Framework Criteria	What to report	Please complete each criterion
1.1 Preanalytical	Preanalytical variables relating to EV sample including source,	See MIFlowCyt report.
variables conforming to	collection, isolation, storage, and any others relevant and available	
MISEV guidelines.	in the performed study.	
1.2 Experimental design	EV-FC manuscripts should provide a brief description of the	See MIFlowCyt report.
according to MIFlowCyt	experimental aim, keywords, and variables for the performed FC	
guidelines.	experiment(s) using MIFlowCyt checklist criteria: 1.1, 1.2, and 1.3,	
2 1 Sample staining	respectively. Template found at www.evflowcytometry.org.	Sample staining was performed as directed in the VEC Protocol. A fresh
details	method used for staining, provide relevant reagent descriptions as	10x vFRed PLUS working solution was prepared from the 100x stock in
	listed in MIFlowCvt auidelines (Section 2.4 Fluorescence	Vesicle Staining Buffer. Staining reactions consisted of 5 ul diluted
	Reagent(s) Descriptions).	sample, 5 ul vFRed 10x Working Solution, and 5 uL 10x antibody added
		to a total volume of 50 uL in Vesicle Staining Buffer. Samples were
		incubated for 1 hour at ambient temperature. Following staining, sample
		was diluted 200-fold for analysis.
2.2 Sample washing	State any steps relating to the washing of samples.	No washing was performed.
details	All methods and stops relating to sample dilution	Samples were subjected to a pro-stein dilution (10.90 fold, as
2.3 Sample dilution	All methods and steps relating to sample dilution.	determined in preliminary experiments), and a 1000-fold post stain
uetalis		determined in preiminary experiments), and a 1000-1010 post stain
2.4 Duffer clans, controls	Ctote whether a huffer only control was analyzed at the same	Duffer only controls showed forwar than 500 Vasials gated events per 100
5.1 Duffer alone controls.	State whether a burler-only control was analyzed at the same	Juli analyzed
	interest. If utilized it is recommended that all samples be recorded	
	for a consistent set period of time e.g. 5 minutes, rather than	
	stopping analysis at a set recorded event count e.g. 100,000	
	events. This allows comparisons of total particle counts between	
0.0.D. ((controls and samples.	
3.2 Buffer with reagent	State whether a buffer with reagent control was analyzed at the	Buffer plus reagent controls showed fewer than 2000 Vesicle-gated
controis.	same settings, same concentrations, and during the same	events per 100 uL analyzed.
	results were	
3.3 Unstained controls.	State whether unstained control samples were analyzed at the	Unstained controls were similar to Buffer-only controls and showed fewer
	same settings and during the same experiment as stained	than 2000 events per 100 uL analyzed.
	samples. If used, state what the results were, preferably in	
3.4 Isotype controls.	The use of isotype controls is applicable to immunofluorescence	A lack of detectable Fc Receptor mediated binding was assessed on a
	labelling only. State whether isotype controls were analyzed at the	subset of samples using an irrelvent IgG1 at a concentration of 5 nM.
	same settings and during the same experiment as stained	I ne median fluorescence of the isotype-stained sample was not
	concentration used, and what the results were (Section 4.2.4.3	undetectable Ec Recentor binding
	4 4) Due to conjugation differences between manufacturers if	
	should be stated if the isotype controls are from the same	
	manufacturer as the matched antibodies	
3.5 Single-stained	State whether single-stained controls were included. If used state	Single stained controls were analyzed as part of optimization and
controls.	whether the single-stained controls were recorded using the same	validation of multicolor assays.
	settings, dilutions, and during the same experiment as stained	
	samples and state what the results were, preferably in standard upits (Section 4.2.4.3.4.4)	
3.6 Procedural controls.	State whether procedural controls were included. If used, state the	No "procedural" controls were identified.
	procedure and if the procedural controls were acquired at the same	
	settings and during the same experiment as stained samples.	
3.7 Serial dilutions.	State whether serial dilutions were performed on samples and note	In preliminary experiment, serial dilutions of samples were performed on
	the dilution range and manner of testing. The fluorescence and/or	selected samples to determine an optimal sample pre-stain dilution,
	scatter signal intensity would ideally be reported in standard units	which showed proportional decrease in events counts with minimal
	(see Section 4.3, 4.4) but arbitrary units can also be used. This	
	events/concentration over a set period of time at different sample	-1.10 - 1.040).
	dilution. The median fluorescence intensity at each of the dilutions	
	should also ideally be plotted on the same or a separate plot	
3.8. Detergent treated EV	State whether samples were detergent treated to assess lability. If	Detergent lability was assessed on a sub-set of samples by treatment of
samples	utilized, state what detergent was used, the end concentration of	stained sample with 0.1% Triton X100 prior to post-stain dilution and
	the detergent, and what the results were of the lysis.	analysis. Greater than 90% of the marker-positive (antibody) events were
4 1 Trigger Channel(s)	The trigger channel(s) and threshold(s) used for event detection	Teliminated by detergent treatment.
and Threshold(s)	Preferably, the fluorescence calibration (Section 4.3) and/or scatter	690/50) corresponding of a diameter of ~95 nm)
	calibration (Section 4.4) should be used in order to report the	
	trigger channel(s) and threshold(s) in standardized units.	
