Supplementary Figures

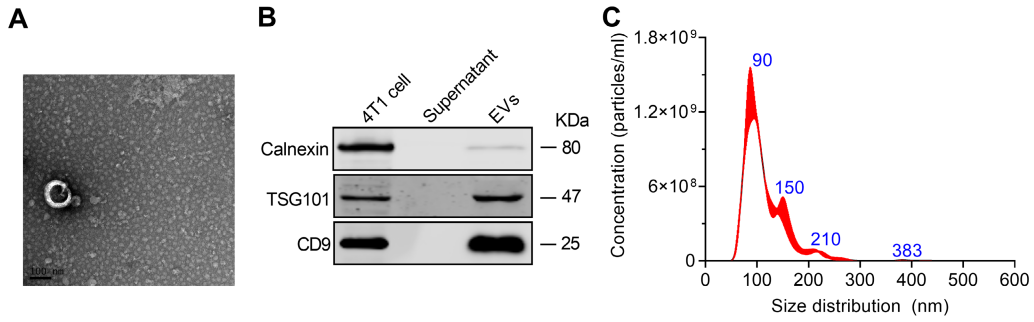

Figure S1. Characteristics of 4T1 cells-derived EVs. (A) Representative electron microscopy images of 4T1 cells-derived EVs. Scale bar = 100 nm. **(B)** Western blot analysis of the EVs markers TSG101, CD9 of 4T1 cells-derived exosomes. **(C)** Measurement of particle size of the 4T1 cells-derived EVs by nanoparticle tracking analysis. EVs, extracellular vehicles.

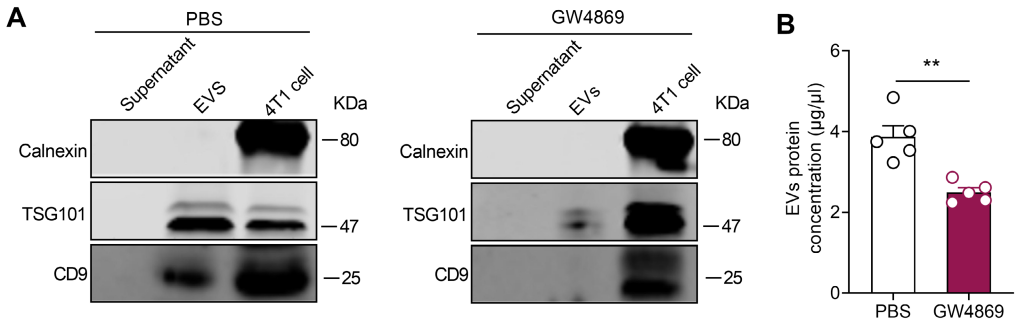

Figure S2. GW4869 inhibits EVs secretion in 4T1 cells. (A) Western blot analysis of the expression of exosomal markers TSG101 and CD9 in 4T1 cells-derived EVs after treatment of GW4869. **(B)** Quantification of the total protein concentration in the 4T1 cells-derived EVs after treatment of GW4869. All data are presented as the mean ± s.e.m., with each point representing data from an individual. $^{**}p < 0.01$ by Student's t test. EVs, extracellular vehicles; PBS, phosphate buffered saline .

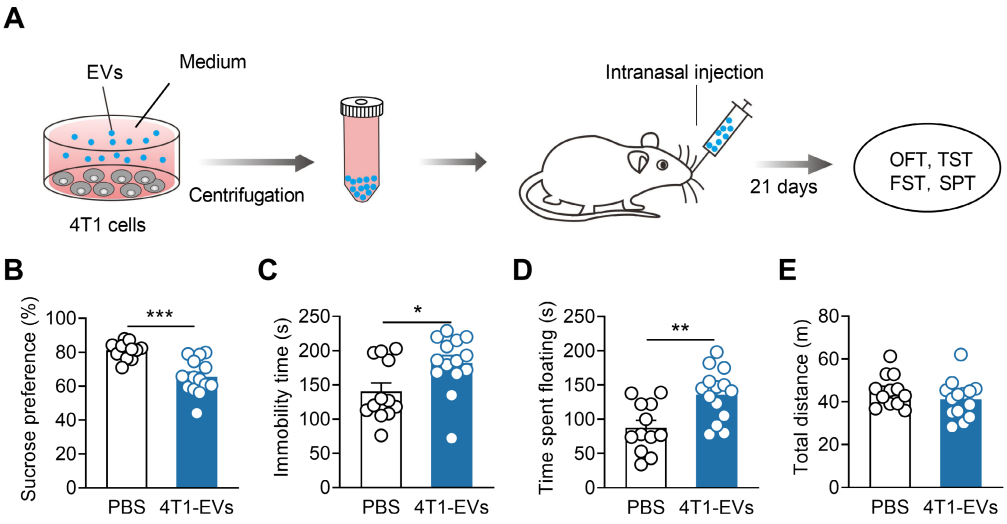

Figure S3. Intranasal administration of 4T1 cells-derived EVs induces depressive-like behaviors in mice. (A) Schematic timeline of 4T1 cells-derived EVs purification, intranasal injection and behavioral tests. **(B-E)** Behavioral consequences of intranasal injection of 4T1 cells-derived EVs in the SPT **(B)**, TST **(C)**, FST **(D)** and OFT **(E)** (n = 12-14 mice per group). All data are presented as the mean ± s.e.m., with each point representing data from an individual. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by Student's t test. EVs, extracellular vehicles; FST, forced swimming test; OFT, open field test; PBS, phosphate buffered saline; SPT, sucrose preference test; TST, tail suspension test.

