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## Bacterially Expressed dsRNA Causes Gene Silencing in EAB

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**Fig 3: Insecticida activity of bacterial dsRNA specific to shi and hsp in EAB larv:**

% mortality @ day 7	Control	dsSHI	dsHSP
Rep 1	10	70	50
Rep 2	20	58.33	50
Rep 3	6.66	80	40
Total	36.66	208.33	140
Mean	12.22	69.44333333	46.66667
SD	6.941556	10.84571959	5.773503
SE	4.007709	6.261779122	3.333333

**Anova: Single Factor**

**SUMMARY**

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>
Column 1	3	36.66	12.22
Column 2	3	208.33	69.44333
Column 3	3	140	46.66667

**ANOVA**

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>
Between Groups	4979.859	2	2489.93
Within Groups	398.2963	6	66.38272
Total	5378.156	8	

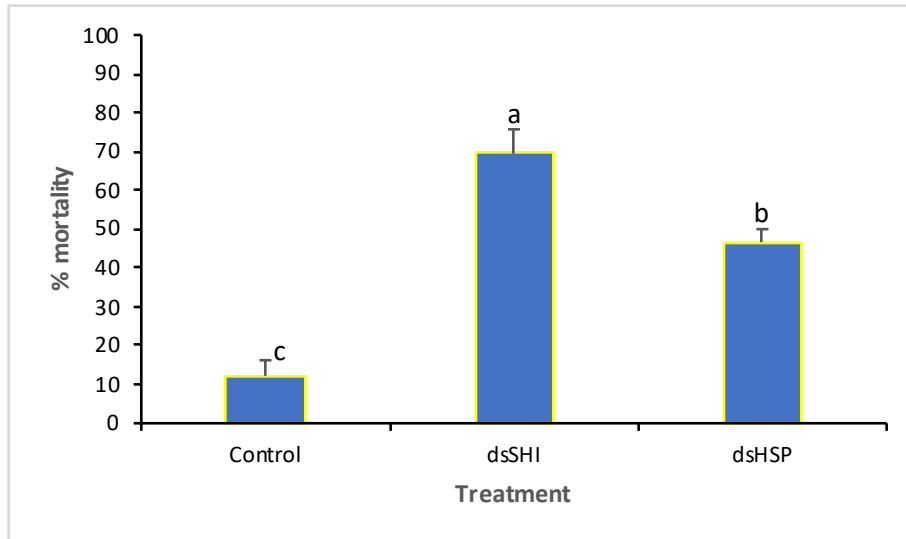
**posthoc T-test**

Group 1	Control	dsSHI
	10	70
	20	58.33
	6.66	80

t-Test: Two-Sample Assuming Equal Variances

	<i>Variable 1</i>	<i>Variable 2</i>
Mean	12.22	69.44333333
Variance	48.1852	117.6296333
Observations	3	3
Pooled Variance	82.90742	
Hypothesized Mean Difference	0	
df	4	
t Stat	-7.69701	
P(T<=t) one-tail	0.000766	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.001533	
t Critical two-tail	2.776445	

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*Variance*

48.1852  
117.6296  
33.33333

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<i>F</i>	<i>P-value</i>	<i>F crit</i>
37.5087	0.000406	5.14325285

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Group 2	Control	dsHSP
	10	50
	20	50
	6.66	40

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	12.22	46.66667
Variance	48.1852	33.33333
Observations	3	3
Pooled Variance	40.75927	
Hypothesized Mean Difference	0	
df	4	
t Stat	-6.60815	
P(T<=t) one-tail	0.001359	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.002718	
t Critical two-tail	2.776445	

