Supplemental data

Supplemental Figure S1. Ssd1$^{1-450}$-NLSΔ fails to localize to the nucleus. Images of three cells expressing Ssd1$^{1-450}$-NLSΔ-GFP and Pap1-RFP (FLY3365 + FLE1211) captured by spinning disk confocal fluorescence microscopy. Left panels: bright field images, middle panel: merged optical sections of GFP and RFP fluorescence (21 x 0.2um sections); right panels: 3-D models of image data generated by Volocity software. The 3-D models reveal that the Ssd1$^{1-450}$-NLSΔ puncta are extra-nuclear or perinuclear in localization. Scale bar = 4 um. The bottom cell is also depicted in **Fig. 3B**.

Supplemental Figure S2. *SRL1* mRNA localization is aberrant in Ssd1-NLSΔ and Ssd1-RBDΔ cells. *SRL1* mRNA localization was analyzed in budded cells, as previously described (Kurischko et al., 2011). Cells (FLY3196) expressing wild type Ssd1 (*SSD1*; FLE1083), Ssd1-NLSΔ (SSD1-NLSΔ-TAP; FLE1277), Ssd1-RBDΔ (SSD1-RBDΔ-TAP; FLE1276) and empty vector (*ssd1Δ;* pRS415) exhibited 5 general patterns of *SRL1* mRNA localization, as depicted in the graph. These include cells with no *SRL1* mRNA spots (no spots), multiple faint spots, and 1-3 bright spots restricted to the mother (M), bud (D) or both mother and bud (M+D). The data for the wild type *SSD1* and empty vector controls are from previously published experiments (Kurischko et al., 2011).

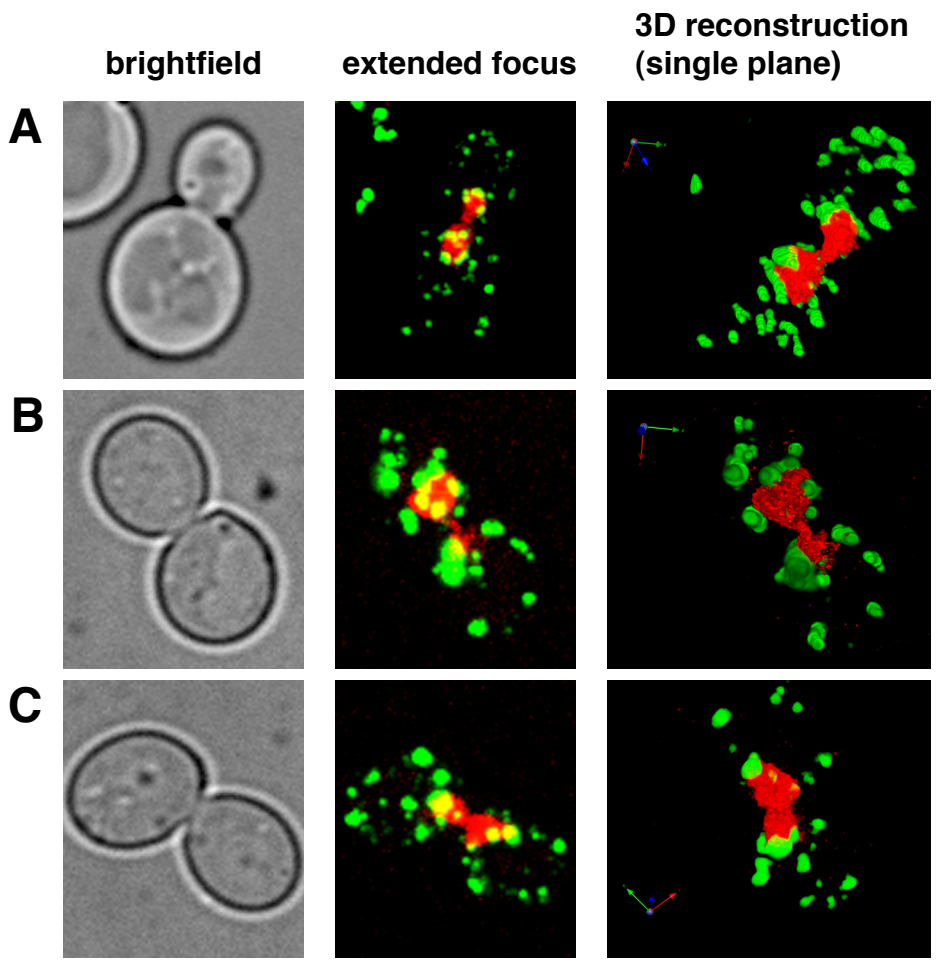
Supplemental Figure S3. C-terminal truncated Ssd1-GFP partially co-localizes with P-bodies in *cbk1*Δ cells. Physiologically expressed Ssd1$^{1-570}$, Ssd1$^{1-670}$, Ssd1$^{1-1014}$ and Ssd1$^{1-1170}$ were monitored in *cbk1*Δ cells that co-express Edc3-RFP. Some cytoplasmic Ssd1 puncta co-localize with P-body protein Edc3, see arrowheads (26.2%