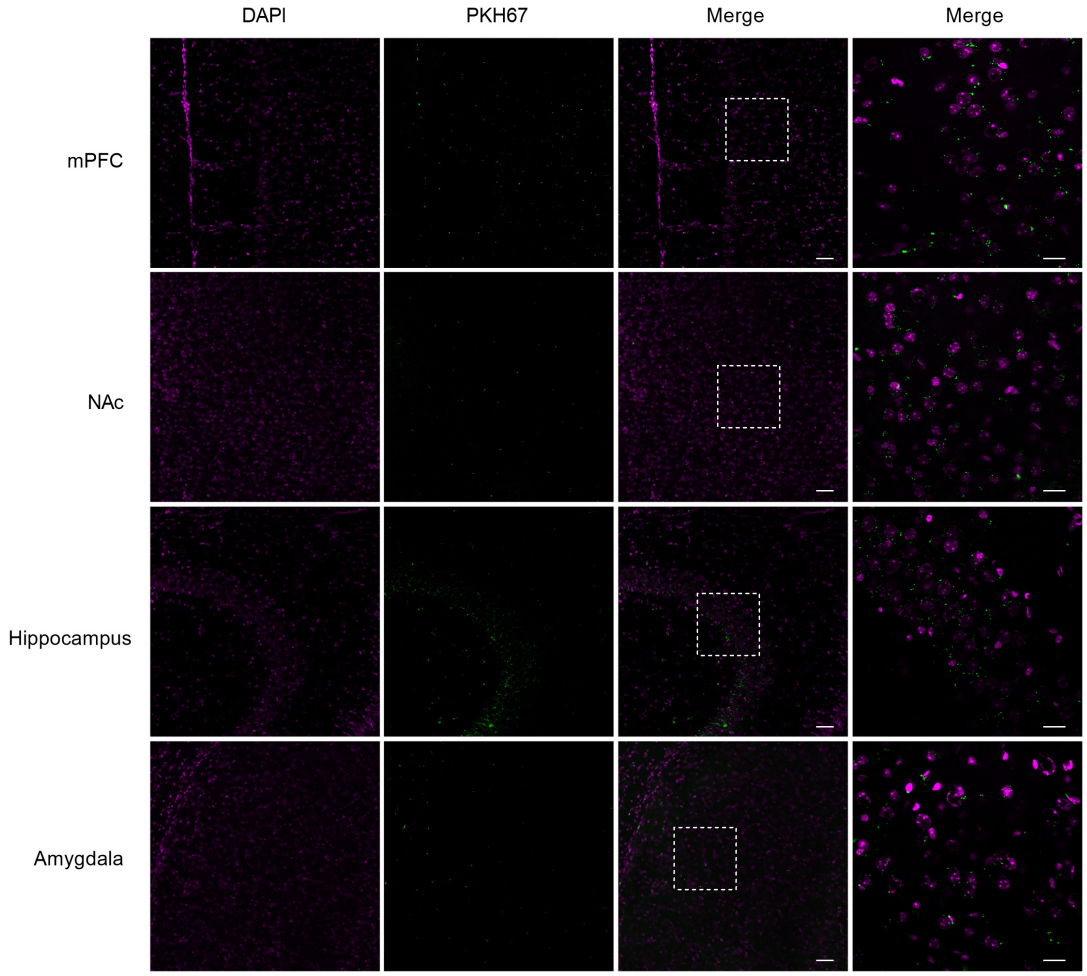

Figure S4. Distribution of intravenously injected 4T1 cells-derived EVs in the brain. Higher magnification views of selected regions (white rectangle). Scale bar = 50 μm (left), 20 μm (right). mPFC, medial prefrontal cortex; NAc, nucleus accumbens.

Supplementary Table 1. Key reagent or resource.

Reagent or resource	Source	Identifier
Antibodies		
4’,6-diamidino-2-phenylindole	Sigma	D9542
Alexa Fluor™ Plus 405	ThermoFisher Scientific	A-48260; RRID: AB_2890271
Chicken-anti-MAP2	Abcam	ab5392; RRID: AB_2138153
IRDye 800CW Goat anti-Mouse IgG	LI-COR	926-32210; RRID: AB_621842
IRDye 800CW Goat anti-Rabbit IgG	LI-COR	926-32211; RRID: AB_621843
Mouse-anti-TSG101	Abcam	ab83; RRID: AB_306450
Mouse-anti-Twist1	Abcam	ab50887; RRID: AB_883294
Mouse-anti-β-actin	Santa Cruz Biotechnology	sc-47778; RRID: AB_626632
PKH67	NoninBio	NW3217
Rabbit-anti-Calnexin	Abcam	ab22595; RRID: AB_2069006
Rabbit-anti-PPAR-δ	Abcam	ab23673; RRID: AB_2165902
Virus strains		
LV-Twist-RNAi (3×109 TU/mL)	Genechem	Cat#133C77A
Chemicals		
B-27 Supplement (50X)	Gibco	Cat#17504-044
Deoxyribonuclease I	Merck	Cat#DN25
Dulbecco's Modified Eagle Medium	Gibco	Cat#C11330500BT
Fetal Bovine Serum	Gibco	Cat#10099141C
Glutamax	Gibco	Cat#25030081
GW4869	MCE	Cat#HY-19363
Lucifer yellow dye	Invitrogen	Cat# L453
Neurobasal medium	Gibco	Cat#21103049
Puromycin	Solarbio	Cat#P3280
RIPA Lysis Buffer	Beyotime	Cat#P0013C
RNAiso Blood	Takara	Cat#9112
RNase A	Takara	Cat#2158
SYBR® Premix Ex Taq™ II	Takara	Cat#RR820A
Triton X-100	Biofroxx	Cat#1139ML100
Critical commercial assays		
Bicinchoninic acid assay	Beyotime	Cat#P0010
PrimeScript™ RT reagent Kit	Takara	Cat#RR037Q

Oligonucleotides		
Twist1: 5'-GAGGTCTTGCCAATCAGCCA-3' (sense)	Invitrogen	N/A
Twist1:5'-CCAGTTTGATCCCAGCGTTT-3' (antisense)	Invitrogen	N/A
GAPDH: 5'-AACGACCCCTTCATTGAC -3' (sense)	Invitrogen	N/A
GAPDH: 5'-TCCACGACATACTCAGCAC -3' (antisense)	Invitrogen	N/A
Software and algotithms		
NF Profession 2.0 software	Nacofcm	https://www.nanofcm.cn/
ANY-maze software	Stoelting Co.	https://stoeltingco.com/
ImageJ	National Institute of Health	https://imagej.net/ij/
SPSS statistical software version 18.0	IBM	https://www.ibm.com/cn-zh/products/spss-statistics
GraphPad Prism software (ver. 7.0)	GraphPad	https://www.graphpad.com/scientific-software/prism/

Supplementary Table 2. Statistical analysis for Figures 1-5 and Supplementary Figure 1-3.