Group 3	dsSHI	dsHSP
	70	50
	58.33	50
	80	40

t-Test: Two-Sample Assuming Equal Variances

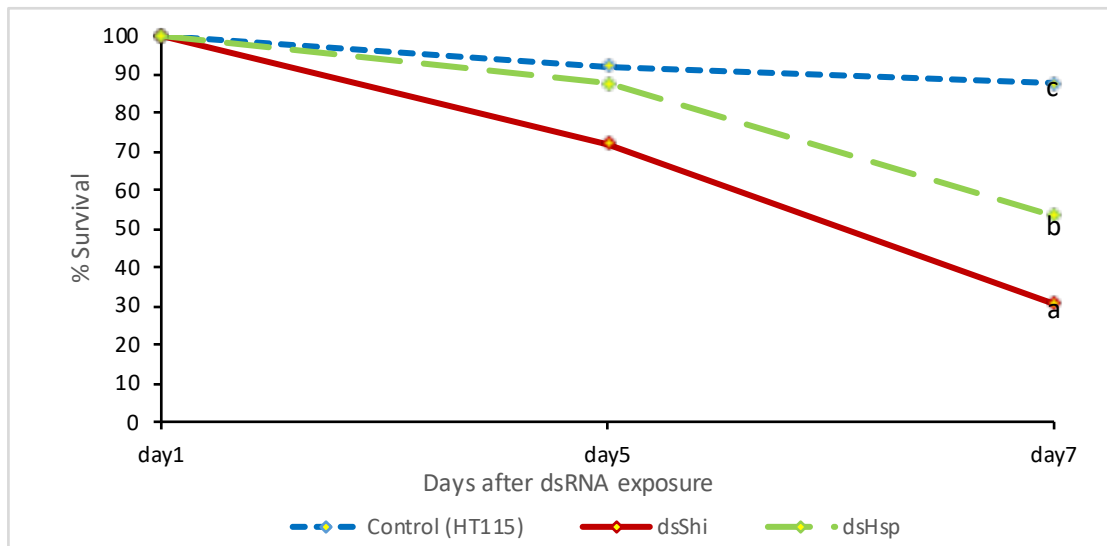
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	69.44333	46.66667
Variance	117.6296	33.33333
Observations	3	3
Pooled Variance	75.48148	
Hypothesized Mean Difference	0	
df	4	
t Stat	3.210817	
P(T<=t) one-tail	0.016282	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.032564	
t Critical two-tail	2.776445	

Fig 4: Effect of dsRNA specific to shi and hsp on EAB neonate larvae: Neonat  
 Observations were taken on day 1, day 5 and day 7 (Value re

	day1	day5	day7
Control 1	100	90	90
Control 2	100	93.33	80
Control 3	100	93.33	93.33
<b>Mean</b>	<b>100</b>	<b>92.22</b>	<b>87.77</b>
dsShi-1	100	70	30
dsShi-2	100	80	33.33
dsShi-3	100	66.66	20
<b>Mean</b>	<b>100</b>	<b>72.22</b>	<b>30.55</b>
dsHsp-1	100	70	50
dsHsp-2	100	93.33	60
dsHsp-3	100	100	66.66
<b>Mean</b>	<b>100</b>	<b>87.77</b>	<b>53.33</b>



	day1	day5	day7
Control	100	92.22	87.77
dsShi	100	72.22	30.55
dsHsp	100	87.77	53.33







the EAB survival (%) 7d after feeding on dsRNA expressing bacteria.  
 presented are mean of 3 individual replications)

day 1		
Control	dsshi	dsHsp
100	100	100
100	100	100
100	100	100

No significant difference @ day 1

Anova: Single Factor

SUMMARY

Groups
Column 1
Column 2
Column 3

ANOVA

Source of Variation
Between Groups
Within Groups
Total

day 5		
Control	dsshi	dsHsp
90	70	70
93.33	80	93.33
93.33	66.66	100

No significant difference @ day 5

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	3	276.66	92.22	3.6963
Column 2	3	216.66	72.22	48.1852
Column 3	3	263.33	87.77667	248.1296333

ANOVA

Source of Variation	SS	df	MS	F
Between Groups	661.7531	2	330.8765	3.308642657
Within Groups	600.0223	6	100.0037	
Total	1261.775	8		

Day 7		
Control	dsshi	dsHsp
90	30	50
80	33.33	60

93.33	20	66.66
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There is significant difference @ day 7

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	3	263.33	87.77667	48.12963333
Column 2	3	83.33	27.77667	48.12963333
Column 3	3	176.66	58.88667	70.31853333

ANOVA

Source of Variation	SS	df	MS	F
Between Groups	5402.464	2	2701.232	48.64811698
Within Groups	333.1556	6	55.52593	
Total	5735.62	8		

Posthoc t-Test

day 7	
Control	dsshi
90	30
80	33.33
93.33	20

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	87.77667	27.77667
Variance	48.12963	48.12963
Observations	3	3
Pooled Variance	48.12963	
Hypothesized Mean Difference	0	
df	4	
t Stat	10.59231	
P(T<=t) one-tail	0.000225	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.00045	
t Critical two-tail	2.776445	

r

<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
3	300	100	0
3	300	100	0
3	300	100	0

