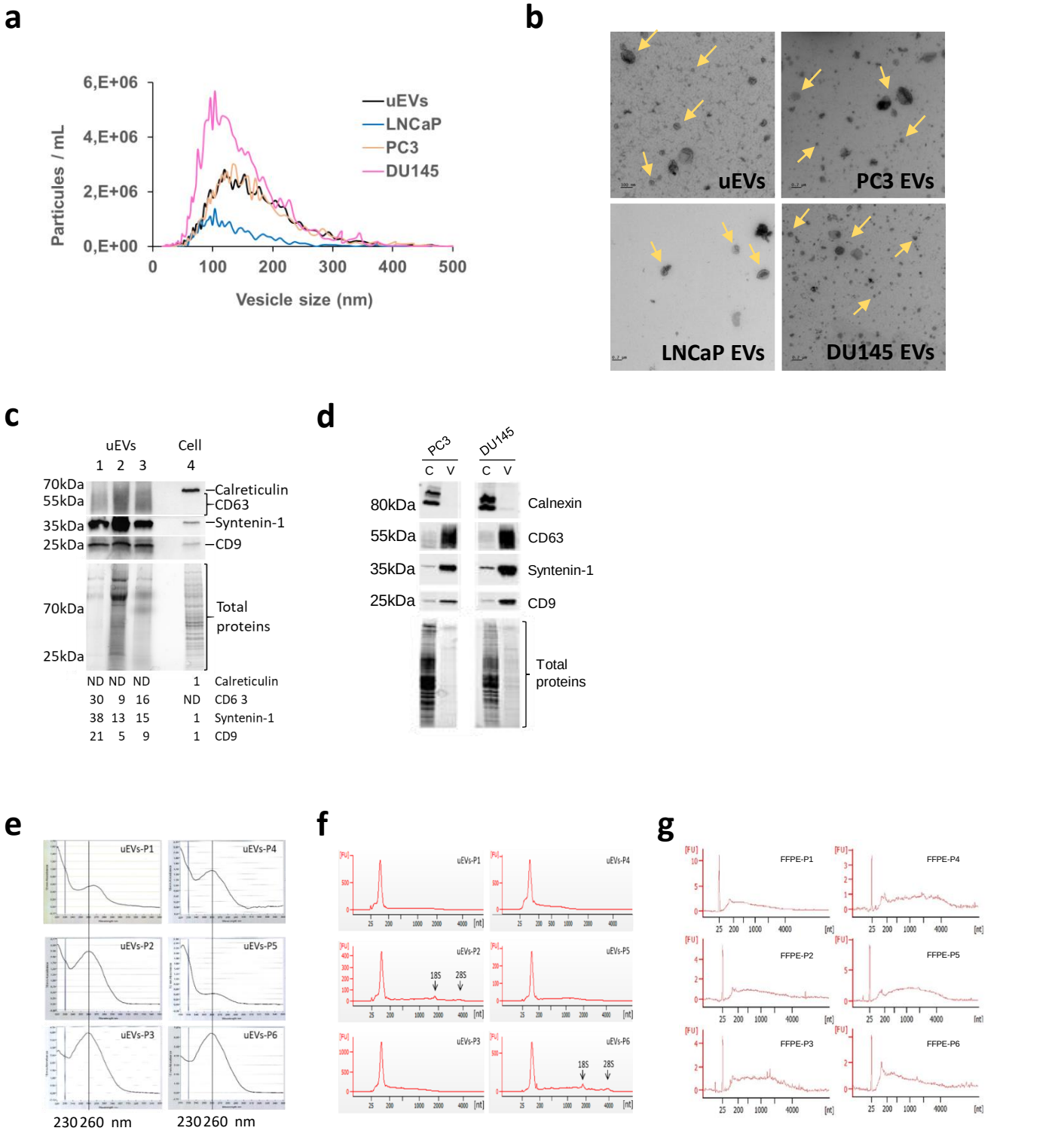
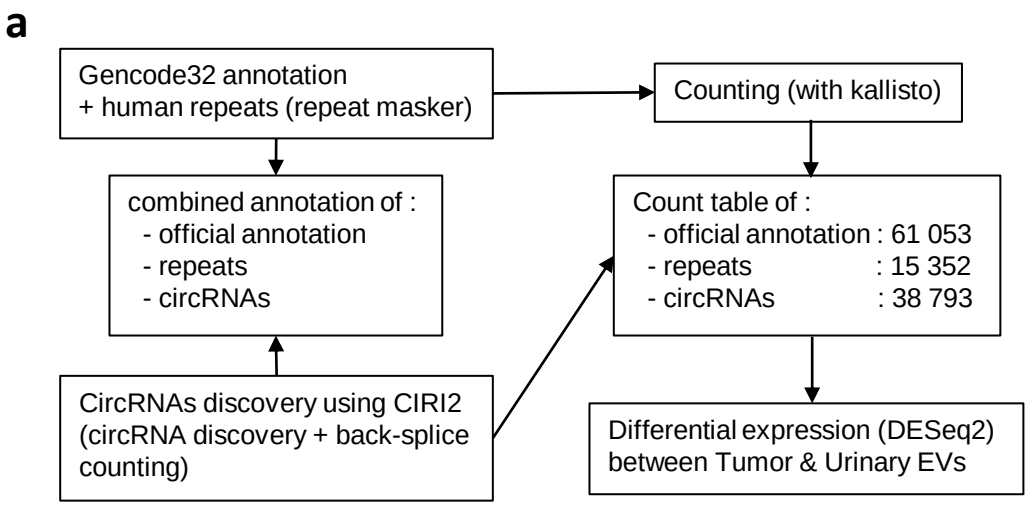
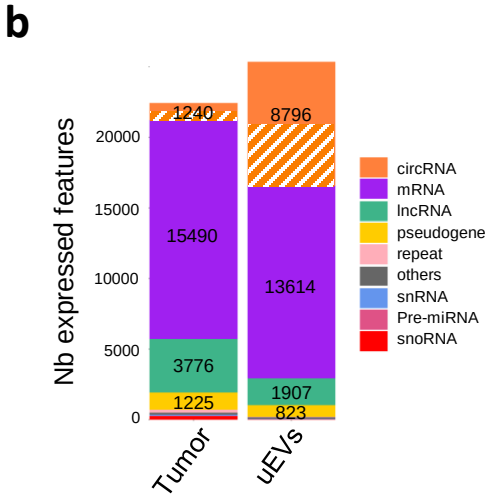
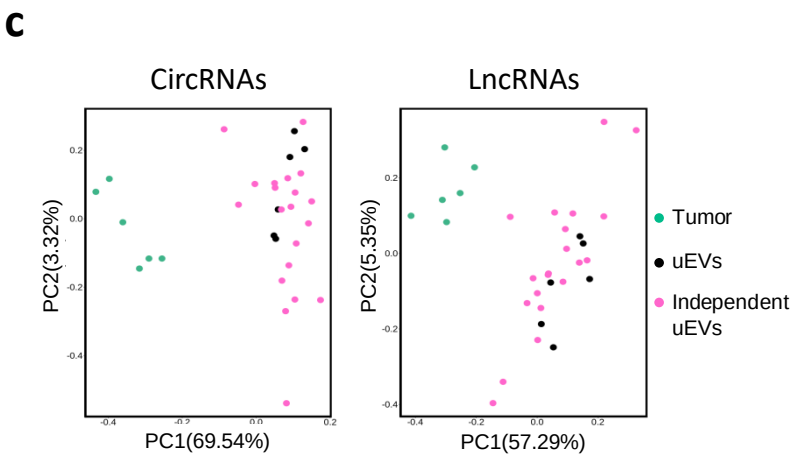


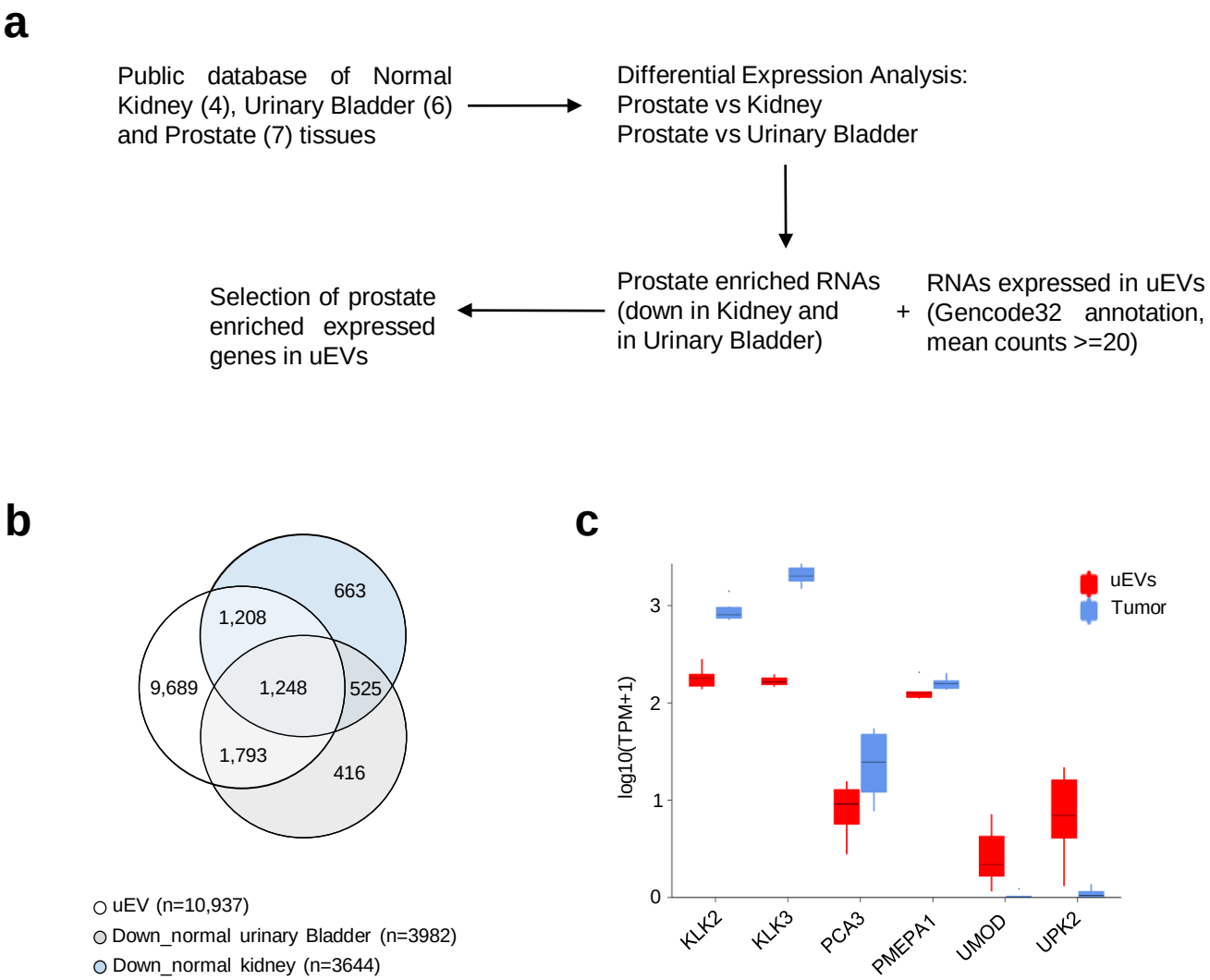
Extended Data Fig. 1. **Characterization of EVs isolated from urine and culture supernatant.** **a**. Characterization of EVs by nanoparticle tracking analysis (NTA) showing average distribution of vesicle size coming from urine (black), PC3 (orange), LNCaP (bleu) and DU145 (pink) cell lines. **b.** Transmission electron microscopy images of EVs from urine, PC3, LNCaP and DU145 cell lines. Scale bar sizes are 100 and 200 nm for urinary EVs and cell EVs respectively. Yellow arrows show some EVs with variable size. **c.** The pellets recovered from 3 ultracentrifuged urines (lines 1,2,3) and HEK293 cell lysate (line 4) were analyzed by Western blot for the indicated proteins side by side. Stain free gel images of total amounts of proteins were used to quantify the relative level of analyzed proteins. **d.** Western blot analysis, for the indicated proteins side by side, of PC3 and DU145 cell lysates (C) and enriched cell-EVs (V). Total amounts of proteins were shown by stain free gel images. **e.** Optical density profiles of RNAs giving their purity. Position of 230 and 260 nm absorbance are shown by black vertical lines. **f and g.** RNA Profiles analyzed by capillary electrophoresis giving their quality. Electropherograms show in the y-axis fluorescence units (FU) and in the x-axis the nucleotide length (nt) of 10 ng of RNA extracted from uEVs (f) and 0.5 ng from FFPE (g) paired samples. Peaks at 25 nucleotides represent internal standards and peaks at 2,000 nt and 4000 nt represent 18S and 28 S ribosomal RNAs, respectively.