4.2 Flow Rate /	State if the flow rate was quantified/validated and if so, report the	The sample volumetric flow rate was calibrated using counting beads
Volumetric quantification.	result and how they were obtained.	(Cellarcus, nanoRainbow), and was found to be within 10% of the
1		Instrument spec

4.3 Fluorescence State whether fluorescence calibration was implemented, and its, provide a scalibrated and its of ABV (antibudes bound per vesciel), which and been cross-calibrated against antibody scale calibration was implemented. 4.4 Light Scatter State whether Huersean and supplet reference units for the standardized units of ABV (antibudes bound per vesciel), which and been cross-calibrated against antibody calibration was implemented. No EV FC Bargs Bio ciences 7540453; and MESF standards Claintain on antibody scale calibration was implemented. 4.4 Light Scatter State whether and how Eyf scatter calibration area integrated to regordize the model. No Eyf scatter calibration was implemented. 5.1 EV diameter/sufface State whether and how Eyf discrete calibration was implemented. No Eyf scatter calibration was performed. 6.1 EV diameter/sufface State whether and how Eyf discrete calibration area and VRed fluorescence and an UFRed fluorescence and UFRed fluorescence	[· · ·		
Calibration incrost the materials and methods used, catalogue numbers, lot numbers, and supplied reference units for the standards. Fluorescence parameters may be reported in standardized units of MESF, FR, or AEC beach. The type of regression used, and another of the standards. Calibrative accession and the standards. Calibrative periods and the standards. State whether the EV refractive index and the rest and the standards. Calibrative periods and the standards. Calibrative perio	4.3 Fluorescence	State whether fluorescence calibration was implemented, and if so,	Fluorescence response was calibrated in units of ABV (antibodies bound
example: numbers, and supplied reference units for the standards. Fourcescence parameters may be reported in standards durits of the second state pict of arbitrary data vs standard data for the resulting state pict of arbitrary data vs standard data for the reference naticines about the supplied reference units for the standard data for the reference naticines about the supplied reference units for the reference naticines about the supplied reference units of nm2, about while the state or calibration was implemented. Light scatter parameters may be reported in standardized units of nm2, along while the state or calibration result of to errors the been calculated using FC measurements. No light scatter calibration was performed. 5.1 EV dismeter/suffex area/olume approximation State whether and how EV dameter, surface area, and/V rRed has been calculated using FC measurements. No light scatter calibration was performed. 5.2 EV refractive index approximation State whether the EV refractive index has been approximated and approximation Immuno mass the been calculated using FC measurements. Immuno mannalian cell plasma membrane (Lipo 100 ¹⁴ , Cellarcus Biosciences, # Cellarcus Disociences, # C	Calibration	report the materials and methods used, catalogue numbers, lot	per vesciel) using hard-dyed nanoRainbow calibration particles
Fluorescence parameters may be reported in standardized units of MESF, FF, or ABC beack. The type of repression used, an inbody cepture beack, BD Biocences 840498 and MESF standards (Quantum FITC, Bangs labs on the same instrument. 4.4 Light Scatter Calibration State whether and how light scatter calibration was implemented. Upt scatter parameters may be reported in standardized units of mn2, along with information required to reproduce here model. No light scatter calibration was performed. 5.1 EV diameter and how Vight Scatter calibration was implemented. ana/volume approximation State whether and how Vight scatter calibration was performed. 5.2 EV transfer/Suffac State whether and how Vight Scatter calibration was performed. 5.2 EV transfer/Suffac State whether and how Vight Scatter calibration was performed. 5.2 EV transfer/Suffac State whether the EV refractive index approximated and has been calculated using FC measurements. 5.2 EV refractive index approximation State whether the EV refractive index has been approximated and how His was done. 5.2 EV refractive index approximation State whether the EV refractive index has been approximated and how His was done. No EV refractive index approximation as approximated and how His was done. 6.3 EV prepor number approximation State whether the EV refractive index has been approximated, and if so, how it was approximated. Immunditurescence intensities are presented in units of ABV (antibidies bound per vesicle), which might be considered tore present the logis gead. 6.3 E		numbers, and supplied reference units for the standards.	(Cellarcus Biosciences, #CBS6) that had been cross-calibrated against
MESF. ERF, or ABC beads. The type of regression used, and the resulting scatter plot of arbitrary data vs standard data for the releance particles should be sumplied. Quantitätte PE beads, BD Biosciences #340439 and MESF standards (Quantum FITC. Bangs labs on the same instrument, Data whether and how jub scatter calibration was implemented. Upth scatter parameters may be reported in standardized units of nr22, along with information required to providue the model. No light scatter calibration was performed. 5.1 EV diameter/surface areavolume approximation State whether and how EV diameter, surface area, and/or volume has been calculated using FC measurements. V size was estimated from the vFRed TM intensity using the linear relationship between the population surface area and vFRed fluorescence distribution surface area and vFRed fluorescence distribution scale calculated of mb mort and lipid composition similar to a namamalian cell plasm amentrane (Lipa tott) TM . Cellerus: Biosciences, # CBS-1), were tained with vFRed TM and ingic composition similar to a namamalian cell plasm amentrane (Lipa tott) TM . Cellerus: Biosciences, # CBS-1), were tained with vFRed TM and measured by flow cytomatry. The Lipo tott Size whether the EV refractive index has been approximated and sproximation No EV refractive index approximation was performed. 5.2 EV refractive index approximation Size whether the EV refractive index has been approximated, and if approximation as scale calculated in the EV refractive index approximation as a performed. No EV refractive index approximation was performed. 6.1 Completion of MEROKYC theokils criteria 1 to 4 using the MIPowCyt evolution tha scale and the invest decatable unit indis of a calibrate and undex approximation. No EV		Fluorescence parameters may be reported in standardized units of	antibody capture beads (nanoCal, Cellarcus #CBS7-MS) and PE
resulting scatter plot of arbitrary data vs standard data for the reference noticides should be sundice Calibration Quantum FITC, Bangs labs on the same instrument. 4.4 Light Scatter Calibration State whether and how light scatter calibration was implemented. Ugbt scatter parameters may be reported in standardized units of nm2, along with information required to reproduce the model. No light scatter calibration was parformed. 5.1 EV diameter/surface area/volume approximation Vs diameter, strate area, and/or volume tas been calculated using FC measurements. No light scatter calibration was parformed. 6.1 EV diameter/surface area/volume approximation State whether and how Ugit scatter calibration strate area, and/or volume tas been calculated using FC measurements. No light scatter calibration was parformed. 5.2 EV information State whether the EV refractive index has been approximated and approximation No light scatter calibration was calculated from the NTA diameter distribution section/anian or sap parformed. 5.3 EV epitope number approximation State whether the EV refractive index has been approximated, and if so, how it was approximated. No light scatter calibration has weer/looper/similated and how this was done. Immunofluorescence intensities are presented in units of ABV (antitodies bound per vesicle), which might be considered to represent the logic budget in Supporting information. 6.1 Completion of AL Completion of AL Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt calculates. Template found at wwer/loopytrum/ty.cn. Immunofluorescence		MESE, ERE, or ABC beads. The type of regression used, and the	(QuantiBrite PE beads, BD Biosciences #340495) and MESE standards