for Ssd1$^{1-570}$, 45.9% for Ssd1$^{1-670}$, 30.3% for Ssd1$^{1-1014}$ and 17.6% for Ssd1$^{1-1170}$ cells; n=100-300 cells for each). The strains used in these experiments were FLY3206, FLY3210, FLY3313 and FLY3316. All cells were monitored by spinning disk fluorescence microscopy and each image represents a single optical section. Scale bar = 8 um.

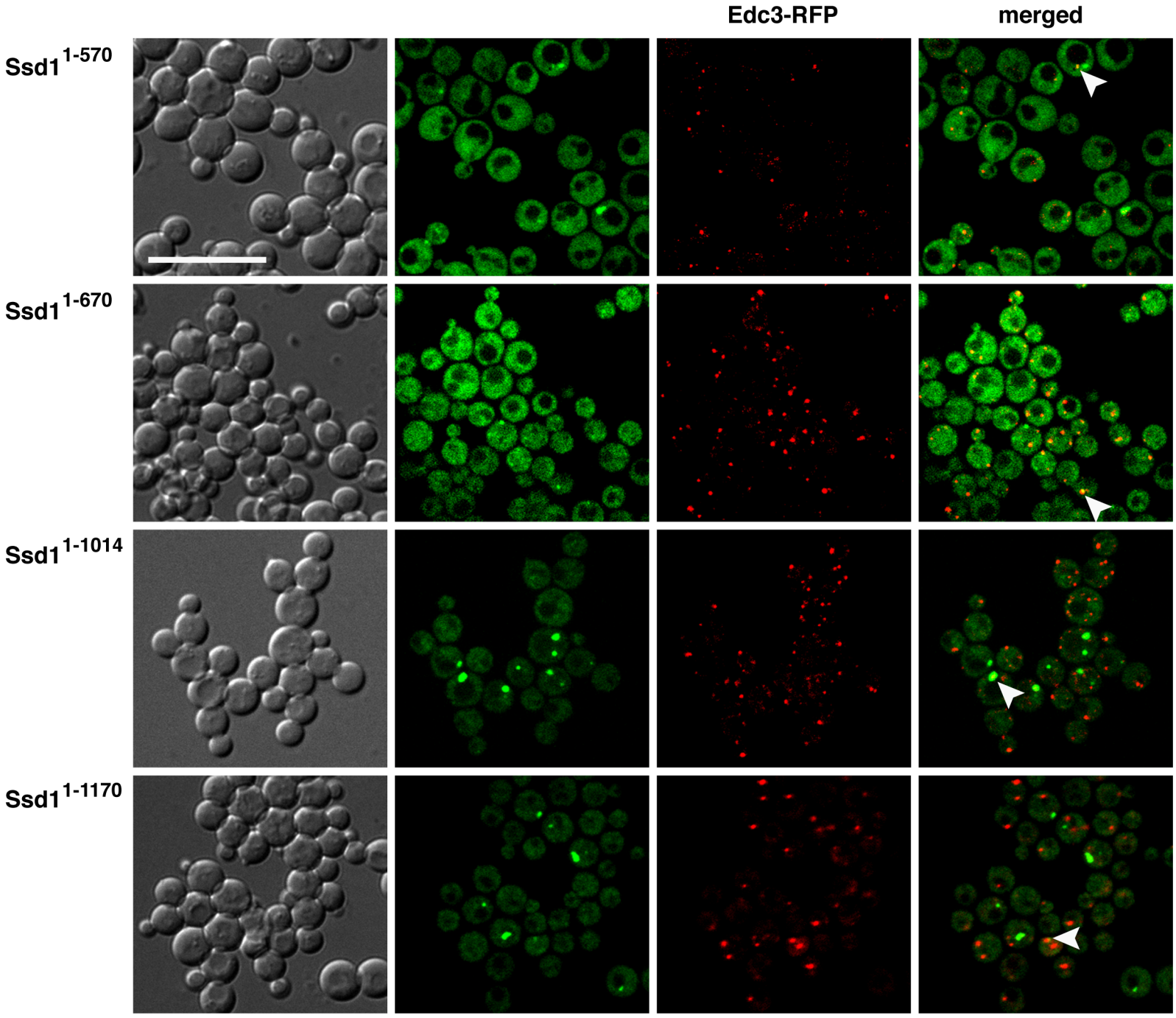
Supplemental movie 1. **3-D model of cells expressing Ssd1$^{1-450}$-NLSΔ puncta.** The movie was generated from the upper 3-D model in **Supplemental Fig. S1** and demonstrates that the Ssd1$^{1-450}$-NLSΔ puncta are extra-nuclear. 3-D projections and movie were generated by Volocity software.

