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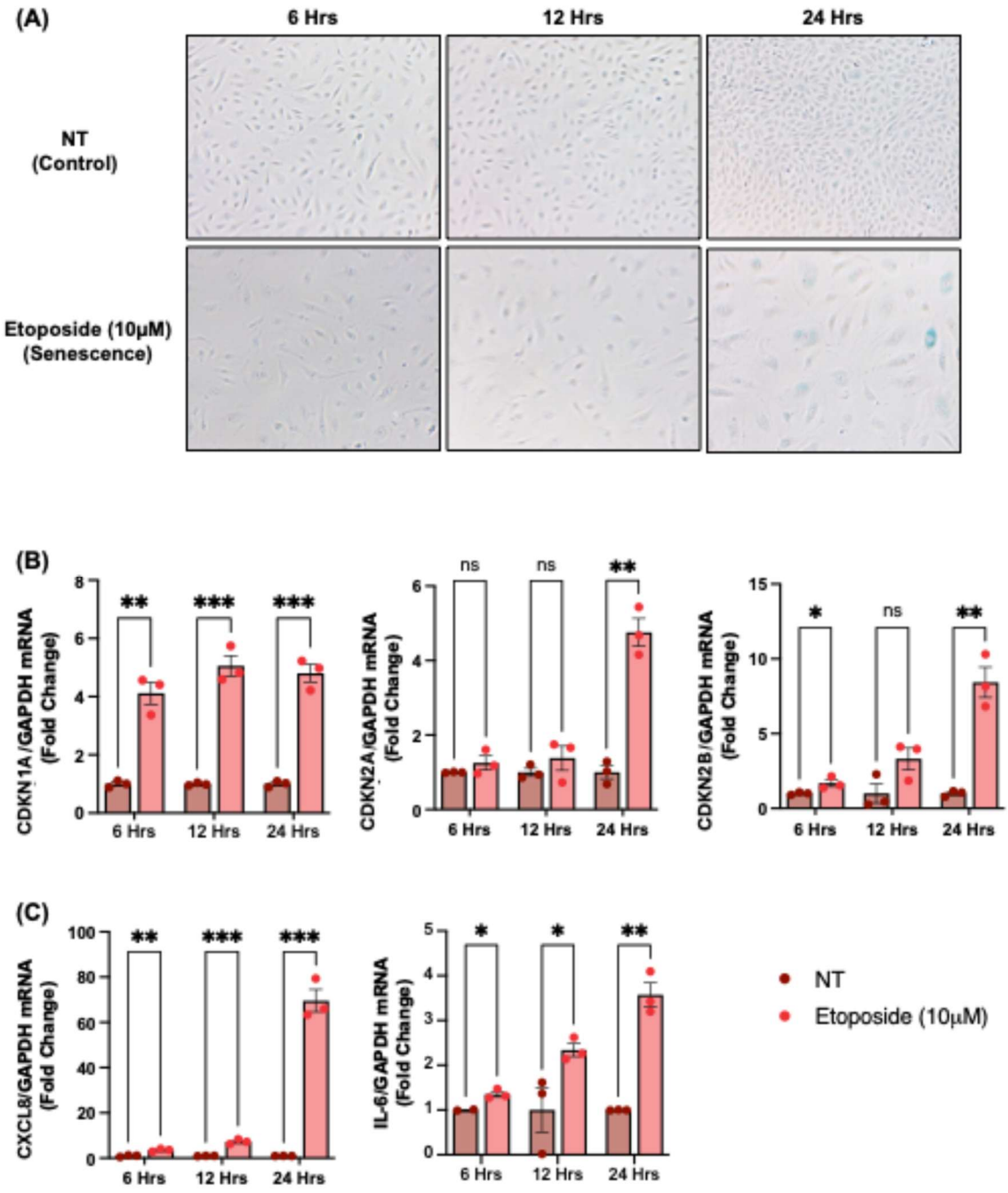
Supplementary Material

