Supplementary Materials

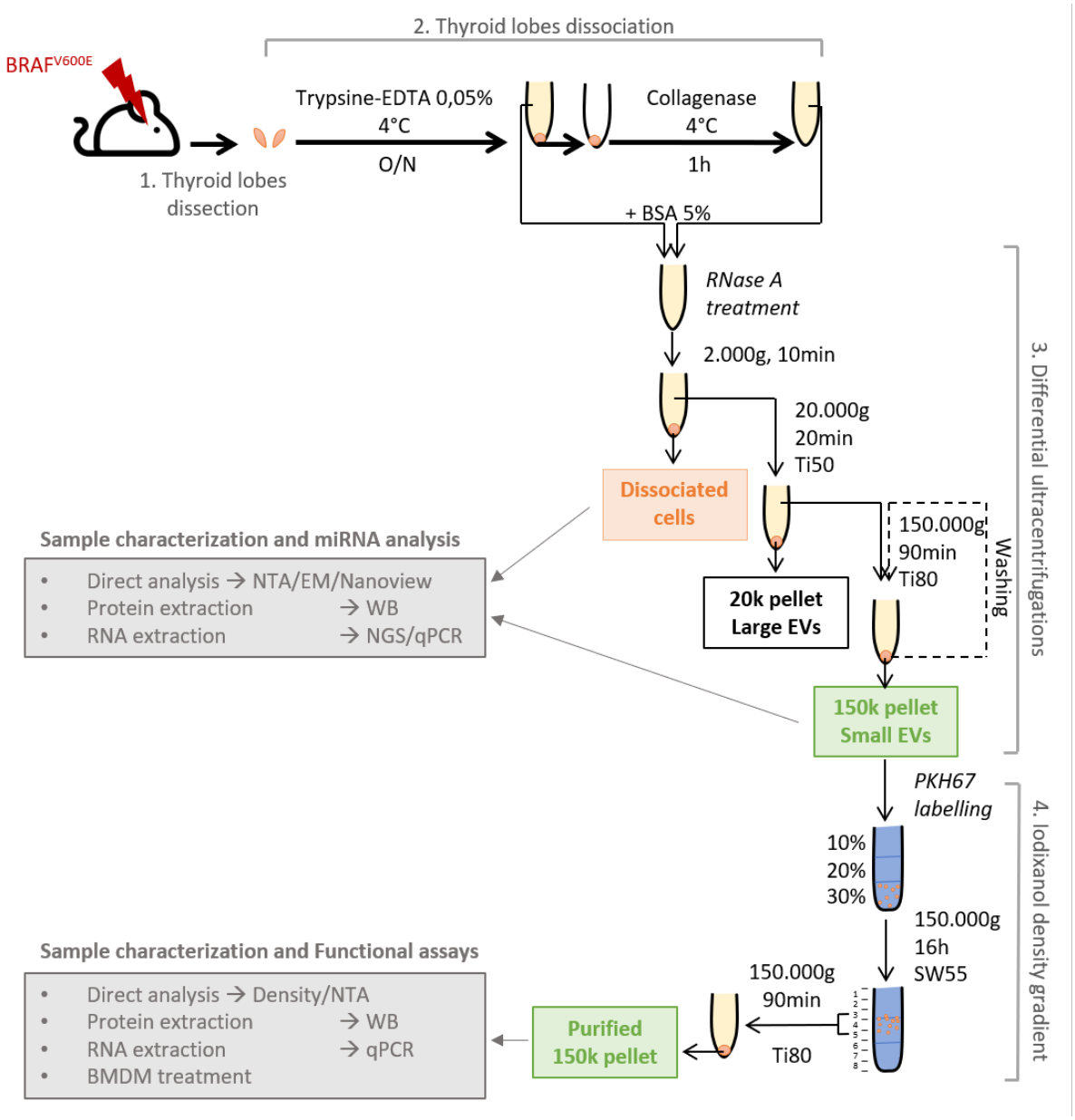


Figure S1: *In vivo* produced EVs are isolated from thyroid lobes by tissue dissociation followed by differential ultracentrifugation and optional iodixanol density gradient. Schematic representation of the procedure for EV isolation, characterization, and downstream analyses.

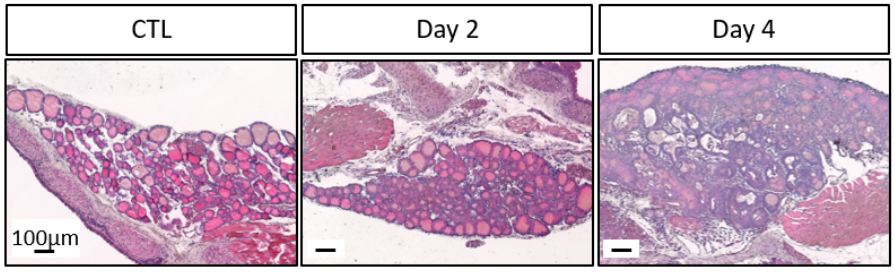


Figure S2: BRAF^{V600E} expression in thyrocytes triggers tissue growth and remodelling Low-magnification view of the haematoxylin and eosin staining of thyroid tissues from control and doxycycline-treated mice, after 2 and 4 days of intraperitoneal injections, shown in Fig.1A.