Supplemental Table 1. Nuclear-cytoplasmic ratios of C-terminal truncated Ssd1. Data were obtained by measuring the average Ssd1-GFP signal in the nucleus and cytoplasm, as described in the Material and Methods. Cells with large vacuoles were not taken in consideration. The data represent N (nucleus), C (cytoplasm), B (background), N-B, C-B, ratio N-B/C-B, number of nuclei (observations), median, mean, standard deviation, p values for pair wise comparisons of ratios between mutants and wild type.

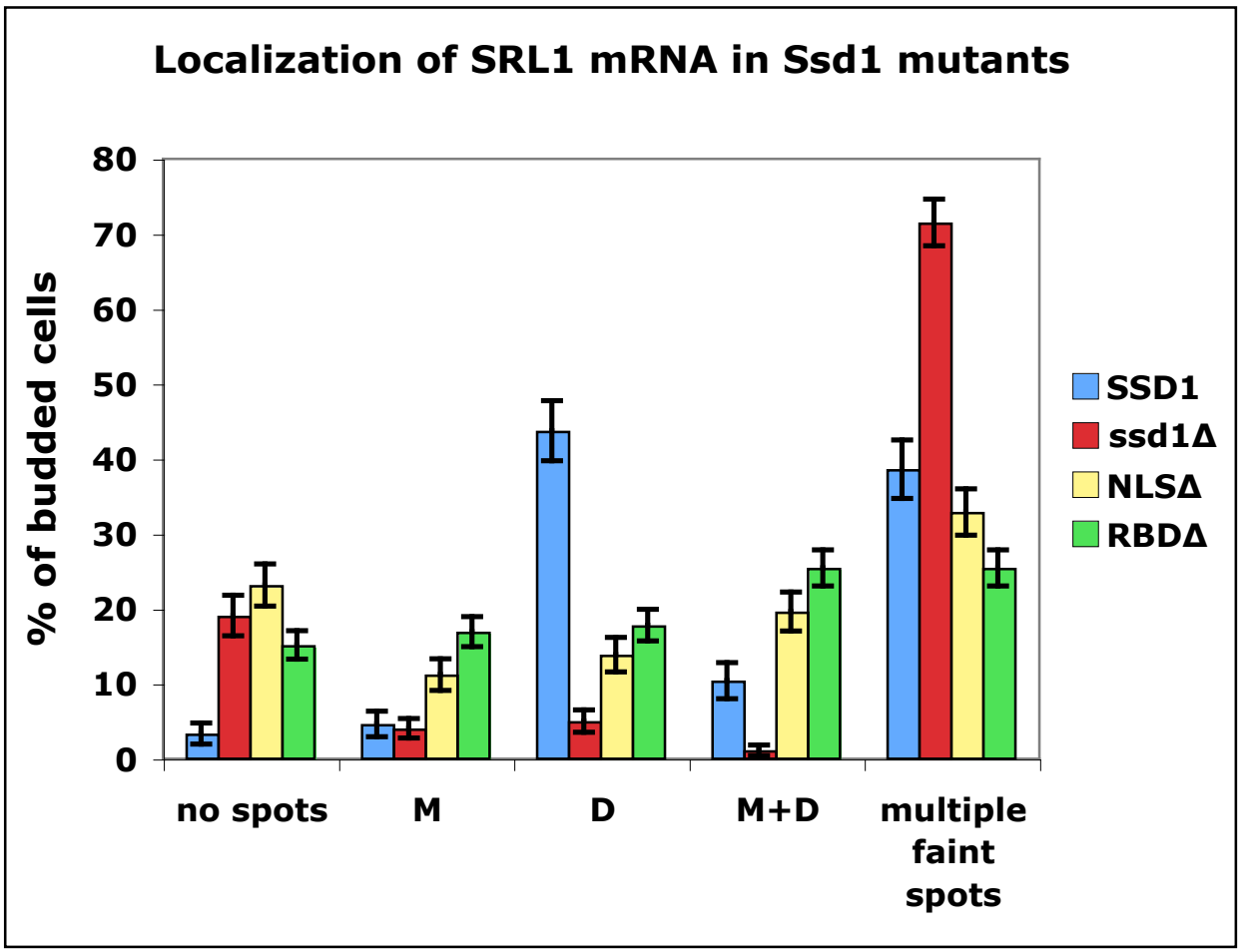
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure S3