Control Control A Light Stater State whether and how bits scatter calibration was implemented. Calibration No light scatter calibration was performed. A Light Scatter Calibration State whether and how EV diameter, surface area, and/or volume approximation EV size was estimated from the vFRed TM intensity using the linear relationship between the population surface area and vFRed fluorescence distribution. Synthesic lipid vesicles with a uniform size distribution (stimated by TR) and a lipid composition surface area and vFRed fluorescence distributions. Synthesic lipid vesicles with a uniform size distribution (stimated by TR) and a lipid composition surface area and vFRed fluorescence distribution systhesic lipid vesicles with a uniform size distribution (stimated by TR) and a lipid composition surface area and vFRed fluorescence distribution systhesic lipid vesicles with a uniform size distribution (stimated by TR) and a lipid composition surface area and vFRed fluorescence distribution systemated from the vFRed TM and measured by flow cytometry. The Lipo100 TM population diameter distribution to marmalian cell plasma membrane (Lipo100 TM). Cellarcus Biosciences, # CBS-1), were statione was calculated from the NTA diameter distribution systemated. 5.2 EV refractive index approximation State whether the EV refractive index has been approximated, and if micrologic hold, within sight or considered to represent the epitope burnoting and and an exact or all biolitics of ABV (antificovicy theoken). The New Cytometry or con- the ledices used. 6.1 Complete MFlowCyt checklet orients 1 to 4 using the MFlowCyt discussed in Section A, 3 and 4, and providing the hipheset unit and scalar and theowethet in unstander population. The lowest unit at which a po		resulting scatter plot of arbitrary data vs standard data for the	(Quantum FITC, Bangs Jabs on the same instrument
44 Light Scatter State whither and how light scatter as implemented. No light scatter calibration Calibration Light scatter parameters may be reported in standardized units of imit. J along atthe information required in standardized units of imit. J along atthe information required in standardized units of imit. J along atthe information required in standardized units of imit. J along atthe information required in standardized units of a standardity devices with a uniform size distribution (satimated by VTA) and a lipid composition similar to a mammalian cell pasam membrane (Lipot10 ¹⁰¹¹ , Cellacrus Biosciences, 4) 5.1 EV diameter distribution assuming a spherical geometry, and in parameter bio standardized from the NTR dameter distribution assuming a spherical geometry, and linear regression performed against the VTRed ¹¹⁸ fluorescence distribution to a spheric index spheric intensities are presented in units of ABV (antenter distribution assuming a spherical) geometry, and intensities are presented in units of ABV (antenter distribution ascience) distribution to the units of ABV (adtenter calibrated transfer during information. 5.2 EV terfactive index State whether EV eptope number has been approximated and if inclower provimation as performed. 6.1 Completion of Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt included in Supportation in the version of the locas due of the units of a calibrated charmed disclose bound per vesiclo, which might is considered to represent the optope source instandardized units. This can bee onacrifed out, authors flow the vesthere V and		reference particles chould be supplied	(Quantum 1110, Dangs labs on the same instrument.
Calibration Light State Using it state of anomation that a prelimination is any being robuiled. 5.1 EV diameter/surface State state parameters may be exposed in standardad units of model. EV size was estimated from the VFRed TM intensity using the linear relationship between the population surface area and VFRed flattores and the VFRed the vessions with a uniform size distribution disenter distribution exposition similar to a marmalian cell plasma membrare (Lipo100 TM cellates) Biosciences, The CBS-11, were stained with VFRed TM and a lipid composition similar to a marmalian cell plasma membrare (Lipo100 TM cellates) Biosciences, The CBS-11, were stained with VFRed TM and measured by flow cytometry. The Lipo100 TM cellates Biosciences, The CBS-11, were stained with VFRed TM and measured by flow cytometry. The Lipo100 TM cellates Biosciences, The CBS-11, were stained with VFRed TM and measured by flow cytometry. The Lipo100 TM cellates Biosciences, The CBS-11, were stained with VFRed TM and measured by flow cytometry. The Lipo100 TM cellates Biosciences, The CBS-11, were stained with VFRed TM and measured by flow cytometry. The Lipo100 TM cellates Biosciences, The CBS-11, were stained with VFRed TM and measured by flow cytometry. The Lipo100 TM cellates biosciences, the the VFRed the EV refractive index has been approximated and if approximation as approximated. 5.2 EV orteractive index whether EV refractive index has been approximated, and if approximation or diverse area approximated. No EV refractive index approximated in culture of the logs used. 6.1 Completion of Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt. Included in Superioring Information. No ESF/ABVs, with inight be conolidered anomellof acolidition for the approximated and by a cal	4.4 Light Scatter	State whether and how light scatter calibration was implemented	No light scatter calibration was performed
Calibitation Up it scatter plainteres may be reported in standardized units of and with information required to reproduce the model. 5.1 EV diameter/surface approximation State whether and how EV diameter, surface area, and/or volume approximation EV size was estimated from the VFRed TM intensity using the linear relationship between the population surface area and vFRed fluorescence distribution, estimated activution. 5.1 EV diameter/surface approximation State whether the EV refractive index has been approximated and the VFRed TM intensity using the linear regression performed against the vFRed TM (fluorescence distribution to S.2 EV refractive index how this was done. State whether the EV refractive index has been approximated, and if the version assuming a sperical pometry, and linear regression performed against the vFRed TM fluorescence distribution to S.2 EV refractive index so how this was done. No EV refractive index approximated in units of ABV (antibiddies bound per vesicle), which migh be considered to represent the epitope abundance to within a factor of 2, given the bivalent nature of the epitope abundance to within a factor of 2, given the bivalent nature of the epitope abundance to within a factor of 2, given the bivalent nature of the epitope abundance to within a factor of 2, given the bivalent nature of the epitope abundance to within a factor of 2, given the bivalent nature of the epitope abundance to within a factor of 2, given the bivalent nature of the epitope abundance to within a factor of 2, given the bivalent nature of the epitope abundance to within a factor of 2, given the bivalent nature of the epitope abundance to within a factor of 2, given the bivalent nature of the epitope abundance to within a factor of 2, given the units of abundance scaled, as discussed in Section 4.3 and 4.4, and prov		Light apotter parameters may be reported in standardized units of	ino light scatter calibration was performed.
