Supporting Information

Negative Modulation of the Angiogenic Cascade Induced by Allosteric Kinesin Eg5 Inhibitors in a Gastric Adenocarcinoma In Vitro Model

Alessia Ricci [1], Marialucia Gallorini [1], Donatella Del Bufalo [2], Amelia Cataldi [1], Ilaria D'Agostino [1], Simone Carradori [1,†] and Susi Zara [1,†,*]

[1] Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, , 66100 Chieti, Italy; alessia.ricci@unich.it (A.R.); marialucia.gallorini@unich.it (M.G.); amelia.cataldi@unich.it (A.C.); ilaria.dagostino1@unich.it (I.D.); simone.carradori@unich.it (S.C.)

[2] Preclinical Models and New Therapeutic Agents Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy; donatella.delbufalo@ifo.it (D.D.B)

* Correspondence: susi.zara@unich.it; Tel.: +39-0871-3554507

† The authors equally contributed to this work.

List of Contents

Dataset for Kinesins topics in PubMed Database

Phylogenetic analysis

Protein-chemicals interaction network

Protein-protein association network

ANOVA and Tukey's multiple comparison for data shown in Figures 5, 6, 7, 8, 9, and 12.

Dataset for Kinesins topics in PubMed Database

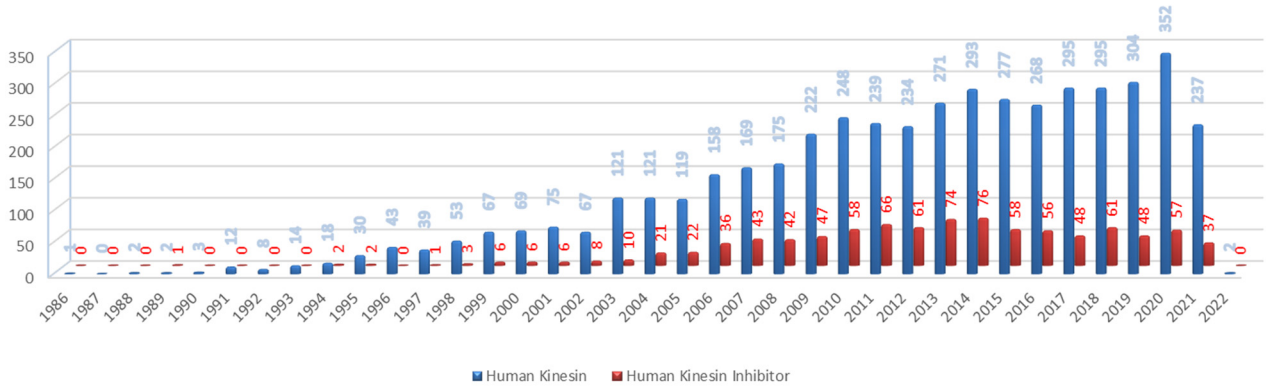

Figure S1. Histogram view of the distribution of datasets found for search queries «Human Kinesin» (blue) and «Human Kinesin Inhibitors» (red) per year (1986-2022) in PubMed Database.

Phylogenetic analysis

A phylogenetic tree, reported in Figure S2, was conducted on the NGphylogeny.fr free service (https://ngphylogeny.fr, accessed on 6 January 2022) [1,2] through the "one click workflow" tool and FastME2.0 program to gain the "distances" among phylogenies based on the balanced minimum evolution approach. Sequences were selected from UniProt Database by searching the following keywords "name:kinesin-like protein" and "organism: *Homo sapiens* (Human) [9606]", filtering only reviewed sequences.