<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>
0	2	0	65535
0	6	0	
0	8		

<i>P-value</i>	<i>Fcrit</i>
0.10753652	5.143253

<i>P-value</i>	<i>F crit</i>
0.000195975	5.143253

day 7	
Control	dsHsp
90	50
80	60
93.33	66.66

t-Test: Two-Sample Assuming Equal Variances

	<i>Variable 1</i>	<i>Variable 2</i>
Mean	87.77667	58.88667
Variance	48.12963	70.31853
Observations	3	3
Pooled Variance	59.22408	
Hypothesized Mean Difference	0	
df	4	
t Stat	4.597736	
P(T<=t) one-tail	0.005024	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.010048	
t Critical two-tail	2.776445	

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<i>P-value</i>	<i>F crit</i>
#DIV/0!	5.143253

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day 7	
dsHsp	dsshi
50	30
60	33.33
66.66	20

t-Test: Two-Sample Assuming Equal Variances

	<i>Variable 1</i>	<i>Variable 2</i>
Mean	58.88667	27.77667
Variance	70.31853	48.12963
Observations	3	3
Pooled Variance	59.22408	
Hypothesized Mean Difference	0	
df	4	
t Stat	4.95104	
P(T<=t) one-tail	0.003878	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.007755	
t Critical two-tail	2.776445	

## SHI -120h

Sample Name	Target Name	Task	Reporter	Quencher	RQ	RQ Min
S1	SHI	UNKNOWN	SYBR	None	0.220421	0.111981
S1	SHI	UNKNOWN	SYBR	None	0.220421	0.111981
S1	SHI	UNKNOWN	SYBR	None	0.220421	0.111981
S2	SHI	UNKNOWN	SYBR	None	0.288151	0.05996
S2	SHI	UNKNOWN	SYBR	None	0.288151	0.05996
S2	SHI	UNKNOWN	SYBR	None	0.288151	0.05996
S3	SHI	UNKNOWN	SYBR	None	0.266996	0.135386
S3	SHI	UNKNOWN	SYBR	None	0.266996	0.135386
S3	SHI	UNKNOWN	SYBR	None	0.266996	0.135386
c1	SHI	UNKNOWN	SYBR	None	1	0.13778
c1	SHI	UNKNOWN	SYBR	None		
c1	SHI	UNKNOWN	SYBR	None	1	0.13778

## SHI-72h

Sample Name	Target Name	Task	Reporter	Quencher	RQ	RQ Min
C	SHI	UNKNOWN	SYBR	None	1	0.006582
C	SHI	UNKNOWN	SYBR	None	1	0.006582
S1	SHI	UNKNOWN	SYBR	None	0.86729	0.043859
S1	SHI	UNKNOWN	SYBR	None	0.86729	0.043859
S2	SHI	UNKNOWN	SYBR	None	0.809811	0.534098
S2	SHI	UNKNOWN	SYBR	None	0.809811	0.534098
S2	SHI	UNKNOWN	SYBR	None	0.809811	0.534098
S3	SHI	UNKNOWN	SYBR	None	0.575306	0.211593
S3	SHI	UNKNOWN	SYBR	None	0.575306	0.211593
S3	SHI	UNKNOWN	SYBR	None	0.575306	0.211593
Control	SHI	UNKNOWN	SYBR	None	1	0.2696
Control	SHI	UNKNOWN	SYBR	None	1	0.2696
Control	SHI	UNKNOWN	SYBR	None	1	0.2696

## HSP 120h

Sample Name	Target Name	Task	Reporter	Quencher	RQ	RQ Min
c1	HSP	UNKNOWN	SYBR	None	1	0.029274
c1	HSP	UNKNOWN	SYBR	None	1	0.029274
c1	HSP	UNKNOWN	SYBR	None	1	0.029274
H1	HSP	UNKNOWN	SYBR	None	0.056055	0.001056
H1	HSP	UNKNOWN	SYBR	None	0.056055	0.001056
H1	HSP	UNKNOWN	SYBR	None	0.056055	0.001056
H2	HSP	UNKNOWN	SYBR	None	0.016234	4.43E-05
H2	HSP	UNKNOWN	SYBR	None	0.016234	4.43E-05
H2	HSP	UNKNOWN	SYBR	None	0.016234	4.43E-05



H3	HSP	UNKNOWN	SYBR	None	0.019219	0.006473
H3	HSP	UNKNOWN	SYBR	None	0.019219	0.006473
C	HSP	UNKNOWN	SYBR	None	1	1.94E-07
C	HSP	UNKNOWN	SYBR	None	1	1.94E-07