Figure and numbers	Statistical analysis	*Post hoc tests*	Means ± s.e.m.
1B	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	
4T1 = 10 Lewis = 10 MC38 = 10	Days: F (4, 133) = 155.2, P < 0.0001 Tumor: F (2, 133) = 5.751, P = 0.004 Days × Tumor = F (8, 133) = 1.128, P = 0.3489	**9:** 4T1 vs. Lewis, P > 0.9999 4T1 vs. MC38, P > 0.9999 Lewis vs. MC38, P > 0.9999 **12:** 4T1 vs. Lewis, P > 0.9999 4T1 vs. MC38, P > 0.9999 Lewis vs. MC38, P > 0.9999 **15:** 4T1 vs. Lewis, P = 0.0555 4T1 vs. MC38, P = 0.2876 Lewis vs. MC38, P > 0.9999 **18:** 4T1 vs. Lewis, P = 0.0024 4T1 vs. MC38, P = 0.0194 Lewis vs. MC38, P > 0.9999 **21:** 4T1 vs. Lewis, P > 0.9999 4T1 vs. MC38, P > 0.9999 Lewis vs. MC38, P > 0.9999	**9:** 4T1 = 16.716 ± 8.301 Lewis = 33.458 ± 11.854 MC38 = 40.474 ± 26.104 **12:** 4T1 = 79.230 ± 28.662 Lewis = 149.382 ± 25.863 MC38 = 102.259 ± 28.193 **15:** 4T1 = 289.421 ± 74.240 Lewis = 548.365 ± 78.258 MC38 = 471.501 ± 48.497 **18:** 4T1 = 661.933 ± 80.847 Lewis = 1034.424 ± 113.907 MC38 = 970.463 ± 96.327 **21:** 4T1 = 1310.960 ± 149.848 Lewis = 1372.199 ± 106.242 MC38 = 1396.151± 107.554
1C	**One-way ANOVA**	**Dunnett's multiple comparisons test**	PBS = 79.80 ± 1.010 4T1 = 41.41 ± 4.909
PBS = 10	F (3, 37) = 14.03	PBS vs. 4T1, P <	Lewis = 64.24 ± 5.595

4T1 = 11 Lewis = 10 MC38 = 10	P < 0.0001	0.0001 PBS vs. Lewis, P = 0.0442 PBS vs. MC38, P = 0.1295	MC38 = 67.35 ± 4.221
1D	**One-way ANOVA**	**Dunnett's multiple comparisons test**	PBS = 95.53 ± 14.98 4T1 = 152.7 ± 12.26 Lewis = 156.6 ± 14.12 MC38 = 147.6 ±13.40
PBS = 10 4T1 = 11 Lewis = 10 MC38 = 9	F (3, 36) = 4.382 P = 0.0100	PBS vs. 4T1, P = 0.0126 PBS vs. Lewis, P = 0.0091 PBS vs. MC38, P = 0.0344	
1E	**One-way ANOVA**	**Dunnett's multiple comparisons test**	PBS = 49.67 ± 9.116 4T1 = 125.55 ± 9.861 Lewis = 105.6 ± 8.884 MC38 = 105.8 ± 11.75
PBS = 10 4T1 = 11 Lewis = 9 MC38 = 10	F (3, 36) = 11.27 P < 0.0001	PBS vs. 4T1, P < 0.0001 PBS vs. Lewis, P = 0.0014 PBS vs. MC38, P = 0.0010	
1F	**One-way ANOVA**	**Dunnett's multiple comparisons test**	PBS = 32.78 ± 2.593 4T1 = 29.73 ± 2.381 Lewis = 32.71 ± 2.950 MC38 = 28.68 ± 1.933
PBS = 11 4T1 = 10 Lewis = 10 MC38 = 10	F (3, 37) = 0.6978 P = 0.5594	PBS vs. 4T1, P = 0.7200 PBS vs. Lewis, P > 0.9999 PBS vs. MC38, P = 0.5174	
1H	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	Vehicle + PBS = 15.32 ± 2.961 Vehicle + 4T1 = 26.000 ± 3.024 GW4869 + PBS = 4.940 ± 0.470 GW4869 + 4T1 = 7.048 ± 0.519
Vehicle + PBS = 4 Vehicle + 4T1 = 4 GW4869 + PBS = 4 GW4869 + 4T1 = 4	GW4869: F (1, 12) = 46.75, P < 0.0001 4T1: F (1, 12) = 8.885, P = 0.0115 GW4869 × 4T1: F (1, 12) = 3.993, P = 0.0689	Vehicle + PBS vs. Vehicle + 4T1, P = 0.0084 GW4869 + PBS vs. GW4869 + 4T1, P >0.9999 Vehicle + PBS vs. GW4869 + PBS, P = 0.0101 Vehicle + 4T1 vs. GW4869 + 4T1, P < 0.0001.	

