Mesenchymal Stem Cell-derived Extracellular Vesicles Reduce Inflammatory Responses to SARS-CoV-2 and Influenza Viral Proteins via miR-146a/NF-κB Pathway

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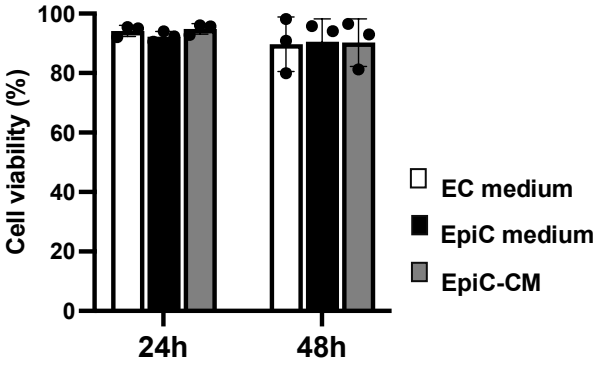
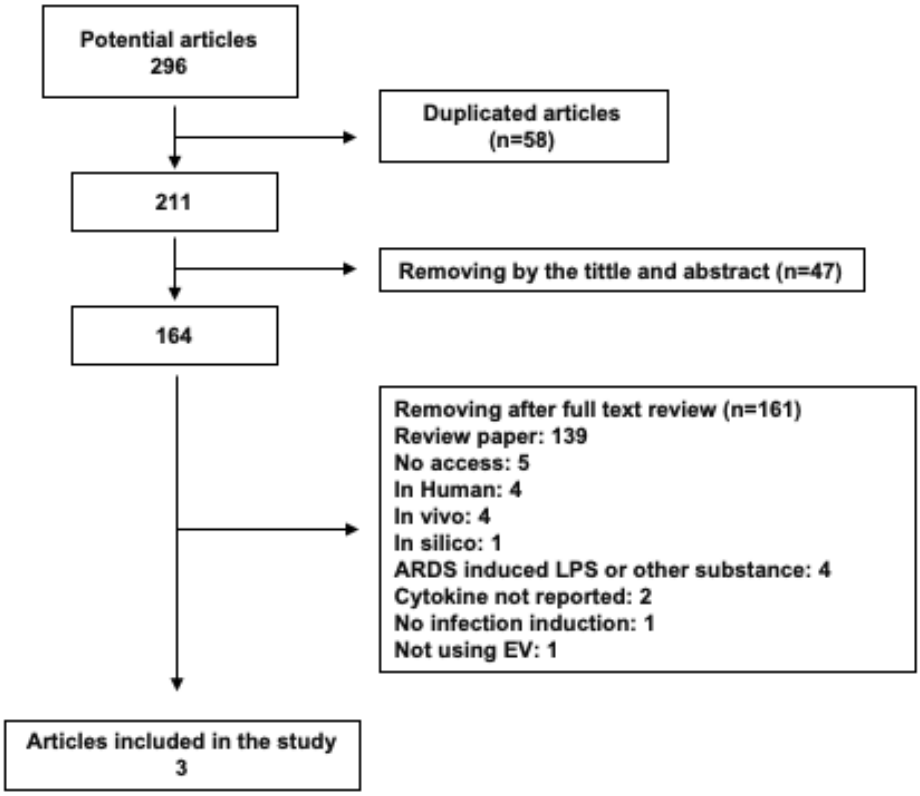
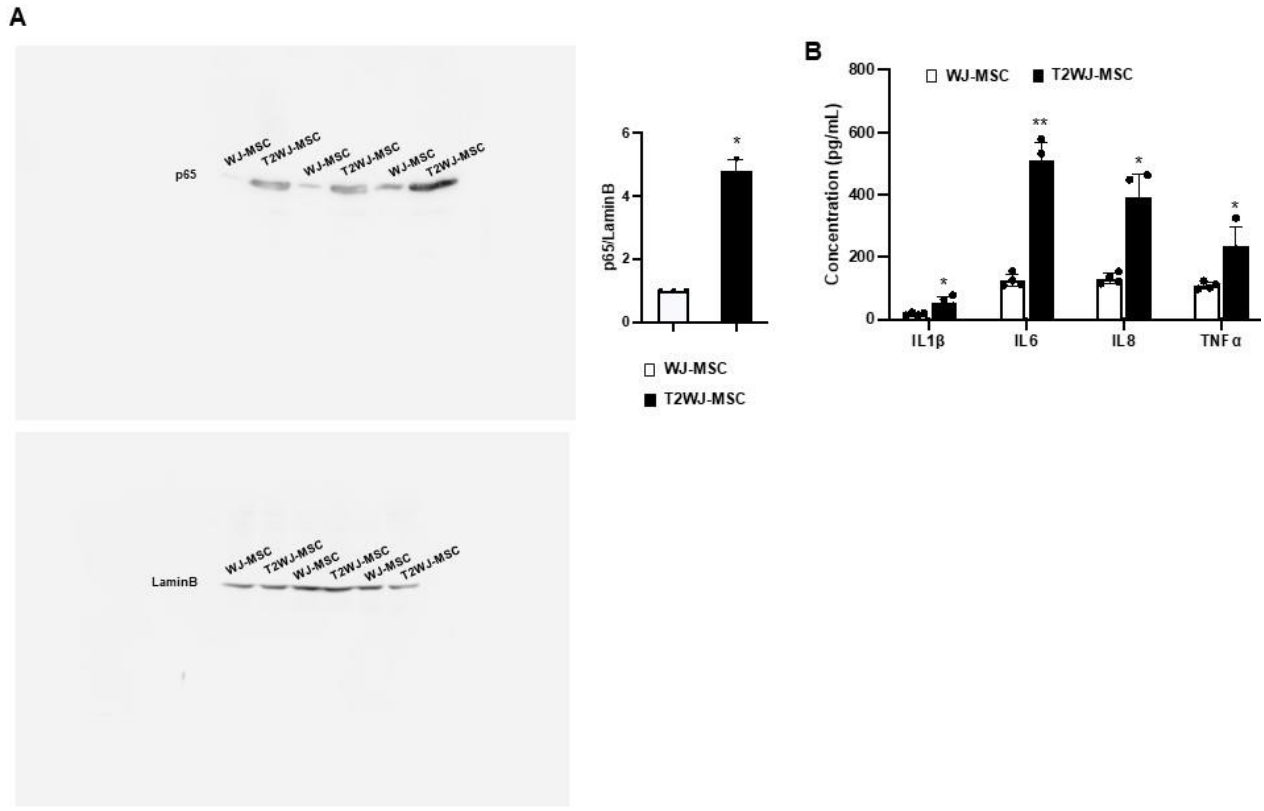


