

## SUPPLEMENT INFORMATION

### CryoGrid-PIXUL-RNA: High throughput RNA isolation platform for tissue transcript analysis

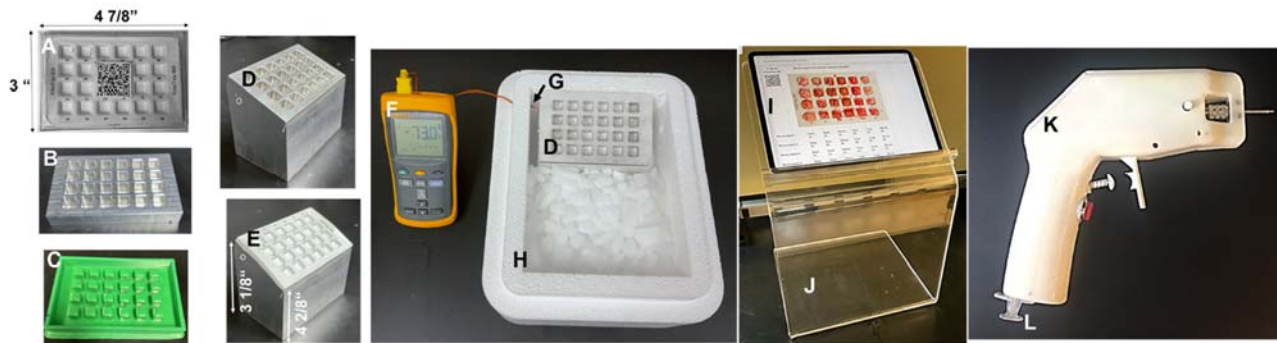
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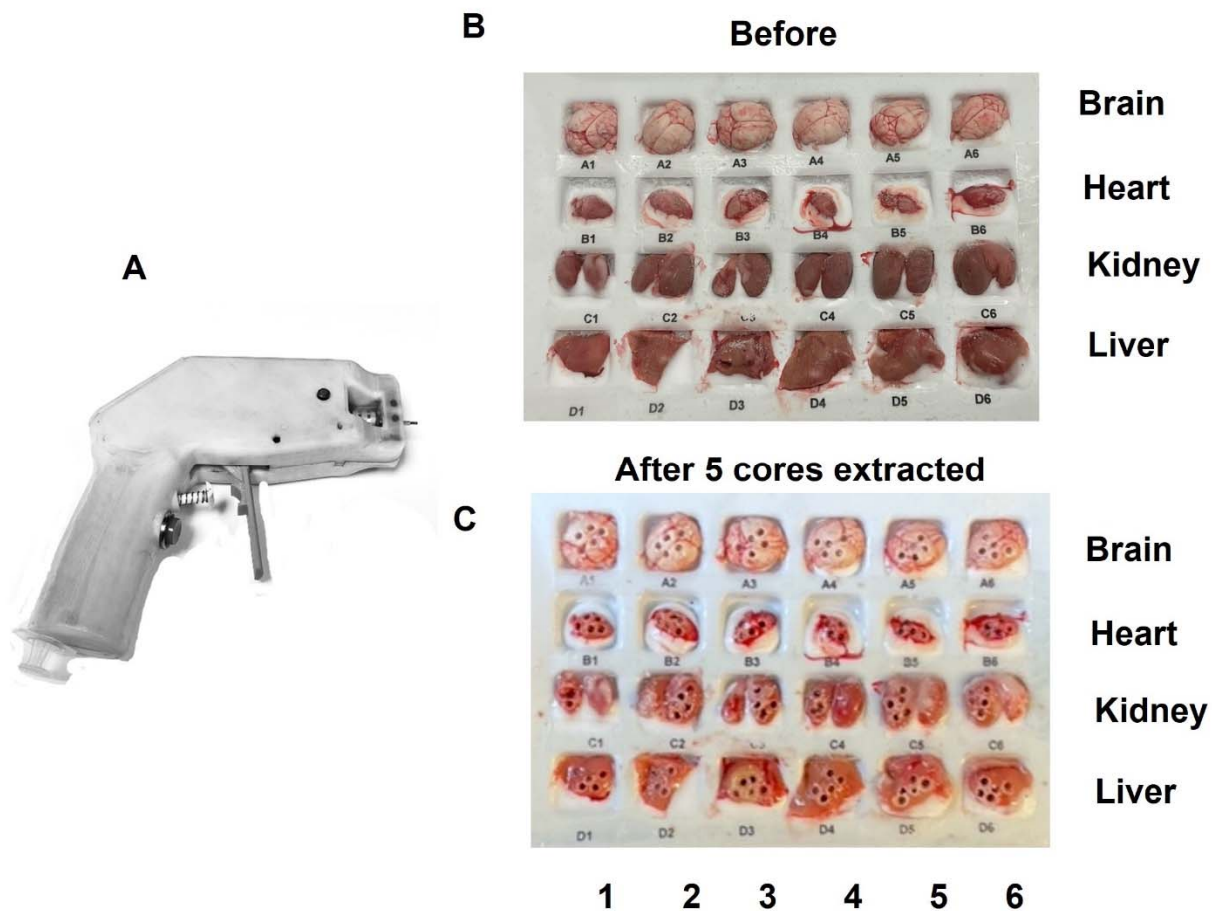
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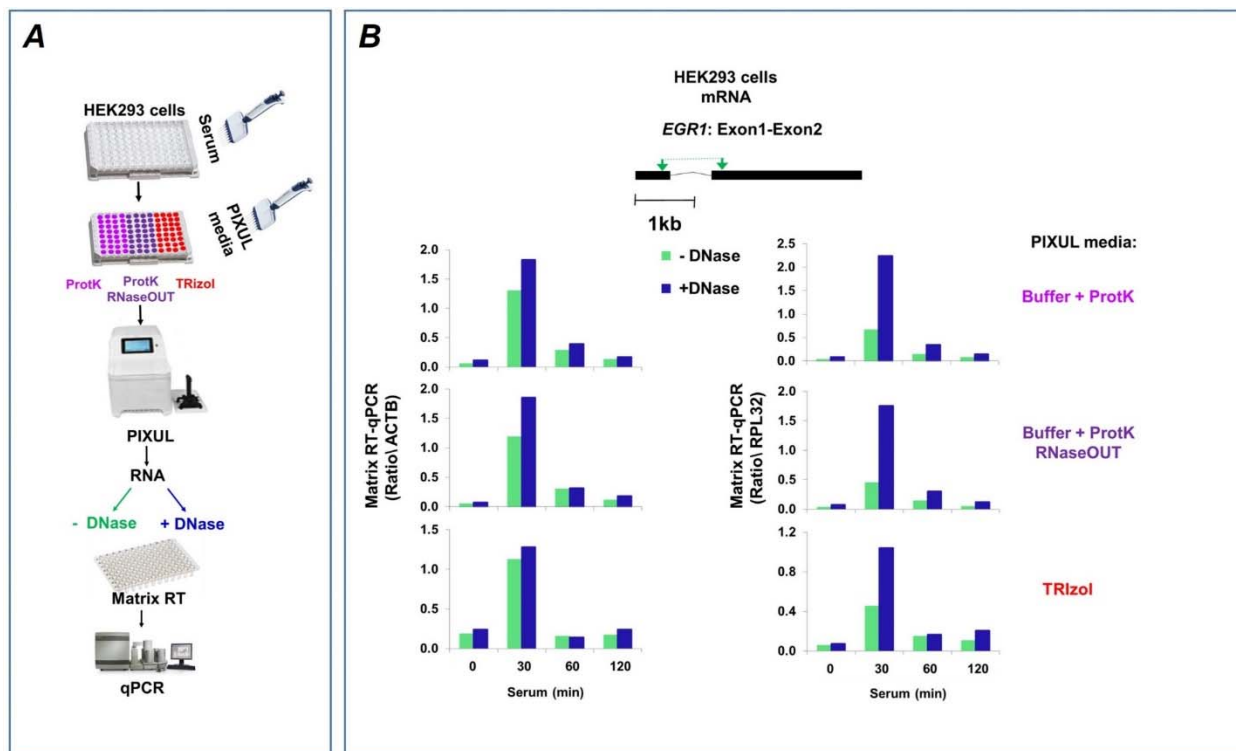
## FIGURES