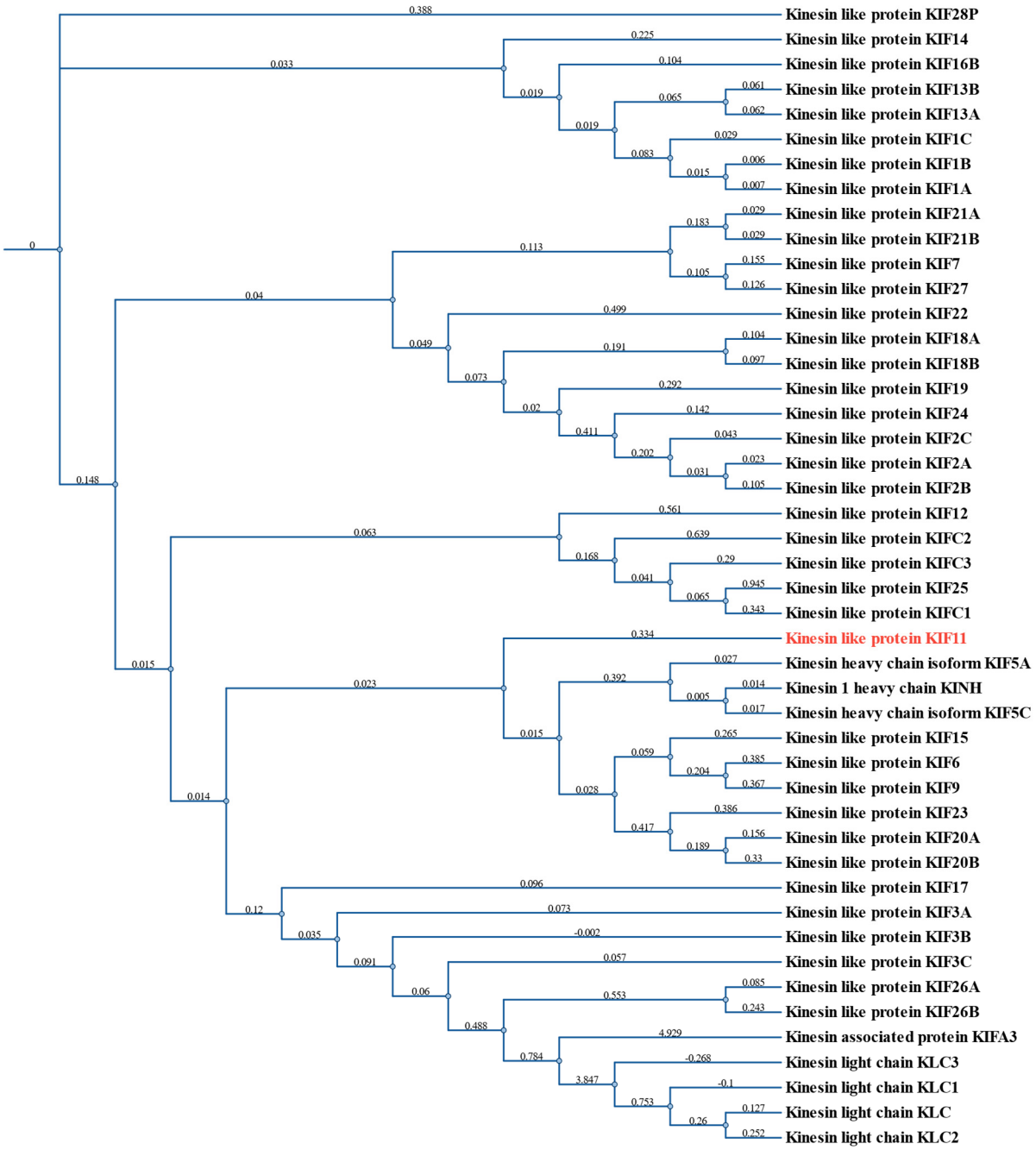

Figure S2. Phylogenetic dendrogram for human Kinesins.

The evolutionary model was determined automatically using NGphylogeny.fr free service. The tree branches are drawn to scale, which represents the number of differences between sequences through evolution. In red, human KIF11. The figure was prepared by means of the Interactive Tree Of Life (iTOL) (https://itol.embl.de/, accessed on 06 January 2022) [3].

Protein-chemicals interaction network

The bioinformatics software platform STITCH (http://stitch.embl.de) was used to predict direct interactions of FIK-11 with compounds.

