Supplementary Table S1. Summary data of EVs extraction experiment for all parasite lines and treatment conditions.

parasite	condition	replicate	sensitivity	parasitemia	mean_kcps	duration	kc_count	df1	df2	df3	kc_total	kc_norm	EV size (nm)	Intensity/100	EVs_count (x10^6)
PfPfVps60_KO	untreated	1	sensitive	4.2	147.9	80	11832	0.25	0.3	0.1	1577600	375619.0476	213.4	0.945	354.96
PfVps60_KO	untreated	2	sensitive	4.4	117.8	90	10602	0.25	0.3	0.1	1413600	321272.7273	192	1	321.2727273
PfVps60_KO	untreated	3	sensitive	4.5	137.4	80	10992	0.5	0.3	0.1	732800	162844.4444	205.4	0.984	160.2389333
PfVps60_KO	DMSO	1	sensitive	4.1	19.9	130	2587	0.2	0.3	0.1	431166.667	105162.6016	281.8	0.838	88.12626016
PfVps60_KO	DMSO	2	sensitive	4.3	25.2	240	6048	0.25	0.3	0.1	806400	187534.8837	160.3	0.723	135.5877209
PfVps60_KO	DMSO	3	sensitive	4.4	24.8	170	4216	0.25	0.3	0.1	562133.333	127757.5758	179.8	0.728	93.00751515
PfVps60_KO	DHA	1	sensitive	0.2	93	50	4650	0.05	0.3	0.1	3100000	3100000	267.6	0.507	1571.7
PfVps60_KO	DHA	2	sensitive	0.1	93.5	90	8415	0.05	0.3	0.1	5610000	5610000	170.7	0.88	4936.8
PfVps60_KO	DHA	3	sensitive	0.2	39.3	180	7074	0.2	0.3	0.1	1179000	1179000	228.5	0.74	872.46
3D7	untreated	1	sensitive	2.4	183.4	70	12838	0.25	0.3	0.1	1711733.33	713222.2222	213.5	1	713.2222222
3D7	untreated	2	sensitive	2.5	82.7	100	8270	0.35	0.3	0.1	787619.048	315047.619	225	0.96	302.4457143
3D7	untreated	3	sensitive	2.1	149.6	80	11968	0.45	0.3	0.1	886518.519	422151.6755	202.6	0.981	414.1307937
3D7	DMSO	1	sensitive	2.2	29.7	100	2970	0.1	0.3	0.1	990000	450000	215.6	0.889	400.05
3D7	DMSO	2	sensitive	2.3	117.9	100	11790	0.5	0.3	0.1	786000	341739.1304	212	0.988	337.6382609
3D7	DMSO	3	sensitive	2.2	219	50	10950	0.1	0.3	0.1	3650000	1659090.909	139.7	1	1659.090909
3D7	DHA	1	sensitive	0.5	49.9	140	6986	0.3	0.3	0.1	776222.222	776222.2222	225	0.88	683.0755556
3D7	DHA	2	sensitive	0.6	74.9	90	6741	0.3	0.3	0.1	749000	749000	248.2	0.935	700.315
3D7	DHA	3	sensitive	0.5	113.6	80	9088	0.2	0.3	0.1	1514666.67	1514666.667	229.5	0.907	1373.802667
R561H	untreated	1	resistant	2.4	152.7	80	12216	0.3	0.3	0.1	1357333.33	565555.5556	231.7	0.967	546.8922222
R561H	untreated	2	resistant	2.9	98.4	110	10824	0.4	0.3	0.1	902000	311034.4828	202.1	0.813	252.8710345
R561H	untreated	3	resistant	2.6	42.6	160	6816	0.2	0.3	0.1	1136000	436923.0769	174.4	0.862	376.6276923
R561H	DMSO	1	resistant	3	20.1	210	4221	0.15	0.3	0.1	938000	312666.6667	166.8	0.785	245.4433333
R561H	DMSO	2	resistant	2.8	65.6	120	7872	0.6	0.3	0.1	437333.333	156190.4762	229.1	0.87	135.8857143
R561H	DMSO	3	resistant	2.6	54.9	130	7137	0.15	0.3	0.1	1586000	610000	225.2	0.872	531.92
R561H	DHA	1	resistant	0.7	56.4	110	6204	0.3	0.3	0.1	689333.333	689333.3333	229.8	0.877	604.5453333
R561H	DHA	2	resistant	0.6	114.6	80	9168	0.2	0.3	0.1	1528000	1528000	250.5	0.913	1395.064
R561H	DHA	3	resistant	0.8	65.5	100	6550	0.3	0.3	0.1	727777.778	727777.7778	220	0.896	652.0888889

