcentrifuge tubes were placed over the fill ports of each cap following by 1 h incubation in 50°C oven. Then, the tubes along with the caps were spun in a microcentrifuge to bring the buffer to the bottom of the tube. Then, DNA fragmentation and library preparation was performed followed by 25 cycles of PCR amplifications. The PCR products were checked by gel electrophoresis **(Figure S3a)**. Amplified DNA with desirable bands was subject to PCR clean up by QIAQuick PCR Purification Kit (Qiagen). DNA concentration was then determined by a NanoDrop™ spectrophotometer and then stored at -80°C before sequencing.

### 1.9.2. Whole exome sequencing

The WGA DNA was first quality checked by the bioanalyzer (2100 Bioanalyzer, Agilent Life Sciences and Chemical Analysis) **(Figure S3b and Table S1)**. DNA library was then prepared using the TruSeq DNA kit (Illumina) followed by exome enrichment using SeqCap EZ Human Exome library (v3.0, Roche). Enriched

exome DNA was then put on Illumina CBot for template enrichment. Sequencing was performed on Illumina HiSeq2000 platform with pair-end 100 bp runs.

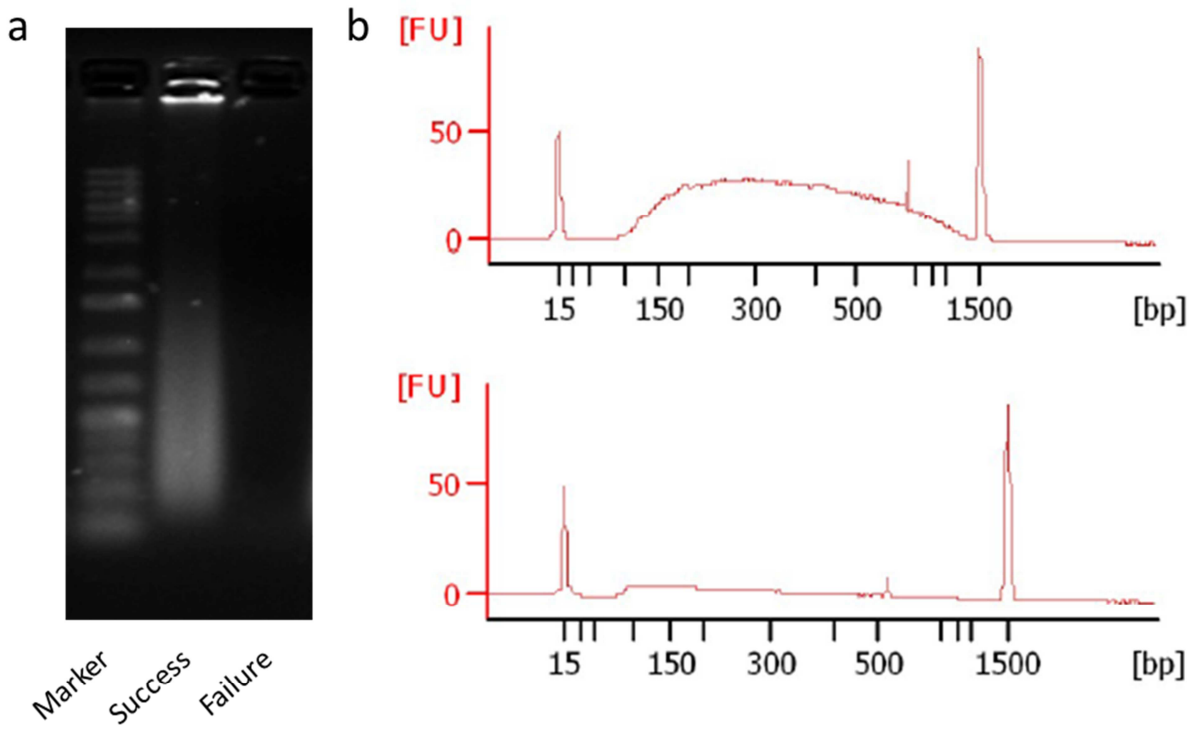



**Figure S3** Quality check of WGA DNA products. a) Gel electrophoresis was utilized to determine if the DNA is amplified; b) bioanalyzer was employed to analyze the distribution of amplified DNA length.