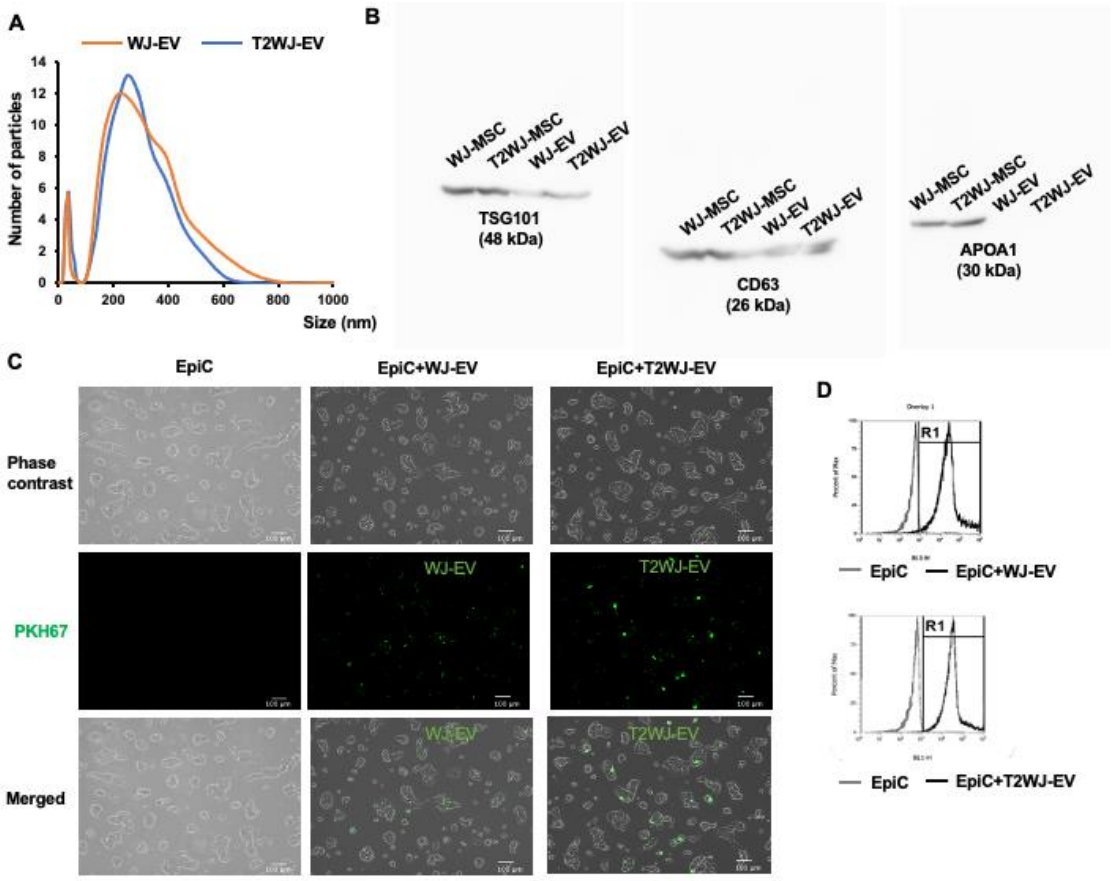
Supplementary Figure 1. Viability of lung EC cultured in different types of medium.



