SUPPLEMENTARY FIGURES

Therapeutic potential of TAF1 bromodomains for cancer treatment

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Supplementary Figure 1. Knock down of TAF1 in KMS11 cells affects proliferation. (a) TAF1 domains and localization of the shRNAs used in this study (b) Western-blot showing the levels of TAF1 in KMS11 cells transduced with the indicated shRNAs and treated with doxycycline for 4 days. (c) Proliferation curves of the transduced cells treated with vehicle or doxycycline.

Supplementary Figure 2. Knock down of TAF1 in H1568 cells affects proliferation. (a) Western-blot showing the levels of TAF1 in H1568 cells transduced with the indicated shRNAs and treated with doxycycline for 7 days. (b) Proliferation curves of the transduced cells treated with vehicle or doxycycline. (c) Crystal violet staining of the transduced cells treated with vehicle or doxycycline for 17 days. Mean, standard deviation and p-value of three replicates is shown.

Supplementary Figure 3. Knock down of TAF1 in H322 cells affects proliferation. (a) Western-blot showing the levels of TAF1 in H322 cells transduced with the indicated shRNAs and treated with doxycycline for 5 days. (b) Proliferation curves of the transduced cells treated with vehicle or doxycycline. (c) Crystal violet staining of the transduced cells treated with vehicle or doxycycline for 19 days. Mean, standard deviation and 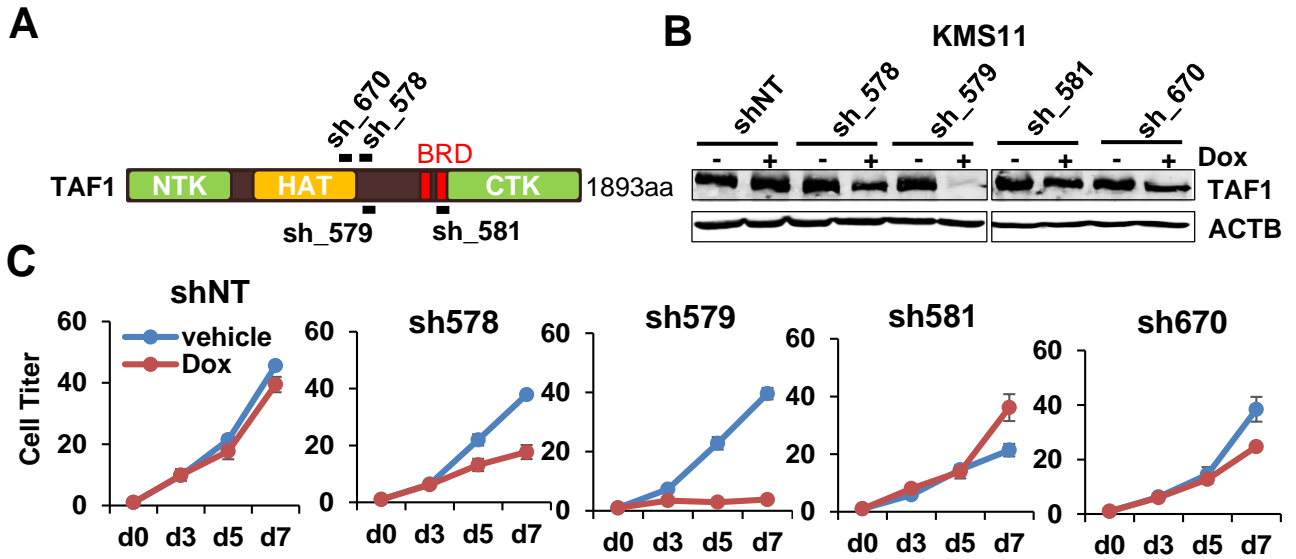p-value of three replicates is shown. (d) Growth competition assays with cells transduced with gRNAs targeting diverse domains of TAF1 and BRD4 including 5' coding region (5'), bromodomains one and two (BD1 and BD2) and N-terminal kinase domain (NTK).
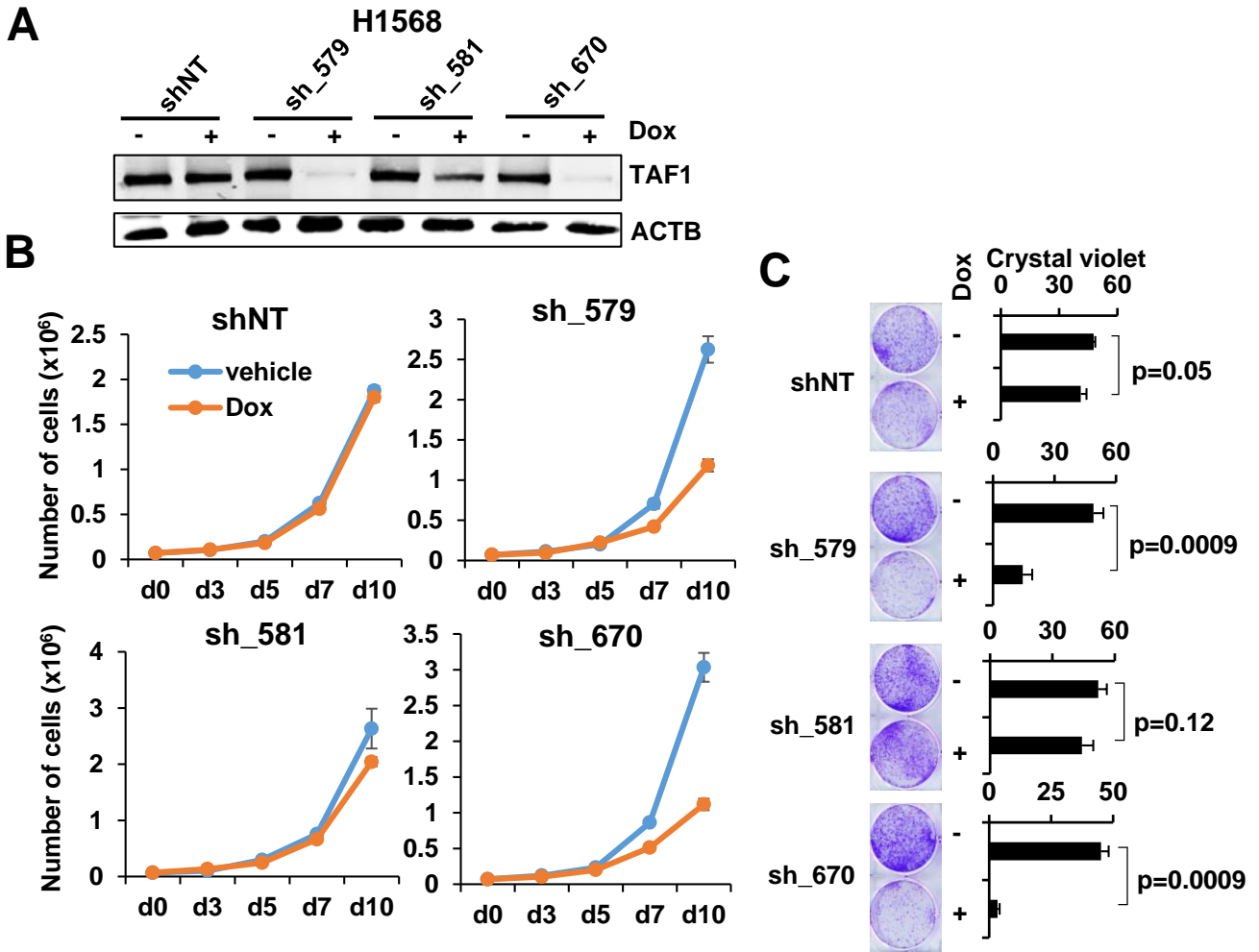
