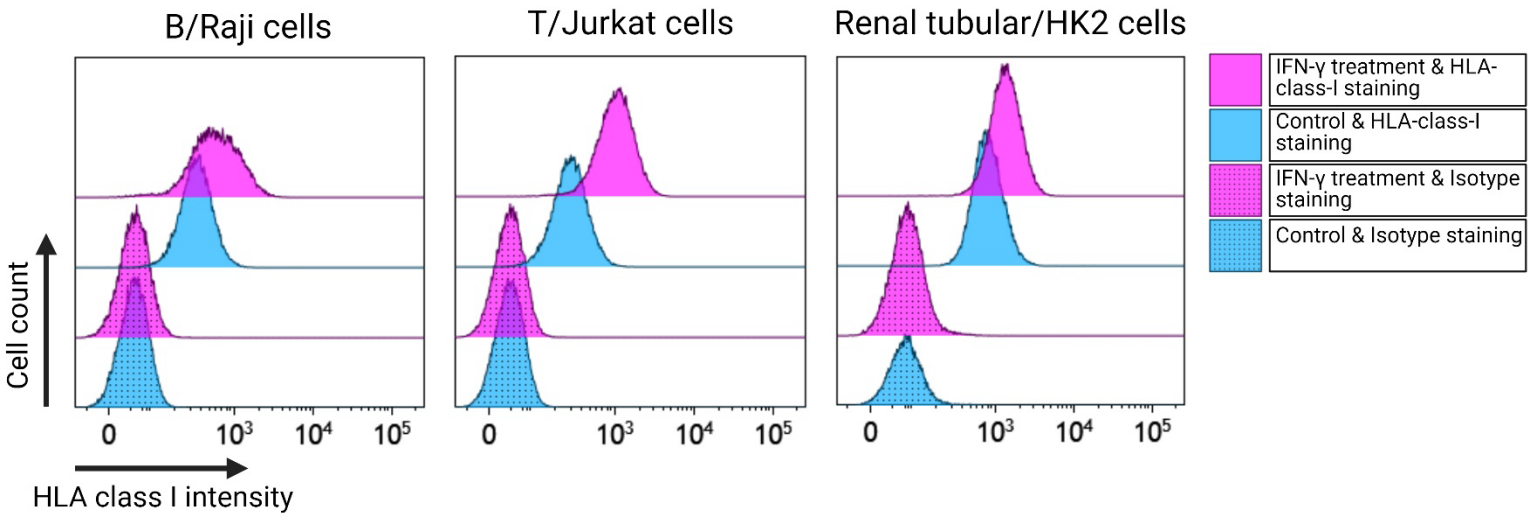
Supplementary Table S1 Clinical parameters of enrolled subjects

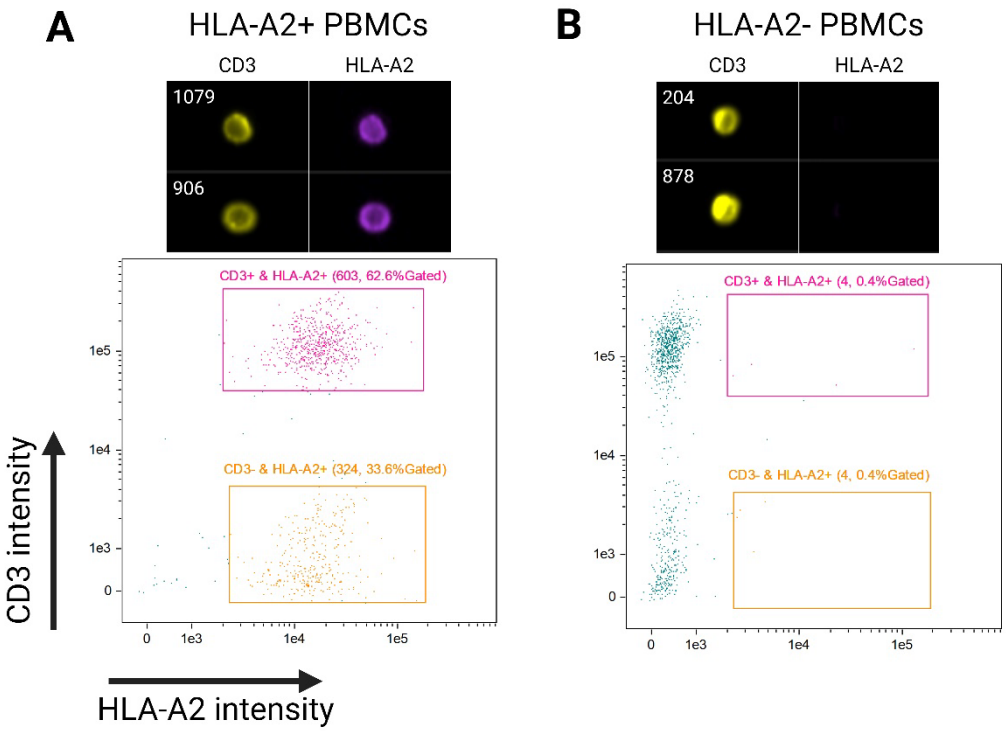
Clinical parameters	Kidney transplant donor (n = 12)	Kidney transplant recipient (n = 12)	Healthy Controls (n = 10)
HLA-A2 typing	Positive	Negative	NA
Sex (male/female; number)	5/7	7/5	3/7
Age (Median, [Q1 – Q3]; year)	52.0 [40 – 58]	61.0 [49.5 – 69.3]	39.0 [27.5 – 47.0]
Urine pH (Median, [Q1 – Q3])	5.5 [5.0 – 6.0]	5.8 [5.5 – 7]	5.5 [5.0 – 6.0]
Urinary total protein concentration (Median, [Q1 – Q3]; g/L)	0.04 [0.02 – 0.07]	0.38 [0.05 – 0.64]	0.05 [0.04 – 0.06]
Urinary creatinine concentration (Median, [Q1 – Q3]; mmol/L)	3.4 [2.2 – 11.3]	4.9 [3.0 – 6.9]	14.0 [7.4 – 15.8]