1I	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	Vehicle + PBS = 491.500 ± 43.119 Vehicle + 4T1 = 734.206 ± 47.781 GW4869 + PBS = 225.004 ± 20.813 GW4869 + 4T1 = 334.398 ± 10.824
Vehicle + PBS = 4 Vehicle + 4T1 = 4 GW4869 + PBS = 4 GW4869 + 4T1 = 4	GW4869: F (1, 12) = 94.61, P < 0.0001 4T1: F (1, 12) = 26.42, P = 0.0002 GW4869 × 4T1: F (1, 12) = 3.787, P = P=0.0754	Vehicle + PBS vs. Vehicle + 4T1, P = 0.0006 GW4869 + PBS vs. GW4869 + 4T1, P = 0.0867 Vehicle + PBS vs. GW4869 + PBS, P = 0.0003 Vehicle + 4T1 vs. GW4869 + 4T1, P < 0.0001.	
1J	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	Vehicle + PBS = 82.89 ± 1.60 Vehicle + 4T1 = 62.96 ± 2.86 GW4869 + PBS = 82.67 ± 1.32 GW4869 + 4T1 = 80.35 ± 2.06
Vehicle + PBS = 12 Vehicle + 4T1 = 13 GW4869 + PBS = 12 GW4869 + 4T1 = 13	GW4869: F (1, 46) = 16.920, P = 0.0002 4T1: F (1, 46) = 28.360, P < 0.0001 GW4869 × 4T1: F (1, 46) = 17.770, P = 0.0001	Vehicle + PBS vs. Vehicle + 4T1, P < 0.0001. GW4869 + PBS vs. GW4869 + 4T1, P =0.8725 Vehicle + PBS vs. GW4869 + PBS, P > 0.9999 Vehicle + 4T1 vs. GW4869 + 4T1, P < 0.0001.	
1K	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	Vehicle + PBS = 136.17 ± 6.11 Vehicle + 4T1 = 184.92 ± 6.10 GW4869 + PBS = 138.17 ± 7.19 GW4869 + 4T1 = 144.85 ± 8.10
Vehicle + PBS = 12 Vehicle + 4T1 = 13 GW4869 + PBS = 12 GW4869 + 4T1 = 13	GW4869: F (1, 46) = 7.179, P = 0.0102 4T1: F (1, 46) = 15.220, P = 0.0003 GW4869 × 4T1: F (1, 46) = 8.766, P = 0.0048	Vehicle + PBS vs. Vehicle + 4T1, P < 0.0001 GW4869 + PBS vs. GW4869 + 4T1, P > 0.9999 Vehicle + PBS vs. GW4869 + PBS, P > 0.9999 Vehicle + 4T1 vs. GW4869 + 4T1, P =	

		0.0004.	
1L	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	Vehicle + PBS = 75.78 ± 4.43 Vehicle + 4T1 = 126.73 ± 7.13 GW4869 + PBS = 94.42 ± 8.41 GW4869 + 4T1 = 91.55 ± 6.20
Vehicle + PBS = 9 Vehicle + 4T1 = 11 GW4869 + PBS = 12 GW4869 + 4T1 = 11	GW4869: F (1, 39) = 1.379, P = 0.2474 4T1: F (1, 39) = 11.65, P = 0.0015 GW4869 × 4T1: F (1, 39) = 14.59, P = 0.0005	Vehicle + PBS vs. Vehicle + 4T1, P < 0.0001 GW4869 + PBS vs. GW4869 + 4T1, P > 0.9999 Vehicle + PBS vs. GW4869 + PBS, P = 0.1467 Vehicle + 4T1 vs. GW4869 + 4T1, P = 0.0018	
1M	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	
Vehicle + PBS = 12 Vehicle + 4T1 = 13 GW4869 + PBS = 12 GW4869 + 4T1 = 13	GW4869: F (1, 46) = 0.003, P = 0.9541 4T1: F (1, 46) = 0.5608, P = 0.4577 GW4869 × 4T1: F (1, 46) = 0.1078, P = 0.7441	Vehicle + PBS vs. Vehicle + 4T1, P = 0.9002 GW4869 + PBS vs. GW4869 + 4T1, P = P > 0.9999 Vehicle + PBS vs. GW4869 + PBS, P > 0.9999 Vehicle + 4T1 vs. GW4869 + 4T1, P > 0.9999	Vehicle + PBS = 50.75 ± 4.47 Vehicle + 4T1 = 54.53 ± 2.46 GW4869 + PBS = 51.70 ± 4.49 GW4869 + 4T1 = 53.17 ± 2.31
1O	**Unpaired t test**		PBS = 78.01 ± 2.410 4T1-EVs = 69.78 ± 2.35
PBS = 12 4T1-EVs = 13	t = 2.444, P = 0.0226		
1P	**Unpaired t test**		PBS = 132.67 ± 8.92 4T1-EVs = 179.92 ± 6.89
PBS = 12 4T1-EVs = 13	t = 4.230, P = 0.0003		
1Q	**Unpaired t test**		PBS = 84.67 ± 8.89 4T1-EVs = 138.15 ± 10.96
PBS = 12 4T1-EVs = 13	t = 3.752, P = 0.0010		
1R	**Unpaired t test**		PBS = 42.02 ± 1.54 4T1-EVs = 43.91 ± 2.14
PBS = 12 4T1-EVs = 13	t = 0.708 P = 0.4861		
2A	**One-way ANOVA**	**Bonferroni's**	PBS+Vehicle = 1.025 ±