Supplementary Figure 4. Knock down of TAF1 in H2291 cells affects proliferation. (a) Western-blot showing the levels of TAF1 in H1568 cells transduced with the indicated shRNAs and treated with doxycycline for 7 days. (b) Proliferation curves of the transduced cells treated with vehicle or doxycycline. (c) Crystal violet staining of the transduced cells treated with vehicle or doxycycline for 16 days. Mean, standard deviation and p-value of three replicates is shown.
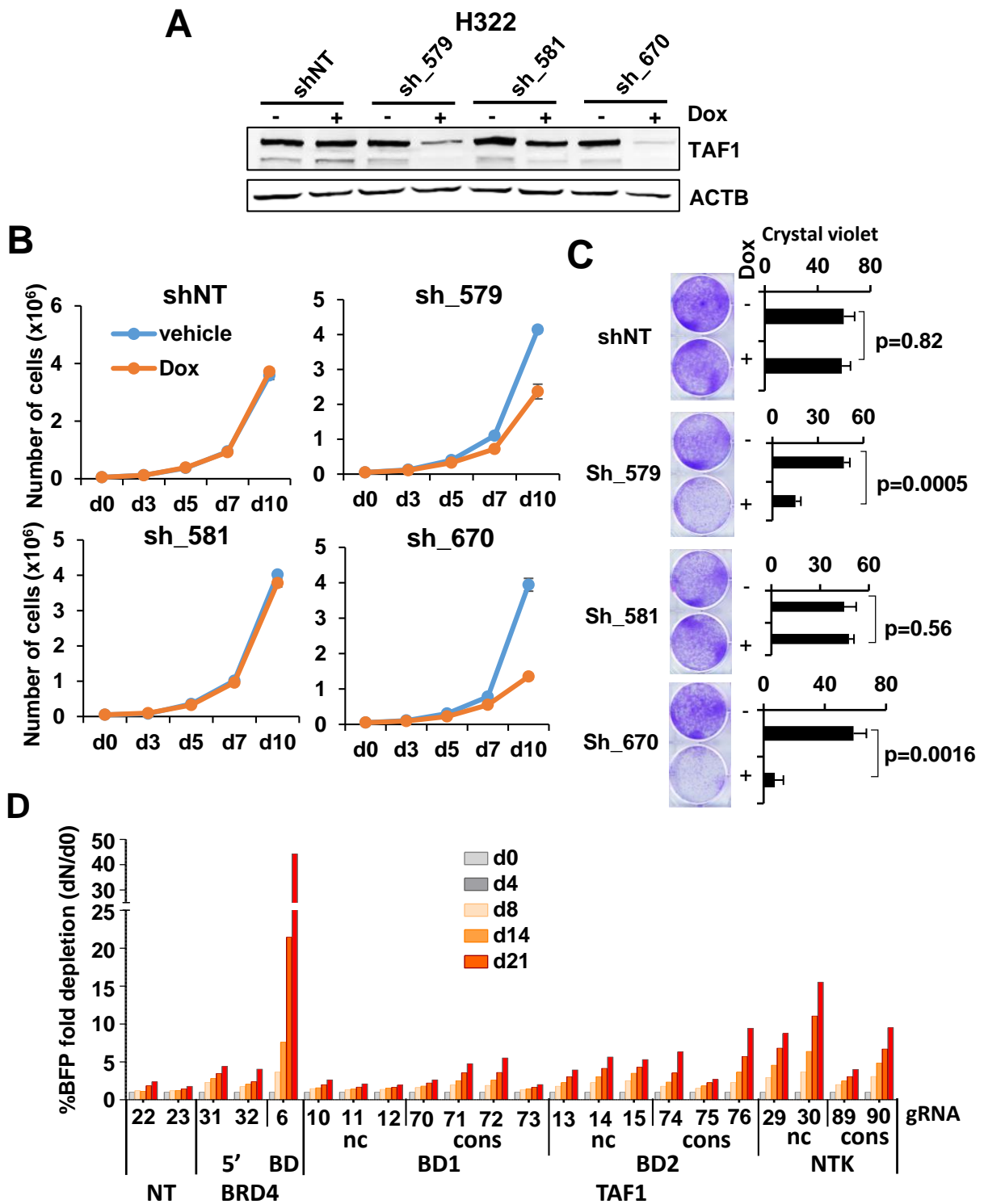
Supplementary Figure 5. Statistical analysis of growth competition assays in K562. (a) ANOVA diamond plots showing the adjusted % of inhibition at day 14 for gRNAs grouped into the indicated categories and considering three independent experiments. (b) Tukey-Kramer statistical test results of data shown in a. Pair comparisons found significant at a p-value<0.05 are shown.