Supplementary Table S2 Antibody usage and EV staining protocol for IFCM

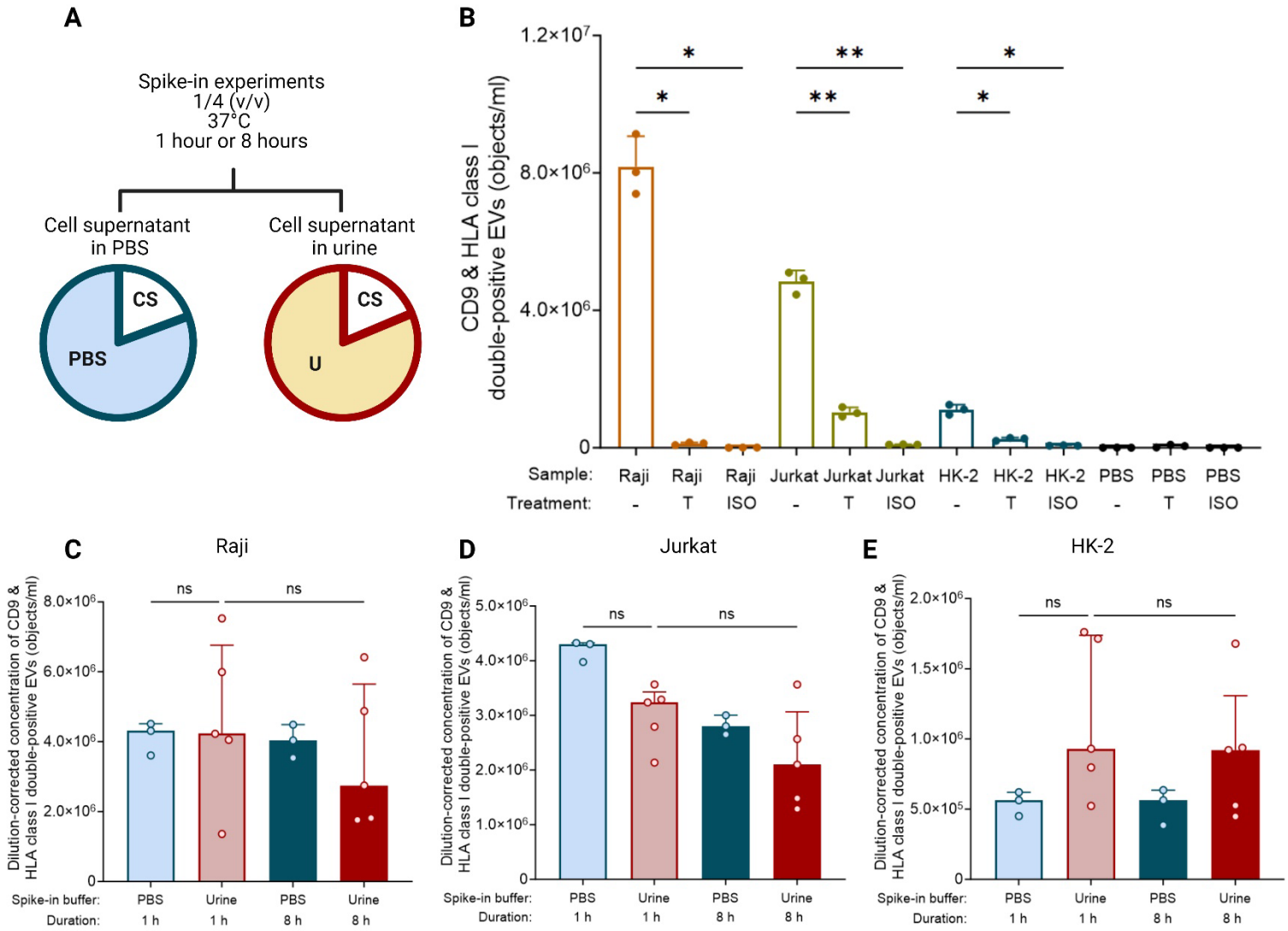
Target EV population	Sample	Antibody	Manufacturer	Clone	In-stock concentration (µg/mL)	Antibody dilution
AQP2+ EVs	Unprocessed urine	CD63-APC	Biolegend (San Diego, CA, USA)	H5C6	200	1:30
		AQP2-Alexa488	Santa Cruz (Dallas, TX, USA)	E-2	200	1:15
HLA-A2+ EVs	Unprocessed urine	CD9-Alexa488	Bio-Connect (Huissen, the Netherlands)	HI9a	100	1:15
		CD63-Alexa488	Biolegend	H5C6	200	1:30
		HLA-A2-VioBlue	Miltenyi Biotec (Bergisch Gladbach, Germany)	REA517	750	1:20
HLA class I+ EVs	Unprocessed urine	CD9-APC	Bio-Connect	HI9a	100	1:15
	100-fold-diluted uEV isolate	HLA class I-Alexa488	Bio-Techne (Minneapolis, MN, USA)	TP25.99SF	730	1:100
	Cell supernatant (Raji, Jurkat, or HK-2)					
	12.7-fold-diluted platelet-poor plasma					



Supplementary Figure S1 HLA class I expression on Raji, Jurkat, and HK-2 cell lines after IFN-γ treatment. 2 million cells were used for staining with 0.5 µL of HLA class I-Alexa488 or 1.8 µL of IgG1-Alexa488 at room temperature for 30 min. Cells were acquired using a BD FACSCanto II flow cytometry system (BD Biosciences). After selecting single cells, the final readout histograms are presented here. The purple peaks denote IFN-γ-treated cells stained by HLA class I-Alexa488 (plane pattern) or isotype (dot pattern). The blue peaks show untreated cells stained by HLA class I-Alexa488 (plane pattern) or isotype (dot pattern).



Supplementary Figure S2 Verification of the HLA-A2-VioBlue antibody specificity using IFCM. One million HLA-A2+ (**A**) or HLA-A2- PBMCs (**B**) were labeled with 0.5 µL of CD3-BV510 and 1 µL of HLA-A2-VioBlue at room temperature for 30 min. CD3 and HLA-A2 double-positive cells are gated in the pink gates, and CD3-negative and HLA-A2+ cells are included in the yellow gates. Each gate's name shows the count and percentage of gated cells. **Abbreviation**: PBMCs, peripheral blood mononuclear cells.



Supplementary Figure S3 Urine matrix effects on the surface HLA class I of EVs from cell lines. (**A**) Schematic overview of diluting cell supernatant containing HLA class I+ EVs into PBS or urine with a volume ratio 1:4 at 37 ºC for 1 h or 8 h. EVs are measured using IFCM. Each cell supernatant is spiked in PBS (repeated 3 times) or healthy urine samples (n = 5). After the incubation, all samples are stained by CD9-APC and HLA class I-Alexa488 and measured using IFCM. (**B**) The concentration of CD9 and HLA class I double-positive EVs in cell supernatant (Raji, Jurkat, and HK-2) measured by IFCM. Data were presented as mean ± SD. (**C** – **E**) Dilution-corrected concentrations of CD9 and HLA class I double-positive EVs in the spike-in experiments using supernatant from Raji (**C**), Jurkat (**D**), and HK-2 (**E**). Data are presented as median [Q1 – Q3]. **Abbreviations**: ISO, isotype staining; T, TritonX-100 treatment. **Marks**: **, $p < 0.01$; *, $p < 0.05$; ns, no significant difference.