| | **Total number** | **WGA success** | **DNA QC pass** | **Exome-Seq** |
|---|---|---|---|---|
| Single CTCs | 25 | 9 | 6 | 2 |
| Pooled CTCs | 7 | 7 | 4 | 1 |
| Single WBCs | 6 | 2 | 2 | 0 |
| Pooled WBCs | 6 | 2 | 2 | 1 |

**Table S1** Summary of sample numbers of our patient in each step

## 1.10. Exome data analysis

To remove the WGA adapter sequences, the reads was trimmed first by Trimmontic[4] for exactly 20 bases before alignment. BWA 0.6.2[5] was used for the alignment to UCSC hg19 reference sequence. After removing PCR duplicates by Picard 1.72, GATK 2.2.3 was used for indel-realignment[6] (**Table S2**). Single nucleotides

polymorphisms (SNPs) were called by SOAPsnp 1.05[7]. In each sample, putative SNPs were filtered by following criteria: 1) a Q20 quality cutoff; 2) covered by at least 6 reads; 3) Rank-sum-test p-value > 0.01; 4) average copy number of nearby region <2; 5) the SNPs had a 5bp away from each other; 6) the SNPs are located in the areas where all 4 exomes have coverage (**Table S3, Figure S4**). The circular visualization map was generated by Circos[8].

| **Sample** | **Number of reads** | **Mapped reads** | **Inside of exome capture area** | | | |
|---|---|---|---|---|---|---|
| | | | Coverage regions | Mapped reads | GC percentage | Mean coverage |
| CTC-1 | 94933687 | 82144903 | 24.67% | 40835940 | 45.66% | 40.63X |
| CTC-2 | 66847027 | 59462273 | 25.05% | 29627847 | 45.32% | 29.28X |
| CTC-pooled | 77017440 | 62872068 | 35.50% | 30585565 | 45.58% | 30.34X |
| WBC | 114649901 | 99090242 | 78.37% | 47225895 | 45.73% | 47.90X |

**Table S2** The statistical summary of the exome data

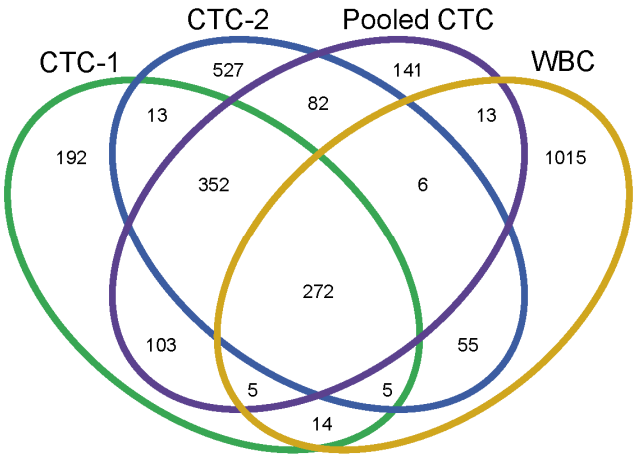


**Figure S4** The Venn diagram of the shared and individual mutations in the four exomes

| | CTC-1 | CTC-2 | CTC-pooled | WBC |
|---|---|---|---|---|
| C:G->A:T | 185 | 136 | 192 | 152 |
| C:G->G:C | 132 | 123 | 127 | 102 |
| C:G->T:A | 214 | 179 | 210 | 244 |
| T:A->A:T | 79 | 82 | 87 | 103 |
| T:A->C:G | 296 | 724 | 308 | 688 |
| T:A->G:C | 50 | 68 | 50 | 96 |
| SUM | 956 | 1312 | 974 | 1385 |

**Table S3.** Summary of SNP patterns in four exomes

## 2. Supporting Reference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