		multiple comparisons test	0.153 4T1+Vehicle = 3.619± 0.577 4T1+RNase = 3.326 ± 0.585 4T1+RNase+Triton-100 = 0.000 ± 0.000
PBS+Vehicle = 5 4T1+Vehicle = 5 4T1+RNase = 5 4T1+RNase+Triton-100 = 5	F (3, 16) = 17.81 P < 0.0001	PBS+Vehicle vs. 4T1+Vehicle, P = 0.0027 PBS+Vehicle vs. 4T1+RNase, P = 0.0077 4T1+Vehicle vs. 4T1+RNase P > 0.9999	
2B	**Unpaired t test**		mPFC: PBS = 1.00 ± 0.06 4T1 = 7.90 ± 0.99 Amy: PBS = 1.00 ± 0.09 4T1 = 1.03 ± 0.06 NAc: PBS = 1.00 ± 0.06 4T1 =1.00 ± 0.07 Hip: PBS = 1.00 ± 0.15 4T1 = 2.11 ± 0.25 VTA: PBS = 1.00 ± 0.09 4T1 = 0.88 ± 0.09
mPFC: PBS = 8 4T1 = 8 Amy: PBS = 6 4T1 = 6 NAc: PBS = 7 4T1 = 7 Hip: PBS = 8 4T1 = 8 VTA: PBS = 6 4T1 = 6	mPFC: t = 6.922 P < 0.0001 Amy: t = 0.229 P = 0.8233 NAc: t = 0.015 P = 0.9885 Hip: t =3.825, P = 0.0019 VTA: t = 0.930, P = 0.3741		
2C	**Unpaired t test**		PPAR-δ: PBS= 1.00 ± 0.14 4T1 = 0.50 ± 0.07 Twist1： PBS = 1.00 ± 0.13 4T1= 1.76 ± 0.13
PBS + Twist1 = 11 PBS + PPAR-δ = 11 4T1 + Twist1 =11 4T1 + PPAR-δ = 11	PPAR-δ: t = 3.196 P = 0.0045 Twist1： t = 4.181 P = 0.0005		
2E	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	
Twist1: PBS + LV-shCon =10 PBS + LV-shTwist1 = 10 4T1 + LV-shCon = 10	**Twist1:** LV-shTwist1: F (1, 36) = 163.8, P < 0.0001 4T1: F (1, 36) =	**Twist1:** PBS + LV-shCon vs. PBS + LV-shTwist1, P = 0.0037 4T1 + LV-shCon vs.	**Twist1:** PBS + LV-shCon = 1.00 ± 0.082 PBS + LV-shTwist1 = 0.56 ± 0.071

4T1 + LV-shTwist1 = 10 **PPAR-δ:** PBS + LV-shCon =10 PBS + LV-shTwist1 = 10 4T1 + LV-shCon = 10 4T1 + LV-shTwist1 = 10	53.22, P < 0.0001 LV-shTwist1 × 4T1: F (1, 36) = 6.445, P = 0.0156 **PPAR-δ:** LV-shTwist1: F (1, 36) = 21.53, P < 0.0001 4T1: F (1, 36) = 6.149, P = 0.0180 LV-shTwist1 × 4T1: F (1, 36) = 1.661, P = 0.2057	4T1 + LV-shTwist1, P < 0.0001 PBS + LV-shCon vs. 4T1 + LV-shCon, P < 0.0001 PBS + LV-shTwist1 vs. 4T1 + LV-shTwist1, P < 0.0001 **PPAR-δ:** PBS + LV-shCon vs. PBS + LV-shTwist1, P = 0.0466 4T1 + LV-shCon vs. 4T1 + LV-shTwist1, P = 0.0003 PBS + LV-shCon vs. 4T1 + LV-shCon, P = 0.0229 PBS + LV-shTwist1 vs. 4T1 + LV-shTwist1, P = 0.8107	4T1 + LV-shCon = 7.75 ± 0.762 4T1 + LV-shTwist1 = 2.37 ± 0.500 **PPAR-δ:** PBS + LV-shCon = 1.00 ± 0.074 PBS + LV-shTwist1 = 1.27 ± 0.045 4T1 + LV-shCon = 0.69 ± 0.044 4T1 + LV-shTwist1 = 1.18 ± 0.131
2F	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS + LV-shCon = 83.056 ± 2.167 PBS + LV-shTwist1 = 87.268 ± 1.619 4T1 + LV-shCon = 65.144 ± 3.352 4T1 + LV-shTwist1 = 79.505 ± 2.742
PBS + LV-shCon =10 PBS + LV-shTwist1 = 10 4T1 + LV-shCon = 10 4T1 + LV-shTwist1 = 10	LV-shTwist1: F (1, 36) = 13.23, P < P = 0.0009 4T1: F (1, 36) = 25.29, P < 0.0001 LV-shTwist1 × 4T1: F (1, 36) = 3.952, P = 0.0545	PBS + LV-shCon vs. PBS + LV-shTwist1, P = 0.5021 4T1 + LV-shCon vs. 4T1 + LV-shTwist1, P = 0.0006 PBS + LV-shCon vs. 4T1 + LV-shCon, P < 0.0001 PBS + LV-shTwist1 vs. 4T1 + LV-shTwist1, P = 0.0766	
2G	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS + LV-shCon = 117.77 ± 13.122 PBS + LV-shTwist1 = 129.25 ± 13.477 4T1 + LV-shCon =
PBS + LV-shCon =10 PBS + LV-shTwist1 =	LV-shTwist1: F (1, 36) = 4.113, P =	PBS + LV-shCon vs. PBS + LV-shTwist1, P	