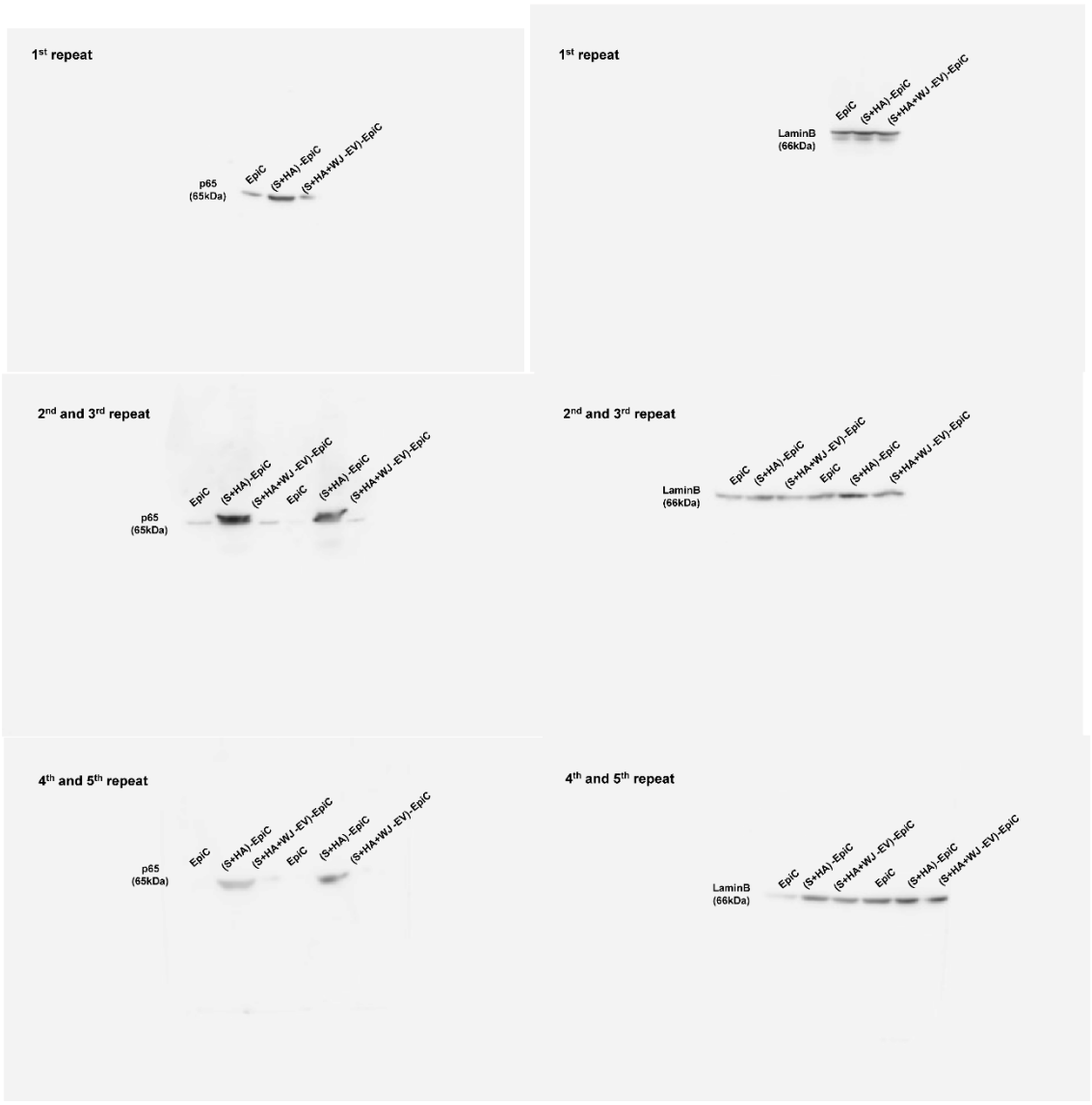
Supplementary Figure 2. Flow chart of systematic review. A systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The search strategy was performed using 3 electronic databases (PubMed, Embase, and Web of Science) from inception to April 11, 2024, using the following keywords: (covid-19 OR sars-cov-2 OR influenza) AND (extracellular vesicles OR exosomes) AND (mesenchymal stem cells OR MSC) AND cytokines.