Supplemental Table 1 (for Fig 8B)

Strain	Nucleus	Cytoplasm	Background	N-B	C-B	Ratio N/B
Ssd1	4160.26	4507.06	1520.92	2639.34	2986.14	0.8838634
	4437.41	5282.24	1520.92	2916.49	3761.32	0.77539
	4458.17	4887.34	1520.92	2937.25	3366.42	0.8725144
	5031.87	5121.29	1520.92	3510.95	3600.37	0.9751637
	4942.49	5753.82	1520.92	3421.57	4232.9	0.8083276
	4221.53	5373.4	1520.92	2700.61	3852.48	0.7010056
	4106.27	5407.42	1520.92	2585.35	3886.5	0.6652129
	3655.39	3714.03	1511.1	2144.29	2202.93	0.9733809
	3623.42	4567.27	1511.1	2112.32	3056.17	0.6911657
	3876.58	4064.39	1511.1	2365.48	2553.29	0.9264439
	3585.33	4576.65	1507.84	2077.49	3068.81	0.6769692
	3343	3720.58	1507.84	1835.16	2212.74	0.8293609
	3723.44	4152.12	1507.84	2215.6	2644.28	0.837884
	4235.42	5111.14	1507.84	2727.58	3603.3	0.7569672
	3862.82	5823.08	1507.84	2354.98	4315.24	0.5457356
	3691.66	4484.42	1507.84	2183.82	2976.58	0.7336675
	3252.54	3569.55	1507.84	1744.7	2061.71	0.8462393
				median	2986.14	0.8083276
				mean		0.79
				stdev		0.12
				n		17

Strain	Nucleus	Cytoplasm	Background	N-B	C-B	Ratio N/B
Ssd1-450	4538.97	3494.62	1518.59	3020.38	1976.03	1.5285092
	5124.94	4042.21	1518.59	3606.35	2523.62	1.4290384
	5291.16	4171.9	1518.59	3772.57	2653.31	1.4218354
	5368.84	3599.85	1518.59	3850.25	2081.26	1.8499611
	4633.03	3403.46	1518.59	3114.44	1884.87	1.6523368
	4661.85	2745.67	1518.59	3143.26	1227.08	2.5615771
	4539.83	3325.32	1518.59	3021.24	1806.73	1.6722144
	4116.14	3216.91	1518.59	2597.55	1698.32	1.5294821
	4515.26	3821.69	1518.59	2996.67	2303.1	1.3011463
	5630.73	3765.75	1518.59	4112.14	2247.16	1.8299276
	4557.49	3499.73	1494.42	3063.07	2005.31	1.5274795
	3944.42	3088.07	1494.42	2450	1593.65	1.5373514
	5274.72	3916.81	1494.42	3780.3	2422.39	1.5605662
	4435.7	3198.22	1494.42	2941.28	1703.8	1.7263059
	4796.82	3596.87	1494.42	3302.4	2102.45	1.5707389
	4410.36	3460.9	1494.42	2915.94	1966.48	1.4828221
				median	1976.03	1.5489588
				mean		1.64
				stdev		0.29
				n		16