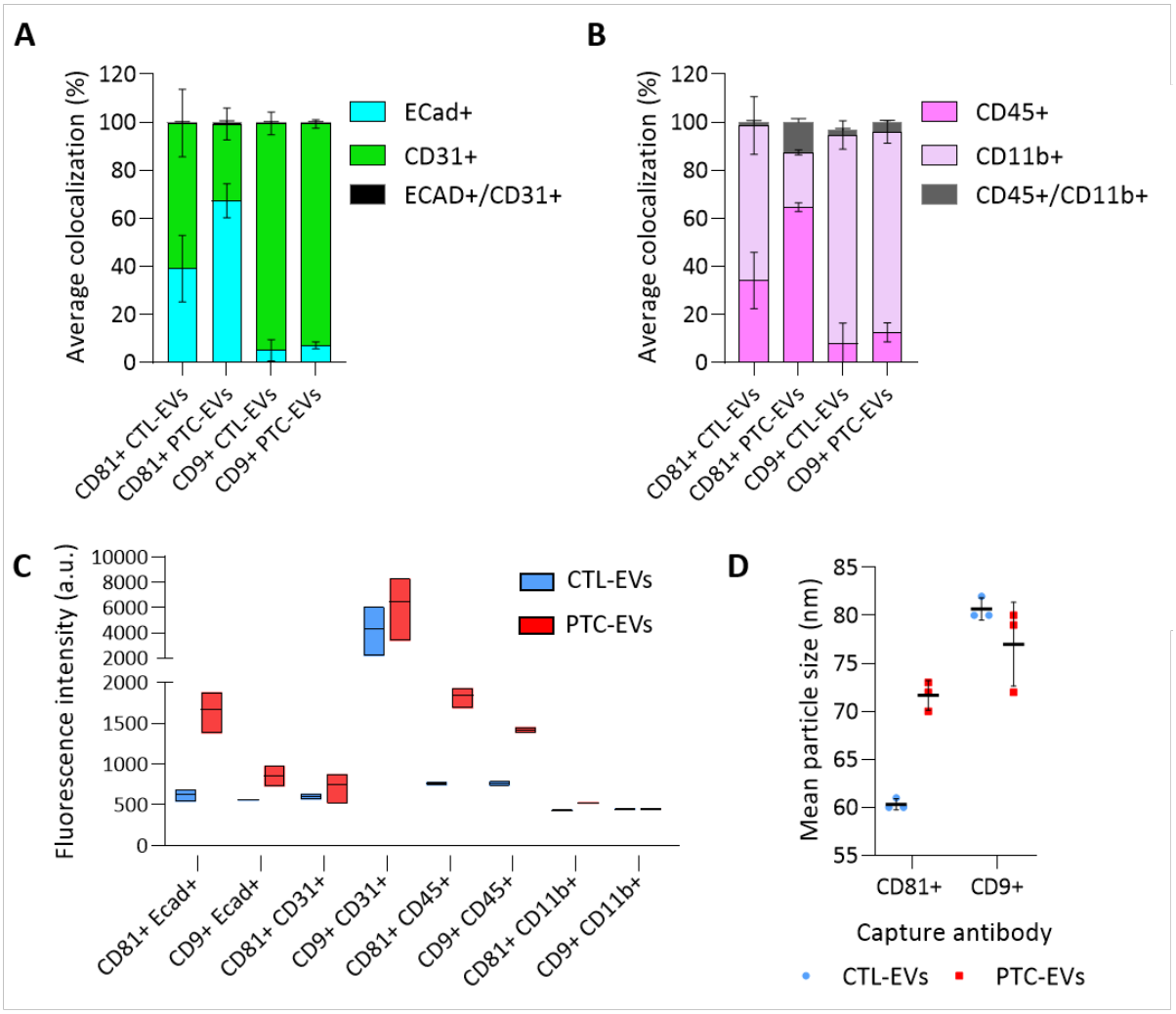


Figure S3: The ExoView platform allows EV population analyses. (A-B) Average percentage of colocalization between Ecad and CD31 detection antibodies (A), and between CD45 and CD11b detection antibodies (B). (C) Fluorescence intensity of detection antibodies measured in CD9+ and CD81+ EV populations in control and PTC- EV samples, reflecting the number of detected epitopes. (D) Mean particles size of CD9+ and CD81+ EVs measured in CTL and PTC-EV samples.