### HSP 72h

Sample Name	Target Name	Task	Reporter	Quencher	RQ	RQ Min
S1	HSP	UNKNOWN	SYBR	None	0.467379	0.189414
S1	HSP	UNKNOWN	SYBR	None	0.467379	0.189414
S1	HSP	UNKNOWN	SYBR	None	0.467379	0.189414
S2	HSP	UNKNOWN	SYBR	None	0.496063	0.111597
S2	HSP	UNKNOWN	SYBR	None	0.496063	0.111597
S2	HSP	UNKNOWN	SYBR	None	0.496063	0.111597
c1	HSP	UNKNOWN	SYBR	None	1	0.029274
c1	HSP	UNKNOWN	SYBR	None	1	0.029274
c1	HSP	UNKNOWN	SYBR	None	1	0.029274
S3	HSP	UNKNOWN	SYBR	None	0.576647	0.335125
S3	HSP	UNKNOWN	SYBR	None	0.576647	0.335125
C	HSP	UNKNOWN	SYBR	None	1	0.417958
C	HSP	UNKNOWN	SYBR	None	1	0.417958



0.057061	21.88945	21.76259	0.179405		3.024364	0.364883		
0.057061	21.63573	21.76259	0.179405		3.024364	0.364883		
5154791	27.84933	27.12078	1.030325		-2.67696	5.182264		
5154791	26.39223	27.12078	1.030325		-2.67696	5.182264		

RQMax	Ct	Ct Mean	Ct SD	$\Delta Ct$	$\Delta Ct$ Mean	$\Delta Ct$ SE	HK Control	HK Control
1.153257	27.87255	27.2256	0.561174		13.67384	0.469323		
1.153257	26.93386	27.2256	0.561174		13.67384	0.469323		
1.153257	26.87039	27.2256	0.561174		13.67384	0.469323		
2.205062	26.33975	27.31973	1.290986		13.58791	0.775173		
2.205062	26.8369	27.31973	1.290986		13.58791	0.775173		
2.205062	28.78254	27.31973	1.290986		13.58791	0.775173		
34.15996	27.27309	29.4899	2.821846		12.57651	1.834805		
34.15996	28.53019	29.4899	2.821846		12.57651	1.834805		
34.15996	32.66643	29.4899	2.821846		12.57651	1.834805		
0.992231	28.93972	28.83149	0.153058		7.490636	0.181978		
0.992231	28.72326	28.83149	0.153058		7.490636	0.181978		
2.392586	28.4845	28.22362	0.368933		6.696395	0.29251		
2.392586	27.96275	28.22362	0.368933		6.696395	0.29251		



5.701323	TRUE	0.275274	TRUE	3	16	1	81.11971	
5.701323	TRUE	0.275274	TRUE	3	16	1	81.11971	
0	TRUE	0.275274	TRUE	3	22	1	80.82147	
0	TRUE	0.275274	TRUE	3	21	1	80.82147	

$\Delta\Delta C_T$	Automatic	Ct Threshold	Automatic	Baseline Sta	Baseline En	Efficiency	Tm1	Tm2
1.097334	TRUE	0.023302	TRUE	3	26	1	83.96832	
1.097334	TRUE	0.023302	TRUE	3	25	1	83.97087	81.87823
1.097334	TRUE	0.023302	TRUE	3	25	1	84.56877	
1.011406	TRUE	0.023302	TRUE	3	24	1	84.419	
1.011406	TRUE	0.023302	TRUE	3	25	1	84.419	
1.011406	TRUE	0.023302	TRUE	3	27	1	80.82672	
0	TRUE	0.023302	TRUE	3	25	1	84.2695	
0	TRUE	0.023302	TRUE	3	27	1	80.98067	
0	TRUE	0.023302	TRUE	3	31	1	80.52776	62.73982
0.794241	TRUE	0.35661	TRUE	3	22	1	76.05703	
0.794241	TRUE	0.35661	TRUE	3	22	1	75.90804	
0	TRUE	0.35661	TRUE	3	22	1	75.61528	
0	TRUE	0.35661	TRUE	3	22	1	75.7643	

Tm3	Comments	HIGHSD	NOAMP	EXPFAIL	MTP
		N	N	N	N
		N	N	N	N
		N	N	N	N
		Y	N	N	N
		Y	N	N	N
		Y	N	N	N
		Y	N	N	N
		Y	N	N	N
		Y	N	N	N
		N	N	N	N
87.85748		N	Y	Y	Y
		N	N	N	N