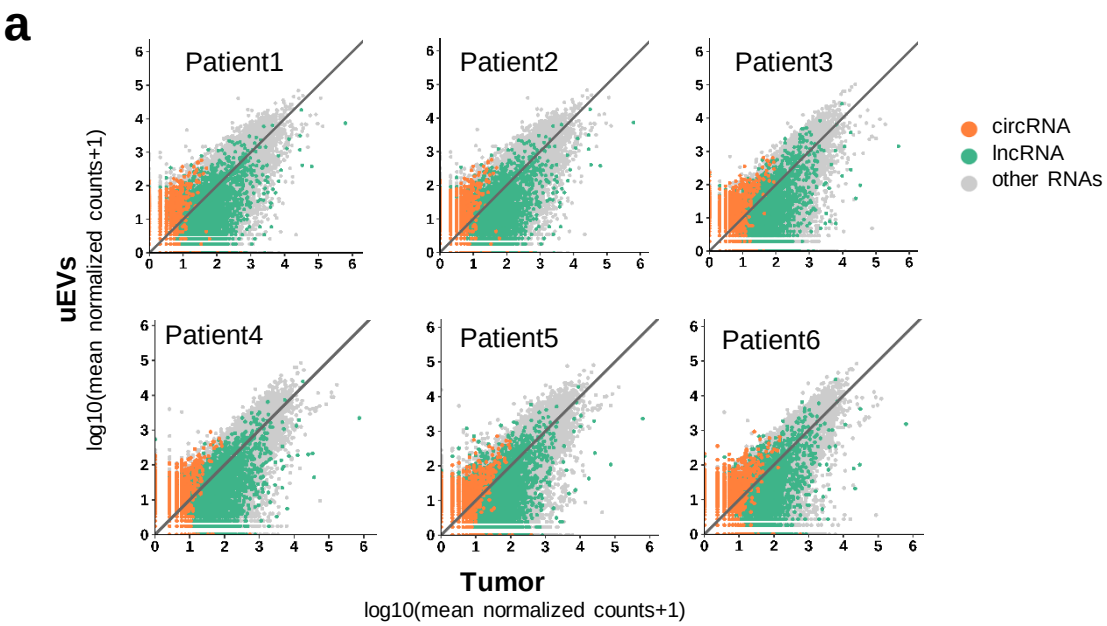


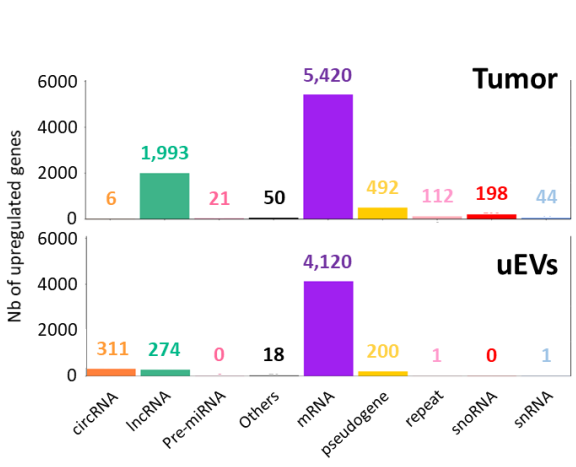


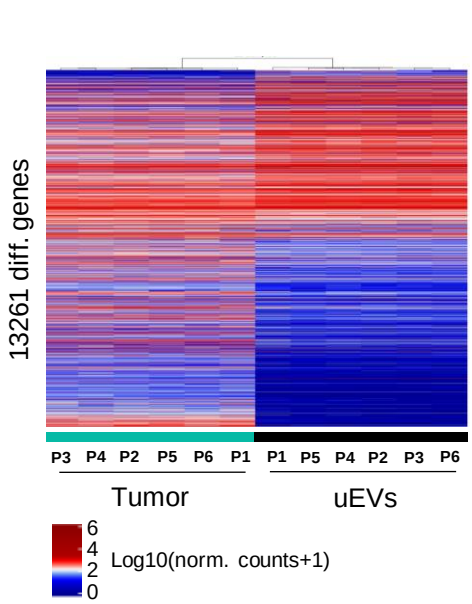
Extended Data Fig. 2. Full transcriptome of paired liquid and solid biopsies of prostate cancer patients. **a**. Bioinformatic procedure for differential RNA expression analysis between Tumor biopsies and uEVs. The number of total official annotated RNAs, repeats and circRNAs found in Tumor and uEvs are indicated. **b.** Number of expressed RNA features in Tumor and uEVs: 1240 and 8796 circRNAs (orange) including respectively 819 (66%) and 4530 (52%) present in circBase (hatched orange), 15490 and 13614 mRNA (violet), 3776 and 1907 lncRNAs (green), 1225 and 823 pseudogenes (yellow), 214 and 43 repeats (pink), 63 and 35 snRNAs (blue), 53 and 7 Pre-miRNAs (dark pink), 245 and 