Strain	Nucleus	Cytoplasm	Background	N-B	C-B	Ratio N/B
Ssd1-570	7193.4	6375.82	1500.01	5693.39	4875.81	1.1676809
	6618.03	6472.11	1500.01	5118.02	4972.1	1.0293478
	6754.34	6125.03	1500.01	5254.33	4625.02	1.1360664
	6376.83	5043.21	1500.01	4876.82	3543.2	1.3763886
	6517.47	5197.67	1500.01	5017.46	3697.66	1.3569284
	4846.82	4472.97	1819.4	3027.42	2653.57	1.1408857
	6201.39	5711.09	1819.4	4381.99	3891.69	1.1259864
	5519.29	4704.86	1819.4	3699.89	2885.46	1.2822531
	4993.39	4698.99	1819.4	3173.99	2879.59	1.1022368
	5107.07	4163.3	1819.4	3287.67	2343.9	1.4026494
	5943.13	4620.13	1819.4	4123.73	2800.73	1.4723768
	5687.72	5644.92	1819.4	3868.32	3825.52	1.011188
	4688.95	3910.46	1819.4	2869.55	2091.06	1.3722944
	4114.98	3701.77	1819.4	2295.58	1882.37	1.2195158
	4166.97	3523.71	1819.4	2347.57	1704.31	1.3774313
	5420.44	4825.96	1487.7	3932.74	3338.26	1.1780808
	4527.89	4338.41	1487.7	3040.19	2850.71	1.0664677
	4319.71	4373.65	1487.7	2832.01	2885.95	0.9813094
				median	3016.17	1.1728808
				mean		1.21
				stdev		0.15
				n		18

Strain	Nucleus	Cytoplasm	Background	N-B	C-B	Ratio N/B
Ssd1-670	5126.86	5113.23	1482.77	3644.09	3630.46	1.0037543
	5635.14	4942.48	1482.77	4152.37	3459.71	1.2002075
	6158.67	5186.5	1482.77	4675.9	3703.73	1.262484
	3151.37	3071.71	1500.68	1650.69	1571.03	1.0507056
	3685.75	3005.9	1500.68	2185.07	1505.22	1.4516616
	3304.31	3055.58	1500.68	1803.63	1554.9	1.1599653
	5134.54	3877.49	1500.68	3633.86	2376.81	1.5288811
	4644.55	3657.72	1500.68	3143.87	2157.04	1.4574927
	4461.6	3218.03	1500.68	2960.92	1717.35	1.7241215
	4876.36	3881.91	1500.68	3375.68	2381.23	1.4176203
	4380.63	4099.07	1500.68	2879.95	2598.39	1.1083594
	4714.67	3413.65	1500.68	3213.99	1912.97	1.6801048
	4647.32	4099.38	1504.65	3142.67	2594.73	1.2111742
	4714.1	3942.35	1504.65	3209.45	2437.7	1.3165894
	4475.04	3592.87	1504.65	2970.39	2088.22	1.4224507
	4167.06	3617.7	1537.82	2629.24	2079.88	1.2641306
	5247.12	4750.49	1537.82	3709.3	3212.67	1.1545848
	3890.11	3368.65	1537.82	2352.29	1830.83	1.2848216
	4018.22	3515.57	1537.82	2480.4	1977.75	1.2541524
				median	2079.88	1.2641306
				mean		1.31
				stdev		0.20
				n		19