5.1 EV diameter/surface State whether and how EV diameter, and/or volume has been calculated using FC measurements. EV size was estimated from the VFRed TM intensity using the linear relationship between the population surface area and vFRed fluorescence distribution. Synthetic ling/or weiseles with a uniform size distribution estimated by NTA) and a lipid composition similar to a mammalian cell plasma membrare (Lipo)100 TM , Cellarcus Biosciences, H CBS-1), were stimated with vFRed TM interescence distribution assuming a spharical geometry, The Lipo100 TM population diameter distribution assuming a spharical geometry, and linaar regression performed against the vFRed TM fluorescence distribution to 5.2 EV refractive index approximation 5.2 EV refractive index approximation State whether the EV refractive index has been approximated, and if approximation No EV refractive index approximated in units of ABV (antibodies bound per vesicle), which might be considered to represent how this was approximated. 6.1 Completion of 6.1 Completion of 6.2 Calibrated to namel is during the MIFlowCyt detection range Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt mutodise spectra celloration than been carried out, authors the done by converting the arbitrary unit scale to a calibrated population of fluorescence or Scale and the lower limits of a calibrated population for fluorescence. The chose been carried out, authors the done by converting the arbitrary unit scale to a calibrated population for fluorescence. The chose and waind you avaing of ways, nucluding reporting the 39th percentile measurement unit of the unstained population for fluorescence. The chose and waind you avaing of ways, nucluding reporting the 39th percentile measurement unit of the unstained population for fluorescence. The chose and the lower term is electeclion r	Calibration	Light scatter parameters may be reported in standardized units of	
S.1 E V diametersuitade Safe whether and how EV diameter, suitade area, and/or volume approximation Soft events Subsection Soft events Soft Soft events Soft Soft events Soft Soft events Soft S		Inm2, along with information required to reproduce the model.	тм
area/outme has been calculated using FC measurements. Inelations by the petween the population surface area and vFRed approximation has been calculated using FC measurements. Inelations by the petween the population surface area and vFRed approximation has been calculated using FC measurements. Inelations by the petween the population diameter distributions. Synthetic lipid vesicles with a uniform size distribution (scimated by NTA) and a lipid composition similar to a mammalian cell plasma membrane (Lipo100 TM , Cellarcus Biosciences, # CBS-1), were stained with vFRed TM fluorescence distribution as sumplay a spherical genetry, and linea spherical genetry, a	5.1 EV diameter/surface	State whether and how EV diameter, surface area, and/or volume	EV size was estimated from the vFRed [™] intensity using the linear
approximation Incressence distributions.Synthetic lipid vesiciles with a uniform size distribution (estimated by NTA) and a lipid composition similar to a mammalian cell plasma membrane (Lipo100 TM , Cellarcus Biosciences, # CBS-1), were stained with VFRed TM and measured by flow cytometry. The Lipo100 TM population diameter distribution was calculated from the NTA diameter distribution assuming a spherical geometry, and linear regression performed agains the VFRed TM Interessence distribution to the white was done. 5.2 EV refractive index approximation State whether the EV refractive index has been approximated and how this was done. No EV refractive index approximation was performed. 5.3 EV eptope number approximation State whether the EV refractive index tables approximated. Immunofluorescence intensities are presented in units of ABV (antbodies bound per vesicle), which might be considered to represent quickelines. Template found at www.erflowcytometry org. 6.1 Completion of AC 2 calibrated found at www.erflowcytometry org. Included in Supporting Information. 6.2 Calibrated connell detection range Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt audicines. Template found at www.erflowcytometry org. 6.3 EV number/concentration. Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt audicines and exact realibrated out, authors abound state whether the upper and lower limits of a calibrated scalad, as discussed in Sated A: and providing the highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit a which a population is deemed positive channel distribution babeen arefront find audition ebud the scale and the lowest det	area/volume	has been calculated using FC measurements.	relationship between the population surface area and vFRed
distribution (estimated by NTA) and a ligid composition similar to a mammalian cell plasma membrane (Lipo100 ^{-M} , Cellarcus Biosciences, # CBS-1), were stained with VFRed TM and measured by flow cytometry. The Lipo100 TM population diameter distribution was calculated from the S2 EV refractive index a peroximation how this was done. 5.2 EV refractive index how this was done. State whether the EV refractive index has been approximated and how this was done. 5.3 EV optiope number approximation State whether EV optiope number has been approximated, and if so, how it was approximated. MIFEWCY, theoklist criteria 1 to 4 using the MIFlowCyt discliper abundance to within a factor of 2, given the bivalent nature of the IgGs usad. MIFEWCY, theoklist criteria 1 to 4 using the MIFlowCyt discliper abundance to within a factor of 2, given the bivalent nature of the IgGs usad. MIFEWCY, theoklist criteria 1 to 4 using the MIFlowCyt discliper abundance to within a factor of 2, given the bivalent nature of the IgGs usad. MIFEWCY, theoklist criteria 1 to 4 using the MIFlowCyt discliper abundance to within a factor of 2, given the bivalent nature of the IgGs usad. MIFEWCY, theoklist criteria 1 to 4 using the MIFlowCyt discliper abundance to within a factor of 2, given the bivalent nature of the IgGs usad. MIFEWCY, theoklist criteria 1 to 4 using the providing the scalet a scalibrated from the channel/fluorochrome. 6.2 Completion of more clipted to matchive the upper and providing the highest unit on this scale and the lowest detectable unit above the unstained population. Mifeword, theore ascale to a calibrated or determining a thein	approximation		fluorescence distributions. Synthetic lipid vesicles with a uniform size
6.3 EV Completion of Completion for fluorescence or scatter calibration has been approximated, authors detection range Image: calibrated calibra			distribution (estimated by NTA) and a lipid composition similar to a
A Section of the section of the section of the section of the section channel with the section channel in standardized units. This can be demond notificated or the section channel in standardized units of a calibrated to the section of the section channel in standardized units. This can be demond notificated in the section channel is called to the originate in the section channel in the section channel in the section channel is called to the section channel in the section channel in the section channel is called to the section channel in the section channel in the section channel in the section channel in the section channel is the section channel in the section channel is the section channel in the section channel in the section channel is the section channel in the section channel in the section channel is the section the sectin the section the section the section the section the section the			memmelien cell pleame membrene (Line100 TM Cellereus Pieceienees, #
CBS-1), were stained with VFRd ^{-III} and measured by flow cytometry. The Lipo 100 ^{-IIII} population dimeter distribution was calculated from the NTA diameter distribution was calculated from the NTA diameter distribution was approximation to S.2 EV refractive index approximation S.3 EV epitope number State whether EV refractive index has been approximated, and if approximation S.3 EV epitope number State whether EV epitope number has been approximated, and if approximation S.3 EV epitope number State whether EV epitope number has been approximated, and if approximation S.3 EV epitope number State whether EV epitope number has been approximated, and if approximation S.3 EV applete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt detection range detection channel scaled, as discussed in section 4.3 and 4.4, and providing the highest unit an used tare detectable unit at which a population is detection range de			