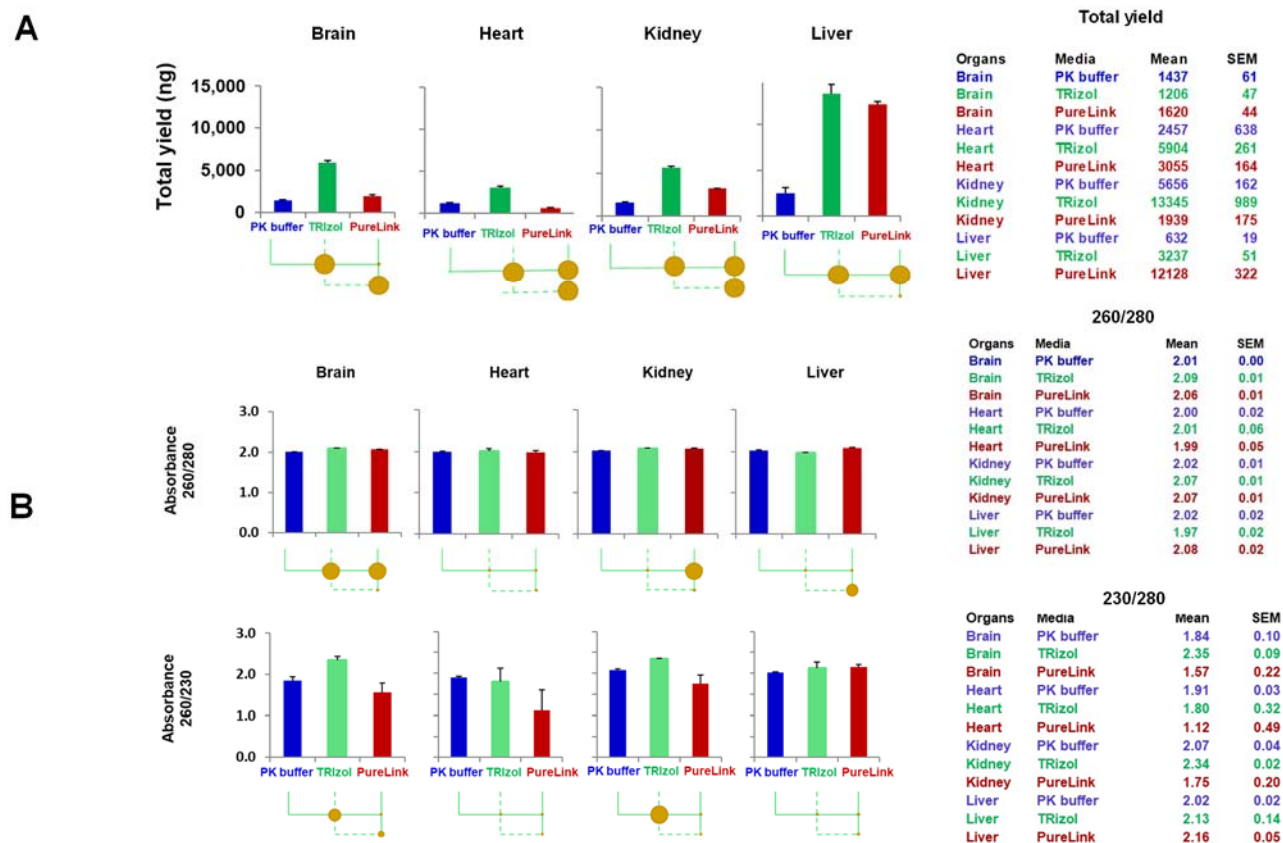
**Fig.S1. CryoGrid system components.** CryoTray dimensions of a standard plate (A) are casted in-house by placing polystyrene sheets (0.4mm) on heated (135°C) 24-cuboid sockets aluminum block (B) and then overlaying and pressing the sheet with a matching 24-cuboid plugs plastic stamp (C). The CryoTray dimensions match an off-the-shelf lid (Cytiva #7704-1001). QR code sticker is secured on the inside of the lid and linked to Google Drive applications. CryoBlock is a 24-pockets aluminum block with a tilted top (30°) to improve ergonomics (D) which serves as a CryoTray receptacle (E) providing access for coring tissues. Fluke thermometer (F) with a thermocouple probe (G) is inserted into the CryoBlock (D) and monitors temperature (<-70°C). CryoBlock is chilled in an off-the-shelf 11"x11"x9" Styrofoam box (CryoBox) containing dry ice (H). iPad (I) on a stand (J) displays a layout of tissues and metadata from an online Google sheet by scanning CryoTray QR code to guide tissue sampling with the CryoCore (K). CryoCore is a miniature coring drill with a trephine burr. Trephine is made from stainless steel tubing shaft (2mm x 30mm) and a smaller diameter tip (1mm x 1-2mm) with axially oriented cutting teeth around the tip. A push-button switch (red) activates the motor to drill into the tissue and then extract a core. Trephine has two holes with axes oriented perpendicular to shaft. A clamping mechanism uses two segments of silicone tubing with semicircular notches at their ends to close around the shaft holes, creating a seal. The clamp is actuated by a cable in a manner similar to a bicycle caliper brake. A pump mechanism utilizes a plunger and cylinder and two check valves to both draw buffer from a 5ml syringe (L) and to generate a fluid jet to eject the extracted core (18,62).