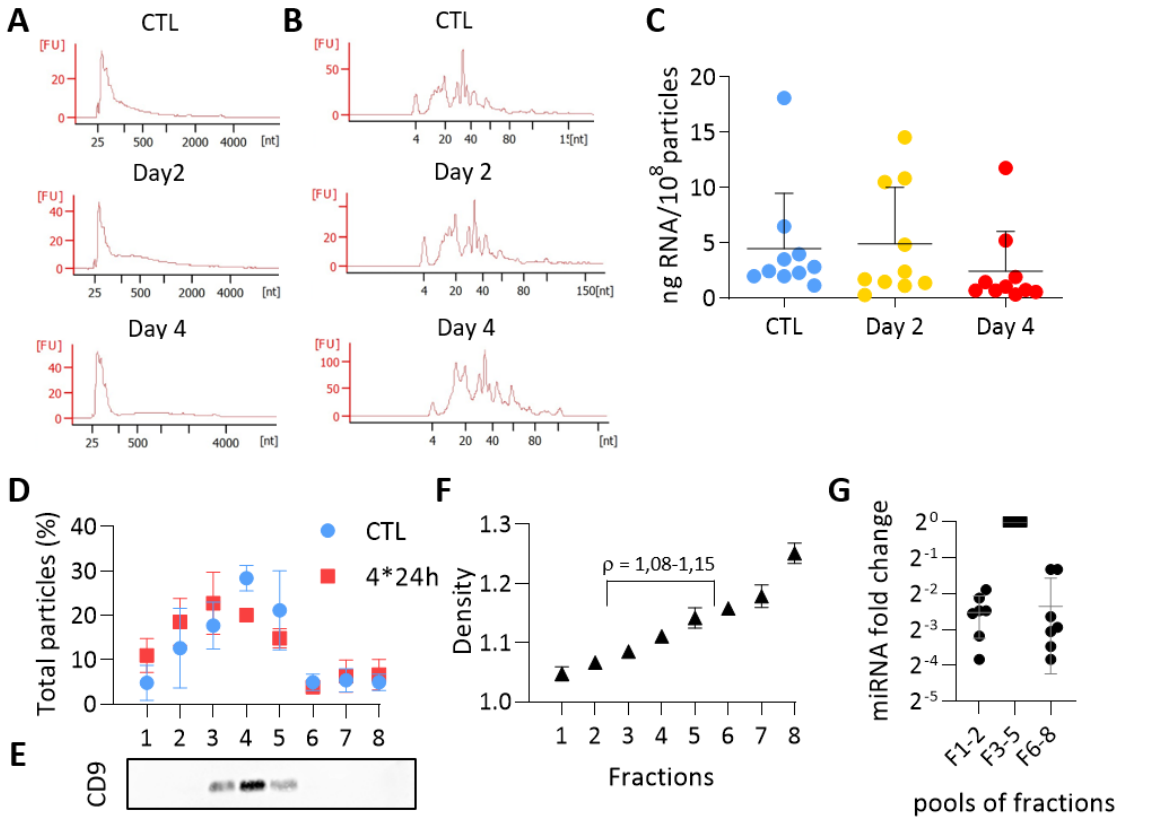
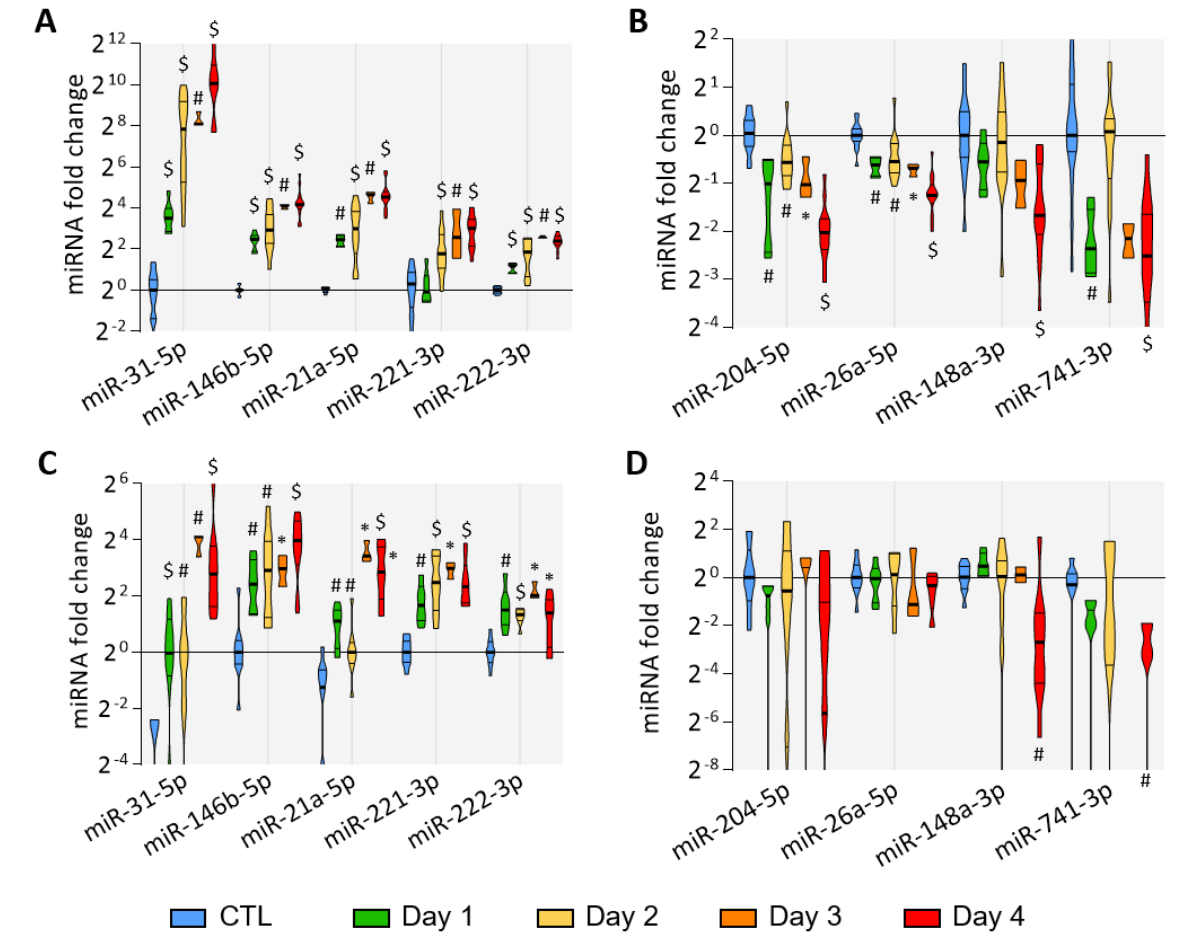