Supplementary Figure S4
Immunogold staining cryo-EM images

A – C: T cell-derived EVs (positive control)

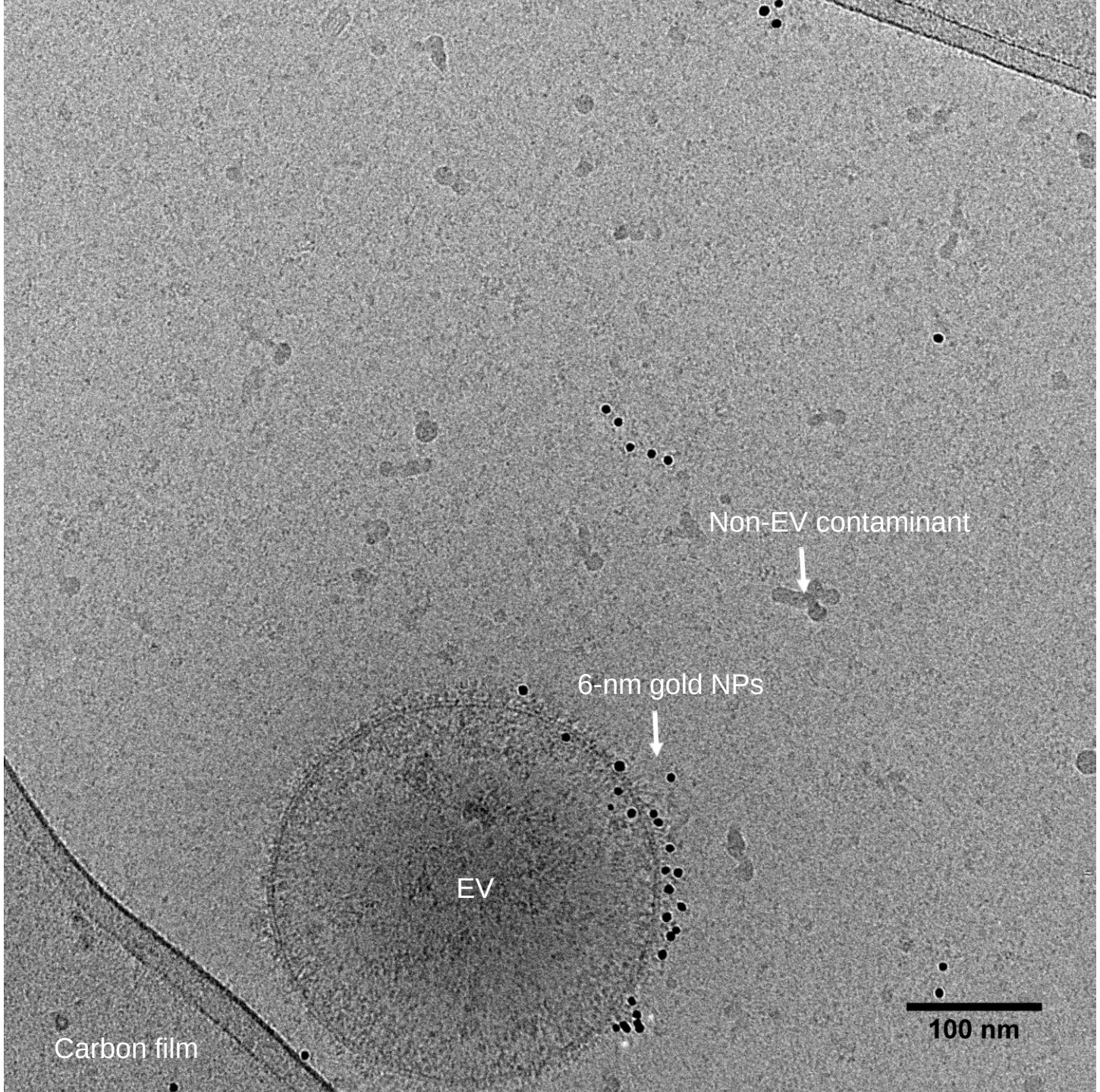
D – G: uEV sample 1

H – K: uEV sample 2

Staining: anti-HLA class I antibody conjugated with 6-nm gold nanoparticles (NPs)

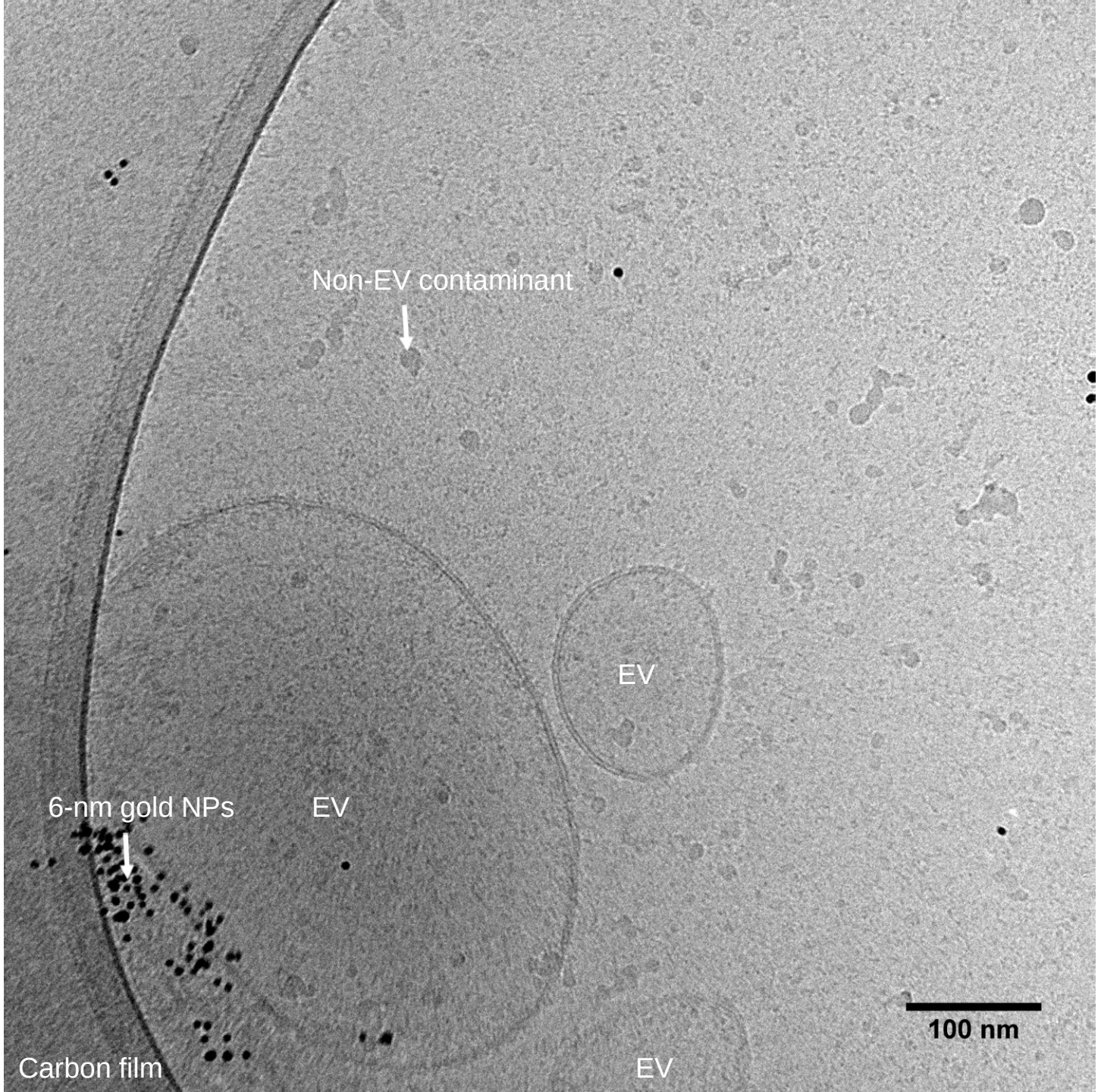
T-cell-derived EVs
(positive control)