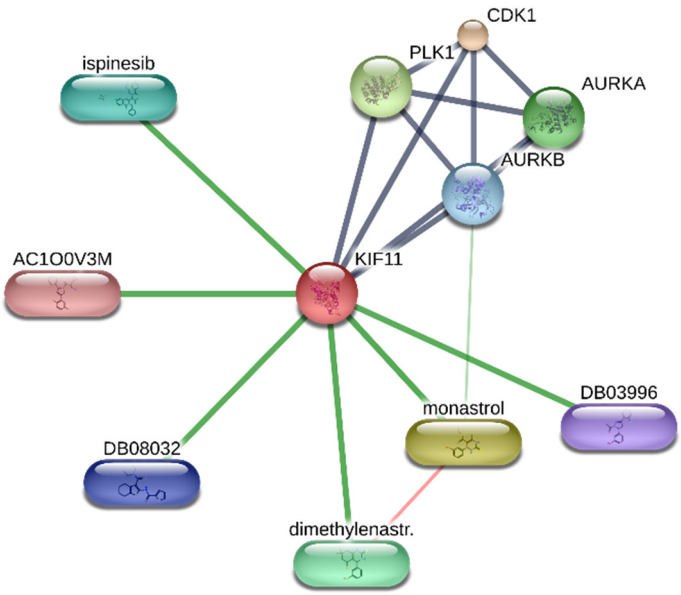

Figure S3. The targets–components analysis performed on the STITCH platform. Lines are related to confidence view: stronger associations are represented by thicker lines. Protein-protein interactions are shown in grey, chemical-protein interactions in green and interactions between chemicals in red.

Proteins are CDK1, Cyclin-dependent kinase 1; PLK1, Polo-like kinase 1; AURKA, Aurora kinase A; AURKB, Aurora kinase B.

Protein-protein association network

The bioinformatics software platform STRING (http://string.embl.de) was used to predict direct interactions of FIK-11 with other proteins.

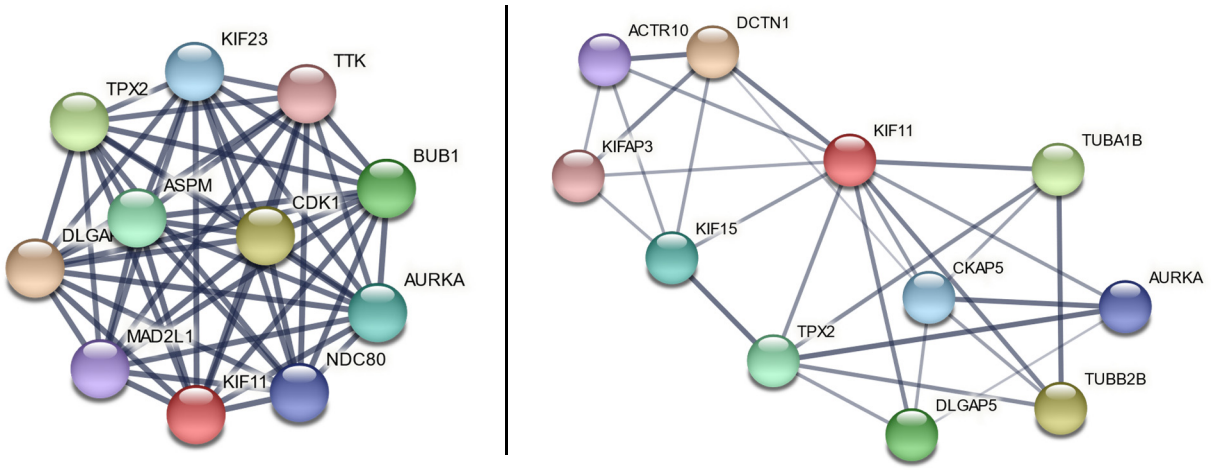

Figure S4. The protein-protein association analyses performed on the STRING platform. On the right, both functional and physical protein associations; on the left, only physical protein association (proteins are involved in a physical complex). Lines are related to confidence view: stronger associations are represented by thicker lines.