kc_count = mean_kcps x duration; kc_total = kc_count/ (df1 x df2 x df3); kc_norm = kc_total/ parasitemia for untreated and DMSO treated conditions; and is equal to kc_total for DHA treated conditions; EVs_count (x10^6) = kc_norm x Intensity/100; kcps – kilo count per second

Supplementary Table 2. Analysis of EVs size distribution by parasite lines.

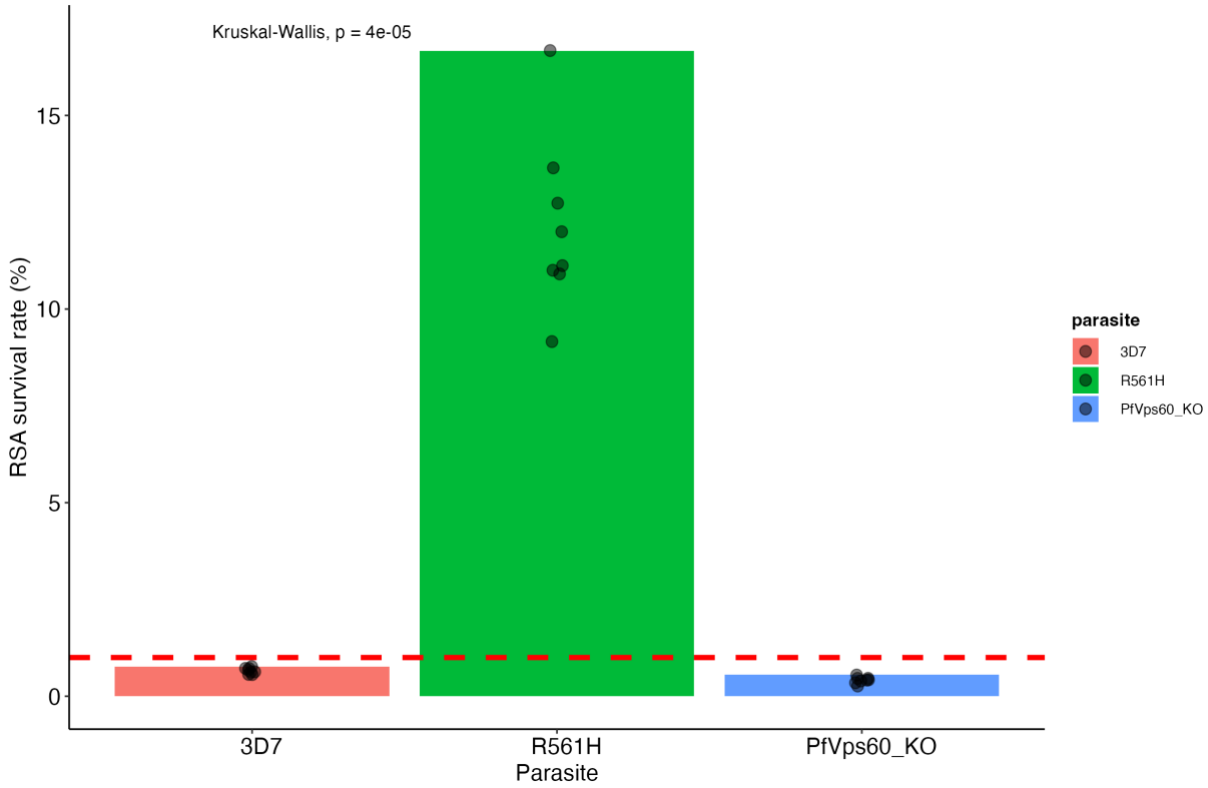
Parasite (n = 9)	mean	Standard deviation	min	Q1	median	Q3	max
PfVps60_KO	211.0556	41.96511	160.3	179.8	205.4	228.5	281.8
3D7	212.3444	30.17549	139.7	212.0	215.6	225.0	248.2
R561H	214.4000	27.88548	166.8	202.1	225.2	229.8	250.5