A



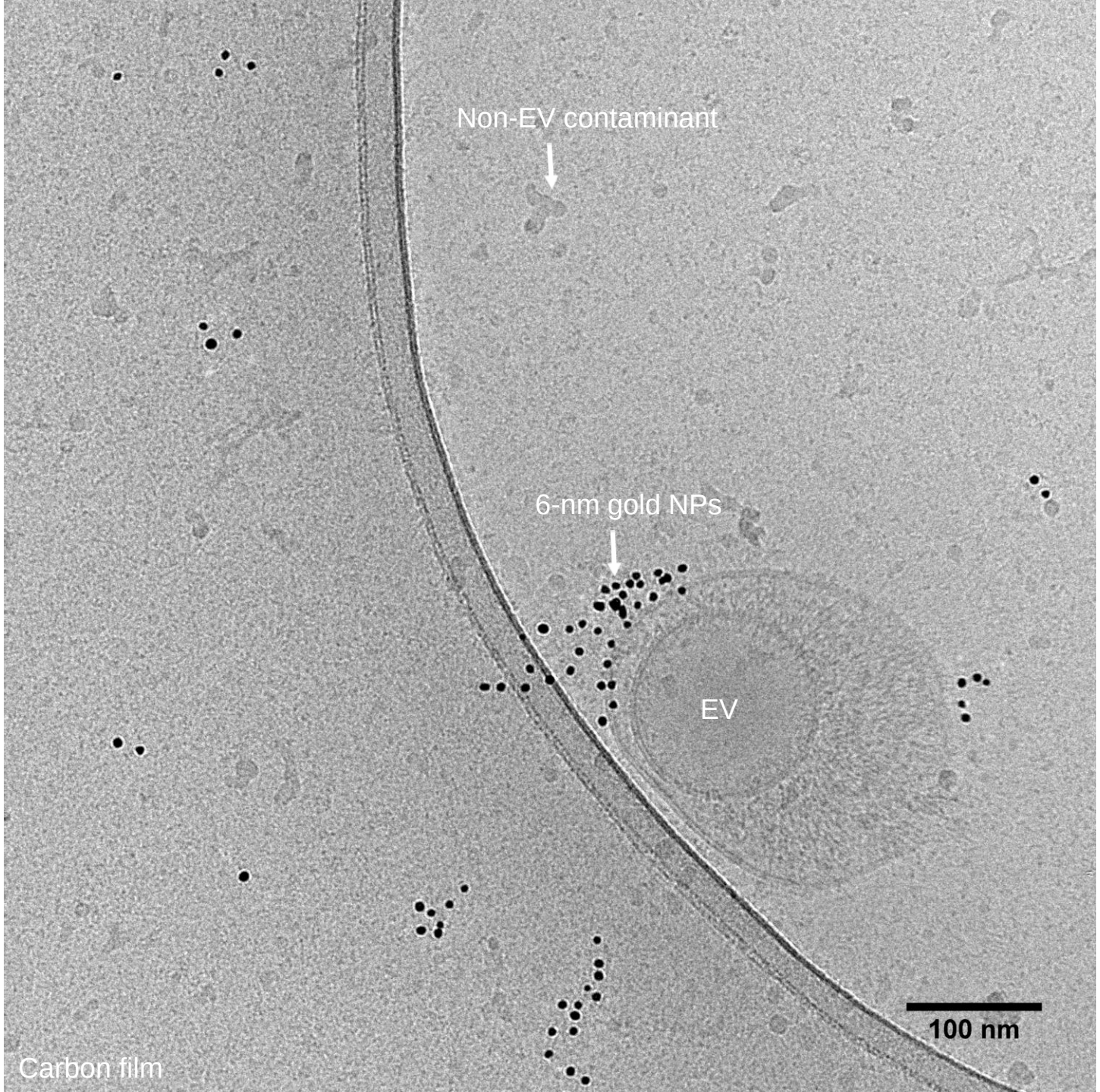
T-cell-derived EVs
(positive control)