Figure S4: miRNAs detected in the high-speed pellets are associated with EV fractions after density-based fractionation. Electrophoretic profiles of (A) total RNA, and (B) small RNA extracted from the 150k pellet isolated from control and DOX-treated thyroids, after 2 and 4 days of injections. (C) Total RNA quantity per 10^8 particles (measured by NTA). (D-G) Characterization of 8 fractions obtained after bottom-up iodixanol density gradient. (D) Distribution of particles measured by NTA. (E) Western blot detection of CD9 marker after purification of the 150k pellet isolated from day 4 DOX-treated thyroids. (F) Measure of the density of each fraction. (G) RT-qPCR analysis of 8 miRNAs in concentrated pools of fractions. Data are expressed as miRNA fold changes in pools of fractions 1-2 and 6-8 compared to their abundance in pool of fractions 3 to 5, considered as EV fractions.



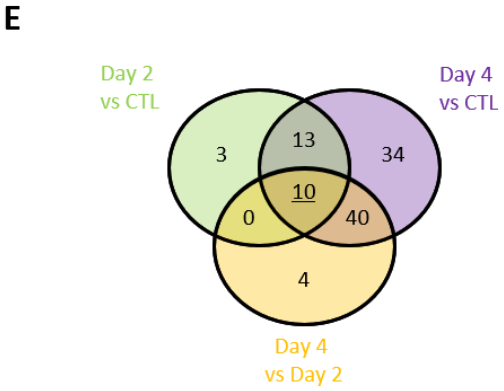


Figure S5: miRNAs display rapid and important changes in their abundance in BRAF^{V600E} tissues and EVs. RT-qPCR analyses of upregulated (A, B) and downregulated (C, D) miRNA in dissociated cells (A, C) and EVs (B, D) from control and doxycycline-treated thyroids after 1, 2, 3 and 4 days of injections (n≥4). MiRNA fold changes are normalized on let-7c-5p expression for cellular samples, and on the geometric mean of miR-126a-3p and let-7b-5p expression for EV samples. Data are expressed as mean ± SD (* p<0.05; # p<0.01; $ p<0.001). (E) Venn diagram illustrating the number of EV-miRNAs with differential abundance in the three groups. Abundance of 10 miRNAs was statistically different between the 3 groups.