Supplementary Table 3. Post-hoc Dunn's test for the factors: parasite lines (3D7, PfVps60_KO and R561H) and treatment condition (untreated, DMSO-treated, and DHA-treated)

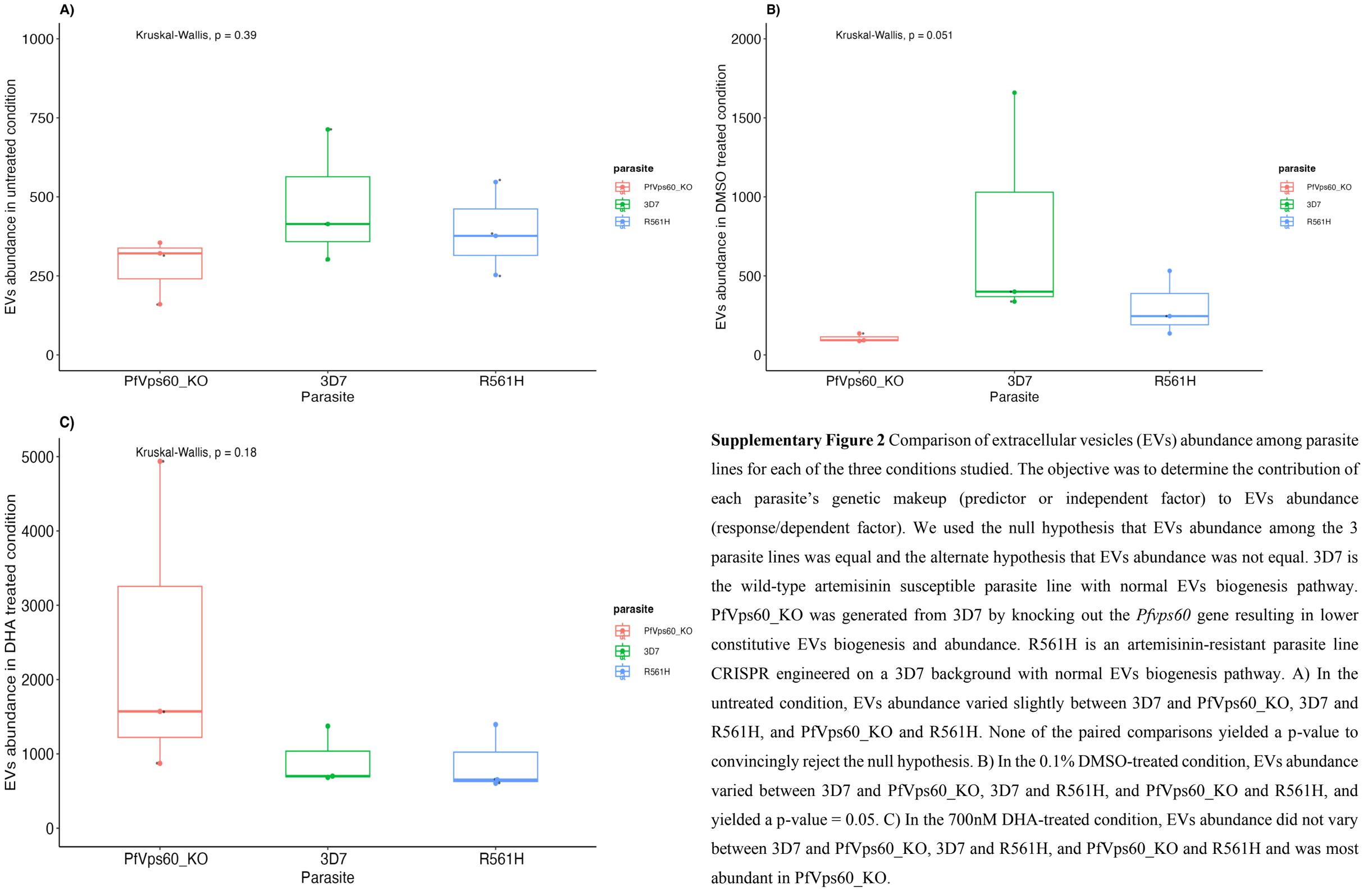
Categorical factor	Comparison	Z	P.unadj	P.adj
Untreated condition	3D7 versus R561H	0.4472136	0.6547208	0.6547208
	3D7 versus PfVps60_KO	1.3416408	0.1797125	0.5391375
	R561H versus PfVps60_KO	0.8944272	0.3710934	0.5566401
DMSO treated condition	3D7 versus R561H	0.745356	0.45605654	0.45605654
	3D7 versus PfVps60_KO	**2.385139**	**0.01707266**	**0.05121798***
	R561H versus PfVps60_KO	1.639783	0.10105026	0.15157538
DHA treated condition	3D7 versus R561H	0.4472136	0.6547208	0.6547208
	3D7 versus PfVps60_KO	-1.3416408	0.17971249	0.2695687
	R561H versus PfVps60_KO	-1.7888544	0.07363827	0.2209148
3D7 parasite line	DHA versus DMSO	0.7453560	0.4560565	0.6840848
	DHA versus untreated	1.0434984	0.2967175	0.8901526
	DMSO versus untreated	0.2981424	0.7655945 5	0.7655945
PfVps60_KO parasite line	**DHA versus DMSO**	**2.683282**	**0.007290358**	**0.02187107***
	DHA versus untreated	1.341641	0.179712495	0.17971249
	DMSO versus untreated	-1.341641	0.179712495	0.26956874
R561H parasite line	**DHA versus DMSO**	**2.385139**	**0.01707266**	**0.05121798***
	DHA versus untreated	1.639783	0.10105026	0.15157538
	DMSO versus untreated	-0.745356	0.45605654	0.45605654