Supplementary Figure 6. Statistical analysis of growth competition assays in H322. (a) ANOVA diamond plots showing the adjusted % of inhibition at day 14 for gRNAs grouped into the indicated categories and considering three independent experiments. (b) Tukey-Kramer statistical test results of data shown in a. Pair comparisons found significant at a p-value<0.05 are shown.
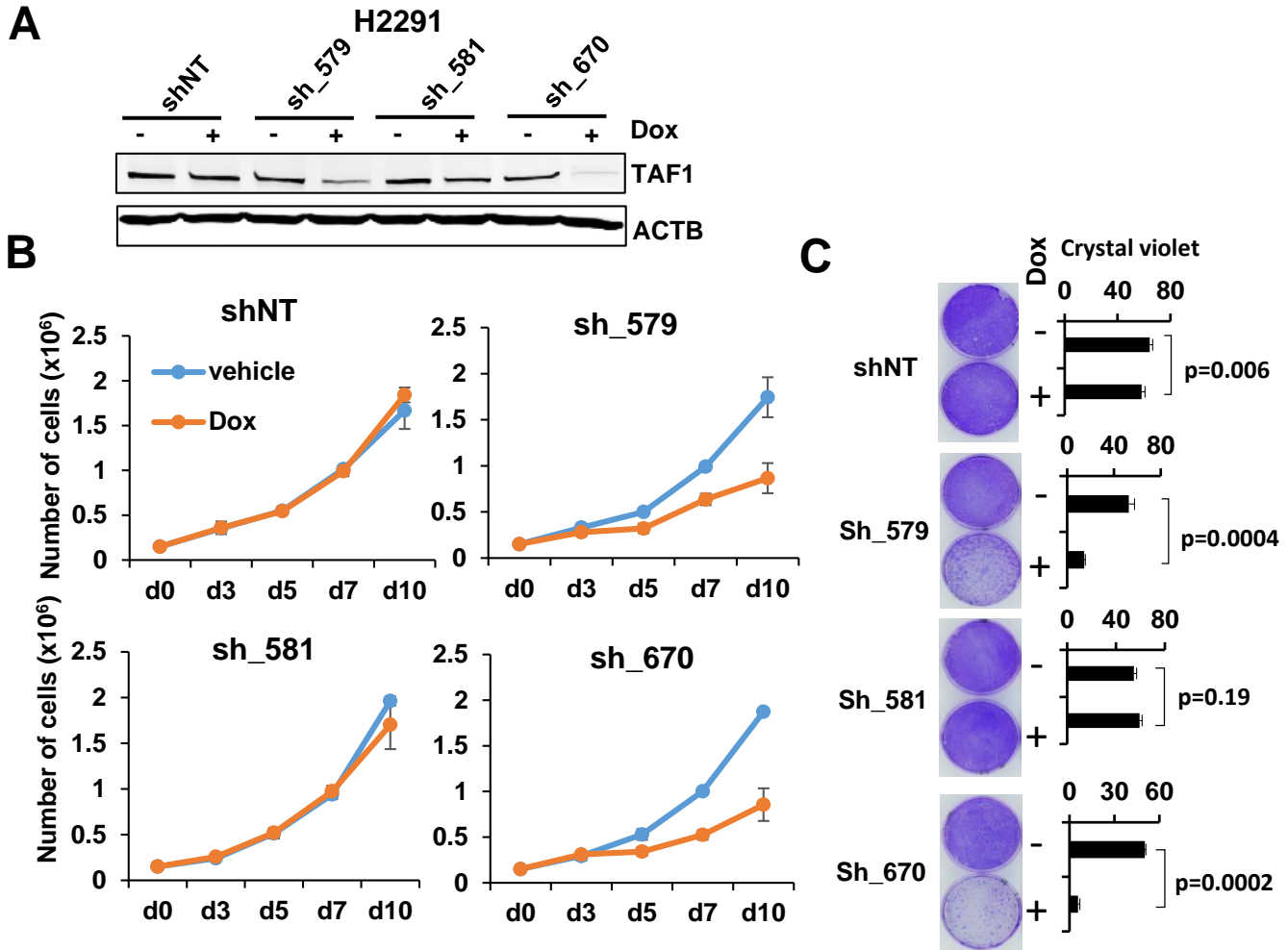
Supplementary Figure 7. Changes in cell cycle caused by BAY-299 treatment and TAF1 knock down in H322. (a) Cell cycle distribution of H322 cells treated with 200nM JQ1 or 2µM BAY-299 or BAY-299N the indicated compounds for 48 and 72 hours (b) Percentage of cells in each phase of the cell cycle. Mean and standard deviation from three replicates are shown. (d) Cell cycle distribution of K562 cells treated with vehicle or doxycycline for 5 days, reseeded at low confluency and harvested at 48 and 72 hours. The mean from three replicates of the percentage of cells in G0/G1 is indicated. *p<0.05, **p<0.005, ***p<0.0005.