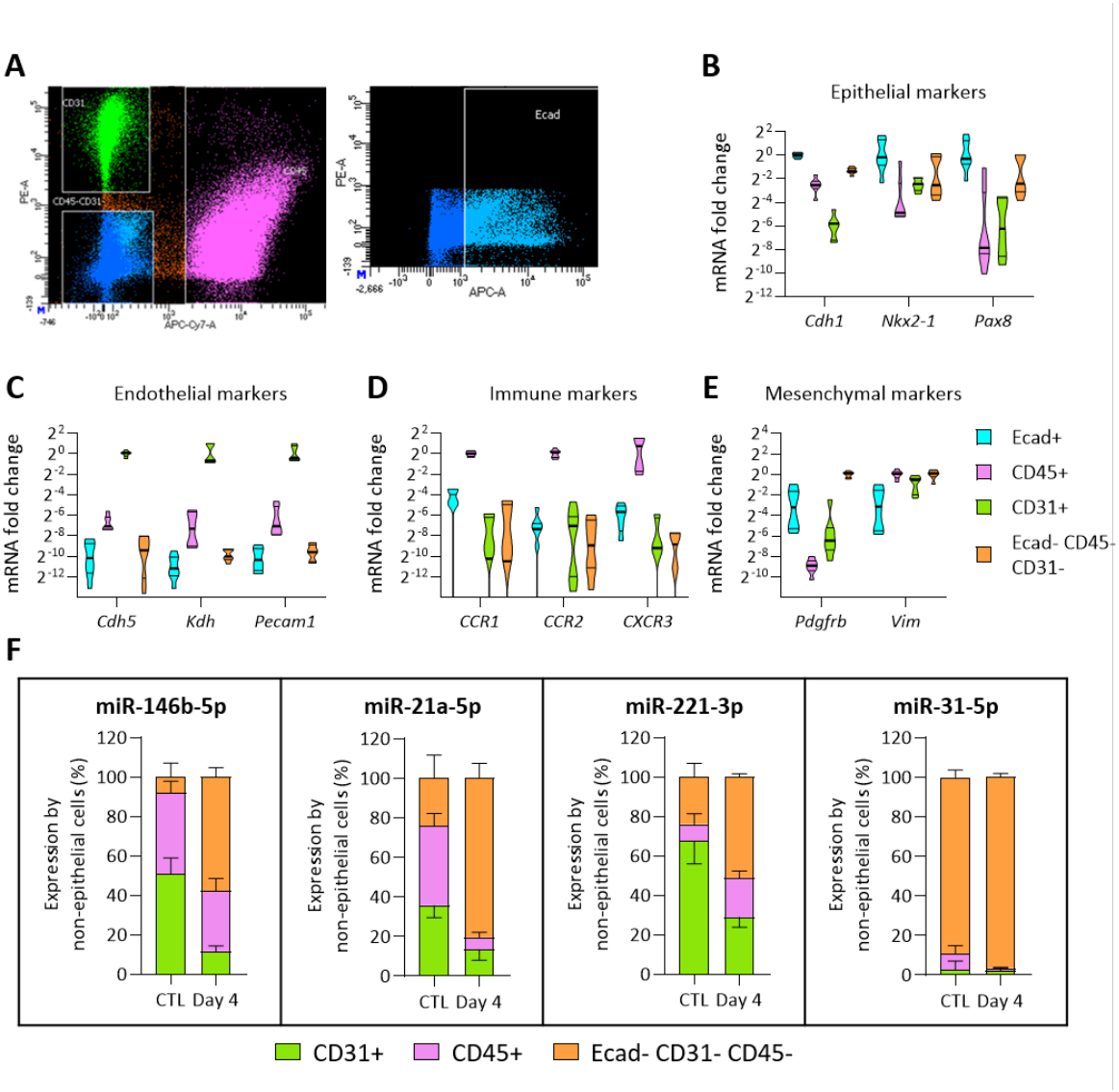


Figure S6: Purification of epithelial, endothelial and immune populations by FACS-sorting of thyroid and tumour dissociated cells using Ecad, CD31 and CD45 markers. (A) Coloured dot plot showing an example of DOX day 4 dissociated cells sorting according to the intensity of fluorescence with CD31, CD45 or Ecad antibodies. (B-E) RT-qPCR analyses of the expression of epithelial (B), endothelial (C), immune (D) and mesenchymal (E) markers in sorted populations. mRNA fold changes are normalized on the geometric mean of *Rpl27* and *Gapdh* expression and compared to the expression in their respective population. (F) RT-qPCR analyses of miRNA expression in FACS-sorted non-epithelial cell populations. Stacked bars show the relative expression of the miRNAs in non-epithelial cell populations normalized by the quantity of RNA extracted from each population. Data are expressed as mean ± SD.