31 snoRNAs (red), 136 and 100 others (miscRNA, ribozyme, rRNA, scaRNA, scRNA, sRNA, tRNA; grey), respectively. **c**. Principal component analysis from expression of 311 circRNAs (left) and 274 lncRNAs (right) upregulated in uEVs (FC>= 1.5, at least 20 counts), in 6 paired tumors (green) and uEVs (black) and 20 independent uEVs (pink), from prostate cancer patients.



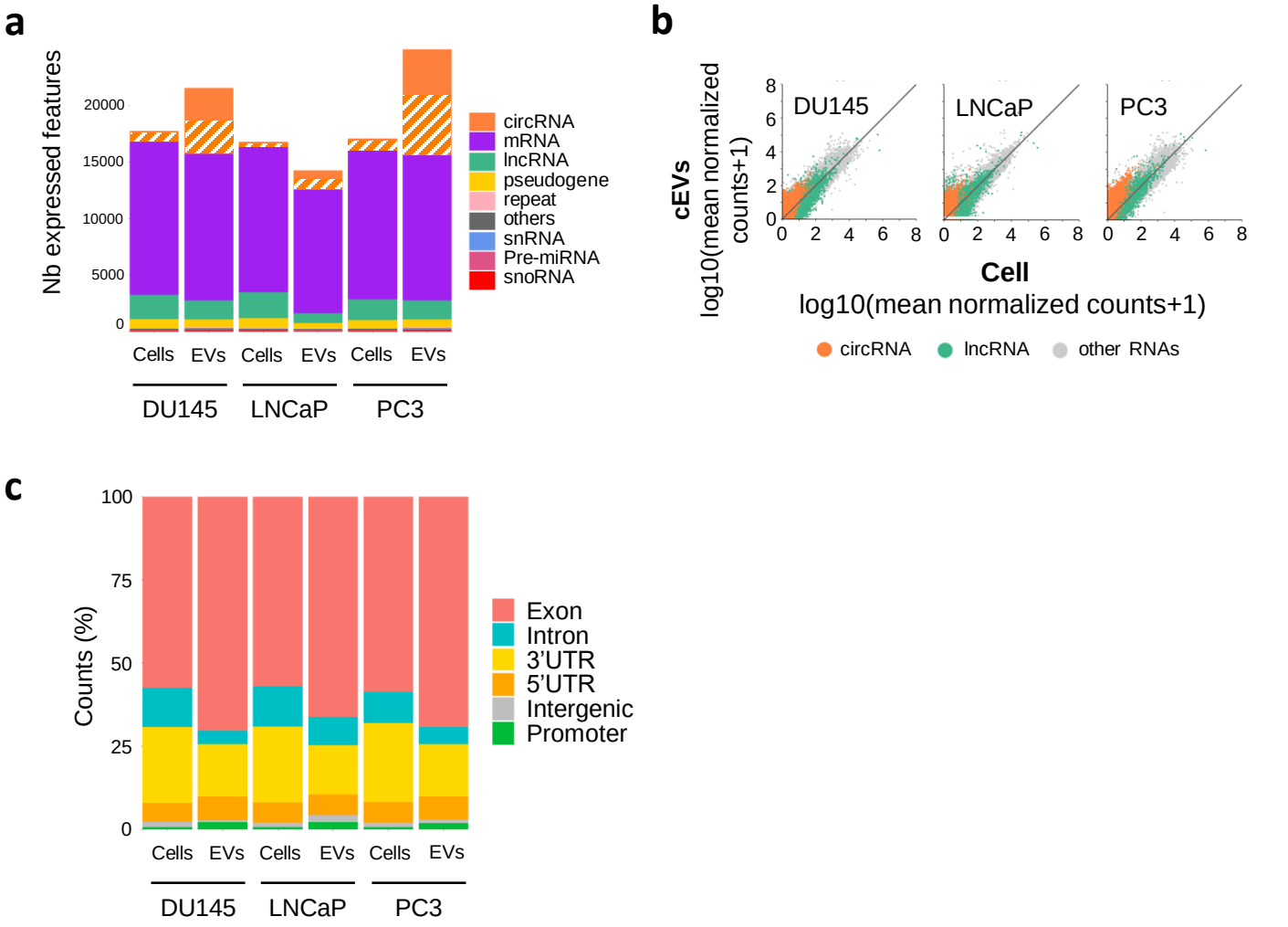
Extended Data Fig. 3. **Selection of genes expressed in uEVs, enriched in prostate tissue**. **a**. Workflow of analysis to select prostate enriched expressed genes in uEVs biopsies. **b**. Venn diagram showing expressed genes in prostate cancer uEVs (white n= 10,937), down regulated genes in healthy kidney (blue, n=3644) and in urinary bladder (grey, n=3982) compared to healthy prostate. **c**. Box-plot