Proteins are DLGAP5, Disks large-associated protein 5 [4]; CDK1, cyclin-dependent kinase 1; TPX2, microtubule nucleation factor [5]; BUB1, Mitotic checkpoint serine/threonine-protein kinase; ASPM, Abnormal spindle-like microcephaly-associated protein; AURKA, Aurora kinase A [6]; KIF23, Kinesin-like protein; NDC80, Kinetochore protein NDC80 homolog; MAD2L1, Mitotic spindle assembly checkpoint protein MAD2A; TTK, Serine/threonine-protein kinase ttk/mps1; DCTN1, Dynactin subunit 1 [7]; TUBB2B, Tubulin beta-2B chain; TUBA1B, Tubulin alpha-1B chain; KIF15, Kinesin-like protein [8]; CKAP5, Cytoskeleton-associated protein 5; ACTR10, Actin related protein 10 homolog; KIFAP3, Kinesin-associated protein 3.

ANOVA and Tukey's multiple comparison for data shown in Figures 5, 6, 7, 8, 9, and 12

Table S1. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test performed by means of the Prism 5.0 software (GraphPad, San Diego, CA, USA).

ANOVA Analysis of Figure 5					
Table Analysed	**G1 Phase-48 h**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	20.99				
R squared	0.913				
ANOVA Table	***SS***	***df***	***MS***		
Treatment (between columns)	2328	7	332.6		
Residual (within columns)	221.9	14	15.85		
Total	2550	21			
Tukey's Multiple Comparison Test	***Mean Diff.***	***q***	***Significant? P < 0.05?***	***Summary***	***95% CI of diff***
DMSO vs 41	31.51	12.26	Yes	***	18.68 to 44.33

Table Analysed	**S Phase-48 h**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	25.51				
R squared	0.9273				

ANOVA Table	*SS*	*df*	*MS*		
Treatment (between columns)	392.5	7	56.07		
Residual (within columns)	30.77	14	2.198		
Total	423.3	21			

Tukey's Multiple Comparison Test	*Mean Diff.*	*q*	*Significant? P < 0.05?*	*Summary*	*95% CI of diff*
DMSO vs HSD	8.02	9.37	Yes	***	3.749 to 12.29
DMSO vs 2	10.37	12.12	Yes	***	6.099 to 14.64
DMSO vs 41	9.68	10.12	Yes	***	4.905 to 14.46
K858 vs HSD+K858	5.903	6.897	Yes	**	1.632 to 10.17
Table Analysed	**G2 Phase-48 h**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	16.62				
R squared	0.8926				
ANOVA Table	*SS*	*df*	*MS*		
Treatment (between columns)	3353	7	478.9		
Residual (within columns)	403.4	14	28.81		
Total	3756	21			
Tukey's Multiple Comparison Test	*Mean Diff.*	*q*	*Significant? P < 0.05?*	*Summary*	*95% CI of diff*
DMSO vs 41	-41.19	11.89	Yes	***	-58.47 to -23.90

ANOVA Analysis of Figure 6					
Table Analysed	**PI3K protein expression 48 h (Fig. 6B)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	36.06				
R squared	0.9404				
ANOVA Table	*SS*	*df*	*MS*		
Treatment (between columns)	0.01639	7	0.002341		
Residual (within columns)	0.001039	16	0.00006492		
Total	0.01743	23			
Tukey's Multiple Comparison Test	*Mean Diff.*	*q*	*Significant? P < 0.05?*	*Summary*	*95% CI of diff*
DMSO vs 2	0.02947	6.335	Yes	**	0.006692 to 0.05225
DMSO vs 41	0.06465	13.9	Yes	***	0.04187 to 0.08743
DMSO vs K858	0.06726	14.46	Yes	***	0.04448 to 0.09004
Table Analysed	**PI3K protein expression 72 h (Fig. 6B)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				