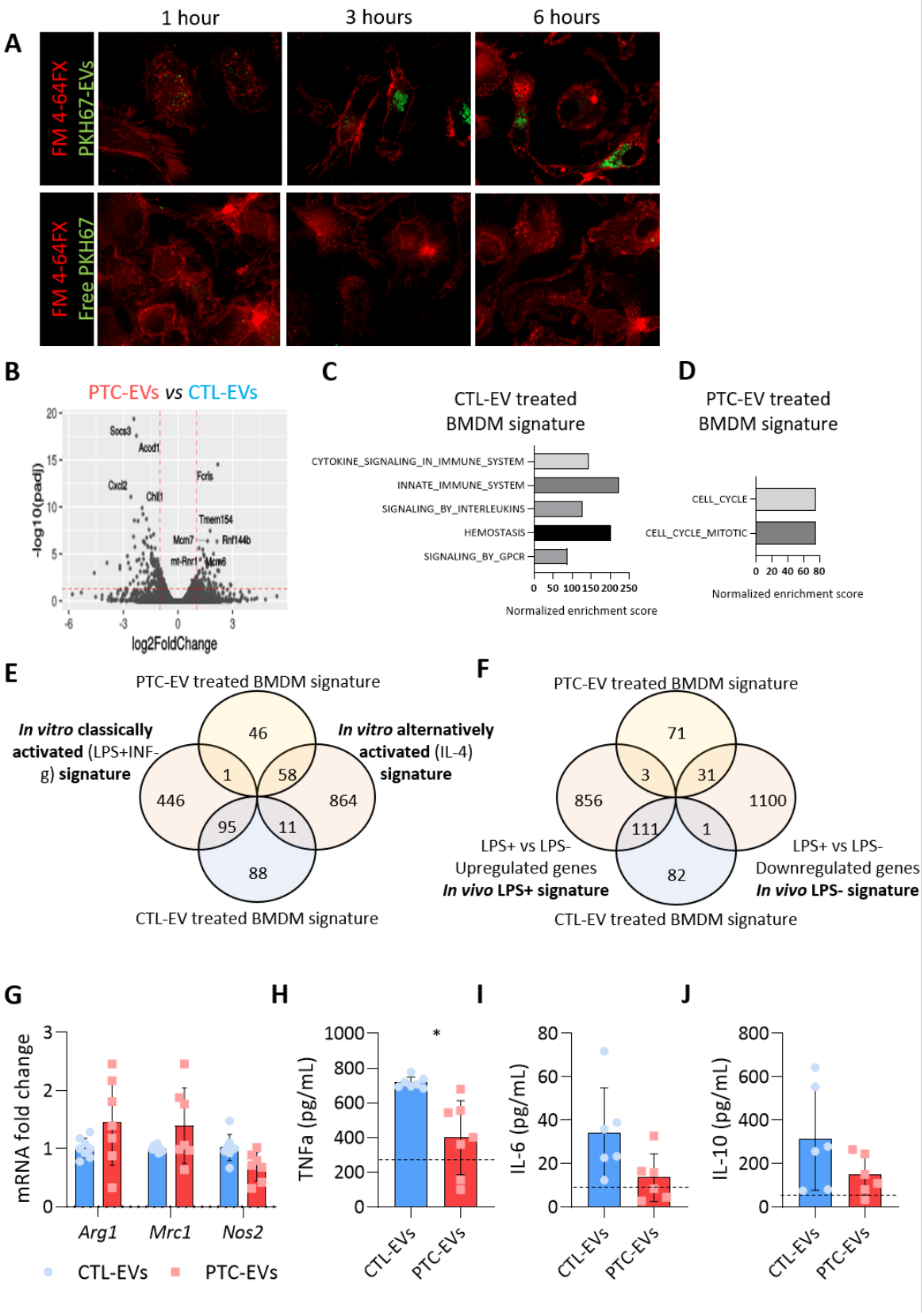


Figure S7: Treatment of BMDM with CTL-EVs or PTC-EVs pools demonstrate internalization but no major effects. (A) Live imaging of BMDM fluorescently labelled by FM 4-64FX, a red membrane dye, and treated with PKH67-EVs or free PKH67 after 1, 3 or 6 hours of incubation. (B) Volcano plot showing

p-adjusted value and fold change relation in differential analysis of mRNA expression by CTL-EVs or PTC-EVs treated BMDM. KEGG pathway analysis of gene upregulated in CTL-EVs treated BMDM compared to PTC-EVs treated BMDM (C) and of gene upregulated in PTC-EVs treated BMDM compared to CTL-EVs treated BMDM (D). (E-F) Comparison of the mRNA signature of EV-treated BMDM with publicly available mRNA signature of (E) *in vitro* (LPS+INFg *vs* IL-4; GSE69607) or (F) *in vivo* (LPS+ *vs* LPS-; GSE38705) polarized macrophages (G). RT-qPCR analyses of *Arg1*, *Mrc1* and *Nos2* expression in non-treated, CTL-EVs or PTC-EVs treated BMDM (n=6). mRNA fold changes are normalized on the geometric mean of *Rpl27* and *Gapdh* expression. Data are expressed as mean ± SD; differences are not significant. (H-J) Concentration of TNFα, IL-6 and IL-10 released in BMDM medium after 24h of treatment with CTL-EVs and PTC-EVs compared to the basal production level in non-treated BMDM (dotted line).

Table S1: Sequences of primers used in mRNAs RT-qPCR.