10 4T1 + LV-shCon = 10 4T1 + LV-shTwist1 = 10	0.0500 4T1: F (1, 36) = 5.435, P = 0.0255 LV-shTwist1 × 4T1: F (1, 36) = 9.369, P = 0.0042	= 0.9398 4T1 + LV-shCon vs. 4T1 + LV-shTwist1, P = 0.0019 PBS + LV-shCon vs. 4T1 + LV-shCon, P = 0.0010 PBS + LV-shTwist1 vs. 4T1 + LV-shTwist1, P > 0.9999	177.70 ± 9.154 4T1 + LV-shTwist1 = 121.140 ± 7.516
2H	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS + LV-shCon = 113.2 ± 13.712 PBS + LV-shTwist1 = 122.5 ± 11.455 4T1 + LV-shCon = 164.5 ± 11.301 4T1 + LV-shTwist1 = 116.4 ± 12.571
PBS + LV-shCon =10 PBS + LV-shTwist1 = 10 4T1 + LV-shCon = 10 4T1 + LV-shTwist1 = 10	LV-shTwist1: F (1, 36) = 2.488, P = 0.1234 4T1: F (1, 36) = 3.377, P = 0.0744 LV-shTwist1 × 4T1: F (1, 36) = 5.446, P = 0.0253	PBS + LV-shCon vs. PBS + LV-shTwist1, P > 0.9999 4T1 + LV-shCon vs. 4T1 + LV-shTwist1, P = 0.0178 PBS + LV-shCon vs. 4T1 + LV-shCon, P = 0.0111 PBS + LV-shTwist1 vs. 4T1 + LV-shTwist1, P > 0.9999	
2I	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS + LV-shCon = 28.321 ± 1.559 PBS + LV-shTwist1 = 25.743 ± 2.115 4T1 + LV-shCon = 26.769 ± 1.404 4T1 + LV-shTwist1 = 24.304 ± 1.860
PBS + LV-shCon =10 PBS + LV-shTwist1 = 10 4T1 + LV-shCon = 10 4T1 + LV-shTwist1 = 10	LV-shTwist1: F (1, 36) = 2.061, P = 0.1597 4T1: F (1, 36) = 0.7256, P = P = 0.3999 LV-shTwist1 × 4T1: F (1, 36) = 0.001, P = P=0.9745	PBS + LV-shCon vs. PBS + LV-shTwist1, P = 0.6124 4T1 + LV-shCon vs. 4T1 + LV-shTwist1, P = 0.6551 PBS + LV-shCon vs. 4T1 + LV-shCon, P > 0.9999 PBS + LV-shTwist1 vs. 4T1 + LV-shTwist1, P > 0.9999	
3B	**Unpaired t test**		4T1/shCon = 1.00 ±

4T1/shCon = 12 4T1/shTwist1 = 13	t = 7.910 P < 0.0001		0.10 4T1/shTwist1 = 0.21 ± 0.02
3C	**Unpaired t test**		4T1/shCon = 1.00 ± 0.14 4T1/shTwist1 = 0.23 ± 0.05
4T1/shCon = 12 4T1/shTwist1 = 14	t = 5.380 P < 0.0001		
3D	**Unpaired t test**		4T1/shCon = 1.00 ± 0.03 4T1/shTwist1 = 0.29 ± 0.02
4T1/shCon = 12 4T1/shTwist1 = 12	t = 22.519 P < 0.0001		
3E	**Two-way ANOVA**	**Bonferroni's multiple comparisons test**	4T1/shCon + Day 9 = 5.87 ± 3.13 4T1/shCon + Day 12 = 26.69 ± 3.57 4T1/shCon + Day 15 = 152.52 ± 21.98 4T1/shCon + Day 18 = 518.57 ± 43.50 4T1/shCon + Day 18 = 1022.27 ± 94.00 4T1/shTwist1 + Day 9 = 5.87 ± 3.13 4T1/shTwist1 + Day 12 = 22.81 ± 3.68 4T1/shTwist1 + Day 15 = 34.75 ± 3.91 4T1/shTwist1 + Day 18 = 60.59 ± 7.52 4T1/shTwist1 + Day 21 = 126.83 ± 26.21
4T1/shCon = 10 4T1/shTwist1 = 10	Days: F (4, 90) = 94.87, P < 0.0001 shTwist1: F (1, 90) = 181.1, P < 0.0001 Days × shTwist1: F (4, 90) = 61.41, P < 0.0001	4T1/shCon + Day 9 vs. 4T1/shTwist1 + Day 9, P > 0.9999 4T1/shCon + Day 12 vs. 4T1/shTwist1 + Day 12, P > 0.9999 4T1/shCon + Day 15 vs. 4T1/shTwist1 + Day 15, P = 0.0917 4T1/shCon + Day 18 vs. 4T1/shTwist1 + Day 18, P < 0.0001 4T1/shCon + Day 21 vs. 4T1/shTwist1 + Day 21, P < 0.0001	
3F	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	Control = 86.63 ± 1.64 4T1/shCon = 66.01 ± 3.61 4T1/shTwist1 = 85.52 ± 1.80
Control = 10 4T1/shCon = 11 4T1/shTwist1 = 11	F (2, 29) = 20.757 P < 0.0001	Control vs. 4T1/shCon, P < 0.0001 Control vs. 4T1/shTwist1, P > 0.9999 4T1/shCon vs. 4T1/shTwist1, P < 0.0001	
3G	**One-way ANOVA**	**Bonferroni's**	Control = 121.40 ± 7.72

		multiple comparisons test	4T1/shCon = 169.00 ± 11.31 4T1/shTwist1 = 112.55 ± 7.70
Control = 10 4T1/shCon = 11 4T1/shTwist1 = 11	F (2, 29) = 11.235 P = 0.0002	4T1/shCon vs. Control, P = 0.0031 Control vs. 4T1/shTwist1, P > 0.9999 4T1/shCon vs. 4T1/shTwist1, P = 0.0004	
3H	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	Control = 89.20 ± 7.76 4T1/shCon = 153.73 ± 9.22 4T1/shTwist1 = 112.64 ± 9.75
Control = 10 4T1/shCon = 11 4T1/shTwist1 = 11	F (2, 29) = 12.990 P < 0.0001	Control vs. 4T1/shCon, P < 0.0001 Control vs. 4T1/shTwist1, P = 0.2377 4T1/shCon vs. 4T1/shTwist1, P = 0.0084	
3I	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	Control = 24.52 ± 2.05 4T1/shCon = 23.46 ± 1.24 4T1/shTwist1 = 24.88 ± 1.45
Control = 10 4T1/shCon = 11 4T1/shTwist1 = 11	F (2, 29) = 0.223 P = 0.8013	Control vs. 4T1/shCon, P > 0.9999 Control vs. 4T1/shTwist1, P > 0.9999 4T1/shCon vs. 4T1/shTwist1, P > 0.9999	
3J	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	Control = 1.00 ± 0.07 4T1/shCon = 3.21 ± 0.22 4T1/shTwist1 = 0.94 ± 0.09
Control = 10 4T1/shCon = 10 4T1/shTwist1 = 10	F (2, 27) = 80.409 P < 0.0001	Control vs. 4T1, P < 0.0001 4T1/shCon vs. 4T1/shTwist1, P < 0.0001	
3K	**One-way ANOVA**	**Bonferroni's multiple**	