Supplementary Table 6. Protein concentration from extracellular vesicles

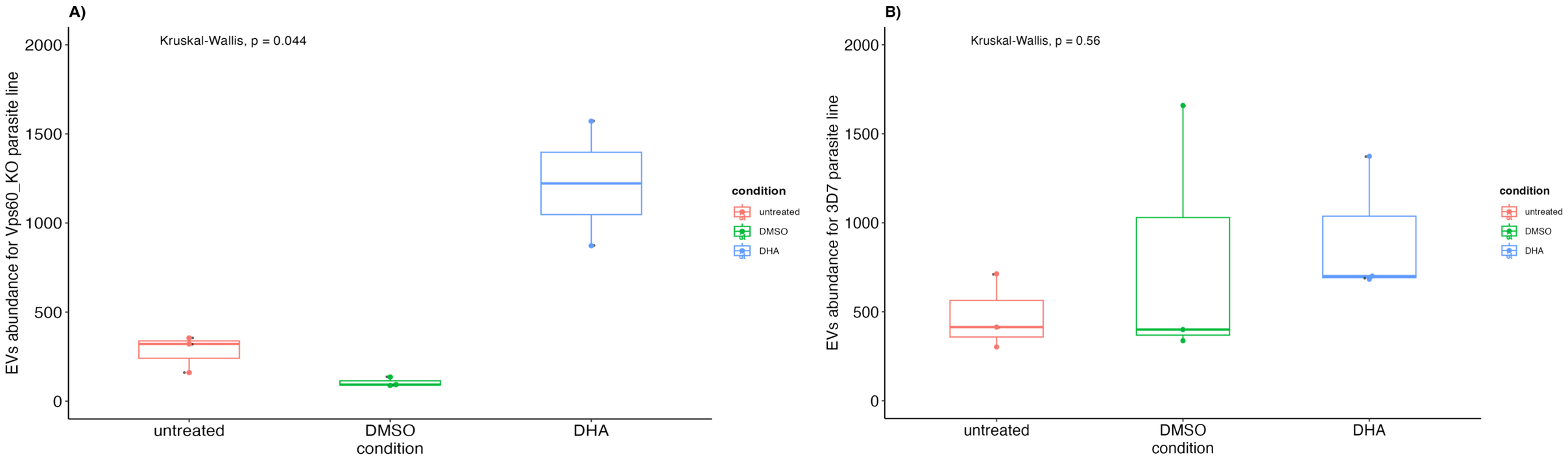
Strain	Treatment	Sample number	Protein concentration (µg/µL)
3D7	untreated	1	0.230555558
3D7	untreated	2	0.146111105
3D7	untreated	3	0.207777781
3D7	DMSO	4	0.086296299
3D7	DMSO	5	0.662222219
3D7	DMSO	6	0.569444444
3D7	DHA	7	0.115000006
3D7	DHA	8	0.170925912
3D7	DHA	9	0.285185174
PfVps60_KO	untreated	10	0.804259232
PfVps60_KO	untreated	11	0.47518519
PfVps60_KO	untreated	12	0.249814826
PfVps60_KO	DMSO	13	0.124814807
PfVps60_KO	DMSO	14	0.250740726
PfVps60_KO	DMSO	15	0.252407407
PfVps60_KO	DHA	16	0.347777778
PfVps60_KO	DHA	17	0.610740749
PfVps60_KO	DHA	18	0.362222218
R561H	untreated	19	0.23037037
R561H	untreated	20	0.242222215
R561H	untreated	21	0.229444442
R561H	DMSO	22	0.272407401
R561H	DMSO	23	0.223148136
R561H	DMSO	24	0.194074066
R561H	DHA	25	0.144444438
R561H	DHA	26	0.153333339
R561H	DHA	27	0.20333333