A

sh_670
sh_578

BRD

TAF1 | NTK | HAT | | CTK | 1893aa

sh_579   sh_581

B

KMS11

shNT   sh_578   sh_579   sh_581   sh_670

- + | - + | - + | - + | - +   Dox

TAF1

ACTB

C

shNT

Cell Titer

vehicle
Dox

60
40
20
0

d0 d3 d5 d7

sh578

60
40
20
0

d0 d3 d5 d7

sh579

60
40
20
0

d0 d3 d5 d7

sh581

60
40
20
0

d0 d3 d5 d7

sh670

60
40
20
0

d0 d3 d5 d7

A

H1568

shNT   sh_579   sh_581   sh_670

Dox
- +   - +   - +   - +

TAF1

ACTB

B

shNT

Number of cells (x10^6)

vehicle
Dox

d0 d3 d5 d7 d10

sh_579

d0 d3 d5 d7 d10

sh_581

Number of cells (x10^6)

d0 d3 d5 d7 d10

sh_670

d0 d3 d5 d7 d10

C

Dox Crystal violet

0 30 60

shNT - + p=0.05

0 30 60

sh_579 - + p=0.0009

0 30 60

sh_581 - + p=0.12

0 25 50

sh_670 - + p=0.0009

A

H322

B

C

Crystal violet

D

A

H2291

shNT   sh_579   sh_581   sh_670

− +   − +   − +   − +   Dox

TAF1

ACTB

B

shNT

Number of cells (x10⁶)

2.5
2
1.5
1
0.5
0

vehicle
Dox

d0   d3   d5   d7   d10

sh_579

2.5
2
1.5
1
0.5
0

d0   d3   d5   d7   d10

sh_581

Number of cells (x10⁶)

2.5
2
1.5
1
0.5
0

d0   d3   d5   d7   d10

sh_670

2.5
2
1.5
1
0.5
0

d0   d3   d5   d7   d10

C

Dox   Crystal violet

shNT

0   40   80
−
+
p=0.006

Sh_579

0   40   80
−
+
p=0.0004

Sh_581

0   40   80
−
+
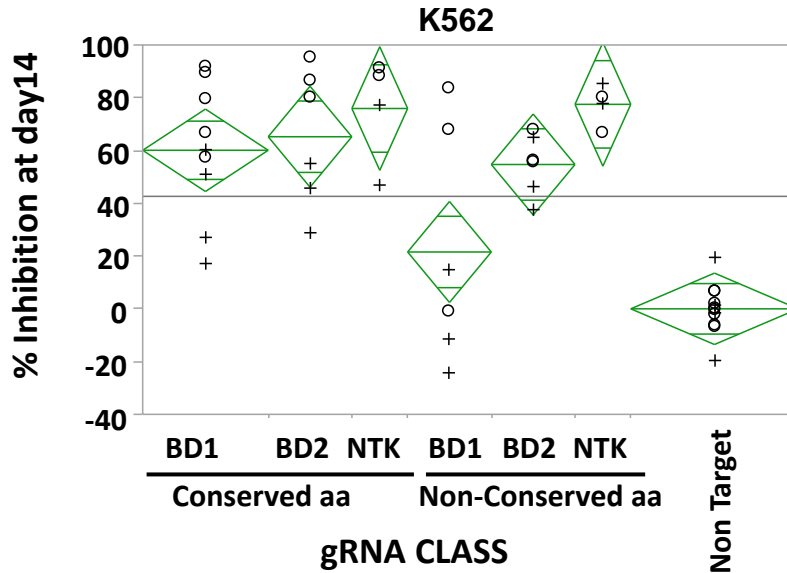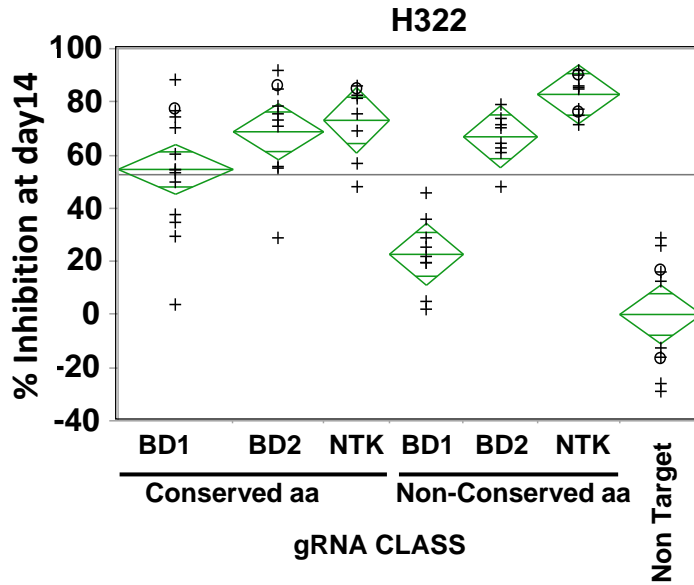p=0.19