Tm3	Comments	HIGHSD	NOAMP	EXPFAIL	MTP
		Y	N	1	
		Y	N	1	
		Y	N	0.86729	
		Y	Y	0.86729	
		N	N	N	
		N	N	N	
		N	N	N	
		Y	N	N	
		Y	N	N	
		Y	N	N	
		Y	N	N	
		Y	N	N	
		Y	N	N	

Tm3	Comments	HIGHSD	NOAMP	EXPFAIL	MTP
		Y	N	N	N
		Y	N	N	N
		Y	N	N	Y
		Y	N	N	Y
		Y	N	N	Y
		Y	N	N	Y
		Y	N	N	Y
		Y	N	N	Y
		Y	N	N	Y

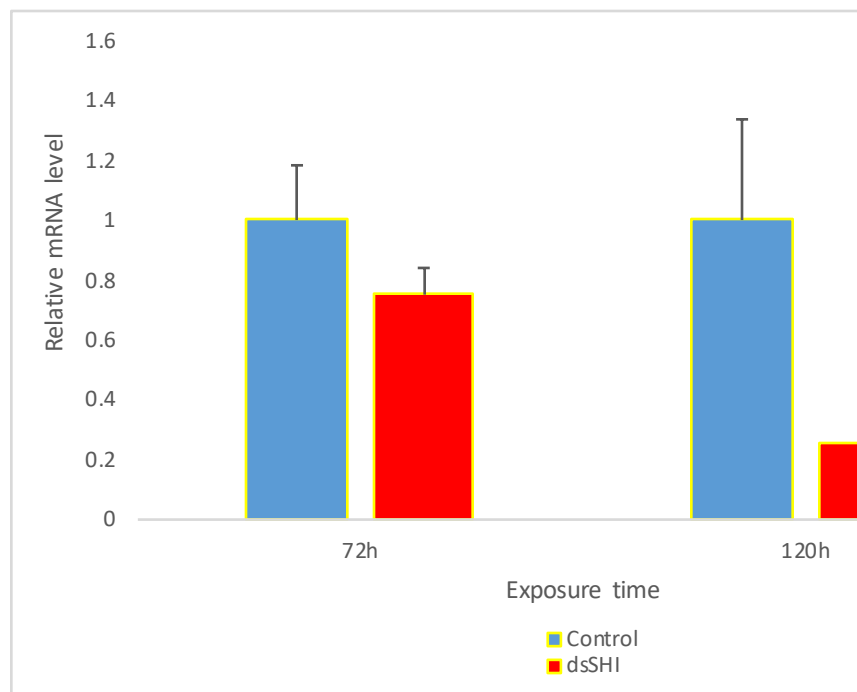
		N	N		
		N	N		
		Y	Y		
		Y	Y		

Tm3	Comments	HIGHSD	NOAMP	EXPFAIL	MTP
		Y	N	N	N
75.30136		Y	N	N	Y
		Y	N	N	N
		Y	N	N	N
		Y	N	N	N
		Y	N	N	N
		Y	N	N	N
		Y	N	N	Y
		N	N	0.576646	
		N	N	0.576646	
		N	N	1	
		N	N	1	

**Fig 6:Quantitative RT PCR analysis of transcript levels after RNAi medi:**

		dsShi			
C		72h	120h		
	1	0.86729	0.220421		
	1	0.809811	0.288151		
	1	0.575306	0.266996		
sum	3	2.252407	0.775568	sum	
Mean	1	0.750802	0.258523	Mean	
SD	0	0.154678	0.034651	SD	
SE	0	0.089303	0.020006	SE	

	72h	120h
Control	1	1
dsSHI	0.750802	0.258523



dsSHi			
Group A	Group B	Group C	Group D
c	72h	c	120h



1	0.86729	1	0.220421
1	0.809811	1	0.288151
1	0.575306	1	0.266996

dsSHI

Group A/Group B (72 h)