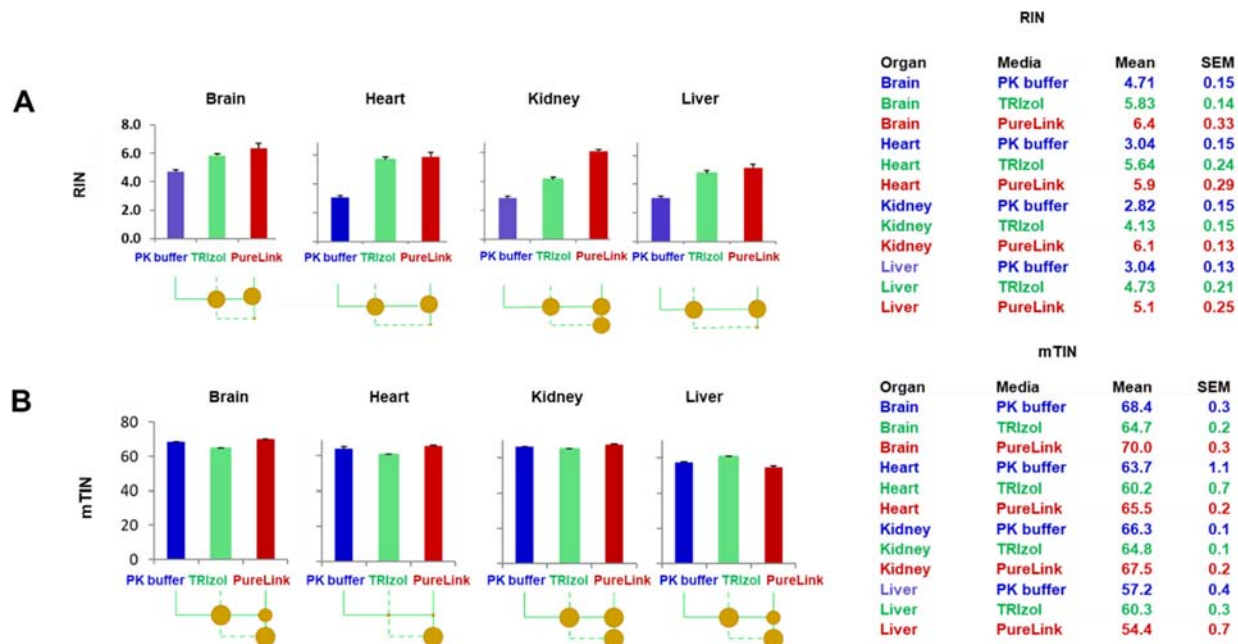
**Fig.S2. CryoTray with tissues before and after multiple CryoCore sampling.** A, CryoCore was used to sample mouse organs. B-C, CryoTray with frozen mouse organs were photographed before and after multiple CryoCore sampling, illustrating the size ( $\sim 1\text{-}2\text{ mm}^3$ ) and the number of cores that can be extracted from an organ as small as the mouse heart ( $<150\text{mg}$ ).