B



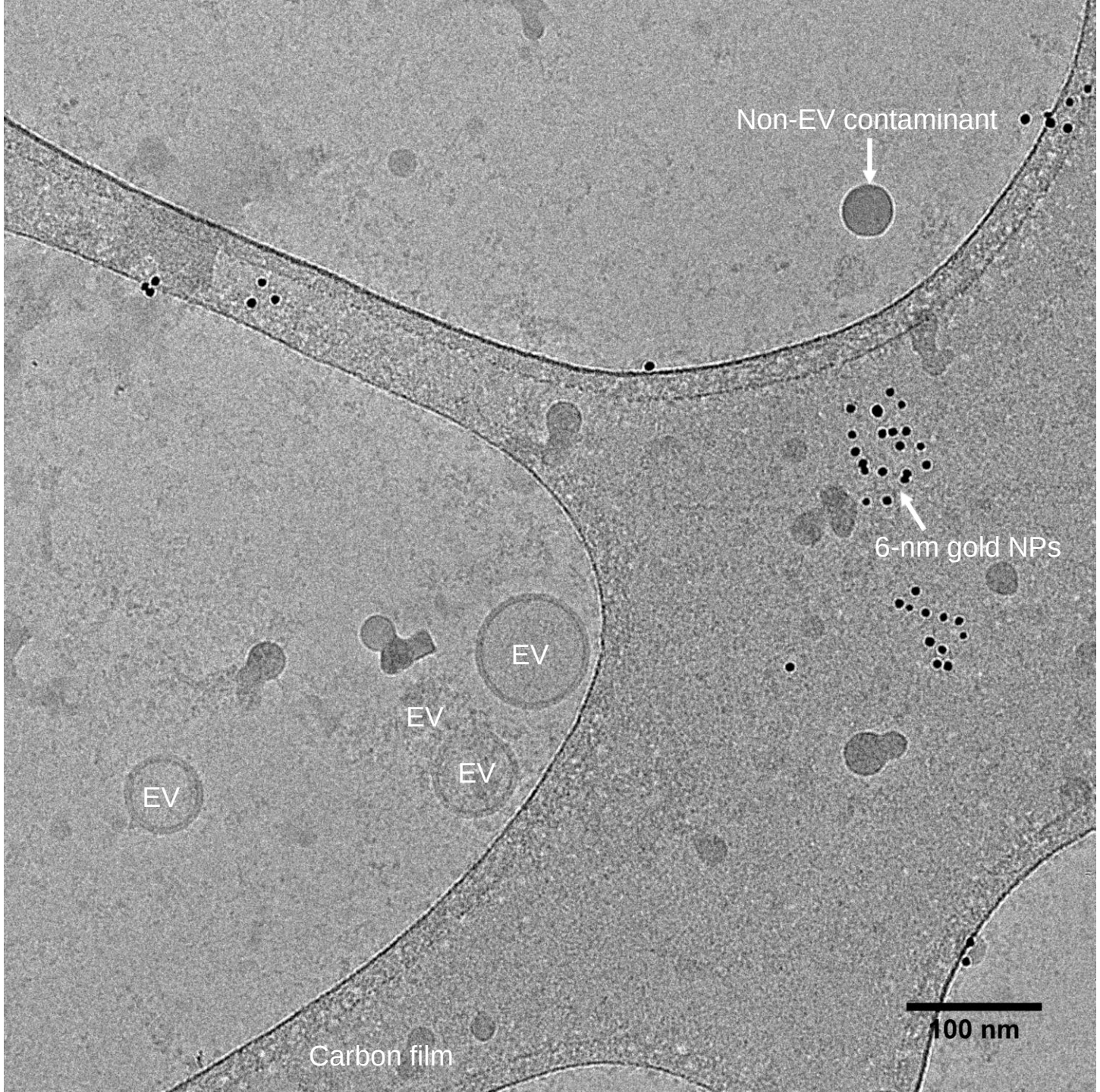
T-cell-derived EVs
(positive control)