Strain	Nucleus	Cytoplasm	Background	N-B	C-B	Ratio N/B
Ssd1-697	7808.61	6495.41	1527.7	6280.91	4967.71	1.2643472
	7630.37	5551.59	1527.7	6102.67	4023.89	1.5166095
	7442.03	5684.66	1527.7	5914.33	4156.96	1.4227536
	5703.97	5023.97	1513.34	4190.63	3510.63	1.1936974
	5470.1	4757.37	1513.34	3956.76	3244.03	1.2197051
	5061.98	4097.57	1513.34	3548.64	2584.23	1.3731905
	4821.65	4336.95	1513.34	3308.31	2823.61	1.1716597
	4231.55	3982.77	1513.34	2718.21	2469.43	1.1007439
	5475.45	4669.89	1509.96	3965.49	3159.93	1.2549297
	4597.91	4629.92	1509.96	3087.95	3119.96	0.9897403
	5678.75	5199.36	1509.96	4168.79	3689.4	1.1299371
	5211.24	4869.28	1509.96	3701.28	3359.32	1.1017944
	4982.46	4630.96	1509.96	3472.5	3121	1.1126242
	6602.66	3993.49	1511.59	5091.07	2481.9	2.0512793
	7279.37	6176.98	1511.59	5767.78	4665.39	1.2362911
	3430.83	3399.47	1511.59	1919.24	1887.88	1.0166112
	3946.93	3513.26	1511.59	2435.34	2001.67	1.2166541
	3426.42	2992.64	1511.59	1914.83	1481.05	1.2928868
				median	3121	1.2067013
				mean		1.26
				stdev		0.24
				n		18

Strain	Nucleus	Cytoplasm	Background	N-B	C-B	Ratio N/B
Ssd1-1014	4173.99	4000.44	1526.88	2647.11	2473.56	1.070162
	3344.86	3132.2	1526.88	1817.98	1605.32	1.132472
	3207.24	3267.7	1526.88	1680.36	1740.82	0.9652692
	3141.51	3067.53	1526.88	1614.63	1540.65	1.0480187
	3822.62	3388.77	1526.88	2295.74	1861.89	1.2330159
	3221.44	3183.24	1526.88	1694.56	1656.36	1.0230626
	3723.31	3090.49	1526.88	2196.43	1563.61	1.4047173
	3657.85	3356.65	1504.45	2153.4	1852.2	1.1626174
	3716.11	3093.79	1504.45	2211.66	1589.34	1.3915588
	4568.03	3732.48	1504.45	3063.58	2228.03	1.3750174
	3727.45	3225.01	1504.45	2223	1720.56	1.2920212
	4069.18	3708.88	1504.45	2564.73	2204.43	1.1634436
	3462.26	3122.74	1504.45	1957.81	1618.29	1.2098017
	4043.93	3148.45	1504.45	2539.48	1644	1.5446959
	3271.2	2979.78	1504.45	1766.75	1475.33	1.1975287
	3392.31	3046.38	1504.45	1887.86	1541.93	1.2243487
	3478.42	2948.44	1504.45	1973.97	1443.99	1.3670247
	3539.3	3078.82	1502.17	2037.13	1576.65	1.2920623
	3654.69	3388.02	1502.17	2152.52	1885.85	1.1414057
	3837.52	3170.53	1502.17	2335.35	1668.36	1.3997878
	3382.4	2892.78	1502.17	1880.23	1390.61	1.3520901
	3624.04	2857.08	1502.17	2121.87	1354.91	1.5660597
	3255.12	3137.56	1502.17	1752.95	1635.39	1.071885
	3039.69	2811.6	1502.17	1537.52	1309.43	1.1741903
				median	1752.95	1.2286823
				mean		1.24
				stdev		0.16
				n		24