**Fig.S3. PIXUL-Matrix-RT-qPCR analysis with and without DNase of serum inducible *EGR1* in 96-well human HEK293 cultures.** **A**, Serum-deprived HEK293 96-well cultures were treated with serum for 0, 30, 60 and 120min. Culture media was aspirated and replaced with either elution buffer-proteinase K, with and without RNaseOUT, or TRIZOL. Plates were treated in PIXUL, and RNA was isolated and either treated (+DNase) or not (-DNase) with DNase I. RNA was used in Matrix RT-qPCR. **B**, Human *EGR1* primer spanning Exon1-Exon2 used is shown. *EGR1* mRNA data are expressed as a ratio to *ACTB* (left) and *RPL32* (right) genes.

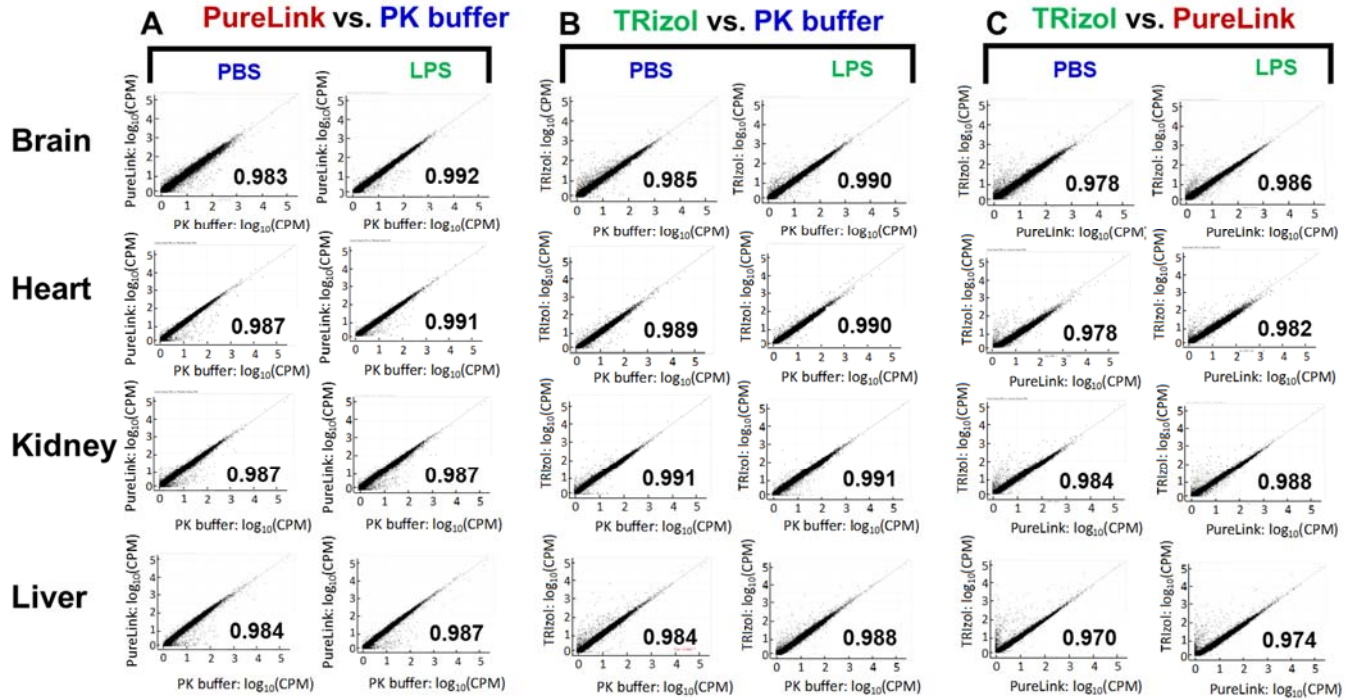


**Fig.S4. Yields and quality of RNA isolated with PIXUL using PK buffer, TRIzol and PureLink column from mouse brain, heart, kidney and liver measured by NanoDrop.** Frozen mouse organs in CryoTrays were sampled with the CryoCore and RNA was isolated using PIXUL with either PK buffer, TRIzol or PureLink column. **A**, RNA yields measured by NanoDrop. **B**, NanoDrop absorbance is shown as 260/280 (upper row) and 260/230 (lower row). Data are represented as mean  $\pm$  SEM, n=4 for each frozen organ. Statistical differences between PK buffer and TRIzol (*P*-value) are shown by the size of the solid circle under the x-axis;  $P \leq 0.05$  by a medium circle  $P \leq 0.01$  by a large circle, the tiny circle indicating the differences are not statistically significant (25).