C



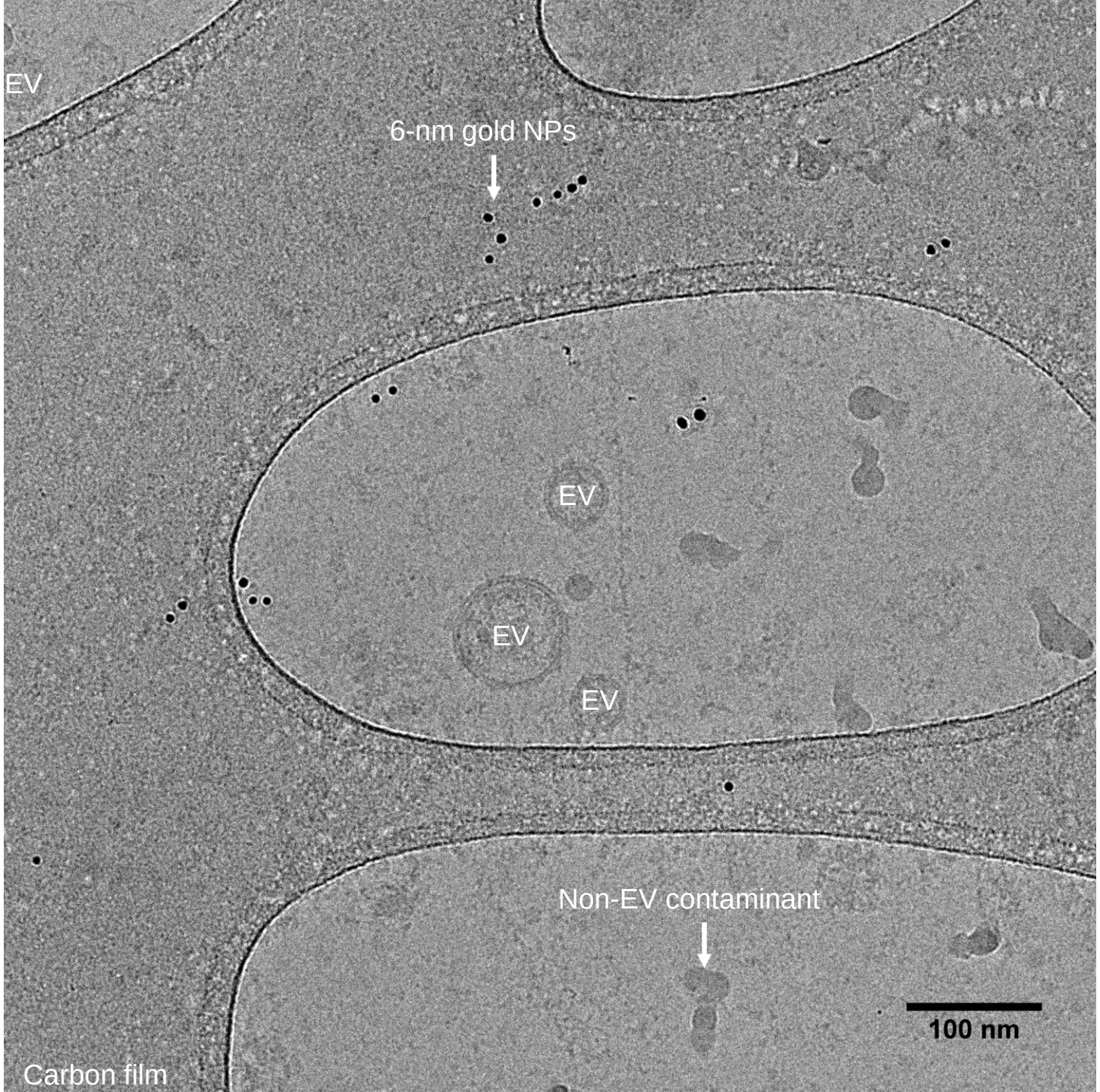
uEV sample 1

D



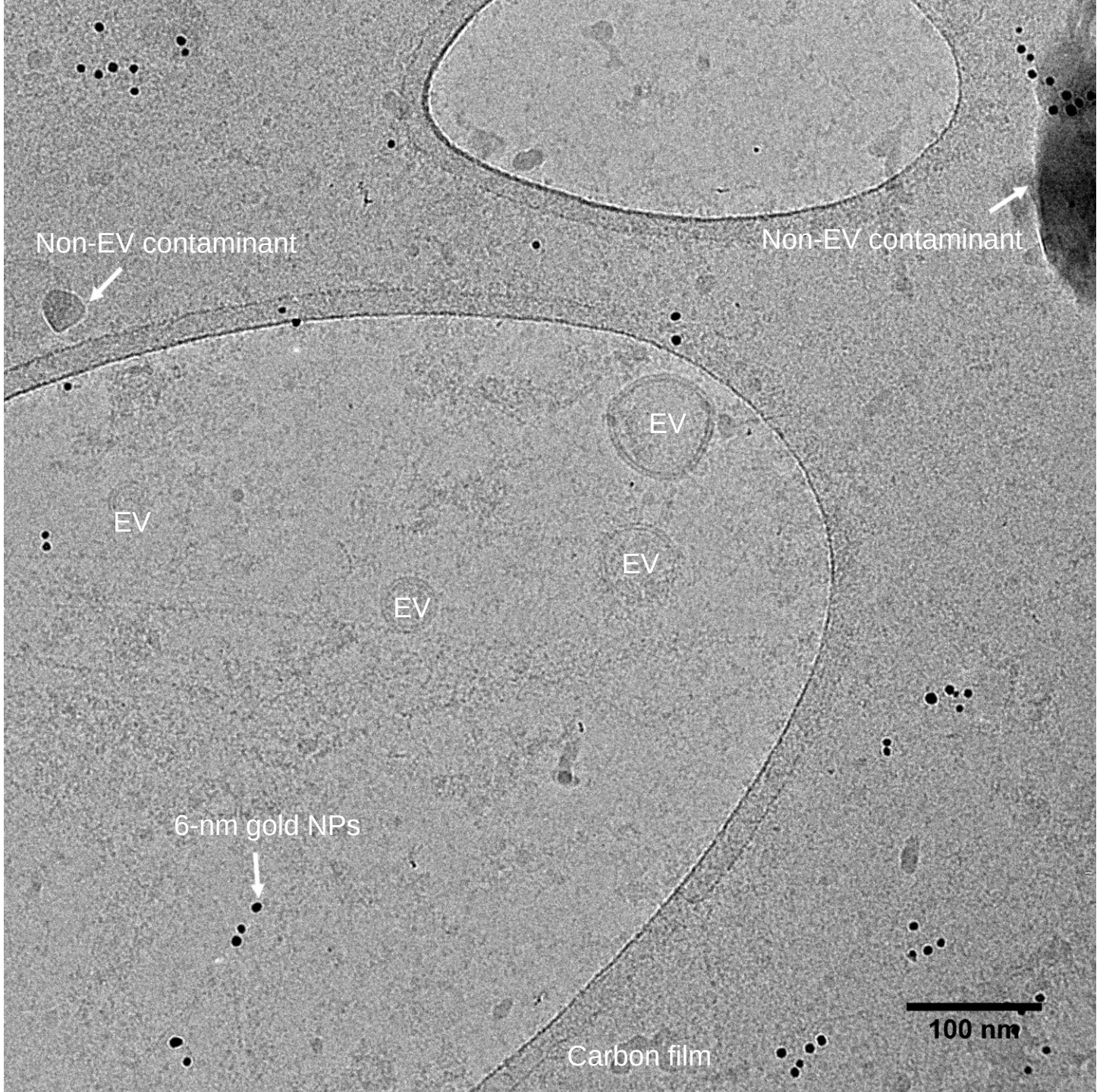
uEV sample 1

E



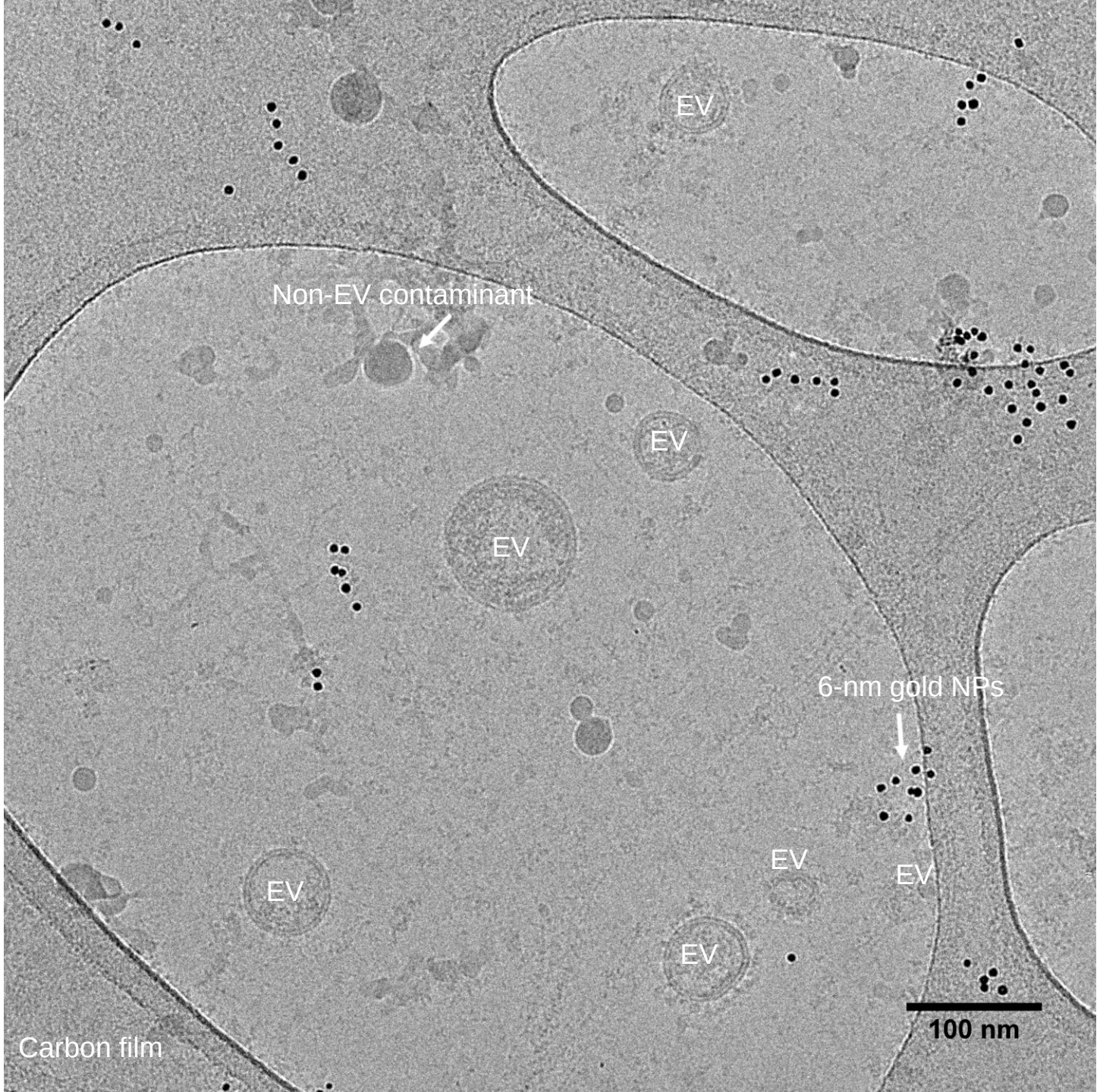