Number of groups	8				
F	60.02				
R squared	0.9633				
ANOVA Table	***SS***	***df***	***MS***		
Treatment (between columns)	0.05414	7	0.007734		
Residual (within columns)	0.002062	16	0.0001289		
Total	0.0562	23			
Tukey's Multiple Comparison Test	***Mean Diff.***	***q***	***Significant? P < 0.05?***	***Summary***	***95% CI of diff***
DMSO vs HSD	0.07024	10.72	Yes	***	0.03815 to 0.1023
DMSO vs 2	0.1224	18.68	Yes	***	0.09034 to 0.1545
DMSO vs 41	0.1149	17.53	Yes	***	0.08276 to 0.1469
DMSO vs K858	0.1571	23.97	Yes	***	0.1250 to 0.1892
Table Analysed	**p-Akt/Akt protein expression 48 h (Fig. 6C)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	230.1				
R squared	0.9902				
ANOVA Table	***SS***	***df***	***MS***		
Treatment (between columns)	0.87	7	0.1243		
Residual (within columns)	0.008642	16	0.0005401		
Total	0.8787	23			

Tukey's Multiple Comparison Test	*Mean Diff.*	*q*	*Significant? P < 0.05?*	*Summary*	*95% CI of diff*
DMSO vs 2	0.1949	14.53	Yes	***	0.1292 to 0.2606
DMSO vs 41	0.4006	29.85	Yes	***	0.3349 to 0.4663
DMSO vs K858	0.3442	25.65	Yes	***	0.2785 to 0.4099
Table Analysed	**p-Akt/Akt protein expression 72 h (Fig. 6C)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	544.6				
R squared	0.9958				
ANOVA Table	*SS*	*df*	*MS*		
Treatment (between columns)	3.401	7	0.4859		
Residual (within columns)	0.01428	16	0.0008922		
Total	3.415	23			
Tukey's Multiple Comparison Test	*Mean Diff.*	*q*	*Significant? P < 0.05?*	*Summary*	*95% CI of diff*
DMSO vs HSD	0.5713	33.13	Yes	***	0.4868 to 0.6557
DMSO vs 2	0.761	44.13	Yes	***	0.6766 to 0.8455
DMSO vs 41	1.168	67.74	Yes	***	1.084 to 1.253
DMSO vs K858	1.06	61.49	Yes	***	0.9760 to 1.145
2 vs HSD+2	0.2231	12.94	Yes	***	0.1387 to 0.3076

ANOVA Analysis of Figure 7					
Table Analysed	**VEGF protein expression 48 h (Fig. 7B)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	74.89				
R squared	0.9704				
ANOVA Table	***SS***	***df***	***MS***		
Treatment (between columns)	0.02415	7	0.00345		
Residual (within columns)	0.000737	16	0.00004606		
Total	0.02488	23			
Tukey's Multiple Comparison Test	***Mean Diff.***	***q***	***Significant? P < 0.05?***	***Summary***	***95% CI of diff***
DMSO vs HSD	0.02875	7.338	Yes	**	0.009564 to 0.04794
DMSO vs 41	0.06718	17.14	Yes	***	0.04799 to 0.08637
Table Analysed	**VEGF protein expression 72 h (Fig. 7B)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				

F	253.5				
R squared	0.9911				
ANOVA Table	***SS***	***df***	***MS***		
Treatment (between columns)	0.05936	7	0.008479		
Residual (within columns)	0.0005352	16	0.00003345		
Total	0.05989	23			
Tukey's Multiple Comparison Test	***Mean Diff.***	***q***	***Significant? P < 0.05?***	***Summary***	***95% CI of diff***
DMSO vs 2	0.07029	21.05	Yes	***	0.05394 to 0.08665
DMSO vs 41	0.07851	23.51	Yes	***	0.06215 to 0.09486
DMSO vs K858	0.1186	35.51	Yes	***	0.1022 to 0.1349
2 vs HSD+2	0.02893	8.664	Yes	***	0.01258 to 0.04528
41 vs HSD+41	0.04408	13.2	Yes	***	0.02773 to 0.06043