Strain	Nucleus	Cytoplasm	Background	N-B	C-B	Ratio N/B
Ssd1-1170	4048.06	3735.85	1598.39	2449.67	2137.46	1.1460659
	3927.44	3876.83	1598.39	2329.05	2278.44	1.0222126
	4292.06	3647.83	1598.39	2693.67	2049.44	1.3143444
	4302.62	4485	1598.39	2704.23	2886.61	0.9368186
	3347.98	2998.79	1598.39	1749.59	1400.4	1.2493502
	3762.95	3781.73	1598.39	2164.56	2183.34	0.9913985
	3561.31	3230.36	1509.11	2052.2	1721.25	1.1922731
	3378.83	3488.47	1509.11	1869.72	1979.36	0.9446084
	3371.34	3319.92	1509.11	1862.23	1810.81	1.0283961
	3808.4	3559.9	1509.11	2299.29	2050.79	1.1211728
	3876.5	4249.05	1509.11	2367.39	2739.94	0.8640299
	4087.75	3540.73	1509.11	2578.64	2031.62	1.2692531
	3878.37	3768.87	1509.11	2369.26	2259.76	1.0484565
	3199.14	3316.29	1509.11	1690.03	1807.18	0.9351752
	3303.93	3111.42	1526.72	1777.21	1584.7	1.1214804
	3656.24	3823.34	1526.72	2129.52	2296.62	0.9272409
	3402.8	3574.49	1526.72	1876.08	2047.77	0.9161576
	3215.02	3523.72	1526.72	1688.3	1997	0.8454181
	3017.69	2776.92	1526.72	1490.97	1250.2	1.1925852
	2841.64	2783.51	1526.72	1314.92	1256.79	1.0462528
	3091.65	3007.84	1502.47	1589.18	1505.37	1.055674
	2894.36	2700.47	1502.47	1391.89	1198	1.1618447
	2724.91	2445.22	1502.47	1222.44	942.75	1.2966746
	2790.36	2691.76	1502.47	1287.89	1189.29	1.0829066
	2701.28	2655.72	1502.47	1198.81	1153.25	1.0395057
	2730.21	2575.42	1502.47	1227.74	1072.95	1.1442658
				median	1198	1.0520652
				mean		1.07
				stdev		0.13
				n		27

statistics

p-values

t-Test: Two-Sample Assuming Unequal Variances

	Ssd1	Ssd1-450
Mean	0.794076	1.6363308
Variance	0.0137117	0.0818973
Observations	17	16
Hypothesized	0	
df	20	
t Stat	-10.941922	
P(T<=t) one-t	3.41E-10	
t Critical one-t	1.7247182	
P(T<=t) two-t	6.821E-10	
t Critical two-t	2.0859634	

t-Test: Two-Sample Assuming Unequal Variances

	Ssd1	Ssd1-570
Mean	0.794076	1.2110604
Variance	0.0137117	0.0230399
Observations	17	18
Hypothesized	0	
df	32	
t Stat	-9.1285903	
P(T<=t) one-t	1.005E-10	
t Critical one-t	1.6938887	
P(T<=t) two-t	2.009E-10	
t Critical two-t	2.0369333	

t-Test: Two-Sample Assuming Unequal Variances

	Ssd1	Ssd1-670
Mean	0.794076	1.3133296
Variance	0.0137117	0.0389191
Observations	17	19
Hypothesized	0	
df	30	
t Stat	-9.7180808	
P(T<=t) one-t	4.434E-11	
t Critical one-t	1.6972609	
P(T<=t) two-t	8.868E-11	
t Critical two-t	2.0422724	

t-Test: Two-Sample Assuming Unequal Variances

	Ssd1	Ssd1-697
Mean	0.794076	1.2591919
Variance	0.0137117	0.0570563
Observations	17	18
Hypothesized	0	
df	25	
t Stat	-7.3759508	
P(T<=t) one-t	4.998E-08	
t Critical one-t	1.7081407	
P(T<=t) two-t	9.995E-08	
t Critical two-t	2.0595385	

t-Test: Two-Sample Assuming Unequal Variances

	Ssd1	Ssd1-1014
Mean	0.794076	1.2417607
Variance	0.0137117	0.0253929
Observations	17	24
Hypothesized	0	
df	39	
t Stat	-10.367607	
P(T<=t) one-t	4.557E-13	
t Critical one-t	1.6848751	
P(T<=t) two-t	9.115E-13	
t Critical two-t	2.0226909	

t-Test: Two-Sample Assuming Unequal Variances

	Ssd1	Ssd1-1170
Mean	0.794076	1.0728293
Variance	0.0137117	0.0175519
Observations	17	26
Hypothesized	0	
df	37	
t Stat	-7.2418203	
P(T<=t) one-t	6.765E-09	
t Critical one-t	1.6870936	
P(T<=t) two-t	1.353E-08	
t Critical two-t	2.0261924	