uEV sample 1

F



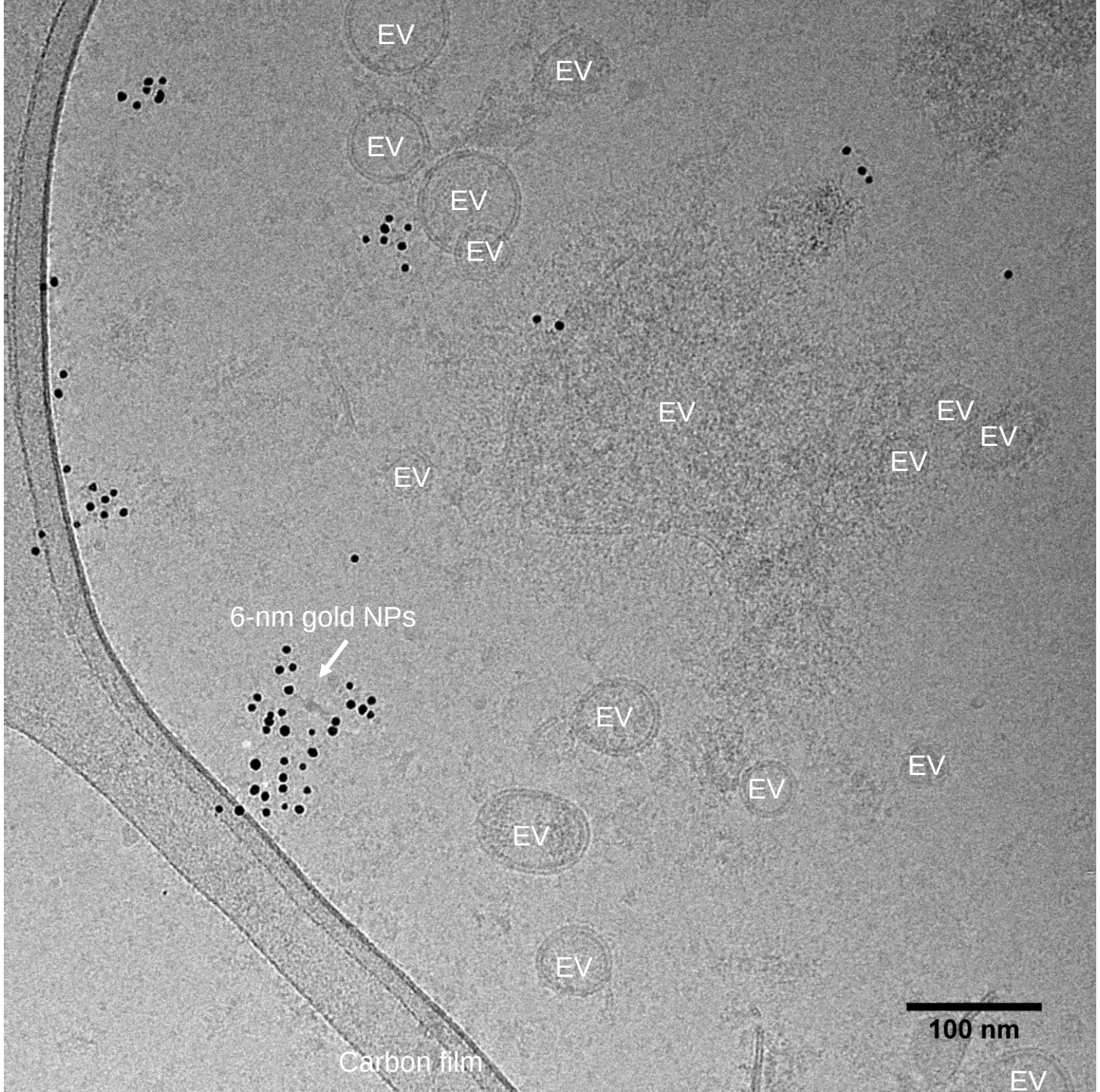
uEV sample 1

G

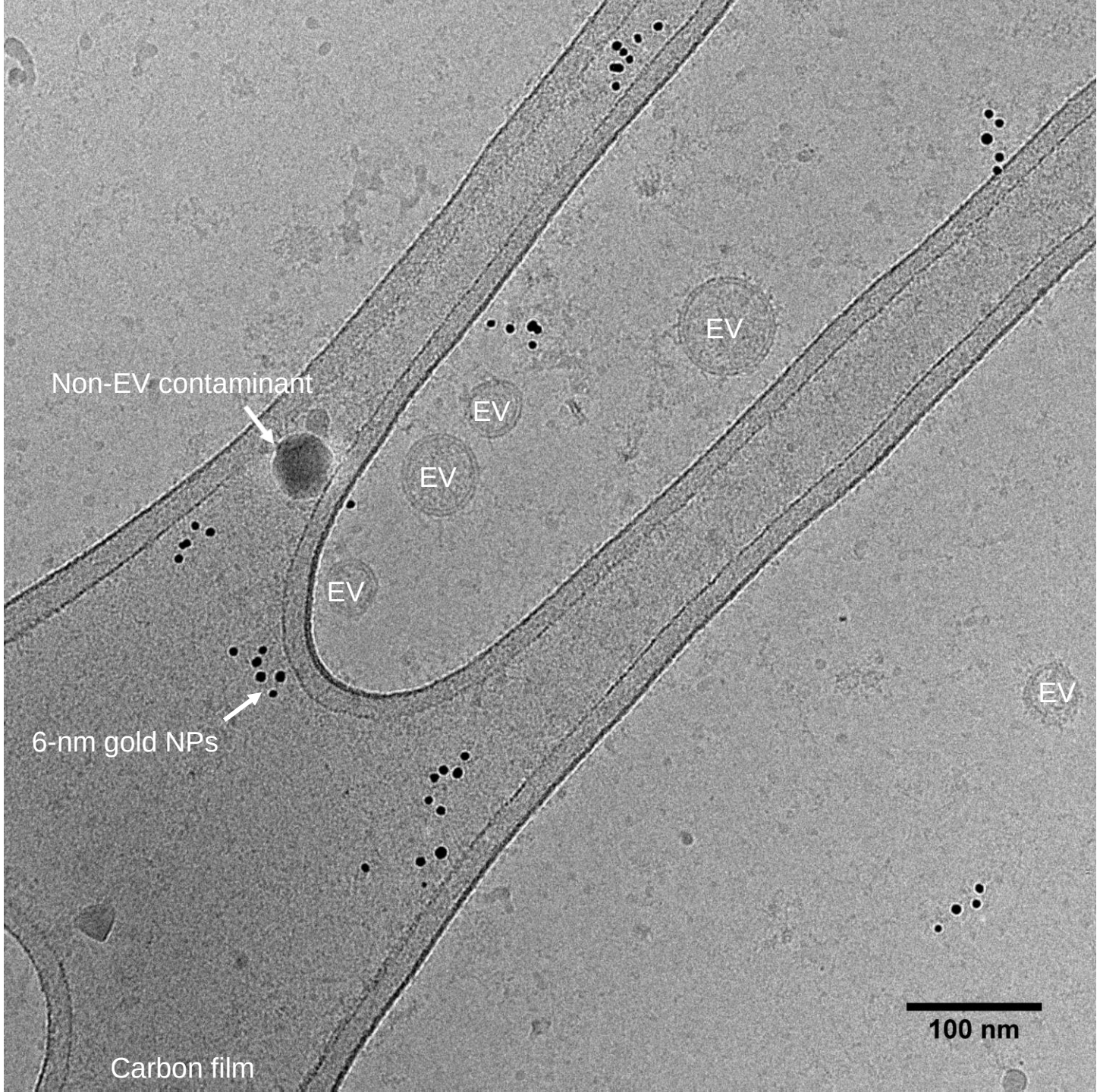


uEV sample 2

H