Supplementary Figure 1. *In vitro* $RSA_{0–3}$ parasite survival rates (%) to artemisinin (ART) treatment for the *Plasmodium falciparum* strains 3D7, R561H and PfVps60_KO. Each bar represents the mean of triplicate experiments. The red dashed line indicates the 1% survival rate above which a parasite line is characterized as ART resistant.



Supplementary Figure 2 Comparison of extracellular vesicles (EVs) abundance among parasite lines for each of the three conditions studied. The objective was to determine the contribution of each parasite's genetic makeup (predictor or independent factor) to EVs abundance (response/dependent factor). We used the null hypothesis that EVs abundance among the 3 parasite lines was equal and the alternate hypothesis that EVs abundance was not equal. 3D7 is the wild-type artemisinin susceptible parasite line with normal EVs biogenesis pathway. PfVps60_KO was generated from 3D7 by knocking out the *Pfvps60* gene resulting in lower constitutive EVs biogenesis and abundance. R561H is an artemisinin-resistant parasite line CRISPR engineered on a 3D7 background with normal EVs biogenesis pathway. A) In the untreated condition, EVs abundance varied slightly between 3D7 and PfVps60_KO, 3D7 and R561H, and PfVps60_KO and R561H. None of the paired comparisons yielded a p-value to convincingly reject the null hypothesis. B) In the 0.1% DMSO-treated condition, EVs abundance varied between 3D7 and PfVps60_KO, 3D7 and R561H, and PfVps60_KO and R561H, and yielded a p-value = 0.05. C) In the 700nM DHA-treated condition, EVs abundance did not vary between 3D7 and PfVps60_KO, 3D7 and R561H, and PfVps60_KO and R561H and was most abundant in PfVps60_KO.



Supplementary Figure 3. Comparison of extracellular vesicles (EVs) abundance between treatment conditions for each parasite line studied. The objective was to determine the contribution of each treatment condition (predictor or independent factor) to EVs abundance (response/dependent factor). We used the null hypothesis that EVs abundance among the 3 conditions was equal and the alternate hypothesis that EVs abundance was not equal. 3D7 is the wild-type artemisinin susceptible parasite line with normal EVs biogenesis pathway. PfVps60 was generated from 3D7 by knocking down the Pfvps60 gene resulting in lower constitutive EVs biogenesis and abundance. R561H is an artemisinin-resistant parasite line CRISPR engineered on a 3D7 background with normal EVs biogenesis pathway. A) In the PfVps60_KO parasite, EVs abundance varied between untreated versus DMSO treated, untreated versus DHA treated, and DMSO treated versus DHA treated. Paired comparisons yielded a p-value to convincingly reject the null hypothesis. B) In the 3D7 parasite, EVs abundance varied between untreated versus DMSO treated, untreated versus DHA treated, and DMSO treated versus DHA treated although none of the paired comparisons yielded a p-value to convincingly reject the null hypothesis. C) In the R561H parasite, EVs abundance varied between untreated versus DMSO treated, untreated versus DHA treated, and DMSO treated versus DHA treated and yielded a p-value to marginally reject the null hypothesis.