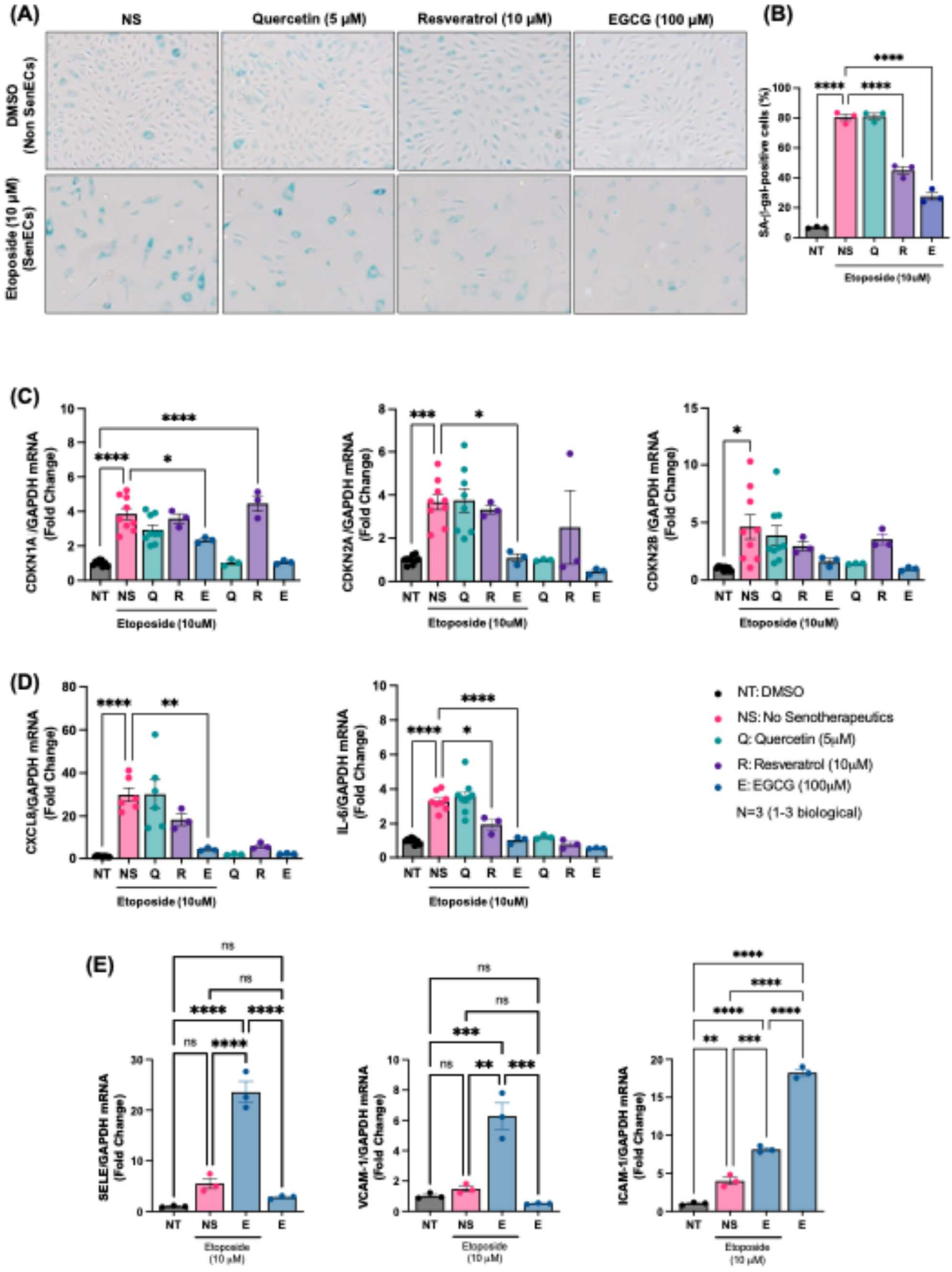
1 Supplementary Figures and Tables

Supplementary Table 1. Primers list for the qRT-PCR.