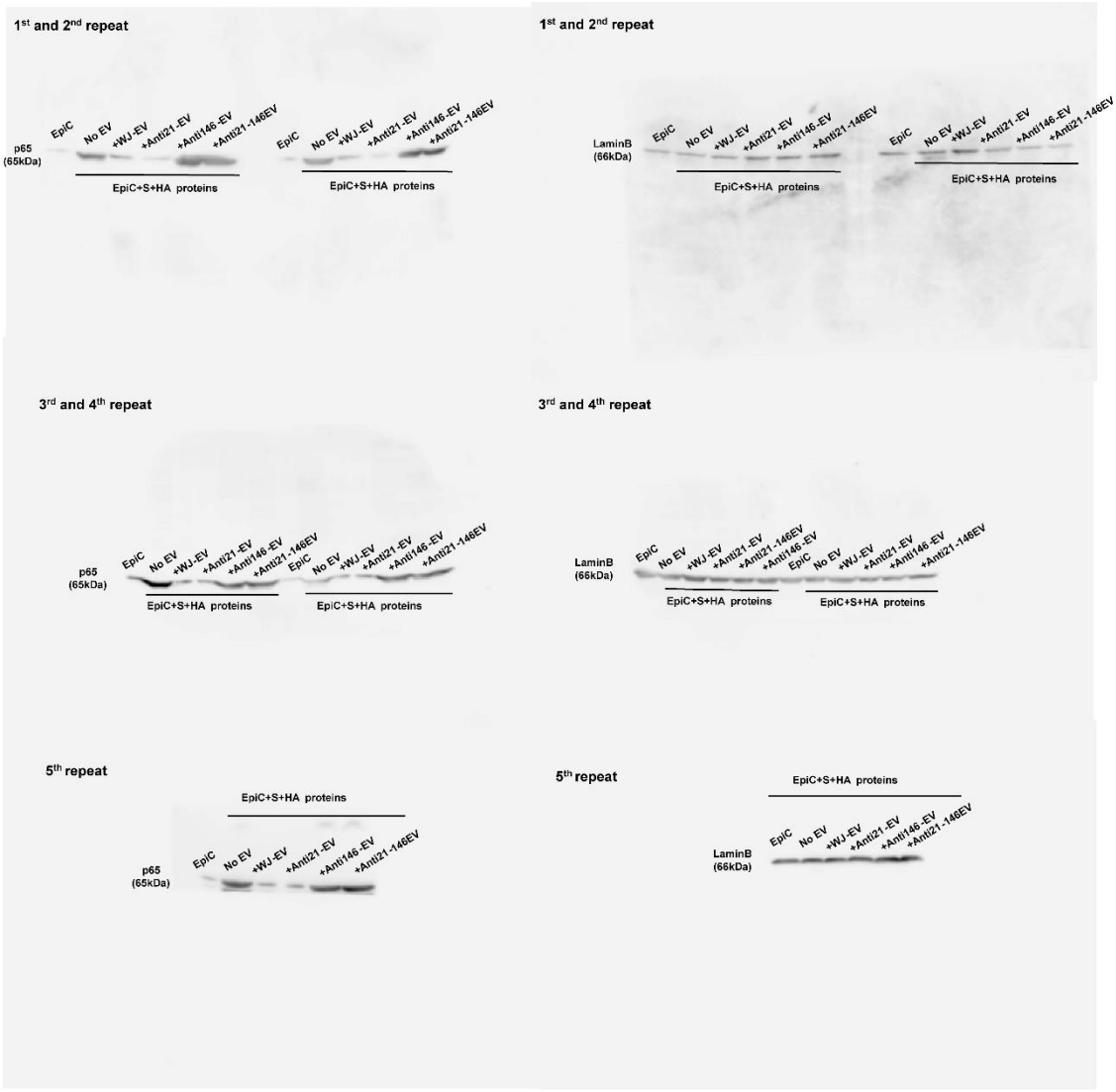
Supplementary Figure 3. A. Expression of p65 in nucleus of WJ-MSC and T2WJ-MSC. **B.** Concentration of inflammatory cytokines secreted from WJ-MSC and T2WJ-MSC. *P<0.05, **P<0.01.