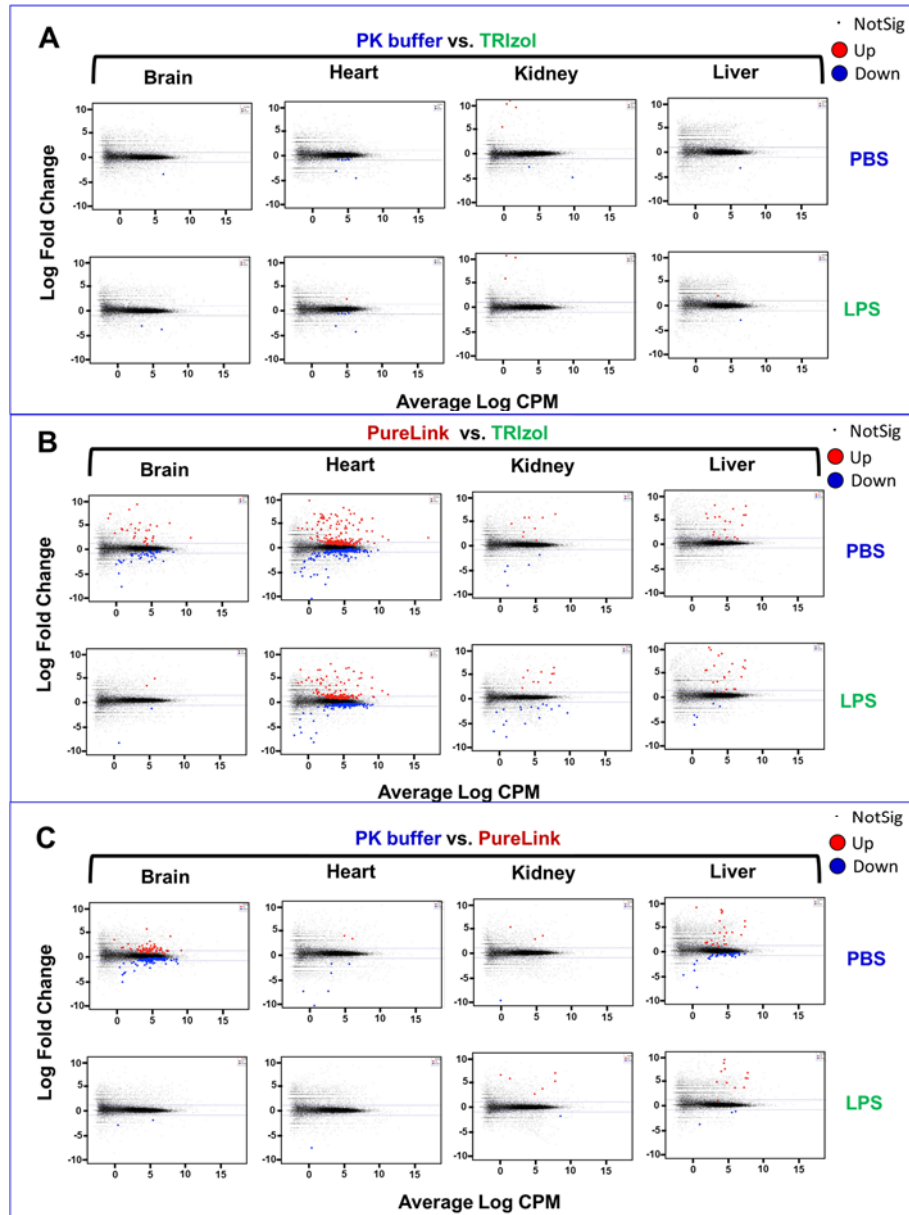


**Fig.S5. Sequencing quality assessed by RIN and mTIN.** **A**, High Sensitivity RNA ScreenTape® was used to assess the RNA integrity numbers (RIN) (50). **B**, Median Transcript Integrity Number (mTIN (28)) for each isolation method. Data are represented as mean  $\pm$  SEM, n=4 for each RNA isolation method.