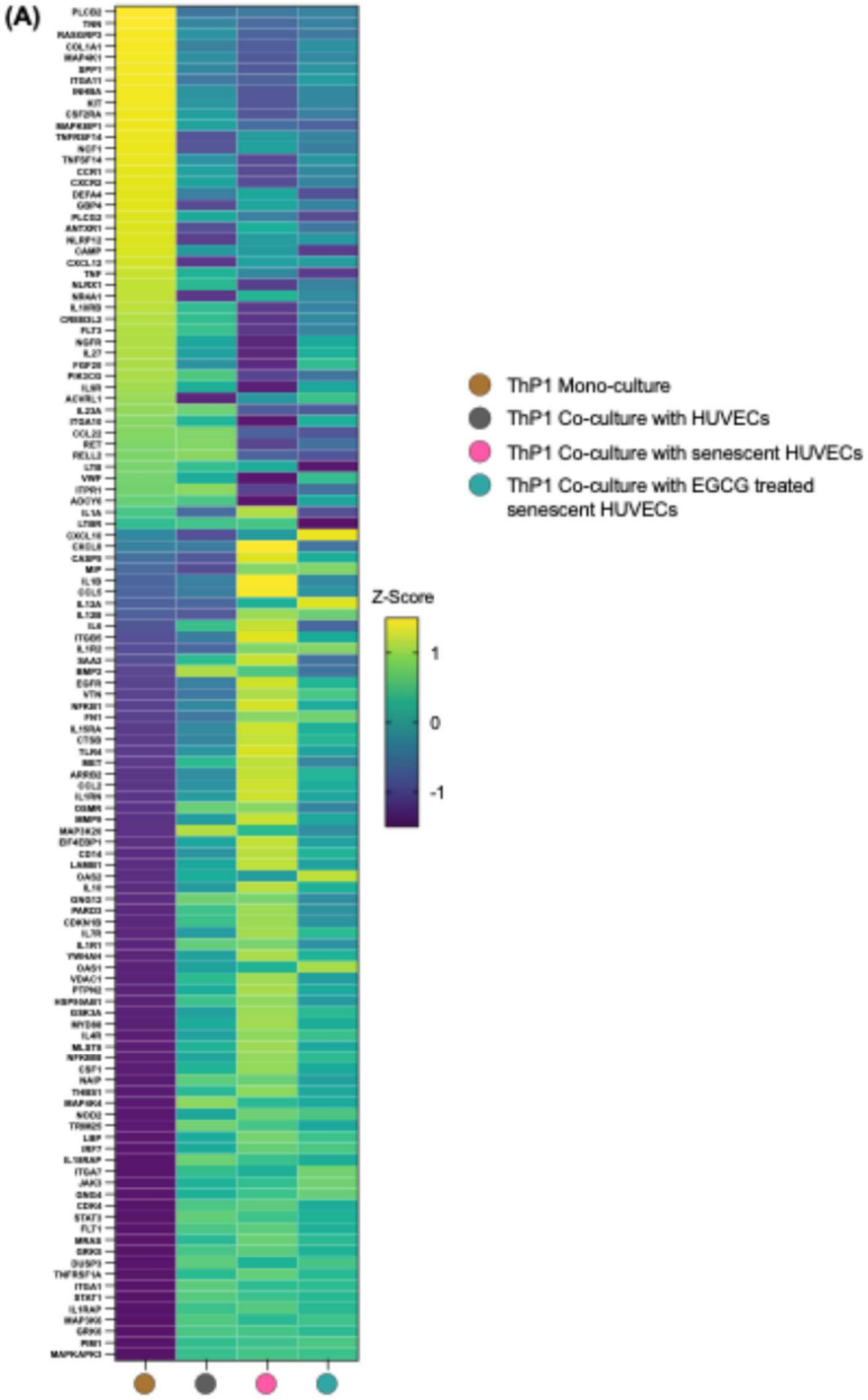
Primers	Sequence	
GAPDH	Fwd (5'-3')	AGG TGA AGG TCG GAG TCA AC
	Rev (5'-3')	GAG GTC AAT GAA GGG GTC AT
TBP	Fwd (5'-3')	TCG GAG AGT TCT GGG ATT GT
	Rev (5'-3')	CAC GAA GTG CAA TGG TCT TT
CDKN1A	Fwd (5'-3')	GCA GAC CAG CAT GAC AGA TT
	Rev (5'-3')	TTA GGG CTT CCT CTT GGA GA
CDKN2A	Fwd (5'-3')	CAA CGC ACC GAA TAG TTA CG
	Rev (5'-3')	CTG CCC ATC ATC ATG ACC T
CDKN2B	Fwd (5'-3')	CAA CGG AGT CAA CCG TTT C
	Rev (5'-3')	GTG AGA GTG GCA GGG TCT G
CXCL8	Fwd (5'-3')	TGC GCC AAC ACA GAA ATT AT
	Rev (5'-3')	ACT TCT CCA CAA CCC TCT GC
IL-6	Fwd (5'-3')	CTG GCA GAA AAC CTG AA
	Rev (5'-3')	ACC AGG CAA GTC TCC TCA TT
IL-1β	Fwd (5'-3')	ACC TCC AGG GAC AGG ATA TG
	Rev (5'-3')	AAC ACG CAG GAC AGG TAC AG
IL-12p40	Fwd (5'-3')	CTG GGA GTA CCC TGA CAC CT
	Rev (5'-3')	CTG AGG TCT TGT CCG TGA AG
IL-23p19	Fwd (5'-3')	CAC AGA AGC TCT GCA CAC TG
	Rev (5'-3')	CAC ACT GGA TAT GGG GAA CA
TNFα	Fwd (5'-3')	GGG CAG GTC TAC TTT GGG AT
	Rev (5'-3')	GGT TGA GGG TGT CTG AAG GA
SELE	Fwd (5'-3')	CTG GCC TGC TAC CTA CCT GT
	Rev (5'-3')	AGC TAC CAA GGG AAT GTT GG
VCAM1	Fwd (5'-3')	GTT GAA GGA TGC GGG AGT AT
	Rev (5'-3')	GGA TGC AAA ATA GAG CAC GA
ICAM1	Fwd (5'-3')	CGG CCA GCT TAT ACA CAA GA
	Rev (5'-3')	GTC TGC TGG GAA TTT TCT GG



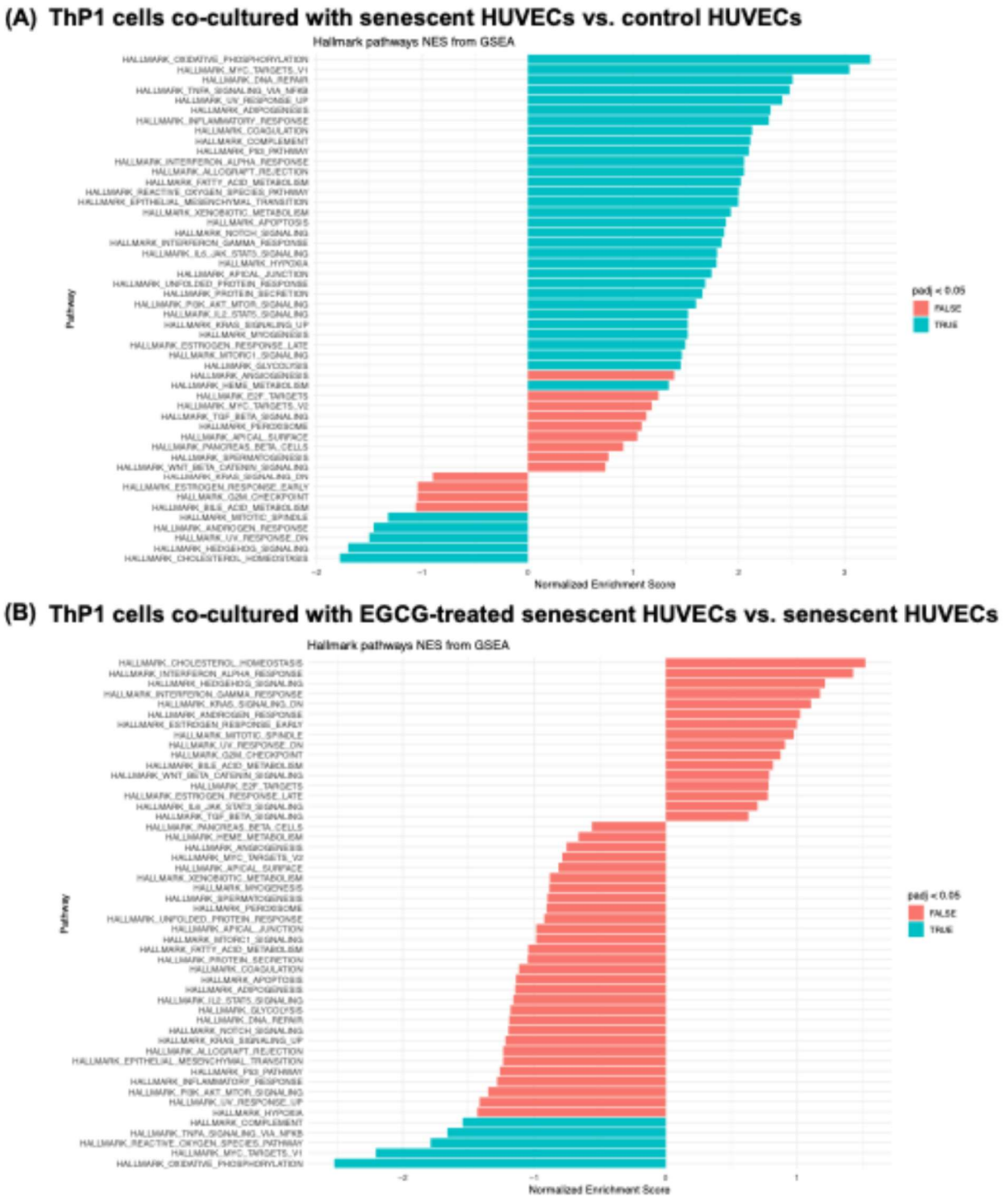