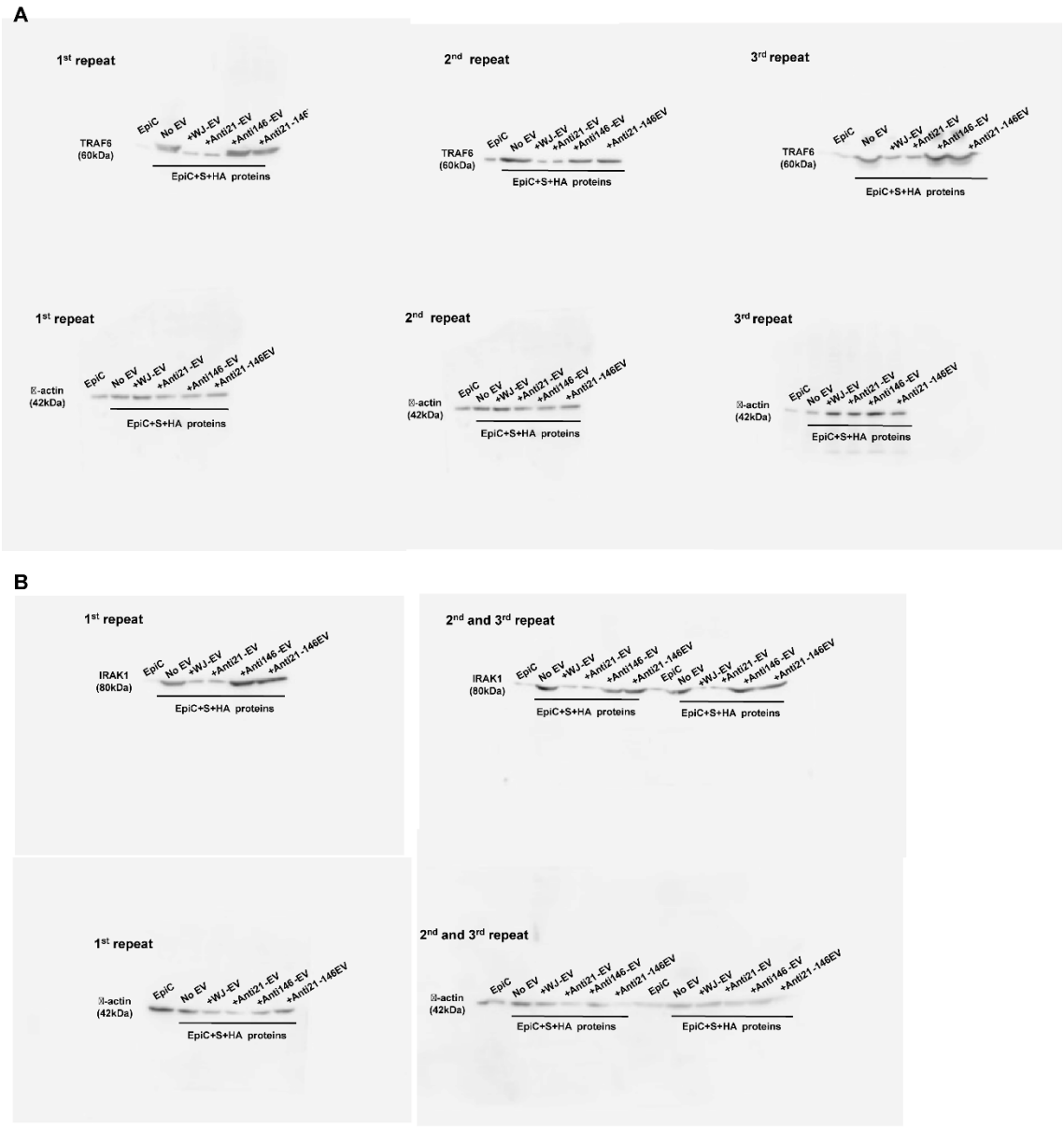
Supplementary Figure 4. EV characterization. A. Size distribution of WJ-EV and T2WJ-EV. **B.** Marker expression of WJ-EV and T2WJ-EV. Both WJ-EV and T2WJ-EV express TSG101 and CD63, but no expression of APOA1. **C.** Incorporation of PKH67-labeled WJ-EV or T2WJ-EV to lung EpiC. **D.** Quantification of EV incorporation by flow cytometry.