ANOVA Analysis of Figure 8					
Table Analysed	**VEGF secretion 48 h (Fig. 8A)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	102.7				
R squared	0.989				
ANOVA Table	***SS***	***df***	***MS***		
Treatment (between columns)	11910000	7	1702000		
Residual (within columns)	132600	8	16570		
Total	12040000	15			
Tukey's Multiple Comparison Test	***Mean Diff.***	***q***	***Significant? P < 0.05?***	***Summary***	***95% CI of diff***
DMSO vs 2	1219	13.39	Yes	***	709.7 to 1729
DMSO vs 41	2237	24.57	Yes	***	1727 to 2746
DMSO vs K858	1733	19.04	Yes	***	1223 to 2242
Table Analysed	**VEGF secretion 72 h (Fig. 8B)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	114.1				
R squared	0.9901				

ANOVA Table	***SS***	***df***	***MS***		
Treatment (between columns)	64470000	7	9210000		
Residual (within columns)	646000	8	80750		
Total	65110000	15			
Tukey's Multiple Comparison Test	***Mean Diff.***	***q***	***Significant? P < 0.05?***	***Summary***	***95% CI of diff***
DMSO vs HSD	1276	6.351	Yes	*	151.5 to 2401
DMSO vs 2	2333	11.61	Yes	***	1208 to 3458
DMSO vs 41	5905	29.39	Yes	***	4780 to 7029
DMSO vs K858	4281	21.3	Yes	***	3156 to 5405

ANOVA Analysis of Figure 9					
Table Analysed	**p-Erk/Erk protein expression 48h (Fig. 9B)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	228.5				
R squared	0.9901				
ANOVA Table	*SS*	*df*	*MS*		
Treatment (between columns)	0.04112	7	0.005874		
Residual (within columns)	0.0004112	16	0.0000257		
Total	0.04153	23			
Tukey's Multiple Comparison Test	*Mean Diff.*	*q*	*Significant? P < 0.05?*	*Summary*	*95% CI of diff*
DMSO vs HSD	0.05397	18.44	Yes	***	0.03963 to 0.06830
DMSO vs 2	0.07823	26.73	Yes	***	0.06389 to 0.09256
DMSO vs 41	0.1372	46.89	Yes	***	0.1229 to 0.1516
DMSO vs K858	0.1333	45.53	Yes	***	0.1189 to 0.1476
Table Analysed	**p-Erk/Erk protein expression 72h (Fig. 9B)**				
One-way analysis of variance					
P value	< 0.0001				

P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	8				
F	984.7				
R squared	0.9977				
ANOVA Table	***SS***	***df***	***MS***		
Treatment (between columns)	0.1829	7	0.02614		
Residual (within columns)	0.0004246	16	0.00002654		
Total	0.1834	23			
Tukey's Multiple Comparison Test	***Mean Diff.***	***q***	***Significant? P < 0.05?***	***Summary***	***95% CI of diff***
DMSO vs 2	0.1293	43.46	Yes	***	0.1147 to 0.1438
DMSO vs 41	0.1908	64.16	Yes	***	0.1763 to 0.2054
DMSO vs K858	0.1864	62.66	Yes	***	0.1718 to 0.2009
41 vs HSD+41	0.06067	20.4	Yes	***	0.04610 to 0.07523
K858 vs HSD+K858	0.02993	10.06	Yes	***	0.01536 to 0.04449

ANOVA Analysis of Figure 12					
Table Analysed	**% Cut width reduction 6 h (Fig. 12)**				
One-way analysis of variance					
P value	0.0599				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	3.751				
R squared	0.5845				
ANOVA Table	***SS***	***df***	***MS***		
Treatment (between columns)	154.8	3	51.61		
Residual (within columns)	110.1	8	13.76		
Total	264.9	11			
Tukey's Multiple Comparison Test	***Mean Diff.***	***q***	***Significant? P < 0.05?***	***Summary***	***95% CI of diff***
DMSO vs HSD+2	9.733	4.545	Yes	*	0.03375 to 19.43
Table Analysed	**% Cut width reduction 24 h (Fig. 12)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	46.34				
R squared	0.9456				