		comparisons test	Control = 1.00 ± 0.15 4T1/shCon = 5.64 ± 1.10 4T1/shTwist1 = 2.38 ± 0.66 Control = 1.00 ± 0.10 4T1/shCon = 0.51 ± 0.07 4T1/shTwist1 = 0.78 ± 0.06
Control = 10 4T1/shCon = 11 4T1/shTwist1 = 11	**Twist1:** F (2, 29) = 9.558 P = 0.0006 **PPAR-δ:** F (2, 29) = 10.26 P = 0.0004	Control vs. 4T1/shCon, P = 0.0006 Control vs. 4T1/shTwist1, P = 0.6532 4T1/shCon vs. 4T1/shTwist1, P = 0.0149 Control vs. 4T1/shCon, P = 0.0003 Control vs. 4T1/shTwist1, P = 0.1662 4T1/shCon vs. 4T1/shTwist1, P = 0.0462	
4B	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	
PBS-SDEVs = 13 4T1/shCon-SDEVs = 14 4T1/shTwist1-SDEVs = 13	F (2, 37) = 12.131 P < 0.0001	PBS-SDEVs vs. 4T1/shCon-SDEVs, P = 0.0005 PBS-SDEVs vs. 4T1/shTwist1-SDEVs, P > 0.9999 4T1/shCon-SDEVs vs. 4T1/shTwist1-SDEVs, P = 0.0004	PBS-SDEVs = 78.31 ± 2.36 4T1/shCon-SDEVs = 64.42 ± 2.53 4T1/shTwist1-SDEVs = 78.56 ± 2.10
4C	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 133.38 ± 10.50 4T1/shCon-SDE = 187.43 ± 7.77 4T1/shTwist1-SDE = 154.85 ± 8.31
PBS-SDEVs = 13 4T1/shCon-SDEVs = 14 4T1/shTwist1-SDEVs = 13	F (2, 37) = 9.532 P = 0.0005	PBS-SDE vs. 4T1/shCon-SDE, P = 0.0003 PBS-SDE vs. 4T1/shTwist1-SDE, P = 0.3003 4T1/shCon-SDE vs.	

		4T1/shTwist1-SDE, P = 0.0392	
4D	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 79.15 ± 9.02 4T1/shCon-SDE = 128.00 ± 12.23 4T1/shTwist1-SDE = 88.77 ± 9.35
PBS-SDEVs = 13 4T1/shCon-SDEVs = 14 4T1/shTwist1-SDEVs = 13	F (2, 37) = 6.304 P = 0.0044	PBS-SDEVs vs. 4T1/shCon-SDE, P = 0.0059 PBS-SDE vs. 4T1/shTwist1-SDE, P > 0.9999 4T1/shCon-SDE vs. 4T1/shTwist1-SDE, P = 0.0330	
4E	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 42.03 ± 2.36 4T1/shCon-SDE = 47.25 ± 2.01 4T1/shTwist1-SDE = 39.92 ± 1.98
PBS-SDEVs = 13 4T1/shCon-SDEVs = 14 4T1/shTwist1-SDEVs = 13	F (2, 37) = 3.239 P = 0.0506	PBS-SDE vs. 4T1/shCon-SDE, P = 0.2649 PBS-SDE vs. 4T1/shTwist1-SDE, P > 0.9999 4T1/shCon-SDE vs. 4T1/shTwist1-SDE, P = 0.0558	
4F	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 1.00 ± 0.05 4T1/shCon-SDE = 2.57 ± 0.10 4T1/shTwist1-SDE = 0.91 ± 0.06
PBS-SDEVs = 10 4T1/shCon-SDEVs = 12 4T1/shTwist1-SDEVs = 10	F (2, 29) = 153.705 P < 0.0001	PBS-SDE vs. 4T1/shCon-SDE, P < 0.0001 PBS-SDE vs. 4T1/shTwist1-SDE, P > 0.9999 4T1/shCon-SDE vs. 4T1/shTwist1-SDE, P < 0.0001	
4G	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 1.00 ± 0.10 4T1/shCon-SDE = 2.44 ± 0.26 4T1/shTwist1-SDE = 1.00 ± 0.14
PBS-SDEVs = 8 4T1/shCon-SDEVs = 8 4T1/shTwist1-SDEVs =	F (2, 21) = 20.986 P < 0.0001	PBS-SDE vs. 4T1/shCon-SDE, P < 0.0001	

8		PBS-SDE vs. 4T1/shTwist1-SDE, P > 0.9999. 4T1/shCon-SDE vs. 4T1/shTwist1-SDE, P < 0.0001	
4I	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 81.57 ± 1.97 4T1/shCon-SDE = 65.53 ± 2.91 4T1/shTwist1-SDE = 79.94 ± 1.88
PBS-SDEVs = 13 4T1/shCon-SDEVs = 13 4T1/shTwist1-SDEVs = 13	F (2, 36) = 14.735 P < 0.0001	PBS-SDEVs vs. 4T1/shCon-SDEVs, P < 0.0001 PBS-SDEVs vs. 4T1/shTwist1-SDEVs, P > 0.9999 4T1/shCon-SDEVs vs. 4T1/shTwist1-SDEVs, P = 0.0003	
4J	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 128.54 ± 8.87 4T1/shCon-SDE = 167.92 ± 8.62 4T1/shTwist1-SDE = 152.00 ± 8.69
PBS-SDEVs = 13 4T1/shCon-SDEVs = 13 4T1/shTwist1-SDEVs = 13	F (2, 36) = 5.152 P = 0.0108	4T1/shCon-SDE vs. PBS-SDE, P = 0.0088 4T1/shCon-SDE vs. 4T1/shTwist1-SDE, P = 0.6159	
4K	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 87.67 ± 9.24 4T1/shCon-SDE = 134.92 ± 8.11 4T1/shTwist1-SDE = 95.25 ± 8.40
PBS-SDEVs = 12 4T1/shCon-SDEVs = 13 4T1/shTwist1-SDEVs = 12	F (2, 34) = 8.969 P = 0.0007	4T1/shCon-SDE vs. PBS-SDE, P = 0.0012 4T1/shCon-SDE vs. 4T1/shTwist1-SDE, P = 0.0070	
4L	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 49.61 ± 3.37 4T1/shCon-SDE = 46.76 ± 2.22 4T1/shTwist1-SDE = 46.87 ± 4.07
PBS-SDEVs = 13 4T1/shCon-SDEVs = 13 4T1/shTwist1-SDEVs = 13	F (2, 36) = 0.2388 P = 0.7888	PBS-SDE vs. 4T1/shCon-SDE, P > 0.9999 PBS-SDE vs. 4T1/shTwist1-SDE, P > 0.9999	