Sector The Lippo101 TM population diameter distribution was calculated from the NTA diameter distribution assuming a sphemical geometry, and linear regression performed against the VFRd TM fluorescence distribution to how this was done. 5.2 EV refractive index approximation State whether the EV refractive index has been approximated and approximation No EV refractive index approximation was performed. 5.3 EV epitope number approximation State whether EV epitope number has been approximated, and if so, how it was approximated. Immunofluorescence intensities are presented in units of ABV (antibodies bound per vesicle), which might be considered to represent the epitope abundance to within a factor of 2, given the bivalen nature of the optope abundance to within a factor of 2, given the bivalen nature of complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt quidelines. Template found at www.evflowcytometry.org. Included in Supporting Information. 6.1 Complete Annel detection range Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt should state whether the upper and lower limits of a calibrated caled, as discussed in Saction 4.3 and 4.4, and providing the highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is deermed 'positive' can be determined a variety of ways, including reporting the 99th percentile neasurement unit of the unstained population for fluorescence. The chosen method for determining at what whether EV number/concentration has been reported. In standardized manner, stating the number/concentration between a standardized manner, stating the number/concentration between a standardized manner, stating the number/concentration between as the optoper stan dil			CBS-1), were stained with vFRed [™] and measured by flow cytometry.
State whether the EV refractive index approximation NTA diameter distribution assuming a spherical geometry, and linear regression performed against the VFRed TM fluorescence distribution to 5.2 EV refractive index approximation how this was done. No EV refractive index approximation was performed. 5.3 EV optiope number State whether EV epitope number has been approximated, and if approximation No EV refractive index approximation was performed. 6.1 Completion of Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt mecklist quidelines. Template found at www.evflowcytometry.orq. Innumonfluorescence intensities are presented in units of ABV (antibodies bound per vesicle), which might be considered to represent the logSu used. MIFlowCyt checklist dista whether the upper and lower limits of a calibrated channel detection channel were calculated in standardized units. This can be done by converting the arbitrary unit scale to a calibrated scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is date whether the chosen method for determining at that unit an eurorus use determined a variety of ways, including reporting the 90th percentilation assumed to events detected in the Vesicles gate and accounting for the calculated, it is preferable to report EV number/concentration has been reported. If andardized manner, stating the number/concentration between a set detection range. EV concentrations are reported in MESF/ABV units where possible and asculated from the number of events detected in the Vesicles gate and accounting for the number of events we calculated from the number of events we			The Lipo100 TM population diameter distribution was calculated from the
6.1 Completion of detection range State whether the EV refractive index has been approximated, how this was done. No EV refractive index approximation was performed. 6.1 Completion of detection range State whether EV epitope number approximation No EV refractive index approximation was performed. 6.1 Completion of detection range Complete MIFlowCyt checklist filluorescence or scatter calibration has been carried out, authors bould state whether the upper and lower limits of a calibrated detection channel were calculated in standardized units. This can be done by converting the arbitrary unit scale to a calibration detection range In Ruorescence or scatter calibration has been carried out, authors detection range In general, fluorescence channels had a calibrated range of ~0-10,000 MESF/ABVs, with limits of detection (LOD, background +3SD) ranging from 10-200 MESF/ABVs, depending on the channel/fluorochrome. 6.3 EV number/concentration. State whether the upper and lower limits of a calibrate detection range. In general, fluorescence channels had a calibrated range of ~0-10,000 MESF/ABVs, with limits of detection (LOD, background +3SD) ranging from 10-200 MESF/ABVs, depending on the channel/fluorochrome. 6.3 EV number/concentration. State whether EV number/concentration has been reported. It whether EV number/concentration has table dompulation. The lowes unit at which ap opulation is deemed 'positive' can be determined a variety of ways, including reporting the sphere contile measurement unit of the unstained population. The lowes conce. The chosen method for determining at table unconstration. EV concentrations are reported in EVs/mL, which is calculated from the number			NTA diameter distribution assuming a spherical geometry and linear
6.1 EV refractive index approximation State whether the EV refractive index has been approximated and how this was done. No EV refractive index approximation was performed. 5.3 EV peitope number approximation State whether EV epitope number has been approximated, and if so, how it was approximated. Immunofluorescence intensities are presented in units of ABV (antibodies bound per vesicle), which might be considered to represent the epitope abundance to within a factor of 2, given the bivalent nature of the logs used. 6.1 Completion of G.1 Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt guidelines. Template found at www.evflowcytometry.org. Included in Supporting Information. 6.2 Calibrated channel detection range Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt guidelines. Template found at www.evflowcytometry.org. In general, fluorescence channels had a calibrated range of ~0-10,000 MESF/ABVs, with limits of detection (LOD, background +3SD) ranging from 10-200 MESF/ABVs, depending on the channel/fluorochrome. 6.2 Calibrated scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale and he lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at the turn an aucut was damed nocitiva exhold he claduid. Util and claduid to its preferable to report EV number/concentration in a standardized manner, stating the number/concentration between a set detection range. EV concentrations are reported in MESF/ABV units where possible and appropriate. </td <td></td> <td></td> <td>r</td>			r
5.2 EV prefractive index State whether the EV refractive index has been approximated and how this was dome. No EV refractive index approximation was performed. approximation No EV refractive index approximated. No EV refractive index approximation was performed. 3.5 EV epitope number approximation State whether EV epitope number has been approximated, and if so, how it was approximated. Immunofluorescence intensities are presented in units of ABV (antbodies bound per vesice), which might be considered to represent the egitope abundance to within a factor of 2, given the bivalent nature of the logs used. 6.1 Completion of Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt in guidelines. Template found at www.evflowcytometry.org. Included in Supporting Information. 6.1 Completion of detection channel were calculated in standardized units. This can be one by converting the arbitrary unit scale to a calibrated scale to a calibrated scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 9th percentine measurement unit of the unstained population for fluorescence. The chosen method for determining at scale and all pre- and post-stain dilutions. Marker-positive events we calculated from the number/concentration in a set detection range. EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesiciegate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gits estandardized rom the experimental data in a public data repository.			regression performed against the VFRed indorescence distribution to
approximation how this was done. 5.3 EV epitope number State whether EV epitope number has been approximated, and if aproximation Immunofluorescence intensities are presented in units of ABV (antibodies bound per vesicle), which might be considered to represent the epitope abundance to within a factor of 2, given the bivalent nature of the loGs used. 6.1 Completion of MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt audelines. Template found at www.evflowcytometry.org. Included in Supporting Information. 6.2 Calibrated channel If fluorescence or scatter calibration has been carried out, authors scale channel were calculated in standardized units. This can be done by converting the arbitrary unit scale to a calibrated scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale and the lowest teletetable unit above the unstained population. The lowest unit at which a population is detection fullorescence. The chosen method for determining at what unit anown was deconced nositive achou determining at value units or export EV number/concentration in a standardized units of scalue and tright expondent outling the value and scalulated, its preferable to report EV number/concentration in a standardized units of scatter and/or fluorescence. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units	5.2 EV refractive index	State whether the EV refractive index has been approximated and	No EV refractive index approximation was performed.