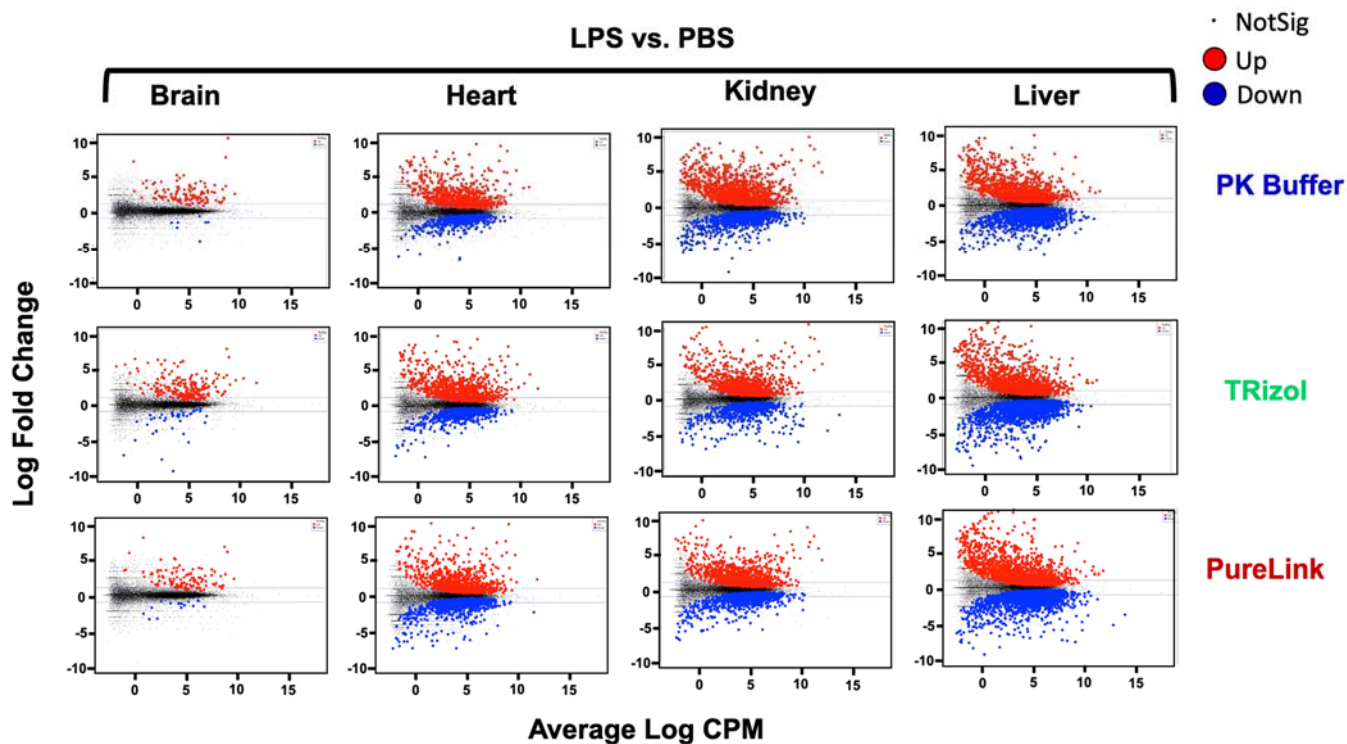


**Fig.S6.** Scatter plots of correlation analysis of PK buffer, TRizol and PureLink columns of brain, heart, kidney and liver of RNA-seq normalized count data from frozen organs of septic (LPS IP injected) and control (PBS IP injected) mice. Results are shown as log<sub>10</sub> counts per million reads mapped (CPM). **A**, x-axis PK buffer and y-axis PureLink column. **B**, x-axis PK buffer and y-axis Trizol **C**, x-axis PureLink columns and y-axis Trizol Numbers in the body of the graphs show correlation coefficients. Each dot represents a mean of two sets of frozen mouse organs.