4M	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 1.00 ± 0.09 4T1/shCon-SDE = 1.86 ± 0.18 4T1/shTwist1-SDE = 0.81 ± 0.11
PBS-SDE = 10 4T1/shCon-SDE = 12 4T1/shTwist1-SDE = 10	F (2, 29) = 16.470 P < 0.0001	PBS-SDE vs. 4T1/shCon-SDE, P = 0.0005 PBS-SDE vs. 4T1/shTwist1-SDE, P > 0.9999 4T1/shCon-SDE vs. 4T1/shTwist1-SDE, P < 0.0001	
4N	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDE = 1.00 ± 0.21 4T1/shCon-SDE = 1.82 ± 0.23 4T1/shTwist1-SDE = 0.87 ± 0.09
PBS-SDE = 8 4T1/shCon-SDE = 8 4T1/shTwist1-SDE = 8	F (2, 21) = 7.760 P = 0.0030	PBS-SDE vs. 4T1/shCon-SDE, P = 0.0147 PBS-SDE vs. 4T1/shTwist1-SDE, P > 0.9999 4T1/shCon-SDE vs. 4T1/shTwist1-SDE, P = 0.0047	
5D	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	
PBS-SDEVs = 20 4T1/shCon-SDEVs = 20 4T1/shTwist1-SDEVs = 20	F (2, 57) = 20.651 P < 0.0001	PBS-SDEVs vs. 4T1/shCon-SDEVs, P < 0.0001 PBS-SDEVs vs. 4T1/shTwist1-SDEVs, P = 0.0984 4T1/shCon-SDEVs vs. 4T1/shTwist1-SDEVs, P = 0.0003	PBS-SDEVs = 23.95 ± 1.05 4T1/shCon-SDEVs = 14.55 ± 1.12 4T1/shTwist1-SDEVs = 20.70 ± 0.98
5E	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-SDEVs = 2785.30 ± 113.55 4T1/shCon-SDEVs = 2094.00 ± 70.57 4T1/shTwist1-SDEVs = 2770.00 ± 94.61
PBS-SDEVs = 20 4T1/shCon-SDEVs = 20 4T1/shTwist1-SDEVs =	F (2, 57) = 17.430 P < 0.0001	PBS-SDEVs vs. 4T1/shCon-SDEVs, P < 0.0001 PBS-SDEVs vs.	

20		4T1/shTwist1-SDEVs, P > 0.9999 4T1/shCon-SDEVs vs. 4T1/shTwist1-SDEVs, P < 0.0001	
5I	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-EVs = 17.97 ± 1.30 4T1/shCon-EVs = 12.38 ± 0.74 4T1/shTwist1-EVs = 16.62 ± 1.06
PBS-EVs = 29 4T1/shCon-EVs = 29 4T1/shTwist1-EVs = 29	F (2, 84) = 7.589 P = 0.0009	PBS-EVs vs. 4T1/shCon-EVs, P = 0.0010 PBS-EVs vs. 4T1/shTwist1-EVs, P > 0.9999 4T1/shCon-EVs vs. 4T1/shTwist1-EVs, P = 0.0173	
5J	**One-way ANOVA**	**Bonferroni's multiple comparisons test**	PBS-EVs = 1462.24 ± 58.11 4T1/shCon-EVs = 1162.48 ± 45.75 4T1/shTwist1-EVs = 1523.12 ± 51.45
PBS-EVs = 29 4T1/shCon-EVs = 29 4T1/shTwist1-EVs = 29	F (2, 84) = 13.777 P < 0.0001	PBS-EVs vs. 4T1/shCon-EVs, P = 0.0003 PBS-EVs vs. 4T1/shTwist1-EVs, P > 0.9999 4T1/shCon-EVs vs. 4T1/shTwist1-EVs, P < 0.0001	
S2B	**Unpaired t test**		PBS = 3.87 ± 0.27 GW4869 = 2.50 ± 0.11
PBS = 5 GW4869 = 5	t = 4.624 P = 0.0017		
S3B	**Unpaired t test**		PBS = 81.05 ± 1.40 4T1-EVs = 65.53 ± 2.74
PBS = 12 4T1-EVs = 14	t = 4.793 P < 0.0001		
S3C	**Unpaired t test**		PBS = 140.2 ± 12.63 4T1-EVs = 181.9 ± 10.94
PBS = 12 4T1-EVs = 14	t = 2.509 P = 0.019		
S3D	**Unpaired t test**		PBS = 87.83 ± 10.42 4T1-EVs = 135.9 ± 9.93
PBS = 12 4T1-EVs = 14	t = 3.334 P = 0.003		
S3E	**Unpaired t test**		PBS = 45.06 ± 2.17 4T1-EVs = 41.18 ±
PBS = 12	t = 1.200		

4T1-EVs = 14	P = 0.242		2.34