5.3 EV peritope number approximation State whether EV epitope number has been approximated, and if approximation Immunofluorescence intensities are presented in units of ABV (antibodies bound per vesicle), which might be considered to represent the epitope abundance to within a factor of 2, given the bivalent nature of the IgGs used. 6.1 Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt audelines. Template found at www.evflowcytometry.org. Included in Supporting Information. 6.2 Calibrated channel If fluorescence or scatter calibration has been carried out, authors bould state whether the upper and lower limits of a calibrated detection channel were calculated in standardized units. This can be done by converting the arbitrary unit scale to a calibrated scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population. The lowest unit at which a population appulation appulation for fluorescence. The chosen method for determining and calibrated from the number/concentration in a standardized manner, stating the number/concentration has been reported. If number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events detected from the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate.	approximation	how this was done.	
approximation so, how it was approximated. (antibodies bound per vesicle), which might be considered to represent the epitope abundance to within a factor of 2, given the bivalent nature of the IgGs used. 6.1 Completion of MIFlowCyt checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist criteria 1 to 4 using the altore 1 and the checklist ander 2 and the checklist criteria 1 to 4 usin	5.3 EV epitope number	State whether EV epitope number has been approximated, and if	Immunofluorescence intensities are presented in units of ABV
6.1 Completion of MIFlowCyt checklist Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt multicluded in Supporting Information. Inducted in Supporting Information. 6.2 Calibrated channel detection range Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt multicluded in Supporting Information. In general, fluorescence channels had a calibrated range of ~0-10,000 miscle channel 6.2 Calibrated channel detection range If fluorescence or scatter calibration has been carried out, authors should state whether the upper and lower limits of a calibrated scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what unit an august was deamed nonsitive should be cleardu outlined standardized manner, stating the number/concentration has been reported. If calculated, it is preferable to report EV number/concentration between a set detection range. EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive versts we calculated for the number of events secaeding an arbitrary gate set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. <td>approximation</td> <td>so, how it was approximated.</td> <td>(antibodies bound per vesicle), which might be considered to represent</td>	approximation	so, how it was approximated.	(antibodies bound per vesicle), which might be considered to represent
6.1 Completion of MIFlowCyt checklist 6.2 Calibrated channel detection range detection range. detection range detection range. detection range. dete			the epitope abundance to within a factor of 2 given the bivalent nature of
6.1 Completion of MIFlowCyt checklist Complete MIFlowCyt checklist criteria 1 to 4 using the MIFlowCyt quidelines. Template found at www.evflowcytometry.org. Included bis/ Dub/ Supporting Information. 6.2 Calibrated channel detection range If fluorescence or scatter calibration has been carried out, authors should state whether the upper and lower limits of a calibrated detection channel were calculated in standardized units. This can be done by converting the arbitrary unit scale to a calibrated detection channel were calculated in standardized units. This can be done by converting the arbitrary unit scale to a calibrated detection. The lowest unit at which a population is deemed 'population. The lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what unit a waret was deemed hoeflive should be clearly outlined. EV concentrations are reported in EVs/mL, which is calculated from the number/concentration in a standardized manner, stating the number/concentration between a set detection range. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate.			the InGe used
6.3 EV State whether EV number/concentration State whether EV number/concentration has been reported. If EV concentrations are reported in EVs/mL, which is calculated from the number/concentration is detection range. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and any scatter and/or fluorescence. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a Provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.	6.1 Completion of	Complete MIFlowCvt checklist criteria 1 to 4 using the MIFlowCvt	Included in Supporting Information
And Doty Or declination If fluorescence or scatter calibration has been carried out, authors In general, fluorescence channels had a calibrated range of ~0-10,000 detection range If fluorescence or scatter calibration has been carried out, authors In general, fluorescence channels had a calibrated range of ~0-10,000 detection range should state whether the upper and lower limits of a calibrated units. This can be done by converting the arbitrary unit scale to a calibrated scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what whether EV number/concentration has been reported. If number/concentration has been reported. If is preferable to report EV number/concentration in a standardized manner, stating the number/concentration between as et detection range. EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 6.4 EV brightness. When applicable, state the method by which the bri	MIFlowCyt checklist	quidelines. Template found at www.evflowcytometry.org	
6.4 EV brightness. When applicable, state the method by which the brightness. When applicable, state the method by which the brightness of EVs EV brightness is reported in MESF/ABV units where possible and accounting for the appropriate.	6.2 Calibrated channel	If fluorescence or scatter calibration has been carried out, authors	In general fluorescence channels had a calibrated range of - 0-10 000
detection range Should state whether the upper and lower immus of actionated detection channel were calculated in standardized units. This can be done by converting the arbitrary unit scale to a calibrated scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what unit an evant was demand neitive should be cleady nutlined State whether EV number/concentration has been reported. If calculated, it is preferable to report EV number/concentration between a set detection range. EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a public adata to a Provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.	detection range	about a state whether the upper and lower limits of a calibrated	MESE/ADVa with limits of detection (LOD, background (2SD) renging
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. Fillio multiplication Provide a link to the experimental data in a public data repository. EV brightness.	detection range	should state whether the upper and lower limits of a calibrated	fine SF/ABVS, with infinits of detection (LOD, background +55D) ranging
be done by converting the arbitrary unit scale to a calibrated scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what unit an event was deemed hositive should be clearly outlined. 6.3 EV State whether EV number/concentration has been reported. If calculated, it is preferable to report EV number/concentration in a standardized manner, stating the number/concentration between a set detection range. EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a Provide a link to the experimental data in a public data repository.		detection channel were calculated in standardized units. This can	from 10-200 MESF/ABVS, depending on the channel/fluorochrome.