Supplementary Figure 5. Full length Western blots. Full length of Western blots shown in Figure 2C.

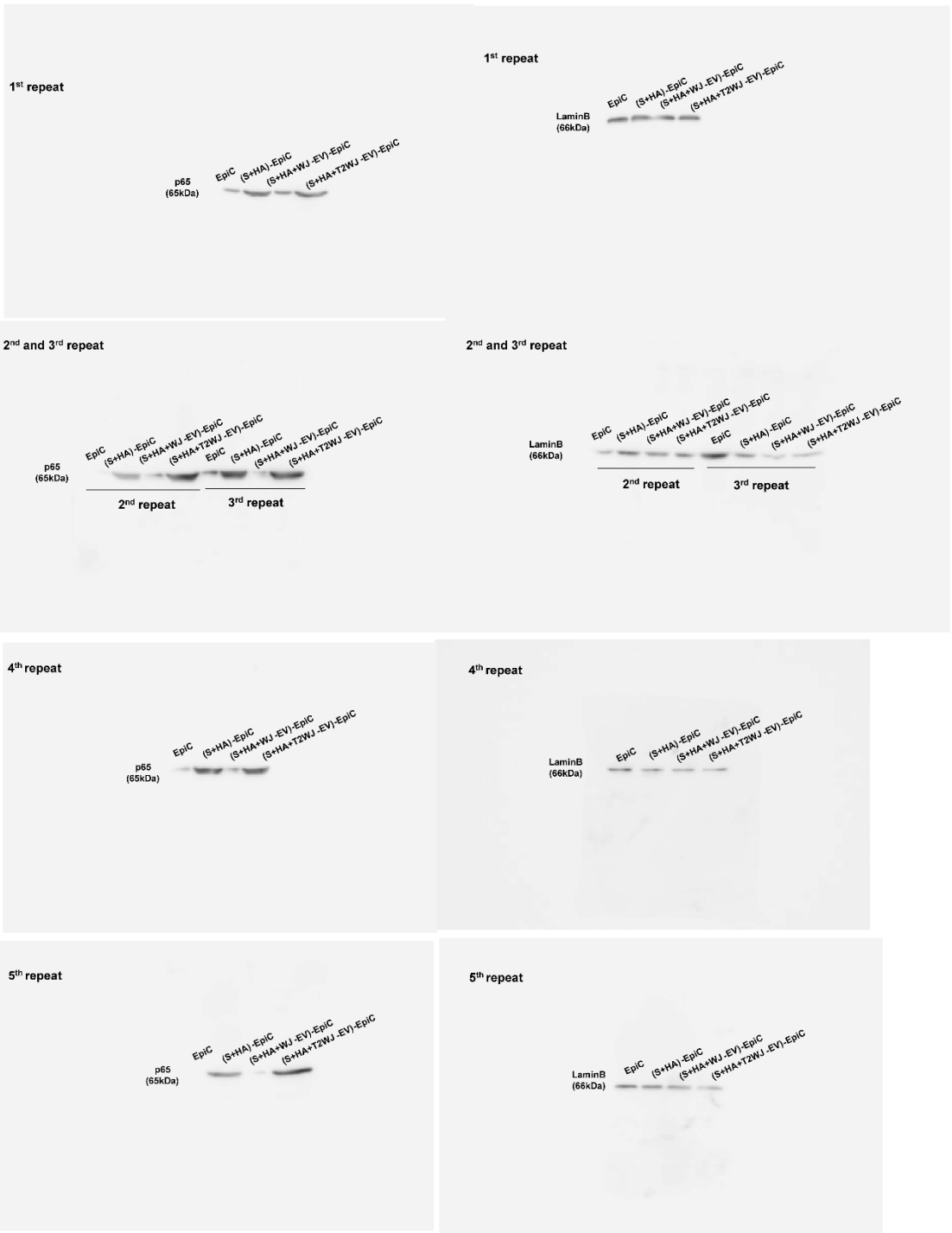


Supplementary Figure 6. Full length Western blots. Full length of Western blots shown in Figure 4A.



Supplementary Figure 7. Full length Western blots. A. Full length of Western blots shown in Figure 5B, **B.** Full length of Western blots shown in Figure 5C.

Supplementary Figure 8. Full length Western blots. Full length of Western blots shown in Figure 5D.



Supplementary Figure 9. Full length Western blots. Full length of Western blots shown in Figure 6D.

Supplementary Table 1. Inclusion and exclusion criteria in systematic review

Inclusion Criteria • Performance in vitro study • Based on SARS-CoV-2 and/or Influenza infection • Reported the inflammatory cytokine levels (TNF-α, IL-6, IL-1β, IL-8 or etc.) • Using exosome or extracellular vesicles from MSC
Exclusion Criteria • Reviews or protocol articles • Using modified-EV (mix with nano/bio particles)

Supplementary Table 2. Selected articles from systematic review. These articles demonstrated the ability of MSC-EV to reduce pro-inflammatory cytokine levels in responses to SARS-CoV-2 or IAV infection/induction using different cells, dose of MSC-EV and MSCEV origins.