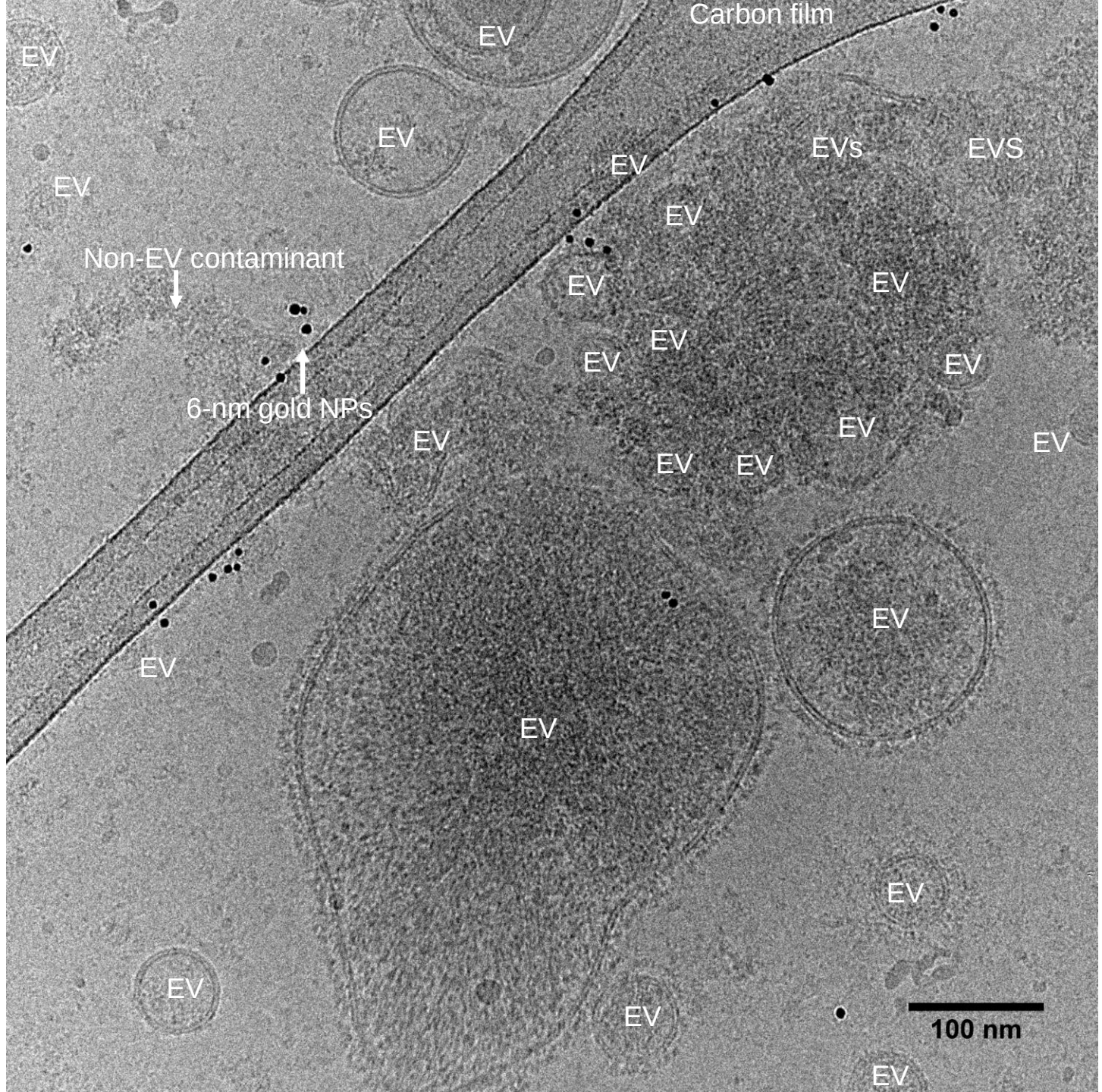


uEV sample 2


Non-EV contaminant
EV
EV
EV
EV
EV
6-nm gold NPs
Carbon film
100 nm

uEV sample 2

J



uEV sample 2

K