scaled, as discussed in Section 4.3 and 4.4, and providing the highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what unit an ouver meand nositive schuld be clearly outload 6.3 EV number/concentration. State whether EV number/concentration has been reported. If calculated, it is preferable to report EV number/concentration between a set detection range. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs r.1. Sharing of data to a Provide a link to the experimental data in a public data repository.		be done by converting the arbitrary unit scale to a calibrated	
highest unit on this scale and the lowest detectable unit above the unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what unit an event was deamed nocitive should be clearly outliedV6.3 EV number/concentration.State whether EV number/concentration has been reported. If calculated, it is preferable to report EV number/concentration between a standardized manner, stating the number/concentration between a set detection range.EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population.6.4 EV brightness.When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence.EV brightness is reported in MESF/ABV units where possible and appropriate.7.1. Sharing of data to a while a link to the experimental data in a public data repository.Pat have been uploaded to the ISAC Flow Repository.		scaled, as discussed in Section 4.3 and 4.4, and providing the	
unstained population. The lowest unit at which a population is unstained population. The lowest unit at which a population is deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what unit an event was chemed positive should be clearly outlined 6.3 EV State whether EV number/concentration has been reported. If EV concentrations are reported in EVs/mL, which is calculated from the number/concentration. State whether EV number/concentration between a standardized manner, stating the number/concentration between a EV concentrations are reported in EVs/mL, which is calculated from the number/concentration. set detection range. EV concentrations are reported in the Vesicles gate and accounting for the output number/concentration between a set detection range. EV concentrations are reported in the vesicles gate and accounting for the output number/concentration between a set detection range. EV built an event was cheered on the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in MESF/ABV units where possible and appropriate. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a <		highest unit on this scale and the lowest detectable unit above the	
deemed 'positive' can be determined a variety of ways, including reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what unit an evant was deamed nositive should be clearly outlined. 6.3 EV State whether EV number/concentration has been reported. If calculated, it is preferable to report EV number/concentration between a standardized manner, stating the number/concentration between a set detection range. EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.		unstained population. The lowest unit at which a population is	
reporting the 99th percentile measurement unit of the unstained population for fluorescence. The chosen method for determining at what unit on event was deemed nositive should be clearly outlined EV 6.3 EV State whether EV number/concentration has been reported. If calculated, it is preferable to report EV number/concentration in a standardized manner, stating the number/concentration between a set detection range. EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a public data to a Provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.		deemed 'positive' can be determined a variety of ways, including	
6.3 EV State whether EV number/concentration has been reported. If calculated, it is preferable to report EV number/concentration in a standardized manner, stating the number/concentration between a set detection range. EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a Provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.		reporting the 99th percentile measurement unit of the unstained	
6.3 EV State whether EV number/concentration has been reported. If EV concentrations are reported in EVs/mL, which is calculated from the number/concentration. State whether EV number/concentration has been reported. If EV concentrations are reported in EVs/mL, which is calculated from the number/concentration. standardized manner, stating the number/concentration between a EV concentrations are reported in the Vesicles gate and accounting for the output using the number/concentration between as et detection range. state whether EV number/concentration between a EV concentrations are reported in the Vesicles gate and accounting for the output using the number/concentration between as et detection range. State whether EV number/concentration between a EV concentrations are reported in the vesicles gate and accounting for the output using the number/concentration between as et detection range. State whether EV number/concentration between a EV concentrations are reported in the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population. State set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a much a link to the experimental data in a public data repository. Data have been uploaded to the		nopulation for fluorescence. The chosen method for determining at	
6.3 EV State whether EV number/concentration has been reported. If calculated, it is preferable to report EV number/concentration in a standardized manner, stating the number/concentration between a set detection range. EV concentrations are reported in EVs/mL, which is calculated from the number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population. 6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a provide a link to the experimental data in a public data repository. Provide a link to the experimental data in a public data repository.		what unit an event was deemed positive should be clearly outlined	
number/concentration.calculated, it is preferable to report EV number/concentration in a standardized manner, stating the number/concentration between a set detection range.number of events detected in the Vesicles gate and accounting for the volume analyzed and all pre- and post-stain dilutions. Marker-positive events we calculated from the number of events exceeding an arbitrary gate set at the ~99.5 percentile of the negative population.6.4 EV brightness.When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence.EV brightness is reported in MESF/ABV units where possible and appropriate.7.1. Sharing of data to a uublic approximeProvide a link to the experimental data in a public data repository.Data have been uploaded to the ISAC Flow Repository.	6.3 EV	State whether EV number/concentration has been reported. If	EV concentrations are reported in EVs/mL, which is calculated from the
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a reported in standardized units of scatter and/or fluorescence. Provide a link to the experimental data in a public data repository.	number/concentration	calculated it is preferable to report EV number/concentration in a	number of events detected in the Vesicles gate and accounting for the
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a public data to a public data to a public data repository. Provide a link to the experimental data in a public data repository.		standardized manner, stating the number/concentration between a	volume analyzed and all pre- and post-stain dilutions. Marker-positive
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a provide a link to the experimental data in a public data repository. Provide a link to the experimental data in a public data repository.		standardized manner, stating the number/concentration between a	evente we esteuleted from the number of evente eveneding on erhitrony
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a number of data to a number of the negative population. Provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.		set detection range.	events we calculated from the number of events exceeding an arbitrary
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a public a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.			gate set at the ~99.5 percentile of the negative population.