of Log10 TPM normalized expression of prostate (KLK2, KLK3, PCA3 and PMEPA1), kidney (UMOD) and urinary bladder (UPK2) enriched RNAs, in Tumor biopsies (blue) and uEVs (red).



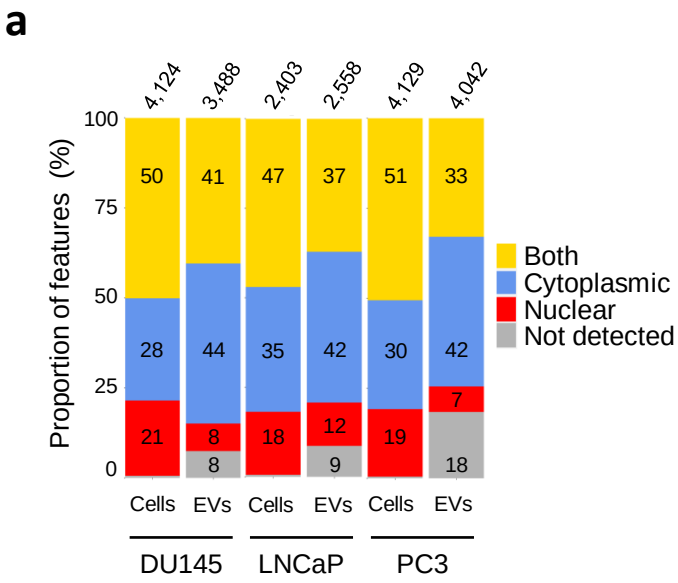


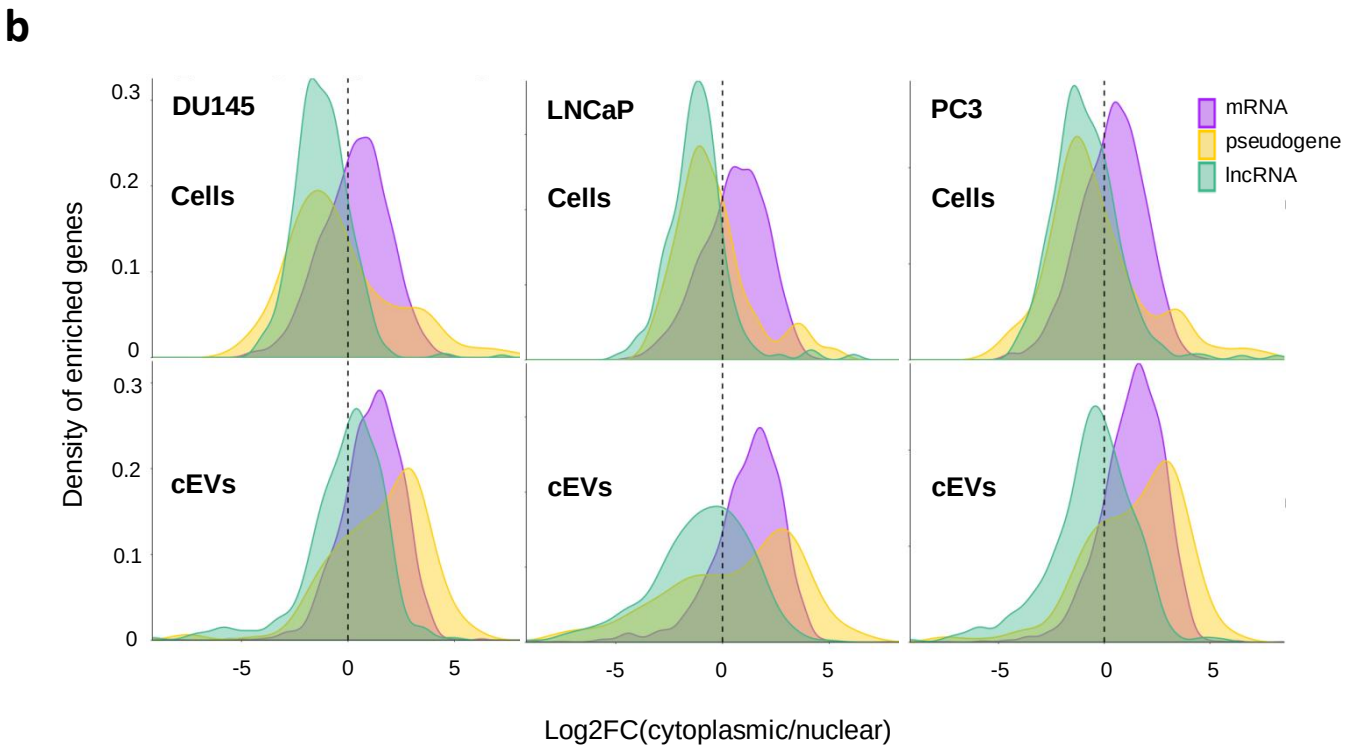


Extended Data Fig. 4. **uEVs are enriched in circRNAs and lncRNAs. a.** Mean gene expression of paired Tumor against uEVs. DEseq2 normalized counts, for each type of RNA are plotted; circRNAs (orange), lncRNAs (green), all others type of RNAs (grey). Each dot represents all transcripts for each gene. Following values are given for at least 5 counts for circRNA expression and 20 counts for all other RNA expression. From 21,633 RNAs of patient 1, R^2=0.007 for 2,310 circRNAs and R^2=0.099 for 2,979 lncRNAs. From 21,147 RNAs of patient 2, R^2=0.095 for 2,334 circRNAs and R^2=0.01 for 2,811 lncRNAs. From 21,487 RNAs of patient 3, R^2=0.02 for 2,369 circRNAs and R^2=0.14 for 3,008 lncRNAs. From 21,737 RNAs of patient 4, R^2=0.027 for 2,296 circRNAs and R^2=0.15 for 3,213 lncRNAs. From 20,565 RNAs of patient 5, R^2=0.03 for 1,714 circRNAs and R^2=0.11 for 2,857 lncRNAs. From 21,156 RNAs of patient 6, R^2=0.033 for 2,633 circRNAs and R^2=0.17 for 2,883 lncRNAs. **b.** Number of over-expressed RNA species in Tumor (top) and in uEVs (bottom). **c.** Heatmap display unsupervised hierarchical clustering with euclidean distance (CED) of all differentially expressed transcripts (n = 13,261, fold change >= 1,5) in each of the individual samples from 6 Tumor biopsies (green) and paired 6 uEVs (black). Color scales represent log10(normalized counts +1).