t-Test: Two-Sample Assuming Equal Variances

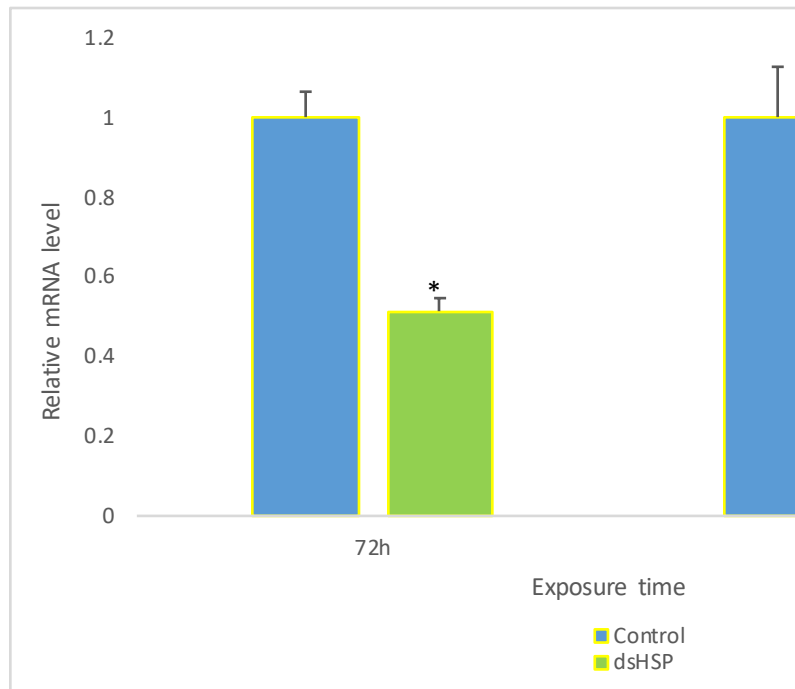
	<i>Variable 1</i>	<i>Variable 2</i>
Mean	1	0.750802
Variance	0	0.023925
Observations	3	3
Pooled Variance	0.011963	
Hypothesized	0	
df	4	
t Stat	2.790464	
P(T<=t) one-tail	0.024644	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.059289	
t Critical two-tail	2.776445	

ated depletion of gene in EAB

C	dsHsp	
	72h	120h
1	0.467379	0.056055
1	0.496063	0.016234
1	0.576647	0.019219
3	1.540089	0.091508
1	0.513363	0.030503
0	0.056651	0.022179
0	0.032707	0.012805

T Test

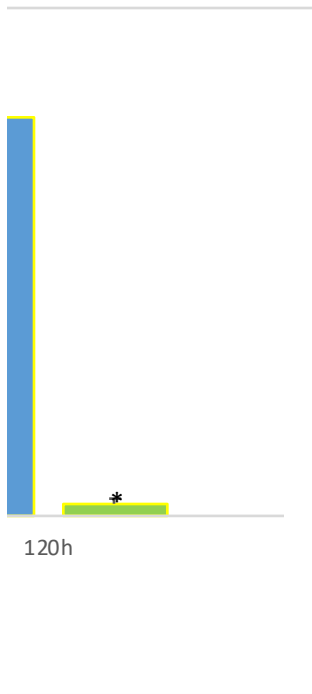
	72h	120h
Control	1	1
dsHSP	0.513363	0.030503



Group C/Group D (120h)

t-Test: Two-Sample Assuming Equal Variances

	<i>Variable 1</i>	<i>Variable 2</i>
Mean	1	0.258523
Variance	0	0.001201
Observations	3	3
Pooled Variance	0.0006	
Hypothesized Mean Difference	0	
df	4	
t Stat	37.0636	
P(T<=t) one-tail	1.58E-06	
t Critical one-tail	2.131847	
P(T<=t) two-tail	3.16E-06	
t Critical two-tail	2.776445	



dsHsp			
Group A	Group B	Group C	Group D
c	72h	c	120h

1	0.467379	1	0.056055
1	0.496063	1	0.016234
1	0.576647	1	0.019219

dsHsp

Group A/Group B (72 h)

t-Test: Two-Sample Assuming Equal Variances

	<i>Variable 1</i>	<i>Variable 2</i>
Mean	1	0.513362835
Variance	0	0.003209298
Observations	3	3
Pooled Variance	0.001605	
Hypothesized Mean Difference	0	
df	4	
t Stat	14.87856	
P(T<=t) one-tail	5.94E-05	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.000119	
t Critical two-tail	2.776445	



Group C/Group D (120h)

t-Test: Two-Sample Assuming Equal Variances

	<i>Variable 1</i>	<i>Variable 2</i>
Mean	1	0.030503
Variance	0	0.000492
Observations	3	3
Pooled Variance	0.000246	
Hypothesized Mean Difference	0	
df	4	
t Stat	75.71079	
P(T<=t) one-tail	9.12E-08	
t Critical one-tail	2.131847	
P(T<=t) two-tail	1.82E-07	
t Critical two-tail	2.776445	