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a public atta to a public data to a public data repository. Provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.			
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a public data to a public data to a public data repository. Provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.			
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a public atta to a public data in a public data repository. Data have been uploaded to the ISAC Flow Repository.			
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in standardized units of scatter and/or fluorescence. EV brightness is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a public atta to a public data in a public data repository. Data have been uploaded to the ISAC Flow Repository.			
6.4 EV brightness. When applicable, state the method by which the brightness of EVs is reported in MESF/ABV units where possible and appropriate. 7.1. Sharing of data to a provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.			
Is reported in standardized units of scatter and/or fluorescence. appropriate. 7.1. Sharing of data to a provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.	6.4 EV brightness.	When applicable, state the method by which the brightness of EVs	EV brightness is reported in MESE/ABV units where possible and
1.1. Sharing or data to a Provide a link to the experimental data in a public data repository. Data have been uploaded to the ISAC Flow Repository.		Is reported in standardized units of scatter and/or fluorescence.	appropriate.
		Provide a link to the experimental data in a public data repository.	Data have been uploaded to the ISAC Flow Repository.

Cytometry Part A

|--|

Requirement	Please Include Requested Information	1			
1.1. Purpose	To measure the concentration, size, and antigen expression on EVs.	1			
1.2. Keywords	extracellular vesice, EV, vesicle flow cytometery, vFC, CD9, CD63, CD81	1			
1.3. Experiment variables	Concentration, antigen expression	1			
1.4. Organization name and					
address	Scintillon Institute, 6868 Nancy Ridge Dr, San Diego, CA 92121				
1.5. Primary contact name and	John Nolan, inolan@scintillon				
email address					
1.6. Date or time period of	March-October, 2022				
1.7 Conclusions		1			
	Instrument performance was characterized using a combination of multi-	1			
1.8. Quality control measures	intensity single fluorophore beads (Quantum FITC, Bangs Labs Quantibrite PE, BD Biosciences) whose intensity had been calibrated in units of MESF, multi- intensity multifluorophore beads (vCal nanoRainbow, Cellarcus), and antibody capture beads (vCal nanoCal antibody capture beads, Cellarcus) calibrated to report results in units of antibodies bound per vesicle (ABV). EV analysis by vesicle flow cytometry (VFC) was conducted and reported as suggested by the MIFlowCvt-EV guidelines (See attached checklist).				
2.1.1.1. (2.1.2.1., 2.1.3.1.)	Media from washed, ionophore-treated cells, centrifuged (2x 1.5kxg, 15') to				
Sample description	pellet cells.				
2.1.1.2. Biological sample	Cell culture media.				
source description					
2.1.1.3. Biological sample					
2.1.2.2. Environmental sample					
location					
2.3. Sample treatment					
description					
2.4. Fluorescence reagent(s) description	See above and Antibody Database record.				
3.1. Instrument manufacturer	Beckman Coulter	Beckman	Coulter		
3.2. Instrument model	CytoFlex	CytoFlex			
	(https://www.beckman.com/techdocs/B49006AP/wsr-168786). Briefly, the Violet 405nm filter is placed in position 2, the Violet 450nm filter in position 3, and an unused filter in position 1. The gain on all scatter channels was set to 100, the gain on all fluorescence channels was set to 1000.				
		I			
		Detector	Parameter Name (SPnN)	EX-EM/BP	Stain Name (SPnS)
		FSC	FSC	488-	FSC
		V-1		405-780/60	
		V-2	Violet SSC	405-405/10	VSSC
		V-3	FL6	405-450/45	V450
		V-4	FL7	405-525/40	V525
		V-5	FL8	405-610/20	V610
3.3. Instrument configuration		V-6		405-660/20	V660
and settings		R-1	FL3	640-660/20	APC
		R-2	FL4	640-712/25	APC700
		R-3	FL5	640-780/60	APC780
		B-1	SSC	488-488/8	SSC
		B-2	FL1	488-525/40	FITC
		B-3	FL2	488-690/50	vFRed
		Y-1		561-561/10	
		Y-2	FL9	561-610/20	PE610
		Y-3	FI 10	561-585/42	PF
		Y-4	FI 11	561-600/50	PE690
		Y-5	FI 12	561-720/50	PE780
			1 L12	501-100/00	FL/OU

	*We recommend all authors to submit their data files to
	http://flowrepository.org and to make them available for the peer-review process. If you have done so, please let us know by inserting the following codes (replace the red text):
4.1. List-mode data files	1) The link for peer-review process:
	http://flowrepository.org/id/
	This link will only be shared with reviewers of your manuscript.
4.2. Compensation description	Single color spectral reference samples were prepared using antibody capture beads (nanoCal, Cellarcus), and run to determine spectra/spectral spillover of each for spectral unmixing/compensation.
4.3. Data transformation	
4.4.1. Gate description	Data were analyzed using FCS Express (De Novo Software). The first 20 seconds of data were discarded via a Time gate to exclue a consistent but unexplained background event anomaly observed on several different CytoFlex instruments. The remaining 100 seconds of data, corresponding to 100 uL of measured sample, and a plot of vFRed-A vs cFRed-H used to set a gate excluding certain background events that could be identified by their lower signal pulse area and widths. These events were further gated to include events with membrane fluorescence and light scatter intensity characteristic of EVS, and to exclude high light scatter intensity background events that have been noted in certain samples.
4.4.2. Gate statistics	EV immunofluorescence data was analyzed to calculate the median fluorescence intensity (MFI) of the entire EV population, the number of EVs with immunofluorescence positive above gate position at the upper threshold of an unstained sample (<0.5% "positive"), and the (MFI) of these positive EVs.
4.4.3. Gate boundaries	EV immunofluorescence data was analyzed to calculate the median fluorescence intensity of the entire EV population, the number of EVs with immunofluorescence positive above gate position at the upper threshold of an unstained sample (<0.5% "positive"), and the (MFI) of these nositive EVs.