Supplementary Figure 1. Effects of Etoposide on HUVECs to induce senescence. HUVECs were treated with Etoposide (10μM) for 6h, 12h and 24h to induce senescence. **(A)** Representative cellular morphology and SA-β-gal staining of HUVECs under various conditions - (i) No treatment (control), (ii) Treated with Etoposide (10μM) for 6h, 12h, and 24h (senescence). **(B)** qRT-PCR analysis of senescence-related genes *CDKN1A*, *CDKN2A*, *CDKN2B*. **(C)** qRT-PCR analysis of senescence-associated secretory phenotype (SASP)-related genes *CXCL8*, *IL6*. Data are given as ± SEM. n=3. Statistical analysis was performed using the unpaired t-test. NT, no treatment; ns, not significance; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.


(A)
NS
Quercetin (5 μM)
Resveratrol (10 μM)
EGCG (100 μM)
DMSO (Non SenECs)
Etoposide (10 μM) (SenECs)
(B)
SA-β-gal-positive cells (%)
NT
NS
Q
R
E
Etoposide (10uM)
(C)
CDKN1A /GAPDH mRNA (Fold Change)
CDKN2A /GAPDH mRNA (Fold Change)
CDKN2B /GAPDH mRNA (Fold Change)
(D)
CXCL8/GAPDH mRNA (Fold Change)
IL-6/GAPDH mRNA (Fold Change)
NT: DMSO
NS: No Senotherapeutics
Q: Quercetin (5μM)
R: Resveratrol (10μM)
E: EGCG (100μM)
N=3 (1-3 biological)
(E)
SELE/GAPDH mRNA (Fold Change)
VCAM-1/GAPDH mRNA (Fold Change)
ICAM-1/GAPDH mRNA (Fold Change)
Etoposide (10 μM)