No	Author (s)	Tittle	Year	Cell	Culture Method	Inducer	Dose	Treatment	Results	Examination Method	Difference (%)	Decrease or Increase	Note
1	Oh, S.J et al	Anti-Viral Activities of Umbilical Cord Mesenchymal Stem Cell-Derived Small Extracellular Vesicles Against Human Respiratory Viruses	2022	Human nasal epithelial cells (HNECs)	ALI culture	IFV A/H1N1 (90 min)	1 MOI	U-exo (UCMSC-EXOSOME)	IL6	supernatant, ELISA	11,52	Decrease*	
									IL8	supernatant, ELISA	17,76	Decrease*	
									Viral genes expression level	RT-qPCR	56,7	Decrease***	
						HCoV-OC43 (90 min)			IL6	supernatant, ELISA	51,6	Decrease**	
									IL8	supernatant, ELISA	3,59	Decrease	
									Viral genes expression level	RT-qPCR	50,82	Decrease***	
2	Cloer, C et al	Mesenchymal stromal cell-derived extracellular vesicles reduce lung	2021	Calu-3	Co-culture	RBD of the S1 SARS-CoV-2	50 nM	BM MSC-EV	IL6	supernatant, Organ on Chip	Data cannot be extracted	Increase	p Value not available

		inflammation and damage in nonclinical acute lung injury: Implications for COVID-19				spike protein (24h)			TNF- α	(Luminex assay)	Data cannot be extracted	Decrease	
									IL1 β		Data cannot be extracted	Decrease	
3	Khanh, et Al	Wharton's Jelly Mesenchymal Stem Cell-Derived Extracellular Vesicles Reduce SARS-CoV2-Induced Inflammatory Cytokines Under High Glucose and Uremic Toxin Conditions	2021	Calu-3	Co-culture	SARS-CoV-2 S-protein (24hr)	6 pmol	WJ-MSC-EV	TNF- α	Cells, qPCR	69,75	Decrease*	
									IL-6		79,59	Decrease*	
									IL1 β		61,61	Decrease*	
									IFN γ		64,11	Decrease*	
								AT MSC-EV	TNF- α		71,3	Decrease*	
									IL-6		81,1	Decrease*	
									IL1 β		59,1	Decrease*	
									IFN γ		60,1	Decrease*	
				Calu-3 high glucose				WJ-MSC-EV	TNF- α		74,5	Decrease**	
									IL-6		74,4	Decrease**	
									IL1 β		60,25	Decrease**	
									IFN γ		61,91	Decrease*	
								AT MSC-EV	TNF- α		23,9	Decrease	
									IL-6		21,81	Decrease	
									IL1 β		35,26	Decrease	
									IFN γ		21,28	Decrease	

				Calu-3 uremic condition				WJ-MSC-EV	TNF- α		55,8	Decrease*	
									IL-6		67,03	Decrease**	
									IL1 β		60,94	Decrease*	
									IFN γ		54,83	Decrease*	
								AT MSC-EV	TNF- α		16,29	Decrease*	
									IL-6		27,82	Decrease*	
									IL1 β		4,69	Decrease	
									IFN γ		29,31	Decrease	
				Calu-3 high glucose + Uremic condition				WJ-MSC-EV	TNF- α		78,89	Decrease**	
									IL-6		64,62	Decrease**	
									IL1 β		70,87	Decrease**	
									IFN γ		62,66	Decrease*	
								AT MSC-EV	TNF- α		21,11	Decrease	
									IL-6		17,42	Decrease	
									IL1 β		24,78	Decrease	
									IFN γ		6,28	Decrease	

* = P value < 0.05; ** P Value < 0.01; *** P value < 0.001

versus control treatment