Gene	Forward primer	Reverse primer
Fosl1	CCGGCCAGGAGTCATACG	CTGCTAGCTTGTTCCGCTCG
Dusp5	CCAGTGTGGAAAGCCCGTT	CGCACTTGGATGCGTGGTA
Pax8	CGGCGATGCCTCACAACTCG	CCGGATGCTGCCAGTCTCGT
Nkx2.1	ATCTGAGCTGGGGTGCTGGG	GCCCTGTCTGTACGCTGCGA
Nis	AGCAGGCTTAGCTGTATCCC	AGCCCCGTAGTAGAGATAGGAG
Tg	TGGGACGTGAAAGGGGAATGGTGC	GTGAGCTTTTGGAATGGCAGGCGA
Tpo	TGCCAACAGAAGCATGGGCAAC	GCACAAAGTTCCCATTGTCCAC
Tshr	CTGCGGGGCAAAGAGTGTGC	AGGGGAGCTCTGTCAAGGCA
Rpl27	GCCCTGGTGGCTGGAATTGACC	AAACTTGACCTTGGCCTCCCGC
Gapdh	TGCACCACCAACTGCTTAGC	GGATGCAGGGATGATGTTCT

Table S2: List of antibodies used.

Antibodies used in western blot					
Antibody	**Isotype**	**Clone**	**Supplier**	**Reference**	**Working dilution**
Calnexin	Rabbit	polyclonal	Enzo	ADI-SPA-860	1:10000
PDI	Rabbit	C81H6	Cell signaling technologies	3501	1:1000
CD63	Rat	NVG-2	Biolegend	143902	1:500
CD81	Rabbit	D502Q	Cell signaling technologies	10037	1:400
CD9	Rat	KMC8.8	eBiosciences	14-0091-82	1:200
Alix	Mouse	C-11	Santa Cruz	sc-271975	1:500
Flotillin-1	Mouse	18/Flotillin-1	BD transduction	610820	1:500
Antibody used in immunohistofluorescence					
Antibody	**Isotype**	**Clone**	**Supplier**	**Reference**	**Working dilution**
CD206	Rabbit	E6T5J	Cell signaling technologies	24595	1:500
Fluorochrome-conjugated antibodies for FACS, flow cytometry and ExoView experiments					
Antibody	**Fluorochrome**	**Clone**	**Supplier**	**Reference**	**Working concentration**
CD3	Alexa 700	17A2	biolegend	100216	10 µg/ml
CD11b	BV510/FITC	M1/70	biolegend	101263/101205	2 µg/ml
CD31	PE	MEC13.3	BD biosciences	553373	2 µg/ml
CD45	APC-Cy7	30-F11	BD biosciences	557659	1 µg/ml
Ecad	Alexa 647	DECMA-1	biolegend	147308	15 µg/ml

Table S3: Sequences of primers and probes used in miRNAs RT-qPCR.

miRNA ID	Sequence	Loop-Reverse primer	Forward primer	Probe
mmu-miR-31-5p	AGGCAAGAUGCUGGCAUAGCUG	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCAGCTATG	ACACTCCAGCTGGGAGGCAAGATGCTG	TCAGTTGAGCAGCTATG
mmu-miR-146b-5p	UGAGAACUGAAUUCCAUAGGCU	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCCTATG	ACACTCCAGCTGGGTGAGAACTGAATTC	TCAGTTGAGAGCCTAT
mmu-miR-21a-5p	UAGCUUAUCAGACUGAUGUUGA	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAACATC	ACACTCCAGCTGGGTAGCTTATCAGACT	TCAGTTGAGTCAACATC
mmu-miR-222-3p	AGCUACAUCUGGCUACUGGGU	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACCCAGTA	ACACTCCAGCTGGGAGCTACATCTGGC	TCAGTTGAGACCCAGT
mmu-miR-221-3p	AGCUACAUUGUCUGCUGGGUUUC	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGAAACCCA	ACACTCCAGCTGGGAGCTACATTGTCT	TCAGTTGAGGAAACCCA
mmu-miR-26a-5p	UUCAAGUAAUCCAGGAUAGGCU	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCCTATC	ACACTCCAGCTGGGTTCAAGTAATCCA	TCAGTTGAGAGCCTATC
mmu-miR-204-5p	UUCCCUUUGUCAUCCUAUGCCU	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGGCATAG	ACACTCCAGCTGGGTTCCCTTTGTCAT	TCAGTTGAGAGGCATAG
mmu-miR-148a-5p	AAAGUUCUGAGACACUCCGACU	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGTCGGAG	ACACTCCAGCTGGGAAAGTTCTGAGAC	TCAGTTGAGAGTCGGAG
mmu-miR-148a-3p	UCAGUGCACUACAGAACUUUGU	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACAAAGTT	ACACTCCAGCTGGGTCAGTGCACTACA	TCAGTTGAGACAAAGTT
mmu-miR-741-3p	UGAGAGAUGCCAUUCUAUGUAGA	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCTACATA	ACACTCCAGCTGGGTGAGAGATGCCAT	TCAGTTGAGTCTACATA
mmu-miR-30d-5p	UGUAAACAUCCCCGACUGGAAG	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTTCCAGT	ACACTCCAGCTGGGTGTAAACATCCCC	TCAGTTGAGCTTCCAGT
mmu-miR-99a-5p	AACCCGUAGAUCCGAUCUUGUG	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCACAAGAT	ACACTCCAGCTGGGAACCCGTAGATCC	TCAGTTGAGCACAAGAT
mmu-miR-10b-5p	UACCCUGUAGAACCGAAUUUGUG	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCACAAATT	ACACTCCAGCTGGGTACCCTGTAGAAC	TCAGTTGAGCACAAATTC
mmu-let-7f-5p	UGAGGUAGUAGAUUGUAUAGUU	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTATAC	ACACTCCAGCTGGGTGAGGTAGTAGAT	TCAGTTGAGAACTATAC
mmu-miR-126a-3p	UCGUACCGUGAGUAAUAAUGCG	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCGCATTAT	ACACTCCAGCTGGGTCGTACCGTGAGT	TCAGTTGAGCGCATTAT
mmu-let-7b-5p	UGAGGUAGUAGGUUGUGUGGUU	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACCACAC	ACACTCCAGCTGGGTGAGGTAGTAGGT	TCAGTTGAGAACCACAC
mmu-let-7c-5p	UGAGGUAGUAGGUUGUAUGGUU	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACCATAC	ACACTCCAGCTGGGTGAGGTAGTAGGT	TCAGTTGAGAACCATAC