Sh_670

0   30   60
−
+
p=0.0002

A

B

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
Non-Cons AA, NTK	Non-Target	77.49523	13.38776	35.9514	119.0390	<.0001 *	
Cons AA, NTK	Non-Target	75.92203	13.38776	34.3782	117.4658	<.0001 *	
Cons AA, BRD2	Non-Target	65.22455	11.59414	29.2466	101.2025	<.0001 *	
Cons AA, BRD1	Non-Target	60.08754	10.22507	28.3579	91.8172	<.0001 *	
Non-Cons AA, NTK	Non-Cons AA, BRD	55.94939	14.96797	9.5020	102.3968	0.0096 *	
Non-Cons AA, BRD	Non-Target	54.70082	11.59414	18.7228	90.6788	0.0005 *	
Cons AA, NTK	Non-Cons AA, BRD	54.37619	14.96797	7.9288	100.8236	0.0128 *	
Cons AA, BRD2	Non-Cons AA, BRD	43.67871	13.38776	2.1349	85.2225	0.0337 *	
Cons AA, BRD1	Non-Cons AA, BRD	38.54170	12.22130	0.6176	76.4658	0.0442 *	

A

H322

B

Means Comparisons						
Comparisons for all pairs using Tukey-Kramer HSD						
Ordered Differences Report						
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Non-Cons AA, NTK	Non-Target	82.81181	7.832423	58.3179	107.3057	<.0001 *
Cons AA, NTK	Non-Target	73.05188	8.307539	47.0721	99.0316	<.0001 *
Cons AA, BRD2	Non-Target	68.74345	7.652343	44.8127	92.6742	<.0001 *
Non-Cons AA, BRD	Non-Target	66.85186	8.047050	41.6867	92.0170	<.0001 *
Non-Cons AA, NTK	Non-Cons AA, BRD	60.18588	8.047050	35.0207	85.3510	<.0001 *
Cons AA, BRD1	Non-Target	54.57846	7.251415	31.9015	77.2554	<.0001 *
UNST	Non-Target	51.96897	9.592720	21.9701	81.9678	<.0001 *
Cons AA, NTK	Non-Cons AA, BRD	50.42595	8.510192	23.8125	77.0394	<.0001 *
Cons AA, BRD2	Non-Cons AA, BRD	46.11752	7.871881	21.5002	70.7349	<.0001 *
Non-Cons AA, BRD	Non-Cons AA, BRD	44.22593	8.256099	18.4071	70.0448	<.0001 *
Cons AA, BRD1	Non-Cons AA, BRD	31.95253	7.482725	8.5522	55.3529	0.0015 *
Non-Cons AA, NTK	Cons AA, BRD1	28.23334	7.251415	5.5564	50.9103	0.0053 *

A

B

C