ANOVA Table	*SS*	*df*	*MS*		
Treatment (between columns)	4044	3	1348		
Residual (within columns)	232.7	8	29.09		
Total	4277	11			
Tukey's Multiple Comparison Test	*Mean Diff.*	*q*	*Significant? P < 0.05?*	*Summary*	*95% CI of diff*
DMSO vs HSD	43.57	13.99	Yes	***	29.46 to 57.67
DMSO vs 2	24.9	7.996	Yes	**	10.80 to 39.01
DMSO vs HSD+2	45.7	14.68	Yes	***	31.60 to 59.81
2 vs HSD+2	20.8	6.68	Yes	**	6.698 to 34.91
Table Analysed	**% Cut width reduction 72 h (Fig. 12)**				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	50.84				
R squared	0.9502				
ANOVA Table	*SS*	*df*	*MS*		
Treatment (between columns)	3373	3	1124		
Residual (within columns)	176.9	8	22.11		
Total	3549	11			
Tukey's Multiple Comparison Test	*Mean Diff.*	*q*	*Significant? P < 0.05?*	*Summary*	*95% CI of diff*
DMSO vs HSD	36.97	13.62	Yes	***	24.68 to 49.27
DMSO vs 2	24.16	8.898	Yes	**	11.86 to 36.45
DMSO vs HSD+2	44.02	16.21	Yes	***	31.72 to 56.32
2 vs HSD+2	19.86	7.317	Yes	**	7.569 to 32.16

References

1. Dereeper, A.; Guignon, V.; Blanc, G.; Audic, S.; Buffet, S.; Chevenet, F.; Dufayard, J.F.; Guindon, S.; Lefort, V.; Lescot, M.; Claverie, J.M.; Gascuel, O. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* 2008, 36, W465–W469. doi: 10.1093/nar/gkn180.
2. Lemoine, F.; Correia, D.; Lefort, V.; Doppelt-Azeroual, O.; Mareuil, F.; Cohen-Boulakia, S.; Gascuel, O. NGPhylogeny.fr: new generation phylogenetic services for non-specialists. *Nucleic Acids Res.* 2019, 47, W260–W265. doi: 10.1093/nar/gkz303.
3. Letunic, I.; Bork, P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 2021, 49, W293–W296. doi:10.1093/nar/gkab301.
4. Liu, J.; Liu, Z.; Zhang, X.; Gong, T.; Yao, D. Examination of the expression and prognostic significance of DLGAPs in gastric cancer using the TCGA database and bioinformatic analysis. *Mol. Med. Rep.* 2018, 18, 5621–5629. doi:10.3892/mmr.2018.9574.
5. Huang, C.; Hu, C.G.; Ning, Z.K.; Huang, J.; Zhu, Z.M. Identification of key genes controlling cancer stem cell characteristics in gastric cancer. *World J Gastrointest Surg.* 2020, 12, 442–459. doi:10.4240/wjgs.v12.i11.442.
6. Dar, A.A.; Zaika, A.; Piazuelo, M.B.; Correa, P.; Koyama, T.; Belkhiri, A.; Washington, K.; Castells, A.; Pera, M.; El-Rifai, W. Frequent overexpression of Aurora Kinase A in upper gastrointestinal adenocarcinomas correlates with potent antiapoptotic functions. *Cancer* 2008, 112, 1688–1698. doi: 10.1002/cncr.23371.
7. Zhang, J.; Goel, A.; Zhu, L. Identification of Novel Alternative Splicing Events Associated With Tumorigenesis, Protein Modification, and Immune Microenvironment in Early-Onset Gastric Cancer. *Front Oncol.* 2021, 11 640272. doi: 10.3389/fonc.2021.640272.
8. Ding, L.; Li, B.; Yu, X.; Li, Z.; Li, X.; Dang, S.; Lv, Q.; Wei, J.; Sun, H.; Chen, H.; Liu, M.; Li, G. KIF15 facilitates gastric cancer via enhancing proliferation, inhibiting apoptosis, and predict poor prognosis. *Cancer Cell Int.* 2020, 20, 125. doi:10.1186/s12935-020-01199-7.