Table S4: List of the most frequently sequenced miRNAs in EVs isolated from control thyroids, with the associated count per million (CPM).

Name	Mean CPM
miR-125b-5p	213400
let-7c-5p	100233
let-7b-5p	83644
let-7f-5p	76620
miR-125a-5p	66392
let-7a-5p	58649
miR-126a-3p	40698
miR-30d-5p	37117
miR-29a-3p	35818
miR-16-5p	34094
miR-148a-3p	31128
let-7i-5p	29743
miR-26a-5p	23394
miR-199a-3p	19919
miR-30a-5p	16369
miR-143-3p	16316
let-7e-5p	13416
miR-200a-3p	11859
miR-191-5p	11082
miR-26b-5p	10077

Table S5: List of the most frequently sequenced miRNAs in EVs isolated from doxycycline-treated thyroids after 2 days of injections, with the associated count per million (CPM).

Name	Mean CPM
miR-125b-5p	147446
let-7f-5p	75838
let-7b-5p	73932
let-7c-5p	72227
let-7a-5p	56754
miR-126a-3p	43233
miR-125a-5p	42283
let-7i-5p	39875
miR-16-5p	39776
miR-29a-3p	37769
miR-148a-3p	30527
miR-30d-5p	25504
miR-21a-5p	21269
miR-199a-3p	20696
miR-26a-5p	19705
miR-143-3p	19673
miR-30a-5p	15666
let-7e-5p	14275
miR-221-3p	10761
miR-200a-3p	10619

Table S6: List of the most frequently sequenced miRNAs in EVs isolated from doxycycline-treated thyroids after 4 days of injections, with the associated count per million (CPM).

Name	Mean CPM
miR-125b-5p	113447
miR-126a-3p	52815
let-7b-5p	52375
let-7c-5p	48836
miR-16-5p	47741
let-7f-5p	47140
miR-21a-5p	41538
let-7a-5p	38777
let-7i-5p	38526
miR-125a-5p	26307
miR-29a-3p	24603
miR-199a-3p	15656
miR-143-3p	13213
miR-221-3p	13163
miR-30d-5p	12642
let-7e-5p	11214
miR-26a-5p	11184
miR-191-5p	9513
miR-146b-5p	8998
miR-23a-3p	8789

Table S7: List of miRNAs with a differential abundance, expressed in logFC, in EVs isolated at day 2 and day 4, as compared to control thyroids. MiRNAs marked with a "*" also present a differential abundance in the 4*24h and 2*24h samples.

Name	2*24h vs CTL	4*24h vs CTL
miR-31-5p *	4.127	5.472
miR-135b-5p *	2.830	3.913
miR-21a-3p	3.110	3.643
miR-21a-5p *	2.465	3.466
miR-146b-5p	3.102	3.360
miR-155-5p *	1.584	3.081
miR-223-5p	2.426	3.066
miR-5099	2.046	2.970
miR-223-3p *	1.783	2.924
miR-142a-5p *	1.354	2.669
miR-20a-5p	1.914	2.582
miR-142a-3p *	1.574	2.533
miR-184-3p	1.877	2.263
miR-221-3p	1.874	2.207
miR-218-5p	1.655	1.917
miR-484	1.477	1.843
miR-674-5p	1.270	1.647
miR-125b-1-3p *	0.825	1.646
miR-222-3p	1.220	1.511
miR-125a-5p	-0.665	-1.303
miR-206-3p	-1.365	-1.490
miR-200c-3p *	-0.639	-1.786
miR-204-5p *	-1.040	-2.773