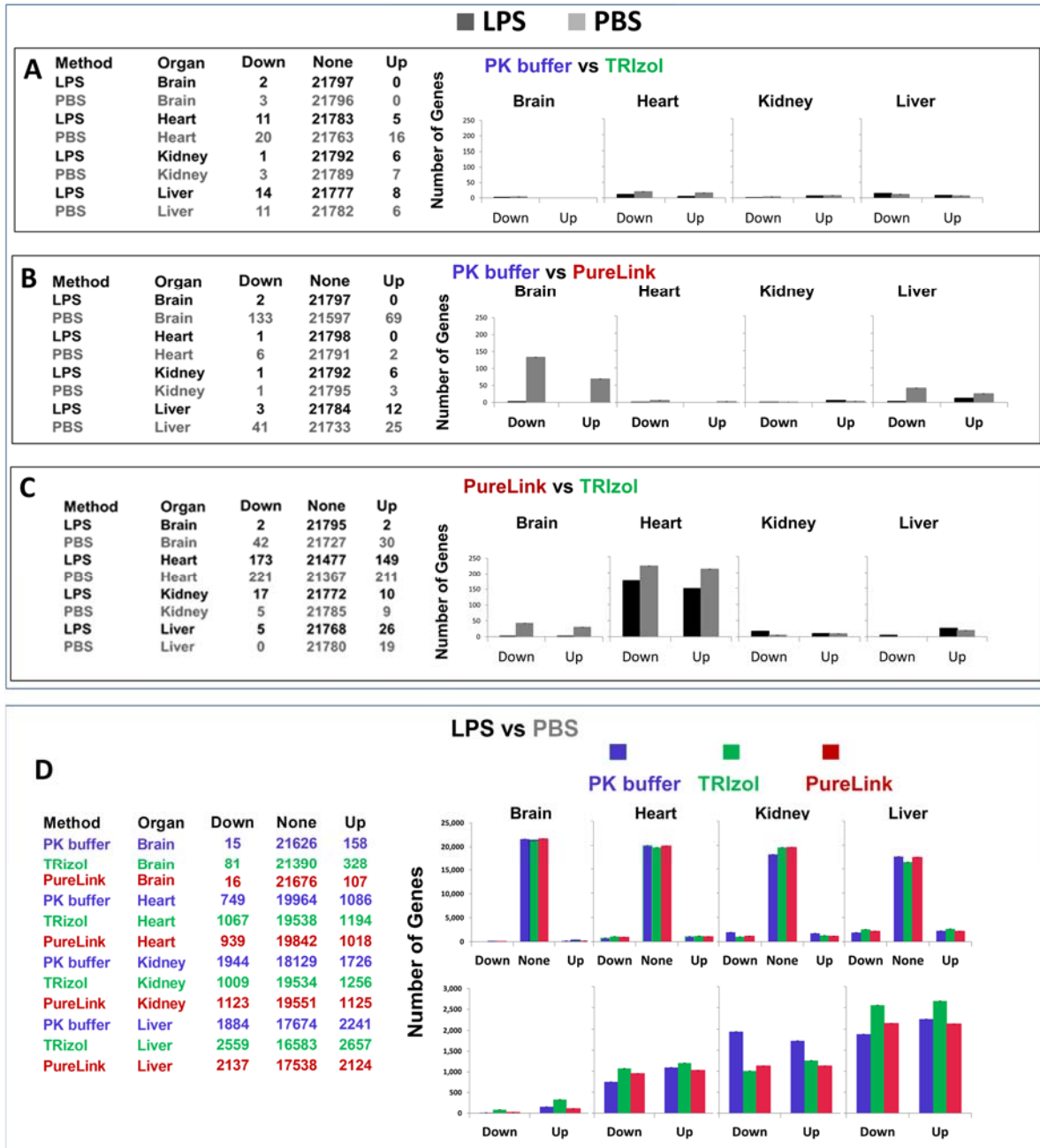


**Fig.S7. ‘EdgeR’ Differential gene expression shown with mean-difference (MD) plots between the different RNA isolation methods.** Comparison of brain, heart, kidney and liver transcripts from LPS treated compared to control (PBS) treated mice. MD plot shows log fold change, y-axis and average log of counts per million (CPM), x-axis. Red and blue dots indicate transcripts that are statistically increased or decreased, respectively. Black dots represent transcripts that were not significantly differentially expressed. **A**, transcripts extracted with PK buffer compared to TRIzol from frozen brain, heart, kidney and liver from PBS- (upper panel) and LPS-treated mice. **B**, transcripts extracted with PureLink column compared to TRIzol from frozen brain, heart, kidney and liver from PBS- (upper panel) and LPS-treated mice. **C**, transcripts extracted with PK buffer compared to PureLink column from frozen brain, heart, kidney and liver from PBS- (upper panel) and LPS-treated mice.





**Fig.S8. ‘EdgeR’ Differential gene expression of LPS vs PBS treated mice shown with mean-difference (MD) plots for the different RNA isolation methods.** Comparison of frozen brain, heart, kidney and liver transcripts from LPS-treated compared to PBS-treated (control) mice, *upper panel* PK buffer, *middle panel* TRizol and *lower panel* PureLink column extracted RNA, respectively. y-axis log fold change; x-axis average log CPM.



**Fig.S9. RNA-seq data of differentially expressed genes in LPS endotoxin and PBS (control) treated mice.** A-C, numbers of transcripts that were different in frozen organs from LPS and PBS treated mice comparing PK buffer vs. TRIZol (A), PK buffer vs. PureLink (B) and PureLink vs. TRIZol (C). D, PK buffer, TRIZol and PureLink columns comparison. Table shows the number of genes data for each group used to generate plots on the right. Right upper panels show comparison of the number of frozen organ transcripts extracted with either PK buffer, TRIZol or PureLink columns from LPS compared to PBS treated mice that were upregulated (Up), downregulated (Down) or were not changed (None). To better show the differences the right lower panels shows comparison of the number of organ transcripts extracted with either PK buffer, TRIZol or PureLink column from LPS compared to PBS treated mice that were upregulated (Up) or downregulated (Down).

## TABLES

**Table S1. Hardware and labware**

| Item Description                                            | Manufacturer/Supplier   | Location         | Part No.    |
|-------------------------------------------------------------|-------------------------|------------------|-------------|
| PIXUL™ 96-well sonicator                                    | Matchstick Technologies | Kirkland, WA     | P01199-01A  |
|                                                             | Active Motif            | Carlsbad, CA     | 53130       |
| CryoTray                                                    | Matchstick Technologies | Kirkland, WA     | P01200-01A  |
| CryoBlock                                                   | Matchstick Technologies | Kirkland, WA     | P01201-01A  |
| CryoCore                                                    | Matchstick Technologies | Kirkland, WA     | P01202-01A  |
| CryoCore trephines                                          | Matchstick Technologies | Kirkland, WA     | P01203-01A  |
| PlateHandle                                                 | Matchstick Technologies | Kirkland, WA     | P01204-01A  |
| Fluke 52-II Dual Probe Thermometer                          | Fluke Corporation       | Everett, WA      | 674689      |
| Fluke 80PJ-1 Bead Probe                                     | Fluke Corporation       | Everett, WA      | 750422      |
| Small Dual Angle Beta Radiation Shield (used as iPad stand) | Universal Medical Inc.  | Oldsmar, FL      | UM3400      |
| iPAD Pro                                                    | Apple                   | Cupertino, CA    | Model A2377 |
| NextSeq2000                                                 | Illumina                | San Diego, CA    | 20038897    |
| 4200 TapeStation                                            | Agilent                 | Santa Clara, CA  | G29991BA    |
| Invitrogen™ Qubit™ 3 Fluorometer                            | ThermoFisher            | Waltham, MA      | Q33216      |
| NanoDrop 1000 Spectrophotometer                             | ThermoFisher            | Waltham, MA      |             |
| Eppendorf ThermoMixer® C                                    | Eppendorf               | Hamburg, Germany | 5382000023  |
| Costar 96-well polypropylene round-bottom (used in PIXUL)   | Corning                 | Oneonta, NY      | 3365        |
| GeneMate 96-well 0.2ml plate semi skirt                     | VWR                     | Radnor, PA       | 490003-794  |
| MicroAmp Optical Adhesive Film (used in PIXUL)              | Applied Biosystems      | Carlsbad, CA     | 4311971     |
| E1-ClipTip™ Electronic Multichannel Pipettes                | ThermoFisher            | Waltham, MA      | 4671090     |
| 7900HT Fast Real-Time PCR System with 384-Well Block Module | ThermoFisher            | Waltham, MA      | 4329001     |