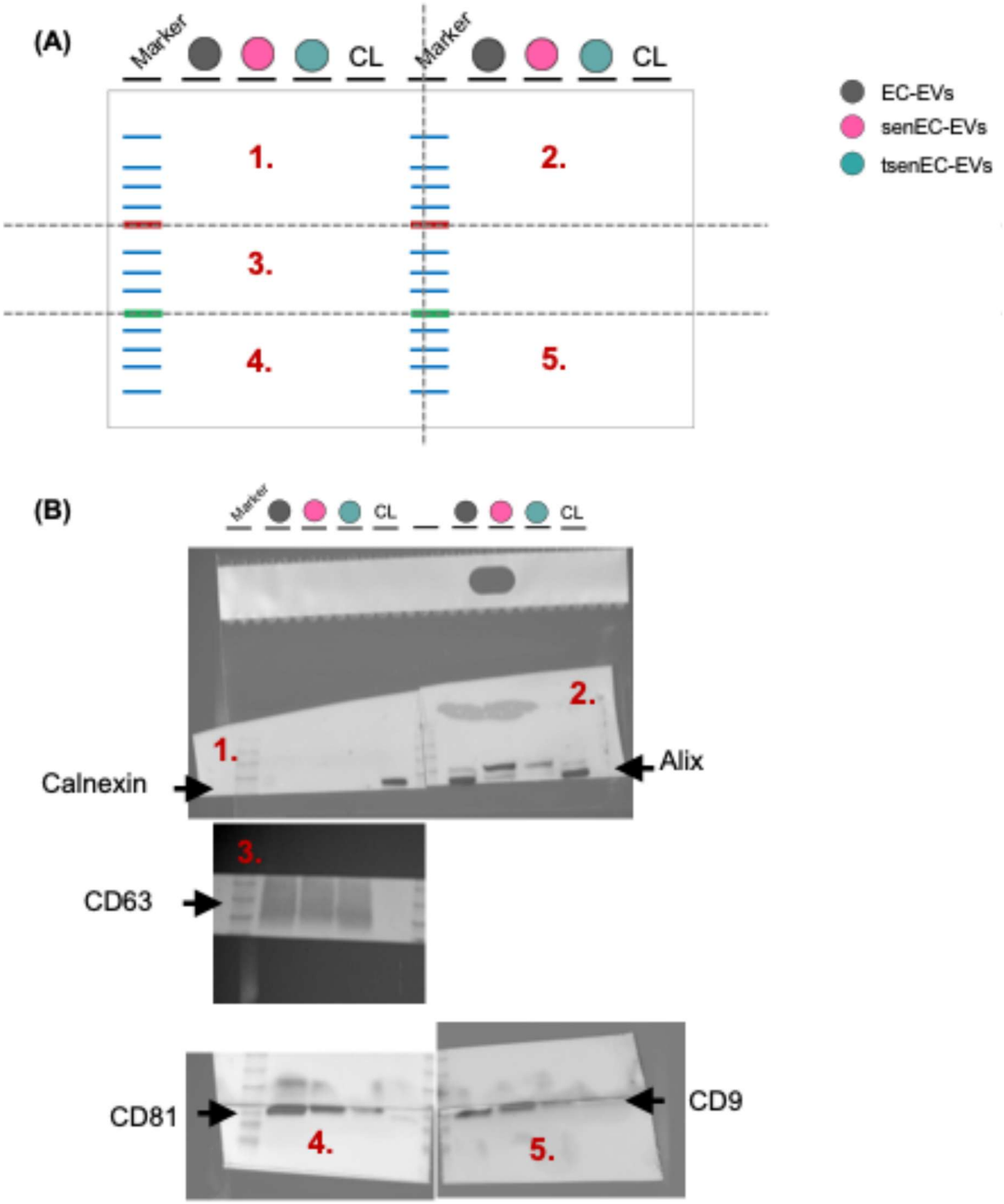
Supplementary Figure 2. Effects of senotherapeutics on senescent HUVECs. HUVECs were treated with Etoposide (10μM) for 24h to induce senescence, followed by treatment with or without Quercetin (5μM), Resveratrol (10μM), or EGCG (100μM) for 24h. **(A)** Representative cellular morphology and SA-β-gal staining of HUVECs under various conditions - (i) No senotherapeutics, Treated with (ii) Quercetin (5μM), (iii) Resveratrol (10μM), or (iv) EGCG (100μM). **(B)** Quantification of the percentage of SA-β-gal positive HUVECs. **(C)** qRT-PCR analysis of senescence-related genes p21, p16, p15. **(D)** qRT-PCR analysis of senescence-associated secretory phenotype (SASP)-related genes *CXCL8*, *IL6*. **(E)** qRT-PCR analysis of adhesion-related markers, including the genes *SELE*, *VCAM1* and *ICAM1*. Data are given as ± SEM. n=3. Two-way ANOVA with a Tukey's multiple comparison test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.



Supplementary Figure 3. Effect of EGCG treatment on the communication between senescent HUVECs and monocyte inflammatory gene profile. The impact of co-culturing THP1 cells with HUVECs for 24h was further assessed using RNA sequencing of THP1 cells. **(A)** Heatmap showing the expression profiles of pro-inflammatory genes. Gene expression was assessed across different experimental conditions to evaluate changes in inflammatory responses. Expression values are represented as colours and range from Yellow (high expression), green (moderate), to dark blue (lowest expression). Enrichment plots of inflammation-related gene sets comparing.



Supplementary Figure 4. Gene set enrichment analysis (GSEA) results of comparison between THP1 co-cultured with different HUVECs. RNA-Seq was performed on samples collected after 24h co-culturing THP1 cells with different HUVECs (control, senescent and EGCG treated senescent). Results of GSEA Hallmark analysis showing enriched gene sets **(A)** in THP1 cells co-cultured with senescent HUVECs vs. control HUVECs **(B)** THP1 cells co-cultured with EGCG-treated senescent HUVECs vs. senescent HUVECs. Bars in blue indicate significant enrichment at adjusted P value < 0.05, bars in red represent gene sets with adjusted P value < 0.05.



Supplementary Figure 5. Western blot analysis of EC-EV markers. (A) Schematic of the Western blot gel used to analyze EV markers, outlining the process for cutting the gel and probing with different antibodies for analysis. **(B)** Images of western blot analysis of different markers of EVs (positive: Alix, CD63, CD81, CD9 and negative: Calnexin)