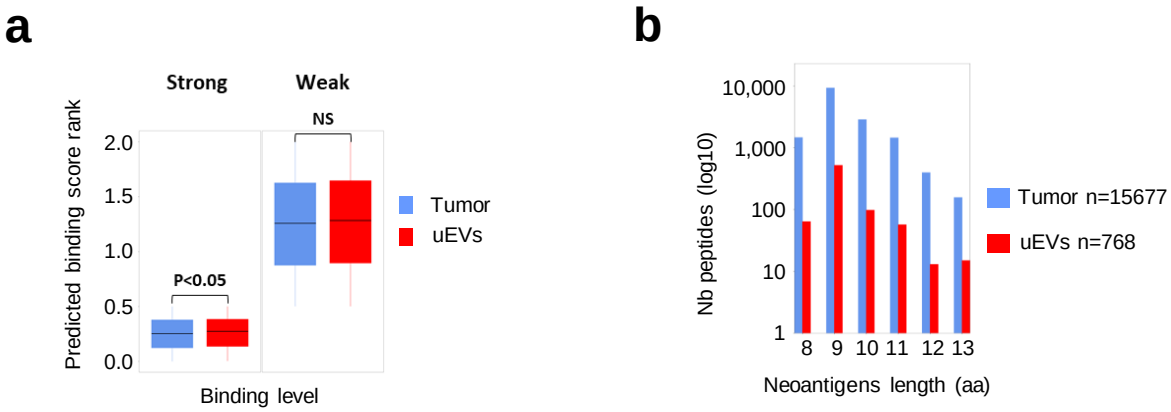


Extended Data Fig. 5. **cell-EVs are enriched in circRNAs and lncRNAs and are depleted in introns. a.** Number of expressed RNA features in DU145, LNCaP and PC3 Cell lines and related cell-Evs. Hatched orange indicate the number of circRNAs found in circBase. **b.** Mean gene expression of paired Cell against cell-EVs. DEseq2 normalized counts, for each type of RNA are plotted; circRNAs (orange), lncRNAs (green), all others type of RNAs (grey). Each dot represents all transcripts for each gene. Following values are given for at least 5 counts for circRNA expression and 20 counts for all other RNA expression. From 17,838 RNAs of DU145 cell line, R2=0.33 for 2,009 circRNAs and R2=0.34 for 1,761 lncRNAs. From 17,666 RNAs of LNCaP cell line, R2=0.2 for 2,384 circRNAs and R2=0.3 for 1,804 lncRNAs. From 19,742 RNAs ofPC3 cell line, R2=0.46 for 4,396 circRNAs and R2=0.45 for 1,546 lncRNAs. **c.** Genomic read counts distribution by percentage across exon, intron, 3'UTR, 5'UTR, intergenic and promoter in DU145, LNCaP, PC3 cell lines and associated cell-EVs.





Extended Data Fig. 6. **Cell-EVs are depleted in nuclear lncRNAs. a.** Stacked barplot distribution, by percentage, of cytoplasmic (blue), nuclear (red), both (yellow) or non-polyA (grey) RNAs of expressed genes in cell and in cell-EVs. Experimental procedure to get the values are presented in fig. 3a. Percentage of expressed RNAs are indicated in each box. Above each barplot are noted the number of total RNAs **b.** Density distribution of log2 (fold change cytoplasmic/nuclear ratio) per RNA types in DU145, LNCaP and PC3 cells and related cEVs (4,048; 2,366; 4,079 RNAs from cells and 3,112; 2,135; 3,157 RNAs from cell-EVs, respectively), mRNA (purple), pseudogene (yellow), lncRNA (green) in Tumor (top) and uEVs (bottom). The left side of dotted line in both graphs corresponds to the nuclear RNAs, the right side corresponds to cytoplasmic RNAs.



Extended Data Fig. 7. **uEVs lncRNAs can be predicted to form strong binding neoantigens. a.** Predicted binding score rank for 65,190 Tumor-neoantigens coming from 255 lncRNA transcripts and for 3,298 uEVs-neoantigens from 16 lncRNA transcripts. 15,677 and 768 strong score lncRNA-neopeptides (score<0.5) were predicted from Tumor (blue, median score= 0.253) and uEVs (red, median score=0.274) samples, respectively. 49,513 and 2,530 weak score lncRNA-neopeptides (score>0.5) were predicted from Tumor (median score=1.258) and uEVs (median score=1.283) samples, respectively. The difference between Tumor and uEVs rank of strong neoantigens is significant ($p<0.05$) but not for weak neoantigens (NS). **b.** Number and lengths of strong predicted neoantigens in Tumor (blue, n=15,677) and uEVs (red, n=768).