**Table S2. Kits and enzymes**

| Item Description                                    | Manufacturer/Supplier | Location        | Part No.         |
|-----------------------------------------------------|-----------------------|-----------------|------------------|
| Zymo-Seq RiboFree Total RNA Library Kit             | Zymo Research         | Tustin, CA      | R3003            |
| Zymo RNA Clean & Concentrator-5                     | Zymo Research         | Tustin, CA      | R1016            |
| PureLink™ RNA Micro Kit                             | Invitrogen            | Carlsbad, CA    | 12183016         |
| SpeedBeads™ magnetic carboxylate modified particles | MilliporeSigma        | Burlington, MA  | GE65152105050250 |
| SuperScript IV Reverse Transcriptase                | ThermoFisher          | Waltham, MA     | 18090050         |
| RNaseOUT™ Recombinant Ribonuclease Inhibitor        | ThermoFisher          | Waltham, MA     | 10777-019        |
| RNase-Free DNase I                                  | Lucigen               | Middleton, WI   | E0013-1D4        |
| DNase I 10X Reaction Buffer                         | Lucigen               | Middleton, WI   | SS000751-D2      |
| Invitrogen™ Proteinase K, recombinant-Invitrogen    | ThermoFisher          | Waltham, MA     | 25530015         |
| Standard Sensitivity Large Fragment Analysis Kit    | Agilent               | Santa Clara, CA | DNF-492          |
| High Sensitivity RNA ScreenTape Ladder              | Agilent               | Santa Clara, CA | 5067-5581        |
| High Sensitivity RNA ScreenTape Sample Buffer       | Agilent               | Santa Clara, CA | 5067-5580        |
| High Sensitivity RNA ScreenTape                     | Agilent               | Santa Clara, CA | 5067-5579        |
| NextSeq™1000/2000 P2                                | Illumina              | San Diego, CA   | 20046811         |
| Colibri™ Library Quantification Kit                 | Invitrogen            | Carlsbad, CA    | A38524500        |
| Quant-it dsDNA High-Sensitivity Assay Kit (Qubit)   | Invitrogen            | Carlsbad, CA    | Q33120           |

**Table. S3. qPCR primers**

| Species | Gene                     | Purpose | Forward                 | Reverse               |
|---------|--------------------------|---------|-------------------------|-----------------------|
| Mouse   | <i>Rlp32</i> Exon2       | RT      | TTAAGCGAAACTGGCGGAAAC   | TTGTTGCTCCATAACCGATG  |
| Mouse   | <i>Actb</i> Exon3-Exon4  | RT      | CTAAGGCCAACCGTGAAAAG    | GCATACAGGGACAGCACA    |
| Mouse   | <i>Alb</i> Exon 15       | RT      | TGAAGACTCAGGACTCATCTTTT | CAGCACAGAGACAAGAAGTC  |
| Mouse   | <i>Fxyd2</i> Exon 6      | RT      | CAGCTTCTCTAACACCCAC     | GATTCATTGAAAACCAGGGGG |
| Mouse   | <i>Tnnt2</i> Exon15      | RT      | CCAATGCAGACTCCTGTTTG    | TGGCTTTTTATTGCTGGCAT  |
| Mouse   | <i>Ngal</i> Exon5-Exon6  | RT      | TCAAGGACGACAACATCATCT   | CACTCACCACCCATTCAGTT  |
| Mouse   | <i>Syn 1</i> Exon13      | RT      | AGGTGAAAGCTGAGACCATC    | TGTCTAGGGGTTTAGGGGTT  |
| Human   | <i>RLP32</i> Exon2-Exon3 | RT      | AGTTCCTGGTCCACAACGTC    | TTGGGGTTGGTGACTCTGAT  |
| Human   | <i>ACTB</i> Exon3-Exon4  | RT      | AGAGCTACGAGCTGCCTGAC    | AAGGTAGTTTCGTGGATGCC  |
| Human   | <i>EGR1</i> Exon1-Exon2  | RT      | TGACCGCAGAGTCTTTTCCT    | TGGTTTGGCTGGGGTAACT   |
| Human   | <i>EGR1</i> Exon2        | RT      | GCTGAGCTGAGCTTCGGTTC    | TCGCCGCTACTCAGTAGGTA  |
| Human   | <i>NR4A1</i> Exon7       | RT      | TGACCCACGATTTGTCTTA     | ACGGCGCTATGTGTTAA     |
| Human   | <i>NR4A1</i> Exon7-Exon8 | RT      | CACAGCTTGCTTGTCGATGT    | CATGCCGGTCGGTGATGA    |